The Use and Care of
ZEISS
MICROSCOPES

CARL ZEISS
JENA

Mikro 421 e, 1938 Edition

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Contents

	Page
Part One	
Design of Stands L (Lu, L, Lg, Lr) .	5
Part Two	
Design of Stand E	11
Part Three	
Further Design Details, and Fundamentals of Microscopy	13
Care and Handling of Microscope . .	34
Alphabetical Index	52

This booklet is written with the object of explaining the fundamentals of the design and manipulation of the microscope. The information presented will unquestionably prove of importance to the beginner who is desirous of acquiring a degree of facility in working with his instrument. The more advanced worker, too, is likely to find forgotten or neglected facts which he can put to good account. No attempt is made, however, to meet the requirements of the expert microscopist or specialist.

That it has occasionally happened that a genius has made important discoveries with a microscope though ignorant of the essentials of its use, should mislead no one into slighting the fundamentals of microscopy. Their mastery increases speed and assurance in working and enables the observer to carry through prolonged investigations in comfort.

Parts of a Zeiss Microscope, Stand L

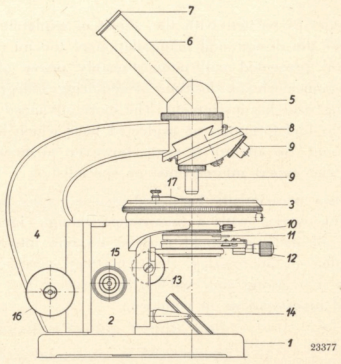


Fig. 1

1 Foot
2 Body
3 Object stage
4 Arm
5 Prism housing
6 Fixed eyepiece sleeve
7 Eyepiece
8 Revolving nosepiece
9 Objectives
10 Condenser
11 Iris diaphragm
12 Iris diaphragm carrier
13 Condenser motion head
14 Illuminating mirror
15 Slow-motion head
16 Coarse-motion head
17 Stage clips

Part One

Design of Stands L (Lu, L, Lg, Lr)

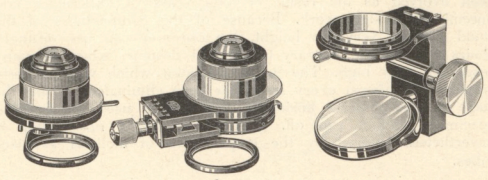
Type L Zeiss microscopes are of a totally novel form which has been evolved as the result of long years of experience and unremitting design work. Because of the compactness of the stand and its very low height, the observer can use ordinary chairs and tables, contrary to the old designs, which in many cases are very high. The inclination joint which was formerly employed in almost every stand has been eliminated, so that the stage always remains horizontal and liquids used for bedding specimens cannot run off. Perfect comfort in observation is nevertheless insured by the employment of inclined eyepiece tubes.

The arm *4*, Fig. 1, is of a fine curved form and serves as a handle for carrying the instrument. The foot has three main bearing surfaces, one at the rear and one at the end of each prong. Two additional bearing surfaces are provided as a safety against upsetting from accidental lateral blows or pressure. These two surfaces are intentionally located in a slightly higher plane than the three main bearing surfaces in order to allow the microscope to stand firmly on an uneven table top. The size and form of the foot are such as to insure amply adequate stability.

The parts designated *10* to *13*, comprising the condenser, iris diaphragm carrier, and condenser motion head, are termed the illuminating apparatus. Its purpose is to effect the most favourable possible illumination for the object with the light available. In each case, several different types of illuminating apparatus are furnished for "L" stands. Illuminating apparatus O is used for the medium stands Lg and Lr, and illuminating apparatus W for the large models L and Lu.

Illuminating apparatus O, Fig. 2, is of simplified design and has the condenser sliding sleeve firmly connected with the condenser slide, Fig. 2c. Vertical adjustment is effected by means of a rack-and-pinion motion. The mirror is plane on one side and concave on the other. Two types of condensers are supplied. One has a fixed iris diaphragm and swing-out filter holder, Fig. 2a. This condenser will serve for the great majority of work. For cases

where it is desired to work also with oblique light, we supply condensers with revolvable and decentrable iris diaphragm and with filter holder, Fig. 2b. This equipment represents a simplified form of the Abbe illuminating apparatus. For dark-field illumination, use may be made of the customary dark-field condensers. All of the various condenser types can be directly interchanged.



a b c

Fig. 2, about 2/5 actual size 25047

Illuminating apparatus W, Fig. 3, for stands L and Lu, departs in its form from the designs hitherto used. It is a kind of universal illuminating device which can be converted into different capacities. This possibility of employing the same condenser in different ways has been attained by designing the sliding sleeve and its carrier, which were hitherto fixed to the condenser motion box, in the form of a "hook-on" substage capable of being taken off altogether from the remainder of the illuminating apparatus and replaced by other substage parts.

Fig. 3 shows three substages:

1. Substage *o*, with fixed sliding sleeve for condensers with iris diaphragm and especially for the pancratic condenser (see Mikro 521e);
2. Substage *d*, which in the form of Model Wd corresponds in all ways to the properties of the Abbe illuminating apparatus;
3. Substage *z* with centrable sliding sleeve and vertical slow motion for the achromatic condenser, for the non-centrable cardioid condenser, for the quartz condenser, and for objectives used as condensers.

In addition, we make a fourth substage, *u*, which when used with stands LW and LuW converts them into an inverted microscope for special work (for details, see Mikro 492e).

Information on the illuminating mirror will be found on page 15.

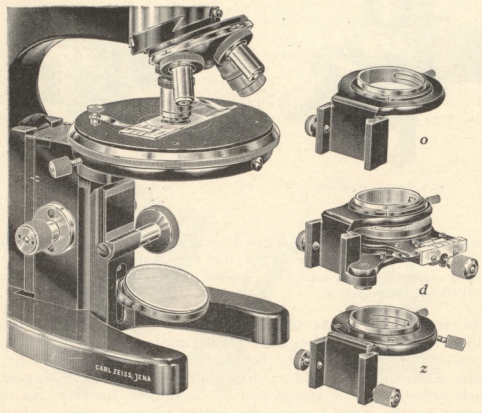


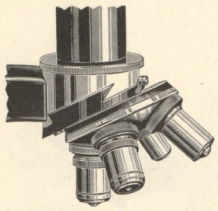
Fig. 3, about 2/5 actual size 23389

Objective Changing Devices

Objectives may be attached either with a triple or quadruple revolving nosepiece, for short turned "revolver", or else with a sliding objective changer. These changing devices are seated in a tube slideway on the tube head.

The changing device most used is the quadruple revolving nosepiece of Fig. 4 (see page 21 for parfocal adjustment). The

revolving nosepiece permits of using the three objectives customarily employed, i. e. a low-power and high-power dry-lens system each and an oil immersion, and in addition a fourth objective. Such an additional objective may be one of extra low



25445

Fig. 4, about 1/3 actual size

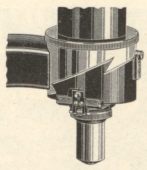


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Fig. 5, about 1/3 actual size

magnification, which can be used to great advantage as a finder to obtain a general survey of the preparation; or it may be found desirable to employ a special objective, for instance for dark-field examination.

The revolver is made with the utmost care and brings each objective into the optical axis of the microscope with sufficient accuracy. The objective to be used is located in position by a knife edge which is held in engagement with a notch opposite each objective by a spring and so accurately registers the objective in the optical axis. To insure proper centring of the objectives, the revolving nosepiece is precisely adjusted and firmly screwed to the tube at the Works. The user is warned against ever removing the revolver, since in so doing it is liable to be brought out of centre. Needless to say, the revolving nosepiece must also be carefully guarded from shocks and blows for the same reason.

The other type of objective changer is the slide fitting with small tube slide of Fig. 5. This fitting takes a single objective mounted on a slide. It gives still higher precision in centring than the revolving nosepiece and allows the change to be likewise made at high speed. This type of objective changer is to be recommended whenever the greatest possible accuracy is required, as for instance and particularly in photomicrography.

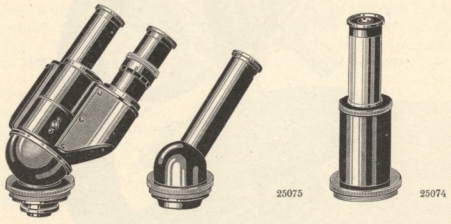
The Eyepiece Tubes

The upper end of the prism head is made with a thread for the reception of any one of three observation tubes which are regularly provided for the stands L. These are:

The inclined monocular tube with fixed eyepiece sleeve for ordinary eyepieces, Fig. 6b.

The inclined binocular tube with dioptric adjustment for one eyepiece and clamp for fixing the eye distance, Fig. 6a. This tube increases the total magnification to 1.5 times that of the normal.

The straight monocular tube without draw tube, for use with a drawing apparatus and with attachment cameras in photomicrography, Fig. 6c.



25075 25074

a b c

Fig. 6, about 1/4 actual size

The inclined monocular tube and straight tube may also be fitted with a draw tube. Besides these three regular tubes, we supply special tubes for special purposes, on which particulars will be found in Mikro 492e.

The eyepieces can be rapidly changed with the aid of an eyepiece revolver with four inclined eyepiece sleeves.

For mechanical tube length, see page 21.

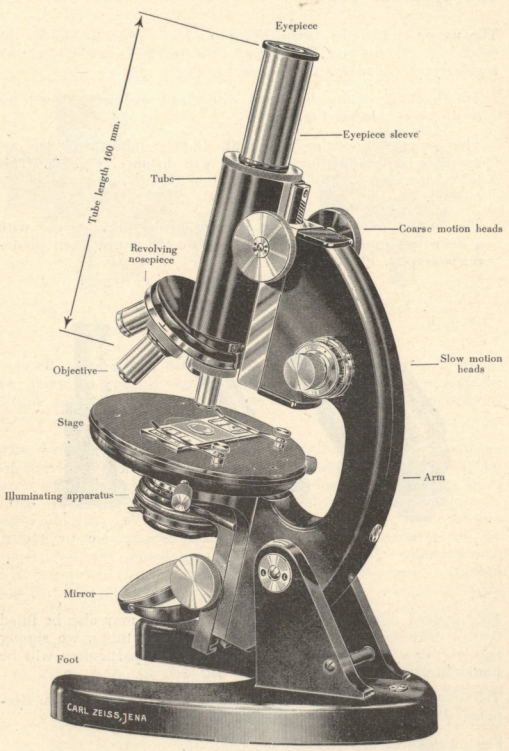


Fig. 7, about 1/2 actual size 12056

Stand EOA

Part Two

Design of Stand E

The type "E" stands are rugged and very strong instruments of a design answering modern requirements. They are of a form having a minimum of corners such as tend to collect dust, Fig. 7.

