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1957

Photomicrography

IN THEORY AND PRACTICE

By

The Late CHARLES PATTEN SHILLABER

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PREFACE

Of the four outstanding laboratory optical instruments — the camera, the telescope, the spectroscope, and the microscope — the microscope with its accessories is by far the least understood, the most inefficiently operated, and the most abused. As Dr. Adrianus Pijper,[1] in his presidential address before the South African Association for the Advancement of Science, truly said, in effect, that there are many microscopes but few microscopists. That this should be so is indeed unfortunate, since in the practice of many professions a diagnosis or report, based on the use of the microscope, may have a bearing on the treatment of a disease, the investment of capital, or the correct or incorrect processing of important material.

The present book has been organized, after no little thought, with the underlying plan of bringing together the technician and the microscope, at the outset. During many years of conducting private instruction in the use of the microscope, I have found this system of approach to be the best way of holding the student's interest and of stimulating his initiative and resourcefulness. The fundamental principles of microscopy are certainly based on optics and to some extent on mechanics, and, although I have made no attempt to treat either subject as such, it has seemed advisable to state some of the problems of each, but only to the extent required to deal with such questions as have been submitted to me by earnest workers, or to encourage clearer understanding of the compound microscope and its operation in the field of photomicrography.

In a discussion of both the practical application of an instrument and its theoretical implications, a strictly logical order of presentation might be clumsy and certainly would be tedious to follow. Accordingly, in this work, though I may occasionally have seemed to sacrifice order for the sake of interest, I hope that in no place has clarity been compromised.

In photomicrography, failure to produce evenly exposed negatives is usually due to poorly aligned apparatus; therefore, in the following

[1] Adrianus Pijper, "The Microscope in Biology," presidential address to Section C, South African Association for the Advancement of Science. *South African Journal of Science*, **36**, 58–72 (1939). Reprinted in the *Journal of the Royal Microscopical Society*, Series III, **42**, 36–50 (1942).

pages, stress is placed on the necessity for exact and precise alignment of all optical parts, and the microscopist is led to judge the accuracy of his own technique independently of all factory or salesroom-made adjustments. He should learn to find the proper balance for his optical equipment, and to select any required lens combination correctly, and from first-hand knowledge to ascertain whether, optically speaking, his microscope is, or is not, out of balance. The extent to which the microscopist is able to do these things will largely determine his success as a photomicrographer. In other words, he must develop a flair for "finding his way around" before he can experience real pleasure and assurance in the use of his microscopical equipment.

The photomicrographs in this book have been reproduced by the half-tone process, and it should be borne in mind that such form of reproduction can never exactly duplicate the fidelity and quality of the original prints; there is an inevitable loss in tonal values, crispness, and detail. The data accompanying the pictures are sufficient for the purposes intended, but figures denoting bellows extension and exposure time have been omitted purposely because they would be of little or no interest to the expert and might serve to mislead the novice.

It has been called to my attention, by reviewers of the manuscript, that discussion of such branches of photomicrography as work with polarized light, color photography, ultraviolet and infrared radiation, stereoscopic pictures, and other highly specialized fields has been omitted. However, rather than unduly increase the size of the present volume it was my thought to add to the treatise from time to time complete information regarding these and other important branches of microscopy. The material included herein is basic and can be applied to most of these fields. Therefore any subsequent discussion of these branches of microscopy will be concentrated chiefly on the use of specialized apparatus and materials.

ACKNOWLEDGMENTS

Because this volume is largely a book of reference, many authorities have been consulted and tables and excerpts from the works of others have been quoted freely, by permission. Many business firms and private individuals have graciously furnished data, made investigations, and lent equipment which have aided materially in compiling the book. Among those to whom I especially wish to express sincere thanks are Miss Mabel A. Hammond, for valuable and timely aid in styling and reading the manuscript and printer's proof; Dr. A. C.

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Bausch and Lomb Optical Co., Spencer Lens Co., E. Leitz, Inc., and Carl Zeiss, Inc., have contributed many cuts and much advice. Eastman Kodak Co., Agfa Ansco, and Defender Photo Supply Co. have been very cooperative and furnished much information, while the Evaporated Metal Films Corp. and General Electric Co. have provided important data.

Companies which permitted the use of many of the pictures are the New York Telephone Co., National Can Co., English Mica Co., Adolph I. Buehler Co., Hoffmann-LaRoche, Inc., Ross and Rowe, Inc., Mallinckrodt Chemical Works, and Artra Cosmetics, Inc.

C. P. S.

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March 1944

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CHAPTER I

THE MICROSCOPE — NOMENCLATURE AND DEFINITIONS

To build and design an instrument as complicated and delicate as the compound microscope, the mechanical and optical talent of the microscope maker is placed under constant challenge, for nothing much short of perfection in mechanical operation and optical excellence should suffice. To this end the utmost precision and attention to detail have been achieved in the manufacture and assembly of the many parts which go to make up the finished machine.

In order to make and maintain proper focus of the microscope, adjustments which are sensitive to considerably less than 1/100,000 of an inch are often required, and the parts involved must move smoothly and exactly over a considerable range and with entire freedom from apparent lost motion. The figure of the surfaces of the best lenses must be accurate to within a small fraction of a wavelength of sodium light; the lens assembly should be faultless; and the precision of the machined surfaces must approach or equal that of gauge blocks.

Anton van Leeuwenhoek, a Dutch scientist who lived from 1632 to 1723, made and used lenses with relatively high magnification, quite comparable in this respect with our modern 2- and 1.5-mm objectives, but according to Rooseboom[1] the numerical aperture of these lenses did not exceed 0.15, the equivalent of our best modern 32-mm lenses. Today, for visual work alone, numerical apertures of 1.25 and 1.3 are common, 1.4 not rare, and 1.6 possible.[2] Objectives, oculars, and con-

[1] M. Rooseboom, *J. Roy. Micr. Soc.*, **59**, 177, 1939.

[2] The history of the microscope and the history of optics as applied to the microscope are thoroughly covered by Alfred N. Disney, editor, in collaboration with C. F. Hill and W. E. Watson Baker in *Origin and Development of the Microscope,* Royal Microscopical Society, London, 1928. Description of the early use of lenses and microscopes up to the invention of the achromatic microscope, about 1820, and a description of the catadioptric or reflecting microscope, which preceded the achromatic microscope, is given by Reginald S. Clay and Thomas H. Court, *The History of the Microscope,* London, 1932. The historical aspect of the microscope objective is discussed by A. H. Bennett, in "The Development of the Microscope Objective," *J. Optical Soc. Am.*, **33**, 123–128, 1943. The paper was presented at a meeting of the Society, Oct. 30–31, 1942. An excellent bibliography is included.

densers are available in almost endless variety for special as well as ordinary work. Each type of lens, however, has its special optical qualities, and the conditions under which it must function to produce its best images vary accordingly. In order to take advantage of the many lenses and combinations of lenses that are available, the photomicrographer must first thoroughly understand the underlying principles of the microscope and its optics; in effect, he must be completely conversant with microscope theory. He must know the possibilities and limitations of his equipment and must cultivate a keen sense of discrimination in adjusting and selecting his lenses for the work in hand. In this way he will learn the meaning of the effects produced on the final image by even slight errors of adjustment in the different optical and mechanical units which make up the complete instrument. The well-informed microscopist will also be able to use to the best advantage all the numerous adjuncts and modern accessories to the microscope, which are second in importance only to the instrument itself.

Sec. 1. The Nomenclature of the Microscope. Before any attempt is made to use the microscope for photomicrography, the technician should become thoroughly familiar with the functions of the various parts of the instrument. This involves the study of fundamental optical principles as they apply to, and affect, the formation of the microscope image, as well as a study of the mechanics by which the various optical adjustments are made and maintained. Without such information the technician is working in an uncertain manner and oftentimes is trying to accomplish something completely beyond the range of his equipment, or even beyond the province of optical microscopy. As a preliminary step the technician should either learn, or refresh his memory by reviewing, the names of the various parts of the microscope as shown in Fig. 1.

The parts of the microscope named in Fig. 1 can be compared with the corresponding parts on the actual instrument; even though parts in the figure may show slight differences in construction and appearance, essentially they are like those on the instrument, and functionally they are the same.

The booklets of instruction, which generally accompany new microscopes, are helpful. Lost booklets will cheerfully be replaced by the manufacturer of the instrument. It is well worth while to read the instruction book carefully, with the microscope at hand, and so become familiar with the various parts and the operating characteristics of each.

The accompanying chart outlines in concise form all the steps, later

described in detail, necessary for setting up the microscope and camera and operating them. Undoubtedly many experienced technicians will be able to use it, at least in part, as a guide in their work, before going

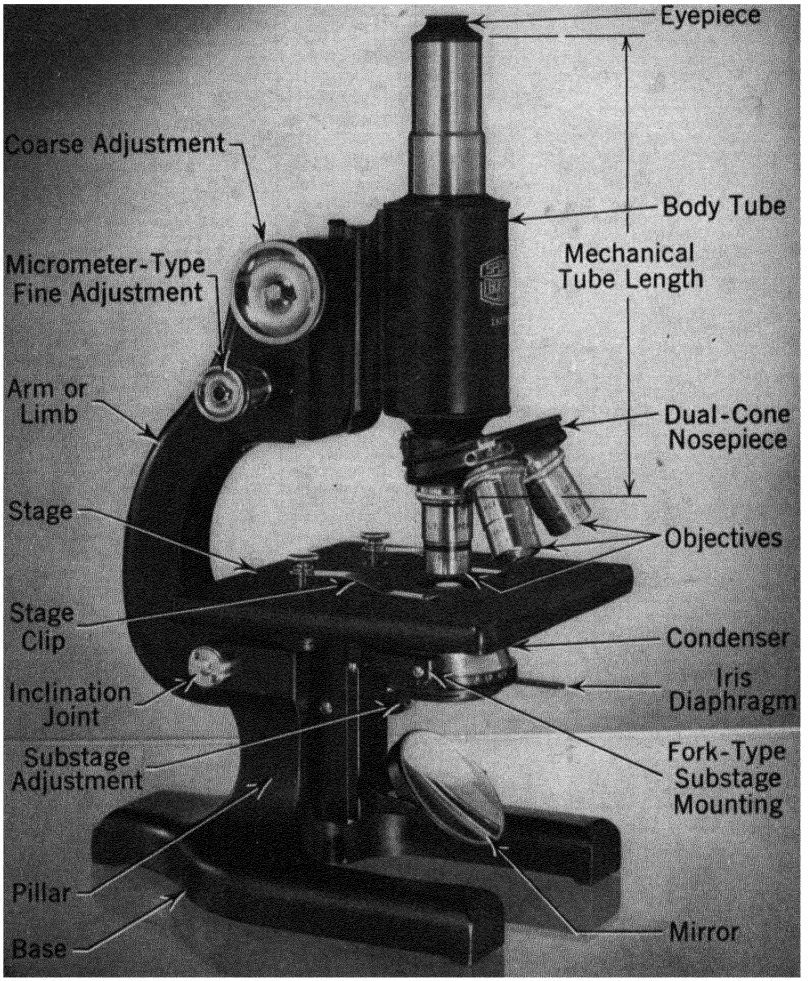
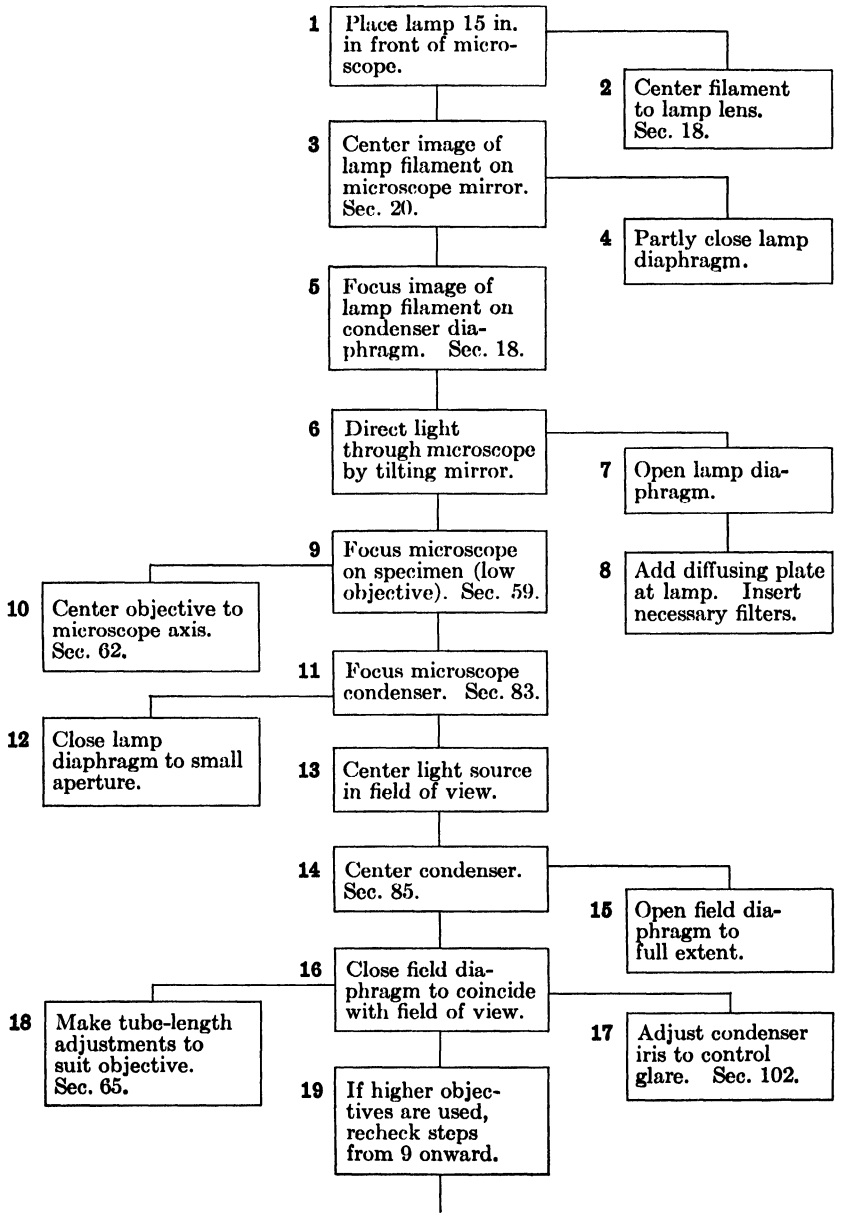


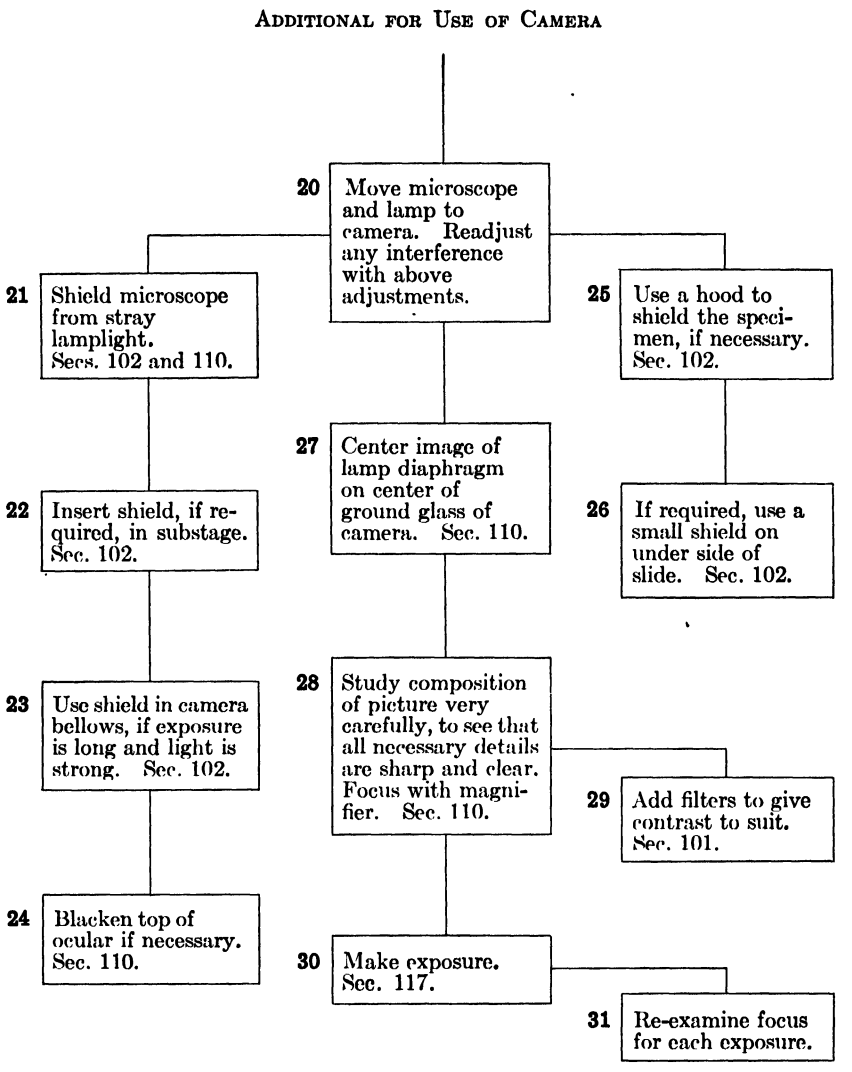
FIG. 1. The mechanical parts and arrangement of a typical microscope stand. (Through the courtesy of Spencer Lens Co.)

deeper into the book. It will also serve, at any time, as a reference for procedure, the steps of which might otherwise have to be looked up separately in the following pages. However, this chart is not intended as a substitute for the information contained in the various

CHART

STEPS IN ADJUSTING AND ILLUMINATING MICROSCOPE.
ILLUMINATION BY METHOD I, SEC. 18





sections, for it is hardly possible to reduce to such a painless level the acquisition of the knowledge essential for the practice of good photomicrography.

The steps as outlined in the chart include many adjustments that will not necessarily have to be repeated each time a photomicrograph is made. A little study of the plan by anyone having some experience with a microscope will indicate the steps to which this refers. However, it is essential that the photomicrographer know the state of

all the adjustments, for too often a picture is spoiled, and time and money wasted, through careless or improper regulation of optical and mechanical arrangements.

For those who are untrained in the use of the microscope, the following abbreviated instructions are offered, so that even the uninitiated will be able to follow the text with the microscope in actual operation. In addition, full advantage should be taken of the information contained in the instruction booklets already mentioned.

1. The microscope lamp is placed about 15 inches in front of the microscope. The lamp filament is focused on the center of the microscope mirror. A diffusing plate (ground glass) is inserted at the lamp, and the iris of the lamp is opened to its full extent. The microscope mirror is now tipped to direct light into the front lens of the objective.

2. A specimen is placed on the microscope stage, and a low-power objective (such as the 16-mm) is turned in. A 10× ocular is inserted in the drawtube. Before any attempt to focus, the tube should be lowered by the coarse adjustment until it nearly touches the slide. A second or third diffusing plate or neutral filter must then be inserted between the lamp and microscope, to protect the eyes. Using the coarse adjustment, the operator can raise the microscope tube slowly while observing the field, thus bringing the specimen into focus.

3. When the microscope is focused the iris diaphragm at the lamp is closed to a small opening and its image is swung into the center of the field of view by manipulating the mirror. While the light source is being centered the condenser of the microscope can be racked up or down to bring the image of the lamp diaphragm into as sharp focus as possible. The iris at the lamp can then be opened to light up the whole field of view.

4. Higher objectives can be used as needed, all preliminary examination and regulation of lighting system and condenser adjustment having been made with the lower objectives. If the objective-changing system is of the usual revolving type, all objectives on the nosepiece should be mutually parfocal; thus as each objective is turned into position only a little movement of the tube, with the fine adjustment, will be required to restore focus. When oil-immersion objectives are used, the microscope tube will of necessity be raised before oiling; refocusing should then be accomplished by lowering the tube very slowly by means of the coarse-adjustment mechanism. When preliminary focus has once been attained, subsequent focusing, for critical examination, should be made with the fine-adjustment mechanism.

Sec. 2. The Mechanics of the Microscope. Fundamentally, the compound microscope is an optical instrument mounting three lenses, two of which form an enlarged image of a specimen while the third illuminates the specimen. From this simple statement it would seem as though the microscope could be made rather inexpensively and with great accuracy. It probably could, if only one set of lenses had to be considered, but complications in design arise because the instrument is used under varying conditions, with a great number of combinations of lenses, and with specimens of various sizes and various light-transmitting characteristics. These factors necessitate the proper adaptation of suitable illuminating devices.

The axis of the condenser lens must be in perfect alignment with the two upper lenses, the objective and ocular. In order to use condensers of different focal lengths, the condensers must not only be interchangeable, but they must also be movable along the microscope axis, so as to focus the light source in the plane of the object.

Provision for holding the specimen must be adequate, so that a flat specimen can be placed in a position normal to the axis of the microscope, and for photomicrographical work some means should be provided for rotating the specimen about the microscope axis. The mechanism for this latter requirement is well met by the rotating stage.

The objective and ocular are mounted in a tube which determines the axis of the microscope. This tube must be made so that the two lenses, objective and ocular, can be placed at a definite distance from each other, and this distance must be subject to control. The objective and ocular must be interchangeable with other objectives and oculars, and the combined units must be focusable with respect to the specimen.

The Base. The base and limb of the microscope are made to provide operation for the above optical parts. The brass base of the modern instrument is usually of a modified horseshoe type, the tripod or claw base having been largely discarded. The extremities of the toes and heel of the base rest on the table, thus affording a three-point contact and so increasing the stability of the instrument when it is placed on a slightly uneven surface.

The large microscope universally has a large heavy base to enable the limb to be swung into a horizontal position without fear of capsizing the whole apparatus. The smaller instrument, however, may not be so stable, owing to less metal in the base and sometimes to poorer design; therefore, if it is intended to be used horizontally its stability should be tested. If such a microscope tends to fall backward when

the tube is tipped to a horizontal position, it should be clamped to the table top. Because there is considerable variation in the size of bases, one should take care, when purchasing an instrument, to select one with a base which is not too large to be accommodated on the base of any camera with which it is to be used.

The Pillar and Inclination Joint. The pillar of the microscope is usually part of the casting of the base and is integral with it. It is machined at the upper end to form part of the inclination joint, the other part of the joint being on the lower end of the limb. On a small microscope, the friction of this joint will generally keep the limb in position; however, on better-class instruments this joint is equipped with a clamping device. In some instruments, this clamp may be operated by a small lever. In others, a small spanner or pin wrench may be used, which can be removed when not in actual use; this has the advantage of giving the design of the limb a more clean-cut appearance. (See Fig. 2.)

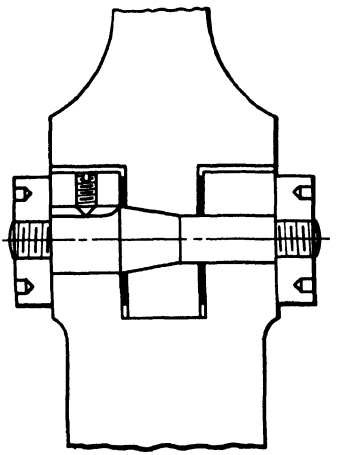


FIG. 2. The inclination joint. Friction against the taper is controlled by loosening the set screw and backing off the side nut on the left while taking up the side nut on the right. (Through the courtesy of Spencer Lens Co.)

The Limb. The limb is the second large brass casting of the microscope. The central part of the limb forms the handle; the lower part is machined for the mounting of the stage and substage apparatus; and the upper part carries the coarse- and fine-adjustment mechanism and the body tube. The shape of the limb, near the level of the stage, sets a limit to the size of objects that can be placed on the stage. As a rule this space is ample, even on small microscopes, and the occasion seldom arises when the size of an object interferes with its examination. The lower end of the limb, carrying the stage and substage, should be very precisely built; in fact, the whole front part of the limb supporting the tube, stage, and substage is virtually an optical track over which the three mentioned parts may travel and be mutually aligned.

The Substage. The substage, in its more elaborate form, consists of a male dovetail fitting carrying a rack, which is engaged by a pinion sunk in the female portion of the dovetail on the limb. The lower end of the substage may have a swinging arm on which the

microscope mirror is mounted; or the mirror may be mounted either in an immovable socket which is part of the substage, or directly on the end of the limb. In any event, the mounting for the mirror should be independent of the vertical travel of the condenser. The socket construction is the more convenient device, and it is used on the better-class microscopes. Although the swinging arm affords a means for obtaining oblique lighting, this is best obtained in other ways. The substage also supports an iris diaphragm which sometimes has a separate carrier ring for holding a diffusing plate, circular filter glasses, polarizing prism, or other special apparatus. Above the iris diaphragm is a slideway or collar for holding a condenser lens. Occasionally, in the older instruments particularly, a second diaphragm may be installed near the microscope stage; this diaphragm is useful only for very low-power photomicrography, where it aids in reducing glare.

The mechanism for changing the condenser is not of great importance provided that it includes an adjustment for centering the lens to the microscope axis. The condenser may be screwed into a holder which is equipped with two centering screws to impart a compound motion to the condenser. The whole is then clamped into a collar in the substage (Leitz). The condenser may be fitted to a slide which is carried in a semicircular plate actuated by two centering screws, and is different from that shown in Fig. 3 (Zeiss); or it may be fitted to a slide mounted on a movable platform of the substage (Bausch and Lomb); or the whole substage apparatus may slip into a yoke, its position in the yoke depending on screw adjustment (Spencer). Four varieties of mounting are shown in Fig. 3. All manufacturers offer numerous types of microscopes having no provision for centering the condenser; such instruments are not adaptable for purposes of photomicrography.

Ordinarily, only a coarse vertical adjustment of the condenser lens is required, as already described, but some research instruments have also a fine adjustment to permit greater accuracy in focusing. This fine motion is by no means a necessity, but a refinement for the most exacting work.

Instead of the rack-and-pinion method for controlling vertical adjustment, some microscopes are provided with a post fitted with a screw thread on which the microscope condenser can be raised or lowered. In its lowered position the condenser can be swung out of the optical path and changed. The swing-out feature is built into other types of substages, to allow the iris with the accessory carrier to be swung to one side.

The iris diaphragm, when built into the substage, is often mounted on a sliding member. so that by means of a small rack and pinion it

can be displaced horizontally, several millimeters, from the center of the system or rotated through an arc of about 270°. The horizontal displacement of the iris is a very important adjustment for the micros-

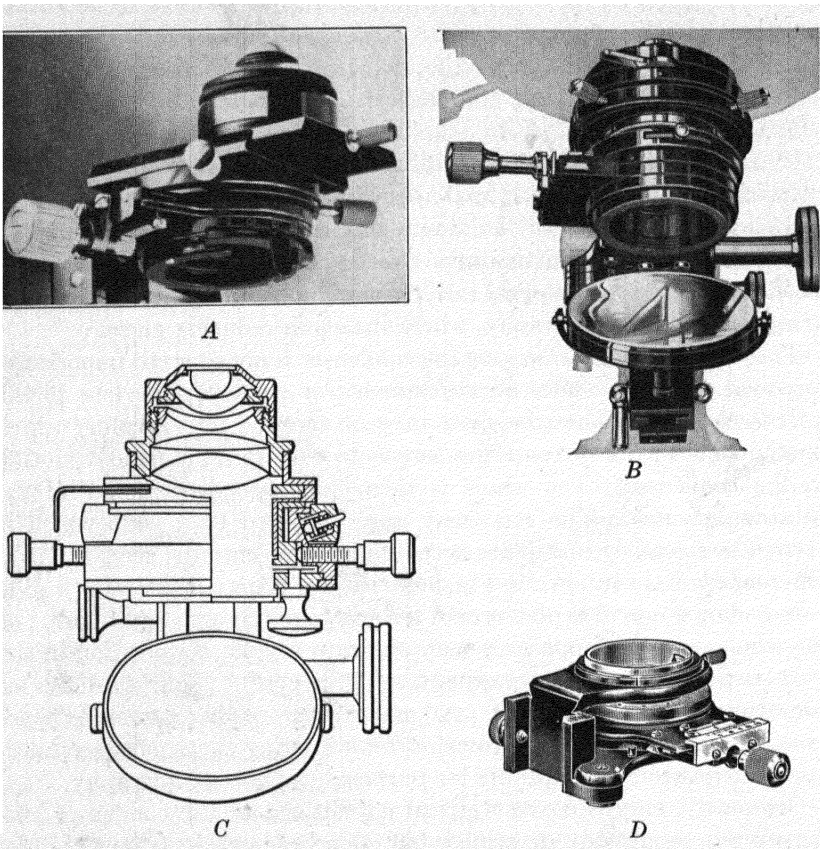


FIG. 3. Various types of substage apparatus. *A*, Bausch and Lomb; *B*, Leitz; *C*, Spencer; *D*, Zeiss. Three of the types shown have centering screws to align the condenser to the microscope axis. The Zeiss substage (*D*), shown in the picture, is not equipped with centering screws. All four makes are equipped with an iris which can be moved horizontally by rack and pinion. The Spencer substage (*C*) is shown with a fine adjustment in addition to the coarse adjustment. (Acknowledgment is made to Bausch and Lomb, Leitz, Spencer, and Zeiss.)

copist who desires to determine refractive index with the microscope; it is of less importance in photomicrographic work. A graduation on the iris mounting to denote the diameter of the iris opening, or the effective numerical aperture of the condenser, is an aid in keeping records, or the scale may be used indirectly to check the numerical

aperture of an objective. Sometimes the iris is mounted between the condenser lenses.

Substage apparatus must be fitted to the limb of the microscope exceedingly well, so that vertical motion of the substage, in all parts of its travel, will be parallel to the axis of the microscope, or tube axis. Unless this is so, the condenser may be in alignment with the objective in a given vertical position but out of alignment in another position. When it is remembered that any misalignment of condenser and objective is highly increased optically by the magnification of the system, it is not surprising to find considerable mechanical error in this part of the assembly when a medium- or high-power objective is used.

The test for the mechanical accuracy of the slideway of this part of the microscope is easy, but it is only comparative. It consists in centering the condenser (see Sec. 85) in one part of its travel and then examining it for alignment after it has been racked to a new position. If misalignment is found in one position and not in another, there is still the question whether the correction should be applied to the tube or to the condenser mounting. Correction for condenser alignment, which varies with the vertical displacement of the condenser, is a problem which should be attended to by the manufacturer.

The Alignment of the Substage Iris Diaphragm. Lenses or diaphragms are said to be mutually aligned, or centered, when their axes coincide. The elements of such a train will appear perfectly concentric with one another when they are in alignment.

The microscope tube axis is the base on which all optical alignment must be made. All lenses, diaphragms, and optical accessories used in the microscope or in the illuminating systems must be referred either directly or indirectly to this axis for alignment. For good visual work, perfect alignment of the optical apparatus is to be desired, and in photomicrographic work it is a necessity. Lack of perfect alignment accounts for uneven light distribution, which spoils so many pictures.

It is customary to center the microscope condenser by moving it until the image of its diaphragm is concentric with the axis of the objective. It is obvious that, if the condenser diaphragm and objective are not correctly centered with respect to the tube axis, all the other optical elements which depend upon them for alignment will be automatically off center. Methods of centering the lenses and apertures which occur elsewhere in the optical train will be discussed later, but two methods will now be described for examining

the alignment or displacement of the condenser iris diaphragm axis with respect to the tube axis.

A pinhole cap is inserted at the top end of the drawtube, and a disc with a central pinhole is placed on the lower end of the body tube (a Davis diaphragm answers nicely; see Glossary), all lenses having been removed previously from the microscope. Under these conditions the two pinholes will serve as aperture sights to line up a third point — in this instance the iris diaphragm of the condenser, which has been closed as far as possible. Usually enough light can be obtained by tilting the microscope mirror toward a window; if a lamp is used, the light should be modified with several diffusing plates or neutral filters. Holding the eye a little distance above the pinhole at the top of the drawtube will aid in establishing a line of sight; the best distance can be determined quickly by a little experimenting.

The Leitz research-model microscopes, and perhaps some others, have three small screws in the diaphragm mounting which can be adjusted to move the diaphragm as required for alignment. Since few other microscopes are designed with adjustment for the condenser iris, this operation may resolve itself into one of mere inspection. If, under such conditions, the iris is found displaced with respect to the tube axis, the microscope should be returned to the manufacturer for repairs, since no apparent eccentricity is permissible.

Another and perhaps more accurate method for centering this diaphragm is to insert a very low-power objective directly into the lower end of the body tube. By using an ocular with cross hairs, the small opening of the diaphragm can be observed after the condenser has been removed. The diaphragm opening should be concentric with the intersection of the cross hairs.

If the complete mount of the diaphragm can be revolved through an arc of 270° or so, the alignment should be made at one particular azimuth setting of the diaphragm. It should then be returned to this azimuth before the condenser is centered.

The Stage. The stage of the microscope, which carries the specimen and various accessories, may be built into the lower part of the limb and be integral with it; or it may be made to slip into a dovetail fitting cut into the limb, so that it can be moved vertically by rack and pinion. When the stage has a vertical adjustment it should always have a lock or clamp so that it virtually becomes part of the microscope limb while the microscope is in use. All microscopes intended for metallography, or for work on thick opaque specimens, should have a stage equipped with vertical adjustments; this is sometimes known as a focusing stage.

It is possible to ascertain whether the stage surface is normal to the microscope axis, by focusing a flat specimen mounted on a flat slide. A high-power objective should be used with a slide of well-dispersed powder, such as test slide 3 described at the end of this chapter. The outer zones of the specimen, as seen in the field of view, should be focused separately, and they should be concentric with the field of view. When they are not concentric it will probably be because the stage of the microscope is tipped, the slide is not lying flat on the stage, or the top and the bottom of the slide are not parallel.

The stage may be either square or circular. The square stage is fixed; the circular one may be made to rotate. The rotation of the stage is important for the orientation of specimens for photomicrography. If means to rotate the stage are provided there should be adequate adjustment for alignment. This is usually accomplished by two screws, at right angles to each other, working in a base which carries a circular fitting into which the lower part of the stage platform rests. If the microscope is ever to be used with polarized light, the circular rotary stage is a necessity. For convenience, every circular stage should be equipped with a locking device; even though a circular scale on the periphery may be used for work in polarized light only, the locking device should always be specified because the additional cost is so little at the time of installation. With a vernier scale, rotation can be measured through angles of 0.1° to 0.05°, or even less.

Aligning the Microscope Revolving Stage. The microscope stage is termed " centered " when the image of a small object, placed under the intersection of cross hairs in the ocular, remains under the cross hairs during a complete revolution of the microscope stage. The stage axis is then in alignment with the tube axis.

There are two kinds of revolving stages, one equipped with centering screws and the other not. Thus two different forms of procedure are required to cope with the problem of centering the stage. They will be given in order.

When the stage can be centered, the intersection of the cross lines in an ocular disc is used as an optical pivotal point. A small object on a test slide is selected as a point of reference and is moved to a position under the cross hairs. If the stage is known to be badly out of center, a low-power objective should be used; slight recorrections can be made later for higher objectives. Otherwise a medium-power objective can be used at first. If the test object moves away from the central position when the stage is rotated, lack of alignment is indicated. In this event, the stage is revolved 180° and the test object is returned to the central position by moving it half of the way with the stage-centering screws

and the remaining distance by hand or by means of the mechanical stage. After one or two trials the point of reference should stay under the cross hairs during a complete revolution of the stage. The stage will then be centered, and its axis will coincide with the microscope axis.

If the microscope is equipped with a non-centerable rotating stage, as some petrographic microscopes are, it will be necessary to center the objectives to the stage rather than to the microscope tube, as explained above. A more detailed discussion of this will be left till later; however, it may be said that, when the microscope is used as a visual instrument rather than as a camera, it is permissible to center the objective to the stage, this being done by moving the objective in its mount instead of moving the stage as described in the last paragraph. If, after this and all other optical centrations have been made, and an unevenly colored image of the lamp diaphragm remains, it indicates that the objective has been moved too far away from the tube axis, which is the axis of the ocular. There is then no choice but to send the instrument back to the manufacturer for correction.

For photomicrographic work the stage should be capable of rotation and centration, for only by rotation of the stage is it possible to take full advantage of the shape and position of the specimen in reference to the dimensions of the plate or film. The rotation of the stage, in conjunction with the movement of the mechanical stage, makes it possible to compose the picture properly and thus to give it " balance."

The Mechanical Stage. Stage refinements include the mechanical or traversing stage. This device may be affixed to any stage at any time. It is designed for making precise horizontal movements of the specimen in two directions at right angles to each other. The moving part, which holds the microscope slide, is actuated by screws set 90° apart, so that the fixture can be moved in two directions. Because of the compound motion, a specimen may be moved a very short distance easily and smoothly in any horizontal direction. In their best form, the screw adjustments are fitted with verniers to permit the reading of displacements of tenths of a millimeter. Thus the operator can put a slide in position, take and record a reading of the verniers on the stage, and return the slide to its previous position, if required for later study. This method of recording orientations is more often needed in photomicrography than in general examinations. Although the experienced microscopist will use such orientation records less often than less experienced operators, the ability to make and record the readings will be found very convenient at times. The mechanical stage also affords a method for making measurements of specimens of irregular shape, such as wool fibers and similar material. A method for doing this is

given by Schwarz.[3] For the photomicrographer the most important feature of the mechanical stage is its slow, easy motion and the facility with which a specimen may be placed in the most advantageous position for photographing.

There are two kinds of mechanical stages which can be attached to the main stage. One is made with small ledges to hold the slide a short distance above the microscope stage, so that immersion oil will not get over the stage platform as the specimen is moved about. However, such a mechanical stage must be extremely well built to be useful; otherwise it is almost certain to tip the slide somewhat, which is a condition that cannot be permitted in photomicrography. The test already suggested for checking the level of the microscope stage can be applied to check the level of the slide held by the mechanical stage. The other type of mechanical stage leaves the slide on the stage of the microscope and holds it in position with an arm actuated by a spring. With this type, the top of the microscope stage may have to be cleaned frequently when immersion oil is used.

Several makes of research microscopes are equipped with the mechanical stage built into the microscope stage. This is no particular advantage; in fact, it may be a disadvantage unless the mechanical feature can be removed completely to permit the photographing of large specimens.

The amount of travel afforded by the mechanical stage in the two directions is of some importance. The length of an average microscope slide is 75 mm, and it is often necessary to examine the whole length of the slide. Thus the lateral motion is generally greater than the fore-and-aft motion, which is seldom more than 25 to 50 mm. Naturally, the smaller, inexpensive stages are designed for less motion than the larger, more costly ones; but it is poor policy to select a mechanical stage which will not be adequate for the work it may be called upon to perform.

The Coarse Adjustment. The upper end of the limb carries the coarse and fine adjustments. The coarse adjustment is actuated by rack and pinion, the gears of which are helical and so distribute the bearing load over more than one tooth. In an endeavor to eliminate lost motion, various types of gearing have been tried, as, for instance, two sets of gears, one to raise the tube and another to lower it; but the conventional helically cut rack-and-pinion type seems to give the most consistently satisfactory results. The fitting of the bearing surfaces is close and should be kept clean for good operation and long life.

[3] Edward R. Schwarz, *Textiles and the Microscope*, McGraw-Hill Book Company, 1934.

Wiping over with a cloth moistened with xylene is generally all that is required. The teeth of the rack can be cleaned with a typewriter brush and xylene, as can those of the pinion.

Two inches is about the maximum amount of travel allowed for the coarse adjustment. As a rule this is sufficient, because long-focus lenses are likely to be set in short mounts, thus leaving plenty of space between the stage and the objective. When the tube has been removed, care should be taken in returning it to its bearings, lest, in forcing it downward, the teeth of the pinion should be jammed against the teeth of the rack and damage them. Nearly always some lost motion is noticeable in the coarse adjustment, but generally it is slight and cannot be completely overcome.

Wear of the coarse adjustment is compensated by moving either the pinion bearings or the rack. When an eyepiece camera is mounted on the tube, movement due to slipping can more often be traced to loose or worn parts of the coarse adjustment than to any fault in the fine adjustment. Instructions for the regulation of the coarse adjustment are generally included in the instruction booklets of the various manufacturers.

The Fine Adjustment. The fine adjustment is decidedly the most-used and the most delicate motion with which the microscope is provided. Its purpose is to make possible very precise and accurate motion of the microscope tube and so to obtain more exact focusing than the coarse adjustment will permit. On nearly all microscopes the fine adjustment carries the objective, the body tube, the coarse-adjustment mechanism, and any eyepiece equipment that may be on the microscope.[4] It is generally built into the upper end of the limb, but in the Leitz research model it is a separate detachable unit. Four different mechanical arrangements are illustrated in Fig. 4*A*, *B*, *C*, and *D*.

The fine motion provided by the Leitz Company can be easily understood by an inspection of the drawing. It has a worm-gear motion, the gear carrying a heart-shaped cam on which the follower wheel rides. The friction of the vertically moving member is reduced by a ball-bearing mounting. The back lash is practically eliminated by a compression spring against which the applied force must do work when the tube is raised. This motion demands more than passing mention.

[4] A notable exception is the Beck microscope (London). In some models this has a fine adjustment carrying only the objective. It has been argued that in such a design the optical tube length is altered with each change in focus. However, on specially built research apparatus it is claimed that the slight changes in optical tube length due to this cause does not necessarily have a noticeable effect on the image formation.

The worm gear is a very effective mechanical device for transmitting motion; it ensures great smoothness and provides large reduction in speed without a gear train. The heart-shaped cam provides a uniform

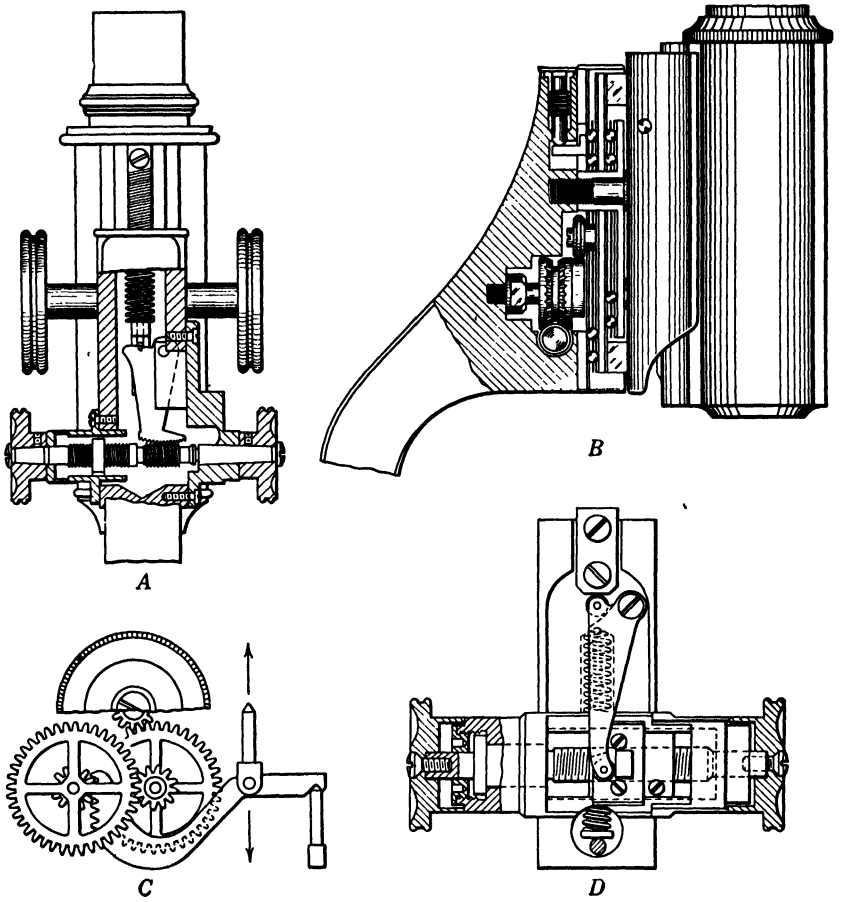


FIG. 4. Fine adjustment mechanism of four different makes. *A*, Bausch and Lomb; *B*, Leitz; *C*, Zeiss; *D*, Spencer. The adjustment by Leitz is the only one which does not include lever construction. (Acknowledgment is made to Bausch and Lomb, Leitz, Spencer, and Zeiss.)

motion; in fact, it is sometimes called a uniform-motion cam. Regardless of the part of travel in which the microscope tube happens to be, a movement of one interval on the drum will produce a standard displacement of the tube.

The motion shown in Fig. 3*A*, *C*, and *D* would indicate a different amount of displacement of the microscope tube for each interval en-

graved on the drum. In fact, any adjustment including a lever motion imparts an unequal tube displacement, for different parts of its travel, for each drum interval.

No type of fine adjustment should show any perceptible lost motion or back lash; therefore a compression spring against which the fine adjustment works is nearly always provided. The travel of the tube, moved by the fine adjustment, should be about 2 mm, and it should hold the tube in any part of its travel without slipping or "creeping." Because of the high gear ratio of most adjustments, 500 : 1 or more, there is little likelihood that the tube will creep after it is once set. For photomicrographic purposes, adjustments of even greater sensitivity than those generally made today would be a boon, but it may be said that those now furnished give very little trouble and remain surprisingly accurate after long years of service.

The fine-adjustment knob is fitted with a drum on which usually 50 or 100 intervals are engraved. The calibrated value of these intervals, for any lever type of fine adjustment, is uncertain since it depends upon which part of the travel the fine adjustment happens to be in at the moment, as already mentioned. If the total travel is, say, 2 mm, then at 1 mm, halfway between its limits, each interval will probably have a maximum value, which decreases as the tube length is moved in either direction. On inexpensive microscopes the travel of the tube may be 4 μ or 5 μ per interval on the fine-adjustment head, when set at its midway point. The better instruments have a lower value than this for each interval. The research instruments have a value of 1 μ per interval. In the best Leitz microscopes, a motion of 1 μ per interval over a range of 2 mm has been developed.

The sensitivity of the fine adjustment is the rate of change of the scale indication of that adjustment to the change in the actual displacement of the tube. Thus, if a change of 1 interval on the fine-adjustment knob produces a change of 1 μ in the position of the tube, the sensitivity of the adjustment will be 1 μ. Adjustments of high sensitivity are always to be desired.

Some makes of instrument have greater stability and rigidity in their coarse- and fine-adjustment mechanism than others. Bearing downward or upward on the adjustment knobs while the eye is at the ocular will usually cause some motion in the field of view, but the fine adjustment should never show perceptible lost motion, and when once the microscope is adjusted for focus it should stay in focus unless moved. If an adjustment is to be tested for creeping over a period of perhaps 15 minutes or longer, the microscope should be set up in a position free from all drafts and temperature changes. If the coeffi-

cient of the expansion of brass is taken as 0.000009 per degree F, then a rise in temperature of 1° F would lengthen a 160-mm brass tube 0.00144 mm. This difference of tube length would throw the microscope completely out of focus with high objectives. In making a test of the adjustment, the heat of the lamp should be considered. The fine adjustment should always be returned to the manufacturer when it is in need of repairs.

An Accessory for Precision Focusing. Certain work may require a series of photomicrographs to be taken at slightly different optical levels. For this the effective sensitivity of the regular fine adjustment may be increased five- to tenfold by the addition of a device described by Hamly[5] and shown in Fig. 5. Essentially it is a screw of fine pitch mounted on an indicating lever, the lower end of which is clamped to the fine-adjustment knob of the microscope. The upper end of the lever serves as a pointer to indicate, on a scale, tube motion in tenths of a micron. In addition to the precise focusing that is attainable by its use, the device can be easily thrown in or out of operation by a specially arranged lock. This positive lock for the fine-adjustment mechanism should make the device a desirable adjunct for the photomicrographer working with high powers.

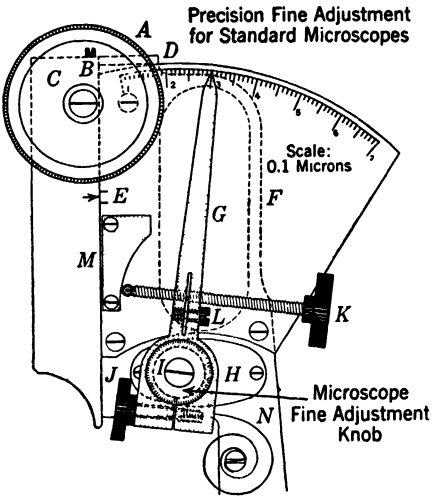


FIG. 5. Precision focusing device after Hamly. Sensitivity, 0.1 μ. (By courtesy of Dr. D. H. Hamly, Department of Botany University of Toronto.)

The Microscope Tube System. Double vision tubes, or binocular tubes as they are generally called, are made with the tube axes either inclined or parallel to each other. Both types are satisfactory. A prism system divides the single image-forming pencil of rays, which leaves the objective, into two separate pencils, and directs them along the axes of the two tubes to the eyes. The part played by the oculars in the formation of the virtual image is the same as with monocular vision, except that both eyes are used with binocular tubes. The prism design and the compensation for tube length are incorporated in the building of the tube and cannot be altered. The tubes should pass an

[5] D. H. Hamly, *Science*, **94**, 263, 1941.

equal amount of light. A test for this is made by looking down first one tube and then the other with the same eye. If the tubes pass an equal amount of light, the fields will appear equally brilliant.

There are adjustments on the binocular tube for interpupillary distance and for compensation when eyes have different focal lengths. The interpupillary adjustment is obtained in one of two ways: either the tubes are swung through an arc of fixed radius, without any alteration in tube length; or the tubes may be extended laterally, in which case the tube length is altered. The type maintaining a constant tube length regardless of interpupillary adjustment is to be preferred. It might be noted that there is no provision for tube-length adjustment on binocular tubes corresponding to the drawtube adjustment so familiar on the monocular tube. With some binocular tubes the oculars may be sprung in the tube, any difference in vision between the two eyes being rectified by slightly withdrawing one of the eyepieces. Sometimes a screw adjustment similar to that mounted in telescope binoculars is installed. This affords better correction and greater latitude than can be obtained with sprung-in eyepieces. Such adjustment is usually made for the right ocular.

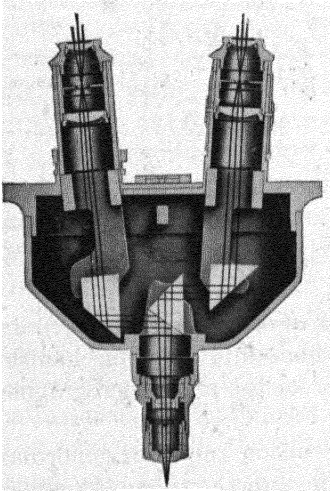


FIG. 6. Illustrating the trace of light rays through the Spencer binocular tube system. The tubes are slightly converging. (Through the courtesy of the Spencer Lens Co.)

Binocular tubes contribute nothing to the excellence of the microscope image, but more comfort is derived from the use of both eyes simultaneously, which may be an advantage, depending on the age, eyesight, and temperament of the individual. The binocular tube should be regarded by the photomicrographer as an accessory. It is a convenience which will aid in prolonged observation, but primarily the image should be studied from the standpoint of the perfectionist who is looking for improvement through the elimination of aberration and glare, and by control of contrast.

The binocular can be used with an eyepiece camera, or with the camera lucida. The Spencer binocular and some others have an arrangement whereby the prism of the binocular tube can be thrown out of position to permit one of the ocular tubes to be shifted over to the axis of the objective. This combination can then be used with any camera. Figure 6 shows the trace of rays through a Spencer binocular system.

The monocular tube, carrying a drawtube, is of considerable importance to the photomicrographer. All monocular tubes intended for photomicrographical work should be of large diameter, that is, about 50 mm. The top of the body tube is fitted with a spring sleeve into which slides a smaller tube called the drawtube; the ocular fits into the upper end of the drawtube. The flange with the spring sleeve should be removable, thus permitting the use of an adapter for the accommodation of amplifying lenses.

The length of the tube assembly, measured from the shoulder of the objective to the top of the drawtube, is called the mechanical tube length. Though there is no recognized standard, the procedure adopted by nearly all microscope companies is to make the mechanical tube length 160 mm for ordinary objectives used on the biological type of microscope. Leitz uses a tube length of 170 mm. For objectives used on uncovered objects, as metallographic and polished petrographic specimens, tubes are longer: 210 mm for Leitz, 190 mm for Zeiss, and 215 mm for Bausch and Lomb objectives. This increase in tube length makes it possible to use vertical illuminators with the ordinary biological microscope, and so compensate in a measure for the length of the illuminator.

The mechanical tube length is of importance only as it controls the optical tube length demanded by the objective in use, and as it serves as a means for rectifying certain lens errors which will be mentioned later. The possible extension of the drawtube should be from at least 10 mm under normal tube length to at least 50 mm over normal tube length, and it should be graduated in millimeters to indicate its setting. If Zeiss Homal eyepieces or Bausch and Lomb Ampliplans are used for photomicrography, the correct mechanical tube-length setting will be 23 mm less than the normal tube length for the Zeiss, and 16.5 mm less for the Bausch and Lomb eyepieces.

In making repairs or alterations involving screw threads, caution should be exercised with regard to the style of thread employed. The angle of most threads on American-made microscopes is 60°; that is, the threading tool has been ground to that angle. On many foreign microscopes the angle of the threading tool is 53°8′, and the pitch is measured in the metric system. This type of thread is called the Löwenherz screw thread. Many repair jobs on microscopes, especially on the tubes, have been badly botched because the mechanic has not thoroughly understood the kind of thread with which he was dealing. The Löwenherz thread should not be confused with the Whitworth thread, which has an angle of 55° and is used on objective mounts.

Microscope tubes formerly made of brass are now often made of aluminum, which places less weight on the tube adjustments and lowers

the center of gravity of the microscope. A black finish on the inside of the tube is always required, and each new microscope should be inspected to see if there are any light spots in the tube which might cause trouble when a picture is taken; if there are, they can be touched out with a little dead black lacquer. A small but very useful tube

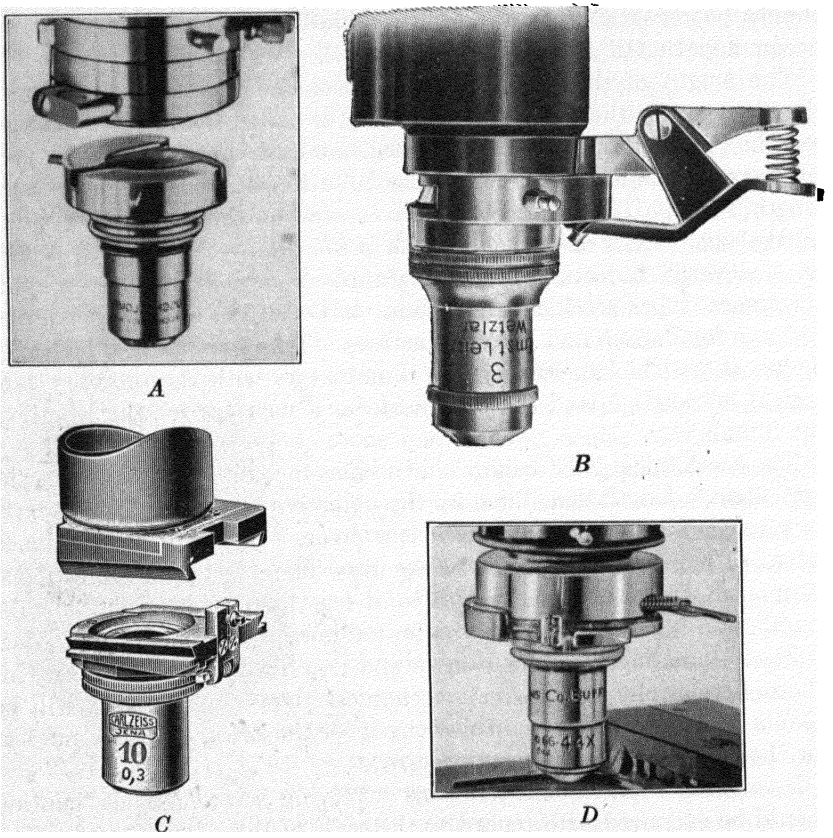


FIG. 7. Different methods of changing objectives. *A*, Bausch and Lomb; *B*, Leitz; *C*, Zeiss; *D*, Spencer. (Acknowledgment is made to Bausch and Lomb, Leitz, Spencer and Zeiss.)

accessory is a lock for the drawtube. This may be built into the main body tube or it may be acquired later. If acquired later it will be in the form of a small split ring which slides over the drawtube and can be clamped in any position, thus ensuring non-creeping of the drawtube when loaded with a heavy accessory, such as an eyepiece camera.

Objective Changing Devices. There are several mechanical systems for changing and centering objectives. The most common, the revolv-

ing nosepiece, may carry openings for two, three, or four objectives. In its simplest form the objectives will have to be screwed into the openings of the nosepiece, and no change in centering is possible. In another form, a small centering collar is provided; this screws into the nosepiece, and the objective screws into the collar. Zeiss has a revolving nosepiece mounted on an intermediate fitting, which carries two screws for centering the whole nosepiece. Leitz provides a revolving nosepiece carrying four objectives, each opening being provided with a means for centering the objective which it carries.

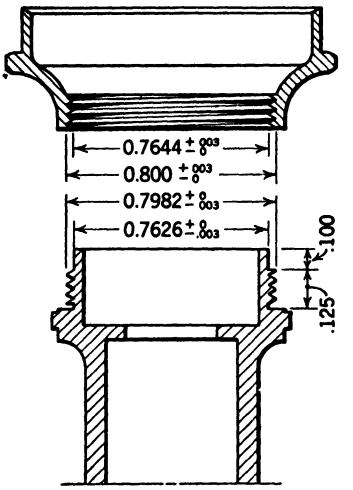


FIG. 8. This drawing, which gives the dimensions of the screw thread of the objective, in inches, as adopted by the Royal Microscopical Society, presents the information necessary for the tool or instrument maker so that he can properly fit accessory apparatus to the microscope. The form of thread is Whitworth, pitch, 36 threads per inch. This standard seems to be the only one to which microscope manufacturers consistently adhere. (By courtesy of The Royal Microscopical Society, London.)

From the standpoint of photomicrography, the most satisfactory form of centering device for objectives is the individual system, whereby each objective is screwed into a slide, or carrier of some sort, which with its centering adjustment is slipped into a holder at the end of the microscope tube. Each objective should have its own centering device, which when once set will keep the objective in proper alignment for a long time. The revolving nosepiece carries, at most, only four objectives; since more than that number is often required, the individual system is to be preferred. Figure 7 shows several kinds of nosepieces and centering devices.

A revolving nosepiece having no means of centration need not be a great handicap if the condenser is centerable. However, there is some advantage in having both objective and condenser on centerable mountings.

Standards of the Royal Microscopical Society. It is well to bear in mind certain mechanical standards in microscope construction. These standards, the originals of which were published in 1858, have been drawn up by committees of the Royal Microscopical Society and have been almost universally adopted by microscope manufacturers. The latest revision was published in the Society's journal[6] in 1936. The

[6] *J. Roy. Micr. Soc.*, **56**, 377–380, 1936.

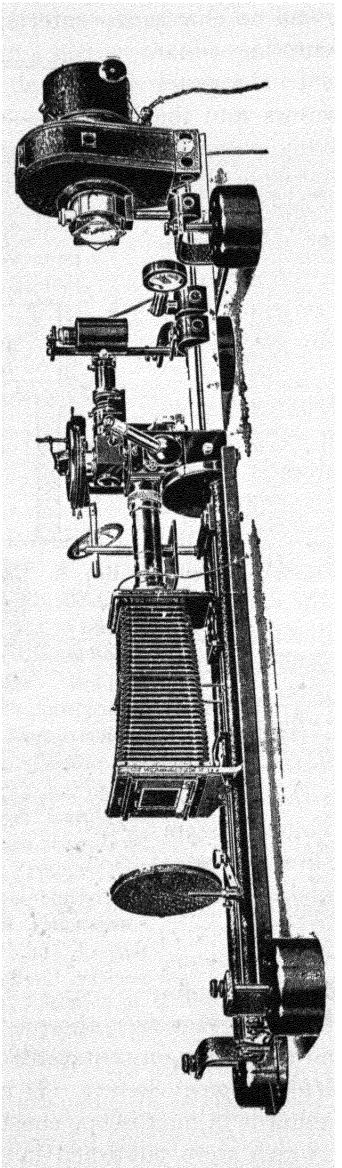


FIG. 9 *A*.

FIG. 9. *A* illustrates the elaborate apparatus of Zeiss for mounting and photographing polished opaque specimens. *B* shows clearly the substage mounting of the objective and the position of the vertical illuminator apparatus, in relation to the specimen. (Through the courtesy of Zeiss.)

most important items in the specifications are the following: pitch of the screw threads of the objective, 36 per inch; form, Whitworth V thread, 55°; diameter, 0.7982 inch or 20.274 mm. For the nosepiece

Table I

Internal Diameter of Drawtube

	Inches	Millimeters
Small	0.917	23.292
Large	1.270	32.258
Extra large	1.410	35 814

to the microscope, the thread is essentially the same as for the objective. The internal diameters of the drawtube for the ocular are of three sizes, as shown in Table I. The internal diameter of the substage tube for the condenser is 1.527 inches or 38.786 mm. Figure 8 gives the dimensions and tolerances for the Standard Objective thread.

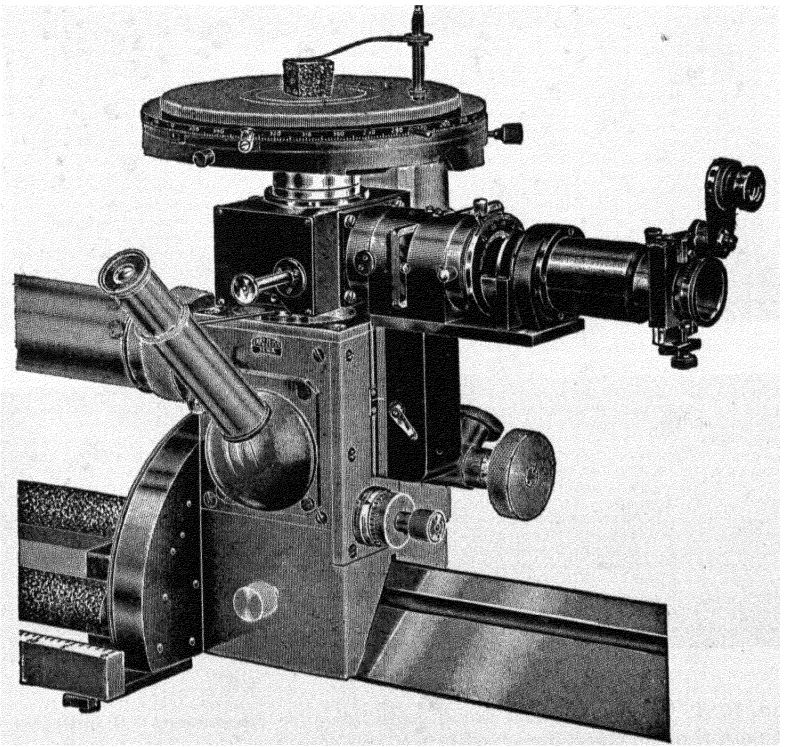


FIG. 9 *B.*

The Inverted Type of Microscope. The above description of the mechanical parts of the instrument covers all microscopes in a general way, although there are individual variations. One of the greatest departures from orthodox microscope construction is found in the inverted type for metallographic work. The French scientist, Henry Louis Le Châtelier (1850–1936), is generally given credit for the original design of this type of instrument. Optically it differs little from the type regularly used for metallographic work with vertical illumination. Mechanically it departs from the standard custom in providing for the

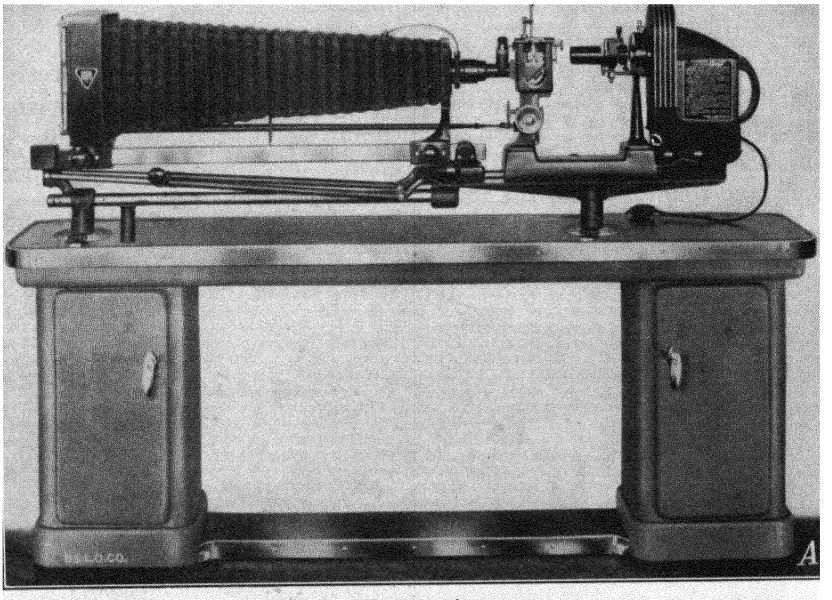


FIG. 10 *A*.

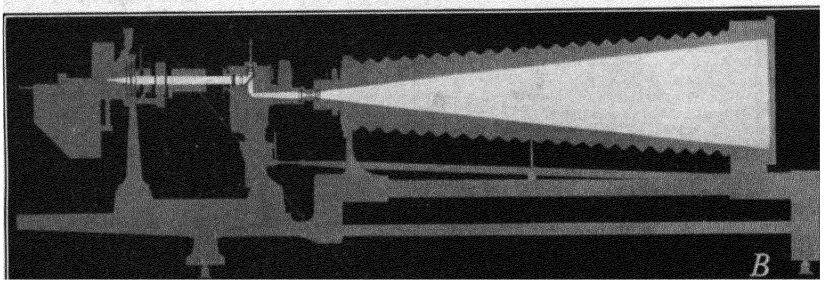


FIG. 10 *B*.

FIG. 10 *A*. The Bausch and Lomb metallographic equipment. *B* indicates the light path through the system, and *C* indicates the physical arrangement for mounting the specimen. This is one of the most modern designs. (Through the courtesy of Bausch and Lomb.)

specimen to be placed face downward on the stage of the microscope instead of face upward.

With one exception, the latest models of the inverted microscope are horizontal; they are used almost entirely for photomicrographic work. Figures 9 and 10 show such apparatus mounted on an optical bed to ensure constant alignment with lamp and camera. The side tube is for the purpose of focusing, for studying the specimen visually, and for selecting a proper field for photography. In this case the fine adjustment carries only the objective. Special illuminators are made to be interchangeable, all in one fitting. Revolving nosepieces are not used, objectives being changed by means of a quick-changing device of some sort working independently of the illuminator. Since the alignment with the camera and illuminator can be made comparatively permanent, considerable speed and accuracy are assured in carrying on the work. In 1942, Bausch and Lomb were the only American manufacturers of metallographic equipment but it was made in England and Germany and in some other European countries.

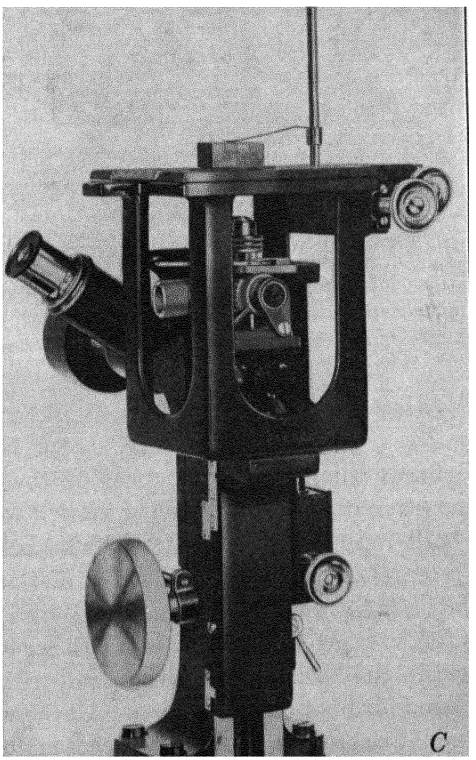


FIG. 10 *C*.

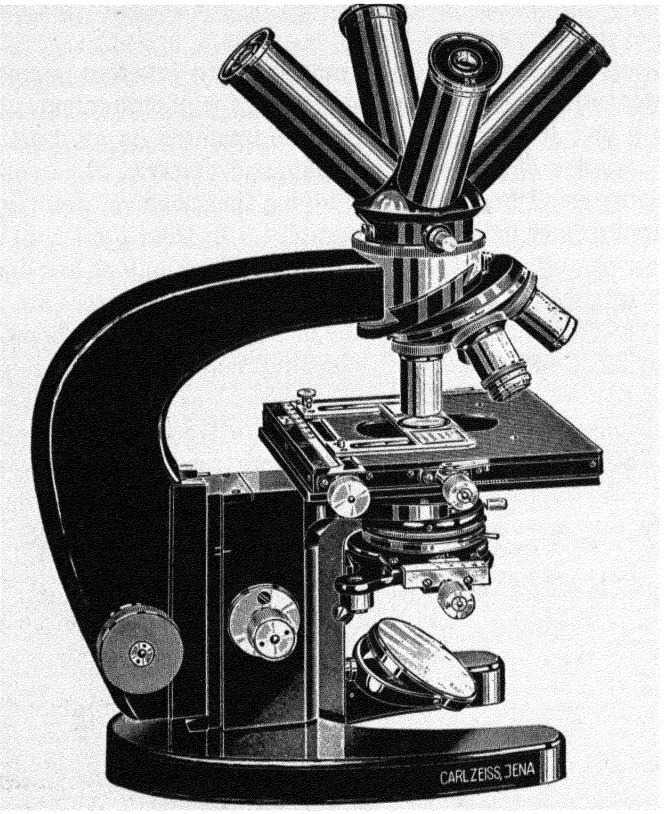


FIG. 11 *A*.

FIG. 11. Innovations in microscope design. *A* shows a revolving type of eyepiece; *B* depicts a microscope with a revolving condenser; both by Zeiss. The idea of a revolving condenser mount is not new. (Through the courtesy of Carl Zeiss, Inc.)

Other Types. Other microscope innovations include several novel constructions by Zeiss, some of which are shown in Fig. 11. The mounting of the fine adjustment in the base of the instrument, the revolving eyepiece attachment, the revolving system for changing condensers, and the built-in illuminating system should be noted. Figures 12 and 13 show research models of Spencer and of Bausch and Lomb. The tube of the latter is toward the rear, leaving the stage unobstructed and convenient for various manipulations. Either of these microscopes can be used in the horizontal position, and with the monocular tube they can be used for photomicrography. All these newer arrangements are designed for greater ease and speed under working conditions, but they offer nothing new in optics as far as increased reso-

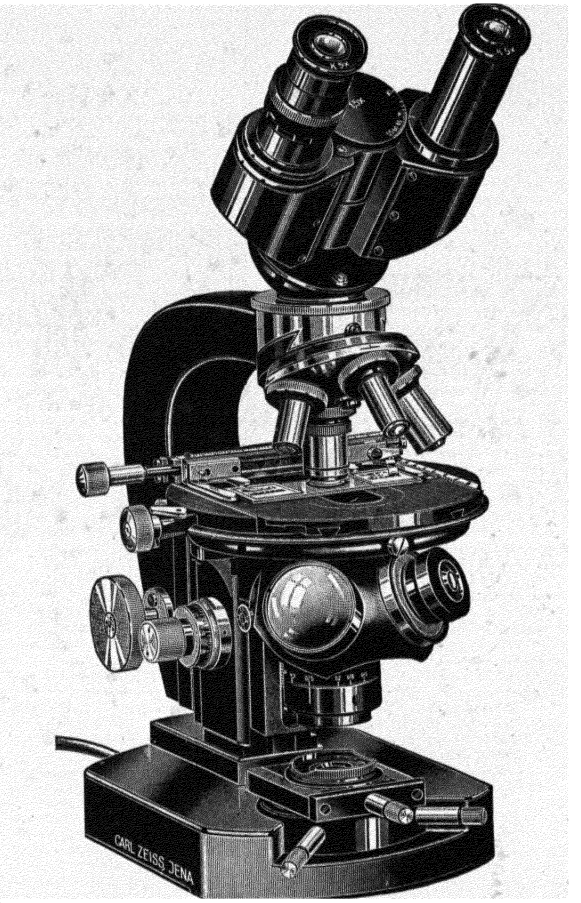


FIG. 11 *B*.

lution or fine images are concerned, and after all those are the main items of importance to the photomicrographer. As a matter of fact, these departures from what may be considered standard orthodox construction have tended to make the instrument less universal and therefore a little more difficult to adapt to special uses.

The construction of the petrographical microscope includes a mount to hold the polarizing prism in front of the condenser. The analyzer is placed in the tube in such a way that it may be removed from the optical train at will. It is mounted in a slider and has two permanent lenses mounted in the tube, between which the analyzer slides. The lenses render the rays parallel for that portion of their travel which passes through the analyzer. By this arrangement, the focus of the

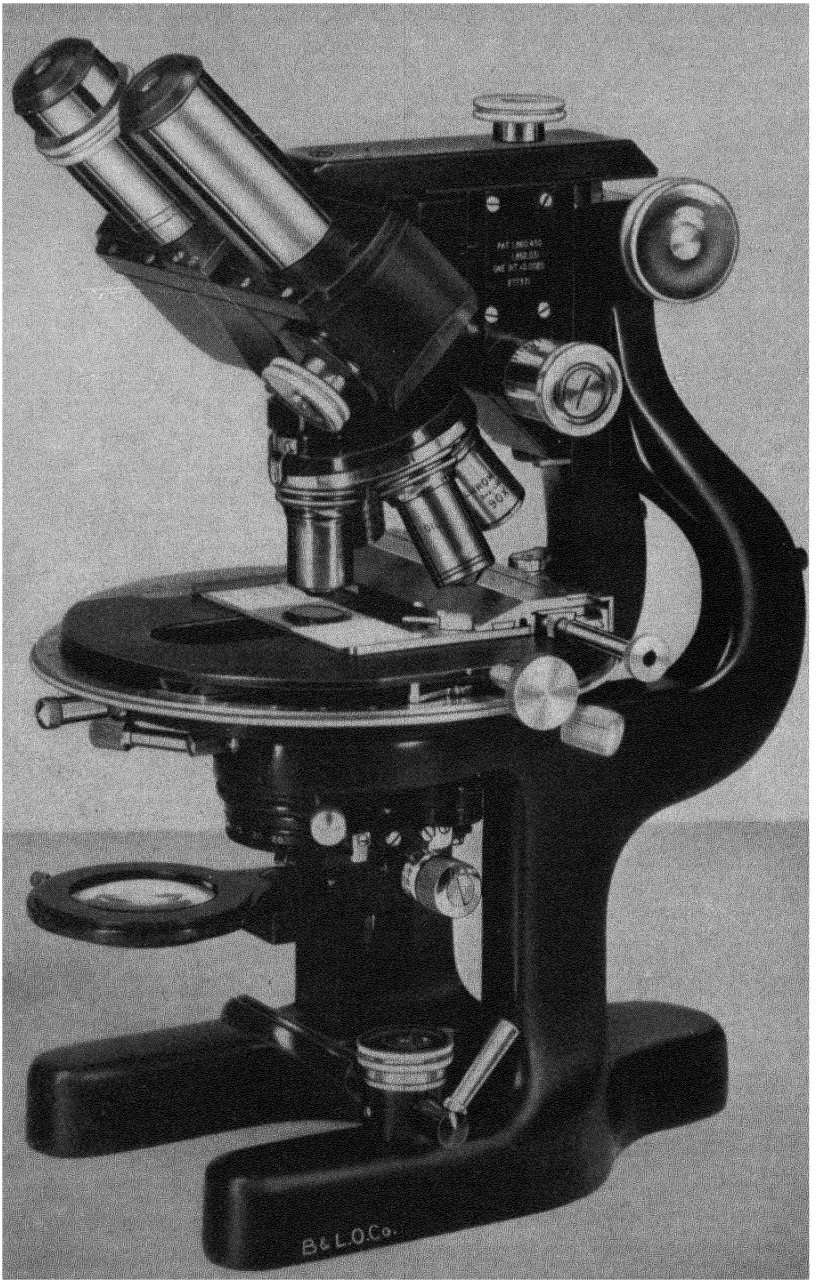


FIG. 12. A new type of microscope design, by Bausch and Lomb. (Through the courtesy of Bausch and Lomb.)

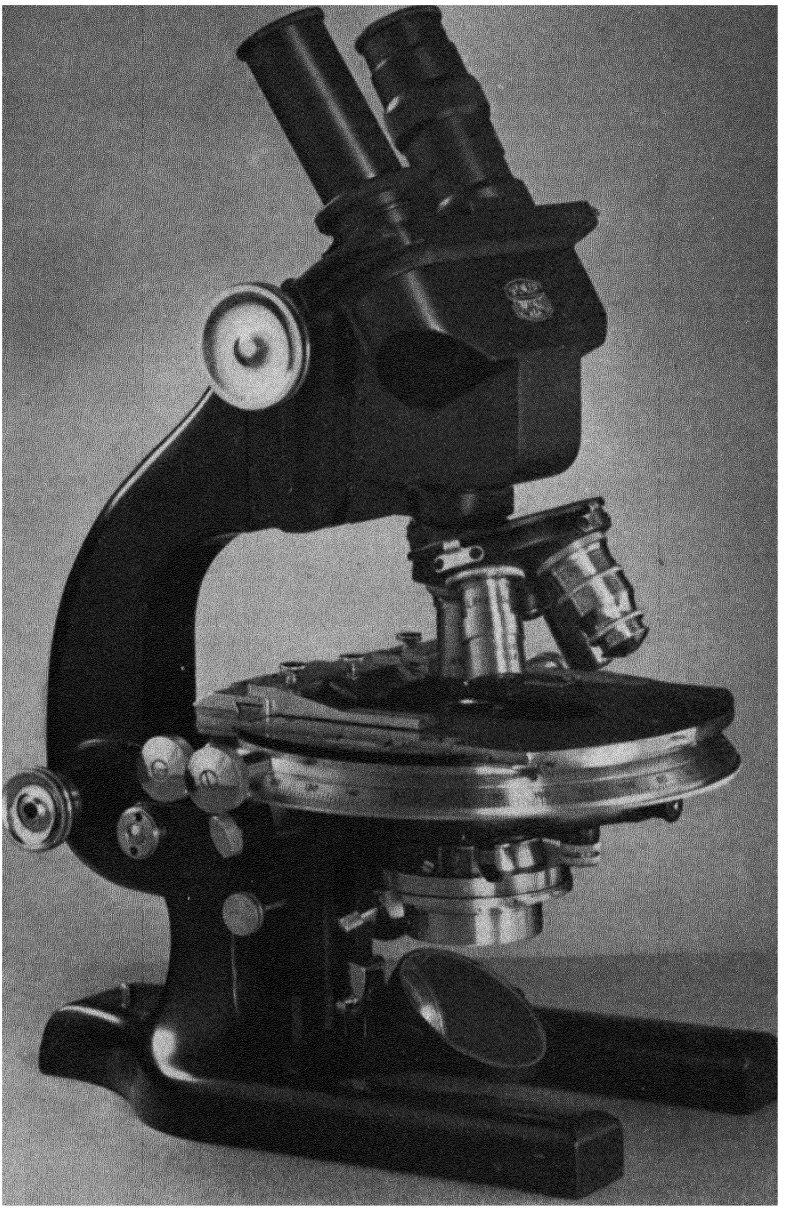


FIG. 13. Spencer research microscope.

instrument is not disturbed when the analyzer is introduced or withdrawn from the path of the image-forming rays. In addition, a slot is provided in the lower end of the tube for the introduction of retardation plates. Figure 14 shows a recent type of petrographic microscope made by Spencer.

Such designations as " student's microscope," " medical microscope," " research microscope," and " fiber microscope " mean little and are

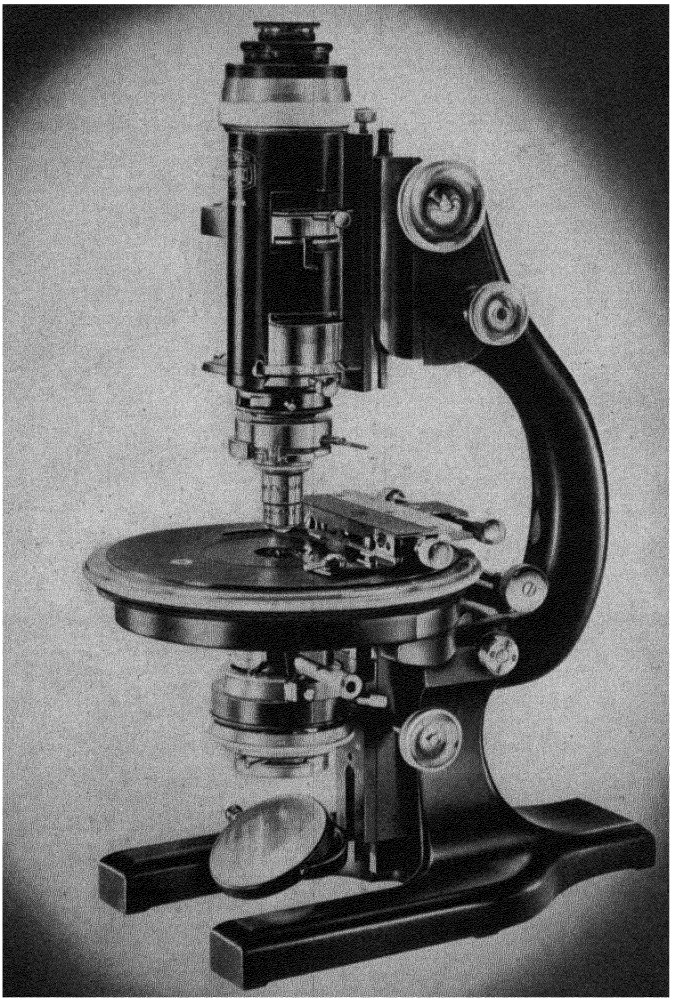


FIG. 14. A recent petrographic stand by Spencer. (Courtesy of Spencer Lens Co.)

largely a convenience for the sales departments of microscope companies. Generally, the student's microscope is a small model made to sell at a low price; the medical microscope is larger and somewhat better equipped and therefore costs more; the research model is the largest and most expensive of all, and probably it is the most adaptable to photomicrographic work. The fiber microscope generally comprises a stand equipped with achromatic lenses; it is moderately priced.

Summary. In considering the various classes of microscopes for photomicrographic work, the salient features desirable in the mechanical design can be summed up as follows:

1. The microscope base should be heavy enough to ensure complete stability of the instrument when used in the horizontal position.

2. It is preferable to have a clamping device for locking the inclination joint rather than to depend on friction to hold the limb in position.

3. The limb should be shaped to give plenty of space from the axis of the microscope at stage level to the handle, thus ensuring the minimum amount of interference with large objects on the microscope stage.

4. The substage should carry adjusting screws for centering the condenser; it should have an iris diaphragm, in a rotary mount, capable of horizontal displacement. A mount, often in the form of a ring which can be swung to one side, or its equivalent should be provided for accessory apparatus. The substage must be focusable by rack and pinion or by screw adjustment.

5. For extended photographic work, the stage of the microscope should certainly be of the rotary type, and it should be equipped with a lock to hold it in any position. The periphery should have a scale marked in degrees, for work in polarized light and for recording orientation. The diameter of the stage should be large, since for work in reflected light the specimen may be of considerable size.

6. The mechanical stage is a refinement of special interest to the photomicrographer; if it is not purchased with the microscope, provision should be made for it to be added later. It should be equipped with two verniers reading to 0.1 mm. Preference should be given to the mechanical stage that permits the slide to be carried flat on the microscope stage.

7. The fine adjustment generally carries the coarse adjustment, but this feature depends on the make of the microscope. The fine adjustment should be considered for rigidity, lack of creep, sensitivity, and uniformity of motion. All fine adjustments of reputable make are mechanically good. The advantages of several have been listed.

8. For photomicrography, the microscope body tube should be of the monocular type, approximately 50 mm in diameter. The drawtube

should have a displacement of at least 60 mm starting at about 10 mm or, better yet, 15 mm less than the usual tube length of 160 mm. There should also be a scale graduated in millimeters to determine its setting.

9. The objective changing device may be of any of the types described. For photomicrographic work, preference might be given to the individual centering slide or clutch changer, with a separate adapter for mounting the various objectives. If the microscope is to be used largely for visual work, a revolving nosepiece should be selected, which, though somewhat lacking in precision, will be found very convenient. Otherwise one of the new revolving nosepieces having centering screws for each objective may be selected.

10. The newer types of microscopes, such as are shown in Fig. 12, have nothing to offer which can be considered specially advantageous for photomicrographic work; but if they are at hand for visual work they can be used for photomicrography. In photomicrography, points to be emphasized are that the microscope stand should be comparatively large, sturdy, and, above all, able to accommodate conveniently the various mechanical adjustments already outlined.

Special types of stand, such as those of the large metallographs which are built for photographic work only, are useful for fast routine work. They are expensive, but they can be counted on to have all the required features for the work that they are intended to do.

Sec. 3. Cleaning and Care of the Microscope. For the most part, modern microscopes are finished in black lacquer which is baked on the metal surfaces of the base, pillar, limb, and tube. This finish is resistant to most solvents, acids, and alkalies, and it is quite durable against mechanical abrasion. The knobs for the adjustments, the mounting of the objectives, the stage fittings, and the verniers and drawtube almost universally have a bright metal finish, of either chromium or rhodium plating over brass.

When not in use the microscope should be kept in its box. If left on the table it should be protected with a cover of cellophane, oil silk, or typewriter ribbon fabric, which is closely woven. The transparent covers are not as strong as the fabrics mentioned, but they look better. The old-fashioned glass bell jars have been replaced by the lighter, cheaper, more convenient, and equally efficient covers mentioned.

For general dusting, a flat camel's-hair or sable's-hair brush is ideal; it can be used on the mirror and around the stage. For special cleaning, a soft cloth can be used, with a little xylene. The vulcanite of the stage can be similarly cleaned. The pinion gears and the gear teeth of the rack of the coarse adjustment can be cleaned with a typewriter brush and a few drops of xylene. The gear teeth should not

be oiled or greased. The fine adjustment is enclosed, and it should not be taken apart for cleaning except by a competent repair man. The drawtube should be wiped occasionally with a cloth and xylene. The inside of the tube also should be wiped, special attention being paid to the top of the tube as far downward as the oculars extend. A cloth and xylene may be used once in a while on the fittings of the substage, especially the fitting that holds the condenser. Any immersion oil that may have run out of place should be wiped off before the microscope is put away. The stage should be kept clean at all times. The drawtube and the main slideways of the tube are the only parts of the instrument that will require the application of a lubricant. After cleaning, the parts can be wiped off with a cloth holding just a trace of some light oil, such as Three-in-One, or the instrument oil made by the Mico Instrument Company.[7]

Sec. 4. Tracing Light Rays through the Microscope System. Figure 15 indicates how the light from a microscope lamp is deflected at the mirror and sent through the microscope. The condenser diaphragm limits the zones of the condenser lens which will receive light. The light, on passing the condenser, is brought to a focus in the plane of the object — the object field — and then passes to the objective. After leaving the objective, the rays are brought to a focus at, or very near to, the ocular diaphragm, the first focal plane of the ocular, where they form a real image.[8]

The distance from the second focal plane of the objective to the first focal plane of the ocular is known as the optical tube length. The rays of light from the image, often called the primary image, formed by the objective at the ocular diaphragm, Fig. 15, pass through the eye lens of the ocular and thence to the eye as parallel or nearly parallel rays. Another image is formed on the retina of the eye; this, like the primary image, is real. Thus the eye is part of the microscope system. The microscope image seen in space is a virtual image; it is analogous to an image seen in a mirror; it has no real existence in space, and, point for point, it lies in a direction coinciding with the direction from which the image-forming rays from each point in the primary image reach the retina. The distance that the virtual image seems to be from the eye is generally taken as 10 inches; this is comfortable reading distance for the unaided normal eye.

[7] Mann Instrument Company, 80 Trowbridge Street, Cambridge, Mass.

[8] A real image is an image as formed by the magic lantern or movie projector. It exists either on a screen or in space where a screen could be placed to receive it. If it exists in space it is called an aerial image. It can then be seen if smoke is blown through the space where it exists.

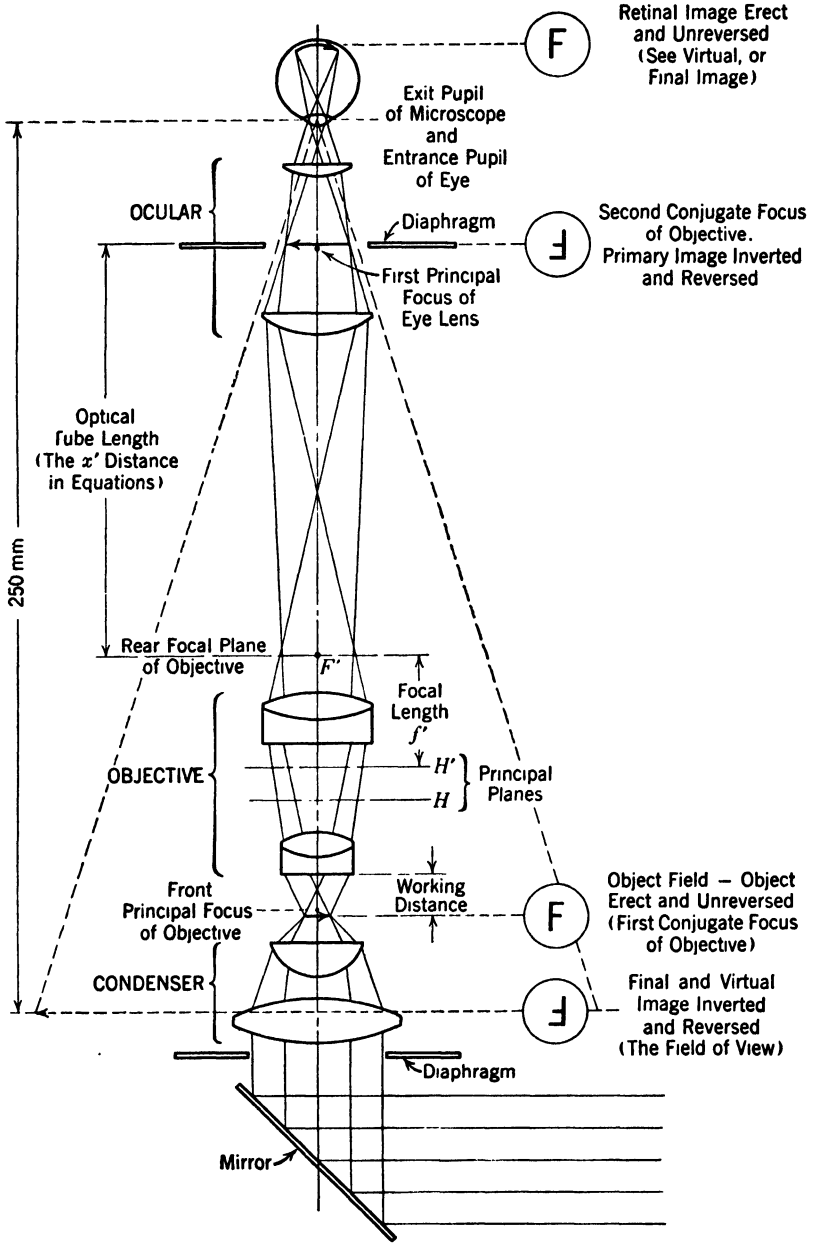


FIG. 15. This drawing illustrates the trace of rays through the optical system of the microscope, from the object to the virtual image. The focal points are shown by the letter *F* and indicate an erect or inverted image.

Sec. 5. Units of Length in Microscopy. In microscopy, measurements of length are denoted by units of the metric system more often than by units of the British system. Dimensions of the microscope are generally given in units of the metric system; lens constants, such as focal length, may be given in both systems; but very long-focus lenses of British or American make are usually designated in the British system while the German companies use the metric system wherever possible. In America and Britain, lamp distances are given in the British system, but in manufacturers' catalogues lamp carbons may be specified by the metric system for diameter and by the British system for length. It is easily seen that there is little or no order for mensuration as universally applied to the art of microscopy, and there is no quick remedy which can be applied to lessen this confusion. However, facility in handling both systems is easily attained, and such familiarity will limit the necessity for making conversions.

The dimension of specimens is usually given in the metric system, three major divisions being recognized. Specimens which are below the size for visual examination with the unaided eye, and are yet large enough to be seen with the bright-field microscope, are said to be microscopic; they are larger than 0.0001 mm. Particles between 0.0001 and 0.000004 mm in size are called submicroscopic, or ultramicroscopic; their presence can be noted in a dark field. Particles less than 0.000004 mm in size are called amicroscopic, for they cannot be seen at all.

In the metric system, the millimeter is the smallest subdivision usually found on rules and scales; subdivisions of the millimeter are obtained by means of verniers or micrometers.

The Micron. Though the millimeter is a convenient unit for measurements made without magnification, such as mechanical tube length, it is altogether too large for measuring microscopic objects, such as the width of a wool fiber or the diameter of a starch grain, because it would necessitate the use of long decimals, as shown above, and lead to confusion and error. For small measurements, therefore, the millimeter is divided into 1000 equal parts, each part being called a micron (μ). The symbol in parentheses is a Greek letter corresponding to our *m* (pronounced mu). A micron, then, is one-millionth of the meter, or one-thousandth of the millimeter, 0.001 mm. Thus a zinc oxide particle may measure 1.5 μ, or a human hair may be 100 μ in diameter.

The Millimicron. An even smaller linear unit is the millimicron ($m\mu$), used in referring to the length of light waves and also in ultramicroscopy in the special branch devoted to the study of colloids. It

Table II

Comparison of Metric, British, and Angstrom Units of Length*

	1 m	1 cm	1 mm	1 μ	1 mμ	1 A
Meter (m)	1	0.01	0 001	0.000,001	0 000,000,001	0.000,000,000,1
Centimeter (cm)	100	1	0.1	0 000,1	0.000,000,1	0.000,000,01
Millimeter (mm)	1000	10	1	0.001	0.000,001	0.000,000,1
Micron (μ)	1,000,000	10,000	1000	1	0.001	0.000,1
Millimicron (mμ)	1,000,000,000	10,000,000	1,000,000	1000	1	0.1
Angstrom (A)	1×10^{10}	100,000,000	10,000,000	10,000	10	1
Inches	39 37	0 394	0 039,4	0 000,039,4	0 000,000,039,4	0.000,000,003,94

* All the numbers in a column represent the same quantity. Thus 1 mm is the equivalent of 1000 μ or 0.001 m, etc.

will be used here mainly in the discussion of light waves of various lengths, with reference to optical filter glasses in particular. The millimicron, as its name implies, has the value of one-thousandth of a micron. However, since microscopic particles cannot be measured directly in units smaller than the micron, or rather large fractions of it, the micron is quite satisfactory for most purposes.

Sometimes, particularly in older writings, mμ was written $\mu\mu$, which actually means one-millionth of one-millionth of the meter, 10^{-9} mm, if the definition of the micron is the same in both cases. Obviously, no such small quantity is intended, since the diameter of the hydrogen molecule is given as 2×10^{-9} mm, and one millionth of one-millionth of the millimeter is 10^{-12}.

The Angstrom Unit. This unit of length was suggested by Anders Jöns Ångström (1814–1874), of Sweden. Its abbreviation is variously Å, Å.U., A, or A.U., but according to the American Standards Association the accents are dropped in both the unit and the abbreviation; its value is one ten-millionth of the millimeter. Thus the expressions 10^{-3} mm, 10^{-6} mm, and 10^{-7} mm refer to 1 μ, 1 mμ, and 0.1 mμ or 1 A.U., respectively, for 1 μ = 1000 mμ = 10,000 A.U. The angstrom unit is seldom used in microscopy.

Table II shows the important subdivisions of the meter and a direct comparison with a similar measurement in the British system. The decimal 0.0000394 in the table is substantially equal to the fraction 1/25,000. In this way it is very easy to remember the British equivalent of 1 μ; roughly, it is 1/25,000 of an inch. Also, 1/1,000,000 of an inch equals 25.4 mμ.

Table III shows the sizes of a few objects of interest to the microscopist.

Sec. 6. Photographic Magnification. In order to carry out the experimental work attached to this chapter it is necessary to consider the practical application of photomicrographic magnification and how it can best be measured. A more theoretical exposition will be found in Chapter III, where it will be considered in reference to some of the cardinal points of a lens and several equations will be presented.

Linear magnification of the photographic image indicates how many times larger than the actual object the real image of a microscopic object is when both are measured linearly. When the distance between any two points in the image is 10 times greater than the distance between the two corresponding points on the object, the lens is said to be magnifying 10 times.

The best method of measuring the magnification at which a picture is taken is to substitute a small scale for the specimen, and to measure

Table III

The Sizes and Methods of Measuring a Few Common Objects

Means of Examination	Material	Approximate Size	Method of Measurement
Ultramicroscope	Colloids*	$<0.1\ \mu$	Generally, by knowing concentration and counting numbers in known volume
High-power microscope	Fine pigments	0.2 to $1.0\ \mu$	Micrometer eyepiece
High-power microscope	Red blood corpuscle	$8.0\ \mu$	Micrometer eyepiece
Medium-power microscope	Low-grade wool (diam.)	$40.0\ \mu$	Micrometer eyepiece or projection
Low-power microscope	Newsprint thickness	$100.0\ \mu$	Toolmaker's micrometer

* Individual discrete particles are sometimes called amicrons, but the term amicroscopic, used occasionally, refers to particles too small to be seen even by means of the ultramicroscope.

the magnified image of this scale on the ground glass of the camera. Such a scale is called a stage micrometer; it can be had with various rulings, but one with a ruling 1 mm long and divided into 100 equal parts is the most convenient for general work. Figure 16 is a photomicrograph of such a ruling. Each interval is 10 μ multiplied by the magnification of the picture, that is, by 100; each space is 1 mm. The accuracy of this statement can be checked by applying a millimeter scale to the picture.

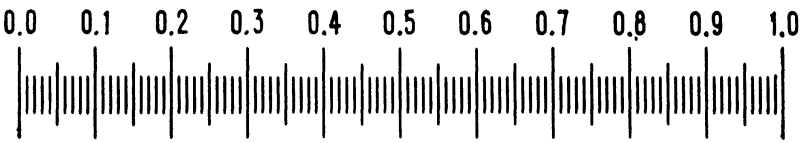


FIG. 16. Photomicrograph of stage micrometer $\times 100$. Length of original scale 1 mm. Each interval in the photograph represents 10 μ. This is a scale made by the photographic process, but at this low magnification it is clear and sharp. Cf. Fig. 19.

Measurement of Magnification. After a picture has been taken, the stage micrometer is placed on the microscope stage, and all camera and microscope adjustments are made exactly as they were when the picture was taken. With the lines of the stage micrometer carefully focused on the ground glass of the camera, the image can be measured with an ordinary millimeter scale.

Suppose that 10 of the stage micrometer divisions measure 25 mm on the ground glass of the camera. Say that each interval of the

micrometer is 0.01 mm, and that 10 consecutive intervals have been measured and found to be equal to 25 mm on the ground glass. The total distance in the object field will be 0.1 mm (10 times 0.01 mm). Then 25 mm on the ground glass will equal 0.1 mm in the object field. If the magnified distance (25 mm) in the image field is divided by the actual distance in the object field (0.1 mm) the result will be the magnification, which in this example is 250×. This can be expressed as 250 diameters, ×250, 250×, or 250 : 1. The most exact expression is certainly 250 diameters, since this obviously refers to linear magnification, whereas the other expressions might refer to areal magnification. However, custom favors the use of the × before or after the number denoting magnification. It is read as "times," and actually there is little chance of ambiguity, since, if areal magnification is intended, it is generally so stated.

As an equation, the magnification formula can be written

$$\text{Magnification} = \frac{\text{Distance in the image field}}{\text{Equivalent distance in the object field}} \qquad [1]$$

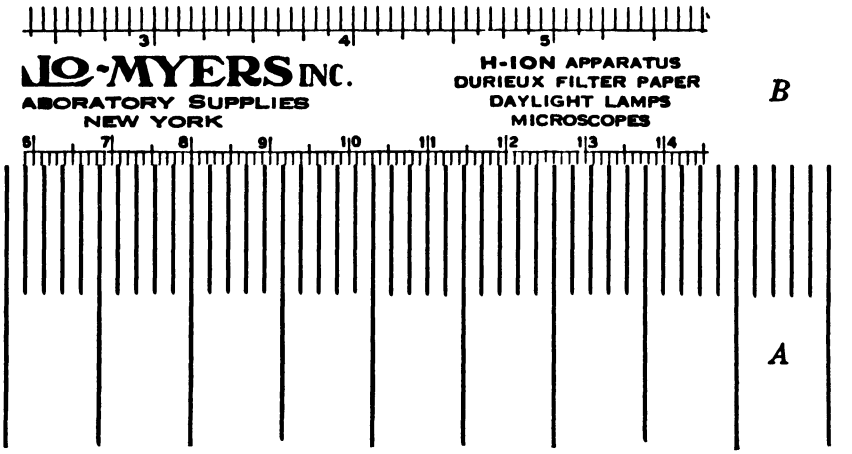


FIG. 17. This illustrates the use of a scale on the ground glass of the camera to measure the image of the micrometer scale. The correct position of the first line, from which a reading is taken, is shown at the figure 8. The line of scale B is on the edge of a line on scale A which is the image, on the ground glass, of the micrometer scale.

Figure 17 shows how the scale lines are placed relative to each other. Measurements are made with the edge of a line of one scale parallel to, and nearly in contact with, a line of the other scale. The figure will probably make the position of the two scales perfectly clear. This

condition is easy enough to attain at the line where measurement begins, as either scale can be moved slightly. However, it may be more difficult to obtain such definite alignment where the measurement ends. This is particularly true in high-power work; because of the fewer lines on the scale image, it may be necessary to estimate portions of an interval. Figure 17 shows the superposed scale *B* which is used to measure the magnified micrometer scale *A*. When using one scale against the other it is easy to detect slight errors in the position of the scales. If the lines cover each other, particularly if one set of lines is wide and the other narrow, there may be doubt about their exact position relative to each other. In this figure, No. 8 and No. 11 of scale *B* are in the correct position for reading.

Ordinarily, it is unnecessary to make more than one measurement to determine magnification, as slight errors of alignment in scale position or errors in estimating fractions of an interval do not make an appreciable difference in the results. However, if an ocular micrometer is to be calibrated for purposes of micrometry it is better to make several readings (at least five) and to take the average.

In the absence of a stage micrometer, the magnification of a picture can be roughly determined by multiplying the magnification of the primary image (the magnification of the objective) by the magnification of the ocular, by the projection distance in millimeters, divided by 250. The projection distance is measured from the exit pupil of the microscope to the focal plane of the camera.

$$\text{Magnification at focal plane of camera} = \text{Magnification of objective} \times \text{Magnification of ocular} \times \frac{\text{Projection of distance in millimeters or inches}}{250 \text{ or } 10} \qquad [2]$$

The 250 represents the distance in millimeters for which the magnifying power of the microscope was originally computed. Visually, the image as seen through the microscope will appear at a distance of about 250 mm for the normal eye. Thus, an ocular capable of magnifying 10 times will give that magnification when used for projection at a distance of 250 mm, or it will magnify the primary image 10 times when the microscope is used visually. For visual work the total magnification of a microscope is

$$\text{Magnification of microscope} = \text{Magnification of objective} \times \text{Magnification of ocular} \qquad [3]$$

Equations 2 and 3 hold fairly well for nearly all makes of microscope

objectives and oculars; however, some manufacturers considerably overrate the magnifying power of their lenses, in which event the equations may err by as much as 20 per cent.

Effect of Camera Extension on Magnification. Figure 18 clearly shows how magnification varies with projection distance. At point E', the exit pupil of the microscope (see Sec. 14), the magnification is 0. At a point 250 mm distant from E', the magnification can be considered equal to that obtained when the microscope is used visually. It follows from the geometry of the figure that

$$\frac{S_1'}{S_2'} = \frac{I_1}{I_2}$$

or, in general, that increasing the projection distance brings about a proportional increase in the size of the image: doubling the distance doubles the size of the image, hence the magnification is doubled.

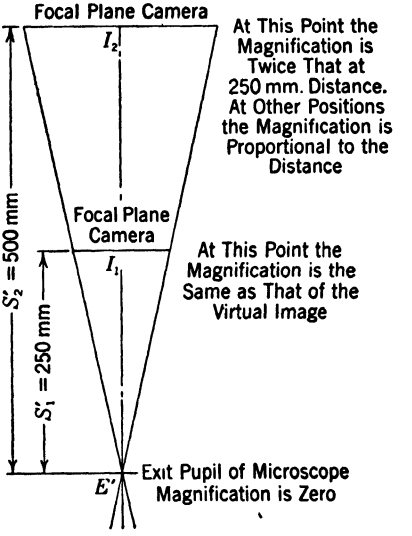


FIG. 18. The effects of camera extension on magnification. All triangles formed by the trace of the focal planes, or bases, and the apex E' are similar. The magnification in all cases will be proportional to the distance of the focal plane from E', the exit pupil of the microscope.

Areal Magnification. This refers to the magnification of the area of the specimen. Its value is the square of the linear magnification. Thus, if an object is magnified 100 times linearly, it is said to be magnified 10,000 times areally. The figures reach rather magnificent proportions very quickly. Nowadays, this notation is seldom used; it has little to commend it, and it is clumsy. It is mentioned here only because it is occasionally found in certain books on microscopy and so should be understood. It is of some importance in the study of the photometry of the microscope.

Recording Magnification. It is highly important to keep careful records of the magnification of photomicrographs. With such records, the actual size of any particle shown in a picture may be determined with some degree of precision by measuring the image with a millimeter scale. Two photomicrographs of different magnification can then be compared intelligently.

When possible, the magnification of the photomicrograph should be

selected to permit a millimeter scale to be used conveniently, in making measurements. This means that the magnification should be such that intervals on the scale represent certain whole numbers. For instance, a magnification of 1000 on the negative makes it possible to use a millimeter scale on a print from that negative, each millimeter on the scale representing the equivalent of 1 μ. For a magnification of 500, each millimeter interval on the scale would be the equivalent of 2 μ on the subject; a magnification of 100 would make each interval on the scale equal to 10 μ on the subject; and so on.

Table IV shows the value of each millimeter on the print for different magnification values.

Table IV

Magnification	Value of 1 mm on the Print in Microns
100	10.0
200	5.0
300	3.3
400	2.5
500	2.0
600	1.66
700	1.43
800	1.25
900	1.10
1000	1.00
2000	0.50
3000	0.33

Standard Magnification. The American Society for Testing Materials[9] has recommended the following standard magnifications for photomicrographs of metal surfaces: 25, 50, 75, 100, 150, 200, 250, 500, 750, 1000, 1500, and 2000 times.

However, within certain rather wide limits, the magnification of a picture is more or less immaterial; the chief aim should be to obtain sharp pictures giving all the resolution needed to stress the desired detail, with sufficient depth of focus and correct contrast of the negative. Judgment must be exercised in choosing magnification, preferably by studying the image on the ground glass, the purpose of the picture being constantly in mind. Too high a magnification is as likely to spoil a picture as one that is too low.

[9] *Tentative Methods of Preparation of Micrographs of Metals and Alloys*, designation E2–39T, Am. Soc. Testing Materials, 1939.

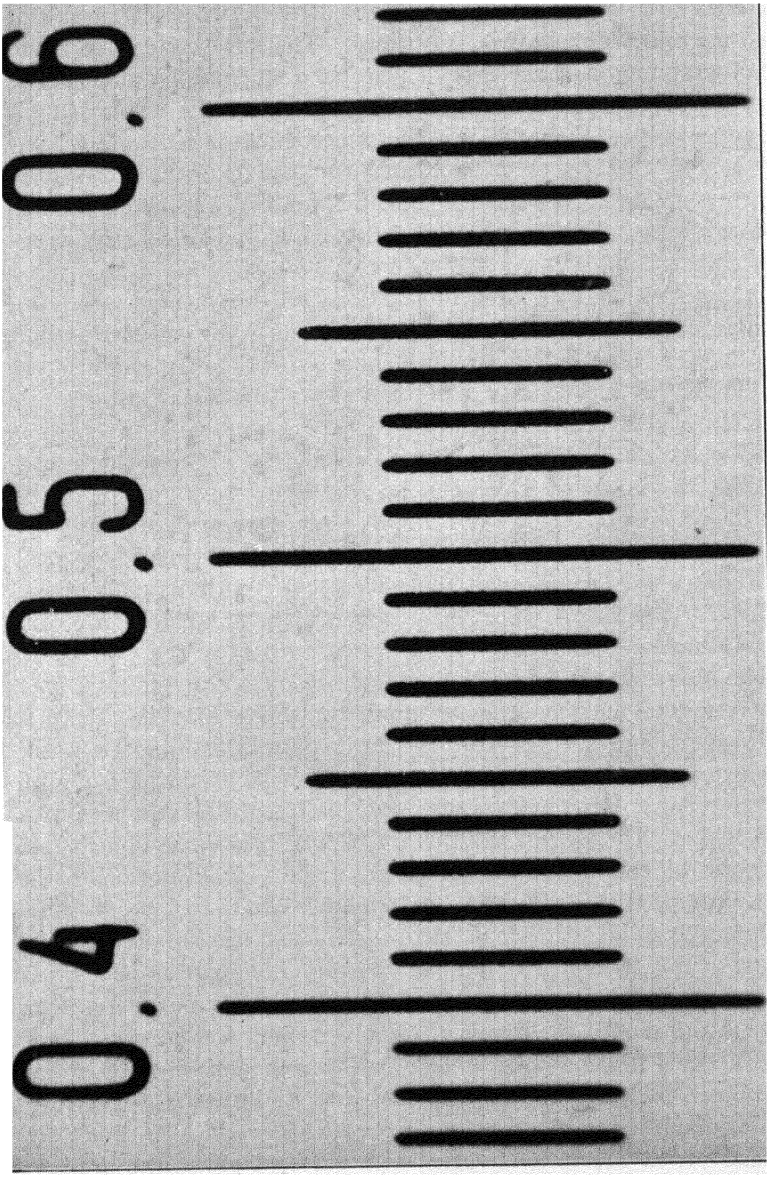


FIG. 19. Photomicrograph of micrometer scale ×600. The lines are wide and fuzzy. Cf. Fig. 20, which is a photomicrograph of a micrometer scale ×1000; the lines are sharp and clear. The scale in this figure was made photographically; the one in Fig. 20 was engraved.

Stage Micrometer. A stage micrometer is strongly recommended as a most useful accessory. One can be had for as little as $2 or $3; better ones cost from $8 to $12. Figure 19 is a photomicrograph of a stage micrometer magnified 600 times. The micrometer was made by photographing a scale onto a sensitized microscope slide. The lines are wide, and silver grains on the edges give the whole scale a fuzzy appearance. Figure 20 is a photomicrograph of a stage micrometer ruled on glass. The magnification is 1000 times, yet the lines are narrow and sharp compared with those of Fig. 19. A good stage micrometer is absolutely necessary for exact analytical photomicrography.

All the scales used in these illustrations are 1 mm long with 10-μ intervals. If needed for low powers, stage micrometers can be had 2 mm, or even more, in length, with spacings of 0.1 mm, one interval being subdivided into intervals of 10 μ each. Such scales are available on either glass or metal surfaces.

Sec. 7. Object Field. The object field is that area on the microscope slide the image of which can be projected into the plane of the ocular diaphragm (see Fig. 15). It is the place where specimens may be mounted and examined. Some authorities have defined it as that part of the field seen through the microscope which actually exists on the microscope slide. This definition is open to the criticism that the field which is visible may be influenced by part of the field which is invisible, for, because of the limits of the ocular diaphragm, only part of the total field as imaged by the objective may be seen. It appears more logical to ascribe the term object field to the whole field which may be projected by the objective, the useful part of such a field depending, of course, upon the objective, the ocular, and, if a camera is used, the size of the plate. In any event, the object field for a given objective is fixed in size, and the visible portion in the field of view is controlled by the ocular diaphragm.

With the focused microscope, if the ocular, or better yet the complete drawtube, is removed and a piece of ground glass is held at approximately the position that was occupied by the ocular diaphragm, the extent and size of the image of the object field can be seen. It is noticeable that, if the source of light is large enough, the full extent of the object field can be seen and also that its image is larger than the ocular diaphragm.

The size of the object field varies with different objectives; a high-power objective may give a very small field; a low-power one may give a much larger field. Certain actual measurements made with an 8-mm apochromatic objective gave a field 0.90 mm in diameter. Through

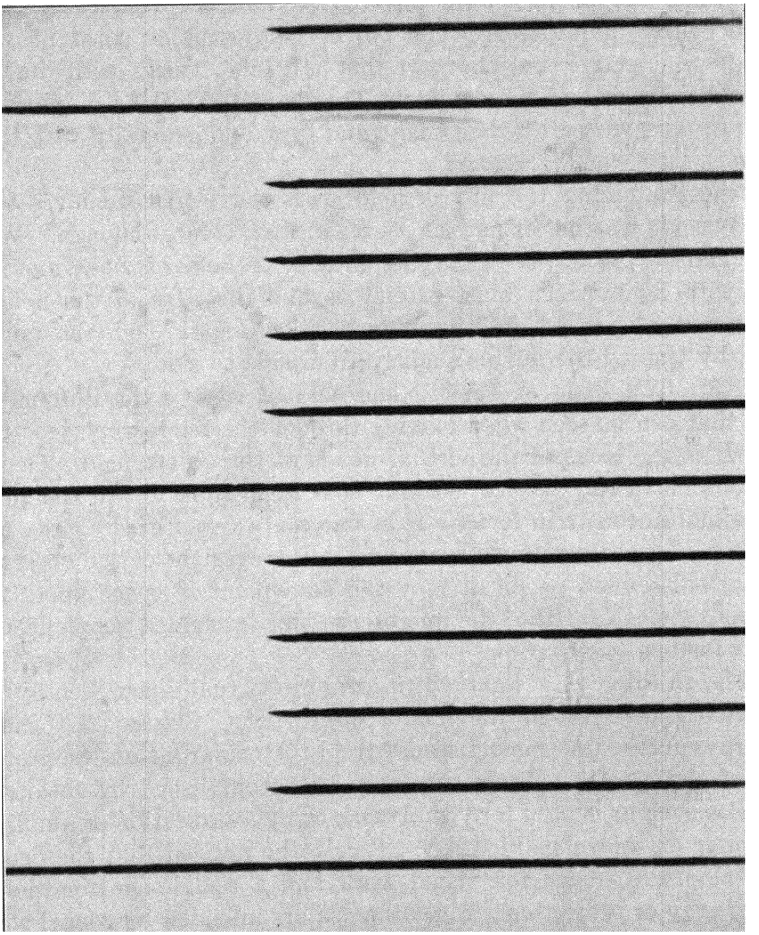


FIG. 20. Photomicrograph of micrometer scale ×1000. The scale was engraved. Cf. Fig. 19.

a 7× ocular, the visible part of the field was restricted to 0.85 mm in diameter, and with a 15× ocular to 0.47 mm. The 3-mm apochromatic objective without an ocular gave a field of 0.28 mm, and with the 15× ocular the visible part of the field measured 0.18 mm. The diameters measured when oculars were not used represent the true sizes of the object fields on the microscope slide; the measurements made when the oculars were inserted showed the restricting effect of the ocular diaphragms. The object field remains constant with any given objective, but the part that is visible varies with the size of the diaphragms of the oculars. The diameters of the diaphragms of the oculars vary with their make and type and generally with their power.

Before examining the object field with the ocular removed, one should decide whether or not the source of light is large enough. With the ocular in place, the ocular diaphragm, or field of view, must be filled with light, which must extend beyond the edge of the field, a condition that can be determined, when looking through the microscope, by tipping the mirror slightly from side to side.

Sec. 8. The Field of View. The field of view is the illuminated circle that can be seen when looking through the microscope (see Fig. 15). It is the locus of the virtual image of the object field. Generally it is noticeably curved, so that it is impossible for all the zones to be simultaneously in focus. It has no real existence, of course, and its position appears to vary according to the eyesight of the observer. For normal-sighted people it may fall at a point in space about 250 mm from the exit pupil of the microscope; for farsighted people it may appear farther away.

In Fig. 15 the rays of light which form the virtual image seen by the eye are traced through the microscope system. Figure 21*A* shows these rays focused at some distance from the ocular, as on the ground glass of the camera. Projection may be accomplished by means of an ocular such as is used for visual work or by a projection or amplifying ocular as shown in Fig. 21*B*. It is to be noticed that the ocular forms a virtual image for visual work and a real image for photomicrography or projection. Since the oculars intended for visual work have no special correction for projection images, the best photomicrographs are usually made with specially designed eyepieces. These are variously called projection eyepieces, photographic eyepieces, or amplifiers, the names having slightly different significance, depending on the manufacturer.

An erect and unreversed image, however formed, is an image of the object exactly as it appears to the eye. If the image is formed by

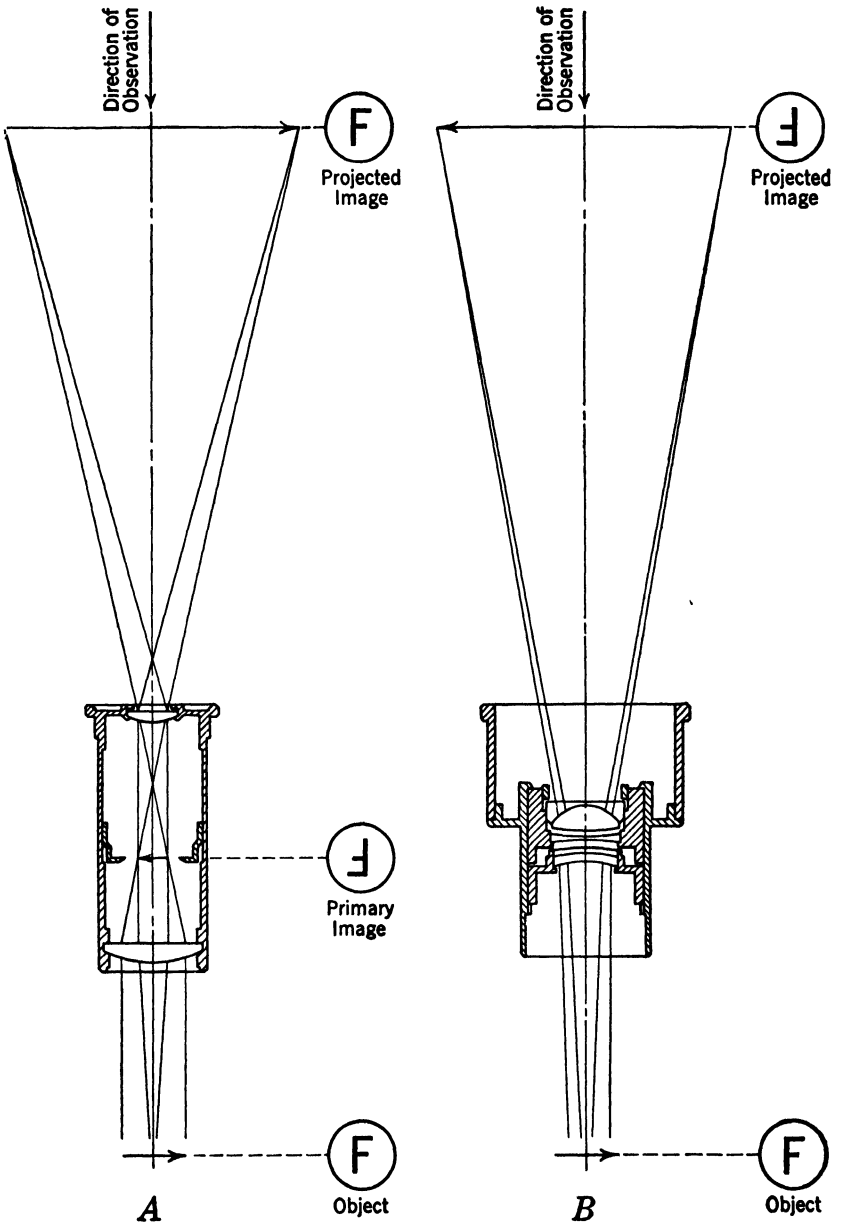


FIG. 21. The drawing at *A* illustrates the projection of an image by an ocular such as is used for visual work. At *B* the projected image is formed by an amplifying lens, acting as if it were part of the objective. No primary image is formed.

a lens and projected onto a screen, it will be a real image inverted and reversed; that is, it will be upside down, and the left side of the object will appear as the right side of the image when viewed from the back side of the screen. This is the familiar camera image. The primary image formed by the microscope objective in the plane of the ocular diaphragm is such an image.

Figure 15 shows the ocular acting in conjunction with the lens of the eye, much as an ordinary hand magnifying lens would do. The inverted and reversed image normally formed on the retina of the eye can be likened to the image formed by a camera lens on ground glass. The different points in the real image on the retina seem to the observer to lie in the virtual image, in directions which correspond to the respective rays incident on the retina. Consequently, the final effect of an inverted retinal image as interpreted by the brain is an erect virtual image. All ordinary vision is composed of inverted images on the retina which, for the above reasons, appear to the observer to be erect and unreversed.

The field of view, then, is really the locus of the virtual image of the real or primary image formed by the objective; it is therefore inverted and reversed with respect to the object. However, if the regular ocular is used to project a real image on the photographic plate, the image will be erect with respect to the object, and unreversed when observed on the side of the screen away from the microscope.

The amplifying ocular, on the other hand, collects the image-forming rays from the objective before an image is formed. This type of ocular acts with the objective and forms the first image on the photographic plate as shown in Fig. 21*B*. Consequently, the image on the plate is reversed and inverted when observed as a camera image. The final print image obtained from either type of ocular is, of course, erect and unreversed.

Sec. 9. Angular Aperture. The aperture of a lens or diaphragm may be expressed in different ways depending largely on how the optical instrument is used. For instance, a diaphragm is usually spoken of as being so many inches or millimeters in diameter. The angular aperture of a diaphragm or lens is the angle subtended by it from some central point on its axis. Customarily the point is at some focal plane. The apertures of telescope objectives are also given in inches or millimeters; the object is at infinity. A photographic lens is rated according to its relative aperture, which is the focal length of the lens divided by the diameter of the entrance pupil of the system, or, roughly, by the effective diameter of the lens. The aperture of the microscope lamp lens is conveniently designated by its diameter,

and the microscope objective and condenser by their so-called numerical apertures. Figure 22 is a graphic representation of angular aperture. An objective is shown focused on an object at *O*. The light rays emerge from a condenser that has been diaphragmed to an aperture equal to that of the objective; the light travels through the object to the objective, and a cone of rays *AOB* is formed. The angle *AOB* is the angular aperture of the objective. If the diaphragm of the condenser had been opened wider, rays of light would have passed outside the objective, as shown by the dotted lines, but the angular aperture of the objective would have remained the same.

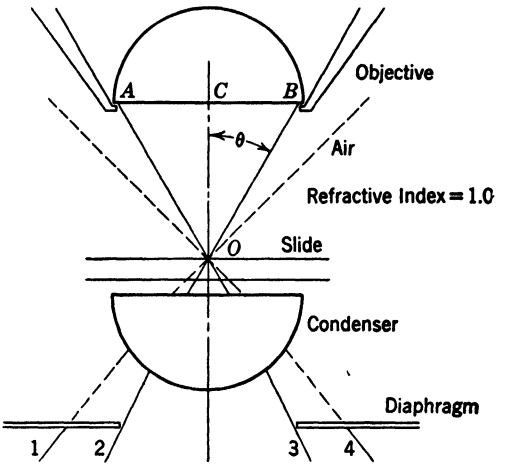


FIG. 22. Angle *AOB* is the angular aperture of the objective. The sine of angle θ times the refractive index of the surrounding medium, in this case air, $n = 1$, is the numerical aperture of the objective.

The angular aperture of a condenser can be controlled by the iris diaphragm inserted at either the lower focal plane of the condenser or between its lenses. Special objectives, made by the Spencer Lens Company and others, have aperture control in the form of a small iris diaphragm, for, as Fig. 22 indicates, the aperture may be controlled at the objective as well as at the condenser. In the next section, under the heading "Numerical Aperture," there will be further discussion regarding the effects of aperture control and the reason for paying particular attention to it.

Sec. 10. Numerical Aperture. Numerical aperture is a lens constant, a function derived from the angular aperture and the refractive index of the material through which the image-forming rays pass. The relationship was discovered by Ernst Abbe (1840–1905). Numerical aperture makes possible the direct comparison of the resolving and light-gathering powers of objectives; it is used in the equation for resolution (Sec. 13). If the numerical aperture, written from here on as N.A., is twice as great in one lens as it is in another, then the first lens will give twice the resolution of the second. In other words, the resolution of a lens is nearly directly proportional to its N.A.

N.A. is the sine of half the angular aperture times the refractive index (Sec. 38) of the substance through which the observation is being made (Fig. 22). Mathematically it is expressed thus:

$$\text{N.A.} = n \sin \theta \qquad [4]$$

in which n is the refractive index of the light-transmitting medium of least optical density (refractive index value), be it air, water, glycerin, oils of various kinds, alpha-monobromonaphthalene, or any other liquid through which the observation may be made. Thus, if an oil immersion objective of high N.A. is used on a dry mount, the air film limits the aperture. The refractive index of air is considered to be 1.00. The angle θ is half the angular aperture, that is, half of angle *AOB* in Fig. 22.

Any lens or system of lenses has N.A. as one of its constants, but in microscopy the term is generally confined to objectives and condensers. Although all lenses, such as bull's eyes and light-collecting lenses for lamps, have measurable N.A.'s, they are used in such a way that other lens constants are of more importance than the N.A.

In Fig. 22 it is clear that, if the iris diaphragm of the condenser had been reduced so that the cone of light *AOB* had been reduced, angle θ would have been less and the objective would not have been filled with light. Then the N.A. of the objective would be reduced to a working aperture equal to about half the sum of the N.A. of the condenser and that of the objective. This working aperture is expressed in an equation as follows:

$$\text{W.A.} = \frac{\text{N.A. objective} + \text{N.A. condenser}}{2} \qquad [5]$$

W.A. represents the working aperture of the complete system, objective and condenser. If the condenser is removed, the second term in the numerator becomes zero and the aperture is reduced by half, provided that normally the condenser has an aperture equal to that of the objective.

N.A. of an objective is a measure of its light-gathering qualities. The light transmitted by a lens varies as the square of its N.A. For this reason it is often possible to increase magnification by changing to an objective of higher power without any material lessening of the illumination of the field of view. As a matter of fact, an objective of higher power sometimes transmits more light, per unit area, than one of lower power, owing entirely to an increase of N.A.

Table V gives some measurements made with a photoelectric cell. It shows the relative light-gathering and -transmitting capacities of seven objectives.

Table V

Light-Gathering Capacity of Seven Objectives

Objective	Focal Length mm	Linear Magnification*	Areal Magnification	N.A.	Relative Light Intensity
No. 1 achro.	30	100	10,000	0.15	69.0
No. 2 achro.	16	200	40,000	0.25	38.5
No. 3 apo.	16	240	57,600	0.30	50.5
No. 4 achro.	8	420	176,400	0.40	31.5
No. 5 apo.	8	460	211,600	0.65	59.0
No. 6 apo.	4	800	640,000	0.95	27.5
No. 7 apo.	3	1200	1,440,000	1.32	18.0

* A 20× ocular was used throughout.

Several comparisons in Table V are worth noting. One is the measured light intensity of the two 8-mm objectives; another is the fact that the relative light intensities of objectives 2 and 7 differ by only 50 per cent, yet the difference in the areal magnification calls for an increase of light intensity of 36 times, for objective 7, in order to make both fields of view equal with regard to lighting.

The areal magnification of the 16-mm No. 3 objective is 57,600, whereas that of the 3-mm No. 7 is 1,440,000, or roughly 25 times as much. This means that to obtain equal intensity of light for these two objectives 25 times more light will be required for No. 7. On the question of magnification alone, the reading on light intensity would be about 2 for the No. 7. The fact that the N.A. of the No. 7 is about 4.5 times as great as that of No. 3 would raise the light-intensity reading from about 2 to about 40, the intensity varying with the square of the N.A. If there is a loss of about 50 per cent of the light, due to absorption and reflection by the No. 7 lens, then the reading of 18 in the relative light-intensity column is just about what could be expected.

Table V also shows that it is not always necessary to increase exposure time in proportion to the increase of magnification. In fact, sometimes the exposure may be even less for magnifications that are actually doubled. Determination of exposure time will be discussed more fully in a later chapter.

In addition to the resolution and light-gathering properties of a lens, N.A. controls the field depth, or vertical resolution, known erroneously as depth of focus (focal depth refers to depth of focus on the image side of the lens). Figure 23 illustrates how field depth is affected by change in N.A. It has been necessary to make these drawings to a

scale of roughly 10 : 1. The front glasses of three objectives are shown at *A*, *B*, and *C*, and the angles of the cones of light collected by them are shown also.

An inspection of the drawing of lens *C*, Fig. 23, shows that the angle

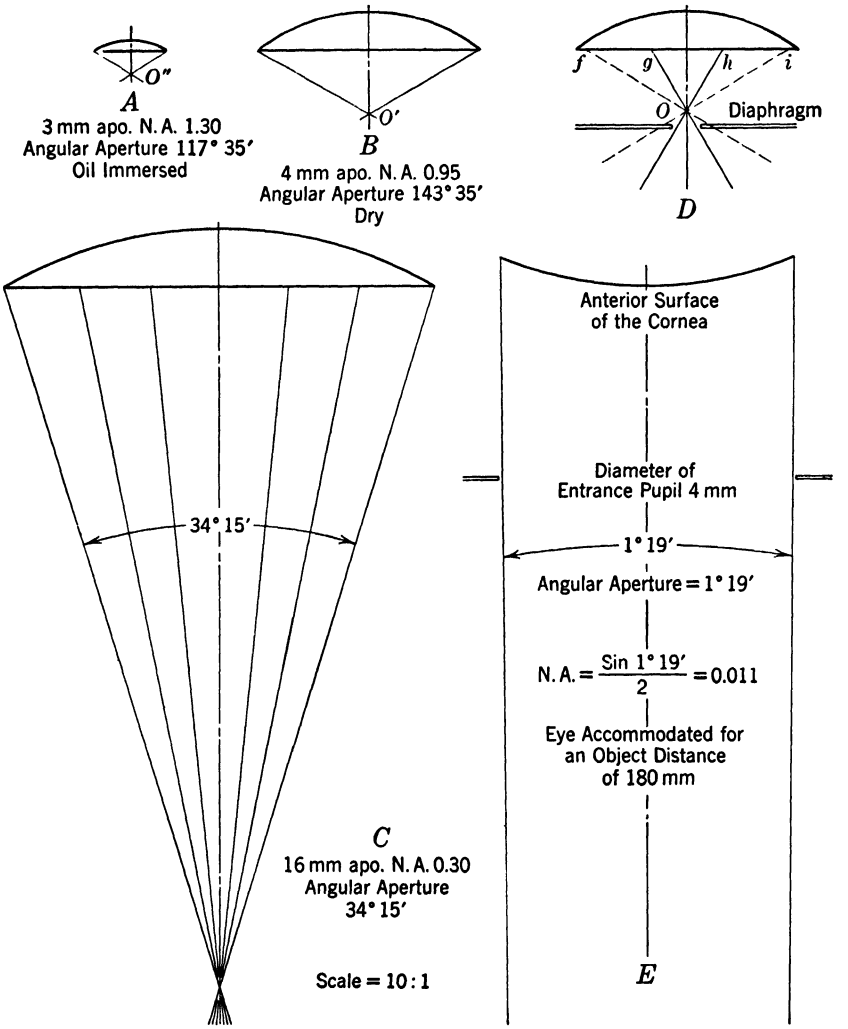


FIG. 23. The comparison of the aperture of several lenses is demonstrated. The scale is about 10 to 1. Diagram *E* illustrates the aperture of the eye accommodated for an object distance of 180 mm. Cf. *C*, a low-power objective.

This drawing demonstrates the tremendous difference between the great obliquity of the light cone used in high-power microscopic vision (lens *A*) and that used in natural vision (eye *E*).

of the light cone proceeding from the object is much more acute than the angle of the cone of lens *B*, or that of lens *A*. Theoretically, there is only one point, or plane, in the object field that is exactly in focus, and this point or plane lies at the apex of the light cone. However, as the angle of the light cone is reduced by using a lens of less aperture, as at *C*, or by diaphragming, there will appear to be an appreciable distance on either side of the apex where the rays are very close together. For this short distance in the object field all detail in the image will appear sharply defined, simultaneously; in other words, there is a certain amount of vertical resolution, or field depth. Field depth is controlled by the aperture of the objective, only. It is independent of focal length or any other lens constant.

For lens *A*, in Fig. 23, the field depth might be on the order of 0.5 μ or less. A field depth of 1/50,000 of an inch, or less, is very little, indeed; yet it must constantly be dealt with in microscopical work.

If the angular apertures of *A*, *B*, and *C* are reduced by a diaphragm, as at *D*, the N.A. of each system has been reduced, but the depth of field has been increased. An observer looking down through the drawtube of the microscope, with the ocular removed, may see an annular patch of gray. This is the unlit part of the objective, as at *fg* and *hi* of *D* in Fig. 23. (Also see Figs. 29 and 30.)

It has been shown that the aperture of an objective can be altered by changing the condenser diaphragm. The N.A. can also be controlled by a small diaphragm built into the objective or placed as an auxiliary slightly above it. A special series of Spencer objectives has such diaphragms between the lenses. An auxiliary iris diaphragm mounted above the objective is known as a Davis diaphragm.

The flatness of the field of view, an important factor in photomicrography, is increased, artificially, by reducing the N.A. However, for photomicrographic purposes there are better ways of reducing field curvature than by diaphragming the objectives. It must be remembered that, whenever an objective is used at less than its rated aperture, it will not be working at capacity, and oftentimes a cheaper lens might serve the purpose of a better one. If the image field is not sufficiently flat it may be expedient to use a longer bellows draw, to use projection lenses specially designed for such corrections, to try an achromatic instead of an apochromatic objective, or to combine a lower-power objective with a longer bellows draw.

Diffraction rings around a photographic image are often caused by too low an aperture. When an aperture is reduced to avoid glare, to get greater vertical resolution, to flatten out a field, or to increase contrast, the more nearly parallel light beam is likely to enhance the

ever-present diffraction rings to a point where they become noticeable. The small size of microscopic objects causes a certain amount of diffraction in interference of light. As the illuminating rays become more nearly parallel, or rather, as the more obtuse rays are eliminated by the process of diaphragming, the interference effect becomes more nearly complete and the diffraction rings are darker and can frequently be seen. (See Fig. 27.) Since a normally bright background has a tendency to mask diffraction rings, it is very common for them to appear in a picture even though they were quite invisible during the visual examination. If fairly large apertures are used the interference is less complete and the diffraction rings may disappear.

As the aperture of an objective is lessened, either by closing the diaphragm of the condenser, or by other means, contrast is increased. This increase in contrast is usually required to some extent. However, it should not be used as a correctional method for glare, because then the aperture may have to be closed beyond safe limits, and image deterioration may be set up. Under such conditions methods suggested in Sec. 102 should be applied before a low aperture is accepted as inevitable.

There are several methods of measuring N.A. of a microscope objective or condenser. The best measuring apparatus is expensive, but such measurements are seldom needed except in examining a lens prior to purchase. Nowadays most objectives have the N.A. values engraved on them, and some of the iris diaphragms of the condensers are marked off in intervals denoting N.A. or diameter of the iris opening. In either case such a diaphragm can be set with great accuracy, and better yet, any particular setting may be noted and filed for later reference, as required.

From the photomicrographer's standpoint, it is usually more important to know how the aperture should be controlled than how to measure it. However, for those who wish to make such determinations, the Abbe apertometer, by Zeiss, is suggested; on this instrument direct readings of N.A. and angular aperture may be made in one operation. Beck[10] has developed a simpler device at much less cost, and Gage[11] describes a method using home-made apparatus. Another way to determine N.A. is by means of the equation given in *Photomicrography*, published by Eastman;[12] this method involves measuring the diameter of the field at the eyepoint of the microscope. The

[10] R. & J. Beck, Ltd., 69 Mortimer Street, London.

[11] Simon H. Gage, *The Microscope*, fifteenth edition, 1932.

[12] Eastman Kodak Company, *Photomicrography*, 1932. Also see later editions.

radius of the eyepoint, R_e, times the magnification of the eyepiece, $M_{ocl.}$, divided by the focal length of the objective, $F_{obj.}$, equals the N.A. of the objective as equation 6 shows. A similar equation is given by Beck.[13]

$$\text{N.A.} = \frac{R_e \times M_{ocl.}}{F_{obj.}} \qquad [6]$$

One of the least expensive methods of measuring N.A. of an objective, though probably not one of the easiest, is to proceed as follows: A piece of ground glass, about 50 or 60 mm square, or slightly larger, should be placed on the microscope stage, and a millimeter scale, of

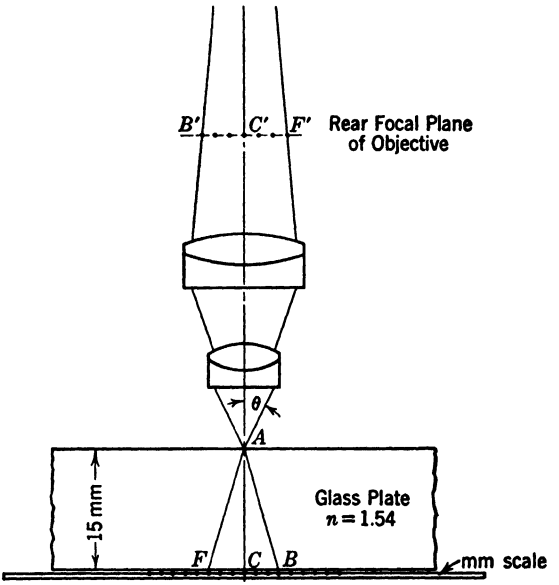


FIG. 24. An extemporized method for measuring the numerical aperture of an objective.

the transparent type if possible, should be placed on the glass directly under the objective. To assure alignment, the objective can first be focused on the scale. The scale should be well lit with a strong overhead lamp, as for oblique illumination, or with strong transmitted light from the mirror, used without the condenser. The tube of the microscope is raised high enough to allow a piece of plain glass about 15 mm thick to be placed directly on the scale. The refractive index of the glass must be known. The objective is focused on the

[13] C. Beck, *The Microscope*, Part II, 1924.

upper surface of the thick glass plate. The ocular is removed, and while the field at the rear focal plane of the objective is observed the scale can be moved, if necessary, until it is in proper position to measure the diameter of the objective circle. The number of millimeters included by the objective circle can now be read. The refractive index of the glass plate, the thickness of the plate, and the number of millimeters that can be observed are all the data needed to compute the N.A. of the objective. Figure 24 shows the geometrical construction and the mathematical steps required for the solution.

The following specific problem is given as an example. The thickness of the glass block is 15 mm; the refractive index is 1.54; the distance FB observed at the rear focal plane of the objective is 6 mm; and the N.A. of the objective is $n \sin \theta$.

$$\sin \theta = 1.54 \ (\sin CAB)$$

$$\tan CAB = \frac{CB}{AC} = \frac{3}{15} = 0.20 = \tan 11°19' = \text{angle } CAB$$

$$1.54 \ (\sin 11°19') = 1.54 \times 0.196 = 0.302$$

Therefore

$$n \sin \theta = 0.302 = \text{N.A. of the objective}$$

In this method of measuring N.A., it should be remembered that no account need be taken of the refractive index of the medium between the glass block and the objective. However, the rear focal plane of the objective should be inspected with the greatest care to ensure that the objective is filled with light. Immersion lenses must be immersed before measurements are taken.

Figures 25, 26, and 27 show effects of aperture control. There is not a great deal of difference between any of the three pictures as far as aperture is concerned, but there is considerable difference in their appearance.

Since the refractive index of air is 1.0, and the maximum sine value is 1.0, the maximum N.A. of a dry objective must be 1.0, indicating an angular aperture of 180°. In practice this is never attained; an N.A. of 0.95 is the highest practical rating for a dry objective.

When a lens is used immersed, its N.A. may be higher than 1.0. Zeiss Company manufactures an objective which has an aperture of 1.6 when used with alpha-monobromonaphthalene. The usual maximum N.A. for apochromatic objectives when immersed is 1.4 or 1.3. For achromatic objectives it is about 1.25.

The N.A. of the condenser should be equal to, or greater than, that of the highest objective with which it is to be used; otherwise there

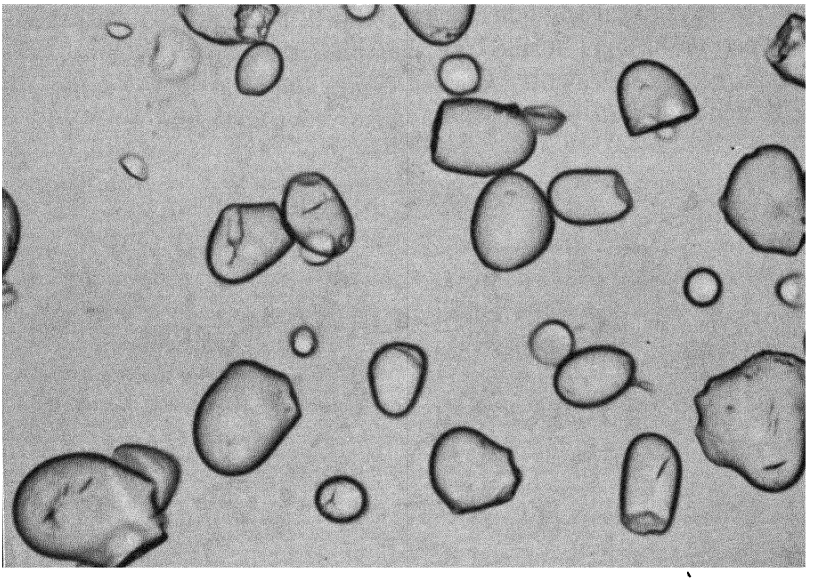


FIG. 25. Photomicrograph of sago flour ×360. The aperture of the condenser afforded about a 9/10 cone of light. Other photomicrographic data are immaterial.

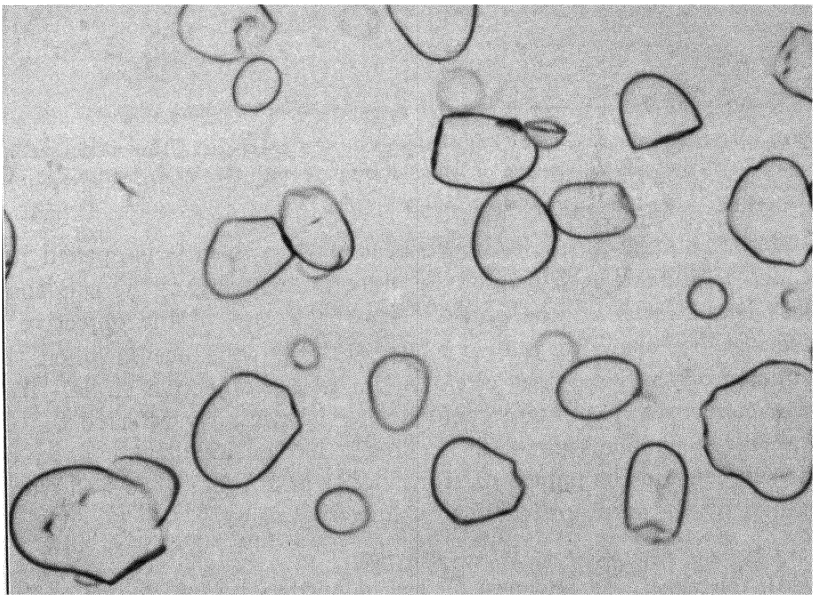


FIG. 26. Sago flour ×360. The aperture of the condenser was larger than that of the objective. Compare with Figs. 25 and 27.

will be loss of aperture on the high-power objectives that cannot be compensated for in any other way.

The cone of light formed by the condenser, with the object at its apex, is often conveniently referred to as a full cone of light, a $^9/_{10}$ cone, a $^3/_4$ cone, and so on. This refers, of course, to the setting of the

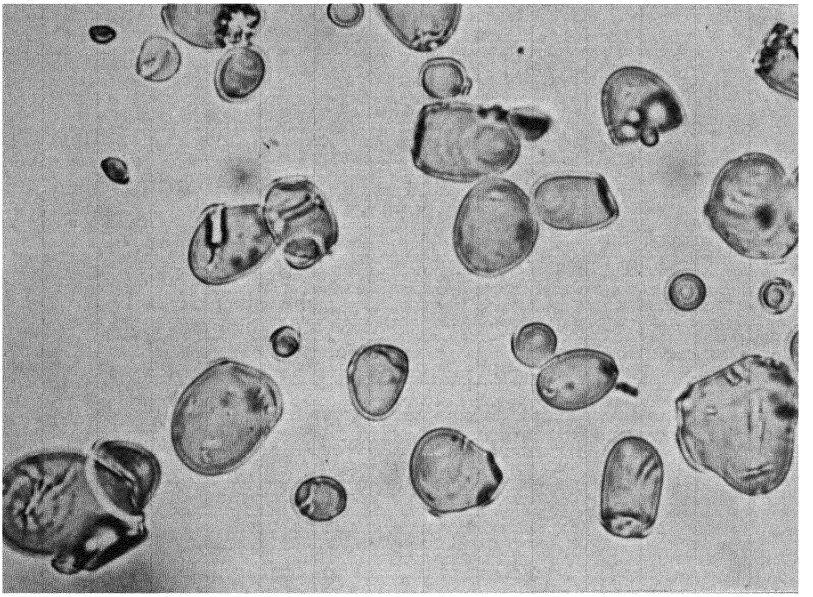


FIG. 27. Sago flour ×360. For this picture the aperture of the condenser was closed, to give about a 2/3 cone of light. Compare with the other pictures of sago flour.

condenser diaphragm; a full cone occurs when the iris is opened just enough to fill the back lens of the objective with light; the condenser must be in focus on the object. The back lens of the objective is observed by removing the eyepiece. A $^9/_{10}$ cone occurs when the diameter of the condenser circle is $^9/_{10}$ of the objective circle. Any other condenser diaphragm opening may be similarly referred to. It is a very convenient method. Belling[14] has an excellent chapter on this subject and the papers of Hartridge[15] and Abbe[16] are well worth reading by those interested in measuring aperture.

[14] J. Belling, *The Use of the Microscope*, 1930.

[15] H. Hartridge, "An Improved Method of Apertometry," *J. Roy. Micr. Soc.*, 1918.

[16] E. Abbe, "Some Remarks on the Apertometer," *J. Roy. Micr. Soc.*, 1880.

Summary. The following essential points concerning N.A. should be borne in mind by every microscopist, and they have special importance for the photomicrographer.

1. Large apertures must be used to preserve detail and maintain the maximum resolving power of the lenses.

2. Changing from lower to higher objectives may not increase exposure time if N.A. is also increased. The intensity of the illumination on the plate varies with the square of the N.A., assuming that other factors remain the same.

3. Field depth is a function of N.A. It can be increased by decreasing aperture.

4. By reducing aperture, glare is decreased and photographic contrast is increased.

5. The projected image may appear more uniformly focused, that is, the field may be flatter, if the aperture is reduced.

6. Greater working distance is obtained with low-aperture objectives than with high-aperture ones.

7. Field depth for high-aperture objectives is very small.

8. Too low an aperture will increase the intensity of diffraction effects until they become objectionable (Fig. 27). This phenomenon will frequently be seen as bands of alternating white and black near the edges of the images. Often only the black line is visible.

Sec. 11. Field Diaphragms. Diaphragms, or stops as they are sometimes called, are openings or ports in an optical system to control either the obliquity of the light rays traversing the system or the size of some field. In the first case, they are known as aperture diaphragms and in the latter case as field diaphragms. They may be fixed in size or they may be adjustable. Adjustable ones are known as iris diaphragms; examples can be found on the camera shutter or lens, on the substage of the microscope, or at the lamp. Diaphragms are usually located in or near the focal plane, or equivalent plane of any lens in any part of an optical system. When found in other parts of a system they serve as shields more than as true diaphragms. Thus a diaphragm halfway down the microscope tube may be acting as a shield to prevent glare from the reflections at the sides of the tube from entering the eye, and so masking detail of the image. In the case of the microscope objective, or a hand magnifier, the mount itself, or some part of it, may act as a diaphragm.

Field diaphragms limit the size of the field under observation. Thus, the diaphragm at the lamp is focused by the condenser, and its image appears in the object field. This image is refocused by the objective, another image (the primary image) being formed at the eyepiece.

The image of the lamp diaphragm is brought to a focus for the third time by the eyepiece and eye acting together as an optical unit, and it can be seen in the field of view. When nearly closed this diaphragm forms a restricted field of light on which the condenser can be most precisely focused. It is a field diaphragm.

As already stated, any diaphragm in such a position that it serves to bound the observed field is a field diaphragm. Consequently, in classifying a diaphragm it is important to note from what point in the optical system the diaphragm in question is examined. For instance, the iris diaphragm of the condenser cannot be seen in the field of view when the condenser and objective are properly focused, because it is acting as an aperture diaphragm and is not at any point in the optical system which is focused in the field of view. If the eyepiece is removed, and the rear focal plane of the objective is observed, the iris of the condenser will be plainly seen as it is opened and closed. When examined in this way it is acting as a field diaphragm. In the first case, it controls the illumination by reducing the angle of the illuminating cone, and in closing, it shuts off some of the light that would otherwise pass to the objective and so to the eye. In the second, it is being observed through the condenser and the objective lenses, and, since the image of the diaphragm is visible and the light source is constant, the illumination over the unit area of the lighted disc will be constant in intensity, irrespective of the opening or closing of the diaphragm. Figure 28 indicates how the diameter of the light circle may vary in size as the diaphragm is opened or closed, but the intensity of light, which is measured over unit area, will remain the same. When examined at the rear focal plane of the objective, the iris diaphragm of the condenser is obviously a field diaphragm, as shown in Fig. 28.

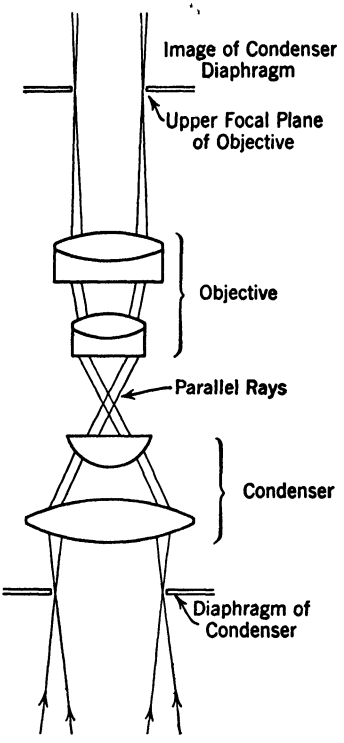


FIG. 28. Diagram of rays passing through the condenser and forming an image of the condenser diaphragm at the second focal plane of the objective. This illustrates clearly how anything at the first focal plane of the condenser is imaged at the second focal plane of the objective and ultimately at the exit pupil of the microscope. These three fields are mutually conjugate.

In the above paragraph, special emphasis is placed on the fact that, when the field of view is being examined, the condenser diaphragm acts as an aperture diaphragm, but when the rear focal plane of the objective is being examined, it acts as a field diaphragm. To avoid confusion, it should be clearly understood that under no circumstances can the function of a diaphragm acting to control aperture be performed by a diaphragm acting to control the size of the field, or vice versa.

Lamp diaphragms in particular, and field diaphragms in general, help to eliminate glare by reducing the size of the observed field and so confining the illumination to a small area. They should be used for this purpose. The image of the field diaphragm of the eyepiece seen in the field of view may be superposed on the image of the field diaphragm of the lamp by opening or closing the latter diaphragm. Anything located at a field diaphragm may be projected into the field of view. Micrometer scales located at the eyepiece diaphragm, dirt on the ground glass at the lamp, or even the structure of the ground glass itself may be so projected.

Without exception, the field diaphragm at the lamp should never be opened wider than is necessary to fill the field of view with light. While the operator is looking through the microscope, the iris diaphragm of the lamp should be slowly closed until its image just commences to cut into the field of view. On very glary material, it may be closed even more, until only part of the specimen in the center of the field is illuminated. Usually too little attention is paid to the regulation of this diaphragm.

When the microscope is used with the camera, particularly when the bellows draw is 15 inches or more, it is possible to close the lamp diaphragm even more than when the microscope is used visually, and yet the image field will not be reduced in size to such an extent that the field diaphragm cuts into the picture. This is an important adjustment for the photomicrographer.

If the lamp is not fitted with an iris diaphragm, small metal or cardboard discs can be made to hang over the lamp lens. Holes of various sizes may be cut through the center of the discs to suit the requirements of the various objectives. Usually, a series of five or six is sufficient. The smallest hole, for the 2-mm objective, might be only 2 mm in diameter, the lamp distance being, say, 14 inches.

Eyepiece diaphragms of the adjustable type are used to mark out the definite size areas in the specimen for purposes of counting or measuring. They have a marked effect on glare and can be used to control it, principally for visual examination rather than for photomicrography. The Ehrlich eyepiece, with an adjustable diaphragm.

is of this type; but a series of thin metal discs made to fit within an ordinary eyepiece at the eyepiece diaphragm will serve the same purpose at much less expense. Holes of appropriate size and shape can be cut in the discs.

Sec. 12. Aperture Diaphragms. Aperture diaphragms, as their name implies, control the aperture of lenses and the obliquity of light cones. Every lens has an aperture diaphragm. It may be interposed, or it may be the limit of the lens itself or part of the mounting. Thus there is an aperture diaphragm for the ocular system, one for the objective system, and one for the condenser system. The effective aperture diaphragms in the microscope system, taken as a whole, are those of the objective and the condenser.

The diaphragm used with the condenser of the microscope limits the angle of the light cone focused on the object. Consequently, if this diaphragm is closed, the condenser aperture will be less and a loss of working aperture (equation 5) will result. There will also be a loss of resolution (Sec. 13), a reduction of light intensity, an increase in field depth, heightened contrast in the image, and a somewhat less curved appearance in the field.

Since the aperture of the objective is generally fixed, the more important aperture for the microscopist is the one located at the condenser. While the microscope is being used visually, particularly at high apertures, the condenser diaphragm should be readjusted almost continuously. As the focus of the microscope is changed, or the eye is shifted from one part of the field to another, even slight alterations in the adjustment of the condenser diaphragm will aid materially in maintaining the best visual acuity. For purposes of photomicrography, the setting of the condenser diaphragm must be at its optimum in order to give the proper contrast to the image and to assist in obtaining sufficient field depth.

Figures 29 and 30 show the appearance of the condenser diaphragm when the eyepiece is removed. The photomicrographs were taken by focusing the camera on the upper focal plane of the objective (see Fig. 28). Figure 29 shows the iris reduced to a small opening; the large opening of the diaphragm is shown in Fig. 30. The larger opening is slightly greater than the normal setting for achromatic objectives.

When the condenser and objective diaphragms as shown in Figs. 29 and 30 are examined it will be seen that both are acting as field diaphragms, although they are still aperture diaphragms as far as the microscope as a whole is concerned. The outer boundary of the gray annulus is formed by the objective mounting; it limits the objective cone; it is the objective circle. The boundary of the spot of

light is the iris diaphragm of the condenser which limits the condenser cone; it is the condenser circle.

In Figs. 29 and 30 the gray hazy ring of partial lighting is glare produced by the subject on the microscope stage. If it is desired to inspect the opening of the condenser diaphragm, a subject producing considerable glare should be used; otherwise the gray ring will not be seen, since the space it would occupy would be perfectly black and it would be impossible to tell where the condenser circle and objective circle are, in relation to each other. A piece of ground glass

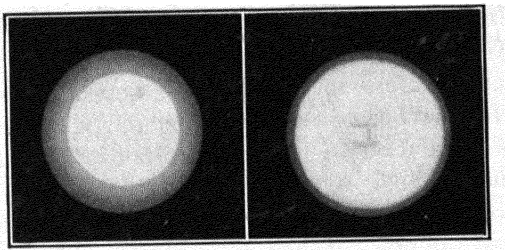


FIG. 29. The condenser and objective circle as seen when looking down the tube of the microscope.

FIG. 30. This is about the same view as in Fig. 29 except that the condenser circle is larger, and approaches very closely the much-to-be-desired 9/10 cone of light.

added to the substage also will enhance the glare and tend to make the difference between the objective circle and the condenser circle more marked. If, in taking a photomicrograph, the gray ring seems too bright, it may be eliminated altogether by using a Davis diaphragm directly over the objective. Otherwise the glare from the unfocused light may cause undue reduction of aperture. In photographing glary subjects, the photomicrographer will find such a diaphragm very useful and in some instances essential for making a good picture.

Central stops are diaphragms which block out the central rays of a lens system. They limit the incident rays of small angle and, therefore are placed in the position of aperture diaphragms. The central condenser stop for low-power dark-field work is an example of this sort.

The discussion of microscope diaphragms can be summed up as follows:

1. There are two classes of diaphragms in the microscope system, field diaphragms and aperture diaphragms.[17]

[17] Every lens has an aperture and field diaphragm. See any book on geometrical optics.

2. Field diaphragms are generally located at the lamp and at the eyepiece. They can be seen in the field of view.

3. Aperture diaphragms are located at the condenser of the microscope and at the objective. They cannot be seen in the field of view.

4. Field diaphragms control the size of fields, and to a certain extent they control glare.

5. Aperture diaphragms control the working aperture of the microscope, resolution, the intensity of the illumination, contrast in the image, depth of field, glare, and to a certain extent flatness of the field of view.

Sec. 13. Practical Considerations of Resolution. The term resolution generally refers to lateral resolution unless otherwise stated. Lateral resolution refers to the distance between discrete particles necessary to form separate and distinct images. The term vertical resolution has been used to indicate depth of field; it refers to the vertical distance through which a microscope objective will form acceptably sharp images. Herein the somewhat more precise term field depth will be used in place of the term vertical resolution.

A lens system with N.A. equal to or less than the aperture of the human eye cannot form a sharp image of particles smaller than can be seen by the human eye, regardless of whether it is used to project a real image, as in photomicrography, or to form a virtual image, as in a visual microscopical examination.[18]

Small objects below the power of the lens to resolve will be seen indistinctly, if at all, and they will have a hazy blurred outline. This difference between the resolving power and visibility should always be borne in mind when using the microscope to study fine detail. A particle that is resolved is clearly and sharply defined and the image should be vivid. Particles below the limit of resolution may be seen as indistinct images; if the power of the eyepiece is increased, these images will be larger but more indistinct than before, yet certainly they will be visible. Thus drawing a line between visibility and resolution is partly a personal problem when considered from the practical standpoint. In any event a very high ocular such as a 30× or even higher will aid in studying the images formed by objectives.

Before considering resolution further, it might be enlightening to turn to Table VI. Here it is shown that usually the lowest-power objective found on a compound microscope is the 16-mm or thereabouts. This gives a primary magnification of about 10 and has a

[18] The limit of resolution of the unaided eye is about 0.0036 inch, or nearly 0.1 mm, which at a distance of 25 cm would subtend an arc of about 1 minute. The N.A. would be 0.004 when the diameter of the entrance pupil is 2 mm.

resolving power of 1.09 μ in green light. Any particle of this diameter can be resolved and clearly seen if it can be properly mounted and if a 10× ocular is used; 100 times 1.09 μ equals 0.109 mm, about 1 minute of arc at 10 inches. The smallest particle that can be resolved with the visual microscope in green light is roughly 0.2 μ, or only a little over 0.9 μ smaller than that resolved by the low-power objective. (More exact figures are meaningless.) In other words, the whole discussion of microscopical resolution revolves around a particle size with a variation of only about 1 μ from the smallest to the largest. It might be noted that this is the maximum figure when the large particle is examined in green light. If both large and small particles are examined in blue light, the difference shown in the table is just 0.67 μ!

In spite of the above rather startling figures, resolution plays a very important part in good photomicrography, for, although more often than not the full resolving power of the system is not utilized because of poor adjustments, it should be possible to plan for the highest resolution desired; then the photographic images must be made perfect enough to justify the resolution. When this is done and when field depth of the objective is sufficient for the specimen, subsequent enlargements are practical and will give pleasing results.

Figure 31 shows a photomicrograph of bacteria. In this picture the resolution is complete, the separate cells appearing clearly as the individual units that they really are. Figure 32 is taken at the same magnification, but the resolution is too low. No single bacterium appears separated from other bacteria unless it is isolated from the main group.

The following mathematical statement connects the different variables involved in resolution:

$$R = \frac{\lambda}{2\,\text{N.A.}} \qquad [7]$$

In words, R, resolution, equals the wavelength, λ, of light used to illuminate the subject divided by twice the N.A. of the lens. If the wavelength of light is given in inches, the resolution will be the least distance apart (expressed in inches) that two particles, or lines, may be distinctly observed as discrete particles or lines.

If equation 7 is written with the fraction inverted, the resolution is given directly in lines per inch. This is sometimes referred to as resolving power, the term resolution being reserved for the value of R as given in the equation. However, this distinction may lead to confusion since it is not generally followed.

Usually tables on resolution have a column showing the objective referred to, another giving the N.A. of the objective, and one or more

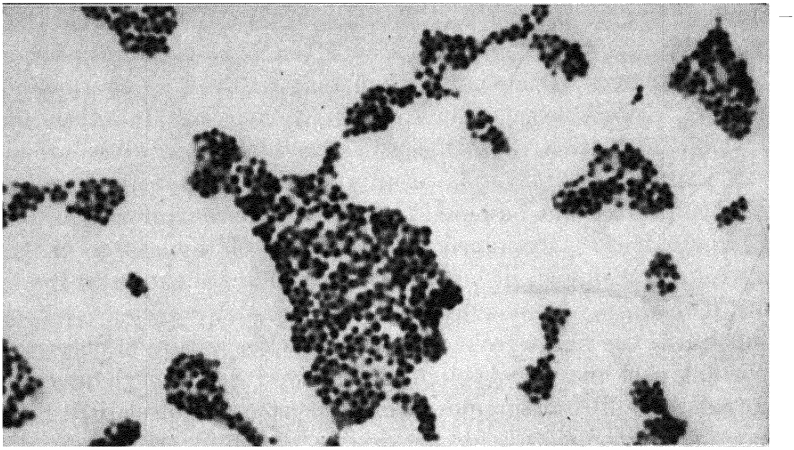


FIG. 31. Photomicrograph of *Staphylococcus albus* ×1600. Compare with Fig. 32.

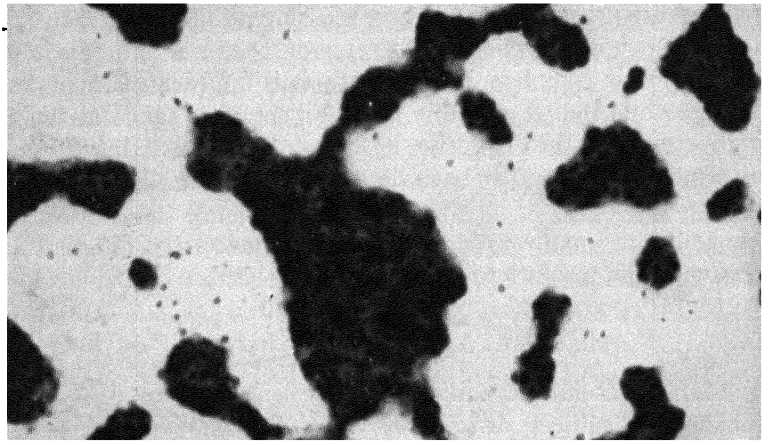


FIG. 32. The same subject as Fig. 31 at the same magnification, but the aperture was too low to resolve the individual cells. The additional separate cells seen in this picture and not in Fig. 31 have been made evident because of the increase in field depth at the expense of low aperture — it is poor technique to stress detail in this way.

columns showing the resolution of the various objectives when used with light of different wavelengths. If no wavelength is given, white light can be assumed; the wavelength at which resolution is figured

under these conditions is, as a rule, 526.9 mμ. It is known as the E line in the spectrum. It is customary in tables of resolving power to give the figures in lines per inch; for example, an 8-mm objective with a N.A. of 0.65 will resolve 62,667 lines per inch in light of wavelength 526 mμ. An additional column in Table VI gives the magnification necessary to make the image of the barely resolvable particle 1 mm long. The camera extension is assumed to be 10 inches. These figures can be reduced proportionately for greater bellows extension.

If equation 7 is applied to check resolution tables, it must be remembered that the value of R will be the distance between lines that is necessary for theoretical resolution and not the number of lines per inch that can be resolved. The reciprocal of R will give the number of lines per inch. Also, it is important to remember when working with equation 7 that while wavelength of light, as a rule, is given in millimicrons (in microscopy), it may also be given in millimeters, microns, or angstrom units. Any of these units may be converted into the British system of inches before the value of R is computed in lines per inch. If this is not done the answer will be in millimicrons, millimeters, microns, or angstrom units, as the case may be.

When speaking of resolving power the term " lines per inch " may be misleading. In some respects it is an unfortunate term to use in connection with resolving power. Actually, lines as drawn on a glass slide can have no significance whatever in referring to resolving power, because obviously the space between such lines might vary according to the width of the lines. In order to avoid such an obvious source of error the geometrical line should always be thought of when speaking of resolution.

Another pertinent point, seldom stressed and sometimes overlooked altogether, is that figures pertaining to resolution are, for the most part, theoretical and seldom subject to proof. The equation for resolution given in this section is based on measurable and precise quantities. However, there are uncertainties and unaccountables with which the equation does not deal, such as variation of individual eyesight, experience in using the microscope, magnification, preparation and mounting of the specimen, visibility, and aberrations of the lenses. All these contribute their share toward the ability to see fine detail with greater or less distinctness. It is futile to select a lens system that theoretically gives the desired resolution for a given photomicrograph, only to find later that the desired detail is entirely lacking in the negative, owing to non-recognition of uncertain contributing factors. Ultimately, for the microscopist, the ability to figure resolution theoretically is of value only as a foundation for the practical part of the work, which

involves many other considerations. Accordingly the selection of lenses for any special work cannot be discussed until later, when more preliminary work has been covered.

As the aperture of the microscope is lessened, the equation

$$R = \frac{\lambda}{2\,\text{N.A.}}$$

does not apply, since the Abbe theory of resolution requires that at least one diffracted ray be included in image formation. If the aperture of the condenser is stopped down, less of the objective will be used, and the necessary diffracted light for resolution will be quickly lost. If the angle of the condenser cone is considerably reduced, the value of R will be doubled, and the equation then becomes

$$R = \frac{\lambda}{\text{N.A.}} \qquad [8]$$

for reduced apertures.

Abbe's theory of image formation, which is widely if not universally accepted, and others are given in Secs. 51 and 52.

Practical points on resolution that it is well for the photomicrographer to remember are:

1. Resolution is a function of N.A. (see Sec. 10).
2. For the utmost in resolution, blue light should be used. (See Table VI.)
3. The limit for resolution with the microscope, using blue light, is about 0.2 μ, or 125,000 lines per inch. This is a good figure to remember when working with small particles, such as paint pigments.
4. Particles that are out of focus must not be confused with particles that are not resolved.
5. Apochromatic objectives, owing to their higher N.A., will give slightly better resolution than the corresponding achromats.
6. The greatest resolution of which the microscope is capable can be realized only under nearly perfect optical conditions.
7. Particles shown in a photomicrograph often seem sharper, and consequently smaller, when blue light is used for illumination. This condition is most marked for particles of about 2 or 3 μ or less.
8. The term " lines per inch " refers to geometrical lines.
9. Equation 7 is applicable only when a full cone of light can be used.

In using Table VI, it should be remembered that the figures given for resolution are seldom attained in actual practice, because it is

Table VI

The Resolution of Nine Objectives Computed for Four Different Wavelengths

Objective	N.A.	Red Light λ = 691 mμ		Green Light λ = 546 mμ		Blue Light λ = 405 mμ		Ultraviolet λ = 365 mμ		Magnification* Required for a 1-mm Image λ = 546
		Lines per inch	Separation for Resolution μ	Lines per inch	Separation for Resolution μ	Lines per Inch	Separation for Resolution μ	Lines per Inch	Separation for Resolution μ	
1	2	3	4	5	6	7	8	9	10	11
16-mm achr.	0.25	18,389	1.38	23,292	1.09	31,216	0.81	34,780	0.73	917
16-mm apo.	0.30	22,073	1.15	27,889	0.91	37,896	0.67	41,666	0.61	1099
8-mm achr.	0.40	29,464	0.86	37,363	0.68	50,191	0.51	56,497	0.45	1471
8-mm apo.	0.65	47,828	0.53	60,152	0.42	81,661	0.31	90,909	0.28	2381
4-mm achr.	0.85	62,500	0.41	79,375	0.32	106,617	0.24	120,773	0.21	3125
4-mm apo.	0.95	69,266	0.36	90,703	0.28	119,812	0.21	133,868	0.19	3571
3-mm achr.	1.25	92,024	0.27	116,510	0.22	157,759	0.16	169,491	0.15	4545
3-mm apo.	1.30	95,479	0.26	120,929	0.21	163,855	0.15	181,818	0.14	4761
3-mm apo.	1.40	102,819	0.24	130,251	0.20	176,657	0.14	196,078	0.13	5000

* This column lists the magnification required to make the image of a particle, at the threshold of resolution, 1 mm in diameter. It is based on the figures given for resolution in green light — Column 6.

generally necessary to reduce apertures for other reasons. Closing the iris diaphragm of the condenser to increase contrast or to avoid glare reduces aperture, which in turn reduces resolution. Objectives of 2 and 1.5 mm were not included in Table VI because their N.A. is no higher than that of the 3-mm lenses. The wavelengths of light selected are easily obtained with the H4 mercury-vapor discharge tube of the General Electric Company when it is equipped with the monochromatic optical glass filters Nos. 2, 5, and 8 by Corning. The table was computed by means of equation 7.

If the figures in column 11 are divided by 10, the quotient will be the total magnification required to give an image sufficiently large to be resolved by the eye. In the field of view it is roughly 0.1 mm. If the figure so found — 0.1 of the figure in column 11 — is divided by the magnification of the objective, the quotient will indicate the magnification of the ocular which will be required to give the total magnification for the resolution indicated.

Columns 4, 6, 8, and 10 give the distance, in microns, by which particles or lines must be separated for resolution by light of differing wavelength. This figure can also be considered the least diameter of a particle that can be resolved under the given conditions. The figures in column 11 can be reduced in the same proportion as the bellows extension is increased, the image size remaining the same in diameter, that is, 1 mm.

Sec. 14. The Exit and Entrance Pupils of the Microscope. The exit pupil of any optical system is the image of the aperture diaphragm in the image space. The image of the aperture diaphragm in the object space is the entrance pupil.[19] The diaphragm may be a special one connected with the lens mount, it may be part of the lens mount, it may be the lens itself that limits the obliquity of the rays at any given point of observation, or it may be a diaphragm in some other position. When a small hand magnifying lens is properly used, the entrance pupil of the eye, which lies slightly in front of the eye lens, is placed at the second focal point of the hand lens. The entrance pupil of the eye is in the image space of the magnifier and is the exit pupil of that lens. Since the exit and entrance pupils are conjugate with each other, the entrance pupil of the magnifier, under the conditions cited, will be at infinity in the object space. The exit pupil of objective and condenser is close to the second focal point of the objective; so the entrance pupil of the microscope lies at the iris of the condenser. (See Fig. 28.) The exit pupil is referred to as the

[19] A. C. Hardy and F. H. Perrin, *Principles of Optics*, Chapter V, McGraw-Hill Book Company, 1932.

Ramsden circle or disc, the eyepoint of the microscope, the Lagrange disc, or the pupil of the microscope. Thus the exit pupil of the microscope is the image of the boundaries of the objective lens. However, the image of the iris diaphragm of the condenser may appear superposed on this image and concentric with it. This diaphragm is subject to manual control, and so to all intents and purposes it becomes the effective aperture diaphragm of the entire microscope system. Likewise this diaphragm exists in the object space and is normally (not necessarily always) the entrance pupil of the microscope. If the condenser is so focused that an image of its diaphragm appears in the field of view, the diaphragm at the lamp becomes the aperture stop and the entrance pupil of the system.

The field at the second focal plane of the objective will appear imaged at the exit pupil of the microscope. If a magnifying lens is held over the ocular, the field of the exit pupil may be examined with great care. With the microscope properly illuminated, the field at the exit pupil will be exactly the same as the field at the rear focal plane of the objective, except that it will appear inverted and somewhat smaller. This method of examination offers an excellent opportunity for centering the condenser with the objective, since the adjustments can be observed very precisely.

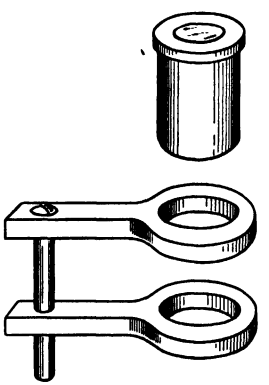


FIG. 33. A magnifier and holder to fit on the drawtube of the microscope and to act as a simple microscope to examine the exit pupil.

A special device for the examination of the eyepoint is made by Beck and others. It consists of a positive ocular mounted in a holder which fits over the drawtube. It has an extension rod and a setscrew for vertical adjustment. Figure 33 is a drawing of the eyepiece holder as designed and made by Mudge.[20] When the auxiliary eyepiece is focused, the eyepoint is clearly shown. Fitting a micrometer scale to the accessory ocular makes it possible to measure the diameter of the eyepoint with precision. To do this, the condenser diaphragm should be opened to correspond with the objective circle (see Figs. 29 and 30), and the diameter of the exit pupil can be measured. Equation 6, Sec. 10, can then be applied.

The N.A. as determined above is the N.A. of the objective only if

[20] Robert S. Mudge, General Manager, Palo-Myers, Inc., 81 Reade Street, New York City.

the back lens of the objective is filled with light; otherwise it is the working aperture.

Oftentimes defects in lighting will be seen at the exit pupil or, when looking down the drawtube, at the rear lens of the objective. Before taking a picture, it is always good practice either to examine the exit pupil or to remove the ocular and examine the back lens of the objective. Dirt on the objective, as well as certain irregularities in lighting, will show very plainly in such an examination.

The height of the exit pupil above different eyepieces varies considerably. With some oculars it is very low, but in special oculars made by Spencer, Swift, and a few others the eyepoint has been raised to afford ease and convenience to those wearing eyeglasses. Some eyepoints are so low that they are particularly troublesome in the summertime. Perspiration and the nearness of the eyepiece to the eye set up a very disagreeable condition when long hours at the microscope are demanded.

Optically it does not seem to make any difference whether the location of the exit pupil is high or low. Its position varies with the make and design of the ocular. Devices used to turn the rays of light after they leave the microscope are best located at the eyepoint. A quick method to locate an eyepoint is to hold a piece of thin paper or ground glass so that its surface is normal to the emerging rays, and then to move it slowly up and down near the ocular; if the microscope has been strongly illuminated and properly focused, the eyepoint is easily seen when the spot of light on the paper or glass has its smallest diameter.

From the foregoing, it can be seen that the position of the eyepoint has little or nothing to do with the excellence of the picture. However, facts worth remembering are:

1. The ocular with a high eyepoint is very desirable for visual work, particularly when glasses are worn.

2. Spencer makes a special line of compensating oculars with high eyepoint. Swift of London also has a special eyepiece of this type called the "Telaugic."

3. The reflecting surface of any device for turning the image-forming rays to a new direction should be placed at the eyepoint.

LABORATORY WORK

Exp. 1. Preparing Test Slides. Numerous materials can be used as test slides, but the reader is urged to make the following six slides, since they will be referred to from time to time throughout the text in relation to various problems.

SLIDE 1. A sample of Carborundum powder 600. Mounted dry. (See Sec. 156 for making this mount and the others which follow.)

SLIDE 2. Red or black magnetic rouge, mounted in balsam, dammar, isobutyl methacrylate, clarite, or diaphane.

SLIDE 3. Finely ground corundum powder (emery powder). Mounted dry, with cover glass.

SLIDE 4. A piece of Dufay color film with the emulsion removed, showing the réseau. Mounted dry.

SLIDE 5. Titanium dioxide mounted in the same way as the red rouge.

SLIDE 6. Potato starch mounted in glycerin jelly. (See Sec. 157.)

Covers for the above slides should be 0.16 mm thick. The slides can be 1.0 to 0.9 mm thick. They should not be over 1.00 mm. The slide of color film can conveniently be 0.8 or even 0.7 mm to compensate for the thickness of the film.

Slides 1, 3, 5, and 6 will be generally useful for testing various microscope adjustments. Slide 2 will be used largely to test for resolution and for dark-field adjustments, although temporary slides of india ink solution are also suggested as test objects for dark-field work with the cardioid microscope. Slide 4 is suitable for testing for color effects, filters, and for other purposes. For specific work, slides of other specimens will be suggested, but the six mentioned here will always be convenient for the microscopist to have on hand for general photomicrographic and microscopic work.

Exp. 2. Determining Magnification. Project the image of a stage micrometer onto the ground glass of the camera. If the camera is of the closed type, without a focusing screen, remove the camera and in its place fix a piece of ground glass the same distance from the eyepiece that the film, or plate holder, of the camera was originally. With a millimeter scale, measure the space occupied by several of the stage micrometer intervals as projected onto the ground glass. Apply equation 1, Sec. 6, and solve for magnification.

Exp. 3. Constructing a Magnification Table. Repeat experiment 2, using various objectives. If the camera has a bellows draw, several convenient magnifications can be obtained with each combination of ocular and objective. Make a chart similar to the one in Table VII, and fill in the spaces with the magnification values obtained. Make the table large enough to include all the objectives with which you are likely to work. The column denoting the maximum diameter of sharp image formation can be referred to the diagonal dimension of plate sizes in Table VIII. Give the camera extension and image circle in millimeters. The diameter of the image circle is to be measured over only the portion which is critically sharp. The tube length may be noted as required in the column listing magnification. Use camera extensions which you find best for your special conditions.

Exp. 4. Demonstrating the Difference between Virtual and Real Images, and the Effect of Projection Oculars. To avoid confusion regarding real and virtual images, it is convenient to remember that real images exist either in space or on some plane reflecting surface. Virtual images, on the other hand,

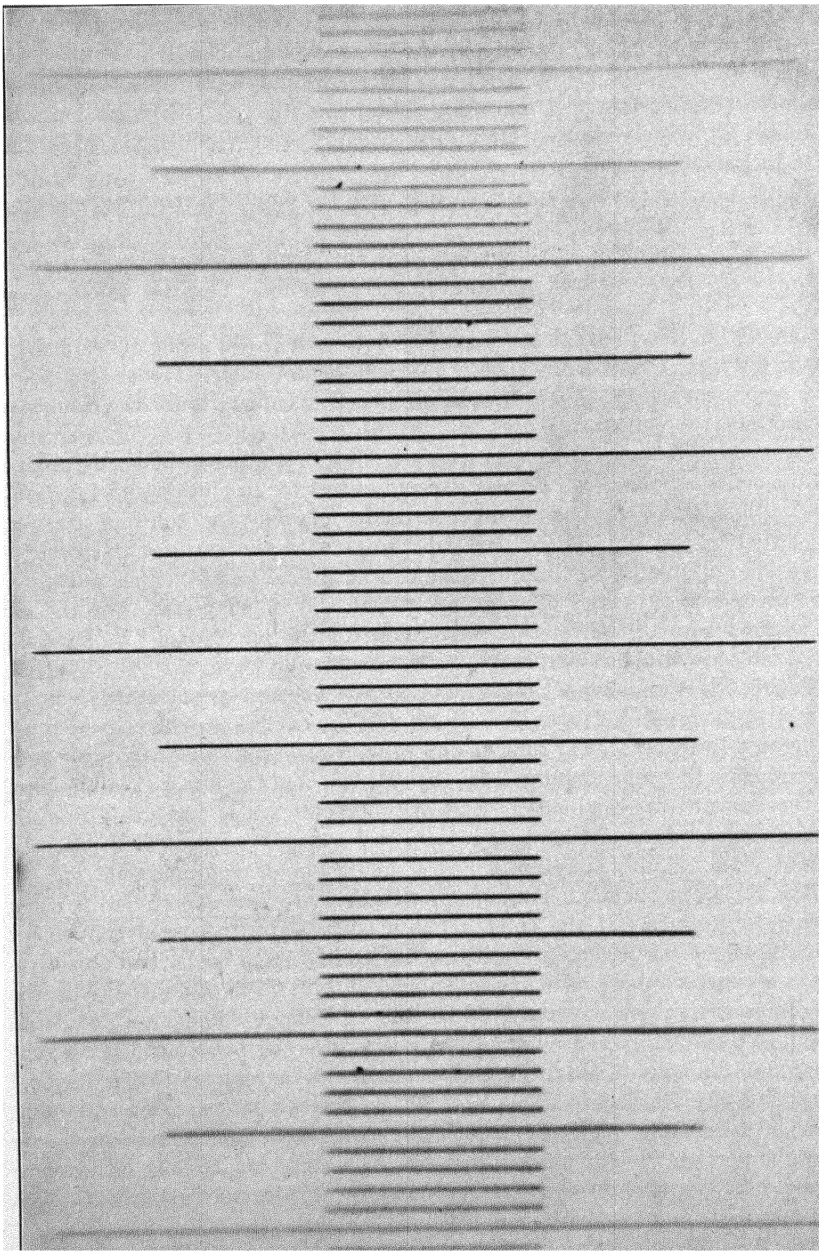


FIG. 34. Stage micrometer ×250. Showing the appearance of over-exposure in the out-of-focus zones.

Table VII

Magnification Table for Photomicrographic Work

To be used in conjunction with Table VIII

Ocular	16-mm apo. obj. — Tube length 170 mm N.A. = 0.3 1600 × N.A. = 480*			8-mm apo. obj.— Tube length 160 mm N.A. = 0.65 1600 × N.A. = 1040			4-mm apo. obj.— Tube length 160 mm N.A. = 0.95 1600 × N.A. = 1520		
	Camera Extension mm	Diam. Image Circle mm	Magnification Measured	Camera Extension mm	Diam. Image Circle mm	Magnification Measured	Camera Extension mm	Diam. Image Circle mm	Magnification Measured
5× Periplan	250 *610*	108 *172*	55 *130*	250 *610*	58 *142*	120 *270*	250 *610*	106 *130*	215 *520*
10× Periplan	250 *610*	108 *172*	108 *270*	250 *610*	85 *153*	220 *525*	250 *610*	140 *210*	420 *1020*
15× Periplan	250 *610*	67 *147*	180 *425*	250 *610*	70 *147*	360 *850*	250 *610*	120 *195*	680 *1650*
15× Comp. Zeiss	250 *610*	67 *156*	175 *415*	250 *610*	70 *165*	350 *830*	250 *610*	117 *300*	660 *1600*
20× Periplan	250 *610*	97 *160*	240 *580*	250 *610*	75 *200*	500 *1150*	250 *610*	114 *280*	950 *2025*
No. I Homal	250 *610*	143 *270*	170 *370*	250 *610*	142 *265*	330 *730*			
No. II Homal	250 *610*	104 *200*	70 *130*	250 *610*	104 *200*	140 *260*			
No. III Homal							250 *610*	124 *260*	630 *1420*

* This figure denotes the highest magnification to be expected from an objective and at the same time retain sharp image formation. The figure is slightly high for low power-objectives. See Sec. 79.

Table VIII

Diagonals of Various Plates

Plate Size inches	Diagonal millimeters	Plate Size centimeters	Diagonal millimeters
3¼ × 4¼	136	6.5 × 9	112
4 × 5	163	9 × 12	150
5 × 7	219	10 × 15	181
6½ × 8½	272	13 × 18	223
8 × 10	326	18 × 24	300

have no real existence and can appear only when the eye is in the correct position to gather the image-forming rays (see Sec. 4). Aerial images are special examples of real images. They can sometimes be seen in space against a light background. The primary image formed by the objective is an aerial image; it is located slightly above the lower focal plane of the ocular.

Remove the ocular from the microscope, and project the image of the numbers or letters of a stage micrometer onto the ground glass. The image will be small. It will be a real image, but it will be inverted and reversed. Insert the ocular, refocus, and examine the virtual image. Notice that it will not be inverted or reversed with respect to the primary image. It will be erect, but it will be inverted and reversed with respect to the object.

With the same ocular, project the image of the test slide onto the ground glass at a distance of about 250 mm. The real image formed there will be inverted and reversed with respect to the primary image, but it will be an erect image of the object. It will also be inverted with respect to the virtual image. All motion will be seen to take place in its true direction, whereas in the virtual image motion will be reversed. This is true of translatory motion. Rotary motion will appear the same in both images. If a microscope with a revolving stage is at hand, test the direction of rotary motion in the two images with respect to its true direction. It is assumed in this experiment that the eye is on the side of the ground glass that is away from the microscope.

If an amplifying ocular is available, show that the real image formed by it on the ground glass is inverted with respect to the image formed by an ordinary ocular and that its directions are the same as in the virtual image. Notice that a print made from a negative taken with either a projection ocular or an ocular for visual use will appear erect and unreversed.

Exp. 5. The Size of the Field on the Ground Glass. Try various objectives, and by projection onto the ground glass decide what factors control the size of the image field. The same bellows extension may be used throughout this experiment.

Exp. 6. Curvature of the Image Field. Using a test slide of some finely powdered material, such as slide 1 or 3, project an image of the preparation onto the ground glass. Notice that a portion in the center of the field is much sharper and has a much clearer image than the surrounding zones. If the

outer zones appear in the picture, they will be indistinct; if they are much out of focus when included in a photomicrograph they will give the appearance of a longer exposure on these parts. (See Fig. 34.)

A normal negative should have correct density in the center of the field, and where the focus gradually loses sharpness, owing to field curvature, the density of the images on the negative will increase. At the edge of the light circle the density will then fall off sharply.

Reducing the iris diaphragm of the condenser will slightly increase the depth of focus and will give the effect of reducing curvature of the field, thus tending to make the field more nearly flat.

Pick out good combinations of oculars and objectives that will give the best and most desirable fields with the least curvature. If possible, vary the bellows draw and make notes of the most desirable combinations. If projection oculars, or the new series of flat field objectives by Zeiss, are used, good coverage can be expected nearly to the edge of the diaphragm circle. (Refer to experiment 3.)

Exp. 7. Effects of N.A. on Brilliancy. Compare the brightness of the field when using different objectives of high and low aperture. Use a finely ground material, such as 600 Carborundum (slide 1), as a test specimen and an 8× or 10× eyepiece. Note that the illumination changes somewhat as the objectives are changed. Do not use diatomaceous earth or other glary substance in this experiment.

If the experiment is to be performed correctly, the iris of the diaphragm of the condenser must be opened to correspond with the aperture of the objective which is being used. This opening can be determined by looking down the drawtube after removing the ocular. It will probably be true that under these conditions glare will be troublesome. Do not expect good images, but simply compare the brilliancy of the different fields as the objectives are changed. This experiment shows the light-gathering powers of high objectives. It will be seen that considerable magnification can be obtained without great loss of light. This, of course, is due to the greater N.A. of the higher objectives. (See Table V, Sec. 10.)

Exp. 8. Loss of Field Depth with High N.A. The test slide used in the last experiment will suffice for this test. Estimate or measure the diameter of one of the particles, say 40 to 60 μ in diameter. Of the two horizontal diameters, adopt the smaller as the depth of the particle. If the particles are fairly uniform in size and shape, there is probably no great error in this assumption. It is desirable to select a particle with at least one sloping edge. With the 16-mm objective, notice how much of the particle can be focused sharply at one time. Turn in an objective of higher aperture, say the 8-mm, and again notice the depth of the particle that can be critically focused at one setting. Repeat, using several objectives, each of higher N.A. than the preceding one.

This experiment conclusively shows the advantage of long bellows draw in obtaining magnification, keeping the greatest amount of the specimen simultaneously in focus. However, a proper balance must be maintained between

the desired detail, resolution, and field depth. There is a limit to the magnification obtainable by long bellows extension, and no attempt should be made to exceed it. More will be said about this limit in a later chapter. The ideal photomicrograph will show everything in the field sharply defined, and the resolution will be as high as the objective is capable of giving.

As the iris diaphragm of the condenser is closed, notice that the depth of field is somewhat increased. This is perfectly good practice provided that no essential detail is lost and that it is not carried to the point of image deterioration.

Horizontal sections through the specimen, which are in sharp focus, are sometimes called "optical sections." Notice that when the highest-power objectives are used an optical section may be considerably less than 1 μ in thickness. The thickness of an optical section should be thought of as equal to the field depth of the objective. On very fine material do not confuse larger particles that are simply out of focus with smaller particles that are below the power of the lens to resolve, and consequently appear hazy and indistinct, irrespective of focus.

Exp. 9. Illustrating the Proper Setting of the Lamp Diaphragm. Focus the microscope on test slide 1. Use a low-power objective. Raise or lower the condenser until the image of the edge of the lamp iris diaphragm can be seen with maximum sharpness. Adjust the lamp diaphragm until it coincides with the field of view. This is the proper setting for this diaphragm. Notice that as the lamp is moved nearer to or further away from the microscope the diaphragm must be reset. Also, if the ocular, objective, or condensers are changed, the opening of the diaphragm should be made to correspond with the altered field of view.

Exp. 10. Illustrating the Proper Setting of the Condenser Diaphragm. Focus the microscope on a test slide, and focus the condenser on the lamp diaphragm. Open or close the diaphragm of the condenser until the test specimen appears without noticeable glare. A preparation of starch is good for this test (test slide 6). Remove the ocular, and inspect the rear focal plane of the objective. Notice the opening of the condenser iris. The condenser circle may show a two-thirds or a four-fifths cone. Under the best of conditions, and with the best optics, it is possible to attain a nine-tenths cone of light. The nine-tenths cone should be attained whenever possible. Section 102 on glare will describe methods of control to permit the greatest possible opening of the condenser diaphragm to be achieved.

Exp. 11. The Effects of Field and Aperture Diaphragms. With the microscope focused on a rather glary test slide, such as No. 5 or No. 6, remove the ocular and inspect the rear focal plane of the objective. Open and close the condenser diaphragm (the condenser should be focused, see Sec. 83), which will be plainly visible because it is acting as a field diaphragm. Open and close the lamp diaphragm, which in this position is acting as an aperture diaphragm. Note that it will control the intensity of the illumination and that the condenser diaphragm will control the size of the field. The effects

will be just the opposite of those produced by these diaphragms when the field of view is being examined through the ocular.

Exp. 12. Resolution of Objectives. Use test slide 2 of rouge and a high-power objective. Focus carefully on a fairly large particle of rouge, and adjust the diaphragm of the microscope for best visual effect. The image of the particle should appear sharp and clear with most objectives. Bring another particle of smaller size into the center of the field, and study the image for the same sharpness of outline as was obtained for the large particle. Repeat, using a yet smaller particle, until one is found that does not give quite as good an image as the previous one. This will indicate the threshold of resolution for the optics employed.

After finding a particle that cannot be quite resolved, insert a higher ocular. Notice that the primary image of the particle can be enlarged, but it cannot be made sharper. In fact, the particle will be less clear as the magnification is increased.

Insert filters of various colors, and notice the effect. The blue filter will probably give the best images. If the particle that was just beyond the power of the lens to resolve is not extremely small, a strong blue filter may possibly give a good image of it.

Another interesting experiment in resolution is to examine slide 3 with a 16-mm apochromatic objective (the experiment is more striking with an apochromat). Select a few very small particles for observation, and bring them to the center of the field. Open and close the condenser diaphragm, noting that, as it is closed beyond a certain point, the sharp outlines of the small particles become fuzzy and resolution is lost. As a rule, the keen definition of the particles will be lost before diffraction rings appear.

QUESTIONS

1. Name the important parts of the microscope, and in a few words describe the function of each part.

2. What is the generally accepted unit for linear measurement of microscopic particles?

3. What is the approximate equivalent in the British system of 1 μ? Express as a decimal and as a fraction of an inch.

4. A photomicrograph is taken of ground mica. Magnification is 500×. A certain particle shown in the print measures 3 mm in diameter. What is its true diameter?

5. An enlargement of 2.5× is made from the above negative. A certain particle in the enlargement measures 7.5 mm. What is its true size?

6. A pollen grain measures 25 μ in diameter. What magnification is necessary to obtain an image of it measuring 10 mm in diameter?

7. If the maximum bellows draw of a certain camera is 20 inches, what objective and ocular would you use to obtain the 10-mm image cited in question 6?

8. With an objective giving a magnification of 10×, and with an 8× ocular, what would be the distance between two adjacent lines of a stage micrometer when projected a distance of 500 mm? It is assumed that the stage micrometer is a millimeter scale divided into 100 equal parts, each interval being 10 μ.

9. Describe in detail how to measure the exact magnification of your photomicrographs.

10. In Fig. 17 what is the magnification of the photomicrograph of scale A? The micrometer scale is similar to the one used in Fig. 16.

11. Describe the main difference between the image projected by the objective and the image formed by the ocular and the eye.

12. Where is the object field located? What controls its size?

13. Where is the field of view located? What controls its size?

14. The angular aperture of an 8-mm objective is 60°. Draw this angle, and show its relation to the objective and a small object in the center of the field.

15. How does resolution vary with N.A.?

16. How does light intensity in the field of view vary with the N.A.?

17. Write the numerical equation expressing N.A., and explain what it means.

18. Draw a diagram and compute the N.A. of an objective whose angular aperture is 60° and whose surrounding medium is air.

19. Using a diagram as in question 18, what would the N.A. of an objective be, if the angular aperture is 85° and the surrounding medium is cedar oil with a refractive index of 1.51?

20. If the N.A. of a condenser immersed in cedar oil ($n = 1.51$) is set at 1.0 by operating the iris diaphragm of the substage, and if it is used with an objective whose N.A. is 1.30, what will be the working aperture of the entire system? The object is assumed to be mounted in balsam, and the objective is immersed.

21. What is the function of a field diaphragm?

22. At what position in an optical system would you expect to find a field diaphragm?

23. Mention two field diaphragms in the microscope and illuminating system.

24. What is the main difference between a field diaphragm and an aperture diaphagm?

25. Mention two aperture diaphragms in the microscope.

26. What optical effects may be controlled by the diaphragm of the condenser?

27. For what specific purpose should the condenser diaphragm not be used?

28. What is the formula expressing resolution?

29. What is the important effect of wavelength of light on resolution?

30. Resolution is generally spoken of in what terms?

31. If the light you are using has a dominant wavelength of 578 $m\mu$ and the objective has a N.A. of 0.65, what is the maximum resolution you may expect?

32. What is the limit of resolution, using blue light and an objective of 0.70 N.A.? State in lines per inch and the distance apart that two objects must be for such resolution.

33. What type of objective gives the highest resolution? Why?

34. Can resolution be figured by counting the lines per inch ruled on a glass slide?

35. Why do small particles sometimes seem sharper in blue light than in red light?

36. Are particles that are blurred in a photomicrograph necessarily out of focus?

37. Which objective will give the greater resolution, a 3-mm apochromat N.A. 1.32 or a 1.5-mm apochromat N.A. 1.32?

38. Where is the exit pupil of the microscope located?

39. When buying oculars would you pay attention to the height of the exit pupil? If so, why?

CHAPTER II

LAMP HOUSES, LAMPS, METHODS OF LIGHTING, AND PHOTOMETRIC UNITS

The illumination of the microscope which was accomplished formerly by means of natural daylight (sunlight, light from the sky or clouds), oil lamps, gas mantles, or spirit vapor lamps is now almost exclusively accomplished by electrically operated lamps. It is fortunate, indeed, that the continuous development of electric lamps has kept pace with the advances made in optics and with the mechanical evolution of the microscope, for to attempt to use a modern microscope without a suitable lamp is a good deal like trying to run a gasoline engine without gasoline. The light source must be powerful, for it is subject to a great degree of modification by means of optical light filters. It should be fairly inexpensive and should be easily controlled. Owing to the foresightedness of various companies, there are now on the market lamps which are nearly universal in character as applied to the microscope; that is, one lamp may be used for many methods of illumination. For difficult work there are numerous highly specialized lamps that will answer all the exacting demands of up-to-date microscopy. For purposes of discussion, electric lamps are conveniently divided into four classes: tungsten filament, gaseous discharge tubes, tungsten arc, and carbon arc.

The fundamental principle of any lighting system involves, of course, the lighting of the specimen with sufficient evenness and with light of the appropriate quality and brightness to ensure negatives of proper contrast, brilliance, and uniformity. The exposure must not be unduly prolonged, and the density of the negative must be correct. The spectral distribution of radiant energy should conform to the demands of the work, and the whole illuminating system, from the microscope condenser to the lamp, must be sufficiently flexible to provide all the adjustments required for modern technique in illumination. These primary requirements are partly provided for by a well-designed lamp house.

LAMP HOUSES AND ACCESSORIES

Sec. 15. The Lamp House. The physical arrangement of the illuminating apparatus should be able to fulfill the following mechanical and optical requirements:

1. A light source sufficiently intense, and large enough to illuminate strongly the front lens of the condenser.

2. A means of controlling the size of the light source, such as a field diaphragm near the source. This is necessary to reduce glare. The adjustment is usually made by a diaphragm on the lamp.

3. An arrangement for holding neutral or colored optical filters, which are used to modify the light as desired. This should usually be placed midway between the lamp and the microscope.

4. The existence of a perfectly evenly lit field at the rear focal plane of the objective.

Figure 35 illustrates an arrangement for illuminating the microscope. It will be seen that the lamp house is large enough to accommodate a 500-watt projection lamp, a tungsten arc, a close-coil tungsten filament, or a tungsten-ribbon filament lamp. With a change of sockets, the mercury vapor lamps or the General Electric photomicrographic lamp could be used. However, for the 6-volt lamps that are generally used with the method described in Sec. 18 as Method II, a rather short-focus light-collecting lens will be necessary, in which case two lamp houses may be indicated, since Method I, which also is referred to in Sec. 18, requires a long-focus light-collecting lens. The lamp house is fitted with a device for centering the filament of the lamp to a light-collecting lens. The focal length of this lens is about 4 inches; its diameter is 2.1 inches; and it can be moved along its principal axis in focusing the lamp. In front of the lens is an iris diaphragm with a maximum aperture to correspond to the free aperture of the lens. In front of the iris is a holder for two diffusing plates. Mounting the iris outside the diffusing plate holder would improve and safeguard the operation of the iris, since the heat would be less in such a position and smoother action of the iris would result. Furthermore, in such a position the iris would give a sharper image when focused by the condenser. Figure 35 shows the old Busch lamp house, now no longer on the market, the nearest approach to it being the large Spencer shown in Fig. 36.

The essential points in a good lamp house are:

1. Ventilation should be adequate, and the ventilating openings should be placed in such a way that no direct light strays from them.

2. The housing should be large enough to accommodate a 250- to 500-watt projection lamp or the General Electric photomicrographic lamp.

3. Adequate means should be provided for aligning the filament of the bulb on the axis of the light-collecting lens.

4. A light-collecting lens in a sliding sleeve should be provided to

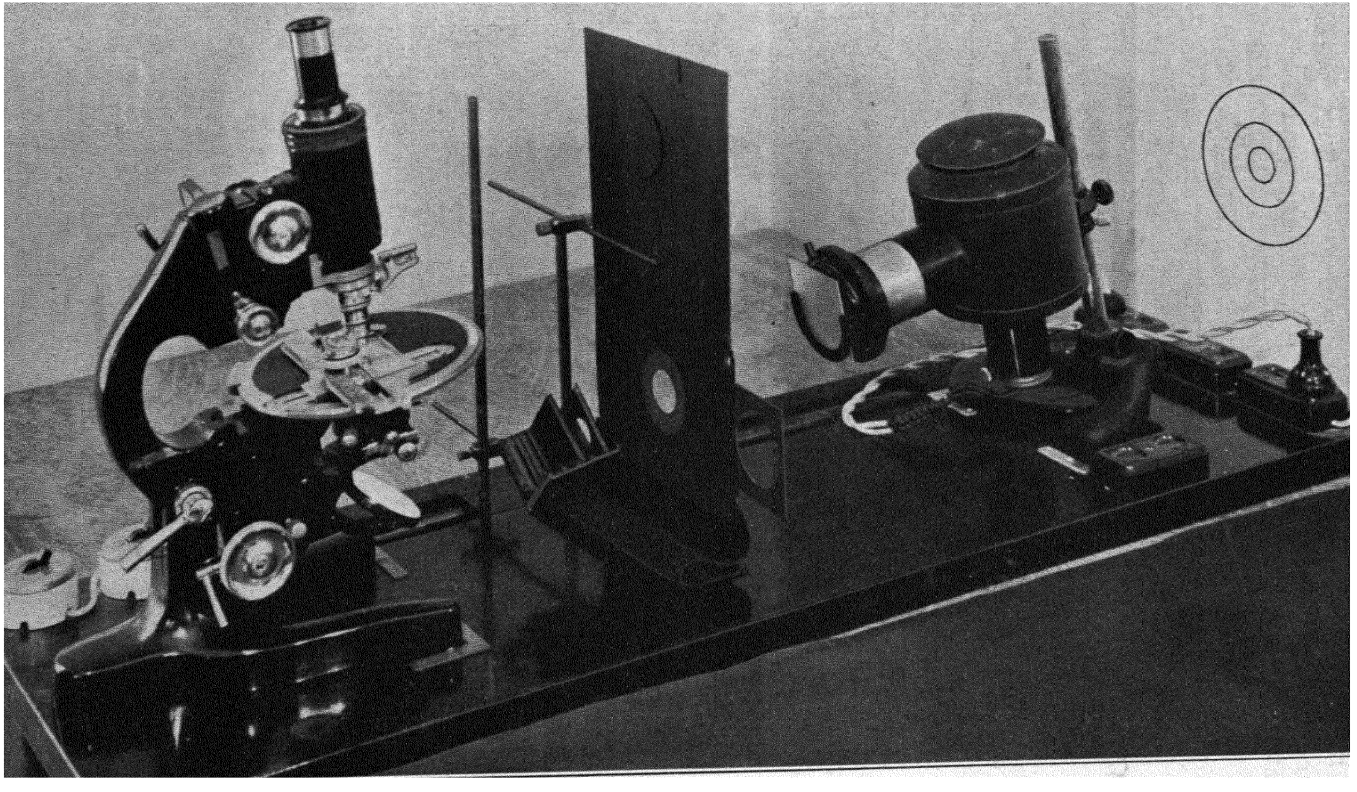


FIG. 35. Microscope aligning board with lamp, shield with adjustable aperture, filter holder, switches, microscope, and on the wall, a target for centering lamp filament.

focus the source of light on the microscope mirror. This can be done by rack and pinion or, more simply, by a sleeve through which the barrel containing the lens can be moved. The lens should have sufficient travel to obtain parallel rays and at the same time should be

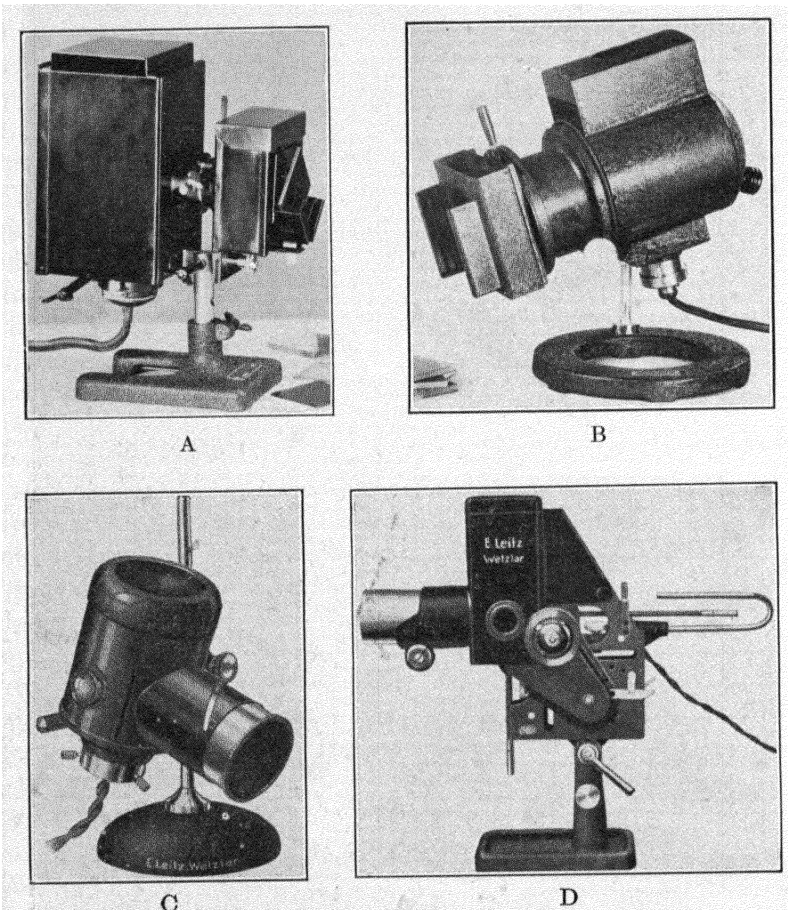


FIG. 36. Four popular types of lamp houses. *A*, Bausch and Lomb research lamp; *B*, Spencer lamp, *C*, Leitz lamp, filament source; *D*, Leitz lamp carbon arc.

capable of focusing the source within 8 or 10 inches from the lamp; this represents a motion of 4 inches. The free aperture of this lens should be at least 2 inches. A focal length of about 4 inches seems to answer all requirements for illumination by Method I.

5. A holder for two diffusing plates about 2 to 2½ inches square should be placed close to the lens.

6. The iris diaphragm should be placed in front of the diffusing plates.

7. The housing should be inclinable through an arc of at least 45° from the horizontal downward.

8. There should be a check of some sort, such as a detent, at the horizontal position. The motion of the housing as it is inclined should be smooth and the locking device positive.

9. It should be possible to raise or lower the lamp through a range of at least 18 inches.

10. The base of the lamp should be heavy, and it should not interfere with the housing even when in its lowest position.

11. If there is a reflector it should be removable.

12. It should be possible to have a well-corrected lens of short focal length (2 to 2½ inches) interchangeable with the regular 4-inch lens mentioned in requirement 4.

At the present time there is nothing on the market that fulfills all these requirements; however, Cargille[1] has a lamp house under construction which from all reports will be a great advance on all existing types.

Sec. 16. Filter Holders and Other Accessories. Figure 35 shows a filter holder placed in front of the microscope mirror. Filters should not be placed near the lamp, but, since microscopes are not provided with means for holding them, a filter holder is a necessity. No accessory of this sort is on the market, but it can be obtained on special order. The one shown in the figure is cast from aluminum; it carries five filters. The slots on the inner side are 6.2 mm wide — this is enough for average filter glasses. If a filter 8 mm thick is to be used, it can rest on the top of the slots, its thickness keeping it from falling.

The aligning board shown in Fig. 35 and in greater detail in Fig. 37, is fitted with angle irons to provide the lamp house and microscope with a means whereby they may always be replaced in the same relative position to each other. Along one side a groove holds the metal bases of the standards which carry the uprights for the filter holder and light shield. Figure 37 gives the dimensions necessary to make this aligning board. When the microscopical work warrants, the arrangement as described here will save much time and trouble. The light shield, shown in Fig. 35, is an important accessory, even for occasional work, as good observations cannot be made with light shining into the eyes. For the same reason, the microscope should be placed in a corner of the room and away from the windows. If daylight is desired for observation, it is much better to make it artificially,

[1] R. P. Cargille, 118 Liberty Street, New York City.

with appropriate filters, than to place the microscope facing a window. (See Sec. 101.)

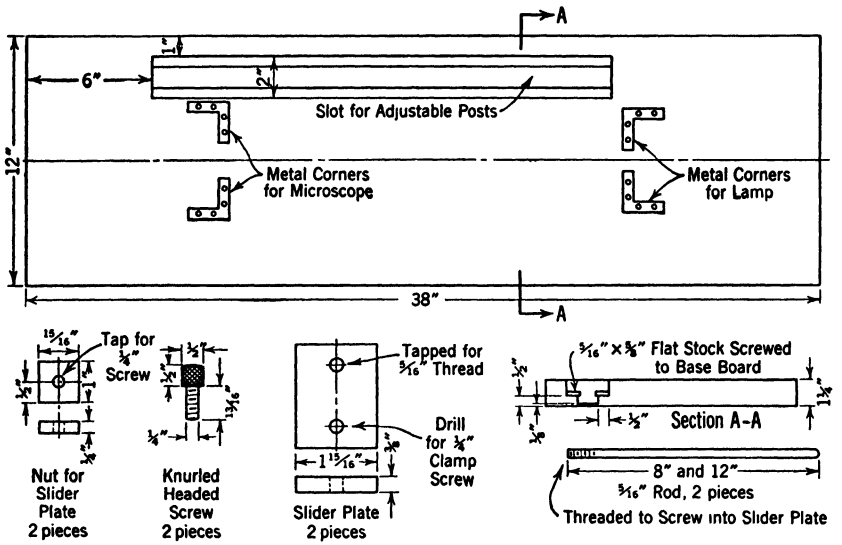


FIG. 37. The plan of the aligning board shown in Fig. 35. The wiring of the board is not shown as this would vary somewhat with individual requirements; the wires should be installed in grooves on the under side of the board and brought to conveniently placed switches. A convenient place for the switch is near the edge of the board, at the left-hand side of the microscope. The metal corners, or stops, for locating the microscope and lamp are the last pieces of hardware to add. The lamp house should be placed in position first, and the metal stops screwed to the board; the microscope can then be lined up with the lamp.

Sec. 17. The Preparation of Diffusing Plates. Small pieces of ground glass to be used as diffusing plates for the lamp, or for other purposes, are easily prepared. The glass is cut to the required size, generally about 2 to 2½ inches square. A small quantity of 600 Carborundum powder, or its equivalent, is mixed on a glass or metal plate with enough water to keep the mixture free flowing. The piece of glass selected to be given a matte surface is rubbed in the Carborundum and water. The position of the fingers should be changed from time to time to equalize the pressure over the plate, and the ground surface of the glass should be examined after it has been rubbed for about a minute. Grinding is a quick process if the glass is flat, but if it is slightly curved it will take longer to get a good surface and a little more pressure will have to be applied in certain spots as indicated by inspection. The term " diffusing plate " has been used in referring

to the ground-glass screen because it is descriptive of its function and is a direct reference to its effect on light.

It is well to keep grinding powder on hand at all times. It is easy to use, and ground-glass screens can be kept in excellent condition by rubbing them with it occasionally; in fact, it seems to improve the surface texture of the plate to clean it in this way whenever it gets dirty. An alternative method of cleaning is to use a good scouring powder such as Old Dutch Cleanser.

Sec. 18. Illumination Technique. *To Center the Lamp in the Lamp House.* In use, the filament of the lamp must be centered with respect to the light-collecting lens of the lamp house. One of the best ways to do this is to place a target of cardboard on the wall or on a stand with the bull's eye level with the center of the lamp lens when the lamp house is horizontal. The coil filament of the lamp, or the incandescent source, is focused on the target. The centering adjustment of the lamp house is moved until the lamp is in such a position that the image of all the filaments appears evenly placed around the bull's eye. The color fringes and any out-of-focus portions in different parts of the image should all be symmetrically placed with respect to the center of the target and to the image itself. When this has been done, a line from the center of the incandescent source to the center of the target should pass through the center of the light-collecting lens; in other words, the line should coincide with the principal axis of the lens. (See Fig. 38.) To test the alignment it is usually wise to partly close the field diaphragm. This increases the contrast of the filament image.

After the lamp has once been made ready for use by this centering process it should not need further attention in this respect during its lifetime. Sometimes, for the sake of economy, it is perfectly good practice to use an ordinary household lamp. This will be more difficult to center properly; but absolutely correct centering will not be so important, since such lamps are used for visual work rather than for photomicrography.

Illumination by Method I. Illumination by Method I involves the use of a diffusing plate at the lamp house which acts as a secondary light source. It should be located about 15 inches from the microscope mirror. The distance 15 inches is suggested because it appears to be the point at which the lessening of glare, due to the diffusing plate, becomes negligible for further increase of lamp distance. If, at this distance, the iris at the lamp has a free aperture of 2 inches, the field of view will be completely filled with light when a 16-mm objective is used with a 15X or higher ocular and a strong condenser.

After centering the lamp filament to the light-collecting lens, all that the operator has to do to put the lamp in operation is to train the image of the filament onto the center of the microscope mirror and then to drop the diffusing plate into the filter holder at the lamp house. The field diaphragm at the lamp house should then be closed to a small opening, and, by turning the mirror, the spot of light is

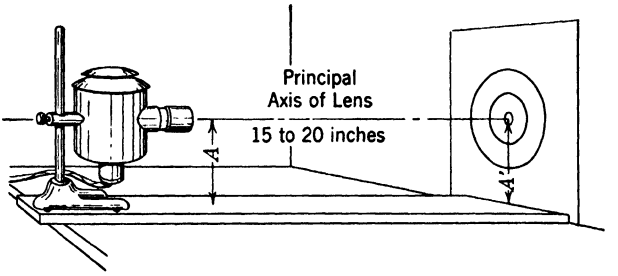


FIG. 38. Illustrating the use of the target for aligning the lamp to the light-collecting lens. Distance A equals A'. The exact distance from lamp to target is not important.

brought into the field of view (see Sec. 8). Before doing this, it is necessary to have the microscope focused on a test slide; the condenser must also be focused until the image of the spot of light is as sharp as possible. The field diaphragm at the lamp is then opened to correspond to the field of view. If the structure of the diffusing plate is visible in the field of view, the condenser should be raised slightly above good focus. Details of this procedure are given in Chapter IV, where the condenser adjustments are explained.

