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THE MICROSCOPE

Its Theory and Applications

By

J. H. WREDDEN

F.R.M.S.

Member of the Institute of the Plastics Industry.
Member of the Society of Public Analysts.
Chief Chemist, The Igranic Electric Co., Ltd.

With an Historical Introduction by
W. E. WATSON-BAKER
A.Inst.P., F.L.S., F.R.M.S.

WITH 298 ILLUSTRATIONS



LONDON
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1947

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FOREWORD

In recent years the scientific control of industrial processes has been steadily developed, and in this field the author has had a great deal of experience. Much of his work has been undertaken with the aid of the microscope, and in microscopy as in other sciences, the correct use of the instrument is of paramount importance. This book covers a very wide range and the author has given detailed instructions regarding the theoretical and practical points which must be observed if the best results are to be obtained. All users of the microscope will find much of interest in this book.

R. K. FLEMING.

Managing Director,

Igranic Electric Co. Ltd., Bedford.

AUTHOR'S PREFACE

My object in writing this book has been not only to provide general information about the theory and construction of the Microscope, but also to set down a number of more unorthodox applications and processes which I have developed as a result of constant use of the instrument.

At present, the microscope is enjoying an expanding popularity in several fields, more particularly in the industrial sphere, but alas, it is too often used without regard to its full capabilities and utility. I have, therefore, allotted the greater part of this volume to the discussion of the underlying theoretical principles governing the functioning and mechanical construction of the instrument. It will, therefore, be seen that the book is primarily intended for those who are either studying the science for the first time, or, having had previous experience of the capabilities of the instrument, desire to take up Microscopy as a serious study.

In order that this object may be accomplished, the first two chapters are devoted to optical principles. These I have endeavoured to present in an attractive and easily assimilable form, taking full advantage of diagrammatic illustration wherever possible.

Next, the instrument has been treated part by part as comprehensively as possible, thus the eyepiece, objective, condenser, stand, etc., are dealt with in separate chapters, in which an attempt has been made to cover both the theoretical and the practical aspects. This was thought to be the best way of presenting the information for ease of reference, and as a means of explaining points (*e.g.*, the reasons for the necessity of a fully corrected condenser), which are somewhat neglected in more advanced works. I believe that if the basic principles are easily grasped at the outset, then future work with the instrument will not be hampered by the necessity to consider the optical principles applying to any particular problem, but will become instead a very real pleasure, apart from opening up numerous avenues of information.

The standard applications of the instrument have been dealt with in a similar manner, and although in some cases I may have been guilty of brevity in this direction, it is solely because space will not permit of more extensive discussion; suggestions for further reading have, however, been included. The greatest emphasis has been laid on those known applications which do not appear to posses an extensive bibliography. For this reason I have not dealt with the metallurgical microscope in any great detail, as this has already been covered by many well qualified authorities. I have,

therefore, kept to the use of transmitted light with a view to stimulating interest in its use in more unorthodox spheres, particularly in industry, where it would appear that (as in medical schools and other similar institutions), the use of critical microscopy is not practised to any great extent.

Stress has also been laid on some new and unusual processes, developed in the laboratories of Igranic Electric Co. Ltd., in the hope that interest in this direction will be stimulated. There would appear to be a vast amount of unexplored territory in the fields of insulating materials, plastics, etc.

From the foregoing it will be seen that an endeavour has been made to cover a very wide field within the scope of one volume, I therefore acknowledge responsibility, beforehand, for any mistakes or inaccuracies, which may have occurred.

Although the subject matter has been kept as simple as possible, it is hoped that more advanced readers, in general, will find something of interest, particularly with regard to newer methods and processes.

The historical aspect of the microscope has been dealt with only briefly, as I had the good fortune to persuade that well-known authority on the subject, Mr. W. E. Watson-Baker, A.Inst.P., F.L.S., F.R.M.S., to write the historical introduction. I would like to take this opportunity to thank him sincerely for undertaking this task.

I would also like to express my gratitude to all those friends who helped during the writing, and in particular to Igranic Electric Co. Ltd., for permission to publish the results of research work carried out in their laboratories, and by whose courtesy the photomicrographs appear. I should also like to express my appreciation of the personal interest taken by Mr. R. K. Fleming in the preliminary editing of the manuscript.

My thanks are also due to Miss C. L. Sharp, who so patiently typed the manuscript and to Mr. G. Campbell who produced all the line drawings and diagrams used as illustrations in the text.

At the same time I have to acknowledge my appreciation to the following publishing houses for permission to quote verbatim from works published by them :—

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Likewise my thanks are due to Messrs. Leitz, Baker, Watson and Bausch & Lomb for the loan of numerous blocks and permission to use illustrations of their apparatus in the text.

J. H. WREDDEN.

BEDFORD.

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HISTORICAL INTRODUCTION

By W. E. WATSON-BAKER, A.Inst.P., F.L.S., F.R.M.S.

No historical survey of any scientific subject can open without a plea for wider instruction in and cultivation of the history of such subjects. The increasing pressure upon those occupied, both academically and industrially, precludes the possibility of an intimate knowledge of such history, but unfortunately, although there is a considerable amount of literature particularly dealing with the history of the Microscope and its applications, it is widely scattered and to be found almost entirely in transactions of Societies', early books, etc., but not in a collected form.

To the student of microscopy, whether from the aspect of brass and glass design or of the application of the instrument, its history is full of suggested avenues of research and constitutes a veritable mine, the treasures of which have by no means been fully explored.

Although to-day the microscope is an accepted weapon in the armamentarium both of the scientist and the industrialist, its advent is relatively new.

There seems no doubt that the properties of burning glasses were known to the ancients. The allusion to these in Aristophanes' comedy " The Frogs " would indicate this, but there seems equally to be no doubt that the use of burning glasses—presumably flasks filled with water—did not lead to the discovery of lenses, for Pliny the younger, in his treatise on eyesight, refers to various ocular diseases and their cures. He mentions presbyopia, but nowhere does he refer to its correction by means of lenses. There is also a mass of other evidence which has been fully explored by Dr. H. Martin (1), which conclusively rebuts any assumptions of the existence and utilisation of lenses in any form. The invention, if it may be so called, of spectacle lenses, is generally attributed to Salvino Degli Armati, while Alexander Spina, a monk of Pisa, is said to have divulged the secret of their construction and use (2). It is stated that on Armati's tomb was the inscription " Here lies the body of Salvino Armati. He invented spectacles ; may God forgive his sins." This inscription is claimed to have existed and was seen in the year 1820.

Roger Bacon, the English Franciscan of Ilchester, who was a contemporary of Armati, was acquainted with the use of lenses, both convex and concave. There is no evidence whether he arrived independently at this knowledge, or through an acquaintance with Armati. From his Opus Majus it might be assumed that he had employed lenses as simple microscopes and, by stretch of imagina-

tion, that he was acquainted with the telescope, but as no directions for the construction of such instruments are given, the question must be regarded as mere speculation. From the lack of evidence or of contemporary work, it may be assumed that at this period both microscopes and telescopes had yet to be discovered. From this time onwards spectacles of various types are figured in portraits of individuals and in paintings, and there is no doubt that they came into relatively general use.

At the Renaissance with its intense mental stimulus the floodgates of enquiry were opened. It was a period of intellectual adventure and it may seem strange to those acquainted with the literature of the time—the questioning attitude of men's minds, the feeling that mankind stood on the brink of a new revelation—that the lenses used for spectacles were not developed further. In a brief chapter it is impossible to do other than refer the reader to other publications, where full information is available. The Essays (4) by Dr. M. Nicholson are comparatively unknown in this country, but merit a wide popularity.

Although the invention of the telescope and the microscope is generally attributed to the brothers Janssen of Middleburgh in Zeeland, it is only just to quote the following from Dr. Nicholson's essay: "The New Astronomy and English Literature Imagination." "So far as England is concerned, there is evidence of the invention of the telescope there, even earlier than on the Continent. It is generally agreed by historians that Leonard Digges, from his study of a manuscript of Roger Bacon, had discovered a principle of the telescope about 1550, although until recently no suggestion has been made that his telescope was designed or used for astronomical purposes." "According to his son, Thomas Digges, 'Bi-concave and convex mirrors and circular and parabolic forms,' Digges "Not only discovered things far off, read letters, numbered pieces of money . . . but also seven miles off declared what had been done at that very instant in private places."

The authority for this statement is Gunther.

Certainly Digges' illustrations justify no assumption that he was acquainted with or employed what would have presumably been an anticipation of the Newtonian form of telescope.

Borelius, who was Ambassador of the Low Countries, in his treatise "De Vero Telescopii Inventore," has left on record a notarial declaration that the telescope was invented by the brothers Janssen, who were spectacle lens makers in Middleburgh and the date is approximately 1560. It is stated that one of the brothers, having polished a spectacle lens, decided to examine its surface by the aid of another lens. To his amazement, the church clock appeared, when seen through the two lenses, both enlarged and nearer. Even if not true, it is a pretty tale and pregnant with the age-old fact that mankind sees but does not observe.

Historically the period was interesting. Motley's epic, " The Rise of the Dutch Republic," provides the necessary background. (5) There is evidence that the Janssens were members of what we to-day call the " Resistance Movement." As spectacle makers they visited all the Provincial and city fairs in the Low Countries, passing news of the anti-Spanish movement and circulating spurious coin so as to undermine confidence in Spanish currency.

On the discovery of the telescope, as good patriots, they showed their invention to the Archduke Maurice and he, recognising its military value, purchased the exclusive right of the use of the instrument for a term of years and it would appear that the Janssens honoured this agreement. The invention of the telescope is, unfortunately, attributed to Lipperchey, but we believe this to be incorrect. At the time of the discovery of the instrument, Lipperchey appears to have been employed by the Janssens as a workman, and not being bound by the undertakings entered into by them, he, on leaving their employ, set up on his own account and commenced the sale of these instruments.

The original telescope consisted of two bi-convex lenses and objects were seen inverted. The first microscopes were in effect very short focus telescopes. It is understandable that with an extremely limited scientific world, the members of which were in correspondence with each other, information of the telescope would spread and that it would reach Galileo, who was already a well-known and famous mathematician. He did not invent the telescope, but he did substitute a concave for a convex eye lens, as a result of which objects were seen erect instead of inverted.

To trace the history of the microscope from this time onwards would necessitate the production of several volumes and it is, therefore, outside the scope of this chapter. The period was one fruitful in ideas and one in which design being in a fluid state, was rich with suggestions for the future. Design was, unfortunately, to become conventionalised in certain channels and the ideas adumbrated during this period of flux were only realised in their ultimate form within the last century, some, in fact, within less than the last fifty years.

From the early seventeenth century to the middle of the eighteenth century there is a voluminous literature, but the books are scarce. A few are in private ownership, the bulk of these, however, are in the libraries of the Scientific Societies, Continental, British and American, and it is difficult to consult them.

The best all-round survey is that to be found in " The Microscope and its Revelations," Carpenter, and the reader is referred to it for amplification of the details given in this chapter.

Passing in rapid review the various developments in design, we would refer to Descartes (Fig. I). This simple microscope designed by Descartes was the forerunner of the Lieberkuhn, which

had a great vogue until the 1880's. The principle was applied by Mr. E. M. Nelson to his reflecting magnifier and subsequently

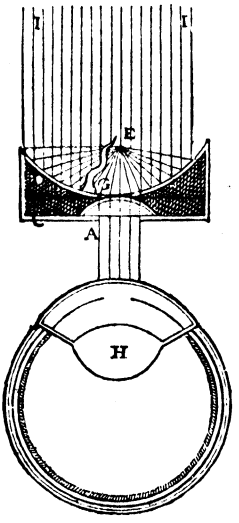


FIG. I.

appeared again on the microscope in the form of ring illuminators, Ultrapak, etc., and is now in frequent use

The microscope of Campani (Fig. II) can be justly termed the precursor of the pocket microscopes, which have been so prominent

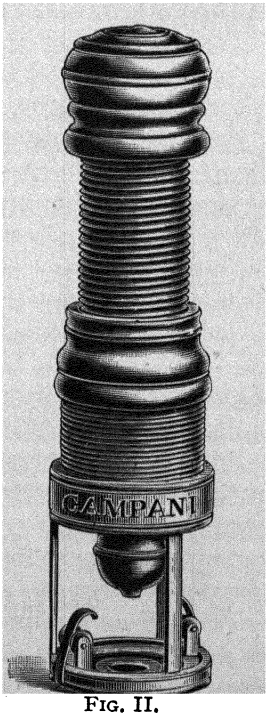


FIG. II.

during the past two decades. It was also the model upon which the Wilson screw barrel and similar instruments were developed.

Of still greater interest is Hooke's compound microscope (Fig. III). For the first time coarse and fine adjustments are

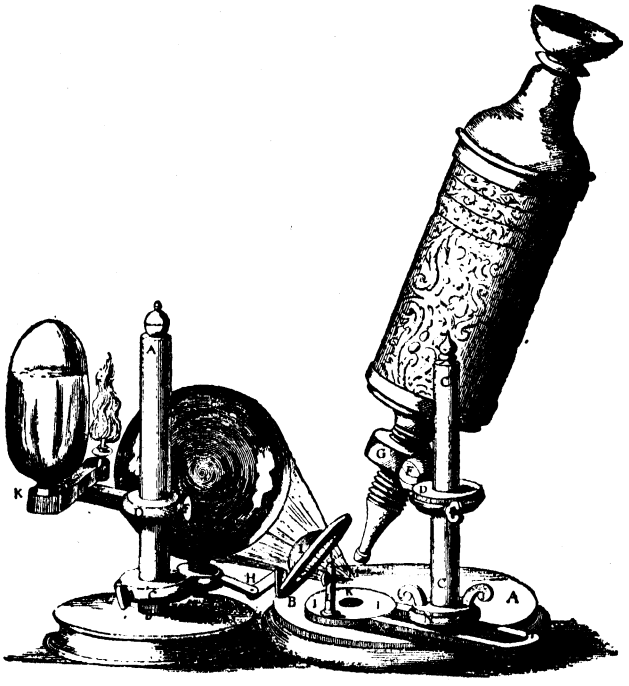


FIG. III.

incorporated, and provision is made for inclination of the body tube, also the object can be orientated for examination at any angle. Admittedly the movements incorporated are rudimentary, but the germ of future design is present. For the first time a lamp with the equivalent of a condensing system is provided, and it will be noted that adjustment to the lamp condensing lens is incorporated. Incidentally, it will be remembered that Hooke was the inventor of Hooke's joint, known to all users of scientific instruments and employed with success up to the present time.

Hooke (Secretary of the Royal Society) was a man of amazing mental activity. He made attempts to overcome the chromatic and spherical aberrations in the lens systems of the time. Actually he introduced a field lens to the eyepiece, but while admitting that there was an improvement in performance, he objected that the field of view was reduced. Another attempt was the introduction of water between the objective and the eye lens system. Again he found that the continuous path of light had its advantages, but the method was inconvenient and the gain insufficient to justify this. He also experimented with the use of colour screens.

The Capucin Priest, Cherubin d'Orleans, introduced the first binocular microscope (Fig. IV). This was a true binocular. Not

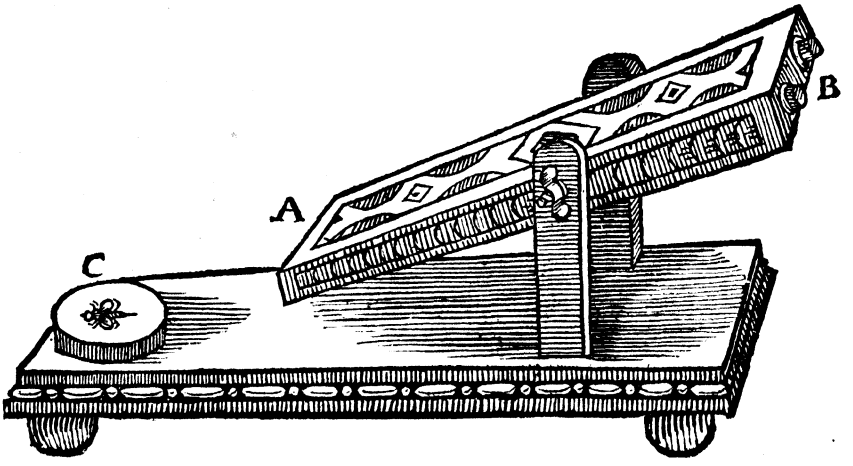


FIG. IV.

only were two eyepieces provided, but also two objectives. In his own book (6), and Zahn (7), figures of these eyepieces appear and it will be seen that they were provided with inter-pupillary adjustments. No coarse focussing adjustment was fitted for this microscope, objects were focussed by moving the stage nearer to or further from the objective—surely the forerunner of the stage focussing used so extensively in the next century and employed to-day on metallurgical microscopes.

The microscopes of Van Leeuwenhoek have so often been figured and referred to that no illustration is necessary, nor—in view of Dobell's publication, in which work the author has been extraordinarily successful in bringing Van Leeuwenhoek to life and making the reader feel that he is actually in mental contact with Dobell's hero—is any reference to Van Leeuwenhoek required.

The next suggestive design in chronological order is that of Bonani (Fig. V), and nothing can be more suggestive or more in advance of its time. The complete instrument is virtually based upon the principle of the optical bench. There is an attempt at rigidity. The microscope body is fitted with coarse and fine adjustments, the coarse being by rack and pinion. The sub-stage or lamp condenser is provided with focussing motion, surely the prototype of the microscope used so successfully for metallurgical research, photomicrography, etc., up to within the last twenty years.

The microscope by Joblot, although it does not appear effectively in the illustration (Fig. VI), incorporates the first objective slide. Joblot mounted his objective in thin brass, dove-tailed slides, and these, some of them incorporating one, others two, objective lenses, were interchangeable and were, what would be known as, objective changers on the microscope.

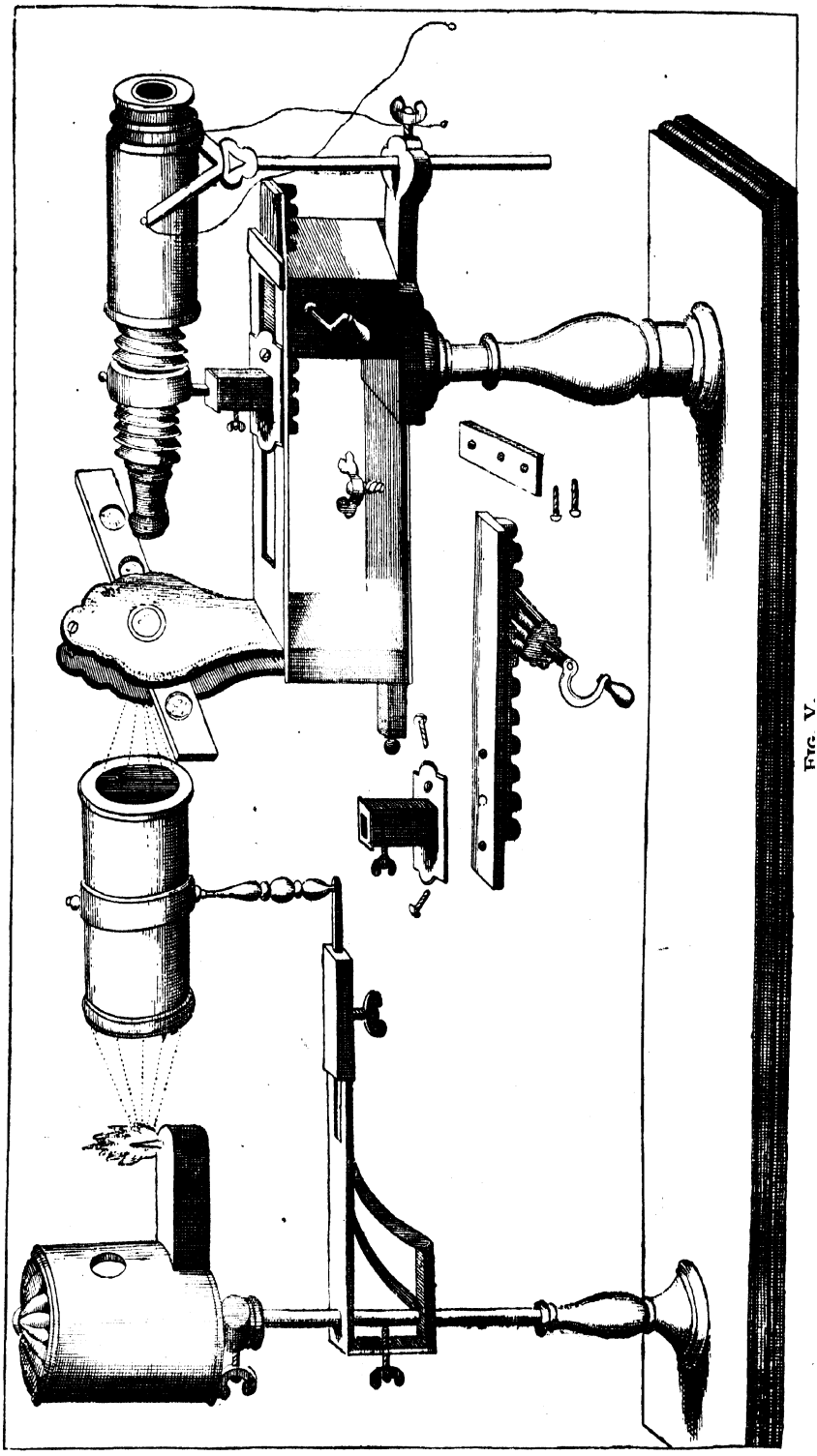


FIG. V.

The first revolving nosepiece was introduced either by Adams or by Martin. These two designers were contemporaries and it is

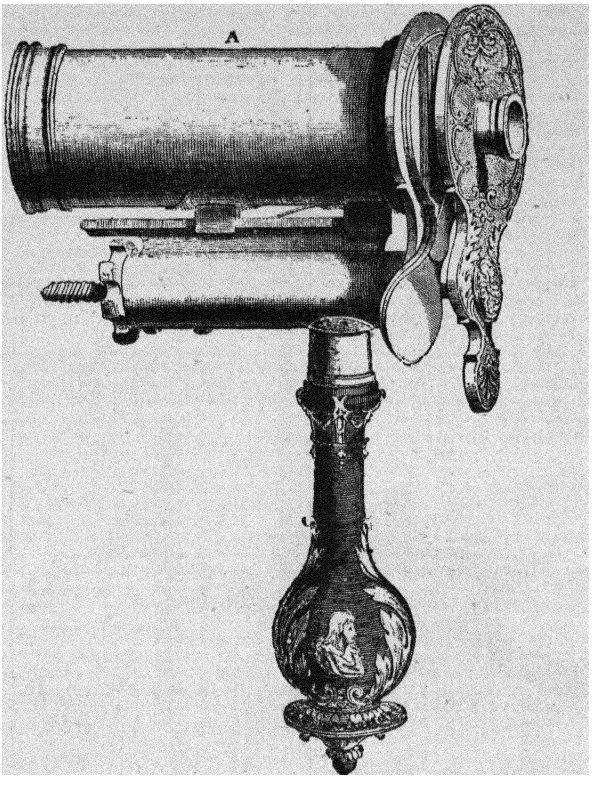


FIG. VI.

sometimes difficult to attribute the various designs for which they are responsible in correct priority the one to the other. It is perhaps convenient at this point to interpolate a note on the first Scientific Society. This, under the name of " The Academy of the Lynx," was founded in Rome and met at the house of the Prince Cesi. Stelluti, Galileo and many others were members. The new knowledge obtained by means of the microscope and the telescope was of such enthralling interest and fitted in with the new outlook of the thinking section of mankind, resultant from the Renaissance. The Church had not fully determined her attitude. The Pope Urbino—he who was interested in Galileo's work—occupied the Papal throne, and both as a compliment and to placate him, the first monograph ever published was on the honey bee. This was selected for study because the bee figured in the papal coat of arms (8). It will be remembered that Milton made the Grand Tour. From his subsequent essay " Aeropagitica " it is known that he met Galileo (M. N.), and the whole of his poetry subsequent to that meeting is instinct with awe of a new knowledge of space. It is interesting to speculate

upon the possibilities of Milton having attended meetings of the Academy of the Lynx and subsequently meetings of the Royal Society, through which there is then a continuous link from the first Scientific Society to the present day, for the Royal Society has the longest continuous history of any scientific society in the world.

To return to the microscopes. Space does not permit a full survey of the various mechanical and optical devices which have been developed over the course of time, but particular reference may be made to those designs which foreshadow present-day practice.

Microscope Stands

Mechanical Features

These were incorporated on microscopes as far back as 1780. An admirable example is that made by Benjamin Martin on his microscope supplied to George III, now in the collection of the R.M.S. Mechanical stages with the milled heads at right angles to direction of movement were used by Tulley and others as far back as 1820. Focussing sub-stages were employed before 1780. Side fine adjustments were introduced by Powell in 1840. In this model, focussing operated on the top plate of the stage. In 1841 Powell introduced a side lever fine adjustment operating upon the objective, subsequently the nosepiece fine adjustment came into general use, an excellent example being the design by James Smith in 1839. To this a recoil spring was fitted. This form of fine adjustment persisted until the 1880's, and on the Society of Arts' type microscope until 1914. It was found, however, that the nosepiece fine adjustment was unsatisfactory in wear, except as fitted in the Powell and Lealand microscope and excepting on the very cheapest instruments it was discontinued. The English designers introduced the horizontal lever form of fine adjustment and this continues either in the horizontal or vertical application of the lever to the present day. The Germans introduced the prism bar type of fine adjustment, with a direct-acting micrometer screw (Fig. VII) which persisted for many years, but had many objections and was ultimately withdrawn in favour of a Cam type. Within the last twenty years Zeiss re-introduced the lever type of fine adjustment on his stands, but this was actuated by means of a train of interlocking clock wheels, rather complicated and clumsy. During this time, except for the very cheapest instruments, the English and American designs have adhered to the lever pattern, either applied horizontally or vertically, which years of use have shown to be the most efficient, reliable and enduring (Fig. VIII). Mention should be made of Swift's " Challenge " fine adjustment (Fig. IX) which, except for its inconvenience, is probably still the most effective as it actuates the whole body only and does not disturb the relationship between eyepieces and objective. The difficulty in

this application is to evolve a design in which the milled head of the fine adjustment is stationary, and in which this milled head is in

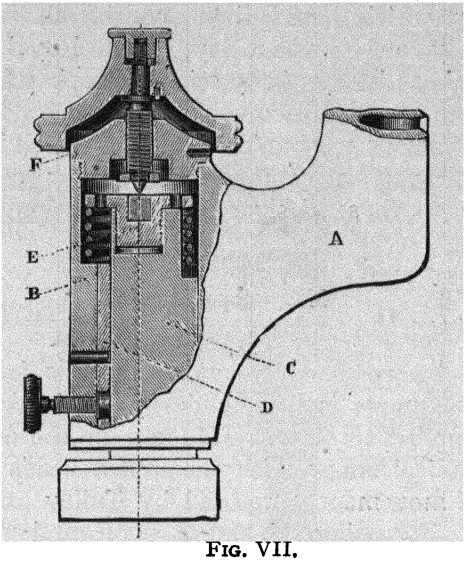


FIG. VII.

a convenient position in relation to the coarse adjustment milled head also to the table upon which the user's hand rests. Another

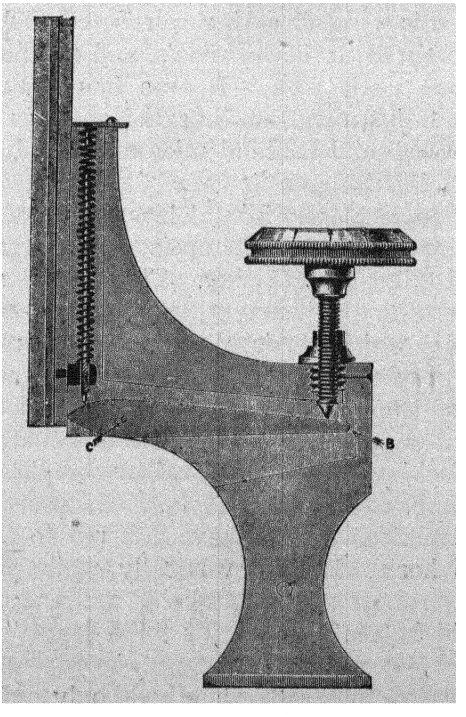


FIG. VIII.

form which has never been fully developed is the differential screw type, 1886.

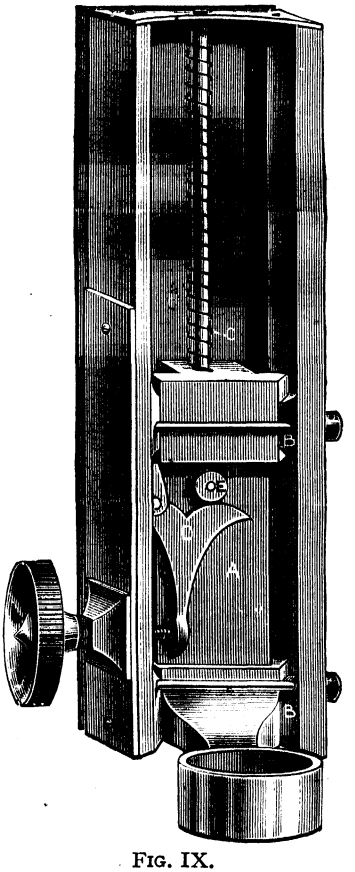


FIG. IX.

The double lever direct-acting fine adjustment employed by Reichert (Fig. X) was not new. Powell actually employed it in

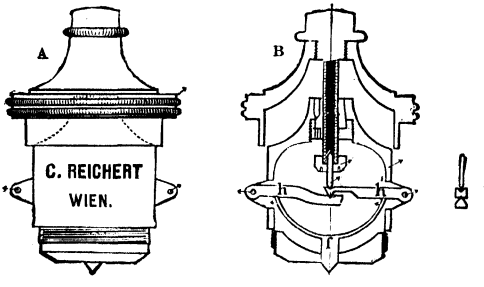


FIG. X.

1830 and it exists on a stand of that period in the writer's collection. The fine adjustment to the sub-stage is still a necessity for the most accurate work. This has never yet been employed by Conti-

nental designers. One American designer has used it, but it is featured more frequently on stands of English manufacture. Probably the reason for this is that only in England was microscopic technique elevated to an art and until recent years only in England was the design and application of the sub-stage condenser given its due importance in relation to objective performance. Anyone examining the English, American and Continental designs will see that only among English designers has the sub-stage hitherto been regarded as an integral adjunct to microscope performance and designed for this purpose. Wrapped up with the design of the sub-stage is that of the mirror. Too often this is treated as a negligible adjunct. The mirror should have universal motion and should be adjustable independent of the sub-stage, both as regards its separation from the back lens of the condenser and its orientation.

Mechanical Stages

There are two distinct schools of design. Undoubtedly for ultimate resolution and the truly efficient employment of oil immersion objectives and condensers in combination, the English design is the best, but it has the serious limitation in that with a moving top plate (Fig. XI) provision must be made for the width of the top

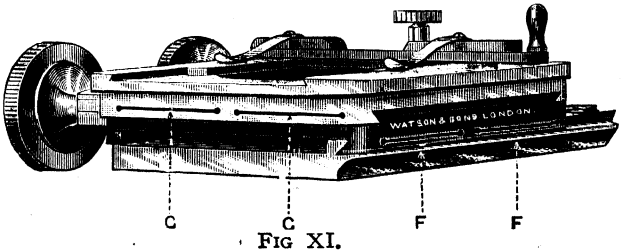


FIG XI.

lens of the condenser, thus the area available for examination in a specimen is, under the most favourable circumstances, limited to $1\frac{1}{2} \times 1\frac{1}{2}$.

For the examination of serial sections and blood smears, longer traverse is essential. This is provided by what may be termed the Continental type of mechanical stage, in which the object is held between two metal fingers and moved progressively across the optic axis. Space does not permit an examination of the advantages and disadvantages of these two types of design. Designers throughout the world now employ both patterns.

Sub-stages

The spiral focussing screw pattern, which at one time was so popular, has been discontinued on all save the very cheapest stands. At best it was a compromise. The rackwork sub-stage ensures rigidity and a basic alignment, provided that it is not arranged to hinge out of the optic axis. This can be achieved by other means.

For serious work, however, some form of centring to the sub-stage itself is essential. It is quite impossible to provide any form of sub-stage with interchangeable condensers, where both condensers will have precisely the same optic axis, unless centring provision is made. During the years following the introduction of the immersion paraboloid, centring nosepieces were used, but these are fragile and of short life. Before the turn of the century, Mr. E. M. Nelson suggested the mounting of condenser optical parts upon slides similar to those used for objective changers. In the 1920's this suggestion was pursued by Mr. Akehurst and the design has since been adopted by all progressive manufacturers. With this method it is possible to render condensers co-axial, but for its ultimate success it is essential that the iris diaphragm should be built into the sub-stage itself and the optical part of the condenser should be a separate element, with its own centring adjustment.

Optical Parts

Eyepieces

Eyepiece design in universal use is that based upon the Huygens formula. During the years many variants have appeared. Towards the end of the eighteenth century design became over-complicated. Euler even evolved an eyepiece comprising ten constituent lenses for this optical system. With the achromatisation of objectives, eyepiece design was simplified and reverted to the Huygens formula. Subsequently the Kellner type became popular, owing to its greatly increased field. For low-power work this field had, and still has, certain advantages, if numerical apertures are low. To-day with the greatly increased numerical apertures of the objective, the Kellner type of eyepiece which persists under many names of orthoscopic, etc., is only preferred for its æsthetic appearance, for it is impossible to achieve anything approaching a flat field over so wide an area.

Ramsden Eyepieces

These are positive eyepieces and are used in connection with screw micrometer and similar apparatus.

Micrometer Eyepieces

Full particulars of these will be found in the following text, but it should be remembered that the eye lens of the eyepiece is the actual magnifier for the eyepiece micrometer, and it is unwise to employ a magnification greater than $\times 6$ As magnification increases, so does the curve of the eye lens. There is, therefore, a decreasing flatness of field with a marginal distortion of the eyepiece micrometer, increasing in relation to the power of the eye lens.

Compensating Eyepieces

These are essential if the full benefits of apo-chromatic or fluorite and similar highly corrected objectives are to be realised. In such

objectives only two functions are corrected in the objective itself, the remaining function being corrected by the eyepiece To be absolutely correct and do justice to the art of the objective designer, users should actually provide themselves with a set of compensating eyepieces for each different make of apo-chromatic or fluorite objective in their equipment. There has been no other real advance in eyepiece design since the introduction of compensating eyepieces. Many modifications for specialised work are available. The "Ehrlich" is of considerable value in blood counting or similar work also for dark ground employing higher apertures than those normally in use. Mention may be made of the Homal series developed by Zeiss. This series is intended for photomicrography only and is useless for visual work. They yield a flat field, but the eyepieces are not universal and can only be employed in conjunction with Zeiss's objectives. Even so, the × 6 Homal eyepiece for a 16-mm. objective would not be efficient if used in combination with a 4- or 2-mm. objective. Separate and complete sets of eyepieces are needed for each objective.

Projection Eyepieces

These are designed both for photomicrography and projection microscopy. The design is such that the diameter of the projected image is reduced to reasonable limits for screen size. Actually, were it not for the æsthetic appearance, eyepieces of corrected negative lenses would be far more satisfactory for projection and photomicrography. Such eyepieces have, in recent years, been introduced by Professor H. Hartridge. The objection to these is that it is impossible to provide the clear-cut margin to the field; although this objection is purely æsthetic it is a deterrent to many who would otherwise find it advantageous.

Objectives

Since the work of Lister there has been a continuous and progressive improvement in the microscope objective. Probably one of the most notable events was in 1854 when Smith, Professor of Mathematics at Cambridge, pursuaded Ross to design and manufacture for him an objective employing quartz with a view to the utilisation of light of a shorter wavelength. This line of research was not further pursued until the commencement of the present century. A notable development was the introduction by Professor Abbe of the apochromatic series of objectives, which by their high chromatic corrections enabled the user to obtain fuller advantage of the apertures with which these objectives were endowed. The subsequent developments under Abbe's direction in the manufacture of a wide range of optical glasses, having varying refractive indices and dispersions, opened an avenue to a wide advance in design and manufacture. It is interesting to note that since 1920 the firm of Chance Bros. & Co. Ltd. in England has produced optical glasses

better in quality and wider in range of those produced by Schott at Jena, and the lead established has been consistently maintained so that even prior to 1939 Messrs. Chance Bros. were exporting optical glasses for lens manufacture all over the world, including the Continent of Europe. In recent years the development of the use of light of short wavelengths, originally adumbrated by Smith in 1854, was pursued in 1906 by Kohler in Germany, and some years afterwards by Barnard and Beck in England.

Condensers

There is considerable misapprehension concerning the use and development of sub-stage condensers. It has been shown that in a rudimentary form these were employed by the early fathers of microscopy. In this country corrected acromatic condensers were known and employed from the time of the achromatisation of objectives. A survey of this will be found in " The Microscope and its Revelations " by Carpenter and Dallinger. Gillett and other condensers anti-date the Abbe illuminator, and it is no exaggeration to claim that the introduction of the Abbe illuminator actually set back microscopy for a very considerable period of years. The Abbe condenser was designed as an illuminator, and those interested are referred to Abbe's original papers of 1878 and 1884 dealing with this subject. This condenser is corrected neither spherically nor chromatically, and is designed for light from infinity. Its nominal N.A. of 1·20 can, of course, only be realised by immersing the front lens, but this N.A. is not aplanatic. It will be found that the maximum N.A. of the best Abbe condensers does not aplanatically exceed 0·65. While therefore the Abbe illuminator (or condenser), owing to its insensitiveness to centring, etc., is probably the best possible design for the student microscope, it becomes an anachronism when employed for more advanced work, particularly remembering the very high standard of modern achromatic objectives.

It is no exaggeration to claim that the standard of microscopical technique was for many years maintained in England alone, Continental design tended more to simplification of the instrument with a view to cheap production. This simplification was achieved by the elimination of movement and fittings necessary, among other functions, for development of sub-stage technique. In England alone, until after 1920, was the problem of the sub-stage condenser thoroughly explored and developed, so that even at the present time the English makers alone offer a range of condensers fully corrected both chromatically and spherically for use with objectives of all powers from the lowest 75-mm. objective up to the 2-mm. oil immersion. Condensers endowed with apertures equal to and of a performance similar to the objectives with which they are to be employed. That this is no idle claim can easily be

checked by those interested, for a fully corrected condenser should be of a quality allowing its use as a microscope objective (9).

Accessory Apparatus

The Nicol prism, named after its inventor Nicol, an Edinburgh man, was made during the opening years of the nineteenth century, and when first introduced was actually mounted as an integral part of the objective, being situated in the objective base. Micrometer stages and eyepieces were introduced by Benjamin Martin before 1770. Sorby introduced the vertical illuminator in the 1870's. Even the material polaroid, so recently produced, is only a development of the Herepath prism known to, and employed by, microscopists between 1870 and 1880. The colour discs introduced by Rheinberg prior to 1900, providing differentiated colour illumination, were re-introduced after 1920 under the trade name of " polychromat" illumination by a German firm.

In the brief space available an endeavour has been made to pass in review some of the outstanding features in the development of microscope design. It will have been seen that practically every modern development has been adumbrated at an earlier date. There is still a wide and fertile field of development, and readers who wish to pursue this are recommended to study the journals of the Royal Microscopical Society and the Quekett Microscopical Club. Many suggestions and outlines of design remain yet to be developed, while much suggested technique remains unexplored. It is hoped that this chapter may stimulate some readers to take up the further development both of the instrument and accessories.

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THE MICROSCOPE

CHAPTER I

ELEMENTARY OPTICS

As the microscope is a scientific precision instrument, it is essential that a working knowledge, at least, of the principles underlying the instrument should be obtained. Accordingly this chapter is devoted to the elucidation of those basic principles of optics which will help the user of the instrument to appreciate the necessity for correct illumination in obtaining a critical image.

The subject is treated from the point of view of explaining in a practical manner the general principles underlying the subject, without going too deeply into the underlying mathematical problems.

Refraction

The microscope and, in fact, all optical instruments from the simple hand lens to the most modern giant astronomical telescope, depend upon the ability of their vital components to refract light ; thus the magnifying power of a microscope depends upon this phenomenon.

Refraction obeys two basic laws :—

(1) A ray of light, which, passing from a rarer medium to a denser medium, makes a certain angle with the perpendicular to the plane at which the two media meet, will on entering the denser medium make a smaller angle with the perpendicular than that which it made whilst traversing the rare medium. Conversely, of course, a ray passing from a denser medium to a rarer medium will make a larger angle with the perpendicular to that which it made whilst traversing the denser medium. In optics, this perpendicular to the plane of junction of two media is called the NORMAL.

Thus, in Fig. 1, we have a plane of junction between a rarer and denser medium at the line AB. The normal to this plane being represented by the perpendicular NN1, a ray of light RR1 traverses first the rarer medium, meeting AB and NN1 at the point O, making an angle θ with NN1. It is then refracted through the denser medium to the line OR1, making an angle $\theta 1$ with NN1, which is less than the angle θ. It will be seen that if the ray travelled in the direction R1OOR, how the angles to NN1 would be $\theta 1$ increased to θ according to the direction of the ray. The ray in one medium is called the incident ray and in the other the refracted ray ; thus, if the direction were to be ROOR1, the ray RO is the incident ray and OR1 is the refracted ray, and the angle

θ_1 is called the angle of refraction, but it must always be borne in mind that the process is reversible and that the incident and refracted rays are always in one plane.

A clearer understanding of the nature of refraction will be obtained if one visualises the case of a ray of light passing from a rare medium, such as air, into a denser medium, such as water. Here we have a ray which may be represented by a wave front of a certain amplitude and angle to the normal, which on entering the denser medium encounters a resistance to its motion and is refracted downwards towards the normal. Perhaps the point will be made clearer by imagining the wave front to be represented by a strip of wood, say 2 feet long by 5 inches wide, the thickness is immaterial. If the wood is travelling towards the surface of the water at an angle to the normal, flat side forward, then it will be seen that when it strikes the water the lower edge of the strip would strike first and be resisted, bringing the upper edge round and so virtually redirecting its path from the original straight line to a new straight line whose angle to the normal would be smaller than the original angle. Although this is not precisely true, it will serve the purpose of illustrating the point.

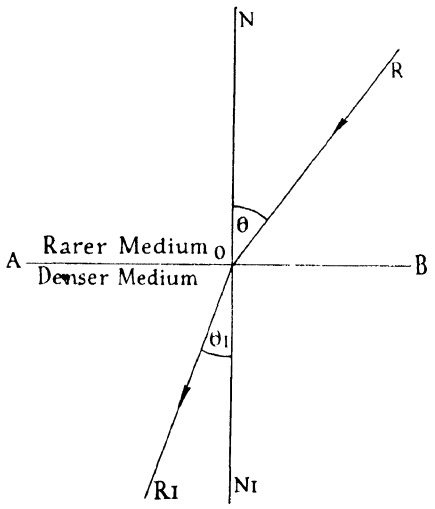


FIG. 1.

(2) The second law may be briefly stated by saying that the sine of the angle of incidence (*i.e.*, the angle of the incident ray with the normal) divided by the sine of the angle of refraction, is a constant quantity for any two given media.

If one of the media is a vacuum, then this ratio of sines is called the absolute refractive index of the medium and it follows that as there is no other medium less dense than a vacuum, the angle of refraction for any other medium paired with it will be less than the incident angle in the vacuum. Therefore the ratio of sines will always be greater than unity, *i.e.*, the refractive index will be greater than unity.

As white light is composed of light of various wavelengths or colours, each colour will present its own wave front, differing in amplitude from the other colours according to their relative positions in the spectrum, to the plane junction of the two media, and in consequence each colour will be refracted to a different degree. This

refraction is the least for the red and greatest for the violet, thus the white light will be split up and virtually dispersed by refraction through a medium, which property is called the dispersive power and which varies according to the degree of refraction or the absolute refractive index of the medium.

This will be understood if reference is made to Fig. 2, which shows us how a ray of light behaves when passing from a rarer to a denser medium, and out again into the rarer medium.

Here we have an incident ray, ROO1R1, which is travelling in the direction indicated by the arrows, making an angle θ with the normal. This is refracted to OO1 making an angle $\theta 1$ with the normal. The plane junction or interfacial is AB.

This, as we know, is all straightforward, but now the ray proceeds to travel out of the denser medium into the rarer medium and we have seen how it is refracted back, so let us look upon the ray O1 as the incident ray with the interfacia plane at CD, and the normal to which is the line, N2N3, parallel to NN1. The angle made by OO1 to N2N3 is of course $\theta 1$, which now becomes the angle of incidence. This ray will now be refracted to O1R1 by the rarer medium, making the angle θ with the normal N2N3.

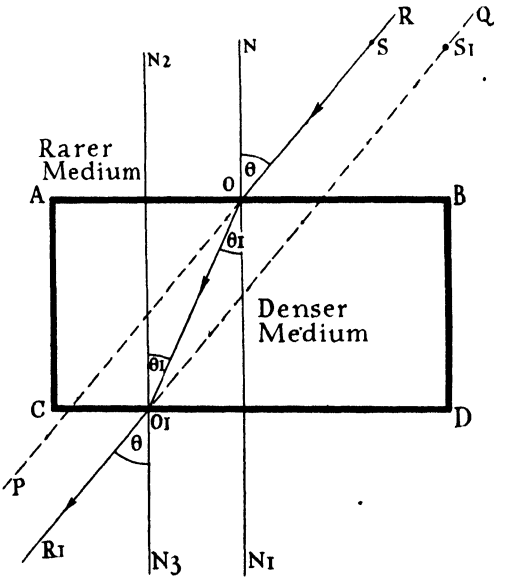


FIG. 2.

It will be seen that this cannot be otherwise, as there are two media which have a fixed refractive index relative to each other; therefore, the ratio $\theta/\theta 1$ is constant, and as the angle of incidence of the ray OO1 is $\theta 1$, then the angle R1O1N3 must be equal to θ, thus restoring the original angle of incidence of the ray, but, which is more important, not its original position. This is shown by the dotted line OP, which would be the path of the ray if there was no refraction.

Thus, if we suppose the rarer medium to be air and the denser medium to be a block of glass whose thickness is BD, if an object such as a pin were placed at the point S and viewed from the side of the glass CD at an angle θ to the normal NN1, then if there was no refraction, the view point would be along the line OP and one

would see the pin at S, but owing to the refraction which takes place, the view point is actually along the line R1O1 and the pin appears to be at the point S1. This is the explanation of the well-known phenomenon of a stick appearing to be bent when placed in water.

In summing up these two laws then the main points are :—

(1) When a ray is incident in a rarer medium, incident light is always refracted towards the normal by the denser medium, and, conversely when it is incident in a denser medium, it is always refracted away from the normal by the rarer medium.

(2) The ratio of the sines of the angles of incidence and refrac-

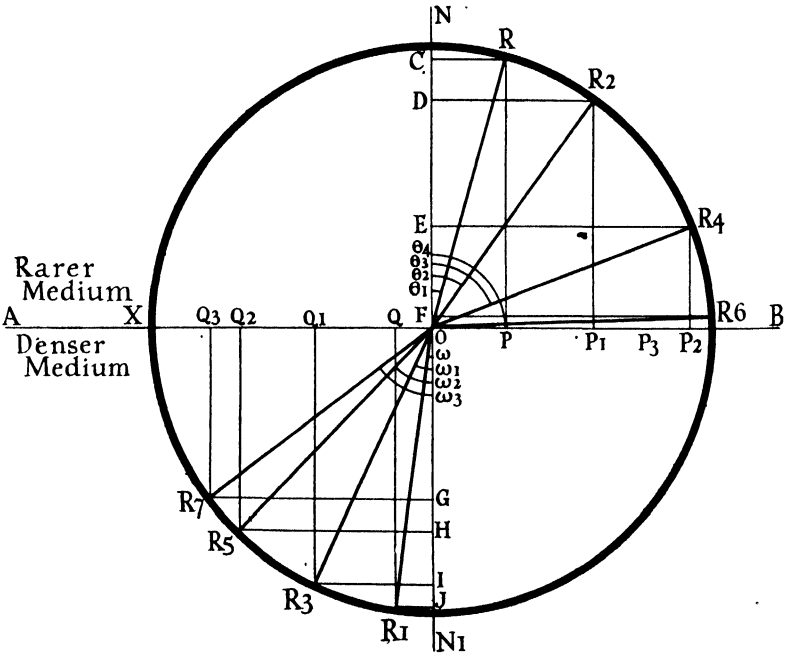


FIG. 3.

tion, in such a system, is a constant. This is known as Snell's law and in our case is given by the expression :—

$$\frac{\text{Sine } \theta}{\text{Sine } \theta 1} = \mu = \text{relative refractive index.}$$

When the angle of incidence θ is for a vacuum, then μ is called the absolute refractive index.

This law is made clear in Fig. 3, which shows a number of incident rays R, R2, R4 and R6, making angles, with the normal, of $\theta 1$, $\theta 2$, $\theta 3$ and $\theta 4$ respectively, and all meeting the interfacial plane where the normal NN1 cuts it at the point O. RO is refracted to OR1, the angle of refraction being ω, R2O is refracted to OR3, the angle of refraction being $\omega 1$, and so on.

Now, if we draw CR perpendicular to the normal and R1J in the same manner, then the ratio CR/OR is the sine of the angle $\theta 1$ and the ratio R1J/OR1 is the sine of the angle ω, and the ratio sine θ/sine ω is μ, the refractive index of the denser medium relative to that of the rarer. In the circle about the point O with radius RO.

$$RO = R2O = R4O = R6O = R1O = R3O = R5O = R7O$$

as they are all radii of a circle. Therefore as the refractive index $\mu = \frac{\frac{CR}{OR}}{\frac{R1J}{OR}}$, OR, and OR cancel out and $\mu = \frac{CR}{R1J} = \frac{DR2}{R3I} = \frac{ER4}{R5H} = \frac{FR6}{R7G}$

Instead of there being several rays, let us imagine just one ray, R, moving to positions R2, R4, etc., then the refracted ray R1 will move to positions R3, R5, etc., in proportion to μ. If at each of these positions of R we drop a perpendicular P, P1, P2 to the interfacial plane, and similarly for the positions of R1, to Q, Q1, Q2, then obviously $\frac{OP}{OQ} = \frac{OP1}{OQ1} = \frac{OP2}{OQ2} = \mu$ and it will be seen that as the point P approaches the periphery it approaches equality with OR6 and when P is coincident with R6 then OP = OR6 and the sine of the angle of incidence is then unity.

Let us see what has been happening to the refracted ray OR1 which has been following the incident ray in proportion to μ. Here Q has been moving along the interfacial plane towards the point X, but owing to its lagging behind P, by the fraction μ, when P has reached its maximum at point R6, Q has only got as far as a point Q3; clearly then, the ratio $\frac{OR6}{OQ3} = \mu$ which shows how, as the angle of incidence grows larger, so does the refraction increase due to the rate of increase of the angle of refraction being less than that for the angle of incidence.

This brings to light a curious phenomenon, for one might ask "What would be the effect of increasing the angle of incidence further?" The answer is that refraction for all practical purposes ceases and one gets total reflection, for it must be borne in mind that while our ray has been undergoing refraction in the denser medium, part of it has also been reflected off the surface at an angle equal to the incident angle; so that if we rotate R a little way past the point R6, the ratio μ still holds until a point below B is reached. When Q has moved past Q3 to a point coinciding with X, when a similar condition is established, to where we had P and R6 coincident. It will be seen how P would now have come back

towards O to a point on AB at P3, so that if R is in any position below OB, it cannot escape out of the denser medium and is reflected back internally from the point O.

Looking at the question in another way, let us imagine the incident ray to be one in the denser medium R1O, Fig. 4, making an angle θ_1, with the normal NN1, then the refracted ray will be

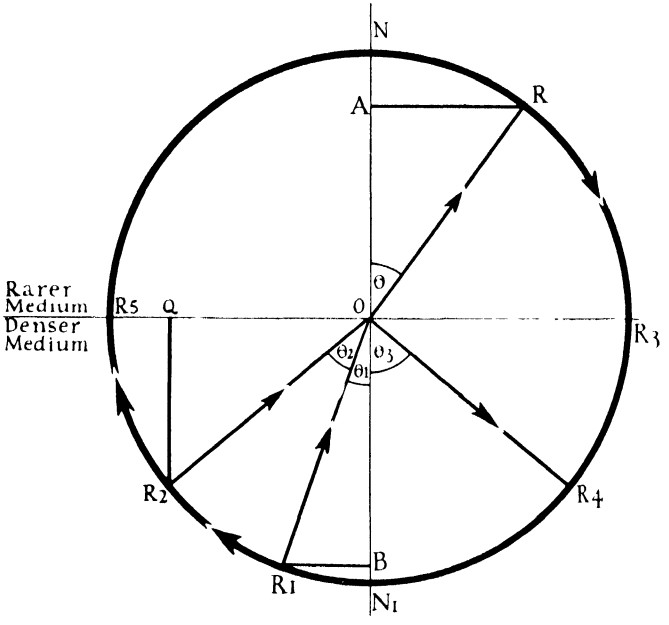


FIG. 4.

OR and the angle of refraction will be θ, the ratio between the sines of the two angles will be μ and the ratio $\frac{AR}{BR_1}$ also $= \mu$

If R1 moves in the direction indicated to R2 such that on dropping the perpendicular R2Q, $\mu = \frac{OR_3}{OQ}$, θ will then be 90° and any further movement of Q will make θ greater than 90°. This, of course, is impossible, and at the position R2 the ray is totally reflected.

It will be seen that in the circumstances the ray cannot leave the denser medium and is consequently totally reflected back from O to a position R4, obeying the laws of reflection, making an angle θ_3 with the normal, which is equal to the angle of incidence θ_2. This angle is called the critical angle.

The condition of total internal reflection is maintained for further movement of R1 until the point R5 is reached, beyond which the ray becomes an incident ray in the rarer medium and refraction takes place as usual in the denser medium.

It will be appreciated from the above remarks that total reflection can only occur in the denser of two media or when the incident ray is in the denser medium, due to the angle of refraction being smaller than the angle of incidence.

As μ may be determined experimentally for any two given media, it is obvious that where one is known the other may be found. For example, if the angle of incidence and μ is known, the angle of refraction can be found. Thus, we see that the path of a ray of light passing through two media of different densities may be accurately determined, and further, that the refraction of red light is less than that of blue light ; for example, the absolute refractive index for red light in crown glass is 1·5124 and for violet light is 1·5288, the difference between these is 0·0471.

The normal to the interfacial plane is always the perpendicular to it, whereas the normal to a spherical surface is the radius of curvature, the angles being measured with the normal in all cases, and never with the surface.

Refraction in Prisms

There is no need to emphasise the importance of the prism in practical optics, so let us straightway see what happens to a ray of light when it traverses a prism. Assume for the moment that the light is monochromatic, that is to say, composed of one wavelength only, such light is produced by a sodium lamp.

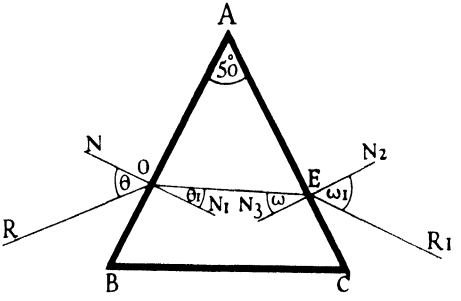


FIG. 5.

Fig. 5 shows the path of a ray of light through a prism, the refracting angle of which is 50°. Now, it can be shown that the connection between the path of a ray through a rarer medium into a denser medium is given by the expression :—

$$\mu \text{ sine } \theta = \mu 1 \text{ sine } \theta 1.$$

Where μ is the absolute refractive index of the first medium and sine θ the sine of the angle of incidence, and $\mu 1$ is the absolute refractive index of the second medium, sine $\theta 1$ being the sine of the angle of refraction.

Suppose the prism be made of dense flint glass with an absolute refractive index of 1·72 for sodium light, then for the ray RO making an angle θ with the normal NN1, which we will say is 45°.

$$\mu \text{ sine } \theta = \mu 1 \text{ sine } \theta 1$$

$$\therefore \text{ sine } \theta 1 = \frac{\mu \text{ sine } \theta}{\mu 1}$$

where sine $\theta 1$ is the sine of the angle of refraction $\theta 1$, μ is the absolute refractive index for air, this being unity, and $\mu 1 = 1{\cdot}72$, we now get

$$\text{sine } \theta 1 = \frac{1 \times \text{sine } 45}{1{\cdot}72} = \frac{0{\cdot}707}{1{\cdot}72} = 0{\cdot}4102$$

$$\therefore \theta 1 = 24{\cdot}25^\circ$$

Thus we see that the refracted ray will take a path OE, making an angle of 24·25° with the normal NN1, through the prism to emerge at the point E.

If we draw the normal to the surface AC through the point E and call it N2N3, then it may be shown that the angle which OE

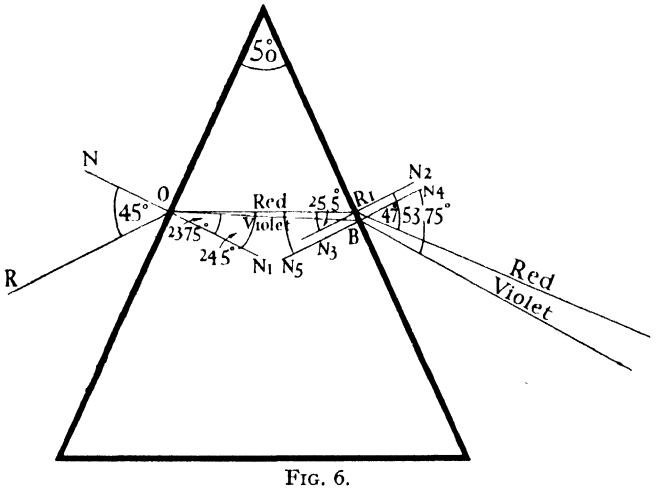


FIG. 6.

makes with this normal is the difference between the refracting angle of the prism and the angle of refraction $\theta 1$, so that we now get :—

$$\omega = 50 - 24{\cdot}25 = 25{\cdot}75^\circ.$$

This ray OE may now be looked upon as an incident ray whose angle of incidence is 25·75° to the normal N2N3 ; on passing out into the air it will be refracted again, but this time away from the normal and the angle of refraction $\omega 1$ will be greater than ω as the refractive indices remain the same we can apply the same expression thus :—

$$\text{sine } \omega 1 = \frac{\mu 1 \text{ sine } \omega}{\mu}$$

substituting we get :—

$$\text{sine } \omega 1 = \frac{1{\cdot}72 \times 0{\cdot}4340}{1} = 0{\cdot}74648$$

$$\omega 1 = 48{\cdot}25^\circ$$

If we take a similar prism and use white light, the refractive index of dense flint glass for red light is 1·7 and for violet light 1·75. Fig. 6 shows the effect of passing this compound ray through the same prism.

In this case the ray of white light R makes the same angle of incidence with the normal NN1, viz. 45°, on entering the glass; however, the red ray will be refracted less than the violet. Thus, from the expression used before we get :—

$$\text{sine } \theta 1 = \frac{1 \times \text{sine } 45}{1{\cdot}7} = \frac{0{\cdot}707}{1{\cdot}7} = 0{\cdot}416$$

$$\theta 1 = 24{\cdot}5° \text{ for red light.}$$

For the violet ray, we get :—

$$\text{sine } \theta 1 = \frac{0{\cdot}707}{1{\cdot}75} = 0{\cdot}404$$

$$\theta 1 = 23{\cdot}75° \text{ for violet light.}$$

Thus we now have a condition where we get two incident rays OR1 and OB in the denser medium emerging into the rarer medium at points R1 and B respectively. Here again we can apply the same formula and obtain the angle of refraction on emergence into air. Thus the angle to the normal at which the red ray emerges is 47°, and that at which the violet ray emerges is 53·75°. We therefore see that the dispersion between the two is 6·75°. Obviously, then, the refraction for rays of the colours between red and blue will lie between these two figures.

In practice, the dispersion of various substances is not measured by an expression which depends on the refraction of a prism, but they are all measured with reference to a selected ray, and for this purpose the bisection of the sodium D lines in the spectrum are chosen. (For an explanation of the special lines, see Watson's "Text-book of Physics.") (1)

Thus, for crown glass the refractive index of the bisection of lines, D $= 1{\cdot}5178 = \mu$, F $= 1{\cdot}52395 = \mu'$.

Line C $= 1{\cdot}51535 = \mu''$ then the dispersive power ω is given by the expression :—

$$\omega = \frac{\mu' - \mu''}{\mu - 1} = \frac{1{\cdot}52395 - 1{\cdot}51535}{1{\cdot}5179 - 1} = \frac{0{\cdot}0086}{0{\cdot}5179} = 0{\cdot}0166.$$

The dispersion of flint glass is given in the same way and comes to 0·0339, so that the dispersion for flint glass is approximately twice that for crown glass.

The Lens

We have now seen how a ray behaves when passing through a prism, supposing we take two similar prisms and place them base to base, Fig. 7, and let four parallel rays of white light A, A1, B and B1 pass through the prisms, as shown. The effect of this combination of prisms is firstly to disperse the rays, and, for purposes of illustration, we will only consider the red and violet.

The red ray leaves the glass at the points O, P, Q and S, and it will be seen how, due to their refraction, they are brought to a

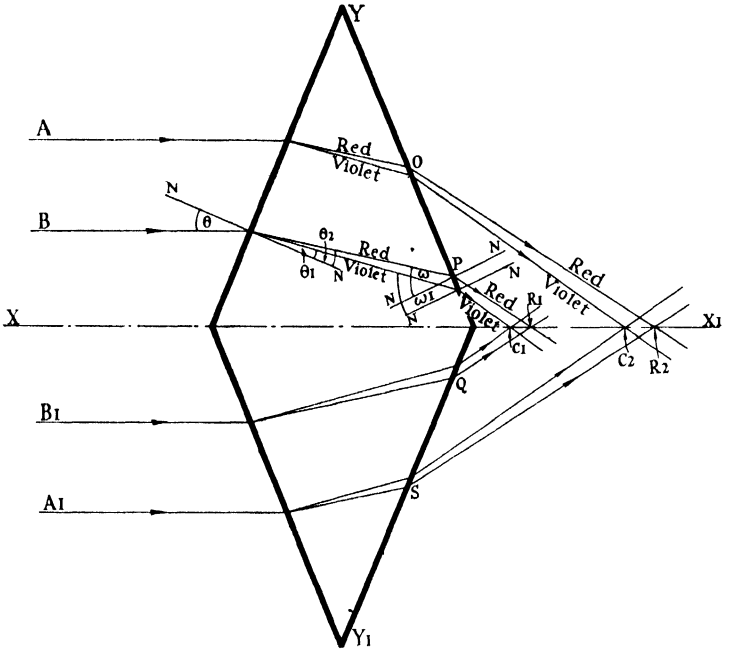


FIG. 7.

focus; thus the red ray from B meets the red ray from B1 at the point R1 on the axis, and similarly the red ray from A meets that from A1 at the point R2 on the axis. These two points are the focal points of the rays concerned.

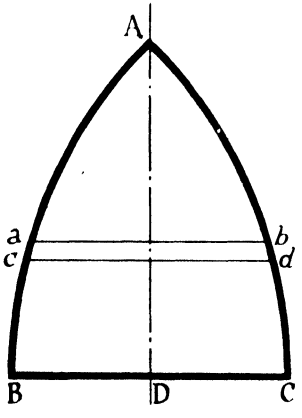


FIG. 8.

In a like manner the violet rays are brought to focal points at C1 and C2, but it must be noted that these focal points do not coincide and, in fact, they cannot coincide. This dispersion is very important as it is one of the faults of lenses which have to be corrected.

It will be seen that the points R1 and R2 can never be made to coincide so long as we use parallel light.

Supposing that instead of a prism of triangular section and fixed refractive angle, we had one whose refractive angle was continuously variable from base to apex, the sides of the prism would then constitute an arc of a circle when seen in section, as shown in Fig. 8, where we have a section similar to that of a lens.

It is only necessary to imagine the surfaces AB and AC to be spherical to produce a lens. This will serve to show how a lens may be considered to be developed from two prisms. To further illustrate the point, if we imagine the section ABC to be made up of a larger number of small elements such as *abcd*, then one can appreciate how the whole section may be looked upon as being composed of a large number of such portions of prisms as *abcd*, whose refracting angles increase from D to A.

We have seen how parallel rays may be brought to meet at a point on the axis of two prisms placed base to base. Those rays which are nearer the base meet at a point nearer to the prisms than those which are further from the base or axis in the first instance. Now let us see how parallel rays behave when passing through a

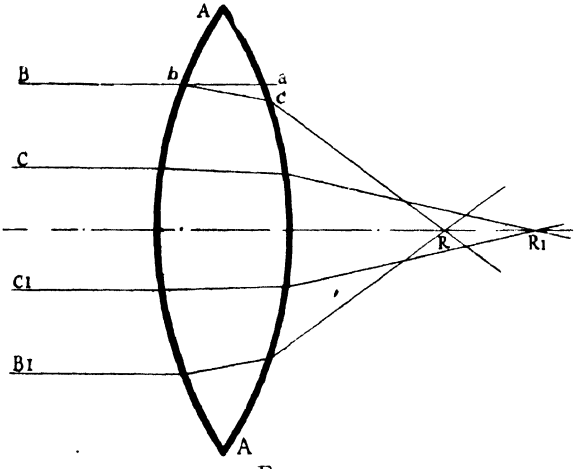


FIG. 9.

lens. Fig. 9 illustrates a lens AA through which parallel rays are passing B.B1, C.C1.

These rays are converged to points R and R1 on the axis, in this case the peripheral rays are converged to a point nearer the lens than the innermost rays. It will be seen that this is the opposite of the effect with two prisms base to base, the reason for this is that the lens has a continuously varying refractive angle, increasing from the centre to the periphery. Thus, if we imagine a ray to pass through the lens coincident with the axis, then it would not suffer any refraction at all but would pass straight through the lens, as it would also be coincident with the normals to the two surfaces of the lens, at the points of entry and exit. If, on the other hand, the rays were to move towards A while still keeping parallel to the axis, it would virtually pass through the elementary prisms described in Fig. 8, and as these will refract the ray more and more as it approaches A, it is clear that the point at which it cuts the axis will get closer and closer to the lens.

This phenomenon is known as spherical aberration and is co-existent with the aberration due to the colour dispersion, called chromatic aberration ; the one causing variations of focus, the other causing colour fringes to appear around an object viewed. Fortunately, these faults can be corrected by means to be described later, but for the present let us consider again the lens in Fig. 9, which is relatively thick. If we increase the radius of curvature so that the lens get thinner, it will be seen that the points of entry and exit of a ray will be brought nearer and at the same time the rate of increase of the refracting angle will become less, which would tend to reduce the angle *abc* so that *ab* approaches *bc* ; when this occurs obviously the lens ceases to exist as such and behaves as a parallel-sided slab.

The net result of this flattening process would be to bring the points R and R1 closer to one another and at the same time move them further away from the lens. With a very thin lens, the difference between these two points is undetectable by the human

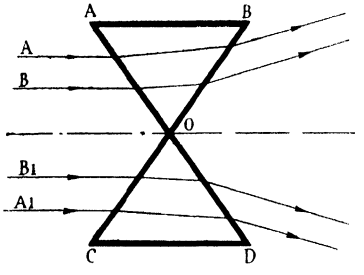


FIG. 10.

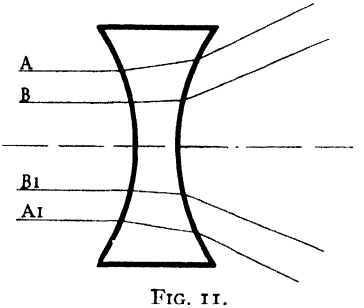


FIG. 11.

eye and may be looked upon as a point of focus where all the rays coincide ; therefore for the present, while discussing lenses, we will consider them of such a thickness that the aberrations may be ignored.

Now let us examine the effect on parallel rays of light when they pass through two prisms placed with their apices together instead of base to base.

Fig. 10 shows two similar prisms so positioned, two rays AA1 and BB1 passing through them. From what we have just seen they will be refracted on their way through the glass, towards the bases of the prisms, being subjected to a further refraction in the same direction on re-entering the air, the net result is to cause the rays to diverge from the axis.

We may build a lens on this arrangement of prisms in exactly the same manner as with those placed base to base, but in this case the surfaces of the lens will be concave and not convex, as in the former case, thus the refractive angle increases from the centre outwards. Fig. 11 shows such a lens called a " diverging lens " as

against the name " converging lens," used for those whose surfaces are convex. The nomenclature is self-explanatory.

In Fig. 11 we see the behaviour of parallel light with a diverging lens, but here we have a slight, but important, modification of the similar case with two prisms, inasmuch as the diverging rays are not parallel to each other, but diverge one from the other, as in Fig. 7. The reason for this is clear when it is realised that the peripheral rays undergo greater refraction than the more central rays.

So far we have only dealt with the case of incident parallel light, so now let us examine the conditions existing when the incident light is at an angle to the face of a prism, that is to say, not parallel to the base. Let us take a prism (Fig. 12) and presume that a ray of light proceeding from some point on the axis (as we are only dealing with one prism, the axis will obviously be the base line) as

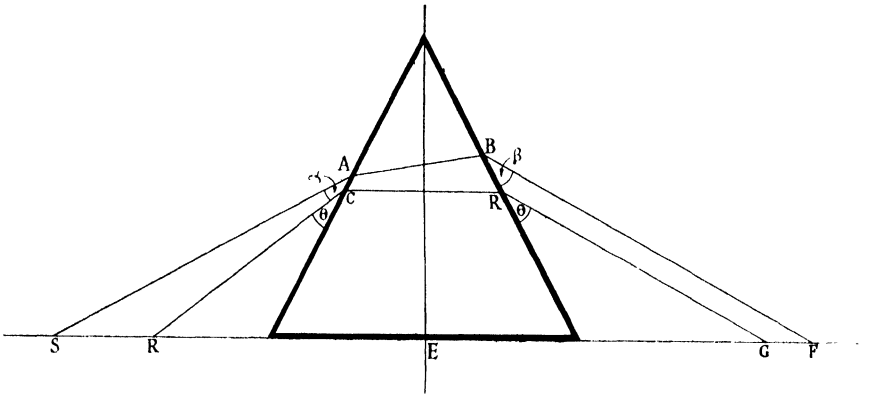


FIG. 12.

shown at SA striking its side at an angle α with the normal, and being refracted through the glass to emerge into the air at B, where it is further refracted to the angle β, thence proceeding to meet the axis at F. It will be seen from this that a ray which proceeds from a point on the axis is so refracted that it is brought back to a point on the axis, and it will also be seen that if this ray strikes the face of the prism at an angle such that the exit angle is equal to it then the distance of the point of origin from the centre of the prism E will be equal to the distance of the point where the exit ray meets the axis, from E.

Thus in Fig. 12, when the angle of incidence θ of another ray such as RC is equal to that of its emergent ray RG then RE will be equal to EG. It can be shown that when this condition exists the angle of deviation is a minimum and is called " the angle of minimum deviation."

Imagine that we have a lens, Fig. 13, with a point source of light S on the axis and at such a distance from the centre line YY that the

angle of deviation is at a minimum, then these rays will be brought to a focus at a point F on the axis, which is the same distance from YY to S. This would produce an image of S at F which would be

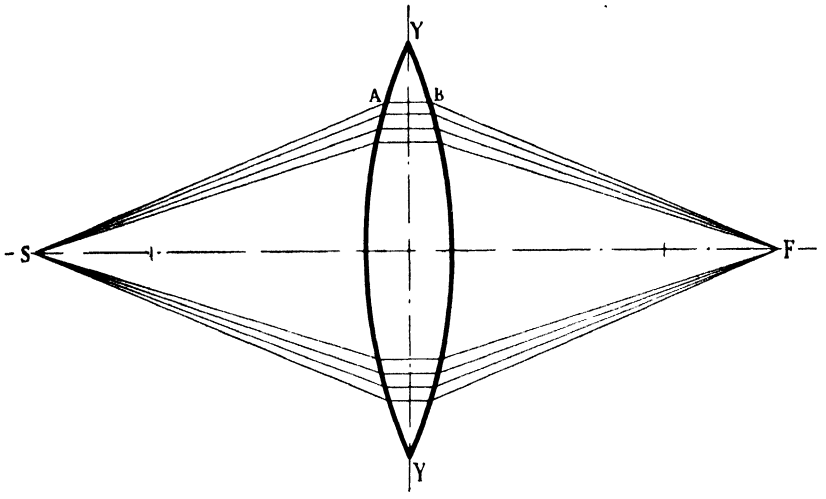


FIG. 13.

the same size as S, but we will examine this condition in greater detail subsequently.

For the moment let us re-examine the case of parallel light, incident on a converging lens; this is illustrated in Fig. 14. Here it is shown how the parallel rays are made to converge to a point on the axis F, at a definite distance from the lens f; this point is

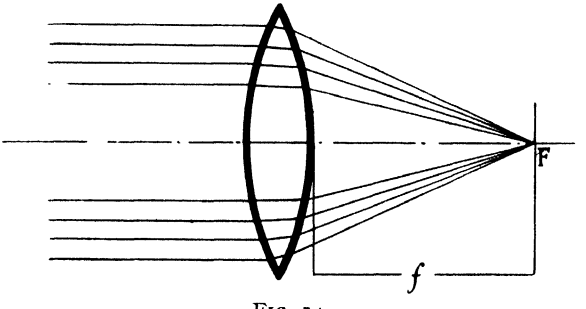


FIG. 14.

known as the principal focus of the lens, and the distance is called the focal length of the lens, and in the case of thick lenses, is taken from the optical centre.

This focus is what is known as a real focus, that is to say, if the rays of light from a distant object, such as the sun, which may be regarded as parallel for all practical purposes, were made to converge by a lens, they would meet at the principal focus of the lens and produce an image of the source capable of being received on a

screen. This is a simple experiment which may be performed by anyone without a great deal of trouble.

Thus we have for a converging lens two equidistant points on either side which are the principal foci, so that if a point source of light were placed at either of these points, then the emergent rays

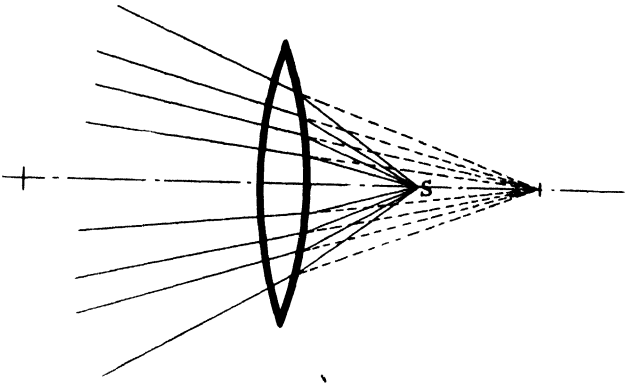


FIG. 15.

would be parallel ; but suppose the source were to be moved further away from the lens, then the outgoing rays would cease to be parallel and would converge to a point on the axis and, as the source moved further from the lens, so would the point of convergence of the emergent rays approach the lens. This point of convergence is known as the conjugate focus of the point of origin. Thus it will be seen how as the source moved away the conjugate focus would approach the lens until a point would be reached when the source and its conjugate focus would be equidistant from the lens. Further movement of the source would bring the conjugate focus nearer the lens until the point of principal focus was reached, when the source would be so far away that its light could be considered to be parallel.

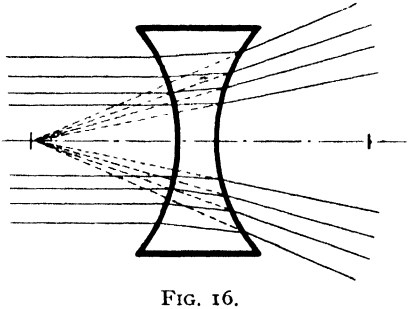


FIG. 16.

It can be shown that when the conjugate foci are equidistant from the lens they are then separated by four times the focal length of the lens in question.

Conversely, it will be seen that the nearer the source is to the principal focus, the further the conjugate focus, and when the source is at the principal focus the conjugate focus is at infinity and the emergent rays are parallel. If the source is moved to a point within the principal focus, then the emerging rays diverge as shown in Fig. 15.

If these diverging rays are projected backwards as shown, they will be seen to meet at a point on the axis coincident with the principal focus. This is known as a virtual focus and cannot be received on a screen. In the same way it may be shown that the principal focus of a diverging lens is virtual, Fig. 16.

The Optical Centre

Consider a ray R, R1, R2, R3 passing through a lens as shown in Fig. 17. Here we have a condition such that after refraction through the lens, the emerging ray is parallel to the incident ray and it is obvious that the lens is now behaving as if it had parallel sides. If we construct another such ray, A, A1, A2, A3, on the other side of the lens, then the point at which they intersect is called the " optical centre " of the lens.

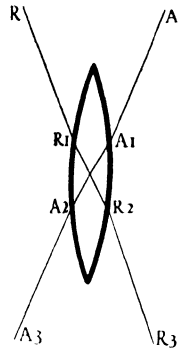


FIG. 17.

The effect of this particular condition is to merely displace the ray slightly in a lateral direction, and it can be shown that all rays whose angle of incidence is such that the emerging ray is parallel to the incident ray pass through the optical centre. It must be borne in mind that the optical centre need not necessarily be the same as the physical centre, but it does coincide in the case where the curvature of the lens is the same on both sides. It will also be seen that when the lens is thin, the lateral displacement will be very small so that the ray may be looked upon as virtually passing straight through the lens ; therefore, while dealing with thin lenses, we will look upon rays which pass through the optical centre as being undeviated.

Types of Lenses

We have so far dealt with the effects produced on rays of light, by lenses of two basic sections, viz. : the convergent lens, which

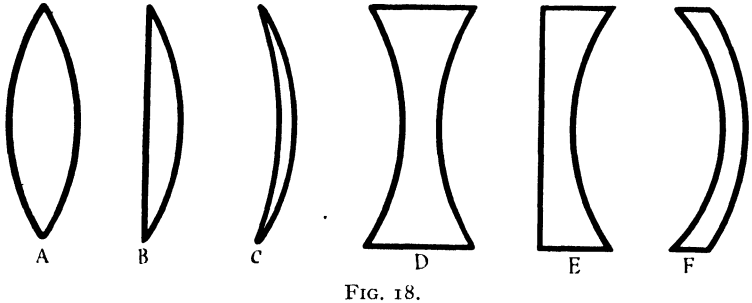


FIG. 18.

may be looked upon as having been developed from two prisms placed base to base, and the divergent lens developed from two prisms placed with their apices together. From these there are

developed six types of lenses, three of the converging types and three of the diverging shown in Fig. 18.

A, B and C are the converging lenses. A is known as bi-convex, B as plano convex, C as converging meniscus; of the diverging types, D, E and F, D is the bi-concave, E the plano concave and F the diverging meniscus.

Spherical Aberration

It has been shown how, in the case of a lens receiving parallel incident rays, those nearer the axis are brought to a focus further along it than is the case with the rays at the periphery of the lens. From this it will be obvious that an image produced under such conditions would appear to have a curved surface, of which either the centre or periphery would be out of focus. This characteristic is known as spherical aberration and is due to the shape of the lens, being least when the lens is bi-convex with the radii of curvature in the ratio of 6 : 1. For those who wish to go into the question in greater detail, an excellent account may be found in " The Microscope " by Carpenter and Dallinger, Vol. I. (2) Spherical aberration is a serious fault, which has to be corrected in order that the images viewed by lenses may be as perfect as possible.

Chromatic Aberration

We have seen that, due to differing degrees of refraction, white light is decomposed during its passage through a lens, with the result that the red rays are refracted less than the violet, in consequence of which they are brought to a focus further along the axis than the violet rays. This fault is called chromatic aberration, and gives rise to colour fringes in an image produced, resulting in a great depreciation of the quality of the image.

If the lens is relatively thin and of large diameter these colour fringes are very much more pronounced in the peripheral portions; for it will be seen that the rays nearer the centre suffer less refraction and consequently less dispersion. There are methods of overcoming the defect, one of which consists of limiting the effective diameter of the lens to a comparatively small portion in the centre by means of an opaque disc with a hole in the centre, which is called a stop, and by means of which a fairly large proportion of the colour produced in an image may be eliminated, albeit the quantity of light passing through the lens is reduced.

Obviously then, the neutralisation of this aberration in an instrument such as the microscope is of prime importance, as the multiple foci produced as a result of chromatic aberration make a perfect image impossible.

Let us re-consider the dispersion of a beam of white light in its passage through a prism. Fig. 6 shows how the red rays are caused

to cut the base line further from the prism than do the violet rays. This also applies to a lens illustrated in Fig. 19, which shows a ray of white light refracted through a lens AB.

The violet rays are brought to a focus at the point F1 inside the point of mean focus F, this being the mean point of the colour foci from violet to red, which latter are brought to a focus at F2. Thus it will be seen that if the image is focussed at the point F1, then the violet will predominate in the colour fringe, while at F2 red will be the dominant colour in the spectral fringe and for any intermediate point, the colour whose particular focus is chosen will dominate the fringe. It is clear that F1 and F2 are the two extreme colours of the visible spectrum and hence are the limiting points of

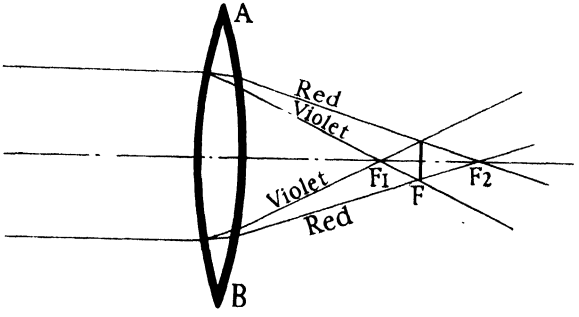


FIG. 19.

the aberration. This is called " the longitudinal chromatic aberration " of the lens.

If we examine the phenomenon of dispersion, we see that it is dependent on two factors :—

(1) The refractive index of the material in question and (2) the refractive angle or angles according to whether a prism or lens is used. For the time being let us consider the case of a prism. If, for example, we take two prisms of the same refracting angle, but let the dispersion of one be half that of the other, then the separation between the red and violet rays due to the one will only be half that of the other. In the same way, if their refractive indices were the same, but the refractive angle of one only half that of the other, then the dispersion due to the one would only be half that of the other.

Imagine two similar prisms placed near each other in the manner shown in Fig. 20, then a ray of white light would be split up on entering the prism A, the emergent beam being dispersed, which in entering the second prism B, is reconstituted due to refraction taking place in the opposite direction to that in prism A, and the beam emerges from prism B in its original form as white light. This would suggest a method of correcting chromatic aberration in lenses, but unfortunately cannot be used as such as it will be observed that refraction has been neutralised as well as dispersion.

The emerging ray from prism B is parallel to the ray incident on prism A ; as all lenses depend upon refraction for their function-

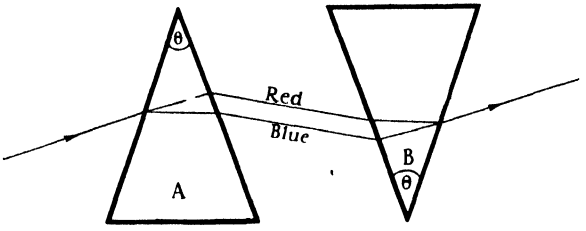


FIG. 20.

ing, a method must be used to procure the desired condition where refraction is not destroyed.

Suppose we have two prisms, one of which (A, Fig. 21) is made of crown glass and the other, B, of flint glass, and whose refractive angles are such that the dispersion is the same in each case, then a

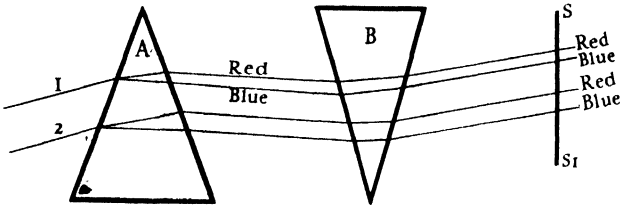


FIG. 21.

ray of white light (1) on traversing the crown prism will be refracted and dispersed downwards. The red ray being less refracted than the violet, these emergent rays on traversing the flint prism will be refracted and dispersed upwards. The dispersion of the two prisms being the same, the rays, when they leave the flint glass, will be parallel to each other, but the mean refraction of the crown prism

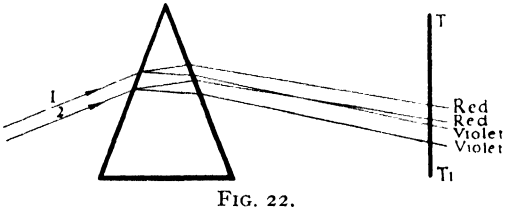


FIG. 22.

is greater than that of the flint prism and the whole of the rays will finally be refracted towards the base of the crown prism A. Let us take another ray, 2, parallel to ray 1, then the emergent rays from B due to ray 2 will be parallel to those due to ray 1, and at a point SS1 the image as seen on a screen would consist of two coloured margins, violet to red at the top and red to violet at the bottom. On the other hand, in the case of a single prism as shown in Fig. 22, the

emergent rays diverge from one another and the image at the point TT1 would consist of a central white portion with broad coloured margins, red above and violet below. The further away TT1 is, the broader will the margins become, while with the two prisms in Fig. 21, the margins will be the same width whatever the distance of SS1.

Therefore, it will be seen that by using two prisms in the above manner, it is possible to produce a combination which will refract light and yet not disperse it; such a combination of prisms is said to be achromatic. This is the principle applied to the correction of chromatic aberration in lenses, but it is not quite so simple as it sounds because the refraction of all colours in the spectrum between any two given materials are not proportional. We will consider this point in greater detail later.

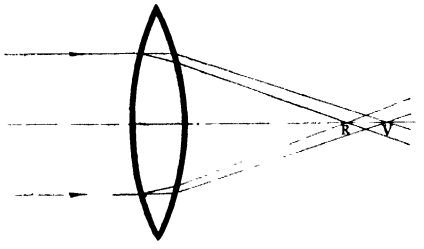


FIG. 23.

The reason for the existence of chromatic aberration has already been explained; now let us examine the problem of correcting it. This is done in the case of lenses in much the same way as described in prisms; it is by building up a compound lens one component of which is composed of flint glass and the other crown.

The spherical lens thus achromatised being a convex of crown and a concave of flint glass, Fig. 23 shows that the chromatic aberration of a bi-convex lens is such that the violet rays are brought to a focus at a point on the axis nearer the lens than the red rays when white light is incident upon it.

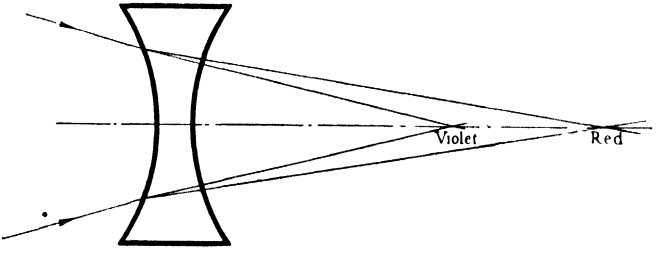


FIG. 24.

With a converging beam incident upon a bi-concave lens (Fig. 24), the violet rays are brought to a focus further from the lens than the red and it is clear that if we combine these two types of lens, some sort of a result will be achieved, in an effort to eliminate chromatic aberration, but it is abundantly clear that if the two lenses are made of the same material, then the refraction would also be destroyed and the resultant combination would behave as

a plain slab ; on the other hand, if the bi-convex element were of crown and the bi-concave element of flint, then it would be possible to so choose their focal lengths that when they are combined the dispersion for the red and violet of one is neutralised by an equal and opposite dispersion in the other, but refraction would not be destroyed, as that of the crown component is greater than that of the flint lens, the combination producing a lens of different focal length from either of its components but free from colour so far as the red and violet are concerned. Thus it is possible to compute a lens corrected for any two colours ; the combination described above would not be achromatised for another colour.

In order to clarify this latter point, let us consider the dispersion of white light. It has been stated that when light is dispersed by a lens, as in Fig. 23, the colours between red and violet lie in their respective spectral order, but suppose we select one, say yellow, which lies approximately midway between red and violet and consider the two prisms in Fig. 21, assuming that the yellow lies midway between the red and the violet in prism A and likewise for prism B, then obviously its dispersion will be neutralised at the same time as that for the red and violet ; that is to say that when the dispersion between any three colours in one medium is proportional to their dispersion in another medium, then, when any two are neutralised the other is neutralised with them.

This is the ideal condition, but it unfortunately does not exist in practice, as there are no two glasses with proportional dispersions. Actually what happens is as follows : the yellow may lie midway between the red and violet for one kind of glass, but in the other it might be much nearer the red ; say, for sake of argument, it lies one-third of the distance between the red and violet from the red, then if the yellow and red were neutralised, the violet would be residual

This disproportionate dispersion in two media is known as the " irrationality of the spectrum," and the residual colours left outstanding in a corrected lens are known as the secondary spectrum. Thus we see that by using two lenses of different materials it is possible to correct for any two colours and by the use of three lenses, whose materials have different dispersive powers, we can correct for three colours in which case the residual colours are known as the tertiary spectrum.

It is desirable to bear this in mind, as the correction for three colours is the basis of the apochromatic objective in which the secondary spectrum has been eliminated.

The use of achromatised lenses was one of the great factors influencing the development of microscopy as an exact science. It was this discovery which brought to light a discrepancy in the performance of these lenses when used with a cover glass ; that is to say, when the object being viewed was covered by a thin piece of glass.

Andrew Ross discovered that the cover glass introduced aberrations into the lenses which greatly affected the quality of the image. Thus, an objective which behaves very well on uncovered objects was adversely affected when used with a cover glass. This is very interesting as it shows how spherical aberration affects the image and led to the development of the aplanatic combination.

The effect of a cover glass is shown in Fig. 25.

It will be seen that the rays of light diverge from the object O,

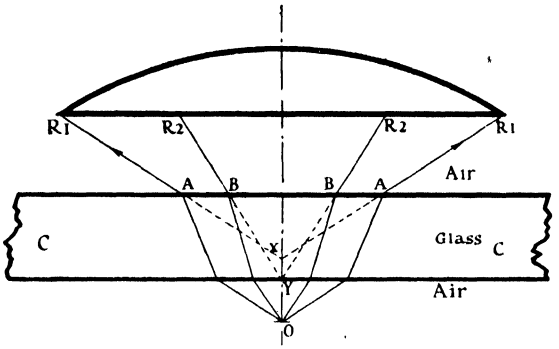


FIG. 25.

two of which, R1 and R2, have been selected to illustrate our point. R1 is refracted through the cover glass CC to a point of emergence A, from whence it proceeds in a direction parallel to the original until it enters the lens. The same happens to the ray R2.

Now, if the portions of the rays between the upper face of the cover glass and the lens are produced back, the ray R1 is focussed at the point X and R2 at Y; thus we see that the portion of the

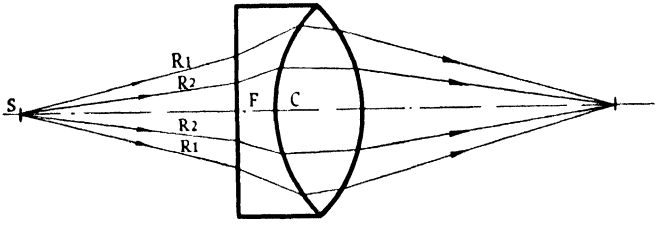


FIG. 26.

object which emitted R1 will appear to be at X and that which emitted R2 will appear at Y, giving two separate foci, and thus detracting from the quality of the image.

If there were no cover glass, all the rays would appear from O (which is what we want) and the objective would have to be corrected to bring all the inner and outer rays to a coincident focus. It would then be " aplanatic " or corrected for spherical aberration; such a system is illustrated in Fig. 26. The rays R1 and R2 are proceeding from a point source S, through the compound lens FC, the bi-convex

component of which is crown glass, the plano-concave component being made of flint glass. The relative curvatures are chosen so that the spherical aberration is corrected, and, of course, the system is at the same time achromatic, the rays being brought to a coincident conjugate focus. Such a lens would perform at its best when used with an uncovered object.

Obviously some sort of compensation is necessary when a cover glass is used; this is achieved by so altering the curvature of the crown lens that the flint lens does not completely neutralise the aberrations. Fig. 27 shows what is called a condition of under-correction, obtained by shortening the radius of curvature of the external face of the crown lens.

Diverging rays from a point source S, outside the principal focus, are brought to foci, such that the focus F2 of the peripheral ray R lies within the focus F1 of the more central ray R1. This is in opposition to the condition set up by the presence of a cover glass, so that if the correct values are chosen for any given thickness of cover, the aberration can be cancelled. It must be emphasised

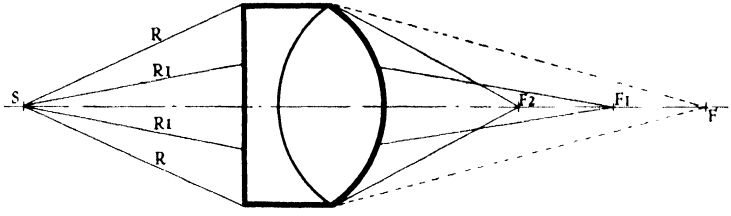


FIG. 27.

that a system of lenses can be corrected for only one thickness of cover glass in this manner, but a more or less universal adjustment is secured by causing the back lens of the system to be movable, so that it may be made to approach or recede from the front of the system. In this way one may pass from a condition of under-correction, through a point of aplanatism to a condition of over-correction, as shown in Fig. 27 at F, where the focus of the peripheral rays is outside that of the more central rays.

Thus we have a method of compensating for varying thicknesses of cover glass, and this is carried out quite simply in the microscope by altering the length of the draw tube in the required direction. This will be described in greater detail subsequently.

Image Formation

Up to the present we have dealt with the elementary principles underlying the functioning of simple lenses, and the expedients adopted to correct their two greatest faults, viz., chromatic and spherical aberration, in the examination of which we have studied the effects produced by bundles of parallel rays and divergent rays

proceeding from a point source of light on the axis of the system under consideration. We have assumed that the lenses were thin enough for us to ignore any deviation to rays passing through the optical centre.

Let us now consider the type of illumination supplied by an area such as a square of white card. The surface of such an object would emit rays of light at all angles to its surface and it may therefore be looked upon as an aggregate of an infinite number of point sources occupying the area of the surface.

If we take a portion of this card and place it at some point outside the principal focus of an achromatised and aplanatised system, then obviously each of our point sources would produce a conjugate focal point somewhere outside the principal focus on the other side of the lens system. Since the production of a focal point in this manner produces an image, it will be seen that each of the point sources would give rise to an image of itself at the conjugate

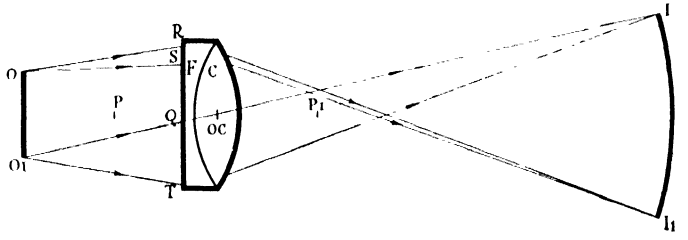


FIG. 28.

focus. In this manner an image of the piece of card would be formed.

It has been pointed out that if an illuminant were placed on the axis, outside the principal focus, the rays are converging to a conjugate focus on the axis on emerging from the lens, this focus being known as a real focus, whereas when the source is placed within the principal focus, the rays are divergent and produce a virtual focus (see Figs. 13, 14 and 15). When considering a multitude of point sources constituting the illuminant, the same principles apply, but instead of obtaining a point on the axis at the conjugate focus, we obtain an area known as an image ; consequently it will be seen that there are two kinds of images, a real image, capable of reception on a screen and a virtual image.

A real image is produced when an object is placed outside the principal focus of a system of lenses, the image produced by cinema projectors, cameras, etc., are real images. The manner in which this takes place is shown in Fig. 28, where OO1 is a portion of our white card in section. FC is a corrected system whose principal foci are P and P1.

