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ELECTRO-OPTICAL PHOTOGRAPHY AT LOW ILLUMINATION LEVELS
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Jaromir Kosar
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PHOTOMICROGRAPHY, Vols. I and II, *Roger P. Loveland*
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PROPERTIES OF THE PHOTOGRAPHIC IMAGE, *Joseph H. Altman*
PHOTOGRAPHY IN THE VISIBLE AND NEAR VISIBLE SPECTRUM, *Allan G. Millikan*

Photomicrography

A COMPREHENSIVE TREATISE

VOLUME I

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Since the cord supplied with 6-V transformers is definitely inadequate, there are two remedies that can be applied.

1. Projector cord may be used. This may be asbestos-covered, size No. 6. To complete the reliability of this system the ends should be soldered to the lamp-socket terminals and heavy lugs provided for any variable resistance or ammeter that is included in the circuit. The variable resistance is to lower the current through the lamp for periods of stand-by and visual use only.

2. The voltage at the power supply may be raised with a variable resistance or transformer plus ammeter to ensure that adequate but not excessive current is carried which would burn out the lamp too quickly. This method really "blasts through" wiring and connection resistance.

Since the 108-W, 115/6-V transformers are relatively inexpensive, two of them may be used with the primary leads plugged into the house circuit in parallel and the two secondaries wired in series.

A variable autotransformer, such as the Variac or Powerstat autotransformer, may be placed in the primary lead to the 6-V transformer. This may require the variable transformer to be set to deliver somewhat more than 120 V from the house supply.

The following arrangement is often used to control a lamp for photometry or quantitative photography (sensitometry). This excellent arrangement allows delicate and stable control but does require two of the more expensive variable autotransformers, one of which acts as a vernier for the other to give smooth voltage adjustment. This second variable transformer uses the whole rotation angle of the Variac or the Powerstat, etc., to add or subtract the voltage increment represented by the secondary voltage of the filament transformer. The latter can be chosen to be adequate for the current carried by the lamp circuit (see Figure 7-9 in which no attempt has been made to suggest the actual circuit of the variable autotransformer). The circuit can be varied somewhat according to the characteristics of the lamp. As shown in Figure 7-9 the lamp should be one operating at or near 100–120 V, since otherwise it could easily be burned out by pushing up the Variac too far. A step-down power transformer can usually be added before the lamp when the latter is a low voltage type, but note the comment at the beginning of this section.

Life of Incandescent Lamps with Current and Voltage Variations

The photographic efficiency of an incandescent lamp goes up very fast with an increase in the current through it or voltage across it. Unfortunately the life of the lamp is shortened at an even more rapid rate

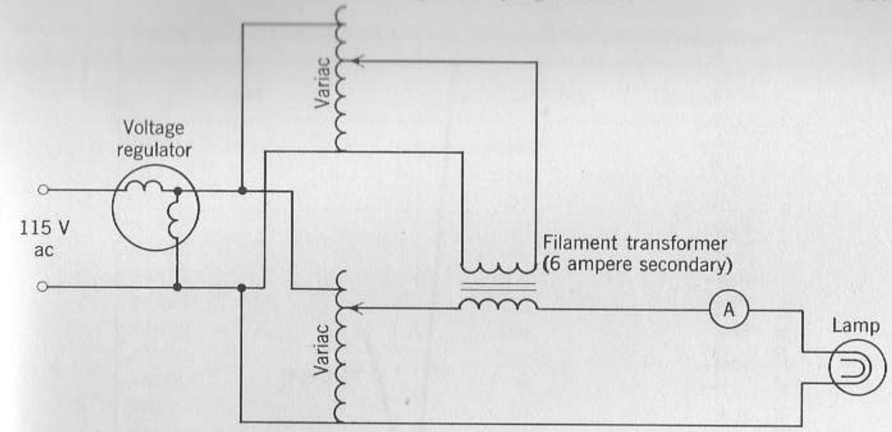


Figure 7-9 Circuit for vernier control of voltage.

