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APPLICATION OF ELECTRON DIFFRACTION TO PROBLEMS IN BIOLOGICAL ELECTRON MICROSCOPY

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APPLICATION OF ELECTRON DIFFRACTION TO PROBLEMS IN BIOLOGICAL ELECTRON MICROSCOPY*

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Berkeley, California

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The role of elastic (Bragg) scattering in relation to contrast and advanced electron microscopy of biological materials has not been investigated in the detail that is now common with inorganic crystals. It is the object of this paper to demonstrate the application of selected area diffraction, dark field imaging, and low angle Fourier contrast to biological materials. Essential differences between the mechanisms of diffraction image-contrast are due to the larger lattice spacings in biological structures, which invariably result in Fourier image contrast. Crystals with spacings greater than 10Å can be expected to show Fourier contrast under normal microscope operations.

Samples of crystalline amino acids were grown by solvent evaporation on formvar-coated specimen grids. Specimen grids were scanned and selected on the basis of appearance in a polarization-optical microscope (see Fig. 1). All crystalline amino acids tested were extremely sensitive to damage in the electron beam. Best results were obtained with ℓ-valine. With both condenser lenses set at near-maximum excitation, and by use of a 100 μm diameter (or smaller) condenser aperture it was observed that the diffraction pattern remained apparently unchanged over periods of 15 min and longer. Figure 2 is one example of a symmetrical spot pattern. After calibration of the camera constant and evaluation of the pattern, the orientation is consistent with the (hk0) reciprocal lattice plane. The values a = 9.70(±0.02)Å, b = 5.31(±0.02)Å are consistent with the reported monoclinic unit cell of ℓ-valine [Tsuboi, Takenishi, and Iitaka: Bull. Chem. Soc., Japan 32, 305 (1959)].

Figures 3 and 4 show non-Fourier diffraction contrast, in the form of extinction contours, obtained by excluding from the image either the diffracted beams (bright field image, Fig. 3) or the transmitted beam (dark field image, Fig. 4). These contrast effects were only photographed at low levels of illumination by using an image intensifier commercially available for the AEI electron microscope. For spacial periodicities much greater than 10Å, it will be impossible to exclude diffracted beams from the image because the reflections fall within the volume of the transmitted beam. In these cases Fourier images always result, and low angle techniques must be used to investigate the diffraction pattern. Examples of such effects will be shown with polystyrene spheres and with striated muscle.

When specimens such as valine are observed by the selected area diffraction mode it is possible to make a semi-quantitative study of the rate of structural deterioration as a function of the level of illumination. In this way it is possible to reveal dynamic changes at a structural scale much less than the resolving power of the electron microscope. This investigation clearly indicated that a major reason why biological specimens such as proteins, viruses, etc. do not show useful contrast at the level of 10-20Å may be that the structure itself can be randomized by high levels of illumination. Thus selected area diffraction provides a useful way to evaluate the potential utility of image intensifiers, high voltage electron microscopes, and other devices which attempt to reduce damage to biological specimens.

