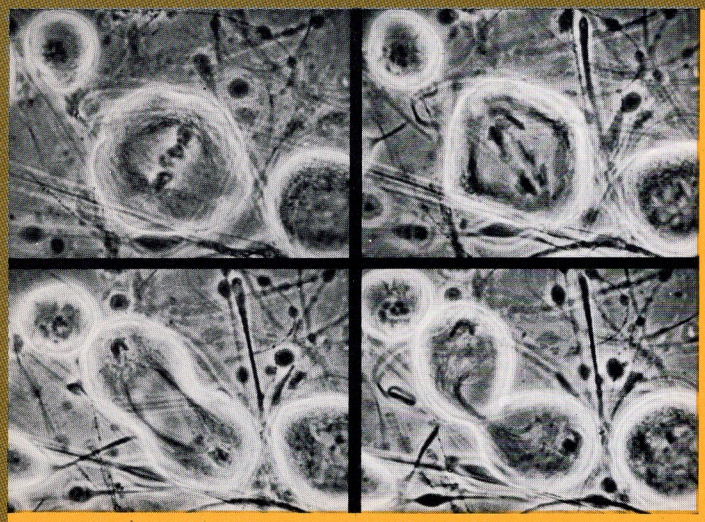
PHOTOGRAPHY THROUGH THE MICROSCOPE

PAILLARD Incorporated, 100 Sixth Avenue, New York 13, New York

BOLEX
TECHNICAL INFORMATION
BULLETIN NO. 35–1/62
PHOTOMICROGRAPHY AND CINEPHOTOMICROGRAPHY
HASSELBLAD



Time lapse sequence of cell dividing.

The microscope has long been one of the most important research instruments in the medical, industrial, and educational fields. As a rule, only one person can view an object at one time through the microscope which is a limitation of visual microscope observation. This can be eliminated by combining the microscope with a still or motion picture camera which photographs or films the specimen under the microscope. The specimen recorded on the film can be viewed as a print, or even more conveniently as a slide or motion picture projected on the screen. The audience which can see and study the microscopic specimen can, therefore, be as large as the auditorium will permit, and the scientist, lecturer, or teacher can describe the object at the same time. Viewing a picture on the screen is certainly more convenient and less tiring on the eyes than visual observation through the microscope itself.

A motion picture camera furthermore presents ways of filming and studying microscopic specimens which are not possible in visual observation. A fast changing specimen can be slowed down with the motion picture camera so that its actions—3 or 4 times slower—can be studied more accurately. On the other hand, specimens which may take hours to change can be filmed so that the change takes place within seconds by a method called time-lapse cinematography.

The equipment for photomicrography consists of the following major components:

1. Light source
2. Microscope
3. Microscope Adapter
4. Camera Stand
5. Camera
6. Time-lapse Unit, if necessary

EQUIPMENT FOR STILL PHOTOGRAPHY

Cameras of almost any film size can be used for this purpose. The Hasselblad is a favorite among the still cameras because the microscopic specimen can be viewed easily and conveniently on its ground glass which, at the same time, permits accurate focusing, so extremely important in microscopic work. It is recommended to equip the Hasselblad Camera with the magnifying hood shielding extraneous light completely and providing a 2½ power magnification of the groundglass image. The square format has proven to be ideal for photomicrography because it is the closest to the circular field obtained in visual viewing through the microscope. The Hasselblad can be equipped with the magazine 12, giving twelve 2¼ x 2¼ images on a roll of 120 film. This large negative can easily be cropped if desired, and still yields excellent enlargements even if only a small part of the negative should be used. One can also use the magazine 16 for sixteen 2¼ x 1⅝ pictures or magazine 16S for 16 Superslides which can be projected on most 35mm slide projectors. The Hasselblad becomes a truly ideal microcamera by equipping it with the adapter for cut film instead of the rollfilm magazine. Many of the emulsions necessary or recommended for photomicrography are available only in sheet film and the Hasselblad cutfilm adapter permits using all of these films. It also permits making test exposures to be developed immediately without wasting an entire roll of film.