For photography, the focusing of the lamp filament should be carried out with considerable care; otherwise shadows may appear in the negative in spite of the fact that ground glass has been used. Figure 39 shows two photomicrographs A and B. The only difference between them is that the lamp filament was focused correctly for A but was thrown out of focus and slightly out of alignment for B. The misalignment is difficult to detect with poorly focused light. It is well worth while to make these adjustments correctly. If the microscope condenser is removed and a piece of ground glass is placed in the ring carrier, or diaphragm of the substage apparatus, and the microscope tube is removed or raised to its full extent, it should be possible to see the image of the lamp filament clearly and to focus it sharply after the diffusing plate has been removed from the lamp house. The image of the filament can now be well centered on the microscope mirror. A diagram of illumination by Method I is given in Fig. 40.

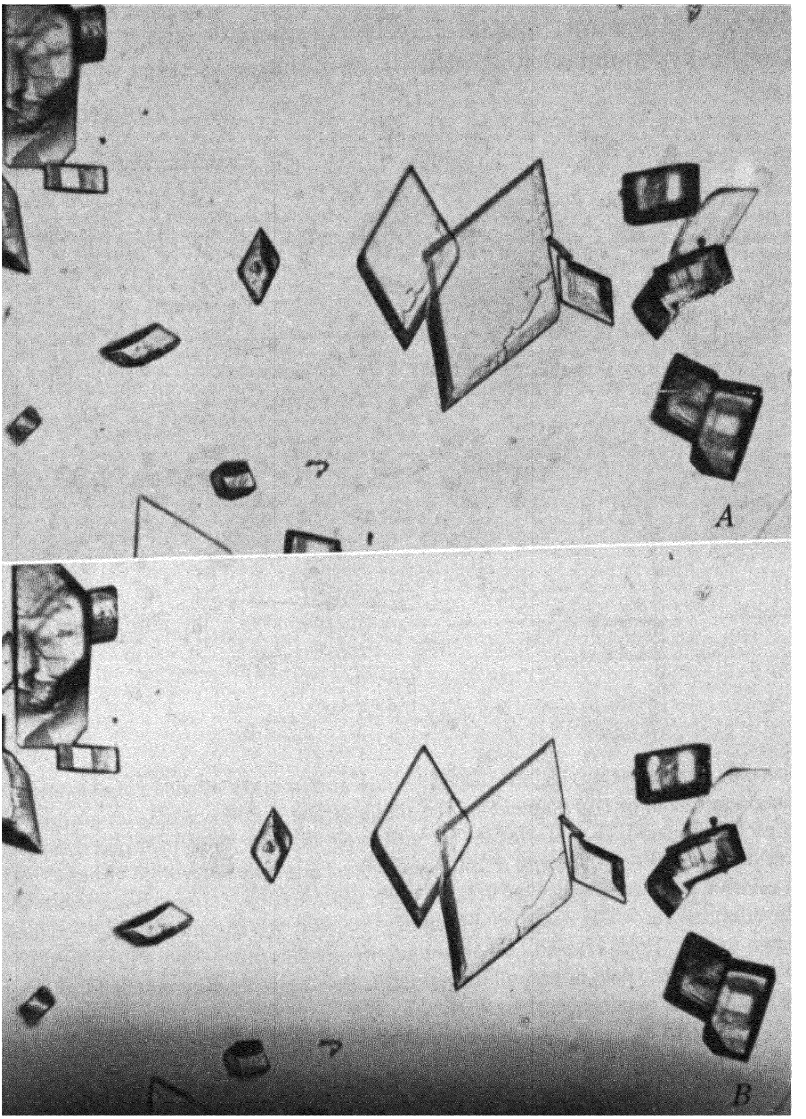


FIG. 39. The specimen is sulphapyridine ×200. At *A*, the lamp lens has been correctly centered and focused on the iris diaphragm of the condenser; the field is evenly lit. At *B* the lamp lens has been thrown out of focus and slightly off center; the diffused edge of the image of the lamp filament is seen at the bottom of the picture. Method of illumination II; ribbon filament lamp.

The projection type of lamp is best for use with illumination by Method I. Other types probably will be found less convenient and more expensive.

With certain subjects there may be considerable glare, particularly when the 4-mm objective is used. If visibility is affected by the glare,

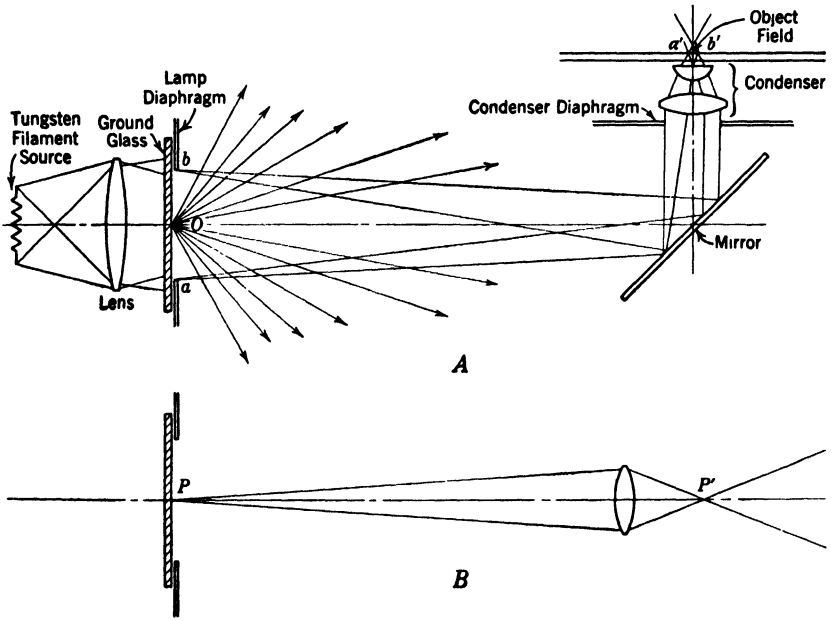


FIG. 40. Illumination by Method I. The preliminary arrangements, such as focusing the image of the filament in the plane of the condenser diaphragm, are the same as for Method II. However, the diffusing plate is then interposed and the surface of this plate at the lamp diaphragm acts as a secondary source of light. The plate gives a good homogeneous field of even illumination, its size being controlled by the diaphragm. The lamp diaphragm is focused by the microscope condenser as shown at *B*. Points *a* and *b* as object points are imaged in the object field at *a'* and *b'*. The object field is a small disc of light, its size depending on the focal length of the microscope condenser, the size of the lamp diaphragm, and the distance of the lamp. The great loss of light at the diffusing plate should be noted.

the field diaphragm can be closed further, thus reducing the size of the illuminated part of the field of view and at the same time reducing glare. This is discussed more fully in Sec. 102. Since the outer zones of the field of view are not often used for photography, loss of the outer area is not important.

The requirements for good illumination have now been fulfilled. (See Sec. 15.) The condenser lens on the microscope is filled with light; the diffusing plate assures this. The plate is acting as a second-

ary source similar to cloud light. The field diaphragm near the plate affords complete control of the size of the source. A place has been provided for the light filters midway between the microscope mirror and the lamp, the distance of about 7 inches from the mirror being sufficiently far from the lamp for the filters to remain cool. With all

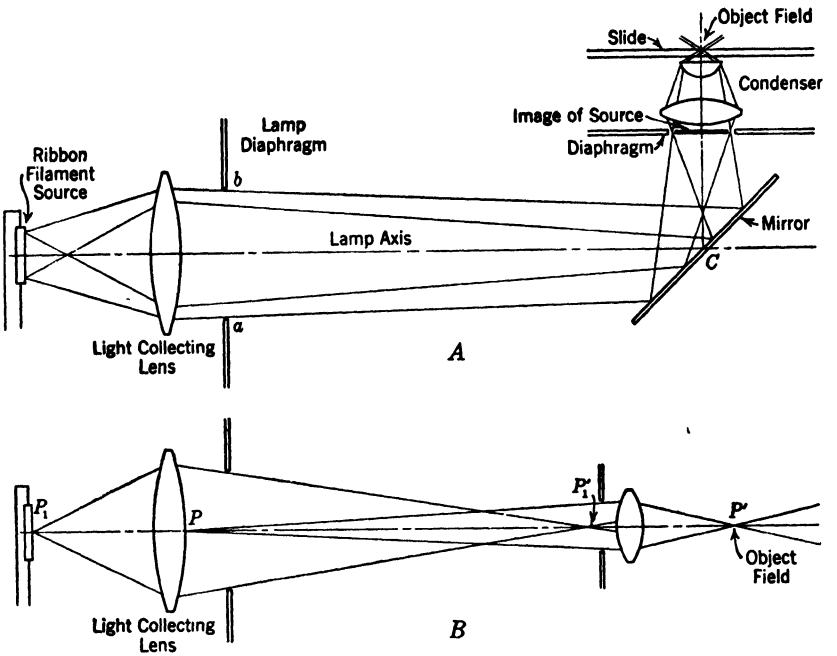


FIG. 41. Illumination by Method II. Compare with Fig. 40, Method I. The surface of the light-collecting lens becomes the secondary source, or for practical purposes the plane at the lamp diaphragm. The loss of light is small compared with that by Method I. *B* shows the conjugate foci of the microscope condenser lens, points *P* and *P'*. Compare Fig. 40 *B*.

these adjustments correctly made, the last requirement, namely, filling the entire aperture of the objective with light of even intensity, will be fulfilled. This condition of even lighting can be seen by inspection through the microscope tube when the ocular is removed.

Illumination by Method II. Figure 41 shows the trace of the light rays in the method of illumination advocated by August Köhler[2] and described herein as Method II.

To understand the Köhler method, or Method II, it is a help to

[2] August Köhler. "Ein neues Beleuchtungsverfahren für mikrophotographische Zwecke," *Zeit. wiss. Mikroskop.*, 443–440, 1893.

remember that if the eye is placed at the focal plane of a lens when it is focused on a light source, no matter how small the source may be, the whole lens will appear to be evenly illuminated. This condition would be well fulfilled if the eye were placed at the object field (at point P', Fig. 41*B*) and the microscope condenser focused on the light-collecting lens at the lamp. This lens has previously focused the source in the lower focal plane of the condenser of the microscope. Thus the light-collecting lens would appear completely filled with light for an observer at P_1' as well as for an observer at P', which is the object field.

For practical application, the procedure is to focus the image of the source in the plane of the iris diaphragm of the microscope condenser, as in Method I. The microscope condenser should then be adjusted to focus the image of the lamp diaphragm in the plane of the object field. The back lens of the objective will be filled with light of even intensity, and an image of the lamp diaphragm can be formed in the field of view whenever this diaphragm is sufficiently closed. Thus it is seen that Method II is identical with Method I except that with Method I a diffusing plate at the lamp acts as a secondary source and in Method II the source is the surface of the lamp lens. For Method II the image of the lamp filament must be made large enough to fill the microscope condenser with a solid field of light. This is essential.

The lamps best suited for illumination by Method II are, in the order of convenience, the tungsten-ribbon filament, the 6-volt single-coil filament, the Pointolite or Punktiform lamp, the German multifilament lamp, the H series of mercury-vapor discharge tubes, the photomicrographic lamp of the General Electric Company; the biplane projection lamp, and the carbon arc lamp.

If Method II is attempted with a light-collecting lens of long focal length, say 4 inches, at a distance of 15 inches from the microscope, or if it is tried with the 250-watt monoplane-filament projection bulb, the front lens of the microscope condenser will not be evenly filled with light, as can easily be seen by holding a card in front of it. Anything located at the front focal plane of the microscope condenser will be imaged in the rear focal plane of the objective. Consequently, if the image of the light source is not large enough to fill the front lens of the condenser completely with homogeneous light, the fact will be shown at the rear focal plane of the objective. Under these conditions the appearance of the black lens of the objective is somewhat as shown in Fig. 42. Only a portion of the objective will be in use, and, as Beck[3] has shown, such a condition tends to produce spurious images.

[3] C. Beck, *The Microscope*, first edition, part II, 1924, London.

An analogous condition would prevail if a small diaphragm with portions cut out to form a silhouette of the lamp filament were dropped onto the rear lens of the objective. To get an image of the filament of a size sufficient to fill the front lens of the condenser with light, a short-focus light-collecting lens must be used. A 2-inch lens at a distance of 14 to 16 inches from the microscope answers very well with

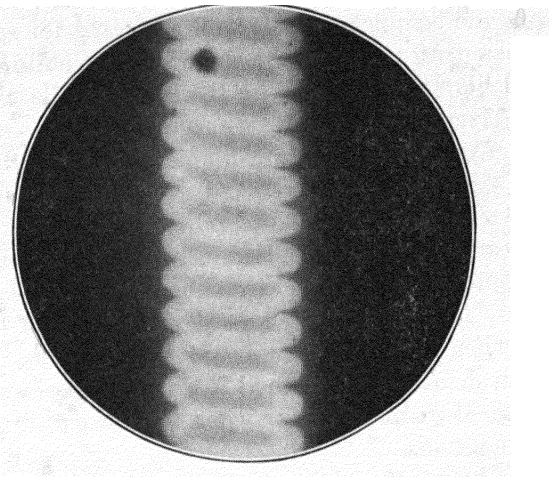


FIG. 42. This photograph of the back focal plane of the objective indicates why the image of the source must fill the aperture of the condenser. The black spot in the filament is an air bubble in the condenser lens. An imperfection of this type, in a condenser, will not impair its usefulness if it is otherwise well corrected. Whatever is imaged at the first focal plane of the condenser will appear at the second focal plane of the objective and also at the exit pupil of the microscope (*see* Glossary).

many lamps, but with the ribbon-filament lamp it will be necessary to increase the distance considerably.

Supposing the diameter of the source to be 4 mm, to fill the front lens of a condenser 32 mm in diameter with light the diameter of the image must be 8 times larger than the source. That is, if the distance from the source to the light-collecting lens is ⅛ the distance from the lens to the microscope condenser, then an enlargement of the image to 8 diameters will have taken place. In the case of the 2-inch light-collecting lens, if the distance of the lens from the lamp filament is 2¼ inches, the distance of the image will be 18 inches, which will be the distance of the lamp from the microscope. Thus the lamp distance, in Method II, is dependent on the size of the incandescent portion of the lamp and on the focal length of the lamp lens.

Since a short-focus lens covers a smaller field than a longer-focus lens, with low objectives (16-mm) it will be necessary to use a long-

focus microscope condenser or to remove the back lens of the regular condenser; otherwise it will be impossible to fill the field of view with light.

Both Bausch and Lomb and Spencer make a lamp house that is well fitted to handle the tungsten-ribbon filament lamp. The light-collecting lens in the Bausch and Lomb lamp house is a compound unit consisting of two lens elements, one of which, at least, has an aspherical surface; thus the complete unit is well corrected for spherical aberration, and a beautiful image of the source can be obtained. If desired, the close-coil filament lamp or the H3 lamp can be used in the same lamp house. The H4 necessitates a special socket.

The possibilities of the biplane filament lamp, with Method II of illumination, should not be overlooked. In the list of lamps just given it was rated last because it is supposed to be used with a forced draft. However, it can be operated successfully in the ordinary lamp house if it is not kept lit too long at any one time. It has a large surface and is the only lamp that can be used with a 4-inch lens and render good service when illumination is by Method II.

Illumination should be by Method II when it is necessary to eliminate the last vestige of visible glare or when fine structure is being studied at high magnification.

Illumination by Method III. A modification of Method II is easily made by using a source of light from a projection lamp. The illumination by Method III is similar in many respects to that obtained by Method II. With a diffusing plate in front of the source, the lamp lens focuses the surface of the diffusing plate in the first focal plane of the condenser. In its best form, this method includes a small diaphragm over the diffusing plate, so made that its image just fills the front lens of the condenser with light. Any increase in the size of the diaphragm will not give additional effective light. This method of lighting may shorten the life of the lamp somewhat by restricting the circulation of air in the lamp house. However, the brightness of the object field is increased by about 200 per cent; it is an excellent method of illumination.

Parallel-Ray Illumination. Illumination with parallel rays of light is shown in Fig. 43. The light-collecting lens at the lamp is focused for infinity, after having first been carefully focused on the microscope mirror. Following this routine will maintain centration of the illuminating system if care is taken not to move the lamp house when the lens is pushed back to infinite focus. The light-collecting lens is then in such a position that the filament of the lamp is at the principal focus of the lens. This position can be found by focusing the

lamp on a wall 20 feet or more distant, thus indicating approximately the proper position of the lens for infinite focus. On some lamps a mark can be made on the sliding sleeve that holds the lens so that the setting can be duplicated at any future time. The microscope

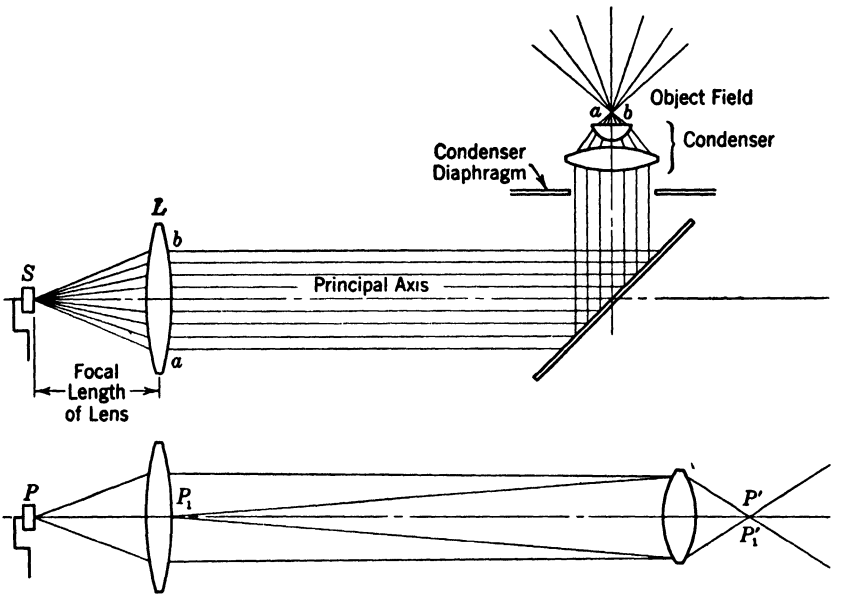


FIG. 43. Illumination by parallel rays. It should be noticed in the lower diagram that P' and P_1', images of the source and the lamp lens, fall so close together that, unless the source image is large enough to give a satisfactory field, poor lighting (uneven lighting) may result as shown in Fig. 42.

condenser is then focused on the field diaphragm, as in Methods I, II, and III. The system is at its best with the lamps advised for Method II or in conjunction with Method III when a very small diaphragm is placed over the diffusing plate.

According to some authorities, all microscope condensers are designed to give their best images with parallel light rays. At the present time this may or may not be so. At any rate, if better results can be obtained with parallel light rays the improvement does not seem to be commensurate with the advantages gained by the use of Methods I, II, or III. Examination and measurement of the light transmitted by Method II compared with the effect of parallel lighting shows a falling off of intensity of 17 to 20 per cent when the rays are parallel. This measurement was made at the exit pupil of the microscope. When light rays are rendered parallel they are said to be collimated. The

term is often used in reference to lens systems which function in this way. A microscope lamp is sometimes said to have a collimating lens; it has, of course, if the source is at the principal focus of the lens. It should be noticed that when the light is collimated P' and P_1', Fig. 43, fall so close together, if the lamp distance is large, that the object field may be poorly lit because of the image of the filament lying in or near that field.

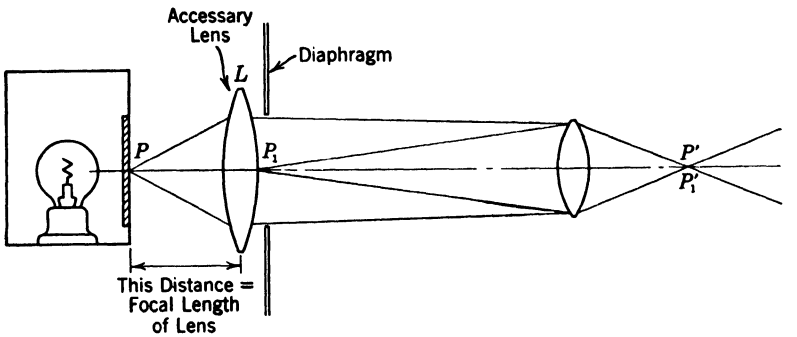


FIG. 44. Illumination by parallel rays from a small substage lamp house used in front of the microscope. This includes the use of an accessory lens L and special diaphragm. The diaphragms may be cut from black paper or cardboard. A 3- to 4-inch lens will answer all requirements.

Other Methods of Illumination. Substage lamps and fixed means of illumination, in which the system is built into the microscope, have their limitations and disadvantages. Substage systems not only set up considerable heat under the microscope condenser but also cause glare. They make the use of standard 2-inch glass optical filters difficult or impossible, and it is generally left to the microscope condenser diaphragm to control the size of the source — an operation for which this diaphragm is not at all suited.

As shown in Fig. 44, the small substage lamp can be used to better advantage if placed at some distance in front of the microscope rather than under it. Since the illumination on the ground-glass surface of these lamps is low, it may not be feasible to place it 14 inches from the microscope, but if it is placed in some intermediate position and used with a bull's eye at its focal length from the ground glass, considerable light will be gathered which would otherwise be lost and the field of view will be evenly lit. This method of using a small two dollar lamp has much to commend it, and, although the light from it is not strong enough for much photomicrographic work, it serves for visual examination, and it may often be

found convenient as an extra lamp when the larger one is set up for use with the camera. It is strongly recommended that diaphragms be placed in front of the diffusing plate of the lamp and behind the bull's eye lens for in this way good illumination for the microscope can be secured at very little expense. The diaphragms can be cut from heavy black paper or cardboard. This method of illumination is essentially the same as Method III when the field diaphragm is focused in the first focal plane of the condenser.

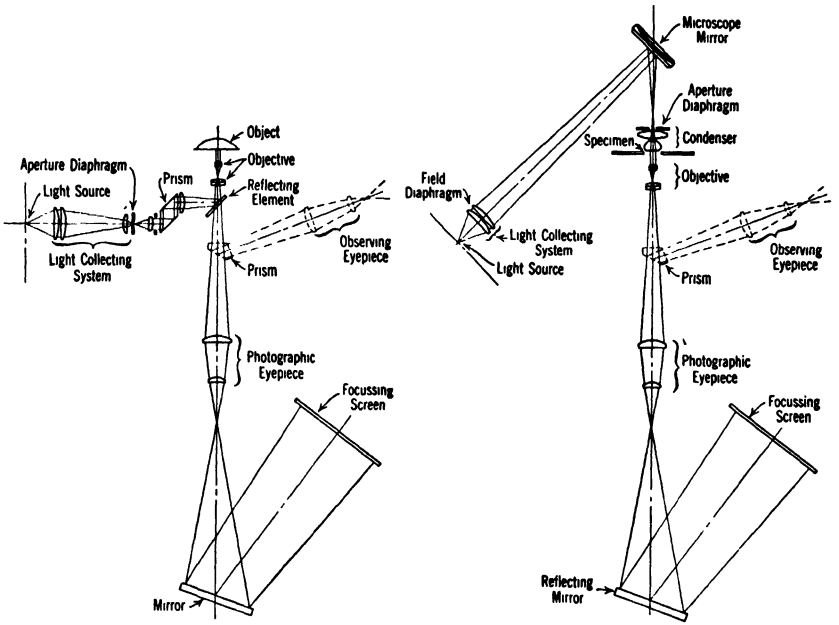


FIG. 45. The trace of rays of light through a universal microscope-camera apparatus as made by Reichert.

Systems with " built-in " illumination are excellent for routine work. They are optically correct and answer all the requirements for good illumination as specified in Sec. 15; but they lack flexibility and the complete control over illumination that is provided by simpler and less costly apparatus. The panceratic system of Zeiss and the universal microscope-camera systems of Reichert and Leitz are examples of built-in illumination. Since all adjustments in these systems are built into the apparatus their use is best explained by booklets of instruction from the manufacturers. Fundamentally, their principle of operation is in agreement with that of the illumination outlined for Method I or Method II. Figure 45 shows the trace of light rays

through such a universal microscope-camera system, as manufactured by Reichert.

Sec. 19. Critical Illumination. Critical illumination is often spoken of as though it were inherent in some particular form of illumination and were the supreme attainment in microscopical illumination in general. For a definition of this loosely applied term, it is necessary to go back to the days when kerosene lamps were used to illuminate the microscope. The image of the flame, either broadside, or edgewise, for high powers, was projected into the object field by the microscope condenser. The image of the source, thus obtained without intervening lenses, assured that the vibrations of the light in the object field would be in the same relative position as when they started their journey from the source. This is known as phase agreement. Such method of illumination was termed " critical illumination." However, since that time, rightly or wrongly, the term has been applied to almost any method of illumination that furnishes good lighting for the microscope, as determined by the excellence of the image in the field of view. It is often applied to the Köhler method of illumination (Method II).

Several definitions of critical illumination have been given in times past. Hind and Randles[4] state in part, " ' Critical Illumination ' . . . and the formation of the ' Critical Image,' are attained when an image of the radiant is projected across the field of view by means of a substage condenser whose back lens is filled with light; the image of the radiant being focused simultaneously with that of the object." Shillaber[5] quotes this and follows with, " In other words, all light impinging on the specimen must be in the same phase; there must be only one wave front at the optical section under examination. However, in practice, critical illumination according to the above standard is seldom, if ever, obtained. Certainly in visual work, and usually in photomicrographic work, phase difference of light at the object makes little or no difference in actual results. It would be difficult or impossible to obtain critical illumination for low powers, and it would be clumsy and impose limitations on high-power work."

Obviously, the illumination systems involving the use of diffusing plates or accessory lenses cannot be classified as critical illumination according to the original meaning of the term. In Methods I and II, the light falling on the object must be completely out of phase, because it is out of phase at the first conjugate focal point of the con-

[4] Hind and Randles, *Handbook of Photomicrography*, second edition, 1927.

[5] Charles P. Shillaber, " Illumination of the Microscope for the Paint Chemist," *National Paint Bulletin*, February, 1938.

denser. However, this does not seem to cause the least trouble in image formation; in fact, from an examination of the field of view, it is impossible to tell whether the light in the object field is in phase agreement or not.

The essential technique in the art of photomicrography involves the proper lighting of the microscope, the criteria for which have already been defined. Without an adequate system of illumination good pictures are impossible. The use of the 250- or 300-watt projection lamp with the diffusing plate as a secondary source should be thoroughly understood; if possible, the Köhler method (Method II) and Method III should also be practiced. These methods can be learned quickly, and the application of any one of them should not take over 30 seconds to 2 minutes, even when the lamp is not already in the lamp house. The lamp can be screwed in, centered with the light-collecting lens, centered on the mirror of the microscope, focused, and be ready for use within that short time. It is necessary to be perfectly familiar with these systems of lighting before taking up the more advanced aspects of photomicrography, all of which have considerable dependence upon the illumination provided for the microscope. Excellence of illumination depends also to a large extent upon the operation of the microscope condenser, as this lens forms a very important part of the illuminating system, but the discussion of this will be reserved for Chapter IV.

Sec. 20. Centering the Image of the Lamp Filament to the Microscope Mirror. Although this operation has already been mentioned, it is so vital to good illumination that it will be discussed here in somewhat greater detail.

By any system of illumination, with the lamp properly centered in the lamp house, an image of the filament, arc crater, anode of the tungsten arc, or quartz tube of glowing gas is projected directly onto the center of the microscope mirror. To do this it is necessary to turn the mirror so that its surface is normal to the optic axis of the lamp lens. When the lamp house is tipped and the lens is focused, an image of the source can be formed on the mirror. The lamp should then be adjusted to bring the center of the image to the center of the mirror. Of course, after this adjustment has been made, neither the lamp nor the microscope can be moved without losing the alignment.

At first thought it might seem that such a refinement in regulation is unnecessary when a ground glass is to be used at the source, but, if the lamp is merely pointed at the microscope mirror without any effort at centering, the mirror must be tipped to reflect the light to the microscope condenser to make up for lack of centration. This may

appear to be satisfactory, but when the intensity of the light is measured with a photoelectric cell at the eyepoint a falling off of intensity of 15 to 20 per cent or more will be found when the lamp house is misaligned, and yet this misalignment may be hardly detectable by the eye. Thus, lack of centration not only decreases the light, which in itself would not be so serious, but, as a further measurement shows,

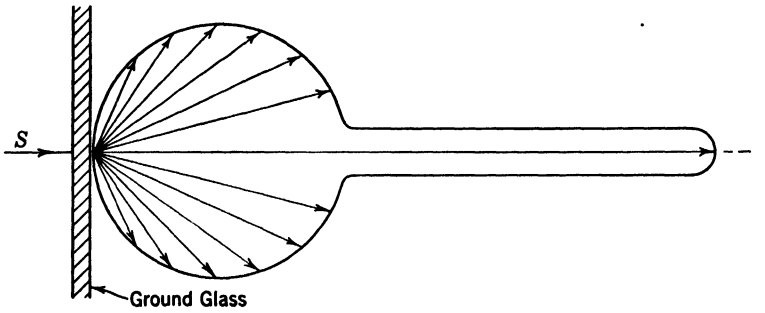


FIG. 46. This diagram indicates how a diffusing plate scatters light. The arrows can be thought of as vectors, representing the light intensity in the directions shown. Each point of the plate, in front of an extended source, affects the light in this way.

it also renders the lighting in the object field uneven; and this condition cannot be permitted in photomicrography. In Method II such a misalignment will be impossible because without the diffusing plate the irregularity will become so evident that it will be corrected at once.

Figure 46 shows the transmission of light through ground glass and explains graphically the reason for keeping the alignment correct. The length of the arrows shows the relative intensity of the light in the directions indicated. The long arrow in the center denotes specular transmission, as through clear glass; the arrows at each side denote diffused light.

Some microscopists have claimed that the best photomicrographs cannot be made on a vertical system and that horizontal apparatus with an optical bed is requisite for good work. The reason for such statements usually lies in the lack of an adequate method for making exact alignment of illuminating apparatus with the vertical camera. Centering the image of the light source on the center of the mirror is not the easiest adjustment to make, although it seems simple enough; and even if it is accomplished, all previous care will be worthless if the mirror is not correctly centered. The center of the mirror is found by methods described in Sec. 88. If when the center is found it is marked

with a dot of india ink, the dot will aid materially in locating the image of the illuminant and will not be detrimental to the work.

Sec. 21. Centering the Source of Light in the Field of View. After all the previously indicated centering operations have been carried out — that is, when the lamp has been centered in the lamp house (Sec. 18), the image of the filament has been centered to the microscope mirror (Sec. 20), and the mirror has been checked for centration with the microscope axis (Sec. 88) — there still remains one more centering operation, the image of the light source to the field of view.

For this purpose the microscope is focused on the specimen or on a test slide, preferably one of a finely ground specimen of powdered material. With a centered and focused condenser (Chapter IV), the iris diaphragm of the lamp is closed to a diameter of 4 or 5 mm, and by means of the mirror the image of this diaphragm can be swung into the center of the field of view.

When the microscope is set up with the camera, the image of the field diaphragm must be brought to the center of the ground glass of the camera. The center of the ground glass should be marked, either with inked-in cross lines or preferably with a small cover glass cemented with balsam to the ground-glass surface. This will leave a clear spot in the center of the field, and, if the cross lines are used also, the field will be divided into convenient quadrants which will serve as landmarks in placing or arranging the specimen and in centering the light.

This adjustment of centering the source to the center of the field of view is an important one for the photomicrographer. He can make it at any time and should check it frequently during his work. Microscope mirrors, unless equipped with a locking device, have an unfortunate way of slipping out of adjustment.

At this point, if all instructions have been correctly carried out, the image of the field diaphragm should be clear and sharp. However, it may be surrounded by color rings, depending upon the adjustment and type of condenser and the filters, and also, in an oil-immersion type, upon whether the condenser is oil immersed. Lack of centration of the lenses that make up the optical train also will cause uneven coloring in the image of the field diaphragm. Later it will be shown how the image of the field diaphragm of the lamp should serve as a check on several important adjustments.

Sec. 22. Illumination for Dark Field, by Substage Condenser. The dark field obtained by substage lighting is divided conveniently into two types; one is for the examination of comparatively large specimens, when images can be formed by reflected light, and the other is for

the examination of colloids, when the images are formed almost entirely by scattered light. With the former, even when photography is to be attempted, illumination may be by Method I, II, or III, but when the specimen is colloidal in nature illumination by Method II or III will be best. Strictly speaking, the high-power dark-field condensers are designed for use with parallel lighting; but, as intensity of light is paramount in work of this type, an arc may be indicated for work with the colloids in order to ensure sufficiently strong illumination. The lighting can be adjusted as for Method II. Because of the dark background and broken effect of the field which is usual in dark-field work, it is often possible, for low-power work, to use the lamps recommended for Method I and to make adjustments as for Method II. However, unless the technician is familiar with dark-field illumination, he is strongly advised to consult Secs. 92, 93, and 94 before proceeding further.

Sec. 23. Vertical Illumination. Opaque specimens with polished surfaces are generally illuminated by means of vertical lighting. The principles of this form of illumination follow very closely those of illumination by transmitted light. Figures 47 and 103 illustrate the two important methods. Figure 47 illustrates how vertical illumination can be attained by means of the glass plate used as a reflecting surface. The fourth diagram in Fig. 103 shows the use of the prism or mirror.

With either the glass plate or the prism, the adjustment of the lamp is essentially the same. The objective will act in the same capacity as the condenser with transmitted light. In order to assure this, the lamp distance denoted as PP_1' in Fig. 47 must be substantially equal to the optical tube length of the objective, the distance being measured from the second principal focal plane of the objective to the iris diaphragm of the lamp or to the surface of the lamp lens (these points being close together). Under these conditions an image of the lamp diaphragm will be formed in the object field. The small iris diaphragm of the vertical illuminator will then act as an aperture diaphragm, just as the iris of the substage condenser does when the microscope is arranged for transmitted light. Both diaphragms can be adjusted in accordance with instructions for the adjustment of their counterparts when transmitted light is employed.

In order to effect the above adjustments, or position of the lamp, in the easiest way and with considerable exactness, a 9- or 12-inch combination square, such as is used by toolmakers, is helpful. This, with a device for carrying a 6-inch scale on the blade, makes a very good height gauge. The working position of the microscope tube is deter-

mined, approximately, and the height of the center of the opening in the illuminator is found by means of the gauge. This is point A in Fig. 47. The lamp is set in front of the microscope with the diaphragm about 160 mm from the tube axis. The height of the lamp can now be adjusted by checking with the gauge until the center of the iris is set at the same height from the table as the center of the

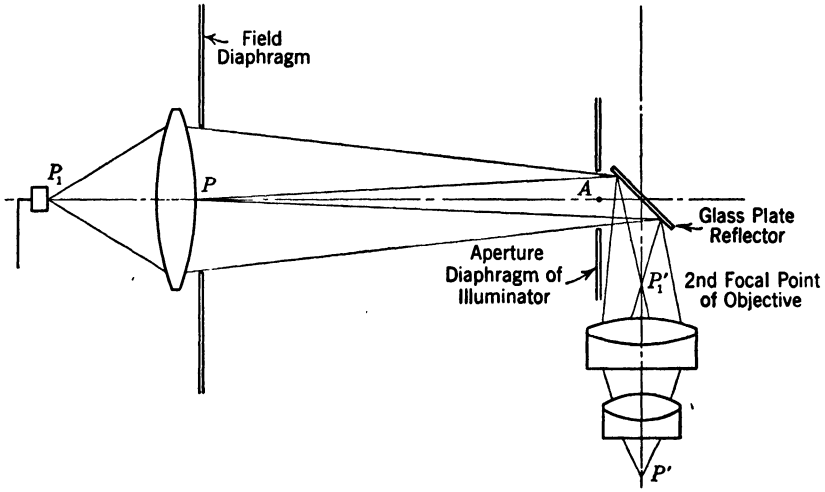


FIG. 47. The vertical illuminator with mirror or glass plate to reflect the light into the objective. The distance PP_1', from the lamp lens to the second principal focal plane of the objective, should equal the optical tube length. Then point P will be imaged in the object field at P'. This system of lighting is analogous to Method II, described for transmitted illumination.

iris of the vertical illuminator. Now, with the lamp lighted, the microscope can be focused, and, with the iris of the lamp fairly well closed to make it visible in the plane of the specimen, the lamp can be moved back and forth, being kept aligned meanwhile with the illuminator, until the iris is sharply focused in the plane of the object.

The lamp may be used with a diffusing plate as in Method I, or it may be used as described for Method II or III.

If high-power objectives are to be turned in, then the preliminary adjustments should be made with a low-power objective. If the microscope is equipped with a stage that can be raised or lowered, all major focusing should be done with the stage adjustment. The small displacement caused by the fine adjustment of the microscope in obtaining exact focus will not disarrange the alignment of the lamp and illuminator sufficiently to cause trouble.

An inspection of the rear focal plane of the objective will show the

proper alignment of apparatus and the opening of the aperture diaphragm, as with transmitted illumination. If the prism is used to deflect the light, the effective N.A. of the objective will be reduced by one-half at right angles to the prism faces. Thus, resolution is at a maximum in one direction, and the lighting is also strongest in this same azimuth.

The foregoing discussion of vertical illumination covers any optical condition which permits the lamp diaphragm to act as a field diaphragm and the diaphragm at the illuminator to act as an aperture diaphragm. When the illuminator is equipped with an accessory lens, conditions are reversed, the diaphragm at the lamp acting as an aperture diaphragm and the diaphragm at the illuminator acting as a field diaphragm. Horizontal outfits, permanently arranged for metallographic work with the inverted type of microscope, may be equipped with an arc lamp, in which case the general practice is to use an accessory lens or two between the lamp and the microscope. This conserves the light and keeps the lamp at a suitable distance, and if the iris of the accessory lens is placed in the first conjugate plane of the objective the diaphragm will act as a field diaphragm; it should then be adjusted accordingly. This is shown in Fig. 48*A* with two intermediate lenses and in Fig. 48*B* with one intermediate lens.

The glass plate used in lieu of the prism to deflect the light at the illuminator sets up considerable glare, or rather the glare is more pronounced than with the prism. This can be partly overcome by means of filters, depending on the subject, and by carefully regulating the diaphragms in the illuminating train.

Only flat surfaces should be illuminated with the devices under discussion. All rough surfaces such as unpolished metal, textiles, and papers should be examined or photographed by light incident at an angle large enough to produce a dark-field effect. The vertical illuminators produce a bright-field effect, and the angle of the light when the glass plate is used is 0°. When the prism or mirror is used the angle of incidence is so small that nearly all the incident light is reflected back into the objective.

Sec. 24. Illumination by Oblique Over-Stage Light Giving a Dark-Field Effect. This method of illumination should be thoroughly mastered because it is applicable to a large and varied class of subjects and can be carried on with little or no special apparatus. The magnification demanded is low, generally less than 100×. Objects illuminated and photographed by over-stage lighting will have a natural appearance that cannot be attained for specimens illuminated by substage lighting or by vertical illumination. Figure 49 demonstrates

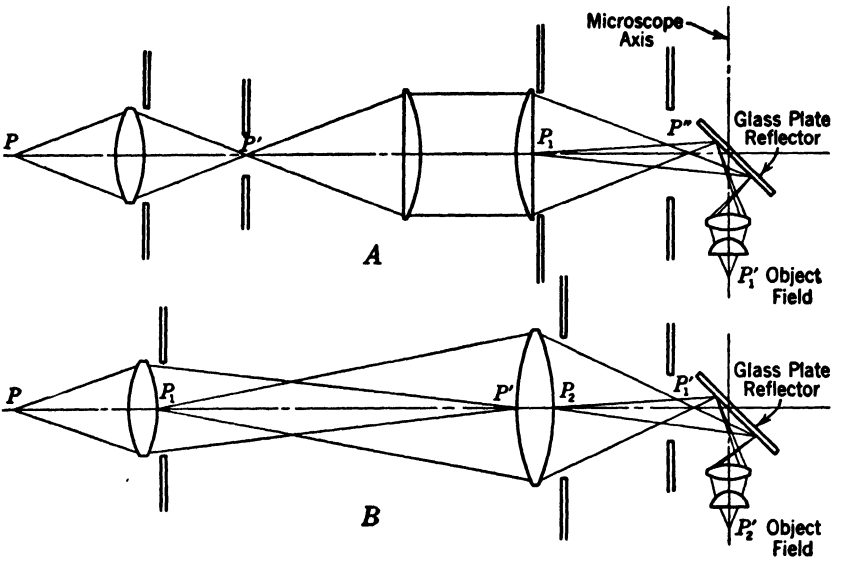


FIG. 48. Two systems using one or more lenses between the lamp and the microscope. Chiefly useful for vertical illumination. In both systems the secondary source is in the same position relative to the objective.

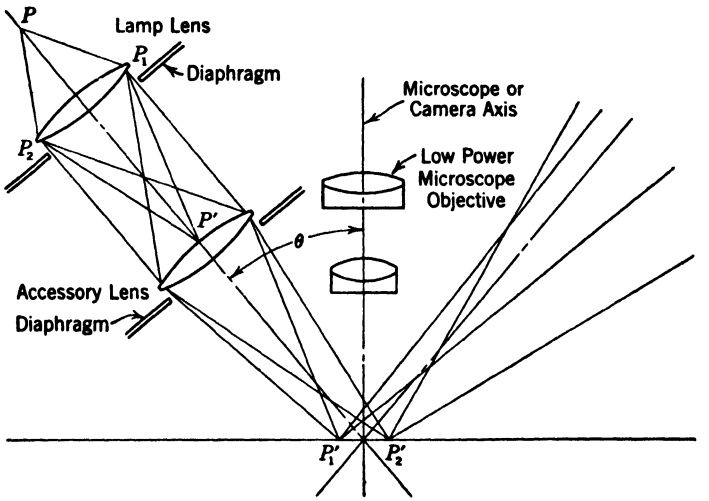


FIG. 49. Illumination by reflected light for low powers. It is, in effect, dark-field illumination. Angle θ may be changed to suit the object structure. The greater the angle, the greater will be the shadow contrast. In this diagram a perfectly flat reflecting surface is shown. No light proceeds to the objective, and the field is black.

how over-stage lamps may be used to illuminate the specimen. The trace of the rays is shown, and it is evident that if the specimen is perfectly flat, as in the drawing, the field of view will be black. However, when the specimen offers a broken surface to the rays, some of these rays will be reflected into the objective and an image will result. Consequently this method of illumination applies largely to opaque or semi-opaque specimens. As the illustrations show, the working distance of the objective must be sufficient for the light rays to reach the specimen. If the 8-mm lens is used, the obliquity of the light rays will be such that much detail will be lost; and if the 4-mm lens is turned in, the illumination will be merely grazing the top of the specimen, and the lighting will be weak and will cast long shadows. It will be found that this method of illumination applies best to objects that can be photographed with the 16-mm objective or those of even lower power such as the microphotographic lens used with or without the microscope.

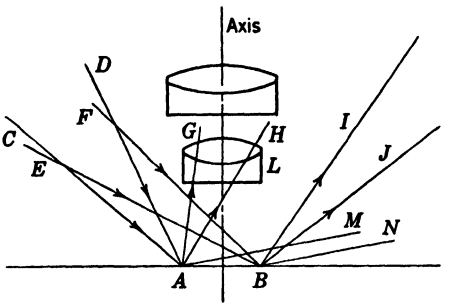


FIG. 50. Illustrating unevenness of illumination produced by using only one lamp. The point A, on surface M, is well lit, while point B lying on a surface N parallel to M, is not effectively lit.

Figure 50 demonstrates clearly the need for more than one lamp for over-stage lighting. Particles at A and B present the same angle to the lens as shown by the lines AM and BN. When cone of light CAD illuminates A and is reflected to the lens as cone GAH a good image of A is formed. Cone of light EBF illuminates B and is reflected as cone IBJ. Thus the light reflected from B is lost. Diffused annular lighting produces the most even field; however, such lighting will give a flat effect. Light directed from several angles will give more detail, and generally it will balance the field illumination satisfactorily.

A typical arrangement for over-stage lighting is shown in Fig. 51. Two lamp houses are shown in conjunction with bull's-eye lenses. The diagram in Fig. 49 indicates how the use of the bull's-eye lens intercepts the light rays and forms a smaller spot of even lighting on the specimen than would otherwise be formed. Thus, the use of this second lens makes the light on the specimen more intense but of less area than when a single lens is used. With the two-lens combination, diaphragm 1 is a field diaphragm and diaphragm 2 is an aperture diaphragm. In order to maintain proper contrast in a picture, and to

show good detail, the lighting must be strong. It may be necessary to use two or even three or more lamps, depending upon the subject, but, as a rule, two lamps will answer. The lamps can generally be placed at either 90° or 180° apart, but there can be no hard and fast rule about this, the structure of the specimen determining the position of the lamps. The effect of uneven lighting resulting from the use of only one lamp is shown in Fig. 52; the even illumination shown in 53 was obtained from two lamps. The specimen may be regular in structure, like a textile having threads running in two directions, but if the surface structure is very rough and has deep fissures that are required to show in the picture, the lamps can be arranged so that the angle of incidence is less.

The fact that the lamps can be used at any angle and from any azimuth makes this system very flexible and easy to adapt to a wide range of subjects. The concentration of the light from certain definite directions gives a contrast to the picture which would be hard to obtain if the lighting were from an annular source around the objective. Figure 53 shows less detail than Fig. 52 taken with one lamp; if more lamps were added detail would be even further suppressed.

Illumination by reflected light demands strong sources. Arc lamps are indicated, but two or three of them might be prohibitive in price. However, if the light flux from small spotlights or ordinary microscope lamps is properly conserved by the use of the accessory lenses as shown, they will generally answer the purpose. Usually the general microscope lamp will serve as a good source. The light can be focused on the bull's-eye lens and the bull's eye then moved to form an image of the lamp lens on the specimen (Fig. 49). The lamp may have to be moved, perhaps several times, to get it into just the right position, or it may merely need refocusing as the bull's eye is moved into position, but, as there is nothing critical about the line-up, the adjustment is easily accomplished. If the small spotlight has no lens that can be focused, it is probably arranged to throw a parallel beam, in which event the spot can simply be used conveniently close to the bull's eye, with the bull's eye focused as above.

Figure 54 illustrates a source of light before which is placed a diffusing plate and a diaphragm (the diaphragm may be on either side of the diffusing plate). According to equation 43, Sec. 53, the distance from the source of light bears a certain relationship to the image of that source as formed by the bull's eye, and is dependent upon the focal length of that lens. Thus, if the bull's eye has a focal length of 4 inches and is placed 4.4 inches from the diffusing plate, as at *A*, Fig. 54, then the image of the plate, or the diaphragm which is close to it,

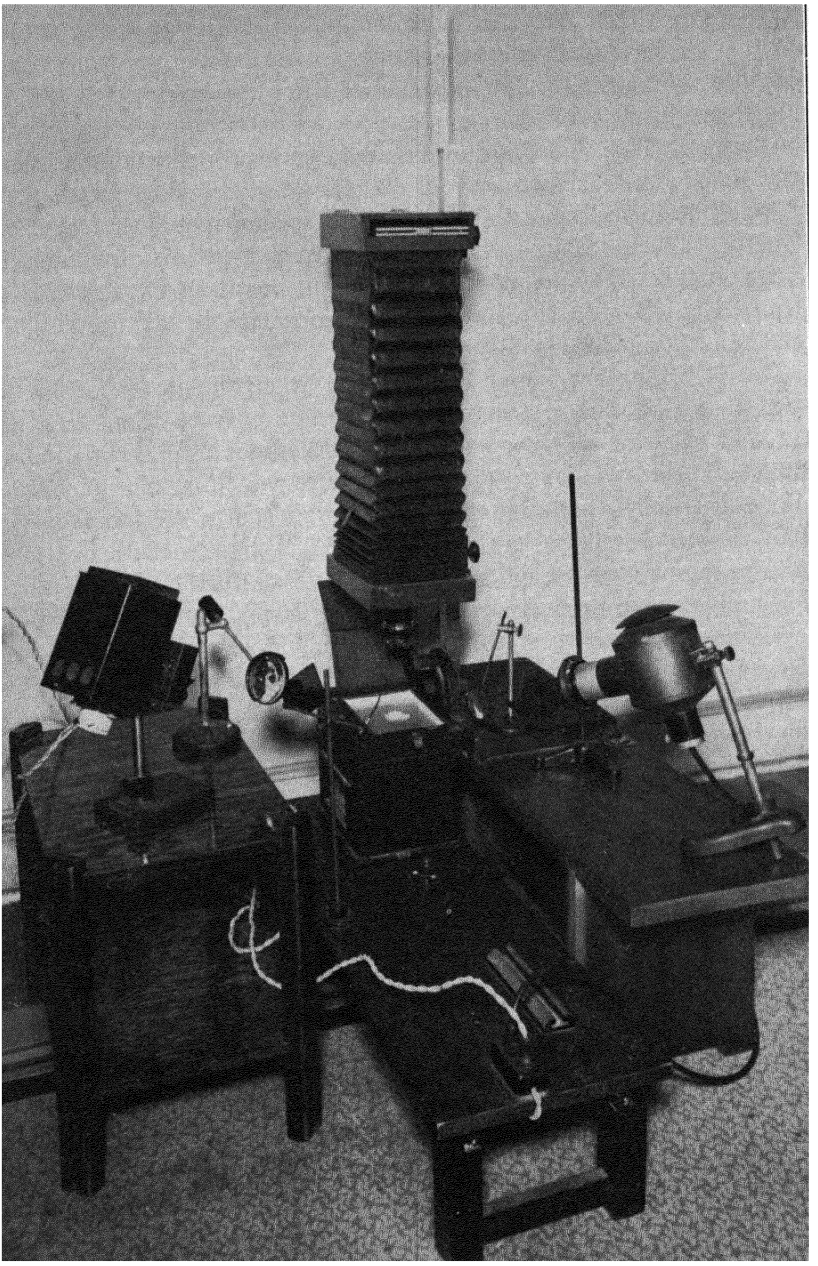


FIG. 51. A simple arrangement for low-power work Two filter holders are shown. One would suffice, as both lights need not be lit simultaneously. The two accessory lenses and lamps are on the " must " list.



FIG. 52. A picture of a painted surface showing cracks. The lighting is uneven, as the result of the use of only one lamp.

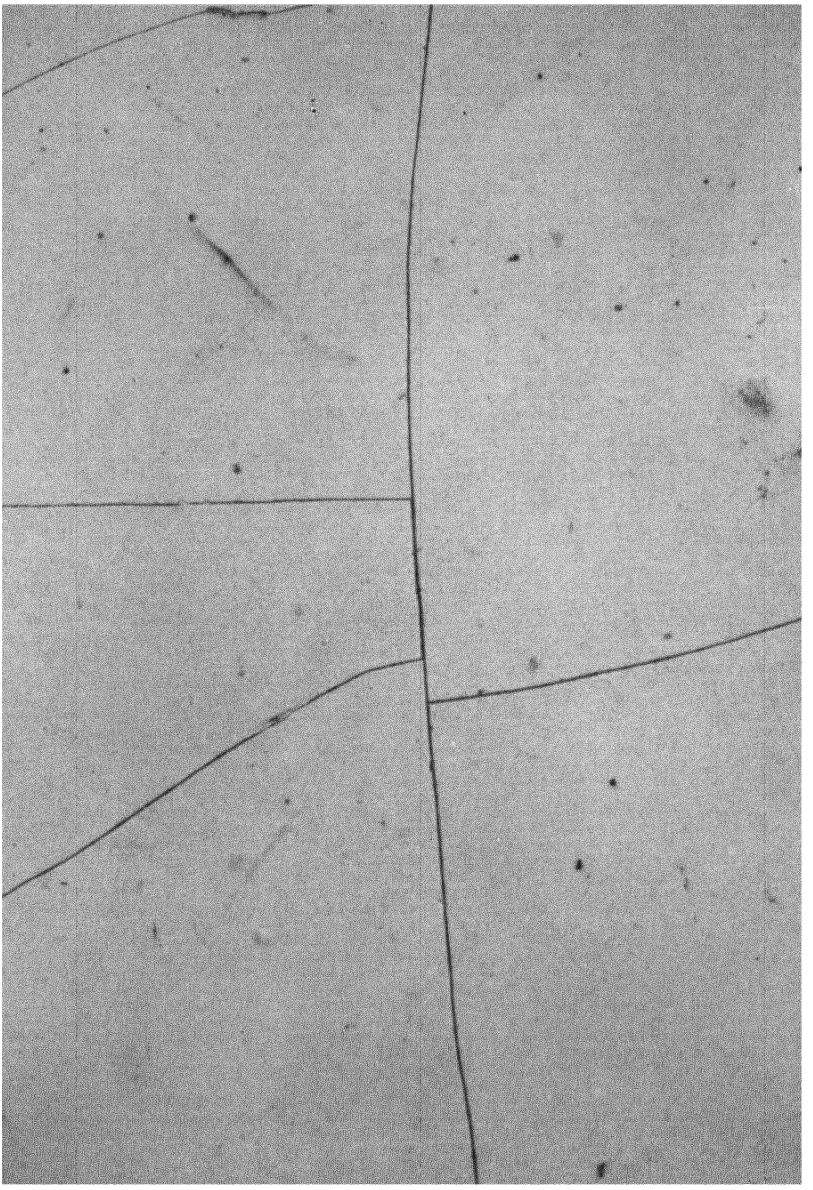


FIG. 53. The same subject as in Fig. 52, lit with two lamps. There is even lighting but less detail.

will be 18 inches from the bull's eye. Since the magnification of such a system is 18.0 : 4.4, the image of the field diaphragm will then be 4.1 inches in diameter when the iris is adjusted to a diameter of 1 inch. If conditions are reversed, and the bull's eye is placed 18 inches from the source, as at *B*, Fig. 54, an image of the source will be formed 4.4 inches from the lens. The magnification will then be 4.4 : 18.0. This is a diminution instead of an enlargement, and the image of the source will be only 0.24 inch, when the diaphragm is adjusted to 1 inch

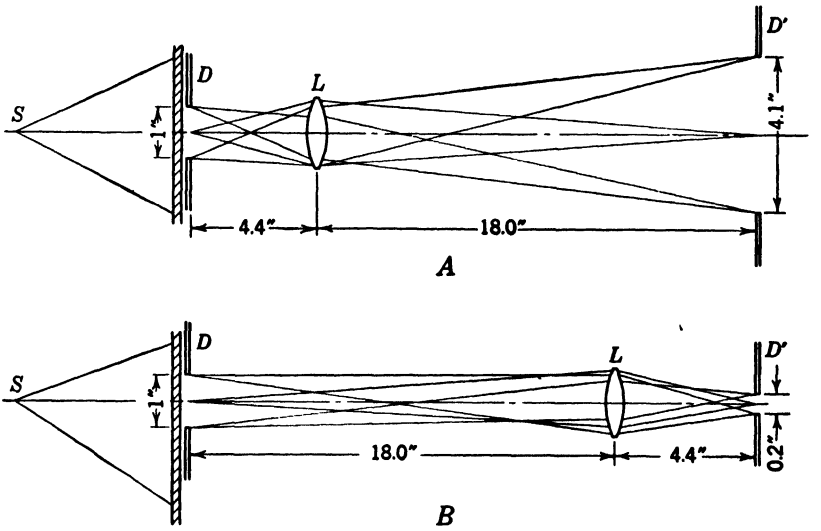


FIG. 54. This illustrates how the size of the illuminated field can be made either small as at *B* or large as at *A*. The light intensity is greatest with illumination arranged as at *B*. See equation 43 for the two positions of the lens. The effective source is the diffusing plate, and all measurements are made from it, as shown.

in diameter. When the bull's eye is placed 8 inches (twice its focal length) from the source, the image will be the same size as the source and will be 8 inches from the lens; there will be neither enlargement nor diminution. Although the theory of lens magnification is taken up fully in Sec. 53, this much of it is cited here to explain roughly the optical principles relating to the use of the bull's eye, in illuminating opaque objects, and to show that the physical arrangements are closely connected with the optics that are involved.

Centering the Light on the Specimen. In order to have the position of the small spot of light formed by perhaps several bull's eyes coincide in the object field, it is necessary to center each lamp and its bull's eye separately. A 3- by 5-inch card will do as a test object. Concen-

tric circles, the outermost having a diameter of about 3 inches, are drawn on the card. The center is marked with a dot, and the card is placed in the object field and focused. It is then moved until the center of the target is in the center of the field, as seen in the microscope or on the focusing screen of the camera. Each lamp in turn can now be trained on the center of the target and the bull's eye inserted in the path of the light rays, with the spot of light focused on the center of the rings. The target, when first examined on the ground glass of the camera, indicates the area to be illuminated in terms of rings. Thus, if it were found to be necessary to illuminate to the fourth ring in order to cover the field, the lamp and bull's eye must be arranged so that the small spot of focused light will extend to at least the fourth ring. In this way it is possible to know exactly how each lamp and bull's eye is placed in relation to the target and so to the specimen. The specimen replaces the target after all the lamp arrangements have been made, and the important part of the specimen is moved to the center of the field; it will then be well lit provided that the lamps are not moved.

In arranging the illumination as described, it must be borne in mind that the specimen must be on the same plane as that occupied by the target; otherwise the lighting effect may be entirely different from what is intended. To appreciate fully the difference that a slight raising or lowering of the object field makes in the illuminating effect, the target can be raised slightly while it is illuminated and the difference noted. If the lamp axes are at a large angle to the surface of the specimen, a very little motion of the card will show that the effect of even illumination is completely destroyed. As the light is incident at a less angle, greater vertical displacement of the card will be necessary to alter the lighting effect.

The Use of Light Filters for Incident Light. Many specimens demand the use of filters in order to increase contrast, and light filters are as suitable for this form of illumination as they are for illumination by transmitted light. Metal specimens, colored objects, and stained subjects must often be photographed in light of definite wavelength. It is not always necessary to provide a set of filters for each lamp house. It is possible to make successive exposures with each lamp. The filter holder can then be moved to the second lamp after the first lamp has been used, and so on; but, if much work is to be done with this system of illumination, it is desirable to provide sufficient filters to permit all the lamps to be used simultaneously.

Orientation of the Specimen. On materials such as textiles, it is surprising how the appearance of the picture is altered when the ori-

entation of the specimen is changed with respect to the lamps. Since it is almost always desirable to have the long lines in the picture running parallel to the long edge of the film, it is best to consider first the placing of the specimen with respect to the shape of the plate or film. The lighting should then be arranged to give the desired effect. One way to do this is to observe the specimen through the microscope or on the ground glass. When the specimen is on a revolving stage, the stage should be turned until a good lighting effect is obtained, the lamps being meanwhile, say, 90° apart. When the best visual effect is attained, the position of the lamps with respect to the specimen is noted. The specimen is then swung back into its prearranged orientation with respect to the photographic plate, and the lamps are moved into the position that was found most effective.

The effect of one lamp should be studied before a second is lighted; the first lamp should then be extinguished and the next one lit, and the effect again studied. In this manner it is possible to know just what effect each lamp is having. The direction of the impinging light should be carried in mind, so that the shadows can be recognized and the effects of a second lamp estimated. The height of each lamp above the specimen can be varied, and, on specimens with considerable depth, this may bring about important and desirable changes in the final appearance of the picture. At this time it may be determined whether the intensity of the illumination is sufficient, or whether other higher-power lamps are needed.

The Addition of Transmitted Light. Lighting by incident illumination is strictly dark-field illumination, and many subjects do not stand out clearly on a dark field. The picture of coffee beans in Fig. 223 would not show up well if the background were dark, but a light background sharply silhouettes each individual bean and makes it stand out strongly and clearly. When the microscope is used, the substage condenser will provide a means for handling transmitted light. With an objective of a power lower than the 16-mm, a spectacle lens condenser will generally be necessary. An extra lamp will be required to furnish the substage illumination, but it can be of low power, an ordinary desk lamp answering all purposes. Sometimes filters are useful to modify the substage light. Sections 83 and 89 describe the use of the low-power condenser.

If the objective is of very low power or is of the microphotographic type, probably the microscope will not be necessary and the objective can be placed directly on the front board of the camera, in a focusing mount. The specimen must then be placed on or above a diffusing plate, and in this position it may be lit from below. A printing box

convenient for this purpose is shown in Fig. 51, this section. It serves to support the specimen at some distance above the ground glass, and it also furnishes the required source of transmitted light. The brightness of the ground-glass surface can be increased easily by arranging several small 100-watt V-shaped filament lamps, mounted in bayonet sockets, around the inside of the box as desired, each connected with an external switch with a master switch on the lead to the box. Before the picture is taken, it is necessary to turn on the substage lights to inspect the lighting of the specimen. This inspection should be made when the over-stage lamps are not lit. If the transmitted light is turned on for only a few seconds the heat from the lamps will be of no importance; but, if much of this low-power work is to be attempted, it is suggested that half-inch holes be bored through the sides of the printing box to allow the escape of the heated air.

The Use of Reflectors. One of the first devices designed for providing over-stage lighting was the Lieberkuhn, a concave reflector that receives light from the microscope mirror, brings it to a focus, and reflects it down onto the object. Since it was attached to the objective, a separate one of appropriate focal length was required for each objective. The Lieberkuhn is not now in general use. A parabolic side reflector can be obtained for use on the stage of the microscope; it is illuminated by a lamp above the microscope stage.

Reflectors in conjunction with direct spot lighting as shown in Fig. 51 are more or less disappointing, but sometimes they help to shorten the exposure time and to lessen deep shadows. A reflector can be arranged extemporaneously as the work demands. It may be in the form of a mirror to reflect the light back onto the object, or, as is more usual, it can be a piece of glazed paper arranged about the specimen in a semicircle. This will pick up the unused light after it has passed the specimen, diffuse it, and redirect it back to the specimen. Inasmuch as the light loss at the reflecting surface is large, and the reflected light is not refocused but diffused, the effect is not very important. The direct lamplight is so strong in comparison that the reflected light is overpowered. However, sometimes such technique may serve to light rather inaccessible parts of the specimen which could not be reached in any other way, and then it is of value; otherwise the addition of an extra lamp will usually give better results.

The Use of Polarized Incident Light. Light is easily polarized by placing a sheet of polaroid between each lamp and the specimen. An analyzer is not needed. In certain photography on moist specimens,

the polarized light will aid in reducing undesirable highlights. The plane of vibration must be at 90° to the plane of vibration of the reflected polarized highlight that it is intended to extinguish; otherwise the addition of the polarizer will not produce satisfactory results. Thus, in effect, the specimen acts somewhat like an analyzer. If, while the specimen is being examined, an assistant is asked to raise or lower the lamp with the polarizer and at the same time to turn the polarizer, the effect on the highlights can be watched. As the loss of light will be large, the source should be adequate to compensate for it.

Photoflood Lamps. The use of photoflood lamps has been mentioned for work with incident light, and such lamps may often be helpful in supplying a source of high intensity. They give off too much heat to last long in a lamp house, but they can be used to advantage in an open lamp house. Even if the specimen is very dark, such illumination may make an arc unnecessary. Since arranging and lighting a specimen may take 15 or 20 minutes or even more, some of this work can be done with other lamps which do not have such a short life and the photoflood lamps can be substituted later. To take full advantage of their light, a bull's eye should be used with each lamp. Since the regular microscope lamp-house equipment is excellent for nearly all work, these higher-power lamps should be resorted to only to enhance detail in a picture of a poorly reflecting or dark subject.

A very good method is to mount a photoflood lamp in a desk lamp holder and while the exposure is in progress to carry it around the specimen in an arc of at least 180°. This technique helps tone down a too contrasty lighting arrangement; in fact, many specimens can be well lighted by this method alone, the only difficulty being that there is no way to judge the final results beforehand.

Sec. 25. The Silverman Illuminator. This device, made by Spencer, is a small annular lamp mounted in a fitting to be clamped to the end of the objective. (See Fig. 55.) The lamp is equipped with a reflector and operates at 6 volts. It can be overloaded slightly while an exposure is being made. It is useful on the 8- and 16-mm objectives and those of lower power. Since it generates a great amount of heat, its use on achromatic objectives, where any damage done will be less costly, is advisable. However, it is an important illuminant; it does not interfere with the regular arrangement for transmitted illumination; and specimens can be examined successively with either incident or transmitted light or both simultaneously. When both methods are used simultaneously, the result is likely to be disappointing unless the stronger illumination is modified by optical filters to

prevent the weaker light from being overpowered. Usually it is the transmitted light that needs to be toned down to a suitable level.

An accessory to the illuminator is a resistance coil with a switch to control the lamp. Another switch makes it possible to cut out some resistance and to step up the light intensity for a photographic exposure. The use of additional resistance of 50 to 100 ohms has proved valuable. This added resistance, if of the slide-wire type, is convenient to modify the light on bright objects, and at the same time it conserves the life of the lamp.

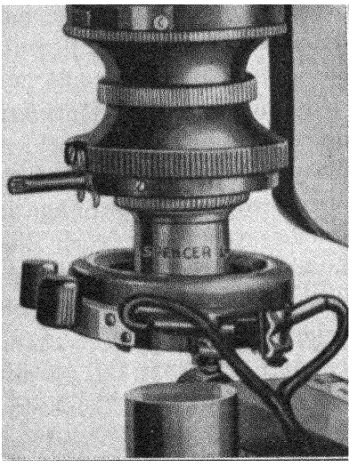


FIG. 55. The Silverman illuminator by Spencer. A handy lighting device for the lower objectives. This illumination has now been taken off the market. Since there is nothing made in this country to take its place, it is to be hoped that after the war its manufacture will be resumed or a similar system provided. (By courtesy of Spencer Lens Company.)

For the examination of textiles, papers, paint surfaces, leathers, metal specimens, and similar materials, the Silverman illuminator is invaluable. In adjusting it care should be taken to see that it is at the optimum height above the object; this can be determined by adjusting the height during observation. The lamps have a comparatively long life and can be obtained in glass of daylight quality if desired. Unilateral lighting can be effected by a small shield which accompanies the equipment.

Sec. 26. Apparatus Requiring Special Objectives, for the Use of Incident Light. Illumination by this means necessitates highly specialized apparatus as developed by the German firms. Leitz, Zeiss, and Reichert have built their systems — the Ultropak, the Epi condensers, and the Epilum, respectively — but because of the situation regarding patents the American firms are not yet in a position to develop similar equipment. Figure 56 shows the apparatus and Fig. 103 illustrates the trace of the light rays in such a system. The effect produced is strictly dark field, but in the Leitz arrangement it is possible to effect a quick change-over from the dark to the bright field by turning in a vertical illuminator. The illumination of such systems depends on an accessory lamp which is supplied with the apparatus. In at least one system, the Ultropak, the lamp

may be separate and distinct from the apparatus. The lamp regularly supplied is removable, making it possible to change to any other light source.

Zeiss has an interesting piece of illuminating apparatus, called the Epi mirror, that fits on the stage of the microscope. It is an annular concave mirror which receives light from below the stage and provides incident illumination. It is excellent for low powers, but the loss of light is large and the illumination is likely to be flat, as with all annular illuminators. The Greek prefix "epi," associated with many of the illuminators of this type, means "upon" or "over" and is therefore very suitable as a prefix in naming this sort of equipment.

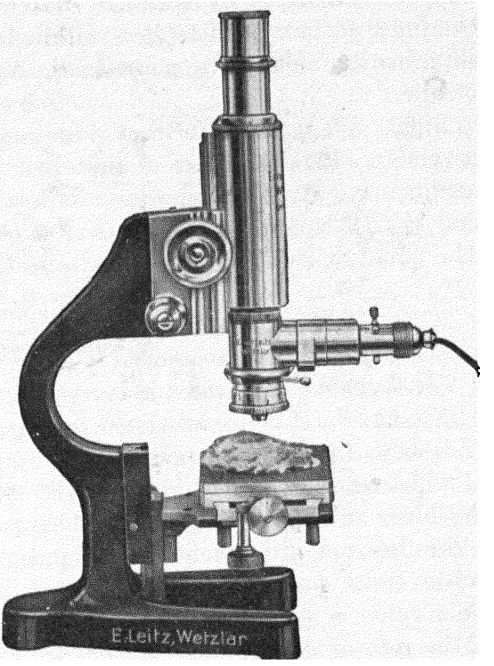


FIG. 56. This illustrates the Ultropak equipment of Leitz. The lamp is attached, making, with the reflector, condenser, and objective, one complete illuminating unit for work with reflected light. See Fig. 104.

Sec. 27. Photometric Units. Comparison of the various lamps intended for microscopy must be made in terms of photometric units commonly used to measure light. Although no unit for measuring lamp illumination can be entirely adequate for microscopical work, there are three from which to choose, the choice depending on the lamp, and the method of illumination selected. They are the lumen (the basic photometric unit), the foot-candle, and the candle per unit area. If the unit is carefully chosen it will offer a fairly satisfactory and logical basis for lamp comparison.

The Foot-Candle. The original standard for measuring light was the British standard candle of spermaceti, which was made to burn at the rate of 7.8 grams of spermaceti per hour. From this arose the term "candlepower." A more modern standard is the pentane standard lamp, which can be controlled to within 0.01 per cent, as compared with a fluctuation of 15 per cent, or even more, for the spermaceti candle.

Other light sources can be compared with the standard by means of a photometer and rated accordingly.

Considering a point source of light equal to 1 standard candle, the source is then said to be the equivalent of 1 candlepower of light. This point source can be imagined as emitting light equally in all directions and as surrounded by a sphere of 1-foot radius. All proportions of the inner surface of the sphere will be bathed with light equally. The illumination at any one point on the sphere is then said to be 1 foot-candle.

Light, or any other form of radiating energy, decreases in intensity inversely with the square of the distance. Thus, a small source of 1 candlepower gives illumination of 1 foot-candle at a distance of 1 foot, and ¼ foot-candle at a distance of 2 feet, or ⅑ foot-candle at 3 feet. The equation, with the foot-candle as the unit of illumination, is

$$\text{Foot-candles} = \frac{\text{Candlepower of the source}}{(\text{Distance from the source})^2} \qquad [9]$$

The Lumen. The radiant energy of a source of light is called the "light flux" (it is analogous to the magnetic flux of a magnetic field and obeys the inverse square law of equation 9), the unit of measure of which is the "lumen." The light flux falling on 1 square foot of the inner surface of a sphere of 1-foot radius from a light source of 1 candlepower at its center is 1 lumen. Thus a source of 1 candlepower emits 4π lumens. The lumen is equivalent to 0.001496 watt. This value is sometimes given in ergs per second; that is, the lumen is the rate of delivery of light energy and is thus a power unit.

In this connection it is interesting to compare the amount of electrical energy received by the lamp with the amount of energy converted into light flux, the useful component. From the above figures, 3000 lumens of light produced from a 200-watt lamp are equivalent to only about 4.5 watts, or $\frac{1}{50}$ of the input of electrical power. The unaccounted-for energy is largely converted into heat, plus the small usual lamp losses.

Illumination of 10 foot-candles at a given point is the equivalent of 10 lumens per square foot; thus lumens per unit area is also a measure of illumination. The equation is

$$\text{Lumens per unit area} = \frac{\text{Total lamp output in lumens}}{4\pi\ (\text{distance})^2} \qquad [10]$$

The lumens emitted by a source through a complete solid angle, or the total lumens emitted, are a measure of the intensity of the source and are numerically greater than the candlepower of the source by 4π times.

The Brightness of a Surface. The brightness of a surface is referred to in candles per unit area. In speaking of the arc lamp, the crater is commonly referred to as having a brightness of so many candles per square millimeter. This unit is also applicable for measuring the brightness of any surface on which light falls, although the unit of area is more likely to be the square foot than the square millimeter. Illuminants of small extended solid area are often rated on the basis of candles per square millimeter. Lumens per solid angle per unit area may also express brightness of a surface.

The relationship between the brightness of a surface in terms of candles per unit area and illumination in terms of foot-candles, or lumens, per unit area is sometimes of interest. It is given by the equation

$$E = \frac{\pi B}{R} \qquad [11]$$

where E is the illumination, B is the brightness per unit area, and R is the reflection factor or the fraction of light that is reflected. If the unit area is 1 square foot, then the value of E is given in foot-candles or lumens per square foot required for a certain brightness, B.

Another important equation for determining the illumination at a surface after the light has passed through a lens, such as the light-collecting lens at the lamp, the condenser of the microscope, or a bull's eye, is given by

$$E' = \pi B \sin^2 \theta' \qquad [12]$$

E' is the illumination at the surface in lumens per unit area; B is the brightness of the source per unit area; and θ' is half the angle subtended at the exit pupil of the lens or half its angular aperture. This equation does not take into account light lost by absorption and reflection in passing through the lens.

To summarize, the *intensity of a light source* may be referred to as candlepower, or the total output of light flux may be given in lumens. *Illumination* at a point or at some particular field or plane may be expressed in foot-candles or lumens per unit area, the lumens per square foot equaling numerically the foot-candles. *Brightness* of a surface is spoken of as candles per unit area or lumens per solid angle per unit area.

Sec. 28. Photometric and Other Lamp Data. Catalogues listing electric lamps may include all or some of the following operating data: the power required to operate a lamp in watts; the required voltage; the type of current; the luminous flux output in lumens per solid

angle; the intensity of the lamp in candlepower; the brightness of the incandescent surface in candles per square millimeter.

In illumination by Method I a diffusing plate lighted by a light-collecting lens is placed near the source of light. It is then obvious that the output in lumens would give a satisfactory comparison of lamps of various power provided that their filament structure is alike.

The filaments of projection lamps are arranged to form a plane of illumination of varying size depending upon the power of the lamp. The power, in watts, to operate the lamp, or the output in lumens, serves as a relative measure of the brightness — candles per square millimeter — that may be expected at the ground glass in each case. The ground glass then becomes the effective source of light for the microscope.

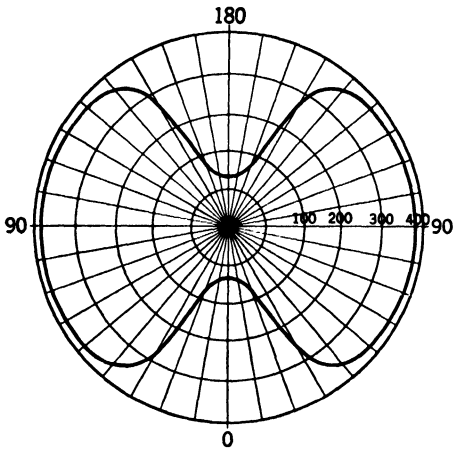


FIG. 57. A polar distribution curve showing the relative candlepower of a lamp, similar to the projection lamps, in a horizontal plane. Each ring interval represents 100 candlepower. The candlepower values should be read only where they intersect the curve. The values have nothing to do with distance from the source, but only with the direction, or azimuth, in which they are read. Distance from the source, of course, is the same for all the candlepower values represented.

The microscopist is interested in the horizontal distribution of light from a lamp rather than in its spherical distribution. The plotting of the candlepower of the projection lamps, with the filament in the form of a plane surface, on polar coordinates, might appear as in Fig. 57. The horizontal direction represents a direction normal to the plane of the lamp filament. This is sometimes called the beam candlepower. Unfortunately, graphs showing the distribution of light from a given lamp are hard to obtain, and only the ordinary published data are easily available.

Table IX shows the relative brightness of the microscope field measured at the exit pupil of the microscope for lamps of various powers. The conditions under which the measurements were made are stated.

Dividing the lumen output of a lamp by the wattage expresses its

efficiency in lumens per watt. This figure will not differ greatly for the various projection lamps likely to be used. Thus, if a lamp is to be chosen for illumination by Method I, it can be selected on a basis either of its power requirements or of its lumen output. The light on the diffusing plate increases as the wattage of the lamp or the lumen output increases.

Table IX

Comparative Light Intensity for Several Lamps — Measured at the Exit Pupil of the Microscope

Lamp		Lumens	Comparative Intensity*			
			Method I	Method II	Method III	Lens at Lamp
C–13–D	400 w. projection	9,600	6 2	100 0	13 8	f = 4 inches
C-13	500 w. projection	13,000	11.0	52 5	13.8	f = 4 inches
C–13	250 w. projection	5,600	3.1	35.0	6.2	f = 4 inches
CC–13	100 w. projection	1,900	2.0	32.5	2.5	f = 4 inches
C–8	108 w. coil fil. 6 v.	2,400		30.0		f = 2¼ inches
SR–6	108 w. ribbon fil. 6 v.	2,000		7.5		f = 2¼ inches

* The intensity of the field at the exit pupil of the microscope was measured comparatively. A 400-watt biplane filament projection lamp was taken as 100. One neutral filter was used throughout the measurements to reduce the intensity to a suitable level. A photoelectric cell was used in conjunction with a long-scale galvanometer.

On the other hand, if the lamp is to be chosen for illumination by Method II, the most useful datum on which a selection can be based, and which permits a fair comparison for microscopic work, is the intrinsic brilliancy of the source in terms of candles per square millimeter of incandescent surface. The lumen output and the power requirements are not very useful figures here. The tungsten incandescent sources, as well as the arcs of all types, can be measured, and their brightness per unit area is generally available. Since Method II involves the use of the image of the source in the first focal plane of the condenser, the size of the source is immaterial provided that its image fills the front lens of the condenser with light.

There is no easy way to measure the relative effectiveness of the ribbon-filament and the mercury-vapor types of lamp because the one has a continuous spectrum while the other has a broken spectrum composed of bright lines; also all the arcs are much richer in the blue light than in the red. Therefore, although lamps can be compared

on the basis of brightness of surface, this should be done only after they have been separated into their various classes. Thus, a mercury-vapor tube may be compared with any other mercury-vapor tube, to some extent with the carbon arc, and to a still less extent with the photomicrographic lamp; but the ribbon-filament and close-coil-filament lamps set up a class by themselves.

If a lamp is to be chosen on the grounds of spectral distribution of energy or on a basis of percentage of energy for given wavelengths, then the proper data sheets must be obtained from the lamp manufacturer. For the photography of stained specimens and for use with chromatic filters of narrow transmission range such data are invaluable. For example, if material of a deep red color is to be photographed to stress the detail in red, then a red filter and a lamp giving much red light should be chosen, probably one of the higher-power projection bulbs being the proper selection. On the other hand, if the work demands the utmost in resolution, strong blue filters will be needed, and the mercury-vapor tube is clearly indicated. Separation in the green can be accomplished with the projection line of lamps, or one of the arcs would be a still better choice. Lamp data are easily obtained from the manufacturers, and greater advantage should be taken of this service.

The microscopist should also be familiar with all the physical dimensional data concerning lamps. These include the type of bulb and its size, the base, and the figures denoting filament shape. The General Electric Company has standardized such nomenclature for its own lamps. Letters G, P, A, S, T, and PS denote the shape of the bulb as shown in Fig. 58. A number following the letter denotes the diameter in eighths of an inch. Thus, T-10 refers to a tubular bulb $1\frac{0}{8}$ inches in diameter.

The type of base must be known if the appropriate socket is to be provided for a lamp. The important bases are the medium screw (generally used for household service), the medium prefocus, and the bipost, which is necessary for heavy current draws. The form of the filaments is designated by the numbers from 1 up. A letter preceding the number indicates that the filament is of straight (S) wire or of coiled (C) wire. CC denotes a filament of close-coil construction such as is used in some of the lamps in the projection series. The wire is first closely coiled, and this coiled wire is again coiled to form the filament. Other data which are available are the burning position of the lamp, its rated laboratory life, the overall length, and the dimensions of the source in millimeters. It is useful to have information regarding the finish of the bulb, that is, whether the glass is clear,

frosted, daylight, or of some other finish. All the above manufacturing specifications, with the photometric data, can be obtained from the manufacturers, and they will aid materially in selecting the right lamp for any given purpose.

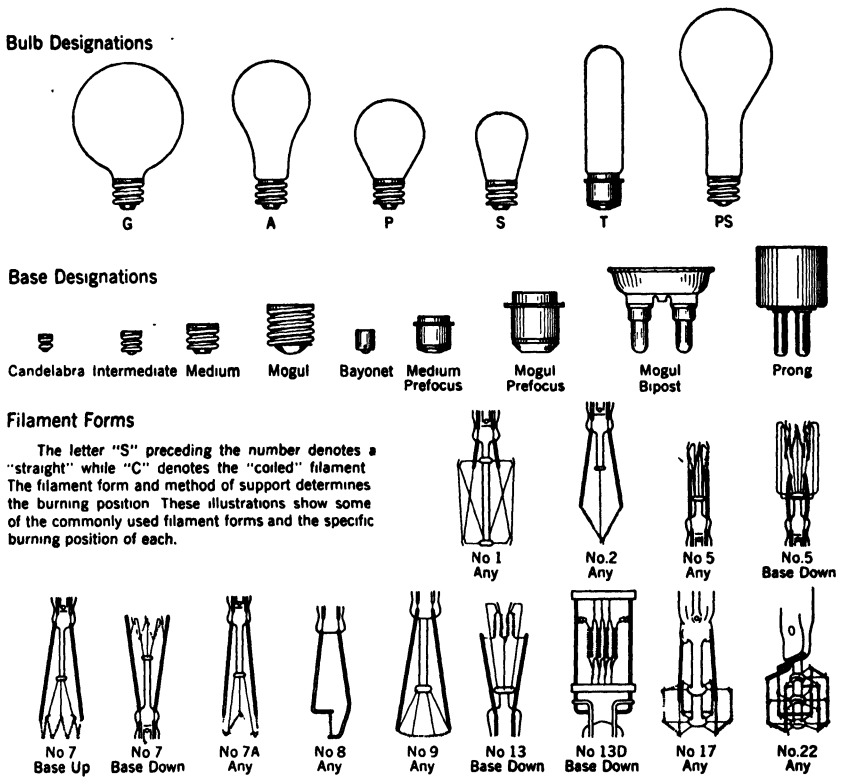


FIG. 58. This collection of drawings represents most of the bulbs, bases, and filaments of lamps suitable for microscopical work. Acknowledgment is made to the General Electric Company.

Sec. 29. Lamps. The following lamps are recommended for photomicrography. All can be used for illumination by Method I or III; those specially suited for Method II are so noted.

When lamp voltage is spoken of as 115 volts, it will be understood to cover any of the normally used voltages of about this figure. Different power lines, depending on local conditions, deliver to the lighting circuits current varying in voltage from about 105 to 120. For this reason many styles of lamps are made with slight changes in their construction to compensate for any differences in electrical pres-

sure that they may encounter. Occasionally lighting circuits may operate on 220 volts, and many of the lamps are made also for this voltage. Table X describes many of the domestic lamps specially suited for microscopic work.

Because of the large number of lamps available, and because one type of lamp may be made for several different voltages and with several different base constructions, it is not always easy to obtain the exact lamp required. Walters Electric, operated by Mr. W. H. Walters,[6] specializes in lamps and as a rule carries in stock all the various lamps the microscopist may need.

The Projection Lamp, 110-volt. The most useful lamp for photomicrographical work is undoubtedly the projection lamp. This lamp, with others, is well described in the booklet "Mazda Lamps" published and sold by the General Electric Company; a more scientific treatment is given by Forsythe and Adams.[7] It requires no auxiliary equipment, and it can be had for various line voltages. The power consumption varies from 100 to several thousand watts. The convenient sizes for microscopic work are from 100 to 500 watts. Probably the most desirable size for general use is the 250- or 300-watt, for either the 115- or 250-volt lighting circuits. The intensity of light from such a lamp is sufficient to permit the addition of a diffusing plate and the filters necessary for separating narrow bands of the spectrum. They are excellent lamps for either visual or photomicrographic work. The filament is C-13. Any source of light of this type, having the incandescent part distributed unevenly over a comparatively large area, should be used with the diffusing plate according to Method I or III. The 250-watt lamp gives 5600 lumens; its spectral distribution of energy is high in the red and low in the blue. When used with a rheostat to control intensity, the relative amount of the red component in the light gains rapidly as the external resistance is increased. It can be operated at an angle of 15° to 25° with no great diminution of life. Laboratory life of the 300-watt lamp is given as 50 hours. Price, $2.00. Base, medium or prefocus. Bulb, T.

The *100-Watt Projection Lamp.* The 100-watt lamp of this series can be had with a small bayonet-type base. It is possible to obtain an adaptor to screw into the regular medium screw-base socket and to use the lamp in the bayonet socket of the adaptor. This makes a very inexpensive source of light, since the lamp costs only 80 cents. The output is 1900 lumens.

[6] Walters Electric, 740 Third Avenue, New York City.

[7] W. E. Forsythe and E. Q. Adams, "The Tungsten Filament Incandescent Lamp," *Denison University Bulletin; Journal of the Scientific Laboratories*, **32**, April, 1937.

Table X

Lamps for the Microscope

Type	Description of Filament	Watts	Lumen Output	Auxiliaries Required	Type of Current	Lab. Life hr.	Base	Bulb Standard	Approx. Cost	Surface Brightness c/mm^2 *	Voltage	Especially Suited for Method
Projection	CC–2	50	825	None	A.c. or d.c.	50	S.c. or d.c. bayonet	T–8	$0.50		110 to 120	I, III
Projection	CC–13	50	810	None	A.c. or d.c.	50	S.c. or d.c bayonet	T–8	0.90		110 to 120	I, III
Projection	CC–13	100	1,900	None	A.c. or d.c.	50	S.c. or d.c. bayonet	T–8	0.90		110 to 120	I
Projection	2CC–8	200	4,700	None	A.c. or d.c.	25	S.c. or d.c. bayonet	T–8	1.50		110 to 120	I, III
Projection	CC–13	200	4,200	None	A.c. or d.c.	50	Med. Screw or prefocus	T–10	2.00		110 to 120	I, III
Projection	C–13	250	5,600	None	A.c. or d.c.	50	Med. screw or prefocus	T–14	2.00		110 to 120	I, III
Projection	2CC–8	300	7,200	None	A.c. or d.c.	25	S.c. bayonet	T–8½	2.25		110 to 120	I, III
Projection	2CC–8	300	7,350	None	A.c. or d.c.	25	Prefocus	T–10	2.70		110 to 120	I, III
Projection (biplane)	C–13–D	300	7,050	None	A.c. or d.c.	25	Prefocus	T–10	3.10		110 to 120	I, II, III
Projection (biplane)	C–13–D	400	9,600	None	A.c. or d.c.	25	Prefocus	T–10	3.50		110 to 120	I, II, III
Projection (biplane)	C–13–D	500	12,200	None	A.c. or d.c.	50	Med. screw or prefocus	T–10	3.50		110 to 120	I, II, III
Projection	C–13	500	13,000	None	A.c. or d.c.	50	Med. screw or prefocus	T–20	2.20		110 to 120	I, III
Projection	C–8	108	2,400	Transformer to 6 v.	A.c.	50	Med. screw or prefocus	T–10	2.05		6	II
Ribbon filament vertical	SR–8	108	2,000	Transformer to 6 v.	A.c.	50	Med. screw or prefocus	T–10	6.25	21	6	II
Ribbon filament horizontal	SR–6A	108	2,000	Transformer to 6 v.	A.c.	50	Med. screw or prefocus	T–10	6.25	21	6	II
Mercury discharge tube	H–4	100	3,200	Special transformer	A.c.	1000	Ad. med.	T–10	9.50	10		II
Mercury discharge tube	H–6	1000	65,000	Transformer and other acc.	A.c.	75	3/16 Sleeve brass	T–2	9.00	300	1000	II
G. E. Photomicrographic	C–2	330		Transformer to 11 v. 30 A	A.c.		Bipost	PS–22	10.50	20–26	11	II

* Candles per square millimeter.

The Biplane Filament Lamp. In overlooking the possibilities of this powerful light source, the photomicrographer really misses something. The 400-watt lamp is rated at 9600 lumens, and this high lumen output can be utilized to good advantage when many filters are required. Because of the staggered arrangement of the two planes of filaments, as shown in Fig. 58, No. 13D, Sec. 28, it is possible to choose any method of illumination with this illuminant even with a long-focus lens at the lamp. Its life is rather short unless used with a forced draft; however, even without a forced draft it can be surprisingly useful if care is taken to snap off the light as soon as the eye is removed from the microscope tube. If long examination of the specimen is required prior to photography, the preliminary work should be done with a lower-power lamp, the biplane lamp being brought into use only for actual photography. The 400-watt lamp is rated at 9600 lumens, and the 500-watt lamp at 12,200 lumens. Both are mounted in a T-10 bulb and so can be accommodated in most lamp houses. They are listed at $3.50.

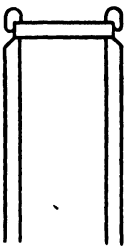
The V-Shape Filament Lamp, 110-Volt. This lamp takes a bayonet socket; it may be had in single or double contact base; it has a spherical or tubular bulb and is somewhat smaller than the 100-watt lamp listed above. This lamp has the advantage over many other smaller lamps that it can be used directly on the 110-volt line. It is inexpensive and convenient to provide illumination by incident light. It may be burned at any angle. As with all lamps, the chief advantage is the low cost of operation and replacement; the main disadvantage is the low lumen output. Laboratory life, about 50 hours. Price, 35–50 cents. Base, bayonet, single or double contact. Bulb, P.

The Household Electric Lamp, 110-Volt. In the interest of economy, the ordinary household tungsten-filament lamp may be successful for illumination by Method I. However, owing to low light intensity it may restrict the use of filters. The filament spreads over a bigger area than in the projection lamp, and less light reaches the lamp lens to be delivered on the ground glass. If the lamp is of high power, it may be too big to fit the lamp house, but in spite of these drawbacks it decidedly has uses in visual work if not for photomicrography.

The 6-Volt C-8 Filament Lamp. Figure 58, Sec. 28, shows a close-coil filament, No. 8. Such a lamp can be operated on the lighting circuit only in conjunction with a transformer. This means that alternating current must be available since it is very wasteful to have such a lamp connected with a rheostat on either direct or alternating current. If desired, it can be operated on storage batteries, or three three-cell batteries of the automotive type will answer very well. They

should be connected in parallel to divide the current draw. This lamp requires a stronger light-collecting lens than the projection lamp for conditions as outlined in Method II, because, under these conditions, the incandescent portion must be enlarged before it can fill the front lens of the microscope condenser with an image of the source. The image of the filament then presents a nearly unbroken ribbon of light to fill the front lens of the microscope condenser. Both Spencer, and Bausch and Lomb, make a lamp house that handles this lamp very nicely. Since the lumen output is low, this lamp does not lend itself particularly well to illumination by Method I; nevertheless, like any of the lamps mentioned, it can be so used if desired. Current required, alternating. Burning position, optional. Lumen output, 2400. Auxiliary equipment, a transformer delivering 18 amperes at 6 volts. Spectral distribution of energy, continuous spectrum similar to the projection lamp. Base, either medium screw or prefocus. Bulb, T. Life, about 50 hours. Price $1.80.

The Tungsten-Ribbon-Filament 6-Volt Lamp. Figure 59 shows the tungsten-ribbon-filament type of lamp. This 108-watt lamp should be used in the same way as the C-8. It will give a good even illumination when used for Method II. The horizontal distribution of radiant energy is similar to that of other incandescent tungsten-filament lamps as shown in Fig. 57, Sec. 28. There will be no trouble from bright spots caused by local heating of the filament. This lamp, and the C-8 filament 6-volt lamp, are excellent for vertical illumination and for dark-field work. However, neither one is brilliant enough for certain work on colloids; for such work an arc lamp is needed. As already stated, the ribbon-filament type of lamp, and some others, necessitate a transformer, and this limits the power source to alternating current; also a light-collecting lens of about 2- to 2½-inch focus is required. A short-focus light-collecting lens with its small diameter, which could be made interchangeable in the lamp house with the long-focus lens with its larger diameter would be a boon to the photomicrographer who may often have occasion to change from one type of illumination to another. Lumen output is given as 2000. Auxiliary equipment, transformer to deliver 18 amperes at 6 volts. Laboratory life, about 50 hours. Cost, $6.00. Burning position, base down or slightly tilted. Bulb shape, T.



Ribbon Filament

FIG. 59. The ribbon-filament type of construction. As shown it is classified as SR-8. If the ribbon is vertical the type is known as SR-6A.

The H4 Lamp, Capillary Mercury-Vapor Tube. The General Electric Company has developed a line of mercury-vapor discharge tubes of the capillary type. The tube is of quartz with an enveloping glass bulb. The generated pressures when the lamps are lit are from 0.9 to 80.0 atmospheres, depending upon the model. These lamps are known as the H series, and the H4 in particular is especially useful for photomicrographical work, or for visual work where light of certain wavelength is needed. Table XI shows the distribution of radiant energy for the H4 lamp. The tungsten filament gives a continuous spectrum; the mercury lamp gives a bright-line spectrum.

Table XI

Spectral Characteristics of the H4 Lamp

Relative Ultraviolet below 3800 A	4358 A Blue		5461 A Green		5780 A Yellow		Continuous Visible		Relative Infrared
	Lumens	%	Lumens	%	Lumens	%	Lumens	%	7600
1.6	25	0.7	1735	49.6	1255	35.9	485	13.8	14.7

In order to define clearly the difference in the radiant emission of the two types of lamp, Fig. 60 draws a comparison between these spectra. As might be expected, the H4 lamp is very rich in blue light and in the near ultraviolet radiation. The spectrum is broken and the positions of the chief points of emission are designated on the abscissa of the curve. The H4 lamp is excellent for fluorescent work using the 365-mμ line. The various lines can be separated rather efficiently by the proper light filters, and the effect is nearly that of monochromatic light. It is possible to use the H3 lamp, the forerunner of the H4, in the socket for the 250-watt projection lamp, that is, a medium screw base. The H3 lamp, although officially off the market, can still be obtained at this date of going to press. This information may be of interest to those who, having bought the original equipment for the H3 lamp, do not care to change to the H4. The H4 lamp can be used in any position. It works moderately well with illumination by Method II, even with a 4-inch light-collecting lens if medium-power objectives are used. For high-power work the 2-inch light-collecting lens will give better results, for the reason that the larger condenser aperture can be filled with light only when the short-focus lamp lens is used. The H4 lamp is shown in Fig. 61.

In order to take full advantage of all the short-wave radiation offered by the H4 lamp, a quartz microscope condenser and a quartz

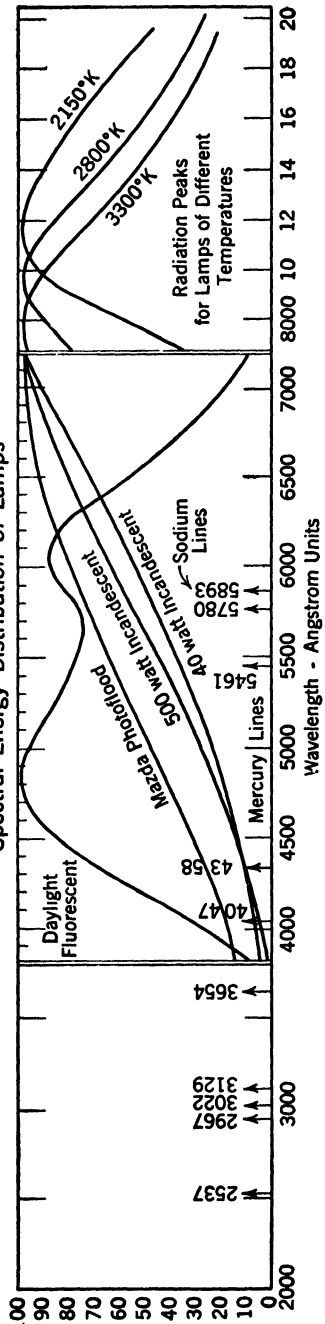


FIG. 60. Spectral energy distribution of lamps. Acknowledgment is made to the General Electric Company.

light-collecting lens are required; also the mirror should be aluminized or be replaced by a quartz prism. As an alternative, the microscope can be placed in a horizontal position. This lamp has one rather serious disadvantage for ordinary work; it takes three minutes or more to heat up and reach a standard operating condition, and when shut off it must be allowed to cool for several minutes before it can be started again. The warming-up process then has to be repeated to relight the lamp. The H4 lamp can be operated on 110-volt alternating-current circuit; it is rated at 3000 lumens; it is provided with a ballast transformer, maintains a voltage drop of 130 volts, draws a little less than 1 ampere of current, has a laboratory life of 1000 hours, and costs $9.00. Burning position optional. Base, ad-medium (an adaptor can be used in the medium socket). Bulb shape, T. Lamp pressure approximately 8 atmospheres.

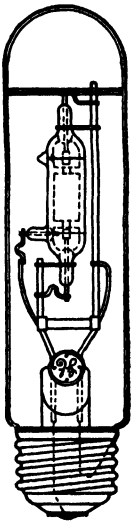


FIG. 61. This drawing shows the construction of the H4 mercury-vapor-tube lamp. The inner tube is made of quartz and the outer envelop of glass. Acknowledgment is made to the General Electric Company.

The H6 Lamp. Capillary Mercury-Vapor Tube. Another lamp of the same series is the H6, shown in Fig. 62, the most recent addition to this important line of illuminants. It is rated at 1000 watts, and it must be water cooled. The lumen output is somewhat greater than that of the 35-ampere low-intensity carbon arc. The surface brightness, though less than that of some of the carbon arcs, is very high, being given by the General Electric Company as 195,000 candles per square inch, one-third the brightness of the sun. Although it undoubtedly has its place in microscopical research work, at the present time it is somewhat expensive for the average laboratory to install, the auxiliary equipment alone amounting to over $100, and because of the need for water cooling it requires special mounting. For high-power microscopical work, high surface brightness is more desirable than a large lumen output; therefore the carbon arc, even of low ampere draw, is recommended as probably a more efficient lamp when only high light intensity is looked for. It would seem that the H6 would be particularly good for projection, since much of the heat is absorbed by the water jacket. At the present time, there is no lamp house on the market to accommodate this lamp; however, Cargille[8]

[8] R. P. Cargille, 118 Liberty Street, New York City.

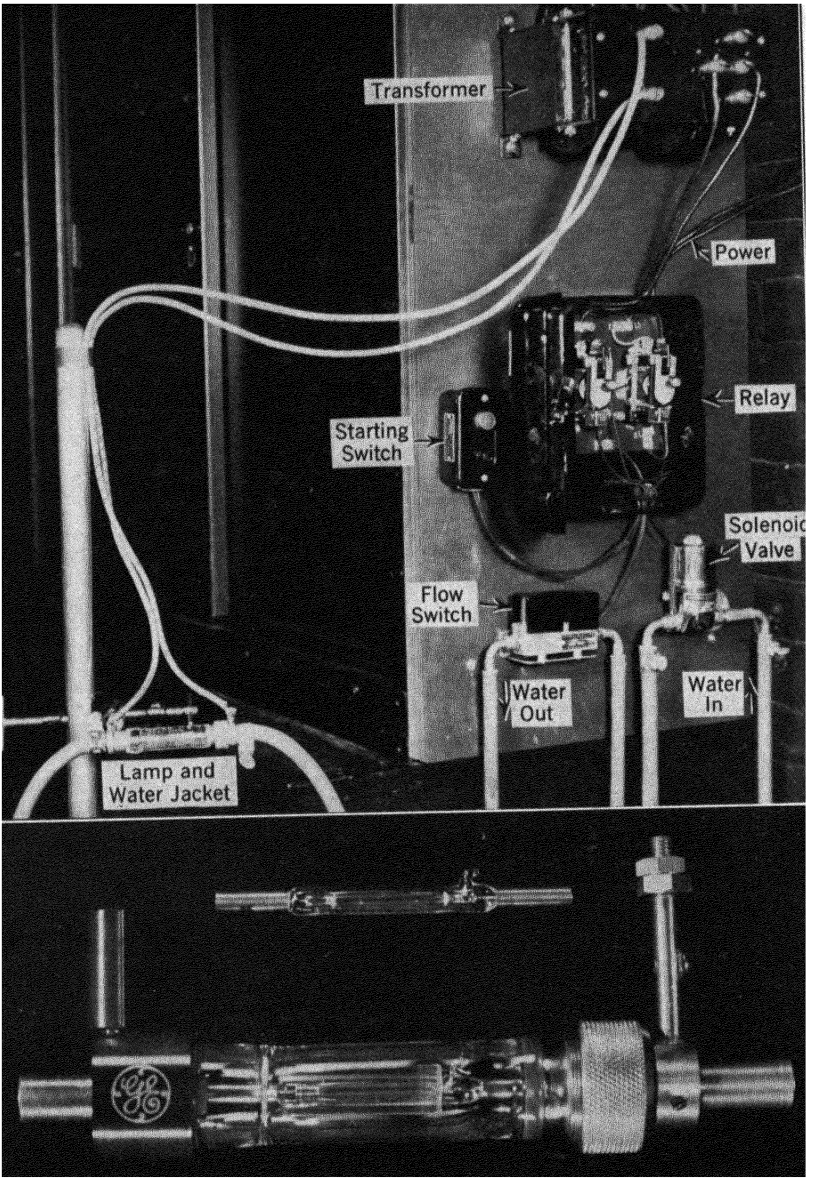


FIG. 62. The H6 lamp and some of the electrical equipment necessary to operate it. Acknowledgment is made to the General Electric Company.

has been experimenting with special apparatus and is developing a specially designed lamp house for it. The H6 is rated at 1000 watts, 65,000 lumens; the distribution of spectral energy is given in Table XII taken from the General Electric booklet "Mazda Lamps." Auxiliary equipment is listed by the General Electric Company. Rated laboratory life, 50 hours. Cost, $9.00. Burning position, horizontal. Base, $\frac{3}{16}$-inch brass sleeve. Bulb, T-2. Vapor pressure 80 atmospheres.

Table XII

Distribution of Spectral Energy of the H-6 Lamp

Band	Lumens	Per Cent
3800–4200 A	25	0.04
4200–4600	960	1.5
4600–5000	2,030	3.1
5000–5400	6,070	9.3
5400–5700	33,700	51.8
5700–6050	19,700	30.4
6050–6500	2,290	3.5
6500–7600	225	0.36
	65,000	100.00

The Photomicrographic Lamp. Figure 63 illustrates a new type of lamp made specially for photomicrographic work by the General Electric Company. It is a combination of mercury-vapor discharge and an incandescent tungsten electrode. It is a 330-watt lamp operating on 11 volts. The current draw is high, and presumably for this reason the company has mounted it on a bipost base, but its large size and the bipost base prevent its use in an ordinary lamp house equipped with a screw base. It is a good lamp for illumination by Method II. Bausch and Lomb use it on some of their metallographic equipment, and they have designed a special lamp house for it. In use the incandescent portion, the anode, which is a small globe of tungsten, gets very hot; in fact, its temperature approaches the melting point of tungsten. It therefore furnishes light of a higher color temperature than the regular tungsten filament lamp.

Owing to its high operating temperature this lamp is rather sensitive to temperature increase, and if it is operated in a poorly ventilated lamp house the electrical resistance will rise. Under such conditions, owing to the electrical characteristics of the transformer, the increased lamp resistance will cause the voltage to rise and the current to drop.

There would probably be considerable trouble in adapting an ordinary microscope lamp house to take this lamp. The figure for the lumen output is not available. The life of the lamp is several hundred hours, depending upon how it is used. Cost, $10.50. Auxiliary equipment is required. It is built to draw 30 amperes at 11 volts. Base, bipost. Bulb, PS.

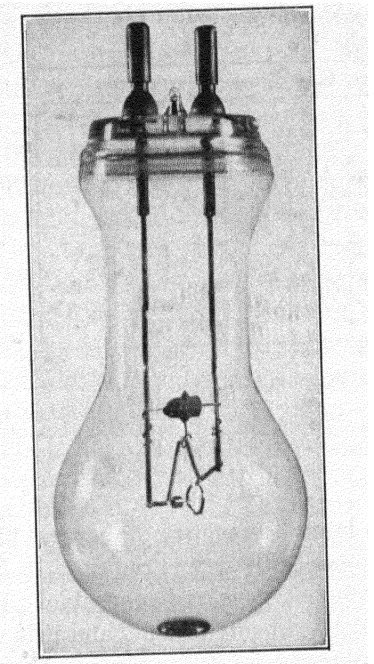


FIG. 63. The photomicrographic lamp. Acknowledgment is made to the General Electric Company.

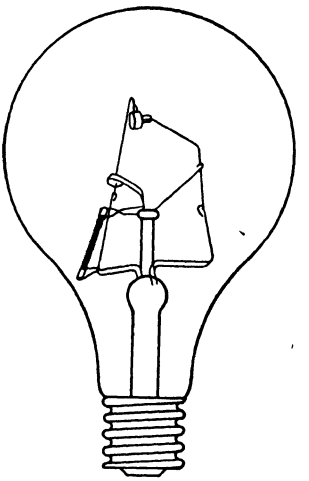


FIG. 64. The pointolite lamp. A tungsten arc.

The Tungsten Arc Lamp. The Pointolite, Punktiform, or tungsten arc, as it is sometimes called, was first made in Germany and later in England. It is similar to the General Electric photomicrographic lamp. The incandescent portion is a small tungsten electrode. It operates as an arc and therefore is best used on direct current. Alternating-current tungsten arc lamps are available, but they are less satisfactory than the direct-current type. Figure 64 is a drawing of the Pointolite lamp showing its similarity to the previously mentioned photomicrographic lamp.

This lamp is excellent for illumination by Method II, and its longer

life makes it cheaper than the tungsten-ribbon-filament lamp. However, like the H lamps, it has the disadvantage that when it is switched off it cannot be relighted until it has had time to cool. It can be supplied by Palo-Myers, Inc.[9] It is sold with a fixed resistance unit for either 115- or 220-volt lighting circuits. The light is very rich in the blue, and even when sharply focused on the specimen it generates very little heat. Auxiliaries, a fixed resistance. Life, several hundred hours. Cost, $11.50. Base, medium screw.

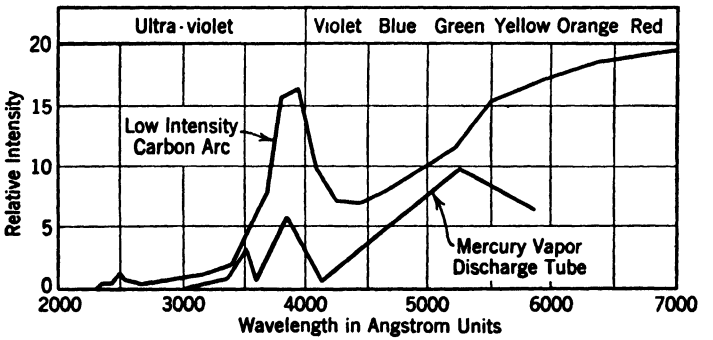


FIG. 65. The relative light distribution of a carbon arc, and also the mercury high-pressure vapor tube. Acknowledgment is made to the National Carbon Company.

The Carbon Arc. The carbon arc lamp is essential for several kinds of microscopical work. Whenever great brilliancy is required to light a very thick or partly opaque specimen, it is indispensable. It is practically a necessity for work with the slit microscope, and it is useful for certain investigations with polarized light, for dark-field work when contrast is lacking between the subject and its surrounding medium, and for fluorescent work below the 3650 line. Although the H4 lamp can be used for work in the near ultraviolet region, the carbon arc lamp is needed for ultraviolet photomicrography with the 2750 A line. It is also used to good advantage with monochromators. Figure 65 indicates the intensity of the carbon arc plotted against wavelength and compared with the mercury-vapor lamp.

There are three kinds of carbon arcs: the flaming, the high-intensity, and the low-intensity arcs. The flaming arc is obtained by means of a carbon electrode charged with flame-supporting material. The principal source of light is the arc stream itself, large in area and relatively low in intensity. Little has been done with it in connection with the illumination of the microscope.

[9] Palo-Myers, Inc., 81 Reade Street, New York City.

The high-intensity carbon arcs are the most brilliant sources of light known. With the negative electrode, or cathode, of the proper size, and in the proper position relative to the positive electrode, or anode, it is possible to build up pressure in the arc crater so that its temperature is increased several thousand degrees. Under these conditions the surface brightness of the arc crater has been increased from about 175 candles per square millimeter for the low-intensity arc to more than 1000 candles per square millimeter for the high-intensity one. However, some serious difficulties arise in adapting this arc to the microscope. It is more expensive than the low-intensity lamps now available for microscopical work, because a current-draw of 35 to 100 amperes is needed, and also because the only suitable lamp mountings on the market have been designed chiefly for projection work in large auditoriums, and therefore their cost is high. But, since the importance of high-intensity light sources for certain types of microscopical work cannot be overestimated, it is to be hoped that the high-intensity arc will be adapted to the microscope.

On the other hand, the center of the crater of the positive carbon in the direct-current low-intensity arc offers a light source of very high intensity, very well adapted to use with the microscope. The electrode tips of the alternating-current type are very brilliant but considerably below the brilliancy of the positive crater face in the direct-current type. These arcs are in general use for microscopical work, and a lamp house suitable for them can be obtained from most microscope manufacturers. The small lamp houses made by Bausch and Lomb, Leitz, Zeiss, and others have proved very satisfactory, and are recommended. One of these is shown in Fig. 36, Sec. 15.

The carbon arcs for the microscope are built to operate on approximately from 5 to 15 amperes of current. If the lower current will not bring the crater of the positive carbon up to the limiting brilliancy of about 175 candlepower per square millimeter, increase of current will give substantial increase of light. However, once the limiting crater brilliancy is reached, further increase of current results only in a slightly larger crater and more rapid carbon consumption without proportionate increase in surface brightness. Increase in current beyond the carrying capacity of the carbon results in "spindling," with actual reduction of crater area and light output.

Since carbon electrodes are consumed during operation they must be mounted in a holder that will permit them to be easily adjusted, or fed forward, as they are consumed. The mechanical type of feed, run by clockwork or motor, is far superior to the hand-fed type, which is extremely inconvenient for photomicrographical work. The rate of

feed in the mechanical type can be synchronized with the consumption of the carbon by installing an ammeter in the line and adjusting the carbons at the right distance apart to enable them to be consumed at the rate at which they are being fed. The reading of the ammeter is taken when the correct setting of the carbons has been determined; this reading can then be used as a standard for future settings of the gap between the two electrodes. The smaller the gap, the higher will the ammeter read.[10]

The unsteadiness once experienced with arc lamps for microscopical work has been overcome by improvements in both the lamps and the carbon electrodes. Modern lamps, with proper ventilation and accurate feeding mechanism and equipped with the improved carbons now available, give a steady light of great brilliancy and uniform quality.

The sizes of the carbons used in several well-known makes of lamps are given in Table XIII.

Table XIII

Size of Carbons for Various Lamps, Current Draw, and Voltages

Make	Current	Arc amperes	Approximate Arc volts	Size of Carbons
Bausch & Lomb	D.c.	5	55	8 mm × 6 in. Cored positive 5.6 mm × 6 in. Cored negative
	A.c.	10	45	6.4 mm × 6 in. Both holders
Zeiss	D.c.	5	59	8 mm × 8 in. Cored positive 6 mm × 4½ in. Cored negative
	A.c.	10	53	7 mm Solid
Leitz	D.c.	10	55–60	7 mm × 150 mm Cored positive 5 mm × 150 mm Cored negative
	A.c.	15	45	6 mm Cored positive 6 mm Cored negative

Since many preparations on the microscope stage may be sensitive to temperature changes, when the carbon arc is used these changes must be guarded against. Overheating can be fairly well eliminated by having a diffusing plate at the lamp and illuminating by Method I, but if the maximum amount of light is desired, Method II must be used.

[10] The ammeter is connected in series with one of the electrodes so care should be exercised to prevent the electrodes from touching each other while trimming or starting the arc. The best way is to install a switch so that the ammeter can be thrown into the circuit only when desired; otherwise the short circuit which is ordinarily used in starting the arc may ruin the ammeter.

To absorb the excess heat, water cells are indicated. These can be inserted in the illuminating train, as far away from the lamp as possible, the usual position being just in front of the glass filters. Such cells can be made from optically flat glass which can be removed for cleaning or to permit the insertion of heat-absorbing glass of the Corning type. This glass has a high coefficient of expansion; when it is mounted in a water cell, its temperature is changed slowly and evenly. Water cells, even without heat-absorbing glass, remove a considerable amount of the ultraviolet radiation and a good deal of the infrared; for this reason they should not be used for fluorescent or ultraviolet work. The larger arc lamps call for two water cells, but for the smaller 4- or 5-ampere arcs one cell is generally sufficient. These cells should be about 5 cm thick, when measured inside from wall to wall. They should hold at least 250 cc of water.

An arc lamp which operates on direct current should be selected, since those for alternating current do not provide as brilliant a source of illumination. If the power line furnishes alternating current it can be rectified by means of a tube rectifier similar to those used in battery repair shops. As the current draw of the small arc is seldom much over 5 amperes and it operates on about 60 volts, a small rectifier, as suggested, will be sufficient. The tube in the rectifier is comparatively long lived, but it will need to be replaced occasionally; the cost of such replacement may be around $8 to $9.

To give a comparative idea of the brilliancy of the light sources of the various carbon arcs and other sources, Table XIV was prepared by the National Carbon Company; thanks are extended to them for the privilege of publishing it here.

The Multifilament 6- to 8-Volt and Other, Less-Used Lamps. The multifilament lamp of Leitz is excellent, but as it has a special base it cannot be used in American lamp sockets without a special adaptor. Since it works on 6 to 8 volts, and this calls for an auxiliary piece of equipment such as a transformer, it must be used on alternating current. Because of the number of coils which give it a large incandescent surface, it is used with a long-focal-length light-collecting lens. It is similar in effect to the biplane 400-watt projection lamp, except that the filaments are all in one plane and the lumen output is low.

For very exact measuring of refractive index or for work with the petrographic microscope a strong sodium light may be needed. Such lamps are made by the General Electric, Reichert, and others. The high-tension spark between cadmium or zinc electrodes is used by Lucas[11] as a source of light of very short wavelength.

[11] F. F. Lucas, *Photomicrography and Technical Microscopy* (see his chapter in Henney and Dudley, *Handbook of Photography*, 1939).

Table XIV*

Comparative Brightness of Light Sources

Light Source	Candlepower per Square Millimeter	Source of Data
Tungsten filament of incandescent lamps 40-watt, clear-bulb vacuum type	2.06	*International Critical Tables*
900-watt, clear bulb, gas-filled special	26.6	*International Critical Tables*
Magnetic arc stream	6.2	Mott
Flame arc stream	8 0	Mott
Positive crater of cored d-c. low-intensity carbon arc	155–175	*J. Soc. Motion Picture Engineers*
Crater of a-c. high-intensity carbon arc	280	*J. Soc. Motion Picture Engineers*
Positive crater of non-rotating, d-c. high-intensity carbon arc	380	*J. Soc. Motion Picture Engineers*
Positive crater of rotating d-c. high-intensity white flame carbon arc	400–800	*J. Soc. Motion Picture Engineers*
Positive crater of d-c. super-high-intensity carbon arc	1000–1200	National Carbon Company
Sun at zenith	920–950	

* Printed with the permission of the National Carbon Company, Inc.

Sec. 30. The Comparison and Selection of Lamps. The selection of a microscope lamp depends largely on the spectral quality of the illumination required and the necessary intensity. The means provided for housing the lamp must also be considered. Since the color temperature of the various incandescent filaments does not cover a great range of values, and the term is scarcely applicable to mercury and carbon arcs, color temperature does not have to be considered when making a selection. The decision should be influenced by the amount of light that will be required at the object field, the chromatic values of that light (that is, whether many filters will be needed to obtain the desired colors), the intensity of the spectral distribution of the light energy, and the expense of maintaining the illumination.

If visual examination with low or medium powers, with occasional recourse to high power, were all that would ever be demanded, then illumination could be effected entirely by Method I, and the simplest and cheapest form of illuminant would be satisfactory. Lamps for such simple work include the 100-watt projection bulb, the small C-2 filament-type lamp, or even an ordinary household bulb of 60 or 100

watts. Monochromatic or any other filters could probably be used successfully for visual work if only low light levels were needed, and the expense of operation would be very low since all these lamps work on 110 volts, without transformer or rheostat.

When photomicrography is to be considered, and it is still deemed possible to employ Method I for illumination, it would be desirable to use a lamp of greater power, such as the 250- or 300-watt projection lamp or even the 500-watt lamp. Both the initial cost and the operating expense will then be greater. When the utmost in resolution is called for, the illumination must be by Method II. This method requires a lamp of the ribbon-filament type (6 volts), C-8 filament (6 volts), 400-watt projection lamp, biplane filament (forced draft necessary), tungsten arc (special transformer or resistance), or possibly the mercury arc (H4 with special transformer). The lamps which operate on voltage other than 115 or 220 always entail the expense of auxiliary equipment. The C-2 filament, 6-volt lamp is the least expensive to buy, and since its power rating is only 108 watts it is comparatively inexpensive to operate. The others mentioned are three or four times more expensive to buy, and they generally use more electric current.

It is quite likely that no one lamp will efficiently answer all the requirements of the microscopical laboratory, and the choice of several should be at hand to take care of the changing demands. A properly arranged lamp house will make it possible to use all the lamps mentioned, and a long-focus lens which is interchangeable with one of short focus will cover the various methods of illumination which are so necessary for good photomicrography. If the interchangeable lens feature is not available two lamp houses will be needed, one to mount the long-focus and one to mount the short-focus lens.

If work is to be done in the ultraviolet, the spectral distribution of light energy must be considered; and if the work is to be carried on at some one definite wavelength, the necessary separation of a narrow band of the spectrum can best be obtained if the source has a bright-line spectrum, such as can be had with the mercury-vapor tube H4. This lamp, which affords a light rich in the blue, is extremely valuable when the utmost in resolution is desired and when deep blue filters are required. It is best for illumination by Method II. Figure 66 compares the spectral distribution of energy of several gaseous discharge lamps which may be of interest.

Sec. 31. The Proper Use of Lamps. Improper operation of an electric lamp will either lengthen or shorten its life. If the lamp is operated at a voltage that is lower than is called for by its rating, its

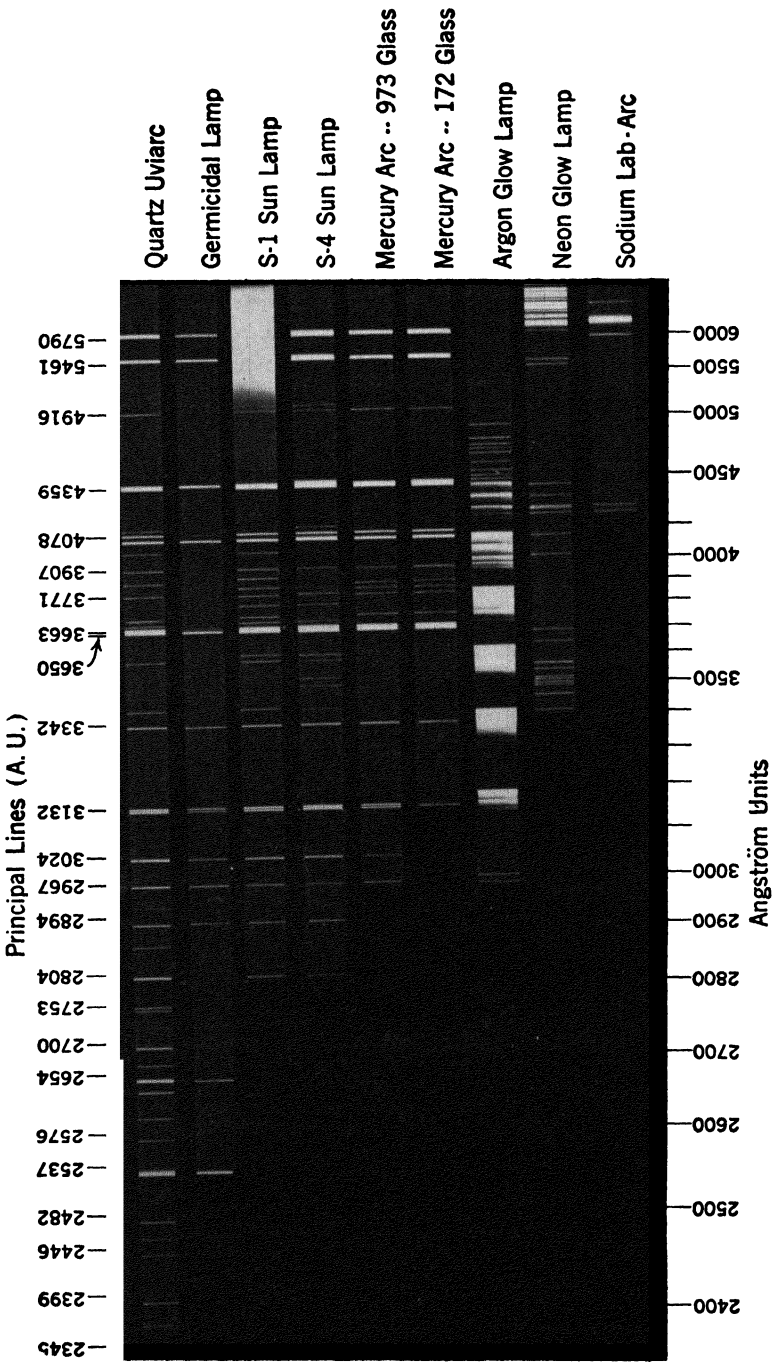


FIG. 66. Spectra of various vapor discharge tubes. (By courtesy of General Electric Company.)

life will be lengthened, but it will develop less light, and the light will be stronger in the red than it should be. When the voltage on the lamp circuit is higher than is necessary, the lamp life will be shortened, the lamp will develop more than its normal rating in light flux, and the light will be overstrong in the blue. To operate an incandescent lamp in a poorly ventilated lamp house will shorten its life; with a gaseous-discharge tube increased resistance due to heat will cause less current to flow, the lamp will not be operating efficiently, and the intensity of the light will be reduced.

It is essential that lamps be operated in the position designated on their container. The projection lamps are intended to be operated base down; if they are mounted at an angle or horizontally, the filament is likely to sag and break. However, this type of lamp, as well as the ribbon-filament lamp, must, as a rule, be tipped slightly for microscopical use. Within certain limits this tipping of the lamp will be necessary in order to use it effectively, but it is advisable to keep it as nearly vertical as possible.

There is probably some advantage in connecting a 100- to 200-ohm slide-wire resistance in series with the lamp circuit when tungsten-filament lamps are used. Failure in such a lamp usually occurs when the current is first turned on; the first surge of current tends to snap the cold filament, electrical resistance being less with cold than with hot conductors. With a resistance unit, the first shock to the filament can be lessened considerably; the slide contact on the resistance can be brought into position for full current flow after the lamp is lit and the filament has been warmed. If expedient, the lamp might be operated at a voltage considerably less than normal while preliminary work is carried on; later, when more illumination is demanded for photomicrographic or other work, it can be increased by operating the rheostat.

The ideal way to install a variable resistance is to place a voltmeter across the lamp terminals; this can be permanently connected. The resistance unit, also permanently connected, is in series with the lamp. Adjustment of the rheostat will then be indicated by the voltage drop across the lamp, and precise control can be maintained. The Variac transformers[12] are particularly good for lamp control.

When selecting any type of resistance, the expected maximum current flow must be known, and the resistance unit must be adequate for it. It is current that burns out a lamp or resistance unit and not voltage. For instance, a 500-watt lamp on a 110-volt circuit will draw 500/110 amperes of current (amperes $\times$ volts = watts). Thus

[12] General Radio Company, Cambridge, Mass.

the 500-watt lamp will require a resistance designed for a flow of about 4.5 amperes of current — say 5 amperes. Such a slide-wire rheostat will handle any lamp with a current flow not exceeding 5 amperes. Temporary overloads should not burn out such a rheostat unless they are excessive, or are maintained too long; therefore a safety factor need not usually be figured, for it is already taken care of in the design of the resistance.

LABORATORY WORK

Exp. 1. Control of Glare by Means of a Field Diaphragm. Focus the microscope on a specimen which gives considerable glare; diatoms mounted in balsam, or some of the fine clays such as are used for filter aids, or test slide 6 would be good for this purpose. Have the field diaphragm at the lamp wide open; use an 8-mm objective; notice that as the diaphragm at the lamp is closed the glare becomes less. The diaphragm may be closed until only a small portion of the field is visible. With a well-restricted field it is often possible to increase the aperture of the condenser diaphragm.

Exp. 2. The Use of the Substage Lamp. Remove the microscope mirror; place substage lamp under microscope condenser. Focus the microscope on test slide 3, using an 8-mm objective and 10× to 15× ocular. Note the strong glare on small particles. Observe that a small cone of light is necessary for a fairly clear image. Replace the microscope mirror, and place the substage lamp in front of the microscope; insert a 3- or 4-inch light-collecting lens in the light train, having the lens at its focal length from the lamp; direct the light beam on the center of the microscope mirror. Examine the field of view having the same specimen as before. Notice that the field of view is improved. Drop a small cardboard diaphragm over the light-collecting lens; have the opening slightly smaller than is necessary to fill the field of view with light. A still further improvement in the clarity of the microscope image will probably then be noticed. The microscope condenser must be focused on the diaphragm at the lens.

Exp. 3. The Difference in Illumination between Method I and Method II. Set up the microscope and lamp for illumination by Method I, using test slide 2. Put a high-power objective on the microscope, and notice the resolution of the finest particles. Change to Method II, and notice that the resolution and clearness of the images of the small particles have improved. The condenser should be oiled to the slide for this test. Also notice that if the condenser is not oiled the resolution will suffer with either method of illumination.

Exp. 4. Reason for Using a Light-Collecting Lens of Proper Focal Length. Illuminate the microscope by Method I; focus on a test slide of finely ground material such as test slide 5. Remove the diffusing plate at the lamp house and examine the rear focal plane of the objective. The filaments of the lamp will be seen, and the back lens of the objective will not be evenly filled with light. Insert a C-8 filament or tungsten-ribbon-filament lamp, and again examine the rear focal plane of the objective. If the light-collecting lens on the

lamp is the one that was used for illumination by Method I it will have a focal length of about 4 or 5 inches, and the image of the coil or ribbon filament will not fill either the back lens of the objective or the front lens of the condenser with light.

Exp. 5. The Effects of Filling the Back Lens of the Objective with Light. Use test slide 1. Focus the slide carefully; illuminate by Method I. Notice the even appearance of the field from side to side. Be sure that the structure of the ground glass does not show in the field of view (Sec. 83). Remove the ground glass at the lamp, and inspect the rear focal plane of the objective; this field is the exit pupil of the condenser and objective taken together. The filament of the lamp will show in the focal plane. The used part of the objective will be only the part lit by the image of the filament. Return the ocular, and note the appearance of the field of view (insert a neutral filter to compensate for the removal of the ground glass). As a rule, the field will appear as though it were streaked with light, and it may appear unevenly lit, depending upon the chance position of the image of the lamp filament. Diffraction rings sometimes noted are due to the lessening of aperture.

This experiment shows that the source of light, be it diffusing plate, incandescent filament, arc, or any other source, must be sufficiently large to fill the effective aperture of the condenser; otherwise the objective will not be filled with light, and its maximum aperture will not be available. Section 90 deals with ways in which this can be done.

Exp. 6. Centering the Source of Light on the Microscope Mirror. Arrange the lamp for illumination by Method I. With the ground glass in place, direct the light at the microscope mirror. Remove the ground glass, and, without disturbing the setting of the lamp, focus it so that the approximate position of the image of the lamp filament can be determined. Frequently it will be found considerably off center from the mirror. Since there is some specular transmission with ground glass, the specimen will not be evenly lighted. Such off-centered illumination cannot be detected by inspection of the microscope mirror unless the ground glass is removed. Measurements made with the photoelectric cell will show definite decrease in effective light intensity unless the image of the lamp is properly centered to the mirror.

Exp. 7. The Effect of Uneven Light Source on the Aperture of the Objective. With the lamp arranged for illumination by Method I, carefully focus test slide 3 and examine the image in the field of view. Remove the ground glass, inserting the necessary neutral filter to compensate. Study the image, and note the different appearance of the images. The field is often streaked with light. Examine the rear focal plane of the objective; note that it is not filled with light but shows the image of the lamp filament. While looking through the microscope tube, drop in the ground glass at the lamp; note the immediate difference at the rear lens of the objective, and, after returning the ocular, again inspect the image. The rear focal plane of the objective must be filled with light for good observation. In this experiment the specimen on the stage of the microscope should be free from glare, so that the condenser diaphragm can be wide open. The condenser should be focused.

Exp. 8. The Necessity for Obtaining Proper Orientation of the Specimen. Use a rather coarsely woven specimen of textile. Illuminate with incident light from above the stage. Examine the specimen first with only one lamp, and, if the microscope has a revolving stage, turn the specimen until the warp lies parallel to the direction of the illuminating beam. Turn again until the filler lies in this direction, then turn 45°, and, while doing so, notice the changes produced in the appearance of the image. Determine which position demonstrates the textile to the best advantage, bearing in mind that a second or third lamp can be used. In this way, by first examining the specimen with only one lamp, the best position for the other lamps can be predicted. For the type of specimen indicated, two lamps are generally enough; their position will probably be 90° apart, and their beams parallel with the threads of the specimen.

QUESTIONS

1. What are the four requirements for good illumination?
2. Why should the illuminating system be capable of filling the rear lens of the objective with light?
3. Why is a strong light source needed?
4. Is the use of filters essential for good illumination?
5. How does the size of the light source affect the microscope image?
6. State the most important requirements for a lamp house.
7. What lamp is the most generally useful?
8. Is the mercury-vapor tube a suitable lamp for routine microscopical work?
9. For what purpose is the H4 lamp specially recommended?
10. When is the arc lamp necessary?
11. What precautions should be taken when using the arc lamp?
12. For what general type of work is the ribbon-filament lamp recommended?
13. What precaution should be observed in using ribbon filament and other high-intensity filament lamps?
14. How can the small substage lamp be used with satisfaction?
15. What is the correct position for the ground glass in Method I?
16. At what point in the illuminating system should filters be used?
17. How many filters may be used at one time?
18. Describe the preparation of a small piece of ground glass.
19. How far from the microscope should the lamp be placed?
20. Why is this a suitable lamp distance?
21. On what basis can microscope lamps be compared?
22. What is a lumen?
23. What would be the candlepower of a lamp that produced 10 lumens of light on a square foot of surface 1 foot from the source?
24. How is the brightness of a surface expressed?
25. What is the essential difference between the spectral distribution of energy of the mercury arc and that of the tungsten-filament lamp?
26. Describe illumination by Method I.
27. Describe illumination by Method II.
28. Describe the use of each on a given specimen, naming the specimen.
29. What is the approximate brightness of the surface of a high-power tungsten lamp?

30. Why should the image of the lamp filament be centered on the microscope mirror?

31. How can the alignment of the microscope mirror with the axis of the lamp lens be determined?

32. How can the lamp be centered in the lamp house, with respect to the lens?

33. What is an excellent lamp for dark-field illumination when high light intensity is needed?

34. Make a small diagram showing the arrangement of two lamps and bull's eyes to illustrate above-stage illumination.

35. Is the orientation of the specimen of importance in arranging illumination by incident lighting? Why?

36. Describe the following three systems of lighting: vertical illumination, illumination with the Silverman illuminator, illumination with the Ultropak. Illustrate by diagrams.

37. How is bright-field illumination obtained by incident light?

38. Describe the arrangement of the lamp for use with vertical illuminators.

39. Should the lamp distance be changed when a high-power objective is used for vertical illumination, rather than one of very low power?

40. If there is variation in the lamp distance indicated in question 39, what is the cause of it?

CHAPTER III

LIGHT, LENSES, IMAGES, AND OBJECTIVES

Before entering very far into the study of apparatus, the successful operation of which is based almost completely on the manipulation of light, it is well to recall some of the fundamental concepts of light. To do this it is necessary, first, to set forth certain postulates upon which subsequent work will depend.[1] These assumptions are:

1. Light travels in a straight path unless reflected or refracted.
2. Light is transmitted by transverse wave motion.
3. Light is reflected from a surface at the angle at which it is incident.

The definitions of the following terms will now be discussed for the purpose of avoiding later ambiguity and possible confusion.

Sec. 32. Light. Light is a form of radiant energy which when it falls on the retina causes stimulation of the tiny nerve endings known as the rods and cones; through them and the optic nerve the visual centers of the brain are stimulated, the result being the sensation of vision. This is at least a physiological and psychological concept of light. Thus, strictly speaking, infrared and ultraviolet and all other forms of radiant energy cannot be classified as light because they are incapable of producing the sensation of vision in the brain of a human being. Such forms of energy, lying just beyond the range of the vision-producing rays, can be specified as infrared or ultraviolet radiation.

The above is a definition of light which is certainly limited in scope, since, if there were no human eyes in the whole world, there could be no vision, yet there certainly would be light. This paradox arises through considering the definition from a subjective rather than an objective point of view. Objectively, light can be thought of as wave motion, or as a disturbance in the ether due to electromagnetic

[1] The following works are excellent sources of reference for the microscopist who feels the need of a thorough understanding of applied optics; at least one of the volumes on geometrical optics should always be at hand. A. C. Hardy and F. H. Perrin, *The Principles of Optics* (geometrical optics), McGraw-Hill Book Company, 1932; James P. C. Southall, *Mirrors, Prisms, and Lenses* (geometrical optics), Macmillan, 1933; Robert W. Wood, *Physical Optics*, third edition, Macmillan, 1934.

waves from a source body. As an example, the electromagnetic radiation received from the sun is partly light and partly heat. That part of the radiation, of limited wavelength, which affects the eye and photographic plates and accounts for the phenomenon of photosynthesis is called light. This definition includes infrared and ultraviolet radiation. To avoid ambiguity, in this book light will be spoken of as limited to the radiation which affects the eye and accounts for vision, while ultraviolet and infrared will be referred to as radiation.

The Origin of Light. Vibration is, in general, the basis on which the modern theory of the origin of radiant energy rests. The magnitude of the wavelengths or frequencies of the radiation is determined by the nature of the vibrating source. An oscillating current impressed on a circuit may give rise to radiation known as radio waves. These are comparatively long, much longer than thermal or infrared radiation rays, and of low frequency. To produce such radiation the external energy must be supplied from a dynamo or battery. Part of the applied energy is lost in the form of heat radiation; more is lost in overcoming the resistance in the circuit; and the useful part is converted into radio waves or electromagnetic radiation, which is the same as visible light radiation in all but frequency and wavelength. Radiation of shorter wavelengths include, progressively, heat, infrared, light, ultraviolet, and finally cosmic radiation. All are included in the electromagnetic spectrum. Radiation which causes heat, infrared, and visible light is due to molecular or atomic vibration, while vibrations of the yet shorter wavelength are caused by excited states within the atom itself. In the visual part of the spectrum there is considerable overlapping; for instance, the bright-line spectra of gases are due to electronic vibration, or, according to the quantum theory, to the emission of energy as an electron passes from a greater to a lesser orbit (see Glossary). Also, when a body is heated, like the incandescent filament of a lamp, thermal radiation takes place, the cause of which is molecular and atomic vibration, which produces both heat and light. The mercury-vapor discharge tubes are so called because there is an electronic discharge at the cathode; the discharge functions as a conductor for current flowing from the anode to the cathode. The continually excited state of the mercury vapor accounts for the visible glow. The bombardment of the mercury atoms by the cathode discharge raises the electrons of these atoms to higher orbital levels; they absorb energy. From these abnormally high levels, or large orbits, the electrons drop to their original orbits while emitting light energy (quanta), the cycle being repeated as long as the external energy is supplied. The difference between the electronic emission of energy and

atomic or molecular emission is largely one of continuity. The vibration of the atom or molecule gives rise to an uninterrupted flow of electromagnetic waves of various lengths, while the discharge of quanta caused by displacement of electrons as in an energized gas gives rise to light rays of intermittent character and of a distinctly limited number of wavelengths. According to some authorities a quantum discharge takes the form of a group of waves.

Sec. 33. Propagation or Transmission of Light Energy. It is possible to conceive of the transfer of energy from one place to another by only two methods: one is some form of wave motion exemplified by water waves; and the other is the actual transfer of particles, body or bodies, exemplified by the lifting or falling of the hammer of a pile driver. Both these theories of energy transmission are of great importance, and both are generally accepted as applicable to light, according to modern theories of its origin as outlined in the preceding paragraph. The idea of wave motion offers a perfectly good basis for a theory for the rectilinear propagation of light, and the phenomena associated with the effects of optical instruments on light are most easily explained by this concept.

Christian Huygens, or Huyghens (1629–1695) developed the hypothesis that light was propagated by longitudinal waves, in the same fashion as sound; but it was not until Thomas Young (1773–1829) and Augustin Jean Fresnel (1788–1827), at about the turn of the eighteenth century, suggested that light was propagated by transverse wave motion, that the theory was adequate to interpret the known facts about light and could be used to predict what would happen under a given set of optical conditions. It is this wave theory, then, which is so useful today in explaining facts concerning lenses and the operation of the microscope, the phenomena of polarization, interference, and refraction, and the behavior of light in general. See Fig. 67.

History repeats itself, and it should be mentioned that, while Huygens was expounding his longitudinal wave theory, Sir Isaac Newton (1642–1727) was advocating his corpuscular theory of light, and that for more than a hundred years these two theories were in conflict with each other, until about 1800 when the theory of transverse wave motion was put forth by Young in England and supported by Fresnel in France. In 1900 Max Planck formulated his quantum theory,[2] the

[2] The quantum theory, which is not entirely reconciled with the theory of electromagnetic radiation in the form of waves, considers light, or other forms of radiant energy, as being emitted at the source in small quantities called "quanta." It is quite beyond the province of this book to discuss the quantum theory, but the interested reader is referred to Wood's *Physical Optics*. Also see Glossary.

groundwork of which was laid by James Clerk Maxwell (1831–1878) and his electromagnetic theory[3] (see Glossary — Light), and by the discovery of the electron by Sir Joseph John Thomson (1856–1940). Today, just as two hundred years ago, there are two theories of the propagation of light, but instead of the longitudinal wave theory and the corpuscular theory we now have the transverse wave theory (electromagnetic wave theory) and the quantum theory. Whether one theory is correct and the other wrong, or whether the two theories can be reconciled in some way, makes no difference from a practical standpoint in the study of the microscope. Obviously, the usefulness of a theory depends more upon the results it produces, and to what degree it can be used to predict ensuing phenomena, than upon its absolute correctness. The wave theory is a perfectly adequate tool for a study of image formation, polarization, interference, and diffraction, and certainly it has proved adequate for the development and design of our present-day optical equipment.

Sec. 34. Wavelength, Amplitude, Period of Vibration, Frequency, and Phase. *Wavelength.* A graphic representation of a series or train of waves developed from simple harmonic motion is shown in Fig. 67.[4] Any point in the wave such as A should be thought of, not as a particle in the ether traveling from A to B along the X axis, but as a point of disturbance in the ether vibrating in the direction of A'. When the light has traveled half a wavelength from point A, shown in the figure, the point of disturbance A will be at A', and not at A'', and any other point in the train, such as P, will vibrate parallel to the Y axis in the direction DE. The distance from A to B is the wavelength; it might equally well be measured between any other corresponding points, such as P and P', in adjacent waves. Wavelength is related to the color properties of the light. Lambda (λ), the Greek letter L, is used to denote wavelength; it will be used in various equations involving light waves.

Amplitude. Distance AC is the amplitude; AA' is the double amplitude. The displacement at any time is measured from the X axis, vertically, to some point of the wave, as at F. The amplitude of a

[3] Electromagnetic oscillations were proved by Maxwell to be the means of energy transfer through the ether, an electric impulse giving rise to a magnetic impulse, and vice versa. It was also shown that light energy was electromagnetic in nature. This electromagnetic theory of light transfer satisfies Young's theory of transverse wave motion.

[4] Although a light wave may never be in the ideal form as depicted here, as a perfect harmonic curve, it can be treated as if it were a combination resulting from several such motions.

light wave controls its intensity; the greater the amplitude, the greater will be its intensity. More exactly, the intensity varies with the square of the amplitude.

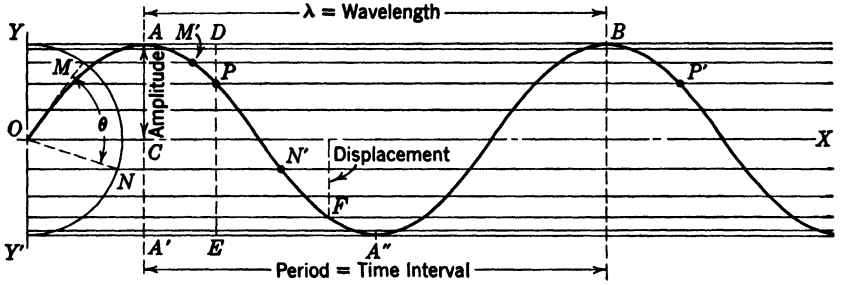


FIG. 67. The development of a wave front from simple harmonic motion. AB is the wavelength; it includes 1 cycle, AC is the amplitude. Period is the time interval for 1 cycle on the OX axis. Displacement is measured at any point on the wave (at any wave front) perpendicular to the axis, as at point F.

Period. The period of vibration is the time interval from A to B in Fig. 67.

Frequency. Frequency is the velocity of light in unit time, divided by the wavelength. Since the velocity of light is 186,327 miles per second,[5] and the wavelength is short, the frequency is enormous. Velocity of light in a vacuum is constant irrespective of wavelength, but in transparent material the longer waves travel faster than the shorter ones, the red light travels faster than blue.

The equation for frequency is:

$$\nu = \frac{v}{\lambda} \qquad [13]$$

where ν represents frequency, v the velocity of the light, and λ the wavelength. It is recorded in cycles per second.

Phase. Phase of any periodic motion refers to the stage to which the motion has progressed. Thus, phase can be referred to wavelength or period, and one train of light waves can be said to be half a period out of phase with respect to another train. However, if, at some point, one train of waves is found to be, say, two full waves behind some

[5] Although absolute velocity of light is of no particular interest here, it is worth noting that the above figures were computed by Simon Newcomb in 1882 and are the equivalent of 299,860 km/sec. Recent measurements made by Wilmer C. Anderson, as reported in the *Journal of the Optical Society of America*, **31**, 187, 1941, give the velocity of light as 299,776 ± 14 km/sec.

other train, the better expression is to say that the first train has been retarded two wavelengths.

In Fig. 68, there is just half a phase difference between two light rays vibrating in the same plane; interference will result, and one

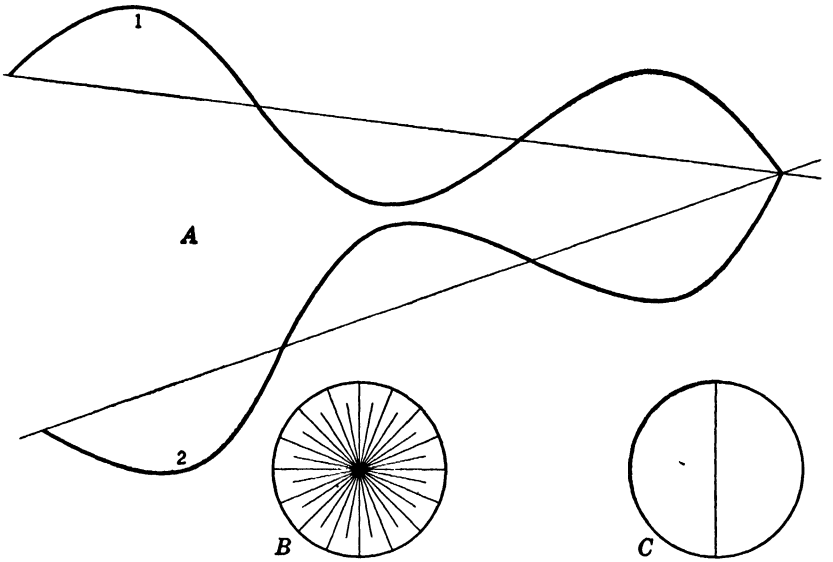


FIG. 68. *A*. The interference of two light rays. They start from a common source and by the action of a lens or by other means are brought together again and interfere, producing darkness. There is a phase difference of half a period between ray 1 and ray 2. If ray 2 were 1 full wavelength out of phase with ray 1, reinforcement would occur and the intensity of the light would be increased.

B. Natural light vibrates in all possible planes.

C. Polarized light, when plane polarized, vibrates in one plane only. The diagram at *A* is shown as polarized light only because of convenience in drawing. Four or five cycles of ray 2, with its axis, can be traced on paper. If the tracing is moved slowly in the direction of the ray 2 axis, interference can be shown to occur at the first ½ phase difference between ray 1 and ray 2 and for every multiple of full phase difference thereafter, as ½ λ, 1½ λ, 2½ λ · · ·.

ray will cancel the other. If the two rays of the same amplitude have zero phase difference, and are vibrating in the same plane, reinforcement occurs, and the amplitude of the ensuing wave is doubled. The interference occurs between two waves when they differ in phase by ½ λ, 1½ λ, 2½ λ · · ·.

Phase can also be denoted in degrees. In Fig. 67 two points are shown at *M* and *N* executing uniform circular motion. They are projected on the wave curve at *M'* and *N'*. Their period and their

maximum amplitude are immaterial, although for convenience they are shown here as equal. Their angular difference is given as the angle θ; this is the phase difference between the two points M and N.

For the sake of clarity, Figs. 67 and 68 are drawn as though the waves were vibrating in one plane only, the plane of the paper. This condition would be true only if the light were polarized (Fig. 68*C*). Natural or unpolarized light vibrates in all possible planes, and a profile view of such a wave would be as shown in Fig. 68*B*. Figure 67 applies perfectly to any ordinary natural light wave, for the curve OAB can be considered as turned about the X axis at will; the drawing may represent any one of the infinite number of directions of wave motion as shown at Fig. 68*B*.

Sec. 35. Color. Light occupies only a very small part of the whole gamut of spectral energy — about 1 octave out of 70 or more. The ether spectrum, so called, comprises all the radiant energy, from the short cosmic rays to the longest radio rays, the whole range extending from about 0.00001 mμ to 3×10^{13} mμ, the figure, of course, referring to wavelength. Light is the visible part of this great range of wavelengths. It exists from about 400 mμ to 800 mμ. The blue end of the visible spectrum has the shortest wavelengths, and the red end the longest. The blue end lies adjacent to the ultraviolet region, and the red to the infrared region.

If a beam of white light, as from the sun, is passed through a prism, it can be shown that the white light is composed of various colors, or wavelengths, in the following order: violet, indigo, blue, green, yellow, orange, red, all of which are included in the visual spectrum. The white light has been resolved into its fundamental components; this is the sun spectrum. Each color has its own distinctive wavelength.

Certain positions in the spectrum are sometimes referred to by the letters A, B, C, D, E, and F, each letter referring to a definite wavelength. A, B, and C refer to wavelengths in the red, D and E to the yellow wavelengths, and F to the blue part of the spectrum. There will be further discussion of this later.

An examination of the spectrum of white light shows it to consist of light of all possible wavelengths from about 400 mμ to about 800 mμ, one color blending imperceptibly into the next. When the light is received on the retina of the eye, the wavelengths produce color stimulus, depending on differences in their wavelength. Long wavelengths produce a color stimulus we recognize as red; we recognize the short ones as blue or violet or indigo. The predominating wavelengths control the color sensation as interpreted by the brain. The eye is most sensitive to green light, $\lambda = 555$ mμ.

Ordinarily, when white light falls on an opaque object, some of the light is reflected to the eye (diffuse reflection) and some is absorbed by the object. Thus, the light which reaches the eye is the original white light minus that part of it which has been absorbed by the object. If the object absorbs a large part of the red light, the light which reaches the eye will stimulate the vision centers of the brain in such a way as to produce the sensation of blue-green, and we say that the color of the object is blue-green; but if the impinging light is absorbed equally for all wavelengths we call the object gray. We say that the object is black when all the light is absorbed, and by like reasoning we say that it is white when it reflects all or nearly all of the incident light. There is yet another class of substances comprising pigments and chemical compounds which reflect the light after a definite penetration of the substance, instead of reflecting it at the surface. For example, to all appearances a crystal of copper sulphate is blue in reflected light under ordinary conditions, but if the surface is scratched the mark will appear whitish. The blue, which is the body color of copper sulphate, is observed only when light passes through a certain thickness of material. When the surface is scratched the tiny chips and angles so formed immediately reflect the light before it has a chance to penetrate the substance, thus indicating the surface color. When substances which appear one color by reflected light and another by transmitted light are properly observed, the body color is usually the complement of the surface color. Under the microscope, body color may virtually disappear if the specimen is very thin. This phenomenon may be carried to the extreme in the examination of fairly coarse white powders under the microscope. The powder grains may appear white by reflected light and entirely black by transmitted light, the absorption of light being complete. Thus, selective reflection produces the surface color of an object and selective transmission produces the body color.

The primary colors are red, green, and blue; in the proper proportions they produce the effect of white. The effect of white can also be produced by the blending of two colors, as, for instance, blue and yellow, or red and green. The two colors are then said to be complementary to each other.

Sec. 36. Rays, Beams, and Pencils of Light. *A Ray.* A ray of light is defined by Winchell[6] as a line from the source to any point on a wave front along which light travels. It may also be thought of

[6] A. N. Winchell, *Elements of Optical Mineralogy*, Part 1, John Wiley & Sons, 1931.

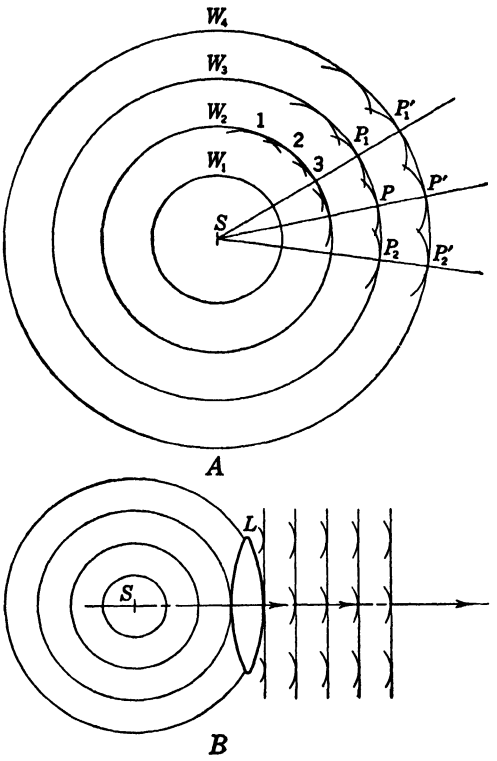


FIG. 69. This figure depicts the wave-front theory according to diagrams based on Huygens' original conception of wave motion. *A* shows successively expanding wave fronts from a point source. *B* shows the development of plane waves by letting the light pass through a lens. The source must be at the focal point of the lens. Plane wave fronts occur in nature, as from the sun or stars, because these sources are at such great distances.

as a line normal to a wave front along which wave motion travels. Because of the rectilinear propagation of light, these two statements have the same meaning.

A Beam. A beam of light is a bundle of parallel rays. The light source, in a searchlight of paraboloid figure, may be placed at the focus of the paraboloid; it will then throw a bundle of parallel rays as will a lens when the source is placed at the first focal point.

A Pencil. A pencil consists of a bundle of converging or diverging rays. It is correct to refer to a cone of light converging to a point, as from a lens, as a pencil of light.

Sec. 37. A Wave Front. A wave front can be defined as a surface tangent to light waves which are all in the same phase. The wave front at any distance from a point source is a sphere; a wave front from a bundle of parallel rays would be a plane surface normal to the rays. Since light travels in a straight line, it will travel normal to its wave front, with the exception of polarized light, which, in general, passing through a crystal, may not travel in a direction normal to its wave front. For further information on the effect of crystals on light the reader is referred to any good book on petrography, such as Winchell, Johannsen,[7] Wright,[8] and other well-known authorities.

[7] A. Johannsen, *Manual of Petrographic Methods*, 1918.

[8] F. E. Wright, *Methods of Petrographic Microscope Research*, 1911.

The classical drawings representing successive wave fronts and the way in which they can be found is attributed to Huygens. Slight modifications of his original drawing are still in use. Figure 69*A* shows a point source emitting light. A wave front at any point can then be considered as a spherical surface as at W_1. If each point of this wave front W_1 is thought of as a fresh source of disturbance, a new wave front at W_2 can be found by drawing the individual wave fronts of each point, as 1, 2, etc.; then the surface tangent to all these new *wavelets* will be the new front, W_2.

A plane wave front is formed when the light rays, as in Fig. 69*B*, are parallel to each other instead of diverging. The source is at the principal focal point of lens L, and successive wave fronts are shown as the light moves in the direction indicated by the arrow.

Thus a wave front is a spherical or plane surface, depending upon whether the light rays are converging or diverging, or are parallel. Under certain conditions, when light is retarded more in one direction of its travel than in another, as it is in anisotropic material, the wave front will be ellipsoidal.

Sec. 38. Refraction and Refractive Index. The refractive index of a substance can be spoken of as the ratio of two velocities: the velocity of light in a vacuum or in air to the velocity of light through the substance. It is a measure of the retarding effect that all transparent substances have on light.

$$n = \frac{V_0}{V_1} \qquad [14]$$

n is the refractive index of a substance through which light passes; V_0 is the velocity of light in a vacuum; and V_1 is the velocity of light in the substance.

The concept of refractive index or refringence of a substance involving terms of absolute velocity is correct, and it is a fundamental principle of refraction; but oftentimes the accompanying effects are of greater importance, as, for instance, the refraction, per se, defined as the change of direction of a light beam as it passes at an angle from one medium to another of different index. Figure 70 shows two conditions: one, when light strikes the surface of a transparent medium at an angle; the other, when it strikes the surface at 0°. In this figure at A, N_1 is a normal to the surface at the point of incidence. The angle of incidence is measured between the ray 1 and the normal. When the angle of incidence is 0°, the ray is perpendicular to the surface S, as at Fig. 70*B*. Where the ray strikes the surface at an angle, it is bent toward the normal; angle r is the angle of refraction, $<$ angle i, the angle of incidence. Angle r is always less than angle i when the ray

travels into a medium of higher refractive index, or greater optical density, as it is sometimes called. When the condition is reversed, and the ray travels into a medium of lower index, the reverse is true, $r > i$. Consequently, after the ray completes its course through the substance, and again emerges into a medium of the same refractive index as that from which it entered the substance, it will resume its former course and travel parallel to its first direction, provided that the two surfaces, that at which it entered the material and that at which it left it, are parallel. As the drawing shows, there will be displacement of the ray, and an eye placed at e will see the source S at S', and angle r_2 will equal angle i.

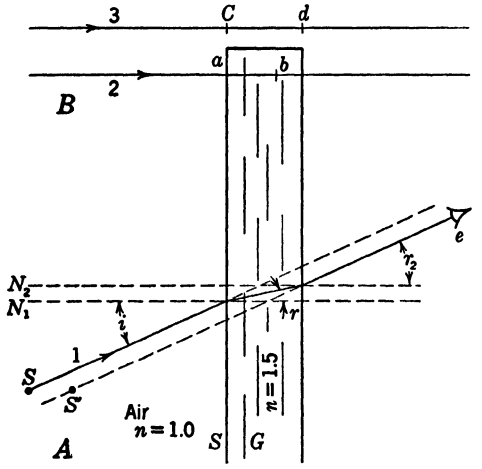


FIG. 70. The effect of a transparent substance on a light ray: *A*, at oblique incidence; *B*, at normal incidence.

If the ray is incident on the surface S at 0°, it is merely retarded, its velocity is lessened, the amount of retardation depending upon the difference of the index of the two media. For instance, ray 2 is incident at a, and reaches b at the same time that ray 3, traveling in air, reaches d. This produces the visual phenomenon known as the shallowing effect. Thus the distance $ab \times n = cd$. Advantage can be taken of this relationship to measure the refractive index of a substance by means of the microscope, as will be shown later.

From the definition of refractive index, as given in the first part of this section, it might seem that the study of refraction would involve figures denoting the absolute velocity of light in a vacuum, or 299,776 km/sec., and its actual velocity through the specimen in question. To avoid this rather clumsy but perfectly correct way of handling the problem, assuming that it could be done, the velocity of light may be thought of as unity in air, and as being always retarded when it passes through a transparent substance. Its velocity through a transparent substance, therefore, will always be less than unity,[9] therefore from equation 14 it will be seen that the index will always have a value greater than unity. Considering the velocity of light in air as unity,

[9] The exceptions to this statement will hardly occur in microscopical work.

or as in a vacuum (actually there is a little difference; $n = 1.000294$ for air), it is possible to figure the index of a substance by knowing the relationship between the angle of incidence and the angle of refraction of a light ray.

In 1621, Willebrord Snell (1591–1626) discovered that there was a definite connection between the angle at which the light was incident at a surface and the angle at which it was refracted. The mathematical deduction which he made states that the sine of the angle of incidence divided by the sine of the angle of refraction equals a constant which is the refractive index of the substance producing the refraction, or

$$n = \frac{\sin i}{\sin r} \qquad [15]$$

where n is the refractive index of the substance, i is the angle of incidence, and r is the angle of refraction.

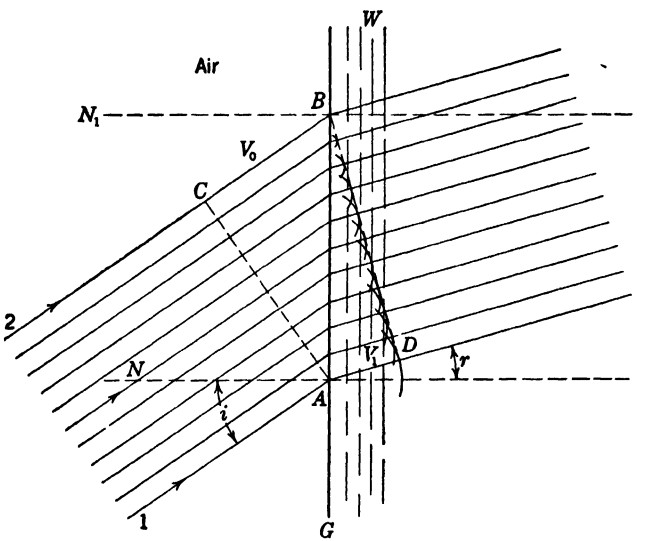


FIG. 71. This diagram illustrates the refraction of light and makes more clear the proof of Snell's equation.

In Fig. 71 let a beam of light be incident at the surface of a glass plate G. The refractive index of the glass is n; V_0 is the velocity of the light in air, taken as 1.0; V_1 is the velocity of light in the glass; N is a perpendicular erected at the point of incidence of ray 1; i is the angle of incidence; r is the angle of refraction. There is a plane wave

front at A at 90° to the beam, and a plane wave front at B at 90° to ray 2 after it enters the glass. DB is tangent to all the new wavelets; therefore it is perpendicular to ray 1, and to all intermediate rays between D and B.

While light is traveling from C to B along ray 2 it is also traveling from A to D along ray 1. V_0 represents the velocity of ray 2 in air, and V_1 the velocity of ray 1 in the glass G; therefore

$$n = \frac{V_0}{V_1} = \frac{CB}{AD}$$

Now,

$$\sin CAB = \frac{CB}{AB} \quad \text{and} \quad CB = AB \sin CAB$$

also

$$\sin ABD = \frac{AD}{AB} \quad \text{and} \quad AD = AB \sin ABD$$

Substituting in the first equation,

$$\frac{V_0}{V_1} = \frac{AB \sin CAB}{AB \sin ABD} = \frac{\sin CAB}{\sin ABD}$$

By geometry, angle CAB = angle i, and angle $ABD = r$; hence

$$\frac{V_0}{V_1} = \frac{\sin i}{\sin r} \quad \text{or} \quad n = \frac{\sin i}{\sin r}$$

Checking through a solution of this sort helps to fix the mathematical side of the phenomenon of refraction more clearly in the mind, and also it shows the relationship between the velocity of light and refractive index as given by the conventional equation 14.

An interesting phenomenon, which is really a concomitant of refraction, is that the wavelength of light is shortened as it traverses a transparent substance. This might be surmised from equation 13

$$v = \nu\lambda$$

for if the value v is lessened because of the retarding effects of a transparent medium the new value of v must be, according to the ratio of v_0/v_1 (see equation 14), or the refractive index of the substance n. Since the frequency remains the same, the wavelength in the substance must be λ/n, where λ is the original wavelength.

Sec. 39. Dispersion and Variation of Refractive Index with Wavelength of Light. The refractive index of a substance varies somewhat for light of different wavelengths. Within the visible range, blue light

is generally refracted more than red.[10] Thus a beam of white light striking a transparent substance will be refracted at the surface as in Fig. 72, the ray being bent (refracted) a definite amount as it enters the glass *G*. Red light would be bent less. The degree of refraction varies with different wavelengths, from red to blue, the difference

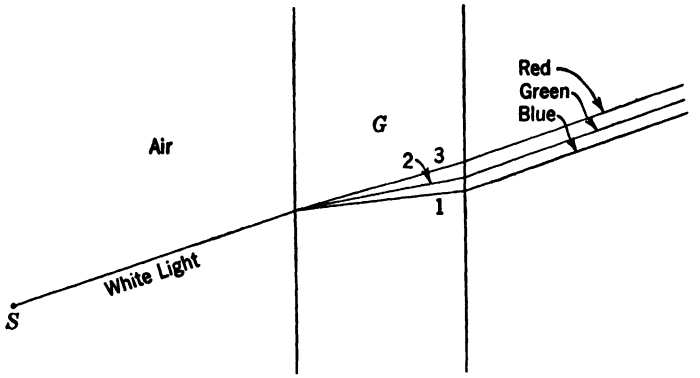


FIG. 72. The dispersion effect of a transparent substance on light. The source *S* emits white light; it is selectively refracted according to its different wavelength components. The amount of the dispersion depends on the thickness of the substance and its refractive index for light of different colors. The transmitted light does not appear in color, because the rays are parallel, and overlapping of the dispersion effects of rays, arising at contiguous points, restores the light to its original whiteness. Cf. Fig. 99 showing dispersion of a prism and dispersion of a lens.

between the refraction for blue light and that for red depending on the molecular structure of the substance. Figure 72 shows the effect of a plate of glass on white light. Although the white light is broken up into its color components, all the rays travel in the same direction after passing the plate and there is such an enormous amount of overlapping of the colors on the back side of the glass that the color separation cannot be discovered by inspection. In the case of a prism, the separation of the colors occurs in the same way but after passing the prism all the reds, greens, blues, and intermediate colors travel in their own separate directions and the familiar rainbow effect is the result. This property of a substance to refract light of different wavelengths by different amounts is called dispersion.

The two spectral lines F (a pure blue light) and C (a pure red light)

[10] This is known as "normal dispersion." "Anomalous dispersion," as it is called, occurs in some substances that absorb certain wavelengths strongly, in which case the longer wavelengths may, in certain positions, have a higher index value than the shorter ones. This happens on opposite sides of the absorption band.

are often chosen in dealing with dispersion (see Sec. 44), the refractive index for the F line being the greater. A substance with a refractive index 1.557 for the F line might have a refractive index 1.555 for the C line. The dispersion would be 0.002; it might be written $n_F - n_C = 0.002$. Similar values could be found by taking the difference in index for the various lines. According to custom, the value $n_F - n_C$ is called the *mean dispersion value.* When the difference of some other refractive index readings is used, it is referred to as *partial dispersion.* Another value called the V value of dispersion is a ratio represented by the refractive index for the D line, minus 1, divided by the mean dispersion. Equations 16 and 17 are in common use in discussing optical problems.

$$\text{Mean dispersion} = n_F - n_C \qquad [16]$$

and

$$V = \frac{n_D - 1}{n_F - n_C} \qquad [17]$$

Refractive indices are measured at some definite spectral line, so that the effects of dispersion are taken into account. Expressing the refractive index of a substance as $N_D = 1.556$ gives a definite figure for the D line of the spectrum. If the refractive index of the substance were given as $n = 1.556$ the reading might have been made at any wavelength or combination of wavelengths (white light); in that event, owing to dispersion, the substance might not have a refractive index value of 1.556 for any of the generally used spectral lines. The wavelength for the D or sodium line is given as 589 mμ. Actually there are two sodium lines very close together at this point, one at 589 and the other at 589.6 mμ, but the figure 589 is sufficiently close for most purposes. Because sodium light can be easily and accurately obtained, and because of its position in the spectrum, refractive index is almost universally referred to this line.

In Sec. 54 it will be shown that the dispersive powers of glass and other materials from which lenses are made give rise to forms of chromatic aberration in a lens which are subject to correction only by the addition of lens elements of different refractive index and shape. Thus, dispersion of one lens may be used to nullify the dispersion of another and so correct it for chromatic errors. On the other hand, advantage can be taken of the dispersive qualities of glass and quartz prisms to increase the separation of the various spectral colors, thus making the prism spectroscope possible.

Sec. 40. Temperature Coefficient of Refractive Index. Refraction of light varies with temperature change; as the temperature rises, the refractive index falls. This change is greater with liquids as the transmitting media than with solids. For many liquids, the rate of change may have a value of around 0.00045 for each degree centigrade of variation; solids may show a change only in the fifth or sixth decimal place. In order to specify the refractive index of a liquid completely, the temperature at which the reading has been made should be taken into account, as well as the wavelength of light used. As an instance, the refractive index of cedar oil might be given as $n_D^{20°C} = 1.515$, meaning that its refractive index is 1.515 when measured at 20° C, for the D line. The rate of change of refractive index for one degree of temperature is sometimes spoken of as the temperature coefficient. In tables it may be written either by that name or as the derivative $-\frac{dn}{dt}$, the minus sign denoting a rise of temperature reducing the index. However, as stated, this change due to temperature is of importance for liquids but not of great moment for solids.

Frequently in microscopical work, particularly with the biological microscope, refractive index of mounting media and specimens is referred to without regard to surrounding temperature or to the wavelengths of the illumination. This procedure, though not exact, is permissible, because the differences produced in refractive index due to changes in working temperature are generally small, and unless measurements that involve exact determinations for purposes of identification are being made, errors introduced by temperature variation can generally be ignored. The same is true with regard to changes in refractive index introduced by the use of color screens. Usually such changes are negligible, so that index values given for the D line are satisfactory for nearly all working conditions. However, if measurements of the refractive index of specimens are required, as in petrographic work, by the immersion method, the temperature and wavelengths of light must be carefully taken into account.

If the refractive index of a liquid at two different temperatures is known, the temperature coefficient can be found by dividing the difference in index by the number of degrees of difference in temperature. Though not always exactly correct, this will usually give a workable value. If the liquid is used at a temperature other than that at which its index was determined, the true index of the liquid for that temperature can be found by multiplying the coefficient by the number of degrees of difference between the two temperatures. If the temperature at which the work is carried on is higher than that at which the tem-

perature was measured, the value, as found above, should be subtracted from the known index reading; if lower, it should be added.

Sec. 41. The Critical Angle. Refractive index is spoken of as the ratio of the sine of the angle of incidence to the sine of the angle of refraction, where angle i is greater than angle r. Since refractive index values are greater than 1, this would indicate that the impinging ray is traveling from a medium of low refractive index into one of higher index. Under such conditions, the ray will be refracted toward the normal; therefore, no matter how great the angle i becomes, up to 90°, the ray will always be able to enter the second medium.

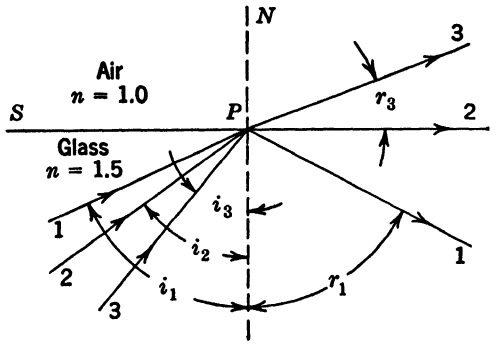


FIG. 73. Diagram showing the critical angle. The refractive index of the glass G is 1.5. In this case the critical angle is about 42°; it is angle i_2 of ray 2.

If, on the other hand, the light ray travels from an optically denser medium to an optically rarer medium, conditions will be just reversed, the light ray emerging at the point of incidence, say from glass, into air (Fig. 73) will be bent away from the normal drawn at the point of incidence. This is shown in the figure by following the trace of ray 3. It would seem that, if the angle i_3 were increased sufficiently, ray 3 would first just graze the surface of the glass, as with ray 2; then, as the angle increased further, as with ray 1, a point would be reached at which the ray would be entirely turned back into the glass G. As a matter of fact, this is exactly what happens. When the incident angle is just sufficient for the impinging ray to travel along beneath the glass surface instead of emerging, as with ray 2, a critical point has been reached; angle i_2 is then called the critical angle for that particular medium, which in this example is glass.

There is a very simple connection between refractive index and the critical angle of the substance. If the refractive index of a piece of glass is 1.5, it is known that the value was obtained by measuring the angle i in, say, air and the angle r in the glass and taking the ratio of the sines of these two angles in the order named. Therefore, if the value of sine i is reversed with, or given, the value of r, as would happen if the light were considered as traveling from glass to air, then the

value of n becomes $1/n$. The equation is

$$\frac{1}{n} = \frac{\sin i}{\sin r}$$

By definition, the critical angle i is critical only when angle r, Fig. 73, equals 90°. The sine of 90° is 1. Putting 1 in place of the sine of r in the above equation,

$$\sin i = \frac{1}{n} \qquad [18]$$

or the sine of the critical angle is the reciprocal of the refractive index of the material in question. In the above example the critical angle of the glass would be about 42°, since the value of n was arbitrarily taken at 1.5.

If water is used as an immersion fluid on a glass surface, the critical angle of the glass will be considerably increased; with glycerin, the angle will be increased still further; and with cedar oil, which has a refractive index the same as that of the glass, the glass and oil will form an optically homogeneous layer, and there will be no critical angle between them. The light rays will travel from one to the other without change in direction. The relationship of the condition in which the indices of both media are known, as well as the angles of incidence and refraction, is expressed by the following equation:

$$\frac{n \text{ of the first medium}}{n \text{ of the second medium}} = \frac{\sin r \text{ in second medium}}{\sin i \text{ in first medium}} \qquad [19]$$

This equation is convenient for tracing a ray of light through various media. Equation 19 is, of course, a more general form of Snell's equation.

The Angle of Total Reflection. Since the critical angle of a substance is the point at which the ray of light is turned back into the substance by reflection at its second surface, it is sometimes called the angle of total reflection. Total reflection accounts for the phenomenon of a prism acting as a mirror. It can exist only when the surrounding medium is less dense optically than the substance through which the light is traveling. As the refractive index of the substance increases, the value of the critical angle decreases.

Sec. 42. Optical Paths. Optical paths are said to be equivalent when light takes an equal amount of time to traverse them. In Fig. 74, rays 1 and 2 are traveling equal geometrical distances from P to P' in air and P_1 to P_1' in glass, but the distances are not equivalent optically because

ray 2 must travel the distance in glass, which has a refractive index of 1.5, and therefore it will travel with one-third less velocity than if it were traveling in air, as application of equation 14 shows. Since ray 2 takes one and one-half times longer than ray 1 to make its journey, its optical path is one and one-half times longer. The optical path has been increased by the addition of the glass prism. For ray 3, the distance

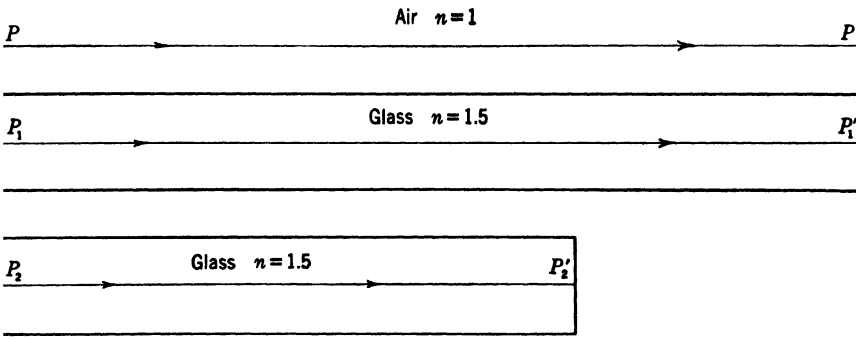


FIG. 74. Equivalent optical paths. The distance P_2P_2' is optically the same as distance PP'. Light takes the same amount of time to travel either path.

P_2P_2' is two-thirds the distance PP'. The light is traveling through a glass prism with an index of 1.5; hence the time required for the light to travel from P to P' is the same as is required for it to travel from P_2 to P_2', since the optical paths are equivalent. All light rays which travel from an object point through a lens to the focused image should have equal optical paths.

If the distance that a light ray travels through an optical substance is multiplied by the refractive index of that substance, the product will be the optically equivalent distance in air. It then follows that if a ray travels a certain distance in air its optical equivalent for a certain substance can be found by dividing the distance in air by the refractive index of the substance.

Figure 75 shows rays of light converging to a focus at F, through air. If a glass plate is introduced into the path of the rays, the course of the rays will be substantially as shown by the dotted lines. Here aberration has been set up. The outer rays 1 and 4 are brought to a focus at F_1, and the inner rays at F_2; there is no sharp focus. The general effect has been to lengthen the optical path by the introduction of the glass plate, but, although the optical paths of the rays 1 and 2 have been lengthened, they have not been lengthened equally. This condition is somewhat analogous to the optical condition prevailing when the glass plate vertical illumination is used, for in that case image-forming rays pass through an optically flat plate at an angle.

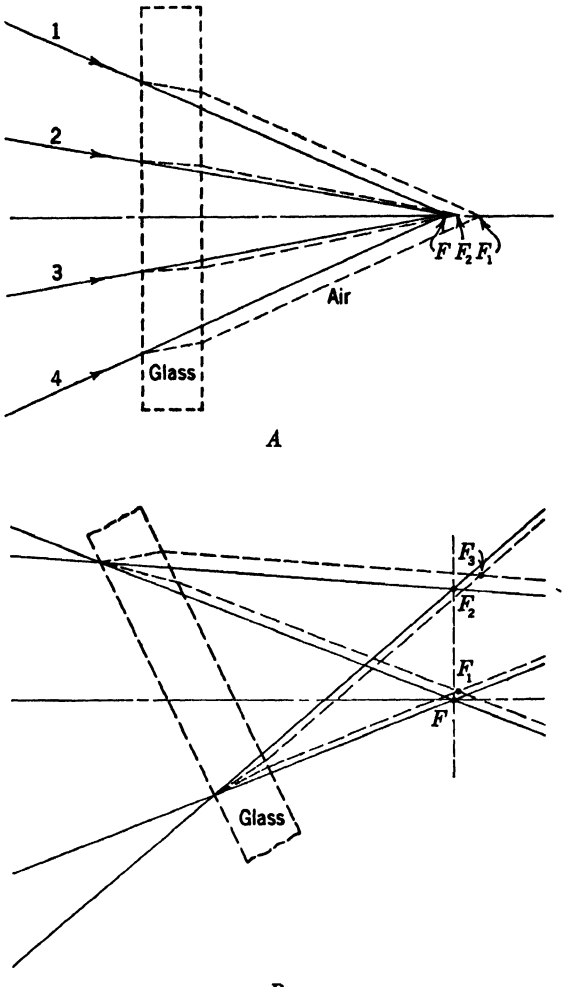


FIG. 75. *A*. Diagram showing the effect produced by altering the paths of converging rays by the insertion of a glass plate. This is a condition commonly met with in microscopy, not only in apparatus but also in the preparation of the specimen, as in cover glasses of varying thickness. The addition of an optically denser region to any optical train has the effect of lengthening the optical path; the total effect for any one ray depends on the distance the ray has to travel through such a region and upon the refractive index of that region.

B. In this case, the glass plate shown in *A* has been set at an angle to the principal axis. The effect on the converging rays has been to shift the points of convergence F and F_2 to F_1 and F_3.

Sec. 43. Diffraction and Interference. In Fig. 69 a small source of light is shown (a point source), the radiation of which is indicated by the Huygens conventional wave development theory; the wavelets are shown at each new wave front to form another and yet another wave front further from the source. The tendency is for any point in the sphere at any wave front to act as a separate and original source, and to send out a new series of wave fronts or wavelets entirely independent of those from the original source, thus creating an infinite number of new light sources. However, if each point of disturbance on a wave front is conceived as the motion of a finite particle of the ether, passing its motion (energy) in turn along to the next particle, and so on, does it not suggest that interference between the particles or wavelets would result in a propagation of motion only in a forward direction? Thus a particle indicating the wavelet at P can act on a particle at P' only, since its motion is somewhat circumscribed or interfered with, at some place, by particles P_1 and P_2, which in their turn are acting on particles which lie ahead of them at P_1' and P_2', thus producing rectilinear propagation. Back wave motion is also eliminated by a self-damping effect inherent in the wave.

In Fig. 76, a cross section of a diaphragm D is shown. It represents a slit less than 0.25 mm wide, measured from a to b, but for purposes of discussion and reference it is shown many times wider than it really is. A beam of light is arranged to enter the diaphragm. The first effect on the impinging light will be to transmit only a very narrow, thin, ribbonlike beam $aa'b'b$. Comparing any point of disturbance in the ribbon of light passed by the slit with a point of disturbance such as P in Fig. 69, it will be seen that in a *vertical* direction the constricting influences around any point in the ribbon of light are less than they are in Fig. 69, it being always remembered that the distance $ab <$ 0.25 mm. As a matter of fact, much light wanders away from the direct path of the main beam, and if the slit is made narrower the percentage of such stray light will increase. This phenomenon is called diffraction. Rather than consider a ray of light as " bent " when passing an edge or through any small opening, it is more accurate to conceive the phenomenon of diffraction as always existing in a beam of light of finite size, and to realize that the diffraction effects become more and more marked as the beam is made smaller. In a wide beam, the interference of wavelets circumscribes their motion.

If the width of the slit is reduced to λ, the light will fan out in a dihedral angle of 90°. The slit will then act through this angle, somewhat as a line source. If the slit is widened to 2 or 3 mm, diffraction effects will be almost impossible to observe.

Diffracted light is evidenced by fringes which, under certain conditions, appear around the main body of images as diffraction rings, or, if the light passes through a slit, by fringes which are formed at certain points on either side of the main beam. Figure 76 clearly shows the effect of diffracted light and the way in which fringes are produced.

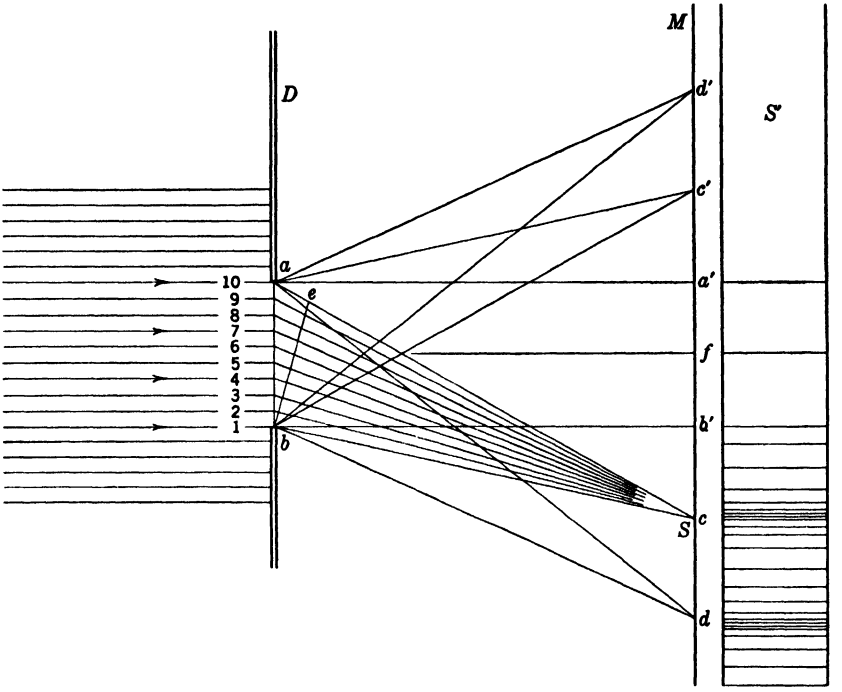


FIG. 76. Diffraction of light at a slit. S' is a plan view of the screen M. A beam of light is shown impinging on the slit ab.

In Fig. 76 the light beam passes through the narrow slit and falls on the screen M at $a'b'$. If the light is coming through the slit in parallel rays, barring diffraction effects, the size of the illumination at M would be equal in area to the size of the slit, and all the rest of the screen surface should be dark. However, since, when the slit is made sufficiently narrow, diffraction takes place, light will wander downward and upward from the main beam. Considering the light that falls below the beam, there will be some position for point S where the distance ac is 1 λ longer than the distance bc, or $ae = 1\ \lambda$. At a certain screen distance, then, interference will take place at c and that point will be dark. Similarly, there will be a point c' above a' where there will be interference, and darkness will result. See Fig. 68.

S' shows the broadside view of the screen and how the light and dark areas blend one into the other. Points at a and b should not be considered as directing light exclusively to c or d but rather as radiating in all directions in an angle included in the total spread of the fringes, in this case, say from d to d'; when five fringes are visible on each side of the slit, the light spreads out as a fan and covers a bigger portion of the screen.

It has been shown in Sec. 34 that interference occurs only when light differs in phase by $\frac{1}{2}\lambda$, $1\frac{1}{2}\lambda$, $2\frac{1}{2}\lambda$, $\cdots$. Now, each point in the lower half of the slit ab, Fig. 76, can be considered to have a corresponding point in the upper half which will be just one-half wavelength out of phase with it when both rays reach a stated point. Thus, for the rays converging to point c on the screen M, ray 1 annuls ray 6; ray 2 annuls ray 7; ray 3 annuls ray 8; ray 4 annuls ray 9; ray 5 annuls ray 10; and similarly all the rays passing through the slit annul each other at point c and produce interference at this point. The phenomenon is symmetrical on both sides of the slit. At any other point between b' and c the light rays will not interfere completely, and this area will receive some light, there will be reinforcement.

In those cases where the outside limiting rays differ in length by $1\frac{1}{2}\lambda$, $2\frac{1}{2}\lambda$, $3\frac{1}{2}\lambda \cdots$, then a portion of the slit can be thought of as producing an interference effect and a portion producing a light band. At a path difference of 2λ, 3λ, $4\lambda \cdots$ for the limiting rays, complete interference will again occur, for every ray will have one ray to match it, the phase difference for which will be just $\frac{1}{2}\lambda$. Thus, on the screen M, bands of darkness will be followed by bands of light in turn followed by other dark bands, and so on, according to the path difference of the limiting rays.

From inspection of the diagram it will be evident that, as the slit is made narrower, c must automatically fall farther from b' in order to make the necessary difference between the path ac and bc so that interference will be produced. Thus, decreasing the width of the slit increases the diffraction effects. Conversely, if the slit is made wider, c, d, and other points of interference where fringes will be seen will be thrown practically into coincidence with b' or fall within the main beam, and no diffraction with interference effects can be noticed.

For convenience, the use of monochromatic light to produce the effects shown in Fig. 76 is assumed. The fringes will then be dark with light of the indicated color in between. If white light were used, color effects would appear; and, since red is diffracted more than blue, a spectrum would be seen from b' to c with red at c, d, and so on, the successive

spectra growing weaker and weaker. These spectra are called the first-, the second-, or the third-order spectrum, as the case may be. If a prism were used to form a spectrum the position of the colors would be reversed; that is, the blue would be refracted more than the red.

The whole theory of diffraction of light as it passes through a slit can easily be kept in mind if it is remembered that at the various points of interference *c*, *d*, and so on the length of the optical paths of ray 1 and ray 10 as shown in Fig. 76 must differ in increments of whole wavelengths, as 1 λ for the first point of interference, 2 λ for the second point, and so on. Also, when white light is used, there will be a tremendous number of such points of interference running through each spectrum formed, the first interference starting with the blue and continuing to the red for each successive wavelength. The process then repeats itself for the spectrum of the next order.[11]

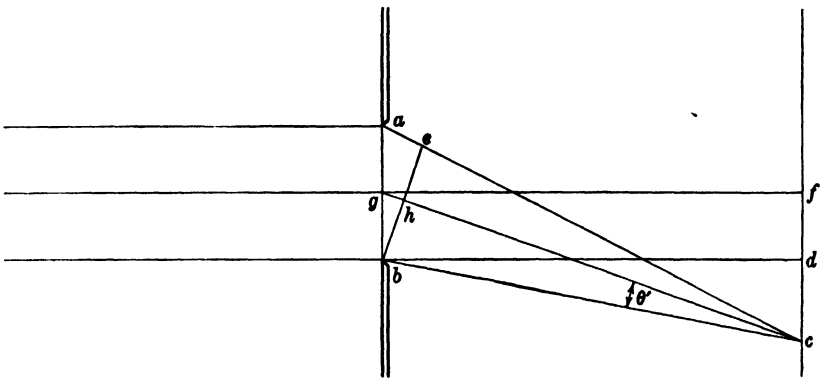


FIG. 77. This diagram illustrates the trigonometrical development of the equation $f_c = \frac{\lambda}{2 \sin \theta'}$; see text.

Figure 77 is a simplified diagram after Fig. 76; many lines which appeared in Fig. 76 have been omitted for the sake of clarity. The distance of the first interference effect from the axis, or the value of *fc*, will be given in terms of wavelength of light and angle θ.

Because *ab* is extremely small compared with *gf*, angle *gbc* is prac-

[11] For experiments in diffraction as related to the microscope the following can be consulted: Carpenter-Dallinger, *The Microscope and Its Revelations*, seventh edition, 1891; F. W. Shurlock, "Experimental Studies in Diffraction," *J. Roy. Micr. Soc.*, 1931; E. Abbe, "Beitrage zur Theorie des Mikroskops und der mikroskopischen Wahrnehmung," *Ges. Abh.*, **1**, 45–100, 1873; Conrad Beck, *The Microscope*, Part II, first edition, 1924.

tically a right angle; then

$$\operatorname{csec} \theta' = \frac{gc}{gb} = \frac{1}{\sin \theta'}$$

Now

$$\frac{fc}{gc} = \frac{gh}{gb}$$

and

$$\frac{fc}{gh} = \frac{gc}{gb}$$

but

$$gh = \frac{\lambda}{2}$$

therefore

$$\frac{fc}{\lambda/2} = \frac{1}{\sin \theta'}$$

and

$$fc = \frac{\lambda}{2 \sin \theta'} \qquad [20]$$

Then $\lambda/(2 \sin \theta')$ is the distance from the center line f to the first dark fringe. fc might be thought of as half the image of a distant point source formed by the slit ab. Sir George Biddell Airy (1801–1892) showed that a lens substituted for the slit will form an image of a distant source somewhat as the slit will, when used with a beam of light. A small disc of light appears surrounded by alternating dark and light rings (fringes). It would seem as though the spot of light in the center, at least theoretically, should be a point, in which event it would be invisible; but this, as we know, is not so because of inevitable lens aberrations.

The central disc of light which is the image of a distant point source is always of finite size. It is called a diffraction disc. About 84 per cent of the light is concentrated at its center. The encircling ring of darkness, surrounded by a ring of illumination, then by another ring of darkness, and so on, constitutes a figure of concentric circles. The dark rings denoting absence of light are called minima; the light circles, maxima. These diffraction and interference phenomena produced by a lens are, of course, analogous to those produced by the slit.

The measurement, then, from the center of the light disc to the first

dark circle, or the first minimum, is an indication of the resolution of the lens; that is, it indicates how closely the image of a distant object approaches a mathematical point and, by the same token, how far apart two points must be to appear imaged as two separate and distinct points. The greater the resolution, the smaller will be the diffraction disc.

If the distance fc in Fig. 77 is half the diameter of the diffraction disc, and if a lens is inserted in place of the slit in Fig. 77, equation 20 becomes

$$fc = \frac{0.61\lambda}{2 \sin \theta'}$$

or, if

$$fc = \frac{D'}{2}$$

where D' represents the diameter of the diffraction disc, the equation becomes

$$\frac{D'}{2} = \frac{0.61\lambda}{2 \sin \theta'}$$

or

$$D' = \frac{1.22\lambda}{2 \sin \theta'}$$

The constant 0.61 is obtained from an equation by Airy; it applies when the opening is circular rather than a slit; it is used only when interference of the first order is considered. The constant varies in magnitude for the maximum or minimum being measured. This phase of the problem is well discussed by Hardy and Perrin.

If the refractive index of the medium from the slit to the screen be other than that of air ($n = 1$), the value n must be inserted in the equation, which then becomes

$$D' = \frac{1.22\lambda}{2n' \sin \theta'} \qquad [21]$$

Several important lessons can be learned by inspecting equation 21. The first is that, if the wavelength of light is decreased, D' decreases and the resolution of the lens is increased. Next, if the cone of light, represented in the equation as $\sin \theta'$, is increased, the value of D' will also be decreased. Finally, if the refractive index of the surrounding medium is increased, the value of D' will likewise be decreased.

It should be noted that the angle θ' has so far been referred to as in the image space. It will later be considered when transferred to the object space.

Sec. 44. Spectra. It was stated in Secs. 38 on refraction and 43 on diffraction that, when white light is refracted, under certain conditions a spectrum is formed, and similarly when diffraction takes place the tendency is for the white light to be arranged to form a spectrum. A spectrum, as formed by a spectroscope, either by diffraction (the grating spectroscope) or refraction (the prism spectroscope), then, is the orderly arrangement or sorting out of the different wavelengths, each of which forms an image of the spectroscope slit. These images are placed side by side in descending or ascending order, or according to wavelength. The short wavelengths are at the blue end of the spectrum; the long ones, at the red end. The gradation from one color to the other formed by the intermediate wavelengths gives a beautiful continuous rainbow effect. There are three principal classes of spectra: the continuous, the bright-line, and the dark-line.

The Continuous Spectrum. The tungsten lamp and other incandescent solid bodies give continuous spectra. A spectroscope will place the slit images so close together that they will merge one into the other, and the effect, when formed from white light, will be a continuous band of colors.

The Bright-Line Spectrum. The bright-line spectrum is formed from an incandescent vapor such as mercury or hydrogen. The mercury-vapor discharge tube is an example. Here the spectrum is broken, bright colored lines appearing only in certain positions. They can be interpreted as representing energy at certain definite wavelengths, and, if the lines are far enough apart in the spectrum and are of sufficient intensity, they may be separated by appropriate filters, without a spectroscopic instrument, to give substantially monochromatic light.

The color of the line will depend on the part of the spectrum in which it occurs; that is, it will depend on the wavelength of the light at that particular point. Sodium light, for instance, gives only two bright lines in the visible part of the spectrum. These lines are yellow and are very close together; one has a wavelength of 589.0 mμ and the other 589.6 mμ. They are generally spoken of as the D line and considered as though they were one. Actually the shorter wavelength is line D_2 and the longer one D_1. Table XV lists some of the important spectral lines and their source.

The Dark-Line Spectrum. This spectrum, obtained in its greatest glory from the sun, is known as the solar spectrum. The continuous

Table XV

Important Spectral Lines

Fraunhofer Line	Wavelength mμ	Source	Color
A	760.0	Atomic oxygen	Red
B	687.0	Atomic oxygen	Red
C	656.3	Hydrogen	Red
D	589.0	Sodium	Yellow
E	527.0	Iron, calcium	Green
F	486.1	Hydrogen	Blue
G	430.8	Hydrogen	Violet

spectrum forms the background. It is crossed by myriads of thin dark lines, which were first discovered by William Hyde Wollaston (1766–1828) and later rediscovered by Joseph von Fraunhofer (1787–1826); they are known as the Fraunhofer lines. There are thousands of these, each representing a wavelength. Some may be formed by the same element; iron in particular accounts for many. Fraunhofer designated some of the strongest lines by letters; these same letters are used today in referring to certain portions of the spectrum, such as the D line. The black lines correspond in all respects to the bright lines of the element or elements which gave rise to them. As an example, if the bright D line of sodium, formed by an independent source, travels through sodium gas which is very much less heated, then on account of the similarity of the period of vibration of the sodium light and the sodium atoms of the gas, resonance will occur, the sodium light will be absorbed by the sodium atoms, and a black line will result. As the sun is surrounded by vapors of many elements at lower temperature, the conditions are just right for the production of the dark spectral lines of the hotter vaporous elements of which it is comprised. Figure 78 shows photographs of the continuous spectrum at *A*, the bright-line spectrum at *B*, and the dark-line spectrum at *C*.

Any spectra formed by material on the stage of the microscope when the spectroscopic ocular is used will be spectra of the light source minus the light that is filtered out by the specimen. The effect is usually a shortening of the spectrum at one end, or gaps may be created, the thickness of the specimen and the source of light playing an important role. For further study of this subject the works of Gage[12]

[12] Simon Gage, *The Microscope*, fifteenth edition, Comstock, 1934. Also see other editions.

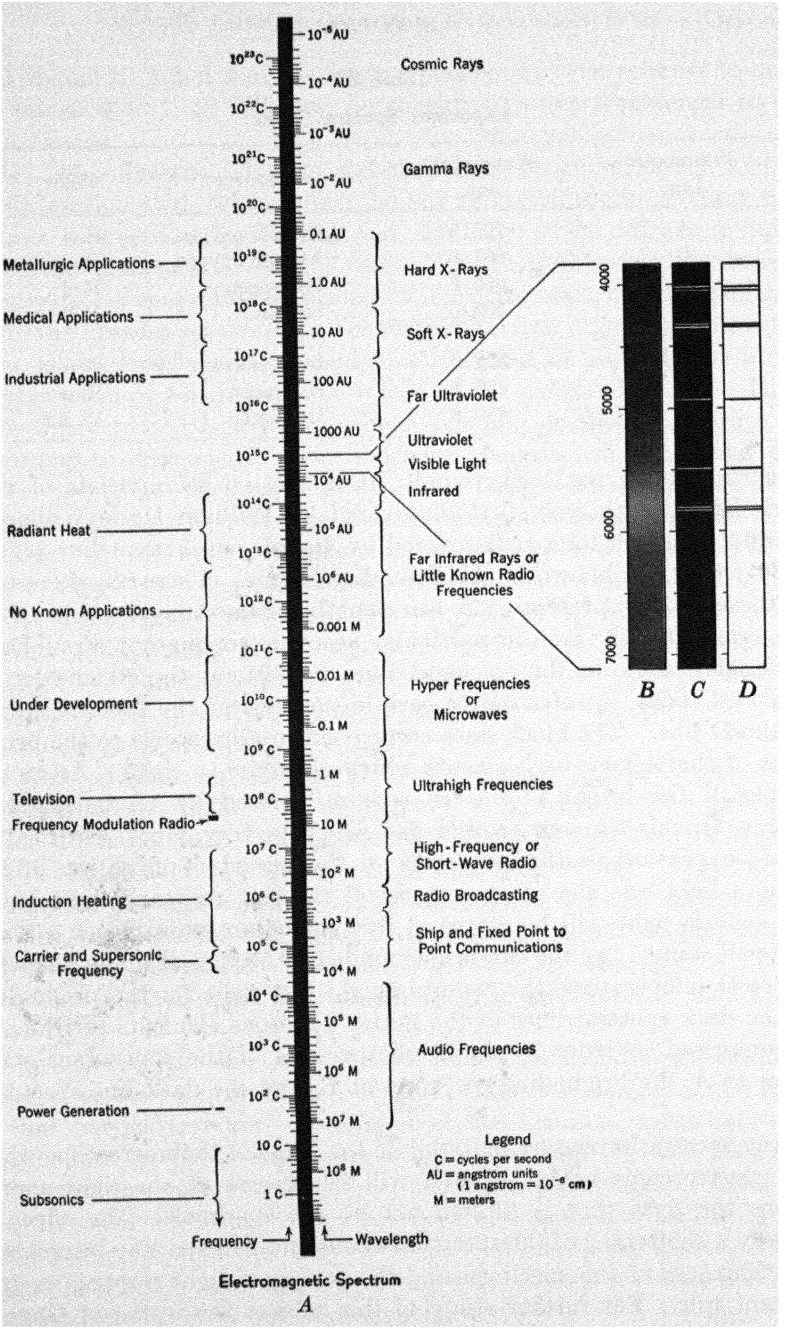


FIG. 78. *A*. The electromagnetic spectrum. *B*. Continuous spectrum. *C*. A bright-line spectrum of mercury vapor. *D*. Dark-line spectrum; the background would be a continuous spectrum as at *B*.

and Chamot and Mason[13] are interesting, and a paper by Jelley[14] describes the use of his grating microspectrograph.

Sec. 45. Color Temperature. The growing use of color plates or film has made it necessary to specify light sources in terms indicating the intensity of the various spectral colors in the light source. This is managed by means of the color temperature scale. When its color temperature is known, a light source can easily be selected or modified for use with color film.

The actinic quality of light can be specified rather exactly. The reading is made in, or converted to, degrees Kelvin, which is the same as the centigrade scale plus 273°; sometimes it is called the absolute centigrade scale. Absolute zero on the Kelvin scale is 0° K, and 0° C = 273° K. The Kelvin scale was named after William Thomson, Lord Kelvin (1824–1907).

Strictly speaking, a source emitting light which is capable of forming a bright-line spectrum, such as the mercury or perhaps the carbon arc, cannot be measured on a color temperature basis. Tungsten-filament lamps are often specified on a color temperature scale, but such a lamp is a gray rather than a black radiating body.

If a hypothetical black body,[15] a perfect radiator, is raised in temperature, it will ultimately become incandescent and go through all stages of color transformation, from the first perceptible dull red to a bluish white. This is a phenomenon often seen when a metal is raised to a high heat. To associate the color of the heated object with the temperature to which it must be raised in order to emit light of that color is a practical and natural mental process, and it often happens that the color temperature and the actual temperature of the object coincide very closely. The actual temperature of a piece of glowing carbon may closely approach the measured color temperature. The color temperature of a tungsten light source is generally about 3000° K, which is very close to that of its filament temperature. On the other hand, the two temperatures of the sky have no agreement

[13] E. M. Chamot and Clyde Mason, *Handbook of Chemical Microscopy*, Vol. 1, second edition, John Wiley & Sons, 1937.

[14] E. E. Jelley, "Application of the Grating Microspectrograph," *Ind. Eng. Chem., Anal. Ed.*, **13**, 196, 1941.

[15] The term black body is likely to be confusing to those not familiar with it. A perfectly black body, irrespective of its composition, would radiate heat perfectly and absorb it perfectly. It would have no reflection whatever. Possibly lampblack approaches as near to it as any substance. Although the ideal black body does not exist, it is frequently referred to as though it were an actual physical standard. It is possible to measure color temperature quite accurately although it is referred to this hypothetical standard for definition.

whatever. The color temperature may be very high — 25,000° K to 30,000° K — yet the actual temperature taken in space may be very low indeed. For work with color plates the color temperature of the illumination is more or less standard at 3200° K.

In a measure, control can be exerted by the use of filters; the actual temperature of the radiating body and the final color temperature so obtained will then have no relation to each other. In lamps a high color temperature can be made lower with a rheostat; the resistance inserted in the power line will cause the light from the lamp to become relatively richer in the red. Raising the voltage will cause the light to become stronger in the blue. The Eastman Kodak Company manufactures a small meter that will make color-temperature measurements to within approximately 50 degrees. For color photomicrography it is essential to consider light in terms of color temperature, but in black-and-white work the frequent use of chromatic filters and mercury lamps with monochromatic separations makes it of less importance.

Sec. 46. Conventions Observed in Speaking of and in Sketching Lens Systems. In speaking of a lens it is often necessary to refer to one particular side. The side toward the light source is referred to as the front or the front surface; the light is traveling from the front to the back. By the same token, the front lens of the condenser is the large lens that receives light from the microscope mirror, the back lens being next to the objective or glass slide. Not all authors have followed this exact terminology, but when this method of designation is used no ambiguity can exist, since the direction in which the light travels through the microscope will always be known.

When lens systems are illustrated graphically, the accepted custom is to show the light as traveling from left to right. The first focal point lies to the left of a positive lens. The object distance measured to the left of the lens is positive, and the image distance measured to the right is positive. A lens radius measured to the right of the vertex is positive.

The notation to be followed in the drawings relating to lenses will, in general, be after that of Hardy and Perrin.[16] F will denote the first principal focal point of the lens, F' the second focal point, the prime denoting quantity or position in the image space. Thus, f and f' will denote the first and second focal lengths of the lens, respectively; x and x', the object and image distance measured from the first and second focal points; s and s', the object and image distance measured from the first and second principal planes; H and H', the first and second principal points or principal planes; V_1 and V_2 the first and

[16] A. C. Hardy and F. H. Perrin, *The Principles of Optics*, 1932.

second vertices of the lens; y and y' will be distances measured from the principal axis; O and I, the object and image points respectively; θ and θ', the angle between the principal axis and a ray. Rays will be numbered. The meaning of other letters or numbers will be made clear from the construction of the diagrams. See Fig. 81.

The Trace of Light Rays. In drawings of lens systems, many lines indicating the trace of light rays through space are often shown. To those unaccustomed to analyzing such drawings, these lines may appear very confusing, particularly when the optical system consists of numerous lenses and diaphragms, but if one or two facts concerning them are kept in mind their meaning will become perfectly clear. All lines (seldom over two) originating from one point on the object, on

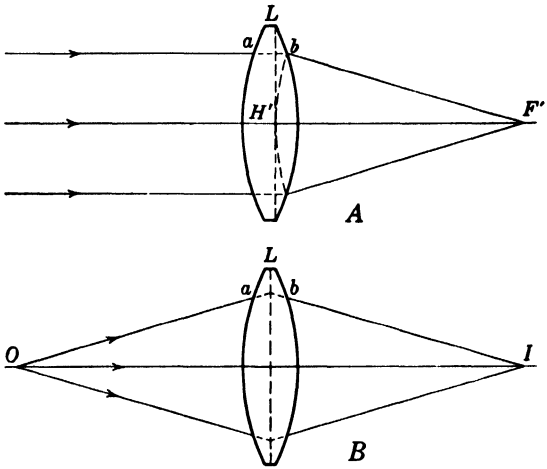


FIG. 79. Rays passing through a lens. It would be impractical to show the trace of a ray through each diagram with a lens pictured in it, and nothing would be gained by it. Instead the rays are shown as meeting at the so-called refracting-plane surface as described in the text.

passing through a lens, will be given a new direction; if the lines then converge, an image of the original point will be shown as existing at their intersection. If the lines are parallel to each other after passing the lens, they are said to form an image at infinity. Such a condition shows that no real image is formed and that the original point was at the focus of the lens. If the lines diverge, no real image is formed. Thus, an image field is indicated wherever two lines intersect, provided that their origin is from a mutual point.

The Equivalent Refracting Plane or Surface. If a ray of light is traced through a lens it will be found to enter it at a certain point as at a, Fig. 79A, and to leave it at the second surface as at b. If these

points of entrance and departure are computed for a number of rays, and their external paths are continued in the same direction within the lens, it will be found that their points of intersection, when connected, will form a curved surface as illustrated in the figure. The radius of the spherical surface will equal the focal length of the lens. The drawings are generally made to show the exterior lines only. However, to simplify matters further, diagrams of lens systems are often drawn as if the equivalent refracting surface were a plane passed through the center of the lens (see Fig. 79*B*), and the point of intersection of the rays is made to fall in this plane. In the finished drawing the lines indicating the spherical surface or the plane surface are erased.

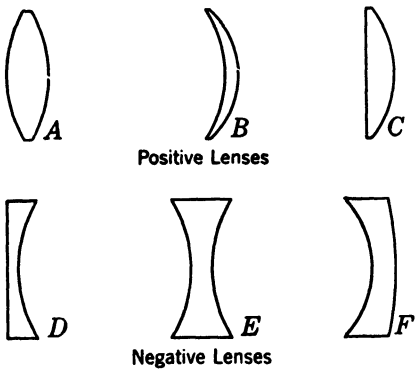


FIG. 80. The six classes of lenses. *A*, double convex; *B*, converging concavoconvex or converging meniscus; *C*, planoconvex; *D*, planoconcave; *E*, double concave; *F*, diverging concavoconvex or diverging miniscus. The first three are positive lenses; the last three are negative lenses.

Sec. 47. Classes of Lenses. Fundamentally there are six principal classes of spherically ground lenses. They are shown in Fig. 80: *a*, double or biconvex; *b*, converging concavoconvex or converging meniscus; *c*, planoconvex; *d*, planoconcave; *e*, double concave; *f*, diverging concavoconvex or diverging meniscus. These are simple lenses. They may be used singly as shown, or they may be combined, two or more elements forming a single lens. The combined elements may be grouped together to form a compound lens, in which event there is usually a separation of the different groups, but the elements of the separate lenses are, as a rule, in optical contact to form distinct lens units. The whole compound system acts together, more or less as a single lens might do. Two lens elements cemented together to form a lens unit are often called a doublet; three elements used together are referred to as a triplet. Some very fine objectives have as many as eleven to fourteen elements

Lenses which are thicker at the margin than at the center are known as negative lenses. The focal length is called negative because it is measured on the side of the lens from which the incident rays originate. Lenses thicker in the center than at the margin are called positive. Lenses may be made of glass, fluorite, quartz, or a combination of glass

and fluorite. The refractive index of glass varies from, say, 1.5 to 1.7; the index of fluorite is 1.43, and that of quartz is 1.54.

Sec. 48. The Essential Features of a Simple Lens. *The Principal Axis and Vertex.* The principal axis of a lens lies on a line connecting the centers of the spheres, parts of the surfaces of which form the two lens surfaces. Figure 81 illustrates the points brought out in this discussion. Measurements in reference to a lens are usually made along the principal axis.

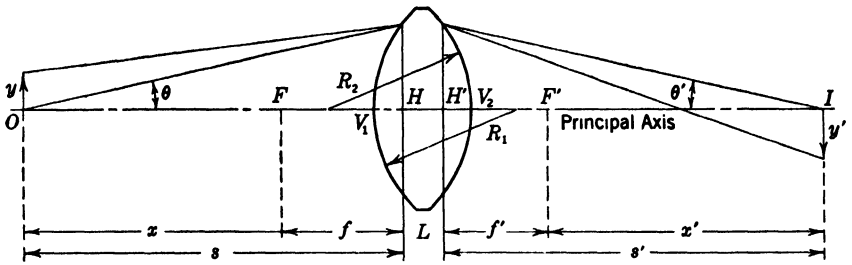


FIG. 81. The important cardinal points of a lens. L, a double convex lens. R_1 and R_2, radii of first and second lens surfaces. V_1 and V_2, vertices of the first and second lens surfaces. H and H', the first and second principal points. The planes passing through these points are the principal planes. F and F' are the first and second principal focal points. f and f' are the first and second focal lengths. The distance $V_2 F'$ is the back focal length. s and s' are the object and image distances from the principal points. x and x' are the object and image distances measured from the focal points. The principal axis is OI. O is the object and I its image. y and y' represent the length of the object and image.

The vertices are the points at which the principal axis intersects the lens surfaces; they are shown at V_1 and V_2. Measurements are sometimes made from these points to locate mechanical parts in the design of instruments.

The Principal Focal Points. Every lens, simple or compound, positive or negative, has two principal focal points; they are located on the principal axis and may be real or virtual. Positive lenses have real focal points. Figure 81 illustrates the relative positions of the focal points of a positive lens. Generally, in a converging system, the first focal point is in the space in front of the lens, which is the side from which the light comes. It is designated by the letter F. The second focal point, F', is at the back of the lens, the side which discharges the light. Occasionally, in some highly complex lens systems, such as high-power microscope objectives, the focal points may lie a short distance within the lens system, but their positions relative to the direction of light transmission are maintained. A beam of light

(Sec. 36) incident on a positive lens will be brought to a focus, all rays converging and intersecting, at some position on the principal axis which is the second focal point of the lens. This phenomenon is beautifully demonstrated by the hackneyed but rather dramatic experiment with the burning glass, where the image of the sun (the rays being substantially parallel) is formed at the second principal focus of a positive lens. Conversely, a point source at the first principal focus of a positive lens will discharge the light from the lens in the form of a beam, the rays being parallel to the lens axis; thus a lens is more or less reversible in action, for no matter which side is toward the light, the second focal point will be found in approximately the same position at the back of the lens. See also Fig. 89.

However, when a beam of light strikes a negative lens, as shown in Fig. 85*C*, the rays diverge as shown, but if the paths of the rays are continued to the front of the lens, as has been done in the drawing, they will be found to converge on the axis at a common intersection which is the second principal focal point of the negative lens. The second focal point, F', of a negative lens is always on the side to which the light is incident. The first focal point of such a system is on the opposite side. These focal points are called virtual because no real image is formed there; this is generally true of any diverging lens system. Thus, with positive and negative lenses the second focal point lies at the back of a positive lens and in front of a negative lens. One interesting and important attribute of any lens is that when a ray of light passes through one of its focal points, or may be extended to pass through one of its focal points, it will have as its counterpart a ray on the opposite side of the lens which will be parallel to its principal axis; the converse of this proposition is also true.

The Principal Planes and Principal Points. The principal planes or Gauss planes of a lens, named after Karl Friedrich Gauss (1777–1855), are shown at H and H', Fig. 81. They establish geometrical positions to which certain lens constants can be referred, and they serve as an extremely useful reference in simplifying the discussions of complicated lens systems. The intersections of the principal axis with the principal planes denote the principal points. The position of the principal points can be determined by the following equations, taken from Hardy and Perrin. t denotes the thickness of the lens.

$$V_1H = -\frac{(n-1)tf}{nR_2} \qquad [22]$$

$$HH' = t - \frac{(R_1 - R_2)t}{n(R_1 - R_2) - (n-1)t} \qquad [23]$$

$$H'V_2 = \frac{(n-1)tf}{nR_1} \qquad [24]$$

The microscopist may never need to use these equations, but they illustrate how the position of the principal points may be found and so make them more understandable.

The principal planes of a lens may lie within the lens as shown in the drawing, Fig. 81; in a complicated lens system such as an objective, they might be in the positions as shown in Fig. 116; or they might be en-

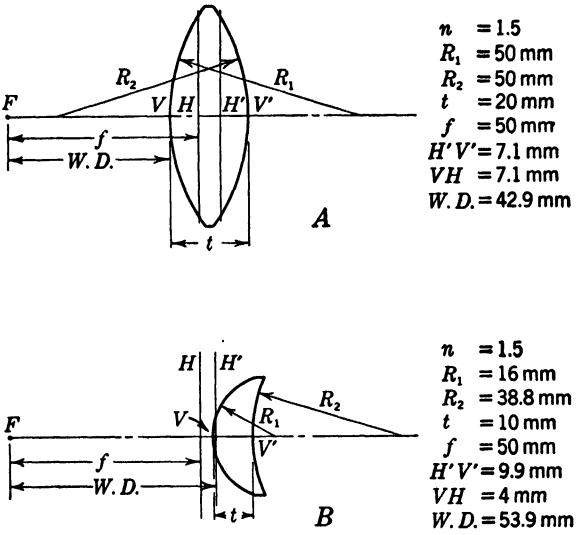


FIG. 82. Two lenses, *A* and *B*, designed to have equal focal lengths but with different working distances, due to the shift in the position of the principal planes according to equations 22 and 24.

tirely or partly outside the physical boundaries of the lens, as in Fig. 82*B*. Figure 82 shows two lenses of equal focal length but with their principal planes in different positions. When a lens is composed of numerous elements, the principal planes of each element, taken separately, can be combined with the planes of another element, and this in turn with another, and so on until the process is completed and the final compound lens will be found to have two principal planes from which other lens measurements can be made. In this way, the complicated compound lens system can be considered as easily as the single lens element used as an example in Fig. 81.

The Equivalent Lens. In the case just cited, Fig. 81, *L* is shown as a relatively thick lens. It might be thought of equally well in terms

of its equivalent lens, that is, as an imaginary lens without thickness. A geometrical analogy would be the geometrical point, line, or surface. This equivalent lens would be endowed with the power to affect light just as the real lens L does, with the exception that, after receiving the light from the object source, it would have to be moved to a slightly different position to discharge it. These positions of the equivalent lens are shown at H and H', Fig. 81, the first and second principal planes. The focal length of the equivalent lens is therefore the same as that of the lens to which it is the equivalent.

The Focal Length of a Lens. A lens has two focal lengths. The first is measured from the first principal point to the first focal point, and the second from the second principal point to the second focal point. Figure 81 shows this relationship. In general, the two focal lengths are equal, irrespective of the shape of the lens, except when the medium on one side of the lens has a different refractive index from that on the other side. The microscope oil-immersion objectives illustrate this point, as does the eye. The equation connecting the two focal lengths is

$$\frac{f}{n} = \frac{f'}{n'} \qquad [25]$$

where f is the first focal length in a medium of refractive index n and f' is the second focal length in a medium of index n'. For an oil-immersion microscope objective the value of n is 1.515 and n' is 1.0. The focal length of a low-power lens is easily measured. The lens can be held on the edge of the blade of a toolmaker's combination square. The head is set at some whole number. The blade of the square is then pointed at the sun, or some distant light source, and when the source is sharply focused on the head of the square the focal length of the lens will be the distance from the head of the square to the point on the blade where the lens is held. This is good approximation.

An equation often used to determine the focal length of a lens when the curvature and refractive index are known is

$$D = \frac{1}{f} = (n - 1)\left(\frac{1}{R_1} - \frac{1}{R_2}\right) \qquad [26]$$

always remembering that the radius is positive when measured to the right of the vertex. Thus R_2 is negative for a double convex lens. The D in equation 26 is the unit of measure by which opticians denote the power of a lens. When the focal length is 1 meter, the lens is said to have a power of 1 diopter; a focal length of 10 cm equals 10 diopters;

5 cm will equal 20 diopters; and so forth.

$$D = \frac{1}{f} \qquad [27]$$

Oculists prescribe eyeglasses by stating the required power in diopters. The term diopter is occasionally used in microscopy; for instance, the accessory lens when combined with the condenser to illuminate a large field for low-power objectives might have a power of -3 diopters; this would denote a negative lens with a focal length of -33.33 cm. A negative lens has a negative focal length.

The Back Focal Length. The distance V_2F', Fig. 81, is called the back focal length of a lens. If the lens is an objective of the microscope, the difference between the back focal length and the focal length may be large. Figure 81 illustrates the comparison of these two quantities in a double convex lens.

The Equivalent Focal Length. This term is used frequently when referring to objectives; it is synonymous with focal length since it refers to the focal length of the equivalent lens. In tables listing objectives the term equivalent focal length is often found, as it is customary to refer a compound system of lenses to its equivalent lens and therefore to its equivalent focal length.

