PHOTOMICROGRAPHY AS AN AID TO TEACHING AND RESEARCH IN BIOLOGY.

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Photomicrographs are the chief support of the instructor in any course in plant anatomy besides the preparations used; they are the very means of illustrating our researches in morphology, in pathological anatomy, in spore germination studies, etc., if the photomicrographs are well made. Undoubtedly it requires fairly good equipment in this work if the best results are to be obtained.

The lighting system may be considered first. By the use of daylight, good photomicrographs can be made but the exposure is long, especially so, if light filters are to be used. Daylight varies considerably. An arc is perhaps the best type of illuminant and on direct current is unusually satisfactory. If the arc is used on alternating current, results will not be worth the effort required to secure a constant and uniform field of illumination. It may be added though, that excellent photomicrographs can be made with a 4 ampere arc and an 8 ampere resistance on alternating current, and that this type gives a very steady and fairly uniform field of illumination. The 108 watt, 6 volt ribbon filament Mazda lamp and transformer used with 110 volt alternating current, is perhaps the best lighting system for general use. An aspheric condenser is best used with this light.

A microscope with the ordinary achromatic objectives and Huygenian eyepieces can be used. Far better results are to be had with apochromatic objectives and hyperplane or periplane eyepieces.

The sub-stage condenser should be aplanatic if a good, uniform field of illumination is expected. This condenser is separable. The lower unit is used for the lower power objectives, and for the higher power objectives the upper unit is replaced. For the very low power objectives such as the 24 mm., 36 mm., and 48 mm. microtessars, summars or planars, no sub-stage condenser is used, since the aspheric condenser in the illuminating stand suffices. A diaphragm with markings is inserted in these objectives by the manufacturer. If the illumination is so bright as to require exposures shorter than one second, a piece of ground glass or several thicknesses of lens paper placed in the light path will give a satisfactory reduction in intensity. For oil immersion objectives, oil is placed on the condenser and in contact with the lower side of the slide. Cedar oil is not to be preferred. Any clear paraffin oil is just as satisfactory. This is true also for objective immersion oil.

The bellows and stand for the camera can be purchased from any of the optical companies (fig. 1). Any plate camera will do, for one need only remove the lens and shutter. The bellows must have a light proof union with the eyepiece.

"Proc. Ind. Acad. Sci., vol. 36, 1926 (1927)."

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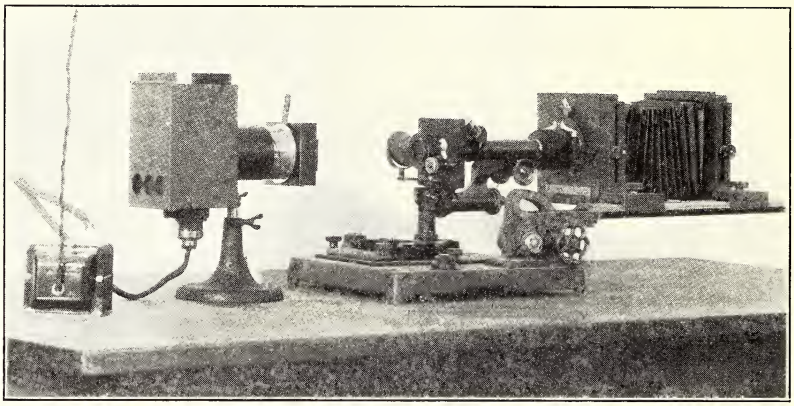

Fig. 1. Photomicrographic equipment assembled.

Any lighting system where artificial light is used must be centered and focused. The light is centered upon the substage condenser and diaphragm so that the area of greatest brilliancy of the light falls upon the substage condenser. The light is moved back and forth to find this place, and the aspheric condenser is properly focused. For the 16 mm. objective, the upper unit of the aplanatic substage condenser is removed (fig. 2). With the diaphragm completely closed and the ocular removed, we examine the light projected on a white piece of paper, held in the position of the ocular and a few inches back from the body tube (fig. 3). The light area should be a perfect circular are of uniform illumination. If it is not, rack the substage condenser back and forth until a perfect area free from any color is obtained. If the lighting system and microscope are in perfect alignment such a light area is soon to be found. A circle of brighter light on this light area aids in focusing the substage condenser. The ocular

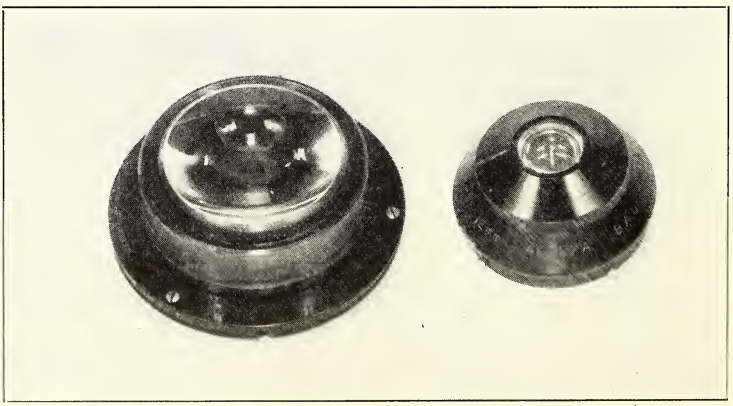

Fig. 2. Aplanatic substage condenser removed and separated. The larger unit is for low power work; as a doublet it is used for high power work.

is replaced and the illumination is examined through it on a white piece of paper in the same manner with a completely closed diaphragm. The object may now be placed upon the stage and focused upon in the same manner again. Then the bellows are drawn into place and the ground glass is focused upon and examined. The field of illumination will be as good as can be had, and can not be improved by examination of the ground glass area, since very slight discrepancies of uniformity over so large an area are not as readily discerned as on a small area just a few inches back of the ocular.