Let us consider firstly two rays, OR and OS, proceeding from

the point O ; these will be refracted by the system and the emergent rays will meet at a conjugate focus at I1. It must be borne in mind that rays of light will radiate from a point, such as the one under discussion, in all possible directions, two of these rays being selected for our purpose which pass through the lens. There will be many others which miss the lens entirely.

In the same way we may take another ray, O1T, proceeding from the point O1, through the lens to a focal point I ; another ray O1Q would proceed through the optical centre to meet IT at I, hence an image of the point O1 would be produced at I and an image of the point O be produced at I1. In this manner conjugate foci of all our points composing the object would produce an image of the object at III1 along the curve shown. It will be noticed that if the object were curved so that it was concave towards the lens, then the curve of the image would be shallower, and *vice versa*. If a

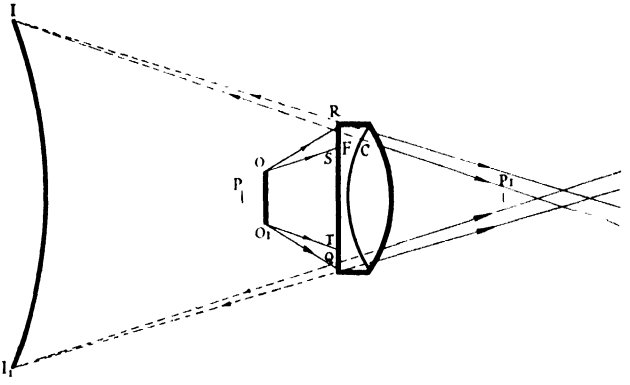


FIG. 29.

screen having the same curvature as III1 were placed to coincide with the curve of the image, an enlarged image of the object OO1 would be seen on it, the enlargement depending on how far away from the lens the object is situated, so that when the object and screen are equidistant on either side of the lens, the image is the same size as the object. If, on the other hand, a flat screen is used and placed so as to coincide with the points III1, then the periphery of the image only would be in focus ; if the screen was moved to the points where the curve cuts the axis, the centre of the image would be focussed. It will also be appreciated that the image produced is inverted.

It has been stated that a virtual image is produced if the object is placed inside the principal focus. This is illustrated in Fig. 29, where we have the same lens system FC, with the object well within the principal focus P ; the effect of this is to cause the emerging rays resulting from OR, OS, O1T and O1Q to be divergent ; that is to say, on emerging from the system the ray OR diverges from OS and O1T

from O1Q. Due to this there are of course no conjugate foci of the points O and O1 on the right-hand side of the system, but there are virtual conjugate foci at the points I and I1 on the left-hand side of the lens, and a virtual image along the curve shown. The image thus formed is not capable of being received on a screen, but if the emergent rays are allowed to enter the human eye, they will be converged by the lens of the eye and form a real image on the retina. In this way the virtual image is made visible and the object appears to lie at III and is of course seen as an enlarged picture of the original. It will be as well to point out that in all optical instruments the eye is the final component in a system and must be regarded as such.

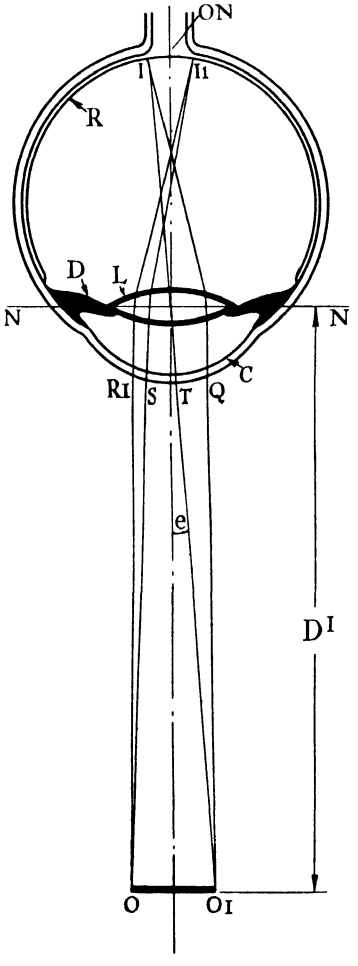


FIG. 30.

Those portions of the structure of the eye which interest us are shown in Fig. 30, which illustrates the method by which an object is seen by the production of a real image on the retina. This section is diagrammatic and the eye is represented as being spherical, whereas in reality it is slightly flattened from front to back, somewhat like an orange. R is the sensitive screen or nerve layer, the retina; from which the optic nerve ON carries the impulses to the brain, L is the bi-convex crystalline lens. This is held in a highly elastic capsule which, when subjected to the pull of muscles attached to it, can either flatten the lens or squeeze it up so that its curvatures are either diminished or increased, so altering the focal length. Thus the eye is equipped with a variable focus lens. The iris, D, serves as a limiting stop in the same way as the diaphragm of a camera, it closes or opens automatically according to the intensity of light entering the eye. The cornea C is the clear membrane protecting the delicate mechanism of the interior of the eye; the space between the cornea and the lens is filled with a clear aqueous fluid called the "aqueous humour," and filling the cavity behind the lens is a clear glassy jelly known as the "vitreous humour." Both these substances are composed mainly of water with a little more than 1 per cent.

solid matter ; the line NN passes through the optical centre of the lens.

Now, suppose we have an object OO1 (the same piece of white card will do) placed at a distance D1 from the optical centre NN, then, if we take two rays proceeding from the point O, such as OR1 and OS, they will be refracted by the lens of the eye and brought to a focus at the point I1 on the retina ; similarly, two other rays, O1T and O1Q, will be brought to a focus at the point I on the retina, in this way a retinal image, II1, of the object is produced. It will be seen that the ray O1T passes through the optical centre of the lens, making an angle θ with the axis. Obviously 2θ will be the angle subtended by the object OO1. This angle ($\theta \times 2$) is known as the visual angle, and it will be seen that to increase the visual angle, the object must be brought nearer to the eye, at the same time the retinal image is increased in size ; thus the size of the retinal image is dependent on the distance of the object and the visual angle and is represented by :—

$$\frac{OO1}{D1} \text{ which is proportional to Tan. } \theta$$

Thus, it will be appreciated that in order to see the object as large as possible, it must be brought as near to the eye as possible compatible with distinct vision. The optimum distance, D1 for distinct vision, is now conventionally fixed at 10 in., or 250 mm., and is called the near point. Those who wish to pursue further the study of the optics of the human eye are referred to any of the standard works. The subject is very well dealt with in Thornton's " Human Physiology," Stage 2.

Suppose we take the eye represented in Fig. 30 and let it function in conjunction with the lens combination of Fig. 29, with the same object placed within the principal focus. We have seen that the image created by this set of circumstances is a virtual image, but Fig. 31 shows how the eye will bring the divergent rays OR, OS, O1T and O1Q to a focus, giving a real image on the retina. It will be seen also that this retinal image subtends a very much larger angle than is the case with the object viewed without the aid of the lens (Fig. 30).

It is clear that the size of the retinal image has been increased hence the apparent size of the object has also undergone enlargement, this latter now appearing to be at V1V11 (Fig. 31). It is clear from this that the lens has magnified the object by virtually decreasing D or bringing the near point closer to the eye. In this way the distance of the near point D may be decreased to values very much smaller than the conventional 250 mm. The use of the lens in this manner is the working principle of the simple microscope and, in fact, such lenses may be referred to as simple microscopes when suitably mounted to give ease of manipulation.

It is clear that the ratio of the size of an object as seen with the naked eye at 250 mm., to the apparent size of the same object

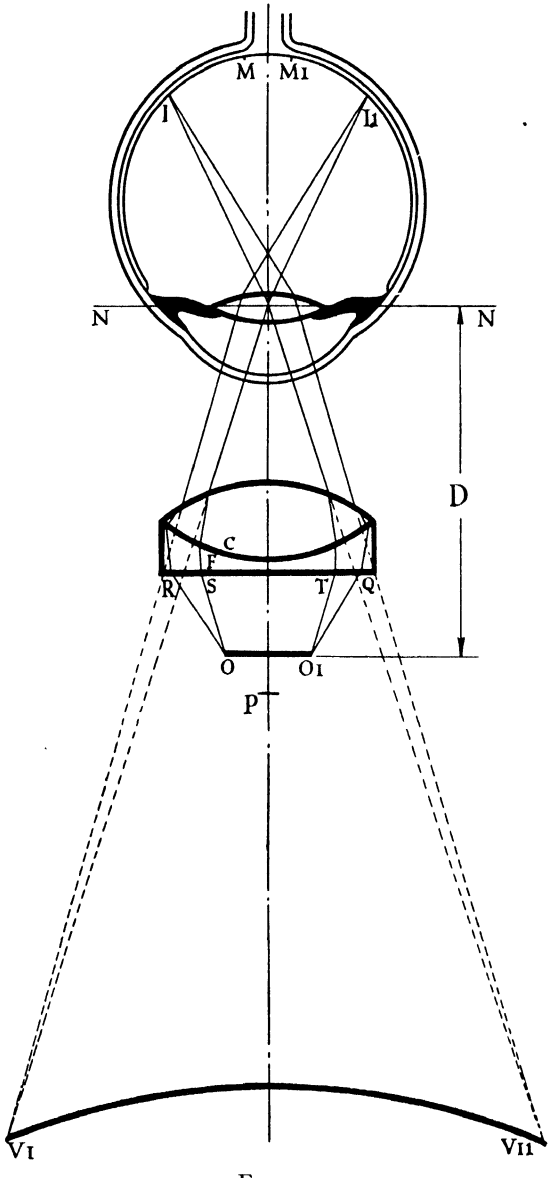


FIG. 31.

when viewed through a lens, will give the magnifying power of the lens thus :—

The magnification of a simple microscope =

$$\frac{\text{The visual angle of the image seen with the lens}}{\text{The visual angle of the image seen with the naked eye at 250 mm.}} = D/f$$

Where D is the distance of the near point and f is the focal length of the lens, so that a lens of 1 in. focal length will give a magnification of 10 diameters, when the resultant image is 10 in. from the eye. Thus, if the focal length of a lens is known, the magnification is obtained by dividing this figure into D. The magnification of the lens shown in Fig. 31 is, of course, given by the ratio of the size of the retinal image II_1 to that of the unaided retinal image MM_1, represented thus :—

$$\text{Magnification} = \frac{II_1}{MM_1}.$$

REFERENCES

(1) WATSON. "A Text-book of Physics." Longmans.
(2) CARPENTER and DALLINGER. "The Microscope and its Accessories." Churchill.

CHAPTER II

THE COMPOUND MICROSCOPE

HAVING examined the principles of the simple microscope and the way in which magnification is produced, we are now in a position to study the arrangement of the compound microscope.

As distinct from the simple microscope, the compound microscope consists of more than one system of lenses. Fig. 32 shows a ray diagram for such an instrument ; as can be seen, the optical system consists of the following train of lenses :—

(1) Condenser, C.
(2) Objective, B.
(3) Occular, A.
(4) The eye.

The object, O, lies between the condenser and objective and is viewed by the objective. Thus we have parallel rays, R1 and R2, entering the condenser, as shown, which are brought to a focus at the plane of the object O, where a real image of the light source is produced. After passing through the transparent portions of the object, the rays which are diverging enter the objective, this latter being a system of corrected combinations, by which they are converged to a focus, producing a real image at I1 in the absence of any following lens.

Up to this point the system is functioning as a simple microscope, giving a certain magnification dependent on the focal length of the objective. Now, if this image is viewed through another lens, 1 (the eye lens), a further magnification will be produced. This method, where the image produced by one lens is viewed and supermagnified by another lens, constitutes the principle of the compound microscope. The resulting image is virtual and is seen at the near point, which is considered to be at 10 in. from the eye.

With the above arrangement, however, only a small fraction of the image I1 will be accepted by the eye lens, so it is usual to interpose another lens, 2, called the field lens, between the objective and the eye lens, the function of which is to bend inwards the rays emerging from the objective so that the primary image is produced at I2 instead of I1, thus reducing the size of I1 somewhat and shortening the focus.

In this manner the rays from the primary image which would have missed the eye lens in the first instance, now pass through it and in consequence the whole of the primary image is seen instead of just a small portion. The rays entering the eye lens, 1, are diverging and continue to do so on leaving it, although the divergence is

The train illustrated in Fig. 32 commences with a condenser system, C, comprising two lenses, 5 and 6. This is an important and indispensable component, if the instrument is to be used at its maximum efficiency. Although an image is produced without any condenser at all, it is very poor in quality as compared with one with which a condenser is used; in fact, it has been shown by many workers that a condenser is essential if critical work is to be carried out. Very broadly, the reason is that any one objective is capable of handling a limited cone of light and if the condenser is omitted the light entering the objective is to all intents and purposes parallel, thereby cutting down the number of rays proceeding from the object to the objective and so seriously detracting from the quality of the image. If, on the other hand, a condenser is used and an image of the light source focussed on the object, the light entering the objective will be in the form of a cone, and for the same area of object, many more rays would enter the objective. Of course, the condenser must be capable of supplying a cone of light equal to that which the objective will accept, then the objective will produce the best image within its capabilities provided that the tube length is correct and it is not over eyepieced. However, this and other points will be discussed in detail subsequently; for the present it is sufficient to point out the necessity for a condenser.

In summarising the above remarks, we see that the complete arrangement consists of an optical train, comprising light source, condenser, object, objective, eyepiece, and the eye. The object is placed between the condenser and objective, which produces a primary image (real), this being viewed through the eyepiece, so producing a superamplified final image (virtual). The object and eyepiece are capable of being moved to and from the object as a unit; they are also capable of an independent movement to and from each other. The former in order to focus the viewing system and the latter to achieve a certain amount of correction.

As efficient working demands an image of the light source in the place of the object, the condenser has a similar movement to and from the object.

Having studied the general principles, we are in a position to proceed to an examination of the various components comprising the complete instrument.

CHAPTER III

THE EYEPIECE

WE have now seen why the eyepiece is necessary to the microscope and why it is used as an integral part of the optical system.

Its chief functions are :—

(1) The formation of a virtual image of the real image produced by the objective.

(2) The formation of a magnified real image of the primary image in cases where projection of the final image is desired, as for example, in photomicrography.

(3) The formation of an image of crosshairs, scales, etc., placed within the eyepiece, so that it is superimposed on the final image, used for measurement, etc.

It has been pointed out that the objective is focussed to produce a real image at the focal point of the eye lens, and also that the field lens, while built into the eyepiece, should be considered as a component part of the objective.

The eyepiece consists of the above two lenses, the field lens being used for collecting the rays proceeding from the primary image, so that the maximum possible number enter the eye lens, thus including the largest possible area of the primary image, although the size of the primary image is somewhat reduced in the process.

The eye lens is used to magnify the real image produced by the field lens, the net result being a magnification of the majority of the primary image.

A further component is the diaphragm, consisting of an opaque disc with a central circular aperture placed at the focal plane of the eye lens. The edge of the central aperture serves to define the limits of the field of view and exclude extraneous rays caused by reflections from the inner wall of the body tube ; it also serves to carry accessories such as micrometer scales, which, when placed on the diaphragm, are in the focus of the eye lens and are, in consequence, superimposed on the final image.

As the focal lengths employed in eyepieces are relatively long compared with those of objectives, interference of their functions by aberrations is not so serious, although a poorly designed eyepiece will put a well-corrected objective under a serious handicap, more particularly when it is used to project a real image, as in photomicrography.

There are many different types of eyepieces, designed to perform divers functions, but the type most commonly used for visual work is the so-called negative or Huygenian eyepiece. This type is

illustrated as a ray diagram in Fig. 33, with Fig. 34 showing two such eyepieces, one by Watson and the other by Bauch and Lomb.

The design of eyepiece lenses depends on the glass used, the thickness, etc., and in theory they should be separated by half the sum of their focal lengths, which latter should be in the ratio 3 : 1, but in practice these conditions are never fulfilled, the general rule being to make the focal length of the field lens four or five times as great as that of the eye lens with the diaphragm situated at the focal plane of the latter.

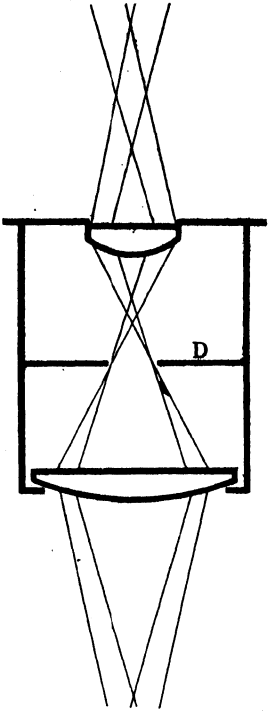


FIG. 33.

The reason for this is that the Huygenian eyepiece has been borrowed from the telescope, having been invented by Huygens for that purpose, and whereas the focal length ratio of 3 : 1 is perfectly satisfactory for the telescope, it has to be modified for use with the microscope, due to the great difference in the tube length of these two instruments; a different formula being

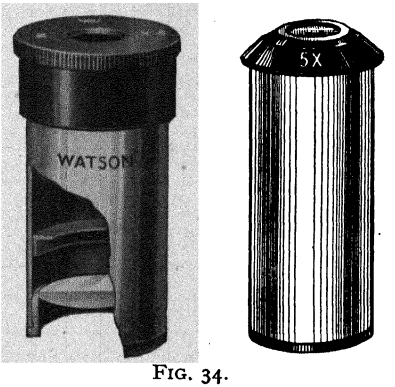


FIG. 34.

required for computing microscope eyepieces, hence a different ratio and separation are used.

The Huygenian eyepiece is very efficient and convenient for super-amplifications up to 10 diameters, but if this latter figure is exceeded the curvature of the eye lens becomes so deep and the focal length so short, that the eye point is then far too close for comfortable working, as the eye all but touches the exterior surface of the eye lens; however, this is its only serious drawback, and for use with any objective, other than the apochromatic type, the negative or Huygenian eyepiece is the most efficient.

Both the eye lens and field lens are normally uncorrected, but this is no drawback as the design of the eyepiece is such that their aberrations tend to cancel each other, and the automatic corrections

so obtained are ample for normal purposes, although in the event of a high degree of perfection being required, greater freedom from aberrations may be obtained by making the eye lens in the form of an achromatic doublet or triplet, but it is questionable whether the small improvement in the quality of the final image is worth the extra expense of achromatising the eye lens.

From the foregoing remarks it will be seen that the main features of the negative eyepiece are, firstly the focal plane is internal, *i.e.*, at the focus of the eye lens, as a result of which the primary image is reduced and brighter. Secondly, that the type of construction automatically corrects the combination to a large extent, and finally, that it has its limits so far as magnifying power is concerned.

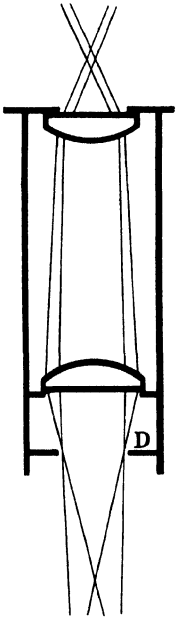


FIG. 35.

As distinct from the negative eyepiece there is the Ramsden or positive type, so called because the resultant focal plane lies outside the lens combination. Fig. 35 is the ray diagram for a Ramsden eyepiece, from which it will be seen that the combination is used as a single lens, as a result of which the diaphragm is outside the lens combination.

Whereas the Huygenian eyepiece consists of two plano convex lenses placed with their plane surfaces facing in one direction, the Ramsden eyepiece consists of two plano convex lenses with both convex surfaces facing inwards, the two together forming a single lens unit.

Positive eyepieces were the only type in use by the early microscopists and they consisted of a single bi-convex lens with no field lens, which must have given very poor definition indeed, and the addition of a field lens, although it was only a bi-convex with focal length ratio and inter lens distance, far from the best theoretical values, must have improved matters so much as to warrant being described as a major advance, until the theoretically perfect Huygenian eyepiece came into use.

All sorts of lenses have been used as eyepieces, including objectives and various forms of simple microscope. Solid eyepieces consisting of achromatised doublets and triplets have been used both in Britain and America, but none of them have yet approached the Huygenian eyepiece in performance.

In the Ramsden eyepiece both lenses have the same focal length and the inter-lens distance is two-thirds of the focal length of one of them. Frequently the eye lens is achromatised, as in Fig. 36, when better results are claimed ; in either case the results are appreciably better than those obtained with a single lens.

This type of eyepiece has one advantage over the negative type, in that the design lends itself admirably for use in micrometry. As the scale is situated on the external diaphragm, the presence of any aberration or distortion, inherent in the eyepiece, will affect both the scale and the image proportionately, thus leading to more

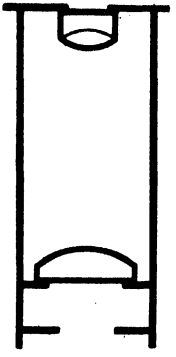


FIG. 36.

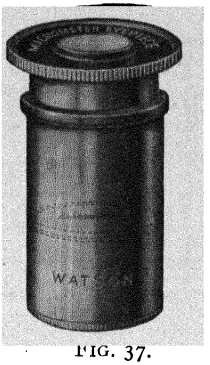


FIG. 37.

accurate results. This is not the case with the Huygenian eyepiece (illustrated by Fig. 37), and in consequence Ramsden eyepieces are usually incorporated in the design of micrometer eyepieces of the best quality.

This is not to say that Huygenian eyepieces are debarred from functioning as micrometers; as a matter of fact, the author habitually uses a Huygenian micrometer such as that illustrated, except in the event of extremely critical accuracy being required. In some respects the Huygenian micrometer is to be preferred to the Ramsden. With a Ramsden micrometer the scale is fixed and built into the unit, whereas with the Huygenian micrometer the scale may be changed in a matter of seconds; thus with various rulings the Huygenian eyepiece micrometer is a universal instrument. A further advantage is the ease with which it may be used in photomicrography.

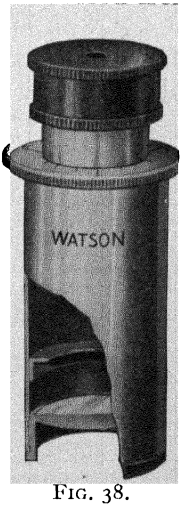


FIG. 38.

It has been stated that various degrees of correction are obtainable with both positive and negative eyepieces. Where they consist of two simple lenses they are called achromatic and are corrected for the red and yellow, the residual blue may be seen as a blue border to the field of view at the edge of the diaphragm aperture. These eyepieces are known as under-corrected.

When apochromatic objectives are used, a different degree of correction is required in the eyepiece, as these objectives are corrected for the secondary spectrum, leaving the tertiary spectrum

residual, which latter has to be corrected in the eyepiece and an over-corrected eyepiece is necessary. They are known as compensating eyepieces and are more usually of the positive type with the lower lens as a triplet combination. This is shown diagrammatically in Fig. 39. These eyepieces are not suitable for use with ordinary achromatic objectives as they introduce aberrations into the image.

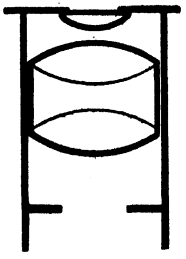


FIG. 39.

Thus it will be seen that where both types of objectives are included in a range of equipment, it is necessary to have two complete sets of eyepieces, one for achromatic objectives, consisting of Huygenian eyepieces, and the other for the apochromatic objectives made up of compensating eyepieces, and in order to overcome this difficulty Messrs. Watson's produced what they term their "Holoscopic" series of eyepieces. These eyepieces are of the Huygenian type, the lenses being two corrected combinations. The eye lens is mounted in a separate tube which slides in the main body tube of the eyepiece, thus the two lenses are capable of being separated. The diaphragm is fixed in the end of the eye lens tube and moves with this lens. Minimum separation gives under-correction and maximum separation gives over-correction. Thus for use with achromatic objectives the eye lens is pushed right in, and for use with apochromatic objectives it is drawn right out, or far enough to correct the residual spectrum of the objective in use.

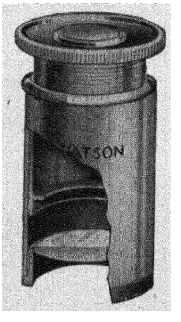


FIG. 40.

In this respect it shows an advantage over the compensating eyepiece inasmuch as one may vary the corrections slightly to suit different makes of objectives, and so obtain the best performance from any particular objective, as apochromatic objectives function best with the compensating eyepieces designed to be used with them. Hence, if one possesses apochromatic objectives of different makes, strictly speaking, a set of compensating eyepieces of the same make should be included with each objective. The "Holoscopic" eyepieces overcome this difficulty without detracting from the performance of the objectives. A "Holoscopic" eyepiece is illustrated in Fig. 40.

The types of eyepieces described above are the most commonly used for ordinary work, and in addition to these there are various types of eyepieces designed to perform special functions, among which is the comparison eyepiece, which enables two separate objects to be viewed simultaneously in the same field. The projection eyepiece, shown in Fig. 38, designed to project the microscopic image and for use in photomicrography. Pointer eyepieces with a

built-in movable pointer, which is focussed at the same time as the image and which may be moved to point at any particular portion of the field. There are also angle eyepieces, which enable the microscope to be used in the vertical position, without the discomfort to the user attendant upon having to look vertically downwards. There are also various forms of drawing eyepieces, all of which afford considerable help in drawing the image as seen in the microscope.*

Eyepieces, like all other optical instruments and components, must be treated with care, otherwise irreparable damage is liable to occur. They should be protected from fumes, acids, alkalis, and other such corrosive substances which are encountered in the laboratory. They should be handled with clean dry hands, care being taken not to touch the exposed lens surfaces, which should be kept perfectly clean and free from dust. These should be cleaned only with special lens paper or a piece of soft cotton cloth which has been repeatedly boiled.

It is permissible to separate the eyepiece for cleaning the internal lens surface, but this must be done carefully as the lenses have to be replaced in the same relative positions and unless they are screwed in carefully there is a big risk of crossing the thread and so upsetting the adjustment, apart from ruining the thread.

The most troublesome effect of dirt in the eyepiece is usually dust on the inner surface of the field lens ; this may be detected by rotating the eyepiece when looking at the image ; if any dust is present in this position it will appear as a number of blurred spots which rotate with the eyepiece.

After having cleaned the internal lens surfaces, it is advisable to replace the field lens first, by screwing it down into the tube so that any particles of the dull black internal coating of the tube, which may have become dislodged, will fall straight through. In any case, it is always advisable to blow out any particles which may be left before screwing in the eye lens, which should be done with the eyepiece in an inverted position, so that it is screwed upwards. The outer surfaces of the lenses may now be finally polished.

For the preliminary examination of an object, the use of a low-power eyepiece is strongly recommended, particularly of a type possessing a large flat field, as a study of the general characteristics of an object will facilitate the examination under high power.

With a monocular instrument both eyes should be kept open, to mitigate any tendency to eye strain. This procedure might seem a little strange at first, but after a little practice it will be found

* There is also the " Homal " eyepiece (Ziess) used for projection ; it produces a real image having a flat field, and as such cannot be used for visual examination, but it is very effective for such uses as photomicrography, in spite of its being purely specialised, one disadvantage is that no individual eyepiece is common to all makes of objectives.

quite easy to accomplish, the image in the vacant eye being practically non-existent to the observer, as the mental functions are concentrated on the microscope image, and if the habit is cultivated together with that of using both eyes alternately, the risk of damage occurring is negligible.

In using the microscope, no attempt should be made to see the image at any definite distance. The image should be focussed with the eye in a completely relaxed condition, and this should be practised until the temptation to help the instrument by focussing the eye when the image is not quite focussed in the microscope is overcome. This can be done by repeatedly throwing the image out of focus and looking into the eyepiece, at the same time relaxing the eye completely and then bringing the image back into focus while still keeping the eye relaxed. In other words focussing should be carried out entirely with the mechanical movements of the microscope and *not* with the muscles of the eye.

The choice of eyepieces depends entirely upon the type of work undertaken, but for routine purposes the ordinary Huygenian eyepiece will serve admirably. The powers chosen should be X6 and X10 together with a X6 micrometer eyepiece and stage micrometer ruled in tenths and hundredths of a millimetre. The eyepiece micrometer scale should be divided into 100 divisions, a pointer eyepiece of X6 is a useful adjunct when any particular portion of the field is required to be shown to others.

CHAPTER IV

THE OBJECTIVE

As the objective is the most important component in the microscope, it behoves us to acquire a thorough understanding of the principles underlying its operation in order to appreciate the necessity for obtaining the utmost quality in the primary image, as the final result is entirely dependent upon this. A poor objective will utterly ruin the performance of an otherwise first-class instrument.

The functions of an objective are threefold :—

(1) To gather the maximum amount of light coming from any part of the object.

(2) To unite this light in a point in the image.

(3) To produce a magnified image of the object.

It has been previously stated that any illuminated object gives off light rays in all directions, and also that, under normal conditions of vision, only a small portion of these rays are included in the visual angle. We have seen how the use of a lens increases the visual angle, it follows therefore that more of the emitted rays would enter the eye, thus more details of the object would become visible, so that it will be seen that as the details required to be examined become smaller, so must the visual angle become greater, in consequence of which the emission angle becomes greater also and the focal length of the lens becomes shorter. Thus we can see that, with a theoretically perfect lens, as the focal length gets shorter, more rays enter the lens from any given point of the object, and in order to see as much detail as possible, the maximum possible number of rays from the object should enter the lens.

For the examination of the coarser features of an object, a small portion of the emitted rays is sufficient, but if we wish to examine the finer details of structure we must use the rays included in as wide an angle as possible. The angle between the most divergent rays which an objective will accept is called the angular aperture of the objective.

Formerly it was thought that the microscopic image was formed in the same way and obeyed the same laws as those apertaining to the formation of the image in a camera or a telescope, and a great deal of confused thought centred around the subject until it was finally elucidated by Abbe, about which Carpenter and Dallinger (1) say : " The delicate and complex structure of an insect's scale or of a diatom were believed to form their images according to the same precise dioptric laws by which the image of the moon or Mars is formed in the telescope. Hence it was taken for granted that every

function of the microscope was determined by the geometrically traceable relations of the refracted rays of light. We would nevertheless remark that visibility of detail in, for example, the moon, depends on the aperture of the telescope. Of course, what is known as its ' aperture ' is simply estimated by the diameter of its object glass, but accuracy appears to require that $n \sin \mu = a$ ought to be applied to the telescope. In practice, the diameter is taken conventionally for the sake of simplicity, as it makes no numerical difference because the sines of small angles such as are dealt with in the telescope are proportional to the angles themselves. The microscope, on the other hand, deals with large angles, consequently the sine cannot be dispensed with."

" But Professor Abbe argues that a close examination in theory and practice of the conditions of vision with microscopic objectives shows that an estimate of aperture is wholly wrong in principle. The front lens of a $\frac{1}{25}$-in. objective may be no more than $\frac{1}{50}$ in. in diameter, while a 3-in. objective may have a diameter of $\frac{1}{2}$ in., yet it is the smaller lens which has by far the greater aperture."

From these remarks it will be seen that the angular aperture of an objective is apparently a measure of its light gathering power, and this was thought to be true until proved otherwise, following the introduction of immersion objectives. However, it is not proposed to discuss this point at any length, suffice it to say the definition of aperture was shown by Abbe to be obtained when the diameter of the emergent pencil of light from the objective is compared with the focal length. He pointed out that a general relation existed between the pencil of light admitted into the front of the objective and that emerging from the back, and that the ratio of half the diameter of the emergent pencil to the focal length of the objective could be expressed by the sine of half of the angle of aperture multiplied by the refractive index of the medium in front of the objective thus :—

$$n \sin \mu = \text{N.A.}$$

Where n is the refractive index of the medium in front of the objective, μ is half the angle of aperture.

Abbe called this product the numerical aperture (N.A.), thus we now have a means of comparing objectives whose N.A. is known, furthermore, as the maximum angle in air is 180° and as $n \sin \mu$ for 180° in air is equal to 1·0, it is quite easy to see whether the objective has an aperture which is smaller or larger than that corresponding to 180° in air.

Thus we now have a measure of efficiency of an objective and as a result we see that the larger the angle of the cone of light accepted by a lens, the better the performance. Obviously then, if we can increase the cone accepted by a lens with air in front of it, the numerical aperture will also be increased or, to put it another

way, if the maximum N.A. of a dry objective is 0·95, this signifies that the lens will accept a cone of 143° in the air. If we now replace the air in front of the objective with a medium of refractive index $\mu = 1{\cdot}5$, the angle of the cone will be reduced to 77°. As the resolution of fine details is proportional to the angle of the cone of light accepted, it will be seen that details much beyond the limit of resolution of such a dry lens will be easily resolved by a lens working immersed in the medium stated, with a maximum N.A. of 1·40, which corresponds to an angular aperture of 134° in the medium, which in turn corresponds with an air angle much greater than 180°.

It was known early on that if an objective was worked with its front lens immersed in water, its efficiency was increased. This was suggested by Brewster in 1813, but it was obvious that if an objective was required to be worked immersed, a radical change in construction would be necessary.

Carpenter and Dallinger (1) say: "As pointed out, the loss of light increases with the obliquity of the rays, so when objectives of very wide aperture are used dry, the advantages of its increase are a great deal nullified by the reflection of a large part of the rays falling very obliquely on the peripheral portion of the lens. Where, on the other hand, rays of the same obliquity enter the peripheral portion of the lens from water, the loss by reflection is greatly reduced and the benefit derived from the large aperture is proportionately augmented."

Thus we see the benefits obtainable by using objectives "immersed," and it follows that the higher the refractive index of the immersion medium, the better the results. These reach a maximum when the slide, the fluid in which the object is mounted, the cover glass, the immersion medium and the lenses composing the objective, all have the same refractive index. It was not until Abbe propounded the theory of the "Homogeneous Immersion System" that the maximum benefit was obtainable.

J. W. Stevenson also discovered the homogeneous immersion system independently, and this is the system in use to-day. It consists of replacing the water or other immersion medium with a fluid medium having the same refractive index as crown glass, hence the term "homogeneous." This condition is very nearly satisfied in practice by the use of cedar wood oil, which has a refractive index of 1·51, while that of crown glass is 1·51 to 1·56.

The point will perhaps be made clearer if we refer to Fig. 41. This diagram shows an object mounted in a medium whose μ is 1·51, above which is a cover glass. Between the object and the lens is a layer of air, thus we have a break in the homogeneity of the system at this point. The maximum cone of rays accepted by this lens is enclosed by the ray R1, and even this is partly reflected

from the surface of the lens. The rays R2 and R3 are totally reflected from the upper surface of the cover glass.

Now it is apparent that if instead of air between the lens and cover glass, we had a medium of higher refractive index, say water ($\mu = 1{\cdot}33$), then the critical angle at the upper surface of the cover glass would be reduced. The effect of replacing air with water is illustrated in Fig. 42. Here we have ray R2 entering the objective while R3 only is reflected back. It will also be noted that the angle of the cone becomes smaller on substituting water for air; this is because the wavelength of light is shortened in its passage through a medium of high refractive index; thus we see another advantage of the immersion system.

The question is of great importance if a thorough understanding of the principles is to be grasped. Carpenter and Dallinger explain

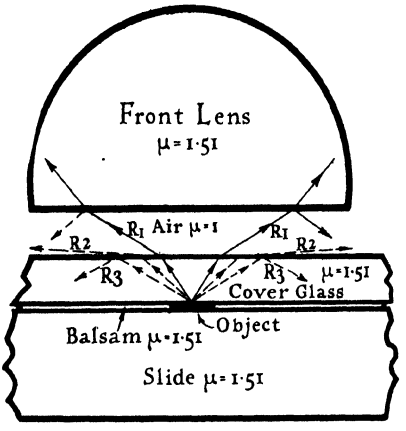


FIG. 41.

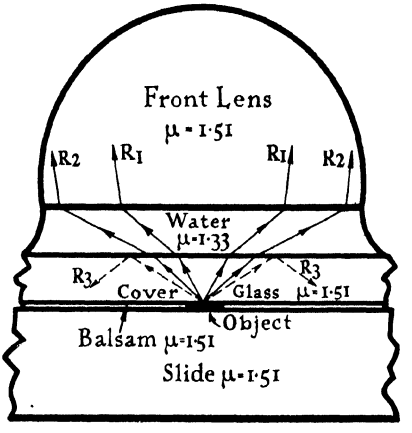


FIG. 42.

this exceedingly well and one could not do better than refer to their work. The following extract will clarify the point :—

" The essence of the idea of ' aperture ' is *relative opening*. Its significance can only be appreciated by taking into account the image forming pencil emergent from the objective and the change in its diameter consequent upon the emission of different cones of light. This diameter affords an indication of the *number of rays* (not mere intensity, which can be readily varied) which are collected to a given area of the image and which must have been gathered in by the lens from the conjugate area of the object. If the diameter of the emergent pencil is seen to be increased, while the magnification of the image and the focal length are unchanged, it is clear that the objective must have admitted more rays from every element of the object, because it has collected more to every element of an equally enlarged image. Thus we get an accurate measure of what is admitted in an objective by being able to estimate what it emits "

" Hence aperture means the greater or less capacity of objectives for gathering in rays from luminous objects."

" A given objective may, in fact, collect the rays from a radiant in air almost to the entire hemisphere, when it utilises a definite opening double its focal length, but when the radiant is in Canada balsam (without any other alteration) the same opening is seen to be utilised by the rays which are within a smaller cone of not more than 82° and rays which are outside this cone require a surplus opening never required by rays in air."

" This holds good whether there be refraction or not at the front surface of the system, the difference is solely based on the difference of medium. Consequently we arrive at the conclusion that the solid cone of 82° in balsam embraces the same rays which, in air, are embraced by the whole hemisphere and every one wider than 82° in balsam conveys more rays from the object than are admitted by the whole hemisphere in air."

" All the rays in balsam outside this cone constitute a surplus of new rays which are never met with in air."

This being due to their total reflection from the upper surface of the cover glass. If, on the other hand, the air is replaced with cedar wood oil, these rays proceed straight to the objective and constitute the additional cone obtained thereby. This is illustrated in Fig. 43, which shows how R3 is now included in the functional cone.

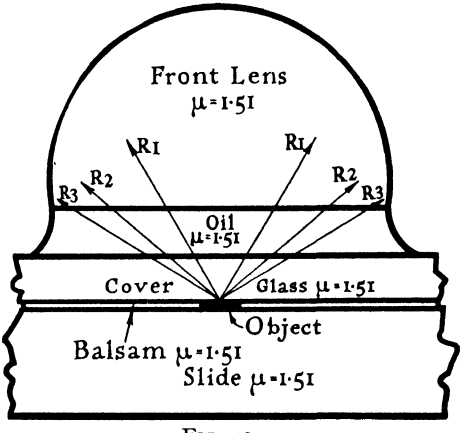


FIG. 43.

After perusing the foregoing explanations, one is apt to say, " but why should an increase in aperture lead to an increase in the efficiency of the objective ? " We do not have to look far, for the answer to it is in the one word RESOLUTION.

The resolution, or resolving power, of an objective is defined by Chamot and Mason (2) as: " Its ability to reveal closely adjacent structural details as actually separate and distinct. Quantitatively it may be expressed as the minimum distance between such details when resolution has been achieved."

Towards the latter half of the last century it was generally admitted that an increase of aperture was followed by an increase in efficiency of an objective. The reason for this is quite independent of the question of immersion systems and any apertures greater than the maximum in air.

It has been known of old that the best results were obtained when the rays emerging from the object were oblique, and this gave rise to the general idea that good results were dependent on the angle at which the light emerged from the object, thus investing the obliquity of the light with some special property and making the whole problem dependent on angle, as such, but we have seen that to attempt to explain the formation of the image by means of numerical aperture by angle alone, is futile. However, Abbe at last decided that the problem was worthy of the closest investigation and proceeded accordingly.

It had been previously demonstrated that a cone of 170° would show much more detail than would one of 80° in the same medium, which resulted in the idea that the delineating power depended on the obliquity of the light.

It was also generally accepted that the image was formed under the dioptric law, as was that in the telescope. However, Abbe succeeded in showing that resolution was not dependent on the obliquity of the rays to the object, but rather to their obliquity to the axis of the microscope.

He showed that coarse objects which only require a few degrees of aperture to resolve them are dependent on " shadow effects." On the other hand, when it is required to resolve minute detail, small apertures are absolutely useless, and an increase in obliquity of the pencil has no effect whatsoever, but an increase in numerical aperture will have the desired effect, thus showing that the obliquity of the rays emitted by an object is not, in itself, the true explanation of the increase in resolution attendant upon an increase in numerical aperture and is not borne out in practice, as with the same objective the angle of the cone supplied by any given object gets smaller, as it is viewed successively in air, water, balsam and cedar oil.

Thus we see that angle is not the live comparison on the following points :—

(1) Its failure with regard to explaining aperture.

(2) Its failure in explaining the number of rays and quantity of light admitted.

(3) Its failure to explain resolution.

From which it follows that in the case of increased resolution obtained from an increase in numerical aperture, something is admitted into the objective which is kept out by those of smaller aperture.

Abbe next showed that microscopic images were not to be compared with ordinary or macroscopic images. The production of microscopic images does not depend on refraction, when it comes to the resolution of minute structure, but rather on *diffraction.*

It is well known that if a narrow opaque object, such as a hair or wire, be placed in the path of a beam of divergent light, then its

shadow on a screen would be bounded on each side by a spectral fringe. If we imagine the hair to constitute one element of a closely spaced grating, we have an object which requires high aperture to resolve. If a beam of light is incident on the grating, perpendicular to its surface, then spectra will be produced on either side of the incident beam or ray (see Fig. 44).

The angular dispersion of the spectral rays R1, R2, R3, R4, etc., depends on the distance between the elements of the grating; thus close spacing causes a wider angular dispersion than a broader one, also red light is dispersed through a greater angle than blue due to its longer wavelength. Similarly, a grating would disperse the spectral rays through a smaller angle when immersed in a high refractive medium, due to the shortening of the wavelength.

Abbe went on to show that the axial beam or one of the spectral beams alone would show only the contour of the specimen, visibility of the structure necessitated the inclusion of at least one spectral beam with the axial beam, thus, as more spectral beams were admitted, so the image became a more accurate representation of the object up to the ideal condition when all the spectral rays were admitted.

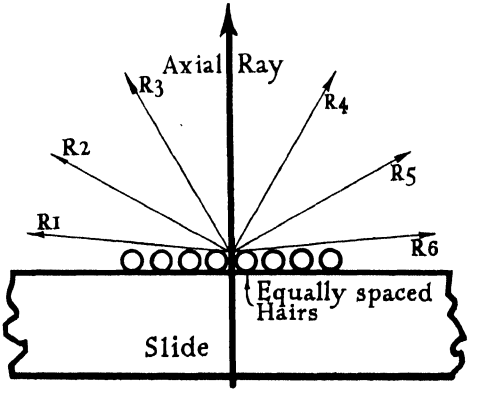


FIG. 44.

Thus the axial ray is quite sufficient to resolve coarse structures and fine periodic structures; that is to say, those whose elements are separated by equal fixed distances. For example, a ruling of hundredths of a millimetre will still show lines equally spaced from one another if viewed with a small aperture, but the difference lies in the thickness of the lines relative to the spaces between them; thus, if only the axial ray and one diffracted ray are admitted to the objective, the spaces are always approximately as broad as the lines, and the contours are lacking in distinctness.

So we can see that in order to get a true representation of fine structures, we must employ as many of the diffracted rays as possible but, as the intervals between any two elements of a given structure become smaller, the angle enclosed by the refracted rays becomes larger, so that a larger N.A. is required to resolve them, and when the intervals become so small that they are reduced only to the dimensions of a few wavelengths of the light employed, the only course open is a shortening of the wavelength, as the angle has now become greater than 180°. This may be accomplished by

using a medium of higher refractive index whose μ is so high that the intervals become large multiples of the wavelength.

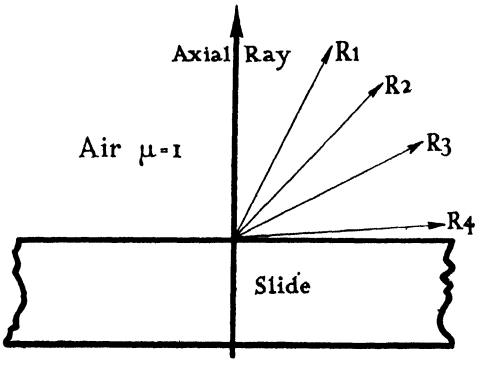


FIG. 45.

The effect of the inclusion of a highly refractive medium is illustrated in Figs. 45 to 47. Here we have, in the first instance, an axial ray, with a fan of four diffracted rays occupying nearly 180° in air. If the air is replaced by balsam with a μ of 1·526, we see in Fig. 46 how the angle enclosed by R4 is closed up and two further rays, R5 and R6, are included in the fan of the same previous angle, whilst if we went still higher up the scale and replaced the balsam with a medium of higher refractive index still, say Realgar, with a μ of 2·549, further extra rays would be included in the fan, such as R7 and R8 in Fig. 47.

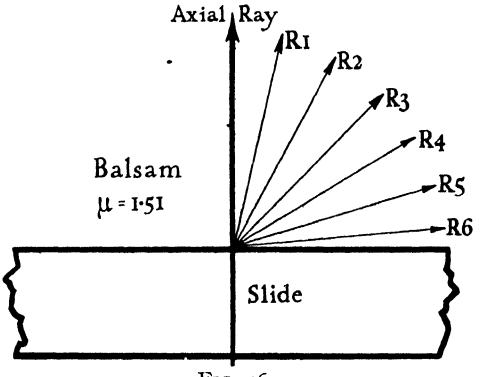


FIG. 46.

Thus, under certain conditions, a dry objective would admit the fan in Fig. 45. In balsam the same fan would be contracted considerably, and for an objective immersed in balsam to admit this fan, the aperture required would be much less than for the dry lens, and it will be seen that if the immersed lens had the same aperture as the dry one, a much larger number of diffracted rays would be admitted, thus increasing the resolution.

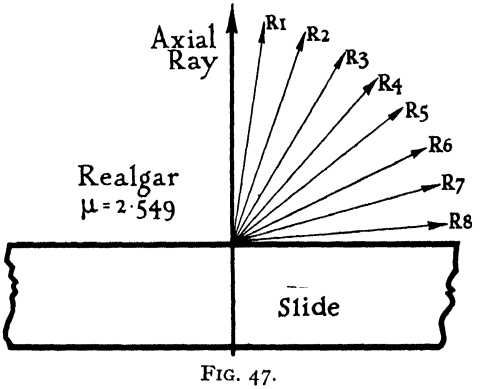


FIG. 47.

From reasoning such as this, Abbe concluded that :—

(1) A wide-angled homogeneous immersed objective possesses an aperture greater than 180° angular aperture in air.

(2) The value of this is fully accounted for and explained by the diffraction theory of resolution.

(3) That dry objectives are only a second best and can never be expected to equal the resolution obtainable with immersion objectives no matter how carefully made.

And we may say further that :—

(1) The true measure of comparison for objectives is the numerical aperture which is the product of half the angular aperture multiplied by the refractive index of the immersion medium, be it air, oil, or any other medium.

(2) That resolution, being dependent on the acceptance of the maximum number of diffraction rays, is therefore dependent on numerical aperture and is much greater in the case of objectives immersed in high refractive media.

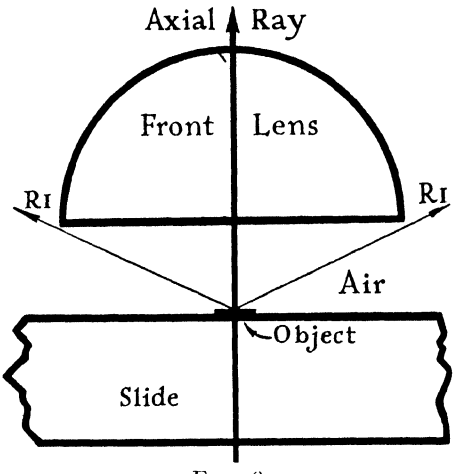


FIG. 48.

We have seen from the foregoing pages that by working the front lens of a suitably designed objective immersed in a highly refractive medium such that the entire space between the objective and the object is maintained at the same refractive index, we obtain vastly superior results. It must be emphasised, however, that the method of preparing and mounting the object, if not carefully carried out, will negative any advantage obtained by the use of an immersion objective, as the medium of lowest refractive index sets a limit to the performance. Thus, if we have an object mounted in, say, water, the effective numerical aperture of the objective is only 1·33 (*i.e.*, the μ of water) in spite of the fact that its designed N.A. may be 1·40 and the cover glass and immersion medium above it may have a refractive index of 1·5.

Apart from the loss sustained by total reflection at the under surface of the cover glass, there is also the loss due to reflection from the leading surface of the front lens of the objective, if the refractive index of the immersion medium is below the rated value of the N.A. of the objective.

So that we see, in order to utilise the advantages of the homogeneous immersion objective to the full, a certain amount of care has to be exercised in the selection of mounting and immersion media; even so, we have still to supply the objective with the maximum possible number of diffracted rays if we are to obtain the best results.

Now, if we take the case of an object mounted in air and viewed with a dry lens, Fig. 48, and illuminated by parallel light, it is obvious that the resolution will be very poor as the first diffracted ray, R1, misses the objective entirely and the image is formed by the axial ray alone ; therefore we must consider means of increasing the resolution (such as it is) so suppose the object is mounted in balsam. We know that this has the effect of closing up the fan of diffracted rays and increasing the number of such rays in the cone. In the case in point, this would probably close the diffraction fan so that the first diffracted ray entered the objective as in Fig. 49, R1, the other rays being totally reflected at the cover glass as shown in Fig. 41 ; it is assumed that the balsam and cover glass have exactly the same refractive index.

We have seen, however, that this condition is not good enough

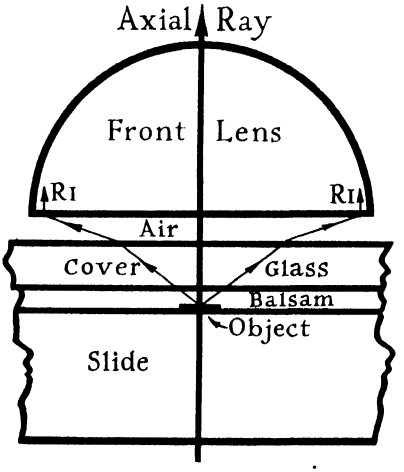


FIG. 49.

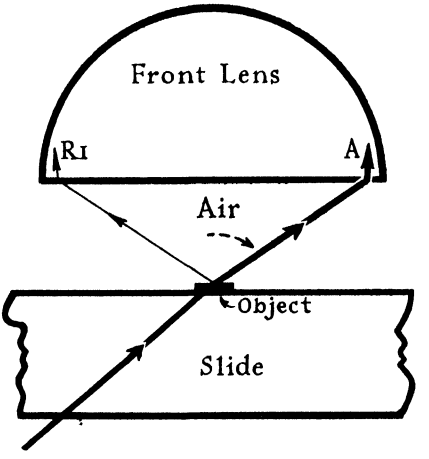


FIG. 50.

for the resolution of fine structure which requires the inclusion of the widest possible diffraction fan, so, in our endeavour to direct more of the diffracted rays through the objective, let us advance another step and see what happens if instead of parallel light we use oblique light. Suppose we tilt the axial ray with the object as the axis so that it enters the objective at the peripheral margin of its aperture, as at A in Fig. 50. It will be seen that the refracted ray R1 now enters the objective, thus increasing the resolution to the same extent as in Fig. 49, without having to mount the object in balsam.

It will be remembered that Abbe claimed that a periodic structure would be resolved if the axial ray and *one* of the first diffracted rays were accepted by the objective. In this case, of course, we have satisfied this condition, although the other half of R1 is a complete loss. It will be seen that this constitutes oblique illumination, in consequence of which one-half of the structural element illuminated

by the axial ray will be in shadow; however, the resolution in the illumination will be much improved.

Although it would appear that we have only obtained the same increase in resolution as we did by mounting the object in balsam, and then only illuminated one half of the object, it will be seen that if we apply this same principle to the object mounted in balsam (Fig. 51) a further gain is obtained, because we can now persuade the second diffraction ray R2 to enter the objective with its attendant increase in resolution.

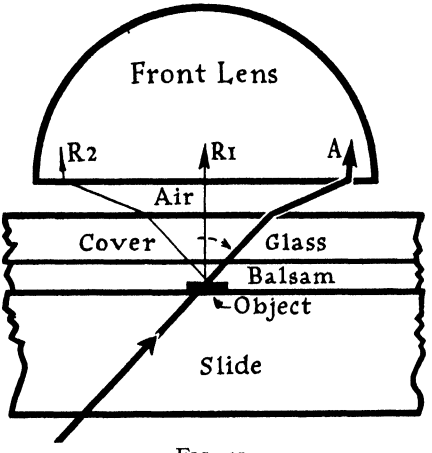


FIG. 51.

Here, what was originally the axial ray is again the ray "A," and it will be seen that, due to the closing of the diffraction fan by the presence of the balsam and cover glass, not only the first diffraction ray, R1, but also the second, R2, is accepted by the objective, the significance of which needs no emphasis.

It is obvious that the permanent illumination of only half the object is intolerable, therefore let us see what can be done to improve this state of affairs. Suppose we use the arrangement in Fig. 51 and illuminate the object from the other side by another ray, B (Fig. 52), which strikes the object at the same angle as does A (as shown), then it follows that we would have the refracted rays R3 and R4 also entering the objective and forming an image of the object illuminated on two opposite sides, thus bringing about a considerable improvement in the image. In the same manner, if we could furnish two more rays opposite each other, to strike the object at the same angle as A and B and at right angles to them, we would get almost complete illumination of the object, improving matters still further. But it would be ridiculous to equip a microscope with the four

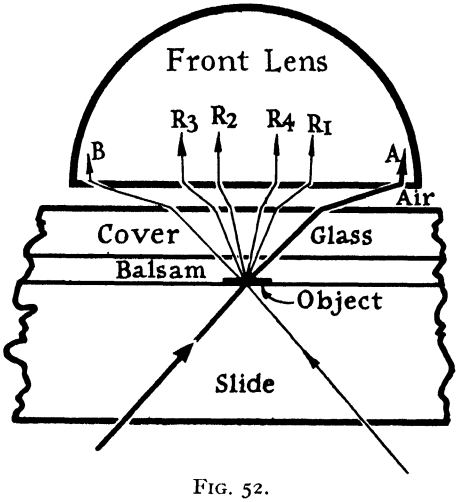


FIG. 52.

separate light sources necessary to accomplish this, so the problem is solved and at the same time conditions are further improved by using a lens under the object to converge light on to it in the form of a cone. Thus we get complete illumination of the object and at the same time increased resolution.

This is illustrated in Fig. 53, which shows the same set of conditions as in Fig. 52, but with the addition of a condenser, which supplies a cone of light to the object (the top lens only is shown).

Thus we see that to get the best out of any objective, be it dry

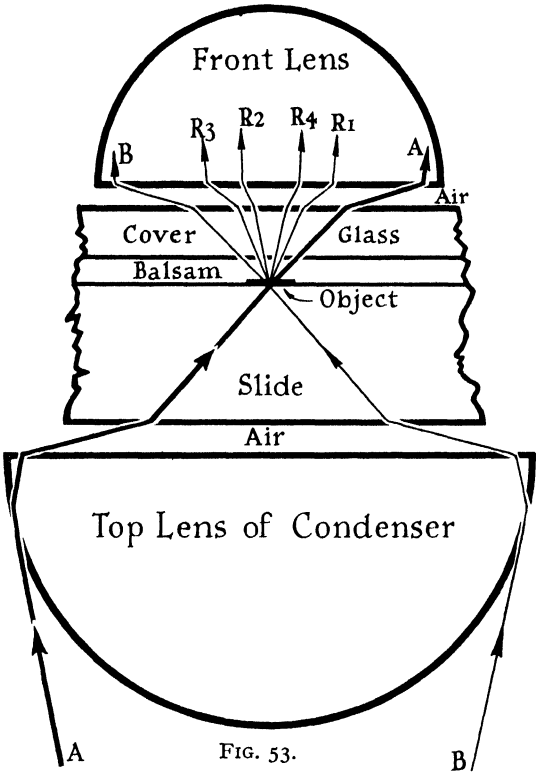


FIG. 53.

or immersed, a condenser is essential, except perhaps in the case of long-focus objectives from 1½ in. upwards, where the resolution only involves the coarser structure and the N.A. is so small that parallel light gives acceptable results; it should, however, be emphasised that as the cone accepted by these objectives is by no means negligible, the use of a suitable condenser does improve the image produced quite considerably; in fact, the author never uses any objective without a condenser and strongly recommends that it should constitute a "golden rule" to all those intent on serious work of any kind with the microscope.

As the focal length of the objective shortens, so the angle of the maximum cone accepted increases; it follows that in order to

efficiently illuminate an object the cone of light supplied by the condenser should also increase. This of course is achieved by shortening the focal length and increasing the N.A. of the condenser and as the maximum N.A. for any lens working in air is 0·95, it also follows that with immersion objectives, whose apertures are greater than 1·0, the condenser must be immersed in order to supply the objective with a cone equal to its own, so that it may work at its maximum efficiency.

Thus far, then, we have seen the importance of the objective in the optical train and have gone into the question of numerical aperture and the advantages of immersion objectives over dry ; we have also discussed the question of resolution and the diffraction theory relating to it, likewise the necessity of a condenser for obtaining maximum resolution has been explained.

It is clear, therefore, that the most desirable characteristic of an objective is its capacity to resolve fine structure. Many objectives of very short focal length have been made, and before the problems relating to N.A. and resolution were explained, these were considered to be the acme of perfection, whereas in reality they only exhibited an enormous amount of empty magnification. An example typical of the times is the old $\frac{1}{50}$-in. water immersion with a N.A. of 1·1. It can be shown that the relation of aperture to power in an objective under ideal conditions is such that the maximum visible resolution of the eye is satisfied when the final magnification is twelve times the initial magnification of the objective, and for this purpose the objective must possess 0·26 N.A. for every hundred diameters of final magnification ; under these conditions an object is seen at its best.

In order that a clearer understanding of the value of numerical aperture, as a means of comparison, may be obtained, Nelson suggested the adoption of the term " optical index " as a supplementary figure. The optical index, or O.I., is the ratio of 1,000 times the numerical aperture to the initial magnifying power, thus :—

$$\text{O.I.} = \frac{\text{N.A.} \times 1{,}000}{\text{Initial Mag.}}$$

This figure is very useful as it enables one to assess the merits of any particular lens almost immediately. Let us take for example three lenses, by different manufacturers, of the same focal length and other details as follows :—

A. has a focal length of 16 mm. and initial magnification of × 10 with N.A. 0·28.

B. ,, ,, ,, ,, magnification of × 10 with N.A. 0·25.

C. ,, ,, ,, ,, magnification of × 15 with N.A. 0·25.

From these figures one would assume that " C " would probably be the best lens, but if we examine the O.I.'s of these lenses we get :—

A. O.I. = 28
B. O.I. = 25
C. O.I. = 16·6.

So that in reality A. is the best lens. Carpenter and Dallinger quote the following comparative examples :—

(1) A Zeiss apochromatic 24 mm. of N.A. 0·3 and initial magnification of × 10.

(2) A Zeiss apochromatic 12 mm. of N.A. 0·65 and initial power of × 21.

(3) A $\frac{1}{8}$-in. oil immersion whose N.A. is 1·4 and initial power × 83.

(4) A $\frac{1}{50}$-in. water immersion whose N.A. is 1·1 and initial power × 550.

These lenses have optical indices as follows :—

(1) O.I. = 30
(2) O.I. = 31
(3) O.I. = 17
(4) O.I. = 2·0,

about which they say " The optical index therefore tells us that the $\frac{1}{50}$-in. water immersion of 1·1 N.A. has a vast amount of empty magnifying power, while on the other hand the 24- and 12-mm. will both stand a higher eyepiece than 10 : may, even require it, before the detail made visible by them is made visible to the eye. It also shows that the $\frac{1}{8}$-in. of 1·4 N.A. will stand a higher eyepiece without arriving at an empty magnifying power than a $\frac{1}{12}$ in. of 1·4 N.A., and whose O.I. is 11·0."

It is the author's opinion that the optical index used in this way gives a much better idea of the value of an objective than the numerical aperture alone, particularly when a choice of one of several different makes of objectives has to be made, or the merits of different combinations compared. Thus we may be confronted with the choice of one of two objectives, both stated to be $\frac{1}{4}$ in. focal length, the N.A. of the one being 0·8 with an initial power of × 60 and the N.A. of the other being 0·9 and initial power 40, the price being the same in each case.

As the desired characteristic is the maximum resolution for the type of lens and price involved, a comparison of their respective optical indices would give an indication as to which lens to choose. The O.I. of the first is 13 and that of the second is 22, and as it is easier to achieve magnifying power than aperture in the design of a lens, we would expect the first lens to be cheaper than the second, but as the price is the same in each case the obvious choice is the second objective.

The limit to which superamplification of the primary image may

be taken is given by the product of the N.A. of the objective used, × 400. Thus, if we call the limiting magnification D_l, then :—

$$D_l = \text{N.A.} \times 400.$$

For example, a 4-mm. objective by Leitz has a N.A. of 0·85 and initial magnification of 45 diameters, the limit to which super-amplification may be taken in this case is :—

$$\begin{aligned} D_l &= 0{\cdot}85 \times 400 \\ &= 340 \text{ diameters,} \end{aligned}$$

and as its initial magnification is × 45, the maximum eyepiece this lens will stand is given by dividing D_l by 45 and is equal to $7\frac{1}{2}$ say × 7.

The converse of this rule may be used to determine the ideal numerical aperture of any objective whose initial magnification is known. This figure is obtained by multiplying the initial power by 0·020 ; as an example let us take the afore-mentioned 4-mm. Leitz objective, the initial power being × 45, the ideal aperture is given by :—

$$\begin{aligned} \text{N.A.} &= \textit{initial power} \times 0{\cdot}020 \\ &= 45 \times 0{\cdot}020 \\ &= 0{\cdot}90, \end{aligned}$$

so that we see that the rated aperture is close to that of the ideal aperture for its focal length : at the same time it is interesting to note that its O.I. is 18·9, and as it can be shown that any lens with which it is required to use a × 10 eyepiece must have a minimum O.I. of 26 if empty magnification is to be avoided, we see that the limiting super-amplification of 7·5 for this lens is confirmed. It is recommended by the author that these rules be proved by practical tests.

So much for the basic principles underlying the production of the best possible image by the objective, and before dealing with the objective itself, let us pass briefly over its history.

As any single lens, be it doublet, triplet, or just a simple chromatic, may be considered to behave as an objective, and its main use is that of procuring magnification, the beginnings of the objective can truly be said to be lost in the mists of antiquity. The earliest known form of lens was the rock crystal discovered by Sir John Layard at Nineveh, its probable date of origin being somewhere in the region of 705 to 721 B.C., while in A.D. 65 a glass globe filled with water and used as an aid to vision was mentioned by the Roman philosopher Lucius Anneus Seneca. The earliest attempts at achromatisation were made by Hooke in 1660, while Chester Moore Hall, in 1740, achromatised the telescope objective ; this knowledge was not however used by him, but by Dolland, at a later date.

However, this early history does not concern us greatly and we

may pass it by, as no noteworthy development in the efficiency of lenses came about until the beginning of the nineteenth century as, in all truth, the modern history of the objective did not begin until its chromatic aberrations had been corrected.

Thus we find, in 1808, one Bernardino Marzoli, Curator of the Physical Laboratory of the Lyceum of Bresica, published a paper on the achromatisation of lenses and in 1811 exhibited examples in Milan. Marzoli's objective was a cemented combination with the plane side of the flint element facing the object.

In 1823 Selligues suggested to Chevalier that a number of achromatised doublets could be superimposed to advantage. Chevalier produced some lenses on this principle, but they had their convex surfaces facing the object and in consequence suffered enormous spherical aberration. Actually this is four times the aberration experienced with the flat side of the lens facing the objective. In 1825 Chevalier realised the mistake which Selligues had made and rectified it.

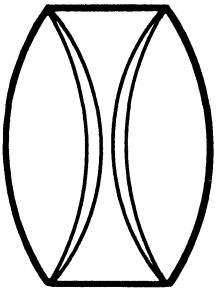


FIG. 54.

It should be noted that the use of superimposed combinations was not a result of applied theoretical knowledge, but rather was it due to a happy accident.

Meanwhile, in this country, Tully, acting on a suggestion by Dr. Goring, in 1824 independently produced an achromatic objective shown diagrammatically in Fig. 54.

This lens was an uncemented triplet, being in fact a miniature telescope objective. He produced two lenses, a $\frac{4}{10}$-in. and a $\frac{2}{10}$-in., both of which were found to be too thick in practice; however, he then produced a $\frac{9}{10}$-in. of 18° aperture (angular) whose performance was stated to be nearly equal to that of the $\frac{2}{10}$-in.

A big step forward was made when he placed a doublet in front of a similar triplet of shorter focal length, the combination possessing an angular aperture of 38°.

In 1844, Professor Amici of Modena visited London and brought with him an objective composed of three doublets and having a large aperture, which created a most favourable impression.

Meanwhile, in England, Lister had discovered the two aplanatic foci of a combination and in so doing had opened an important epoch in the evolution of the objective. In January 1830 he read a paper to the Royal Society in which he showed how the aberrations of one doublet may be neutralised by another. At first the importance of this discovery was not appreciated, and in 1831, after he had tried in vain to interest the practical opticians in further experimental work, he taught himself the art of lens grinding and produced an objective which consisted of a meniscus pair for the

front, with a triple combination following and having a plano-convex doublet for the back. It had a working distance of 0·11 in., and was declared to be the best lens of the time.

The reading of the paper and the production of Lister's objective resulted in an immediate awakening of the professional opticians, who started rapid production of achromatic objectives. Lister's data proved of great value and progress was rapid compared with what it had been up to then.

These objectives were manufactured by Andrew Ross in 1833, Powell in 1834, and Smith in 1839, and in order to illustrate the rapid progress made, the following are a few of the objectives made by Ross :—

1834	—$\frac{1}{4}$ in., 55°, composed of three pairs.	
1837 to 1841	—1 in., 22°, ,, ,, triple front and two double backs	To Lister's data.
	—$\frac{1}{8}$ in., 63°, ,, ,, ,, ,, ,,	
1842	—$\frac{1}{4}$ in., 63°, ,, ,, ,, ,, ,,	
,,	—$\frac{1}{8}$ in., 74°, ,, ,, ,, ,, ,,	

It was in 1837 that Ross discovered that owing to the perfection of the corrections in his lenses the presence of a cover glass was sufficient to upset their performance. He overcame this difficulty by making the combination separable, whereby the lenses in the combination were mounted so that the front lens could be moved towards or away from the back lens at will, thus either over-correcting or under-correcting, as previously explained.

This type of COLLAR correction was in vogue for some considerable time and consisted of a separate tubular mount for the front lens, which is capable of sliding on the tube holding the remaining lenses of the combination, the graduations consisting of two horizontal lines and the words "Corrected" and "Uncorrected." However, as the demands on the instrument increased due to work of greater delicacy being undertaken, two drawbacks to this method of correction became apparent.

Firstly, the calibration was much too coarse, this leading to the development of the screw collar by Smith, graduated in fifty divisions.

The second and more serious defect was in the movement of the front lens, while the back lens of the combination remained stationary relative to the optical train. This resulted in the, by no means unusual, occurrence of pushing the front lens through the cover glass when using a short focus objective with a small working distance Smith's screw collar moved the back lens while the front lens remained stationary.

In general, the early half of the nineteenth century up to about

1840 saw the birth and rapid development of the achromatic objective, and it may be said of the lenses of that time that in the lower powers the corrections were well balanced and apertures moderately high, but with regard to the higher powers the lack of aperture was painfully apparent. However, 1844 saw the production of a $\frac{1}{2}$-in. objective of 112° by Amici, who brought it to England. The increase in aperture was brought about, it is understood, by the use of extra dense flint glass, which, unfortunately, is unstable. Ross, after modifying Amici's construction, succeeded in increasing the aperture of a $\frac{1}{8}$-in. objective to 85°, or 0·68 N.A., and that of a $\frac{1}{12}$-in. to 135°, or 0·93 N.A.; this latter objective was said to possess the largest aperture obtainable in objectives. The lens train of a typical $\frac{1}{4}$-in.

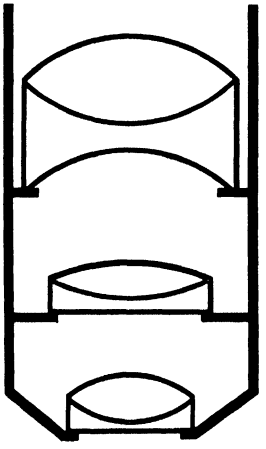


FIG. 55.

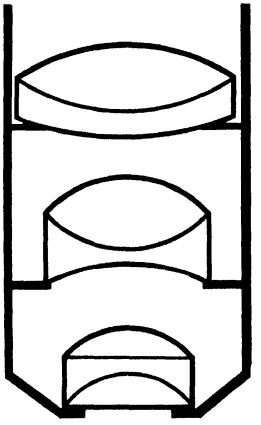


FIG. 56.

Ross objective of the time is shown in Fig. 55; as can be seen, this lens consists of three doublets.

The year 1850 heralded the production of objectives having a triplet back lens said to be first produced by Lister, although others attributed this combination to Amici. This point is justifiably debatable as this design potentially brought the dry objective to its peak of perfection and it is doubtful if there is anything to better the British achromatic objective with the triple front lens, a double middle component and a double back lens, as shown in Fig. 56. In 1854, Smith (Mathematics Professor at Cambridge) pursued the development of the objective by combining quartz with glass.

So far we have seen the development of the objective up to the high state of perfection reached in Lister's or Amici's triplet-doublet combination. Meanwhile the search for higher aperture (or angle) went on and in the same year Wenham produced a combination having a single front lens; this, however, was not equal to the Lister objective.

The next advance of importance occurred with Amici's develop-

ment of the water immersion objective, although it should be remembered that Brewster had suggested water immersion in 1813, in spite of the fact that the principles underlying the improvement were not understood at the time.

Prazmowski and Hartnack, in Paris, developed these objectives to a high degree, and in 1868 Powell and Lealand adopted it and bettered the products of the Parisian makers, producing some of the finest water immersion objectives ever made. In 1877 the system reached its peak, apertures as high as 1·23 being obtained, Spencer, Tolles and Wales, in America, also producing some fine lenses of high aperture at this time.

In 1873 Tolles produced a $\frac{1}{5}$-in. glycerine immersion objective with a duplex front lens, *i.e.*, a front combination consisting of two uncorrected lenses instead of an achromatised pair; this lens had a 110° balsam angle.

At this time water immersion objectives were being produced to an increasing degree of perfection, Abbe's diffraction theory being as yet unknown, we have seen how false was the value put on aperture as then interpreted. This resulted in wholly useless lenses being produced, as power alone was considered to be the characteristic to be aimed at. For example, lenses of $\frac{1}{25}$ in. with an N.A. 1·2 were common, and focal lengths of $\frac{1}{35}$ in., $\frac{1}{40}$ in. and $\frac{1}{50}$ in. were produced in this quest after high magnification.

This state of affairs existed up to 1877, the year in which Abbe's diffraction theory was made public. This led to a great advance, inasmuch as the "homogenous immersion" system was evolved as distinct from the "heterogeneous immersion" systems previously in vogue, the latter using mainly water as an immersion medium. The significance of the "homogeneous" system has already been pointed out.

Actually, Tolles in New York produced the first "homogeneous" system in 1873, with his $\frac{1}{5}$-in. objective designed to work in soft Canada balsam, but, unhappily for him, this idea was not developed beyond the stage of an initial trial by Dr. Woodward of the U.S. Army Medical Department. This system was, however, unwieldy in use, and the credit for the development of a homogeneous system of universal application lies entirely with Abbe.

The introduction of the homogeneous system led to an immediate development of the higher powers in general; for example, Powell and Lealand increased the aperture of a $\frac{1}{12}$-in. objective from 1·25 to 1·43 N.A., and many other lenses developed similarly.

It has been shown that achromatism obtained by flint and crown glass is not complete in that only two colours are achromatised, leaving a residual secondary spectrum.

In 1886 Abbe, working in conjunction with Schott and Zeiss, produced new vitreous compounds, by the use of which three

colours could be corrected and the secondary spectrum eliminated, although there was a small residual tertiary spectrum which, as has previously been explained, is corrected in the special compensating eyepieces used with these " apochromatic " objectives.

In this way Abbe was able to produce an objective with an aperture of 1·63, which he designated " Apochromatic." This objective, however, had no practical advantages and was not a commercial proposition.

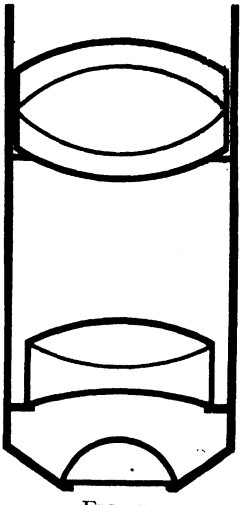


FIG. 57.

At the present time the majority of manufacturers produce apochromatic objectives and there is very little to choose between them.

In the apochromatic objective the removal of the secondary spectrum depends upon the use of the mineral fluorite, thus in visual examination and in photomicrography the freedom from colour is a very big asset, as the high quality of the results achieved by apochromatics testifies. Thus we see that in these objectives chromatic aberration is corrected for three colours and spherical aberration for two colours, so that practically all the images produced by the spectrum lie in the same plane and are equally focussed.

The lens train of a typical apochromatic objective is shown in Fig. 57. As will be seen, it consists of a hemispherical front lens followed by a doublet and triplet. Modern apochromatic objectives



FIG. 58.

are illustrated in Figs. 58 and 60, the former showing a range of " apochromats " produced by Bausch and Lomb. The correction collar on the 4-mm. objective will be noticed, and as a comparison Fig. 59 is a photograph of an early ¼-in. objective of unknown make, having the sliding tube method of correction incorporated, although

this is better than the early ones produced by Ross, inasmuch as the back lens is the movable component. A sectional view of a modern 2-mm. aprochromat by Watson is shown in Fig. 60.

When apochromatic objectives were first introduced, the majority of microscopists were sceptical, the images produced by them were so white and devoid of secondary colour, which seemed to give contrast and character to the images of the old achromats that this great advance might have been partially obscured had it not been for the early enthusiasts, such as Nelson, who helped to popularise them.

As a result of the general acceptance of the superiority of the apochromatic objective, rapid development occurred in the making of optical glass, and all objectives today, even the cheapest, are made of superior glasses that were not available before the advent of apochromatism.

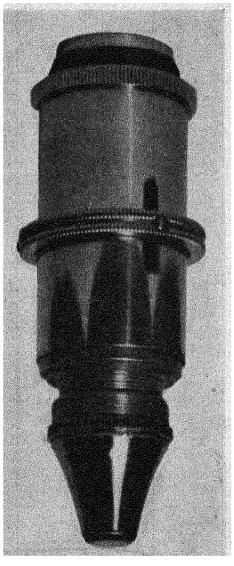


FIG. 59.

However, in spite of the undoubted superiority and high price of apochromatic objectives, it would be absurd to suggest that first-class work cannot be done without them. Good results with

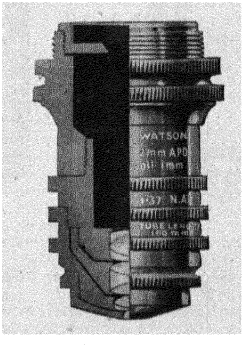


FIG. 60.

the lower grade lenses depends more upon the skill of the user than the possession of superior equipment.

The very nature of the corrections and the materials used in obtaining them must necessitate a high price for apochromatic objectives, and soon after their advent it became apparent that there was an increasing demand for something better than the standard achromatic objective and yet not so costly as the "apochromatic," and so by the use of fluorite most manufacturers produced objectives which are to this day termed semi-apochromatic. These lenses give an image closely approaching that of the apochromatic, although the secondary spectrum is not completely eliminated, but to offset this slight disadvantage these objectives possess, in a great number of cases, a higher aperture than the apochromatic and,

in fact, in certain cases, as in that of Messrs. Watson's $\frac{1}{4}$-in. semi-apochromatic, which they term holoscopic, the N.A. of 0·95 is greater than that of their $\frac{1}{6}$-in. apochromatic of N.A. 0·85, which serves to point out the perfection to which the modern semi-apochromatic lens may be brought and indeed, when skilfully used, the difference in performance is only detected with difficulty by the expert.

A pair of fluorite objectives by Bausch and Lomb are shown in Fig. 61, while one of Watson's holoscopic series is illustrated in Fig. 62.

So much for the high-quality objectives, now let us examine the modern achromatic objective. These objectives (as has been previously stated) were the precursors of all modern objectives and the modern equivalent of the original achromats is such a vast improvement that the pioneers of their development would be astonished if confronted by them, due to the production of new optical glasses and improvement in manufacturing methods, modern

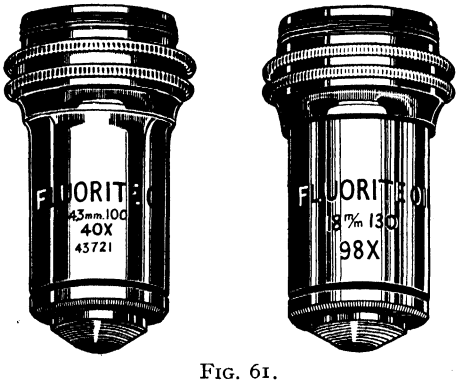


FIG. 61.

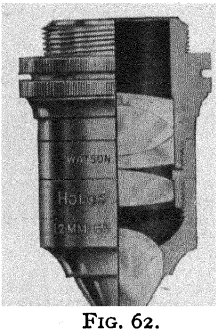


FIG. 62.

achromatic objectives of reputable make do not fall so far short of the apochromatic as one would be led to believe. For example, a usual N.A. for an achromatic $\frac{1}{6}$ in. is 0·8, whereas that for an apochromatic is 0·8 to 0·9 N.A., and the spherical and chromatic corrections are such that they will withstand high eye piecing without breaking down ; furthermore, in most good achromats, the whole of the aperture is capable of utilisation provided always that there is sufficient difference between the refractive indices of the mounting medium and specimen. This rule of course applies, in a more or less degree, to all types of objectives.

This type of achromat is the most popular objective in use at present as they are comparatively inexpensive and very efficient, and for general work are hard to surpass. There are of course many types of cheaper achromats on the market, but these are generally much inferior and much less expensive, from which arises the question of identification. If an objective is suspected of being inferior in quality, and there is no maker's name or N.A. engraved on it, then

it is far better to leave it alone as usually in such cases the lens is of inferior manufacture and low efficiency. When the term " achromatic objective " is used in succeeding pages, it should be taken to refer to the high-quality lens. The following table will give some idea of the difference in performance of the various types of objectives:—

Details	Apochromatic			Semi-apo			Best achromatic			Cheaper achromatic		
Focal length .	$\frac{2}{3}''$	$\frac{1}{6}''$	0·1 mm $\frac{1}{12}''$	$\frac{2}{3}''$	$\frac{1}{6}''$	0·1 mm $\frac{1}{12}''$	$\frac{2}{3}''$	$\frac{1}{6}''$	0·1 mm $\frac{1}{12}''$	$\frac{2}{3}''$	$\frac{1}{6}''$	0·1 mm $\frac{1}{12}''$
N.A. .	0·3	0·85	1·37	0·45	0·95	1·37	0·28	0·7	1·28	0·17	0·6	1·15
O.I. .	20	14·1	11·4	30	15·8	11·4	18·7	12·3	9·4	11·3	9·25	8·3

The figures for the best achromatic are taken from Messrs. Watson's range of " parachromatic " objectives, likewise those for

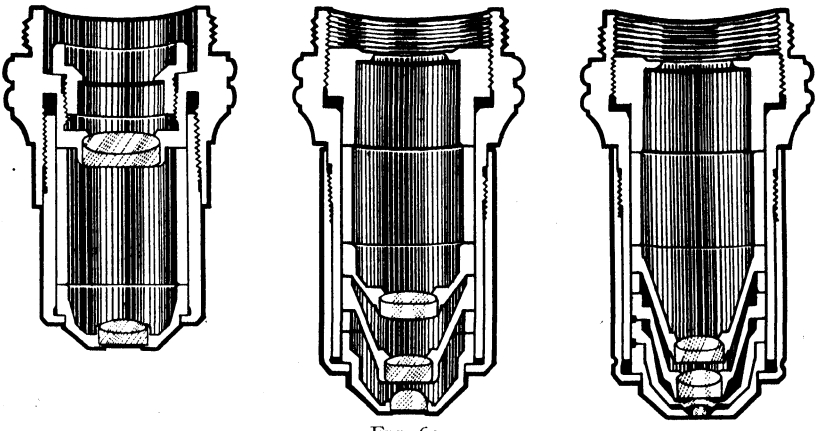


FIG. 63.

the apochromatic and semi-apochromatic. This table shows the vast difference between the two types of achromatic objectives (the figures for the cheaper type being taken from inferior continental objectives) ; at the same time it shows how close to the apochromatic is the high-grade achromat, though the impossibility of obtaining apochromatic performances from these latter must not be overlooked, due to their being corrected for two colours only.

The optical trains of representative modern achromats are shown in Fig. 63, which is a sectional drawing of three of Bausch and Lomb's achromats. The illustration also shows the modern method of positioning the various components in the mount. It will be seen that the low-power objective consists of two doublets, the medium power has a single front lens followed by two doublets, while the high-power has a duplex front, consisting of an uncemented pair, the front component of which is hyper-hemispherical, followed by two doublets.

So far, then, we have seen how the objective was developed and the erroneous ideas which existed during the early days up to the time of the diffraction theory of resolution, from which time the greatest strides in lens design were made and which led to the development of the apochromat and all the highly efficient modern objectives.

We know the meaning of numerical aperture to be the all-important factor for efficient working, which figure, coupled with Nelson's Optical Index, enables one to pass judgment on any particular objective.

We also see that of modern objectives there are three main types, the apochromatic, corrected for three colours and giving a superb image, the semi-apochromatic, whose corrections are not quite as good as the apochromatic, but which, nevertheless, closely approach the apochromatic in performance if intelligently used, and thirdly, the achromatic objective which has been brought to a high standard of perfection.

Apart from the three main types of objectives outlined above, there are, of course, those designed for special purposes. It has been shown that resolution is increased with a shortening of the wavelength of the light used. This may be accomplished for visual purposes by the use of colour filters transmitting light in the blue or blue-green region of the spectrum, and the resolution obtainable with white light may be nearly doubled by the use of ultra-violet light and the camera. For this latter purpose the complete optical train of the microscope must be constructed in quartz, as the ordinary glasses offer too much resistance to the passage of ultra-violet light. Such quartz objectives are corrected strictly for this wavelength (275 mu), and hence are called monochromats. In this way it can be shown that the resolving power of a monochromat of 1·25 N.A. is equivalent to an effective N.A. of approximately 2·5.

Another important characteristic of objectives is " penetrating power," or " depth of focus." This term is generally interpreted as meaning the ability of a lens to focus more than one plane simultaneously ; this is theoretically impossible, but in practice it is found that the image appears to be sharp over a certain depth, depending on the focal length of the objective, inasmuch as the longer the focal length the greater the depth of focus. However, this does not signify a contradiction of the theoretical considerations of the phenomenon ; its apparent existence does, on the other hand, depend upon the inability of the eye to appreciate minute differences of focus in the image. Thus, let us assume that we have a portion of some object in focus ; we know that the image is built up of rays of light converged to a point, the whole forming an assembly of an infinite number of such points, but actually this does not occur (except at the exact plane of focus) ; these points instead take the

form of a " disc of confusion," which is a point at the point of focus and becomes larger as we move away from the focal point ; but for lenses of long focus and small apertures, the rate of increase in the diameter of the disc of confusion is smaller than with lenses of short focal length and high aperture.

This will perhaps be made clearer if we refer to Fig. 64. Here we have two rays, R1 and R2, made to meet at a point on the axis P, and it is assumed that they originate from a point of an object and

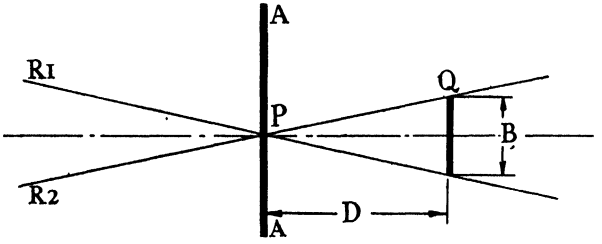


FIG. 64.

pass through a system of lenses ; at the point of focus is a screen AA. Now, it is clear that at this point, theoretically, the rays unite to a point, suppose the screen be moved backward a distance D to the point Q, then, instead of the rays producing a point image, it is clear that they will produce a disc of light of diameter B. This is our disc of confusion.

Now let us assume that the diameter B of the disc of confusion is the minimum diameter capable of perception by the human eye. Clearly then, for all positions of AA between the points P and Q, the difference in diameter of the disc will not be appreciated; in other words, as the diameter D is the minimum perceptible, the eye could not distinguish any difference in sharpness of the image between P and Q.

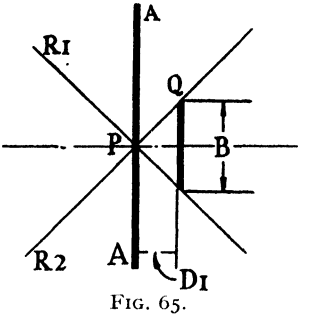


FIG. 65.

The rays shown in Fig. 64 would be produced by a lens of long focal length and small aperture, hence the angle between them is small and the distance comparatively long. If we now refer to Fig. 65, it will be seen that the rays make a much greater angle, such as would be produced by a short focus, large aperture lens, in consequence of which the corresponding distance D1 between P and Q for the same minimum diameter of the disc of confusion is considerably reduced. This distance is of course the depth of focus of the lens. Thus we see that " depth of focus " is in reality a physiological misrepresentation of the actual condition and exists only in the imagination, anent which the accommodating power and acuteness

of vision of the observer will play their part in determining its value. The refractive index of the mounting medium will obviously also have an effect.

As the aperture of any particular lens has a big influence on its depth of focus, it is necessary to take these two constants into account when designing objectives, as they tend to negate each other thus making it impossible for any objective to possess a maximum of each property, so that for ordinary lower power work, where high resolution is not so necessary, depth of focus is to be preferred to high aperture, as then objects which are relatively thick may be seen in their entirety. On the other hand, when we come to high powers, where good resolution is of the highest importance, a large depth of focus is sacrificed to a high numerical aperture. For example, an objective of 1·40 N.A. has, at a magnification of 1,000 diameters, a depth of focus of no more than 0·0005 mm., or half a micron, so that objects which are thicker than this figure are viewed by the examination of a series of optical sections by successively focussing through it. By studying a specimen at different levels, as it were, a true idea of the general structure might be obtained, but this necessitates some practice.

For photomicrographic work, however, a succession of superimposed images of the object at different levels throughout its thickness is obviously impossible, so special objectives are made which incorporate an iris diaphragm, thus enabling the aperture to be reduced and the depth of focus increased. Of course it must be understood that in the use of such a diaphragm resolution is affected owing to the reduction in aperture, and a compromise must be effected so that the minimum sacrifice of aperture is made for a maximum increase in depth of focus.

Other characteristics of objectives are illuminating power, which governs the brightness of the image and depends on the aperture and the amount of light emitted by the object; it varies as the square of the numerical aperture, all other factors being constant, and the light transmitting power, which varies with the material, thickness and number of surfaces in the combination and whether the objective is dry or immersed. The chief factor affecting it is loss by internal reflection in the system, thus it is possible that objectives of the same focal length and aperture will give images differing in brightness.

The distance between the front lens of an objective and the object when it is focussed is known as the working distance of the objective. This distance is, in practice, considerably less than the focal length of the objective. This is due to the focal length of an objective being measured from its two effective centres, as such a lens is thick and these do not coincide with the physical centre, as is the case with a thin single lens; and as part of the lens system

lies between the front effective centre and the object, it will be seen how the working distance is less than the focal length.

When a series of objectives is so designed that on interchanging there is no, or very little, alteration required of the focussing adjustment of the microscope, they are said to be parfocalled. This is very convenient when making quick changes of objective.

As the objective is the heart of the microscope and its efficiency depends upon the maintenance of the distances separating its components, it must be treated with the greatest of care. The use of a revolving nose piece to carry three or more objectives is strongly advised as a means of minimising the handling of objectives, by enabling the user to rapidly change objectives without removing them from the instrument.

Apart from the external surfaces of the front and back lenses, objectives should on no account be taken apart for cleaning, as there is a grave risk of upsetting the inter-component distances. If they are of reputable make, the cleanliness of the internal surfaces may be taken for granted, but if by some unhappy chance these surfaces are suspected of being dirty, in which case the image will be hazy and far from clear, then the lens in question should be returned to the manufacturers for cleaning.

The precautions outlined in Chapter III apply equally well to objectives, in addition to which the front lens should always be examined after having been used on uncovered liquids, and in the event of its having been in contact with the liquid under examination, it should be immediately wiped dry and carefully cleaned as more than momentary contact may cause irreparable injury.

In the case of immersion objectives, the immersion medium should be cleaned off immediately after use, particularly in the case of immersion oil, which is the most widely used immersion medium, as this eventually becomes hard on exposure to air and the risk of loosening the front lens when removing it is great. Cedarwood oil should never be removed with a solvent, for example xylol or benzene, as these substances are also solvents for the cement with which some front lenses are secured. Even if it is known that an immersion objective has its front lens fixed in with a metal bezel, solvents should not be resorted to, as it has been known for them to creep through the bezel and so reach the cemented joints of the internal combinations.

The best method of cleaning an immersion objective is to commence by wiping off the majority of the oil with a piece of dry lens tissue. The front of the objective may then be polished with the aid of the tongue and a clean soft handkerchief. If this rule is adhered to there is no risk of loosening the front lens of an expensive objective.

Immersion oils should not be allowed to become too viscous as

they then become unmanageable and a risk of moving the cover glass is incurred; on the other hand, they should not be used if too thin, as then the difficulty of maintaining homogeneity in the system becomes apparent. If a sample of oil has become too thick, no attempt should be made to thin it down with any solvent, as this will upset its refractive index and lead to poor results. *Anent this, it is always advisable to use the oil supplied by the makers of the objective as they adjust its refractive index to suit the lens and thus ensure its efficient working.*

On commencing the microscopical examination of an object, it is advisable to adopt the following procedure :—

(*a*) Examine with a low power objective to obtain a general idea of the structure. Suitable objectives are 1½ in., 1 in. and ⅔ in.

(*b*) Having decided on the portion of the object to be more closely examined, change to a higher power, say ½ in. or ⅓ in. This will indicate the presence of any finer structures.

(*c*) If (*b*) shows the necessity for higher magnification, then the high powers may be used in succession, such as ¼ in., ⅙ in. and finally the $\frac{1}{12}$ in. oil immersion.

In this way the object is developed progressively and much more is learned about its structure than if it was examined straight away with a high or medium power.

Care should be exercised in focussing any objective from about ½ in. downwards, as the working distance gets progressively smaller. The general method adopted is to lower the objective, while carefully watching it, until it almost touches the cover glass then, by looking into the instrument and racking the objective slowly upwards, the object can be brought into focus without the risk of going past it and pushing the front lens of the objective through the cover glass, which tragedy has frequently occurred when the focussing has been carried out in the reverse direction. This is also a very good reason why the thinnest cover glasses available should be employed, as nothing is more calculated to annoy than to find that after having spent much time and trouble over mounting an object for high power examination, too thick a cover glass has been used and the ⅙ in. or $\frac{1}{12}$ in. objective will not focus through it. Therefore always use the thinnest cover glass possible.

The choice of suitable objectives is dictated by the type of work involved, and for general work as wide a range of powers as possible is advisable The following sizes are recommended as a useful range: 1½ in., 1 in., ½ in., ⅔ in., ⅓ in., ⅙ in., $\frac{1}{7}$ in. O.I. mm., $\frac{1}{12}$ in. O.I. mm. These objectives with suitable eyepieces cover a very wide range of powers from 17 to 2,000 diameters. The author uses this range for general work, all the lenses being of the high-grade achromatic type, in addition to which a ½ in., ⅓ in., ⅙ in. and $\frac{1}{12}$ in. of the apochromatic or semi-apo-type are extremely useful for extra

critical work and photography, although the achromatic, which are Watsons' parachromatic objectives, have produced some excellent photomicrographs, up to 500 diameters, which bear close comparison with those produced from apochromatic objectives.

Finally, it should be pointed out that unless one has thoroughly trained oneself to the recognition of aberrations which are present, even in lenses of good quality, the expense of high-class objectives is

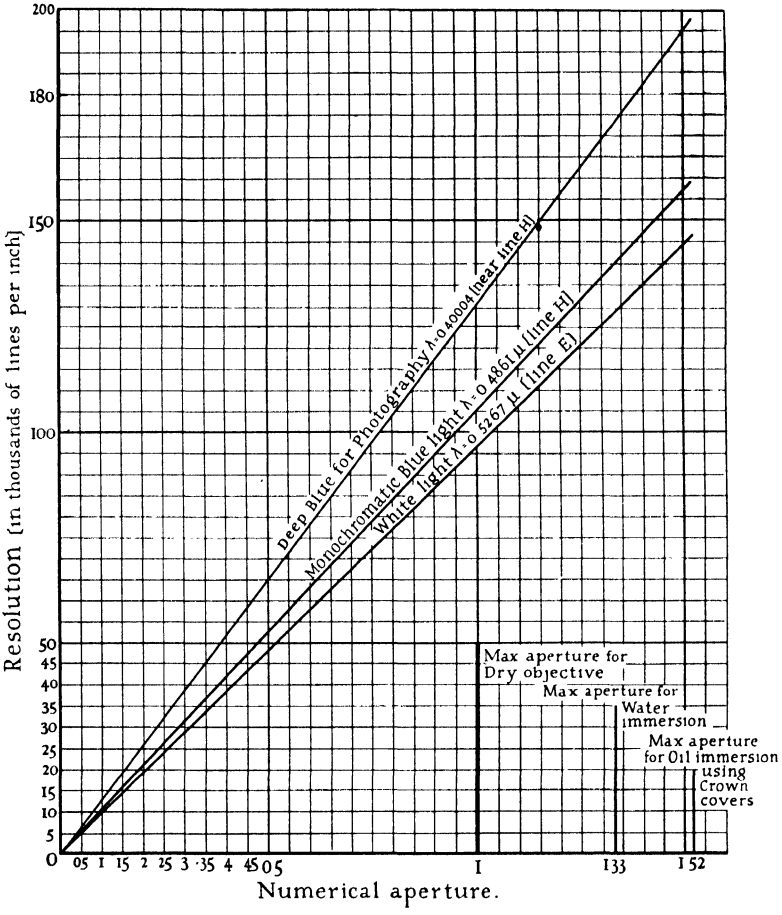


FIG. 66.

not justified, as the fine points of superiority will pass unheeded by one whose powers of discrimination are undeveloped. The more obvious differences are of course noticeable by the veriest novices, but the detection of the important and more subtle differences require careful training. Therefore a word to the novice: train yourself not only to look at the image but to understand and appreciate the significance of the smallest details and whenever possible try comparing different objectives, so that in time you will learn to detect the minute differences between the types of high-quality objectives.

