THE USE AND CARE OF YOUR CARL ZEISS JENA MICROSCOPE

by

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CARL ZEISS / JENA

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I. Construction of the Microscope

A microscope is usually provided with a wooden cabinet for its safe keeping. In order to securely hold the instrument in position during transportation, a heavy bolt is passed through the bottom of the cabinet and into the underside of the microscope foot. This bolt, of course, must be removed before attempting to withdraw the microscope from its cabinet. A special flat key is supplied for manipulating the bolt; in its absence a screwdriver will serve.

Handle

Placing the microscope on a table in front of the observer, it will be noted the main body consists of a single piece of metal, in shape roughly the segment of a circle, the middle part of which has been cut out and thus forms a handle (Fig. 1). This handle is the convenient and safe place to grasp when carrying a microscope or moving it about. Near its lower end the main body is attached by means of a pin to the foot.

Inclinable Joint

The pin is mounted so as to constitute an inclinable joint, permitting the microscope to be inclined at any angle between the vertical and horizontal positions. If necessary, a key can be obtained for adjusting the tension of the joint should it be too tight or too loose. Generally it will be found best to work with the microscope in its vertical position. If it is used inclined, however, care should be taken to ascertain that the inclinable joint holds the body of the instrument securely at the angle at which it is placed. This is especially important when ocular attachments are employed, such as photomicrographic ocular Phoku (Mikro 373)*, binocular attachment Bitumi (Mikro 355), etc., since the added weight of these attachments is sufficient to produce a momentum which will topple the microscope over if the body sinks at a fairly rapid rate.

* These numbers refer to bulletins published by Carl Zeiss, Jena. Copies will be supplied upon request.

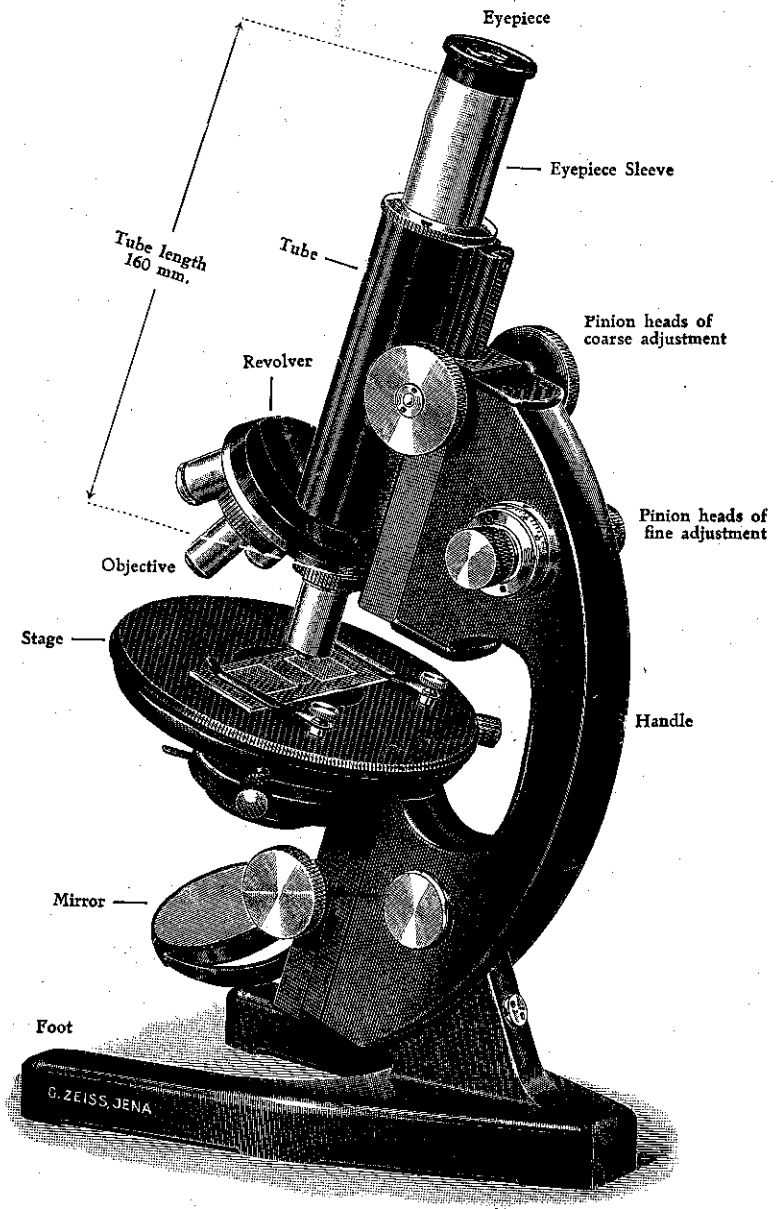


Fig. 1, about 1/2 actual size
Microscope Stand ESA

Foot

The foot is provided with three main bearing surfaces, one behind and one at the end of either prong in front. Two additional bearing surfaces come into play when the microscope is subjected to a lateral blow or pressure. These two surfaces are intentionally placed at a slightly higher plane than the three principal bearing surfaces, in order to permit the microscope to stand firmly on an uneven table top. The foot is proportioned so as to give adequate stability without unduly increasing its weight.

Stage

The microscope stage, which serves as a support for the specimen under examination, is attached about on a level with the lower part of the handle. A properly constructed stage is sufficiently rigid to prevent its yielding enough to interfere with focusing of the specimen under examination. It is bad practice, however, to rest the full weight of the observer's hand on the stage. The elbow or forearm should rest securely on the table, and the hand manipulating the slide rest lightly on the stage.

The stage may be entirely of metal, or it may be covered with hard rubber, or some other composition. If this becomes gray, its dark color can be restored by rubbing in a small amount of oil, and thoroughly wiping off the excess with a clean cloth.

A large opening at the center of the stage allows ample room for the condenser. For many purposes the opening is unnecessarily large, and a stage ring is provided for appropriately reducing the size of the opening.

Stage Clips

Specimens for examination are usually mounted on a glass slide and covered with a cover glass. A pair of spring metal stage clips serves to hold the slide in position. In order to function properly, the clips should be fully inserted into the holes of the stage provided for the purpose. Care should be taken to keep the stage holes clean, likewise the pins of the clips, so that the clips can be readily inserted or removed; a trace of lubricating oil helps.

Much greater freedom of movement for examining a slide is obtained if the clips are dispensed with altogether. With some patience the beginner will quickly acquire the knack of accurately displacing a slide with the free hand, and the patience required to form this habit is well worth the effort. On the other hand, the clips should be used when it is necessary to securely hold a slide in position for a longer period, as in drawing or photographing.

Mechanical Stages

For systematic searching of a specimen and for displacing it mechanically, special devices — mechanical stages — are employed. The microscope illustrated in Fig. 1 is equipped with a simplified form of mechanical stage. The movable stage plate is displaced by means of two screws at the right and left operating against a buffer spring (not shown) in front. Occasionally a small drop of non-corrosive oil which does not gum (such as Three in One oil) should be placed on the projecting part of the buffer pin and also on the accessible parts of the screw threads in order to keep them moving freely. When the two screws are simultaneously turned clockwise, the stage plate moves forward, and in the opposite direction when turned counter-clockwise. Turning the two screws simultaneously in opposite directions, results in a transverse movement of the stage plate.

The range of movement of the simplified mechanical stage is about 10 mm. in either direction. If a larger range of movement is required, and if the position of objects is to be recorded, an attachable mechanical stage may be employed, equipped with vernier scales, and having a range of movement 50×30 mm. (Fig. 2). The microscope stage is regularly provided with three holes for receiving the attachable mechanical stage. The central hole is threaded for engaging the clamping screw (b Fig. 2) of the mechanical stage, the other two holes are smooth walled and receive two guide pins of the mechanical stage which serve to secure it in a fixed position. These holes, of course, should be kept clean at all times. In attaching the mechanical stage, care should be taken that it rests flatly on the microscope stage, and that the guide pins are fully inserted in their holes before engaging the clamping screw.

