16. Microscope Construction, Use, and Care

The microscope is probably the most indispensable of the instruments used in the biological sciences. Intelligent purchase and effective utilization of a microscope require an understanding of at least the elements of its optical and mechanical construction. It is an expensive instrument, built to the highest standards of precision and having possibilities of performance that are not fully utilized by many users. Although having some structural features that are delicate or even fragile, the microscope has adequate durability to give many years of useful service.[1]

The function of a microscope is to produce an enlarged image of an object. This is accomplished by a system of lenses. A lens may be defined as a transparent body having at least one curved surface. A simple lens, consisting of one piece of glass, may be used to illustrate how a lens produces an enlarged image by bending or refracting light. A ray of light coming from the object enters the upper portion of the curved face of a lens and is bent downward. Similarly, a ray entering the lower portion of the lens is bent upward. The rays which pass through the lens converge and then continue as a diverging cone. If a sheet of paper or ground glass is placed to intercept the rays which pass through the lens, an enlarged image of the object is produced on the screen. A photographic plate can be placed in the cone of light and a photographic image obtained. A hand lens or the lens on a simple dissecting microscope produces an image on a screen in this manner (Fig. 16.1 *A*). The objective or lower lens of a microscope consists of two to nine lenses which act as a unit to produce an image as described above. There are certain limitations on the magnification and quality of image obtainable with the objective alone. The primary image produced by the objective is intercepted

[1] The author has drawn freely on the catalogues and service leaflets of the leading optical manufacturers.

and magnified, and improved in quality by an eyepiece. The eyepiece or ocular consists of two or more lenses working as a unit and having a fixed magnification. If a ground-glass screen, a sheet of paper, or a photographic plate is placed at any plane above the eyepoint of the ocular, an image is produced (Fig. 16.1 *B*). Note that the primary image is inverted and the projected image is erect.

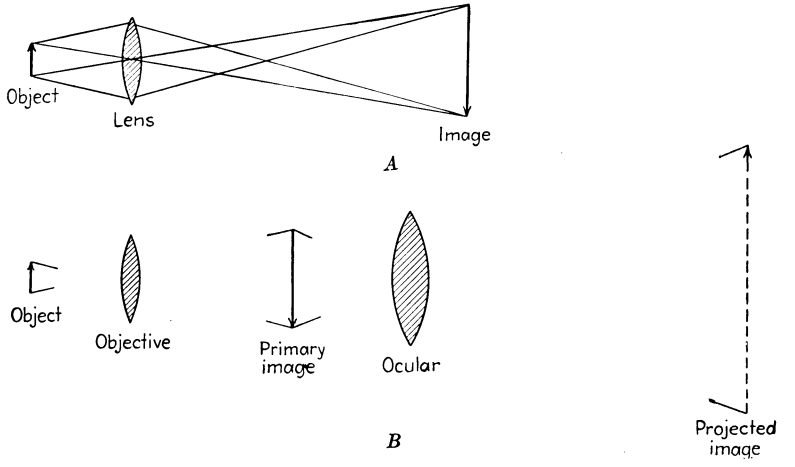

FIG. 16.1—Formation of projected images by the microscope: *A,* simple microscope; *B, compound microscope.*[2]

With a given objective and ocular, the size of the image varies with the distance of the screen from the ocular. If the screen is placed approximately 10 in. (254 mm.) from the eyepoint, the size of the image will be approximately equal to the product of the designated magnifications of the objective and ocular. Thus, an objective having a designated magnification of 10×, used with a 10× ocular, gives a total magnification of approximately 100×. Exact values must be determined by micrometry.

The foregoing discussion does not take into account the operation of the human eye working in conjunction with the microscope. However, most microscopic work is done by direct visual observation with the eye held at the eyepoint of the ocular. Let us turn for a moment to a consideration of the eye as an optical instrument. The lens of the eye operates as a simple lens, and the curved retina is

[2] The illustrations in this chapter are highly diagrammatic and simplified and are intended only to show the approximate relative positions of the object, the optical elements, and the images.

the receptive surface on which the image is formed. If an object is held at a given distance in front of the eye an inverted image of definite size is produced on the retina. If a larger object is substituted at the same distance, or if the original object is moved closer to the eye, the *visual angle,* or the angle of the cone of rays between the object and the eye, is increased, the size of the retinal image is increased, and the object appears to be larger. In Fig. 16.2*A* compare the two objects shown in solid and dotted lines, respectively, their respective visual angles Va_1, Va_2 and the retinal images Ri_1, Ri_2.