with increase of current and voltage. This is illustrated by Figure. 7-10, which shows the effect of voltage variation in terms of percent variation. This can be applied to most incandescent tungsten lamps if their rated voltage is known. The rated life of a household lamp is usually 1000 hr, that of projection lamps, much used in photomicrography, 25-50 hr, whereas miniature lamps are most dependent on the particular lamp. Some rated life values are quoted in Table 7-3. A few are also given in Table 7-2. It must be remembered that this is a statistical concept, not followed exactly by any one lamp but closely enough for the relation to be useful.

Usually a lamp of known quality for color photography is precious for the inherent calibration involved in it. There is normally little reason for the lamp to be kept at its photographically optimum current value except during photography; a lamp thus used will last a long time. Figure 9-9 shows the life of the lamp as affected by current and voltage variation and as related to color quality (color temperature).

Means of modulating the current through the lamp have just been discussed. It should be so convenient that the lamp will promptly be turned down – except during photography.

A somewhat more extensive review of light sources, especially of incandescent lamps, is given by F. E. Carlson and C. N. Clark[2. Kingslake (Ed.), 1965, Vol. 1, Chapter 2].

Heat Filters

In photomicrography by transillumination a large diverging cone of radiation emerging from the light source is redirected by the optical

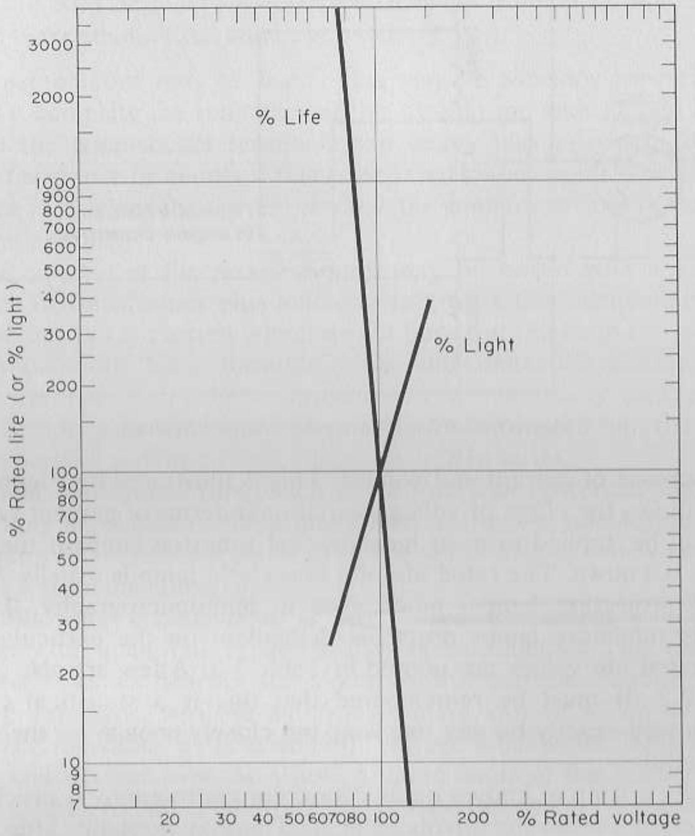


Figure 7-10 Life of incandescent lamps as affected by voltage.

system to a convergent beam, transmitted by the optical system itself and the specimen. Moreover, the intensity level is normally brought up so that photographic exposures in the final image plane are conveniently short. The concentration at the plane of the specimen, therefore, is tremendous, that is, the square of the image magnification more than in the image plane; for instance, at 1000X magnification the radiation is one million times more intense in the object plane, assuming no losses. It is still very strong going through the substage condenser and the objective. The objective, at least, is a valuable lens with cemented components. In the older ones the cement can be presumed to be balsam; now other resins are also used.