Objective Changing Devices

The changing device most commonly used is the revolving nosepiece, to which applies that which has already been said in Part One, page 8. On the stands E, the revolving nosepiece screws to the lower end of the tube. The standard nosepiece gives room for four objectives, although models for but two or three objectives will also be supplied. When no revolving nosepiece is used, the objective is screwed to the lower end of the body tube by an intermediate fitting. This fitting is of the same length as the revolving nosepiece, so that the mechanical tube length (page 21) remains the same with either mode of attachment.

A third possibility is to use a sliding objective changer, of the same general design as that of the device shown for the "L" stands, Fig. 5. This device also screws to the end of the tube.



Fig. 8, about 1/7 actual size 22612

The Tubes

On the Stands E, either a straight fixed extension tube or a draw tube may be used (for mechanical tube length, see page 21). Ideal comfort in observation is had by the use of inclined monocular and binocular observation tubes. The binocular models, Fig. 8, are tubes with two eyepieces for standard mon-objective microscopes. They

are of particular advantage in prolonged examinations by permitting both eyes to be simultaneously used.

Arm

On the broad food is seated the one-piece arm, which carries the illuminating apparatus, the stage, and the tube.

The arm is in the general form of a segment of a circle with recessed central portion. This portion serves as a handle and is so shaped as to give a great depth of throat, so that relatively large plates, dishes, and other objects may be examined all over while in position on the stage. The lower end of the arm which carries the illuminating apparatus is inclinable around a pin, around which the arm can be tilted by any angle to the horizontal position of the tube. Because of the width of the foot, the microscope will stand firmly on the table also in this latter position, unless it is too heavily loaded by large eyepiece attachments. The bearing surfaces on the foot are of exactly the same kind as in the "L" stands (see page 5).

The Illuminating Apparatus

For stand E, two types of illuminating apparatus are provided. The simpler of these, designated "B", consists of a condenser sliding sleeve attached to the stage and of a universally adjustable combined plane and concave mirror (see page 15). The other, designated "O", is a simplified illuminating apparatus with rack-and-pinion vertical adjustment, Fig. 2, page 6.

Part Three

a) Further Design Details, and Fundamentals of Microscopy

The microscope is supplied with a wooden cabinet for safe keeping. In order to hold the instrument securely in position during transportation, a heavy screw is passed through the cabinet and into the under side of the microscope foot. This screw must, of course, be withdrawn before it is attempted to take the microscope from the cabinet. A flat key is included with the microscope for turning out the screw, though a screwdriver may also be used.

Stage

The stage provides a support for the specimen under examination. It is bad practice for the observer to allow the full weight of his hands to come on the stage. The elbow or forearm should rest securely on the table and the hand manipulating the slide be allowed to bear on the stage but lightly. It is only in this way that there is certain to be no interference with the focusing of the specimen.

We supply both all-metal stages and stages with a hard-rubber covering. If this covering should become gray, its dark colour may be restored by rubbing in a small amount of oil and wiping off the excess with a clean cloth.

A large opening in the centre of the stage allows ample room for the condenser. When this opening is too large for the work in hand, it can be reduced by means of a stage ring which we supply with the microscope.

Stage Clips

The specimens to be examined are usually mounted on a glass slide and covered with a cover glass. A pair of spring stage clips are supplied for holding the slide in position. In order to fulfill their purpose, the clips must be pushed with their studs all the way into the matching holes in the stage. The clip studs and the holes in the stage should always be kept clean so that the clips can be readily inserted and withdrawn. A trace of lubricating oil will be found helpful.

Much greater freedom of movement for examining the specimen is obtained if the clips are dispensed with altogether. With some patience, the beginner will soon acquire the knack of accurately displacing the slide with the free hand. The stage clips should, however, always be used when it is necessary to hold a slide securely in position for some length of time, as in drawing or photographing.

Mechanical Stages

For systematically searching a specimen and displacing it mechanically, special devices, called mechanical stages, are employed. The microscope illustrated in Fig. 7 is equipped with a simplified mechanical stage. The movable stage plate ist shifted by means of two screws, at the right and left, which act against a spring (not visible in the illustration) in front. Occasionally, a small drop of noncorrosive and nongumming oil should be applied to the projecting end of the spring and also to the accessible parts of the threads in order to keep the screws moving freely. When the two screws are simultaneously turned clockwise the stage plate moves forward, and when they are turned counterclockwise it moves backward. By turning both screws simultaneously in opposite directions the stage plate is traversed sideways.

The range of movement of the simplified mechanical stage is about $\frac{3}{8}$ in. in either direction. If a larger range of movement is required, it is to be recommended to use an attachable mechanical stage, Fig. 9. This stage may be fitted with a scale and vernier for ease in relocating given points in the specimen.

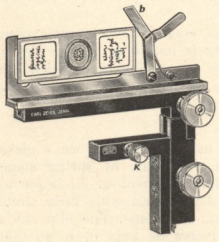


Fig. 9, about $\frac{1}{2}$ actual size 13455
Attachable mechanical stage

In applying the attachable mechanical stage, lever *b* is pressed aside and on being released holds the stage in position by spring pressure. The object stage is regularly provided with three holes for the reception of the attachable mechanical stage. The central one of these holes is threaded for the clamp screw *K*, Fig. 9. The other two holes are plain and receive the pins which hold the attachable stage against rotation. It goes without saying that these holes must always be kept clean. In applying the attachable stage, care must be taken that it bears all over and that the locating pins are engaged all the way in their holes before the clamp screw is drawn up.

The forward and backward movement of the attachable mechanical stage works somewhat more stiffly than the lateral movement. The procedure in systematically searching a specimen is to begin either at the top or at the bottom, traverse the specimen sideways through its entire width, then move forward by the width of the field of view, again traverse sidewise throughout the entire width of the specimen, and so on. With this mode of operation, the forward and backward movement is used relatively little and having it work more stiffly prevents the specimen from being inadvertently displaced.

The large mechanical stage *E* permits of traversing specimens by to 2×3 in. (50×75 mm.). The displacements so produced are directly indicated on scales which can be read to $^1/_{20}$ mm. with the aid of verniers. In this way it becomes possible, by accurately centring the stage with the aid of the screws provided for the purpose, and with the specimen in a definite position noting the readings for certain points in it, to relocate these points at any later time. Moreover, another observer residing at a distance can in this way be enabled to find the designated spots with the aid of these data if the preparation is sent to him. It is, of course, necessary to this end that the second observer have a stage and optical equipment of the same type as the first. For details on such measurements and on the centring of the stage, see Mikro 71 a.

Mirror

The mirror is plane on one side, concave on the other, and mounted so as to be movable in all directions. If it is used without a condenser, either surface may be employed. The concave mirror yields more intense illumination. Condensers should always be employed in conjunction with the plane mirror.

If a specimen is illuminated with the mirror only and without the aid of a condenser, then a diaphragm should be used for reducing the area illuminated to approximately the size of the field of the objective employed. This is accomplished either by means of an iris cylinder diaphragm, Fig. 10, or of a cylinder diaphragm with a set of three interchangeable stops with fixed apertures, Fig. 11. This diaphragm is less liable to get out of order, and in practice it often suffices to use a single size aperture, so that

the inconvenience of frequent changes of stops is avoided. The mount of either cylinder diaphragm has an outside diameter of 36.8 mm. and fits into a sliding sleeve which, in the simplest stands, is attached directly to the under side of the object stage.

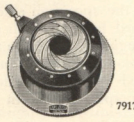


Fig. 10, about 1/2 actual size

Iris Cylinder Diaphragm

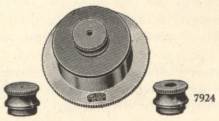


Fig. 11, about 1/2 actual size

Plain Cylinder Diaphragm

When a diaphragm is used, its upper surface should be level with the upper surface of the object stage.

Condensers

Examinations with oil immersion objectives require the aid of a condenser in order to obtain adequate illumination, and a condenser is likewise of advantage in working with all other kinds of objectives. When used with low-power objectives, the front lens of the condenser should be screwed off as shown in Fig. 13.

Condensers are available in considerable variety. The ordinary type, with a numerical aperture of 1.2, is in most common use. For immersion objectives having a numerical aperture of 1.3 or more, a condenser with a numerical aperture of 1.4 would usually be employed. The illuminating aperture of over 1.0 is, however, attained only if the front lens of the condenser, too, is connected with the object slide by a drop of immersion oil. With highly corrected objectives it is advantageous to use also highly corrected condensers, such as aplanatic condenser 1.4, Fig. 12, or an achromatic condenser. It is to be noted, however, that the working distance of a condenser and the area illuminated decrease as the numerical aperture increases. The working distance of an aplanatic condenser is less than that of an ordinary condenser having the same numerical aperture.

Most microscope condensers are built up from a number of lenses. Usually the upper lens or group of lenses can be unscrewed, Fig. 13. The remaining portion then constitutes a condenser of lower aperture and longer focus. The optical components are

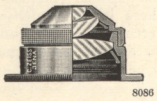


Fig. 12, about 1/2 actual size

Aplanatic Condenser

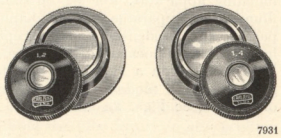


Fig. 13, about 1/2 actual size

Condensers with front lens taken off

usually housed in a cylindrical mount of a standard diameter of 36.8 mm., the same as the cylinder diaphragm, and fits into the sliding sleeve previously mentioned. To focus the condenser, it is slid up and down in its sleeve if this is attached to the object stage (illuminating apparatus B). Focusing is greatly facilitated, however, if the condenser sleeve is mounted on a carrier actuated by a rack-and-pinion motion. In this case the condenser sleeve is provided with a clamp screw which is released to remove the condenser and tightened after a condenser is in place, care being taken to insert the condenser up to its flange. The condenser motion is so adjusted at the factory that in its highest limit position the upper surface of the condenser will lie about 0.1 mm. below the level of the upper stage surface. This is essential because it is desirable to allow the condenser to be brought up as close as possible to the slide, while at the same time the condenser must be prevented from touching the slide, since this would endanger objectives with short working distance and would also interfere with maintaining proper immersion if the condenser is used immersed.