When the eye is held at the eyepoint of the microscope, it intercepts the image-forming cone which has a definite angle established by the microscope, and a retinal image of definite size is produced (Fig. 16.2 *B*). The observer sees a magnified virtual image, which appears to be near the level of the microscope stage, approximately 10 in. from the eye. The retinal image is erect, the virtual image is

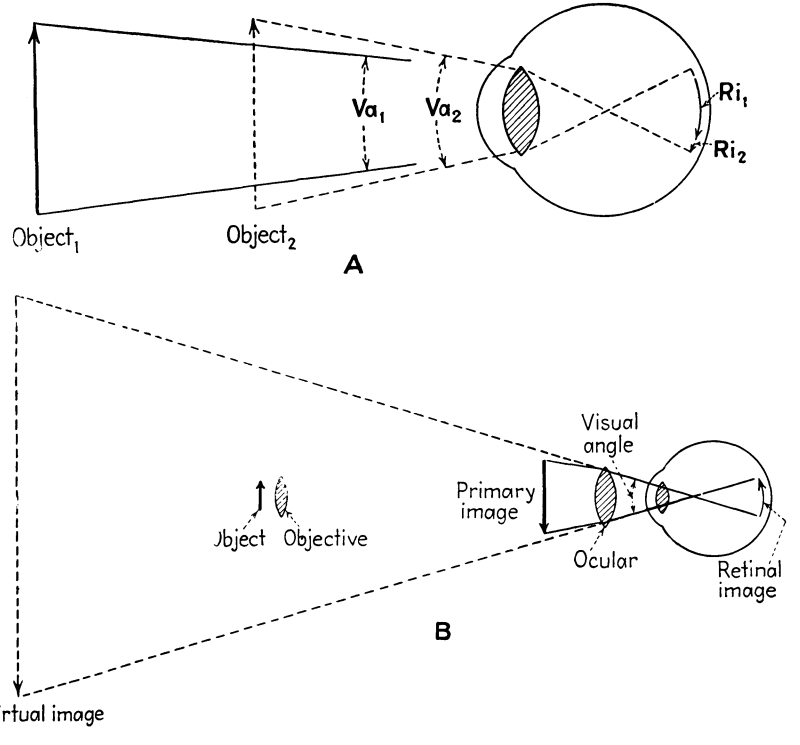

FIG. 16.2—*A,* Formation of images by the eye showing relative size of retinal image in relation to visual angle; *B,* retinal and virtual images obtained with a compound microscope.

inverted, and the direction of motion of the object is reversed. The apparent size of the virtual image is the same as if the observer viewed the projected image on a screen 10 in. from the ocular. As a specific case of magnification, let us view an object 0.1 mm. long with a 100× microscope. This produces a retinal image of the same size and the same impression of magnitude as if we looked at the 10-mm. image projected by the same microscope on a screen 254 mm. from the eyepiece.

Properties of Objectives

MAGNIFICATION

The most obvious property of objectives is magnification, which is a fixed value under the conditions outlined in a preceding paragraph. The objective magnifications used most commonly on standard monobjective microscopes range from 3.2× to 100×. Magnifications below this range are used on paired-objective stereoscopic prism binocular dissecting microscopes. Objectives above 100× have rather limited uses. The conventional low-power objective is 10×. The lower powers, from 3.2× to 6×, are not fully appreciated and deserve more serious consideration.

WORKING DISTANCE

Free working distance is the distance between the objective and the cover glass, using a cover glass 0.18 mm. in thickness. The catalogues of the manufacturers give the working distances of their objectives. A few selected illustrations show the relation between magnification and working distance: 10×, 7.0 mm.; 43×, 0.6 mm.; 45×, 0.3 mm.; 95×, 0.13 mm. It is obvious that for an elementary class the most desirable high-power objective has a magnification in the forties and the longest available working distance. Objectives of high magnification and short working distance must be used with care to avoid damaging the front lens and the slides.

FOCAL LENGTH

If a beam of parallel rays is projected through a simple lens, the rays converge at a point. The distance from this point to the optical center of the lens is the focal length. In an objective consisting of several components, the situation is somewhat more complex, and a different value is used. The manufacturers engrave on the mountings and list in their catalogues a value known as the equivalent focus. At the standard projection distance of 250 mm., an objective

that has several components and an E. F. of 16 mm., will give an image of the same size as a simple lens of 16 mm. focal length. The E. F. should not be confused with working distance. The equivalent focus decreases as the magnification increases. The experienced worker is in the habit of speaking of an objective as a 4-mm. objective, for instance. For class use it is much better to speak in terms of magnification, which is 43× in a certain 4-mm. objective. In the past the manufacturers have paid undue attention to computing their objectives so that the equivalent focus is an even number, and a series of objectives will have the equivalent focus in the orderly progression 16, 8, 4, 2 mm., etc. A magnification may turn out to be some awkward fractional number like 3.2 or 5.1. A more practical series would be a sequence of magnifications such as 3, 5, 10, 20, 40, 60, etc. There is a trend toward the use of the latter system.