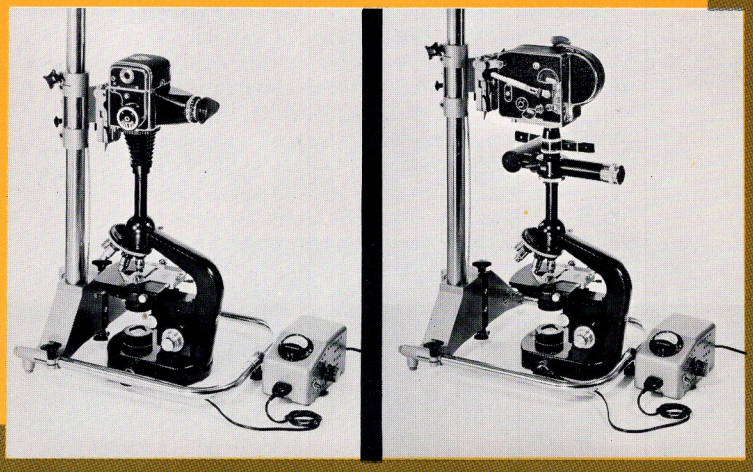
MICROSCOPE ADAPTER FOR THE HASSELBLAD CAMERA

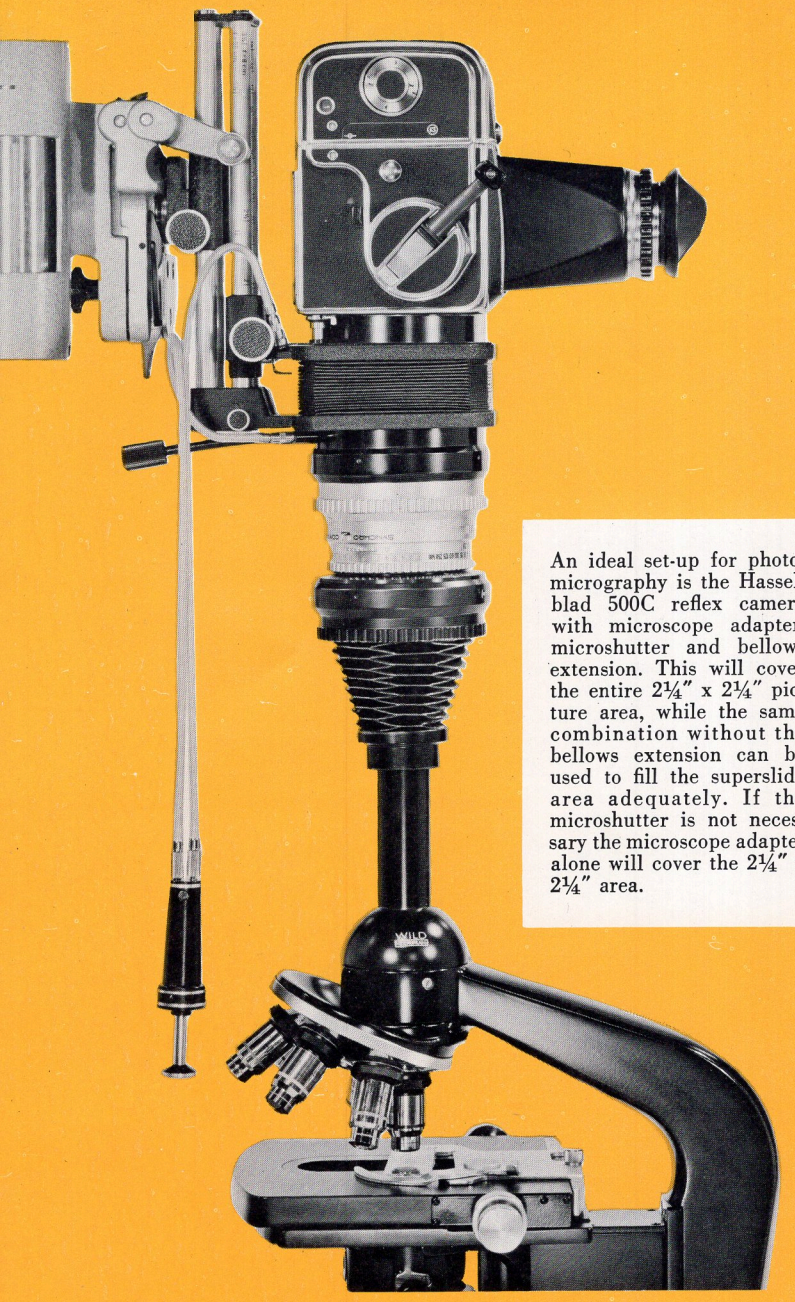
For photomicrography the Hasselblad 500C is mounted on the Bolex Super Universal Titler or a similar camera stand, such as the Multipurpose Camera Stand manufactured by Wild. To yield a photographic picture of the same magnification power as observed through the microscope, the camera is set above the microscope tube so that the distance between the microscope-photo eyepiece and the film plane is about 10″. The photo eyepiece should be of the adjustable type, set to 10″ or 25cm. Accurate focusing and framing is done on the groundglass of the camera. No lens is used in the camera; the photo eyepiece, being inserted into the Hasselblad microscope adapter on the microscope tube, serves as camera lens.