The condenser is accurately centred by us so as to come in the optical axis of the microscope, and will remain centred unless the instrument receives a severe blow or other mechanical injury. In such an event it is best to return the microscope to our representative to have it readjusted. Care, of course, should be taken not to use force when displacing the condenser in its sleeve;

it should be slid up and down with a rotating movement while the sleeve is kept supported with the other hand.

The aperture of condensers is regulated by means of an iris diaphragm. In microscopes equipped with the Type Wd Abbe illuminating apparatus, this diaphragm is mounted on the illuminating apparatus. For use on stands equipped with a simplified illuminating apparatus, the iris diaphragm is attached to the lower part of the condenser itself (except in some special condensers in which it is located between lenses). These latter condensers have in addition a swing-out ring for the reception of a ground-glass disk for moderating the illumination, special diaphragms, or filters (see Figs. 2a and b).

Load on Illuminating Apparatus

Since, with the new illuminating apparatus types O and W, the load on the motion greatly varies with the condenser used (the pancratic condenser, for instance, weighing from six to seven times as much as a simple condenser with iris diaphragm and

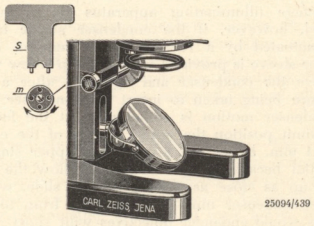


Fig. 14

filter holder), the pinion bearing is equipped with an adjustable brake. By turning nut *m* with wrench *s* in or opposite to the direction of the arrow, the motion can be made to work harder or easier, respectively. *The wrench must not be used for adjusting the coarse motion g.*

Coarse Motion

The body tube can be rapidly raised and lowered by means of a rack-and-pinion motion by turning the coarse-motion heads, Figs. 1 and 7. Turning the heads clockwise lowers, and turning them counter-clockwise raises the tube.

In the Type L stands, the coarse motion is disposed close to the foot in order to allow the observer to rest his arms on the table to relieve the motion mechanism. In these stands, the arm is raised and lowered together with the tube.

The coarse motion should work freely enough to prevent the hand from becoming tired from its continued manipulation, yet must have enough friction to hold the tube firmly in any position so that it will not sink of its own weight or from slight pressure. When binocular eyepiece tubes, camera attachments, and other accessories are used on the tube, it may happen that the tube sinks imperceptibly of its own accord. The movement manifests itself, however, in that the image drifts out of focus without the focusing mechanism being operated by the observer and that this trouble recurs soon after refocusing with the slow motion.

The degree of stiffness can be easily adjusted whenever necessary. In older types of instruments (A, B, and C) two special screws are provided for this purpose, as shown at S_2, S_1, Fig. 15. By

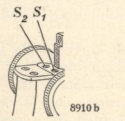


Fig. 15, about 1/4 actual size

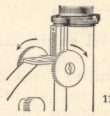


Fig. 16, about 1/4 actual size

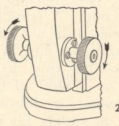


Fig. 17, about 1/2 actual size

Adjustment of friction of coarse motion

A, B, and C — E (and U, D, F, G, H, K, S, V) — Lu, L, Lg, and Lr

turning both these screws in a clockwise direction the friction is increased, and by turning them in the reverse direction it is decreased. The two screws should be tightened simultaneously, and each should be turned through the same angle (a quarter

turn at a time), until the correct amount of friction on the spindle has been reached. A screwdriver of the proper size must be used on these screws, and it must be placed accurately in the slots in order to avoid damage to the heads.

Microscope stands of the newer and newest types are not provided with these adjusting screws but have an even simpler friction adjustment. By holding the left focusing head against rotation with one hand while turning the other in the direction of the arrow, Figs. 16 and 17, the friction is increased, and by movement in the opposite direction it is decreased.

The coarse motion works with great precision, but its movement is too rapid for conveniently focusing objectives of high magnifications. For this reason, the microscope stand is equipped with a second, so-called fine or slow-motion adjustment, Figs. 1 and 7, which is employed for the final focusing when specimens are examined at higher magnifications, approximate focusing having first been effected by means of the coarse motion.

Slow Motion

The fine adjustment operates by means of a system of gears, and is characterized by complete absence of "play". The mechanism is securely housed within the main body of the microscope, only the heads for operating it protruding from either side. The left-hand head is provided with an index and graduated drum, one division of which in stands E, Lg, and Lr equals 0.002 mm. A complete revolution of the motion head displaces the body tube 0.1 mm.
In stands Lu and L, one interval on the drum corresponds to a shift of the tube by 0.001 mm., and one full revolution of the head to a shift of 0.05 mm. In the first-named stands, the fine adjustment is limited at each end of its range by a stop, while in stands Lu and L over-turning is prevented by a ratchet in the motion heads. Two white index lines on the right side of the body mark the limits of the motion range.

Turning the pinion heads clockwise lowers the body tube and turning them counter-clockwise raises it, the direction of movement being the same as for the coarse adjustment. No attempt should be made to lubricate the slow motion, and it must never be tampered with. Ordinarily, it will require no attention; should something go wrong with it, then the safe way is to send the microscope to our representatives. The mechanical devices for focusing a compound microscope are second in importance only

to the optical system. Not only does an imperfect focusing mechanism endanger the more powerful (and more expensive) objectives, but it also hinders, if it does not wholly prevent, adequate detailed examination of the specimen. The proficient microscopist will, therefore, insist that both the coarse and the fine motions of his instrument are in good working order.

160 mm. Mechanical Tube Length

At its upper end, the body tube carries an arrangement for holding the eyepiece, which may either be a draw tube or else a fixed-extension ocular sleeve. The latter is generally to be preferred, since it insures maintenance of the correct mechanical tube length of 160 mm*), a point of large importance. The draw tube is rarely required but can be supplied at any time and is readily interchangeable with the fixed-extension ocular sleeve. Draw tubes are provided with a millimeter scale. The newer draw tubes have a conspicuous line at the 160 mm. mark. This marking of the draw tube is correct provided the body tube is equipped at the lower end with an objective changer (revolving nosepiece or the like) of the standard length of 15 mm. It is to be noted that, in marking the draw tubes for stands of older design such as A, B, and C, an objective changer was not taken into consideration. These tubes, when set at 160 mm., give a mechanical tube length of 160 mm. from the top of the draw tube to the lower end of the body tube. If an objective changer is attached, it is necessary to subtract its length from the reading on the draw tube scale. Many old style revolvers, for instance, have a length of 18 mm., so that the old style draw tube would have to be set to 142 mm. in order to obtain the mechanical tube length of 160 mm. with such a revolving nosepiece.

Parfocal Adjustment

The mounts of the "dry" objectives from Achromat 6/0.17 and Apochromat 10/0.30 on are so interadjusted or "parfocalized" that the image remains visible in passing from one objective to another on the revolving nosepiece or the sliding changer. Exact

*) By "mechanical tube length" is to be understood the distance from the upper edge of the ocular sleeve or draw tube upon which the ocular rests, to the shoulder of the objective thread.

focusing then requires but a very slight movement of the slow-motion head. This parfocal adjustment applies, however, only to normal-sighted observers working with their accommodation relaxed. Short-sighted observers must raise the tube in passing to a stronger system, while far-sighted observers must lower it, if their defects in vision are at all marked and the several objectives differ considerably in strength. Finally, the parfocal adjustment can obtain only when the tube length equals the presribed figure of 160 mm. Otherwise, similar disturbances will arise as those experienced by observers having defective eyesight.

Lower power objectives cannot be parfocalized because of their length or the magnitude of their object distance. Immersion systems are not parfocalized because the application and removal of the immersion fluid prevents rapid transition to a dry system anyway.

The changing of eyepieces likewise does not require a change in adjustment when a normal-sighted observer works with his accommodation relaxed. Here again, however, short-sighted observers must raise and far-sighted observers must lower the tube in passing to a stronger eyepiece.

Objectives and Eyepieces

The eyepiece or ocular is seated in the upper end of the tube, next to the eye. It consists of several lenses mounted in a metal tube which slips freely into the upper end of the eyepiece sleeve or draw tube.

The objective derives its name from being situated next to the object or specimen. It, too, usually consists of a number of lenses placed within a cylindrical mount. At its upper end, this mount is provided with a standard thread for attachment to the objective changer or revolving nosepiece. The same thread has been adopted as a standard by all the better known microscope manufacturers. It is to be noted, however, that all manufacturers do not correct their objectives for the tube length of 160 mm.

Magnification

The magnified image of the specimen under observation formed by the objective is located near the upper end of the microscope tube, and is there picked up and magnified again by the eyepiece.

For this reason, the instrument is known as the "compound microscope", the image examined by the eye being twice magnified, in contrast to the single magnification produced by a magnifier.

The degree of magnification obtained is readily found by placing a glass rule, known as a stage micrometer, Figs. 25 and 26, on the stage of the microscope, and projecting its magnified image on a flat white surface placed 250 mm. away from the eyepiece and perpendicular to the microscope axis to avoid distortion. To see the image well, it will usually be necessary to darken the room partly. If, for example, it is found that 30 divisions of a stage micrometer with 1 mm. divided into hundredths and therefore having a length of 0.30 mm., measures 120 mm. in the projected image, then the magnification equals $\frac{120}{0.3}$, equal to 400 diameters or, as it is commonly written, 400×. If the image were projected a greater distance, the magnification would be increased. A distance of 250 mm. has been arbitrarily set as a standard for rating the magnification of a microscope because this is the generally accepted distance of distinct near vision for the normal human eye.

In actual practice, it is not necessary to measure the magnification when modern Zeiss objectives and eyepieces are employed, since they have their individual magnifications engraved on the mounts. The total magnification of the microscope is the product obtained by multiplying the individual magnifications of the objective and eyepiece used. As an example, objective 40 (4 mm.) with eyepiece 10× gives a total magnification of 400×. The same objective with eyepiece 15× yields a total magnification of 600×. These values are correct if the standard mechanical tube length of 160 mm. is adhered to. For the limits of useful magnification see pages 25 and 26.

Spherical and Chromatic Corrections

To understand the optical performance of a microscope, it is essential to bear in mind the fundamental problems involved in producing a relatively faithful magnified image of an object. If the image is produced by a simple lens having spherical surfaces, two main kinds of defects will be present. On the one hand, it will be found that a point of the object does not appear as a point but as a disk, and on the other that the image shows colours which are not present in the object. Both of these defects,

the spherical and chromatic aberrations, are corrected to a high degree in Zeiss objectives.