DEPTH OF FIELD (DEPTH OF FOCUS)

A minute body or a very thin section has thickness or depth. If a deep cell is being viewed with a 10× objective and the lens is focused on the upper wall of the cell, the bottom wall may also be in focus. If a 45× objective is focused on the top wall, the bottom wall may be completely out of focus and practically invisible. If the lens is focused on the bottom wall, the top wall becomes obscured. The vertical extent of the zone of sharp focus is the depth of field. The term "depth of focus" is in common use. For instance, some camera lens mounts have depth of focus scales. Depth decreases as the magnification increases. There are mathematical limits to the depth encompassed by a given objective. Magnification and other factors being equal, objectives of the several manufacturers have the same depth of field.

RESOLVING POWER

Resolving power is that property of a lens which makes possible the recognition, as distinctly separated bodies, of objects that are exceedingly close together, or subtended by a small visual angle. The simplest illustration of resolving power is the visibility of double stars. Although the two stars may be separated by a vast distance, the visual angle reaching the eye is very small, and the stars appear to be close together. Many individuals can see but one star. Other persons, whose eyes have better resolving power, can see the two stars distinctly. Applying this principle to the microscope, a lens of poor resolving power will show a slender chromosome as a single thread, whereas a lens of good resolving power will show the chromo-

some as two interwound threads. The question to ask concerning an objective is not "how small a thing can you see?" but "what is the minimal separation between two objects that the lens can resolve?"

The mathematical derivation of the formula for determining resolving power can be found in textbooks of optics or physics. The formula contains the following factors:

n = the lowest index of refraction in the path of the rays, *i.e.*, the index of refraction of water, glass, air, cedar oil, balsam, etc.

u = half of the angle made by the effective cone of rays entering the objective. This value can be obtained from a table in Gage (1941) or from the manufacturers.

N.A. = numerical aperture, a number that is indicative of relative resolving power.

The formula is

$$\text{N.A.} = n \cdot \sin u$$

The value of the numerical aperture is engraved on most modern objectives and is given in the catalogues of the manufacturers. This number is 0.25 in a 10× objective, for example, and increases through progressively higher magnification, attaining the value 1.4 in an expensive 90× objective.

Knowing the numerical aperture, we can make a simple computation and arrive at a tangible value of resolving power. Assume that we are using an objective of N.A. 1.0 and using light having a wave length, in round numbers, of 0.0005 mm. The formula is

$$\frac{\lambda\ (= \text{wave length})}{2\ \text{N.A.}} = \frac{0.0005}{2} = 0.00025\ \text{mm.}$$

This means that if two bacteria or two chromomeres on a chromosome are separated by a space of 0.00025 mm., they can be seen as two distinct bodies. As the numerical aperture increases, the resolving power increases, the working distance and depth of field decrease, and the cost increases.

The practicable upper limit of N.A. 0.95 is obtainable with dry lenses, used with an air space between the objective and the cover glass. In accordance with the foregoing formula, the N.A. can be increased by increasing the value of n or of $\sin u$ or both. If the angle of the ray that passes from the glass slide to air is greater than 41°, the light is totally reflected back into the glass. This phenomenon limits the angle that determines n. If a drop of cedar oil or synthetic

silicone immersion fluid is used to connect the immersion objective with the slide, the values of n and $\sin u$ are both increased, and consequently the resolving power is increased. An N.A. of 1.10 can be obtained with a water immersion lens, and N.A. 1.40 using cedar oil.

OPTICAL CORRECTIONS

The foregoing discussion of the properties of objectives does not take into account the quality of the image produced. A simple lens produces a decidedly imperfect image. Rays of white light which enter the lens are broken up to some extent into a band of colors, a spectrum. These colors are not brought to a focus at a common point; blue is converged at a point closer to the lens than is red. Consequently, the colors of the object are not reproduced accurately, and a color fringe or "rainbow" is visible along the boundaries of objects in the microscopic image. This is known as chromatic aberration.

Spherical aberration is a defect that produces poor definition in the center of the field. This defect is aggravated by a cover glass that is not within the maximum thickness range of 0.15-0.21 mm. Image quality also is impaired by variation from the standard tube length of 160 or 170 mm. designated by the manufacturer. Certain objectives have an adjustable correction collar on the objective mount, calibrated for variations in cover-glass thickness.

Curvature of the field is another structural defect in the microscopic image. If the center of the field of view is in sharp focus, the margins may be out of focus. With some objectives, the image may be distinctly dome-shaped. The degree to which objectives are corrected for the above color and structural defects of the image will be indicated in the discussion of the optical categories in which objectives are classified.

PARFOCALIZATION

Two or more objectives are parfocal with each other when it is possible to focus one objective on an object, turn to the next objective without focusing, and see the object in more or less sharp focus. This feature is extremely important with large classes of elementary students. If the conventional 10× low power and the 40 to 45× high power are not parfocal, the student must refocus with the latter lens, which has a short working distance, small field, and shallow depth of field. Excessive breakage of slides and scratching of objectives occur if the objectives are not parfocal. Adjustment of the old-

style objectives should be left to the manufacturers or to a skilled instrument mechanic. The new Bausch & Lomb objectives have an internal adjustment, with which the student cannot tamper but which can be easily adjusted with a special wrench.