Working Distance. The preceding paragraphs indicate why a 16-mm objective may have only slightly more than 5-mm working distance although its equivalent lens might have a full 16-mm working distance. An object lying only a little over 5 mm from the front glass of a 16-mm objective might still be beyond the principal focus of the objective, and a real image of the object could be formed at the proper position at the front focal plane of the ocular. By examining the formula for the location of the principal points and thereby the location of the focal points, it will be seen that the shape and thickness of the lens have much to do with the location of the first principal point. Lenses can be designed with an equal focal length but with varying distances of the principal focus from the vertex of the first surface as Fig. 82 demonstrated; in this way, in a measure, the working distance of the lens can be controlled. Figure 82 shows two lenses A and B of the same focal length, 50 mm; the distance from the vertex to the object, which is at the first focal point of the double convex lens, is 42.9 mm, and in the case of meniscus lens B it is 53.9 mm; the working distance has been increased. The change in the position of the principal planes due to the change in the design of the lens is shown. See Fig. 116.

Many microscope objectives are of complicated construction and sometimes involve many individual lenses, singles, doublets, and triplets. The distance between the units which make up the complete lens can be varied, and this spacing is a vital factor in the location of the principal planes. Thus, the difference in working distance between two similar objectives is accounted for. For work involving the use of the haemacytometer and similar apparatus, lenses of great working distance must be chosen.

In the example cited above, the working distance of 53.9 mm was spoken of as measured from the front surface of the front lens of the objective to the object. In tables this figure is often given without explanation. Most authorities, and at least two of the major optical companies, measure the working distance from the top of the cover glass to the nearest part of the objective, generally the metal of the lens mount. A standard cover-glass thickness must be assumed, and presumably the object must be mounted directly on the under side of the cover rather than on the slide, for if it were mounted on the slide the thickness of the mount would introduce variations and give misleading results. Even high-power objectives, N.A. 1.4, may have the front plano surface of the front lens set slightly back of the front surface of the metal mount.

Conjugate Points. Points O and I, Fig. 81, are a pair of conjugate points. Since by definition, conjugate means joined with, any change at O implies a corresponding change at I, the image of O. Conjugate focal points are so situated with reference to a lens that light rays from one of them, after passing through the lens, converge to the other point. These points lie farther from the lens than the principal focal points when real images are concerned; however, the cardinal points of a lens, which consist of pairs of points, are also conjugate with each other. There may be any number of conjugate points. A point object and its image represent a pair of conjugate points. These positions are sometimes called "conjugate foci." Planes which pass through conjugate points and are normal to the principal lens axis are said to be conjugate planes.

The Anti-Principal Points. For low-power photomicrography of petri dishes, some textiles, and other material, when the magnification must be 1.0×, it may be convenient to know how such a magnification can be obtained. From equation 38 it will be seen that magnification will be unity when the object and image distances are equal. If distance equal to the focal length of a lens is measured on the lens axis from the two focal points, in a direction opposite to the correspond-

ing principal points, then the positions so found will be the anti-principal points and the lateral magnification will be unity and negative. Thus, any photomicrographic lens can be placed at twice its focal distance from a specimen and an image will be formed at an equal distance on the back side of the lens at a magnification of $-1\times$. The minus sign, denoting inversion of the image, is usually dropped in speaking of microscope magnification. In order to cover a 5 by 7 inch film, at a magnification of 1, it is usually necessary to employ a lens with a focal length of 6 to 8 inches.

Nodal Points and Optical Center. The nodal points of a lens correspond to the principal points and are found to coincide with them when the lens is used with a medium of the same refractive index on both sides, as for instance when the microscope lens is operated in air. Considering the eye, air is on one side of the eye-lens system in the object space, and the vitreous humor occupies the image space from the eye lens to the retina. Thus, the nodal points of the eye do not correspond in position with the principal points. The same condition is true of the oil-immersion objective. Here the space in front of the lens is filled with an oil, and the space from the back of the objective to the conjugate image is occupied by air. However, with these exceptions and when the condenser is used immersed, all other microscope lenses have the nodal points coinciding with their principal points.

Figure 83 shows how the nodal points can be drawn. If the two radii R and R' are drawn from the centers of curvature of the two lens surfaces, parallel to each other, and the points of the intersection of the radii with the lens surfaces are connected, the connecting line will cross the principal axis at C, the optical center of the lens. At the intersection of the radii with the lens surfaces, tangent planes can be drawn which will be parallel to each other. If these planes are parallel, a ray of light passing from one to the other will suffer displacement after it passes through the lens, but its new direction will be parallel to its first direction. Under these conditions the lens is acting as a glass plate. The path of light from one point of tangency to the other will, of course, lie along the

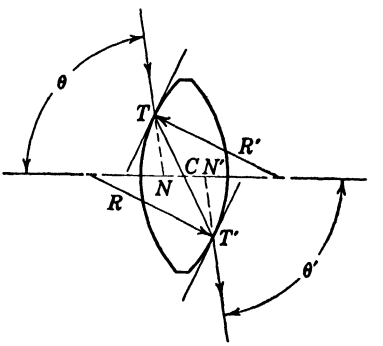


FIG. 83. Illustrating the geometrical position of the optical center of the lens, and the nodal points.

line intersecting the principal axis and will pass through the optical center of the lens. The center of any simple lens may be found in this way.

If the ray incident at T is extended graphically to intersect the axis at point N, N will be the first nodal point, and N' will be the second.

The angular magnification of the ray under these conditions is unity, $\theta = \theta'$. If the lens is revolved through a small arc about a perpendicular axis at the second nodal point, no displacement of the image will take place. The nodal slide, which is a special device for making such determinations, will quickly locate the nodal points after the manner described.

A complicated lens of many elements has only two principal points, two nodal points, two principal focal points, any number of conjugate foci, and one equivalent lens; it may have one or more optical centers. The simple lens will have but one optical center as shown, but, as the optical system increases in complexity, the ray shown in Fig. 83 may cross the principal axis more than once. The permanent characteristic points of a lens from which measurements can be made are, in general, called the cardinal points.

Ordinarily, drawings depicting various effects of the lens on light show what is called a thin lens, that is, one so thin that the principal points can be considered coincident with a transverse plane passing through the center of the lens. In practice, nearly all the lenses used in the microscope, such as the objective or condenser, are thick. If such lenses were drawn, the principal planes would have to be referred to, and the whole exposition would become much more involved. For this reason, in drawings showing the effect of the lens on light, the aberration of a lens, and other interesting and pertinent facts, the thin lens will be used as an example.

Sec. 49. Tracing Light Rays through a Lens or Series of Lenses. This is really a function of the lens designer. The problem as given generally is to determine the image distance when the object distance, the angle of the ray, the radii of curvature, and the refractive index of the lens are known. This process is repeated for each lens surface in turn, the image formed by the previous surface being taken as the object for the next surface; thus the object may be real or virtual, in succession, until the procedure is completed. Although no attempt is made to consider this branch of optics, several important equations will be given which may occasionally be of use. One connects the object distance to the image distance when the thickness of the lens can be

ignored. It is

$$\frac{1}{s} + \frac{1}{s'} = (n - 1)\left(\frac{1}{R_1} - \frac{1}{R_2}\right) \qquad [28]$$

and

$$\frac{n}{n'} = \frac{\sin i}{\sin r} \qquad [29]$$

Equation 29 will be recognized as Snell's equation, which is useful in tracing a ray through a piece of glass plate when the angle of incidence is known. An equation which is often of value is

$$ny \sin \theta = n'y' \sin \theta' \qquad [30]$$

This is a modified form of Lagrange's equation, after Joseph Louis Lagrange (1736–1813). Another, including one surface, is

$$\frac{n}{s} + \frac{n'}{s'} = \frac{n' - n}{R} \qquad [31]$$

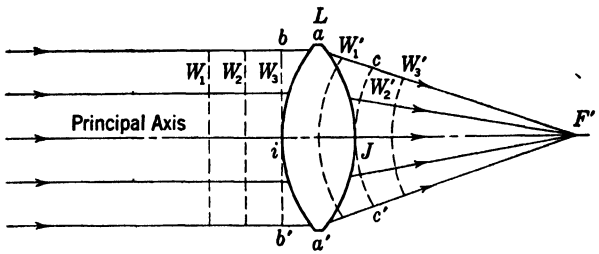


FIG. 84. The effect of a lens on a beam of light (a plane light-wave). The emerging wave is spherical and converges at F', the second principal focus of the system.

Sec. 50. The Effects of a Lens on Light. A lens is an instrument which for its functioning depends purely on its ability to refract light according to prearranged plan. If the surfaces of the lens are properly ground to the required curvatures, and other conditions are suitable, light from a point source on the object side of the lens will be brought to a focus in the image space. The optical path for all rays will be the same, irrespective of the zone of the lens they traverse. The phase of each ray as it arrives at the focus will coincide with that of every other ray.

Considering the double convex lens L, Fig. 84, from the standpoint of the mechanics of wave motion, light is striking the lens surface as a beam parallel to the principal axis. The wave fronts will be plane surfaces, as shown at W_1, W_2, and W_3. The first point of contact is at i; the last portion of the plane wave to meet the lens surface is at the mar-

gin of the lens at a and a'. The refractive index of the glass is, say, 1.5. Now it can be shown that it takes the same length of time for the light to travel from i to j, the distance through the center of the lens, as it does from b to c and b' to c'; the optical paths are equal. See Sec. 42. Consequently, when the wave emerges from the lens it is concave, owing to uniformly decreasing retardation effects acting from the center of the lens to the periphery. Since the surfaces of L are spherical, the emerging wave front will be spherical and will focus at F', and optical path bF' will equal iF'. Under these conditions, all rays will converge and will meet at F', the focus of lens L. The radii to which the lens is ground (both sides of the lens are not always ground on the same radius), and the refractive index of the glass being known, it is very simple to compute the focal length of the lens and to tell just where the rays from a parallel beam will converge to a focus, by means of equation 26

$$\frac{1}{f} = (n - 1)\left(\frac{1}{R_1} - \frac{1}{R_2}\right)$$

Roughly, the above is a general outline of the manner in which rays are affected by a lens. By tracing the rays backward, from right to left, there seems to be evidence to conclude that, if the source were within the principal focus, the final effect of the lens on the rays would be different from that shown, and that if the source were at point F' the rays would leave the lens as a plane wave to the left. As a matter of fact this is what actually does take place under the changed conditions. From studying Fig. 84 it is also plain that, if the lens were thicker on the edge than at the center, the effect of the lens on the parallel rays would be to make the rays diverge instead of converge. The different effects, due to the position of the source with reference to the focal point of the lens, are shown in Fig. 85. At C, when the rays are parallel, the negative lens gives a virtual image; but it will give a real image with converging rays, as shown at D. This presupposes another lens to furnish the converging rays. Compare with Fig. 21, p. 49. The positive lens also will give a virtual image when the object is within focus of the lens, as at A. With parallel light, the microscope condenser will act as in Fig. 84; the objective, as at B, Fig. 85; the ordinary ocular, as at A, Fig. 85; the negative projection ocular, with converging rays, as at D, Fig. 85; and the front lens of the Huygenian ocular as at E. A hand magnifier would act as at A, Fig. 85. From this it can be learned not only that the shape of the lens controls the direction of the rays leaving the last lens surface, but also that the manner in which the rays are arranged — parallel, con-

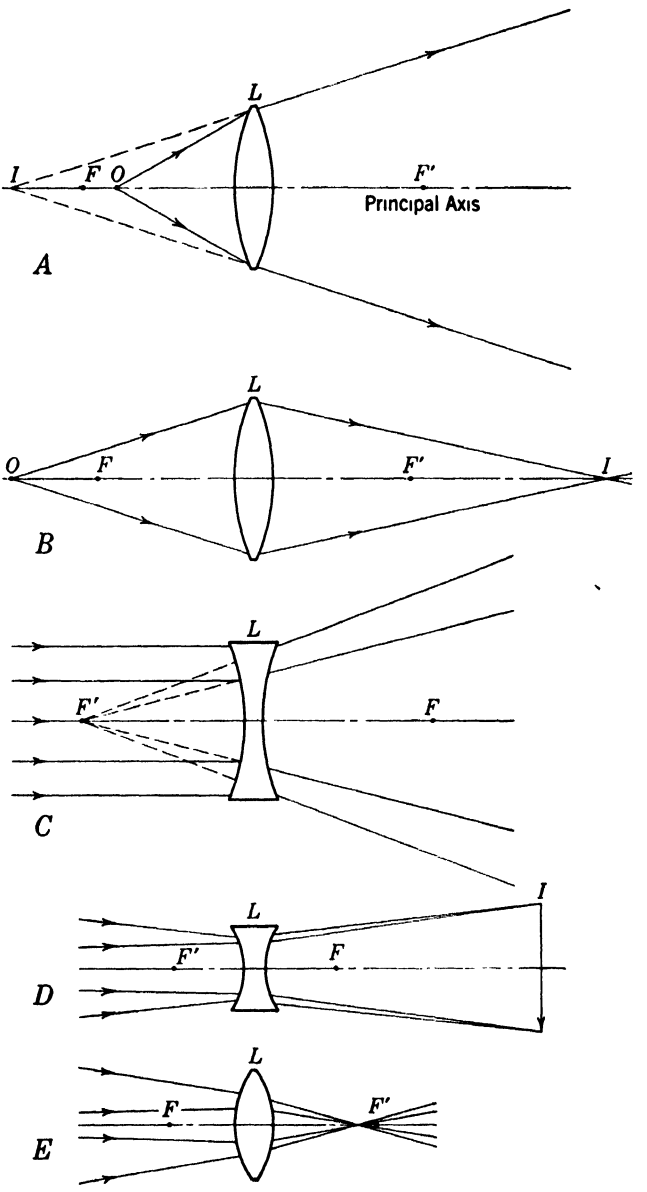


FIG. 85. The way lenses affect light when the object or the light source is in different positions relative to the first focal point of the lens. The effect of converging rays is also shown at *D* and *E*.

verging, or diverging — when they reach the lens, is an equally important factor, as is the position of the object, relative to the focal point.

Sec. 51. Image Formation by a Lens. The way in which the image is formed by a lens is now easily explained. The image of a point which lies on the principal axis has already been considered in speaking of the effects of a lens on light rays, Figs. 84 and 85. If the object lies partly off the principal axis of the lens and beyond the principal focus, an image of the object will be formed on the back of the lens, the exact position of which will be determined later. It should

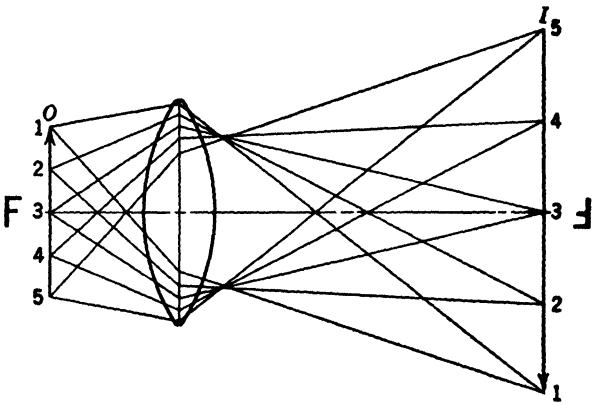


FIG. 86. Image formation of a lens as built up point for point. The image is inverted and reversed.

be borne in mind that each point of the object can be treated separately, that all points are treated in a similar manner, and that each of these innumerable points is represented by a corresponding point in the image. Also, while any one point in the drawing is shown as emitting only two light rays to two different points on the first surface of the lens, actually, each point on the object is radiating light to all possible points on the lens surface, and because of refraction and curvature of the lens each of these rays is directed to a focus to the corresponding point of the image. In Fig. 86 the image construction of an arrow as an object is shown. Each image point is the focus of all the light rays which reach the lens from the corresponding point at the object. The image of the arrow *I* is the graphic summation of all the light rays from all points of the object brought to a focus, point for point, at *I*.

The Geometrical Construction of the Image. The geometrical construction of the image can be carried out as follows: From any point

on the object O, such as P, in Fig. 87, a line is drawn parallel to the principal axis of the lens L and continued to point A on refracting plane H; see Sec. 46. From this point the line is drawn through the principal focus of the lens, F', and continued indefinitely at the back of the lens. A line is then drawn from P through the center of the lens C, until it meets the continuation of the line AF' at P'. The intersection of these lines is the focus of the image of point P. Point P_1' can be found in a like manner. Connecting P' and P_1' locates the locus of all other image points of the arrow O. The lens is assumed to be positive with the object beyond its principal focus F. In considering

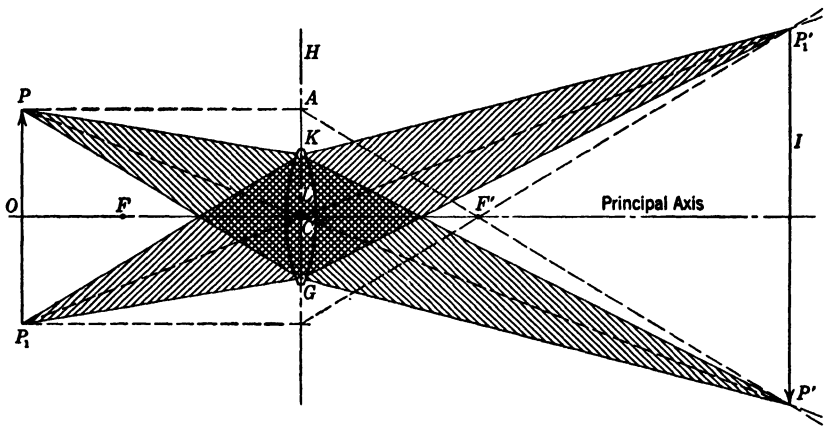


FIG. 87. The geometrical construction of image formation, when the object is beyond the first focal point.

The shaded portions indicate the path of light flux from the points P and P_1 of the object O; the broken lines indicate the geometrical construction necessary to locate the image points P' and P_1' of image I. This same method of showing the paths of light flux and geometrical construction is followed in Figs. 88, 89, and 90.

the lines as representing rays, the first line drawn (the one parallel to the principal axis) is called the *parallel ray*. The second line is called the *undeviated ray*; it passes through the center of the lens, and, as has been shown in Sec. 48, its direction is not changed, although it may be slightly displaced when it emerges from the lens. The dotted lines in the drawing are intended for construction purposes only. After the image points are found the lines indicating the effective rays can be inserted; they are, for image point P', $PKP'G$, and are shown by solid lines. All rays within this area from point P pass through the lens; all the lens area is utilized.

When the object is of some size, limiting points of the image can be found and connected. Any number of such points may be so found.

As the object is moved away from the lens, the conjugate focal plane I moves toward the lens until the object is at infinity, when all the rays come to a focus at F'.

Figure 88 shows the construction of the image when the object lies within the focus of a positive lens; a virtual image results. The geometrical construction is similar to that in Fig. 87 except that the lines are continued to form the image on the front side of the lens. The image is erect. The entrance pupil of the eye is depicted as coin-

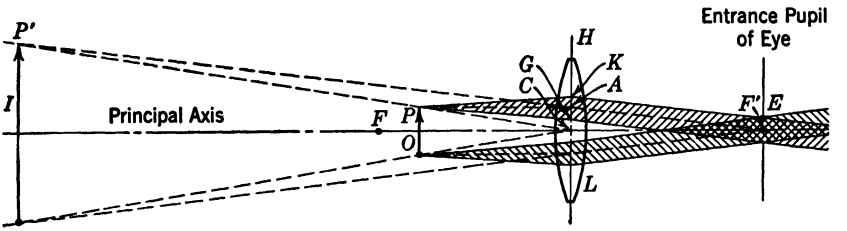


FIG. 88. The geometrical construction of the image when the object lies within the focus of the lens. The shaded portion shows the trace of the light flux from two points at the object O.

cident with F' at E. The effective rays are indicated by solid lines and the diagram shows the reason why for optimum use of a hand magnifier, the eye should be placed at the second principal focal plane. It is also shown that only a portion of the lens is used for a point object when the lens is larger than the pupil of the eye; thus the entrance pupil of the eye coincides with the exit pupil of the lens.

If the object is at the principal focus of the lens as in Fig. 89 the emerging rays (drawn exactly as directed for the two preceding diagrams in Figs. 87 and 88) will not meet but will be parallel to each other. If the object is a point on the principal axis, all emerging rays will be parallel to the principal axis, and a parallel beam of light will result. With a large object, as, for instance, the ribbon filament of a lamp, the parallel and undeviated rays will be parallel to each other, and the result will be called parallel light. However, only the rays are parallel to each other; the beam is diverging, as smoke blown across its path will show and as exemplified by Fig. 89. A microscope lamp adjusted for parallel light will always show beams diverging from the lens because the source can never be a mathematical point. When focused for parallel light, the lamp is sometimes said to be focused for infinity, since parallel lines are said to meet at infinity.

To return to Fig. 88. If the object lies within the focus of the lens, the lens will be acting as a hand magnifier, as shown. The undeviated

and parallel rays are drawn as before but when extended they will be found to meet on the front side of the lens, and a virtual image is formed at *I*. However, the system is not completed unless another lens or an eye lens and retina are shown. A real image is then formed on the retina by the diverging rays, and, since they seem to originate at *I*, the visual sensation is as if the object were at *I*. *I* is in the plane of the virtual microscope image. Figure 88 also indicates why the field

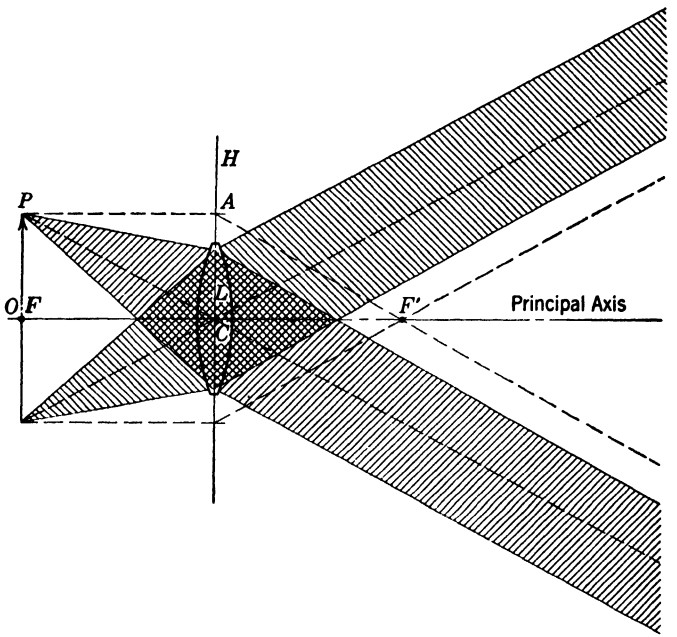


FIG. 89. The geometrical construction of the image when the object lies at the first focal plane. This results in the series of parallel rays. If the object were a point the resulting rays would form a beam on the back of the lens.

of view is decreased in size when the eye is moved away from the exit pupil of the lens which is coincident with exit pupil of the eye, at *E*.

The diverging or negative lens is shown in Fig. 90. The virtual image is found by extending the *p* ray through the focus on the front side of the lens. It is an erect image. A real image is formed by the negative lens only when it is receiving converging rays. In microscopy it is used largely in combination with other lenses to modify their action, and for the purpose of introducing corrections in a positive system. The negative lens, when used as in Fig. 90, gives a virtual erect image smaller than the object. Added to a positive lens, the negative lens forms a combination of lower power than when the posi-

tive lens is used alone. The relationship is shown by

$$\frac{1}{f} = \frac{1}{f_1} + \frac{1}{f_2} - \frac{a}{f_1 f_2} \qquad [32]$$

where f equals the focal length of the combination, f_1 and f_2 the focal lengths of the respective lenses, and a stands for the distance between the lenses when measured between adjacent principal points. If a is so small that it is negligible the last term of the equation is omitted. It should be noted that in the series of diagrams in Figs. 87 to 90 the lens

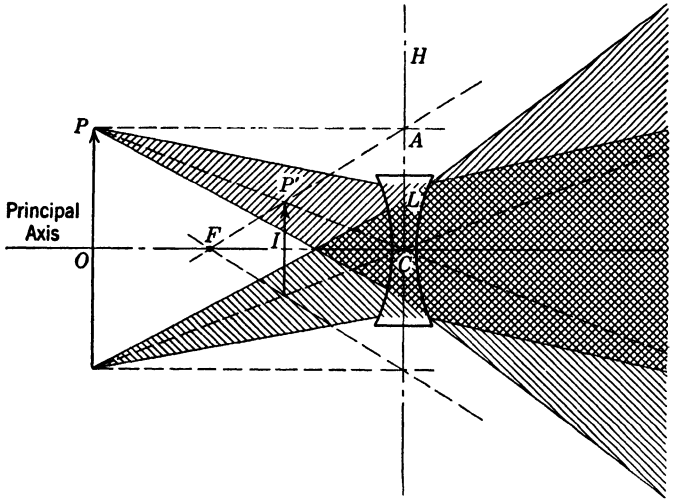


FIG. 90. The construction of the image when the lens is negative in character and the object lies beyond the principal focus of the lens.

was considered to be thin, and the principal planes coincided. If the lens is thick enough to make the position of the two planes important, the drawings can still be made as before after locating the cardinal points and leaving the space, between the lines representing the planes, blank.

It is of more than passing interest to observe from equation 32 that a lens placed at the focal point of another produces a combination with a focal length equal to that of the second lens. For this reason, an eyeglass worn at the first focal point of the eye will not alter the normal magnifying power of the eye.

Sec. 52. Resolution Theories. Resolution of a lens was defined from a practical standpoint in Sec. 13. In that section it was discussed as the power of a lens to form separate images of closely related detail in the object. Abbe's equation for resolution was taken for

granted and served its purpose for the argument in hand. The development of two theories, that of Abbe and that of John William Strutt, Lord Rayleigh (1842–1919), will now be given.

Rayleigh's thought on microscopical resolution was to the effect that, if the object were illuminated with a cone of light large enough to fill the aperture of the object glass, then Airy's equation as stated in Sec. 43 would apply. In equation 21

$$D' = \frac{1.22\,\lambda}{2n' \sin \theta'}$$

D' represents the diameter of the diffraction disc, or image of a small point source. If there are two such points to be imaged separately by the lens, their images must be separated, measuring from their centers, by the distance D'. They would then be resolved and would appear as two separate diffraction discs. Obviously such a statement is more or less arbitrary because of the lack of well-defined terms, for it might be perfectly evident that there were actually two points even if their images or diffraction discs overlapped by a half or even more. However that may be, in photomicrography, complete separation of the images of contiguous detail in the object is always to be desired. Therefore equation 21, indicating the smallest size of an object or the least distance apart two small objects may be for resolution, is of great importance to the microscopist.

Equation 21 has been used when speaking of the image, but it can be shown that it is also applicable to measurement in the object space. From equation 30, Sec. 49,

$$ny \sin \theta = n'y' \sin \theta'$$

$$\sin \theta' = \frac{ny \sin \theta}{n'y'}$$

assuming $n' = 1$

$$\sin \theta' = n \sin \theta \frac{y}{y'}$$

$$= \frac{n \sin \theta}{m}$$

Substituting in equation 21

$$D' = \frac{1.22\,\lambda m}{2n \sin \theta}$$

D' is still the diameter of the image but n and θ are values in the object space. By dividing the right-hand member by m, D will represent the

diameter of the object required to give an image the size of D'. Equation 33 will then follow, with all values in the object space.

$$D = \frac{1.22\,\lambda}{2n \sin \theta} \qquad [33]$$

From equation 4, N.A. $= n \sin \theta$, and angle θ is the angle θ of equation 33; therefore equation 33 can be written as

$$R = \frac{1.22\,\lambda}{2\,\text{N.A.}} \qquad [34]$$

where R equals the resolution of the system.

Equation 34, when applied to the microscope, assumes that microscopic resolution can be figured in the same way as resolution of other optical instruments, and, indeed, this was first stated by J. F. W. Herschel (1792–1871),[17] nearly half a century before Abbe developed his theory of resolution.

Using a fine grating as an object, Abbe sought proof for his theory of resolution by showing that the diffraction of light at the grating plays a part in image formation, and that, to resolve an object, the first-order and zero-order spectra must be included by the objective; also, that the " perfect image " is formed only when all the diffracted light is included or takes part in image formation.

Figure 91 illustrates more clearly the meaning of the above paragraph. O, the objective, embraces the first diffracted ray at A. This is the light that would go to make up the spectrum of the first order. Consequently the grating can be said to be resolved, and the two adjacent lines can be defined as such.

In examining the drawing, several facts are apparent. One is that it should be possible, to some extent, to control the amount of diffracted light entering the objective. This can be done by using light of shorter wavelength, it being remembered that blue light is diffracted less than red. The drawing would then appear as in B, Fig. 91. Or the aperture of the objective might be increased as shown at C; or, if the system can be immersed, the effect would be as at D; or, if the light is made oblique, the effect would be as at E.

Experimental proof of the Abbe theory can be attempted by taking closely ruled lines as an object, because the spectra of the different orders may be observed at the rear focal plane of the objective. If the aperture of the objective is reduced by a diaphragm, the resolu-

[17] See H. Moore, " Theories of Image Formation in the Microscope," *J. Roy. Micr. Soc.*, Series III, **60**, 140, 1940.

tion of the lines will vanish when the aperture has been reduced to eliminate the first-order spectrum. The Abbe equation (7), for resolution, was given in Sec. 13; it is

$$R = \frac{\lambda}{2\,\text{N.A.}}$$

2 N.A. may be thought of as being the sum of the apertures of the objective and the condenser. If the aperture of the condenser is much

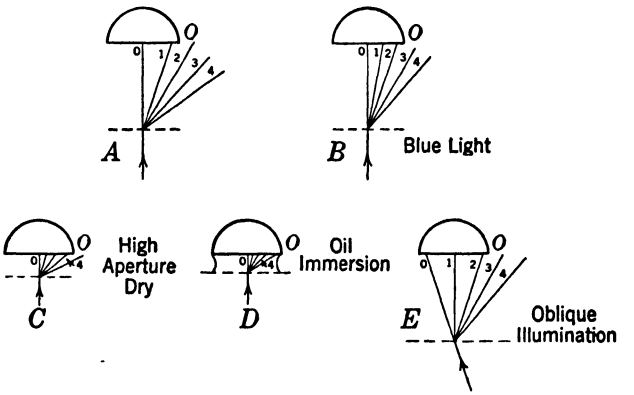


FIG. 91. Illustrating Abbe's theory of image formation. One undiffracted ray and at least one diffracted ray are required to form an image. *A*. The dioptric pencil, shown by ray 0, and the diffracted ray 1 are forming the image. *B*. Ray 0 and two diffracted rays enter the objective because blue light is used. *C*. A high-aperture objective grasps three diffracted rays. *D*. The addition of oil makes possible the use of four diffracted rays. *E*. Resolution is increased by oblique lighting, making use of two diffracted rays.

reduced, it is about the same as putting its value at 0; thus, leaving the N.A. of the objective alone, the 2 can be omitted from the denominator, and the formula becomes

$$R = \frac{\lambda}{\text{N.A.}}$$

This was given also in Chapter I. It would apply for central lighting of very narrow cones or for axial lighting.

Abbe's theory of resolution has been long and widely accepted. It has furnished an approach to a subject which otherwise might be difficult to understand, and, above all, it has been reduced to an easily handled equation. However, before Abbe's time and since then, other physicists have offered theories of resolution which have proved more tenable than that of Abbe. In effect, Abbe stated that image forma-

tion by the microscope differed from image formation under other conditions, as by the telescope, for the reason that a microscopic object was, in general, not self-luminous; but Rayleigh assumed that the Airy equation applied when the objective was filled with light by a proper condenser adjustment and that the image would then be formed as if the object were self-luminous.

Other theories of resolution and image formation have been suggested, and from time to time a great deal has been written on this subject. A recent theory of image formation is that of Max Berek, published in 1926 and described by Moore in 1928 and 1940, wherein the effects of consonant and dissonant light, components of coherent

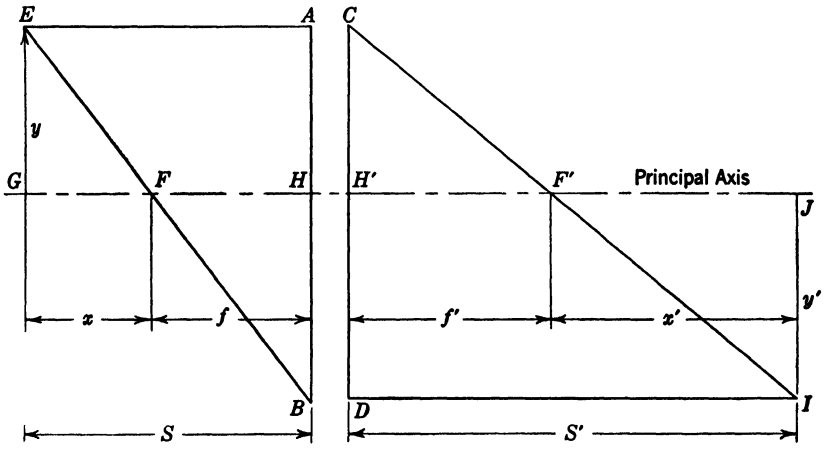


FIG. 92. Geometrical illustration of the relationship of various lens quantities. (After Hardy and Perrin.)

light, are discussed. However, all the theories of microscopic imagery agree that the greatest resolution is obtained, and the best image formed, when the exit pupil of the objective is filled with light, that is, when the condenser and objective apertures are about equal. The fact that such a condition cannot always be attained is another matter. As far as making a photomicrograph is concerned, it makes little difference what theories the photomicrographer holds regarding resolution or image formation. The question is largely an academic one, since the excellence of the photomicrograph depends entirely upon the perfection of the optical equipment and the manner in which it is used.

If the N.A. of a certain objective is 0.71, by means of equation 7, Abbe's equation of resolution, it can be shown that the resolving power in terms of lines per inch is 64,993 lines. If equation 34 is used the

resolving power will be 53,365 with the same objective and $\lambda = 0.555\ \mu$. This is a difference of 22 per cent between the two methods. However, the first method shows that the lens can resolve a particle 0.39 μ in diameter, and that by the second method it can resolve a particle 0.47 μ in diameter. The difference of 22 per cent still exists, but, since direct microscopic measurement of particle size cannot take into account such a small size difference as 0.08 μ, it is at once seen that, experimentally, the difference is beyond the power of the microscope to determine.

Sec. 53. Lens Magnification. Figure 92 shows the geometrical relationship between the lateral size of object and image as formed by a lens. AB and CD represent the principal planes of a lens, y the object, and y' its image (y being negative below the axis). The position of the planes is such that their magnification is unity; the rest of the drawing is self-explanatory. Triangles EFG and FHB are similar, as are triangles $CF'H'$ and $F'JI$. EA and DI are parallel to the principal axis. Thus from the figure the following equations can be written:

$$m = \frac{-y'}{y} \qquad [35]$$

$$m = \frac{-x'}{f'} \qquad [36]$$

$$m = \frac{-f}{x} \qquad [37]$$

$$m = \frac{-s'}{s} \qquad [38]$$

$$m = \frac{s'}{f'} - 1 \qquad [39]$$

$$m = -\frac{f}{s - f} \qquad [40]$$

$$m = -\frac{s' - f'}{f'} \qquad [41]$$

$$xx' = ff' \qquad [42]$$

$$\frac{1}{s} + \frac{1}{s'} = \frac{1}{f} \qquad [43]$$

The essential relationships shown in these equations are exemplified particularly by equations 38 and 43. Both should be memorized. Equation 38 is especially useful when working with microphotographic objectives, for it so clearly indicates the importance of realizing the connection between object and image distance and magnification. No matter how the object and image distances are controlled, by altering the camera distance, changing the lens for another of different focal length, using a telephoto combination, or altering the bellows draw, the ratio s'/s will equal the magnification of the picture. However, it is not intended to suggest that the magnification can be increased without limit, because a point will soon be reached where image deterioration sets in, and few microphotographic lenses give crisp images at bellows extensions greater than, say, 30 to 50 inches. Equation 43 can be used in conjunction with equation 38 to find the required focal length of a lens for any given situation. For high-power work, or even for work with a low-power objective when an ocular is used, neither of these two equations has much importance. Again, a little consideration of equations 36 and 38 shows why a longer-focus lens is resorted to, in ordinary photography, to give a higher magnification, and why a shorter-focus lens accomplishes the same purpose in photomicrography.

As already mentioned, the value x' corresponds to the optical tube length of the microscope. The magnification of the microscope is often given as tube length (the mechanical tube length being assumed because the optical tube length is seldom known) divided by the focal length of the objective. From an examination of Fig. 92 it is easy to see the relative amount of error introduced as the focal lengths of the objectives become longer. Thus, with a 16-mm objective, the error might not be large, but if the objective had a focal length of 32 mm the optical tube length might be 130 mm, while the optical tube length of the 2- and 3-mm objectives would be about 180 mm. For intermediate objectives the optical tube length would have intermediate values. There is no counterpart for mechanical tube length in optical formulae. For accurate computation of the magnification of the microscope under any particular conditions the technician is strongly advised to make actual measurements as described in Chapter I. After the magnification of an objective is determined the optical tube length can be discovered by applying equation 36.

Equation 36 can be applied to both the objective and the eyepiece, assuming that the distance of the virtual image is 250 mm and that the optical tube length of a 2-mm objective is 180 mm. If f_1 is the focal length of the objective and f_2 the focal length of the eyepiece,

the magnification of the microscope is

$$M = -\frac{180}{f_1} \times \frac{250}{f_2} \qquad [44]$$

the minus sign indicating a reversed and inverted image. Considering the microscope as a whole, dropping the minus sign, and letting f represent its focal length, equation 44 can be written

$$\frac{250}{f} = \frac{180}{f_1} \times \frac{250}{f_2}$$

Simplifying

$$\frac{1}{f} = \frac{180}{f_1 f_2}$$

or

$$f = \frac{f_1 f_2}{180} \qquad [45]$$

In the microscope, the aperture of a lens and the exit and entrance pupils are really so closely involved with the mechanics of the instrument and with its adjustments that the description already given in Chapter I will be sufficient, and they will not be discussed again at this point. Those who feel the need for a more thorough understanding of microscope optics are recommended to consult a textbook on geometrical optics or a recent edition of *The Microscope*, Part II, by Conrad Beck. See also the Glossary, under "Entrance pupil" and "Exit pupil."

Sec. 54. Aberration of Lenses. There is a great difference in the performance of lenses. As an example, one of two objectives of equal focal length may give excellent images and definition over the complete field of view, while the other may give only a mediocre image in the central part of the field. The first lens may cost perhaps $135 and the other only $35. What then is the difference to account for so much variation in performance and price? Is the higher price warranted by the results obtained in visual work or photography? Is it possible to select one lens from others and be able to give a logical reason for the selection? An attempt to answer these and other questions will be made in the next few pages, to the end that the selection and use of photomicrographic optical equipment can be predicated on rational lines.

Figures drawn of a simple lens focusing a point or an arrow are not actually correct; in reality they are impossible of attainment. They

are included only for the purpose of demonstrating certain facts. Some of the figures have been redrawn to show errors inherent in a simple lens.

Outstanding errors in lenses are usually due to the presence of one or more of the seven important aberrations. Five of these can be classed under the general heading of spherical aberration, although this term is often reserved more specifically for one of the lens errors included in the general category of aberrations. The five are spherical aberration, coma, astigmatism, curvature of field, and distortion. The other two are chromatic errors and may be referred to as lateral and longitudinal chromatic aberrations.

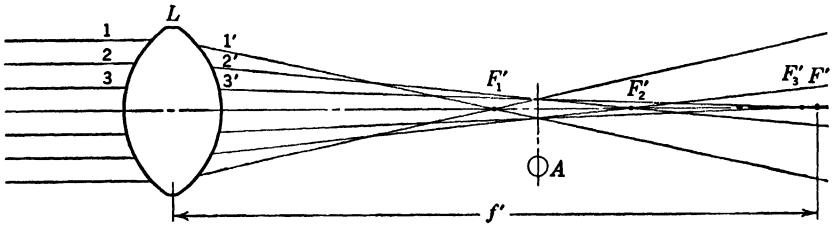


FIG. 93. The effect of spherical aberration; the reason is given in the text. The focal length for the lens L is f' for the central zone with the focal point falling closer to the lens, at F_1' and F_2' for the outer zones. The best point of observation is at A, where the cone of light is a small circle, instead of being at F', where, if the lens were well corrected, the image would appear as a small point.

Spherical Aberration. Spherical aberration occurs in the center of the field. If accuracy of shape is paramount, a lens can be ground to only a spherical or plane surface. The spherical surface may be either convex or concave. When a simple positive lens is made under these conditions, the rays of light impinging on it from a point source on the principal axis, beyond the principal focus, will not all be concentrated at a point on the back of the lens. Those rays which pass through the center of the lens are brought to a focus at a point farther from it than the rays passing through the outer zones. This is the condition spoken of as spherical aberration; it is shown in Fig. 93. The difference in position of the focal point for the various lens zones results in an image with a series of overlapping unfocused images each of which tends to give a fuzzy appearance to the main image. The circle at A shows how the light spreads out, away from any particular focal point, to cause a rather large circle of confusion. This condition is true even when the light is of one wavelength, but it must not be confused with the interference rings set up around the diffraction disc.

It is obvious that, when the above condition exists, the angles at which the light enters and leaves the lens in the different lens zones must be incorrect, and that if the angle can be altered so that all the light rays striking any part of the lens will be brought to a common focus then the lens will be free from spherical aberration. If the angle of incidence is changed by altering the curvature of the lens, and the ray in the image space has its angle of emergence from the lens also changed by the same process, then it would seem possible, by careful figuring of lens surfaces, to have all the rays that strike the lens surface, in any zone, emerge to a common focus on the principal axis. Actually, this is what is attempted when a lens is corrected for spherical aberration. The spherical aberration of an uncorrected negative lens is just the reverse of that of a positive lens; that is, the central rays unite nearer to the lens than the rays passing through the outer zones. Thus, if a negative lens of the proper strength is added to a positive lens, the spherical aberration of one cancels that of the other.

From the above paragraph it is logical to ask how is it possible to make a positive and a negative lens with errors of spherical aberration as great in one as in the other, and yet have them act as a lens when used together, and not as a piece of plane glass, giving zero magnification? The answer is that the shape of the lens is the important factor in controlling spherical aberration, and that relatively the aberration in one lens may be greater than in another, although both lenses may have the same focal length. This fact makes it possible to introduce corrections in a doublet and still have it maintain its lenticular qualities. Thus, a positive lens with a certain amount of under-correction may become a corrected doublet if a negative lens of much longer focal length and the proper amount of over-correction is added to it.

Considering Fig. 93, it would also seem that if the lens zones could be ground to different radii it would be possible to exert control over spherical aberration and to eliminate it from a lens by careful selection of the radii for the different zones. This is actually done in certain cases. The ensuing lens surfaces are said to be aspherical. The outer zones are made with a little less curvature than the inner zones. It is not possible to produce such corrections as precisely by this method as can be done by the addition of a second or even a third lens unit, but the aspheric lens is very satisfactory for many purposes and can be used to good advantage for condensers, light-collecting lenses, and bull's eyes. Some microscope lamps are equipped with condensers made of aspherically ground lenses so that the heat from the lamp will

not melt the cement which holds a doublet together. Under these circumstances a single lens unit is more practical.

Spherical aberration can be measured by finding the distance between the intercepts of an outer and a central ray on the principal axis, and converting this figure into per cent of f', which is the focal length of a paraxial ray. This gives the spherical error for one lens zone. In this way the correction of a lens can be defined very clearly. If the central rays focus at a greater distance than the outer rays, the lens is said to have positive aberration; if the reverse is true, the aberration is said to be negative. The term under-correction is used in microscopy to describe positive spherical aberration. The lens is said to be over-corrected when the aberration is negative.

A high-power lens can be well corrected spherically for only one object distance. This makes little difference in the microscope, because the optical tube length, and thus the image distance, are well established. However, the cover glass between the lens and the object is part of the optical path and must be taken into account when the lens is corrected. Since it has the effect of lengthening the optical path, corrections must be made not only for one object distance but also for one cover-glass thickness. A cover of less than standard thickness introduces under-correction; one of more than standard thickness introduces over-correction. If the objective is to be used oil immersed, the oil must have the proper refractive index; otherwise under- or over-correction will ensue. Correction for such aberration can be made on the microscope, to a certain extent. Increasing or decreasing the tube length will necessitate a change in the object distance. This or any other adjustment designed to permit the use of a lens at the object distance for which it was made is called correcting the lens. It is an essential adjustment for all medium- and high-power lenses.

Coma. Coma is a form of aberration caused by differences in magnification in the various lens zones, the difference being due, not to the position of the focal point, as in spherical aberration, but to the position of the second principal point. It occurs in the portions of the visible field away from the center. When coma is present, the aberration of a point will look something like a comma with the tail lying either toward, or away from, the center of the field of view. The term "coma" is from the word "comet," meaning long haired, with a tail, from the Greek κόμη. The comatic condition is said to be positive when the tail is toward the center of the field; negative, when the tail lies away from the center.

Figure 94 is the regular conventional drawing intended to denote the

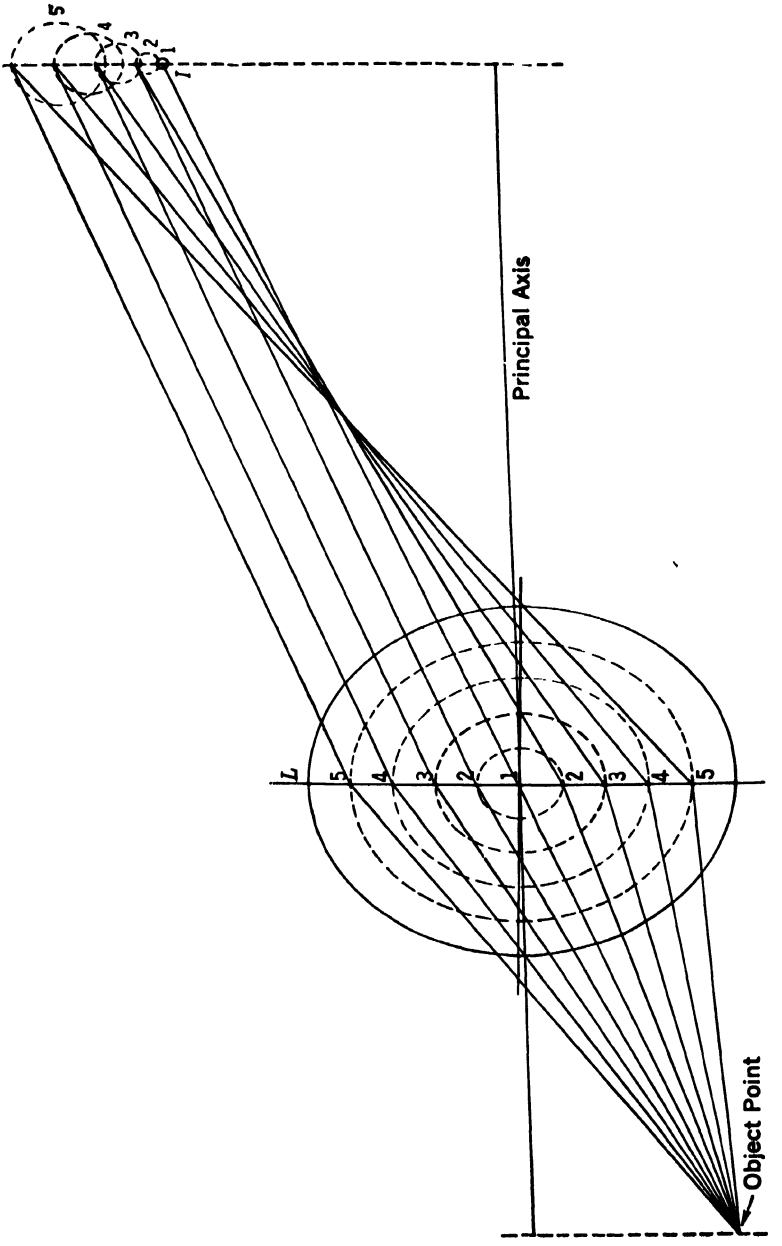


FIG. 94. A graphic representation of coma. This drawing was made from a photograph of a model. Black thread was used to simulate the rays, and a disc of transparent plastic represented the lens.

presence of coma. The central rays in zone 1 are shown converging to a point and forming an image correctly. The outer rays in zones 2, 3, 4, and 5 focus in different positions, either closer to or farther from the principal axis. In this figure they are shown away from the principal axis. This phenomenon is caused by the increased or decreased focal length of those particular lens zones. The difference in focal length of the outer zones is due to the faulty position of the second principal plane for these extra-axial points.

The rays shown in the drawing lie on one lens diameter. If all the rays of all the other diameters could be similarly shown in the drawing, the ensuing image would have a point as at I-1, and each successive zone of the lens would have its series of points placed in a circle. Because increased focal length has increased the magnification of the outer zones, each successive circle will lie farther and farther from the principal axis and be larger than the previous one.

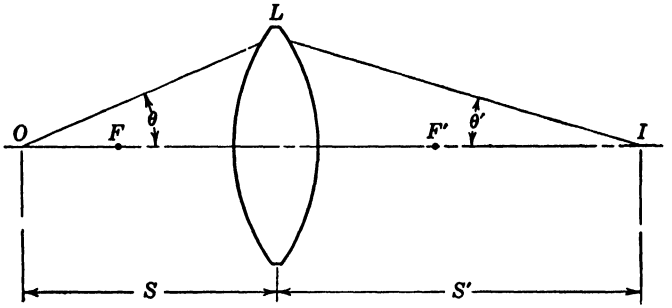


FIG. 95. To satisfy the sine condition of Abbe: $\frac{\sin\theta}{\sin\theta'} = \frac{s'}{s}$.

The higher the degree of coma, the farther from the principal axis will any extra-axial point appear. The final appearance of the figure is not at all as shown, except in outline, because the image is a composite of overlapping images of an infinite number of zones. The result is a rather fuzzy tapering figure.

Abbe has shown that when a lens is corrected for coma the ratio of the sines of the angles of incident rays to the refracted rays equals a constant, and that this constant is the magnification of the lens when the same medium for light transmission exists on both sides of the lens. When the magnification of the different lens zones for the extra-axial points in the field is equal, there is no coma. Figure 95 makes the mathematical expression

$$\frac{\sin\theta}{\sin\theta'} = \frac{s'}{s} \qquad [46]$$

quite clear. It might be noted that the fraction s'/s expresses the magnification of the lens. The correction for coma was referred to by Abbe as the *sine condition.* When the sine condition is not fulfilled, the lens suffers from coma, although the spherical aberration existing at the center of the field may be well corrected and in this small part of the field the images may be excellent.

As with spherical aberration, some one object distance must be selected at which the lens must be corrected; the lens cannot be correct for more than one object distance at one time. The second conjugate focal length, which is the optical tube length of the microscope, must always be considered as constant for purposes of design.

Aplanatism. A lens is called aplanatic when it is corrected for coma for, say, two-thirds of its field diameter and is nearly free from coma for the remaining third, and is at the same time free from spherical aberration. A lens can be aplanatic for only one definite object distance. Condensers and other lenses are sometimes said to be aplanatic, meaning that they are well corrected for spherical aberration and coma when used under certain conditions. Methods for discovering what these conditions are and when they are attained in practice are of vital importance to the photomicrographer. This more practical part of the work will be handled in detail in Sec. 65. A condenser lens when stopped down to eliminate errors in its outer zones is said to give an aplanatic cone of light.

Astigmatism. In a lens, astigmatism deals with unequal magnifications in different azimuths; in an eye, it refers to defective shape of the cornea. The Greek στίγμα means spot or point; the addition of the prefix *a* makes it mean not a spot or point. Thus a lens giving a stigmatic image would show images of points as points, while an astigmatic lens would give images of points as blurred lines or discs. The addition of yet another negative prefix *an* forms the word anastigmat, which indicates that the astigmatic condition of a lens has been corrected. Since uncorrected lenses are normally astigmatic, this term and the term anastigmatic are often used, the former referring to the uncorrected and the latter to the corrected condition. Astigmatism is very difficult to show graphically and more difficult to visualize clearly. However, it is well worth while to try to form a clear idea of the subject, for other errors and their corrections will then be made more understandable.

Astigmatism in a lens, in its highly developed form, is found in the outer field zones, beyond the errors commonly called coma. When it exists, a point as O, in Fig. 96, is imaged as a disc, at I_3, which does not appear particularly sharp. At I_1, the image of the point is a line

lying in a position tangent to the field. This image plane is called the primary or tangential plane. At I_2, the image of the point is a line lying in a radial position in the field; it is variously called the secondary plane, sagittal plane, radial plane, or it may be known by other names. When the primary plane lies nearer to the lens than the secondary plane the astigmatic condition is said to be positive; if it lies beyond the secondary plane it is said to be negative.

The object O is shown as below the axis. The rays passing through the lens in the vertical plane come to a focus at I_1, and those passing through the lens in the horizontal plane focus at I_2. Rays through other planes pass through these two line images. The primary and secondary fields, shown at I_1 and I_2, are curved, and astigmatism does not exist at the center of the field because at the center the curved surfaces coincide. Hence correcting a lens for astigmatism attains, as far as possible, coincidence of the two curved surfaces. All the points I_1, I_2, and I_3 will coincide, and a stigmatic image of O will be formed in the outer as well as in the inner field zones. Continuing reasoning along this line, it would seem as though a properly placed stop, which causes, more or less effectively, a flattening of the image field, would also reduce astigmatism. In fact this is a control which has long been recognized in the photographic art, but in order for it to be successful, the equation well known as the Petzval condition must be satisfied, that is

$$n_1 f_1 + n_2 f_2 = 0 \qquad [47]$$

When there are two lenses, the sum of the product of their refractive indices and focal lengths must equal zero.

It is possible to evolve formulae for the lenticular conditions which produce the three aberrations mentioned: spherical aberration, coma, and astigmatism. By placing the value of zero on one side of the equation which represents the numerical value of the error, it is possible to solve for optimum lens shape, refractive index, and separation of the lens elements.

Curvature of the Field of View. The two image surfaces shown in Fig. 97 illustrate astigmatic conditions. When correction is applied, the result is a curved surface C which is the locus for the sharpest image formation. It is intermediate in position between the primary and secondary image surfaces A and B. With photomicrographic objectives and some other forms of lenses this final result of a curved image surface is a serious drawback to the proper functioning of the lens. A compromise in correction is effected which appears to flatten out the image surface somewhat, but at a sacrifice of extreme sharpness

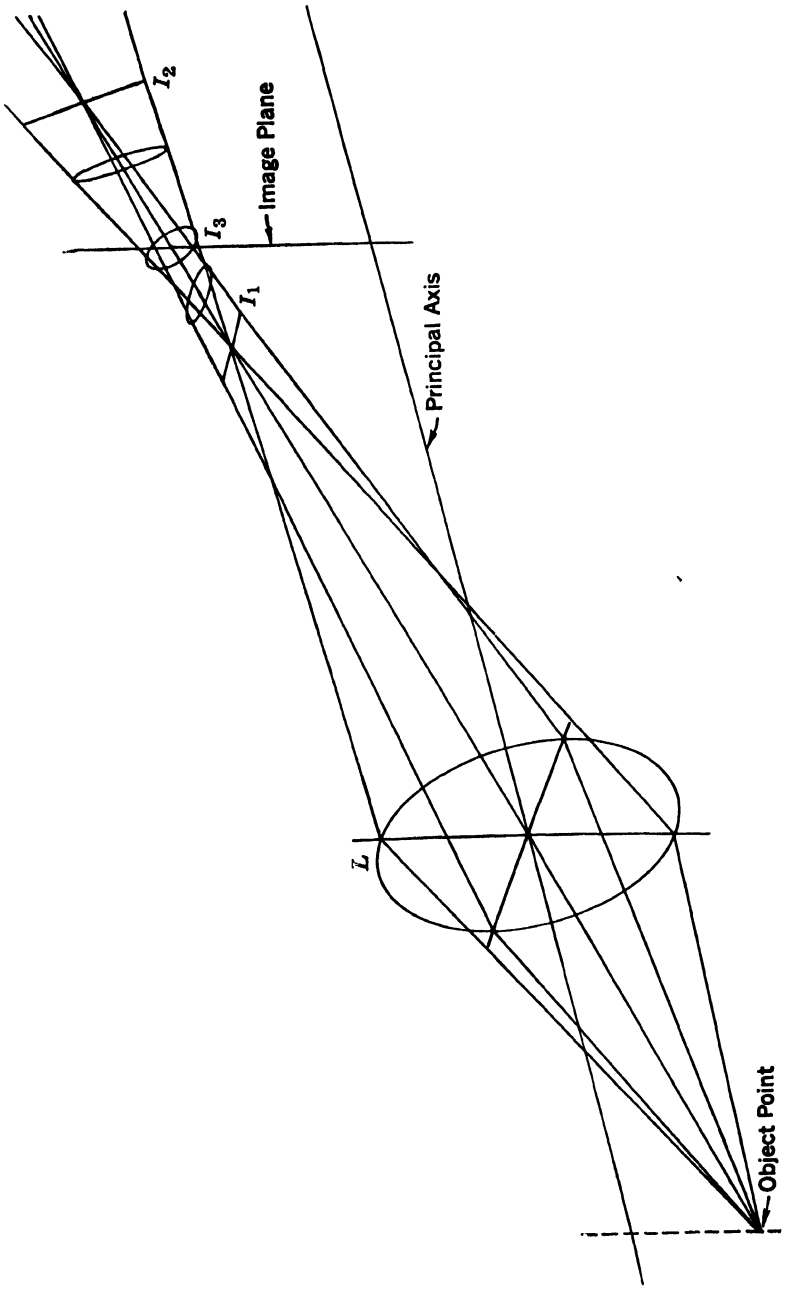


FIG. 96. Astigmatism is a form of aberration in the outer lens zones and is due to unequal magnification in the different azimuths.

of image. A diaphragm often aids in this process, for it can be shown that a diaphragm placed in the correct position tends to eliminate the more oblique rays and to give the appearance of a flatter image field.

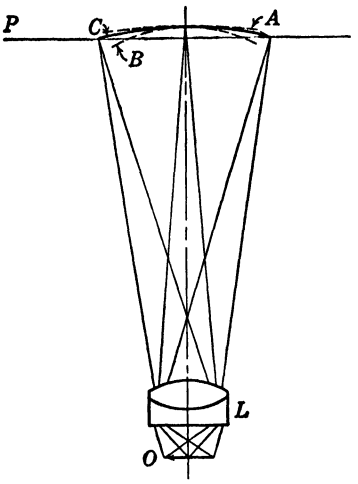


FIG. 97. Field curvature. Another form of aberration that exists in the field of view, or in the image field; it is particularly objectionable in photomicrographic work.

In Fig. 97, only the points of the surface C which intersect the plane of the plate P are in focus.

Owing to demands for an extremely sharp primary image at the ocular diaphragm, microscope objectives have, as a rule, severe curvature of the image field. In general, it has not been found practical or even possible to maintain high apertures and sharp images and simultaneously have a large flat field. Therefore, little attempt has been made to correct this error of field curvature. In fact, the best high-aperture apochromatic objectives have a highly curved image field. This curvature is no handicap for visual work because the focus can always be slightly shifted to compensate for it, but for photomicrographic work it introduces complications. It has been found that the microscope may be made to give a fairly flat image field for photographic purposes, with but little or no loss in the excellence of the image if a negative lens such as a Homal or Ampliplan is substituted for the ordinary ocular.

Lower-power objectives generally, and the special flat-field objectives of Zeiss in particular, give comparatively flat fields, but in the first instance the magnification is low and in the second instance the numerical aperture is low.

Distortion. This error is rare in objectives, but it occurs more frequently in oculars. Figure 98 is a photomicrograph of an Abbe test plate. A straight edge laid along the upper and lower bars shows a decided inward curve at the center. This is an example of positive distortion. Negative distortion would produce curvature in the opposite direction. Distortion is caused by unequal magnification in various zones in the visual field. It can be noted that the lines lying near the center of the field have less distortion, and that at the center distortion is completely absent. It is very easy to test for distortion, and it can generally be eliminated by changing the ocular.

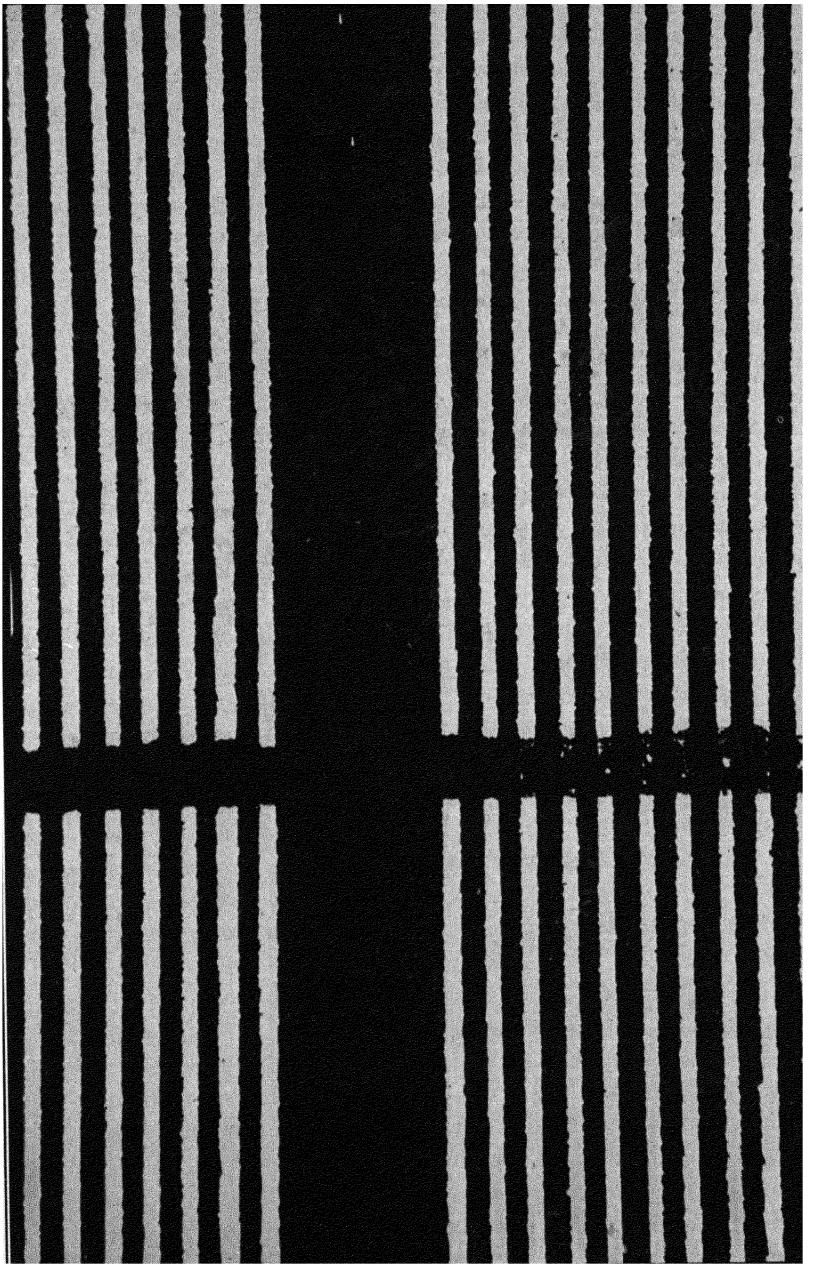


FIG. 98. Distortion. This is an example of positive distortion. The outer lines of the ruled silver film are curved.

On small particles, imaged at the periphery of the field, distortion would not be detectable, but it might interfere a little with accurate micrometry if the points between which measurements were being made were far enough apart. Distortion seldom introduces serious errors, even in the most exacting work.

Longitudinal and Lateral Chromatic Aberration. In the discussion of spherical aberration, the effect of a lens upon light of various colors was not taken into consideration. Monochromatic light must be assumed when tracing a light ray because of the dispersion of the glass, and to attain most exact corrections for spherical aberration the index of refraction of the glass must be known within four or five decimal places for the several colors. The study of chromatic aberration takes into account the variation of the index of a lens for light of different colors; it shows the optical errors arising therefrom and indicates how they may be corrected.

Two kinds of serious chromatic errors are possible, one longitudinal and the other lateral. Figure 99 illustrates longitudinal error. It is analogous to spherical aberration and shows that light of short wavelengths, at the blue end of the spectrum, is brought to a focus at a position closer to the lens than the light of longer wavelengths, at the red end of the spectrum. The remaining colors are focused at intermediate positions according to their spectral order. Thus, the focal points of heterochromatic light in an uncorrected system lie between F_B' and F_R' and are comparable to the range of colors on the screen S, at A, between B and R. As the microscope is focused first slightly too high and then slightly too low, the different colors can be brought into view. *Longitudinal chromatic aberration, then, is caused by the separation of the focal points for light of different wavelengths, the blue light focusing closest to the lens.* This is a normal condition of an uncorrected lens.

If the focal points of the above lens system are made to coincide, chromatic error still persists. This is lateral chromatic aberration, caused by unequal magnification for light of different colors. That is, the focal length of the lens is not the same for all colors of light. While the focal points were made coincident to eliminate longitudinal error, the focal length for light of different wavelengths varies because *the position of the principal planes of the lens are different for each color of light.* The effect is to give the unequal magnification already mentioned. The focal length for the red light is greater than that for blue light; that is, if an object is examined in white light with an uncorrected lens, lateral chromatic error will result in the formation of an image surrounded by a red outline which in turn is surrounded by a green

and then a blue outline, the result being a fuzzy image. The red image is the smallest and the blue the largest.

Some lateral chromatic error occurs in apochromatic objectives, because to neglect it will permit finer correction for spherical error. For this reason the compensating eyepiece is designed to give a shorter focal length for the red than for the blue light, thus compensating for the residual chromatic error of the apochromatic objective.

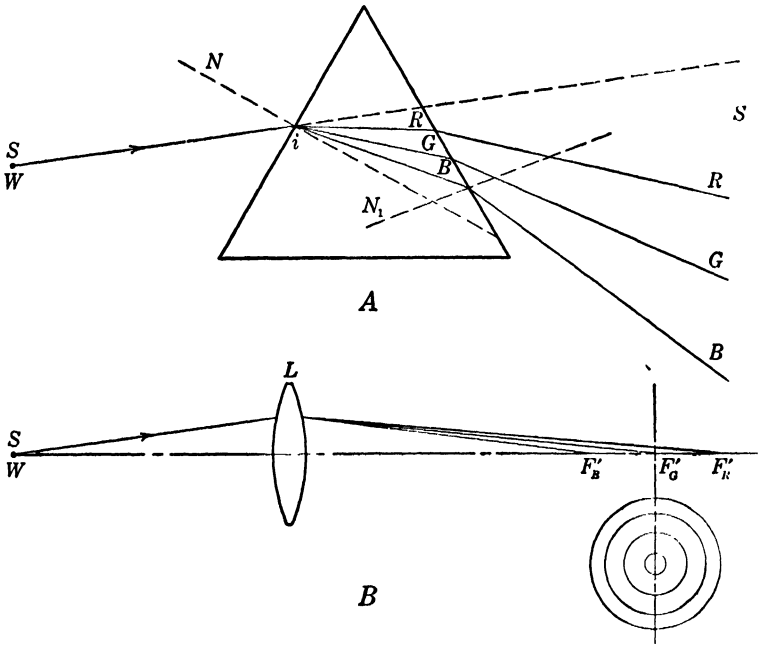


FIG. 99. Chromatic aberration of a lens is compared to the aberration produced by a prism. A prism can be made achromatic as well as a lens. It should be noted that if lens L is moved toward S, the source of white light, an image of S will be formed at F_G', and this image will be sharp if a blue filter has been inserted in the optical path. The blue image will then be larger than if the lens is focused for red or green light.

In the construction of a lens it has been found that, by using materials of different dispersion values and by properly spacing the different lens units, a lens can be well corrected for three colors simultaneously, so that the final optical effect for practical work will be complete achromatism from the blue end of the spectrum to the red. Mathematically, in such a correction, the ratio of the dispersion of the glasses (lenses) is set equal to the ratio of their focal lengths. This can be done without interfering with the correction for the other aberrations.

A lens corrected spherically for two colors, and having the other corrections mentioned, was figured by Abbe in 1886. It was a microscope objective called an apochromat. Previous to 1886 the best lenses available were achromats. These are still the most common type; they have color correction for two wavelengths and spherical correction for only one. Fluorite, as first used by Abbe, proved to be the material with optical properties which made the finer corrections possible.

The wavelengths for which an achromatic lens is generally achromatized are the F and C spectral lines. In the apochromat the corrections are extended to the G line. Special-purpose lenses may be made with very exact chromatic and spherical corrections for one color only; they are called monochromats. Such is one of the flat-field objectives in the new Zeiss series. It is corrected for use with the mercury line 546 mμ. A series of objectives made by Bausch and Lomb, but now discontinued, were corrected for the 365 mμ line, ultraviolet radiation, and for the green line for focusing. Doubtless other monochromats may be expected in the near future.

Color corrections are never complete, and under certain conditions this gives rise to a color fringe called the secondary spectrum (achromats). A little yellow or yellowish green usually shows within the focus, and a little red or light pink above the focus. The tertiary spectrum is still fainter, but it can be seen as a residual color error for the apochromatic objectives. When the color corrections are intermediate, the lens is said to be semi-apochromatic. That, in the high powers, one type of lens verges very closely on the other type in the matter of corrections is well shown. To attain maximum spherical corrections, an appreciable amount of chromatic error is neglected in both apochromatic and achromatic objectives in the high powers and the compensating oculars, specially designed to rectify this residual error, can be used to advantage with either achromatic or apochromatic lenses. However, the over-correction of the compensating eyepiece will cause color fringes when it is used with an achromatic objective of low power.

The foregoing may give rise to the following question. When a lens is corrected spherically for one color and chromatically for two, of what use is the second color correction if the spherical corrections are limited to one color? Strictly speaking, the achromatic objective is almost perfectly corrected spherically for light of one color, even over a large field, and well corrected for light of two colors in the center of the field. The apochromatic objective is well corrected in the center of the field for light of three colors. The art of lens making has advanced to such a degree that the modern achromats can be said to be exceedingly well

corrected spherically and chromatically for two colors and the apochromatic objectives for three, particularly if the aperture of the condenser is somewhat reduced. This was recently demonstrated by the author in certain color work using the three-color process. The color images were measured, and, as near as the eye could detect, they were of equal size and all fell in the same focal plane. The objective used was a Zeiss 8-mm achromat.

Methods of measuring the lateral chromatic errors of apochromatic objectives are given by Gardner and Case.[18] Also included is a method for measuring the distortion of oculars.

The chief points in the foregoing discussion of lens aberrations can be briefly summed up as follows:

1. A simple lens has seven important aberrations which are inherent, namely, spherical aberration, coma, astigmatism, curvature of the field, distortion, longitudinal and lateral chromatic aberration. Chromatic errors and spherical aberration are the most important, since, unless suitable correction is applied, only a very small portion of a lens will be useful for visual work.

2. Corrections in a lens can be made by slightly altering the curvature of the different lens zones. The lens is then aspheric. Good lens systems, such as fine microscope objectives, are corrected by the addition of other lens elements which have errors that compensate for those already in the system. The first lens unit of an objective usually affords the magnification; the successive units afford correction.

3. A lens is said to be aplanatic when the spherical aberration and comatic error are abolished for one object distance.

4. An achromatic objective is said to be corrected spherically for one color and chromatically for two.

5. An apochromatic objective is said to be corrected spherically for two colors and chromatically for three. This statement and that in item 4 are tending to have less and less significance.

6. Corrections for microscope objectives should be carried well toward the periphery of the field. This is not always done.

7. The performance of an objective can often be improved by proper selection of the ocular.

8. A highly curved field may, and usually does, exist when the finest lenses are used. The curvature of the field is immaterial for visual work; in fact, it aids in keeping the attention fixed on a small part of

[18] I. C. Gardner and F. A. Case, "The Lateral Chromatic Aberration of Apochromatic Microscope Systems," *Research Paper* 316, Bureau of Standards, 1931.

the field. When the camera is used, the curvature can be fairly well compensated for by means of an amplifying lens such as the Ampliplan or Homal.

Sec. 55. Objectives. There are many kinds of objectives made by the various companies, and to a very large extent they can be used with eyepieces of a different make; sometimes the best results are obtained in this way. However, satisfactory combinations are likely to be due to chance rather than to plan. Consequently such matings of objective and eyepiece must be carried on in an empirical manner. Certain assumptions can always be safely made regarding the selection of objectives, and a few fundamental rules will serve as guides.

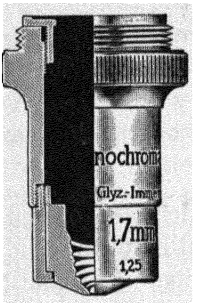


FIG. 100. Sectional view of quartz monochromatic lens by Zeiss.

The mechanical tube length for which the objective was made must be observed. If the objective is an achromat of high power, or an apochromat of any power, it will probably deliver its best images when served with a compensating or other well-corrected ocular. Low- and medium-power achromatic objectives should be used with Huygenian oculars of low or medium power or with oculars of intermediate correction. Some objectives are made for use on an uncovered specimen, and this correction generally necessitates a tube length in excess of 160 mm. All objectives made for immersion should be immersed in use. The condenser should always have a maximum aperture at least equal to that of the objective. Monochromatic objectives are made for use only with light of one color. Figure 100 illustrates the quartz monochromatic objective made by Zeiss. It might be compared with the achromatic and apochromatic series of objectives shown in Fig. 116.

Sec. 56. Achromatic Objectives. The achromat is the most widely used and most popular objective. Its price is moderate, being about one half to one third the price of a corresponding apochromat. Table XVII at the end of this chapter lists many of the available focal lengths. Fluorite or semi-apochromatic lenses as they are sometimes called, have superior achromatic corrections, but they are generally classed as achromatic objectives. They are made especially in the shorter focal lengths. Cross sections of achromatic objectives and of corresponding apochromats are shown in Fig. 116.

The achromatic lens is made in a much wider range of focal lengths than the apochromatic type. It may be had in focal lengths of 55 mm or even longer, down to about 1.8 mm, the corresponding magnifications being from 1× to 100×.

The achromatic objectives of low power must be used with Huygenian eyepieces. As far as the author has had opportunity to determine, compensating eyepieces will give color fringes when used with low-power achromatic objectives. The medium-power objectives work well with low-power Huygenian eyepieces or with those of medium power if they have intermediate correction, such as the Periplan ocular of Leitz, the Hyperplan of Bausch and Lomb, the Orthoscopic of Zeiss, or the Planoscopic of Spencer. The eyepieces of the Huygenian type may have a magnification of 8 or 10; with better corrected oculars it may be around 12 or 12.5.

The high-power achromatic objectives, particularly those of fluorite, are best used with compensating eyepieces, if they are very good lenses; otherwise eyepieces with intermediate corrections should be used. The final magnifications so obtainable are never as high as can be had with apochromatic systems. In general the achromats work best with an eyepiece of 10 power or lower.

It is to be expected that if a lens is corrected for spherical aberration in the yellow-green, for best results it should be used with a yellow-green filter. Certainly the best images are formed when such screens are used. However, with all lenses below the 4-mm, the images are excellent for even photomicrographical purposes when used with light of daylight quality. When the subject is colored, and it is desired to register the color gradations, a daylight screen is essential. Under these conditions the achromatic objectives may work well if the magnification is not too high. There is some difference in the quality of various makes, and to a certain extent in individual lenses, these differences being more marked in the high powers. Each lens must be judged on its own merits. Critical visual observation is required in making a decision between two lenses.

Practically all very low-power work must be done with either achromatic long-focus microscope objectives or with short-focus photographic lenses. Probably the longest-focus apochromatic objectives listed at the present time are made by Beck and Swift. They have a focal length of 40 mm; Zeiss manufactures one of 25-mm focus superseding one of 30-mm focal length by the same company. With these exceptions, the 16-mm lenses are the longest-focus lenses of apochromatic quality obtainable for the microscope. In the achromatic series, as already stated, and as the table of lenses shows, much longer focal lengths can be had.

Sec. 57. Apochromatic Objectives. This series of microscope object glasses represents the best of the lens maker's art. There is nothing finer in optics than an apochromatic objective of high aperture like

N.A. 1.40. Because of the high prices that such objectives bring, they are not used as much as they deserve to be. It is important to treat these objectives with the greatest of care, guarding them not only against accidental physical injury, which in common applies to all lenses, but also against misuse on the microscope. Care must be taken to see that all microscope adjustments are made correctly; otherwise a very fine apochromatic lens may give an image that is inferior to that of a lens of poorer quality. Unless the technician knows how to use his microscope to the best advantage, it would be better for him to buy a cheaper lens. This applies to both photomicrographic and visual work.

When using apochromatic objectives glare must be eliminated not by reduction of aperture alone but as far as possible by other means as described in Sec. 102. The condenser of the microscope must be critically focused, and for the ultimate resolution it is necessary to employ Method II for illumination.

Unfortunately, the apochromats give a field which is considerably more curved than that given by the corresponding achromats, and photomicrographs made with apochromats may vary a good deal in the diameter of the sharply focused part of the field. Figure 101 shows two photomicrographs taken with different objectives of equally rated focal length, one an achromat, at *A*, and the other an apochromat, at *B*. No eyepiece was used. The strongly curved field of the apochromat can be noted. The pictures should be studied with a hand magnifier.

Since resolution has been shown to be a function of wavelength and N.A., the photomicrographer should pay a great deal of attention to these independent variables when he uses apochromats of the higher powers. As a rule, with these objectives light of daylight quality can be used, modified as required with a light green filter, such as Wratten No. 66 or Corning No. 428 or 430. With the mercury arc lamp, the monochromatic blue filter of Corning or Wratten is recommended for the highest degree of resolution.

The apochromatic series of lenses does not include as many different focal lengths as the achromatic series. In the apochromatic series most manufacturers supply a 16-mm, 8-mm, 4-mm, 3-mm, 2-mm, and 1.5-mm. The 3-mm and 2-mm are generally made in two different apertures, N.A. 1.3 and 1.4. The Zeiss Company furnishes a 25-mm apochromat for a long-focus lens. This lens deserves to be more popular that it is. The same company lists for photomicrographic work two special apochromatic objectives having focal lengths of 5 mm and 2.9 mm, with apertures of 0.85 and 1.0, respectively.

Figure 116 shows the assembly of a 3-mm apochromatic objective. It has one triplet, two doublets, and two single lenses — nine elements in all.

Two apochromatic objectives fitted with correction collars are worth discussing; they are the 4-mm and 3-mm. Their use is often neglected on account of ignorance about their proper adjustment. The N.A. of

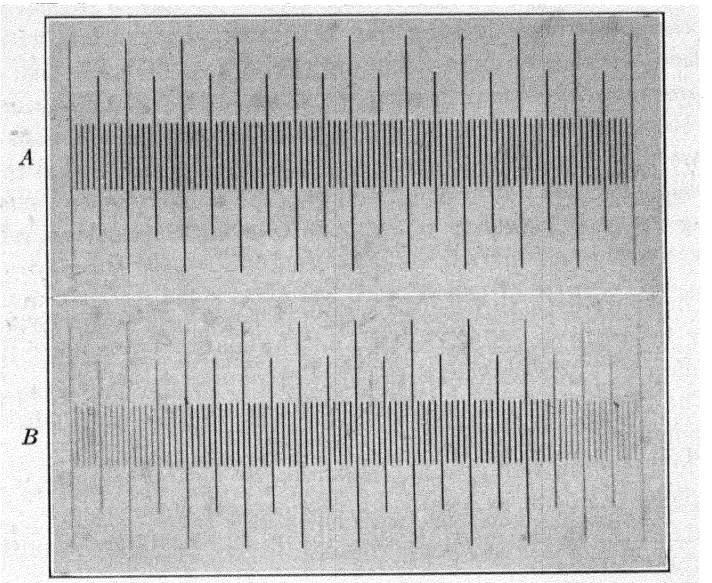


FIG. 101. *A* is a photomicrograph of a micrometer scale taken with an 8-mm. achromatic objective. *B* is a similar photograph taken with an apochromatic objective. Both photographs were taken without an ocular. They clearly show the stronger field curvature of the apochromatic lens.

these lenses is 0.95; this is as high as can be had with a dry system. Since they are apochromats with a high aperture, their images are noticeably affected by any departure from standard cover-glass thickness, and their field depth is very small. The correction collars are provided to separate the two rear lens combinations from the front elements or to bring them closer together. This adjustment controls the focal length of the lens and the positions of the principal points and so affects the spherical corrections. The collars are marked with numbers ranging from 10 or 12 to 20 or 22. These numbers read as hundredths of a millimeter show the setting required for cover glasses of corresponding thickness. With a cover thicker than normal, the additional glass lengthens the optical path from the object to the lens and

causes over-correction, but turning the collar to a high number introduces under-correction to compensate the over-corrected condition caused by the thick cover. It will be shown later how the correction collar can be quickly and properly set to give the optimum image with various thicknesses of cover glass. The 4-mm lens of this series is specially recommended, but probably the 3-mm oil-immersion is a better choice than the 3-mm dry objective.

Apochromatic objectives, as already mentioned, work best with compensating eyepieces, unless the projection lens is used for photomicrography. The compensating ocular should be of high power, preferably 15× or 20×. The ability of these lenses to take a high-power eyepiece should be fully utilized; otherwise the fine detail that they are capable of bringing out in a photomicrograph is likely to be lost.

Some essential points to remember regarding the use and selection of objectives are as follows:

1. The achromatic series of lenses offers a much wider choice in focal lengths than can be obtained with apochromats. One maker lists 27 achromats to 13 apochromats in a corresponding series.
2. The N.A. of apochromats is 4 or 5 per cent greater than that of corresponding achromats.
3. The magnification attainable by the use of apochromats is somewhat higher than can be attained by the use of achromats.
4. Green filters aid in reducing glare in achromats and tend to improve their images.
5. An achromatic lens with a magnification of 1 can be obtained for very low-power work.
6. Unless high-power apochromats are used with considerable skill the image they produce may be inferior to that from a much cheaper lens.

Sec. 58. Special Objectives. *Dark-Field Objectives.* There are numerous special-purpose objectives designed for special optical tasks. One of the most important is a type, made by most optical companies, for work with high-power dark-field condensers. Since this work does not demand high magnification, these objectives are generally made with a focal length of about 3 mm. They are either equipped with an iris diaphragm so that the aperture may be stopped down to suit the condenser, or built with a small aperture of, say, 1.0 or less. They can be had in either the achromatic or the aplanatic series and can be used for bright-field visual work also. When the lens is equipped with an iris diaphragm the image for bright-field work will be at its best with the iris open, since the images formed by objectives equipped with a

diaphragm deteriorate very rapidly if the iris is closed beyond a certain point. Such special objectives can also be used for photomicrography, with excellent results.

For high-power dark-field work of the most exacting kind, a quartz cell is commonly used to mount the specimen, which is often of a colloidal nature. Thus all the ultraviolet radiation reaching the object field is passed to the objective. It is customary to use an immersed objective for this high-power work. If the immersion medium is cedar oil it will fluoresce with ultraviolet radiation, and the dark-field effect will be lost. To avoid this difficulty, special objectives have been made for glycerin immersion. They generally have a magnification of about 60 times.

Objectives for Use with Vertical Illumination. Special lenses both achromatic and apochromatic are made for use with metallographic equipment. In order to eliminate as much glare as possible, the mounts are short and the tube is longer than usual, measuring 190 mm to 210 mm or more, depending upon the make. This tube-length correction is made to permit the lens to be used with the various vertical illuminators. Care should be exercised that the tube length is correct. These lenses should be used, as a rule, without a cover glass. The 8-mm or 4-mm ones are of special value for making preliminary examination of smears or pigment mounts before the cover is put on. It is wise to have one or two of these lenses always on hand for such work.

Objectives Corrected for Infinite Tube Length. Figure 102 shows how a glass plate, when inserted in the path of image-forming rays from the objective, causes spherical aberration of the negative type, or over-correction. If the rays pass through the plate as parallel rays, their direction is the same after passing the plate as it was before, although they suffer some displacement. No spherical aberration or comatic error is caused by the reflector under these conditions. Objectives with accessory lens systems are made to carry the image-forming rays as beams of parallel rays through the glass reflector of vertical illuminating systems. Such objectives are said to be corrected for infinite tube length.

Several firms, notably Zeiss and Leitz, are using objectives corrected for infinite tube length for their metallographic equipment. Bausch and Lomb, and Spencer, have not at the present time designed such objectives.

The objectives of Zeiss and Leitz corrected for infinite tube length carry the first accessory lens in the objective mount. It renders the image-forming rays parallel. The second lens element, which receives the rays after they pass through the glass reflector, causes them to con-

verge toward the focal plane of the ocular and focus there. A system of this sort, slightly modified, is installed in the tube of the better-class petrographic microscope. It carries the rays through the analyzer in a strictly parallel formation. This is sometimes called a " telecentric " system.

The diagram, in Fig. 102, is divided, the upper portion showing the plate in front of the lens and the lower portion the plate in the image space. Over-correction in the image space results in both. It was

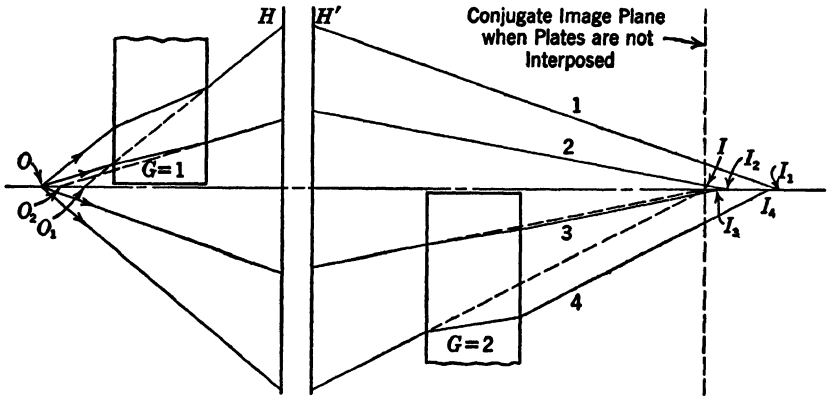


FIG. 102. The glass plate as used in vertical illuminators will cause over-correction of a lens if the rays are converging. In the drawing, for the sake of simplicity, the plates are shown as normal to the lens axis. At an angle, the plate may develop coma and astigmatism; see Fig. 75*B*. The plate shown between the lens and the object is also a source of over-correction unless the lens is designed specially for it, as for a cover glass of definite thickness.

necessary to draw the diagram considerably out of proportion in order to emphasize just how the over-correction is caused. The condition as shown on the front side of the lens, in the object space, is particularly applicable because it demonstrates clearly why a cover glass may introduce spherical aberration. In addition it shows how under-correction may exist simultaneously in the object space with over-correction in the image space. It should also be noted that over-correction results when a reflector plate is placed in front of a lens for very low-power work.

In Fig. 102 the object is at *O*. Ray 1, the outer ray, leaves the object and passes to the glass plate *G*1, where it is refracted according to Snell's equation 29 and proceeds above the plate to the lens, represented by its planes *H* and *H'*. The second refraction at the second surface of the plate gives the ray a new direction parallel to the first direction but offset with respect to it. If an eye were placed in the position of

the lens, this last refraction would make the object appear as at O_1. Likewise, ray 2, after refraction, will make the object appear as if at O_2. Now, on the image side of the lens, the virtual object O_1 will focus at I_1, and O_2 will focus at I_2. Both are images of the object O. The outer ray 1 is focused farther from the lens than ray 2 — the condition already described as over-correction or spherical aberration of the negative type. See Sec. 54.

The glass plate at $G2$ on the image side of the lens can similarly be shown to produce the same kind of aberration. From inspection of the diagram it is evident that, the thicker the glass plates and the greater the angle of the impinging ray, the greater will be the displacement of a ray after passing the plate, and thus the greater will be the error introduced. Thus when a plate is employed for vertical illumination it is best to use it in a system corrected for infinite tube length. It should be very thin, of the order of cover-glass thickness, for aberration will be intensified by a thick plate. Glass plates in ocular diaphragms become a source of over-correction and may give rise to serious image deterioration in photomicrography.

Flat-Field Objectives. In the achromatic series, several special lenses with a very flat field are on the market. Most of them require a specially designed eyepiece. These objectives are of low power and are useful for "survey" purposes, that is, for photographing a large portion of the specimen at one time. In addition, the Planachromats have recently been placed on the market by Zeiss. These are a new series of lenses of the achromatic type, which differ somewhat from other achromats because they are intended for use with compensating oculars only and when so used will give a perfectly flat field for photomicrographical work. The N.A. is smaller and the cost is the same as for apochromats, but on the other hand they offer the advantage of a flat field. These lenses, described in Zeiss booklet Mikro 538e, are obtainable in five different focal lengths, from 8.2 to 1.88 mm. The highest power can be used only with monochromatic green light, wavelength 546 mμ. The front lens of the 3-mm objective is concave. For cleaning, these objectives are mounted on a special revolving disc.

Objectives for Illumination by Incident Light. Three very important series of objectives for illumination by incident light are made by Leitz, Zeiss, and Reichert. These systems employ an annular condenser above the objective. Above the annular condenser is a reflector which receives light from a special lamp, the light being directed to the mirror above the condenser from a small built-in lamp. The condenser focuses the light at a spot which coincides with the position of the specimen when the microscope is in focus. The angle of incident light is such

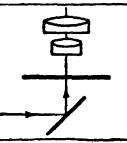
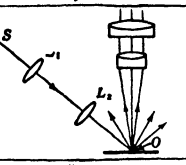
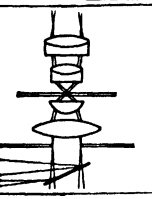
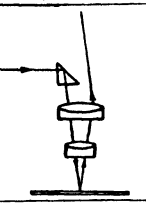
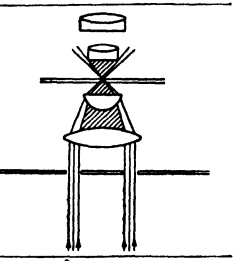
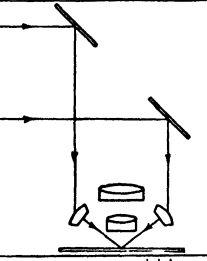
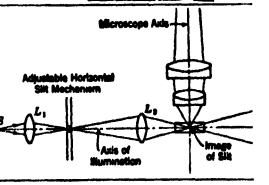
Method-Source	Apparatus	Suitable for	Graphic Examples
Bright field — axial transmitted light. Parallel rays or diffused by ground glass.	Microscope mirror — flat side. No condenser.	Lowest-power objectives, 35-mm. focal length or greater. Transparent or translucent material.	
Oblique overstage illumination. Projection lamps or photoflood lamps for dark specimens. Image of L_1 focused by L_2 on object O.	Lamphouse equipped with light-collecting lens. Auxiliary lens. Microphotographic or low-power microscope objective.	Low-power work on opaque specimens usually having unpolished or rough surfaces.	
Bright field — central or oblique transmitted light. Any source to fill front lens of condenser with light of even intensity.	Concave mirror or any condenser that insures the back lens of the objective being filled with light.	All objectives from 35-mm. focal length or less. Transparent or translucent material. Will not show structural detail of opaque material.	
Bright field — reflected light. A source sufficiently large to light object field.	The glass plate used with any vertical illuminator, giving centered illumination. Or the prism or mirror giving slightly inclined illumination.	Flat polished surfaces—metal specimens, petrographic specimens which are opaque.	
Dark field — strong illumination, Method II. Arc lamp. Light directed from beneath specimen.	Dark-field stops used in substage. Dark-field condensers. Hollow cone of light is produced.	The objective must have a lower N.A. than the lower N.A. of the condenser; otherwise a bright field will be produced. No lower limit for objectives, highest objective N.A. 1.3. Any size material.	
Dark field — the light is incident at such an angle that it would be reflected away from the objective unless intercepted by a specimen. Light above specimen.	Ultropak system of Leitz, Epilum of Reichert, Epi condenser of Zeiss, Silverman illuminator of Spencer, etc. Any overhead lamp and bull's-eye.	Any material, particularly opaque specimens. Smooth surface not essential. Low- or medium-power objectives only unless the German systems are used, then special objectives can be had of all powers.	
Dark field — the light is incident at an angle of 90° to the microscope axis. Arc lamp.	The ultramicroscope or slit microscope. Any microscope can be used with a specially designed slit used in the path of illumination.	Any colloidal material gas, liquid, or solid. Image is formed from light scattered by the specimen. Medium-power magnification is used.	

Fig. 103

that a dark field is produced; this is the reverse of the effect produced by the usual type of vertical illuminator. Figure 103 shows the general direction of the light rays which are required for different types of microscopical illumination. The Leitz system is called Ultropak, the Reichert system the Epilum, and the Zeiss system the Epi. The Leitz system is shown alone in Fig. 104.

The objectives for all these systems are achromatic, and each has been designed for its own specific work. They are built for water immersion, oil immersion, or to work in air. Leitz offers fifteen objectives of different focal lengths. The mechanical tube length required is 185 mm. The directions accompanying these systems must be strictly carried out to ensure centration.

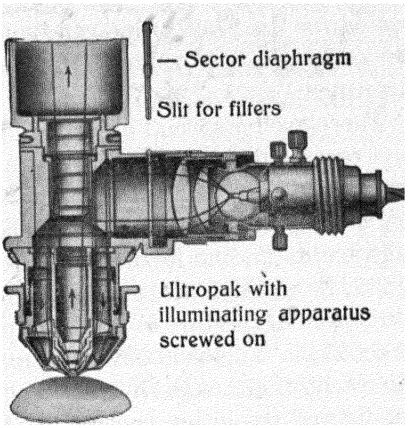


FIG. 104. The Ultropak equipment of Leitz, and similar equipment of Zeiss and Reichert, make possible the use of reflected light at high magnification.

Objectives for Work with Polarized Light. For all work with the petrographic microscope the objective must be free from polarization effects. Glass is generally perfectly isotropic if properly annealed, but any residual internal strain will cause it to polarize. A condition of strain may be induced in a lens through improper mounting. It is usually attributable to unequal or too great pressure on the mount at the time of assembly. Thus, objectives to be used with polarized light should be examined for strain by placing the lens between crossed nicol prisms. If more light is apparent when the objective is in place, then the plane of vibration of the polarized light has been slightly shifted and the presence of strain is indicated. Achromatic lenses have fewer elements than apochromats, and therefore they lend themselves better to work with the petrographic microscope. Most lenses, even those selected for the petrographic microscope, will show a slight pseudo interference figure, but it should not be very marked.

Monochromatic Objectives. Several firms make a series of monochromatic objectives which are used, for the most part, with ultraviolet radiation. They may be made of quartz and be corrected for the 2750 A line (Zeiss), or they may be corrected for two spectral lines, the 5461 A and the 3650 A (Bausch and Lomb); these may also

be made of glass. With glass objectives a green filter is used during focusing to separate the 5461 A line; then the green filter is replaced with one for ultraviolet to separate the 3650 A line. The picture can then be taken. For work with light or radiation with a wavelength shorter than 3650 A quartz oculars must be used, but pictures can be taken with radiation of wavelength of 3650 A and the entire optical system may be of glass. Sandalwood oil is sometimes used as an immersion medium. It has an index of about 1.52 at 20° C. Its fluorescing qualities are somewhat variable.

The work done by Lucas in ultraviolet photomicrography is particularly outstanding. He and others have shown that a lens corrected for the 2750 A line can be used with nearly as good results with the 2573 or 2800 A line. His method of focusing is exact and well described.[19]

There are no special objectives, as far as the author is aware, which are corrected for monochromatic illumination in the visible range, except the Zeiss high-power, flat-field achromat.

The Monobromonaphthalene-Immersion Objective of Zeiss. This lens is outstanding for its high aperture, which is 1.6. Its focal length is 2.5; its magnification at the ocular is given as 74. Resolution at its unrestricted aperture is 0.17 μ, when the wavelength of light is taken as 0.555 μ. This is about the utmost in resolution obtainable in visual microscopy, although, if blue light is used with wavelength of 0.468 μ, the figured resolution becomes 0.14 μ, or a little more than that.

Water-Immersion Objectives. This series of object glasses is useful for the botanist and biologist. They can be had in varying focal lengths, in the Ultropak series of Leitz and others. Zeiss supplies them in magnifications of 6 to 90 in the achromatic series, and with a magnification of 70 in the apochromatic series.

Microphotographic Objectives. These are special objectives intended for use without an ocular. They are similar to the photographic lenses used for macro work. Their anastigmatic corrections are designed to give a good image when the object distance is short and the image distance is long, this being just the opposite from the condition applying to the usual photographic procedure for scenic photography. Microphotographic lenses are made in focal lengths from about 16 mm to 125 mm. Those of shorter focus can be mounted on the microscope tube since they are threaded with the Standard objective thread, but those of longer focus are larger and therefore are mounted on the camera lens board. The apertures of microphoto-

[19] F. F. Lucas, contributor, Keith Henney and Beverly Dudley, *Handbook of Photography*, 1939.

graphic lenses are usually stated in relative aperture numbers, the maximum being generally about f:4.5. The actual number has little significance, however, because the bellows extension with which such lenses may be employed varies. An iris diaphragm mounted between the lens elements can be used in the same way as the condenser diaphragm on the microscope; it will reduce aperture when contracted and stimulate photographic contrast. It is hardly worth while to experiment with the short-focus photographic lenses, such as those found on candid cameras, because the portion of the image field which can be made sharp and clear will be very small.

Sec. 59. Focusing an Objective. It is surprising how many broken front lens elements are found in second-hand equipment. Even experienced users of a microscope seldom focus their instruments cleverly; therefore, a few simple rules will be given which, if observed, will save time and prevent abuse of objectives.

1. *To Focus with the Low-Power Objective (16-mm or Lower).* With the specimen in position, and the condenser diaphragm closed to give a narrow cone of light, the microscope tube is lowered to within the working distance of the objective, say 3 mm. While the field is observed, the mirror is turned until the field is bright. The tube can then be raised with the coarse adjustment until the specimen is in focus. Preliminary examination of an unknown specimen should always be made with a low-power objective.

2. *To Focus with a High-Power Objective after Examination with One of Low Power.* The low-power examination has shown some material in the field. A narrow cone of light still being used, the high-power objective is turned in, immersed if necessary, and the microscope tube is raised just above the position of the focus or until the drop of immersion oil is ready to break. With the coarse adjustment the tube can be slowly lowered to focus, preferably with both hands, while observation is made through the ocular.

3. Always, after the focus has been established with the coarse adjustment, the fine adjustment should be used exclusively, unless the lens is of a power lower than the 16-mm.

Sec. 60. Chemical, Photographic, or Actinic Focus. If all the light forming a lens image were focused in one plane, the visual and chemical foci would coincide. The term "chemical focus" is applied to that part of the light or radiation (generally invisible) that is focused in a plane that may not coincide, respecting focus, with the visibly focused image. Thus, if a camera is focused visually with an achromatic objective using white light, focal planes will

exist beyond the visual one; they will be composed of deep blue light merging into ultraviolet below the visual focal plane, and of deep red above it. Since the photographic plate is very sensitive to blue light, the general effect obtained may be a blurred image. Apochromatic objectives, with their complete range of corrections, avoid in part errors due to the above conditions. However, the performance of an achromatic objective is improved sensibly by the use of filters working entirely in some nearly monochromatic visual range.

The term "actinism" is applied to the action of light on chemical substances; in the early days of photography it referred, in general, to the shorter wavelengths of light, since the emulsions were sensitive only to those rays. The term "actinic focus" is still used interchangeably with chemical or photographic focus. Light is said to be actinic when it is composed of a goodly proportion of short wavelengths and is therefore very active on film or plate. Formerly, light was thought of as actinic or non-actinic, according to its ability to affect a photographic emulsion, but with the use of panchromatic material, having nearly the same sensitivity to blue, green, and red light, the term is gradually falling into disuse.

Sec. 61. The Immersion of a Lens. On the axis of a completely spherical lens there are two, naturally aplanatic, conjugate points; one of these points is within the lens. Wood apparently credits Amici with designing the first microscope objective utilizing such points; presumably the lens was immersed in water. Gage, however, quoting Mayall's *Cantor Lectures on the History of the Microscope,* gives credit unreservedly to R. B. Tolles for the formula and design of an oil-immersion objective in 1874, when these points were then taken into account. At about this same time Abbe designed an oil-immersion system for Zeiss. Aside from the interesting historical discussion, the importance of the discovery of the oil-immersion objective can hardly be overestimated, for it not only permitted the formation of an image free from spherical and chromatic error, by the front lens element of the objective, but it also increased the resolving power of the microscope by more than 50 per cent.

The front lens of a high-power microscope objective is approximately hemispherical. If the lens is used so close to the object that it can be connected to it with a drop of oil, water, or other liquid, it is said to be immersed. Under the conditions of immersion, then, it is as if the object were embedded in a completely spherical lens, the immersion liquid forming the lower hemisphere of the lens. When thickened cedar oil is the connecting medium between the lens and the object, the optical path is practically homogeneous since the refrac-

tive index and dispersion value of the glass and oil are about the same. With the front lens element properly designed and immersed, the focused object is at one of the aplanatic points and the image, which is virtual, is at the other. The succeeding lens elements of the objective, being of the correct curvature, can then bring the light rays toward the microscope axis where they are focused at the ocular diaphragm. The last lens elements usually are doublets or triplets to avoid introduction of chromatic error.

The immersion objective thus accomplishes two important missions: first, it permits the employment of the naturally aplanatic points of a lens and so aids in obtaining better images; and secondly it increases the available N.A. aperture from about 0.95 to 1.4 or even 1.6, which accounts for the greater resolving power, as already mentioned. See Sec. 52.

Objectives such as the Ultropak of Leitz and others are designed for water immersion or for immersion in glycerol, α-monobromonaphthalene, cedar oil, or sandalwood oil. The most commonly used is cedar oil. Immersion oil will also be used for the condenser since naturally cones of light of apertures greater than 1.0 cannot be obtained without immersing the condenser. The effect of immersion on the aperture of the cone of light which reaches the objective from the condenser is shown in Fig. 105. The greatly to be desired optical condition is attained when the back condenser lens, immersion liquid, slide, specimen, cover, immersion liquid, and front lens of the objective are of the same optical density. It will be shown later that to get the desired contrast for good photography the mounting medium will often have to have a much higher or lower refractive index than that of the immersion liquid, but this does not necessarily cause error which cannot be otherwise rectified. See Sec. 65 under "Inspection for Correct Tube Length."

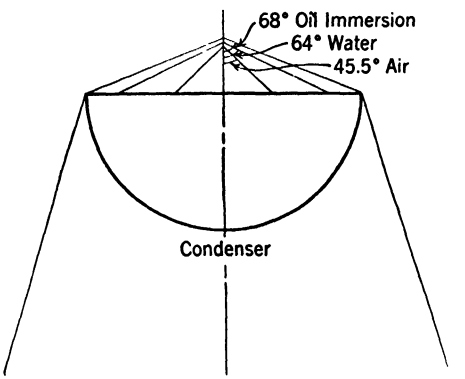


FIG. 105. The effect of air, water, glycerol, and immersion oil on the light cone. Increasing the refractive index of the medium in the image space increases the focal length of the condenser. Thus, when immersion oil is added, the condenser should be lowered to maintain a good focus of the light source. See equation 25.

To make the immersion, it is best to have the microscope vertical.

This is not always necessary, but it helps to keep the oil in place on the slide. The drop can be picked up on the end of a glass rod and placed in the proper position on the slide. The objective is then lowered into contact with the drop. The contact should be made slowly; otherwise bubbles may form. The tube should be raised slightly to be sure that it is above focus, and then lowered slowly into focus by the coarse adjustment, the eye observing the field attentively for the first signs of image formation. Also see Sec. 59.

Because of the extremely short working distance of the oil-immersion objective, the front lens is in danger of being broken if immersion oil is allowed to dry on it. The remaining drop may become quite hard, and, if it is not discovered in time, the lens may be broken or displaced when it is used later. Special care should be taken to see that the lens is always wiped with a piece of lens paper after use, and that the directions for cleaning an objective, as given in Sec. 67, are carefully followed. Before immersing a lens it is always a good plan to glance at the surface of the front element to make sure that it is clean. It should then be wiped with a piece of lens paper moistened with xylene to help avoid the formation of bubbles.

Sec. 62. Centering the Objective. Irrespective of the objective-changing system on the microscope, there must be some standard of centration that can be referred to from time to time, so that the technician can always be assured of a properly centered system. In general, this base line is the axis of the microscopic tube as stated in Sec. 2.

In any microscope system there are generally five optical parts which should be kept mutually centered: the mirror, the condenser, the iris diaphragm of the condenser, the objective, and the ocular. The ocular is a fairly long-focus lens compared with the objective. Slight errors in centering the ocular and objective can be kept within the errors of the lathe work on the microscope tube and objective. These errors in lathe work are easily discovered but relatively unimportant; each microscope furnishes its own standard of centration. The centering of all other lenses can be referred to the correctly centered objective and ocular, which, disregarding lathe errors, is substantially the axis of the microscope tube. This standard can be used for the indirect centering of the condenser.

After the revolving nosepiece, or any quick-changing device which may be installed instead of the revolver, has been removed, the objective can be screwed directly to the microscope tube. This procedure may involve the use of a 15-mm intermediate spacing collar, since otherwise it may be impossible to focus the objective on account of the shortening of the tube. If the collar is necessary, a slight addi-

tional error of lathe work may be introduced, but it can be ignored. A micrometer ocular having a cross-hair disc is then inserted, or such a disc can be placed in an ordinary eyepiece; the field lens or eye lens of the ocular is unscrewed, and the disc is dropped onto the diaphragm. The microscope is focused on a test object, preferably crossed lines, with the point of the intersection of the cross hairs of the ocular superposed on the intersection of the crossed lines.

With the test slide in place, the next step is to remove the microscope tube. The objective is removed; the revolver or quick-changing device is replaced by the objective; and the tube is returned to the microscope. On refocusing the microscope, if the centration of the objective is correct, the test object will still be under the cross hairs. The success of the whole operation depends upon the test slide's staying exactly in place during the test.

Supposing that the objective is attached to the microscope tube by one of the quick-changing devices and that when the microscope is focused the second time it is found that the objective is not centered with respect to the ocular; then the correction is obvious. The centering screws on the objective holder can be adjusted and perfect centration can be obtained by moving the objective to align the test object with the cross hairs in the ocular.

With a revolving nosepiece it will generally be found more difficult to make the required corrections. Some types of nosepieces have the openings marked for certain objectives. These markings should be observed unless it can be established that better centering can be attained by changing the objectives from one opening to another. On some makes, it is mechanically possible to change the position of the revolving nosepiece slightly to effect alignment of the objective. For more precise work, centering collars are obtainable. These can be inserted between the objective and the nosepiece, and centration obtained by adjusting the collar. If centering collars are used, corrections must be made for tube length. Zeiss makes a revolving nosepiece in which a device is built to permit centering, as required. Leitz builds a quadruple nosepiece with aligning adjustment for each objective.

Sometimes the necessity for so much preliminary adjustment is not immediately obvious, particularly if the microscope has been recently purchased, but it should be remembered that in microscopy it is certainly true that without meticulous attention to detail the best work is impossible. Each adjustment contributes its part to the making of the final image, and, even though any departure from perfection on the part of any one adjustment may not, in itself, be particularly

noticeable, if many such errors are present appreciable degradation of the image is sure to result.

It is often asked whether all objectives have to be centered. The answer is yes. All objectives, particularly those of high power, should be examined for centration in order to avoid later trouble. If the centering process has been performed first on a high immersion lens, it is quite permissible to center all other lenses with that one as a standard. It is then unnecessary to screw the other objectives directly on the microscope tube. On a revolving nosepiece there is little likelihood of the lenses' becoming uncentered once they have been put into correct position, but if a quick-changing adapter is part of the equipment it is necessary to check frequently for centration with the tube axis, for when objectives are subjected to frequent handling the adjustments may change. Sometimes when it is necessary to center the condenser to the objective the objective is adjusted instead of the condenser. The reverse, of course, is the correct method; it is the condenser, and not the objective, which should be moved to effect centration.

With ball-bearing stages, there is an exception to the method of centering objectives as outlined. Since the various manufacturers have made these stages without centering adjustment, the objective has to be centered to the axis of the stage. This is a regular feature of many petrographic microscopes, but it is not so common on biological microscopes. If such a stage is to be used, it is wise to see that the axis of the stage coincides fairly well with the tube axis; otherwise the microscope should be sent back to the manufacturer for readjustment.

When examining the centration of the objective with the tube axis, and so with the axis of the ocular, it should be noted that any lathework error is multiplied by the magnifying power of the objective and the ocular; this makes the test so sensitive that it will be only a coincidence if two objectives are found to have an axis displacement equal in magnitude and direction.

Sec. 63. Parfocal Objectives. Objectives are said to be parfocal one with another when their image planes coincide. When a quadruple revolving nosepiece is used, the objectives are said to be parfocal with each other when one setting of the microscope will serve to focus all four objectives. As a rule parfocalization can be attained for all microscope objectives except those of great focal length. Parfocalization of objectives should always be demanded from the manufacturer if a revolving nosepiece is to be used. It is not so important on other types of objective-changing systems.

Sec. 64. The Designation of Objectives. To be fairly well specified, an objective should be designated according to its focal length, magnification, numerical aperture, and its class — that is, achromatic or apochromatic. If immersion liquid is to be used it should be so stated. Tube-length data to a certain extent depends on make. The former practice of listing objectives according to letters or numbers or a combination of both had nothing to commend it and is being gradually replaced. The magnification of the objective often is the only means of identification available to a purchaser, all other data going by the board. Too frequently, an objective is ordered merely according to the power required, as, for instance, a 10 times or 60 times objective, and the salesman is left to fill the order at will. This is an unfortunate condition and not far from universally true. The purchaser should be able to form his own opinion as to his needs and buy accordingly. Table XVII, p. 264, lists objectives of manufacturers in the United States, Great Britain, and Germany.

The make of the objective should be considered first. The choice must be governed in a measure by the microscope on which the objective is to be used; otherwise a lens may be purchased with a mechanical tube length requirement of 160 mm for use on a microscope with a fixed tube length of 170 mm or more. To some extent oculars with which the lens is to be used will influence the selection of the make, although it is often possible to combine oculars and objectives of differing makes; a trial will be necessary to determine the best combination. The magnification is largely specified in stating the focal length of the lens, although here there may be quite a little variation in the low powers of different makes. With the high and medium powers, the N.A. of the lens must be taken well into account; otherwise a lens may be purchased with a N.A. of, say, 0.65 when one with an aperture of 0.85 or even perhaps 0.95 should have been bought. The purchaser is strongly urged to make his own decision as to what he needs and to make his own selection if possible, to avoid disappointment in the performance of his lenses.

Formerly the focal length of objectives was generally specified in fractions of an inch. Some companies have maintained this awkward designation, but others now use the millimeter. Table XVI compares the British and metric systems.

Sec. 65. The Evaluation of Microscope Objectives. It is entirely possible for the microscopist to learn to examine critically his own objectives or any that he may be desirous of purchasing. Examining a few objectives in a methodical manner should develop skill and give experience on which the technician can confidently base his opinions.

Table XVI*

Focal Length of Objectives in Inches and Millimeters

Inches	Millimeters	Inches	Millimeters
3	76.2	$\frac{1}{6}$	4.23
2	50.8	$\frac{1}{7}$	3.64
$1\frac{1}{2}$	38.1	$\frac{1}{8}$	3.17
1	25.4	$\frac{1}{10}$	2.54
$\frac{1}{2}$	12.7	$\frac{1}{12}$	2.12
$\frac{1}{3}$	8.46	$\frac{1}{14}$	1.82
$\frac{1}{4}$	6.34	$\frac{1}{16}$	1.59
$\frac{1}{5}$	5.08		

* It is interesting to note that, on special order, objectives have been made with focal lengths as short as about 1/40 in. or 0.6 mm. With an optical tube length of 180 mm, this short-focus lens would have a magnification of 300 times in the focal plane of the eyepiece.

Before deciding on the purchase of an objective that may cost a considerable amount of money, the purchaser is entitled to know certain facts about the lens, and these he should be able to ascertain for himself. Individual lenses of high aperture vary considerably in corrections and sometimes in focal length, the latter affecting the magnifying power. The N.A. of such objectives is often lower than is called for by the rating on the lens. Experience is necessary in testing microscope object glasses. Therefore, before the technician attempts to test new or second-hand objectives, he should carefully examine those with which he is accustomed to work and, if possible, compare them with any others he may have the opportunity of handling, as only in this way can he acquire the necessary background to judge the variation in performance of new object glasses. A lens should be judged systematically and inspected for certain definite characteristics, good or bad. Findings should be jotted down one at a time; then when the examination is finished there will be some pertinent facts at hand from which a conclusion can be drawn.

Before attempting the inspection of an objective, Sec. 62, dealing with the centering of the objective, and Chapter IV on the condenser and ocular should be read carefully, for unless all the optical parts are properly adjusted the objective may not be operating under conditions which permit full utilization of its capacities.

Tests are conveniently made in approximately the following order:

1. General examination.
2. Inspection for correct tube length, and application of the star test.
3. Inspection for centration of the lens elements.

4. Measurement of magnifying power.
5. Measurement of focal length.
6. Measurement of N.A.
7. Inspection for spherical aberration.
8. Inspection for chromatic aberration.
9. Inspection for resolution; computation of resolution.
10. Estimation of field depth.
11. Inspection for lateral chromatic aberration of objectives intended for color work.
12. General survey of the field of view.

1. *General Examination.* An objective should be examined with a hand magnifier before it is put on the microscope. The barrel should show no evidence of rough handling, corrosion, or dirt. The lens surfaces can be inspected for cracks, scratches, air bubbles in the balsam, and chemical changes as evidenced by corrosion or oxidation; the lens elements should be firmly seated. Inclusions found in the fluorite do not impair the lens. A correction collar to compensate for under- or over-correction must work smoothly and not be loose, and all moving parts should function properly. If the lens is of old construction the front and rear portions can probably be unscrewed and the interior can be examined critically with a magnifying glass. The interior of the barrel should be black. The screw threads should be unbroken, and, for the sake of interchangeability, the pitch must be 36 threads per inch, which is the standard adopted by the Royal Microscopical Society, and is in universal use. See Sec. 2. Springs in 4-mm objectives with correction collars should not be broken. When the lens has been much used, the mechanical parts subjected to wear may have to be replaced. This preliminary investigation is practical and quick, and objectives should always be tested first in this way, since imperfections revealed by such an examination may often be sufficient to rule out the lens from further consideration.

To illuminate a lens sufficiently to examine its glass surfaces thoroughly is something of a problem; it is not always easy to direct light into the lens mount and at the same time hold a magnifier over it. Oil-immersion lenses are particularly difficult to illuminate. A practical method is to mount the lens on the nosepiece and focus it on a specimen. The specimen is removed and the light modified to protect the eyes. If the body tube of the microscope is detachable from the nosepiece it can be removed and the lens surfaces can then be examined very critically with a hand magnifier. However, not all microscopes are built so that the tube can be removed and still leave the objective

in place. To examine the back lens of the objective with a magnifier at the exit pupil of the microscope is not sufficient, nor is it satisfactory to use an accessory lens on the end of the drawtube. Neither of these methods makes it possible to examine the lens at an angle and this is necessary to stress the poorly visible figures of incipient chemical action.

2. *Inspection for Correct Tube Length and Application of the Star Test.* Objectives are made to form their best images only under certain definite optical conditions, and these conditions must be adhered to precisely for high-aperture objectives, if the finest results are to be expected. There are six important points to be remembered all of which can be controlled in the laboratory.

1. Mechanical tube length must be correct (see manufacturer's catalogue), generally 160 mm measured from the shoulder of the objective where the thread ends to the top of the microscope tube with eyepiece removed.

2. Correct cover-glass thickness must be observed; this is about 0.17 mm. (See manufacturer's catalogue.)

3. If the lens is of the immersion type the immersion liquid must have the proper refractive index. The objective may be corrected for water, glycerol, or oil immersion.

4. The specimen mount must be of negligible thickness; otherwise the tube length will have to be readjusted or the correction collar reset as examination is shifted from the lower level of the preparation to the upper level.

5. The correction collar on an objective so equipped, must be properly set, according to the thickness of cover glass.

6. If the objective is monochromatic, the wavelength of light used must be suitable for it.

Since variation from standard of any of the above six conditions will cause degradation of the image formation by producing under- or over-correction, and since it may be impossible to have them all correct all the time, a way must be found to determine whether error does exist and a means must be provided to compensate or correct it. Obviously, each of the six items mentioned could be measured separately and the appropriate correction applied, but this is unnecessary because, within working limits, an over-correction on one of the items results in compensation for under-correction in any one of the others, and vice versa. The star test affords a means for detecting poor adjustment in any of the six items measured but it will not indicate where the maladjustment occurs. Adjustment of tube length is usually pos-

sible, and by lengthening the tube for under-correction and shortening it for over-correction while checking also with the star test, any condition of over- or under-correction attributable to any of the items listed can usually be found and nullified.

Figure 106 is a diagram of the light rays passing through an under-corrected system. In the first position shown, the lens would form an image disc of the object, at *I*, the size of which is shown at *B*. If the lens is moved to the second position, downward, or toward the object, the image will recede and point *A* will occupy the space at *I*.

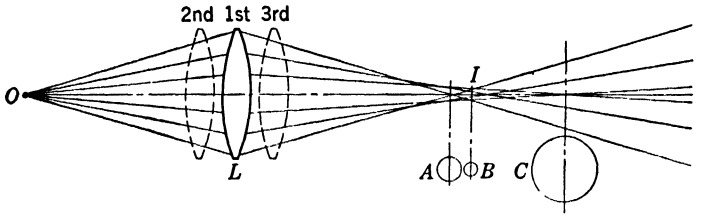


FIG. 106. A modified form of the star test adapted to the microscope. This important test can be quickly learned and should be often applied. Misalignment of mirror or condenser is also often indicated.

This out-of-focus image will appear larger than the first image but not so clear. Similarly, the point at *C* may be examined by raising the lens, which is the equivalent of moving it toward the right, as in the third position. This would bring point *C* to the original position at *I*. The comparative size of the out-of-focus images is shown as the circles *A* and *C*. Circle *I* represents the best image of *O* that can be obtained with this under-corrected system. Circle *A* represents the appearance of the out-of-focus image when the lens is within the proper focal distance. Although the illumination is the same, image-circle *A* will appear lighter than image-circle *C* because *C* is larger than *A* and there is an equal amount of light for both. Circle *A* will also have an appearance described as "hard"; sometimes a diffraction ring may show around it. Circle *C* is described as "soft"; it has a fuzzy outline.

When a good objective is properly corrected, the appearance of the out-of-focus image should be about the same whether the objective is lowered or raised. If, when the objective is lowered slightly, the image is hard, with a ring around it, then the objective is under-corrected. The condition would be the same as shown in Fig. 106 in the second position. If the out-of-focus image *A* is soft and fuzzy the objective is over-corrected.

To apply the star test, focus should be directed on a very small

object which has been selected to give as much contrast with its surroundings as possible. The fine adjustment should be turned downward slightly and the image observed; it should then be turned slightly above the proper focus and the image again observed. Rather rapid shifting of the focus is often an aid because it seems to stress the visual difference between the two out-of-focus positions.

After determining whether the system is under- or over-corrected, tube length can be adjusted, or the correction collar, if the objective has one, can be turned to the proper position. If the error is not very large, the most convenient method of correction can be chosen. However, if the cover happens to be of very great thickness, say 0.3 mm, or the refractive index of the immersion liquid abnormally high or low, then correction for these points should be made separately.

The suggestions for adjustment can be tabulated as follows:

To adjust an under-corrected system:

1. The drawtube may be lengthened.
2. The correction collar can be set at a lower number.
3. A thicker cover glass can be used.
4. Immersion liquid of higher refractive index can be used.

To adjust an over-corrected system:

1. The drawtube can be shortened.
2. The correction collar can be set at a higher number.
3. A thinner cover glass can be used.
4. Immersion liquid of lower refractive index can be used.

The star test is of great importance for optimum imagery, and it should be used much more frequently than it is, especially on objectives in the apochromatic series of 8-mm focal length and less. In the achromatic series it can be used to good advantage on the objectives of 4-mm focal length and less. On objectives of longer focal length, coverglass thickness has less effect, and an approximately correct tube length is generally sufficient. It will be found in examining a number of individual high-aperture lenses that although their listing may be identical, they are likely to have slight optical differences built into them. Thus, they will demand slightly different tube-length adjustments in order to develop the best images of which they are capable. When making this test it is a great help to use high oculars.

Originally, the star test was used for inspection of telescope lenses, where the test object could be an infinitesimally small spot of light on a black background. This black background and small point of light would be the ideal condition if it could be applied to the microscope.

Applying the test with the bright-field illumination reverses the original procedure. A small black spot must be used on a bright field, but on the bright field slight image differences may be hard to detect on account of the presence of glare. However, the test will accomplish its purpose rather successfully as described, and the results assure formation and recognition of the optimum image, which is so necessary in good photomicrography. Figure 107 is a photomicrograph of a small particle showing the appearance of the image correctly focused, and slightly above and slightly below the correct focus.

An objective should be tested under standard conditions as far as possible; that is, the light, the filters, ocular, and the condenser should be those which will be used later with the objective. If, in the interest of obtaining better imagery, any departure from these standards is made, it should be so noted for future reference. For instance, to obtain the best images with a certain 2-mm objective an increased tube length of, say, 6 or 7 mm may be indicated. Measurements of magnification, focal length, and other constants can then be made at this setting, since it is the one that will be habitually used later.

3. *Inspection for Centration of Lens Elements.* To detect errors in the centration of the elements of a lens, a plate coated with aluminum by the evaporation process is useful. The aluminum coating can be applied to a microscope slide at very little expense; such a slide is also a very valuable test plate for the star test. A search for a hole in the metal film of such a plate will generally reveal one which is, to all intents and purposes, close to the threshold of resolution. It can be even smaller than the lens can resolve. The out-of-focus image of such a hole may show concentric rings around the central portion. The image will be a diffraction disc. If the rings are not concentric, some of the elements of the objective have loosened and slipped from their proper setting, or perhaps they were never properly centered. In making this test it is sometimes convenient to over-correct and then under-correct the lens, to enhance the diffraction rings, thus making the test more sensitive. Very strong illumination should be used; Method II is to be preferred. If the elements of the lens are found not correctly centered, there is no recourse but to send the lens back to the manufacturer.

Occasionally, it is found that the out-of-focus image appears as an ellipse with its long diameter in one direction when the lens is within its focus and at right angles to this direction when the lens is beyond its focus. This condition, known as *twist,* can be rectified only by the manufacturer (probably by replacing certain parts).

A lens should pass this test perfectly before it is accepted.

4. *Measurement of Magnifying Power.* Magnification of an objective is easily measured with an ocular micrometer scale and positive eyepiece, or more exactly with a Zeiss 15× compensating filar microm-

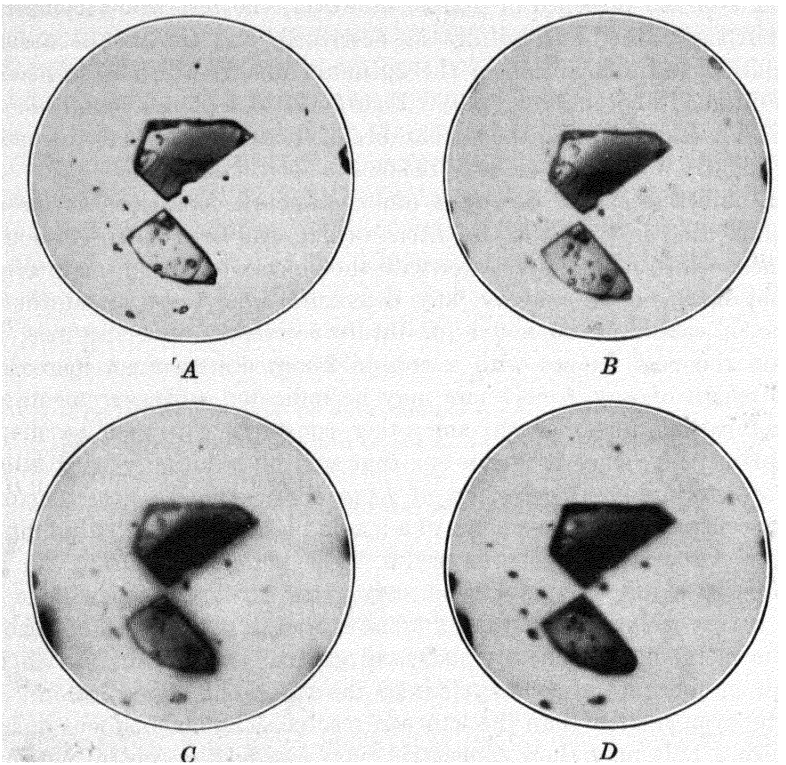


FIG. 107. Photomicrograph of several particles of Carborundum 600, test slide 1, ×950. For *A*, the microscope system was corrected by using the star test. For *B*, the microscope was made under-corrected; detail is lessened. *C* was taken within the focus of the objective; the corrections were the same as for *B*; it illustrates the appearance of an under-corrected system. *D* was taken with the microscope tube slightly raised above the correct focus; the system was under-corrected and should be compared with *C*. This illustrates the use of the star test. When correction has been attained, the image should appear about the same when examined either slightly above or below the focal plane. An over-corrected condition would appear the opposite of that shown. Objective; 4-mm apochromatic, Zeiss; ocular; 15× compensating, Zeiss; condenser; achromatic-aplanatic, Leitz, oiled; filters: Wratten 45 + 47; illumination: H_3 mercury-vapor discharge tube; film: Eastman Contrast Process Panchromatic; developer: D-11, 6 minutes.

eter. The ocular scales are generally 5 mm long and divided into 50 equal parts, each interval being 0.1 mm. If the image of a stage micrometer scale is projected onto the scale of the ocular, the number

of spaces of the eyepiece scale that equals a certain number of intervals on the stage micrometer can be determined. Suppose that 29 spaces on the ocular scale equal 40 spaces on the stage micrometer scale. That is, 2.9 mm equals 0.4 mm $\times$ 7.3; 7.3 is a constant which is the magnification of the objective at a certain tube length. Here the magnification of the objective would be 7.3. If a positive ocular is not at hand, fairly good results can be obtained by using a negative ocular with the field lens removed. The only precaution to be observed is that the measurements should be made at the standard tube length, or at a modified tube length which may have been decided on in advance. An alternative is to project an image of the stage micrometer for a distance equal to that of the mechanical tube length minus 12 mm and to measure the size of the image on a piece of ground glass. This method is less accurate than the former.

It is quite common to find that objectives give a lower magnification than their rating calls for, perhaps as much as 15 per cent or more. However, unless magnification is of vital interest to the microscopist, the importance of this in the evaluation of a lens is not very great, and the test may often be made solely for the purposes of record. A magnification lower than the rating calls for indicates a longer focal length, and probably lower N.A. of the objective, than would otherwise be the case, and this low aperture might be of importance with a high-power objective.

5. *Measurement of Focal Length.* Focal length is quickly measured at the time the magnification of the objective is being determined. Two measurements of the magnification of the objective are taken at extreme tube lengths, one short and one long. If the difference between the two tube lengths is divided by the difference between the two magnifications, the quotient will be the focal length of the objective. l_1 and l_2 represent the tube lengths; m_1 and m_2, the corresponding magnifications. The formula then is:

$$f = \frac{l_2 - l_1}{m_2 - m_1} \qquad [48]$$

As in the test for magnification the fact that a focal length differs from specification is, in itself, no reason for turning down an objective. However, focal length is an important lens constant. If it is greater than it should be, a low N.A. may result, magnification will be less, and resolution may suffer. As a rule it is well to continue testing the lens to see how it behaves otherwise before rejecting it on faulty rating of focal length.

6. *Measurement of N.A.* The N.A. of an objective can be measured

with considerable exactness by the method described in Sec. 10 or with any of the apertometers. When buying a lens it is wise to examine it for this quality, because it is the main feature on which the price depends. Resolution also depends upon it. Individual lenses by the same maker may vary somewhat in N.A. although they may all have the same listed rating. Thus, a lens with N.A. 1.40 inscribed on the barrel may actually measure as low as 1.36 or even 1.35, whereas a lens with a rating of 1.32 may have this full rating. The difference in price between the two is, say, $50. To pay $50 for an increase of maybe only 0.03 in aperture is hardly reasonable. The older objectives, and all second-hand ones, should be carefully examined for N.A. The cheaper lenses in the achromatic series may sometimes fail so much in this respect that the purchase even at a low price may prove disappointing. It is well to remember in this connection that, unless the technician is very experienced in selecting lenses, he may not be able to distinguish between a lens with N.A. of 1.4 and one of 1.25, if he relies on observation of test slides only. N.A. cannot be guessed; it must be measured.

A quick method for comparing the aperture of two objective lenses is to focus one on a non-glary specimen and to open the diaphragm of the condenser to fill the rear focal plane of the lens with light. The condenser circle must just coincide with the objective circle. The second lens is inserted, and, if the condenser circle appears within the objective circle, the second lens has a larger aperture than the first, or if the condenser iris is graduated in values of N.A. the numerical value of the objective may be read directly.

Just when a lens should be rejected because of low aperture is hard to say. If it measures up to other expectations and gives excellent optical and photographic images, and if it is well suited to the eyepieces with which it is to be used, it has much in its favor, and great latitude might be permitted in over-rating. A limit might reasonably be set at $+0.02$ for a lens which otherwise is good.

7. *Inspection for Spherical Aberration.* The Abbe test plate was specially designed to determine spherical and chromatic aberration of lenses. It is not practical to use it for measurements that must be put into exact mathematical terms, but it does help in giving some standard of comparison for the corrections made on objectives. However, some authorities claim that, because of the spreading out of light at the surface of the silver film, the use of only one zone of the lens is not sufficiently assured by this test.

Essentially the test plate is a glass slide measuring 25 by 75 mm with a long rectangular, wedge-shape cover glass on the under side

of which a silver film has been deposited chemically. Lateral and transverse lines are drawn on this film. The cover is about 43 to 45 mm long and is marked off in sections corresponding to different cover thickness. Figures show the thickness of the cover at the points indicated. The thinnest part of the wedge is usually about 100 μ, and the thickest section about 200 μ to 210 μ. Thus, when the silver lines are focused, the slide can be moved to any cover-glass thickness within the range of No. 1 covers.

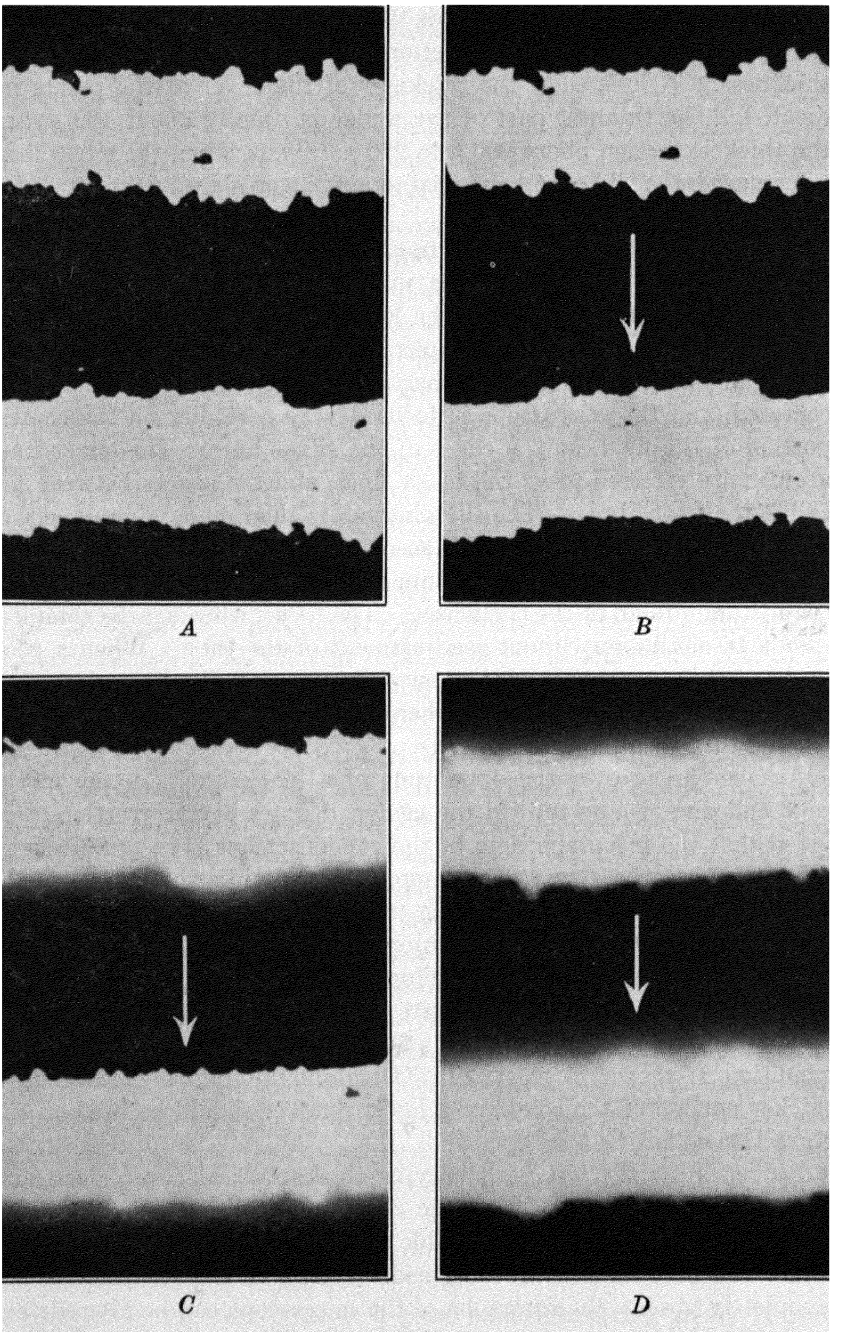
Abbe test plates can be had from optical companies for about $8 each. Some of the older slides may mount a series of covers of various thicknesses, instead of the long wedge cover. The directions accompanying test plates are complete and easy to follow.

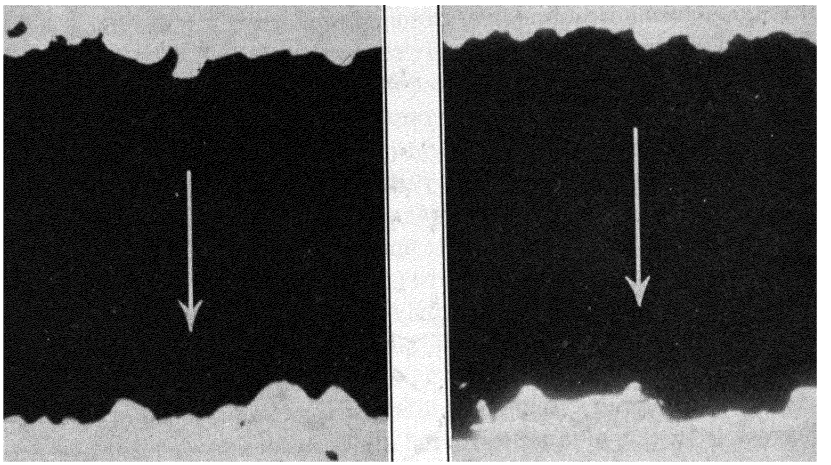
For detecting errors due to spherical aberration, the illumination is centered, and the microscope is focused very carefully on the central portion of the field, on the edges of the silver lines. The silver lines are usually referred to as the black lines, and the spaces between are called the white lines. The illumination is then made oblique, either by means of a sector stop, which accompanies the plate and is dropped into the carrier on the substage apparatus, or by laterally displacing the iris diaphragm of the condenser. The focus, when again examined, should be optimum without readjustment of the tube. When a good lens and the correct part of the test plate are used, there should be but little difference in the images when examined first by central lighting and then by oblique lighting.

A haze spread over the upper side of a black line, starting at the edge and diminishing toward the center, denotes over-correction, provided that the iris diaphragm of the condenser has been moved away from the observer, toward the lamp. A result like this indicates the test should be continued, moving the slide to a position where the cover glass is thinner, or, if the objective is equipped with a correction collar, this collar should be set to a higher number.

When the hazy condition appears on the under side of a black line and is accompanied by a falling off in definition, an under-corrected condition is shown to exist. The slide should then be moved to a thicker portion of the cover, or a lower number should be used on the correction collar.

By repeating the test with varying thicknesses of cover glass, and by making adjustments with the correction collar when objectives are so equipped, it will be possible to find the position on the slide where the best images are obtained. If spherical aberration is not completely absent, the drawtube of the microscope can be altered: extended for an under-corrected condition of the lens and shortened for an


A
B
C
D



E *F*

FIG. 108. The attainment of the optimum image by correcting the system with an Abbe test plate as standard ×900. *A*, the image of a black line with central lighting. *B*, the image of a black line with oblique lighting; the system has neither under- nor over-correction. The direction of lighting is shown by the white arrow. *C*, taken at thick end of cover glass to show over-correction. *D*, taken at thin end of cover to show under-correction. Objective: 4-mm apochromatic correction collar; ocular: Homal III; condenser: Leitz, achromatic-aplanatic; filters: daylight; illumination: 400-watt biplane filament projection lamp, method I; film: Eastman Contrast Process Pan; developer: D-19.

E and *F* illustrate slight under-correction introduced when immersion oil is of too low an index ×1600. *E*, black line of test plate with oblique lighting when the oil is of correct index. *F*, the under-corrected condition produced by an oil with an index of refraction 0.002 lower than at *E*, the lighting being oblique and in the direction of the arrow. Objective: 3-mm apochromatic; ocular: Homal IV; condenser: Leitz, achromatic-aplanatic; filters: daylight; illumination: 500-watt projection lamp, method I; film: Eastman, Contrast Process Ortho; developer: D-19.

over-corrected condition. It should now be possible to say whether an objective is free from spherical aberration and whether it works well with a normal tube extension. The cover-glass thickness for which it is best suited and the setting of the correction collar that gives the best correction for a cover of standard thickness can also be learned.

Oil-immersion objectives are not as sensitive to cover-glass thickness as dry objectives are. The hazy condition along the edge of the black line may not be so marked as it is with the dry lenses. Figure 108, a series of photomicrographs taken with a 4-mm apochromatic objective, illustrates the points brought out in this discussion.

The behavior of the lens under test may be modified by chromatic filters. Achromatic objectives respond well to a strong green screen which aids in the reduction of aberration. Red and blue filters sometimes aid in determining whether a lens is achromatic or apochromatic, but as a general rule an examination with the test plate should be commenced with light of daylight quality.

The test can be influenced by the selection of the eyepiece; for exact work it is wise to try several makes and magnifications. However, in general, the microscopist must work with the equipment he has on hand, and when an objective is to be added to it a limited assortment of eyepieces is not necessarily a drawback, since the objective he has under consideration will be tested with the eyepiece with which it would have to be used.

Another means of testing an objective for the presence of spherical aberration is the aluminum-coated plate recommended for the test to determine centration of lens elements (Test 3). Several small covers of thicknesses varying from 0.10 to 0.20 mm should be mounted on the plate. The cover thickness recommended by the manufacturer of the objective can be expected to give the best images, but if the result is not good another cover, of different thickness, may be tried.

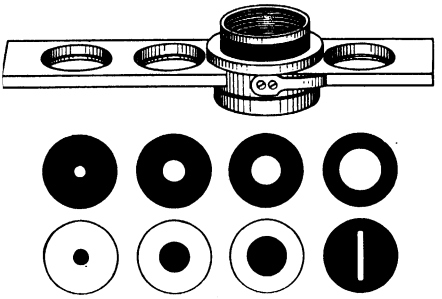


FIG. 109. A set of stops and diaphragms which can be used for testing the different zones of an objective. Courtesy R. and J. Beck, Ltd., London.

Figure 109 shows a set of stops and apertures of various sizes that can be inserted over the objective. Beck, of London, makes a device to be screwed onto the objective; through this device a plate that carries the required stops and apertures can be slipped. In this way, only a very restricted portion of the lens is brought into play, and by proper selection of the different stops the whole surface of the lens can be explored at will, systematically, zone by zone. It is used in conjunction with either the Abbe or the aluminum-coated test plate.

When the aluminum plate is used, a small hole in the film can be found and the star test applied. If the lens shows spherical aberration due to improper cover-glass thickness, incorrect tube length, or any other cause, either under- or over-correction of the lens will be at once apparent. The test is so sensitive that actually an objective

is seldom found which gives exactly the same image beyond and within its focus, although some of the good apochromats closely approach this ideal condition when correctly adjusted. Whether the lens is achromatic or aplanatic, it should be tested with the oculars with which it is to be used, and also with blue, green, and red filters, and with light of daylight quality.

It is significant to note that all directions for testing objectives for spherical and chromatic aberration state that the test should be made only for the center of the field. This would not indicate that a very strong aplanatic condition should be expected. Since objectives differ greatly in this respect, it is a good plan to make the test from the center of the field outward, toward the periphery, thus learning how far from the center of the field the corrections have been carried. High oculars can be used to magnify errors. For photomicrography, where a large field is essential and the edge of the picture may be equal in value to the center, such a comparison of two or more objectives is desirable. Probably such a test is better made with the Abbe plate than with the aluminum-coated plate; with the Abbe plate the image of the lines may be examined in several different azimuths without moving the plate.

Since zonal errors can be detected only by actually blocking out sections of the lens, the set of stops mentioned is required for such work. Coma and astigmatism, as already stated, exist at the outer portions of the field and may be masked by light from other portions when the lens is in general use. Thus the effect of one error may obliterate the effect of other errors.

In making a test to determine relative amounts of spherical and chromatic errors in lenses, the microscopist must be exceedingly critical in his observations. The focusing must be done in a very precise manner, and the exact appearance of the image must be remembered for comparison with the image formed by a lens which is known to be well corrected.

8. *Inspection for Chromatic Aberration.* In the opinion of many, chromatic error which is more easily detected with the Abbe test plate than spherical error, may do more harm to image formation than the various kinds of spherical aberration. With oblique lighting, it appears in the form of color fringes along the edges of the silver lines. An achromatic objective gives complementary colors of the secondary spectrum on each side of the lines, with blue and red showing when the lens is not corrected. The apochromatic objective under like conditions shows only slight traces of the tertiary spectrum. In other

words, the better the lens the weaker the color fringe. In fact a very good high-power lens may not show any trace of color fringe as far as the normal eye can detect.

Chromatic aberration may be determined by using the star test and the aluminum-coated glass plate. The amount of chromatic error can be roughly estimated by inspecting the color fringe around the outer part of the diffraction rings as the out-of-focus image is examined when the illumination is central. If a bluish ring is seen when the image is examined above the focus, and a reddish one when it is examined below the focus, then two different focal points for red and blue are indicated. This is an example of longitudinal chromatic aberration. A lens in which this aberration is noticeable should not be used for color photomicrography. The reason for this can be demonstrated by holding first a red and then a blue filter in the path of the light beam and noticing the change in the size of the two different colored images as first one filter is used and then the other. A change in image size which accompanies a change in the color of the light (for the same image plane) is lateral chromatic aberration. The image can be projected in the camera ground glass and the sizes of the color images measured. To make such tests satisfactorily, the eyes must react normally to the different colors and magnification must be high.

9. *Inspection of a Lens for Resolution.* In the stereotype test for the resolution of a lens the diatom is used as a specimen, various species being recommended. Hind and Randles recommend *Amphipleura pellucida, Navicula rhomboides, Surirella gemma, Navicula lyra, Pleurosigma angulatum* among others. When the valves of the diatom have been resolved, the lens is said to have resolution for that particular diatom. Such a statement, however, has little meaning in industrial microscopy. Diatoms make poor criteria for reference because they show so much individual variation and they lend themselves to so many different methods of treatment. Two similar specimens of diatoms, equally well mounted, may vary in index of mounting media and cover-glass thickness; they may be illuminated by different methods and quality of light; the magnification of the eyepieces used in their examination may have almost any value up to 30×; and, above all, the individual eyesight and experience of the microscopist are uncertain factors upon which to a large measure the ultimate performance of the objective depends.

For comparing the resolution of one lens with that of another, the rouge slide (test slide 2) is fairly satisfactory. Material can always

be found that will be near the resolving power of the lens. Particles just below resolution will appear as small, rather hazy discs, while those within the resolving power of the lens will show sharply and have well-defined outlines. If the approximate size of some of the particles in the specimen is ascertained, it will be easy to make a comparison after a little experience in examining this slide with various lenses. A good procedure is to look for two small particles that can just be separated optically by one of the lenses but cannot be separated by the other. Bacterial smears also are useful for this purpose because the cells are small and will take a strong stain, and some that are very close together can always be found. In such a specimen individual cells can be recognized when changing from one objective to another. Because the examination of the resolution of objectives is largely a matter of comparison, each microscopist will naturally adopt his own objectives as standards and compare lenses of a similar grade with them. He will then be in a position to decide for himself the value of his own lenses.

It is an advantage to place a micrometer disc in the eyepiece. In this way guesses can be made of the actual resolution of the lens which can then be compared with the resolution figures from equation 7 or 34. Oculars have a great influence over the appearance of the visual image. As higher oculars are used, it will be seen that the images grow proportionately more hazy. This haziness should not necessarily be laid to the ocular as it is probably due to the fact that the errors of the objective have been magnified enough to be seen. Around every image that is formed by the lens there is what is called a circle of confusion (not diffracted light). No lens can be so accurately made that it will pick up all the direct and diffracted rays and focus them exactly in their proper position. The circle of confusion on a good lens should be less than $\frac{1}{250}$ inch, somewhere near the threshold of visibility; in fact, it should be enough less so that after magnification by the ocular the diameter of the circle will still be $\frac{1}{250}$ inch or less. This means that a geometrical point of light should give an image of finite size not to exceed $\frac{1}{250}$ inch. This would be the diffraction disc as already defined. When the magnification of the microscope is too high this circle becomes evident and very sharp images cannot be attained. Poor lenses will give large circles of confusion, and good lenses will give small ones. For this reason oculars when used with good objectives can be of higher power than when used with poor ones.

In studying the resolution of one lens as compared with that of another, a variety of colored filters and oculars should be experimented

with, and the tube length must be corrected for each filter. If the oculars are of different make, the tube length should be examined by the star test each time the oculars are changed.

If a lens to be tested is of high power and high aperture, illumination should be by Method II, since this will give slightly better images with less glare. The lamp diaphragm should be closed until its image can just be seen in the field of view. The condenser diaphragm should be adjusted to give a $\frac{9}{10}$ cone for apochromatic and something less than this for achromatic lenses.

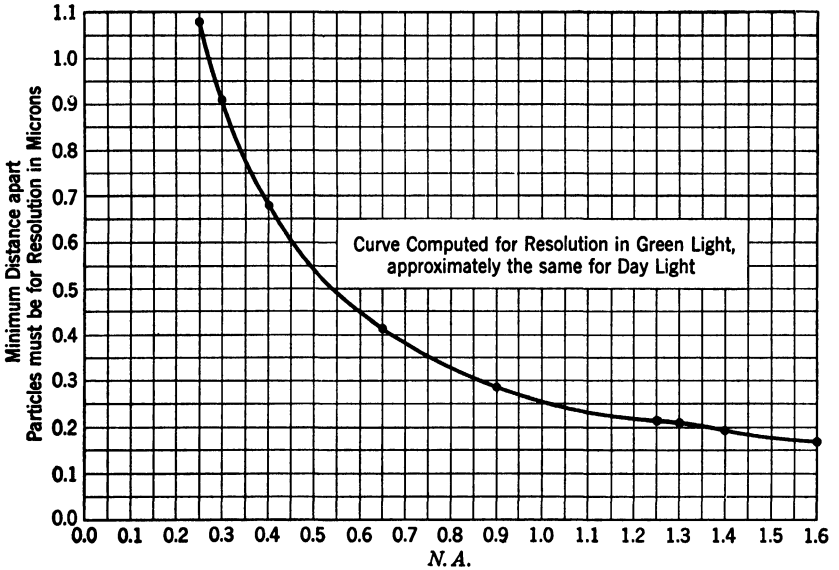


FIG. 110. The change of resolution with respect to numerical aperture.

Thus, it is evident that the resolution of which a lens is capable can be demonstrated only when the microscope as a whole is used in critical manner. As the lenses approach a N.A. of 1.4 only the greatest care and skill will suffice for a fair comparison. The technician must learn to recognize the best conditions for his individual lenses, and he must set up his own standards. In a way, this accounts for the conflicting opinions among microscopists concerning equipment.

The resolving power of medium- and low-power lenses is seldom of great importance. As a rule, when photomicrographs are to be taken at magnifications up to 600 or 700 diameters, other qualities in a lens are of more importance than resolution. Pictures of pigments of small size, bacteria, or small precipitated particles are more likely to need

high resolution. Replicas of diffraction gratings may make good test objects for low-power objectives. The space between the lines should be carefully determined. From the curve in Fig. 110 it is apparent that, with the low-power lenses or those with low aperture, the rate of change of resolution with respect to aperture is very much greater than with high-aperture lenses.

10. *Estimation of Field Depth.* This test is more applicable to low-power objectives and short-focus photographic lenses than to optics of higher power. When using long-focus lenses it is an excellent idea to know just how much depth of field can fairly be expected from them. For instance, assuming that a photomicrograph is to be taken of a specimen which measures 50 or 100 μ thick, if all the specimen is to be in sharp focus, a lens must be selected which will give the required depth in the picture. A lens can be measured for field depth with considerable accuracy.

To determine the field depth of a low-power lens (32-mm focus, or greater) by direct measurement, a fine line or a scratch can be made on a cover glass; or a fine carbon black mounted on a cover glass can be used. The carbon black can be dispersed in collodion; the aggregates which will probably be left are desirable. The cover glass should be broken, and a piece about 10 or 12 mm long and 4 or 5 mm wide should be selected. A tiny right-angular glass prism, with a base about 5 mm long and one angle somewhat less than 30°, should be obtained. The cover is mounted on the prism face opposite the 90° prism angle. The scribed line, or the carbon black, should be on the upper side of the cover; see Fig. 111. Assuming that the line is used, it should run lengthwise of the prism face as shown in the figure. A prism specially made for this work can be obtained from Nichols.[20]

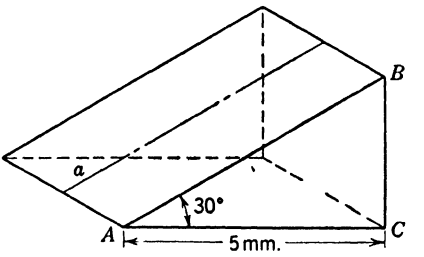


FIG. 111. A prism for measuring the field depth of a low-power lens. The angle of 30° measured in the principal section, as above, can be made less for measuring the shorter-focus photomicrographic objectives.

With the prepared prism on the microscope stage, the line can be focused. The rear element of the condenser should be removed and the source focused as nearly as possible on the front lens of the objective. The part of the field which is critically sharp can then be

[20] Lyman Nichols, 111 Vreeland Avenue, Nutley, N. J.

measured. Figure 112 is a photomicrograph of such a field. It might be noticed that the out-of-focus images are about the same on either side of the focus.

The prism angle BAC, Fig. 111, and the length of the line a (which is satisfactorily sharp, as seen by inspection through the microscope), being known, the geometrical figure as shown in Fig. 113 can be drawn.

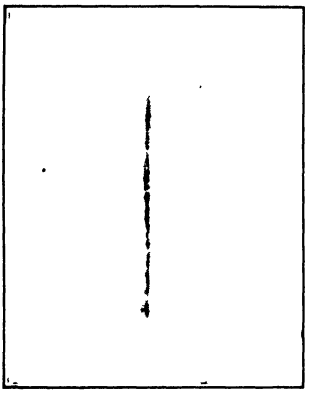


FIG. 112. Photomicrograph of the line as shown on the prism in Fig. 111. The portion which is acceptably sharp is the equivalent of the distance $A'B'$ in Fig. 113.

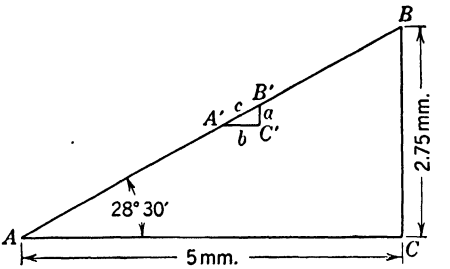


FIG. 113. The geometrical relation of the focused line $A'B'$ to the prism section ABC.

The measurement of the line c gives the length of b which is a projection of c on a horizontal plane. Tan $A' = a/b$. Substituting actual values and solving for a gives the depth of focus for the objective in question. Triangle $A'B'C'$ is, of course, similar to triangle ABC.

The measurement of field depth of high-power lenses is of so little importance that it is hardly worth making. The 2-mm apochromat has a field depth of something less than 0.5 μ, a very small distance to measure. If any of this work is attempted, it should be done only with a microscope which has a fine adjustment calibrated in not more than 1 μ per division. By focusing the objective very carefully on a flat surface such as a small hole in the aluminum film of a test plate, the microscope tube can be raised slightly by the fine adjustment until the focus is disturbed. It will be seen that an almost inperceptible motion of the tube will destroy the focus.

Figures for field depth obtained in the above manner will always be larger than a corresponding photomicrographic method will indicate. This is due to the natural accommodation exerted by the eye. In other

words, the eye sees greater depth than can be registered on a photographic plate, the exact difference between the two methods of recording field depth depending upon the condition of the visual apparatus of the individual. Thus, for photomicrographic work the following equations may be useful.

For the camera

$$\text{Depth of field} = \frac{\lambda\sqrt{n^2 - (\text{N A.})^2}}{(\text{N.A.})^2} \qquad [49]$$

For visual work[21]

$$\text{Depth of field} = \frac{250 \text{ mm}}{M^2} \qquad [50]$$

For low-power objectives when the apertures are rated in f values, the N.A. may be found by this equation:

$$\text{N.A.} = \frac{M}{2f(M + 1)} \qquad [51]$$

where f is the f number denoting relative aperture.

The focal depth at the focal plane of the camera is

$$\text{Depth of focus} = \text{Depth of field (equation 49)} \times M^2 \qquad [52]$$

From equation 49 the field depth of a 16-mm apochromat N.A. 0.3, using light with a wavelength of 555 mμ, is only 0.00588 mm, while if the magnification is 300 the focal depth is 529.2 mm, a rather startling result. For field-depth values of other objectives see curve Fig. 114.

11. *Inspection for Lateral Chromatic Aberration of Objectives Intended for Color Work.* An objective that has successfully passed the test for chromatic aberration is not necessarily a satisfactory objective for color photomicrography. It was stressed in that test that observations were to be made in the center of the field. Photomicrography demands a large image field. Therefore, corrections must extend at least three-quarters of the way across the field of view in order to cover a 5 by 7 plate satisfactorily when the bellows extension is about 570 mm. The National Bureau of Standards has worked out a method for measuring the lateral chromatic aberration in different parts of the field; it is described in *Research Paper* 316.[22]

For most practical work, a somewhat simpler method for measuring

[21] As Hardy and Perrin prove, this equation is for persons able to accommodate for an object distance of 250 mm, *op. cit.*, p. 512.

[22] I. C. Gardner and F. A. Case, *Research Paper* 316, National Bureau of Standards.

lateral chromatic aberration than that given in the research paper will suffice. In the center of the field, color images will probably coincide in size fairly well when proper eyepieces are used, but, as the distance of the image from the center of the field increases, it will be found that the focal length of the objective changes for these different positions

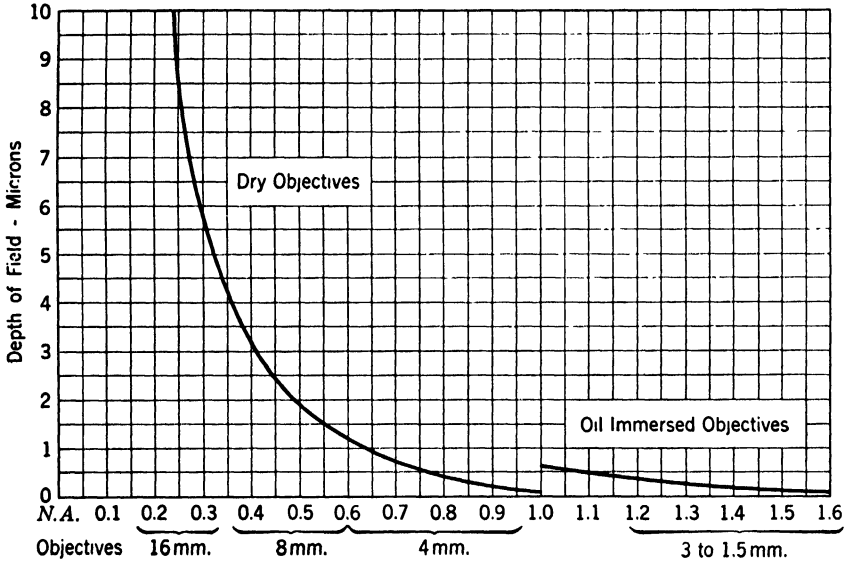


FIG. 114. The field depth of objectives plotted against N.A.

as the color screens are changed. This change necessitates refocusing, and consequently the size of the different color images varies. The image formed with the red screen would be found to be the smallest, the green the next in size, and the blue the largest (the second principal plane for the blue light is closer to the F' point than the principal plane for red light). The fact that small objects often appear smaller in blue light than in red is not often due to lateral chromatic error but rather to resolution.

If three-color separation negatives are made with a lens showing difference in magnification due to color the result will be a picture hazy in outline; the haziness will increase from the center outward, and the bigger the negative the more evident will this effect become.

The difference in size of these color images can be roughly measured on the ground glass of the camera, particularly if the ground glass is 5×7 inches or, better yet, 8×10 inches. The larger plate may not be used in actual work, but the objective that comes nearest to having a constant focal length for the different colors near the edges of the

bigger plate will surely give more equal magnification near the center. The image of a test object, such as a stage micrometer, should be projected at the approximate distance at which the work will be carried on. With a pair of dividers, measurements can be made of the distance between the intervals, as shown on the ground glass. Since the Eastman filters A, B, and C will probably be used later, measurements should be made successively with them, and the differences noted.

The results so obtained will indicate the actual amount of overlapping of the different images. A study of a lens in the manner described is very interesting. The measurements must be precise to within 0.2 mm, and the objective should be tested first without an eyepiece. The 4-mm objective is a good one to try. The bellows must be well extended. The tests should be made by every worker in photomicrography, whether he expects to do color work or not. If they are carried out in white light, and if several oculars are tried, it will be possible to select the proper combination to give the best images over the entire field.

12. *A General Survey of the Field of View.* A general examination of the behavior of an objective is often very enlightening. It can be carried out as follows: With a mount of finely ground material, such as corundum (test slide 3), for low objectives, and black magnetic rouge (test slide 2), or its equivalent, for high objectives, the field should be critically studied from the center outward. The star test can be applied at first to ascertain the best tube length, the eyepieces can be changed, and the resulting effect on the images noted. Color screens can be introduced, and, since the images are nearly colorless in white light, any change in the performance of the objective will be due to its chromatic corrections and to eye conditions. The field should be examined for failure of definition, progressing from the center to the periphery; the general flatness of the field and the size of the area that is in focus should be taken into account. Any eyestrain experienced when examining particles in the center of the field should be noted; it may increase as the outer portions are examined. If an immersion condenser is used, it should be immersed, even though the objective may not demand it for purposes of filling the back lens with light.

With the appropriate eyepiece, any objective can reasonably be expected to give a critically sharp image in the center of the field, with absolutely no eyestrain. Eyeglasses, if worn, should be for distant vision (Belling). If the lens is achromatic, the strength of the ocular probably must not exceed 12.5× under the best of conditions, and it may be an 8× or even 5×. An apochromatic lens should be capable of taking a 15× ocular easily; often it will take a 20×, particularly in the

lower and medium powers. A superficial examination like this should be made with light of daylight quality and later with the red, green, and blue filters. If the lens is of high power it is best, though not necessary, to illuminate by Method II.

One valid objection might be raised to all the above tests. It may be said that the objective is never tested alone but always in conjunction with an eyepiece. This is perfectly true, but such precise testing as is indicated by testing an objective alone properly comes within the province of the optical companies or an optical laboratory, where more elaborate apparatus is available. All the tests cited here can be carried out by the technician with his own equipment, with his own eyepieces, and under his own operating conditions, with little or no expense for accessories and with small expenditure of time. From information he can so gain he will be in a position to select lenses best suited to his purpose and to compare and evaluate their respective merits. Certain tests can be eliminated as the occasion warrants, but the better and finer the lens, the more exhaustively should it be tested.

Sec. 66. Summary. 1. A lens can be evaluated for work at hand by systematic inspection for some of its constants and for its general mechanical condition.

2. Such an examination is probably of more value to the user than an inspection and report by an optical laboratory.

3. Examinations for spherical and chromatic errors are largely comparative and depend to a great extent upon the experience of the technician.

4. Low-power lenses need only simple tests, such as tests 1, 2, 3, 4, 5, 12, and perhaps 6.

5. The test for N.A. is important for expensive lenses and those with a high N.A. rating.

6. It is important to observe chromatic errors if the lens is to be used for color photomicrography, particularly if the three-color process is employed.

7. Color corrections should be more carefully examined in apochromatic lenses than in achromats. Any color fringes around an image in the central part of the field should disqualify a lens for any class of work, assuming that the lens has been used correctly.

8. The examination of objectives should be carried out with appropriate oculars and proper tube length.

9. Useful information can be obtained by using filters of different colors (red, green, and blue) when inspecting for spherical errors.

10. The tests for field depth, for curvature of the field, and for aplanatic condition are not of major importance for a lens which is to

be used for visual work alone. In fact, lenses which have little field depth and great field curvature, and which offer small central areas where the image can be made very sharp, may give comparatively high lateral resolution. However, an objective to be used for photomicrography should have the minimum field curvature and should give sharp images well toward the periphery of the field of view.

11. Poor centration of lens elements, poor spherical corrections in the center of the field, and poor mechanical condition should be considered contributing causes in rejecting a lens.

12. For purposes of testing an objective of high power, illumination should be by Method II. The condenser should be achromatic in quality and properly adjusted; otherwise the tests may be misleading, particularly the test for resolution.

Sec. 67. The Care and Cleaning of Objectives. When not in use the objectives should be kept on the microscope or in the boxes provided for them. Generally they should be stored in a dry place to restrain chemical action and to discourage the growth of mould on the glass surfaces. The growth of mould is more likely to take place in the tropics than in northern latitudes. A desiccator is an excellent place to store objectives.

To clean an objective a solvent is generally indicated. Water may answer, but more often a fat solvent, such as xylene or benzine, is required. Benzine is much to be preferred because it has a lower boiling point and will evaporate more quickly than xylene; therefore there is less time for it to enter between the lens elements and dissolve the balsam cement. If the lens has been immersed, the surplus oil can be wiped off with a piece of dry lens paper, then with the lens paper dampened with benzine; the glass surface should be lightly rubbed several times and dried. If the lens is to be used again within a few hours, this will be sufficient; but if it is to be put away for an indefinite period it should be examined with a pocket magnifier and the last traces of oil removed. It can then be swabbed with distilled water and dried.

A good swab can be made from a stick with a tuft of cotton twisted around the end, such as physicians use to treat the throat. The swab can also be made from a piece of lens paper cut about 12 mm wide and 4 to 6 cm long. Figure 115 illustrates how it can be rolled around the end of the stick, with just enough paper overlapping the end to form a cushion. This will keep the stick from injuring the glass. The upper portion of the end of the paper can be moistened with the tongue just before the roll is finished; the moisture will act as an adhesive and hold the roll together until it is discarded. These paper rolls seem

to be much more efficient than cotton swabs since they leave less fiber on the glass.

In cleaning an objective, a fair-sized rubber bulb is indispensable. It should be used to blow a blast of air over the lens surfaces and so free them from adhering grit before cleaning with the swab. The rubber bulb can be used again to remove any fiber particles that may remain after cleaning with the swab. A bulb is much superior to the breath for this purpose, since the breath is sure to cause some condensation, which, unless completely wiped off, will dry and leave a slight film on the lens surface. Also, it seems to act as a mild adhesive, tending to anchor lint particles on the glass.

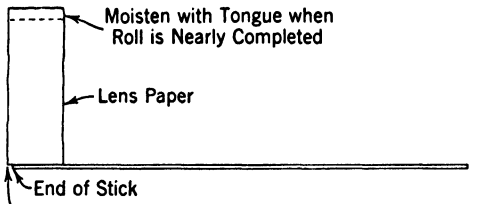


FIG. 115. Small sticks (throat swabs), obtainable at the drugstore, provide an easy method for rolling a piece of lens paper for cleaning objectives.

The front lenses of dry objectives do not need a great deal of cleaning, but they should be examined frequently with a magnifier, and, if they are found greasy or dusty, some of the dirt can be blown off with the bulb, the rest being removed as described for immersion lenses.

The back lenses of objectives are more likely to collect dirt than those at the front, because they are in a position to catch any of the settling dust and dirt particles admitted to the microscope tube when oculars are changed. Each time the condenser is examined for centration an opportunity is offered to inspect the surface of the back lens of the objective This inspection should become a habit with the photomicrographer. Although it is surprising how much dirt may collect on the back lens of the objective without degrading the image as seen through the microscope, nevertheless, the lenses should be kept clean because dirt offers opportunity for the gathering of moisture and so enhances the chances of chemical change on the glass surface. Any chemical change generally shows as grayish filmlike patches under the magnifier. It cannot be removed easily, and the lens must be sent to the maker for repairs.

The metal parts of objectives can be wiped off with a soft cloth dampened with xylene. Alcohol should never be allowed to come in contact with the lens surface, since shellac may have been used to set the lens elements. Modern objectives, particularly those of foreign make, usually should not be taken apart for cleaning; most of them are put together with special instruments and should be opened only under

factory conditions. Factory care in assembly assures better centration of the lens elements and greater resistance to shock. However, there are certain low-power lenses that are intended to be unscrewed,

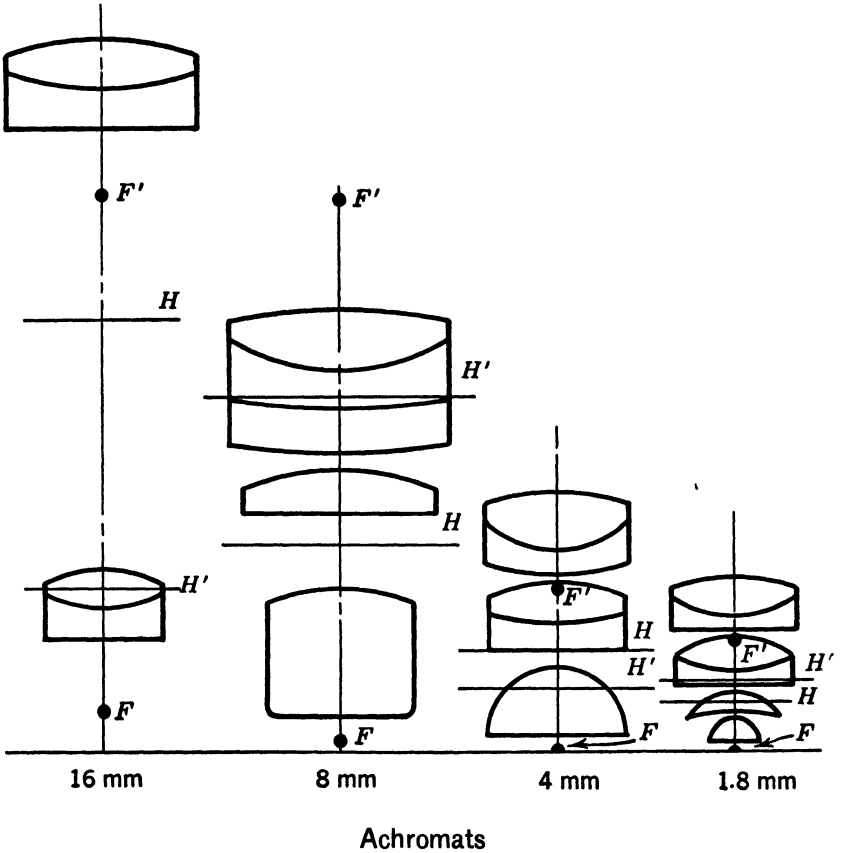
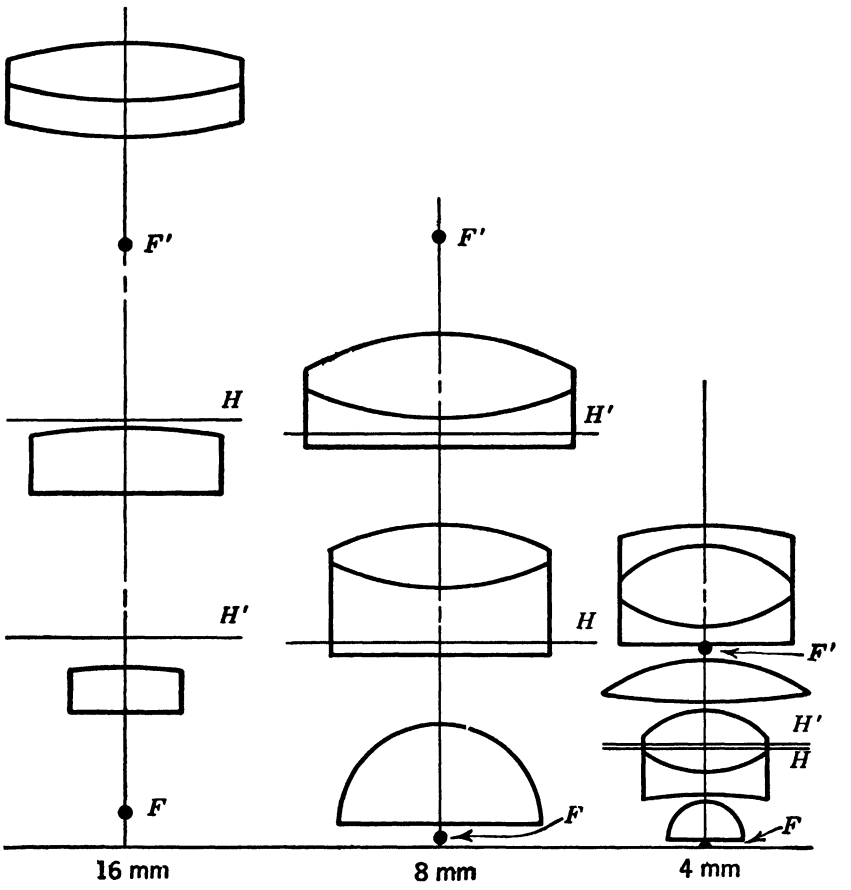


FIG. 116A.

FIG. 116. This figure illustrates the lens elements of a series of achromatic and apochromatic microscope objectives. Since the focal length of a lens is measured from its principal plane, the drawings show why the front of the objective glasses can be closer to the object than the focal length of the lens would seem to permit. It is also shown that the second focal point is often very low in the objective mount, that the first and second focal lengths are not equal in the oil-immersion systems, and that the principal planes may be, and often are, crossed. In order to make this drawing it was necessary to work from certain assumptions which may not have been correct; accordingly the positions of the focal and principal points of the systems shown may be in considerable error. However, that does not lessen the value of the drawings for the purpose for which they are intended.

so that the various elements may be separated to form a longer-focus lens. These and the older types of lenses may need to be taken apart for cleaning purposes. Scratches on the surface of a lens, unless very bad, are as a rule rather harmless as far as image formation is concerned. So, too, are the air bubbles in the glass of large lenses.

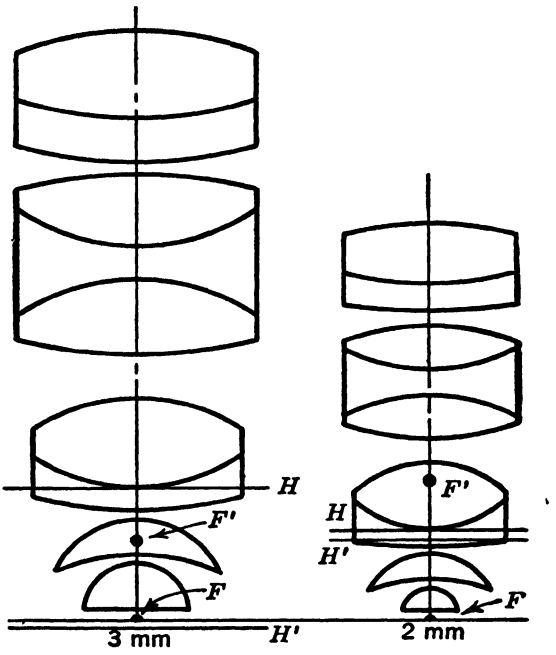


Apochromats (Dry)

FIG. 116*B*.

Bubbles often occur in some of the best photographic lenses. In small lenses, bubbles increase lenticular glare and should not be tolerated. The fluorite or fluorspar used in lenses of both the achromatic and apochromatic series hold many inclusions which at first glance might seem detrimental to the performance of the lens, but in actual work it

is difficult to prove that the image formed by such a lens suffers. As a matter of fact, it is almost impossible to find pieces of fluorite entirely free from such inclusions.



Apochromats (Oil Immersion)

FIG. 116C.

Corrosive fumes, particularly those of hydrofluoric acid, should be kept away from all microscopical equipment at all times.

Figure 116 indicates the change in position of the principal planes and focal points for two series of objectives, and Table XVII lists objectives of leading optical companies.

Table XVII

Objectives

Bausch and Lomb

Achromatic Objectives. Tube Length, 160 mm
Cover-Glass Thickness, 0.18 mm

Equivalent Focal Length, mm	Magnification	N.A.	Working Distance, mm	Immersion	Remarks
48.0	2.0	0.08	53.0	Dry	
40.0	2.6	0.08	43.5	Dry	
28.0	3.2	0.08	12.5	Dry	
32.0	4.0	0.10	38.0	Dry	
22.7	6.0	0.17	15 5	Dry	
16–32	10–4	0.25–0.10	7–38	Dry	Elements divisible
16 0	10 0	0.25	7.0	Dry	
8.0	21.0	0.50	1.6	Dry	
8.0	21.0	0.50	1.6	Dry	Iris diaphragm
7.0	26.0	0.50	2.0	Water	
5.5	31.0	0.65	0.9	Dry	
4.0	43.0	0.65	0.6	Dry	
4.0	45.0	0.85	0.3	Dry	
4.0	43.0	0.65	0.6	Dry	Iris diaphragm
3.0	60.0	0.85	0.2	Dry	
2.2	81.0	1 10	0.15	Water	
2.2	80.0	1.25	0.2	Oil	
1.8	97.0	1.25	0.13	Oil	
1.8	97.0	1.25	0.13	Oil	Iris diaphragm

Bausch and Lomb

Achromatic Fluorite Objectives. Tube Length, 160 mm
Cover-Glass Thickness, 0.18 mm

4.3	40.0	1.00	0.27	Oil	
4.0	43.0	0.85	0.34	Dry	
1.8	98.0	1.30	0.13	Oil	
1.8	98.0	0.80	0.35	Oil	

Bausch and Lomb

Apochromatic Objectives. Tube Length, 160 mm
Cover-Glass Thickness, 0.18 mm

16.0	10.0	0.30	4.85	Dry	
8.3	20.0	0.65	0.50	Dry	
4.0	45.0	0.95	0.18	Dry	Correction mount
3.0	62.0	0.95	0.13	Dry	Correction mount

Table XVII (*Continued*)

Bausch and Lomb

Apochromatic Objectives. Tube Length, 160 mm.
Cover-Glass Thickness, 0.18 mm.

Equivalent Focal Length, mm	Magnification	N.A.	Working Distance, mm	Immersion	Remarks
3.0	61.0	1.40	0.12	Oil	
2.2	78.0	1.00	0.135	Glycerin	
2 0	90.0	1.30	0.12	Oil	
2 0	90.00	1.40	0.07	Oil	
1.5	120.0	1.30	0.08	Oil	

Bausch and Lomb

Achromatic Objectives for the Petrographic Microscope.
Cover-Glass Thickness, 0.18 mm.

40.0	2.6	0.08	43.5	Dry	
32.0	4.0	0.10	38.0	Dry	
22.7	6.0	0.17	15.5	Dry	
16.0	10.0	0.25	7.0	Dry	
8.0	21.0	0.50	1.6	Dry	
4.0	45.0	0.85	0.3	Dry	
4.0	36.0	0.95	0.25	Dry	
1.8	97.0	1.25	0.14	Oil	
1.8	98.0	1.30	0.13	Oil	Fluorite in construction

Bausch and Lomb

Achromatic Objectives. Tube Length, 215 mm.
For use without a cover glass.

32.0	5.0	0.10	38.0	Dry	
32.0	5.75	0.12	21.0		
24.0	18.0	0.20	14.0	Dry	
16.0	12.5	0.25	7.0	Dry	
10.25	20.0	0.40	2.80	Dry	
8.0	26.0	0.50	1.60	Dry	
5.5	37.5	0.65	0.90	Dry	
4.0	54.0	0.85	0.30	Dry	
1.8	120.0	1.25	0.32	Oil	

Table XVII (*Continued*)

Bausch and Lomb

Fluorite Objectives. Short Mounts. Tube Length, 215 mm.
For use without a cover.

Equivalent Focal Length, mm	Magnification	N.A.	Working Distance, mm	Immersion	Remarks
4.0	52.5	0.85	0.34	Dry	
4.3	50.0	1.0	0.45	Oil	
2.75	78.0	1.25	0.44	Oil	
1.8	116.0	1.30	0.13	Oil	

Bausch and Lomb

Apochromatic Objectives. Short Mounts. Tube Length, 215 mm.
For use without a cover glass.

4.0	60.0	0.95	0.18	Dry	
3.0	75.5	1.40	0.29	Oil	

Spencer

Achromatic Objectives. Tube Length, 160 mm.
Cover-Glass Thickness, 0.18 mm.

*48.0	2.2	0.08	52.5	Dry	
*40.0	2.8	0.08	35.2	Dry	
*32–14	4–12	0.12–0.24	31.5–6.0	Dry	Elements divisible
32.0	4.0	0.10	21.0	Dry	
30.2	3.5	0.09	24.1	Dry	
24.0	3.5	0.08	24.0	Dry	
*25.0	5.0	0.17	21.0	Dry	
16.0	10.0	0.25	4.5	Dry	
16.0	10.0	0.25	4.5	Dry	Elements divisible
8.0	20.0	0.50	1.44	Dry	
*5.0	36.0	0.60	0.8	Dry	
4.0	44.0	0.66	0.63	Dry	
4.0	45.0	0.85	0.2	Dry	
*3.0	60.0	0.85	0.2	Dry	
3.0	60.0	1.25	0.25	Oil	
1.8	95.0	1.25	0.13	Oil	
*1.5	115.0	1.25	0.10	Oil	

* Suspended.

Table XVII (*Continued*)

Spencer

Fluorite Objectives. Tube Length, 160 mm.
Cover-Glass Thickness, 0.18 mm.

Equivalent Focal Length, mm	Magnification	N.A.	Working Distance, mm	Immersion	Remarks
4.0	44.0	0.85	0.52	Dry	
1.8	97.0	1.30	0.10	Oil	

Spencer

Apochromatic Objectives. Tube Length, 160 mm.
Cover-Glass Thickness, 0.18 mm.

16.0	10.0	0.30	5.2	Dry	
8.0	20.0	0.60	0.75	Dry	
4.0	44.0	0.95	0.20	Dry	Correction mount
3.0	60.0	0.95	0.16	Dry	Correction mount
3.0	60.0	1.30	0.20	Oil	
3.0	60.0	1.40	0.17	Oil	
2.0	90.0	1.30	0.10	Oil	
2.0	90.0	1.40	0.05	Oil	
1.5	120.0	1.30	0.08	Oil	

Spencer

Achromatic Objectives for the Petrograph. Tube Length, 166.4 mm.
Cover-Glass Thickness, 0.18 mm.

48.0	2.2	0.08	52.5	Dry	
40.0	2.8	0.08	35.2	Dry	
32.0	4.0	0.10	21.0	Dry	
25.0	5.1	0.17	21.0	Dry	
16.0	10.0	0.25	4.5	Dry	
8.0	20.0	0.50	1.44	Dry	
8.0	20.0	0.85	1.00	Dry	Iris diaphragm
4.0	44.0	0.66	0.63	Dry	
4.0	45.0	0.85	0.20	Dry	
3.0	60.0	0.85	0.20	Dry	
1.8	95.0	1.25	0.13	Oil	

Table XVII (*Continued*)

Zeiss

Achromatic Objectives. Tube Length, 160 mm.
Cover-Glass Thickness, 0.17 mm.

Equivalent Focal Length, mm	Magnification	N.A.	Working Distance, mm	Immersion	Remarks
55.0	1–1.5		64–47	Dry	
45.0	1.5–2		32–25	Dry	
50.0	2.0		60.0	Dry	
	1.2–2.4		33–7	Dry	
36.0	3.0		29.0	Dry	
25.0	5.0		12.0	Dry	
23 5	6.0	0.17	9.0	Dry	
24.0	6.0	0.11	36.0	Water	
18.0	8.0	0.20	9.0	Dry	
15.6	10.0	0.30	7.50	Dry	
8.3	20.0	0.40	1.6	Dry	
4.4	40.0	0.65	0.55	Dry	
4.3	40.0	0.75	1.9	Water	
3.5	50.0	0.85	0.40	Oil	Dark-field
3.0	60.0	1.00	0.12	Glycerin	Quartz cover 0.75 mm.
2.0	90.0	1.18	0.07	Water	Correction mount
2.0	90.0	1.25	0.15	Oil	
2.0	90.0	1.25	0.16	Oil	Iris diaphragm

Zeiss

Fluorite Objectives. Tube Length, 160 mm.
Cover-Glass Thickness, 0.17 mm.

4.4	40.0	0.85	0.32	Dry	
4.4	40.0	0.85	0.32	Dry	Correction mount
2.9	60.0	0.90	0.12	Dry	
2.9	60.0	0.90	0.12	Dry	Correction mount
2.0	90.0	0.90	0.09	Dry	Correction mount
1.8	100.0	1.30	0.10	Oil	

Zeiss

Apochromatic Objectives. Tube Length, 160 mm.
Cover-Glass Thickness, 0.17 mm.

25.0	6.0	0.15	7.3	Dry	
16.2	10.0	0.30	5.0	Dry	
8.3	20.0	0.65	0.7	Dry	

Table XVII (*Continued*)

Zeiss

Apochromatic Objectives. Tube Length, 160 mm.
Cover-Glass Thickness, 0.17 mm.

Equivalent Focal Length, mm	Magnification	N.A.	Working Distance, mm	Immersion	Remarks
5.0	35.0	0.85	0.25	Oil	
4.3	40.0	0.95	0.12	Dry	Correction mount
2.9	60.0	0.95	0.07	Dry	Correction mount
2.9	60.0	1.0	0.22	Oil	Iris diaphragm
2.9	60.0	1.30	0.15	Oil	
2.9	60.0	1.40	0.13	Oil	
2.5	70.0	1.25	0.11	Water	
2.0	90.0	1.30	0.11	Oil	
2.0	90.0	1.40	0.05	Oil	
1.5	120.0	1.30	0.08	Oil	

Zeiss

Achromatic Objectives in Short Mounts for Metallographic Work.
Tube Length, 190 mm. No Cover Glass.

36.0	4.0		29.0	Dry
25.0	6.0		19.0	Dry
23.5	7.3	0.17	11.0	Dry
18.0	9.0	0.20	9.0	Dry
15.6	12.0	0.30	7.5	Dry
8.3	21.0	0.40	1.6	Dry
4.4	40.0	0.65	0.6	Dry
3.5	53.0	0.90	0.57	Oil
2.0	95.0	1.25	0.32	Oil

Zeiss

Achromatic Fluorite Objectives for Metallography.
Tube Length, 190 mm. No Cover Glass.

4.4	40.0	0.85	0.32	Dry
2.9	60.0	0.90	0.12	Dry
1.8	100.0	1.30	0.27	Oil

Table XVII (*Continued*)

Zeiss

Equivalent Focal Length, mm	Magnification	N.A.	Working Distance, mm	Immersion	Remarks
Apochromatic Objectives for Metallography. Short Mounts. Tube Length, 190 mm. No Cover Glass.					
16.2	10.0	0.30	5.0	Dry	
8.3	22.0	0.65	0.7	Dry	
4.3	40.0	0.95	0.12	Dry	
2.9	62.0	0.95	0.04	Dry	
2.9	64.0	1.30	0.30	Oil	
2.9	64.0	1.40	0.30	Oil	
2.5	74.0	1.25	0.11	Water	
2.0	94.0	1.30	0.28	Oil	
Zeiss					
Monobromonaphthalene-Immersion Objective. Tube Length, 160 mm.					
2.5	74.0	1.60	0 07	Monobromo-naphthalene	No cover
Zeiss					
Flat-Field Objective. Achromat. Low Power. Tube Length, 160 mm.					
18.0	8.0	0 20	15.0	Dry	Special ocular required
Zeiss					
Achromatic objective for Epi Lamp No. 3. No Cover.					
8.3	20.0	0.40	1.6	Dry	
4.3	40.0	0.65	0.53	Dry	
Zeiss					
Achromatic Objectives for Epi Condenser W					
	5.3	0.14		Dry	
	5.7	0.17		Dry	
	7.5	0.23		Dry	
	9.0	0.30		Dry	
	17.0	0.42		Dry	
	21.0	0.65		Dry	
	30.0	0.75		Water	
	33.0	0.55		Dry	Fluorite
	33.0	0.85		Oil	
	50.0	1.00		Oil	

Table XVII (*Continued*)

Leitz

Achromatic Objectives. Tube Length, 170 mm.
Cover-Glass Thickness, 0.16 to 0.18 mm.

Equivalent Focal Length, mm	Magnification	N.A.	Working Distance, mm	Immersion	Remarks
56.0	1.0	0.05	55.0	Dry	
42.0	2.7	0.08	40.0	Dry	
40.0	3.2	0.12	34.5	Dry	
32.0	4.3	0.15	27.0	Dry	
29–16	4.5–10.0	0.15–0.25	5.8–26.0	Dry	
24.0	3.2	0.08	3.2	Dry	
24.0	6.0	0.20	16.0	Dry	
20.0	8.0	0.30	7.7	Dry	
16.0	10.0	0.25	0.65	Oil	
13.0	14.0	0.40	3.2	Dry	
9.0	22.0	0.45	2.0	Dry	
8.0	22.0	0.65	0.45	Oil	
6.0	30.0	0.65	0.75	Dry	
4.0	45.0	0.85	0.32	Dry	
4.0	45.0	0.65	0.60	Dry	
3.6	50.0	1.00	0.40	Water	
3.0	62.0	0.85	0.28	Dry	
2.1	90.0	1.20	0.10	Water	
1.8	90.0	1 25	0.13	Oil	
1.8	100.0	1.30	0.11	Oil	

Leitz

Fluorite Objectives. Tube Length, 170 mm.
Cover-Glass Thickness, 0.16 to 0.18 mm.

4.2	42 0	0.85	0.38	Dry	
3.45	54.0	0.95	0.20	Oil	
3.2	58.0	0.85	0.28	Dry	
2.6	70.0	0.90	0.25	Dry	
2.6	70.0	1.30	0.18	Oil	
2.2	85.0	0.90	0.13	Dry	
1.95	95.0	1.32	0.11	Oil	
1.6	114.0	1.32	0.08	Oil	

Table XVII (*Continued*)

Leitz

Apochromatic Objectives. Tube Length, 170 mm.
Cover-Glass Thickness, 0.16 to 0.18 mm.

Equivalent Focal Length, mm	Magnification	N.A.	Working Distance, mm	Immersion	Remarks
16.0	12.0	0.30	5.0	Dry	
8 0	23.0	0.65	0.85	Dry	
4.0	46.0	0 95	0.20	Dry	Correction collar
3.0	65.0	0.95	0.15	Dry	Correction collar
3.0	65.0	1.32	0.13	Oil	
3.0	65.0	1.40	0 13	Oil	
2.0	92.0	1.32	0.11	Oil	
2.0	92.0	1 40	0 05	Oil	

Leitz

Achromatic Objectives in Short Mounts for Metallographic Work.
Tube Length, 215 mm. No Cover Glass

40.0	5.5	0.12	Dry
32.0	8.5	0 15	Dry
24.0	12.0	0.20	Dry
16.0	20.0	0.25	Dry
16.0	20.0	0.25	Oil
14.0	25.0	0.40	Dry
9.0	37.0	0.45	Dry
8.0	39.0	0.65	Oil
6.0	55.0	0.65	Dry
4.0	78.0	0.85	Dry

Leitz

Fluorite Objectives. Same Series.

4.0	73.0	0.85	Dry
3.5	96.0	0.95	Oil
2.6	125.0	1.30	Oil
2.0	170.0	1.32	Oil

Leitz

Apochromatic Objectives. Same Series.

8.0	44.0	0.65	Dry
4.0	73.0	0.95	Dry
3.0	103.0	1.32	Oil
3.0	103.0	0.95	Dry
2.0	170.0	1.32	Oil
2.0	170.0	1.40	Oil

Table XVII (*Continued*)

Leitz

Achromatic Objectives for Ultropak System. No Cover Glass.

Equivalent Focal Length, mm	Magnification	N.A.	Working Distance, mm	Immersion	Remarks
	3.8	0.12	33.0	Dry or water	
	5.0	0.15	26.3	Dry or water	
	6.5	0.18	16.2	Dry or water	
	11.0	0.25	5.8	Dry or water	
	11.1	0.25	5.8	Water	
	22.1	0.45	2.2	Water	
	22.0	0.45	2 2	Dry	
	23.0	0.55	0.65	Water	
	50.0	0.65	0.70	Dry	
	55.0	0.84	0 57	Water	
	75.0	0.90	0.45	Water	
	90 0	1.00	0.42	Water	

Leitz

Fluorite Objectives. Similar Series

	23.0	0.55	0.65	Oil
	60.0	0 85	0.57	Oil
	75.0	1.00	0.51	Oil
	100 0	1 00	0 48	Oil

Beck

Achromatic Objectives. Tube Length, 160 mm. Cover-Glass Thickness, 0.18 mm.

60.0	1.3	0.07	Dry	
50.0	2.0	0 08	Dry	
32.0	4.5	0.12	Dry	
32.0	4.5	0.15	Dry	
25.0	6.3	0.12	Dry	
16.0	11.0	0.17	Dry	
16.0	11.0	0.28	Dry	
8.0	20.0	0.54	Dry	
6.0	30.0	0 85	Dry	
4.0	49.0	0 85	Dry	
4.0	49.0	0.65	Dry	
3.0	65.0	0.95	Oil	
3.0	65.0	1.20	Oil	Stop to reduce aperture
2.0	90.0	1.0	Oil	
2.0	90.0	1.2 or 1.3	Oil	

Table XVII (*Continued*)

Beck

Apochromatic Objectives. Tube Length, 160 mm.
Cover-Glass Thickness, 0.18 mm.

Equivalent Focal Length, mm	Magnification	N.A.	Working Distance, mm	Immersion	Remarks
40.0	3.3	0.16		Dry	
16.0	10.0	0.35		Dry	
14.0	14.0	0.35		Dry	
8.0	19.2	0.65		Dry	
4.0	47.5	0.95		Dry	
4.0	47.5	0.95		Dry	Correction collar
3.0	63.5	1.2		Oil	Stop to N.A. 0.95
2.0	88.5	1.2		Oil	Stop to N.A. 0.95
2.0	88.5	1.3		Oil	
2.0	88.5	1.4		Oil	

Swift

Achromatic Objectives. Tube Length, 160 mm.
Cover-Glass Thickness, 0.17 mm.

70.0	1.4	0.08		Dry	
60.0	1.7	0.09		Dry	
45.0	3.0	0.10		Dry	
45.0	2.7	0.17		Dry	
32.0	4.2	0.12		Dry	
32.0	4.2	0.20		Dry	
25.0	5.7	0.16		Dry	
24.0	6.2	0.25		Dry	
16.0	9.2	0.17		Dry	
16.0	8.3	0.30		Dry	
12.0	13.4	0.50		Dry	
8.0	20.2	0.50		Dry	
6.0	32.3	0.80		Dry	
4.0	41.0	0.72		Dry	
4.0	42.5	0.85		Dry	
3.0	51.5	0.85		Dry	
3.0	52.0	0.92		Dry	
4.0	41.5	0.92		Oil	
3.0	56.0	0.92		Oil	
3.0	56.0	1.30		Oil	
2.0	87.0	1.20		Oil	

Table XVII (*Continued*)

Swift

Achromatic Objectives. Tube Length, 160 mm.
Cover-Glass Thickness, 0.17 mm.

Equivalent Focal Length, mm	Magnification	N.A.	Working Distance, mm	Immersion	Remarks
2.0	92.5	1.33		Oil	
1.5	121.0	1.30		Oil	
1.2	162.0	1.30		Oil	

Swift

Fluorite Objectives. Tube Length, 160 mm.
Cover-Glass Thickness, 0.17 mm.

3.0	57.0	0.92		Oil	
3.0	57.0	1.32		Oil	
2.0	92.6	1.32		Oil	
1.5	120.0	1.32		Oil	

Swift

Apochromatic Objectives. Tube Length, 160 mm.
Cover-Glass Thickness, 0.17 mm.

40.0	3.0	0.12		Dry	
25.0	5.5	0.25		Dry	
24.0	6.5	0.30		Dry	
16.0	10.7	0.30		Dry	
12.0	16.2	0.65		Dry	
8.0	21.0	0.65		Dry	
6.0	31.2	0.92		Dry	
4.0	40.0	0.92		Dry	
4.0	40.0	0.95		Dry	Correction collar
3.0	56.2	1.30		Oil	
3.0	56.2	1.40		Oil	
2.0	87.5	1.30		Oil	
2.0	87.5	1.40		Oil	
1.5	112.0	1.30		Oil	

Table XVII (*Continued*)

Baker

Achromatic Objectives. Tube Length, 160 or 250 mm.

Equivalent Focal Length, in.	Magnification 160-mm tube	N.A.	Working Distance, mm	Immersion	Remarks
4.0	1.4	0.08		Dry	
3.0	2.0	0.08		Dry	
2.0	2.8	0.10		Dry	
2.0	2.8	0.15		Dry	
1½	3.9	0.197		Dry	
1.0	6.0	0.197		Dry	
⅔	11.5	0.15		Dry	
⅔	11.5	0.25		Dry	
¾	10.5	0.25		Dry	
½	15.0	0.35		Dry	
⅓	17.0	0.30		Dry	
¼	34.0	0.75		Dry	
⅛	44.0	0.82		Dry	
⅛	63.0	0.85		Dry	
1.0	6.5	0.11		Water	
⅛	40.0	0.75		Water	
⅛	61.5	0.95		Oil	
1/12	101.5	1.30		Oil	
1/12	101.5	1.20		Oil	

Watson

Parachromatic (Achromatic) Objectives. Tube Length, 160 mm.
Cover-Glass Thickness, 0.18 mm.

Equivalent Focal Length, mm	Magnification at 10 in.	N.A.	Working Distance, mm	Immersion	Remarks
100.0	3.0	0.08		Dry	
75.0	4.0	0.09		Dry	
50.0	6.0	0.15		Dry	
35.0	8.0	0.17		Dry	
25.0	12.0	0.21		Dry	
16.0	15.0	0.28		Dry	
12.0	20.0	0.34		Dry	
6.0	42.0	0.68		Dry	

Table XVII (*Continued*)

Watson

Parachromatic (Achromatic) Objectives. Tube Length, 160 mm. Cover-Glass Thickness, 0.18 mm.

Equivalent Focal Length, mm	Magnification at 10 in.	N.A.	Working Distance, mm	Immersion	Remarks
4.0	65.0	0.70		Dry	
4.0	65.0	0.70		Dry	For uncovered
4.0	65.0	0.80		Dry	objects
3.0	83.0	0.88		Dry	
3.4	77.0	0.94		Oil	
1.8	140.0	1.25		Oil	
1.8	140.0	1.28		Oil	
1.5	160.0	1.30		Oil	

Watson

Holoscopic Objectives. Tube Length, 200 mm. Cover-Glass Thickness, 0.18 mm.

75.0	4.0	0.11		Dry
50.0	5.0	0.17		Dry
35.0	8.0	0.19		Dry

Watson

Holoscopic Objectives. Tube Length, 200 and 160 mm. Cover-Glass Thickness, 0.18 mm.

25.0	10.0	0.30		Dry
16.0	15.0	0.45		Dry
12.0	20.0	0.65		Dry
8.0	30.0	0.65		Dry
6.0	45.0	0.95		Dry
4.0	60.0	0.95		Dry
2.0	120.0	1.37		Dry

Watson

Apochromatic Objectives Corrected on Order to Any Tube Length. Cover-Glass Thickness, 0.18 mm.

16.0	15.0	0.30		Dry
8.0	30.0	0.65		Dry
4.0	60.0	0.85		Dry
2.0	120.0	1.37		Dry

NOTE. Magnifications at other distances than 10 inches can be found by applying equation 36 and substituting for X'.

Table XVII (*Continued*)

Reichert

Achromatic Objectives. Tube Length, 160 mm.
Cover-Glass Thickness, 0.18 mm.

Equivalent Focal Length, mm	Magnification	N.A.	Working Distance, mm	Immersion	Remarks
44.0	2.3		60.0	Dry	
34.6	3.2		40.0	Dry	
32.8	4.0	0.17	30.0	Dry	
32.8	3–4		22.5–9.2	Dry	Variable
26.6	4.6		20.0	Dry	
27.9	4.6	0.17	20.0	Dry	
16.2	10.0	0.28	7.2	Dry	
10.8	16.0	0.43	1.7	Dry	
5.1	34.0	0.65	0.55	Dry	
4.0	44.0	0.81	0.42	Dry	
4.0	45.0	0.65	0.55	Dry	
3.2	60.0	0.75	0.40	Dry	
2.8	71.0	0.82	0.24	Dry	
2.9	66.0	0.85	0.25	Dry	
2.9	66.0	0.85	0.25	Dry	
30.4	7.0	0.10	33.00	Water	
2.0	87.6	1.12	0.20	Water	
2.7	74.0	1.00	0.49	Oil	
2.7	76.0	1.24	0.27	Oil	
1.8	100.0	1.30	0.16	Oil	
1.8	101.0	1.26–1.30	0.20	Oil	
1.5	128.0	1.30	0.12	Oil	

Reichert

Apochromatic Objectives. Tube Length, 160 mm.
Cover-Glass Thickness, 0.18 mm.

16.0	11.5	0.32	4.9	Dry	
8.0	20.0	0.60	0.74	Dry	
4.0	45.0	0.90	0.21	Dry	Correction collar
3.0	61.5	0.95	0.16	Dry	Correction collar
2.0	96.0	1.33	0.11	Oil	
2.0	96.0	1.40	0.11	Oil	
1.5	124.0	1.30	0.12	Oil	

Table XVII (*Concluded*)

Fuess

Equivalent Focal Length, mm	Magnification	N.A.	Working Distance, mm	Immersion	Remarks
Achromatic Objectives					
30.0		0.1		Dry	
24.0		0.1		Dry	
15.2		0.2		Dry	
11.0		0.3		Dry	
7.5		0.5		Dry	
5.0		0.75		Dry	
4.0		0.85		Dry	
1.8		1.30		Oil	
1.4		1.30		Oil	
Fluorite Systems					
4.5		0.85		Dry	
3.0		0.90		Dry	
1.8		1.30		Oil	
Apochromatic Objectives					
16.0		0.30		Dry	
8.0		0.60		Dry	
4.0		0.95		Dry	
3.0		0.95		Dry	

LABORATORY WORK

Exp. 1. Comparing the Image Field Formed by the Objective with That Formed by the Objective and Ocular in Combination. Project an image of the stage micrometer on the ground glass screen of the camera. Use the microscope objective without an ocular. Notice the small diameter of the field and the low magnification. Now, replace the ocular, and notice the size of the field and the increase in the magnifying power. If the camera is of the 35-mm eyepiece type, it may be necessary to hold a temporary screen 12–15 inches above the microscope by a clamp and ring stand.

Exp. 2. Difference in Image Fields Produced by Achromatic and Apochromatic Objectives. Study the field of view produced under the following conditions while using test slide 3. With an achromatic objective of about 8-mm focal length and a Huygenian ocular, focus the center of the field sharply; then lower the tube slightly to bring in the outer zones, making them as sharp as possible. Notice that only the central zones can be made sharp, and that the images get progressively poorer toward the periphery of the field irrespective of focus. Repeat with higher oculars. If compensating oculars are at

hand, try them with the 4-mm achromatic objective, and decide whether they give an image, in the center of the field and at the margins, that is superior to that given by the Huygenian oculars. If a photographic ocular is available, use it in conjunction with the achromatic objective and light of daylight quality. Decide whether the green screen gives an improved image or whether the best image is obtained with light of daylight quality.

Repeat the experiment with an apochromatic objective and compensating oculars. Prove to yourself that better and more extensive fields are obtainable with the apochromatic microscope. In the center of the field the definition of the two systems will probably be about the same, even with light of daylight quality. Probably a difference will easily be noticed about halfway to the edge of the field.

In performing this experiment it is a help to project the image onto a ground glass, forming a circle of light at least 4 or 5 inches in diameter. As the different pieces of optical equipment are used, a paper can be laid across the image field and the part of the field useful for photographic purposes marked off. The images can be studied with a hand magnifier, and notes on the actual sizes of the fields can be filed for future reference. The camera extensions should be included in the notes.

Exp. 3. Lateral Chromatic Aberration. Focus the microscope on a test slide. Select a large particle, place it in the center of the field, and measure it. Illuminate with light of daylight quality, and use an achromatic objective. Record the measurement and the conditions of lighting and color screens. If the objective is of low or medium power, it may be well to project the image 30 inches or more, to increase the differences in measurement which may be found as various colored screens are used.

Add an optical filter very strong in the red. A monochromat or Wratten No. 25 will probably answer. Repeat the measurements. Repeat with a strong green filter, Wratten 56, and with a blue one, Wratten 45. Shift the object toward the edge of the field, say halfway, and repeat the measurements in the new position. Place the object, say, four-fifths of the way from the center of the field to the edge. Repeat the measurements.

At the completion of this experiment, the notes which have been taken should make it possible to say definitely whether the objective in question is suitable for color work, and a fair idea will be obtained of how the objective will perform on ordinary work. If the magnifications are much less for the red than for the blue images, the objective will probably be unsatisfactory for color work, although an examination has shown the image to be clear and sharp for any one color. Since this would indicate good spherical corrections for the colors separately, the objective would probably be satisfactory for ordinary work where the light is of daylight or monochromatic quality. Daylight quality is mentioned because oftentimes a lens having different focal lengths for red and blue light will give an excellent picture if the variation in focal lengths for the different colors is not too great.

Few objectives and eyepieces will give good correction at the outer zones or even for points more than halfway to the periphery. Only the best lenses and correct eyepieces will give good images near the edge of the field. That

oculars play a very important part in this may be proved by trying a Leitz apochromatic objective with a Leitz periplan ocular 15×, changing to a Zeiss 15× compensating ocular, and noting how much more of the field can then be made sharp and clear. The field will be far from flat with either ocular, and only small zones can be examined at one time. Amplifying oculars would be required to give flat fields.

Keep notes from this experiment for future reference. Make note of the objectives, oculars, color screens, the extent of the correction of the lateral color aberration, and also the camera extension.

Exp. 4. The Influence of the Iris Diaphragm of the Condenser on the Outer Zones of the Field of View. With test slide 1 or 3, focus the microscope sharply and study the effects produced by opening and closing the iris diaphragm of the condenser. To do this most precisely, focus on the center of the field and determine the optimum setting of the condenser iris diaphragm; then determine it for a position somewhere near the edge of the field. Try to find the best setting for three different zones, and notice that no one position of the diaphragm seems to be optimum for more than one zone. When the outer zones are used, the iris will have to be closed more than when the central part of the field is under observation; the light cone becomes smaller and its angle less. This experiment can be carried on better by visual examination than by projection on the ground glass. Full cones of light (Sec. 52) are, of course, necessary to maintain resolution.

Exp. 5. Field Depth of an Objective. Mount a preparation of lampblack on a cover glass, or rule a very fine line on a cover. Mount the cover, with the preparation facing upward, on a tiny prism of glass, so that light can be seen through it from the condenser. See Fig. 111 for details. Angle A can be determined with a toolmaker's protractor or an ocular goniometer, or better and more accurately on the revolving stage of the microscope. With the whole device on the microscope stage, focus on the top part of the cover glass. If the line is used, it will appear as in Fig. 112; if carbon black is employed it will show a zone where it appears to be fairly sharply imaged while the rest of the field is out of focus. Measure the distance across the zone which appears sharp and well defined or, better yet, photograph it. Enough data are now at hand to compute the depth of focus of the objective used in this test (it is suggested that the objective be of very low power, such as a microphotographic objective). Tan $A = a / b$; the equation can be solved for the value of a, which represents the depth of field of the objective. See Fig. 113.

If the field depth is measured visually, and then computed from photographic data, the computed figures will be much smaller than the measured ones. This effect is due to the natural accommodation of the eye, which, of course, cannot be duplicated by the photographic process.

This experiment is rather impressive as it shows how very small is the field depth possessed by even low-power objectives, and how very important is the proper preparation of a specimen for photomicrography.

Exp. 6. Measuring the Magnification of an Objective. Use a stage micrometer divided into 0.01 mm. The Zeiss compensating filar micrometer eye-

piece is recommended for this determination because it is a well-corrected positive ocular and can be used for long periods without any appreciable eyestrain. However, it is a rather expensive piece of equipment, and if it is not available use any positive ocular with a micrometer disc at the diaphragm. Measure the distance between any convenient number of intervals on the stage micrometer, and put this measurement in terms of millimeters as represented by the intervals on the eyepiece disc. Thus, 0.1 mm (10 intervals) on the stage micrometer may equal 1.28 mm on the eyepiece disc (a convenient ruling on the disc is 5 mm divided into 50 equal parts); the fraction 0.08 is estimated. Then the magnification by the objective is 12.8. See equation 1. Test the magnification of several objectives.

If a micrometer disc for the eyepiece is not available, project an image of the stage micrometer on a piece of ground glass. Do not use an ocular. The projection distance should be 160 mm measured from the objective, or whatever tube-length distance is required by the objective, minus 12 mm. The image may be very difficult to see. Measure the imaged scale as shown on the ground glass and apply equation 1. A buff-colored screen may give better results than a white one. It is possible to make the projection distance larger if desired, but a proportionately larger figure for the magnification power of the objective will be obtained, and it must then be reduced to the magnification existing at regular tube-length projection distance.

Exp. 7. Measuring the Focal Length of an Objective. Measurement of the focal length of an objective can be easily and quickly made with a positive micrometer eyepiece. Take any objective at hand and determine its magnification at two different tube lengths. Select tube lengths as extreme as possible, one very short and one very long. Divide the difference in tube length by the difference in magnification at the two tube lengths; the result will be the focal length of the objective. See equation 48.

Exp. 8. Using the Abbe Test Plate. Use a strong light source. Modify the light, by means of a filter, to daylight quality. Focus the microscope condenser. Mount an 8-mm apochromatic or 4-mm achromatic objective, and focus the extreme end of the test plate, the end with the thin cover. A 15× ocular can be used with the apochromatic objective or a 10× with the achromatic objective. Adjust the tube length so that it will be normal for the objective in use. The system under these conditions will be substantially undercorrected. Place a black line in the center of the field, running from east to west, and make the focus very sharp in the center of the field. Remove the ocular and close the iris diaphragm of the condenser to give a 9/10 cone of light, or a little less. Replace the eyepiece. You are now ready to start making the test.

It is assumed that the lamp has been placed directly in front of the observer so that the axis of illumination will be at right angles to the lines on the test plate. Make the illumination oblique by displacing the iris of the condenser, or use the sector diaphragm, which is part of the test-plate equipment, in the ring holder of the condenser. Inspect the rear focal plane of the objective when first making this adjustment. The movement of the iris should be made

toward the lamp, or the opening of the sector should be turned toward the lamp. When inspecting the rear focal plane of the objective, the illumination can now be seen to be entirely on the side of the objective toward the observer. This spot of light within the objective circle indicates the sector of the objective which is under test. It should appear somewhat like the sketch in Fig. 117.

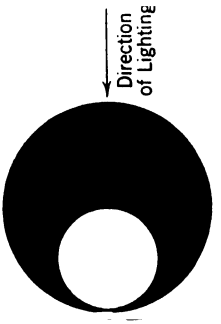


FIG. 117. The appearance of the back focal plane of the objective when the light is made oblique for the illumination of the test plate. The iris diaphragm of the condenser is moved toward the light source. It is always best to have some regular method for obtaining oblique illumination; there will then be less opportunity for error.

Observation of the test plate, after the lighting has been made oblique, should show the lower edge of the central black line to be hazy, the haze extending inward from the edge. The top edge will be poorly defined. Color effects may be strong, but they do not have to be considered at this time. The hazy lower edge of the black line and the poor definition of the upper edge are the criteria for discovering under-correction. The conditions have purposely been somewhat exaggerated.

Now move the test plate and repeat the experiment using the thick end of the wedge cover glass. The appearance of the black-line image, with oblique lighting, should now be reversed; the top edge of the line will appear hazy and the lower edge less poorly defined than before. This indicates over-correction.

Move the test plate so that a position near the center is in focus. Repeat the experiment with central and oblique lighting. After one or two trials a position on the plate may be found that will give an image which, when illuminated by oblique lighting, will be nearly as sharp and well defined as when illuminated with central lighting. This condition will indicate that the objective is free from spherical aberration for the thickness of cover glass at which the observation is made, and for the tube length employed.

If no position of the test plate indicates freedom from spherical aberration, find the position where it appears at a minimum and then adjust the tube length of the microscope. If a little under-correction is noted, lengthen the tube; conversely, shorten it for signs of over-correction. After this has been done, a very good idea can be formed of the excellence of the objective, the tube length for which it is best suited, the cover-glass thickness which permits it to perform best, and whether or not it can be made free from spherical aberration.

Repeat this experiment, using a 4-mm apochromatic objective with a correction collar. When examining the thin end of the wedge-shaped cover glass, try to obtain correction by adjusting the collar to a low number, thus giving over-correction to compensate for the under-correction introduced by the thin cover glass. Note that partial correction can be attained but that the image is not as good as when the proper cover-glass thickness is used and the collar is

turned to the number that corresponds to that thickness. A really good, well-corrected 4-mm objective should show practically no image difference at all between central and oblique illumination.

Repeat the experiment with an oil-immersion objective. Preferably it should be an apochromat or an achromat of high aperture. Notice that tube length makes a great difference and that the position of the test plate is not as important as it was for the dry objectives. If immersion liquids of different index, differing by 0.002, are available, show that this small difference or at least a difference of 0.004 is clearly demonstrable with the test plate.

The above experiments indicated presence or absence of spherical aberration only. The color fringes which were probably noted indicated the need for chromatic correction to a greater or less degree. Notice that when the spherical aberration was reduced to a minimum the color fringes also were less conspicuous. The apochromats are so well corrected for color that those of high power may give an image, in oblique lighting, which to all intents and purposes is free from color fringes. The reddish colors will usually appear on the top of the black line, if the foregoing method of obtaining oblique light has been followed, and the complements of those colors will appear on the bottom of the line. A good apochromat will show very weak spectral colors of the third order, and the achromats will show colors of the second order. Sometimes the colors of a good lens are so weak that they cannot be seen on the strong white background. The rare event when the blue colors appear on the top of the black line indicates over-correction for those colors. Such an objective may be better for photomicrographic work than for visual work.

QUESTIONS

1. What objection is there to using, for photomicrography, the objective without the benefit of an eyepiece?

2. What is meant by equivalent focal length?

3. Define the term spherical aberration.

4. Define the term chromatic aberration.

5. Why does an achromatic objective give its best images with light predominately green?

6. When is it necessary to use lenses with the finest color corrections?

7. What is meant by difference in magnification due to color? Give an example.

8. What is one of the most commonly used mechanical tube lengths?

9. How can depth of focus of an objective be determined? Is there any particular need for making such a determination?

10. How can the focal length of an objective be determined?

11. What is the best way to measure the magnifying power of an objective?

12. What is the best solvent for cleaning an objective?

13. How should objectives be stored?

14. For what is the Abbe test plate used?

15. How can an objective be examined for resolution?

16. What is the most important test for an objective that can be made by the photomicrographer?

17. Should all the twelve tests that have been suggested be made for all objectives? What are the important ones for high-power objectives? For low-power objectives?

18. Compute the magnification of a 25-mm lens when the image is real and its distance is 250 mm; also, when the image is virtual and its distance is 250 mm.

19. What type of eyepieces should be used with achromatic objectives?

20. What are two chief disadvantages in using achromatic objectives?

21. Are achromatic objectives suitable for photomicrography?

22. Why are apochromatic objectives specially desirable?

23. Mention three qualities of the apochromatic objective which make it more difficult to use than the achromat of similar power (focal length).

24. What type of eyepiece should be used with the apochromatic objectives?

25. Mention four kinds of microscopical work for which special lenses have been made.

26. Describe in detail the procedure in centering an objective to the microscope axis.

CHAPTER IV

OCULARS, ILLUMINATING APPARATUS, SLIDES, COVER GLASSES, AND THE EFFECT OF DIRT ON THE OPTICAL SYSTEM

For visual work the microscope ocular, or eyepiece, serves to magnify the real image formed by the objective and, in conjunction with the eye, to form the virtual image (see Fig. 15), thus acting more or less as a hand magnifier. For photomicrographic work the operation differs somewhat. A real image must be formed on the photographic plate; the eyepiece then acts as a projection lens.

Oculars may be used to carry accessory glass plates, or discs, on which scales may be engraved. These plates should always be placed in the plane of the primary image in the erect position as they are intended to appear in the field of view. Then, when the microscope is focused, the image of the scale, superposed on the image of the specimen, will be erect and unreversed. Sometimes these scales and the mechanical devices for using them may be built into the ocular; for quantitative work under visual conditions they are practically indispensable. However, in Fig. 102 it is shown that the insertion of a glass plate into the path of the image-forming rays will cause over-correction in the image space. For this reason their use in photomicrography, to project a scale into the image field, is not to be recommended when the sharpest possible pictures are desired.

Oculars may be divided into three main groups: Huygenian, or negative; Ramsden, or positive; and the amplifying ocular which can hardly be termed an ocular at all since it is not used for visual work but only for photomicrographic work or projection.