The fact that the heat component of the radiation from an incandescent tungsten lamp is many times that of the visible component is obvious

Table 7-3 The Rated Life of Some Incandescent Lamps

Lamp	Rated Life (hr)	Rated Volts
100G16½/29SC	200	115
CDS	50	115
CMV	25	115
#1634	200	20
#1630	100	6.5
#1497	100	6.5
#1493	100	6.5
#1460	100	6.5
#1130	200	6
#870	50	8
#419	50	8
#88	300	6
#55	500	6
CPR	50	6
#14	15	2.47
Horizontal ribbon filament-108 W	150	6

from such graphs as that of Figure 7-11. Only that portion of the spectrum below the limit of about $\lambda 700$ mμ (or 0.7 μ) is used by the eye or most photographic films; the rest, namely, the infrared, is most effective, however, in carrying heat and raising the temperature of receiving material. The quantity of the energy from the source is given by the area under the curves. Water is the most effective single material for filtering out the heat radiation without affecting the visible and photographic spectrum. This is shown by its spectral transmission curves in Figure 7-12. It will be noticed, however, that reasonably thick layers of water do not absorb much of the heating infrared below about 1.15 μ, leaving an important window above the end of the visible spectrum and about $\lambda 1.2\ \mu$. This gap can be plugged by using a water solution of a copper salt (such as copper sulfate), more effectively with less absorption in the visible spectrum than with any other material. The concentration of the copper sulfate will depend on how much far red with consequent cyan coloration one can accept. For black-and-white photography or for color photography, in which a cyan color filter is needed anyway, a minimum of a 2.5% solution of crystal copper sulfate* should be used. Copper sulfate is an ideal cyan and in very strong solution can be made a complete minus red.

*For chemists this is 0.1 molar $CuSO_4$ or 2.5% $CuSO_4 \cdot 5H_2O$

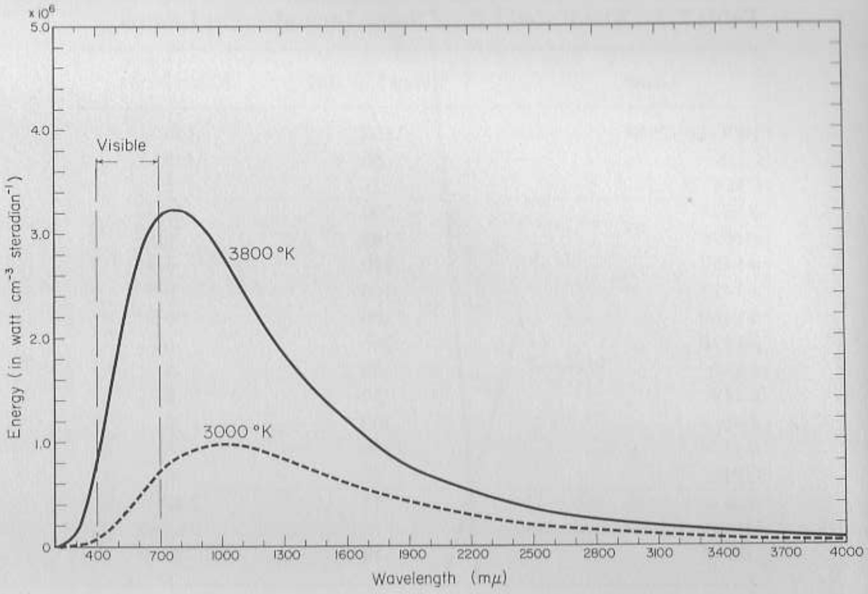


Figure 7-11 Spectral distribution of radiation from tungsten lamps.

Although the absorption-concentration relation is expressed in percent-salt solution in a 1-cm layer, a thicker cell such as the common 3-cm cell or any other thickness can be used to obtain the same absorption by dividing the concentration per centimeter by the layer thickness that is to be used. It can be put into the common water cell with a gain in the absorption by the water. A 3-cm ($1\frac{1}{8}$-in.) layer of water effectively absorbs all significant infrared from lamps beyond 1.5 μ (Figure 7-12).

