17. Photomicrography

The use of photomicrographs for illustrations in teaching and research has become a firmly established practice. A choice between drawings and photomicrographs should be based on an understanding of the limitations and possibilities of these methods and upon the method of reproduction to be used. A drawing may be said to expound and explain the subject, while a good photograph is an accurate, impersonal reproduction of the subject. A drawing may be a routine diagrammatic record of rather gross structures, or it may represent the *interpretations* of the microscopist, either in full detail or in idealized, semidiagrammatic form. The routine type can be made by an artist; the interpretation drawing can be made only by the investigator sitting at his microscope. Photographs have similar characteristics and range from mere routine recording to the most critical probing of structural details.

Instead of arguing the relative merits of drawings and photographs, the experienced and versatile worker simply decides which method will best serve a specific need and uses such talent as he has or can hire. A few simple examples will illustrate the criteria by which a choice can be made between methods of scientific illustration. A cross section of a corn stem, or the corn kernel in the frontispiece contains several thousand cells. To make a drawing which would purport to be an accurate cell-for-cell representation would be an almost incredibly laborious task (for someone else to do). A photomicrograph of such subjects reproduces with acceptable accuracy the number, distribution, shapes, and sizes of the numerous cells and, furthermore, reproduces texture in a way that can only be remotely approached by the most talented artist. Photomicrographs of this type can be made only by a photographer who is familiar with plant materials.

Controversial subjects or new and striking discoveries deserve photographic illustrations. The reader has greater confidence in a

description if it is evident that the investigator had presentable preparations. In illustrating some materials the very act of making an ink drawing on paper exaggerates magnitude, visibility of details, and texture. For instance, protoplasm does not consist of discrete dots and sharp lines. A photomicrograph accompanied by an interpretation drawing affords much more convincing illustrations of many subjects than does either method alone.

The making of record photomicrographs is often an essential part of diagnostic routine in clinical, chemical, criminological, and many other studies. Under standardized conditions, especially if there is some uniformity in the character of the subjects, such photomicrographs can be made by a well-trained technician.

In some fields of research the investigator is the only one who can locate and recognize the structures being studied. He must determine the proper focal level, the correct magnification, color filters, and other factors. It may be necessary to make several negatives at different foci in the same field of view. The investigator must personally decide from a contact print whether the photograph shows the desired structures. Research photomicrography of this type is clearly an inseparable part of the research and must be done by the investigator in person, with his research microscope and frequently without disturbing the slide that has been under scrutiny.

It is a common fallacy that a photomicrographer must be primarily a photographer, who can easily and quickly "pick up" what he needs to know about the microscope. On the contrary, he must be a skilled and critical microscopist, furthermore he must be familiar with the structure of the material that is to be photographed. He can learn the processing of negatives much quicker than he can gain a mastery of microscopy. Given a good negative and some supervision by the scientist, the commercial photographer can make excellent contact prints and enlargements.

This chapter was written for the research worker or teacher who has modest facilities for making photomicrographs and wishes to utilize them to the best advantage. It will be assumed that the advanced worker who has more elaborate facilities has studied both photography and microscopy beyond the elementary scope of this manual.

Attachment Cameras

Photomicrography can be done with a standard microscope and a camera attachment that rides on the ocular tube without other support. The largest camera of this type uses 9- by 12-cm. or 3¼- by 4½-inch plates or films. The projection distance is constant, and magnification is equal to the product of the magnifications of the objective and ocular. Other models use smaller plates or sheet film, or 35-mm. or Bantam roll film. Examples of such attachments are the Erb and Gray Visicam, Leitz Makam and Micro-Ibso, Zeiss-Winkel, and the Bausch & Lomb Model N (Fig. 17.1). Precise focusing is possible with the lateral observation ocular or screen. If the collar that fastens the camera assembly to the ocular tube of the microscope does not hold the assembly rigidly, press or solder a brass sleeve to the outside of the ocular tube, and turn the sleeve on a lathe until it makes a tight, turning fit inside the clamping collar of the camera.

The Leitz, Zeiss, and similar attachments are advertised to be used with expensive miniature cameras like the Leica or Contax on the grounds that the camera can be used for "other purposes." This can be a handicap, rather than a virtue, especially if the camera must be shared by several workers. When one person wishes to use the camera for photomicrography, it is sure to be out on a field trip or a vacation trip, or it is loaded with the wrong film. The same criticism applies to the Exakta and similar reflex cameras. A stripped camera body[1] that is merely a holder for a spool of film can be purchased from Brinkmann,[2] Kessel,[3] and other dealers for use with the Leitz Micro-Ibso or the Zeiss-Winkel attachments (Fig. 17.1). By having several of these inexpensive film holders, several emulsions can be kept available.