Dry objectives between 10× and 60× can be made parfocal in any combination. The older 4 to 5× objectives cannot be so adjusted, but the American Optical Company now makes a 3.5 and a 5.1× objective, and Bausch & Lomb makes a 3.2 and a 6× objective which can be made parfocal with the 10×, and parfocal with the 43× within a quarter turn of the fine adjustment. With a combination of 3.2, 10, and 43× objectives students should be taught to change magnification up or down in that sequence, thereby minimizing damage to slides and lenses.

Types of Objectives

ACHROMATIC OBJECTIVES

These are in the lowest price class and are used on classroom microscopes and for routine work in research. In these objectives chromatic aberration is corrected for two colors and spherical aberration for one color. Achromatic objectives have undergone relatively greater improvement in recent years than have other types.

FLUORITE OBJECTIVES

In these objectives the mineral fluorite is used in conjunction with special optical glasses. The corrections are of a higher order than those of the achromatic series. Fluorite objectives are particularly useful for photomicrography by virtue of excellent color correction. They are available only in magnifications over 40×.

APOCHROMATIC OBJECTIVES

These objectives have chromatic aberration corrected for three colors and spherical correction for two colors, affording brilliant images, presented in their true colors and without distortion of shape. The highly actinic violet rays are brought to the same focus as the longer visual rays, making these objectives highly desirable for photography. Apochromatic objectives are expensive because of their complex construction and the scarcity of suitable fluorite.

Oculars (Eyepieces)

Oculars have distinctive optical characteristics that must be understood in order to use the correct ocular, and the correct combination of ocular and objective under specific conditions. An ocular has a

definite equivalent focal length. This value may be obtained from the catalogues, but a more useful designation, which is engraved on modern oculars, is the magnification value, which ranges from 4 to 30×. For routine work and for classwork 10× is the most satisfactory magnification. The lower magnifications are likely to have marked curvature of the image. Higher magnifications cause increasingly greater eyestrain, which is very pronounced with the 30×. Furthermore, there is an upper limit, beyond which the ocular produces only empty magnification, with no gain in the revealing of detail.

The maximum effective ocular magnification, which may be used with a given objective, can be computed easily. Assume that a 43× objective of N.A. 0.65 is being used; the formula is

$$\frac{1{,}000 \text{ (N.A. of objective)}}{\text{Magnification of objective}} = \frac{(1000)\ (0.65)}{43}$$

$= 15\times$, the approximate maximum ocular magnification.

It is evident that with a microscope on which the 43× objective is the highest power used, an ocular magnification of over 15× is of no value with respect to resolving power but of possible value for counting or drawing by projection. This simple calculation will enable a purchaser to specify the most useful lens combinations. Modern oculars are parfocal, making it possible to interchange oculars of different magnifications without requiring much change of focus.

OPTICAL CATEGORIES OF OCULARS

Huygenian oculars are of comparatively simple two-lens construction. They are designed for use with achromatic objectives and yield inferior images with apochromatic objectives.

Compensating oculars are designed to correct certain residual aberrations inherent in apochromatic objectives. It is, therefore, imperative to use compensating oculars with apochromatic objectives, and oculars and objectives must be of the same make. These oculars may be used with achromatic and fluorite objectives having magnification over 40×.

Flat-field oculars are of the noncompensating type and yield images in which curvature has been considerably reduced. These oculars have various trade names, Hyperplane and Planascopic being the best-known. A serious objection to some oculars of this type is that the eye must be held rigidly at a restricted eye position. The

slightest lateral motion of the head cuts off part of the field, and prolonged use produces marked fatigue.

Wide-field oculars (noncompensating) have an exceptionally wide field and good correction for curvature but may have a restricted eye position as in the flat-field type. This objection may be raised concerning high-eyepoint oculars, which are designed to permit the use of spectacles by the observer.

Workers who must use spectacles with low-eyepoint oculars find that the lenses of the spectacles and oculars become scratched after some use. A simple remedy is to paste a narrow ring of felt over each ocular. This permits the user to press his glasses against the ocular and to utilize the full field, without damage to the glasses or the ocular even after years of use.

Illumination

The most common method of illuminating a slide or other transparent object is by transmitted light. The light is projected through the hole in the stage and passes through the preparation. The simplest device for projecting light through the specimen is a concave mirror under the stage, designed to focus a converging cone of rays at the level of the specimen. Regardless of the character of the light source, whether daylight or artificial light, the *curved* mirror should be used if the microscope has no condensing lenses under the stage. The intensity of the illumination is controlled either by an iris diaphragm, or by a rotating disk having a series of holes of different sizes.

Microscopes that are used for advanced work are usually equipped with a condenser. A condenser is a system of two or more lenses under the stage, designed to receive a beam of parallel rays from a *flat* mirror or a prism and to converge the light at the level of the stage.