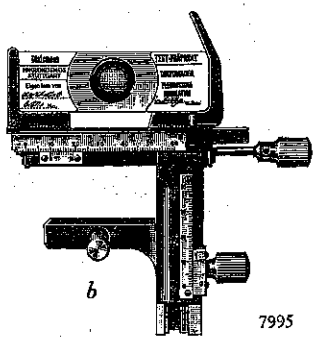


Fig. 2, about 1/3 actual size
Attachable mechanical stage

The forward and backward movement of the attachable mechanical stage is made somewhat stiffer than the lateral movement. The procedure in systematically searching a specimen is to begin either at the top or bottom, traverse it sidewise through its entire extent, then move forwards to the extent of the field of view and again travel laterally over the entire width of the specimen. In this way the forward and backward movement is used relatively little, and having it operate more stiffly prevents unintentional dislocation of the specimen.

From the standpoint of freely and rapidly displacing a slide, the same objections apply to the use of a mechanical stage as were made against stage clips. It is good practice, therefore, to restrict the use of a mechanical stage to those examinations which actually require its aid.

The appliances situated below the stage of a microscope are called collectively the substage equipment. It serves to illuminate the specimen by means of transmitted light, and consists essentially of a mirror (Fig. 1), a diaphragm, and often in addition a condenser.

Mirror

The mirror is plane on one side, concave on the other, and mounted so as to be movable in all directions. If used without a condenser, either surface may be employed, the concave mirror yielding more intense illumination. Condensers are constructed for the use of parallel light, and, therefore, should primarily be employed in conjunction with the plane mirror. In practice, however, when a small source of light is used, the concave mirror at times is of advantage also in conjunction with a condenser permitting the illumination of a larger field.

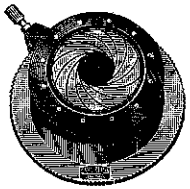


Fig. 3, about 1/2 actual size
Iris Cylinder Diaphragm

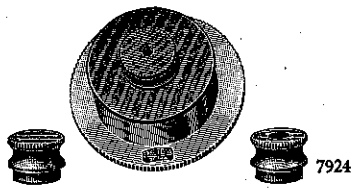


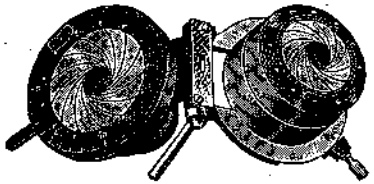
Fig. 4, about 1/2 actual size
Cylinder Diaphragm with 3 stops

If a specimen is illuminated by means of the mirror only without aid of a condenser, a diaphragm should be used for reducing the area illuminated to approximately the size of the field of view of the objective employed. This is accomplished either by means of an iris cylinder diaphragm (Fig. 3), or a cylinder diaphragm with a set of three interchangeable stops with fixed openings (Fig. 4). The latter is less liable to get out of order, and for practical purposes it often suffices to use one size opening only, so that the inconvenience of frequent interchange 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 microscope stage. When in use, the upper surface of the diaphragm should be level with the upper surface of the microscope stage.

Condensers

Examinations with oil immersion objectives require the aid of a condenser, in order to obtain adequate illumination, and a condenser likewise is of advantage in working with high power dry objectives. In fact, with proper manipulation, a condenser also yields fairly satisfactory illumination for low power objectives, so that there is a

tendency to employ a condenser for illumination of the specimen throughout. Meticulous workers, however, will substitute a cylinder diaphragm for the condenser when employing objectives with a numerical aperture of less than, say, 0·2. The passing from one to the other is facilitated if so-called swingout condensers are employed (Fig. 5).



7935

Fig. 5, about ½ actual size
Swingout Condenser

7919 a

Fig. 6, about ½ actual size
Condenser n. a. 1·2 with Iris

These have the condenser attached to the mount of an iris cylinder diaphragm in such a manner that, when the iris is fully opened, the condenser can be swung into position for use. When the iris cylinder diaphragm is to be used, the condenser is simply swung down and out of the way. A disadvantage of swingout condensers is that they easily get out of alignment, a matter of some importance as will appear presently.

Condensers are available in considerable variety. An ordinary condenser with a numerical aperture of 1·2 is in most common use (Fig. 6). For immersion objectives having a numerical aperture of 1·3 or more, a condenser with a numerical aperture of 1·4 would usually be selected. For use with highly corrected objectives it is advantageous to also employ highly corrected condensers, such as

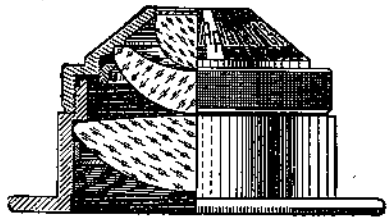


Fig. 7, about actual size 7912
Aplanatic Condenser

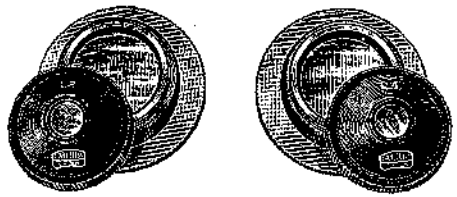


Fig. 8, about ½ actual size 7931
Condensers showing front lens unscrewed

aplanatic condenser 1·4 (Fig. 7). It is to be noted, however, that the working distance of a condenser decreases, likewise the area illuminated, 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.

The majority of microscope condensers is built up from a number of lenses. Usually the upper lens or group of lenses can be unscrewed, the remaining portion constituting a condenser of lower aperture and longer focus. (Fig. 8). The optical components are usually housed in a cylindrical mount which has the 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 microscope stage. Focusing is greatly facilitated, however, if the condenser sleeve is mounted on a carrier actuated by rack and pinion movement. In this case the condenser sleeve is provided with a clamping screw which is loosened when the condenser is to be exchanged, and drawn tight after a condenser is in place, care being taken to insert the condenser up to its flange. The condenser rack and pinion movement is so adjusted at the factory that, when it is carried to its highest position, the upper surface of the condenser (provided it is inserted up to its flange in the sleeve) will lie about 0·1 mm. below the level of the upper stage surface. This is essential, since it is desirable to provide the possibility of bringing the condenser close to the slide, and at the same time provision must be made that the condenser cannot actually touch the slide, since this would endanger an objective of short working distance, and would also interfere with maintaining proper immersion if the condenser is used immersed.

The condenser is accurately centered by the manufacturer so as to lie in the optical axis of the microscope, and will remain centered unless the instrument receives a severe blow, or other mechanical injury. In that event it is best to return the microscope to the manufacturer's 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, and the sleeve supported with the other hand at the same time.

In order to regulate the aperture of a condenser, an iris diaphragm is provided. In the larger research microscope stands, equipped with the complete Abbe illuminating apparatus, this diaphragm is separately mounted on the illuminating apparatus. For use on stands equipped with a simplified illuminating apparatus, the iris is attached to the lower part of the condenser itself (excepting in some special condensers in which it is located between lenses). In these latter stands there also is mounted below the condenser a swingout ring which is intended for the reception of a ground glass disc (employed for moderating the illumination), special diaphragms, etc.

Coarse Adjustment

Turning our attention now to the portion of the microscope above the stage, it will be noted that the upper part of the main body carries

the body tube which can be rapidly raised and lowered by means of a rack and pinion movement, the so-called coarse adjustement, operated by turning the pinion heads (Fig. 1). Turning the pinion heads counter-clockwise raises the tube and, after the end of the rack has been reached, the tube with attached rack may be slid out of its bearing by exerting an even upward pull upon it. However, there rarely is any occasion for doing this, and it should be avoided as much as possible, since, unless done with care, the rack or pinion is liable to be damaged. In returning the tube, care must be taken to start the rack bar evenly in its bearing, and to lower it gently until the teeth of the pinion are engaged.

The coarse adjustment should operate sufficiently freely so that the hand does not become tired from its continued manipulation, at the same time it must be firm enough to securely hold the microscope tube in position. Needless to say, the tube must not sink of its own weight, nor yet in response to a slight pressure. The degree of firmness in the coarse adjustment is readily adjusted. In microscope stands of older construction, two screws are provided for this purpose (S_2 S_1 Fig. 9). Turning these screws clockwise tightens the movement, turning them counter-clockwise loosens it. The screws should be turned a little at a time and each by the same amount until the desired degree of firmness in the coarse adjustment is obtained. In manipulating the screws, care should be taken, of course, to employ a screw driver of appropriate size, and to keep it inserted well to the bottom of the slot while turning the screws, in order to avoid marring them.