As far as the microscope adapter is concerned, two possibilities exist with the Hasselblad 500C. One can use either the Hasselblad microscope adapter (Item #8209) alone. In this case, the exposure time is controlled by the rear shutter of the camera and is limited to 1/25 sec., or time exposures, or for shorter exposure times the camera can be connected to an electronic flash unit which is automatically synchronized with the rear shutter.

For more versatility in exposure times, the Hasselblad microscope adapter can be combined with the Microshutter Adapter (Item #8213) which includes a synchro compur shutter with speeds from 1 sec. to 1/500 sec. plus B completely M&B synchronized at all shutter speeds with any

Left: Hasselblad 500C with microscope adapter mounted on Bolex Titler over Wild M-20 microscope. Right: A set-up for Cinephotomicrography with Bolex H Camera and Wild Cinetube microscope attachment.





An ideal set-up for photomicrography is the Hasselblad 500C reflex camera with microscope adapter, microshutter and bellows extension. This will cover the entire 2¼" x 2¼" picture area, while the same combination without the bellows extension can be used to fill the superslide area adequately. If the microshutter is not necessary the microscope adapter alone will cover the 2¼" x 2¼" area.

type of flash. When using the micro shutter, the rear shutter may be opened before exposure with the quick release button, and the exposure made with the micro-shutter alone, eliminating all vibrations. The bellows extension may also be introduced between micro shutter and camera giving an uninterrupted transition from the magnification provided by the microscope eyepiece. The bellows extension, like the microscope adapter provides a vibrationless microscope attachment. The Hasselblad double cable release for the bellows extension opens the rear shutter before the micro-shutter makes the exposure.

EQUIPMENT FOR MOTION PICTURE PHOTOGRAPHY

16 mm film is favored generally as it is the film size used for auditorium projection. As far as the taking is concerned, however, 8mm or 16mm can be used and the 8mm Bolex H-8 Rex Camera offers the same features as the H-16 Rex which is the favorite camera for micro-cinematography. Both feature through-the-lens reflex viewing which enables the operator to frame and view the object while filming.

All Bolex Cameras permit single frame exposures and together with the built-in frame counter are therefore ideal for time lapse sequences by means of a cable release or a complete time lapse unit. The use of electronic flash is often desirable in cinephotomicrography and the Bolex H Models are ideal for this important application. These cameras can be set for time single frame exposures and an electronic flash can, therefore, easily be synchronized so that it flashes while the shutter is open without the need for an internal camera synchronizing mechanism. The H-8 and H-16 Rex Models, furthermore, have the extremely valuable variable shutter for shortening the exposure times without increasing the running speed. Short exposure times are especially desirable for time lapse sequences of moving specimens.

The running speeds of the Bolex H Models are from 12 fps to 64 fps and, therefore, accelerated and slow motion studies are possible. 24 fps is the standard speed for films which are used commercially or which are to be equipped with an optical soundtrack. For films which are only used within an organization, either silent or with a magnetic sound track, the slower 16 or 18 fps speed can be used. Both 8mm and 16mm Bolex H Cameras take standard 100-foot rolls of film, the H-8 being the only 8mm camera in the world with this large film capacity. A 100-foot roll of 16mm film has 4000 frames, a 100-foot roll of 8mm has 8000 frames on each side or 16000 after the film is slit.

The Bolex H-16 Rex also has a filter slot for using gelatin filters behind the lens, a convenient and important advantage when one considers that any type of filter for regular, special or scientific purpose is available in gelatin squares.