Although there is no ideal heat absorber with a sharp cut at λ 700 μ that absorbs all longer wavelengths, and a solution of a copper salt is quite good, there are times when a solid filter is required. A glass filter is available and is now used in many slide and movie projectors. One of these is the 2043 glass filter made by the Pittsburg Plate Glass Company. Like a copper sulfate solution, its absorption is proportional to its thickness, which must be specified. Its absorption is shown in Figure 7-13. The Corning Glass Filter 1-69 is quite similar.

Because a heat absorber, such as the glass, comes to equilibrium and reradiates heat as fast as it absorbs it (at a longer wavelength at ordinary temperatures), it should not be placed against the substage condenser. **Its absorption ceases beyond λ 900–1000 mμ at which that of water is starting, so that the latter is still really needed** for many light sources. It is good practice to put the heat-absorbing glass directly into the cooling water.

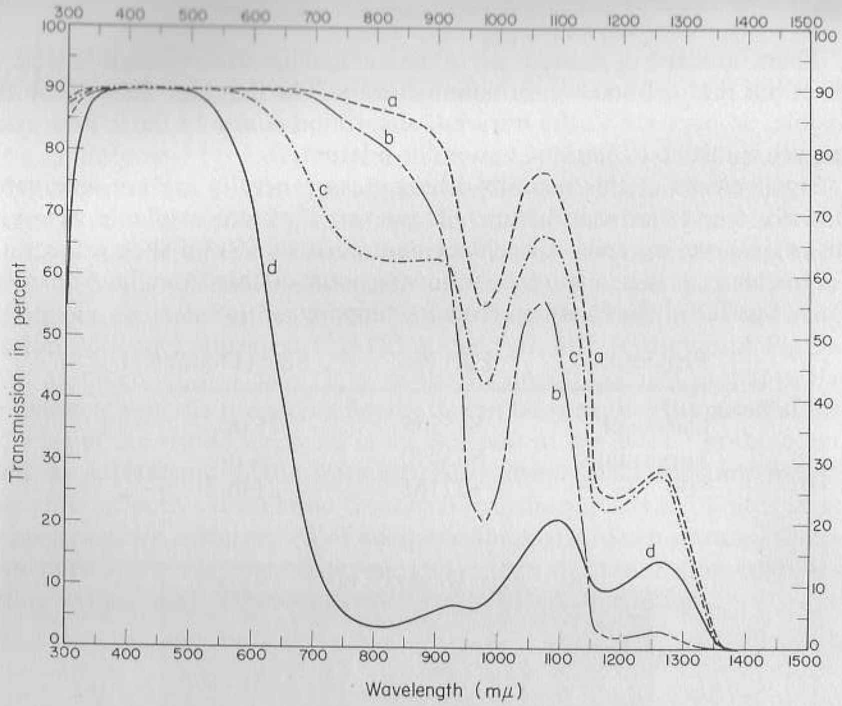


Figure 7-12 Spectral absorption of liquid heat filters:
(*a*) water, 1 cm; (*b*) water, 3 cm; (*c*) $CuSO_4 \cdot 5H_2O$–0·25% water solution, 1 cm; (*d*) $CuSO_4 \cdot 5H_2O$–2·5% water solution, 1 cm.