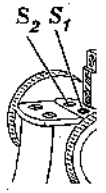


Fig. 9 8910b

In microscope stands of most recent construction, the just mentioned adjusting screws are not present. Regulation of the coarse adjustment in these stands is an even simpler procedure. Grasp the left hand pinion firmly with one hand, and at the same time turn the right hand pinion head with the other hand, clockwise to tighten the movement, counter-clockwise to loosen it. After this has been done repeatedly, it may become necessary to tighten a set screw which is visible at the center of the pinion head. A special key can be supplied for this purpose.

Ordinarily it will not be necessary to lubricate the coarse adjustment; should it become desirable, a small amount of non-corrosive vaseline may be employed. Oil is not to be used for lubricating a sliding bearing.

The coarse adjustment works with great precision, but its movement is too rapid for conveniently focusing objectives of high magnifications. Therefore, the microscope stand is equipped with a second, the so-called fine, or micrometer, adjustment (Fig. 1), which is employed for the final focusing when specimens are examined under higher magnifications, the approximate focusing having first been accomplished by means of the coarse adjustment.

Fine Adjustment

The fine adjustment operates by means of a system of cogwheels, and is characterized by a complete absence of lost motion. The mechanism is securely housed within the main body of the microscope, only the pinion heads for operating it extend out on either side. The left hand pinion head is provided with an index and graduated drum, one division of which equals 0·002 mm. A complete revolution of the pinion head displaces the body tube 0·1 mm. The fine adjustment movement is arrested at each end of its range by means of a stop, index marks on the right side of the body mark the extent of the range. Turning the pinion heads clockwise, lowers the body tube, 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 or in any way to tamper with the fine adjustment. Ordinarily, it will require no attention, should something go wrong with it, the safe procedure is to send the microscope to the manufacturer's representative. The mechanical devices for focusing a compound microscope are secondary 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 quite prevent adequate detailed examination of the specimen. The proficient microscopist will, therefore, insist that both the coarse and fine adjustments of his instrument are in good working order.

Revolver

Objectives are attached to the lower part of the body tube, usually by means of an intermediary device which facilitates a rapid exchange of objectives. The revolving nosepiece or revolver (Fig. 1) is the most commonly used objective changer. In its standard form it accomodates four objectives, although revolvers holding only three or two objectives are also made. There is ample room on a properly designed revolver for four objectives, and it is obviously advantageous to have space for another objective in addition to the usual three (low and high power dry, oil immersion). Frequently, a fourth objective of exceptionally low magnification can be used to great advantage in obtaining a general survey of preparations; or it may be found desirable to provide later on one of the special objectives intended more particularly for dark field examinations.

The revolver is made with precision and brings each objective into the optical axis of the microscope with sufficient accuracy for most practical purposes. A knife edge is held against the revolver by a spring, and engages a notch opposite each objective. The notch is accurately placed so that the objective is in the axis of the microscope when the knife edge snaps into position. To insure proper

centering of the objectives, the revolver must be attached securely and accurately to the body tube. The user is cautioned against removing the revolver, since in so doing its centering may suffer. Should he none the less decide to do so, a key should be obtained for manipulating the locknut.

Needless to say, the revolver is to be carefully guarded against blows. Knocking against it with a bell jar, for instance, is almost certain to ruin its accuracy.

Mechanical Tube Length 160 mm.

At its upper end the body tube carries an arrangement for holding the eyepiece, either a draw tube or else an ocular sleeve (Fig. 1) of fixed extension. The latter is generally to be preferred, since it insures maintenance of the correct mechanical tube length of 160 mm.*, an important matter (footnote p. 20 and p. 33), and since there rarely is occasion for employing a draw tube. If required, a draw tube can be supplied at any time, it is readily interchangeable with the ocular sleeve of fixed extension. Draw tubes are provided with a scale marked in millimeters; in tubes of current construction a conspicuous line is drawn about the tube 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 having a standard height of 15 mm. If, for any reason, objectives are to be screwed directly into the body tube, an extension ring 15 mm. in length is supplied. It is to be noted that, in the marking of draw tubes of older construction, 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 deduct its height from the reading on the draw tube scale. For example, many old style revolvers had a height of 18 mm., so that the old style draw tube would be set at 142 mm., in order to obtain with such a revolver the mechanical tube length of 160 mm.

There is no inherent reason that objectives must be corrected for a tube length of 160 mm., they could be corrected for other lengths. As a matter of fact, for special purposes objectives are supplied corrected for tube lengths of respectively 190 and 250 mm.

Objectives and Eyepieces

It now remains for us to consider the objectives and eyepieces, or oculars, the two lens systems which are directly concerned in producing the image of a compound microscope. The eyepiece or ocular (Fig. 1), is situated at the upper end of the tube, next to the

* By mechanical tube length is meant 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 (Fig. 1).

eye, hence its name. 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 (Fig. 1), derives its name from being situated next to the object or specimen. It also usually consists of a number of lenses placed within a more or less cylindrical mount. The objective mount at its upper end is provided with a screw of standard thread for attaching to the objective changer, etc. 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 same tube length.

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 a compound microscope, the image examined by the eye being twice magnified, in contrast to the single magnification produced by a simple magnifier.

The degree of magnification obtained is readily ascertained by placing a glass rule, known as a stage micrometer (Fig. 17, p. 24) on the stage of the microscope, and projecting its magnified image on a flat white surface placed 250 mm. from the eyepiece, and perpendicular to the microscope axis, in order to avoid distortion. To see the image well, it will usually be necessary to partly darken the room. If, for example, it is found that 30 divisions of a stage micrometer ruled in 1/100th of a mm., and, therefore, actually having a length of 0·30 mm., measure 120 mm. in the projected image, the magnification will be $\frac{120}{0{\cdot}3}$ or 400 diameters or, as it is commonly written, 400×. If the image were projected a greater distance, the magnification would be increased, but 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 Carl Zeiss objectives and eyepieces are employed. The values for the component magnifications of each are engraved on the metal mounts, and the total magnification of the microscope is the product obtained by multiplying the individual magnifications of the objective and eyepiece employed. Thus objective 40 (4 mm.) with eyepiece 10× yields 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 maintained. For the limits of useful magnifications see pages 18 and 22.

Spherical and Chromatic Corrections

To better understand the optical performance of a microscope, it is essential to have in mind the rudiments of the problems involved in producing a relatively faithful magnified image of an object. If the image is made by a simple lens having spherical surfaces, two main groups of defects are present. On the one hand, it is found a point of the object is not pictured as a point but as a disc; this type of distortion may be grouped under the heading of spherical aberrations. On the other hand, it will be found that the image shows colors which are not present in the object, this type of defect in the image being known as chromatic aberration. By rather complicated means, it has been possible to correct both spherical and chromatic aberrations to a high degree.

Generally speaking, this result is obtained by building up an objective with a number of lenses made of glasses (in some instances transparent minerals are also used) which differ appropriately in their optical properties, and also differing in the curvatures given to their surfaces. Extreme precision is required in the proper mounting of the component lenses of a highly corrected objective, and it goes without saying the user should never attempt to take the objective apart. For many years color correction was restricted to two colors of the spectrum, but, because of the great improvement as compared to single lens objectives, these corrected objectives were called achromatic objectives, although, according to present day standards, the image produced by them is far from being free of color defects. It was not until new glasses with hitherto unknown optical properties were produced, as the result of the joint labors of Abbe and Schott, that it was possible to carry out more complete corrections. The culmination was reached in Abbe's apochromatic objectives which embody chromatic as well as spherical corrections of an entirely new order; as many as twelve lenses are required in some apochromatic objectives to attain the desired results. The new materials 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 their correction and in price.

Flatness of Field

It is to be noted, in aiming at a high degree of correction in a microscope objective, flatness of field is not a primary consideration. In fact, the field is conspicuously curved in some of the best corrected objectives, so that the center and the margin will not be simultaneously in focus. It is relatively unimportant to have the entire field

of view simultaneously in focus, since only a portion of the field 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, which is occupying the center of interest, into sharpest focus. It is important, however, that the definition be good over the entire field, so that a good image may be obtained in all parts of the field of view. The degree of correction of an objective can best be ascertained by means of an Abbe Test Plate (Fig. 10), (Micro 116).

