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Publication Date

2017-12-07

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DEVELOPMENT OF METHODOLOGY FOR LOW EXPOSURE, HIGH RESOLUTION ELECTRON
MICROSCOPY OF BIOLOGICAL SPECIMENS

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Specimen damage in the electron microscope due to the inelastic scattering of the incident electrons is one limitation to obtaining high resolution image data of biological specimens. For most organic molecules, when the electron exposure reaches 10^{-3} Coulomb/cm², further observation will provide no information concerning the original structure. Therefore, in order to obtain high resolution data, the exposure must not exceed this value. At this exposure, the resulting image will be a statistically noisy one. In our work, we investigated the feasibility of improving the signal to noise ratio in the image of a periodic specimen, by spatially averaging the statistically noisy images of all the unit cells.

Using a carbon replica of an optical diffraction cross grating (54800 lines/inch) as our test specimen, the statistically noisy image was recorded with an image intensifier at the magnification of $\times 1600$ and at the exposure of 3×10^{-4} electron/ μm^2 on the image plane (Fig. 1a). The image data was stored on a magnetic tape and processed in a CDC 7600 computer. The calculated diffraction pattern shows distinct peaks (Fig. 1b). Spatial averaging of the noisy image was done in the computer by first setting the value of each diffraction spot equal to the average of all points within an area chosen around the spot, and setting to zero all other points. This was followed by an inverse Fourier transform to yield the spatially averaged image (Fig. 1c).

Because of the small number of incident electrons, the best recorder of statistically noisy images is one that has a high electron detection efficiency, and a low level of recorder noise. The detection efficiency of the image intensifier was found to be only 20%. Since some of the commercially available fast emulsions are very sensitive to electron radiation, we experimented with photographic methods for recording statistically noisy images. For exposure not exceeding 10^{-3} Coulomb/cm² at the specimen and magnifications above 40,000, the optical densities of the developed emulsions are too low to be detected by a scanning microdensitometer. Infectious development was used to increase the optical densities of the developed emulsions. This effect is caused by local fogging around the developed silver grains in the emulsion (1). By far, the best results have been obtained with Kodak NTB2 emulsion. The NTB2 emulsion was first developed in D-19 at 10°C for 20 minutes, and then developed in D-8 at 4°C, into which 0.25 gm/1000cc of hydrazine dihydrochloride ($\text{N}_2\text{H}_4 \cdot 2\text{HCL}$) has been added to cause infectious development. Time of development in D-8 varies with the exposure, and it is stopped when the O.D. reaches a desired value. For exposures as low as 10^{-3} electron/ μm^2 on the image plate, it is possible to reach O.D. values as high as 3 while the fog level remains insignificant (Fig. 2).

Figure 3 shows a statistically noisy image of a uranyl acetate stained catalase crystal recorded on NTB2 at the magnification of $\times 40,000$, and at the exposure of 5×10^{-3} electron/ μm^2 on the plate. The diffraction pattern (inset in Fig. 3) was taken over an area of 2cm in diameter. Due to the irregular stain distribution over the crystal, only small usable image areas were available for recording optical diffraction pattern and for spatial averaging. We expect a significant improvement in the attainable resolution, when a better specimen preparation technique is implemented with the use of this method.

This work has been supported in part by USPHS Training Grant No. 5 T01 GM00829 from the National Institute of General Medical Sciences and by research grant number GM19452 from the NIH.

1. Stauffer, R. E., Smith, W. F., and Trivelli, A.P.H., J Franklin Inst., 238, 291 (1944).

Fig.1 (a) The Z-modulation display of a statistically noisy image of a carbon replica of an optical diffraction cross grating, recorded with an image intensifier. (b) The power spectrum of the noisy image. (c) The spatially averaged image.

Fig.2 Optical density of NTB2 emulsion vs the time of infectious development. (A) Exposure on the plates is 10^{-2} electron/ μm^2 . (B) 10^{-3} electron/ μm^2 . (C) 10^{-4} electron/ μm^2 . (D) Fog.

Fig.3 The statistically noisy image of a uranyl acetate stained catalase crystal taken at the exposure of 5×10^{-3} electron/ μm^2 and at the magnification of 40,000.

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