THE MICROSCOPE ADAPTER FOR THE BOLEX CAMERAS

Although the groundglass of the H-8 and H-16 Rex Camera is ideal for framing and viewing the specimen, it is recommended to mount between camera and microscope a microscope adapter with its own viewing and focusing arrangement. This is because the Reflex finder in the camera does not give sufficient magnification to permit accurate focusing on the delicate details of a microscopic specimen. Even if the groundglass were replaced with a clear glass with cross hairs, focusing would not be entirely satisfactory. Instead, focusing must be done by observing the image through the eyepiece of a microscope adapter. Therefore the Bolex H Cameras without reflex viewing can be used equally well for cinephotomicrography in cases where the variable shutter and filterslot are not required. An ideal microscope adapter for the Bolex Cameras is the Wild #7600 attachment for cinephotomicrography, usually referred to as "Cinetube." It clamps onto the microscope viewing tube and is equipped with a focusing telescope with two semi-transparent prisms, one permitting 50% of the light to reach the camera while the other 50% is deflected into the focusing telescope. This prism provides a bright viewing image and is therefore used for framing and focusing as for the projection tube. The second prism lets 95% of the light reach the camera while only 5% is deflected into the focusing telescope. This prism is used during filming where it provides sufficient light for watching the specimen. The focusing telescope is provided with a reticle of the cross-hair type plus two rectangular fields. The larger shows the area covered when a 16mm camera is equipped with a 50mm, or an 8mm camera with a 25mm focal length lens. The smaller rectangle corresponds to a 75mm lens on a 16mm camera or a 36mm lens on an 8mm camera. The Wild Cinetube, furthermore, is equipped with a photoelectric cell to determine correct exposure. Available as an accessory is a projection tube which attaches to the Cinetube and which permits printing identification marks on the 8 or 16mm film while the picture is filmed. This makes scientific evaluation of the finished footage much easier especially in time lapse sequences. For use with the Wild Cinetube attachment, the Bolex Camera is equipped with a taking lens of 50 or 75mm focal length in the case of 16mm or 25 or 36mm in the case of 8mm. This arrangement has the advantage that it does not require any additional optical devices, photographic eyepieces with adjustable lens or mechanical devices to fix the distance between microscope and camera. The taking lens on the camera is always set at infinity and at its largest "f" opening. The eyepieces to be used are fixed photo eyepieces. The distance between camera lens and cinetube is not critical. The connection between microscope and camera is not rigid to avoid possible vibrations from the camera being transferred to the microscope. The Bolex lenses can be used with their sunshades mounted, and a special light exclusion ring is provided to produce a light trap.

THE CAMERA STAND

For the Bolex H-8 and H-16 Cameras, the Bolex 16mm Super Titler, in the vertical position, can be used as a camera stand. Since the titler however does not have a special heavy base, it is highly recommended to place it so that the supporting rod rests against a wall to limit vibrations. In a permanent setup, the rod could even be attached firmly to the wall. Microscope manufacturers like Wild have available special camera stands for photomicrography of a much heavier construction than the titler and which, can be set up on a table or fixed into a wall. The Bolex 16mm Super Titler, or the special camera stands are also used for photomicrography with the Hasselblad. The Bolex 8mm titler with the extension legs can be used as a stand for the small Bolex 8mm cameras.

THE MICROSCOPE

The microscope and its light source are of major importance in photomicrography, and one cannot expect to obtain good still or motion pictures through a microscope without knowing the fundamentals of microscopy. The Koehler lighting principle provides virtually glare free and even illumination over the entire field and should be used for all photographic purposes.

This lighting principle consists in imaging the filament of the lamp on to the lower focal plane of the condenser.

For maximum illumination, the image should just fill the bottom lens of the condenser. When this is accomplished, the field diaphragm is stopped down, and while viewing through the microscope, the substage condenser is moved until a sharp image of the diaphragm is observed in the object plane (the microscope should be focused on the specimen.) The field diaphragm should also be centered if necessary. Then the field diaphragm is opened just slightly larger than the entire microscope field seen in the eyepiece or on the camera groundglass. This eliminates glare, as only the light beam participating in the image formation is permitted to reach the object plane.

Just as in regular microscopy, various investigation methods, such as transmitted or incident light, bright field or dark field, phase contrast, fluorescence and polarization can be used for photographic purposes. Especially phase contrast has gained importance since it enables investigating living objects with a maximum contrast and good optical resolution without chemical influence (vital coloration). It is the classical method for cytologic research (mainly in cell dividing process).