Fig. 10, about 2/3 actual size
Abbe Test-Plate

The situation as to flatness of field is somewhat different in photomicrography, since there ordinarily the focus cannot be changed during an exposure. In order to obtain a larger portion of the field simultaneously in focus, when photographs are to be made, special lens systems called Homals (Micro 390) are employed instead of eyepieces. Homals cannot be used for direct ocular 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 obtaining a large image is to see greater detail. If the image produced by an objective does not contain a given detail of structure characteristic of the object, obviously that detail cannot appear in the final image after further magnification by the eyepiece. Therefore, interest lies primarily in ascertaining which qualities of an objective determine its capacity for imaging minute details of structure, its resolving power, as it is called. Now the factors determining the resolving power of an objective are quite a separate matter from its spherical and chromatic corrections, although these, of course, must be of a high order if a useful image is to be obtained.

The resolving power of an objective is found to be proportional to its numerical aperture (see page 18), and inversely proportional to the wave length of the light employed. Or, stated in other words, the greater the numerical aperture and the shorter the wave length of light employed, the smaller the structure which can still be imaged

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 (micron, abbreviated μ, = 0·001 mm.). Accordingly, the smallest structure which can be imaged by, say, achromatic objective 40 with a numerical aperture of 0·65 is $\frac{0{\cdot}55\ \mu}{0{\cdot}65}$ or 0·85 μ. That means, if the objective were used to examine a structure consisting of closely drawn parallel lines 0·5 μ apart, the image produced would not contain separate lines, the structure would not be resolved. Employing powerful oculars in conjuction with the objective under these circumstances is of no avail. Even a 20× ocular yielding a total magnification of 800× cannot reveal the lines, since they are not present in the image made 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 imaged is $\frac{0{\cdot}55\ \mu}{1{\cdot}25}$ or 0·44 μ, and it will be found the lines of our hypothetical specimen are brought out in the image, the observer's eye will see them even with a relatively low power ocular yielding 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 ascertain more precisely what this term means, how it is arrived at, since in so doing an insight is obtained in some of the underlying conditions upon which the performance of an objective depends.

The resolving power of an objective increases with the angle at which light enters it. The increase, however, is not directly proportional, but rather is proportional to the sine of ½ this angle. Take two objectives for which the maximum angle at which light can enter is respectively 80° and 120°. The sine of ½ of 80° or 40° is ·64, the sine of ½ of 120° or 60° is ·87, and the resolving powers of the two objectives bear the ratio of 64 : 87, not 80 : 120. Furthermore, the resolving power increases with an 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

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

33 % greater than if it is air (with a refractive index of 1·00), and if it is cedarwood oil with a refractive index of 1·51, the resolving power will be 51 % greater than with air.*

Abbe now introduced the term numerical aperture (abbreviated N. A. or N. AP.) to include both these factors, that is, the numerical aperture is the product of the sine of ½ the maximum angle at which light enters the objective and the index of refraction of the medium between the front lens of the objective and the cover glass. In practice, numerical aperture is directly measured by means of a simple instrument, the Apertometer (Fig. 11), (Micro 114). Because of its importance, the numerical aperture is engraved on Zeiss objectives having an n. a. exceeding 0·15.

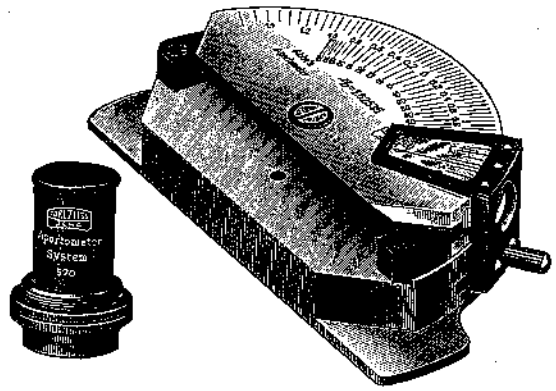


Fig. 11, about ⅔ actual size 8250

Apertometer

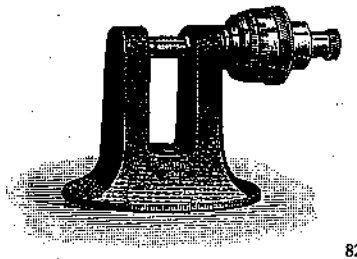
Correction for Thickness of Cover-Glass

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 highest n.a. is most suitable for all purposes. As the numerical aperture increases, it becomes increasingly difficult to adequately carry out chromatic and spherical corrections and, unless this is accomplished, the larger aperture is practically useless. The more complicated means required to secure adequate correction necessarily increase the cost of the objective. The conditions under which the objective must be used, in order to obtain best results, are also likely to be more exacting as the aperture is increased. This is particularly noticeable

* This does not mean that the resolving power of a dry objective can be increased 50% by the simple expedient of placing cedarwood oil between its front lens and the cover glass. The refractive index of the medium between front lens and cover glass enters into the formula for constructing objectives, and a given objective will perform properly only with the medium for which it has been calculated.

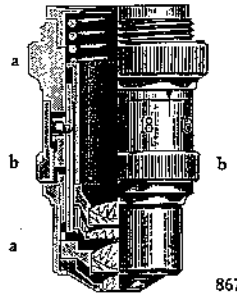
in dry objectives in respect to their sensitiveness to variations in cover glass thickness. The spherical correction of an objective is influenced by the thickness of cover glass, if the refractive index of the medium between objective and cover glass is different from that of the glass. Therefore, objectives, with the exception of homogeneous immersion systems, are corrected for a definite thickness of cover glass, usually 0·17 mm*. However, with objectives having a numerical aperture not exeeding about 0·65 considerable departures can be made from the prescribed cover glass thickness without seriously impairing the image. But a deviation of a few hundredths of a millimeter from the prescribed cover glass thickness suffices to seriously impair the image of an objective having an n. a. of 0.85 or 0·95. The image obtained with the highly corrected fluorite objective 40 n. a. 0·85 or with 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.

Two procedures are available for meeting the situation. The cover glasses may be measured before being used by means of a cover glass gauge (Fig. 12), using only those of the prescribed thickness. The alternative is to provide the objective with an adjustable correction collar (Fig. 13). By means of this, the objective



8231

Fig. 12, about 1/3 actual size
Cover-Glass Gauge



8678

Fig. 13, 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 the mount aa.

can be set for cover glasses of various thicknesses the range usually extending from 0·10 to 0·20 mm., which covers the variations in thickness likely to be encountered. When working with objectives provided with correction collar, it is a convenience to know the

* 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 14).

thickness of cover glass employed, since then it is 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 somewhat annoying procedure, especially if a considerable number of slides is examined in rapid succession.

A further characteristic of practical importance is that the working distance of objectives becomes less as the n. a. increases (assuming the focal length to remain constant). Full data regarding working distances, numerical apertures, initial magnifications and focal lengths of Carl Zeiss objectives are contained in Micro leaflet 367.

Choosing Objectives

From a consideration of the foregoing paragraphs, it is 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 the requirements. If the cost is to be kept low, the 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 and 2. By substituting a 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 outfit is increased at relatively small additional expense, and without greatly adding to the care required in the operation. Generally, however, the use of apochromatic objectives is to be recommended, if their price does not exclude them. These objectives are to be valued not only in the examination of difficult specimens, but perhaps even more on account of the greater ease with which the eye works with them in routine examinations, thanks to their superior definition, a consideration of no little importance to a microscopist whose eyes may be employed daily for hours at a time at the microscope. Generally speaking, it is to be noted that the use of more highly corrected objectives also makes greater demands upon the observer in regard to the care required in giving heed that the somewhat more exacting conditions are met for securing the results of which these objectives are capable. Microscopy, however, is a precision occupation, and in taking up the subject, the beginner should from the outset determine to master the fundamental requirements of his equipment, and to meet these requirements as a routine procedure. This in no wise necessitates the wasting of time on irrelevant fussy details.

A favorite selection of apochromatic objectives consists of 10 n. a. 0·3, 20 n. a. 0·65, oil immersion 90 n. a. 1·3. For many practical purposes apochromat 40 n. a. 0·95 is omitted on account of its short working distance, and the necessity of properly adjusting the correction

collar if the advantages of this exellent objective are to be realized. Instead, apochromat 20 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 intended to meet the most exacting requirements. They are made in two focal lengths, namely, 3 mm initial magnification 60, and 2 mm initial magnification 90. The latter has its front lens mounted with extreme delicacy, the slightest contact with a cover glass is liable to dislodge it. This, together with its short working distance, necessitates 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 also is less danger of coming in contact with the cover glass. With reasonable care, it can be used with entire safety in daily routine work and, because of the greater ease with which it is employed, is generally to be preferred to apochromat 90 n. a. 1·4.

