A MASKING TECHNIQUE FOR CONTRAST CONTROL IN ELECTRON MICROGRAPHS

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The technique of "dodging" photographic prints for electron microscopy is almost universally practiced. One merely interposes a hand or some suitable object in the light path during enlargement in order to produce prints of even density from negatives of uneven density. There are occasions when the intricate shape of areas to be dodged render such a simple procedure inadequate. This is especially true of negatives of tissues in which irregular patches of very dense material are adjacent to structures of moderate or low density. Dense areas may black out completely in prints made to obtain optimal tonal separation in light areas. Conversely, areas of low density often become "washed out" when details in dense areas are desired. If one attempts to correct excessive large-area contrast by using low contrast paper, the resulting fine-detail contrast is often too low, especially for photomechanical reproduction.

A printing instrument that uses a scanning light source has been described (1). It automatically corrects for excessive large-area contrast while retaining, or enhancing, fine-detail contrast. Light from the flying spot is monitored by a photomultiplier tube after it has passed through the negative. By means of negative feed-back, the spot is dimmed as it passes over light areas in the negative. The amplitude of the feed-back signal determines the degree of contrast correction in the final print. The size of the flying spot determines the minimum size of areas, the contrasts of which will be altered. Strictly photographic methods for dealing with this problem have been known for a long time. The reader is referred to Yule (2) for an introduction to the pertinent literature.

Aerial photographs (3) and roentgenograms (4) have been printed by exposing prints through an unsharp positive transparency (mask) superimposed in register on the negative. This produces effects on prints similar to those produced by the electronic instrument described above. Making the mask unsharp is like defocusing the flying spot; increasing the contrast of the mask has the same effect as increasing the amplitude of the negative feed-back signal.

A method for visualizing fine details in large areas of radically different densities on the same negative is an important aid in obtaining maximum information from a given negative. It is also desirable to minimize unevenness in the nega-

tives due to errors in technique such as uneven lighting, uneven development, compression marks in the section, etc. Since few electron microscopists are familiar with masking techniques for contrast control, it has seemed of value to report the following method which has proved very helpful in our work.

Prints made through a *sharp* positive mask, in perfect register with the negative, reveal a reduced over-all contrast; *i.e.*, the contrast of both large-area and fine-detail is reduced. If the sharp mask is out of register, the print will show a pseudo-bas-relief effect. Prints masked by an *unsharp* positive image show a reduction in large area contrast but no reduction in fine-detail contrast because fine details are not resolved in the unsharp mask. Furthermore, the unsharp positive image is easier to superimpose in register with the negative. The minimum size of areas, the contrast of which will be affected by the masking, is determined by the degree of sharpness of the mask. The degree to which such areas will be affected depends upon the contrast of the mask.

In our initial efforts, negative, diffusing material, and Kodak Fine Grain Positive Film[1] were arranged with their emulsions facing each other and with the diffusing material between them.

We have used the enlarger as a light source, although other light sources could be used (2). It is important that the negative, diffusion material, and unexposed film be kept in intimate contact since it is desirable to have the positive mask and the negative as nearly the same size as possible. This is done by simply placing a ¼-inch piece of plate glass over the stacked material. Originally we used two or four layers of a fine lens paper as the diffusing "screen." Four thicknesses of Kodapak Diffusion Sheets[1] are equally satisfactory and have the advantage of being more uniform and structureless.

A second method for making the mask depends on a diffuse light source and a clear separator, which is interposed between the negative emulsion and the unexposed emulsion. This may be carried out on the enlarger base-board by arranging the negative film and Kodak Fine Grain Positive material back-to-back so that the combined thicknesses of their supports serve as a clear separator between their emulsions (Fig. 1) and by diffusing the enlarger light with opal glass or opal plastic held between the enlarger lens and the base board. Emulsions that have antihalation backing cannot, of course, be exposed through the support. Film exposed back-to-back with glass negatives yields excessively unsharp masks because of the thickness of the glass. A proper mask for a glass negative can be obtained by interposing a clear plastic sheet about 0.01 inch thick between the emulsion of the negative and the emulsion of the unexposed masking material as shown in Fig. 2. The second method for making masks is preferred and is currently in use in our laboratory.