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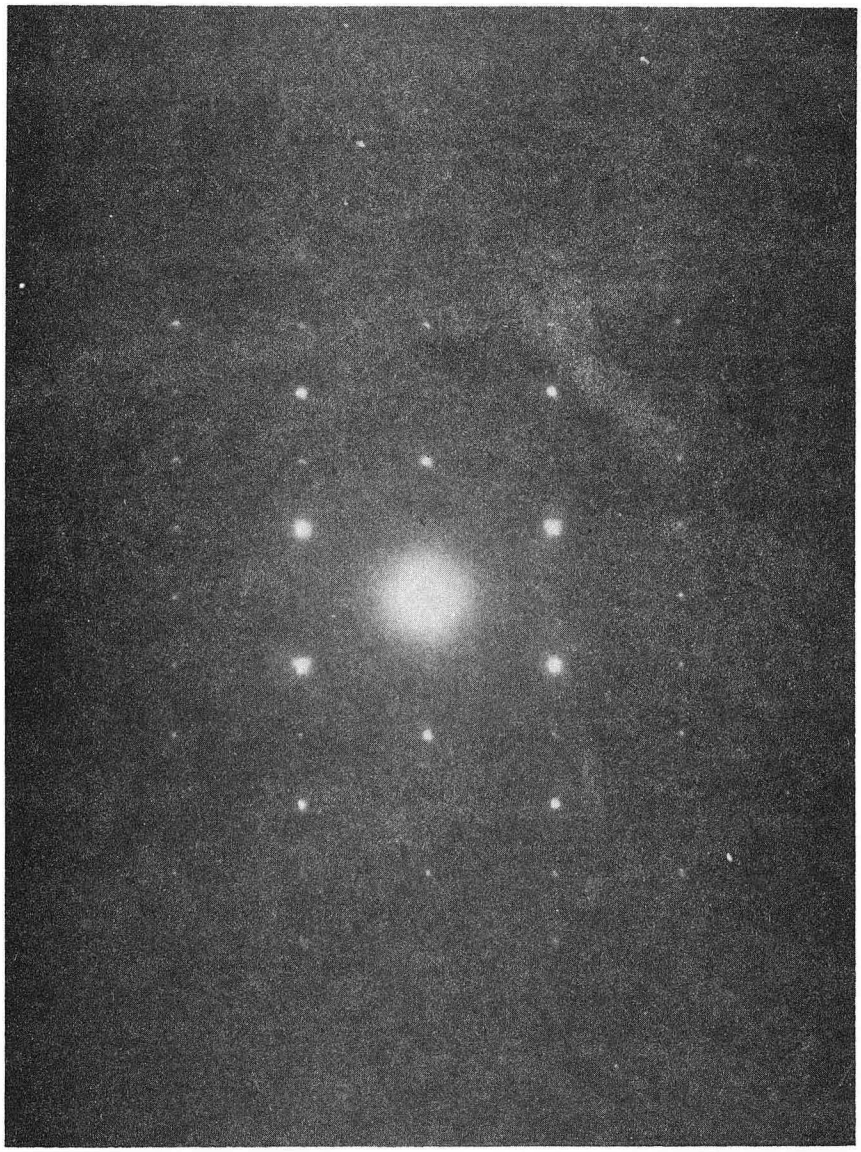
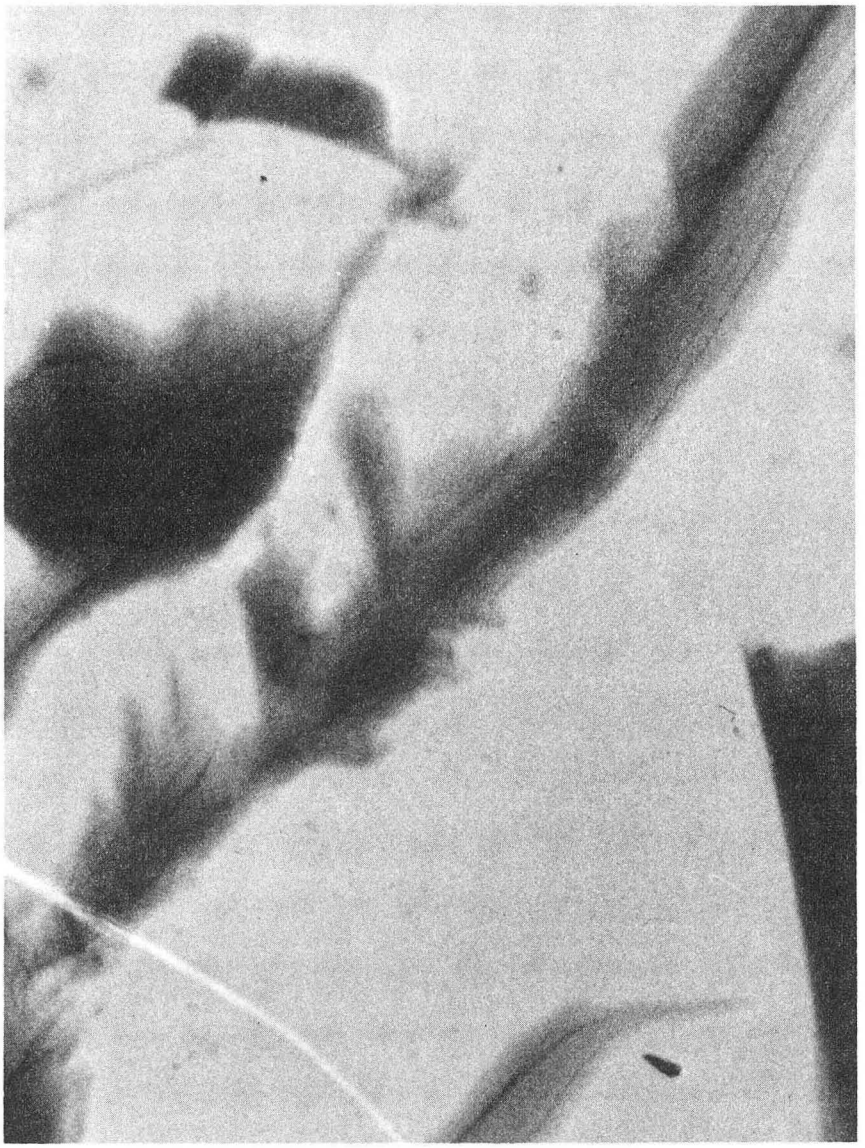
Fig. 1. Photomicrograph of crystalline l-valine mounted on an electron microscope specimen grid. Polarization optics.