The simplest type of condenser, known as the Abbe condenser, consists of two lenses. Although Abbe condensers are not corrected for color or curvature, they are adequate for classwork and for much of the routine work in research. The N. A. is 1.20 or 1.25. The upper lens may be unscrewed (not in an elementary laboratory!); the lower lens then serves as a long focus condenser of N. A. 0.30, suitable for use with objectives of 10× (N.A. 0.25) or less. On some Leitz models the upper element of the condenser is on a swinging yoke, whereby the upper lens can be swung aside, leaving the lower lens as a long focus condenser that fills the field of the lowest powers. A three-lens condenser with N.A. 1.40 is available for use with objectives having an

N.A. greater than 1.25. One or both upper lenses are removable, giving N.A. 0.70 and 0.40 respectively.

Aplanatic and achromatic condensers, made by several manufacturers, have excellent corrections for color and curvature. The elements, usually in 3 units, are separable, affording combinations with N.A. ranging from 0.20 to the full 1.30 or 1.40 of the complete condenser.

The resolving power inherent in an objective can be utilized only if the illuminating system has a numerical aperture equal to that of the objective. A curved mirror has an approximate N.A. of 0.25; therefore, it meets the aperture requirements of a 10× (16 mm.) objective. Microscopes having objectives of over N.A. 0.25 should be equipped with a condenser, provided that the users are sufficiently skilled to use the condenser properly. An improperly adjusted condenser is worse than having no condenser. Some teachers prefer not to have condensers for large elementary classes in which thorough training in microscopy and close supervision are difficult.

The conventional high-power objective on elementary class microscopes is a 4–mm. objective, 43× or 44×, N.A. 0.65 or 0.66. Many thousand instruments of this type are in use, equipped with an Abbe condenser of N.A. 1.20 or 1.25. If this condenser is not focused accurately it is a handicap, furthermore it does not cover the field of objectives below 10×. Removal of the condenser or of its upper element, a common practice among advanced workers when using low powers, is a most undesirable practice in large classes of beginners. The need for a condenser designed specifically for low and intermediate powers has been met by the American Optical Co. (Spencer Lens Co.) and the Bausch & Lomb Optical Co. These condensers have numerical apertures of .66 and .70 respectively, and therefore meet the aperture requirements of 4 mm., 43× or 44× objectives, and also illuminate the field of a 3.2× or higher power objective.

A maximum N.A. of 1.00 can be obtained with a condenser if the condenser lens and the slide are separated by a layer of air. Obviously, an oil-immersion objective of N.A. 1.30 does not yield maximum performance unless the condenser, as well as the objective, is connected to the slide with cedar oil. Research workers who wish to obtain maximum resolving power make a routine practice of immersing the condenser. There are some practical objections to this practice for classwork.

Dark-field illumination is a neglected, but useful method of observation. In this method the light that reaches the eye from the

object does not pass through the object but is reflected from the surface of the object. None of the light from the illuminant reaches the eye directly. The object thus appears to be self-luminous against a black background. Illumination of the object is obtained by either a standard condenser provided with an adapter or by means of a special dark-field condenser.

The simplest form of adapter consists of a wheel-shaped metal disk inserted into the slot below the condenser. The center of the disk cuts off the central rays of light and illuminates the object with the oblique marginal rays. A more effective adapter is a unit that replaces the upper element of the Abbe condenser.

The much more expensive dark-field condensers are of two principal types. Refracting condensers provide an oblique cone of light by refraction through the marginal regions of the condenser lenses. A disk below the central region of the condenser shuts out light from that portion. Reflecting condensers produce an oblique cone by total reflection from internal surfaces of the condenser lenses. Diagrams and descriptions of the various types of condensers can be found in the catalogues.

Dark-field illumination is recommended for the study of filamentous or unicellular algae and fungi, as well as for unstained sections of tissues. The cytoplasmic strands and nuclei of *Spirogyra* and cytoplasmic streaming in leaves of *Elodea* and filaments of *Rhizopus* make striking and instructive demonstrations.

The discussion of sources of light for the microscope has been deferred to this point, where the source can be discussed in conjunction with the condenser and the other optical components. Illumination is said to be *critical* when the source of light is superimposed on the object. This means that if an unfrosted tungsten coil bulb is the source, the coil is sharply defined upon the object. It is true that the portions of the object that coincide with the coil are under critical illumination, but only a very small part of the field may be so illuminated, and it is obvious that a naked coil cannot be used in this manner.

A frosted bulb is some improvement, but the granularity of the bulb is visible under critical conditions, as defined above, and the curvature of the bulb is visible under lower powers. If the condenser is lowered to obscure the granularity and curvature, the resolving power is decreased.