An obsolete model, Exakta or similar reflex camera, stripped of lenses and other superfluous parts, can be bought for a small sum. Fastened permanently to a swinging bracket like the one in Fig. 17.3, the camera is always at hand. If the reflex device registers accurately with the emulsion plane, and if the shutter operates without jarring, excellent photomicrographs can be made at all magnifications.

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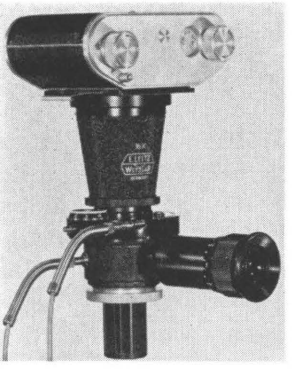
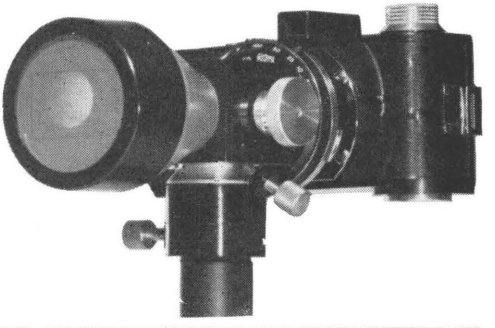
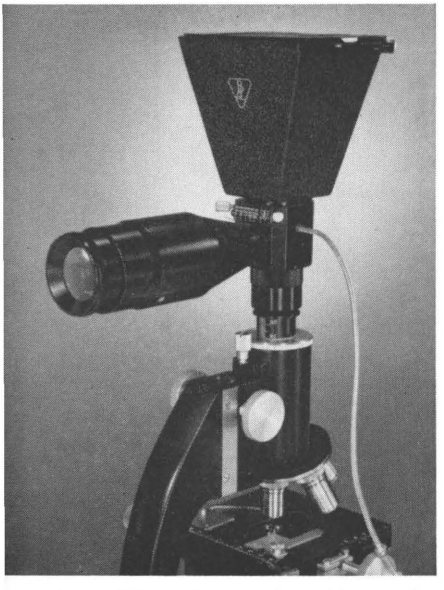
[1] Based on the Argus, Eastman Pony, Bower, and similar cameras.
[2] Brinkmann Instrument Co., 115 Cutter Mill Rd., Great Neck, L. I., N. Y.
[3] W. H. Kessel & Co., 510 N. Dearborn St., Chicago, Ill.







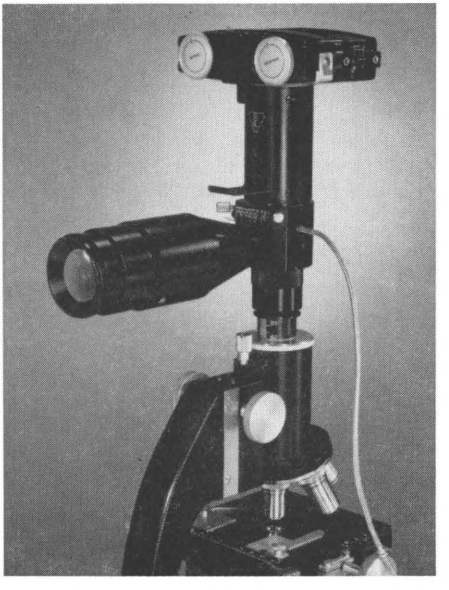


FIG. 17.1 — Cameras that ride on the ocular tube: upper left, Leitz Makam 9- by 12-cm. fixed-length camera; upper right, Leitz 35-mm. Micro-Ibso (=Mikas), with simple film holder; right center, Viscam 35-mm. camera with Argus body; lower left, Bausch & Lomb 2¼- by 3¼-inch camera; lower right, B & L with 35-mm. film holder, interchangeable with 2¼- by 3¼-inch box.

Pillar-Type Cameras

This type of apparatus carries the camera on a vertical post, which is attached to a heavy metal base. A simple version has a bellows camera, which my be used either with a compound microscope (Fig. 17.2), or with Micro Tessar lenses that are carried in a focusing mount on the shutter. Excellent work can be done with such an apparatus if the components, including the separate light source are correctly and rigidly aligned. The microscope cannot be used conveniently for visual study because the camera front must be raised and the pillar swung away from the ocular. In order to do both visual and photographic work, much time is spent in dismantling and reassembling the apparatus.