More about Eyepieces

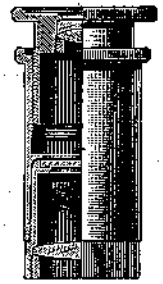
One of the main functions of eyepieces is to magnify the image produced by an objective to such an extent that the observer's eye can readily perceive the details of that image. Generally, it will be found that, if the total magnification exceeds about 500 times the numerical aperture of the objective employed, the human eye will see all the detail imaged by the objective. For convenient observation, it may be desirable to extend the magnification somewhat further. There is a certain amout of variation in eyes. A coarser spacing of perceptive elements in the retina of the observer requires a proportionally greater magnification of an image, in order to perceive its details. Also, 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 required for an objective with a numerical aperture of, say, 0·65 would be approximately 600×. If the objective has an initial magnification of 40, that would mean an eyepiece magnification of 15×. As a matter of fact, a 10× eyepiece would probably be found ample in many instances. The old prejudice which existed against high eyepiece magnification is no longer justified, provided well corrected objectives are employed, and the total magnification does not exceed the values just indicated.

For use with achromatic objectives, Huygens eyepieces are usually employed, although, if the numerical aperture is 0·65 or more, compensating eyepieces may be used to advantage also with achromatic objectives. Orthoscopic eyepieces are especially to be recommended if high eyepiece magnifications are desired for achromatic objectives with apertures below 0·65. Orthoscopic eyepieces 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 color correction, it was found possible to give them a magnification difference for the individual colors of the spectrum just the opposite of that obtaining in the objectives. Consequently, the two must be used together, in order to obtain the properly corrected image.

Full data as to field of view, magnification, etc. for the various oculars are contained in Micro leaflet 367.

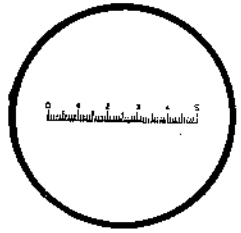
Micrometer Eyepieces

For measuring purposes, specially mounted eyepieces, usually magnifying 7×, are employed, known as focusing eyepieces, or micrometer eyepieces if they are equipped with the measuring scale. In these eyepieces, the upper "eye lens" is adjustable, either by means of a screw thread, or a simple sliding sleeve. (Fig. 14). The scale is a ruled glass disc which is dropped (ruled side down) into the eyepiece after removing the sliding sleeve, if the eyepiece is of this



8744

Fig. 14, about 2/3 actual size
Micrometer Eyepiece K 7×



7903

Fig. 15, about 1½ actual size
Eyepiece Micrometer Disc
5 mm. divided into 50 parts

type. The disc should lie evenly on the eyepiece diaphragm. If the eyepiece is of the type equipped with a screw focusing mount the lower end of the eyepiece can be unscrewed, and the measuring disc is then laid upon the diaphragm. A standard ruling for micrometer discs is 5 mm. divided into 50 (Fig. 15), but other rulings can also be supplied. The micrometer eyepiece is inserted into the microscope tube in the customary manner, and the eyelens is adjusted until the scale is in focus.

Before the scale of an eyepiece micrometer can be used for measuring in "absolute" units, it is calibrated by means of a stage micrometer (Figs. 16 and 17), which is viewed the same as a specimen would be. Supposing a stage micrometer ruled in 0·01 mm. is employed and that ten of its divisions coincide with 5 divisions of the eyepiece micrometer, then 5 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, abbreviated $\mu = 0{\cdot}001$ mm). It is necessary, of course, to calibrate the scale for each objective employed and, 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 the tube length will alter the value of the eyepiece scale.

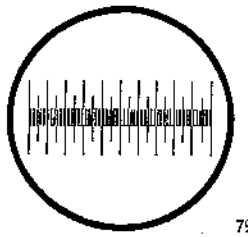
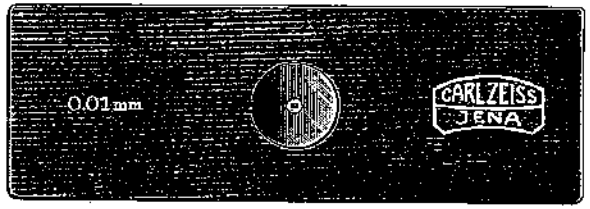

7902

Fig. 16,
Scale magnified about 15 ×

The draw tube offers a means of obtaining convenient integral values for the eyepiece scale. If, for instance, one division for a given objective and the standard tube length of 160 mm. represent 11·4 μ this value can be reduced to 10 μ by appropriately increasing the tube length. The method has its limitations, however. Deviation from the standard tube length must not be sufficient to impair the image to the extent of making measurement unreliable. A changeable tube length is also a fertile source of errors, great care is required that the length is not changed unintentionally.



8329

Fig. 17, actual size
Stage Micrometer, 1 mm. divided into 100 parts

Binocular Eyepieces Bitumi and Bitukni

In conclusion a word should be said in regard to several special eyepieces which have wide fields of application. Binocular eyepieces for use in conjunction with standard monobjective microscopes are a great aid in prolonged studies, since both eyes are employed. Furthermore, they yield a correct stereoscopic image (three dimensional) of the specimen under examination. The additional information regarding the actual spatial relations of the various structural elements in a specimen which at once is thrust upon the observer's attention when using a binocular is little short of startling, and cannot be appreciated until personally experienced. The construction of these binoculars requires great skill, if the image formed by a highly corrected objective is not to be impaired. Carl Zeiss binocular attachments Bitumi (Fig. 18) and Bitukni (Fig. 19) fully meet these requirements, and can be attached to your microscope at any time. (Micro leaflets 355 and 412.)

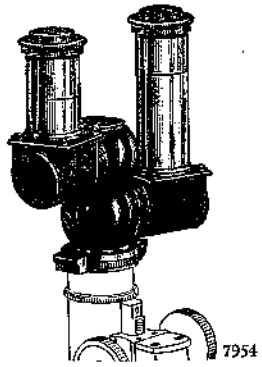

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Fig. 18, about 1/3 actual size
Bitumi

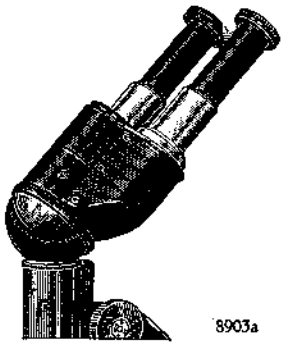
8903a

Fig. 19
Bitukni

Photomicrographic Eyepiece Phoku

Photographic eyepiece Phoku (Fig. 20) offers a simple and convenient device for obtaining photomicrographs of specimens under examination, living as well as fixed. Observation is not interrupted during exposure of the photographic plate, and special provisions are made for insuring exceptionally sharp definition over practically the entire plate. This eyepiece likewise can be attached at any time to your microscope. (Micro leaflet 373.)

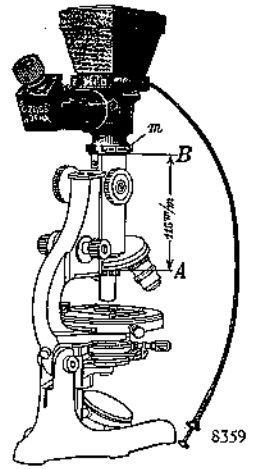

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Fig. 20, about 1/7 actual size
Phoku

II. The Routine of working with the Microscope

a) Care of the microscope

Having become familiar with some of the main considerations involved in the construction of a microscope, there remains now to be taken up those details in the method of use, habits of working, which to a considerable extent determine the comfort and efficiency with which an observer carries out his investigations.

At the outset, it is to be kept in mind that, as previously emphasized, 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 well made microscope properly used will literally last a lifetime. Proper use, as far as the welfare of the instrument is concerned, consists largely of two factors: keeping it clean and avoiding mechanical injury.