Both the density and contrast of the mask obviously are important. It is essential to work within the straight line portion of the characteristic curve of the Kodak Fine Grain Positive material. Furthermore, the contrast of the mask should be less than that of the negative, *i.e.*, the slope, or gamma, of the characteristic curve of the mask should be less than 1. Gamma is a function of development. Longer development and/or higher

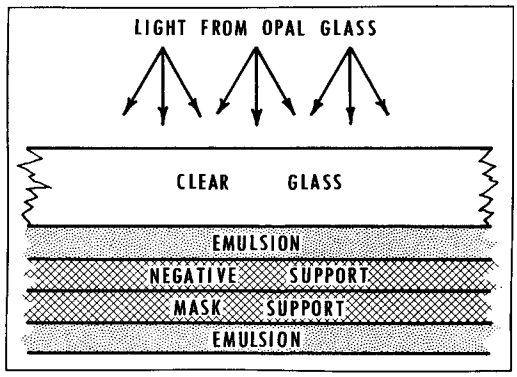


FIGURE 1

Stacking arrangement for negatives on film.

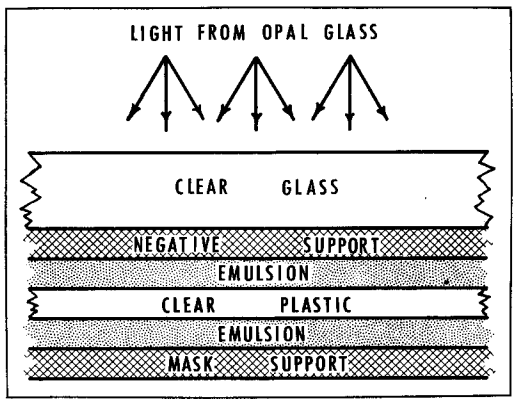


FIGURE 2

Stacking arrangement for negatives on glass.

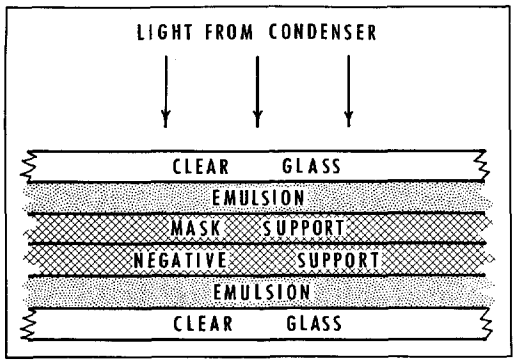


FIGURE 3

Arrangement of mask-negative pack.

temperatures result in higher gamma. At a gamma value of 1, the density ratios between different areas of the negative are the exact reciprocals of density ratios in corresponding areas of the mask. By using such a mask one would level all contrast differences in large areas of the negative. For example, the density within a lipid droplet would be the same as that of the background in the final print. The lipid droplet would disappear were it not for two properties of the unsharp mask method; *i.e.*, (*a*) fine detail appears in high contrast and (*b*) the method introduces an artifact at abrupt edges of large areas. This edge effect is due to the unsharpness of the mask. An abrupt change in the density of a large area in the negative is rendered as a gradual change in density in the mask. A print made with an unsharp mask shows a halo immediately outside of sharp edges in large, dense areas and a diffuse density immediately inside of such areas. This effect is accentuated with a mask of high gamma and of a high degree of unsharpness.

OUTLINE OF UNSHARP MASK TECHNIQUE

A. Procedure for Negatives on Film

1. Kodak Fine Grain Positive Film is cut slightly larger than the negative.

2. The cut, unexposed film is placed with the emulsion side *down* on the base-board of the enlarger.

3. The negative is placed on the unexposed film, emulsion side *up*.

4. A piece of clean, strain-free plate glass (¼ inch thick) is placed on top of the film to insure intimate contact.

5. Diffusing material is interposed between the enlarger lens and the base-board, about 3 inches above the film. ⅛-inch opal plastic was used.

6. The exposure must be determined from case to case. As a starting point, exposures are first made at about 20 seconds, with a 105 mm lens at F:16. The enlarger contains a No. 212 (150 w) enlarging bulb and its lens is about 18 inches above the base-board. With this set-up, an illumination intensity of about 2 footcandles was obtained beneath the opal plastic, without a negative in the enlarger.