In order that the effect of increase on aperture may be further understood, Fig. 66 shows the curves of N.A. against resolution, for light of three wavelengths. This also shows the effect of shortening the wavelength of the light used very clearly; thus we find that by shortening the wavelength of a light from 0·526 μ to 0·4861 μ, by using monochromatic blue instead of white, with an N.A. of 1·2, the resolution is increased by 9·712 lines per inch from 115,692 to

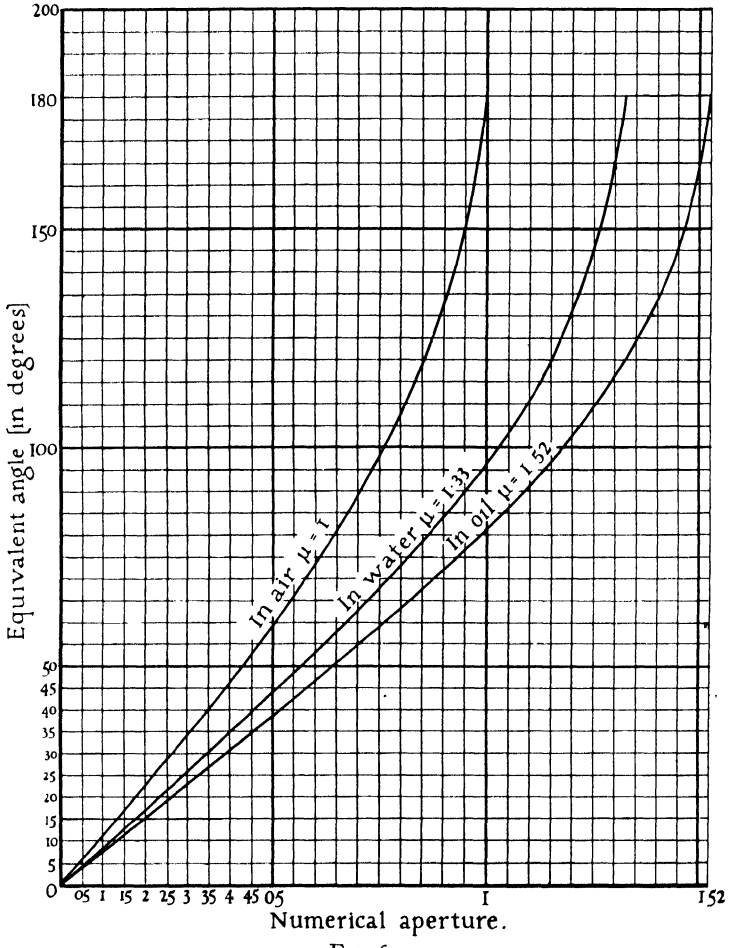


FIG. 67.

125,404. Similarly, by shortening the wavelength still further from 0·4861 μ to 0·4000 μ, by using photographic deep blue, we get a further increase of 26,993 lines per inch from 125,404 to 152,397. This example will show the importance of using the shortest possible wavelength commensurate with any particular set of working conditions.

The three curves in Fig. 67 show the advantage of homogeneous immersion; the curves represent the relation of the total

angle of the cone of light accepted by an objective, plotted against N.A., from which we see that if an objective accepts the maximum theoretical cone of 150° in air, its maximum theoretical aperture, if designed as a dry lens, is 0·95 N.A. When the lens is designed as a water immersion system, the maximum aperture is increased to 1·26 N, provided that it accepts a cone of 150° in water. In the same way, this objective would have an aperture of 1·46 if it accepted a cone of 150° in oil and was designed as a homogeneous immersion system.

Now let us see what effect this will have on the resolving power of the lens in question. By referring back to Fig. 66 we find that for an aperture of 0·95 N.A., we may expect a resolution of about 91,500 lines per inch for white light ; as a water immersion system, this objective would then give a resolution of approximately 120,180 lines per inch at the N.A. of 1·26, whereas when used as a homogeneous immersion system the resolution to be expected at 1·46 N A. will be 141,000 lines per inch, which shows an increase of 49,500 lines per inch over that for the dry objective.

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(1) CARPENTER and DALLINGER. "The Microscope and its Accessories Vol. I. Churchill

(2) Reprinted by permission from; "Handbook of Chemical Microscopy" by Chamot and Mason, published by John Wiley and Sons, Inc., New York.

CHAPTER V

THE SUBSTAGE CONDENSER

If we intend to use the microscope as a scientific instrument, the condenser is an absolutely indispensable part of the instrument. We have seen, in the previous chapter, how essential it is that the objective be supplied with a cone of light possessing an angle equal to that of the maximum cone it will accept, thus ensuring that the greatest possible number of diffracted rays enter it.

The effects of restricting the entry of diffracting rays on the resolving power of the objective were gone into and the reason for the use of a condenser explained. Indeed, the microscope used without a condenser is nothing more or less than a magnifying glass and as such has no value as a scientific instrument of the highest precision.

We know very well that the highest degree of correction is required in objectives, for critical work, and therefore it follows that for the objective to give of its best, the condenser must be included and used. Thus, let it be repeated again, that the optical train of the compound microscope must include this lens, commencing with which we have the following series of lenses :—

(1) Condenser.
(2) Objective.
(3) Ocular.
(4) The eye.

The condenser was used very early in the history of the microscope, although it is impossible that its importance can have been realised at that time, in view of the very elementary state of the knowledge of the underlying principles of the instrument ; but in spite of this an early horizontal microscope produced by Bonanni in 1691 was fitted with a condenser of sorts, showing that even then the improvement due to the inclusion of a condenser had been discovered, though it was only used as a means of increasing the intensity of illumination. The improvement in resolution was, in all probability, credited to the improved lighting, and it is of interest to note that in 1694 Hartsoeker improved on Bonanni to the extent of fitting a condenser as part of his instrument produced in that year ; apparently he had been able to effect some improvement by so doing, and although it could not have been so important then it is of the utmost importance to-day.

No improvement of note appeared until 1829, when the lens system known as " Wollaston's Doublet " was used as a condenser, with a marked improvement in the performance of the instrument.

Pritchard further improved it by altering the inter lens distances and placing a diaphragm between them. Good results were claimed for this arrangement, but something better was required.

With the discovery and use of achromatic objectives and the understanding of their theory, it was soon realised that in the compound microscope, lenses in the system were of equal importance; thus Sir David Brewster, in 1831, said: "I have no hesitation in saying that the apparatus for illumination requires to be as perfect as the apparatus for vision, and on this account I would recommend that the illuminating lens be perfectly free from chromatic and spherical aberration, and that the greatest care be taken to exclude all extraneous light, both from the object and from the eye of the observer." This line of thought has since been fully confirmed in practice.

The discovery of the importance of the ability to focus the condenser seems to have occurred with the use of Wollaston's doublet in this capacity, anent which Carpenter and Dallinger (1) say: "In 1829 Wollaston recommends the focussing of the image of the diaphragm by means of a plano convex lens of $\frac{3}{4}$ in. focus upon the object," and in 1832 Goring says concerning it, "There is no modification of daylight illumination superior to that invented by Dr. Wollaston"; but Sir David Brewster objected to this, contending that the source of light itself should be focussed upon the object. He preferred a Herschelian doublet placed in the optic axis of the microscope; but whilst there is a very clear difference between these authorities, we can now see that both were right.

Goring, who was also a leader in the microscopy of his day, used diffused daylight, and as the lens he employed was a plano-convex of $\frac{3}{4}$ in. focus, the method of focussing the diaphragm was as good as any other, because the diaphragm was placed at a distance from the lens of at least five times its focus, so that the difference between diaphragm focus and "white cloud" focus, or the focussing of the image of a white cloud upon the object was not very great.

But Brewster was writing of a flame from a saucer of burning alcohol and salt, when he insisted on the bringing of the condenser to a focus on the object, and in this he was beyond all doubt, right.

In 1839 Andrew Ross gave some rules for the illumination of object in the *Penny Cyclopædia;* these were:—

"(1) That the illuminating cone should equal the aperture of the objective and no more.

(2) With daylight, a white cloud being in focus, the object was to be placed nearly at the apex of the cone. The object was seen better sometimes above and sometimes below the apex of the cone.

(3) With lamplight a bull's-eye is to be used to parallelise the rays so that they may be similar to those coming from a white cloud."

Apart from showing the early appreciation of the necessity for a condenser, the foregoing extract also summarises the method of employing it. Ross's three points hold good to-day.

Let us examine these points. The first one refers to the necessity for the illuminating cone to be equal to the maximum cone accepted by the objective.

This point has already been dealt with and is obvious, as cutting down the illuminating cone virtually cuts down the aperture of the objective. It should also be remembered that the cone supplied should be aplanatic, that is all the rays should be brought to a focus at the same place. Suppose, for example, we take a condenser which has an aplanatic cone of 0·50 N.A., and suppose that we are using it with a 1/12 oil immersion objective of 1·20 N.A., whereas we would get some sort of result, it will be seen that the objective cannot function at an aperture higher than 0·5 under these conditions, and we would be losing nearly 25 per cent. of the resolving power of the lens, which latter is approximately the sum of the numerical aperture of the objective and the aplanatic cone of the condenser, divided by two. Thus in our example we have :—

$$\frac{\text{(N.A. Objective plus N.A. Condenser)}}{2} = \text{Effective N.A. of objective.}$$

$$= \frac{1{\cdot}20 + 0{\cdot}50}{2} = 0{\cdot}85.$$

This substantiates the first of Ross's points and Brewster's remarks regarding the quality of condensers. We should, therefore, use a condenser having an aplanatic cone large enough to accommodate the largest apertured objective we are likely to use.

The second point, dealing with daylight illumination, may be dealt with briefly. Artificial illumination is so general to-day owing to its greater efficiency and convenience, that daylight is now little used because, as Ross says, " a white cloud has to be focussed on the object," and apart from being able to find a cloud in the right position and at the right time, clouds have a disconcerting habit of moving at some considerable speed. The annoyance of having to suddenly find another cloud, or move the microscope so as to keep the same cloud in focus, needs no emphasis, hence the general use of artificial light nowadays.

Ross's second point does, however, bring one important factor—which forms the third point—to the foreground. This is the necessity for the use of *parallel light* with the condenser, and the rays of light emanating from a white cloud are, to all intents and purposes, parallel. This gives the next golden rule for the microscopist : *Never, in any circumstances, use the concave side of the mirror when using the condenser.* This point will be dealt with in greater detail in subsequent pages.

The need for parallel light entering will be appreciated when it is realised that if the condenser is to supply its maximum cone, the image of the light source has to be focussed at its principal focus, because in this position the angle of the cone is at its maximum and the rays at their greatest obliquity, for we have seen in Chapter I how, as the focal point moves away from the principal focus, the angle becomes smaller and smaller, and as it moves inside the principal focus this latter becomes virtual. So in order to obtain the maximum efficiency from the condenser, the light entering it must be parallel, and this is obtained from an artificial source placed at the principal focus of a bull's-eye or similar lens.

Thus we may say, for the ideal condenser, that :—

(1) It should be capable of furnishing the maximum full cone free from spherical aberration.

(2) This cone should also be devoid of chromatic aberrations.

(3) It should have a working distance long enough to focus through ordinary slides. (This thickness is usually taken at 1·5 mm.).

For conditions of ideal illumination with transmitted light :—

(1) The illuminating cone should be equal to the aperture of the objective used.

(2) The object should be placed at the apex of this cone.

(3) Parallel light entering the condenser is absolutely necessary.

These conditions are the ideal. In practice it is impossible to produce a condenser which is not more or less undercorrected, with the result that the central rays do not entirely coincide with the peripheral rays.

In some condensers the undercorrection is so great as to reduce the central portion of the cone to mere annular form when use for transmitting a wide cone. This of course is an undesirable quality.

It is one of the anomalies of microscopic optics that it is virtually impossible to use an objective at its full aperture, they usually break down under conditions of ideal illumination ; hence we must put up with the sacrifice of the ideal, or more likely, if the object lacks sufficient contrast, the ideal must be modified ; but assuming we have a suitable object and a perfect objective, it may be taken that as we increase the aperture of the cone, the perfection of the image increases until the illumination is equal to the full aperture of the objective. It is the author's experience, however, that even with an opaque black object, the very best of objectives show a certain amount of glare round the edge of the object when used at full aperture, and supplied with a full cone equal to its aperture, the best results being obtained at about 0·8 of the full aperture, although the ideal may be realised with the best apo- and semi-apochromatic objectives up to 0·4 N.A. (beyond which power no improvement seems possible).

A condenser which has enjoyed a wide popularity for many

years and even to some extent at the present time, is Abbe's *chromatic condenser.* The position of this condenser needs clarifying; and perhaps the best method is to quote Carpenter and Dallinger, (1) who deal with the matter in a manner difficult to improve upon ; they say :—

" The optical productions of Abbe are too well known and too valuable as a rule to make it needful to be other than perfectly frank concerning so important a piece of apparatus as this, and there can be no doubt that the wide popularity of this instrument is due, not so much to intrinsic merit as to the fact that it has been employed much by those who, previously ignorant of the value of any condenser, have at once perceived the enhanced value of the results yielded by its means.

" To those who have made the scientific use of the microscope a careful study in England it has been a persistent source of regret that it was so long and pertinaciously taught that the correct histological microscope (author's note, the term ' histological ' means the study of the micro structure of animal and plant tissues), must be of the Hartnack type and that it should be used with narrow-angled dry lenses, perhaps a $\frac{1}{7}$ in., and no illumination but that afforded by a small concave mirror, the focal point of which is extremely doubtful and unknown, and in practice wholly disregarded. No doubt a student instructed on these lines would be astonished indeed when he exchanged such a practice, for the illumination and improved image afforded by an Abbe condenser.

" The fact is that a large part of the admiration that has been expressed for this condenser has resulted, not from a comparison of its results with those of other high-class condensers, but of images obtained without any substage optical arrangements at all, placed in contrast with the results obtained by using this condenser against the same objective when used without its aid. But that even these images are entirely inferior to the images obtained by the higher order of achromatic condensers, we only require the practical testimony of Prof. Abbe to prove ; for he has since produced an achromatic condenser of much merit."

With regard to the chromatic condenser, they continue :—

" The power of this condenser is low and its aperture is very large (1·36), hence, beyond the fact that it is not achromatised, it has enormous spherical aberrations. The distance between the foci of the central portion and of a narrow annular zone whose internal diameter is $\frac{5}{8}$ in. is $\frac{1}{40}$ in. Its aplanatic aperture is therefore only 0·5. Now, whilst it is a gain of no inconsiderable character to have an achromatised condenser, yet the point of vital importance is that it should be *aplanatic ;* the best condenser is always that which will transmit the largest aplanatic cone."

We see then that, although Abbe's chromatic condenser consider-

ably improves the image as obtained without any condenser at all, it is really unsatisfactory for work of any consequence, owing to its being entirely uncorrected. Its only use is a means of producing an image of better quality than with the mirror alone, for purposes of very elementary instruction in schools and laboratories, being useful as a means of concentrating the light, and nothing more, but it is

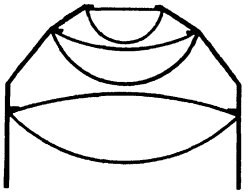


FIG. 68.

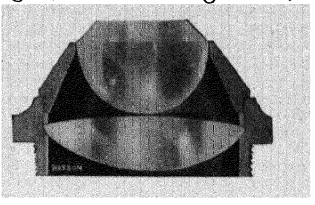


FIG. 69.

effective for dark ground illumination for the lower powers and serves as a cheap method of attaining this end.

It has been shown that with the chromatic Abbe condenser objectives having an aperture greater than 0·5 N.A. cannot give their full resolving power. In research work, therefore, or in any work where unknown structures are to be investigated and objectives are required to work at their maximum efficiency, the employment of a condenser whose aplanatic aperture is equal to that of the objective is essential. It is not sufficient to use apo- or semi-apochromatic objectives or even modern high-class parachromatics, in which the colour corrections reach a high state of perfection and expect the chromatic beam given by Abbe's condenser to help them produce optimum results. It is obvious that, no matter how well corrected an object is for colour, it will not eliminate it when it is in the beam of light supplied to it. The corrections of objectives are necessarily based on the assumption that the beam of light supplied to them will be corrected.

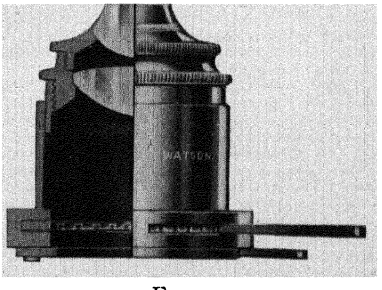


FIG. 70.

A diagram of the lens train of Abbe's chromatic condenser is shown in Fig. 68. It will be seen to consist of three lenses, the front being hemispherical, while the two lower lenses form a Herschelan doublet. This is the most perfect form of this type of condenser, a simpler form is that produced by Messrs. Watson and illustrated in section in Fig. 69, which shows the lens train, while Fig. 70 shows the complete apparatus with its associated fittings.

Actually, the Abbe condenser was not produced until the latter part of the nineteenth century, much better achromatised condensers having been produced before it.

About 1865 Powell and Lealand produced an excellent achromatic condenser with an angle of 170° (air). Its power was $\frac{1}{5}$ in. and as such it was suitable for objectives of $\frac{1}{4}$-in. focus and higher powers, but it could also be used for powers as low as 1 in. by the simple expedient of removing the top lens, which left a still corrected combination of reduced power and angle which was more suitable for the lower-powered objectives. This lens seems to have been the forerunner of the modern corrected condenser, and this method of extending its usefulness is used to-day, although the removal of the top lens might have appeared to be a compromise evolved to overcome the necessity for two corrected condensers, one for high, another for low powers, the general tendency then was to use two lenses.

The author has found that there is no appreciable difference between the use of a specially designed low-power condenser and a high-power modern condenser used with the top lens removed, provided, of course, that the aplanatic aperture is the same in each case. Under these conditions, there is no detectable difference in the corrections of the two condensers, which would seem to indicate that there is no relative loss in efficiency by adopting the principle of removing the top lens.

The next advance came with the advent of the dry apochromatic condenser, and until the development of the homogeneous immersion system, the aperture of these achromatic condensers (which were of course dry) could not exceed N.A. 1·0, which figure was quite common and a lens with a total aperture of 1·0 with an aplanatic aperture of 0·9 was not a rarity.

When the homogeneous immersion system appeared with objectives having apertures as high as 1·40 to 1·50, something had to be done by way of supplying these objectives with a cone of light of similar aperture, and Powell and Lealand produced the first homogeneous immersion chromatic condenser which was quickly superseded by an achromatic lens on the immersion system. This combination consisted of a duplex front lens followed by two doublets, it had an aperture of 1·40 and was capable of working through a slide 1·5 mm. thick.

Thus we see that condensers in general fall into three main classes, those of the chromatic or Abbe type, which are not of much use for serious work, form the first group, which is followed by the more important achromatised high-power condenser possessing a large aplanatic aperture and capable of accommodating the complete series of dry objectives from 1 in. upwards, the top lens being removed for all powers below $\frac{1}{4}$ in. The greatest possible aperture of these condensers is 1·0. The final group consists of the oil immersion condensers having apertures as high as 1·40 immersed and 1·0 when used dry; with the top lens removed a good

condenser of this type is capable of supplying an aplanatic cone of N.A. 0·6.

The two condensers illustrated in Figs. 71 and 72 are in the second group. The first, which is Messrs. Watsons' " Universal " condenser, is designed on their well-known holoscopic principle. It has a total aperture of 1·0 with an aplanatic aperture of 0·95, although the makers claim that if it is used with slides of 1 mm. thickness, its aplanatic aperture is equal to its total aperture. With the front lens removed, this condenser supplies an aplanatic aperture of 0·4, thus it is capable of being used with low-power objectives from 1 in. to ½ in. and with the top lens in position it will satisfy the demands of the best dry apochromatic objectives.

The removal of the top lens is due to the necessity for obtaining a large angle for the illuminating cone when the condenser is used with the higher powers, the result of which is a short focal length, which in turn decides the physical dimensions of the image of the light source ; thus the shorter the focal length the smaller the image,

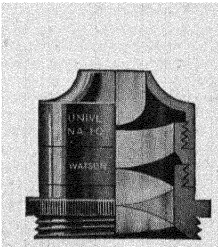


FIG. 71.

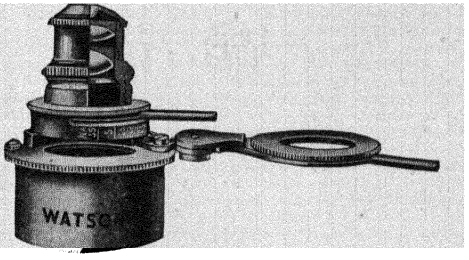


FIG. 72.

and if the condenser is used with objectives below ¼ in. with the top lens in position, the size of the image is not sufficient to cover the field of the objective, with the result that only a part of the field is illuminated, which state of affairs can be very trying at times. Though some workers advocate the use of the top lens, the argument being that only the wanted portion of the field is illuminated, thus concentrating the attention on that portion, it is the author's opinion that to have the outer portion of the field in semi-darkness is more of a hindrance than a help, apart from which no optical advantage is obtained.

The lens illustrated in Fig. 72 is Watsons' parachromatic condenser, having a total aperture of 1·0 with an aplanatic aperture of 0·9, with the top lens removed, the aplanatic aperture is 0·4. This lens is in the same class as the universal condenser, but it is the author's experience that the last-mentioned condenser is slightly the better ; however, both are capable of accommodating a complete range of dry objectives.

If we now refer to Fig. 73, we see an illustration of a condenser

representing the peak of perfection in so far as condensers for transmitted light are concerned. This is Messrs. Watsons' " Holoscopic " oil immersion condenser ; it is constructed on the same plan as their well-known " Holoscopic " (semi-apo) objectives in which the spherical and chromatic aberrations are corrected by means of a triple back combination, as shown. Its total N.A. is 1·37 and it will supply an aplanatic cone to the limit of its aperture, apart from which it may be used with slides up to 1·3 mm. thick. Used dry, it will supply an aplanatic cone of 0·92 and with the top lens removed with the aplanatic cone is 0·55. Thus it will be seen that this lens is sufficient to take care of the utmost requirements of modern objectives of the greatest aperture and where a battery of lenses includes oil immersion objectives, be they apochromatic or otherwise, one condenser of this type will serve for them all. The focal length of this lens is 0·22 in. dry or immersed and 0·55 in. with the top lens removed.

It is interesting to note the remarks passed by Carpenter and Dallinger (1) with regard to the original version of this lens, they say :—

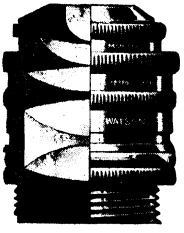


FIG. 73.

" One of the most valuable condensers introduced by any maker lately is an oil immersion one by Messrs. Watson & Sons. It has special claims upon the attention of those who work with high powers, for we know of no similar instrument that yields so large a solid cone of illumination. (Author's note : Their term ' solid cone ' signifies the largest aplanatic cone.) The construction is an unusual one, the corrections for both spherical and chromatic aberrations being effected by means of a cemented triple back lens.

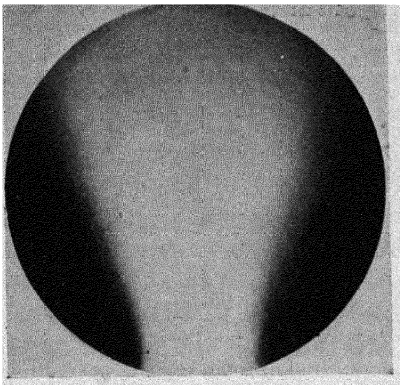
" The only flint glass used in it is the middle of the triple back. The total N.A. is 1·33, the aplanatic aperture being in excess of 1·25.

" The magnifying power is $\frac{1}{4}$ in. and the clear aperture at the back of the lens is $\frac{6}{10}$ in. working through a slide of 0·073 in. thick.

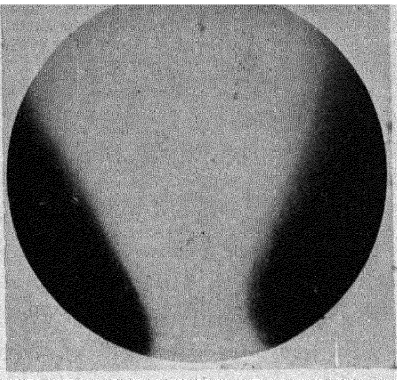
" With the front lens removed it is an efficient dry condenser for medium powers, magnifying $\frac{2}{7}$ in., with a total N.A. of 0·56, the aplanatic aperture being over 0·5 in."

The interest in these remarks lies in the fact that they were written in 1901, and after forty odd years the only improvement is that the aperture has been increased from 1·33 to 1·37 which, although not inconsiderable, goes to show that with the present materials and methods of manufacture the limit has nearly been reached.

Thus we see that, so far as work with transmitted light is concerned, a good oil immersion condenser will cover a complete range of objectives ; indeed, where this range includes oil immersion



(*a*)



(*b*)

FIG. 74.

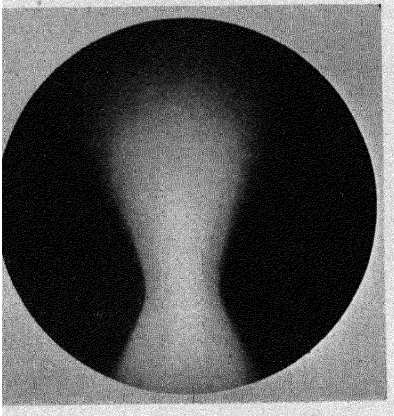


FIG. 75.

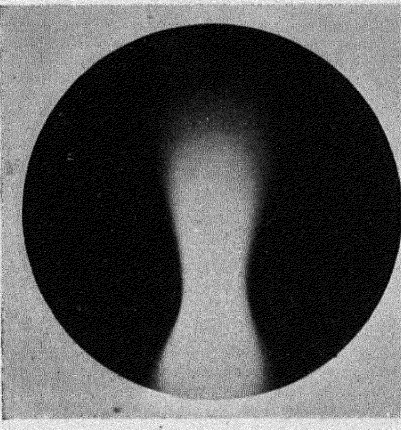


FIG. 76.

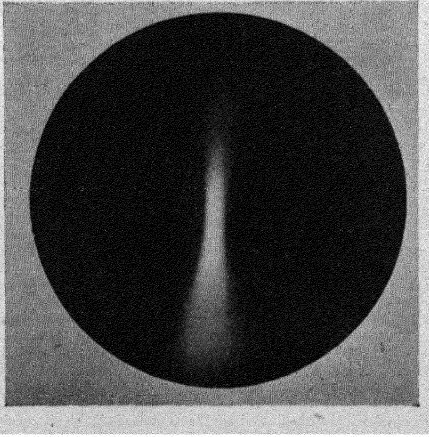


FIG. 77.

objectives with apertures greater than unity, this type of condenser is essential ; if, on the other hand, the range of objectives is entirely dry, then a good achromatic or apochromatic condenser is necessary.

Chromatic condensers of the Abbe type should be left severely alone unless it is impossible to obtain anything better, as condenser-less illumination should not be resorted to even as a last resort. It would be far better to forego microscopic examination if no condenser is available, as the images under these conditions are capable of producing misleading results and wrong interpretations.

In order that this question of cones will be further understood, ray path photographs of two condensers made by Messrs. Watson are reproduced in Fig. 74, where (*a*) is that of the Abbe condenser at the maximum aplanatic aperture, (*b*) the ray path for the " Universal " condenser. In each case the lens is working at its maximum aplanatic aperture, the difference in the angle of the

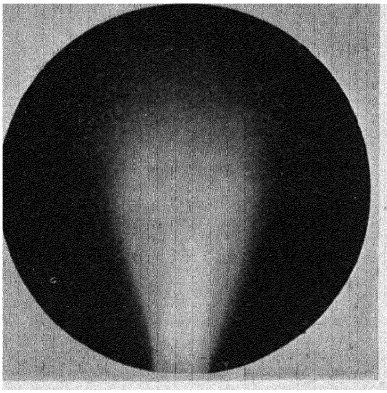


FIG. 78.

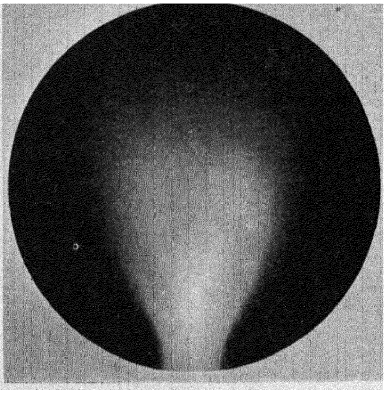


FIG. 79.

cones supplied will be readily appreciated and in order to show the effect of closing the diaphragm, on the angle of the cone accepted by the objective Figs. 75, 76 and 77 show successively the cone supplied by the " Parachromatic " condenser at full aperture, approximately half aperture and finally with the diaphragm shut down to roughly one-tenth of the aperture of the lens. It will be noticed how the angle of the upper cones is progressively decreased until finally it is almost a parallel ray of light, and bearing in mind the necessity for very oblique light to procure the best resolution, it will be appreciated how the resolution is destroyed as the effective aperture of the objective is reduced by a corresponding reduction of that of the condenser by use of the diaphragm. Similarly, the effect of immersing the condenser is shown in Figs. 78 and 79, which are the ray paths of a Watson " Holoscopic " oil immersion condenser. The increase in aperture due to immersion is clearly seen.

The term " diaphragm " has been frequently used in the fore-

going pages ; in all cases this refers to the iris diaphragm, which is built in precisely the same manner as that in a camera and is fitted to the substage mount carrying the condenser. This can be seen in position relative to the condenser in Fig. 72. A diaphragm itself is illustrated in Fig. 80. It will be seen that opening and closing the diaphragm the diameter of the beam of parallel light entering the condenser is varied, as a consequence of which the aperture of the condenser is varied with the effects on the cone which we have just seen. These accessories are supplied by all makers of microscopes and are in universal use. They are very ingenious pieces of workmanship and with reasonably careful handling should keep free from trouble indefinitely ; however, when selecting one it is as well to see that it works quite freely over the whole of its range from wide open down to a mere pinhole. In the fully closed condition there should be no tendency for the central portion to bulge in either

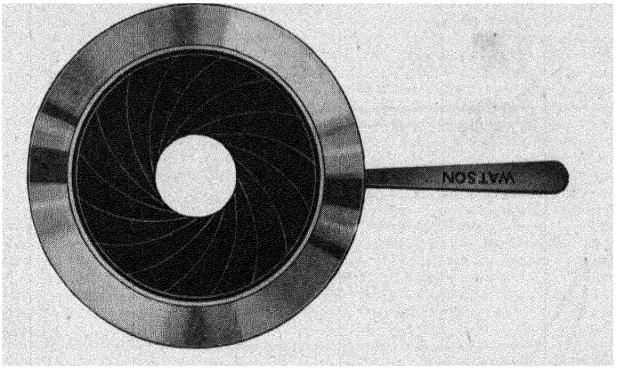


FIG. 80.

direction of the axis, unless of course excessive pressure is applied to the operating lever, in which case there is a risk of causing irreparable damage.

This is not the only position in which iris diaphragms are used, as we shall see subsequently, and they vary in size from about $\frac{1}{4}$ in. diameter to 3 or 4 in. A very useful type of illumination is obtained with a condenser if a suitable stop is placed immediately underneath it, such that only an annular ring of light is allowed to enter the lens, thus causing the object to be illuminated by the very oblique peripheral rays of the cone. Matters have to be adjusted so that the cone used is larger than that which the objective will accept, and the stop has to be of such a size as to just shut out of the condenser's cone one of the size corresponding to the aperture of the objective. By this means a transparent object whose refractive index differs from that of the mounting medium is rendered brilliantly illuminated on an intense black background.

The application of this type of illumination is very useful and at

times necessary where colourless transparent objects are to be examined; therefore, it will be agreed that a short study of the development of the modern dark ground condenser will not come amiss.

Microscopists of the nineteenth century must have noticed often that when an object was illuminated by rays of light at an angle so oblique that they missed the objective, the object appeared to become self-luminous on a dark background. Reade became sufficiently interested in the phenomenon to develop a method of producing it at will and published his findings in 1837.

The method consisted of converging the light on to the object from one side and at such an angle that the rays missed the objective by means of a condensing lens, as shown on Fig. 81. This method

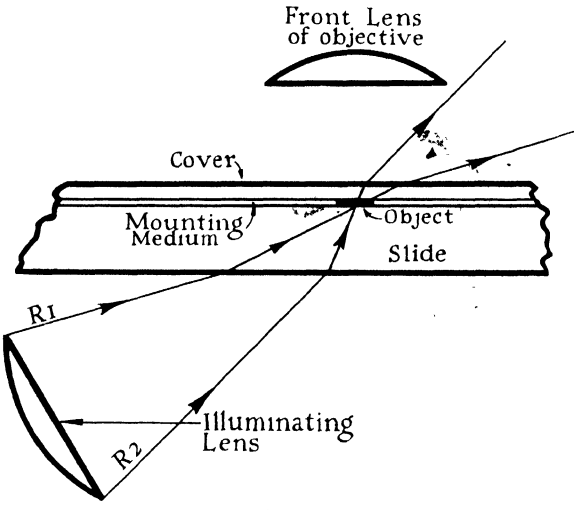


FIG. 81.

of illumination being from one side, possesses inherent disadvantages; for example, a spiral fibril would appear as a discontinuous structure, the only luminous portions being those which were almost perpendicular to the rays, and the benefits which would result from an annulus of oblique rays, in which case the object would receive light from all directions, are apparent, because obviously it will be seen that to supply such an arrangement a cone of light is required wherein the obliquity of the innermost rays would have to be such that they missed entering the objective. The object, of course, would have to lie at the apex of the cone.

In 1851, Wenham described an illuminator illustrated in Fig. 82, which provided a hollow cone of rays converged on the object. The apparatus consisted of a truncated hollow parabolic reflector of silver, the central ray being stopped out by a dark well. The internal reflecting surface was designed so as to ensure that when the entering

light was parallel the rays would be converged to a point on the object. A meniscus lens was fitted below the focus in order to correct for the thickness of the slide.

The production of an annular prism by Shadbolt, followed soon after, the upper and outer surface of which reflected the rays obliquely towards the axis. The disadvantage of this apparatus, however, was that as all the rays were deflected at the same angle there was no true focus. He realised the shortcomings of this method and suggested an improvement based on constructing Wenham's parabolic reflector in glass. Wenham took it up and produced an apparatus which became the most popular illuminator ever made,

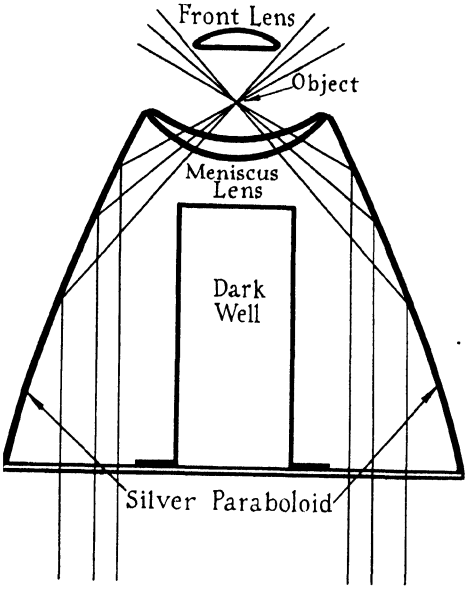


FIG. 82.

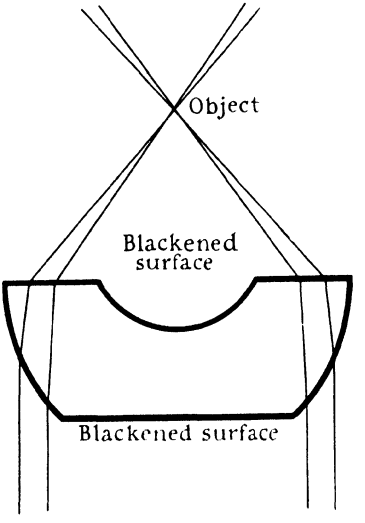


FIG. 83.

known as Wenham's paraboloid, being generally preferred to any other ; for all except the highest powers.

There were, of course, other illuminators ; Ross and Robert produced the spot lens, which consisted of a thick plano convex lens, the plane surface of which was towards the object. The central portions of both surfaces were ground away and blackened, so that the more central rays were shut off, as shown in Fig. 83 ; but this lens was only suitable for the lower powers down to about $\frac{2}{3}$ in.

All these appliances were known and in use in 1855, by which time achromatic condensers had reached a high state of perfection and were being supplied as standard, with a series of patch stops, by the use of which they could be made to supply a hollow cone, thus procuring dark-ground illumination with objectives of suitable power.

Until this time, dark-ground illuminators were only suitable for use with the lower powers, and this type of illumination could not be obtained with the higher-powered objectives, including immersion objectives, and Wenham produced a new apparatus in 1856 which was capable of being used with the highest powers. This instrument was an immersion paraboloid as shown in Fig. 84, and consisted of a paraboloid with a flat top which was in oil contact with the bottom of the slide.

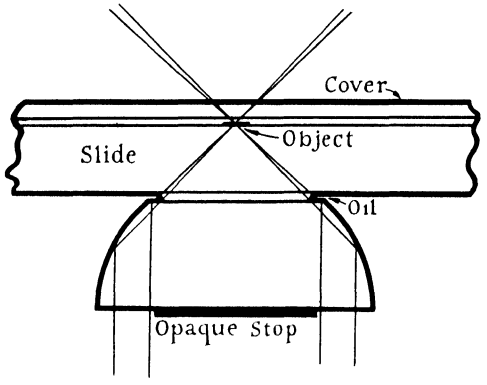


FIG. 84.

By the use of a greater thickness of glass either in the paraboloid or the slide, the focus of Wenham's flat-topped paraboloid may be made to coincide with the object, the rays striking it with greater obliquity than is possible with a dry illuminator. Fig. 85 shows the ray path through this illuminator and demonstrates how the light is reflected back on to the object by the under surface of the cover glass, thus illuminating the object, which appears luminous on a black background.

It has been stated that all condensers were supplied with patch stops (Fig. 86) as standard equipment, but unfortunately these achromatic condensers were of small size and as, under dark ground conditions, they are very sensitive to centring and only the smallest

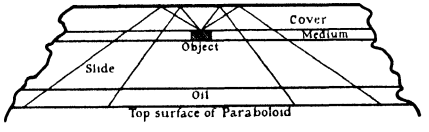


FIG. 85.

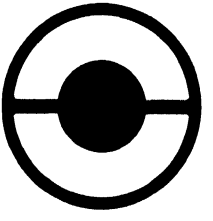


FIG. 86.

movement was sufficient to ruin the image, this constituted a drawback which was overcome by the production of larger condensers. For example, a well-corrected condenser such as Watsons' " Universal " of 1·0 N.A. will provide dark ground with objectives up to 0·8 N.A., and even Abbe's condenser will satisfy objectives up to 0·70 N.A., but the initial setting up must be carried out very carefully if successful results are to be obtained with objectives of the higher numerical apertures. For the lower-powered objectives, quite successful results are obtained with achromatic condensers if

the top lens is removed. This enables a larger field to be covered by the same size stop as that required for the higher powers.

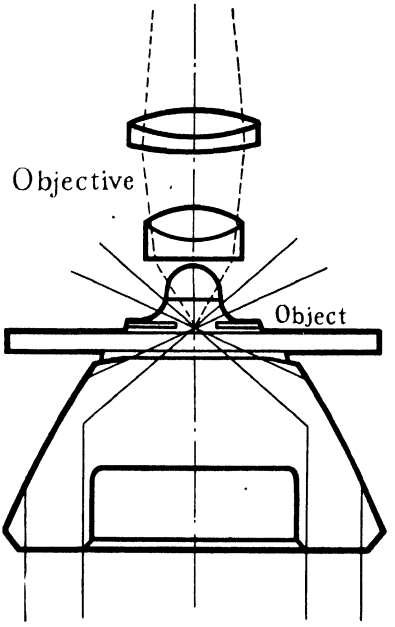
As the aperture of any dry condenser or any immersion condenser used dry cannot exceed 1·0 and also as the more oblique rays lose in intensity before reaching the object, it seems to be asking much of an illuminator, under these conditions, to give really successful dark ground with objectives above about 0·70 N.A. ; in fact, the author does not recommend the use of dry condensers for dark ground work. With objectives of not higher power than $\frac{1}{2}$ in. an achromatic condenser, or an Abbe used with a stop of suitable dimensions, is to be preferred to any other apparatus. They are highly efficient when used for this purpose, and obviate the necessity of extra accessories such as a spot lens or paraboloid.

So far, then, we see that dark ground illumination is obtained when only those rays which are refracted by the object enter the objective, and we have discussed the early development of the dark ground illumination, but up to now the achievement of this aim with really high-power objectives with apertures greater than unity has not entered the problem. In 1877 Wenham, with his immersion paraboloid, and Stevenson with his caloptric illuminator, tried to solve the problem in an unsuccessful endeavour to apply the technique to the examination of bacteria and Koch's development of successful staining methods which utilised full transmitted (*i.e.*, light passing through the object) light for purposes of examination, caused the dark ground method to be neglected. Even the demonstration in 1884 of the *Bacillus tuberculosis* by Nelson on dark ground failed to revive interest, and it was not until 1903 that the work of Siedentopf and Zsigmondy gave the question of high-power dark ground illumination a real start. They showed that the existence of ultra-microscopic particles (*i.e.*, particles whose size is below the limit of visibility) could be demonstrated by causing them to be illuminated by a narrow intense beam of light at right angles to the axis of the microscope. For example, the particles of gold in its colloidal solutions, fine smoke particles in air, etc., each of which appears as a spot of light on a black background.

In 1903 Siedentopf described an improved method of employing an axial cone which was intercepted by a stop within the objective. In 1907 the Wenham immersion paraboloid and the Stevenson caloptric reflector were revived, followed in 1909 by Siedentopf with an instrument on a similar principle which he called the cardioid condenser. The improvement on the Wenham paraboloid followed this latter and became very popular, thus high-power dark ground illumination became a reality.

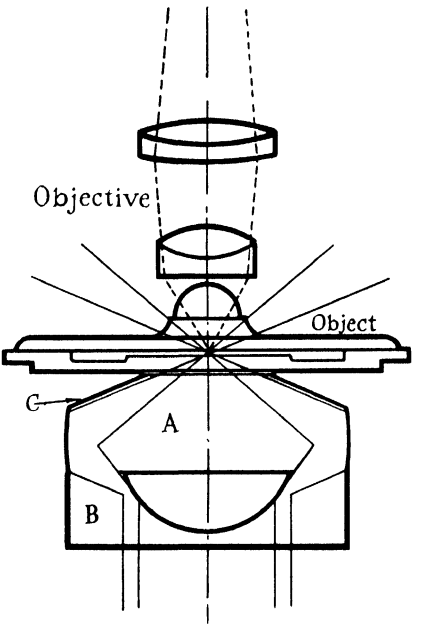
As the total aperture of an objective has to be stopped out to procure a dark ground, it is clear that the functional aperture of any dark ground illuminator to be used with it must be greater

than that of the objective by as wide a band as possible. In those days high-power dark ground was used almost exclusively for the examination of organisms in watery media so that the maximum obliquity of the rays which could pass through the mounting medium would be represented by a numerical aperture of 1·33, thus fixing an upper limit to the maximum aperture of the illuminator and in order to obtain a reasonable margin of illumination, the lower limit was usually fixed at unity. This, of course, necessitated the stopping down of the aperture of the objective, at least to unity, but it was found that if the objective aperture was reduced to about 0·9 a much blacker background was obtained. This was done by the use of a



PARABOLOID CONDENSER.

FIG. 87.



CARDIOID CONDENSER.

FIG. 88.

funnel stop which was placed in the top of the objective, but the modern method is to build a small iris diaphragm into the objective which is operated by a knurled ring on the outside, the positions of which are calibrated in figures representing numerical aperture.

Among modern high-power illuminators is an immersion paraboloid produced by Bausch and Lomb, a cross sectional ray diagram of which is shown in Fig. 87. They claim for this condenser a numerical aperture band width extending from N.A. 1·24 to 1·33, recommending that, for the best results, the objective should not have an aperture greater than N.A.1, any reduction being accomplished by means of a funnel stop supplied as a standard accessory. The immersion medium may be either cedar oil or glycerine. The

author recommends the use of glycerine owing to the ease with which it is cleaned off, although its refractive index is slightly lower

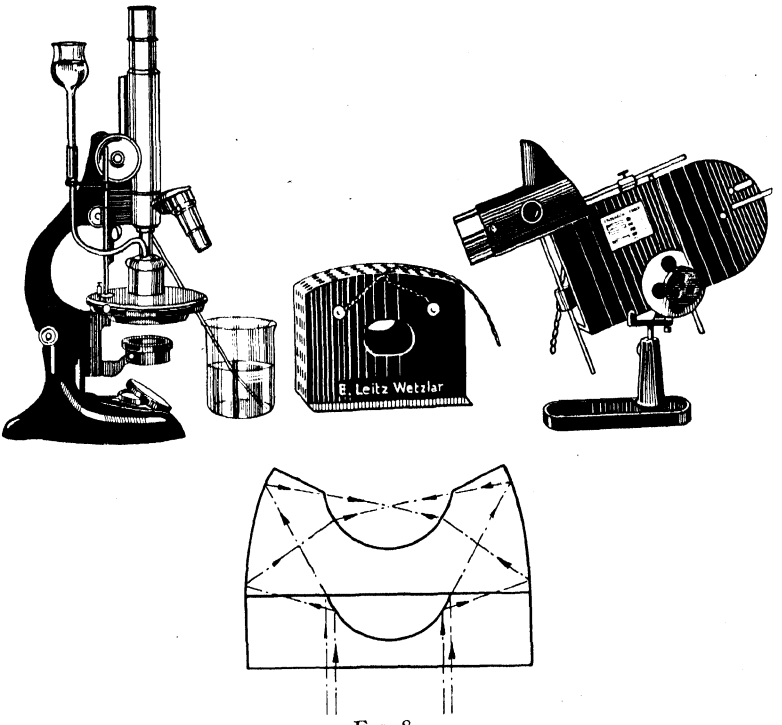


FIG. 89.

than that of cedar oil, the performance is not affected to any noticeable extent.

The same makers also produce a very excellent reflecting condenser in the form shown in Fig. 88. This apparatus is a cardioid

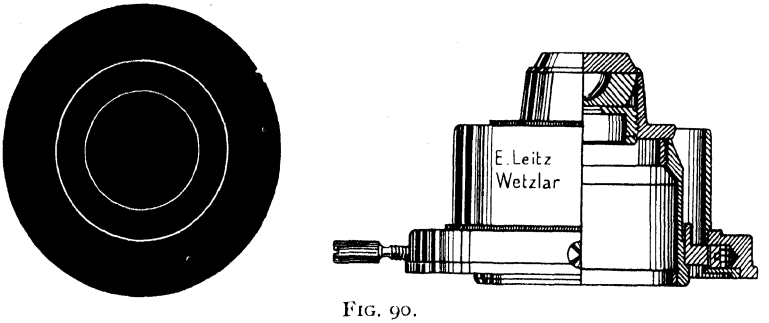


FIG. 90.

condenser and is exceptionally free from both spherical and chromatic aberrations and the efficiency is such as to enable it to be used for the detection and examination of ultra-microscopic particles. A similar type of reflecting condenser by Leitz is shown in Fig. 89, though this is designed for use with the lower powers only and in

conjunction with the special apparatus shown in the same illustration, the N.A. band width is from 0·85 to 0·99; methods of setting up and manipulating dark ground illuminators will be discussed subsequently.

An excellent all-round dark ground illuminator is that illustrated in Fig. 90, which shows the lens in section and also an enlarged view of the two circles engraved on the top lens to facilitate centring. For the unskilled user, this lens can be supplied in a centring mount, where none is fitted on the microscope. The N.A. band width is 1·20 to 1·33, which of course necessitates a reduction in aperture, to unity, for all lenses whose aperture is greater than one.

British manufacturers also produce very good dark ground illuminators.

Messrs. Watsons' immersion paraboloid (shown in Fig. 91) being an excellent one of its type, it has a wide N.A. band width from unity to N.A. 1·45. An interesting feature is that the use of the iris diaphragm cuts out the rays of lower N.A., first so that the illumination becomes more and more oblique, thus attaining, it is claimed, the greatest resolving power possible in all circumstances, for all objectives with an aperture exceeding 0·48, but perhaps their best illuminator is the one termed the Zonal, shown in Fig. 92A. The author can vouch from experience of this instrument as to its efficiency. It has a long working distance which is extremely valuable and an intense black background is obtained, and although the light concentration is very high the resolution is very satisfactory and unmarred by halation. While in Fig. 92B is shown the hollow cone supplied by a Baker Nelson immersion illuminator.

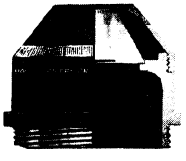


FIG. 91.

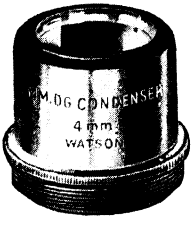


FIG. 92A.

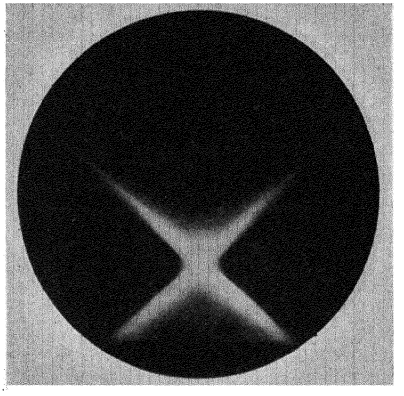


FIG. 92B.

In general there is a very little to choose between the three different types in the British, American and Continental, all three being very much the same and designed on the same general principles.

If an intense beam of light is caused to pass through a suspension of one substance in another, then the path taken by the light beam

will become visible due to the small suspended particles scattering the light encountered by them. In this way is a ray of sunlight made visible in a darkened room which contains a suspension of fine dust particles in air. This phenomenon is called the " Tyndall " effect and is the principle upon which the slit microscope of Siedentopf and Zsigmondy was based.

In foregoing pages, mention has been made of the slit ultramicroscopes, the modern counterpart of the first instrument produced by Siedentopf and Zsigmondy is a very efficient instrument and is used chiefly for the examination of colloidal particles of ultramicroscopic size. An up-to-date instrument of this type is illustrated in Fig. 93, it consists briefly of a microscope mounted on an optical bench, which carries the necessary apparatus to supply a very intense and narrow beam of light at the focal point of the objective, and at right angles to the axis of the microscope. The illustration clearly shows the arc lamp light source, the collimating system, and

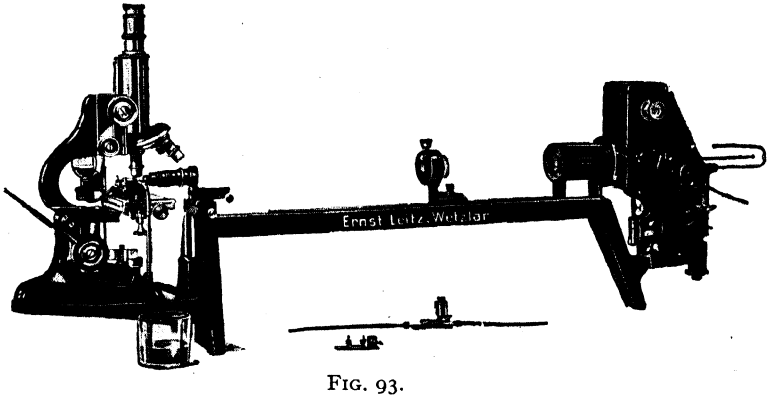


FIG. 93.

the objective which focusses a very small and intense beam of light on to the suspension under examination. By this means particles far below the limit of visibility are shown by virtue of the light scattered by them, which enters the objective. The main beam, however, being at right angles to the optic axis of the microscope, does not enter the viewing objective and consequently the particles in suspension appear as bright spots of light on a black background.

In dealing with the apparatus used for illuminating the object, we must not overlook the illumination of opaque bodies.

In this case obviously, transmitted light cannot be used and the object has to be illuminated from the top. For objectives down to about $\frac{2}{3}$ in. the working distance is sufficiently large to enable a beam of light to be directed on to the object from the lamp itself, in which case the light will strike it at an angle and from one side. However, as the magnifications under these conditions are not high, and furthermore, as a large number of objects are best illuminated in this manner, this is no disadvantage. As a matter of fact, the

contrast, more often than not, is improved and a far better idea of the structure of the surface under examination is obtained. It will be frequently found that one particular angle for the light will produce the best results ; this, of course, must be ascertained by trial.

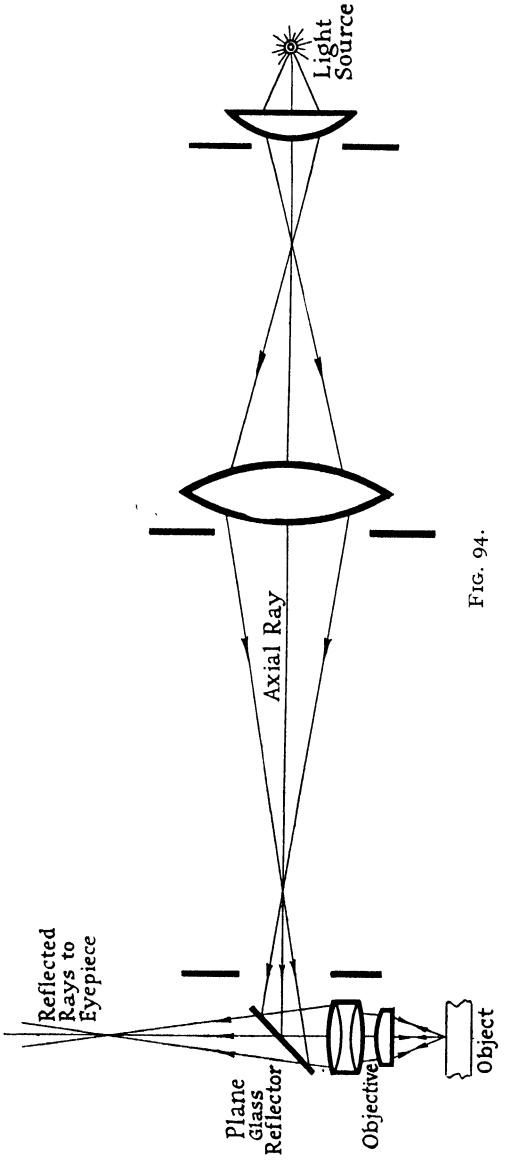


FIG. 94.

When it is necessary to illuminate opaque bodies for examination under high powers, the solution is not so simple, as the short working distances of high-powered lenses preclude the method of illumination from the side and accordingly other means have to be adopted. The solution to the problem lies in supplying the surface to be examined with a beam of light directed on it, vertically. Suppose we had an opening in the body of the microscope just above the objective and that inside the tube a thin sheet of optical glass was fixed (Fig. 94) at 45° to the optic axis of the instrument in such a way that a beam of light entering the opening in the tube at right angles to the optic axis would be confronted by a reflecting surface at 45° to itself, then the greater portion of this beam would be reflected downwards along the optic axis and through the objective which would bring it to a focus at the surface of the object, in the form of a cone of light, perpendicular to it. This same cone will be reflected back through the objective, but it will be seen that total reflection will only be obtained when the surface is at right angles to the cone, any irregularities in the surface will only partially reflect back, thus giving light and shade.

Vertical illumination is suitable only for objects mounted dry, or if covered they must be in optical contact with the under surface of the cover glass and specially designed lenses must be used. The great majority of the work done with these instruments is in the examination of metallurgical specimens, which, of course, are not covered ; they are, however, extremely useful for the examination of other than metal specimens on occasion.

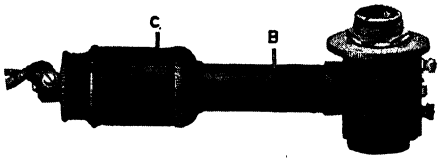


FIG. 95.

The instrument was originated by Smith in America and at the present time has reached a high degree of perfection. Some makers still use the thin glass reflector which was modified from the small silvered speculum used by Smith, a notable example is the Watson-Conrady illuminator illustrated in Fig. 95, and may be classed as a typical British product. The three main disadvantages of vertical illumination have been overcome by this appliance ; that is to say, glare, accurate centration and focussing of the light source, the whole unit is self-contained, incorporating the lamp, optical system

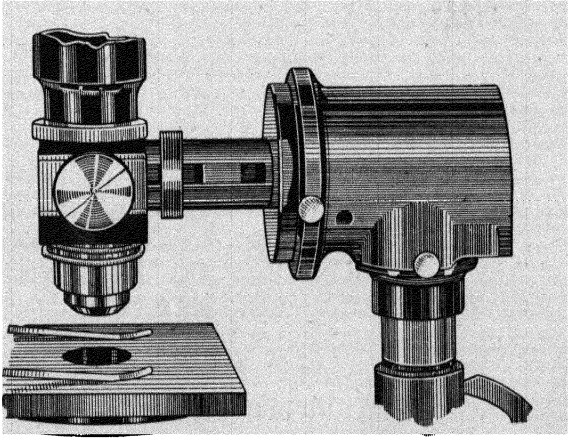


FIG. 96.

and iris diaphragm. It is possible with this instrument to have the same complete control over the illumination that is obtained with transmitted light, thus obtaining the maximum illumination from a small source of light.

The illuminator fits into the objective thread of the microscope and the objective is screwed into it ; the inner iris acts as a field diaphragm, regulating the size of the field under observation ; the outer iris is the regulator for the illuminating cone.

The illuminator shown in Fig. 96, by Bausch and Lomb, may be taken as representative of American design. Here again a plane glass reflector is used, this latter being mounted on a shaft carrying

a knurled knob on which the orientation of the reflector is shown by means of an engraved line. This is a simple instrument which, however, gives excellent results and may be adapted to any microscope very easily. One advantage of this instrument is the adjustment on the reflector which may be set so that it is at right angles to the optic axis; thus the microscope may be used for transmitted light without removing the vertical illuminator.

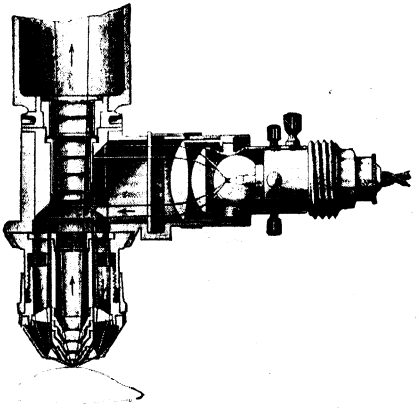


FIG. 97.

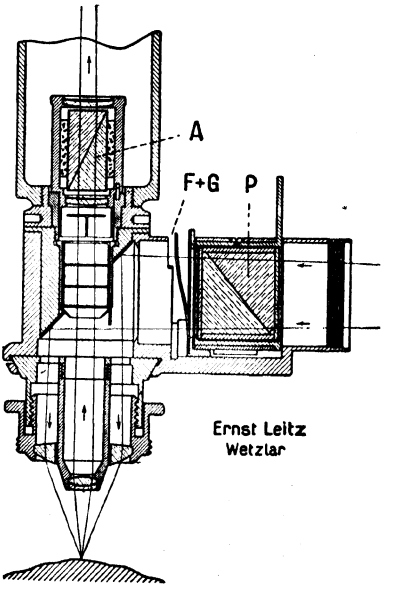


FIG. 98.

It would perhaps be as well to stress, at this point, the necessity for using the special short-mounted objectives when vertical illumination is employed on uncovered specimens. These objectives are corrected for use with incovered specimens and are designed for very short mounts to obviate glare under these conditions.

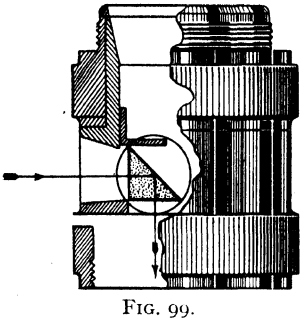


FIG. 99.
(After Chamot & Mason)

Continental design, on the other hand, has taken another trend; this is ably represented by the "Ultropak Illuminator," by Leitz, shown in Fig. 97. This cannot be called a true vertical illuminator, as the light does not pass through the objective in the first instance; instead, it passes through an annular condensing lens which can be seen in Fig. 98 being converged on to the object by this latter. The reflected light then passes through the objective in the usual way. Fig. 98 shows the construction and ray path of the "Polarising Ultropak," the only difference being the inclusion of Nicol prisms in the optical train by means of which images entirely free from glare reflexes are obtained. This is a first-class instrument and can be highly recommended; the only drawback is the non-

standard fitting of the objectives; these are specially designed and built to function with the instrument and standard objectives cannot be used with it.

There is another type of vertical illuminator which uses a tiny 45° prism situated to one side of the objective, as shown diagrammatically in Fig. 99. This type gives very bright light but is analogous to the use of a substage condenser for transmitted light, used with a stop blocking out the whole of the back lens except for a small portion at the periphery, thus giving an oblique beam from one side, in consequence of which the illumination is only from one side; thus on a rough surface sharp contrasts are in evidence, but as only a part of the total N.A. of the objective is used, maximum resolution cannot be obtained. However, if the magnification is not too high, relief is shown in a remarkably clear manner. Thus we see that with vertical illumination the objective itself functions as its own condenser and under the proper conditions it focusses an image of the light source at the apex of its own illuminating cone, at its own focal point. In this way it is quite possible to obtain a critical image and the maximum resolution which the objective is capable of giving.

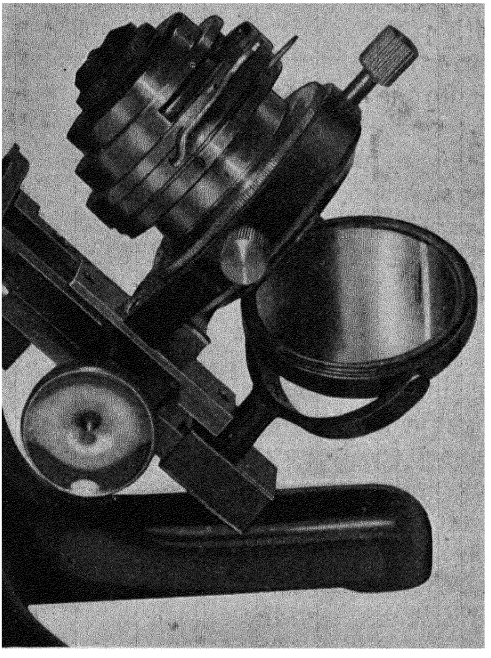


FIG. 100.

As the great majority of microscopes are used in an upright or nearly upright position, we must have some means of reflecting the beam of light into the condenser, and accordingly all microscopes of conventional design are provided with a mirror mounted below the substage condenser in such a manner that it can be rotated in two directions. This is achieved by mounting a circular mirror, generally about 2 to 2½ in. in diameter, in a gimbal. The mount carrying the complete assembly must be capable of movement along the optic axis.

The mirror on all high-class instruments invariably consists of a plane mirror on one side and a concave mirror on the other. A typical mirror and fittings is illustrated in Fig. 100.

The plane mirror can obviously have no modifying effect on the light supplied to it ; that is to say, if parallel light is supplied then parallel light is reflected.

The concave mirror, on the other hand, has a focal point, as a result of which it is capable of supplying a cone of light in much the same manner as the condenser, although this cone is only equivalent to an N.A. of 0·25 or under, hence the necessity for being able to move it along the optic axis so that the apex of its cone may be made to coincide with the plane of the object ; in other words, we have to be able to focus the concave mirror and with objectives whose aperture does not exceed N.A. 0·25. The concave mirror will give as good a result as a condenser provided always that it is used with parallel light only. Illumination by means of the concave mirror is not suitable for magnifications above about 100 diameters, due to the limiting numerical aperture and the lack of control of the direction and intensity of light. For low magnifications, however, and when used with daylight, it is quite useful for purposes of checking or rough examination as the setting up is quickly achieved and does not involve much time spent in adjustments, but the author recommends the use of a condenser at all times and for all powers above about 30 diameters, in view of which he keeps an equipment permanently set up for critical illumination with a fully corrected condenser. In this way intital low-power examinations are carried out under the best conditions. As the field of the condenser is too small at powers much below $\frac{2}{3}$ in., it is only a matter of seconds to remove the top lens of the condenser to obtain a sufficiently large field and ample aperture to satisfy the most exacting objectives.

It is well known that a plane mirror will give multiple images of the light source due to reflections occurring off the front surface of the glass, as well as the silvered surface ; this, of course, has an adverse effect on the critical nature of the illumination. There are three methods of overcoming this defect. Firstly the mirror is made in the form of a wedge so that the multiple images are widely separated, thus allowing only one to be used. This is by far the most common method, and all instruments of good quality are fitted with wedge mirrors as standard.

The second method is by the use of a surface silvered mirror, but these are delicate and require very careful handling ; furthermore, cleaning must be carried out with the utmost care. However, in spite of the most careful treatment, the surface soon wants re-silvering, as a result of which this type of mirror is hardly ever used ; instead, the use of a polished optical flat of stainless steel is often advocated, which is much more stable, although the efficiency of reflection is lower than for silver.

In the author's opinion, the best method of obtaining a single image is by means of a 45° prism (shown in Fig. 101), which gives

total internal reflection without a multiplicity of image and no loss of light, although it must be borne in mind that the light path from the illuminant to the condenser must proceed through an angle of exactly 90°, because if the light deviates from the normal through the prism, refraction will take place and spectral colours will become apparent. In consequence of this the setting up of an equipment employing a prism is critical and must be carried out with much care, but the results amply justify the little bit of extra time and care needed in the initial adjustments. However, it is not intended to convey the idea that first-class critical work cannot be done with a

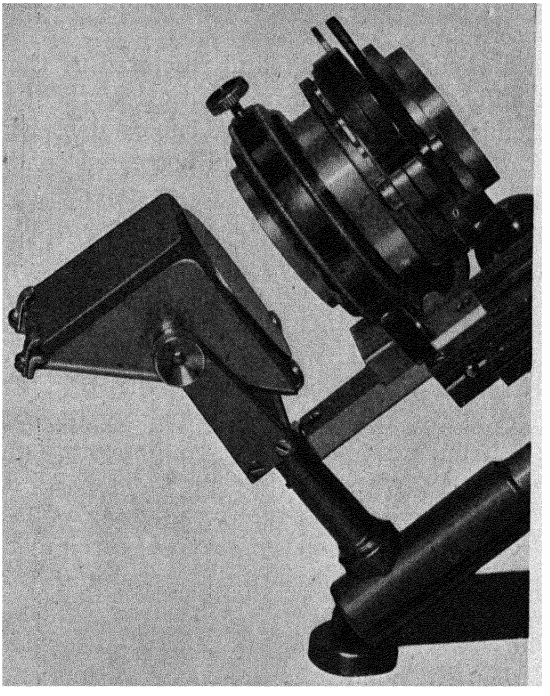


FIG. 101.

good wedge mirror. As a matter of fact, the mirror is by far the most common reflecting apparatus employed and the use of a prism should be regarded more in the light of a refinement required by a hypercritical user than as a necessity, as the difference in the final image would only be noticed by the expert as being slightly better in quality.

The remarks on the care of objectives in Chapter IV apply equally well to condensers. The exposed lens surface should be kept scrupulously clean, and where one condenser serves a multiplicity of purposes, such as removal of the top lens for low powers and immersion for high powers, these changes should be carried out

with all due care and caution. If the condenser is used, with the top lens removed, for any length of time, on reverting to its use with the top lens in position, this latter must be replaced carefully after having made sure that the upper surface of the lower lens, which was previously exposed, is quite clean and free from dust. The top lens should be screwed on quite squarely and right home, as if not, then the focal length of the combination will be altered and the corrections will suffer accordingly.

A good condenser is a very valuable piece of apparatus, therefore treat it with the same care as you would the best objective and never look upon it as of secondary importance. As we have seen, the best objectives cannot work at maximum efficiency without a good condenser.

The choice of condensers is mainly dictated by the type of work undertaken and where routine and critical work of a very varied nature is intended it is as well to have one condenser for low powers and one for high, whereas one good corrected condenser such as Watson " Holoscopic Oil Immersion " is capable of covering the entire range of objectives from 1 in. to $\frac{1}{12}$ in. O.I., it is convenient to have a low-power condenser in the equipment when work on low powers is carried out for longish periods ; this frees the high-power condenser for use with another instrument. The author is fortunate in having at his disposal two microscopes, one of which is kept permanently set for visual work and the other being incorporated in photomicrographic equipment and frequently the need has arisen for the two instruments to be in use simultaneously, and as the low-power condenser is a Watson holoscopic of 0·5 N.A., it will satisfy a wide range of objectives up to $\frac{1}{2}$ in. focal length. This arrangement has proved very useful many times and is recommended wherever possible. However, as the majority of users in all probability have only one instrument, one good high-power condenser is recommended. Of course, if the range of objectives includes a $\frac{1}{12}$-in. oil immersion, then the condenser should be an immersion type with aplanatic aperture in excess of that of the immersion objective. Removal of the top lens will ensure an adequate field and aperture for the lower powers.

Finally, avoid using an Abbe condenser unless there is no possibility of obtaining a corrected lens, if you wish to do work of real value. The Abbe condenser is, we know, very popular, but the reasons for this mistaken popularity have been given in preceding pages and should be carefully noted.

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CARPENTER and DALLINGER. "The Microscope and its Accessories." Churchill.

CHAPTER VI

ILLUMINATION

WE have seen in previous chapters the importance of using correct illumination with the microscope in order to obtain the best results. This point cannot be stressed too strongly as far too many users ignore this problem, with the result that the most minute and very often most important structures go entirely unnoticed. Therefore let us always realise that the finest microscope and accessories are reduced to the level of a mere magnifier if they are not used with proper illumination. It must be appreciated that no serious work can efficiently be carried out without due regard to this aspect of the problem.

As it is impossible to obtain the best results without a thorough understanding of the basic principles underlying the use of correct illumination, before proceeding further it would perhaps be as well to take notice of what Munoz and Charipper * say about the overlooking of this point. In their excellent monograph they say :—

" Important as this subject is, it is surprising how little attention is paid to it by the majority of people who work with the microscope. We do not refer to careful and experienced microscopists who, naturally, do use proper illumination correctly, but rather to the vast number of earnest scientists, technicians and students who simply have never bothered to be careful about this point and who because of this are not very likely to see all that their microscopes can show them."

" The following is an actual case which illustrates the question quite well. A graduate student working for his Ph.D. saved, after considerable effort, enough money to purchase a beautiful research binocular microscope with apochromatic objectives, etc. When he got it he was very disappointed because he did not see the details in his slides any better ; as a matter of fact, he did not see them as well as when he used the old monocular previously. It was found upon investigation that the young fellow was using the same small 15-watt substage lamp with his new binocular. Also, he was not focussing his condenser carefully because he never had found it necessary with his old monocular, but his new research microscope was properly equipped with a highly corrected achromatic and aplanatic condenser which required much more careful focussing than the simple Abbe condenser he had used previously. When these two points were corrected and he learned something about

* Munoz and Charipper. " The Microscope and its Use." Chem. Pub. Co., N.Y. By kind permission of the Authors and Publishers.

proper illumination, our budding Ph.D. soon found that he could see more details better and with more comfort."

The foregoing example will serve to illustrate and drive home the importance of having an understanding of and the ability to use, illumination to its best advantage.

In the past, daylight was extensively employed as a means of illumination, but we have seen in a previous chapter how unreliable this is, especially in the country, and as the majority of work with the microscope is carried out indoors and preferably in a darkened room, artifiical light of one sort or another is universally used nowadays. This is one of the reasons why manufacturers lay such emphasis on illuminating equipment, although the main reason is that they realise that their objectives cannot function at their best unless correctly illuminated.

Basically there is only one type of illumination, this was demonstrated by that eminent English microscopist E. M. Nelson, who showed that the best results were obtained when the light source was focussed in the plane of the object. This type of illumination he called " critical," and it is the author's opinion that critical illumination based on this fundamental fact is the only method by which the best results may be obtained, although Munoz and Charipper recommend three types, (*a*) an approximation to critical illumination, (*b*) critical illumination, and (*c*) Kohler illumination ; this last being a modification of critical illumination which they contend is more suitable for photomicrography. This point will be discussed later ; for the present, however, let us see what critical illumination means and why it should be superior to other types. At the same time let us modify slightly our views on the constitution of the optical train of the microscope, so that it includes every optical component between and including the light source and eye, as in reality it commences with the light source.

We see from Fig. 13 (Chapter I.) that if a light source is placed on the axis of a lens, the rays diverging from it passing through the lens will be brought to a focus at some point on the axis, the other side of the lens at a distance from it, dependent on the distance of the source from the lens. That is to say, the further away the source is from the lens, the closer will the focal point and image of the source lie, until a point is reached where this latter coincides with the principal focus (Fig. 14), the source then being, theoretically, at infinity, and the rays from it are parallel. We already know that in order to obtain maximum resolution we want high aperture in the objective, which in turn has to be satisfied by a similar aperture in the condenser, also that aperture in the condenser may be best interpreted in terms of the angle of the cone of light produced. Thus, from the facts just stated, we see that by removing the light source to infinity the angle of the cone produced by the lens in ques-

tion increases progressively to a maximum at the principal focus.* This immediately shows us one important fact, viz. that the image of the light source which is focussed in the plane of the object must lie at the principal focus of the condenser, otherwise the maximum cone from the condenser cannot be obtained. In practice, Nelson found that 8 to 10 in. from the mirror gave the best results ; this, of course, means some 10 to 14 in. from the back lens of the condenser.

As the light source in those days consisted usually of an oil lamp, with the flame turned edge on, to facilitate focussing, only a narrow band covering about one-tenth of the area of the field was illuminated, and for years some of the finest work of the early microscopists was carried out under these conditions, criticism of which was answered by remarks relating to the adaptability of the eye and that anyway the centre of the field produced the finest

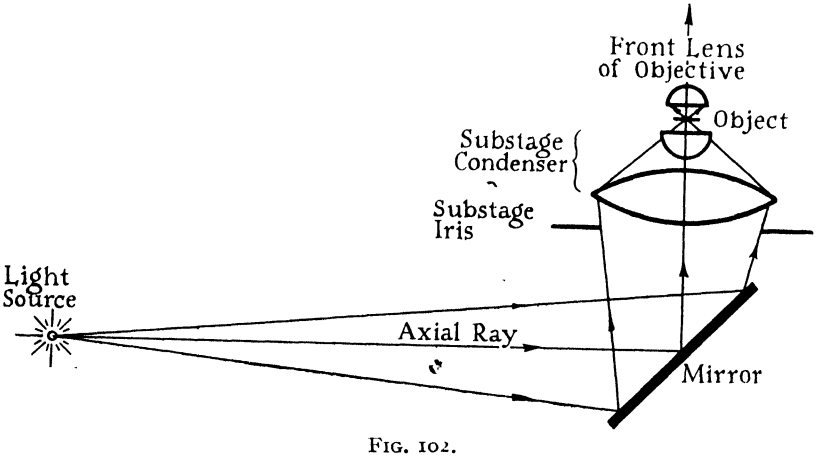


FIG. 102.

image. In which respect they were, of course, perfectly correct ; thus we see Nelson's basic critical illumination illustrated diagrammatically in Fig. 102, which shows the light source from which is proceeding the axial ray and two diverging rays which are brought to a focus at the object.

Excellent results were claimed and no doubt achieved by this means, the chief difficulty being in striking a compromise between the distance of the source from the mirror and the size of its image in the field, in consequence of which it was soon realised that matters could be much improved by supplying the condenser with truly parallel light, which is comparatively easy to achieve by the use of an auxiliary lamp condenser in the form of the familiar plano convex bull's-eye.

Now, if we take a plano-convex lens and place the source of light at the principal focus, with the flat side of the lens towards the

* It should be noted that the most modern condensers are corrected for use with a point source of light at 10 in. from the back lens.

source, then the rays issuing from the curved surface will be parallel and possess minimum aberrations. Thus we have a convenient source of parallel light in the lens itself, for if a lamp flame is placed edge on in the centre and at the principal focus of the lamp condenser, it is expanded and fills the lens. This can be seen by looking directly into the lens, when the appearance will be similar to that shown in Fig. 103; if, on the other hand, the flame is within the principal focus it appears as in Fig. 104, and if it is outside the focus, as in Fig. 105, one point to bear in mind is that the diameter of the disc in Fig. 103 depends upon the diameter of the lens, but the intensity depends upon the focal length; that is to say, the shorter the focal length the more intense the light.

By the use of the lamp condenser, virtually "two birds have been killed with one stone." Firstly, we have a source of parallel light which, secondly, may be brought close enough to give the largest

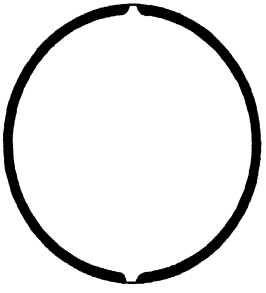


FIG. 103.

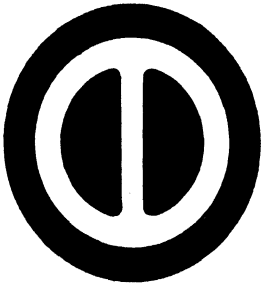


FIG. 104.

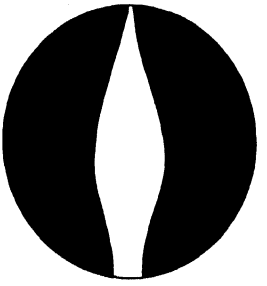


FIG. 105.

field of which the condenser is capable, thus giving us a full field and maximum cone of light.

Thus, if we use the lamp condenser as the source of light and focus its image in the plane of the object, we have improved matters considerably. The use of the lamp condenser is shown diagrammatically in Fig. 106; here we have the source of light at the principal focus of a plano-convex lamp condenser, which is placed with its plane side towards the source, as in this position the aberrations are at a minimum. Just in front of the lamp condenser is a diaphragm (lamp iris) which acts as a field diaphragm regulating the size of the field to that of the objective and thus mitigating glare in the body of the microscope. It is also useful as an aid to focussing the convex surface of the lens, although the author has found that a bristle, mounted in a handle, the end of which is brushed over the surface of lens when it is focussed, is the best method for accuracy, as in this way the surface of the lens may be brought into exact focus, whereas a diaphragm has, of necessity, to be mounted a short distance in front of the lens. The parallel rays emerging from the lamp condenser are reflected

by the mirror and proceed through the substage condenser to a focal point at its principal focus in the usual way.

Before proceeding further, let us examine the question of illuminants. The lowly oil lamp was dealt with first because it was the original light source and is not to be scorned even to-day, as there must be many a case where oil is the only illuminant. As has been stated, the wick is usually used edge on, as in this position focussing is easier and the brilliancy is slightly greater than when the flat of the flame is used ; likewise, the source of light is absolutely textureless. Admittedly the flame is yellow, but a surprising improvement can be effected if the best quality oil is used, to which has been added a small quantity of camphor. These expedients,

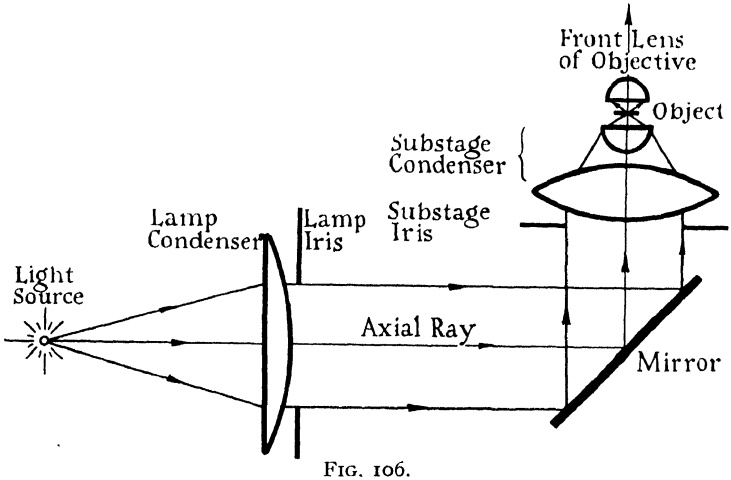


FIG. 106.

together with the use of the correct filter, will produce illumination almost equivalent to daylight in co our.

Owing to its convenience and popularity, electricity as a means of obtaining illumination has at the present time largely superseded the other types, such as gas, incandescent lime, etc. There are several types of electric illuminants from the humble gas-filled metal filament lamp to the powerful carbon arc, which is still used extensively, by a number of workers, for photomicrography. There are, for instance, the ribbon filament, the tungsten arc, popularly known as the " Pointalight," the high-pressure mercury vapour lamp giving almost monochromatic light rich in ultra violet, of much use in ultra-violet microscopy, for which purpose the high-voltage spark * has also been used. The subsidiary apparatus is very elaborate and cumbersome, however, which factor has led to a decline in its popularity in favour of the high-pressure mercury vapour lamp. For supplying monochromatic light, the high-pressure sodium vapour lamp is also very good.

* Usually between cadmium electrodes.

The author, after trying first one and then another of these illuminants over a period of years, was finally driven to the conclusion that the best illuminant of them all is the gas-filled metal filament lamp in an internally frosted (pearl), or preferably opal envelope. For normal work, this source of light is almost ideal as it gives a large area of practically uniform luminosity which is textureless and in the author's opinion is unsurpassed for even the most critical work. The intensity, however, is not sufficient for photography, and after some experimenting the author overcame this difficulty by using one of the popular photographic lamps known as " Photo-floods." These are of the metal filament type with an overrun filament and have a comparatively short life, but the author uses one of these in his photomicrographic apparatus run through a variable resistance which gives admirable control over the intensity, besides prolonging the life of the lamp considerably. Seldom, if ever, has the lamp to be run at its full intensity, approximately half intensity has been found ample for the majority of purposes.

These lamps cannot be obtained in an opal envelope, they are instead internally frosted, which, of course, would give a grainy background if the lamp itself was focussed sharply. However, as the author always uses a lamp condenser with the luminous surface of the lamp approximately at its principal focus, thus obtaining parallel light from the lamp condenser, and uses this condenser as the source of light, when this latter is in focus the texture of the source is not evident. Due to the lamp being the merest shade outside the principal focus, this of course makes the emergent rays very slightly convergent, but the error is so small as to be indistinguishable from the true parallel condition.

In this way true critical illumination can be used for photomicrography, thus ensuring the optical system giving of its best.

Thus critical illumination may be briefly defined as an arrangement which will cause the substage condenser to produce an image of the source of light at its principal focus, which point must be coincident with the plane of the object under examination. Under these conditions, the substage condenser is supplying its maximum possible cone for any opening of its associated iris diaphragm, and the maximum possible resolution is attained for any given objective working in conjunction with any given condenser. It must be emphasised that in no other way can this condition be obtained, more particularly when dealing with high powers.

When a point source of light such as an arc or " Pointalight " is used, it is not possible to obtain a large evenly illuminated field, such as is obtained with the metal filament lamps just described; the same applies to metal filament lamps in a clear envelope. This led to the development of a modified form of critical illumination known as Kohler illumination, which used the same apparatus

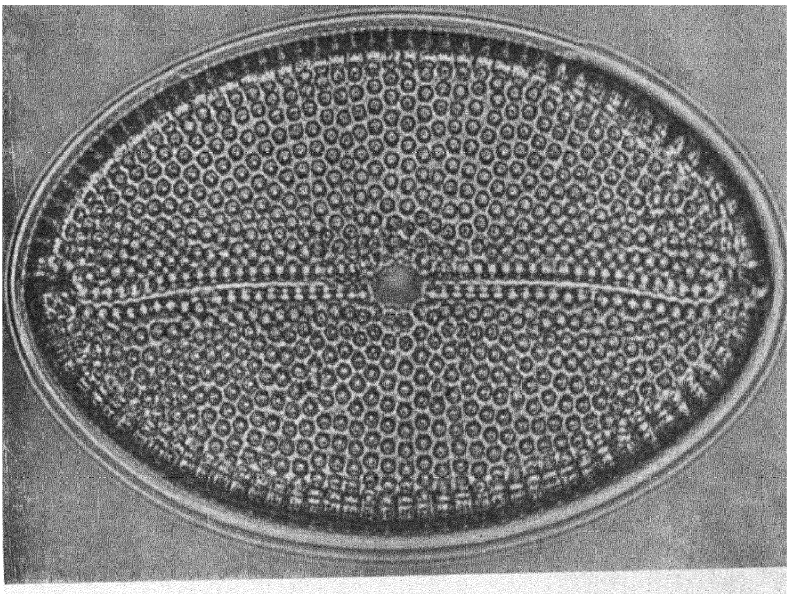


FIG. 107. × 815.

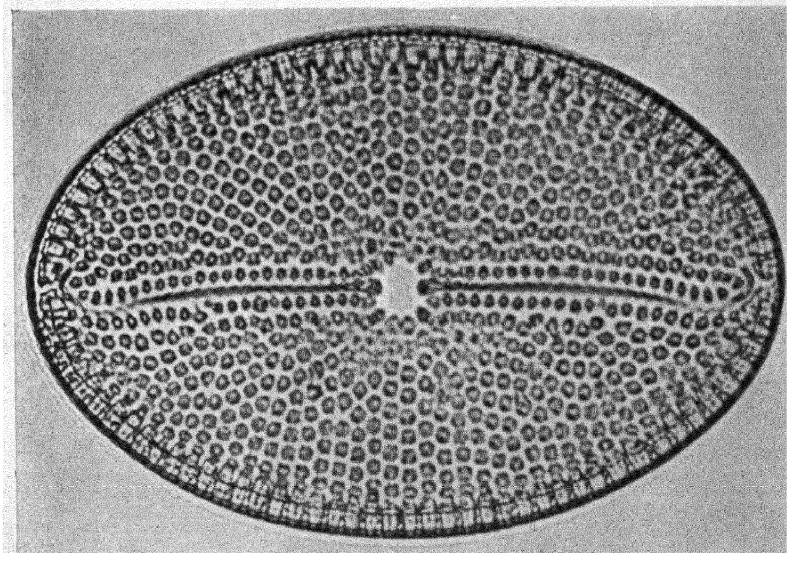


FIG. 108. × 815.

[*To face p.* 104.

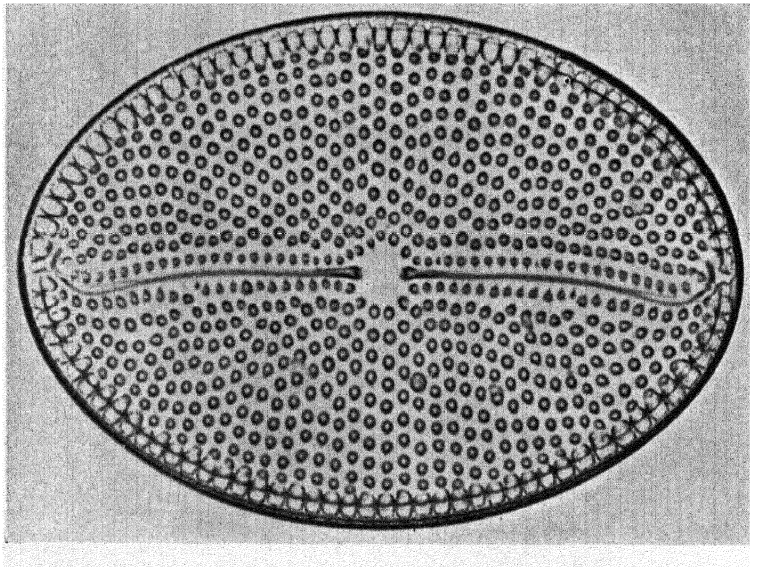


FIG. 109. × 815.

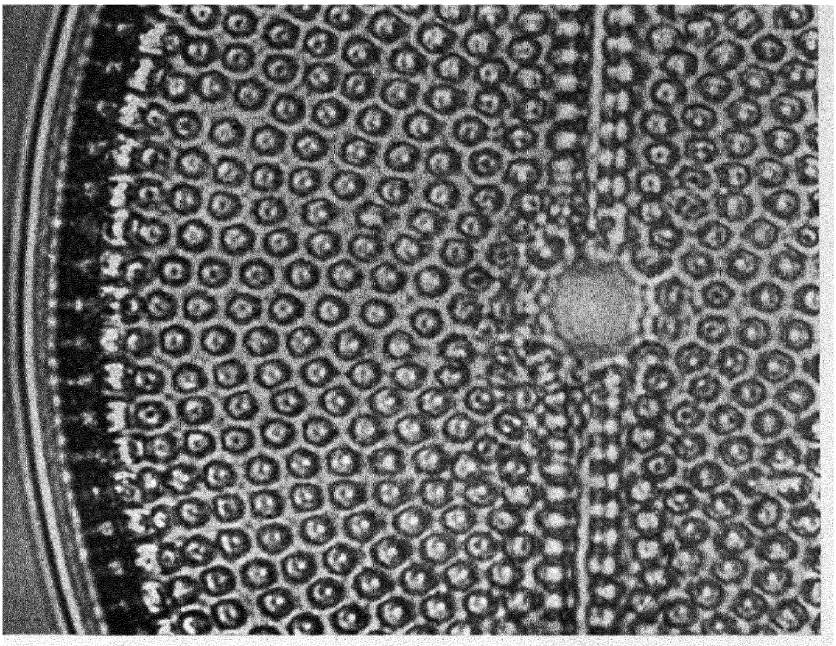


FIG. 110. × 1600.

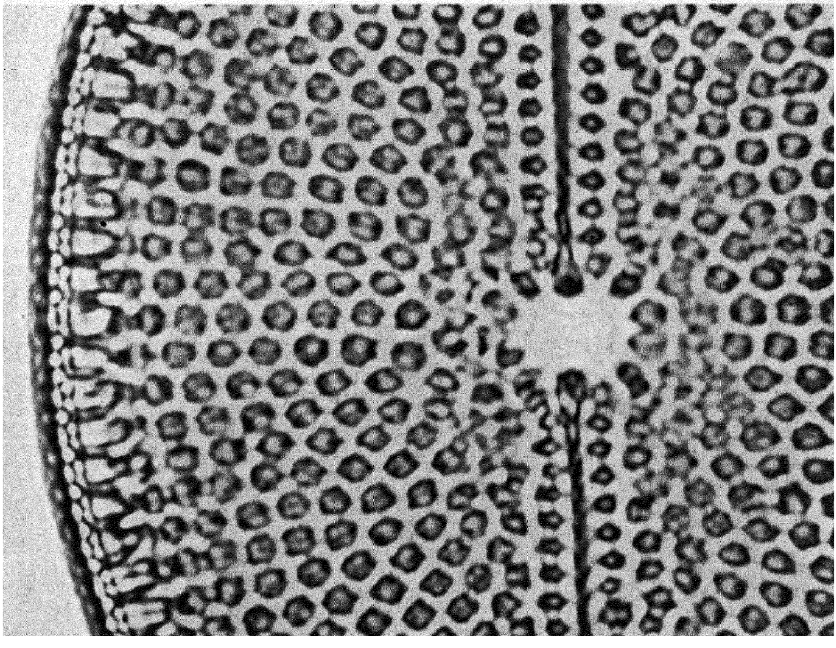


FIG. 111. × 1600.

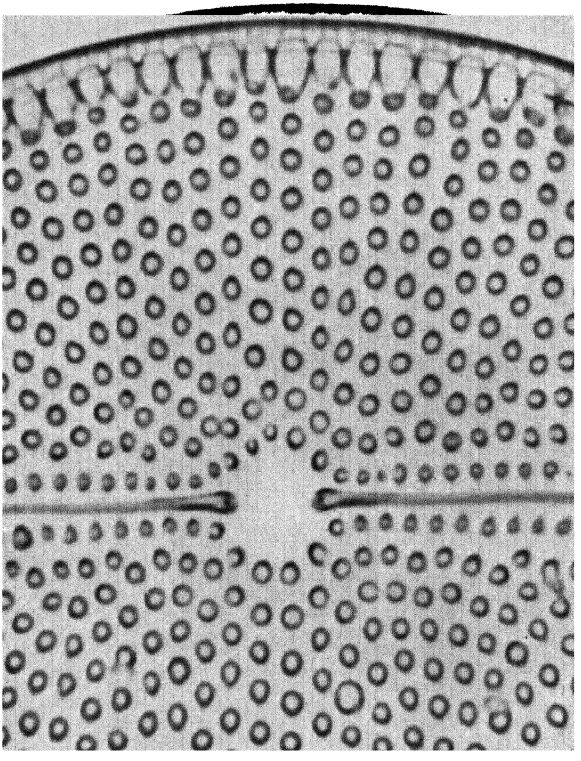


FIG. 112. × 1600.

described in Fig. 106, viz., a lamp condenser with iris and the usual substage condenser with iris. As was suggested for critical illumination, the lamp iris is focussed in the plane of the object by the substage condenser, but instead of the source being fixed at the principal focus of the lamp condenser, it is moved outside it until an image of the lamp is produced at the plane of the iris of the substage condenser.

With the type of illuminant mentioned, this method gives an evenly illuminated large field ; it is not, however, strictly critical, and the absolute maximum cannot be got out of the optical system, but its popularity for photomicrography is without question. The author, however, prefers to use true critical illumination in the manner previously described, and recommends its use on all occasions, particularly when maximum resolution is required.

Thus we see the absolute necessity for correct illumination if we intend to use the microscope to the best advantage, and in order to make this quite clear, let us examine some photographs taken with different types of illumination, critical and uncritical. Figs. 107–109 inclusive show three photographs of the same diatom, " Othonеis Splendida " ; in each case the magnification is 815 diameters, the objective being a fluorite semi-apochromatic, 0·82 N.A., with a X6 compensating occular ; in each case the focus of the objective has not been altered, but the lighting conditions have. In Fig. 107 the illumination was accomplished by the plane mirror only, without any substage condenser. This image is, of course, altogether wrong, as it was obtained with parallel light. Spurious images and diffraction effects, which lead to wrong interpretations, are only too apparent ; in fact, one could say the whole picture is false. Compare this with Fig. 108, in which case the illumination was by an Abbe condenser which was carefully centred but not critically focussed. Here we have the effect of not utilising the maximum aperture of the objective. It is a big improvement on the previous photograph, most evident in the central orifice and junction line of the two halves of the valve, the structure becoming clearer, although the lack of sharpness should be noted. This is not due to the objective being out of focus but entirely to the indifferent illumination. The true picture of the diatom is seen in Fig. 109, where the illumination was with fully corrected substage condenser critically centred and critically focussed and with sufficient aperture utilised to give as perfect an image as possible at the selected focus. Here, of course, we see the vast difference from the two previous illustrations, the structure standing out clearly.

Now let us look more closely at what we know (from Fig. 109) to be clean round holes in the body of the diatom. Fig. 110 is a portion enlarged up to 1,600 diameters, and we see that the holes, particularly near the edge, appear to be more in the nature of

craters, which is entirely false and due mainly to diffraction effects. In Fig. 111, however, they begin to look something like holes but their shape is very irregular; this would, of course, lead one to a wrong interpretation of the image, which is seen in Fig. 112. Here we can see that the holes are mainly circular in shape, showing the great difference from the other two enlargements. These photographs should remove any doubts as to the necessity for critical illumination, although it should be borne in mind that they are at high magnifications; this does not mean to say that for the lower powers critical illumination should be neglected. It is the author's opinion that the best possible illumination should be used with all objectives, and he habitually uses a fully corrected condenser for all objectives from 1 in. down, as the improvement in the image,

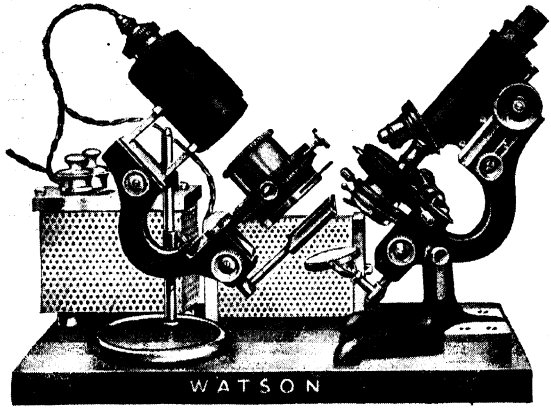


FIG. 113.

even with a 1-in. objective, is surprising when attention is paid to the illuminating conditions.

Thus we see that for the best illuminating conditions the substage condenser has to function at its designed principal focus. This applies equally well to dark-ground condensers, in which case the focus and centring is, if anything, more critical, particularly in the case of high-power D.G. illuminators; also vertical illuminators of better quality must of necessity be critically set up. If they are to give of their best, the same method is employed for vertical illumination; that is to say, an image of the light source is focussed on the surface under examination. All good vertical illuminators have the necessary optical equipment to achieve this end.

Now let us look into the question of lamp condensers. The ordinary plano-convex lens, which is most widely used, may be built into the lamp housing, as shown in Fig. 113, which illustrates a very useful design built around a microscope limb. The lens is adjustable by rack and pinion and is also fitted with centring

screws. The lamp itself is also adjustable, or it may be mounted as a separate component like that illustrated in Fig. 114; in this case the lens is mounted on a stand which is adjustable for height, the lens mount itself being capable of tilting around the horizontal axis and fitted with a centring device. This type of mounting is, however, not very widely used, as the self-contained unit, consisting of lamp housing, carrying the lamp condenser, iris and filter holders, is much more compact and easy to manipulate than an assortment of individual components.