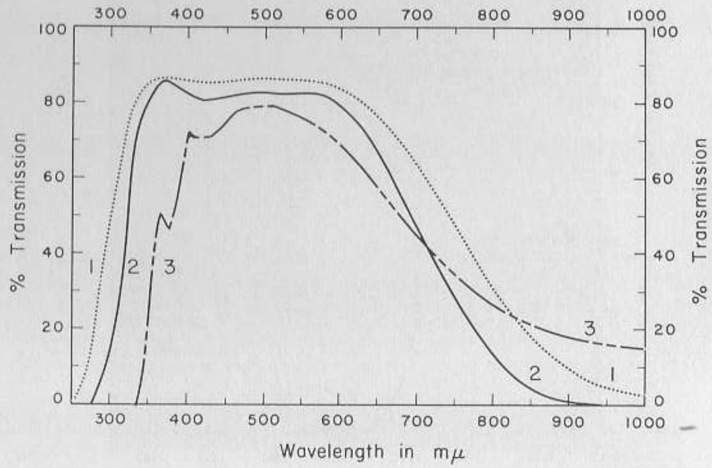


Figure 7-13 Spectral absorption of Pittsburgh Plate Glass heat filters: (1) 2043, 2-mm (1) 2043, 2-mm thick; (2) 2043, 6-mm thick; (3) Solex, 1/4-in. thick.

There are also a number of other companies that make this type of glass but that sell mostly to manufacturers. The depth of the color in the visible, as opposed to the infrared, absorption is due to ferric iron as an impurity; this characteristic varies considerably.

Small pieces of this heat-absorbing glass generally are not obtainable directly from the manufacturer. However, they are available as round disks by ordering spare or replacement parts of 35 mm slide projectors; for instance, at this writing the following are available from the Apparatus Parts Service of the Eastman Kodak Company.

Projector	Part No.	Size (Diameter)
Carousel	835596	$2\frac{1}{2}$ in.
Supermatic	835490	$2\frac{1}{16}$ in.
500	827161	$2\frac{3}{4}$ in.

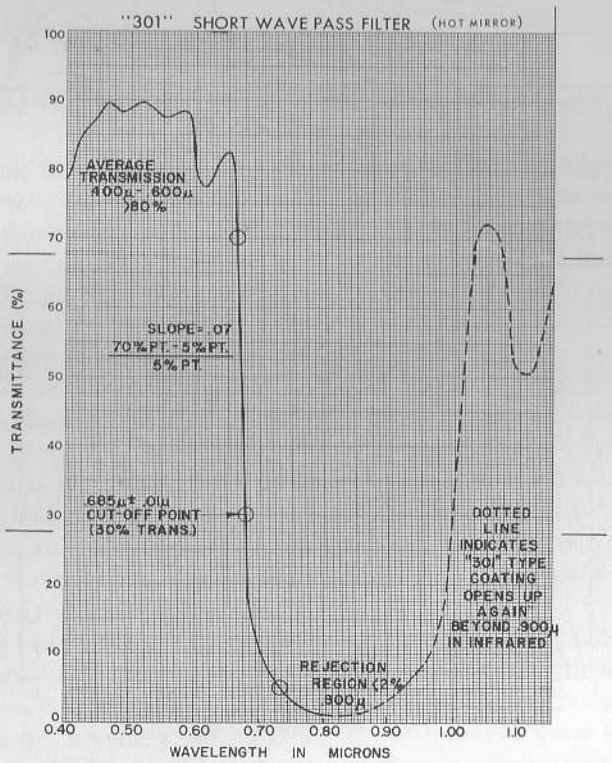
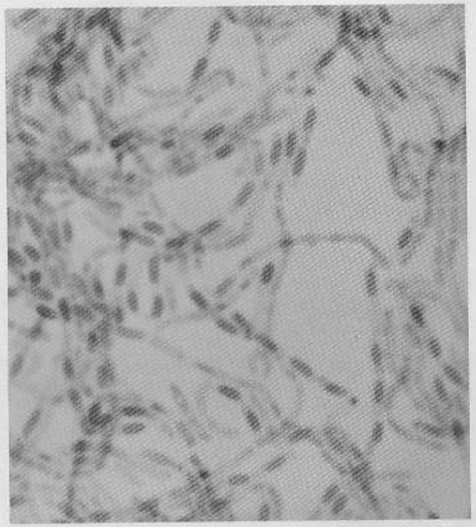


Figure 7-14 Spectral absorption of Kodak Infrared Cut-Off Filter.