7. The film is developed at 68°F for 45 seconds with vigorous agitation in Dektol[1], diluted 1:2. This yields a gamma of about 0.7.

8. The mask is fixed until it clears (1 to 2 minutes), and is washed in rapidly running water for about 2 minutes. It is then dried with a hair dryer.

9. The negative is superimposed, emulsion side *out*, in register with the mask and the two are taped together along opposite edges. Registration is best accomplished by using opposite corners of the negative as reference marks.

10. The negative-mask pack is put between glass and into the enlarger with the negative on the bottom and its emulsion side *down* (Fig. 3). Lantern slides as well as prints can now be made without manual "dodging."

B. Procedure for Negatives on Glass

The procedure is the same, except that in readying the negative and film for exposure, the film should be put on the base-board emulsion side *up* and the negative should have its emulsion side *down*. A clear sheet of plastic (0.01 inch thick) is interposed between negative emulsion and unexposed emulsion of the fine grain positive material (Fig. 2).

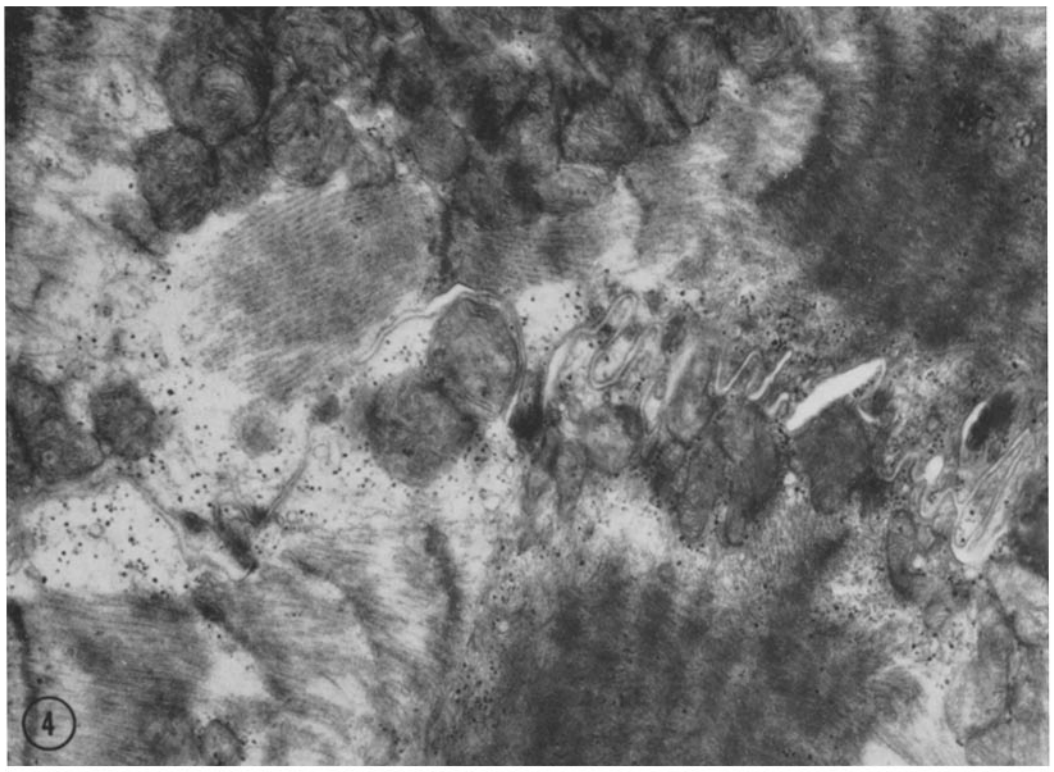
RESULTS

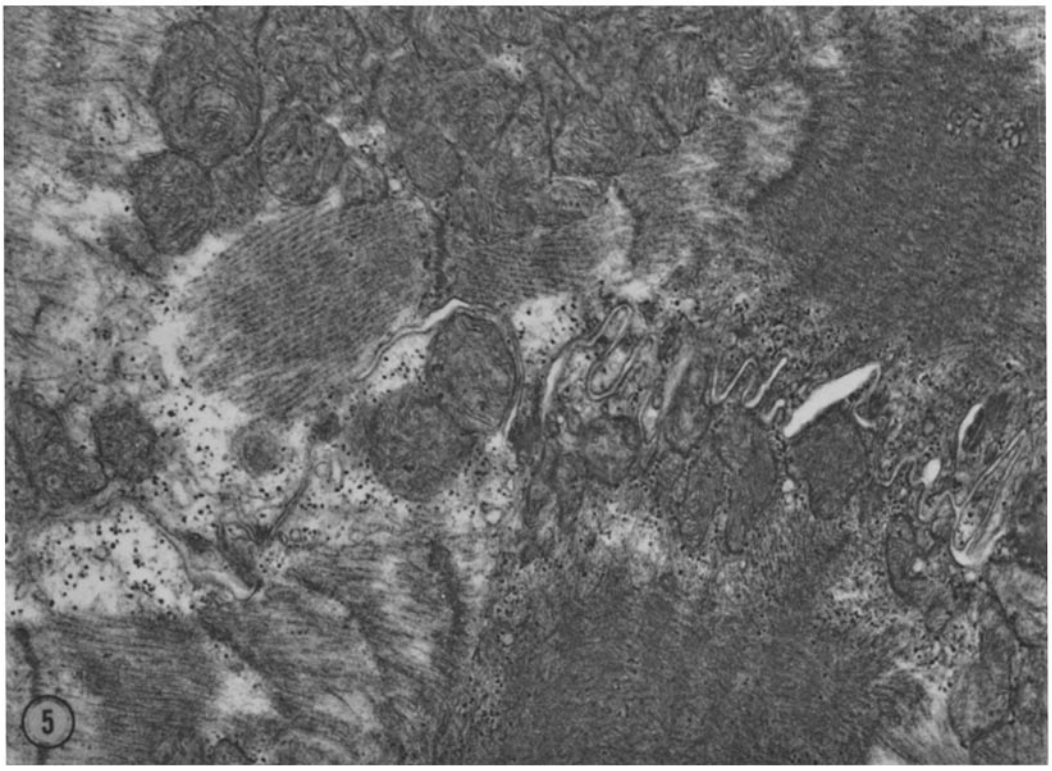
By the use of this method, details in the dark areas and light areas of micrographs are more clearly

[1] Eastman Kodak Company, Rochester, New York.

FIGURES 4 AND 5

Electron micrographs of contracted heart muscle cells of snake. Both prints were made from the same negative. Fig. 4 was made without dodging or masking. Fig. 5 was made without manual dodging, but with an unsharp positive mask superimposed on the negative. × 28,000.





revealed. Furthermore, faults in negatives, such as streaking, or in sections, such as compression marks, are minimized.

Figs. 4 and 5 are prints made from the same negative. The micrograph is of parts of two contracted muscle cells of snake heart. Contraction bands can be seen at the same level in both cells. At this level the plasma membranes are thrown into tight, interlocking folds. Mitochondria are tightly packed between the myofibrils.