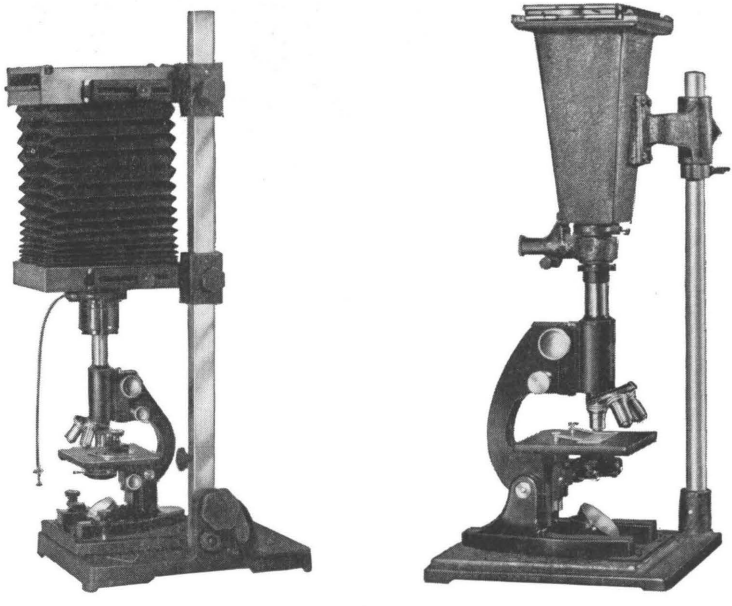


FIG. 17.2—Pillar-supported cameras: *left,* Bausch & Lomb bellows camera on hinged pillar; *right,* Bausch & Lomb fixed-length camera with observation eyepiece. Camera swings on post.

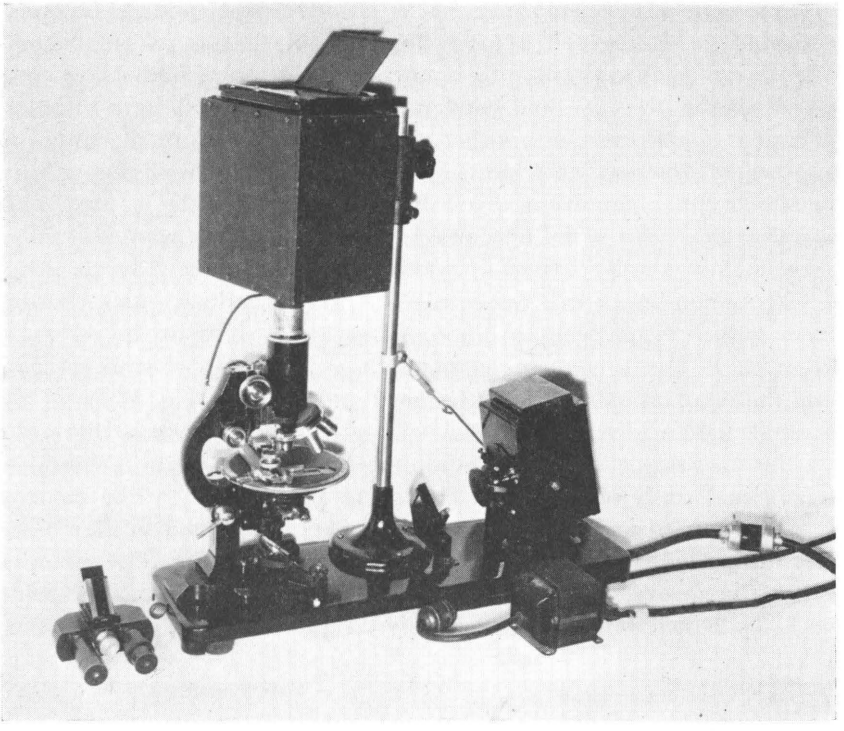


FIG. 17.3—Combination visual and photomicrographic apparatus, permanently assembled on commercial (B & L) metal base. The home-made plywood camera swings on a post and can be removed.

The swing-out type of camera permits free use of the microscope for visual work, and the camera can be swung into position accurately. The lateral observation tube permits precise focusing (Fig. 17.2). A compact and rigid unit can be made by bolting a length of channel iron to the metal base of either style shown in Fig. 17.2, and fastening the lamp permanently to the channel iron.

Combined Visual-Photographic Apparatus

Experienced research workers know that the taking of a photomicrograph is inseparably associated with critical visual study. For example, let us assume that a wet acetocarmine preparation has been

studied with a fine binocular research microscope, and it becomes desirable to photograph a loose floating cell that is in satisfactory orientation. It is impossible to remove the slide from immersion contact with the objective and condenser, transport the slide to another part of the laboratory or another part of the city, set up the slide on another microscope, and locate the specific cell and photograph it in the original condition. Even if a permanent slide is used, the investigator — who is the only one who knows what is wanted — must personally locate the desired field and focus at the desired level.