Generally speaking, this result is obtained by building up an objective from a number of lenses made of glasses, and in some instances transparent minerals, differing both in their optical properties and in the curvatures given to their surfaces. Extreme precision is required in mounting the component lenses of a highly corrected objective, and it goes without saying that the user should never attempt to take the objective apart.

For many years, chromatic correction was restricted to two colours of the spectrum, and because of the great improvement as compared to single-lens objectives, these corrected objectives were called "achromatic", although according to present-day standards, the image produced by them is far from being free from colour defects. It was not until new glasses with until then unknown optical properties were produced as the result of the joint labours of Abbe and Schott, that it was possible to carry out more complete correction. The culmination was reached in Abbe's apochromatic objectives, which embody chromatic as well as spherical correction of an entirely new order. As many as twelve lenses are required in some of these apochromatic objectives to attain the desired results. The new material, however, made it possible also to improve the achromatic objectives, so that these yield relatively good images and are preferred for many purposes because their simpler construction makes them less expensive. So-called fluorite or semi-apochromatic objectives occupy an intermediate position between achromats and apochromats, both in respect to correction and to price.

Flatness of Field

If the best possible correction is to be aimed at in a microscope objective, flatness of field must be considered a secondary consideration. In fact, the field in some of the best corrected objectives is conspicuously curved, so that the centre and the margin will not be simultaneously in focus. Usually it is not absolutely necessary to have the entire field of view simultaneously in focus, since only a portion can be attentively observed at a time. The trained microscopist continuously operates the fine adjustment of the microscope during observation, and automatically brings the portion of the field occupying the centre of his interest into the sharpest focus. It is important, however, that the definition be good over the entire field, so that a good image may be obtained

anywhere in it. The degree of correction of an objective can best be ascertained by means of an Abbe Test Plate, Fig. 18 and Mikro 116 e.

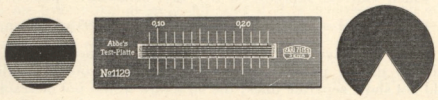


Fig. 18, about $^2/_3$ actual size 8263

Abbe Test Plate

Flatness of field is more important in photomicrography, since there the focus cannot ordinarily be changed in exposure. In order to have a larger portion of the field simultaneously in focus when photographs are to be made, special lens systems called Homals (Mikro 390 e) are employed instead of eyepieces. Homals cannot be used for direct visual observation.

Resolving Power

A microscope is employed in order to obtain a magnified image of an object, and it is important to understand to what limits magnification may advantageously be carried. The aim in magnification is to see greater detail. If the image produced by an objective does not contain given structure details characteristic of the object, then obviously these details cannot appear in the final image, after further magnification by the eyepiece. The principal point of interest therefore are the qualities of an objective which determine its capacity for reproducing minute details of structure, or resolving power. The factors which determine the resolving power of an objective have nothing to do with its spherical and chromatic corrections, although these must, of course, be of a high order if a usable image is to be obtained.

The resolving power of an objective ist proportional to its numerical aperture (see below), and inversely proportional to the wave length of the light employed. Or, in other words, the greater the numerical aperture and the shorter the wave length of the light, the smaller will be the structure which can still be reproduced by the objective. Written in the form of an equation we have:

Smallest structure visible $= \frac{\text{wave length of light}^*)}{\text{numerical aperture.}}$ The average wave length of white light may be taken as roughly 0.55 microns (1 micron, symbol μ, = 0.001 mm.). Accordingly, the smallest structure which can be reproduced by, say, achromatic objective 40 with a numerical aperture of 0.65 is $\frac{0.55}{0.65}\ \mu$ or 0.85 μ. This means that if the objective were used to examine a structure consisting of closely drawn parallel lines 0.5 μ apart, the image produced would not show separate lines; the structure would not be resolved. Employing powerful eyepieces in conjunction with the objective under these circumstances is therefore of no use. Even a 20× eyepiece yielding, in this case, a total magnification of 800×, cannot reveal the lines since they are not present in the image produced by the objective. In order to resolve the structure in question, employing central illumination and white light, it is necessary to use an objective of greater aperture, say, achromatic objective 90 with a numerical aperture of 1.25. In this case the smallest structure which can be reproduced is $\frac{0.55}{1.25}\ \mu$ or 0.44 μ, and it will be found that the lines of our hypothetical specimen are brought out in the image. They can be distinctly perceived even with a relatively low-power eyepiece giving a total magnification of about 600×.

Numerical Aperture

In the foregoing discussion, the term "numerical aperture" played a prominent part. It is worth the effort to define more precisely what this term means and how it is arrived at, since in so doing an insight is obtained into some of the basic conditions upon which the performance of an objective depends.

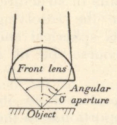


Fig. 19 22767

The resolving power of an objective increases with the angle at which light enters it. The increase is not, however, directly proportional to this angle, but is proportional to the sine of half this angle σ. By way of an example, let us take two objectives in which the maximum angle at which light can enter is 80° and 120°, respectively. The sine of one half of 80°, or 40°, is 0.64; the sine of one half of 120°, or 60°, is 0.87. The resolving powers of the two objectives therefore bear the ratio of

*) This formula holds for central illumination; if extremely oblique illumination is employed, the resolving power is approximately doubled.

64:87, and not 80:120. The resolving power also increases with every increase in the refractive index of the medium intervening between the front lens of an objective and the cover glass over the specimen. If this medium is water with a refractive index of 1.33, the resolving power will be 33 per cent greater than if it is air (refractive index 1.00), and if it is cedarwood oil with a refractive index of 1.51, the resolving power will be 51 per cent greater than with air. This does not mean that the resolving power of a dry objective can be increased 50 per cent by the simple expedient of placing cedarwood oil between its front lens

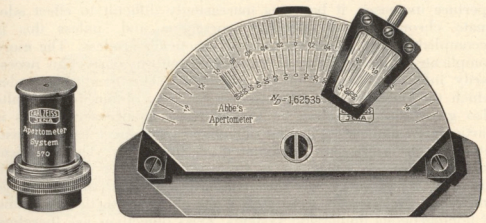


Fig. 20, about 2/3 actual size 22205

Apertometer

and the cover glass. The refractive index of the medium between the front lens and cover glass enters into the formula for objectives, and a given objective will perform properly only with the medium for which it has been calculated.

Abbe introduced the term "numerical aperture" (abbreviated N. A.) for the product of the sine of half the maximum angle σ at which light enters the objective, and the index of refraction n of the medium between the front lens of the objective and the cover glass:

$$A = n \times \sin \sigma.$$

The brightness of the microscopic image is directly proportional to the square of the numerical aperture, and inversely proportional to the square of the magnification.

In practice, numerical aperture is directly measured by means of a simple instrument, the Apertometer (Fig. 20, and Mikro 114 e). Because of its importance, the numerical aperture is engraved on all Zeiss objectives when it exceeds 0.15.

Correction for Thickness of Cover Glass

It goes without saying that microscope manufacturers have made every effort to push the numerical aperture to the utmost limits. It would be a mistake, however, to conclude that an objective of the highest N. A. is the best for all purposes. As the numerical aperture increases, it becomes increasingly difficult to effect adequate chromatic and spherical correction and, unless this is accomplished, the larger aperture is practically useless. The more complicated means required to secure uniform correction necessarily increase the cost of the objective. The conditions under which the objective must be used in order to obtain best results also grow more exacting as the aperture is increased. This is particularly noticeable in dry objectives in respect to their sensitiveness to variations in cover glass thickness. The spherical correction of an objective is influenced by such variations, if the refractive index of the medium between the objective and cover glass differs from that of the glass. With the exception of homogeneous immersion systems, Zeiss objectives are therefore corrected for a standard cover glass thickness of 0.17 mm. Objectives are also corrected for a definite tube length, and a deviation of a few millimeters from the prescribed tube length suffices to impair the image of a highly corrected objective with large numerical aperture (see page 21). With objectives having a numerical aperture not exceeding about 0.65, the cover glass thickness may sensibly depart from the prescribed figure without seriously impairing the image. But a deviation of a few hundredths of a millimeter from the prescribed cover glass thickness suffices to depreciate the image of an objective of N. A. 0.85 or 0.95. The image obtained with a highly corrected fluorite objective 40, N. A. 0.85 or with an apochromatic objective 40, N. A. 0.95 may actually be markedly inferior to that obtained with an ordinary achromatic objective 40, N. A. 0.65, if the former are used with cover glasses of incorrect thickness.

There are two ways to avoid difficulties of this kind. One is to measure the cover glasses by means of a Cover Glass Gauge (Fig. 21), and use only those of the prescribed thickness. The other way is to provide the objective with an adjustable correction

mount, Fig. 22, by means of which the objective can be set for cover glasses of different thickness, the range usually extending from 0.12 to 0.20 mm., which covers the variations in thickness likely to be encountered. When working with objectives provided with correction mounts, it is a convenience to know the thickness of the cover glass employed, since it is then only necessary to set the collar to the known value. If the thickness is not known, the collar must be adjusted back and forth while observing the image until it is at its best. This is a troublesome procedure, especially if a considerable number of slides are to be examined in rapid succession.

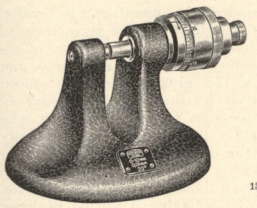


Fig. 21, about 1/2 actual size

Cover Glass Gauge

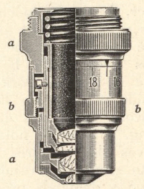


Fig. 22, actual size

Objective with Correction Mount

The correction collar bb serves to alter the distance which separates the two upper doublets from the lower lenses secured within mount aa.

If measured cover glasses are employed and the correction collar of the objective is set for the corresponding value, the adjustment will be correct only if the specimen is in close contact with the cover glass. An intervening layer of Canada balsam, for example, will have the same effect as an increase in cover glass thickness.

A further characteristic of practical importance is that the working distance of objectives becomes less as the numerical aperture increases (assuming the focal length to remain constant). Full data regarding working distance, numerical aperture, initial magnification, and focal length are contained in Mikro 367 e.

