IN THE LABORATORY

Determination of Exposure Time in Color Photomicrography

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The determination of exposure time in color photomicrography is a problem which is of utmost importance to the microscopist. The technique of "trial-and-error" exposure, which is almost universally used and recommended, has serious drawbacks. This method is time consuming and inexact, since the criterion of good exposure is determined by the subjective discrimination of the particular worker, and the exposure times are not of wide applicability, necessitating test exposures for different slides and stains. The method of choice would be one in which the criterion of a proper exposure is objectively and accurately determined, and one which can be used for a wide range of microscopic preparations.

The procedure devised employs an electronic photometer[1] to measure the intensity of the light, Wratten filters number 55 (green) and 38A (blue), and an "Omag" R-I (red) filter, to calibrate the instrument. The scale of the photometer is graduated from 0 to 100% transmission and can be used as an arbitrary measure of the light intensity in the microscopic field. The technique involves the calibration of the instrument for exposure time at various light intensities, using physical methods, all of which are available to the average laboratory.

In order to calibrate the photometer scale, a filtered light source is set up, using one of the filters and a number 2 photoflood lamp. The 4-mm objective is set at the distance from the stage that it would be were it focused on a microscopic slide. A 35-mm camera adapter is set into the body tube of the microscope, and a series of at least three test exposures are taken of the colored field for each light intensity. The light intensity, which is measured with the photometer "seeker" placed in the adapter with the camera removed, can be varied by manipulating the iris diaphragm of the substage condenser. This procedure is repeated with each of the above-mentioned filters at specific light intensities.

Qualitative inspection of the processed transparencies will show that they run in a series. For a given intensity, one transparency will be underexposed, one will be overexposed, and an intermediate transparency between the two former will seem to be properly exposed when compared with the filter used.

[1] The instrument used was a model 500 electronic photometer manufactured by the Photovolt Corporation, New York City.

The criterion of the proper exposure is not the actual color of the transparency but its percentage transmission of white light as compared with the original filter. We have found that the shapes of the spectral transmission curves are essentially the same, and the wave length which permitted maximal transmission is about the same, regardless of the over- or underexposure of the transparency. The variable, then, which serves to define the best exposure is the percentage transmission of a beam of light passed through the transparencies. This can be measured using the same photometer which is being calibrated. The apparatus measures per cent transmission directly from the photometer scale, and the transmittance of the filters and the test transparencies are determined. The proper exposure for a given filter (and hence for a given spectral range) at a given light intensity will be that exposure given to the test transparency which has a transmittance most similar to that of the filter used.

A sample series of the calibration data is presented in Table 1.

TABLE 1

Intensity reading	Exposure (sec)	Transmission of transparency (%)
70	1/5	31.1
70	2/5	35.0
70	4/5	38.2*
Transmittance of "Omag" R–I (red) with neutral film base		40.0

* Indicates best exposure time.

TABLE 2

Intensity reading	Exposure time (sec)			
	Red or Blue		Green	
	t	1/t	t	1/t
25	2.24	0.44	1.12	0.89
35	1.60	0.63	0.80	1.25
50	1.12	0.89	0.56	1.78
70	0.80	1.25	0.40	2.50
100	0.56	1.78	0.28	3.57

If the data in Table 2 are represented graphically, as in Fig. 1, a straight-line curve in the form

$$I = K \cdot (1/t)$$

results, I being the intensity of illumination (densitometer reading); t, the exposure time in seconds to produce a satisfactory transparency; and K, a constant. This equation is of the same form as the Bunsen-Roscoe reciprocity law and indicates that the exposure times are based on sound physical principles (*1*, *2*).

In a test series of photomicrographs of histological material, transparencies of excellent color rendition and density were produced, and the percentage of improper exposures was reduced to zero.

