EDGE-PROJECTION TOMOGRAPHY

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A biological structure can be reconstructed in three dimensions from a series of transmission electron microscope (TEM) images taken as a support grid is tilted over a large angle. A back-projection (tomographic) algorithm using a radially weighted Fourier transform of each tilted image can be used for this purpose. The accuracy of reconstructing a nonsymmetric structure is limited by the number of micrographs that can be taken, and by the total tilt angle that can be achieved. Thirty independent views of a nonsymmetric structure (taken over a total tilt angle of ~180 degrees) are needed to resolve a nonsymmetric structure to ~3nm. The total tilt angle is usually limited by geometric constraints in the microscope to ~120 degrees. The number of micrographs that can be taken is limited by the integrated electron dose a structure can withstand without damage. We are using edge-projection TEM imaging to overcome these difficulties.

A conventional TEM image of a biological structure is formed by passing an electron beam through the structure and through a thin, underlying support film placed on a grid. In edge-projection TEM imaging (EPTEM), a biological structure is supported on the highly curved apex of a slender, needle-like "tip" that is positioned at right angles to the imaging direction. An EPTEM image shows the profile of a structure on the edge of the tip apex seen in silhouette. An EPTEM tomographic reconstruction is made from a series of sagittal EPTEM images generated by rotating the tip through 360 degrees about its axis (Fig. 1). A commercial side entry goniometer stage has been modified to provide full tip rotation without tilting the stage (Fig. 2). Substrate contrast does not contribute to the contrast generated by a structure in a series of EPTEM images (Fig. 3).

We are investigating several ways of displaying structures that have been reconstructed from a series of EPTEM images. A back-projection algorithm provides a series of density maps that can be combined, and viewed in perspective in the usual way. If each micrograph in a 360° rotation series is digitized and treated as an opaque profile of a structure at a given angle, simply adding the digital images together in the proper perspective may be sufficient.

References
7. Hitachi bulk translation specimen stage, model HS001LT.
8. TEM support by Mary A. Raymond (UNM School of medicine).
Fig. 1.—Edge-projection (TEM) tomography.

Fig. 2.—Hitachi H5001LT specimen stage (modified) with tip at $0^\circ$ (1), $120^\circ$ (2), $180^\circ$ (3).
Fig. 2.—Phi-X virus (rotary shadowed with tungsten) at $0^\circ$ (4), $120^\circ$ (5), $180^\circ$ (6). Bar = 50nm.