The desirable source is a flat, luminous, grainless surface of sufficient size to cover the field of the lowest power objective. When

such a source is superimposed on the object field, uniform illumination is obtained. For elementary class use, and for many routine tasks in research, the nearest approach to optimum illumination seems to be an opal glass disk in a suitable lamp housing, with a 50 to 60 watt frosted blue mazda bulb. Place the lamp 8–12 inches from the microscope and manipulate the mirror until the field of view, with an object in focus, is uniformly illuminated. If the microscope has a condenser, place the point of a pencil against the lamp disk and adjust the height of the condenser until the pencil point is in focus. If finely ground glass, or a plano-convex lens with a ground flat surface is used instead of opal glass, the condenser must be moved out of optimum position to eliminate the granularity in the field of view. The use of a lamp that has a condensing lens system and a diaphragm is discussed in the chapter on photomicrography, and the worker who wishes to do critical visual work should consult that chapter.

Mechanical Operation

A microscope usually has a set of two to four objectives permanently installed on a revolving nosepeice. The objective are centered and parfocalized, each screwed into its designated opening in the nosepiece. The older nosepieces have adjustable stops for lateral centering of individual objectives. Improvements in manufacturing methods have made possible the quantity production of nosepieces of such precision that no adjustments for centering are required on the nosepiece. The removal of objectives should be strictly forbidden in the classroom.

The body tube of the microscope on which the objectives and ocular are mounted is moved up and down by two mechanisms, a coarse adjustment which produces rapid displacement, and a fine adjustment which moves the body tube very slowly. The coarse adjustment is actuated by a rack and pinion. This device is practically identical in the several leading makes. The tightness of the action can be adjusted easily by tightening or loosening the split bearing block against the pinion shaft by means of the readily accessible screws. In the Zeiss instrument the action is tightened by grasping the pinion heads firmly and screwing them toward each other.

The fine-adjustment mechanism differs radically in the different makes. One type employs a gear-and-sector device in which only a few teeth are in contact. This action, though very smooth and responsive, is rather delicate and easily damaged. The most rugged

type is actuated by a split nut which has numerous threads in permanent contact with a worm gear. The threads are almost impossible to strip, and this action has excellent responsiveness. Details of construction of the various makes may be obtained from the illustrations and description in the catalogues. The repair of fine-adjustment actions should be entrusted only to a highly skilled mechanic or to the manufacturer.

The normal procedure in using the microscope is to locate the object with a low-power objective and then turn to the next higher power. Objectives of 10× or less are the most satisfactory finder lenses because of their large field of view, considerable depth of field, and long working distance. Microscopes for elementary work should be equipped with a safety stop on the body tube which prevents contact between the slide and the low-power lens. With an objective of 10× or less in position, it is safe to rack the body tube down until it is stopped by the safety stop. With the body tube in this position look into the ocular and manipulate the mirror until the field of view is uniformly illuminated. Move the body tube upward with the coarse adjustment until the image is visible, then bring the image into sharp focus with the fine adjustment. Search the section by moving the slide, using the fine adjustment freely to bring into sharp focus structural features at different depths in the specimen.

When it is necessary to turn to a higher magnification, center the desired structure in the field of view and bring it into sharp focus with the lowest power. *Without changing the focus,* turn the objective of *next higher* magnification into position. A properly parfocalized objective has ample clearance. The image should now be visible, and it should require not more than a quarter turn of the fine adjustment to bring the image into sharp focus.

The safety stop provided on the barrel does not prevent pressing the high-power objective upon the slide. Therefore, the high-power objective should never be used for locating the object. If an objective of 3 to 5× is used, do not change from this low magnification to 43×, but go progressively up through the range of magnifications. Similarly, go down the range progressively. The manufacturers can furnish safety stops for installation on the tubes of older microscopes.

Some teachers prefer to have the objectives adjusted so that when the object is located with the low power, and the high-power objective is swung into position, a slight *upward* movement brings the object into sharp focus. The objection to this arrangement is that, if the user inadvertently moves the body tube downward, he is moving it farther

out of focus and may not stop until the slide is smashed. As an alternative arrangement the high-power objective may be parfocalized so that, when it is swung into position, the image is visible and a slight downward movement brings it into sharper focus. An accidental movement in the wrong direction, upward, will then do no harm. Students should be told firmly that there is no excuse for turning a fine-adjustment knob more than a half revolution in either direction. On the best modern microscopes very little pressure is exerted on the slide when the body tube is lowered upon it with the fine adjustment.

The condenser and illuminant are introduced again at this point. Assume that a grainless disk of a lamp serves as the immediate source of light. Adjust the height of the condenser until the surface of the disk is in view simultaneously with the focused specimen. The object is then within a disk of light of uniform luminosity. Obviously, the condenser does not project a point of light, but a disk of light in the plane of the specimen. Although a ground-glass source approaches the requirements for correct illumination, the condenser must be lowered to move the granularity out of focus. An opal glass disk permits a closer approach to correct illumination.

