A METHOD OF CALCULATING EXPOSURES FOR PHOTOMICROGRAPHS[1] *

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INTRODUCTION

The task of making photomicrographs of reproducible quality often confronts many investigators in the fields of biological science as well as in other fields of endeavor. Usually such undertakings involve rather extended trials and errors, with the hope that some usable prints may ultimately result.

The techniques of photographic processing, formulae for solutions, and present day photographic equipment approach near perfection. Even the guesswork of exposure determinations has been largely eliminated by the photronic exposure meter. It is mainly in the field of photomicrography, where it is impossible to measure directly the light transmitted by, reflected from, or emanating from an object of sub-visual proportions that difficulty is encountered in obtaining a negative of optimal density and contrast.

Several works on photomicrography have appeared during the past decade, among which are those of Allen, 1942, and Shillaber, 1944. Despite the general excellence of these books, both decry the use of a photometer as an instrument for determining photomicrographic exposures. These and other works present good methods for determining exposures based on a set of trial exposures, but some unfortunately are indirect methods that involve mathematics beyond the training of many biologists. Such methods are not only time consuming, but expensive as well, especially when color work is being executed.

Hefley and Smith, 1942, advocated the use of the photronic exposure meter for determinations of photomicrographic exposures with readings taken in the plane of the image. A Weston Master Exposure Meter Model 715 was used and a set of relatively high image intensity values (0.1 to 50 foot candles) was obtained. These values were combined with the Weston speed ratings of several films to give exposure values. The results were incorporated into an exposure table.

Subsequent use of the method has shown it to be valid. Unfortunately the range of Weston speeds of emulsions and light intensities given are insufficient to take care of many situations encountered in photomicrography. The method of calculating photomicrographic exposures presented here is an attempt to extend, elaborate and modify the previously reported technique.

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MATERIALS AND METHODS

The materials used included a Photovolt Photometer Model 512 calibrated to read from 5 foot candles to 0.00002 foot candles, a Leica camera with copying attachment, various light sources with color temperatures approximately 3200 degrees Kelvin equipped with diaphragms so that the intensity of the light was controlled without affecting the color temperature, and a target (object) of slightly roughened light gray asbestos. This object was used to insure an even density over the entire emulsion field, so that subsequent measurements of light transmission would be as constant as possible.

A 50 mm color corrected f.3.5 Leitz Elmar lens was focused on the object by means of extension tubes. Various magnifications were used because the use of image intensity values, properly measured, eliminates the necessity of considering the inverse relation of intensity and magnification (see Hefley and Smith, 1942).

The exposed negatives were developed in a tank and carefully controlled, with a variation of ± 30 seconds in time of development, and a variation of ± 0.5° C. Edwal developer No. 12 compounded of reagent quality chemicals and weighed to an accuracy of ± 0.01 gm per liter of solution was used on all emulsions having a Weston speed of 8 or above. Slower films were developed in either Eastman D-11, Eastman D-72, or Eastman Dektol. Continuous agitation of 276 oscillations per minute was used.

After processing, the percentage of light transmitted by the negatives was read on a densitometer.

Afterwards, a photomicrographic camera was set up, using a Zeiss research microscope equipped with apochromatic objectives, compensating oculars, and an achromatic condenser. Various photomicrographs were taken using the method explained below. Test films included indoor type Kodachrome as well as black and white emulsions.

The image intensity was measured after the image was focused sharply upon the frosted glass screen of the camera. The frosted glass was then *removed*, the sensitive surface of the photoelectric cell was placed in the plane of the image, and the reading taken. It should be remarked that valid measurements *cannot* be made through a frosted glass focusing screen, because different pieces of glass vary considerably in optical characteristics, moreover, they are seldom frosted to the same density or fineness.

DISCUSSION

An examination of the data presented by Hefley and Smith, 1942, revealed that the values for exposures given in their table satisfy the equation $E = \frac{1}{I_i W}$, where E is the exposure in seconds or fractions thereof, I_i is the intensity of the image in foot candles, and W is the Weston speed of the emulsion on the film or plate being used.

Or, stated another way: *the exposure in seconds is equal to the reciprocal of the product of the image intensity in foot candles and the Weston speed of the photosensitive material being used.*

Assuming that the formula would hold for image intensities below 0.1 foot candle, series of calculated test exposures were made with image intensities down to 0.001 foot candle. This intensity approaches the lower limit of the author's eyes (when partially dark-adapted) to distinguish clearly the different portions of a sharply focused image on a frosted glass screen. About 300 exposures were made in the intensity range given above, on film with Weston speeds of 0.6, 1.5, 4, 6, 8, 16, 32, and 64. These ratings are for Tungsten light, and include Kodachrome color film balanced for light at 3200° Kelvin. Color transparencies were processed by the Eastman Kodak Co. at Rochester, New York, and were of excellent projection quality.

The negatives were developed according to the directions given with the developer formulae by the originators. In general, development times and temperatures are balanced with emulsions to give a gamma of approximately 1 with a correct exposure. Such a film density will transmit 10% of the light falling upon it, and is considered to be of optimal printing quality for No. 2 paper.

The negatives varied ± 1.33% from the expected 10% transmission. The majority of them transmitted 10% of the light from the densitometer. The transmission range was from 8.5% to 11.8% inclusive. Errors in timing the exposures probably accounted for most of the discrepancies, because the majority of errors were in the fractional second range where the times of exposure were not those for which shutters are calibrated. Longer exposures, and short exposures that could be timed mechanically varied little from the expected transmission of 10%.

Exposures calculated by means of the formula $E = \frac{1}{I_i W}$ have been used on emulsion with Weston speeds from 0.6 to 64. Films and plates used in photomicrography usually fall within this range of speed values. It was found that low image intensities give more accurate exposures, since longer times minimize small errors in timing.

It should be emphasized that only Weston speed values for emulsions should be used in substituting in the above formula. Other film ratings will not give accurate results by this method, although in ordinary photography they are quite satisfactory.

SUMMARY

A method for calculating exposures for photomicrographs has been presented that has proved to be accurate and workable. It is hoped that it may be an aid to those who infrequently take photomicrographs, and who otherwise might waste considerable amounts of time, energy, film, and enthusiasm trying to obtain properly exposed negatives by trial and error.

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