Various specialized oculars for measuring, colorimetric work, and goniometer eyepieces, spectroscopic eyepieces, or any eyepiece intended for visual work, are all adaptations or modifications of the Huygenian or Ramsden types and therefore may be either negative or positive.

The term " positive " when applied to an ocular means that it performs as any positive lens might be expected to do. It can always be used as a hand magnifier provided that the object can be placed near enough to its lower lens. The term " negative," applied to any ocular

except the amplifying lens, does not refer to the shape of the lens, as described in Chapter III, because the two lenses in the Huygenian ocular are positive, but rather to the fact that it does not behave in the same way as a positive lens. It cannot be used as a magnifier, for, if an attempt is made to use it in this way, a small reduced image of the object will be seen. The image will be real and close to the eye lens, so that, to see it, the head must be held some distance from the ocular. Thus, it acts neither as a positive nor as a negative simple lens would be expected to do. The amplifying lens is a true negative lens combination.

Sec. 68. Ocular Designation and Dimensions. The Huygenian or Ramsden ocular may be modified and corrected to almost any extent, but, unless the under-corrected system is referred to, the term Huygenian or Ramsden is usually dropped. Thus, compensating eyepieces having the maximum correction, when used with apochromatic or high achromatic objectives only, may be of the negative or positive type. Usually the lower powers are negative and the higher powers are positive.

Oculars with intermediate corrections give rise to various trade names such as Orthoscopic, Hyperplane, Periplan, and Holoscopic. These eyepieces are not completely compensating, but they are more fully corrected than either the simple Huygenian or Ramsden types; they may give excellent results with achromatic objectives and fair results with apochromatic objectives.

It is common practice to refer to oculars by their magnifying power; thus, a 5× ocular will magnify the primary image for visual purposes 5 times at a field of view distance of 250 mm. Gage has drawn attention to the fact that, in the designation of oculars, the earlier the letter in the alphabet, or the lower the number, the lower will be the magnification of the eyepiece. Amplifying lenses are designated by Zeiss according to their focal length. They are called Homals. Corresponding lenses by Bausch and Lomb known as Ampliplans are designated by their magnification, presumably at 10 inches projection distance. These are the only two makes of amplifying lenses that are procurable in the United States.

For the most part, eyepieces have long focal length because the magnifications are generally comparatively low. The 3× compensating eyepiece of Zeiss has focal length of 83 mm; that of the 30×, in the same series, has a focal length of 8.4 mm. The magnifications may vary from 2× or 3× to as high as 50× and 100× for special work. Beck lists a 100× ocular, but 30× is considered to be quite high. The most commonly used magnifications are from 6× to 15×. Low eye-

pieces are popular because many microscopes are never adjusted well enough to permit the use of those of high power.

Apertures of oculars are relatively low, and the N.A. on the image side is very low indeed. However, even for photomicrographic work when extreme resolution is demanded, final resolution is not limited by the optics of the ocular but by the working aperture of the system as a whole, which is limited by the objective and condenser.

The outside diameter of the ocular barrel is fixed by the diameter of the drawtube. This is given in Table I. The diameter of the small or ordinary size is 0.917 inch. In closeness of fit, there is sometimes much left to be desired, but in general the system affords an interchangeability which is important. Oculars for petrographical microscopes are larger than those used on the biological instruments, and they have a correspondingly larger field of view attributable to larger diaphragms. The tube with which they are used is 1.270 inches in diameter, but polarizing microscopes requiring this large size are often equipped with an adaptor to take the smaller size. Usually the amplifying lenses demand a larger barrel than those used for visual work; therefore these lenses also must be used with adaptors.

Sec. 69. The Huygenian or Negative Ocular. Figure 118 is a drawing of the Huygenian ocular. It was invented by Huygens in the latter part of the seventeenth century, and for a long time it was the only type in use. It consists of two simple planoconvex lenses, mounted in a sleeve, with the convex sides of the lenses toward the objective. A diaphragm is located between the lenses at the first focal point of the second lens. The lens near the objective is called the field lens; the one near the eye is called the eye lens. Before the image-forming rays can be brought to a focus by the objective, they pass through the field lens and are focused by it very near to or slightly above the ocular diaphragm. Since the path of light has been altered by the field lens so that the image is formed at a point somewhat nearer to the objective than it would otherwise be formed, the magnification of the objective is slightly reduced. This is true of all negative oculars. The field lens acts in conjunction with the objective to form an image at the eyepiece diaphragm. The trace of the rays is clearly shown in Fig. 118. The eye lens acts as a simple magnifier of the image formed by the objective and field lens acting together. Thus, the primary image must lie at or slightly within the principal focus of the eye lens in order to form a virtual image with the aid of the eye, and this position of the primary image is attained when the microscope is focused. When the primary image is at the first focal point of the eye lens, the rays to the eye are parallel, and the virtual image is formed about 250 mm from the eye for people with normal vision.

It might appear at first glance that the Huygenian ocular would form a very inferior image because of seeming lack of correction. In the outer portions of the field of view this is so, but in the center of the field the images are bright and sharp. Lack of chromatic error accounts for the good image formation as well as for the fact that Huygenian eyepieces are usually of comparatively low power and have small diaphragms. The field lens, which is uncorrected, forms the primary

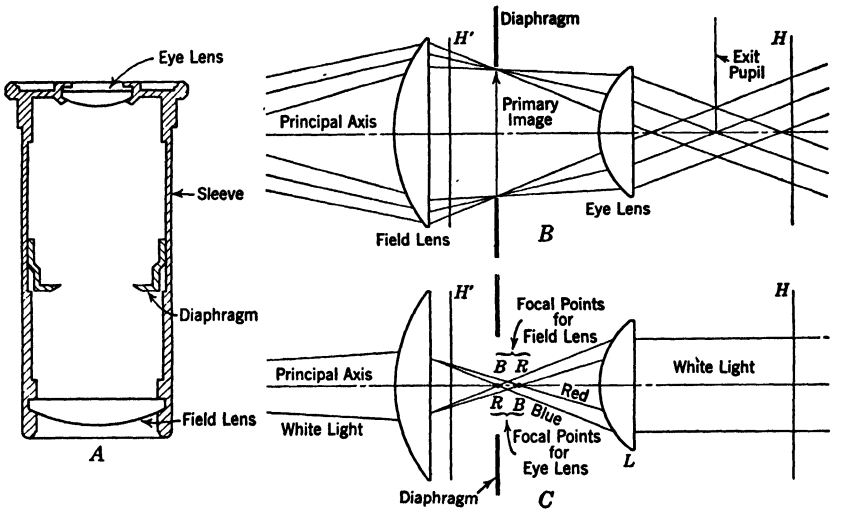


FIG. 118. Huygenian ocular. *A*, the essential parts; *B*, a trace of the image-forming rays, also the crossing of the principal planes; *C*, the way chromatic error is corrected by an Huygenian system. The second focal point is very close to the exit pupil. The second focal point of the field lens lies beyond the primary image.

image in several planes according to its focal length for the colors involved. This is easily verified by removing the eye lens of the ocular and using the field lens alone. As the tube of the microscope is moved slightly above and below the proper focus, the color fringes to the images will show in greater or less distinctness, the outer portions of the field showing the strongest ones. In attempting this experiment the greatest care must be exercised lest the ocular drop down the microscope tube.

Since the blue image will lie nearer to the field lens than the red, and the reverse will be true with respect to the eye lens, the errors of one lens can be made to compensate for those of the other. Thus, good chromatic correction can be attained for a Huygenian ocular if the respective lenses are of correct focal length and properly separated. However, under no conditions can this ocular in its simple form give

a good image over the whole field of view. Figure 118*C* shows the microscope focused on a point object. The blue and red images are shown as separated because of longitudinal chromatic error, and they are of different size. The eye lens combines these two images, forming one image free from chromatic error. This correction is possible when the errors of the field lens are balanced by the errors of the eye lens.

To attain correction, Huygenian oculars must observe a certain relationship of focal length between their two lenses. This ratio lies between 3.5 : 1 and 1.5 : 1, the field lens having the longer focal length. In addition, the separation of the two lens elements must be the sum of their focal lengths divided by 2. Dwight[1] has constructed an interesting nomogram showing at a glance the focal lengths of the lenses and the distance apart they must be to form a Huygens system.

Because of the way the Huygenian system is planned, it must be clear that, if the ocular is used for projection, to form a real image on the photographic plate the primary image must fall at a point somewhat below the diaphragm. This, of course, is attained by refocusing the microscope. Under this condition the correction of the chromatic error of the field lens may not be entirely compensated for by the eye lens. However, for low-power photographic work, and particularly when a deep-green screen is used, the Huygenian ocular will give very good pictures. In this connection it is also true that the displacement of the microscope tube, necessary to focus a real image rather than a virtual one, will make the objective work farther from the object and thus introduce under-correction in the primary image and so in the final image.

Sec. 70. The Ramsden or Positive-Type Ocular. The positive oculars are represented by a lens combination in which the field lens is above the diaphragm. The rays from the objective are brought to a focus in the diaphragm plane and are then refocused to form the virtual image by the field lens, eye lens, and eye, all acting together as an optical unit.

Ramsden oculars were named after Jesse Ramsden (1735–1800), an English optician.

Figure 119 is a diagram of the Ramsden ocular, the simplest type of positive ocular. Such an ocular is especially well suited for measuring the magnification of an objective, since a micrometer scale dropped onto the diaphragm will give a direct measure of the primary image. It should be noticed that the lenses used in the Huygenian and Ramsden oculars are of the same class, planoconvex, but the field lens in the

[1] C. Harrison Dwight, "Nomogram on the Huygens Ocular," *J. Opt. Soc. Am.*, **30**, 140, 1940.

Ramsden eyepiece has the convex surface uppermost whereas in the Huygenian ocular it is placed downward. The eye lens in both types has the convex part downward.

It is often possible to distinguish between a positive and a negative eyepiece by simply trying to focus the complete unit as a hand magnifier. If it can be focused to give a virtual image it is of the positive

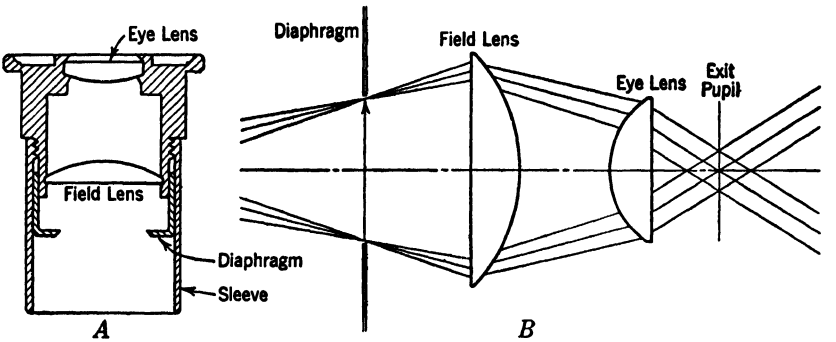


FIG. 119. *A* shows the essential parts of the Ramsden or positive ocular; it should be compared with Fig. 118*A*. *B* indicates the trace of the image-forming rays. The combination of both field lens and eye lens functions about the same as the single eye lens of the Huygenian system. Fine corrections are obtained by the addition of other lens elements as shown in Fig. 120.

type; if it cannot, it is negative. It is not always possible to make this test; for instance, with the 15× compensating positive ocular of Zeiss the whole lens system is so far within the sleeve that it is difficult to place the specimen in a suitable position. In this particular system it is evident that the diaphragm is below the field lens; consequently, the ocular must be positive.

Sec. 71. Compensating Oculars. It has been shown that, to obtain the best possible spherical corrections, apochromatic objectives are not fully corrected but are left with a certain amount of *chromatic* under-correction. To offset this residual color aberration, inherent in all lenses of this type, compensating oculars must be employed. The compensating ocular is designed with the necessary amount of over-correction to compensate for the under-correction of the objective; this accounts for its name. Because of the ever-present over-correction for color, compensating oculars show a yellow ring around the ocular diaphragm which can be seen when inspecting the field of view. Other types of oculars, usually under-corrected, show a bluish ring around the diaphragm. This shades off so easily into white that it is often

unnoticed, and it may be very difficult to see at all. There are one or two kinds of special objectives which demand specially made oculars of the compensating type because the amount of residual under-correction is not the same as for the customary apochromatic objective. As a rule, all apochromatic objectives can be said to carry equal amounts of under-correction for color, and can thus be used indiscriminately with any compensating ocular of either high or low power. Most opticians claim that their own individual line of apochromatic objectives can give the finest results only when employed in conjunction with oculars of like make. This is a question which technicians can solve best by the trial method. Figure 120 shows the construction of a compensating ocular of high power.

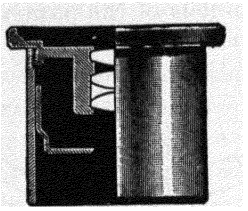


FIG. 120. Modification of a positive ocular to form a compensating eyepiece. A similar modification is possible with negative oculars to form higher-quality lenses.

On account of their fine corrections, and the equally fine corrections of the objectives with which it is customary to combine them, compensating eyepieces can be of very much higher power than the Huygenian or any other less well-corrected ocular, but the optical qualities of any microscope image cannot be expected to be at an optimum beyond about 1000 × N.A. of the objective.

The field of view of compensating oculars is decidedly more curved than with achromatic objectives and Huygenian oculars. The photomicrographs in Fig. 121 were taken under different conditions as explained in the legend. They show the actual field size and the size of the circle that is critically sharp. *C* and *D* in the same figure illustrate particularly well that there is a distinct difference between lack of

FIG. 121. This series of pictures indicates the size of the image field and how well different ocular systems and combinations of ocular and objective have eliminated curvature of field. The camera length was constant throughout the series except for *H*. The objective was changed as noted.

A. Zeiss 7× Huygenian ocular; 8 mm apo. objective, Leitz; ×130.

B. Zeiss 7× Huygenian ocular; 8 mm achro. objective, Zeiss; ×100.

C. Leitz 15× Periplan ocular; 8 mm apo. objective, Leitz; ×290. Focus was made on the outside zones of the field; should be compared with *D* and with *H*.

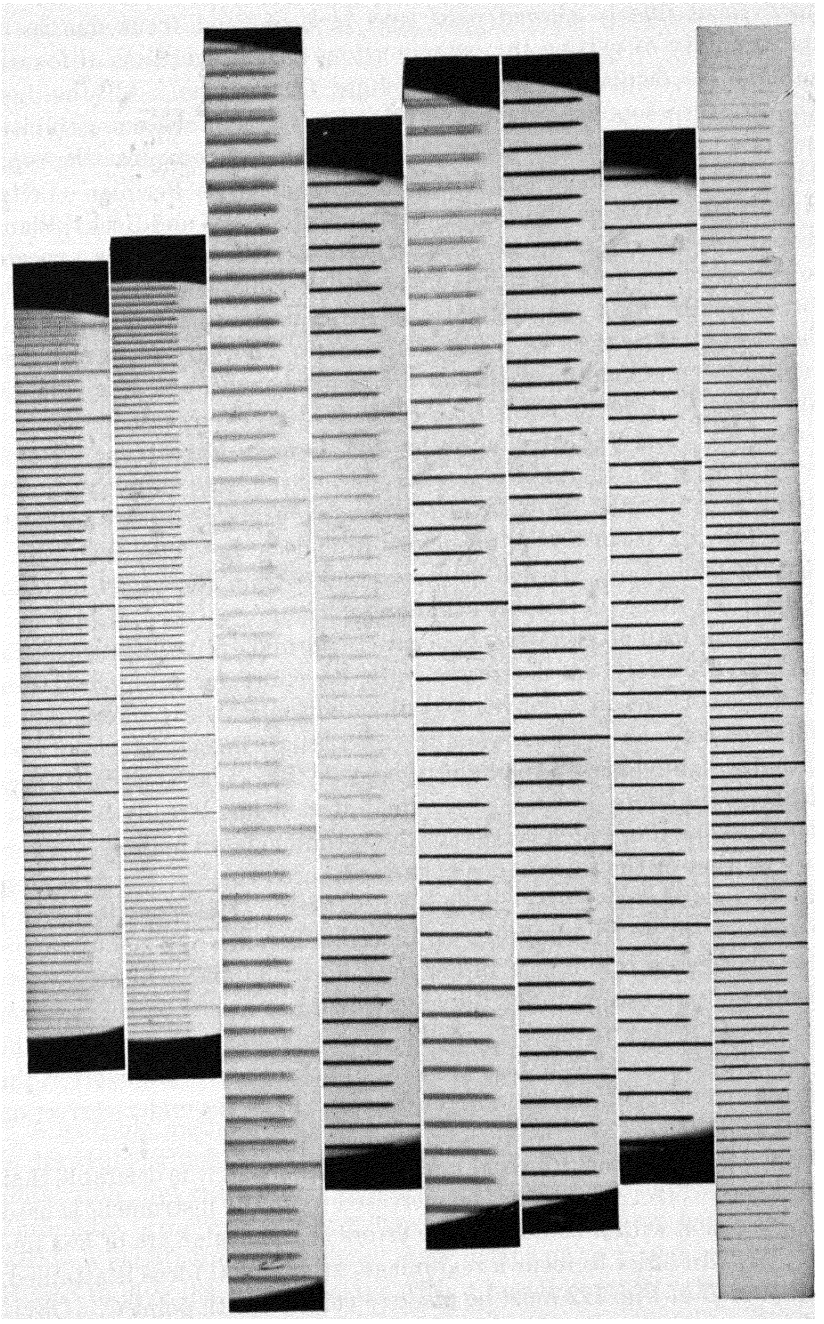
D. Zeiss 15× compensating ocular; 8 mm apo. objective, Leitz; ×280. Focus was made on the outer zones of the field.

E. Winkel-Zeiss 18× Photo-ocular; 8 mm apo. objective, Leitz; ×330.

F. Winkel-Zeiss 18× Photo-ocular; 8 mm achro. objective, Zeiss; ×300.

G. Zeiss No. 1 Homal amplifying lens; 8 mm apo. objective, Leitz; ×310.

H. Leitz 15× Periplan ocular; 8 mm apo. objective, Leitz; ×160. Cf. *C*.



A *B* *C* *D* *E* *F* *G* *H*

good focus due to curved field, and lack of good focus due to the impossibility of getting the outer portions sharp regardless of focus.

Sec. 72. Oculars with Intermediate Corrections. All the best-known microscope manufacturers furnish a series of oculars with intermediate corrections in addition to Huygenian and compensating types. They are known under special trade names such as Periplan (Leitz), Hyperplane (Bausch and Lomb), Orthoscopic (Zeiss and Beck), Planoscopic (Spencer), and Holoscopic (Watson). They can be expected to give a flatter field than the compensating oculars; they are intended for use with either apochromatic or achromatic objectives; probably they are at their best with the achromats. They are frequently recommended for photomicrography, particularly with eyepiece cameras. They cost considerably less than compensating eyepieces.

Sec. 73. Errors Introduced by the Camera-Microscope. Before investigating the photomicrographic, projection, and amplifying eyepieces, it is in order to discover the image defects which are produced when the microscope with an ocular intended for visual operation is used for projection. It will be shown that certain steps may be taken to keep the image defects at a minimum.

A person with an emmetropic or nearly normal eye will focus parallel light rays exactly on the retina with no effort for accommodation. This, then, is the ideal physiological condition which the designers of eyepieces consider. Thus, when the microscope image is focused by a normally sighted person the light rays leave the ocular as parallel rays. Although the finite distance of the field of view has already been mentioned as 250 mm, which is true for the accommodated normal eye, very little motion of the microscope tube is required to shift the field from infinite focus to a virtual distance of 250 mm. However, to move a microscope image from infinity to the finite distance of a photographic plate, to form a real image rather than a virtual one, will necessitate raising the microscope tube (the object distance must be made greater to decrease image distance), and this produces under-correction in the whole system, as shown in Fig. 122. Furthermore, the shorter the bellows extension of the camera, the greater will be the under-correction at the image field.

The objective is the principal microscope lens, and it is desirable that the objective errors should not be increased when the instrument is used in conjunction with a camera. The errors of the ocular are of less importance. In order to focus a real image, after visual focus is attained, point *A* or *B* of Fig. 122 must be made to coincide with point *C*. Obviously there are two ways of doing this: either the objective can be refocused by raising it slightly; or the ocular itself can be raised, which

is equivalent to an increase in tube length. The latter method produces less error in the final image because the perfectly formed image at point A or B is left undisturbed and becomes the object for the eye lens of the ocular. In the sketch, an Huygenian ocular is shown, but this ocular may be of any type.

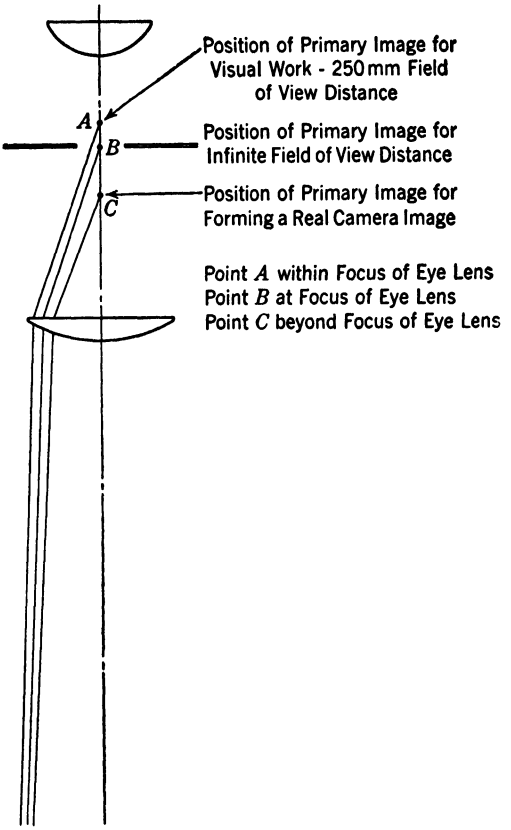


FIG. 122. Demonstration of how a real image can be formed by focusing with the ocular, thus leaving the objective in its normal position.

A suitable method for focusing the ocular as described will require a drawtube which is actuated by rack and pinion. Such a device is obtainable, but the method involved is awkward and clumsy, and some chromatic error will be introduced by the ocular. A better way to attain a satisfactory real image is by means of an ocular specially designed to give the desired image with a normal optical tube length for the objective.

In Fig. 122, it may be noted that, when the tube length is extended

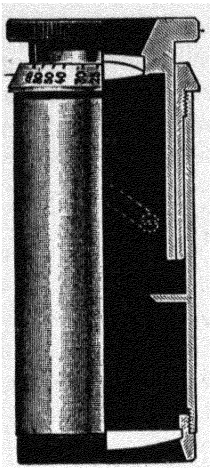


FIG. 123. Photographic ocular of Zeiss. The numbers on the top flange refer to camera extension.

to focus a real image, over-correction is not produced because the objective is not moved, but when focus is obtained by moving the objective, the system will become under-corrected as already stated because the optical tube length is shortened. The three types of oculars now to be discussed will give image fields that are flatter than can be attained by any other type; they will obviate under-correction inherent in a long object distance or in the formation of a real image.

Sec. 74. Photographic and Projection Oculars. Several firms manufacture a line of adjustable oculars which permit the camera image to be focused without disturbing the tube setting. The adjustment is made by turning the eye-lens mount. These oculars can be used in one of two ways. The microscope can be focused as for visual work and moved to the camera where further focusing is done by the ocular adjustment; or the ocular diaphragm can be focused, in the image plane of the camera, by moving the eye lens, then focusing the specimen in the usual way. In any event, if the bellows extension is altered after setting the ocular, the ocular must be reset. Oculars of this type are shown in Fig. 123. They are known as photographic oculars.

Projection oculars are made by only a few firms. They are used when the projection distance is 10 or 15 feet or more. They are designed for direct microscope projection, for demonstrations, and like purposes. Unlike the photographic oculars which can be had in many powers, projection eyepieces can be had in low or medium powers only.

For the photomicrograph shown in Fig. 124, a compensating ocular was used, but in printing the picture a small diaphragm was required to exclude the part which was out of focus. The center

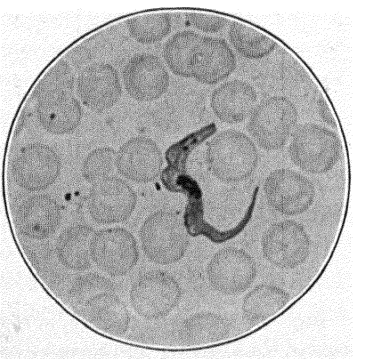


FIG. 124. Photomicrograph of *Trypanosoma brucei* ×1000. Obj. 3 mm apo. Zeiss; Zeiss compensating ocular 15×; Zeiss aplanatic condenser, 9/10 cone; illumination by method II; daylight filter; camera extension 284 mm Eastman film, Pan. X; dev. D-19. The small diaphragm was used in printing to stress the small flat field obtainable under these conditions.

of the picture is good but it would have been larger if a photographic or amplifying eyepiece had been used.

Sec. 75. Amplifying Lenses. Amplifying lenses are designed for photomicrographic purposes, but they are also useful for projection, particularly if the throw is short. They are negative in character.[2] Thus when used as hand magnifiers they act as reducing glasses. As they are negative, the exit pupil of the microscope is well within the tube, at the point E' in Fig. 125. Because the field of view of amplifying lenses is so small they are not suitable for visual work; however, the microscope image can be very sharply focused when looking through them.

Table XVIII gives the focal lengths of two makes of amplifying lenses, the Ampliplan, by Bausch and Lomb, and the Homal, by Zeiss. It is not feasible to specify the magnifying power of amplifiers. This may be discovered for any bellows draw by applying equation 36 and letting the image distance equal x'. It will be seen that each amplifier is corrected for use with only one, or at the most two, objectives. This is because of the great difference in field curvature of objectives of different powers. Amplifying lenses are corrected primarily for apochromatic objectives, but they can be successfully employed with achromats, particularly with a strong green screen; they are also

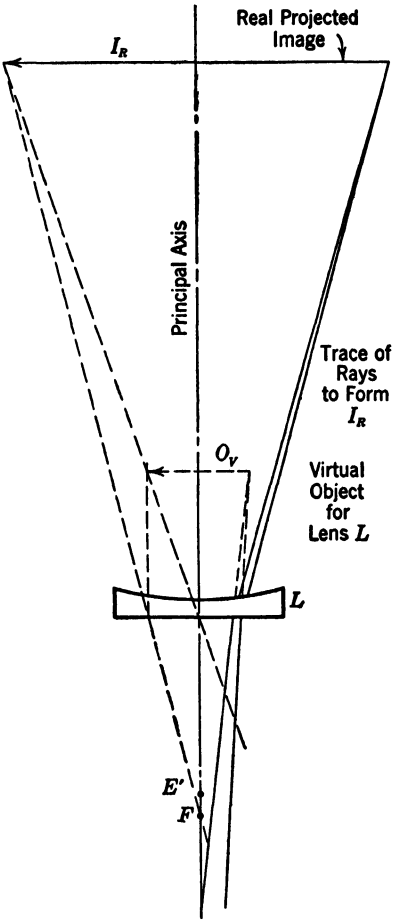


FIG. 125. The development of the real image by geometric methods, to illustrate the trace of rays when an amplifying lens is used. The dotted lines show the construction necessary to locate the real image. See Sec. 51.

[2] The term negative here is intended to denote the character of the lens as explained in Sec. 47. It should not be confused with the same term used to denote Huygenian eyepieces. Amplifying lenses have a negative focal length.

Table XVIII

Amplifying Lenses

Make	Lens	Focal Length, mm	Objective to be Used
Zeiss	Homal I*	− 20	16-mm, 8-mm
Mechanical tube length reduced 23 mm	Homal II	− 70	16-mm
	Homal III	− 20	4-mm
	Homal IV	− 20	3-mm, 2-mm
	Homal VI	− 37.5	16-mm, 8-mm
Bausch and Lomb	Ampliplan low-power	− 51	16-mm
Mechanical tube length reduced 16.5 mm	Medium power	− 47	8-mm
	High power	− 42	4-mm 1.9-mm

* This eyepiece, although one of the best in the opinion of many, has been discontinued. Used with the 16-mm objective it gave good results that can hardly be duplicated; any specimen found in good second-hand condition should be added to the laboratory equipment if possible.

interchangeable with different makes of objectives. The correction of amplifiers for achromatism is equal to that of compensating oculars. A reduced tube length will be required when these lenses are used. A reduction of 23 mm for Homals and 16.5 mm for Ampliplans is indicated. When a set of amplifiers is installed, consideration must be given to the diameter of their sleeve; it is larger than the average microscope tube will accommodate. The usual practice is to remove the drawtube and the sleeve through which this tube slides, and to attach an adapter provided by the manufacturer of the eyepiece. This adapter will automatically alter the tube to the required length.

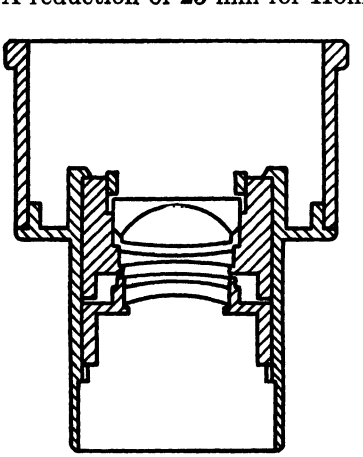


FIG. 126. The construction of an amplifying lens.

Figure 125 shows the geometrical construction of the image projected by the amplifying lens. The normal position of the primary image is illustrated at O_v, by dotted lines. On the introduction of the negative lens, the primary image no longer exists; it becomes the virtual object of the negative lens. Considering the image formation of a lens of this type, as already explained, it is clear why the reduction in tube length is needed. It is also fairly evident that the amplifier must be rather sensitive to this tube-length reduction, since it depends on this for proper object distance. The shorter-focal-length

eyepieces will give images with but little or no under-correction when set at one tube length, the changes required for different bellows extension being very slight. The longer-focus amplifiers, however, can be used at varying tube lengths. They have a scale engraved on the sleeve to indicate the correct setting for various image distances. Figure 126 shows the construction of a Homal amplifying lens of −37.5-mm. focal length.

Sec. 76. The Micrometer Ocular. The micrometer ocular is by far the most important of the many special eyepieces. It is useful in photomicrography when an image of its micrometer scale is projected into the image field. Figure 127 shows the construction of such an ocular of very simple design; the optical principle employed is the same for all.

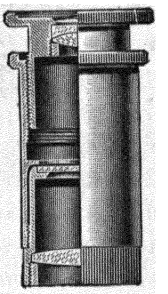


FIG. 127. A Huygenian ocular showing the adjustment of the eye lens by screw threads. The micrometer disc with scale is shown at the diaphragm between the field and eye lens.

A micrometer disc as in Fig. 127 is mounted on the diaphragm of the eyepiece. The disc is inserted with the scale erect; that is, it should appear in the virtual image as it appears naturally to the eye, with the numbers erect and unreversed. The scale is generally engraved on the disc and covered with a thin glass, balsam being used as a cement. When the two glasses are not of even thickness the scale is usually engraved on the thicker disc. Thus, to keep the disc face uppermost, the thin glass must be on top. A hand magnifier will generally show how the disc should be mounted. Various types of scale may be had, with networks, cross lines, and other geometrical designs, but the most important is the regular micrometer scale 5 mm long and divided into 50 equal parts.

The eye lens is movable and can be focused on the disc. Since the primary image lies in the same plane as the disc, both the image of the specimen and the micrometer scale will fall together in the field of view.

The micrometer eyepiece shown in Fig. 127 is of the negative type; it might equally well be of the positive type. In order to facilitate the making of measurements the scale is sometimes so mounted that it can be moved laterally across the field. The amount of travel is read on a scale attached to the drum of the micrometer head. The total motion is usually about 5 to 6 mm. Figure 128 shows clearly the working parts of this screw-type micrometer eyepiece, or filar micrometer.

The positive-type micrometer eyepiece permits the magnification of

the object to be read directly on the eyepiece scale. This is a great advantage since it is then necessary only to mount a stage micrometer and to focus it with a positive micrometer eyepiece. Each interval on the disc being 0.1 mm, the magnification produced by the objective can easily be determined. This micrometer is very easy on the eyes, since

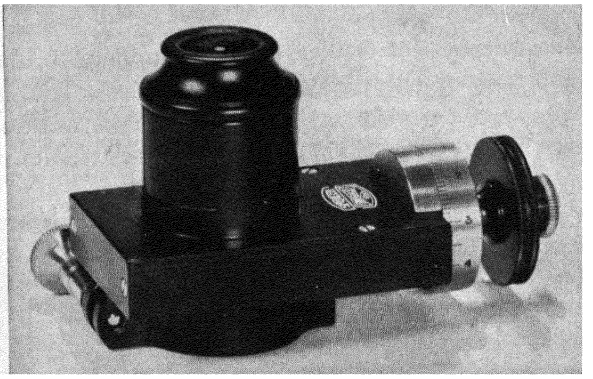


FIG. 128*A*. Spencer filar micrometer.

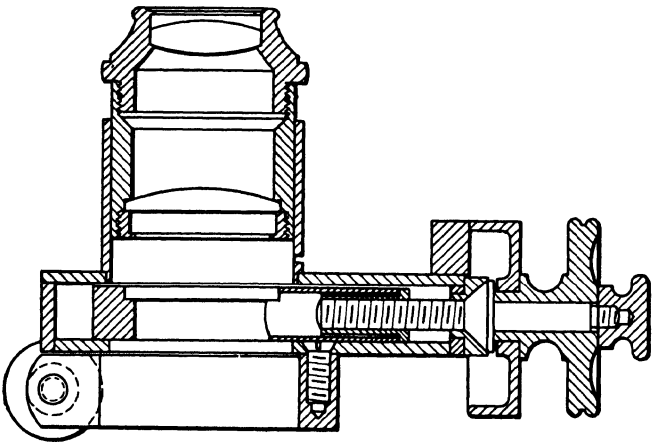


FIG. 128*B*. Cross section of the micrometer shown in Fig. 128*A*. The micrometer screw actuates the plate carrying the scale and moves it across the field of view.

but little aberration seems to be introduced by the glass disc, and it is generally of low power.

Micrometer eyepieces may be of any magnification, but two predominate. In the Huygenian class the magnification is generally about 7; for compensating oculars it is about 10. The Zeiss compensating filar micrometer has the exceptional magnifying power of 15 times.

This is very desirable. For intermediate magnifications it is possible to improvise by using any ordinary ocular which allows the micrometer disc to be placed at the eyepiece diaphragm; the eye lens can then be screwed out a little way to ensure focus of the disc. When the eye lens is properly adjusted, the microscope can be focused on an object, either in the center or on the side of the field, and the object and scale will be perfectly sharp, simultaneously. If the eye is held several inches above the eyepoint and the head is moved laterally the scale and the object on which the microscope is focused should stay in perfect alignment. If the scale should seem to move independently of the object, parallax exists, indicating that either the scale or the microscope is not in proper focus.

Before a micrometer ocular can be used for measurement, it must be calibrated. For any style of micrometer eyepiece this is accomplished by comparing the intervals on the stage micrometer with the intervals on the eyepiece scale. If 10 intervals on the eyepiece scale equal 15 intervals on the micrometer on the stage, obviously 1.0 interval on the eyepiece scale equals 1.5 intervals on the micrometer scale. The number of intervals counted on the stage micrometer, divided by the number of intervals which they represent on the eyepiece scale, gives as a quotient the number of stage micrometer intervals which equal 1 interval of the eyepiece scale. If 1.5 intervals on the stage micrometer equal 1 interval on the eyepiece micrometer, and the value of the 1 interval of the stage micrometer is 10 μ, then 1 interval of the eyepiece micrometer has a value of 15 μ. See Sec. 6 for the proper position of one scale in reference to another when making measurements as described above. The equation for calibrating an ocular micrometer is

$$\text{Value in microns of 1 interval of ocular micrometer} = K \cdot \frac{\text{Number of stage micrometer intervals}}{\text{Number of ocular micrometer intervals}} \quad [53]$$

K is the actual value of 1 interval, in microns, on the stage micrometer.

In photomicrographic work it is often desirable to have a micrometer scale superposed on the picture of the specimen. This can be done with one of the micrometer eyepieces if it is carefully selected to go well with the objective. Photoeyepieces are often positive in character and are good for such work. Amplifying lenses cannot be used with an eyepiece micrometer disc. It is always best first to focus the image of the scale onto the ground glass, the bellows having been extended to their desired position. After the ocular scale is focused by adjustment of the eye lens, the microscope must be focused. The specimen and

scale images will then be sharp simultaneously. Figure 129 shows the use of a network micrometer. The calibration of the scale can be made either before or after the picture is taken.

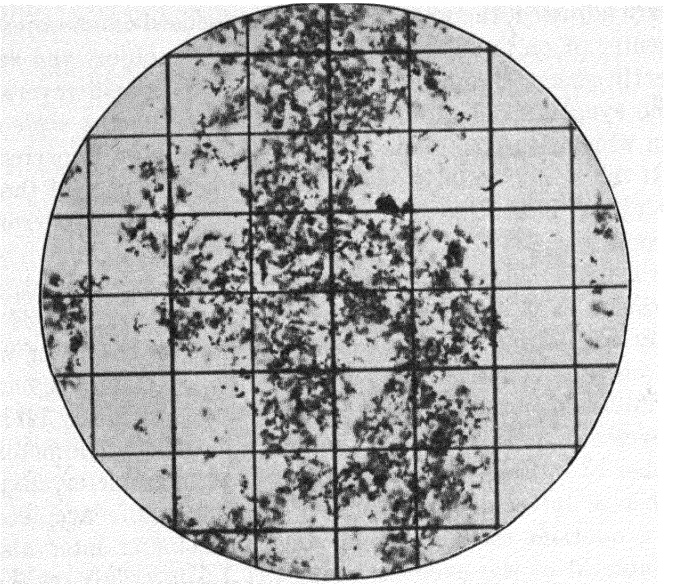


FIG. 129. Crankcase engine drainage ×560. This illustrates the use of a network micrometer disc.

Sec. 77. Other Special Oculars. For work requiring the measurement of angles the goniometer ocular is useful. This ocular is shown in Fig. 130. It has a scale with a vernier; when the disc is turned to measure an angle, the degrees can be read on a graduated circle. Reichert has made an ocular to measure color by matching with a standard that can be turned into the field at will. The Ehrlich ocular is particularly convenient for cutting down the size of the observed field until only a definitely measured area is in view. It is made with screw adjustments and gives a square field. Demonstration oculars can be arranged for two observers; a small pointer in one ocular can be set at a certain point to mark special detail to be studied simultaneously by both observers. Paired oculars are necessary for

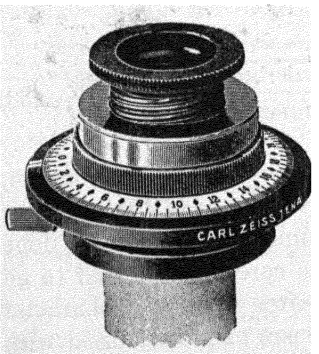


FIG. 130. The goniometer ocular. Courtesy of Zeiss.

binocular microscopes. They are specially selected, having similar optical properties, and are so chosen that the image will appear the same for either eye; that is, magnification is exactly the same for each unit of the ocular pair. Other special oculars may be had for spectroscopic work and for work with polarized light.

Sec. 78. Ocular Distortion. Eyepieces of any type may introduce a certain amount of distortion near the periphery of the field of view, which may become quite noticeable.[3] On the other hand, if the subject is small particles with a broken field, quite possibly it may pass unnoticed. Figure 98 is a photomicrograph of a test plate showing distortion. To avoid distortion or to reduce it to a minimum, a change of optics, magnification, or bellows length may be tried. When it is particularly desirable to avoid distortion, a plate similar to the one shown in Fig. 98 can be used as a check before taking the photomicrograph. Another method is to compare the image of the lines of a stage micrometer on the ground glass with a straight edge.

Sec. 79. Selecting an Ocular on the Basis of Magnification. Not only is an objective corrected to give its best image at a certain tube length and cover-glass thickness, but there is also a range of magnification at which the combination of objective and eyepiece will give the best performance. This range is between 500 and 1000 times the numerical aperture of the objective, or about four times higher than is required to see all detail clearly. Theoretically, if an objective has a N.A. of 1.3, it should give good images at a magnification of 1300×. These figures are better criteria for work with the camera than for visual work. Taking two extremes, if the 16-mm N.A. 0.3 apochromatic objective is used with a 20× eyepiece, the magnification will be only 200×. For visual work, a magnification much higher than this will not be successful with this objective, but with a compensating ocular, and the image projected to a photographic film, the effective magnification may go to 300 or even slightly higher with the best of results. This magnification is 1000 times the N.A. With the 3-mm apochromatic objective, the maximum magnification which will be comfortable for visual work will generally lie around 800× to 900×. This entails the use of a 15× eyepiece. Homal or Ampliplan lenses should be able to give excellent projected images at magnifications of at least 1500 to 1600 times the N.A. of the objective, although these lenses are corrected for optimum image formation at 1000 times the N.A. of the objective.

[3] I. C. Gardner and F. A. Case, "The Lateral Chromatic Aberration of Apochromatic Microscope Systems," *Research Paper* 316. Natl. Bureau of Standards, 1931. Ocular distortion is discussed in the latter part of this paper

Achromats with the same type of ocular give best results with a somewhat lower range of magnification. However, with a green screen the magnification of such objectives can be made to approach that which can be obtained with the apochromats, and the picture may still be perfectly satisfactory.

In speaking of magnification in photomicrography, the total magnification is always assumed unless otherwise specified. It is the magnification of the microscope plus the magnification due to bellows extension, which is the total effective magnification at the image plane.

No objective will form a perfect image. It is only necessary to examine the primary image with a sufficiently strong ocular, to uncover some deficiencies due either to aberration or diffraction. As a rule, the accompanying diffraction phenomenon will be evident; in particular, the image will appear fuzzy. When the ocular is selected on a basis of 500 to 1000 times the N.A. of the objective, all the detail which the objective is capable of resolving will be magnified enough to be visible to a person with eyesight even below normal. The fact that a magnification of 1000 times the N.A. is approximately four times higher than is necessary to see all detail is shown by the following discussion.

When the exit pupil of an optical system coincides with the entrance pupil of the eye, in position and size, there is no loss of resolution between the two. Equation 30 was given as

$$yn \sin \theta = y'n' \sin \theta'$$

If the right-hand member of the equation refers to quantities within the eye, the equation can be rewritten as

$$\frac{y'}{y} = \frac{n \sin \theta}{n' \sin \theta'}$$

or

$$M_1 = \frac{y'}{y} = \frac{n \sin \theta}{n' \sin \theta'} = \begin{matrix}\text{Magnification of retinal image} \\ \text{with the instrument}\end{matrix}$$

If an instrument is not used the magnification is given as before

$$y_2 n_2 \sin \theta_2 = y_2' n' \sin \theta'$$

and

$$M_2 = \frac{y_2'}{y_2} = \frac{n_2 \sin \theta_2}{n' \sin \theta'} = \begin{matrix}\text{Magnification of retinal image} \\ \text{without an instrument}\end{matrix}$$

The ratio then of $M_1 : M_2$ is the magnification obtained by the use of

the instrument. Thus

$$M = \frac{M_1}{M_2} = \frac{\dfrac{n \sin \theta}{n' \sin \theta'}}{\dfrac{n_2 \sin \theta_2}{n' \sin \theta'}}$$

or

$$M = \frac{n \sin \theta}{n_2 \sin \theta_2} \qquad [54]$$

The value M is called the normal magnifying power of the instrument.

With strong lighting the entrance pupil of the eye is probably 2 mm in diameter, and for normally sighted people the distance for close

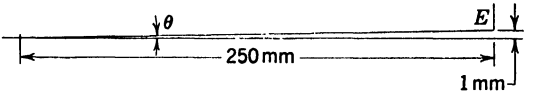


FIG. 131. The relationship of radius of entrance pupil of eye, object distance, and angle θ; N.A. = 0.004 when the radius of pupil is 1 mm for the emmetropic eye.

scrutiny of an object is about 250 mm. A sketch of this is shown in Fig. 131. The entrance pupil of the eye, then, has a radius of 1 mm and a N.A. of 0.004. From the figure

$$\tan \theta = \frac{1}{250} = \sin \theta \quad \text{(angle } \theta \text{ being small)}$$

Angle θ having the same value as θ_2 in equation 54, by substitution

$$M = \frac{n \sin \theta}{1/250} \qquad (n_2 = 1)$$

or

$$M = 250n \sin \theta$$

But $n \sin \theta$ = N.A. of the instrument. Therefore

$$M = 250 \text{ N.A.} \qquad [55]$$

This means that 250 times the N.A. of the microscope will give a figure that represents the magnification required to see all the detail the instrument can resolve.

If equation 34 is applied to an objective with a N.A. of 1.3 used with green light, the resolution is 0.25 μ. Now, if the N.A. 1.3 is multiplied by 250, the product is 325, which will be the magnification necessary to see all detail. If this statement holds, the resolution of the objective, 0.25 μ, times 325 will give a product in microns which is just about the

limit of resolution of the eye. Actually the product is 81.25 μ, or 0.081 mm, very close to the figure given for resolution with the unaided eye in Sec. 13.

The value 250 times the N.A. of an objective may seem a very low figure for the magnification of an optical system capable of giving four times that magnification with satisfactory clarity. Although the theoretical figure 250 times the N.A. may show all the detail, well resolved, to a person of perfect eyesight, 500 to 1000 times the N.A. is more practical, and for photomicrographic work the ocular can often be selected, in conjunction with the bellows extension, so that the magnification is several hundred per cent higher than the normal magnifying power.

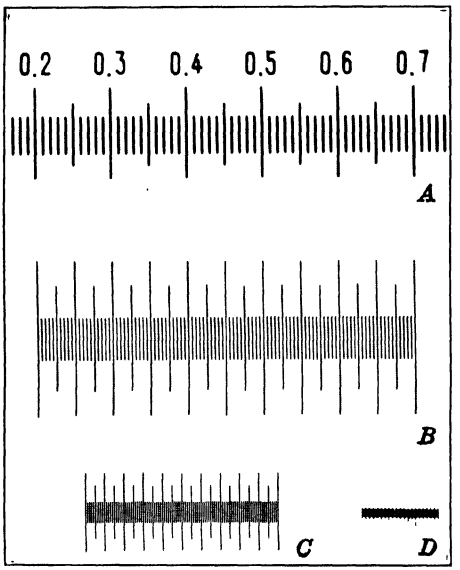


FIG. 132. Series of pictures illustrating the necessity for sufficient magnification to render detail. *A*, stage micrometer ×100; distance between interval 1 mm. *B*, stage micrometer ×50; distance between each interval 0.5 mm. *C*, stage micrometer ×25; distance between each interval 0.25 mm. *D*, stage micrometer ×10; distance between each interval 0.1 mm. The limit for the distance between resolved detail, in the finished print, cannot be much less than 0.25 mm, as the above series shows. The negative from which the print marked *D* was taken shows the closely spaced lines clearly but they are entirely lost in the printing. At *C*, the dot structure of the halftone process indicates limits for the degree of fineness of line which can be reproduced by this process.

A low limit on magnification for a photomicrograph is illustrated in the series of scales depicted in Fig. 132. The scale, a stage micrometer divided into 0.01 mm, was first photographed at a magnification of 100, thus spacing the lines 1.0 mm apart. In the next picture it was magnified 50 times; in the next, 25 times; and in the one marked *D*, 10 times. It should be noted that picture *D* is not resolved in the print although it is resolved in the negative; almost any emulsion will resolve 10 lines per millimeter. The photomicrograph marked *C* with the quarter-millimeter spacing is difficult to see although well within the threshold of visibility; the one marked *B*, with a line spacing of half a millimeter, is easy to study.

Considering the appearance of the various results in the scale series, it would seem that the minimum spacing for fine detail completely resolved should be half a millimeter. One-quarter millimeter will resolve the individual particles in a print, and they will clearly be recognized as separate entities, but they will be difficult to see. If the specimen had been well-stained bacteria the individual cells would show more clearly and discretely than the evenly spaced lines of the scale. A good rule is to try to attain a magnification in a photomicrograph so that the minimum separation of important detail is from a quarter to a half millimeter.

Aside from the rule-of-thumb limit asserting that the magnification should not exceed 1000 times the N.A. of the objective, the limit for overall high magnification depends on the specimen and the extent to which fuzziness may be tolerated in the image. For high magnifications, under optimum conditions, the specimen must be almost optically flat, and then magnifications of 3000, 4000, or even 5000 diameters may sometimes be used. The microscope adjustments must be practically perfect, and generally monochromatic light is essential. When a very small field is permissible, a 20× ocular may be used with a long bellows extension; however, in many high-power pictures, the 15× ocular or short-focus amplifying lens, for the oil-immersion objective, coupled with a long bellows draw, may give the best results. Another difficulty encountered in running up the magnification is that vibration may spoil the picture, for without a little exploring it is almost impossible to tell whether fuzziness in a highly magnified image is due to vibration, poor focusing, or diffraction. Magnifications of 2000 and even 2500 are easily obtained, and are close to the top limit for sharp pictures.

Summary on Oculars

1. Oculars may be listed in their natural order of importance for good photomicrography approximately as follows:

   a. Amplifying lenses.
   b. Photoeyepieces.
   c. Compensating oculars.
   d. Oculars with intermediate correction.
   e. Huygenian oculars.

2. For visual work compensating oculars should always be used with apochromatic and high achromatic objectives.

3. Huygenian oculars should be used only with low-power achromatic objectives.

4. Oculars with intermediate correction can be used with all achro-

5. Photoeyepieces are excellent for the eyepiece camera with achromatic objectives, or with apochromatic objectives and a strong green filter.

6. Ocular and bellows extension should give magnification not exceeding about 1600 times the N.A. of the objective. For visual work magnification should be about 1000 times with the high-power objectives and less with the lower powers and achromatic objectives.

7. Oculars, as a rule, may be selected, by trial, independently of the make of objective with which they are to be used.

8. Curvature of the field is a normal condition that will exist in varying degree according to changes in optical combinations. Often the best images will be obtained in strongly curved fields.

Sec. 80. Microscope Illuminating Systems. The three fundamentally different systems for illuminating the specimen on the microscope stage are:

1. *Bright-Field Illumination by Transmitted Light.* In this type of illumination the light reaches the specimen from below and passes directly to the objective. It is transmitted by transparent objects. Condensers for this method are known as bright-field condensers.

2. *Dark-Field Oblique Illumination.* In typical dark-field illumination the light may come from below stage or from above stage, but it must reach the specimen at such an angle that only light reflected from the specimen enters the objective. Substage illuminators for this system are known as dark-field condensers. Over-stage illuminators include the Silverman illuminator; Epi condensers, of Zeiss; Ultropak, of Leitz, and others. The slit microscope is a special example of dark-field illumination. The lighting is at 90°.

3. *Vertical Illumination.* This is a special form of bright-field, but it can hardly be placed in class 1 because the system is quite different. Here, the light enters the microscope above the objective and is then directed downward; the objective acts as a condensing lens, focusing the light on the specimen. The specimen is opaque and as flat as it can be made by grinding and polishing. Consequently the light is reflected back to the objective. The effect is that of a bright field. Vertical illuminators are classed according to the means by which they reflect the light in the microscope: a glass plate, a prism, or a mirror. Also see Fig. 103.

Sec. 81. Principles of Substage Illumination. When an object is examined without a lens, the image on the retina has a certain size and brightness. The size depends on the distance of the object, and the brightness depends mainly on the reflecting qualities of the object, on the intensity and distance of the light source, and on the size of the

pupil of the eye. If a simple or compound microscope is used to aid in examination of the object, the image on the retina will be large and its brightness will depend on the N.A. of the optical system and on magnification. Thus it might be assumed that, if the N.A. of the lens system is large enough and the magnification small enough, a balance can be reached where the magnified image will appear equally bright when compared with the image formed by the eye alone.

Assuming that the entrance pupil of the eye and the exit pupil of the magnifying instrument are the same size and in the same position, all the light leaving the microscope will enter the eye. Now, if the square of the N.A. of the lens equals the square of the product of the N.A. of the eye and the magnification of the image, the brightness of the enlarged image on the retina (to all intents and purposes the virtual image) will equal that of the image formed without the lens. Theoretically, then, the brightness of the virtual image, when the microscope is used, is proportional to the square of the N.A. of the system and inversely proportional to the square of the magnification. Thus N.A. is a measure of the light-gathering qualities of a lens.

Microscope objectives must have large apertures and the specimen must be strongly lit in order to be seen. As a typical example, with the 4-mm objective and a magnification of 400,

$$\overline{\text{N.A.}}^{2} = (400 \times 0.004)^2$$

$$\text{N.A.} = 1.6$$

The N.A. of the 4-mm objective would have to be impossibly high in a dry system in order to collect enough light from the object to make it appear as brilliant when magnified as when observed naturally. In fact, a N.A. of 1.6 would not suffice; it would have to be perhaps three or four times as high on account of light losses at the lenses and for other reasons. This difference of light-gathering power, between such a high aperture and one that is attainable, such as, say, 0.95, must be made up by increased illumination. To apply artificial illumination economically and well, at least two lenses are required, a light-collecting lens at the lamp and a lens of short focal length, say 10 mm or thereabouts, at the microscope (the microscope condenser).

Table XIX shows how the arrangement of a lamp and lenses affects illumination. The first value indicates the illumination at the surface of the lamp lens, or approximately the illumination received on a ground-glass surface placed close to the lamp lens. Since the area of a 4-inch lamp lens (diameter = 2 inches) seldom exceeds 3.1416 inches,

Table XIX

Photometric Computation of the Microscope Illuminating System

The source of light is assumed to be a 5600-lumen lamp of small source area. The focal length of the lamp lens was assumed to be 4 inches and that of the condenser 0.4 inch. The distance from the source to the microscope axis is 15 inches and that to the specimen 18 inches. When the lamp lens is introduced it is assumed to be 15 inches from the microscope axis. No account is taken of reflection or absorption of light.

Conditions	Illumination, lumens per square inch
1. At the lamp lens. 4 in. from source.	27.9
2. Without lenses at the microscope mirror. 15-in. dist.	1.97
3. Without lenses at the specimen. 18-in. dist.	1.37
4. With lens at lamp, without condenser lens, at microscope mirror.	188.2
5. With lens at lamp, without condenser lens, at specimen.	131.2
6. Without lens at lamp but with microscope condenser lens, at specimen.	36,038
7. With both lamp lens and microscope condenser lens, at specimen.	36,756*

* This figure was arrived at by knowing the illumination at the entrance pupil of the condenser, taken from item 4, and then computing it for unit area of the illuminated circle at the microscope slide. The apparent difference between items 6 and 7 is due to errors in making the above estimates or measurements.

of the 5600 lumens furnished by the lamp only about 88 lumens are available for the microscope.

The important point to be gathered from the last two values in the table is this: In the first case an image of the source is in the object field. Probably it would not be large enough to fill the field of view with light; certainly it would not do so at low powers. In the second case the field at the lamp lens acts as a secondary source (Method II of illumination), and although the illumination is equal in both cases a much larger field is obtained when the lamp lens is used.

The use of the lamp lens is thus justified. It does not increase the illumination at the specimen, but it does increase the size of the illuminated area, and, with a diaphragm, the size of the effective source can be controlled. Thus the function of the lamp lens is mainly to furnish a large controllable field of light, and the function of the microscope condenser is to furnish illumination of the highest possible intensity at the specimen. In addition, the condenser must form a cone of light of sufficient aperture to fill the objective with light. That is, the N.A. of the condenser should equal or exceed that of the objective.

Sec. 82. Types of Bright-Field Condensers. Optical companies generally refer to their condensers by N.A. rating rather than by focal length. Thus a condenser with a N.A. of 1.2 could be used only with objectives having an aperture of 1.2 or less; otherwise only the central portion of the object glass would be lit. Table XX lists condensers made by Watson.[4] Some of the other companies may not manufacture quite such a complete line. It should be noted that Watson supplies an aplanatic low-power lens of long focus.

It is nearly always possible to adapt condensers to any make of instrument. The regular standard diameter size set by the Royal Microscopical Society[5] is 38.786 mm. However, as not all manufacturers have adopted the Society standards, it may be necessary to have the fitting taken care of by the repair shop.

The Spectacle Lens Condenser. Spectacle lens condensers range in focal length from about 3 inches to about 1 inch. In their best form they are aplanatic, and the N.A. may be as high as 0.4 or 0.5. These lenses are very useful, and for objectives of focal length greater than 16 mm they are essential. In making a selection to suit any given objective, it is necessary to have the N.A. of the condenser as large as, or slightly larger than, that of the objective. The free aperture of the light source is more or less fixed at a maximum of about 2 inches, and, if a strong, high-power condensing lens is used to illuminate the low-power objective, the field of view will probably not be filled with light. The wisest course is to select a weaker, longer-focus condenser. It is therefore apparent that, if a great range of low-power photomicrographic work is to be attempted, condensers of various focal lengths and apertures should be available, each selected to serve an objective having an aperture slightly less than its own.

The Condenser of Medium Power. The selection is more limited for medium-power condensers than for low powers, the reason being that high-power condensers are so made that they can be taken apart to form others of a medium or even a moderately low power. Table XX shows the change in aperture and focal lengths brought about by removing the top lens from the Watson high-power condensers. However, it is questionable practice to screw and unscrew the back lens of a high-quality condenser frequently, and according to Swift the apochromatic condensers made by them should not be used in this way. The Zeiss Company makes an aplanatic condenser with a N.A. of about 0.6 that should be very useful for medium-power work. The

[4] W. Watson & Sons, Ltd., 313 High Holborn, London, England.

[5] R.M.S. (1936), Royal Microscopical Society's Standard Specifications.

Table XX

Set of Condensers of Various Powers

Condenser	Full Aperture	Aplanatic Aperture		Equivalent Focus, mm	
		Complete	Top Lens Removed	Complete	Top Lens Removed
Macro illuminator or spectacle lens condenser				50	
Aplanatic low-power	0.50	0.48		17	
Universal	1.00	0.95	0.40	10	25.4
Parachromatic	1.00	0.90	0.40	7.5	10.0
Abbe	1.20				
Oil-immersion	1.30 to 1.40	Full	0.55	5 6	14.0

greatest use for a medium-power condenser is undoubtedly with the 16-mm objective.

The High-Power Condenser. High-power bright-field condensers are conveniently divided into five classes which considerably overlap each other with regard to corrections and usefulness. They serve all objectives with focal length less than 8 mm. The classes are given in ascending order of their optical corrections.

1. Abbe condenser, two-lens type, Fig. 133*A*. No attempt at optical correction.

2. Abbe improved condenser, three-lens type, Fig. 133*B*. Correction of spherical aberration for one color.

3. Aplanatic condenser, Fig. 133*C*. Correction of spherical and comatic errors for one color.

4. Achromatic condenser. Chromatic aberration corrected for two colors.

5. Achromatic-aplanatic condenser, Fig. 133*D*. Spherical and comatic correction for one color. Chromatic correction for two colors.

The Abbe condenser of the two-lens type was originally designed by Abbe;[6] it was described by him in 1873. Owing to its low cost and the ease with which it can be used, and also because it was the best condenser obtainable for many years it has enjoyed greater popularity than it actually deserves. It is an under-corrected combination and emphatically not suited for objectives with apertures above approximately 0.4. The cone of light is not aplanatic, and its rated N.A. is

[6] Abbe, "Über einen neuen Beleuchtungsapparat am Mikroskop," *Ges. Abh.*, **1**, 101–112, 1873.

generally 1.2. An 11-mm two-lens condenser of this type is listed by Zeiss. With the upper lens removed it has a focal length of 32 mm and an aperture of about 0.4. As is true for many uncorrected lens systems, a strong green screen improves the performance of this lens.

The three-lens condenser, which several firms now call the improved Abbe condenser, is made with a N.A. of 1.4. It has a somewhat shorter

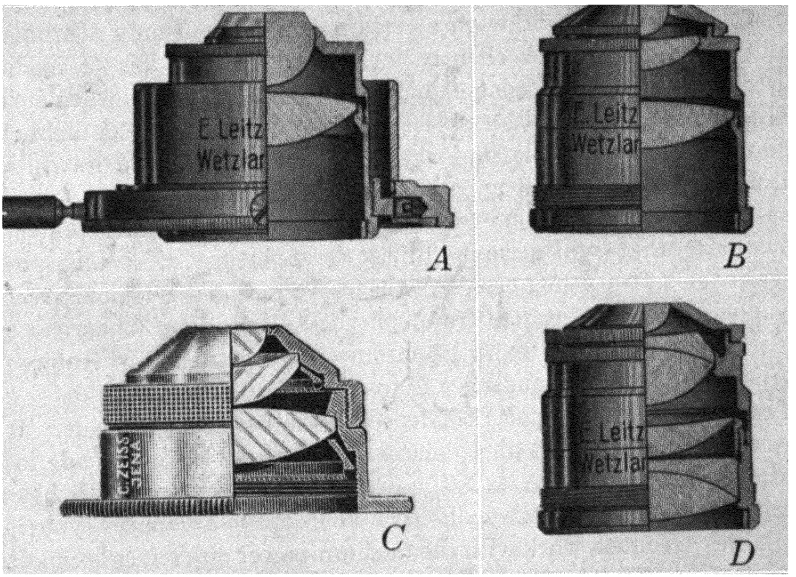


FIG. 133. *A*, Abbe condenser. *B*, an improved Abbe condenser. *C*, an aplanatic condenser. *D*, an achromatic-aplanatic condenser.

focal length than the two-lens Abbe, but like that lens it is not adequate for photomicrographic work.

Several firms make a good three-lens aplanatic condenser. The top surface of the lower lens is given an aspherical surface. In its best form this condenser is a most effective condensing lens. It has a focal length of about 10.0 mm and a N.A. of 1.4. It can be used with the two upper lenses removed; the N.A. of the remaining lens is 0.4, with a focal length of about 40 mm, depending upon the make. Good photomicrographic work can be done with this condenser. In fact, when a green screen is used and the condenser is immersed there is little choice between this and the achromatic-aplanatic combination. It gives an excellent aplanatic cone at high aperture and can be focused through a fairly thick slide. A cross section is shown in Fig. 133*C*.

Both Spencer, and Bausch and Lomb, make lenses of this type. The corrections for spherical errors are excellent. For photomicrographic work such a lens can well be used with immersion oil, even with medium-power objectives.

Most present-day manufacturers claim that their achromatic condensers are also aplanatic. As in all lenses, achromatism in a condenser is a very desirable quality, but the corrections for spherical aberration and coma are necessary in order to get a large aplanatic cone of light. Thus, although a certain condenser of only achromatic quality may perform excellently for an objective of low or medium power (low N.A., small cone of light), it is also true that, when such a lens is opened up to fill an objective of high aperture with light, the cone will not be aplanatic, and when examined through the drawtube it will not give the clearcut appearance to the back lens of the objective that it should. It will be found impossible to make any condenser adjustment that will permit filling the aperture of a high-power objective in a proper manner. For the above reason it is logical to select a condenser for its freedom from spherical aberrations (spherical and comatic errors), as well as for its chromatic corrections. Therefore the achromatic-aplanatic condenser is to be preferred.

The achromatic-aplanatic condenser is the best that is made. It is a necessity for the finest photomicrographic work. If it is made to be used dry (Zeiss), its N.A. is 1.0; corrected for oil immersion, it has an aperture of 1.4. Thus, two different achromatic-aplanatic types are available. If much work with the medium-power objectives is expected the dry type of condenser might be selected. For best results with the immersion type, it must be oiled to the slide.

Figure 133*D* shows a cross section of the achromatic-aplanatic condenser by Leitz. This condenser is intended for oil immersion, and when so used it has an aperture of about 1.4. Its focal length is not given. It can be used with the top lens or the two top elements removed to form a longer focal lens. The working distance of any highly corrected condenser is not great, and slides used with it should not measure more than 1.0 mm in thickness.

Sec. 83. Focusing the Bright-Field Condenser. Before being centered and otherwise adjusted, a condenser must be focused on the light source. It will then be put into some definite relationship with the objective with which it is to be centered. The condenser system can be termed focused when its position is such that an image of the light source (either the ground glass at the lamp or the lamp diaphragm) can be seen in the field of view when the microscope is focused. An

image of the light source must then lie in the object field. Figure 134*A* is a diagram of a focused high-power condenser.

Focusing the Medium- and High-Power Condenser. The microscope is first focused on, say, test slide 1, of well-dispersed discrete particles.

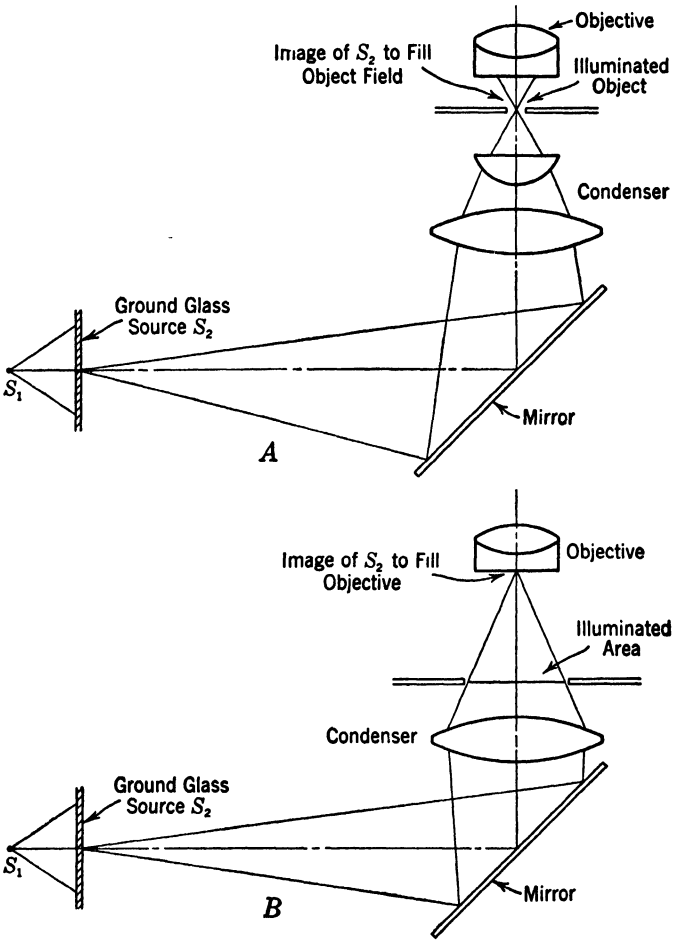


FIG. 134. Diagrams of focused high- and low-power condensers. At *A*, illumination is by method I, commonly used for medium- and high-power illumination. At *B*, the image of the source has been raised to focus at the entrance pupil of the objective, or very close to the front element of the objective.

With one hand on the microscope mirror and the other on the focusing adjustment of the condenser, the observer can tip the mirror slightly until the edge of the field diaphragm comes into view. At the same time the edge of this diaphragm can be focused by manipulating the

condenser substage adjustment. If the condenser is at the upper end of its travel, it will only be necessary to lower it in order to focus the lamp diaphragm sharply. This is the simplest and quickest way to focus the bright-field condenser.

An object on which the condenser can be focused is also obtained by closing the diaphragm of the lamp to 2 or 3 mm in diameter. The image of this small spot of light is then swung into the center of the field of view by manipulating the mirror, and focused sharply by adjusting the condenser. After focusing, any further motion of the condenser, either up or down, will make the image of the source increase in size.

The condenser stop at the end of the upward travel of some substage apparatus may increase the difficulties in making the above adjustment. The reason for the stop is obvious, but it is often so placed that the condenser cannot be raised enough for sharp focus with a lamp distance of 14 or 15 inches. In such a case, the lamp may be moved nearer to the microscope, but this would not be good practice because it increases glare. The alternative is to remove or readjust the stop to give greater travel to the condenser, so that it may be brought closer to the microscope slide. Adjustment of the stop must be made with the greatest of care, for with good condensers the clearance between the top lens of the condenser and the under side of the slide is seldom more than 0.1 mm. Another method to overcome a very short working distance is to unscrew the top lens of the condenser slightly; this will have the effect of increasing focal length and so give a little additional distance. A method for increasing the working distance of a high-power condenser, which mounts a −2- or −3-diopter lens in the condenser substage, is being adopted by some of the microscope companies for long lamp distance on horizontal camera stands. This very convenient method is coming into considerable favor, for the accessory lens can be placed in a swing-out arm, and it adapts the high-power condenser for reasonably low-power objectives. However, any makeshift method of increasing focal length by altering the optics of the condenser will introduce aberrations, and since, in purchasing a high-grade condenser, the first consideration is the elimination of aberration in the illuminating cone, it would seem that the logical recourse would be to fit the condenser to the microscope so that it can be focused without tampering with the lens separations and without adding another lens.

In the event that the limit of the working distance is too great to permit the source to be focused in the object field, methods can be evolved to obtain a shorter working distance. For instance, the lamp

can be moved farther away, or excessive working distance can be reduced by filling up the space between the condenser and the slide with bits of broken cover glass oiled together.

When a condenser is oiled to the slide the optical path and the actual working distance are increased. Actual measurements made on an achromatic condenser showed that when oil was used there was a difference in vertical displacement of 0.16 mm; that is, when immersed, the condenser had to be lowered by that amount in order to keep the light source focused in the object field.

Precision of focus is of less importance for uncorrected and low-power condensers than it is for those of high power and good correction. Low-power condensers tend to show the structure of ground-glass surfaces at the lamp more plainly than those of high power, since the aperture of such condensers is less and the contrast of the ground-glass detail is enhanced. If the ground-glass structure interferes with the background of the picture, there are two recourses: one is to raise the condenser approximately 50 μ; the other is to add a second piece of finely ground glass at the lamp. If the extra piece of glass is used, the ground surface should be lightly oiled to increase transmission and give the desired homogeneous effect. Both ground surfaces should face the microscope, and one glass should be about $\frac{1}{8}$ inch in front of the other. If the field of view is inspected with a perfectly focused condenser, only very little motion, if any, will be noticed in the background when the second ground glass is moved in its holder. With the high-power condenser, the addition of the lightly oiled piece of ground glass is generally sufficient to give a smooth background, even for very exacting work; but with a low-power condenser the background may even yet be not sufficiently free from structural detail of the ground surface for photomicrographical work, so that it may be expedient also to raise the condenser slightly.

It is sometimes questioned whether a condenser focused for one objective is still in focus when an objective of perhaps much higher power is substituted. The answer is not entirely obvious, for if the condenser is first focused with, say, the 16-mm objective, and properly adjusted, the diaphragm will have been well closed. If a 2-mm objective is turned in the condenser diaphragm will have to be opened to nearly its full extent to fill the objective with light. Under these conditions the effective focal length of the condenser may have been altered slightly on account of the use of the outer zones of the lenses. Thus, refocusing may be required. Refocusing might be required also if the color of the light was changed, the condenser having a shorter focal length for blue than for red light.

A Special Method for Focusing Spectacle Lens Condensers. Naturally it is much more difficult to illuminate a large object field evenly than a small one. Partly for this reason, the operation of the spectacle lens condenser differs somewhat from that of higher-power condensers. The spectacle lens condenser must be focused on the front lens or entrance pupil of the objective instead of in the object field. This can be accomplished in practice by holding a small card close to the front lens of the objective and focusing the condenser accurately on the card. The light is centered later. It is unnecessary to " stoop, squat, and squint " in an endeavor to try to see the front of the card, for if the card is thin and oiled the spot of light will show through to the back. When the condenser is focused as described the rear exit pupil of the objective will appear evenly filled with light. Figure 134*B* is a diagram of the properly focused low-power condenser.

The significance of this method of focusing the condenser lens may not be at once apparent, even with the help of the diagram in Fig. 134. To go back a little, it has already been shown in Sec. 18 that, when using Method I, II, or III for illumination, if the iris diaphragm of the condenser is evenly filled with light, the exit pupil of the objective will also be filled with light. The condenser referred to was focused on the object field, and the iris diaphragm of the condenser controlled the angle of the illuminating cone. Considering the condenser and objective acting together as one lens, the entrance pupil would be in the plane of the condenser diaphragm and the exit pupil of the system would very nearly coincide with the rear focal plane of the objective. Now, when the spectacle lens condenser is focused on the front lens of the objective or practically in the plane of the entrance pupil of that lens, the exit pupil will be evenly filled with light. The size of the spot of light in the entrance pupil of the objective is controlled by the iris diaphragm at the lamp because it is an image of that diaphragm. Therefore the aperture diaphragm of the illuminating system under these conditions is the diaphragm at the lamp. To sum up, an image of the lamp diaphragm is formed in the entrance pupil of the objective and so (because entrance and exit pupils are conjugate with each other) in the exit pupil of the objective. The lamp diaphragm then controls the angle of the illuminating cone when the low-power condenser is focused as outlined. A demonstration of this arrangement is made by inspecting the exit pupil of the objective, when the spectacle lens is carefully focused.

Sec. 84. To Move the Condenser a Stated Distance Vertically. Occasionally it may be necessary to move the condenser vertically after it has been focused. It may also be convenient to know the amount

of such displacement. This measurement can be made by first raising or lowering the focused objective the same distance that the condenser is to be moved. The amount of the displacement of the objective is measured on the scale of the fine adjustment. When the microscope tube is in its new position the condenser can be refocused; it will then have been moved the required amount. The microscope is then refocused. When it is desirable to raise the condenser a certain amount, say 50 μ or 75 μ, to eliminate the structure of the ground glass, this method provides a way to do it precisely.

Sec. 85. To Center the Bright-Field Condenser. The importance of the mutual alignment of the condenser and the objective cannot be overrated when photomicrography is to be attempted. A certain amount of misalignment may not be evident in visual work. The camera, however, can register differences in field intensity that the eye cannot see, and that frequently result in light spots or shadows across the film. Three methods for aligning the condenser with the objective axis are described: Method A is the fastest and most convenient; Method B is the most accurate; and Method C should not be used unless it is rechecked by Method A or B.

At this point it is assumed that the alignment of the objective with the microscope axis has been achieved, and so under no circumstances should the objective be moved into alignment with the condenser. It is always the condenser that should be moved to agreement with the objective.

Method A. With a focused microscope and focused condenser, the first step in centering the condenser is to remove the eyepiece and insert a pinhole cap; next, inspect the rear focal plane of the objective where the image of the condenser diaphragm will be seen. The iris diaphragm at the lamp should be wide open. The iris of the condenser should be closed until the condenser is giving about a $\frac{9}{10}$ cone of light, then, by making the condenser circle concentric with the objective circle by manipulating the condenser centering screws, the condenser can be centered to the axis of the objective. Once this operation has been performed, the condenser will stay centered until it is removed or another objective is inserted. After objectives have been changed or any change in the vertical position of the substage has been made, it is always wise to remove the ocular and glance down the tube to see that the centration of the condenser has not been disturbed. If it has been disturbed, the condenser should again be centered before proceeding with the photomicrograph.

Method B. A somewhat more accurate method for centering the condenser is to examine the eyepoint of the focused microscope with a

magnifier, since anything appearing at the rear focal plane of the objective will be shown at the exit pupil of the microscope. Figure 33 shows a device for holding an eyepiece or other magnifier of about 15×. Such an accessory is very convenient; with it centration will be more exact because errors will be magnified, and the condenser circle will be brought into greater contrast with the objective circle. After the exit pupil is focused by the auxiliary lens, the condenser iris can be observed and centration effected. With either Method A or B, care should be taken to select a specimen for the test slide that will not distort the image of the condenser circle. Large pieces of material, such as a bundle of fibers lying across the object field, are likely to cause much distortion by partially covering the image of the condenser diaphragm with glare in certain azimuths. Some excellent materials that set up sufficiently well-dispersed glare are corundum, clays, pigments, and starches; test slide 6 is recommended. Sometimes there may be some advantage in mounting a diffusing plate below the condenser.

Method C. Since the iris diaphragm of the condenser is generally a little below the focal plane of the condenser, it is possible to form an image of the iris in the plane of the object field by lowering the condenser. This image can then be seen in the field of view when the microscope is focused. On account of this change of position of the condenser its diaphragm has become a field diaphragm, and under these conditions the diaphragm at the lamp will be acting as an aperture diaphragm. It is easy to prove this by inspecting the rear focal plane of the objective and noticing the way in which the illuminating cone is controlled by the lamp diaphragm. This method of centration is best with the high-power condenser.

After this change in the position of the condenser has been made, the diaphragm can be closed to something less than the aperture of the eyepiece diaphragm, and as the observer looks through the microscope the condenser can be centered perfectly. This method has the disadvantage that the condenser must be lowered considerably, or the microscope tube must be raised a like amount, in order to focus the diaphragm image. If all microscopes were perfectly made, with tube and condenser fittings so good that the travel of the substage apparatus would always be exactly parallel with the microscope axis, this system of centration would always produce good results. However, slight errors in alignment of the substage and tube track may exist, and, since such errors will be magnified by the microscope, a condenser centered by this method and then rechecked by one of the other methods mentioned may show faulty alignment after it is brought to

its working position. The usefulness of this third method of centration then depends upon the precision in manufacture of the individual microscope, but since a way has been shown for detecting errors of travel of the tube and substage condenser it is for the microscopist to select the method best suited to his own particular instrument.

After a few attempts have been made to center the condenser by any method, it will be discovered that the operation is aided by manipulating the iris diaphragm; that is, after the adjusting screws have been turned slightly to increase or decrease the opening of the iris, any eccentricity of the condenser and the objective circles will be made more evident.

Sec. 86. A Quick Test for the Alignment of the Condenser. One of the most useful and at the same time one of the quickest and simplest tests for the centration of the condenser is to close the lamp diaphragm to a small opening and examine its image in the field of view. If the condenser is well centered with the objective, the spot of light will have a uniform fringe of color around it. The fringe may be gray, blue, green, or red, depending on the corrections, but the color should be continuous without breaks in any azimuth. If the condenser is fully corrected by being oiled to the slide, and the slide is of the correct thickness, the image will be gray or black. If a certain quadrant shows a color fringe it indicates misalignment; the color-free image of the field diaphragm is to be desired.

Once in a while it may happen that an objective carrier becomes thrown so far off center that the above test persists in showing a fringe of two colors and no amount of condenser adjustment will fix it. This indicates misalignment of the objective with the eyepiece; it must be corrected before further work is attempted.

An advantage of this color-ring test is that it can be carried on after the microscope is mounted on the camera stand without disturbing the camera arrangement. The field diaphragm of the lamp can be examined on the camera ground glass or by looking through the ocular.

Sec. 87. The Microscope Mirror. The flat side of the microscope mirror affords the simplest method of obtaining bright-field illumination; the light rays are merely directed through the object to the objective. This method may suffice for some photographic work when the magnification is less than 10 or 12 times, but if the rear focal plane of the 16-mm objective is examined when it is so lit, it will be found that only the center of the lens is in use. The effective N.A. of the objective has been so reduced that any image formed under these conditions will have diffraction lines and depth of field to an extent

that any dirt on the cover glass or even on the bottom of the microscope slide will inevitably show in the negative and spoil the picture.

Parallel rays of light from the flat surface of the microscope mirror used without a condenser would naturally travel through the specimen as substantially parallel rays; they should be parallel to the microscope axis. This condition might be called axial illumination. If the mirror is tipped slightly, without destroying the bright-field condition, the illumination becomes unilateral. When the mirror can be tipped sufficiently to afford a dark-field effect, the illumination is generally termed oblique. Central lighting, as applying to illumination with a condenser lens, occurs when the condenser is centered with the objective and the light source is centered in the field of view, the cone of light being symmetrical with the axis of the microscope. With either exactly central or axial illumination, the image of a particle in the center of the field should remain centered as the focus of the microscope is raised or lowered. It should not sway in any direction. See Fig. 103.

The flat side of the mirror should always be used when the microscope condenser is in place. Close examination of the illumination when using this side of the mirror will show that several images of the source of light may be formed in the field of view. This is seen when the field diaphragm is closed sufficiently to appear in the field of view. It is due to the double or triple reflection from the first and second mirror surfaces. Normally, the mirror is silvered on the second surface to preserve the reflecting film of metal; the main image of the source is formed by the condenser of the microscope, in the center of the object field, by reflection from the silvered surface. In addition some light is reflected at the first surface, at a slightly different angle, forming a secondary image after passing through the condenser lens. If the source of light is sufficiently intense, some of the reflected light from the silvered surface may be reflected back again from the first surface to form even a third image of the source. Figure 135 shows how such multiple images appear in the field of view, and Fig. 136 shows how the image of a small source, as the diffusing plate, should appear in the object field.

When the contrast of the specimen is high, and low apertures are used, the extra images cause little or no trouble; but when large apertures are used, on subjects prone to glare, the presence of the secondary and tertiary images may add just enough scattered light to spoil the chances of a good picture. The fact that the extra images may lie beyond the field of view, when the source is made large enough, makes no difference; glare can still be produced within the visible field.

There are two ways of overcoming the objectionable extra reflections from the microscope mirror. The first method, in use for a long time, is to discard the mirror and substitute a right triangular prism of glass or quartz. Quartz transmits and reflects the shorter wavelengths of radiation and is useful for work in the ultraviolet below the 3650 A line. The second method, and by far the more practical for nearly all work, is to substitute a first surface mirror. The quartz prism is expensive and difficult to mount, and it is supplied by only a few firms, but the first surface mirror can be adapted from the mirror accompanying the microscope at a negligible cost. The metal coating, applied by the vacuum process, can be done by a number of firms.[7] If aluminum or one of its alloys is used, the percentage of reflected ultraviolet radiation will be high.[8] There will probably be a little less light than when the silvered mirror is used, but this varies. Taylor[9,10] made a study of the reflectivity of metal surfaces which should be of interest to the microscopist, and Koller[11] measured the transmission and reflection of ultraviolet for many substances.

Some objections have been raised to the use of the aluminum-coated mirror. It has been pointed out that good microscope mirrors are nearly optically flat, and there is no assurance that the reflecting surface will be as flat after the metal coating has been applied. The argument is sound in theory, but actual practice does not show that the image formed by the microscope is in any way affected by the slight optical errors introduced by coating the mirror in this way.

The concave side of the mirror will light up the object field with a very acute pencil of light similarly to a low-power condenser, the acuteness of the angle depending on the focal length of the mirror. The focal length of a spherical mirror is given by the equation

$$f = \frac{R}{2} \qquad [56]$$

The N.A. of such mirrors is less than 0.1, and the used aperture of the objective is not always circular. Figure 137 shows the appearance of the back focal plane of the objective when the concave mirror is used

[7] The Evaporated Metal Films Corp., 116 Summit Ave., Ithaca, N. Y., coats glass with metal and various alloys by the vacuum process.

[8] J. D. Edwards, *Trans. Illum. Eng. Soc.*, **29**, 5, 1934.

[9] A. H. Taylor, "Light and Ultraviolet Reflection by Various Materials," *ibid.*, **30**, No. 7, 1935.

[10] A. H. Taylor, "Reflection-Factors of Various Materials for Visible and Ultraviolet Radiation," *J. Opt. Soc. Am.*, **24**, 7, 1934.

[11] L. R. Koller, "Production, Transmission, and Reflection of Ultraviolet Radiation," *General Electric Review*, pp. 232–236, May, 1936.

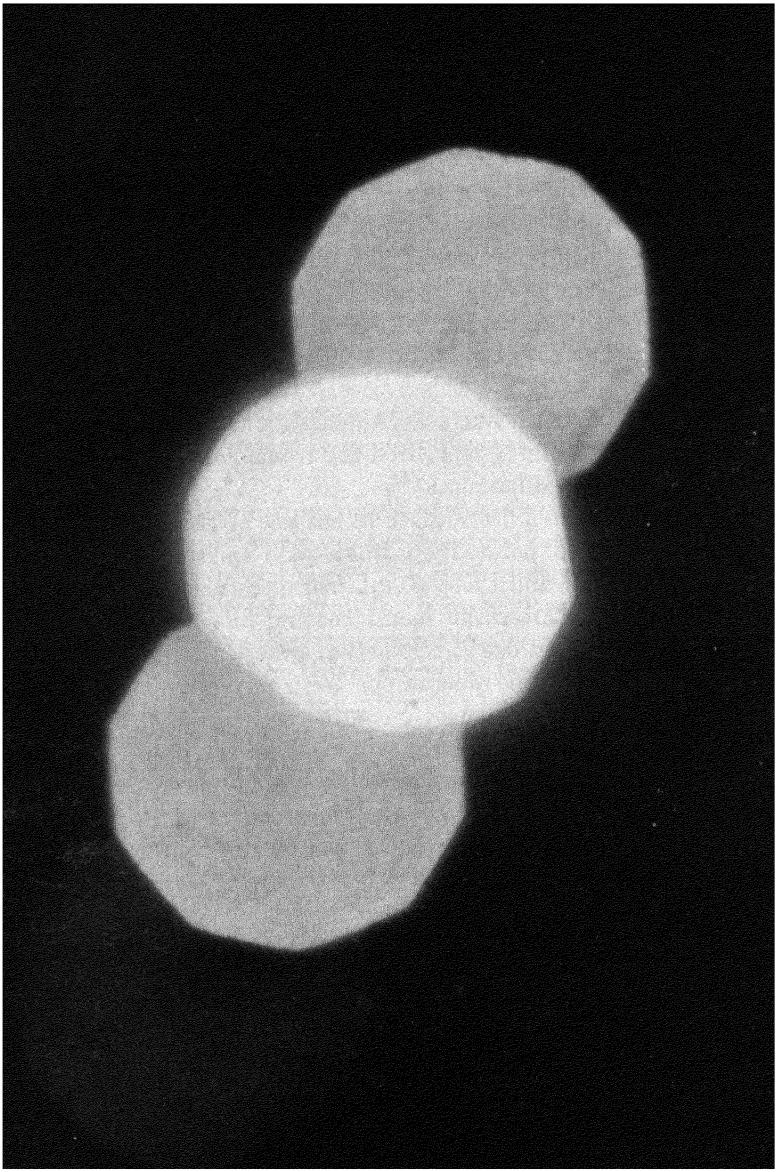


FIG. 135. The image of the lamp diaphragm in the field of view when the ordinary microscope mirror is used. The additional images, caused by reflection, cause glare.

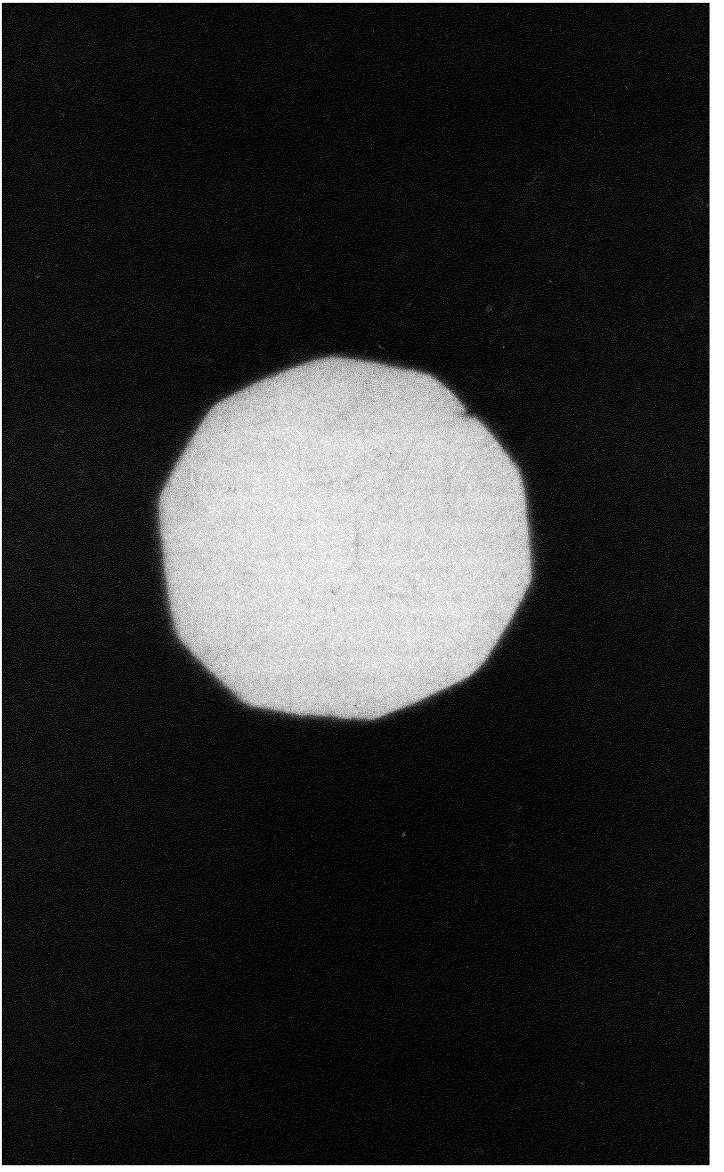


FIG. 136. The image of the lamp diaphragm when using a first surface mirror.

as a light-condensing system. The spherical mirror has no application in photomicrography, and the plane mirror alone is useful chiefly when parallel rays are necessary, as in certain careful determinations with the petrographic microscope.

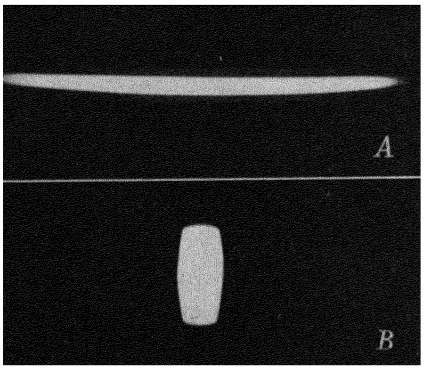


FIG. 137. The appearance of the rear focal plane of the objective when using a spherical surface mirror. At *A*, the first focal line is shown; the second focal line is shown at *B*. This picture was taken with a low-power condenser in place. The camera objective (24 mm) was focused on the exit pupil of the microscope. If the condenser lens had not been used the images would not have been as sharp and *B* would have appeared more like *A*, although perpendicular to it.

The optical theory of the spherical microscope mirror is not, as a rule, clearly understood, but it is simple and well worth considering. Equation 56 will determine the focal length of a spherical mirror, and, according to the rule to be followed for lenses, this focal distance is measured on the principal axis of the mirror. The axis of a spherical mirror, like that of a lens, is a geometrical line intersecting the surface of the mirror at its vertex and passing through the center of curvature. The axis of the mirror, then, does not coincide with the microscope axis, but it bisects the angle formed by the microscope axis and the axis of the lamp lens. This is true whether the mirror is flat or spherical, provided that the center of the mirror is on the microscope axis.

If the spherical mirror were pointed at the lamp with the mirror axis coinciding with the lamp axis, the light, if far enough removed, would be brought to a focus at a point identified by equation 56. Naturally the microscope would not be lit, but a minified image of the light source would be formed which would be circular in shape, corresponding to the original source, and would be on the mirror-lamp axis.

When the spherical mirror is tipped to send rays through the microscope, the lighting at the mirror surface is oblique with respect to the mirror axis. An inspection of the mirror, under this condition of oblique lighting, will show that only part of the mirror surface is in use, and this part will appear as a narrow strip of light which can be seen in the mirror after the microscope tube is removed. The length and breadth of the strip of light will be governed by the diameter of the

lamp diaphragm. If an image of the source is now received on a piece of ground glass held closely over the mirror it will appear as a long strip of light lying in an east-to-west direction. It will also be discovered that as the ground glass is moved away from the mirror another line image which is wider than the first and at right angles to it will be formed in a north-to-south direction.

This phenomenon of the focal lines of a spherical mirror, as explained here, are well accounted for by Wood (third edition), but the explanation is clearer when accompanied by the demonstration just cited. In forming a concept of the focal line images, it is a great aid to remember also that the law of reflection states that the angles of the reflected and incident rays must be equal, and that these angles are measured against perpendiculars erected at tangents, incident at the points where the rays intersect the mirror. It is then obvious that only a narrow strip of the mirror can be used at one time, from one position of observation. The primary focal line indicates a shorter effective focal length for the mirror across the illuminated portion than lengthwise of it; in other words, the reflected wave front is not spherical but has two radii of unequal length. The illuminated portion referred to is the narrow strip of light which is seen when looking down the microscope tube.

Sec. 88. To Center the Mirror to the Microscope Axis. Alignment to the microscope axis is as important for the mirror as it is for any lens or diaphragm, for, like the proverbial chain, the microscope illuminating system is only as good as its poorest element or the adjustment of that element. It is too often taken for granted that any microscope coming directly from the factory must be ready for use without checking for maladjustment, but this is not true. The mirror should be examined for centration, and any errors should be rectified once and for all; from then on, any difficulties due to uneven lighting can be looked for elsewhere. The results of mirror misalignment are shown in Fig. 138*B*. Here the mirror is shown misaligned with the microscope axis; at *A*, correct alignment is shown. The alignment of the mirror can be examined in three ways:

Method A. This method depends on the fact that, with most microscopes and condensers, the surface of the mirror is brought to a focus at the exit pupil of the objective and therefore is plainly visible. The method is indirect since it assumes a perfectly centered objective and condenser.

The procedure is first to ensure alignment of the objective with the tube axis (Sec. 62) and the alignment of the condenser with the objective axis (Sec. 85). The flat side of the mirror should be marked

with cross lines at the center. This can be done conveniently with a toolmaker's square with center gauge attachment, and a sharply pointed pencil specially made for marking on glass. With a low objective in place, the microscope is focused on a test slide such as 1 or 3. The back focal plane of the objective is observed; the image of the cross lines on the mirror should be easily seen. The point of intersection of the lines will be in the center of the field when the mirror is centered.

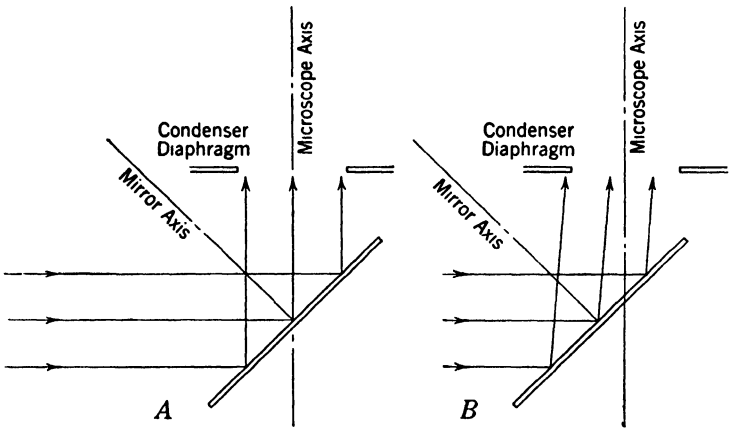


FIG. 138. The angularity of light rays when passing through the condenser diaphragm from a poorly centered mirror.

If the iris diaphragm of the condenser is partly closed it will aid in determining the alignment. Thus, when the iris diaphragm of the condenser is properly aligned, the mutual alignment of the objective, condenser, iris, and mirror can be examined at one time.

Mirrors will be found, as a rule, to be approximately correctly centered regarding an east-to-west direction; error, if any, will usually lie in a north-to-south direction. The amount of error can be estimated by holding a millimeter scale across the mirror surface while it is being examined. Any error in an east-to-west direction must be corrected at the repair shop or by the manufacturer. When the error lies in a north-to-south direction, the technician can make his own corrections. When the center of the mirror lies north of the microscope axis, away from the observer, a careful inspection of the mirror pin and yoke should be made to determine which part is acting as a stop. It may be the end of the mirror pin striking the bottom of the socket, or the outer end of the socket striking the mirror yoke. Metal should be filed or ground from the part acting as the stop. When properly followed out this procedure will bring the mirror into alignment. If the mirror lies too far

south, thin washers can be used on the mirror pin to act as shims between the mirror yoke and the socket.

Method B. This is a direct method to be used alone or as a check on Methods A and C. The mirror is marked at the center as in Method A, or a paper disc, cut to the exact size of the mirror glass, is marked at the center with an ink dot, and then fastened to the mirror with a drop of balsam. All lenses are removed from the microscope, and the iris diaphragm of the condenser is closed to a small opening. A pinhole eyepiece in the upper end of the drawtube, and the closed iris, constitute two aperture sights on which a third point, the center of the paper disc, can be aligned. A desk lamp placed in front of the microscope will afford enough illumination. The mirror is swung to a horizontal position for adjustment by this method. Figure 139 illustrates the arrangement.

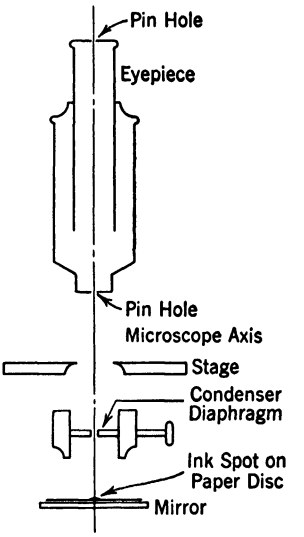


FIG. 139. Arrangement of mirror and pinhole stops to align mirror. Either the pinhole at the lower end of the tube or the one at the condenser diaphragm can be eliminated.

If misalignment is discovered, corrections can be made as indicated in Method A.

Method C. This method is not a direct method of alignment but it may be required under certain conditions; it applies when the iris diaphragm of the condenser is mounted between the condenser lenses. It is based on the fact that, when the microscope tube is raised a given amount, the image of the mirror surface comes into the field of view.

The objective and condenser must be previously centered; the microscope is then focused on a test slide such as No. 1; the mirror is marked as for Method A; and the tube of the microscope is raised above its focus until the image of the mirror surface appears. Displacement of the mirror from correct alignment will be plainly evident. To make this test a little more delicate, an eyepiece fitted with cross hairs can be used; adjustments of the mirror as for Method A can then be followed very closely.

The above methods of centration for the mirror will cover all cases and may be applied to the mounting of a prism reflector. If the mirror is of the first surface type, it should not be marked with a pencil; Method B should be used. When the mirror is mounted on the end of a swinging arm pivoted to the lower part of the substage, the arm

must be placed in vertical position before proceeding with the aligning process. Errors in an east-to-west direction can then be corrected by moving the arm in the indicated direction. The swinging arm mount is a great nuisance for photomicrography, and when the microscope is so equipped, tightening the screw on which the arm pivots may help to lock the mirror in place.

The exactness with which the mirror is centered in its mountings can be quickly checked by tipping it in various directions, meanwhile watching the central mark. If the mirror is correctly mounted and centered, the mark will not move from the central position, no matter how the mirror is tipped. But, if the center of the mirror does not coincide with the axes of the yoke pivots and the mirror pin, the image of the cross lines will sway as the mirror is tipped.

Sec. 89. Selection and Use of Low-Power Condensers. All objectives with a focal length greater than 24 mm when used for work with transmitted light should be served with a condenser of similar aperture and suitable focal length. If this rule is followed, the lighting will be high in intensity and glare will be at a minimum. Several companies make a special condenser to accompany each of their long-focus microphotographic lenses.

Since the spectacle lens condenser is focused on the entrance pupil or the front lens of the objective, it is desirable that the image of the source be large enough to fill the objective lens with light. This is easily calculated by taking into consideration the size of the light source and the focal length of the condenser. Assuming a lamp distance of 14 inches and 2 inches for the focal length of the condenser, equation 43 will show that if the diameter of the source is 2 inches the diameter of its image will be $\frac{1}{7}$ of that, or a little more than 7 mm. This would be large enough to cover the front lens of many low-powered objectives; microphotographic objectives often have comparatively large front lenses.

There is quite a wide choice in selecting spectacle lens condensers. Many are uncorrected simple lenses, and others have aspherical surfaces giving an amount of freedom from spherical aberration. However, on account of mounting difficulties, since some of the lenses are quite large, it is generally a good policy and saves trouble to choose the low-power condensers made by the manufacturer of the microscope with which they are to be used.

To center low-power condensers to the objective is not as important as it is to center those of medium and high power. If the light is centered, and if there is an absence of swaying of the image as the objective is moved up and down, the centration is probably sufficiently good for low-power photomicrography.

Often with the low powers the substage diaphragm must be swung to one side and it cannot be used. Under such conditions, an additional diaphragm may be needed if there is considerable glare from the specimen. A diaphragm made from a metal disc or from a piece of black paper can be dropped into the filter carrier, and the carrier moved to the appropriate distance from the microscope. With the diaphragm placed at approximately the front focal plane of the condenser, the ocular can be removed, and the back lens of the objective examined. The diaphragm can then be seen and one of suitable size can be selected and centered.

The adjustment of aperture of the low-power condenser is not nearly as critical as it is with the higher-power illuminators. A $\frac{9}{10}$ cone is always to be desired, but on much low-power work the full aperture of condenser can be used. The objective may be fitted with an iris diaphragm, which alone, will give adequate light control, but if the condenser is focused in accordance with the suggestions in the first part of this section, then the lamp diaphragm will control the condenser aperture. See Sec. 83.

Sec. 90. Selection and Use of Medium-Power Condensers. If an attempt is made to use the 16-mm objective with a high-power condenser, difficulty will be experienced in filling the field of view with light. The image of the source is made so small by such a condenser that even when magnified by the objective it is not large enough to fill the eyepiece diaphragm.

When the condenser is of too high a power for the objective, the substage diaphragm must be closed to an opening so small that it will seriously interfere with the optical corrections of the condenser. Additional lens diffraction is then set up; consequently, images formed under such conditions will be poor. This can be easily tested. As the difference in the images is being studied, note should be made of the seemingly lower resolving power of the objective when the high-power condenser is stopped to a low aperture. Actually, the resolving power may be in no way changed, but owing to increased glare, which is present with the high-power condenser, fine detail will be masked. The condenser should be selected so that the diaphragm can be maintained at, roughly, one-third the diameter of the front lens of the condenser or more. If the condenser is selected to permit this, the illumination in the object field will be more brilliant.

If the field of view is not filled with light the following steps can be taken:

1. The top lens may be removed from the high-power achromatic-aplanatic condenser, thus making a condenser of medium power.

2. A medium-power condenser with aplanatic corrections can be installed.
3. An ocular of high power may be used.
4. An accessory lens of −2 to −4 diopters mounted in the substage will convert the condenser into one of lower power.
5. The lamp can be moved nearer to the microscope.
6. A larger source of light can be used.
7. A diffusing plate can be placed in the substage.
8. The concave side of the mirror may be turned in.

Suggestion 1 is probably the most practical and the most-used method, although not necessarily the one that is recommended. The high-power achromatic-aplanatic condensers are often as well corrected with the top lens removed as are special low-power condensers of intermediate focal length. If the N.A. of the condenser is 1.4, it will be around 0.6 to 0.8 without the top lens. This aperture would be high enough for even 8-mm objectives.

Suggestion 2 is the best, but if a medium-power condenser is selected it should be a good one and perfectly aplanatic for its full cone of light. A cheap medium-power condenser would be less satisfactory for photomicrography than the achromatic condenser with its top lens removed. Suggestion 3 depends largely on existing conditions; occasionally it may be useful.

Suggestion 4 is suitable for either visual work or photomicrography, but only a few microscopes are equipped with accessory lenses. Moving the light source nearer to the microscope as in suggestion 5 is fairly practical for photomicrographic work provided that the specimen is not of a very glary nature.

Suggestion 6 involving the large source of light may not always be possible of fulfillment. Suggestion 7 is not practical if the subject is glary; otherwise it may be satisfactory. Suggestion 8 is a poor adjustment and should be used only for temporary visual inspection.

It is poor practice to fill the field of view with light by lowering or raising the condenser. Such an adjustment is likely to afford the poorest illumination attainable, for when the image of the aperture diaphragm appears, the diaphragm has lost its function and is acting as a field diaphragm, so that control of aperture is practically lost.

When the 16-mm objective is used merely to search for a field or for similar work of a temporary nature, the small field of view may not be a factor of importance, since the real work will be done later with a high-power objective. Under such conditions it is feasible to turn in the concave mirror. This will often light up the whole field,

even with the high-power condenser. However, the mirror must be turned again before the high-power objective is used, since the concave side should never be employed for photomicrography.

After a suitable condenser has been focused and centered, the appearance of the field should be carefully studied and the condenser diaphragm adjusted to give the contrast desired in the picture. When the microscope is used visually it is customary to adjust the diaphragm as observation is shifted from one part of the field to another, choosing the aperture best suited to the portion under examination. For photomicrographic work a definite setting of the diaphragm must be decided on, and always it should be borne in mind that photomicrographs should be taken at as high apertures as possible. The $^9/_{10}$ cone has already been referred to as the goal for which to strive. The conditions which make possible optimum aperture adjustment are freedom from glare (Sec. 102), good lens adjustments, and good illumination.

For photomicrographic work, only aplanatic or, better yet, achromatic-aplanatic condensers should be considered. Achromatic and apochromatic objectives respond equally well to good illumination.

Sec. 91. Selection and Use of High-Power Condensers. For photomicrographic work with objectives higher than the 16-mm, the regular high-power condenser should be used. The condenser should have full corrections; it may be of either the immersion or dry type. Several manufacturers make an achromatic-aplanatic condenser to be used dry. Its limiting aperture is, of course, a little less than 1.0; consequently it is suitable only for objectives of apertures lower than 1.0. It is always convenient to have a condenser that does not need to be oiled for use with the 8- or 4-mm objectives. If the regular high-power oil-immersion achromatic condenser is used without being immersed, the corrections for spherical aberration and coma, originally built into it, cannot be realized. Thus it is necessary to immerse it for work even with the medium-power objectives.

Whether or not the dry condenser will be satisfactory depends in large measure on the specimens with which it is to be used. Immersion reduces glare at the first surface of the slide; consequently, with a specimen that tends toward glare, the immersion condenser will give the best results, and the photographic images will be a little more contrasty. It is important to remember that the immersion type will do almost anything that the dry type will do, and in addition it can be used with any objective, whereas, although the dry type will have full corrections when operated dry, its use is limited to medium-power objectives.

Condensers such as the Abbe or modified three-lens Abbe are not recommended for photomicrographic work. Good pictures can sometimes be taken with them, but if the work is at all varied there are likely to be too many failures which can be traced to illumination faults. The aplanatic is a good condenser, but as Belling has pointed out it works best with a green screen; it is then practically on a par with the fully corrected condenser. The green screen imposes limitations, and the achromatic-aplanatic condenser, since it is not much more expensive than the aplanatic, is always first choice.

In actual practice it will be found convenient to use a low-power objective for preliminary examinations. As is customary when surveying the field, a preliminary focusing and centering of the condenser can then be carried out. When the high-power objective is turned in only slight adjustments should be needed; if objectives are changed during work, the focusing and centration of the condenser should be rechecked. A glance down the microscope tube will indicate whether or not the centering has been disturbed.

The working distance of a good high-power condenser is slight and very sensitive to slide thickness. When the lamp distance is 14 or 15 inches, the second conjugate focus of the condenser is very near its principal focus; in other words, the working distance is about as short as it can ever be. This phase of the operation of a condenser and control of working distance was explained in Sec. 83 under the heading of focusing.

The iris diaphragm assumes considerable importance in high-power work. Up to this point, there has been no particular question of resolution; as the aim has been to obtain sharp images, the iris could be closed more or less to suit. With high-power work, resolution is of great importance, and apertures will have to be large to get the utmost from the objectives. The $^9/_{10}$ cone of light which was mentioned before as desirable now becomes a necessity if high resolution is to be attained. Chapter V, discussing the use of filters and the control of glare, will give suggestions for control to make high apertures possible. It should be remembered that a well-corrected condenser is itself important in high-aperture work, and that each optical adjustment of the microscope contributes its share toward the perfect optical functioning of the whole apparatus without which high apertures are not attainable. The operation of adjusting the iris of the condenser now assumes a good deal more importance than the mere opening or closing to get a good image; it becomes a final test for good adjustments throughout the system.

Not all specimens are by any means good subjects for high-aperture work. In fact, the use of a high-power objective, N.A. 1.4, with a $^{9}/_{10}$ cone of light means that the flatness of the specimen must be comparable with an optical flat, if sharp images are to be attained. Even a well-stained contrasty bacteria smear may show an unevenness of surface which requires reduction of the iris opening, with its resulting loss of resolution, before the images will be sharp and clear. For visual work this great precision in adjustments is less necessary, and the iris diaphragm of the condenser may be made to cover many errors in technique. Also, the eye has a greater accommodation for field depth; it is about ten times greater than can be recorded photographically.

The advantage of a well-corrected condenser is fully demonstrated by means of a cube of uranium glass or any plastic material containing a pigment that promotes a translucent, rather than a transparent, effect. The side of such a cube should measure about 2 or 3 cm. It can be oiled to a thin glass slide. When light is directed through it, the light path will be plainly visible, just as it is when smoke is blown across a beam of light. With the Abbe condenser the light path will show a rather ragged cone of light unless it is well stopped down. Even when the condenser is oiled, it is necessary to reduce the aperture if the sides of the light cone are to appear fairly sharp.

Various condensers, as available, can be tried with the cube, and the different results compared. Condensers should be tried both dry and oiled; the light can be made oblique, and the manner in which the displacement of the condenser or the iris diaphragm affects the light cone can be noted. The extent to which focusing the condenser affects the aperture can be demonstrated as the condenser is raised or lowered from its normal position.

Because low- and medium-power condensers are neither as sensitive nor as well corrected as the high-power achromatic-aplanatics, special methods for testing them are not required. The quickest and best way to test the adjustments of the high-power condenser is to examine the back lens of the objective. When the condenser is being used correctly, the appearance of the field at the rear focal plane of the objective will be the same whether the condenser is slightly raised or lowered from its proper focus.

To make this test, the light should be first diaphragmed to a small opening, about 3 mm, and if the condenser is made for immersion it should be oiled to the slide. The test is best made with a 2-mm objective focused on a slide of well-dispersed discrete particles, such as

test slide 2. The condenser should be sharply focused, and the slide should not be more than 1 mm thick. Belling[12] and Beck[13] discuss this method. Belling's method of illumination by transmitted light is based on the fact that microscope condensers are made to be used with parallel light rays, and that only when so used will they give their best performance. Accordingly, he suggests installing an achromatic lens in the substage under the microscope condenser. This accessory lens has a focal length of approximately 23 cm and a diameter of 30 mm; it is an achromatic[14] meniscus. The light is placed at the first principal focus of this lens; consequently the condenser is served with parallel light rays. However, the accessory lens does not seem to be necessary in order to make a satisfactory condenser test.

The back lens of the objective is observed, and the condenser iris is opened to a full cone. If a light ring appears around the objective circle as the condenser is raised (this can be referred to the star test, see p. 239), the condenser is under-corrected. If the ring occurs when the condenser is lowered, an over-correction exists. Under-correction will be almost universal for immersion condensers that are used dry; this can be seen by repeating the test with the immersion oil removed. Using immersion liquid of too low a refractive index (for instance, glycerin instead of cedar oil) will also give an under-corrected condition. Over-correction will probably not be troublesome unless the slides are very thick. Lamp distance, as stated by Belling, will have an effect if the condenser is used according to his method, with an accessory lens; otherwise variation of lamp distance, within reason, makes little difference.

It is always a good idea to inspect a new condenser, as directed above, to make sure that it is possible to get a nearly perfect cone of light. Unless this is done, if the condenser is used on a high-aperture objective such as 1.4, and if proper correction is lacking, there will always be a slight portion of the objective that is never well illuminated, irrespective of the adjustment of the condenser diaphragm. The objective circle in this test should appear *sharply defined* when the condenser diaphragm is opened to give a full cone of light; it should not have a fuzzy, indistinct appearance.

If the substage apparatus is equipped with an iris diaphragm directly over the top lens of the condenser, the diaphragm should be kept wide

[12] J. Belling, *The Use of the Microscope,* p. 88, McGraw-Hill Book Company, 1930.

[13] C. Beck, *The Microscope,* first edition, p. 118, R. & J. Beck, Ltd., London, 1924.

[14] Private communication, Feb. 29, 1932.

open while the microscope is being used for high-power work. However, for low-power or photomicrographic work on particularly glary material, it acts as a shield and is useful with the low-power condensers for shutting off stray light that otherwise might interfere.

Sec. 92. Dark-Field Substage Condensers. Condensers which have the central rays shut off or stopped by some means form a hollow cone of light with the object at the apex. If these rays of light which form the cone pass beyond the limits of the front lens of the objective, the object field will be perfectly black. Condensers which operate in this way are known as dark-field condensers. If there is a specimen in the field, some of the light will be reflected from it to the objective, and thus the bright image of the specimen will become visible in the field of view, on a black background. Images of this type are reflection images.

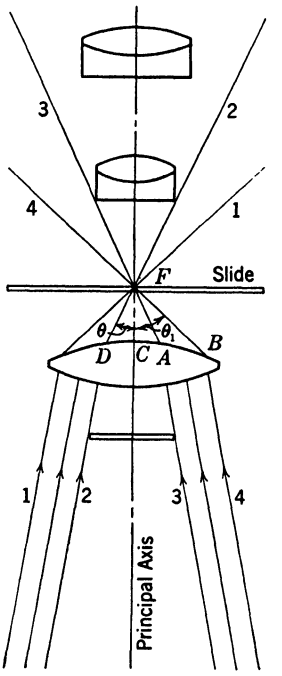


FIG. 140. Formation of a dark field by using a central stop in conjunction with a bright-field condenser. Angle θ is bounded by rays 2 and 3 (although this is not clear in the drawing because of its small size); the enclosed cone is dark. Rays 2 and 3 set the lower limiting aperture for the condenser. Angle θ_1 is bounded by rays 3 and 4; this is a section of the outer shell of the light cone formed by rays 1 and 4. Rays 1 and 4 set the upper limits of the condensers aperture. The larger angle θ_1, the greater will be the amount of light focused on the specimen.

Figure 140 shows the hollow-cone effect produced by the dark-field condenser; since there is no specimen in the field, no light is passing to the objective. Transparent objects in the dark field will show dark centers and bright outlines; opaque objects show their surfaces bright much as they would do with ordinary lighting. Dark-field effects are exactly opposite to bright-field effects in every respect; an object appearing bright in one field will appear dark in the other, and vice versa. Figure 141 shows two photomicrographs of the same subject. *A* was illuminated by a bright-field condenser and *B* by a dark-field condenser. It might be noticed that the finer the detail the better it appears by dark field.

Dark Field by Use of a Central Stop. There are two distinct methods of obtaining dark-field illumination from beneath the microscope stage,

and combinations of these methods are the basis for specialized forms of dark-field illuminators. The first and simplest method consists of blocking out the central rays which pass through the bright-field condenser by means of a central stop. The second method is with a con-

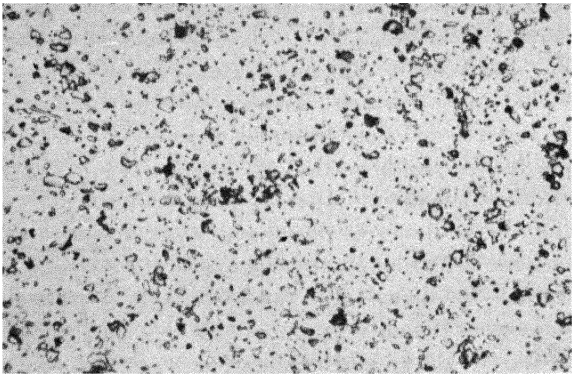


FIG. 141*A*. Photomicrograph of ground mica ×360. Obj. 16 mm apo. Leitz; ocular, 15× Zeiss Compensating; condenser, Leitz achromatic, top lens removed; illumination, method I; daylight filter; Eastman Pan. X film; developer, D-19; 9 by 12 eyepiece camera. Compare with *B*, which is the same subject, same data as for *A* except the dark field which is obtained by use of central stop.

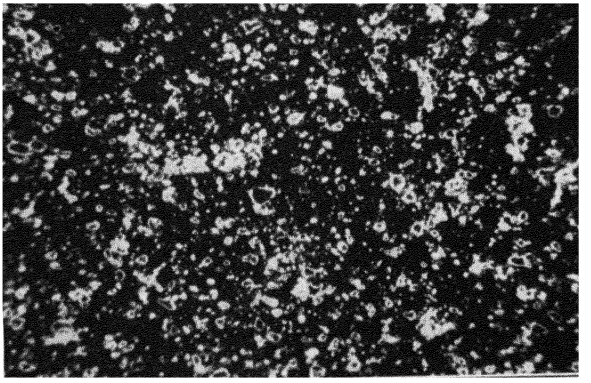


FIG. 141*B*.

denser having one or two reflecting surfaces to focus the hollow cone of light upon the object.

Figure 140 shows an ordinary low-power bright-field condenser with the central rays shut off with a stop. The resulting cone of light is therefore somewhat as shown in the figure by the limiting rays 1 and

2. If the N.A. of the objective is 0.3 and that of the bright-field condenser 0.8, and if the stop which is introduced is of such size that it shuts off all the light rays corresponding to a N.A. of 0.3, then no light will pass directly to the objective; the field will be perfectly dark as shown in Fig. 140. The remaining rays, those between the limiting rays 1 and 2, and 3 and 4, form a conical shell of light converging to an apex at *O*. Ray 2 will graze the objective, and the lower limiting aperture of the dark-field condenser is therefore 0.3. Ray 1 will form the upper limiting aperture of the lens; it is 0.8. Light reflected from the object at an angle which corresponds to a N.A. of less than 0.3 will pass to the objective, and that part of the object which reflects light to the objective will be seen in the microscope.

The above system has the advantages of being simple and inexpensive and utilizing equipment generally at hand, but it is limited to low-power objectives. Figure 141*B* is a photomicrograph taken at a magnification of 360 diameters. With the exception of the stop to produce the dark field, the condenser in this picture was the same as that in the picture in Fig. 141*A*.

The Paraboloid Condenser. One of the oldest and best known of the dark-field condensers is the paraboloid. It consists of a solid piece of glass ground as accurately as possible into the form of a paraboloid. A point source of light at the focus of a paraboloid reflector will be reflected from that surface as a parallel beam. Conversely, parallel rays incident on a paraboloid reflector will all be brought together at the focal point of the reflector. If the paraboloid is cut so that the focal point is slightly beyond the top of the segment, parallel light rays of the illuminating beam will be brought to a focus at the position which is correct for lighting up the specimen. Immersion oil on the top of the condenser, together with the microscope slide, represents the optical equivalent of that portion of the paraboloid which is cut away. Ordinarily, under these conditions, the central rays would form a bright field; to make the field dark a central stop is used to block off these rays. The other rays meeting the side of the paraboloid, generally at an angle greater than the critical angle, are reflected to the principal focus of the condenser. The glass slide, mounting medium, and immersion oil all go to complete the optical homogeneity of the paraboloid shape. The paraboloid dark-field condenser made by Bausch and Lomb is shown in Fig. 142*C*.

The paraboloid condenser is excellent for dark-field effects with the medium-power objectives. Since it generally has a lower limiting aperture of around 0.85 it may be used with medium objectives with apertures lower than this. Figure 142*A* shows how the outer rays of

a beam incident on the paraboloid condenser become the inner rays of the emerging pencil.

The Cardioid Condenser. Figure 142*D* illustrates the trace of rays through a doubly reflecting condenser system. (Leitz publishes a

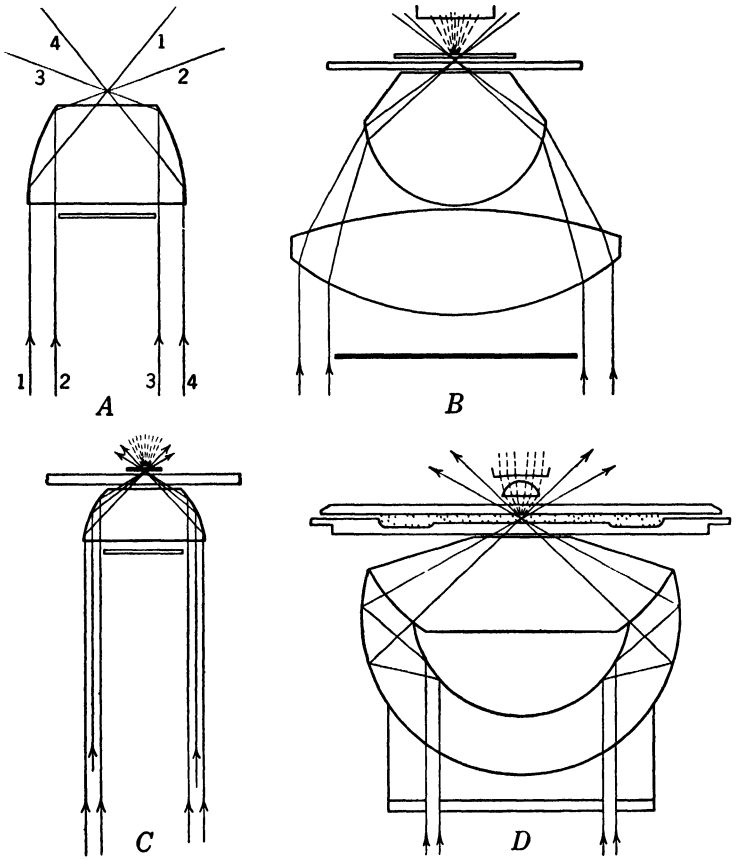


FIG. 142. *A* illustrates how the inner incident rays of dark-field condensers, of certain types, become the outrays after transmission. *B* shows an Abbe condenser arranged for dark field. *C* is a diagram of a paraboloid condenser. *D* illustrates a cardioid condenser. (After Bausch and Lomb.)

booklet[15] giving a table of various dark-field illuminators.) The hemisphere in the center, as shown in the figure, is silvered on the under side to reflect the light to a second reflecting surface, and from this surface it is brought to a focus at the object field. The first reflecting surface acts not only to reflect the light, but also as a central stop.

[15] E. Leitz, *Reflecting Condensers for Dark Ground and Ultra-Microscopy*, 1931.

One important difference between bispheric, bicentric condensers, or, as they are sometimes called, bireflectors, although not all of them have the second reflecting surface ground to a cardioid figure, is that these reflecting types furnish a stronger illumination at the object field than those of the purely refracting type.

If the shape of the second reflecting surface resembles a cardioid of revolution, the condenser is known as a cardioid condenser. Such a condenser has a lower limiting aperture of about 1, and when used immersed and with a 4-mm dry objective it becomes a very useful piece of equipment. Like all higher-power condensers the cardioid condenser is rather sensitive to adjustment and is therefore more difficult to operate than the paraboloid, or the refracting central stop type, but it offers the advantage of focusing the light sharply and with little loss, which makes it a popular choice when sols or gels are to be examined without a slit microscope.

The glare when using this condenser may be very great, owing to its high aperture and to the difficulty of making preparations glare free. Its focal length is so short that it may give poor illumination on particles which exceed a few microns in size.

Theoretically, the cardioid condenser is one of several naturally aplanatic optical systems. The combination of a spherical reflecting surface and a cardioid reflecting surface gives a condenser which is free from spherical aberration, coma, and chromatic aberration. Practically, however, it is very difficult to form a perfect cardioid surface; therefore these condensers may depart somewhat from complete perfection, but since their performance is usually so highly satisfactory one hesitates to find fault with them on this account.

The Quick-Change-Over Condenser. To simplify the transition from the bright-field method of observation to dark-field observation, a quick-change-over condenser has been made. It is a rather good dark-field lens but a less efficient bright-field illuminator. It is shown in Fig. 143. It is equipped with a central stop which can be swung out of the path of the light beam. When in place it shuts off rays 3 and 4; the light can then be passed only to the reflecting surface S and diffused upward to light the object, as in bright-field illumination. When the central stop is swung out of the light path and rays 1 and 2 are shut off by an iris diaphragm, the condenser becomes a dark-field illuminator, the trace of the rays being easily seen. This condenser is not suitable for photomicrographic work when used for bright-field illumination.

The Spot-Ring and Cassegrain Condensers. The luminous spot-ring condenser by Zeiss and the Cassegrain by Watson are special forms of

high-aperture dark-field illuminators. The Cassegrain is said to have a lower limiting aperture of 1.4, and the spot-ring of Zeiss can be used with objectives of N.A. 1.3. The dark field of extreme angle is obtained by reflection. No part of the optical path from the top of the condenser to the objective may have an index less than glass, or the oblique rays will be blocked.

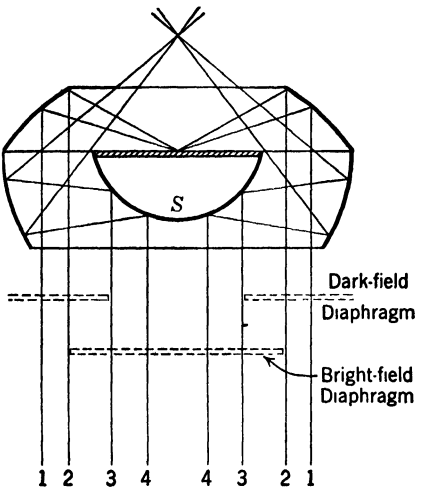


FIG. 143. A quick change-over condenser. Courtesy of Leitz.

Sec. 93. Focusing and Centering the Dark-field Condenser. These two operations are carried on simultaneously. The focus is exactly the same for either dark field or bright. An image of the lamp diaphragm is formed in the object field, but centering is done when the condenser is slightly off focus.

At first the field diaphragm is opened until the preparation is focused by the microscope. It may then be partly closed during focusing and centering. Later it is adjusted as for bright-field operation.

With the dark-field condenser formed from the bright-field condenser by the use of a central stop, extreme care in centering is not as important as with the higher-power dark-field illuminators. It is best to drop in the central stop after the bright-field condenser has been focused and centered. Then, if the condenser is slightly raised or lowered while an observation is being made, the black shadow caused by the stop will be in the center of the field.

Low-, medium-, and high-power dark-field condensers are all occasionally made with a small circle engraved on the top of the back glass. To center such a condenser it can be illuminated from above by incident light; an ordinary desk lamp will suffice. A low-power objective, of 16-mm focal length or greater, should be used. Care should be taken to ensure the mutual centration of the low-power objective and the objective to be used later on for observation. The rings on the top of the dark-field condenser will be visible with the low-power objective, so that the condenser can be centered by making the rings concentric with the microscope field. After oil is added, for observation, the rings will be invisible.

A further examination of the adjustment of the high-power dark field should be made in a somewhat more critical manner. This is best done after the high-power objective has been turned in, and, if required, the condenser is oiled to the slide. Figure 140, showing the way the light passes from the condenser to the object, would indicate that when the condenser is slightly raised or lowered a dark spot should appear in the center of the field and, further, that the dark spot should be exactly in the middle of the field of view. With a test slide of a well-diluted india ink solution, and a 16-mm objective in position, the field can be studied. The 16-mm will be a very low objective to use with many dark-field condensers, but it will show up the condenser adjustments well. As the condenser is slightly raised or lowered to a position of focus, a point will be found where the appearance of the field is comparable to that shown at *A*, Fig. 144. On either side of this position the appearance of the field should be as at *B*, if the condenser is centered; when it is off center the condition may appear as at *C* or at *D*. The condition as shown at *C* merely indicates that the condenser must be centered by the screws provided for that purpose. If the condition is as shown at *D*, not only must the condenser be centered, but also the mirror must be adjusted to center the light.

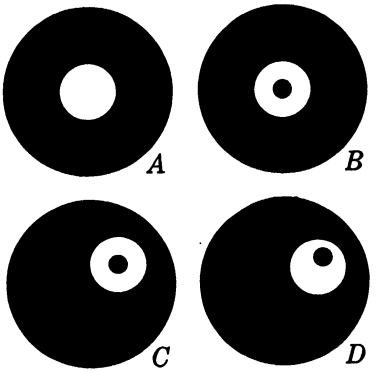


FIG. 144. Diagram showing appearance of field of view during different steps in centering a dark-field condenser.

Adjustment of the mirror will align the dark spot in the center of the cone of light, but the centering screws of the condenser should be used to center the field of light in the field of view. These two adjustments, the mirror and centering screws of the condenser and the focusing adjustment (to enhance contrast of the central black spot), can be worked alternately until complete centration has been attained. The condenser is then brought to focus. While a dark-field condenser is in use it is well to check its centration occasionally, as the position of the mirror may shift. Sometimes a change can be noted by the unilateral lighting effects produced on the specimen. In any event, it is not necessary to return to the low-power objective; the condenser can be examined by slightly racking it out of focus, when any error in centration will be plainly visible.

Condensers that do not have a central mark inscribed must of necessity be centered entirely by the method illustrated in Fig. 144. The greater the obliquity of the light cone given by the condenser, the greater will be the need for most precise centration of both the light and the condenser in order to obtain a good field. Before any actual attempt is made to center the dark-field condenser it is urged that Sec. 94 be read.

Sec. 94. Selection, Use, and Adjustment of the Dark-Field Condenser. Dark-field condensers are selected entirely with regard to the material under examination. Large particles and pieces of specimen, such as fibers and large crystals, can be examined with a dark field produced by a central stop in combination with the lower part of the bright-field condenser. On such specimens, a high-power dark-field condenser would not give a large enough field and would produce too much glare for good observation. Particles smaller than those mentioned can be examined with the paraboloid or one of the medium dark-field bispheric condensers of Leitz and others. This class of subject may include large pigment particles, coarse emulsions, and material in general which does not reflect enough light to be examined by the central-stop dark field. The cardioid and other high-power dark-field condensers are used for the examination of yet smaller particles — bacteria, blood smears, and, in general, particles which are difficult to observe by bright-field methods. Colloids (particles less than 0.1 μ) can be examined by dark-field condensers, although the results are not as good as with a regular ultra or slit microscope. With a slit microscope the images are formed by scattered light, the particles being very small.

After any dark-field condenser is centered and focused no attention is paid to the condenser diaphragm. It should be wide open when the condenser is first put into the substage and left that way during the examination. The illumination can be by Method I, II, III, or a parallel light beam, depending upon the amount of light required. If the arc lamp is employed it is well to have it adjusted for parallel rays, particularly for the paraboloid and cardioid condensers which are certainly figured for parallel light. The smaller the microscopic particles, the more intense must be the light, and for colloids Method II may be necessary. Particles of 0.2 μ or less, below the resolution of the microscope, are made visible only as diffraction discs rather than as images by reflection. This means that the impinging light on each particle is scattered so completely that the particles act as self-illuminating sources. Therefore when these very small particles are being examined the limit of visibility, as far as size is concerned, depends upon the bril-

liance of the illumination and the power of the particle to scatter light. For this reason, the light source should be very strong for ultramicroscopic sizes of material, but it need not be as strong for particles that are seen by light reflected in the usual way. Soap sols, most of the body fluids, beers, gelatins, and india ink need high light intensity for examination. Emulsions, pigments, and most mechanical suspensions can be examined at a much lower light level and often with a low-power condenser.

Magnification, as a rule, is not a very important factor when using a dark field; extreme contrast is relied upon to render detail visible. High-aperture lenses can be employed with the spot-ring or Cassegrain illuminators, and resolution is in accordance with the formula for bright-field illumination; but, if indication of particles alone is sought, the diffraction disc can be seen by either low- or high-power objectives.

The stopping down of an objective is quite possible. Thus an aperture of 1.3 can be stopped down to less than 1.0 and the objective can then be used, for instance, with the cardioid condenser which has a lower limiting aperture of, say, 1.0. Some objectives of high power are equipped with a small iris for this purpose. The reduction in N.A. may also be accomplished by means of an accessory diaphragm at the back of the objective, or by taking the objective apart and inserting a small funnel with an opening of the appropriate size. With the funnel system, the objective, generally a 3-mm oil-immersion, can be taken apart above the back lens, so that there is no danger of getting the different lens elements out of alignment by repeated use of the system. The dry 4-mm apochromat is a fine objective for dark-field work requiring high magnification. With a 15× eyepiece it will always give good results. Sometimes it can be used with the 20× eyepiece. With condensers of over 1.0 for a lower limiting N.A., any of the 4-mm objectives can be used without stopping down. The ideal condition exists when it is possible to use the 4-mm objective corrected for use without a cover glass, thus eliminating considerable glare.

Dark field is essential for photographing many of the crystals. Thus vitamin B_1, hydrochloride, a very thin acicular crystal occurring in clusters, is nearly invisible in the liquid from which it crystallizes, but it is easily photographed with the simplest of dark fields. Figure 145 is a photomicrograph of this vitamin illuminated with the lower part of a bright-field condenser and a central stop to give a dark-field effect. Figure 146 is a photomicrograph of a blood specimen. With the exception of parasitic specimens, photomicrographs of blood are usually taken to demonstrate anatomical or histological structure. This



FIG. 145. Photomicrograph of vitamin B_1 hydrochloride ×360. This subject is well adapted for dark-field work. Obj. Leitz 16 mm apo.; ocular, Zeiss Homal I; condenser, Leitz achromatic, top lens removed, central stop; illumination, method I; daylight filter; camera extension, 570 mm.; Eastman Pan. X; developer D-19

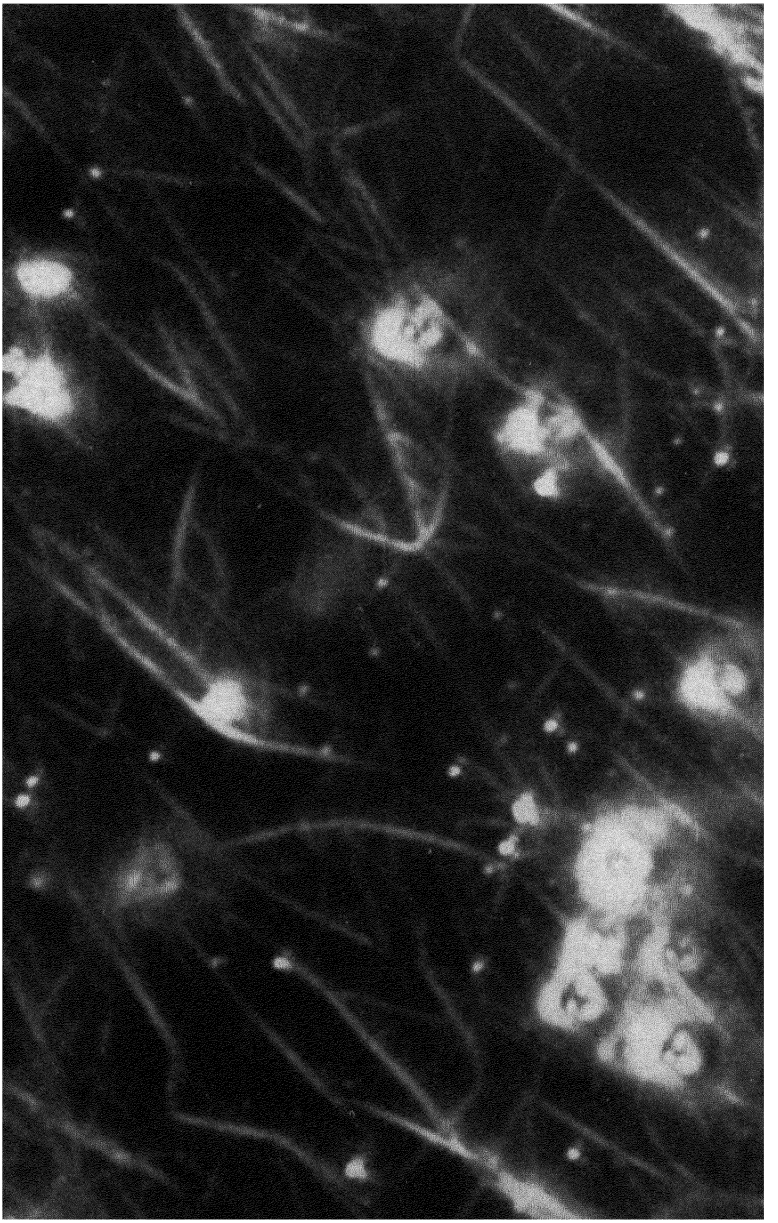


FIG. 146. Blood fibrin ×4500. Objective, 4 mm apo. Zeiss; ocular, Homal III, Zeiss; condenser, cardioid, Leitz; illumination, 400-watt biplane filament lamp, method II; filter, Corning 306; film, Defender, Fine Grain Pan; developer, D-19; magnification, microscope and camera 1000×; enlargement, 4.5×.

necessitates the use of three distinctly different microscopical techniques; medium to high magnification with bright-field arrangements for photographing the blood cells which have been stained or otherwise treated; high magnification and high-intensity dark-field illumination are required for recording constantly moving chylomicrons; high-power dark-field optics are best for studying fibrin structure. Even with the advantages of staining, it is best to treat the blood components separately, for they usually occur at different optical levels, and therefore the field depth of a high-power objective cannot focus them all, simultaneously.

Dark-field illumination is not difficult to arrange as far as the manipulation and alignment of the dark-field condenser is concerned, but a frequent and aggravating source of trouble comes from dirt on the slide or cover glass. Inclusions in the glass, artifacts, dust, or any form of dirt tends to spoil the dark-field effect. Dirt on the top of the cover glass may be rather far from the focal point of the objective and so may not show as individual particles but, rather, will cause the whole field of view to be veiled with a haze of light, making it impossible to obtain a completely black background. Figure 147 is a photomicrograph of a slide. The usual cleaning methods have not proved effective, and the bright spots are probably due to devitrification of the glass. Such a slide would be unfit for dark-field work, although it might be used for the most exacting bright-field work. Therefore, in photomicrography, it pays to examine the slide on which the specimen is about to be mounted, using the same dark-field condenser that will be used for illuminating the specimen.

A slide of fused quartz is more free from inclusions than one of glass, and the surface is carefully polished. Special dark-field chambers that will maintain a liquid specimen at a definite thickness, from 2 μ to 10 μ, are excellent for mechanical suspensions of small particles.

A later section will deal in detail with general methods for cleaning slides.

Sec. 95. Special-Purpose Condensers. In addition to the bright-field and dark-field condensers mentioned above, there are numerous condensers for special purposes.

For ultraviolet work or for demonstrating fluorescence, most of the condensers already discussed can be obtained in quartz, but the cost is usually at least 50 per cent higher. If the condenser is of quartz, the light-collecting lens at the lamp and the microscope slide and cover should also be of quartz.

The dissecting condensers by Zeiss have a long focal length and are intended for work with low-power objectives. They are built with a

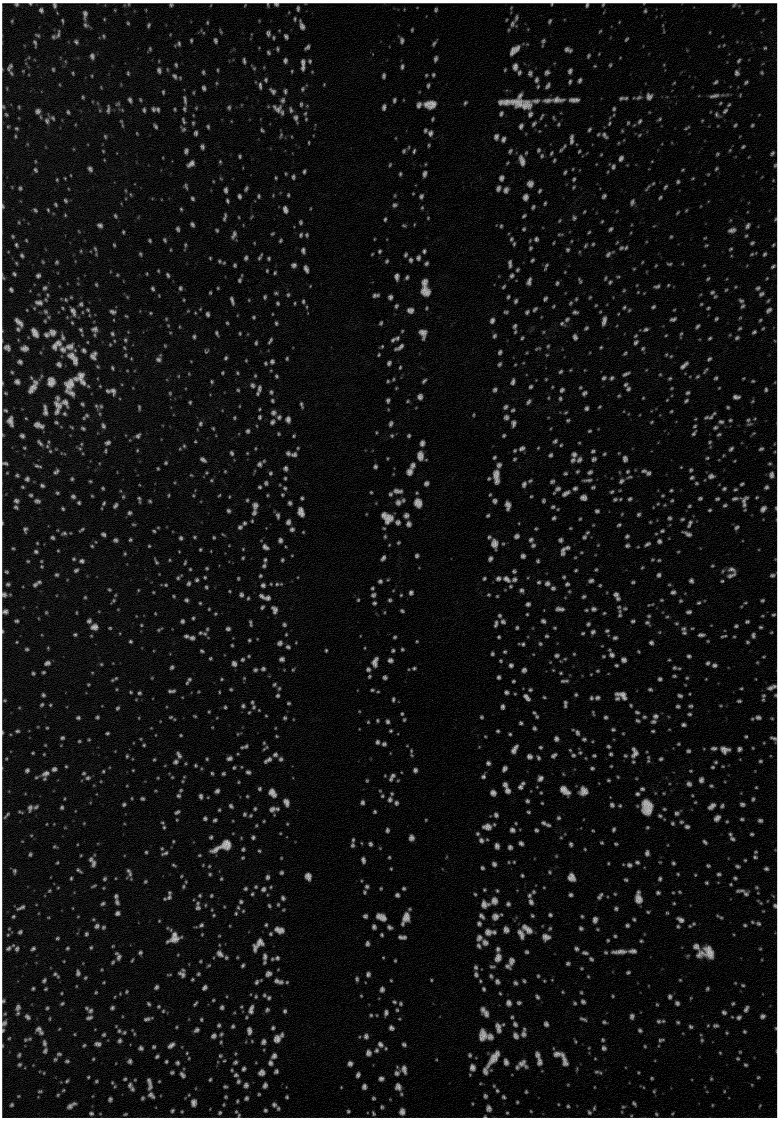


FIG. 147. Photomicrograph of a microscope slide ×360. The white spots are devitrified areas (?). Examination shows that they all occur on the surface, or at least in one optical plane. Obj. 16 mm apo., Leitz; ocular, Homal I, Zeiss; condenser, achromatic-aplanatic, top lens removed, central stop, Leitz; illumination, method I, no filters; camera extension 570 mm; Eastman film, Pan process; developer, D-19.

central stop which may be swung in or out to give bright-field or dark-field effects.

At least one spectroscopic condenser (Zeiss) disperses the light to form a spectrum in the object field. By swinging the mirror it is possible to illuminate the specimen with pure colors.

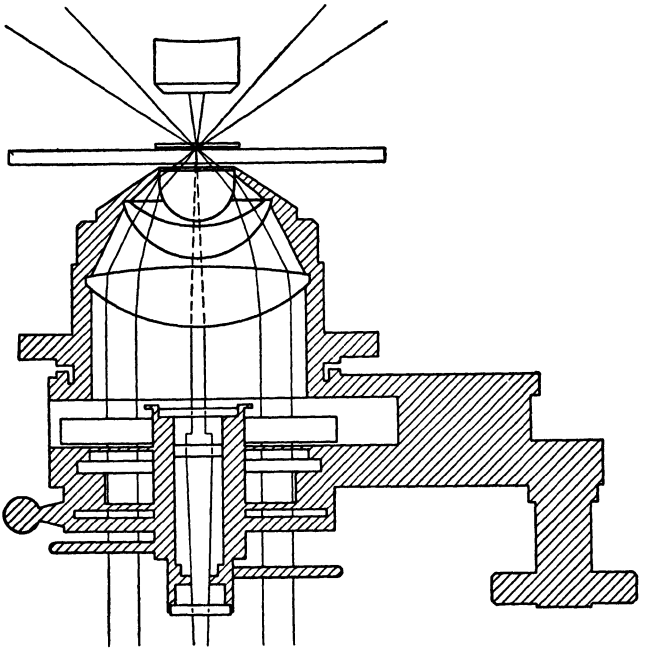


FIG. 148. Polychrome condenser of Zeiss. By the use of two color filters, the transmitted light is of one color, say blue, and the light impinging on the specimen at a great angle is red. The effect on a colorless body is to make it appear one color on a background of another color.

The Wratten-Rheinberg differential color filters[16] are accessories serving the same purpose. The polychromatic condenser by Zeiss is a special development of substage and condenser to make dichromatic illumination possible.

Figure 148 shows how the polychromatic condenser operates. The central rays pass through the center part of the filter disc, these rays forming the background in the field. Assuming the central part to be red and the outer zone of the filter to be blue, the blue light passes through the outer zones of the condenser and is analogous to the light rays from any dark-field condenser. The objects are then illuminated

[16] Eastman Kodak Company, Rochester, N. Y., supplies these filters in the form of discs 33 mm in diameter.

by blue light, and they will appear blue with a red field. The effect is striking, but it is a question whether any additional information can be obtained by such a method of examination. However, in exceptional cases it may give the specimen the contrast necessary for a good picture.

There are special devices that will carry low-power apochromatic objectives and center them with the substage to permit them to be used as condensers. Such use of an objective would seem to have little to commend it, since all the fine corrections of the objective will be vitiated by the spherical aberrations introduced by the slide. In any event, the aperture would of necessity be low, and the specimen would have to be mounted on a slide of cover-glass thickness.

Summary. Useful Facts Concerning Condensers.

1. The first surface mirror obtained by depositing aluminum or an aluminum alloy by vacuum distillation is inexpensive. Such a mirror can be used to eliminate glare or to reflect ultraviolet radiation.

2. The substage equipment has several adjustments, the use of which should be thoroughly understood.

3. A bright field is obtained by transmitted light by means of a mirror used alone or in conjunction with low-, medium-, or high-power condensers.

4. The achromatic-aplanatic is the best type of condenser, but the aplanatic is almost as good, particularly when used with green light.

5. The dark-field condenser may be had in two entirely different types. The most common is formed by means of a central stop with the ordinary condenser of appropriate focal length. All the light is brought to a focus by refraction. The specially made dark-field condensers have one or more reflecting surfaces.

6. The Wratten-Rheinberg system of differential color filters may give added contrast to certain specimens.

7. The condenser should be focused before it is centered.

8. The centration of a condenser is always important, but considerably more so for photomicrography than for visual work.

9. Dark-field condensers of the higher powers should be centered with great exactness.

10. A bright-field condenser should be selected with reference to the objective with which it is to be used.

11. After a condenser of any type has been aligned the source of light must be recentered in the field of view.

12. A dark-field condenser should be selected with regard to the material it is intended to illuminate.

13. The most effective way to fill an objective with light is by means

of a condenser of the proper focal length. This will also increase the light intensity by permitting an aperture of greater diameter.

14. If much work is to be done with a high dry objective, the dry achromatic condenser with a N.A. of 1.0 is recommended.

15. The correct setting of the high-power condenser is easily checked by examining the image of the diaphragm of the light source and the rear focal plane of the objective.

16. Condensers built for immersion oil should be immersed, if the best illumination is to be expected. This is so regardless of the objective.

17. To get good dark-field effects, the sides and cover glasses must be scrupulously clean.

Sec. 96. Cleaning and Selecting Slides and Cover Glasses. *Slides.* The selection of slides presents no great problem. The slight greenish color which may be observed in many slides when viewed edgewise is not harmful. The thickness of the slides should be very close to 1 mm. Since they usually come packed edgeways and half a gross to a box, a quick check for average thickness can easily be made. If a millimeter scale laid over the slides shows that they occupy more than 72 mm, their average thickness can be considered to be more than 1 mm. If half a gross, so measured, total over 80 mm, they should certainly be rejected. The limit for slide thickness is established by the working distance of the condenser. A slide of too great thickness tends to over-correct the condenser. The sensitive, high-power, dark-field condensers cannot be used with slides that measure over 1 mm. Few if any of the best achromatic-aplanatic condensers will focus through a slide that measures much more than that, particularly if the light source is 14 or 15 inches from the microscope.

If an important preparation must be mounted, the slide should be tested for flatness. It should be placed on the microscope stage and pressed gently, first on one corner and then on the opposite corner. Any tendency to rock will indicate that the slide is not flat. After one side has been tested for flatness, the slide can be turned over and the other side can be tested similarly.

The optical qualities of the slide can be tested by reflecting the light from a straight edge. The casement of a window may furnish a straight edge sufficiently well lit. A wavy reflection of the edge of a straight line indicates an uneven surface of the glass slide. Two images of the casement appearing where only one should show indicates that the two faces of the slide are not parallel, but only exceptionally, where the work is very exacting, is it necessary to discard a slide because of such aberrations.

All the slides can be cleaned at one time when purchased, and stored for future use; or they can be cleaned as needed. If the first method is to be followed, they can all be placed in a bowl and washed with a soapy sponge and warm water to which a little ammonium hydroxide has been added. They should then be well rinsed in running water and finished with a little swishing in distilled water. After being wiped with tissue paper once, they can be stored in alcohol until needed. When removed for use, they should dry by evaporation and not by wiping. The treatment with strong acids or cleaning solutions is necessary only for slides for dark-field work.

If the slides are to be cleaned just before use, they are best washed under the tap with warm water and soap, and then either rubbed between the fingers or sponged. After a good rinsing, if a wet slide is tipped, the water should run off in a curtain formation rather than in streaks or tiny rivulets. This is a good test for the cleanliness of any glassware. It practically ensures chemical and optical cleanliness. A further test for cleanliness can be applied when the slide is used by placing a drop of water on it. If the slide is clean and free from grease, the water will flow freely; otherwise it will stay in globular formation. The slide, if clean, can be wiped free of water with one stroke. Toilet tissue, to be used once and discarded, is preferable to even the proverbial soft linen rag and is much superior to facial tissue. The paper, held between the thumb and forefinger, is pressed against both sides of the slide and then pulled over the slide lengthwise. The little remaining water on the corners of the slide will dry quickly, or it can be removed with a corner of the paper. The surface of the slide should not be wiped again before it is used. For general work, the practice of cleaning the slide just before it is needed has much to commend it.

Slides for dark-field work may need special treatment, although occasionally practically flawless work can be done when the slides are simply washed as above. If the treatment is to be more rigorous, a quartz slide is recommended. This slide can be dipped into a very hot solution of bichromate cleaning mixture, swished around or left for several minutes, removed, rinsed in distilled water, finished in alcohol, and allowed to dry, carefully protected from dust. The slide can be suspended for easy handling by twisting a piece of platinum wire around it. The cleaning solution may be any that is in general use in the laboratory, or it can be made by the following formula:

CLEANING SOLUTION

Sulphuric acid, gr. 1.834	400 cc
Potassium bichromate, sat. sol.	250 cc

Care must be observed in making up this solution, as considerable heat is generated.

Figure 147 shows the appearance of the surface of a slide that no amount of cleaning can change. Thus it seems advisable to examine the slides after cleaning and before they are used for dark-field work. If successive slides from the box have the appearance shown in the picture, a new source of supply should be sought.

The size of slides varies considerably, the standard being 25 by 75 mm. The important sizes are the 26 by 45 or 46 mm, for petrographic work; the 25 by 75 mm for most biological and general work; the 50 by 75 mm for occasional use; and the 50 by 114 mm, which is used in dairy and other industrial laboratories. For general classification in merchandising, slides are sorted into three classes, thin, medium, and thick. The uniformity of thickness in each class depends upon the manufacturer, slides classed as thin being about 0.9 to 1.2 mm, the medium 1.3 to 1.5 mm; anything over 1.5 mm would be classified as thick.

Cover Glasses. Cover glasses are best bought by the ounce; they are usually put up in one-half ounce boxes. They may be either round, square, or rectangular, the size of the round cover running from 11 to 22 mm in diameter. In purchasing, preference should be given to the smaller sizes, for they are less likely to have warped surfaces and a larger number can be mounted on one slide. This is quite an advantage in photomicrographic work, since the slide is usually discarded after the picture is taken. When many fields with various mounting media have to be examined, comparisons can thus be easily made. The small square slide is almost as useful as the small round one. The shape is not of much importance unless the mount is to be permanent; then, for purposes of sealing, the round slide is more convenient. For very large specimens, an inch or so long, the rectangular cover is required. It may be obtained up to 60 mm in length and longer on special order.

Since the thickness of the cover glass is a vital factor in good microscopical work, covers are put up in a large range of thicknesses. The various classes under which cover glasses are sold are designated by numbers from 0 to 3, No. 0 being the thinnest and No. 3 the thickest. The thickest may be 0.4 mm or more, and the thinnest may be less than 0.1 mm. Covers included in class 1 are the most useful. The desirable range of thickness for a cover has been determined by the microscope companies as from 0.16 to 0.18 mm, and objectives have, in general, been corrected for such covers. The range of sizes in any one class is large, and for exact work each cover should be measured

before use. Those which fall below standard thickness and those which exceed it are useful only on work where thickness of cover glass makes little or no difference, as in low-power photomicrography. For exact measurement of cover thickness, a toolmaker's micrometer measuring in thousandths of an inch or in hundredths of a millimeter is most convenient.

Covers can be inspected for flatness and aberration exactly like slides. Owing to the thinness of the glass, perfect covers are rare; perhaps more than 50 per cent show plenty of aberration when examined as above. Since the slide is placed between the specimen and the condenser, and the cover between the objective and the specimen, aberrations in the cover are more likely to cause trouble than aberrations in the slide. Like slides, covers intended for exact and critical work should be free from aberration. In oil-immersed systems aberrations are not very important.

The refractive index of cover glasses is far from uniform. For the best work, and to give optical homogeneity when immersion oil is used, the glass should have a refractive index of 1.515, since the immersion oil is standardized at that index. The results of measuring seven covers, Japanese, German, and American, for refractive index are given in Table XXI.

Table XXI

Refractive Index of Seven Different Makes of Cover Glasses

Specimen	n_D
1	1.519
2	1.522
3	1.523
4	1 525
5	1.526
6	1.526
7	1.531
*	

* All the above covers will tend to over-correct the objective if the corrections are based on a cover glass with an index of 1.515.

In most work, either visual or photomicrographic, slight departure from the standard index 1.515 will not be noticeable. However, for the most exacting work at high apertures, the index of the oil and the glass should be about the same.

It is advisable to clean cover glasses just before they are installed. They may be either washed with warm water and soap, as directed for

slides, or it may be sufficient to run a few drops of alcohol on both sides of a cover and wipe it clean with tissue. This operation may have to be repeated several times. For dark-field work the cover can be rigorously cleaned as was suggested for slides.

Table XXII

Dimensions of Cover Glasses as Listed by Arthur H. Thomas Co., 1942

Squares mm	Circles mm	Rectangles mm
12	12	22 × 30
15	15	22 × 32
18	18	22 × 36
22	22	22 × 40
25	25	22 × 50
		24 × 30
		24 × 36
		24 × 40
		24 × 50
		24 × 60
		35 × 50
		35 × 62
		43 × 50
		43 × 70
		48 × 60

THICKNESS OF COVER GLASSES* (slightly more or less)

No. 0 : 0.10 mm
No. 1 : 0.12 to 0.18 mm
No. 2 : 0.18 to 0 25 mm
No. 3 : 0.25 to 0 50 mm

* Taken from Simon H. Gage, *The Microscope*. Measured by Edward Pennock. These measurements can be used only as a rough guide.

For years, the best sources of cover glasses were Germany and Japan. When those avenues of supply were closed the American manufacturers made little effort to supply the demand. The result was the substitution of lacquer or thermoplastics for the glass cover. The medical sciences in particular tried many substitutes, a very satisfactory one being found by Penny,[17] who discovered that a special lacquer made by Canadian Industries, Ltd.,[18] gave good results, did not discolor easily, and had an estimated index 1.4835.

[17] Stuart F. Penny, "A Substitute for Cover Glasses in Mounting Pathological Sections," *Canadian Journal of Medical Technology*, **2**, March, 1940.

[18] Slide Lacquer, BM-397, Canadian Industries, Ltd., West Toronto, 9, Ontario, Canada.

Another substitute for cover glasses, very recently introduced, is made by Hubbs.[19] It is truly a glass substitute, the covers being cut to conventional sizes and used in the customary way. This substitute is said to have an index of 1.528 and to be unaffected by alcohol.

Sec. 97. The Effects of Dirt on Parts of the Optical System. Many defects in photomicrography are caused by dirt and dampness. Small particles of dust or globules of condensed moisture may cast shadows on parts of the microscope image which show as blurs in the finished picture. There are at least seven places in the optical train where dirt may collect, and in four of them it is likely to cause image deterioration. The seven places are:

1.* The eye lens or field lens of the ocular.
2. The mirror.
3. The screens or ground glass, or lamp lens.
4.* The cover glass and slide.
5. The condenser.
6.* The objective.
7.* The specimen, or the mount.

The starred numbers show where the dirt is specially harmful.

Dirt on the Ocular Lenses. Dirt particles on any of the ocular lenses are likely to show in the negative as blurred shadows; even a small speck will make a rather large blurred spot. The remedy is to clean the eye lens, and perhaps the field lens also. This may involve unscrewing the upper or lower lens to permit cleaning on both sides. An inspection of the lens surfaces with a hand magnifier will often locate the dirt. Dirt seems to be especially objectionable on projection lenses.

Dirt on ocular lenses is easily discovered while the ocular is on the microscope. By reducing the iris of the condenser to a small opening, or by raising the tube until the objective is well above its proper focus, the dirt can be seen. Either method will make the light rays passing through the tube more nearly parallel, enhancing the shadow cast by the dirt particle. The ocular can be turned to prove the position of the dirt. Another way to discover dirt is to light up the microscope and remove the objective.

Dirt on the Mirror. Dirt on the mirror can be seen by slightly raising the tube or by inspecting the rear focal plane of the objective. Dirt is generally easy to see in this position if the high-power condenser is in place. Moving the mirror in and out in its socket will bring it to the attention of the observer. The mirror should be kept

[19] Charles F. Hubbs and Company, 389 Lafayette Street, New York City.

clean, but actually dirt on it will not cause image deterioration. However, a very dirty mirror may absorb an appreciable amount of light.

Dirt on the Filters and Diffusing Plate. Like dirt on the mirror, dirt on the various filters and ground glass will not cause trouble unless the condenser is focused on the screen. The proper position of the screen, however, is usually at some distance from any focal plane of the condenser. Dirt on the ground glass at the lamp will cause trouble if the condenser is closely focused on this point, but, since the structure of the ground glass is often eliminated by racking the condenser slightly upward, dirt would likewise be thrown out of focus. With illumination by Method II, dirt on the collecting lens at the lamp may be evident, but it can be thrown out of focus by the condenser or, better yet, the lens can be cleaned.

Dirt on the Cover Glass and Slide. With a long-focus objective, trouble may occur from dirt on the cover glass or the bottom of the slide, for the depth of focus of the objective will be sufficient to give an out-of-focus image of the dirt particle. In a picture, the defect will appear very similar to that produced by dirt on the ocular. With a short-focus objective, the effects of dirt on the cover glass or the slide are not so evident, but since the image formation will be affected the slides and covers should be scrupulously clean.

If a dark field is used, slides and cover glasses must have extra care. Attention is again called to Fig. 147, showing the defective slide. Imperfections of glass cannot be removed by cleaning; therefore slides defective in this respect must be discarded. It may be nearly impossible to make a surface so clean that the dark field will not show some dirt, or to find one entirely free from artifacts; but the slide surface must certainly be very much better than that shown in the picture.

Dirt on the Condenser. Dirt on the condenser has no effect whatever on the image. Naturally, an oily or greasy film over the lens surfaces is undesirable, but it is unlikely that the effect of such a film on the condenser lens would be noticed in the picture. However, such dirt may set up a certain amount of glare, and where extreme resolution is demanded the iris of the condenser may have to be closed more than it would otherwise. A few dust particles on the lens surfaces of the condenser will not, as a rule, cause trouble, but it is always desirable to remove all dirt from all microscope parts.

Dirt on the Objective. The objective too can stand a large amount of dirt before image deterioration sets in, but it is considerably more sensitive in this respect than the condenser. An oily, watery film on the surface of objective glasses will certainly cause trouble, as will large pieces of dust. Glancing down the microscope tube will

often locate any particles of extraneous matter, but it will not show up a greasy film on a glass surface. A hand magnifier is an excellent device for examining the objective, and it should be used frequently. As the upper end of the drawtube is occasionally opened to change eyepieces, dust is likely to settle on the back lens of the objective. Whenever dirt is noticed it should be removed. Specimens under observation with the 16-mm objective are often examined without a cover glass. This frequently exposes the objective to oils or liquids, particularly when much chemical microscopy is undertaken. The front lens of all objectives should be kept clean, but the popular 16-mm objective deserves special notice because of its frequent exposure to liquids on the microscope slide. The method of cleaning the lenses of the objective was described in Sec. 67.

Dirt on the Specimen. Specimens which are poorly mounted will often carry dirt with them. Artifacts, sometimes formed by crystallization or chemical change, may stay attached to the specimen or float off into the mounting medium. Such dirt is very objectionable in a picture. Even if it does not actually cover up an interesting part of the specimen it will detract from the general appearance and may even cause false interpretations. Great care should be taken to have the specimen and the area around it free from dirt. Dirt in this sense might include anything that is not desirable in the picture; even parts of the specimen structure not needed to complete the photographic story are as undesirable as foreign matter. Carefully looking over the slide in order to find a field free from dirt and undesirable material will often make all the difference between a mediocre picture and an excellent one.

LABORATORY WORK

Exp. 1. The Effects of Immersing the Condenser. Set up the microscope for illumination by Method I as shown in the chart. Use a test slide of fine black pigment, such as black magnetic rouge, test slide 2. The pigment should be well dispersed on the slide; see Sec. 155. If the specimen is mounted dry, focus with the 4-mm objective; otherwise, with the 3-mm oil immersion. Have nearly a $\frac{9}{10}$ cone of light. Notice that, when the condenser is used dry, the image of the smaller particles is more or less spoiled by glare, and that when the condenser is immersed the images of the same particles are very much improved.

Before immersing the condenser, study the color fringe around the image of the iris diaphragm of the lamp in the object field. It will be best if the ground glass at the lamp can be placed on the side of the diaphragm away from the microscope. Note the superior image of the diaphragm when the condenser is immersed.

Exp. 2. To Judge the Centration of the Condenser. With the medium-power condenser (if the top lens of the regular high-power condenser is removed, the resulting lens will be of medium power), note the color fringes around the image of the field diaphragm of the lamp. Adjust the condenser so that it will be off center. Note that the color fringe is uneven. Learn to judge the centration of the condenser by the evenness of this color fringe. The usual condenser corrections will demand a direction of the centering motion toward the reddish side of the color fringe; this is followed by tipping the mirror to realign the diaphragm image.

Exp. 3. Controlling the Size of the Illuminated Circle in the Object Field. With the high-power condenser and a low-power objective, focus on a slide such as test slide 3 illuminated by Method I. Close the lamp diaphragm to a small opening so that it can be seen in the center of the field of view. Remove the back lens of the condenser, or use one of medium power. Refocus the condenser, and again examine the field, noting the larger image of the lamp diaphragm. Now insert a spectacle lens condenser, and note that the size of the image of the diaphragm is again increased. The lower the power of the condenser, the larger will be the image of the lamp diaphragm. With higher-power objectives the diaphragm at the lamp can be smaller. Unless otherwise stated, the condenser should always be carefully focused for all experiments.

Exp. 4. Other Methods of Increasing the Size of the Illumination at the Object Field. With the microscope focused on test slide 1 or 3, and using the 16-mm objective and high-power condenser, close the iris at the lamp to an opening to permit the field to be about half illuminated. Now turn in the concave mirror. The field will probably be filled or almost filled with light. Reduce the iris at the lamp to a much smaller opening and notice the shape of the image. It will be elliptical, with the long axis north and south. If the condenser is lowered the long axis of the image will be east and west. Turn back to the plano mirror. Then insert a piece of ground glass in the microscope substage. Notice that the field is again completely filled with light, but the glare is increased. This will be more noticeable with a glary specimen, such as a slide of diatoms.

With the plano mirror, and the lamp diaphragm nearly closed, note the effect produced on the size of the diaphragm image by oculars of different powers. The high oculars tend to enlarge the image so that it may even fill the entire field of view. The same effect, of course, can also be produced by making the lamp distance less.

Exp. 5. Two Methods of Controlling Aperture. With the 4-mm objective focused on test slide 2, illuminate the specimen with the plano mirror without using a condenser. Notice the diffraction rings around the images. Insert a spectacle lens condenser and examine the images again. Notice that they are somewhat improved. Repeat, using a high-power condenser. The image should now be good. Each time a change is made in the method of lighting, inspect the rear focal plane of the objective, and note the aperture of the objective. Optical results as obtained above can be duplicated by using the same condenser throughout and closing the aperture diaphragm as required.

Exp. 6. The Effects of an Uncentered Condenser or Poorly Centered Light Source. If the microscope is fitted with a complete Abbe substage, throw the iris diaphragm out of center by means of the rack and pinion; otherwise, either use the centering screws, or, with a large source of light, tip the mirror slightly. Notice that, as the microscope tube is raised or lowered from its proper focus, the image of a particle in the center of the field sways as it goes out of focus. This is the effect of poorly centered illumination; it is caused by unsymmetrical lighting. Recenter the condenser, or make any other indicated correction, and notice that the image of the particle in the center of the field does not sway as it is racked out of focus.

Exp. 7. Operation of the Iris Diaphragm of the Condenser. Focus a slide of medium-size particles; test slide 1 is about right. Choose the 16-mm objective and a high-power condenser. Fill the field of view with light by inserting a high eyepiece or by opening the diaphragm at the lamp. Close the diaphragm of the condenser to about a $\frac{1}{5}$ cone. Pull the condenser diaphragm to one side and notice the diameter of the opening. Study the field of view. Insert a medium-power condenser; open the condenser iris to a $\frac{1}{5}$ cone. Pull the diaphragm to one side and note the larger diameter of the opening with the lower condenser. Study the field of view, noticing the great intensity of illumination and the greater excellence in image formation. Condenser diaphragms should not be closed too far or image deterioration will result. This is irrespective of the diameter of the light cone as seen in the rear focal plane of the objective.

Exp. 8. The Effects Produced by a Poorly Centered Dark-Field Condenser. With any properly focused and well-adjusted dark-field condenser, and a specimen giving an image by either reflection or diffraction, notice the effect produced as the condenser is slightly shifted out of center. The image is going to suffer, and the unilateral illumination will give spurious effects. Slight differences in centration are much more marked with high-power condensers and very much more detrimental to the image than with bright-field condensers.

Exp. 9. Increased Visibility with the Dark-Field Condensers. If a mount of face powder is made in balsam, the talc particles will be nearly invisible by bright-field illumination, but visibility will be greatly increased by a dark-field method. Make such a test, using both a low-power dark-field condenser, and a high-power one, such as a paraboloid or a short-focus bicentric dark field. Focus on the cover glass and notice the glare caused by dirt; focus on the bottom of the slide and undoubtedly dirt will be seen. The increased visibility given by dark-field condensers can often be used to advantage when a photomicrograph has to be taken of material which gives poor contrast.

A blood smear is a good specimen for examination by dark field. Place a cover glass on a drop of fresh blood and examine it with a fairly high-aperture dark-field condenser; the 4-mm objective will give a good magnification for this object. Notice that the fibrin is invisible by bright field but perfectly visible by dark field. The chylomicrons, although fairly numerous as rule, cannot be seen at all by bright field, but they are perfectly visible by dark field.

Exp. 10. Demonstrating the Proper Use of Oculars. Focus test slide 3 carefully with a 16-mm achromatic objective and a 10× Huygenian ocular. Change the ocular for one of the compensating type and notice that the image has not been improved and perhaps that it is poorer. Try the same thing with a lower-power objective, and notice that the difference is even more marked. Repeat the experiment using a 16-mm apochromatic objective. Try an achromatic objective of high power, 3 mm or higher, and notice that the image then is improved with the compensating ocular. Prove to your own satisfaction that you can use oculars of higher power if they are of the compensating type than if of the Huygenian or some other intermediate type, and that the image formation will sometimes be excellent to the edge of the field.

Exp. 11. To Discover Dirt on the Ocular. (*a*) Focus the microscope on any test specimen. Open the iris of the diaphragm of the condenser until it coincides with the rear aperture of the objective when looking down the microscope with the eyepiece removed. Notice whether or not dirt can be seen when the eyepiece is revolved. Close the iris of the condenser until diffraction lines can be seen around the test object, and revolve the eyepiece again, noting how much easier it is to see the dirt under these conditions.

(*b*) With the iris diaphragm open, raise the tube of the microscope half an inch or more from its proper focus, revolve the eyepiece again, and notice the dirt particles. Remove the eyepiece and clean the eye lens; return the eyepiece to the microscope, and notice the result. Then clean the field lens and examine as before.

Exp. 12. Dust Spots on the Microscope Mirror. Locate dust spots on the microscope mirror, and prove that they are on the mirror by moving it slightly in and out of its socket while you look through the microscope. It will be necessary to raise the tube of the microscope to locate the dust spots, or they can be seen in the rear focal plane of the objective.

QUESTIONS

1. What advantage has a quartz prism over the ordinary microscope mirror?

2. What advantage has the first surface aluminized mirror over the glass or quartz prism?

3. What factors should be considered when selecting a substage condenser for a given job?

4. If the spot of light at the lamp is too small to fill the field of view with light, what steps can be taken to remedy the condition?

5. What is learned by inspection of the rear lens of the objective?

6. How may the objective aperture be filled with light?

7. When should the concave mirror be used?

8. When should the plano side of the mirror be used?

9. With what objectives should the spectacle lens condenser, the medium-power condenser, and the high-power condenser be used?

10. What advantages has the achromatic-aplanatic condenser over the Abbe type?

11. How can the aplanatic condenser be made to give nearly as good illumination as the achromatic-aplanatic type?

12. Describe the principles of dark-field illumination.

13. What is the simplest form of dark field for substage illumination?

14. Is the so-called quick-change-over condenser equally good for dark- and bright-field work?

15. What is the first consideration in selecting a dark-field condenser?

16. In selecting an objective to be used with a given dark-field illuminator, what factors must be considered?

17. Can an objective with an aperture of 1.25 be used with a paraboloid condenser? If so, how?

18. What dark-field condenser would be required by an objective with a N.A. 1.3?

19. What dark-field condenser could be used with good advantage on a specimen of blood? On a specimen of cotton fibers? On a specimen of 600 Carborundum? On a colloid specimen?

20. When is a quartz condenser necessary?

21. On what principle does the use of differential color filters depend?

22. Describe the centering of a bright-field condenser.

23. Describe two different methods for centering the dark-field condensers.

24. What is the preliminary step in centering the condenser?

25. Upon what should the condenser be focused?

26. What effect has slide thickness upon the use of a condenser?

27. How can the image of the structure of the ground glass at the lamp be eliminated when the condenser is focused upon it?

28. In what way does the Ramsden type of ocular differ from the Huygenian type?

29. Are compensating oculars negative or positive?

30. On a basis of magnification, how should an ocular be selected for a given objective?

31. What types of oculars could be used advantageously with the following objectives: achromatic 32- and 16-mm; achromatic 16- and 8-mm; achromatic 4-, 3-, and 2-mm; apochromatic 32- and 16-mm; apochromatic 16-, 8-mm; apochromatic 4-, 3-, and 2-mm?

32. What special care must be observed in using the projection lens type of ocular?

33. What is the special advantage of a projection ocular?

34. Can good results be expected if projection oculars are used in conjunction with achromatic objectives?

35. Is an ocular likely to cause distortion at the periphery of the field?

36. Describe in detail the centering of the microscope revolving stage.

37. Will dirt on the eye lens or on the field lens of the eyepiece show in the finished picture?

38. Name the different positions in the microscope system where dirt may cause trouble in photomicrography.

39. What care would you take to eliminate undesirable effects of ground glass at the lamp?

CHAPTER V

OPTICAL LIGHT FILTERS AND THE CONTROL OF GLARE

The terms filter, light filter, or light screen, as applied to microscopical work, refer to the optical device designed to modify or alter the quality of the light for purposes of illumination. The filter or screen placed in the path of the light beam affects the light passing through it by absorbing some of its energy. If the absorption is equal, or nearly so, for all wavelengths of white light, the filter is called a *neutral filter.* The light is substantially the same color before and after transmission; only the intensity is reduced. If, on the other hand, the absorption is selective, that is, greater for some wavelengths than for others, the transmitted light will appear colored, and the filter is then known as a *chromatic* or *color filter.* If the transmitted light is of only one color, of a narrow band of the spectrum, then all the remaining wavelengths of light will have been absorbed, and the filter is called a *monochromatic filter.* In another form the chromatic filter absorbs the infrared and heat rays from the lamp; this special filter is known as a *heat-absorbing filter.*

The transmission of the above filters is understood to be *specular;* that is, the filters are made of clear glass and light rays pass through them unaltered in direction with respect to each other and without diffusion. If the filter has a ground or matte surface, so that the transmitted light is *diffused* and also partly absorbed, it should be spoken of as a diffusing plate to avoid confusion with the more orthodox clear glass filter. Diffusing plates are placed close to the lamp as a secondary light source. They are usually made from white glass. The best position for filters in the optical train is midway between the lamp and the microscope; this does not apply to a diffusion plate used at the lamp house.

Filters are used primarily for three purposes:

1. To modify and regulate the intensity of the light. Neutral filters and diffusing plates fall into this class.

2. To increase or decrease contrast in a subject by selective filtration of the light. Chromatic and daylight filters for visual and photomicrographic purposes, particularly when the specimen is colored, belong in this group.

3. To aid in the performance of the objective, the eyepiece, the condenser, and the eye. Chromatic filters used when the characteristics of the various lenses can be taken into account, or for increasing resolution, are in this group.

4. To furnish polarized light. This includes the "Polaroid plates." Heat-absorbing, ultraviolet, and infrared filters are all special examples of the above and do not need separate mention.

Sec. 98. Classes of Optical Light Filters. The three important types of light filters, grouped according to their construction, are:

1. Solid glass-plate filters.
2. Filters made from stained gelatin sheets mounted between glass plates, including Polaroid plates.
3. Flat-sided glass cells filled with plain water or a colored liquid.

Solid Glass Filters. Solid glass filters are made, or sold, by most of the microscope manufacturers and by the various companies manufacturing glass. Corning,[1] Jena,[2] and Chance-Parsons[3] are outstanding in this line. It is well worth while to write for the catalogues of these companies.

The Corning filters are available in what is known as a molded finish or with a highly polished surface. The filters with a polished surface are desirable because the reflection at each surface is thereby reduced and the light transmission is increased. Molded, unpolished filters will diffuse the light somewhat, and often this may not be objectionable, as when illumination is by Method I; but for illumination by Method II or III, the polished surfaces are advised. The polishing process raises the price of the filter two or three times.

The glass of which the filters are made is generally of optical quality, although plate glass may also be used. The stock sizes run from about 1½-inch squares to 6-inch squares. The 2-inch size is satisfactory for nearly all microscopical purposes.

Solid glass filters come in a great variety of colors and in glass of a very light blue-green with high heat absorption. If overheated locally, these latter filters are sometimes very fragile, for the glass has a high coefficient of expansion. In use they may be immersed in

[1] Corning Glass Works, Corning, N. Y., or 718 Fifth Avenue, New York City.

[2] Jena Glass Works, U. S. agents, Fish-Schurman Corporation, 230 East 45th Street, New York City.

[3] Chance Brothers and Company, Ltd., 10 Princes Street, Westminster, London, S. W. 1. New York agent, The Ednal Company, Inc., 95 Madison Avenue, New York City. The Chance-Watson light filters are sold by W. Watson and Sons, Ltd., 313 High Holborn, London, W. C. 1.

a water cell, if the occasion warrants. This aids in distributing the heat throughout the glass and so minimizes the danger of breakage. Not all the manufacturers provide neutrally tinted filters, but Bausch and Lomb makes them of glass of several optical densities.

Unlike the filters made from sheets of gelatin, solid glass filters affect light according to both thickness and color. Consequently, the specification for thickness is as important as the color when ordering a filter for a certain purpose; the thicker the filter, the more deeply saturated will the color be. In the Corning filters stock sizes are often carried in several thicknesses. The filter manufacturer should be advised of the light source with which the filter is to be used, and the quality of the light desired. He will then be in a position to recommend the correct filter thickness. The range of thickness of glass filters is from about 1.0 mm to over 10.0 mm. Monochromatic filters are quite thick.

Gelatin Filters. Gelatin filters are made from sheets of gelatin as thin as about 0.004 inch, and stained with appropriate dyes to give the color effect desired. They are known as Wratten filters, after the company which originally made them. They are excellently described in a booklet[4] published by the Eastman Kodak Company, which manufactures them. In addition to more than 100 spectrophotometric curves, this book gives considerable general information about filters for many optical purposes. Gelatin filters are sold either mounted between solid glass plates or unmounted, but the unmounted ones are inclined to curl and are clumsy to use, so that the mounted ones are well worth the very small additional cost.

The gelatin filter is not as durable, mechanically, as that of solid glass. Exposure to heat may soften the sealing cement and permit air bubbles to form; shock may loosen the cement at the edges and admit air; and moisture from the air or water used in cleaning may get between the plates of a mounted filter, causing the gelatin to swell. It is necessary to protect the edges well if organic solvents are used for cleaning. However, Wratten filters have some very important advantages. They have great uniformity of transmission — a quality obtainable only in a lesser degree in the solid glass filter because of the difficulty of duplicating properties of light transmission in glass.

Optical glass of high quality is used for mounting Wratten filters. The B grade is made with plane parallel surfaces, and this is all that is necessary for the most exacting photomicrographic work. The A grade is of thicker glass with an optically flat surface — a type of finish which would be very desirable if the filter were to be placed be-

[4] *Wratten Light Filters,* Eastman Kodak Company, Rochester, N. Y., 1940.

tween the specimen and the objective. But with the microscope the filter can always be placed between the specimen and the light, so that the finish has no effect on image formation. The filters mounted in A glass cost several times more than those mounted in the B glass.

Wratten filters mounted in B glass have a uniform thickness of about 4 mm. As the absorption of light depends upon the degree of stain in the gelatin sheet, only the color of the light desired and the source of illumination to be used need be specified in ordering these filters.

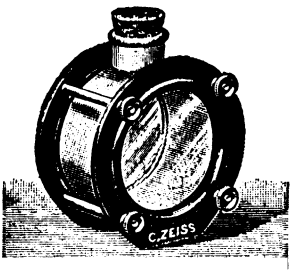


FIG. 149. A filter cell holding about 275 cc of liquid. It is 55 mm thick with an effective diameter of 80 mm. Courtesy, Carl Zeiss.

Liquid Filter Cells. Although liquid filters, or filter cells, are nearly obsolete in modern microscopy, they are useful occasionally for absorbing the longer heat waves from the lamp light and for other special purposes. Used for selective filtration of light, the cells are in their best form when made with plane parallel sides that can be detached. The body of the filter is usually made of porcelain; cross rods with nuts at each end hold the flat glass sides in place. Figure 149 shows the appearance of such a filter cell. The cell when filled with colored water as required makes a good chromatic filter, but it is comparatively bulky; it is hard to place in position without a specially designed holder; and several such cells might be needed, the cost of one of which would buy many glass or gelatin filters. Furthermore, when light of different color is needed the solution must be changed, and considerable space may be required to keep a full range of colored solutions. In order to use any filter scientifically it is necessary to learn its transmission. To learn the transmission of chromatic liquid filters, facilities must be at hand for measuring the transmission spectrophotometrically, or at least to examine it with a spectroscope.

The water cell type of filter has one outstanding advantage, and in that respect it is unsurpassed: it absorbs heat from light. When an arc lamp is used, the liquid filter cell filled with plain water for heat absorption is almost a necessity, since the focused light from the arc may be excessively hot. The ideal way of using such a cell is to place a piece of Corning heat-absorbing glass inside before it is filled with water. This glass is generally so poor a conductor of heat that it might break if overheated locally, but the water will prevent this by conduction and convection, and will tend to keep the filter glass cool. The heat capacity of the glass added to that of the water increases the

efficiency of the whole unit. Another good method is to have the sides of the cell made of heat-absorbing glass.

The small glass filter cells mounted, on some lamp houses, near the lamp lens, are not recommended. The light from the lamp soon heats the small amount of water, partly by conduction through the filter supports and partly by radiation, and certainly such cells are far too small to be of much heat-absorbing value.

If a diffusing plate is used in conjunction with an incandescent source, a water cell filter is unnecessary. If a tungsten ribbon filament lamp is used with Method II of illumination, the heat rise at the object field will not be great. In a recent experiment, using a 250-watt projection lamp and Method I of illumination, without filters, and with a high-power condenser, the rise in temperature due to lamp light was measured in the object field with a mercury thermometer. The bulb of the thermometer was blackened. After a period of 15 minutes, the temperature rise was only 5° C. When the diffusing plate was removed at the lamp, the rise in temperature was 11° C. Such a measurement should rightly be made with a thermocouple, but the experiment is sufficient to indicate that temperature rise at the focal point of the condenser is small when a tungsten lamp is the light source, and that therefore a water cell is not generally required.

A copper sulphate solution with a little ammonia makes a good blue liquid for a filter. For a green solution Hind and Randles[5] suggest 1 volume of saturated copper sulphate solution plus 3 volumes saturated potassium bichromate solution plus a few drops of sulphuric acid. Directions for making solutions of other colors will also be found in the same work, and in addition a table is given showing the color value of the light that such solutions will transmit. It should be remembered that the transmission of liquid filters, like that of solid glass filters, depends largely on thickness.

A Photographic Plate or Film as a Filter. It might be well to mention the possibility of using ordinary photographic material in lieu of a regular light filter. Either the plate or film can be so used. The sensitive material is exposed in steps with a geometrical ratio of 2 for the exposure intervals, then it is developed. After fixing and drying, it can be trimmed to size. The light transmitted by a filter of this sort is much more diffuse than specular. The effect will simulate ground glass except that there is considerable control over density, which, with the ground glass, can be achieved only by means of additional pieces of glass. The film may be dyed any desired color. This technique may be of some advantage if a very large filter is required

[5] Hind and Randles, *Handbook of Photomicrography*, 1927.

for a special purpose. Such a filter would be rather expensive to buy but inexpensive to make in this way. As a rule, only rough approximations can be made of the transmission of such a home-made contrivance, unless rather elaborate apparatus is at hand; but there are times when exactness of light transmission by a filter is more or less immaterial, only a general effect being sought.

Cellophane stained with appropriate dyes would seem to offer a source of cheap light filters. However, the du Pont Company says that their colored cellophane is not particularly stable, and they do not provide it mounted between glass. In addition, the chromatic transmission of different batches of cellophane is apt to vary considerably, and no attempt is now made to standardize it spectrophotometrically. Filters made from plastics, particularly Lucite, may be developed satisfactorily later on, but it should be borne in mind that plastic surfaces can never be made as flat or as optically correct as glass surfaces unless they attain a greater degree of hardness than they possess at present. It would seem that the future use of plastics or cellophane for optical light filters would entail their being mounted between glass plates, as gelatin filters are; otherwise their use will be restricted to rather uncritical operations.

Sec. 99. Theory of the Light Transmission of Filters. If a piece of glass plate 1 mm thick transmits $\frac{9}{10}$ of the light falling on it, the reflected light at both surfaces being neglected, then a plate 2 mm thick will transmit $\frac{9}{10}$ of the impinging light through the first millimeter and $\frac{9}{10}$ of the remaining light through the second millimeter. If the plate is thicker than 2 mm, each additional millimeter of thickness will transmit $\frac{9}{10}$ of the light reaching it. This can be put into the form of an equation as follows:

$$I_t = I_u{}^T \qquad [57]$$

or

$$\log I_t = T \log I_u$$

where I_t represents the fraction of transmitted light; I_u, the transmitted fraction through unit thickness; and T, the total thickness of the transparent substance.

If a neutral filter 2 mm thick transmits, say, $\frac{1}{10}$ of the light, and it is desired to find the thickness that will transmit $\frac{1}{150}$ of the light, then the equation would appear as

$$\log \frac{1}{150} = \frac{T}{2} \log \frac{1}{10}$$

the thickness of the filter would then be 5.6478 mm. Equation 57 applies when the light is monochromatic, also to neutral filters or any transparent optical material having non-selective transmission.

The transmission of two neutral filters used together is the product of their separate transmissions, to a good approximation. Thus, two filters each transmitting $\frac{1}{10}$ of the incident light would, if used together, transmit $\frac{1}{100}$ of the light. This also holds for chromatic filters if transmission of each wavelength is considered separately (always remembering that reflection at each filter surface has been disregarded and that it may amount to 4 or 5 per cent at each filter face).

Optical, neutral filters may be referred to in several ways. They may be rated according to transmission, opacity, or density.

Transmission. A filter that transmits $\frac{15}{100}$ of the light falling on it, in a direction normal to its plane, and absorbs or reflects $\frac{85}{100}$, is said to have a transmission of 15 per cent.

Opacity. The opacity of a filter is the reciprocal of the transmission value. A filter transmitting $\frac{1}{10}$ of the incident light has an opacity value of 10.

Density. This is not to be confused with optical density which refers to the refractive index of a substance. The density number of a filter is the logarithm, to the base 10, of the opacity, or, as often stated, it is the logarithm to the base 10 of the reciprocal of the transmission.

Occasionally a filter is spoken of as passing $\frac{1}{2}$, or $\frac{1}{3}$, etc., of the incident light but as a rule the ratings are based either on transmission values in terms of percentage or on density ratings.

Although usually the above terms are applied to neutral filters, they may be applied to chromatic filters if the transmission of each wavelength is taken separately. Tables sometimes give the transmission of chromatic filters as above, listing the values for important wavelengths.

Sec. 100. Spectrophotometric Curves and Their Use. Chromatic filters are generally referred to in terms of the color of light transmitted by them, but such designation is incomplete. To make classification of color filters more accurate, the amount of light that they transmit at certain definite wavelengths is measured and put into terms of per cent of transmission or density. A curve can then be plotted, with wavelength (generally millimicrons) as abscissa and density or transmission values or both as ordinate. Such a curve is shown in Fig. 150. It represents the transmission of Wratten No. 58, the well-known B filter.

The curve in Fig. 150 is called a spectrophotometric curve. The

dark portion shows the light waves to which the filter is partly or totally opaque; the border line shows the density of the filter for any wavelength. To make such a series of measurements on a filter is the function of the manufacturer. Roughly, it is done by interposing the filter in a beam of light passing to a spectrophotometer. The light is broken up into its numerous component wavelengths, and photometric

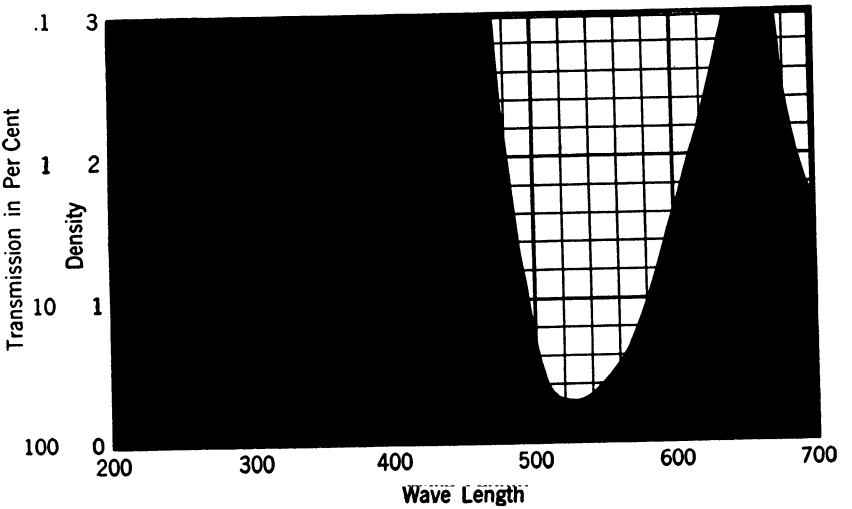


FIG. 150. A typical spectrophotometric curve. It represents the transmission of Wratten filter No. 58, the well-known B filter. Courtesy, Eastman Kodak Company.

measurements are made of the required number of wavelengths at selected points in the spectrum. Thus, to specify a chromatic filter as accurately as possible it is necessary to refer to its spectrophotometric curve.

Figure 151 is a spectrophotometric curve of Wratten No. 96, a neutral filter. The curve is nearly straight with a sharp cut in the blue just beyond the visible range; at this point the absorption of ultraviolet by the gelatin is large. The ideal neutral filter would have a curve, between wavelengths 450 and 700 mμ, parallel to the abscissa, substantially as shown by Wratten filter 96. The ideal monochromatic filter would have a curve somewhat as shown in Fig. 152 (Wratten filter 15 plus 45), but it would be so narrow that only one wavelength of light would pass. At the present time, however, it is impossible to make such a filter, and if it could be made the light intensity would probably be so low that it would be nearly useless for photomicrographic purposes. Every demand is served by making the transmis-

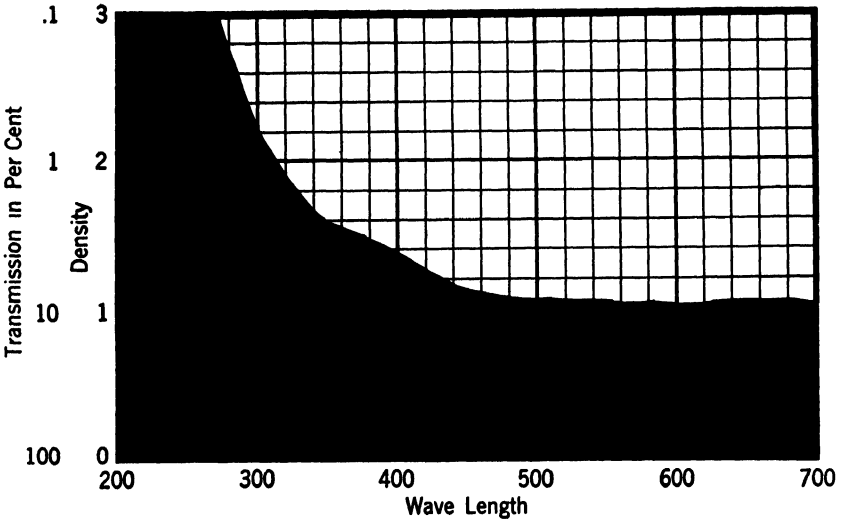


FIG. 151. This curve illustrates the transmission of a neutral filter, Wratten No. 96. It should be noted that the absorption of light is about the same for all parts of the visible range. Courtesy, Eastman Kodak Company.

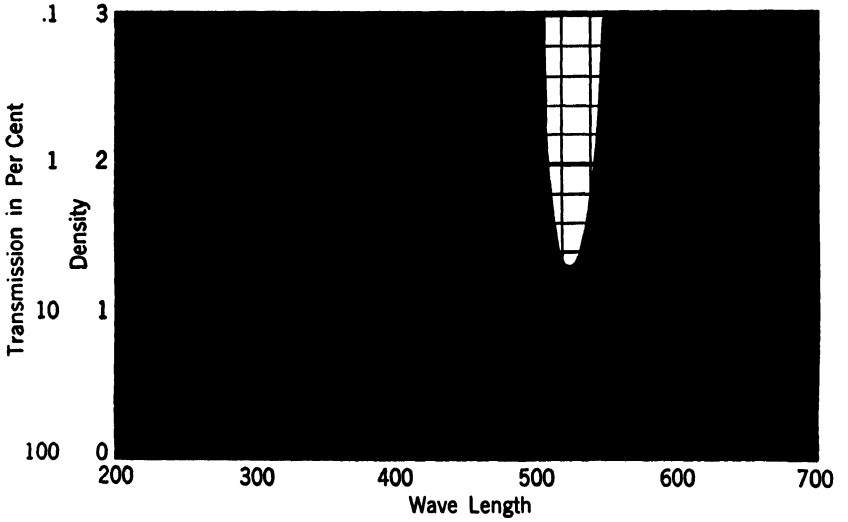


FIG. 152. The spectrophotometric curve of two Wratten filters, Nos. 15 and 45, used together. This combination gives a good approximation to monochromatic green light. Courtesy, Eastman Kodak Company.

sion band of a monochromatic filter only as narrow as that shown in Fig. 152.

Eastman's book on photomicrography[6] should be owned by all microscopists. It gives particularly good suggestions for the use of filters. Belling[7] devotes a whole chapter to the consideration of filters, largely for visual work; he stresses the use of the green filter. Both these books are good collateral reading and valuable for reference. The catalogues with their spectrophotometric curves are replete with information of special value to those considering the purchase or use

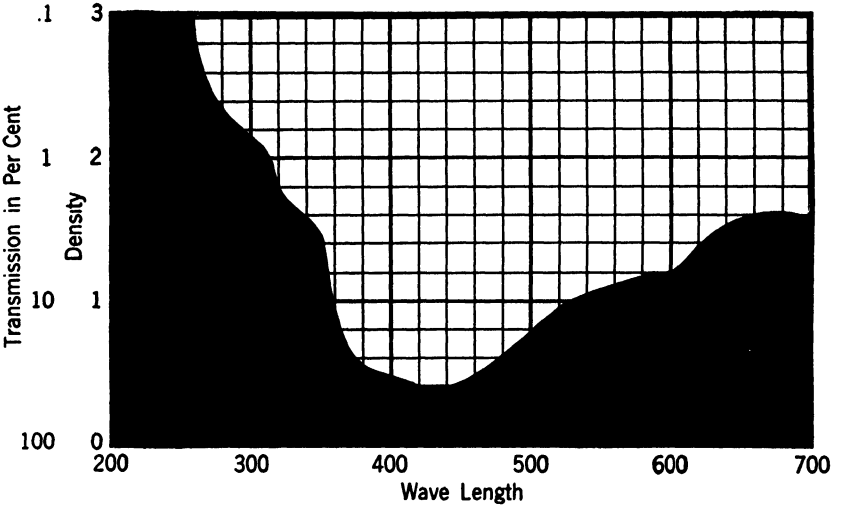


FIG. 153. The spectrophotometric curve of a so-called daylight filter, Wratten No. 78, to be used to give daylight effects from a tungsten source. Courtesy, Eastman Kodak Company.

of filters. They tell in graphic form the results which can be expected from any filter or combination of filters. The Wratten filters 51 to 69 inclusive are especially good examples of the value and use of spectrophotometric curves. The nineteen green filters show widely different characteristics when their transmission curves are studied. If the duty of the green filter is to give as narrow a band as possible, including the mercury green line 546, the selection might fall on Wratten No. 62. On the other hand, such a narrow band might shut out too much light, in which event a study of the spectrophotometric curves would make it possible to select some other green filter having

[6] Eastman Kodak Company, *Photomicrography*, 1941.
[7] John Belling, *The Use of the Microscope*, 1932.

perhaps added transmission in some other chosen part of the spectrum, or one with a wider transmission band.

Quick filter cuts (a point in the wavelength scale where the filter abruptly ceases to transmit light) are desirable in many filters. A filter cut is represented in the spectrophotometric curve when a portion of the curve becomes nearly vertical in certain positions along the abscissa. Figure 152 shows two abrupt cuts. With some filters this might not be a desirable condition. For instance, in neutral filters, the curve should be horizontal, and in daylight ones it should show a slight rise in the red, as shown in Fig. 153.

In the absence of spectrophotometric curves, information about a filter can be gathered from published data, which generally come in the form of a table giving the percentage of light transmission at stated wavelength and also a general overall transmission value. However, this is not of special significance unless carefully studied in connection with the transmission at different wavelengths. The value of such data depends upon the ability of the individual to visualize the stated transmission values in the form of a spectrophotometric curve, this in turn involving experience in studying such curves. With all data at hand, the final test is in the actual use of the filter. Data concerning the light transmission of the specimen are not available; therefore the performance of any filter is, to this extent, unpredictable. A little experimentation with various filters is invaluable.

Sec. 101. Selection, Use, and Care of Light Filters. The diffusing plate at the lamp acts as a neutral filter and serves to decrease the light intensity. If one piece is insufficient to reduce the intensity to a comfortable level, others may be added as required. The glass may be ground on one or both sides and lightly oiled to increase the light transmission and to lessen the effects of the ground-glass structure which may interfere with the image or introduce an undesirable effect in the background.

Either opal or flashed opal glass, obtainable from Semon Bache and Company,[8] may replace the ordinary diffusing plate. The properties of diffusion in the opal glass are due to colloidal particles formed by devitrification or by a component added to the melt. Opal glass should give good diffusion since the diffusion takes place through the whole thickness of the glass, but with flashed opal, formed by fusing a plate of clear glass to a thin layer of opal glass, the milky or diffusing part is on the surface only. This flashed opal produces less color change in the transmitted light than the other variety.

If illumination is by Method II, clear neutral filters must be used.

[8] Semon Bache and Company, 636 Greenwich Street, New York City.

As the neutral filters of Eastman have a very even transmission, several may be used together without disturbance of the neutral quality. Some so-called neutral filters are so selective in transmission that two or more used together will result in light that is noticeably colored; it is usually made reddish. A good test for a neutral filter is to see if there is any tendency to transmit more light in one part of the spectrum than in another; this tendency will usually be cumulative in effect and will become apparent when several filters are used together. This undesirable characteristic seems to be absent with the Wratten neutral filters.

Since the light intensity should be modified entirely by means of filters and not by the iris diaphragm of the microscope condenser, it goes without saying that a set of neutral filters is a very important adjunct to the microscopical outfit. A useful set would have densities of 0.6, 0.9, 1.2, and 2.0.

Neutral wedges, for modifying the light source without introducing a color effect, are satisfactory but rather expensive. Two wedges of neutral quality are used, the thin end of one being placed over the thick end of the other. As the wedges are slid apart in the direction of their long axis, transmission increases. A special stand is necessary to manipulate a device of this sort. It is not recommended for general laboratory work as it is clumsy and expensive, and it will not accomplish anything that cannot be done more easily by providing a few extra filters of the ordinary type.

Next in importance comes the daylight filter. The name is rather meaningless because the filter has no special optical specifications, and, as with all filters which are used chromatically, the results depend partly upon the light source. A daylight filter which gives a good match for daylight when used with a 250-watt tungsten lamp would be entirely inadequate with an arc lamp, or even with the 250-watt tungsten operated at a different voltage. The most satisfactory way to match the light from any lamp with light of daylight quality is to place the microscope and lamp near the window in such a position that the microscope mirror can be used to change the illumination from the lamp as a source to the window as a source. Appropriate filters can then be placed in front of the lamp and its light checked for a visual match with the light obtained from the window. It is seldom, however, that the best match for daylight can be obtained by one filter only; almost invariably several are required.

Daylight from the northern sky is the ideal light to which artificial sources should be matched. As northern sky light changes in quality with the seasons, the time of day, the latitude, and climatic and local

smoky or dusty atmospheric conditions, the exact color temperature referred to as daylight is probably not known for a filter giving daylight quality to the light from a certain lamp. The reader wishing more precise information along this line might consult the papers of Priest[9] and Abbot,[10] or the book by Hardy and Perrin.[11]

As Belling and others have pointed out, long-continued work at the microscope can be carried on efficiently only when eyestrain is at a minimum. Among the best ways to lessen eyestrain are providing light of either daylight quality or slightly green, having all microscope adjustments correct, and selecting lenses suitable for the work in hand. The light of the ordinary tungsten source is rather unpleasing to the eyes, being too strong in the red for comfort. By using a daylight filter in conjunction with Wratten No. 8 or a Corning Aklo heat-absorbing filter about 3 mm thick, two different shades of light can be obtained, one greenish and the other bluish. Even for long periods of work the eyes should not feel tired with light of this quality. These modifications of the tungsten light seem to be best obtained by first matching the light to daylight and then adding another filter, such as the green Wratten filter 66, as mentioned by Belling. This is not a deep green; it would not be satisfactory for photographic work or to aid in the correction of lenses, except under certain circumstances, as for instance to stress a red stain slightly. However, it is an excellent filter for visual work, and it can be used for this purpose intermittently with light of daylight quality during long periods of investigation. After one has worked a short while with light green or light blue filters all sense of the colored background is completely lost. This does not happen if the light is predominantly red.

In connection with the use of green filters for visual work, it is well to point out that the maximum sensitivity for the eye, regarding the chromatic range, is at 555 mμ. This part of the spectrum can be fairly well separated by the Wratten No. 53, and better yet with the No. 54. It might also be noted that No. 62 with a maximum transmission near the mercury green line, 546 mμ, could undoubtedly be used interchangeably with No. 53 or No. 54, without prejudice to the visual sensitivity of the eye.

[9] L. G. Priest, "A Proposed Scale for Use in Specifying the Chromaticity of Incandescent Illuminants and Various Phases of Daylight," *J. Opt. Soc. Am.*, **23**, 41, 1933.

[10] C. G. Abbot, F. E. Fowle, and L. B. Aldrich, "The Distribution of Energy in the Spectra of the Sun and Stars," *Smithsonian Misc. Collections*, 74 No. 7, Pub. No. 2714, 1923.

[11] A. C. Hardy and Fred H. Perrin, *The Principles of Optics*, Chapter IX, 1932.

Apochromatic objectives are well corrected for three colors, and the condenser, if of achromatic quality, is well corrected for two. Under such conditions if a photomicrograph is taken of an object field showing in black and white, little advantage will be realized in using a green screen, unless some other conditions are unusual, as, for instance, a magnification reaching 2000 times the N.A. of the objective. If, however, the entire microscope system is achromatic there is very likely to be a noticeable improvement with the use of green light. Likewise, if an aplanatic condenser without achromatic correction is used with an apochromatic objective, a green filter is indicated. If an Abbe condenser is used with an achromatic objective, a strong green filter will always improve image formation; it will increase the sharpness of the image and reduce the glare. Under these circumstances, resolution will be somewhat greater and objectives of high N.A. can be utilized to better advantage. With low magnifications, from 200 downward, the benefits derived from such a filter are not so marked. If the system is apochromatic, there should be no difference at all in the clearness of the images, whether they are made through green, red, or blue filters.

A green filter for photomicrography should be one that is as nearly monochromatic as possible, as Wratten No. 62 for use with the mercury-vapor discharge tube. This combination would give light with a predominant wavelength of approximately 546 mμ. The Corning filter for the same spectral line would be No. 5; it is actually a combination of three filter elements, Nos. 351, 512, and 430. When ordering Corning filters the lamp in use should be specified, because the Corning filters are made in thicknesses to suit the intensity of the source. The Jena filter that passes the 546 line in great purity is composed of three elements, OG-1, BG-20, and BG-18.[12] Table XXIV lists filters from three optical companies which give essentially monochromatic light with the mercury-vapor discharge tube. If the light source is a tungsten filament, the above combination of filters can be used, but the transmitted light will not be as pure as with the mercury lamp.

When a monochromatic effect is not expected, the Wratten B No. 58 might be chosen, or the combination No. 15 plus 45. All Wratten filters are numbered, and the ones commonly used are designated by letter. The A, B, and C are the well-known tricolor filters, their numbers being 25, 58, and 47. Wratten No. 58 plus 47 (B plus C), the

[12] Elizabeth M. Staats, "The Design of Monochromatic Filters for the Type H-3 Mercury Lamp," *J. Opt. Soc. Am.*, **28**, 3; "An Improved 365 mμ Mercury Monochromatic Filter," *J. Opt. Soc. Am.*, **29**, 221–222, 1939.

No. 45, will give a deep green. Other combinations will suggest themselves. In selecting filters for a narrow range of the spectrum, the spectrophotometric curves are invaluable and should be resorted to frequently. The Corning No. 401, about 5 mm thick, is a good green filter for the tungsten lamp. As many green filters pass some red light, their performance is improved if used in combination with a light blue, such as Corning No. 430 or 428. The Jena VG-2, 2 mm thick, goes well with tungsten light; it gives a nice green at approximately 546 mμ.

In addition to aiding the performance of the lenses of the microscope and the eye, green filters are useful for increasing contrast, particularly of objects which have been stained red. Eastman[6] gives a list of about forty stains with the proper Wratten filter or combination of filters to enhance their contrast, the general rule being to choose a filter complementary to the color of the stain. With the permission of the Eastman Company, a copy of their list is reproduced here as Table XXIII. For objects which are colored naturally, the filters must be selected experimentally, it being remembered that the observed colors may not be always as pure as they seem and that it therefore pays to test several filters or several sets of filters combining entirely different colors.

Filters that pass light similar in color to the object increase the visibility of detail within the object; however, in a black-and-white print, the surrounding background will appear very weak since it will lack contrast with the subject. If the object is large, possibly none of the background will appear; then a filter of the same color as the object may give the best results.

Very small objects are best photographed in blue light. The finer pigments and such material will be more easily resolved, but the individual particles may look smaller, when photographed in the blue range. Figures 154, *A* and *B*, show bacteria photographed in blue light and in light of a daylight quality, the resolution being obviously greater with the blue filter. Objects which have been stained yellow can be given increased contrast with a blue filter, blue being complementary to yellow. Blue light is very important in photomicrography, but it is very tiring on the eyes for visual work. There is always a certain amount of radiation in the near ultraviolet which will reach the retina. The eye lens is "highly fluorescent" (Gage), and although a glass system may shut out all the shorter wavelengths of ultraviolet radiation it will pass a large portion of the longer ones — not enough to cause eye injury, perhaps, but enough to cause a nervous fatigue which increases with the length of the observation.

Table XXIII

Biological Stains*

(Published by permission of the Eastman Kodak Co.)

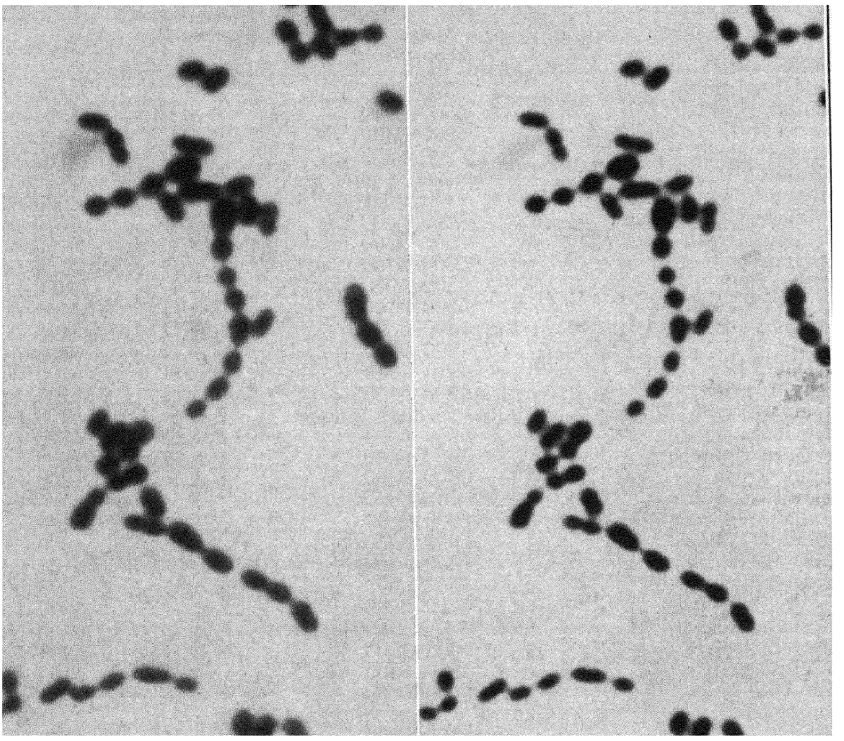
Stain	Spectral Absorption‡	Screens Recommended	Band Used
Acid Fuchsine†	5300–5600	B & G	5100–6000
Aniline Blue	5500–6200	B & E	5600–6000
Azure I†	5800–6400	B & E	5600–6000
Basic Fuchsine†	5200–5500	B & G	5100–6000
Bismarck Brown§	General in blue and violet	C	4000–5100
Brilliant Cresyl Blue	5700–6400	B & E	5600–6000
Brilliant Green	6000–6400	F	6100–6800
Carmine†	5000–5700	B & G	5100–6000
Congo Red	4800–5200	B & E	5600–6000
Crystal Violet	5500–6100	B & G	5100–6000
Cresyl Violet	5500–6300	B & G	5100–6000
Eosine B	4800–5500	G & H	5100–5400
Eosine Y†	4900–5300	G & H	5100–5400
Ethyl Eosine	4900–5400	G & H	5100–5400
Haematoxylin (Ehrlich)†	Gradual through green	B & G	5100–6000
(Heidenhain)	5600–6000	B & E	5600–6000
Indigo Carmine	5600–6500	B & E	5600–6000
Janus Green	5600–6400	B & E	5600–6000
Light Green S. F.	6000–6600	F	6100–6800
Malachite Green	5900–6400	F	6100–6800
Martius Yellow	3800–4500	C	4000–5100
Methyl Green†	6200–6500	F	6100–6800
Methyl Violet†	5500–6000	B & E	5600–6000
Methylene Violet	5600–6200	B & E	5600–6000
Methylene Blue†	6000–6200 & 6500–6800	D & G	6400–6800
Methyl Orange	4300–5000	C & H	4200–5100
Neutral Red	4800–5500	G & H	5100–5400
Nile Blue	5600–6500	B & E	5600–6000
Nigrosine	General with maximum at 5800–6000	B & E	5600–6000
Orange II†	4600–5100	C	4000–5100
Orange G	4700–5000	C	4000–5100
Phloxine	5100–5500	G & H	5100–5400
Pyronine	5300–5600	B & G	5100–6000
Safranine O†	4800–5400	G & H	5100–5400
Sudan III†	General in blue and green with maximum at 5000	G & H	5100–5400
Sudan IV†		G & H	5100–5400
Thionine	5600–6100	B & E	5600–6000
Toluidine Blue	5500–6500	B & E	5600–6000

* The most commonly used stains are marked with a dagger. Gentian Violet belongs to these stains, but it is not officially recognized by the National Commission on Standardization of Biological Stains. It is a poorly defined mixture of violet rosanilins, nearly synonymous with Methyl Violet. The same filters as recommended for Methyl Violet may be tried.

‡ The spectral absorption of the stained object may differ from that of the stain in watery or alcoholic solution. Such object should therefore be investigated separately.

§ When photographing by transmitted light for insects and yellow sections generally, photograph for *contrast* with a C filter; for detail in the section with an F filter.

Such a condition is more marked with the mercury lamp and less with the tungsten. Corning makes a special glass for absorbing the ultraviolet radiation completely; it may pay to use it in conjunction with



A *B*

FIG. 154. *Streptococcus salivarius* ×3600. *A*. Some of the cells are not completely resolved; they all appear hazy. This picture was taken with light of daylight quality. *B*. The resolution here is much better than at *A*; the cells are better defined and appear smaller than in the first picture. A strong blue filter was used. Objective, 1.5 mm apo. Zeiss; ocular, Homal IV; condenser, achromatic-aplanatic, Leitz; illumination, ribbon filament lamp, method II; filters, Bausch and Lomb daylight plus a neutral filter density 0.6, for *A*; Wratten No. 45 plus 47 for *B*; Defender film, Fine Grain Pan; developer, D-19.

other filters when separating lines of the mercury spectrum. Wratten 2A and Corning Noviol No. 306 will cut off the 365 line, and Corning No. 038 will permit the use of the 436 but not the 405 line.

Comparatively pure ultraviolet radiation can be obtained from the mercury-vapor lamp by means of Wratten filter 18A, Corning filter

986 plus 428, or Jena filter BG-10 plus UG-2 plus a 1-cm water cell. The 365-mμ line is obtained with any of these combinations. It is an important wavelength because it passes through most kinds of glass, and fluorescent effects can be obtained with it for photomicrography or for visual work. Figure 66 illustrates the lines available in the mercury-vapor spectrum, filters being available for separating the important lines with considerable purity.

Infrared separations may be made from heterochromatic light. They are important for certain classes of photomicrographic work. Insects, metal surfaces, dark-colored foodstuffs, and other materials often require infrared radiation for their illumination; that is, they naturally reflect or transmit the long wavelengths so much more freely than the short waves that detail is enhanced on the photographic plate when infrared radiation is resorted to. One of the old classics as a specimen for microscopists is the tongue of the blowfly; when this is photographed with infrared the result will be better than when other lighting is used. Wratten No. 87 is suggested for such work. Long exposure is required, but the picture will be taken actually with infrared and not with just a deep visible red. The Eastman Company furnishes prepared plates and films for various ranges in the infrared, for all parts of the visible spectrum, and for ultraviolet, each emulsion having the maximum amount of sensitivity for the range of radiation it is designed to handle. The range of the infrared plates is from about 800 mμ to 1200 mμ. A booklet[13] on infrared materials, and another on emulsions for scientific photography,[14] published by the Eastman Company, are well worth owning, as is the work of Clark[15] on infrared. A good source for infrared is the 250-watt tungsten lamp. Figure 221 shows the surface structure of a piece of steel. Ordinary lighting recorded no surface details at all, but with the No. 87 filter and a long exposure the result obtained was as shown.

Table XXIV illustrates the various filters that may be used with the mercury-vapor discharge tube to effect a separation of the useful lines. The various important Fraunhofer lines are given by letter, and the nearest corresponding wavelengths in the mercury spectrum are listed in the third column. The remaining columns give the appropriate filters of various manufacturers which will separate the desired lines.

[13] Eastman Kodak Company, *Infrared Photography with Kodak Materials*, 1940.

[14] Eastman Kodak Company, *Photographic Plates for Use in Spectroscopy and Astronomy*, 1937.

[15] W. Clark, *Photography by Infrared, Its Principles and Applications*, 1939.

It may be noticed that although green and blue light has been discussed in detail little has been said of other colors. Other colors are used primarily for contrast effects, but the greens and blues are of special importance because they aid in the critical formation of the

Table XXIV

Filters for Separation of the Important Mercury Lines

Fraunhofer Line	Angstrom Units	Color	Mercury Line, angstrom units	Wratten Filter	Corning Combination Number, Filter Element, and per cent Transmission	Jena Filter
A	7600	Red				
		Red	6908		#2 241 50%	
B	6870	Red				
C	6563	Orange	6234	25	#3 428 245 7.7%	
D	5893	Yellow	5790	22	#4 348 430 2.2%	VG–3 BG–18 OG–2
E	5270	Green	5461	62 or 77 or 77A or 15 + 45	#5 351 512 430 14.1%	OG–1 BG–20 BG–18
F	4861	Blue	4916	45 + 47	#6.1 338 503 5.4%	
G	4308	Blue	4358	50	#7 038 511 21.8%	BG–2 BG–7 Corning 038
H	3968	Violet	4047		#8 306 597 428	GG–13 BG–12 Corning 597
		Ultra-violet	3650	18A	986 428	BG–10 UG–2

The combination of Corning filters is listed by number in the catalogue; their percentage of transmission is also given.

The Jena list is taken from the papers by Staats to which reference has already been made.

A 1-cm water cell should be added for use with all the Jena combinations.

microscope image and in resolution. Trials should be made with various filters or combinations of filters to determine which will make the images appear at their best. Often with bacteria a blue-green combination is superior to either color alone. If panchromatic sensi-

tive material is used for photomicrography without filters, the final photographic contrast will be very close to that observed by the eye.

In connection with photomicrography it is well to remember that because filters usually have low transmission values they may increase the time of exposure tremendously. A filter used to give ultraviolet radiation to promote fluorescence might extend the exposure from several seconds to several hours. This increase in time is not so marked with filters within the visible range.