XBB 684-1969

Fig. 2. Selected area electron diffraction pattern showing the symmetrical hk0 plane of reciprocal space, 1-valine.



XBB 684-1975-A

Fig. 3. Bright-field illumination electron micrograph of crystalline 1-valine, showing extinction contours due to Bragg scattering.

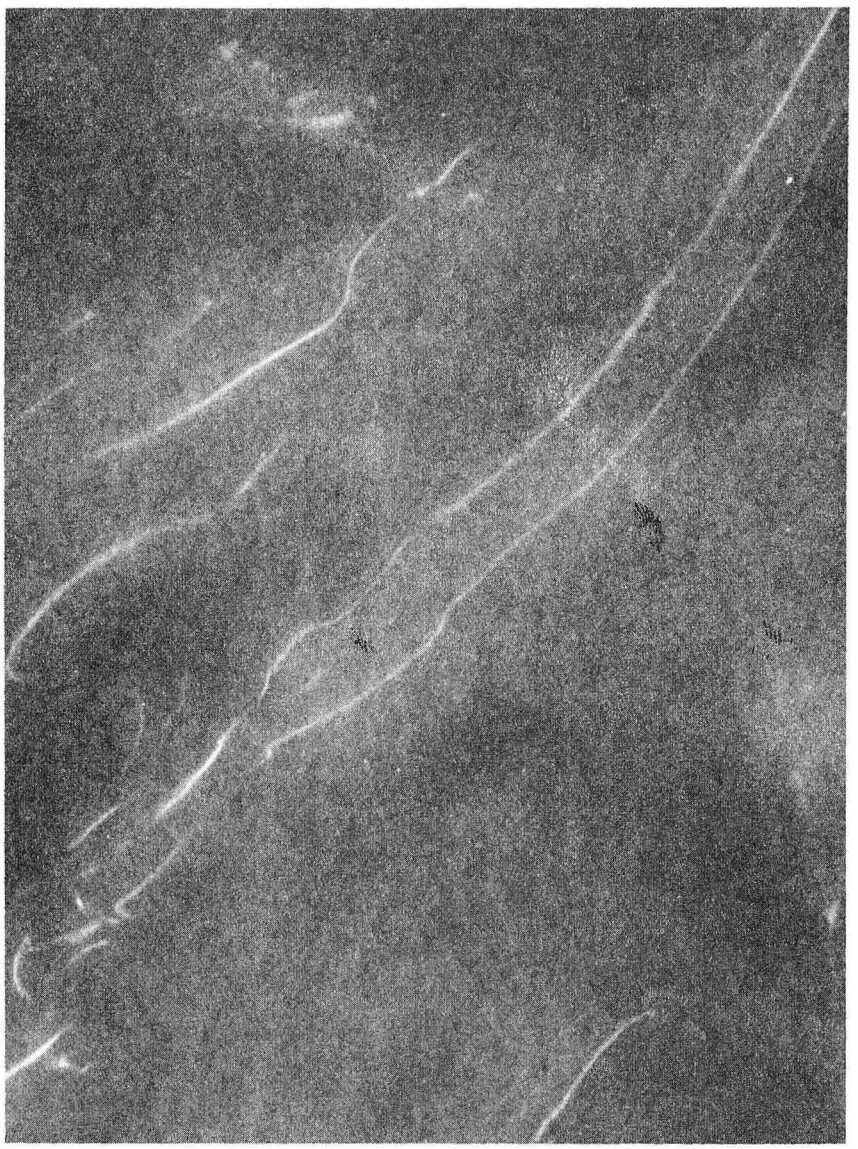


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Fig. 4. Dark-field illumination electron micrograph of crystalline 1-valine using the corresponding Bragg reflections excited in Fig. 3. Note reversal of contrast.

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