The usual condenser, however, is entirely uncorrected, although by using it with its plane surface towards the source of light, the aberrations are reduced to a minimum; the aperture is small and it cannot be too strongly recommended that a corrected lamp condenser be used. This should be achromatised and aplanatised to ensure a solid and colour-free beam reaching the microscope condenser, enabling both that and the objective to develop their maximum capabilities. In photomicrography it ensures images free from colour and reduces the exposure, as the large aperture of such a lens utilises all the available rays from the light source, being very effective in the case of small high intensity sources such as the pointalite and carbon arc, but it is doubly effective in the case of large area light sources like the opal lamp.

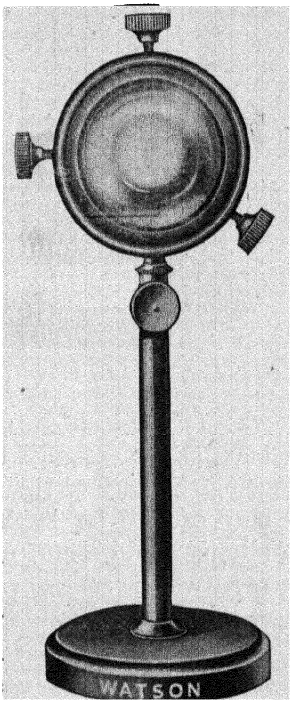


FIG. 114.

After much experimenting, the author has found the Watson-Conrady achromatised aplanat to be the best, so far; the difference in the image when using one of these lamp condensers, from that produced with an uncorrected lens, must be seen to be believed. The corrected lamp condenser is a big improvement on the ordinary " Bull's-eye," and should be used in preference to the latter.

In dealing with the theoretical aspects of critical illumination, very little has been said of the actual construction of the apparatus used, so let us see what a combination of modern ingenuity and skill has to offer the microscopist of to-day.

Let us commence with the oil lamp. Fig. 115 shows a simple type of lamp by Watson; it is fitted with a flat glass reservoir, thereby allowing the light to be brought close to the table. The lamp is fitted with an arm carrying a bracket, which in turn slides on a square section upright pillar, on which it may be securely fixed

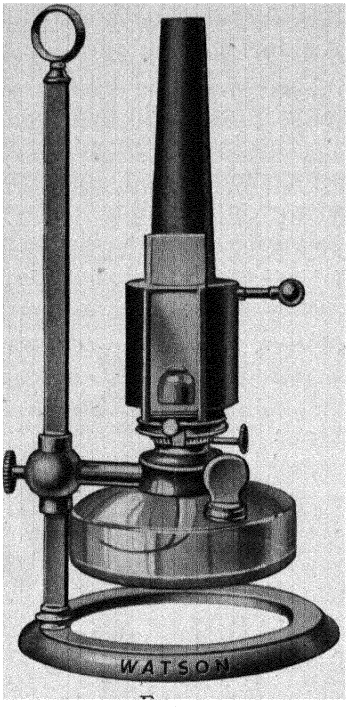


FIG. 115.

at any height, avoiding the possibility of its swinging round. The metal chimney is fitted with a slot to take glass slips 3 in. × 1½ in. which, if coloured, may act as correcting filters. As a further refinement and aid to photography, an aplanatic lamp condenser to Nelson's formula and mounted as shown in Fig. 116, can be used in conjunction with this lamp to produce excellent result. A notable feature of this design is the broad, ring-type base, which gives the

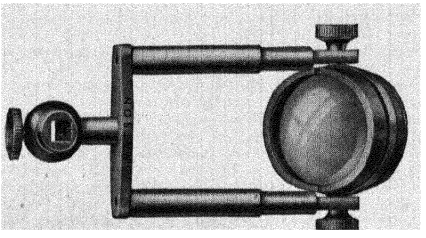


FIG. 116.

whole instrument a remarkable stability. Perhaps the only drawback in the design is the lack of a ready and convenient means of using the edge of the flame instead of the flat. However, this has been catered for in the more ambitious product illustrated in Fig. 117, which shows an oil lamp of the highest class by the same makers. It has rack work and screw movements fitted to the upright bar, giving movement in horizontal and vertical directions by means of which the light may be exactly adjusted. It is fitted with a rotatable burner by means of which the edge or flat of the flame may be used, requiring only a simple movement of the finger and thumb. This lamp can also be fitted with a lamp condenser of the Nelson aplanat type, mounted on an arm attached to the reservoir and having a sliding focussing adjustment. A lamp of this type

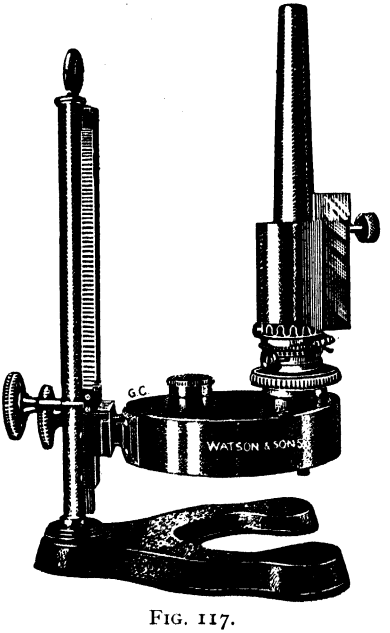


FIG. 117.

is an extremely useful piece of apparatus, and in situations where there is no other means of obtaining illumination it is unsurpassed, as with its aid it is possible to undertake the most critical and painstaking work in complete independence. This type of lamp still enjoys a well-deserved popularity, limited though it may be.

So much for oil as an illuminant. It is quite necessary in certain circumstances, but in the vast majority of cases there is a ready and reliable source of energy available in the form of the electricity supply, which is clean, easy to manipulate and odourless. Makers in general have given much attention to the design of electric microscope lamps, with the result that there are literally hundreds of different sorts to choose from (or is this an indication of the lack of fixed ideas ?), but as a general rule lamps can be divided into roughly three main classes: those of simple construction and intended for elementary use, those of more elaborate construction carrying a number of accessories for the use of the research worker, and the third group consisting of the specially designed high-intensity lamps for special applications, such as photomicrography, dark-ground illumination, etc. Of recent years, what may be classed as an additional group has made its appearance in the miniature substage fitting.

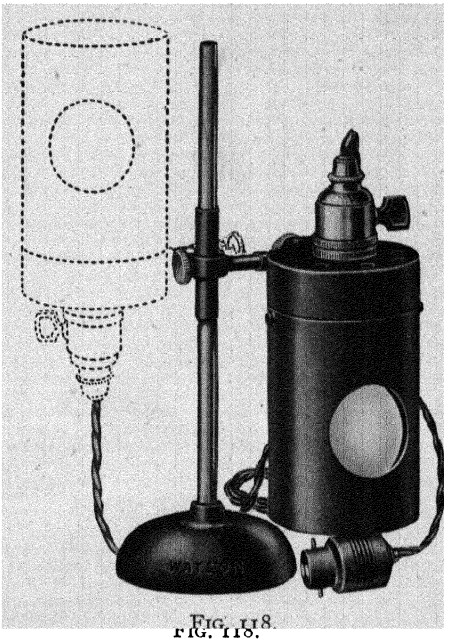


FIG. 118.

In this quick survey of modern electric microscope lamps, let us briefly glance at the simpler ones before passing on to the more elaborate types. Figs. 118, 119 and 120 show three representative patterns; the first, by Watson, is a simple but very efficient fitting, useful for general and elementary work. The housing is large enough to accommodate a 60-watt opal lamp, thus giving ample intensity. The movements are such as to enable it to be used for a variety of purposes; it can be correctly adjusted for angular relation to the mirror and it may be used for work with transmitted light, opaque and dark-ground illumination; a noteworthy feature is the heavy base.

The next lamp, Fig. 119, is a simple and inexpensive illuminant produced by Bausch and Lomb. It consists of a housing holding 10-watt lamp, which is fitted with a frosted lamp condenser of simple

design. This arrangement, in conjunction with a whitened interior to the housing, is capable of producing the same amount of illumination with less heat than is commonly found with fittings having larger lamps. It is possible to fit a daylight filter to this little lamp. A very well-made lamp of clean design is that by Leitz, shown in Fig. 120; it is a simple fitting carrying a 60-watt opal lamp, having provision for ventilation and mounted on a solid stable base. There is no lamp condenser, but a most useful feature of the design is the tubular hood projecting from the front which limits the light to the narrow beam required by the microscope without illuminating the workroom or the laboratory.

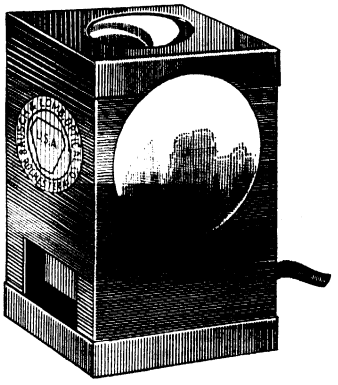


FIG. 119.

The author thinks it necessary to stress this point with regard to illuminants. It is his belief that, if at all possible, effort is made to suppress extraneous light, then far better results will be obtained due to increased accuracy of vision and greater comfort in working.

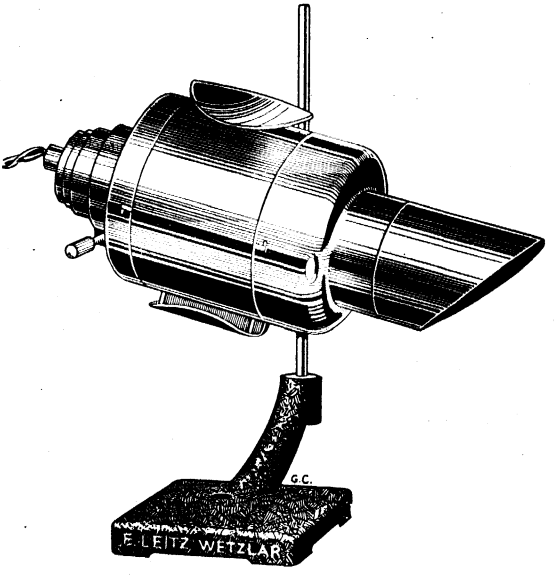


FIG. 120.

Anent which great care is taken in his laboratory to suppress all sources of extraneous light when the microscopes are in use, even to the extent of putting the instrument room into darkness, which steps are fully justified by the results.

Means of excluding extraneous light is a feature common to all,

the lamps are made by Messrs. Leitz, which feature alone makes them useful appliances.

Now let us look at the rather more elaborate type of lamp, with which we may obtain really critical illumination. Referring back to Fig. 113, we see Messrs. Watsons' complete set up for critical work. As we have seen, the research type of lamp is designed round a microscope limb which virtually constitutes an optical bench; both lamp condenser and lamp have independent rack-work movement, the whole being mounted on an upright pillar by means of an adjustable clamping device, enabling adjustments for height and tilt about the centre of the limb to be obtained. The condenser mount has its own centring arrangement and iris, and a filter carrier is mounted on the limb in front of the condenser. Altogether this is a very excellent lamp, capable of use in the most critical circumstances and embodying features such as the rack and pinion focussing arrangements, and a housing so designed that the change from a high-intensity lamp like the "pointalite" to the textureless source of the opal lamp is very simply effected. This is not found among the continental or American designs. Perhaps the only point on which this lamp may be criticised is the lack of any light-tight flexible coupling between the housing and the condenser, as a result of which the extraneous light is enormous; the inclusion of a field diaphragm on the lamp housing would also be an added advantage. If used with a fully corrected lamp condenser, such as the Watson-Conrady, the illumination from this lamp is sufficient to satisfy the most exacting requirements.

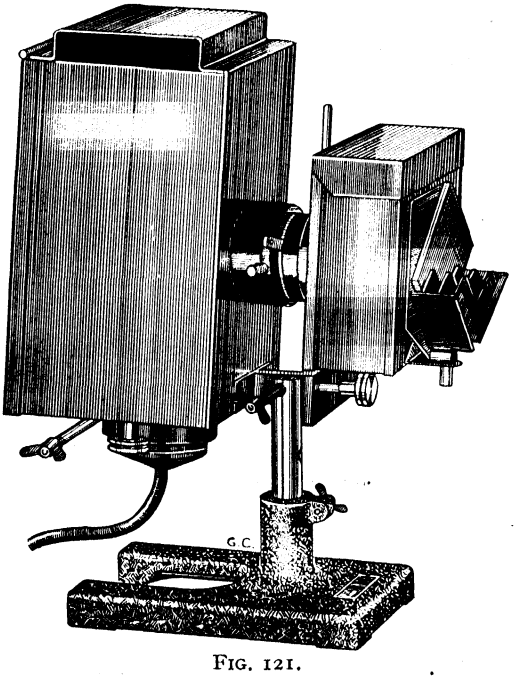


FIG. 121.

If we now turn to Fig. 121, we see a research lamp by Bausch and Lomb. As will be seen, the usual movements are incorporated and include those for height, tilt, lamp and condenser focussing; they are all sliding movements fixed by thumb screws. The equipment of this lamp is very complete, the source is a 100-watt prefocus, ribbon filament lamp, working through a specially corrected

condenser capable of satisfying a substage condenser of 1·40 N.A. Its aperture is adjustable by a diaphragm ; both lamp and condenser iris diaphragms are fitted together with holders or filters and a water cell, this latter being necessary when using Wratten " 17 " filters in contrast photography.

Messrs. Leitz produce a lamp of this type, which is illustrated in Fig. 122. The illuminant is a 100-watt lamp, the mounting of which is provided with centring mechanism. The lamp condenser is focussed by twisting it, and is provided with an iris diaphragm and filter carrier. This lamp is suitable for practically all microscopic work,

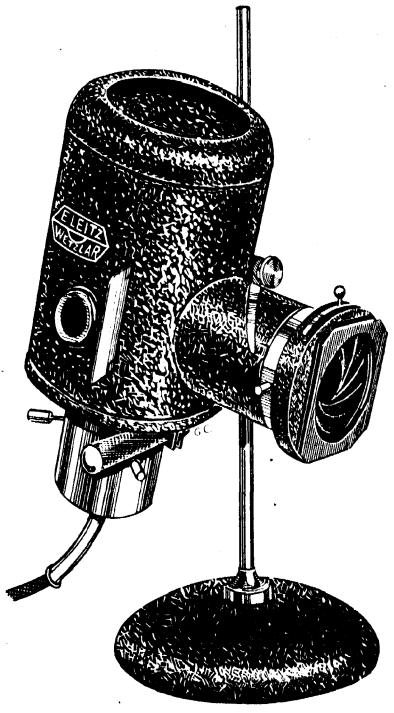


FIG. 122.

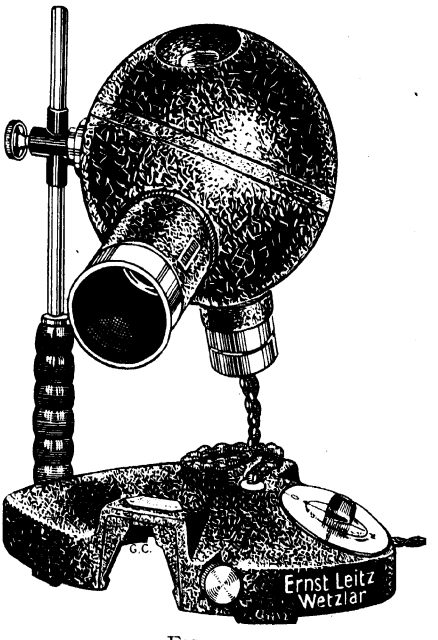


FIG. 123.

including dark ground and polarised light, and it is therefore a useful accessory to the critical worker.

Where intense monochromatic illumination is desired, the lamp illustrated in Fig 123 is very useful This is designed round a high-pressure sodium vapour lamp and is capable of producing excellent results in photomicrography.

Another very good lamp for monochromatic light is the mercury vapour lamp by Watson, illustrated in Figs. 124 and 125, which provides an absolutely steady source of monochromatic light of high intrinsic brilliancy for general observations, photomicrography and visual work of all kinds. Its intensity and monochromatism make it particularly suitable for photomicrography, as filters can be

utilised under almost perfect conditions. It is also a source rich in ultra violet, and with the correct filter is extremely useful in research work in the invisible region of the spectrum. The lamp itself consists of a fused quartz burner which is vented to the atmosphere ;

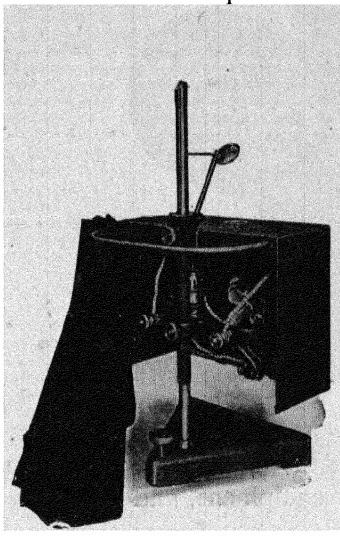


FIG. 124.

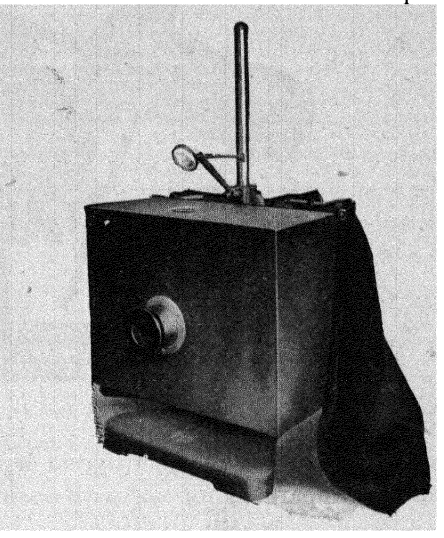


FIG. 125.

it can be raised, lowered, or rotated on a vertical axis, and is also capable of being tilted. A small silvered mirror fitted above an aperture in the housing enables both vertical and horizontal beams to be employed ; the housing is fitted with a focussable lamp condenser and iris diaphragm.

For use in ultramicroscopy, special cases of photomicrography and micro-projection, or any work where a particularly intense or richly actinic source of light is required, it is usual to use a carbon arc. Three forms of microscope arc lamps are shown in Figs. 126, 127 and 128, representative of British, Continental and American practice. It will be seen that they are all designed and built on much the same lines, being mounted on a heavy base, which provides stability, with provision for adjustment to any elevation or tilting to any angle which is required. The condenser, which is focussable, is mounted in a tube, the arc itself is enclosed, but is provided with ventilation to keep down overheating, the feed being automatically controlled by a clockwork mechanism, which ensures the proper rate of travel for the carbon holders.

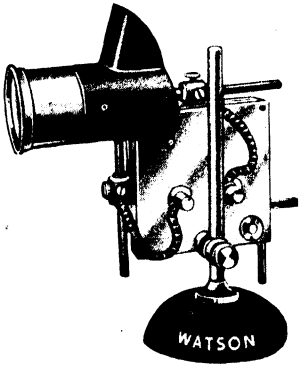


FIG. 126.

From the foregoing we can see the great importance of having

correct critical illumination in order to secure the maximum resolution from the optical train used, in consequence of which microscope

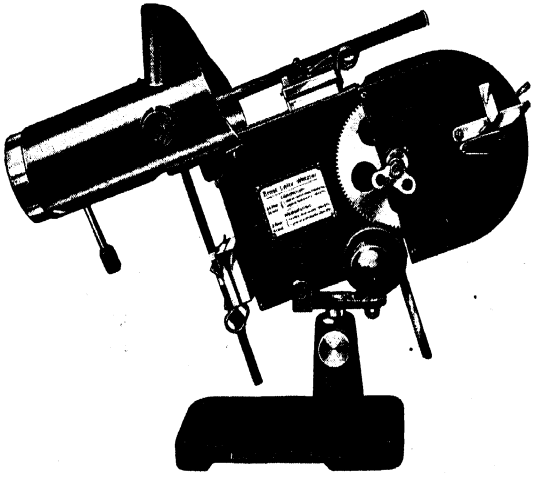


FIG. 127.

manufacturers have gone to great lengths to produce suitable illuminating apparatus for use with their equipment, and it cannot be

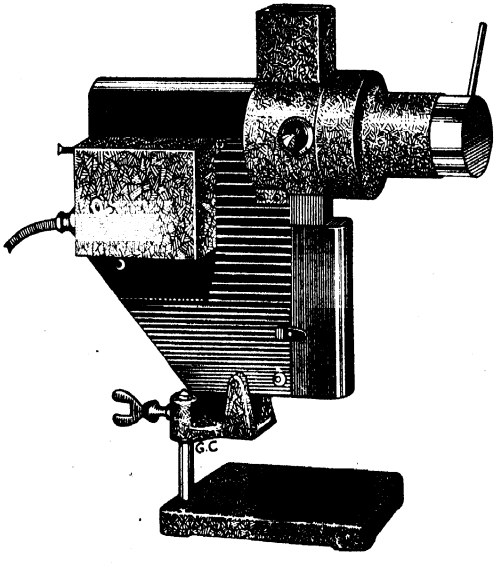


FIG. 128.

too strongly urged that when using the microscope the utmost attention must be paid to the illumination. If this be done, and the optical equipment is beyond reproach, then the best results will be achieved.

CHAPTER VII

THE STAND AND MECHANICAL PARTS

HAVING dealt with the optical principles of the microscope, both simple and compound, let us now consider the mechanism required to put the optical apparatus into operation. Let us commence with the simple microscope. This consists of a single lens with some sort of holder, by means of which it may be held in position and



FIG. 129.

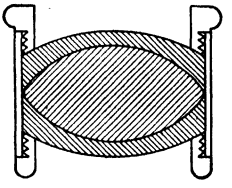


FIG. 130.

focussed, thus leaving both hands free for any manipulations to be carried out on the object being viewed.

The lenses are usually put up in a simple metal mounting of standard dimensions; sometimes they consist of a doublet as shown in Fig. 129, but the best type consist of a cemented triplet based on the Steinheil formula, which gives a large and very flat field, exquisite definition and a long working distance, but it should be stated that the diameter of the lens is no criterion of the size of field or magnification. They usually possess large apertures and yield brilliant images. One such lens is illustrated diagrammatically in Fig. 130. They are made in various magnifications from X10 up to X30, but the author does not recommend using this type of lens at a greater magnification than X20, as even then the working distance is becoming too short and many manipulations which have to be carried out on the object become increasingly difficult.

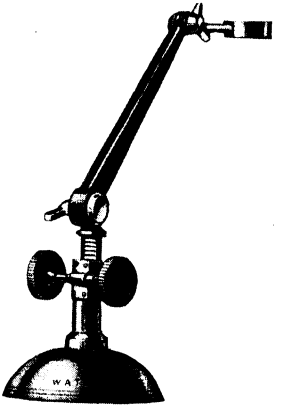


FIG. 131.

The simplest form of stand with which this type of lens may be used at all satisfactorily consists of a massive cast base, on which is mounted a short pillar carrying another one which can be raised or lowered by a rack and pinion motion, and from the top of which extends a jointed rod with the lens holder at the free end, as shown

in Fig. 131, while one of the lenses which fits into the holder ring is shown in Fig. 132.

Another pattern which is more useful, in that it is portable, is shown in Fig. 133, although in this case precise focussing is slightly more difficult. These simple forms of lens holders have, of course,



FIG. 132.

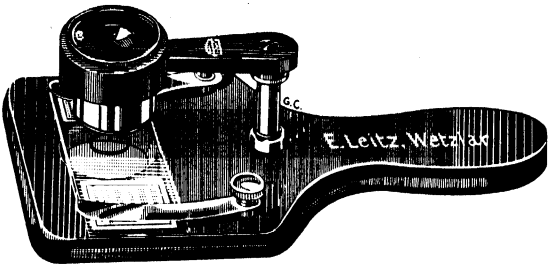


FIG. 133.

only a limited usefulness; nevertheless they are not to be scorned, but in certain circumstances something more elaborate is called for, which gives a greater degree of control, in which case the stand illustrated in Fig. 134 is very efficient.

As will be seen this type of instrument is much more elaborate;

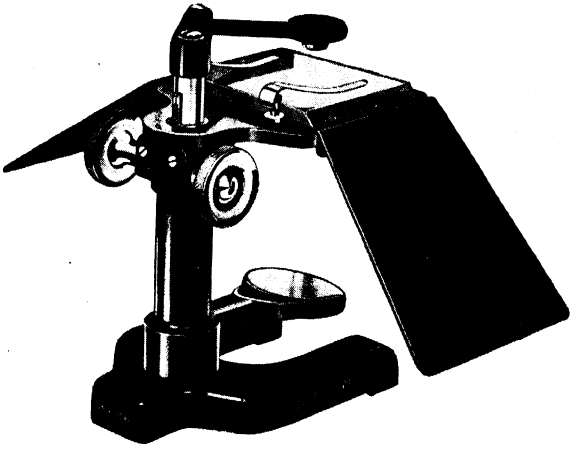


FIG. 134.

made with a jointed arm to carry the lenses, it is equipped with a large horseshoe foot which, together with the pillar, forms one casting, giving great stability. At the top is fixed a large stage fitted with a glass plate and spring clips, and to either side are fitted hand rests which are detachable, thus enabling any dissection or separation to be carried out in comfort. Below the stage and fixed to the base is a bracket carrying a large mirror, on the reverse side of which is a

glass disc. Focussing is by rack and pinion adjustment to the lens carrier.

With the help of a simple instrument such as this, much useful work can be done in the way of preliminary examination and a great deal of time saved in unnecessary high power examination. The most essential points to be catered for are weight and stability in the base, which makes for steadiness, smooth working of the rack and pinion focussing adjustment, which should be free from all backlash, a firm and secure attachment to the stage, which must be of such a rigidity as to be able to withstand quite a considerable pressure without an undue amount of spring.

So much for the simple microscope; now let us consider the compound microscope. We have seen that the instrument consists of an optical train comprising :—

(1) Light source.
(2) Lamp condenser.
(3) Substage condenser.
(4) Objective.
(5) Eyepiece.
(6) The eye.

But for our present purpose we may ignore the first two and the last items on the list and examine the mechanical arrangements necessary to co-relate the third, fourth and fifth items into a unified whole, which comprises the compound microscope as it is to-day. Fig. 135 illustrates a diagrammatic section of a modern microscope. The diagram is annotated with the names of the various parts clearly shown and from which it will be seen that we now have a much more complicated piece of apparatus than in the case of the simple microscope.

The modifications and variations of this basic compound instrument are many and various, all of which it has been necessary to develop to meet special needs, such as metallurgical microscopes, stereoscopic, binocular, polarising, comparison and even special stands for dark ground illumination only; but let it be clearly understood that they are all either developments of, or modifications to, the basic type shown in Fig. 135. Therefore, let us examine it in detail so that the specialised types will be more readily understood and we will immediately obtain a great deal of help by asking ourselves the question: "What are the essential attributes of the microscope to meet the rigorous demands of modern conditions?"

So let us examine the diagram; we see that the instrument has a foot or base on.which is carried a pillar or other means of affording a pivot to the limb which in turn carries the stage, beneath which is fixed the mirror and the substage mechanism, carrying the substage condenser, by means of which this latter may be moved up and

down so that it may be focussed and also centred. The upper part of the limb carries the coarse and fine focussing adjustments to the body tube in the upper end of which is a sliding draw tube carrying the eyepiece, the lower end of the body tube is fitted with a dustproof revolving nosepiece capable of accommodating two or more objectives, as shown. Attached to the lower end of the limb is the tailpiece which carries the mirror, or prism, as the case may be, in its gimbal.

Thus we see the multiplicity of mechanical parts necessary to

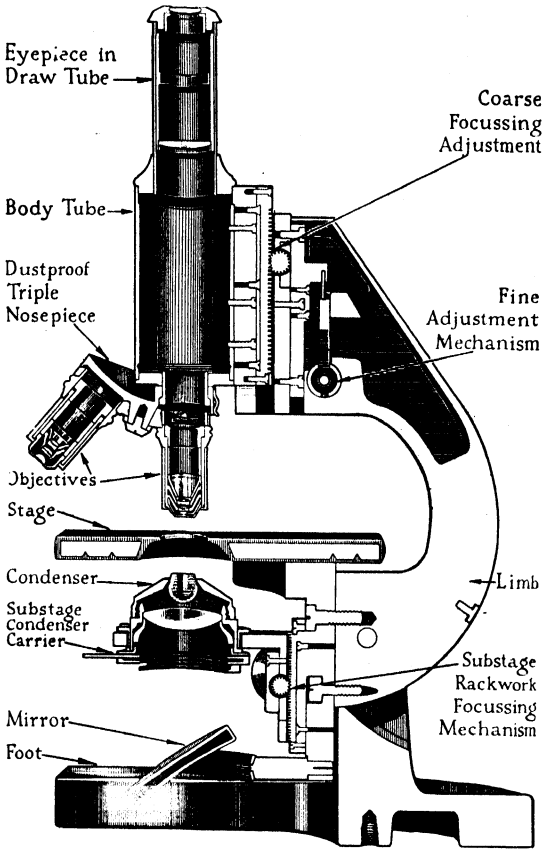


FIG. 135.

(Courtesy Bausch and Lomb)

get our optical train into operation, and as the efficiency of the whole instrument depends on the design of, and accuracy with which these are made, it behoves us to examine the individual requirements of each of these parts.

The first requirements are obviously stability and rigidity, because if our stand were to topple over with the slightest push, or there was a lack of rigidity, then no matter how fine an optical system it possessed, it would be worthless as an instrument; therefore it is agreed that steadiness is an indispensable quality.

Steadiness in a microscope is mainly obtained by two basic designs of foot :—

(1) The horseshoe or " Continental " pattern, and
(2) The " Tripod," or English, foot.

For a number of years there has been a controversy over which is the better, the Continental and American manufacturers favouring the horseshoe type, which is admirably shown in Fig. 136 (although by a British maker in this instance, the illustration was chosen for its clarity), the British manufacturers favouring the tripod type of foot shown in Fig. 137. Nowadays the

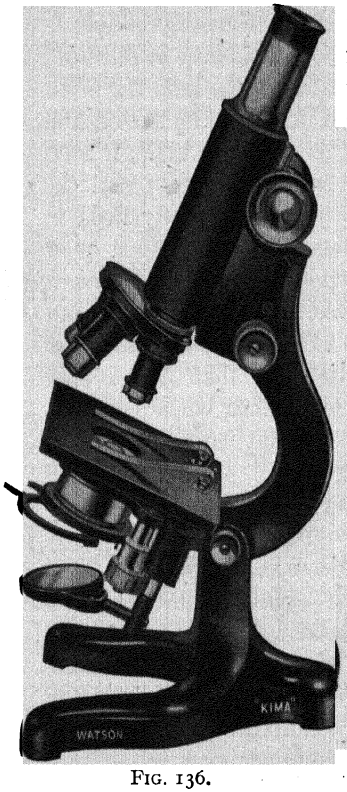


FIG. 136.

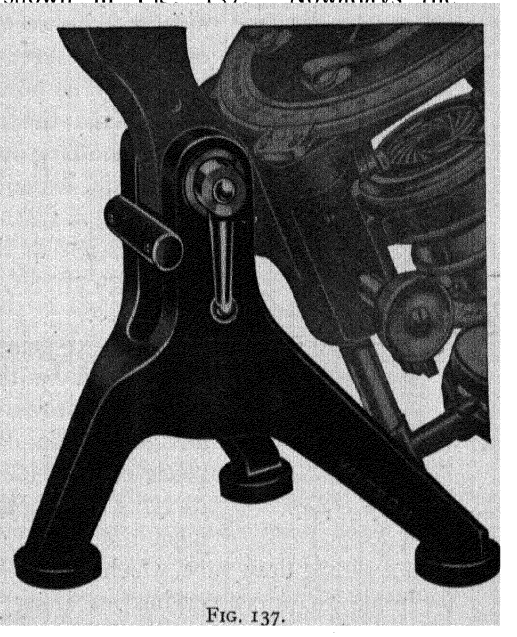


FIG. 137.

British manufacturers also use the horseshoe foot, so designed that the load is taken on three points.

It is claimed by the supporters of the tripod foot that, in the Continental type, the instrument is fixed to a massive horseshoe foot, the ratio of whose weight to that of the rest of the instrument is so high that the whole is usually steady ; whereas, it is claimed for the tripod foot that steadiness is obtained by design, the ratio of weight of the foot to that of the limb being much lower.

That this difference in weight ratios exists is a debatable point, as in modern instruments with the horseshoe foot the casting is usually hollowed out to a considerable extent, with the result that the weight ratio has been greatly reduced (thus making the whole

instrument less unwieldy); so much so, that it is doubtful if there is very much difference from one to the other.

If we examine the shapes of these two types of feet more carefully, it will be seen that the load-bearing points in each case are at the apex and the three corners of the base of a three-sided pyramid and, therefore, for such a pyramid of any given dimensions, a foot of either type and having the same weight could be designed to give exactly the same stability to any given limb; in fact, it is the author's opinion that the stability of a microscope does not depend so much on the design of foot as on keeping the centre of gravity as low as possible (which, after all, is one of the fundamental laws of physics). This is dependent on how far up the centre line of the limb the pivot point is fixed. Obviously the higher it can be taken the better, and in his experience there is very little, if any, difference between one type of foot and the other. An argument has been advanced in favour of the horseshoe foot inasmuch as it was said that the tripod was more inconvenient for the handling of the substage controls; but here again, experience has shown that there is nothing in it, so it seems that there is only one question in the balance with regard to the design of foot, and that is the question of appearance.

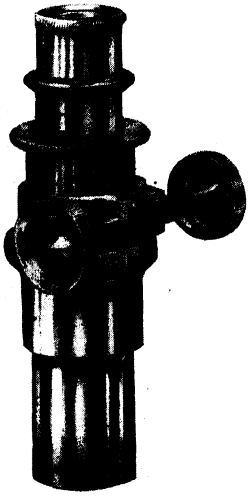


FIG. 138.

The next point to come under consideration should be the body tube. The body tube carries the objective, or objectives, and auxiliary equipment such as multiple nosepiece, etc., on its lower end, the upper end carrying the eyepiece. In a monocular instrument the body tube should always be fitted with a sliding draw tube, which should be calibrated in millimetres for some 10 or so centimetres along its upper portion so that at any given reading, using the upper edge of the body tube flange as an index, the mechanical tube length is known. This is important, as we have seen that the better quality objectives are very sensitive to tube length and cannot function at their best unless this is correct, particularly is this the case with apochromatic objectives.

In the highest grade stands this draw tube is moved by a rack and pinion motion. An attachment by Watson whereby an instrument having an ordinary sliding draw tube may be converted into one possessing rack and pinion motion is shown in Fig. 138, which also clearly shows the method of mounting the rack and pinion on the front of the body tube. Failing this, tube length correction may be very easily and most efficiently carried out optically by the Jackson

tube length corrector shown in Fig. 139. This very efficient piece of apparatus was invented by Sir Herbert Jackson; it can be fitted between the objective and the body tube, the adjustment being carried out by a simple turning of a captive ring. By this means a range of tube length covering 100 to 300 mm. may be obtained.

Formerly there were two tube lengths, the British makers fixing their standard at 250 mm., or 9·8 in., measured from the top edge of the draw tube to the end of the nosepiece into which the objective is screwed, whereas the continental manufacturers took as their standard, a tube length of 160 mm., or 6·3 in., but nowadays the general trend is for microscopes and objectives to be produced with a working tube length of 160 mm., although the majority of instruments possess draw tubes capable of extending the tube length to 250 mm. to allow for cases where objectives which have been corrected for this length are used, but it is worth while noting that a small difference in tube length produces a greater resultant effect with a short body than a long one, and hence the adjustment of tube length in the case of the short body is more critical and not so easily managed as with the long body.



FIG. 139.

The end of the draw tube should be equipped with a diaphragm carrying the standard R.M.S. objective thread, the length of the draw tube being so arranged that this aperture is neither too large nor too small. As in the former case, glare will result and in the latter case some of the rays forming the primary image will be cut off, this virtually having the effect of reducing the aperture of the objective where this is high. Improvements in the reduction of glare are also effected by the use of a large-diameter body tube, which minimises side reflection.

After considering the body tube, the next points to be examined are the focussing arrangements attached to the body. In all high-grade instruments two focussing mechanisms are fitted; these are, firstly, a relatively fast motion achieved by means of rack and pinion, called the "coarse adjustment," and secondly, a very slow and delicate movement, called the "fine adjustment."

A first-class coarse adjustment is a feature of prime importance to the instrument, and all reputable manufacturers pay a great deal of attention to this part of their stands. The usual method is to fix the body tube along a V slide which carries a rack on the other side. The slide fits into a dovetail groove in the upper portion of the limb, and the rack engages with a pinion fitted into the limb and which is operated by a knurled knob on either side. Fig. 140 illustrates a body tube complete; here we have a complete picture of this part,

D.T. is the draw tube suitably engraved for tube length, N.P. is the nosepiece into which the objective or the multiple nosepiece is screwed and which carries a standard Royal Microscopical Society objective thread. C shows very clearly the V slide and its associated rack.

One important point about this fitting is the necessity for absolute rigidity, and whereas hitherto, and even in many cases at the present time, the body tube consisted of the outer tube with a fitting which screwed into the lower end and was threaded to receive the standard R.M.S. objective thread, this being fixed to the bar on which the V slides were machined to form the coarse adjustment, the fixing of the body tube to the bar being accomplished either by screws alone or by screws and solder. Messrs. Watsons, in producing the tube shown in Fig. 140, are responsible for a noteworthy advance in the design of the stand inasmuch as the tube and V slide are made from one solid piece of metal. R, Y and N.P. are one piece, thus the machining operations may be carried out so as to give perfect alignment of the slides and axis of the tube, and doing away with all risk of the separate components of the built-up body parting company, which latter has been known to happen in the past.

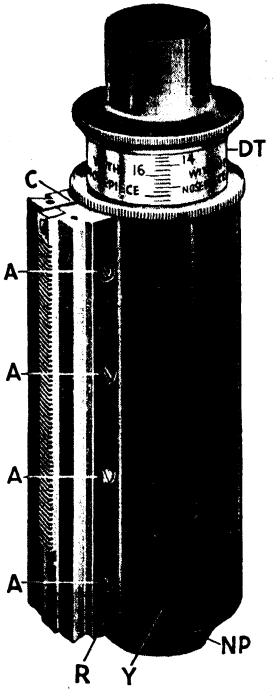


FIG. 140.

It will be noticed that the V slide has a longitudinal slot indicated also at C; this is to allow for adjustment for wear of the slide, this adjustment being carried out by the screw marked A, for it will be appreciated that as the slide wears it will inevitably become slack in the dovetail groove, with the result that the higher-powered objectives are liable to slip out of focus. Therefore, on all reputable stands, this adjustment is fitted as standard.

The rack shown in Fig. 140 is a diagonal rack which meshes with a helical pinion. This form of rack and pinion was introduced by Swift about 1880 and has since become the universal mechanism for the coarse adjustment. Formerly, the rack and pinion were both straight cut, but were given up in favour of the diagonal rack and helical pinion, due to greater freedom from backlash, the importance of which is obvious.

The body tube V slide works in a corresponding dovetail groove, in a block which carries the pinion bearings; thus it is capable of movement relative to the block, which in turn has a V slide fitting

into a dovetail groove in the upper portion of the limb proper, shown in Fig. 141. The dovetail groove is seen at A; it will be noticed that again we have a slot which allows for adjustment of the dovetail by means of the screws B. The movement of the block in this slide will obviously move the whole body tube up and down, this action being carried out very slowly and with greatest precision by the fine adjustment mechanism, which will be discussed subsequently.

Hence we see that the coarse adjustment must be well designed and made with ample provision for taking up any wear that might take place, so that it may work with velvet smoothness and, so far as is possible, be absolutely free from any signs of backlash. Modern methods of manufacture have so improved matters that, for all practical purposes, backlash is non-existent. A coarse adjustment when well made should be such that all the lower-powered objectives are capable of being perfectly focussed with it, and with the highest powers; objects should be capable of being brought to the point of clear visibility.

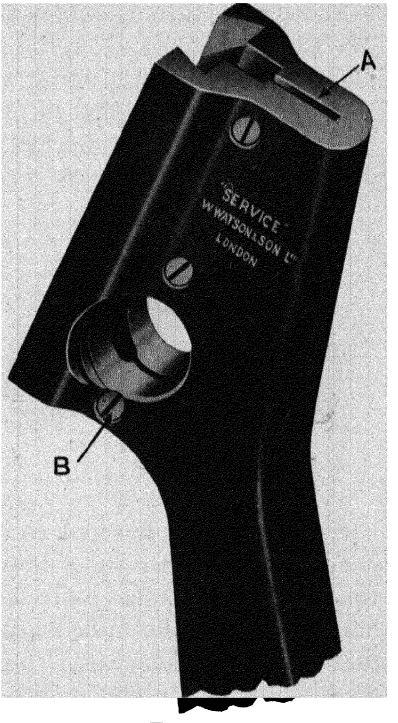


FIG. 141.

Of equal, if not greater, importance is the fine adjustment, for if this is poor the instrument is useless for critical work with high powers of magnification. When it is realised that, with a $\frac{1}{12}$-in. oil immersion objective, a movement of three or four microns is sufficient to throw the object completely out of focus, it becomes clear that the fine adjustment must work with the utmost precision and be quite definite in operation; backlash and its associated tendency towards slipping cannot be tolerated, hence much thought and ingenuity have gone into the design of the fine adjustment, as a result of which two main types have become the most popular in this country. They both function by virtue of raising the complete assembly consisting of the body tube and its associated lenses, together with the whole coarse adjustment mechanism, as was explained in reference to Fig. 141.

Of these two types, perhaps the most popular to-day is the vertical lever shown in Fig. 142, which design blends admirably

with the modern type of limb and due largely to a demand for a fine adjustment operated from the side of the limb. In the illustration "A" is the block which is fixed to the V slide on the body block and which moves with this slide in the square portion of the dovetail groove shown in Fig. 141. It has ample clearance and does not touch any portion of the groove in which it moves. The pivot of the lever B is fixed to the limb itself, so that its distal point is in contact with the face of the wheel C.

This, together with the shaft and fine screw thread is made in one piece, the whole arrangement is assembled in a tube, which carries the nut for the screw at one end, shown in Fig. 143, and an aperture in the centre, through which the actuation of the lever takes place. The tube is firmly fixed in the aperture in the limb, so that its axis is at right angles to the optic axis of the instrument. The lever is shaped and placed so that its pressure on the block is always vertical; thus by rotating the milled heads the wheel C is moved in a horizontal direction, so that the point of the lever B in contact with the block A

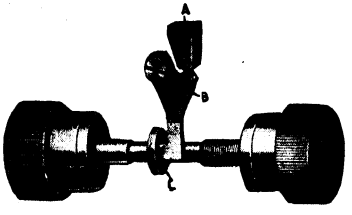


FIG. 142.

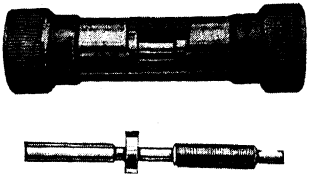


FIG. 143.

is either raised or lowered according to the direction of movement, hence, of course, raising or lowering the block and its attached body tube. Needless to say, all load-carrying surfaces in contact are of hardened steel, the milled head on one side is usually graduated, the calibration being as a rule in microns. This is very useful in cases where measurements are required.

The older type of fine adjustment was first evolved about seventy or eighty years ago, and owing to its inherent sensitivity and robustness, is still quite popular to-day and is still thought to be more efficient than the vertical lever by some workers. As a matter of fact, the author has used a microscope fitted with a horizontal lever fine adjustment for a number of years, and has still to find another type to better it for accuracy and sensitivity. This type is shown diagrammatically in Fig. 144, and it will be seen that the whole body of the instrument is supported on the shorter end of the lever C, which is boxed in the limb; the long end rests on the point of a micrometer screw actuated by a large milled head, whilst double spiral springs exert their force along the plane of movement, thus keeping the body pressed closely against the lever. It will be seen

that the fulcrum D is very close to the body end of the lever, giving a leverage ratio of 3·25 to 1, thus minimising the screw pressure at the actuating end. This ratio, combined with an actuating screw of 70 threads per inch, impart to the body a movement of 100 microns per revolution of the fine adjustment milled head. The head itself is calibrated into 100 divisions; there one is able to read directly to one micron and estimate without any difficulty to ½ micron, the divisions being approximately 2 mm. apart. Also shown in the sketch is the pinion bearing BB of the coarse adjustment, the eyepiece fitting at the top of the draw tube E, the objective thread F in the lower end of the draw tube, and the objective thread G in the nosepiece of the body tube.

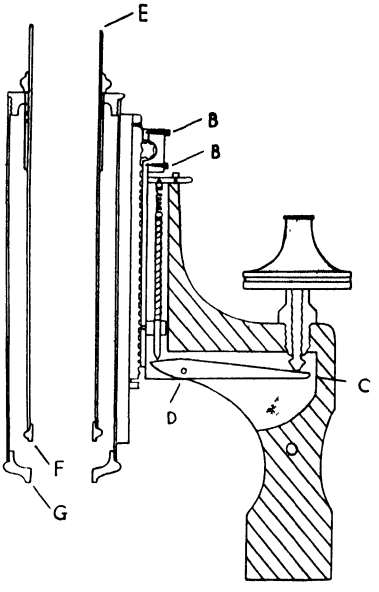


FIG. 144.

The fine adjustments just described are those designed by Messrs. Watsons, and it is not intended to convey the idea that all other manufacturers use the same methods to actuate either the vertical or horizontal lever, but rather to give some idea of the general principles of operation of the two types most generally employed, irrespective of the precise method of operation; however, the sensitivity, rigidity and workmanship must all be up to the very highest standard for both coarse and fine adjustments, no matter what method is chosen to achieve the desired end; in fact, the fine adjustment should be such that a $\frac{1}{12}$-in. O.I. objective, having been focussed with the microscope in a vertical position, is still in focus when the instrument is turned to the horizontal. A further very important point is reliability over long periods, which dictates that the best materials be used in order to ensure that the absolute minimum of wear occurs for the maximum amount of use over any given period. If all these points are not conformed to, then at some time or other the instrument will become partly useless and its scope restricted, due to a falling off of mechanical sensitivity, etc., and consequent incapability of use for critical work with high-powered objectives.

The foregoing remarks regarding the slides and rack and pinion movement apply equally well to the movements of all other parts on the instrument; in fact, it could be said that whenever movement is required of one part relative to another, the mechanism to achieve

this end must be efficiently designed and embody workmanship of the highest grade to ensure the utmost precision in working. Sliding fits such as V slides, etc., should be made without having to use the wear adjustments to take up slack; in other words, slides and screws, etc., should fit as perfectly as possible and the provision for taking up wear be left for that purpose. Movements should work with velvet smoothness without detectable backlash and be free from all tendency to jerkiness or stickiness. Conformity with these requirements will ensure the accuracy, sensitivity and reliability necessary to allow a first-class optical system to give of its best.

The next portion to come under consideration is the limb; one such component which is typical of modern design is illustrated in Fig. 145. The big advantage of this type over the older designs is that it consists of one casting or forging carrying the body on the upper portion and the substage on the lower portion with the bracket to which the stage is fixed, an integral part of the casting lying immediately above the substage. Thus it is possible to machine the dovetail grooves for carrying the body and the substage in one continuous operation, ensuring basic alignment throughout, at the same time and at one setting the stage bracket may be machined to ensure correct orientation of this surface with those of the dovetail grooves. In this way a degree of accuracy hitherto unknown is achieved, which calls for the minimum of adjustment to obtain perfect optical alignment. It will be seen that the limb virtually constitutes an optical bench, and Messrs. Watson, the originators of this type of limb, have called it their "Optical Bench Limb." When compared with the older types of limb, which were built up of separate parts screwed together, this method is obviously a big step forward in the design of this component, and at the present time this type of limb is universally popular in this country as well as on the Continent and in America. The arm portion should have as deep a curve as possible in order that there will be ample room for all manipulations on the stage. It should, of course, be of substantial section to ensure maintenance of alignment.

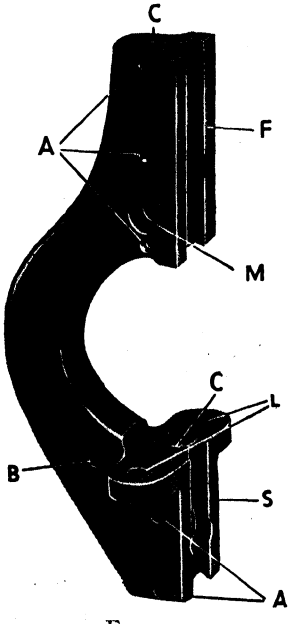


FIG. 145.

Having dealt with the limb, let us take a look at the substage. As the name suggests, this is fitted below the stage and used for carrying the illuminating equipment, and is intended to enable us to use this latter in the most efficient manner possible with the various

powers of objective. Therefore, let us examine those requirements of the substage which will enable it so to perform.

Firstly, it must possess a means of lowering or raising the condenser with a smooth and positive motion in order that it may be correctly focussed. Formerly, with the cheaper type of stand, and also in some of the more pretentious instruments, this was accomplished by means of a multiple start helical screw, which, however, was never very satisfactory as it was always subject to an excessive amount of backlash among other faults, and accordingly it is not now used to any great extent, except perhaps on elementary stands of the student's type, where it serves a useful purpose, as it introduces the novice to the method of using a substage and is, of course, infinitely preferable to no substage at all; an arrangement of this type is clearly shown in Fig. 136.

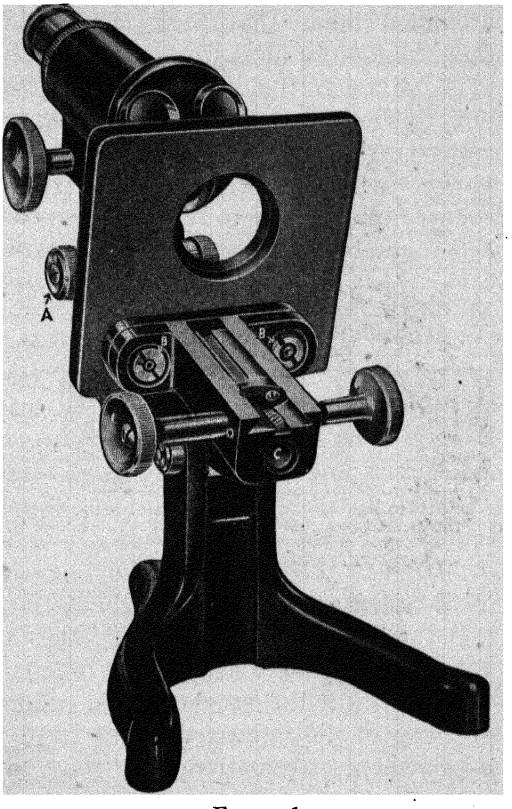


FIG. 146.

The more usual method nowadays, and one which is universally adopted, is to focus the substage by rack and pinion motion in the same way as for the body, and the remarks already made about rack and pinion motions apply equally well to that for the substage. Therefore we see that the foremost requirement is a reliable focussing arrangement of the rack and pinion type whose delicacy of operation and precision must be every bit as good as that for the body tube. Fig. 146 shows the dovetail groove in the limb and the pinion together with its associated milled heads, to which the substage slide is fitted in exactly the same way as the body slide.

The next most important necessity is an arrangement whereby the condenser may be centred relative to the optic axis. The exacting requirements of modern research work and high aperture lenses demand the utmost accuracy in the alignment of the optical train.

This in turn demands means of centring because without such means, condenser, objective and eyepiece cannot be made to conform to a common optic axis; therefore, some means of centring the condenser is essential, respecting which it is worth mentioning that for years the Continental and American manufacturers were at variance with their British contemporaries over this point. The British designers appreciated from the beginning that this movement was necessary but the others disagreed, with the result that the majority of Continental and American microscopes, even to-day, are entirely without any means of centring the condenser, their argument being that their instruments are so accurately made that there is no need whatever for provision for aligning the optic axis, although they have supplied high-power dark-ground illuminators into whose mounts a centring arrangement has been built for many years, thus in some measure contradicting their own argument. However, the author, in common with many other workers in this country, prefers not to rely alone on the skill of the craftsman, and hence insists that the condenser centring motion is a fundamental necessity. It is significant to note that of late years both Continental and American manufacturers fit centring arrangements if required.

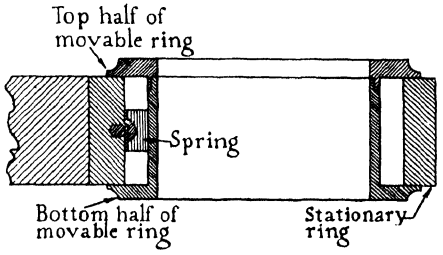


FIG. 147.

The centring movement is usually obtained by making the ring, which actually takes the condenser mount, with an annular groove on its periphery, which fits over the ring attached to the slide, so that the horizontal faces of both rings are in contact and provide a sliding fit and there is sufficient difference between the diameter of the bottom of the groove in the condenser ring, and the internal diameter of the ring fixed to the slide, to allow the condenser ring a movement of something like $\frac{1}{8}$ in. to $\frac{3}{16}$ in. At a point on the bottom surface of the annular groove, which is nearest the slide, a flat piece of clock spring is fixed to the ring at its central point so that the two ends press against the fixed ring. Two screws working through the fixed ring are placed in such a way that their axes are at right angles to one another in the horizontal plane, and intersect at the centre of the fixed ring, each being also at 45° to the diameter obtained (in the same plane) by joining the fixing point of the flat spring and the centre of the fixed ring. They are located on the opposite side of the ring to that on which the spring appears. The whole arrangement is illustrated diagrammatically in two views shown in Figs. 147 and 148, the first being a vertical section and the second a horizontal section · the method of obtaining the

annular groove by screwing one half of the condenser ring into the other is also shown.

In the early part of the present century, when achromatic condensers of high aperture were coming into regular use, it was realised that the focussing of these lenses was very critical, and Nelson suggested the fitting of a fine adjustment to the focussing arrangements of the substage, and whereas it is not strictly a necessity, one has only to work a $\frac{1}{12}$-in. O.I. apochromatic objective to the limit of its aperture and with high superamplification to realise how useful is such a feature. However, as the majority of workers seldom if ever resort to such high powers, we need not go into any great detail on this subject; suffice it to say that it becomes in the nature of a necessity in certain circumstances, but may more generally be regarded as something of a luxury. The modern completely equipped stand, however, usually carries a fine adjustment to the condenser focussing as standard, this being necessary for high-power dark ground illumination.

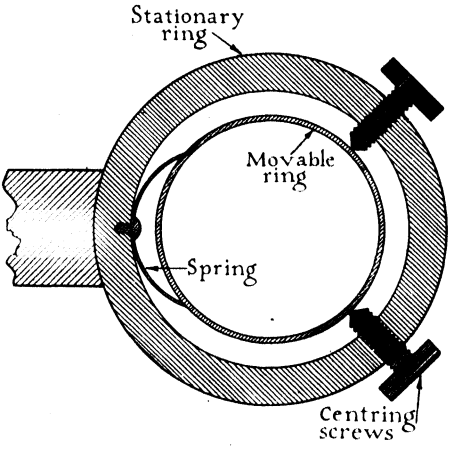


FIG. 148.

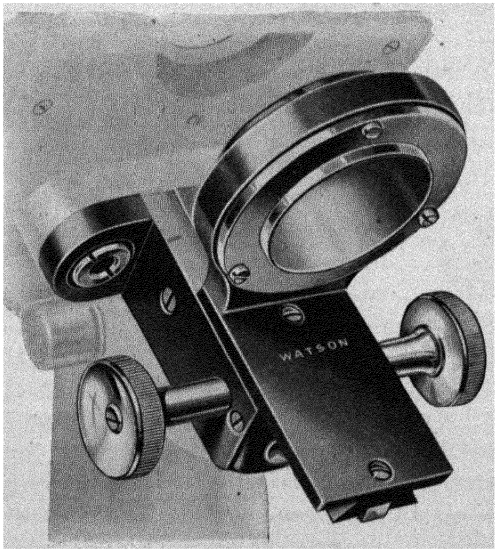


FIG. 149.

A typical simple type of substage which has replaced the spiral screw focussing type for general laboratory stands is shown in Fig. 149. This type is focussed by a rack-and-pinion motion and consists of a solid casting to carry the condenser in its mount. A certain amount of latitude is given to the sleeve fitting so that at any subsequent date if, after rough handling in the laboratory, it is found that the condenser is no longer central, the necessary centring can be carried out by releasing

the three screws shown; the adjustment screws to the slide are also shown. This substage can only be used with a simple condenser such as the Abbe, due to the lack of centring arrangements, and hence limits the scope of the instrument to simple uses

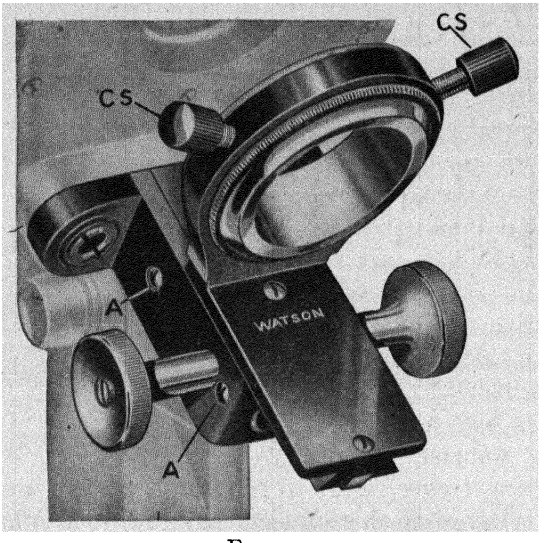


FIG. 150.

such as preliminary checking of preparations and more or less cursory examinations.

A type of fitting which is more complete, and being the least with which an instrument intended for serious work of any kind should be fitted, is shown in Fig. 150. It will be seen to be similar to the previous fitting (Fig. 149) but possessed of centring screws CS for the condenser, thus enabling any form of condenser to be aligned to the optic axis of the microscope.

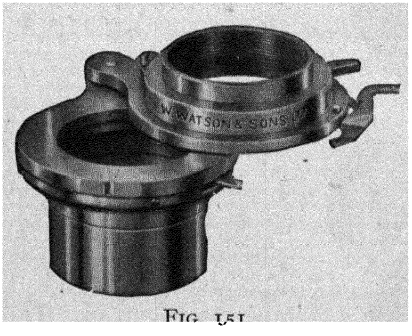


FIG. 151

In this type of fitting it is necessary to rack the entire fitting right out, and to overcome this difficulty Messrs. Watson have designed a condenser mount shown in Fig. 151, in which the optical part, complete with condenser and filter holder, is capable of being swung right out of the axis of the microscope. This movement greatly facilitates the removal and changing of the condenser, as one only has to lower the substage sufficiently for the condenser to clear the stage, when it may be swung out to one side of the microscope and the necessary operations carried out. It is also useful in cases where very low-powered objectives are employed and the

condenser is not needed, when it may be swung out of the way, and as the diaphragm is left *in situ*, it may be used for controlling the illumination. It will be seen that a trigger is fitted to the movable part of the mount carrying the condenser and filter holder. This is made of case-hardened steel and is furnished with a blunt endpiece which engages in a corresponding slot in the lower section of the mount, also made in a piece of case-hardened steel, let into the main body of the mount ; disengaging the trigger enables the condenser to be swung out.

Perhaps the most efficient substage ever designed is the Akehurst pattern shown in Fig. 152; this is about the most fully equipped substage for research work. It is designed to save much time and trouble by enabling a quick change of illuminating apparatus. For example,

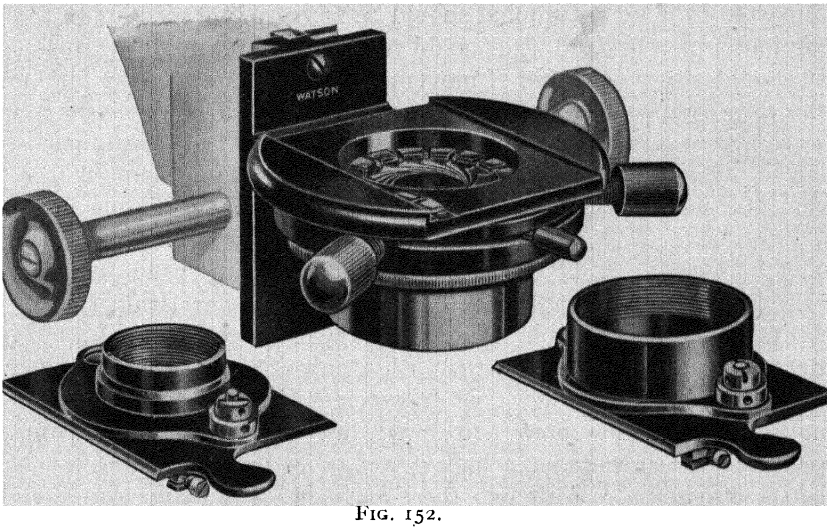


FIG. 152.

in photomicrography where the microscope is mounted as part of the complete apparatus and where a wide variety of magnifications ranging from 10 diameters to 2,000 are used, this type of substage comes into its own ; also the changeover from transmitted to dark-ground illumination is carried out in a fraction of the usual time. It will be seen that each condenser is mounted on a separate slide which has its own preset centring arrangements, thus rendering it co-axial and interchangeable with the other condensers. In this way a series of photomicrographs may be taken at different magnifications without the necessity of dismounting and fitting a different condenser and realigning the optical train.

The substage itself incorporates the usual centring arrangements, in addition to which the iris diaphragm is mounted in the substage ring, above which, and cast in one piece with it, is the dovetail groove to take the condenser slides. This design is novel and unique

and constitutes a big improvement in the conventional swing-out substage, the only feature in which it is not complete is the inclusion of a fine focussing adjustment.

Thus we see that if we are to carry out work of a useful and critical nature with the microscope, the substage work must be just as carefully designed and made as the body tube, the movements must be equally precise and smooth and there must be the same provision for taking up wear. We have seen that for use with high-aperture condensers a centring arrangement is absolutely essential; this must work smoothly and remain rigidly where set; a fine adjustment is not essential but is a great help when high powers are used.

The mirror has already been dealt with, and at this stage needs only passing reference; sufficient to say that the movements imparted to it by the gimbal should be smooth and yet require the minimum of effort and also should remain firmly in place when set. It should also be capable of movement up and down in the line of the optic axis. The best mirrors are made with a wedge-shaped section to eliminate multiple reflections, and if full advantage is to be taken of this property the mirror should be mounted in a rotatable mount so that the best position of the wedge may be used.

The next part of the instrument to call for attention is the stage. Nowadays far too many instruments are fitted with no other means of manipulating the slide under examination than a flat plate possessing a hole, coaxial with the optic axis, through which the condenser works, and fitted with a pair of spring clips in order to hold the slide down. This arrangement is only satisfactory if nothing but the low powers are used, but when it comes to manipulating the slide under a high-power objective, something much better is required. Although there are those who claim that they have so educated their sense of touch as to be able to move a slide about under a 1/12 oil immersion objective with ease, these people are in the minority; for the great majority of workers this would be impossible without the waste of much valuable time in training oneself to do this operation. It does seem rather futile when for a comparatively small cost the operation may be performed with perfect smoothness and precision by mechanical means. However, since this type of stage is so abundant, let us consider its requirements, because, after all, it does form the basis of more elaborate stages.

Firstly, then, the stage has to possess a smooth and perfectly flat surface which must be fixed to the microscope quite rigidly with the upper surface at a true right angle to the optic axis. It must possess an aperture coaxial with this latter of sufficient diameter to allow of all manipulations likely to be met with. An aperture with a diameter of about 1½ in. is sufficient; it must not have any tendency

to warp or move in any way with changes of temperature, and its surface should be proof against attack by chemical substances, solvents, etc. The usual practice in the case of reputable manufacturers is to make the stage as a casting with its under surface ribbed in such a way as to give it dimensional stability. The whole is then covered with either vulcanised rubber or an inert plastic material. This is usually mounted on and is continuous inasmuch as the upper and lower surfaces are connected by means of holes drilled through the metallic portion, thus giving a rigid member whose surface may be machined to the required degree of accuracy and which when finished will not suffer from the faults outlined above. A modified form of this simple stage is made in the rotating stage shown in Fig. 153, where the upper surface is capable of rotation round the optic axis, and relative to the lower surface, this type of stage is useful when using polarised light. In the case of plain stages, these should be of ample size to accommodate a slide, that is, not less than 3 in. square or 3 in. diameter for the rotating type.

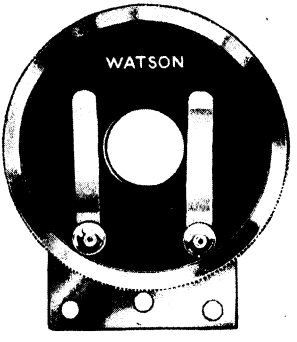


FIG. 153.

If serious work of the precision and delicacy required by high-class high-power objectives is to be undertaken, a mechanical stage is essential, because it must be remembered that apart from the size of the object being magnified, movement will also be magnified to the same extent. Thus when an object is magnified let us say 1,000 diameters, any movement made by or imparted to it appears to be 1,000 times faster and farther than it actually is and the difficulty of moving a slide about with the fingers at magnifications such as this will be readily appreciated; therefore we must have some precise and definite means of controlling this movement. This is obtained by means of the mechanical stage. The first type of mechanical stage to come under our consideration is that which is known as the "built-in stage," or true mechanical stage, in which the slide is carried in two directions and not slid along the surface of the fixed stage. A typical example of a true mechanical stage is shown in Fig. 154. This type of stage is so superior for delicate observations under high power that it may be said no one who has used one would ever consider using another type. Such a stage is actually an integral part of the microscope and is built and fitted in the same way as the other mechanical movements. Its precision depends on the fitting of metal dovetail slides working in corresponding grooves and bearings accurately machined, at right angles to the optic axis.

In the case illustrated, following modern practice, the movements are controlled by rack and pinion for the vertical and screw for the horizontal movement. The type of bearing used is shown at A and will be seen to consist of a ball upon which the screw works; the groove acting as a dirt and grease collector will be noticed. The assembly shown is capped by the part B, adjustment for wear being

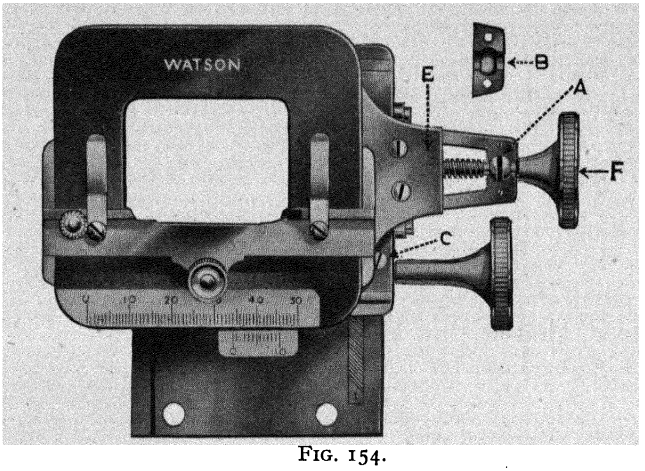


FIG. 154.

provided. At C is seen the adjustment screw for the vertical slide, and the method of fixing the verniers is indicated at D. These verniers are very useful for making coarse measurements and also for logging the position of an object on any particular slide, so that it may be easily found at a later date. E carries the screw which is driven by the horizontal movement actuating head F;

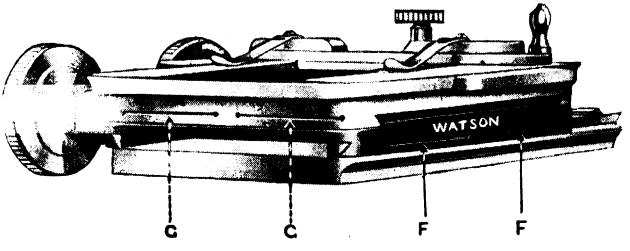


FIG. 155.

this milled head is interesting inasmuch as it is stationary and does not move with the traversing plates of the stage. The plate slides are shown in Fig. 155, and the method of allowing for wear being clearly seen as saw cuts through which the adjusting screws pass at FF and GG so that any wear may be immediately taken up. For the highest precision in working and accuracy of movement, this type of stage is unquestionably the most satisfactory pattern made. The object is placed on the top plate and when moved in either

direction the slide and plate move as a complete unit. The advantage of this is that if an oil immersion condenser be used in conjunction with an oil immersion objective, the resistance of the oil is overcome and the slide is positively carried by the travelling top plate of the stage. With other forms of mechanical stage where the slide is moved over the surface of the stage, the immersion oil is apt to offer quite considerable resistance to the movement of the slide.

Besides the built-in type of mechanical stage, there are the deservedly popular attachable stages. As the name implies, these are so designed that they may be fixed to or removed from the microscope at will. Formerly this type of stage was only a make-

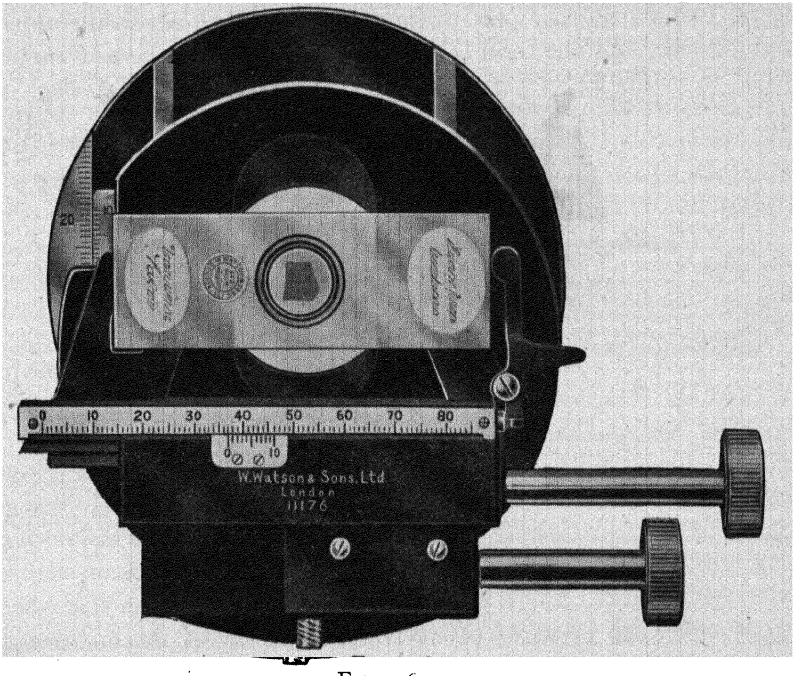


FIG. 156.

shift, but in recent years they have had so much thought and care put into their design and manufacture that they bid fair to rival the built-in variety. This will be seen by reference to the attachable stage shown in Fig. 156, which will be seen to be every bit as good as the built-in type previously illustrated and, except for the minor point of the slide being made to move over the surface of the top plate, there is nothing to choose betweeen them. As a matter of fact, the detachability is probably an asset, as a microscope with a plain stage which is in every other respect a high-grade instrument may be converted into a really first-class piece of apparatus by the addition of a stage such as this. A simpler type of attachable stage is shown in Fig. 157.

The types of mechanical stage just mentioned are typical of

general practice. Needless to say, whichever type is chosen, the materials and workmanship must be beyond reproach, and the same remarks apply to the movements as applied to the other movements on the stand.

Having dealt with the most important points of the microscope stand, let us now turn our attention to an accessory which of late years has become so popular and universally used as to almost be classed as part and parcel of the stand proper. The accessory referred to is the multiple nosepiece, by which it is possible to obtain a rapid change of objectives, up to as many as four in number, and in any preselected order.

The term " nosepiece " is defined by Carpenter and Dallinger as being " primarily that part of the microscope into which the objective screws ; but the term is also applied to various pieces of appa-

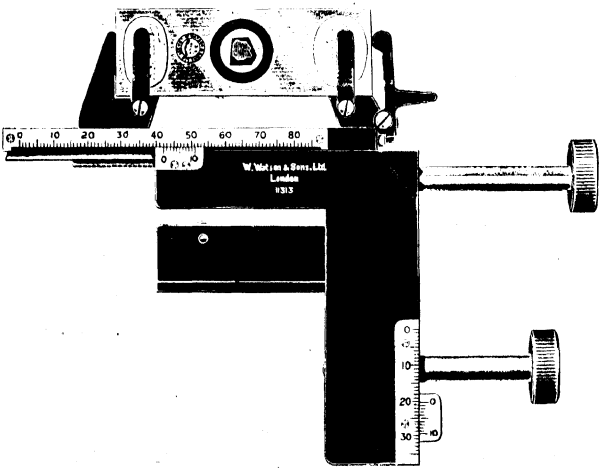


FIG. 157.

ratus which can be fitted between the nosepiece of the microscope and the objective." Thus the modern multiple nosepiece comes under the latter part of the definition and is correctly classed as an accessory. However, the majority of high-class instruments are to-day fitted with this apparatus as a standard fitting, some manufacturers even going to the extent of calibrating the draw tube to allow for the extra length due to the nosepiece. It is therefore a wise precaution to ascertain if this calibration allows for the nosepiece or not when purchasing an instrument.

With regard to the advisability and utility of this accessory, Carpenter and Dallinger (1) seem to have no doubt, as the following extract shows. They say : " It is continuously desirable to be able to substitute one objective for another with as little expenditure of time and trouble as possible, so as to be able to examine under a high magnifying power the details of an object of which a general view

has been obtained by means of a lower ; or to use the lower for the purpose of finding a minute object (such as a particular diatom in the midst of a slide full) which we wish to submit to higher amplification." So that it will be seen to be a great time saver apart from reducing wear and tear on objectives and threads due to constant handling and screwing in and out.

The next point raised by the same authors is the question of additional weight. They say (1): "The one drawback to the use of a rotating nosepiece is the extra weight it throws on the fine adjustment. A double nosepiece is to be preferred to a triple, and a quadruple need not be entertained for a delicate instrument, when made of ordinary metal, unless it is to find out in how short a time a fine adjustment may be ruined, for let it be noted that a 2 in., 1 in., ½ in. and ¼ in. objective of English make, weigh together 8½ oz., without any nosepiece." The modern stand, however, is quite capable of bearing this extra weight; the chief difficulties in this direction having been experienced with the old nosepiece fine adjustments. Apart from this objection, they continue to stress the importance of the nosepiece being dust tight, which would appear to be the more important of the two points. As they say, there is nothing to guard the back lenses of the objectives in the older types of nosepiece if these are left accidentally so that there is no objective in the optic axis.

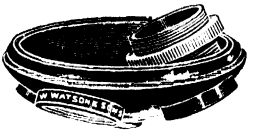


FIG. 158.

The modern nosepiece, shown in Fig. 158, is, however, properly designed and made to eliminate this trouble. They usually consist or two circular plates having spherical surfaces which fit into one another (in the same way that two watch glasses would fit together) with a smooth working bearing at the centre designed so that one plate may be rotated relative to the other, and in their interfacial plane, without being capable of separation from the other by so doing. On a circumference whose diameter is somewhat smaller than that of the concave discs, the upper and stationary plate is fitted with a short tube, having a standard positive objective thread and carrying a locking ring. The axis of this tube being normal to the concave surface of the disc. The lower and movable disc is fitted with two, three or four (according to the number required, but more usually three) short tubes with negative objective threads on the same circumference. These tubes receive the objectives and are of course equally spaced on their circumferential centre line. The tube on the upper disc is screwed into the nosepiece of the body tube and locked with the locking ring, so that the whole assembly points away from and at right angles to the optic axis. In this way the axis of the short upper tube and that of any of the short lower tubes which happen to be lined up underneath it are coincident with the

optic axis of the microscope. Thus, a simple rotary movement is sufficient to bring any of the short lower tubes with its associated objective into line with the optic axis, the upper disc keeping the back lens protected from the ingress of dust at all times. As a further precaution against dust, the edge of the upper disc carries a rim which projects over the edge of the lower disc. Correct alignment is indicated by a built-in precision spring catch action. Thus we can see the method of eliminating any trouble due to dust on the back lenses of the objective; the question of excessive weight is answered by efficient design and manufacture of the stand in general, and the fine adjustment in particular, though much is achieved by making the multiple nosepiece of a light metal such as aluminium or a light alloy. In some cases a further improvement is obtained in the accuracy of the accessory by fitting the objective screws with individual centring arrangements, so that all the objectives carried by it may be exactly lined up.

From the foregoing remarks we can see that the microscope should have the following characteristics: it must be supported on a foot of broad dimensions in such a manner that it is perfectly stable at all inclinations of the limb, from the vertical to the horizontal. The mechanical stage, which nowadays is regarded as more or less of a necessity, should be built in as an integral part of the instrument, although in view of the mechanical perfection of the modern attachable stage, there is very little to choose between them. The substage must be adjustable by rack and pinion, with fine adjustment preferable; it must also possess centring movements for the correct alignment of substage and dark-ground illuminators. The body tube should be large in diameter and provided with considerable extension, preferably by means of two draw tubes, one of which is adjustable by rack-and-pinion motion. The coarse and fine adjustments should be of the highest mechanical efficiency and sound design; all movements must have ample allowance for taking up wear.

A stand possessed of these general characteristics should last the user for a lifetime and give no trouble if used with reasonable care. The wear adjustments should seldom, if ever, need attention, and the accuracy of the instrument will be maintained over a considerable period.

Having discussed the salient features of the stand individually, we are now in a position to examine the complete article. Microscopes in general may be classed under four main type headings:—

(1) The simplest effective stand such as those used by beginners and found mainly in school laboratories, etc.

(2) The student's stand, which is slightly more advanced than No. 1.

(3) The complete research stand suitable for the most critical research work at very high magnification.

(4) Stands of special design intended for work of a specific character.

The stands under No. 1 are of the simplest design and cannot as a rule be used for exacting work. They are made by most manufacturers and are intended only to be used for instructional work of the most elementary character.

As a rule they lack any form of substage, or if possessing one, it

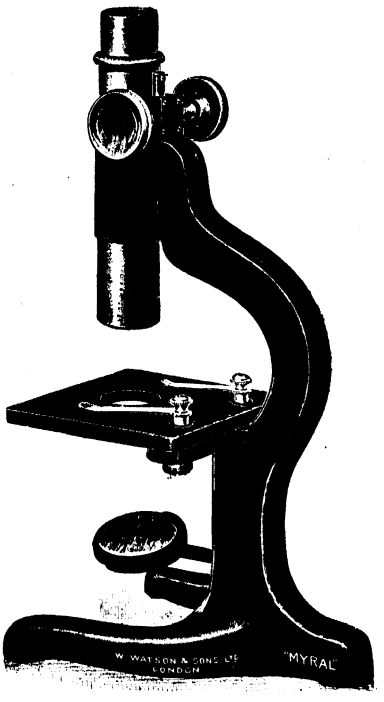


FIG. 159.

is usually of the most rudimentary character. A typical elementary stand is shown in Fig. 159; as will be seen, there are no substage arrangements and no fine adjustment to the body tube. Bakers' elementary stand is shown in Fig. 160. Here we have a rather more elaborate instrument possessing a coarse and fine adjustment, a triple nosepiece and a plain tubular understage fitting to carry a fixed condenser is supplied. This stand is an advance on the one shown in Fig. 159, but the value of the fixed understage fitting is dubious, except perhaps for the purpose of carrying an iris diaphragm by means of which the light may be controlled to a certain degree.

Continental practice is very much the same in this type of stand, as shown in Fig. 161, which illustrates a small microscope by Leitz. Here again there is no fine adjustment and no substage arrangement; in fact, the main characteristic of this type seems to be an entire lack of any substage, or if this is included it is vestigial in nature and may therefore be left out altogether. They are generally incapable of being added to, so that they cannot be considered as the base on which to build a more elaborate instrument.

We now come to the second type, namely the student's stand, which is more complete than the elementary stand, as shown in Fig. 162, which illustrates Watsons' "Service" stand. As can be seen, it is rigidly built having a large diameter body

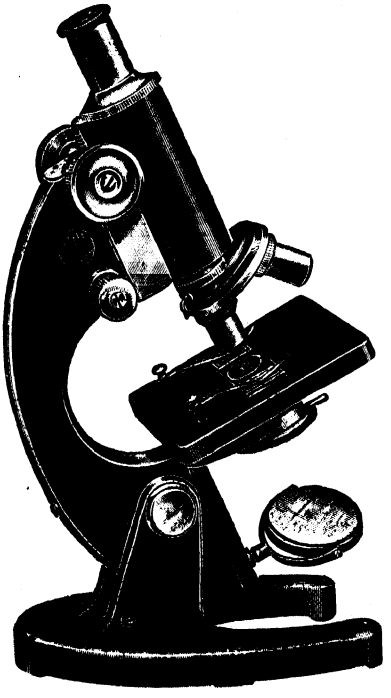


FIG. 160.

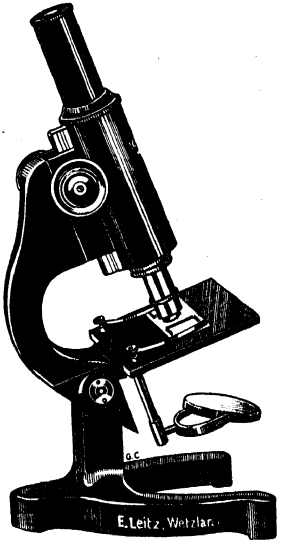


FIG. 161.

tube with coarse and fine adjustment, a large plain stage which is a casting covered with moulded ebonite; a rack-focussing substage of the plain type is fitted having no centring arrangement and below which is a large-diameter double mirror of the usual pattern. All the movements have adjustments for wear, and, in fact, the whole instrument is designed and built to a high degree of perfection. This is necessary, because it is intended to act as the basis of a high-precision research instrument by the addition of the necessary subsidiaries.

In the same way Bakers' newest student's stand, shown in Fig. 163, is intended to act as a foundation on which a better microscope may be built by additions from time to time. The new

design of limb will be noticed which promises to be very rigid, and coupled with the new foot should produce a very stable instrument. It will be noticed that both this instrument and the one illustrated in Fig. 162 are possessed of optical bench limbs.

Indicative of American practice is the microscope shown in Fig. 164 by Bausch and Lomb. It is much the same as the British models, and the illustration shows the way in which the mechanical stage is built on, if this is desired at a later date. There is one point in the design with which the author does not agree, this being

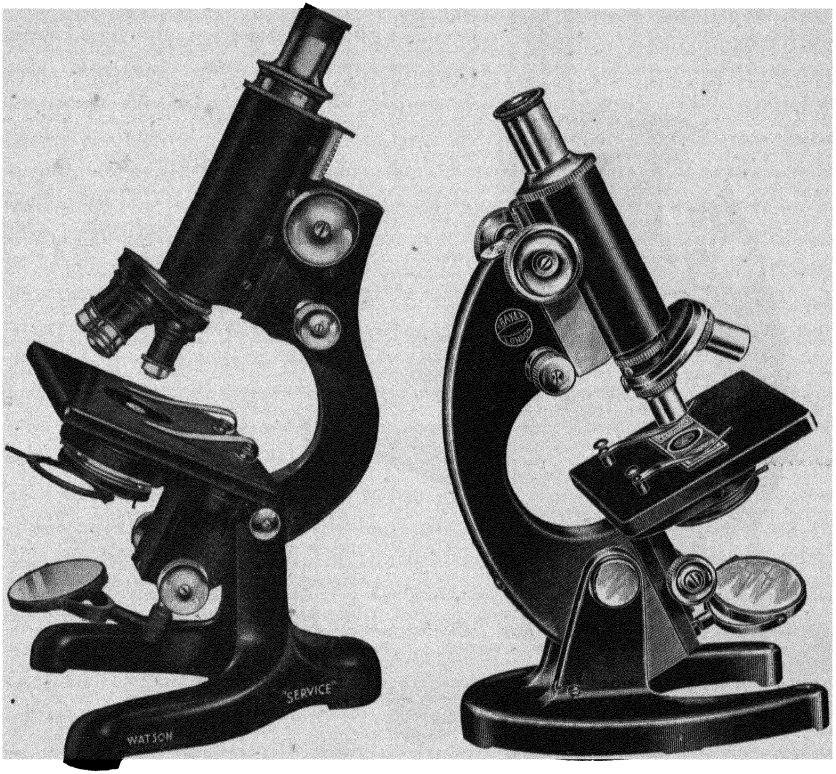


FIG. 162. FIG. 163.

the absence of an extensible draw tube. This is characteristic of most American manufacturers and, in fairness to them, it should be stated that their reason for using a fixed tube length is due to the liability of several people using the microscope and causing damage if a variable draw tube is used.

American opinion on this question of the draw tube expresses the view that only the hypercritical worker, checking every step as he proceeds, is liable to feel the need of a variable draw tube, this fitting being at a disadvantage in situations such as in schools and similar institutions, where several people may have to use the same instrument.

The argument advanced by Munoz and Charipper (2), for instance, being that as objectives are generally parfocalled for a definite tube length, it is conceivable that the microscope may be left with its draw tube too much extended or shortened. Thus the following user runs the risk of hitting the slide with one of the objectives when rotating the nosepiece and possibly causing much damage.

In view of these possibilities they strongly advocate the use of a fixed draw tube in the aforementioned situations.

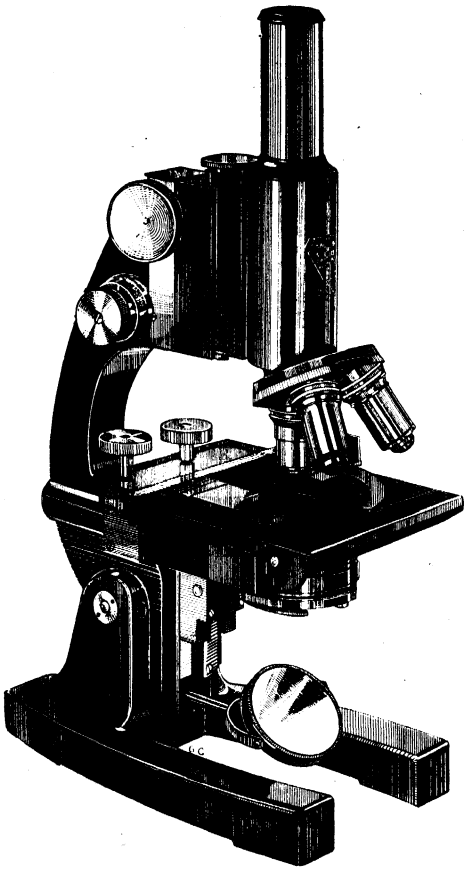


FIG. 164.

Now this argument would be justified if the fixed tube length were adopted for instruments which were intended for this type of rough work only, but even so, it is the author's opinion that students in schools and colleges where this type of work is done should be taught the elementary principles and practice of the microscope before ever being allowed to handle one. In far too many cases the student is rushed into the study of microscopic structure without any, or at the most only a fragmentary, knowledge of the instrument he is about to handle, in many cases leading to expensive mistakes on his part; but in the absence of a course of microscopy from the average curriculum, it would seem that there is a certain amount of justification for the adoption of a fixed tube length for school and college microscopes.

Suppose we examine this problem from another aspect. The great majority of this type of microscope, more particularly in schools, where there are usually a number of commercial microscopes, are equipped with a range of objectives consisting of 1 in., $\frac{2}{3}$ in. and $\frac{1}{6}$ in. and, for the sake of argument, let us suppose that they are all made by the maker of the stand (which latter may be of the first or second class, but is more frequently of the first class) we may expect them to be reasonably parfocalled. Now the modern $\frac{1}{6}$-in.

achromatic objective of 0·65 N.A. has a working distance of approximately $\frac{3}{64}$ in., or 1191·0 μ. The effect of draw-tube extension may be quite easily checked on any microscope possessing a graduated fine adjustment. The author has done this and found that for a $\frac{1}{6}$-in. objective of 0·8 N.A. working through a No. 1 cover glass, the amount by which the objective is required to be focussed downwards for an extension of the draw-tube from 140 mm. to 200 mm. was only a matter of 16 μ, which is only about 15 per cent. of the working distance of the lens. Thus it can be seen clearly that if an object covered with a No. 1 cover (0·17 to 0·18 mm. thick) is focussed with the $\frac{2}{3}$-in. objective with the draw tube right in at 140 mm., after which the draw-tube was pulled out to its fullest extent at 200 mm., then the $\frac{1}{6}$-in. objective could be rotated into position without the slightest possible risk of its fouling the slide, all of which seems to point to there being no justification at all for deleting the draw-tube. On the contrary, the necessity for its inclusion is very well shown by Eliot Merlin who, in an article entitled " Hints to Beginners " in Watsons' *Microscope Record* (No. 18, September, 1929) says concerning the resolution of the diatom P. Angulation, " The perforated structure covering the surface of its valves can be seen with object glasses of about 0·52 N.A., and should be quite distinctly rendered into clear round dots by object glasses of 0·60 N.A. working at full aperture with strictly axial light. The laboratory dry $\frac{1}{6}$ in. of about 0·8 N.A. should show the structure of this diatom very distinctly working at full aperture with strictly axial light in whatever medium the valves are mounted. With no diaphragm in the condenser, and the edge of the lamp flame sharply focussed on to and forming the background of the diatom, the distinctness of the dotted structure will only be rendered at its best at one very definite tube length, which may be found by trial. There is hardly any better way of demonstrating the necessity of correct tube length to develop defining quality. If all has been arranged as it should be to attain this maximum of definition (or resolution), a $\frac{1}{2}$-in. variation of tube length (Author's note : this is approximately 12·7 mm.) in either direction will prove sufficient to render the image markedly indistinct."

Anent the question of tube length, Munoz and Charipper (2) say : " Although it is true that, all other things being equal, an objective designed for use with a tube length of 160 mm. will give better results on a tube having such a dimension than if it is used with a tube 170 mm. long, nevertheless there are so many other factors to be considered that we feel this point may have been somewhat over-emphasised by the majority of persons who have written about the microscope. Since the objectives are not only designed for a certain tube length but also for a definite cover glass thickness, it follows that if we use the wrong thickness of cover glass we would

have to change the length of tube. *If the cover glass is too thick we would have to shorten the length of the tube, if it is too thin we must elongate it.* Actually this question of cover glass thickness used is more important than the tube length, for each 0·01 mm. difference in the ideal thickness of a cover glass requires a change in tube length of about 10 mm. We do not hesitate to state that for all practical purposes it is perfectly satisfactory to use, for instance, a Leitz objective (for a 170-mm. tube length) on a Spencer microscope which has a tube 160 mm. long. The ideal, of course, is the right tube length with the right cover glass."

The significant point in this statement is the sentence printed in italics: "If the cover glass is too thick we would have to shorten the length of the tube, if it is too thin we must elongate it." Now these authors' justification for the deletion of the draw tube was the damage resulting in someone having pulled it out too far, but as the use of No. 1 covers in schools is unheard of, much thicker glasses being the rule, it is difficult to see how the necessity for extending the draw tube arises, because if it is used at all it will be required to be shortened. Assuming, of course, that no unofficial experimenting with the instrument has taken place.

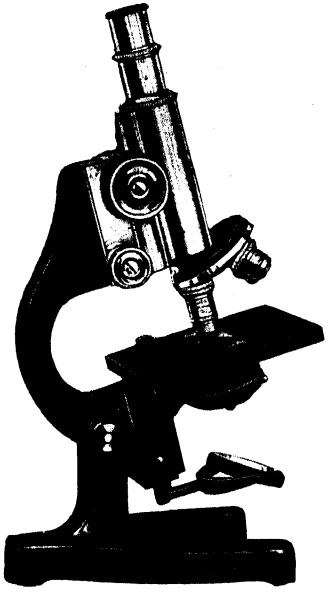


FIG. 165.

However, as the stand shown in Fig. 164 is intended to be used as a school and students' stand on which may be built a more complete equipment, it is the author's opinion that the lack of an extensible draw tube considerably detracts from its usefulness for critical work of any nature.

Continuing with the Class 2 stand, Fig. 165 shows one by Leitz; this is a typical Continental design, the substage is fitted with spiral focussing but here again with no centring devices, so we see that apart from the American practice of eliminating the extensible draw tube, the main features of this class include a focussing substage, without centring arrangements, a coarse and fine main focussing adjustment but no mechanical stage, and as a rule the stand may be added to from time to time, ultimately becoming a first-class instrument.

It is a well-known fact that the image seen with both eyes simultaneously is far better than that seen with one eye. This is

the reason why the modern tendency in microscope design is to arrange for ways and means whereby the primary image produced by the objective may be viewed by both eyes at once, in effect to produce a binocular microscope. The success achieved by manufacturers in this direction can only be judged by actual practice; suffice it to say that the beauty of the image and the comfort in using a binocular instrument have to be experienced to be believed. Monocular instruments such as have been dealt with hitherto are and can be very good, but in spite of all that has been said to the contrary, it has been the actual experience of the author to suffer

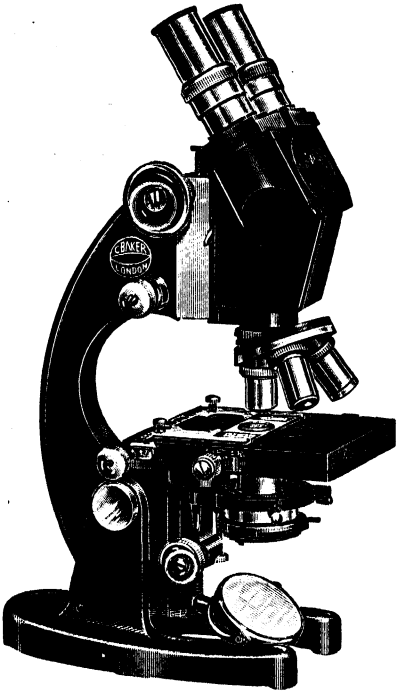


FIG. 166.

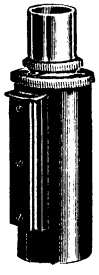


FIG. 167.

damage to the eyes by constant use of a monocular instrument; albeit, the damage was only slight, amounting to severe overworking of the ciliary muscle of the eye, recovery being rapid on changing to a binocular instrument, and he has no hesitation in recommending the use of a binocular instrument for visual work wherever possible.

In the modern binocular microscope the rays from the objective are equally reflected in two directions by a Swann cube, or some similar prism, each half then being taken by separate paths to each of two eyepieces, which of course have to be paired. The eyepieces are capable of being moved towards or away from each other to allow for the natural variations in pupillary distance, also one of the eye-

pieces is capable of a limited focussing movement along the optic axis which allows for adjusting the eyepieces to match up with eyes that are not quite equal. Thus the two eyes may be brought into play simultaneously, and working together eliminate any tendency to overwork either one, also the comfort in viewing the image in this manner is incomparably more than when viewing it with one eye only.

So we come to Class 3 stands, which comprise fully equipped research stands of the highest grade and with which it is possible

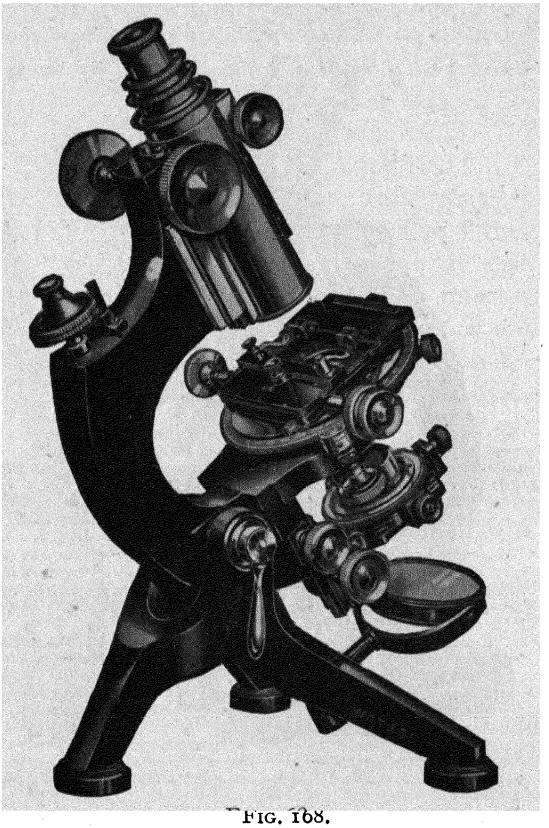


FIG. 168.

to do any normal type of work from simple routine examination to the most critical research observations. Nowadays these are nearly always in the form of a binocular instrument such as that by Baker, shown in Fig. 166; this instrument is an example of the finest design and workmanship found to-day. As will be seen, it possesses a coarse and fine adjustment arranged in the usual manner, a rack-work focussing and centring substage, a circular rotating stage with limited centring motion; has a built-in mechanical stage, the binocular body, fitted with a dustproof triple nosepiece, has adjustments for pupillary distance and odd focussing. It is usual to

supply binocular stands with an interchangeable monocular body for use in photomicrography. Fig. 167 shows the monocular body which is interchangeable with the binocular head.