The microscope must, of course, be equipped with high quality optics. It is recommended that the microscope has an interchangeable condenser system for low and high power magnification. Many different types of objectives and eyepieces are available and each has its specific application where it provides best results. Only the best corrected microscope lenses such as the Wild Fluotar and Plane Fluotar are satisfactory for color photography, where also the microscope condenser must be achromatically corrected. The aperture used for illumination (condenser aperture) must be as close as possible to the aperture used for taking the pictures (numerical aperture of objective) so that the colors retain their natural luminosity.

An adjustable and centerable cross-stage is recommended. For photographing warm blooded tissues, an adjustable heating stage is necessary.

In still photography with the Hasselblad where a camera lens is not used, it is recommended to equip the microscope with an adjustable eyepiece which permits focusing for the eyepiece to film plane distance. In cinephotomicrography with Bolex Cameras, where a camera lens is used, an adjustable eyepiece should not be used but for best results with regular achromatic and Fluotar objectives the microscope should have a photo eyepiece which is corrected specially for photographic purpose rather than visual observation.

Sweetwaterpolyp filmed in dark field with Bolex 8mm Camera.

Technician engaged in Cinephotomicrography with Bolex H Camera mounted on Wild camera stand.

With Plane Fluotar objectives, only a compensating eyepiece is used since these objectives are already corrected for photography.

The magnification in still photography without a camera-lens is:

$$M = Mo \times Me \times \frac{D}{250}$$

M = Total magnification
Mo = Magnification of objective
Me = Magnification of eyepiece
D = Distance eyepiece to film plane in mm

In motion pictures where a camera lens is used, the magnification is:

$$M = Mo \times Me \times \frac{f}{250}$$

f = focal length of camera lens in mm

LIGHT SOURCES

A low voltage lamp with a special filament bulb of 30 watts, used on AC current is a good universal light source for black and white photography. It is not recommended for color due to great changes in the color temperature. The lamp can be overloaded by 50% for short periods. A heat absorbing filter should be inserted between lamp and microscope. Gas discharge lamps used on AC current have a considerably higher luminous density but are not recommended for motion pictures above 8 f.p.s., since the image frequency interferes with the AC frequency causing fluctuation in exposure. Arc lamps, including mercury vapor high pressure burners, are also satisfactory for black and white work. Recent developments created a lamp which is ideal for photographic and cinematographic purposes in color and black and white. It is a lamp featuring a Xenon high pressure burner which has a color temperature similar to daylight and permits variations in intensity without change

of color temperature. This lamp uses DC current only and should be used together with ultraviolet-excluding and heat-absorbing filters. The lamp housing should be equipped with an adjustable field diaphragm and have the possibility of attaching liquid or glass filters.

Electronic flash offers another possibility in still photography as well as time lapse motion picture studies. The color temperature of electronic flash is about 6000°K, very similar to daylight, and daylight type color film is therefore used, generally without a filter. Sometimes an 81A color filter is recommended.

EXPOSURE DETERMINATION

The only accurate exposure determination is by means of a meter. The Wild Cinetube used for motion pictures is equipped with a built-in photocell which is connected to a galvanometer such as the one available from Photovolt Corp. The galvanometer shows the subject brightness in foot candles, and test exposures will determine the correct number of foot candles for each type of film. This record should be kept by the photographer for future reference.

For still photography, a photocell especially designed for miscroscpopic work and availabe from Photovolt Corp. or Wild Heerbrugg Instruments, Inc. can be placed over the microscopic eyepiece. This photocell is again connected to a galvanometer.

An extremely sensitive exposure meter such as the Lunasix can also be used. It is simply held behind the groundglass of the Hasselblad camera and the correct setting for each lightmeter reading determined by test exposures.

In cinematography, the light intensity or exposure can be regulated only by:

a. Closing the variable shutter in the Bolex Camera.
b. Using neutral density filters.
c. Using a pair of polarizing filters placed into the path of light. Turning one polarizer against the other produces the desired variation in intensity, but may also change the color and characteristics of the specimen, especially in color work.
d. Changing the current fed into the light source. With filament lamps this can be done only for black and white film since changing the lamp voltage changes the color temperature of the light.

Controlling exposure in still photography is no problem. In addition to the above possibilities, b, c, and d, the Hasselblad 500C can be combined with the Hasselblad microshutter adapter which permits shutterspeeds from 1 to 1/500 sec. plus B. Without this adapter, the rear shutter of the camera provides a 1/25 sec. exposure. Both shutters are synchronized for flash.