The filter factor is the number by which the normal exposure time is multiplied to determine the correct exposure time when the filter

Table XXV

Filter Factors

Film	Filters								
	A 25	B 58	C5 47	G 15	H 45	B + G 58 15	B + H 58 45	C5 + H 47 45	G + H 15 45
East. Tri X Pan.	2	6	10	2					
Panatomic X	3	8	10	2					
Ortho X		4.5		3					
Pan. Proc.	5	14	9	3.5	16	40	500	32	480
W. & W. M (plate)	38	6	11	1.7	14	8	95	40	135
W. & W. Met. (plate)		5	6	4	8	9	80	28	105
Agfa Triple S Pan.	3	8	5	1.6	15	18	190	48	750
Iso Port.	7	11	7	1.6	15	22	380	64	722
Super Plena. Press		6	6	3	6	16	96	22	270
Process					7			18	
Defender Fine Grain Pan.	4.5	6	14	2.2	24	16	600		1000
F-X Ortho Press		4	5	3	10	8	200		300
Pentagon		9	4 5	9	8	24	450		1000
Proc. Pan.	4 5	6	14	2.2	30	16	200		800

is added. A few filter factors are listed in Table XXV, but, because of the impossibility of reproducing film emulsions with identical sensitivities, they are only approximately correct. Each box of film usually includes its own table of filter factors.

It is best to purchase optical filters with some specific purpose in mind rather than to attempt to cover a wide range of colors, some of which may prove not particularly useful. The most convenient neutral and daylight filters have been mentioned. The selection of others should depend upon the type of work to be done. If much work is to be done with finely ground material affording discrete particles for examination, about three blue and three green filters would be indicated; for work of a histological nature such filters as the Wratten

Nos. 15, 45, 47, 58, 25, 8, and 38 would be a wise selection. Table XXIV lists filters giving substantially monochromatic light with the mercury-vapor tube. If the laboratory is devoted largely to petrographic work, filters which can separate the C, D, and F lines would be of first importance. If careful measurements with a micrometer scale are required, the Wratten filters 22 or 62 may be used, or the Corning filters 348 plus 430. These will give a good strong yellow or green when used with tungsten as the source. For refractive-index work, measurements will probably be made with light of daylight quality or with filters giving either the C, D, or F line. Wratten filter 66 has already been mentioned as excellent for miscellaneous visual work; it should always be included in the filter box. For tricolor work, the A, B, and C Wratten filters are necessary. For other color work the filter and illuminant will probably be suggested by the manufacturer of the plates in use.

The chromatic quality of the film must be suitable for the quality of the lighting arrangement employed. Many times in laboratories red filters may be seen in use with orthochromatic film, the intention being to enhance the detail in a red object. This is impossible, but a red filter used with panchromatic film will give good detail in subjects stained red, while a green filter will yield the maximum amount of contrast. For the most part, it is wise to use panchromatic film, relying on the filters to produce the required contrast or detail as needed. At any time, the effectiveness of panchromatic film in rendering color can be reduced to the level of orthochromatic or regular film by simply subtracting the red or the red and green components of the light by means of filters, but orthochromatic film material cannot be given the characteristics of panchromatic film by any combination of filters.

Filters are just as important for low-power as for high-power work. The ideal position for them, between the lamp and the object, may be difficult to arrange when several lamps are in use. The natural inclination is to place them between the object and the lens, as in macrophotography, but this is not good practice because it produces over-correction of the lens, as already shown. In ordinary scenic photography, the rays on the object side of the lens are practically parallel and the aberration produced by the filter glass is therefore less than it would be for photomicrography.

When an exposure calls for several lamps and only one set of filters or one filter holder is available, the lamps can be switched on in succession, and the filters moved to each lamp in turn, until the exposure is completed. Background lighting can be white light, as a rule, regardless

of the color of the incident illumination. The exception might arise when it is desirable to use a colored background to contrast with a colored transparent object. The specimen might then be placed directly on the selected filter, which could be Eastman Wratten or a Corning polished glass. Sometimes the microscope stage is employed for low-power work; the transmitted light can then be controlled in the usual way, with the filter in the path of the illuminating beam.

The Cleaning of Filters. The cleaning of the solid glass filter requires no special care. Any of the solvents can be used, but generally warm water and soap is all that is needed. All manner of abrasives should be kept away from these and all other types of filter, and when the filters are not in use they should be kept in the dark.

The Wratten mounted filters should be kept where it is dry and moderately cool. They usually come from the manufacturer in small boxes which are ideal receptacles for them. These filters need special care in cleaning. Liquid, particularly hydrocarbon solvents, should be used sparingly and kept away from the binding at the edges. Envelopes of proper size will store those which are not provided with cases. The gelatin sheets can be conveniently stored in a small negative booklet and kept in a dry, cool, and dark place.

Examination for deterioration due to fading or other causes can be made by the Eastman Company for a special fee, or, if a hand spectroscope is available, the transmissions can be compared roughly with the curves in the filter book.

Some important facts concerning filters may be summarized as follows:

1. There are three distinct types of filter: glass, gelatin, and liquid.
2. The transmission of two or more superposed neutral filters can be determined with accuracy if the percentages of their transmission are known.
3. Liquid filters are valuable mainly to absorb heat and radiation when an arc lamp is used.
4. The filter is introduced into the path of the light beam in such a position that its optical corrections are not of paramount importance.
5. The transmission of a filter is stated in terms of percentage, fractions, or density.
6. The selection of chromatic filters can be based on the study of the spectrophotometric curves and on visual inspection of their effects on the specimen.
7. The neutral filter can be replaced by one or more diffusing plates at the lamp when the illumination is by Method I.

8. Filters other than diffusing plates should not be mounted at the lamp house.

9. Three or four neutral filters of varying density should be available for regulation of light intensity.

10. Eastman's book on Wratten filters has especially complete filter data; a copy should be in every microscopical laboratory.

11. The four important uses of optical filters should be thoroughly understood; see p. 364.

12. Light of daylight quality, and light green, are easy on the eyes for long periods of observation.

13. Satisfactory illumination by monochromatic light can be obtained by using the indicated filters, and the mercury-vapor discharge tube as a source.

14. The poorer the optics of the microscope, the more importance should be attached to the use of the deep green filter.

15. Filters of the same color as the object will give the greatest degree of transparency in the image. Filters complementary in color will give the greatest contrast.

16. Blue light should be used in photographing very small particles.

17. Ordinarily, chromatic filters are best selected by trial. If the specimen is colored, various filters should be experimented with, and the one giving the best optical effect should be selected.

18. All filters should be kept in a dark, dry place when not in use.

19. The filter catalogues of the various glass companies give a considerable amount of data of general interest.

Sec. 102. Causes, Effects, and Control of Glare. It has been said that glare is light out of place. In a microscope system, where light must be handled with precision and exactness, extraneous light from the surroundings or light which has got off its appointed course within the microscope is very likely to cause trouble. Light reflected from the sides of the microscope tube, from glass surfaces, cover glasses, and so forth, may find its way to the eye or to the photographic plate, where it will register as a light spot, or, as is more common, it may veil the image with an undesirable haze that will inevitably mask detail and lessen visual and photographic contrast.

Glare can be conveniently classified according to the source whence it arises, and recognition of the source will generally suggest the remedy.

The following may produce glare:

1. Many lens surfaces. Glare from this cause is known as lenticular glare.

2. The cover glass, or the surface of the microscope slide.

3. A light source that is too large.
4. Diffusing plate placed too close to the microscope.
5. Lack of proper optical light filters.
6. The ordinary second surface microscope mirror.
7. An improper adjustment of the iris diaphragm of the microscope condenser.
8. The improper adjustment of the microscope condenser, or a poor or uncorrected condenser.
9. A condenser not suited to the objective.
10. An improperly prepared specimen.
11. Maladjustment of tube length, an under- or over-corrected objective.
12. Extraneous light from the lamp or other light source.
13. Light reflected from the inside of the camera bellows or from the microscope tube.
14. Halation. Likely to be a cause when plates and not film are used.
15. A diffusing plate at the lamp.

Glare may affect the field of view or the image on the film in any or even all of the following ways:

1. The image may be flooded with light and visibility reduced. A flat negative or a very weak field of view may result.
2. Small detail and even small particles may be rendered entirely invisible.
3. Color effects may be made uncertain.
4. Unequal distribution of light over the film or plate may give a spotty or uneven appearance to the negative.

Lenticular Glare. This type of glare is always present; it is caused by the unavoidable reflection of an appreciable amount of the incident light at each lens surface. An aggravated form of lenticular glare is exemplified by certain photographic lenses when the reflected light may appear on the negative and show in the print as white spots or flares, sometimes known as " ghosts." In microscope objectives, lenticular glare is, as a rule, so weak in comparison with the focused light that it is scarcely noticeable; glare from the ocular is negligible.

With a constant light source the total amount of light flux reflected from the various lens surfaces to produce glare will depend upon the obliquity of the surfaces to the incident rays — the refractive index of the glass — upon the number of the surfaces involved, and thus, in general, upon the N.A. of the lens. The remedy indicated is to use

lenses of lower power and lower N.A. Since but little lenticular glare arises at the ocular, most changes should be made at the objective. Substitution of an achromatic objective for an apochromat is suggested, or the use of an objective of lower power in conjunction with a higher ocular, if it is necessary to hold the magnification to a certain value. On the other hand, when an objective such as the 4-mm apochromat is used, glare may be reduced by changing to a 3-mm immersion lens even though it has a higher N.A. The reason, of course, is that the immersion oil forms an optically homogeneous system from the condenser lens to the front surface of the first objective element. If for any reason it is desirable, the N.A. of the 3-mm may be reduced to that of the 4-mm with a small diaphragm, or stopping down the condenser may suffice.

Vertical illuminators set up glare which falls into the lenticular class. The prism, mirror, or glass plate which is inserted into the path of light directly over the objective will always produce glare. Poorly prepared specimens not only contribute their own share of scattered light but they also increase that of the illuminator and objective; consequently the image is weakened. It should be noted that, with vertical illuminators, the light is passing through the objective in two directions, and naturally the tendency for an objective to form glare is increased under such conditions.

With the source constant, the intensity of the focused light on the specimen is lessened when the glass plate is used, thus proving that light losses due to the plate are greater than with the prism or mirror, and much of this dissipated light becomes a source of glare. The simpler vertical illuminators seem to give the least glare; those made without an accessory lens are generally the most satisfactory. In all cases, there should be a diaphragm to limit the size of the spot of light on the reflecting surface of the illuminator, and it should act as an aperture diaphragm for the objective. In actual practice it is difficult to install such a diaphragm in the proper position and it seldom acts exactly as it should for either a field or an aperture diaphragm, yet, in spite of that, with the vertical illuminator, glare is largely controlled by such a diaphragm.

Lenticular glare is seldom bothersome at apertures of less than 0.65. If present to an objectionable degree when the lower powers are being used, it is more likely to arise from some cause other than reflection at the surface of a lens.

The work done by Blodgett[16] on thin films, and her original studies relating to the destruction of reflected light by interference may lead

[16] Katharine B. Blodgett, *Science*, **89**, 60, 1939.

to important developments[17] regarding the control of glare in the microscopical field. High-aperture lenses, if treated for glare by the application of a film only a few molecules thick, might increase the resolution of the microscope on certain material by making it possible to work at an aperture higher than had heretofore been thought possible. It seems particularly feasible to decrease the glare set up by the vertical illuminator systems by the application of thin films.

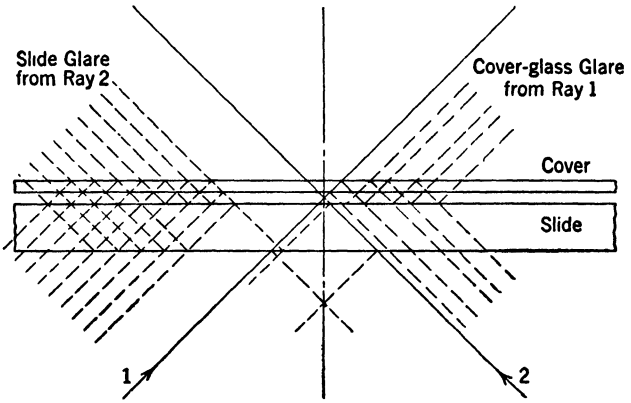


FIG. 155. Diagram showing how glare originates at the surfaces of slide and cover glass. Rays due to reflection are shown as broken lines. For the sake of clarity the reflections are shown as the result of only two light rays striking the glass surfaces. The greater the angle of the light cone, the greater the glare is likely to be, particularly in a dry system.

Methods are now being perfected to form these films on glass by chemical treatment. They seem to give substantially the same effects[18] as the molecular films formed by Blodgett. The chemically formed film may be silica, the index of which is relatively high, and the thickness is about the same as the Blodgett films. The principle underlying the making of such a film is that the refractive index of it shall be the square root of the index of the glass on which it is applied, and that its thickness shall be $\lambda/4$ of the light with which it is to be used. In fact, photographic lenses can be obtained already coated, for certain types of work.

Glare from Cover Glasses and Slides. Figure 155 shows how glare may be started at the under surface of the cover glass when the prepara-

[17] Editorial, "Coated Lenses Bring New Beauty to the Screen," *Ind. Eng. Chem., News Edition*, **18**, 3, 1940.

[18] F. L. Jones and H. J. Homer, "Chemical Methods for Increasing the Transparency of Glass Surfaces," Paper read before the Optical Society of America, Abs., *J. Opt. Soc. Am.*, **30**, 654, 1940.

tion is mounted dry. The reflected light might travel from the cover to a particle, lighting up what would otherwise be a black boundary line and so making it grayish in appearance. Thus contrast is lost, and very small detail may be completely obliterated. When the particles themselves have a high reflecting value, cover glass and slide glare is most pronounced. When examining fine pigments that have a refractive index around 1.55, the tendency is to mount them dry because (from a standpoint of visibility) many small particles may be lost to view if a mounting medium is used. If the specimen should set up undue glare when mounted dry, this can be reduced only by changing to a liquid mount, such as alcohol or monomethyl glycol for a very low index medium, methylene iodide for a high index; other media and melts are suggested in Chapter VII.

Glare from the under side of the microscope slide can be controlled by immersing the condenser. However, since light may be reflected from either slide surface the best condition for its elimination is attained when the condenser and objective are immersed and the specimen is mounted in a liquid with a refractive index similar to the immersion liquid. There is then entire optical homogeneity from the condenser to the rear surface of the front lens of the objective. Although the condition of optical homogeneity reduces glare due to reflection, and is very desirable for good imagery, it also reduces visibility on a transparent specimen to zero, and it is therefore practical only if the specimen is highly colored or very large.

Glare caused by reflection from the surface of the slide is at its worst, or rather it is more noticeable, when dark-field illumination is used. In fact, if the slide is not optically clean, on both the top and bottom, enough glare may be set up to ruin entirely any attempts at observation. The cover must be free of all dirt, grease, and anything that might deflect and scatter the light. For many dark-field effects when the specimen is mounted dry, the cover glass can be omitted; if it is possible to do this, it is always good procedure, especially for photomicrography. Section 96 describes a method for selecting and cleaning a slide for dark-field work. If these instructions are carried out properly, glare, which is always present with dark field, will be at a minimum. For high-power dark-field work, it is frequently necessary to use the completely immersed system; the magnification may be unduly high, but this can hardly be avoided, and the results may justify it. When examining a specimen expected to be colloidal, it is absolutely necessary to eliminate every last vestige of glare from the cover glass and slide, as far as is possible. Otherwise it may completely

mask the disperse phase and a colloidal specimen might easily be falsely reported as non-colloidal.

Glare from too Large a Light Source. When illumination is by Method I, glare may be set up because of the large size of the light source. For low-power objectives the source must, in general, be larger than for high-power work, and when the top lens is removed from the condenser, the 16-mm objective can stand considerable reduction of the diaphragm at the lamp before the image of the diaphragm encroaches upon the field of view. If the maximum aperture at the lamp diaphragm is 2 inches, it is generally possible to reduce the aperture 1½ to 1 inch in diameter when the 16-mm objective is used under the above conditions. The high-power lenses with the high-power condenser can fill the field of view with light when the diaphragm at the lamp is only a few millimeters in diameter.

If the camera is used with bellows extension of, say, 50 cm or more, it is generally possible to close the iris at the lamp to an even smaller aperture than when the microscope is used for visual work and yet have the image field completely lit. This adjustment has two advantages. Not only does it reduce the size of the spot of light in the object field, but it also shuts off a large amount of light that would otherwise illuminate the inside of the camera.

For visual work, the diaphragm at the lamp can be closed until it affords illumination for only a selected part of the field of view. A circular patch of light in the center of the field will then be seen where the desired portion of the specimen can be placed and studied with minimum interference from surrounding light.

If illumination is by Method II, the same reduction of the source field is often desirable. However, irrespective of the method of illumination and the amount of glare present, the diaphragm at the lamp should never be larger than is required to fill the field of view with light.

Diffusing Plate too Close to the Microscope. Ground glass has the effect of dispersing the light passing through it and making it appear very similar to light from a white cloud. If the source is some distance from the microscope, the light will have become scattered by the time it reaches the microscope mirror. Since the mirror is comparatively small, and the diametrical aperture of the condenser is yet smaller, the light that is actually used will be traveling nearly parallel to the axis of the condenser. This is a desirable condition. If a diffusing plate is placed directly under the condenser, in the ring carrier, or if a substage lamp is used in conjunction with the diffusing plate, the

more oblique rays from its surface will find their way into the microscope condenser. Illumination is then by completely scattered and diverging light. This form of illumination gives rise to glare. As the diffusing plate is moved away from the microscope, the glare decreases noticeably. With a glary specimen, it can easily be shown that there is a constant decrease of glare due to the diffusing plate as it is moved from the microscope to a point approximately 15 inches from the mirror.

Occasionally it will be found necessary to dispense with the diffusing plate entirely and to illuminate by Method II. This recourse, to rid the image of the last vestige of glare, is, as a rule, required only when the magnification is high.

Chromatic Glare from the Lack of Proper Filters. On stained or naturally colored subjects, glare can often be controlled by means of the proper filters. Not only are delicate histological sections and small weakly colored particles made more contrasty (visible) by the use of appropriate filters, but also glare is lessened. If the microscope is achromatic and white light is in use, an indefinite amount of unfocused light due to chromatic aberration will be present; it might be styled "chromatic glare." Generally, it is negligible, and the strong white light with the dark boundaries of the particles will make it unnoticeable. Occasionally, however, the specimen serves to accentuate this type of glare and it must then be dealt with.

For all practical purposes a deep green filter will improve chromatic glare. If the color of the object is such that a deep green illumination cannot be used, a strong yellow, blue, or red can be tried, with the idea of filtering out or absorbing the unwanted colors. Modern objectives, even achromatics, are so well corrected that they handle light of nearly the full range of the visible spectrum exceedingly well, and all their corrections can be utilized to good advantage when the light is monochromatic. If the system is apochromatic, a filter similar to Corning 430 or 428, which absorbs the red rather effectually, tends to reduce glare. The intensity of the illumination is not seriously interfered with when such filters are used and exposure time is but little increased. In like manner, the blue end of the spectrum, or any other section of it, may be absorbed. A few trials will quickly determine the most advantageous part of the spectrum to use for any given specimen or set of optics.

The Use of a First Surface Mirror to Control Glare. In Sec. 87 mention is made of a first surface mirror or a prism as a means of overcoming glare caused by the secondary and tertiary images of the light source in the regular type of mirror. Figures 135 and 136 illustrate the appearance of these reflections and show just how the source may

be imaged in the object field by the condenser. Figure 136 shows the ideal condition: only one image is present and no part of the specimen is illuminated except that directly under the microscope objective. Glare that is started under conditions as shown in Fig. 135 may be rather weak and seldom noticed, but if the best conditions of illumination are to prevail only one image of the source should be present in the object field. If the specimen is of very finely ground material and the ultimate in resolution is to be attained, the first surface mirror is practically a necessity.

The Use of the Iris Diaphragm of the Condenser in Respect to Glare. A source of glare that is seldom neglected, and the remedy for which is very much overworked, is too large an aperture of the condenser diaphragm. To close down this diaphragm is frequently one of the first steps taken to reduce glare. As a matter of fact, this is one of the last steps that should be taken, because reducing the aperture of the condenser reduces the aperture of the microscope as a whole, and resolution is lost in proportion to the closing of this diaphragm. Only after all other steps have been taken to control glare is it correct procedure to close down the condenser iris to obtain the proper contrast.

In the directions given for setting up the microscope for visual work, the closing of the condenser iris to a $\frac{9}{10}$ cone is mentioned as one of the last adjustments, and for visual work, where the iris can be constantly adjusted to suit successive portions of the specimen as they are scrutinized, it is correct to use it in this way. When the microscope is set up for photomicrography and every part of the field must be registered simultaneously and under optimum conditions, then one setting of the condenser iris must answer for the whole field and the excellence of the resulting picture depends very much on this setting. Therefore, a $\frac{9}{10}$ cone of light should be attained whenever possible, and all pains should be taken to eliminate glare by other methods before closing the condenser diaphragm excessively.

It might be noted, with benefit, that closing the condenser diaphragm reduces not only glare due to less obliquity of the light cone but also that due to all the first ten causes mentioned. Undoubtedly it is for this reason that closing the aperture diaphragm has come to be popularly regarded as a sort of panacea for all glare troubles. Great obliquity of light on the specimen naturally tends to cause glare, but, if the $\frac{9}{10}$ cone is taken as a desired achievement and is to be used, all other sources which give rise to glare must be taken well into account and corrections must be applied.

Improper Adjustment of the Condenser. This refers to the correction and centration of the condenser as well as to its focus. If the

condenser is out of focus the objective may not be entirely filled with light. The illumination striking the specimen and passing to the objective will be more nearly in the form of parallel rays when the condenser is above or below its proper focus. A method has been given for testing the correction of a condenser. Unless properly corrected a condenser will produce glare, particularly if its position is such that the ring of light formed by under- or over-correction can be seen in the rear focal plane of the objective. With high powers a poorly adjusted condenser produces a relatively large amount of glare, for the aplanatic cone is much reduced in size as the condenser is thrown out of adjustment. In preparations susceptible to glare, there is a visible difference between the effect produced when the condenser is in a state of proper adjustment and when it is not.

The centration of the condenser should be as nearly perfect as it is conveniently possible to have it. When considerably off center, it may set up unilateral illumination. Although this may or may not be the direct cause of glare, it may give false appearances and be the source of false interpretations. The centration is so easily checked and corrected that an uncentered condenser is an inexcusable error.

Since glare produced by condenser maladjustments is not easy to distinguish from other forms of glare, it is always safe to assume its existence at high magnifications when the condenser is used uncorrected, unfocused, or not centered.

The Use of a Condenser Not Suited to the Objective. Unless low-power condensers are used with low-power objectives, unnecessary glare is sure to result. When the high-power condenser is adjusted for use with the 16-mm objective, the diameter of the condenser iris diaphragm will appear very small. This condenser is intended to be used habitually with a larger stop, since the small stop will cause a large falling off in illumination, and also glare will not be reduced as much as it would be with a longer-focus condenser.

Glare Occasioned by the Improper Mounting of the Specimen. It frequently happens that most of the glare present is occasioned by improper preparation of the specimen. Clays, abrasives, flours, precipitates, chemicals, pigments, starches, and foodstuffs are all extremely inclined to reflect a considerable amount of light and so to cause glare. Clearly, the remedy indicated is to have the particles as well dispersed as possible, and to select a mounting medium with a refractive index as near to the specimen in question as can be used without impairing visibility. The preparation of material is an extensive subject, and detailed descriptions for mounting specific material will be left to a

later chapter. The point to be stressed here, in respect to glare, is that, when it arises in the object field from the specimen itself, a great deal can be done to lessen it. A field with fewer particles, suitable refractive index of the mounting medium, the reduction of the field diaphragm at the lamp, and the use of proper filters are the main points for attention. The surface characteristics of the specimen will influence the degree of glare produced by high apertures or any of the other factors mentioned. Actually, nearly all forms of glare are aggravated if the specimen has high reflectivity.

The size of the specimen particles is also an influence on the amount of light reflected from them. As an instance, large crystalline particles produce glare by dispersing the light incident upon them, largely by reflection; the amount of light reflected varies with the surface structure of the particles, the angle at which the light is incident upon them, and the reflection factor of the substance. As the average size of the particle becomes smaller, the amount of light causing glare by reflection is reduced for any one particle, but the number of particles and the total amount of surface that may reflect light and cause glare are substantially increased. Thus glare is increased by reduction in the size of the particles, and this continues until the particles are so much reduced in size that their diameter approaches or is less than a wavelength of light. At this point glare begins to lessen, from the causes which have already been mentioned, and diffraction and scattering of light begin to play an important part. Interference rings (the formation of which was outlined in Chapter III) have been formed at the expense of light which should have been used entirely on image formation of the central disc. Since usually there are several such rings, not all of which may be visible, the general result will be glare. When the particle becomes submicroscopical in size, it appears entirely as a diffraction disc as in work with colloids. On yet further reduction, the particle approaches the size of the molecule, which is its limit. It has then no marked effect on light except to retard or absorb it. Under certain conditions, molecular scattering of light takes place. The blue of the sky, formerly considered to be caused by scattering of light by dust, is now known to be largely due to molecular scattering.

Diffracted light from small particles is noticeable only under special conditions as when the aperture of the microscope is very low in relation to its magnification. High apertures and strong lighting make it difficult or impossible to see the rings caused by diffraction because the adjacent rods and cones of the eye are irradiated by strong back-

ground light, and the weak image of the diffraction ring is lost. Thus, it is often possible to record, photographically, diffraction effects and faults in illumination which would not otherwise be visible.

Improper Tube Length. When the tube length is not correct for the objective, the objective will be under- or over-corrected, and glare will be produced. Particular care must be taken when the 4-mm N.A. 0.95 objective is used. The correction collar or the tube length must be carefully adjusted to suit the prevailing conditions; otherwise the image will be veiled with glare. In fact, glare from this source is of the worst kind. It may actually make particles that should be visible become completely invisible. With the 16-mm objective it may be difficult to establish proper tube length within 10 to 15 mm, but with a 2-mm objective a difference of even 1 mm in tube length is generally noticeable. Section 65 deals with methods for making tube-length corrections.

Stray Light. Extraneous light from any source whatever, falling on the specimen or on the front lens of the objective, may cause glare; and stray light on the microscope mirror, if of sufficient intensity, may find its way into the optical system and cause trouble. The main precaution to take is, of course, to shield the microscope from all strong adventitious light. Figure 177 shows how the stand can be protected from light from the lamp by means of interposed screens. Small screens as shown can be set up around the microscope while an exposure is being made. A lamp hanging from the ceiling may cause glare which will appear as a bright spot in the ocular when the microscope is used for visual work. This glare disappears if the light is moved to another location.

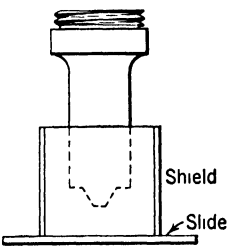


FIG. 156. Shield for objective.

If the back lens of the condenser is removed for work with the low-power lens, the substage may be racked down so low that, in many microscopes, light will be admitted between the clamping ring which holds the condenser and the microscope stage. As one looks downward, it should not be possible to see stray light which may strike the microscope slide. If such light can be seen, it can be blocked out by screening with a piece of black paper. The piece of paper, about 3 by 5 inches, should have a hole cut in the center and then be slipped over the condenser holder to block off effectually stray light from below.

A small collar can be placed around the objective to shield the specimen from direct light from a window or lamp. This collar also aids in preventing dust from settling on the specimen during the time

the specimen is on the stage. See Fig. 156. Another useful shield is made by cutting a hole a millimeter or so in diameter in a piece of black paper about 2 inches square. Placed over the specimen it will be found quite effective in blocking out stray light.

Glare from within the Camera. When the bellows extension of the camera is long, the cone of light from the ocular may strike the sides of the bellows considerably below the plate holder; for the distance between the plate holder and the point where the light strikes the bellows, the sides of the camera become a source of light of low intensity which is reflected to the plate or film. This light, when it strikes the film, will often be completely overpowered by the stronger focused light and no harm may result, for the folds of the camera bellows act as a baffle to absorb some of this light. However, a certain critical condition may arise when the intensity of the focused light forming the images on the sensitive material is so low that the light from the camera sides causes flooding and glare and thus reduces the contrast of the negative. It might be thought that, as the focused light on the film becomes less intense, the scattered light from the sides of the bellows would do the same. This is true as a rule in ordinary pictorial photography, but the photomicrographic picture may be of, say, fibers, with low light transmission. Therefore, irrespective of the background lighting, which causes the reflection on the bellows, the exposure must be long because the light passing through the fibers, on which exposure depends, is weak. Thus, the light useful for taking the picture may be relatively weak, and yet the reflection on the camera sides may be strong.

It is easy to control such a condition. A shield can be placed in the camera bellows in a position to block off the side light and yet not interfere with the light passing to the film. A piece of black cardboard with a rectangular hole, of the same proportions as the plate or film, cut in it can serve as the shield. Care should be taken each time the bellows are shortened or lengthened that the shield does not interfere with the image on the ground glass.

Halation. Inasmuch as halation is " light out of place " it can certainly be classified as glare. It is caused by light reflected from the back surface of the plate or film, forming a second image or a halo around the first image. Halation is often seen in ordinary photography when a picture is taken of an interior showing a window. The strong light from the window may be outlined by a soft nebulous effect. Modern plates, and to a greater extent film, have generally overcome this trouble; nowadays, they can be had with a backing to absorb the superfluous light which has made its way through the emulsion, the

small amount left to be reflected back to the sensitive area of the film being negligible. Figure 157 shows the course of the light rays when halation is present.

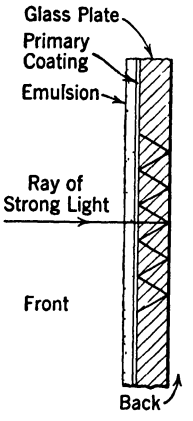


FIG. 157. Halation is caused by the reflection of the light passing through the silver salts emulsion and striking the back of the plate or film, where it is reflected back to the emulsion.

Photomicrography is similar to ordinary photography when the camera is pointed directly at the sun. Under these conditions halation is at its maximum. However, in photomicrography the illumination falling on the sensitive material is of very low intensity; it may not exceed 1 foot-candle or it may even be considerably less. The high magnification accounts largely for the normally low illumination level at the film. The high N.A. aperture of an objective, giving great light-gathering power, is measured in the object space, the N.A. in the image space being very low by comparison.

Plates and film specially treated to reduce halation are prominently marked "anti-halation." On account of their thickness unbacked plates offer more opportunity than films for light to spread out before it strikes the emulsion for the second time; therefore they cause greater halation effects. However, there need be very little trouble from this cause in photomicrography. It is something to be understood rather than feared. In photomicrography literature, formulae have been given for making backing with which to coat plates, but with the abundance of modern sensitive material now available homemade coatings are practically obsolete.

Control of Glare by Selecting the Method of Illumination. Under Sec. 18 it was stressed that Method II or III will afford better resolution than Method I, since the glare is less. When the specimen demands the utmost resolving power the slight glare introduced by the diffusing plate at the lamp becomes objectionable and must be overcome. Illumination by Method II is recommended for this purpose. The nucleus of living yeast cells will become very clear by this method of illumination particularly when a blue filter is employed.

Summary. In photomicrography there are at least fifteen sources from which glare may arise, and its effect depends upon its intensity. With high-power large-aperture objectives, either dry or immersed, control of glare plays an important part in securing a clean, brilliant negative. Low-power objectives are less sensitive than high-power

ones to conditions which may produce glare, partly because with the low power the image is so much stronger than the glare that it is not materially affected. In seeking to control glare some of the essential points to remember are:

1. The greatest source of glare probably lies in the specimen and its preparation. Improper tube length, any of the various condenser adjustments improperly made, and ground glass inserted too close to the microscope are of importance in about that order.

2. The iris diaphragm of the condenser should not be used to control glare arising from other sources. If photomicrographs are to be attempted, the sharpness of the image on the film will depend to a large extent on the aperture of the microscope system. A $\frac{9}{10}$ cone of light is always to be desired.

3. With the camera arranged for photography, all adventitious light should be blocked out, particularly from around the microscope stage.

4. Trouble may arise from strong extraneous light when using low powers.

5. Method II should be used for illumination when the last vestige of glare must be removed and the highest resolution is desired.

6. Modern plates and films are nearly halation proof. There is some advantage in using film rather than plate material.

7. Filters afford an important control over glare, and their use should always be considered.

LABORATORY WORK

Exp. 1. To Demonstrate Increased Visibility and the Lessening of Glare When a Blue Filter is Used. Use the slide of titanium dioxide or red rouge, test slide 5 or 2. Focus sharply with a 4-mm or an 8-mm objective. Have the objective well corrected. Have diffusing plates at the lamp or neutral filters to cut down the light intensity. Under these conditions notice the maximum aperture which can be employed, the amount of glare present, and the general visibility of the smallest of the particles.

Insert filters to simulate daylight. Compare the effect so obtained with and without these filters, always maintaining the same light level.

Insert a monochromatic green filter; it may be Wratten 62 or Wratten 15 plus 45. Compare the effect of the 62 with the effect of the combination. Make the comparisons on the smaller particles.

Insert a monochromatic blue; any selection from Table XXIV will answer. Notice that the smaller particles are now easier to see, the glare is less, and the contrast is greater than before.

Exp. 2. Proof that the Distance of the Diffusing Plate from the Microscope Influences Glare. Use test slide 2 and the 4-mm objective. Set up illumination for Method I. Have light of daylight quality, and the diffusing plate

in the ring carrier of the substage condenser. Adjust for a 9/10 cone of light or larger. Notice the glare on the specimen. This test might be repeated with test slide 5 or 6.

Move the diffusing plate away from the microscope and toward the lamp, in steps of 3 or 4 inches at a time. When the diffusing plate is at the lamp the glare should have become negligible; naturally the contrast will have increased.

Exp. 3. The Use of Chromatic Filters to Increase or Decrease Contrast. Use the 16-mm objective. Place test slide 4 in the field of view with one edge of the specimen showing.

Examine the specimen when only the diffusing plate and neutral filters are used. Next, cut in a daylight filter. Notice that there appears to be but little difference in the relative color values of the lines; try to keep the level of the illumination about the same as the filters are changed.

Insert the red filter Wratten 25. The most noticeable effect of the addition of this filter will be the almost complete loss of contrast between the background and the red lines. With the red filter, the green which formerly appeared more brilliant than the blue will now approach black; the blue will be lighter in tone than the green and will give a distinct sensation of redness. The blue lines appear reddish because they are passing an appreciable amount of red light and are not a pure blue. If Corning filter 348 is at hand, insert it in place of Wratten 25. Notice that although this filter is red also it gives a substantially different effect; the blue lines take on more of a red cast than before, and the green lines appear not nearly so dark.

The above series of filter changes shows how easily the contrast of colored specimens may be controlled by means of chromatic filters, and also why it is advisable to have numerous filters of the same general color, and why the trial method is necessary. Contrast produced by means of complementary colors is stressed, and detail is increased in a given section by using light of the same color as the section under observation.

If the Wratten filter 58 is inserted, the red lines will still show a decided trace of red and both the blue and green lines will appear greenish. Look at the spectrophotometric curve of filter 58, and note the transmission of red near the 800-mμ line which accounts for the showing of the residual red in the red lines. Insert Corning filter 430 with Wratten 58, and note the complete removal of the red; the red lines will be black and at the same time the green lines will be vivid, but the contrast between the background and the green lines will be nil.

In like manner, study the effects produced by blue light and by filters of other colors. Observe particularly the changes that can be made in contrast by using relatively weak filters, that is, those with a wide range of transmission. Notice the effect produced by heat-resisting glass.

If there is difficulty in remembering slight difference in color as filters are changed, the two filters to be compared can be held together, edges abutting, then moved together into position so that each filter affects about half the field of view. In this way it will be possible to note simultaneously the effects produced by each.

Exp. 4. To Demonstrate Glare Produced by an Uncorrected Lens. Use test slide 5, of titanium dioxide, and the 2-, 3-, or 4-mm objective. Have the objective perfectly corrected for tube length. Use a rather large cone of light, and notice that as the tube length is extended the glare is increased.

If blue light is used, it should be possible to detect an increase in glare on a change of tube length of 5 mm or even much less. This is true with apochromatic objectives; with achromats the distance may have to be doubled. This is a very striking experiment when performed with the 4-mm objective equipped with a correction collar. Unless the lens is well adjusted certain small particles may be entirely invisible.

Exp. 5. To Demonstrate the Reduction of Glare When Illumination is by Method II. Using test slide 5 and a high-power oil-immersion objective, illuminate the microscope by Method I. Arrange appropriate filters to obtain a good image. Change the illumination to Method II.

The comparison of the two methods of illumination will show that there is less glare by Method II.

In the experiments on glare, high-power objectives have been indicated because glare is a serious problem when examining fine detail. Often, on large particles, although much glare may be present it may go unnoticed because of the size of the images and because of the greater image contrast and general brilliance.

In this experiment it will probably be convenient to set up the illumination first as for Method II and then simply add a diffusing plate to obtain Method I.

QUESTIONS

1. What are the three important classifications of optical light filters?
2. For what are liquid filters especially suited?
3. What is the chief characteristic of a neutral filter?
4. What is shown by the spectrophotometric curve of a light filter?
5. If a filter passes 17 per cent of the light incident upon it, what is its density value?
6. Can monochromatic light be obtained by selective filtration of white light?
7. With the Wratten system what filter or combination of filters would pass sodium light in substantial amount? The spectrophotometric curves in the Wratten filter booklet may be consulted.
8. What is the advantage of using monochromatic light?
9. What would constitute a good set of neutral filters for general work with the 250-watt tungsten projection lamp?
10. What is a good quick test for neutral filters to determine the regularity of the transmission to light of different wavelengths?
11. Is the thickness of a filter of importance? If so, when?
12. Do Wratten filters need any special care?
13. What should be guarded against in storing all filters?
14. Do filters fade?
15. Over what range of wavelengths can filters be used?
16. What is a good color to use in long optical examinations?

17. How can light of daylight quality be obtained?

18. In selecting a filter for monochromatic qualities, is it necessary to consider the source with which the filter is to be used?

19. What is the best position for the filters?

20. Describe the best method for using Corning heat-absorbing glass.

21. What is meant by using a filter for contrast?

22. On what basis is a contrast filter selected?

23. Should sensitive material be considered when filters are selected for a particular photomicrograph?

24. If neutral filters are not at hand, what may be substituted?

25. What are the three important uses for filters?

26. What is meant by lenticular glare?

27. What is the reason for preferring a first surface mirror to the regular second surface mirror?

28. Describe what precautions you would take to avoid glare from the lamp or other sources?

29. Does mounting medium affect glare?

30. What is the effect of a diffusing plate placed close to the microscope?

31. What size would you make the source in order to avoid glare?

32. What care is required to avoid glare when mounting finely divided powdered material?

33. To what extent would you reduce the diaphragm of the condenser to avoid glare?

34. List all the factors that may produce glare in the microscope system.

35. What is the effect of N.A. on glare?

36. Does the cover glass produce glare? If so, how can you prove it?

37. Why should a green screen aid in the production of a better image?

38. Would you use light filters to reduce glare?

39. What effect does glare have on a photomicrograph?

40. What effect does a totally immersed system have on glare?

41. What is probably the greatest source of glare?

CHAPTER VI

CAMERAS, PHOTOSENSITIVE MATERIAL, FORMULAE AND PHOTOMICROGRAPHIC TECHNIQUE

The microscope and the illuminating system can be considered the most important parts of the photomicrographic apparatus, if it is possible to regard any essential part as more important than another. If these two components of the equipment are adequate and properly adjusted, the film or plate which receives the photographic image may be held in position in the crudest sort of way and the resulting picture be no less perfect than if it were taken with an expensive camera. However, although crude, makeshift camera apparatus undoubtedly can be made to work, the operation and assembly will usually consume much time and may call for an outlay of a surprisingly large amount of money. The smoothly operating, neatly designed camera outfits now available are most satisfactory, and as a rule they are well worth the price asked for them.

The camera equipment is fundamentally simple. It usually includes the bellows, or light-tight box, a holder for the plate or film, a front board carrying a light trap to fit over a female portion attached to the microscope drawtube, and sometimes a shutter. The support for the whole is generally a steel post fixed to a heavy cast-iron base. This post may be horizontal or vertical, or adjustable to either position. On some outfits, a vibration-damping device is used. Lenses are not generally part of the regular equipment, since either the microscope or microphotographic lenses are used in place of the camera lens, and shutters are not usually included.

Less recent books speak of using an ordinary camera for photographic work. Good results can be obtained with this equipment. The optical principle of such a combination depends upon the fact that the image-forming rays from the microscope ocular are made substantially parallel; consequently the camera lens will form an image from such rays at its focal plane when the camera is focused for infinite object distance. It has been shown by Hardy[1] that, when a camera is so arranged independently of the microscope, vibration of the camera will

[1] Arthur C. Hardy, *J. Soc. Motion Picture Engineers*, **17**, 216, 1931.

not blur the image on the film. This may be of considerable value in cine-photomicrography.

To a large extent, the amount and range of the photomicrographic work to be attempted should act as a guide in the selection of a camera for the microscope, regardless of whether it is bacteriology, petrography, metallography, textile, or any other work. If large numbers of routine photomicrographs are to be taken for record, a camera permanently connected with a microscope set aside specially for the purpose may be used, or perhaps one of the eyepiece cameras taking 35-mm film will be found very convenient. In a metallographic laboratory, one of the completely equipped metallographic camera outfits is most desirable, particularly if speed in the completion of a large number of photographs is demanded. If the work is of a general nature, a vertical camera 5 by 7 inches, with long bellows extension, will probably be found most convenient. Microphotographic lenses can be used with it for low-power work.

Sec. 103. Cameras. *The Vertical Camera with Bellows Extension.* Figure 158 shows the vertical camera. The base is heavy, being made from cast iron. The camera post is of stainless steel graduated in millimeters. The bellows extension is about 20 to 24 inches, which is hardly sufficient to permit the covering of a 5 by 7 inch plate because the height of the microscope is included in this figure. It should be noted that, when a vertical camera with extension bellows is listed in a catalogue, the makers often disregard the height of the microscope. To determine the useful bellows extension, the height of the exit pupil of the microscope should be measured from the table on which the microscope stands. The front board of the camera should be adjusted to this same height from the camera base. The bellows can then be fully extended and measured to find the effective bellows extension.

As a rule, cameras of this type are made so that the microscope can be used in either the horizontal or vertical position. They should be so arranged that the bellows can be swung aside to permit visual examination to be made without disturbing the microscope. The camera post is mounted on a short support on the cast-iron base and equipped with a clamping device. Loosening this clamp will permit the post to be lowered to a horizontal position and locked in place. The camera is equipped with a front board holding part of a light-tight fitting for the microscope. The other portion of the fitting is slipped over the drawtube of the microscope as shown in Fig. 159. The base can be made to support a low stand for macro objects when long-focus lenses are used.

Shutters are sometimes mounted on the front board of the camera

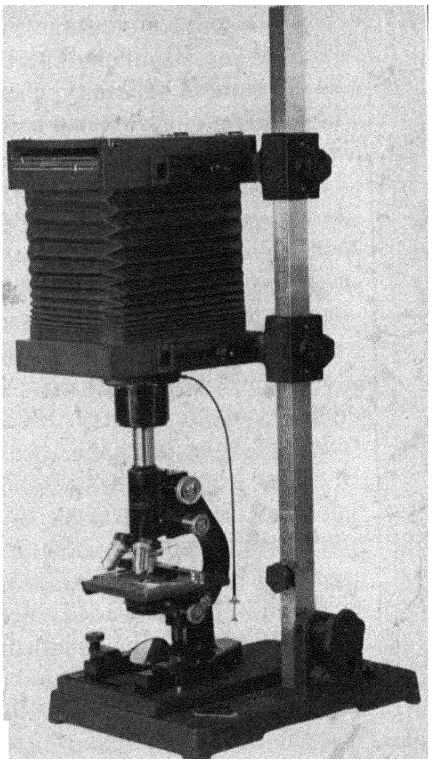


FIG. 158. Bausch and Lomb camera, type H. One of the most practical all-around cameras made. Although the post and bellows are too short, these can be lengthened on special order. The stock camera, in 5 by 7 in. size, will fall far short of covering a 5 by 7 in. plate satisfactorily. Courtesy, Bausch and Lomb Optical Company.

and a light trap fitted to the shutter, but the usual method of making the exposure is to place a dark slide or opaque black card in the path of the light, between the microscope and camera. The dark slide is pulled out at the film holder and the exposure made by removing the card. For some seconds, before removing the card, the plate will be free to receive what reflected light it

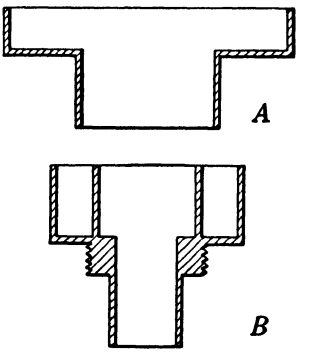


FIG. 159. A light-tight fitting for microscope and camera. Part *A* is attached to the camera front board and fits into part *B*, which is attached to the microscope tube.

may from windows or the other room-lighting sources. The exposure will have commenced, but the use of screens or baffles will help control the effect of this unfigured exposure time. With high-power objectives, the surrounding light will not be strong enough to cause much trouble, but it should be guarded against carefully when low-power microphotographic objectives are employed.

A camera of this type is very adaptable and is a well-nigh universal instrument. The bellows extension makes it possible to vary the magnification from that obtained on the microscope to two to

four times this figure. The fact that it may be adapted to film sizes from 3¼ by 4¼ inches up to 5 by 7 inches is a great advantage. A large 6½ by 8½ inch or 18 by 24 cm camera of this type can be had from at least one firm; it is equipped with a 40 inch bellows. Nearly all microscope cameras are provided with plate holders, so sheaths will have to be bought separately when cut film is used. Small sheaths are available for film smaller than the holder is made to carry. Kits can also be utilized to convert a plate holder to one of smaller size. However, kits do not hold the sheaths securely in place, and the method recommended by Beals[2] is to lay the sheath in the center of the plate holder and fasten it in place with scotch tape or surgical tape. This method is very satisfactory; it forms a solid and rigid fastening. Figure 160 illustrates how the sheath lies in the plate holder. For use with full-size film, regular film holders can be had in place of the plate holders accompanying the camera.

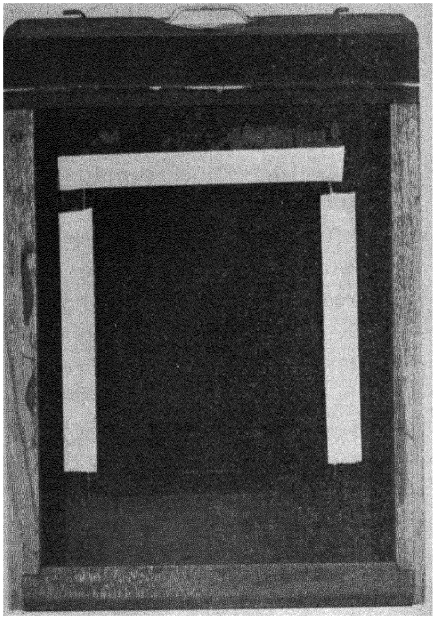


FIG. 160. A 5 by 7 in. plate holder equipped with a 9 by 12 cm film sheath The sheath is held in place by surgical or scotch tape. The suggestion of Mr. A. Tennyson Beals.

With a free camera extension of 20 to 24 inches, measured from the microscope exit pupil, only very short-focus oculars will cover a 5 by 7 inch film, from corner to corner, with a sharply focused image. With an ocular such as Zeiss Homal I (focal length 20 mm), an extension of at least 570 mm, or 22½ inches, will be required, and a further extension will be needed for Homal II (focal length 70 mm). It might be noted that Homal I, one of the most useful projection oculars ever made, has been replaced by Homal VI (focal length 37.5 mm); therefore the bellows extension required for this projection lens, in order to cover a 5 by 7 inch film evenly, would be more than 22½ inches. The eyepieces used for visual work can scarcely cover a film of this size be-

[2] A. Tennyson Beals, 5833 85th Street, South Elmhurst, Long Island, N. Y.

cause, to obtain it, the bellows extension would have to be so long that image deterioration would result. If such lenses are used a circularly diaphragmed print that should be expected. See Chapter I, Sec. 6, and experiment 3 of that chapter, also Sec. 112 on the selection of magnification and size of the finished picture.

Improvement can be made in stock equipment as ordinarily supplied by having the camera post lengthened by about a foot and the bellows extended to correspond. This extra extension may be the cause for a little additional vibration, but probably it will not be troublesome, and with the added length there will be no difficulty in obtaining complete coverage of the 5 by 7 inch film.

Negatives of the 5 by 7 inch size offer greater possibilities in processing than smaller ones; contact prints can be made for reproduction; enlargements can be made of the whole negative, or only parts of it, as required, or the whole picture can be reduced. A camera carrying a 5 by 7 inch film, and a sheath to carry smaller sizes as required, will serve for the most exacting research work, for routine photomicrography, when a large number of either small or large negatives may be needed, or for the occasional photomicrograph for general purposes. Such a camera may be mounted in a space even as small as 1½ square feet. The microscope must generally be carried from the table to the camera stand, as it is not convenient to operate the microscope visually while it is set up in conjunction with the camera. But, if the microscope and camera are set up together continuously, it may be worth while to install a second

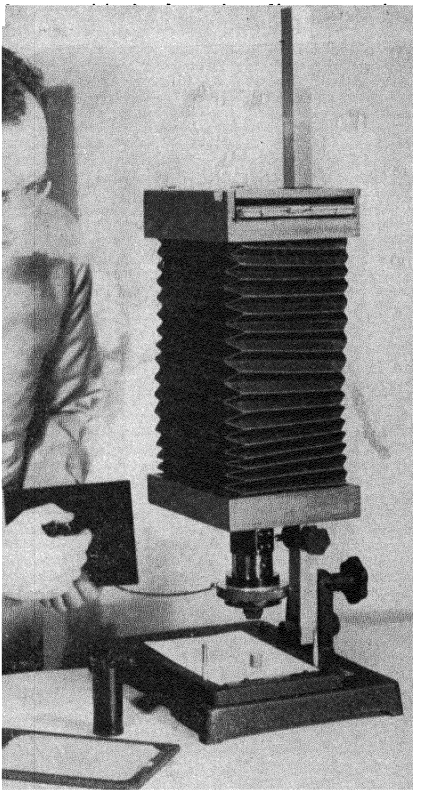


FIG. 161. The vertical camera arranged to take pictures at low magnification, with microphotographic lenses. Courtesy, Bausch and Lomb Optical Company.

microscope for visual work and to have the microscope on the camera stand bolted in place.

For work with reflected light, when microphotographic lenses are required, the vertical camera arranged as shown in Fig. 161 is practically a necessity. Magnifications as high as 30 diameters are easily

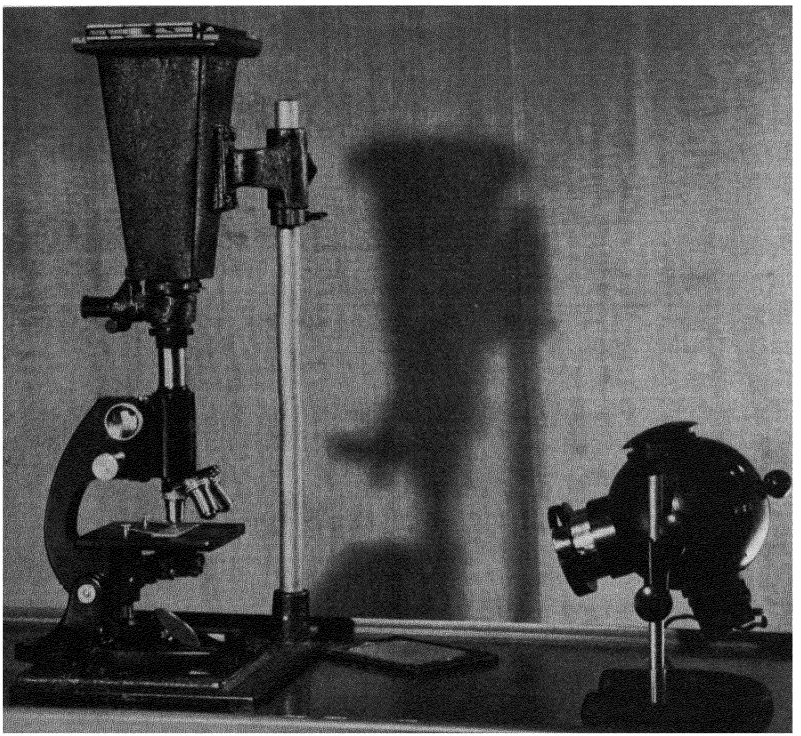


FIG. 162. A camera with fixed plate distance and side tube for observation up to the moment of taking the picture. The camera is fitted with a shutter. The one shown in the picture is 3¼ by 4¼ in. This is a very satisfactory camera for industrial concerns requiring occasional photomicrographs. The magnification at the focal plane is generally the same as that obtained in visual examination. Courtesy, Bausch and Lomb Optical Company.

possible with a microphotographic objective of 24-mm focal length, mounted directly on the camera front board, while with a longer-focus lens — say 7 inches — such a camera can conveniently handle a picture of a standard size petri dish at a magnification of 1 : 1.

Sec. 104. Vertical Camera with Fixed Plate Distance. This type of camera is shown in Fig. 162. It is not as adaptable as the camera just cited, but on the other hand it is often more convenient. The

plate or film size is generally 3¼ by 4¼ inches, 9 by 12 cm, or 4 by 5 inches. The camera box is usually of metal with a fixed plate distance of 10 inches; thus the magnification on the plate is the same as that observed in the microscope. The light-tight feature is essentially the same as in Fig. 159, but the fitting on the camera is attached to a side tube with an ocular. Above this device is a shutter. The side tube with a Swan cube, or a lightly silvered prism, directs some of the light rays to the ocular where the image can be examined exactly as it is focused on the plate. The arrangement of the side tube is very suitable for certain kinds of work, such as the photographing of crystals during growth, or any small moving object. When the side-tube ocular is correctly adjusted, focusing will be much easier and more exact than when the projection is on ground glass. The working theory of the side tube is given in Sec. 105.

Since the extension of such a camera is only a short distance, not over 10 inches, the intensity of the light which falls on the plate makes fast exposures possible, and for this reason the camera is equipped with a shutter giving several instantaneous exposure settings in addition to bulb and time. It should be noted that cameras with fixed bellows extension cannot be used to advantage with microphotographic lenses since either the specimen or the entire camera must be raised or lowered to attain focus.

The magnification of the pictures obtained by this camera will be limited, but, in general, the negatives will have sharp images because of the short plate distance. If the finished pictures are needed larger than the camera permits, the negatives can be enlarged; the small initial magnification will make enlargements particularly crisp and clear.

The camera occupies about the same space as the one previously described, and as before the microscope must be taken to the camera. However, the camera should be set up on a table because the side-tube ocular can be used for focusing, whereas the camera mentioned previously must be set on the floor or on a low bench. It will be found very convenient in industrial or plant laboratories, since it lends itself well to the rapid production of a large number of pictures, and it is particularly handy for routine work. As the 4 by 5 inch negatives are large enough to make useful contact prints, for most purposes enlargements will not be necessary. The short bellows extension, however, does not permit complete coverage of the 4 by 5 inch plate, and it will be necessary to diaphragm the prints to approximately a 3-inch circle. Figure 163 illustrates an installation made for the Fibre Conduit Company of Orangeburg, N. Y.

Sec. 105. The Eyepiece Camera. This small camera can be carried to the microscope and clamped to the end of the drawtube, and then the picture can be taken without disturbing the microscope arrangement. The side-tube attachment with which most of these cameras

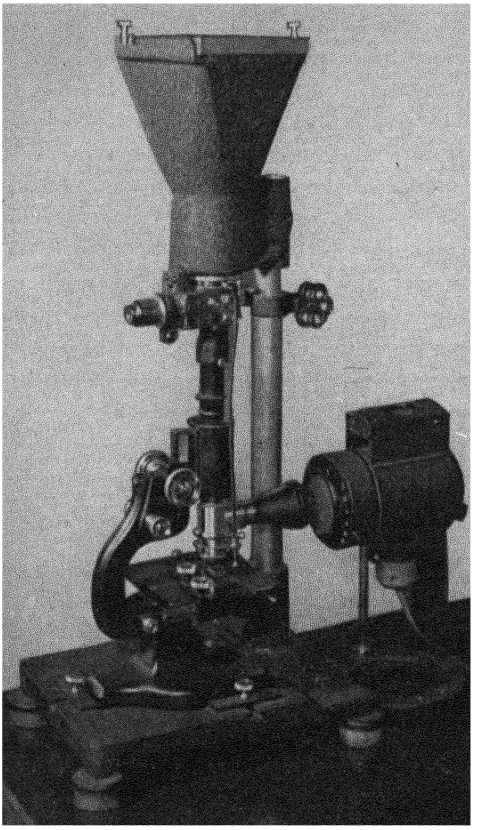


FIG. 163. The arrangement of a Spencer camera and microscope installed for an industrial firm. The Leitz Ultropak system of illumination has been adapted to the Spencer microscope. Courtesy, Mr. E. J. Wall, Chief Chemist, Fibre Conduit Company, Orangeburg, N. Y.

are fitted makes it possible to examine specimens, change slides, and select a field, all without removing the camera. Because the rays from the microscope ocular are normally parallel, it is easy to design the side tube to operate on the principle of a telescope so that the virtual image appears to be about the same size as the image projected onto the film.

Eyepiece cameras are obtainable in various sizes. One of the most popular is the 35 mm, but the film for this must be either projected or enlarged for subsequent study. However, for a matter of routine photomicrography or for purposes of record this may not be a serious obstacle. An attachment for mounting a Contax or Leica camera is available, in which a shutter and telescope side tube are included. At photographic supply houses, special holders to take single film for

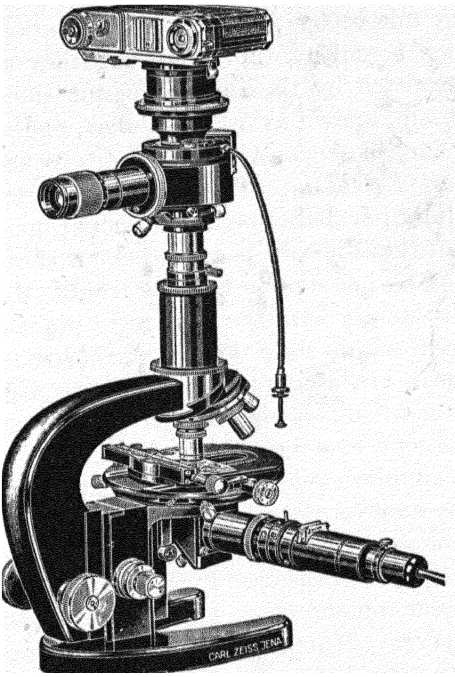


FIG. 164. A small eyepiece camera using the contax camera head. This is the Miflex attachment of Zeiss. Similar cameras are not made in this country. Courtesy of Carl Zeiss.

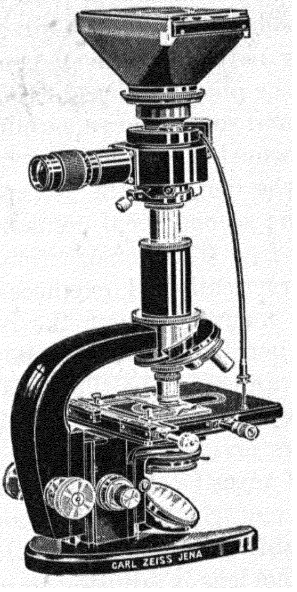


FIG. 165. The Miflex attachment of Zeiss using a camera box taking 6 by 9 cm film. Courtesy, Carl Zeiss.

Contax and Leica cameras are available, which will enable test exposures to be made conveniently. Unfortunately the cost of such an outfit may equal or exceed that of the larger 5 by 7 inch equipment first mentioned, but it is offset, in part, by the low cost of the sensitive material and the rapidity with which the small camera can be operated.

Figure 164 shows an eyepiece attachment carrying a Contax camera connected to the microscope. Figure 165 shows a slightly different eyepiece attachment. Here, the camera is larger and will take a 6 by

9 cm plate holder. Although eyepiece cameras can be had to take plates as large as 4 by 5 inches, with a distance of 10 inches from eye-point to plate the most usual and useful size is probably 9 by 12 cm.

Either one of these eyepiece cameras is useful for work in water laboratories, or the like, or for occasions when, to avoid moving the microscope, it is desirable to take the camera to the microscope.

Figure 166 shows the position of the focal planes and the optical arrangement of an eyepiece camera; the primary image is shown in the focal plane of the eye lens of the microscope ocular at I_1. The light rays leaving the ocular must then be substantially parallel; they are partly reflected and partly transmitted by the camera prism (shown in the cut by the half-silvered prism, known as a Swan cube), or they may be wholly reflected to the side telescope, where they are focused by the objective lens of the ocular (L_3) at I_2. They then pass to lens L_4 and so to the eye as substantially parallel rays, and a virtual image is formed in the usual way. That the side tube is a telescope is shown by the fact that the objective lens L_3 has no optical tube length; that is the second focal point of L_3 coincides with the first focal point of lens L_4. This construction places it within the field of telescope optics.

Practically all eyepiece cameras are equipped with an accessory lens mounted above the reflecting prism. This lens serves to focus the parallel rays from the microscope eyepiece in the focal plane of the camera. The theory is very simple. When the image is sharp at I_2 it will be sharp at I_3. This is easy to accomplish because the two lenses involved, L_3 and L_5, can be set in predetermined positions and need never be changed as long as the camera is used in the normal way. The eye lens of the telescope ocular, on the other hand, must be adjustable to suit the eye of the observer. If image plane I_2 is fixed, and the eye lens is adjusted to make that field sharp, then any object at I_1 will also appear sharp and be imaged at I_2. These are the conditions which will make the image sharp at I_3, the focal plane of the camera. So to focus one of these cameras it is necessary only to adjust lens 4 to make the cross lines inserted at I_2 appear sharp, then to focus the microscope which makes the primary image fall at point I_1. The camera will then be in perfect focus, for the image of I_1, which will then fall at I_2 and be superposed on the cross hairs at that point, will also be focused at I_3. Lens 5 is shown in Fig. 166 as positive, but a negative lens could be substituted. Under these conditions the rays from the eyepiece of the microscope must be slightly convergent. They are then brought to a focus, as before, in the focal plane of the camera. The difference between the two systems lies chiefly in the fact that with a negative lens the resulting camera image is enlarged, and so the

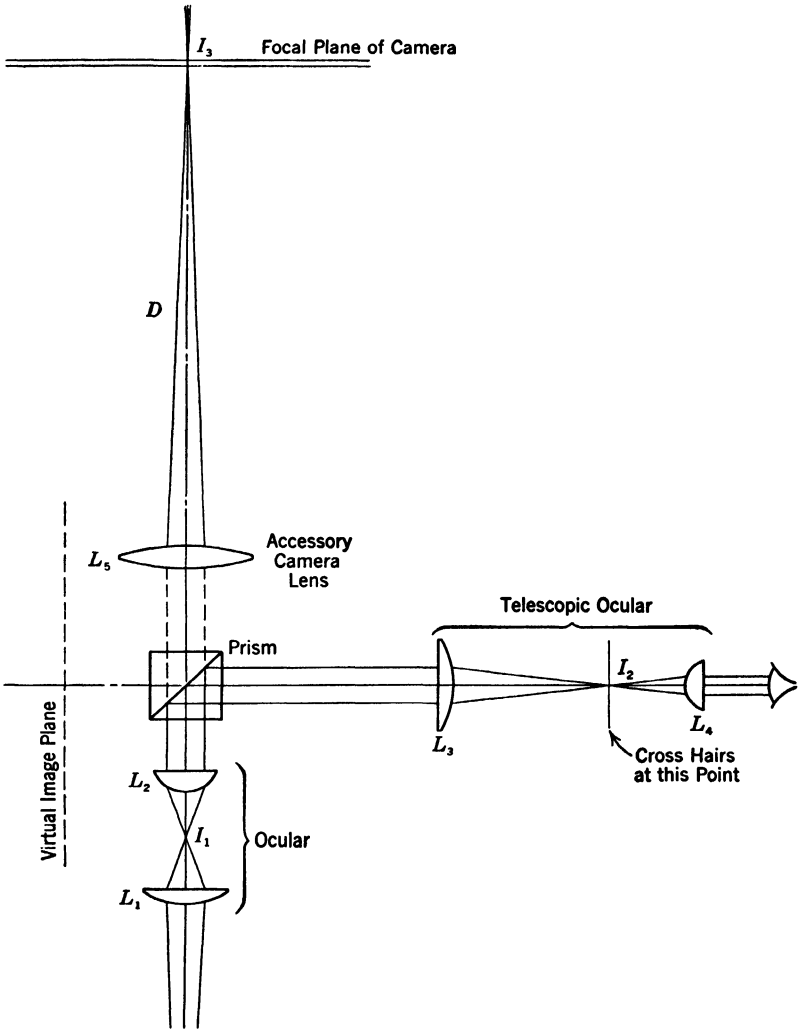


FIG. 166. Diagram of an eyepiece camera showing the trace of light rays when a telescopic eyepiece is used. An image of the specimen is focused simultaneously at I_2 and I_3, I_3 being in the focal plane of the camera. The accessory lens may be either positive or negative in character.

camera distance D, as shown in the figure, can be a little shorter than when a positive lens is used. There seems to be no difference in the quality of pictures taken with either equipment. The accessory lenses are not designed to aid in giving a flat field but merely to ensure sharp focus at I_3 when the image is sharp on the cross lines at I_2.

With the negative lens as an accessory camera lens, normally a certain amount of under-correction would be introduced; this, however, can be avoided by increasing the tube length of the microscope, the exact amount being determined by the focal length of the ocular employed. The figures for proper adjustment of tube length can be obtained from the manufacturers of the eyepiece camera.

It may be stressed, further, that, to take advantage of the speed afforded by the eyepiece camera with a Leica or Contax head, roll film should be used and developed after, perhaps, thirty-six exposures. Although the single small plate holder available for the Contax or the similar device for the Leica affords a convenient means of making test exposures, both systems work to their best advantage when a large number of photomicrographs are needed, as, for example, when changes in a specimen are to be recorded at set intervals.

Sec. 106. The Horizontal Camera. This camera has been most highly developed by Leitz, Zeiss, and Bausch and Lomb as well as by Beck and Watson, both of London. Figure 167 illustrates the Leitz horizontal camera, and Figs. 168 and 169 show one by Bausch and Lomb; others were shown in Figs. 9 and 10. The cost of the cameras described heretofore ranges from approximately $100 to $200, but these more elaborate outfits may run into much larger figures, even above $1000. The large horizontal combinations include a quantity of equipment, sometimes even the microscope. The separate optical units are mounted on an optical track for easy alignment, and the stands are practically vibration proof. Long bellows extension is provided, that of Leitz being about 4 feet and one by Zeiss 6 feet. However, excessively long bellows extension is gradually being given up in favor of shorter extension and lower magnification, which results in sharper images. Larger prints can be obtained from enlargements. One of the latest developments in horizontal camera equipment is that by Bausch and Lomb already mentioned and shown in Fig. 168.

To work with the microscope in the horizontal position is a distinct handicap with many specimens and impossible with others. Oil is inclined to collect at the lower side of immersed objectives, leaving the upper surface of the lenses bare, and at best the adjustment of the condenser, when it is oiled to the slide, must be carefully watched lest uneven lighting results.

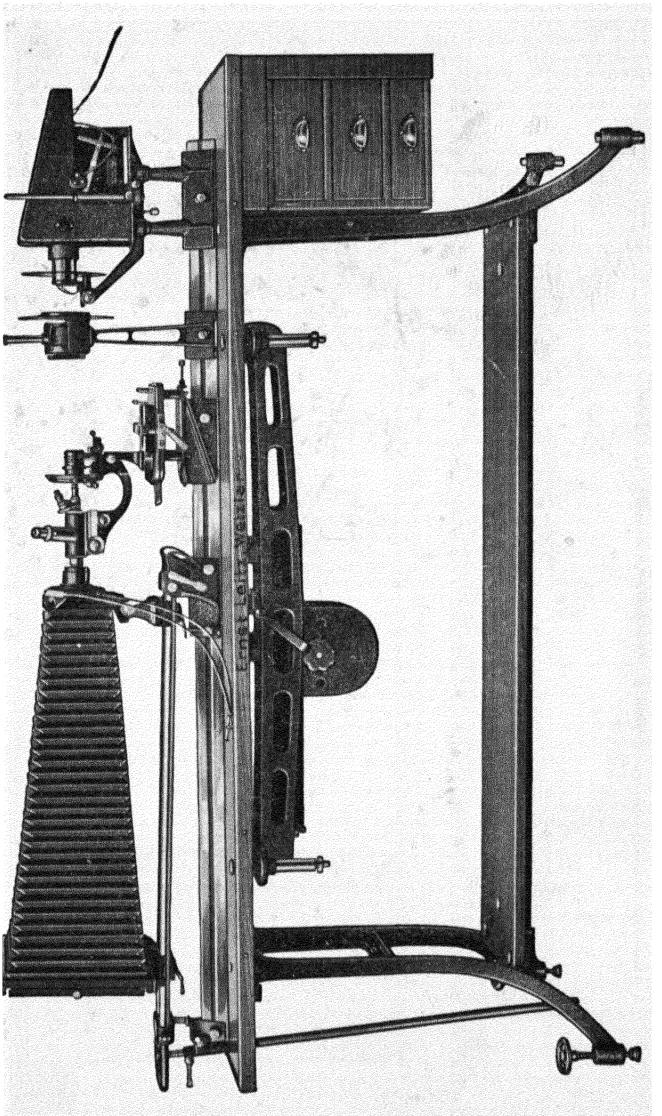


FIG. 167. A Leitz horizontal camera showing the anti-vibrational device mounted under the long table. Courtesy, E. Leitz.

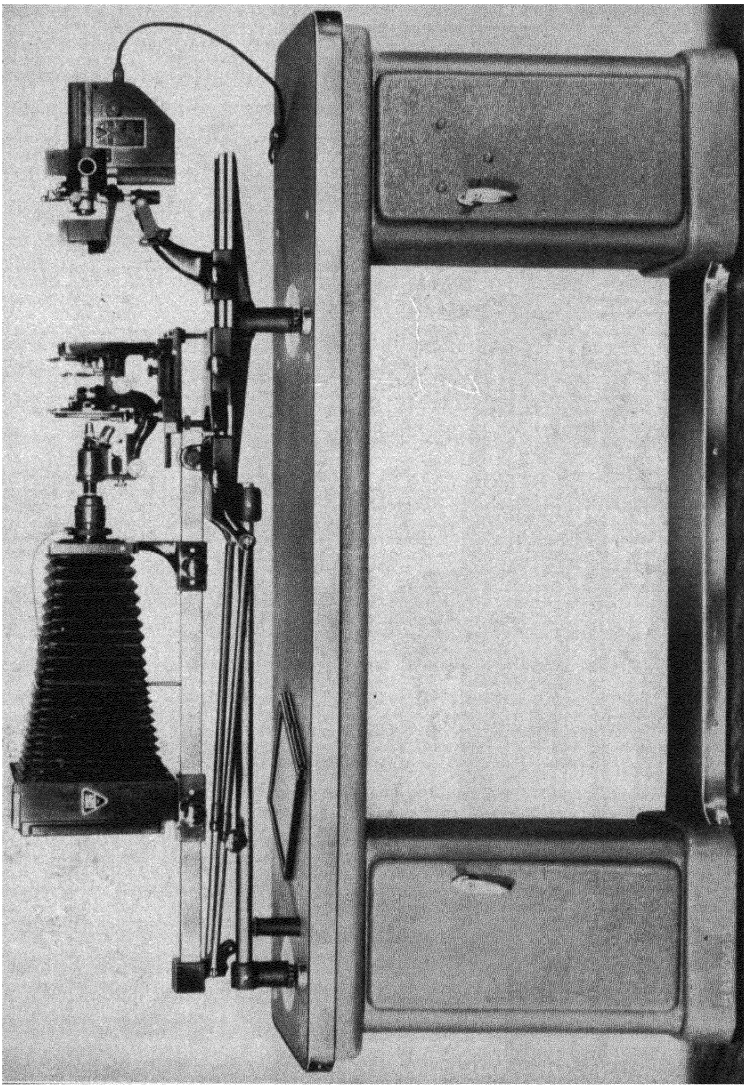


FIG. 168. Bausch and Lomb latest development of a photomicrographic outfit.
Courtesy, Bausch and Lomb Optical Company.

The horizontal camera stand is very convenient to manipulate, since all the apparatus is at bench level and the parts are easily accessible. It is unexcelled for speed and convenience in metallographic or petrographic work with vertical illuminators. For such use, an inverted microscope can be had which is interchangeable with a biological microscope, all being mounted on the same optical track. All phases of photomicrographic work can be done on one instrument of

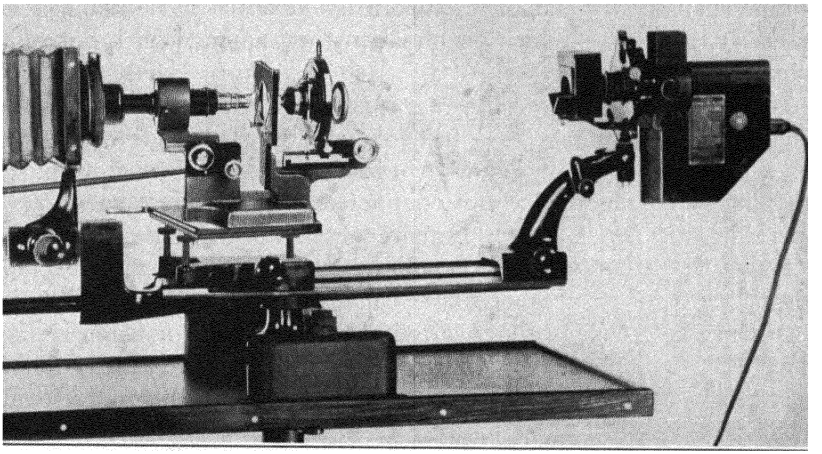


FIG. 169. A Bausch and Lomb photomicrographic camera; the microscope has been replaced by a tube and condenser, and a stage specially designed for photomicrographic work. Courtesy Bausch and Lomb Optical Company.

this type since the camera is often designed for use either in the horizontal or vertical position. The addition of a side telescope makes focusing easy. Remote control is provided for adjustment of the focus. Although the camera will sometimes accommodate a plate as large as 8 by 10 inches, full advantage can seldom be taken of such a large plate. The 5 by 7 inch kits are customarily used in the 8 by 10 inch plate holders.

A distinctive feature in one of the horizontal cameras as supplied by Bausch and Lomb is the special photomicrographic microscope which can be made interchangeable with the regular type on the same bed. This photomicrographical microscope is shown in Fig. 169; it is used only for photography.

The light source usually supplied with these large stands is a carbon arc, but Bausch and Lomb supplies one of its stands with the General Electric photomicrographic lamp. Accessory lenses may be placed in the lighting train. The trace of the rays is shown in Fig. 10. Other

lenses may be added, their purpose being principally to control the size of the illuminated field.

The horizontal camera stand is advantageous in metallographic and petrographic laboratories. Polished metal surfaces and mineral rock sections which are nearly optically flat are ideal subjects for this instrument. However, any work performed by the horizontal camera can also be accomplished by the vertical microscope and the vertical camera. Many preparations are much easier to handle on the vertical equipment; thick specimens, temporarily mounted in a liquid of low viscosity, are particularly troublesome when handled on a horizontal microscope.

Horizontal cameras occupy a rather large amount of floor space. There is one instrument, 6½ feet long and 1½ feet wide which takes 9¾ square feet of space, exclusive of that needed by the operator. The installation of such a piece of equipment, with sufficient room for an operator, calls for at least 100 square feet of working space.

Sec. 107. Metallographic Equipment. In its most highly developed form, this consists of a horizontal camera stand, with the addition of a special microscope of the inverted or Châtelier type as described and shown in Figs. 9 and 10. As the specimen must be optically flat for best results with vertical illumination, the use of metallographic equipment is restricted almost entirely to metals and other opaque specimens, such as ores with a polished surface. The Reichert universal camera has the inverted system and an additional method of applying transmitted illumination.

The objectives in the metallographic camera outfits are universally corrected for use on uncovered objects, and the shorter-focus objectives are corrected for infinite focus, made necessary by the introduction of the glass plate used in the vertical illumination. The eyepieces are generally corrected for projection of the image.

All the methods that have been previously cited for centration can be applied to the metallographic outfit. Correction for tube length should be carefully checked, but the latitude of the adjustments may be limited. However, the tests suggested should be applied wherever possible to ensure that the apparatus is optically correct.

Sec. 108. Universal Microscope Cameras. One of the latest developments in photomicrographic apparatus is the so-called universal camera. Four or five foreign firms make them, but as yet they have not been produced in the United States. Basically, they consist of a camera and an illuminating system with the necessary mechanical arrangements for connecting special microscopical equipment all built into one compact assembly. Figures 170, 171, and 172 show the

Ultraphot of Zeiss, the Panphot of Leitz, and the Universal Camera Microscope MeF of Reichert. Polarizing equipment and all kinds of illumination are available. The Leitz and Zeiss outfits have a carbon arc lamp in addition to provision for illumination by the tungsten light.

Cameras of this type are very expensive. Completely equipped, they may cost $4500 or more, but they will certainly measure up to their specifications. If directions are followed closely, anyone, even without special training, can operate such a camera satisfactorily. The type is particularly convenient in a laboratory where in addition to general work much metallographic work is undertaken. The Reichert camera, with the inverted system, is specially well arranged for metallographic or petrographic work. With the other makes, illumination is vertical as on the regular microscope. The universal cameras occupy but very little space; they demand scarcely more than the microscope itself.

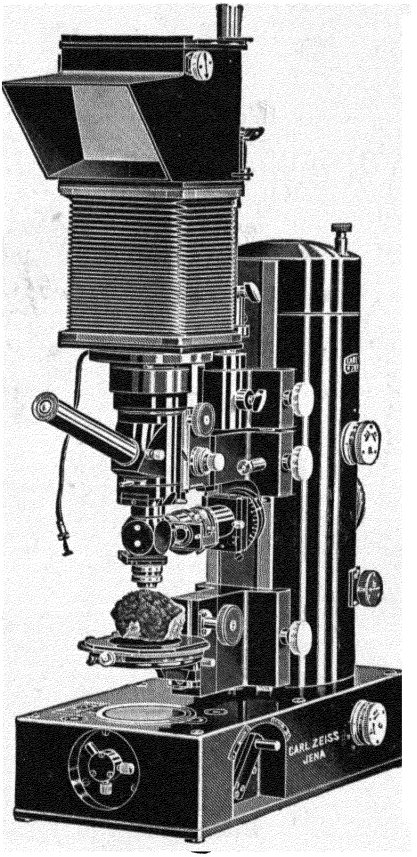


FIG. 170*A*. The universal microscope-camera of Zeiss. Here it is shown arranged for photomicrography, using a 9 by 12 cm plate.

The standard plate or film carried by this equipment is only 9 by 12 cm. This small size sets up limitations; for instance, a 3½-inch petri dish cannot be photographed at a magnification of 1 : 1, and preparations of textiles and petrographic or metallographic specimens, where the view of a large area is often particularly desirable, have to be decidedly reduced in magnification. However, at a special price, the 5 by 7 inch camera back can be had on models of at least two makes. It is true that small pictures can be enlarged, and, by using a slightly lower magnification, an area can be included that is just as large as it would be on a larger plate with the higher magnification. But, as a rule, contact prints are easier and quicker to make, and enlargement

apparatus may not always be at hand. This type of photomicrographic equipment is suited to an institution such as a hospital rather than to the research laboratory, which demands great flexibility in equipment to cover a wide range of work.

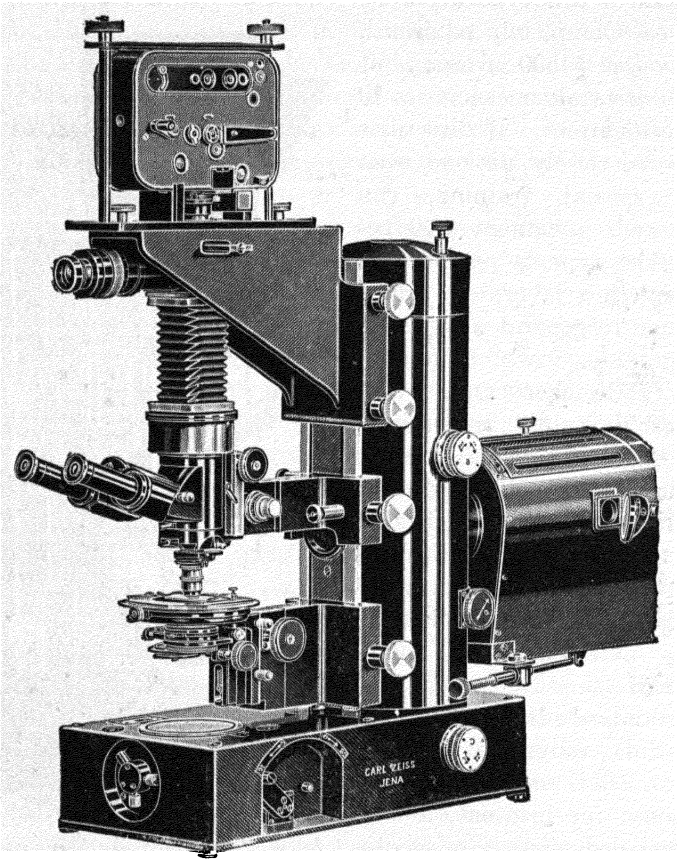


FIG. 170*B*. The microscope-camera equipped with a moving-picture camera for cine-micrography. Courtesy, Carl Zeiss.

Sec. 109. Cameras for Special Purposes. Several cameras are made to accompany specially designed microscopes or eyepieces. Examples are the camera used with the spectrographic eyepiece and the stereographic camera used with the Greenough binocular stand, both of Zeiss manufacture. In the United States, Bausch and Lomb manufactures the stereographic camera shown in Fig. 173. Figure 174 illustrates the spectrographic camera connected to the spectroscopic eyepiece. The plate used is small, only 4½ by 6 cm. Both cameras have

been available for a number of years, but little use has been made of them. The value of the work accomplished by either of them must be decided by the individual user.

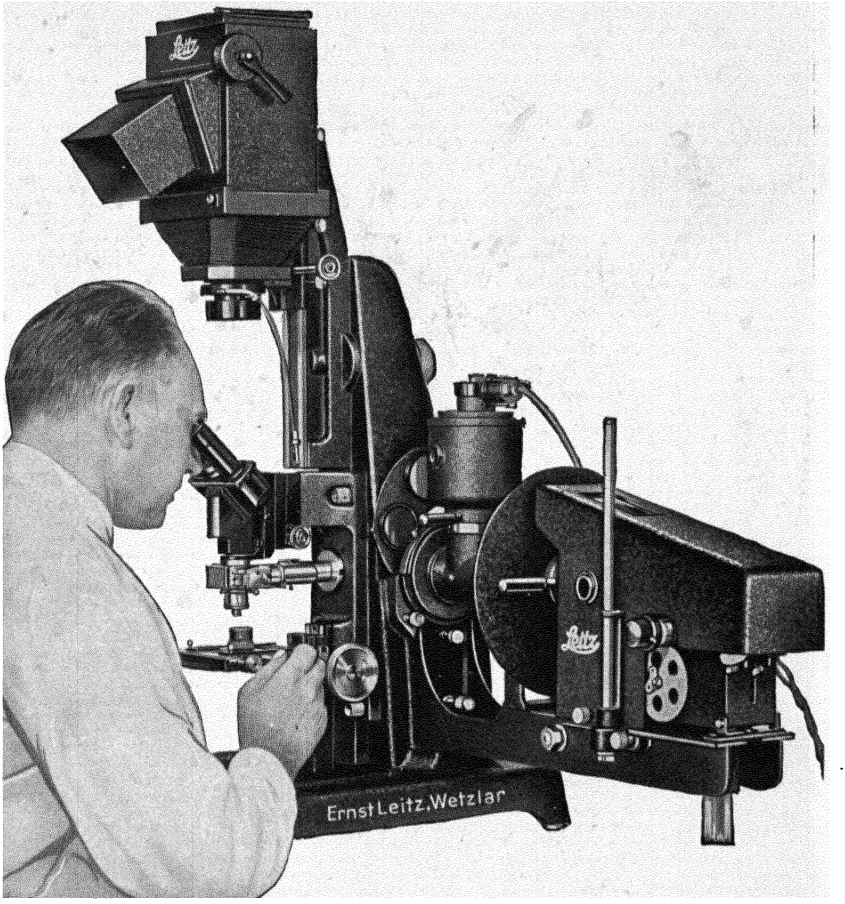


FIG. 171. A camera invented by Leitz, similar in many respects to the universal camera of Zeiss. Courtesy, E. Leitz.

Dr. Jelley[3] of the Eastman Kodak Company has developed a new spectrographic camera. The dispersion is obtained by means of a grating at the eyepoint rather than by prisms. The camera is shown in Fig. 175.

Cameras for cine-micrography are made by Leitz, Zeiss, Bausch and Lomb, and others. Figure 170*B* shows the method of using the Zeiss

[3] Edwin E. Jelley, "Application of the Grating Microspectrograph to the Problem of Identifying Organic Compounds," *Ind. Eng. Chem., Anal. Ed.*, **13**, 196, 1941.

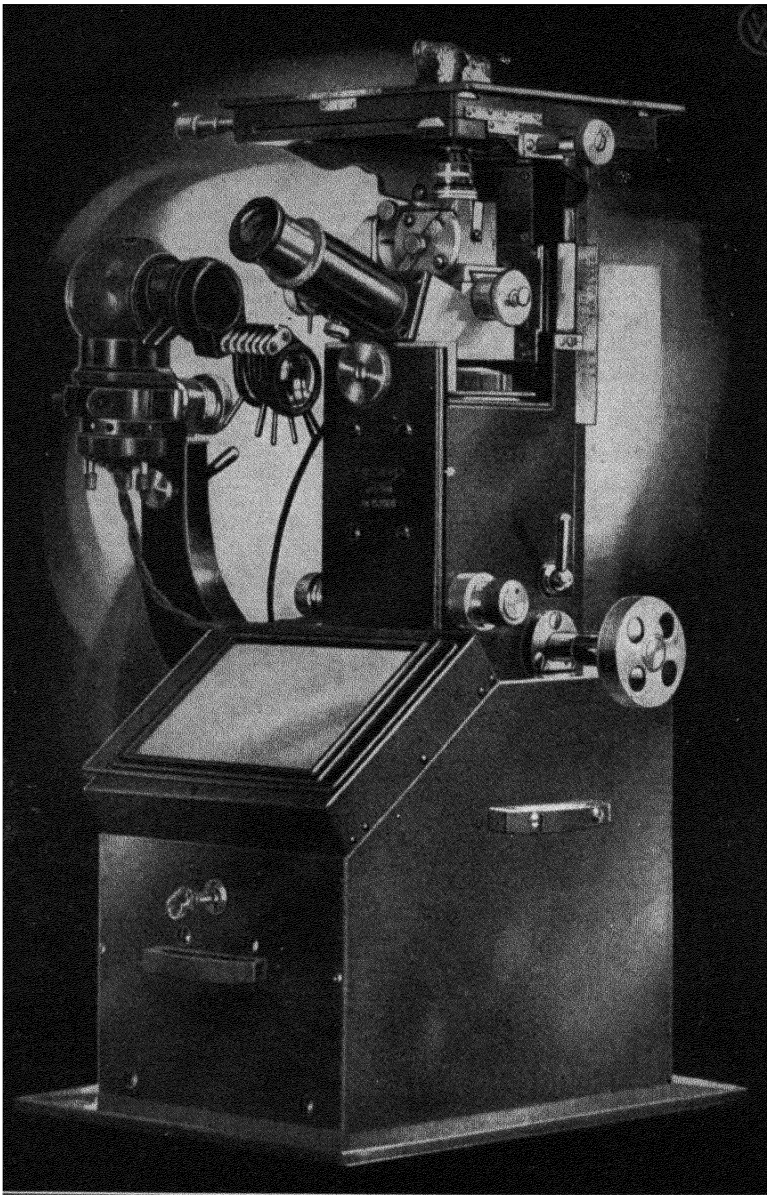


FIG. 172. Reichert Universal microscope camera.

cine-camera on the universal microscope stand. Relatively little work has been done in this field, and more research on equipment and technique is required. It would seem that one profitable line of development would be along the lines of scanning large surfaces. A microscopical field in the plane of the object is a very small area indeed. It

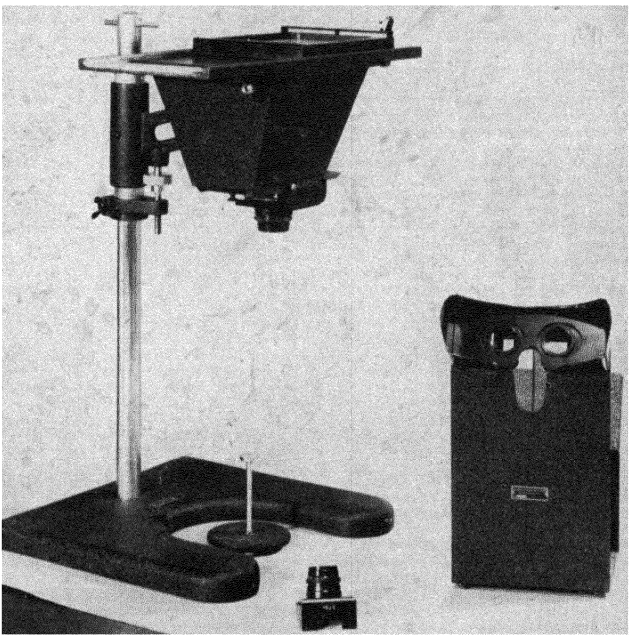


FIG. 173. A stereoscopic camera. Courtesy, Bausch and Lomb Optical Company.

would seem feasible to develop equipment that would photograph the specimen in long paths as the subject is passed beneath the objective on a perfectly flat surface. An observation or telescopic eyepiece could be used to ensure constant focus.

Automatic exposure equipment is available and has been used successfully at the Rockefeller Institute. It is possible to set such apparatus and, by electrical operation, to take pictures on 35-mm film at desired intervals. The growth of yeast cells and other material has been studied in this manner.

The Graton-Dane Precision Microcamera. This horizontal camera, Fig. 176, of which only two have been built, is noteworthy for its extreme precision in focusing, its great adaptability, and its novel and

precise mechanical arrangements for using standard optical parts. It is the outgrowth of years of experience and experimentation on the part of Professor L. C. Graton, of Harvard University, and was brought to its present finished state by Dr. Graton in collaboration with Dr. E. B. Dane, Jr.[4]

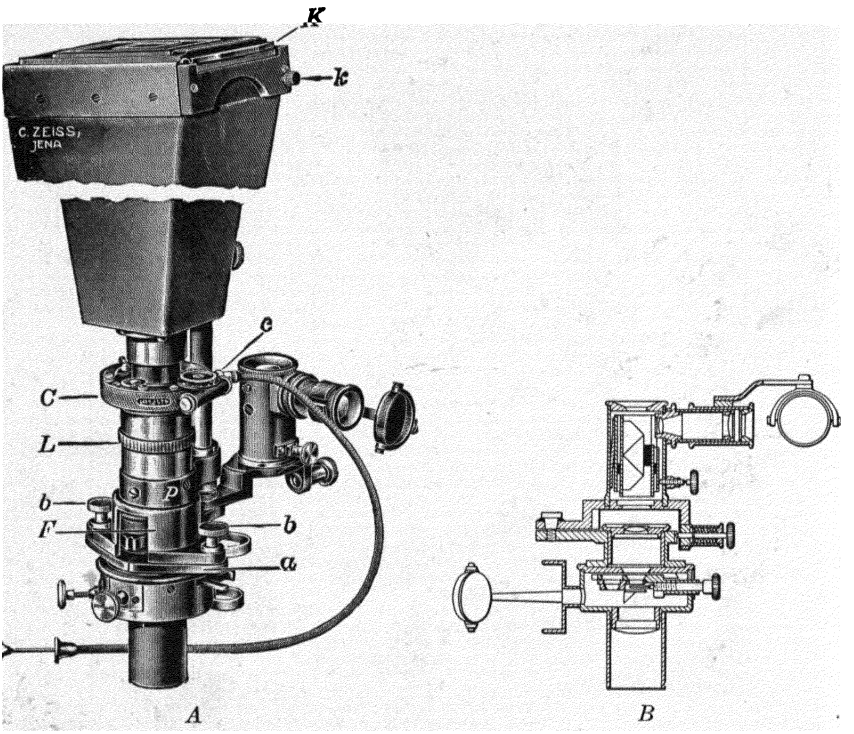


FIG. 174. *A*. Spectrographic camera of Zeiss. *B*. Details of the spectroscopic eyepiece. The spectroscope is of the direct-vision type. The eyepiece can be used independently of the camera. Courtesy, Carl Zeiss.

In a private communication, Dr. Graton stresses the following points which his design has made possible. The following is quoted from his letter:

1. The datum point in the system is the fixed axial center of the mechanical stage. Provision has been made for adjustment of all essential parts with respect to this datum point.

2. Massiveness of design and construction, which insures against misalignment or change, once adjustments are established.

3. Provision for transmitted, dark-field and reflected illumination with the

[4] L. C. Graton and E. B. Dane, Jr., "A Precision, All-Purpose Microcamera," *J. Opt. Soc. Am.*, **27**, 355, 1937.

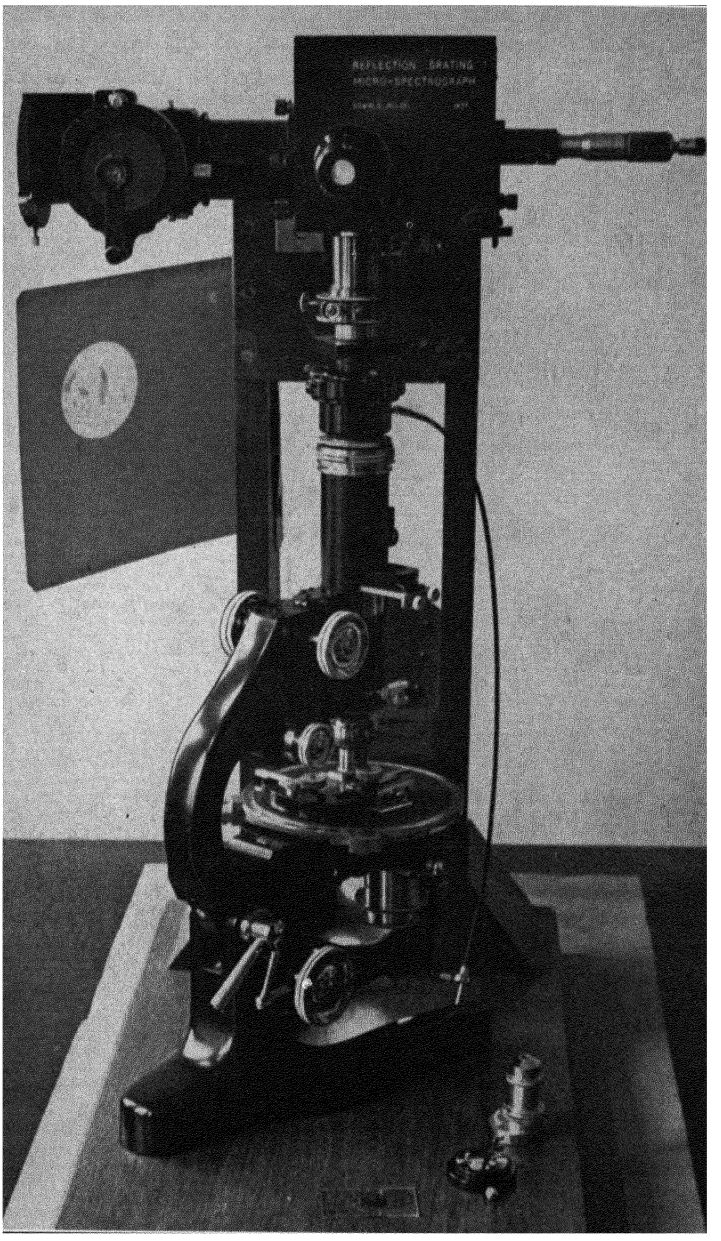


FIG. 175. The Jelley spectrographic camera. The Jelley camera obtains dispersion by the use of a grating, while the Zeiss system uses a prism system. Acknowledgment is made to the Eastman Kodak Company, but the photograph was obtained through the kindness of Dr. E. E. Jelley.

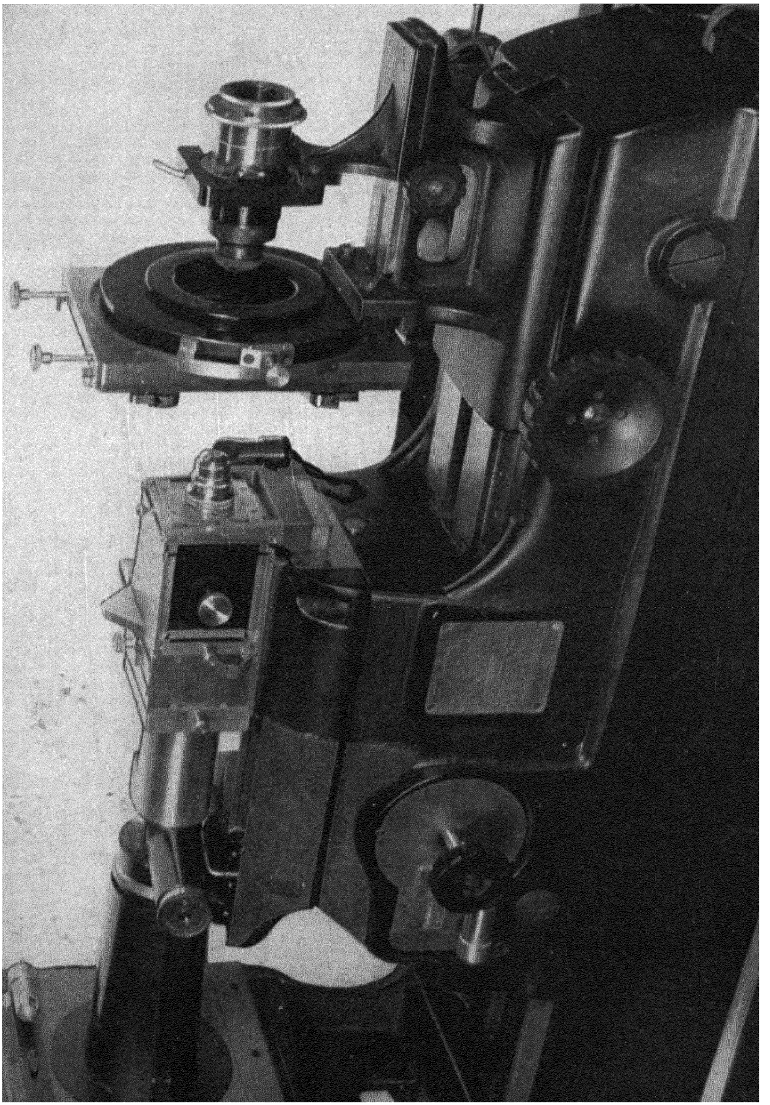


FIG. 176. The Graton-Dane precision microcamera. This camera has an especially sensitive fine adjustment. Courtesy, Professor L. C. Graton, Harvard University, Cambridge, Mass.

availability of polarized light for each one of these methods. Transmitted and reflected light may be employed simultaneously so that the transparent and the opaque portions of the preparation may be examined or photographed on the same plate, each with its appropriate type of illumination.

4. Unusual precision and unusual slow motion for the fine focusing mechanism. This I believe for the first time permits a higher-power lens to be placed invariably at the truly optimum focus position. Partly in consequence of this control we find that high-grade lenses have a higher resolving power than has hitherto been supposed possible, and we also find that they have a substantially shallower depth of field than has been indicated hitherto when reliance has to be placed entirely upon computation from certain assumptions.

In order to build a fine focusing adjustment of sufficiently high degree of sensitivity, it was found necessary to actuate it by motor power; otherwise the time required to attain focus would have been altogether too long. The coarse adjustment is also motor driven.

The results attained from the use of this microcamera, with its precise methods of focusing and centering the optical system, certainly seem to justify the care and meticulous attention to centration, focusing, and the correct use of lenses so strongly advocated in this book. The instrument indicates that even the most sensitive focusing mechanisms on stock microscopes are not nearly good enough, and that, when the finest lenses are used, differences as small as 500 A (0.05 μ) in focusing will make a perceptible difference in the picture. At present the Graton-Dane instruments are available only on special order.[5]

Sec. 110. The Use of the Camera with the Microscope. *Installation and Adjustment of the Vertical Camera with Bellows Extension.* Once the use of the large vertical camera has been mastered, there need be little or no trouble in setting up and operating cameras of other types. Certain fundamental points regarding the operation of photomicrographic cameras are common to all and are particularly well emphasized in the operation of the vertical type. Consequently, the description of the method of using this camera will be thorough, and it is suggested as somewhat basic photomicrographic procedure although the manipulation of the 5 by 7 inch and smaller cameras cannot include all the problems inherent in the larger ones.

The 5 by 7 inch vertical camera is the most useful type for covering a wide range of work. It can be set up for operation in several ways. While the microscope is being used, the camera normally is in an upright position, though for certain types of work, such as taking

[5] The Graton-Dane Precision Microcamera was built by the Mann Instrument Company of Cambridge, Mass. Its successor is the Mico Instrument Company, also of Cambridge.

a number of photomicrographs at magnifications which permit the use of dry lenses throughout, the horizontal position may be more convenient. However, a thick section in a low-viscosity liquid mount requires the vertical arrangement.

Figure 177 illustrates the use of the vertical camera, but some of the shields occasionally required are not shown. After the microscope is properly adjusted and illuminated, it is moved to the camera. The appropriate ocular having been selected, and the light trap for connecting the microscope to the camera having been installed, the front board of the camera is lowered, and other necessary adjustments can then be made. These adjustments include rearranging the illumination to suit the new position of the microscope. If the lamp distance is the same as when the microscope was used visually, all illumination can be aligned by focusing the image of the source at the center of the mirror and making the spot of light central on the ground glass at the camera back.

In order to return the microscope always to the same position relative to the camera, the microscope may be pressed against the stops provided for the camera base. If stops are not provided by the manufacturer, it is easy to take two flat and rather thick metal angle pieces, and, after the microscope has been placed in its correct position under the camera to screw these angle irons down to the base holding the camera. The angles are most conveniently placed at the two toes forming the microscope base; it is not necessary to place one at the heel. If the camera base is made of cast iron, holes will have to be drilled and tapped in order to take the screws.

In any arrangement for photomicrography by which the microscope has to be carried to the camera, either a second lamp house must be provided, or the lamp for visual work must be used. Unless considerable work is to be done, both visually and photomicrographically, one lamp will probably be sufficient. However, an elaborate outfit might necessitate a second microscope as well as a second lamp, in which event the camera-microscope apparatus should become a permanent unit.

When only one microscope and one lamp are available, the position of the lamp can be fixed as accurately as that of the microscope by having a stop against which the lamp can be placed. Sometimes the camera base is provided with a special device, like that which accompanies the Model R camera of Bausch and Lomb; here a short piece of optical track is provided on which the lamp is mounted. Various arrangements for alignment of the lamp, microscope, and camera will suggest themselves to the ingenious worker. In making the alignment

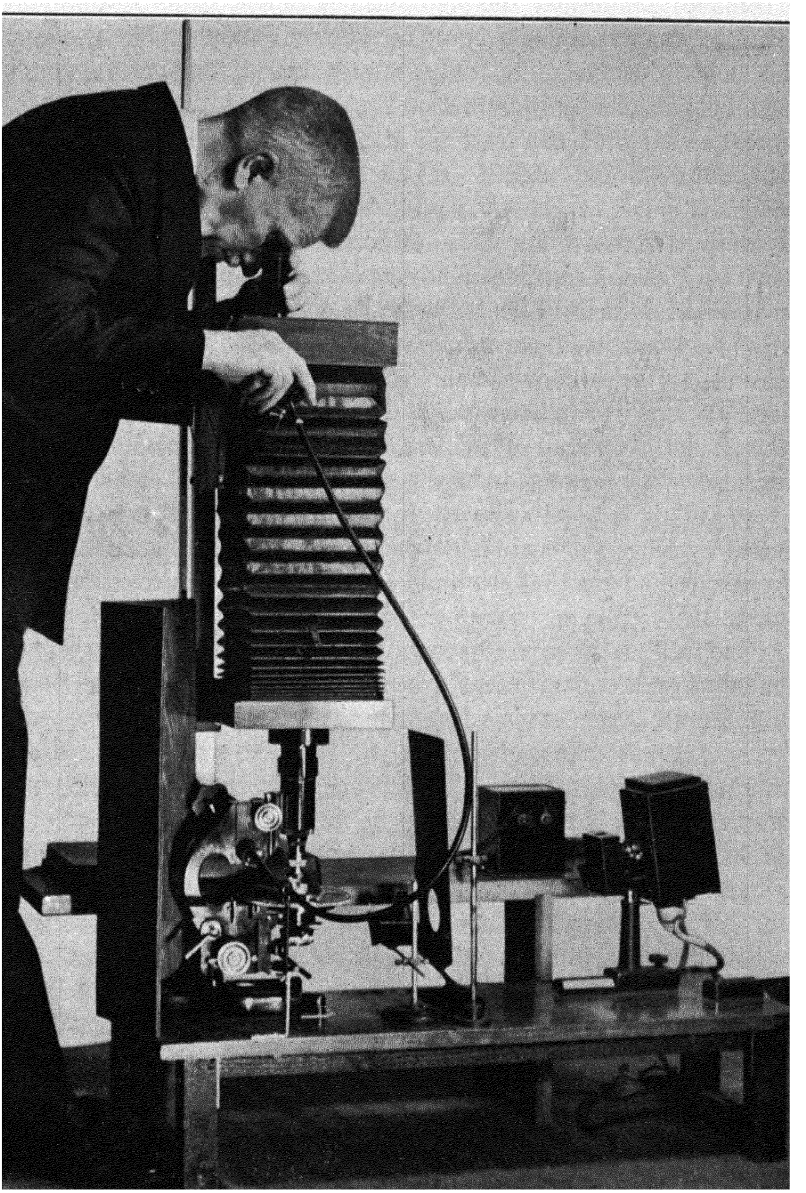


FIG. 177. A modified stand, using the Bausch and Lomb camera. The bellows have been lengthened to give an extension of 31 in.; the camera post is 38 in. long. The light shields and filter holder are in place for illumination by transmitted light. The remote-control focusing attachment was made from an automobile radio control cable. It was assembled by Mr. P. J. Speth, Belrose, L. I., N. Y.

for camera illumination, the plan outlined in Sec. 18 for setting up the microscope for visual work should be followed regarding lamp distances, filter holders, screens, etc. The camera installation should be such that all conditions for illumination that existed on the table for microscopical visual work can be duplicated when the camera is added.

Probably the best place to set the vertical camera outfit is on the floor or on a low bench. A good arrangement of camera, microscope, and lamp is shown in Fig. 177. Here the camera is placed on a bench about 14 inches high; the microscope and lamp are set on another bench about 7 inches high. Placing the various pieces of apparatus in this way makes it possible to utilize more of the camera bellows extension. The camera shown in the figure is a Bausch and Lomb Model R, with extra bellows extension and extra long post made to special order. The top of the post measures 38 inches from the camera base. Since the post sits in a socket near the lower set screw, as shown in Fig. 161, it is possible to swing the entire camera away from the microscope when a picture is not being taken. This feature is a great convenience and one that should be considered when purchasing camera equipment.

As the two benches are adjacent, it is advisable to connect them with two metal eye screws, one attached to each bench in such a position that one eye screw is directly under the corresponding one on the other bench. A pin with a head of suitable size can be dropped into the eye screws, coupling the benches so that they will stay in alignment.

When the camera and microscope are being installed, the camera back carrying the film holder should be made parallel to the microscope stage. The microscope can be leveled by using shims and testing in several directions with a small spirit level. The camera can be tested by placing a plate holder in position and holding a small level on the top edge of the holder. When the microscope is resting on the cast base of the camera, the camera can be leveled first and the microscope then be made level with it; leveling screws are often provided on the camera base for this purpose. Leveling the equipment is an adjustment that should not be overlooked when setting up an outfit, not only for optical reasons, but also because dishes containing liquids may often be placed on the microscope stage, and specimens mounted in a thick layer of mounting fluid may slowly slip toward the low side.

Vibration. There is likely to be more trouble from vibration with the long-bellows vertical camera than with any other type of camera. The fact that the camera and microscope have no mechanical connection makes even the slightest vibration very objectionable. Any move-

ment whatever that is visible on the ground glass of the camera will certainly spoil a picture. It is possible to study the vibrations of the building in which the microscope may be mounted by means of the microscope and camera as a makeshift seismograph; they make a rather delicate instrument for this purpose. In order to investigate these customary building vibrations, a few drops of water can be placed in a small cell cemented to a glass slide; the depth of the cell is immaterial, but the water should be built up to almost overflow the cell to produce an unstable condition. The surface is sprinkled with a very small amount of carbon black, and the microscope is then focused on the small aggregates of carbon floating on the surface. The surface of the drop is so sensitive to the slightest vibration, as shown by the motion of the carbon particles, that it is rare to find a location where no vibration is indicated. This test is interesting, but it should not be relied on in determining vibrationless locations; it is altogether too sensitive to be practical for general work.

A somewhat less sensitive but efficient test is to focus the microscope on a slide surface and to swing the image of the edge of the field diaphragm at the lamp across the center of the image field. This will show any vibration in respect to the lamp and ground glass. With a few pieces of carbon black on the dry slide, the motion of the slide with respect to the ground glass of the camera can be seen. This test should be absolutely negative. If any vibration is indicated a good picture cannot be expected. It is useful to remember that the visual effect of vibration is magnified by the microscope, and that vibration of a certain amplitude at 100 diameters will be 10 times greater at a magnification of 1000 diameters.

In eliminating the effects of vibration the underlying theory is to interpose material between the source and the microscope so that the vibrational energy can be absorbed. Rubber, or any such resilient material, is recommended. Sometimes it may be necessary to have blocks of rubber or cork at each corner of the board on which the equipment is mounted, and to place a heavy piece of lead or iron plate, each weighing perhaps 100 pounds or more, across each pair of blocks. The baseboard is then mounted on the metal plates. The two rather large metal masses offer considerable inertia, which has to be overcome by the vibrational force, already weakened on account of absorption by the rubber blocks. If a more complete system of vibration damping is required the Leitz system is recommended. This is mentioned in the section dealing with horizontal cameras and illustrated in Fig. 167. It is a very efficient system. Another method is to mount the camera and baseboard on what is called a rubber-in-

shear vibration dampener. This consists of properly cured rubber blocks secured on either side by iron or steel supports. One side of each shock-absorber unit is fastened to a base which rests on the floor; the other side of the unit is fastened to the apparatus. The apparatus to be freed from vibration can be supported at as many points by the shock-resisting units as required. The weight of the apparatus produces a shearing force on the rubber blocks, and when the weight is correctly adjusted, by the addition of lead plates if necessary, the rubber-in-shear mounting is very satisfactory. The Graton-Dane Precision Microcamera is so mounted.

The Use of Shields. It is only rarely that all the shields suggested in Sec. 102, on glare, are required at one time. The exigencies of each case must determine the requirements. However, no direct or strong reflected light other than that from the object field should be allowed to enter the front lens of the objective. Appropriate methods have been described for controlling such troubles, and their sources were indicated. Perhaps one of the most useful shields for low-power work is that placed about the objective; it is shown in Fig. 156. A shield for the top of an ocular with a bright finish (Homal) can always be used to advantage. It is necessary to ensure good focusing conditions. All manner of glare from within the microscope tube must be eliminated.

Figure 177 shows a tall screen of plywood. These screens are very convenient for use with microphotographic lenses, and for all photomicrographic work. When properly placed they will shut off a considerable amount of stray light from around the microscope and from entrance into the objective and thus reduce the chance of unintentional exposure.

Centering the Light Source. In all photomicrographic work the image of the source should be carefully examined on the ground glass of the camera each time an exposure is made; it should fall exactly in the center of the plate. With cameras which are equipped with side tubes, this centration can be accomplished by adjusting the mirror to bring the field diaphragm of the lamp into the center of the field of view. If there is no side tube the image of the diaphragm at the lamp should lie in the center of the image field.

In centering the field diaphragm of the lamp to the ground glass of the camera, the camera and microscope must previously have been leveled and aligned so perfectly that if the light circle is centered on the ground glass back of the camera it will also be perfectly centered in the field of view. This is always a good test for alignment. Thus the center of the field of view and the center of the image field at the

focal plane of the camera should coincide completely with the center of the ground glass at the camera back.

Focusing the Camera Image. In focusing, the first step is to examine the field roughly in the ground glass and make the image as sharp as possible. The focus should then be completed with the aid of a focusing glass (essentially a hand magnifier) of 4× to 12× magnifying power. The glass need not be held exactly in the image plane, but it should be near to it. Better images are obtained with the focusing glass when the ground glass back of the camera is removed. It may take some time to learn to pick up the aerial image easily and quickly in this way, but it can be learned with a little practice. It is a very essential part of photomicrographic technique.

Special methods of focusing, such as examining for parallax between the focal plane and the plane of the film surface, are not recommended. If the procedure with the focusing glass is carried out carefully and unhurriedly, the focus will probably be satisfactory, but it will be necessary to guard against other errors which may occur to offset and vitiate good focusing. The specimen may move; the microscope tube may slip; there may be vibration; or the camera may be accidentally moved during exposure. The camera will record sharply much less field depth than the human eye; this fact alone may account for certain disappointments and the inability to obtain critically sharp negatives.

The Use of the Vertical Camera with Fixed Plate Distance. Since, in its method of operation, the vertical camera with fixed plate distance does not differ substantially from the eyepiece camera, the following notes regarding the operation of the eyepiece camera will apply equally well to the camera with fixed plate distance.

The Use of the Eyepiece Camera with the Microscope. In preparing to work with the eyepiece camera, the microscope should first be set up as for visual work. The camera can then be clamped in place. The functioning of the side-tube eyepiece was explained in Sec. 105. This eyepiece may be used interchangeably with several cameras of various image distances, in which event it must be properly set for each distance. To do this, the back of the camera should be removed and the aerial image sharply focused with the hand magnifier. The side ocular can now be adjusted so that, without moving the focus of the microscope, the image will be sharply defined. On the regular standard camera equipment that accompanies the side ocular, a disc with cross lines is inserted at the proper position, as shown in Fig. 166, to indicate when the image is sharp. If the ocular is adjusted for some other camera image distance, as mentioned, the cross lines and the camera image will not be sharp simultaneously.

When it has been necessary to correct an objective by lengthening the drawtube, Sec. 65, it may be found that the weight of the camera is too great for the drawtube to support it in its raised position. The friction of the push fit, which supports the drawtube, is limited by the split collar through which the drawtube slides, and consequently it varies with the resiliency or spring in the metal of this collar and with the surface conditions of the contacting parts. When the tube is clean and slightly oily, there will be less friction, and the drawtube will be more susceptible to accidental movement. The device shown in Fig. 178 is a split ring with a screw to hold the ends together. It may be slipped over the drawtube and clamped at any point; it will act as a positive stop, and the tube will then support any eyepiece apparatus without slipping.

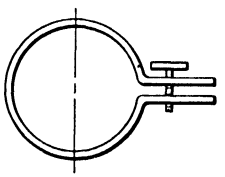


FIG. 178. A clamping ring to go over the end of the drawtube which when locked will prevent the drawtube from slipping during an exposure.

Since the weight of a heavy eyepiece camera may be more than the coarse adjustment of the microscope can support without showing perceptible creep, it may be necessary to tighten the adjustment or to clamp the main tube in place. Sometimes a short wooden or metal rod of the appropriate length can be used to support the tube, but this will put the coarse adjustment completely out of action. When such a prop is employed, one end may be placed on the stage with the other end against the lower part of the tube, or it may extend from the top of the limb to a small plate on top of the coarse-adjustment rack. A small clamp can be made to fit around the base to which the rack is screwed. In applying the clamp, care should be exercised to see that the jaws do not come in contact with a bearing surface. If the microscope is equipped with a vertically adjustable stage there is an easier method of holding the body tube firmly in place. The body tube is first racked downward until it is resting on its stop. The stage adjustment is then used for coarse focusing, thus leaving the body tube firmly seated at all times. In any event, before the body tube is locked the fine adjustment should be set midway in its travel, to give a possible focusing motion of about 1 mm either upward or downward.

If the fine adjustment creeps because of a heavily loaded tube, the maker of the microscope should be consulted.

The focus can be tested on the ground glass with a magnifying lens, and any adjustments should be made when the prism, which is used to direct part of the light into the side-tube ocular, is out of position. This prism is thrown out of position in either one of two ways:

automatically when the shutter is tripped, or manually with a release similar to the shutter release just prior to making an exposure. The prism should always be removed from the light path during an exposure.

After the camera has been set on the microscope it is always possible to check on the centration of the light source through the telescopic tube in the usual way. The centration of the condenser can be learned by the third method described in Sec. 85, that is, by lowering the condenser until the iris is brought into focus in the field of view. The side tube is of particular advantage when crystal growth is being watched, or in water examination, when photomicrographs may be wanted of some organism appearing only infrequently in the field.

The inside of the camera box must be kept very clean. The slightest speck of dust may ruin a picture, since it may fall on the accessory lens and register as a shadow on the film. The plate or film holders, also, are likely to pick up lint and small dust particles which may drop off onto the camera lens when the holder is placed in the camera.

For the most part, exposures with eyepiece cameras can be made with the shutter at some instantaneous setting. Such a quick exposure will probably prevent the picture from being spoiled by stray light. The shields recommended for the larger cameras will not usually be needed with the eyepiece camera.

Inasmuch as these cameras are fastened solidly to the microscope, any vibration to which the microscope is subjected will be transmitted to the film or plate. However, if the camera has very short focal distance from the eye point to the image plane, both the camera and the microscope may vibrate in unison, and no trouble will be experienced. On the other hand, if the camera has a rather long extension, say 10 inches, the phase of vibration at the image on the plate may differ greatly from the phase of vibration at the microscope stage, and then the image is likely to be blurred. If the work cannot be moved to a sufficiently stable foundation, it may be possible to minimize the effects of vibration by mounting the microscope and other units on a small wooden platform, such as the board used for alignment in Fig. 35. The lamp, microscope, and other units are then all on one base, and this base can be insulated against vibration rather effectively as directed in the instructions for use of the vertical camera.

The Use of the Horizontal and Universal Cameras. Directions for using these cameras should be carefully followed. The manufacturers have taken care that the directions which accompany each instrument are most precise, and faithful observance of the instructions ensures good results. However, it is always well to inspect for the centra-

tion of the various optical parts; this technique has already been described in detail.

In using a horizontal camera the absence of the microscope mirror simplifies the centering of the optical components between the lamp and the microscope. Universally, the lamp can be focused on the center of the substage and the image of the lamp diaphragm will be formed on the ground glass of the camera back. From then on, the introduction of an accessory lens, diaphragms, or filters should not cause disruption of this first step in centration. Since the use of an optical track makes lateral alignment very easy, the only adjustments of importance are those of vertical displacement, or displacement along the axis of illumination. Most of the adjustments along the axis of illumination have been figured by the manufacturer, who has also prepared scales which give definite settings for various accessories. Slight changes can then be made as they are warranted.

Horizontal optical beds are comparatively long, and the light source is very strong. This combination makes it possible to place the light several feet from the microscope and, by a properly selected system of intermediate lenses between the lamp and the microscope, to light a very large field. In an optical train of this sort, the light is often carried as a parallel beam for most of the distance, the last lens in the system acting as the secondary source. For large fields, large lenses, 5 inches or more in diameter, may be used.

In analyzing such a train of lenses and the function performed by each individual lens, it is well to bear in mind the effects of light on lenses of differing shape. By inserting a black card or diffusing plate in the beam or pencil of light, focal points can be determined; and from the negative or positive character of the lens its general effect on the impinging pencil of light can be foretold.

Horizontal cameras should be placed in good natural light or under a suspended electric lamp. Care must be exercised that the objective and observation eyepiece are not directly in the path of light from lamps or windows. Vibration dampeners being included as part of this equipment, preliminary investigations regarding local vibration need not be as searching as when a camera is mounted without such dampeners. Cameras of the universal type are not normally equipped with vibration dampeners.

It is absolutely necessary to level universal microscope cameras, to facilitate the handling of liquids on the stage of the microscope, but it is not so important to level horizontal cameras unless they are to be used occasionally in the vertical position. The legs of some hori-

zontal models have leveling screws which make adjustment very simple. In the two-table equipment of Zeiss, both tables must be level.

Sec. 111. Steps in Setting up the Microscope and Camera. A complete series of steps involving the use of the microscope and camera can now be listed; they will be found to correspond to the chart in the front of this book and with the detailed and separate description of each operation as dealt with up to this point. A quick glance at the items of operation will show that some may be omitted occasionally, whereas others must be carried through with the greatest attention. Centering operations are always critical; attention to diaphragm openings and the manipulation of the filters depend upon the individual conditions and to a certain extent on the adaptability or accommodation of the eye of the observer. However, each adjustment has already been carefully explained in detail with reference to the effect that can be expected from its observance or non-observance.

STEPS IN SETTING UP THE MICROSCOPE FOR CRITICAL OPERATION

1. *The Lamp Distance.* See Sec. 18. For illumination by Method I, 15 inches is suggested as an excellent working distance between the diffusing plate of the lamp and the microscope axis. If illumination is by Method II, the lamp distance must be such that the image of the source fills the working aperture of the front lens of the microscope condenser. Thus the distance will depend in a measure on the focal length of the lamp lens. It is suggested that such adjustments be made that the lamp distance will always be a minimum of 15 inches for illumination by transmitted light. If vertical illuminators are used, see Sec. 23, the lamp distance will be corrected accordingly.

2. *The Filament of the Lamp Centered to the Lamp Lens.* See Sec. 18. This is an important adjustment if illumination is to be by Method II; it is of only slightly less importance with other methods of illumination. After adjustment has been made the lamp should stay in alignment until removed.

3. *The Image of the Lamp Filament Centered to the Microscope Mirror.* See Sec. 20. For illumination by Method II, the image of the source must be carefully adjusted to be cocentral with the mirror. For photographic work this adjustment should be carefully made irrespective of the type of illumination. For purely visual work slight differences in centration will not be detected.

4. *Diaphragm of the Lamp Partly Closed.* This aids in obtaining a sharp image of the lamp filament on the mirror (step 3), and also in carrying out step 5.

5. *The Image of the Filament Focused on the Microscope Diaphragm.* See Sec. 18. This adjustment is vital for illumination by Method II, and for photomicrographic work in general when illumination is by Method I or III.

6. *Light Directed through the Microscope by Tilting the Mirror.* This step is performed in conjunction with steps 7 and 8. Its purpose is merely to light up the object field sufficiently to make focusing of the microscope possible.

7. *The Lamp Diaphragm Opened Wide.* This is done to aid in the quick adjustment of the mirror. Exact centration of the light in the focal plane of the camera is not attempted at this time.

8. *The Diffusing Plate and Required Filters Inserted.* The addition of filters at this time is merely a precaution to protect the eyes from strong light. No attempt is made now to select filters to promote contrast or for other photographic needs.

9. *The Microscope Focused on the Specimen.* See Sec. 59. A preliminary examination is usually made with a low-power objective, 16 or 8 mm.

10. *The Objective Centered to the Microscope Axis.* The objective in use and all other objectives on hand should be centered to the tube axis; see Sec. 62. With individual centering systems for each objective, close attention should be paid to this detail.

11. *The Microscope Condenser Focused.* See Sec. 83. Focusing at this stage may be only approximately correct for any part of the field, but slight errors have little or no bearing on subsequent adjustments.

12. *The Field Diaphragm at the Lamp Reduced to a Small Aperture.* See Sec. 83. This operation aids in focusing the condenser (step 11); focus can also be made on the rim of the lamp diaphragm. The image of the lamp diaphragm is brought into the field of view by tilting the mirror as required.

13. *The Light Source Centered in the Field of View.* See Sec. 21. It is nearly always essential to reduce the lamp diaphragm before its image can be centered in the field of view. It is easier to proceed to the next step if the light has been centered.

14. *The Condenser Centered.* See Sec. 85. For all photomicrographic work this is a most important adjustment and should never be neglected. Each change of objective demands rechecking on this centration.

15. *The Field Diaphragm Opened to Its Full Extent.* This step is part of step 14 and should be carried out as part of that adjustment. It is fully explained in Sec. 85.

16. *The Field Diaphragm Reduced to Coincide with the Field of View.* This adjustment is necessary when objectives of large aperture are used, but since it aids in the control of glare it is always a wise precaution to take.

17. *The Condenser Iris Adjusted to Control Glare.* This regulation should be made only to promote contrast (see Sec. 102) or to adapt the aperture of the condenser to that of the objective. A $^9/_{10}$ cone is desirable but not always attainable because of the glary nature of the specimen.

18. *Corrections Made to Suit Objective.* See Sec. 65. Regulation of the tube length or any correction of the objective should be postponed until all other adjustments have been made in order to get the best image which the optical arrangement of the microscope is capable of giving.

19. *When Higher Objectives are Used.* When high-power objectives are turned in after adjustments have been made for low powers, or when any change of objective is necessary, a quick recheck of steps 9 to 18 is required. These adjustments are especially necessary in photomicrography, particular attention being paid to the centration of the condenser, its focus, diaphragm adjustment of lamp and condenser, and correction of the objective.

ADDITIONAL STEPS WHEN THE CAMERA IS ADDED

20. *The Microscope and Lamp Removed to the Camera.* Any previous adjustment that has been disturbed by resetting the microscope must be corrected.

21. *The Microscope Shielded from Stray Light.* See Sec. 102. For low-power work the front lens of the objective is large and comparatively far from the specimen, and offers opportunity for the gathering of extraneous light. Such light can be blocked by means of shields.

22. *A Shield Required on the Microscope Substage.* See Sec. 102. A shield is sometimes necessary when the top lens of the condenser has been removed and the base of the microscope is reflecting light past the condenser into the objective.

23. *A Shield in the Camera Bellows.* See Sec. 102. If the exposure is long and the lighting strong, a shield in the camera bellows will increase contrast slightly.

24. *The Top Rim of the Ocular Black.* See Sec. 110. The top rim of the ocular should be black to reduce glare while focusing; this adjustment can be classed as an essential.

25. *A Hood to Shield the Objective from Stray Light.* See Sec. 102.

The lens hood is always advisable for low-power work; it helps to keep dust from the specimen during photography.

26. *A Small Shield under the Slide Which Holds the Specimen.* See Sec. 102. This refinement may be beneficial on very glary specimens, particularly at low and medium powers. The hole in the shield should be only slightly larger than the object field.

27. *The Light Source Centered on the Focal Plane of the Camera.* See Sec. 110. This is an important adjustment; unless attended to it may be a source of uneven illumination on the film.

28. *The Composition of the Picture Carefully Studied and Focused.* The picture cannot be any better than the image on the ground glass of the camera. Focusing should be carried out very carefully with a hand magnifier. The composition of the picture is of great importance, for, unless details are properly shown and stressed, by both position and focus, the picture may be a loss. Composition in this sense does not refer to the pictorial value of the finished print as in ordinary photography.

29. *Filters Added to Give Contrast.* See Sec. 101. It is not always possible to forecast the results from a given filter, even with panchromatic sensitive material. "Cut-and-try" methods may be the best way to obtain satisfactory effects.

30. *The Exposure Made.* See Sec. 117. The pilot or trial exposure should generally be in the form of a series of exposures in steps with a time ratio of 2. After development of the film, the correct exposure time can be estimated.

31. *Focus Re-examined for Each Exposure.* This precaution is always required.

Sec. 112. The Favorable Regulation of Bellows Extension. The choice of a magnification for a photomicrograph was discussed in Sec. 79, in relation to the selection of an ocular. In that section it was shown that good definition could be had for photomicrography when the magnification at the image plane was 1500 to 1600 times the N.A. of the objective, and that it was immaterial how this magnification was obtained, whether by high eyepiece and short bellows draw or long bellows draw and low eyepiece. Now particular attention will be paid to the influence of bellows draw on the finished picture, and the following statements should not be construed as contradictory to the statements in Sec. 79.

Full advantage of a long bellows draw is undoubtedly best realized when the specimen has considerable field depth and when resolution of less than 1 μ is not of paramount importance. Under such conditions

an objective of low power can be used and the total magnification can be obtained by properly balancing the magnification of the ocular and that which is produced by the bellows extension. This balance is fairly well explained by consulting Table VII, p. 77, where the size of the plate is taken into account. As shown, a 5 by 7 inch film is about the largest that can be covered sharply by standard microscope optics; smaller sizes permit considerable latitude in bellows extension balanced against ocular magnification. When the finished picture may be diaphragmed by a circular printing mask of small diameter, there is great choice of oculars and bellows draw. Since high oculars give a larger field than those of low power, when low ones are used there is likely to be a long bellows draw not only to obtain magnification but also to afford sufficient plate coverage. It is the ocular diaphragm that limits the size of the image field and not the focal length of the objective.

Preliminary study of the specimen will give information concerning the required field depth and indicate the focal length of the objective to be used, but the ultimate magnification will depend on the separation of fine detail that will be needed in the print to make it easily visible (Sec. 79). How the magnification is obtained depends on the desired size of the finished print and on how bellows draw and eyepiece power can be balanced one with another (Table XXVI and Table VI).

Table XXVI

Influence of Objective, Ocular, and Bellows Draw on Magnification

Objective Magnification	Ocular Magnification	Camera Magnification	Total Magnification
90	10	1	900
30	10	3	900

High magnification must be confined almost entirely to objects of small field depth, polished specimens, bacteria smears, very fine pigments, and similar material. Long bellows draw is always indicated in such circumstances, 30 to 40 inches being very near the upper limit. Thus ¼ to ¾ of the total magnification may depend on the camera alone. For most work it is nearer ¼ to ½, assuming that the magnification of the microscope is figured at 10 inches. High oculars and high eyepieces with a long bellows must be used for high magnification, and since there is less initial crispness of image for a high objective than for a low one, all microscope, illumination, and camera adjustments must be made very exactly.

Table XXVI does not present any new information but it stresses the advantages accruing to a photomicrographic system equipped with a long bellows extension.

The technician may feel that the combination in the last row of the table offers considerable advantage over the first combination.

If the picture is to be used for publication, it is suggested that it be taken at a magnification appropriate for use without reduction in size. The picture can be trimmed to fit a certain space, but if it is reduced or enlarged the resulting appearance will be uncertain. Unless otherwise stated, all photomicrographs in this book are reproduced from contact prints, and the reproduction is 1 : 1. Pictures made by enlargement or projection printing have great latitude in magnification at the negative owing to the different projection distances that can be used in printing. In lantern slide work, if a certain projection distance, professionally known as the "throw," is, say, 20 feet, an object should have a diameter of at least 9 mm to be clearly visible to people with normal eyesight; this is the approximate size of the Snellen test letter used by ophthalmologists. The photographic image on the film should then have a diameter of 0.23 mm if it is to be successfully projected 20 feet. The projection lens must have a focal length of 6 inches. Gage[6] gives a good account of projection.

Under normal conditions, the eye, owing to its small aperture, subtends a very small angle from any point on an object (see Fig. 23), but a microscope objective subtends a very much larger angle. This difference of aperture, which makes the high resolution of the microscope possible, gives to an object in a photomicrograph an appearance which is never duplicated by natural sight. When magnification is increased without increase in aperture, no new detail is seen; thus the slightly false appearance which is always present in a photomicrograph for the reason just stated will become even more unreal according to natural standards of vision as magnification increases. Experience and the cultivation of judgment are essential in determining the effects of magnification, camera extension, and N.A. on the finished picture. Nothing can take their place, and the significance of each factor must be thoroughly understood if photomicrography is to be rapid and accurate. With a clear conception of these factors and their relation to each other in their effect on the picture, it is easy to interpret a photomicrograph and so avoid erroneous conclu-

[6] Simon H. Gage, *The Microscope*, 17th ed., 1941. A more complete account is found in S. H. Gage and H. P. Gage, *Optic Projection*, Comstock Publishing Company, Ithaca, N. Y., 1914.

sions. Figures 31 and 32 show the effect of high magnification with too low an aperture.

Sec. 113. Photosensitive Material Especially Adapted to Photomicrography. In times past, glass plates to carry the light-sensitive emulsion were considered necessary for the best results in photomicrography. Considerable opposition was offered to the use of film for photomicrographic purposes. It was claimed that film would not lie evenly in the holder and so would cause distortion of the image, or else would throw part of it out of focus. It was also claimed that film would shrink and therefore the measured magnification on the ground glass would not correspond with the magnification of the image on the negative.

Both these arguments have been refuted by experimental evidence. As far as the question of the flatness of the film is concerned, the best way to check this condition is to pull out a dark slide and examine the film beneath. It will probably be found to be lying perfectly flat with no evidence of buckling. To avoid wasting film, this examination can be carried on in the dark room. However, if the holder has a hinged end piece, this should be held down during the examination, because this is its normal position when in the camera. With regard to the second objection, see Fig. 20. This picture was taken on film at a magnification of 1000 diameters; an application of a millimeter scale will show that a correct result has been attained. The photomicrograph shows no sign of distortion, and measurement of the spaces shows them to be just about the same as they were on the ground glass. Even the swelling and contracting of the printing paper have not materially lessened the technical accuracy of the picture. Film has proved so satisfactory that plates are fast becoming obsolete.

Modern cut film can be had from stock with almost any of the existing emulsions, and in all sizes. Film is light, easy to handle, and less fragile than glass, and it occupies less space in storage. When feasible, film holders should be employed to obviate the handling of sheaths. Film pack is not suitable for photomicrographic work except under special conditions. As a rule, it is more economical in both time and money to develop each picture as it is taken, and so to learn whether a retake is necessary.

The speed of a film is a good indication of the contrast which can be expected from it. Slow emulsions take a long exposure and give high contrast; fast emulsions with shorter exposures give less contrast. The suggested negative material for a wide range of photomicrographic work is listed in Table XXVII. As a rule, it pays to choose panchromatic emulsions since filters of suitable color may be

added to promote contrast, or for other purposes, and the photographic effects translated into black and white will be similar in effect to the contrast seen by the eye. The table also lists orthochromatic material because advantage can be taken of its limited chromatic sensitivity to make certain subjects which have been stained with red dye appear more contrasty. With a green filter the red values of the specimen will be rendered more nearly in the black.

The M plates made by the Eastman Company and the metallographic plates for photomicrographic work are still listed and are used chiefly in metallography, but this company is also listing Panatomic X, one of the later films, for photomicrographic work. Figure 179 shows the sensitivity range of Eastman plates available for scientific work.

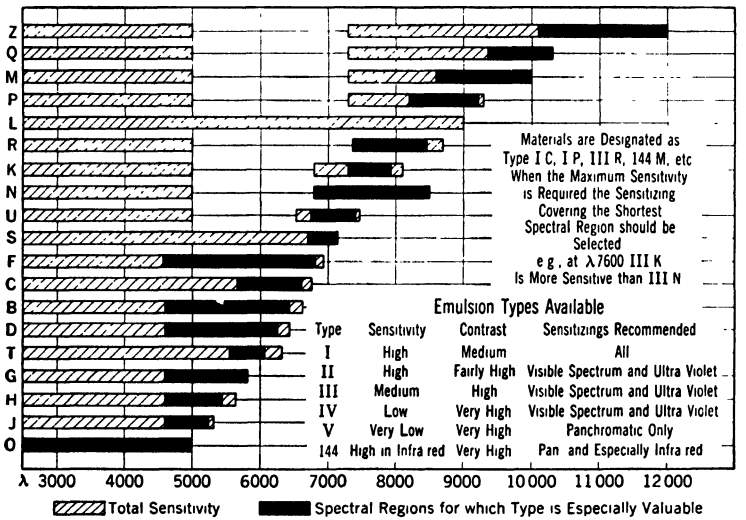


FIG. 179. This chart shows the sensitivity ranges of specially prepared Eastman plates. The emulsions can be had on film at extra cost. These plates are coated only on order. Courtesy, Eastman Kodak Company.

Sec. 114. Identification of Film in the Dark Room and Recognition of the Emulsion Side. The marks of identification which are stamped into the edge of the film are listed in the various manufacturers' catalogues. This notched edge serves as a means of identification. When working in the dark room one often needs to know which is the emulsion side. It can be easily recognized by noticing the position of the notches when running the finger along the edge of the film. When this notched edge is at the upper right-hand corner, the film will be emulsion side up. In the absence of specific marking, the emulsion

Table XXVII

American Films and Plates Suitable for Photomicrography*

Manufacturer			Contrast	Weston Rating Tungsten Light
Eastman	Agfa Ansco	Defender		
	Cut Film			
Tri X Pan.	Triple S Pan.		Soft	64
Panatomic X	Iso Port.	Fine Grain Pan.	Medium	16
Process Pan.		Process Pan.	Hard	East. 1, Hamm. 2, Def. 2
Contrast Process Pan.				8
Ortho X	Super Plenachrome Press	X–F Ortho Press	Soft	64
		Pentagon	Medium	16
	Miniature Camera film			
Panatomic X	Fino Pan.		Medium	16
Super XX Pan.	Ultra Speed Pan.		Soft	64
	Plates			
W. & W. M			Hard	8
Eastman Metallographic			Hard	4

* For special-purpose plates for infrared and ultraviolet sensitivity see Fig. 179.

side can be determined in the dark room by placing the film between the teeth; the emulsified surface tends to stick to the teeth as the mouth is opened. If the material is a glass plate, the emulsion side can be recognized by sliding the finger or thumb over the surface. There is a distinct difference in the friction set up by the back of a glass plate and the front or emulsion side.

Sec. 115. Gamma. Gamma is a mathematical term denoting the relationship between the density of a negative and the time of exposure. It is well described by Henney and Dudley,[7] and also by Neblette.[8] For a full discussion of the subject one may refer to these authorities or to a description of sensitometry by Hardy and Perrin.[9] An apparatus for making exposures to determine the gamma of negative material is excellently described by Dudley,[10] and the catalogues of various manufacturers offer suitable photometric devices for measuring the densities obtained on step tablets. A recent book by Mees[11] on the photographic process has four chapters on sensitometry and is a valuable addition to any library.

The gamma values of different emulsions, as well as time-gamma curves made with various developers, are part of the information furnished by the film manufacturers for the benefit of the consumer. A short description of how the gamma of a film may be measured is offered to make the meaning of the term plainer rather than to develop a technique for making such measurements. Although the photomicrographic laboratory cannot be expected to enter into research on its material and supplies, it should take note of such work carried on by the manufacturers of plates and films, and all data supplied with the sensitive material should be carefully studied and filed for reference.

If a film is exposed in steps or sections, so that the exposure time of each step differs from the preceding one by a certain ratio, a negative showing useful gradations of density will be obtained. The time steps are in geometrical progression. The ratio usually selected is $\sqrt{2}$. A negative exposed in this way is known as a step tablet. Since additive exposures do not give the same results on the film as continuous exposures, it is customary to have special apparatus for mak-

[7] Keith Henney and Beverly Dudley, *Handbook of Photography*, 1939.

[8] C. B. Neblette, *Photography, Its Principles and Practice*, fourth edition, 1943.

[9] A. C. Hardy and F. H. Perrin, *The Principles of Optics*, Chapter XI, 1932.

[10] Beverly Dudley, "Intensity Scale Sensitometer," *Photo Technique*, **2**, 5, pp. 18–21, 1940.

[11] C. E. Mees, *The Theory of the Photographic Process*, The Macmillan Company, New York, 1942.

ing individual exposures for each step. For this and other reasons, step tablets for the determination of gamma are best made by the manufacturer of the plate or film in question.

After the film has been exposed, each step differing in appropriate increments of time, it is developed according to a definite formula and the density of each exposure is carefully measured on a densi-

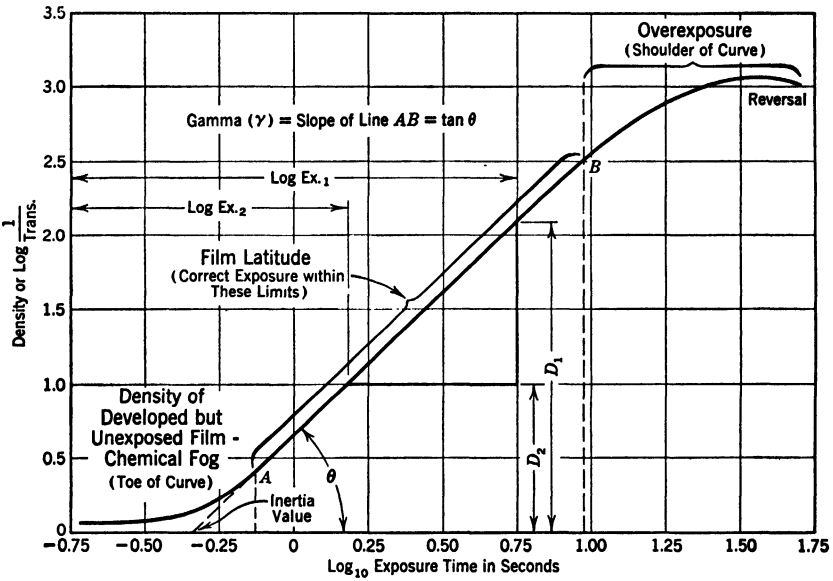


FIG. 180. A typical gamma curve. The greater the angle θ, the greater will be the developed contrast in the negative. Any type of film may be made to give curves of varying slope, depending on development and the other factors involved in processing. If the scales on the abscissa and ordinate of the curve are of equal numerical value, the slope of the straight portion of the curve can be found by measuring the angle θ with a protractor, reading the degrees, and then taking the tangent of the angle so found. The slope on the curved portion, at any point, is the slope of the tangent at that point. Several methods of determining the slope of the *H* and *D* curve are given in *Photo Technique*, January and February, 1940, in an article " Film Characteristics," signed B.D.

tometer. The transmitted light is then measured and converted into figures representing density. If the density of each step of such a negative is plotted against the logarithm of the time of the exposure, and the density values taken on the ordinate and the exposure values on the abscissa, then a curve can be drawn, a typical example of which is shown in Fig. 180. Such a curve is spoken of as an H and D curve, after the two Englishmen, Ferdinand Hurter and Vero C.

Driffield, who originated the method, or simply as the D-$\log_{10} E$ curve or gamma curve.

The toe of an H and D curve shows the under-exposed portion of the negative, and the shoulder shows the over-exposed portion. The straight-line portion represents the exposure latitude of the film emulsion. Other parts of the curve are noted in the figure. In the straight-line portion the same relationship would exist between the light and dark rendering of the negative; that is, good prints could be made from negatives having any exposure time between A and B.

The straight-line portion of the H and D curve, if continued, would form an angle θ with the abscissa. In mathematical terms the tangent of angle θ is called the slope of the line AB. The tangent value of angle θ is called the gamma (γ) of the film. As the tangent of 45° is 1, a curve the straight portion of which makes an angle of 45° with the abscissa has a slope of 1, and

$$\tan \theta = 1 = \text{the gamma of the film}$$

or, in a more general form, referring to Fig. 180:

$$\gamma = \frac{D_1 - D_2}{\log E_1 - \log E_2} \qquad [58]$$

If the development time is increased, tangent θ is increased and the gamma rises; if the time of development is shortened, tangent θ decreases and the gamma lessens. Thus, gamma can be controlled by regulating the time of the development, by changing the temperature or nature of the developer, by changing the character of the emulsion on the film, or by changing the spectral quality of the light.

A gamma of 1 signifies that the gamma of the negative corresponds with the natural contrast conditions prevailing at the point where the picture was taken. If a certain contrast prevailed at the specimen, that same contrast will have been preserved in the negative. For most photomicrographic work, particularly where the detail or particles are discrete, a gamma higher than 1 is indicated. On the other hand, the tendency is to build up, or to try to build up, too high a gamma, especially in histological work.

To avoid ambiguity, it should be noted that the terms gamma and contrast are not synonymous. If a picture were taken of a specimen of raw cotton fiber, the image would be formed almost entirely by refraction and the interior of the fiber would appear to be nearly as light as the background. The picture might be developed to a gamma of, say, 1. Suppose that the cotton were stained with a black dye and the negative again developed to a gamma of 1, the contrast in the pic-

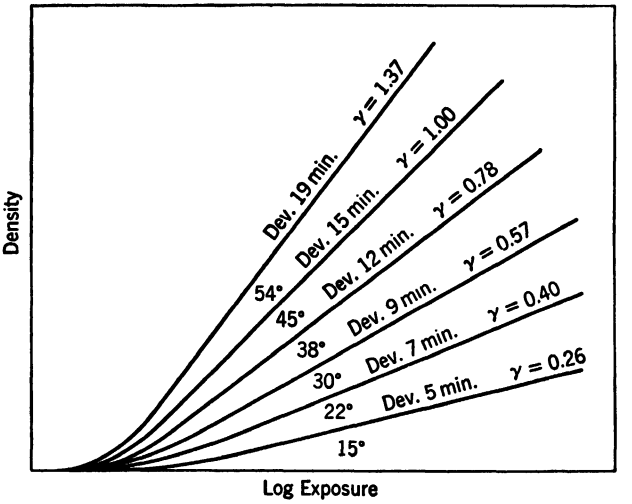


FIG. 181. A group of gamma curves obtained from one kind of emulsion by varying the time of development.

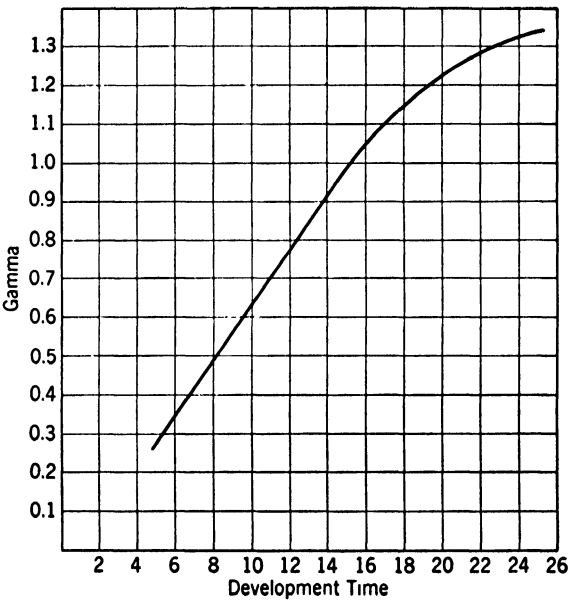


FIG. 182. A gamma-development time curve. Using one developer, gamma can be predicted with considerable certainty. This curve is the outgrowth of those shown in Fig. 181.

ture would be increased tremendously but the gamma would be the same. On the other hand, when gamma is increased, contrast increases also, for density of the negative is built up in the highlights faster than in the shadows.

If a step tablet is made and plotted as in Fig. 180, other step tablets may be made under the same conditions and developed for varying lengths of time. A family of curves such as shown in Fig. 181 will result. If a sufficient number of the curves are made, and the gamma of each determined, a curve of time of development against gamma values may be plotted, such as shown in Fig. 182. The emulsion

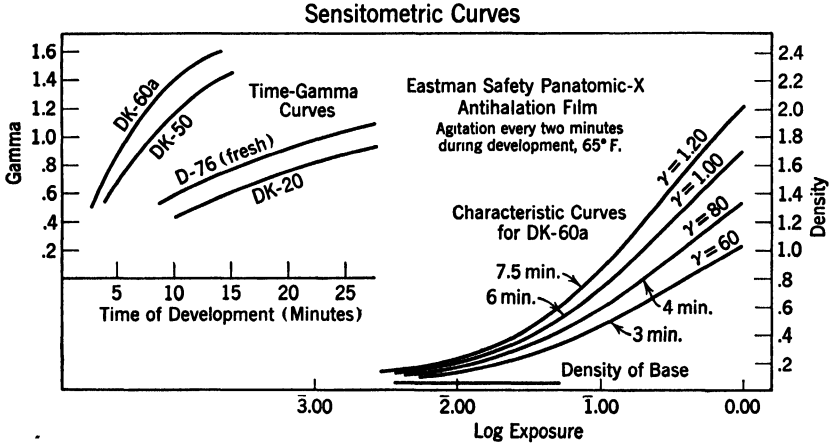


FIG. 183. Information regarding the processing of films, such as the above, can be had from the Eastman Kodak Company and other companies.

material, the temperature, and the formula for development are, of course, the same throughout. Such a curve as this indicates how the gamma may be varied for a given film by controlling the time of development.

Certain kinds of film lend themselves very readily to increase in gamma by development, and great contrast can be attained with them. For instance, it is much easier to increase the gamma of Panatomic X film by longer development than to increase the gamma of Commercial Panchromatic film. This sensitivity to increase in gamma is important in selecting sensitive material for photomicrographic work. The information furnished by the manufacturers of photographic materials generally includes not only the gamma curves of the film but also the time-gamma curves for various developers. Figure 183 is a copy of the time-gamma and gamma curves of Panatomic X film, showing the length of development time needed to obtain various gammas when

using developers DK-60*a*, DK-50, D-76, or DK-20. Particular attention is directed to the rapid increase in gamma for developer DK-60*a*. This difference in the effectiveness of different developers in controlling gamma is an important point for the photomicrographer, as it gives him an added tool for strengthening detail and improving visibility.

Sec. 116. Selecting the Type of Film. A preparation having been placed on the stage of the microscope, and a desirable field selected for photographing, the specimen must be studied before the proper sensitive material is decided upon. Consideration must be given to the degree of contrast that is wanted in the finished picture and to the way in which it can best be obtained. Assuming the specimen to be transparent and colorless, the images on the plate will be formed largely by refraction. Assuming also that the material is similar to that shown in Fig. 197*a* there will be a large number of particles which decrease in visibility rapidly as their size approaches and becomes less than 1 μ. Even though proper mounting medium has been chosen a certain number of small particles will be lost in the photographic process unless contrasty film is used. It is claimed that with proper exposure the detail on soft emulsion will equal that registered by a contrasty film. However, in actual practice it will be found that the tone of the finished picture from a negative so made will be very unpleasing and some detail that might have been registered on the more contrasty film will very likely be lacking. Figure 184 and Fig. 185 show clearly the effect produced by the selection of sensitive material with proper regard to the visibility of the specimen.

Figures 186, 187, and 188 illustrate the effect produced by controlling contrast in the picture by means of appropriate filters and film. The specimen is a piece of Dufay color film showing the réseau. The réseau is a color filter screen with minute squares dyed green and blue with a continuous line of red. A panchromatic emulsion was selected, and the first picture was taken with a daylight filter to render the colors in appropriate black and white tones. Panatomic X was used, and the resulting print is reproduced in Fig. 186, the colored lines being indicated.

The red line may be presented more strongly against its background by making it appear black or white. It can be made black either by filtering out all the red component from the illuminating light or by using an emulsion non-sensitive to red, as in Fig. 187. The red line can be made to appear white by using an emulsion sensitive to red and introducing a filter to subordinate the other colors as in Fig. 188. In Fig. 187, Ortho X film and a daylight filter were used. Figures 188

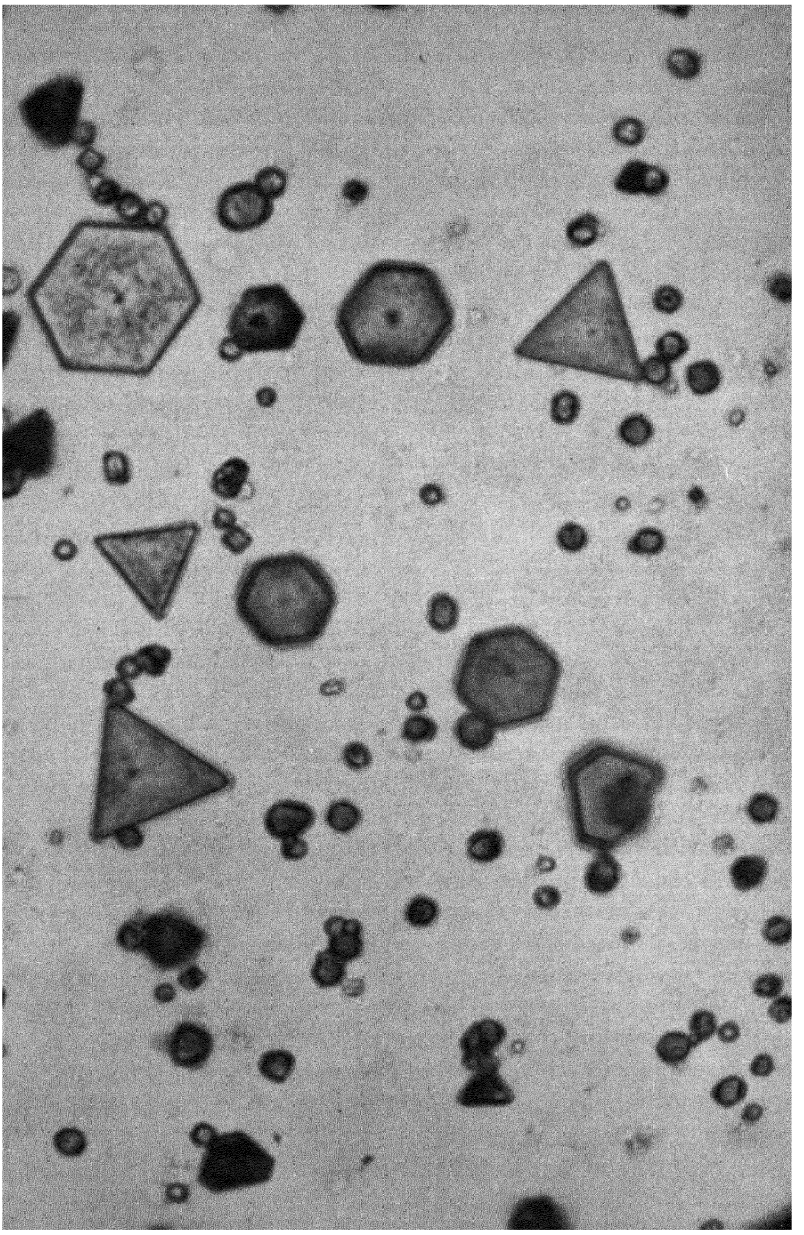


FIG. 184. Silver salts from Agfa Superpan Press film, ×3600. The salts were recrystallized from an ammoniacal solution. Cf. Fig. 185. Objective, 1.5-mm apochromat, Zeiss; ocular, Homal IV; condenser, achromatic-aplanatic, Leitz; illumination, tungsten-ribbon-filament lamp, method II; filters, Wratten 45 plus 47; Eastman Tri X Pan film; developer, D-19.

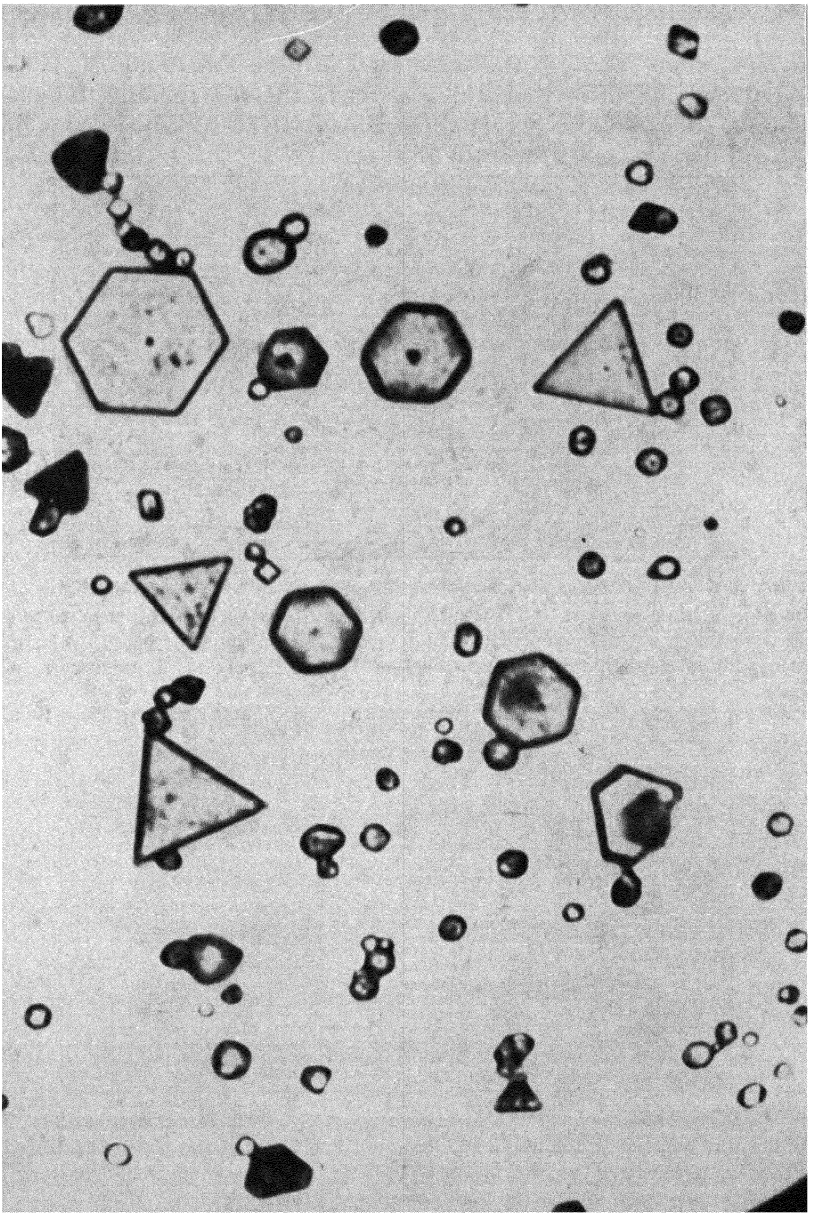


FIG. 185. Silver salts from Agfa Superpan Press film, ×3600. Cf. Fig. 184. All optical arrangements were the same as for Fig. 184 except that in this picture Defender Fine Grain Pan film was used. There appears to be a slight difference in size of the images in the two pictures, the softer film giving slightly fuzzy image outlines.

and 186 were taken on the same kind of film, Panatomic X, but for Fig. 188 a red filter was added to stress the red (making it appear white), to make the green appear black, and to darken the blue.

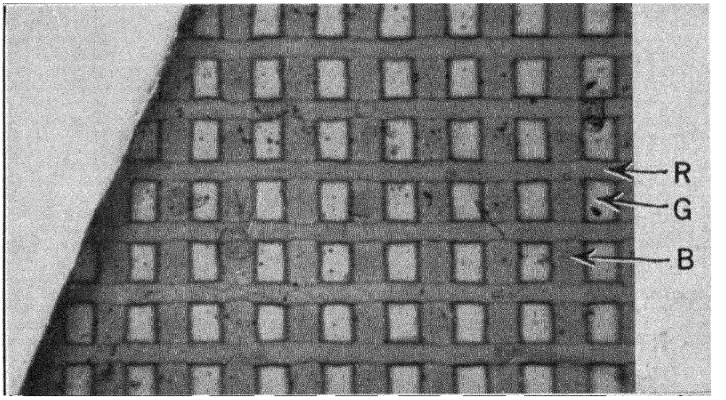


FIG. 186. Dufay color film showing réseau ×180. There is a deep shadow along the edge of the specimen. Cf. Figs. 187 and 188. This picture and the two following were taken with an eyepiece camera. Obj., 16 mm apo.; ocular, 15× compensating; filters, daylight; illumination, method I; film, Panatomic X; developer, D-19.

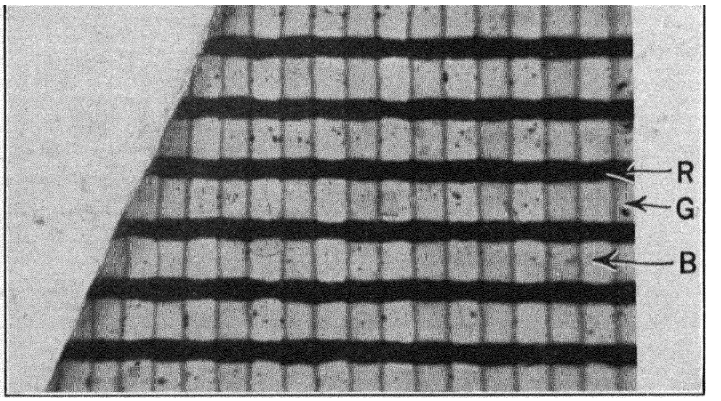


FIG. 187. Dufay color film showing réseau ×180. Cf. Figs. 186 and 188. The picture was taken with the daylight filter as in Fig. 186, but the film was changed to Ortho X non-sensitive to red. It might be noted that the shadow shown along the edge of the specimen in Fig. 186 does not appear as strongly here

By a like process of selecting film or filters to enhance or subdue certain colors, almost any photographic contrast can be obtained. For instance, a strong blue filter and a film with a regular emulsion, that is, one sensitive to neither green nor red, would make blue lines

appear white on a black background. The same thing can also be done fairly well with the regular emulsion without the blue filter. However, since the colors in the specimen probably will not be pure colors, the blue filter will aid materially in increasing contrast.

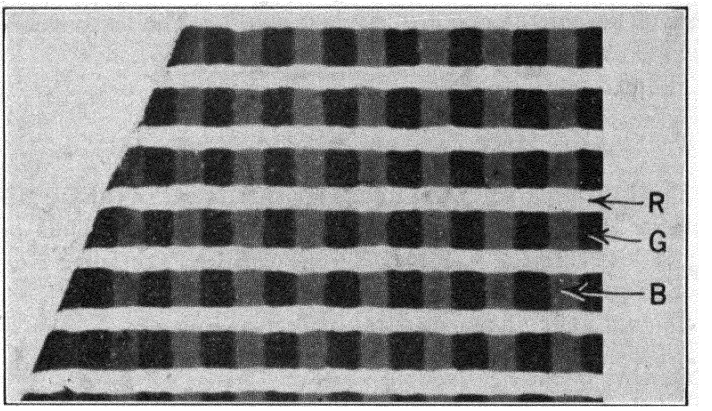


FIG. 188. Dufay color film showing réseau ×180. Cf. Figs. 186 and 187. Wratten filter 25 was used.

Sec. 117. Determining Exposure Time. The effects of varying the time for any given exposure can be studied on a carefully made test film or step tablet. The appearance of the picture may be entirely changed by increased or lessened exposure, and only exposure within the latitude of the plate will give results from which satisfactory prints can be made. Figure 189 should be carefully examined.

A good photomicrograph shows the required detail to the best advantage. The ability to judge the proper time of exposure from a film with a series of graded exposures is quickly acquired by experience. This method will save time and expense over mathematical calculations or methods involving the use of exposure meters. For those who wish to base exposure time on mathematical equations, the method of Hardy and Perrin[12] is suggested, but even here the authors say, "The illumination on the plate in this case is therefore approximately . . . in the clear areas of the specimen." Of necessity, the valuable detail of a specimen lies in areas which are not optically clear; there is certain to be absorption of light through any area of the specimen which may be selected, and this absorption cannot be taken into account mathematically. Briefly, Hardy and Perrin work out a

[12] A. C. Hardy and F. H. Perrin, *The Principles of Optics*, p. 509. McGraw-Hill Book Company, 1932.

method as follows, starting with equation 12

$$E' = \pi B \sin^2 \theta'$$

E' is the illumination in the image plane for unit area, B is the brightness of the source in candles per unit area, and $\sin \theta'$ is the numerical aperture of the microscope in the image space. The last value may be obtained from equation 30, and equation 12 then becomes

$$E' = \pi B \frac{\overline{\text{N.A.}}^2}{m^2} \qquad [59]$$

where N.A. is the N.A. in the image space. Now by taking 100 times the inertia of the film, which is in terms of lumen-seconds per unit area, and knowing the value of E', one can find the correct exposure time for a certain gamma.

The factors controlling exposure time are as follows:

1. Intensity of the light source.
2. Means of illumination or spectral quality of the light source (arc lamp, mercury lamp, tungsten lamp, etc.).
3. Modifying light filters.
4. Focal length, N.A., and type of condenser.
5. Focal length, N.A., and type of objective.
6. Focal length and type of the eyepiece.
7. Distance from the eye point to the image field.
8. The sensitivity and nature of the film emulsion.
9. The nature of the specimen.
10. Total absorption of light by all the lenses of the system.
11. Adjustment of focus and aperture diaphragms of lenses.

It is seen in the foregoing list that some of the items might be lumped up as total magnification, but obviously this would be erroneous because identical magnifications could be had with systems absorbing or reflecting different amounts of light energy. Thus, to reduce the timing of an exposure to a mathematical equation is not as simple in photomicrography as in photography, where only one lens is involved. The empirical method of making a test film, although far from satisfactory for several reasons, is certainly the most direct way of learning the proper exposure time.

Exposure meters have never been entirely successful when applied to photomicrography. In the first place, the light intensity at the image field is so weak that oftentimes an ordinary meter will not register. Moreover, on many specimens, most of the light flux that

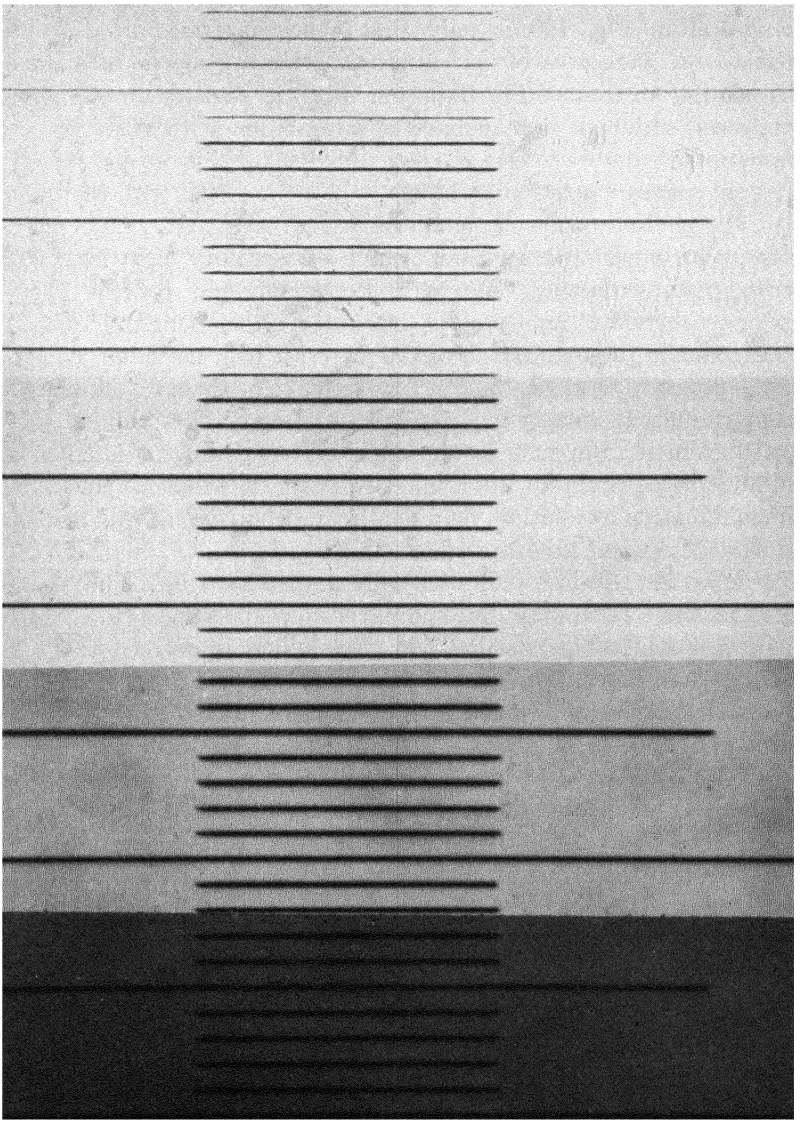


FIG. 189. Photomicrograph of micrometer scale ×330. The exposure was made in steps and demonstrates not only how a test exposure appears but also the effect of exposure time on the width of the lines.

strikes the photoelectric element is only background lighting and therefore has nothing whatever to do with the image of the specimen. The part of the illumination which is useful in making up the photomicrograph in Fig. 190 is only that which is transmitted by the particles. A large area of useless illumination appears in most image fields similar to that of Fig. 190, and this will register on the photoelectric cell although it may have nothing to do with the subject of the picture.

If light measurements must be made in conjunction with the microscope, the photoelectric cell should have high sensitivity. The galvanometer or recording meter should have a sensitivity of at least 0.035 microampere, or better. Filters and various light sources have a bearing on the use of light meters, and unless all such factors are taken into account in the calibration of the meter, it is of no value. Taking all this into consideration, the test film seems to be the most satisfactory means of judging correct exposure time. Many other things can be learned from it also, such as the distribution of the specimen in the field and the coverage of the film. Moreover, it will serve as a check on magnification, resolution, field depth, and contrast, as well as determining development time and other values.

To make the test exposure with 4 by 5, or 5 by 7 inch film, the accepted practice is to draw out the dark slide and expose the film for a period considerably shorter than that which is deemed to be the ideal exposure time. The dark slide is then returned to the film holder and another exposure is made with about three-quarters of the 4 by 5 inch film or four-fifths of the 5 by 7 inch film exposed. The time of this second exposure is always the same as that of the first one. From then on, the balance of the film is exposed in steps, the time of each being double that of the preceding exposure. Test exposures, on the same film, can be made with various filters by covering up half the film parallel to its long dimension with a piece of stiff black paper, of appropriate size, dropped into the back of the camera before the film holder is inserted. The exposures are then made as before. After one side of the film is exposed the dark slide is pushed all the way in, the film holder is taken out, and the paper is moved to the opposite side of the camera back; the film holder is then returned, another filter is selected and placed in the illuminating train, and the exposures are repeated. Four or five exposures of graduated timing, or even more, can be made on one 4 by 5 or 5 by 7 inch film, the steps being in geometrical ratio. It may be necessary to make eight or ten exposures, either as a continuation of the series or as a repetition of the series under different lighting conditions.

In making test exposures it should always be remembered that a suitable portion of the specimen must be included in each step; otherwise it will be impossible to form an adequate opinion of the correct timing.

With film as small as 3¼ by 4¼ inches there may not be sufficient space to show four different timing steps unless it is divided lengthwise as suggested above, because small areas are not always sufficiently enlightening. With yet smaller size, it may be necessary to use several films for the desired results, and one complete frame may be required for each exposure when working with 35-mm size.

Approximately the same thing can be done a little differently and more quickly by means of the Goldberg wedge. This neutral wedge is placed in front of the sensitive material and the exposure is made, care being taken that the exposure is long enough. From a study of the developed film and from the density in different parts of the wedge, the correct exposure can be calculated. This method is recommended by Zeiss.

After exposure, the test film is taken to the dark room for development. This offers an opportunity to test for the optimum development time and to study control or contrast in the film. If the regular developing time is, say, 8 minutes, the film can be removed at the end of that time and cut lengthways with a pair of scissors; a portion is then put back for further development, say for 4 additional minutes. The best developing time can be estimated from the two strips.

Although inspection of a test film shows a number of important facts, the focusing adjustment cannot be judged from such a film because there is almost certain to be a slight change in position of the plate holder in successive exposures. Correct timing is indicated only approximately, because the effect of a continuous exposure on sensitive material differs somewhat from the effect of the same total exposure time applied at intervals. It is generally necessary to make the final exposure time somewhat shorter than that deemed to be correct by inspection of the test film. For instance, if the correct time on the test strip appears to be 8 seconds obtained in perhaps three steps, the time of a continuous exposure to give the same photographic density might be only 6 seconds.

Exposures for the test film should be carried on in a methodical manner. The light switch should not be used because the lag due to the heating of the filament would account for a large proportion of the short exposure time but only a very small proportion of the longer time. It is a good idea to use a shutter or else to chop the light beam

with a dark slide held in the hand — a method that has proved very satisfactory.

A test film is an ideal means for studying the effect of exposure time on the width of the boundary lines. Figure 189 shows that measurements are remarkably constant between intervals when measured from the side of one line to the corresponding side of another, although the lines vary tremendously in width, being wide for the under-exposed and narrow for the over-exposed portions. The true appearance of the lines is shown at the center section; the section exposed for the shortest time and that exposed for the longest time do not give a true idea of the appearance of the lines or an accurate measure of their width. In order to select the time of exposure which renders the lines with the greatest fidelity, it will be necessary to study their appearance by actual inspection through the microscope, for only by comparing the appearance of the field of view with the appearance of the negative is it possible to judge the accuracy of the photographic process in any given circumstance. In this respect, it might be remembered that the width of the lines shown in an image formed largely by refraction is controlled also by the mounting medium and by the numerical aperture of the system. The fact that the lines constitute single discrete objects, as in Fig. 189, and happen to be absorption images, does not make the example of any less value. The boundaries of a transparent crystal with image lines formed by reflection and refraction would show the same results as far as sensitivity of line thickness to exposure time is concerned. Thus, since the correct registering of image lines is an important factor in a photomicrograph of high fidelity, the optimum exposure time is vital to a good picture.

No one plate or film is capable of dealing with all the gradations of contrast that may arise in general photomicrographic work; neither can one developer or one time of development satisfactorily handle all negatives. Therefore, the interpretation of the test film is the photomicrographer's greatest aid in determining exposure time, a proper developing time, and the appropriate developer.

Sec. 118. Graininess. Freedom from graininess is often a basis for selecting certain sensitive material, particularly in work requiring a high magnification. The use of the 35-mm cameras, with their necessary accompanying negative enlargement in printing, has brought forth much discussion on the cause and control of grain size. Many developer formulae have been offered to the public as panaceas with the claim that they will banish all troubles due to grain.

Basically, the crystals of silver salts composing the emulsion of negatives are very small, only a few microns in diameter. They are

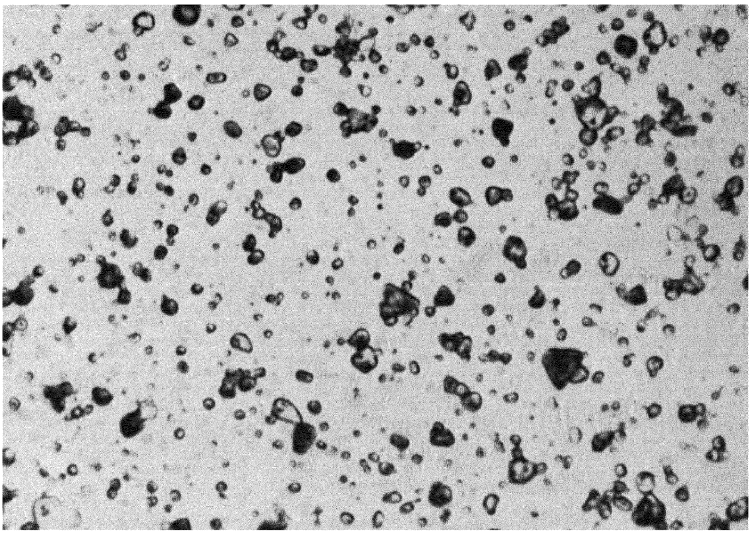


FIG. 190. *A*. Photomicrograph of silver salts used in the emulsion of Agfa film, ×2500. It is possible to find occasional well-formed crystals, but for the most part the silver salts appear as shown in this picture and the two following. Objective, 2 mm apo., N.A., 1.4, Bausch and Lomb; ocular, Homal IV; condenser, achromatic, Leitz; illumination, tungsten-ribbon filament, 6-volt lamp, method II; filters, Wratten 45 plus 47; Defender Fine Grain Pan; developer, D-19.

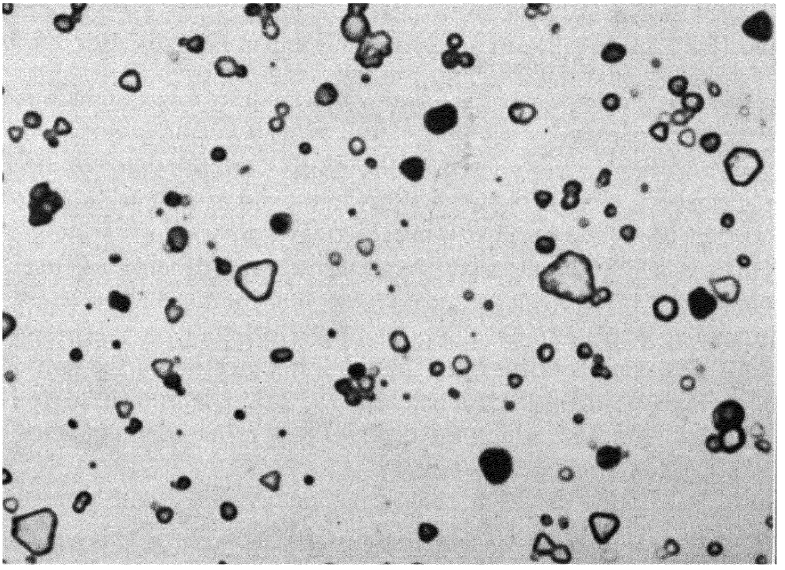


FIG. 190. *B* is the same subject at a magnification of 3500×, the extra magnification being attained by using a longer bellows extension.

shown in Fig. 190, taken at a magnification of 2500. After the negative has been exposed and developed, the small grains of metallic silver as shown in Fig. 191 lie dispersed through the thickness of the gelatin layer making up the emulsion. These perfectly discrete particles of

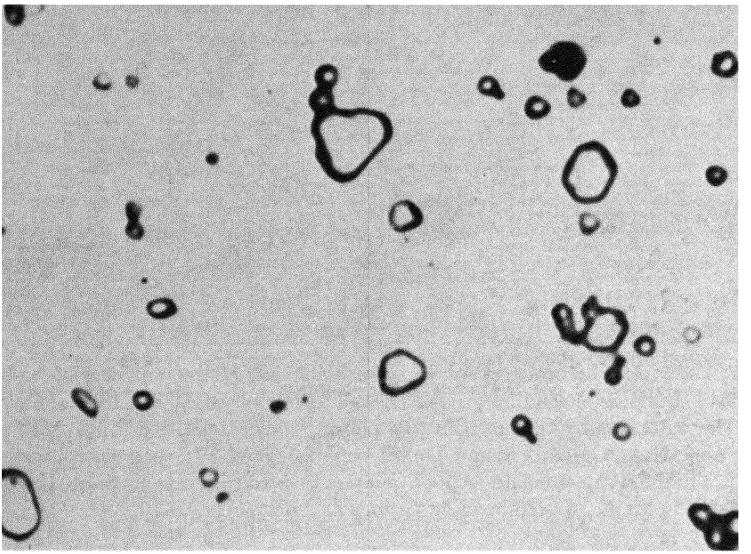


FIG. 190. *C* is the same subject at an even higher magnification, 5000×. In *C* a change was made to a 1.5-mm apochromatic objective, with a N.A. of 1.3, Zeiss. The reproduction at *B* is probably the best picture of the series; it is only part of the original 5 by 7 negative which is completely and sharply covered from corner to corner.

silver are in themselves so small, and they overlap in the emulsion to such an extent, that in a dense film they present a layer almost impenetrable to light.[13] These small grains, the size of which varies but little with different negative material and is only slightly influenced by development procedure, do not in themselves cause the graininess so objectionable in the enlarged print or on the projection screen. The trouble arises from the clumping together of the individual metallic silver particles. An example of grain is shown in the enlargement in Fig. 193, where the graininess is so much in evidence that the effect of the whole print is ruined.

Grainy structure is, as a rule, much in evidence with fast emul-

[13] A study of silver grains has been made by C. E. Hall and A. L. Schoen, of the Eastman Kodak Company: "Application of the Electron Microscope to the Study of Photographic Phenomena," *J. Opt. Soc. Am.*, **31**, 281, 1941. Photomicrographs showed the developed silver grain to be filamentous rather than a solid mass.

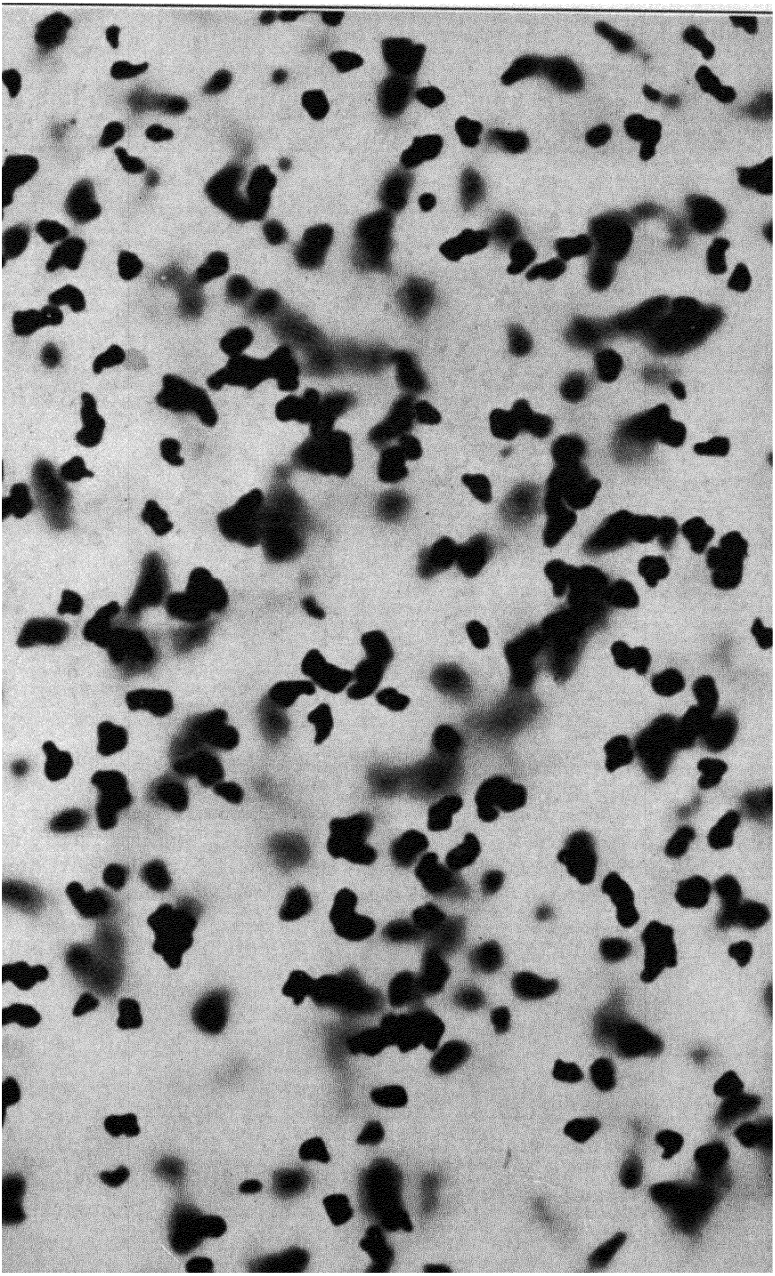


FIG. 191. Photomicrograph of silver grains near the surface of a negative, ×3600.

sions and dense negatives; the processing of the film also has considerable influence on the grain since fast or over-development and the use of developers intended to promote high gamma tend to enhance graininess. The newer emulsions, particularly those on 35-mm film, however, seem to be extremely free from grainy appearance. Cer-

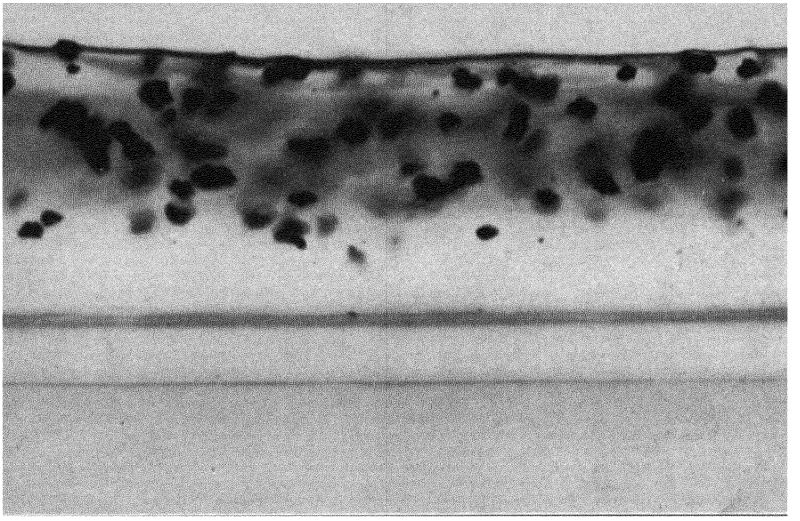


FIG. 192. Cross section of photographic negative film ×1200. The top portion is the gelatin layer; it shows the metallic silver grains. The middle strip is a foundation layer found on most film. The bottom of the picture represents the film base. Objective, 4-mm apochromatic, Zeiss; ocular, Homal III; condenser, achromatic-aplanatic Leitz; illumination, ribbon filament lamp, method II; filters, Wratten 15 plus Corning 428; Eastman Contrast Process Pan; developer, D-19.

tainly, it seems unnecessary to resort to so-called soft, fine-grain, or grainless developers simply to avoid this trouble. Faster developers with more alkali will produce better results for photomicrographic work, since contrast in negatives usually needs to be built up rather than toned down.

Sec. 119. The Theory of Photographic Development. *The Film.* Successful photomicrography depends to a great extent upon the selection of film emulsions and developers that will attain optimum results under given conditions. In many arts and sciences, photography is a specialized tool of rather narrow scope; often all that is needed is one type of plate or film, one light source, one developer formula, and one grade of paper. In fact the whole procedure often can be so standardized that all the photographic work can be considered a cut-

and-dried routine. With photomicrography the situation is different. The technician must be readily conversant with various photosensitive materials and methods of processing them. Films with several different photographic characteristics should be at hand, and the operator should be familiar with many different developers suitable for special circumstances. Many good books and articles on separate phases of the subject are available,[14] some of which have already been mentioned.

The active component of the emulsion of photosensitive negative material is composed largely of silver bromide with a small proportion of silver iodide; the positive emulsions, which act more slowly and are used on photographic paper, are composed largely of silver chloride with various mixtures of silver bromide. Figure 190 illustrates the halide salt crystals as they appear in a photographic emulsion before development. Gelatin is the medium used to carry the silver salts and to disperse them evenly over the transparent plate or film. This layer of gelatin with the silver salts constitutes the light-sensitive emulsion. Gelatin, which is permeable to water and so to the various developing agents and fixing solutions, is transparent and can be easily handled to coat any base.

In addition to the silver salts, other chemicals can be employed to promote sensitivity and restrain fogging. The emulsion is generally treated to withstand a certain amount of heat, so that the film is easier to handle and store and its keeping qualities are thereby improved.

For a film, the base on which the emulsion is applied is generally either cellulose nitrate or the less inflammable cellulose acetate. Other coatings may be used in addition to the emulsion. The most important is probably a backing applied to the side free from emulsion to render *the film* halation proof; it serves to absorb the light which having passed through the emulsion layer would otherwise be reflected back again into the emulsion. Figure 192 shows another coating applied between the outer layer of emulsion and the base. Non-curling properties may be given to a film by an additional coating applied to the back, thus ensuring equal shrinkage on either side of the film.

Action of Light on the Emulsion. Light rays from the specimen, after passing through the lens, fall on the photosensitive emulsion,

[14] L. P. Clerc, *Photography, Theory and Practice,* 1937; A. P. H. Trevelli and S. E. Sheppard, *The Silver Bromide Grain in Photographic Emulsions,* 1921; L. A. Jones, *Photographic Sensitometry,* Eastman Kodak Company; *Elementary Photographic Chemistry,* Eastman Kodak Company; E. R. Bullock, *Chemical Reactions of the Photographic Latent Image,* Eastman Kodak Company.

producing a pattern of light and dark areas corresponding to the formation of the image. In those portions where the light strikes the emulsion an invisible change takes place on or within the minute individual silver salt grains. Not a great deal is known about this change. Perhaps it can best be described as a physicochemical action. The tiny points of alteration on the silver salts can be thought of as incipient nuclei without which development cannot proceed. The emulsion which has been acted upon by light in this way constitutes the latent image. Those areas of the latent image which have received the most light produce more metallic silver when developed than those portions which have received less light or radiation. Thus the latent image follows in detail the image pattern as projected by the lens, and the developing process only serves to complete and make visible, in the negative, the image detail already recorded.

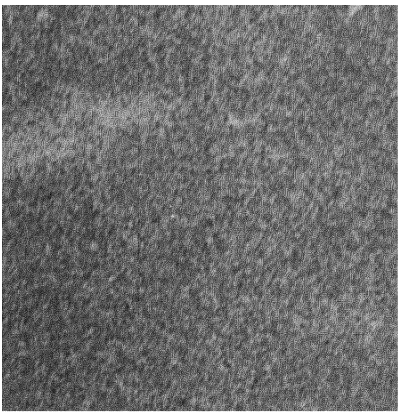


FIG. 193. This picture shows the difficulties caused by a grainy negative. The silver particles have clumped together to such an extent that a good print would be impossible to obtain from such a negative.

Development. The chemical action of the developer on the silver salt grains varies somewhat with the active agent in the developer, but the important product of the reaction is always the metallic silver precipitate from the silver salt. This silver precipitate is black, not a white lustrous silver. It is analogous to platinum black and other pure metals which can be obtained in similar form, the black form indicating a submicroscopic spongy surface structure. By reflected light, the black metallic silver deposit on a negative appears as tiny silvery bright points, when inspected under the microscope.

After the film has been treated with the developing solution, in darkness, it generally contains much active material which is still photosensitive and which must be removed before exposure to the light. Accordingly, the film is passed to a second solution containing sodium thiosulphate (hypo), which dissolves and washes out the portions of silver salts which have not been acted upon previously by light. The hypo solution is then removed from the film by washing the negative in water.

Below are listed a few of the many chemicals capable of reducing the silver salts; they are the most important ones for general use.

1. Amidol. A strong developer. Used alone, not in combination with other developing agents.
2. Elon (metol). A soft working developer, generally used with hydroquinone.
3. Pyrogallic acid (pyro). A medium working developer giving good contrast. The developed negative is stained a light yellow — deeper in the denser portions. This developer tends to increase contrast. Keeping qualities are poor when mixed with other chemicals. Can be used alone or with hydroquinone.
4. Glycine. A fine-grain developer; can be used alone.
5. Hydroquinone. One of the most popular developing agents, generally used with elon.
6. Paraphenylenediamine. A soft-acting fine-grain developer, used largely for small film.

These developing agents are listed in descending order of their ability to act as reducers.

Many other chemicals act as developing agents, as any comprehensive book on photography will show, but for photomicrographic work they are unnecessary. Those listed above are the old stand-bys and usually are all that are needed in the photomicrographic laboratory. Since there are many trade names for these six developers the chemical names of each are given:

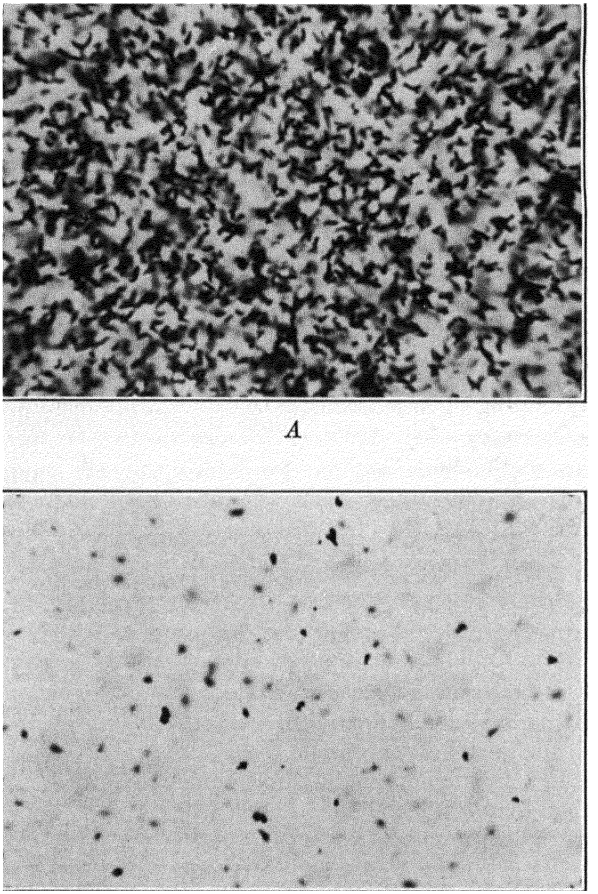
Elon: *p*-methylaminophenol sulphate.
Hydroquinone: *p*-dihydroxybenzene.
Pyro: 1,2,3,-trihydroxybenzene.
Amidol: 2,4-diaminophenol hydrochloride.
Glycine: hydroxyphenyl aminoacetic acid.
Paraphenylenediamine: the same.

It is necessary to add other chemicals to the developing agent in order to promote the indicated chemical reactions. As the bath must be alkaline, either sodium carbonate or borax is usually added to establish a satisfactory *p*H value and hasten development. If great developing energy is required the formula may call for sodium hydroxide. Because the alkaline chemical accelerates the developing action, it is often termed the *accelerator*.

The proper *p*H of a developing solution is important from a chemical standpoint, but the accelerator also is important because it softens the gelatin layer and makes it easier for the developing solution to soak completely through it. Figure 192 shows the typical thickness of the

gelatin layer of a process film, and also to what extent the developing solution must penetrate.

A third component for a well-balanced developer is a chemical to



A

B

FIG. 194. *A*. Photomicrograph of negative ×900. The film was not exposed, but it was developed in a developer without potassium bromide. This development of an unexposed portion of a film would ordinarily be called chemical fog. Compare with *B*, where all conditions were the same with the exception of the addition of a proper amount of bromide to the developer.

act as a preservative. All developing agents are reducing agents. Consequently they themselves are easily oxidized. In order to prevent rapid oxidation of the developer from air-borne and dissolved oxygen, sodium sulphite is added. This chemical, itself something of

a reducing agent, acts chiefly to prevent the rapid oxidation of the developer. It is called a *preservative.* The only other preservative of importance is sodium bisulphite, which sometimes is included in the formulae for standard developers; it is called for in formula D-61*a*.

Potassium bromide is a fourth component found in nearly all developing formulae. Bromide is a *restrainer,* restraining development of that portion of the film which has not been acted on by light. The whole process of development depends upon the selective action of the developing solution. If the solution were to develop all parts of the film alike, irrespective of the portions which have received light, then the developing process would simply produce a perfectly homogeneously darkened film without a trace of image formation. Many chemicals act in just this way. It is easy enough to reduce the silver salts to metallic silver, but for photographic purposes the reduction must be selective, the development must occur only where the light pattern has fallen, and only in proportion to the amount of light received in the various areas. The main action of potassium bromide is to promote selective development by controlling chemical action on the silver salts which have not been affected by the light. Thus, chemical fog, which is always present to a greater or less degree, is considerably lessened when the proper amount of potassium bromide is contained in the developer. However, when it is added the time of development must be increased. Two strips of film were developed without being exposed. They were photographed and are shown in Fig. 194. Strip *A* was developed in a solution containing no restrainer; strip *B* was developed in a solution with the restrainer added. The amount of fog produced because of lack of bromide is tremendous.

Sec. 120. Mixing and Storing Developer. If a considerable amount of photographic work is to be attempted, chemicals for the developer, short stop, and fixing bath should be bought in sufficient quantity. Since, sometimes, two or three different developers may be required to suit varying conditions, it is convenient and economical to have all the dry chemicals at hand and to mix the solutions as wanted, to ensure freshness. The essential chemicals for processing film are listed below; tentative amounts are suggested for a balanced stock. All the chemicals listed have good keeping qualities.

In powder form, chemicals can be kept almost indefinitely in a dry place. It is suggested that they be removed as needed from the container in a small scoop made of plastic material, procurable from a five-and-ten-cent store. As all formulae call for the weighing of the ingredients, a set of scales should be included in the equipment. A

CHEMICAL	POUNDS
Elon or metol	1
Hydroquinone	1
Pyrogallic acid	1
Sodium sulphite	5
Sodium carbonate	5
Sodium bisulphite	1
Potassium bromide	¼
Borax	5
Additional for Fixing	
Sodium thiosulphate	15
Potassium alum	5
Chrome alum	5
Glacial acetic acid	5

set somewhat larger than is usually associated with photographic work is recommended. The dishes on the pans may be of plastic, which is in no way attacked by the chemicals and is very easily cleaned.

Photographic formulae are always given in a sequence which should be carefully observed in mixing. Directions for mixing chemicals for either the developer or the fixing bath sometimes call for water at a temperature of 125° F. This temperature should never be exceeded. Photographic formulae are given in two systems of measure, the British and the metric. It makes little difference which is employed provided that it is followed consistently. For convenience and to eliminate error, all graduates and thermometers should conform to the system that has been adopted.

After photographic solutions have been mixed, they can be kept in glass bottles in a cool place until needed. Developer once used should not be returned to the stock solution but should be discarded.

Brown glass bottles are frequently recommended as proper receptacles for storing photographic solutions because they afford protection from the light. They are, however, difficult to see through, and the condition of the contained solution cannot be conveniently ascertained. Also it is not easy to tell when such bottles are really clean. For practical work, the white glass bottles will be found more convenient in many ways and quite satisfactory if kept where the light is not very strong.

Sec. 121. Developer Formulae. The formulae suggested for Eastman film used according to the directions specified are given below. However, these formulae have become so well known and are so satisfactory that they are recommended for film of any make.

EASTMAN FORMULA D–1

Stock Solution A	*Avoirdupois*	*Metric*
Sodium bisulphite	140 grains	9.8 grams
Pyro	2 ounces	60.0 grams
Potassium bromide	16 grains	1.1 grams
Water to make	32 ounces	1.0 liter
Stock Solution B		
Water	32 ounces	1.0 liter
Sodium sulphite, desiccated	3½ ounces	105.0 grams
Stock Solution C		
Water	32 ounces	1.0 liter
Sodium carbonate, desiccated	2½ ounces	75 grams

In all these formulae desiccated sodium carbonate is specified. In the list of chemicals suggested for stock, monohydrated sodium carbonate was included. The monohydrated is a more stable form, and in formulae calling for the desiccated sodium carbonate the figures for the carbonate can be increased by 14.5 per cent and the monohydrated carbonate substituted.

Formula D-1 keeps well when made up in the three solutions as above, but it oxidizes rapidly when the solutions are combined and exposed to air. For consistent results, a small quantity should be mixed fresh for each film. For a 5 by 7 inch film, 30 cc of each of *A*, *B*, and *C*, with the addition of 210 cc of water, makes plenty of solution to be used in a 5 by 7 inch tray. After use it is discarded. The developing time may vary from 5 to 7 or more minutes.

This developer gives a stained image because of the presence of the pyrogallic acid. The stain is deeper in the part of the image which is the densest; therefore the effective gamma of the negative can be increased by increasing the developing time. This is true also of other developers. However, D-1 has the additional advantage of the stained image.

EASTMAN FORMULA D–9

Stock Solution A	*Avoirdupois*	*Metric*
Water at about 125° F, 52° C	16 ounces	500.0 cc
Sodium bisulphite	¾ ounce	22.5 grams
Hydroquinone	¾ ounce	22.5 grams
Potassium bromide	¾ ounce	22.5 grams
Water to make	32 ounces	1.0 liter
Stock Solution B		
Cold water	32 ounces	1.0 liter
Sodium hydroxide	1¾ ounces	52.5 grams

The bottles should be well shaken before using. Equal parts of each are taken to make up the developer. This developer gives high

contrast and is intended for process film, but it can be used to advantage whenever the contrast produced by developer D-19 is not sufficient.

EASTMAN FORMULA D–11

	Avoirdupois	*Metric*
Water at about 125° F, 52° C	16 ounces	500.0 cc
Elon or metol	14 grains	1.0 gram
Sodium sulphite, desiccated	2½ ounces	75.0 grams
Hydroquinone	130 grains	9.0 grams
Sodium carbonate, desiccated	360 grains	25.0 grams
Potassium bromide	70 grains	5.0 grams
Cold water to make	32 ounces	1.0 liter

This developer is used without dilution for high contrast; to reduce the contrast it can be diluted with an equal volume of water. The Eastman Company recommend it particularly with process film, but it is good with any film for photomicrographic purposes. The time of development is from 5 to 8 minutes.

EASTMAN FORMULA D–19

	Avoirdupois	*Metric*
Water at about 125° F, 52° C	64 ounces	2 liters
Elon or metol	128 grains	8.8 grams
Sodium sulphite, desiccated	12 ounces, 360 grains	384.0 grams
Hydroquinone	1 ounce, 75 grains	35.2 grams
Sodium carbonate, desiccated	6 ounces, 180 grains	192.0 grams
Potassium bromide	300 grains	20.0 grams
Cold water to make	1 gallon	4.0 liters

This developer, like D-11, can be used undiluted for maximum contrast. It is recommended particularly for process film, but for photomicrographic work it is an excellent developer with any film. If the film is to be subjected to enlargement, D-11 or D-61*a* may give better results because of less grain. The developing time varies from 3½ to 7 or 8 minutes, depending on the contrast desired.

EASTMAN FORMULA D–61*a*

	Avoirdupois	*Metric*
Water at about 125° F, 52° C	16 ounces	500.0 cc
Elon or metol	45 grains	3.1 grams
Sodium sulphite, desiccated	3 ounces	90.0 grams
Sodium bisulphite	30 grains	2.1 grams
Hydroquinone	85 grains	5.9 grams
Sodium carbonate, desiccated	165 grains	11.5 grams
Potassium bromide	24 grains	1.7 grams
Cold water to make	32 ounces	1.0 liter

This is an excellent developer for many classes of work and for many kinds of film. It should be diluted with an equal volume of water for tray development. The time of development can be varied over a considerable period, from 7 to 14 minutes, depending on the contrast desired. Panatomic X can be made to give relatively high gamma by long development in D-61*a*. It is convenient to have D-61*a* as stock solution always in readiness, since its keeping qualities are excellent.

EASTMAN FORMULA D–76

	Avoirdupois	*Metric*
Elon or metol	116 grains	8.0 grams
Sodium sulphite, desiccated	1¼ ounces	400.0 grams
Hydroquinone	290 grains	20.0 grams
Borax	116 grains	8.0 grams
Water to make	1 gallon	4.0 liters

This is a so-called fine-grain developer. It may be used when enlargements are needed and a rather soft negative is not objectionable. It is used without dilution.

The above six developer formulae should easily take care of all average work. The most important and useful to have on hand are D-61*a* and either D-11 or D-19. The other developers apply only under the special conditions noted.

During the winter there is little trouble in maintaining the accepted optimum developing temperature, which is 65° F. As a rule, the workroom is but little warmer, and if necessary the time of development can be adjusted to suit slight difference of developing temperature. As the temperature of the developer rises, the time of development should be reduced. Table XXVIII gives a scale of reductions and increases of developing time for temperatures other than 65° F. The developing solution should be brought to 65° F and turned into the tray. If, during work, the temperature of the solution should rise above 70° F, the small tray can be placed within a larger one containing water and ice cubes. A few trials will indicate about how much ice is needed to keep it at approximately 65° F. If more convenient, the developer in the tray can be placed in an ice chest and cooled as required. Since developer in the tray generally needs cooling rather than heating, it is sometimes rather difficult to keep it at a constant temperature. In a large photographic laboratory it is possible to have facilities for temperature control that are entirely beyond the scope of a small establishment; but even in the small laboratory it is quite possible to keep the developing temperature

Table XXVIII

Change in Developing Time to Compensate for Change in Developing Temperature

(Reproduced through the courtesy of Henney and Dudley, *Handbook of Photography*.)

t	Time at Temperature t / Time at 65° F
62	1.15
63	1.10
64	1.05
65	1.00
66	0.95
67	0.91
68	0.87
69	0.83
70	0.80
71	0.77
72	0.74
73	0.71
74	0.68

within practical working limits, by exercising a little ingenuity. That it is important to make this effort is beyond question, for time and temperature control are paramount factors in processing modern sensitive material.

If the developing temperature is 70° F instead of 65° F, the correct developing time at 65° F should be multiplied by 0.80, as the table shows, to find the correct time of development at 70° F. The authors of the book from which this table is taken state that the table is fairly accurate for hydroquinone and paraphenylenediamine developers.

Since developers can be used practically interchangeably with any make or grade of film, the choice of a developer depends on the desired gamma and visibility of the specimen. The film selected may be Panatomic X, as chosen for the photomicrograph reproduced in Fig. 195. Here, the cross section of hair is moderately contrasty and has good visibility. The photomicrographic procedure was therefore as follows. After a field had been selected the camera with the microscope was set up and a magnification of 360 diameters was chosen. A trial exposure was then made in the form of a step tablet or test film. See p. 455. Since no particular difficulty in attaining contrast seemed apparent, developer D-61*a* was chosen. The test film was split lengthwise, and both parts were put into the developing bath together. One strip was removed after a period of 8 minutes; the other remained 3 minutes longer. After fixation, the two parts were compared, and the third exposure time of 8 seconds was decided upon, with an intermediate developing time of 9 minutes.

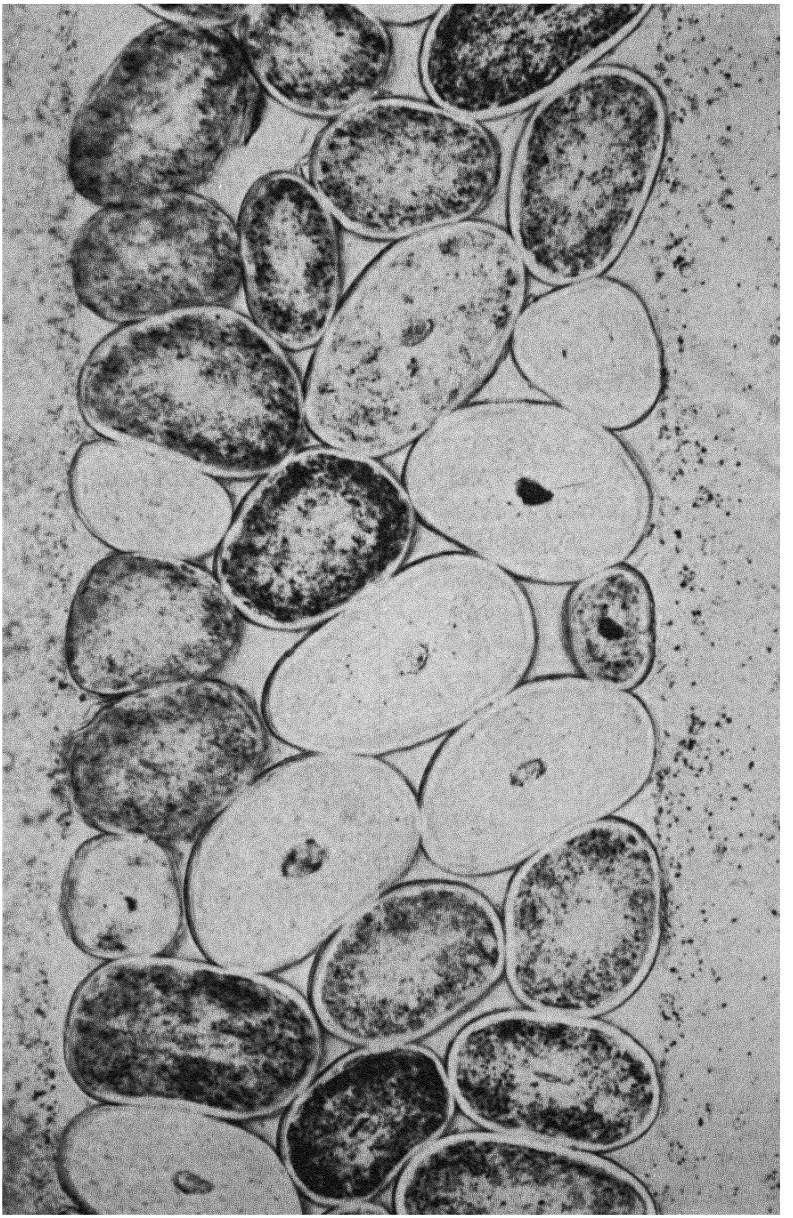


FIG. 195. Cross sections of human hair ×340. Obj., 16 mm apo.; ocular, Homal I; condenser, Leitz achromatic, top lens removed; illumination, 250-watt projection lamp, method I; filter, Wratten 66 plus Corning 306; Eastman Panatomic X film; developer, D-61a.

The above example has been discussed because it exemplifies average conditions of contrast. It shows that if a series of trial exposures is made, an appropriate film selected, and developing time determined experimentally, each picture can be made to emphasize any desired detail. It also shows that there can be no special optimum time of exposure or development as there can be for a photograph of, say, a street scene. In a street scene, the subjects may be varied. A horse and cart, a man, or an automobile may form a part of the picture, and yet the correct exposure time can be found for the general view. However, taken separately as individual close-ups, each subject might demand a different exposure. Photomicrographs are analogous to the close-up in ordinary photography. In photomicrography, the exposure must be made to show the nature and detail of all parts of the specimen.

Sec. 122. Short-Stop and Fixing Baths. After development the film is transferred to a basin or tray of water, swished around in it for a few seconds, drained, and passed to the short-stop or hardening bath. The water for washing should be freshly drawn for each film, or, if convenient, the film can be held under the cold-water tap for 3 or 4 seconds, being reversed occasionally to let the water run over both sides. The sudden difference in temperature between the tap water and the developer does not seem to cause trouble such as reticulation, or cracking of the gelatin layer. A formula for a stop bath for negatives follows:

STOP BATH

	Avoirdupois	*Metric*
Water	32 ounces	1 liter
Chrome alum	½ ounce	14 grams
Acetic acid (glacial)	¼ ounce	8 cc

In the summer time, or when the wash water is likely to be warm, the film should stay in the stop bath for at least 2 minutes; a little more than this does not seem to harm it. Otherwise it can be removed at the end of about 30 seconds and passed to the fixing bath. The use of the short stop is urged. It increases the useful life of the fixing bath by neutralizing the alkalinity of the developer and by soaking out and displacing a considerable part of the developer carried over in the gelatin emulsion. The fixing bath for both films and papers can be made according to the following Eastman formula. The stock quantity of 1 liter is especially convenient.

EASTMAN FIXING BATH F-1

Solution A	*Avoirdupois*	*Metric*
Water	32 ounces	1 liter
Sodium thiosulphate	8 ounces	240 grams
Solution B		
Water at about 125° F, 52° C	2½ ounces	80 cc
Sodium sulphite, desiccated	½ ounce	15 grams
Acetic acid, 28%	1½ ounces	43 cc
Potassium alum	½ ounce	15 grams

After solution *B* has cooled, it can be added to solution *A*.

Fixing a negative consists of washing out from the emulsion all the by-products formed by the chemical action of the developer on the silver salts, and of dissolving the remaining silver salts that have not been affected by the light. When the unaffected silver salts have been removed by the fixing solution, the negative loses its milky appearance. If the fixing bath is strong and fresh, fixing is completed as soon as the milky appearance has disappeared. However, if the fixing bath has been used once before and is therefore of uncertain strength, it is a good idea to let the negative remain in the fixing tray as long again as it took the milky effect to disappear. This is always a safe rule to follow, because, if fixation is not complete, the negative will not keep well, and a sojourn in the fixing bath a little longer than is necessary cannot harm it.

Of the various tests for checking the strength of the fixing bath, measuring its electrical conductivity or its change in specific gravity is suggested. Probably the most practical test is to drop a few pieces of film into a bath which is thought to be in poor condition. An undue length of time taken to clear the film is an indication of exhaustion of the bath. Since there is considerable difference in fixing solutions and in the length of time they take to clear film of different make, it is a good idea to standardize on one particular formula and on one particular kind of film for testing. The film need not be exposed and developed. It can be taken from the box and dropped into the freshly made fixing bath. A standard time for the disappearance of the milky condition is then noted. If the bath has reached a point of exhaustion when the clearing time is, say, 10 minutes, this can be set as the limit for clearing, and as soon as the bath is weakened to this extent it should be discarded. A chemical test given by Henney and Dudley is to wash the test strip thoroughly in running water, then to immerse it in a 1 to 3 per cent solution of sodium sulphide. Discoloration of the strip in the sulphide solution indicates a spent fixing bath.

The stop bath should be discarded after one day's use, or sooner if it is used frequently enough to lose its acidity quickly. The fixing bath can be kept indefinitely and used until exhausted.

After fixation, the film must be washed, preferably in running water. If the water is used freely, and some of the overflow escapes through the bottom of the tray or washing box, the hypo should be fairly well removed in about 15 minutes; longer washing will do no harm. A well-hardened film will stand a wash water of 80° F or even higher without frilling. Drying can be hastened by hanging the film in a draft of warm air.

Sec. 123. Dark-Room Technique for Film. Film is best loaded in the dark room without the aid of a safe light. The technician soon learns to manipulate both the film and the holder without loss of time. A set of prearranged moves is easily learned; its methodical performance soon becomes so familiar that an expert can load the holder as quickly in total darkness as in the light.

Since developing is chiefly a time and temperature process, it may as well be carried on in total darkness without even a green safe light. The method recommended is to place the film for development in a 5 by 7 inch tray and to set this tray into another, larger one to catch any of the solution which may slop. After immersion, the film is held by the thumb and forefinger of the right hand while the first three fingers of the left hand are passed across the surface of the film to break loose any adhering air bubbles. A stop watch is started, and a focusing cloth or other cover is thrown across the tray holding the film. The tray can now be rocked gently. Agitation of the film in the developer should be carried on systematically and in a similar way for each film; otherwise unaccountable differences in gamma may appear. If needed, a low-power lamp can be turned on. However, this is risky because there may be a few pinholes in the cloth cover. From time to time the stop watch can be consulted by means of a small pocket flash lamp. When the indicated time has elapsed, the film is taken from the tray and quickly dipped into a basin of wash water, then transferred to the stop bath and fixing bath.

When development is carried on in complete darkness, there may be some difficulty in determining the length of time necessary to complete the process. To a large extent this will have to be determined by empirical methods. If tests for developing time, based on information supplied by the makers of the film, have been made on the step tablet, as recommended in Sec. 117, they will serve as a guide in determining the optimum developing time.