This is a particularly difficult negative to print because of the very high density of the packed mitochondria and the contracted myofibrils. If one makes a print from this negative on Kodabromide[1] No. 3 paper without dodging or masking, one loses details either in the dark areas or in the light areas depending on the exposure given the print. Fig. 4 was made to show detail in the dark areas. Note the loss of detail in the light areas. A print made on No. 2 paper shows detail in both light and dark areas, but the contrast is very low.

Fig. 5 was made by the masking method described above. The print was made without manual dodging on No. 5 paper. Note that this print has a more even quality and that detail is revealed in both light and dark areas without loss of contrast.

COMMENTS

In addition to the described methods of contrast control, (photographic and electronic masking), a third possible technique depends on one of the minor properties of photographic emulsions. A latent image in a blue-sensitive emulsion can be destroyed or weakened by exposure of the emulsion to far red and near infra-red light. This is the "Herschel effect" (5). It should be possible to expose a print with white light and then re-expose with red light, and thereby to obtain the same effect as that obtained by masking.

In preliminary experiments we found that white light, filtered with a Wratten A filter, did indeed weaken the latent image on Kodabromide paper. However, the exposure times required to show this effect were inordinately long.

In order to avoid misinterpretation of micrographs, the user of this technique should be aware of the edge artifact introduced by the unsharp mask. This artifact is minimized by using masks developed to low values of gamma. Other contrast changes introduced by use of this method are essentially the same as those resulting from manual "dodging" of prints.

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