A very complete research stand by Watson is shown in Fig. 168. This stand is in the highest class and possesses beside the usual movements a fine adjustment to the substage and a rotating centring circular stage, to which is built in a mechanical stage of sound design. The stage is capable of complete rotation and has a range of 2 in. in the horizontal and about 1¼ in. in the vertical directions. The milled

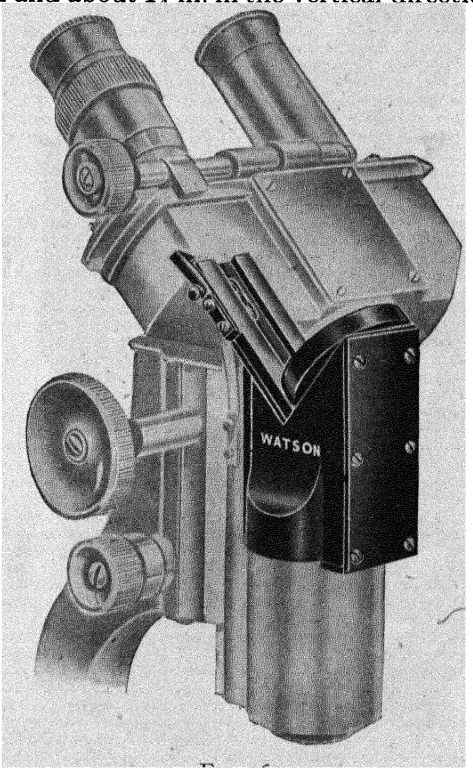


FIG. 169.

heads controlling it work on a common centre line and, if desired, may both be worked simultaneously, imparting a diagonal movement to the object. The stage is of large diameter (5 in.) and has concentric rotation through 360°. It will readily be seen that the shape of the limb gives great freedom to the stage surface and incidentally acts as a convenient handle. A noteworthy refinement is the doubly extensible draw tubes, one of which is actuated by rack and pinion. The instrument shown is a monocular, which may be fitted with a binocular body such as shown in Fig. 169. In the majority of cases, binocular instruments may be obtained with inclining eyepiece units, but the advantages of this type are debatable.

Another high-power binocular instrument by Watson is shown in Fig. 170. This stand has a horseshoe foot of great stability and the usual refinements to be found on a microscope of this type. The body is interchangeable with the monocular body (A), both fitting into the basic body tube. The interocular adjustment is by means of a horizontal spiral screw, a divided scale being provided to show the exact measurements; the right-hand eyepiece has an adjustment for odd focussing.

As representative of American practice, we have Bausch and

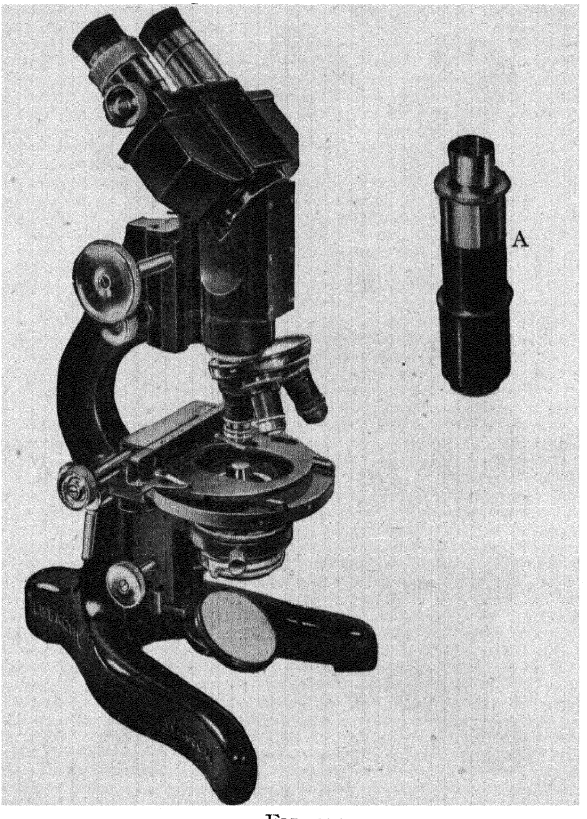


FIG. 170.

Lomb's inclining binocular instrument shown in Fig. 171. This is seen to be an instrument of clean design and first-class workmanship, possessed of the movements necessary in such a one, with the notable exception of centring movements to the substage. This, in the author's opinion, is a mistake, seriously handicapping the utility of the instrument, as without this movement it is impossible to get the best out of a combination of apochromatic objectives and highly corrected condenser; therefore, as it stands, the instrument is hardly capable of being used for work of the most critical nature.

The same fault is to be found in the Continental models, typical of which is the one by Leitz shown in Fig. 172, which shows a binocular stand with inclined body completely equipped except for centring arrangements to the substage; instead it is fitted with a rack-and-pinion motion which moves the condenser at right angles to the optic axis, by which means oblique illumination may be obtained.

Thus we see that the modern trend in the complete research instrument is for a binocular body, together with as many refine-

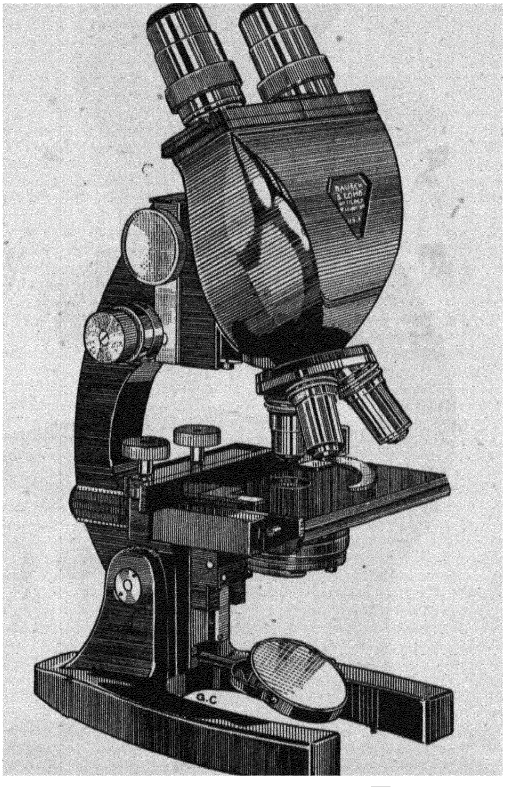


FIG. 171.

ments in the way of additional mechanical movements as possible. There is one criticism which may be levelled at binocular bodies in general, and this is the lack of any provision for tube-length adjustment. The author realises the difficulty in providing for this, but suggests the inclusion in the optical systems of binocular bodies some such apparatus as the Jackson tube length corrector, shown in Fig. 139, whereby the tube length is corrected by optical means. This and the regrettable lack of substage centring arrangements on the American and Continental models are the only two criticisms of note.

The instruments which have been considered up to the present are all basic ; that is to say, they may by suitable additions be used for special purposes, such as metallurgy, the examination of colloidal substances, the examination of heating effects and the use of polarised light, etc. However, manufacturers in general produce microscopes designed to do specific work and in some cases to perform a multi-

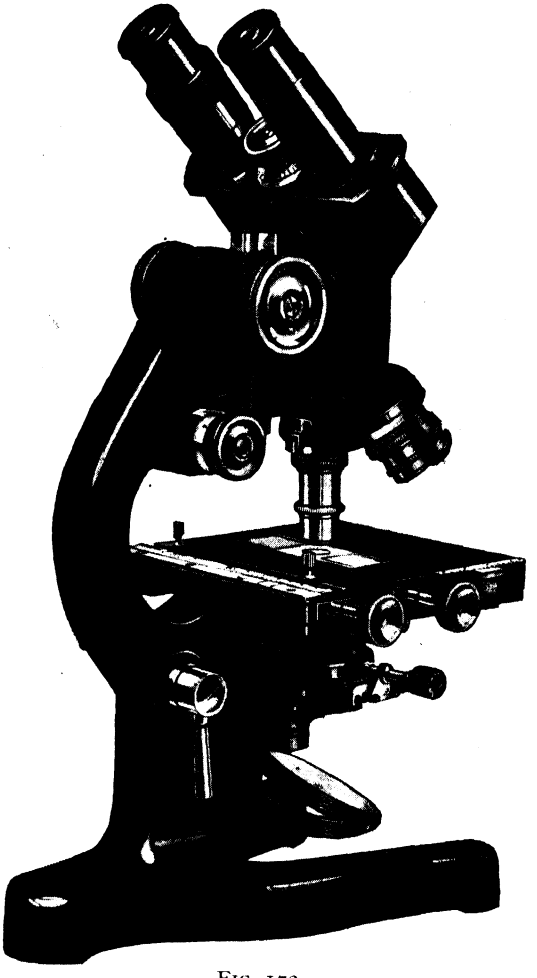


FIG. 172.

plicity of functions. This we see in Figs. 173 and 174, metallurgical microscopes by Baker and Leitz respectively. It will be seen at once that the basic stand has been modified to the extent that the substage rack and pinion is now used to move the entire stage up or down and no substage fitments such as are necessary for transmitted light are fitted ; obviously these are not necessary as the objects are in every case examined by incident light from a vertical illuminator or similar apparatus.

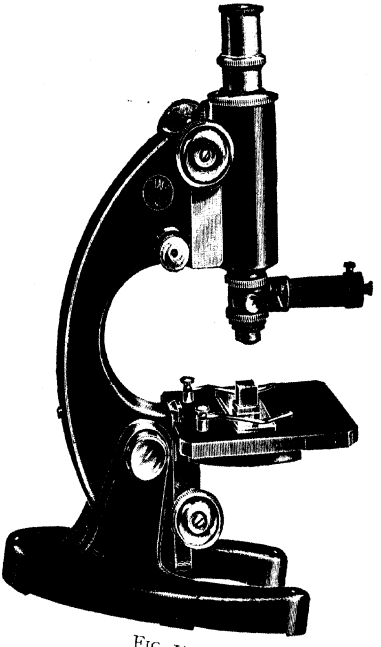


FIG. 173.

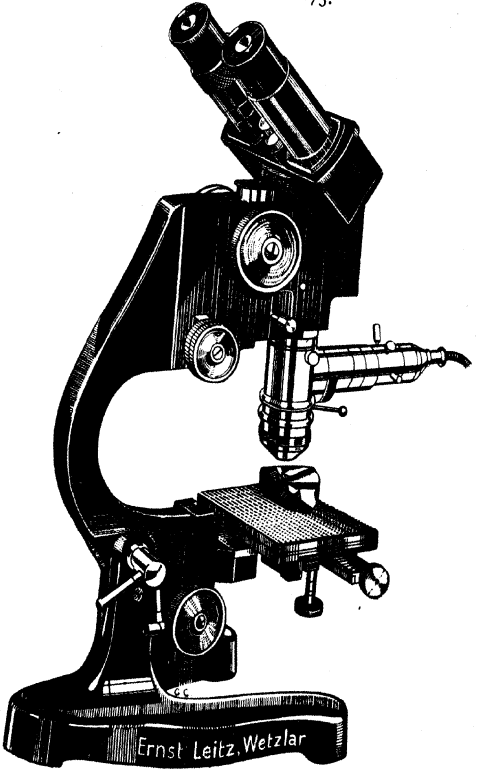


FIG. 174.

These special metallurgical microscopes, together with polarising or "Petrological" microscopes, are perhaps the most common special microscopes met with. Figs. 175 and 176 show two instruments of this type, the number of special fittings which are additional to those found on the basic stand will be noticed. It is not proposed to discuss these microscopes in detail at this point, but they will be dealt with later.

In any laboratory or institution where microscopy is practised, a very desirable addition to the equipment consists of a low-powered instrument for preliminary examination and manipulation; this

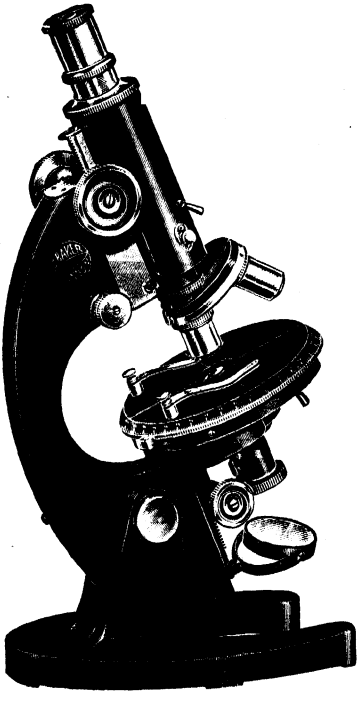


FIG. 175.

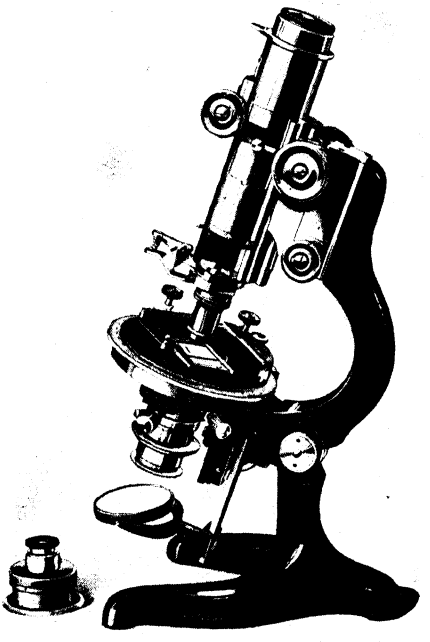


FIG. 176.

need is admirably met by the "Greenough binocular" microscope. It is a low-powered instrument not capable of magnifications exceeding 100 diameters, but its usefulness lies in the fact that, by means of paired objectives and eyepieces, it gives a truly stereoscopic and erect image, and by reason of its additional characteristics, of wide field of view and long working distance. This type of binocular instrument has found increasing favour among all microscopists; there is, in fact, no science in which the microscope is used where additional information in the elucidation of problems cannot be obtained by its employment. Two models of this type of instrument are shown in Figs. 177 and 178. These two special types together

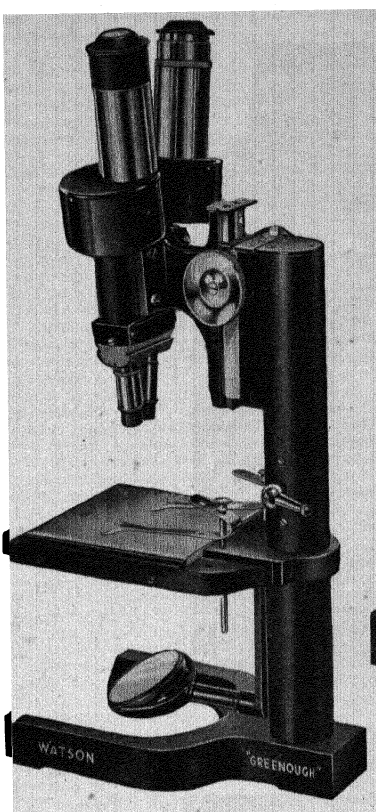


FIG. 177.

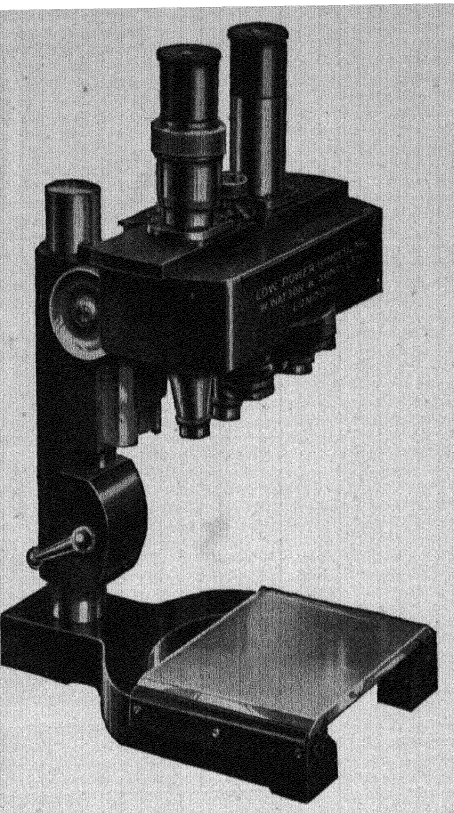


FIG. 178.

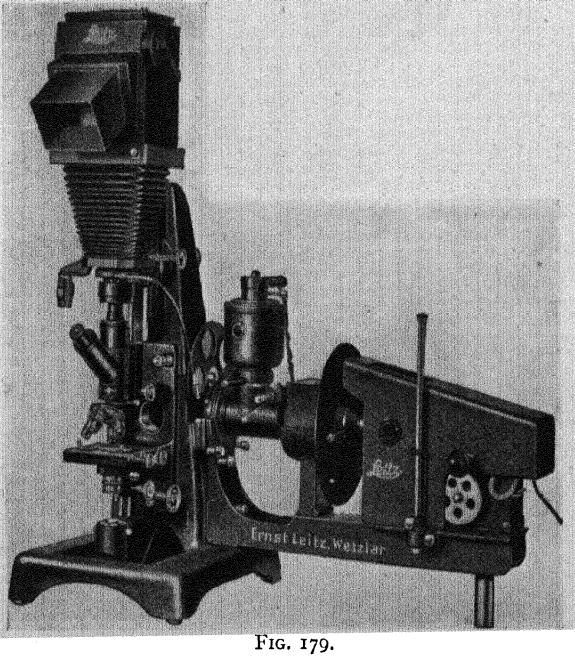


FIG. 179.

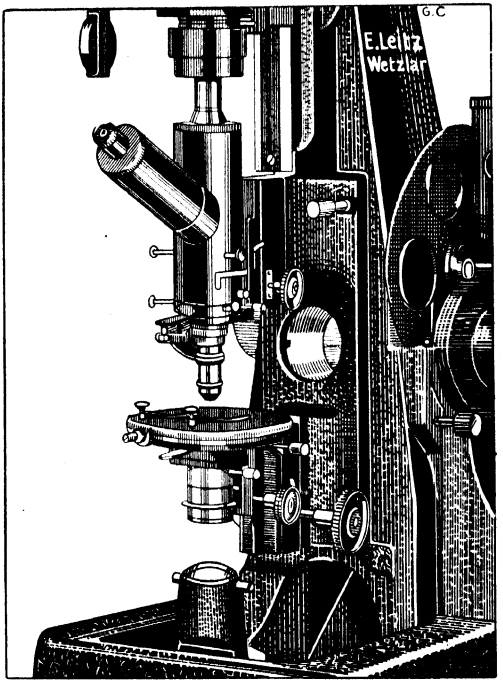


FIG. 180.

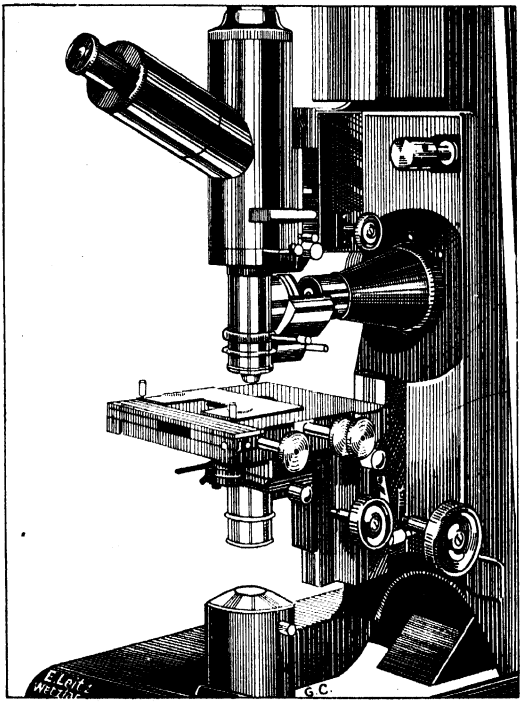


FIG. 181.

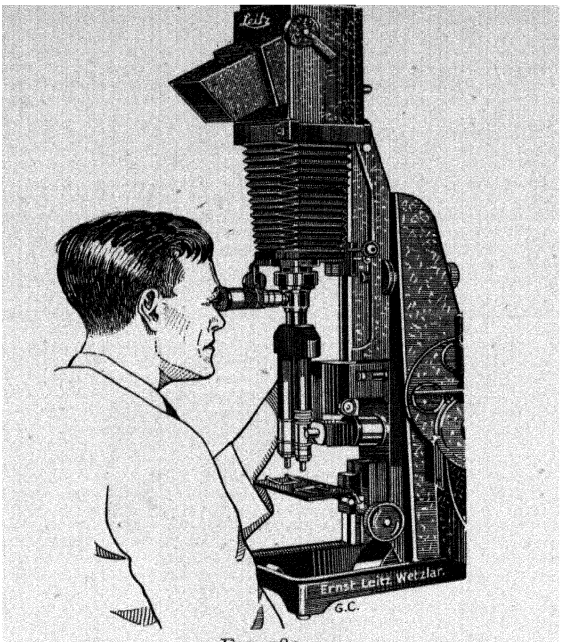


FIG. 182.

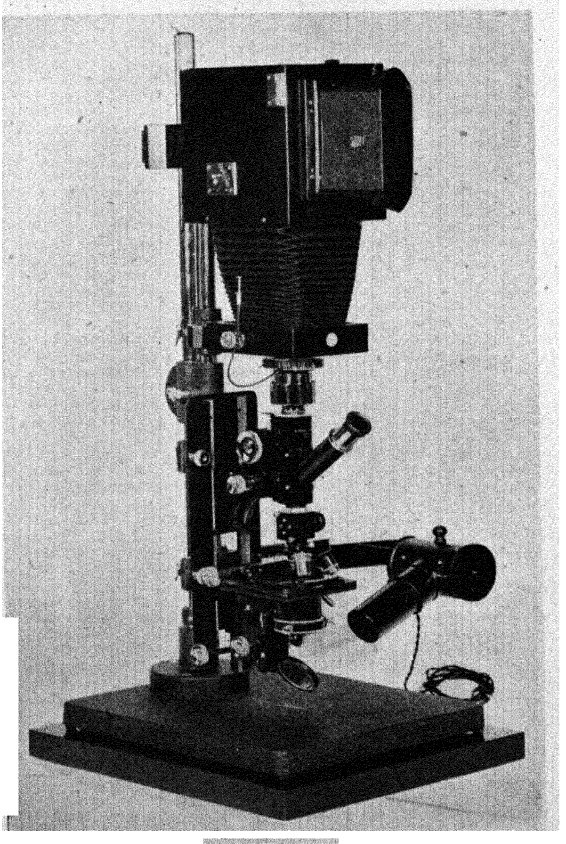


FIG. 183.

with others make up a complete range of microscopes in themselves.

Of late years many manufacturers have successfully produced instruments designed to perform many functions and hence may be termed universal microscopes. Fig. 179 shows such an instrument produced by Leitz. As can be seen, it is a very impressive piece of

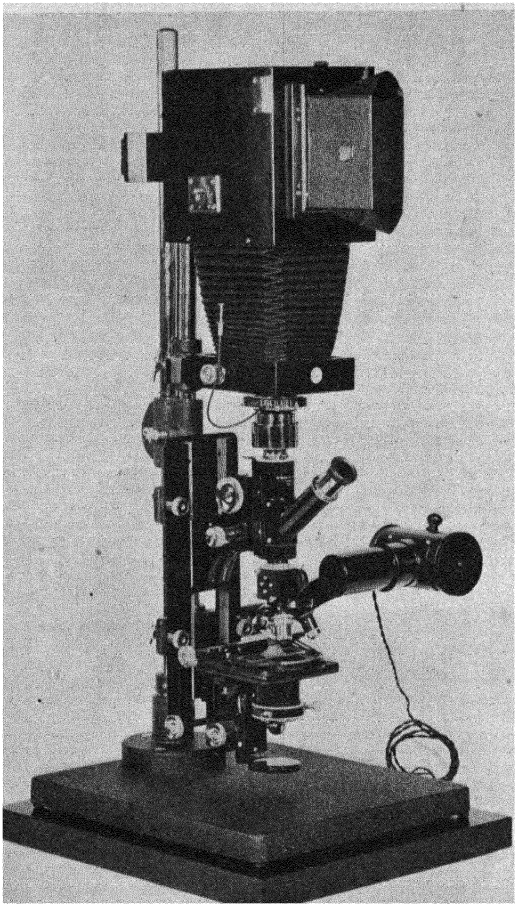


FIG. 184.

apparatus, most completely equipped. The elaborate lighting system capable of supplying illumination by both arc and filament lamps is built in together with appropriate filters ; the microscope itself is of the research pattern fully supplied with mechanical movements, although no centring arrangements are fitted to the substage condenser. The built-in camera is a noteworthy innovation, as the instrument is ready for photomicrography at all times. The universal nature of the instrument is shown by

Figs. 180 and 181, which show it set up for polarised light and incident light.

Arrangements are also included for projection and photomicrography, the set up for this latter is shown in Fig. 182.

A British version of this type of instrument is shown in Fig. 183, which is Watsons' "Holophot." Here again we have a similar universal instrument, in this case set up for either photomicrography

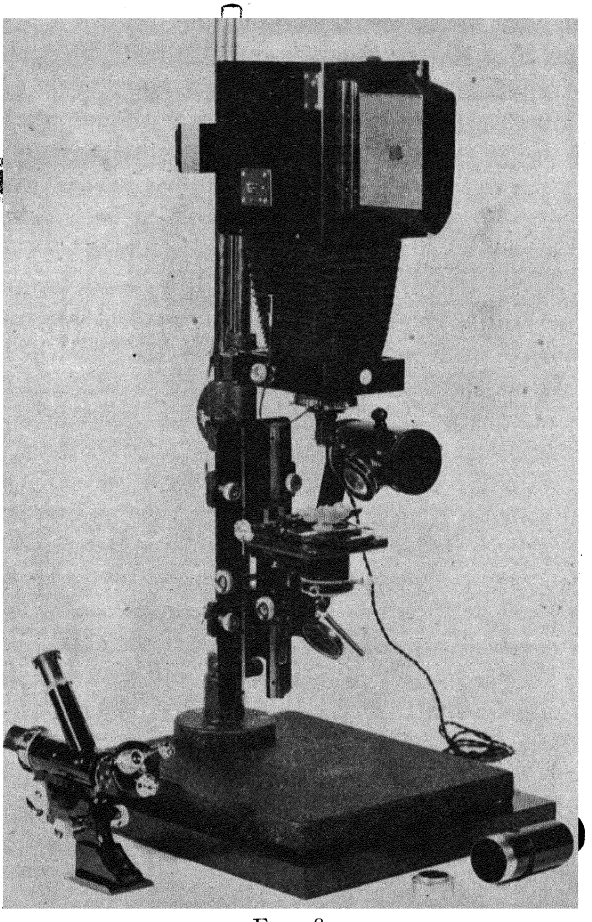


FIG. 185.

or visual examination by transmitted light; a notable feature is the simple but effective lighting system, the one fitting being used for all purposes. For instance, it only has to be raised to a level with the aperture of the vertical illuminator to convert the instrument to a metallurgical microscope, as shown in Fig. 184; here again at the same time it is ready for photography. In a similar manner, removal of the microscope body and the substitution of a macro lens converts the instrument into a macro camera, usually

used at a magnification of about 8 to 10 diameters. The instrument set up for photomicrography is shown in Fig. 185; it is made with the same precision and excellent workmanship typical of this maker, and in certain respects shows improvements on the Continental model. For example, the inclusion of a special " Compur " shutter fitted to the camera and the simplified lighting system all go to make for efficiency and compactness. Perhaps the only criticism which may be made of these two instruments is that they are not equipped with binocular bodies for visual examination, but the author is given to understand that Messrs. Zeiss produce an instrument designed on similar lines which is so equipped. Thus it appears that the microscope of the future will take some such form, making for the utmost convenience in use and conserving the maximum amount of space.

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(2) MUNOZ and CHARIPPER. " The Microscope and its Use." Chemical Publishing Co., N.Y. Extract included by kind permission of the authors and publishers. *N.B.* The italics in the extract are the author's.

CHAPTER VIII

THE USE OF THE MICROSCOPE

HAVING completed our study of the underlying theory and construction of the microscope, we are now in a position to put our knowledge to practical use, as we know exactly the capabilities of our instrument.

We have seen how sensitive the objectives are to correct setting up and handling, and the development of a definite technique in using the instrument is a logical result of this sensitivity. Therefore, let us examine this technique. We will assume at the commencement that the instrument to be used is a fully equipped research microscope with a binocular body, together with a research type lamp and accessories. In this way more modest equipments are catered for, the handling of which will mean a few quite obvious omissions from the ensuing descriptions.

Firstly let us look at the instrument itself; we see that it possesses a triple nosepiece, into which we screw the $\frac{2}{3}$-in., $\frac{1}{6}$-in. and $\frac{1}{12}$-in. oil immersion, fluorite objectives, taking great care not to touch the front lenses with the fingers in the process, these latter having been carefully examined to see that they are quite clean and do not possess any scratches or other blemishes on the front surface. If dirty, they should be lightly polished with a soft cotton cloth, such as a well-washed handkerchief. (It is a sound policy to keep one or two clean handkerchiefs, which have finished their useful life as such, for this purpose.) The objectives are screwed into the nosepiece in such a manner that they may be brought into the optic axis in the order shown above, the multiple nosepiece is then revolved completely once or twice to make sure that it works smoothly and that the spring catch locator is quite positive in action and free from any wobble.

Having done this, the next step is to make certain that there are two eyepieces of the same magnification in the tubes of the binocular body. It is advisable to ensure that an eyepiece is always in position in the tube in order to prevent any dust from dropping in, as it is surprising how much dust can collect on the back lens of an objective with an open tube above it, in spite of the fact that the instrument might have been kept in its case or under glass. For our general purpose, we will select a pair of universal X7 eyepieces of the " Holoscopic " type; thus we are in a position to adjust for the residual spectrum in the objectives.

The body tube is now complete with its optical equipment and, before leaving it, we rack it up and down once or twice to test the

coarse adjustment, after which we try the fine adjustment in a similar manner, and at the same time make a mental note of the calibration. This is usually two microns per division, one complete revolution of the milled head moving the body 100 microns.

After having decided that all is in order with the body, we may now transfer our attention to the substage. It is as well to try all the mechanical movements in the same way as for the body, after which the condenser, which in our case will be fully achromatised and aplanatised, may now be inspected to see that the lens surfaces are quite clean, following which it is screwed into the condenser mount.

The mount also carries the diaphragm and filter holder, which may be swung out for the purpose of changing the filter. Having made sure the condenser is screwed right home, the substage is racked out and the condenser and mount pushed into the substage ring, in which it must be a snug fit with no tendency to movement relative to the ring, because if this is so, the difficulties involved in keeping the substage condenser in line with the optic axis are greatly increased. Having found everything to be in order, we may gently introduce the slide into the V groove and rack the condenser up a short way, where we leave it for the time being.

Before leaving the microscope we inspect the movements of the mechanical stage and unlock the body hinge ; if a locking device is provided, to see if the action is smooth and free. If no locking arrangement is provided for the hinge, the movement must be smooth and yet tight enough to ensure that the microscope might be left at any inclination from the vertical to the horizontal, without the possibility of its altering under its own weight.

Now let us examine the lamp. We see that the housing contains a 60-watt opal bulb, one side of which is against an aperture in the side of the housing which is fitted with a diaphragm touching the surface of the bulb. This is the lamp diaphragm, mounted in front of which, and in such a manner that it may be moved towards or away from the lamp diaphragm, is a cell containing the corrected lamp condenser, which has another iris diaphragm mounted just in front. We see that the whole lamp is capable of being raised or lowered and tilted through a large angle, each of which movements are capable of being locked.

As we are setting the apparatus up for critical work, it is obvious that we will require a beam of parallel light from the lamp condenser. This is produced when the surface of the bulb, which in this case is the source of light, is at the principal focus of this lens. The quickest method of achieving this object is to shut down the lamp iris to a mere pinpoint and open up the lamp condenser iris fully, then the lamp is switched on and pointed at a flat wall or similar body which can act as a screen, some 30 or 40 ft. distant. The lens mount may

be moved backwards and forwards until an image of the lamp iris is sharply focussed on the wall. If the lamp condenser is then locked and the position marked, the setting for parallel light is permanently known, as in this position the beam will be parallel, for all practical purposes.

As we now know that we have a source of parallel light, we may proceed to set up the complete apparatus. The microscope is placed on the table, which should be of solid construction and stand quite firm so that it is comparatively insensitive to vibration, the limb being tilted to an angle which ensures a comfortable posture when looking into the eyepiece. There should be no tendency to discomfort when doing this, because when lengthy observations are undertaken in an uncomfortable position, fatigue very soon becomes evident with the consequent falling off of acuity of vision.

Having adjusted the instrument to the best position to suit our individual requirements, we now place the lamp opposite the microscope and roughly adjust it, so that the optic axis of the lamp strikes the centre of the mirror, whose surface is horizontal, the diaphragm of the lamp condenser being 4 or 5 in. from the surface of the mirror.

The next step in the procedure is to move the mirror about until we see the top lens of the substage condenser filled with light. Having accomplished this, we are now in a position to place a slide on the mechanical stage and proceed with our final adjustments. For this purpose the author recommends a well-stained histological specimen, such as a kidney or muscle section, and advises keeping this preparation for this purpose, because after a time the individual gets to know the various portions of the slide and automatically selects the best portion to help him set up.

Having placed the slide on the stage, we move it by means of the stage movements so that the centre of the specimen is thereabouts over the centre of the top lens of the substage condenser (having previously racked the body tube well clear of the slide), we now see that a small circular patch of the specimen is illuminated and by further manipulation of the mirror find, in all probability, that we can increase the brilliance of this disc of light to a maximum, which position indicates correct orientation of the optic axis of the condenser with respect to the reflecting surface of the mirror.

We now rotate the $\frac{2}{3}$-in. objective into the optic axis and rack the body downwards, using the right hand, and at the same time looking along the upper surface of the slide from left to right, the eye of course being on a level with this surface. We continue racking the objective down until the leading surface of its front lens is nearly touching the upper surface of the cover glass, the gap being about $\frac{1}{32}$ in. We now look down the eyepieces and will see a diffused disc of light probably noticeably smaller in diameter than the total field

and very likely considerably eccentric to the field. We know by this that at least part of the object in the field is illuminated and we ignore any eccentricity for the time being.

The operation immediately following the observation of this diffused disc of light is to slowly and steadily rack the objective upwards by means of the coarse adjustment. If this is done correctly, the object will come into focus quite suddenly. The whole secret in performing this operation, which is known as "focussing upwards", successfully, is in carrying out the upward movement slowly and steadily. This is the method to be adopted for focussing all objectives, but in the case of parfocalled objectives, after the lowest power has been focussed upward and replaced by the next higher power, it is seldom if ever found that the object is in correct focus, and as one does not know whether the error lies upwards or downwards, the course to adopt is to try racking upwards to commence with. If the object does not come into focus when this is done, then it is obvious that the parfocal error lies downwards and the complete aforementioned process of focussing upwards must be carried out. It cannot be too strongly emphasised that in no circumstances should an objective be focussed downwards no matter what the power, as in this way lies catastrophe, because it is amazingly easy to flash past the "focal point" and continue racking the objective down until the front lens has been pushed through the cover, thereby causing expensive damage to the objective, apart from ruining what might easily be an irreplaceable specimen. In fact, the author has personal knowledge of one case where an enthusiastic beginner in a misguided attempt to demonstrate to a friend his "expert" technique, actually pushed a $\frac{1}{6}$-in. objective right through the cover glass and slide, so that both the front lens of the objective and the top lens of an expensive achromatic substage condenser were irretrievably ruined and both pieces of apparatus had to be returned to the manufacturer forthwith, leaving him virtually without a microscope and a costly repair to pay for when the lenses were returned; but what grieved the young man more than anything was the fact that he had utterly ruined a superbly mounted slide of diatoms which was quite irreplacable, having been mounted by one of the old masters of this art, long since dead. Needless to say, this young man has never employed the focussing-down method since.

Having brought the object into focus in the correct and proper manner, the next phenomenon to be brought to our immediate attention is a feeling of strain in the eyes, which is almost unbearable. This must be immediately rectified, as it is due to the focus of the eyepieces in the binocular head being mismatched to that of the eyes. This is best carried out by looking down the right-hand eyepiece, which is usually the fixed one, with the right eye only, the

left-hand eyepiece being blacked out by a piece of black cloth or similar opaque screen, and bringing the object into sharp focus with the coarse adjustment. One particular portion (such as a clearly defined cell nucleus) is chosen as the datum and finally sharpened by a touch of the fine adjustment. After having accomplished this to our satisfaction, the focussing adjustments are left alone for the time being. We next reverse the blacking out process so that the left eye looks down the left-hand eyepiece, the right-hand eyepiece being inoperative. We now find the datum to be much out of focus and rectify this by using the independent eyepiece focussing arrangement usually built on to the left-hand eyepiece tube and generally carried out by a twisting motion imparted to a knurled portion of the tube by the thumb and forefinger of the left hand. Having focussed the datum to its optimum point in this way, we may now use both tubes simultaneously and find that there is no feeling of strain in the eyes, but in all probability we would still feel somewhat uncomfortable while looking into the eyepieces, this being due to the necessity for adjusting the interocular distance of the eyepieces so that they match that of the eyes.

It will be found that when this adjustment is considerably less or more than it should be, the feeling of discomfort becomes acute and the image of the field is doubled, the two fields overlapping somewhat. The optimum position for this adjustment is reached when the brightest single image is seen, whereupon any final adjustment required of the focussing of the eyepieces may be carried out.

We now have our object in correct focus and may turn our attention to the illumination. The first step is to centre the substage condenser to the optic axis of the microscope. This is best accomplished by closing the substage iris right down and racking the objective slowly upwards, meanwhile looking into the eyepieces until an image of the aperture of the iris is seen in the field. If there is any doubt about this, it is quite easily confirmed by opening and closing of the iris, when, of course, the image will behave likewise. Having found and focussed the iris, it is opened until the aperture is approximately three-quarters of the field, when it may be centred by means of the centring screws in the substage ring. The centring operation is best carried out in two steps, the first step (just described) being more or less a rough centring, after which the iris is opened so that its aperture is very nearly equal to the diameter of the field when any slight inaccuracies immediately become apparent and may be rectified in the same way. After completing this operation, the object is brought into view again by the usual process of focussing upwards.

An alternative method of centring the substage condenser, which has the advantage that the original focussing of the object is not disturbed, consists of setting the condenser so that its top lens is

nearly touching the slide, in the first place, and before bringing the object into focus. Then after having focussed the object and carried out the eyepiece adjustments, the substage iris is closed and the condenser racked slowly downwards until the image of the diaphragm is superimposed upon that of the object, and the necessary centring operations may be carried out without having moved the objective.

We next close down the lamp condenser iris and rack the substage condenser up until the aperture of this latter is sharply focussed. Here again this image will be superimposed upon that of the object. We now open this diaphragm until it nearly fills the field (opening the substage iris as well, if there is not sufficient light); the lamp condenser iris may now be centred by moving the mirror or the lamp.

We now come to what is perhaps the trickiest part of the whole business of setting up the microscope for critical illumination; this consists of lining up the lamp and microscope so that the source of light, lamp condenser, mirror, substage condenser, objective and eyepieces all lie with their centres on the optic axis. To do this, obviously the optic axes of the lamp and microscope must line up together at a point which is the centre of the reflecting surface of the mirror, perfect alignment being obtained when the angle through which the axial ray is reflected is a right angle.

So far we have achieved this alignment for the lamp condenser, mirror, substage condenser, objective and eyepieces, and we now have to bring the source into line. We have an image of the lamp condenser iris superimposed on that of the object and we now close it until its aperture occupies about two-thirds of the field, after which we showly close the lamp iris. Now this is an aperture diaphragm and as such diminishes the cone of light and not the size of the field, like the lamp condenser iris. Nevertheless, we will see a poorly focussed image of the lamp iris inside the aperture of the image of the lamp condenser iris if we close the former down far enough, and in all probability it will be much out of centre with the latter. If it so happens that the two images are concentric, then we will be well advised to leave well alone, as it signifies perfect alignment; if not, then the centring of the two images with each other and field is carried out by movements of the microscope, the lamp, and, if necessary, the mirror. It is impossible to give any routine for carrying this out, as it depends entirely on the relative positions of the microscope and lamp and is best adjusted by a method of trial and error. It is, however, possible after some practice to obtain this adjustment very quickly. The author recommends that this should be practised until the worker is quite familiar with the method, the resulting quality of image and comfort in working amply justifying the short time required to set the apparatus up.

We are now in a position to put the final touches to the critical illumination, which we have seen consists of focussing a source of

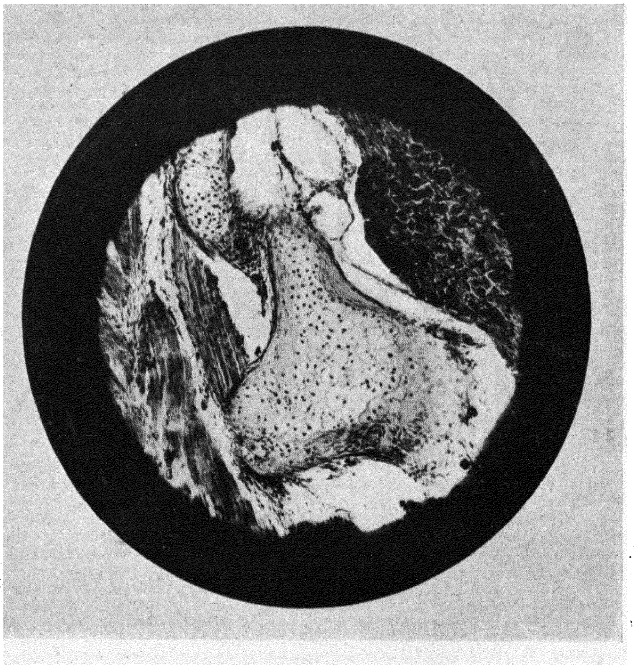


FIG. 186.

[*To face p.* 165.

parallel rays, by means of the substage condenser, in the plane of the object. Now, we have set the lamp condenser so that the lamp is at its principal focus ; therefore the front surface of this lens, *i.e.*, the surface nearest to the mirror, is the source of parallel light, because it is at this interface of glass and air that the rays become parallel. We now open up the lamp condenser iris to its fullest extent and taking a fine camel hair brush, gently pass the tip of the hairs over the front surface of the lamp condenser, at the same time looking into the microscope and racking the condenser very gently upward, until the tips of the hairs in the brush come into focus, at which point the source of parallel light is accurately focussed and the object is under the most critical conditions of illumination.

As a rule the lamp condenser iris is placed very close to the front surface of the lens and consequently the majority of workers ignore the slight inaccuracy involved when the image of this diaphragm is focussed instead of the front surface of the lens. However, when the utmost accuracy is desired, as when using apochromatic objectives at high superamplifications, it is essential to focus the front surface of the lamp condenser, but for all practical purposes it is sufficient to focus the image of its iris, as shown in Fig. 186.

Having carried out the above operations to our satisfaction, we must open the lamp iris until it is the same size as the field, and no more. This keeps down glare in the objective, after which we are in a position to proceed with an examination of a slide of some diatom, say *P. Angulatum*. We remove the slide used for setting up and next place a blue green filter in the filter ring of the substage, the slide is then cleaned on both sides with a soft clean cloth and placed on the stage and moved so that the disc of light from the substage condenser appears roughly in the middle of the cover glass. We next close the substage iris to nearly its full extent and proceed with operation of focussing the $\frac{2}{3}$-in. objective upwards ; having brought the diatoms into focus, we set the substage iris so that the image is not blotted out by glare. This can be checked by opening it until the image is made very indistinct by the glare and then closing it slowly until a point is reached when there is a crisp image of the diatoms on a bright field. This point represents the maximum aperture which the particular objective in use will stand with the object under examination.

We are now able to examine the diatoms at leisure and note their general shape and the coarser details of the structure. We would perhaps notice a suspicion of a finer structure, whereupon we would prepare to examine the preparation under higher power, the next highest power on the triple nosepiece being the $\frac{1}{6}$-in. objective.

So let us prepare to examine the slide with this objective. As the microscope under discussion is a binocular instrument, there is no

provision for the mechanical alteration of tube length; but this is arranged for optically by the fitting of a Jackson tube length corrector between the body tube and the triple nosepiece. The first step is to see that this is set at its minimum; then rotate the $\frac{1}{6}$-in. objective into position and leaving the lighting as it is, we proceed to bring the specimen into focus by the usual method of focussing upwards. We shall have to be a little more careful with this objective, as owing to its shorter working distance it must initially be taken somewhat closer to the slide than was the case with the $\frac{2}{3}$-in., and likewise the racking operation must be carried out more slowly; but it is surprising how rapidly the whole operation can be carried out after a little practice. Having got the specimen in focus we next close the lamp condenser iris so that its aperture occupies about two-thirds of the field. It will probably be out of centre and is brought into the correct position by the use of the substage centring screws, thus ensuring the correct alignment of the optical system. The lamp condenser iris is next accurately focussed and opened so that its aperture is equal to the diameter of the field.

We may now proceed with the examination and correction for tube length by observing a black speck of dust or some other extraneous matter, which is usually to be found in any preparation, and noting whether the image alters in the same manner when thrown out of focus either upward or downward. Thus it might happen that in throwing the image of the speck out of focus upward, the image breaks up with the formation of a series of concentric rings. This signifies that the tube length requires shortening and on monocular instruments is carried out mechanically. If, on the other hand, when focussing upwards the image breaks up by an even diffusion and the rings appear when focussed downwards, it is a sign that the tube length requires lengthening. The final adjustment is reached when the image breaks up in the same manner, whether by forming rings or by diffusion, when thrown out of focus in either direction.

In the instrument under consideration, this adjustment is of course carried out by the rotation of the Jackson tube length corrector, the main point to aim at being to get the break up of the image of the speck exactly the same in either direction, or as near to this as possible, after which we may proceed with the examination of the specimen.

Suppose that after examining the specimen with the $\frac{1}{6}$-in. objective we desire to look at it under a still higher magnification, using the $\frac{1}{12}$-in. oil immersion objective which occupies the third place in the multiple nosepiece. To do this we will have to rack the body of the microscope well away from the stage, leaving ourselves plenty of room between the front lens of the $\frac{1}{12}$-in. objective and the stage. We next proceed to rotate the $\frac{1}{12}$-in. objective into position. As this objective

has a total N.A. greater than unity, the condenser will have to be immersed in order to supply a cone large enough to satisfy the objective. We therefore proceed to remove the slide from the stage and place a drop of immersion oil on its under surface, rapidly inverting it and placing it back in position on the stage. If the stage and condenser have not been moved, the top lens of the condenser will be now in oil contact with the under surface of the object slide, where it is left for the time being.

The next step in the procedure is to place a drop of oil on the cover glass approximately on the point of light and then proceed to rack the objective down in the same manner as for focussing until its front lens touches the oil. Immediately this happens it will be seen to spread over the surface of the lens, due to capillary attraction. We now proceed to very gently rack the objective down until its front lens just touches the cover glass and no more. This is best judged by carefully watching the edge of the oil film, which gradually gets larger until the point is reached when the lens touches the glass surface, when no further movement takes place. Focussing upward may then be carried out with great care. When the object is brought into focus the image is finally sharpened with the fine adjustment, after which the lamp condenser iris is closed down until its aperture is somewhat smaller than the field, in the usual way, and its image brought into sharp focus by a touch of the substage focussing adjustment. (This is where one appreciates the inclusion of a fine adjustment to this movement.) After the lamp condenser iris has been focussed, it is centred and opened up to the necessary extent. As usual the substage iris is then adjusted to give the brightest image consistent with the absence of glare and the homogeneous immersion is complete and adjusted to perform at its maximum with the object under examination.

When using the microscope, the fine adjustment should be used as little as possible, the coarse adjustment should be sufficiently smooth and sensitive to focus all the low and medium powers and even the initial focussing of the high powers, including the $\frac{1}{12}$-in. oil immersion objective, if the correct procedure of focussing upward is adopted. The fine adjustment should not be used any more than is necessary while observing the object under high power, while it is not used at all for objectives of lower powers than $\frac{2}{3}$ in. The characteristics of a careful and well-trained microscopist include the avoidance of using the fine adjustment as much as possible and a rigid adherence to the practice of focussing upward for all objectives.

When using homogeneous lenses of any kind it is essential that there are no air bubbles in the film or films of immersion medium, as these will obviously have a deleterious effect on the image.

So far we have only dealt with objects viewed by transmitted light. In the case of incident light on opaque objects the foregoing remarks apply equally well, the main difference being that with a vertical illuminator the objective functions as its own condenser; nevertheless, it is quite possible and highly desirable to obtain strict critical illumination in these circumstances.

High-power dark-ground illumination, however, involves a slightly different technique from that for ordinary transmitted light. One of the chief factors affecting this type of illumination lies in the preparation of the specimen, the perfection of which exercises a determining influence on the success of the method. The slide and reflecting condenser should be scrupulously clean and entirely free from scratches and other flaws, more particularly in the case when "dry" lenses are used. The thickness of the slide is fairly critical and should lie within 0·9 and 1·1 mm. If the slide thickness is engraved on the illuminator, one should make every endeavour to use slides of the specified thickness as the illuminator is designed to produce the best results at this figure. Departure from this optimum thickness of slide is one of the causes of poor results with a high-power dark-ground illuminator, as it prevents the instrument from being adjusted to its optimum position. If the slide is too thick the object will not lie at the apex of the hollow cone supplied by the illuminator, in consequence of which azimuth errors will be introduced. There is no remedy for this fault except the use of a slide of the correct thickness. If, on the other hand, the slide is too thin, the distance between the top surface of the illuminator and the lower surface of the slide will be too great for the surface tension of the oil and consequently the oil film usually breaks in the endeavour to obtain the correct adjustment. This difficulty may be overcome by oiling a cover glass to the top surface of the illuminator and the establishing oil contact between the lower surface of the slide and the upper surface of the cover glass, oiled to the illuminator.

Faulty dark ground occurs when the slide, the object stratum or the cover glass are not plane and parallel, thus giving rise to unilateral deformations or diffraction phenomena; the only cure for this trouble is a fresh preparation.

If the preparation is too dense, the resulting large excess of diffraction elements will give rise to greatly increased halation which destroys the contrast produced by a dense black background, and may in some cases be so bad as to make the specimen worthless for high-power dark-ground examination. Here again the cure is a fresh preparation if possible.

An important point which must not be overlooked when using a $\frac{1}{12}$-in. O.I. objective with a high-power immersion dark-ground illuminator is the reduction of the aperture of the objective to unity; this, as we have seen, is accomplished by the use of a funnel stop, or

preferably an iris built into the objective; it serves the following purposes:—

(1) It reduces the aperture of the objective in the event of it exceeding the aperture ratio of the illuminator.

(2) It may be used for increasing the contrast in the image thus mitigating, somewhat, the adverse effects of a dense specimen.

(3) It reduces the effect of any residual spherical aberration in the objective.

(4) It increases the depth of focus to a certain degree.

The setting up and adjustment of a high-power dark-ground illuminator requires special care, as even microscopists of considerable experience are sometimes confronted with certain difficulties in the adjustment of the illumination, these being more likely to occur when a changeover from ordinary transmitted illumination to dark ground is required, with altering the position of the specimen.

Therefore it behoves us to examine the setting up of a high-power dark-ground illuminator with a certain amount of care. Suppose we have examined an object with transmitted light and desire to look at it under dark ground; we will obviously require to change the substage condenser, so we remove the mirror and rack the substage right out, leaving everything else as it is. If the instrument is fitted with a swing-out substage, we will only require to rack the substage condenser out far enough to enable us to swing the complete assembly clear of the stage. We may then proceed to replace the substage condenser with the dark-ground illuminator, replacing the mount in the slide or swinging it back into position. We next rack the illuminator right up in order to make certain that it can touch the under surface of the slide. This is important, as in the event of it not being possible we will not be able to maintain oil contact with the slide, and the illuminator becomes useless. (It is as well to make sure of this point when purchasing a high-power dark-ground illuminator.) However, assuming that we are quite safe with regard to this eventuality, we next proceed to rack the illuminator down well clear of the stage so that we may place a drop of immersion oil on its surface, whereupon it is racked up and oil contact is established between this surface and the under surface of the slide.

Now we replace the mirror in approximately the correct position and rack the objective well out of the way so that the whole surface of the specimen is exposed to view when looking at it from the side. Then, by moving the mirror with one hand while we gently raise and lower the illuminator with the other, by means of the rack and pinion, we adjust the conditions so that the smallest spot of light appears in the centre of the preparation, as near as can be judged. The size of this spot of light does not depend so much on the quality of the corrections in the illuminator as on its focal length. Illuminators of short focal length, and in particular the homogeneous

immersion type, will give the smallest spot. Whereas these latter are only designed for work with high-power objectives, those required for use with the lower as well as the higher powers must of necessity be of longer focal length and hence will produce a larger spot of light at the level of the specimen. However, in all cases, when this spot is of a minimum diameter, the illuminator is correctly adjusted.

We are now in a position to put the final touches to the adjustment of our illuminator and next focus the image with a low-power objective, say 1 in. or ⅔ in., and if everything is as it should be, we shall see the object brightly illuminated on an intense black background. We now close the lamp condenser iris down until the illuminated area is only a small patch in the centre of the field. This is the image of the lamp condenser iris which is now brought to a sharp focus in the usual way, after which it is opened up slightly so that any eccentricity may be corrected by a final adjustment to the mirror. The centring of the illuminator may now be checked by closing the substage iris, whereupon the image may either darken from the centre outwards or *vice versa*, whichever way the darkening

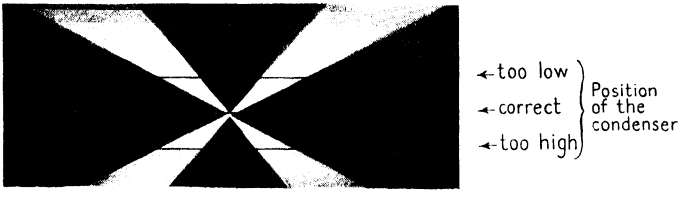


FIG. 187.

takes place it will be concentric with the field if the illuminator is centred correctly. If it is eccentric, then this can be corrected by the substage centring screws.

The setting up is now complete and we are ready to examine the preparation with the high-power objectives, which is done in the usual way, by focussing up. If the light appears to be more brilliant towards one side of the field when viewed with the high power, the condenser requires adjustment for centring by a touch on the appropriate screws.

By a careful following out of this setting up routine an intense and uniform dark-ground illumination can be fairly well guaranteed, and the results more than justify the little extra care bestowed on the setting up.

Some workers insist that only a very powerful light source will produce the best results, but it is the author's experience that for visual purposes the ordinary lamp housing and a 60-watt opal bulb is quite sufficient for the majority of immersion illuminators. He only resorts to the use of a high-intensity lamp for photography.

An idea of the correct appearance of the spot of light, when the

dark-ground illuminator is correctly set up, will be gained by reference to Fig. 187, which shows an enlarged view of the ray path of a high-power illuminator when looked at from the side; the three horizontal lines indicate the position of the plane of the specimen in focus by the objective. Obviously, if the illuminator is focussed either too high or too low the spot will take the form of an annular ring of light, only becoming a spot or disc at the correct point.

So much then for the actual technique of using the microscope; of course it must be realised that the foregoing description is in great detail and deals with the setting up and handling of the instrument for the first time. Obviously many of the steps such as checking the mechanical movements are not required to be carried out every time the instrument is used, as they would merely constitute a waste of valuable time. The steps more directly affecting the quality of the image should, however, be carried out at every sitting; for instance, if the apparatus has been set up correctly and used in the morning and then not used again until, say, the afternoon, before carrying out any examination the second time, the alignment should always be checked, as it is astonishing how easily this shifts, a matter of a movement amounting to thousandths of an inch being sufficient to upset the delicate adjustment, and in situations where the instruments are subject to appreciable vibration, such as in a laboratory attached to a factory, the need for checking and carrying out any necessary readjustments to the alignment becomes more imperative. However, as in most things, " practice makes perfect," and it is surprising how quickly the process of setting up may be accomplished after a short period of practical experiment, more so when a knowledge of the theory underlying the use of the apparatus has been gained.

In order to obtain the utmost out of the microscope, practice is required in its use and the hands and fingers have to be virtually trained to undertake delicate operations and adjustments; in the same way the eyes have to be trained to observe and function correctly and must also be taken care of, although there is very little risk of permanent damage to the eyes, if the instrument is properly used. It is possible to damage the eyes seriously if the simple rules are deliberately ignored. This applies more particularly to the monocular type of instrument where only one eye is used at a time. Five basic rules (1) may be stated to apply to the use and care of the eyes; they are :—

(1) **Use the Correct Amount of Light**

This is more important than at first realised, the light must not be too bright nor too dull. If it is too dull, the eye tends to overreach itself in looking for fine details which are not sufficiently powerfully illuminated to stimulate the nerve endings on the retina, with the

result that the automatic mechanism operating the opening of the iris becomes strained, due to it tending to open too far. Admittedly the resolution and aperture of the eye is thus increased, but at the expense of an unnecessary amount of nervous energy which will eventually have its effect on the general health. Much the same argument applies to having the light too bright, by this is meant too intense, as obviously the illumination may be made too bright by opening up the substage iris, but in this event the image would be ruined by glare and nothing will be gained. What is meant by " too bright " is looking at an object correctly illuminated by means of the usual opal lamp on the one hand, and by, say, an arc lamp on the other hand ; it is a matter of relative intensity. Some authorities say that bright light is bad for the eyes, while others say that, within limits, bright light is actually beneficial as the eye was primarily designed to function in bright sunlight. However, this question is of no great importance in our problem. What really does concern us is the fact that, the brighter the light, the more the iris of the eye closes down, in consequence of which the aperture and resolving power of the eye is reduced, so it would be defeating our object if, having a perfectly aligned collection of apparatus with the best lenses, we proceed to use such an intense source of illumination that the resolution of the optical train is negated by the destruction of the resolving power of the eye. However, nature comes to our aid inasmuch as we are warned of either an excess or lack of light by a feeling of discomfort and simple experiments such as moderating the intensity of light by filters or screens will soon find the optimum conditions to meet our individual case.

Perhaps the best method of moderating too high an intensity is by the use of colour filters. It is well known that the maximum sensitivity of the human eye lies in the yellow-green region of the spectrum, the peak being reached at a wavelength of approximately 5,500 Angstrom units ; therefore it follows that if we use a filter whose transmission band covers this point, we may moderate the intensity of the illumination and at the same time keep the sensitivity of the eye unimpaired in actual practice. The best filter to use for maximum resolution in visual work is a blue-green, whose transmission band lies slightly below the 5,500 mark, thus the light is still further reduced in intensity and we have the added advantage of a general shortening of the wavelength of the light used. The author has found a Chance-Watson blue-green or an Ilford 303 filter eminently suitable for visual work, apart from the question of modifying the intensity of illumination.

(2) **Keep Both Eyes Open when Using Monocular Instruments**

If strain is to be avoided, this operation is absolutely necessary. When first attempted it appears almost impossible, as at first the

field will come into view at one moment and fade out to give way to a view of the top of the table which in turn will fade after a short while only for the field to come back into view, but the very fact that one sees these two views alternately and seldom, if ever, simultaneously, is the proof that one is able to so educate the eyes that either may be held to the exclusion of the other, and it is only practice which will develop the ability to hold the field in view at will, keeping the other eye psychologically blind. When practising this, one should never try it continuously for more than two or three minutes without a rest, and certainly never more than fifteen minutes at a sitting, only undertaking two or three sittings during the day at well-spaced intervals, so that the eyes have every chance of recovery from the effort involved, for it must be realised that the effort is mainly mental and must not be overdone.

If the effort appears too much, it is advisable to give up trying to obtain the desired result by direct means and resort to other measures which consist chiefly of using a shield. These are made by most manufacturers, but are quite simple to construct for oneself, the general construction being a black disc of opaque material held in front of the vacant eye by a wire support which is fixed round the top of the eyepiece. This simple device will be found to be exceedingly helpful in the early stages of the education of the eyes, but must be discarded as soon as possible.

Facility in this operation is a sign of the well-trained microscopist, as only the beginner or poorly informed worker takes the risk of severe eye strain by persisting in the habit of shutting one eye, as apart from eye strain the muscles of the eyelid are fatigued and strained unnecessarily. One excellent method of avoiding the use of the disc is to cover the vacant eye with the appropriate hand, cupping the hand over the eye so that it may be kept open without the discomfort of having the eyelashes rubbing against the palm of the hand.

(3) **Use Both Eyes Alternately**

This rule applies only to monocular instruments. Just as a careful and well-trained worker may be recognised by the way in which he handles his instrument and refrains from squinting, so he may also be recognised by his habit of never using any one eye for more than a few minutes, but instead he uses both eyes alternately for about the same length of time. This rule is perhaps more important than the preceding one, as it will be realised that constant use of one eye will eventually have a physical effect which will become more or less permanent. As a matter of fact, the development occurs of what is known as " microscope eye," in which the eye loses its sensitivity to intensity of light but gains in its ability to perceive detail, which condition does not balance with the other eye, and the likelihood of treatment by an oculist results.

Rule 3 is not operative if one uses a binocular body which is properly adjusted, and the author recommends the use of a binocular instrument for visual purposes whenever possible, but would add that one should make very sure that the prisms and optical system are in perfect alignment, as to force the eyes to look into an instrument which is badly adjusted is liable to cause extreme discomfort, if not actual pain. Here again, nature gives her own warning that things are not quite right, in spite of which it is possible to use a binocular instrument which is only slightly out of adjustment by an unconscious effort of the eyes to correct for the error in the instrument, thus leading to a cumulative building up of the effects of strain. However, this slight error is unmistakable when first looking into the instrument, when it should immediately be put right by the manufacturers or else refused.

(4) **Never Make a Habit of Prolonged Observations**

By this is meant that one should alternate visual study with the microscope with some other operation. Corrington (1) puts the case admirably, as is shown by the following extract from his "Working with the Microscope." He says: "It is neither advisable nor necessary in most cases to look steadily into the microscope for more than a few minutes at a time. One naturally turns from a scrutiny of, say, a new protozoan to looking him up in a book, making a sketch or drawing of what is seen, or writing notes, then look back into the microscope again to check the first observations, and so on indefinitely. This procedure is not only excellent to prevent eyestrain, it is the natural and efficient method of working. Once in a while it may be necessary to make prolonged observations, as when one catches a cell in division or watches fertilisation or some other significant happening, but such occasional sessions do no harm."

(5) **Always Relax when Using the Microscope**

Never try to force the eye to do that of which the instrument is capable. This means to say that when focussing the object let the eyes be completely relaxed, as they would be if one were looking at a distant object, then use the focussing adjustment to bring the object clearly into view, at the same time exerting no effort to focus it with the eyes. Corrington describes this very well, he says: "If one can get himself into the frame of mind that he is looking down into a deep well, and that the object is far away, which is actually the case if you consider relative sizes, the eye will accommodate itself to focus at a distance in a naturally relaxed position and the lens muscle will not be strained in a futile attempt to accommodate to a close-up view." If this rule is adhered to and practised it becomes automatic; the microscope may be used without any fear of straining the eyes,

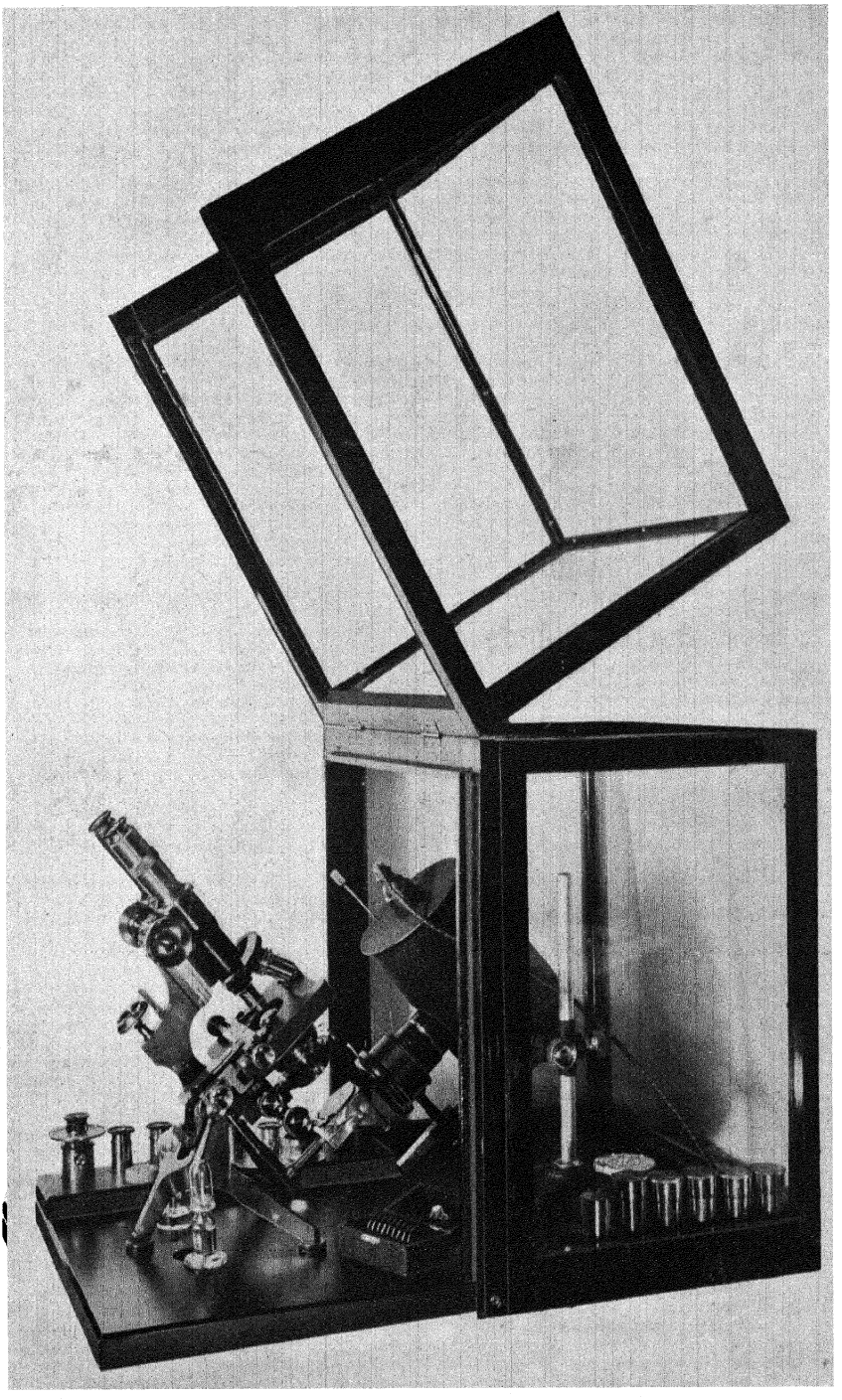


FIG. 188.

[*To face p.* 174.

particularly so if the preceding rules are borne in mind and used in the same way.

It should be remembered at all times that a microscope of reputable make is a scientific instrument of the highest precision and workmanship, and if treated with the care and respect due to it, will give a lifetime of trouble-free service. Therefore it behoves us to handle the instrument with the greatest of care and use it as it was meant to be used. For example, in many laboratories and scientific institutions it is standard procedure to use the microscope on the laboratory bench, where it occupies any convenient space amidst an array of chemicals, specimens and the like. This practice is to be deplored, as fumes and vapours deleterious to the delicate mechanism may quite easily do irreparable damage, apart from the grave risk of the instrument being knocked right off the bench. The best method to adopt in these circumstances is to relegate a bench, preferably along a wall away from windows, for this purpose, and let no other type of work be carried out on it. Thus a laboratory catering for students of biology, for example, would have one bench carrying a number of microscopes each allotted a space and fitted with a lamp, so that critical illumination may be employed or as near critical as possible, and it would be the rule that the bench would be used for the purpose of examining specimens only, all preparatory work such as staining and mounting being done on the standard laboratory benches. We could elaborate on this scheme and have each instrument covered by its own bell jar, therefore affording added protection from fumes which are always present. Actually the best practice would be to have the microscopes fitted up in a separate instrument room.

With regard to small individual laboratories, it is always advisable whenever possible to use the microscope, and for that matter other scientific apparatus such as balances and the like, in a room by themselves, away from the laboratory proper. This is more necessary in small qaurters, where the danger from fume attack is much increased.

In order to avoid having to set the microscope up with respect to the lamp and align the optic axis every time it is desired to use the instrument for a spell, it is very desirable to have some arrangement whereby the whole of the apparatus may be left set up and protected when not in use. The author has designed an arrangement which performs this function admirably ; it is shown in Fig. 188. As will be seen, it consists of a flat base, in this case a 1-in. thick slate 24 in. by 18 in., on to which is fitted a glazed case having a wooden frame which is divided vertically ; the rear half is fixed to the slate, while the front half is hinged to the rear half along the top. In this way the front half of the case may be swung up on top of the rear half, thus leaving the microscope exposed and ready for use ; the lamp is

situated in the rear half of the case. It will be seen that the base is large enough to accommodate racks carrying accessories such as spare eyepieces and objectives, etc. Thus the microscope is initially set up accurately for critical illumination and may be left without attention; when it is required to be used, all that is necessary is to swing the front half of the case upward and back so that it rests on top of the rear half and everything is ready for use. This is a simple but effective method of protecting the apparatus and at the same time affording the convenience of having it permanently set up for critical illumination and ready for immediate use.

In cases where a person wears spectacles, it depends on the kind of eye weakness as to whether he can wear them, or not, when looking into the microscope. If he is merely short or long-sighted it is quite in order for him to dispense with the glasses when using the microscope. The only point to remember is to replace them when he turns away from the instrument to make a note or check on a drawing; as a matter of fact, it will be found that he will be unable to do these things without replacing them. However, some workers thus handicapped prefer to leave their spectacles on all the time; in any event, it is immaterial whether they are worn or not, provided there is no other trouble present, and individual opinion should be the guiding factor.

We have a rather different state of affairs where the use of spectacles is dictated by the presence of astigmatism. In this case the glasses must be worn while using the microscope, and it is advisable in cases where an astigmatic condition is suspected to make quite certain by consulting an oculist. When glasses are worn it is desirable to afford means of protecting them from damage due to rubbing on the metallic mount of the eye lens; this is quite easily accomplished by fitting a rubber cap over the eyepiece. Munoz and Charipper recommend the fitting of a rubber band round the raised edge of the mount as a simple and effective cure. It is also possible to fit subsidiary lenses over the eye lenses which have been computed to counteract the astigmatism in the observer's eyes, although it must be borne in mind that this while being a good method is inclined to be costly and is really only efficient when applied to a binocular instrument, because it is quite possible that the astigmatism will not be the same in each eye, and if subsidiary lenses are used with a monocular instrument, obviously they will have to be changed when changing the active eye, added to which is the necessity for replacing the spectacles every time we look away from the instrument to make notes, etc.

In general, therefore, it would appear that the best plan to adopt is to educate oneself to using the microscope while wearing spectacles, except in cases of mild, short or long sight; however, a little experi-

ence will soon indicate to the individual the most suitable method of solving his own problem.

We have seen, in previous pages, explanations of the delicate and sensitive nature of the microscope in general, and perhaps a few words on the handling and general care of the instrument will not be amiss. To commence with, when moving the instrument never pick it up by any other part except the curve of the limb, as apart from forming the base of the optical bench, this part of the microscope is usually designed to afford a convenient hand hold ; and when actually transporting the instrument, it is always advisable to support it under the foot with the one hand while holding the limb with the other.

Dust is the greatest enemy of the microscope and should be rigidly guarded against. If it is not possible to keep the instrument permanently under glass, as previously described, and one has to put up with setting it up for each session, it should be an inflexible rule always to keep the instrument in its case when not in use, thoroughly cleaning it free from dust after having used it. The same applies to pieces of accessory apparatus, which should have individual cases of their own ; if not, then it is a good plan to make or have made a case to hold accessories unequipped with cases of their own.

If it is required to use the microscope after it has been stored away in its case for any length of time, it is always advisable to check the mechanical movements, wipe all V slides dry and lubricate them afresh with the minimum amount of pure petroleum jelly, though it is not necessary to use petroleum jelly only, there being various opinions as to the best lubricant to employ. For instance, Carpenter and Dallinger (2) recommend the use of fine watchmaker's oil, while Munoz and Charipper (3) find petroleum jelly the most suitable. Other authorities prefer a mixture of half and half, petroleum jelly and lanoline, while still others use a mixture of Russian tallow, beeswax and petroleum jelly ; this latter is the basis of the well-known " optician's grease " and is very stiff, its effect being to impart a hydraulic quality to the feel of the movement. Many authors have objected strongly to its use, on the grounds that unscrupulous manufacturers use it to camouflage slack fitting slides and bearings, which in fact it does, by virtue of its stiff texture, but it is the author's experience that when applied to a sound movement it does definitely improve the handling qualities ; therefore it seems rather incomprehensible that the material should be condemned because in certain cases the qualities which make it so useful are grossly misapplied. There is one point, however, which is worthy of consideration. Owing to its composition it is naturally acid, and after having been applied for some time it is apt to react with the brass and turn green, but this should not cause undue alarm as the acids are all weak organic acids with only a very slight action on

the fitted surface amounting to a slight etching, and this only after some considerable time. As a matter of fact, the author has a microscope which has been lubricated with a grease of this type for many years and the effect of its acidity on the surfaces of the slides is scarcely detectable even under a X15 hand lens, they are still as good a fit as ever. Therefore it would seem that in spite of warnings to the contrary, this type of grease may be used without giving rise to any anxiety due to possible disintegration of slides and mechanical movements. On the other hand, we do obtain a degree of control and smoothness over the movement not obtainable by the other types of lubricants. The whole question does not depend so much on the type of lubricant used as on the quantity, for obviously a properly fitted slide can only hold a given amount of lubricant and any excess is merely squeezed out on the outside where, if it is left, it acts as a beautiful dust collector, eventually forming a most efficient abrasive paste which can be guaranteed to ruin any slide in the shortest space of time. So let us see to it that we use only the merest trace of lubricant, taking the greatest care to remove any excess completely.

At the same time as re-lubricating the stand it is advisable to check the mechanical movements for wear and take up any slackness in the slides and bearings by the means provided. It is not proposed to describe this in detail, as each manufacturer has his own method of wear adjustment. Suffice it to say that in most cases it is carried out by screws, whose application is quite obvious ; in any event, these should seldom if ever require touching if the instrument is properly looked after.

One important point to remember is that when setting up the microscope (after it has been stored in a cold room) in a warm room it should be left until it has reached the higher temperature, otherwise much moisture is liable to condense on the delicate mechanisms from the warmth of the hand, and particularly from the breath. This moisture will condense on the inside as well as the outside and is impossible to remove, short of stripping the stand down. If the difference in temperature levels is very great, moisture will condense out of the atmosphere, and this should be prevented by letting the instrument reach the higher ambient gradually.

Before commencing a session with the microscope one should examine all exterior lens surfaces liable to be contaminated, for finger marks, residual oil, dust, etc., and if found to be dirty should be properly cleaned. The methods to adopt for cleaning have been dealt with in detail in previous chapters. At the same time, do not forget to clean the object slide thoroughly if a permanent preparation is to be used ; if a temporary preparation is to be examined then make quite certain that all the glass surfaces are scrupulously clean. We shall go into this in greater detail subsequently.

Most mechanical stages and rotating stages are these days fitted with vernier scales. These scales are very useful, but as at first their method of operation is a trifle obscure (to the uninitiated), let us examine the method of using them.

As will be seen from Fig. 189, the vernier scale is a small scale engraved on the fixed portion of the movement. In this case there are two, one for the cross traverse and the other for the up-and-down traverse of a mechanical stage. It will be seen that it virtually consists of an extended datum line from 0 to 10, but this extension is not haphazard; the total distance of the extension, that is to say from 0 to 10 in each case, is equal to the distance occupied by nine divisions of the moving or master scale. Thus, for example, the total distance of the vernier of the cross traverse is the same as that occupied by the graduations numbering say 30 to 39 in the cross traverse scale.

Now, suppose that on reading either one of these scales the 0 of the vernier happened to be exactly coincident with, say, the 30 mark on the scale, then the reading would be 30 plus or minus nothing. Further, as the vernier is 0·9 the length of ten divisions of the scale, it follows that one division of the vernier is nine-tenths of a division of the scale, therefore it will be clear that when the zero of the vernier is exactly coincident with any whole number on the scale, in this case 30, then the first division of the vernier will occupy a position one-tenth of a division short of the 31 mark. Similarly, the second division of the vernier will occupy a position two-tenths of a division short of the 32 mark, and so on, each succeeding division of the vernier being one more tenth of a division shorter than the preceding one, until at the tenth vernier division we see that it coincides with the 39 mark.

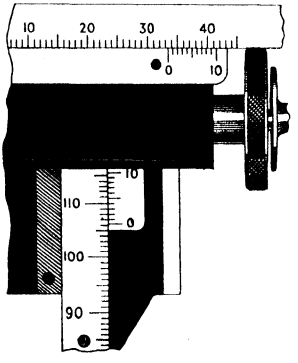


FIG. 189.
(After Munoz and Charipper.)

Keeping the same example in mind, let us advance the scale a little so that the first division of the vernier exactly coincides with the 31 mark, then if we look back at the zero on the vernier we see that the zero is slightly in advance of the 30 mark. This distance is obviously one-tenth of the distance from 30 to 31; similarly, if we advance the scale still further so that the second division of the vernier corresponds exactly to the 32 mark, we see that the zero has advanced two-tenths of the distance between the 30 and 31 mark, and so on, up to the point when the 10 on the vernier corresponds with the 40 mark on the scale, then the zero will be found to be coincident with the 31 mark. Thus we see that we have a means of accurately finding the position of the zero when it lies at any point

between any two whole numbers on its associated scale to an accuracy of one-tenth of a division.

From the foregoing remarks it will be seen that the cross transverse scale in Fig. 189 is set to read 33·2 as the zero is a short way past the 33 mark and the second division of the vernier scale is the only one of the ten which is coincident with a mark on the moving scale. Thus to read the vernier we have to do two things, first to note the position of the zero with respect to the nearest whole number below it, in this case the 33, and then glance along the vernier scale until we find a line on it coinciding with one on the moving scale. Suppose the zero happened to be very near the 34 mark but not quite up to it, then the nearest whole number below the zero would still be 33 and on glancing along the vernier scale we should, in all probability, find that its ninth division is coincident with a line on the moving scale, thus indicating a reading of 33·9. It should be remembered when reading verniers that, of the two coincident lines, the value of that which is on the moving scale is immaterial, but the value of the line on the vernier scale is the one which completes the answer.

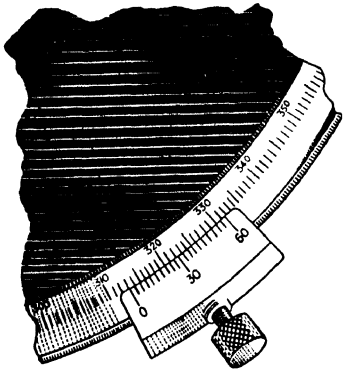


FIG. 190.
(After Munoz and Charipper.)

The movements of mechanical stages are usually calibrated in millimetres, and in the stage shown the cross transverse is set at 33·2 mm. and the up-and-down movement is set at 106·3 mm. Now these measurements are very useful as we may use them to make overall measurements of objects to 0·1 mm. A further use is the calibration of permanent preparations ; for example, we might have a particular slide which contains a number of interesting points and much time and trouble is saved in finding these same spots again and again if the slide is calibrated when first examined. Now suppose that at this setting of the stage a particular slide showed something worth further study, then a note would be made of the subject together with the slide number and the numbers 33·2 × 106·3, so enabling this particular spot to be immediately placed in the field of the microscope on future occasions.

In the same way verniers are fitted to the calibration of circular stages, but in this case they are calibrated in degrees and minutes, the vernier illustrated in Fig. 190 being calibrated in divisions of three minutes of arc and readings are capable of being directly read to 3 minutes and estimated to one minute.

We have studied the construction of the microscope and the methods of using it to the best advantage, and before closing this

chapter it would perhaps be as well to look briefly at the question of interpreting that which we see with the instrument.

The objects examined may be divided broadly into two classes, those which are coloured, either naturally or artificially, and those which are not coloured. On examining either of these types it is necessary to be able to distinguish the false from the true, particularly so with uncoloured objects, therefore it behoves the inexperienced to gain some experience at first hand on the spurious effects produced by many objects. Simple experiments may be carried out by examining such objects as air bubbles in water and canada balsam, etc., noticing the various appearances produced by change of focus and refractive index of mounting medium. The same tests can also be applied to a filament of glass wool and may be further extended by using different methods of illumination; all these tests will demonstrate the necessity for care in deciding the nature of an object examined, particularly in the case of an object whose structure is unknown.

If any doubt exists at all, it is always a wise precaution to examine the object under various types of illumination before making any pronouncement as to the structure and constitution of any particular specimen, the main point to bear in mind being to search for the truth and having apparently obtained it prove it true to your entire satisfaction before making a statement. If in the light of further knowledge one subsequently proves oneself wrong, there should be no hesitation in correcting the first impression.

It is advisable to lay down for oneself some guiding principles to work to and it is suggested that the following may help; they are :—

(1) Cultivate powers of correct observation.

(2) Gather and note all the observed facts, no matter how irrelevant they may seem.

(3) Interpret these facts correctly.

(4) Co-relate them to form the complete picture.

(5) Perhaps the most important, keep an entirely open mind.

REFERENCES

(1) CORRINGTON. "Working with the Microscope." McGraw Hill. By kind permission of the author and publishers.

(2) CARPENTER and DALLINGER. "The Microscope and its Revelations." Churchill.

(3) MUNOZ and CHARIPPER. "The Microscope and its Use." Chemical Publishing Co.

CHAPTER IX

AN INTRODUCTION TO THE POLARISING MICROSCOPE

POLARISED light was first used with the microscope about 1840, when Hartnack constructed an instrument employing this illumination. It was used mainly for showing the beautiful colour effects obtained by some crystalline minerals and for a period remained as such.

It was not until 1877 or thereabouts that polarised light was used seriously, when it was found to be of great help in the science of minerology, from which time its use has been extended to other fields until at the present time there are few, if any, branches of science where the polarising or " Petrographic " microscope is not of benefit.

Therefore, let us briefly examine the functions of a polarising microscope and some of the uses to which it may be put. It is not proposed to go into any great amount of detail on the subject as each particular field of application necessitates its own technique and as many volumes have already been written on this subject alone, by the most competent authorities, the reader is referred to the standard works. If further study is desired, details of these will be found in the bibliography.

Before proceeding with the subject of the polarising microscope, it would perhaps be as well to go into the question of polarised light, as a thorough understanding of the basic principles involved is essential if the instrument is to be used to the greatest advantage.

What then is polarised light and how is it produced ? To answer this question it will be necessary to touch briefly on the history of its discovery and on the nature of light itself. The polarisation of light was first discovered by Huygens while examining the refraction of light through a crystal, and resulted in the discovery of double refraction or " bi-refringence " ; from which beginning it was soon discovered that tourmaline and many other crystalline substances exhibited this phenomenon. Further work brought to light the fact that bi-refringent substances were capable of modifying a beam of light transmitted through them in certain directions ; for example; it was found that plates of tourmaline, when cut and polished along the axis of the crystal, as shown in Fig. 191 at 1 and 2, are orientated in various directions relative to each other, then at certain relative positions the light passed through the first plate encountered by it, but not through the second, the direction of the beam being along the axis OO.

Thus in the position shown in Fig. 191 where the long axes of the plates are coincident the beam of light passes through both crystals without visible alteration, except for tinting by the natural colour of the tourmaline, but when the No. 1 plate is rotated on its horizontal axis through any angle, the light coming through it from the No. 2 plate is seen to have been diminished in intensity

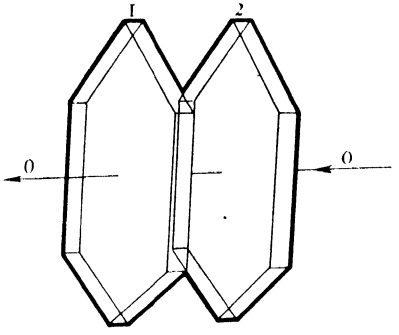


FIG. 191.

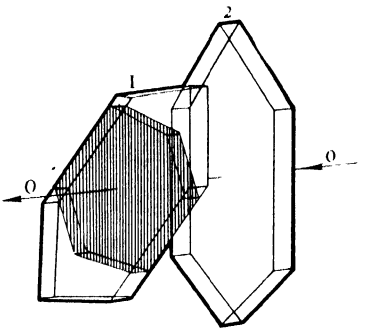


FIG. 192.

and the image of the No. 2 plate as seen through the No. 1 takes on a greyish tinge (Fig. 192) which gradually becomes darker until a point is reached where there is a complete blackout when the angle is 90° as shown in Fig. 193. On increasing the angle past 90° the image of the No. 2 plate becomes lighter up to a maximum, when the long axes of the plates are coincident again.

The observation of this and similar phenomena led to speculation on the nature of light and the experiment with the tourmaline plates was taken to demonstrate the transverse direction of the vibrations.

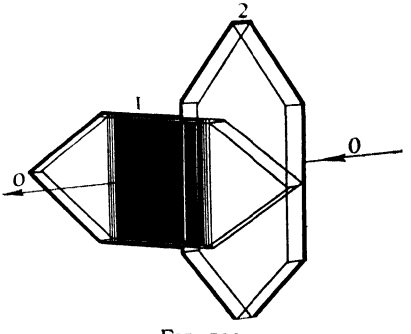


FIG. 193.

Up to the present we have not considered the nature of the vibrations in a beam of light apart from a general assumption that light is vibratory in nature, but in order that we may understand something about the manner in which polarisation is accomplished, it is necessary to look a little more closely into the matter. For the purpose under consideration we may take it that a ray of light is in vibratory motion, this latter being in a direction transverse to the direction of propagation. Thus it will be appreciated that wave motion is present.

Now, if we imagine our ray of light to be in the form of a cylinder, along whose long axis lies the direction of propagation, then on

viewing a transverse section of the wave we could very well imagine something like the structure shown in Fig. 194, where the point O represents the propagational axis of the ray, the vibrations taking place in planes composed of diameters of the circle. Thus if we take the plane AB, the vibrations take place in the directions indicated by the arrows and along the axis, the same applying

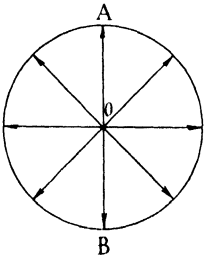


FIG. 194.

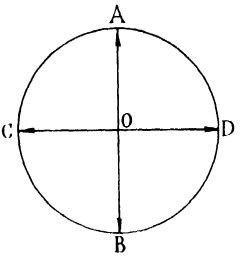


FIG. 195.

to any diameter of the circle, four of which are shown in the figure.

We will take two of these planes as AB and CD in Fig. 195 at right angles to one another. We know that their axis of propagation lies in a direction perpendicular to the plane of the paper, therefore let us see what this wave would look like if we could view it from the side.

Assuming that the wave had a definite mass, we could imagine

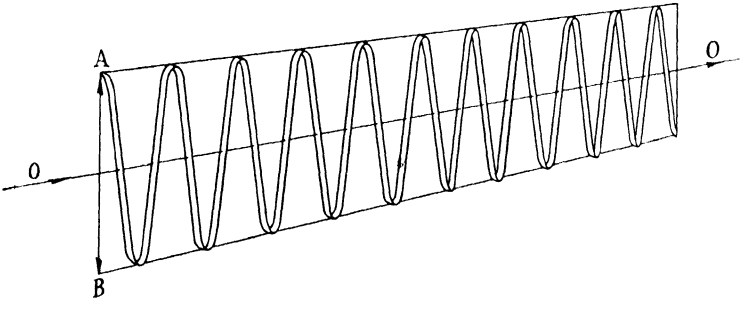


FIG. 196.

it appearing as a wavy tape as shown in Fig. 196. It represents the wave in the vertical plane AB of our ray of light, which for purposes of clarity is shown as being three dimensional, although it must be remembered that this and subsequent diagrams are only broad graphical analogies used by the author in offering a simple explanation of the phenomenon of polarisation and should be regarded only as such. However, to return to our ray in Fig. 196, we see the vertical component in perspective, in a similar manner we may imagine the horizontal component or the plane of vibration CD as

shown in Fig. 197. In both cases the axis is shown as OO on which is marked the direction of propagation. Now let us assume that our ray contains only these two components and assemble them in their relative positions so that their axes OO coincide and thus give us a common axis for both components, which in turn are set at right angles to one another in the manner shown in Fig. 198. We now have a broad concept of an elemental ray of light

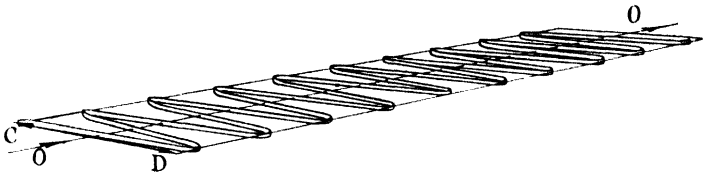


FIG. 197.

made up of only two planes of vibration at right angles to one another.

On examining the phenomenon of the tourmaline plates a little more closely, it would soon be apparent that on its passage through the first plate a ray of light is modified in some way so that it can proceed through the second plate unhindered, so long as the major axes of the crystals lie in the same direction, but it is completely stopped from traversing the second crystal when the major axis of

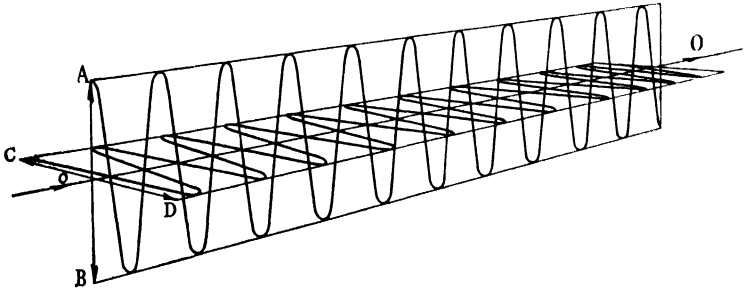


FIG. 198.

this latter is so placed that it lies at right angles to that of the first crystal. A little thought would show that this modification might take the form of a re-orientation of the planes of vibration so that they all lay in the same direction as the major axis of the first crystal, then when the crystals were crossed the orientation of the vibratory plane proceeding from the first crystal would be entirely contrary to the orientation of the planes in the second crystal, thus producing the blackout.

All bi-refringent substances exhibit this characteristic when handled and orientated correctly, and we may proceed a step

further with our analogy by imagining that these substances act as a slotted grating; thus Fig. 199 represents such a hypothetical grating in the form of a block of such a substance. PL having vertical slots through it as shown. If these slots were only wide enough to permit the passage of one plane of vibration when placed in the path of our elemental ray (Fig. 198) (only the planes of vibration are hereafter shown, the waves being omitted in order to avoid complicating the diagram), this latter will come up against a certain amount of obstruction, the effect of which is shown in Fig. 200. Here we find the ray ABCD trying to proceed through our hypothetical block, but when the ray reaches the block it finds that only half of it can proceed through the block, this half being the vertical component AB, by virtue of the fact that the slots in the block are

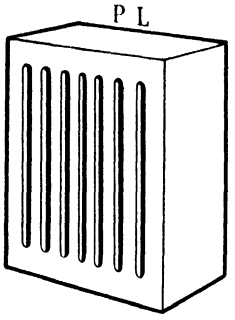


FIG. 199.

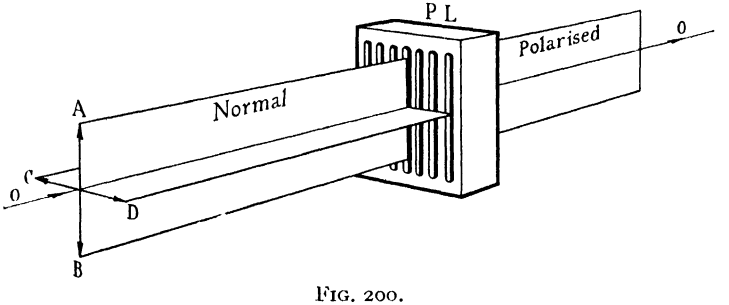


FIG. 200.

also vertical, the horizontal component, on the other hand, cannot proceed through the block as there are no slots lying in a horizontal

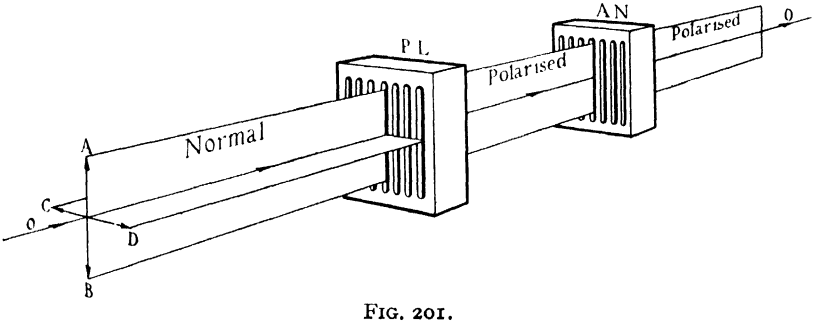


FIG. 201.

direction; therefore we find that the ray appearing on the far side of the block consists of only one component, viz. AB, while the horizontal component CD has been stopped and we could say that the single component ray was polarised, and in any system using polarised light the component occupying the position of the block

PL (in the case of the tourmaline crystals it would be the first plate) would be called the polariser.

Now suppose we take another block such as PL and place it in the path of the polarised ray so that its slots are vertical, then it will be clear that the polarised ray will pass through the block as the slots in AN (Fig. 201) are orientated in the same direction as the plane of the polarised ray. Fig. 201 shows how this would take

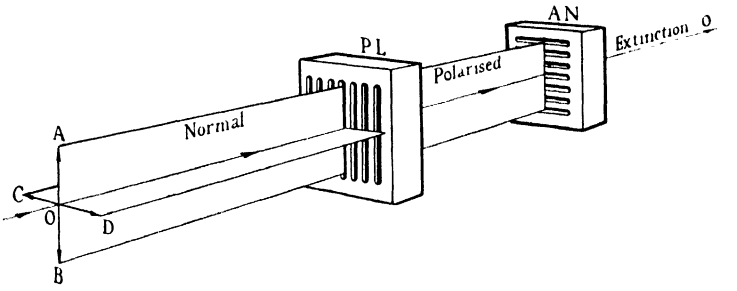


FIG. 202.

place and we can now see the similarity between this analogy and the case of the tourmaline plates when their long axes are coincident.

If we now refer to Fig. 202 we see the result of turning the block AN through 90° so that its slots are at right angles to those in the block PL and hence also to the plane of the polarised ray. In this case the polarised ray meets a similar obstruction to that met by the plane BC in the normal ray (when it reaches the block PL) and

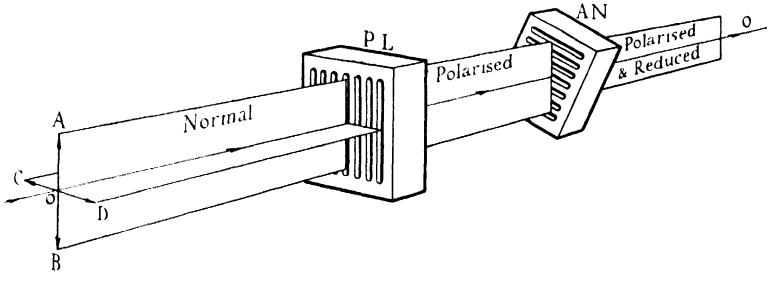


FIG. 203.

hence it is stopped from further progress; it is clear that here we have a similarity in the case of the crossed tourmaline plates which produce a blackout.