Some idea of the rapidity of development of various parts of the

image can be gained by watching the process of development when a print is made from a negative. A perceptible decrease in the rate of development will be evident throughout the process until finally no further change is noted over a period of half a minute or so. To force development beyond this point will result in lost detail in the dark portions, analogous to over-development of the negative. As with the print, there is a time in the development of every film when the image will seem to remain at its best for a space of half a minute or so, but the period at which this takes place will vary with even slightly differing conditions.

Each time the film is transferred from one bath to another, with the exception of the wash bath prior to the stop bath, the surface of the film is gently rubbed with the fingers. This surface agitation is of considerable help in promoting even chemical action in both the stop bath and fixing bath. Those who may be susceptible to metol poisoning should use a cotton swab held with film tongs to wipe over the film surface. Rubber gloves are often worn in the dark room to prevent staining the fingers.

The hypo can be used repeatedly when the film has been processed as directed. It may become a bluish color from a carry-over of chrome alum from the stop bath, though this discoloration can to a large extent be avoided by washing the film between the stop bath and the hypo bath. However, this is hardly necessary.

The stop bath also should be agitated somewhat, and the film wiped over as previously directed. If the stop bath is fresh, the film should be sufficiently hardened in about a minute. Longer soaking, unless excessive, will do no harm. The fixing bath requires but little agitation provided that the film was wiped over when immersed. As fixing baths become exhausted, a scum may form on the surface of the film; but, if a relatively small number of negatives are being processed, the bath should remain in good condition long enough to complete the process. Fifteen minutes will usually be sufficient for the film to become perfectly fixed. If longer than this is required the bath should be renewed. Methods for estimating the fixing time were discussed in Sec. 122 as well as a method for checking depletion of the bath.

If several negatives are to be washed at a time in running water, care should be taken that each remains separate. Negatives have an undesirable way of collecting into a corner of the wash tank, one film slipping over another, so that the under film is likely to receive less fresh water than the top one.

Medium-size glass tubing can be bent to form a U with one arm

longer than the other. A little ingenuity will indicate how this can be used to siphon the water from the bottom of the tank. Fifteen to twenty minutes' washing time under running water should be sufficient, but longer will do no harm.

After washing, but while still in the wash tank, the film should be wiped over carefully on both sides with a tuft of cotton. If quick drying is wanted, the film can again be wiped over with a piece of cellulose sponge or chamois after removal from the wash tank, then hung up to dry on a line by means of a clip. Wiping to avoid spots does not seem to be as important for the cut film as for the roll film. For some reason the 35-mm roll film seems to be particularly susceptible to water marking unless it is wiped before it dries.

Trays several sizes larger than the film are generally the most convenient to work with, especially for short-stop, fixing-bath, or wash tank. The solutions required in photography are not expensive, and generous use of them is advised. For most photomicrographic work the 5 by 7 inch tray will be ample for developing; the 9 by 11 inch size is advised for stop bath and fixing.

Sec. 124. Papers and Printing. Printing papers for photomicrographic purposes are rather easily selected. For the most part there is not much choice in surface; nearly everyone agrees that it should be glossy. The glossy surface with its fine rendering of detail lends itself exceptionally well to scientific work.

The glossy-surface papers are obtainable in six grades of contrast: 0, 1, 2, 3, 4, and 5, and in double and single weights. All these grades in the desired weight should be carried in stock by the laboratory planning to do photomicrographic work. The No. 0 grade has the fastest emulsion and therefore tends to give soft effects; the No. 5 is the slowest and gives contrasty effects. Ordinarily No. 0 paper should be used with negatives of high contrast, No. 5 with those of low contrast, and intermediate numbers with negatives of intermediate contrast. So-called normal contrast is obtained with No. 2 paper. It is considered that this grade used on a properly exposed negative will render the same contrast in the print as was originally present in the specimen. Under photomicrographic conditions, however, it may sometimes be desirable to depart from the customary rules governing the choice of printing paper. As an instance, a contrasty negative having a stage micrometer as a subject could well be printed on a No. 4 or 5 paper to give additional contrast. Thus it is apparent that choice of an appropriate grade of printing paper and its developer offers an opportunity for further control of contrast.

Although the selection and manipulation of the print concludes the

photomicrographic process, it is nevertheless important because incorrect procedure at this point may completely ruin what would otherwise be a perfectly good picture. The results of entrusting a photographic negative to a regular photographic studio for printing have often verified this statement. Unless the photographer is familiar with the purpose and aims of photomicrography, he may not understand what is wanted, and so it will be only by chance that he is able to turn out a satisfactory print. If it is necessary to have extra prints made by a photographic studio, a sample print should accompany the negative.

In photomicrography, experience is, after all, one of the prime necessities, and nowhere is its absence or presence more evident than in making prints. Fortunately, in this particular part of the work, experience can be gained cheaply and quickly. It is well to follow a regular routine and, by empirical methods, discover just what grade of paper will give the best results for any given negative. Since there is no way to place a mathematical value on a finished print, its excellence must be judged entirely by the technician's opinion. His opinion, in turn, will automatically be based on his knowledge of the subject in question and his knowledge of photomicrography in general. The procedure can be explained, but it is the man who does the work who must decide on the end point.

Exposing the Paper. Printing can be done either in a printing box or in a printing frame. The frame is to be preferred because, with it, " dodging " can be resorted to, to intensify or to hold back certain areas of the negative. Even for scientific photographs this practice is perfectly permissible. The printing frame should be at least one size larger than the negative to make the use of shields or masks easier. Often, the circular mask may be used, and if the printing frame is large enough it is possible to move the mask to various positions on the negative.

The light used for printing should be of about 100 watts, placed at a distance of 3 or 4 feet. Nearly all negatives made by professional photographers are favored in the printing in one way or another. A popular device is a knitting needle with a piece of paper on the end, trimmed to conform to the shape of the section which it is desired to hold back. At a time in the printing determined by trial, the paper on the end of the needle is moved between the light source and the negative, casting a shadow on the negative. The more experienced photographer will get the same result by shading the negative with his finger. Since the image is not interfered with in any way by such a practice it is perfectly permissible.

Test Prints. Strips can be cut lengthways from various grades of paper, and test exposures can be made and developed. The grade of paper and the exposure time which seem to give the desired results can then be selected. It is not wise to economize here. Paper is inexpensive; much time, labor, and money may have gone into the picture up to this point; therefore, a few additional cents spent in experimenting with various grades of paper may make just the difference between an excellent job and a mediocre one.

Developing the Print. The developing can be carried on in rather a strong red or yellowish red light, so that the appearance and final development of the image can be easily watched. Simply drawing the ordinary yellowish window shades is often all the darkening a room needs in order to print.

If portions of the print appear to be under-exposed, it is possible to intensify them by swabbing over with undiluted or warm developer during the developing process.

The difficulty of maintaining the developer for printing at a sufficiently low temperature can be overcome by placing the tray in the ice box for a few minutes, or the developing tray can be placed in a larger tray with ice cubes and water, as required. The temperature of the developer should always be known within a degree or two.

The developing tray for paper should be larger, in proportion, than that used for film, because, usually, a large number of prints are made at one time, whereas perhaps only one or two films are developed at one session. The 9 by 11 inch trays are recommended. The larger amount of developer ensures more uniform development of each print and the solution will not become exhausted so quickly.

The two following developing formulae for paper will probably suffice for most types of work, and frequently one will be found sufficient.

EASTMAN FORMULA D–72

Stock Solution	*Avoirdupois*	*Metric*
Water at about 125° F, 52° C	16 ounces	500.00 cc
Elon or metol	45 grains	3.1 grams
Sodium sulphite, desiccated	1½ ounces	45.0 grams
Hydroquinone	175 grains	12.2 grams
Sodium carbonate, desiccated	2½ ounces	67.5 grams
Potassium bromide	27 grains	1.9 grams
Water to make	32 ounces	1.0 liter

Use 1 part developer and 2 parts by volume of water at 65° F.

EASTMAN FORMULA D–73

Stock Solution	*Avoirdupois*	*Metric*
Water at about 125° F, 52° C	16 ounces	500.0 cc
Elon or metol	40 grains	2.8 grams
Sodium sulphite	1 ounce, 140 grains	40.0 grams
Hydroquinone	155 grains	10.8 grams
Sodium carbonate, desiccated	2½ ounces	75.0 grams
Potassium bromide	12 grains	0.8 gram
Water to make	32 ounces	1.0 liter

Use 1 part of developer to 1½–2 parts of water, by volume, at 65° F.

The directions for the above developers call for a time of about 45 seconds; however, the time is largely a matter of judgment. As the developing progresses, a stage appears to be reached where further sojourn in the bath does not produce a further darkening of the image. At this point, when all possible detail has been developed, the print should be removed.

After development, the print is passed directly to the stop bath. Here, also, it is possible to control locally some of the developing action. A corner of the print that has not developed as far as the rest can be given additional treatment by being held back for a moment or two before the whole print is slid into the stop bath.

The stop bath is composed of water and acetic acid, according to the following formula:

EASTMAN FORMULA SB–1

	Avoirdupois	*Metric*
Water	16 ounces	500.0 cc
Acetic acid, 28%	¾ ounce	24.0 cc

The print can remain in the stop bath for 2 or 3 minutes, the minimum time being 15–20 seconds. From the stop bath it goes to the fixing bath, or hypo bath, a formula for which was given on p. 477. An ample supply of the hypo solution should be used. The minimum size for trays to take 5 by 7 inch prints is 9 by 11 inches, and a larger tray is better. The prints can stay in the hypo for some time without harm. Special care should be taken to agitate them from time to time. The common tendency is to slip ten or twelve prints into the hypo in such a way that the prints first introduced do not come into contact with a sufficient amount of fresh solution and consequently do not receive an equal amount of fixation.

Washing the Print. After fixing, which generally takes about 15 minutes, the prints must be washed. The technique observed for

washing the negatives applies here. As before, at least part of the water should be drawn off from the bottom of the tank. Regular print washers provide sufficient change of wash water, but, if the washing is to be done by placing the prints in a tray or basin and running in a hose, care must be taken that the bottom water does not become stationary. Because of the nature of the base upon which the sensitive emulsion is placed, prints need to be washed much longer than film to rid them of the fixing solution. About 1 hour in the wash tank will be needed for prints made on single-weight paper; double-weight paper will require about ½ hour longer. To test wash water for traces of hypo the following solution can be used:

REAGENT FOR TEST FOR RESIDUAL HYPO

Stock Solution	*Avoirdupois*	*Metric*
Potassium permanganate	4 grains	0.3 gram
Sodium hydroxide	8 grains	0.6 gram
Distilled water to make	8 ounces	250.0 cc

One cubic centimeter of the solution is added to 250 cc of water, and a few drops of the wash water are allowed to fall into the solution. If even a small amount of hypo is present, the solution will turn orange; otherwise the color will remain unchanged.

The fact that the fixing solution is an electrolyte makes it possible to test the wash water of either films or prints by measuring the electrical resistance, but this measurement is not as simple to make as the chemical test. Several small kits for the chemical test are on the market, among them the Hypo-Sharp,[15] which is on sale at most photographic supply stores. The general procedure is to add to a given amount of water some of the chemical supplied with the kit. Into the solution a few drops of the wash water are allowed to drip from the corner of the print. A color reaction determines the result.

Drying the Print. In order to get full benefit from the glossy surface of the paper, the prints must be dried on ferro-type tins. The old-fashioned kind with a coating of enamel has now been generally superseded by a type finished in chromium plate. The cost of these modern plates is considerably higher, but they need practically no care. Before use the surface can be swabbed off, under running water, with a piece of cotton. The prints are then applied, and rolled or squeegeed on. The backs of the prints should be wiped carefully with a towel to remove surplus water and hasten drying. Small portable

[15] Hypo-Sharp can be obtained from the manufacturer, R. P. Cargille, 118 Liberty Street, New York City.

electric heaters may be useful to shorten the process, but care should be taken that the prints are not overheated or they may have to be soaked off the plates. It is well to let the prints dry a short time on the tins at room temperature before they are subjected to additional heat.

LABORATORY WORK

Exp. 1. Method of Making a Test Exposure. With test slide 3 on the stage of the microscope, make a test exposure as follows: Withdraw the dark slide from the film holder until the edge of the slide is even with the edge of the holder. If a picture is made while the dark slide is in this position, the whole surface of the film will be exposed but a small strip of the edge of the dark slide will still be inserted in the holder.

With a scriber or the point of a knife, scratch a line across the dark slide at the point where it enters the holder. Divide the remaining portion of the slide into even parts. For the 5 by 7 inch holder, the slide can be divided into five intervals. Counting the part of the slide already in the holder this will make six intervals in all.

For a 4 by 5 inch, a 3¼ by 4¼ inch, or a smaller holder, the slide can be divided to suit. Each interval must be large enough to make sufficiently big areas for the purpose of comparison. With 35-mm film, one complete frame can be used as a single exposure area.

When ready, withdraw the slide from the holder and make the exposure for, say, 2 seconds. With the scribed side uppermost, push the slide back into the holder until the second scribed line coincides with the edge of the holder. Repeat the exposure, using the same timing, 2 seconds. Again push in the slide to the third scribed line, and make an exposure of 4 seconds. Continue in this way, making each exposure twice that of the preceding one, until the dark slide is finally pushed home. On a 5 by 7 inch film, exposed at the above ratio, five areas would be obtained with an exposure time of 2, 4, 8, 16, and 32 seconds, respectively. With this wide range, there is an excellent chance that the correct timing will appear at some interval on the film, so that the required exposure can be judged approximately. Figure 189 shows how a test film should appear.

The film can now be developed all in one piece, or, even better, it can be developed for its normal time, say 7 minutes, and then removed from the bath and cut lengthways with a pair of scissors. One portion is then returned to the tray to develop 50 per cent longer, making the whole developing time for this half 10½ minutes.

After development and fixing, examine the different sections of the negative in a good light, and select as correct for timing and developing that portion which imparts the most information, especially considering contrast and detail. The personal judgment of the technician is vital in obtaining a good picture. He must know beforehand what is to be shown, and he must be able to recognize it in the negative. The background should be dark enough to

appear nearly white in the print, and detail in the specimen must not be blocked out by over-timing.

In making the exposure, do not turn the light switch but rather interpose a piece of black cardboard, if the camera has no shutter, and use a stop watch if one is available. If the correct time of exposure seems to be, say, 16 seconds, it may be found necessary to cut this time by even as much as 4 or 5 seconds, because continuous exposure time has a greater effect on the sensitive material than intermittent timing; this must be decided in individual exposures. In different sections of the film, note that the images formed by refraction have lines of varying width, the under-exposed portion of the film having the wider lines. Also, the images often show a fuzziness which is sometimes caused by motion of the film holder as the dark slide is pushed into the different positions. Do not confuse this with poor focusing.

Exp. 2. Use of the Focusing Glass. Focus the microscope on the ground glass of the camera, using any convenient test slide.

With the ground glass of the camera removed, focus the aerial image by means of the focusing glass. Hold the focusing glass in the focal plane of the camera, and, when the image has been made sharp, move the focusing glass along the axis of the system. Considerable depth of focus will be found. If possible, double the camera extension, and notice that the focal depth has increased.

Theoretically, there is only one point at which the microscope image will be at its sharpest; but practically there is a wide vertical range over which the eye fails to see any difference in image sharpness. This is due to the small angle subtended by the image rays at the focal plane; as the bellows extension of the camera is increased, this angle becomes smaller, and the apparent focal depth is increased. See Sec. 65 and equation 52.

Exp. 3. Selection of Film. To demonstrate the comparative amount of fines in a powder material, take a preparation of evenly dispersed, finely ground material, such as test slide 3. This material is suggested because it is moderately fine, contains some opaque and some transparent particles, and is sensitive to the use of color filters.

Study the preparation under the microscope. Notice that it has good visibility when mounted dry. Observe its appearance when mounted in paraffin oil or balsam.

Select a magnification to show good comparison between the percentage of fine and large particles. Photograph the specimen with Tri X Pan and W & W. M plates or contrast film. Use a medium developer such as D-61*a*. Take particular care to determine the best setting of the condenser diaphragm to give proper detail to the large transparent particles.

Use Method I for illumination. Try a second slightly oiled ground glass at the lamp to help eliminate the effects of the glass structure in the final image. If the 16-mm objective is being used, remove the top lens of the condenser, or else oil the condenser to the slide. As an example, refer to the photomicrograph in Fig. 184 which has been taken on Tri X Pan and to Fig. 185, taken on Defender Fine Grain Pan film.

In a picture of slide 3, as large a field as possible is desirable, the aim being to show many representative particles, each standing out separately and distinctly. A good magnification seems to be around 350 diameters; less would make the detail very small. Such a picture does not present a problem in resolution, but it does show the necessity for keeping the magnification high enough to afford easy and correct interpretation of the finished picture.

This particular subject begins to present a problem in field depth with the use of lenses of aperture greater than 0.65. In fact, sharp definition is just beginning to be lost on some of the large particles. However, with the eyepiece camera, good pictures can be made with the achromatic 8-mm objectives. Those who are working with the eyepiece camera may find it difficult or impossible to duplicate results attained with the long-focus camera, but they should have no trouble in making negatives which can be enlarged to give comparable results.

Exp. 4. Demonstrating the Value of Panchromatic Material. With Panchromatic-X film, take a photomicrograph of test slide 4, which has the Dufay color film as a specimen. The magnification is immaterial. Arrange the illumination to have approximately daylight quality. Take another photomicrograph of the same slide using Ortho-X film. Compare the two finished pictures. Consider the reason for the difference in appearance. Take a third picture using any film with little or no color sensitivity except to blue light.

Exp. 5. Advantages of Selecting an Adequate Developer. Take two photomicrographs of test slide 6 on Panatomic-X film; develop one in D-76 and the other in D-19. Compare the two negatives, and note the superior printing qualities (greater contrast) obtained with developer D-19.

Exp. 6. The Observation of Vibration. Fill a small cell of any depth with water. Any of the various types available for the examination of fluids will answer. Build up the depth of water in the cell until the drop becomes unstable. The vertex of the drop will then be above the cell walls. On the surface of the drop place a few grains of carbon black, and observe it at low or medium magnification. Note that under ordinary conditions vibrations originating within the building or from the street become evident as they are translated to the tiny particles on the surface of the water.

QUESTIONS

1. What type of camera is the most convenient for general photomicrographic work?
2. When is the horizontal camera most useful?
3. When can the inverted type of microscope be used, and with what type of camera?
4. When is the use of the 35-mm camera indicated?
5. Why are 5 by 7 inch negatives generally more useful than smaller ones?
6. How is the side-tube telescopic eyepiece adjusted for use?
7. Can the side-tube attachment be used for a camera of any size?
8. What are some of the advantages of the eyepiece camera?
9. Describe the installation of a vertical camera.

10. What steps can be taken to guard against vibration?

11. Are any cameras equipped with vibration-elimination devices?

12. Is a long bellows draw essential?

13. What is the advantage of a long bellows draw?

14. Is there a limit to the length of the bellows draw?

15. How would you test a microscope-camera assembly for vibration?

16. What care should be exercised in protecting the microscope from stray light when taking a picture?

17. Describe the purpose and position of some of the shields used with the microscope and camera.

18. Is it always necessary to use all the shields mentioned?

19. What care should be observed in focusing the image with Homal lenses or other lenses with bright tops?

20. Describe what care should be taken with reference to the light source when taking a picture.

21. If the drawtube of the microscope slips during exposure, what remedial steps can be taken?

22. If the microscope body tube slips during exposure, what should be done?

23. What are the ten essential steps in setting up the microscope?

24. What other nine steps are called for when the camera is used?

25. In selecting bellows extension and magnification of the picture, what points should be observed?

26. What is the maximum magnification for which nearly all objectives are figured?

27. Is it permissible, in photomicrographic work, to exceed the theoretical limit of optimum magnification?

28. Are glass plates necessary for photomicrography?

29. Is it practical to make all your negatives on films or plates of one grade?

30. What are some of the important films or plates for photomicrography?

31. What is the relationship of the speed of a film to contrast?

32. What is meant by the term gamma?

33. Is gamma synonymous with contrast?

34. As a rule, should photomicrographs be of low or high gamma?

35. How can the gamma of a negative be controlled?

36. How would you proceed in making a negative with a gamma of 1.0?

37. How would you set about increasing the contrast of a picture?

38. Describe different ways of increasing contrast.

39. Are exposure meters of value in photomicrographic work?

40. If light measurements are to be made with the microscope, what care should be taken in selecting a galvanometer?

41. Describe in detail the method of making trial exposures on 4 by 5 inch film; on 35-mm film.

42. What can be learned from the inspection of a trial exposure?

43. What do you understand by the term graininess?

44. How can graininess be controlled?

45. What is meant by the following photographic terms: reducer, accelerator, preservative, restrainer?

46. Is it practical to attempt general photomicrographic work with the intention of using only one developing formula?

47. Why are several developing formulae suggested?

48. When is the use of a formula such as D–11 or D–19 indicated?

49. When is freedom from graininess especially desirable in a film?

50. Name at least one important chemical in each of the following photographic groupings: reducer, accelerator, preservative, restrainer.

51. What is the purpose of the fixing bath?

52. What is the correct temperature for developing?

53. What is the best method for testing the strength of the fixing bath?

54. Describe the different general methods of controlling contrast in the finished print.

55. How many grades of paper are generally available for printing?

56. Describe the full photographic process, in general, from the exposure of the film to the drying of the finished print. Mention all steps involved, and roughly outline the reason for each.

CHAPTER VII

MOUNTING MEDIA, STAINS, REAGENTS, AND SOLVENTS; THEIR USE AND APPLICATION IN PHOTOMICROGRAPHY

In this chapter, the subject of mounting media is treated rather fully, but stains, reagents, and etching agents will be less completely discussed. The reason for the difference is that the selection and use of mounting media fall entirely within the province of the photomicrographer, whereas exhaustive treatment of the other topics mentioned may properly be left to experts in those particular subjects. However, the photomicrographer should have a general bird's-eye view of the possibilities of all mounting methods as applied to various branches of medicine, mineralogy, metallography, and other scientific studies, to the end that he will be able to adopt or adapt, wholly, in part, or in combination, the various techniques of staining, etching, chemical reaction, or crystallization for his own purposes. A few basic references are provided to serve as starting points to a more thorough knowledge of microscopical technique relating to various separate branches of science.

Sec. 125. The Purposes of Mounting Media. Liquids, resins, or melts are used as mounting media in photomicrography for the following purposes:

1. To increase the visibility of the specimen. Visibility will increase as the difference in index between the mounting medium and specimen increases.
2. To increase the transparency of the specimen, as will occur as the indices of mounting medium and specimen approach each other.
3. To give maximum field depth. Field depth of the mount is increased by a mounting medium; it will vary directly with the index of the medium.
4. To aid in decreasing glare through the elimination of reflection.
5. To make the use of high apertures possible.
6. To increase the viscosity of the liquid phase of a specimen. A mounting liquid may also be used as a diluent of a liquid specimen and as a means of slowing down or stopping the motion of small bodies in mechanical suspension. Glycerol is particularly suited for this purpose.

7. To act as a preservative of certain specimens, as in prepared slides, for record or for future study.

8. To determine refractive index. It is then generally known as a refractive-index liquid.

Mounting media are naturally divided into two major classes: those suitable for temporary mounts and those suitable for permanent mounts.

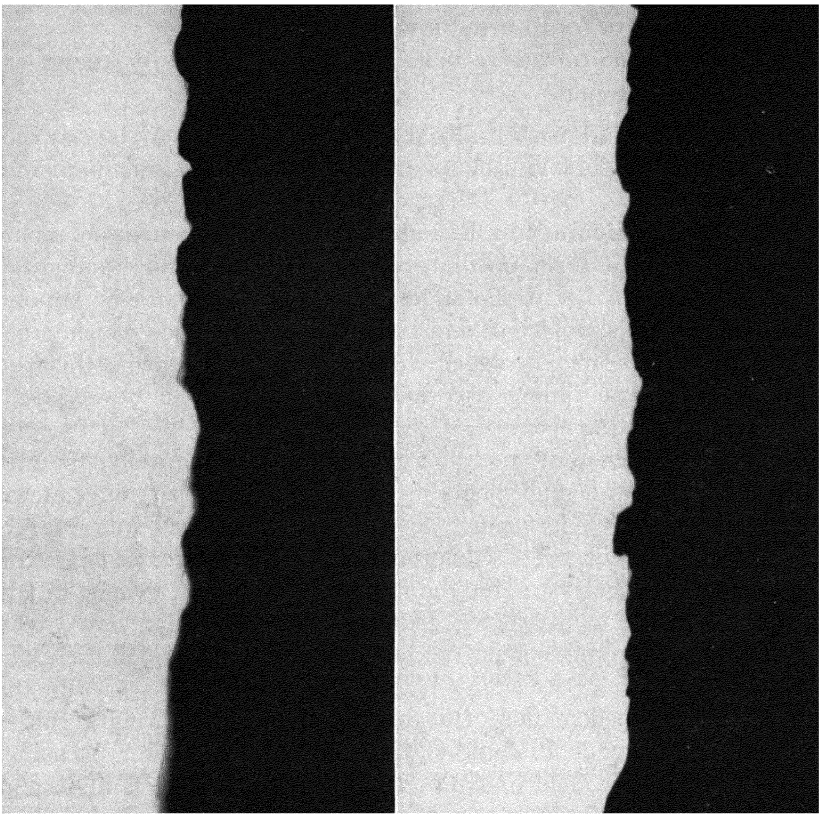
Sec. 126. Temporary or Examination Media. Temporary mounts are useful mainly for immediate examination, and for photography when it is expected that the slides will soon be discarded. Aqueous solutions, alcohols, volatiles, and in general, any liquids which cannot be permanently sealed, or which will react after a time with the specimen and so cause fading or other deterioration, can be classed as temporary mounting agents.

In temporary mounts made for purposes of photography, the great desideratum is often high visibility and strong contrasty images with good field depth. Glare must be at a minimum, and quiescence in the specimen is required. Thus the medium must differ in index from the subject sufficiently to give the necessary visibility; it must be high enough in index to give good field depth; and it may have to be viscous enough to prevent particles from moving around in the mount and flocculating.

Because of the demands of a good temporary mounting agent, recourse is often made to some of the media classified for permanent mounts. Thus the field of temporary mounting agents is almost unlimited, but that of good permanent mounting agents is decidedly restricted.

Sec. 127. Permanent Mounting Media. Mounting media which have the greatest degree of permanency are those which harden, or set, after the mount is made. Such are the resins, either natural or artificial; they will protect the specimen for the longest possible time. The ideal permanent medium should be chemically inert to the glass, to the specimen, and to the atmosphere, and it should not cause stains to fade. It should be colorless, and over a period of time should not form granules or crystals.

The histologist usually prefers a medium of the same index as the specimen. His specimens are usually stained and he wants absorption images only. Generally such a requirement is impossible of fulfillment because various areas of a specimen usually differ slightly in index. The belief that the maximum transparency of the specimen ensures the maximum visibility of stained detail is a matter to question but hardly to discuss here.



A *B*

FIG. 196. Two views of a sharp edge, ×200. The specimen was a piece of thin-gauge metal foil cemented to a cover glass. The cover glass bearing the foil specimen was mounted in a small cell 2 mm deep. One end of the specimen was rested on two thicknesses of cover-glass bits to impart the out-of-focus effect as seen at the lower end of the black line, in *A*. *A* was taken while the specimen was mounted in air; at *B*, the mount was the same except that methylene iodide had been added to give increased field depth. The black line shows clear and sharp its entire length. The optical arrangement is immaterial in a demonstration of this sort. In each exposure, focus was made sharp for the portion of the line showing at the top of *A* and *B*.

On the other hand, many specimens which are to be preserved permanently often appear at their best when mounted in a liquid of high index in order to increase field depth. Figure 196 illustrates the same subject in two different media. At *A* the specimen line is mounted in air and only the upper portion is clearly seen, whereas at *B*, with the specimen mounted in methylene iodide, much more of the line is sharp and clear. The relationship of the field depth of one liquid to

that of another is now shown in terms of the index of refraction of the two media.

If the field depth of a lens in air is represented by D, then its field depth in a liquid of refractive index n is $D \times n$. When the field depth of an objective is computed by equation 49 to be D_1 on a specimen mounted in a medium of index n_1, and it is desired to know the field depth in some other medium having an index of n_2, then the new field depth, D_2, is given by the equation

$$D_2 = D_1 \frac{n_2}{n_1} \qquad [60]$$

Sec. 128. Characteristics and Classification of Various Media. The characteristics and physical features of a medium for mounting specimens will depend not only upon whether the mount is to be permanent or temporary but also upon the chemical properties of the specimen and its physical condition. The infinitely varied materials useful for mounting may be conveniently classified as follows:

1. *Air and Other Gases.* Specimens mounted in air, with or without covers, are called dry mounts. For certain work, chambers have been devised for using other gases,[1] perhaps under pressure, under heat, or in a vacuum. The dry air mount is easy to make, permanent, and satisfactory for many specimens that lend themselves to examination in a medium of low index. Since the index of air can be considered 1.000, high-aperture objectives will have their apertures reduced to slightly less than 1.00 when they are used with a dry mount.

2. *Water, Alcohols, and All Volatile Liquids.* These are chiefly of service for temporary mounts. When sealed with wax, oil, shellac, glue, lacquer, resin, latex, or other sealing compound, specimens so mounted will often keep without spoiling for many days. These media are useful for specimens that have been fixed in place or must be put into the form of a mechanical suspension. As a rule they promote Brownian motion. Most of these liquids have fairly low indices, but there are some exceptions, as, for instance, the solution of iodide salts in water.

3. *Glycerol, Heavy Oils, Non-Drying Liquids, Aroclors, and Others.* These are excellent for temporary mounts and many can be used for permanent mounts. Motion of free floating particles or unfixed specimens will be reduced but not stopped. In stained specimens there

[1] Taisia Stadnichenko, Geologist, Geological Survey, Washington, D. C., has designed a cell, or micro furnace, which permits various gases to be used under temperature and pressure control.

may be fading. This is a very important group of liquids for the photomicrographer. The indices vary from about 1.46 to 1.64.

4. *Natural and Synthetic Resins and Thermoplastics.* The old standby, Canada balsam, is only one of other natural resins mentioned in Table XXIX. Some of the synthetic resins are also listed in this table. Many of these materials are used normally with a solvent, the evaporation of which will cause the mount to become perfectly hard. Some of the media are added to the specimen in a melted state and they solidify on cooling. The synthetics may be frequently used in this way; some of them may polymerize on setting or under the influence of low-temperature heating. All the media can be used for permanent mounts, some being more suited to this than others. All can be employed to good advantage in photomicrographic work. The indices vary from about 1.5 to 1.7.

5. *Melts.* These are all solids at room temperature; they must be heated for use, some of them to several hundred degrees Fahrenheit. Most of them are mixtures of one or more compounds or elements. They are typified by selenium and sulphur. Generally, the color is reddish or brownish-straw to yellow. The indices run from 1.9 to 3.0. Some melts crystallize after standing and so must be reheated before the slide on which they have been used can be examined. Melts are valuable chiefly for petrographic work.

6. *Liquids of High Refractive Index.* These are suitable for temporary mounts only; they have a tendency to attack the specimen. They include methylene iodide, which is one of the most innoxious of all, phosphorus-sulphur-methylene iodide, selenium monobromide, and others. In a thin film they are rather light in color. The indices vary from about 1.76 to 2.05.

Sec. 129. Description and Use of Some Important Mounting Media. *Air: $n = 1.0$.* Mounts made in air, or dry mounts, are very useful. They may be used with an objective of N.A. even greater than 1.0. An air mount furnishes the maximum amount of contrast when a medium of an index lower than that of the specimen must be used. In the best form of air mounting, no cover glass is used either for visual examination or for photography; objectives with a focal length of less than 16 mm should be corrected for use without a cover glass. If covers are necessary, considerable glare may be introduced; this will be quite unavoidable, and it is especially objectionable when the specimen is a fine powder having high reflectivity. Many pigments offer an excellent example of the value of air mounts. If particles are small and fairly transparent, and reflect light, yet are hard to see in certain liquids, good results may be obtained with an air mount.

On the other hand, fine detail may be lost in air mounting, for such images are generally formed by refraction, and the very heavy image lines may mask the smaller detail in the specimen and prevent it from becoming visible. On materials to be examined by reflected light, it is specially desirable to avoid the use of a cover; if one is required to preserve the specimen, it should be removed if possible just before the photograph is made. Field depth will be at a minimum with a dry mount — a feature which may, in itself, preclude the possibility of obtaining a good picture.

Water: n = 1.33. Water mounts are easy to make although they may not always clear very well. Their low index is a very desirable feature. They are not suitable for very small material unless it is anchored to the slide; however, more frequent use could be made of them to good advantage. To ensure non-drying of the mount the cover can be ringed with melted paraffin, latex, or isobutyl methacrylate. Heavy oil has been recommended, but it is very troublesome to handle and, if the slide is set aside for subsequent work, the oil is almost sure to spread over the face of the cover. Ringing with paraffin can be done easily with a small brush. The paraffin hardens the instant it touches the cold slide and so forms an effective seal.

Other Aqueous Media: n = 1.33–1.80. When water seems to be a desirable mount, but a medium of greater viscosity is indicated, the viscosity of the water can be raised by the addition of glycerol. Glycerol and water, 1 : 1, has an index of about 1.4. If good photomicrographic conditions are to be maintained, the use of a little glycerol with the water is advisable unless the optical conditions are such that no rise in index can be permitted. With the glycerol added the evaporation will be so much retarded that probably the cover will not require to be sealed. Commercial Karo is a sugary syrup which is sometimes useful. Its refractive index can be reduced by the addition of water, but before a picture is taken the specimen should be thoroughly examined for sugar crystals. Levulose, also, is useful, and it will not crystallize (Lee[2]). For specimens which demand aqueous mounts of low refractive index, the above liquids should answer well, but aqueous mounts of higher refractive index can be made, for temporary use, by a saturated solution of the two salts mercuric and potassium iodide. This solution is said to have a refractive index of 1.68.[3] When the salts are mixed with glycerol, the solution is said to have an index of 1.78–1.80. Either can be diluted with water to suit (Lee).

When working with unsealed liquid mounts, it may become neces-

[2] Lee, *The Microtomist's Vade-Mecum*, ninth edition, 1928.

[3] *J. Roy. Micr. Soc.*, N. S. ii, 167, 1882.

sary, on account of evaporation, to add more of the mounting solution. This can be done easily by placing a drop from a tiny pipette near the edge of the cover, allowing it to run under the glass. Capillary attraction will carry it to all uncovered parts of the specimen.

The action of water as a solvent or as a swelling agent on many chemicals, fibers, and other materials must be watched for and guarded against. When it is noticed to an objectionable degree, some other medium must be used. However, when water will act as a solvent but a water mount seems desirable, the following suggestion may be practical. A saturated solution is made of the specimen and this liquid is then used as a mounting medium. The mount should be sealed to prevent evaporation. The specimen particles must be carefully watched. Any crystalline material showing around the edges of the mount indicates that equilibrium is not being maintained and that crystallization is taking place. On the other hand, if the corners and edges of particles in the specimen appear rounded, again equilibrium is not being maintained for the specimen is being taken into solution.

All aqueous liquids when used as mounting agents can be handled like water, unless the liquid is or contains a volatile acid, in which case, care must be taken to guard the objective, the microscope tube, and the stage of the microscope from injury by chemical action.

Chemical microscopy demands the use, on the stage of the microscope, of chemical compounds many of which are highly corrosive and injurious to all metal and optical parts of the microscope. It was mentioned in Chapter III that objectives should be protected from corrosive compounds and their fumes. This will present no great difficulty since nearly all chemical microscopical work can be carried on at a low magnification and therefore with objectives having relatively long working distances. The front surface of the lens can be protected by a cover glass. A cover may or may not be used on the preparation itself, but one can always be used on the objective. The front lens of most microscope objectives is plano; consequently, if a small drop of cedar oil is placed on a very small cover glass, the cover can be applied to the objective lens and very little additional aberration will be noticed. A thin cover should be used and only very little oil.

The bright metal upper parts of the microscope can be easily protected by a coating of vaseline. If hydrofluoric acid is to be used, the condenser must be protected on the top surface, but on account of the difficulty of protecting the under surface it is advisable not to have a valuable condenser on the microscope under such conditions. The lower surface can be protected to some extent on nearly all assemblies by the

addition of a plane window which can be screwed to the lower part of the condenser and mounting. Care in operation will preserve the stage of the microscope, but, if it has a considerable amount of metal inlay, the whole stage may be covered with a sheet of plastic or a thin piece of hard rubber with a hole pierced in the center. Some microscopes, particularly those intended for chemical work, have a slot in the lower end of the tube located above the objective for the purpose of inserting retardation plates. The ends of such a plate should be covered with a coating of vaseline. If no plate is used, the slot should be closed.

Glycerol: $n = 1.47$. Glycerol is an important mounting medium. It has a refractive index sufficiently low to give good contrast to many classes of material, and it makes a mount that keeps well for a long time, even when the cover is not sealed. Air bells may be difficult to get rid of, but if the glycerol is slightly diluted with water, or if the slide is gently heated, a little pressure on the cover will make it possible to move the bubbles to the edge of the cover. Breathing on the specimen, or moistening it with a little water, will tend to increase the wetting power of the glycerol and help to avoid the formation of air bells.

Glycerol can be used with water in all proportions. The water will lower its index and make is less viscid; as already mentioned 1 part water and 1 part glycerol has an index of about 1.4. The refractive indices of various percentages of glycerol and water are listed in many chemical handbooks. For permanent mounts glycerol must be undiluted and the mounts should be sealed; for temporary mounts the mixtures will stay for a comparatively long time without change, and therefore it will not be necessary to seal the mounts for purposes of photomicrography.

Glycerol is a good preservative agent. Its comparatively high viscosity reduces the chance of motion in the specimen. Often it can be used in place of water, and it is less likely to act as a solvent. It can be used indiscriminately on damp or dry specimens.

Glycerin Jelly: $n = ca.\ 1.48$. Glycerin jelly is excellent for both temporary and permanent mounts. Mounts that are to be kept for a long time should have the covers well sealed; several coatings of shellac will protect them for years. If exposed to considerable heat the jelly will soften and the gelatin is likely to change to meta-gelatin. If this happens, jelly will not form again on cooling, and the mount will remain in a liquid state. Arc lamps used without water-cooling cells for photomicrography are likely to spoil glycerin jelly mounts.

Glycerol and glycerin jelly have the great advantage that they make

it unnecessary to dehydrate a moist specimen by passing it through alcohols, as would have to be done in order to avoid cloudiness if the specimen were mounted in a resin. A specimen that may flocculate in a fluid mount can be dispersed in warm glycerin jelly, and since the jelly sets rather rapidly the small particles do not have time to move through the viscous liquid and form flocculi.

Lee[4] gives eight formulae for making glycerin jelly. The following is taken from Gage.[5]

Soak 25 grams of gelatin in cold water until soft.
Drain off superfluous water.
Melt the gelatin over a water bath.
Add 5 cc egg albumen; stir, and warm for 30 minutes at 75° C.
Filter while warm.
Add an equal volume of glycerin.
Add 5 grams chloral hydrate, and shake thoroughly.

A small amount of the jelly is taken on the end of a spatula, placed on a slide, and warmed very slightly over the microscope lamp or warm plate. When the jelly is melted, the specimen is added. Fibers should be arranged by means of needles. Granules can be dispersed by sufficient gentle pressure on the cover to move them into position. For histological sections, where the jelly is placed on top of the specimen, it is more convenient to melt the jelly in a watch glass and transfer a drop to the section. If air bubbles persist, they may be eliminated by gently remelting the jelly and pressing slightly on the glass cover in the place indicated.

Isobutyl Methacrylate: $n = 1.47$. This thermoplastic has been suggested for use as a mounting medium by O'Brien and Hance.[6] It can be obtained in small lumps to be dissolved in benzine or xylene. O'Brien and Hance recommend dipping a slide into this solution, letting it dry, and using it as a substitute cover glass. This, however, does not constitute good optical practice. A slide prepared in this way is almost certain to introduce aberration, which will be particularly undesirable with high-aperture objectives.

For permanent mounts, Groat[7] found that this plastic is not satisfactory (presumably for biological subjects). Its index is too low; it does not stick well to glass; there is a rapid fading of certain stains;

[4] Bolles Lee, *Microtomist's Vade-Mecum,* ninth edition, p. 288, 1928.

[5] Simon H. Gage, *The Microscope,* fifteenth edition, 1932.

[6] H. C. O'Brien and R. T. Hance, "A Plastic Cover Glass, Isobutyl Methacrylate," *Science,* **91,** 412, 1940.

[7] Richard Groat, "Evaluation of Isobutyl Methacrylate Polymer as a Mounting Medium," *Science,* **92,** 268, 1940.

and it softens and decomposes at low temperatures. However, for photomicrographic use when the mounts are not permanent it is of great value.

Paraffin Oil: n = 1.45 to 1.50. This is a very convenient mounting medium. It can be used on practically all large material, and on all small material that has an index for which it is suited. It is obtainable with various viscosity values. It is inert chemically to most specimens; it is water white and very easy to handle; it seldom entrains air bells; and it is non-drying. The acid value of a good paraffin oil is practically nil.

Turpentine: n = 1.475. This is a splendid examination medium. It can be had water white, and when triple-distilled it is excellent for microscopical use. One of its good features is its great wetting power; air bubbles are seldom entrained in turpentine mounts. It can be used to dilute other media to obtain mixtures of different indices.

Turpentine is also valuable as a fixative. Finely ground powders can be rubbed out on a slide with a little turpentine, and, by continuing the rubbing until the turpentine evaporates, a good even distribution of particles can be obtained. The slide can be warmed to drive off volatile residues and the mount can then be examined dry or with a medium of appropriate index.

Exposure to air will thicken turpentine considerably. It becomes rather sticky and when it is used as a mounting medium in this condition the increased viscosity will slow down or stop the Brownian motion of fine particles. This is the turpentine of commerce as taken from American pine, from the Georgia and loblolly pine in particular.

Venice Turpentine: n = 1.469. Venice turpentine is obtained from the European larch. It is viscid and of a yellow or straw color. In the past, it has been used to a great extent by microscopists and has been a favorite of many.

Canada Balsam: n = 1.53. Other Balsams. Balsams are the natural exudates from certain trees. They are aromatic, tend to flow more or less easily, and contain essential oils and resin. When the oils dry out, the hard amberlike resin remains. This property of forming a hard transparent mass which is not easily dissolved and is fairly inert chemically makes the resins valuable for permanent mounting of microscopic specimens.

Canada and other balsams can generally be obtained commercially in several grades. The refined and paper-filtered balsam is clear, light in color, and, as the supply houses say, commercially neutral. It is of good optical quality. Balsams can also be purchased in the resinous state in the form of small hard yellowish lumps, which must

be either heated or dissolved in a solvent. Xylene balsam is also on the market. It is made commercially by drying out the natural oils from Canada balsam and taking the resin into solution in xylene. This is the grade that is generally sold for microscopical purposes. For petrographical work, the paper-filtered variety is used. It is cooked until it reaches a hard and slightly sticky condition. A method for its preparation is described, and its use by heating is explained, by Head.[8] Balsam should not be used in its natural state, for, according to Lee, it would be uncertain in viscosity and would contain an amount of water which would cause turbidity. Probably, also, it would carry considerable dirt, and its acid value would be high enough to cause fading in many stains. A method for making a neutral balsam is described by Lee.[9]

The source of Canada balsam is the balsam fir, *Abies balsamea,* a tree native to North America. Other balsams and resins useful for mounting media include dammar, sometimes called agathis, obtained from trees of the *Dammara* genus. Sandarac, from the tree *Callitris quadrivalvis,* is native to Morocco; sandarac resin is also known as juniper resin. Styrax or storax or liquidambar, as it is variously known, is obtained from the tree *Styrax officinalis,* which is well distributed over the world. This resin has become well established as a mounting medium, owing to its rather high refractive index. Gum thus is taken from the spruce fir, genus *Boswellia,* of which there are several species. Presumably it is the frankincense of Holy Writ. Balsam of Peru comes from Central America, from *Myroxylon pereirae.* Balsam of Tolu, which is more like a resin than a balsam, also comes from Central America; the tree is *Myroxylon balsamum.* This balsam has one of the highest indices for the resins; it is listed at 1.64. Colophony or colophonium is the common rosin left after distillation of turpentine. It is obtained from various pines, firs, and larches. These resins and balsams are those usually encountered in microscopical work, Canada balsam and styrax being by far the most common.

Although, according to Findlay,[10] Canada balsam was introduced as a mounting medium by J. T. Cooper in 1832, the technique of its use has altered but little during the hundred years and more. Probably the greatest change has been in the solvents in which it may be dissolved. Almost any organic solvent is suitable; and the alcohols,

[8] R. E. Head, "The Technique of Preparing Thin Sections of Rock," *Technical Paper* 8, U. S. Bureau of Mines, 1929.

[9] Bolles Lee, *Microtomist's Vade-Mecum,* ninth edition, p. 231, 1928.

[10] G. M. Findlay, "The Debt of Medicine to Microscopy," *J. Roy. Micr. Soc.,* Series III, **60,** 36–40, 1941.

chloroform, and turpentine are sometimes used. The solvent determines the drying time. Turpentine balsam stays liquid for a long time; chloroform balsam dries out quickly. Probably the most useful and best-known combination is xylene balsam. This mixture solidifies fairly rapidly, and the cover glass becomes well anchored in a few days after the mount is made.

The refractive indices of the natural resins are given in Table XXIX. The figures apply to the dried resin only; in liquid form the resins always have a lower index because practically all their solvents have an index considerably lower than that of the resin. When the refractive index of Canada balsam is stated as approximately 1.532, this figure does not refer to the balsam (the viscous liquid) but to the resin (the solid). As measured on the refractometer xylene balsam has an index of 1.526; Lee quotes it at 1.524. Thus, as the resin sets and becomes hard from its loss of solvent or essential oil, the index rises slowly. Usually, the mount also darkens. One or two of the resins may form small crystals or become granular after a considerable period and spoil the preparation. However, balsam mounts made from Canada balsam have stood through years with very little change. Slides so mounted have been in the possession of the author's family for over 80 years, and they show no granulation or cracking. The specimens have been perfectly preserved.

As mounting media, resins are a little more difficult to use than liquids and jellies. Almost without exception, the specimen material must be dry for a permanent mount. Histological sections can be dehydrated through alcohols; other specimens may simply be dried slowly in an oven. Many classes of materials such as chemicals, clays, pigments, and fibers are sufficiently moisture-free to be mounted immediately without further drying. The importance of dehydrating a specimen is not always apparent at the time the mount is made, since the ensuing cloudiness, due to a slight amount of entrained moisture, may not be manifest for some months. If the slide is intended for temporary use only, dehydrating may not be necessary unless there is immediate evidence of cloudiness.

In use, Canada balsam is generally dropped onto the specimen after a distribution has been effected. A cover is then pushed down rather firmly with forceps. If the balsam seems a little too thick and sticky it can be warmed slightly to increase the flow and to remove any air bells. Some preparations are particularly prone to entrain air bells since the wetting power of balsam is poor; it helps if the specimen is flooded with one of the solvents. When xylene balsam is used, the specimen can be flooded with xylene, but, unless the excess xylene is

nearly dry before the balsam is added, the balsam will not flow quickly and easily over all parts of the preparation. A little experience in making mounts will soon indicate the proper amount of medium, which will vary, of course, with the size of the cover glass. A mount made with a xylene resin will dry out around the edges of the cover glass in a few days; it can then be sealed with cement if desired.

If the specimen cannot be mounted directly on the slide but must be distributed in the balsam, it should be placed on the slide first and the resin then added, the distribution being effected by pressing on the cover and moving it around slightly. This is a better procedure than stirring the specimen into a drop of the medium on the slide.

Occasionally there may be reasons for using the solid balsam as a mounting medium. If a small lump of the resin is placed on the slide and a little heat gently applied, it will melt at about 61° C; then the specimen and the cover glass can be added. This procedure can be reversed. The specimen may be placed on the slide and a lump of balsam added; the cover is then laid on the balsam, and a little heat will finish the mount. However, air bubbles may be difficult to eliminate when the mount is made in this way.

Balsam will keep indefinitely, but a small quantity kept always at hand should occasionally be inspected for change in viscosity. Balsam that has become too sticky should be thinned with a little solvent. The resinous solution can be heated a little to aid in dissolving, but the heating must be done with care. If the solution is overheated it may become " burnt," losing its fresh smell and turning dark.

Dammar: $n = 1.54$. Dammar, having an index slightly higher than Canada balsam, can be worked in the same way, and it is specially recommended as a good resin for sealing cover glasses. If it is put into solution as described for Canada balsam, and brought to a consistency to flow easily from a brush, it can be applied to the cover to make a very effective seal. It has a melting point of about 105° C, but it is said that crystallization or granulation may occur after a long period.

Clarite: $n = 1.54$; Clarite X: $n = 1.57$. Clarite, a cycloparaffin or naphthene polymer, is made by the Neville Company[11] in two forms: Clarite, $n = 1.54$; and Clarite X, $n = 1.57$. Both have very desirable properties which make them useful as mounting media. One of the important and outstanding characteristics which indicate Clarite as a mounting agent of some consequence is its high melting point — about 150° C. This is an important factor if slides are used for projection or photomicrographs are to be taken with the arc lamp.

[11] The Neville Company, Neville Island, Pittsburgh, Pa.

Melting mounting medium has spoiled many good slides by producing air or gas bubbles under the cover which are difficult or impossible to remove without ruining the specimen. The acid number of Clarite is given as zero, and its refractive index is very close to that of balsam. It would seem that, because of its permanency in color and its neutrality, Clarite or a similar synthetic might profitably replace Canada balsam, especially since most natural resins have inherent undesirable physical properties, such as high acidity, yellowing with age, cracking, and even slow crystallization or granulation. Synthetic media are more stable and easily controlled.

Cedar Oil: n = 1.515. This is the regular standard immersion oil. It is obtained from *Juniperus virginiana*. Mounts made in this liquid, when photographed or examined with a high-power objective, are seen to have nearly complete optical homogeneity from the condenser to the objective. This condition means that there will be the minimum of glare from all the intervening glass surfaces. Cedar oil will clear a specimen well, and will to a certain extent absorb water without clouding. It is ideal for many temporary mounts for which its index is suited, and it can be used for a permanent mount by simply setting the slide away for a short time and letting the oil dry around the edges of the cover glass. In a day or two the mount can be ringed with any sealing compound.

Cedarwood oils offered by the trade may have an acid value of 40–60 milligrams of potassium hydroxide per gram. If such oils are used on stained specimens for permanent mounts, fading is likely to result. If desired, unthickened cedarwood oil can be obtained with an index of about 1.510. Either thickened or unthickened cedarwood oil is good for temporary mounts since both are very penetrating. For permanent mounts and general use, the thickened oil will probably prove the more satisfactory.

Cassia Oil: n = 1.6. This oil is of value to the microscopist not only because its index is reasonably high but also because of its good wetting power for many preparations. However, it is more active chemically than cedar oil and so must be used with caution. Balanced against this poor feature is the fact that the photomicrographer, when he prepares his own specimens, generally makes temporary rather than permanent mounts, and for these the cassia oil may often serve.

Aroclor: n = 1.63. The trade name Aroclor refers to a series of artificial resins not very well known in microscopical circles. They are chlorinated diphenyl compounds made by the Monsanto Chemical Company,[12] and they can be had as permanently viscid liquids or as

[12] Monsanto Chemical Company, St. Louis, Mo. New York City Office, 30 Rockefeller Plaza.

solids. The liquids can be used without further preparation; the solids must be melted in the same way as glycerin jelly, but a few minutes after the mount is made the aroclor will set and be perfectly solid. The rather high index of refraction of the aroclors makes them very valuable as both mounting and examination media. The index is given as 1.627 to 1.667, the low value being for the liquids and the high value for the solid resin.

The aroclors are soluble in nearly all proportions in the hydrocarbons, alcohol, and other solvents. The solid resin can be put into solution with xylene, and for temporary work this is a satisfactory method. As the index of refraction of the aroclor solution is lower in this form, and a precipitate has been noticed after the mixture has been allowed to stand for some weeks or months, its use in this manner for permanent mounts is not recommended.

The aroclors are very slightly acid: 0.01 to 0.02 milligrams KOH per gram. Since the aroclor resins 1242, 1248, and 1254 are liquids and non-drying, they can be used to great advantage for examination media, whereas the solid resin is suitable for permanent mounts.

α-Monobromonaphthalene: n = 1.6557. This is a very desirable medium for temporary mounts whenever it can be so used. It penetrates the material quickly, and because of its low viscosity few air bubbles are formed. It can be mixed with oils to lower its index. As it evaporates rather fast, permanent mounts are hardly possible with it. The index of this chemical varies considerably; the above figure was measured from a fresh commercial lot, and presumably it is about right for average samples. As the substance ages, the index rises and the color may darken; the darkening varies with different makes. One lot after five years shows considerable darkening and some turbidity; it has an index of 1.6567. Another lot, after twenty-three years, is clear, shows no turbidity, and has an index of 1.6578. The dispersion of α-monobromonaphthalene is about $v = 40.0$.

Hyrax: n = 1.8(?). This synthetic resin, invented by Dr. G. Dallas Hanna,[13] is described by Meakin.[14] In the liquid form it is supplied in a solvent which can be driven off by heat. A slide may be mounted with it and put to one side to dry. It will be dry around the edges in a few hours, and in a day or so it will be ready to be ringed with a sealing cement, such as shellac, dammar, gold size, or lacquer. After a period Hyrax mounts will darken, but no crystal formation has been

[13] G. Dallas Hanna, Department of Paleontology, California Academy of Sciences, San Francisco, Calif.

[14] S. H. Meakin, "Mounting Diatoms in Hyrax," *Watson's Microscope Record*, September, 1933.

noticed. Air bubbles are very hard to remove; it is better to avoid them in the first place by flooding the slide with xylene, as is occasionally necessary with some of the natural resins. Since Hyrax is quite viscid when purchased, it may have to be thinned somewhat, preferably with xylene.

Owing to the way this material is marketed at the present time, its index is very uncertain. On the bottle in which it is sold the index is stated to be 1.8, but actual measurement has shown it to be as low as 1.66. Since there was very little solvent in the resin when it was measured, the index of the solid may be considerably below 1.8. Hyrax may be had from Palo Myers, Inc.[15]

Methylene Iodide: n = 1.74. Because of its high index, methylene iodide is one of the most important liquids for microscopical mounts. It flows very freely, has a medium vapor pressure, and is colorless. Mounts made with a small quantity of this liquid can be sealed with paraffin immediately, but any surplus on the slide must be wiped off before sealing, or the slide must be laid aside until the surplus liquid evaporates. Methylene iodide is somewhat unstable. Free iodine may form and darken the liquid, but this darkening can be prevented by adding a few small strips of bright copper to the bottle in which it is kept. This mounting agent may also be found to have a slightly lower index after being kept a while. It is soluble in all proportions in alcohol or ether, but only slightly soluble in water.

Methylene iodide will dissolve sulphur, the refractive index of the mixture being raised to 1.78 for a saturated solution. The sulphur is easily put into the iodide in excess by heating some of the liquid with the sulphur in a test tube. Larsen[16] mentions other combinations of methylene iodide with indices up to about 1.87.

Selenium Monobromide: n = 2.05. The refractive index figure of this liquid is about as high as is possible for liquid media. The liquid is clear but its dark brown-red color is a disadvantage because blue light is necessary for the utmost resolution when liquids of high index are used to increase visibility. However, in a thin film the color is not so noticeable and the effect of the liquid on light is correspondingly less.

Phosphorus-Sulphur-Methylene Iodide: n = 2.05. This combination is the basis for a series of liquids the refractive index of which ranges from about 1.74 for methylene iodide to a maximum of 2.05 when the mixture contains phosphorus 8 parts, sulphur 1 part, and

[15] Palo Myers, Inc., 81 Reade Street, New York City.

[16] Larsen and Berman, "The Determination of Non-Opaque Minerals," *Geological Survey Bulletin* 848, Superintendent of Documents, Washington, D. C.

methylene iodide 1 part, according to West.[17] This concoction has an objectionable odor and is highly inflammable when exposed to air; therefore it must be kept under water. It is very light in color and so is a valuable liquid for work with small particles when blue light must be used. Before making or using it, the technician is urged to consult West's original paper.

Selenium-Sulphur Melt: n = 2.0–2.7. Larsen and Berman[16] give a good account of several melts which can be used for specimens requiring a very high-index mounting medium. The refractive index of sulphur is 2.038 and that of selenium 2.92. A mixture of these can be made by heating together weighed portions of each. The resulting index will follow the curve worked out by Larsen, or it will be in accordance with the table given by Winchell.[18]

Melts are likely to be disappointing since small opaque bodies will often appear in the field. In the selenium-sulphur melts these have been identified as selenium dioxide by Brownmiller,[19] who offers a method for making optically clear selenium by distillation in the presence of carbon dioxide. The sulphur also should be pure; it can be obtained from the first and second crop of crystals from carbon disulphide. Selenium is soluble in sulphuric acid and carbon disulphide, melts at 220° C, and boils at 688° C. Sulphur melts at 120° C.

It is a good plan to make up several of these melts in advance, taking the proportion of each element that seems to be desirable. In use, a small fragment is placed on the slide with the specimen, a cover glass is dropped on, and heat is applied to melt. Then the cover can be pressed in place with the tips of a pair of forceps to effect dispersion.

The mount, when finished, will be yellow to brownish yellow and red, depending on the proportion of the mix. If the melted layer of the selenium-sulphur is thin, the color will be light and blue filters can be used, but with thick mounts the preparation will act as its own filter, transmitting red light only.

Arsenic Disulphide or Realgar: n = 2.3. Like the selenium-sulphur this melt is likely to be not optically clear. The melting point is given at 267° C, which is also a transition point. Very small material mounted in arsenic disulphide is quite likely to be confused with the impurities of the melt. It is possible to distil the melt onto a slide

[17] C. D. West, "Immersion Liquids of High Refractive Index," *American Mineralogist*, **21**, 245, 1936.

[18] Alexander N. Winchell, *Elements of Optical Mineralogy*, 1933.

[19] L. T. Brownmiller, "The Preparation of Optically Clear Selenium for Use in Index Media," *American Mineralogist*, **12**, 43, 1927.

carrying the prepared specimen, but it is still better technique to deposit it by evaporation in a vacuum as will be described. Originally, arsenic disulphide was used to mount diatoms, and some of the best pictures of diatoms have been made in such mounts, but it has never been used to any extent in industrial laboratories.

Sec. 130. The Use of Vacuum Evaporation for the Deposition of Melts. The difficulty of making clear mounts can be entirely overcome by the use of the vacuum process, by which the melt is evaporated rapidly in a vacuum and deposited on the specimen. Essentially the system is the same as that for depositing aluminum on glass. It was suggested by Hanna and Grant,[20] and it seems to offer remarkable results. Figure 197*A* illustrates a fine pigment, calcium carbonate, $n = 1.52$, mounted in balsam; at *B* the specimen is prepared on a slide coated with selenium by the evaporation process and then mounted in balsam. The smallest particles can be clearly seen. It would seem impossible to duplicate the result by other means, for, if the preparation were mounted dry to get the necessary differential in refractive index, glare would be increased; large apertures could not be used even with an oil-immersion objective, and also the large aperture of such an objective would be wasted because of the air between the slide and the cover. The most oblique rays from the condenser would, of course, be stopped at the glass-air interface.

It was found, in experimental work carried on in collaboration with Evaporated Metal Films Corporation,[21] that the thickness of such a film could be controlled by successive layers of mounting material. Apparently to get good results, the film thickness must be greater than that of the wavelength of light with which the specimen is observed. Small holes in such a film are not of great importance because the photomicrographic work on such material is carried on at high magnification and the holes can be avoided easily.

Sec. 131. Selecting a Mounting Medium for Photomicrography. The selection of a mounting agent for any particular purpose must be largely empirical because, even when the refractive index of the specimen is known, its size, color, reflectivity coefficient, and ability to absorb light, will, in general, be unknown. All the above characteristics have a bearing on the proper choice of a medium in which a microscope examination or photographic procedure can best be carried

[20] G. D. Hanna and W. M. Grant, "Preliminary Note on a Technique for Mounting Diatoms in Realgar and Other Substances," *J. Roy. Micr. Soc.*, Series III, **59**, 174, 1939; "Apparatus for Mounting Diatoms in Realgar and Other Substances," *ibid.*, Series III, **60**, 152, 1940.

[21] Evaporated Metal Films Corporation, 436 West State Street, Ithaca, N. Y.

on. Thus, it is impossible to state that the index of the medium should differ from that of the specimen by any specified figure. Some agents

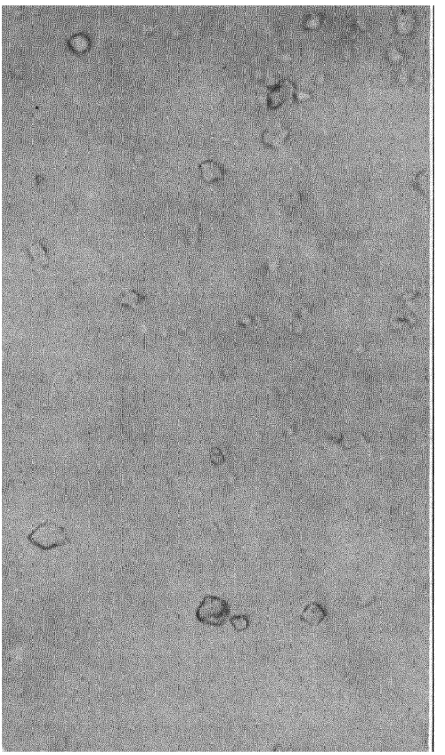


FIG. 197*A*.

FIG. 197. *A*. The three pictures in this series *A*, *B*, and *C* are all of calcium carbonate ×800. It is a very fine precipitate with an index of refraction close to that of Canada balsam. At *A*, the mount was made in Canada balsam. At *B*, the calcium carbonate was distributed on the slide as for *A* but the vacuum distillation process was used to coat the particles with a thin layer of selenium metal. Balsam was then added and a cover glass dropped on the mount. In *A*, many particles are invisible; in *B*, visibility is high. *C* illustrates a specimen imperfectly coated with the selenium. In addition to the bare areas around each particle, the unevenness of the coating is evidenced by the shadowlike areas pervading the whole picture. The Evaporated Metal Films Corporation, 436 W. State St., Ithaca, N. Y., gave their generous cooperation in obtaining this set of pictures. Objective, 8 mm apo., Leitz; ocular, Homal I; condenser, achromatic-aplanatic, Leitz; illumination, 400-watt biplane tungsten-filament lamp, method I; filters, Wratten 25; Defender Fine Grain Pan film; developer, D-19.

will clear better than others. Usually an aqueous solution will clear many specimens much more readily than a resinous agent. The clearing action is important, for, unless it is well carried out, mis-

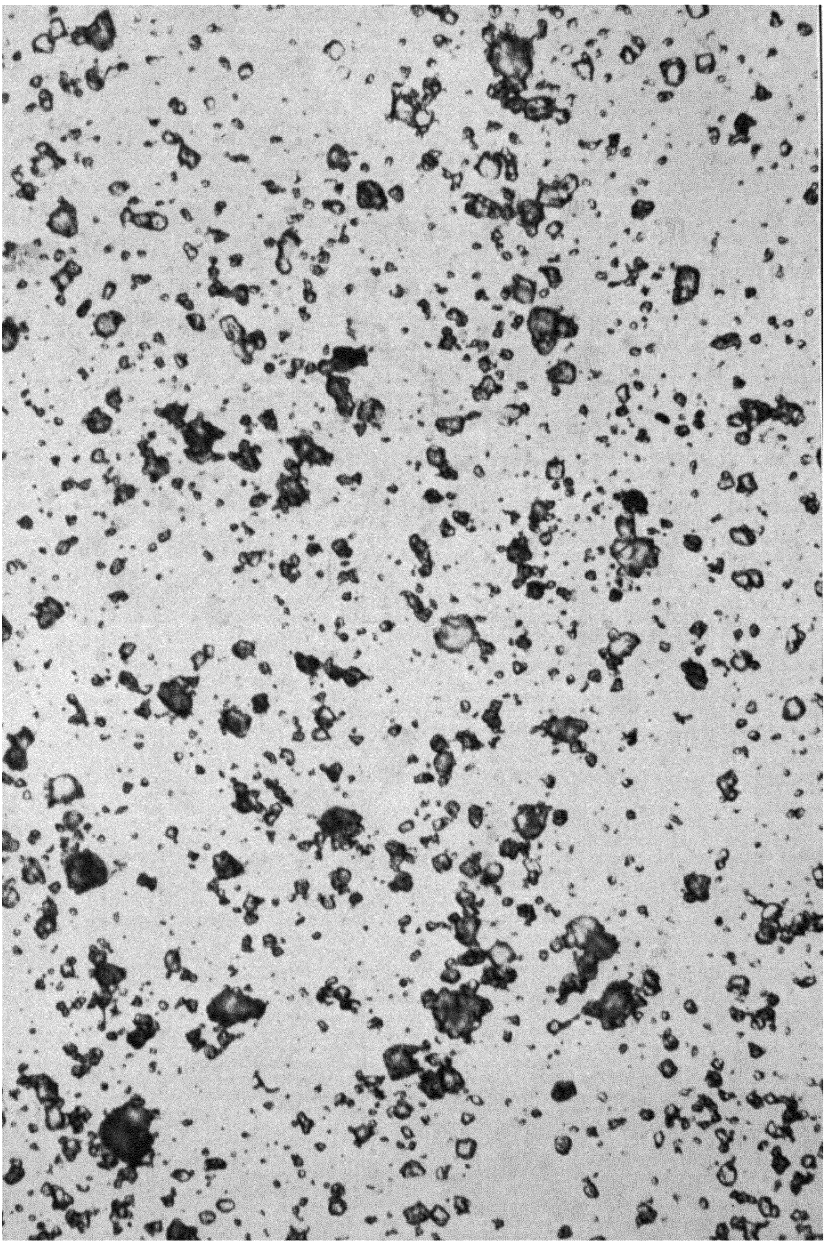


FIG. 197*B*.

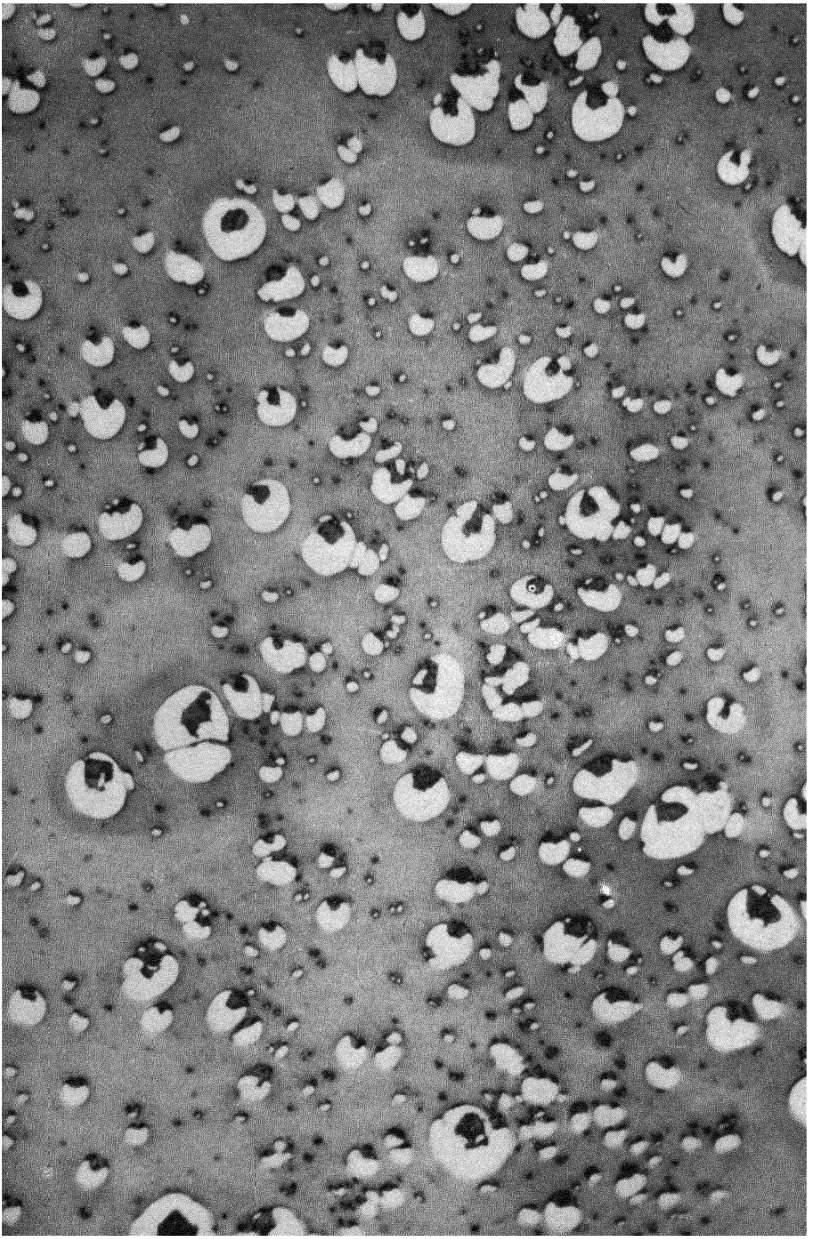


FIG. 197*C*.

leading images may appear in the photomicrograph. Clearing involves the complete displacement of the former surrounding substance, air or liquid.

There is always an advantage in first examining a specimen in its natural condition, that is, as it is when received by the microscopist. From then on, various mounting media can be applied and the changes in appearance noted carefully. Although no definite rules can be applied in making a decision which is vital to the success of the photomicrograph, yet one or two fundamentals should always be borne in mind. Any mounting agent which is likely to cause chemical or physical change in the specimen should be avoided unless such change is desired. Agents of high volatility must be carefully watched to see that they do not evaporate before the exposure is made; media of low viscosity may permit considerable Brownian motion, or, if volatile, they may set up currents which move the specimen during exposure. The remedy is to choose a liquid of higher viscosity if possible. The internal structure of a transparent specimen is best emphasized by a medium of like index, provided that the structure, or the inclusions, differ from the specimen in index. Too great a difference between the index of the mounting medium and that of the specimen will cause the refraction images of large particles to have outlines which are too broad. If the material is smaller than 2 μ, care must be taken to select a mounting medium that differs from the specimen sufficiently to ensure good photographic contrast. The smaller the particle the greater must be the difference in index between specimen and mounting image.

If covers as small as approximately 10 to 13 mm, either square or circular, are available, the effects of a number of mounting media may be conveniently tested at one time. A sufficient quantity of the material to be photographed is placed on a slide in such a way that at least three, four, or even five mounts can be made at the same time. Three mounts may be very significant. A medium with an index close to that of the specimen can be used for the center mount. A liquid of lower and one with a higher refractive index than that of the central mount can be used for the two side mounts. A rapid examination of the three mounts in succession will serve as a basis for selecting, if necessary, a fourth mounting medium which will give the desired contrast. For the two media of higher and lower index, liquids which differ considerably from the central mount should be chosen.

Table XXIX lists a number of materials suitable for mounting agents. Those marked with a dagger should be on hand at all times. Many of the values have been determined especially for this table,

Table XXIX

The Refractive Index of Some Important Mounting Media

(The refractive indices of many substances listed in this table are somewhat variable, particularly those of the oils and resins)

Alphabetical Listing		Listing According to Ascending Values of n	
n Temperature 25° C	Name	n Temperature 25° C	Name
1.000294	Air*	1.000294	Air*
1.405	Alcohols, amyl (iso)	1.323	Methyl alcohol
1.397	butyl (*n*)†	1.333	Water†
1.358	ethyl†	1.358	Ethyl alcohol†
1.323	methyl†	1.397	Butyl alcohol†
1.583	Aniline oil	1.400	Ethylene glycol monomethyl ether†
1.515	Anisol		
1.6375	Aroclor (1254)†	1.405	Amyl alcohol
2.6 (circa)	Arsenic sulphide (realgar)	1.430	Ethylene glycol monoethyl ether
1.640	Balsam of Tolu	1.430	Triacetin
1.594	Bromoform	1.446	Kerosene
1.535	Canada balsam (hard)†	1.452	Paraffin oil (light)†
1.620	Cassia oil	1.463	Glycerol†
1.477	Castor oil	1.463	Palm oil
1.515	Cedarwood oil (thick)†	1.468	Olive oil
		1.472	Turpentine†
1.584	Cinnamon oil	1.477	Castor oil
1.54	Clarite	1.48 (circa)	Glycerin jelly
1.57	Clarite X	1.481	Linseed oil
1.529	Clove oil	1.480	Nujol
1.545	Colophony	1.491	Xylene†
1.430—	Ethylene glycol monoethyl ether	1.508	Parlodion (dry)
		1.515	Anisol
1.400—	Ethylene glycol monomethyl ether†	1.515	Cedarwood oil (thick)†
1.48 (circa)	Glycerin jelly	1.52	Sandalwood oil
1.463	Glycerol†	1.521	Gum dammar
1.521	Gum dammar	1.529	Clove oil
1.635	Halowax	1.535	Canada balsam (hard)†
1.7 (circa)	Hyrax†		
1.446	Kerosene	1.54	Clarite
1.480	Linseed oil	1 545	Colophony
1.737	Methylene iodide†	1 57	Clarite X
1.656	α-Monobromo-naphthalene†	1.583	Aniline oil
		1.584	Cinnamon oil
1.508	Parlodion (dry)	1.594	Bromoform
1.481	Nujol	1.620	Cassia oil
1.468	Olive oil	1.62+	Styrax
1.463	Palm oil	1.635	Halowax
1.452	Paraffin oil (light)†	1.6375	Aroclor (1254)†
2.05 (max.)	Phosphorus-sulphur-methylene iodide	1.640	Balsam of Tolu
		1.656	α-Monobromo-naphthalene†
1.52	Sandalwood oil		
1.62+	Styrax	1.7 (circa)	Hyrax†
1.430	Triacetin	1.737	Methylene iodide†
1.472	Turpentine†	2.05 (max.)	Phosphorus-sulphur-methylene iodide
1.333	Water†		
1.491	Xylene†	2.6 (circa)	Arsenic sulphide (realgar)

* The index of air is based on that of a vacuum as unity; it is 1.000294. The index of refraction of a substance other than air is usually based on air with an *assumed* index of unity. To convert an index based on air to an index referred to a vacuum as unity, multiply by 1.000294.

† These media should be on hand at all times.

from supplies at hand. Although the index readings were carefully made and should be correct to 0.0005, the values may vary slightly from the published data because of inherent variations in some of the substances and because samples were taken from the usual laboratory stock.

Sec. 132. Importance of Suitable Index of Mounting Medium When Photographing Very Small Particles; Characteristics of Mounting Agent for Cellular Specimens. Very small transparent particles, such as clays, pigments, ceramic material, and fine natural abrasives, are always more difficult to see when mounted in a liquid than when

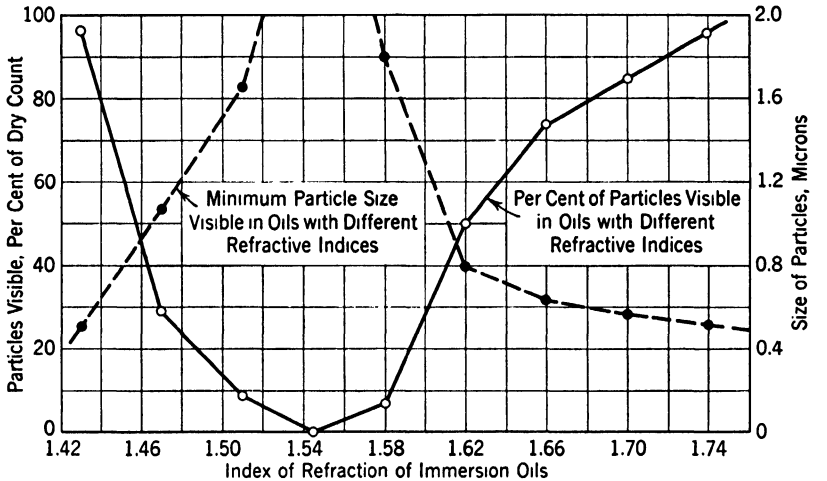


FIG. 198. These curves indicate the minimum particle size and the percentage of particles visible in media of different indices. The subject is quartz. Taken by permission from Foster and Shrenk, Bureau of Mines Paper R. I. 3368, 1938.

mounted in air. This is one of the first problems for the photomicrographer working with finely ground substances. A mounting medium usually gives the effect of a reduction of very small particles in the field, and the absence of a mounting medium is likely to cause trouble in obtaining a good image. Foster and Schrenk[22] showed that not all transparent particles ranging in size close to 0.55 μ (geometric mean) and having a refractive index of 1.54 can be seen when liquids with refractive indices between 1.43 and 1.74 are used as mounting agents. Figure 198 is a curve plotted to show the results obtained with quartz particles mounted in liquids of various indices.

Inspection of Fig. 198 impresses one with the necessity for care-

[22] W. D. Foster and H. H. Schrenk, "Petrographic Identification of Atmospheric Dust Particles," *Bureau of Mines, R. I.*, 3368, 1938.

fully inspecting the specimen before as well as after a mounting agent has been added. The size of the particles is of paramount importance and must be taken into account; the larger the particle, the smaller will be the range of the refractive index of the liquid which will cause it to become invisible. Large particles, unless very thin, do not require a mounting liquid of either a very high or a very low index in relation to that of the particle. Colored particles will give purely color images when immersed in a liquid of their own index. Very thin (1 μ or less) particles of carbon may become light brown, and they will be less easy to see than thick ones. If dark field is to be used on particles above colloidal size (0.1 μ), the images become largely reflection images; but even with reflection images the mounting medium plays an important part, and the reflection can be increased by increasing the difference in the index between the subject and the liquid in which it is mounted.

On delicate cellular structures fresh isotonic media should be used to avoid mechanical damage or undue swelling or contracting of the cell walls from osmotic pressure. Animal tissue can be mounted in the body fluids obtained from the specimen. Lee[23] gives an excellent account of a number of liquids useful for examining and mounting biological material. The normal salt solution of the biologists is 1000 cc of water and 8 grams of sodium chloride; this is modified somewhat by different workers. It is to be noted that small colorless particles, whether of organic or inorganic origin, are seen in such solution only with difficulty, if at all. If they are to be photographed the specimen should be fixed, and generally stained, or the relative index should be increased.

Sec. 133. The Refraction Image. An image of a transparent colorless object, as formed by a lens, is the summation of the total effects of the lens on the light that is refracted, reflected, or diffracted from all possible points of the object. Considering a very small area of a microscopic object, and assuming that all the rays which are refracted, reflected, and diffracted from that area reach the objective, then they will again be united and form an image of that area at the second focal plane of the lens. However, if all or some of the rays are refracted or otherwise directed from any area of the specimen so that they do not reach the objective, then the corresponding area of the image will appear darker than it would otherwise, owing to the absence or reduction of light from that particular point of the object. For this reason the boundaries of a transparent particle will generally appear as black lines, since they refract or reflect the light

[23] A. B. Lee, *Microtomist's Vade-Mecum*, ninth edition, 1928.

so that the rays from these regions are not included in the image-forming rays which proceed to the lens. Because refraction plays a much larger part in image formation of transparent objects than either reflection or diffraction, images so formed are known as refraction images. For further details of image formation see Sec. 51.

In measuring the index of refraction of a microscopic object, the underlying principle of the usual technique consists essentially of selecting a mounting medium, the index of which is known, so that the medium and the specimen will have the same index and will be optically homogeneous. Under these conditions a refraction image cannot exist since refraction and reflection at an interface depend upon the difference in index of the two phases, and the specimen, if visible at all, will be seen by color effects, or it may be recognized by inclusions. There are various methods by which it is possible to detect slight differences in index between the mounting liquid and the specimen, and which indicate when the relative index is 1.0, meaning that the indices of both specimen and mounting liquid are the same.[24] These procedures based on optical phenomena constitute several well-known tests for determining the index of refraction of microscopic specimens.

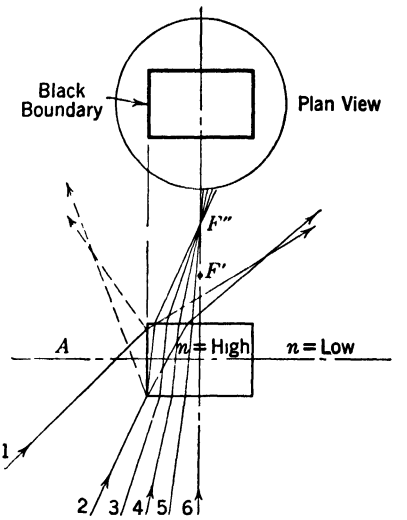


FIG. 199. The diagram illustrates the origin of the outlines of an image of a transparent colorless solid, mounted in a liquid of lower index. In this and following figures, only rays on one side of the specimen have been drawn to avoid confusion. At the optical level *A*, the width of the black boundary line would be less than at a higher level. All rays between rays 1 and 2 are lost to the objective. A projection of the cube on the horizontal plane is shown in the plan view.

Microscopic specimens are of many shapes and colors, and their effects on light are as varied as the specimens themselves. However, for purposes of discussion, a specimen can be thought of as a geometric cube as in Fig. 199, where its index is higher than that of the mounting medium. The specimen is highly transparent. The light rays from

[24] The term "relative index" is seldom given in absolute figures because it is likely to be misunderstood. It is actually the index of the specimen divided by the index of the mounting agent. However, it is a convenient term.

the condenser are numbered from 1 to 6; the focal point of the condenser is F', which is raised to F'' by the action of the specimen on the pencil of light included by rays 2 and 6. The light in this pencil is refracted toward the side of the cube, thus tending to light up the cube interior completely to its edge. Light striking the side, as rays 1 and 2, is refracted toward the center of the cube and tends also to produce more light on the inner side of the cube than on the outer. Rays 1 and 2 are partly reflected, but as this light is very weak a dark edge at the side of the cube appears. The image of these dark edges, due largely to refraction, accounts for the refraction image under the given conditions.

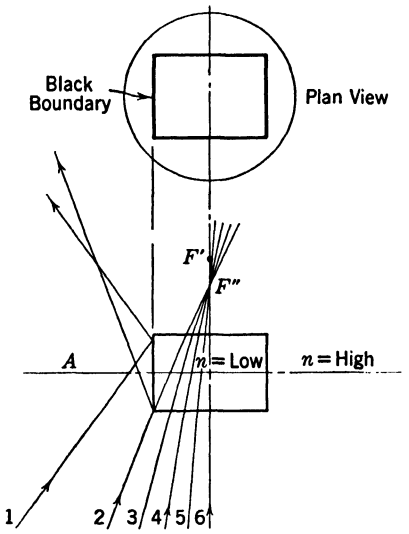


FIG. 200. This diagram is to be compared with that of Fig. 199. Here the refractive index of the mounting medium is higher than that of the specimen. A black line boundary is again shown as forming at any optical level.

When the cube is of lower index than the mounting liquid, as in Fig. 200, its action on light is somewhat different. The interior pencil formed by rays 2 to 6 is refracted toward the center of the cube, and the light impinging on the side of the cube between rays 1 and 2 suffers complete reflection at the cube surface. Ray 2 in both drawings may be thought of as a double ray striking the corner of the cube; part is refracted and part is reflected. This second example differs from the first inasmuch as the point F'' is below F', and owing to the total reflection on the side of the cube there is more light at that point than within the cube adjacent to the side. The dark boundary line tends to lie within the geometrical solid. The reflection plays a very important part, but the image can still be thought of as a refraction image, because the reflection is due to the light striking the cube side at an angle greater than the critical angle. See Sec. 41.

These two examples of the refraction image are fundamental and should be thoroughly understood to aid in subsequent image interpretation. If the sides of the cube slant, or if the top and bottom are uneven, the light rays can be traced through by applying the rules of equation 29 and by remembering that light traveling from a rarer to a denser medium is refracted toward the normal. With a little prac-

tical experience it is surprising how easily and quickly the true shape of a particle can be deduced from its refraction image.

A little consideration of the diagrams of the ideal hypothetical example of the refraction image will show that the position of the condenser, or point F', has little bearing on the subject. In any event a black line image of the specimen will be formed, and the lower the N.A. of the condenser the wider will be the black boundary line. Also, point F', in actual practice, will be the image of the light source, or secondary source, and normally it will cover an area completely engulfing the specimen. In a well-adjusted system it will be approximately in the position indicated. Diagrams so drawn would be confusing to study and would offer nothing of value that is not shown in the more simple and direct methods adopted here. For similar reasons the condenser and objective lens units are omitted from the drawings. The only mental reservation to be made because of this is that the plan view would be reversed and inverted when appearing in the field of view.

Sec. 134. Methods for Judging Relative Refringency. *The Becke Line.* Again referring to Figs. 199 and 200 it is seen that the concentration of light within and above the cube depends upon the level at which the inspection is made. At the bottom of the cube, the light appears fairly evenly distributed; at higher levels it appears to be concentrated more toward the center. If the condenser were to be focused lower, this effect would be still more marked. When the microscope objective is raised from a position of focus, say as at the optical section *A*, the concentration of light (particularly observing the paths of rays 1 and 2) can be plainly seen as a white or light ring around the border of the cube or particle. As the drawing of the rays indicate, the white line moves toward the material of higher index. When the microscope tube is lowered the line moves in the opposite direction, or toward the material of lower index. This line is the well-known Becke line. When the specimen and the mounting liquid are of equal index the Becke line does not exist and the specimen is not visible. When a transparent, colorless specimen has low visibility and is neither very small nor very thin, it is generally safe to assume that its refractive index is very close to that of the mounting liquid. Winchell[25] gives an excellent description of the observation of the Becke line and allied phenomena.

Figure 201 is a photomicrograph of a crystal of vitamin C, ascorbic acid, $n = 1.588$, in a liquid of $n = 1.76$. The focus is high to show the Becke line.

[25] Alexander N. Winchell, *Elements of Optical Mineralogy*, fourth edition, 1931.

If the specimen is of lower index than the mounting medium, the Becke line will still move toward the material of higher index. In the example cited it will move outward as the tube is raised. Figure 200 shows why.

The occurrence of the Becke line is variable, and conditions are not always favorable for its perception. It is often quite marked when the specimen is of a crystal nature and has vertical, straight sides.

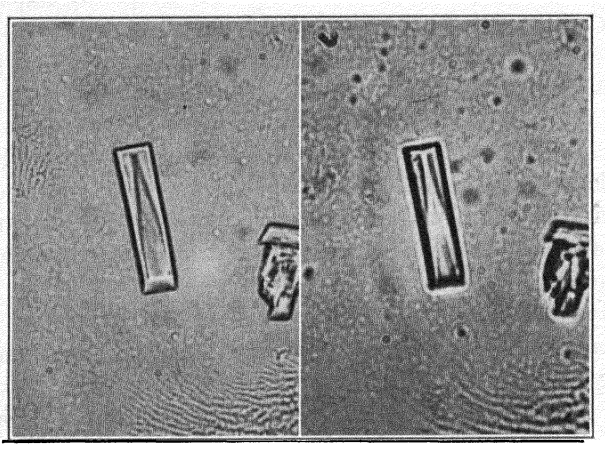


FIG. 201. Photomicrographs of crystal of vitamin C, $n = 1.588$, in a liquid $n = 1.74$. At A, the specimen is within the focus of the microscope; at B, it is beyond the focus of the microscope. The position of the Becke line (the white line) is shown in both. The line is seen nearer the substance of higher index as the focus of the microscope is raised.

Sometimes it can be seen best when the microscope tube is lowered and sometimes when it is raised, and again the nature of the specimen has a bearing. A strong light-absorbing specimen would probably show the Becke line best when the line is brought within the image of the specimen. The selection of an objective best suited to bring out the Becke line strongly depends on the specimen. Perhaps the 8-mm achromat, with a weak condenser giving a ⅔ to ½ cone, will be the most generally useful. A wide cone of light may make observation of the Becke line impossible. On the other hand, strictly axial lighting (parallel rays — no condenser) will usually set up diffraction effects which mask the true Becke line.

Determination by Noting Concentration of Light by Specimen. Most microscopic objects vary considerably in shape, but one feature is common to many: the edges of the specimen are usually much

thinner than the center. This suggests a lenticular configuration; in fact, nearly all discrete particles affect light rather imperfectly, like either a positive or negative lens. Figures 202 and 203 illustrate

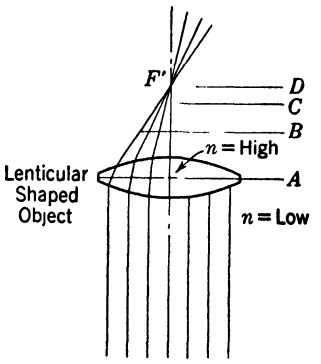


FIG. 202. This figure indicates how transmitted light is brought to a focus above the specimen by a transparent particle. All particles may not have the exact lens shape shown in the figure, but they are usually inclined to be thinner on the edges than in the centers, and thus they set up a lenticular condition wh ch focuses parallel light rays as shown in the cut. If the impinging rays were converging, the focal point would fall within F', which is the principal focal point of the particle. As the microscope tube is raised from focus at level A to level B, the same number of lumens are present at B as there were at A, but the area at B level is smaller than that at A level, so that the area at B appears brighter per unit area. Successively higher levels appear brighter per unit area, as at C and D. Here the particle is surrounded by a medium of lower index than itself.

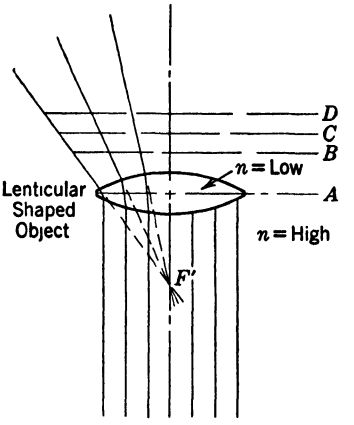


FIG. 203. This diagram is the reverse of Fig. 202; that is, the particle is immersed in a medium of higher index than itself. Consequently, at levels higher than A, as B, C, and D, the light intensity per unit area decreases, and the center of the particle appears darker at the higher stages. Conversely, if the microscope tube be lowered, the center of the particle would appear brighter as the point F' is approached, for the doubly convex particle will act like a negative lens because it is in a medium of higher index than itself.

clearly how parallel light behaves under such conditions. Although, for the sake of conforming to the geometry of the refraction of light, the drawings show parallel rays reaching the particle, in actual work the results can be duplicated by means of a condenser, particularly

one of low power, well stopped down. In fact, the use of a low-power condenser is advocated because superior images can be obtained with it and it suppresses diffraction.

In Fig. 202 the particle shown is of high index compared with the mounting medium. As when a positive lens works in air, the parallel rays are brought to a focus above the specimen at its principal focus. Therefore when the tube of the microscope is raised from focus on the particle at optical section *A*, to focus on succeeding sections as *B*, *C*, and *D*, the center of the particle appears to grow bright. Although the amount of light at the *D* level is the same as at *A*, it is concentrated in a smaller area.

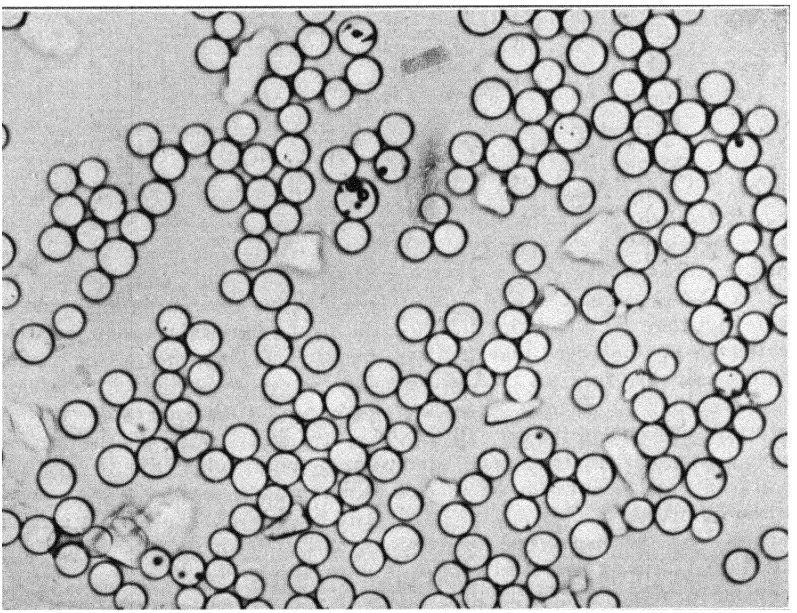


FIG. 204. Glass spheres ×650. The index of the spheres is about 1.468, and they are mounted in ethylene glycol, $n = 1.40$. In this figure the focus of the microscope was made as nearly perfect as possible, to give a true idea of how the spheres appear under these conditions. This picture should be compared with Fig. 205, where the focus of the microscope has been raised, and with Fig. 206, where it has been lowered.

When conditions are reversed, as in Fig. 203, and the particle is of lower index than the surrounding medium, the specimen behaves as a negative lens with its principal focus located on the side from which the light comes. Therefore when the microscope tube is raised the center of the particle appears to grow darker at successively higher levels, as at *B*, *C*, and *D*.

If the tube of the microscope is moved downward instead of upward the effects cited above will be reversed and the center of the particle will grow darker in the first instance and lighter in the second.

As with the Becke line, there should not be any difference in appearance of the image on lowering or raising the tube when the specimen and medium are of similar index, for then all lenticular effects disappear.

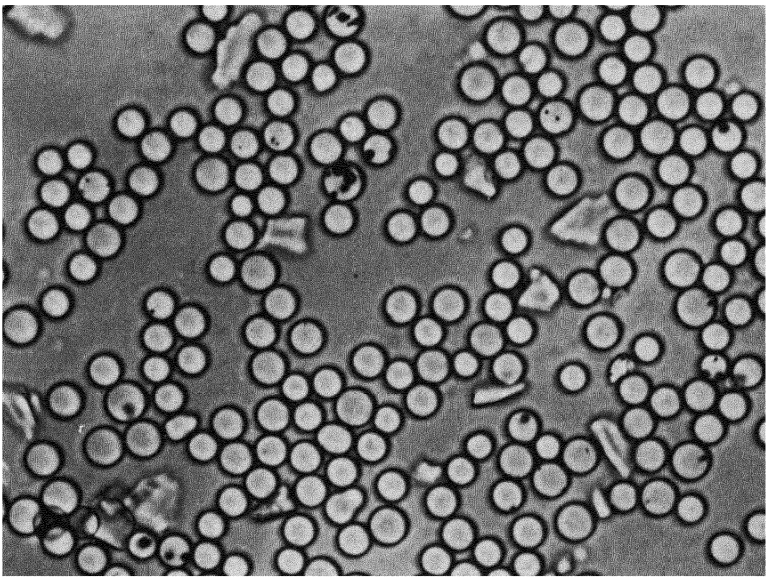


FIG. 205. Glass spheres ×650. This is the same subject as in Fig. 204, but microscope tube has been raised from the optimum position of focus. It shows how the centers of the individual particles get brighter when mounted in a liquid of lower index. Cf. Figs. 204 and 206.

Figures 204, 205, and 206 comprise a series of photomicrographs of tiny glass spheres. Their index is about 1.5, and the mounting medium is monomethyl glycol with an index of 1.41. In Fig. 204 the spheres are in good focus; in Fig. 205 the tube of the microscope has been raised and the centers of the spheres appear brighter than before; in Fig. 206 the tube has been lowered and the spheres appear darker. The spheres were furnished by Drs. C. R. Bloomquist and A. Clark, of the Battelle Memorial Institute. The method of making the spheres is ingenious and is described in the Jan. 15, 1940 edition of Industrial Engineering Chmistry, analytical edition.

This test can be applied while a search is being made for the Becke

line, one test serving as a check on the other. In conducting tests of this sort, it saves time if all preliminary observations are made in a certain order. Thus, if the tube is always raised first, it will then only be necessary to remember that, when the object is of higher index than the medium, the center will grow lighter and the Becke line will move toward the material of higher index.

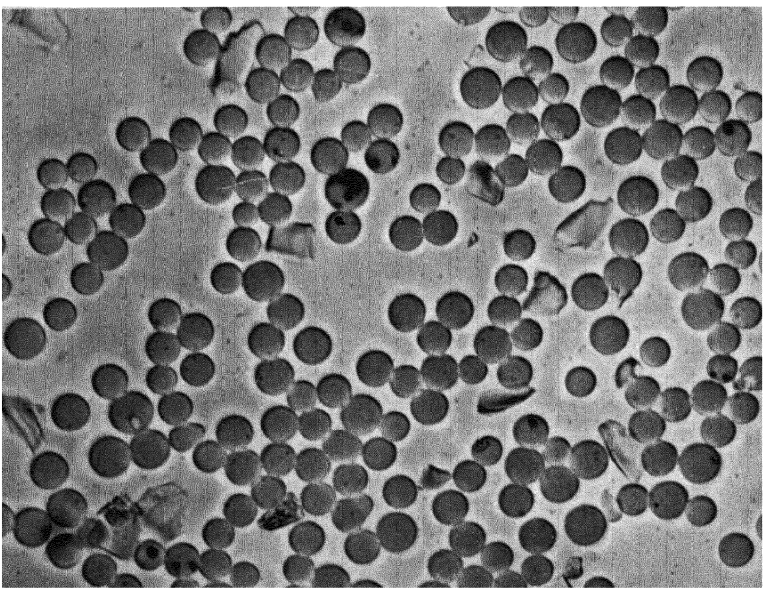


FIG. 206. Same subject as Fig. 204 but taken at a slightly lower optical level. The centers of the spheres appear darker than the surrounding field.

Good results can usually be expected from this test irrespective of the objective in use. However, like all tests in refractive index work, there is a certain gain in speed and accuracy if the technician is accustomed to the different optical phenomena produced under a given set of conditions. Except for work with pigments, the 16-mm objective can generally be used for all tests, the 8-mm being turned in only as needed to confirm the Becke-line test. The medium-power condenser, with the iris closed sufficiently to give the greatest contrast in the final results, can be used throughout.

Determination by Oblique Illumination. The Becke-line test for relative refringence, and the test for noting the concentration of light within the specimen, are always reliable but they cannot be applied as universally as can the oblique illumination method. Figures 207

and 208 show the effects of oblique illumination. The amount of light in pencil 1 and in pencil 2 is the same. At A, in Fig. 207, the light is spread over a larger area than at B, and so point A appears darker than B. Under these conditions one side of the specimen will appear shaded and the opposite side bright. The shaded side of the specimen will be on the side from which the light comes when the index of the object is higher than that of its surroundings. The opposite is true

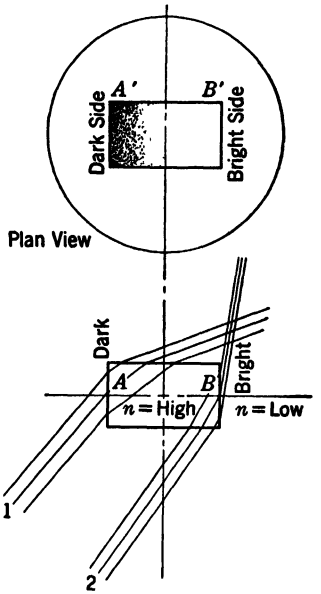


FIG. 207. The effect of a specimen on oblique light rays when the specimen is of higher index than its surroundings. The plan view indicates the shaded side.

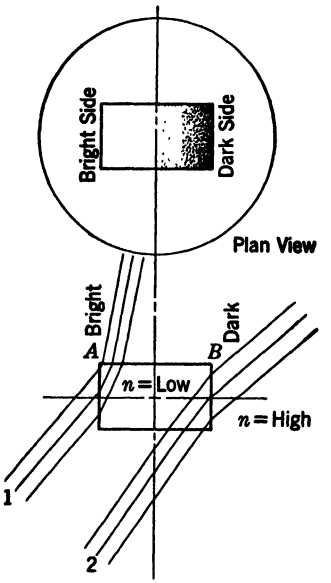
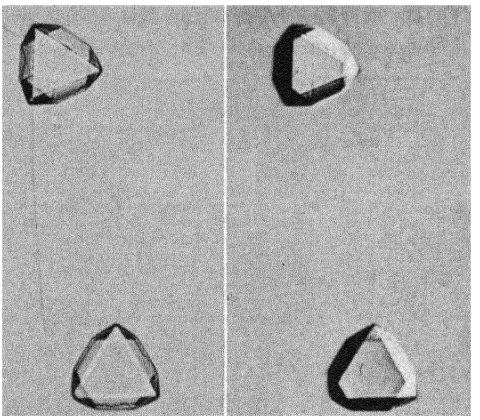


FIG. 208. In this diagram the specimen is shown as having a lower index than its surroundings, the lighting being oblique. The shadow effects are indicated in the plan view.

when the specimen is of lower index than the mounting liquid; then the shaded side of the specimen will be on the side away from the direction of the light. As before, when specimen and mounting liquid are of like index, the specimen will be invisible for it will have no refractive effects on the light rays.

The mounting of the condenser, its focal length, and the position of the iris diaphragm greatly influence the sensitivity of this test. Generally, results are best and clearest cut when the top of an achromatic-aplanatic or other good condenser is removed and the condenser carefully focused, and the iris can be closed to give about a ⅔ cone.

Displacement of the condenser iris in its adjustable mount by rack and pinion is the ideal way to obtain oblique lighting. If the iris is too far from the first focal point of the condenser the results will be less clear than when it is at, or near, this point. Shutting off the light in one direction with the finger or a card is a clumsy method, and results are never as good as when the light is sharply cut off with the properly placed diaphragm. The effect of oblique lighting is shown in the photomicrograph in Fig. 209. At *A*, the crystals are illuminated by central lighting; at *B*, they are illuminated by oblique lighting.



A *B*

FIG. 209. Photomicrograph of crystals of $NaBrO_3$, $n = 1.617$, in a liquid, $n = 1.51$, ×50, with central lighting at *A* and oblique lighting at *B*.

Study of the diagrams in Figs. 207 and 208 will show that the trace of light rays through the specimen, and the final appearance of the specimen, indicate that no account is taken of the reversal of directions which a lens would introduce. If an objective were used, the plan view of the specimen would be reversed; the plan view would become a field of view, and the bright side would appear in the place of the dark side. A condenser, if introduced, would not make any difference because it could be shaded on either side and, being served with nearly parallel lighting, would maintain the direction of rays 1 and 2 as shown in the figures. Thus, when the bright side of the object is toward the direction from which the light comes, the object is of higher index than the surrounding medium.

Other Methods of Determining Refractive Index. A few years ago Emmons[26] developed the now well-known method of "double variation," which utilizes the fact that the index of a liquid varies with the temperature whereas that of a solid varies chiefly with the wavelength of light used in its examination. A liquid which has an index known to be slightly above that of the solid is first selected. Heat is applied by means of a special stage, and, as the specimen and its mounting

[26] R. C. Emmons, "The Double Variation Method of Refractive Index Determination," *American Mineralogist*, **14**, 482, 1929.

medium grow warm, the index of the liquid drops. Tests are applied by any of the three methods just described, and when the index of the liquid becomes the same as that of the specimen the temperature is noted; from the temperature coefficient of the liquid the index of the specimen can be computed. This process can be repeated with monochromatic filters for the C, D, and F spectral lines. Thus a curve can be drawn, showing the variation of index with wavelength, from which dispersion values ($n_F - n_C$) can be calculated.

As an example, suppose that a liquid having an index slightly above that of the specimen is selected. The index of the liquid is $n_D^{25°C} = 1.546$, with a temperature coefficient of 0.0004. The temperature of the mount is raised until liquid and specimen coincide in index. This may be found to be, say, 36° C, as read on the thermometer in the specially constructed warm stage, or a rise of 11° C above the temperature at which the index of the liquid was 1.546. Thus the index of the liquid has been lowered 0.0044, or 11×0.0004, and at 36° C it is therefore $1.546 - 0.0044$, or 1.5416. At this temperature the specimen and the liquid have the same index; therefore the index of the solid is 1.5416 for the D line.

Emmons' method requires special equipment for heat control but fewer liquids than other methods which do not utilize thermal variation.

Bausch and Lomb has developed an elaborate thermal-variation method utilizing a refractometer and monochromator. The temperature of the refractometer is controlled by a circulating liquid so that it will keep in complete agreement with that of a cell holding the specimen on the microscope stage. When the mounting medium and specimen have been warmed sufficiently to agree in index, a sample of the medium is mounted on the refractometer and its index is read.

Leitz has a special apparatus for a modified form of the Emmons method. A special hot stage warms the specimen and mounting liquid until they coincide in index. The stage with the specimen can then be revolved (like the turning of the prisms in the Abbe refractometer) about a horizontal axis until the critical angle is reached. The accessory stage is a simplified form of the Universal or Fedorow stage. The index of the specimen is determined by referring the reading of the stage to an accompanying table.

All the foregoing methods for determining the index of a specimen are indirect. They are referred to generally as *immersion methods;* the index of X, the unknown, is found in terms of A, the index of which is known. However, it is also possible, by microscopical methods, to find the index of a solid or liquid directly. The specimen should be

fairly transparent and flat, and preferably should be mounted in air. Its thickness is first determined on the microscope by using the fine adjustment and measuring, generally, at the edge of the specimen; the apparent thickness is then measured by focusing first on the lower side of the specimen, then on the top, while looking through it. The index will be the true thickness divided by the apparent thickness, or

$$\text{Index of specimen} = \frac{\text{True thickness}}{\text{Apparent thickness}} \qquad [61]$$

Sec. 135. Sensitivity of the Various Methods and Conditions Leading to Erroneous Conclusions. All indirect methods for measuring refractive index involve tests for relative index, and the precision of the result cannot be greater than the operator's ability to estimate when the relative index has been made 1.0. Thus the microscopist must possess not only power of critical observation but also great skill in all details involved in optical and mechanical technique. The most universally sensitive test for relative index is undoubtedly that which makes use of oblique illumination. Under ideal conditions and with a flat specimen say, 50 μ to 100 μ in thickness, it is possible to detect easily differences in relative index of less than 0.0005. Even under conditions that are not conducive to easy interpretation, differences of that amount can often be detected. As a rule the Becke-line test and the test for concentration of light above the specimen will not be nearly so critical as that which uses oblique illumination.

The Bausch and Lomb apparatus using a refractometer makes it possible to *estimate* the index of liquid to within one or two figures in the fourth decimal place. This is about equal to the sensitivity afforded by oblique illumination. The Leitz method will be correct to about one figure in the third place.

Sensitivity is influenced considerably by the immersion liquids employed. Pure chemical compounds are not likely to have any special relationship in index. In a given set, one liquid is bromoform, $n = 1.5940$; another, quinaldine, $n = 1.6088$; and a third, cinnamaldehyde, $n = 1.6190$. The difference between the first two is 0.0148; between quinaldine and cinnameldehyde, 0.0112. On the other hand, the index difference between two other adjacent liquids in the same set is 0.0018. Obviously readings falling between the latter liquids would be correct to within 0.0018, whereas between bromoform and quinaldine they would be correct to within 0.0148 and between quinaldine and cinnameldehyde they would be correct to within 0.0112, estimations being closer. If the index of the specimens falls between two of the first-mentioned liquids, the sensitivity of the oblique illumination test will

be more than ten times greater than the interval between the liquids, whereas the ideal liquids should have intervals at least equal to, or preferably less than, the sensitivity of the test. Probably results will be most accurate if the thermal coefficient of the liquids is known and the double variation method is followed, for then intervals between the indices of the liquids will be of less importance. A simpler method which will reduce error in reading to less than 0.002 makes use of a set of liquids which are stable mixtures having a constant index difference of 0.002 between each two units. These liquids may be used with the thermal variation method; then estimation between two units is eliminated, and the maximum sensitivity is that of the particular test for relative index which may be employed.

In learning the refractive index of a specimen, the first consideration is the refractive index of the liquid with which the index of the specimen most closely agrees. Therefore careful attention should be paid to the recorded index values of that liquid. α-Monobromonaphthalene was shown in Sec. 129 to vary in index between 1.657 and 1.656, and a specimen of xylidine taken from a set of refractive index liquids and measured on a recently calibrated refractometer had an index of 1.5585 against a value of 1.5559 as marked on the bottle by the manufacturers. Changes are more likely to occur in the pure compounds than in mixtures, provided that the mixtures have been properly made. Errors in refractive index values of such liquids should not exceed 0.0002.

There are several important sources of error which may occur singly or together when determining refractive index. Trouble may be experienced when a specimen has a range of indices due to various causes. Natural minerals often show a range of index as do some chemicals. Experience and knowledge concerning the material, and a complete study of it with the petrographical microscope, may sometimes be required in order to correlate the findings with the published data. Small-size material may often lead to erroneous readings. When, in an immersion method, the index of the liquid is made to approach that of the specimen, the visibility factor becomes so low for very small particles that estimations of relative index are very difficult to make. Figure 198 demonstrates how easily the smaller components of a mixture may be overlooked as their relative index approaches 1.0. Errors from this source may lead to a report on one component only, whereas actually two or more may be present. As a rule, high-index substances also are difficult to determine with the same degree of accuracy as is possible with material of low or medium index of refraction. Here, the chief difficulty lies in the fact that the index of the immersion medium is often given to only one to two significant

decimal places. In addition, when melts are used in a mount changes in their index are unavoidable on account of the high temperatures required in handling them. Sensitivity may then be less than one figure in the second decimal place.

Readings should always be made in the center of the field, and, to avoid errors arising from unilateral lighting in an uncentered system, all care must be taken in making lighting adjustments, and in arranging the condenser for strictly central illumination. The aperture should generally be low for both condenser and objective. The 30- or 32-mm objective, with an aperture of 0.15 or less, gives fine results when the specimen is large enough to permit its use.

Determinative tables, as used by mineralogists, and similar tables found in many chemical handbooks, as a rule, list more than one value for the index of refraction for anisotropic specimens; two values are given for uniaxial, and three values for biaxial, material. In general, anisotropic substances, on the stage of the microscope, pass light which vibrates, horizontally, in two planes at right angles to each other, thus separating the transmitted light into two components. Now one characteristic of an anisotropic crystal is that it will retard the passage of light traveling normal to these planes of vibration differently for the different planes; thus the specimen will have a different index for each component of light. Since there are three planes of vibration for biaxial crystals and two for uniaxial crystals, these two categories are listed with three and two values of index of refraction, respectively. Measuring these indices accurately depends on the optical orientation of the specimen. It is work which comes within the province of the petrographer, with a polarizing microscope. With a biological microscope and natural or unpolarized light the specimen will give an index value which is generally midway between the highest and lowest values found with a petrographical microscope. It might also be remembered that the maximum and minimum values of index are not necessarily present in the haphazard orientation of a crystal, but intermediate values may be shown. Thus, several readings on anisotropic biaxial crystals oriented at random, when taken with the biological microscope in natural light, generally give an index value very close to the β index as measured on the petrograph, which corresponds to a vibrational direction of the crystallographer. In value, it is about halfway between the highest and lowest index of the crystal. If the crystal is uniaxial, an average taken from several readings in natural light may correspond to an average of the ω or ϵ value or the α and γ values of index. The average of several readings works out well when the birefringence of the specimen is low and when the crystal can be presented in

many different orientations. If the crystals are similar to those obtained from iodoform, crystallized from xylene, they will always lie on the slide in only one optical orientation, in which case only the ω value of index can be determined. A large number of anisotropic specimens have a birefringence less than 0.01; on a still larger number it is even lower, quartz, for instance, being 0.009. In general, the lower the index the lower the birefringence.[27]

In the literature, refractive index is often quoted for only one value on anisotropic material, particularly fibers and very fine pigments. Thus, although wool fiber and all hair polarize and have in general two values of refractive index, the usual custom is to report only one.

The definition of dispersion was given in Sec. 39 as the difference in refractive indices of the substance for light of two different wavelengths, generally the difference between the index for the red and violet spectral lines. Color fringes sometimes are encountered when the dispersion of the specimen is much less than the dispersion of the liquid. As a rule liquids have a much higher dispersion than solids. When the color fringes appear, the refractive index of the specimen for blue light will be less than the refractive index of the mounting liquid for blue light; and the index of the specimen for red light will be greater than the index of the mounting liquid for red light. When the specimen is viewed in a liquid of equal index and with oblique illumination, the image appears tinged with red on one side and blue on the other. In this case, the specimen will have an index for an intermediate color, for example, green or yellow, which will be the same as the refractive index of the liquid for light of that color. The color fringes around the specimen will disappear when optical light filters are used to give monochromatic light of the desired intermediate value. Two Corning filters — 428 and 348 — pass a strong yellow light comparable to the D line of the spectrum. Refractive index values obtained by means of the above set of filters are very nearly the same as those obtained with natural daylight.

Sec. 136. Lighting for Refractive-Index Work. If the work is very exacting and of a petrographical nature, the lighting should be monochromatic, and three readings should be taken using the C, D, and F lines. The tungsten lamp with appropriate filters will answer well.

[27] The biological microscope, even when fitted with a polarizing and analyzing prism, is not particularly well adapted to carrying on work involving polarized light. The determination of refractive index is exacting, precise, quantitative work, while accessory polarizing equipment on a biological microscope is best suited for qualitative work only.

If the work is of a general nature, the tungsten lamp with a daylight screen will generally suffice. For a little better grade of work, filters giving the D line can be used; the reading obtained will probably not differ greatly from that obtained by daylight, but the results may be more accurate because dispersion effects will be eliminated. Illumination may be by Method I. For most of this work, as far as the photomicrographer is concerned, only quick relative readings are required so that he may select his next liquid with a view to obtaining contrast for his picture. The method of illumination is then unimportant.

Sec. 137. Sets of Liquids for Refractive-Index Work. To facilitate the measuring of refractive index, sets of liquids can be obtained which vary in index by small increments. These sets, although originally devised for mineralogists, apply equally well to the work of the photomicrographer, for they provide him with a ready means of controlling contrast in his specimen. They are also useful as a quick and ready means of identification for many substances.

When the liquids comprise pure compounds, their indices will not be under control. Sets composed of liquid mixtures with predetermined indices are much to be preferred. Generally they will be less volatile than the pure compounds, and their uniform difference in index is a very desirable feature.[28] The Eastman Kodak Company list thirty-five liquids for refractive-index work. For the most part these are pure compounds and are volatile. Winchell[29] gives two lists, including that of Emmons.[30] Chamot and Mason[31] give a table of liquids which are mixtures and cover a range from 1.37 to 1.96. A list of pure liquid compounds and one of solids of varying index are also given.

The photomicrographer may not need a large number of liquids for examination or mounting media; 10 or 12 may be enough, unless, of course, his work deals extensively with the identification of materials.

[28] A set of liquids prepared by R. P. Cargille, 118 Liberty Street, New York City, comprises 91 non-volatile liquids differing in index by 0.002 ± 0.0002. The range is from 1.460 to 1.640. The whole set may be ordered, or any part of it, as desired. In this way it is possible to have on hand, as a nucleus, the liquids required for immediate use and to add the remainder as needed until the set is completed.

[29] Alexander N. Winchell, *Elements of Optical Mineralogy,* Part I, John Wiley & Sons, 1931.

[30] R. C. Emmons, "The Double Dispersion Method of Mineral Determination," *American Mineralogist,* **13,** 1928; "The Double Variation Method of Refractive Index Determination," *ibid.,* **14,** 482, 1929.

[31] Chamot and Mason, *Handbook of Chemical Microscopy,* Vol. 1, second edition, 1938.

The liquids should be kept in uniform bottles each with its individual applicator mounted in the stopper. Such bottles, if no larger than about 15 to 30 cc, can be stowed away in small compass and will be convenient on the work table. Bakelite caps with flat tops are recommended to enable stickers to be affixed to indicate the index of the contained liquid. The sticker can be covered with transparent tape and so become practically permanent.

Sec. 138. The Mixing of Mounting Liquids. The index of any liquid can be raised or lowered by the addition of another liquid with which it is miscible. The resulting index can be foretold with a fair degree of precision by means of the following equation:

$$n\ (v_1 + v_2) = n_1 v_1 + n_2 v_2 \qquad [62]$$

n is the desired index; n_1 is the index of liquid 1; n_2 is the index of liquid 2; v_1 is the volume of liquid 1 that is to be used; v_2 is the volume of liquid 2 that is to be used. The application of this equation should give results to within aproximately 0.001. Though the equation is reasonably reliable, for exact work the mixture must be checked by a refractometer. Kaiser and Parrish[32] discuss the use of equation 62 and methods for final adjustment.

Sec. 139. Determination of the Refractive Index of Liquids by Means of the Microscope. Equation 61 is suggestive of a method for determining the index of refraction of liquid specimens by means of the microscope. At the bottom of a cell, fused or cemented to a microscope slide, a line is inscribed. The line is then filled with finely divided carbon black, all surplus being wiped from the bottom of the cell. A cover glass also having an inscribed line should then be placed on the top of the cell with the side bearing the line placed downward. The depth of the cell can now be measured with the vertical adjustment of the microscope or by any other method. If measured by the microscope, it will be necessary to have the cell less than 2 mm in depth for this is about the limit of the range of a fine adjustment. If the cell is then filled with the liquid the index of which is to be measured, and the cover glass applied, a new reading can be made of the cell depth by focusing first on the line scribed on the slide and then on the line scribed on the cover. The difference in the reading of the fine adjustment in the two positions will give the apparent depth of the liquid. Equation 61 can now be applied and solved for the index of the liquid. There is no limit to the range of index values to which this method can be applied.

[32] Kaiser and Parrish, "Preparation of Immersion Liquids," *Ind. Eng. Chem., Anal. Ed.*, **31**, 560, 1939.

The Nichols micro-refractometer[33] is a microscope accessory for measuring the index of refraction of liquids. It is an adaptation of the Clarici cell, which consisted originally of one prism. The Nichols device consists of two tiny, right-triangular prisms mounted side by side in a glass or metal cell. A line is scribed across the bottom of the cell, directly under the prisms. The prisms are in a reversed position in respect to each other, and the hypotenuse planes intersect above the scribed line, so that an image of the line on the slide is refracted in one direction by one prism and in the opposite direction by the other. If the liquid in the cell is of the same refractive index as the prisms, there will be no deviation of the line image by either prism; but if the index of the liquid differs from that of the glass prisms, the light rays proceeding from the line will be deviated in one direction by one prism and in the opposite direction by the other. The refractive index of the liquid is determined by measuring the separation of the broken-line image. This device must be calibrated for the optics with which it is to be used and with a set of liquids of known index. It has been operated with an amount of liquid as small as 0.004 cc. The method has been investigated by Alber and Bryant.[34]

Other methods for the measuring of refractive index of liquids by means of the microscope suggest themselves. Many are already recorded in the literature on the subject. The method of Jelley,[35] developed by Leitz, and similar apparatus by Fisher,[36] though not applicable to the microscope, are capable of operating with microscopic amounts of material. Perhaps one of the most useful methods from the standpoint of the photomicrographer is to reverse the principles of determining the index of refraction of solids, as outlined in Sec. 134. A series of fine powders of varying known index is used, immersions being made to find a powder which has the same index as the liquid.

Sec. 140. Compounds for Sealing Cover Glasses. Circular cover glasses are best sealed on a revolving table to which the slide can be secured. A small brush saturated with the sealing compound is held over the edge of the cover, and the table is spun. After one or two trials it should be possible to make perfect seals. If the cover is square or rectangular, the sealing compound must be painted on freehand. The brush should not be too small, for it must be able to hold a substantial

[33] Lyman Nichols, 111 Vreeland Avenue, Nutley, N. J.

[34] H. K. Alber and J. T. Bryant, "Systematic Qualitative Organic Microanalysis," *Ind. Eng. Chem., Anal. Ed.*, **12**, 305, 1940.

[35] E. E. Jelley, Eastman Kodak Company, Rochester, N. Y.

[36] Fisher Scientific Company, Pittsburgh, Pa.

amount of the compound, which should be applied in such a way that the seal will lap over both sides of the joint for some distance. The sealing on some of the Moeller slides is an excellent example of overlap.

Covers can be sealed with many kinds of material, as Table XXX shows, but some are suitable for temporary mounts only, as, for instance, castor oil on a water mount. Sometimes the sealing com-

Table XXX

Sealing Compounds*

For Permanent Seals	For Temporary Seals
Shellac	Any heavy oil
Gum dammar	Glue
Gold size	Latex
Varnish	Aroclor, the liquid
Lacquers	Vaseline
Asphaltum	Paraffin
Bakelite curing at 100° C	Beeswax
Paraffin, melted	Plasticine
Sealing wax in alcohol	Narrow strips of Scotch tape
Parlodion in amyl acetate	
Aroclor, the solid, warmed	
Celluloid in acetone	
Isobutyl methacrylate in xylene	
Clarite	

* This list suggests only a few of the many materials suitable for sealing slides.

pound of a temporary or semi-temporary mount can be made permanent by a further coating of another compound, for example, shellac over paraffin. The list of materials in Table XXX includes substitutes for permanent and temporary mounts. Their selection for each specific mount must be left to the judgment of the user.

An ideal sealing compound would be easy to apply, mechanically strong, heat resistant, impervious to moisture, and chemically inactive with the glass and mounting medium. It would resist the action of any solvents that might be used for cleaning the slide, and it would adhere well to glass to protect the mount against evaporation or oxidation by the atmosphere. At present there is nothing which can be easily applied and which also fulfills all other requirements. A good seal should not crack, even with age; cracking can be avoided in part by applying several thin sealing coats rather than one thick one, and considerable strength will be added to the seal by making it laminated through successive applications. Valuable specimens should be given additional coats of compound from time to time during storage.

Sec. 141. Staining for Photomicrographic Work. For nearly all work in industrial laboratories, stains are used to augment, in images, the contrast which has already been partly obtained by refraction. Stains may be used to good advantage with appropriate filters to differentiate selectively between two components. For example, if a mixture of fibers has a red stain for one component and blue for another, oftentimes the difference can be enhanced for photographic contrast by the judicious use of filters. For observational purposes, the color difference alone would be sufficient; but for photomicrographic work the difference will probably have to be converted into photographic contrast. When it is desired to render such differences, it often helps to resort to a plate or film with a regular emulsion, which will render the blues a light shade of gray on the finished print, and the other colors comparatively darker.

The histologist, working with either animal or vegetable material, is accustomed to use his staining material very critically. As a rule, he knows what effect to expect before he commences to work, and so he can anticipate results. His specimens are put through a definite course of preparation — killing, fixing, and hardening — then stained according to a well-planned formula. By contrast, when material comes to the photomicrographer it has to be worked with " as is," and it is his problem to do the best he can with it.

For those who wish to make a more thorough study of the technique of staining, the works of Lee,[37] Conn,[38] McClung,[39] and Belling[40] are all excellent. The *Journal of Biological Stains,*[41] Chamberlain's[42] book on botanical methods, and the more recent work by Johansen[43] are recommended. Lawrie's[44] book on textiles, though not very recent, is quite thorough, and the paper on woods by Koehler, Gerry, and Weinstein[45] is still useful.

[37] A. B. Lee, *Microtomist's Vade-Mecum.* This book is wonderfully complete in original references. It describes the theory and practice of staining, and the making of stains for the biologist.

[38] H. J. Conn, *Stain Technology.*

[39] C. E. McClung, *Handbook of Microscopical Technique,* 1929.

[40] John Belling, *The Use of the Microscope,* 1930. This book does not describe the preparation of stains, but it has a short chapter on their application to plant material and to certain biological specimens.

[41] *Journal of Biological Stains,* Geneva, N. Y.

[42] Chamberlain, *Methods in Plant Histology.*

[43] D. A. Johansen, *Plant Microtechnique,* 1940.

[44] L. G. Lawrie, *Textile Microscopy,* 1928.

[45] A. Koehler, E. Gerry, and A. I. Weinstein, " Preparing Woody Tissue for Making Microscopical Mounts," U. S. Dept. of Agriculture, Forest Products Laboratory, Madison, Wis., 1927.

As will be seen, it is necessary to turn to the literature covering any given profession in order to keep informed of the stains which are most useful in that field. Merritt[46] has described some highly specialized stains for paper fibers, and Lofton[47] describes the photomicrography of paper pulp and the use of several stains.

Every photomicrographer in any plant laboratory will eventually develop his own methods of handling the material in which he is individually interested. This will often include the use of special stains and special staining technique adapted to suit only the work in hand. Consequently, with such diversified demands and multiple uses of stains on such a wide range of material, the technique of staining cannot be discussed here comprehensively. However, some of the more common uses of stains in industrial work will be cited, and certain rather unorthodox methods of using them will be mentioned later, with the thought that, in photomicrography, stains may be used just a little differently from the way they are used in other branches of science, and often for different purposes. The technician must develop his own skill along this line as his work progresses. As an instance, he will find that sometimes, although a specimen may have good visibility and good contrast without stains, the application of a weak solution of Lugol's solution or gentian violet may add tremendously to the photographic rendition. Two or three stains kept on hand and used frequently for experimental work may considerably improve the general level of the work of the photomicrographic laboratory.

Some Useful Stains. The paper industry has developed several well-known special stains which are quite selective. One of the best known is the *Herzberg stain,* which stains lignified cellulose to a light or dark yellow, pure cellulose to a brownish red, and chemically purified cellulose to light or dark blue. The formula for making it is as follows:

HERZBERG STAIN (MERRITT PROCESS)[48]

Solution A

50 g zinc chloride, fused sticks
25 cc distilled water

The specific gravity of this solution should be adjusted to 1.8.

[46] Muriel F. Merritt, "Pulp and Paper Fiber Composition Standards," *Natl. Bureau of Standards Tech. Paper* 250, 1924.

[47] R. E. Lofton, "Photomicrography of Paper Fibers," *Natl. Bureau of Standards Tech. Paper* 217, 1922.

[48] Muriel F. Merritt, "Improvements in Making the Herzberg Stain Used in Fiber Analysis," *Paper Trade J.,* Aug. 24, 1922.

Solution B

2.25 g potassium iodide
0.25 g iodine
12.5 cc distilled water

Solution *B* is added to solution *A*, stirred well, and kept in the dark. After twenty-four hours the clear portion is pipetted into another receptacle. If a tall cylinder has been used this separation should be easy. A leaf of crystal iodine is then added. Merritt's directions are to dilute the stain by one-half for photomicrographic purposes. This stain does not keep well, and for continuous critical work it should be freshly made every two weeks.

Another stain for determining unbleached sulphite from unbleached sulphate is the *Lofton-Merritt stain.*[49]

LOFTON-MERRITT STAIN

Solution A

2 g malachite-green crystals
100 cc distilled water

Solution B

1 g basic fuchsine
100 cc distilled water

One part of solution *A* is used with 2 parts of solution *B*. The specimen can be stained on the slide. After staining, the specimen is washed with 4 drops of hydrochloric acid solution made by adding 1 cc HCl, sp. gr. 1.19, to 1 liter of water. The acid is left on the specimen for 10 seconds and then washed off with water. This stain keeps well.

The *Shaffer* method distinguishes bleached sulphite from bleached sulphate by means of a stain made of brazilin.

SHAFFER-BRAZILIN STAIN[50]

1.0 g sodium carbonate to 175 cc distilled water; add 1 gram C. P. brazilin. Keep protected from air.

Bleached sulphite fibers are colored wine red while bleached sulphate fibers are dyed purple with this stain.

In using these stains care must be exercised that the specimen is not over-stained. Such precaution is particularly necessary in using

[49] R. E. Lofton and M. F. Merritt, "Methods for Differentiating and Estimating Unbleached Sulphite and Unbleached Sulphate," *Natl. Bureau of Standards Tech. Paper,* 189.

[50] Ralph M. Shaffer, "A Rapid Method for Distinguishing Bleached Sulphate from Bleached Sulphite," *J. Ind. Eng. Chem., Anal. Ed.,* Jan. 15, 1933.

Herzberg's stain in photomicrography. Obviously, any blocking out of detail will be detrimental to the photograph.

Lugol's solution, the formula for which follows, is a useful iodine stain. When used for starch, it should be well diluted. To get an idea of its action a small drop can be placed in contact with the edge of the cover glass of the starch slide. As it diffuses through the specimen the appearance of the grains can be carefully watched. There should be a gradation in intensity of staining. The color should be deep at the edge of the cover, progressing to a light tint near the center of the specimen, where the solution should be considerably diluted.

LUGOL'S SOLUTION[51]

45 cc 90 per cent alcohol
0.5 g iodine
To which is added
5.0 g potassium iodide
5.0 cc distilled water

Or the solution may be made entirely with water:
100 cc distilled water
6 g potassium iodide
4 g iodine

This solution will color starch blue, glycogen brown, cellulose a reddish or wine color, and mercerized cotton black. The mounts can be made in the diluted stain solution.

Neocarmen is a selective staining agent which gives quite a range of colors, depending upon the fiber on which it is used. It will show oxycellulose as red spots on a blue field. This stain can be obtained from the Fisher Scientific Company.

Schultze's solution will color cellulose violet. It is made as follows:[52]

To a saturated solution of metallic zinc in HCl, evaporated to the consistency of a thick syrup, is added all the potassium iodide and all the iodine that will dissolve.

DELAFIELD'S HAEMATOXYLIN

400 cc saturated solution ammonium alum
4 g haematoxylin crystals dissolved in 25 cc alcohol

Expose for three or four days to light and air, and then filter. Add
100 cc methyl alcohol

The solution is allowed to stand till sufficiently dark, then is filtered. This stain will last almost indefinitely. It improves with age; in fact, it should not be used until it has stood for at least two months after it is made. It can be procured made up ready for use from any

[51] From Lee.

[52] Formula taken by permission from E. R. Schwarz, *Textiles and the Microscope,* 1934.

supply house; this is probably the best way to obtain it. It is a very strong clear nuclear stain that can be used with or without dilution. The color varies from violet to deep purple.

Safranin stain[53] is made of a mixture of equal parts aniline water and a saturated solution of alcohol and safranin. It is one of the most convenient stains to have at hand. It colors to a deep red if desired, or it can be used diluted. It has excellent keeping qualities.

Gentian violet also is a very convenient stain to have available. It can be made up in a 1 per cent solution and diluted about 1 : 1 for use.

Malachite green can be made from the crystals in the proportion 1 part crystals to 100 parts distilled water.

Bismarck brown can be made in the form of a saturated water solution. It is useful for paper fibers.

The formula for methylene blue follows:

METHYLENE BLUE (LOFFLER'S SOLUTION)

10 mg sodium hydroxide
100 cc water
30 cc saturated alcholic solution methylene blue

After the nuclei of cells have been stained with haematoxylin, and differentiated, they can be stained with a solution of *eosin*. The haematoxylin will color the nuclei a bluish purple, and the eosin will make the protoplasm of the cell pink or pinkish red. When used in this way, the eosin is acting as a counterstain.

Eosin may be made up into aqueous or alcoholic solutions. It can be used in strengths of 1 to 5 per cent.

Sudan III or IV is a stain for fats. It is oil soluble and so is used in alcohol. It is made by dissolving the dye to the point of saturation in 70 per cent alcohol.

A 2 per cent solution of cyanin can be made with 80 per cent alcohol (Koehler). It can be used as a counterstain on woody structure. It imparts a blue color.

Gram stain is one of the important stains of the bacteriologist. It is quite frequently encountered in photographing bacteria. It stains certain types of bacteria a very dark violet, nearly black, and other types a pink color. Because of the weak pink and the deep violet, two conditions are often presented in the same slide, one component having great contrast and the other very little. The Gram technique is somewhat as follows:

The specimen is stained first with gentian violet and then with the Gram iodine solution, which consists of 1 g iodine, 2 g potassium iodide,

[53] Directions for making from Lee.

and 300 cc of water. The slide is washed in alcohol, then in water, and finally stained for a few seconds with safranin.

Sec. 142. Staining Technique. In this discussion it is not intended to give more than an elementary outline of staining technique; more complete directions are left to the authorities on the various subjects, some of which have already been cited. The chemistry of staining is also beyond the province of this book. Since it is for the photomicrographer to utilize stains as best suits his purpose, the greater his fund of information on this subject, both botanical and biological, the better chance will he have of applying the art of staining successfully to obtain the contrasts he must have in his finished picture.

Dyes can be bought as crystalline powders in small quantities of 10 grams or so. They are then prepared for use by dissolving in water, alcohol, mixtures of the two, or other solvents as indicated. As already shown, many stains contain several ingredients, and directions for making them should be followed carefully. "Water" always means distilled water, and when alcohol is directed, ethyl alcohol is understood. For the most part the solutions are comparatively weak —1 or 2 per cent. Generally the solubility of the dye is low when water is the only solvent. After a stain is made it should usually be filtered through a filtering crucible.

Usually the photomicrographer will find it least expensive to obtain his stains already made up, since he requires only small amounts.

There is not much preparatory work in the use of stains, but, if a specimen has been immersed in a liquid which is not miscible with the stain, it will have to be cleared of the liquid with which it was formerly impregnated and be brought into a liquid which is miscible with the stain. When the specimen has been fixed to the slide, it is covered with a drop or two of the stain and then allowed to stand for a few minutes as required, the length of time being determined largely by inspection and experience. The excess solution is removed with filter paper, and perhaps a mount is then made directly in water, or water and glycerin; otherwise the slide can be immersed in running water and mounted. If the specimen must be mounted in a high-index medium other than an aqueous solution, it may have to be dried, after washing, by gently heating or by passing the slide through a series of alcohols of varying strength until absolute alcohol is reached. The absolute alcohol can be cleared with cedar oil or aniline oil, followed by xylene. The specimen is then ready for the mounting medium.

Because hundreds of dyes are available, and the number of ways in which they can be used is almost infinite, the photomicrographer is likely to feel confused. He is advised to select a few stains, make a

study of their use in histology and in bacteriology, and then apply his knowledge to the best advantage on his own problems in photomicrography, perhaps on subjects entirely unrelated to those for which the stain was originally intended.

Differential staining is not of primary importance from a photomicrographical standpoint unless the work involves the preparation of histological specimens. The term refers to the way in which certain stains act. For example, after nuclei have been stained with haematoxylin, the specimen has been washed, and the excess stain removed from the cytoplasm and other parts of the cell, the nuclei still retain the stain. This is called regressive staining, or differentiation. If the specimen takes up the stain in certain parts more rapidly than in others, the process is known as progressive staining.

The use of counterstains goes hand in hand with differentiation. After the first stain is removed from all but one structure, the specimen is stained a second time to color the surrounding unstained material. This type of staining is seen in its most common form when eosin is used after staining with haematoxylin.

Selective stains usually consist of two or more dyes in one solution. They act to stain part of the specimen one color, and other parts another color. An example is Herzberg's stain; when it is used on a mixture of pulp containing ground wood and sulphite fiber the mechanical pulp is stained yellow and the sulphite pulp blue. Such staining can be utilized to good advantage in stressing differences in composition of mixtures. Usually it makes possible the rendering of one of the colors nearly black and the other a much lighter gray, or even white, in the finished print.

Anything which has been cut or sectioned should be stained after cutting if the purpose is to stress internal structure. The staining can be done on the slide when the specimen is fixed in place, or the section can be floated in the stain. Some material is easier to handle and control in macro than in micro pieces. Scraps of paper can be stained before mounting and then shredded on the slide in the mounting medium. Other material may best be handled by simply being mounted in the stain and sealed with wax or latex. Fine sugars may be examined and photographed if mounted in a saturated sugar solution, a little Sudan III or IV being added. In this preparation there is no apparent staining action but the visibility of the sugar is increased. Whether this increased visibility is due to the fact that the alcohol in the stain gives added reduction of refractive index over water and sugar mounts, or whether there is some absorption of dye by the sugar particles, is hard to say. Delafield's haematoxylin can

be used in the examination of paper. When the paper is stained, its surface will show much greater detail and glare will be lessened. Viscose rayon stained with haematoxylin and mounted in a medium of high index will show its surface figure beautifully. α-Monobromonaphthalene is a good mounting medium for this purpose. Hair and other keratin material can be stained with gentian violet and also with safranin.

Many specimens which are difficult to see, or which do not lend themselves to mounting in a medium of the required index, if stained may give fine photographic results through the combination of absorption and refraction image. The counterstaining of the histologist can be resorted to if necessary, but this two-color technique is more striking visually than in photomicrography. Unless one color is subordinated by a filter that will make it come out black or nearly black, the two-color effect is likely to be disappointing when translated into black and white. Cotton fibers and similar subjects at high magnification give better pictures when slightly stained. High apertures reduce contrast, and a little judicious staining will build up contrast without blocking out detail.

The length of time required to stain a given subject is, of course, dependent on the nature of the subject, the kind of dye, and the results desired. If only very little staining is required, the time can be quite short. Safranin will color vegetable fibers deeply in a very few minutes.

Sec. 143. The Use of Reagents as an Aid in Photomicrography. The discussion here will be confined to the use of chemicals on nonmetallic specimens. Their purposes are listed under the following heads:

1. To dissolve some portion of a specimen, generally a component of a powdered mixture, to leave a residue for further study or for photomicrography.

2. To swell certain fibers to determine structure, oxycellulose, bacterial attack, damage, or chemical constitution (Schwarz).

3. To produce a fine precipitate, useful to fill in cracks or junctures or to make certain elements more visible.

4. To decolorize the specimen, or to soften it for further arrangement.

5. As in chemical microscopy,[54] to analyze substances on the stage of the microscope, and to identify them through the formation of

[54] Émile M. Chamot and Clyde W. Mason, *Handbook of Chemical Microscopy*, 2 vols., second edition, 1940.

typical crystals brought about by the action of a reagent. Oils, fats, and waxes have been so treated by Greene[55] and by Mehlenbacher[56] and Butcher.[57]

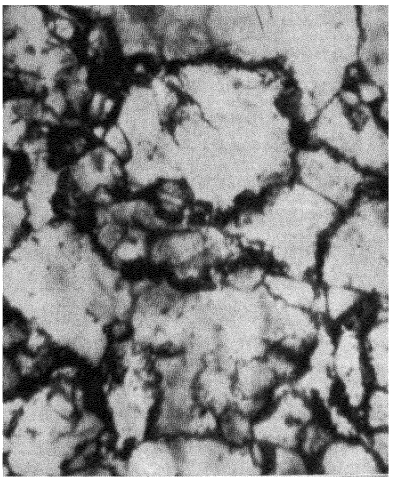


FIG. 210. Mica-coated paper ×330. Directions for preparation are in the text. It may take several trials to prepare a field suitable for photographing. Objective, 16-mm apo, Zeiss; ocular, Homal I; condenser, aplanatic, Zeiss; illumination, carbon arc, method II; filters, Wratten No. 57 plus No. 15; Eastman Commercial Pan film; developer, D-1.

Animal fibers may be dissolved by warming or boiling in a 5 per cent solution of sodium hydroxide. Silk, cotton, wool, and other fibers may be swollen by various reagents, and often they can be dissolved. Lists of such reagents, together with their formulae, will be found in modern handbooks on textiles. Frequently such treatment will make it possible to photograph characteristic features which would otherwise pass unnoticed.

Winchell[58] gives suggestions for the use of chemicals in studying rock sections, as does Short.[59]

Many of the suggestions and descriptions of reactions may be turned to good account by applying them to other fields. As an instance, Fig. 210 shows a photomicrograph of paper coated with mica powder. When the paper was examined under the microscope, regardless of the method of lighting, very little could be seen of the mica particles, and even their presence seemed uncertain. When the paper was dipped into a 5 per cent solution of silver nitrate and, after a quick wash, into a 10 per cent potassium chromate solution, finely divided silver chromate was deposited. After another quick wash, part of the precipitate

[55] L. Wilson Greene, "The Chemical Microscopy of Essential Oils," *Perfumery and Essential Oil Record,* **30,** 309-316, 1939, London; "The Animal and Vegetable Waxes in 1937," *Oil and Soap,* **15,** 317-325; "Chemical Microscopy of Fats and Waxes," *ibid.,* **11,** 31, 1934.

[56] Virgil C. Mehlenbacher, *Oil and Soap,* **13,** 277-282, 1936.

[57] C. Butcher, "Industrial Microscopy," *Ind. Chemist.,* **10,** 1935.

[58] Alexander N. Winchell, *Elements of Optical Mineralogy,* Part I, 1931.

[59] Maxwell N. Short, "Microscopic Determination of the Ore Minerals," *Geological Survey Bulletin* **825,** Superintendent of Documents, Washington, D. C.

remained around the edges of the mica particles, causing them to appear clear and distinct.

Dried entomological specimens from foodstuffs can be softened and made more transparent by soaking in a weak solution of sodium hydroxide. This treatment is often necessary in order to avoid breaking the specimens.

It must again be emphasized that, whenever chemicals are used in conjunction with the microscope, care must be taken not to stain or corrode any part of the instrument. See Sec. 129. Microscope stages are to some extent inert chemically, but the metal parts and lenses are not. When a chemical action is being watched and bubbles arise from the specimen, a gas is being formed which may damage the instrument or its accessories. All lenses must be specially well protected when hydrofluoric acid is being used, and the fumes should be prevented from entering the tube of a petrographic microscope. For working with hydrofluoric acid, ordinary glass slides should be coated with a thermoplastic or celluloid, or a celluloid slide 1 mm thick or more to prevent curling can be used.

For convenience, chemicals can be kept near the microscope in small reagent bottles. Regular glass-stoppered bottles without droppers have been found satisfactory. The reagent can be removed with a pipette, or a drop can be taken up with a glass rod; after being dipped into a beaker of distilled water the rod is ready for use again. Beakers of 500- or even 1000-cc capacity are about the right size to hold the distilled water for washing the rods or pipettes; smaller ones may become contaminated too quickly. For exact control over the amount of liquid reagent a pipette is required. Pipettes can be pulled out from glass tubing as needed. The diameter of the bore can be made so small that a very minute amount of liquid can be dispensed if desired. A rubber bulb slipped over the large end of the pipette is practical enough, even without flaring the tube end to hold the bulb in place. Dropping bottles are convenient for many of the standard mounting agents, particularly inert ones, but for chemical reagents in liquid form bottles with a solid glass stopper will probably be found best.

These few remarks about reagents as an aid to photomicrography are intended to indicate only some of their possibilities and to demonstrate that they have a very real value. The quality of picture obtained is related to the skill and knowledge with which the specimen is prepared. The knowledge, the ingenuity, and the resourcefulness of the worker should be developed to suggest ways

in which chemical reactions may be employed in his own particular work.

For chemical microscopy, the technician is referred to the original works of Behrens[60] and to the second edition of Chamot and Mason. The reagents required for this work are listed in those books. To avoid needless expense to many students who would like to make up their own sets of chemicals, it should be mentioned that vials larger than ½ dram would be clumsy and wasteful, since a greater quantity of material would deteriorate or become contaminated long before all of it could be used. The Chamot and Mason list of chemicals for microscopy calls for sixty ½-dram bottles in each of two sets. Cargille[61] supplies two sets closely following this list. The bottles are convenient in size, and any vials lost or broken can be replaced at small cost.

Sec. 144. Reagents Used in Metallography. The value of chemical compounds and mixtures used for metallographic purposes depends largely on their ability to oxidize. Their purpose is to etch and roughen an optically flat metal surface by outlining segregations, eutectics, and the larger crystalline formations which denote separate chemical compounds that may be present in metals and alloys especially after heat treating, processing, or fabricating. Since the metallographer will naturally be a specialist in this form of specimen preparation, which includes methods of grinding, polishing, and mounting, this brief discussion is directed to the more general practitioner of photomicrography. A few provocative suggestions may lead him to a broader consideration of the aspects of treating his specimen to show, perhaps indirectly, detail which he has found difficulty in seeing or photographing directly.

Some of the standard works of reference on this subject are Sauveur,[62] Stratton,[63] Rawdon and Scott,[64] and Ellinger and Acken.[65]

As will be seen, almost any compound that will act chemically on the metal may serve as an etching agent. The important point to

[60] H. Behrens, *Mikrochemische Methoden,* 1882, Amsterdam; *Mikrochemische Analyse,* edited by P. D. C. Kley, 1921, Leipzig.

[61] R. P. Cargille, Laboratory Supplies, 118 Liberty Street, New York City.

[62] A. Sauveur, *The Metallography and Heat Treatment of Iron and Steel,* McGraw-Hill Book Company, 1935.

[63] S. W. Stratton, "Structure and Related Properties of Metals," *Natl. Bureau of Standards Cir.* 113, Washington, D. C., 1921. (Still available.)

[64] H. S. Rawdon and Howard Scott, "Microstructure of Iron and Mild Steel at High Temperatures," *Natl. Bureau of Standards Scientific Paper* 356, 1920.

[65] G. A. Ellinger and J. S. Acken, "A Method for the Preparation of Metallographic Specimens," *Trans. Am. Soc. Metals,* **27,** 382, 1939.

remember is that it pays to try several agents before selecting any particular one. A certain solution may attack two or more constituents of a specimen in about the same way, and, even though it may form identical chemical compounds, there may be a decided difference in the appearance of the products after the action is completed. Table XXXI suggests several of the better-known etching agents and the purposes for which they are generally used. This table is reprinted by special permission from the American Society for Testing Materials, from paper E3–42T, revised 1942. Paper E2–39T, 1939, of the same society, also has considerable information for the photomicrographer.

Table XXXI

Some Important Etching Agents

Etching Reagents for Aluminum and Its Alloys

Etching Reagent	Composition	Remarks	Use
No. 1. Hydrofluoric acid	HF..... 0 5 ml H_2O.....99.5 ml	Swab with soft cotton for 15 sec.	General; microscopic.
No. 2. Sodium hydroxide	NaOH..... 1 gram H_2O.....99 ml	Swab for 10 sec	General; microscopic.
	or		
	NaOH.....10 grams H_2O.....90 ml	Immerse for 5 sec at 70° C (160 °F), rinse in cold water.	Can be used for both micro- and macroetching.
No. 3. Sulfuric acid	H_2SO_4.....20 ml H_2O ..... 80 ml	Immerse for 30 sec at 70° C (160° F), quench in cold water.	Aluminum-copper-iron-manganese from aluminum-iron-manganese or aluminum-copper-iron.
No. 3A. Sulfuric acid	H_2SO_4.....10 ml H_2O.....90 ml	Immerse at 60 to 70° C.	$FeAl_3$
No. 4. Nitric acid	HNO_3.... 25 ml H_2O.....75 ml	Immerse for 40 sec at 70° C (160° F), quench in cold water.	α Aluminum-iron-silicon from $FeAl_3$; microscopic.
No. 5. Keller's etch	HF..... 1.0 ml HCl..... 1 5 ml HNO_3..... 2.5 ml H_2O..... 95.0 ml	Immerse for 10 to 20 sec, wash in stream of warm water.	Microstructure of duralumin-type alloys.
No. 6. Flick's etch	HF.....10 ml HCl .....15 ml H_2O . .. 90 ml	Immerse for 10 to 20 sec, wash in warm water followed by dip in HNO_3.	Macroscopic etching.
No. 7. Tucker's etch	HF.....15 ml HCl.....45 ml HNO_3.....15 ml H_2O.....25 ml	Etch by immersion.	Macroscopic.
No. 8. Vilella's etch	HF..... 2 parts HNO_3..... 1 part Glycerol... 3 parts		General.
No. 9. Double etch	Solution A HNO_3.....25 ml H_2O.....75 ml Solution B NaF..... 0.5 gram HNO_3..... 1.0 ml HCl..... 2.0 ml H_2O..... 97.0 ml	Immerse for 60 sec in Sol. A at 70° C (160° F), quench in cold water. Then immerse in Sol. B for 15 to 30 sec, wash in stream of warm water.	This method is used for determining quality of heat treatment of duralumin alloys and presence of $CuAl_2$ precipitate at grain boundaries.

Table XXXI — *Continued*

Etching Reagents for Copper and Its Alloys

Etching Reagent	Composition	Remarks	Use
No. 1. Ammonium hydroxide-hydrogen peroxide	NH_4OH ... 5 parts H_2O..... 5 parts H_2O_2 (3 per cent). 2 to 5 parts	Peroxide content varies directly with copper content of alloy to be etched. Immersion or swabbing for about 1 min. Fresh H_2O_2 is desirable for good results.	Generally used for copper and many of its alloys. Film on etched aluminum bronze removed by weak Grard's solution.
No. 2. Ammonium hydroxide	Diluted solutions.	Immersion.	Polish-attack etching of brass and bronze.
No. 3. Ammonium hydroxide - ammonium persulfate	NH_4OH ... 1 part H_2O ..... 1 part $(NH_4)_2S_2O_8$ (2.5 per cent).... 2 parts	Immersion.	Polish-attack of copper and some alloys.
No. 4. Ammonium persulfate	$(NH_4)_2S_2O_8$ 10 grams H_2O.. ...90 ml	Use either cold or boiling. Immersion.	Copper, brass, bronze, nickel silver, aluminum bronze.
No. 5. Chromic acid.	Saturated aqueous solution (CrO_3).	Immersion or swabbing.	Copper, brass, bronze, nickel silver (plain etch).
No. 6. Chromic acid-hydrochloric acid	CrO_3 (10 to 15 per cent)....50 ml HCl ...1 to 2 drops	Add HCl at time of use. Immersion.	Same as reagent No. 5. Color by electrolytic etching or $FeCl_3$ reagents.
No. 7. Chromic acid-nitric acid	HNO_3.....50 ml CrO_3.....20 grams H_2O.....30 ml or HNO_3..... 5 ml CrO_3.....20 grams H_2O .....75 ml	Immersion.	Aluminum bronze; film from polishing removed by 10 per cent HF
No. 8. Copper ammonium chloride - ammonium hydroxide.	10 per cent aqueous solution of copper ammonium chloride plus NH_4OH to neutrality or alkalinity.	Immersion. Wash specimen thoroughly.	Best for darkening large areas of beta in alpha-beta brass. Copper, brass, nickel silver.
No. 9. Ferric chloride	Parts: $FeCl_3$ / HCl / H_2O 5 / 50 / 100 19 / 6 / 100* 5 / 10 / 100† 25 / 25 / 100 1 / 20 / 100 8 / 25 / 100 10 / 1 / 100‡ 3 / 10 / 100§	Immersion or swabbing. Etch lightly or by successive light etches to required results.	Copper, brass, bronze, aluminum bronze; darkens beta in brass; gives contrast following dichromate and other etches.

* Grard's No. 1
† Grard's No. 2
‡ Plus 1 part CrO_3.
§ Plus 1 part $CuCl_2$ and 0.05 part $SnCl_2$.

Table XXXI — *Continued*

Etching Reagents for Copper and Its Alloys — Continued

Etching Reagent	Composition	Remarks	Use
No. 10. Nitric acid...	Various concentrations.	Immersion or swabbing. $AgNO_3$ (0.15 to 0.3 per cent) added to 1:1 nitric acid solution gives a brilliant, deep etch.	Deep etching.
No. 11. Potassium dichromate.....	$K_2Cr_2O_7$... 2 grams H_2SO_4..... 8 ml NaCl (saturated solution)... 4 ml H_2O.....100 ml	NaCl can be replaced by 1 drop HCl to 25 ml solution added just before using. Immersion.	Copper, copper alloys of beryllium, manganese, silicon, nickel silver, bronze, and chromium copper. Followed by $FeCl_3$ or other contrast etch.
No. 12. Electrolytic etch	$FeSO_4$.....30 grams NaOH..... 4 grams H_2SO_4....100 ml H_2O.....1900 ml	Use 0.1 amp at 8 to 10 volts. Generally not over 15 sec. Do not swab surface after etching.	Darkens beta in brass, gives contrast after H_2O_2 etch. Nickel silver, bronze, and other alloys.
No. 13. Electrolytic etch.....	HNO_3.....10 ml Glacial acetic acid.. 5 ml H_2O.....85 ml		Is very satisfactory for etching high-nickel alloys such as 20 to 30 per cent cupro-nickel and monel. It tends to minimize the striations which appear after etching due to coring effect.

Etching Reagents for Macroscopic Examination of Iron and Steel

No. 1. Hydrochloric acid.....	HCl.....50 ml H_2O.....50 ml	Used hot or boiling for about 10 to 15 min, depending on the steel.	Shows segregation, porosity, cracks, depth of hardened zone in tool steel, etc.
No. 2. Mixed acid...	HCl.....38 ml H_2SO_4.....12 ml H_2O.....50 ml	Recommended by Yatsevitch. To be used as above for 15 to 45 min.	Same as for reagent No. 1.
No. 3. Nitric acid...	HNO_3.....25 ml H_2O.....75 ml	Used cold for large surfaces such as split ingots which cannot conveniently be heated.	Same as for reagents Nos. 1 and 2.
No. 4. Ammonium persulfate.....	$(NH_4)_2S_2O_3$ 10 grams H_2O.....90 ml	Surface should be rubbed with absorbent cotton during etching.	Brings out grain structure in cases of excessive grain growth, recrystallization at welds, etc.
No. 5. Stead's reagent (No. 1)....	$CuCl_2$..... 2.5 grams $MgCl_2$.....10.0 grams HCl..... 5 ml Ethyl alcohol. Up to 250 ml	The salts are dissolved in the HCl with the addition of the least possible quantity of hot water.	Brings out phosphorus-rich areas and phosphorus banding.

Table XXXI — *Continued*

Etching Reagents for Macroscopic Examination of Iron and Steel — Continued

Etching Reagent	Composition	Remarks	Use
No. 6. Fry's reagent.	$CuCl_2$.....90 grams HCl.....120 ml H_2O.....100 ml	Most useful for mild steel, particularly bessemer and other high-nitrogen steel. Before etching, sample should be heated to 200 to 250° C (302 to 482° F) for 5 to 30 min, depending on the condition of the steel. During etching the surface should be rubbed with a cloth soaked in the etching solution. Wash in alcohol or rinse in HCl (1:1) after etching to prevent deposition of copper.	Shows up strain lines due to cold work.
No. 6A. Fry's reagent	$CuCl_2$.....45 grams HCl. ...180 ml H_2O. .. 100 ml	Same as for reagent No. 6 but modified by Wazau; may give more contrast. Specimen can be washed in water without depositing copper.	Same as for reagent No. 6.
No. 7. Nital.....	HNO_3. ... 5 ml Ethyl alcohol.... .95 ml	Etch 5 min, followed by 1 sec in HCl (10 per cent).	Determines cleanness and increases contrast.
No. 8. Humfrey's reagent	Copper ammonium chloride 120 grams HCl .. ...50 ml H_2O 1000 ml	Slight abrasion of surface after etching is recommended.	Develops dendritic segregation.

Etching Reagents for Microscopic Examination of Steels and Irons

(Only reagents of higher analytical purity should be used)

I. General Reagents for Irons and Steels (Carbon, Low- and Medium-Alloy Steels)

No. 1. Nitric acid (Nital).....	HNO_3, colorless...1–5 ml Ethyl or methyl alcohol, 95 per cent or absolute,....100 ml (also amyl alcohol)	Etching rate is increased, selectivity decreased, with increasing percentages of HNO_3. Reagent No. 2 (picric acid) usually superior. 4 per cent in amyl alcohol useful for grain boundary and contrast of low-carbon materials. Etching time, a few seconds to a minute.	In carbon steels: (1) to darken pearlite and give contrast between pearlite colonies, (2) to reveal ferrite boundaries, (3) to differentiate ferrite from martensite.

Table XXXI — *Continued*

Etching Reagents for Microscopic Examination of Steels and Irons. Group I. — *Continued*

Etching Reagent	Composition	Remarks	Use
No. 2. Picric acid (Picral)	Picric acid . 4 grams Ethyl or methyl alcohol, 95 per cent or absolute 100 ml (Use absolute alcohol only when acid contains 10 per cent or more of moisture.)	Superior to reagent No. 1 except for those specific uses listed thereunder. More dilute solutions occasionally useful. Does not reveal ferrite grain boundaries as readily as No. 1. Etching time a few seconds to a minute or more.	For all grades of carbon steels: (1) annealed, (2) normalized, (3) quenched, (4) quenched and tempered, (5) spheroidized, (6) aus-tempered. For all low-alloy steels attacked by this reagent.
No. 3. Hydrochloric and picric acids	HCl. . . . . 5 ml Picric acid . 1 gram Ethyl or methyl alcohol, 45 per cent or absolute, 100 ml	Best results are obtained when the martensite is tempered for 15 min at 400 to 475° F (205 to 245° C).	For revealing the austenite grain size in quenched, and quenched-and-tempered steels.
No. 4. Chromic acid.	CrO_3. . . . . 10 grams H_2O. . . . . 100 ml	Used electrolytically, the specimen as anode, the stainless steel or platinum as cathode, ¾ to 1 in. apart; 6 volts usually used. Time of etching 30 to 90 sec, depending on specimen.	For various structures except grain boundaries of ferrite. Attacks cementite very rapidly, austenite less rapidly, ferrite and iron phosphide very slowly if at all.
No. 5. Heat tinting	Heat only.	Clean, dry, polished specimen heated face upon hot plate to 400 to 700° F (205 to 370° C). Time and temperature both have decided effects. Bath of sand or molten metal may be used.	Pearlite first to pass through a given color, followed by ferrite; cementite less affected, iron phosphide still less. Especially useful for cast irons.
II. General Reagents for Alloy Steels (Stainless and High-Speed Steels)			
No. 6. Ferric chloride and hydrochloric acid	$FeCl_3$. . . . . 5 grams HCl. . . . . 50 ml H_2O. . . . . 100 ml		Structure of austenitic nickel steels.
No. 7. Aqua regia	HCl. . . . . 75 ml HNO_3. . . . . 25 ml	Mixture should stand 24 hr before using. Used full strength for rapid work, but requires careful handling.	Structure of stainless steel.
No. 8. Chrome regia.	HCl. . . . . 25 ml CrO_3 solution (10 per cent) in H_2O. 5 to 50 ml	Activity is controlled by amount of chromic acid.	Heat treated 18 per cent chromium, 8 per cent nickel stainless steels.

Table XXXI — *Continued*

Etching Reagents for Microscopic Examination of Steels and Irons. Group II. — *Continued*

Etching Reagent	Composition	Remarks	Use
No. 9. Ferric chloride and nitric acid.....	Saturated solution of $FeCl_3$ in HCl, to which a little HNO_3 is added.	Use full strength.	Structure of stainless steel.
No. 10. Mixed acids in glycerol.....	A. HNO_3 . . 10 ml HCl. . 20 to 30 ml Glycerol 30 to 20 ml	Warm specimen in water before etching. For best results use method of alternate polishing and etching. If given sufficient time, will etch totally austenitic alloys, but better results are obtained by using reagent No. 10-C.	Structure of iron-chromium base alloys, high-speed steels, and austenitic manganese steel. Etches nickel-chromium alloys satisfactorily.
	B. HNO_3 . . 10 ml HF.....20 ml Glycerol 20 to 40 ml	Amount of glycerol may be varied to suit metal.	Structure of high-silicon alloys of the Dur-iron type.
	C. HNO_3 . . 10 ml HCl. . . . . 20 ml Glycerol. . 20 ml H_2O_2.....10 ml	Amount of HCl may be varied if reagent acts too rapidly or slowly. For best results employ method of alternate polishing and etching.	To etch iron-chromium-nickel, iron-chromium-manganese, and all other austenitic iron-chromium base alloys.
No. 11. Oxalic acid....	Oxalic acid . 10 grams H_2O.....100 ml	Used electrolytically, the specimen as anode, stainless steel or platinum as cathode, about 1 in. apart; 6 volts usually used. Precipitated carbides in stainless steels may be revealed in 10 to 15 sec, the general structure in about 1 min. For study of carbides, 1.5 to 3 volts may be used, thus increasing the etching time and improving control of etch.	For austenitic stainless steels and high-nickel alloys. Carbides and general structure revealed depending on etching time.
No. 12. Mixed acids and cupric chloride.....	HCl.....30 ml HNO_3.....10 ml Saturate with cupric chloride and let stand 20 to 30 min before use.	Apply by swabbing.	For stainless alloys and others high in nickel or cobalt.
No. 13. Nitric and acetic acids.....	HNO_3.....30 ml Acetic acid . 20 ml	Apply by swabbing.	For stainless alloys and others high in nickel or cobalt.

Table XXXI — *Continued*

Etching Reagents for Microscopic Examination of Steels and Irons. Group II. — *Continued*

Etching Reagent	Composition	Remarks	Use
No. 14. Hydrochloric and nitric acids in alcohol	HCl.....10 ml HNO_3..... 3 ml Methyl alcohol...100 ml	Etch 2 to 10 min.	To reveal the grain size of quenched, or quenched and tempered high-speed steel.
No. 15. Ferricyanide solution	Potassium ferricyanide.....30 grams KOH.....30 grams H_2O.....60 ml	Must be fresh. Use boiling.	To distinguish between ferrite and sigma phase in iron-chromium, iron-chromium-nickel, iron-chromium-manganese and related alloys. Colors: sigma phase, light blue; ferrite, yellow.
No. 16. Cupric sulfate.	$CuSO_4$..... 4 grams HCl.....20 ml H_2O.....20 ml	Marble's reagent.	Structure of stainless steels.
No. 3. Hydrochloric and picric acids	HCl..... 5 ml Picric acid. 1 ml Ethyl or methyl alcohol, 95 per cent or absolute....100 ml		To etch many steels of the iron-chromium, iron-chromium-nickel, and iron-chromium-manganese types.
III. Phosphorus Segregation, Phosphides, and Strain Lines			
No. 17. Cupric chloride	A. $CuCl_2$ .. 1 gram $MgCl_2$..... 4 grams HCl..... 1 ml H_2O.....20 ml Alcohol, absolute ..100 ml	Dissolves salts in least possible quantity of hot water. Etch for about 1 min, repeating if necessary. Stead's reagent.	For showing segregation of phosphorus or other elements in solid solution; copper tends to deposit first on areas lowest in phosphorus.
	B. $CuCl_2$ .. 5 grams HCl.....40 ml H_2O.....30 ml Ethyl alcohol.....25 ml	May be used cold. Etching time, about 10 sec. Fry's reagent.	To reveal strain lines and their microstructure, and precipitation hardening in steel.
No. 18. Sodium picrate (neutral)	Sodium picrate..... 1 gram H_2O.....100 ml (Wash salt well with alcohol to remove excess acid or alkali)	Use boiling. Etching time 20 min.	Shows difference between phosphides and cementite; iron phosphide attacked, cementite unattacked.
No. 19. Chromic acid and heat tinting	CrO_3..... 8 grams H_2O.....100 ml Followed by heat tinting.	Etch first in picric acid (No. 2) then for 1 min in chromic acid· heat tint by heating face upon hot plate at about 500° F (260° C) for 1 min.	Distinguishes between iron phosphide and cementite in phosphide eutectic of cast iron; iron phosphide is colored darker.

Table XXXI — *Continued*

Etching Reagents for Microscopic Examination of Steels and Irons. — Continued

Etching Reagent	Composition	Remarks	Use
	IV. Structure and Depth of Case of Nitrided Steels		
No. 20. Cupric sulfate and cupric chloride..	$CuSO_4$....1.25 grams $CuCl_2$....2.50 grams $MgCl_2$ .....10 grams HCl..... 2 ml H_2O..... 100 ml (Dilute above solution to 1000 ml with 95 per cent ethyl alcohol)	Proportions must be accurate. Etch by immersion to avoid confusing edge effects.	For showing total depth, structure, and various zones of nitrided chromium-vanadium steels and nitralloy.
No. 21. Picric and nitric acids.....	Picric acid (4 per cent) (No. 2).....10 parts HNO_3 (4 per cent) (No. 1).. 1 part	Best results are obtained when the specimen is annealed in lead at 1475° F (800° C) before etching.	For depth of case and structure of nitralloy.
No. 1. Nitric acid (nital).....	HNO_3..... 2 ml Ethyl or methyl alcohol, 95 per cent or absolute....100 ml		For structure and depth of case of nitrided steels.
No. 16. Cupric sulfate.	$CuSO_4$..... 4 grams HCl.....20 ml H_2O.....20 ml	Marble's reagent.	Total depth of nitrided case.
	V. Carbides, Nitrides, Tungstides, and Their Differentiation		
No. 22. Sodium picrate, alkaline.....	Picric acid . 2 grams NaOH.....25 grams H_2O.....100 ml	Use boiling, 5 to 10 min, or preferably, electrolytically at room temperature; for the latter, specimen is anode, platinum or stainless steel is cathode; with 6 volts, about 40 sec is usually sufficient.	Colors cementite, but not carbides high in chromium. In tungsten steels, iron tungstide (Fe_nW) and iron tungsten carbide (Fe_4W_2C) are colored more rapidly than cementite, but tungsten carbide is unaffected. Attacks sulfides. Delineates grain boundaries in hypereutectoid steels in slowly cooled condition.

Table XXXI — *Continued*

Etching Reagents for Microscopic Examination of Steels and Irons. Group V. — *Concluded*

Etching Reagent	Composition	Remarks	Use
No. 23. Hydrogen peroxide and sodium hydroxide.....	H_2O_2.....10 ml NaOH 10 per cent solution in water. 20 ml	Must be fresh. Etching time 10 to 12 min.	Attacks and darkens iron tungstide in carbon-free iron tungsten alloys. When carbon is present this solution darkens the compound (FeW WC?) in proportion to the amount of carbide present; tungsten carbide is darkened.
No. 24. Ferricyanide solution.....	A. $K_3Fe(CN)_6$ 1–4 grams KOH .... 10 grams H_2O..... 100 ml	Must be freshly made; etch 15 min in boiling solution.	Differentiates between carbides and nitrides. Cementite is blackened, pearlite turned brown, and massive nitrides remain unchanged.
	B. $K_3Fe(CN)_6$ 10 grams KOH..... 10 grams H_2O.....100 ml	May be used cold, but preferably hot, should be freshly made, etching time 5 to 10 min. Murakami's reagent.	Darkens carbide containing chromium, carbides and tungstides in tungsten and high-speed steels. At room temperature colors ternary carbides (Fe_3W_3C or Fe_3W_2C) in a few seconds, iron tungstide (Fe_3W_2) in several minutes, and barely colors cementite.
No. 25. Sodium cyanide.....	NaCN.....10 grams H_2O.....90 ml	Used electrolytically, the specimen as anode, cathode, similar material about 1 in. apart; 6 volts (not less than 5). Etching time 5 min or more.	Darkens carbides without attacking austenite or grain boundaries.
No. 4. Chromic acid..	CrO_3.....10 grams H_2O.....100 ml	See No. 4 in Section I.	Attacks carbides in stainless steels very rapidly, austenite less rapidly, and ferrite very slowly if at all. For various structures of stainless steels.
No. 11. Oxalic acid....	Oxalic acid. 10 grams H_2O.....100 ml	See No. 11 in Section II. If strongly etched general structure is revealed; therefore for study of carbides reduced voltage is used for etching, giving better control of etch.	Reveals carbides in stainless steels.

Courtesy of American Society for Metals.

Table XXXI — *Continued*

Etching Reagents for Lead and Its Alloys

Etching Reagent	Composition	Remarks	Use
No. 1. Ammonium molybdate	Molybdic acid (85 per cent) 100 grams NH_4OH .. 140 ml H_2O ..... 240 ml Filter and add to HNO_3 .....60 ml	Alternately swab specimen and wash in running water.	Rapid etch. Very suitable for removing thick layers of worked metal.
No. 2. Mixed acids	Glacial acetic acid .. 3 parts HNO_3 ..... 4 parts H_2O .....16 parts	Use freshly prepared solution at 40 to 42° C (104° to 108° F). Immerse 4 to 30 min, depending on depth of worked metal layer. Clean with wet cotton in running water.	Gives excellent detail for photographing.
No. 3. Mixed acids in glycerol	Glacial acetic acid .. 1 part HNO_3 ..... 1 part Glycerol ... 4 parts	Use freshly prepared solution at 80° C (176° F).	Used for alternate polishing and etching.
No. 4. Ammonium molybdate - mixed acids	See reagents Nos. 1 and 2.	Swab with reagent No. 1 until structure is clearly visible. Immerse in reagent No. 2 for about 6 min. Clean by swabbing in running water.	Combines advantages of reagents Nos. 1 and 2.
No. 5. Acetic acid-hydrogen peroxide	Glacial acetic acid .. 3 parts H_2O_2 (9 per cent). .. 1 part	Etch 10 to 30 min, depending on depth of worked metal layer. Clean in HNO_3 (sp. gr. 1.42) if necessary.	Recommended for antimony alloys up to 2 per cent antimony.
No. 5A. Acetic acid-hydrogen peroxide	Glacial acetic acid .. 3 parts H_2O_2 (30 per cent).... 1 part	Etch for 6 to 15 sec.	For lead-antimony alloys.
No. 5B. Acetic acid-hydrogen peroxide	Glacial acetic acid .. 2 parts H_2O_2 (30 per cent).... 1 part	Etch 8 to 15 sec by immersion.	For pure lead and lead-calcium alloys.
No. 6. Perchloric acid	$HClO_4$ .....60 ml H_2O .....40 ml	Electrolytic etching; specimen cathode, platinum anode.	Recommended for antimony alloys over 2 per cent antimony.
No. 7. Silver nitrate	$AgNO_3$ 5 to 10 grams H_2O .95 to 90 ml	Use by swabbing.	For anti-friction metals.
No. 8. Nitric acid	HNO_3 .....50 ml H_2O .....50 ml	Etch in boiling solution 5 to 10 min.	Macroscopic etching; welds, laminations, etc
No. 9. Nitric acid	HNO_3	Alternate in acid and running water.	Pure lead.

Table XXXI — *Continued*

Etching Reagents for Magnesium and Its Alloys

Etching Reagent	Composition	Remarks	Use
No. 1. Acetic acid....	10 per cent aqueous solution.	Swab with cotton for ½ to 2 min.	Macroetching.
No. 2. Oxalic acid....	2 per cent aqueous solution.	Swab for 2 to 5 sec.	Cast and wrought magnesium and most alloys in cast form.
No. 3. Nitric acid....	2 per cent aqueous solution.	Swabbing.	Develops coring, and is used to etch some casting alloys.
No. 4. Malic acid-nitric acid.....	Aqueous solution, 5 per cent malic and 2 per cent nitric acids.	Etch 10 to 30 sec.	For wrought alloys.
No. 5. Tartaric acid..	Aqueous solution (2 per cent.)	Etch 10 to 20 sec.	For magnesium-manganese wrought alloys and cast and heat-treated magnesium-aluminum-manganese-zinc alloys.
No. 6. Glycol etch...	Diethylene glycol 75 parts by vol. Distilled water 24 parts by vol. HNO_3.. 1 part by vol.		
No. 7. Citric acid.....	5 per cent aqueous citric acid.	Swabbing.	For all-around etchant, particularly good for magnesium and manganese both in same alloy.

Etching Reagents for Nickel and Its Alloys

No. 1. Flat solution.	HNO_3.....50 ml Glacial acetic acid..50 ml	Make up fresh daily; use colorless HNO_3 to avoid staining. Etch by immersion at room temperature for 5 to 20 sec.	For nickel; monel and other nickel-copper alloys. Dilute with 25 to 50 per cent acetone for alloys of less than 25 per cent nickel.
No. 2. Electrolytic-contrast.....	Glacial acetic acid.. 5 ml H_2O.....85 ml HNO_3.....10 ml	Electrolytic etch for 20 to 60 sec using 1.5-volt dry cell and platinum wires.	Very satisfactory for grain-size studies. Stains less than flat solutions.
No. 3. Electrolytic-sulphuric.....	H_2SO_4..... 5 ml H_2O.....95 ml	Electrolytic etch for 5 to 15 sec using 2 or 3 1.5-volt dry cells and platinum wires.	For nickel and Inconel (nickel-chromium-iron).
No. 4 Aqua regia...	HNO_3..... 5 ml HCl.....25 ml H_2O.....30 ml	Etch by immersion 30 sec to 2 min.	Inconel.

Table XXXI — *Continued*

Etching Reagents for Nickel and Its Alloys — Continued

Etching Reagent	Composition	Remarks	Use
No. 5. Nitric-hydrofluoric	HNO_3.....20 ml HF (48 per cent)....15 drops	Warm specimen in bunsen flame or boiling water and etch by immersion 15 to 45 sec.	Inconel.
No. 6. Nitric acid	HNO_3.....30 ml H_2O.....70 ml	Etch by immersion.	Macroetch for nickel silver.
No. 7. Potassium cyanide	KCN..... 5 grams H_2O.....95 ml H_2O_2.....few drops	Etch by immersion.	For low-zinc nickel silver.
No. 8. Ammonium hydroxide-hydrogen peroxide	NH_4OH...85 ml H_2O_2. ....15 ml	Etch by immersion.	For high-zinc nickel silver.
Etching Reagents for the Precious Metals			
No. 1. Potassium cyanide - ammonium persulphate*	*A*. KCN (5 per cent solution). 1 part $(NH_4)_2S_2O_8$ (5 per cent solution). 1 part	*A*. Etch 1 to 2 min.	*A*. Pure silver.
	B. KCN (10 per cent solution) 1 part $(NH_4)_2S_2O_8$ (10 per cent solution). 1 part	*B*. Addition of 2 per cent KI will produce more rapid attack. Etch ½ to 3 min. Make up fresh each time. The etching rate may be increased by warming solution. KI additions may cause staining.	*B*. Gold, nearly all karat golds.
	C. KCN (20 per cent solution). 1 part $(NH_4)_2S_2O_8$ (20 per cent solution). 1 part	*C*. 3 per cent KI may be added	*C*. A slow etch for palladium or the complicated dental alloys.
No. 2. Chromate	*A*. HNO_3 (1 : 1)..100 ml $K_2Cr_2O_7$. 2 grams	Dilute *A* to 20 vol, add equal amount of *B*. Apply with camel's-hair brush. Non-adherent film of red silver chromate should form. If film adheres, add more of *A*; if non forms, add *B*.	Used for silver alloys.
	B. CrO_3 ...20 grams Na_2SO_4.. 1.5 grams H_2O....100 ml		
No. 3. Chromic acid-sulphuric acid	Aqueous solution, 0.2 per cent each CrO_3, and H_2SO_4.	Etch 1 min.	Silver alloys.

* Developed for a somewhat different purpose by M. C. Jewett.

Table XXXI — *Continued*

Etching Reagents for the Precious Metals — Continued

Etching Reagent	Composition	Remarks	Use
No. 4. Electrolytic etching	*A.* 1 per cent solution of HF plus small amount of $SnCl_2$.		*A.* Used for silver-tin alloy with over 73 per cent silver.
	B. Dilute solutions of HCl, KCN, or KCN + KI.		*B.* Occasionally used for gold.
	C. KCN (5 per cent)		*C.* Used particularly for silver where silver is in contact with other metals as in plated materials.
No. 5. Iodine	50 per cent solution of U.S.P. tincture of iodine in aqueous KI solution.	Film of silver-iodide may remain on surface of gold-silver alloys; can be removed with KCN solution.	Gold alloys.
No. 6. Aqua regia	*A.* Slightly diluted.	*A.* Etch 5 min in warm solution.	*A.* Pure platinum.
	B. Concentrated.	*B.* Use hot.	*B.* Platinum alloys. Also used for gold alloys but will form chloride film when much silver is present. Ammonia or KCN will remove film.
	C. In glycerol.	*C.* Use cold.	*C.* Palladium alloys.
No. 7. Potassium sulphide	K_2S	Use hot.	Gold-nickel alloys.
No. 8. Fused salts	KOH + 10 per cent KNO_3 or $KHSO_4$	Etch in fused salt.	Used for platinum if aqua regia is ineffective.
No. 9. Nitric acid	HNO_3.	Use hot.	Palladium.
No. 10. Ammonium hydroxide-hydrogen peroxide	NH_4OH ... 5 parts H_2O_2 ... 1 to 3 parts	Immersion.	For silver or silver-palladium alloys and silver soldered joints.
No. 11. Dichromate etch	$K_2CR_2O_7$ (saturated solution) 100 ml NaCl (saturated solution) .. 2 ml H_2SO_4 ..... 10 ml	Use 1 part solution and 9 parts water. Apply with swab.	Silver and silver alloys.

Table XXXI — *Continued*

Etching Reagents for Tin and Its Alloys

Etching Reagent	Composition	Remarks	Use
No. 1. Nital	HNO_3.. 2 to 5 ml Ethyl alcohol. 95 to 98 ml	Swab or immerse for several minutes.	Tin-cadmium or tin-iron alloys.
No. 2. Potassium dichromate	Acidified, diluted solution.		Tin-cadmium alloys.
No. 3. Mixed acids in glycerol	*A*. HNO_3.. 1 part Acetic acid..... 1 part Glycerol . 8 parts	*A*. Immerse ½ to 10 min. at 38 to 42° C.	*A*. Tin-lead alloys.
	B. HNO_3 . 1 part Acetic acid.. .. 3 parts Glycerol . 5 parts	*B*. Same as for *A*, above.	*B*. Pure tin.
No. 4. Hydrochloric acid	*A*. HCl	*A*. Immerse for several seconds.	*A*. To remove surface flow.
	B. HCl 10 to 20 ml H_2O 90 to 80 ml	*B*. Immerse ½ to 5 min following *A*.	*B*. Follows *A*.
	C. HCl... 10 ml H_2O.....90 ml	*C*. Electrolytic etch at low current density.	*C*. Tin-iron alloys.
No. 5. Ferric chloride	$FeCl_3$ ... .10 grams HCl. ..... 2 ml H_2O.....95 ml	Immerse ½ to 5 min at room temperature.	Microetching tin-rich babbitt metal.
No. 6. Nitric and picric acids	Alcoholic solution.		Tin-iron alloys.
No. 7. Ammonium polysulphide	Concentrated solutions.	Immerse 20 to 30 min at room temperature.	Macroetching of tin-rich babbitt metals.

Solutions of silver nitrate or 10 per cent nitric acid plus 5 per cent chromic acid are also occasionally used.

Etching Reagents for Zinc and Its Alloys

No. 1. Palmerton reagent	CrO_3 (99.95 per cent)200 grams Na_2SO_4(c.p.) 15 grams H_2O.....1000 ml	Immersion with gentle agitation. Follow with rinse in solution of: CrO_3..... 200grams H_2O..... 1000 ml	General. (Reduce Na_2SO_4 to 7.5 grams when using solution to develop grain structure in alloys containing coppers.)
No. 2. Diluted Palmerton reagent	CrO_3 (99.95 per cent) 50 grams Na_2SO_4(c.p.) 4 grams H_2O.....1000 ml	Immersion for 2 to 3 sec. Follow with rinse in solution of: CrO_3..... 200grams H_2O..... 1000 ml	Structure of die castings, also contrast between the same and plated coating.
No. 3. Hydrochloric acid	HCl		Macrostructure of pure zinc.

NOTE: Table XXXI is reprinted by permission of the American Society for Testing Materials from "Tentative Methods of Preparation of Metallographic Specimens," A.S.T.M. Designation E3-42T.

CHAPTER VIII

ANALYSIS OF PRACTICAL PHOTOMICROGRAPHICAL PROBLEMS

The art of photomicrography is naturally divided into three steps: lighting technique, manipulation of the microscope, and the mounting and handling of the specimen on the microscope stage. The first two items have been treated with what is hoped will prove to be adequate thoroughness. The last item is more difficult to discuss except in a general way. To cover the ground, an effort has been made to select typical examples from industrial and scientific fields and to choose photomicrographic subjects of widespread interest as standards around which more specialized techniques may be built. Therefore, all intricate and involved methods of preparation are referred to the experts or specialists in the respective fields.

Sec. 145. Classification of Specimens. The arranging, mounting, and photographing of specimens can best be discussed if the various specimens are divided into categories, the grouping depending mainly upon the microscopical technique involved. Preparation of the material depends upon the physical characteristics of the specimen. It is necessary first to consider the preparation of the specimen, then to select a suitable lighting method and make proper mechanical and optical arrangements of the microscope and camera.

To a certain extent there is an inevitable overlapping in the classifications. For example, textiles can be considered as manufactured cloth or as fibers — two entirely different groups, since the handling of the specimen on the stage of the microscope will be quite different in the two classifications. The same may be said of leather, paper, and many other materials to which both low- and high-power photomicrography may be applied.

The eight groups into which specimens may be divided are as follows:

Group I. Specimens Photographed at Magnifications of 50 Diameters or Less, Generally with Reflected Light. Included in this group are textiles, paper and leather surfaces, coarse granular material, and sections of joints. The microscope may or may not be used, depending upon individual circumstances. When the microscope is not used,

the objective must be of the photographic type and must be attached to the front board of the camera. See Sec. 146 et seq.

Group II. Finely Powdered Materials or Subjects Offering a Distribution of Small Discrete Particles. Pigments, cosmetics, and bacterial smears can be included in this class. The magnifications will be from 50 to 3600 diameters. Illumination will generally be by either transmitted light or substage dark-field condenser. See Sec. 155 et seq.

Group III. Metallographic and Petrographic Specimens and Other Flat Surfaces Photographed at Magnifications Greater than 50 Diameters. These specimens are thick and are often prepared with polished surfaces which, if the specimen is metal, are also etched. The illumination is generally by vertical lighting, although overstage dark-field illumination is sometimes used. See Sec. 164 et seq.

Group IV. Histological Specimens and Thin Sections of Any Material. Magnifications are generally less than 600 diameters, and illumination is usually by transmitted light. See Sec. 168 et seq.

Group V. Crystal Formations Obtained from Chemical Reactions on the Slide and from Slower Crystallization. Magnifications are generally less than 350 diameters. Illumination is by transmitted light, with occasional use of the substage dark-field condenser. This group is of interest to the chemical microscopist and microchemist. See Sec. 172 et seq.

Group VI. Fibers. Animal, Plant, Paper Pulp, and Synthetic Material. The magnification will seldom be over 600 diameters, and illumination will usually be entirely by transmitted light. See Sec. 175 et seq.

Group VII. Sharp Edges. Magnifications are usually from 500 to 1000; exceptional magnifications may be only 250 diameters. The system as outlined is particularly adaptable to razor blades, but can be applied to any sharp-edged tool. See Sec. 181 et seq.

Group VIII. Emulsions. The magnifications for this group are generally less than 500 diameters, 350 diameters being suitable for a large class of this material. See Sec. 184 et seq.

GROUP I

MATERIAL TO BE PHOTOGRAPHED AT MAGNIFICATIONS OF LESS THAN 50 DIAMETERS

Sec. 146. Description of Material. The following material is discussed in this group.

EXAMPLES

Wire screening, Sec. 147.
Textiles, Sec. 148.
Foodstuffs, Sec. 150.
Small metal and mechanical pieces, Sec. 149.
Paint films, Sec. 154.
Coarse powders, seeds, Sec. 150.
Leather, Sec. 154.
Joints, rivets, and cans, Sec. 151.
Paper, Sec. 154.
Plate cultures, Sec. 152.
Surface structures, Sec. 154.
Writing, Sec. 153.
Botanical specimens, Sec. 150.

Illumination. Large evenly lit fields are required. Illumination may be by overstage lamps, Silverman illuminator, Ultropak, Epi condenser, or other specialized form of reflected lighting device. Substage light is sometimes used to give the proper background, and infrared radiation is often a necessity. The small diagrams accompanying many of the low-magnification pictures indicate the position of the lamps in relation to the specimen. The small circle *O* marks the position of the object; the circles *S* indicate the azimuth of the light sources; and the letters *H, M,* and *L* indicate the angular position of the light source above the plane of the object *O, H* representing an angle greater than 45°, *M* an angle of about 45°, and *L* an angle of less than 45°. The wattage of the lamps is placed over the circles *S,* and the filter, if any, is indicated by the initial of the color; *D* and *T* indicate, respectively, light of daylight value and the unmodified tungsten light. In order to control the shadow effects and employ them to the best advantage, the lamps included in a system of illumination by reflected light may be of various wattage, or the effect of different wattage can be obtained by placing the lamps at different distances from the specimen.

The following pictures, illustrating subjects in Group I, show the lighting arrangements in detail. However, it should not be inferred that any other arrangements must necessarily be incorrect. For instance, lamps of different wattage may be used, and filters and sensitive material may be changed, but the final pictorial effect may, or may not, be altered thereby. The technician is therefore urged not to accept any detailed photomicrographic procedure as exclusively correct, but rather to build up his own methods by experimentation to suit his particular work and equipment.

Exposure time, so dependent on illumination, bellows extension, and other factors, is not included in the photographic data because it is likely to lead to confusion. Concerning exposure time in general, it might be said that a long exposure with weak lighting will not give the same effects as a shorter exposure with strong lighting. The strong lighting is more likely to yield satisfactory results. Most

subjects can be illuminated well enough to produce a good negative with an exposure of 3 to 5 seconds, occasional longer exposures being allowed for subjects reflecting a good deal of green and blue light. For microphotographic lenses, the bellows should seldom be extended above 30 or 36 inches. In special circumstances this distance may be exceeded, but ordinarily it is better practice to use a shorter-focus lens and to maintain the bellows draw within the 30-inch figure.

Apparatus. Lateral resolution is seldom of importance in photographing any material that would fall into this class. The lenses indicated are the 32-mm objective with low ocular, or any one of the microphotographic objectives used in the same way as a camera objective. The various focal lengths of available lenses were listed in Table XVII. Eyepiece cameras can be used with microphotographic objectives if the objectives can be mounted on the microscope tube provided that sufficient working distance can be obtained. These long-focus objectives can be used on cameras of fixed film distance, being screwed directly to the front board of the camera. However, it will be necessary to move the whole camera, lens, box, and film, in order to focus, unless the specimen can be raised or lowered to suit. Equation 43 will give the object distance, which is measured from the lens to the object; it will be substantially the equivalent of the working distance. The object distance for any particular lens is thus easily ascertained. Supposing that a 48-mm microphotographic lens is to be used with a fixed image distance of 10 inches; by reducing to millimeters and substituting in equation 43, the distance is

$$\frac{1}{s} = \frac{1}{48} - \frac{1}{254}$$

On solving, $s = 59$ mm; this is the required distance from the object to the lens. More specifically, the value of s is from the object to the first equivalent plane of the lens, and not to the vertex of the lens; also, as the lens mount invariably extends beyond the lens, the actual working distance is very slightly less than the computed value of s.

It will be seen from the above that any camera, or microscope and eyepiece camera, that can satisfy the above conditions of holding the 48-mm objective at a distance of approximately 2 inches from the object can be used to give an image 10 inches from the second principal point of the lens. The general arrangement of the illumination and camera is shown in Figs. 49 and 51.

The degree of field depth is a vital factor in obtaining pleasing photomicrographs of specimens listed in this group. The actual depth

of the specimen should not be in excess of the field depth of the objective. Field depth may be increased by reducing the aperture or by using a longer-focus lens; the apparent depth of the specimen may be lessened by choosing a mounting medium of high refractive index. Equation 49 can be applied in special problems. Lamps for overstage lighting have been described in Sec. 24.

General Procedure. The difficulties that most often arise in dealing with the subjects in this group are usually found to be due to one or all of the following causes.

1. Insufficient depth of field.
2. Undesirable highlights due to poor lighting arrangements.
3. Undesirable shadows due to insufficient lighting. (This trouble and the previous one might be summed up as poor photographic contrast.)
4. Improper balance of the transmitted and incident illumination when both are employed.
5. Poor arrangement of the material.
6. Lack of proper filters.
7. Lack of contrast due to improper selection of film.

Screening or small mesh (Fig. 213) offers an excellent example for study of field depth, for without considerable depth of field much detail in such a picture will inevitably be lost. The two views in Fig. 196 give some idea of control of field depth by means of mounting media of different indices. At *A* the specimen was mounted in a medium with an index of 1.0 (air); at *B* the index was 1.74.

The actual *f* number of a lens, as engraved on the barrel, has little or no significance in photomicrography because bellows extension will vary, as will the size of the entrance pupil. In practice, the iris diaphragm of the lens will be closed, to give acceptable contrast and to reduce glare, as appears necessary from studying the image on the ground-glass back of the camera. This same adjustment also affords a marked control over field depth, and it is proper so to use it, for resolution is seldom of importance in pictures made with microphotographic objectives. Lighting from below can be used concurrently with top lighting, or the two exposures can be made in succession if more convenient. The specimen is placed in position, and the top lights are turned on. Sharp focus is established, and the specimen is turned 45° and re-examined for any difference produced by the new direction of lighting. An examination of the specimen in two azimuths 45° or more apart, with the lamps set at 90° apart, will indicate the best position for avoiding shadows and highlights. A test

with different methods of illumination, mounting material, and orientations is essential procedure with every new specimen, for only by constantly experimenting with conditions can good technique be learned. The handling of a given condition for one specimen may be totally inadequate even for a similar specimen.

The surface of paint films, such as that in Figs. 52 and 53, may often approach specular reflection, and this lack of diffusion may produce on the negative an effect of uneven lighting. This trouble can be corrected by multiple lighting. Three lights may be required to cover a big field, or one photoflood bulb can be used in a desk lamp which during exposure can be moved around, above the specimen, in an arc of 120°.

Highlights on metal objects, such as machine parts, small tools, or large powder grains, may generally be overcome by immersing the specimen in a liquid. On transparent subjects, highlights can be completely eliminated in this way. A diffused light will lessen prominence of highlights, as will the use of fast plates. Figure 222 shows the even illumination obtained with diffused light, the specimen being a coarse white grain of considerable size.

Deep shadows are as undesirable as highlights; both will mask detail. Generally, the addition of extra lamps or the use of more diffused light will eliminate shadows. Shadows are particularly undesirable with textiles, since the weave of the cloth may be completely obscured unless the illumination is properly directed. Figure 214 is a picture of rayon. The background was made black because the contrast of white on black is essential to give the picture a pleasing appearance. The shadows have been nearly eliminated by placing two lamps at 90° to each other and well above the specimen. Good lighting arrangements may be suggested by critically studying the image in the ground glass of the camera.

Several lamp houses on the market are so made that they will produce equal intensity of illumination from all sides. They are toric in shape and are mounted over the objective. However, it is not always desirable to eliminate all shadow effects, for a shadowless picture is likely to have a flat appearance with little sense of depth, and so appear uninteresting. In making the picture shown in Fig. 223 the problem of shadows arose. The coffee beans cast shadows which fell largely on the glass plate supporting the beans, but when the transmitted light was turned on the shadows were lost. If they had been allowed to remain, that is, if the picture had been taken with a background of white paper without the transmitted light, the black figures of the shadows would have proved distracting; attention would have

been directed partly to the shadow and partly to the specimen that caused it. The proper distribution and depth of shadow are best determined by carefully examining the image when the ground glass back of the camera is in place.

With most granular material the grains must be placed in position individually. Even such small objects as grains of granulated sugar need to be placed carefully to avoid an uneven distribution. A needle is generally all that is needed to accomplish this, but in dealing with a subject mounted in a liquid, the particles may move and bunch together, and it will then be necessary to anchor them to the slide before they are immersed. They can be fixed to the slide with celluloid solution, or a coating of turpentine can be spread on the slide and the particles distributed on it and allowed to dry. After the slide has been gently heated the particles will be found to be fairly well anchored. It is sometimes possible to get an even distribution of very small particles by tapping on the edge of the slide or by tapping directly under the particles. The slide can be examined on the table by means of a magnifier, or a dissecting microscope can be used to ensure a satisfactory final arrangement.

The field should be interesting but not crowded, and large vacant spaces of background should be avoided if possible. This does not mean that pictorial effects are to be specially strived for, but simply that the best use is to be made of the film area. Certainly, a pleasing picture is of greater credit to the photomicrographer than an uninteresting one, although the scientific value of the two may be equal. Any increase in magnification will also increase the area between the particles as well as magnify the particles themselves. Therefore pictures taken at high magnification frequently show a comparatively wide separation of the particles which cannot be avoided, particularly since there are other factors at high magnification which contribute to this. At low magnification the particles or specimens should be grouped fairly closely together for best effects, for, provided that there is no overcrowding, the greater the amount of material in the field, the better will be the chance of obtaining a picture of value.

Metals, insect parts, seeds, textiles, and colorless or seemingly colorless specimens may all respond favorably to the use of an appropriate filter. It is not possible to lay down rules regarding the selection of filters, and the photographic effect may not always be easy to evaluate on the ground glass of the camera. Filters of various colors must be tried before it can be decided which filter will give the best results. As a rough guide, it might be said that for many dark reddish objects, or those bordering on the black, the longer wavelengths should be used

and the shorter ones suppressed. That means that the effect of red, orange, and yellow filters may be tried first. On dark specimens, a strong red filter will generally indicate whether efforts are being made in the right direction. To make a reliable test of the effect produced on large objects by colored filter glasses, several photomicrographs may have to be taken with a variety of filters and the negatives compared.

In order not to overemphasize the use of the filter for low-power photomicrography, it might be said that a large proportion of such work probably will not require any filter at all, even for better than mediocre results. Unless there is an apparent reason for the filter, the unmodified light source can be expected to give a good picture on many subjects. Low magnifications, and the low apertures of microphotographic objectives, do not call for the extremely good correction which is built into high-power microscope objectives. Therefore the use of green light to obtain optimum image formation is, as a rule, uncalled for.

As far as possible, cover glasses should not be used with either vertical or oblique lighting, as they are sure to introduce excessive glare. On certain objects it may be impossible to avoid them; if so, low-power, low-aperture lenses are indicated.

Numerous aids of one sort or another will be discovered in handling various objects of sufficient size to fall into group 1. Test tubes photographed to indicate turbidity of their contents can be mounted with a wire or string stretched behind them, or on a white card on which a line has been drawn. The turbidity of the solution is measured by the degree of visibility of the wire or line showing through the solution in the tube. Tiny objects may be supported by still smaller drops of shellac which have been allowed to become partially dry, and Plasticine can be used to good advantage to hold some of the larger objects in position, but these supports should never be visible. Certain specimens can be mounted on double-pointed short pins stuck into a cork or soft wood base covered with black paper. A black paper which serves for a background should be mounted far enough beneath the object to be completely out of focus in the picture. A white paper background may not prove a success on account of unevenness in the lighting and the formation of shadows. The method of back lighting, already described, is more likely to produce good results if a white background is desired. When objects have to be immersed in a liquid to obtain adequate field depth or to deaden highlights, it may be necessary to anchor them to a paper support or to the bottom of the glass dish itself in order to keep them in place during exposure. Various

liquids such as paraffin oil, aroclor, or water are all good for this purpose. As a rule it is not hard to find a liquid which is chemically inert with the object and which will not dissolve it.

Immersed specimens should be freed from entrapped air bells either by mechanical means or by gently warming the liquid. Large mats made of stiff black paper with the center cut out to the size of the object field can be placed in the object plane to shut off extraneous reflected or transmitted light. Screens 12 to 18 inches high, as shown in Fig. 177, serve the same purpose. Coarse granular material should generally be freed from all unneeded small particles, since dustlike specks give the picture an untidy appearance and often detract from the main purpose of the photomicrograph. Scales can be laid in the object field to afford a direct means of measuring the specimen, or they can be photographed separately and either added to the mount as a second picture or printed with the picture.

Stains play a very minor role in large specimens; they are seldom, if ever, needed. Etching with acid will make the outline of rivets or other metal joints stand out more clearly. Such surfaces as cross sections of glued joints can generally be made suitable for photography with a knife or plane if the sectioned surface is finished off by rubbing smooth with very fine sandpaper. Any abrasive which collects in the joint should be removed with a coarse stiff brush before the specimen is photographed. Specimens of cross sections of hose or tubing should be cut as thin as an eighth of an inch or less, and, if practicable, the lighting should be from beneath as well as overhead.

Thick sections of foodstuffs can be cut by hand for low-power photomicrographs; many nuts, vegetables, and berries can be so handled. Cake and bread can be embedded in a wax with a low melting point, the blocks of material being about 1 cm square; and the sections are cut freehand with a razor blade. Parasitic material, if not too small, can be easily handled and arranged on a slide. Larvae and the adult insects that infest food products can be washed and spread out for photography, or they may be mounted on a pin and dried. Infrared radiation is a great help in photographing insects, particularly adults of the order Coleoptera.

Sec. 147. Wire Mesh and Similar Material. Figure 211 shows a photomicrograph of brass wire mesh. It is intended to be studied in connection with Figs. 212 and 213. The series clearly shows the effects produced by different methods of lighting. In Fig. 211 transmitted illumination only was used; in Fig. 212 the overstage lighting produced a dark-field effect; the picture shown in Fig. 213 was made with the combination of incident and transmitted light. The position

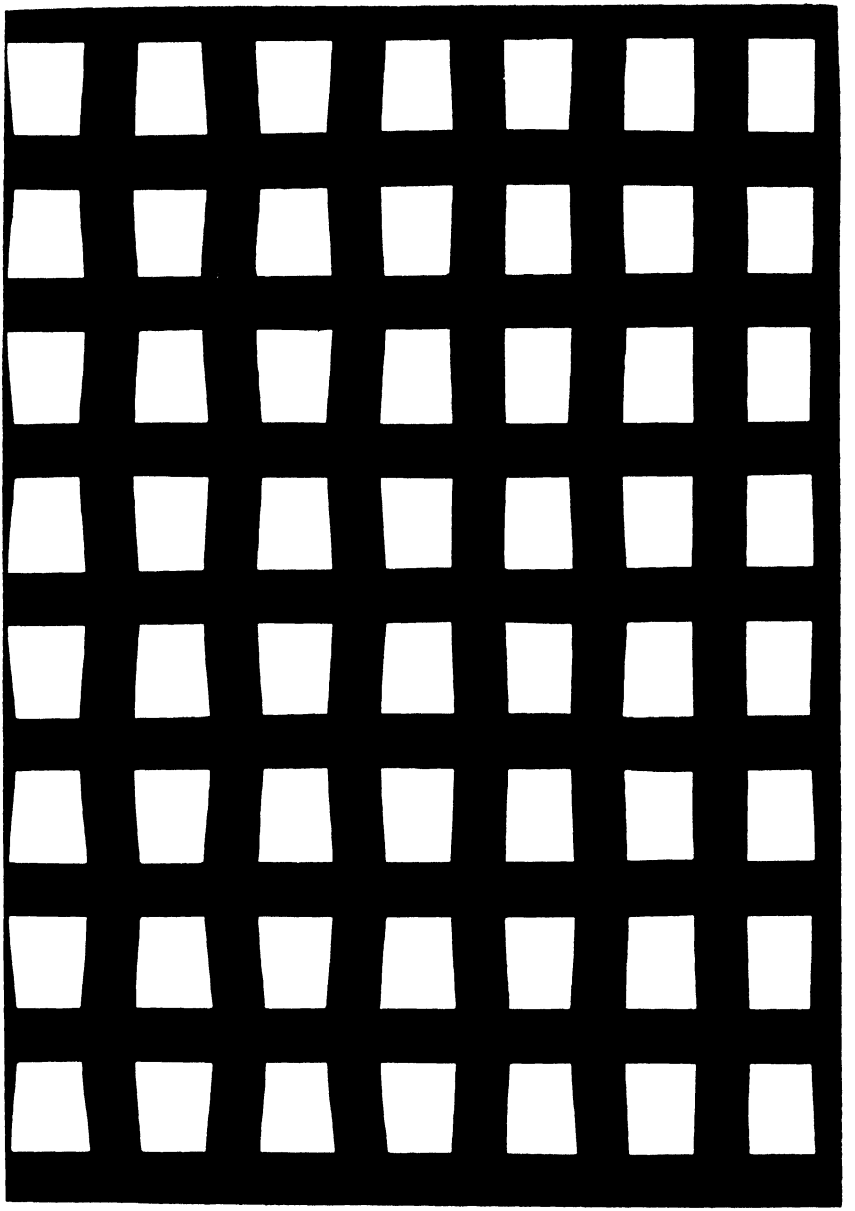


FIG. 211. Wire mesh ×30. Objective: Leitz, 24-mm micro-summar, mounted on microscope; condenser, Leitz, 3-inch focus; lighting, 100-watt tungsten projection lamp close to condenser, diffusing plate; Eastman Commercial Pan film; developer, D-11.

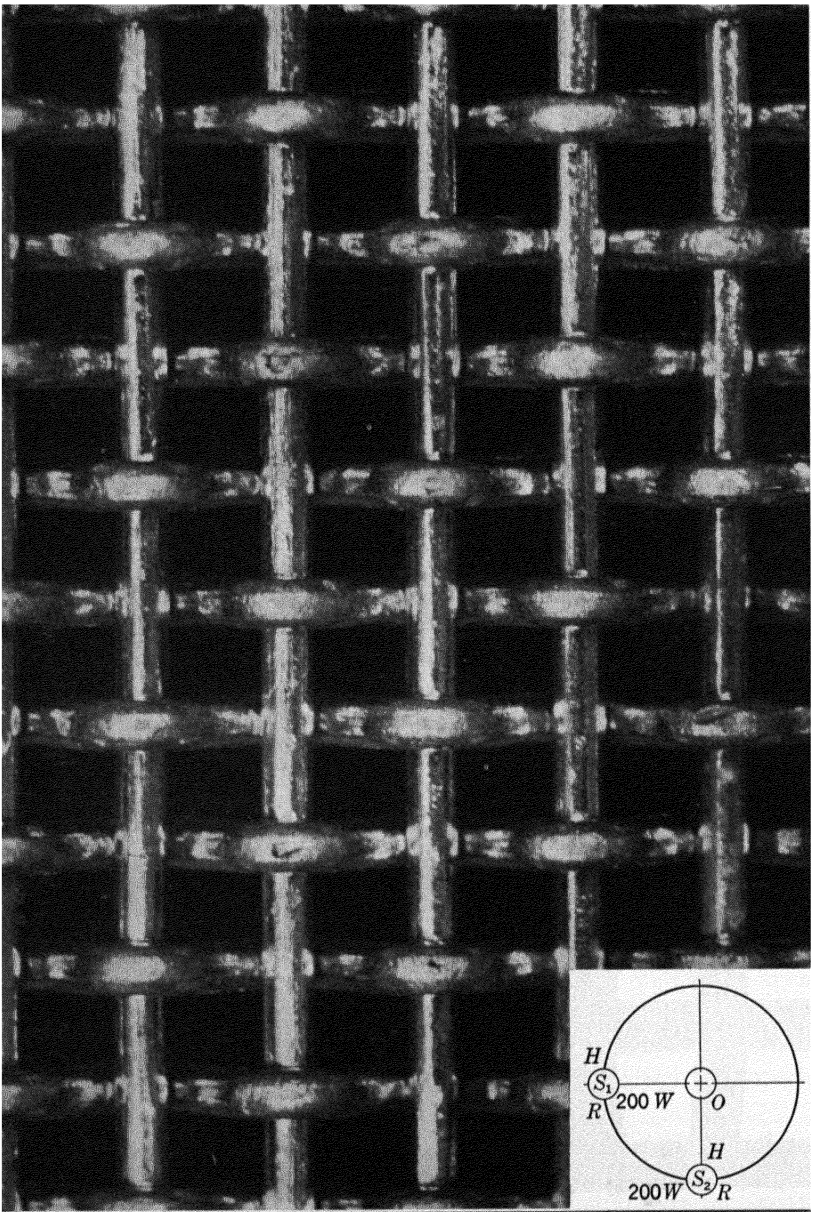


FIG. 212. Wire mesh ×30. Objective, Leitz, 24-mm micro-summar; Wratten filter No. 25; black card placed over microscope condenser. Projection lamps placed close to stage. Eastman Commercial Pan film; developer, D-61. It should be noted that a developer giving greater contrast was used for Fig. 211 than for this figure.

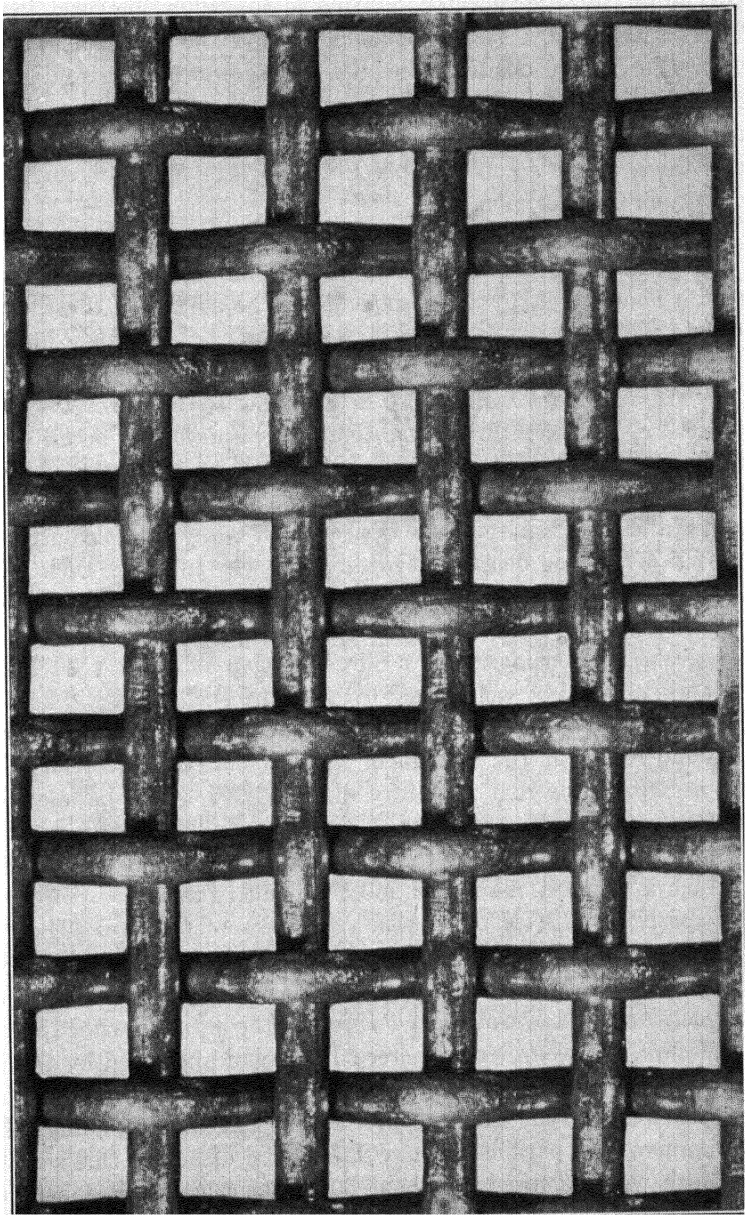


FIG. 213. Wire mesh ×30. This picture is the result of combining the two lighting methods used in Figs. 211 and 212. All other arrangements are the same as for those pictures. The specimens for the three pictures were different.

of the lamps for the reflected lighting is shown in the inset in Fig. 212. The lamps were placed about 50° above the specimen. To determine the correct position, one light was switched on and placed so that the minimum amount of highlights and shadows appeared. The second lamp was then placed at an azimuth angle of 90° to the first. The position of the second lamp could be predicted by observing the symmetry of the specimen.

The three pictures of the mesh are entirely different in effect, Fig. 213 being the most pleasing, but none can be said to be exclusively correct. That taken by transmitted light alone shows the mesh in silhouette with no details of surface structure. This might be adequate for certain purposes, as for measuring the size of the wires and the size of the intervals or for determining the " mesh size." The next picture, taken with top lighting only, shows the detail which was lacking in the previous picture. The last of the series does not give any new information about the mesh, but it is the most attractive picture of the three and might be desirable for many special illustrative purposes. Thus, the decision regarding the way in which such a picture is to be taken must depend to a large extent upon the purpose for which it is required. A company interested in making wire mesh would probably consider that a large portion of their photomicrographic work would be well served by a picture such as Fig. 211, which is the easiest of all to make. On the other hand, if interest lay in finding and recording the amount of wear that a certain screen would stand, it is probable that a picture like Fig. 212 would be adequate. An advertising department or sales division would look for pictorial value, and undoubtedly they would select a picture made as in Fig. 213, which is unquestionably the most difficult to take.

The specimen of wire mesh was very easy to prepare. The first step was to clean the mesh. After being brushed with a typewriter brush to free it from dust and large particles of lint, it was held under the faucet, and water was forced through the meshes with appreciable pressure. It was then immersed in alcohol and dried by waving in the air a few times; next it was dipped into zylene, swished around a little, and removed. To prepare the specimen for photography, it was immersed in paraffin oil in a small Petri dish. In this way the focal depth was increased by about 50 per cent. The dish with the specimen was placed on the stage of the microscope and illuminated as already described. Before the exposure was made, the portion selected for the photograph was examined for extraneous matter, either adhering to the mesh or floating on the top of the oil. Fre-

quently, particles loosened from an immersed specimen may float to the surface of the immersing liquid and escape notice unless the surface of the liquid is brought into focus. If allowed to remain such particles will record as a shadow on the negative quite capable of spoiling the picture.

For the picture in Fig. 212 a red optical filter was used. This filter was selected while the specimen was being examined visually under the microscope. It seemed that Wratten filter 25 increased the detail of the yellowish red structure of the wire. Accordingly, that filter was added when the picture was taken. Other filters were tried, but for the most part they seemed to increase unduly the contrast between the highlights and the shadows. If a red filter had not been available, probably a strong red, oil-soluble dye could have been added to the paraffin with equally good results. The transmitted or bright-field lighting was obtained by making use of the microscope and its concave mirror, but the same result could have been attained equally well with the back lighting from a printing box. The microscope is usually more convenient if circumstances permit its use, that is, if the specimen is small enough and the magnification high enough to permit the specimen to be lit adequately with the microscope mirror. To get even lighting for the transmitted beam, a large piece of ground glass was placed in front of the lamp and the light from this surface was reflected through the specimen. The concave side of the microscope mirror was used.

Sec. 148. The Arrangement of Textiles. Like wire mesh, textiles can be photographed in three important ways: with transmitted light, with overhead light alone, or with a combination of the two. Overhead lighting alone is shown in Fig. 214. The subject is white silk, and the dark-field illumination alone is very appropriate. In order to obtain a dark field free from shadows and glare, the specimen was mounted with water on a microscope slide, and, when a satisfactory arrangement had been arrived at, a second slide was placed over the first, the two slides being held together with rubber bands until the water evaporated. To hasten evaporation, the slides were placed over a lamp, and the slight heat on one side caused the specimen to adhere to it. The other slide was then removed, leaving the specimen perfectly flat, as can be seen from the picture. The anchored specimen was then placed on the stage of the microscope and illuminated from above with two lamps placed at 180° to each other. The orientation of the specimen with respect to the lamps differed somewhat from that of the wire screen specimen. The under side of the

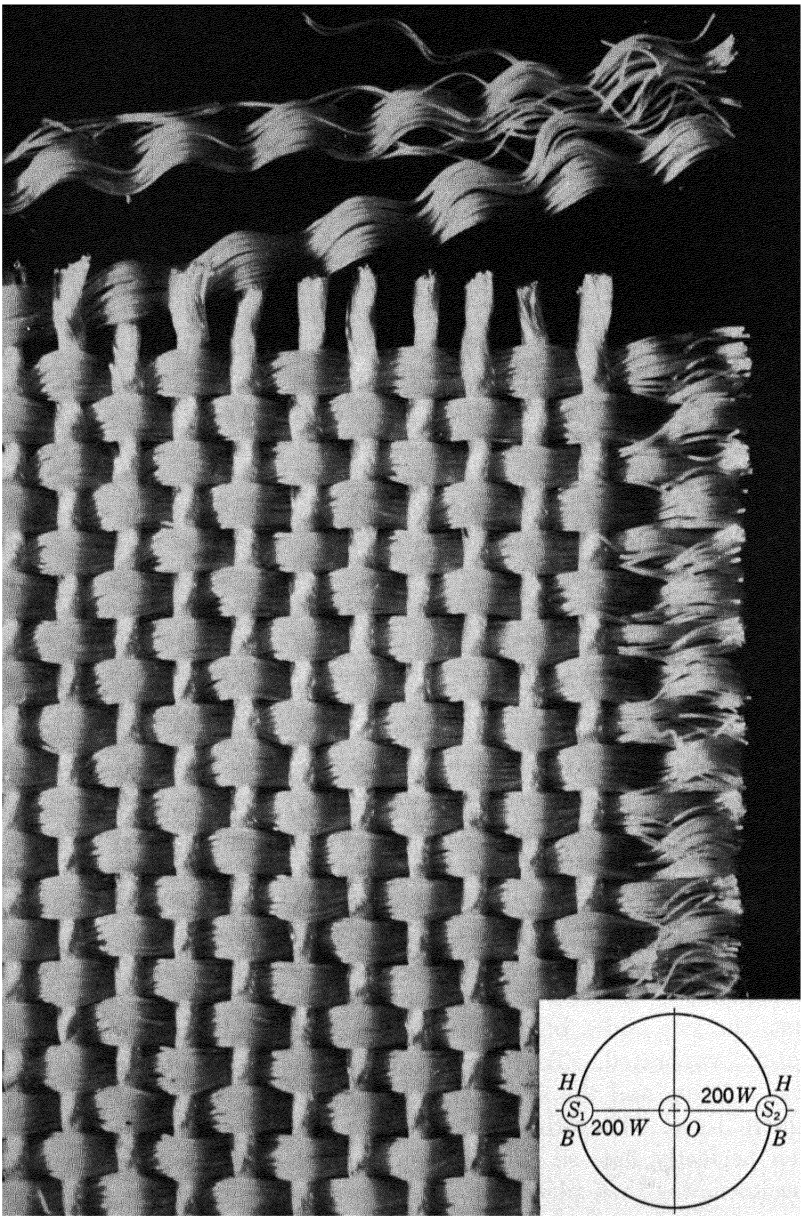


FIG. 214. Silk textile ×24. Leitz 24-mm micro-summar objective; Wratten filter No. 45. Lamps used without light-collecting lenses. Specimen mounted on microscope stage. Eastman Commercial Pan film; developer, D-61a.

stage was blocked off at the mirror with a black card, the condenser having been first removed. This technique ensures a textureless background.

When the specimen was examined through the microscope, it was discovered that a blue filter seemed to give an image with good tonal values, as is often true with white objects and many bright metals. Accordingly Wratten filter 45 was chosen.

A blue textile specimen will require a long exposure and perhaps, to avoid photographic flatness, process plates or film. Better photomicrographs of such specimens may be obtained with the mercury-vapor discharge tube and a blue filter; the time of exposure is thus reduced to the minimum.

Figure 215 shows a textile photographed with a combination of bright-field and dark-field lighting which gives a certain depth to the pattern of the specimen that would be impossible to attain if the lighting were either transmitted or incident alone. This specimen was mounted differently from that of Fig. 214. The textile was first cut to fit over a small metal ring about 2½ inches in diameter and ½ inch in height. The sides were pulled down over the ring and secured in place by a rubber band. This permitted the whole to be placed over the opening in the stage of the microscope. After being illuminated as desired, it was ready to be photographed. The special purpose of this photomicrograph was to emphasize the defect in the specimen. Without the slight pressure on the mesh, applied equally in all directions, to support the fabric, this defect could hardly have been demonstrated.