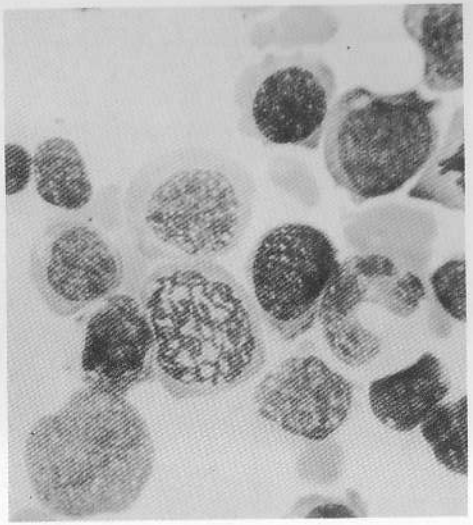
"Solex" heat-absorbing glass, made by the Pittsburgh Plate Glass Co., is sold by the square foot by dealers for that company. Its spectral absorption curve is included in Figure 7-13.

An entirely new type of dry-heat filter was introduced with the advent of **interference filters**. This type of optical filter is *discussed in Chapter 9*. The edge of its transmission band is even sharper than that of any absorption filter with all of the radiation being reflected that is not transmitted. These filters can be made to transmit the visible, reflecting the infrared or conversely, to make either a "cold mirror" or "hot mirror." The transmission of Kodak Infrared Cut-Off Filter No. 301 is shown in Figure 7-14, as taken from a data sheet of the Eastman Kodak Company. By comparison with the preceding figures, it can be seen that **the cutoff at the red edge of the visible spectrum is the sharpest of any filter**. On the other hand by λ 1000 mμ in the infrared these filters again transmit freely. Hence for effective protection from heat radiation this type of filter must be protected by a water cell of adequate thickness. Such infrared filters have been made on heat-absorbing glass; then the absorption edge lies further in the visible, but is sharper than that of the glass alone.

Petrographic section of anorthosite rock, a feldspar rock without quartz, under polarized light, crossed prisms. Original magnification 12x.



Spore-stained bacteria, *Bacillus subtilis*. Spores stained red, with the bacteria filaments counterstained blue. Original magnification 3000x.



Human blood cells in bone marrow smear, showing mitosis (prophase). Original magnification 1000x.

Crystallized citric acid, by polarized light. Original magnification 12x.

All figures are reduced about a third in reproduction

PHOTOMICROGRAPHY

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TORONTO LONDON

NEW YORK

monochromatic light of great intensity, but in the yellow, through the double D line of the solar spectrum at 580 mμ. No filter is needed with the sodium lamp, but one must always be used with the mercury-vapor lamp, for passing *either* the 365 mμ or 546 mμ line, but not both at the same time as would happen if no filter were employed.

All of these light sources find their primary application in the photography of transparent objects, by transmitted illumination, light or dark field. Only the most intense of them are suitable for vertical