For general class use, the most practical light source is a lamp with a grainless or nearly grainless diffusing disk. It is preferable to have the lamp fastened to the table in constant relation to the position of the microscope. Under such conditions, the condenser, especially the low N.A. condenser described on page 192, can be mounted in simple ring mounts that are not adjustable by the students.

The position of the microscope in use depends to some extent on the height of the available table and chair in relation to the physical

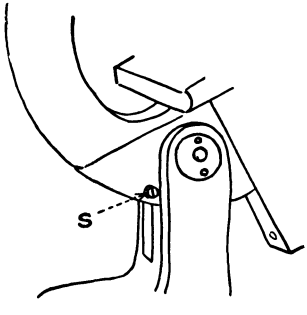

FIG. 16.3—Hinge stop for classroom microscope.

build of the user. Hard and fast rules of posture are ridiculous in a classroom having tables and chairs of fixed, uniform height, and students of diverse build. A very short person should certainly tilt the

microscope for most work. However, if a fluid mount is used on a tilted stage, disturbing currents are likely to be set up in the liquid, and the liquid might drain into the diaphragm; therefore, it is advisable to use wet preparations on a horizontal stage. To forestall the progressive trend of weary students toward a reclining position, a hinge stop can be installed on modern microscopes, preventing tilting beyond 30° (Fig. 16.3).

Micrometry

The measurement of minute objects by means of the microscope is an interesting and valuable feature of microscopic study. Although the procedure is simple and rapid, the method does not receive adequate attention in teaching. The simplest form of measuring device is an eyepiece micrometer, a disk of glass having an engraved scale, a series of accurately spaced lines. The spaces do not have a standard value, and each disk must be calibrated for each given ocular and set of objectives. Place the disk upon the metal diaphragm in the ocular. If the diaphragm is in the correct position, the lines on the disk will be in sharp focus. Occasionally, these diaphragms become displaced, but they can be pushed back and forth with a softwood stick until the eyepiece micrometer is in focus.

The stage micrometer with which the calibration is made is a slide bearing an engraved scale with known values, usually in tenths and

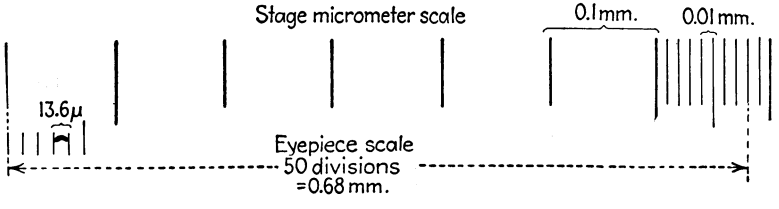

FIG. 16.4—Calibration of an eyepiece micrometer disk and measurement of a minute object.

hundredths of a millimeter, but scales in hundredths of an inch are obtainable. When the stage micrometer is brought into focus, the scale of the eyepiece will be seen superimposed on the scale of the stage micrometer. Shift the stage micrometer and revolve the ocular until the two scales are in such position that the values may be compared. A specific case using a 43× objective and a 10× ocular is shown in Fig. 16.4. It will be seen that the 50 small divisions of the

ocular scale, only five of which are shown in Fig. 16.4, are equivalent to 6.8 large divisions or 68 small divisions of the stage micrometer scale. The computation is:

50 eyepiece divisions = 0.68 mm.
1 eyepiece division = 0.0136 mm. = 13.6 microns (μ)

The curved spore in Fig. 16.4, occupies one space on the ocular scale, and is 13.6 μ long.

The loose eyepiece disks described above are easily lost if they are not kept permanently in the ocular. In a large department it is an economy, over a period of years, to buy special micrometer eyepieces instead of disks. These eyepieces have a built-in disk, and the eye lens is adjustable to focus the scale sharply for the eyes of different individuals. Consult the catalogues for descriptions of micrometric devices.

Microprojection

The discussion of image formation showed that an image is produced if an intercepting screen is placed above the eyepoint of the ocular. With a sufficiently darkened room, a brilliant light source such as an arc lamp, and a good screen, an acceptable image can be obtained with the highest powers of the microscope. However, the most satisfactory results are usually at low and moderate magnifications. An image can be projected on drawing paper and a diagrammatic or detailed drawing made with considerable accuracy. Calibrations must be made for each lens combination and projection distance. This is done by projecting the image of a stage micrometer on the screen, measuring this image with an accurate ruler, and computing the magnification.

The catalogues and service leaflets of the manufacturers furnish detailed descriptions of a wide range of types and price classes of microprojectors.

Types of Microscopes

In the foregoing discussion of the elements of microscopy, the various types and makes of microscopes were not specifically discussed. A *simple* microscope is one that uses only one lens unit to magnify the object. The lens unit may be a single lens. A pair of lenses in fixed relation to each other comprise a *doublet*; a *triplet* consists of three lenses in a mounting. The most useful magnifications range from 6

to 12×. Magnifications up to 20× are available, but, as the magnification increases, the size of the field and the working distance decrease.