The diaphragm aperture at which the exposure is made has much to do with definition. If too small an aperture is used the finest definition is destroyed. It follows also that a loss of depth results with

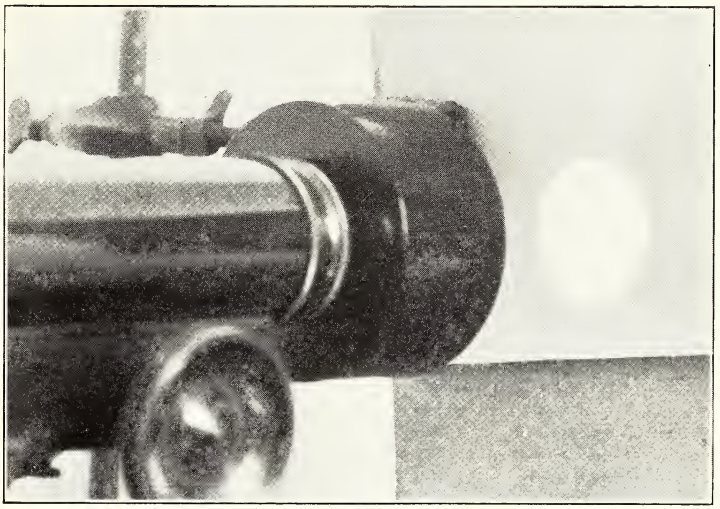

Fig. 3. Focusing the light through the ocular on a piece of paper held back of the ocular.

too wide an aperture. So a medium aperture is to be desired and the examination of the ground glass will determine what aperture one wishes.

Exposure varies with the aperture used and with the distance the ground glass is extended beyond the ocular. It is both desirable and necessary to have the bellows extension bar ruled off in units of length. The camera stand of most optical companies is marked in centimeters. On the other hand, the diaphragm is never marked, and so one must either judge by the intensity of the illumination on the ground glass each time to determine the exposure or stops must be marked for the diaphragm, so that exposure in relation to aperture can be standardized. These stops can be fixed since there is a lever that actuates the diaphragm of the substage condenser (fig. 4). If there is no lever, some other means can be devised. If correct exposures are found for one's favorite brand of plate, record can then be kept from the mark-

ings both on the diaphragm and the bellows draw bar. For the same substage condenser unit, and the same aperture of the diaphragm, exposure varies as the square of the magnification. If the upper unit of the condenser is replaced for a 4 mm. objective or a 1.9 mm. objective, then the exposure must be ascertained at some magnification and diaphragm aperture again, and the variations of exposure above or below that magnification accounted for on the rule as stated, i. e., exposure varies with square of the magnification.

In order to have relatively good contrast or a contrast greater in monochrome value than appears in polychrome on the triple stained slide, Wratten or similar light filters may be used. So then, it follows that a green filter on a safranin stained cell wall will cause the red to be darker. However, in xylem cell walls detail is thus frequently lost,

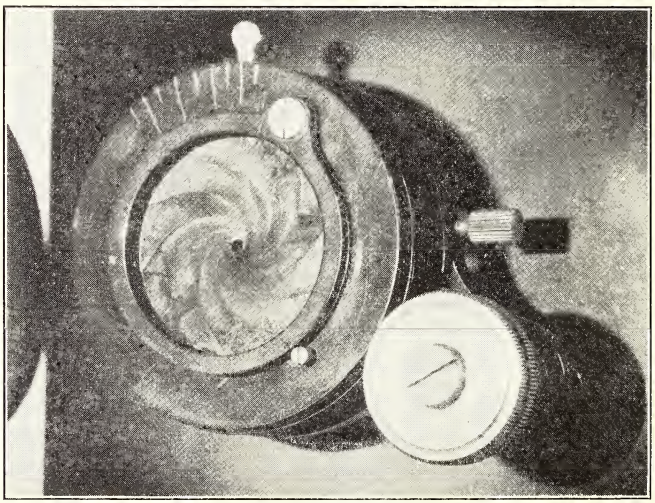

Fig. 4. The substage diaphragm closed. Stops are marked on the plate.

due to too great a contrast in monochrome, and so an orange or even a light red filter is frequently desirable. The required contrast is to be had by a prolonged development if necessary. The ordinary orthochromatic plate or panchromatic plate emulsions do not give quite sufficient contrast even with prolonged development, so as to be of absolutely satisfactory use in photomicrographic work, although fairly good work can be done on these plates. The Wratten "M" plate is far superior in that it is panchromatic and a contrast plate of good speed.

The developer to be used for Wratten "M" plates is given in the directions accompanying the plates. The following developer was found to be particularly good. It keeps well and can be used repeatedly. However, after development the developer used is discarded, even if only one plate is developed, in order that a standard practice may be

maintained. The developer[1] is as follows: hot water (100° F.), 2 liters; Elon (Metol), 0.6 grams; sodium sulphite, anhydr., 80 grams; hydrochinone, 12 grams; sodium carbonate, anhydr., 38 grams; potassium bromide, 1.8 grams; citric acid, 1.4 grams; and sodium bisulphite, 3 grams. Use at full strength at 65° F. For printing on a No. 2 contact paper about six minutes is the correct time of development, although if less contrast is desired even four and one-half or five minutes will not result in any great flatness. Development and fixation are done in total darkness, directions for the use of a green safelight from the manufacturer nothwithstanding. It is not possible to determine a relatively sufficient end point during development by means of a green safelight; so none is used.

[1] This formula was furnished through the kindness of Dr. M. E. Diehmer, University of Wisconsin, Madison, Wis.