These conditions call for equipment that permits a quick change from critical visual work to photomicrography, right in the research laboratory. Use a commercial metal base on which the microscope and the lamp are permanently fastened and aligned (Fig. 17.3). This arrangement, which can be used with attachment cameras that ride on the microscope, as well as with a pillar camera, permits comfortable visual study and a quick change to photography. The camera can be removed and used by another worker who has a similar base, microscope, and lamp. The versatile apparatus in Fig. 17.4 permits quick change-over from visual study to a 35-mm., Bantam, Polaroid, or 4- by 5-inch camera. The massive, rigid Bausch & Lomb reflex

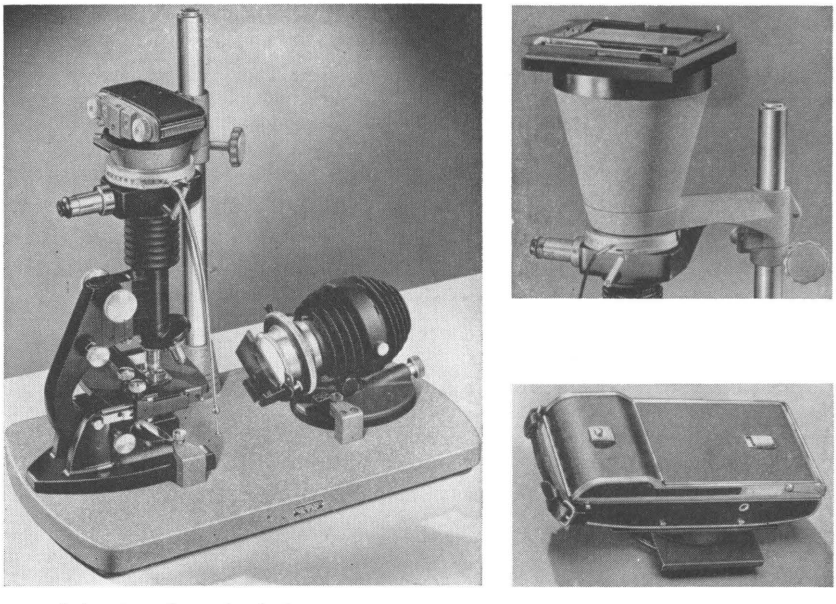


FIG. 17.4 — American Optical Co. apparatus, convertible from 35 mm. or Bantam (left), to 4 by 5 inch (upper right), to Polaroid (lower right).

model L has the above desirable features, as well as convertibility to Micro Tessar and gross photography (Fig. 17.5). The microscope and lamp are on a base that is slid as a unit from under the camera for visual study. This apparatus could be improved by providing a separate base to which the removable microscope-lamp unit could be transferred so that someone else could use the pillar-base, pillar, and camera.

The remarkable Polaroid Land camera will greatly influence and perhaps revolutionize photomicrography. Used on the Central Scientific Co. assembly (Fig. 17.6) or the versatile American Optical Co. apparatus (Fig. 17.4), this camera produces black-and-white transparencies in minutes. From the transparencies, positive prints can be made for dissertations or publication. It remains to be seen whether such prints can compete with contact prints made from large, fine-grain negatives for producing half-tone cuts.

Optical-Bench Cameras

The optical-bench photomicrographic apparatus has long been considered the ultimate in precision and rigidity. The three principal components, the camera, the microscope, and the arc lamp, are

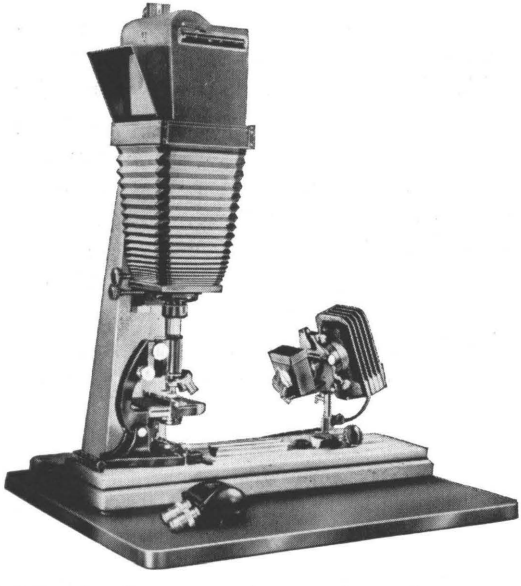


FIG. 17.5—Bausch & Lomb reflex camera that permits maximum versatility for visual study and photography, with Micro Tessar lenses as well as with all powers of the compound microscope.