In Fig. 215 there appears to be a slight shadow that masks all the detail on certain parts of the threads, but it in no way detracts from the value of the picture, the purpose of which was to demonstrate the defect in the center. However, for advertising or other purposes, it might be better to eliminate the shadow entirely. This could be done by the addition of another lamp or by shifting the position of one of those already in use. Figure 216 shows a specimen in which the highlight and shadows have been particularly well balanced. This balance was attained partly by the proper selection of the film, Tri X Pan.

When photographing threads or textiles, practically all highlights can be eliminated by immersing the specimen in a liquid of identical index. This method of mounting has the disadvantage that individual fibers which would otherwise show as projecting from the threads may become completely lost to view; however, field depth is greatly increased.

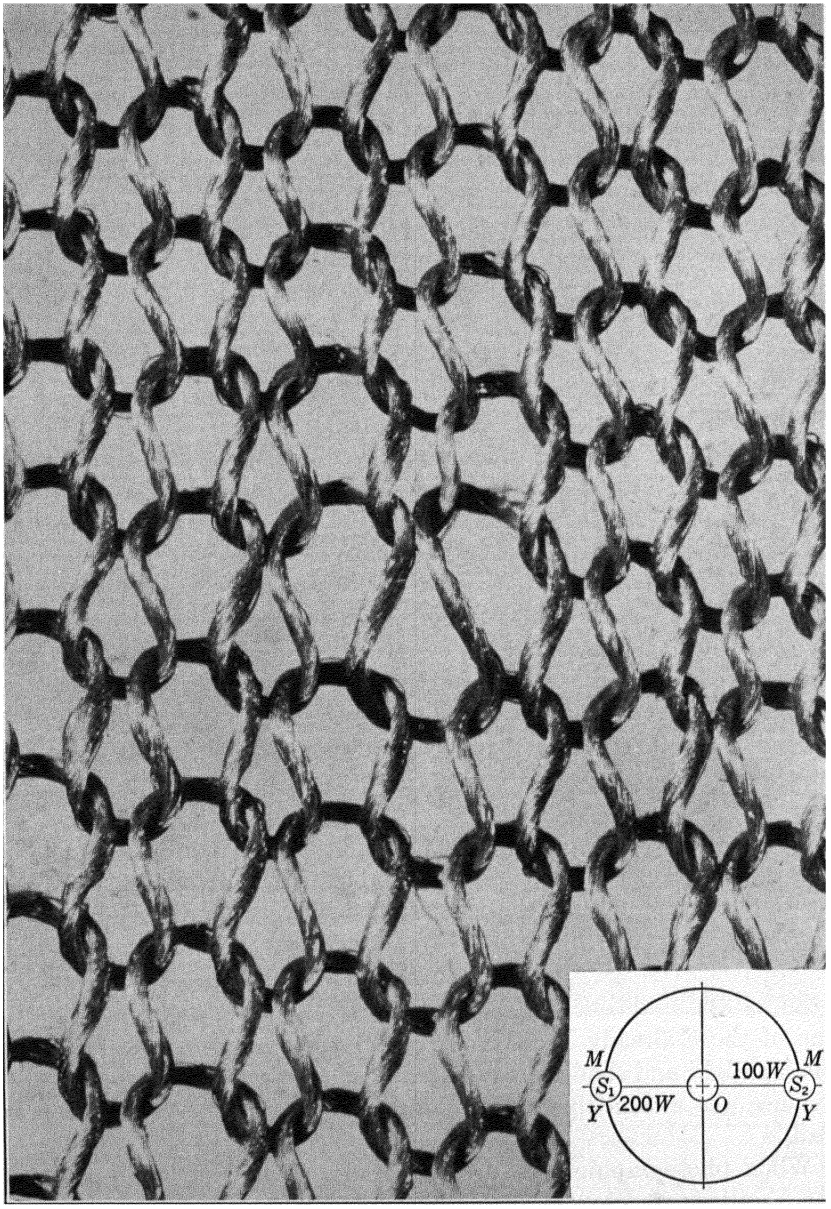


FIG. 215. Rayon textile ×28. Leitz 24-mm micro-summar objective; Wratten filter No. 15 (the specimen was yellow); condenser, none; the transmitted light was received on a diffusing plate placed in the substage ring, the lamp being arranged for parallel rays. The lamps for the reflected lighting might have been better arranged if they were placed 90° apart. Eastman Commercial Pan film; developer, D-76.

Black specimens can be photographed as they are, or they can be bleached. As a rule, black specimens require a great deal of light and a long exposure in order to give sufficient detail. Fast film can be

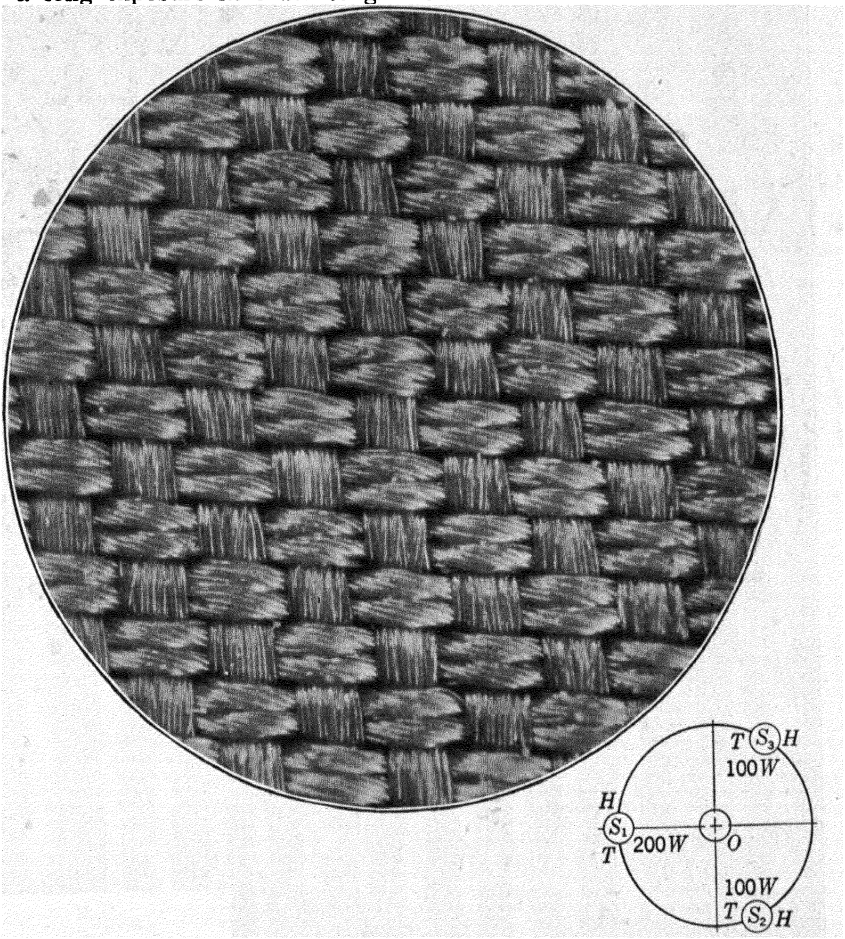


FIG. 216. Nylon textile ×70. The objective used in taking this picture had an aperture of 0.15, high enough to give adequate resolution of the individual elementary fibers. When the weave is close and the yarn is fine enough, this objective and ocular make an excellent combination for pictures of textiles. Objective, 32-mm apo. Zeiss; ocular, Homal II; Eastman Tri X Pan film; developer, D-19.

used, particularly if the subject is of rather solid construction needing no background, but if the weave is open better pictures can be had from a bleached specimen.

Individual threads of sufficient length can be wound around a microscope slide. The first end is held against the slide with one finger

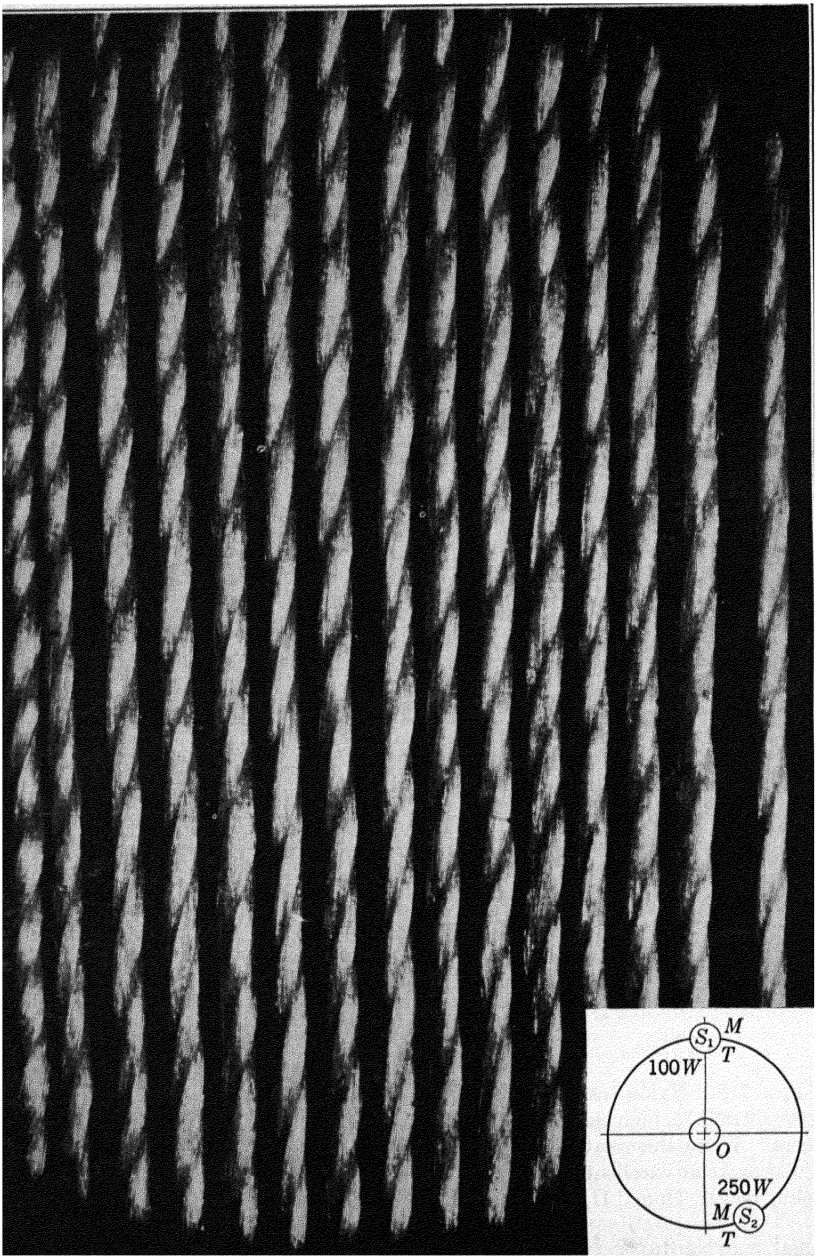


FIG. 217. Thread ×20. Leitz 24-mm micro-summar. The lamps were used with diffusing plates. No special effort or care was required to space the individual coils of thread. The resolution of the individual fiber elements is not nearly as satisfactory as that shown in Fig. 216, where the aperture of the system was higher. Eastman Panatomic X film; developer, D-11.

while about a dozen turns are made. Then, after the coils are secured to each edge of the slide with a little melted paraffin applied with a small brush, the threads on the under side of the slide are cut away with a razor blade, leaving a few threads parallel and close to each other convenient for study or photography. Figure 217 shows a slide prepared with parallel threads. If a single thread is to be photographed, each end may have to be anchored to the slide with a drop of melted paraffin. If the thread is so twisted that it will not lie sufficiently flat, even when stretched between the paraffin anchors, it can be placed between the cover glass and slide of a specimen compressor, several types of which are made by Beck and other London firms. Sometimes a thin cover can be used with an ordinary dark-field slide. When the specimen is placed in the chamber, slight pressure exerted by the clamping device will straighten it without distortion, as in Fig. 218.

Figure 219 shows a photomicrograph of a rayon textile with defective weave. This picture was taken with a 48-mm lens. The diagram accompanying it should be studied because the lighting is different from that employed hitherto. All previous pictures of textiles were taken with a 24-mm objective of the microphotographic type, which, as a rule, is the best focal length for textiles.

Textiles can be photographed without



FIG. 218. Dirty filler ×25. Rayon 3-ply yarn showing a dirty filler thread. The specimen was mounted in a cell with pressure on the cover to maintain, as nearly as possible, one optical level. The position of the lamps gave illumination to create an appearance of depth. Leitz 24-mm micro-summar objective; the microscope stand was used; Defender Pentagon film; developer, D-1.

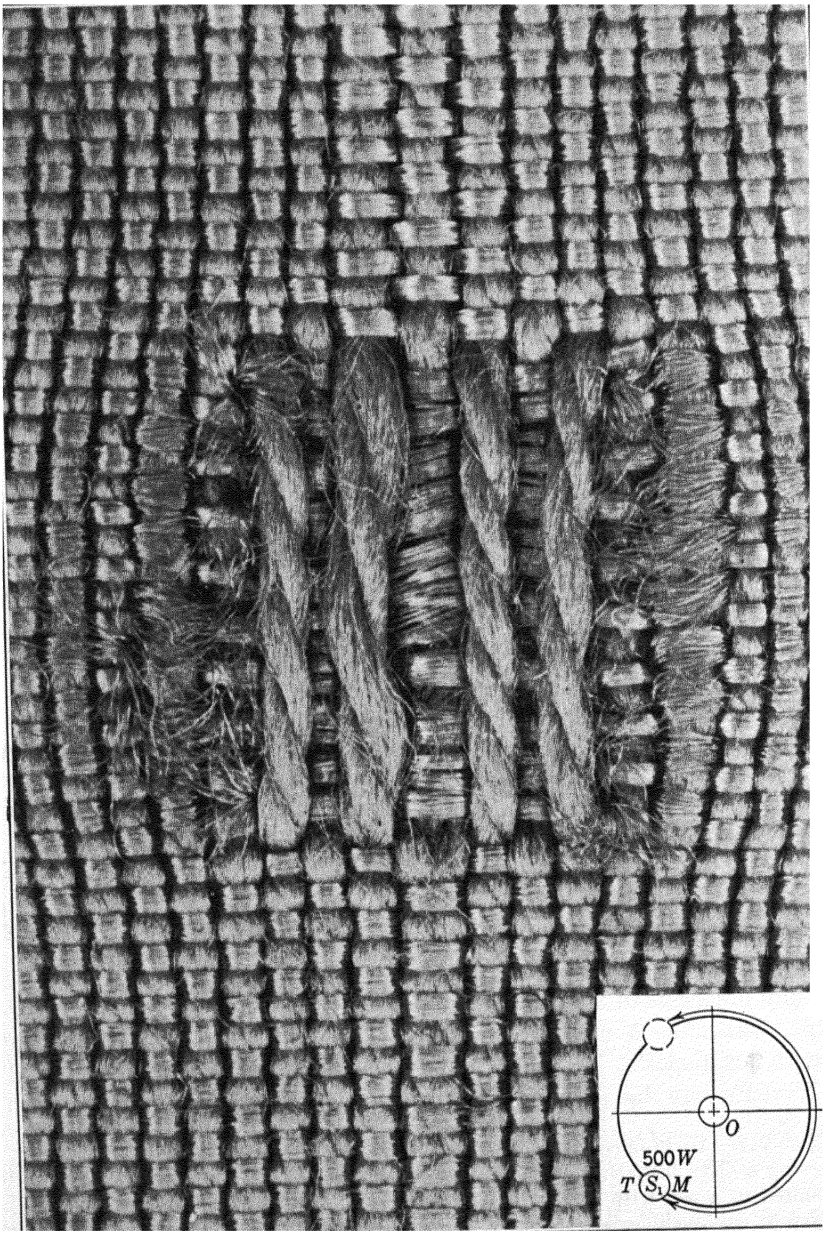


FIG. 219. Rayon textile ×12. Defective weave is illustrated. Bausch and Lomb 48-mm Tessar Ic; a photoflood lamp was used, it was carried around the specimen, during exposure, as indicated by the arrow in the diagram. Defender, Pentagon film; developer, D-19.

any top lighting, in the same way as the wire mesh shown in Fig. 211, when the weave is sufficiently open to allow considerable background lighting to show through. Such a picture may or may not be of value to the textile technologist, but from the standpoint of the microscopist, whose aim should generally be to show all possible detail in a picture, results obtained by this method can never be more than mediocre, irrespective of the perfection of the technique. Such a picture gives the impression of being only half finished because, under normal conditions, a textile is inspected by reflected light against a light background, and its appearance is familiar that way.

Occasionally, it may be necessary to photograph a section of carpet or rug to show the depth of the pile or the manner in which the threads are woven. Figure 220 shows such a section. The carpet was cut with a heavy microtome knife so extremely sharp that it could not only cut a thin section but also cut it so that there would be the least possible disarrangement of the threads, which in the specimen were distinct and separate from each other. As the material was of many colors, chromatic filters were undesirable. The only filter used gave the tungsten light a daylight quality, so that with panchromatic film correct color values were obtained.

To make the photograph, the section was laid on the glass top of a printing box, and both transmitted and reflected light were employed.

Sec. 149. Arrangement of Small Metal Pieces and Mechanical Parts. The photographing of a small piece of metal is well exemplified in Fig. 221. In this particular problem many difficulties were encountered. The specimen was a nearly black and much-corroded piece of steel, which had been removed from a man's eye after an explosion. The aim of the photograph was to show all the detail possible, stressing the jagged appearance. The specimen had considerable depth as shown, and had to be photographed on a bright field because of its dark color. In white light, little or no detail or surface structure was visible, but with a strong red filter and a plate registering infrared the texture of the surface could be photographed. The depth of the piece, even when lying flat, was considerably greater than the field depth of the 48-mm B. & L. Tessar lens which was selected. In order to have the top of the specimen sharp while the bottom edge was in focus, the specimen was mounted in aroclor to get the necessary shallowing effect, and even when the specimen was turned edgeways, good depth was obtained in the picture.

In this particular piece of work no trouble was experienced from highlights. Shadows were completely done away with by the sub-

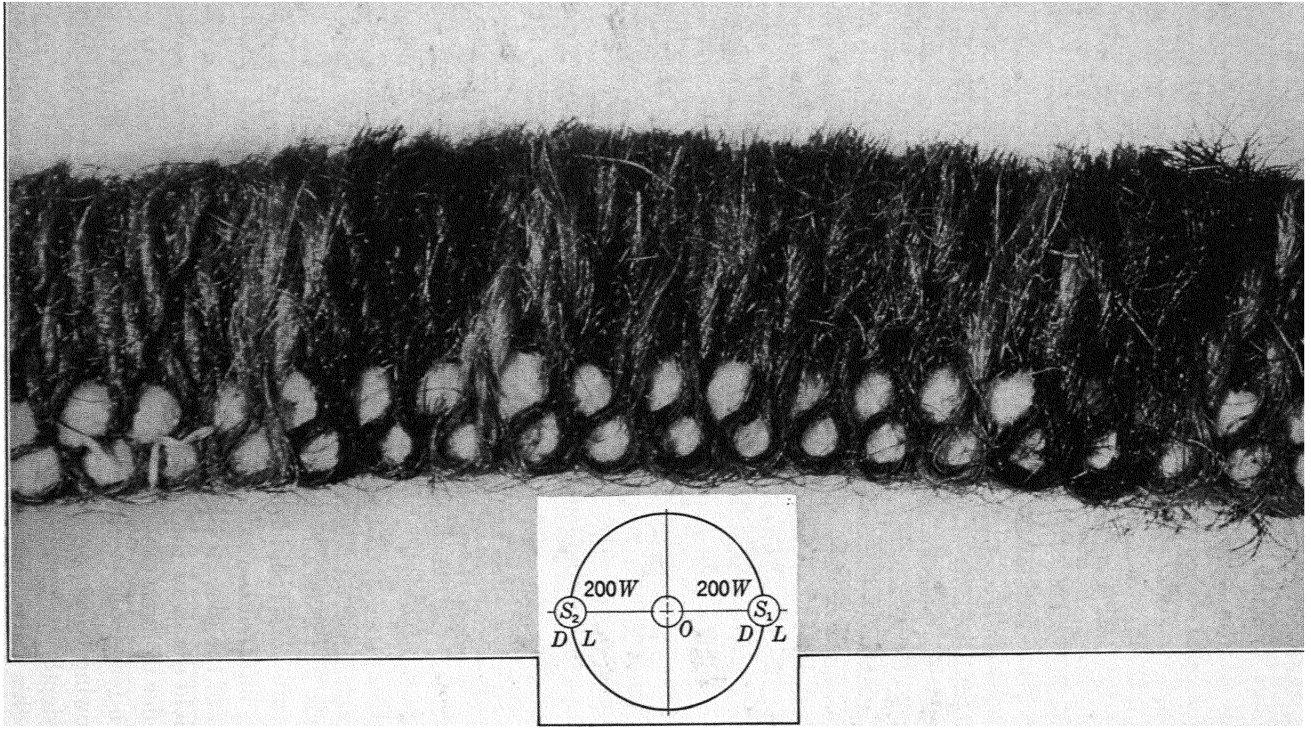


FIG. 220. Carpet in cross section ×2. Objective used was an old Bausch and Lomb 5-inch rapid rectilinear lens. The transmitted light was obtained by mounting the specimen on a printing box. Eastman Commercial Pan film; developer, D-61a.

stage light obtained by employing the microscope as a stand and holder for the photographic lens and illuminating from beneath by the concave mirror. (Note that in this book it is only seldom that the use of this mirror is advocated.)

The edge view of the specimen was more difficult to obtain. It was found necessary to balance the specimen carefully on its edge and to hold it in place with a small piece of Plasticine. The Plasticine was

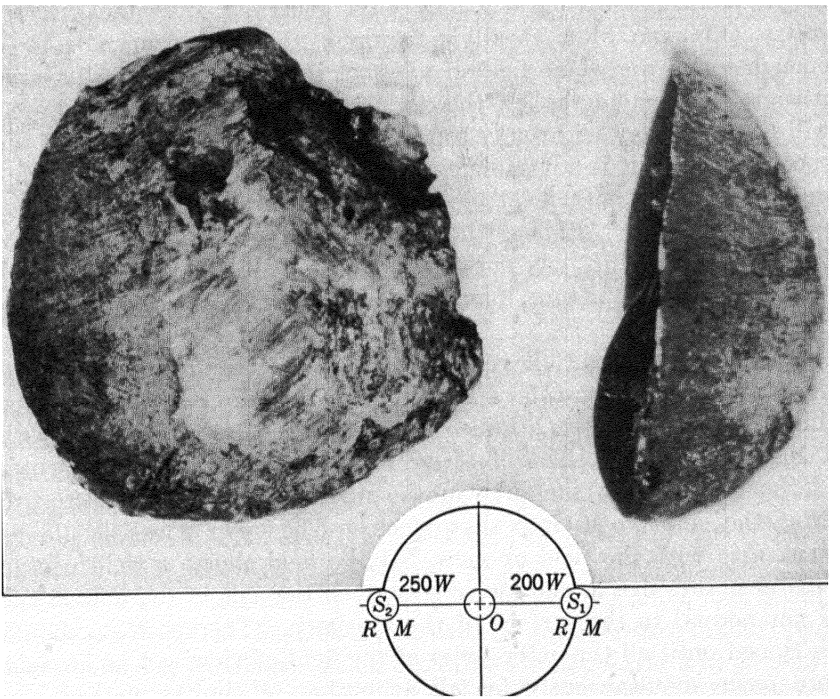


FIG. 221. Steel fragment ×12. Bausch and Lomb 48-mm Tessar Ic; transmitted light, 100-watt lamp and concave mirror used as a condenser. Written filter No. 25 Eastman Infrared film; developer, D-61a.

kept well back from the edges of the metal so that it was not visible in the finished picture. Much depth of focus was possible with the specimen arranged in this way.

The overstage lighting presented no difficulties because the piece of metal seemed to have nearly complete symmetry (if the term may be used) as far as its light-reflecting powers were concerned. Therefore, with two lamps placed in any position, the piece of metal might have been oriented in nearly any azimuth without making much altera-

tion in the final result. Shadows would have spoiled the picture, and even detail of the Petri dish which supported the specimen would have been undesirable. Also, had the specimen been mounted on a white card or paper, the texture of the paper would inevitably have showed and some shadow would have been cast.

Small mechanical parts, screws, needle points, type, and similar objects may all be successfully handled in the manner just described for the specimen of steel. A red filter may not be needed, and on bright polished steel parts a blue filter generally gives the best results. Often no filter at all is required. If the specimen is large enough to fill the whole field of view, or if only a portion of a large mass is being photographed, the complication of the transmitted light will be done away with; only top lighting need be considered. If the specimen is flat it is advisable either to have two lamps for top lighting, or to use a photoflood bulb moved in an arc of about 120° as explained before. Although the image on the ground glass may seem to be evenly lit with only one fixed lamp, the appearance may be deceiving, and the picture may be disappointing because of uneven lighting.

Often it is required to have the surface of the specimen parallel with the stage of the microscope. If there is difficulty in determining when this condition has been attained, inspection can be made easily with a 16-mm objective. The objective is focused sharply on the center of the object field; the tube of the microscope is then lowered to bring the outer zones into sharp focus. The outer zones should be concentric with the field of view. If the field shows a well-focused area near the edge in one sector only, then the axis of the microscope is not normal to the surface of the specimen. The specimen should be tipped until all the outer zones of the field of view can be brought into focus simultaneously for all azimuths. A similar method was suggested in Chapter I for determining whether the microscope axis is normal to the stage.

Textiles, papers, and like material will lie flat and give a perfectly horizontal field without any trouble, but small objects that show a flat surface toward the lens and are irregular in shape on the opposite side may cause some difficulty. To facilitate the mounting and placing of such objects in the correct position, the circular opening in the stage of the microscope can be utilized. A steel ball of appropriate size can be ground down to a hemisphere and mounted with the spherical side downward in the stage opening. The specimen can then be placed on the upper part of the hemisphere. By turning the hemisphere and specimen in the mounting, the top surface of the speci-

men can be correctly placed. The condenser should have been previously removed or lowered.

Sec. 150. Photographing Coarse Powders, Seeds, and Similar Material. Specimens of this sort usually present a problem of field depth. The highlights are often difficult to control, and each particle must, as a rule, be placed individually. A crowded field will show one particle overlapping another, but a scattered one will not show enough material. The field must be painstakingly arranged.

Figure 222 illustrates the care with which the particles must be placed. Each particle is almost isolated, yet there is no appreciable waste of space. The background is dark because the particles are white; real advantage is gained by this added contrast. The depth of the particle, though considerable, is recorded with a lens of long focal length with its diaphragm well closed.

This class of material would include large seeds and the roasted coffee beans shown in Fig. 223. In Fig. 224 a cacao bean is shown in longitudinal section. Each portion has been placed individually in position, with the definite aim of showing it to the best possible advantage. After any manipulation of the specimen there should be no evidence of careless or unintentional arrangement. Each part must be in its proper position relative to other parts, and any special arrangement must be made to show some definite detail. Nothing should be left to chance.

In Fig. 224 the halves of the cacao bean were secured in place on the glass plate with Plasticine. A printing box furnished the substage light. The Wratten filter 25 seemed to give the greatest amount of detail in the subject and to bring out the yellows, reds, and browns satisfactorily. The bean was cut with a safety-razor blade. Such a blade held in the fingers has a decided advantage over a large microtome knife, or even a razor, for much botanical work. The safety-razor blade, being very thin, will not force the specimen apart before it is actually cut. Thus the specimen will not be torn or have a ragged edge. Soaking the specimen in water before it is cut will give it the needed softness without undue swelling.

Sec. 151. Rivet, Can, and Glue Joints. Rivet and can joints, and indeed any kind of joint, can be photographed easily enough after it has been sectioned, but there is usually difficulty in getting a good section that can be trusted to show the actual condition as it existed before the sectioning process. When the specimen is delivered ready to photograph, there is little to be done except to take the picture; but oftentimes the specimen will arrive at the laboratory as a complete unit which must be sectioned or surfaced at the appropriate place.

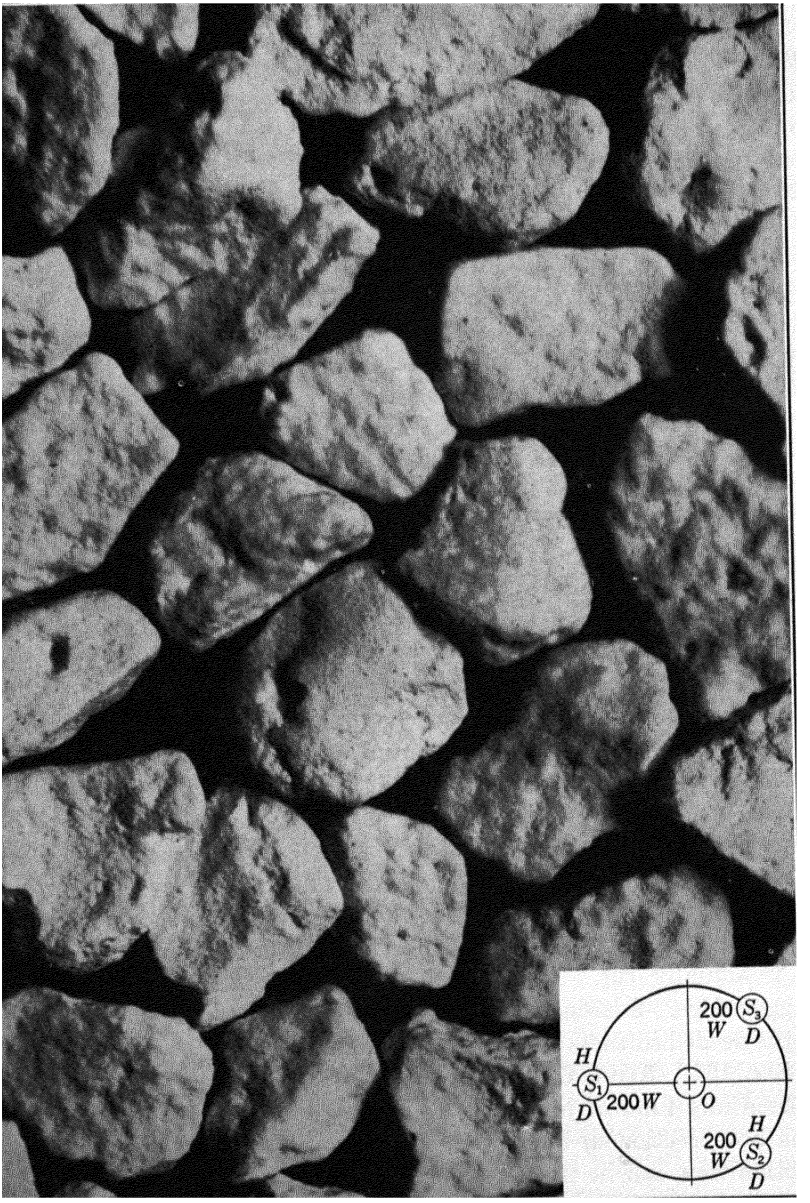


FIG. 222. Soda lime crystals ×6. Bausch and Lomb 5-inch rapid rectilinear lens. Two photoflood lamps were used; Eastman Panatomic X film; developer, D-61a.

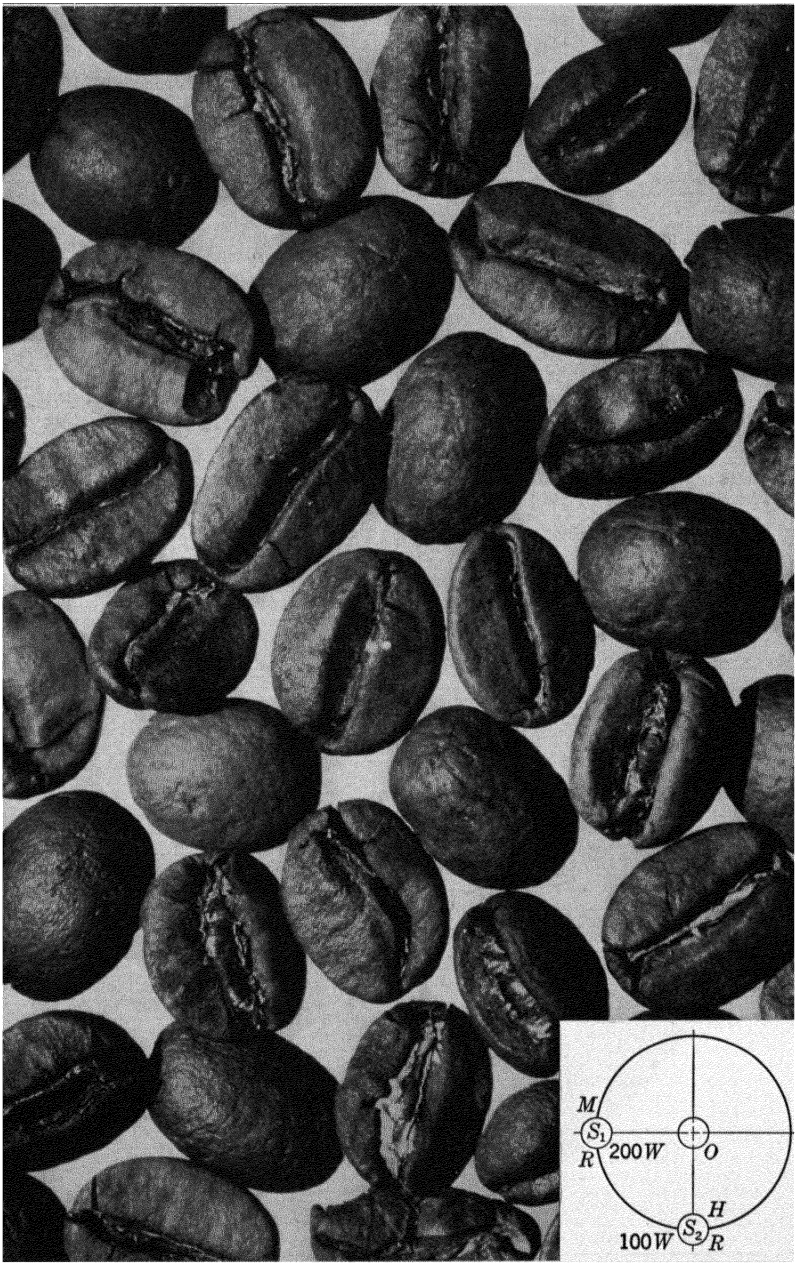


FIG. 223. Roasted coffee beans ×2.5. Bausch and Lomb 5-inch rapid rectilinear lens. Specimen mounted on clear glass plate above diffusing plate of printing box. Eastman Commercial Pan film; developer, D-61a.

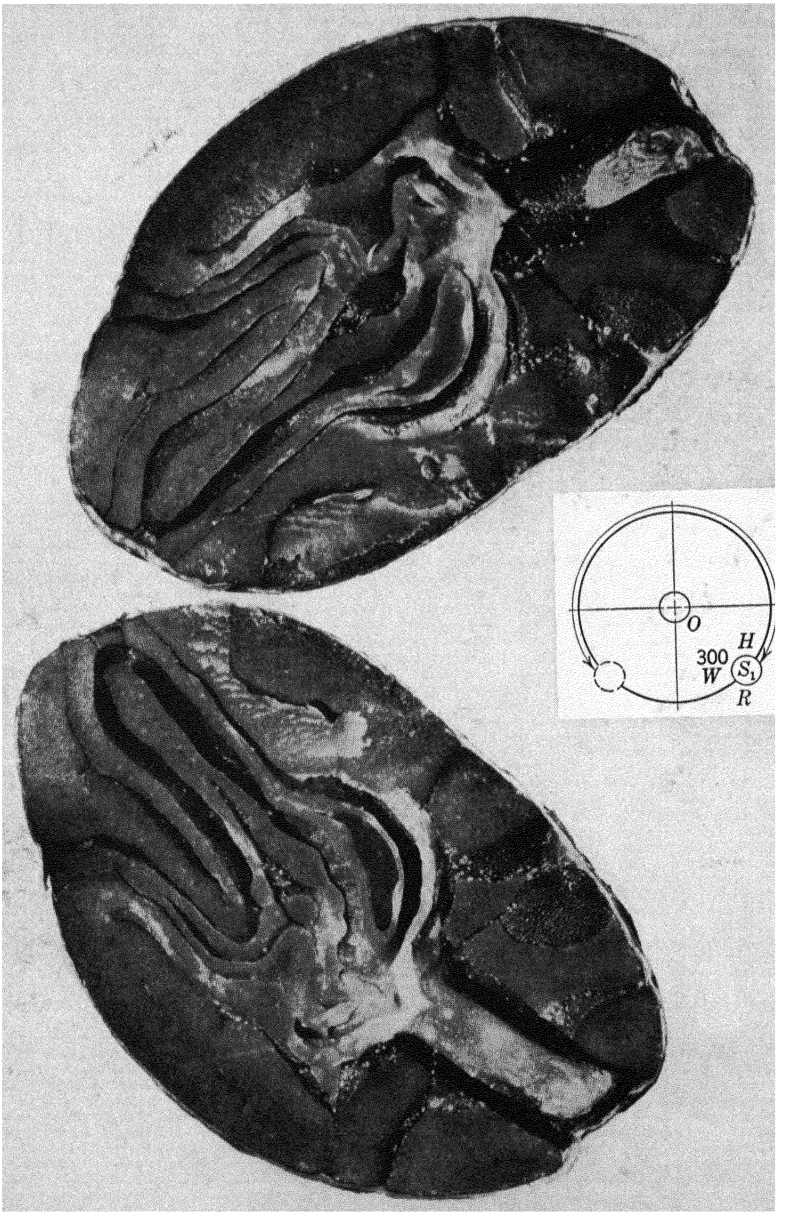


FIG. 224. Split cacao bean ×2.5. Zeiss 120-mm mikrotar objective; photoflood lamp moved about specimen as indicated by arrow in diagram. Wratten filter No. 25; Eastman Commercial Pan film; developer, D-76.

A metal part with a riveted joint to be examined can probably be cut longitudinally with a saw, or it may be filed or ground on a coarse wheel. The entire length of short rivets can be photographed, but long rivets may require two or more photographs to show the true condition of the joint. In grinding a section, the surface should be carefully watched to avoid carrying the grinding process too far; otherwise the rivet may drop out of position after a portion of it has been ground away. When most of the unwanted metal has been removed with the coarse wheel, the surface should be smoothed with a fine wheel and then rubbed by hand over a flat plate covered with fine emery paper.

Emery papers, fine files, and coarse- and fine-grit grinding wheels are essential in the preparation of metal surfaces for photomicrography. The grades of emery paper are listed according to the size of the grit. In the Behr-Manning line, No. 1G, ½, 0, 00, 000, and 0000 are good sizes to have on hand. The last four or five sizes are used only for polishing. In preparing large metal areas for photography, a highly polished surface, as a rule, is neither needed nor desirable. The final operation can generally be attained with the No. 1G cloth or for finer work with the No. ½. Nothing finer than No. ½ will be needed unless the specimen is to be polished and etched, and photographed at a magnification much higher than is being considered at this time.

After the grinding operation is finished, the specimen should be well washed to free it from all gritty particles. A small piece of emery cloth of appropriate size is then placed on a flat surface, such as a heavy piece of plate glass, and the specimen is rubbed across it until all the heavy grinding marks have been removed. After the specimen has been washed again to remove the final powder and dust, it is ready for photography.

When a fracture or a cross section of a rivet or joint is to be shown, or when a photomicrograph is to be taken of a piece of metal so large that it will fill the whole field of view, the principal precaution in mounting is to see that the upper surface of the specimen lies at an angle of 90° to the microscope axis. After it has been so mounted, the photography proceeds as usual. If the specimen is a joint from a can or other thin piece of metal, it should be trimmed so that the thickness is reduced to less than ⅛ inch. Then it can be placed on a slide and illuminated from below. Thus the surrounding field will be bright, and the outlines of the metal will show clearly.

Sometimes it may be required to show, in a soldered joint, just where the flow of solder has stopped. Recourse may then be had to etching. On can joints, a dilute solution of hydrochloric acid

is often useful. The joint is immersed for only a few seconds, after which the specimen is rinsed in running water. Treatment will differ, however, with the various metals used in the joints. Nitric acid may be tried on copper rivets.

Wooden joints which have been glued can be sawed and smoothed with fine sandpaper. All dust from the sanding should be carefully cleaned away with a stiff brush before the photograph is made. If sanding seems objectionable, a good surface may be obtained by planing, or a small specimen can be held in the hand and pulled against the knife in the bottom of the plane.

Figure 225 illustrates a can joint; Fig. 226 a rivet joint; and Fig. 227 a wood joint. The rivet joint shows the head and the peaned end of the rivet; these will show only if the rivet is short.

Sec. 152. Petri Dishes. A standard method of photographing Petri dishes is easily worked out. The standard size dish is 100 mm in diameter. If one is photographed on a scale 1 : 1, the image will fall within a 5 by 7 inch film. A 7- or 8-inch lens will do nicely for this work. From equation 43, putting s equal to s', it is seen that the value for the object distance must be twice the focal length of the lens when a magnification of unity is required. This establishes the anti-points mentioned in Sec. 48. Thus, the object and image lie at the anti-points of the lens. A lens of at least 5-inch focal length is necessary, and longer is desirable, because otherwise the diameter of the field may not be great enough to embrace the diameter of the dish with a little additional room to spare.

Since any mold or bacteria colonies that may be on the dish will, in general, be whitish, transmitted light is neither necessary nor desirable. Figure 228 illustrates how such a picture may appear. To obtain a perfectly black background it is essential to mount the dish on a glass plate which has been raised a few inches from its support; a printing box answers this purpose very well. If other means are used to support the plate, it is advisable to place a piece of black paper beneath, and a few inches away from, the glass supporting plate. In this position the black paper will be so far out of focus that its structure will not register on the negative. Extraneous light is excluded by the shields. As shown in the diagram, illumination was obtained with one photoflood lamp.

For stationary lighting two lamps should be sufficient, for the area to be covered is so large that the accessory lenses generally used with this sort of illumination are not needed, or, if used, they should be in the first position as described in Sec. 24. Without an accessory lens, sufficient brilliance on the plate can be obtained by means of

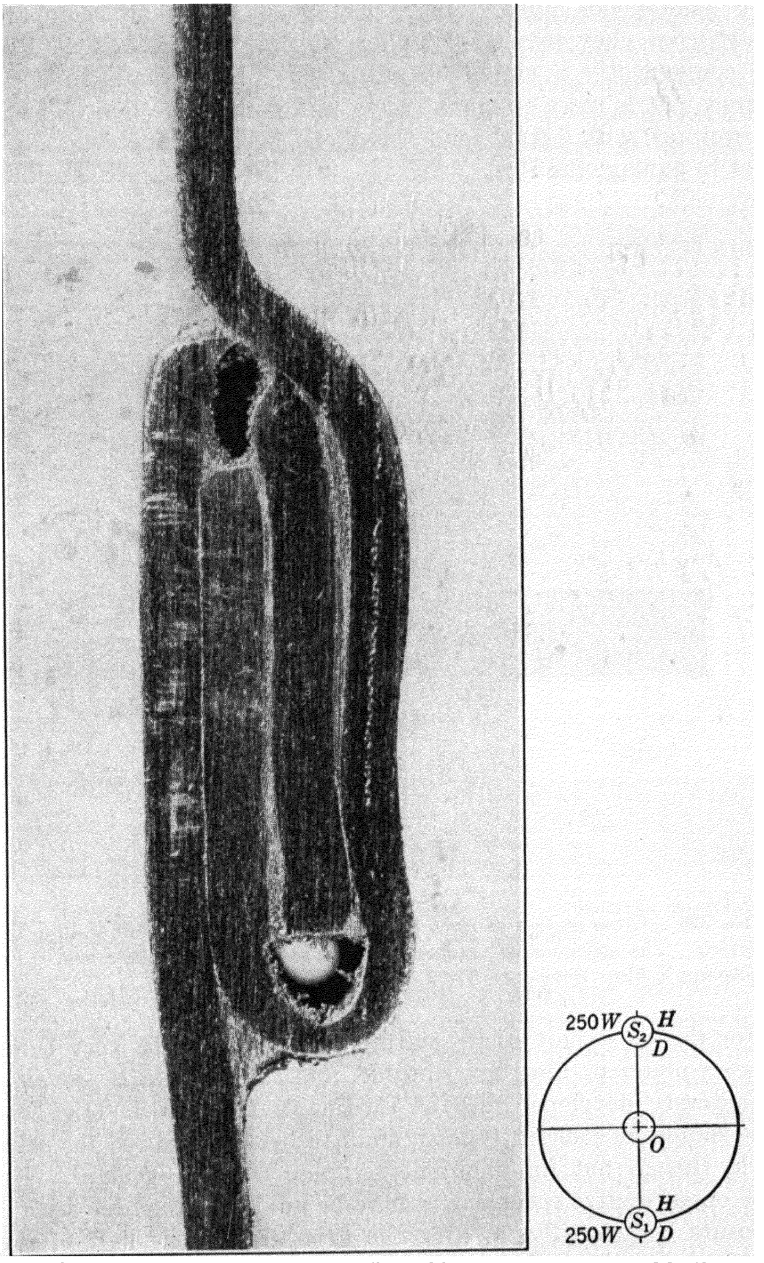


FIG. 225. Cross section of can joint ×25. Leitz 24-mm micro-summar objective. The specimen was illuminated with a 100-watt lamp for transmitted light; a long-focus condenser was used. It was necessary to etch the specimen with 1:1 hydrochloric acid. Eastman Process Pan film; developer, D-19.

two photoflood bulbs. Since photoflood bulbs are comparatively short-lived, they should not be left burning unnecessarily. They can be conveniently mounted on either side of the plate, in desk lamp stands. It is risky to mount them in a microscope lamp house which is equipped with a good condensing lens, because they generate enough heat to damage the lens.

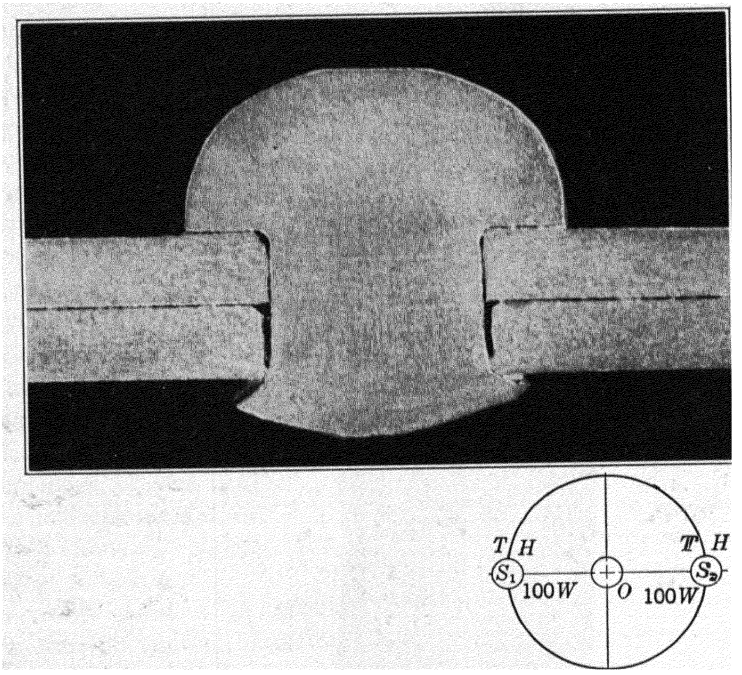


FIG. 226. Cross section of rivet joint ×5. Bausch and Lomb 48-mm Tessar Ic objective. The specimen was etched with a 1:1 solution of nitric acid. Eastman Panatomic X film; developer, D-19.

For the most part, plate culture pictures must be very contrasty. Process plates or films are suitable, and they should be developed in a contrasty developer such as Eastman's D-19 or D-11. Exposure time should be sufficiently long, and can be standardized for petri-dish work, though not for photomicrographic work in general. To save time on successive specimens it may be advisable to check with a trial exposure occasionally, making the exposure on one half of the film double that on the other half. Unless trials are made, the very small colonies of bacteria may be lost.

Highlights are very objectionable because they may easily be con-

fused with the subject itself. The bacteria may grow well up on the sides and the rim of the dish, or they may lie at the bottom in peculiar formation, so that after the picture is made it may be difficult to distinguish between bacteria and highlights unless all due precautions have been taken. By careful spacing and arrangement of the lamps,

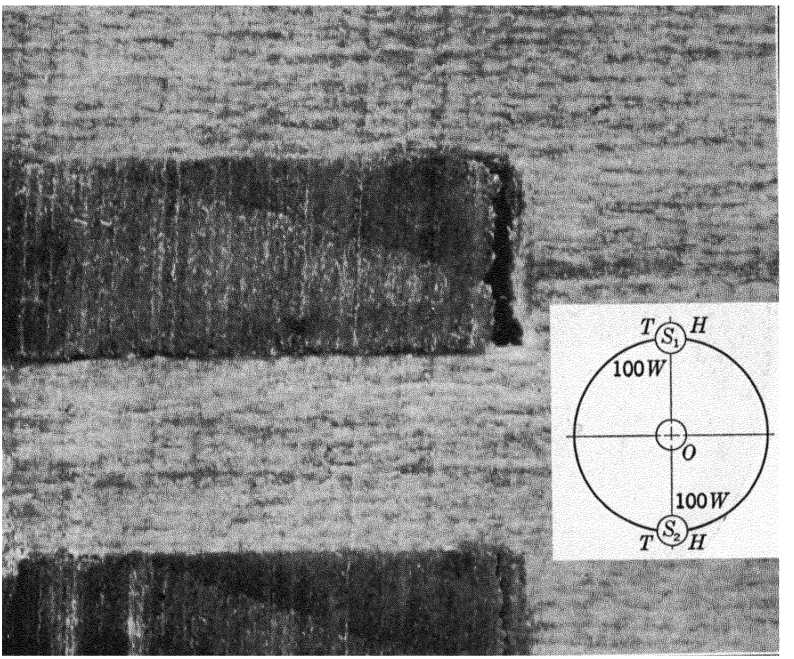


FIG. 227. Cross section of glued wooden joint ×12. Objective, Bausch and Lomb 48-mm Tessar Ic. Eastman Panatomic X film; developer, D-61a.

highlights can be reduced to a minimum. A thorough study of the image in the ground glass of the camera is an essential preliminary step.

If good pictures are to be obtained, all covers must be removed from Petri dishes before they are photographed. Usually, this is perfectly safe. If it should be inadvisable to remove the cover, a dish with a plano cover should be used. Such dishes can be had at a special price. Whether or not the bottom of the dish is plano, the under side should be oiled to the supporting plate with paraffin oil to help eliminate the last vestige of glare due to reflections at the two surfaces.

Filters will make but little difference; with panchromatic sensitive material they will tend to decrease the light intensity and make a longer exposure necessary. Resolution is not a vital factor in a pic-

ture of this type. The fine tracery of a mold pattern is easily brought out under almost any optical condition that is likely to exist. The picture in Fig. 228, taken without the benefit of a filter, shows both bacteria and mold colonies clearly and with good contrast.

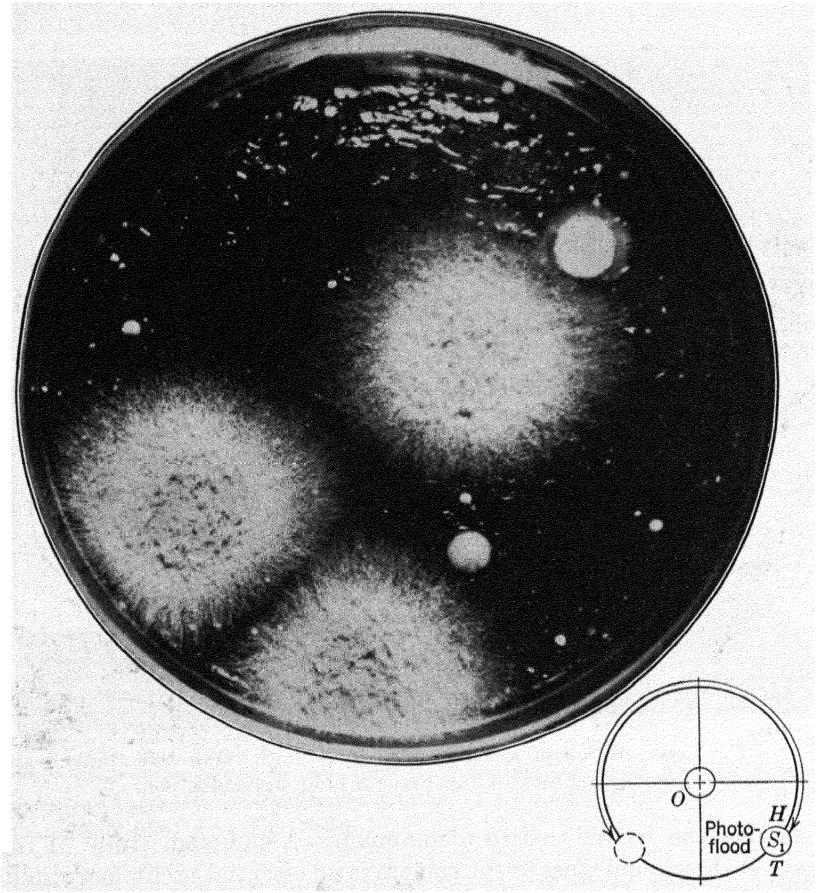


FIG. 228. Petri dish culture ×1. Goerz Dagor 8-inch objective. Light filters are generally unnecessary in a picture of this sort; however, a light blue or daylight filter may occasionally be used to advantage. Eastman Process Pan film; developer, D-11.

Sec. 153. The Use of Infrared to Show Differences in Material. Figure 229 shows a photomicrograph of writing. The words "was" and "took" have about the same appearance as far as blackness is concerned. Figure 230, taken with an infrared film, brought out a great difference between the two inks in which the words were written.

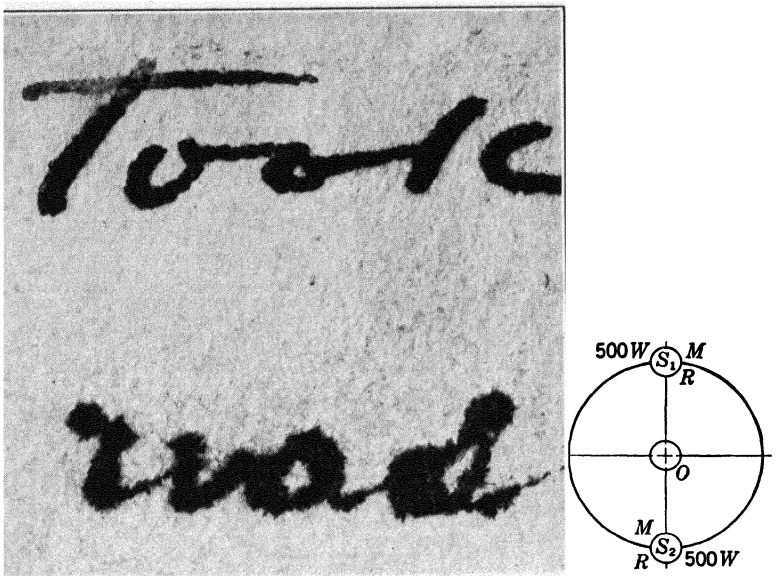


FIG. 229. Handwriting ×4. Bausch and Lomb 5-inch rectilinear lens. The picture was taken using panchromatic film. The word " was " seems to be written with the same ink as the word " took." Eastman Panatomic X film; developer, D-61a. Through the courtesy of Mr. A. Tennyson Beals.

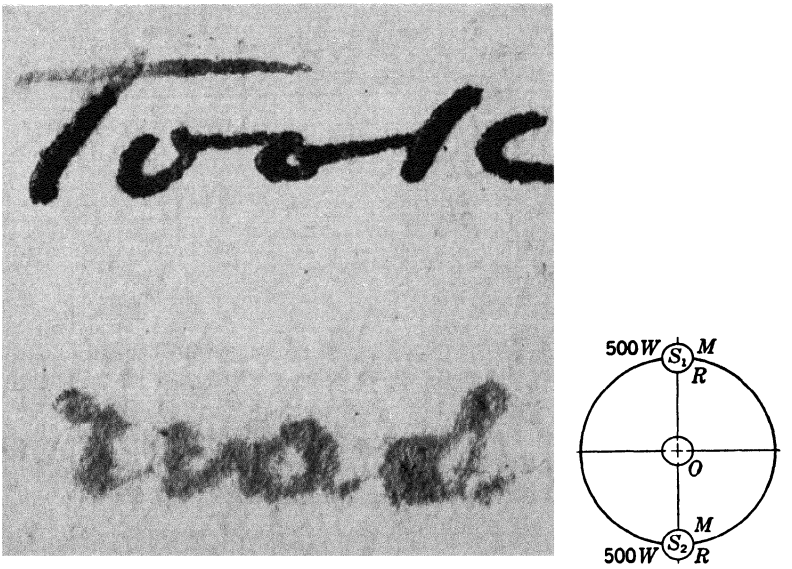


FIG. 230. Handwriting ×4. The same as Fig. 229 except that Wratten filter No. 25 was used in conjunction with Eastman infrared film. The word " was " is shown to be written with a different ink from that used to write the word " took." Through the courtesy of Mr. A. Tennyson Beals.

When using Wratten filter 25, considerable visible light was present, but filter 87 was not required. Infrared film permitted rapid registration of "was" and showed that the word was written in ink which reflected infrared radiation strongly; it also indicated that the word



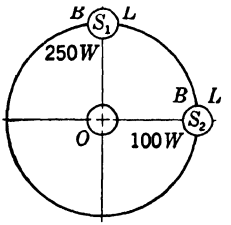
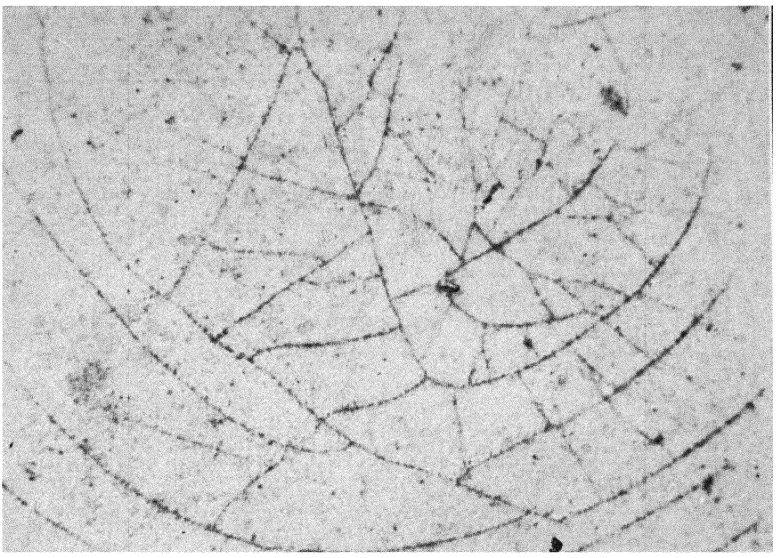


FIG. 231. Toilet tissue ×20. Objective, Leitz 24-mm micro-summar. The very low lighting stressed the texture of the specimen. Eastman Panatomic X film; developer, D-19.

"took" was written in a different ink. As a rule, infrared radiation is used to stress detail which otherwise would be invisible, but it is equally valuable in determining such differences as shown in Figs. 229 and 230. It might be noted that the results obtained by using infrared film without a filter, to exclude all light of shorter wavelength than the red, will be very similar to those obtained by using a panchromatic film with ordinary lighting. A strong red filter is required

with an infrared emulsion in order to show the details which only infrared radiation can register.

Sec. 154. The Use of Lighting to Stress Surface Textures. Figure 231 shows a photomicrograph of paper. The illumination was arranged to graze the surface — the incidence is greater than 80° be-



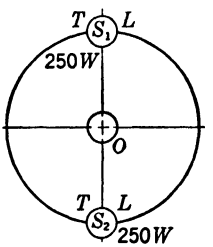


FIG. 232. Paint film showing cracks ×12. Low lighting is generally required on subjects similar to this. Bausch and Lomb 48-mm Tessar Ic. Eastman Contrast Process Pan film.

cause, if the angle of the incident light is less than this, the picture will appear flat and so lose detail.

Materials other than paper can be photographed with light at grazing incidence, but the effect is more striking with material having a moderately rough surface. If the surface is perfectly smooth, the field will, of course, appear black; if the surface is very rough, as some leather surfaces may be, the angle of the light will be too great and the shadows will seriously interfere.

Paint films are a case in point. When minute cracks are to be demonstrated the angle of the light plays a very important part. Figure 232 is an example of lighting on such a surface. In the photographing of painted surfaces, filters play an important part, and, as usual, they must be selected largely by the trial-and-error method. Many red

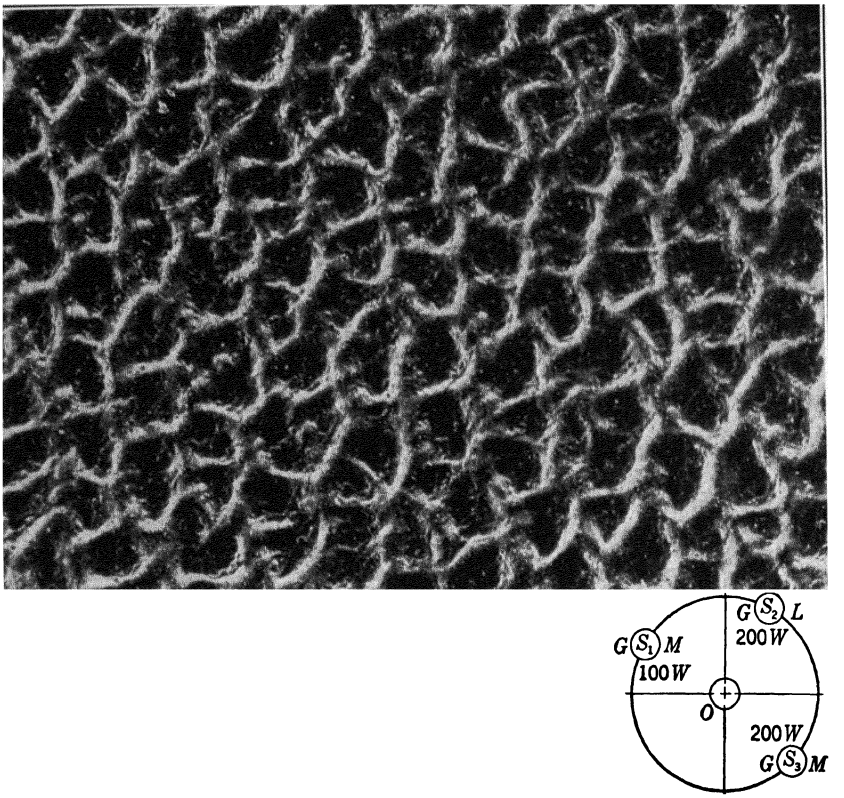


FIG. 233. **Leather surface** ×10. Bausch and Lomb 48-mm Tessar Ic. Filters may be needed on subjects of this sort which are highly colored. As this was a green leather, a green filter was used. Eastman Panatomic X film; developer, D-11.

paints transmit a large amount of blue light which passes unnoticed because of the preponderance of red, but the relative visibility of both may be about the same. The possible effect of filters which transmit a narrow band of the spectrum may therefore be difficult to judge, but a good estimate can usually be made after filters of the three primary colors, red, blue, and green, have been tried.

Leather can be fastened to a slide with cement to ensure a flat

surface, or it can be held in place by a heavy metal ring. Figure 233 shows a leather surface illuminated by three lights arranged as shown in the inset.

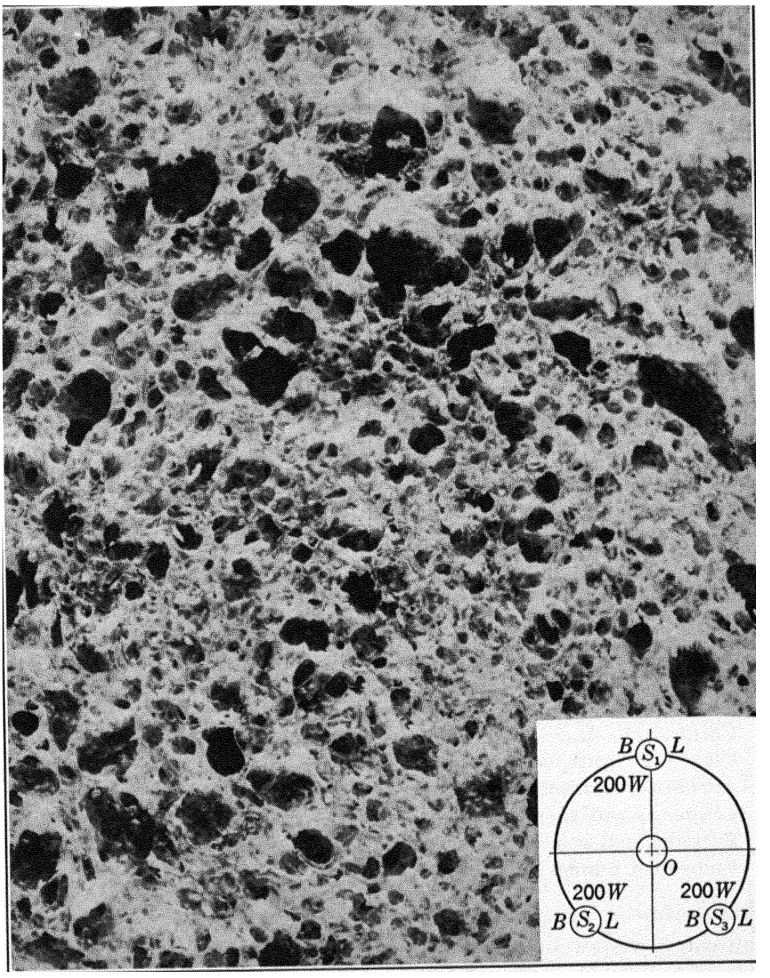


FIG. 234. Bread sliced for table ×5.9. Objective, Zeiss 95-mm Protar. An example of low lighting. Eastman Commercial Pan film; developer, D-11.

A section of bread as sliced for the table is shown in Fig. 234. The illumination was by lights placed at a very small angle.

Regular, closely spaced surface structure of a periodic nature may produce color effects which can be photographed at low magnification, but the detail of such surface structure, if it can be photographed

at all, will require a high magnification. Pits in a specimen may be difficult to distinguish from elevations. The best way to deal with them is to illuminate the specimen from one side and then study the shadows. When a pit is indicated, the shadow will appear in the image on the side opposite to the lamp. This same system can be used with colored lights on opposite sides of the specimen. Thus, if a pit is illuminated with red and blue light from lamps placed 180° apart, the blue side of the pit will show in the field of view as on the same side as the lamp giving a blue light, whereas for an elevation the blue side will show in the field of view as on the side away from the blue lamp.

GROUP II

POWDERS, PIGMENTS, AND SMALL DISCRETE PARTICLES

Sec. 155. Description of Material and Procedure for Photographing. This group comprises a large class of material in powder form. All the members are distinguished by their existence as discrete particles on the microscope slide, each particle being a distinct and separate photographic entity which must be treated as such. Bacterial smears, blood specimens, and similar material are included in this group. The magnifications required are in excess of 50; otherwise these specimens would be placed in Group I.

EXAMPLES

Pigments, fillers, and extenders, Sec. 156 et seq.
Foodstuffs such as starches, sugars, flours, stock feed, ground spices, vegetable drugs, etc., Sec. 157 et seq.
Cosmetics — rouges, face powders, detergents, etc., Sec. 158.
Clays — cements, ceramic materials, filter aids, Sec. 159.
Abrasives — Carborundum, emery powder, polishing powder, etc., Sec. 160.
Chemicals and chemical drugs in powder form, precipitates, etc., Sec. 161.
Bacterial and other smears, Sec. 162.
Embedded powder grains, Sec. 163.

Illumination. The illumination for this group is almost universally by substage, either bright-field or dark-field. For low and medium powers either Method I or Method III is suitable. For critical work demanding the utmost in resolution, Method II or Method III is required.

Filters are a great aid in bright-field illumination because colored subjects lend themselves well to the stimulation of contrast by such means. In colorless specimens resolution is enhanced by filters, which, with achromatic lenses, serve also as corrective modifiers of the light.

Apparatus. Since magnification may vary from 50 to several

thousand times, it is obvious that work in this group may demand the whole gamut of equipment available for bright-field and dark-field work. At high magnifications, the better the lenses the better will be the picture. At low and medium powers, covering 3¼ by 4¼ inch film, achromatic optics will give good results. The performance of 2-mm and 1.5-mm objectives will vary with the make and with the individual lens. Attention has already been directed to this in Sec. 65. Certainly, if large fields are to be covered with these short-focus lenses, the lenses must be of apochromatic quality. The ocular should be of the amplifying type, but if the objective is of the Spencer make, a good field which is large and flat can be obtained with the Spencer compensating ocular, as Fig. 249 shows. On some materials, to be selected by experience, better images will be obtained with a low aperture of the objective. To attain this low aperture with high-power objectives, the Davis diaphragm is more effective than any adjustment that can be made by the condenser diaphragm alone. Since a high aperture is required for the ultimate in resolution, the N.A. 1.4, 2-mm lens is a "must." However, it should be ascertained by a most careful comparison with the effects of a lens of 1.3 aperture whether or not the superior qualities of the higher-aperture lens are actually contributing as they should toward the making of a better picture. It is generally possible to form a correct opinion by visual examination with a rather high ocular, such as 15× or 20×, or by a critical examination of the image at the focal plane of the camera.

A camera with long bellows extension is practically a necessity; without it, high magnifications will be unattainable. Eyepiece cameras will be suitable for making records if small pictures can be used without enlargement.

General Procedure. In mounting material included in Group II, the aim will be to show each particle distinctly, separately, well defined, and with all the detail that may be required. Depth of field is important when handling powders which vary in size, but it is of less importance in dealing with blood smears and most bacterial preparations. Liquids of high refractive index are useful for increasing visibility and apparent field depth.

Many foodstuffs are conveniently mounted in glycerol and water or in monomethyl glycol. To obtain and maintain particle separation, pure glycerol or a heavy inert oil like paraffin is often advantageous. High-viscosity paraffin oil is useful because, after distribution is once attained, the particles are not likely to gather together in clumps. It is worth while first to examine the specimen in air and then to observe the changes that occur by the addition of a

mounting liquid. Often the difference in effect caused by changes in the refractive index may suggest a suitable liquid.

Many variations in technique are required in handling the subjects in this group. Regarding foodstuffs, and to a lesser extent pigments and ceramic materials, considerable information can be had from the literature. Trade and technical journals on special subjects are very helpful and should be consulted freely. Papers that have been read before trade and scientific societies often contain last-minute information of vital interest to those who are engaged in work on the particular subject dealt with. Secretaries are always willing to furnish a copy of papers read before their societies or to give information as to how they can be obtained. Handling of specific kinds of material is discussed in detail in the succeeding sections.

Sec. 156. Pigments. *Mounting and Photographing Fine Pigments.* The microscopy of fine pigments, especially typical of Group II, has been discussed by many.[1] The method of Green[2] is exceptionally efficient for small-sized pigments, particularly if the mounts are to be photographed. In this method a small drop of redistilled turpentine is placed near the center of a microscope slide, a very small amount of pigment is added, and the whole is rubbed into a thin layer with a glass rod moved slowly and with a circular motion from one end of the slide to the other. This compound motion of the rod will cause a greater concentration of pigment at some points than at others so that a thin layer of the pigment is obtained in varying degrees of concentration. The rubbing motion should be repeated until the turpentine has started to dry, in order to prevent the pigment from forming flocculi.

[1] The following references deal with the mounting and photographing of pigments:

G. Fearnley, "Microscopy in the Paint Industry," *Chem. Met. Eng.*, **20**, 38, 1936.

Henry Gardner, *Physical and Chemical Examination of Paints, Varnishes, Lacquers and Colors*, ninth edition, 1939, and other editions.

Hans Wagner, *Z. angew. Chem.*, **44**, 665, 1931.

C. H. Butcher, "The Microscopical Character of White Pigments and Extenders," *Ind. Chem.*, **2**, 400, 1926.

A. M. Munro, "The Photomicrography of Pigments," *Chem. Eng. Mining Rev.*, **19**, 36–41, 1926.

New Jersey Zinc Company, "The Microscopy of Paint and Rubber Pigments," *Research Bulletin.*

[2] H. Green, "A Photomicrographic Method for the Determination of Particle-Size of Paint and Rubber Pigments," *J. Franklin Inst.*, **192**, 637, 1921; "Application of Microscopy to Study of Pigments," *Paint, Oil and Chemical Rev.*, **83**, Nos. 10, 15, 21, 25, 1927, **84**, 1, 1928; "Microscopy of Paint and Rubber Pigments," *Chem. Met. Eng.*, **28**, 53, 1923; *Ind. Eng. Chem.*, **16**, 677, 1924.

Modifications of the above method consist of varying the vehicle to carry the pigment. A weak solution of celluloid, or, if the particles are very small, amyl acetate, either in its pure form or with ½ to 2 per cent parlodion[3] added, may be used. The smaller the particle to be dispersed, the smaller should be the percentage of the adhesive component in the solution.

After the dispersing liquid has dried, gentle warming of the slide will aid materially in fixing the pigment to the slide, particularly if the turpentine method has been followed. Immediately the slide has dried, and before the cover glass is in place, the slide can be examined with a low-power objective. This examination will indicate whether or not the pigment has been satisfactorily dispersed. If not, and if it must be redispersed, the appropriate solvent can be added to flood the slide and loosen the pigment, and then the rubbing process can be repeated. For photographic purposes, the ability to establish a gradient of concentration on the slide makes it possible to select a portion where the field is most representative. Care must be observed to avoid a field composed entirely of small particles or one which includes too many large particles.

To examine the pigment mount with magnifications higher than the 16-mm objective the specimen must be covered unless the objective is corrected for use without a cover. The covered mount indicates the use of a mounting medium to avoid glare and for other reasons, but unless the proper medium is selected, much of the pigment material may not be visible. If the index of refraction of the pigment is unknown, a mount can be made with a liquid of intermediate refractive index in the center of the slide, and another mount on each side with a liquid of higher and lower index respectively. A comparison of these three mounts should make it possible to determine quickly the index which will be required for the best visibility. The correct choice of a mounting medium also will help to make dark-colored pigments more transparent.

Pigment of small particle size and of low or intermediate refractive index is very difficult to photograph. If the mounting medium is air, the best objectives will be the 4-mm or the 3-mm dry, corrected for use without a cover glass. If a cover glass is used without a mounting liquid, the objective can be of the oil-immersion type, but this arrangement is likely to be unsatisfactory because the glare produced by the cover on a dry mount is usually very objectionable and the high aperture of the objective is not attainable. An alternative is to

[3] Parlodion is obtainable from laboratory supply houses or from E. I. du Pont de Nemours and Company, Parlin, N. J.

mount such pigment in one of the melts as described in Sec. 129 or in the phosphorus-sulphur-methylene iodide compound also mentioned or in selenium monobromide. When the melts or selenium monobromide are used it should be remembered that the effectiveness of the blue light will be partly lost because of the light-absorption characteristics of the melt; therefore photomicrographic recording of a pigment mounted in this way may be unsatisfactory. However, very small size pigment may have a certain opaqueness which will permit the use of liquids of decidedly lower refractive index. Advantage can also be taken of the chromatic quality of the images. Suppose the particles to have a reddish tinge, or to show absorption of blue light; a white mounting medium and a blue or green filter, with an orthochromatic or regular plate, will give the specimen good contrast for resolution under the best of conditions. Blue or strong green light is advised for high magnifications. The resolution required for pigments is high, and for sharp images short wavelengths are necessary. With a filament lamp the intensity of the blue light may be low, particularly if a narrow band in the extreme blue, such as that given by the Wratten filters 45 plus 47, is used. A mercury lamp or a high-wattage projection lamp is recommended. For the photomicrograph of zinc oxide shown in Fig. 235, Wratten filters 45 plus 47 were used, and the illuminant was the 6-volt 108-watt ribbon filament lamp, the predominant wavelengths passed by the filters being from 440 to 480 mμ with a maximum transmission of about 10 per cent at 460 mμ.[4]

Occasionally it is required to photograph pigments in the vehicle in which they are used in order to show how they flocculate. The flocculi may be large enough to be easily discernible. Gardner shows examples of such pigments in various vehicles. When the pigment is carbon black no great difficulty need be anticipated. Some of the sample can be dropped on the microscope slide, covered, and examined. It may be diluted with an appropriate thinner. If there is objectionable motion, the slide can be set aside for a while to give some of the particles an opportunity to settle out; otherwise the picture must be taken with the particles in motion. A high-intensity light source with a fast exposure and fast plate will be needed, or a fast exposure at low magnification can be made and subsequently enlarged. A combination of these two methods might be advisable. Motion may be reduced somewhat if the vehicle can be made more viscid with some heavy oil such as the No. 32 Standard Viscous Oil of the Standard Oil Company. The refractive index of this oil is 1.456.

If pigment particles are so small as to approach the limit of resolu-

[4] Eastman, *Wratten Light Filters*, sixteenth edition, p. 65, 1940.

tion, or if they are of low visibility, it may be necessary to photograph them by dark-field illumination in order to register all the small ones. When counts are to be made this method is particularly helpful in obtaining registration of all particles. If the particles are of various

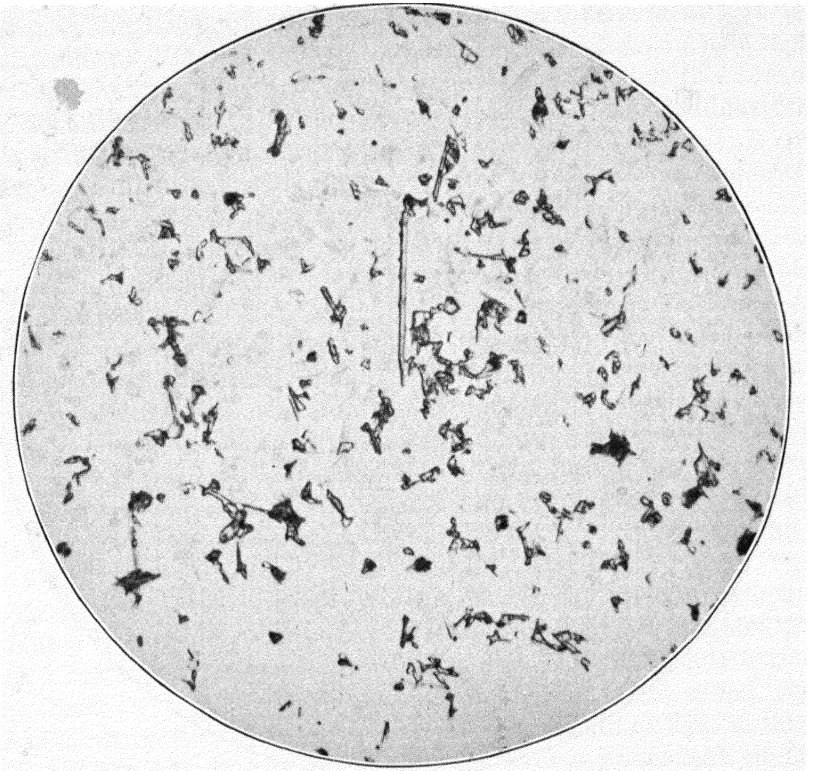


FIG. 235. Acicular zinc oxide ×1000. The specimen was dispersed in pure amyl acetate and mounted in ethylene glycol. Blue filters necessitated a long development time to give the required contrast. Objective, Zeiss 4-mm apo.; ocular, Homal III; condenser, Leitz achromatic-aplanatic, 4/5 cone; illumination, 108-watt tungsten-ribbon-filament lamp, method II; filters, Wratten No. 45 plus 47; Eastman Kodatron film; developer, D-19, 9 minutes.

shapes, and their form has a bearing on the usefulness of the pigment, a very high magnification is demanded in the photomicrograph. Some of the oxides may be mentioned in this connection. Zinc oxide, shown in Fig. 235, is made by a process to produce long acicular crystals. Titanium dioxide, ×3500, shown in Fig. 236, illustrates the value of high magnification. For this picture the illumination was nearly monochromatic to ensure the best results, and the H3 mercury-vapor

discharge tube was used with Wratten filters 45 plus 47. For such work apochromatic objectives are a necessity. A magnification of 3500 would seem to be stretching the limits of good imagery of any objective, but if the object field is flat, and the subject lends itself to

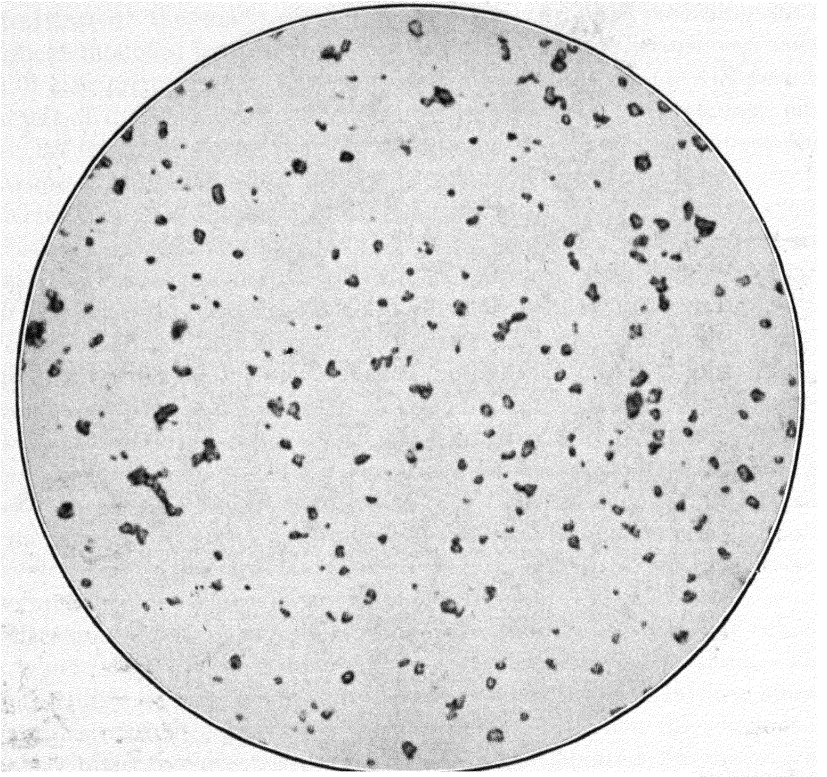


FIG. 256. Titanium dioxide ×3500. The specimen was dispersed on slide in pure amyl acetate. No cover, immersion oil mount. The refractive index of titanium dioxide is given as 2.54. At this high magnification the actual diameter of the field as shown in the picture is only about 0.028 mm. No crystal formation is evident. Objective, Zeiss 1.5-mm apo.; ocular, Homal IV; condenser, Leitz achromatic-aplanatic, 4/5 cone; illumination, General Electric mercury vapor discharge tube H3, method II; filters, Wratten No. 45 plus 47; Eastman Kodatron film; developer, D-19.

the requirements of high-power lenses, fair results may be obtained. Many of the resolved particles shown in this picture are less than 0.3 μ in diameter. It is interesting to note in this picture that the actual diameter of the field is only about 0.028 mm. For such a picture the subject should have good contrast, and it must be small and thin.

The difficulties of field depth are considerably enhanced in high-power work, and since an objective with N.A. of 1.3 has very little field depth, which is at a minimum with strong blue light, it is apparent that the object field must be particularly suited and well prepared for such work. Separation of discrete particles is increased to such an extent by high magnification that the field is likely to appear but poorly filled. However, no attempt should be made to include more particles than would appear normally for work at magnification of, say, 1000 times the N.A. of the objective, because to do so will increase glare and give a poor effect to a high-power picture.

Pigments Mounted in Media of High Refractive Index. Any of the melts can be used to advantage for mounting very fine pigments of medium or low index and for material such as finely ground mica where the individual flakes may be extremely thin. For white pigment, the red color of the melt will not be objectionable, unless for physiological reasons during a visual examination, and good photomicrographs can be made under such conditions if the particles are not too small. Of course, resolution will be reduced, but this disadvantage will be more than offset by the increased visibility of the particles. However, if the melts are used according to the method described by Hanna, Sec. 130, that is, by vacuum distillation, then much of the color effect will be avoided by reason of the thinness of the film.

At *A* in Fig. 197 is shown the appearance of calcium carbonate mounted in Canada balsam, and at *B* the same subject mounted first with a coating of realgar, and then with a coating of Canada balsam. The thickness of the deposed film is a factor for consideration, since for best results it must be more than one wavelength of light in thickness. If the film is only a little thicker than a wavelength of blue light, a strong blue filter may increase visibility, because the wavelength of the transmitted light will have about the same value as the thickness of the film, or possibly it will be a little less, as it should be.

Figure 237 shows a sample of finely ground mica mounted in selenium monobromide.

The above techniques can be applied to many subjects, particularly to very small particles or to very thin plate-like particles, of which the ground mica is an example.

Mounting and Photographing Coarse Pigment and Extenders. Work with pigment having great variation in particle size always presents a problem. A balance must be found between suitable resolving power and magnification. It is a simple matter to use a low magnification and thus to show a field well focused, but this pro-

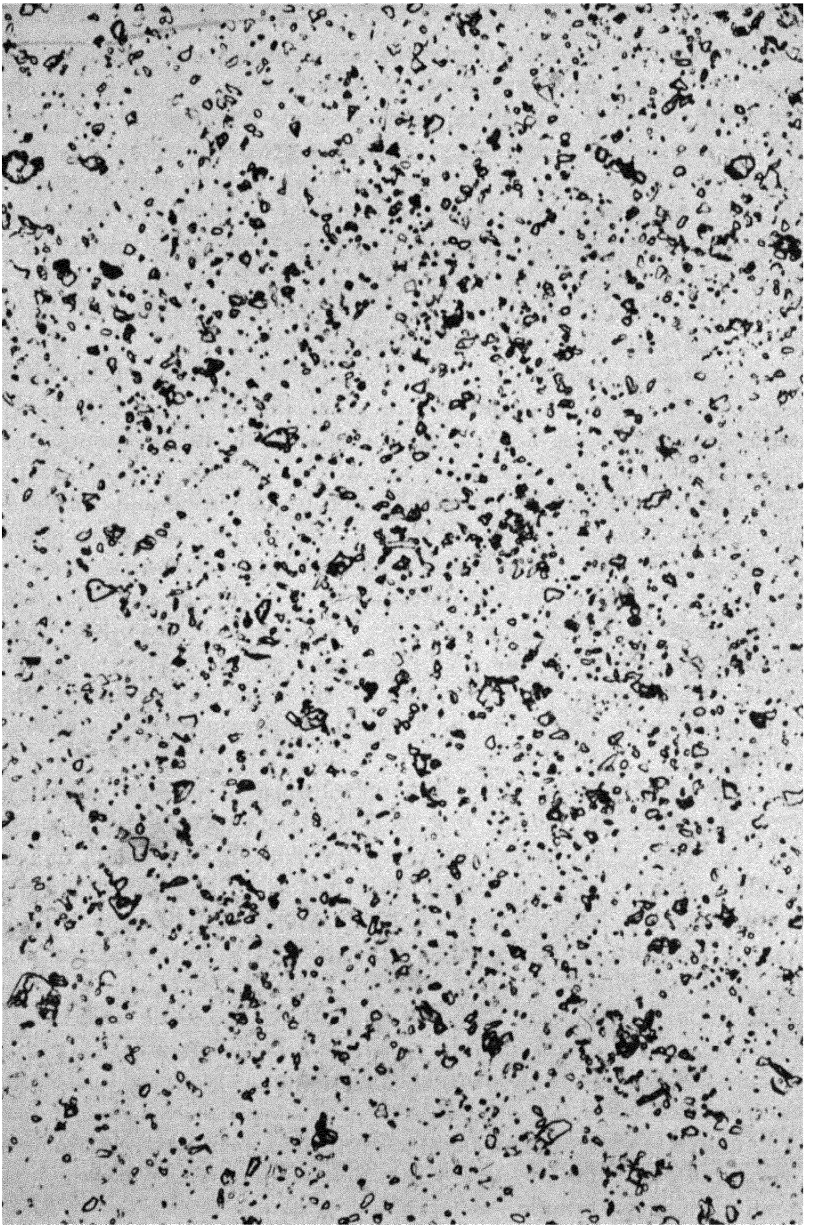


FIG. 237. Finely ground mica ×360. The mount was made in selenium monobromide. The size and shape of even the smallest particles are clear and distinct. Objective, Leitz 16-mm apo.; ocular, Homal I; condenser, Leitz achromatic-aplanatic with top element removed, 9/10 cone; illumination, 250-watt projection lamp, method III; no filters; Eastman Commercial Pan film, developer, D-19

cedure is likely to over-emphasize large particles and under-emphasize smaller ones. With material such as barytes, composed of both large and small particles, trouble lies in obtaining sufficient depth of field to have both sizes sharply focused simultaneously. The mounting medium selected should have as high a refractive index as is consistent with good imagery. With small oxide pigments the object field is nearly flat, but with the larger pigments it is very far from flat. If a particle about 40 μ in diameter, and generally spherical in shape, is mounted in air and photographed with the 16-mm objective, by equation 49 the depth of field for an objective with a N.A. 0.3 will be about 5.9 μ. If the condenser is stopped down to give a working aperture of 0.25, the computed field depth will still be only 8.5 μ. If the index of the mounting medium is increased from 1 to 1.74, and the same formula is applied, the field depth will be more, or, to be exact, it will be 11.6 when the N.A. is 0.25. If equation 49 is solved for N.A. when the field depth is given as 40 μ, it will be found that the N.A. required is 0.115 when the material is mounted in air. The 30-mm achromatic objective with a N.A. of about 0.1 will be found to have a field depth of approximately 55 μ, which will be sufficient for the material in question.

When mounting a powder in which particle size varies greatly, one must be careful that the small and large particles are not segregated by the glass rod or spatula with which they are dispersed. If the particles can be felt under the rod and impart a gritty sensation, it is advisable to distribute them with a very flexible spatula. The spatula can be held nearly flat so that the individual particles instead of being pushed about will be carried along the slide by a light rolling motion between the spatula and the glass. The small particles will be distributed by the motion of the liquid accompanying the large particles.

Figure 238 is an example of the photomicrography of particles of greatly varying size. The magnification is low, and the difference between the refractive index of the subject and the mounting medium is not so critical as it would be with small particles alone.

Pigment Photographed by Dark-Field Illumination. Figure 239 illustrates the use of dark-field illumination. The material is "Fish silver" pigment; the particles are very thin and flat and have low visibility. Therefore many of the smaller particles would be lost to view if illumination were by bright field.

A picture taken with dark-field illumination must have sufficient exposure or the smaller particles will not be registered, but the magnification may be rather low even when the particles are very small for

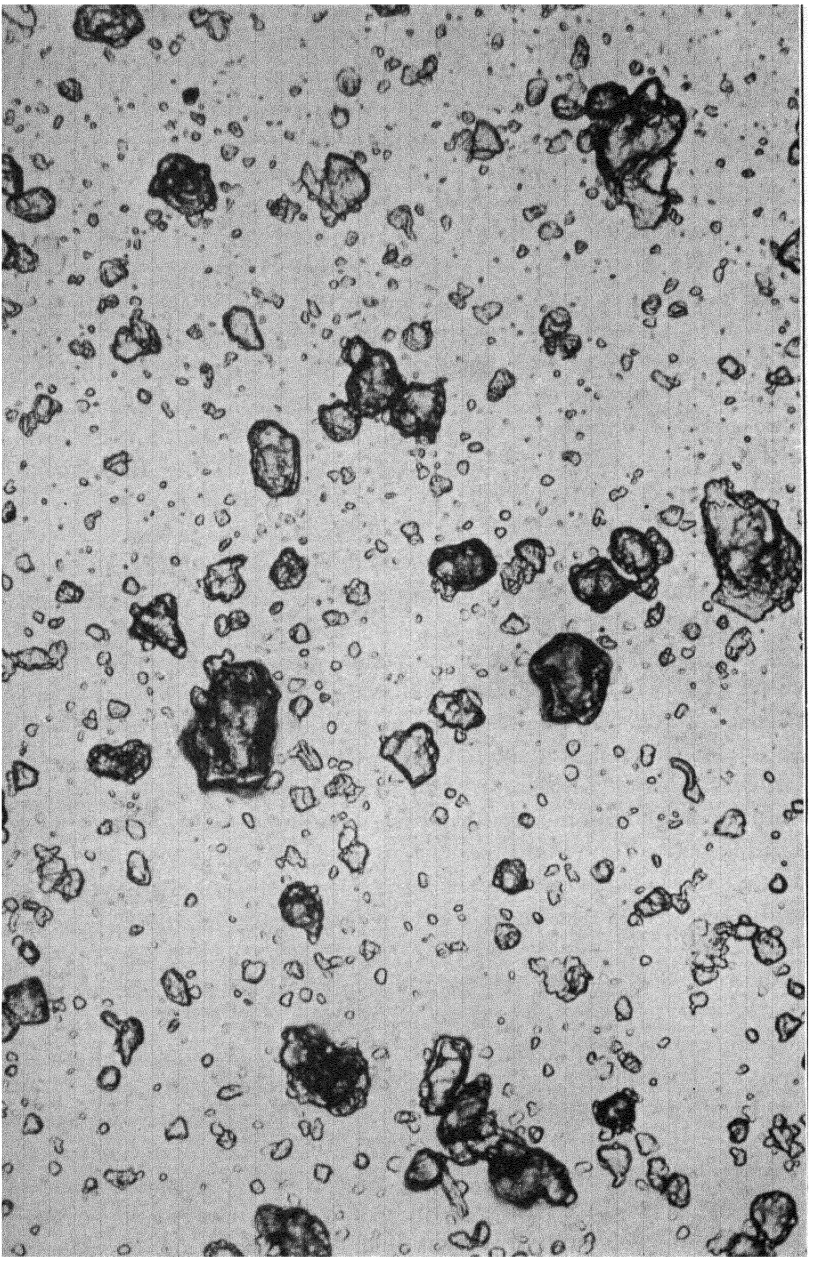


FIG. 238. Barytes ×270. This pigment has an index of 1.637 and so may be mounted in any low-index liquid. Objective, Zeiss 32 mm apo.; ocular, Homal I; condenser, Leitz low-power; illumination, 250-watt projection lamp, method I; filters, Wratten 58 plus Corning 428; Defender, Pentagon film; developer, D-61.

they will have high visibility. A dry mount without a cover glass will tend to make the field contrasty when medium-power dry objectives are selected. For higher magnifications either a cover must be used, or preferably, if conditions warrant, the specimen may be mounted directly in the immersion oil.

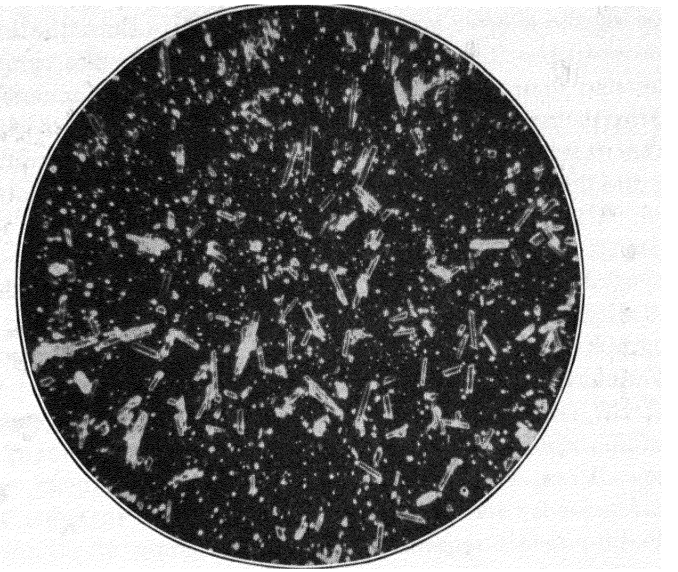


FIG. 239. Fish silver ×208. This pigment, used in lacquers, has a very low visibility even when mounted in air. Dark-field illumination is the easiest means of increasing its naturally low contrast. Objective, Leitz 16-mm apo.; ocular, Winkel-Zeiss 18×; condenser, Leitz achromatic-aplanatic, top element removed and central stop added for dark-field effect; illumination, 400-watt projection lamp, method II; Eastman Proc. film; developer, D-19; Miflex camera.

The arrangement for dark field which consists of a central stop and a condenser of medium focal length is suitable for the 16-mm and lower-power objective. The paraboloid or medium-power dark-field condenser will take care of the intermediate magnifications nicely; the higher-power cardioid condensers will be required with the oil-immersion systems. In photographing pigments, the dark field is chiefly used to form images by directly reflected light and not by diffracted or scattered light which is generally associated with work on colloids. For this reason the strong contrasty images are not so likely to be overpowered by glare from the slide as are images from scattered light only.

Pigments Photographed with Ultraviolet Radiation. The special objectives required for ultraviolet work are described in Sec. 58. Ultra-

violet may be useful on account of its selective effect on different materials, as shown by Sawyer[5] and others. The two components of lithapone can be differentiated by their action on ultraviolet radiation, which will also enhance detail in small particles. Increase in resolution is an important factor in certain classes of pigment work, and added resolution can be obtained only by means of the radiant energy of the shorter wavelengths. However, since the image is invisible until the film has been developed, the results cannot be predicted, and fluorescence of the specimen, slide, or immersion oil will spoil the picture. These may be the reasons why such work has not become more general, for the cost of installing the equipment for using the 3650 A line is small, even when a quartz condenser is included. Ultraviolet may be useful at any magnification; it need not be restricted to fine materials.

Lucas[6] describes the use of ultraviolet radiation in the photography of metals and other materials. He explains his apparatus for photomicrography with quartz lenses and his source of radiation, and he gives numerous examples of work using the 2750 A line.

Sec. 157. The Photography of Foodstuffs and Vegetable Drugs. Foodstuffs which naturally fall into Group II are many. All the starches, flours, ground materials generally, fine sugars, spices prepared for foods, and many other materials are included. We shall not go into detail regarding the photographing of all the different food materials, for the combinations of microscopical technique necessary to cover the whole field are innumerable.[7] However, single examples can be cited to cover large groups of materials.

[5] Roscoe H. Sawyer, A paper before the American Chemical Society, April 14, 1936.

[6] F. F. Lucas in Henney and Dudley, *Handbook of Photography,* McGraw-Hill Book Company, 1939; "Ultraviolet Microscopy of *Hevea* Rubber Latex," *Ind. Eng. Chem.,* **30,** February, 1938.

[7] For detailed information consult the following:

A. Schneider, *The Microanalysis of Powdered Vegetable Drugs,* 1921. A general discussion and specific directions for mounting numerous drugs and rye and wheat flour.

Andrew L. Winton and Kate B. Winton, *The Structure and Composition of Foods.* In four volumes, 1932–1939. An authoritative work on vegetables, fruits, cereals, starches, oil seeds, nuts, oils, forage plants, animal products, syrup, honey, tea, coffee, cocoa, spices, extracts, yeast, and baking powder. Practically no information on photomicrography, the majority of the illustrations being made with the camera lucida.

H. G. Greenish, *The Microscopical Examination of Foods and Drugs,* 1923.

L. Rosenthaler, "Chemical Identification of Drugs," *Pharm. Acta Helv.,* **12,** 7, 1937.

For the most part foodstuffs have an index of refraction lying somewhere around 1.5 ± 0.08, and generally the particles are large. Thus, starches, ground spices, sugars, flours, drugs, and much other material can be examined and photographed at magnifications of 50 to 360 diameters.

Glycerol and glycerin jelly are excellent media for many mounts. They hold the particles well in place, and since the particles in this group are generally large the visibility is good. The dispersion can be made by simply dropping some of the specimen into a little melted jelly or glycerol on a microscope slide and putting the cover glass in place. As a rule this treatment will give sufficient dispersion to the particles; if not, slight pressure can be exerted on the cover before the jelly sets.

Sometimes glycerol and water ($n = 1.4$) is a better mounting medium than pure glycerol. High-refractive-index liquids can occasionally be used to good advantage, but in general low-index media give better results.

Mounting and Photographing Starch. Many excellent methods are cited in the literature for preparing and mounting starch for microscopical examination. Methods are given for identification by examining the position of the hilum and the size of the grain, or by the iodine test, but in the literature little can be found regarding the actual photography of starch. The size and shape of the grains and the figure of the hilum, if present, are the three important features to be stressed in every photomicrograph of starch taken in natural light. Between crossed Nicols, the starch cross can be seen; the hilum is located in the center of the cross, and, under special methods of lighting, the successive rings become visible in some specimens.

Figure 25 shows a photomicrograph of sago flour. The magnification is 360 times. Generally speaking, this magnification is suitable for the starches found in industry, and it is seldom necessary to exceed it. Starches stained with Lugol's solution show little or no additional detail due to the action of the stain, but the starch will be nicely differentiated from other material. The refractive index of starch is about 1.53. Samples composed of small grains will require a greater differential in refractive index between the mounting medium and the specimen than samples composed of larger grains, but the difficulties encountered with some pigments having flat, thin particles will not arise in handling small-grain starches, which are more or less spherical. The specimen can be mounted in the same way as fine pigments. A few of the grains may become broken, but most of the small and moderate-sized ones will remain intact. When the grains are fixed on

the slide the mount can be finished with glycerin or water and the cover can be dropped on. Under these conditions there will be no motion of the particles, and the effects of various mounting media can be tested on one slide.

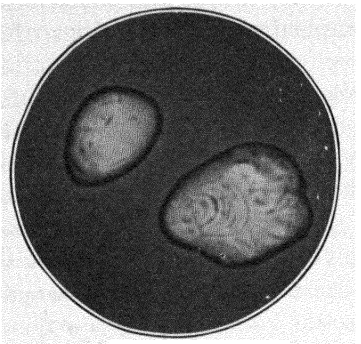


FIG. 240. Potato starch ×300. The mount was made in Niglytine, thus reducing glare. Objective, Zeiss 8-mm achro.; ocular, Zeiss 15× compensating; condenser, Leitz achromatic-aplanatic, top element removed; illumination, 400-watt biplane filament projection lamp, method II; filters, Wratten No. 45, plus polarizing plate oriented to give maximum contrast to subject; Eastman Panatomic X film; developer, D-19.

When glycerin jelly is the mounting medium, the specimen may be placed on the slide and the melted jelly added. The cover glass is put in place while the slide is slightly warm, and pressure is applied with the points of a pair of forceps by a gentle rolling motion on the cover. However, if the specimen includes large particles that may become broken it is probably advisable to distribute them in a fixative solution before the glycerin jelly is added.

Large starch particles will cause much glare; they have a partly globular shape and the lenticular quality is no help in the examination. If a medium of very high or very low refractive index is used, the outlines of the starch will appear altogether too heavy; but if the extremes in index are not used the surface detail will not be apparent. The best intermediate effect is probably obtained by choosing a mounting material of an index lower than that of the starch, and perhaps making the illumination slightly unilateral by decentering the iris diaphragm of the condenser. Figure 240 shows the effects produced, and how the surface structure may be shown, when the starch is mounted in Niglytine;[8] see Glossary.

It might be said that only a few of the starches in commercial use show the ring formation sufficiently clearly for photography. The best procedure under such circumstances is to place the particle in the center of the field and to concentrate on focusing it to good ad-

[8] J. B. McLair, "The Differential Analysis of Starches," *Field Museum of Natural History Publication* 275, Vol. IX, No. 1, June, 1930.

V. Poschl, *Technische Mikroskopie, ein Lehrbuch der Mikroskopischen,* Verlag von Ferdinand Enka, Stuttgart, 1937.

James Scott, *The Microscope in the Mill,* 1920.

vantage to show the surface detail. In printing the negative the field can be diaphragmed down to a small diameter. Blue light will probably be of some advantage. In the picture shown in Fig. 240, a polarizing plate was placed in the substage ring carrier.

The Photography of Sugars. Granulated sugars, when photographed to demonstrate particle size, are spread on the slide in the same way as coarse powder, directions for handling which were given on p. 609. Fine sugars can be handled as coarse pigments; the mount can be made in some rather high-refractive-index liquid, like aroclor, monobromonaphthalene, or hyrax. Even the small particles will show up well in hyrax. Magnifications are generally low to medium, 360 being the most useful. All the finer sugars, such as those used in the confectionery trade, are easily within the scope of Group II, and in photographing them it will be found that a deep green or blue filter will render the detail clearly. Any difficulty in obtaining sufficient depth of field can generally be overcome by choosing a mounting medium of high refractive index. An achromatic objective and a green light might be used when great depth is required. The condenser diaphragm should not be closed to much less than a $\frac{1}{5}$ cone if it can be avoided, or the delicate outlines of the crystal particles will surely be lost in the heavy outline of the refraction image.

If the photomicrograph is to be taken for purposes of identification, the technique of Quesne and Dehn[9] can be followed. Their paper is of particular interest since it contains photomicrographs of many sugars showing the varying crystal formation from several solvents. Figure 241 illustrates crystals formed from sucrose. A saturated solution of the sugar was made in a test tube, and alcohol was added to produce precipitation. A drop of the solution containing crystals was then placed on a slide and covered. A paraffin or latex seal will retard evaporation, and the crystals will be clear and distinct in the mother liquor.

The Photography of Flours. It is best to handle flours by distributing them in glycerin and water. The starch particles, on which the identification depends to a large extent, can be easily studied and photographed in this medium. A paper by Silberberg,[10] revised by

[9] J. A. Quesne and W. M. Dehn, "Microscope Identification of Sugars," *J. Ind. Eng. Chem., Anal. Ed.*, II, 555, 1939.

[10] B. H. Silberberg, "Microscopical Examination of Feeds and Feeding Stuffs," *Publication* 5, 1940, revised by George L. Keenan, Microanalytical Division, U. S. Dept. of Agriculture, Washington, D. C.

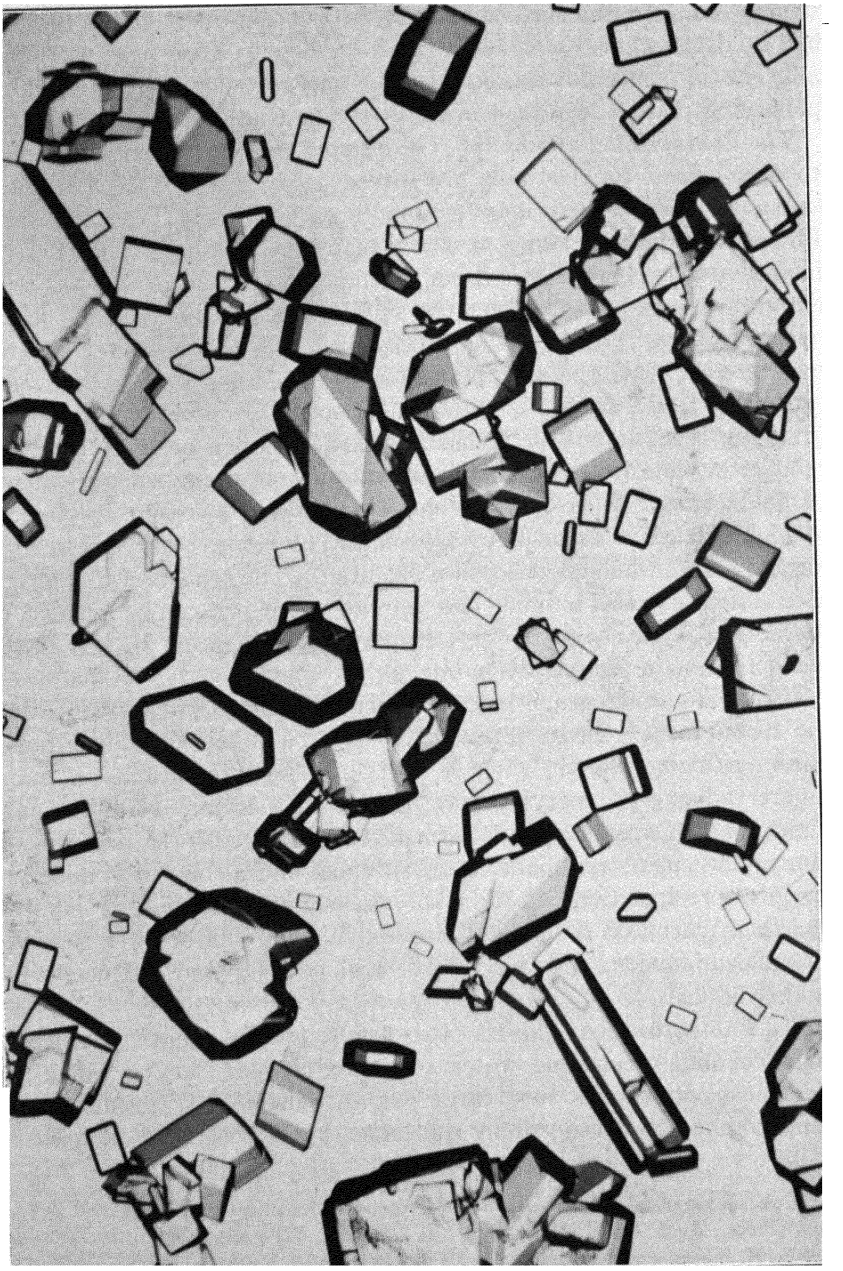


FIG. 241. Sucrose crystals ×150. Objective, Zeiss 30-mm apo., ocular, Homal I; condenser, Leitz achromatic-aplanatic, top element removed; illumination, 250-watt projection lamp; filters, Bausch and Lomb daylight plus Wratten No. 15; Eastman Panatomic X film; developer, D-19.

Keenan, gives a table for the identification of a number of meals and flours.[11]

There is little information in the literature relating to the actual photography of flours and meals, but a good flour or meal picture is easily obtainable after a good distribution has been attained. For such a picture a correct exposure time is vital. Some of the particles will prove quite transparent, and some only partly so. The exposure should be such that the actual appearance of the flour, as seen under the microscope, will be maintained in the picture. Each particle should be separate and distinct so that the different seed elements may be recognized. The picture of banana flour in Fig. 242 shows starch aggregates which are typical of the flour; therefore their presence is desirable, if in a representative quantity. Hertwig's solution[12] is particularly efficient as a mounting medium for it clears the specimen well and has about the right refractive index for flours, meals, and ground vegetable drugs.

It is not always practicable to mount the specimen of flour directly in a drop of the mounting medium. Often it is better practice to place some of the flour in a small crucible and to add the mounting medium while stirring until the whole mass is well mixed. The mixture can then be allowed to stand for a few mintues, or hours, until the specimen seems clear of air bells when examined under the microscope. If air bells are hard to dislodge, slightly warming the crucible may help to dispel them. The mount is made by removing a drop of the mixture from the crucible and placing it on a slide. The preparation should be covered and should be sealed for photomicrographic purposes if Hertwig's solution is used.

The Photography of Vegetable Drugs. Like foodstuffs, ground herbs are likely to have low or medium refractive indices. Many of them contain a great deal of starch, and frequently identification is made from seed hairs, vessels, fibers, cells of different sorts, and spiral vessels. Since the demand is sometimes to show traces of adulteration, the mounting methods should have some degree of uniformity for the specimens so that small differences between various herbs may be shown consistently.

Staining is of value, but it must not be deep enough to mask detail.

[11] B. J. Howard, "Estimation of Insect Excreta in Flour," Microanalytical Division, U. S. Dept. of Agriculture.

W. G. Helsel and Kenton L. Harris, "Method for the Recovery of Filth and Foreign Matter from Wheat Meal (Tentative)," 1940, Microanalytical Division, U. S. Dept. of Agriculture; "Method for the Recovery of Filth from Corn Meal (Tentative)," 1939, Microanalytical Division, U. S. Dept. of Agriculture.

[12] This is an old formula by Hertwig: ⅓ alcohol, ⅓ glycerol, ⅓ acetic acid.

Several attempts may have to be made before a suitable depth of stain is obtained on the specimen. Lugol's solution is useful; it will turn the starch grains blue and the cellulose yellowish brown, and it will also affect other substances.

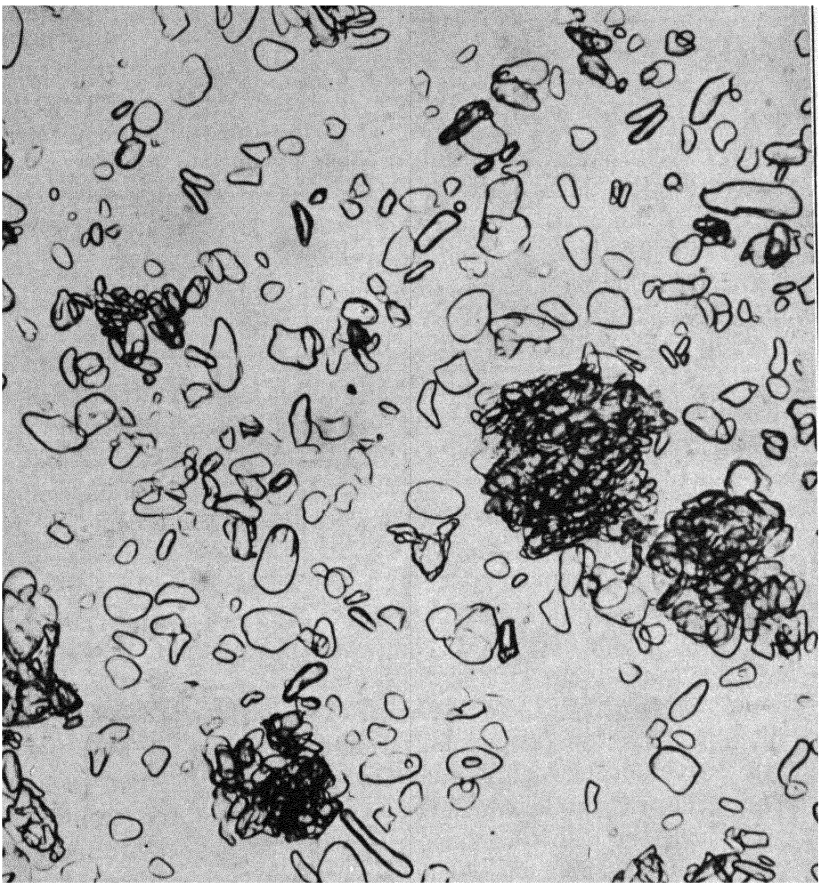


FIG. 242. Banana flour ×200. Specimen mounted in Hertwig's solution. The clumping of the starch grains is characteristic of this flour. Objective, Zeiss 32-mm apo.; ocular, Zeiss Homal I; condenser; Leitz low-power; illumination 300-watt projection lamp, method III; filters, Wratten No. 58 plus 15; Eastman Panatomic X film; developer, D-19.

The proper selection and skilful use of the mounting medium will, in itself, often produce the desired results and make further manipulation of the specimen unnecessary. For temporary mounts, when the picture can be taken within a few hours or at most in a few days, the

specimen can be mounted in aroclor or other high-refractive-index medium without being dehydrated. It will be more transparent than a stained specimen. Figure 243 shows a photomicrograph of an unstained specimen of aconite, the mounting medium being Hertwig's solution, with an index of 1.441 at 25° C.

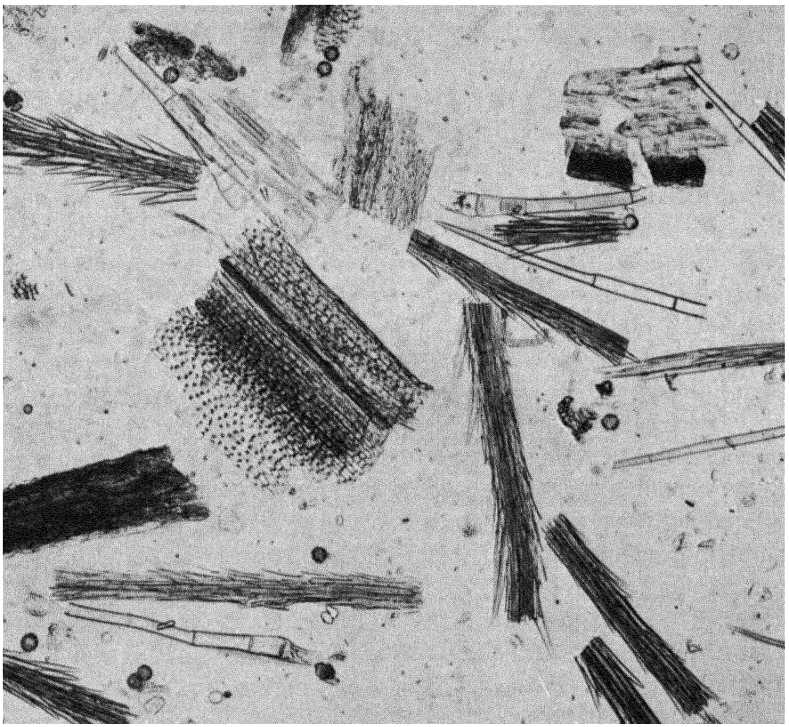


FIG. 243. Vegetable drug, aconite, ×112. Specimen mounted in Hertwig's solution, not fixed to slide. Objective, Zeiss 32-mm apo.; ocular, Homal II; condenser, Leitz long-focus; illumination, 500-watt projection lamp, method I; filters, Wratten No. 58 plus 15; Eastman Contrast Process Ortho film; developer, D-19.

Sec. 158. Mounting and Photographing Cosmetics and Detergents. Many of these materials can be handled and photographed in the same way as the pigments used in the paint industry. Face powders may be composed of five or more components, each being subject to mounting and photographing as described for paint pigments, since, in fact, many of the materials are essentially pigments.

Face powders, if coarse, should be mounted in some liquid after distribution on the slide as outlined for coarse pigment particles. The

refractive index of the liquid may be either high or low, depending, to a large extent, upon the size of the particles of the various components. If the average particle is small, a good picture may be secured with any 16-mm or 8-mm objective without either a mounting liquid or a cover glass. With higher objectives, a mounting medium and cover glass are generally necessary. Figure 244 shows a photograph of a fine face powder taken with an 8-mm objective. A green screen was used on account of the combination of an achromatic objective and a Homal eyepiece.

Within the last few years, talc, one of the chief ingredients of face powder, has been ground finer and more uniformly than previously by a specially developed apparatus, the average particle size thus being reduced. Some powders contain several ingredients of a similar nature which can be identified under the microscope only with a great deal of difficulty. Therefore, occasionally it may be advantageous to photograph the ingredients separately, before they are mixed, and to compare the photographs. Since the purpose of nearly every photomicrograph of face powder is to study the size of the particles and the percentage of components, the value of the above plan is at once evident. By treating each ingredient separately, a larger quantity of each constituent can be shown and therefore more can be learned from the photographic work. A series of pictures such as this should be accompanied by a picture of the complete product. Some of the important ingredients found in face powders are talc, kaolin, zinc oxide, titanium oxide, starch, rouge, and zinc stearate.

Lipstick and pastes can often be distributed on the slide by an appropriate solvent, and, since solvents generally have low indices of refraction, they may often serve as mounting media. Lipstick, rouge, and face powders in general, if merely rubbed on the slide for examination or photography, are almost sure to give disappointing results. Material of this kind must be well extended with a solvent to separate the various solid ingredients and so to make them visible. Extraction of the oils and fats will leave a residue which is easily mounted and examined.

Tooth powders, though generally coarse, can be treated as face powders, but the pastes must be mounted in about the same way as lipstick. Glycerol is a good vehicle to carry such material, but there will be a good deal of clumping together of the particles, and difficulty may be experienced in breaking up the aggregates, perhaps because of the cohesive action of any soap that may be present.

To be photographed satisfactorily, salves and ointments carrying solids in mechanical suspension must, as a rule, go through a wash-

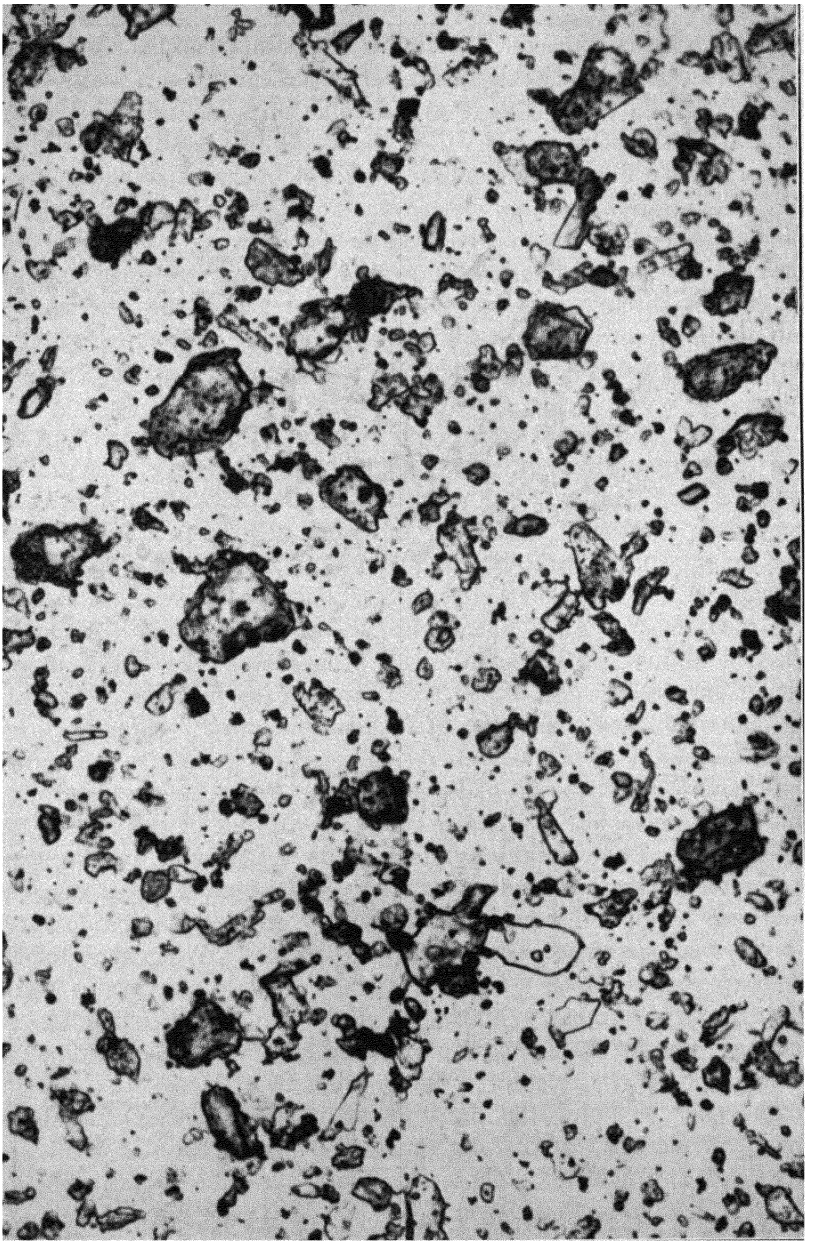


FIG. 244. Face powder ×600. Mounted dry and photographed without a cover glass. Dispersion was in Parlodion-amyl acetate sol. Objective, Zeiss 8-mm achro.; ocular, Homal I; condenser, Leitz achromatic-aplanatic; illumination, 400-watt projection lamp, method II; filters, Wratten 45 plus 15; Eastman Panatomic X film; developer, D-19.

ing or extracting process either in extraction apparatus or by repeated washings and settlings. Sometimes the demand is for a picture of the material to be taken "as is"; the value of a picture taken without separating the components will probably lie in a study of the dispersion of the solids to be compared with competitive products. Work like this is often done at low power, and the field should include as much material as possible. In the preparation of this material there may be a certain amount of unavoidable breaking of original aggregates, but through a series of such pictures this will average itself out, so that, on the whole, the final results will not be misleading but will give a good idea of comparative conditions in a series of preparations.

Commercial cleaners can usually be photographed by simply mounting them in a drop of ethylene glycol monomethyl ether (methyl "Cellosolve") or any of the higher alcohols that do not evaporate very fast. However, if circumstances permit, it is best to photograph the components separately. Figure 245 illustrates the appearance of volcanic ash, an ingredient of certain commercial cleaners. The mounting medium was air. The material was dispersed in 0.5 per cent Parlodion-amyl acetate solution. In this picture the field is uninteresting, largely because of the sparseness of the particles. The effect is unavoidable with material of this sort mounted as described, however, because a greater concentration of grains will cause the material to flocculate faster than the dispersing agent evaporates. Naturally all individual detail of the separate particles will then be lost.

Sec. 159. Photographing Cements, Clays, Ceramics, Insulating and Refractory Materials, and Filter Aids. These materials, if they have not been molded and baked, are easily examined and photographed by the methods already described for pigments. When they must be photographed after they have been made up into chinaware, insulating material, refractory bricks, or other forms of merchandise, thin sections must be made of the specimen to permit it to be examined and photographed by transmitted rather than by reflected light.

The clay particles may be uniform in size, or they may be extremely irregular. If the size range of irregular clays is great, sufficient depth of field for a photomicrograph cannot be obtained at high or even medium magnification. The large particles may sometimes be separated from the smaller ones by sifting or centrifuging. In mixtures, if the components vary enough in the specific gravity, they may be separated by elutriation and the different sizes may then be photographed separately.

Oftentimes, it is claimed that a certain clay is colloidal, and a pic-

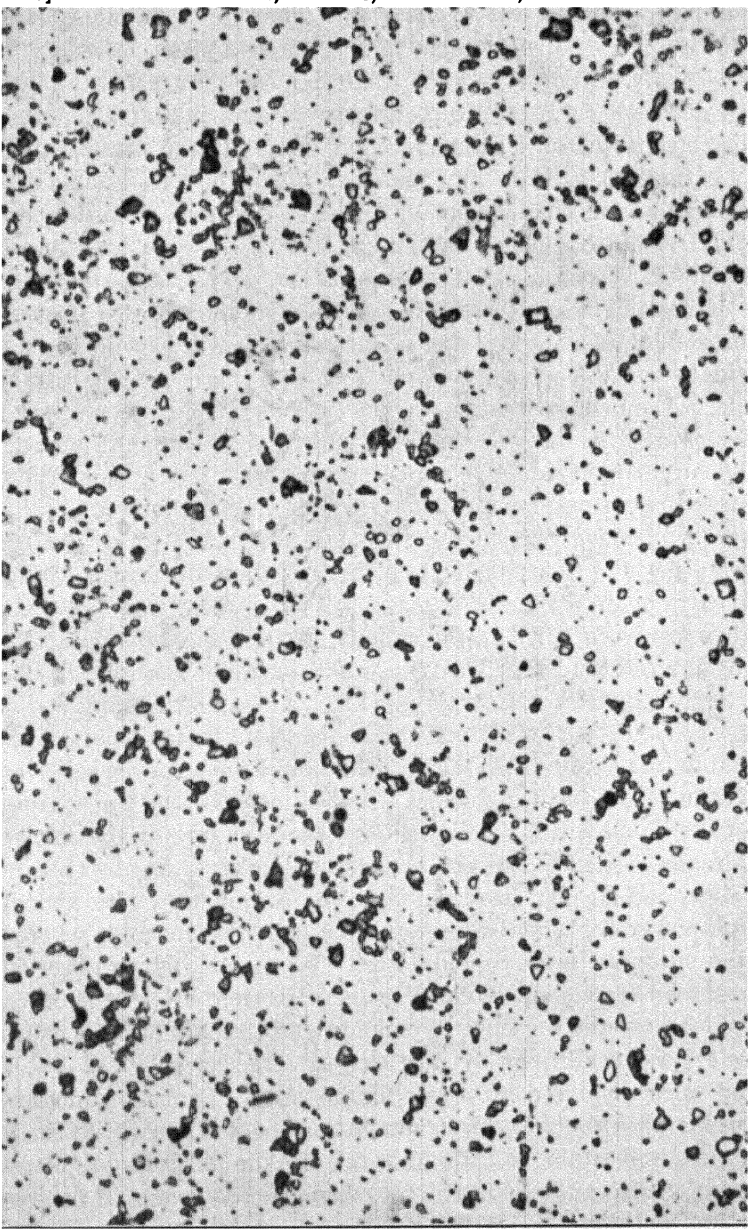


FIG. 245. Volcanic ash ×700. Dispersion of this substance is difficult. The particles tend to move into chain formation as the solvent of the dispersing medium dries. Specimen was mounted dry. No cover glass. Objective, Zeiss 8-mm achro. short mount; ocular, Homal I; condenser, Leitz achromatic-aplanatic; illumination, 400-watt projection lamp, method I; filters, Wratten No. 45 plus 15; Eastman Panatomic X film; developer, D-19.

ture is desired to show this condition. The term " colloidal," without careful definition, means little to the microscopist. Formerly it referred chiefly to non-crystalline material like gum arabic or gelatin, which does not form a true molecular solution in water but remains in discrete particles of submicroscopic size. At the present time the term is used to indicate any material which may be ground or precipitated to form particles of 0.1 μ or less. Thus a clay 100 per cent colloidal would consist of particles all of which were less than 0.1 μ in diameter. On the basis of this criterion, only a very small proportion of ceramic clay is actually of colloidal dimensions, irrespective of the claims made, and, of those clays which can be said to be truly colloidal, only a very small portion of any one is less than 0.1 μ, as a rule. A quick estimation of the colloidal condition of any clay mount can be made by noting the difference between the appearance of the bright-field and the dark-field images. For a more thorough examination, a small amount of the sample can be mounted in a liquid; all small adhering particles washed loose from the larger grains will show in the field as separate particles, and their size can be estimated as above or below 0.1 μ.

When the work permits, the colloidal matter can be separated from other material by suspension in water or other appropriate liquid. The larger particles will settle out after a few hours or maybe a few days. Since Stokes' law does not apply to very small particles, it is advisable to leave the specimen in the liquid for a considerably longer time than might seem necessary. A drop can then be taken from the top of the liquid with a small pipette without disturbing the clay which has already settled. The fine material so gathered can be transferred to a slide, and a cover glass applied. An examination with a medium- or high-power dark-field condenser will usually determine whether or not the specimen is of colloidal size. When making an observation, it should be remembered that Brownian motion, or pedesis, does not of itself denote the presence of colloids, since such motion is exhibited under certain conditions by particles as large as 10 to 12 μ — well-ground mica dispersed in water, for instance. Brownian motion can be considerably controlled by evaporating the drop nearly to the point of dryness and adding a little glycerol. If the particles are of nearly microscopic size and very brilliant, they may be mounted in glycerin jelly; but as this jelly is in itself colloidal it should not be used unless the particles under examination are large and brilliant enough to overpower the colloidal effect of the jelly.

Sometimes particles that are not moving very rapidly may be photographed with a very strong light source, such as an arc lamp, and

with a short bellows extension of 5 to 10 inches, but large and small particles of clay cannot be photographed in the same field by this system because the intensity of the high-power dark-field illumination will cause a flooding of light from the large particles which will result in loss of contrast.

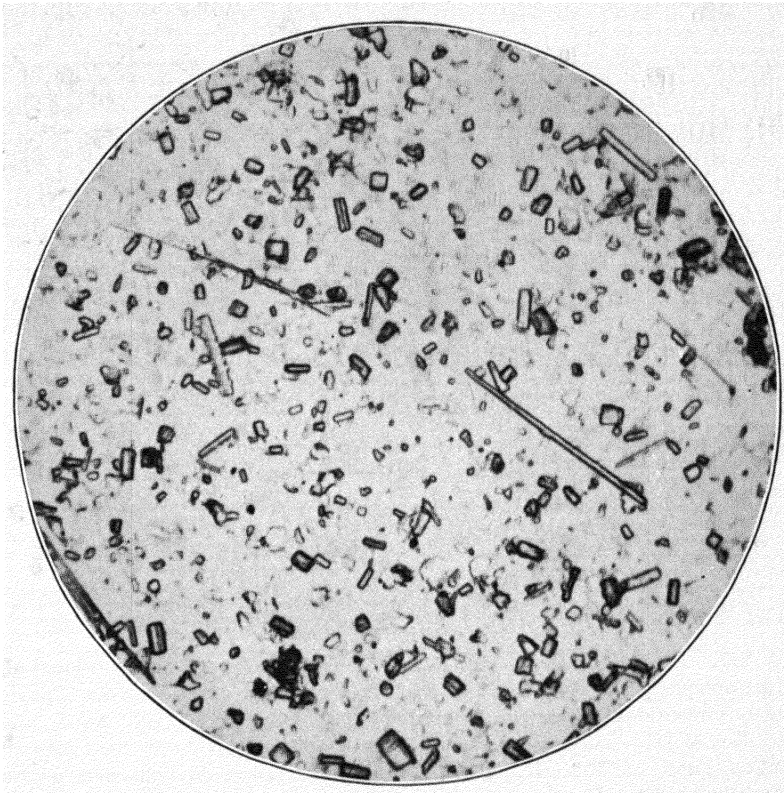


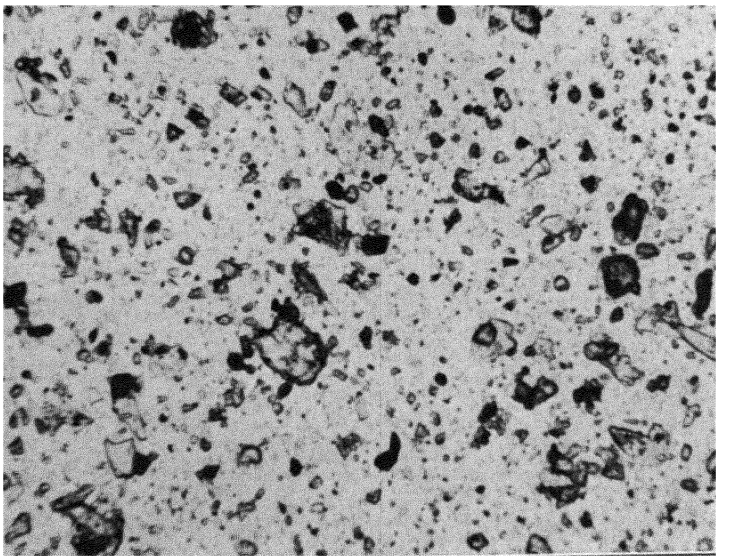
FIG. 246. Ground asbestos ×600. Dispersed in turpentine and mounted dry. Objective, Zeiss 8-mm apo.; ocular, Homal I; condenser, Zeiss aplanatic; illumination, Pointolite lamp, method II; filters, Wratten 58 plus 15 Eastman Commercial Pan film; developer, D-61a.

Figure 246 shows a photomicrograph of ground asbestos, a mineral of the amphibole group. In order to obtain the contrast shown here, the powder was mounted dry and photographed with a lens corrected for use without a cover glass.

Sec. 160. Abrasives and Geological Specimens. Natural and synthetic abrasives to be photographed in powder form are handled as pigments (Sec. 156) or as clays and cements (Sec. 159). No great difficulty should be experienced in either mounting or photographing

most of this material, although some of the polishing powders are so fine and transparent that good visibility may sometimes be a little difficult to attain. Figure 247 shows powdered emery, or corundum — a typical natural abrasive.

Geological specimens consisting of fine sands, silt, and similiar deposits are not difficult to photograph if it is permissible to run them



some are opaque. The range in particle size is large; some of the particles approach colloidal dimensions. Speciman mounted dry. Objective, Zeiss 4-mm apo.; ocular, Homal III; condenser, Leitz achromatic-aplanatic; illumination, 500-watt projection lamp, method III; filters, Bausch and Lomb daylight plus neutral, density 0.6; Eastman Panatomic X film; developer, D-19.

through a sieve and to make separate photographs of the coarse and fine particles. If this may not be done, a low magnification will be required for sharp, well-defined pictures. Fine sands lend themselves well to the embedding method, Sec. 163, but a photomicrograph of sand so treated will be distinctly different from one of sand in its natural state, as can be seen by inspecting Fig. 250.

Sec. 161. Chemicals in Powder Form. Figure 248 is a photomicrograph of magnesium stearate, the virtue of which lies largely in the regularity of size and the fineness of its particles. These features are well emphasized in the picture by means of perfect dispersion and by sufficient material in the field to show a representative sample.

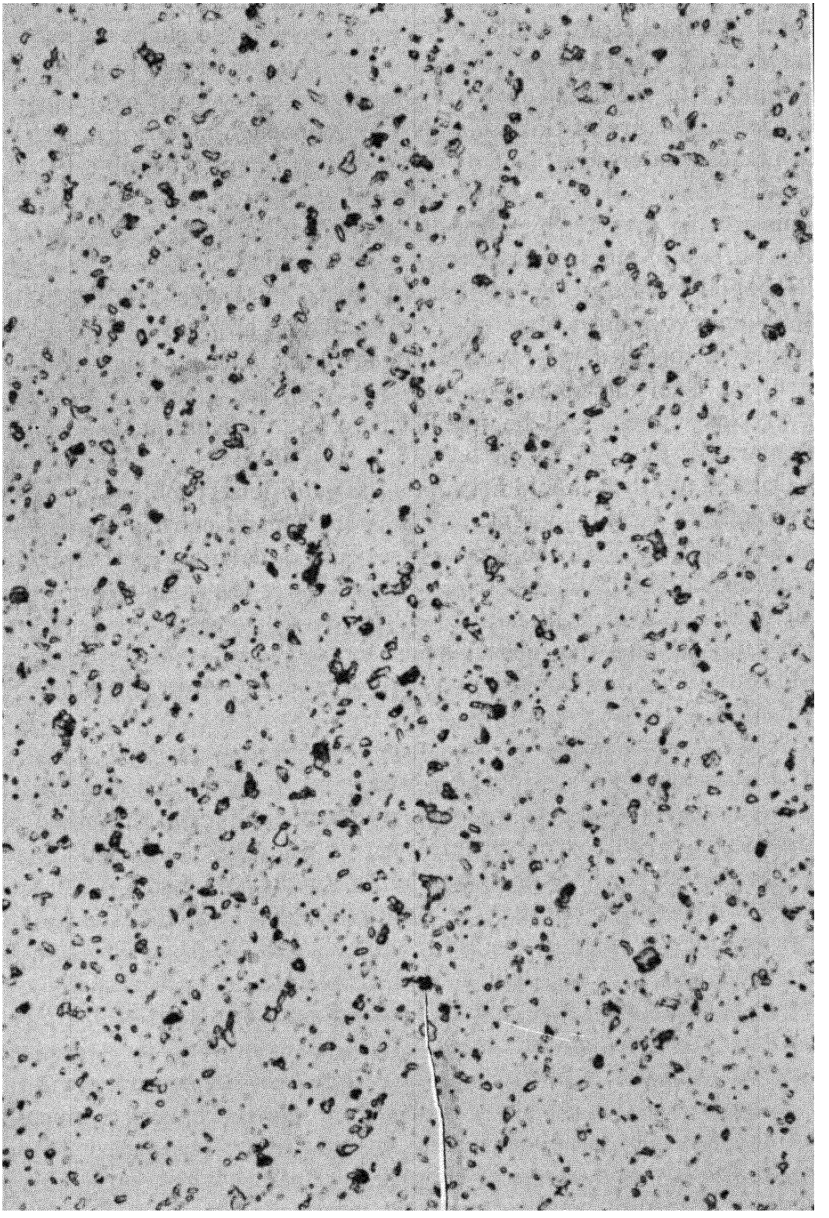


FIG. 248. Magnesium stearate ×360. Objective, Zeiss 16-mm apo.; ocular, Homal I; condenser, Zeiss aplanatic; illumination, 200-watt projection lamp, method I; filters, Wratten No. 57 plus 15; Eastman Proc. Pan film; developer, D-61a.

For advertising purposes, chemicals are often photographed in powder form to demonstrate uniformity of size or particle shape. Such pictures must be easy to reproduce by the half-tone process. The technique of mounting this material has already been explained. A successful picture of this type must carry enough material in the field to make it interesting and of value, but it is quite possible to select a field which will give an erroneous impression of the true condition. This can be avoided by carefully studying the field and the slide in general before making the picture. It is a good plan to make several slides and to study them to find out what constitutes a representative field, since it is not always easy to decide just how many big particles should be included in proportion to the little ones. When the specimen has been distributed on the slide with the glass rod, comparatively large areas will be found where the distribution is even and regular.

Colored powders should not present an additional problem, since they are likely to be transparent, and so great control can be exercised by means of filters. If the powder is strongly colored, the detail may be well rendered by the use of a filter of nearly the same color. Small particles, however, will naturally have less strong color effects, and they may become too inconspicuous in the picture unless made more contrasty with the background. Here a contrasting filter may be indicated.

As with any powder, the mounting media for powdered chemicals and drugs should be carefully selected, with regard not only to the optimum index but also to complete chemical inertness toward the specimen. No solvent or chemical action whatever should take place. If there is any doubt as to the action of a mounting medium, observation through the microscope at the time that the medium is applied will usually disclose any tendency of the specimen to go into solution or evolve gases. The edges of crystals should be watched; any that are dissolving will have rounded edges, and any that are growing will have sharp, clear-cut edges. With pigments and most cosmetic materials, the action of the mounting liquids on the specimen will not be a major question. These materials are relatively inert chemically, and are insoluble in any mounting medium likely to be used. However, solubility and chemical activity are factors which must always be very carefully guarded against when working with powdered chemicals in general.

Chemicals which need to be recrystallized on the stage of the microscope present a different problem which will be handled separately; they fall into Group V.

Sec. 162. Bacterial Smears. For the most part, material of this nature is very easy to handle. The magnification is usually high, 2000 to 3000 diameters, but the contrast is, or can be made, strong. Owing to the high magnification and the high resolution demanded, the mercury-vapor discharge tube is a good light source. Illumination should be by Method II or Method III. For most of the specimens already discussed illumination could have been by Method I or by Method III, except that for the smaller pigments strong blue light is of value.

Figure 249 shows a photomicrograph of *Staphylococcus pyogenes aureus*. The magnification of 3000 is high enough to register all the detail and to maintain a fair degree of sharpness. The connection between many of the individual cells is shown.

These high magnifications can be obtained best with a moderately long bellows draw. Oculars giving magnifications of 25, 30, or even 50 diameters will give a maximum magnification equivalent to as high as 6000 diameters, when used with a 1.5-mm objective at a distance of 10 inches, but the 15× or 10× ocular will produce images sufficiently sharp to permit a bellows draw that can yield final magnifications of 2000 to 3000 while retaining a measure of crispness in the image.

Sec. 163. Embedded Powder Grains. Sometimes, for identification work with the petrographic microscope, sand or any finely divided mineral is embedded in a plastic or natural resin. Thin sections are made according to standard petrographical procedure.[13] The section is ground to the standard thickness of 30 μ. The relative retardation of quartz at this thickness is such that it appears white between crossed Nicols.

The usefulness of this method of dealing with miscellaneous material depends to a great extent upon the size of the grains. If the thickness of the grains is less than that of the section, photographic efforts are likely to be disappointing; but if their size is sufficient for a section of every grain to appear at the optical level at the surface of the plastic mount, photographic results should be satisfactory, although the true size of the original grains will not be known. Figure 250 is a photomicrograph illustrating the appearance of embedded material, in this case mine tailings. The large vacant space at the top is a break in the section where the embedding resin was torn

[13] George Rév, Grinding Laboratory, Columbia University, New York City, and W. Harold Tomlinson, 114 Yale Avenue, Swarthmore, Pa., are equipped to do this kind of work, and their fees are very reasonable.

R. E. Head, "The Technique of Preparing Thin Sections of Rock," *Tech. Paper* 8, Utah Eng. Experiment Sta., University of Utah, Salt Lake City, Utah.

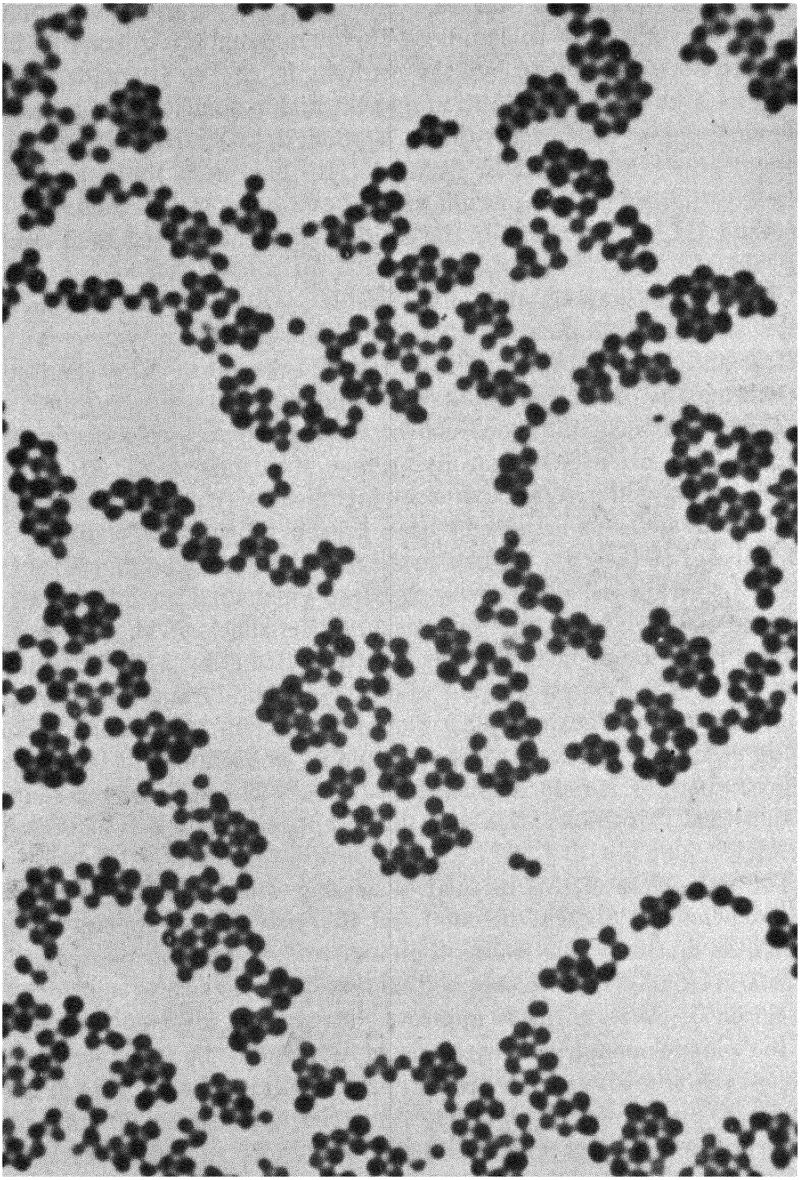


FIG. 249. *Staphyloccocus pyogenes aureus* ×3000. Although great resolution is required in this picture a strong green filter was used to gain contrast. Objective, Spencer 1.5-mm apo.; ocular, Spencer 15× compensating; condenser, Leitz achromatic-aplanatic, 9/10 cone; illumination, mercury-vapor lamp H3, method II; filters, Wratten No. 56 plus Corning 428; Eastman Commercial Pan film; developer, D-19.



FIG. 250. Mine tailings ×240. The specimen of fine grains was mounted in a block of transparent Bakelite, and a thin section was then made. Objective, Zeiss 16-mm apo.; ocular, Homal II; condenser, Zeiss aplanatic; illumination, 200-watt projection lamp, method I; filters, Bausch and Lomb daylight; Eastman Commercial Pan film; developer, D-1.

away in grinding. This particular picture shows comparative freedom from grinding or polishing compound. In making pictures of fine material care must be taken that the field is as clear of grinding compound as possible.

GROUP III

OPAQUE SURFACES

Sec. 164. Description of Material. There are three distinct classes of material in this group. The first includes specimens that demand polished and etched surfaces, such as metals and alloys. The magnification may be high or low. The art of preparing and photographing this material is known as metallography. The second includes specimens of opaque ore minerals prepared with a polished surface; this surface is seldom etched for photographic purposes. As a rule, the magnification is low to medium; it is seldom very high. Slight differences in colored areas are of importance. This is known as petrography. The last class is miscellaneous and includes the surface of unpolished opaque specimens, such as paint film and plastic and ground metal surfaces. The magnification seldom exceeds 150 diameters.

EXAMPLES

Metal specimens: ground, polished, and etched surfaces, Sec. 165.
Petrographic specimens: polished ore surfaces, Sec. 166.
Other specimens with rough or unpolished surfaces, generally opaque, Sec. 167.

Illumination. The illumination for polished surfaces is generally by means of a vertical illuminator. Dark-field illuminators such as the Ultropak, Epi condenser, or Silverman illuminator would give too much prominence to the details of the polish and not enough to the detail of the specimen. Vertical illumination stresses the desired detail and, partly by inevitable glare, suppresses defects due to polishing.

As most metallic specimens are colorless and act as mirror surfaces, strong green screens can be used. Fortunately, they make possible the use of achromatic objectives which are essential for the elimination of the excessive glare arising with vertical illuminating systems. The mercury-vapor tube, the ribbon filament lamp, or the General Electric photographic lamp are good light sources, but if a ground glass is to be a secondary source, the 500-watt projection lamp is recommended for additional light.

Colored specimens of ore can be given considerable contrast by means

of filters which should be utilized to full advantage since some of the colors, which are diagnostic, may be very weak. Light pinks can be intensified by an orthochromatic or regular-type emulsion; light blues can be thrown into contrast by a light yellow filter and a panchromatic emulsion.

Apparatus. Objectives should be corrected for use without a cover glass and should have short mounts. There is probably some advantage in having the vertical illuminator and the objective of the same make. For work with the standard type of objective, condensers and oculars can be used indiscriminately regardless of make, but when a short-mount objective is used with a vertical illuminator, it should be remembered that the position of the diaphragm of the illuminator and its aperture are designed to fit the optical and mechanical characteristics of objectives of like make.

The prism or mirror-type illuminators will give the most contrasty images, but they should not be used with objectives of focal length greater than 16 mm, and, as a rule, the high and medium objectives should be served with the glass plate illuminator. When the vertical illuminator is cut in on a microscope which is habitually used with an objective corrected for tube length of 160 or 170 mm, allowance must be made for the increase in tube length brought about by the attachment of the illuminator, and adjustment may have to be made to compensate for the tube-length requirements of the short-mount objective. However, when the illuminator and objective are of like make, the increased length of tube due to the addition of the illuminator will probably be correct for the additional tube-length requirements of the objective.

In metallographic microscopes the stage is adjustable vertically by rack and pinion. Biological microscopes should have this same adjustment; otherwise the lamp must be lowered or raised to correspond with each position of the focused microscope tube. When the adjustable stage is part of the equipment all coarse focusing is done by manipulating the stage, and final focusing by the fine adjustment will not seriously affect the lighting alignment. The large metallographic stands of the Le Châtelier type with their full complement of accessories simplify vertical illumination work tremendously. They make possible automatic alignment of the specimen on the microscope stage, so that the flat surface will be exactly normal to the axis of the microscope. Moreover, once the lamp and illuminator on a metallographic stand are aligned, they will stay in alignment until interchanged.

For occasional use, the biological microscope, with the addition of a vertical illuminator for metallographic or petrographic work, is

not only practical, but it is frequently the only equipment available. It is possible to use the 16-mm or 8-mm objective with a vertical illuminator, and good results can be obtained with it if a moderate magnification will suffice. True, the pictures so taken may not be quite so professional in appearance as those made with short-mount objectives, but they may answer all purposes, and the only additional expense involved will be the purchase of the vertical illuminator. If

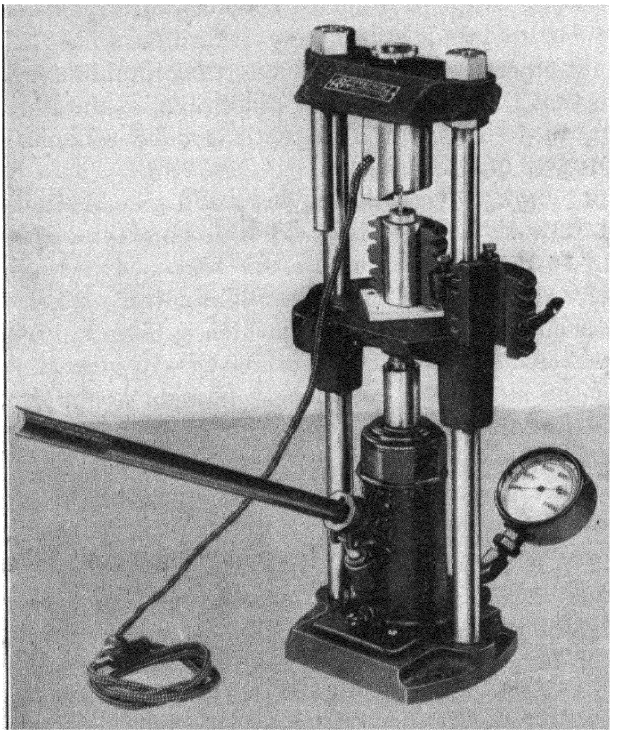


FIG. 251. An electrically heated molding press for mounting small specimens for microscopical examination. By special permission, Adolph I. Buehler, Chicago.

pictures are to be taken with objectives corrected for 160- or 170-mm tube length, every effort should be made to keep the microscope tube as near to the standard length as possible, plus a suitable allowance for necessary over-correction. Flat-field oculars are always a help and should be used if possible. A green screen should be inserted when achromatic objectives are in use.

Considerable equipment of a rather expensive sort is required for the rapid mounting and polishing of metal and mineral specimens.

Formerly, the polish was imparted by hand, but the manual method has now been superseded by large automatic machines which can handle a number of specimens at one time and keep them properly supplied with abrasive and water. This development has undoubtedly accounted for some of the recent rapid strides in heat treatment of metals and the perfection of alloys.

The specimen is first mounted in a standard-size mold, filled with powdered resin; the mold is placed in an electrically heated press, see Fig. 251, and subjected to appropriate heat and pressure. Thermoplastics solidify at low temperatures so that but little heat is necessary in working with them; Bakelite, however, requires higher temperatures and pressure, and also enough time must be allowed for polymerization. The mold is then removed and cooled. The use of soft metals for mounting purposes has been discontinued in favor of the artificial resins which are much more resistant to etching agents. Figure 252 illustrates the appearance of the finished mounts.

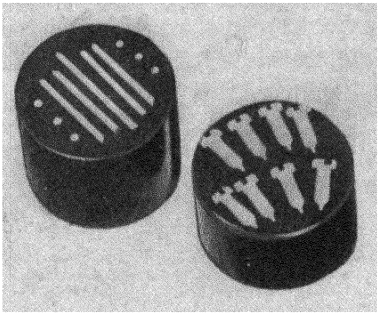


FIG. 252. Small, short cylinders molded on the press shown in Fig. 251. By special permission, Adolph I. Buehler, Chicago.

Specimens mounted as described above are then prepared by grinding and polishing. Essentially the apparatus consists of one or more horizontal flat wheels, 8 to 14 inches in diameter. The wheel when used with a polishing compound is generally termed a lap, for the polishing is essentially a lapping process. The lap is driven at speeds which can be controlled by step or cone pulleys, motor, or both. Laps may be of lead, aluminum, or cast iron; a wheel covered with cloth is referred to as a cloth lap.

The first cutting away of excess material can be done by grinding on a comparatively coarse wheel before the specimen is brought to the polishing machine. The next step is also a grinding operation, and as actual cutting is done the grinding compound for this stage should be very uniform in size and very small; 600 Carborundum or fine emery powder is recommended. The object, namely, to leave the surface scored by a series of very fine and regular lines, can be accomplished in a very few minutes. The final polish is imparted by easy transitions to a very fine polishing powder or by going directly

from the 600 Carborundum powder to the final polishing material. The final polish is attained with magnesium oxide, red rouge, black magnetic rouge, levigated aluminum, or one of the specially prepared powders sold under various trade names.

The more elaborate polishing machines have several spindles and are built to hold several specimens on one lap. Each specimen moves constantly across the face of the lap and also automatically turns in its holder, thus assuring good distribution of the lapping compound and an evenly polished specimen. The lapping compound may be fed



FIG. 253. Three lap polishing tables, for polishing metallographic or petrographic specimens. By special permission, Adolph I. Buehler.

automatically with water from a container where it is kept in constant agitation. When much metal polishing is required, a number of spindles or laps should be at hand so that wheels will not have to be interchanged in going from a coarser to a finer compound. Each lap should be in a state of readiness for its work. Figure 253 shows a three-unit polishing table.

Ideally, after final polishing, the metal would be entirely free from scratches when examined under the microscope. However, if a few scratches still persist on a specimen otherwise successfully prepared, a field can usually be found which will be sufficiently clear to give a good picture.

Soft metals are troublesome; the various components are likely to "flow" and make the demarcation between the grains indistinct. To

avoid this, Lucas[14] developed a method of surfacing soft metal specimens with a very sharp microtome knife.

After being polished the specimen is etched. Etching solutions were mentioned in Sec. 144. The specimen should be photographed as soon as possible after etching. If the prepared specimen has to be set aside for a few hours before being photographed, it may be necessary to repeat the last polishing process and to re-etch as oxidation of polished surfaces is rapid and often will ruin a specimen in a very short time. Preservation by means of lacquer has been attempted, but it is necessary to dissolve the lacquer before photography and even then repolishing may be necessary.

Petrographic specimens are likely to be softer and have components of more widely varying degrees of hardness than metals. However, the apparatus used for the preparation of metal specimens is applicable to the preparation of opaque ore minerals. The etching is usually omitted for minerals. Some of the leading references for the preparation of metal and mineral specimens are listed below.[15]

[14] F. F. Lucas, "Microtome Methods for the Preparation of Soft Metals for Microscopic Examination," *Mining and Metallurgy,* February, 1927.

[15] M. N. Short, "Microscopic Determination of the Ore Minerals," *Geological Survey Bull.* 914, second edition, 1940. Describes polishing processes used at the Geological Survey Laboratories, Harvard University, and the University of Minnesota.

R. E. Head, "The Technique of Preparing Thin Sections of Rock," *Tech. Paper* 8, University of Utah, 1929. Gives a method for making a homemade polishing device.

R. E. Head and Morris Slavin, "A New Development in the Preparation of Briquetted Mineral Grains," *Tech. Paper* 10, University of Utah, 1930. Describes a homemade device for embedding mineral grains in Bakelite.

F. F. Lucas, "Photomicrography and Technical Microscopy," in Henney and Dudley, *Handbook of Photography,* McGraw-Hill Book Company, 1939.

F. F. Lucas, "Photomicrography and Its Application to Mechanical Engineering," *Mechanical Engineering,* **50,** 3, 1928.

H. S. Rawdon, "Structure and Related Properties of Metals," *Natl. Bureau of Standards Cir.,* 1922.

A. Sauveur, *The Metallography and Heat Treatment of Iron and Steel,* McGraw-Hill Book Company, 1939. This is one of the most important books on the subject.

U. S. Dept. of Commerce, "Metallurgy: Publications by Staff of the National Bureau of Standards," *Cir. Letter* 522, 1938. Contains several hundred references.

George A. Ellinger and Joseph S. Acken, "A Method for the Preparation of Metallographic Specimens," *Transactions of the A.S.M.,* 1938. A good discussion on polishing.

American Society for Testing Materials, "Tentative Methods of Preparation of Metallographic Specimens," Designation E3-39T, 1939. A very complete paper on etching agents.

(Footnote is continued on page 638.)

Usually the expense for acquiring the apparatus described for the preparation of metal and mineral specimens is warranted only in large laboratories. However, it is possible to take good photomicrographs of metals and minerals which have been prepared entirely by hand. The apparatus required includes hacksaws, one or two grinding wheels of coarse and fine grit, files, and a good assortment of polishing papers.

Suitable wheels may have a 60 and 200 grit. The specimen may be roughed out partly by coarse grinding and partly with the hacksaw, whichever is the more convenient. A comfortable working size for the specimen, which is to be held in the hand, is about ¾ inch in diameter — little larger than for the completely mechanized method already mentioned. The 200-grit wheel is used to smooth up the specimen. The initial cost of the two wheels and the grinding head, exclusive of the motor, need not exceed $15 to $20. It might be noted that good machine shop practice demands a surface speed of about 5000 feet per minute for fine grinding.

The next step is polishing by hand on emery papers laid flat on a piece of glass or cast iron. An economical size to cut the paper is about 4½ by 2⅞ in. The required grits are 1G, ½, 0, 00, 000, and 0000, in the Behr-Manning line. Not all the various grades will necessarily have to be used on any one specimen, but the cost is so small that it is a good plan to have some of each on hand. For a great deal of work, only the 1G, 0, and 000 may be required, the operation being reduced to three steps after the specimen comes from the 200-grit wheel.

General Procedure. After the specimen reaches the stage of the microscope, it will be necessary to ascertain that the polished surface is level, or at right angles to the microscope axis. This is a very important adjustment on all microscopes not equipped with the inverted system. Methods for discovering whether the specimen surface is level in all azimuths have already been described. Raising and lowering the microscope tube from focus and watching for the concentricity of focus of the outer zones are easy and quick. A simple way to mount a specimen that may have to be tipped is to place it on a small wad of Plasticine. It can then be adjusted as required; with a little pressure on one edge, it will stay in its new position quite well.

The prism illuminator will give a more contrasty image than the plate illuminator, and it can be used to good advantage on all low-power or medium-power work; the glass plate will generally be used

American Society for Testing Materials, "Tentative Methods of Preparation of Micrographs of Metals and Alloys," Designation E2-39T, 1939. Particularly good on standards for particle size and for standard photomicrographs.

for high-power work. This plate will give considerable glare with a consequent loss of light. To reduce the exposure time to reasonable limits high-intensity light sources are a necessity. Often in metallographic work small fields are satisfactory, but for petrographic work large fields should be striven for. The magnification on the microscope can be made low, and a long bellows extension can be employed.

The use of green screens for metallography will suggest Ortho X film on account of its high sensitivity to green. Contrast process film is also very effective, particularly when a plate illuminator is required. It may be desirable to lengthen the exposure time for this slow film in order to get the contrast of the picture at a high enough level; otherwise faster film is suggested. There is now no need for insisting on plates rather than film even for very exacting work, although many metallurgists are still depending on W. & W. M plates, which have excellent contrast value. However, equally good results can be attained with the newer contrast process emulsions on cellulosic film.

Sec. 165. Photographing a Metal Specimen. Figure 254 is a photomicrograph of a polished section of beryllium bronze, unetched. The specimen was prepared by hand. A piece about ½ inch thick was sawed from a cast bar about ¾ inch in diameter. The first grinding was made with plenty of water on the 60-grit wheel, the specimen being held very lightly. As soon as the saw marks were ground out, the specimen was transferred to the 200-grit wheel and, with plenty of water, ground lightly until all the marks of the previous grinding were eliminated. It was then polished by hand on the 1G grade of paper, dry. The next step was to the 0 paper, then to the 000, and finally to the 0000, the whole procedure taking less than an hour. The time spent on each paper was determined by examining the specimen under the microscope. At each change to a paper of finer grade, the specimen was lapped with a motion across the paper at 90° to the motion used on the previous paper; in this way all the marks of abrasion were running in one direction before the specimen was passed to the next paper. The final lapping left only slight marks, which were scarcely visible on the metal surface.

The next step, etching, was carried on with the potassium dichromate solution listed in Table XXXI. The results are shown in Fig. 255. When dry, the specimen was mounted on a small wad of Plasticine on a microscope slide. It was examined at low magnification to select a field and photographed at 500×. The optics used are stated in the legend under the picture.

Sec. 166. Photographing a Polished Ore Specimen. This specimen of nickel ore shown in Fig. 256 was prepared on the Mann polishing

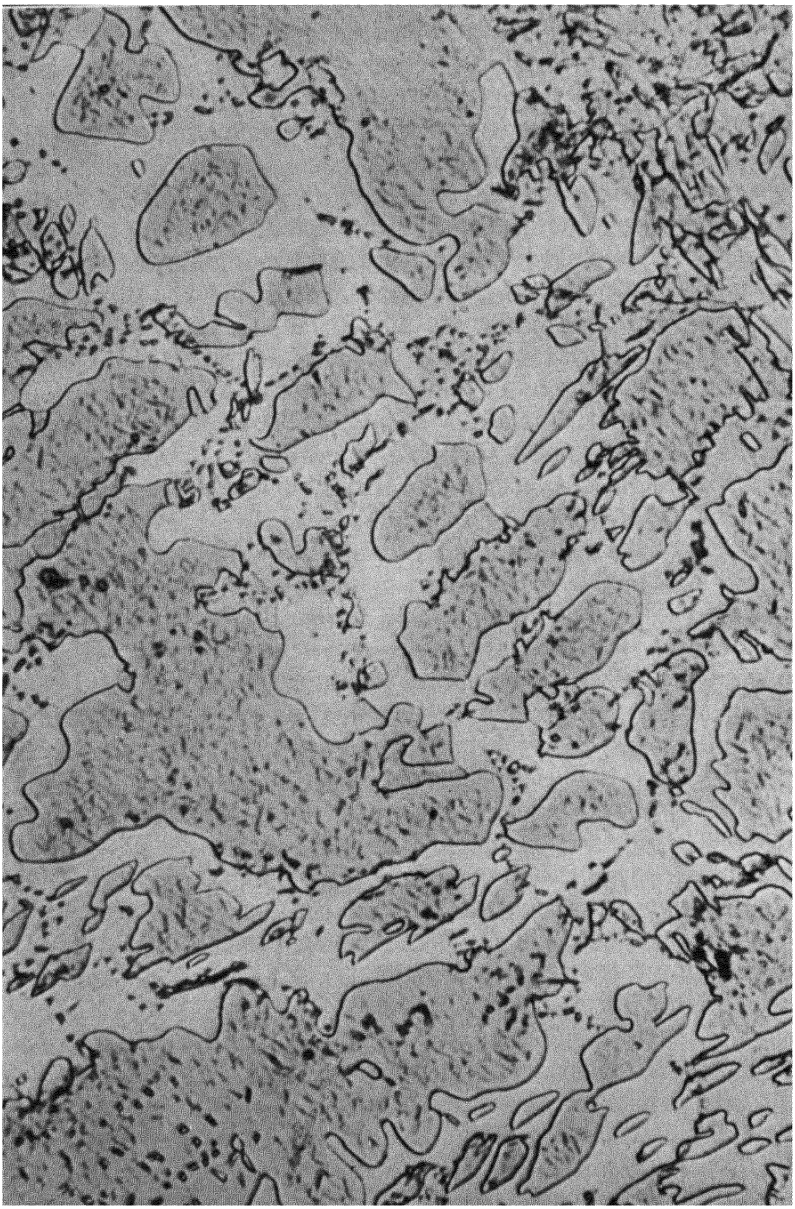


FIG. 254. Polished but unetched 2¼ per cent beryllium bronze, ×500. Cf. Fig. 255. Objective, Zeiss 8-mm achro. short mount; ocular, Homal I; prism-type vertical illuminator; illumination, 500-watt projection lamp, ground glass. Filters, Wratten No. 58; Eastman Commercial Pan film: developer, D-61a.

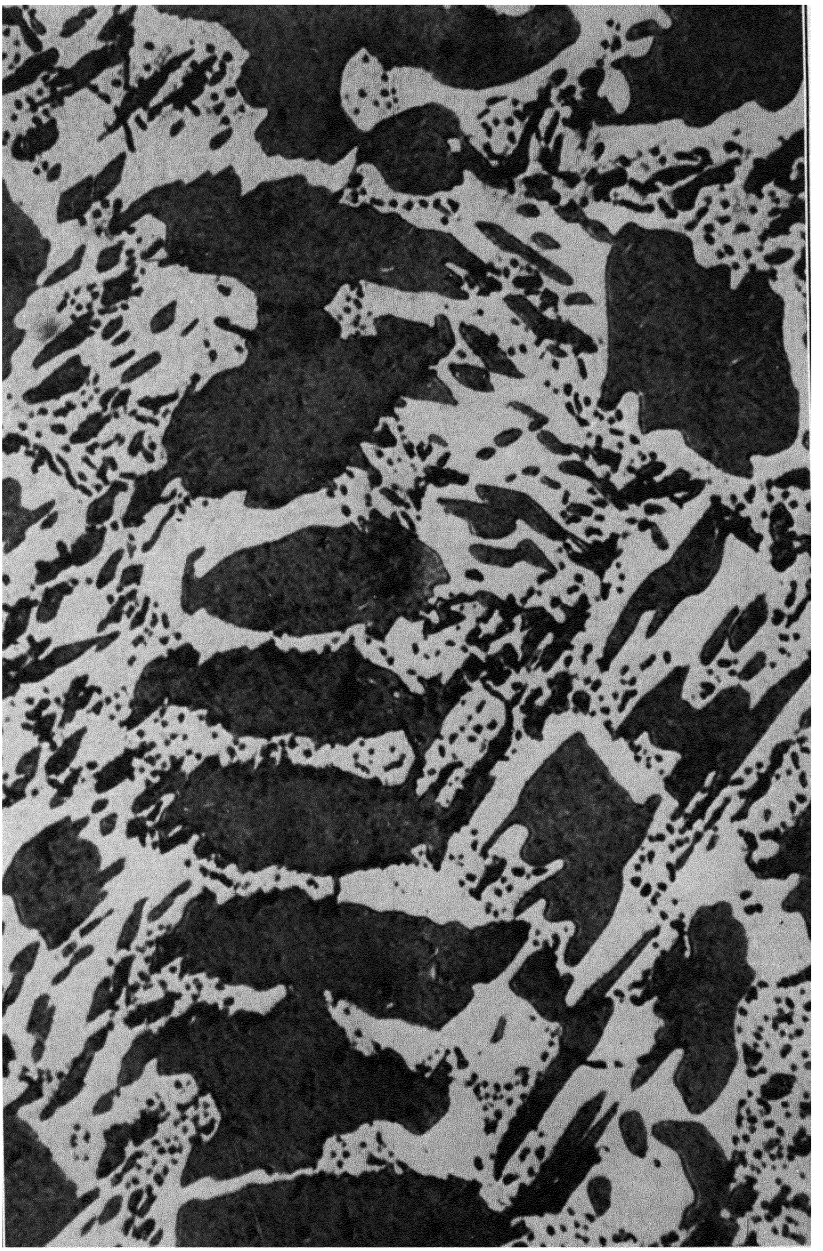


FIG. 255. Etched 2¼ per cent beryllium bronze, ×500. The same data as for Fig. 255.

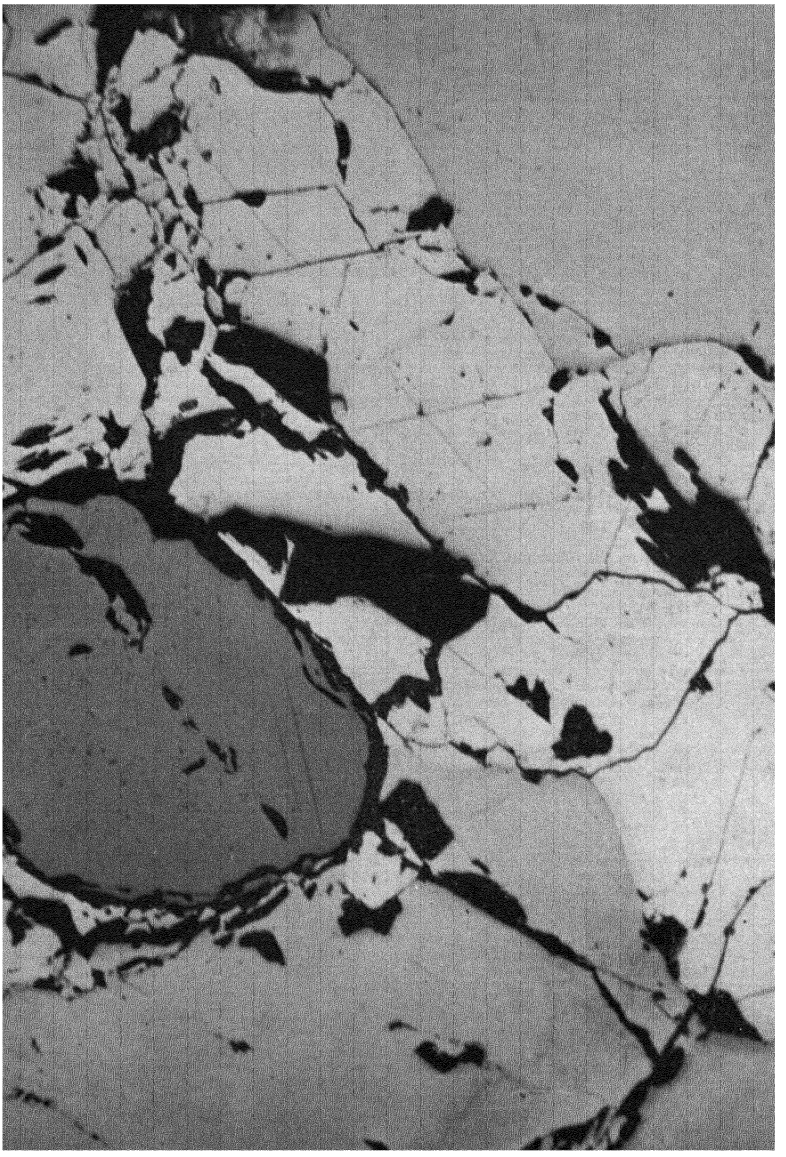


FIG. 256. Nickel ore, polished surface, ×300. Objective, Zeiss 8-mm achro.; ocular, Homal II; prism-type vertical illuminator; illumination, 500-watt projection lamp, ground glass; filters, Wratten No. 8; Eastman Panatomic X film; developer, D-61a.

apparatus at Columbia University. It was obtained through the courtesy of Professor Chas. A. Behre. Through the center of the field runs a streak of pentlandite with pyrrhotite on the right and left. The large dark mass at the bottom, slightly left of the center, is magnetite, the crystalline formation of which shows five sides of the octahedral crystal. The black areas in the pentlandite are principally holes; some of the black areas around the magnetite are quartz. Faint traces of the lapping process are visible.

Ore specimens present a problem. The textbooks describe light-colored yellowish or bluish areas or slight tinges of other colors, all of which are not only difficult to recognize by the uninitiated, but also troublesome to photograph. In the present specimen the pyrrhotite, under the illumination used, showed as a very light blue, the magnetite as a light steel blue, and the pentlandite as white; the color of pentlandite in a polished section is given as yellow. A light yellow filter aided in the contrast between the pentlandite and pyrrhotite. The picture was taken on panchromatic film. It would be interesting to see a comparison picture on a regular emulsion.

Sec. 167. Photographing Smooth but Unpolished Surfaces. Figure 257 shows a photomicrograph of a piece of steel taken by vertical illumination, giving the effect of bright field. The specimen was part of a steel scale as it came from the machine shop; the final grinding marks are clearly shown. The blotchy appearance is caused by staining. Figure 258 is the same subject, but the illumination was by a Silverman illuminator to produce a dark-field effect. In this particular case the bright-field illumination probably shows the greater amount of detail.

For specimens with surfaces rougher than that shown, and for transparent specimens such as glass surfaces, the Silverman illuminator, Ultropak, or Epi condenser are by far the best means of illumination, but the error of installing systems of vertical illumination instead of oblique lighting methods is frequently encountered. It is a good rule to use the Silverman illuminator, or its equivalent, for all unpolished surfaces.

GROUP IV

HISTOLOGICAL SPECIMENS

Sec. 168. Description of Material. Material included in this group consists of botanical and biological specimens which have been cut into thin transparent sections on a microtome. In addition, it includes any material which can be so cut, such as sections of paint film and similar



FIG. 257. Surface of a steel scale ×150. Cf. Fig. 258. Objective, Leitz, 16-mm apo. not corrected for use without a cover; ocular, Homal II; prism-type vertical illuminator; illuminator, 300-watt projection lamp; filters, Wratten No. 58; Defender, Pentagon film; developer, D-61a.

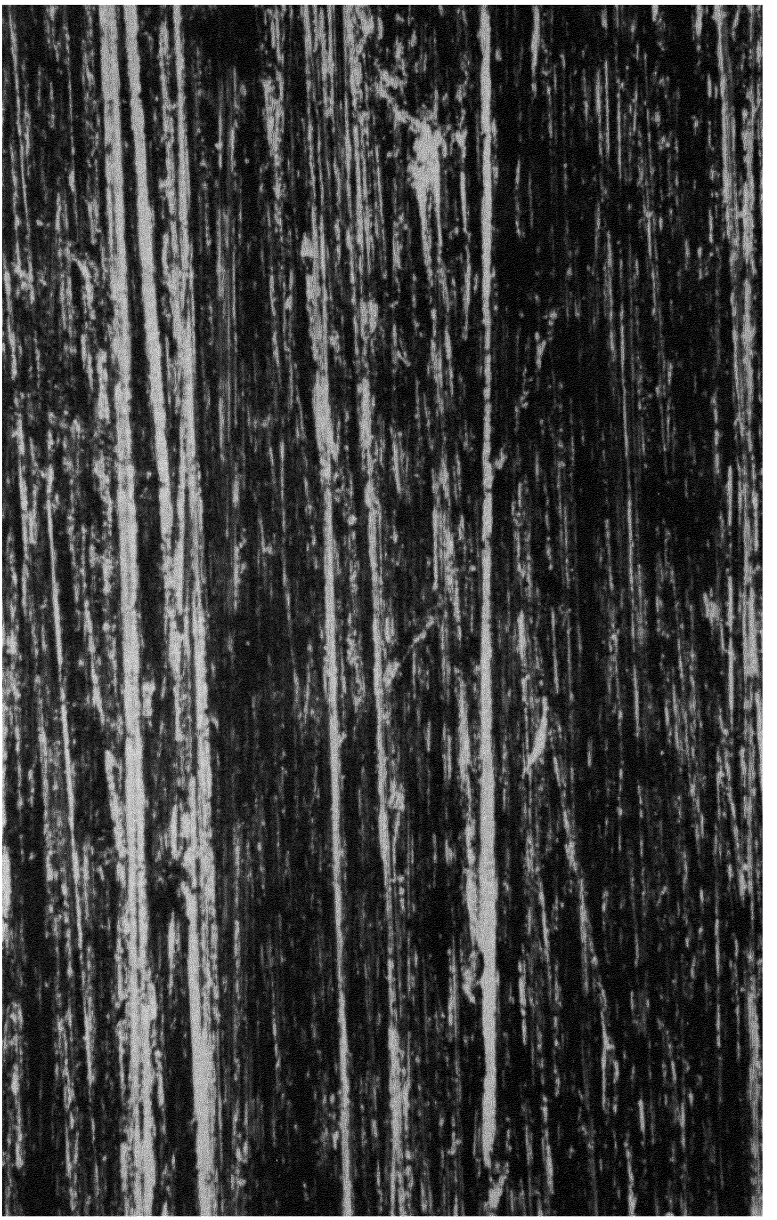


FIG. 258. Surface of a steel scale ×150. Same data as for Fig. 257 except that a Silverman illuminator was used to give a dark-field effect.

substances. The sections vary in thickness from about 1 μ, which is rare, to about 100 μ. As a rule, a section is thicker than the field depth of the objective used to photograph it, but in estimating the thickness of the specimen the tendency is to consider it as too little rather than too great. Biological and botanical specimens are generally stained. This treatment produces absorption images. These specimens are often mounted in a medium of like index to give the maximum transparency. The stained parts show up well, but anything not taking the stain, or any minute parts of the specimen from which the stain has been washed, may go unnoticed, as for instance, the starch particles stored in the large cells in the cross section of *Ranunculus acris*, Fig. 263. For photomicrographic purposes, thick sections can be handled better if mounted in a medium of higher index because field depth is thereby sensibly increased.

EXAMPLES

Biological specimens, Sec. 169.
Botanical specimens, Sec. 170.
Sections of other material, Sec. 171.

Illumination. The lighting can be by Method I or III. Ordinarily either is completely satisfactory since the magnification is usually low, 150 to 500 diameters. Chromatic filters play an important part in the photomicrography of stained sections. Subject to special conditions, the mercury-vapor discharge tube furnishes one of the best sources of light, because it can be modified to light of daylight quality without great loss of intensity and because it is strong in the green and blue components. If Method II is used the loss of intensity will, of course, be much less than when a diffusing plate is in the light train. High-intensity sources, such as the 400-watt projection lamp, are useful for thick and closely organized specimens.

Apparatus. Any biological microscope having the necessary refinements for centering the lenses will suffice for this sort of work. Large cameras will naturally be needed for large fields, and in this connection it might be said that 5 by 7 inch prints made by contact are much to be favored over the same size arrived at by enlargement. In addition, the long bellows draw on the larger cameras is a great advantage because it makes possible the use of relatively long-focus objectives, with their accompanying greater field depth, and yet adequate magnification is assured.

Apochromatic objectives and the best condensers are certainly suggested, as are flat-field oculars of the Homal or Hyperplane type. As a rule, the specimens are covered, and the higher objectives should be

carefully corrected by means of the star test. If dark field should be desirable, the central stop system can be used for low-power and the paraboloid dark-field condenser for medium-power work.

The preparation of the specimen needs much highly specialized apparatus and accessories for extended and serious work. A critical discussion of this subject is entirely beyond the bounds of this volume, but several important reference books on microtomy are mentioned.[16]

For serial sections, rotary microtomes are generally recommended; but for general and diversified work a microtome with a knife clamped in a heavy carriage sliding on broad V-section ways, similar to the carriage on a lathe bed, is to be preferred. The small microtome of Spencer, which clamps to a table top and has a hand-operated knife, does remarkably well on many miscellaneous subjects; several of the pictures in this book were made from specimens prepared on this type of microtome.

No microtome, whatever its cost, will perform well unless the condition of the knife is nearly perfect. It takes considerable care and skill to hone and strop a knife expertly enough for it to have a clean sharp edge, free from nicks when examined under the microscope. (See the photomicrograph of the edge of a safety-razor blade magnified 500 diameters, Fig. 287.) A knife must be in such condition that it will cut a section cleanly and without mutilation. The safety-razor-blade holders, which can be used on some microtomes, make it possible to avoid the long tedious care that is demanded by a good microtome knife, but even the safety-razor blade will cut better if given some attention. First it should be washed free of anti-rust compound, then stropped on a simple type of stropper. Running the edge of the blade between the thumb and forefinger several times puts it in splendid condition; the human skin seems to be especially efficacious in giving the final edge to a sharp tool. Knives should always be honed after use, and the edges should be wiped with a soft chamois before they are put away.

In contrast to the highly precise and smoothly working microtomes, it is surprising how much good work can be done by freehand sectioning. Very useful sections can often be obtained by simply holding the specimen in the hand and cutting it with a knife or safety-razor blade. Sections of many substances less than 100 μ are easily cut in this manner.

Additional apparatus, always in evidence in the purely histological

[16] A. B. Lee, *Microtomist's Vade-Mecum*, ninth edition, Blakiston, 1928.
McClung, *Microscopical Technique*, Hober, 1929.
S. H. Gage, *The Microscope*, seventeenth edition, Comstock, 1941.

laboratory, is a suitable collection of dishes and glass containers for specimens and solutions, since many of the solutions can be used many times. A collection of stains and fixing, clearing, and killing solutions is always at hand.

Clarity of detail is the main distinguishing mark of a good photomicrograph, and generally it is evidence of faultless technique in the preparation of the specimen. Nowhere is the technique of preparation more noticeable than in photomicrographs of histological specimens. In the interests of good photomicrography, therefore, the technician is earnestly advised to see to the proper preparation of his specimen or, if the specimen is such as to demand special apparatus or the application of an unfamiliar technique in its preparation, to consult an expert who has the necessary equipment and knowledge. The specialist can so mount a specimen that the points which the photomicrographer desires to stress will be brought out in the photomicrograph.

General Procedure. With a well-prepared specimen, the choice of a field is of next importance. If the photomicrographer is not sufficiently familiar with the nature of the specimen to be able to select a suitable area for photographing, he must have advice. It is a good idea to invite the client to select his own field in order to avoid later arguments. However, discretion should be exercised, since the field selected by the interested party may prove to have poor photomicrographic possibilities. Compromises may have to be made regarding what constitutes an acceptable field.

The selection of proper optical filters is vital to the success of photographs of stained sections. A study of the specimen may show deep staining, which, in a thick section, is likely to produce harsh contrasty negatives, lacking in detail. Prints giving strong black-and-white effects with a lack of intermediate tones usually come from poor negatives, for they indicate blocking-out of detail. Fast film, with its soft rendition, is of great assistance in softening up contrasty subjects.

Sec. 169. Biological Sections. Figures 259 and 260 are sections of guinea-pig skin.[17] The first picture was taken to show the appearance after the hair stubble was removed by shaving; the second, after a depilatory was used. Both sections were cut very thick to ensure having some of the hairs in their natural state. The magnifica-

[17] The following references may prove useful in addition to those already mentioned:

Aram A. Krajian, *Histological Technique,* Mosby.

Bailey (Smith), *Histology,* tenth edition, Williams and Wilkins, 1940.

Harvey Ernest Jordan, *Textbook of Histology,* eighth edition, Appleton-Century, 1940.

Karl A. Stiles, *Handbook of Microscopic Characteristics of Tissues and Organs,* Blakiston, 1940.

tion of 100 was obtained with a 30-mm objective, a 5× ocular, and a sufficiently long bellows. Staining was not resorted to because the strong contrast would have blocked out all detail of the hair.

The photomicrograph of striated muscle, Fig. 261, is a good example

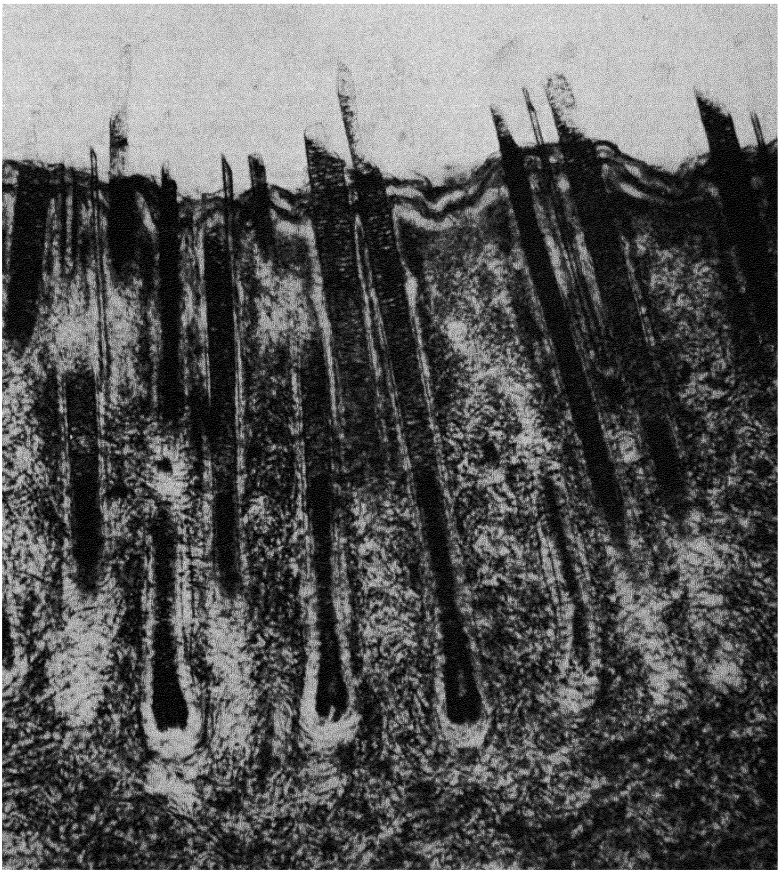


FIG. 259. Section of guinea-pig skin ×100. The hair has been removed by shaving. Objective, 30-mm apo. Zeiss; ocular, Leitz 5× periplan; condenser, Leitz long-focus; illumination, 250-watt projection lamp, method I; filters, Wratten No. 58 plus 15; Eastman Panatomic X film; developer, D-11.

of the handling of a contrasty subject. The specimen was stained with iron-haematoxylin, which shows the tissue in great detail. With light of daylight quality and Panatomic X film, the negative would have had poor printing qualities, but with a fast film, Tri X Pan, a good negative was obtained. A red filter was used, but it is probable that a yellow one would also have given a good negative. Sometimes, on

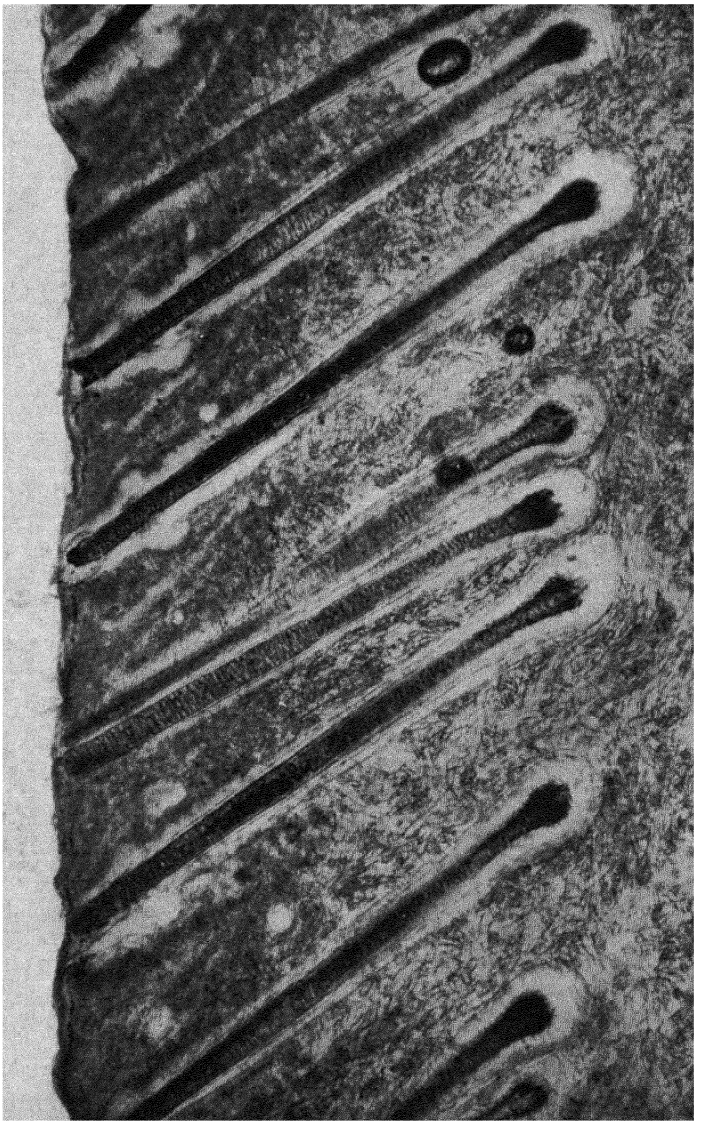


FIG. 260. Section of guinea-pig skin ×100. The hair has been removed with a depilatory; cf. Fig. 259. The microscopical and photographic arrangements were the same as for Fig. 259.

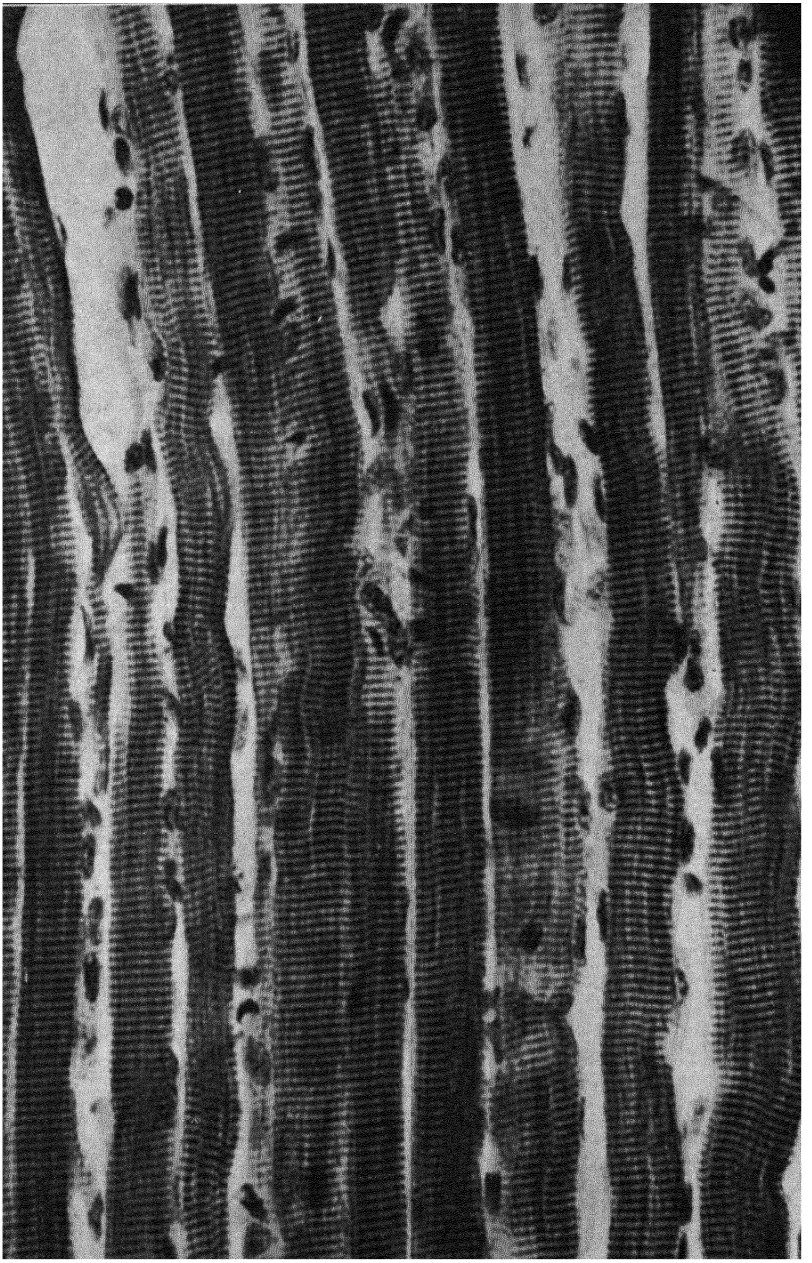


FIG. 261*A*. Striated muscle fibers ×600. Objective, Leitz 8-mm apo.; ocular, Homal I; condenser, Leitz achromatic, oiled to slide; illumination, 500-watt projection lamp, method I; filters, Corning No. 348; Eastman Tri X Pan film; developer, D-1.

stained subjects, there may be a fairly wide range of suitable filters, for the stain passes a wide band of the spectral colors. In addition, personal preference plays a part, and effects in a print pleasing to one person may be displeasing to another, even though the detail may be equally plain in both pictures.

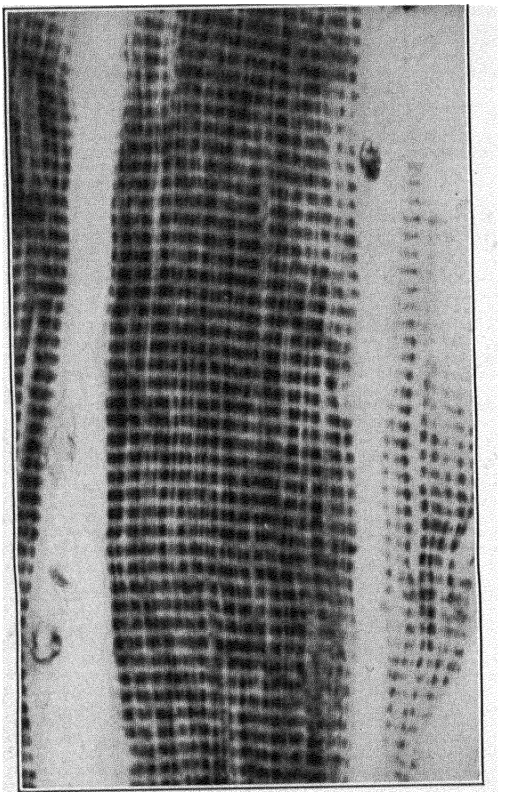


FIG. 261*B*. Striated muscle fiber ×1200. The striae are well resolved. Objective, 3-mm apo., Leitz; ocular, Homal IV; condenser, achromatic-aplanatic, Leitz; illumination, General Electric mercury-vapor discharge tube, H3, method II; filters, Wratten No. 45 plus 47; Eastman Contrast Process film; developer, D-19.

In the picture shown in Fig. 261, it is evident that the muscle fibers were absorbing a good deal of light; in fact, a 500-watt lamp had to be used in conjunction with illumination by Method I. This strong light on the dense fibers would ordinarily give a very displeasing contrasty effect and would mask considerable detail unless proper steps were taken to counteract it. In addition to the fast film and red filter, a developer such as formula D-76 giving softer effects than D-1 might have been used.

Sec. 170. Botanical Sections. All the soft woods and pulpy material can be sectioned without softening, but the hard woods can be cut only after they have been subjected to a softening process. Rather complete directions for sectioning hard woods and the preparation of other plant material for the microscope are given in the references listed below.[18] Steaming the specimen is recognized as good technique for softening hard woods, as is cooking it in a pressure cooker with alcohol or alcohol and water. However the specimen is softened, it must be soaked long enough in dilute hydrofluoric acid to dissolve the crystals of calcium oxalate and other mineral compounds generally associated with woody material. Some woods can be cut immediately after the softening process; others of open cell structure need the added support given by infiltration with celloidin (Koehler). Figure 262 shows a cross section of *Pinus strobus* (white pine). The picture was taken to show a growth ring. The specimen was stained and mounted in balsam, but it might have been left in its natural state and mounted in a high-refractive-index liquid or resin. Pictures of this sort are not troublesome to take unless the specimen is rather thick and does not lie flat. When the sections are freshly cut, it is sometimes advantageous to mount them in a compressor type of cell before photography so that any tendency to curl is under complete control. In Fig. 262, additional contrast, obtained by means of contrast filters, was unnecessary; in fact, it would have been poor technique. The magnification is sufficient to show all detail, and yet a large enough area of the specimen is included to demonstrate the growth ring nicely. Only occasionally is a magnification higher than this required on this type of subject.

Figure 263, a cross section of the root tip of *Ranunculus acris* (buttercup), was taken with the same filter as was used for the picture in Fig. 262, both specimens being stained red. All detail in the cell walls is lacking. The substitution of a green filter, as in Fig. 264, for the daylight filter has a peculiar result. It might be expected that a filter complementary in color to the specimen would produce additional contrast, but instead the opposite occurred: the contrast was reduced, showing that much green light is transmitted by the stain.

[18] Arthur Koehler and Eloise Gerry, *Preparing Woody Tissues for Making Microscopic Mounts*, Forest Products Laboratory, U. S. Dept. of Agriculture, 1927.

C. J. Chamberlain, *Methods in Plant Histology*, fifth edition, 1932.

"Microscopic Examination of Woody Material," *Watson's Microscope Record*, May, 1933.

E. C. Jeffery, "Improved Methods of Softening Hard Tissue," *Botanical Gazette*, 1928.

D. A. Johansen, *Plant Microtechnique*, McGraw-Hill Book Company, 1940.

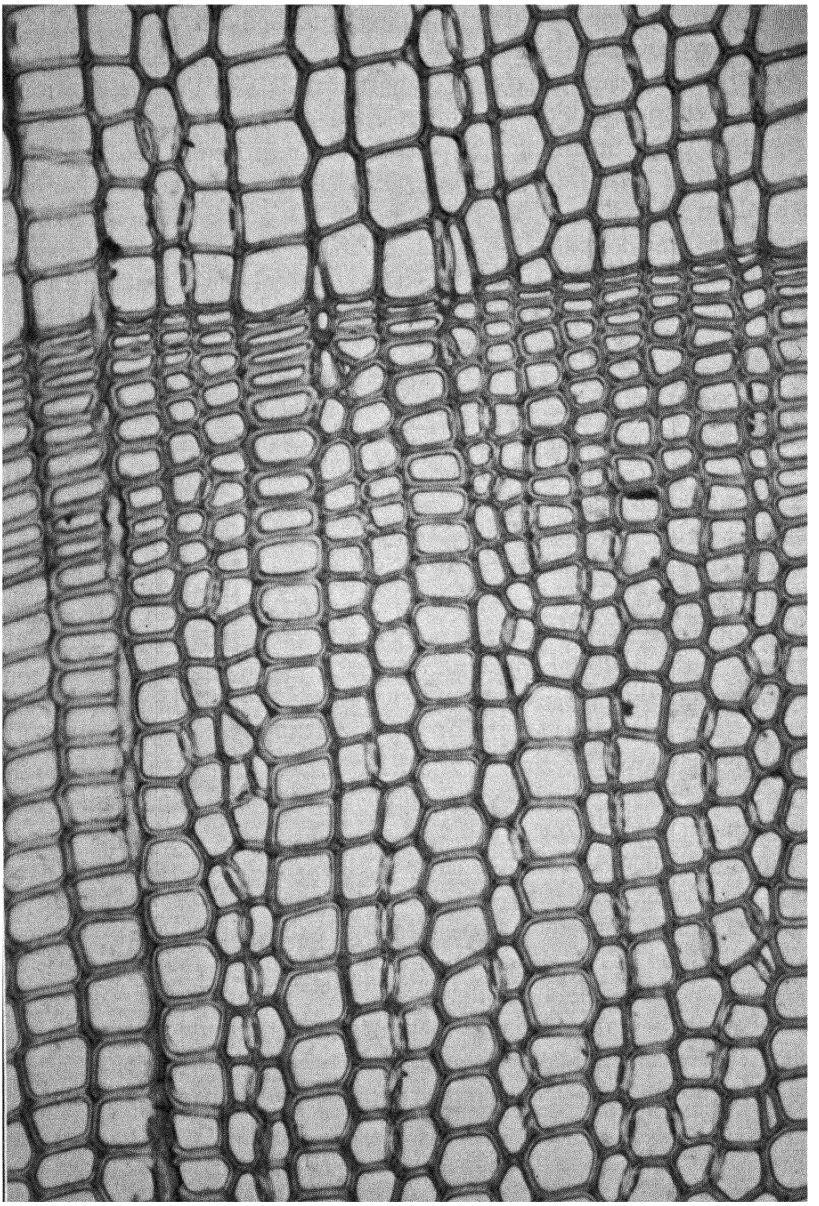


FIG. 262. *Pinus strobus*, cross section ×360. Objective, Leitz 16-mm apo.; ocular, Homal I; condenser, Leitz achromatic, top lens removed; illumination, 500-watt projection lamp, method III; filter, Bausch and Lomb daylight; Eastman Panatomic X film; developer, D-19.

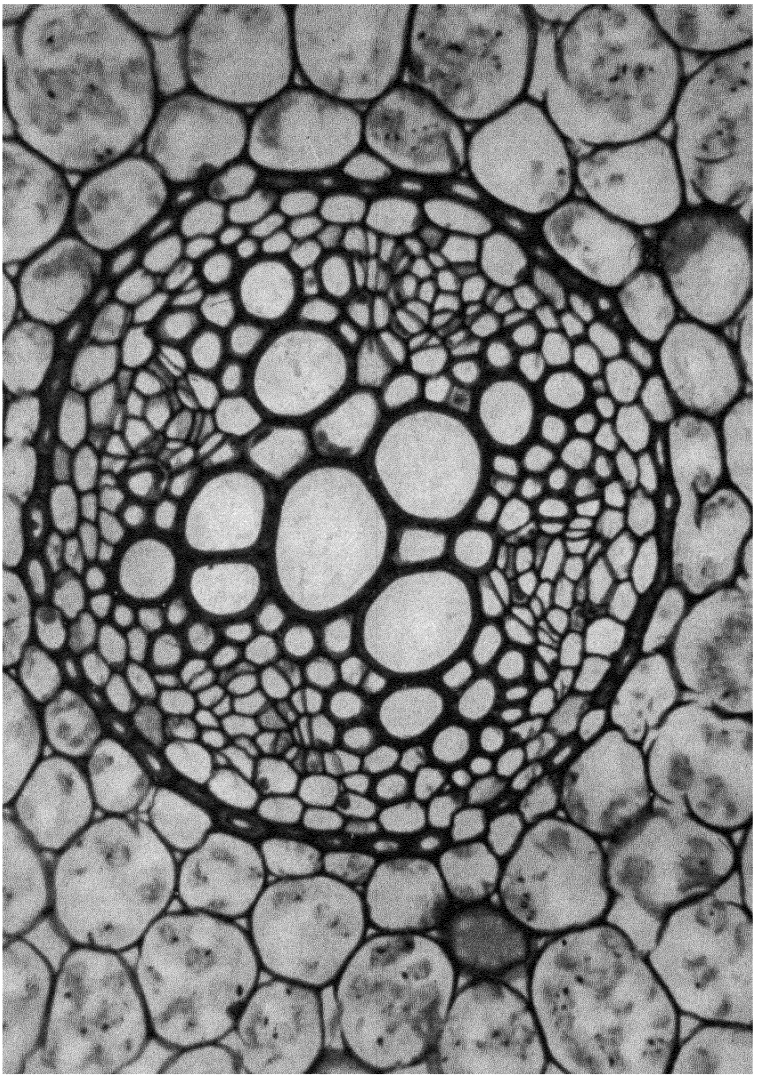


FIG. 263. *Ranunculus acris*, cross section of root tip ×360. Cf. Figs. 264 and 265. Objective, Leitz 16-mm apo.; ocular, Homal I; condenser, Leitz achromatic, top lens removed; illumination, 500-watt projection lamp, method III; filter, Bausch and Lomb daylight; Eastman Panatomic X film; developer, D-19.

Figure 265, taken with a yellow filter, shows still greater detail in the cell walls. It might be noted that the storage of starch is seen in the large cells shown in Fig. 263, nearly as plainly in Fig. 264, but

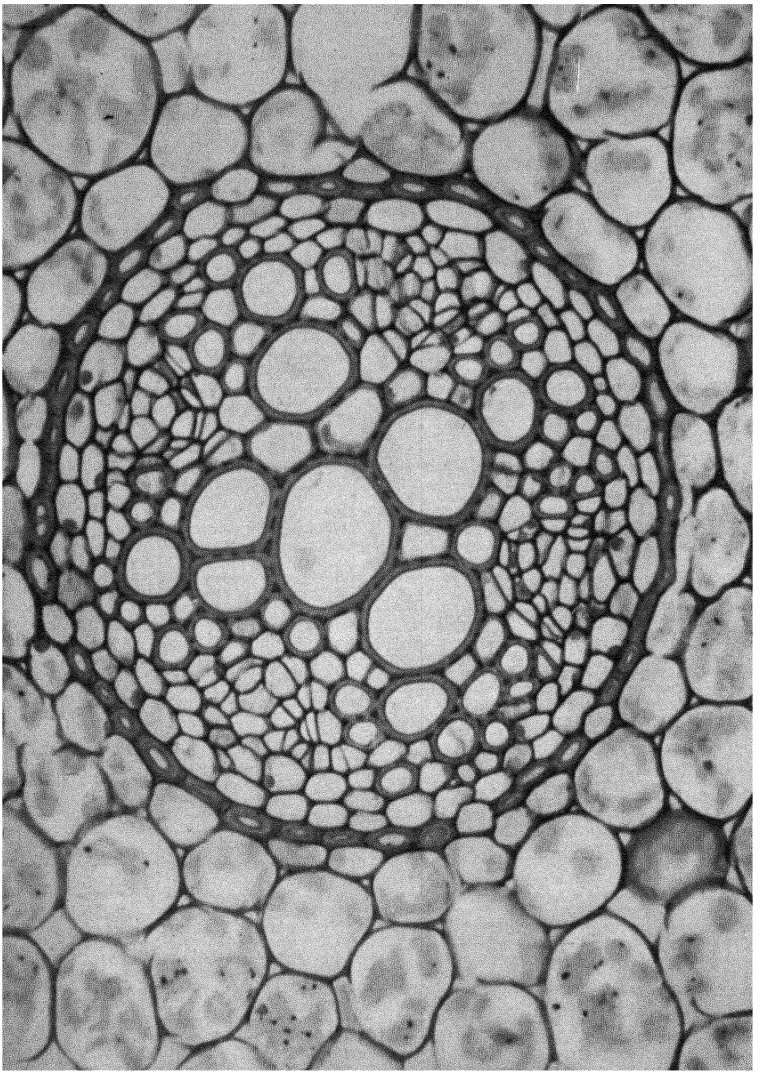


FIG. 264. *Ranunculus acris*, cross section of root tip ×360. Cf. Figs. 263 and 265. The microscopical and photographical arrangements were the same for this as for Fig. 263 except that the daylight filter was changed for a Wratten No. 57.

hardly at all in Fig. 265. The starch is identified by its characteristic cross in polarized light.

Sec. 171. Sections of Miscellaneous Material. The art of making thin sections, either with the microtome or by grinding, can be ap-

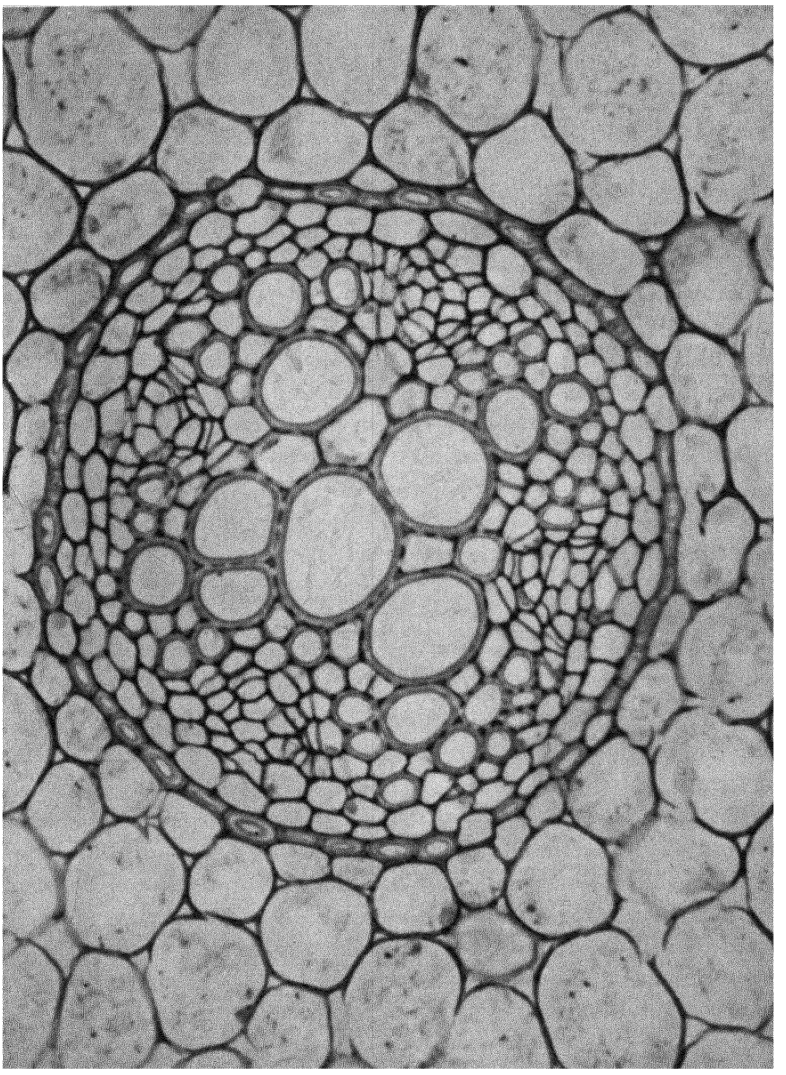


FIG. 265. *Ranunculus acris*, cross section of root tip ×360. Cf. Figs. 263 and 264. The microscopical and photographical arrangements were the same for this as for the two preceding figures except that a Wratten No. 15 filter was used here.

plied to many subjects which would be very difficult or even impossible to photograph if prepared by any other method. Figure 192 shows a section of photographic film cut on a small table microtome. The film was cemented to a block of white pine and cut in the usual way.

The underlying theory on which such technique depends is that the supporting medium, whether for metal, wood, or any other substance, should always be harder than the substance to be sectioned. Infiltration with wax, resin, or celloidin is usually required for porous specimens in order to support cell walls and prevent the collapse of tiny cavities.

The specimen for Fig. 266 was dried bread. It was infiltrated with balsam and then ground as a petrographic specimen. The specimen, brought from Egypt by an expedition headed by Ambrose Lansing and William C. Hayes and sponsored by the Metropolitan Museum of Art, New York, dates back to the eighteenth dynasty and is nearly 3500 years old.[19] The material as received at the laboratory was in the form of small pellets, somewhat like small balls of well-dried clay and just about as hard. It was decided to impregnate them with balsam, mount them in Bakelite, and grind them to a thin section. This sectioning work was carried out at Columbia University by Bird.[20] The sections were successfully made, and Fig. 266 illustrates the structural detail of an article of diet prepared for the table in 1494 B.C.

GROUP V

MICROSCOPIC CRYSTALS

Sec. 172. Description of Material. For purposes of discussion, this group can be divided into two parts. One is concerned with the photomicrography of crystals which form as precipitates as the result of the addition of a reagent to a test drop which holds some other chemical in solution. This technique is standard procedure for the chemical microscopist, who bases his analysis of a substance upon the recognition of elements through new compounds formed by the reaction of certain reagents. The other part is the photomicrography of crystals formed slowly from a solution as it evaporates. The technique includes recrystallization; a new product is not usually formed.

EXAMPLES

Part I, Rapid precipitation of crystals as in chemical microscopy, Sec. 173.
Part II, Slow precipitation of crystals as from evaporation, Sec. 174.

Illumination. The method of lighting will depend a great deal on whether the crystals under examination are the product of chemical

[19] Charles A. Glabau and Pauline F. Goldman, "Some Physical and Chemical Properties of Egyptian Bread," *Cereal Chem.*, **15**, 295, 1938.

[20] Bird has since left the grinding laboratory, which is now in charge of George Rév.