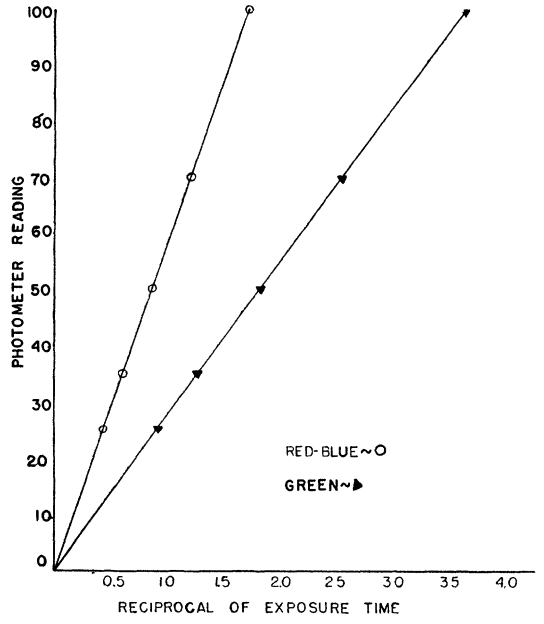

FIG. 1

This physical, nonsubjective method of exposure time determination in color photomicrography can be routinely used over a wide range of biological material.

References

1. GLASSER, O. *Medical physics.* Chicago, Ill.: Yearbook Publishers, 1944. P. 1146.
2. LOVELAND, R. P. *Kodachrome and Ektachrome exposure in photomicrography.* Rochester, N. Y.: Eastman Kodak Company, 1947.

A Simple Electronic Electrolytic Drop Recorder

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The majority of drop recorders or drop counters in use today for the measurement of salivary secretion, urine output, and bile flow are of two fundamental types (*2*). One, the electromechanical type, which is available commercially, allows the drop to overbalance a bucket-ended lever, which in turn makes an electrical contact. With careful adjustment and continuous supervision, these drop recorders can be made to work satisfactorily with thin, watery fluids, like urine, but viscous secretions tend to stick to the bucket so that the system does not return to normal for ensuing drops. The other system is far more satisfactory. The drop serves to make contact between two electrodes, and a sensitive relay serves to actuate a writing lever (*1, 4, 5*). However, the very sensitive relays needed are liable to stick, and the high voltage requirement is hazardous. The current needed for more rugged relays corrodes ordinary electrodes rapidly so that they are soon inoperable.

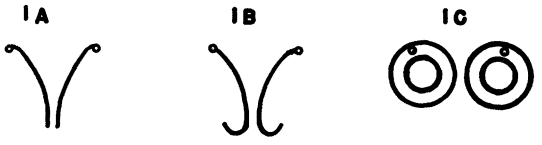

FIG. 1

These undesirable attributes may be easily overcome by proper electrode design and by using an electronic relay between the electrodes and an ordinary "physiological signal magnet" or one of the more elaborate integrating signal magnets (*6*). Biskind and Dan (*1*) suggested the use of curved platinum-wire electrodes (Fig. 1a) that would catch a drop, even though the drop source was not accurately centered, and yet not hold the drop after momentary contact was made. We have had excellent success with similar electrodes made of nichrome wire (Fig. 1b), but they are too frail for general student use. At present we are using stainless-steel washers (Fig. 1c). The eccentric hole facilitates adjustment to any desired size gap. The electrodes are attached to Johnson stand-off insulators with 6–32 hex nuts, although thumbscrews could be used. By placing a medicine dropper orifice at sufficient height above these electrodes, a drop of even a very viscous fluid will have enough velocity to fall through the electrodes without sticking after making momentary contact.

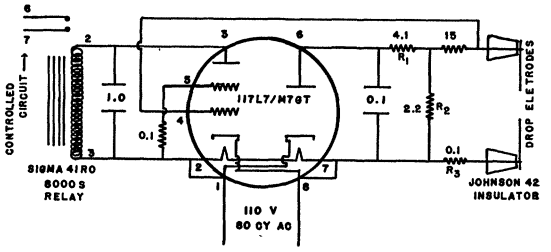

FIG. 2. All resistances in megohms; all resistors, ½-watt size; all condensers in microfarads; all condensers, 200-v paper type.

Although there are several hard-tube electronic relays on the market at present, we found it convenient to design our own device, which has certain advantages in that it is sturdy, simple, economical, and dependable. The wiring diagram in Fig. 2 is self-explanatory. The 117L7/M7GT electronic tube was used, since it combines both rectifier and tetrode in one envelope and requires no filament resistor or transformer. A 117N7 or 117P7 could be used with appropriate socket changes. The Sigma relay[1] was used because it has adequate sensitivity

[1] Sigma Instrument Company, 70 Ceylon Street, Boston, Massachusetts.