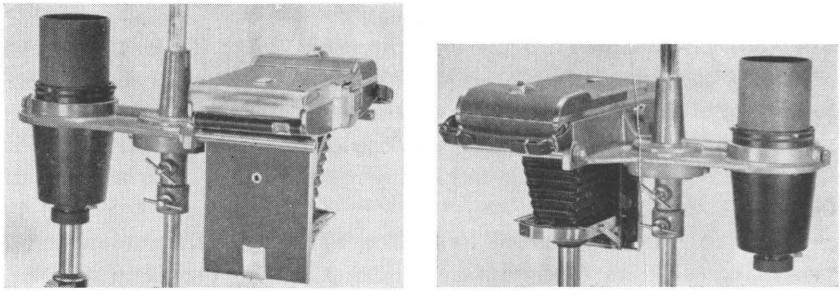


FIG. 17.6 — Central Scientific Co. apparatus, with focusing device over ocular (left) and Polaroid camera swung into position over ocular (right).

mounted on a heavy metal track on which the units may be slid back and forth in accurate alignment. If the microscope is removed for visual study, the replacement and re-alignment are very time-consuming. There is a temptation to keep an expensive microscope, possibly a binocular, permanently on the apparatus where it is not available for visual use, and may be used for photomicrography only a fraction of the time (Fig. 17.7). For low magnifications, Micro Tessars are used in conjunction with special substage condensers. This requires removal of the microscope assembly and the installation and alignment of an entirely different optical system.

The sequence of operations for setting up and using these elaborate outfits is identical in principle with the procedures outlined for simpler apparatus. A lateral observation and focusing tube is available in some makes, or a ground-glass screen may be used for focusing. A limitation of present models is that they use large and expensive plates, 5- by 7-inch or 8- by 10-inch sizes. Reducing kits make possible the use of 3¼- by 4¼- inch and 4- by 5-inch plates. Continued improvement of fine-grain film of high resolving power will undoubtedly lead to the use of much smaller negatives, especially for expensive color work. The rapid trend to smaller cameras that can be used at the research bench in conjunction with visual study will probably reduce optical bench cameras to a minor role in photomicrography.

There is a continuing trend toward "trinocular" microscope heads, an inclined binocular to which a third, vertical tube has been added. The latter can carry a permanently attached 35-mm. camera, or a post-supported large camera can be positioned over the third tube.

In the ideal photomicrographic camera, nothing intervenes between the ocular and the emulsion during exposure.

Light Sources

The character of the light source and the method of illuminating the object are important factors in photomicrography. Artificial light is in almost universal use because of its constant intensity and ease of control. A 6-volt, 108-watt coil filament or ribbon-filament lamp furnishes a steady, fixed source of adequate intensity. A transformer furnishes 6-volt current from the 110-volt alternating-current line. A rheostat may be used to control the intensity if color temperature is not critical. The tungsten-arc and zirconium arc also are excellent illuminants. The carbon arc has a brilliant, homogeneous crater, but the crater shifts as the carbons burn away, and it is difficult to keep the crater exactly in the optical axis.

The lamp must be provided with an adjustable condenser and an iris diaphragm. A one-lens spherical condenser or the slightly more expensive aspheric condenser will give good results, but a better corrected condenser with two or more components is preferred.

Focusing Aids

The focusing panel in commercial apparatus is usually made with sufficient precision to place the ground glass in the same plane as the photographic emulsion. If correct register is not obtained, and

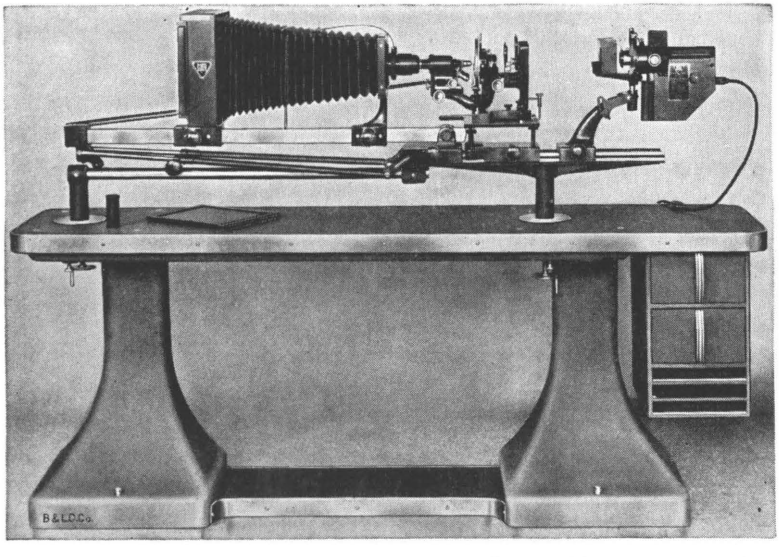


FIG. 17.7 — Bausch & Lomb optical bench photomicrographic apparatus with research microscope in place.

inaccurate positioning of ground glass is suspected, the easiest remedy is the use of a plate holder as a focusing panel. Remove the partition that separates the plates in a double holder. Insert a ground glass into the plate grooves. This places the ground surface in the same plane as the emulsion. Take the photographs with a plate or film holder of the same make as the one used as a focusing panel.