FIG. 266. Egyptian bread in thin section ×330. Objective, Zeiss 16-mm apo.; ocular, Zeiss 15× compensating; condenser, Zeiss aplanatic; illumination, 108-watt tungsten-ribbon filament; method II; filters, Wratten No. 15 plus 60; Eastman Commercial Pan film; developer, D-61a.

reaction (spoken of under Part I) or whether they are of the type that forms slowly (mentioned under Part II). In the first case the new compound is insoluble, or nearly so, in the test drop on the stage of the microscope, and consequently the formation of the crystals is for the most part rapid. The crystals may be in motion, or they may be at rest on the slide with other crystals floating over them on the surface of the test drop. Obviously, under such conditions instantaneous exposures may be required to " stop motion "; high-intensity lamps are thus indicated. The best and most convenient seems to be the 400- or 500-watt biplane tungsten-filament projection lamp used according to Method II; all the pictures shown in this group were taken with a lamp of this type. An alternative would be a carbon arc, or perhaps, if available, the mercury-vapor discharge tube H6. However, the projection lamp is much easier and simpler to handle than other high-intensity sources, and it will permit pictures to be taken with exposures of $\frac{1}{25}$ to $\frac{1}{100}$ second, or possibly even faster. Since filters may be required for certain colored crystals, all the light from the lamp may not be useful, but, even so, exposures can generally be made at $\frac{1}{10}$ to $\frac{1}{25}$ second, which is sufficiently fast for many subjects.

For crystals obtained through evaporation, less light is needed. The crystals settle out on the slide and grow slowly. Oftentimes they can be evaporated to dryness, and with a mounting medium added the slide can be made permanent or semi-permanent. Since a short exposure time is no object, almost any lamp can be used. The method of illumination would be either I or III.

Filters, as already mentioned, play a part in the illumination. All strongly colored crystals should be photographed with a chromatic filter of like color. If the crystals are very small, a contrasting filter may be useful, but as a rule these crystals will be neutral in color. Strong blue filters will seldom be needed to aid in resolution because the size of the most minute detail is generally well within the resolving power of the lens system. Most chemical microscopists work with either a petrographic microscope or its simplified form, the chemical microscope. The upper end of the drawtube of the chemical microscope may be fitted for an ocular of larger diameter than that used on the biological instruments. As the diameter of the larger standard size will probably be 30 mm, eyepiece cameras will need to have an adaptor built to fit into the large drawtube for the benefit of the camera fitting and the small standard-size ocular. When achromatic objectives are used, a green filter will be an optical asset to the whole system.

Apparatus. An eyepiece camera of the 9 by 12 cm size is strongly recommended as best for chemical microscopical work. Such a camera permits the microscope to be used in the regular way and does not interfere with the analysis. After the exposure time has once been established for a given set of conditions, it pays to have half a dozen to a dozen plate holders at hand all loaded and ready to use. Fast plates or film are often convenient, particularly where the contrast of the subject is great; they decrease the time of exposure and give negatives from which good prints can be made on number 3 or 4 paper.

For photographing crystals obtained by slow evaporation, any kind of camera will answer. The long bellows draw of the 5 by 7 inch size is always useful. Since time is not of the essence, the picture can be composed to suit the shape of the film, and the bellows can be regulated to modify the magnification.

For photographing crystal formation over a long period of time, which calls for a picture every few minutes, an eyepiece camera with a Leica or Contax head is an excellent choice. The spool will hold film for 36 exposures. The image distance being very short serves to conserve light. However, the pictures will have to be projected or enlarged for inspection, so that for any work other than this the larger film will be preferable.

Any microscope is suitable if it has the adjustments that are required for critical work, as previously discussed. The optics recommended for chemical microscopy are good achromatic objectives with oculars affording large flat fields. Eyepieces with intermediate corrections will do well, but it pays to try out the combination of objective and ocular on a 5 by 7 inch film before it is used on an eyepiece camera. Film 9 by 12 cm or 3¼ by 4¼ inches is so small that as much of it as possible should be made use of. Undeniably, apochromatic objectives and compensating eyepieces give beautiful pictures, but it is scarcely worth while to risk damaging them by using them over acid solutions which may often be quite warm. For crystal formations which have been obtained from evaporation as discussed in Sec. 157, apochromatic objectives and amplifying oculars can be used to good advantage.

General Procedure. Boričky,[21] Behrens,[22] Behrens-Kley,[23] and Chamot[24] are some of the earlier names connected with chemical

[21] Emmanuel Boričky, *Elemente einer neuen chemische-mikroskopischen Mineral- und Gesteins-Analyse*, 1877.

[22] H. Behrens, *Mikrochemische Methoden*, Amsterdam, 1882.

[23] Behrens-Kley, *Mikrochemische Analyse*, L. Voss, Leipzig and Hamburg, 1915.

[24] Émile Monnin Chamot, *Elementary Chemical Microscopy*, New York, 1921.

microscopy. Short[25] gives a little résumé of this history of the science by mentioning the pioneers, many of whom were mineralogists. In addition, various journals have published numerous papers concerning specific tests for organic as well as inorganic compounds.

The technique of chemical microscopy — not to be confused with microchemistry, which consists, roughly, of the adaptation of ordinary chemical analysis to a micro scale — involves, as previously stated, the identification of elements and ions according to their reaction when treated with reagents on the stage of the microscope. For the most part crystalline precipitates are formed, and they may be photographed. The different methods of taking the material to be tested into solution, obtaining a test drop, and the reagents, and the application of the reagents are thoroughly discussed by Chamot and Mason[26] and by Short.[25] Both these works, particularly Chamot and Mason, devote considerable attention to the mechanics and optics of the microscope. The discussion of the work here will be confined merely to its photomicrographical aspects.

When a test drop on the microscope slide is treated with a reagent the preciptitation of crystals is generally fairly rapid. The function of the photomicrographer is to photograph the crystals in their most characteristic stage of development. As a test drop evaporates, crystals other than the diagnostic ones are sure to form, and a photograph at this stage would have but little value. Thus fast operation becomes important. All microscope adjustments should first be made with a test slide on the stage; the eyepiece camera should be mounted; and plate holders should be filled and ready for use. The first few films should be developed immediately after exposure to allow correction for timing or to make other adjustments as indicated. Thereafter several exposures may be made of one test drop and all developed at one time. The test drop should be small at the start, say 2 mm in diameter, and the reagent, if a solution, should be about the same size. The reaction should be carefully studied; the size and the concentration of the test drop can be varied to give fields which will photograph to the best advantage. Small quantities of water can be added to the standard-size test drop by means of a tiny pipette, the bore of

[25] M. N. Short, "Microscopic Determination of the Ore Minerals," *U. S. Geological Survey Bull.* 825, pp. 115, 116, 1931. A later edition was brought out in 1940.

[26] Chamot and Mason, *Handbook of Chemical Microscopy,* Vols. I and II, second edition, John Wiley and Sons, 1939 and 1941. The first volume deals largely with microscopy, in theory and practice, the formation of crystals under the microscope, and their recognition with polarized light. The second volume is concerned with the chemistry involved. In addition to tests for many elements it includes a scheme of systematic analysis as carried on under the microscope.

which is very small. Regulating the size of the reagent drop will also influence the distribution and formation of the crystals. In this way, with plenty of patience, it will become possible to find the optimum condition for crystallization to give an acceptable field. The crystals in the field should be well formed, and, since a photomicrograph is documentary evidence, the crystal formation as seen in the picture should not be in doubt. Fields selected to show the general characteristic appearance of a certain reaction may give almost no idea of the true appearance of the ideally formed crystals.

Clean slides are essential for chemical microscopical work. On a clean slide the crystals are more likely to be precipitated evenly, and, above all, the test drop will be spread out in such a way as to present a flat surface to the lens. A drop of aqueous liquid on a greasy slide will be lenticular in shape and may give the appearance of misalignment of optical parts. Crystal formation may follow scratches on the surface of a slide. Such scratches not only interfere with crystal growth but they may even show in the picture.

Magnification must be correct. Optics which give a suitable magnification with a long bellows draw will be inadequate for use with the 9 by 12 cm film on an eyepiece camera. There are two ways to make the images of the individual crystals large enough to be pleasing. One is to obtain the proper magnification on the microscope; the other is to search for fields with larger crystals. Owing to the depth of field required in these pictures, particularly those which show stars, crosses, and masses with long radiating needle-like structure, low objectives are recommended. High oculars can be used with them, and, with a little patience in finding large enough individual crystals, considerable control can be exerted over the size of the final images.

The working aperture of the system will be low on account of reduction by the condenser diaphragm. This will aid in obtaining field depth and good coverage of the plate or film. Resolution will probably not be a factor, but if it is, a special picture had better be taken for the one purpose of showing the desired detail. A separate picture can be taken to show a more comprehensive field. The reduced aperture will unfortunately increase the effect of shadows moving across the field and of any debris floating on top of the drop. However, with the side telescope arrangement it is easy to watch the field up to the moment of exposure and to choose a moment when the liquid around the crystals is comparatively clear. Many specimens will yield crystals which settle out easily and quickly on the slide after the reagent has been introduced to the test drop. In others the crystals will float over the surface of the drop for some time before coming to rest, and they

may have to be photographed in motion. The first trial or two should certainly be made with the edge of the test drop and the edge of the reagent drop in the field at the same time; then, when they are merged with a glass rod drawn out to a fine thread, the first reaction is easily visible. As the crystals grow in size, and the drop starts to evaporate, the appearance of the field will change, sometimes rapidly. The reagent itself, if in excess, will crystallize around the edges, and finally, as the crystals are exposed to air, they will become black around the exposed portion. The drop should then be discarded and another one started. After watching two or three tests, and knowing what kind of crystals to expect, one can easily search for and find good fields which lend themselves well to photography.

The procedure in handling crystals obtained from evaporation is considerably different from the above. There is plenty of time to carry on the work, and the slides may even be covered, as they seldom can be when the crystals are formed by chemical reaction. A drop of solvent can be placed on the slide and material added to it; then as the material goes into solution it can be watched under the microscope. After saturation has been reached a lowering in temperature, slight evaporation, or seeding will start crystallization. At this point a cover can be dropped on, and the whole process will be considerably retarded. Since there will be sufficient time to manipulate the microscope and camera, the larger camera may be used and the picture can be composed on the ground glass. Figure 241 shows a picture of sugar crystals — sucrose. A solution of sugar and water was first brought up to the saturation point in a test tube, a little alcohol was added to start crystallization, and a drop of the solution with the tiny well-formed crystals was transferred to a slide, covered, and photographed. Such mounts when sealed with a heavy oil or vaseline will keep for some time.

Recrystallization of this sort can be carried on in many ways. However, when the work can be carried on in a test tube and the crystals precipitated by the addition of another liquid which is miscible in the mother liquor, though not a solvent for the chemical in question, great control can be had over the fields and the size of the crystals. In addition, a supply of specimen is obtained which can be called on as required.

Sec. 173. Photomicrography of Crystals Obtained from Chemical Reaction. *Testing for Cobalt.* Reagent: potassium mercuric thiocyanate in solution. New product: cobalt mercuric thiocyanate. References: Chamot and Mason, Short. To obtain the result shown in Fig. 267, a drop of the reagent was placed close to the test drop

of 1.0 per cent cobalt nitrate solution; while the two drops were observed through the microscope they were merged together with a very fine glass rod. The flow of precipitated crystals was watched, and the slide moved to find the regions in which good crystal forma-

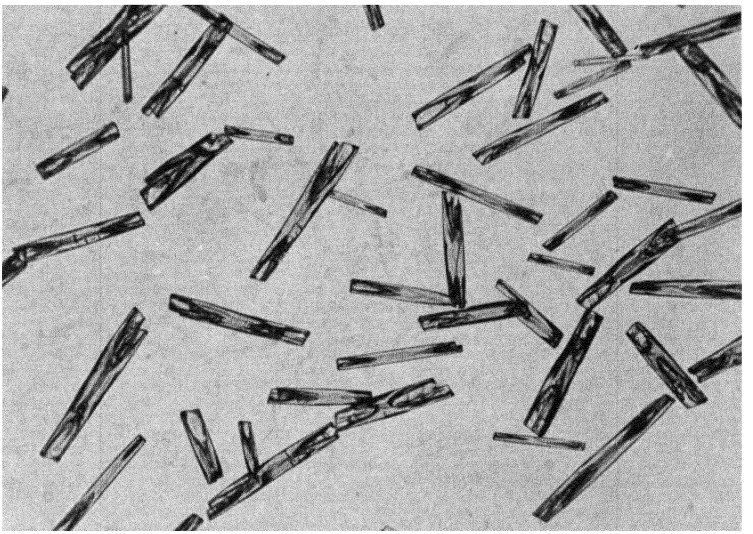


FIG. 267. Cobalt mercuric thiocyanate ×180. Objective, Leitz 16-mm apo.; ocular, Zeiss 15× compensating; condenser, Leitz achromatic, top lens removed; illumination, 400-watt biplane-filament projection lamp, method II; filters, Corning 428, Eastman Panatomic X; developer, D-1; exposure 1/10 second; Miflex camera attachment.

tion might be expected. This procedure was carried out several times, the size of the test drop being increased by the addition of water until, by estimation, it contained about 0.2 per cent of cobalt nitrate, the size of the reagent drop being kept the same as before. Thus a large drop of the solution to be tested was treated with about one-fifth as much of the reagent. A search then led to the discovery of the field shown in Fig. 267.

Crystals of cobalt mercuric thiocyanate are light to dark blue, depending on their size, thick crystals naturally being the darker. In preparing to make the picture shown in Fig. 267, Wratten filter 45, a fairly deep blue one, was tried first, but the effects of this would have been to make the crystals appear white in the finished print because the background and the body color of the crystals would have been approximately the same. Corning filter 428, a much lighter blue with

a much wider transmission band, was then substituted, and the results were as shown in the picture. Other fields might have been selected which would have been more representative of the effect observed through the microscope during one of these reactions.

Testing for Potassium. Reagent: perchloric acid. New product: potassium chlorate. Reference: Chamot and Mason. (Fig. 268.) A weak aqueous solution of potassium salt was tested with perchloric

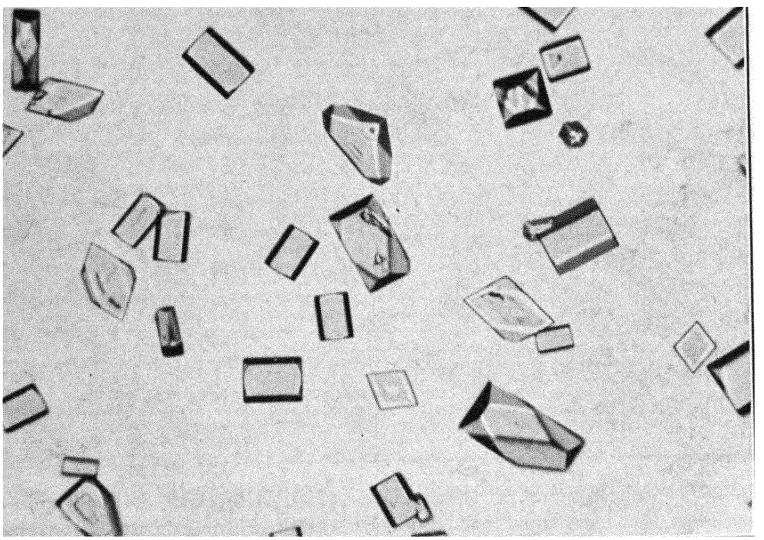


FIG. 268. Potassium chlorate ×180. Objective, Leitz 16-mm apo.; ocular, Zeiss 15× compensating; condenser, Leitz achromatic, top lens removed; illumination, 400-watt biplane-filament projection lamp, method II; filter, Bausch and Lomb daylight glass; Eastman Panatomic X film; exposure, 1/25 second; developer, D-1; Miflex camera attachment.

acid by the same technique as was followed for cobalt. The solution was diluted two or three times before satisfactory crystals were obtained. These colorless crystals are very little disturbed by other free-floating crystals, after a good field is found there is generally plenty of time to operate the shutter. The filter was a Bausch and Lomb daylight glass, and this might have been dispensed with. With an achromatic objective a strong green screen would have helped. If the addition of a green screen slows up the time of exposure too much, a faster plate or film can be used. Kodatron film and Eastman's Ortho X are very sensitive to green, and with a contrasty developer, such as D-11 or D-19, no trouble should be experienced in getting enough contrast.

Testing for Lead. Reagent: potassium iodide. New product: lead iodide. References: Chamot and Mason, Short. (Fig. 269.) The test drop must be of just the right dilution to get the best crystals for

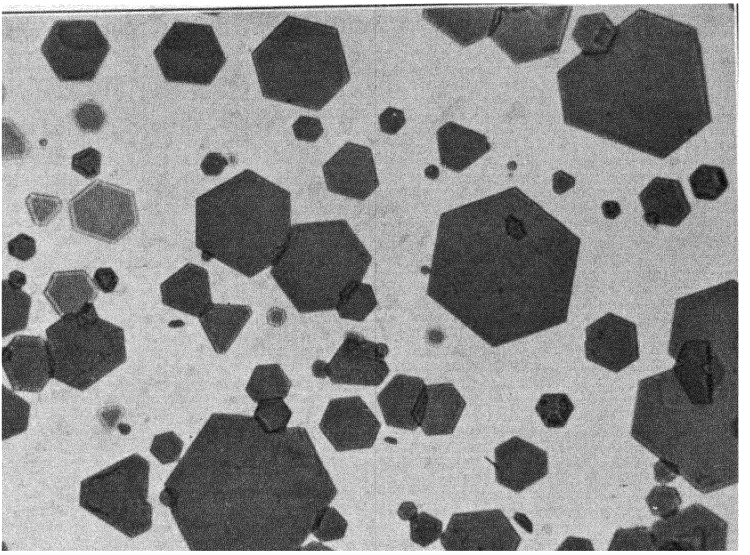


FIG. 269. Lead iodide ×180. Objective, Leitz 16-mm apo.; ocular, Zeiss 15× compensating; condenser, Leitz achromatic, top lens removed; illumination, 400-watt biplane-filament projection lamp, method II; filter, Corning 428; Eastman Panatomic X film; exposure 1/25 second; developer, D-1; Miflex camera attachment.

photography. The reagent can be applied as a liquid, or in solid form (Short). As the drop dries, the double iodide may be formed, the hexagonal plate-like crystals of lead iodide completely disappear, and long acicular crystals are formed of the double salt. The diagnostic yellow hexagonal plates of lead iodide, being of varying thicknesses, are of different shades of yellow, some quite deep and others only faintly tinged with color. A yellow filter would make them all appear white in the finished print. They can be photographed successfully either with or without a chromatic filter. For the picture included here, a light blue filter was chosen to give a little added contrast. A deep blue, of course, would have rendered them entirely too dark and not at all as they actually appeared in the microscope. The choice of filter is often entirely a matter of personal opinion and taste. If it is desired to show the crystals standing out strongly and sharply against a light background, then the blue filter is necessary.

It should be noted that the blue cobalt crystals and the yellow lead

ones were photographed successfully with the same light blue Corning 428 filter. The copper crystals shown in Fig. 270 have about the same color as many of the lead iodide crystals, but a red filter was used with infrared plates. It must be emphasized that although the filters and sensitive materials chosen for this series of pictures have given

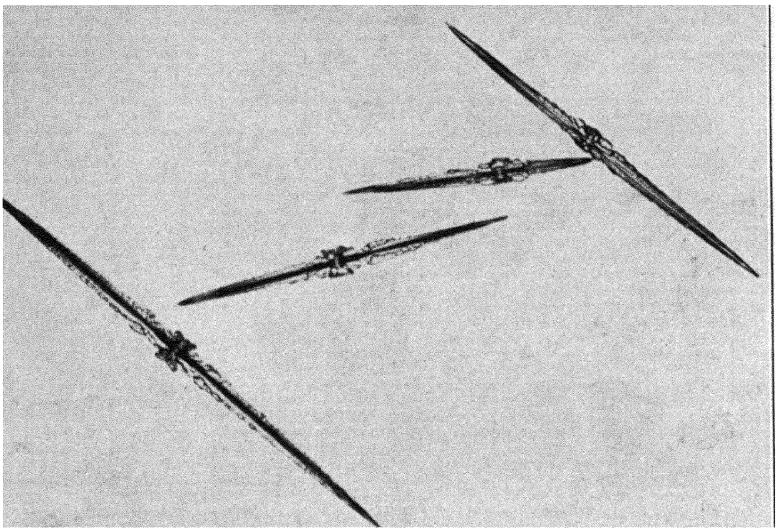


FIG. 270. Copper mercuric thiocyanate ×180. Objective, Leitz 16-mm apo.; ocular, Zeiss 15× compensating; condenser, Leitz achromatic, top lens removed; illumination, 400-watt biplane-filament projection lamp, method II; filter, Wratten No. 25; Eastman infrared film; exposure, 1/10 second; developer, D-1; Miflex camera attachment.

good results they are not necessarily the only satisfactory combinations; further experimentation might yield combinations that would be even more suitable.

Testing for Copper. Reagent: potassium mercuric thiocyanate. New product: copper mercuric thiocyanate. References: Chamot and Mason, Short. (Fig. 270.) A general survey of a test drop to which this reagent has been applied shows a very different appearance from that recorded here. Most of the crystalline formation consists of tree-like forms, clumps of needles, and a few of the well-formed crystals as shown in the picture. The crystals may continue to grow for some time after they first form so that there is plenty of opportunity to select a field where the crystals are large enough to photograph. The difficulty is experienced in finding well-formed crystals sufficiently removed from dendritic growths so that only the perfect crystals will appear in the picture. For purposes of chemical microscopy this

separation of the well-formed crystals might not be desirable, and then the field would certainly be easier to select. Wratten filter 25 was used.

Testing for Silver. Reagent: ammonium bichromate. New product: silver bichromate. References: Chamot and Mason, Short. (Fig. 271.) The reagent is added to the test drop in the form of a very small fragment; too large a piece will make the search for a good photographic field futile. The test drop should be acidified

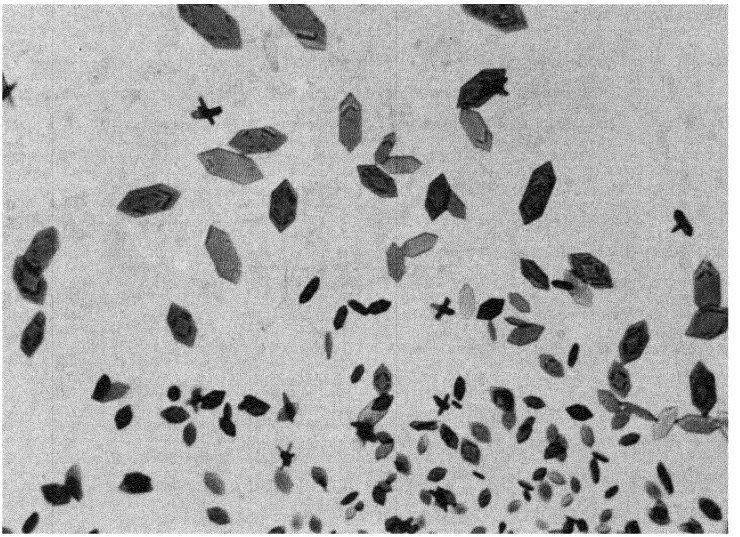


FIG. 271. Silver bichromate ×290. Objective, Zeiss 8-mm achro. short mount; ocular, Zeiss 15× compensating; condenser, Leitz achromatic, top lens removed; illumination, 400-watt biplane-filament projection lamp, method II; filter, Wratten No. 15; Eastman Panatomic X film; exposure 1/25 second; developer, D-1; Miflex camera attachment.

with nitric acid. The crystals are red. A strong red filter will eliminate all contrast. For this particular field, a Wratten 15 filter was selected, but a light red such as Corning 348 might be required for larger crystals of deeper color. In fact, in all these pictures, if the crystal size had been different from that shown, it probably would have been necessary to select filters offering either a more restricted transmission band or a much freer transmission. Each problem must be decided on its own merits, and of course the chromatic characteristics of the plate or film must be taken into account also.

Sec. 174. Photographing Recrystallized Compounds. *Vitamin C (Ascorbic Acid).* (Fig. 272.) This compound has been selected as

an example because it presents many difficulties. The vitamin is water soluble. When it crystallizes it is prone to form many dendrites with a few very tiny well-shaped crystals. A good-sized drop of water was placed on a clean slide and enough vitamin added to produce a saturated condition. As soon as crystallization started it was observed carefully under the microscope with a low power. Before complete dryness was reached, the slide was scratched in several places with a glass rod drawn out to a small thread and the end somewhat flattened. The crystals obtained along the scratched areas were similar to the small scattered crystals except that they grew to a much greater size. The drop was then allowed to dry out completely, and the slide was flooded with methylene iodide. Other mounting media were tried, but a high index was indicated, and, as the picture shows, the contrast obtained was very suitable. No change in the crystals due to the addition of the iodide was noted for several hours; the slides were then discarded. An ultraviolet-absorbing filter was used. As the crystals are perfectly white, if the achromatic microscope had been employed a green screen would not have been amiss.

Recrystallized Cocoa Butter. (Fig. 273.) The cocoa butter was dissolved in amyl alcohol in a test tube. The sample was allowed to stand at room temperature after being slightly heated. The crystals were rather slow to start forming. As the liquid became cloudy a drop was removed to a microscope slide and a cover applied. The growth of the crystals was watched and a good field selected; as the crystals approached a suitable size, the microscope was removed to the camera stand and the picture was taken. There seems to be no hurry in handling this class of material, and several exposures can be made from one preparation, even allowing enough time to develop, fix, and examine each negative.

Among the research workers who have spent considerable time on the study of oils, waxes, and fats under the microscope, might be mentioned Greene,[27] Lewkowitsch,[28] and Mehlenbacher[29]. The photographic procedure is simple and perfectly independent of the way in

[27] L. Wilson Greene, "Chemical Microscopy of Fats and Waxes," *Oil and Soap,* **11,** 31–32, 1934; "The Animal and Vegetable Waxes in 1937," *Oil and Soap,* **15,** 317–325, 1938 (there is no information regarding microscopy in this paper but it contains an extensive bibliography); "Chemical Microscopy," *The Drug and Cosmetic Industry,* **43,** No. 2, 1938; "The Chemical Microscopy of Essential Oils," *Perfumery and Essential Oil Record,* London, **30,** 309–316, 1939.

[28] J. Lewkowitsch, *Technology and Chemical Analysis of Oils, Fats and Waxes,* London, 1921. An essential work for those interested in oils and fats.

[29] Virgil C. Mehlenbacher, "Fat and Oil Microscopy," *Oil and Soap,* **13,** 277–282. This paper includes numerous photomicrographs for identifying crystals.

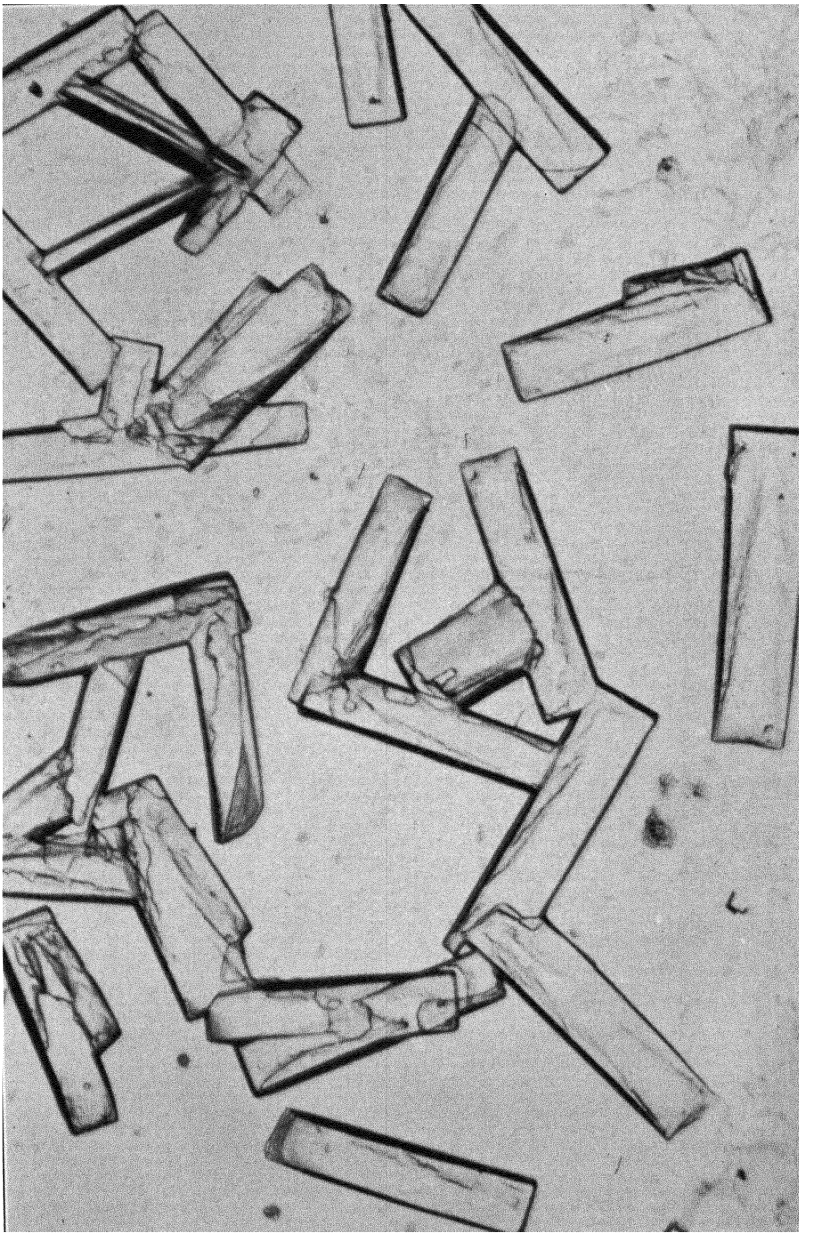


FIG. 272. Vitamin C ×360. Objective, Leitz 16-mm apo.; ocular, Homal I; condenser, Leitz achromatic, top lens removed; illumination, 250-watt projection lamp, method I; filter Wratten No. 57; Eastman Commercial Pan; developer, D-61a.

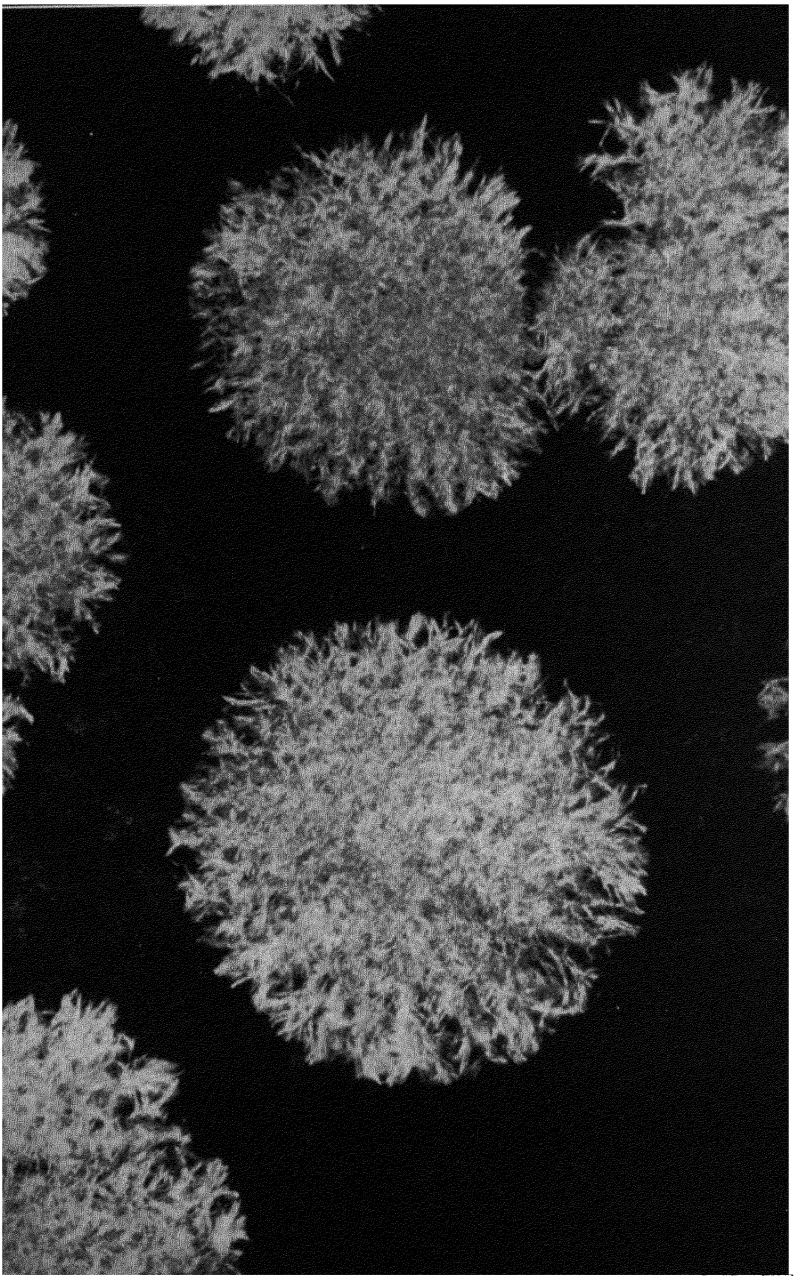


FIG. 273. Cocoa butter ×360. Objective, 16-mm apo.; ocular, Homal I; condenser, Leitz achromatic, top lens removed, stop for dark field; illumination, 250-watt projection lamp, parallel rays; no filter; Eastman Commercial Pan; developer, D-11.

which the crystals are obtained, whether from crystallization from an appropriate solvent or from chemical reaction, as when a soap is formed by the application of an alkali to a saponifiable oil. The slide can be ringed with a semi-permanent sealing compound and kept for some time. Since photomicrographs of these crystals may be numerous, the smaller camera, as recommended for chemical microscopy, may be best, most convenient, and most economical. Probably the soap crystals will not grow very large; the fat crystals from a hydrocarbon solvent will, in general, grow large if allowed enough time. Dark field is often the best means for giving the image high contrast, especially if the crystals are obtained by evaporation and are small, flat and thin, or finely acicular. The reason is that such crystals generally have an index very close to the solvent, and the state of saturation of the mother liquor in which they exist tends to keep the index level of the liquid phase close to that of the solid crystal phase.

GROUP VI

FIBERS

Sec. 175. Description of Materials. This group includes all the natural and artificial fibers, either before or after they are made into commercial products: felts, roofing materials, all textiles, packing and insulating products, papers, wall board, and fiber-bonded synthetic resins indicate the wide scope of this interesting field.

EXAMPLES

Natural hair of animals, Sec. 176.
Plant fibers, Sec. 177.
Synthetic fibers, Sec. 178.
Feathers, Sec. 179.
Fibers used in the manufacture of paper, Sec. 180.

Photomicrographs of these materials often supply documentary evidence of identification in civil suits and in criminal court trials. The identification is made under the microscope, and the photographs, with proper explanation, serve as proof. Both forensic medicine and forensic chemistry may make wide use of photomicrographic evidence.

Illumination. Moderately strong tungsten projection lamps are all that are needed for fiber work; illumination can be by Method I or Method III. Dark field is rarely used. Polarized light aids in identification, but pictures are seldom taken with it, and ultraviolet radiation has found little application either for the examination or for the

photography of fibers. The illumination of fiber specimens by overstage lighting has been advanced by the creation of the Epi systems, and at least one authority believes it of great promise in the study of the surface of natural hairs. The high opacity characteristic of many fibers is generally due to dye; consequently, in order to obtain greater penetration it is better to bleach the specimen than to try to increase the light intensity.

Filters are indicated for some of the stained or dyed specimens. When there is need for stressing the contrast between weak or partly washed out colors, filters are particularly useful; otherwise they may be selected merely as an optical aid, the choice depending on the lenses employed. Resolution is seldom a factor in fiber work, unless the work is of a research nature. In nearly all photomicrographs of fibers visibility is of greater importance than high resolving power. Blue filters and high apertures are less to be desired than maximum field depth and strong contrast.

Apparatus. There are several so-called fiber microscopes on the market, but they have little or nothing to offer that is new or that is not provided in most good instruments. Excellent fiber work can be done with any microscope that is acceptable for photomicrographic work. The gadgets and accessories specially provided for fiber work are small devices mounted on the microscope stage useful for holding and turning the specimen. Optical devices may be those common to all microscopical work in general, such as micrometer systems, ocular goniometers, polarizers, and analyzers. The condensers selected for fiber work should be of medium and high power. The useful objectives will be found to be the 16-mm and 8-mm, with occasional recourse to high powers, for which the 3-mm oil immersion is suggested. The most useful camera will be found to be the 5 by 7 inch vertical type with a generous bellows extension. When a large number of pictures are needed for record, the 9 by 12 cm eyepiece camera may serve. Good fiber pictures can be made with this camera, but it is not particularly suitable for photomicrographs of cross sections. Comparisons may be made of the pictures taken with both types; for instance, Fig. 281 can be compared with Fig. 282. The small camera has given a very well-balanced picture of the longitudinal views of the rayons, but such a camera would have been inadequate to produce the pictures of cross sections shown in Fig. 275.

In addition to the above, fiber work demands special apparatus for the preparation of the specimen; special fiber microtomes and simple apparatus for chemical treatment, staining, and bleaching are all helpful.

General Procedure. Fibers may be prepared for photomicrographic work in three ways: the view may be longitudinal; the view may be cross-sectional; or occasionally the fiber may be macerated mechanically or with the aid of a chemical process, the aim being to separate the fiber cells, plastids, pigment, or other elementary body. Longitudinal views are easy to arrange. In general the idea is to have the specimen transparent; a specimen that is not naturally transparent will usually have to be bleached. In animal hair the medulla will appear, but the scales will not be seen unless specially mounted in a way to be described later. In vegetable fibers of any origin, irregularities will show to best advantage if the individual specimens are as long as possible, but specimens of synthetics can be cut to a millimeter or less. With certain exceptions, such as dirty animal hair, the specimens can generally be mounted directly into any suitable liquid; glyceryl triacetate ($n = 1.4297$) has been particularly recommended. It will seldom be necessary to arrange the fibers parallel to each other, except for large hairs. Teasing needles will be required to place and arrange the specimen. The individual fibers should be separated to show characteristic features, and threads must be untwisted completely.

The mounting medium for unstained and highly transparent specimens may be of high index (methylene iodide, aroclor, monobromonaphthalene, etc.) or of low index (water and glycerol, monomethyl glycol, or light paraffin oil). For large or well-colored specimens, such as some of the coarse natural hairs or deeply stained textiles and fibers, immersion oil or any medium of an index similar to the fiber may be indicated. The index of keratin, the chief constituent of hair, is about 1.550, and that of cotton, the chief constituent of which is cellulose, is 1.551.

Sectioning. Cross sections of fibers are of major importance in identification with the microscope. The Hardy sectioner[30] is a very convenient and inexpensive microtome for making cross sections of fibers. It is a delicate piece of apparatus and must be used with care.

Figure 274 is a photograph of the Hardy microtome. The base of the instrument, which is the same size as an average microscope slide, 25 by 75 mm, is jointed transversely at the center and has a very narrow slot cut longitudinally into one of the halves. A sample of the specimen is placed in the slot and fed through it by means of a

[30] Can be obtained from G. E. De La Rue, 3406 Longfellow St., Hyattsville, Md. Invented and patented in 1935 by Dr. J. I. Hardy, Senior Animal Husbandman, Bureau of Animal Industry, U. S. Dept. Agriculture. See J. I. Hardy, "A Practical Laboratory Method of Making Thin Cross Sections of Fibers," *Circular* 378, U. S. Dept. Agriculture, 1935.

plunger actuated by a screw. As the specimen protrudes from the slot it is covered with a celluloid or collodion solution which acts somewhat as an embedding agent. When the solution is quite dry a cut can be made.

In using the Hardy sectioner, the directions accompanying the instrument should, for the most part, be followed in detail, but a mixture of a good clear white nail polish and ether will be a better aid in the sectioning operation than the collodion mentioned in the direc-

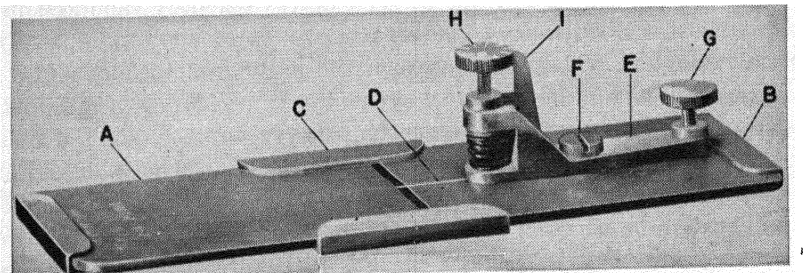


FIG. 274. Fiber microtome as designed by Hardy. Courtesy Mr. G. E. De La Rue.

tions. The section is cut with a safety-razor blade. Failures with any microtome are usually the fault of either a dull knife or poor manipulation of the knife; the same applies to the Hardy machine. The safety-razor blade should be wiped with a solvent and then stropped before use. The blade should be held so that the first finger presses evenly and steadily on it to guide it over the plate. For the best work, only one cut should be made with any one part of the blade. A fresh edge of one blade can be presented to the specimen for four separate cuts. Blades used once can be put to one side to make initial cuts as demanded by the directions accompanying the machine, or they can be used to make medium or thick sections.

Photomicrographs of sections are made at magnifications of 150 to 600, but the high limit is seldom required. The depth of field will be a less important factor than in pictures of the longitudinal view. Many of the sections will be about 10 μ thick, which is thicker than the field depth of the 16-mm objective can cope with. When a satisfactory section is found it may pay to transfer it immediately to a dark-field slide, so that the pressure applied to the cover will help to hold the specimen in one plane. Figure 195 is a photomicrograph of human hair in cross section.

On well-dried plant and synthetic fibers, little or no preparation is required before sectioning with the Hardy microtome. Good technique lies in judging just when the right amount of fiber is in the

slot. If too few, they will bend as they are pushed through and diagonal sections will result; if packed too closely, they may be crushed out of shape or broken. A little experience with the device will soon show the best conditions for operation.

Animal fibers need to be washed in ether before cutting. Wool fibers, especially, are greasy, and so are mohair and camel's hair. Thinner sections may be obtained if the fibers are mounted in celloidin or wax and cut by certain other methods, but the Hardy sectioner is faster, and although the sections are slightly thicker they may give a better idea of the true physical composition of the fiber than a section that is, perhaps, only 1 or 2 μ thick There could be too little of the specimen to examine or photograph.

Other methods of embedding and sectioning with a large microtome are available, but most are time-consuming and entail considerable expense. In general the Hardy device will make satisfactory sections, and its use is recommended. Some methods of sectioning fibers are described by Schwartz[31] and by Smith and Glaister.[32] Schwartz's book has an excellent bibliography of original papers. Matthews'[33] book is also well worth consulting, many kinds of fibers being discussed in detail.

Sec. 176. Animal Fibers. The industrial microscopist may be required to make photomicrographs of the hair of any living animal. Sometimes such specimens come to hand as evidence of contamination in foodstuffs, and they commonly occur in all branches of clothing manufacture and textiles. Some animal hairs are listed in Table XXXII; others will be found described by Bachrach.[34]

Human hair and hair of animals play an important part in criminology, according to Else and Garrow.[35] Figure 195 illustrates the anatomy of a hair, showing the main divisions clearly. The central portion is the medulla; next to it lies the cortex which carries the pigment; and the cuticle with the lenticular scales as shown in Fig. 277 constitutes the outer portion.

It is unnecessary to make longitudinal sections for an average study of the medulla and pigmentation. The index of keratin being 1.55, all that is necessary is to mount the unsectioned hair in a medium

[31] Edward R. Schwartz, *Textiles and the Microscope,* McGraw-Hill Book Company, 1934.

[32] Smith and Glaister, *Recent Advances in Forensic Medicine,* London, 1931.

[33] J. M. Matthews, *The Textile Fibers,* John Wiley & Sons, New York and London, 1924.

[34] Max Bachrach, *Fur,* Prentice-Hall, 1930.

[35] Walter M. Else and James M. Garrow, *The Detection of Crime,* published by the *Police Journal,* London, 1934.

Table XXXII

Some American Fur Fibers

Badger	Marmot	Sable
Bear	Marten	Seal
Beaver	Mink	Sheep
Cat	Mole	Skunk
Coyote	Muskrat	Squirrel
Fisher	Opossum	Weasel
Fox	Otter	Wild cat
Goat	Pony	Wolf
Horse	Rabbit	Wolverine
Lynx	Raccoon	

of that index to attain the greatest transparency and nullify the lenticular effect of the hair (due to its shape). A picture can be taken at any optical level in the specimen, and under these conditions no detail will be distorted. The smaller hairs, such as fine rabbit hair, if nearly colorless, may suggest a medium of higher index, and then, unless a high, large-aperture objective is used, the results should be good.

When making pictures for the purpose of comparison, it is a good plan to arrange the fibers parallel to each other if possible. Of course, it will be more difficult to arrange small hairs in this way than large ones. When possible, a picture of animal hair should include the tip, the root, a guard hair or two, and some of the under fur.

Color effects may have to be taken into account when photographing hair. If the hair is large or of medium size, from 50 μ up, a filter of like color will aid in rendering detail. If this reduces the contrast unduly, a contrasty film such as Contrast Process Ortho or Contrast Process Pan should be used, and if necessary a strong alkali developer. Grouping of pigmentation is often of interest. It can be examined in longitudinal views but even better in cross section.

Washing the hair before sectioning, in ether (sulphuric), chloroform, or alcohol, and then passing it through xylene before immersing in a resin, will increase the apparent wetting power of the mounting agent in addition to removing the dirt. When the hairs are so treated the final appearance of the image will be sharper and there will be less tendency for air bells to form.

Hairs are much easier to section than either vegetable or synthetic fibers. The structure of most hair is quite firm, and the keratin will cut cleanly without tearing. Nevertheless, like the fibers, some kinds of hair are soft enough to be distorted in the slit of the Hardy microtome plate, and the necessary precautions as already described should

be taken to guard against this. In sectioning, cells of the medulla the contained air or gas will be released leaving but little of the medulla cells to show in the picture. When this occurs the medulla may sometimes be emphasized by making the mount with a liquid of unusually high index. When making such cross sections for photography much time will be saved if several mounts are put on one slide to afford a choice of fields. Because the sections have a tendency to curl when rather thick or not properly covered, each section will probably require a cover of its own. The advantage of using small-diameter covers is evident, since three or even four mounts can thus be made on one 75-mm slide. Figure 275 illustrates cross sections of wool, and Fig. 276 cross sections of cashmere.

A Method for Photographing the Surface Structure of Animal Fibers. Animal hair is covered with a scaly structure, which often can be seen to extend from the root to the tip of the hair. On most hairs it lies very flat and close enough to the cuticle to be part of it, so that under certain conditions it may not be visible. On some specimens it may be sufficiently visible to photograph if the specimen is immersed in a medium of rather high index. Since hair is composed almost entirely of keratin, there is no visibility due to difference of index within the structure.

Various methods have been offered for increasing the contrast of the scale outline, but none are entirely satisfactory; if, by control of the refraction index of the mounting medium, the outline of the scale is increased sufficiently to be evident in the picture, the outline of the fiber as a whole will be a great deal too broad.

For visual examination, the scaly structure stands out more clearly when the specimen is examined in air. The scales on human hair are easily seen under these conditions, but a good picture cannot be obtained because the hair is cylindrical and has great depth.

To overcome many difficulties in studying and photographing scale structure, Hardy and Plitt[36] have worked out a method for taking an impression of the scales in a thermoplastic and studying or photographing the impression so obtained.

In the paper describing the process, one valuable advantage of the system is stressed: impressions are obtained on both sides of the fiber simultaneously and for its full length. Roughly, the technique consists of placing a fiber between two sheets of thermoplastic 0.002 to 0.003 inch thick; two other sheets of thermoplastic 0.02 inch thick are

[36] J. I. Hardy and Thora M. Plitt, "An Improved Method for Revealing the Surface Structure of Fur Fibers," Fish and Wildlife Service. U. S. Department of the Interior, Washington, D. C.

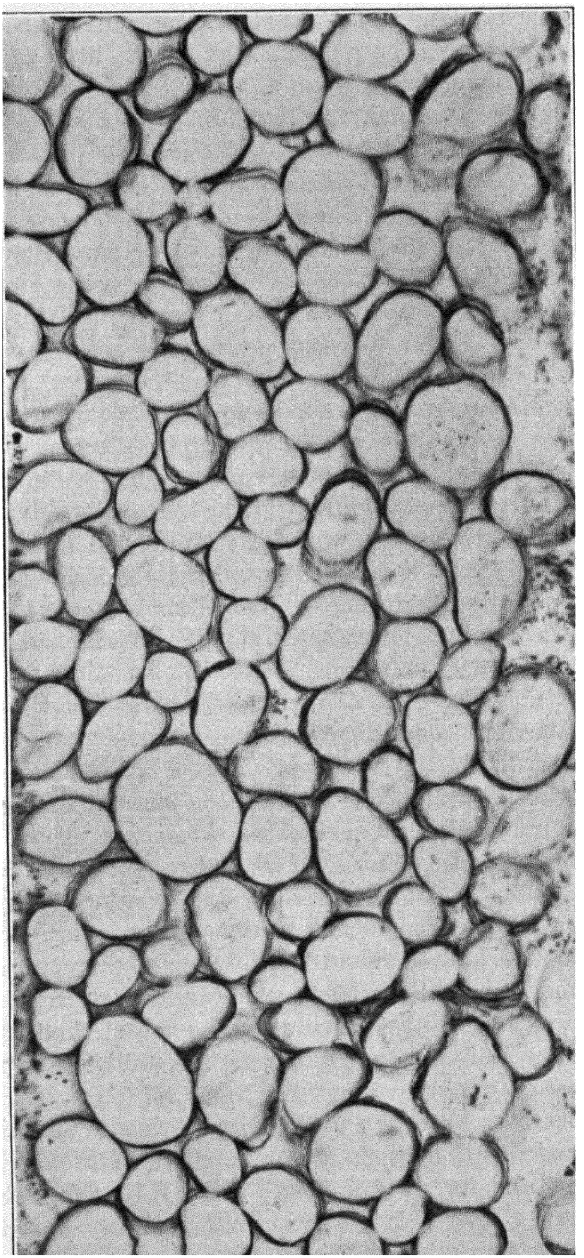


FIG. 275. Cross section of wool fiber ×360. Objective, Leitz 16-mm apo.; ocular, Homal I; condenser, Leitz achro. oiled to slide; illumination, 250-watt projection lamp, method I; filters, Wratten No. 60; Eastman Pan Commercial film; developer, D-61a.

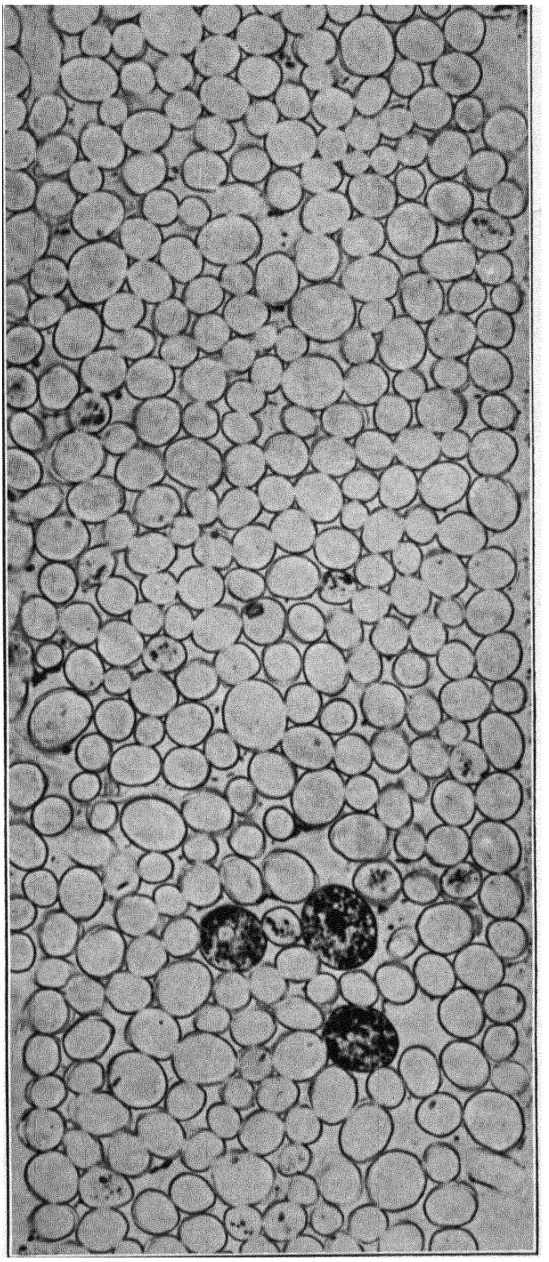


FIG. 276. Cross section of Kashmir ×360. Same data as for Fig. 275.

added as a cushioning backer to the thinner sheets; the whole is placed between two sheets of pressboard ⅛ inch thick. The pack is then clamped together and placed in an oven at a temperature just sufficient to soften the thermoplastic. Hardy and Plitt give the correct temperature for Ethofoil[37] as 90° to 95° C. After being heated for 15 minutes the pack is opened, the hairs are removed, and the molds are examined. The clamps should not be screwed down too tightly

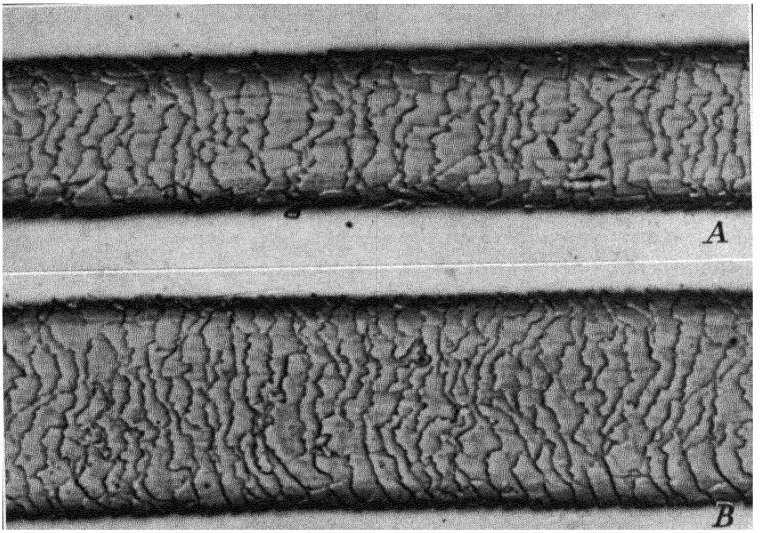


FIG. 277. Longitudinal view of wool fiber ×700. Dry mount. Impression on Ethofoil. Pictures are of the same fiber taken at different points. *A* shows part of the hair near the tip, and *B* part nearer the root. Objective, Zeiss 8-mm achro. short mount; ocular, Homal I; condenser, Leitz achro. oiled to slide; illumination, 400-watt biplane-filament projection lamp, method I; Eastman Contrast Process Ortho film; developer, D-1.

or the hair may be split or distorted. A few trials will soon show about how much pressure the specimen will stand without flattening out. It is suggested that new pieces of the heavy-gauge foil be taken for each mount. Each piece of Ethofoil should be wiped with a damp chamois before use to discharge static electricity and to clean the foil. Unfortunately, however, the foil will never become microscopically clean; the surface will have scratches and abrasions that are sure to show in the picture. If the field is large, a number of mounts may have to be made before a field can be found sufficiently free from dirt

[37] Ethofoil is made by the Dow Chemical Company, Midland, Mich. The New York City Office is at 30 Rockefeller Plaza.

and scratches to be useful. This trouble is not mentioned in the Hardy and Plitt paper.

Good results have been attained by mounting the molds dry. Figure 277 is a photomicrograph of a portion of such a mold. It would seem that this method offers a new technique for obtaining good pictures of many surface conditions of various substances which could be attained otherwise only with considerable difficulty if at all.

Sec. 177. Vegetable Fibers. A partial list of vegetable fibers with which the microscopist is often engaged is given in Table XXXIII.

These fibers may be found separately or in combination. Usually a genus has a number of species, and in the samples received at the laboratory it may be difficult to tell the species apart. Fibers are often referred to by the manufacturer as bast fibers, leaf fibers, or seed hairs, depending on the part of the plant from which they are taken.

Table XXXIII

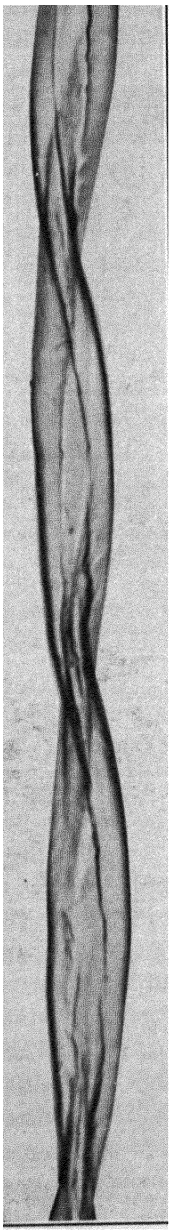
Commercial Fibers from a Vegetable Source

Cotton	Redwood	Corn	Manila
Italian hemp	Tampico grass	Linden	Chingma
Henequin	(see Tula)	Lanatin	Sisal
Coir	Kapok	Flax	Mulberry
Palma	Jute	Cantala	Raffia
Ramie	New Zealand hemp	Russian hemp	Tula ixtle
Yucca	Esparto	Jaumave ixtle	Pita ixtle
Peanut	Phormium	Pineapple	Ife
	Sida	Cocoanut	
	Solidonia	Imitation hair	
	Milkweed	(Palma-Pita-Tula)	

Cottons, the commonest of all commercial fibers, are grown in many parts of the world, and at the present time there is no sure way of identifying the different species microscopically. The hemps are another widely used class of fibers the species of which are difficult or impossible to identify from the average laboratory sample.

Figure 278 illustrates a specimen of cotton fiber. Since cotton is nearly pure cellulose and has a refractive index of 1.551, the mount was made in hyrax, $n = 1.7$. In cotton, the twist of the fiber is an important characteristic, and it should be shown if present. Absence of twist might indicate mercerizing or a " dead " cotton. When mercerized (treated with a caustic) the fibers swell and look somewhat like smooth inflated tubes instead of like flat or deflated twisted tubes as is normal. As the picture shows, magnification of such material can be invariably low to medium; 500 to 800 diameters is generally the upper limit.

The main difficulty in photographing any small fiber longitudinally is exemplified in cotton. The depth of field necessary to ensure a uniformly sharp picture is sometimes very difficult to attain. It is undoubtedly better to select a field with small field depth than to experiment with low apertures and high-index media.



It is not good practice to cut very short any vegetable fibers which are to be photographed, because characteristic features may be lost.

As a rule the fibers are placed on a medium-sized drop of the mounting medium and teased apart with dissecting needles. Care must be exercised to avoid the formation of bubbles; if they occur, they must be broken with a hot needle or driven off by heating the mount gently over an alcohol lamp. The cover should be pressed well down. Presumably the 16-mm or 8-mm objective will be employed to take the picture; from an examination of the mount it should be possible to tell at once whether the field depth of the objective will take care of the overlying fibers. If depth of field is insufficient, a little pressure may be exerted on the cover with a specimen compressor, or the mount might be made very conveniently in a cell such as is generally used for the dark-field microscope. As the fibers, unless they have been stained, will be colorless or nearly so, any system of filters may be chosen. The natural selection would be a system eliminating the strong reds and the strong blues. For colorless objects, when resolution is not an important factor, the combination of Wratten 15 and 57 with Corning 430 or 428 gives a nice green. Wratten filter 15 effectively cuts off any ultraviolet that might pass 57, and Corning filter 430 or 428 cuts off any stray red. When a stronger or purer green is desired, nearly monochromatic effects can be obtained as described on p. 382.

FIG. 278. Cotton fiber ×800. Egyptian cotton grown in Arizona; mount hyrax. Objective, Leitz, 8-mm apo., ocular, Homal I, condenser, Leitz achro. oiled to slide; illumination, 400-watt biplane-filament projection lamp, method II; filters, Corning 428, Wratten No. 57 plus 15; Eastman Ortho X film, developer, D-1.

For the most part, there will be sufficient photographic contrast if the picture is taken with Panatomic X film and developed with D-19. Glare is not particularly troublesome with this class of material, as a rule.

Figure 279, of esparto, shows a characteristic picture of fiber that has been soaked in water, warmed, and teased apart with needles. Such a specimen can be stained with safranin or Delafield's haematoxylin, which was used for this picture. The small comma-like cells serve to identify this fiber. Before making any effort to work on plant fibers it is wise to consult some botanical authority, for the technician needs to become aware of all known differences and minutiae of detail with which the botanist is familiar and which he desires to see in a picture. Unless the large fibers, which are composed of bundles of long needle-shape cells, are broken down to isolate the individual members, a photomicrograph in a longitudinal plane will probably mean but little. Figure 280 shows a picture of Tampico grass. The individual cells have been isolated by a short sojourn in warm chromic acid (10 per cent solution), then teased apart with needles.

Table XXXIV

Some Important Synthetic Fibers

Rayons — cellulosic origin	Cuprammonium
Viscose	Nitrate
	Acetate
Other Synthetics	
Aralac	Cisalfa
Lanital	Celta
Nylon	Soybean
Vinyon	Vistra
Glass	Saran

Sec. 178. Synthetic Fibers. This class of fiber is expanding so rapidly that to keep a complete list up to date would entail constant revision; however, some of the fibers of this group which are of commercial importance at the present time are listed in Table XXXIV. The *Rayon and Staple Fiber Handbook* describes many of them.[38]

Figure 281 illustrates typical examples of synthetic fibers. The longitudinal striations which show as lines in the pictures are due to the shrinking of the liquid cellulose compound as it hardens after leaving the spinneret. The contracting is so typical that often a fiber can be identified by studying the cross section. The longitudinal

[38] E. W. K. Schwartz and H. R. Mauersberger, editors, *Rayon and Staple Fiber Handbook*, third edition, 1941.

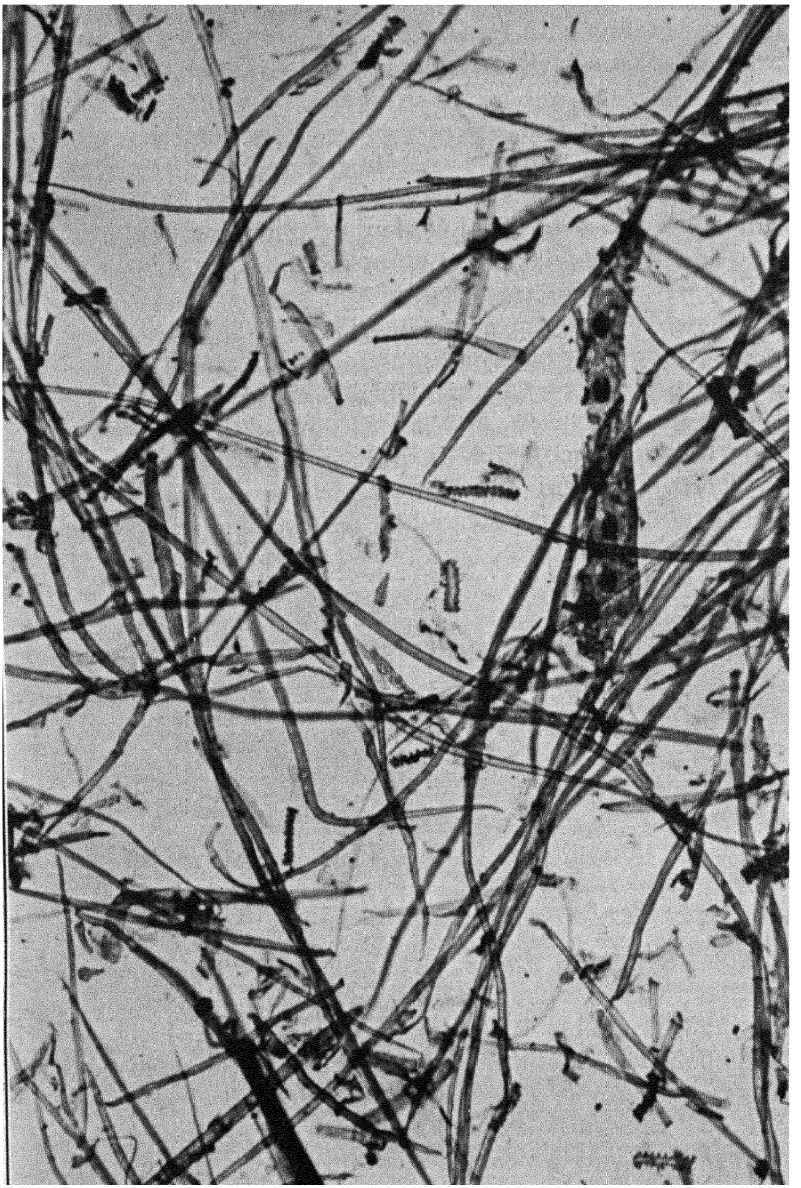


FIG. 279. Esparto fiber ×125. The comma-like particles seen throughout the picture are characteristic of this fiber. The specimen was stained with Delafield's haematoxylin. Objective, 30-mm apo., Zeiss (this objective has been replaced by Zeiss with one of shorter focus 25 mm); ocular, Homal I; condenser, Leitz achro., lower element only; illumination, 400-watt biplane-filament projection lamp, method III; filters, Wratten No. 57 plus 15; Eastman Ortho X; developer, D-19.

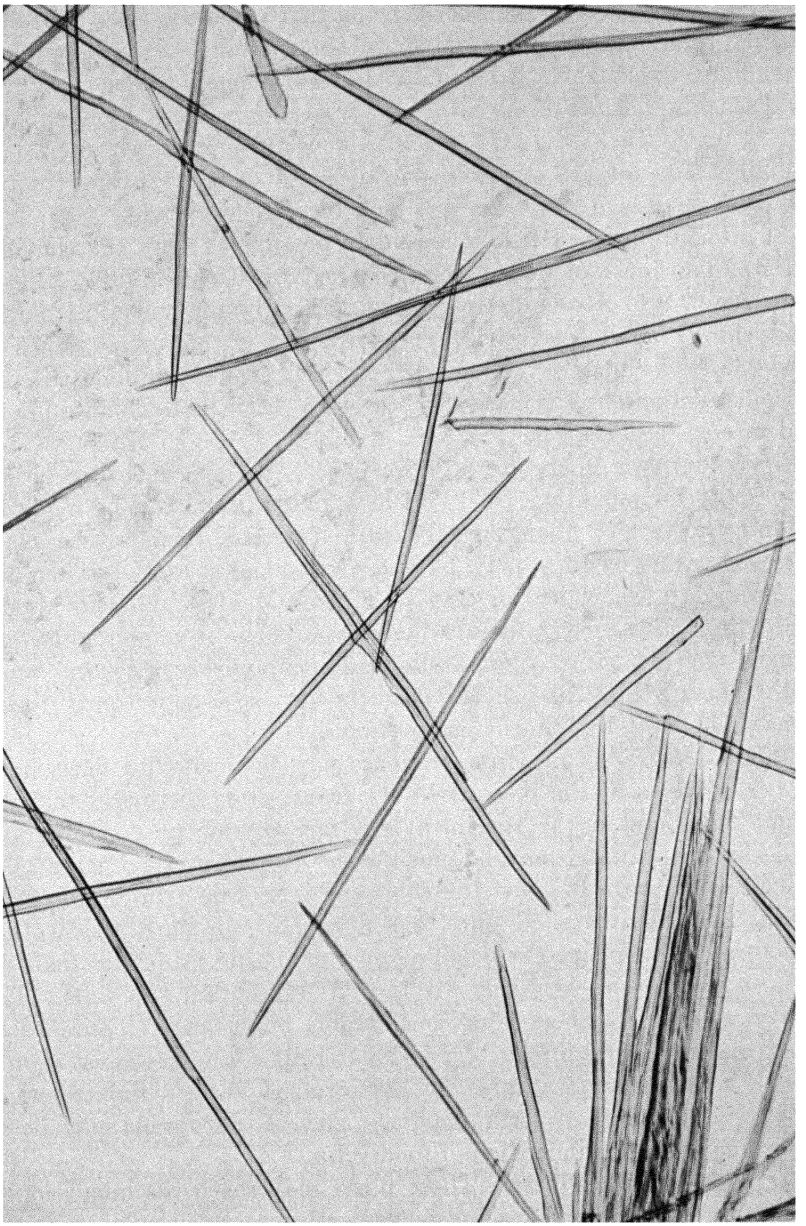


FIG. 280. Tampico grass ×65. Staining is unnecessary with these large easily seen fiber cells. Objective, Zeiss 30-mm apo.; ocular, Homal II; condenser, Leitz long-focus; illumination, 300-watt projection lamp, method I; filters, Corning 430, Wratten No. 57 plus 15; Eastman Panatomic X film; developer, D-19.

mounts require a liquid of higher or lower refractive index than that used for animal fibers, the reason being that synthetic fiber is usually smaller than natural fiber and more transparent, so that high contrast must be sought. Aroclor and monobromonaphthalene are good mounting liquids, as is glycerol and water ($n = 1.4$). For a higher index, methylene iodide ($n = 1.76$) is useful. It is of interest to examine some of the synthetics in a liquid of very high refractive index, even though it may be too high for a good picture.

In a longitudinal picture it is seldom possible to show the convolutions of the figure of the fiber which are formed as the fiber solution is extruded into a coagulating liquid. Usually the convolutions appear simply as lines, without detail, but an exception is shown in Fig. 282. The fiber is viscose rayon. To become familiar with the various synthetics it is necessary to study them both longitudinally and in cross section.

Since synthetic fibers are uniform in size, the specimen can be cut very short for mounting. Cuts of less than 1.0 mm can be made by holding the tuft of fibers on a board and cutting them with a sharp knife or razor, or they may be cut with sharp scissors. When the pieces are cut short in this way, each individual strand will appear in the field only once, whereas when the specimen is composed of long strands one piece may twist around another and occur several times in the same field. Also short-cut fibers have less opportunity to increase field depth by lying across one another.

Photomicrographs of cross sections may be made by dark field. The boundaries of the pieces will stand out more distinctly than by bright field, and if the specimen is a mixture the photomicrograph made by a dark field may be much easier to interpret. Figure 283*A* is such a picture. *B* shows the same specimen taken by bright field.

Sec. 179. Feathers. Figure 284 illustrates, in part, the feather structure. The higher alcohols are good mounting media for feathers because they are less likely to form bubbles than the oils. The identification of feathers is of considerable importance to purchasing departments dealing in this class of material. A microscopist should be able to recognize feather fiber, particularly down, without confusing it with the hair of certain rodents, such as mice; this may be of importance in the examination of foodstuffs.

Sec. 180. Paper Fibers. These fibers may reach the microscopist in the form of botanical specimens, as partly processed pulp, or as the finished paper. In any event, the procedure indicated is to soften the fibers and distribute them so that characteristic features may be recognized in the picture. Figure 285 is a sample of ground pulpwood

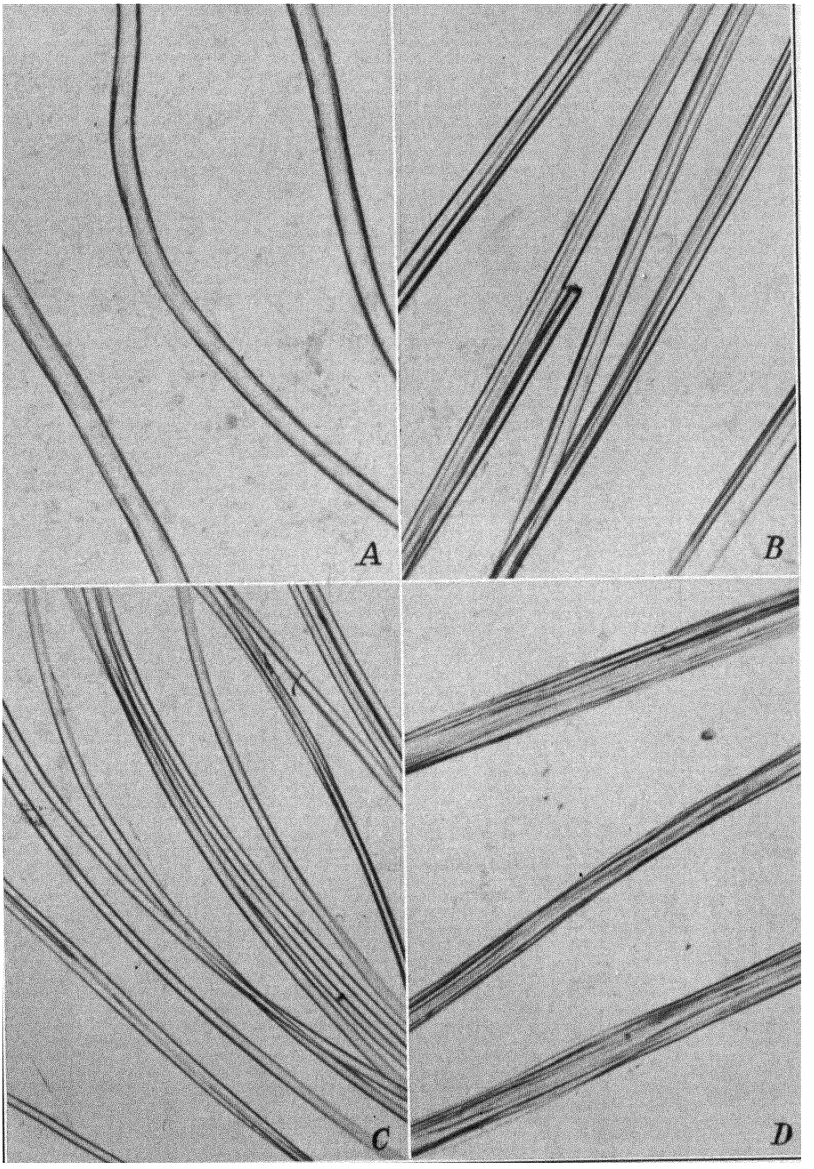


FIG. 281. *A*, an Italian casein fiber (synthetic wool) ×130. *B*, Nitrocellulose rayon ×130. *C*, cuprammonium rayon ×130. *D*, viscose rayon ×130. Cf. Fig. 282. Objective, Leitz 16-mm apo.; ocular, Leitz Periplan 10×; condenser, Leitz achromatic-aplanatic, top lens removed; illumination, 250-watt projection lamp, method I; filters, Bausch and Lomb daylight, Wratten No. 58; Eastman Pan Commercial film; developer, D-61a. These pictures were taken with an eyepiece camera, 9 by 12 cm.

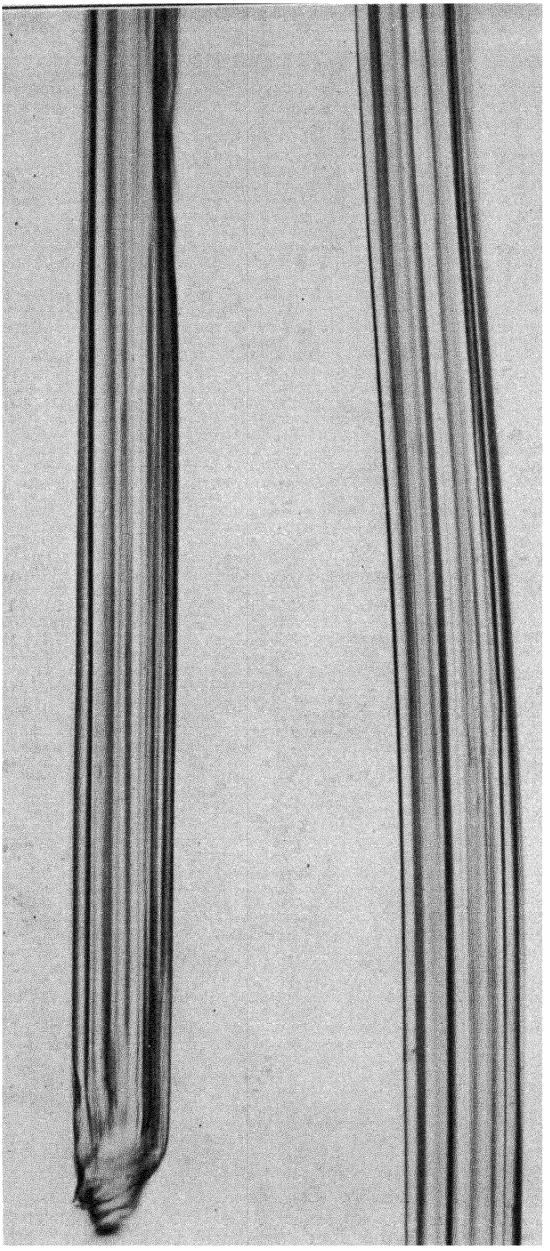
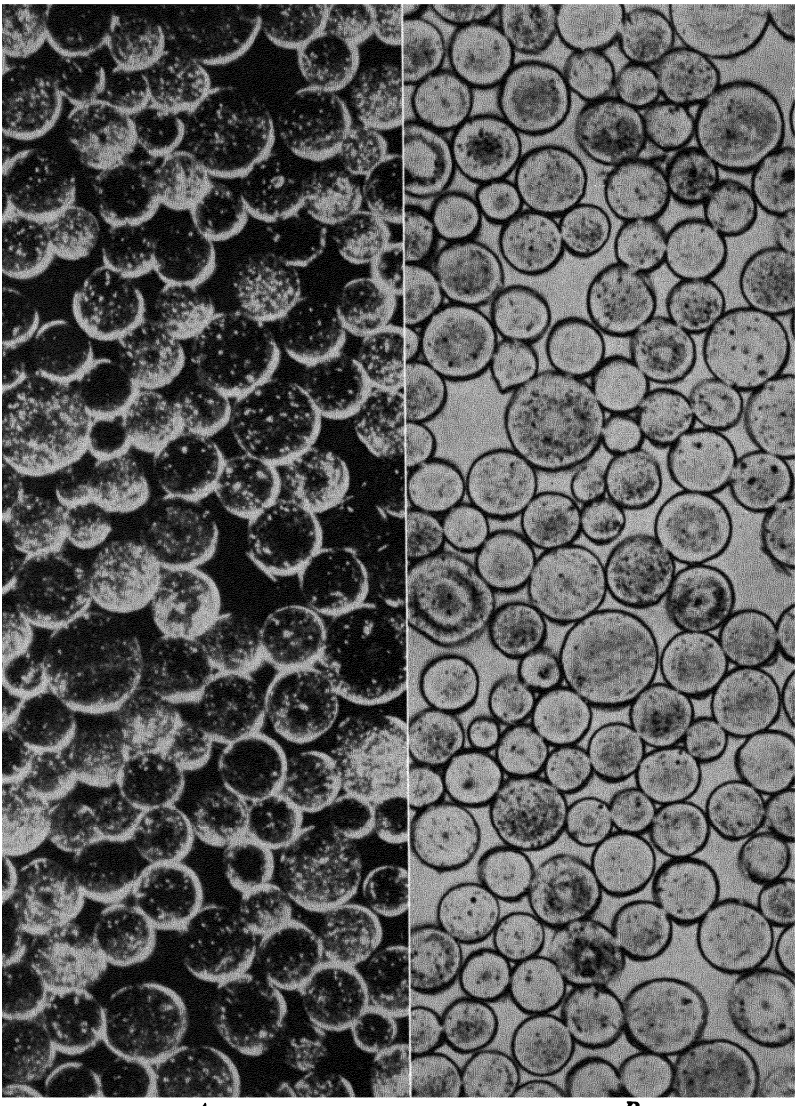


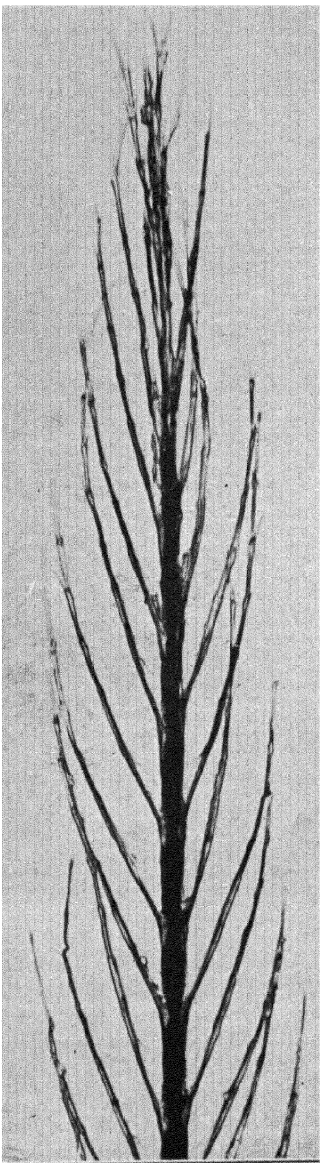
FIG. 282. Longitudinal view of viscose rayon fiber ×800. Mount was made in methylene iodide. Objective, 8-mm apo. Leitz; ocular, Homal I; condenser, achromatic-aplanatic Leitz, oiled to slide; illumination, 300-watt projection lamp, method I; filters, Wratten No. 58 plus 15; Eastman Ortho X film; developer, D-19.



A *B*

FIG. 283. Cross section photomicrographs of Aralac fiber ×360. Dark-field illumination was used for *A* and bright-field for *B*. The dark-field illumination stresses the presence of pigment. The great range of sizes may or may not be diagnostic of this fiber; the author has not examined enough samples to know this. Objective, 10-mm. apo. Zeiss; ocular, Homal I; condenser, achromatic-aplanatic, Leitz, top lens removed; illumination, 500-watt projection lamp, method III; filters, none for *A*, Corning 428 plus Wratten 15 for Defender Fine Grain Pan films; developer, D-19. Dark-field stop inserted for *A*; otherwise data are applicable for both pictures.

stained with malachite green. After softening in water the specimen was transferred to a slide and teased apart with dissecting needles. A good field was chosen and the picture made. The mounting medium, glycerol and water, might equally well have been any one of a number of media with a moderate refractive index. The images are a combination of absorption and refraction. It was not considered necessary to use a filter for the purpose of increasing or decreasing contrasts. The combination Corning 430 plus Wratten 15 was selected to eliminate strong reds and blues. Resolution was not a consideration, the main object being to get nice gradations of shade to render all the detail possible.



A parti-colored specimen presenting detail stressed by gradations of color gives rise to the problem of how best to deal with color values so that they will be truly represented when translated into black-and-white values in the print. In nearly all such pictures the best results are obtained by using panchromatic film and light of daylight quality.

Rag pulp can be detected by the absence of woody cellular structure. The pulp may be either cotton or linen. If linen, the nodes or slip planes will be seen occasionally, and several should be in evidence in a picture of this paper fiber. A cotton fiber is free from characteristics, and the twist will usually be seen.

FIG. 284. Feather from hermit thrush ×360. The tip of a barb with barbules is shown. Mounted in butyl alcohol. Objective, Leitz 16-mm apo.; ocular, Homal I; condenser, Leitz achro. oiled to slide; illumination, 400-watt projection lamp, method I; filters, Corning No. 428, Wratten No. 57 plus 15; Eastman Panatomic X; developer, D-1.

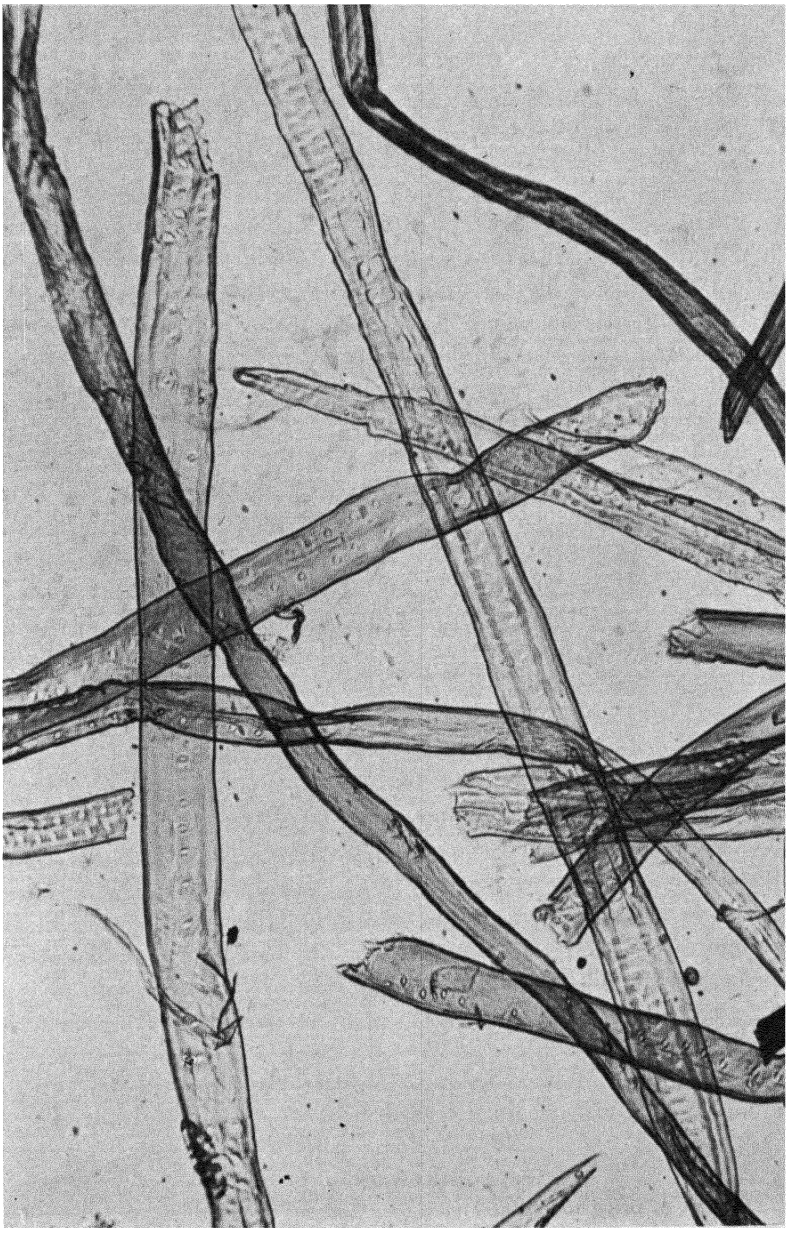


FIG. 285. Unbleached pine sulphate paper pulp ×200. Objective, Zeiss 30-mm apo., ocular, Homal I; condenser, Leitz achromatic-aplanatic, lower lens element used alone; illumination, 400-watt biplane-filament projection lamp, method I; filters, Wratten 430 plus 15; Eastman Panatomic X film; developer, D-1.

Ground wood pulp is easily recognized by the cellular structure characteristic of coniferous woods. Such specimens turn yellow with Hertzberg's stain.

Chemically prepared pulps of the sulphate or sulphite process, when bleached, can be recognized by staining with the Shaffer brazilin stain; the unbleached pulp can be differentiated with the Lofton-Merritt stain. See p. 536. Many other tests and descriptions of paper fibers are given by Sutermeister.[39]

Photographic contrast of slight color differences will generally be aided by differences in the shape of cellular structure. Sometimes, when the color differences are but slight, the best effects will be obtained by using light of daylight quality or, if the color differences are somewhat stronger, by means of moderately strong selective filters. The greater the color differences in the specimen, the more selective can the filters be in order to increase this difference. It would seem that, to record or increase slight differences in color in the specimen, filters passing only a narrow part of the spectrum would be indicated, but practice does not always bear this out. Good contrast between light blue and yellow may sometimes be obtained without any filter.

Figure 286 shows the structural details of lens paper. The paper, a very light tissue, was mounted in immersion oil. The individual fibers have photographed well.

GROUP VII

SHARP EDGES

Sec. 181. Description of Material. Observation, or more particularly a photograph, of the edge of cutting tools is likely to be disappointing or even misleading unless considerable care is taken in mounting the specimen, and in carefully adjusting the optical arrangement of the microscope to the work in hand. Razor blades are taken as an example, but the same or similar procedure can be applied to any sharp-edged specimen such as knives, cutting bits for machine tools, such as lathes, millers, shapers, planers, or drill presses, though some of them may require a little ingenuity to mount and illuminate.

EXAMPLES

The edge of razor blades, face view, Sec. 182.
Cross section of edge of razor blade, Sec. 183.

[39] Edwin Sutermeister, *The Chemistry of Pulp and Paper Making*, John Wiley and Sons, third edition, 1941.

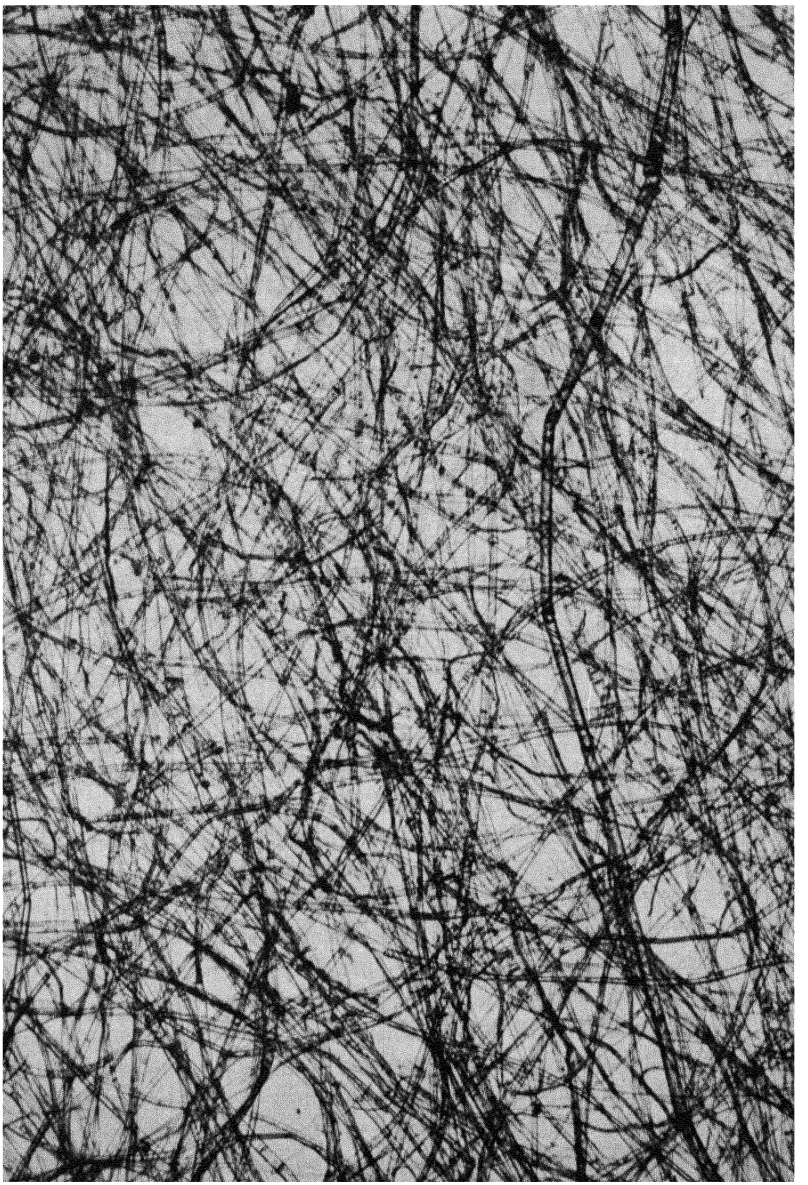


FIG. 286. Lens paper ×65. Mounted in immersion oil. Objective, Zeiss 30-mm apo.; ocular, Homal II; condenser, Leitz long-focus; illumination, 400-watt projection lamp, method I; filters, Corning No. 428, Wratten No. 57 plus 15; Eastman Panatomic X film; developer, D-19.

Illumination. Two lamps are required: one to furnish light for a vertical illuminating system; the other, for the microscope condenser. Thus the picture is taken by bright-field illumination. The lamp for the vertical illumination must be of high power, preferably a 400- to 500-watt projection lamp, but for the substage illumination 100 to 200 watts will suffice.

Two filters are needed: a strong red such as Wratten 25 for the transmitted illumination; and a blue like Wratten 45 for the vertical illuminating system. It has been found that the chromatic effect afforded by this combination of filters gives a very nice negative when the exposure is correct. The blue light on the surface of the steel blade gives a softness and, paradoxically, at the same time a strengthening of the grinding and honing detail which is otherwise unobtainable. The red filter brings out the background in strong contrast with the edge of the blade, panchromatic film being very sensitive to red light.

Apparatus. In addition to the illuminating equipment already enumerated, a special holder for the specimen will be necessary. If work is to be done entirely with one kind of blade, such as a razor or knife blade, a specially designed holder should be built with a clamp to hold the blade in a way that will permit the edge to be turned to the proper position for photographing. The face of the blade should be lying in a plane normal to the microscope axis. Since there is but little width at the outer bevel on a razor blade, the blade must be adjusted while it is being observed through the microscope; a method for doing this may be found in Sec. 2, which describes a procedure for testing the microscope stage. Larger knives can often be placed in small toolmaker's clamps, or they can be strapped to a short rod supported by V blocks. Light blades can be mounted on a small wad of Plasticine on a microscope slide; the edge under observation can then be tipped or forced into place by pressure applied judiciously with the aid of a teasing needle.

The microscope should be equipped with a vertically adjustable stage. A long-focus condenser must be used for the transmitted illumination, but the aperture of the condenser should be large enough to fill the objective with light.

Because a long-focus condenser is to be employed, the objective should be of the achromatic type and of low aperture. It should be of 16- or 8-mm focal length and corrected for use without a cover glass. The best objectives for this work have short mounts and are of low aperture, such as those intended for use in metallography. The 8-mm objective will easily give magnifications up to 500 diameters, which

is often sufficient for this type of work, but the 16-mm objective will give a larger view of the object and include more of it. This is very desirable for coarse sharpening methods when the edge of the blade may be rough. Eyepieces can be of the amplifying type, if the objective is a good one; otherwise an eyepiece of intermediate correction should be chosen.

Illumination for the overstage lighting is given by a prism or mirror-type vertical illuminator. It might also be obtained by any of the Epi systems. There would then be a combination of light field and dark field and the dark-line effects (see p. 698) would be reversed. A plate illuminator would probably give a much softer negative than anything else, but it would have to be counterbalanced by using a more contrasty film and developer or by some other method of strengthening the photographic contrast.

A film similar to Panatomic X is suggested; it should be developed in formula D-19.

Sec. 182. Photographing the Edge of a Blade. The microscope is set up with a prism vertical illuminator, a long-focus condenser, and a 16- or 8-mm objective. The blade is mounted on the stage in approximately its correct position, and the light for the vertical illumination is switched on and roughly adjusted. After the microscope has been focused on the bevel of the blade nearest the edge, the specimen is moved until the whole field is occupied. The lamp can now be adjusted to the best advantage. The specimen is turned until the beveled edge lies perfectly normal with the microscope axis and then is moved until the edge of the blade coincides with a diameter of the field of view. The next step is to rotate the microscope stage until the edge of the blade is at right angles to the edge of the illuminating prism or mirror, the position of which can be seen by inspecting the rear focal plane of the objective. Unless this is done the objective may be used with its diameter of greatest resolution (see Sec. 23) parallel with the blade edge instead of across it, and the edge of the blade might have one or more diffration lines running parallel with it. This adjustment is vital for either visual work or photomicrography.

The vertical illumination is given final adjustment, the blue filter is inserted, and this illumination is then switched off. The substage light is turned on; illumination can be made by Method I. It will probably not be necessary to remove the specimen to adjust the substage lighting. The red filter is inserted, and when vertical illumination is switched on again the edge of the blade should show plainly, illuminated with a blue light against a red background.

In making the exposure, it is undoubtedly best to get the correct timing first with one lighting system and then with the other. The exposure by the transmitted light system should be long enough to assure complete opaqueness of the negative, since there is no reason for having a slightly darkened background. The overstage illumination should have an exposure to give a nicely graded negative with proper contrast rendering all visible detail.

The interpretation of a picture taken under the above conditions is very simple. Any dark line, such as that on the edge of the blade in Fig. 288, is caused by a slight rounding of the edge. Where the edge is broken, the defect will be surrounded by a dark line, varying in width, caused by the reflection of the vertical light component away from this part. Thus, only when a blade shows a clear surface of the bevel as far as the edge is it in good condition. That is, the edge must have practically a geometrical angle, for, no matter which way the edge may be turned, either upward or downward, light from the vertical illuminator will be reflected away from the objective, thus causing the dark line to appear if the blade is in imperfect condition. Only the best and most perfect of edges will appear as shown in Fig. 287. Wire edges, unless perfectly flat, will appear as a dark line. The focus of the microscope is important in searching for this dark line; even when present the line may not be visible at all positions of focus. It might be argued that a blade could be broken parallel to the edge and that, with the top surface overhanging, the black line would not appear. This is true. Nevertheless the author, after examining several hundred blades, has noted that, whenever a break occurred or the edge was otherwise defective, the dark line showed to a greater or less degree or the break was otherwise plainly evident.

In general, razor blades have two bevels at the blade edge. The one toward the center of the blade is formed by rather coarse grinding, as an inspection will show. It is the bevel at the edge which shows in the pictures. Therefore, this surface is of importance, and care should be taken that as much of it as possible will appear in the picture. It is here that any carry-over of the coarser grinding compound from the first grinding will be indicated. A coarse particle of grit carried over from the primary grinding will leave a long scratch across the honed surface, and at the point where it is discharged from the edge of the blade there will generally be found a defect or break in the steel.

Sec. 183. Photographing the Cross Section of a Blade Edge. To make the cross section an ordinary blade can be broken by bending it in the hands until it snaps. Blades of heavy construction will have

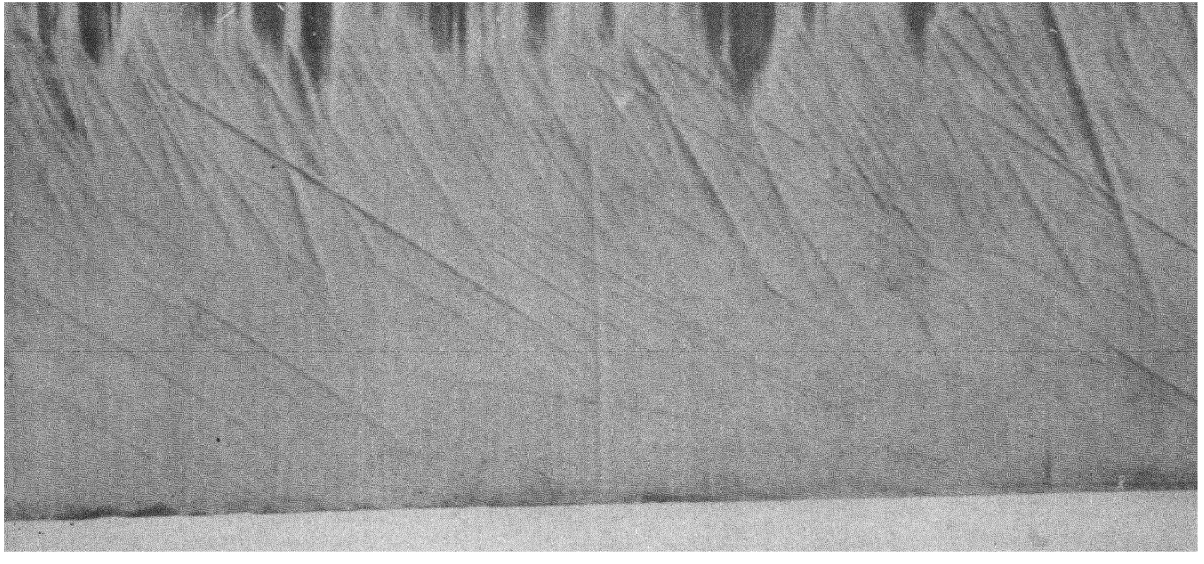


FIG. 287. Edge of safety-razor blade ×500. The edge of a sharp blade should be as free from nicks as this picture shows; also with this system of illumination a dark line should not show on the edge of the blade. The end of the first grinding marks are seen at the top of the picture. Objective, Zeiss, 8-mm achro., short mount; ocular, Homal I; vertical illuminator, Zeiss, prism type; condenser, Leitz, about 20-mm focus; illumination, 400-watt biplane-filament projection lamp for vertical illuminator, 200-watt projection lamp for substage condenser; filters, Wratten No. 45 on vertical illuminator, Wratten No. 25 on substage condenser; Eastman Pan, Commercial film; developer, D-61a.

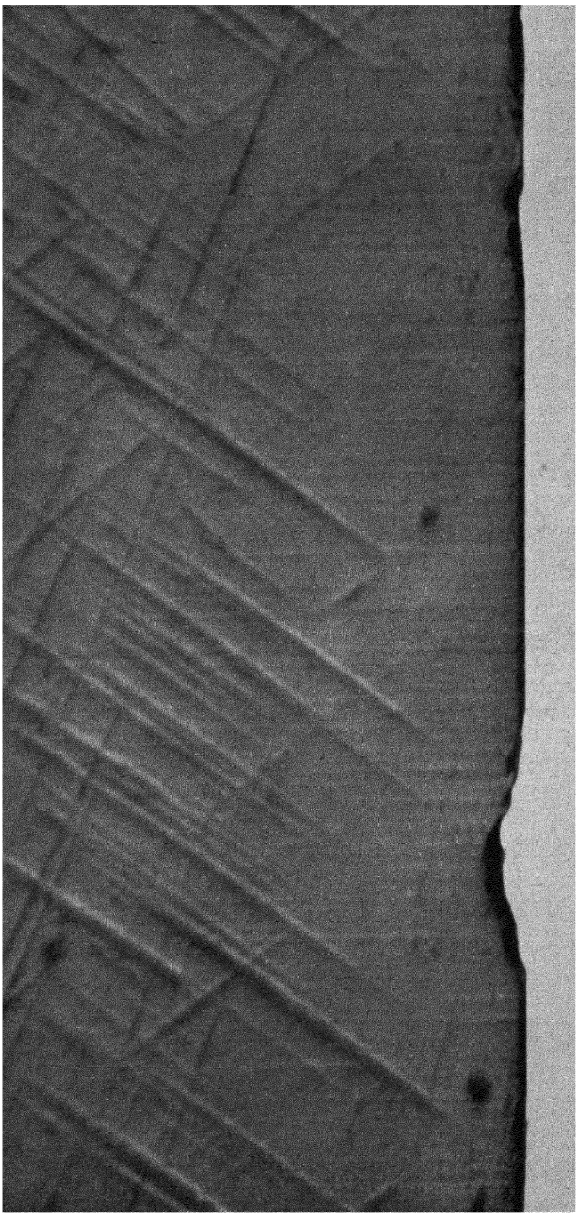


FIG. 288. Edge of safety-razor blade ×500. This blade is defective. Compare the grinding marks with those shown in Fig. 287. The data for taking this picture are the same as those for the photograph shown in Fig. 287.

to be held in a vise and struck with a heavy hammer. The edge of the broken blade should be examined with a hand magnifier to observe whether or not the break is clean; if not, another attempt should be made. When a satisfactory break has been obtained it may be necessary to break the piece again to make it shorter. If possible, the piece should be less than 5 mm long. Small blade fragments can be mounted directly on a microscope slide in a little Plasticine. The Plasticine must not appear in the picture. See Fig. 289.

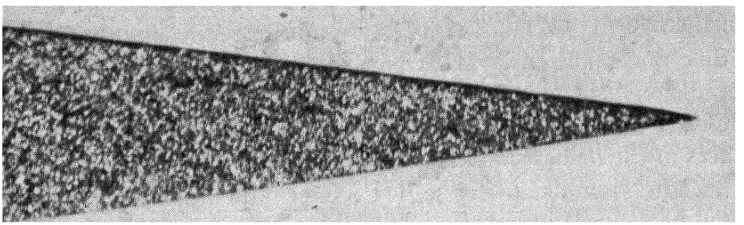


FIG. 289. Cross section of safety-razor blade ×250. The angle at which the cutting edge of the blade is ground can easily be determined from a picture of this type, but it tells practically nothing about the condition of the edge of the blade. The data for this picture are the same as for the two preceding figures except that a 16-mm objective was used in place of the 8-mm. In a picture of this sort a green filter might have been substituted for the blue.

Low-power optics are generally employed in photographs of this sort, a final magnification of 250 diameters being about right. The purpose of the picture will be to show the angle at which the blade is ground; the condition of the cutting edge will not be indicated, as it may be slightly distorted when the blade is broken. The arrangements for lighting can be the same as for the picture of the face of the blade, that is, a prism vertical illuminator with a blue screen, and a long-focus substage condenser for transmitted light with a red screen.

Difficulty may be experienced in placing the specimen for a view of this sort in a strictly vertical position so that sides of the blade will not cast shadows. However, it can be done with a little care and patience. Visual inspection will show when the last trace of shadow on the side of the bevel has disappeared.

The angle at which the blade is ground can be measured in the picture by means of a protractor, or by a visual observation with an ocular goniometer while the specimen is on the microscope stage, or by inserting a cross-line disc in the eyepiece and revolving the micro-

scope stage. In the last method the stage must be graduated in degrees on the periphery.

Further work on blades and sharp edges falls within the province of the metallographer; it involves a polished surface and etching. See Group III, Sec. 164.

GROUP VIII

EMULSIONS

Sec. 184. Description of Material. Foodstuffs, cosmetics, pharmaceuticals, insecticides, and many other industrial products come in emulsified form. When two or more non-miscible liquids are brought together and sufficiently stirred or otherwise agitated they will form an emulsion in which one liquid exists in another as tiny droplets or globules. The continuous phase refers to the liquid which holds the droplets of the dispersed phase in suspension. If the dispersed phase is a solid, the " emulsion " is called a mechanical suspension. Several liquids may constitute the dispersed phase of an emulsion, as in salad dressing and pharmaceuticals of various kinds. Milk is a simple form of emulsion. The dispersed phase consists of the butterfat, and the continuous phase is largely water containing casein, salts, and protein matter. Butter is, in a way, mechanically the reverse of milk; the fat is the continuous phase and the dispersed phase is mostly water. Nearly all emulsions have one characteristic in common: the small globules and perhaps even the large ones are in a state of constant motion; this is Brownian motion or pedesis.

In natural emulsions the dispersed phase consists of reasonably small droplets. The smaller the droplets, the more stable the emulsion is likely to be. In artificial or man-made emulsions the aim is to make the dispersed phase as small as possible and thus increase its stability. For this reason, the purpose of most photomicrographic work on emulsions is to determine the diameters of the droplets of the dispersed phase, to compare the range of their size with a prearranged standard. Special care must therefore be taken to obtain characteristic fields and to see that the droplets are not distorted during the mounting procedure.

If the size of the droplets of the dispersed phase is less than 0.1 μ the emulsive mixture is said to be colloidal, the photomicrography of which is best carried on with a slit or ultramicroscope. Examples of this kind of work are not given here.

EXAMPLES

Natural emulsions, milk, Sec. 185.
Manufactured emulsions, hand lotion, Sec. 186.

Illumination. As might be easily imagined from the above description, very strong light sources are required to photograph an emulsion successfully. The motion of the particles, although of small amplitude, must be "stopped" during the exposure. An exposure of about $\frac{1}{25}$ second can often be made. This indicates the use of either a carbon arc lamp or a 400- or 500-watt biplane filament projection lamp, adjusted for Method II. Occasionally, a filter of light color can be used, but its value is doubtful. Wratten 8 or Corning 328 might be employed to eliminate the shorter wavelengths, but they are likely to reduce the exposure time unduly.

Apparatus. The microscope can be of the simplest construction provided that it has the necessary adjustments for the centration and focusing of the lenses. A small square stage is suitable.

The special apparatus required is a cell or two of sufficient depth to mount the specimen. The regular dark-field cell supplied by microscope companies may be all that is required. These cells come in various depths, depending on the make, ranging from about 2 μ for the Zeiss up to nearly 10 μ. Cells will successfully handle emulsions with particle size up to and slightly larger than the cell depth. Under these conditions there will be little or no distortion of the large droplets, and the smaller droplets will appear in correct proportion to the larger ones. For emulsions with very large droplets, the cell may have to be deeper than 10 μ; the haemacytometer cell, the depth of which is 0.1 mm and which was designed for blood counting, will answer nicely. Other cells with intermediate depths may be found occasionally.

Objectives can generally be of the achromatic type because of the increased field depth over apochromatic objectives afforded by their low aperture. Although the low aperture of the objective reduces its light-collecting power, it is almost a necessity with the haemacytometer cell, which is equipped with a cover much too thick to lie within the range of cover-glass correction by means of shortening the drawtube. The apochromatic objective would thus be seriously overcorrected, and good imagery at high apertures would be completely lost. Even if the thick cover glass provided with the haemacytometer cell is replaced by a thin cover, the great depth of the cell will be further beyond the field depth of an apochromatic than that of an achromatic objective.

Accordingly, 16-mm, 8-mm, and 4-mm achromatic objectives, with apertures of 0.2 to 0.25, 0.4 to 0.5, and 0.6 to 0.7, respectively, are recommended. Magnifications will, for the most part, have to be medium to high, that is, about 500 to 1000. To get this magnification

with the objectives mentioned, it will be necessary to use a camera with a long bellows draw and a high ocular, 12× or 15×. If a 5 by 7 inch plate is to be covered, an amplifying ocular will have to be included as part of the equipment. Unless a contrasty film is used, pictures of emulsions are likely to appear flat, or at least to have a dark background, which gives a very unpleasing effect. The strong light source furnished by the 400- or 500-watt biplane filament projection lamp makes short exposures possible.

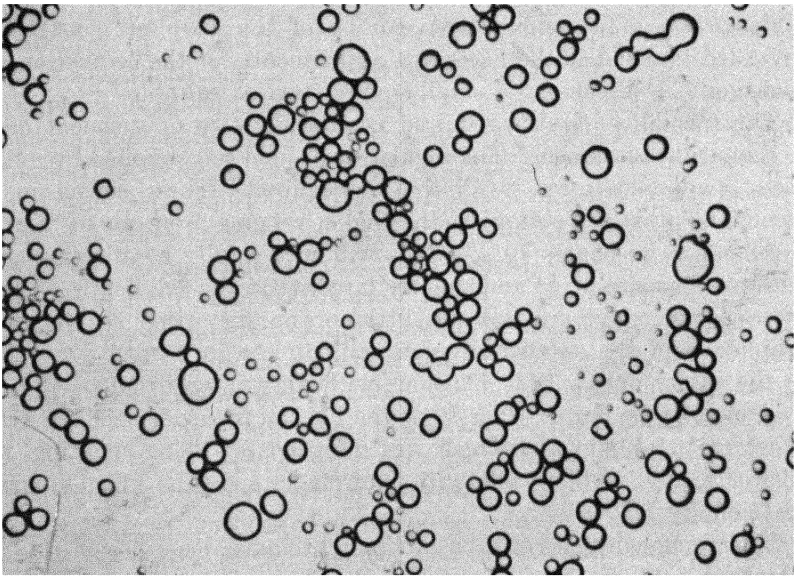


FIG. 290. Milk ×750. The dispersed phase is butterfat. All the smaller particles were in motion at the time the exposure was made. Objective, 8-mm apo; ocular, Homal I; condenser, Leitz achromatic, oiled to slide; illumination, 400-watt biplane-filament projection lamp, method II; no filters; Eastman Contrast Process Ortho film; exposure, 1/25 second; developer, D-19.

To make an exposure of $\frac{1}{10}$ or $\frac{1}{25}$ second, a shutter will be required for exact timing. The shutter can be attached to the camera, and it may be of the automatic, multiblade type; or, better yet, it may be of the focal plane type, mounted between the light source and the microscope independently of the camera. In this position a smooth-working shutter will not cause enough vibration to affect the picture. In lieu of a shutter, short exposures can be made by holding two pieces of black cardboard ¼ inch or more apart in such a way that the light is blocked off by one of them. As both are moved across

the light path, the light will be blocked off again by the second cardboard. This method is clumsy; it will take several trials and a little practice to get the correct exposures, and they will be difficult to duplicate. However, if other means are not at hand, or if only little of this sort of work is to be carried out, the cardboard system will be found practical and inexpensive.

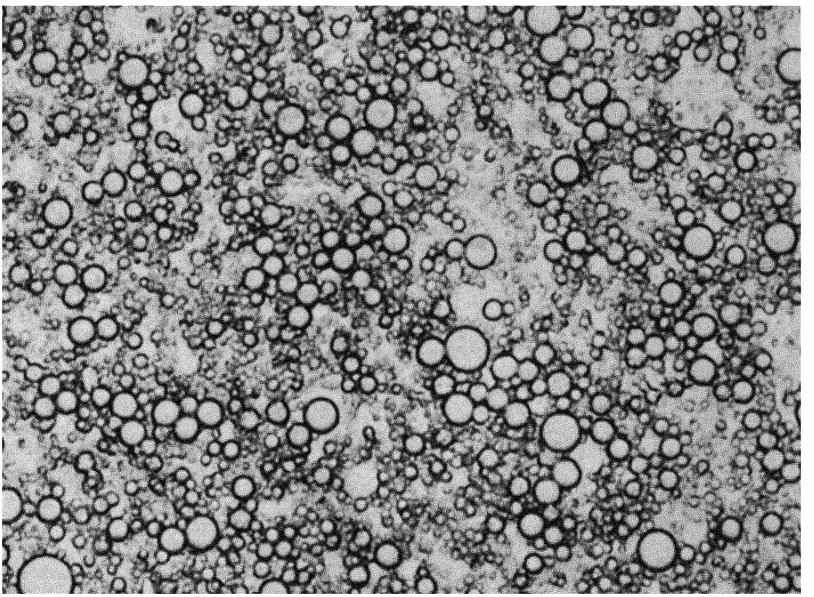


FIG. 291. Hand lotion ×700. Little or no motion, owing to close packing of the disperse phase. Objective, Zeiss 8-mm achromat, short mount; ocular, Homal I; condenser, Leitz achromatic, oiled to slide; illumination, 400-watt biplane-filament projection lamp, method I; filters, Wratten 15 and 58 plus Bausch and Lomb daylight; Eastman Contrast Process Ortho; developer, D-19.

Sec. 185. Photographing Milk. Figure 290 illustrates the size and distribution of the butterfat particles as they occurred in this sample of milk. Other specimens might have a different appearance since the size of such particles and their uniformity of distribution may vary, or they may show even more tendency to collect together. The necessary data accompany the picture.

When an emulsion appears as in Fig. 290 the particles are likely to have the maximum amount of motion; the space between the droplets of the dispersed phase is rather large so that great freedom of motion is permitted. Careful observation, either on the ground glass

of the camera or through the microscope, will show that butterfat particles up to several microns in size are never entirely still. The specimen was mounted in a cell with a depth of 7 or 8 μ. This depth was great enough to take the larger particles with little or no distortion. The smaller particles floated up to the under side of the cover glass. Thus fairly sharp images of both small and large droplets were possible.

Sec. 186. Photographing a Hand Lotion. The photomicrograph in Fig. 291 presents a condition slightly different from that depicted in Fig. 290. The dispersed phase shows the particles packed closely together, so that pedesis is slowed down or prohibited. This state of quiescence makes possible the utilization of long exposure times. In this particular picture, an achromatic objective was used. It might be noted that both pictures of emulsions were taken on contrast process film.

GLOSSARY

Some Optical Terms used in Microscopy

Abbe (ăb' ē), **Ernst** (1840–1905). A man who accomplished more to further the interests of scientific microscopy than any other individual. He was a German mathematician and physicist, professor at Jena, and inventor and originator of much optical apparatus at the Zeiss works. His inventions include the apochromatic objective, the compensating ocular, the Abbe condenser, a well-corrected oil-immersion achromatic condenser, the immersion objective, Abbe apertometer, Abbe refractometer, and the drawing camera; he evolved the Abbe theory of resolution and microscope imagery, the numerical-aperture formula, and other optical theories.

Abbe apertometer. A device for measuring, simultaneously, the numerical and angular aperture of an objective or microscope condenser. The back focal plane of the objective is viewed with an auxiliary lens, and the device is set to show the position of an indicator just cutting into the edge of the field.

Abbe condenser. Originally a two-lens combination designed by Ernst Abbe, it is not a corrected lens and has only a very low-angle aplanatic cone. It may be rated with a numerical aperture as high as 1.3. It is not suitable for use with oil-immersion objectives or with the high-aperture 4-mm objective.

Abbe substage apparatus. A type of substage invented by Abbe at the Zeiss works. It includes a rack and pinion for horizontal displacement of an iris diaphragm to obtain oblique lighting.

Abbe test plate. A long wedge-shaped cover glass about 0.20 mm thick at one end and 0.10 to 0.12 mm thick at the other end, coated chemically with a silver film, on which are ruled horizontal lines, and at each variation in thickness of 0.01 mm there are vertical lines. By means of oblique illumination, and by focusing on different portions of the plate, it is possible to determine the optimum cover-glass thickness for any objective, and also the tube length at which the objective gives its best performance. The approximate freedom from spherical and chromatic aberration can also be estimated. Small isolated bits of silver near the edges of the lines form good objects for the star test.

Abbe theory of image formation. Abbe's theory is based on the fact that a particle which is not self-luminous, but is illuminated by an extraneous source, gives rise to diffracted light rays, in addition to the dioptric pencil. He stated that to form a good microscope image as many of the diffracted rays as possible should be intercepted by the objective. With closely ruled lines, his theory is easily demonstrated by observing the back lens of the objective, for here the diffracted rays produce interference. It can be shown that, when the illumination is arranged to exclude the diffraction phenomenon, resolution is lost.

Aberration. In an optical system, the aberration of light refers to the straying away of rays from their appointed courses. Instead of all light rays which arise from a definite object point converging to a conjugate image point, some rays passing through certain extra-axial lens zones and in different azimuths may

converge at other points. Dispersion of the lens glass causes light of different colors to be focused in separate planes and also causes a shift in the focal and Gaussian points for light of different wavelengths. All these errors, unless corrected, tend to produce a hazy image and give rise to spherical aberration, coma, astigmatism, curvature of field, distortion, and longitudinal and lateral chromatic error. A simple lens or single lens element will have all the above errors although it may have faultless spherical surfaces. *See* Correction of an objective; Correction of lenses.

Aberration, chromatic. The natural dispersive power of glass causes a simple lens to focus blue light at a shorter distance than red light. That is, a simple lens has different focal lengths for light of different colors, that for blue light being shorter than that for red light. An image produced by such a lens, particularly one of large aperture, will show color fringes around the border of the image for the lens is affected with chromatic aberration. The difference in position, along the axis, of the focal points for light of different wavelength is called longitudinal chromatic aberration. The difference in magnification due to differences in position of the principal points for light of different wavelengths (also a difference of focal length) is known as lateral chromatic aberration.

Aberration, spherical. A specific term applied to the zonal aberrations of a lens regarding an axial point. When rays from a point on the axis passing through the outer lens zones are focused closer to the lens than rays passing the central zones, the lens is said to suffer positive spherical aberration. If the condition is reversed, and the outer zones have a longer focal length than the inner zones, the lens is said to have negative spherical aberration. In the first instance, the lens is uncorrected or under-corrected; in the second, it is over-corrected. Spherical aberration is also a generic term applying to spherical aberration as described above, as well as to comatic and astigmatic error.

Absolute temperature scale. A scale sometimes known as the Kelvin scale in honor of its inventor, Lord Kelvin. Its zero is $-273.1°$ C or $-459.6°$ F. Thus $0°$ C $= 273.1°$ K. At $0°$ K all thermal motion is said to cease. There is complete absence of heat. The value of 1 interval on the absolute scale is the same as that of 1 interval on the Centigrade scale.

Absorption. Light passing through a transparent substance is partly absorbed (generally accompanied by a rise in temperature), partly transmitted, and partly reflected. If a substance absorbs selectively, it will appear colored in transmitted white light. A red filter absorbs blue, green, and yellow light, allowing only the red light to pass. A neutral filter absorbs all colors equally, and so will merely reduce the intensity of the light without changing its chromatic quality. Since each layer of a transparent substance absorbs the same proportion of light incident upon it as any other layer of equal thickness, the equation for transmission is

$$I_1 = Itx$$

where I is the intensity of the incident light, I_1 the intensity of the transmitted light, t the fraction of transmitted light for unit thickness, and X the total thickness of the substance. The difference between the incident and transmitted values indicates the amount absorbed plus reflection losses, say 4 per cent at each surface.

Accommodation. This is the attribute which is possessed by the normal human eye to focus objects at various distances correctly. Muscular action changes the focal length of the eye lens by altering the curvature of the lens surfaces. The range through which accommodation can be made is, roughly, from a point at infinity to a point about 8 inches distant. Age has much to do with the ability to accommodate.

Achromatic. Literally, color-free. It is said of a lens or prism which passes white light without resolving it into its separate color components. An image formed by an achromatic lens will not be surrounded by color fringes. A doublet composed of a positive and negative element can be made achromatic for two colors. Achromatizing corrects for chromatic aberration.

Achromatic-aplanatic condenser. Properly used, this condenser should give practically perfect results. For oil immersion it has a maximum numerical aperture of 1.4, and for the dry system it is rated at 1.0. For achromatism, it may include two doublets over which is a positive meniscus element. The short focal length (usually less than 10 mm) is obtained by means of a strong planoconvex top lens. The oil-immersion system must always be immersed in order to take advantage of the full corrections. This is true irrespective of the objective in use.

Achromatic condenser. This is practically synonymous with the achromatic-aplanatic condenser since all firms consulted claim that their achromatic condensers are also aplanatic.

Achromatic objective. Objectives are said to be achromatic when they are corrected chromatically for light of two wavelengths (generally the C and the F lines) and spherically for light of one color (usually in the yellow-green part of the spectrum). This series of objectives is made in all powers. The higher powers have the mineral fluorite included in their construction to aid in achromatizing. The better lenses of this series are sometimes called semi-apochromats. Good achromatic objectives will give a fine image in the central portion of the field when used with appropriate oculars. However, it is not possible to get as sharp an image at the edge of the field as can often be obtained with apochromats. Usually achromats have a lower N.A. than the corresponding apochromatic objectives and take decidedly lower oculars.

Achromatic prism. A prism composed of two elements, the dispersive effects of one being counterbalanced by the dispersive effects of the other. Such a prism will pass white light without forming a spectrum.

Achromatism. The state of being achromatic.

Achromatize. To make achromatic.

Ahrens prism. A polarizing or analyzing prism as used in a petrographic microscope. The ends are so cut that they are at 73° to the microscope axis. The more important form is a three piece prism with end faces normal to the microscope axis. *See* Nicol prism.

Airy (âr′ ĭ), Sir George Biddell (1801–1892). English astronomer and mathematician. Originator of the Airy theory of resolution. *See* Airy disc.

Airy disc. A diffraction pattern, or the image of an infinitely distant point, as focused by a lens. With monochromatic light, it consists of a central point of maximum intensity surrounded by alternate circles of light and darkness caused by the reinforcement and interference of diffracted rays. The light areas are called maxima and the dark areas minima. The distribution of light from the center to the outer areas of the figure was investigated mathematically by Airy.

The diffraction disc forms a basis for determining the resolving power of a lens system. The diameter of the disc depends largely on the aperture of the lens. See equation 21.

Alcohols for mounting media. The use of alcohols as mounting media and for obtaining recrystallization has several advantages. They are of low index and can be mixed with various solvents. The higher alcohols have a low index combined with a relatively high boiling point. The higher alcohols are essential mounting media for many specimens.

Alignment, optical. The arrangement of optical parts — lenses and diaphragms — so that all their axes are mutually coincident.

Alpha-monobromonaphthalene. A liquid chemical compound with a refractive index of about 1.66. It is used as a mounting medium as well as an immersion fluid for the high-aperture objective (N.A. = 1.6) made by Zeiss. It is soluble in alcohols and hydrocarbons.

Amici (ä mē′ chē), **Giovanni Battista** (1784–1863). An Italian astronomer who made several contributions to optical science, including the Amici prism (*q.v.*).

Amici prism. A prism, invented by G. B. Amici, which disperses light without deviation and so can be used to make a direct-vision spectroscope. Amici prisms are used in certain spectroscopic eyepieces.

Amicron. Colloidal matter. Any small particle a few millimicrons in diameter visible in the ultramicroscope.

Amicroscopic. Too small to be seen in the ultramicroscope (less than 0.005 μ in diameter).

Amplifier. A lens, used in place of the usual microscope ocular, designed to give flat fields for projection and especially for photomicrography. It is a negative combination with all the corrections of the compensating ocular; therefore it should be used with apochromatic objectives or high-power achromats. It is made by at least two firms, Bausch and Lomb (the Ampliplan) and Zeiss (the Homal). Owing to the differences in field curvature of objectives of different power, amplifiers are selected according to the objective with which they are to be used. There are four lenses in the present Zeiss series. Their focal lengths are from 70 to 20 mm. Amplifiers being negative, the microscope tube length must be shortened a specified amount; the objective can then be used at its calculated object distance.

Amplitude. A dimension of wave motion or vibration. Amplitude is the distance from the point of rest to the limit of the oscillation. Double amplitude is the total distance traveled by the elementary vibrating particle. See Fig. 67.

Analyzer. The upper Nicol prism (*q.v.*) used in a petrographic microscope. Its position is usually within the microscope tube. When Nicol prisms are added to a biological microscope, the analyzer may be screwed on to the lower end of the drawtube or may be mounted above the ocular. The function of the analyzer is to discover the optical effects produced by the specimen on polarized light from the lower Nicol or polarizer.

Anastigmatic lens. A photographic or microphotographic (*q.v.*) lens free of astigmatism. It is a better and more expensive lens than the rapid rectilinear.

Angle of deviation. The extent to which a ray, incident at the surface of a transparent medium, is refracted from its course depends on the relative index of the two media. The angle of deviation is the angle between the course of the ray after it enters the second medium and its original course.

Ångström (ōng' strûm), **Anders Jöns (1814–1874).** A Swedish physicist after whom the angstrom unit (*q.v.*) is named.

Angstrom unit. A small unit of linear measure named after A. J. Ångström (*q.v.*). It is 1×10^{-10} of the meter; $1\ \mu = 10{,}000$ A. It is variously abbreviated: A, A., A.U., Å., or ÅU. It is used chiefly in spectroscopy. See Table II.

Angular aperture. The angle subtended by a diameter at the entrance pupil of a lens, from a centrally located point in the object field. *See* Aperture.

Angular magnification. The angular ratio made by a ray of light passing through a lens from an axial point to its conjugate point. The first angle is measured in the object space between the ray and the lens axis; the second angle is measured in the image space between the ray and the lens axis. The angle in the image space is the numerator of the fraction. The angles are usually represented by their tangents.

Anisotropic. *See* Birefringence. Any material which shows polarization colors between crossed Nicols is anisotropic. The material may be crystalline or amorphous. Opposed to isotropic, which indicates that the substance has no polarization effects on light. In certain orientations, anisotropic material can be shown to have two refractive indices, one for each direction of vibration.

Annular illumination. A stop placed in the first focal plane of the condenser to produce a cone of light with all the light flux near the surface of the cone. The central part of the cone will be dark. This arrangement is often used for a dark-field effect in low-power work. The condenser must be correctly focused. Above stage, annular illumination is attained with the Silverman illuminator or similar lighting arrangement. Annular illumination implies that the object is lit from all sides.

Anti-principal points. These are conjugate points for which the magnification is unity. They occur at equal distances from the focal points but on opposite sides from the respective principal points. Their distance from the focal points is equal to the focal length of the lens. Thus an 8-inch lens will produce an image of natural size (magnification $1\times$) on a screen 16 inches distant when the object distance is 16 inches.

Aperture. *The aperture of a diaphragm* is a measure of the diameter of its opening. *The aperture of a telescope* refers to the diameter of its objective lens. *Relative aperture* of a camera lens is the ratio of its focal length to the diameter of its exit pupil; it controls the brightness of the image. *Angular aperture* of a microscope objective is the angle subtended by a diameter of its front lens from a centrally located point in the object field. *Numerical aperture* (N.A.) of a microscope objective is the sine of half the angular aperture times the index of refraction of the light-transmitting medium (1.0 for air, 1.515 for immersion oil). The aperture of an objective is a measure of its light-gathering capacity and of its resolving power as well as of its field depth. However, not all additional transmitted light due to increase in aperture may be effective; the light absorption of the higher-aperture lens may be much greater than that of the lower-aperture lens, owing to additional lens elements and greater surface reflection. *Free aperture* of a lens or diaphragm is measured as the unrestricted diameter of the lens or diaphragm opening.

Aperture diaphragm. *See* Diaphragms. A diaphragm placed in a position to limit the angular aperture of a lens, as the condenser diaphragm of the microscope condenser.

Aperture of condensers. Apertures of condensers vary from about 0.15 or less for spectacle lens condensers to 1.4, in terms of numerical aperture. A controlling iris diaphragm is usually placed in the front focal plane of the condenser. When the condenser is properly adjusted, this position coincides with the entrance pupil of the microscope. Thus the aperture of the complete microscope system is limited by the condenser or objective aperture, depending on which is the smaller, the condenser aperture, of course, being adjustable. Under normal conditions the available maximum condenser aperture should always exceed that of the objective by at least a small amount. *See* Aperture.

Aplanatic. Corrected for spherical aberration and coma.

Aplanatic condenser. A substage bright-field condenser consisting of three lenses. The front lens is corrected by grinding to an aspheric surface (*see* Aspheric lens). Used with a strong green screen, it is an excellent condenser.

Aplanatic points. With a completely spherical lens, a glass sphere, sometimes called the aplanatic sphere, there are two conjugate points for which the lens is aplanatic. They lie on the principal axis. Advantage can be taken of this naturally aplanatic condition in the design of an oil-immersion objective. The drop of oil serves to complete the spherical shape of the front lens of the objective and so includes an aplanatic point in the object space. The object lies at the first aplanatic point, and the image, which is virtual, is at the second aplanatic point and serves as the virtual object for the second lens element.

Aplanatic sphere. *See* Aplanatic points.

Aplanatism. The state of being aplanatic (*q.v.*).

Apochromatic objective. Objectives corrected chromatically for three colors and spherically for two colors are called apochromats. These corrections are superior to those of the achromatic series of lenses. All apochromats are under-corrected for color and therefore must be used with compensating oculars which are over-corrected the proper amount to produce compensation. This inherent under-chromatic correction makes possible the fine spherical corrections of these objectives. Apochromatic objectives were first designed by Abbe, in 1886. Great spherical correction was finally attained by the use of fluorite and new glasses having the requisite optical properties, which were first available to Abbe.

Aqueous humor. A watery fluid between the cornea and the crystalline lens of the eye. *See* Vitreous humor.

Aspheric lens. In a measure, a lens can be made aplanatic by grinding the outer zones to a greater radius than the inner zones A surface of this sort is obviously not spherical; it is called aspheric. Aplanatic condensers can be made in this way. Bull's-eyes sometimes have aspheric surfaces.

Astigmatism of the eye. A dot examined through a cylindrical lens, such as a glass rod, 5 or 6 mm in diameter, will appear as a line at right angles to the rod axis, because there is magnification only in this direction. By like reasoning, if the refracting surfaces of the eye are deformed, and, departing from a true spherical surface, tend to become cylindrical, the eye will suffer from astigmatism. Dots will appear as lines, and lines radiating from a common center will appear sharp only in certain azimuths.

Astigmatism of a lens. An aberration detected in the outer portions of the field of view or image field. It shows itself by making radial or tangential lines appear sharp in different focal planes. A point appears as a radial or tangential line depending upon whether the examination is made beyond or within the average focal plane. It results in two curved image surfaces being formed,

primary and secondary. When the primary surface, or the one with the tangential image formation of a point object, is nearer the lens, the error is said to be positive; otherwise it is negative. This aberration is usually associated with coma, and both aberrations may co-exist, one masking the effects of the other. Astigmatism in a lens is due to different magnifications in different azimuths; compare with astigmatism of the eye.

Axis, optic. The optic axis usually refers to the axis on which several principal lens axes may lie. It also refers to the axis of the eye which extends through the center of the crystalline lens. In petrography, it refers to the direction through an anisotropic crystal in which natural or unpolarized light may proceed without suffering the effects of polarization. Uniaxial crystals have one optic axis; biaxial crystals have two optic axes.

Axis, principal. A line conceived as passing through the center of a lens to connect the centers of curvature of the lens surfaces is the principal axis of the lens. The focal points of a lens lie on the principal axis.

Azimuth. When referred to a lens, the azimuth angle is the angle subtended by an arc measured between two points on the lens circumference, or, by extension, it may refer to a single radial position, measured circumferentially, in an image field. Because a lens circumference usually has no reference points, this term is rather vague, and has not the precise definition attributed to it in astronomy.

Back focal length. The back focal length of a lens is measured on the principal axis, from the second lens vertex to the back focal point of the lens. It is not the equivalent of the focal length, although the term is often so used.

Back focal plane. The plane, normal to the lens axis, situated at the back focus of a lens.

Back focal point. The second principal focal point of a lens. It may be called the second focal point. It is measured along the principal axis from the second principal point. *See* Principal point; Focal point.

Back lens. In any compound lens (a lens system composed of more than one lens element), the last lens through which the light passes is called the back lens. It may be a single simple lens, a doublet, or triplet.

Balsam, Canada. A turpentine from the balsam fir (*Abies balsamea*) found within the limits of the United States and Canada. It is processed, and the residue, a sweet-smelling resin, dissolved in a quick-drying solvent such as xylene, toluene, or benzol, is used as a medium for making permanent microscopical mounts. It discolors and darkens with age. Excessive heat will produce a dark yellow color in the resin accompanied by a strong pungent odor. The refractive index of the dried resin is 1.535 (Lee). Melting point, 61° C.

Base, microscope. The microscope base is now usually a modified form of the horseshoe type. When properly made, it provides three points of contact with the table, one at the heel and one at each toe. The pillar is, as a rule, part of the base casting.

Beam of light. Strictly speaking, a bundle of parallel light rays obtained by placing a source at the focal point of a lens or reflector. The beam from a paraboloid reflector is substantially parallel in all parts when the source is very small and is at the focus of the reflecting surface. The beam from a microscope lamp, when adjusted for parallel ray illumination, generally fans out in the form of a cone because of the extended source. However, all the rays arising

at any one point in the object will travel parallel to each other after passing the lens.

Becke line. When the liquid phase of a microscopical mount is of different index from that of the solid phase, a narrow white line can be observed around or just within the outlines of the specimen as the microscope tube is raised or lowered from its position of best focus. The presence of the line indicates the difference in index referred to, and its absence, therefore, indicates similarity of index between the specimen and its mounting fluid. The Becke line is useful in determining the index of small transparent particles. See p. 517.

Benzene. Also benzol, C_6H_6. A liquid with a boiling point of 80.1° C, slightly soluble in water. It should not be confused with benzine (*q.v.*).

Benzine. A mixture of the lighter ends from the refining of petroleum. Its boiling point is lower than that of benzene, in some grades being between 40° and 60° C. Because of its fast evaporation it is an excellent solvent for cleaning objective lenses. It is also known as petroleum ether.

Bertrand lens. A small, low-power lens screwed into the lower end of the draw-tube or otherwise inserted at the proper position to be used in conjunction with the ocular. It is used principally for petrographic work to examine interference figures. The principle is also utilized in the biological microscope to aid in observing the back focal plane of the objective. The true Bertrand lens effect is microscopic, therefore forming with the ocular a secondary microscope system; this system is apt to be strongly astigmatic.

Bicentric condenser. A dark-field condenser with two reflecting surfaces, sometimes called a bispheric condenser.

Binocular attachment. A double-tube attachment to fit the top of the regular monocular tube, to convert a monocular monobjective microscope into a binocular instrument, thus permitting the use of both eyes.

Binocular microscope. A microscope fitted with a double tube for vision with both eyes. The low-power dissecting microscopes are usually binocular, and the image is erect; two objectives are used (after Greenough). The monobjective microscope may also be binocular. In image formation, no additional clarity or resolution is gained, but the simultaneous use of both eyes reduces nerve strain, and for protracted observation the binocular is to be recommended. The binocular tube is nearly always interchangeable with a monocular tube. The double-tube construction may furnish converging axes or, in some makes, parallel tube axes. For excellence of image, there is little to choose between the converging or parallel tube systems; it is largely a question of personal adaptation.

Birefringence. Anisotropic substances possess birefringence. They are doubly refracting. A birefringent crystal polarizes light in two directions at right angles to each other. The two light rays travel at different velocities through the crystals. Thus the crystal exhibits two refractive indices, one for each ray. This phenomenon is called birefringence. Numerically, it is the difference in index between the greater and lesser index values of any anisotropic material.

Bispheric condenser. *See* Bicentric condenser.

Black-body radiation. *See* Color temperature.

Body color. The color of a transparent body as seen by transmitted light. It is not necessarily the same as surface color (*q.v.*).

Bright-field illumination. Bright-field illumination is the ordinary form of microscope illumination as furnished by means of a substage condenser and mirror.

The image of the specimen appears on a bright, well-lit field. In effect, it is the opposite to dark-field illumination.

Brightness. The brightness of a surface is measured in candles per unit area. It equals the product of the illumination per unit area and the reflectivity of the surface (reflection factor, *q.v.*) divided by π. It is also measured in lamberts; 1 lambert is the equivalent of 1 lumen per square centimeter.

Brownian motion. Also known as pedesis. Very small particles (usually less than 5 μ in diameter) mounted in a liquid are often in a state of constant oscillation. It is a complex motion composed of several separate and distinct movements. It was named in honor of one of its discoverers, the English botanist Dr. Robert Brown.

Bull's-eye. A bull's-eye lens is usually a simple lens, either double convex or planoconvex. The plane side should be toward the light pencil of greatest angle. The lens is of low power; those used in microscopy generally have a focal length of about 4 inches. They are used as light-collecting lenses at the lamp or for spot lighting objects from above the stage. They can sometimes be bought in the form of well-corrected doublets of long focus.

Calcite. A doubly refracting mineral used in the manufacture of polarizing prisms. As Iceland has been largely the source of supply, the name Iceland spar is often applied to it. It is uniaxial, negative, trigonal division of the hexagonal system of crystals; $\epsilon = 1.486$; $\omega = 1.658$; sp. gr. = 2.715 (Larsen).

Camera, photomicrographic. Any camera, box or bellows type, can be adapted for microscopy. The use of the regular camera lens in conjunction with the microscope has been practically abandoned. Thus a photomicrographic camera is essentially nothing more than a light-tight box to hold the photosensitive material. However, home-made makeshifts, although they may work, are not recommended. The nicely made, smoothly operating camera stands, with all accessories conveniently provided, not only save time in operation but also have more adjustments for alignment, and so forth, than can be easily provided on home-made apparatus.

Camera lucida. A prism attachment which fits on top of the drawtube. When properly adjusted, it affords a means for examining the microscope image with the superposed image of the field lying conveniently near the microscope at the same time. Tracings can thus be made of microscopic objects, or the image of scales can be superposed for purposes of measuring or estimating quantities and percentages of mixtures. The camera lucida is also useful in measuring the magnifying power of a magnifier or ocular.

Candle, standard. A standard source of light, the illumination from which is 1 foot-candle at a distance of 1 foot. The total emitted light flux is 4π lumens. Originally the source of light was a ⅞-inch sperm candle burning at the rate of 120 grains per hour; the candle has been supplanted by the pentane lamp.

Candlepower. A rating of light intensity applied to light sources; 1 candlepower gives 1 foot-candle of illumination at a distance of 1 foot.

Capacity of objective. The power to resolve and give a clear, well-defined image of fine detail. It is not a very definite term and might well be discarded. This term is also used to denote the light-gathering capacity (*q.v.*) of a lens.

Cardinal points. The focal points, principal points, nodal points, and anti-principal points of a lens.

Cardioid dark-field condenser. A condenser designed with two reflecting surfaces; the first, a spherical surface which reflects the rays to a second, cardioid surface. The virtue in such an arrangement is that, if the cardioid surface is of true figure, the lens is both achromatic and aplanatic. It has a limiting aperture of about 1.0. Thus objectives of greater aperture cannot be used successfully with it.

Cassegrain dark-field condenser. A high-power, dark-field condenser which can be used with objective apertures as high as 1.3. It is sometimes called the luminous spot ring condenser (Zeiss).

Cedarwood oil. An essential oil from the cedar tree *Juniperus virginiana*. It is processed by clearing and evaporation, and is useful in microscopy as an oil for the immersion of high-power lenses. At the temperature at which it is used for this purpose its index should be 1.515. A less perfectly refined form is used as clearing agent for histological work.

Center of lens. *See* Optical center of a lens.

Centering device. There are three well-known mechanical methods of centering such optical parts as objectives, condensers, diaphragms, and lamp lenses. One includes the use of two screws with their axes at right angles to each other, by the adjustment of which a sliding member holding the optical part can be moved to any position within the range of the screw motion, the movement being always in a plane normal to the axis of the lens or diaphragm. Another system employs two screws as above, but the screws are mounted in the moving part and their ends act as adjustable stops, the moving part sliding into position against the stops. A third system, applicable alike on objective changers and condensers, utilizes a ring to carry the optical part which is mounted eccentrically with respect to the ring; by revolving the ring and also its mount, one can easily center the optical part.

Centering the microscope lenses. This procedure is possible only when the lenses are equipped with centering devices. For photomicrographic work, careful centering of lenses is of the utmost importance. *See* Alignment. The objective should be centered to the microscope axis and the condenser lens aligned to it. It is also essential that the mirror and condenser diaphragm be aligned to the microscope axis.

Central lighting. Obtained when a condenser is properly centered together with the rest of the illuminating train. Under these conditions the cone of light at the specimen will be perfectly symmetrical.

Central stop. A stop placed in the ring carrier or diaphragm carrier of the substage apparatus. It excludes the central rays and is used for dark-field work at low magnification. The better-made stops can be contracted or expanded as desired.

Chalet microscope lamp (after Gage). A lamp house, made by Spencer, shaped somewhat like a Swiss chalet, with overhanging eaves which shade the eyes from direct light. It has ground-glass windows on two or four sides, as desired. It has no light-collecting lenses. It is suitable for general student use and for low magnifications where the lighting is not especially critical; it does not give enough light for high-power dark-field work. It can be used by several microscopists simultaneously.

Changing devices. The mechanical contrivance on the lower end of the microscope tube, for mounting the objective. The usual device is the well-known revolving nosepiece (*q.v.*) which may carry from two to four objectives, a late

development of which makes it possible to center each objective separately (Leitz). Individual centering rings can also be had for the revolver without the centering feature. For microscopes on which it is expected to do a great deal of photomicrographic work, a slide changer mounted on the end of the tube in place of the revolving nosepiece is recommended. Each objective is then equipped with a slide fitting which engages the part mounted on the microscope tube. Each objective can be permanently centered on its own slide.

Chemical focus. An old term which originated in the days when photosensitive material was sensitive to only blue light or the near ultraviolet. Unless specially corrected (*see* Aberration, chromatic), a lens will ordinarily focus blue light at a nearer point than light of longer wavelengths. Thus, although the eye may focus a camera lens correctly for white light and the strong visual colors, the picture may be taken by the hardly distinguishable blue light and the result will be an out-of-focus picture. However, the advent of panchromatic photosensitive material and lenses well corrected for the entire visual range have largely made the term chemical focus obsolete.

Chief ray. The theory of geometrical image formation, by a lens, is based on the point-by-point correspondence of object and image. Thus, in the object space, each object point is the apex of a cone of rays, the base of which is the entrance pupil of the system. Likewise, in the image space, each image point is the apex of a cone of rays the base of which is the exit pupil of the optical system. Rays of light coinciding with the axes of these light cones in the object and image spaces are called chief rays, and they will pass through the centers of their respective pupils; each object and image point will have a chief ray and all the chief rays in the object space converge to the center of the entrance pupil, and in the image space diverge from the center of the exit pupil.

Chromatic aberration. *See* Aberration, chromatic.

Chromatic light filter. *See* Color filter; Optical light filters.

Circle of confusion. The image of a small point of light registered on a plate by a good lens will appear as a very small disc. That is, an immeasurably small object point will be registered by a lens as a measurable point. The better the lens, the smaller will be the point image. With lenses of poor quality, the small image disc becomes larger and less sharply outlined; it develops a circle of confusion which is seen as an indistinct image. For purposes of enlargement the circle of confusion should be so small that even after enlargement the image will appear sharp. The photographic image can be thought of as composed of many such circles. The degree of confusion (fuzziness) of the circles, or image, depends on the quality of the lens forming the image, the amount the lens is stopped down, the distance the point image may lie from the effective focal plane (the amount that an object may be out of focus), and the ability of the photosensitive material to register fine detail. The circle of confusion, or as it is sometimes called the blur circle, should not be mistaken for the diffraction disc.

Coarse adjustment. A manual control of the microscope tube for preliminary focusing, obtained by means of a rack-and-pinion mechanism. The rack is mounted on the tube, and the pinion on the microscope limb.

Collimating lens. Strictly, a lens which is operated to throw a beam of light (*q.v.*) rather than a pencil of light. Thus the source must be at the first focal point of the lens. Its use as a light-collecting lens for microscope illumination is largely restricted to dark-field illumination.

Colloids. Any small ultramicroscopic particle. This definition limits the size of the particle to 0.1–0.005 μ. Since colloids exist below the limits of microscopical resolution, they are studied by dark-field illumination, particularly with the ultramicroscope where the limit of visibility is controlled by the intensity of the light source and the contrast of the field.

Colophony. A natural resin, the rosin of commerce. It may be refined and used in microscopy as a mounting medium. It has an index of refraction of about 1.545 (Lee). It is sometimes called colophonium.

Color filter. The more common term applied to optical chromatic filters. Any filter which produces a chromatic effect when placed in the path of the illuminating rays.

Color temperature. The temperature, on the Kelvin or absolute temperature scale, to which an ideally perfect radiating black body must be raised to radiate light. A low temperature is indicated by a deep dull red, which with rising temperature changes progressively until it approaches white. The color temperature of a tungsten lamp is around 2800° to 3000° K or slightly higher. The color of blue sky is given as about 25,000° K, and that of the sun as a good deal less. It might be noticed that the sky is intensely cold, and there is no connection between color temperature and actual temperature. However, the actual temperature of incandescent tungsten closely approaches that of its color temperature.

Coma. A lens aberration which occurs in that part of the image field which is slightly away from the principal axis of the system. It results from different magnifications in the various lens zones. It causes extra-axial object points to appear as short comet-like images, with the tail either toward the center of the field (positive coma) or away from the center (negative coma). Coma is fundamentally due to the faulty position of the principal points of the lens.

Compensating oculars. The compensating ocular may be of either the positive or negative type. The negative types are usually used for the low-power oculars and the positive types for the high-power ones. The corrections of this kind of eyepiece are of a high order. They are intended for use with apochromatic objectives of all powers. The residual under-correction for color associated with apochromats is compensated for by the over-correction of the compensating ocular (hence its name). For this reason a ring of yellow appears around the image of the ocular diaphragm in the field of view.

Compound lens. A lens combination of two or more simple lenses. Corrected lenses, such as microscope objectives, composed of four to twelve elements, are therefore compound lenses. Also, combinations of separate simple or compound lenses mounted on a common axis, forming one or more images, as the compound microscope.

Compound microscope. *See* Microscope, compound.

Condenser circle. The image of the iris diaphragm of the microscope condenser, as seen in the back focal plane of the objective.

Condensers. In microscopy, the condenser is the lens, mounted under the microscope stage, which furnishes illumination for the objective. There are two main categories of condensers: (1) bright-field and (2) dark-field condensers. Bright-field condensers are of three distinct types: (*a*) the Abbe or uncorrected condenser composed of two lenses; (*b*) the aplanatic condenser, a three-lens condenser corrected for aplanatism; (*c*) the achromatic condenser which has full corrections for color and spherical aberration. The dark-field condenser for low

powers may be nothing more than a low-power bright-field condenser with a central stop. Medium- or high-power dark-field condensers are usually of the cardioid or paraboloid type. The lamp lens is sometimes called a condenser lens, but light-collecting lens is a more definite term. Microscope condensers must always be carefully focused and aligned for the best results.

Cone of light. Usually, the light cone formed by the microscope condenser. A full cone is obtained when the focused condenser is adjusted to just fill the objective aperture with light. A half cone, a 9/10 cone, etc., is obtained when the diameter of the condenser circle (*q.v.*) is ½, 9/10, etc., the diameter of the objective circle (*q.v.*)

Conjugate points. Two points in an image-forming system are said to be conjugate with each other when a displacement of one causes a corresponding displacement of the other, one being in the object space and one being in the image space. The amount of movement of the object point is usually different from that of the image point. The principal points of a lens are mutually conjugate, as of course all object points and their corresponding image points are. Conjugate planes pass through conjugate points.

Contrast micrometer. A micrometer composed of alternate black and white squares which replace the lines in an ocular disc scale. The additional contrast makes measuring easier and faster on many subjects.

Corex glass. A glass, made by the Corning Glass Company, quite transparent to ultraviolet radiation. It is used for microscope slides and cover glasses intended for fluorescent work. In a measure it replaces the more expensive quartz. Corex is also used as a filter glass. It should not be subjected to contact with water.

Cornea. The comparatively hard covering on the outside of the front part of the eyeball.

Correction collar. A screw adjustment, generally under constant spring tension, which affords a method for controlling under- or over-correction by altering the spacing of the two rear lens combinations in the 3- and 4-mm objectives. It makes possible the adaptation of the lens to various cover-glass thicknesses, usually from 0.10 to 0.20 mm. The adjustment is turned until the optimum image is obtained, as determined by the star test. The setting for covers of definite thickness is marked on the collar.

Correction of an objective. This term is often used by the expert microscopist to indicate that he has observed certain rules in the adjustment of his instrument. An objective is made to function at its best only under a predetermined set of optical conditions. Those which are controllable by the microscopist are: tube length, setting of correction collar, cover-glass thickness, refractive index of immersion oil, proper qualities of light (wavelength), and proper adjustment of the illuminating apparatus. When all these factors are suitably coordinated to answer the demands of the objective, the objective is said to be corrected. The same term applies to the adjustment of the condenser, or for that matter to the working conditions of any lens, although the demands of most lenses are generally less, and therefore corrections can be less rigorously applied than for microscope objectives.

Correction of lenses. When this term is not applied as defined under "Correction of objective" (*q.v.*), it refers to the corrections of a lens according to its design. Roughly, it includes the following: the selection of glass combinations on the basis of refractive index, dispersion values, etc., the computation of radii

of surfaces, and the spacing of elements so that under conditions of service the required results may be expected. To obtain a well-corrected lens, the aim of the designer is to build, from simple lens elements, a compound lens system as free from errors as the situation warrants. This involves tracing many rays and often eliminating positive errors by the introduction of negative ones. Some lenses, such as spectacles, reading glasses, and bull's-eyes, require but little rigorous attention, but on the other hand microscope lenses demand great thought and patience in their creation.

Counting ocular. An ocular of the micrometer type. In place of a scale it is fitted with either a set of interchangeable discs with square openings of various sizes or with an adjustable diaphragm. It is then possible, by proper selection of magnification and diaphragm size, to make the area seen in the field of view have some convenient value. Counts of particles in such an area can be easily used for purposes of computing percentages of components. To avoid the expense of a special ocular, the regular micrometer ocular can be used with a disc of cross-ruling forming a reticule, sometimes called a network micrometer disc.

Cover glasses. Used to cover the microscopic preparations. They may be either rectangular or round. Thickness varies but should not be less than 0.1 mm or more than 0.2 mm. A thickness of 0.16 to 0.18 mm has been adopted as standard by a number of microscope makers; accordingly their objectives are corrected for covers within these limits of thickness. Covers are classified according to their thickness, the numbers running from 0 for the thin covers to 4 or 5 for the thick ones. Packages put up as number 1 usually contain many covers of desirable standard thickness. Covers can be measured after the mount is made or, better yet, before the mount is made, by means of a toolmaker's micrometer calibrated in 0.01 mm.

Cover-glass substitutes. Owing to shortage of cover glasses, lacquers and synthetic resins have been tried as substitutes. Although some have proved fairly satisfactory, the idea is not optically sound unless the synthetic is first cast and cut to size, so that its thickness is under control. Plastic covers of suitable thickness and of any desired size have recently been placed on the market.[1]

Critical angle. The maximum angle of incidence that can be formed by a light ray in passing from a high medium to one of lower index. The angle is measured beween a ray and a perpendicular erected at the intersection of the ray with the surface of the medium. The sine of this angle is the reciprocal of the refractive index of the medium. It is the angle of total reflection (*q.v.*).

Crown glass. Most ordinary window glass is crown glass. Optical, alkali-lime glass is also crown. It is known as a soft glass and has low dispersive power. An achromatic doublet is composed of two lens elements, a negative lens made of flint glass and a positive lens made of crown glass.

Crystalline lens of the eye. The small lens which focuses light rays on the retina — the inner part of the back wall of the eyeball. The lens has a variable focal length of 14 to 20.7 mm (after Helmholtz's Schematic Eye; see Hardy and Perrin). The short focal length is for the far point and the long focal length for the near point. The change in focal length is attained by muscular effort which effects alteration in the radii of curvature of the crystalline surfaces, thus producing accommodation for the far and near points.

[1] Obtainable from Charles F. Hubbs and Company, 383 Lafayette Street, New York City.

Curvature of field. The image field focused by a lens is naturally curved. When the convex side is away from the lens, the curvature is said to be positive; when toward the lens, it is said to be negative. Judicious use of a properly placed diaphragm produces what might be described as an artificial flattening of the field. A highly curved field is not at all incompatible with good images, but the portion of the field of such a lens having all particles simultaneously sharp and clear will necessarily be small. The outer zones can be made sharp only by refocusing. *See* Astigmatism of a lens.

Dammar. A natural resin obtained from evergreen trees in the East Indies, especially from *Agathis australis*. It is closely allied to the kauri gum or copal of the paint manufacturers. In microscopy it is used like Canada balsam (*q.v.*) for mounts. It is also used to seal mounts. Occasionally, however, it is said to form crystals.

Dark-field condenser. *See* Condensers. The ordinary bright-field condenser of low power, used with a central stop, makes a good dark-field condenser. Special dark-field condensers are the paraboloid, cardioid, and Cassegrain. They form a dark field by illuminating the specimen with a hollow cone of light, the lower limiting aperture of which must be greater than the N.A. of the objective with which it is to be used. Thus, no direct light enters the objective; the specimen is seen by reflected or scattered light on a dark background.

Dark-field illumination. Any method of illumination which illuminates the specimen but does not admit light directly to the objective. It may be by substage (*see* Dark-field condensers); it may be by over-stage spot lighting, by Ultropak or Epi condensers, or by the slit or ultramicroscope.

Dark-field objective. Certain objectives for high-power dark-field work equipped with an iris diaphragm so that their apertures may be reduced to correspond to the dark-field condenser with which they are to be used.

Dark-field slides. Owing to the exacting demands of dark-field illumination, not only must the microscope slide be especially clean and free of extraneous dirt, but also the glass of which the slide is composed must be optically clear under dark-field conditions. The glass should not fluoresce. For much work, choice, specially selected glass slides of good grade may answer the purpose. They should not exceed 1.0 mm in thickness, and for some condensers they may have to be thinner. For more exacting work and for work with the fluorescent microscope, quartz or Corex slides may be required.

Dark-field stop. A central stop for obtaining a dark-field effect for low-power objectives. It is customarily used with a low-power bright-field condenser.

Davis diaphragm. A small iris diaphragm mounted between the objective and microscope tube.

Daylight, artificial. Artificial daylight may be obtained by placing the microscope and lamp near a window and examining the field of view alternately with daylight and lamplight. Optical filters can be added until the lamplight simulates a daylight effect. Results obtained in this way may be considerably different from those obtained by the stereotype method of merely inserting a "daylight filter glass."

Definition. The clarity, sharpness, distinctness, and brilliancy of the microscope image.

Density, optical. A term applied particularly to the rating of neutral filters (*see* Opacity) and to the measurement of the density of negatives. Mathematically it is the logarithm of the reciprocal of the transmission.

Developer. A selective reducing chemical which in solution, and with other chemicals, reduces the silver salts of the photographic film to very tiny particles of pure silver. Only the individual halogen salt grains which have been exposed to light are subject to development. Many chemicals act as developers, but hydroquinone, elon, and pyrogallic acid are among the best known.

Diaphragms. Diaphragms, sometimes called stops, are apertures in the illuminating and image-forming systems of the microscope. They may be fixed in size, as the diaphragm of the objective, or adjustable, as the iris diaphragm of the condenser or lamp. They are usually placed in a focal plane of some lens. Thus they serve either to restrict the angular aperture of the system as a whole, like the iris diaphragm of the microscope condenser, or to limit the visual field, after the fashion of the lamp or ocular diaphragm. The diaphragms which limit the angular aperture of a system are called aperture diaphragms; those which limit the field of view are called field diaphragms. Whether a given diaphragm is classed as an aperture or as a field diaphragm depends upon its final effect on the system as a whole, and not upon its effect at any one field within the system.

Diffraction. Rays of light passing through a small opening, such as the slit of a spectroscope, suffer diffraction, thus causing the light beam to fan outward on both sides of the slit. Red light is diffracted more than blue, hence the long-wave components of white light passing a slit will fan out more than the short-wave components, thus producing a spectrum. When the slit is made narrow enough, and the paths of rays to certain points on a receiving screen can be made to differ in length by ½ λ, 1½ λ, 2½ λ, etc., reinforcement occurs between the points on the screen, and destructive interference occurs at those points. With white light illuminating the slit, the result is a series of colors giving a rainbow effect. In monochromatic light, the result is a series of dark and light bands, or fringes. The part of the fringe where the light is concentrated is called a maximum; where interference occurs the area is dark and is called a minimum. A lens focusing an indefinitely distant light source will have similar results, giving rise to the diffraction disc of Airy (*see* Airy disc). The position at which interference occurs denotes the order of the spectrum so produced; thus, the first-order spectrum is produced where the first interference is caused by wavelength differences of ½ λ, and the second, third, and higher orders occur at the successive positions of interference, 1½ λ, 2½ λ, etc. As the phase differences which cause the interferences get larger, and the order of the spectrum higher, the demarcation of the colors gets weaker and the colors paler.

Diffraction disc. *See* Airy disc.

Diffusion plate. A ground-glass plate used at the lamp house or occasionally in the substage condenser.

Diopter. An optical unit representing the reciprocal of the focal length of a lens, in terms of the meter. A 1-diopter lens has a focal length of 1 meter; a 2-diopter lens has focal length of ½ meter, etc. The diopter expresses the power, as in spectacles or other weak lenses.

Dioptrics. An old term, relating to the study of the image formation of a lens due to refraction. It is now included in the general term geometrical optics.

Dispersion. The dispersion of white light passing through a prism causes the well-known spectral or rainbow effect. The reason for the dispersion is that the refractive index of the prism is greater for the shorter than for the longer wavelengths, and so forms a gradient for intermediate wavelengths. The difference in refractive index of the glass prism for the red and blue light (say, the F

and C lines) gives the mean or average dispersion figure. *See V* value of dispersion.

Dispersion, mean. *See* Dispersion. The difference in refractive index of a transparent substance between the C and F lines. That is, $n_F - n_C$.

Distance of virtual image. The distance of the virtual microscope image from the eye of the observer will certainly vary a little for different observers. For those with normal eyesight it is considered to be at a point 10 inches from the entrance pupil of the eye, or at the near point. For others, it may lie slightly farther away, or even nearer to the eye than the conventional figure.

Distortion. A lens aberration which produces different magnification in different parts of the image field. If the magnification increases with the distance from the center of the field, the distortion is said to be positive — it gives a pincushion effect to a square object; if the magnification decreases with the distance from the center of the field the distortion is termed negative — it makes a square object appear bulging in the center, or barrel like.

Dominant wavelength. A term used to describe the transmission of an optical light filter. It may refer to the preponderance of transmitted light according to chromatic effect — blue, yellow, green, etc., or it may refer to the preponderance of light intensity according to wavelength.

Doublet. Two simple lens elements cemented together, for the purpose of giving spherical and chromatic corrections. An achromatic doublet may be composed of a positive crown-glass lens and a negative flint-glass lens. The errors of one element are made to compensate those of the other element.

Drawing prism. The prism of a camera lucida which permits both the field of view and the drawing surface to be examined simultaneously. Also a reversing prism placed at the exit pupil of the microscope to direct the image-forming rays onto a drawing board or vertical screen.

Drawtube. The smaller of the two tubes on a monocular microscope. The drawtube carries the ocular; it can be adjusted to control tube length and so effect corrections for the objective lens.

Dry objective. Any microscope objective designed to be used dry, without immersion liquid (*q.v.*).

Electromagnetic theory. Before the days of wireless, James Clerk Maxwell formulated a hypothesis for the transmission of electrical energy through space from the consideration of a spark gap, across which an alternating discharge of great frequency was maintained. At the making and breaking of an electric circuit a magnetic field is generated, and conversely the collapse of a magnetic field gives rise to an electric field. The lines of force of the fields are at right angles to each other, and the direction of propagation of energy is normal to the electric and magnetic lines. This, very briefly, is the electromagnetic wave hypothesis of Maxwell, and explains the transmission of electrical energy through space (ether). This work was later confirmed experimentally by Hertz, who showed the similarity between the long-wave electrical impulses and the short light waves. From then on, the electromagnetic hypothesis became the generally accepted *theory* of transmission of radiant energy of any wavelength. It can be used to explain polarization, interference, and other light phenomena.

Entrance pupil. When referred to the microscope, the entrance pupil is at the first focal plane of the condenser lens, practically at the iris diaphragm. More generally, it is the image of the aperture diaphragm of a system as formed in the

object space. In the absence of a condenser, the entrance pupil usually lies within or near the lens system of the objective. The entrance pupil of any lens system should be thought of as the common base of cones of light proceeding from all possible object points. Whether or not it is an actual stop, or the image of the aperture diaphragm, it is evident from the preceding sentence that contraction of the pupil will restrict the angle of the light cone equally, for all image points, and so make the image field uniformly darker. Likewise, if the pupil is expanded, more light will enter the lens system and the image field will be brighter. Thus the iris diaphragm of the microscope condenser is effective in controlling the effective aperture of the system and regulates the brightness of the image plane at the ground glass of the camera.

Equivalent focal length. The focal length of a thick lens referred to a simple lens element of like power. Usually used to designate the focal lengths of objectives. The true focal length of the objective is, of course, equal to its equivalent focal length.

Equivalent thin lens or equivalent lens. Any compound lens can be thought of as consisting of two thin lenses, one to receive the rays and the other to discharge them. The position of these hypothetical lenses would be at the principal points of the thick-lens combination. An equivalent lens is a hypothetical thin lens that can be thought of as above. The above hypothesis calls for two identical lenses, and, since they are identical, the proposition really calls for only one lens which can be imagined as moved along the axis as required. It is assumed to be at the first principal point when considering the object space, and at the second principal point when considering the image space; it is referred to as the equivalent lens.

Eye lens. The lens nearest the eye in any ocular. Also the crystalline lens of the eye.

Eyepiece. *See* Ocular.

Eyepoint. *See* Exit pupil.

Exit pupil. The exit pupil of the microscope is situated practically at the second focal point of the ocular. It is the point on the axis where all the chief rays cross. It is also called the Lagrange disc, eyepoint, Ramsden circle, etc. The exit pupil is conjugate with the entrance pupil. It is the common base for all cones of rays proceeding to their respective image points. It is the image, in the image space, of the aperture diaphragm of the system. In the microscope system, this means the image of two diaphragms, that of the condenser and objective.

***f*: number.** A measure of relative aperture of a lens, particularly a photographic or microphotographic lens. It is the ratio of the focal length to the diameter of the exit pupil of the lens. *See* Aperture.

Far point of eye. For the normal eye, the far point is at infinity. The rays of light from an infinitely distant point source are parallel and can be focused with the accommodation muscles of the eye entirely relaxed. *See* Accommodation; Near point of the eye.

Field depth. Synonymous with penetration. The longitudinal distance in the object space within which objects focused by a lens will all appear in good focus simultaneously.

Field diaphragm. *See* Diaphragms. Any diaphragm the image of which limits the field of view. In the microscope, the diaphragm at the ocular is a field

diaphragm, as is the diaphragm at the lamp, when adjustments are properly made.

Field lens. The lower lens in an ocular; the lens nearest the object field.

Field of view. The image field as seen through the microscope. It is considered as existing about 10 inches from the eye of a normally sighted person.

Filar micrometer ocular. A micrometer ocular with cross hairs in the lower focal plane which can be focused simultaneously with the image and moved across the field by means of a micrometer screw. The amount of displacement can be read to 0.01 mm on the micrometer drum head. This makes a very delicate measuring device, since calibration of the micrometer scale by means of a stage micrometer gives values for each interval on the drum head which are much less than the resolving power of the highest-aperture objectives.

Film speed. This is of interest to the photomicrographer more from a standpoint of obtaining photographic contrast than from a necessity of making fast exposures, although such exposures are occasionally required. The slowest type of film, usually termed "Process," gives the greatest amount of contrast. Very fast film such as Eastman Triple X Pan, is apt to be too soft for photomicrographic work. A good medium speed film, which has considerable latitude and can be developed to a high gamma by prolonged developing, is the best type to select for general work; a film that answers these requirements fairly well is Eastman Panatomic X. The commercial speed ratings assigned to photosensitive material is not of great assistance in photomicrography, except as a means of preliminary comparison — because it is not feasible to use exposure meters in connection with photomicrographical work.

Filters. *See* Optical light filters.

Fine adjustment. All good microscopes have a fine-adjustment mechanism in addition to the coarse adjustment; it is needed for precise focusing. One full turn of the fine-adjustment knob is usually equivalent to a vertical motion of the microscope tube of 0.1 mm. Thus, if the knob is engraved with 100 equally spaced marks, the sensitivity of the adjustment will be 1 μ.

Fine motion. *See* Fine adjustment.

Fixing bath. A solution of sodium hyposulphite, or more correctly sodium thiosulphate, commonly called hypo; usually a hardening agent such as alum, and an acid are added. It removes the undeveloped silver salts.

Fluorchromes. Dyes which have little coloring effect under ordinary lighting conditions but which fluoresce when irradiated with ultraviolet radiation.

Fluorescence. The irradiation of certain substances — oils, waxes, some minerals, pigments, etc. — with ultraviolet radiation (invisible) produces a visible radiation. The short-wavelength emanations are changed to longer wavelengths of scattered light. The scattered light may be of almost any color and is, under certain conditions, diagnostic of the substance. Bloom on oil is a fluorescent effect.

Fluorescent microscopy. The fluorescent microscope should not be confused with the ultraviolet microscope. The former renders a visible phenomenon; the action of the latter is recorded only by photography. Fluorescent microscopy is best carried on with a quartz light-collecting lens, quartz mirror, quartz prism, and quartz slide, in place of the usual glass apparatus. Ultraviolet microscopy demands, in addition, a quartz cover glass, objective, and ocular.

Fluorite. A mineral used in lens manufacture; *see* Fluorite objective. It has a refractive index of 1.434 (Larsen); it is isotropic and so belongs to the cubic

or isometric crystal system. It has a hardness of 4 and specific gravity of 3.18.

Fluorite objective. All apochromats of perhaps all manufacturers contain at least one fluorite element, and the high powers may contain three such elements. The optical properties of fluorite make possible the attainment of a high order of correction. The lenses, however, which are separately classed as fluorite objectives are achromatic objectives of high power; they are sometimes called semi-apochromats. Fluorite generally has many inclusions, which when the mineral is incorporated into a lens system can frequently be seen by inspection with a hand magnifier. These inclusions do not seem to harm the performance of the lens.

Flux, light. Sometimes called luminous flux; the visible portion of the radiant energy emitted by a light source. It is measured in lumens per solid angle. In electrical engineering, it is analogous to the lines of force in a magnetic field, spoken of as magnetic flux.

Focal depth. Strictly, the depth or distance in the image space through which the focused image will appear uniformly sharp and clear. Oftentimes this term is used erroneously to denote field depth (*q.v.*).

Focal length. The focal length of a lens is measured from the second principal point to a point on the axis where rays from an infinitely distant source converge to a common point or focus. Sometimes, for commercial purposes, the focal length refers to the back focal length (*q.v.*). A lens operating in a medium of like index in both the object and image spaces has two focal points at equal distances from their respective principal points.

Focal point. The point on the principal axis of a lens at which impinging parallel rays converge to a common point or focus. The point is virtual for a negative lens.

Focusing glass. A hand magnifier, used at the focal plane of a camera, usually with the ground glass removed. Its purpose is to examine the image for critical focus. It is, as a rule, a low-power magnifier so mounted that it can be placed on a piece of clear glass at the focal plane of the camera, for studying the image. It operates equally well on the aerial image.

Foot-candle. A measure of illumination. The illumination received at a surface 1 foot from a standard lamp of 1 candlepower; 1 foot-candle = 1 lumen per square foot.

Free aperture. The free aperture or a diaphragm or lens is a measure of its effective diameter. It is the diameter of the unrestricted surface between the edges of the mount of the lens, or of the unrestricted area of the diaphragm. Telescope objectives are rated according to the diameters of their free apertures.

Frequency. The frequency of electromagnetic wave motion is the number of complete waves, or cycles, per second.

Fresnel (frā nĕl'), **Augustin Jean** (1788–1827). A French mathematician and optician. Designer of the lens which bears his name.

Fresnel lens. A lens built up, progressively, in zones or steps, each zone being ground on its own individual radius. Considerable spherical correction is attained, and the weight of the lens is not excessive. Fresnel lenses were originally designed for lighthouses but they are now attainable for small spotlights, automobile headlights, and similar uses.

Front lens. The front element of a compound lens system; the first lens element which the entering light encounters. In the microscope condenser, it is the lower lens element, the upper lens element being the back lens (*q.v.*).

Gamma. The contrast of a negative or print as controlled by developing time, or otherwise. Numerically, it is the tangent of the angle of the straight-line portion of the D log E curve.

Gauss (gous), **Karl Friedrich** (1777–1855). A German mathematician and optician. Inventor of the Gaussian points, addition and subtraction logarithms, the probability curve, etc.

Gauss points. The principal points of a lens, after Karl Friedrich Gauss.

Glare. Stray or scattered light within the microscope system; extraneous light from windows or lamps; light scattered by the specimen that masks image detail and prevents thorough observation. An under- or over-corrected objective or condenser also may cause glare.

Glycerol immersion. A few lenses, chiefly those for dark-field observation, are corrected for glycerol immersion.

Goniometer ocular. A special type of ocular fitted with cross hairs and a graduated head which revolves the cross hairs to facilitate the measuring of angles, as in crystallography.

Greenough binocular microscope. First manufactured by Zeiss (?). It is equipped with erecting prisms; it has an objective for each tube and so is truly stereoscopic.

Haemacytometer. A specially built cell with a reticle for counting blood corpuscles in a given volume. The cells are 0.1 mm deep and are made with several types of ruling, Neubauer ruling being common. These cells are convenient counting chambers for many suspensions.

Hanging drop slide. A slide with a concavity ground in the center, or with a built-up cell which allows a drop of culture to be placed on a cover glass inverted over the cell. It makes possible the examination of freely moving Protozoa, confined only by the limits of the drop and the bottom surface of the cover glass.

Heat-absorbing glass. Particularly some of the Corning filter glasses which have a high heat capacity. They are placed in the path of the illuminating beam, to absorb heat. They are quite fragile.

Homal. A negative lens combination made by Zeiss, to be used in place of the regular microscope ocular for projection and photomicrography. A preparation can be examined visually through the Homal but only a very small part of the object field is visible at one time. *See* Amplifier.

Homogeneous immersion of objective. For oil immersion, the front glass of the lens of an objective is so chosen that it will have substantially the same index of refraction and dispersion value as the immersion oil with which it is to be used. With an immersed condenser, slide, and specimen all of similar index, and with correct immersion liquid, a homogeneous optical condition exists for this part of the light path, which is particularly advantageous for the elimination of glare and for full utilization of the corrections built into the objective. It also makes possible utilization of the aplanatic points of the front lens element.

Huygenian ocular. An ocular invented by Huygens, about 1680, consisting of two planoconvex lenses with the convex sides placed downward. The lenses have a focal-length ratio of 3.1 to 5.1 : 1 (Hardy and Perrin) and a separation equal to one-half the sum of their focal lengths, so that the combination is achromatic. This is known as a negative type of ocular.

Huygens or Huyghens (hī' gĕnz), **Christian** (1629–1695). Dutch mathematician, astronomer, natural philosopher, inventor of the Huygenian ocular, and originator of the wave theory of light. His diagrammatic scheme of showing the advance of wave fronts, and how each wave front consists of points which can each be considered as individual original sources for the development of successive wave fronts, is still in general use.

Hyperfocal distance. The nearest point to a lens at which objects will appear sharp in the image space when the lens is focused for objects at infinity.

Hypo. A solution of sodium thiosulphate, sometimes called sodium hyposulphite, used to dissolve the silver salts of photosensitive material which have not been affected by light. It is a bath used immediately after the negative has been developed.

Iceland spar. *See* Calcite.

Illumination, critical. Strictly, the formation of an image of the original light source in the object field. The term is often used to denote the Köhler method of illumination, referred to in this book as Method II of illumination.

Illumination, Köhler method. A method of microscope illumination discovered, or invented, by A. Köhler, in which an image of the source is focused in the lower focal plane of the microscope condenser, and the condenser, in turn, focuses an image of the lamp lens in the object field. It is referred to in this book as Method II of illumination.

Image, aerial. A real image formed on a screen or any surface becomes an aerial image when the screen is removed. It actually exists in space, as can be proved by blowing smoke across the space it occupies.

Image, real. An image as formed by a lens on a screen, plate, or any plane surface, as a magic lantern. In the absence of a receiving screen, the image will exist in space, as may be proved by blowing smoke into the area where it exists; this is sometimes called an aerial image.

Image, virtual. A virtual image has no real existence. It is the image seen when looking into the mirror. The field of view of the microscope is a good example of a virtual image. When the eye operates in conjunction with a lens to form an image on the retina, the visual sensation is as if the image existed in space, each image point lying in a direction coinciding with the direction of the image-forming rays as they reach the retinal surface of the eye.

Image field. Any field showing a focused image. There are a number of such fields in the complete microscope system. The term may also denote the field of view, or the image field at the focal plane of the camera, generally the field where the final image is formed.

Image space. The space about an optical system each point of which is conjugate to some point in the object space (*q.v.*). Thus image space and object space may be thought of as infinite in extent, yet under a certain given set of conditions they may be more precisely defined. Properties relating to the image are said to be in the image space, and properties relating to the object are considered as in the object space.

Immersion liquid. Any liquid occupying the space between the object and microscope objective. Such a liquid is usually required by objectives of 3-mm focal length or less. It is also used between the high-power condenser and the microscope slide. Immersion objectives are usually designed for immersion with cedar oil, glycerol, water, etc., the refractive index of the liquid being the deter-

mining factor. The great desideratum is that the liquid and the front lens of the objective should coincide in index and in dispersion value. *See* Oil immersion.

Immersion of a lens. With nearly all high-power lenses, it is intended that the spaces between the condenser and the slide, and the specimen and the front lens of the objective, be filled with an immersion liquid, since, owing to the limitations imposed by the critical-angle phenomenon, high apertures would be impossible without immersion. In addition, immersion makes possible the use of the naturally aplanatic points of the front lens element of the objective.

Incidence, angle of. If a perpendicular is erected at the point of incidence of a light ray, at a surface, the angle between the perpendicular and the light ray is the angle of incidence. *See* Plane of incidence.

Incident light (illumination). Sometimes denotes any over-stage lighting not included by methods of vertical illumination (*q.v.*).

Index of refraction. The ratio of the velocity of light in a vacuum to its velocity through a transparent or translucent substance. For optical materials the light velocity is always less than in a vacuum. Thus, for this class of material the index is always greater than 1. Because of the change of direction of a beam of light when it strikes the surface of a transmitting medium, Snell was able to prove the following relationship of the sine of the angle of incidence and the angle of refraction. Snell's law is:

$$\frac{n'}{n} = \frac{\sin i}{\sin r}$$

where n' is the refractive index of the medium in which the light is refracted, n is the index of the medium in which the light is incident, i is the angle of incidence, and r is the angle of refraction.

Infrared radiation. For photomicrographical purposes, the energy radiation extending beyond the long visible red rays of the spectrum, from about 800 mμ to 1200 mμ. The image is invisible. Thus it must be studied by photographic recording.

Intensity. Intensity of a light source is measured by its output of luminous flux, in terms of lumens per solid angle. It is reduced to candlepower by dividing by 4π. Intensity of a source is also spoken of in terms of brightness (candles) per unit area (*see* Brightness). The latter term usually applies to sources with extended surfaces.

Interference. If rays which have diverged from a point source are brought together again at another point by methods of refraction, reflection, or diffraction, and there exists a certain definite phase difference between them, then reinforcement, destructive interference, or partial reinforcement or interference occurs. The amplitude of the resulting ray is the algebraic difference of the amplitudes of the converging rays at the instant of union. Thus, if the phase difference between two sets of rays is $\lambda/2$, the resulting amplitude will be zero and darkness will result at the point of intersection. If the phase difference is zero or multiples of λ then reinforcement occurs, the resulting amplitude is increased, and greater light intensity occurs at the point of intersection. Light rays which may interfere are said to be coherent. Diffraction (*q.v.*), as by means of a slit, produces interference of light waves. When the light is heterochromatic, the interference results in a beautiful spectrum or rainbow effect. If the light is monochromatic, alternate dark and light areas result.

Interpupillary distance. The distance between the centers of the pupils of the eye. The binocular microscope tube must be adjustable for this distance.

Iris diaphragm. A diaphragm (*see* Diaphragms) with thin metal leaves so arranged that the rotation of an actuating lever or ring will bring the edges of the leaves together until only a very small circular opening remains, or, in the reverse direction, will withdraw the leaves to make a larger opening.

Isotropic. *See* Birefringence. Substances which have no polarizing effects on light and therefore remain dark between crossed Nicols are isotropic. The cubic system of crystals is isotropic. Such crystals have only one refractive index value, irrespective of the direction of the light passing through them.

Jelley microspectrograph. A spectrograph for use on the microscope, which utilizes a grating to obtain dispersion. It was invented by Dr. E. E. Jelley.

Köhler method of illumination. *See* Illumination, Köhler method.

Lagrange disc. The exit pupil of the microscope. *See* Exit pupil.

Lambert. A photometric unit for describing the brightness (*q.v.*) of a surface. One lambert is the equivalent of 1 lumen per square centimeter.

Lens, simple. A glass disc which is ground and polished with a spherical figure on one side and is plano, concave, or convex on the other. There are six possible shapes: double convex, or biconvex; planoconvex; converging concavoconvex or converging meniscus; planoconcave; double concave or biconcave; and diverging concavoconvex or diverging meniscus. The first three lenses are positive; the last three, negative.

Light. Light is radiant energy of such wavelength that, falling on the retina, it stimulates the nervous system of the eye and produces the sensation of vision. The foregoing is a physiological definition of light which tells what light does, under certain conditions, rather than what it is. According to the Maxwell theory, all radiant energy is electromagnetic (*q.v.*) in character, the generation of the radiation depending upon the portion of the ether spectrum under consideration. For that part of the spectrum lying within the visual range — 400 to 800 mμ — the release of light energy from a source, which has been externally stimulated, may be thought of as due to atomic or molecular vibration or to the passing of electrons from high to lower energy levels accompanied by the spasmodic release of energy as the electronic orbits decrease in diameter. *See* Quantum theory.

Light filters. *See* Optical light filters.

Light flux. *See* Flux, light.

Light-gathering capacity. The amount of light flux gathered by one objective as compared with that gathered by another is proportional to the squares of the numerical apertures of the objectives. The percentage of transmitted light depends upon the number of the lens elements in the system, the amount of reflection at each interface, and the light-transmitting qualities of the materials used to make the individual lens elements.

Light sources. Three main light sources are available to modern microscopy: (*a*) the tungsten-filament lamp in any of its many forms, the most useful of which is the projection lamp and the ribbon-filament lamp; (*b*) capillary gaseous discharge tubes of the mercury-vapor type, such as the H series of the General

Electric Company; (*c*) arcs — tungsten, carbon, iron, and cadmium. Natural daylight is unsuited to photomicrography.

Limb, microscope. This is the upper part of the microscope; it is pivoted to the microscope pillar at the inclination joint. It carries the microscope tubes and the coarse- and fine-adjustment mechanisms.

Longitudinal magnification. A certain distance, measured axially, in the object space is referred to the homologous distance in the image space. The ratio of the distance D' in the image space to the distance D in the object space equals the square of the linear magnification of the system. Thus,

$$M^2 = \frac{D'}{D} \tag{63}$$

Lumen. A measure of light flux. A source of 1 candlepower emits 4π lumens; 1 foot-candle = 1 lumen per square foot; 1 lumen = 0.0015 watt, $\lambda = 555$ mμ.

Luminous flux. *See* Flux, light.

Luminous spot ring condenser. *See* Cassegrain dark-field condenser.

Magnification. The number of times by which the size of the microscope image exceeds the original object. Linear magnification is usually referred to. It is the ratio of the distance between two points in the image to the distance between the two corresponding points in the object. It is universally more than 1 for microscope images and usually less than 1 for macrophotographic images. Areal magnification, the square of the linear magnification, is the ratio of an area in the image to the corresponding area in the object. *See* Angular magnification; Magnification, empty.

Magnification, empty. Any magnification, whether obtained by the microscope or by subsequent enlargement, which is greater than the minimum requirements to show the resolvable detail.

Marker, object. A small diamond set in a rotating holder mounted on the lower end of the drawtube. The specimen is placed in the center of the field, and the diamond point is pressed against the slide or cover, and revolved. It describes a tiny circle about the object field.

Maximum. As used in diffraction and interference phenomena to denote those parts of the diffraction pattern where the light energy is most concentrated.

Mechanical tube length. The mechanical tube length of the microscope is measured from the shoulder of the objective to the upper end of the drawtube. The generally accepted length for biological microscopes is now 160 mm, although the Leitz Company uses a 170-mm tube length. Metallographic objectives and some others are corrected for use with longer tube lengths — 190 to 215 mm or more. *See* Correction of lenses; Correction of an objective; Optical tube length.

Melt. A mixture of several elements or compounds for use as a mounting medium. The components are of high index, such as sulphur and selenium, and are often solid at room temperature. The mixtures must be melted for use.

Mercury-vapor discharge tube. The mercury-vapor lamp. Several types are manufactured by the General Electric Company. Those known as capillary discharge tubes, or the H series, are the best suited for microscope illumination. They are known as cold discharge lamps; the current is passed between the electrodes by gaseous conduction. Traveling electrons raise the mercury-

vapor-atom electrons to higher energy levels (energy is absorbed), and, on the return of the electron to its normal or lower level, light energy is emitted.

Mercury-vapor lamp. *See* Mercury-vapor discharge tube.

Micrometer, stage. A small scale of natural size engraved or photographed on glass or metal. It usually carries divisions as small as 0.01 mm. It cannot be used for direct measurement but is useful in calibrating ocular micrometers.

Micrometer disc. A glass disc engraved with a suitable scale, used at the diaphragm of a micrometer ocular. The scale can be focused by the eye lens and seen in the field of view.

Micrometer ocular. An eyepiece with a focusable eye lens which carries a suitable scale for measuring. *See* Micrometer disc. The scale must be calibrated by means of a stage micrometer for each objective with which it is used.

Micron. 0.001 mm. The abbreviation is μ (mu).

Microphotographic lens. Similar to the photographic lenses for macrophotography, except that it is specially designed to give its best images with a short object distance and a long image distance. Microphotographic lenses are made in focal lengths from about 16 mm to 120 mm. The low powers, up to about 75 mm, can be used on any large tube (50-mm diameter) microscope. No ocular is required with these lenses.

Microphotometer. A photometric device made by several companies to fit onto the microscope tube. It measures the intensity of light reflected or transmitted from the specimen.

Microscope, compound. An optical instrument wherein a primary image is formed by the first lens, or objective, and focused by a second lens to form a virtual image with the aid of the eye, or a real image in projection. The refinements and developments of the compound microscope include apparatus for illuminating the specimen, mechanical means for holding the specimen, adjustments for centering the different lens systems, diaphragms for controlling aperture and field diaphragms, and interchangeability of optical and mechanical parts and accessories. *See* Binocular microscope. There are several distinct microscope types: the low-power or dissecting microscope; the two-objective binocular microscope; the biological microscope; the petrographic microscope; the metallographic microscope; the dark-field microscope; the ultramicroscope; the fluorescent microscope; the ultraviolet microscope, and others. Adaptation, the interchangeability of mechanical parts and lenses, makes possible, for the most part, the converting of a single adequate microscope stand into any one of the foregoing specialized instruments.

Microscope, simple. *See* Microscope, compound. A simple lens, sometimes a doublet or triplet, which acts as a hand magnifier.

Microscope mirror. The mirror, mounted under the substage, is as a rule plano on one side and concave on the other. The flat side is habitually used unless the objective is of very low power. The mirror should be so mounted that the concave side can be focused on the front lens of the objective. For the best work, a first surface mirror is necessary.

Microspectroscope. A spectroscopic eyepiece (*see* Jelley microspectrograph) which makes possible the analysis of light transmitted by or reflected from a specimen. The better spectroscopic oculars are fitted with a wavelength scale, and one is in the form of a direct-vision spectroscope. A camera attachment can be used with them.

Millimicron. 0.001 micron (*q.v.*), or 0.000001 mm.

Minification. *See* Magnification. A reduction of the lens image from the true size of the object. The ordinary macrophotograph image is usually smaller than the object.

Minimum. *See* Diffraction. Part of the diffraction pattern where destructive interference has caused darkness. The dark rings surrounding the central disc of a diffraction pattern are minima.

Monobjective binocular microscope. A microscope with one objective and two tubes, for binocular vision.

Monochromatic light. Light of substantially one wavelength, obtained in greatest purity from a monochromator, a dispersing instrument which makes possible the segregation of very narrow parts of the spectrum. However, for practical microscope work, the sodium or mercury vapor tubes in conjunction with a monochromatic filter give good separation of the various available spectral lines. For nearly all microscopical purposes, a tungsten-filament lamp with a monochromatic filter gives good results.

Monochromatic light filter. An optical light filter passing only a very narrow spectral band. It is best used with a gaseous discharge lamp where the energy sources are concentrated into a few narrow bands of very restricted wavelengths.

Monochromatic objective. An objective corrected for use with monochromatic light. The quartz objective corrected for the 2750 A line is an excellent example.

Monochromator. A light dispersing instrument which is used to obtain light of substantially one wavelength, or at least of a very narrow band of the spectrum. The light is apt to be weak because of its great purity. The use of monochromatic light filters is usually satisfactory for microscopical work.

Monocular microscope. A microscope with one objective and one tube for monocular vision.

Mounting media. Any liquid, resin, melt, or even gas used to mount microscopic specimens before examination. The chief attribute of a mounting medium is its refractive index.

Mu. The Greek letter *m*. It is used as the abbreviation for micron (*q.v.*).

Muscae volitantes. Dead cells floating in the vitreous humor of the eye. They are sometimes troublesome in monocular microscope vision because they cast shadows on the retina. However, their disagreeable optical effects can be largely eliminated by substituting binocular vision, or by using a larger aperture and higher ocular.

Myopia. Nearsightedness.

Naphrax. A new mounting medium, described in the *Journal of the Royal Microscopical Society,* March and June issue, 1943, by Colonel William D. Fleming, Medical Corps, U. S. A., the inventor. It has a high refractive index, circa 1.70. The suffix "rax" implies something that can be stretched; it is connected with the names of several other resins used in microscopy, such as Hyrax and Styrax. Naphrax can be had from R. P. Cargille, 116 Liberty St., New York, N. Y.

Near point of the eye. The nearest point to the entrance pupil of the normal eye at which focus is attained without strain; 10 inches is the generally accepted distance. In very young people and in those suffering from myopia, this distance is less. The near point rapidly recedes with age, causing a farsighted condition, presbyopia.

Negative focal length. *See* Focal length. The focal length of a negative lens is said to be negative. Parallel rays impinging on a negative lens can be traced to a virtual focus which exists on the same side of the lens as the impinging rays. The distance from the second principal point of the lens to this second focal point is measured on the same side of the lens as is the object, consequently it is said to be negative.

Negative lens. A lens with a negative focal length. The edge of a negative lens is thicker than the center. The three negative lenses are, according to their figure: planoconcave; double concave, or biconcave; and diverging concavo-convex, or diverging meniscus. *See* Lens, simple.

Negative ocular. A Huygenian ocular, or one based on the Huygenian principle.

Nicol (nĭk' ol), **William** (1768[?]–1851). A Scottish physicist, inventor of the Nicol prism (*q.v.*).

Nicol prism. A prism invented by William Nicol in 1828. It is of calcite; the end faces are ground to an angle of 68° between one vertical side and the end face. The prism is sawed in two diagonally, the cut being made at right angles to the shorter diagonal of the end face. The parts are then cemented together with Canada balsam. Natural or unpolarized light striking an end face is polarized in two directions. The extraordinary ray passes through the crystal, and the ordinary ray is reflected to one side at the calcite-balsam interface and is lost. There are two prisms in the petrographic microscope; the lower one is called the polarizer, and the upper one the analyzer (*q.v.*). Modifications of this prism are common. The Nicol prism makes possible the analysis of the effects of a substance on light.

Niglytin. A black liquid, nearly opaque even in a thin film. It will give a dark-field effect with a bright-field condenser. It is especially recommended for the mounting of algae, which will appear in their true colors on a dark field. Before the opening of World War II it was made in Germany by Dr. K. Hollborn & Sohne, Leipzig.

Nosepiece, revolving. A device to be screwed on to the end of the microscope tube to permit the mounting of two to four objectives, any of which may be swung into place, ready for use, by turning the nosepiece into the desired position. The nosepiece usually occupies a mechanical-tube-length space of 15 mm.

Nodal points. Two points on the axis of a lens so situated that an external ray directed at one of them will emerge as if from the other with its direction unchanged; that is, the incident and emerging rays will be parallel to each other. The nodal points having an angular magnification of unity are coincident with the principal points when the refractive index of the surrounding medium is the same on both sides of the lens.

Numerical aperture. Generally abbreviated N.A. A mathematical relationship discovered by Abbe which directly connects the resolving power and the light-gathering power of an objective with its aperture. Numerical aperture is the product of the sine of half the angular aperture of a lens, and the index of the medium through which the light passes. It is a very important constant for high-power lenses. Microscope objectives and condensers are largely designated by the N.A. value.

Object field. A position lying in the first conjugate plane of the objective, on the microscope slide, directly under the objective, which is focused in the upper end of the microscope tube, forming the primary image.

Object space. *See* Image space. A space infinite in extent existing around a lens system. Properties relating to the object lie in the object space.

Objective. In any optical instrument, the lens nearest to the object in the image-forming system. In the microscope system, the lens on the lower end of the microscope tube. Good high-power microscope objectives are of very complicated construction and computation, often containing ten or more separate lens elements. The lower element, or front lens, affords the magnification and is of high power. The subsequent lens elements serve to bend the image-forming rays back to the lens axis and bring them to a focus without introducing undue aberration. The front lens is usually a single lens element; the back lenses may be doublets or triplets according to the intentions of the lens computer. The front lens of a high-power system is quite free of aberration, and, since it is oil immersed, the object can be at one of the aplanatic points.

Objective circle. When the focused objective is examined by withdrawing the ocular and viewing its back focal plane through the microscope tube, the limiting boundary of the objective, the circumference of the white spot of light, is the objective circle. The condenser must be focused and operated to give a full cone of light.

Oblique lighting. Oblique lighting may be obtained with a substage condenser by blocking out part of the condenser lens in a certain azimuth, as by throwing the condenser diaphragm out of alignment. It is regularly obtained with over-stage lighting when a bull's-eye is used. In order not to confuse unilateral lighting with oblique lighting, it is convenient to think of oblique lighting as being so oblique as to cause a dark-field effect, whereas unilateral lighting is only slightly oblique and does not necessarily cause a dark-field effect.

Ocular. Essentially, an ocular of any optical instrument is nothing more than a hand magnifier; it serves to magnify some primary image, be it real or virtual. It operates with the eye to form a virtual image, or, in photomicrography it forms a real image of the primary microscope image, the difference in operation being attained by simply focusing the microscope to bring the primary image into the proper position. The two main types of oculars are the Huygenian and Ramsden types. They can be obtained with varying degrees of correction. In their best form they appear as compensating oculars. Huygenian oculars are of the negative and Ramsden oculars of the positive type. Amplifying lenses (*see* Amplifiers) used in place of oculars are truly negative and are suitable only for projection or for photomicrography.

Oil immersion. The immersion of a lens in a liquid of a similar index makes possible the use of the naturally aplanatic points of the front objective lens to the best possible advantage. *See* Immersion liquid.

Opacity. Opacity of a transparent material is the reciprocal of its light-transmitting value. *See* Density, optical.

Optical axis. *See* Axis, optical.

Optical center of a lens. A ray of light passing through a lens so that its angular magnification is unity (*see* Nodal points) will pass through the optical center of the lens, which lies on the lens axis. There may be more than one such crossing in a thick lens system. A method for plotting the optical center of a simple lens is to draw the lens to scale, drawing parallel radii from each center of curvature to the corresponding circumference at the lens surface. Connecting the points so found will give a line crossing the axis at the optical center of the lens. The optical center does not necessarily lie within the lens, as a little experimenting with the meniscus forms will show.

Optical density. *See* Density, optical.

Optical flat. Usually, glass or quartz plates or discs, the thickness of which should be at least $\frac{1}{10}$ their diameter. They are ground until both faces are what is termed optically flat, that is, until any remaining unevenness can be measured only by interferometric methods. Their maximum departure from flatness usually is less than $\frac{1}{10}$ of a wavelength of sodium light.

Optical glass. An especially fine glass made under the most carefully controlled conditions. There are many kinds, some with low index and high dispersion values and some with high index and low dispersion. Good lenses or prisms are always made from optical glass.

Optical index. A constant applied to objectives for purposes of comparison. It takes into account the focal length or magnifying power of the lens and also the N.A. It was offered by Nelson and by Coles, but little use has been made of the optical index figure, probably because the equations of the two men differed and the resulting figures have been at variance.

Optical light filters. Filters made to modify the light source either chromatically (chromatic filters), in intensity (neutral filters), or thermally (heat-absorbing filters). They may be of solid glass as made by Corning, Chance, and others; or they may be stained gelatin sheets mounted or unmounted between glass plates as made by Wratten; or they may be glass cells filled with an appropriate liquid. Their correct position in the light train, for microscope use, is midway between the source and the microscope.

Optical tube length. The distance from the second principal focal point of the objective to the first focal point of the ocular. This distance is about 180 mm for high-power objectives, less for those of low power. It is controlled by varying the mechanical tube length.

Over-correction. An over-corrected lens focuses the light rays passing the central zones at a point nearer to the lens than rays passing the outer zones. It is a negative aberration, the reverse being under-correction, a positive aberration.

Paraboloid dark-field condenser. A lens of paraboloid shape. The vertex end is ground back so that its focus can be brought into coincidence with the specimen on the slide. A central stop is provided to block the central rays. It is used chiefly for medium-power work.

Parallel rays. *See* Beam of light.

Paraxial. Referring to the space or rays closely surrounding the principal axis of a lens system.

Parfocal lenses. Lenses which can be used interchangeably and are so mounted that the second conjugate plane will fall at the same position on the microscope axis, for each lens, as it is moved into position, motion of the microscope tube being unnecessary to retain a focus. Objectives mounted on a revolving nosepiece are generally parfocal, as are oculars. Parfocalism is not of much importance with individual objective-changing devices.

Penetration. The distance on the axis in the object space through which a point could be moved without destroying good focus in the image space.

Petzval condition. This is based on a theory which states that the sum of the product of the refractive indices and focal lengths of two thin lenses must equal zero in order to attain a fairly flat field free from astigmatic conditions.

Photomicrography. This term has been much twisted and misused. It should not be reversed into microphotography. A photomicrograph is a photograph of

a small object, the image of which is magnified. A microphotograph is a small photograph, requiring an enlargement or a lens system in order to view it; the image is minified.

Photosensitive material. Usually, films or plates sensitive to light action; also, refers to printing paper or any surface treated to produce a photographic effect.

Pillar, microscope. The post; often part of the microscope base. To the pillar is attached the limb of the microscope, mounted on the inclination joint.

Planck (plangk), **Max** (1858–). A German mathematician and physicist, originator of the quantum theory.

Planck constant. Mathematically, 6.55×10^{-27}. The constant is associated with the quantum theory and is represented by h. The value $h\nu$ is the unit of energy emitted by a body, where ν equals the frequency of the atomic or molecular vibration. The theory applied to that part of the spectrum which is light producing refers to light quanta. *See* Quantum theory.

Plane of incidence. The plane containing a ray incident to a surface, and the normal to that surface, erected at the point of incidence.

Plano. In optics, an optical surface which has been made substantially flat, the degree of flatness depending upon the work required from the part to which it refers. A planoconvex lens is a positive lens with one surface flat and the other convex. In the dictionary, plano is given only as a combining form, but in practical optics it is often used alone to denote any particularly flat surface — one that has been worked flat. *See* Optical flat.

Polarized light. Light that is vibrating in one plane (plane-polarized light), light with a rotary vibration (circular polarized light), or light that is vibrating elliptically (elliptical polarized light). Moonlight is polarized, as is much reflected light; cloud light is polarized under certain conditions. However, naturally polarized light is, on the whole, rather imperfectly polarized. Nicol prisms are the accepted means for obtaining polarized light for instrumental use.

Polarizing microscope. The petrographic microscope which is normally equipped with two Nicol prisms and a Bertrand lens. It is an instrument for measuring and analyzing the effects of a transparent specimen on light.

Polarizing prism. *See* Nicol prism. The lower prism in a petrographic microscope.

Positive focal length. Any lens which converges parallel rays to a focus on the back of the lens is said to be a positive lens and have a positive focal length. *See* Negative focal length. The focal length is measured from the second principal point of the lens to the point on the lens axis where the rays from an infinitely distant point are brought to a focus.

Positive lens. Any lens with a positive focal length. Such lenses are thicker in the center than around the circumference. There are three types of positive lenses: double convex or biconvex; planoconvex; converging concavoconvex or converging meniscus.

Positive ocular. A Ramsden ocular or any modification of it. The diaphragm is below the field lens.

Presbyopia. Farsightedness due to age; inability to focus nearby objects.

Principal plane. A plane normal to the principal axis of a lens, passing through a principal point (*q.v.*).

Principal point. The principal points or Gaussian points of a lens lie on the principal axis and are so situated that they are mutually conjugate and have a magnification of unity. They coincide in position with the nodal points when

the refractive index of the surrounding medium is the same on both sides of the lens. They are cardinal points; every lens system has two. Focal length, object, and image distances are measured from the principal points. Their positions are of great importance in a thick lens system.

Printing paper for photomicrography. Printing paper with a glossy surface usually gives the best results for photomicrographic negatives. The light is reflected from such a surface with less diffusion than from a matte or semi-matte surface; this tends to give a clear outline of the details and is distinctly better for purposes of reproduction. Printing paper with a glossy finish is obtainable in six degrees of contrast, No. 0, 1, 2, 3, 4, and 5. No. 0 is the fastest and gives the least contrast; No. 5 is the slowest and gives the greatest contrast.

Projection lamps. These are the most useful types of lamp for general microscopical work and are very satisfactory for photomicrography. They may be had in many wattages, and the biplane lamp presents a large, nearly unbroken luminous surface which can be used for the Köhler method of illumination. A diffusing plate should be used with the single-plane lamps.

Projection ocular. Strictly, low-power oculars which are designed for projection purposes at distances of 16 feet or more. Homal lenses are not classed as projection lenses but as amplifiers (*q.v.*).

Pupil of the eye. Usually, the entrance pupil; according to Helmholtz's schematic eye (see Hardy and Perrin), it is 3.05 mm back of the vertex of the cornea; for the accommodated eye it is 2.69 mm back of the vertex.

Quantum theory. According to this theory, after Max Planck, radiant energy is given off from atoms or molecules in small discrete lots called quanta. It is absorbed in a like manner. The Bohr conception of the atom in relation to its emissivity ties in the discharge of the energy with the passing of an electron from a high energy level to a lower level (from a larger to a smaller orbit), and the passage of an electron from a smaller to a greater orbit is accompanied by an absorption of energy. The energy discharged, at one time, is given by the formula $h\nu$, where h is the Planck constant and ν is the frequency of the radiated energy. *See* Planck constant.

Quartz. A transparent mineral with an index of refraction of 1.553 for the extraordinary ray and 1.544 for the ordinary ray. Quartz is used in microscopy in the manufacture of lenses, slides, and covers for fluorescent work and for the ultraviolet microscope, because it is quite transparent to these wavelengths. It is a uniaxial mineral, positive in character, and belongs to the trigonal division of the hexagonal system of crystals. It has a hardness of 7 and a specific gravity of 2.66 (Larsen).

Quartz slides and covers. These are necessary for work with the ultraviolet microscope in the short wavelengths. However, for work with the 3650 A line, Corex glass can be substituted and is much cheaper.

Quick-change-over condenser. *See* Condensers.

Ramsden (rămz' dĕn), **Jesse** (1735–1800). An English optician and inventor of the Ramsden ocular.

Ramsden circle. *See* Exit pupil.

Ramsden ocular. A positive type of ocular made with two planoconvex lenses with the convex sides facing each other. It is a particularly good type of ocular for micrometry.

Real image. *See* Image, real.

Reflection factor. The ratio of reflected light from a surface to the incident light. This is sometimes called the coefficient of reflection. Unless specially stated it takes into account both specular and diffuse reflection.

Refractive index. *See* Index of refraction.

Relative aperture. The ratio of the focal length of a lens to the diameter of its entrance pupil. This gives a number known as the f : number, usually written $f : 8$, $f : 16$, etc. Thus, if the focal length f is divided by the number, 8, 16, etc., the result will be the diameter of the entrance pupil of the lens, or if the diaphragm of the lens is wide open it will be very nearly the diameter of the free aperture of the lens.

Relative refraction, index of. The ratio of the refractive index of a substance to the index of its surrounding medium.

Resolution. The ability of the eye or a lens to register small detail. The resolution of the human eye is said to be 1 minute of arc. However, this is not the limit of visibility, for a strong light between two closely spaced wide lines can be seen when the width of the slit is much less than the above figure. According to the best authorities, the resolution of the microscope is now placed at

$$\frac{1.22\,\lambda}{2\text{ N.A.}} \quad \text{or perhaps} \quad \frac{0.8\,\lambda}{2\text{ N.A.}}$$

Retina. The receiving surface on the back part of the inner side of the eyeball. It is lined with tiny nerve endings called the rods and cones. The image-forming rays from the eye lens are focused on the retina, and through the rods and cones, via the optic nerve, the sensation of vision is transmitted to the brain.

Retinal image. The image formed by the lens of the eye on the retina (*q.v.*). The image is reversed and inverted.

Reversing prism. Used at the exit pupil to direct the rays in a direction of 90° to the microscope axis. The image is reversed.

Revolving nosepiece. *See* Nosepiece.

Screen. *See* Optical light filter.

Screw micrometer. *See* Filar micrometer ocular.

Semi-apochromatic objective. Sometimes called fluorite objective. An objective intermediate in cost and quality between an achromat and an apochromat.

Short-stop bath. An acid bath for film or paper to stop developing action quickly. It is used as soon as the film is taken from the developer.

Silverman illuminator. A small annular lamp, made by Spencer, used with a reflector, and mounted on the objective by means of a small three-jaw chuck. It furnishes over-stage illumination particularly suited to low powers. As it generates considerable heat, care must be exercised in its use.

Sine condition. Sometimes referred to as the Abbe sine rule. It must be observed in the design of a lens if the aplanatic condition is to be attained. It states that the ratio of sines of the incident rays and the refracted rays must be a constant, that constant being the magnifying power of the lens.

Slit microscope. *See* Ultramicroscope.

Snell (snel), Willebrord (1591–1626). Dutch mathematician and discoverer of the law of refraction which bears his name.

Sodium lamp. A gaseous discharge lamp made by the General Electric Company. Excellent as a source of monochromatic yellow light. The visible sodium lines are at 589.0 mμ and 589.6 mμ on the wavelength scale.

Spectrophotometry. The study of the intensity of spectral lines, also the measurement of light transmission of filters according to the separate color components.

Spectrum. The dispersion of light into its color components according to wavelength. When the light is white light, or natural sunlight, a beautiful rainbow effect is obtained. The dispersion effect is attained by an optical instrument known as a spectroscope which makes use of a prism or finely ruled grating. A well-lit narrow slit is placed at the focus of a collimating lens; the resulting beam of light is passed through the dispersing prism and then focused on a camera plate by another lens or observed with an ocular.

Specular. A smooth mirrorlike surface; by extension, the action of such a surface on light, as a specular reflection, meaning a mirrorlike reflection, opposed to diffuse reflection. Also used in light transmission, as specular transmission, denoting transmission of a pencil of light without diffusion.

Spherical aberration. *See* Aberration.

Stage, mechanical. A small fixture, either built into the regular microscope stage or applied separately; it holds the specimen slide and has two horizontal screw adjustments at right angles to each other. The screw motions permit the specimen to be moved as desired. The better types have vernier scales for reading the amount of displacement to 0.1 mm. This stage is known under several names; it is sometimes called the traversing stage.

Stage, microscope. The platform under the microscope tube is called the stage. It carries the specimen and is usually mounted permanently on the microscope pillar, although in some research instruments it is mounted on a rack and pinion. For metallographic or petrographic work it should be movable along the microscope axis; it is then called a focusing stage. For photomicrographic work, it should be of the focusing type and should always be supplied with a strong clamp to permit it to be locked in position after it is once set.

Star test. This test is well described in the text. It affords a means for determining whether under- or over-correction exists in the microscope system. Its intelligent use ensures the attainment of the optimum image of which an objective is capable.

Stop. *See* Diaphragm.

Stop, dark-field. *See* Central stop.

Submicroscopic. Particles which, although visible in the ultramicroscope, are too small to be resolved by bright-field methods. This places their size between 0.2 μ and 0.005 μ.

Surface color. The color of a body seen in reflected light; it is due to selective reflection, the light not having penetrated the surface.

Swan cube. A glass cube split diagonally into two pieces. One face is partly silvered and the parts are then cemented together. A beam of light striking the silvered surface will be partly reflected at an angle of 90° from its course, and part will be transmitted without deviation.

Telaugic oculars. Oculars with an extremely high exit pupil. During their use spectacles can be worn with comfort. These oculars are made by Swift.

Thick lens. Any lens or system of lenses which, because of its thickness, cannot be treated by the ordinary lens formula which disregards thickness. A doublet,

a triplet, a microscope objective, or even the whole microscope instrument itself should be considered thick-lens systems. The Gaussian points for such a system are determined and measurements are made from them for focal length, lens position in mounting, etc.

Thickness of cover glasses. The so-called standard thickness of a cover glass (not sponsored by any scientific society) is from 0.16 to 0.18 mm. This thickness is included in No. 1 covers. *See* Cover glass.

Thin lens. Any simple lens of low power can usually be considered as a lens without thickness, and measurements of focal length can be made as from the center of the lens, or sometimes the back focal length (*q.v.*) value is used. The formula for handling a thin-lens problem assumes that the front and back surfaces exist in the same plane; this is satisfactory for much work.

Toluene. One of the hydrocarbon solvents used in microscopy, also called methyl benzene; $C_6H_5 \cdot CH_3$; boiling point 110.8° C (Lange).

Total reflection. Occurs when a ray of light is passing from a material of high index to one of a lower index. When the angle of incidence (*see* Incidence, angle of) is sufficiently great, total reflection occurs at the interface. *See* Critical angle.

Transmitted light. The usual method for illuminating transparent microscopic specimens. The light is brought to a focus on the specimen by the substage condenser. The light cone should be perfectly aligned with the objective axis. Objects appear in outline (refraction images) or colored on a bright field (color images).

Transparent. Any optical substance or other material which is so optically clear that a pencil of light passing through it may be focused without undue injury to the image. A piece of clear glass is transparent; a piece of opal glass is translucent.

Triplet. A combination of three simple lenses, cemented together. Usually two positive lenses with a negative lens between them, affording a well-corrected system.

Tube length. *See* Mechanical tube length; Optical tube length.

Tube-length adjustment. The drawtube of the microscope can be extended beyond or pushed within the standard setting demanded by the objective in use. It is normally set at 160- or 170-mm mechanical tube length, which is correct for nearly all modern biological-microscope objectives. Extending the tube beyond the normal setting produces an over-correction in the microscope system and is useful to offset under-correction due to various causes. Conversely, shortening the tube length tends to correct for an over-corrected system.

Tyndall blue. The blue color produced by light scattered by extremely fine particles suspended in a liquid or a gas. Often observable through the dark-field microscope. Also called the Tyndall effect.

Ultramicroscope. A microscope so arranged that the specimen, which is usually a solid-liquid colloid or suspensoid, is illuminated by a strong pencil of light at right angles to the microscope axis. The visibility is limited by the intensity of the light source. It is used to examine particles within the range of about 0.005 μ to 0.2 μ.

Ultraviolet filter. Filters which pass ultraviolet radiation and are substantially opaque to longer wavelengths.

Ultraviolet radiation. That part of the spectrum extending from 120 to 4000 A. For photomicrography the most useful wavelengths are 3650 A and 2750 A.

Ultraviolet sources. The H series of mercury-vapor discharge tubes made by the General Electric Company are the most convenient sources. Carbon and cadmium arcs are also used.

Under-correction. The optical condition which normally exists in a simple lens resulting in spherical aberration. The rays from the outer zones are brought to a focus closer to the lens than the rays from the central portion. The condition is the opposite of over-correction.

Unilateral illumination. Usually, a form of illumination with the substage bright-field condenser when part of the condenser aperture is blocked out or when the condenser is misaligned. Over-stage lighting (except vertical illumination and annular illumination) is unilateral illumination but is seldom referred to as such. If the unilateral lighting is carried to such an extreme that dark field results it is called oblique illumination.

***V* value of dispersion.** A ratio given as follows:

$$V = \frac{n_D - 1}{n_F - n_C}$$

where n_D is the refractive index of a substance for the D Fraunhofer line; n_F the index of the substance for the F line; n_C, the index for the C line.

Velocity of light. 299,776 ± 14 kilometers per second (Anderson), 1941.

Vertex. The center of a lens surface. The point on either lens surface which lies on the principal axis.

Vertical illumination. Vertical illumination is attained by means of a special illuminating device mounted between the objective and microscope tube. The light is received, as a beam, at right angles to the microscope axis and reflected downward through the objective by means of a prism, mirror, or glass plate. The objective performs the function of a condenser lens and focuses the light source on the object. Cover glasses are not used. The objective plays a dual part, that of condenser and that of objective. It is equivalent to bright-field illumination because if the surface is optically flat all the light is reflected back through the objective, only the detail which reflects light away from the objective being made visible. There is considerable glare due to lenticular reflection in all forms of vertical illumination. The higher the aperture of the objective, the greater will be the glare.

Virtual image. The image seen in a looking glass or through a magnifier is a virtual image. It has no real existence.

Visibility limit. For the normal eye, the limit of visibility is considerably below the limits of resolution (*q.v.*). It depends largely on contrast.

Visual angle. The angle at the eye subtended by the limits of the object. If the object is at the threshold of resolution, the angle is said to be 1 minute of arc.

Vitreous humor. An aqueous jelly-like mass which occupies the space between the eye lens and the retinal wall.

Wave front. A surface at which all vibratory motion is of like phase concurrently.

Wavelength. In wave motion, the distance between a point on one wave and a homologous point on the next wave. Or the distance between the crest of one wave and the crest of the next wave.

Wave number. The number of waves or cycles of light flux or radiant energy, measured through a distance of 1 cm.

Wide-field oculars. Oculars with a wide field of view. Used chiefly on petrographic microscopes.

Working distance. The distance from the specimen to the nearest point of the lens or its mount, usually its mount.

Xylene. One of the hydrocarbons especially useful in microscopy. Often incorrectly called xylol; $C_6H_4(CH_3)_2$; boiling point about 140° C.

ADDENDUM

NEW AND OLD FILTER NUMBERS OF CORNING OPTICAL FILTERS

New Glass Code	Old Glass Code	Name
014	014	Clear Blue Fluorescing
3389	038	Noviol Shade A
2403	241	H.R. Pyrometer Shade Red
2404	242	H.R. Dark Red
2408	243	H.R. Signal Red
2412	244	H.R. Lantern Red
2418	245	H.R. Traffic Red
2424	246	H.R. Lighthouse Red
2540	254	Heat Transmitting
2550	255	Sextant Red
3060	306	Noviol Shade O
3307	330	Signal Yellow
3385	338	Noviol Shade C
3390	339	Akloweld
3421	346	H.R. Amber Shade A
3480	348	H.R. Red Shade Yellow
3482	349	H.R. Lantern Shade Yellow
3484	350	H.R. Traffic Shade Yellow
3486	351	H.R. Yellow Shade Yellow
3384	315	Noviol Shade D
3387	368	Noviol Shade B
3750	375	Fluorescent Canary
3780	378	Dark Lemon Yellow
3850	385	Greenish Nultra
3966	395	Extra Light Shade Aklo
3965	396	Light Shade Aklo
3962	397	Medium Shade Aklo
3961	398	Dark Shade Aklo
4010	401	Sextant Green
4060*	406	H.R. Disc Green
4084	408	H.R. Emerald Green
4308	428	Light Shade Blue Green
4305	429	Medium Shade Blue Green
4303	430	Dark Shade Blue Green
4407	440	Signal Green
4441*	444	H.R. Lantern Green
4500	450	H.R. Yellow Green
5031	502	Light Theater Blue
5030	503	Dark Theater Blue

* New Glass Code numbers 4060 and 4441 require an AA-1 priority rating or better.

New Glass Code	Old Glass Code	Name
5070	507	Amethyst
5113	511	Violet
5120	512	Didymium
5330	533	H.R. Furnace Door Blue
5433†	543	H.R. Projector Blue
5543	554	H.R. Lantern Blue
5551	555	Signal Purple
5562	556	Signal Blue
5572	557	Signal Lunar White
5840	584	Red Ultra
5850	585	Blue Purple Ultra
5860	586	Violet Ultra
5874	587	H.R. Red Purple Ultra
5900	590	Daylite
5920	592	H.R. Illusion Pink
5970	597	Red Purple Ultra
738	738	H.R. Nultra
774	774	H.R. Clear Chemical Glass
791	791	High Silica UV Transmitting
9700	970	H.R. Clear Corex D
9780	978	Colorimeter Blue Green
9863	986	Red Purple Corex A

† AA-5 rating or better.

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