Under no circumstances, either for movies or stills should one attempt to change the opening of the condenser diaphragm which must remain a function of the objective aperture, the object contrast, and the depth of focus.

FILMS

Since detail is a major requirement in microscopic work, it is always recommended to use a film with high resolution. In black and white, these are usually the films of the lowest sensitivity. For example, a film like Plus-X with an ASA Tungsten rating of 40 will record better detail than TRI-X with ASA Tungsten of 160. This rule does not apply entirely for color films where Kodachrome #2 with ASA Tungsten 40 is known for a resolution superior to other emulsions even with lower sensitivities. Only if the microscopic illumination does not permit using these relatively slow films should one change to a faster film.

RECORDS

It is highly recommended that accurate records be made of all photo-micrographic work. They will be invaluable as a future reference. The records should include the following information:

Date, specimen, camera lens (if used), microscope objective, eyepiece, condenser, illumination lamp, filters, exposure reading, film, shutter speed in still photography or running speed of movie camera, variable shutter setting of movie camera.

SLOW MOTION CINEMATOGRAPHY

Bolex Cameras are ideal for this type of work, since they permit running speeds up to 64 fps. The increased running speed causes increased vibrations and special care must be taken that these are not transferred to the microscope. Filming at higher speeds also shortens the exposure, and therefore the illumination must be increased accordingly or the camera must be loaded with a film of higher sensitivity. Only top performance lamps should be considered for this work, for instance xenon high pressure or carbon arc lamps.

TIME LAPSE CINEPHOTOMICROGRAPHY

With Bolex Cameras, time lapse sequence can be produced without any special accessories since the cameras permit single frame exposures. With Bolex H Cameras, either instantaneous exposure or time exposures are possible. Since time lapse sequences, however, generally extend over long periods, a time lapse unit which automatically trips the camera at regular pre-determined intervals, is a highly valuable accessory. Time lapse units of various types especially designed for Bolex H Cameras are available from various companies. The light intensity and the exposure times must remain absolutely constant within ±5% throughout the time lapse sequence. Focusing should be checked rather frequently. The specimen should not be moved between exposures. The number of frames and time intervals must be pre-determined, based on the length of the sequence being filmed and the desired length of the projection. The time interval is determined with the following formula:

$$1 = \frac{Z_1}{Z_2 \times B}$$

1 = Time interval in seconds
Z_1 = Effective duration of process in seconds
Z_2 = Desired projection time in seconds
B = Projection speed
The acceleration is determined from:
Acceleration = 1 x B

Examples:

Zoology: Development of a tick
(episcopic light, dark field)
Duration of process—
10 days (= 864,000 sec.)
Duration of projection—
5 minutes (= 300 sec.)

$$1 = \frac{864{,}000}{300 \times 24} = 120 = 2 \text{ minutes}$$

(one frame every two minutes)
Acceleration = 120 x 24 = 2880 times.

Cytology: Mitosis in fibroblasts
(transmitted light, phase contrast)
Duration of process—5 hours
Duration of projection—3 minutes

$$1 = \frac{18000}{180 \times 24} = \text{approximately 4 sec.}$$

Acceleration: 96 times

Electronic flash can also be used for time lapse motion pictures and provides an ideal light source for many applications. It provides a bright, constant light of short duration, much shorter than the shutter speed of the camera.

Bolex H Cameras are ideal for time lapse sequence with electronic flash and are about the only motion picture cameras which do not require internal synchronization. This is because the Bolex H Cameras permit single frame *time* exposures by setting the single frame lever on the Camera to T (instead of the normal 1 setting). This makes it possible to use the "open shutter" method, where the light is flashed while the camera shutter is open. Tripping the electronic flash is by means of a solenoid similar to the one used for tripping the camera.

SOUND

8 or 16mm motion pictures can be equipped with a soundtrack narrating the microscopic sequences. This is done after the film is developed and edited, by means of a magnetic sound projector such as the Bolex S-221 for 16mm or the Bolex Sonorizer for 8mm. The edited film is equipped with a magnetic stripe along one edge on which the narration is recorded with the projector or Sonorizer in the same way as recording on tape with a tape recorder. In 16mm, it is recommended to use single perforated film for this purpose, since this will permit applying the wider 100 mil track for better sound quality.