Protection against Dust and Lubrication

A microscope should be protected from dust as much as possible. If impracticable to return it to its case when not in use, it may to advantage be covered with a bell jar, or with 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 protected, it will be found in most locations that dust nonetheless accumulates on the instrument sooner or later. This should be removed by first dusting with a soft, clean brush (a 1½ 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 polish of lens and mirror surfaces. Hence the importance of dusting with a clean brush before wiping with a cloth. The brush can be kept clean if after use it is dusted out against a clean piece of wood or glass rod, and stored in an envelope or other dust-excluding place. A separate, smaller brush, preferably camels hair or sable, should be provided for the optical parts. The metal portions of the instrument are likely to be somewhat oily, and oil should be kept from lenses and mirrors as much as possible.

To maintain a good finish on the metal parts, it is desirable occasionally to use a trace of good non-corrosive oil (such as Three in One Oil). This is lightly applied by placing a few drops on a soft

clean cloth, rubbing, and wiping off all excess with a clean cloth, the object being to have an invisible protective film, but not enough to rub off appreciably on to the user's hands. Similarly, a minute droplet of oil may at times be applied (a toothpick is a convenient applicator) to screws and joints to prevent corrosion, and to keep movable parts freely moving. The excess again should be thoroughly wiped off. Occasionally rubbing in Three in One Oil on the wooden case and wiping off the excess will improve its appearance and maintain its protective coating against atmospheric conditions.

Oil should not be employed for lubricating bearing surfaces such as slideways of condenser or coarse adjustment rack and pinion, bearing surfaces of mechanical stages, etc. These are to be lubricated with a grease; non-medicated, acid-free vaseline will answer. If the grease of bearing surfaces has become hardened, it may be removed with the aid of xylol, benzine, or Three in One Oil, the oil in turn being thoroughly wiped off before applying a thin layer of vaseline. No attempt should be made to oil or clean the fine adjustment of a microscope. This will rarely require attention, but if it does, the microscope should be returned to the manufacturer's agent.

It goes without saying if in the course of an observation liquids are spilled on the microscope, these should be thoroughly 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 cleaning a microscope. Although modern finishes are relatively alcohol resistant, some of them are nevertheless more soluble in alcohol than in xylol, and, on the other hand, Canada balsam, etc. is more readily removed by means of xylol.

Cleanliness in handling Optical Parts

Cleanliness in all matters pertaining to a microscope is essential, and 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. Similarly with binoculars, keeping eyepieces in the eyepiece sleeves prevents dust from falling upon the upper prism face. See to it that oculars not in use are protected from dust, and, when interchanging oculars, be particularly careful not to set an ocular down on a dusty surface and then later replace it in the ocular sleeve of the microscope. There are few surer methods for scraping dust into the microscope tube and depositing it on the back lens of an objective.

Glass surfaces should not be handled with the fingers. They will probably leave oily markings and more damaging corrosive sweat. If a glass surface requires cleaning, first dust it with a small

camel's 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 (flicking it against a clean piece of wood or glass rod), and store it away from dust in an envelope. If dusting does not sufficiently clean the glass surface, it may be wiped with a clean, soft linen cloth, or with lens paper, moistened, if necessary, with distilled water. If this does not suffice, xylol may be tried. In any event, avoid unnecessary flooding the surface with liquid, and avoid undue rubbing.

Cleaning Objectives and Eyepieces

Cleaning of objectives and eyepieces on the part of the user should be confined to the exposed upper and lower lens surfaces. If dust should appear on the inner lens surfaces of eyepieces, attempts to remove it are likely to make matters worse. It usually is better to send the eyepiece to the manufacturer's agent for cleaning. Under no circumstances should an attempt be made to take an objective apart; to do so is likely to impair its performance. Users of Carl Zeiss microscopes are also cautioned against sending objectives in need of cleaning or repairs to anyone excepting the authorized representatives of Messrs. Carl Zeiss. Almost every year a number of objectives are received which have been seriously damaged as a result of attempts to repair them elsewhere. The user then should confine his cleaning of objectives and preferably also of eyepieces to the two exposed lens surfaces. If the lenses are recessed, as in some oculars, and the back lens of many objectives, the cleaning process is facilitated by wrapping clean, soft and lint-free cloth or lens paper about the end of a rounded, soft wooden stick, and wiping with a slight twirling motion. Alcohol should not be used as a cleaning fluid. (See also page 38.)

b) The procedure of making observations with a microscope

Microscopy is a delightful but also an exacting occupation. With proper precautions, it can be followed for many hours daily in comfort and without injury to the observer. In fact, properly carried out, the use of a microscope is a beneficial exercise for the eyes, strengthening them rather than the reverse.

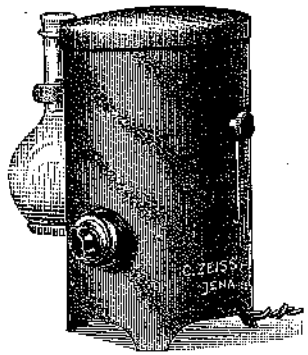
The first requirement is to select a table and chair so adjusted that the observer can maintain a fairly erect position. There commonly is a tendency to have chairs relatively too high, or the microscope pushed in too far from the table edge. As a result, a stooped posture is assumed which is not conductive to the health and long life of the observer, and which, with a little care at the outset, is easily avoided.

Having provided for a comfortable, erect position of the observer, the microscope is placed squarely before him, taking care that it stands firmly on the table top. Preferably the microscope is employed in an erect position, there rarely is occasion to incline it. An inclined microscope greatly hampers the easy, free displacement of the slide, an

essential for rapid and thorough searching, and it also interferes with the examination of liquid preparations. It should, therefore, be made a practice to habitually employ the microscope in the vertical positon.

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 at the same time is close enough to the window to afford adequate illumination of its mirror. It is to be remembered the image in a microscope often reveals details which are only faintly visible, and their observation is rendered more difficult if strong light from without enters the eye at the same time. Neither should bright light fall from without upon the eyelens 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 in that direction. Direct sunlight should not fall upon the mirror hence a north window is preferred, since it supplies fairly even illumination for the greater part of the day. Because of the uncertainty of daylight and the desirability of having a uniform source of light, many observers work entirely with artificial light. For most purposes, an incandescent electric lamp (Fig. 21), provided with a boiling flask to serve as condenser and filter, 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. It is convenient to prepare a concentrated stock solution of copper sulphate in ammonia water and this, a drop at a time, is added to distilled water in the flask until the desired intensity of color is obtained. Many observers prefer a slight blue tinge to the light. An excess of ammonia water should be added to the contents of the flask. If the solution turns greenish, the blue color can be restored by adding more ammonia water. For work with low magnifications, the lamp may be fitted with an ordinary lighting bulb. If this does not furnish sufficiently bright light, special concentrated filament 100 Watt bulbs should be used.



11015

Fig. 21, about 1/5 actual size
Incandescent Electric Microscope Lamp

Mounting of Specimen

Specimens for examination are usually mounted on a glass slide and covered with a cover glass. Temporary mounts may be made in

water or watery solutions. Permanent slides are most often mounted in Canada balsam, or in some other medium of high refractive index more or less approaching that of glass.

Examination with 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 under low magnifications in order to become acquainted with its general features before proceeding with the more detailed examination of some special part.

Having swung the lowest power objective into position over the slide, and employing a low power eyepiece, say 5× or 7×, the microscope tube is lowered by means of the coarse adjustment until the objective almost touches the cover glass. This is ascertained by observing the objective from the side, the eyes, being placed almost on a level with the microscope stage. Then, looking into the eyepiece, the coarse adjustment is turned upwards until the magnified image of the specimen appears. If inadvertently the focal plane should be passed by without being noticed, the procedure is repeated. Under no circumstances should the observer focus down in finding the specimen while the eye is applied to the eyepiece. Failure to observe this simple precaution has ruined many slides as well as objectives. When the specimen is found, it is accurately focused by moving the coarse adjustment back and forth until the image appears sharpest. If fairly high magnifications are employed, final focusing can to advantage be carried on with the fine adjustment. The fine adjustment should not be used, however, in first finding a specimen, but only in controlling the final focusing after the specimen has been approximately brought into focus with the coarse adjustment. During observation with any exepting the very lowest magnifications, one hand of the microscopist continuously operates the fine adjustment, bringing succussive 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 particular detail of the specimen which occupies the center of interest. In a short time the fine adjustment is manipulated without the observer paying any attention to it whatsoever. Usually the right hand will be used.