The ground-glass surface provides a satisfactory image for orienting the subject, but not for critical focusing. For maximum sharpness, use the clear window method. Make a fine X mark on the ground glass with India ink, on the diagonals of the glass. Allow the ink to dry, place a drop of balsam or cover glass resin on the mark and lower a cover glass on the resin. This will make a clear window in the ground surface. A focusing glass may be purchased, but an inexpensive one can be made by fitting a 3 to 5× magnifier into a metal tube of such length that when the tube rests on the clear area of the ground glass, the X mark is in focus. Bring the image into approximate focus on the ground surface, view the image through the magnifier and bring into sharp focus.

Exposure Meters

An extensive literature has accumulated on the subject of exposure control in photomicrography. The most accessible sources are the indexes of Stain Technology and the *Journal* of the Biological Photographic Association. Only a brief survey of the principal methods can be given here.

The photoelectric meters used for general photography will register a significant reading with some photomicrographic apparatus. This makes possible the calibration of the apparatus and fairly satisfactory exposure control. Several highly sensitive, but expensive, electronic meters are available. These meters give good readings in the plane of the emulsion, in fact permit probing of small areas of the image. Consult the advertisements of scientific journals for the currently available meters.

If an exposure meter is not available, an experienced photographer who can judge negative densities can obtain good negatives with a little expenditure of film and time. Assume that previous experience with a certain magnification suggests an exposure of 15 seconds. Draw the dark slide halfway out of the film holder and make a 10-second

exposure. Remove the dark slide and expose for another 10 seconds. The two halves of the film have had 10 and 20 seconds respectively. Develop the negative and decide whether the next exposure must be less than 10 seconds, more than 20 seconds, or an intermediate interval.

Negative Materials

Orthochromatic emulsions can be used for photomicrography. These emulsions are sensitive to green, blue, and ultraviolet. A black object or one that is rich in green or blue may be rendered accurately in monochrome with such emulsions. Representative emulsions in this category and Eastman's ortho, process ortho and Verichrome films, D. C. Ortho plates, and Agfa Plenachrome film.

Noncolor sensitive emulsions such as process plates have not been given adequate attention for photographing such objects as black-stained chromosomes.

Historically, the best-known emulsion for photomicrography is the Wratten M plate. This is a panchromatic plate having comparatively coarse grain and slow speed, producing negatives of high contrast. The more recent fine-grain panchromatic emulsions may well bring about a radical revision of photomicrographic techniques. These emulsions are fast, they have a wide range of color sensitivity, and, because of the fine grain of the negative, enlargements of many diameters can be made. This makes possible the use of relatively low microscope magnifications, with greater depth of field and a large, comparatively flat field. A negative will yield a contact print or lantern slide of a large field, and selected portions of the negative may be greatly enlarged to exhibit finer details of structure. Films in this category are Eastman Panatomic X and Agfa Finopan. The speed ratings of emulsions can be obtained from the manufacturers or from the frequently revised tables of makers of exposure meters.

The choice between plates and films depends on the size of film, the microscope magnification being used, the type of negative holder, and the focusing method. Large sheet film has considerable concavity, whereas a glass plate is flat over its entire area. With the lower magnification ranges, up to 100×, the lack of perfect flatness of the emulsion does not seriously influence focusing, but, if much of the area of a large negative is to be utilized with high magnifications, the use of plates may be necessary. Sheet film holders designed to hold

the film along all four sides are superior to separate adapters that fit into plateholders. Some of these adapters do not hold the emulsion of the film in the same plane as when a plate is used in the same holder, therefore the focusing screen or observation tube is not in accurate register with the emulsion, resulting in inaccurate focusing. The foregoing sources of error should be tested for the available apparatus and accessories.

Roll film is useful only if the conditions are so well standardized that the length of exposure can be estimated accurately. The smaller sizes lie sufficiently flat for moderate magnifications. Pack film has some advantages over roll film. Individual films can be removed from the pack for development, making it possible to establish exposure time with one or more trial exposures, developing the films at once. Subsequent exposures under similar conditions can then be made in rapid succession. In the larger film-pack sizes the film has considerable curvature along the edges, but the central portions are adequately flat.