Now let us rotate AN through 45° on the axis OO, then the slots will be at an angle of 45° to the direction of vibration of the polarised ray and hence also to the slots in PL. This condition will not stop the passage of the polarised ray entirely, but instead some of it will go through the block, as shown in Fig. 203, resulting in a diminution of the intensity, visibly detected by the decrease in intensity. Thus it will be seen that if we commence with the blocks

PL and AN placed as in Fig. 201, then the whole of the polarised ray from PL passes through AN. If we now start rotating AN on the axis OO, say in a clockwise direction, PL remaining fixed meanwhile, then as AN approaches a position where the slots are at 90° to those in PL as shown in Fig. 202, the polarised ray which emerges from the block AN will suffer a gradual decrease in intensity until AN has been rotated through 90° when extinction occurs, as in Fig. 202, if the rotation is carried past the 90° the emergent ray comes into being again and gradually brightens to a maximum when the slots in AN are again vertical (although upside down) and so on right through the complete circle we would get a cycle of fading out to extinction and brightening to the maximum every 180° of rotation. Thus it will be seen that in our three dimensional analogy we have traced step by step the experiments with the tourmaline crystals, reproducing a set of hypothetical conditions corresponding to the phenomena produced by the tourmaline plates. Thus reasoning

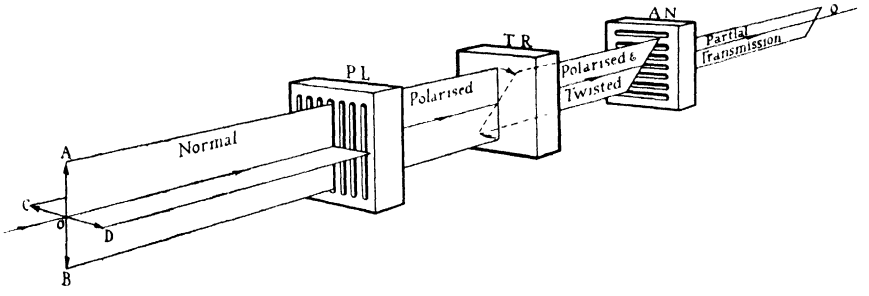


FIG. 204.

back from the original example of the tourmaline, we see that the suggested concepts of the structure of our hypothetical ray and the bi-refringent blocks are admirably suited to the resultant theoretical explanation of the mechanism of polarisation. In an optical train the block AN is known as the " Analyser."

It has been stated that all bi-refringent substances exhibit the phenomena produced by the tourmaline plates, but at the same time it must be stated that the degree of bi-refringence varies from substance to substance. This characteristic is most useful for purposes of identification.

Now suppose we have a polariser and analyser set as at PL and AN in Fig. 204 (*i.e.*, in the crossed position) and between them we interpose a block of some other bi-refringent substance TR, then it will be seen that this latter block will have some modifying effect on the polarised ray (unless its orientation corresponds to that of PL). If its bi-refringence is such that when placed with its long axis at right angles to OO and in the same direction as that of PL, its hypothetical slots are not coincident with those in PL, but displaced

by some small angle (indicated by the arrow in the diagram), then obviously it will offer a resistance to the polarised ray in the same way as that shown by the analyser AN in Fig. 203. Thus the ray as it emerges from the block TR will be apparently twisted and decreased in intensity, depending on the amount by which the orientation of TR differs from that of PL.

On reaching the analyser AN this ray now encounters a certain amount of resistance due to the orientation of the slots and only a portion of it is allowed through AN in exactly the same way as in Fig. 203. Now imagine that the block TR is rotated so that the angle shown in Fig. 204 is reduced to zero then it will be appreciated that the polarised ray will pass straight through TR without being subjected to any modification as we have now brought its slots into a position where they coincide with those in the polariser PL and on reaching the analyser the ray will now suffer extinction as its plane of vibration is at right angles to the slots in the analyser.

Thus we have seen in a very elementary way something of the mechanism of polarisation, and when used in conjunction with the microscope it extends the usefulness of the instrument to a considerable extent and may even prove to be the only means of achieving a desired point, but before going further into this question let us examine an example of its application.

Imagine the same arrangement as in Fig. 204, but in this case applied to the microscope. The polariser PL would be fitted in the substage, the specimen TR would be placed on the stage with the objective between it and the analyser, on the other side of which would be the eyepiece. This in the case of the microscope would be horizontal. Now let us suppose that TR is a tourmaline crystal and that the polariser and analyser are crossed ; on looking into the eyepiece with the tourmaline removed the field would be black or we would see nothing. If we now place the tourmaline crystal so that its long axis is parallel to the slots in the polariser, this is done by parallelising the polariser and analyser so that the polarised ray can pass right through the system, when the field of course becomes luminous and we are able to see the tourmaline crystal through the microscope, the eyepiece of which is fitted with cross hairs and has previously been aligned with the polariser and analyser so that complete extinction occurs when the cross hairs are vertical and horizontal when seen in the field. Thus if we rotate the analyser so that the system is parallelised we may align the tourmaline crystal in the field, so that its long axis lies parallel to and superimposed on the vertical cross hair, then the crystal may be said to be orientated with respect to the polariser ; if the system is crossed then we get the same condition shown in Fig. 204 and the crystal will appear self-luminous on a black background, albeit not very bright. Now, assuming the instrument to be fitted with a graduated

rotating stage, if we note the setting of the vernier and then rotate the stage until the crystal disappears and then read the angular vernier again, we have measured the angle through which the ray has been apparently twisted by the tourmaline.

This data is very important as by its use the refractive indices of bi-refringent substances may be calculated very accurately. Thus we have one example of the increased use of the microscope ; as a matter of fact there is hardly a single branch of science where its increased usefulness is not felt.

The uses of the polarising microscope are unlimited, as there is hardly any branch of science wherein it is not found to be of some use. For instance, in medical microscopy the use of polarised light is playing an increasingly important *rôle*. The changes undergone by tissues during the exercise of their normal functions can be studied to advantage, as also can pathological or post-mortem changes. Owing to the bi-refringent nature of most tissues, and indeed the basic protoplasm itself, the structure of these tissues including such as muscle and nerve fibre and the delicate enclosing membrane of the red blood corpuscle, may be studied and investigated. Likewise the presence of mineral matter in the tissues in the form of crystals may be detected and the substance identified.

The polarising microscope is also of great use in geological studies, particularly in the petroleum industry, where the mineral deposits of various locations may be readily identified, district by district, and thus help considerably in locating the best sites for sinking wells.

In the ceramic industry the polarising microscope plays an invaluable part, for the course of a particular process may be closely followed for the development of various materials in the products, as a result of which one is enabled to establish stricter control over processes. It is also of great use in the manufacture of glass, for with its aid minute flaws are rapidly detected and the trouble stopped at its source. It is also useful for the strain testing of glass.

In the textile industry the use of the polarising microscope is of very great importance ; the knowledge which has been gained as a result of its use in the microscopic study of various textile fibres is enormous. Both the natural and synthetic fibres exhibit unmistakable characteristics under polarised light, by means of which their identification is greatly facilitated. For example, their cross-sectional shape and particular surface markings, together with any internal inclusions, etc., are all clearly exhibited under the polarising microscope.

In a similar manner the paper industry makes use of the polarising microscope for the study of fibres and the identification of adulterants and contaminating substances. The behaviour of the various fibres

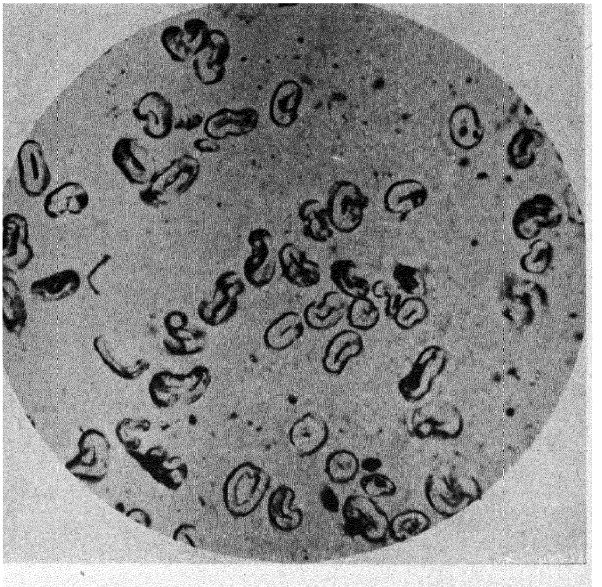


FIG. 205. × 105.

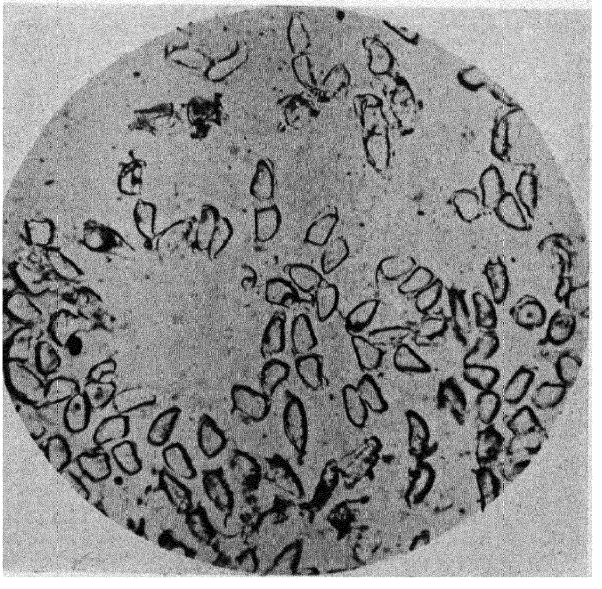


FIG. 206. × 105.

[*To face p.* 190.

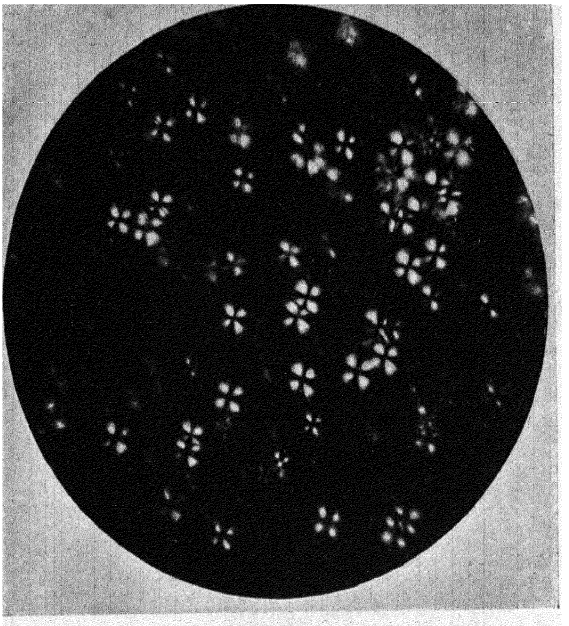


FIG. 207. × 420.

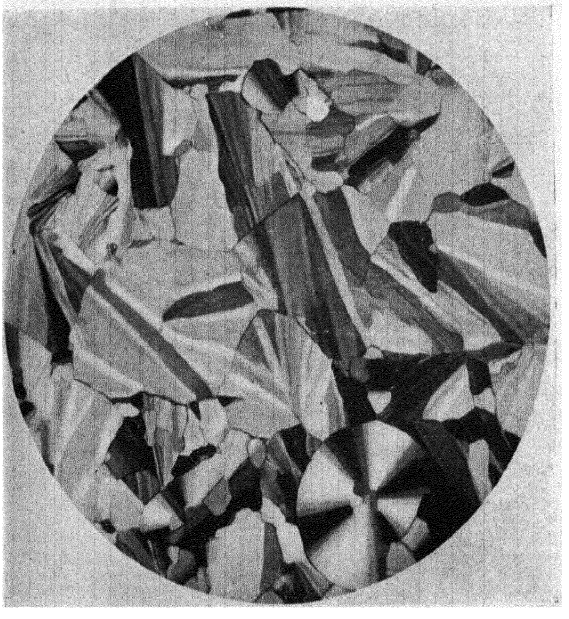


FIG. 208. × 105.

used under the manufacturing processes to which they are subjected is also easily studied.

Thus one may continue describing uses of the polarising microscope indefinitely ; there are the fields of botany, biology, industrial hygiene, tropical medicine, food and drugs, and toxicology, to mention but a few. The general trend to a wider and more scientific use of this instrument during recent years is very noticeable, and with the broadening of knowledge on the subject, the future outlook is very encouraging.

The polarising microscope was of great use to the author some time ago in connection with an investigation into varnished fabrics as used for electrical insulators. In one instance it was desired to show clearly the tubular nature of the cotton fibres and it was found that the preparations were so transparent as to necessitate the use of a very small aperture, which of course destroyed the resolution. However, when the polarising apparatus was fitted up the condenser was enabled to be used with a 75 per cent. cone thus giving a good photograph with high resolution. Two photographs taken at the same time with the system parallelised are shown in Figs. 205 and 206. Fig. 205 shows a cross section of cotton fibre in which the tubular nature of the fibre is very clearly shown, whereas Fig. 206 clearly demonstrates the solid nature of the natural silk fibre ; both photographs are at 150 diameters.

In addition to the foregoing may be mentioned its usefulness in the investigation of waxes and allied material in connection with which the author had occasion to use the polarising microscope in an investigation of the new plastic material " Polythene." Fig. 207 shows a photomicrograph of one of the grades of this material in the form of a thin film which has been shock-cooled under pressure. The photograph was taken with the system crossed, thus demonstrating clearly the presence of small crystals represented by the " Maltese cross " shaped light portions ; the magnification is 420 diameters.

In a similar manner the beauty of polarised light is very well shown in Fig. 208, this being a photomicrograph at 105 diameters of a chlorinated naphthalene, with a melting point of 134° C. in the form of a thin film which has been very slowly cooled.

We have now studied in a very elementary way the mechanism of polarised light and have touched briefly on the many and diverse uses of the polarising microscope ; therefore let us now examine briefly the construction of the polarising microscope.

The simplest polarising microscope is obtained by the addition of a polariser and analyser to an ordinary stand, the polariser fitted beneath the substage condenser and the analyser in a convenient position above the object.

Polarisation of light may be obtained in one of several different ways :—

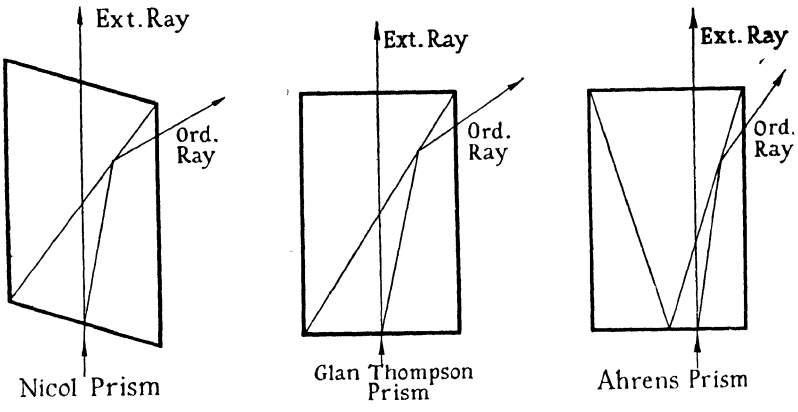
(1) By the use of a black glass reflector, for if light is reflected off a polished black surface at the polarising angle, the reflected light is plane polarised.

(2) By the use of tourmaline plates, or plates constructed of some other bi-refringent substance.

(3) By transmission through one of three specially shaped prisms made from natural calcite.

(4) By the use of the comparatively recently introduced patented sheet material known as " Polaroid."

Of the four methods the last two are more generally used, although the value of a black glass reflector is becoming more and more obvious for low-power work. Of the last two methods the prisms are generally taken to be more effective, although the author must confess that since first using " Polaroid " sheet some three or four years ago, he has become so enamoured of it that he now uses



(After Munoz and Charipper) FIG. 209.

nothing else. The photographs discussed elsewhere in this section were all taken with the aid of " Polaroid " sheet, and he is convinced that this material has a big future.

With regard to the prisms, there are three types shown in Fig. 209, viz., the Nicol, the Glan Thompson and the Ahrens, but before going into an explanation of their functions it would perhaps be as well to define bi-refringence. A bi-refringent substance is one which under certain conditions exhibits the property of possessing two refractive indices. Of the natural substances, calcite exhibits this property strongly and is the material usually used in the aforementioned prisms, each of which consists of two prisms in the case of the first two, and three in the case of the third, which are cemented together with Canada balsam.

A ray of light striking the prism is first of all split into two portions by the double refraction of the prism encountered; these two parts are known as the ordinary and extraordinary rays. On

encountering the cemented face, the ordinary ray is totally reflected out of the field of view as shown, while the extraordinary ray proceeds straight through the assembly and is plane polarised. The most popular type of these three is the Nicol, but some workers prefer the Ahrens prism because it gives a larger field of view. At the same time it should be pointed out that with the sheet material (" Polaroid ")

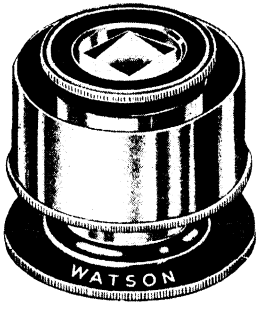


FIG. 210.



FIG. 211.

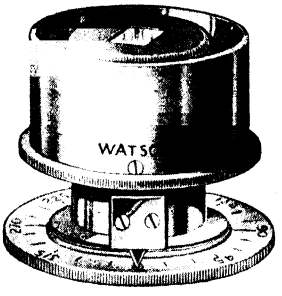


FIG. 212.

the full normal field of the microscope is available, whereas even the Ahrens prism does offer some restriction.

In order to adapt an ordinary stand for use with polarised light it is necessary to have a simple type of polariser and analyser as shown in Figs. 210 and 211. The prism in the former is mounted in a rotatable mount which fits the substage condenser ring and the

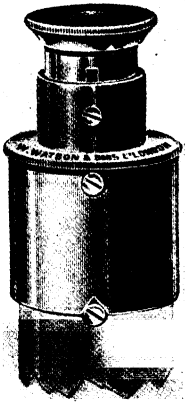


FIG. 213.

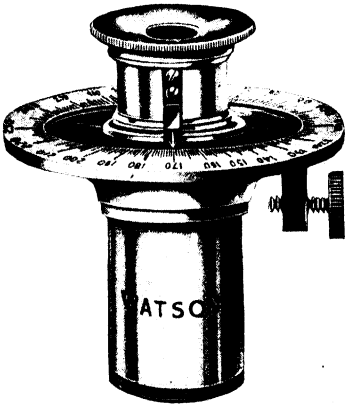


FIG. 214.

analyser is built to screw into the R.M.S. thread in the bottom of the draw tube. With these two accessories fitted to an ordinary stand, one is capable of utilising polarised light to a limited extent; however, this arrangement should not be scorned because of its simplicity, as it is surprising how much can be achieved with the barest minimum of apparatus.

In Figs. 212 and 213 we see rather more elaborate forms of

these accessories, the former being the polariser whose rotating movement is graduated and which is fitted with a large prism, to give as large a field of view as possible ; the latter illustration shows an eyepiece analyser. Now these are very convenient fittings when a quick change from polarised to ordinary light is desired, as one only has to slip the polariser into place in the substage ring and the analyser on to the eyepiece to convert the microscope from one to the other; but this type of analyser suffers from a big disadvantage inasmuch as the prism lies above the eye lens of the eyepiece and in consequence one cannot get the eye right to the Ramsden disc, as a result of which the field is greatly restricted.

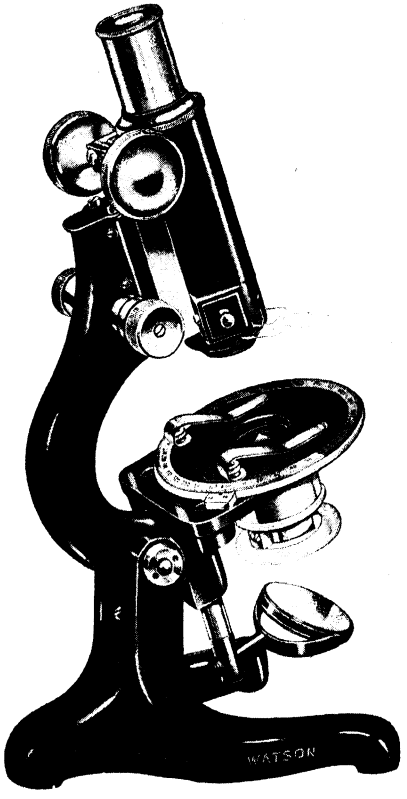


FIG. 215.

Another and more refined type of eyepiece analyser is shown in Fig. 214. Here the eyepiece (which is fitted with cross hairs) and analyser are built into one complete unit so that one would call it an analyser eyepiece rather than an eyepiece analyser. The prism is again mounted above the eyelens and its mount is free to rotate relative to the rest of the apparatus, which in turn carries a circular scale calibrated in degrees ; thus one is able to measure any angle through which the prism is turned. These accessories have been described in order to demonstrate the methods used for bringing about a quick conversion from one type of illumination to the other, but if serious work is to be undertaken, involving delicate measurements, it is by far the better policy to use a specially built polarising microscope.

It has already been stated that development of the science of petrology was responsible for the development of the polarising microscope. We have also seen that microscopical examination by means of polarised light is not only of use in the study of minerals, but has many important applications in the technology of rayon and other textiles, papers, chemistry, glass, abrasives, cements, sands and many other technical industries as well as in the biological

sciences. While the use of polarised light for the examination of such materials as textile fibres does not require all the special features found in advanced petrological microscopes, it is often found to be beneficial and indeed sometimes necessary to provide some of the special facilities with which the latter instruments are provided.

An example of the polarising microscope in its simplest form is shown in Fig. 215, which shows a simple stand consisting virtually of a Class 1 ordinary stand with the barest necessities for elementary petrological work built in, instead of functioning as accessories. For example, it is possessed of a built-in plain circular stage which is rotatable and which is also calibrated on its periphery in degrees which are read by an indicator on the stage bracket. The body tube is fitted with a slot, going diametrically across it at 45° to the field horizontal, to carry compensators above the objective; an analyser slides into the body above the compensator and may be made inoperative by withdrawing it from the optic axis. The polariser is carried in a tubular fitting below the stage from which it may be easily withdrawn.

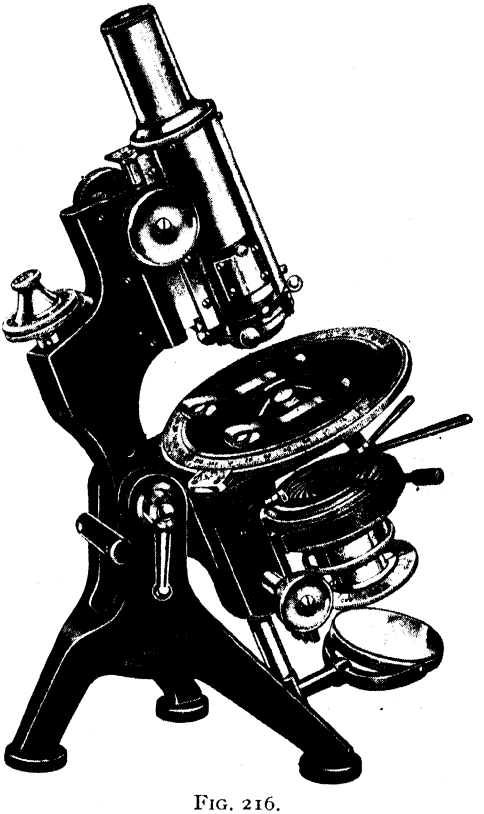


FIG. 216.

This class of instrument is suitable for students in technical colleges, industrial chemical laboratories and public analytical laboratories; it will also be found useful to beginners in the study of minerology, for the examination of crystals and the determination of adulterants in manufactured products.

A more complete stand suitable for the advanced worker is illustrated in Fig. 215, which shows Messrs. Watsons' "School of Mines" microscope. As can be seen, it is very completely equipped; apart from the usual movements it possesses a polariser with a large prism, fitted in the substage, the rotary motion of which is calibrated at every 15° and arrested by means of a spring check every 90°. It has a large circular stage fitted with spring clips (there is no

mechanical stage) the edge of which is calibrated in degrees and reading by means of a vernier to 15 minutes of arc. The body tube is fitted with a centring motion to the nosepiece; this is a most important fitting when critical work is undertaken, and a diagonal slot above it to take compensators, above which is the slide carrying the Bertrand lens, used in the examination of interference figures at high magnifications. The analyser is completely enclosed, possessing a square-ended prism having sliding movements in and out of the optic axis. The eyepiece is fitted with cross hairs and has a focussing eye lens; the substage is fitted with the special form of condenser used for examinations with polarised light. Its elements are capable of being immediately swung out of the optic axis by means of the levers shown, either singly or simultaneously.

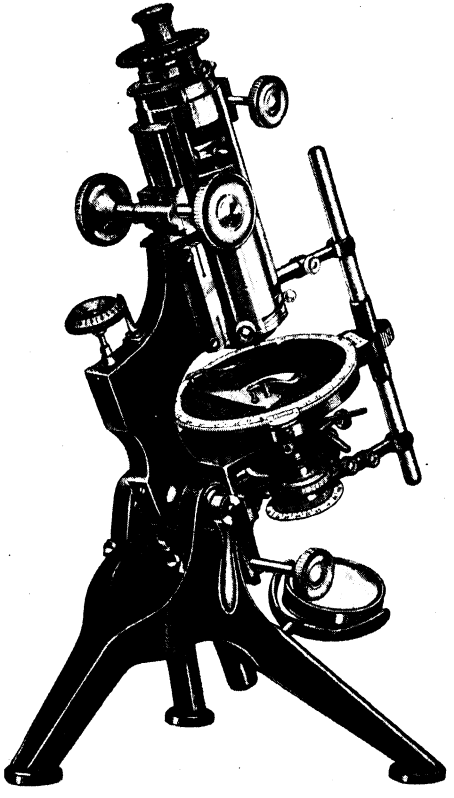


FIG. 217.

On referring to Fig. 217, we see illustrated an advanced petrological microscope. This is an instrument representative of the highest class and is suitable for the most exacting demand of accurate research work and photomicrography; its size and rigidity enable it to carry, without whip, every kind of special accessory required for modern methods of petrological examination. The body tube is of large diameter and carries a centring nosepiece and slots for compensators; it also has an analyser fitted in a rotating mount which may be coupled at will with the rotation of the polariser, thus enabling either simultaneous or independent rotation of these two components. The Bertrand lens in the body is focussed by a rack and pinion movement to the draw tube, the eyepiece is fitted with cross hairs and has a calibrated scale and a large calibrated circular stage carries a sliding bar; the substage is of standard dimensions capable of receiving illuminators of all kinds and is fitted with centring screws The polariser is a large nicol prism whose rotation may be made independent of, or simultaneous with, the analyser

by means of the fitting illustrated ; its rotation is calibrated every 15° and checked every 90°.

We have now briefly studied polarised light and its effects and also seen how it may be used to advantage on numerous occasions. We have also touched briefly on the construction of specially built polarising microscopes and enumerated their various additional parts, several of which have been mentioned by name but not described, and perhaps a short description of the functioning of these accessories will not be amiss.

These descriptions will only be brief as it is not intended to make this section a treatise on the use of polarised light but rather as an introduction to the subject, which is very completely dealt with in works devoted to it, to which the earnest student is recommended to refer.

For the observation of interference figures, a condenser is used with the polarising microscope, and the figures may be observed directly by removing the eyepiece and looking at the back of the objective (this is sometimes known as conoscopic observation). If, however, a low-power lens is inserted beneath the eyepiece in the optic axis, such as by screwing it into the bottom of the draw tube, this latter is converted into a low-power secondary microscope by means of which the back lens of the objective, and consequently the interference figures may be observed. A lens such as this is known as a Bertrand lens, and it should be remembered that it is only effective with high-power objectives from $\frac{1}{6}$ in. onwards. These lenses are sometimes fitted with a diaphragm when used for work on small crystals.

When it is desired to carry out ordinary routine work with the polarising microscope which is not very critical, the normal objectives may be used ; but if the work is at all critical it is essential that these objectives be constructed in such a manner that the lenses are entirely free from strain, as this will upset critical investigation and lead to false results. Therefore it behoves the user to make certain that his objectives are strain-free before embarking on work of this nature, as it is the bi-refringence of the glass or flourite used in objectives which sometimes gives rise to polarising properties in them, which effect, if present, is easily observed by using a strong illuminant and crossing the system, whereupon a dark and light pattern will be observed on viewing the back lens of the objective while at the same time rotating it in its mount. The appearance of this pattern will obviously preclude the use of this objective when it comes to critical work with polarised light, although it may be excellent with ordinary illumination.

Another phenomenon which has to be taken into account when pursuing critical investigations is that brought about by the insertion of lenses between the polariser and analyser ; this, of course, includes

the objective, taking the form of an incomplete blackout when the system is crossed. This phenomenon is mainly due to the light being only partially polarised owing to the presence of refractive media in its path from polariser and analyser, in consequence of which a faint interference is seen with the system crossed and no object on the stage. This fault occurs to a greater extent with high-powered objectives whose curved surfaces are numerous and in which the curvature is high. It should be pointed out that it is impossible to eliminate this defect in objectives and condensers possessing a high numerical aperture.

Compensators are used where it is desired to study the interference effects produced by slow and fast components of a bi-refringent substance interfering with one another. In order to understand this a little more fully, we will of necessity have to modify our earlier elementary conception of the polarised ray after it has passed through the polariser and a bi-refringent object. If we consider the object to be slotted in the same way as the polariser and analyser, then, when its slots are parallel to those in the polariser, the polarised ray will obviously proceed straight through it unhindered and will be stopped by the analyser. Assuming the system to be crossed, in this position the field will be dark and the object invisible ; if, on the other hand, the object is rotated say through 45°, then the polarised ray on leaving it will be resolved into two planes of vibration at right angles to one another, thus resembling the original ray in our analogy.

This is the peculiar property by all anistropic and bi-refringent materials, and is due to the possession of two refractive indices. This orientation of light occurs whether the entering light is polarised or not. These mutually perpendicular directions corresponding to the planes of vibration of the substances are known as vibration axes, therefore we may look upon them as having two sets of slots at right angles to one another ; thus we can now visualise the ray emerging from the object as possessing two mutually perpendicular planes of vibration, when the object is at an angle to the plane of vibration of the polariser. Under these conditions the object will be visible in the field of the microscope, because when these components pass through the analyser they are reduced to one plane of vibration. So that in summing up this phenomenon we have the following sequence of events. Ordinary light enters the polariser where it is resolved into light having one plane of vibration ; this polarised ray next enters the bi-refringent object where the single plane is resolved into two component planes mutually perpendicular to each other, each component of which is then re-orientated so that the planes of vibration of both components are in the same direction.

The two component planes of vibration which emerge from an anistropic substance in this way possesses different velocities, due

to the different values of the two refractive indices, and the slower wave or component is said to suffer retardation to the faster. It will be clear that under these conditions, even if the retardation is such that the two component waves completely oppose one another or are exactly anti-phased, then between the object and the analyser, destructive interference is not possible because the planes of vibration are at right angles to one another, but after passing through the analyser the planes are resolved to a common direction of vibration as a result of which interference or reinforcement are possible, depending on whether the component waves are in or out of phase.

The destructive interference is complete when certain thicknesses of the bi-refringent substances are used and the illumination is by monochromatic light, in which case the slow and fast component will be exactly anti-phased. Thus where the thickness corresponds to this condition the object will appear dark, for different thicknesses the antiphasing will diminish to a minimum condition where the components are exactly in phase, this producing a maximum brightness. Thus we have two limiting conditions, that of exact anti-phase and that where the component waves are exactly in phase; these produce either complete darkness or maximum brightness for certain definite minimum thicknesses of material. It will be clear that certain multiples of these minimum thicknesses will produce these same results depending on the wavelength of the light used. Thus we see that with monochromatic light we get variations from blackness to a maximum brightness, according to the thickness of the object.

If instead of monochromatic light we use white light, then we get different colours for variations in thickness of the specimen. This is due to the fact that of the two component waves, certain frequencies will obliterate one another, thus leaving the remainder of the component minus those colours; these remaining frequencies or wavelengths go to constitute the polarisation colour of the material at that thickness.

Certain colours are due to reinforcement—that is when the two components are in phase—and others due to subtraction when the components are anti-phased. These have been carefully charted and are of the utmost value in investigations by polarised light; they may be used for the measurement of retardation and indirectly to measure thickness, also as the retardation is directly proportional to the difference in the refractive indices of the material, they may be used to measure these latter. We see, therefore, that the measurement of retardation is important. This is best obtained by the use of compensators, which are generally made in the form of a wedge in such a way that the thickness and consequently the retardation varies uniformly to produce polarisation colours of the first three or four orders. The wedge usually occupies a position above the

objective so that it may be inserted into the optic axis at an angle of 45° to the field perpendicular ; thus the wedge is superimposed on the image of any object on the stage and if this latter is orientated so that its slow direction of vibration is at right angles to that of the compensation, which is usually in a direction across the width of the wedge, then the wedge may be used as a uniformly varying standard and a point found on it where its retardation compensates exactly for that of the specimen. Thus we see the useful nature of this accessory, its beauty lying in its simplicity of use and the accuracy with which measurements appertaining to polarised light may be made.

CHAPTER X

MICROMETRY

THE term " Micrometry " signifies the science of measurement with the microscope. The wide field of usefulness covered by it needs no emphasis, for it is not only of use in the determination of dimensions of microscopic objects, but it may also be called in, in the identification and even the analysis of substances, as most objects possess more or less characteristic dimensions.

Micrometry is of great usefulness in the examination of such substances as paper and textile fibres, starches, abrasives, fibres, ceramics, dust, pigments, photographic emulsions, micro-organisms and many other subjects in the examination of which measurements may constitute a valuable aid to identification, so let us delve into the ways and means by which we may use the microscope to these ends.

The first consideration is obviously the question of units of measurement. Clearly if we took the unit of measurement to be the millimetre, for example, then microscopic measurements would involve the use of cumbersome decimal fractions, and in order to simplify matters as much as possible, the unit of micrometric measurement is taken as the thousandth part of a millimetre (0·001 mm.) and is called " the micron." The abbreviation of this is the Greek letter μ (pronounced mu) ; a unit known as the millimicron and representing the thousandth part of a micron is also sometimes encountered, and is designated by the symbol $m\mu$; this latter unit is more frequently employed as a measurement of the wavelength of light or very short electro-magnetic waves of similar nature. Thus we have as our unit of measurement, the micron, being 0·001 mm.

Now let us consider the question of accuracy of measurement. It will immediately be apparent that there must be some definite unit of accuracy to which micrometric measurement may be carried out. This is governed by several factors, viz., the quality of the engraving of the micrometer, the sharpness of the image, particularly so at high magnifications. This point is very well dealt with by Chamot and Mason (1) in the following extract from their " Handbook of Chemical Microscopy," Vol. I. ; they say :—

" The coarseness of the image of the markings on the micrometer scale is governed by their actual fineness and by the extent to which they are magnified. The outlines and details of the object vary in apparent width, depending on the focus, the illumination, the refractive indices of the preparation, and most of all the resolving power of the microscope. Only under the best conditions of illumina-

tion with a minimum of diffraction patterns and with delicate well-defined structural details is it possible to achieve the utmost accuracy of microscopic measurement. The limit is ultimately dependent upon the breadth of the diffraction power of the optical system. Hence the true position of any boundary which is to be measured is uncertain, to this extent. If an objective of high resolving power is used, the absolute error may be very small; for example, circa $\pm$ 0·2 μ for an objective of 1·40 N.A. This may be negligible in measuring the width of a textile fibre which is 25μ in diameter, but it becomes much more important when pigment particles less than 1μ in diameter are measured."

Thus again we see that it behoves us to secure the best possible image if we are to undertake micrometric measurements of an accurate nature, at the same time bearing in mind that an average of a large number of measurements will help considerably in increasing the accuracy of the final results; also, a further increase in accuracy may be gained by taking measurements between centres rather than between the edges of the structures under consideration.

We have seen in earlier sections that the microscopic image appears at the visual distance of the eye and we know that this distance is conventionally taken at 250 mm.; therefore, if an object appears in the field to be 1 cm. in diameter, and the magnification is known to be 250 diameters, then, clearly, the actual size of the object is $\frac{1}{250}$ cm., or 0·025 mm., always bearing in mind that the apparent increase in size of the object is measured in linear units of diameter. This method of estimating the size of an object as it appears in the field, is micrometry in its simplest form, and as such is very inaccurate and not to be encouraged.

The more accurate and scientific methods, however, demand certain accessories whose importance is such that without them it is virtually impossible to carry out micrometric measurements of any accuracy or usefulness. The general method of obtaining accurate measurements consists of comparing the object to be measured against a standard whose dimensions are known. This standard usually consists of a slide whose operative surface bears accurately engraved rulings divided into intervals of $\frac{1}{10}$ and $\frac{1}{100}$ mm.; or if measurements are to be made with reference to inches, the engraving is in $\frac{1}{100}$ and $\frac{1}{1000}$ in. In the former case the ruling usually occupies 1 mm. ruled into tenths, one of which is further subdivided into ten; in the case of the inch micrometer the ruling usually occupies $\frac{1}{10}$ in. divided in ten with one of these divisions further subdivided into ten equal divisions. The rulings are more usually protected by a cover glass which is sealed down. The slide thus formed is known as a stage micrometer, and as its name implies, is used on the stage of the microscope, the rulings being the object of which the dimensions are known. The appearance

of a stage-micrometer ruling in the field of a microscope is shown in Fig. 218.

It will be clear that something more than the stage micrometer is required to help us in making accurate micrometric measurements, because after focussing the ruling it has to be removed so that the object to be measured may take its place in the field; thus we no longer have our known object with which we can compare the unknown. This difficulty is quite easily overcome by using an eyepiece micrometer, which little accessory, as implied by its name, is fitted to the eyepiece. The simplest form of eyepiece micrometer consists of a disc of glass one face of which is engraved with an arbitrary scale, usually divided into fifty or 100 equally spaced divisions; every tenth division carries an appropriate number. The appearance of a micrometer scale in the field of a microscope is shown in Fig. 219. This particular scale has fifty divisions and is

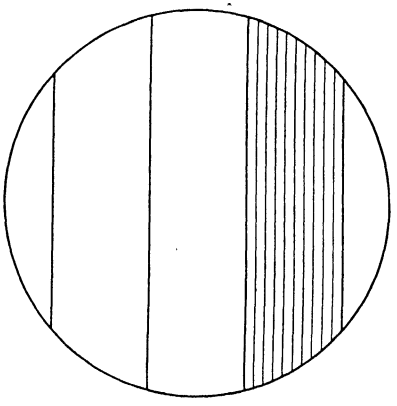


FIG. 218.

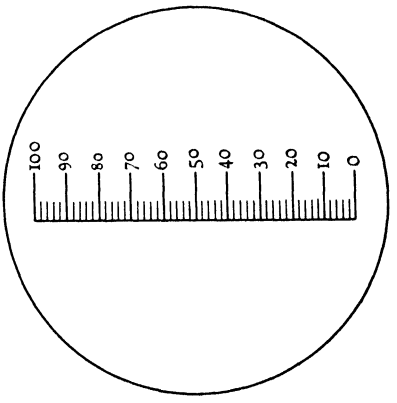


FIG. 219.

calibrated from 0 to 100, thus each division of the graduation represents two units of the scale.

The simplest method of using this eyepiece is to drop it on to the diaphragm of the eyepiece after having carefully unscrewed the eye lens in such a manner that it lies on the diaphragm face downward, then on screwing the eye lens back into place and inserting the eyepiece into the body tube of the microscope and looking into the instrument with no object in the field, the scale should be perfectly in focus and appear in the blank field as shown. If the scale appears slightly out of focus and the figures are reversed from left to right, then the micrometer disc is lying face upward, in which case it should be removed and gradually replaced correctly. If, on the other hand, the figures are not reversed and yet the scale is out of focus, then this signifies that the diaphragm is not in the correct position for the observer's eye. This fault constitutes one of the objections to using the scale in this manner, as in the majority of

eyepieces the diaphragm is immovable, being fixed at the focal point of the eye lens. This condition cannot be altered, the slight discrepancy due to variations in individual eyes is normally of no account, but when the eyepiece is required to be used with a micrometer scale it then becomes useless. This difficulty is overcome by the use of a micrometer eyepiece, such as that shown in Fig. 38, where the scale lies in the plane of the diaphragm, both being fixed, but the eye lens is mounted on a separate tube which slides in the main tube of the eyepiece, thus forming a focussing mount by means of which it is a simple matter to focus the scale correctly to suit any individual eye. These eyepieces are sometimes made with the scale built in, but the author recommends the type which has a loose micrometer disc which can be removed and replaced with one of another type; in this way one eyepiece performs a number of

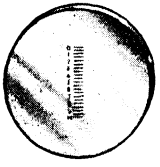


FIG. 220.

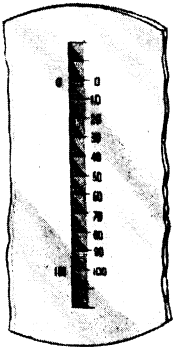


FIG. 221.

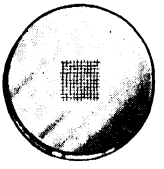


FIG. 222.

functions, doing away with the necessity of having a number of eyepieces.

The micrometer eyepiece just described is also capable of being used with a binocular instrument, in which case one must have two eyepieces one of which carries the scale. In using them care must be taken to see that the blank eyepiece has its eye lens pulled out to the same extent as that necessary to focus the scale in the eyepiece carrying it; failure to do this will cause the eyepieces to be mismatched, in consequence of which eyestrain will result. Three types of eyepiece micrometer scales are shown in Figs. 220 to 222; that shown in Fig. 221 is a very clear scale to use and should be used in preference to the one shown in Fig. 220.

Now let us examine the method of using the stage micrometer and eyepiece scales; in order that we may make use of the known dimensions of the stage micrometer rulings we have to use it to calibrate the eyepiece. This is accomplished by focussing the rulings with the objective which we intend to use, but previous to this we have examined the object intended to be measured under the

most critical conditions of lighting and tube length adjustment, after which all preset adjustments are left alone, particular attention being paid to the tube length, which must not be altered. The object slide is next removed and replaced with the stage micrometer, and the eyepiece scale is then placed in position and focussed sharply. The stage micrometer rulings are then focussed, this results in the image of the rulings being superimposed on to that of the eyepiece scale, as shown in Fig. 223. Now, by comparing these two scales and interpreting one in terms of the other, we are able to calibrate the eyepiece scale. Thus it will be seen from Fig. 223 that ten divisions of the eyepiece scale correspond to three hundredths of a millimetre as represented by the stage micrometer ruling (assuming the stage micrometer to be ruled in tenths and hundredths of a millimetre), thus ten eyepiece scale divisions equal thirty microns, from which we are enabled to develop a factor of the eyepiece scale, for the particular objective in use working at the tube length already set ; thus we may say that :—

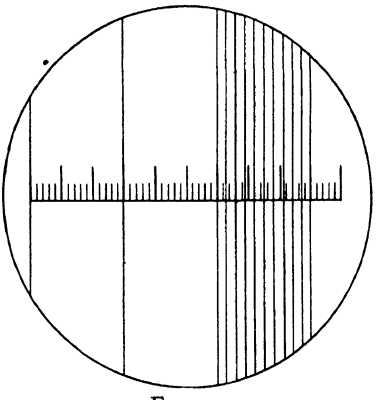


FIG. 223.

$$10 \text{ divisions of the eyepiece scale} = 30\ \mu = x$$

$$\therefore \text{ Factor} = \frac{x}{10} = \frac{30}{10} = 3 \text{ (for this particular instance).}$$

Therefore it will be clear that to express the eyepiece scale in terms of microns all we have to do is to multiply any readings taken with it by 3. Suppose, for example, we have found an object to occupy 47·5 divisions of the eyepiece scale, then the actual measurement of this object is obtained by multiplying this figure by 3, thus :—

$$47{\cdot}5 \times 3 = 141{\cdot}5,$$

so that the object measures 141·5 μ.

Some workers recommend using the draw tube to align the stage micrometer ruling with the eyepiece scale, but this practice is to be deprecated as being inefficient and leading to inaccurate results. As we have seen in an earlier part of this section that, unless the image is critical, the accuracy of measurements cannot be relied upon, so that by using the draw tube to align the two scales we destroy the critical nature of the image and impair the results, as it is seldom that, at the critical tube length, the scales will be in alignment and the little extra time and trouble taken in working out the calibration factor is amply justified. Thus it behoves us

to take this extra trouble in making measurements, for it is the observance of little points like this in all aspects of the science of microscopy which hallmark the true microscopist and produce results which can be relied upon.

It will be obvious from the foregoing remarks that each objective will have its own calibration factor for any particular value of tube length; this means that every time the tube length is altered, possibly with a change of object, the eyepiece scale will have to be re-calibrated.

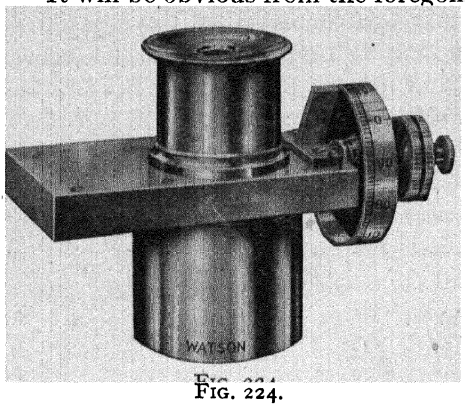


FIG. 224.

It will be appreciated that although the foregoing method of making measurements is quite useful and very accurate, if carried out properly, there will inevitably occur instances when greater accuracy still is required. It was this necessity for extreme accuracy, apart from the natural tendency to greater perfection, which resulted in the screw micrometer eyepiece, two types of which are shown in Figs. 224 and 225. In principle this eyepiece is very similar to the previous type, but somewhat different in operation. As can be seen, they are a self-contained unit and replace the ordinary occular in the microscope, hence they cannot be used with a binocular instrument. In construction they contain an arbitrary scale, just as in the simple eyepiece micrometer, but in this case the scale is movable, this movement being carried out by the knurled head projecting from the side of the instrument, the movement of which is calibrated in a complete revolution. As a rule, each division of the internal scale is exactly equal to the lateral movement caused by one revolution of the external drum, that is to say, one complete revolution of the drum moves the internal scale in a lateral direction across the field for a distance corresponding to one division of the latter. As the drum is usually calibrated into one hundred divisions, it will be clear that we are

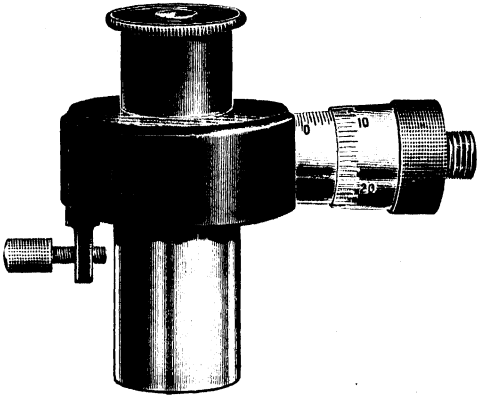


FIG. 225.

enabled to read to the one-hundredth part of one of the divisions of the internal scale.

Thus, if having calibrated the scale and found the factor for the objective in use (in exactly the same way as previously mentioned), we wish to measure an object with the screw micrometer eyepiece, the procedure to follow is to place the object so that one of the edges to be used in the measurement coincides with a graduation representing any whole number on the eyepiece scale, it will in all probability be found that the other edge to be measured lies somewhere between two divisions of the scale, indicating a fractional measurement. This fraction we can now measure by using the external drum, rotating it and thus moving the internal scale until one or other of the divisions, between which the edge lies, is made to coincide with it, the fraction is then obtained to an accuracy of one hundredth of a division by reading off the amount of movement required by the drum and indulging in a little subtraction.

This type of micrometer is very useful for exact measurement and should be included in the equipment of all those intending to use the microscope in a scientific manner.

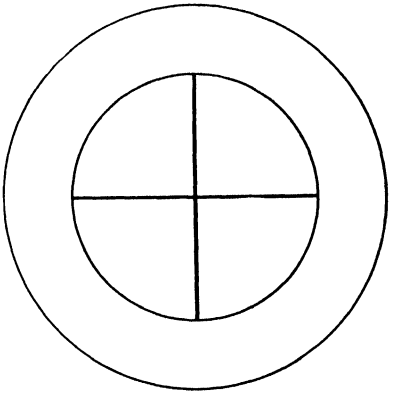


FIG. 226.

Apart from the simple discs previously mentioned, there are many other rulings designed for special purposes which increase the utility of the micrometer eyepiece; there is the "Howard" disc, used for mould counting in the examination of foodstuffs. This disc is ruled in squares, the area of each square being equal to a known fraction of that of the field of view; they are used in conjunction with the "Howard" counting chamber, a specially constructed slide which receives the substance under examination.

A different type of squared ruling is met with in the "Whipple disc," which is used for counting bacteria and dust particles and possessing a large square divided into four smaller squares, each of which is further divided into twenty-five smaller squares, one of these latter is again divided into twenty-five squares.

Another type of disc, having a circle divided into quadrants by cross lines, is known as the "milk smear" disc (Fig. 226) and, as its name implies, is used in the examination of milk smears for the purpose of counting bacteria.

The ordinary "net" ruling, of course, is often met with and is of great usefulness when carrying out analyses by area measurement.

This disc usually consists of one large square divided into 100 smaller ones, of which those comprising two adjacent sides of the large square are numbered; this disc is very useful for general use, particularly when it is required for counting the coarser materials, such as abrasives. This ruling is shown in Fig. 227.

It will be appreciated that the foregoing methods are only applicable to fairly high magnifications; when it is desired to carry out measurements of comparatively large objects, this is best accomplished by the use of the vernier on the mechanical stages used in conjunction with a simple eyepiece disc ruled with cross lines, this latter being necessary as a datum from which to work.

In carrying out measurements under the microscope by any of the foregoing methods, the magnification under which they are made should not be chosen at random for, as we have seen, in order to obtain the maximum accuracy it is necessary to have as perfect an image as possible, in fact a critical image, so that it is entirely unnecessary to magnify the object beyond that figure which will make its measurement possible to the tolerances decided upon, for by so doing we would inevitably sacrifice a certain amount of accuracy. This correct magnification is simply determined if we draw on our knowledge of the mechanism by which magnification takes place. Obviously in magnifying an object we must enlarge it to such an extent that we may, metaphorically speaking, measure it in comfort with an ordinary rule. This immediately gives us a pointer in the right direction, for we know that it is quite possible to measure 1 mm. with the naked eye, therefore it will be apparent that an object whose length is, say 1 micron, must be magnified at least 1,000 diameters in order that we may measure it; this, of course, would make its apparent size to be 1 mm. and therefore capable of accurate measurement. If we increased the magnification to 2,000 diameters, then it would be much easier to measure, its apparent size now being 2 mm., but as the image would deteriorate somewhat we could not measure it to the same accuracy as at 1,000 diameters. If, on the other hand, we only magnified it to 500 diameters the measurement would be very difficult if not impossible, as the apparent size is now only $\frac{1}{2}$ mm., therefore it will be seen that there is an optimum value for the magnification of an object intended for measurement. The following table gives an idea of these values :—

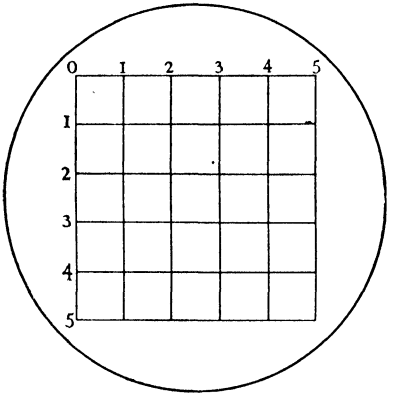


FIG. 227.

Actual size of object.	Optimum magnification.
25 to 30 microns . .	100 diameters.
2·5 to 5 microns . .	400 to 500 diameters.
1 to 2 microns . .	600 to 1,000 diameters.

It is not suggested that these figures are the absolute values, but are given as a guide, as the variability of the human eye must be taken into account when deciding which figure to use.

Other methods of measuring direct are assuming an ever-increasing importance in industrial applications, where the increase in accuracy offered by the use of optical means is becoming more and more apparent, and indeed for some purposes essential, the apparatus employed usually consists of a specially built instrument designed to perform the desired function. They range from the simple measuring magnifier shown in Fig. 228 illustrating a magnifier mounted in an inclined mount and having a built-in scale, the whole instrument being pre-calibrated. Its main use is in the measuring of ball impressions made with a Brinell hardness-testing machine, but a little thought will bring to light many other applications of this useful little instrument in the



FIG. 228.

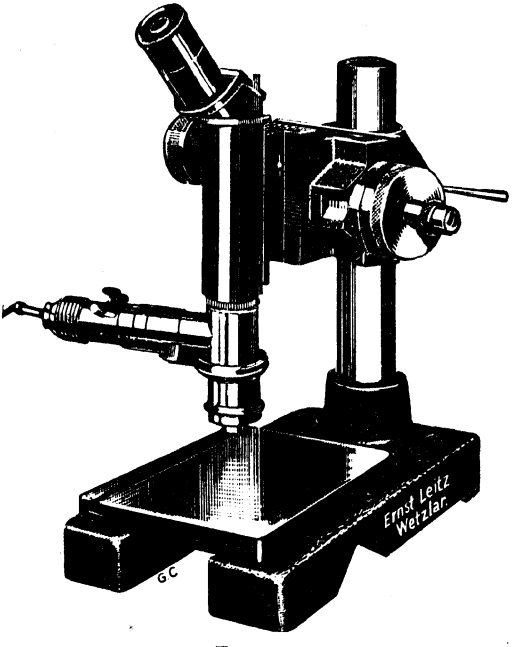


FIG. 229.

workshop. The magnification is eight diameters and therefore limits its use to comparatively large objects.

A more accurate instrument for measuring ball depressions and small machined parts, etc., is shown in Fig. 229, consisting of a solid base carrying a heavy pillar on which is mounted the main casting carrying the body of the microscope. This casting is capable of vertical adjustment by means of a clamp, the microscope itself is focussed by rack and pinion in the usual way and is provided with an incident light vertical illuminator. The whole assembly of the microscope is capable of horizontal movement by means of the calibrated micrometer shown, which is capable of providing a horizontal displacement of 25 mm., the accuracy of the calibration being to $\frac{1}{100}$ mm. by direct reading and $\frac{1}{1000}$ mm. by means of a

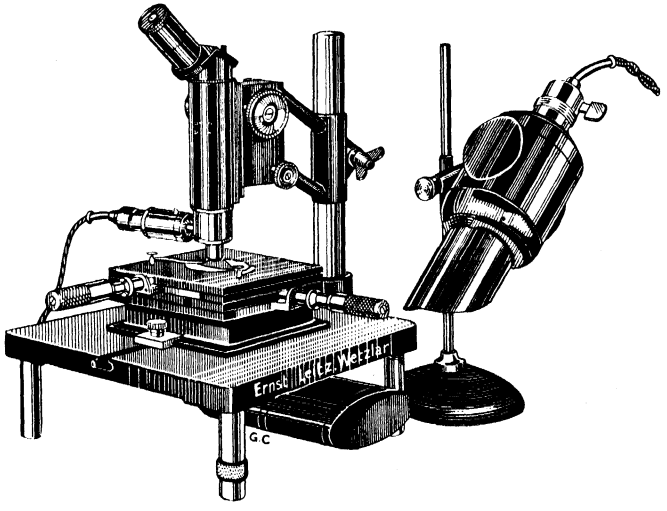


FIG. 230.

vernier. The overall magnification of the instrument is forty diameters; it is unnecessary to point out the directions in which an instrument such as this may be made use of.

Another application of the same principle is shown in Fig. 230, which illustrates a microscope designed for the checking of small bores. In this case the instrument is supplied with both incident and transmitted light systems, the measuring being accomplished by means of calibrated movement to a well-made mechanical stage reading to $\frac{1}{100}$ mm. and affording a 25-mm. traverse; the magnifications of this instrument are 50 and 160 diameters.

The instrument shown in Fig. 231 is a most useful piece of apparatus, being capable of measuring comparatively large parts with extreme accuracy. It consists of a heavy base on which is a slide track, along which two microscopes are capable of being moved in a horizontal direction; the track is engraved with a

millimetre or inch scale, the two microscopes have the usual rack and pinion focussing mechanism together with rack and pinion fine adjustments; each has its individual illuminating apparatus, this latter being a mirror mounted on a ball-jointed arm. The measuring range of the instrument for length is 2 to 16 in., or 50 to 400 mm., and is capable of carrying a specimen with a maximum permissible thickness of 9·15 in., or 240 mm. The magnification of the microscopes is 100 diameters, one of which carries a cross line eyepiece disc, the other carrying a screw eyepiece micrometer by means of which measurement may be made to an accuracy of $\frac{1}{10,000}$ in., or $\frac{1}{2000}$ mm. In using the instrument the specimen is placed on the base plate, which is a large mechanical stage having a lateral movement and moved laterally until one of its extremities coincides with the cross lines on the eyepiece disc of the right-hand microscope,

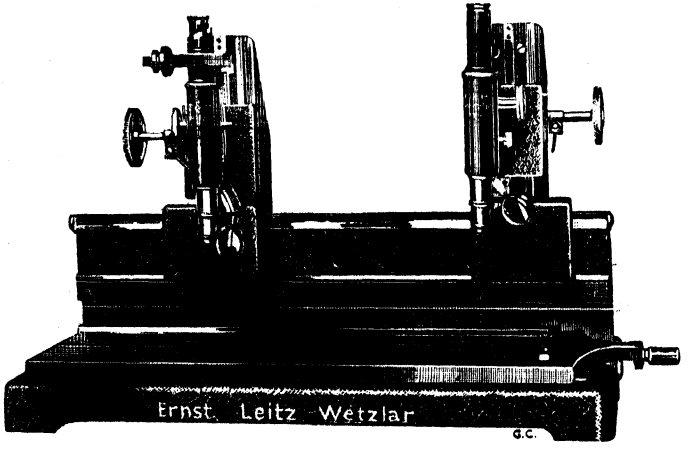


FIG. 231.

the other microscope is then moved along the slide to the other extremity of the specimen and the length read off on the slide scale, any fractional discrepancies are then measured with the screw micrometer eyepiece. As can be seen, an instrument such as this would prove of inestimable benefit in such locations as a tool room and, in fact, anywhere where extreme accuracy is required of fairly large parts, and tends to show the trend of modern precision manufacture in the adoption of optical aids for procuring the necessary accuracy in fabrication.

For many years now much time and thought has been expended on the production of accurate screw threads, especially in the finer sizes, and various optical methods of checking machined threads have been devised. One of the most efficient little instruments is illustrated in Fig. 232; this consists of a low-power microscope, magnification 30 diameters, mounted on a solid stand possessing

levelling facilities and carrying underneath an illuminating device capable of being used with any existing light source. On the stand is fitted a revolving stage whose movement is calibrated into 360°, the stage itself has dual horizontal movements at right angles to one another, these being operated by micrometrically calibrated screws, and also has two adjustable centres between which the specimen is placed. The microscope, focussed by rack and pinion, is mounted on a tilting mount, thus enabling it to be inclined to the angle of the helix if so desired, the whole assembly being capable of vertical displacement along the pillar mounted on the stand. The eyepiece is of novel design, having incorporated into it a revolving

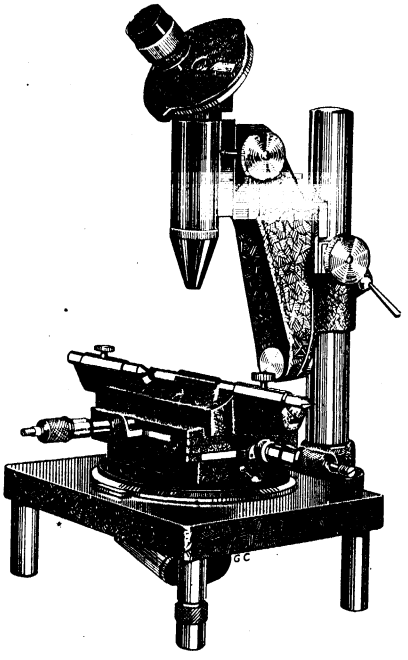


FIG. 232.

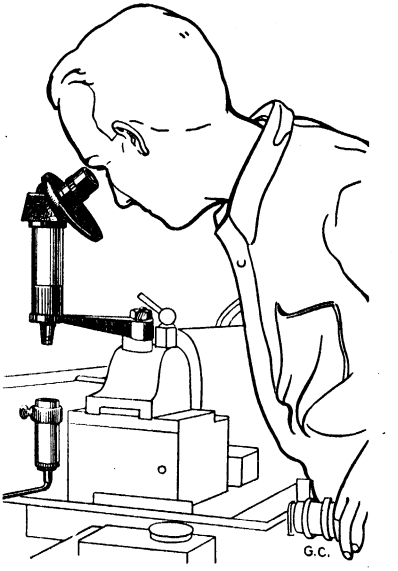


FIG. 233.

template carrying silhouettes of a number of different threads, metric or English, as required, against which the specimen thread is checked, by which means a number of different threads may be rapidly and conveniently checked. Altogether this is a most useful instrument for rapid checking and controlling manufacturing processes involving the production of screw threads, wherein the use of a modified form of this instrument, shown in Fig. 233, where it is part of the machine tool enabling the thread to be checked as it is being cut, should also prove of inestimable benefit.

So much then for methods of measuring by direct observation of the specimen ; there are other methods involving what might be termed indirect observation by which is meant one or other of the

methods of projecting the image of the object on to a screen. The simplest method of accomplishing this is by the use of the super-ocular prism which merely consists of a 45° prism clamped on to the ocular of the microscope, thus turning the optic axis of the instrument through 90°, so that with the microscope in a vertical position the image may be projected on a vertical screen placed at any convenient distance. If to commence with the image of the ruling of a stage micrometer is projected and the intervals marked on the screen, then by the simple expedient of applying a rule to the markings, the magnification may be obtained or the dimensions of an object measured by comparison. This method, which is the basic principle upon which the operation of all projection measuring apparatus depends, of course demands a fair amount of space and

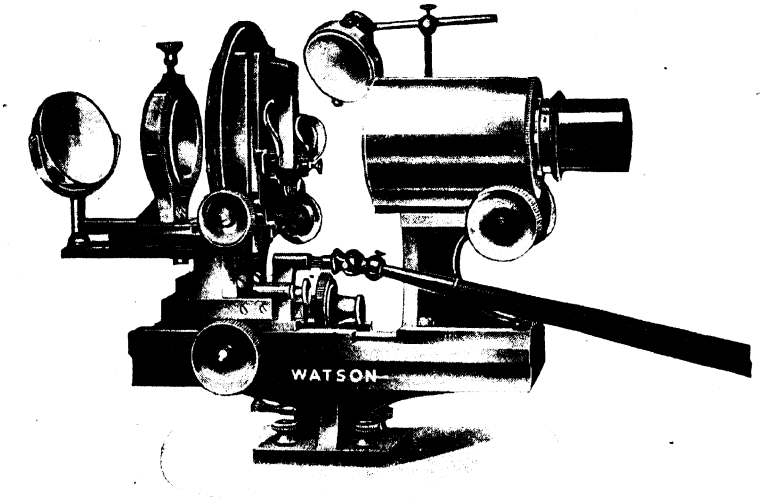


FIG. 234.

either darkness or semi-darkness to be effective. It also requires a powerful light source.

One method of cutting down the amount of space required and the intensity of the light source is to place the microscope in the horizontal position, in which case the screen will be horizontal and may consist of a sheet of paper on the table. The same procedure for carrying out measurements may then be adopted; this is also a very useful method to adopt when making drawings under the microscope.

An instrument specially designed for projection purposes is shown in Fig. 234, from which it will be seen to consist of a microscope functioning in the horizontal position; it is of very rigid construction, both the body and stage being supported on massive pillars so as to give the maximum stability. Focussing is effected by the usual methods, in addition to which a remote control is

fitted to the fine adjustment in instances where it is required to be operated from a distance, as in photomicrography. The construction of the fine adjustment is rather unorthodox inasmuch as it moves the stage instead of the body tube, apart from this the design of the other movements follows conventional practice.

As a result of the use of the foregoing methods of measurement, it was quickly realised that a self-contained compact piece of apparatus giving the same results would be much simpler to operate than is the case when several pieces of apparatus have to be aligned,

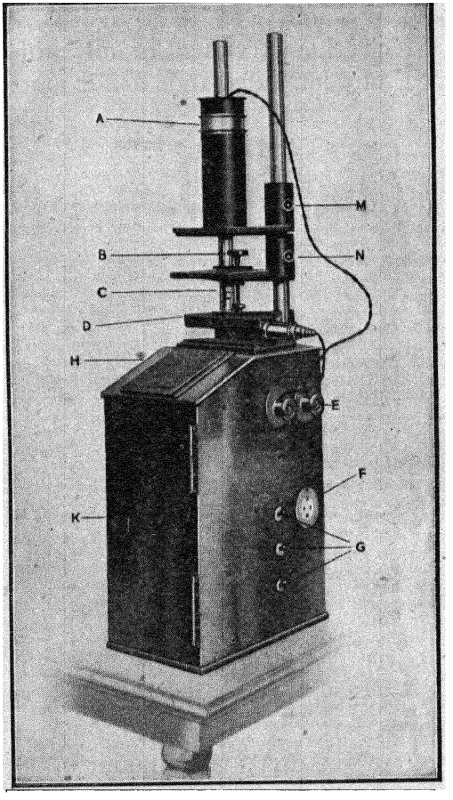


FIG. 235.

requiring much more floor space and remote controls, etc.; hence a number of manufacturers produced the self-contained projection micrograph mainly as a result of the demands made by industry for some method of accurate comparison of profiles. As a rule these were screw threads, and this type of instrument has been in constant use in industrial organisations for a number of years. One such instrument by Watson is illustrated in Fig. 235; it is known as the "Ramsden" micrograph, and may be used for the examination of structures by projection, for the measurement of structure, crystals, etc., for profile comparison with suitable templates, and for producing

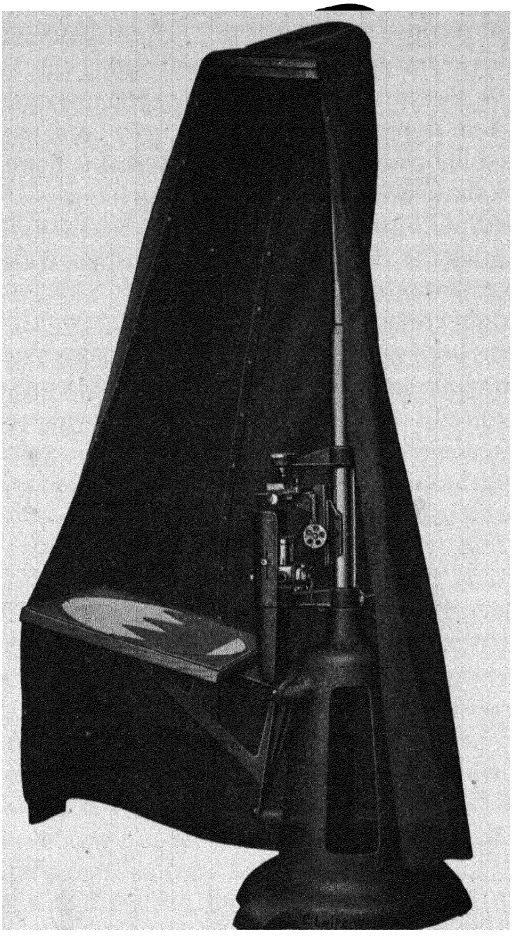


FIG. 236.

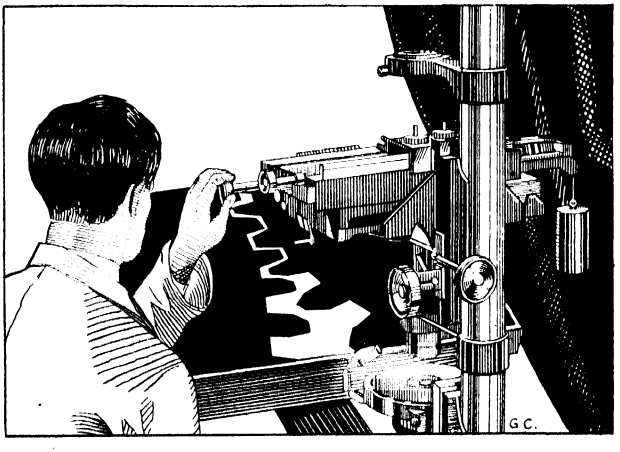


FIG. 237.

photographic records of the above. The instrument is capable of use with both transmitted and incident light, the image appearing on the glass screen in the sloping front of the instrument. As will be seen, the whole apparatus is very compact and portable ; having self-contained lighting systems of both types, it does not necessitate a completely darkened room for operating it.

A larger type of profile projector by Leitz is shown in Fig. 236, which illustrates the ray path through the apparatus, while Fig. 237 shows the method of using the apparatus.

This method of measurement is essential if the correct magnification and scale is to be stated on photomicrographs ; put briefly, if the magnification of any particular photomicrograph is to be accurately stated, then after having taken the photograph, the rulings of the stage micrometer are focussed on the focussing screen of the camera and the intervals measured with a transparent rule, after which the magnification is quite easily found.

REFERENCE

(1) CHAMOT and MASON. "Handbook of Chemical Microscopy," Vol. I. Wiley. By kind permission of the authors and publishers.

CHAPTER XI

PHOTOMICROGRAPHY

THE modern method of recording the image in the microscope is to photograph it, and the science or art of making photographic records of the microscopic image is termed photomicrography.

It is only of recent years that the art has become generally applied ; its beginnings, however, date back to the very beginnings of ordinary photography, so let us delve for a while into the history of photomicrography.

As is well known, Thomas Wedgewood (1771–1805) is generally considered to be the first person to use the effect of light on silver salts to produce an image in the form of a photograph at about the year 1800. The man who was later to become Sir Humphrey Davy became interested in Wedgewood's experiments and considered them of sufficient importance to warrant the reading of a paper to the Royal Society on the question. This was done under the title " An account of a method of copying paintings upon glass and of making profiles, by the agency of light upon nitrate of silver, Invented by T. Wedgewood, Esq., with observations by H. Davy."

This occurrence is very well commented on by Oakden in Watsons' *Microscopic Record*, No. 21, about which he says : " From this it appears that the woody fibres of leaves and the wings of insects were ' pretty accurately represented ' by being placed upon the sensitive material ; the images formed by means of a camera obscura were found to be too faint to produce a satisfactory image in any moderate time, but images of small objects produced by means of the solar microscope were easily obtained provided the sensitive material was placed at but a small distance from the lens. A difficulty arose from the fact that they could not fix the image obtained. Davy concludes : ' Nothing but a method of preventing the unshaded parts of the delineation from being coloured by the day is wanting, to render the process useful as it is elegant.' "

From this extract we see that the beginnings of the two sciences of photography and photomicrography were coincident ; indeed, since a camera is nothing more or less than an elaborate photographic camera obscura, we may be justified in saying that photomicrography came before photogrpahy as we know it to-day, for Davy and Wedgewood gave up attempting to produce images from the camera obscura in favour of those produced by the solar microscope.

It was not until round about 1838, however, that real development began with the use by Reade of gallic acid as a developer and

sodium hyposulphite as a fixing medium. The results of his early work on these lines were exhibited at the Royal Society on April 23rd, 1839. From that time up to the present day, photomicrography has developed simultaneously with photography, becoming almost the universal method of recording results over the last fifty years or so.

The process involved in producing a photomicrograph consists, briefly, of projecting the microscope image on to the sensitive surface of a photographic plate or film, protected from extraneous light, for a sufficient time to expose it correctly. The plate or film is then developed and fixed in the usual way, producing a negative image from which any number of positive prints may be produced.

The field of photomicrography is very extensive and indeed many volumes have been written upon this subject alone, therefore this section is only intended to be an elementary introduction. Those intending to pursue the subject further are referred to the standard works on the subject. However, in reviewing the subject briefly it will be appreciated that the usefulness of the art is unbounded, as anyone can view a photomicrograph and get a very good idea of what the actual image looks like, be he the most inexperienced of workers. The same of course applies to the projected image ; since the advent of direct colour photography it is possible to reproduce the microscopic image in its natural colours.

In a broad sense, photomicrography may be classed under two headings, that section known as " Photomacrography " and that called " Photomicrography proper," or more usually just " Photomicrography."

Let us deal with photomacrography first. This should never be confused with its partner, as more often than not it does not require the use of the microscope at all but only uses a camera equipped for unusual extensions and special lenses, for the purpose of taking photographs of objects at magnifications not exceeding abour twenty diameters, from which we see that it is more or less the little brother of photomicrography ; nevertheless, it should on no account be scorned as it has repeatedly proved itself to be of great use, particularly in industrial spheres. It is surprising how often a photograph at 10 diameters tells one much more than one at, say, 250 diameters. Fig. 238 illustrates the point very well ; the photograph is a section of a coil used in an investigation of the characteristics of a new winding machine in which the author was engaged some time ago. The machine was in the course of development, and wound coils for electrical purposes of enamelled copper wire with paper insulation between each layer. In the early stages of development the winding was not all that was desired and so it was decided to examine a sectional coil in order to ascertain where the faults lay. Examination of the coil with the naked eye was impossible as the wires were of a

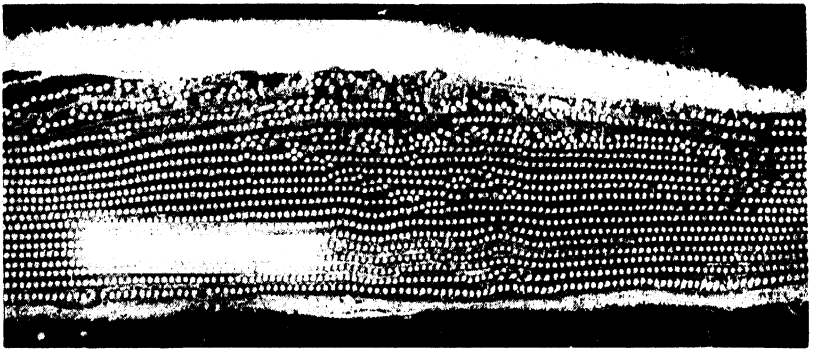


FIG. 238. × 3.

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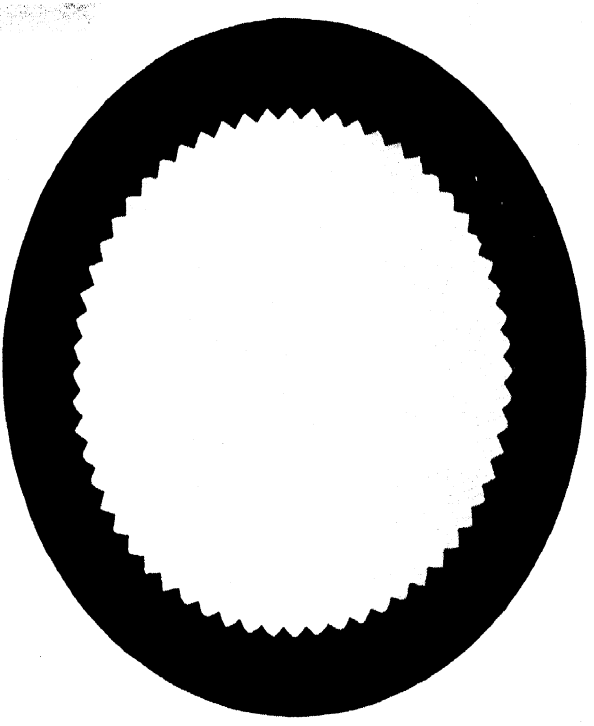


FIG. 240.

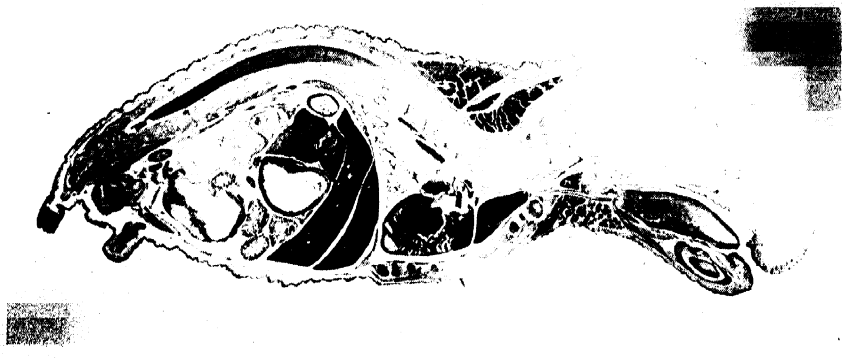


FIG. 241.

small gauge and were difficult to distinguish. If one used a hand lens the general layout was lost. The author decided to photograph the section at a slight enlargement, with the result shown in Fig. 238, which shows the sectional surface of the coil at a magnification of approximately three diameters. Here one sees the entire coil in section at one and the same time, the individual wires and their relative positions one to another are quite easily discernible. It is by means such as this that much important information is obtained,

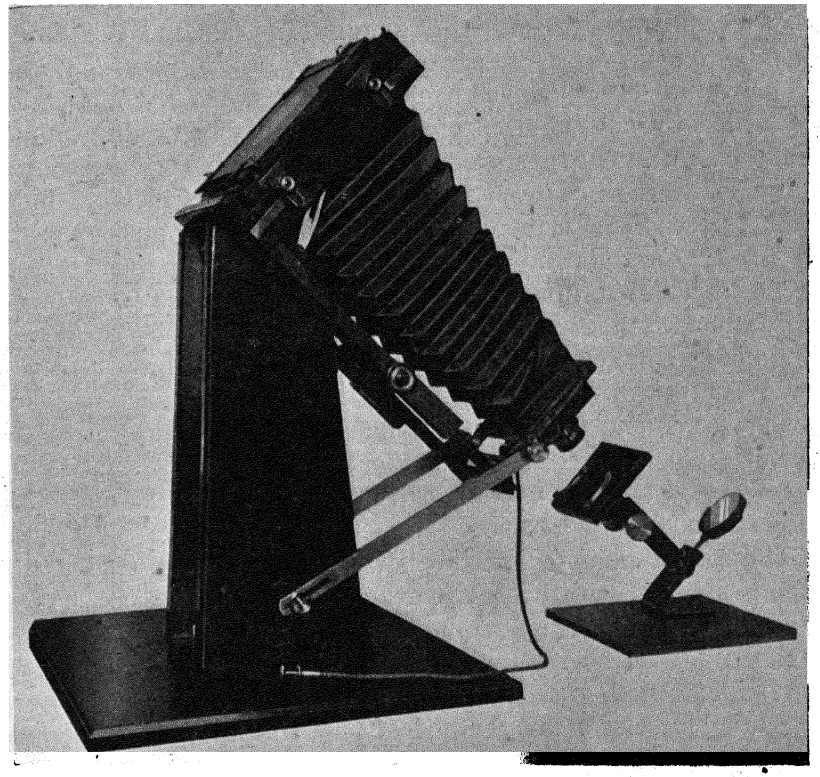


FIG. 239.

and it is the author's contention that much more use could be made of photomacrography, particularly in industry.

The apparatus need not be at all elaborate, in most cases a double or triple extension camera with a lens of 75-mm. focus is all that is required. Of course it may be necessary to mount the camera so as to protect it from vibration when working at long extensions, but this can usually be accomplished quite simply by some such method as that shown in Fig. 239, which illustrates the macroscopic camera used by the author. As will be seen, it consists of an ordinary half-plate camera having a double extension to the bellows; the front carries a Thornton-Pickard shutter which has a number of

front panels, the lens most used is a Zeiss Tessar f3·9 of 75 mm. focus ; but when, as sometimes happens, higher magnifications are required, the front panel is changed for one fitted to receive microscope objectives or the special macroscopic lens fitted with the standard R.M.S. thread, but more often than not the author has found the 75-mm. lens to be ample for macrophotography.

The arrangements for mounting the camera are of the simplest, yet are very effective. The stand consists of a rectangular section column of heavy-gauge sheet metal firmly fixed to a slate base, the top of the column slopes at 45° and carries a table of phenolic resin-bonded paper firmly fixed to the column. The camera is next firmly mounted on this table, the front steadying ties, so necessary when working a full extension, will be noted ; they are heavy brass straps by means of which the front of the camera may be rigidly clamped to the base column, thus protecting it from the effect of vibration. This apparatus has proved itself to be quite as efficient as much more expensive and elaborate commercially made appliances ; it is probably easier to operate owing to its being set at an angle.

The focussing arrangements on the camera proved to be inadequate when working at magnifications higher than about 3 diameters so the focussing specimen stage shown in the illustration was devised. This consists of the pillar of a simple dissecting microscope having a rack and pinion motion mounted at an angle of 45° ; on top of the pillar is fixed the stage which carries the specimen, thus the specimen may be quite easily held at the correct angle, final focussing being carried out by means of the rack and pinion.

The mirror mounted at the bottom of the pillar enables the stage to be used with transmitted light by placing a piece of glass on the stage large enough to project well beyond the top edge, the specimen is then held in position on the projecting portion of the glass and the mirror used in the usual way.

The usefulness of this method of photography is demonstrated by Fig. 240 ; this is a photograph of a small die used for broaching a ratchet, the overall diameter of which was only $\frac{1}{4}$ in. and which had to possess teeth on its periphery, each of which was to be the same as its neighbour. There was a question as to whether the teeth in the die were evenly cut or not, and the author was requested to exhibit the die under the " microscope " so that those concerned might view it under "high" magnification. After a certain amount of argument and explanation, it was agreed to leave him to produce a photograph which would show the teeth in the die quite clearly. On the production of the photograph (Fig. 240) together with the information that the magnification was only eight diameters, the surprise exhibited by the devotees of " high " magnification was something to behold ; however, the illustration shows very clearly that the teeth in the die were not even. The photograph is, of course,

a view looking through the aperture of the die taken with transmitted light by means of the aforementioned apparatus.

This type of work is not by any means of use only in industry ; in the field of biology a macro-photograph more often than not reveals more of the structure of a specimen than microscopic examination, as evidenced by Fig. 241, showing a section of a fœtal mouse magnified four diameters. The various organs are clearly seen, but what is more important, the orientation of the organs within the whole structure is shown much more clearly than would be the case if the section were magnified say 100 diameters and only a portion of it visible in the field. All of which goes to show that this branch of photography should not be ignored.

There are, of course, numerous cases when higher magnifications are required, such as, for example, when it is necessary to study the relative position of single cells on a tissue or investigate the micro structure of certain waxes, then it is necessary to use the microscope in conjunction with the camera, which science, as we have seen, is

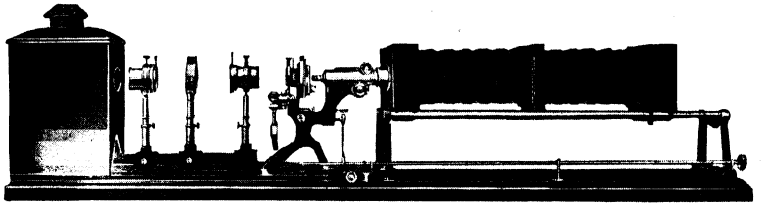


FIG. 242.

called photomicrography. Before proceeding any further, it will be as well to explain a point regarding nomenclature, which is very frequently misquoted. The results of photomicrography are known as photomicrographs and not, as so many people state, " micro-photographs." A little thought on this point will soon make clear that a " microphotograph " is really a very small photograph, whereas a photomicrograph is the permanent record of a microscopic image produced through the medium of photography.

Now let us look at this question of photomicrography in general. The basis of the science involves the use of the microscope in conjunction with some sort of camera, so that the image produced by the former may be impressed on the sensitive surface of the plate and so result in the production of a permanent record of the image.

Modern apparatus designed with this end in view takes divers forms, from a simple attachment whereby any ordinary camera may be used, to elaborate and expensive pieces of apparatus such as those used for metallography ; such a piece of apparatus is shown in Fig. 242. As will be seen, it consists of camera, microscope and illuminating system, mounted together on a common base board ;

the front portion of the baseboard carries a revolving table mounted on which is the microscope and illuminating system. The object of this is to enable this table to be swung at right angles to the main optic axis, so that visual focussing and initial setting up may be completed in comfort, after which the system is turned back and the flanges coupled up to the camera. The object needs no further adjustment ; beneath the camera is a steel rod by means of which the fine adjustment of the microscope is controlled from the viewing screen end. The camera itself is carried on metal end supports connected by steel rods on which the camera bellows can be extended and clamped in any desired position, the bellows extension being ascertained by means of a scale on the baseboard. The front of the

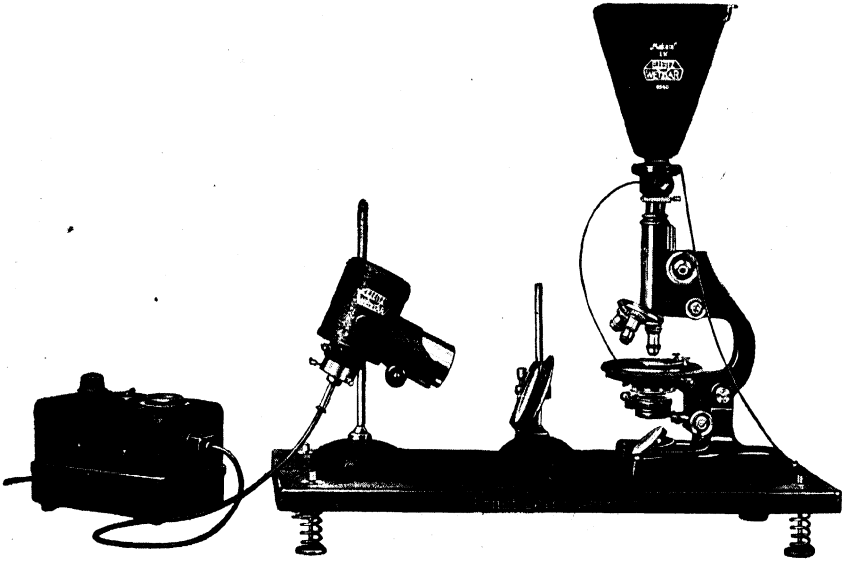


FIG. 243.

camera carries no lens but is fitted with a shutter ; this type of camera was in extensive use in the early part of this century and indeed still has its devotees, but the modern tendency is toward something simpler to operate and which occupies less floor space, for it is no exaggeration to say that the type of photomicrographic camera depicted in Fig. 242 requires a room to itself. For modern requirements, however, the camera must take up as little room as possible so that it may be placed in a corner of a laboratory or some such location. This demand for a small compact apparatus led to the development of the eyepiece camera ; one such example by Leitz is shown in Fig. 243, which depicts the camera in position on the microscope, together with illuminating apparatus all set up on a sprung baseboard, this latter to minimise the effects of vibration. It will be seen that the camera possesses its own shutter and has a

photographic eyepiece; that is to say, an eyepiece fitted at right angles to the optic axis so that the image may be viewed whilst it is being photographed. This refinement is particularly useful in cases where the object is a living organism which is moving about, thus necessitating a photograph of the nature of a snapshot. Needless to say, this type of apparatus can be quickly and easily set up for use and just as easily dismantled and packed away when not required. The author has used an apparatus of this type, and in his experience has found it to be capable of producing quite as good work as the more elaborate and cumbersome apparatus, and with greater ease of working, provided the necessary care is taken in setting up and handling.

Another type of camera designed to work in a vertical position is shown in Fig. 244. This is a simple form of photomicrographic camera by Watsons' designed to take up the minimum amount of space and yet function efficiently. The long extension to the bellows is useful inasmuch as photographs at relatively high magnifications may be made with low-powered objectives. The microscope is placed on the base and the illumination arranged in the usual way. For initial setting up and focussing of the image, the camera is swung to one side, being replaced when everything is ready to take the photograph, the final adjustments to bring the image into focus on the screen can then be made and the exposure made by using the shutter built into the camera front, which is also capable of carrying a low-power lens in a focussing mount, thereby converting the instrument for photomacrography. This type of instrument is very useful and has the advantage of a long and variable bellows extension, but there is a disadvantage which, in the author's opinion, is worthy of serious thought, and that is the height of the instrument, for it will be clear that at long and even moderate extensions of the bellows one would have to stand on a chair or some such article in order to focus the image on the viewing screen: either that, or put up with the alternative of using an abnormally low table for the instrument. However, whichever way we use it, there is still the comparatively great distance between the viewing screen and the fine adjustment with its attendant operational difficulties.

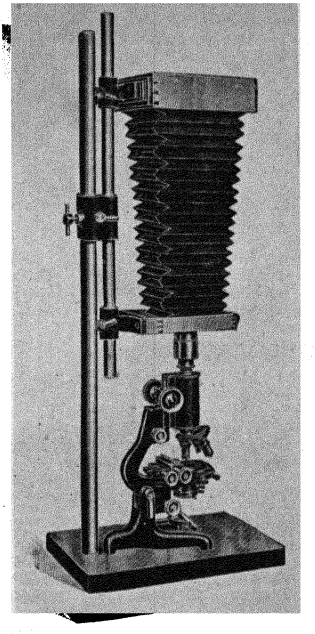


FIG. 244.

A more elaborate and improved type of vertical camera is shown in Fig. 245, in which we have the advantage of the built-in

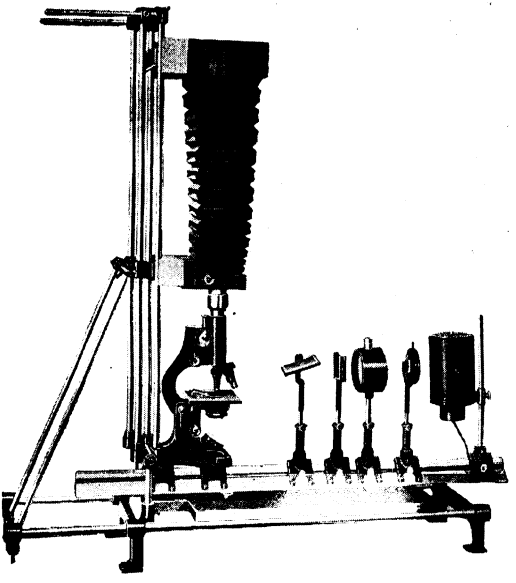


FIG. 245.

illuminating system of the large horizontal stands, together with the low space factor of the vertical stands; furthermore, this instrument may be easily converted into the horizontal type, as shown

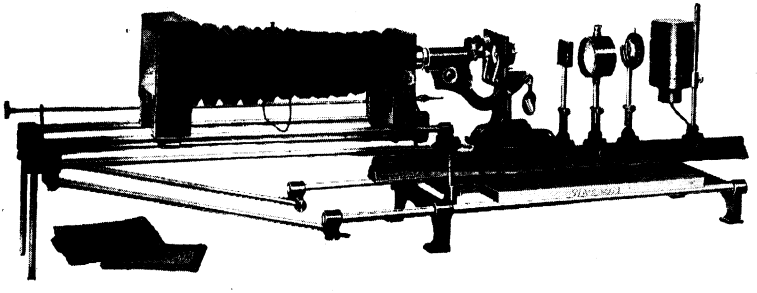


FIG. 246.

in Fig. 246, but we are still faced with the difficulty of height when it is used vertically and space factor when used horizontally, and in both positions the difficulty of working the fine adjustment while looking at the image on the viewing screen, still exists.

Since the modern tendency to use the eyepiece and a standard screen distance has proved so efficacious, it is the author's opinion that photomicrographic cameras should be designed in as compact a form as possible, because after all if one intends to indulge in

critical photomicrography, that is to say, photography of a critical image, then one must be in a position to take the same care over producing an image on the viewing screen of the camera, every bit as critical as that produced for visual examination, for what is the use of going to great lengths to produce a truly critical visual image when the photographic reproduction is only a travesty of the original? Therefore, let us so design our apparatus that we may carry out the entire routine for producing a critical image (*i.e.*, adjusting the illuminant and centring the complete system, adjusting

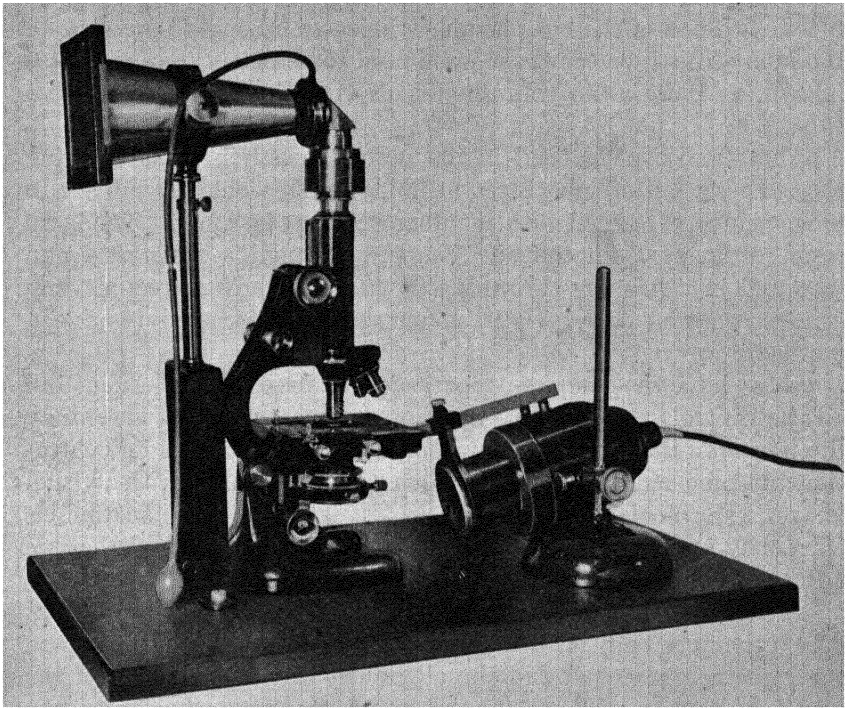


FIG. 247.

for correct tube length, etc.) on the screen, without having to stand on chairs while examining the screen and at the same time having to stretch downward to reach the fine adjustment, the discomfort involved in which operation has to be experienced to be believed.

It was in order to overcome the foregoing objections that the author designed the apparatus shown in Fig. 247. As can be seen, it is very simple, consisting in this case of the conical tube of a simple eyepiece camera, on to the small end of which is fitted a shutter taken off an old pocket camera. On the front of the shutter is a brass housing holding a right-angled prism in position, so that the optic axes of the microscope and the camera form a right angle On the

horizontal face of the prism mount is one of a pair of ordinary flanges which form a light trap (the prism was taken out of an old super-ocular mount used for projection); the whole camera assembly, which incidentally takes a lantern size of plate ($3\frac{1}{4}$ in. $\times$ $3\frac{1}{4}$ in.), is fixed firmly to the movable portion of a telescopic stand, which, in turn, is rigidly fixed to the bracket mounted on the base, which consists of a slate 24 in. $\times$ 14 in. $\times$ 1 in. The camera may be adjusted for height by means of the telescopic stand and firmly locked into position, or, for initial examination, may be removed altogether, thus leaving the microscope free for visual examination, or swung out of the optic axis of the microscope. The shutter is fitted with one of the old pneumatic releases having a long tube to the bulb; this type of release was found to be better than the cable release, as there is no transmission of vibration along the rubber tube.

The microscope is used in the vertical position, thus enabling the examination of substances in fluids which would be subject to movement if examined with the microscope at an angle. The lamp is an ordinary "Photoflood" worked through a resistance and mounted in a housing carrying a Watson-Conrady corrected lamp condenser fitted with a field diaphragm and focussed to give a parallel beam.

So we have the complete apparatus on a base 24 in. $\times$ 14 in. and all the controls at the finger tips, for one may sit down and study the image on the screen, meanwhile making adjustments for centration and tube length, etc., in the same way as if dealing with a visual image. The final focussing, particularly at high magnification, is extremely easily accomplished with a focussing magnifier, the fine adjustment being in almost the normal position.