Choosing Objectives

From a consideration of the foregoing paragraphs, it will be evident that the selection of suitable objectives for a given purpose is not an altogether simple matter. Certain standard combinations have, however, gradually been developed which meet a large percentage of requirements. If the cost is to be kept low, a good selection would consist of achromatic objectives 8, N. A. 0.20, 40, N. A. 0.65, oil immersion 90, N. A. 1.25, and perhaps in addition an exceptionally low-power objective, such as achromat 3 or 2. By substituting fluorite oil immersion 100, N. A. 1.3 or apochromatic oil immersion, 90, N. A. 1.3, for the ordinary achromatic oil immersion, the efficiency of the equipment is increased at relatively small additional expense and without greatly adding to the care required in operation. Generally, however, the use of apochromatic objectives is to be recommended if their price does not exclude them. These objectives are of advantage not only in the examination of difficult specimens, but perhaps even more because of the greater ease they afford the eye in routine work on account of their superior definition, a consideration of no little importance to a microscopist who must work for hours at a time at the microscope. Generally speaking, it is to be noted that the use of more highly corrected objectives also requires the observer to exercise greater care in order to secure the results of which these objectives are capable. Microscopy demands precision, and in taking it up the beginner should from the outset determine to master the fundamental requirements of his equipment and meet them as a routine procedure.

A favourite selection of apochromatic objectives consists of 10, N. A. 0.3, 20, N. A. 0.65, and oil immersion 90, N. A. 1.3. For many practical purposes, apochromat 40, N. A. 0.95 may be omitted on account of its short working distance and the necessity of exactly adjusting the correction collar if the advantages of this excellent objective are to be realized. Instead, apochromat 20, N. A. 0.65 serves very well as a high-power dry objective. It will be noted that its N. A. is identical with that of achromatic objective 40, N. A. 0.65. If the selection of apochromatic objectives is to be further extended, one of the oil immersions, N. A. 1.4, would primarily come into consideration, either as an additional objective, or as a substitute for apochromat 90, N. A. 1.3. They possess the greatest resolving power of the entire series of standard objectives, and one of them should be included in every equipment which is required to meet the most exacting demands. They are made in two focal lengths, namely 2.9 mm., initial

magnification 60×, and 2 mm., initial magnification 90×. The latter has its front lens mounted with extreme delicacy so that the slightest contact with a cover glass is liable to dislodge it. This, together with the short working distance, renders it imperative to apply exceptional care in its use. The front lens of apochromat 60, N. A. 1.4, on the other hand, is more securely held, and on account of its greater working distance there is also less danger of collision with the cover glass. With reasonable care, it can be safely used in daily routine work and, because of its greater ease of manipulation, is generally preferred to apochromat 90, N. A. 1.4.

More about Eyepieces

One of the main functions of the eyepiece is to magnify the image produced by an objective to where the observer's eye can readily perceive the details of that image. Generally, it will be found that, if the total magnification equals about 500 times the numerical aperture of the objective employed, the eye will see all the detail reproduced by the objective. For convenience in observation, it may be desirable to extend the magnification somewhat further. Eyes differ, and a coarser spacing of the perceptive elements in the retina of an observer requires a proportionally greater magnification of the image in order to permit its details to be perceived. Moreover, an untrained observer is likely to perceive details of the image more readily if it is magnified somewhat beyond what would be adequate magnification for a skilled observer. In any event, the upper limit of useful magnification for direct visual examination will generally be reached at about 1000 times the numerical aperture of the objective employed. Accordingly, the maximum magnification admissible for an objective with a numerical aperture of, say, 0.65 would be approximately 650×. It must always be remembered that this useful magnification, and not the possible magnification, determines the value of the microscope.

For measuring and counting, on the other hand, it will often be desirable to exceed the useful magnification. In such cases, however, the point is not to make out the finest details but to obtain a convenient survey over intervals and particles.

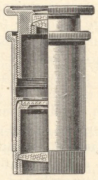
With achromatic objectives, Huygens eyepieces are usually employed, although if the objective has a numerical aperture of 0.65 or over, compensating eyepieces may also be used to advantage. Orthoscopic eyepieces are especially to be recommended if high eyepiece magnifications are desired with achromatic objectives of

apertures below 0.65. Orthoscopic eyepices are also characterized by an exceptionally large field of view. Apochromatic objectives are used exclusively in conjunction with compensating eyepieces. Their name is derived from the fact that, in order to obtain the most perfect colour correction, they are made with a magnification difference for the several colours of the spectrum just the opposite of that obtaining in the objectives. Consequently, the two must be used together in order to obtain a properly corrected image.

Full data as to field of view, magnification, etc. for the various eyepieces are contained in Mikro 367 e.

Micrometer Eyepieces

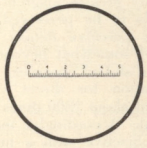
For measuring purposes, use in made of specially mounted eyepieces, usually magnifying 7× and known as focusing eyepieces, or as micrometer eyepieces if they are equipped with a scale. In these eyepieces, the upper "eye lens" is adjustable by means



8744

Fig. 23, about $^2/_3$ actual size

Micrometer Eyepiece K 7×



7903

Fig. 24, about $1^1/_2$ actual size

Eyepiece Micrometer, 5 mm. divided into 100 parts

of a screw thread (Fig. 23). The scale is a ruled glass disk. The lower end of the eyepiece can be unscrewed and the micrometer laid upon the diaphragm, ruled side down. A standard ruling for micrometers is 5 mm. divided into 50 parts (Fig. 24), but other rulings can also be supplied. The micrometer eyepiece is inserted into the microscope tube in the customary manner and the eye lens adjusted until the scale is in focus.

Before the scale of an eyepiece micrometer can be used for measuring in "absolute" units, it must be calibrated by means of a stage micrometer (Figs. 25 and 26) which is viewed in the same way as a specimen would be. Supposing that a stage micrometer ruled in 0.01 mm. is employed and that ten of its divisions coincide with five divisions of the eyepiece micrometer, then five divisions of the eyepiece scale correspond to an actual length of 0.10 mm. in the specimen, and one division of the eyepiece scale represents 0.02 mm. or 20 µ (1 micron, symbol µ, = 0.001 mm.).

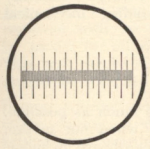


Fig. 25 7902

Scale magnified about 24×

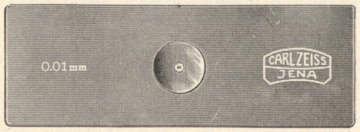


Fig. 26, actual size 8329

Stage Micrometer, 1 mm. divided into 100 parts

It is necessary, of course, to calibrate the scale for each objective employed. If the microscope is equipped with a draw tube, care must be taken that it is set for a definite tube length, since any change in tube length will alter the value of the eyepiece scale.

The draw tube offers a means of obtaining convenient integral values for the eyepiece scale. If, for instance, for a given objective and the standard tube length of 160 mm., one division represents 10.4 µ, then this value can be reduced to 10 µ by increasing the tube length. This method has its limitations, however. Deviations from the standard tube length must not be so great as to impair the image and render measurement unreliable. A changeable tube length is a fertile source of errors, and great care is required to insure that the length is not changed unintentionally.

b) Care and Handling of Microscope

Having become familiar with some of the main considerations involved in the design of a microscope, there remain now to be taken up the more important details in handling and working procedure.

In all circumstances, it must be borne in mind that a microscope is a precision instrument which requires considerate care if it is to perform reliably and give the long service of which a well-made microscope is capable. A good microscope properly used will literally last a lifetime. Proper use in a microscope essentially means two things: keeping it clean and avoiding mechanical injury.

Protection from Dust and Oil

A microscope should be protected from dust as much as possible. If it is impracticable to return it to its case when not in use, then it is best covered with a bell jar or a cardboard cylinder. A further good practice is to provide a cloth of soft, close-woven fabric which is loosely thrown over the microscope when not in use. No matter how well it is protected, it will be found in most locations that dust none the less accumulates on the instrument. This should be removed by first dusting with a soft, clean brush (a $1\,^1/_2$-inch flat paint brush will answer) kept for the purpose, and then wiping with a soft clean cloth. Dust is apt to contain minute sharp particles, — concrete and plaster are prolific sources, — and if a cloth is used for wiping the instrument before these particles are removed, they will act as an abrasive, dulling and in time removing the finish of the metal parts, and impairing the lens and mirror surfaces. Hence the importance of dusting with a clean brush before wiping with a cloth. The brush can be kept clean by dusting it out against a clean piece of wood or glass rod each time it has been used and storing it in an envelope or in some place where it is safe from dust. A separate, smaller brush, preferably camel's hair or sable, should be provided for the optical parts. Oil should be kept from lenses and mirrors.

To maintain a good finish on the metal parts, it is desirable occasionally to use a trace of good non-corrosive oil (fine machine oil). The oil is lightly applied by rubbing on a few drops with a soft rag and wiping off all excess with another clean but dry rag. When properly done, this will leave an invisible protective film on the instrument but not enough to rub off appreciably on

to the user's hands. A minute droplet of oil should occasionally be applied to screws and joints to prevent corrosion (a toothpick is a convenient tool), and to keep movable parts from rusting. The surplus should again be thoroughly wiped off. Occasionally rubbing in some oil on the wooden case and wiping off the excess will improve its appearance and protect the lacquer coating against atmospheric influences.

Oil must not be employed for lubricating bearing and registering surfaces such as those on the condenser, the coarse motion rack and pinion, the bearing surfaces of mechanical stages, etc. These parts should be lubricated with nonacid vaseline. If the grease on bearing surfaces has become hardened, it may be removed with the aid of xylol or benzene. No attempt should be made to oil or clean the slow motion of a microscope. This will rarely require attention, but if it does the microscope should be returned to our representatives.

It goes without saying that if liquids are spilled on the microscope during work, they must be carefully removed before setting the instrument away. Cedarwood oil, Canada balsam, or similar substances are best taken off with xylol or chloroform. Alcohol should never be used for cleaning a microscope. Although modern finishes are relatively alcohol-resistant, some of them are nevertheless more soluble in alcohol than in xylol. On the other hand, Canada balsam is more readily removed by means of xylol.

Cleanliness in Handling Optical Parts

Cleanliness in all matters pertaining to a microscope is essential, and is doubly essential with its optical parts. Avoiding the soiling of glass surfaces is preferable to cleaning them, and to this end a few simple precautions should be observed. An eyepiece should always be kept in the eyepiece sleeve. This prevents dust from entering the tube from above and settling on the back lens of the objective. In all cases, it will of course be advisable to keep the whole instrument covered with a bell.