A *compound* microscope is one in which a lens unit, the objective, produces a magnified image, which is in turn magnified by a second lens unit, the ocular. By far the most common type of compound microscope employs one objective and one ocular in working position at one time. This is known as a *monocular monobjective* microscope. This type is durable, has a wide range of usefulness, and permits full use of the performance capacities of the optical system. The principal objection is that the user employs one eye at a time, and the tendency to use one eye more than the other causes excessive eyestrain and fatigue.

A *binocular monobjective* microscope uses a matched pair of oculars with a single objective. A system of prisms in the binocular body tube splits the beam coming from the objective and produces two images of identical magnification and intensity. The use of both eyes diminishes eyestrain and fatigue, and there is an impression of depth and perspective to the visual image. Ocular tubes of the binocular body are parallel in the majority of the principal makes. The tubes of American Optical Co. binoculars converge, but this firm will furnish parallel tubes. Convergent tubes present the image to the eye as if the image were at ordinary reading distance. When using parallel tubes the eyes are relaxed, as in looking at an object at a considerable distance. Some microscopists are convinced that they can use only one or the other of these two types of binocular with comfort, whereas other workers can use either type effectively. The binocular body has adjustments for separating the ocular tubes for the interpupillary distance of the observer. One ocular tube has a vertical adjustment for correcting slight differences of focus of the two eyes. To make this adjustment, select a minute structure in the specimen, close the eye over the adjustable tube and focus on the object with the fixed tube. Now close the eye over the fixed tube and bring the image into sharp focus in the adjustable tube with the focusing device on this tube.

The quality of the image obtained with binocular bodies is equal to that obtained with the single tube. Supplementary binocular bodies that are designed to be placed upon older monocular microscopes, have the tube length increased by the superimposed binocular body. A reducing lens system must therefore be used to bring the magnification back to the standard designated value. The most modern, and

in many ways most desirable, binocular body has the eyepiece tubes inclined. This permits the head to be held in a comfortable position and greatly reduces fatigue.

An important category of binocular microscopes utilizes matched pairs of objectives. This type is customarily known as the dissecting binocular or *stereoscopic binocular*. These instruments show true perspective and depth. The image is erect, thus facilitating dissection, isolation, and other manipulations of the object. The practical range of total magnifications is from 10 to 150×. Two or more pairs of parfocal objectives can be installed on a nosepiece of either the revolving or sliding shuttle type. In one A. O. C. model a set of objectives may be permanently installed on the objective changer, a desirable arrangement for class use. For research work, each pair of objectives may be obtained in a removable mounting, readily interchangeable on an objective changer, which, in the several makes is either a rotating drum, a rotating disk, or a sliding shuttle.

Several categories of noncompensating oculars are available for twin-objective binoculars. The standard Huygenian type is the least expensive and probably the most satisfactory for classwork. Wide-field oculars are well worth the greater cost. Two manufacturers produce a good junior-wide-field ocular, intermediate in cost and performance between Huygenian and wide-field oculars. High eye-point oculars also are available, but they require that the eyes must be held at restricted eye position, making these oculars objectionable to some workers.

This chapter would be incomplete without a few words concerning the durability and life span of the microscope. It must be obvious that the period of service obtainable from a well-constructed microscope depends upon the skill and care with which it is used, the amount of use, and certain environmental conditions, such as atmospheric conditions, extremes of temperature, and corrosive chemical fumes. An outstanding illustration of durability is afforded by an occasional microscope that seems to be in excellent mechanical and optical condition after 30 years of continuous research service. On the other hand, a classroom instrument may be in poor condition after 10 years of use. Serious scratching and corrosion become evident first on the 4-mm. dry objective, the oil-immersion objective, and on oculars, especially the type having a raised eye lens. The lower power objectives should show no contact wear or corrosion, especially if the instrument has a safety stop on the body tube. Examination of large numbers of class microscopes has shown that the serviceable

period of a microscope is approximately 20 years. Replacement of the ocular and high-power objectives after 15 years is a good investment which may extend the life of the microscope for another 15 years. Periodic mechanical overhauling and refinishing of metal parts should be done by a competent fine-instrument mechanic. Major repairs and lens work should be entrusted only to the manufacturer. Considering the first investment, the low cost of upkeep, the large trade-in allowances, and the many generations of students served during a normal life span of a microscope, this instrument is the least expensive item of laboratory equipment.

The foregoing brief discussion of the principal types of microscopes and of the essential optical and mechanical features can be supplemented by a study of the well-illustrated descriptive catalogues of the leading manufacturers. Details of construction of specific models are available in leaflets provided by the manufacturers.

The belief in the superiority of the continental European optics may have been well founded 50 years ago, but is no longer a prime factor in purchasing an instrument. A choice among the better-known makes is now largely a matter of personal preference. The prospective purchaser should examine and, if possible, use various models and base his preference on mechanical and optical features and specifications that meet his needs.