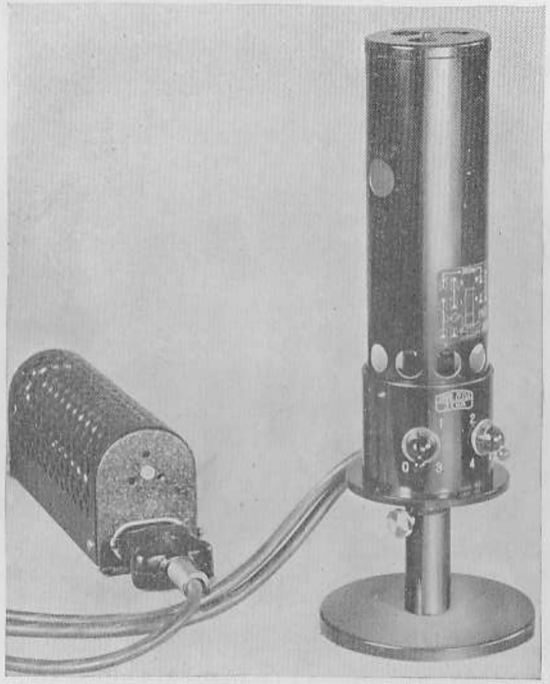

FIG. 59. Zeiss Electric Sodium Lamp

illumination – metallography, etc. The same is true of low-power incident illumination with oblique light. For this purpose an ideal arrangement is an arc lamp with focussing condenser, or other concentrated light source, mounted on a portable floor stand and adjustable as to height – say, from 3 to 5 feet.

Such equipment does not seem to be standard with the microscope manufacturers, but the floor stands are easily procurable and it is a simple matter to mount the arc or other high-intensity lamp, com-

plete in its housing, on the adjustable support. Figure 60 illustrates the general idea.

A very practical illuminating device for low-power photomacrography with either transmitted or incident light, which can also be used for copying and reducing, is the Macro Stage of Zeiss, shown in Figure 30. For opaque work the individual top lamps are used, as desired, the object being supported, with a suitable background, on the flat glass stage. When the object is to be photographed by transmitted light, illumination is effected by lights located under the glass stage, each of which is separately controlled.

Other forms of illuminating devices for low-power work are available from the various manufacturers. The Macro-Ring illuminator and light box of Leitz are shown in Figure 61. Manufacturers are continually bringing out new modifications of this type of equipment, but the general designs remain the same.

Miscellaneous Accessory Equipment

In addition to the cameras, optical benches, and light sources, which constitute the essentials of a photomicrographic outfit, most of the larger manufacturers supply numerous accessories, some of which are almost as important for certain classes of work as the more basic elements. Chief of these is the cool-

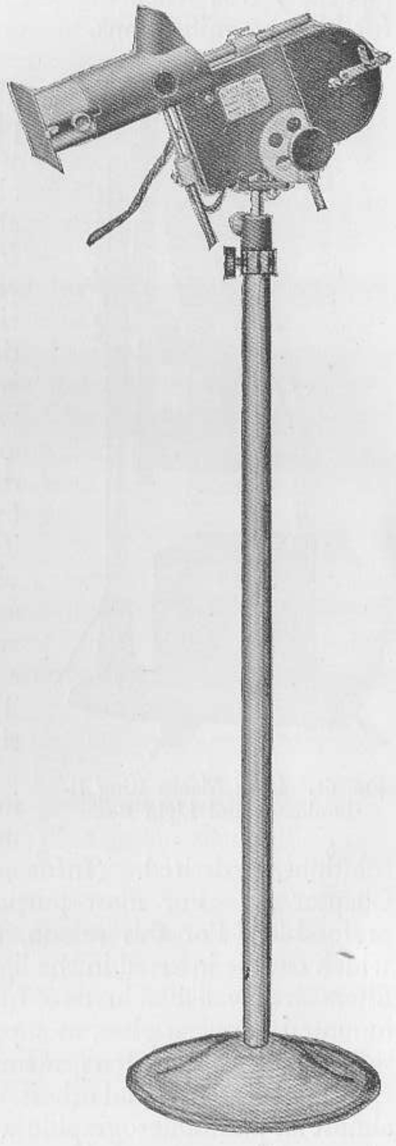

FIG. 60. Arc lamp on floor stand

ing cell, an absolute essential when arc lamps are employed. Some other lamps high in heat rays are also likely to cause damage to lenses or objects unless a cooling cell is inserted in the light train. This is especially true when the so-called Köhler illumination is employed, for high magnifications.