With binocular microscopes, too, the eyepieces should be kept in the eyepiece sleeves to prevent dust from settling on the upper prism face. See to it that eyepieces not in use are protected from dust, and, in changing them, be particularly careful not to set an eyepiece down on a dusty surface and later replace it in the eyepiece sleeve of the microscope. Otherwise, dust will be conveyed into the tube and deposited on the back lens of the objective.

Lens and other glass surfaces should not be touched with the fingers, as this will leave oily markings and what is worse, corrosive sweat. If a glass surface requires cleaning, first dust it with a small camel hair or sable brush kept especially for cleaning the optical parts. See to it that the brush is kept free from grease, that it is well dusted out (by flicking it against a clean wood or glass rod), and store it away in an envelope. If dusting does not sufficiently clean the glass surface, it may be wiped with a clean soft linen cloth or with Japanese rice paper, moistened, if necessary, with distilled water. If this, too, does not suffice, then xylol may be tried. In any event, unnecessary flooding of the surface with liquid and undue rubbing should be avoided.

Cleaning Eyepieces and Objectives

Eyepieces

The optical parts of the microscope must at all times be kept scrupulously clean. In the eyepieces, the presence of dust on the lens surfaces becomes readily noticeable on narrowing down the diaphragm of the illuminating apparatus and, with the specimen focused, shifting it until it completely disappears from the field. Any dust, particularly on the condenser lens, will then be seen to stand out as dark points or specks. In turning the eyepiece in the tube, the specks will participate in the rotation. When such specks are noticed, the outside of the lens must be dusted with a clean camel hair brush or a spotless rag of fine soft cloth such as washed linen. Failure of such cleaning to eliminate the specks indicates the presence of dirt on the inner surfaces of the lenses. When this is the case, the eyepiece must be taken apart for cleaning. In this operation, the following points should be borne in mind:

Always take apart **only one eyepiece at a time,** and clean and completely reassemble it before starting in on the next. Carefully note order and position of lenses. Do not screw the condenser lens to the end of the eyepiece tube to which the eye lens belongs. Be equally careful to avoid displacing the diaphragm in the interior of the tube. In either eventuality, the edge of the diaphragm will no longer appear sharply defined when the eyepiece is screwed together again. If the lenses are removed from their cells, care must also be taken to place them with the proper surfaces facing out and in. When the eyepieces become fogged at low temperatures, they should be carefully and slowly warmed up by placing them near a radiator.

Objectives

Uncleanness of objectives is not as a rule so readily apparent. Isolated sharply bordered dust particles do not affect the image at all but merely cause a negligible loss of illumination. Larger blotches covering portions of the lens aperture of some size but still passing light, betray themselves by "dead" images lacking contrasts and veiled by a fog of light. In this case, the objective must be examined with a magnifier. The front lens should be viewed in incident light while letting a bright surface, such as a window, reflect itself in it. The rear lens must be observed in transmitted light by holding the objective towards and with its front lens facing a window.

In objectives, cleaning should be restricted to the outside surfaces, and in particular to the external surface of the front lens. If this surface is dirtied by a reagent, then it must be immediately cleaned with distilled water. **Alcohol must not be used under any circumstances.** (For procedure in removing immersion fluids, see page 50).

In no circumstances must the oil be allowed to resinify on the front lens. When this happens, the film so formed hardens and reduces the small object distance by enough to cause collision with the preparation in focusing, at the risk of loosening and decentring the front lens in its cell. By far the greater proportion of the repairs required on oil immersions are due to this cause.

The cleaning of oil immersions is greatly facilitated when the observer makes it a practice not to use too much oil from the outset. If the microscope has been kept protected from dust, then cleaning of the rear lens of the objective will hardly ever be required. If this should nevertheless become necessary, the dust must be removed with a soft and well cleaned brush which has been kept stored safe from dust, and by blowing with a rubber bellows. Now wrap the end of a round-pointed softwood stick of about the diameter of a match with a strip of clean linen, leaving no wood protrude, and wipe the rear lens lightly with a rotary motion. Perfect cleaning can, however, be effected only by an expert with the necessary auxiliaries available in an optical shop, where also the internal surfaces which are not accessible from the outside can be got at. **We expressly warn users against taking objectives apart.** Any attempt to do so is almost certain to lead to damage of the lens system and is sure at least to reduce its performance.

In systems containing fluorite, examination with a magnifier sometimes reveals small dark specks which cannot be removed

by cleaning. As a rule, these specks are small inclusions in the fluorite which must be regarded as totally innocuous blemishes. In view of the rarity of the material, pieces of proved optical perfection cannot be excluded from use because of such specks. For this reason, we cannot admit claims based on the presence of blemishes of this kind.

Objectives which are not needed on the microscope should always be kept in their boxes. Similarly, objectives should be packed in their boxes when they are sent in for repairs. The boxes are engraved on the bottom end to permit the objectives to be inserted into the cover with the front lens up, so as to protect the back lens from dust (Fig. 27).

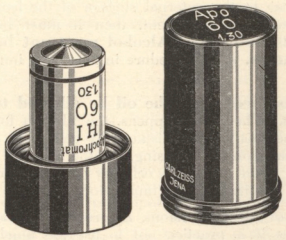


Fig. 27, about actual size 25093

Objective box

Daylight and Artificial Light

If possible, the observer should avoid facing a window, unless the upper part is effectively shaded so that no bright light falls directly upon the eyes and the microscope is at the same time close enough to the window to afford adequate illumination of its mirror. It should be remembered that the microscope image often reveals details which are only faintly visible and that their observation is rendered more difficult if light from without enters the eye at the same time. Neither should bright light from without fall upon the eye lens of the eyepiece, since this may produce disturbing reflections. Generally, it is preferable to have the window at the observer's side, inclining the mirror accordingly. Direct sunlight should not fall upon the mirror. A north

window is to be given preference since it supplies fairly even illumination for the greater part of the day. Since daylight is subject to constant change while it is desirable to have a uniform source of illumination, many observers work entirely with artificial light. For most purposes, an incandescent electric lamp, Fig. 28, provided with a boiling flask for a condenser and filter,

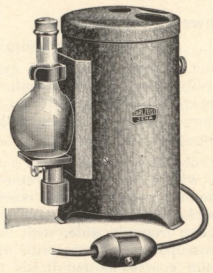


Fig. 28, about $^1/_8$ actual size 13692

Microscope Lamp Model I

will be found satisfactory. If the flask is to serve merely as a heat filter, it is filled with distilled water. The light will then have a slightly yellowish tinge. An ammoniacal copper sulphate solution, on the other hand, supplies an almost white light approximating daylight. A good plan is to prepare a concentrated stock solution of copper sulphate in ammonia water and add this a drop at a time to the distilled water in the flask until the desired intensity of colour is obtained. Many observers prefer a slight blue tinge to the light. In this case, an excess of ammonia water should be added to the distilled water in the flask. If the solution turns greenish, the blue colour can be restored by adding more ammonia water. For work with low magnifications, the lamp may be fitted with an ordinary filament bulb (about 25 c. p.). If this does not furnish sufficiently bright light, special 100-watt filament bulbs should be used, especially in dark-field work.

Mounting the Specimen

Specimens for examination are usually mounted on a glass slide and covered with a cover glass. Temporary mounts may be made by placing the specimen in water or watery solutions. Permanent specimens are most often mounted in Canada balsam, or in some other medium of a refractive index more or less approaching that of glass.

Examination at Lowest Magnification

A beginner will best start with the lowest power objective with which his microscope is provided, and the experienced observer likewise will generally begin the examination of an unfamiliar specimen at low magnifications in order to become acquainted with its general features before proceeding with the more detailed examination of some special part.

The lowest power objective having been swung into position over the slide, and with a low-power eyepiece, say 5× or 7×, in the tube, lower the tube by means of the coarse motion until the objective almost touches the cover glass. This is ascertained by observing the objective from the side, with the eyes about on a level with the microscope stage. Now, with the observer looking into the eyepiece, the coarse motion heads are turned to raise the tube until the magnified image of the specimen appears. If the focal plane should inadvertently be passed by without being noticed, the procedure is repeated. Under no circumstances should the observer shift the tube downwards to find the specimen with his eye applied to the eyepiece. Failure to observe this simple precaution has ruined many slides as well as objectives. When the specimen has been found, it is accurately focused by shifting the tube with the coarse motion until the image appears sharpest. If fairly high magnifications are employed, final focusing is best carried out with the slow motion. The slow motion should therefore not be used for finding the specimen, but only for sharpening the final adjustment after the specimen has been approximately brought into focus with the coarse motion. During observation with any excepting the very lowest magnifications, one hand continuously operates the slow motion, bringing successive levels of the specimen into sharp focus, and making the necessary changes in focus as the attention shifts from one part of the field to another. The eye itself can make considerable adjustments in focus, but much unnecessary fatigue is avoided if the observer from the outset insists upon accurately focusing with the microscope the detail of the specimen which

occupies his interest. In a short time he will learn to manipulate the slow motion without paying any attention to it whatsoever. As a rule, the right hand will be used.

It is well to have the slow motion approximately at the middle of its range, as indicated by the marks at the right side of its housing, before beginning work. If this is done, the observer will not have to fear that he will suddenly come to the end of the fine adjustment during the course of an examination.

Adjusting the Illumination

After a specimen has been found and focused with a low power objective, the next step is to adjust its illumination. The mirror is tilted back and forth until the entire field is evenly illuminated. When a window is employed as a source of light, the sash cross bars should be avoided whenever possible. If an artificial source of light is used, the illuminating beam should fall upon the centre of the mirror. A piece of paper may be held on the mirror in order better to locate the position of the illuminated area. If the microscope is equipped with a condenser, the distance of the lamp and its condensing means (boiling flask) should be such that an image of the lamp filament is formed *in the plane of the iris diaphragm* of the microscope condenser. (Some of our microscope lamps are furnished with a connecting bar, Fig. 29, which registers the lamp in the correct position). This is

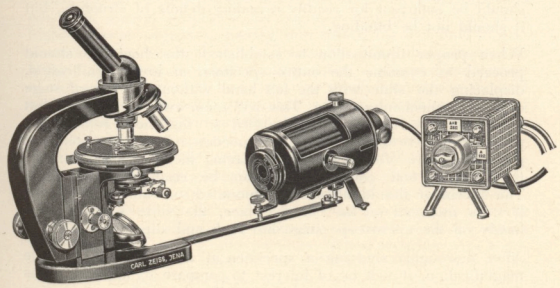


Fig. 29, about 1/5 actual size 23212

readily ascertained by removing the ground glass from the lamp and holding a piece of paper over the diaphragm of the microscope condenser. If necessary, the microscope condenser is then focused so that an image of the lamp filament appears sharply defined together with the specimen. Before proceeding with the study of the specimen, the ground glass of the lamp should be replaced in order to obtain more uniform illumination of the field of view. Actually, it will be found in working with low-power objectives that the microscope condenser, when focused, does not illuminate the entire field. With many condensers, this condition can be remedied by unscrewing the front lens or lens set, the portion of the condenser so left having a longer focus and illuminating a larger field. This, however, is a somewhat troublesome procedure, and for most practical purposes it will suffice to focus the condenser down until the entire field is covered (see also page 16), although the condenser diaphragm may thereby lose its effect as aperture stop. Having obtained an even illumination of the entire field, it remains to regulate its intensity. To a certain degree this can be accomplished by means of the iris diaphragm below the microscope condenser.