It is well to see to it that the fine adjustment is approximately at the middle of its range as indicated by the guide marks at the right hand side of its housing before beginning work. If that is done, the observer will not be subjected to the inconvenience of coming to the end of the fine adjustment movement during the course of an examination.

Adjusting 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. If a window is employed as a source of light, cross bars of the sash are avoided as much as possible. If an artificial source of light is used, the illuminating beam should fall upon the center of the mirror, a piece of paper may be held on the mirror in order to better locate the position of the illuminated area. If the microscope is equipped with a condenser, the distance of the lamp and its attached condenser (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. This is readily ascertained by removing for the time being the ground glass from the lamp and holding a piece of paper over the diaphragm of the microscope condenser. The microscope condenser should then be focused so that an image of the lamp filament appears at the same time the specimen is in focus. Before proceeding to a study of the specimen, the ground glass of the lamp is replaced in position, in order to eliminate the appearance of the lamp filament in 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 of view. The front lens or set of lenses of many condensers can be unscrewed, the remaining portion of the condenser will then have a longer focus and illuminate 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 10.) Having obtained an even illumination of the entire field, it remains to regulate its intensity. To a certain degree this is accomplished by means of the iris diaphragm situated below the microscope condenser. Its operation will be taken up more fully in connection with the use of higher power objectives. Another means of reducing the intensity of illumination is to place a ground glass disc (ground surface up) in the carrier provided for the purpose below the condenser (or in the iris diaphragm carrier of the complete Abbe illuminating apparatus with which the large microscope stands are equipped).

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 employ too bright light. This is especially the case since artificial illumination has come into more general use. The illumination for any given magnification should be sufficient to readily reveal details of structure, but it should not be blinding. The latter is particularly liable to

occur when low magnifications are employed, and there is danger of it at night time with all magnifications, unless the work place is brightly illuminated.

When satisfactory illumination is established, the beginner should proceed to examine the entire specimen under 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 persistence is rewarded by awkwardness giving place to a high degree of skill in quickly and without effort moving the slide as desired. 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 surfaces can readily be distinguished, and the specimen should be mounted on the convex surface. If mounted on the concave surface, the slide will not rest firmly on the microscope stage, but rock back and forth.

After thoroughly studying a specimen under the lowest magnification available, it will be of interest to compare its appearance with eyepieces of greater magnification before passing on to more powerful objectives. It will be observed 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 larger, no additional detail is revealed (page 22). For most purposes, eyepiece magnifications not exceeding 10× or 15× will be found ample.

Using Both Eyes

The microscopist should from the outset accustom himself to keepig both eyes open, and to alternate from one eye to the other in working with a monocular microscope. The slight effort required at the outset to acquire these habits is well worth while. The eyes undergo an elaborate training as the skill of the microscopist progresses, and it is invaluable to have both eyes participate in this from the outset, both as an insurance if one eye should become temporarily or permanently incapacitated, and as a means of relief during prolonged investigations. In this connection, the growing recognition of the importance of binocular monobjective microscopes may be emphasized. Since these instruments are available in a mechanically as well as optically satisfactory construction, it may be assumed they will, before another decade has passed, be in general use for visual microscopy. Binocular attachment Bitukni (page 25) is particularly serviceable, not least because — due to its inclined observation tubes — it for the first time offers an arrangement which insures an erect and comfortable posture for the observer, and at the same time the stage of the microscope remains horizontal, and thus permits a free and convenient manipulation of the specimen.

Examination with objectives of higher magnification

Having acquired some familiarity with the manipulation of a specimen and the microscope under the lowest magnification objective and with several eyepieces, the beginner may turn 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 is brought as nearly into the center of the field as possible and focused. Then, without disturbing the focus, 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 the microscope stand is purchased are made parfocal, that means, 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 the first time, the user should ascertain whether the objectives actually are parfocal, before depending upon their clearing the cover glass as the nosepiece is revolved. It is to be noted that objectives are made parfocal for the standard tube length of 160 mm., and for observers with normal vision. Deviations from these standards destroy the parfocality. It is not necessary that succeeding objectives should swing exactly into focus, all practical purposes are served if a bare outline of the specimen appears. A slight manipulation of the fine adjustment then quickly adjusts the focus exactly.

In passing from a lower to a higher magnification, it is to be remembered the field of view decreases as the magnification increases. That means, if a specimen lies slightly out of the center of the field under a low magnification, it will lie relatively much closer to the margin of the field or even entirely outside of the field with a higher magnification. Needless to say, care must be taken to have the slot in the nosepiece opposite the objective in use actually engage the knife edge of the arresting spring. Should the nosepiece be inadvertently turned a trifle farther, or not far enough, the field of view would shift. Well made revolving nosepieces are sufficiently well centered to meet practical requirements (page 13). For greatest precision of centering, as sometimes required in photo-micrography and in ultra-microscopy, sliding objective changers are employed (Mikro 82).

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 immersion objectives. The latter present a few complications which, however, are readily mastered, especially if the microscopist is already fairly at home in the use of high power dry objectives, and has acquired the ability of manipulating the slide with assurance under high magnifications. Oil

immersion objectives usually are of high magnification and large numerical aperture, as a result their working distance and depth of focus is so small that it is impracticable to make them parfocal with the other objectives. Instead, they are usually made somewhat shorter, so as not to strike the cover glass if accidentally swung around over the slide in place of a dry objective.

Finding the Specimen with Oil Immersion Objective

Oil immersion objectives owe their specific advantageous characteristics to the employment of an immersion fluid — special thickened cedarwood oil of specific refractive index and dispersion — between front lens of the objective and cover glass. They cannot be used without this immersion fluid, and to obtain the best results of which Carl Zeiss objectives are capable, only Carl Zeiss cedarwood oil should be used. A convenient procedure for applying the oil and focusing an oil immersion objective is as follows: Locate the specimen accurately in the center of the field under high magnification with a dry objective, raise the microscope tube by means of the coarse adjustment so as to have ample space above the slide, swing the oil immersion objective into position, place a small drop of immersion oil on the cover glass over the field under examination, being careful not do disturb the slide, promptly 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), then raise the tube a trifle, but not enough to break the immersion contact. The object of this last step is to make certain the objective is above its plane of focus. Now apply the eye to the eyepiece, slowly and steadily focus down with the coarse adjustment until the outlines of the specimen begin to appear. Stop at that moment and complete focusing with the fine adjustment. The working distance of the high power oil immersion objective is so short that attempts to bring the objective below its plane of focus and then focus up would probably result in coming into contact 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 adjustment is preferable for this because the process would be so long drawn out with the slowly moving fine adjustment that the observer's attention might wander for a moment with the possibility of passing the plane of focus unnoticed. If this happens with the fine adjustment, the hand operating it is less likely to detect contact with the cover glass than if the coarse adjustment is used. Contact with a cover glass should be and usually can be completely avoided. (See also foot note page 35.)

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

In finding the focal plane for difficult specimens, the microscopist with a hand well trained in the manipulation of slides has a valuable advantage. While focusing down with the coarse adjustment, the left hand lightly moves the slide back and forth through a narrow amplitude, displacing the slide by less than a field of view with the immersion objective, so that there is no danger of losing the selected part of the specimen. If in descending the plane of focus should be passed, the hand moving the slide will at once detect the slightest pressure on the slide.

Protecting devices for the fine adjustment, which are intended to prevent injury when the objective comes in contact with a cover glass, may prevent a slide from being broken, but are not adequate to prevent injury to the objective, since sufficient force must be given to the downward movement to insure its functioning, and under proper conditions the force is sufficient to dislocate the delicately mounted front lens of an objective if it is brought into contact with a cover glass. It, therefore, is best not to employ such devices.