The Setting Up and Operation of the Apparatus

Before outlining the procedure used in taking photomicrographs, some suggestions are offered concerning the choice of objectives and oculars for any given subject. The ultimate aim of the photographer is a finished print on paper, or a lantern-slide (transparency) image on a screen. The image should convey to the observer the intent of the photographer: a low-power survey of a large area, with little emphasis on cell detail; a rendering of texture and tone in black and white, without much cell detail; an accurate reproduction of details within a cell or within a minute object; or the sharp outlining of an object against a contrasting background, without detail within the object. The worker may have other aims and may combine them, with emphasis placed where needed.

When using the standard oculars that are used for visual work, the best results are obtained with oculars of moderate magnification, 8 to 12×. The major manufacturers advertise special photographic oculars that produce a flat field. These oculars cannot be used for visual study.

The objective to use is one that covers the desired area of the object generously, especially when using visual oculars, so that the

important area will be in focus simultaneously and the out-of-focus marginal region can be masked out in the finished product. In addition to adequate coverage, the objective should have adequate resolving power to show the *necessary* detail. Keep in mind that, as the magnification and resolving power increase, depth of field decreases. It may be advantageous to obtain a sharp negative covering the necessary area and depth of the object – but having relatively low magnification – and to enlarge a few diameters in making the positive. However, the positive must show the detail that the photographer intended to show. Some workers prefer to keep the negative image of such size that lantern slides may be made by contact, or that contact prints will be of the correct size for publication in a journal. Wider use of the fine-grain methods of miniature photography will promote the use of excellent objectives of comparatively low magnification, large field, and good resolving power. Examples are the Bausch & Lomb oil immersion, 40×, N.A. 1.00, and several makes of oil-immersion objectives, with magnifications of 60 to 65×, N.A. from 1.30 to 1.40.

The sequence of operations leading up to making the exposure will now be described. It will be assumed that the slide, all lenses, the mirror, and the filters are perfectly clean, and that all units are firmly fastened in place. The procedure varies with the type of illumination being used.

When using an ordinary mazda bulb behind a sheet of ground glass or grainless opal glass the operations are as follows:

1. Locate and focus the object as in visual study.

2. Place a thin wedge of black paper against the diffusion glass of the lamp, and focus the condenser until the paper marker is in focus simultaneously with the specimen. Remove the marker. If ground glass is used, the grain of the glass will be visible, and the condenser must be displaced slightly to eliminate this grain.

3. Remove the ocular and adjust the substage diaphragm until the back of the objective is just filled with light.

4. Replace the ocular, bring the camera into position, and adjust the angle of the mirror until the illumination on the focusing screen is centered.

5. Focus the image sharply on the focusing screen and make the exposure.

The use of the foregoing equipment and procedure may be regarded as amateur photomicrography, which nevertheless affords valuable training and may yield results that meet some needs.

For serious and critical work, the lamp should have a concentrated filament bulb, a condenser system of one or more components, and an iris diaphragm.

Two systems of illumination are possible with suitable lamps. *Critical* illumination is obtained when the condenser system focuses the incandescent light source (filament) upon the plane of the specimen on the stage. This superimposed filament image must be of adequate area to cover the field of the objective and must be of uniform brilliance. Many laboratories do not have a lamp suitable for this system, and it is not used extensively.

The *Köhler* system of illumination is the most practical and widely used method. The image of the filament is focused on the substage condenser diaphragm, and the image of the lamp diaphragm is focused in the plane of the specimen. The operations usually are performed in the following order:

1. Direct the beam of light upon the mirror, with no filters or other screens in the beam. Open the substage diaphragm completely, reduce the lamp diaphragm aperture, and manipulate the mirror until the light reflected back from the lower lens of the substage condenser is projected by the mirror as a spot of light, exactly centered on the lamp diaphragm. This position of the mirror must not be altered. If the filter holder is adjustable, insert any dense filter and adjust the holder until the light that is reflected from the back surface of the filter is centered on the lamp diaphragm.

2. Open the lamp diaphragm, close the substage diaphragm and focus the lamp condenser until the filament image is sharply defined on the substage diaphragm.

3. Bring the object into focus with the objective that is to be used to take the photograph.

4. Open the substage diaphragm completely and partly close the lamp diaphragm. Rack the substage condenser up and down until the lamp diaphragm, with its edges sharply defined, is superimposed on the sharply focused specimen.

5. Replace the ocular with a pinhole ocular, look down into the tube and bring the spot of light in the back lens of the objective

into the exact center by manipulating the centering screws of the substage condenser. (Not by moving the mirror.)