It was found by experience that it simplified matters a great deal if the initial adjustment was carried out visually, that is to say, the camera is removed and the alignment of the optic axis of the visual system completed in the usual manner, whereupon the camera is replaced and any adjustment for tube length which may be required made while viewing the screen image; in this way it is a comparatively simple matter to obtain a truly critical image on the screen. As in all such instruments there must be some form of protection from vibration, the slate base is mounted on four sponge-rubber cushions situated at its corners. The possibility of transmitting a shock, sufficiently severe to upset the adjustment of a $\frac{1}{12}$ O.I. objective, from the camera to the microscope when inserting the dark slide is obviated by the flanges, whereby we obtain a light-tight joint, at the same time keeping the camera and microscope out of mechanical contact. However, the author agrees with other workers that this question of vibration is very much over emphasised. We may quote Garner (1), who says: "The vibration bogey is,

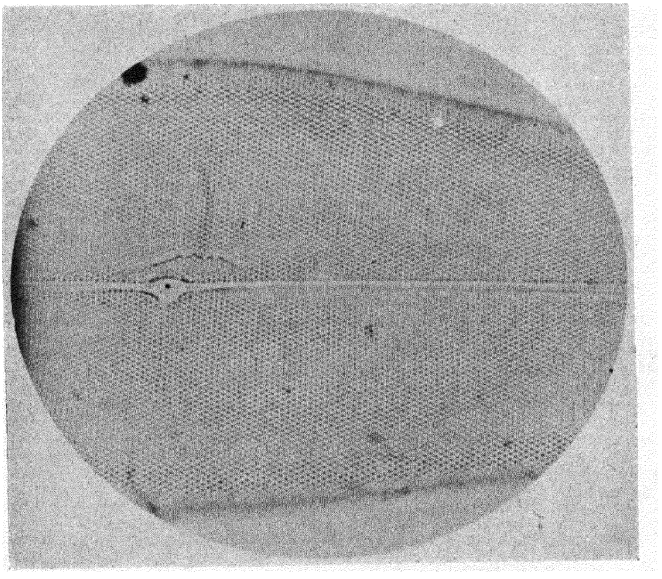


FIG. 248.

[*To face p.* 227.

however, greatly exaggerated. Vibration due to trams or traffic on a road outside the laboratory, for example, will have a negligible effect ; on the other hand, a book dropped on the table may cause trouble by moving the slide closer to the stage. For the same reason it is always wise to focus upwards for photographic work, as there is then no chance of the body tube dropping slightly during exposure." So we see that the main fear of vibration comes from completely controllable sources, and if people are cautioned to remain quite still during the exposure, no ill effects should accrue. However, the sponge rubber used by the author has proved itself to be eminently satisfactory, as demonstrated by Fig. 248, which shows a view of the well-known diatom, Pleurosigma angulation, photographed at 1,200 diameters direct ; that is to say, the screen image was at 1,200 diameters, a $\frac{1}{12}$ O.I. objective was used together with an O.I. condenser. It was decided to use this object as a test for vibration, the object being to see if the apparatus would hold the black dot resolution of the diatom for a long exposure (20 minutes) while people walked about the laboratory in the normal way. The result speaks for itself, the resolution has not altered and the focussing is quite sharp, thus amply supporting the views expressed by Garner.

In certain cases it is desirable to take photographs without the aid of the eyepiece, but by using the objective alone. Under these conditions the diaphragm in the end of the draw tube will undoubtedly be responsible for intercepting some of the image-forming rays, in this way reducing the size of the screen image. Therefore the draw tube should be removed and for the same reason the microscope should possess a large-diameter body tube which should be lined with black velvet or some similar non-reflecting material in order to eliminate internal reflections off the wall of the tube.

These conditions as a rule only appertain to photomicrography at comparatively low powers, in which case the ordinary substage condenser will not be suitable owing to the limited size of field obtainable with it ; an appropriate condenser should be used and focussed in the usual way to ensure a fully illuminated field.

So much for the apparatus used in photomicrography. Now let us consider the problems involved in using the apparatus to achieve the desired end. The first problem encountered is that of focussing the image on the viewing screen so that when this latter is replaced by the sensitive surface the image will be sharp. When producing a photograph of a magnified image the orthodox method of focussing by visual inspection is not good enough, this is due to the increased sensitivity imparted to the mechanism used, particularly so in the case of high and medium powers where the slightest touch on the fine adjustment is sufficient to throw the whole image out of focus entirely, apart from which the fine detail, which in most cases it is

desired to show, cannot be seen on the screen no matter how finely it is ground, although some slight advantage is gained by slightly oiling the screen, thereby making it more transparent. The presence of the grain is still obvious; we must therefore use some other means whereby this process will be simplified, and make certain this is accomplished, by an auxiliary hand lens called a focussing magnifier which consists of a simple lens mounted in a focussing mount so that the operative surface of the focussing screen may be viewed through it. In this way we obtain a magnified view of the screen image, but if we use this lens on the ground-glass screen we are no better off because we magnify the grain of the screen as well as the image and the fine detail is still unresolved. There is, however, one way out of the difficulty and that is to view the image direct with no screen in place, so getting an uninterrupted view of the image. The great difficulty here, however, is to view the image at the exact plane, in space, corresponding to that occupied by the sensitive surface when the plate or film is *in situ*. This is quite simply overcome by using a clear-glass screen and pre-setting the focussing magnifier to focus the correct surface (*i.e.*, the inner surface) of the glass. The best method of accomplishing this is to rule diagonals on the surface of the screen with Indian ink and then focus these lines sharply with the magnifier. In this way the image and the lines are seen together and we know that when we have focussed the image to our requirements it is focussed in the correct plane, also the point of intersection of the lines gives a means of locating any desired portion in the centre.

The author has found it to be more convenient to use two screens, one of finely ground glass which is used for the initial setting up, the other being clear glass used in the afore-mentioned manner. This arrangement might, however, be inconvenient to some workers, but there is a method of combining the two in one screen, which consists of making a small portion of the ground-glass screen transparent by the simple expedient of cementing a cover glass about $\frac{7}{8}$ in. diameter to the ground face. This has the effect of creating a clear disc in the centre of the ground screen, which is used in the same manner as the clear screen. In order to be sure that the focussing magnifier is set correctly, it is advisable to rule diagonals on the ground face of the screen in pencil before cementing the cover glass in position, thus the clear portion of the screen will show the two pencil lines which can be accurately focussed in the usual way. Thus with the aid of these simple appliances the image may be focussed in the correct plane and with an accuracy unobtainable by unaided visual inspection.

A further advantage of using the focussing magnifier is the increase in brilliance obtained in the image when viewed in this way. The light lost in the minute reflecting surfaces of the ground glass

in the ordinary way results in a considerable diminution of the intrinsic brilliance of the projected image and at high and even medium powers the image is very difficult to see, even in a completely darkened room, but when the focussing magnifier and clear screen are used the image is very nearly as bright as when seen during ordinary visual examination. It must be borne in mind that a lens must be used, as the image on the clear screen is invisible to the naked eye, due to its being a real image ; the lens produces a virtual image which of course becomes visible.

Having focussed our image, the next step is to decide on the exposure to be given so that we may produce a good negative. This is probably the most discussed and debated question in the whole science of photography, and it seems with ample justification, for there are a formidable number of variable factors affecting the exposure, a variation of any one being sufficient to detract from the ideal. It has been said that there is no such thing as a perfectly exposed photograph, because if the shadows are correctly exposed, then the high lights are necessarily over-exposed, and it is very probable that this is true. Rodman, (2) in the section on photomicrography in " Photography as a Scientific Implement," gives nine separate factors influencing exposure of the negative as follows :—

(1) The speed of the photographic emulsion used.
(2) The intensity of the illuminant.
(3) The efficiency of the collecting and condensing system.
(4) The presence or absence of a colour filter.
(5) The numerical aperture of the objective.
(6) The magnification at the screen.
(7) The distance of the screen from the Ramsden disc.
(8) The magnification of the eyepiece.
(9) Characteristics of the object, *i.e.*, colour, density, etc.

These nine factors, all of which are variable in themselves, must be taken into consideration when deciding on the exposure.

It is not proposed to discuss the problems of exposure as this would require a chapter to itself, and indeed many volumes have already been written about this subject, but rather to give some sort of guide to the novice commencing work in this field. For detailed instruction it is recommended that the standard works be referred to ; we may, however, consider very generally the question determining a suitable exposure.

Taking into account the nine variables involved in the determination, the most effective plan to adopt is to measure the background intensity of the image. This is quite easily carried out by the use of one of the small extinction-type photometers which made their appearance on the market a few years before the war. This type of meter is used with the clear screen and will give a reasonably accurate reading for an average exposure to produce a good negative.

One method which the author uses is to focus the image in the usual way and then put it out of focus until the illumination is evenly diffused, and then take the reading with an extinction photometer ; this has never failed to produce a well-exposed full negative.

It must be remembered when using this method that the exposure indicated by the meter must be increased somewhat, but this difficulty is quite easily overcome by calibrating the instrument beforehand for each particular objective. The work involved in doing this is well worth while, and amply justified by the results. We see, therefore, that the difficulties confronting us with regard to exposure are really quite simply overcome.

Let us now examine the question of negative material, the choice of which is dictated by the nature of the specimen, which might come under one of two broad classifications, one being those specimens which are coloured, by which is meant stained specimens, and which constitute the majority when the subject is looked at as a whole. They are chiefly of histological and botanical subjects, consisting of stained sections and may contain as many as three or four different colours.

The object of staining is to differentiate the various types of tissue and so make the structure of the tissue clear. The other heading includes those subjects which are not artificially coloured ; in the majority of cases the specimen has only one colour, the object then being to show detail rather than differential contrast, as in the previous case.

The choice of the correct sensitive medium is important if the best results are desired. The first consideration applying to all types of specimens to be photographed under magnification, more particularly at high power, is that of grain size. It is obvious that if we go to a great deal of trouble to obtain high resolution and then proceed to use a coarse-grained emulsion, the result is going to be far from satisfactory. As graininess in a negative may also be produced by careless processing, it behoves us also to pay attention to this side of the question, especially if it is decided to use negatives of small size, necessitating printing by enlargement.

Another factor affecting the use of plates more than films is that of halation. The user of plates must use anti-halation material if he intends to produce good photomicrographs, and although not absolutely necessary in the case of films, it is to be strongly recommended.

Negative material may be classified under three main groups, thus :—

(1) Those sensitive to blue and yellow light only (ordinary).

(2) Those sensitive to blue, yellow and green (orthochromatic).

(3) Those sensitive to all colours (panchromatic).

The foregoing headings are general, and should not be regarded as so many watertight compartments, as it were, because the

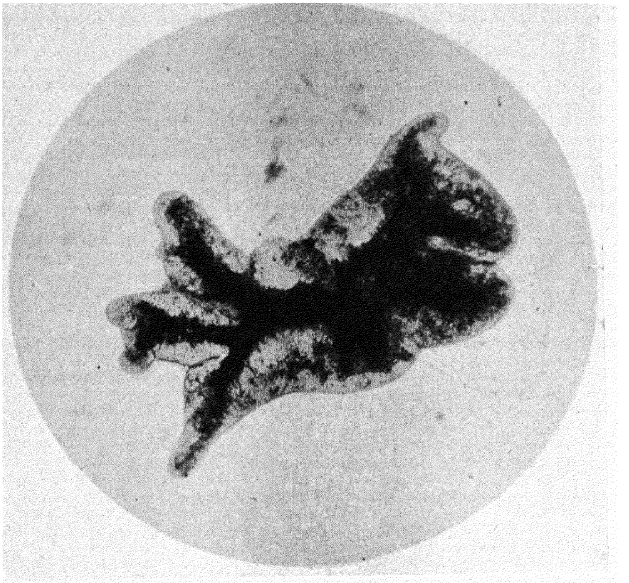


FIG. 249.

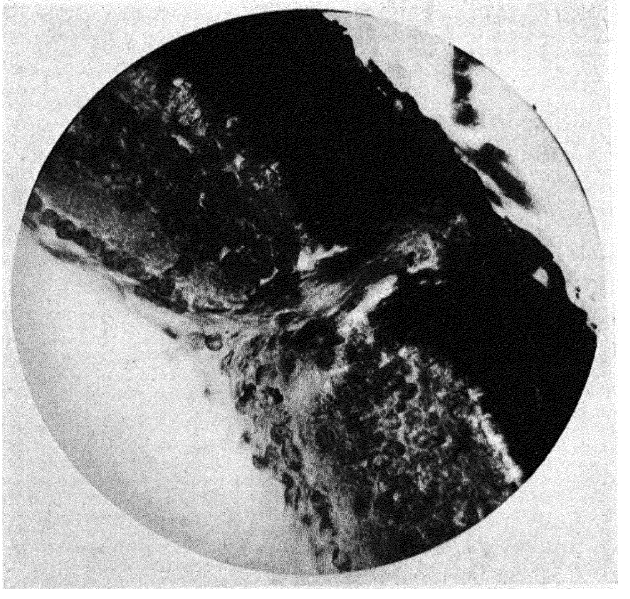


FIG. 250.

[*To face p.* 231.

number of different emulsions in existence to-day is so great that overlapping from group to group is bound to occur, also special emulsions such as those of X-ray and infra red photography cannot be strictly placed in any of the three main groups.

Thus it will be seen that for the first type of specimen, viz. the coloured, a panchromatic emulsion is the best to use so that all the colours may be recorded in their true relative tones when converted to black and white. It should, of course, be a fine-grained material in order that fine detail may not be obscured. For the uncoloured or self-coloured type the obvious choice is an ordinary plate or film, as the recording of detail is of primary importance. It should be borne in mind that the speed of the emulsion increases greatly from groups (1) to (3), therefore for ordinary work where long exposures do not cause any inconvenience, the slower the emulsion the better the photograph, as the slower emulsions have very fine grain sizes not met with in material of higher speeds such as those in Group (3).

From the foregoing it would appear that we would have to use a different type of negative material for each of the different specimen groups ; as a matter of fact, that is not strictly necessary, as a good orthochromatic plate used in conjunction with the correct filter will produce excellent results for all normal specimens, coloured or uncoloured. The author uses an orthochromatic emulsion for all types of specimens except where a moving object is to be photographed, which case calls for a faster emulsion, as the exposure has to be of the nature of a snapshot of not more than $\frac{1}{25}$ second duration. This, of course, requires a panchromatic emulsion.

Examples of the result of using an orthochromatic emulsion on coloured subjects are shown in Figs. 249 and 250 ; the former is a photomicrograph of the well-known organism *Amœba Proteous*, taken from a stained specimen at a magnification of 200 diameters. The specimen was stained with fuchsin and the object of this was to demonstrate the structure of the protoplasm of the cell. As can be seen, this is differentiated into two distinct zones, an outer zone which is not so heavily stained, possibly because it is not so dense as the inner zone, which is more heavily stained.

The fine fibrillar structure of the outer zone, or ectoplasm, will be noted. The photograph was taken with a 12-mm. holoscopic objective used in conjunction with a X10 holoscopic ocular, the lighting being supplied by the " Photoflood lamp," previously described, used in conjunction with a Watson parachromatic condenser and Chance-Watson green filter, the former working about three-quarter full aperture ; the exposure required was 45 seconds. This example, of course, contained only one colour of varying depth, but the example shown in Fig. 250 is a double-stained section of the eye of a three-weeks'-old tadpole, the portion shown being the

point of insertion of the optic nerve into the retina. The photograph is at a magnification of 480 diameters ; the specimen was very lightly stained with hœmatoxylin and eosin. Actually this particular specimen was unsatisfactory as the staining was insufficient, but it was very useful for demonstrating the effect of the green filter in increasing contrast as the cell nuclei in the retina can be clearly seen in photomicrograph, whereas they were very difficult to distinguish by visual examination. The details of Fig. 249 are as follows : Watson $\frac{1}{6}$ parachromatic objective with X8 Zeiss ocular, parachromatic condenser at $\frac{3}{4}$ aperture with Chance-Watson green filter ; lighting was as for Fig. 249.

The foregoing remarks bring us to the subject of filters, the use of which in ordinary photography is too well known to need detailed discussion. In photomicrography the filter is all important and is best described by T. Thorne Baker in an article in Watsons' *Microscope Record,* No. 28 ; he says : " If we look at a penny stamp through a piece of pure green glass it will appear black. If we place the stamp on a piece of emerald-green velvet and again look at it through the green filter it will give the appearance of a black stamp on a ' white ' background. The same subject looked at through a piece of red glass would make the velvet appear black and the stamp white. In each case we have by the use of a suitable light filter thrown one of the two objects into relief or made it appear in the strongest possible contrast to the other. It can thus be easily recognised how, when we are dealing with *B. tuberculosis* in a methylene blue stained tissue, the use of a coloured filter will help to throw the bacilli into strong relief.

" There is an old saying ' there is a cure for every ill.' In photomicrography we may say that there is a contrast filter for every stain."

Thus we see how the filter may make or mar the final result. The correct filter to give the maximum possible contrast in any particular specimen is easily chosen if we bear in mind the following simple rule, that the filter must be of a colour complementary to that of either the object or the background. The following table from the same article will serve as a guide to the correct choice of filter and negative material to produce the best results.

Stain	Colour	Filter	Negative material
Hæmatoxylin.	Reddish pink.	Blue green.	Panchromatic
Gentian violet.	Blue violet with some deep red.	Yellow.	Orthochromatic.
Methylene blue.	Violet blue.	Orange.	,,
Fuchsin.	Blue violet and red.	Green.	Panchromatic.
Malachite green.	Blue green.	Red.	,,

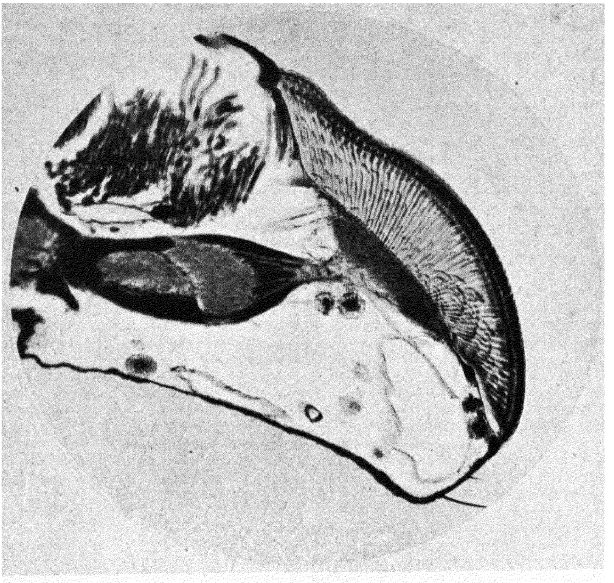


FIG. 251.

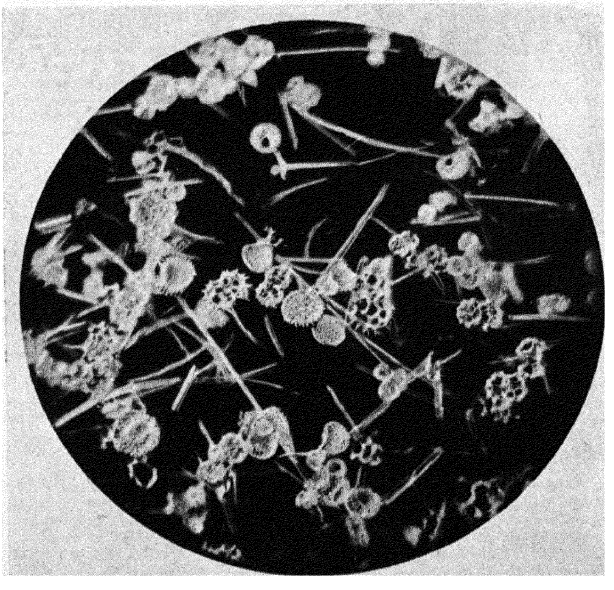


FIG. 252.

[*To face p.* 233.

This table gives a good general idea, but for more definite information a table showing the filter to use with any given colour, so that the colour in question appears black when looked at through the filter, will be of use. So the following table of eight colours commonly met with gives the correct filter to use for securing maximum contrast :—

Colour	Filter
Red.	Green.
Orange.	Blue.
Yellow.	Indigo.
Greenish yellow.	Violet.
Green.	Red.
Blue.	Orange.
Indigo.	Yellow.
Violet.	Greenish yellow.

Thus, by the use of these tables, it will be possible for us to show any particular portion of a specimen in contrast to the remainder ; a good example is that given by Fig. 241, showing the fœtal mouse. This section was stained in three colours, blue, violet and red ; the photograph was taken through an apple-green filter on an orthochromatic emulsion and the effect has been to produce the red-stained portions as black or very dark, the violet portions as a dark grey and the blue portions from light grey to nearly white, as a result of which the general structure of the animal is very clearly shown.

So far we have only dealt with filters used for producing areas of contrast in the final result. It may be desired to reproduce fine detail rather than contrast, in which case a filter of the same colour as the specimen should be used together with a fine-grained emulsion, which consideration points to the use of either an ordinary plate or an orthochromatic. For cases where the specimen is unstained a green filter used with an orthochromatic is the best choice ; two examples of this kind are shown in Figs. 251 and 252, the former being a photomicrograph of a section through the eye of a house fly at a magnification of 80 diameters ; the lenses and their associated nerves are clearly seen. A group of polycystina at the same magnification are shown in Fig. 252 ; the fine details of the structure are easily seen. This latter was, of course, taken by dark-ground illumination, and is a good example of the possibilities of this type of illumination. The filter in both cases are of a Chance-Watson green.

Sets of filters of the correct size for the standard substage filter ring are made by most reputable manufacturers of photographic supplies. Messrs. Ilford, for example, produce a set of nine filters

designed to cover very nearly all requirements. Messrs. Kodak also supply a similar range.

It is obvious that by interposing a filter in the optical train the intensity of the light reaching the sensitive surface of the plate will be diminished and accordingly the exposure necessary will be extended. Therefore we must have some knowledge of how much extra exposure is required for any given filter. The manufacturers have obtained this knowledge and issue it in the form of an exposure factor. Typical examples are given in the following table taken from Messrs. Ilford's literature on the subject and applying to the set of nine micro filters made by them :—

Filter No.	Colour	Use	Exposure factor
1	Blue violet	Contrast for chitin, diatom markings, yellow stains, *e g.*, saffranin.	X35
2	Blue .	Contrast for chitin, diatoms and for saffranin, orange G., carmine, etc.	X15
3	Green .	Contrast for hæmatoxylin, methylene blue, carmine, eosin, saffranin, etc.	X7
4	Yellow (very deep)	Contrast for hæmatoxylin, methylene blue, gentian violet, methyl violet, etc.	X1½
5	Orange (deep)	Contrast for hæmatoxylin, methylene blue, gentian violet, also for infra red.	X2½
6	Purple .	Contrast for light green, methyl green, saffranin, chitin, orange G., etc.	X11
7	Magenta .	Contrast for light green, methyl green, saffranin, etc.	X3
8	Yellow .	Compensating to absorb ultra violet.	X1½
9	Yellow (pale)	Compensating to absorb violet and ultra voilet. Contrast for gentian violet, and methyl violet.	X1½

Thus we see that the chief difficulties involved in the production of photomicrographs are really not insurmountable, and therefore the beginner need not approach the subject in fear and trembling. It will be obvious that some experience in photographic manipulations is highly desirable, but lack of this need not be a deterrent.

Further examples of photomicrography, this time by incident light, are shown in Figs. 253 to 256. That shown in Fig. 253 demonstrates the value of the technique in industrial applications ; it is actually a surface view of the middle layer of an electrical coil wound with 40 S.W.G. enamelled wire, the whole coil being impregnated in a varnish, the object of which is to fill up the space, in the winding, originally occupied by air. The illustration is at a magnification of 106 diameters and clearly shows the thick film of varnish covering the conductors. This thickness is demonstrated in places

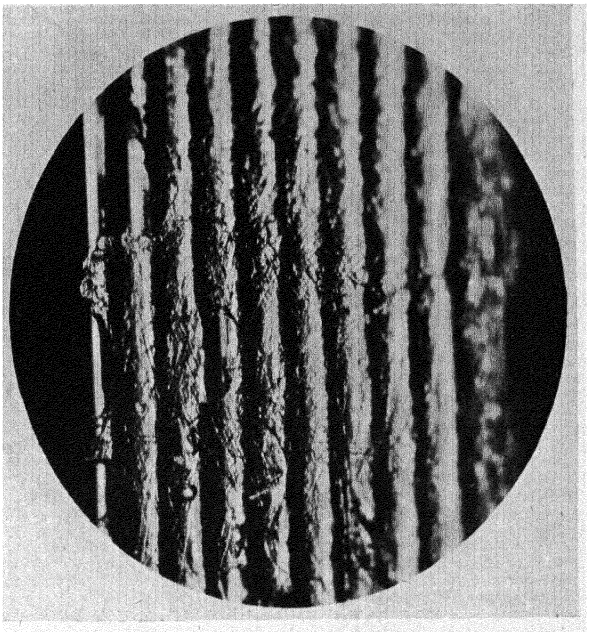


FIG. 253.

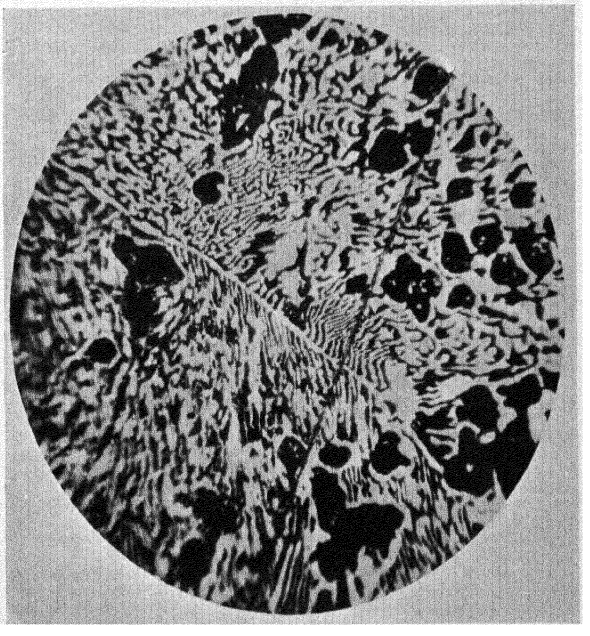


FIG. 254.

[*To face p.* 234.

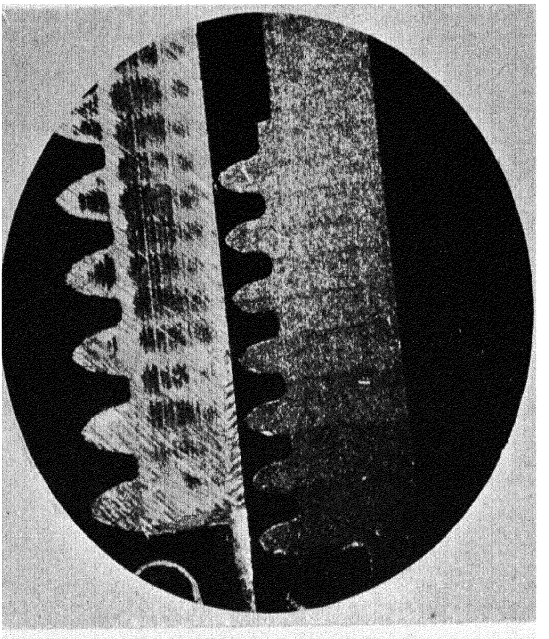


FIG. 255.

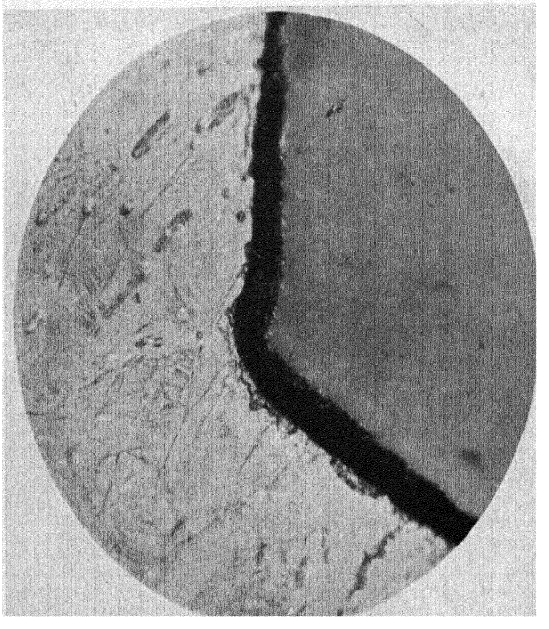


FIG. 256.

where the varnish has been removed to reveal the conductor underneath.

To proceed to an entirely different subject, Fig. 254 shows an etched section of a bismuth-tin alloy photographed at 700 diameters. The structure of the alloy is revealed clearly, and to those with a knowledge of metallurgy needs no explanation. It does, however, give some idea of the wide field of usefulness of this method of examination and recording.

A further example is given in Fig. 255, which is a photomacrograph of two small racks taken at 4 diameters. These were supposed to be similar in tooth profile and the discrepancy was only realised when the photograph was taken. In the same way it was desired to know the thickness of a graphite film applied to the working surfaces of a small-toothed wheel similar to a clock gear. A section was made so that the graphite film was undisturbed, and the photomicrograph, shown in Fig. 256, taken of this illustration, shows the tooth root of one tooth at a magnification of 490 diameters with the graphite film adhering to the surface ; by this means the thickness of the film was accurately measured.

The foregoing pages will serve to introduce the subject to those who intend to take it up ; it will also give some idea of the enormous scope and value of photomicrography in the furtherance of serious researches and development, particularly so in its industrial applications, for we have in this art a means at our disposal of permanently recording the visual impression gained on normal examination, further study of which will invariably bring to light fresh facts and configurations of structure which has been missed in the visual examination.

REFERENCES

(1) GARNER. " Industrial Microscopy." Pitman. By kind permission of the authors and publishers.
(2) RODMAN. " Photomicrography," " Photography as a Scientific Implement." Blackie.

CHAPTER XII

THE PREPARATION OF SPECIMENS

In order that the structure of any given specimen may be clearly seen under the microscope, it is necessary to prepare and mount it in one of several different ways, particularly so when it is desired to keep the preparation permanently. It is not intended to make this section a treatise on the preparation and mounting of objects, but rather to give a general summary of the more common methods, going into more detail on some rather less orthodox processes developed by the author in the course of research on various subjects, whereby it is hoped some help may be given to those undertaking similar work.

It would perhaps be convenient to commence with the operations involved in mounting. We have seen that for the great majority of subjects the specimen should be mounted in a medium of high refractive index, so that the maximum resolution may be obtained. This applies particularly to colourless objects such as diatoms and the like, but where the object is stained as in the case of biological and botanical tissues, the stained portions are the sections required to be seen, while the unstained portions are, as a rule, required to be invisible. This indicates the use of a mounting medium having the same refractive index as the unstained parts of the specimen.

In some cases, for example when examining living organisms, the mounting medium is of an aqueous type and as such possesses a lower refractive index than the specimen which is usually colourless. This is useful, inasmuch as the difference between the refractive index of the medium and that of the specimen serves to show up the latter, but it would be far better if we could have a medium whose refractive index was higher than that of the specimen, as then the resolution would be increased due to a larger number of the rays of the diffraction fan entering the objective. However, as it is impossible to use such a medium without killing the specimen, we must put up with the loss of resolution.

There are cases where the specimen is mounted in air, or dry, as with pollen grains and similar substances, which are mounted on an opaque black background for examination by incident light ; metallurgical specimens also come into this category.

So much then for the various conventional methods of mounting objects for examination, now let us consider the requirements for making a mount. The first item to come under consideration is obviously the slide, or slip. This is usually a piece of fine plate glass, whose size is conventionally fixed at 3 in. × 1 in., although for certain cases such as large embryo or brain sections, larger sizes of 3 in. × 1½ in. are used. Carpenter and Dallinger refer to the glass used for

slides as " patent plate." They are made as a rule from two types of glass, that known as crystal, or water white, and the more commonly used, Chance's " non-corrosive," which has a greenish tinge when viewed edge on. They should be accurately cut and have all edges ground, and should also be free from flaws such as veins and air bubbles, the deleterious effects of which are obvious.

Microscopic slides are made in various thicknesses, but there is a tendency to-day to standardise on a thickness of about 1 mm., which enables them to be used with most oil immersion condensers and darkground illuminators, and it is advisable to specify a thickness of 1 mm. when purchasing slides, as most manufacturers grade their slides according to thickness.

One of the hallmarks of a well-trained microscopist is the care which he takes in cleaning slides preparatory to using. Time spent in this way always produces its own reward in a clean mount, which in its turn is a pleasure to examine, as nothing is more annoying than having to examine a dirty mount in which the presence of bits of dust and fluff, etc., is only too obvious, in many cases obscuring the very portion of the structure which it is desired to examine. Therefore let us take great care in cleaning our slides before using. There have been many methods described from time to time for cleaning and storing slides, all of which are quite effective. The author, personally, stores his slides in concentrated sulphuric acid ; this removes all adherent organic material, particularly in the form of grease, and when required for use they are removed with the aid of tongs and dropped into a bowl of running water. They may then be handled by the edges and held under the tap for a second or two in order to thoroughly wash off the acid, after which they are rinsed in alcohol and dried on a clean cotton cloth. From the time of the rinsing in alcohol the slide should never be handled with the bare hands, except by the edges ; if this rule is adhered to there should be no trouble with dirty slides.

In all cases, except in that of metallurgical specimens, the object has to be covered. In the early days of microscopy this was accomplished by means of thin pieces of mica, thus the early microscopists were enabled to use objectives of short focal length without damaging the object. However, there were many objections to the use of mica, which has since been superseded by thin glass made by a special process by Messrs Chance Bros. In the process this glass is not annealed, consequently it is somewhat brittle ; but when one considers that it is possible to obtain it in thicknesses down to $\frac{1}{500}$ in., the slight objection to the brittleness is more than offset, as it is obvious that glass as thin as this would have to be handled carefully whether it were brittle or not. However, a small amount of practice will soon show results.

Cover glasses are usually made in four thicknesses and are

graded as No. 0, No. 1, No. 2 and No. 3. The average thicknesses are: No. 0, 0·10 mm.; No. 1, 0·15 mm.; No. 2, 0·20 mm. and No. 3, 0·25 mm. The general opinion is that the No. 0 covers should be used for objects to be examined under the highest power O.I. objectives, No. 1 for high-power dry objectives, the No. 2 for medium-power objectives, and the No. 3 for low powers. This is very nice and orderly, but it is difficult to think of any object, with perhaps the exception of some of those mounted dry, which, scheduled as for examination by low powers, will never be used with medium or high, in which case if the No. 3 cover is used, it is highly probable that a $\frac{1}{6}$-in. objective of high aperture will not focus through it. Therefore the author recommends the use of the thinnest cover glass possible, consistent with the nature of the specimen; that is to say, for thick specimens such as insects it is advantageous to use a thicker cover, say No. 2; likewise in cases where the specimen is mounted in a cavity or cell a thick cover is advantageous as a thin one would in all probability "cave in" in the centre, thus weakening the whole structure and spoiling the quality of the image. Of course, in the case of cell or cavity mounts, high- and medium-powered objectives are seldom used (except where specially small cavities are used with this purpose in mind), and No. 3 cover will be found to answer very well.

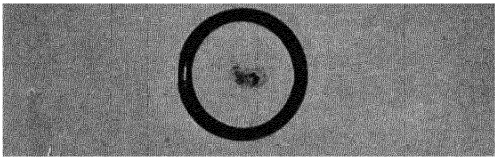


FIG. 257.

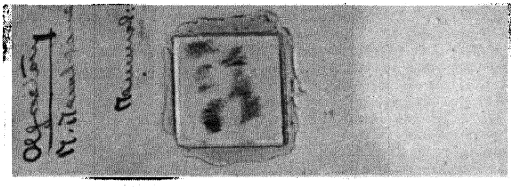


FIG. 258.

Cover glasses are made in various shapes, such as discs (or circles), squares and rectangles. The disc cover glass is the best to use as it is much easier to finish the mount off by a sealing ring, thus making a neat workmanlike job in which one can take a pride. Apart from the finished appearance, a sealed cover will make a much more permanent mount, particularly when the mounting medium is Canada balsam, as this medium is very apt to yellow with age due to very slow oxidation, and in mounts which are not sealed the whole mount will turn yellow whereas, if the sealing is carried out as soon as the mount is hard enough, it will remain quite white indefinitely. A completed slide properly sealed up is shown in Fig. 257; the neat appearance of the whole will be noticed, particularly the sealing ring, which finishes off the mount proper. Now contrast this with Fig. 258, which depicts a typical medical

student's slide, using a square cover glass for the mount, which is not even cleaned let alone sealed ; an examination of this illustration will immediately point out the difficulty of sealing a square cover ; however, this subject will be discussed in greater detail subsequently.

There are cases where a specimen must of necessity be covered by a glass other than round, such as, for instance, the case of long insect mounts or serial sections, when a rectangular cover *must* be used. In such cases it is still possible to make a presentable job of the mount, as shown by Fig. 259. It will be seen, however, that the round cover glass is preferable wherever possible, the limiting diameter being about $\frac{7}{8}$ in., this allows $\frac{1}{16}$ in. all round for the sealing ring. If the specimen is too large for a circular cover, a rectangular glass is called for. It is a good plan to choose the size of the cover glass so that there is plenty of room all round the specimen, in this way the covering up of portions of the object by the sealing ring is avoided.

There are many types of varnishes and cements which may be used for sealing mounts, such as Japanner's gold size, Ashphaltum varnish, Bells' cement, etc., each of which have their own adherents. The author always uses a good quality cellulose enamel of spraying consistency ; however, there are certain quite definite characteristics required of the sealing medium for it to be effective, they are as follows :—

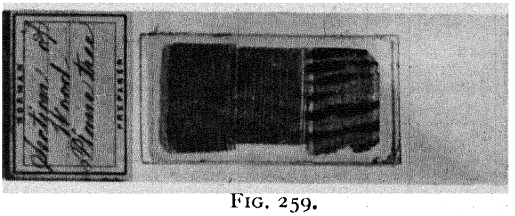


FIG. 259.

(1) The medium must adhere to glass.

(2) When dry it must not show any signs of brittleness, but should rather be tough.

(3) This toughness should be a lasting quality, not subject to alteration with age.

(4) It should be resistant to immersion oil, that is to say, the oil should not attack it in any way.

(5) It should flow rapidly to facilitate application and should be easily thinned if accidentally allowed to become too thick.

From the foregoing it will be seen that whatever the medium, it has to possess certain characteristics, without which it is useless.

Apart from making the finished mount look pretty, the sealing medium acts as a hermetic seal to the contents of the mount by forming a continuous film extending from the surface of the slide to the upper surface of the cover. When it is considered that the finished slide will frequently be polished, thus putting a stress on the mount, tending to push the cover glass off, it will be appreciated that the film will have to stand up to quite rough treatment. These cements are used also for making shallow cells on slides by building

up a ring of cement on the slide itself, they are also used for attaching the deeper cells, made from metal or glass tube, to the slides.

Before applying the sealing ring, all surplus mounting medium must be removed from around the edge of the cover glass. This may be accomplished by the judicious use of a razor blade or (if the mount has been properly prepared with only the right amount of medium) the small surplus may be removed with a small quantity of a solvent for the medium, applied on a piece of cotton wool, or other similar substances; whichever method is adopted, the edge of the cover should be quite free from any surplus medium before sealing is attempted.

The sealing ring is applied by means of a turntable and fine camel hair brush; a typical ringing turntable is shown in Fig. 260. The slide is held firmly by the two spring clips and the cover glass is centred by rotating the table slowly and moving the slide slightly until the cover rotates without any "wobble."

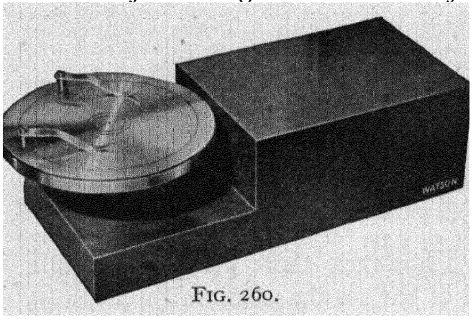


FIG. 260.

The ring is now applied by means of a fine camel hair brush in the following manner. First ascertain by a trial the amount of sealing varnish which the brush is capable of holding, this will vary with the medium and is governed largely by viscosity and surface tension. Having done this, take a brush full and drain off approximately half the varnish by touching the brush on the side of the varnish bottle, then spin the turntable so that it rotates at approximately 80 revolutions per minute, this will be found to give the best results with a $\frac{1}{2}$-in. diameter cover glass and a cellulose paint of spraying consistency. Of course for larger covers the peripheral speed will be greater and the speed of the turntable will have to be reduced accordingly, this is quite easily found by a little experimenting. The next step is to just touch the edge of the cover glass with the loaded brush, which immediately develops a thin ring of paint. If a slight pressure is now exerted on the brush it will spread out and apply a band of paint extending from the surface of the slide and a little way over the edge of the cover in the form of a thin continuous film. No attempt should be made to "paint" the sealing ring on to the slide, but rather must the brush be held quite still and the paint allowed to flow off it on to the mount, the brush merely serving as an instrument for directing the flow of paint and keeping it within bounds. With a little practice a high degree of control is soon acquired. The ring should not be more than about

$\frac{1}{8}$ in. in width with $\frac{1}{32}$ in. or less on the surface of the cover ; if the ring is made much broader than this it begins to look somewhat clumsy and the mount lacks that little extra finish of the craftsman.

After the first application the ring is allowed to dry, and this is where the cellulose paints are very suitable, for apart from being permanent, the film drys rapidly, requiring little more than three or four minutes, and successive coats may be applied in quick succession, the whole operation being completed in from a quarter to half an hour. Fig. 261 shows a section through a completed mount indicating the amount of sealing paint that should be applied.

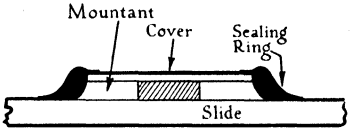


FIG. 261.

After completing the sealing the slide is placed in a dust-free atmosphere for two or three days, so that the ring may dry and harden off, thus leaving the mount in a condition fit for handling.

One point to be considered in connection with the use of cellulose paints is the question of the solvent action of the diluent on the mounting medium. Most cellulose paints employ very powerful solvents, and unless the mounting medium is quite dry and hard, these solvents will attack it and the enamel creep into the mount in the manner shown in Fig. 262, which illustrates the results of trying to rush the completion of a slide and seal it down before it was ready.

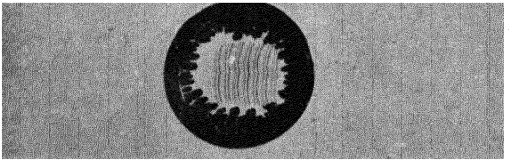


FIG. 262.

When the object to be mounted is too thick for the conventional method of covering with a cover glass in the ordinary way, a cell of some sort must be used for small objects. A very efficient cell is made by painting a series of coincident rings on to the slide, one on top of the other, but where this type of cell is still not deep enough a cavity slide may be used. These are slides which have cavities ground and polished into one surface, they are produced with cavities of various sizes and depths. As a rule the cavities are circular in shape but slides may also be obtained with oval cavities, when long thin objects are to be mounted ; a cavity slide of the circular variety is illustrated in Fig. 263. These slides are of necessity appreciably thicker than the standard 1 mm., the difference in thickness depending on the depth of the cavity ; thus as a rule they cannot be used for high powers owing to the impossibility of focussing a high-powered oil immersion condenser through them, but as the objects generally mounted in them are too thick for high-power

examination, and are usually intended to be examined with low powers only (in which case they would be used with a dry condenser of relatively long focal length), this extra thickness is of no real consequence. A very useful type of cell is that of the plate type, which merely consists of a piece of plate glass with a hole in it cemented to the surface of a standard slide. As illustrated in Fig. 264 the depth of the cell is, of course, the thickness of the plate, and this

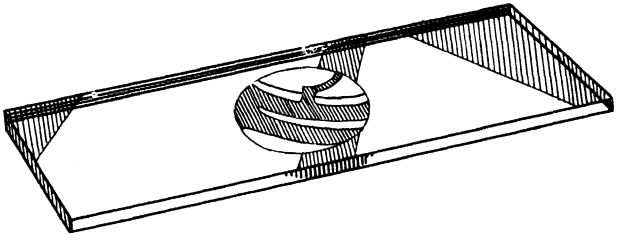


FIG. 263.

type of cell has one advantage over the cavity slide, inasmuch as it may be constructed on a standard 1-mm. slide, thus enabling the use of a high-powered condenser and a larger illuminating cone.

So much then for the types of slides and cover glasses used in preparing, in a permanent manner, specimens for examination, and before proceeding with the discussion on mounting, let us examine briefly the completed permanent mount. It is obvious that if we intend to carry out our work in a scientific and methodical manner

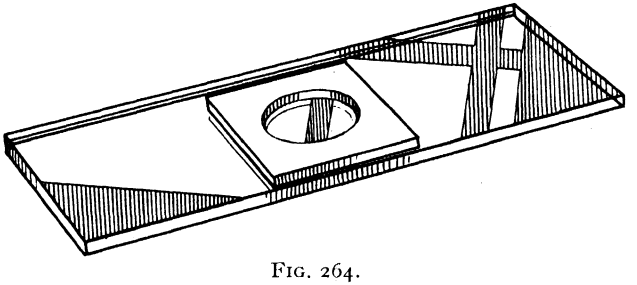


FIG. 264.

the finished slide must bear some legend as to the type of subject concerned and the methods employed in preparation, and this is best accomplished by the use of labels, with regard to which it is to be regretted that far too many slides are prepared and labelled with the bare name of the subject, all information regarding processing methods, mounting medium, etc., being non-existent. One very important item which is seldom, if ever, seen on a slide is the date of preparation. It is the author's opinion that one cannot put enough information about the preparation on the slide itself, as space is so limited; nevertheless there are certain items which may be included, conveying a wealth of information; thus one should

always use a label at each end of the slide, as large as is conveniently possible. The author always uses labels 1 in. square at each end, thus leaving a space of 1 in. square in the centre for mounting, and with the exception of very large objects, this space will be found ample for most requirements. It is advisable to have labels printed specially for oneself so that the information may be laid out to suit. The author usually uses plain 1 in.-square labels on the right-hand side of the specimen, ruled with seven dotted lines spaced $\frac{1}{8}$ in. apart, for the purpose of recording the subject matter relating to the specimen, and it is surprising how much information may be put on a label of this type.

The left-hand label is illustrated in Fig. 265, and as can be seen, contains spaces for a filing number, which incidentally is essential, staining details, description of mounting medium, thickness of cover glass, and the date. The information relating to the mounting medium immediately tells one the limit of resolution which may be obtained. As we have previously seen, the higher the refractive index of the mountant the higher the resolution obtainable. Also a note may be made as to whether the mount is a fluid mount or not, thus immediately indicating that in the former case the slide should be handled with greater care than is necessary for a solid mount. This does not mean to say that solid mounts may be handled carelessly, but rather that one should pay more particular attention to the handling of fluid mounts, as they are much more easily disrupted than those of the solid variety.

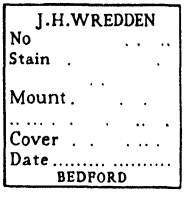


FIG. 265.

The usefulness of information regarding the thickness of the cover glass will also be appreciated, as one may immediately judge whether the higher-powered objectives are capable of focussing through it or not. It also gives a guide as to the correct tube length to employ; it is not essential to state the actual thickness, although this would make the information more useful. The group number is sufficient indication, *i.e.*, whether the cover is No. 1, 2 or 3, etc. The usefulness of a date, in any circumstances, of course needs no emphasis whatever, particularly in this instance.

In the case of stained specimens information regarding the type of stain is of paramount importance, as one is immediately made aware which structures are differentiated and which not, thus before even examining the slide we have quite a wealth of information placed before us. Therefore, let us consider this question of labelling slides as being of prime importance and resolve to place as much information as possible on the finished article.

Having collected a number of slides, labelled with the maximum amount of information, we must obviously have some orderly

method of storing them. This is best accomplished by the use of special slide cabinets, made by all reputable manufacturers of scientific supplies ; three types are illustrated in Figs. 266 to 268. As will be seen from Fig. 266, the cabinet contains a number of trays holding six slides in each, the front of the cabinet being

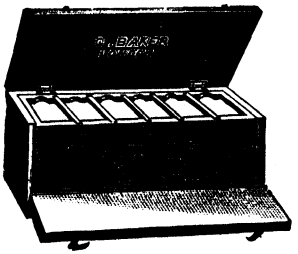


FIG. 266.

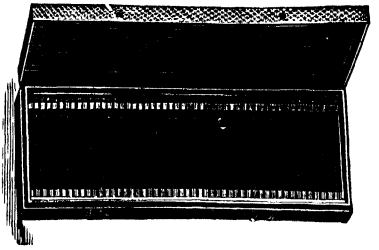


FIG. 267.

hinged so that it lets down, exposing the entire stack of trays, any one of which may be easily removed. This type of cabinet is very popular, as it is possible to pack quite a large number of slides into a relatively small space ; furthermore, if the trays are numbered it is a simple matter (with the aid of numbers on the slides) to start, and keep up to date, a card index system with additional information about each slide on its appropriate card, together with cross indexing arrangements, so that at any time any slide may be found rapidly and with the minimum of information. Another type of cabinet is that shown in Fig. 267, which possesses slotted racks in which the slides are placed on edge. The slots are numbered and, as a rule, there is an index in the lid. This type of cabinet has one slight disadvantage over that shown previously, inasmuch as the slides are stored on edge when the cabinet is laid flat, consequently the contents of any fluid mounts which are free to move would, in time, sink to the bottom of the cell, which in this case means the lower edge. However, this is only a minor point and is quite easily overcome by storing the cabinet itself in an upright position on one end, like a book.

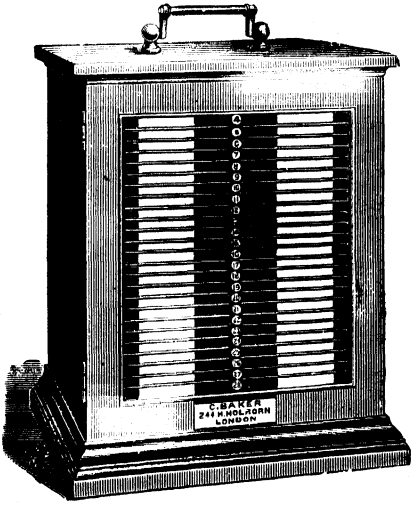


FIG. 268.

A more elaborate cabinet is that shown in Fig. 268, being constructed to hold anything up to 1,000 slides in drawer-type trays, each of which is numbered and possesses two white mat

surface tablets, on which indications as to the contents may be written ; a glass-panelled door fitted with a lock makes the whole collection secure.

Now let us examine the question of mounting objects for examination. We have already seen that if we surround the object with a transparent medium of high refractive index, the resolution obtainable from the preparation increases as the refractive index of the medium rises, but there are certain cases when we have to put up with a medium of lower refractive index than we would like to use in order not to damage the specimen, and it is for this reason, amongst others, that one is compelled to use say a fluid medium instead of a solid medium. We may therefore look upon mounting media as being divided into four classes, thus : solid, gelatinous, liquid, gaseous. The last mentioned, which of course covers the dry mounts when the mounting medium is the atmosphere, we may ignore, as this type of mount is seldom used these days, except in metallurgy. We are then left with the other three types, of which the solid media generally consist of a resinous substance, natural or synthetic in solution in a solvent, although there are some solid media which are used in the solid state and are applied by melting.

The gelatinous media are usually based on gelatine and require a slight degree of heat in use ; the liquid media are numerous and include such substances as liquid glucose, glycerine and water. However, it is not proposed to go into descriptions of the various media, as these are exhaustively dealt with by the authors indicated in the bibliography at the end of the chapter, but rather to deal with the technique generally applicable to each group.

The first group to come under consideration is the solid group. In general, these media are resinous in nature and usually of fairly high refractive index, when compared to the media in the other groups. They are used in solution in a volatile solvent, such as benzene or toluene, thus by nature they are not miscible with water, hence the object to be mounted has to be thoroughly freed from water, a process known as dehydration, after which it is soaked in some of the pure solvent or other similar substance, which by virtue of its high refractive index makes the object somewhat transparent. As a result of this the process is known as clearing, although it should be understood that the object of the process is really to permeate the specimen with a solvent for the mounting medium, so that this latter may rapidly take its place in the object. Thus we see that the object is to be thoroughly impregnated with the mounting medium for the best results, and this principle applies to all mounting media irrespective of the group to which they belong.

Assuming in the first instance that the object is fixed to the slide during processing (as would be the case in a histological section),

the first step in mounting is to remove the slide from the vessel of clearing medium, dry the back surface and place it on the mounting tablet, after which a drop of the mounting medium is run on to the object, before the clearing medium has had an opportunity to evaporate. (This is important as the section must not be allowed to dry before the application of the mountant.) A small drop of the mountant is next placed in the centre of a previously cleaned cover glass which is then inverted and gently lowered on to the object until the mountant on the slide just touches the drop of mountant on the cover, which may then be released. Gentle pressure on the cover, or its own weight, will serve to spread the mountant out in an even film ; this should be applied until the medium just exudes from under the edges of the cover and no more. If the amount of mounting medium used is correct (the only way to judge this is by gaining experience, as obviously the size of the object will dictate the amount of medium to use), the object should now be embedded in the minimum quantity of mountant. The slight excess of the medium exuding from under the edge of the cover is to allow for shrinkage due to evaporation of the solvent ; thus it will be seen that for relatively thick objects there should be more excess, as the shrinkage due to the greater volume of mounting medium used will be greater than for thin objects.

The slide is now set on one side to dry. The time for this may vary from one or two days to as many weeks, depending on the volatility of the solvent used with the mounting medium. However, the longer a mount can be left at this stage the better, as the presence of solvent in the medium generally has the effect of lowering the refractive index and the more chance the medium has to develop its maxiumm refractive index the more efficient will the mount become from an optical standpoint. Some workers advocate the use of a spring clip applied to the cover glass during this drying period in order to ensure the thinnest possible mount, but it is the author's experience that, with the exception of tough springy objects such as flat insect mounts, the spring pressure applied to the mount during this drying period is liable to do more harm than good, even going so far as to disrupt the object.

The author has found that polystyrene dissolved in benzene functions is an excellent mounting medium ; objects so mounted are ready for ringing in a matter of one or two days. One advantage of this medium is the ease with which the surplus may be removed prior to ringing ; this is accomplished by slitting the surplus medium across from the edge of the cover to the outside edge of the overflow, then if the surplus is lifted with the edge of a razor blade on one side of the slit it may be peeled off in its entirety leaving the edge of the cover quite clean and ready for the application of the sealing ring.

Polystyrene is not a new medium, having been tried in the early days of the development of plastic materials, and at that time there were many objections raised regarding its poor adhesive properties. Actually, the adhesion of the pure resin is bad, but this is greatly improved if it is plasticised, and it is the author's experience that if the mount is sealed, after one or two days' drying no trouble is evident in this direction, the reason being that whereas the surplus resin is hard and relatively brittle, the resin in the mount itself still contains an appreciable quantity of the solvent which has the effect of plasticising it, and if it is sealed in this condition, the solvent is prevented from evaporating and the resin never becomes quite hard. The poor adhesive properties of this medium are an advantage when it comes to cleaning the finished mount, as evidenced by the ease with which the overflow may be stripped away. Its refractive index of 1·59 is quite satisfactory. Furthermore it is neutral, and if the solvent used is also acid free, the objection to acidic media is done away with.

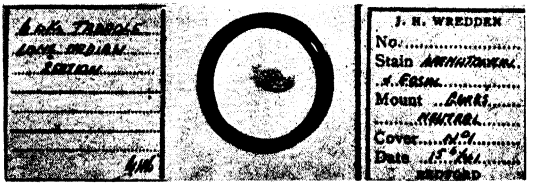


FIG. 269.

Thus we have gone over the process of mounting a prepared object up to the point of cleaning and sealing the mount, all that remains to be done is to label the slide when the ring is quite dry and index the subject. As the labelling is the last operation and takes place after much handling of the slide, we must have some method of identifying the slide so that the labels may be filled in correctly. Some workers recommend that identification particulars should be scratched on the slide with a diamond point, and this of course is the best method, as the identification is permanent, and if, as sometimes happens, the label comes off, the slide may be re-labelled without the chance of a mistake occurring, However, for those who do not possess a diamond, a very good method consists of writing the details on the slide in Indian ink, after having thoroughly cleaned the surface. It is surprising how much rough handling this will stand before becoming unreadable. Having labelled the slide, the finished article should look something like that illustrated in Fig. 269.

So much for the mounting of thin objects. When it is desired to mount thick objects without undue pressure, as in the case of insect mounts, the technique is the same but the objects will need to be mounted in a cell deep enough to take them. In this case it becomes necessary to fill the cell with the medium, cover it so that the covered cell is completely full and contains no air bubbles.

Perhaps the best method of carrying this out is to first fill the cell with the object in place, then place a small drop of the mountant in the centre of the cover and gently lower the cover on to the cell. It must be remembered that the outside diameter of the cell must be the same as that of the cover glass.

The gelatinous media are as a rule based on the use of gelatine, the most widely used being the well-known "glycerine jelly." This is set at ambient temperatures but liquefies at about 40° or 50° C., hence for mounting in this medium heat must be employed. Albeit, the temperature is low and not likely to damage most objects, although a number of delicate objects are damaged by it; it is, however, an excellent medium for quick preparation as all one requires is a hot plate kept at the required temperature, upon which the bottle of jelly is placed, likewise the slide. Objects may be mounted direct from glycerine or dry, but the author recommends that dry objects be given a preliminary soak in the molten medium before mounting, this avoids the production of bubbles. As soon as the mount is made it may be put aside to set overnight, after which it may be ringed and finished off in the usual way. Glycerine jelly is a very useful medium to use in cases where the solid media are unsuitable owing to the solvent affecting the object. For example, the author was confronted with the problem of mounting some varnished silk yarns and it was found that the resinous media affected the varnish on the yarn, thus producing misleading results. The use of glycerine jelly, however, solved the problem successfully.

It is frequently found, particularly with delicate aquatic organisms, etc., that a liquid medium is th only means of mounting if the organism is not to be damaged. There are numerous liquid mounting media, some used for temporary preparations and others for permanent fluid mounts. Here the technique is much the same, with the exception that the ring is applied immediately the mount is made, and greater care must be taken to use only sufficient of the medium to just reach to the edge of the cover glass. In this way the difficulty of cleaning away surplus fluid is avoided and the application of the ring made easier. Care must also be taken in the actual applying of the ring, as a slight excess of pressure with the brush will move the cover and probably ruin a fine mount.

A very useful medium is obtainable from most chemists in the form of liquid glucose. It has a refractive index of $\mu = 1{\cdot}49$ and is soluble in water; it also possesses a characteristic of developing a relatively hard surface film on exposure to air. This is a help when it comes to ringing the slide.

This medium has been of use to the author in the mounting of wax films. These were made on the slide and cooled very slowly,

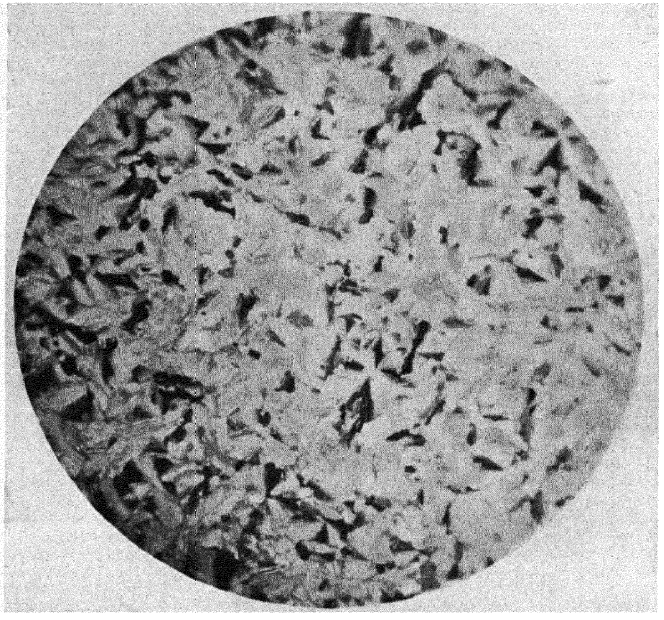


FIG. 270.

[*To face p.* 249.

as a result of which the film was very delicate. The ordinary solid media were unsuitable as the solvents affected the film, the gelatinous media were unsuitable because of the heat necessary for their use and the fluid media had too low refractive indices, with the exception of the liquid glucose, which proved to be admirably suited to the making of permanent preparations of these films. An example of these wax films is illustrated in Fig. 270, which shows the effect of slow-cooling chlorinated naphthalene ; the photomicrograph is at a magnification of 115 diameters and was taken by polarised light with the system crossed.

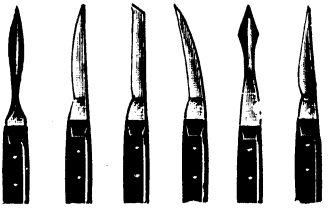


FIG. 271.

So much then for the mounting of objects, now let us consider briefly the question of the preparation. All objects have to be prepared in some way or other before mounting, the method of preparation depending on the object and the structure which it is desired to show. Complete treatises have been written on the subject of preparation alone, and it is not proposed to go into any great detail, but rather to indicate the processes involved for the standard methods ; for more detailed descriptions the reader is referred to the bibliography.

The first consideration when commencing to mount objects for microscopical examination is that of tools. Most workers naturally develop their own particular technique and evolve tools for the purpose, but there are a number of instruments which are fundamentally necessary. A variety of knives such as those shown in

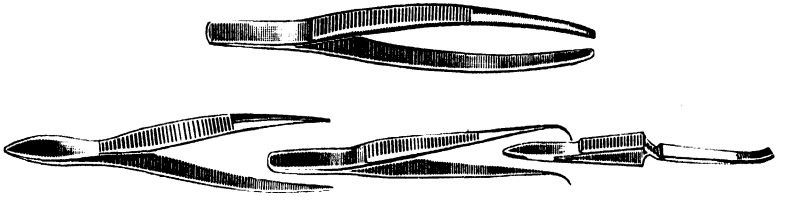


FIG. 272.

Fig. 271, for example, are always useful, likewise it is useful to keep a supply of blade-shaped surgical needles at hand, together with screw-grip holders for them, as these needles may quite easily be ground into small knives of various shapes and uses. An assortment of forceps or tweezers, such as those shown in Fig. 272, are most necessary for different purposes, as also are various types of scissors, some of which are shown in Fig. 273.

A very useful implement which will be found to be constantly in demand is a pair of bone forceps Although these were originally designed for cutting bone, the shape and strength enables them to

be used for divers purposes. These are illustrated in Fig. 274. A number of seekers of the type shown in Fig. 275 will also be found useful.

For handling small organisms such as protozoa, etc., a number of dipping tubes of various kinds, such as those shown in Fig. 276,

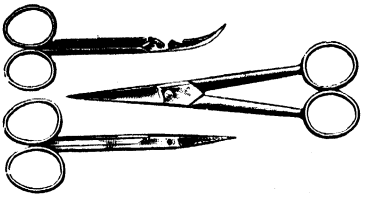


FIG. 273.



FIG. 274.

are necessary. These are used by placing the finger over one end of the tube and immersing the other end in the liquid containing the organisms. If the immersed end is then brought close to one of the animals and the finger rapidly removed from the other end and replaced, some of the liquid will be forced up the tube, taking the organism with it. The tube may then be removed from the liquid,

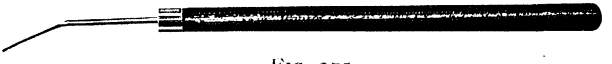
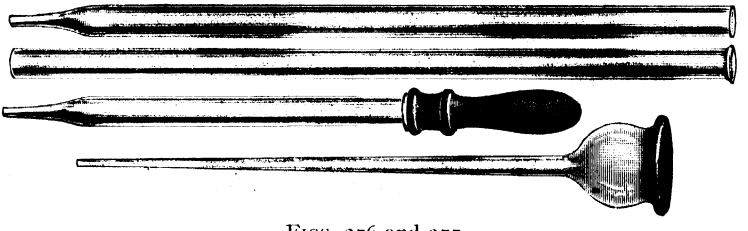


FIG. 275.

together with the contents, which may be emptied into another vessel, by the simple expedient of removing the finger from the upper end. In this way a single amœba or paramœcium may be transferred from solution to solution in perfect safety. Another type of dipping tube is shown in Fig. 277; this consists of a thistle funnel with a fine drawn-out stem. Over the mouth of the funnel



FIGS. 276 and 277.

is stretched a piece of thin indiarubber which acts as a diaphragm. Slight pressure of the finger is sufficient to draw up enough fluid to pick up single organisms. It is advisable to press the diaphragm down and immerse the tip of the tube, not releasing the pressure until the wanted organism is in the required position. Other accessories include such things as test tubes, drop bottles, watch glasses, slide holder, brushes, section lifters, etc., and for those undertaking

histological work a set of dissecting instruments is necessary, and indeed it is surprising how useful is a set of these instruments in other sections of the science. The foregoing list is not intended to be taken as complete, but merely as an indication of the type of appliance required. As has been stated, most workers develop a liking for certain particular implements, and very often either develop their own designs or improve on the standard types.

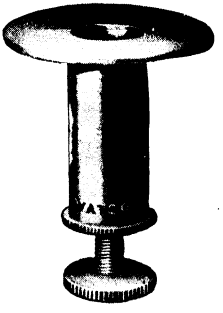


FIG. 278.

The various techniques adopted as standard for mounting various objects may be classified under three main headings, thus :—

(1) That involving the cutting of thin sections applied mainly to animal and plant tissues.

(2) The mounting of entire objects, with or without pressure, such as insects.

(3) The cleaning and mounting of diatoms, foraminifera, radiolaria, etc.

The first-mentioned method is probably the most widely used, as the majority of biological preparations consist of sections. For preparing these specimens it is necessary to use some method of cutting very thin slices of the tissue to be examined. This is accomplished by means of a piece of apparatus known as a microtome.

There are many types of microtome; the simplest consists of a piston screwed into a tube whose free end is fixed into a flat plate,

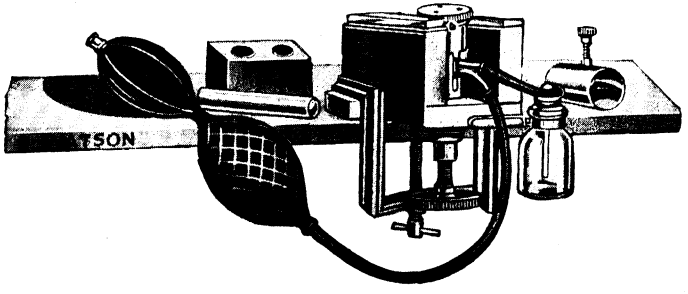


FIG. 279.

such as that illustrated in Fig. 278. The knurled head at the bottom of the instrument moves the piston up or down the central tube when rotated in the appropriate direction. The specimen is fixed to the flat top of the piston and sections are cut by drawing a razor over the flat upper surface of the instrument. This is a simple hand microtome, but is nevertheless quite useful when very thin sections are not desired.

A more efficient instrument is to be found in the " Cathcart " microtome. Fig. 279 shows such an instrument, together with its

accessories. It is all metal, is comparatively cheap and simple to operate. With it, sections may be cut from specimens embedded in pith, carrot or paraffin ; it also has an ether freezing attachment for cutting frozen sections. It will be noticed that the razor is held in a carrier which slides on two rails, the thickness of section is regulated by the " click " register on the knurled head used for raising the specimen.

A modification of this instrument by Watson is shown in Fig. 280, illustrating the Cathcart-Darlaston microtome. This instrument, while possessing the simplicity and excellence of the Cathcart

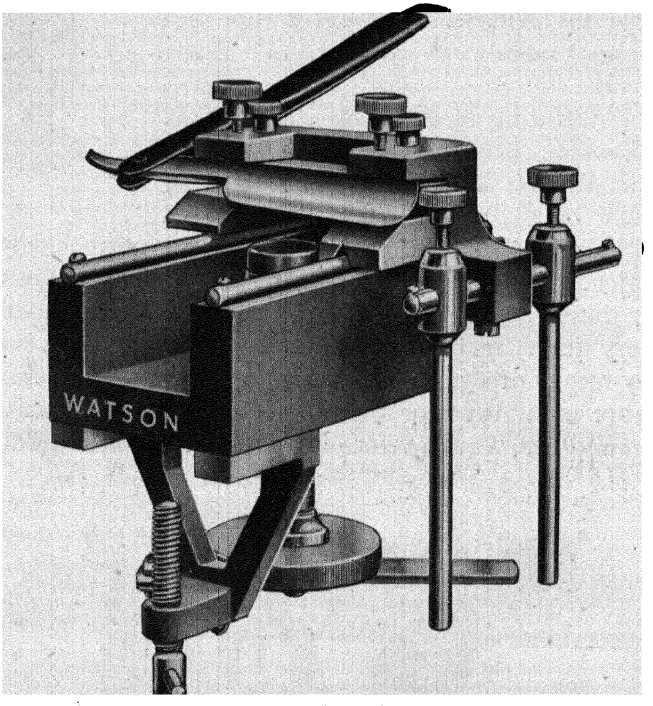


FIG. 280.

pattern, yet enables the microtomist to cut sections of a definite thickness. The modifications consist of the addition of a mechanism for automatically advancing the specimen for any pre-determined distance. This is accomplished by the forks attached to the knife carrier which, in being drawn back, causes one of the arms to engage with the lever which works on a milled head through a spring pawl, thus raising the specimen a given distance, depending on the position in which the fork has been set. The forward cutting stroke engages the other arm of the fork with the lever, which is now returned to its original position, so that all one has to do after mounting and setting the fork is to draw the knife carriage backwards and forwards and sections of a definite and uniform thickness are cut.

The microtomes just described are of the simple type, and not really suited to the rapid production of large numbers of sections. In cases where much section cutting is carried out, such as in the pathological departments of large hospitals, something more robust and reliable is required. This has been well catered for by various manufacturers in the types of fully automatic mechanical microtomes now procurable. Perhaps the most popular of these instruments is the well-known "Cambridge" rocking microtome, illustrated in Fig. 281. It is deservedly popular on account of its simplicity of construction and efficiency in action; its accuracy and reliability are due to the avoidance of the use of slides and bearings which might wear or become loose. The material to be sectioned is carried downwards across the knife, which is held with its edge upwards, by means of a rocking movement. The rocking arm to which the

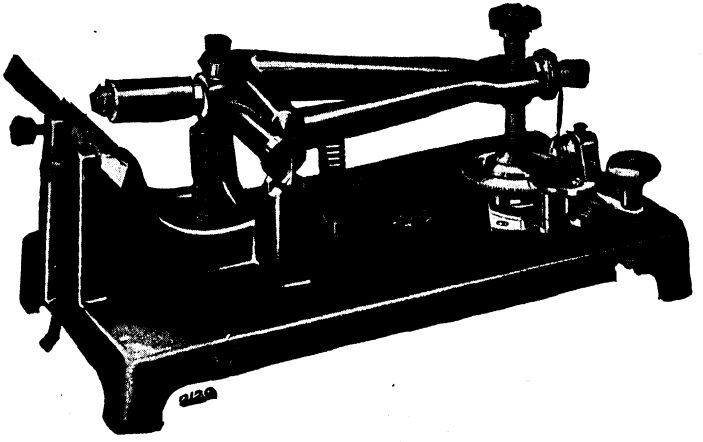


FIG. 281.

material is fixed turns on knife-edge bearings, ensuring an exact and smooth action to the arm carrying the bearings just mentioned. The operating handle which actuates the rapid movement of the material past the knife also controls its forward advance through a pawl and screw movement. The advance of the specimen for each section is controlled by a stop, which can be set for any desired section thickness by means of a scale having twelve divisions, each division causing an advance of 2 microns.

Owing to the rocking movement of the arm carrying the specimen, it will be seen that it travels past the knife edge on the arc of a circle, therefore the section is cut on this arc and is not truly flat, and in order to overcome this slight objection the "Cambridge" flat-cutting microtome was produced. This instrument, illustrated in Fig. 282, was designed for cutting perfectly flat sections from material embedded in paraffin. It is capable of cutting sections

3 cm. square, the thickness being variable from zero to 20 microns, the production of ribbon serial sections being quite simple. Automatic compensation for wear in the essential mechanism ensures the instrument remaining in adjustment over considerable periods.

The mechanism is similar to that of the rocker microtome, the specimen being fed forward automatically by the rotation of a micrometer screw, effected by the action of a pawl on a milled disc fixed to the base of the screw, the pawl being operated by a reciprocating handle.

In this instrument, rotation of the micrometer screw raises one end of a horizontal arm pivoted on knife edge, thus feeding the specimen forward by an amount proportional to the rotation of the screw. The adjustment for this is obtained by adjusting the time

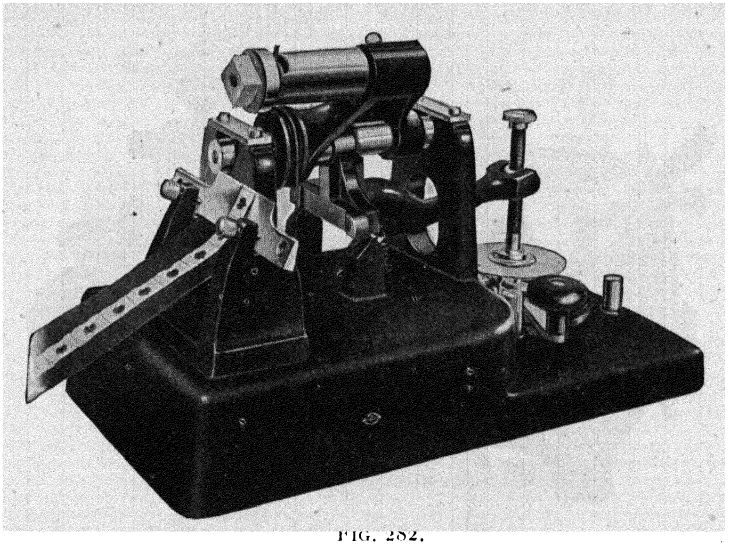


FIG. 282.

of duration of contact between the pawl and milled head, by means of an index pointer moving over a scale divided in microns, the entire mechanism is free from backlash, and the knife holder is of very rigid design. This instrument shows an advance on the original rocker, and is fast becoming as popular.

Another type of high-grade instrument working on a somewhat different principle is illustrated in Fig. 283; this is known as "Rotatome" and is typical of rotary microtomes in general, although this particular instrument is of rather novel design inasmuch as the whole mechanism rotates. The rotating system which revolves in phosphor bronze bearings is balanced, and in operation the specimen is carried past the knife edge in a circular path and the section is cut without vibration or sudden jar. The thickness of the section is regulated by an adjustable cam on the rotary mechanism and may be varied from zero to 36 microns, indicated on a direct reading scale.

Altogether this instrument is of first-class manufacture and is capable of withstanding considerable use without going out of adjustment.

Before sectioning, the material must be prepared in a suitable manner. In the case of living tissues, this involves much processing, commencing with killing and fixing, followed by dehydration and clearing, thence to the embedding medium, in which it is cast so that it may be properly cut. The object of this processing is to thoroughly impregnate the tissues with the medium, usually paraffin wax or "celloidin," so that the delicate structures are supported during the cutting, ensuring that there is no tearing action as would occur during the cutting of untreated tissue. After cutting, the sections are flattened and stuck to slides ready for after treatment, which might involve gradual hydration down to water when staining operations are carried out, followed by gradual dehydration and

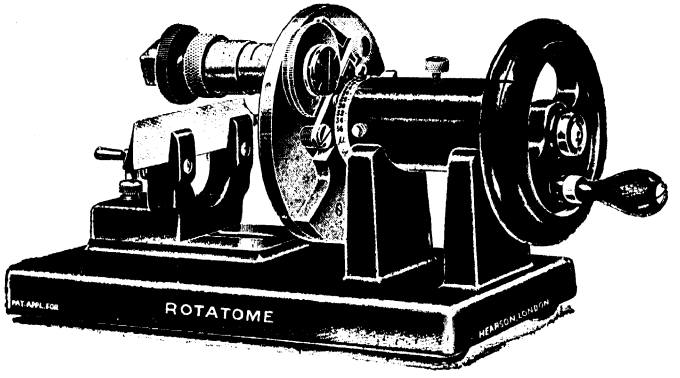


FIG. 283.

clearing, after which the section is mounted in one of the resinous media.

All these processes of dehydration and hydration must be very gradual in order to avoid damaging the tissues by the violent shrinkages set up if the process is rapid. The usual method is to commence with a 50 or even 25 per cent. alcohol/water mixture and pass the material through, gradually ascending strengths of alcohol up to 100 per cent. The material is then transferred to a medium which is mixable with paraffin, the most popular being xylene, which removes the alcohol and at the same time makes the material relatively transparent, hence the term "clearing." The tissue is next placed in molten paraffin at a temperature of about 55° to 58° C.; the maximum permissible temperature is 60° if damage to the tissues is to be avoided.

After the tissue is thoroughly impregnated, it is cast into a solid block of paraffin by means of the L-shaped moulds shown in Fig. 284. The moulds are first placed on a glass plate or some similar material

and set to form a base of the required dimensions, which is filled with molten paraffin, after allowing sufficient time for the formation of a reasonably thick layer on the bottom; the object is next placed in the mould and orientated by means of warm needles. The next step is to blow on the top of the molten paraffin until a skin appears on the surface of such a thickness that when the mould is tilted slightly the liquid paraffin under the skin does not break through. When this is successfully accomplished, the entire assembly, consisting of the mould, together with its contents, and the glass plate on which it rests, are plunged as quickly as possible into a large volume of cold water. When immersing the mould it is advisable to tilt it slightly so that the water flows into the box from one end, levelling out and completing the immersion in one steady movement. When the paraffin is quite solid it is easily removed and the block trimmed ready for the microtome. The reason for cooling the paraffin suddenly is to cause it to solidify rapidly, thus avoiding the large crystalline structure consequent upon slow cooling. This is the most generally used method, but there is a school of thought which advocates the use of slow cooling, that is to say, the mould is allowed to set at room temperature. The devotees of this method claim that the wax cuts much better after this treatment, but the author's experience is that this latter method leads to excessive shrinkage and a less compact mass than that produced by shock cooling.

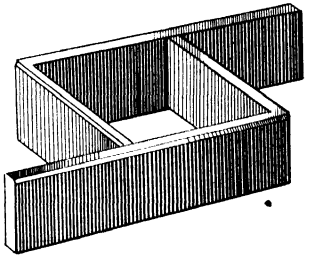


FIG. 284.

The foregoing remarks describe, very briefly, the processes involved in the paraffin embedding technique. Another method of obtaining sections of fresh tissue fairly quickly is by the freezing technique. This may be applied either to freshly excised tissue or previously hardened material; they are then soaked in a gum syrup (Johnson, "Microscope Objects" (E.U. Press), gives 2 pints gum acacia, 1 pint syrup), of which there are many formulæ, and frozen on a microtome equipped with the necessary apparatus, such as the ether apparatus of the "Cathcart" microtome, or one of the special instruments made for the purpose which use carbon dioxide. The method of cutting is as follows: the plate of the microtome is covered with a layer of syrup and the material transferred to the plate, the whole is then subjected to freezing action, adding more syrup as it freezes, until the material is embedded in a frozen mass of syrup.

The tissue is now ready for sectioning in the usual way. This method requires a little more care than other methods, as it is just

as easy to over-freeze, when the sections either roll up tightly or break up, due to excessive brittleness, as to under-freeze, when they are torn by the knife instead of being cut cleanly. These faults are, however, more easily remedied than with other methods, the first-mentioned requiring the material only to be allowed to thaw out until the sections come off clean and flat, the second fault obviously requires further freezing, the main point being that somewhere between the two conditions lies the correct one. It is quite easy, after some practice, to be able to freeze the mass to the right consistency every time.

After cutting, the sections are allowed to soak in distilled water until all the gum has come out. It is advisable to change the water several times, using a little and often rather than a lot with only a few changes. After soaking they may be stained immediately after fixing to the slide or stored in alcohol. If they are immediately stained, the subsequent treatment is dictated by the type of mount to be used, *i.e.*, whether solid or gelatinous, or fluid ; usually they are mounted in either solid or gel mounts, fluid mounts being seldom used in these days. If a solid mount is desired, then the section must be dehydrated and mounted as previously described. If, on the other hand, the section is to be mounted in glycerine jelly, for example, then dehydration is unnecessary, but care must be taken to ensure that the section never becomes dry during the process. A good plan is to transfer it from water to glycerine and thence to the mounting medium.

This method of sectioning is perhaps the most beautiful of them all and is certainly productive of some of the finest results, as the treatment of the material causes little if any damage due to shrinkage, and furthermore, the finished result may be produced in a matter of a few minutes. For example, sections of diseased tissue may be examined by the surgeon only a few minutes after excision, thus helping in an immediate diagnosis during the course of an operation. These temporary preparations are quite easily made permanent by mounting them in the glucose mounting medium previously mentioned. Perhaps the only criticism is the inability to cut sections much below 5 microns by this method.

The third embedding method is that employing " celloidin." This substance is a pure form of cellulose nitrate of a low degree of nitration, it is soluble in a mixture of ether and alcohol and solutions of the medium in this solvent are used for impregnating and embedding the material to be sectioned. The solid material may be purchased either in the form of shreds or slabs ; in the latter case the slabs have to be shredded, therefore it is advisable to purchase the shredded material which is used to make up two solutions, the one containing 6 per cent. and the 3 per cent. celloidin. These are dissolved in half the required solvent volume of ethyl

alcohol, the other half of the volume consisting of ether is added after about 24 hours.

The processing for celloidin embedding is much the same as that for paraffin ; that is to say, the material is carefully dehydrated up to absolute alcohol, whence it is transferred to a mixture of alcohol and ether in equal proportions for a period of 24 hours, after which it is transferred to the 3 per cent. celloidin solution where it is left as long as possbile, preferably not less than 48 hours. The material is then transferred to the 6 per cent. celloidin solution, where it is left for a period of several days ; some material may even require weeks or months before it is completely impregnated. In this case it is again better to leave the material to soak for the longest possible time. When this process is complete the material is ready for sectioning and is now placed on a small block of wood, which has previously been prepared with some of the 6 per cent. solution, and left exposed to the atmosphere for a short time, after which more celloidin is added and allowed to dry slightly, the process being repeated until the specimen is well covered, just as in the preparation for the freezing method. After the celloidin has dried somewhat, the block together with the attached material is placed in a 60 per cent. alcohol/water mixture in order to harden the celloidin ; this mixture is also useful for storing blocks so prepared.

To cut sections, the block is fixed in the microtone and the sections cut in the usual way. There are one or two points to bear in mind when cutting cellodinised material, the knife must be orientated so that it takes an oblique cut and should be kept well moistened with 60 per cent. alcohol. Each section, as it is cut, should be placed in 60 per cent. alcohol until required for mounting, when it is fixed to the slide with albumen and stained and mounted in the usual way.

So much then for the methods adopted to prepare thin sections of tissues. The foregoing descriptions are very brief and intended merely to convey some idea of how these sections are cut and the processing involved. The subject is dealt with in detail by many experts in this particular line, and for the reader who is desirous of pursuing the subject further, the bibliography at the end of this chapter gives suggestions for further study.

Before proceeding to the next section, the author would like to point out that although the methods of sectioning material just described are generally looked upon as belonging to the field of medical and biological work, he has found them very useful in the industrial sphere.

Examples of the application of wax embedding technique to industrial uses will not be amiss, the following being an indication of the type of work in which wax embedding will be found of use.

The example concerns the examination of some of the insulating tubing used so much in the electrical industry, in particular by the manufacturers of radio apparatus. The question affecting the issue was the water absorption of this material: one type consisting of rolled oiled silk over which was a cotton braid; another type being the conventional varnished cotton braid. In the case of these two types the moisture absorption was greater than expected, and it was decided to try and find the reason for this by means of an examination of the structure as seen in transverse section.

In the case of the braided rolled silk, the cotton braid appeared to have been treated with some kind of binding agent such as a lacquer or size. Although bearing in mind the purpose for which the tubing was designed, it was highly improbable that this agent would be composed of such a hygroscopic substance as a starchy size. However, whatever the composition of the material, its purpose was to produce an homogeneous moisture-proof outer covering for the inner tube of rolled varnished silk, at the same time forming a comparatively strong mechanical sheath and a ready means of applying distinctive identification colours. The result of sectioning this material is shown in Fig. 285, which shows a photomicrograph of a portion of a transverse section at a magnification of 48 diameters. The braid, together with its filling, is quite clearly seen, likewise the varnished silk in section. The reason for the excessive moisture absorption becomes apparent on examining this photograph; firstly the layers of varnished silk are separate, which indicates that the roll of silk (which consists of three layers) is loose inside the braid tube, whereas the varnished silk should be made with a slight surface tack which would result in the layers of material adhering firmly together, due to the rolling. In this way there would be no spaces between the layers which would allow ingress of moisture, as in the case of the specimen under consideration. The next point is the condition of the braid; here the filling medium which is seen in the dense black areas is anything but evenly dispersed through the structure of the braid. One large gap is seen where the filler has penetrated the braid and is adhering to the surface of the outermost layer of the silk material in two places, with a large gap in between them. The presence of this gap would allow considerable quantities of moisture to enter the structure of the sleeving

The filler itself is pigmented, hence its opacity, and this serves a useful purpose inasmuch as it shows up the condition of the cotton yarn of which the braid is made up. These are seen as large relatively light areas in the mass of the filler, thereby showing that the filler has not penetrated the interstices of the braid. The cotton itself, being a very absorbent medium, is thus unprotected and free to absorb moisture, so that we see here the reason for the excessive moisture absorption of this material.

In the same manner a section of the other type of sleeving (varnished cotton), shown in Fig. 286 at a magnification of 48 diameters, served to point out the weaknesses of the material. In this case the varnish was applied to the outside of the braid, and the illustration clearly shows that this lies and functions solely as an external coating of no great depth, while the cotton masses of the braid are by no means impregnated with the varnish but left entirely free, except for a thin portion of the exterior surface. This of course results in excessive moisture intake both by absorption and capillary attraction, and it will be appreciated that if the cotton masses were completely impregnated and embedded in a solid layer of varnish, then moisture could not enter the structure of the material due to the varnish barrier.

Apart from the foregoing remarks, a further interest in these two illustrations lies in the processing methods evolved to produce the sections. As has been stated, they were made by the wax-embedding technique, but the standard technique had to be considerably modified in order to produce the results shown. It was not possbile to dehydrate the specimens in the normal manner, using alcohol of varying strengths followed by clearing in xylol, because these substances would have attacked and destroyed the varnish film in both cases and in the case of the first example the pigmented filler would have suffered, therefore the samples were dehydrated by the stoving at a temperature of 110° for twelve hours (this long time was chosen in order to make quite certain of the drying), after which they were transferred direct to the paraffin bath running at 60° C., and in order to facilitate the thorough impregnation with the wax, the impregnating vessel was exhausted with a water pump to 29 in. of mercury and impregnation was considered complete when bubbles ceased to emerge from the specimens ; this was accomplished in about two hours. The specimens were then cast into blocks and sections cut in the usual way, finally being mounted in a solution of polystyrene in benzene, and finished off as described previously.

We see how by modification and adaptation, a technique generally recognised to belong almost exclusively to one section of the science may be usefully applied to help in the work of another section. The paraffin-embedding technique, if handled well, is capable of producing beautiful results, as shown by the structures illustrated in Figs. 287 and 288. The former is a transverse section of a portion of the stem of a flowering plant of the sunflower variety, photographed at 100 diameters, while the second illustration is that of a transverse section of a leaf of an acacia tree at the same magnification. Both of these structures are delicate, particularly that of the leaf, but they do not seem to have suffered damage due to the processing, the wax embedding medium giving ample support during the cutting of the sections. It is always sound policy to use

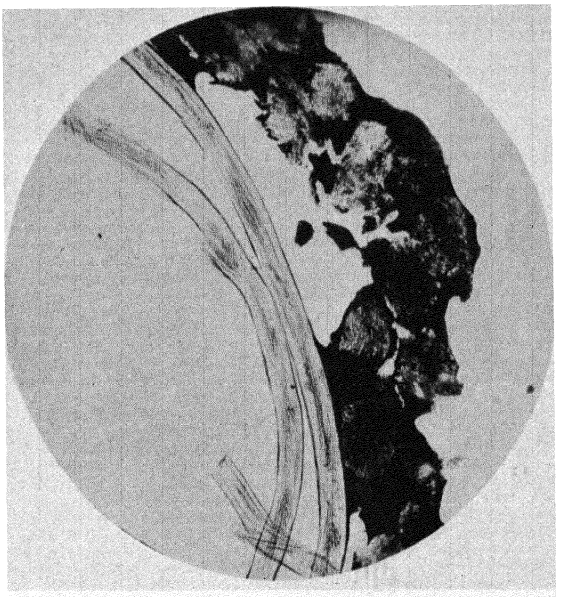


FIG. 285.

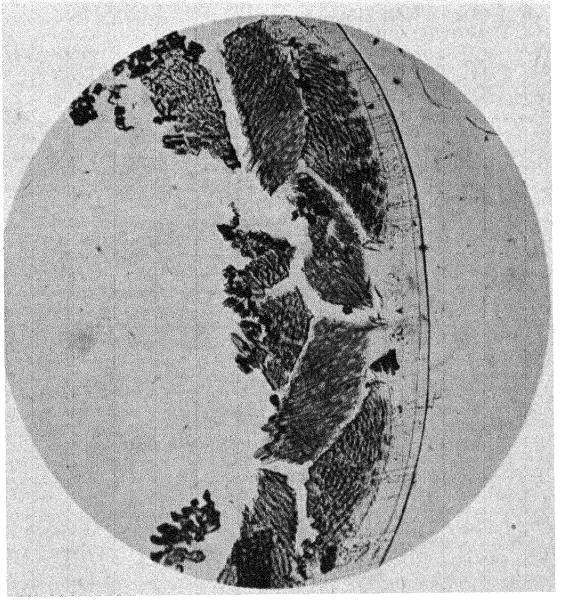


FIG. 286.

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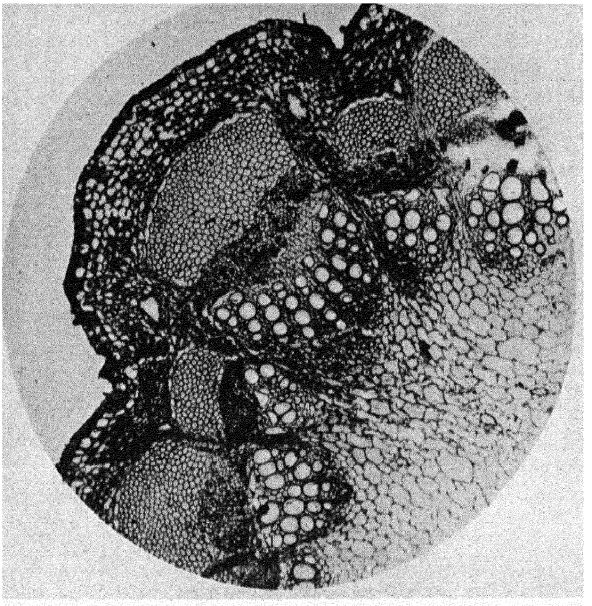


FIG. 287.

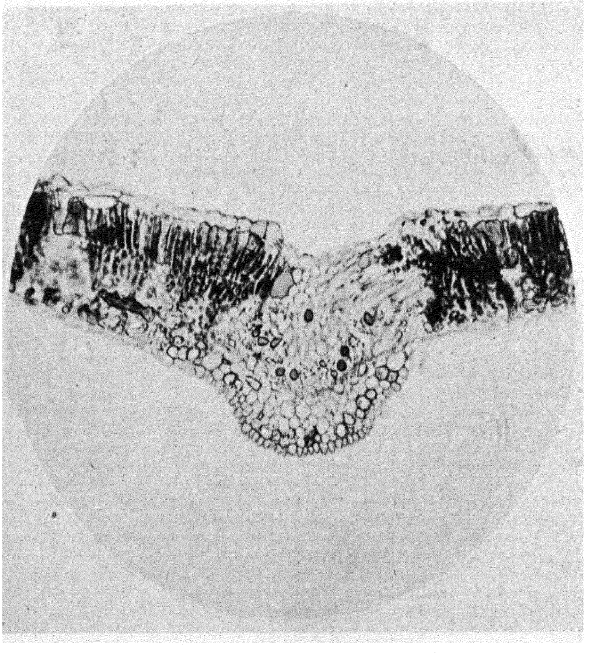


FIG. 288.

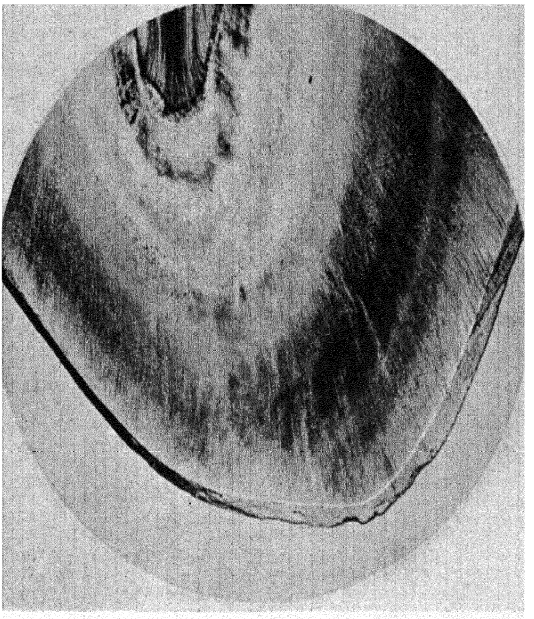


FIG. 289.

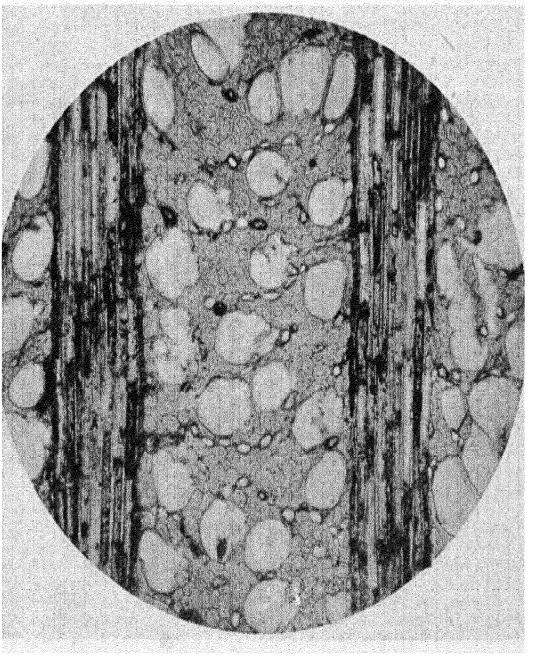


FIG. 290.

the wax technique whenever possible, although there are certain occasions when the heat is undesirable ; for example, some embryological tissue containing much yolk material. In such circumstances one is forced to employ other methods, such as celloidin technique, but with these other techniques it must be remembered that it is not possible to obtain such thin sections as with the wax method.

In many cases it is desirable to make thin sections of hard or brittle substances which are too hard to cut on a microtome ; these include such things as fossils, coal, rocks, bones, teeth and the like. In these circumstances a different technique has to be adopted to attain the desired result.

The most efficient method of making thin sections of hard substances is by grinding down comparatively thick sections until they become so thin that they are transparent to light. In general, the first step is to obtain as thin a section as can be safely cut with a fine hack-saw. Most substances will respond to this treatment, but in cases where the specimen is too hard for the saw it is necessary to use an instrument such as the lapidary's disc (this consists of a thin disc of soft iron whose edge is charged with fine diamond dust), or similar apparatus ; however, having obtained our basic section, as it were, we next proceed to face up one surface. This is accomplished by cementing the specimen to a slide with Canada balsam and rubbing the uncemented face down on succeedingly finer grades of emery cloth, giving a final polish with one of the metallurgical polishes, such as " Diamantine." The next step is to remove the specimen from the slide by dissolving away the balsam, after which it is recemented to the slide with the polished face towards the glass surface. The specimen is now ground down and polished in the same way as just described, great care being taken when the section becomes really thin, as it is liable to commence breaking up if the grinding is taken too far. The best plan is to watch the section carefully during the process, using the microscope if necessary, stopping the grinding when the first signs of transparency become apparent. The specimen is then finished off by polishing with " Diamantine " and water on a sheet of plate glass. When the desired thickness is obtained, the specimen is carefully dried, a drop of mounting medium applied to its surface and covered in the usual way, the mount being completed as previously described.

In cementing the specimen to the slide face, the most satisfactory medium is dried Canada balsam. This has to be melted and the specimen cemented to the slide while the balsam is still molten. Care must be taken during this process to avoid any bubbling of the balsam due to overheating ; if this occurs it is best to remelt and cement afresh.