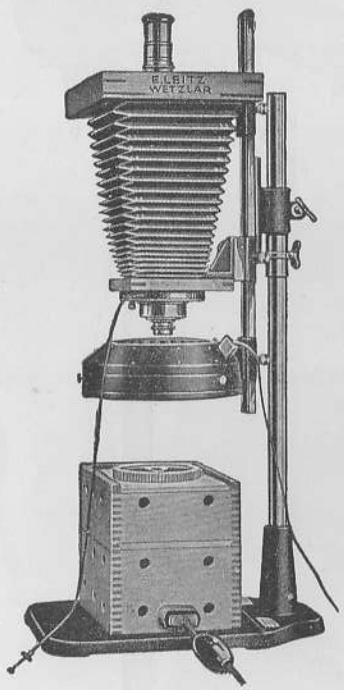

Fig. 61. Leitz Macro Ring Illuminator and Light Box

The cooling cell takes the form of a glass container with parallel sides of plane surface glass, capable of holding water or an aqueous solution of salt, alum, or other chemical having a strong absorption for infra-red rays. The Zeiss form, used either singly or in pairs, as required, is shown on its individual dual rider, in Figure 62. This is a very practical design. The body of the container is made of heavy porcelain, the sides of which are ground flat. Plain glass disks are held in place on each side by means of clamping rings; leaking is prevented by the use of rubber packing rings. The cells can be easily taken apart for cleaning or to replace a broken or scratched glass disk. For ultra-violet work the glass disks can be replaced by a pair made from fused clear quartz. These latter, however, are quite expensive.

In addition to their function for cooling purposes, cooling cells can also be utilized for holding aqueous color filter solutions, if desired. (Information about such solutions is given in Chapter 4.) For most purposes, however, commercial filters are preferable. For this reason, manufacturers provvide some means, which can be inserted in the light train, for holding such filters. The filters are available in two forms, the Wratten dyed gelatin films mounted between glass, as supplied by the Eastman Kodak Co., and solid colored glass filters, manufactured by Schott & Gen, Jena, the Corning Glass Co., and others. The two-inch-square size is ample for almost all photomicrographic work, although larger sizes are available

at a higher price. Neutral tint and heat-absorbing glass filters are supplied by Bausch & Lomb. These are available in different densities.

In special equipment for use in the light train on the large outfits, the Zeiss Co. leads all others, on account of the employment of the one-meter optical bench and the unit design of each piece of apparatus. It must be noted, however, that the advent of the universal Ultraphot has more or less placed such apparatus on the "special" or discontinued list, together with all forms of their larger research photomicrographic models. This special equipment includes special condensing lenses from 7″ in diameter down, of various focal lengths; centering condensers; condensers with associated iris diaphragms; large and small iris diaphragms; object-supporting tables; shutters; filter holders; cooling cells of various types; polarizing prism, etc. Each of these is provided with its own individual rider; separate riders are also provided, on which the photomicrographer may mount any special equipment of his own. To anyone working with one of the large outfits, this represents flexibility in the extreme, but at the same time, it assumes a theoretical knowledge capable of setting up and using the various pieces of apparatus, which is often not in evidence.

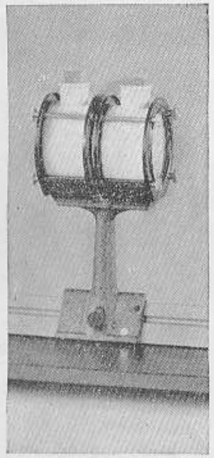

Fig. 62. Zeiss Cooling Cells on Optical Bench Rider

Another piece of auxiliary apparatus supplied by Zeiss, and which should be made available by every manufacturer of photomicrographic equipment, is the Multiplicator Back, shown in Figure 63. This back fits on the camera in place of the regular plateholder, and the latter then mounts on the Multiplicator. It enables the plateholder to be shifted, throughout its length, across the centerline of the camera. Then, by means of metal masks, mounted in the camera in front of the Multiplicator, having openings of predetermined widths, a whole series of exposures of an identical field can be taken at various exposure times, or with various filters in the light train, for determining ideal exposure conditions. This is a valuable adjunct for eliminating guesswork and making direct comparisons.

It is impractical to attempt to cover all the various pieces of equip-