Too Intense Illumination Dangerous

Great care should be exercised to employ the correct intensity of illumination. This will not be the same for all observers. Generally speaking, there is a tendency for beginners to use too much light. The illumination for any given magnification should be sufficient for readily revealing details of structure, but it should not be blinding.

When proper illumination is established, the beginner should proceed to examine the entire specimen at low magnification, displacing the slide with the left hand without the use of stage clips or a mechanical stage. This will seem awkward at first, but the patient worker will be gratified to find how rapidly his persistence is rewarded. In this connection it is to be noted that glass slides are often warped. By placing them on a flat surface, the concave and convex faces can be readily distinguished. The specimen should always be mounted on the convex face. If it is mounted on the concave face, the slide will not rest firmly on the microscope stage but rock and slip about.

After thoroughly studying a specimen at the lowest obtainable magnification, it will be of interest to compare its appearance as viewed with eyepieces of higher magnification before passing on

to more powerful objectives. It will be observed that as the eyepiece magnification increases the image loses in illumination, although, with modern powerful sources of light, this is not a serious disadvantage. A point is reached, however, where although the image is further enlarged, no additional detail is revealed (see pp. 25 to 28). For most purposes, eyepiece magnifications not exceeding 10× or 15× will be found ample.

Binocular Observation

The microscopist should from the outset accustom himself to keep both eyes open, and to alternate from one eye to the other in working with a monocular microscope. The slight effort which it takes to acquire this habit is well worth while. The eyes undergo a steady training as the skill of the microscopist progresses, and it is invaluable to have both eyes participate in this training from the outset, both as an insurance against one eye becoming incapacitated, and as a means of relief during prolonged investigations. In this connection, the growing recognition of the advantages of binocular monobjective microscopes may be emphasized.

Examination with Objectives of Higher Magnification

Having acquired some familiarity with the manipulation of a specimen and of the microscope at the lowest objective magnification and with several eyepieces, the beginner may pass to the next higher power objective, having first inserted a 5× or 7× eyepiece into the eyepiece sleeve. Before revolving the nosepiece to bring the next higher objective into position, a conspicuous element of the specimen should be brought as nearly centre into the field as possible and focused. Now, and without disturbing the focal adjustment, the higher power objective is swung into position. In doing this the first time, the observer should watch the objective from the side and make certain that it will not strike the cover glass as it is swung into place. Dry objectives supplied at the same time as the microscope stand are "parfocalized", on the revolver, so that when a specimen is focused with one objective, it will also be approximately in focus with the others when they are swung into position. When working with a microscope for the first time, the user should ascertain whether the objectives actually are parfocal, and should not depend upon their clearing the cover glass as the nosepiece is revolved. It is

to be noted that objectives are parfocalized for the standard tube length of 160 mm. and for observes with normal vision. Deviations from these standards destroy the parfocal adjustment. It is not necessary that succeeding objectives should swing exacty into focus; all practical purposes are served if a bare outline of the specimen appears. A slight manipulation of the slow motion then quickly adjusts the focus exactly.

In passing from a lower to a higher magnification, it should be remembered that the field of view decreases as the magnification increases. This means that if a specimen is slightly out of the centre of the field at a low magnification, it will lie relatively much closer to the margin or even entirely outside of the field with a higher magnification. It goes without saying that care must be taken to have the slot in the nosepiece opposite the objective in use actually engage the arresting spring tongue. If the nosepiece is inadvertently turned a trifle farther or not far enough, then changes will unavoidably take place in the field of view.

Oil Immersion Objectives

It is well for the beginner to familiarize himself with the use of high-power dry objectives before going on to oil immersions. The latter present a few complications which, however, are readily mastered, especially if the microscopist is already fairly well versed in the use of high-power dry objectives and has acquired the ability of manipulating the slide with assurance at high magnifications. Oil immersion objectives usually have high magnifications and large numerical apertures, and accordingly their working distance and depth of focus is so small that it is impracticable to parfocalize them with other objectives. Instead, they are usually made somewhat shorter, so that they will not strike the cover glass if they are accidentally swung around in place of a dry objective.

Finding the Specimen with Oil Immersion Objectives

Oil immersion objectives owe their specific advantages to the employment of an immersion fluid — thickened cedarwood oil of specific refractive index and dispersion — between the front lens of the objective and the cover glass. They cannot be used without this immersion fluid, and to obtain the best results of which Zeiss objectives are capable, only Zeiss cedarwood oil

should be used. A convenient procedure for applying the oil and focusing an oil immersion objective is as follows: Using a high magnification and a dry objective, locate the specimen accurately in the centre of the field. Raise the microscope tube by means of the coarse motion by enough to give ample space above the slide, and swing the oil immersion objective into position. Place a small drop of immersion oil on the cover glass over the spot under examination, being careful not to disturb the slide, and lower the microscope tube until the objective just touches the oil drop (it is well to observe this from the side with the eyes about on a level with the stage). Finally raise the tube a trifle, though not enough to break the immersion contact, in order to make sure that the objective is above its plane of focus. Now apply the eye to the eyepiece, and slowly and steadily lower the tube with the coarse motion until the outlines of the specimen begin to appear. As soon as this point is reached, stop and complete focusing with the slow motion. The working distance of high-power oil-immersion objectives is so short that attempts to bring the objective below its plane of focus and then "focus up" would probably result in collision with the cover glass. Hence it is necessary to reverse the rule laid down for dry objectives and start from above, focusing down. The coarse motion is preferable for this because the process would be so long drawn out with the slow motion that the plane of focus might be passed unnoticed if the observer's attention wandered for a moment.

A valuable aid in locating the focal plane with an immersion objective is observation of the back lens of the objective. As long as the objective is still above its plane of focus, a reduced-scale image of the preparation can be seen on its back lens. If there is any doubt as to whether any portion of the specimen is within the field of view, then the slide can be shifted until a definite portion of the specimen appears. The eyepiece is now inserted and the tube lowered for focusing with the coarse motion. This method can, of course, be employed only if the object contains relatively large structures. It would fail in cases such as are constituted, for example, by preparations with small bacteria spaced apart by wide intervals. Even in such specimens, however, there are often inclusions of coarser materials which may be put to use as "landmarks" for finding the focusing plane. It is, of course, necessary to avoid the error of mistaking scratches or spots on the lower face of the slide for objects located between the slide and the cover glass. For this reason, scratches on the lower face of the slide should be avoided as far as it is possible to do so.

A special difficulty is presented by small structures scattered at wide intervals through the preparation, so that it is doubtful whether anything will be in the field of view. If there are large particles in the vicinity, these should be focused and then the smaller structures sought, their position relative to the larger masses having previously been noted.

In finding the focal plane for difficult specimens, the microscopist who is well trained in the manipulation of slides has a valuable advantage. While he is focusing down with the coarse motion, the left hand lightly moves the slide back and forth through a short distance, displacing the slide, however, by not over the width of the field of view with the immersion objective so that there is no danger of losing the selected part of the specimen. If the plane of focus should be inadvertently passed in descending, the hand moving the slide will at once detect the slightest pressure of the objective on it.

Protective devices for the slow motion which are intended to prevent injury when the objective comes in contact with a cover glass may prevent a slide from being broken, but cannot prevent injury to the objective, since sufficient force must be given to the downward movement to insure its functioning. This force is apt to suffice for dislocating the delicately mounted front lens of an objective if it is brought into contact with the cover glass. It is therefore best not to employ such devices.

Further Control of the Illumination

When the specimen has been found with an oil immersion objective, the next step before proceeding with its study is to ascertain whether the illumination is all it should be. For this purpose, the eyepiece is removed and the back lens of the objective observed by looking down the microscope tube, care being taken not to disturb the focus. The back lens should be evenly illuminated. If air bubbles are seen in the oil, they should be removed, either by moving the slide back and forth (replacing the eyepiece for the time being and observing the specimen in order not to lose the selected spot), or else by drawing a clean hair through the oil between the cover glass and objective. If it is not possible to remove the air bubbles from the field of view, then the oil must be wiped off and the process repeated by relocating the specimen and applying a new drop of oil. Air bubbles should not be tolerated, since they impair the image and in fact make it quite unfit for use if they are present in any size or quantity.

Observation of the back lens of the objective indicates at once whether the mirror is properly placed to secure even illumination, as well as how far the condenser iris diaphragm is opened. It will be found that, even with the diaphragm open all the way, the peripheral portion of the back lens of oil immersion objectives is not illuminated (unless it is one of the special oil-immersion objectives intended primarily for dark field observation, with a numerical aperture of less than 1.0). The explanation is that the illuminating beam thrown by the condenser upon the specimen under examination must have the same aperture as the objective if the full aperture of the latter is to be utilized.

Immersing the Condenser

Condensers in common use have a numerical aperture of 1.2 or 1.4, but as long as air intervenes between the condenser and slide, the aperture of the illuminating beam falling upon the specimen cannot exceed 1.0. It is therefore necessary to immerse the condenser also if it is desired to utilize the full resolving power of objectives with a numerical aperture exceeding 1.0. Condensers are less finely corrected than objectives of the same numerical aperture, so that most of them may be used dry or immersed as desired. To immerse a condenser, a few drops of cedarwood oil are placed on its upper lens surface, care being taken to avoid air bubbles. The condenser is then lowered a few millimeters and, with a slide in position on the stage, is raised again until immersion contact is established.