Many substances, such as sedimentary rocks, are too soft and crumbly to be handled in this way, but successful sections may be

produced if the specimen is thoroughly impregnated with balsam before cementing, the object of this being to support the structure in much the same way as in wax embedding. This is best accomplished by soaking the specimen in benzene after which it is transferred to a solution of the dried balsam in benzene and left to soak until thoroughly impregnated. The specimen is then placed on a slide, covered with some of the solution of balsam and put on one side until all the solvent has evaporated; the specimen is then gently baked until the balsam is quite hard. The grinding and polishing operations may then be carried out in the normal manner.

The foregoing method of making sections is capable of producing very beautiful results. One good example is shown in Fig. 289, which illustrates a photomicrograph of the tip of a human canine tooth at a magnification of 67 diameters. This is a portion of a transparent section through the entire tooth made by grinding down. The result of the grinding treatment applied to a soft tissue is shown in Fig. 290; this illustration is a photomicrograph of a portion of a thin section of wood from a plane tree. It is one of the structures which requires impregnation with balsam before sectioning can be attempted; the result of careful processing is well shown in the excellent preservation of the structural details of this delicate tissue.

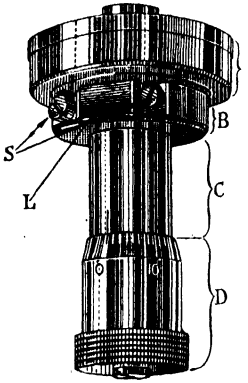


FIG. 291.

This method of sectioning is used extensively in petrological studies and is looked upon by many as being characteristic of that branch of the science but, just as the pet histological technique was made use of in other fields, so the grinding technique may be used to advantage by other than petrologists. The author has used this method to advantage for the examination of plastic materials and succeeded in developing a technique using what might be termed a "grinding microtome," whereby sections of known thickness may be obtained. This little instrument is illustrated in Fig. 291. As will be seen, it consists of a modified engineer's micrometer action, the main parts being a flat circular head-piece "A," which is made in two pieces. The upper disc, made of hardened high-speed steel, is fixed to the lower portion of the head by screws; immediately below the head is the collar "B," which is followed by the barrel "C," this latter is fitted internally with a nut having a standard micrometer screw thread, which takes the micrometer screw "D"; to the free end of which is fitted a plunger "P," which works in a hole bored in the head and collar. Thus, by screwing the micrometer screw right out, the upper surface of the plunger is brought to a position some $\frac{1}{8}$ in. or so above the

upper surface of the hardened steel head. Both the surface of the plunger and the head are ground with the micrometer screw locked in position by the locking device " L," actuated by the screws " S.," so that the zero mark on the micrometer head coincides with the datum line on the barrel.

To use the instrument, a slice of the specimen about $\frac{1}{16}$ in. thick is cemented to the surface of the plunger with balsam and allowed to thoroughly cool. The plunger is then screwed back until the surface of the specimen projects slightly above that of the head, the plunger is then firmly locked and the whole instrument now functions as a convenient jig and holding device, by means of which the specimen may be accurately rubbed down to a pre-determined level. This is done by rubbing on a medium-cut file until the file appears to be slipping on the hardened steel surface of the head, then the rubbing is continued on a fine-cut file until this slips on the head, thus indicating that the surface of the head and the specimen are coincident. If it is so desired, the specimen may be finally polished by raising it a fraction of $\frac{1}{1000}$ in., as indicated on the micrometer head, after unlocking the micrometer screw. It is then re-locked and very gently the specimen is polished on rouge paper fixed to a glass plate. Great care must be exercised in this as the rouge paper will also take off some of the hardened steel surface of the head, thus upsetting the calibration of the instrument (the files of course have no effect on this surface), instead the author relies on using a fine file which has seen some wear, as this produces a reasonably good polish. However, as the after-treatment of the specimen includes a polishing process, the use of rouge paper with the instrument may be left out entirely.

Having completed this process the specimen possesses one flat and smooth surface and it must now be removed from the plunger, preferably by dissolving the balsam away, and re-cemented to it with the treated surface adjacent to the surface of the plunger. After the instrument has cooled, the specimen is treated in exactly the same way as just described, except that the fine file may be left out for the time being, the medium cut file only being used until it slips, at which point the plunger is unlocked and the specimen raised slightly and relocked, the rubbing process being repeated until the slip occurs again, when the specimen is raised further. It will be seen that we are taking off pre-determined amounts of the specimen in this way and at all times have the thickness under our direct control. The micrometer head gives the thickness of the specimen at the completion of any rubbing down operation, directly in thousandths of an inch and when it comes down to five thousandths, care must be exercised as the specimen is by this time becoming somewhat delicate. Therefore, we now resort to the fine cut file and take off one thousandth at a time until the specimen

is about two thousandths of an inch thick, in terms of metric measurement this is about 50 μ, and if it is desired to mount the specimen at 30 or 40 μ (as is sometimes required) the rubbing down process should not be carried any further, but the specimen should be removed and finally polished and rubbed down with diamantine, after having been mounted on a slide, as previously described, finally it may be washed and mounted in the usual way.

If, as frequently happens, the secimen is to be mounted at about 10 or 15 μ, or in some cases even as low as 5 μ, then the rubbing down with the micrometer may be taken to a thickness of one thousandth of an inch or less. Although the author has succeeded in taking a specimen down to half a thousandth on the micrometer, he recommends a minimum thickness of one thousandth with this instrument, as one has to proceed with extreme caution at these thicknesses for fear of breaking up the specimen. It is far better to stop at one thousandth or even one and a half, and finish the rubbing down in the orthodox way, the great advantage of this being that after the final polishing the specimen is not disturbed, but mounted in situ, as it were.

This instrument was originated by the author as a means by which sections of plastic materials such as synthetic resin, bonded paper and fabric, and phenolic mouldings and the like may be made and studied in a short space of time. The instrument is simple to use and requires very little skilled handling, sections may be prepared with it in a matter of an hour or so after a little practice. In this respect, the instrument has proved itself to be of great use in the study of the aforementioned material, in the course of which some excellent sections were produced.

In one instance a particular phenolic moulding was giving trouble by breaking on assembly and the filler in the moulding powder was suspected. This was called for as a pure cotton filling, but on examining a section of the moulding it was found to be filled with a mixture of cotton and wood flour. This is clearly shown in Fig. 292, illustrating a photomicrograph of the section under consideration at a magnification of 100 diameters the cotton fibres may be quite clearly seen in longitudinal and horizontal section, together with large woody masses, thus proving definitely that the filler was not as called for and at the same time indicating the reason for the mechanical failure. This illustration shows the value of this method in checking the composition of bakelite mouldings the one or two hours spent in preparing the sections being amply justified.

The usefulness of this method when applied to the study of resin bonded paper commonly known as "paxolin," is illustrated in Figs. 293 and 294. These are photomicrographs of thin sections of a high grade and low grade material, taken at 100 diameters.

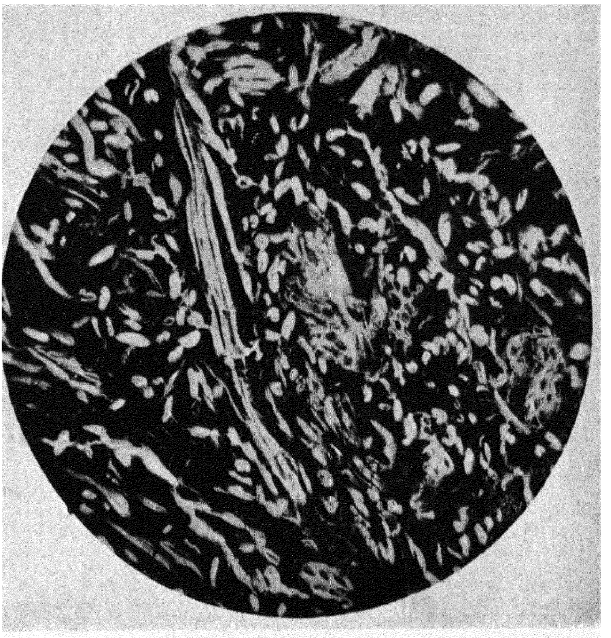


FIG. 292.

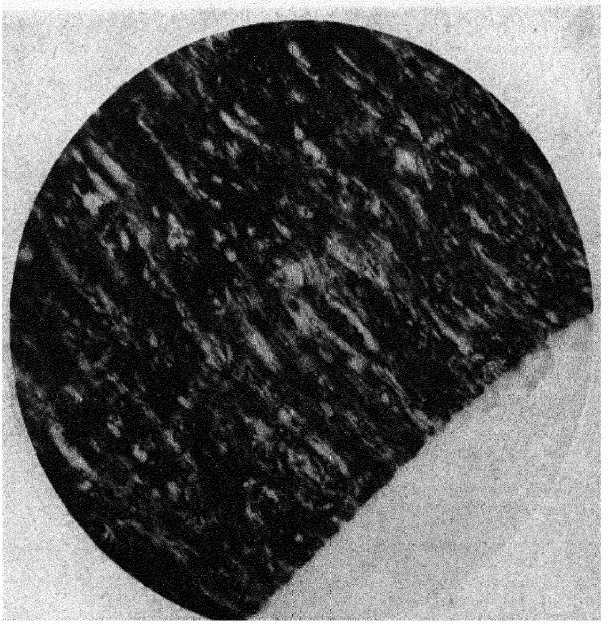


FIG. 293.

[*To face p.* 264.

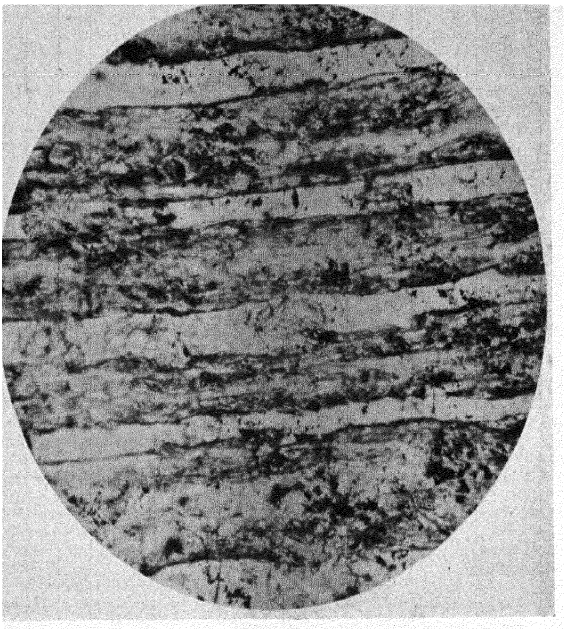


FIG. 294.

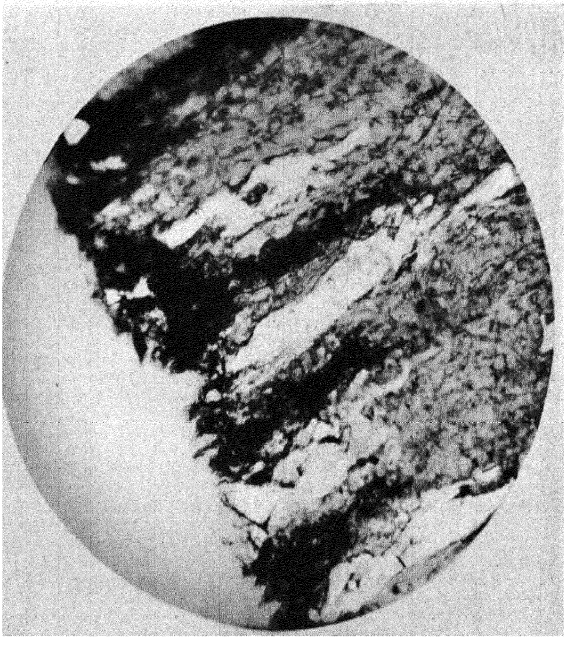


FIG. 295.

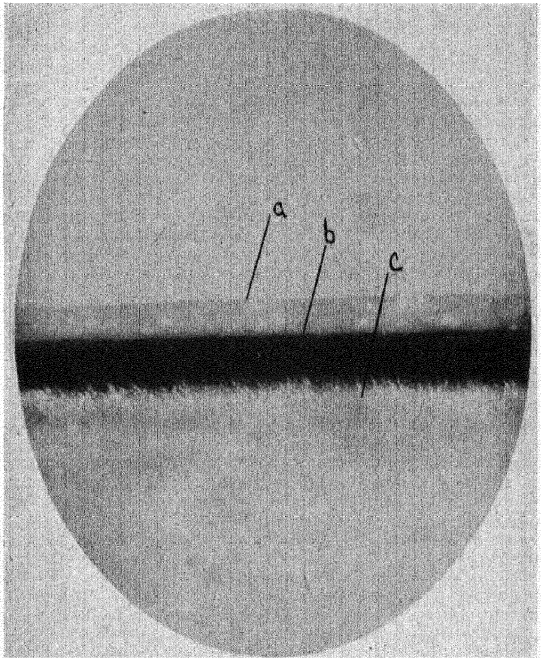


FIG. 296.

That shown in Fig. 293 is a high grade material made by curing under heat and pressure. As can be seen the material is compact and as homogeneous as possible, the layers of paper are so closely packed as to be almost indistinguishable one from the other. In direct contrast to this, we have a low grade material in the form of a tube, a transverse section of which is shown in Fig. 294. This material is heat cured only, no pressure of any consequence being used. Here we see the layers of paper quite distinctly separate, with large spaces between them, another important point brought to light in this illustration is the presence of large bubbles in the resin masses between the paper layers. Needless to say these have a deleterious effect on the electrical properties of the material, the open structure is, of course, bound to have a pronounced effect on the moisture absorption of the material, as the paper being in such a loose state will absorb more moisture than would be the case in the compact structure. In the study of this subject, it was decided to try and show that the moisture was preferentially absorbed by the paper and not the resin, accordingly a specimen of the material was immersed in a 10% solution of methylene blue in distilled water for twenty-four hours, and a section made. The result is shown in the photomicrograph at Fig. 295, again at 100 diameters. Here the depth to which the dye has penetrated can be clearly seen, also the fact that it has penetrated entirely by way of the paper layers, thus conclusively proving the point.

The foregoing examples are only a few of many showing how use has been made of a processing method developed originally for other purposes. It has been described in detail in the hope that other workers may be led to develop and originate methods of processing to suit particular circumstances.

We cannot leave this subject of sectioning without mention of the processes adopted for metallurgical specimens, although this process does not involve the preparation of a thin section, it does deal with the preparation of a surface and as such is justifiably included under the grinding technique. Briefly, the process consists of rubbing down and polishing the surface of the piece of metal to be examined in the same manner as explained for ground sections, the object being to produce a flawless polish on the surface, and in order that the surface of the metal may be disturbed as little as possible, the polishing is carried out wet.

In the great majority of cases, the metal is of a convenient size to grip comfortably in the finger, if not it should be cut down to the appropriate size. The surface is then rubbed down on files of increasing fineness. In such cases the rubbing should be carried out in one direction only, that is to say when starting with the coarse file, the specimen should be rubbed down without twisting it, until an evenly scored surface is obtained. The rubbing on the

next grade of file should be in one direction, but with the specimen turned through a right angle. Thus the new score marks will cut those of the previous file at 90°. This rubbing is continued until all the marks due to the previous rubbing are obliterated, this process is repeated for each grade of file or emery cloth right down to No. ooo emery. The specimen is then polished on a wet lap polishing machine using diamantine or magnesium oxide, until a flawless polish is produced. The lap should be kept wet with a liberal supply of water so that the specimen may be polished with the least disturbance of the surface due to heating.

When the desired polish has been reached, the specimen is ready for etching. This consists of treating the surface with a chemical reagent which reacts with certain constituents and not with others, thereby rendering the structure of the metal clearly visible. It is not proposed to go into the question of the technique of etching, as this is a science on its own and the reader is referred to the many specialised works on the subject for details of the technique, sufficient to say that without etching or treating the polished surface in some way or other, the structure of the metal is not seen.

In certain circumstances where the specimen is small and difficult to hold in the fingers, it may be embedded or moulded into a block of " Bakelite " although the use of one of the polymethyl-methacrylate moulding powders will probably be found to be more beneficial as it is glass clear and the specimen is quite easily seen and its orientation noted. This technique of moulding a specimen into a plastic was modified slightly by the author to produce some interesting results dealing with the examination of the enamel on enamelled wire. It was decided to use the aforementioned clear plastic in the form of " Diakon Granules "*. As the straightforward moulding technique was unsuitable due to the pressure and heat (1·5 tons at 130° C.) involved tending to disrupt the enamel film, a moulding technique was evolved employing no pressure and very little heat, using a mixture of " Diakon " granules and the monomeric liquid methylmethacrylate, which when mixed form a thick syrup. The wire suitably held was immersed in this syrup in a crucible and stoved at 60° C for some three or four hours, this resulted in the solidification of the contents of the crucible with the wire firmly moulded in situ. This method does not result in a clear block of the plastic but a block containing masses of small bubbles, this is a useful factor as will be explained subsequently.

The block of material is removed and treated exactly as for a metal specimen until either a transverse or longitudinal section of the wire is obtained, depending on how the specimen is orientated. Thus the relationship of the enamel film to the conductor is easily

* This is the trade name of a product supplied by Messrs. I.C.I.

examined. This is demonstrated in Fig. 296 which is a photomicrograph of a longitudinal section of some enamelled wire taken at 490 diameters. The enamel coating in this specimen is made of two coats of different materials, the coat shown by the dark band is a conventional enamel covered by a coating of " Nylon " shown by the light band in the centre. The broader bright band at the bottom of the illustration is the surface of the copper, the diffraction at the crystal faces of which may be seen by the splashes of light along its edge. So once again we see how one of the more or less exclusive processes has been adapted to meet the needs of another branch of the science.

Having dealt with the first heading of our mounting methods, we may now proceed to the second which deals with the mounting of entire objects with or without pressure, the objects to which this type of mounting is applied are generally insects and microscopic flora or fauna, both terrestial and aquatic.

A large number of insects are mounted in Canada balsam, after having their soft internal organs entirely removed, thus leaving only the external chitinous skeleton structure. This is flattened with its various components such as legs, head and antennæ arranged in the correct position. The processing is somewhat lengthy but the results amply justify the time and trouble expended in preparation.

Briefly, the insects are stored in alcohol, after killing, until wanted, from whence they are transferred to water and left to soak until the alcohol is removed. They are then placed in a 10% solution of caustic potash until they are quite soft, this depends entirely on the size of the insect and is best learned by experience. After this softening treatment the potash is removed by repeated washing in successive changes of clean water (it is important that all the potash be removed as the presence of even minute quantities in the finished mount will give rise to the formation of crystals which will spoil the mount). After washing the potash out the specimen is transferred to glacial acetic acid where it may be left in store until required for further treatment, which consists of transferring it again to a dish of water and, using dissecting needles and camel hair brush, carefully positioning the limbs and other parts without breaking up the specimen. The next step is to expel the contents of the thorax and abdomen by gentle pressure downwards towards the tail. When the body cavities are quite empty the specimen is carefully transferred to a slide and all excess water drained off, after which the limbs, antennæ, etc., are carefully arranged in their final position, a glass slide is next placed on top of the specimen and pressed down until the insect has been flattened. The slides are next tied together and immersed in alcohol until the specimen is dehydrated, here again the time required is best learned

by experience, but the longer one can leave the specimen the better.

After dehydration the slides are removed from the alcohol and very carefully separated, the specimen is then floated in a dish of alcohol, in which it is allowed to remain for an hour or two to make quite certain that dehydration is complete. The specimen is then placed in a dish of clearing medium, such as Xylol, Cedar Wood Oil, Oil of Cloves, etc. (extreme caution must be exercised in handling the specimen at this stage as it is in a particularly brittle and friable condition, it is advisable to employ a camel hair brush and section lifter. This is one of the processes requiring a certain amount of skill which is only acquired by practical experience).

Having cleared the specimen it is only necessary to transfer it to a slide and mount in balsam or polystyrene in the usual way, exercising the required degree of careful handling; in fact, in dealing with this type of specimen it is advisable to adopt as a maxim that all mechanical processes involving the actual handling of the specimen be carried out with extreme caution, right up to the final operation of covering. After mounting, the slide is put away to harden off and then sealed and finished off as previously described.

The method just described does not take into account occasions when it is desired to mount the entire insect without flattening it, for this a different technique is required, and the author would like to say that in his opinion this is the best method of mounting insects, as it does not distort the specimen in any way, if properly carried out, thus enabling the subject to be studied more easily and in truer perspective.

For this method the specimen has to be very carefully set out and dried, which process alone requires some skill and patience. It is then transferred to a dish containing absolute alcohol, great care being taken not to disturb the setting out in any way. The specimen is left to dehydrate in the alcohol for three or four hours and then taken to a phenol-xylol solution for clearing (this solution consists of a 25% solution of absolute phenol crystals in xylol) which is accomplished in from one to two hours, after which time the solution is replaced by pure xylol, from which the specimen is mounted in balsam or polystyrene.

It will be noticed that the treatment prior to mounting is somewhat simpler than that in the previous process. The crucial point of the process in this method is the actual mounting, which requires practice and skill to accomplish successfully. Put briefly, the specimen is carefully transferred from xylol and positioned in the centre of a clean slide, the greatest care has to be exercised in this operation owing to the risk of damaging the specimen (which is extremely brittle and delicate) by breaking off legs or wings. Very carefully place two or three drops of the mounting medium on

the specimen, it is advisable to slightly warm the slide before applying the medium as this helps it to run under the specimen. The next step is to place three small pieces of glass, whose thickness is very slightly less than that of the specimen, around the specimen and in the mounting medium so that when the mount is covered the under surface of the cover glass just touches the top of the object with the glass supports within the periphery. In this case the cover is prevented from damaging the specimen by excessive pressure and yet the object is lightly held and prevented from moving out of centre.

The mount is now left to stand for twenty-four hours, more medium being added under the edge of the cover to make up for shrinkage after which the mount is set aside to harden off and then finished off in the usual manner.

Those interested in this method of mounting will find that the time and trouble expended in acquiring the comparatively high degree of skill required will be amply repaid by the beauty of the final results.

The next heading to come under consideration is the cleaning and mounting of diatoms. This is classed under a separate heading as the technique of handling these beautiful specimens has developed into a science of its own, impossible to describe fully in a short paragraph or two, therefore we will discuss the subject briefly, dealing with the more salient features, referring to the many comprehensive works on the subject for further details if required.

Diatoms may be described as minute unicellular plants inhabiting both fresh and salt water. They secrete a silicious skeleton, the structure of which has been the cause of much conjecture and argument, as they are most beautifully patterned with apparent perforations of exceedingly small dimensions. The mounting of these skeletons after they have been thoroughly cleaned of all organic matter, constitutes the art of handling diatoms.

The first point to consider when dealing with this subject is the mounting medium. The refractive index of diatom silex is 1·43 and that of Canada balsam is 1·526, and we have seen that in order to get maximum visibility and resolution the refractive index of the medium must be as high as possible above that of the object, therefore, we see that when mounted in balsam the diatoms will be nearly invisible due to the small difference in refractive indices. This is quite easily shown by the " index of visibility " which is obtained by multiplying the difference of the two refractive indices by one hundred, thus :—

$$I_v = 100\ (\mu m - \mu o)$$

where I_v is the index of visibility.

μo ,, ,, refractive index of the object.

μm ,, ,, ,, ,, ,, ,, mounting medium.

Thus for the case under consideration

$$I_v = 100\ (1{\cdot}526 - 1{\cdot}43)$$
$$= 9{\cdot}6$$

On comparing this with a substance of high refractive index such as Realgar ($\mu = 2{\cdot}549$) we get an index of visibility of 111·9. Thus we see the reason for the search for mounting media of higher and higher refractive index; in many cases, as with realgar, the process is difficult and dangerous hence these substances have not come into general use. However, a few comments on the more common media will not be amiss:—

Canada Balsam ($\mu = 1{\cdot}526$).

This medium is not very much used as its refractive index is too low.

Styrax ($\mu = 1{\cdot}582$ European) ($\mu = 1{\cdot}63$ American).

The two types of this material are natural resins, the European variety being obtained from the true *Liquidambar Orientales* and the American type from *Liquidambar Styraciflua*, it is used in the same way as Canada balsam, *i.e.*, in solution in benzene, or xylol and is as easy to use, producing permanent mounts. The American variety with an index of visibility of 20 is slightly better than its European counterpart, which has an index visibility of 15·2. These resins are useful inasmuch as the refractive indices (particularly that of the American) allow the resolution of most diatoms.

Hyrax ($\mu = 1{\cdot}71$).

This is a synthetic product suitable for diatoms of somewhat finer structure, its index of visibility of 28 gives it a decided advantage over the previously mentioned media. It is used and applied in the same way, the only drawback being that great care must be taken to have no impurities in the mount as then the mount is not permanent.

Syrax ($\mu = 1{\cdot}8$).

This is another synthetic product and is perhaps the best of the more easily worked media, its high refractive index and index of visibility ($I_v = 37$) being eminently suitable for the mounting of diatoms. It is slightly more difficult to use, having a tendency to bubble when heated, if not carefully watched, this is one of the newer media and up to the present appears to produce permanent mounts.

Realgar ($\mu = 2{\cdot}549$).

This medium is an arsenical compound and is both difficult and dangerous to use, but in spite of this its high refractive index

has made it of great value in the study of very fine diatom structure and expert mounters prefer it to any other.

The foregoing brief survey of mounting media will serve to convey some idea of the time and trouble expended in the study of diatom structure, and an equal amount of energy has gone into the question of preparatory treatment.

Before commencing to prepare a batch of diatoms it is always advisable to check and see whether the batch is worth further treatment, the best and quickest method is to incinerate a small portion of the diatomaceous material on platinum foil or talc in a bunsen flame until all organic material is burnt away, leaving the diatoms clean and white. These may then be examined under the microscope and general conclusions drawn as to whether the remainder of the batch is worth proceeding with. If this is the case the batch of material should be placed in a porcelain evaporating dish and boiled up with hydrochloric acid until the contents turn a dark brown colour. Effervescence of the contents of the dish indicates the presence of chalk and other mineral substances and these must be put into soluble form by the addition of acid until all effervescence has ceased, indicated by no reaction on the addition of a small quantity of acid, which will now be in excess. When this stage has been reached the diatoms should be left in the solution for twenty-four hours longer, in order that any debris still adhering may be mascerated away, after which they are stirred up and strained into a boiling tube, using a coarse straining material such as muslin.

The specimens are next treated with sulphuric acid, but before doing this they must be thoroughly washed free of all inorganic chlorides, as these, if present in the form of calcium chloride (from chalk or limestone in the original material) during the sulphuric acid treatment, will be precipitated as insoluble sulphates on the diatoms and ruin the specimens. Therefore the washing has to be very thorough and is best carried out by decantation; the boiling tube is three parts filled with distilled water and the contents stirred up, they are then allowed to settle, after which the supernatent fluid is carefully poured off and the tube refilled, repeating the process until such time as a small portion of the liquid shows no cloudy white precipitate upon the addition of a drop or two of tenth normal silver nitrate solution. This indicates that the solution is now free of soluble chlorides, at this point the tube is allowed to stand overnight to allow the contents to thoroughly agglomerate at the bottom, after which the liquid contents are decanted off leaving the diatoms in a mass at the bottom of the tube. Concentrated sulphuric acid is now carefully poured drop by drop down the side of the tube on to the mass at the bottom. At this stage the mass at the bottom of the boiling tube consists chiefly of the

diatoms and sand, together with some organic matter and the sulphuric acid is used to remove the last traces of this latter, which is indicated by the colouring of the acid which chars the organic matter. The addition of a small crystal of potassium bi-chromate will destroy the charred organic matter leaving a solution greenish-yellow in colour. As a result of the reaction a considerable rise in temperature will be noticed in the contents of the tube, which should now be stood on one side to cool, after which the tube is emptied into a large volume of cold water in a beaker or some such vessel, the washing by decantation is then carried out as before until the water is quite free of acid, as shown by the absence of a white precipitate when tested with a drop or two of barium chloride solution. It will be found that at least six washings are necessary to effect this. The diatoms should now be clean and seen as a glistening white layer on the bottom of the beaker, after having been allowed to settle, they may now be stored in distilled water or alcohol until required.

The foregoing description applies to fresh or live material, a large part of the study of diatoms is carried out on fossilised material usually in the form of diatomaceous earths, which require somewhat different preliminary treatment. The earth first of all has to be broken up into fine particles without destroying the fossil diatoms; obviously this cannot be done with a pestel and mortar, so other means have to be used and perhaps the best method is the use of a super saturated solution of sodium acetate. A small portion of the earth is placed in a strong flask (preferably of Pyrex glass) together with approximately six times its volume of crystalline sodium acetate to which is added a small quantity of water in the proportion of about five parts water to 100 of the sodium acetate. The contents are then thoroughly boiled up until all the sodium acetate is dissolved, this forms a highly super saturated solution of the salt which will remain liquid if cooled carefully while protected from vibration. When cold the addition of a small crystal of sodium acetate will cause the contents of the flask to solidify accompanied by the evolution of much heat and considerable shrinkage after cooling, this process should be repeated by reheating the flask and re-crystalizing until the diatomaceous earth has been completely broken down into a fine powder.

When the breaking down process has been completed, the flask is three parts filled with water and the contents thoroughly stirred and allowed to settle, after which the supernatant liquid is decanted off and the sludge washed with several changes of water, as previously described. The sludge is then treated with dilute hydrochloric acid to remove impurities and washed again, after which the sediment is treated with concentrated sulphuric acid added by pouring it down the side of the flask drop by drop

and shaking the whole vigorously so as to thoroughly mix the contents, which are then bleached by heating the flask and adding crystals of potassium chlorate one by one, until the mixture is colourless. The heating is continued until the mixture fumes, it is then allowed to cool, followed by the careful addition of distilled water a drop at a time down the side of the vessel. The whole is now well stirred and the solid matter allowed to settle.

The sediment is now washed as before and a small quantity of distilled water added (about 50 c.c.) followed by the addition of about ten drops of a 5% solution of caustic potash. The mixture is boiled up as rapidly as possible, the diatoms are allowed to settle and the liquid poured off. The flask is then filled with distilled water and rapidly stirred for some time and allowed to stand. This has the effect of putting the debris into suspension while allowing the diatoms together with any sand which might be present, to settle to the bottom of the vessel. The suspension is next poured off and the sediment washed again, after which it is passed through a 300 mesh sieve which separates the diatoms from the sand.

Thus we see that the treatment of diatoms both fresh and fossil requires a fairly involved technique requiring practice and skill in its application. The foregoing descriptions are of necessity brief and merely indicate the type of work involved; there are other methods of obtaining the same ends, some more difficult than others, but all lead to the same ultimate objective—that of producing the diatoms in a perfectly clean condition free from any extraneous matter and ready for mounting.

Early mounters used to frequently mount diatoms as dry mounts but this method does not lend itself to high resolution for obvious reasons, and has accordingly been superseded by mounting in one of the aforementioned media. To mount in this type of medium the phial in which the diatoms are stored should be well shaken up before they have had time to settle again, a small drop containing some of the specimens is taken up in a fine dipping tube and released on to the surface of a perfectly clean cover glass where they will spread out evenly. The fluid is then dried off by the application of gentle heat, keeping the cover glass quite still the while. This is best accomplished by placing the cover on a hot plate immediately after the diatoms have been deposited thereon from the dipping tube. After the cover has become quite dry it is gently transferred to the surface of a piece of flat talc or platinum and with the aid of a bunsen flame brought to a dull red heat for a short time, this effectively fixes the diatoms to the cover.

To mount in any of the resinous media some xylol is placed on the burnt cover and allowed to thoroughly penetrate the diatoms so that all air is removed. While this is soaking a drop of the medium

is placed in the centre of a clean slide, the cover is then gently lowered on to the drop of medium, after which the mount is transferred to a hot plate whose surface temperature is at about 60° C. where it is left for from twelve to twenty-four hours, after which time it may be removed and the mount completed in the usual manner.

The foregoing methods of mounting result in what are known as strewn mounts, that is to say the diatoms whether mixed or of a single variety are strewn more or less evenly over the surface of the cover glass, the expert mounters go further than this and mount selected diatoms. Thus there may be two or three diatoms in the centre of the mount with a small ring painted on the cover glass to show their position. This process obviously requires the use of an auxiliary microscope and a good deal of patience and skill besides a fairly wide knowledge of the various type of diatoms (running into thousands) from which the specimens have to be identified.

Another method of mounting employed by the older specialists was to mount groups of selected diatoms arranged in various patterns on the slide. This practice has unfortunately, more or less, died out and these slides mounted by the old masters are rapidly becoming rare.

We have now examined the three main methods of preparing permanent mounts, there are of course numerous slight modifications of these where the demands of special cases have to be met, regarding which it would perhaps be as well to examine methods used for the examination of substances in thin films produced otherwise than by sectioning methods. This type of mount is probably more widely used in the examination of blood and similar substances than for anything else.

The method of preparing the film is simple, a drop of the material is placed towards one end of a clean slide and the cleaned edge of another slide is brought into contact with it, so that this latter makes an angle of about 45° with the surface of the slide on which the drop is placed. The immediate effect of touching the drop with the edge of the spreading slide is to cause the fluid to spread out along this edge and all that remains is to draw the edge along the surface of the slide, which process results in the spreading of a very thin film on this surface. The film is then ready for further treatment such as drying, fixing, staining and mounting, depending on the nature of the specimen.

The examination of substances in thin films was applied to the study of waxes by the author with some quite interesting results. It was desired to examine the structure of various waxes and the modifications brought about by different conditions of cooling. The examinations were made in thin films, with the aid of polarised light, which showed the crystalline structure quite clearly.

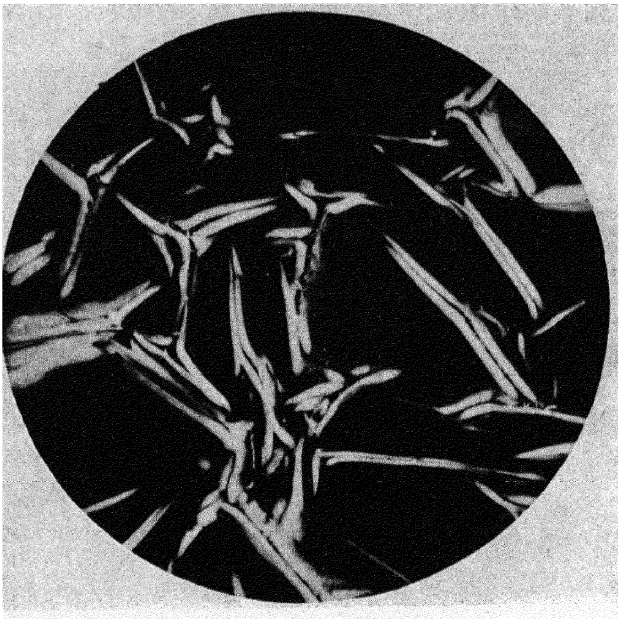


FIG. 297.

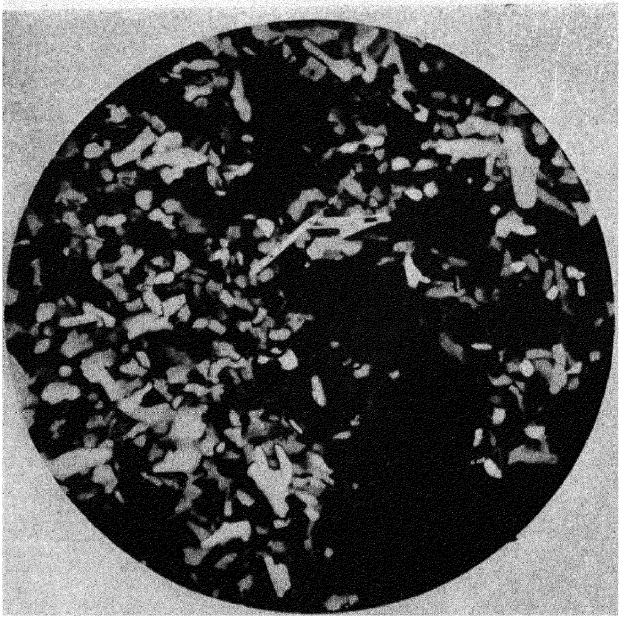


FIG. 298.

[*To face p.* 275.

For example Fig. 297 shows a thin film of paraffin wax (melting point 52° C.) which was cooled on the surface of the slide from a temperature of 150° C. down to 40° C. very slowly over a period of eight hours. The photomicrograph is taken at 100 diameters by polarised light, with the system crossed, the large crystals tending towards a three-pointed star shape are clearly seen, together with the way in which they appear to join up with one another. The film was produced by placing a perfectly clean slide in a petrie dish in an oven running at 150° C., one end of the slide was rested on the edge of the dish so that its surface was sloping at an angle of about 30°. A test-tube containing a small amount of the wax was also placed in the oven and when the whole had attained a temperature of 150° C. the wax was poured on to the slide, the cooling process was then commenced and took place as stated. In this way the wax was cooled very slowly in the form of a very thin film, without any extraneous forces hampering the crystalisation.

In direct contrast to the slow cooling, Fig. 298 is a photomicrograph of the same wax photographed under the same conditions as Fig. 297, but the film of wax was cooled under very different conditions, which may be termed shock cooling under pressure. This was accomplished by placing a clean slide on a hot plate running at 150° C., a small piece of wax was then placed on the slide and when this had melted a cover glass was dropped on to the wax, after which a spring clip was applied to the centre of the cover glass, the whole was then replaced on the hot plate and allowed to attain the aforementioned temperature, when it was rapidly transferred to the surface of a block of brass which had previously been cooled to about 10° C. This of course resulted in a very rapid cooling of the wax film, while still under the influence of the cover glass and spring clip. The effect of this treatment on the crystallisation of the wax is very well shown, the crystals are no longer large and of more or less characteristic shape, but rather has the treatment tended to produce very much smaller crystals which have no characteristic shape.

These films were mounted in glucose and sealed in the usual way, thus making the preparations permanent and enabling examinations to be conducted without the risk of damaging the film.

Thus we have dealt with the mounting and preparation of specimens in a brief manner, we have seen that the actual mounting and finishing off of a slide involves not inconsiderable skill which is by no means impossible of attainment with a certain amount of practice, and it is strongly recommended that those sufficiently interested, make a habit of producing slides of the best possible finish as a matter of routine, as the constant practice in attending to the small details so necessary for the production of the best results will develop to such an extent as to make the observance of

the fundamental principles of cleanliness and care in all operations a habit, which comes into operation automatically. In this way one will produce slides to be proud of, which are well finished and clean. As has been previously stated, this question of a clean mount is of paramount importance as nothing can cause more annoyance to an observer than bits of extraneous matter included in the mount, too often this will be found to be in such a position as to obscure entirely the very portion desired to be examined.

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APPENDIX

TABLE OF REFRACTIVE INDICES OF VARIOUS SUBSTANCES

(To three places of decimals)

In alphabetical order.

	μ
Acetic acid (glacial)	1·372
Acetone	1·364
Air	1·0003
Albumen	1·350
Alcohol, n-butyl	1·399
ethyl (absolute)	1·367
iso-propyl	1·378
methyl	1·323
normal propyl	1·386
Alum (sat sol)	1·457
Aniline	1·586
Antimony bromide (approx.)	1·680
Bees wax	1·553
Benzene	1·503
Benzylaniline	1·621
Blood serum (human)	1·354
Borax	1·515
Calcite (Iceland spar) ordinary ray	1·657
Carbon-di-sulphide	1·630
Cedrene	1·539
Cellosolve	1·406
Chloroform	1·446
Creosote	1·538
Diamond (sp. gr. 3·4)	2·407
Diaphane	1·483
Diatom Silex	1·430
Dioxane	1·423
Ether (60° F.)	1·357
Ethyl ether	1·354
Felspar	1·764
Fluorite	1·434
Glycerine (absolute)	1·473
,, 50%	1·397
Gum arabic	1·512
Iso-butylmethacrylate	1·477
Lead borate	1·866
carbonate	1·01 – 2·08
chromate	2·5 – 2·97
Meta-cinnamene	1·597
Methanol	1·392
Methylbenzoate	1·517
Methyldiphenlylamine	1·616
Methylene-di-iodide	1·743
Methyl salicylate	1·530
Monobromonaphthalene	1·658
Naphtha	1·475
Naphthyl-phenyl-ketone	1·669
Nitro-benzene	1·558
Oil, almond	1·478
ambergris	1·368
aniline	1·586
aniseed	1·55
bergamot	1·464
cajeput	1·460
cassia	1·578
castor	1·490
cedar ((thick)	1·520
,, (thin)	1·510
cinnamon	1·619
cloves	1·533
fennel	1·544
lemon	1·527
linseed (sp. gr. 0·932)	1·485
olive (sp. gr. 0·913)	1·476
origanum (cretan)	1·494 - 1·504
sandalwood	1·510
thyme	1·483 – 1·510
turpentine (sp. gr. 0·885)	1·474
Paraffin (liquid)	1·471
Petroleum	1·457
Phenylthiocarbamide	1·654
Quinidine	1·617
Quinoline	1·633
Rock crystal	1·545
salt (sp. gr. 2·143)	1·555
Saliva (human)	1·339
Salt (sat. sol)	1·375
Sea water	1·343
Spermaceti	1·503
Stannous chloride	1·503
Sugar (sucrose)	1·535
Sulphur in methylene-di-iodide	1·778
(molten)	2·148
Toluene	1·495
Tourmaline (ordinary ray)	1·668
Trichlorethylene	1·478
Turpineol	1·484
Water (distilled)	1·336
Water (tap)	1·334
Xylene	1·497
Zircon	1·950

REFRACTIVE INDICES OF VARIOUS SUBSTANCES

(To three places of decimals)

In ascending order of magnitude of refractive Index

μ	
1·0003	Air.
1·323	Methyl alcohol.
1·334	Tap water.
1·336	Distilled water.
1·339	Saliva (human)
1·343	Sea water.
1·350	Albumen.
1·354	Blood serum (human).
1·354	Ethyl ether.
1·357	Ether (60° F.).
1·364	Acetone.
1·367	Ethyl alcohol (absolute).
1·368	Oil of ambergris.
1·372	Acetic acid (glacial).
1·375	Salt (sat. sol.).
1·378	Iso-propyl alcohol.
1·386	Normal-propyl alcohol.
1 392	Methanol.
1·397	Glycerine 50%.
1·399	Normal-butyl alcohol.
1·406	Cellosolve.
1·423	Dioxan.
1·434	Fluorite.
1·446	Chloroform.
1·457	Alum (sat. sol.).
1·457	Petroleum.
1·460	Oil of cajeput.
1·464	,, ,, bergamot.
1·471	Paraffin (liquid).
1·473	Glycerine.
1·474	Oil of turpentine (sp. gr. 0·885).
1·475	Naphtha.
1·476	Oil of olives.
1·477	Iso-butylmethacrylate.
1·478	Oil of almonds.
1·478	Trichlorethylene.
1·483	Diaphane.
1·484	Turpineol.
1·485	Oil of linseed.
1·490	Oil of castor.
1·495	Toluene.
1·497	Xylene.
1·503	Spermaceti.
1·503	Stannous chloride.
1·503	Benzene.

μ	
1·504	Oil of origanum (Cretan).
1·510	,, ,, thyme.
1·510	,, ,, sandalwood.
1·510	,, ,, cedar (thin).
1·512	Gum arabic.
1·515	Borax.
1·517	Methyl benzoate.
1·520	Oil of cedar (thick).
1·527	,, ,, lemon.
1·530	Methyl salicylate.
1·533	Oil of cloves.
1·535	Sugar (sucrose).
1·538	Creosote.
1·539	Cedrene.
1·544	Oil of fennel.
1·545	Rock crystal.
1·550	Oil of aniseed.
1·553	Beeswax.
1·555	Rock salt (sp. gr. 2·143).
1·558	Nitro benzene.
1·578	Oil of cassia.
1·586	Aniline.
1·527	Meta cinnamene.
1·611	Benzylaniline.
1·616	Methyldiphenylamine.
1·617	Quinidine.
1·619	Oil of cinnamon.
1·630	Carbon-di-sulphide.
1·633	Quinoline.
1·654	Phenylthiocarbamide.
1·657	Calcite (Iceland spar) ordinary ray.
1·658	Monobromonaphthalene.
1·668	Tourmaline (ordinary ray).
1·669	Naphthyl-phenyl-ketone.
1·680	(approx.) Antimony chloride.
1·743	Methylene-di-iodide.
1·764	Felspar.
1·778	Sulphur in methylene-di-iodide.
1·866	Lead borate.
1·950	Zircon.
2·08	Lead carbonate.
2·148	Sulphur (molten).
2·407	Diamond.
2·5 – 2·9	Lead chromate.

TABLE OF REFRACTIVE INDICES AND INDEX OF VISIBILITY

(Alphabetical)

Of substances capable of being used as mounting media, these include some of the newer synthetic resins.

The index of visibility Iv is calculated on the refractive index of Diatom Silex taken as 1·43 and is obtained by multiplying the difference between the refractive index of the mounting medium and that of Diatom Silex, by one hundred. In those cases where the refractive index of the medium is lower than that of the Silex, the calculation is carried out in the same way, but the result is expressed as a negative quantity. This is necessary in order to avoid a false visibility index; for example, the refractive index of Dammar is 1·520 which gives a visibility index of 9·0, this is obviously more efficient as a mounting medium than sea-water with a refractive index of 1·343, which latter, however, gives a visibility index of 9·7, but it will be seen that this figure is really below that for a substance having the same refractive index as Silex, with a corresponding visibility index of zero. Therefore, the visibility index of sea-water must be expressed as a negative value.

Substance	μ	Iv (Diatom Silex)
Camsall balsam	1·478	4·8
Canada balsam	1·526	9·6
Cedarwood oil	1·510	8·0
Colophonium	1·545	11·5
Dammar	1·520	9·0
Diaphane	1·483	5·3
Distilled water	1·336	— 9·4
Euparal (Flatters and Garnet)	1·483	5·3
Glycerine	1·470	4·0
Monobromonaphthalene	1·658	22·8
Phosphorus	2·224	79·4
Phosphorus in methylene-di-iodide	1·944	51·4
Piperine	1·681	25·1
Piperine balsam	1·657	22·7
Piperine and picric piperine	1·681	25·1
Realgar	2·549	111·9
Sandarac camphloral	1·485	5·5
Sea water	1·343	— 8·7
Styrax (liquidamber orientalis)	1·582	15·2
Styrax (liquidamber Styraciflua)	1·63	20·0
Synthetic resins:		
Cellulose acetate	1·50	7·0
Cellulose acetate-butyrate	1·49	6·0
Cellulose nitrate	1·50	7·0
Clarite poly-cyclo-paraffins	1·544	11·4
Claritex (American)	1·567	13·7
Ethyl cellulose	1·47	4·0
Hyrax	1·710	28·0
Isobutylmethacrylate	1·477	4·7
Methylmethacrylate (Perspex)	1·50	7·0
Phenol-formaldehyde (casting)	1·600	17·0
Polystyrene	1·59	16·0
Polyvinyl-alcohol	1·53	10·0
,, butyral	1·49	6·0
,, chloride acetate	1·53	10·0
Polyvinylidene-chloride	1·615	18·5
Sirax (Stafford Allen)	1·80	37·0
Synthetic neutral (Flatters and Garnet)	1·515	8.5
Urea formaldehyde	1·55	12·0

TABLE OF REFRACTIVE INDICES AND INDEX OF VISIBILITY

(In ascending order of μ)

μ	Iv (Diatom Silex)	Substance
1·336	− 9·4	Distilled water.
1·343	− 8·7	Sea water.
1·47	4·0	Ethyl cellulose.
1·47	4·0	Glycerine.
1·477	4·7	Isobutylmethacrylate.
1·478	4·8	Camsall balsam.
1·483	5·3	Diaphane.
1·483	5·3	Euparal (Flatters and Garnet).
1·485	5·5	Sandarac camphloral.
1·49	6·0	Cellulose Acetate-butyrate.
1·49	6·0	Polyvinylbutyral.
1·50	7·0	Cellulose acetate.
1·50	7·0	Cellulose nitrate.
1·50	7·0	Methylmethacrylate.
1·510	8·0	Cedarwood oil.
1·515	8·5	Synthetic neutral (Flatters and Garnet).
1·520	9·0	Dammar.
1·526	9·6	Canada balsam.
1·53	10·0	Polyvinyl alcohol.
1·53	10·0	Polyvinyl chloride-acetate (co-polymer).
1·54	11·4	Clarite.
1·545	11·5	Colophonium.
1·55	12·0	Urea formaldehyde.
1·567	13·7	Clarite X.
1·582	15·2	Styrax (liquidamber orientalis).
1·59	16·0	Polystyrene.
1·600	17·0	Phenol-formaldehyde.
1·615	18·5	Polyvinylidine chloride.
1·63	20·0	Styrax (liquidamber styraciflua).
1·657	22·7	Piperine balsam.
1·658	22·8	Monobrono naphthalene.
1·681	25·1	Piperine.
1·681	25·1	Piperine and picric piperine.
1·710	28·0	Hyrax.
1·80	37·0	Syrax (Stafford Allen).
1·944	51·4	Phosphorus in Methylene iodide.
2·224	79·4	Phosphorus.
2·549	111·9	Realgar.

TABLE OF REFRACTIVE INDICES AND DISPERSIONS OF GLASSES

Substance		Refractive Index μ.D.	Dispersion $\frac{\mu - 1}{8\mu}$
Crown		1·51 − 1·56	59·0 − 46·0
Plate		1·516	−
Extra light flint		1·541	49·2
Light flint		1·574	41·0
Dense flint		1·622	36·5
Extra dense flint		1·650	34·2
Double extra dense flint		1·710	30·0
Borosilicate crown	Jena Glass	1·51	64·0
Phosphate crown	Jena Glass	1·51 − 1·56	70·0 − 67·0
Barium silicate crown	Jena Glass	1·54 − 1·60	59·0 − 55·0
Borosilicate flint	Jena Glass	1·55 − 1·57	49·0 − 47·0
Borate flint	Jena Glass	1·55 − 1·68	55·0 − 33·0
Barium phosphate crown	Jena Glass	1·58	15·2
Very heavy silicate flint	Jena Glass	1·963	19·7
Antimony glass		2·216	−

EQUIVALENT TABLES FOR VARIOUS MEASURES

Length.

1 in.	= 2·539998 cm.
1 ft. = 12 in.	= 3·047997 decimetres.
1 yd. = 3 ft.	= 0·914399 metres.

Area.

1 sq. in.	= 6·45159 sq. cm.
1 sq. ft. = 144 sq. in.	= 0·92903 milliares.
1 sq. yd. = 9 sq. ft.	= 8·36126 milliares.

Volume.

1 cu. in.	= 16·387 cu. cm.
1 cu. ft. = 1,728 cu. in.	= 2·83168 cm.
1 cu. yd. = 27 cu. ft.	= 7·64553 decimetres.

Weight (Avoirdupois).

1 Grain (gr.)	= 6·479892 centigrammes.	
1 Drachm (dr.) = 27·34375 gr.	= 1·77185 gm.	
1 Ounce (oz.) = 16 dr.	= 2·83495 decagrammes	= 28·3495 gms.
1 Pound (1 lb.) = 16 oz.	= 4·5359243 hectogrammes	= 453·59243 gms.
1 Stone (st.) = 14 lb.	= 6·35029 kg.	
1 Quarter (qr.) = 28 lb.	= 12·70059 kg.	
1 Hundredweight (cwt.) = 4 qrs.	= 50·80235 kg.	
1 Ton (tn.) = 20 cwt.	= 1,016·05 kg.	

CONSTANTS USEFUL TO THE MICROSCOPIST

Area (English to Metric).

Square $\frac{1}{8}$ in.	= 10·08061 sq. mm.	
,, $\frac{1}{10}$ in.	= 6·45159 ,, ,,	
,, $\frac{1}{12}$ in.	= 4·48027 ,, ,,	
,, $\frac{1}{100}$ in.	= 0·06452 ,, ,,	= 61515·9 sq. microns.
,, $\frac{1}{1000}$ in.	= 645·159 sq. μ.	

Area (Metric to English).

Square centimetre	= 15·5 sq. $\frac{1}{10}$ in.
,, millimetre	= 15·5 sq. $\frac{1}{100}$ in.
,, 100 μ	= 15·5 sq. $\frac{1}{1000}$ in.
,, 10 μ	= 0·15500 sq. $\frac{1}{1000}$ in.
,, 1 μ	= 0·00155 sq. $\frac{1}{1000}$ in.

Multiples of these values may be calculated by multiplying the value given by the square of the multiplier, thus: If we require the value for a sq. $\frac{4}{10}$ in. the multiplier is 4 and as the square of 4 is 16, the answer is 16 × 6·451596 = 103·2254 sq. mm.

Volume (English to Metric)

Cubic $\frac{1}{8}$ in.	= 32·00589 cu. mm.
,, $\frac{1}{10}$ in.	= 16·38702 ,, ,,
,, $\frac{1}{12}$ in.	= 9·48323 ,, ,,
,, $\frac{1}{100}$ in.	= 0·01639 ,, ,,
,, $\frac{1}{1000}$ in.	= 16387·02 cu. μ.

Volume (Metric to English).

Cubic centimetre	= 61·0239 cu. $\frac{1}{10}$ in.
,, millimetre	= 61·0239 cu. $\frac{1}{100}$ in.
,, 100 μ	= 61·0239 cu. $\frac{1}{1000}$ in.
,, 10 μ	= 0·06102 39 cu. $\frac{1}{1000}$ in.
,, 1 μ	= 0·0000610239 cu. $\frac{1}{1000}$ in.

Multiples of these values may be calculated by multiplying the values given by the cube of the multiplier, thus: If we require the value of 2 cu. mm. the multiplier is 2 and as the cube of 2 is 8, the answer is 8 × 61·0239 = 488·1912 cu. $\frac{1}{1000}$ in.

Areas of Circles (English to Metric).

$\frac{1}{8}$ in. diameter	= 1·22718 sq. $\frac{1}{10}$ in.	= 7·91726 sq. mm.
$\frac{1}{10}$ in. ,,	= 0·78539816 sq. $\frac{1}{10}$ in.	= 5·06706 ,, ,,
$\frac{1}{12}$ in. ,,	= 0·545415 sq. $\frac{1}{10}$ in.	= 3·51879 ,, ,,
$\frac{1}{100}$ in. ,,	= 0·78540 sq. $\frac{1}{100}$ in.	= 50670·6 sq. μ.
$\frac{1}{1000}$ in. ,,	= 0·78540 sq. $\frac{1}{1000}$ in.	= 506·7 sq. μ.

Areas of Circles (Metric to English).

1 mm. diameter	= 0·78539816 sq. mm.	= 12·17372 sq. $\frac{1}{100}$ in.
100 μ ,,	= 78540·0 sq. μ	= 12·17372 sq. $\frac{1}{10000}$ in.
10 μ ,,	= 78·54 sq. μ	= 0·12175 sq. $\frac{1}{10000}$ in.
1 μ ,,	= 0·7854 sq. μ	= 0·00012175 sq. $\frac{1}{10000}$ in.

Multiples of these values may be obtained in the same manner as for volumes

Volumes of Spheres (English to Metric).

$\frac{1}{10}$ in. diameter	= 1·02266 cu. $\frac{1}{10}$ in.	= 16·75835 cu. mm.
$\frac{1}{10}$ in. ,,	= 0·52360 cu. $\frac{1}{10}$ in.	= 8·58024 ,, ,,
$\frac{1}{12}$ in. ,,	= 0·30301 cu. $\frac{1}{10}$ in.	= 4·96543 ,, ,,
$\frac{1}{100}$ in. ,,	= 0·52360 cu. $\frac{1}{100}$ in.	= 0·00858 ,, ,,
$\frac{1}{1000}$ in. ,,	= 0·52360 cu. $\frac{1}{1000}$ in.	= 8580·24 ,, μ

Volumes of Spheres (Metric to English)

1 mm. diameter	= 0·52360 cu. mm.	= 31·952 cu. $\frac{1}{100}$ in.
100 μ ,,	= 523600 cu. μ	= 31·952 cu. $\frac{1}{10000}$ in.
10 μ ,,	= 523·6 cu. μ	= 0·03195 cu. $\frac{1}{10000}$ in.
1 μ ,,	= 0·52360 cu. μ	= 0·00003195 cu. $\frac{1}{10000}$ in.

USEFUL CONSTANTS AND FORMULÆ

Areas and Volumes.

Area of triangle = base × half the perpendicular height.
Volume of a wedge = area of base × half the perpendicular height.
Volume of a cone or pyramid = area of base × $\frac{1}{3}$ the perpendicular height.
Surface of a cone = circumference of base × $\frac{1}{2}$ the length of side.
Area of a parabola = base × $\frac{2}{3}$ height
Velocity of light = 186,377 statute miles per second.
Wavelength of yellow light = 1/43100l in.
Frequency of yellow light = 508,961,293,000,000 cycles per second.

PROPERTIES OF CIRCLES AND SPHERES, ETC.

$\pi = 3{\cdot}14159265$ plus
$\log \pi = 0{\cdot}49715$
$\pi^2 = 9{\cdot}8696$
$\sqrt{\pi} = 1{\cdot}77245$
$\frac{1}{\pi} = 0{\cdot}31831$
$\frac{1}{\pi^2} = 0{\cdot}10132$
$\frac{1}{\sqrt{\pi}} = 0{\cdot}56419$
$\frac{\pi}{4} = 0{\cdot}785398$
$\frac{\pi}{6} = 0{\cdot}5236$
$\sqrt{2} = 1{\cdot}41421$
$\sqrt{2g} = 8{\cdot}02496$

Circumference of a circle C $= 2\pi r = \pi d$ (r = radius)
Area of a circle A $= \pi r^2$
Surface of a sphere S $= \pi d^2$ (d = diameter)
Volume of a sphere V $= \frac{\pi d^3}{6}$
Diameter of a circle d $= \frac{c}{\pi}$
Side of square equal in area to a circle $= r\sqrt{\pi}$
Side of inscribed square $= r\sqrt{2}$
Area of ellipse = $\frac{1}{2}$ maj. axis × $\frac{1}{2}$ mi. axis × π
Volume of spheroid = polar axis × (equatorial axis)^2 × $\frac{\pi}{6}$

CONVERSION TABLE. MICRONS TO FRACTIONS OF AN INCH

(After Nelson)

μ	inches	μ	inches	μ	inches	μ	inches
1	0·000039	19	0·000748	36	0·001417	80	0·003150
2	0·000079	20	0·000787	37	0·001457	90	0·003543
3	0·000118			28	0·001496	100	0·003937
4	0·000157	21	0·000827	39	0·001535	200	0·007874
5	0·000197	22	0·000866	40	0·001575	300	0·011811
6	0·000236	23	0·000906			400	0·015748
7	0·000276	24	0·000945	41	0·001614	500	0·019685
8	0·000315	25	0·000984	42	0·001654	600	0·023622
9	0·000354	26	0·001024	43	0·001693	700	0·027559
10	0·000394	27	0·001063	44	0·001732	800	0·031496
		28	0·001102	45	0·001772	900	0·035433
11	0·000433	29	0·001141	46	0·001811	1,000 = 1 mm =	
12	0·000472	30	0·001181	47	0·001852		0·039370
13	0·000512			48	0·001890		
14	0·000551	31	0·001220	49	0·001929		
15	0·000591	32	0·001260	50	0·001969		
16	0·000630	33	0·001299				
17	0·000669	34	0·001339	60	0·002362		
18	0·000709	35	0·001378	70	0·002756		

CONVERSION TABLE. FRACTIONS OF AN INCH TO MICRONS

(From $\frac{1}{1000}$ in) (after Carpenter and Dollinger)

inches	μ	inches	μ
$\frac{1}{1000}$	25·399978	$\frac{1}{8000}$	3·174997
$\frac{1}{2000}$	12·699989	$\frac{1}{9000}$	2·82222
$\frac{1}{3000}$	8·466659	$\frac{1}{10000}$	2·539998
$\frac{1}{4000}$	6·349994	$\frac{1}{15000}$	1·693332
$\frac{1}{5000}$	5·079996	$\frac{1}{20000}$	1·269999
$\frac{1}{6000}$	4·23333	$\frac{1}{25000}$	1·015999
$\frac{1}{7000}$	3·628568		

CONVERSION TABLE. LINES PER INCH TO LINES PER mm

(After Carpenter and Dallinger)

Lines/inch	Lines/mm	Lines/inch	Lines/mm	Lines/inch	Lines/mm	Lines/inch	Lines/mm
5,000	197	75,000	2,953	190,000	7,480	450,000	17,717
10,000	394	80,000	3,153	200,000	7,874	500,000	19,685
15,000	591	85,000	3,346	210,000	8,268		
20,000	787	90,000	3,543	220,000	8,661	Lines/micron	
25,000	984	95,000	3,740	230,000	9,055	25,400	1
30,000	1,181	100,000	3,937	240,000	9,449	50,800	2
35,000	1,387	110,000	4,331	250,000	9,843	76,200	3
40,000	1,575	120,000	4,724	260,000	10,236	101,600	4
45,000	1,772	130,000	5,118	270,000	10,630	127,000	5
50,000	1,968	140,000	5,512	280,000	10,024	152,400	6
55,000	2,165	150,000	5,906	290,000	11,417	177,800	7
60,000	2,362	160,000	6,299	300,000	11,811	203,200	8
65,000	2,559	170,000	6,693	350,000	13,780	228,600	9
70,000	2,756	180,000	7,087	400,000	15,748	254,000	10

DETAILS OF NOBERT'S TEST PLATE RULINGS

19 *Band Test Plate.*

The difference between each band is 5629·75 lines/inch.

Band No.	Lines/inch	Band No.	Lines/inch
1	11259·5	15	90076·1
5	33778·5	19	112595·1
10	61927·3		

20 *Band Test Plate.*

The difference between each band is 11259·5 lines/inch.

Band No.	Lines/inch	Band No.	Lines/inch
1	11259·5	15	168892·7
5	56297·6	20	225190·3
10	112595·1		

Resolution.

The resolution of the human eye in light of approximately 5,000A wavelength = 0·1 to 0·2 mm.

Abbe formula for microscopic resolution

$$R = \frac{K\lambda}{NA}$$

Where R = the smallest resolvable distance between two resolvable points.
K = illumination constant usually about 0·5.
λ = wavelength of the light employed.
NA = numerical aperture of the objective used.

LOGARITHMS AND ANTI-LOGARITHMS

LOGARITHMS

	0	1	2	3	4	5	6	7	8	9	Proportional parts. 1 2 3 4 5 6 7 8 9
10	0000	0043	0086	0128	0170	0212	0253	0294	0334	0374	4 8 12 17 21 25 29 33 37
11	0414	0453	0492	0531	0569	0607	0645	0682	0719	0755	4 8 11 15 19 23 26 30 34
12	0792	0828	0864	0899	0934	0969	1004	1038	1072	1106	3 7 10 14 17 21 24 28 31
13	1139	1173	1206	1239	1271	1303	1335	1367	1399	1430	3 6 10 13 16 19 23 26 29
14	1461	1492	1523	1553	1584	1614	1644	1673	1703	1732	3 6 9 12 15 18 21 24 27
15	1761	1790	1818	1847	1875	1903	1931	1959	1987	2014	3 6 8 11 14 17 20 22 25
16	2041	2068	2095	2122	2148	2175	2201	2227	2253	2279	3 5 8 11 13 16 18 21 24
17	2304	2330	2355	2380	2405	2430	2455	2480	2504	2529	2 5 7 10 12 15 17 20 22
18	2553	2577	2601	2625	2648	2672	2695	2718	2742	2765	2 5 7 9 12 14 16 19 21
19	2788	2810	2833	2856	2878	2900	2923	2945	2967	2989	2 4 7 9 11 13 16 18 20
20	3010	3032	3054	3075	3096	3118	3139	3160	3181	3201	2 4 6 8 11 13 15 17 19
21	3222	3243	3263	3284	3304	3324	3345	3365	3385	3404	2 4 6 8 10 12 14 16 18
22	3424	3444	3464	3483	3502	3522	3541	3560	3579	3598	2 4 6 8 10 12 14 15 17
23	3617	3636	3655	3674	3692	3711	3729	3747	3766	3784	2 4 6 7 9 11 13 15 17
24	3802	3820	3838	3856	3874	3892	3909	3927	3945	3962	2 4 5 7 9 11 12 14 16
25	3979	3997	4014	4031	4048	4065	4082	4099	4116	4133	2 3 5 7 9 10 12 14 15
26	4150	4166	4183	4200	4216	4232	4249	4265	4281	4298	2 3 5 7 8 10 11 13 15
27	4314	4330	4346	4362	4378	4393	4409	4425	4440	4456	2 3 5 6 8 9 11 13 14
28	4472	4487	4502	4518	4533	4548	4564	4579	4594	4609	2 3 5 6 8 9 11 12 14
29	4624	4639	4654	4669	4683	4698	4713	4728	4742	4757	1 3 4 6 7 9 10 12 13
30	4771	4786	4800	4814	4829	4843	4857	4871	4886	4900	1 3 4 6 7 9 10 11 13
31	4914	4928	4942	4955	4969	4983	4997	5011	5024	5038	1 3 4 6 7 8 10 11 12
32	5051	5065	5079	5092	5105	5119	5132	5145	5159	5172	1 3 4 5 7 8 9 11 12
33	5185	5198	5211	5224	5237	5250	5263	5276	5289	5302	1 3 4 5 6 8 9 10 12
34	5315	5328	5340	5353	5366	5378	5391	5403	5416	5428	1 3 4 5 6 8 9 10 11
35	5441	5453	5465	5478	5490	5502	5514	5527	5539	5551	1 2 4 5 6 7 9 10 11
36	5563	5575	5587	5599	5611	5623	5635	5647	5658	5670	1 2 4 5 6 7 8 10 11
37	5682	5694	5705	5717	5729	5740	5752	5763	5775	5786	1 2 3 5 6 7 8 9 10
38	5798	5809	5821	5832	5843	5855	5866	5877	5888	5899	1 2 3 5 6 7 8 9 10
39	5911	5922	5933	5944	5955	5966	5977	5988	5999	6010	1 2 3 4 5 7 8 9 10
40	6021	6031	6042	6053	6064	6075	6085	6096	6107	6117	1 2 3 4 5 6 8 9 10
41	6128	6138	6149	6160	6170	6180	6191	6201	6212	6222	1 2 3 4 5 6 7 8 9
42	6232	6243	6253	6263	6274	6284	6294	6304	6314	6325	1 2 3 4 5 6 7 8 9
43	6335	6345	6355	6365	6375	6385	6395	6405	6415	6425	1 2 3 4 5 6 7 8 9
44	6435	6444	6454	6464	6474	6484	6493	6503	6513	6522	1 2 3 4 5 6 7 8 9
45	6532	6542	6551	6561	6571	6580	6590	6599	6609	6618	1 2 3 4 5 6 7 8 9
46	6628	6637	6646	6656	6665	6675	6684	6693	6702	6712	1 2 3 4 5 6 7 7 8
47	6721	6730	6739	6749	6758	6767	6776	6785	6794	6803	1 2 3 4 5 5 6 7 8
48	6812	6821	6830	6839	6848	6857	6866	6875	6884	6893	1 2 3 4 4 5 6 7 8
49	6902	6911	6920	6928	6937	6946	6955	6964	6972	6981	1 2 3 4 4 5 6 7 8
50	6990	6998	7007	7016	7024	7033	7042	7050	7059	7067	1 2 3 3 4 5 6 7 8
51	7076	7084	7093	7101	7110	7118	7126	7135	7143	7152	1 2 3 3 4 5 6 7 8
52	7160	7168	7177	7185	7193	7202	7210	7218	7226	7235	1 2 2 3 4 5 6 7 7
53	7243	7251	7259	7267	7275	7284	7292	7300	7308	7316	1 2 2 3 4 5 6 6 7
54	7324	7332	7340	7348	7356	7364	7372	7380	7388	7396	1 2 2 3 4 5 6 6 7
	0	1	2	3	4	5	6	7	8	9	1 2 3 4 5 6 7 8 9

LOGARITHMS

	0	1	2	3	4	5	6	7	8	9	Proportional parts. 1	2	3	4	5	6	7	8	9
55	7404	7412	7419	7427	7435	7443	7451	7459	7466	7474	1	2	2	3	4	5	5	6	7
56	7482	7490	7497	7505	7513	7520	7528	7536	7543	7551	1	2	2	3	4	5	5	6	7
57	7559	7566	7574	7582	7589	7597	7604	7612	7619	7627	1	2	2	3	4	5	5	6	7
58	7634	7642	7649	7657	7664	7672	7679	7686	7694	7701	1	1	2	3	4	4	5	6	7
59	7709	7716	7723	7731	7738	7745	7752	7760	7767	7774	1	1	2	3	4	4	5	6	7
60	7782	7789	7796	7803	7810	7818	7825	7832	7839	7846	1	1	2	3	4	4	5	6	6
61	7853	7860	7868	7875	7882	7889	7896	7903	7910	7917	1	1	2	3	4	4	5	6	6
62	7924	7931	7938	7945	7952	7959	7966	7973	7980	7987	1	1	2	3	3	4	5	6	6
63	7993	8000	8007	8014	8021	8028	8035	8041	8048	8055	1	1	2	3	3	4	5	5	6
64	8062	8069	8075	8082	8089	8096	8102	8109	8116	8122	1	1	2	3	3	4	5	5	6
65	8129	8136	8142	8149	8156	8162	8169	8176	8182	8189	1	1	2	3	3	4	5	5	6
66	8195	8202	8209	8215	8222	8228	8235	8241	8248	8254	1	1	2	3	3	4	5	5	6
67	8261	8267	8274	8280	8287	8293	8299	8306	8312	8319	1	1	2	3	3	4	5	5	6
68	8325	8331	8338	8344	8351	8357	8363	8370	8376	8382	1	1	2	3	3	4	4	5	6
69	8388	8395	8401	8407	8414	8420	8426	8432	8439	8445	1	1	2	2	3	4	4	5	6
70	8451	8457	8463	8470	8476	8482	8488	8494	8500	8506	1	1	2	2	3	4	4	5	6
71	8513	8519	8525	8531	8537	8543	8549	8555	8561	8567	1	1	2	2	3	4	4	5	5
72	8573	8579	8585	8591	8597	8603	8609	8615	8621	8627	1	1	2	2	3	4	4	5	5
73	8633	8639	8645	8651	8657	8663	8669	8675	8681	8686	1	1	2	2	3	4	4	5	5
74	8692	8698	8704	8710	8716	8722	8727	8733	8739	8745	1	1	2	2	3	4	4	5	5
75	8751	8756	8762	8768	8774	8779	8785	8791	8797	8802	1	1	2	2	3	3	4	5	5
76	8808	8814	8820	8825	8831	8837	8842	8848	8854	8859	1	1	2	2	3	3	4	5	5
77	8865	8871	8876	8882	8887	8893	8899	8904	8910	8915	1	1	2	2	3	3	4	4	5
78	8921	8927	8932	8938	8943	8949	8954	8960	8965	8971	1	1	2	2	3	3	4	4	5
79	8976	8982	8987	8993	8998	9004	9009	9015	9020	9025	1	1	2	2	3	3	4	4	5
80	9031	9036	9042	9047	9053	9058	9063	9069	9074	9079	1	1	2	2	3	3	4	4	5
81	9085	9090	9096	9101	9106	9112	9117	9122	9128	9133	1	1	2	2	3	3	4	4	5
82	9138	9143	9149	9154	9159	9165	9170	9175	9180	9186	1	1	2	2	3	3	4	4	5
83	9191	9196	9201	9206	9212	9217	9222	9227	9232	9238	1	1	2	2	3	3	4	4	5
84	9243	9248	9253	9258	9263	9269	9274	9279	9284	9289	1	1	2	2	3	3	4	4	5
85	9294	9299	9304	9309	9315	9320	9325	9330	9335	9340	1	1	2	2	3	3	4	4	5
86	9345	9350	9355	9360	9365	9370	9375	9380	9385	9390	1	1	2	2	3	3	4	4	5
87	9395	9400	9405	9410	9415	9420	9425	9430	9435	9440	0	1	1	2	2	3	3	4	4
88	9445	9450	9455	9460	9465	9469	9474	9479	9484	9489	0	1	1	2	2	3	3	4	4
89	9494	9499	9504	9509	9513	9518	9523	9528	9533	9538	0	1	1	2	2	3	3	4	4
90	9542	9547	9552	9557	9562	9566	9571	9576	9581	9586	0	1	1	2	2	3	3	4	4
91	9590	9595	9600	9605	9609	9614	9619	9624	9628	9633	0	1	1	2	2	3	3	4	4
92	9638	9643	9647	9652	9657	9661	9666	9671	9675	9680	0	1	1	2	2	3	3	4	4
93	9685	9689	9694	9699	9703	9708	9713	9717	9722	9727	0	1	1	2	2	3	3	4	4
94	9731	9736	9741	9745	9750	9754	9759	9763	9768	9773	0	1	1	2	2	3	3	4	4
95	9777	9782	9786	9791	9795	9800	9805	9809	9814	9818	0	1	1	2	2	3	3	4	4
96	9823	9827	9832	9836	9841	9845	9850	9854	9859	9863	0	1	1	2	2	3	3	4	4
97	9868	9872	9877	9881	9886	9890	9894	9899	9903	9908	0	1	1	2	2	3	3	4	4
98	9912	9917	9921	9926	9930	9934	9939	9943	9948	9952	0	1	1	2	2	3	3	4	4
99	9956	9961	9965	9969	9974	9978	9983	9987	9991	9996	0	1	1	2	2	3	3	3	4
	0	1	2	3	4	5	6	7	8	9	1	2	3	4	5	6	7	8	9

ANTI-LOGARITHMS

	0	1	2	3	4	5	6	7	8	9	Proportional parts. 1	2	3	4	5	6	7	8	9
·00	1000	1002	1005	1007	1009	1012	1014	1016	1019	1021	0	0	1	1	1	1	2	2	2
·01	1023	1026	1028	1030	1033	1035	1038	1040	1042	1045	0	0	1	1	1	1	2	2	2
·02	1047	1050	1052	1054	1057	1059	1062	1064	1067	1069	0	0	1	1	1	1	2	2	2
·03	1072	1074	1076	1079	1081	1084	1086	1089	1091	1094	0	0	1	1	1	1	2	2	2
·04	1096	1099	1102	1104	1107	1109	1112	1114	1117	1119	0	1	1	1	1	2	2	2	2
·05	1122	1125	1127	1130	1132	1135	1138	1140	1143	1146	0	1	1	1	1	2	2	2	2
·06	1148	1151	1153	1156	1159	1161	1164	1167	1169	1172	0	1	1	1	1	2	2	2	2
·07	1175	1178	1180	1183	1186	1189	1191	1194	1197	1199	0	1	1	1	1	2	2	2	2
·08	1202	1205	1208	1211	1213	1216	1219	1222	1225	1227	0	1	1	1	1	2	2	2	3
·09	1230	1233	1236	1239	1242	1245	1247	1250	1253	1256	0	1	1	1	1	2	2	2	3
·10	1259	1262	1265	1268	1271	1274	1276	1279	1282	1285	0	1	1	1	1	2	2	2	3
·11	1288	1291	1294	1297	1300	1303	1306	1309	1312	1315	0	1	1	1	2	2	2	2	3
·12	1318	1321	1324	1327	1330	1334	1337	1340	1343	1346	0	1	1	1	2	2	2	2	3
·13	1349	1352	1355	1358	1361	1365	1368	1371	1374	1377	0	1	1	1	2	2	2	3	3
·14	1380	1384	1387	1390	1393	1396	1400	1403	1406	1409	0	1	1	1	2	2	2	3	3
·15	1413	1416	1419	1422	1426	1429	1432	1435	1439	1442	0	1	1	1	2	2	2	3	3
·16	1445	1449	1452	1455	1459	1462	1466	1469	1472	1476	0	1	1	1	2	2	2	3	3
·17	1479	1483	1486	1489	1493	1496	1500	1503	1507	1510	0	1	1	1	2	2	2	3	3
·18	1514	1517	1521	1524	1528	1531	1535	1538	1542	1545	0	1	1	1	2	2	2	3	3
·19	1549	1552	1556	1560	1563	1567	1570	1574	1578	1581	0	1	1	1	2	2	3	3	3
·20	1585	1589	1592	1596	1600	1603	1607	1611	1614	1618	0	1	1	1	2	2	3	3	3
·21	1622	1626	1629	1633	1637	1641	1644	1648	1652	1656	0	1	1	2	2	2	3	3	3
·22	1660	1663	1667	1671	1675	1679	1683	1687	1690	1694	0	1	1	2	2	2	3	3	3
·23	1698	1702	1706	1710	1714	1718	1722	1726	1730	1734	0	1	1	2	2	2	3	3	4
·24	1738	1742	1746	1750	1754	1758	1762	1766	1770	1774	0	1	1	2	2	2	3	3	4
·25	1778	1782	1786	1791	1795	1799	1803	1807	1811	1816	0	1	1	2	2	2	3	3	4
·26	1820	1824	1828	1832	1837	1841	1845	1849	1854	1858	0	1	1	2	2	3	3	3	4
·27	1862	1866	1871	1875	1879	1884	1888	1892	1897	1901	0	1	1	2	2	3	3	3	4
·28	1905	1910	1914	1919	1923	1928	1932	1936	1941	1945	0	1	1	2	2	3	3	4	4
·29	1950	1954	1959	1963	1968	1972	1977	1982	1986	1991	0	1	1	2	2	3	3	4	4
·30	1995	2000	2004	2009	2014	2018	2023	2028	2032	2037	0	1	1	2	2	3	3	4	4
·31	2042	2046	2051	2056	2061	2065	2070	2075	2080	2084	0	1	1	2	2	3	3	4	4
·32	2089	2094	2099	2104	2109	2113	2118	2123	2128	2133	0	1	1	2	2	3	3	4	4
·33	2138	2143	2148	2153	2158	2163	2168	2173	2178	2183	0	1	1	2	2	3	3	4	4
·34	2188	2193	2198	2203	2208	2213	2218	2223	2228	2234	1	1	2	2	3	3	4	4	5
·35	2239	2244	2249	2254	2259	2265	2270	2275	2280	2286	1	1	2	2	3	3	4	4	5
·36	2291	2296	2301	2307	2312	2317	2323	2328	2333	2339	1	1	2	2	3	3	4	4	5
·37	2344	2350	2355	2360	2366	2371	2377	2382	2388	2393	1	1	2	2	3	3	4	4	5
·38	2399	2404	2410	2415	2421	2427	2432	2438	2443	2449	1	1	2	2	3	3	4	4	5
·39	2455	2460	2466	2472	2477	2483	2489	2495	2500	2506	1	1	2	2	3	3	4	5	5
·40	2512	2518	2523	2529	2535	2541	2547	2553	2559	2564	1	1	2	2	3	4	4	5	5
·41	2570	2576	2582	2588	2594	2600	2606	2612	2618	2624	1	1	2	2	3	4	4	5	5
·42	2630	2636	2642	2649	2655	2661	2667	2673	2679	2685	1	1	2	2	3	4	4	5	6
·43	2692	2698	2704	2710	2716	2723	2729	2735	2742	2748	1	1	2	3	3	4	4	5	6
·44	2754	2761	2767	2773	2780	2786	2793	2799	2805	2812	1	1	2	3	3	4	4	5	6
·45	2818	2825	2831	2838	2844	2851	2858	2864	2871	2877	1	1	2	3	3	4	5	5	6
·46	2884	2891	2897	2904	2911	2917	2924	2931	2938	2944	1	1	2	3	3	4	5	5	6
·47	2951	2958	2965	2972	2979	2985	2992	2999	3006	3013	1	1	2	3	3	4	5	5	6
·48	3020	3027	3034	3041	3048	3055	3062	3069	3076	3083	1	1	2	3	4	4	5	6	6
·49	3090	3097	3105	3112	3119	3126	3133	3141	3148	3155	1	1	2	3	4	4	5	6	6
	0	1	2	3	4	5	6	7	8	9	1	2	3	4	5	6	7	8	9

ANTI-LOGARITHMS

	0	1	2	3	4	5	6	7	8	9	Proportional parts. 1	2	3	4	5	6	7	8	9
·50	3162	3170	3177	3184	3192	3199	3206	3214	3221	3228	1	1	2	3	4	4	5	6	7
·51	3236	3243	3251	3258	3266	3273	3281	3289	3296	3304	1	2	2	3	4	5	5	6	7
·52	3311	3319	3327	3334	3342	3350	3357	3365	3373	3381	1	2	2	3	4	5	5	6	7
·53	3388	3396	3404	3412	3420	3428	3436	3443	3451	3459	1	2	2	3	4	5	6	6	7
·54	3467	3475	3483	3491	3499	3508	3516	3524	3532	3540	1	2	2	3	4	5	6	6	7
·55	3548	3556	3565	3573	3581	3589	3597	3606	3614	3622	1	2	2	3	4	5	6	7	7
·56	3631	3639	3648	3656	3664	3673	3681	3690	3698	3707	1	2	3	3	4	5	6	7	8
·57	3715	3724	3733	3741	3750	3758	3767	3776	3784	3793	1	2	3	3	4	5	6	7	8
·58	3802	3811	3819	3828	3837	3846	3855	3864	3873	3882	1	2	3	4	4	5	6	7	8
·59	3890	3899	3908	3917	3926	3936	3945	3954	3963	3972	1	2	3	4	5	5	6	7	8
·60	3981	3990	3999	4009	4018	4027	4036	4046	4055	4064	1	2	3	4	5	6	6	7	8
·61	4074	4083	4093	4102	4111	4121	4130	4140	4150	4159	1	2	3	4	5	6	7	8	9
·62	4169	4178	4188	4198	4207	4217	4227	4236	4246	4256	1	2	3	4	5	6	7	8	9
·63	4266	4276	4285	4295	4305	4315	4325	4335	4345	4355	1	2	3	4	5	6	7	8	9
·64	4365	4375	4385	4395	4406	4416	4426	4436	4446	4457	1	2	3	4	5	6	7	8	9
·65	4467	4477	4487	4498	4508	4519	4529	4539	4550	4560	1	2	3	4	5	6	7	8	9
·66	4571	4581	4592	4603	4613	4624	4634	4645	4656	4667	1	2	3	4	5	6	7	9	10
·67	4677	4688	4699	4710	4721	4732	4742	4753	4764	4775	1	2	3	4	5	7	8	9	10
·68	4786	4797	4808	4819	4831	4842	4853	4864	4875	4887	1	2	3	4	6	7	8	9	10
·69	4898	4909	4920	4932	4943	4955	4966	4977	4989	5000	1	2	3	5	6	7	8	9	10
·70	5012	5023	5035	5047	5058	5070	5082	5093	5105	5117	1	2	4	5	6	7	8	9	11
·71	5129	5140	5152	5164	5176	5188	5200	5212	5224	5236	1	2	4	5	6	7	8	10	11
·72	5248	5260	5272	5284	5297	5309	5321	5333	5346	5358	1	2	4	5	6	7	9	10	11
·73	5370	5383	5395	5408	5420	5433	5445	5458	5470	5483	1	3	4	5	6	8	9	10	11
·74	5495	5508	5521	5534	5546	5559	5572	5585	5598	5610	1	3	4	5	6	8	9	10	12
·75	5623	5636	5649	5662	5675	5689	5702	5715	5728	5741	1	3	4	5	7	8	9	10	12
·76	5754	5768	5781	5794	5808	5821	5834	5848	5861	5875	1	3	4	5	7	8	9	11	12
·77	5888	5902	5916	5929	5943	5957	5970	5984	5998	6012	1	3	4	5	7	8	10	11	12
·78	6026	6039	6053	6067	6081	6095	6109	6124	6138	6152	1	3	4	6	7	8	10	11	13
·79	6166	6180	6194	6209	6223	6237	6252	6266	6281	6295	1	3	4	6	7	9	10	11	13
·80	6310	6324	6339	6353	6368	6383	6397	6412	6427	6442	1	3	4	6	7	9	10	12	13
·81	6457	6471	6486	6501	6516	6531	6546	6561	6577	6592	2	3	5	6	8	9	11	12	14
·82	6607	6622	6637	6653	6668	6683	6699	6714	6730	6745	2	3	5	6	8	9	11	12	14
·83	6761	6776	6792	6808	6823	6839	6855	6871	6887	6902	2	3	5	6	8	9	11	13	14
·84	6918	6934	6950	6966	6982	6998	7015	7031	7047	7063	2	3	5	6	8	10	11	13	15
·85	7079	7096	7112	7129	7145	7161	7178	7194	7211	7228	2	3	5	7	8	10	12	13	15
·86	7244	7261	7278	7295	7311	7328	7345	7362	7379	7396	2	3	5	7	8	10	12	13	15
·87	7413	7430	7447	7464	7482	7499	7516	7534	7551	7568	2	3	5	7	9	10	12	14	16
·88	7586	7603	7621	7638	7656	7674	7691	7709	7727	7745	2	4	5	7	9	11	12	14	16
·89	7762	7780	7798	7816	7834	7852	7870	7889	7907	7925	2	4	5	7	9	11	13	14	16
·90	7943	7962	7980	7998	8017	8035	8054	8072	8091	8110	2	4	6	7	9	11	13	15	17
·91	8128	8147	8166	8185	8204	8222	8241	8260	8279	8299	2	4	6	8	9	11	13	15	17
·92	8318	8337	8356	8375	8395	8414	8433	8453	8472	8492	2	4	6	8	10	12	14	15	17
·93	8511	8531	8551	8570	8590	8610	8630	8650	8670	8690	2	4	6	8	10	12	14	16	18
·94	8710	8730	8750	8770	8790	8810	8831	8851	8872	8892	2	4	6	8	10	12	14	16	18
·95	8913	8933	8954	8974	8995	9016	9036	9057	9078	9099	2	4	6	8	10	12	15	17	19
·96	9120	9141	9162	9183	9204	9226	9247	9268	9290	9311	2	4	6	8	11	13	15	17	19
·97	9333	9354	9376	9397	9419	9441	9462	9484	9506	9528	2	4	7	9	11	13	15	17	20
·98	9550	9572	9594	9616	9638	9661	9683	9705	9727	9750	2	4	7	9	11	13	16	18	20
·99	9772	9795	9817	9840	9863	9886	9908	9931	9954	9977	2	5	7	9	11	14	16	18	20
	0	1	2	3	4	5	6	7	8	9	1	2	3	4	5	6	7	8	9

NATURAL SINES.

Degrees	0′ 0°·0	6′ 0°·1	12′ 0°·2	18′ 0°·3	24′ 0°·4	30′ 0°·5	36′ 0°·6	42′ 0°·7	48′ 0°·8	54′ 0°·9	Mean Differences. 1	2	3	4	5
0	·0000	0017	0035	0052	0070	0087	0105	0122	0140	0157	3	6	9	12	15
1	·0175	0192	0209	0227	0244	0262	0279	0297	0314	0332	3	6	9	12	15
2	·0349	0366	0384	0401	0419	0436	0454	0471	0488	0506	3	6	9	12	15
3	·0523	0541	0558	0576	0593	0610	0628	0645	0663	0680	3	6	9	12	15
4	·0698	0715	0732	0750	0767	0785	0802	0819	0837	0854	3	6	9	12	15
5	·0872	0889	0906	0924	0941	0958	0976	0993	1011	1028	3	6	9	12	14
6	·1045	1063	1080	1097	1115	1132	1149	1167	1184	1201	3	6	9	12	14
7	·1219	1236	1253	1271	1288	1305	1323	1340	1357	1374	3	6	9	12	14
8	·1392	1409	1426	1444	1461	1478	1495	1513	1530	1547	3	6	9	12	14
9	·1564	1582	1599	1616	1633	1650	1668	1685	1702	1719	3	6	9	12	14
10	·1736	1754	1771	1788	1805	1822	1840	1857	1874	1891	3	6	9	12	14
11	·1908	1925	1942	1959	1977	1994	2011	2028	2045	2062	3	6	9	11	14
12	·2079	2096	2113	2130	2147	2164	2181	2198	2215	2232	3	6	9	11	14
13	·2250	2267	2284	2300	2317	2334	2351	2368	2385	2402	3	6	8	11	14
14	·2419	2436	2453	2470	2487	2504	2521	2538	2554	2571	3	6	8	11	14
15	·2588	2605	2622	2639	2656	2672	2689	2706	2723	2740	3	6	8	11	14
16	·2756	2773	2790	2807	2823	2840	2857	2874	2890	2907	3	6	8	11	14
17	·2924	2940	2957	2974	2990	3007	3024	3040	3057	3074	3	6	8	11	14
18	·3090	3107	3123	3140	3156	3173	3190	3206	3223	3239	3	6	8	11	14
19	3256	3272	3289	3305	3322	3338	3355	3371	3387	3404	3	5	8	11	14
20	·3420	3437	3453	3469	3486	3502	3518	3535	3551	3567	3	5	8	11	14
21	·3584	3600	3616	3633	3649	3665	3681	3697	3714	3730	3	5	8	11	14
22	·3746	3762	3778	3795	3811	3827	3843	3859	3875	3891	3	5	8	11	14
23	·3907	3923	3939	3955	3971	3987	4003	4019	4035	4051	3	5	8	11	14
24	·4067	4083	4099	4115	4131	4147	4163	4179	4195	4210	3	5	8	11	13
25	4226	4242	4258	4274	4289	4305	4321	4337	4352	4368	3	5	8	11	13
26	·4384	4399	4415	4431	4446	4462	4478	4493	4509	4524	3	5	8	10	13
27	·4540	4555	4571	4586	4602	4617	4633	4648	4664	4679	3	5	8	10	13
28	4695	4710	4726	4741	4756	4772	4787	4802	4818	4833	3	5	8	10	13
29	·4848	4863	4879	4894	4909	4924	4939	4955	4970	4985	3	5	8	10	13
30	5000	5015	5030	5045	5060	5075	5090	5105	5120	5135	3	5	8	10	13
31	·5150	5165	5180	5195	5210	5225	5240	5255	5270	5284	2	5	7	10	12
32	5299	5314	5329	5344	5358	5373	5388	5402	5417	5432	2	5	7	10	12
33	·5446	5461	5476	5490	5505	5519	5534	5548	5563	5577	2	5	7	10	12
34	·5592	5606	5621	5635	5650	5664	5678	5693	5707	5721	2	5	7	10	12
35	5736	5750	5764	5779	5793	5807	5821	5835	5850	5864	2	5	7	10	12
36	·5878	5892	5906	5920	5934	5948	5962	5976	5990	6004	2	5	7	9	12
37	·6018	6032	6046	6060	6074	6088	6101	6115	6129	6143	2	5	7	9	12
38	·6157	6170	6184	6198	6211	6225	6239	6252	6266	6280	2	5	7	9	11
39	·6293	6307	6320	6334	6347	6361	6374	6388	6401	6414	2	4	7	9	11
40	·6428	6441	6455	6468	6481	6494	6508	6521	6534	6547	2	4	7	9	11
41	·6561	6574	6587	6600	6613	6626	6639	6652	6665	6678	2	4	7	9	11
42	·6691	6704	6717	6730	6743	6756	6769	6782	6794	6807	2	4	6	9	11
43	·6820	6833	6845	6858	6871	6884	6896	6909	6921	6934	2	4	6	8	11
44	·6947	6959	6972	6984	6997	7009	7022	7034	7046	7059	2	4	6	8	10

NATURAL SINES.

Degrees	0′ 0°·0	6′ 0°·1	12′ 0°·2	18′ 0°·3	24′ 0°·4	30′ 0°·5	36′ 0°·6	42′ 0°·7	48′ 0°·8	54′ 0°·9	Mean Differences. 1	2	3	4	5
45	·7071	7083	7096	7108	7120	7133	7145	7157	7169	7181	2	4	6	8	10
46	·7193	7206	7218	7230	7242	7254	7266	7278	7290	7302	2	4	6	8	10
47	·7314	7325	7337	7349	7361	7373	7385	7396	7408	7420	2	4	6	8	10
48	·7431	7443	7455	7466	7478	7490	7501	7513	7524	7536	2	4	6	8	10
49	·7547	7558	7570	7581	7593	7604	7615	7627	7638	7649	2	4	6	8	9
50	·7660	7672	7683	7694	7705	7716	7727	7738	7749	7760	2	4	6	7	9
51	·7771	7782	7793	7804	7815	7826	7837	7848	7859	7869	2	4	5	7	9
52	·7880	7891	7902	7912	7923	7934	7944	7955	7965	7976	2	4	5	7	9
53	·7986	7997	8007	8018	8028	8039	8049	8059	8070	8080	2	3	5	7	9
54	·8090	8100	8111	8121	8131	8141	8151	8161	8171	8181	2	3	5	7	8
55	·8192	8202	8211	8221	8231	8241	8251	8261	8271	8281	2	3	5	7	8
56	·8290	8300	8310	8320	8329	8339	8348	8358	8368	8377	2	3	5	6	8
57	·8387	8396	8406	8415	8425	8434	8443	8453	8462	8471	2	3	5	6	8
58	·8480	8490	8499	8508	8517	8526	8536	8545	8554	8563	2	3	5	6	8
59	·8572	8581	8590	8599	8607	8616	8625	8634	8643	8652	1	3	4	6	7
60	·8660	8669	8678	8686	8695	8704	8712	8721	8729	8738	1	3	4	6	7
61	·8746	8755	8763	8771	8780	8788	8796	8805	8813	8821	1	3	4	6	7
62	·8829	8838	8846	8854	8862	8870	8878	8886	8894	8902	1	3	4	5	7
63	·8910	8918	8926	8934	8942	8949	8957	8965	8973	8980	1	3	4	5	6
64	·8988	8996	9003	9011	9018	9026	9033	9041	9048	9056	1	3	4	5	6
65	·9063	9070	9078	9085	9092	9100	9107	9114	9121	9128	1	2	4	5	6
66	·9135	9143	9150	9157	9164	9171	9178	9184	9191	9198	1	2	3	5	6
67	·9205	9212	9219	9225	9232	9239	9245	9252	9259	9265	1	2	3	4	6
68	·9272	9278	9285	9291	9298	9304	9311	9317	9323	9330	1	2	3	4	5
69	·9336	9342	9348	9354	9361	9367	9373	9379	9385	9391	1	2	3	4	5
70	·9397	9403	9409	9415	9421	9426	9432	9438	9444	9449	1	2	3	4	5
71	·9455	9461	9466	9472	9478	9483	9489	9494	9500	9505	1	2	3	4	5
72	·9511	9516	9521	9527	9532	9537	9542	9548	9553	9558	1	2	3	3	4
73	·9563	9568	9573	9578	9583	9588	9593	9598	9603	9608	1	2	2	3	4
74	·9613	9617	9622	9627	9632	9636	9641	9646	9650	9655	1	2	2	3	4
75	·9659	9664	9668	9673	9677	9681	9686	9690	9694	9699	1	1	2	3	4
76	·9703	9707	9711	9715	9720	9724	9728	9732	9736	9740	1	1	2	3	3
77	·9744	9748	9751	9755	9759	9763	9767	9770	9774	9778	1	1	2	3	3
78	·9781	9785	9789	9792	9796	9799	9803	9806	9810	9813	1	1	2	2	3
79	·9816	9820	9823	9826	9829	9833	9836	9839	9842	9845	1	1	2	2	3
80	·9848	9851	9854	9857	9860	9863	9866	9869	9871	9874	0	1	1	2	2
81	·9877	9880	9882	9885	9888	9890	9893	9895	9898	9900	0	1	1	2	2
82	·9903	9905	9907	9910	9912	9914	9917	9919	9921	9923	0	1	1	2	2
83	·9925	9928	9930	9932	9934	9936	9938	9940	9942	9943	0	1	1	1	2
84	·9945	9947	9949	9951	9952	9954	9956	9957	9959	9960	0	1	1	1	2
85	·9962	9963	9965	9966	9968	9969	9971	9972	9973	9974	0	0	1	1	1
86	·9976	9977	9978	9979	9980	9981	9982	9983	9984	9985	0	0	1	1	1
87	·9986	9987	9988	9989	9990	9990	9991	9992	9993	9993	0	0	0	1	1
88	·9994	9995	9995	9996	9996	9997	9997	9997	9998	9998	0	0	0	0	0
89	·9998	9999	9999	9999	9999	1·000	1 000	1·000	1 000	1·000	0	0	0	0	0
90	1·000														

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