Further Control of the Illumination

Having found the specimen 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 present in the oil, they are readily seen and 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, it is necessary to wipe off the oil and repeat the process of locating the specimen, applying a new drop of oil, etc. Air bubbles should not be tolerated, since they impair the image, in fact, make it quite useless if they are of considerable size or abundance.*

Examining the appearance of the back lens of the objective indicates at once whether the mirror is properly placed to secure even illumination. Furthermore, the extent to which the iris diaphragm of the condenser is opened can be seen at a glance. It will be found that, even with the diaphragm fully opened, the peripheral portion of the

* Observing the back lens of the objective also affords a valuable aid in finding the focus with an oil immersion objective. As long as the objective is still above its plane of focus, a miniature image of the preparation will be seen upon the back lens. If there is doubt whether any portion of the specimen is in the field of view, the slide can be moved about until a part of the specimen appears. Then the eyepiece is placed in position and the tube focused down by means of the coarse adjustment, as described on page 34. This method, of course, is applicable only when the specimen contains relatively large structures. It would fail, for instance, with a preparation consisting of widely separated minute bacteria. But even in such specimens there are often accidental inclusions of coarser materials which will serve as guides for finding the plane of focus. Care must, of course, be taken not to mistake scratches or stains on the lower surface of the slide for objects lying between cover glass and slide. Needless to say, such defacements on the lower slide surface should be avoided as much as practicable.

back lens of oil immersion objectives is not illuminated (unless indeed it should be 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, therefore, is necessary to immerse the condenser also if it is desired to fully utilize the resolving power of objectives with a numerical aperture exceeding 1·0. Condensers are less highly corrected than objectives of the same numerical aperture, so that it is permisible to employ most of them at will, either dry or immersed. 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 lowered a few millimeters and, after placing a slide in position on the microscope stage, is raised again until immersion contact is established. The specimen is first found and focused with a dry objective, the condenser likewise is focused as previously explained. If now an oil immersion objective is put into use, it will be found — looking down the tube of the microscope — that the back lens of the objective is completely illuminated, assuming a condenser with a numerical aperture of 1·4 is being employed with its diaphragm fully opened and that the illumination is properly adjusted. If the condenser has an n. a. of 1·2, there still will be a narrow margin which is not illuminated if the objective has an n. a. exceeding 1·2. With objectives having an n. a. of 1·25, this may be considered negligible for most practical purposes. If objectives with an n. a. of 1·3 or over are employed, it will generally be advisable to provide a condenser with an n. a. of 1·4, so that, if occasion arrises, the full aperture can be utilized, even if in routine work there may seldom be occasion for this.

Size of Opening of Iris Diaphragm to be employed

The extent to which the iris diaphragm of the condenser should be opened often troubles a beginner. It is to be noted at the outset that under no circumstances should the illuminating beam have a greater aperture than the objective, otherwise the image will be flooded with light. Usually, it will be advantageous to reduce the illuminating aperture below that of the objective. The lower the aperture, the greater the depth of focus and the greater the contrast of component elements in the image. The lowest permissible aperture

is reached when diffraction bands become evident about the borders of objects imaged. The actual aperture which is best suited for the examination of a given specimen is determined, therefore, 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 a field of view diaphragm. This is placed close in front of the condensing lens which accompanies the source of light. When the lamp with its condenser is in the correct position, and the microscope condenser is properly focused, an image of the field of view diaphragm appears in the plane of the preparation under examination. As the diaphragm is opened and closed, the intensity of the illumination does not change, 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 placed directly below the microscope condenser (or, in some special condensers, between the condenser lenses). The importance and utility of a field of view diaphragm is appreciated in photomicrography and in the study of opaque specimens by means of vertical illuminators (Mikro 89). The extension of its use generally in critical microscopy is only to be recommended. By restricting the illumination to the area actually under observation, extraneous light is avoided which otherwise falls over the image and more or less obscures its details. 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 are in evidence when working with a field of view diaphragm. The margins of the diaphragm are sharply depicted 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 quickly appreciate the advantages of this type of objective. The first feature likely to arrest attention is the great brightness of the image. It is instructive to compare in this respect, for example, an oil immersion objective 90 with eyepiece 7× yielding a total magnification of 630×, and a dry objective 40 with eyepiece 15× yielding a total magnification of 600×. If the observer has had experience with dry objectives having an n. a. of 0·85 or more, he will be impressed with the 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. Furthermore, considering the high initial magnification, the working distance is

considerably greater than for a dry objective. These advantages are made possible by employing cedarwood immersion oil. With a few precautions, the use of this oil will be found simple and free from trouble, so that oil immersion objectives will be used as a matter of course, and for most purposes will be found more convenient than dry objectives with an n. a. above 0·65.

Care of Immersion Oil

First and foremost in the successful use of immersion oil is a certain amount of cleanliness. The oil is supplied in vials holding about 7 1/2 gm. A small quantity of this is poured into the inner container of the double oil bottle (Fig. 22) first seeing to it that the container is dry and clean. After an oil vial is once opened, it should again be stoppered and have a piece of paper wrapped or tied over the stopper, first wiping off adhering oil. In this manner, a supply of clean immersion oil is assured. The depth of oil in the inner container of the double oil bottle should be about 15 mm., and the glass rod extending from a wire in the cap of the bottle is adjusted so that it will carry 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 strike 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 should be carefully wiped with a lintless cloth moistened with xylol or benzine.

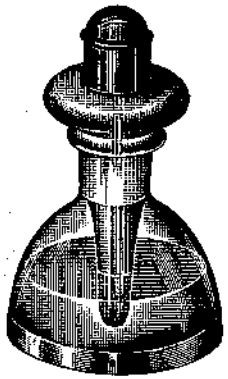

8649

Fig. 22, about 1/2 actual size
Double Bottle for Cedarwood Oil and Cleaning Fluid

When the cap with its attached glass rod is once removed from the oil bottle, the droplet 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 on the oil bottle. Under no circumstances should it be laid down. Failure to observe this precaution will result in collecting dust, etc. in the oil, and the oil should be a perfectly clear, homogeneous medium.

Removing Immersion Oil from Objectives

Immersion oil should be removed once a day from objectives, when the day's work is completed. Ordinarily, there is no advantage in doing it oftener and, as previously mentioned, it is desirable to avoid unnecessary polishing of lens surfaces. Xylol or preferably benzine

(petroleum ether) are safe cleaning fluids, a supply being kept in the outer container of the double oil bottle. As mentioned on page 28, alcohol should never be used for cleaning objectives.

Lens paper

Japanese lens paper is to be recommended for wiping objectives. It is well to store a supply, cut into pieces of about 1 1/2" square, in a clean glass jar with overhanging lid, to prevent dust from settling into the jar. In cutting the paper, scrupulously avoid contaminating it with dust. To clean an objective, remove a single piece of lens 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 lens 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 front, first passing a dry corner of the lens paper across the oil to remove the bulk of it, then the portion moistened with cleaning fluid, and finally a clean, dry part of the paper. In this manner, with a single circular sweep, practically all of the oil is removed with a minimum of friction. The inner container of the oil bottle, by means of which the cleaning fluid is transferred to the lens paper, should be immediately returned to its position in the outer container, not laying it down elsewhere, since this would bring dust into the cleaning fluid.

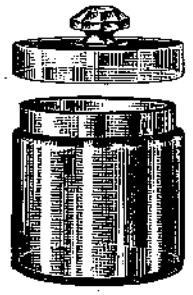


Fig. 23 about 1/4 actual size
Lens Paper Jar

Removing Immersion Oil from Condenser

If the condenser is immersed, it requires cleaning before it is again used dry, and in any event once a day. Because of its larger surface, it is preferable to use a clean cloth for removing the oil, but, if there is any question about the cloth being clean and free from dust, it is better to employ lens paper for the condenser also. Particular care is to 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 lens paper for this purpose. First wipe off the bulk of the oil with a single stroke with a dry part of the cloth, then wipe over once with a fresh part 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 — for most purposes an unnecessary refinement, which those who are so inclined may carry a step further by substituting for

breathing on the glass a moistening of the cloth with distilled water. Cedarwood oil should be removed from a slide before using a high power dry objective on it, aside from its likelihood of interfering with the image, there is danger of its coming in contact with the front lens of the dry objective. It is a not uncommon experience to find dry objectives greatly impaired in their performance by a small amount of immersion oil which became attached to the front lens unknown to the user.

Benzine has the advantage over xylol as a cleaning fluid for slides in that Canada balsam is less soluble in the former, and hence there is less danger of dissolving and dragging the mounting medium over the cover glass. For this reason, benzine is to be preferred as a cleaning fluid. It is advisable to keep a separate bottle of it for cleaning slides; in so doing, the supply for cleaning objectives will be kept cleaner.