6. Replace the ocular. Open the substage diaphragm fully. If the disk of illumination – which represents the lamp diaphragm – does not cover the desired area of the object, remove the upper element of the substage condenser and repeat operation 4. It may be necessary to remove the upper two elements of a three-element substage condenser to obtain a large enough illuminated field.

7. Place a 3× to 6× magnifier above the ocular, adjust the magnifier until the back lens of the objective is in focus. Close the substage diaphragm and open it slowly until the rim of the diaphragm coincides with the rim of the back lens of the objective. The full numerical aperture of the objective is utilized only under these conditions. In practice, the aperture may be reduced by means of the substage diaphragm, but not more than one-sixth of the diameter of the back lens of the objective.

Up to this point the operations are identical for visual study and photography, and the foregoing operations can be performed with the binocular body. This is the time to try Wratten filters – usually in pairs – to obtain the desired contrast or detail.

8. Connect the camera with the microscope. Arrange the composition of the image on the screen by means of the revolving stage or revolving camera. Focus critically with a magnifier over the clear window of the screen. Close the shutter, insert the film holder, and make the exposure. With cameras that have a side-ocular, composition and focusing can be done after the dark-slide has been withdrawn from the film holder. The prism of the side-ocular can be swung aside and the exposure made.

Low Power Photomicrography With Micro Tessar-Type Objectives

The lowest power objectives, such as the 3.2× and 4×, do not cover a large enough field, and have too much magnification for some subjects. The objectives of stereoscopic binocular microscopes have the desired specifications, but such objectives are not adapted for use on a single-tube microscope of correct tube length. The compound system is therefore not suitable for photomicrographs in the 5× to 30× range (See frontispiece). Such photographs are taken with special objectives that produce a flat, well corrected image and are

never used with an ocular. Objectives of this type are the Micro Tessars of Bausch & Lomb and the Micro-Teleplats of Spencer (American Optical Co.). Leitz and Zeiss also make excellent objectives of this type. Each Micro Tessar must be used with a condenser that has the same focal length as the objective. The manufacturers furnish matching condensers for their objectives. The illuminant must have a flat ribbon filament or a homogeneous arc, and a condenser lens.

The mechanical set-up and the operation of a particular commercial apparatus should be obtained from the directions supplied by the manufacturer. The principles will be outlined on the basis of the apparatus shown in Fig. 17.8. The usual sequence of operations is as follows:

1. Adjust the lamp condenser to give a beam of parallel rays. This can be done with adequate practical accuracy by focusing the filament upon a wall 8–10 ft. away. Lock the lamp condenser.

2. Remove all optical components from the stage and camera and center the filament image on the ground glass of the camera back. The filament will not be in sharp focus, but do not change the setting of the lamp condenser.

3. Insert the Micro Tessar objective and center it into the filament image by shifting the objective (lens board), not the light beam.

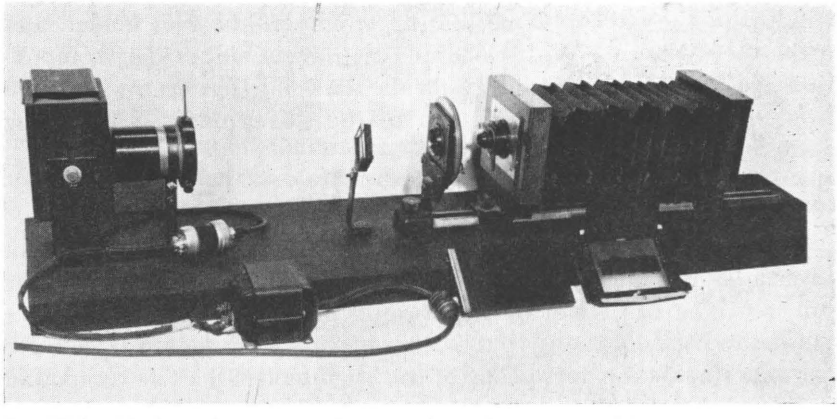


FIG. 17.8 — Horizontal apparatus for use with Micro Tessar objectives. Components, from left to right: ribbon filament lamp; filter holder; revolving stage with condenser holder; bellows camera with removable lens board, which carries the focusing mount and a behind-the-lens shutter. The commercial spring-back focusing screen is removable.

4. Insert the substage condenser that has the same focal length as the objective. Center the condenser into the beam by moving the condenser, not the light beam.

5. Place the slide on the stage and bring the object into sharp focus on the ground glass.

6. Try various Wratten filters and use the combination that gives the desired balance between contrast and detail.

7. Make the exposure.

The above optical system transmits enough light, if the filters are removed, to register adequately on a good exposure meter. Exposure factors can be worked out for a given optical system, filter combination and type of negative material.