Aperture of Iris Diaphragm

The width to which the iris diaphragm of the condenser should be opened often troubles the beginner. It is to be noted at the outset that under no circumstances should the illuminating beam have a greater aperture than the objective, since otherwise the image will be flooded with light. Usually, it will be advantageous to reduce the illuminating aperture below that of the objective. The smaller is the aperture, the greater will be the depth of focus and the greater the contrast between the various elements in the image. The lowest permissible aperture is reached when diffraction bands become visible about the borders of the object. The actual aperture which is best suited for the examination of a given specimen is therefore determined by observation rather than by any hard and fast rule.

Field-of-View Diaphragm

A further aid in obtaining good definition in critical examinations is afforded by the field-of-view diaphragm of the microscope lamp. This diaphragm is located right in front of the condensing lens of the lamp (see Fig. 29). When the lamp and microscope condenser are correctly set, an image of the field-of-view diaphragm appears in the plane of the preparation. Opening and closing the diaphragm causes no change in the intensity of the illumination, but instead the area illuminated becomes larger or smaller, hence the name "field-of-view diaphragm" to distinguish it from the aperture diaphragm, which is situated directly below the microscope condenser (or, in some special condensers, between the lenses). An increased use of the field-of-view diaphragm in critical microscopy is only to be recommended. By restricting the illumination to the area actually under observation, the diaphragm excludes extraneous light which would otherwise fall on the image and obscure its details more or less. Incidentally, specimens sensitive to light (and heat) are less subject to injury if the area illuminated is restricted by a field-of-view diaphragm. The advantages of an aplanatic condenser, again, become evident when a field-of-view diaphragm is employed. The margin of the diaphragm will be sharply delineated only when a well corrected microscope condenser is employed.

Advantages of Oil Immersion Objectives

Returning from this excursion into the realm of illumination, to the study of the specimen by means of an oil immersion objective, the observer will readily appreciate the advantages of this type of objective. The first thing which will probably arrest his attention is the great brightness of the image. In this respect it is instructive to compare, for example, an oil-immersion objective 90 with 7× eyepiece, giving a total magnification of 630×, and a dry objective 40 with 15× eyepiece, giving a total magnification of 600×. If the observer has had experience with dry objectives having a nominal aperture of 0.85 or more, he will be impressed with another advantage of the oil-immersion objective in that, within the range permitted by its working distance, it is not sensitive to variations in cover glass thickness. Moreover and in view of its high primary magnification, the working distance is considerably greater than for a dry objective. These advantages are made possible only by employing cedarwood immersion oil. With a few precautions, the use of this oil is so simple and free from trouble that it is no wonder that oil-immersion

objectives are used as a matter of course. Actually, immersion objectives will be found more convenient for most routine work than dry objectives of a numerical aperture above 0.65.

Care of Immersion Oil

The first condition for the successful use of immersion oil is a certain amount of cleanliness. The oil is supplied in vials of different sizes. A small quantity is poured into the inner container of the double oil bottle (Fig. 30), care having first been taken to see that the container is dry and clean. After an oil

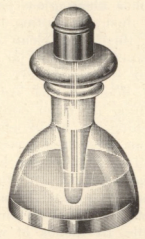


Fig. 30, about 1/2 actual size 8649

Double Bottle for Cedarwood Oil

vial is once opened, it should at once be closed again, any adhering oil wiped off, and a piece of paper wrapped or tied over the stopper. In this manner, a supply of clean immersion oil is assured. The oil should fill the inner container of the double oil bottle to a depth of about $\frac{5}{8}$ in. The cap of the bottle bears a flexible wire with a glass rod which takes along just the right sized drop as it is lifted out. Do not stir the oil, for this introduces air bubbles. In removing the rod and replacing it, take care not to touch the sides of the oil well. If the sides are kept free from oil, there will be no trouble from having oil creep over the edges of the bottle, making a sticky, dust-collecting mass. If this happens, the mouth and cap of the bottle must be carefully wiped with a lintless cloth moistened with xylol or benzine.

When the cap with the attached glass rod has once been removed from the oil bottle, the drop of oil should immediately be deposited on the cover glass (or on the condenser, as the case may be), and then at once returned to its place in the oil bottle. Under no circumstances must it be laid down. Failure to observe this rule will result in introducing dust and dirt into the oil, whereas to function properly it should be a perfectly clear, homogeneous medium.

Removing Immersion Oil from Objectives with Rice Paper

Japanese rice paper is to be recommended for wiping objectives (see also p. 37). It is well to store a supply, cut into pieces about 1½ in. square, in a clean glass jar with overhanging lid to prevent the entry of dust. In cutting the paper, scrupulously avoid all contact with dust. To clean an objective, remove a single piece of rice paper from the jar, holding the lid in the other hand and replacing it immediately without setting it down. Moisten — but do not flood — one corner of the paper with the cleaning fluid by lifting the inner container out of the double bottle and barely touching its lower end to the paper, and at once wipe across the lens face, first passing a dry corner of the rice paper across the oil to remove the bulk of it, then the portion moistened with cleaning fluid, and finally a clean, dry spot of the paper. In this manner, practically all of the oil is removed with a minimum of friction by a single circular sweep. The inner container of the oil bottle, by means of which the cleaning fluid is transferred to the rice paper, should be immediately returned to its position in the outer container and not be laid down elsewhere, since this would bring dust into the cleaning fluid.

Removing Immersion Oil from Condenser

If the condenser has been used immersed, it must be cleaned before it can again be used dry, in any event once a day. Because of its larger surface, it is preferabe to use a clean cloth for removing the oil, but if there is any question whether the cloth is clean and free from dust, it is better to employ rice paper for the condenser also. Particular care must be taken to remove any oil which may have run down the sides of the condenser, as well as oil which may have reached the stage.

Removing Immersion Oil from Slides

Immersion oil should be removed from slides immediately upon completing the examination. Soft cloth is preferable to rice paper for this purpose. First wipe off the bulk of the oil at a single stroke with a dry part of the cloth, then wipe over with a fresh portion of the cloth moistened with benzine. If desired, the cover glass may be further polished by breathing on it and rubbing with a clean, dry portion of the cloth. Cedarwood oil should be removed from slides before using a high-power dry objective, for aside from its likelihood of interfering with the image, there is danger of oil coming in contact with the front lens. It is a not uncommon experience to find dry objectives greatly impaired in their performance by a small amount of immersion oil which has lodged on the front lens without the user being aware of it.

Benzine is preferable to xylol as a cleaning fluid for slides, in that Canada balsam is less soluble in the former, so that there is less danger of dissolving and dragging the mounting medium over the cover glass. It is advisable to keep a separate bottle of benzine for cleaning slides in order to keep the cleaning medium for the objectives in the cleanest possible condition.

Alphabetical Index

	Page
ABBE Test Plate	25
Achromatic Objectives	23, 30
Apertometer	27
Aperture, numerical	26
Apochromatic objectives	23, 30
Arm, microscope	5, 12
Attachable mechanical stage	14
BELL COVER	34
Binocular observation	11, 43
CABINET	13
Canada balsam	31
Cedarwood oil	49
Changing devices, objective	7, 11
Chromatic correction	23, 32
Cleaning eyepieces	36
Cleaning objectives	37
Cleanliness in working	33
Clips, stage	13
Coarse motion	19
tightening	19
Condenser, condensers	16
immersion of	47, 56
motion, tightening	18
Compensating eyepieces	32
Cooling filter	39
Correction, chromatic	23, 32
of cover glass thickness	28
mounts, objectives with	29
spherical	23
Cover, bell	34
Cover glass gauge	29
Cover glass thickness, correction for	28
Cylinder diaphragm	16
DESIGN DETAILS	13
Design of microscope	5, 11
Diaphragm, cylinder	49
field-of-view	48
iris	16
Draw tube	21, 22, 33
Dust, removing	34
EYEPIECE, Eyepieces	22, 31
cleaning	36
compensating	32
Huygens	31
micrometer	32
micrometers	33
orthoscopic	31
photographic	25
FIELD-OF-VIEW DIAPHRAGM	48
Filter solution	39
Finding the object	20
Fine adjustment	20
Flat-field objectives	25
Flatness of field	24
Fluorite objectives	24
Focusing high-power objectives	43
low-power objectives	40
Foot	5, 12
Free working distance	30
HANDLING the microscope	34
Homals	25
Huygens eyepieces	31
JAPAN PAPER	37, 50
ILLUMINATING APPARATUS B	12
O	5, 12
W	6
load on	18
Illumination, adjustment of	41
Illumination, control of	46
danger from too intense	42
Immersion of condenser	47, 50
fluid	49
oil	49
oil on objectives	44, 48, 50
LAMPS	38, 41
Lamp connection rail	41
Lens paper	37, 50
Light, artificial	38
natural	38
wave length of	26
Lubrication	34
MAGNIFICATION	22
determining	22
difference	32
examination with lowest	43
limits of	28
Mechanical stages	14
Micrometer eyepieces	32
Micrometer, object	32
Microscope, lamps	38, 41
Mirror	15
NUMERICAL APERTURE	26
OBJECT, finding	44
Object micrometer	40
slide stage	13
Objective, objectives	22
achromatic	23, 30
apochromatic	23, 30
changing devices	7, 11
flat-field	25
fluorite	24
front lens of	31
high-power, focusing	43
immersion oil on	44, 48, 50
low-power, focusing	40
oil immersion	44
parfocal adjustment of	21
with correction mount	29
Observation, binocular	11, 43
Oiling	24
Orthoscopic eyepieces	31
PARFOCAL ADJUSTMENT	21
Photographic eyepieces	25
Protecting microscope	34
RESOLVING POWER	23
Revolver	7, 11
Revolving nosepiece	7, 11
SLIDE, object	40
Slow motion	20
Specimen, mounting	30
Spherical correction	23
Stage, stages	13
attachable mechanical	14
clips	13
mechanical	14
micrometer	33
Stands E	15
Stands L	11
TEST PLATE, Abbe	25
Tilting axis	12
Tubes	9, 11
Tube length, mechanical	21
Tube, load on	19
WAVE LENGTH of light	26
Working distance	30

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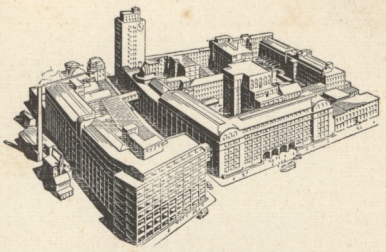

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