

Elemental Tomography of Biological Structures

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Advances in energy-filtered electron microscopy (EFTEM) including the availability of charge-couple device (CCD) detectors with high efficiency and algorithms that can correct for specimen drift make it reasonable to consider the feasibility of elemental tomography. In such experiments, maps of an element are recorded in a tilt series and then the three-dimensional distribution of that element is reconstructed within the specimen volume [1-3]. Recently, this approach has been applied to determine the three-dimensional elemental composition of inorganic materials [4-6]. Here we investigate the feasibility of performing elemental tomography of biological structures.

It was decided to test the technique of elemental tomography by mapping phosphorus in the nematode, *Caenorhabditis elegans*. To map phosphorus by EFTEM, special consideration must be given to the specimen preparation. Specifically, the presence of heavy metals strongly interferes with the P L_{2,3} edge in the energy-loss spectrum at 132 eV, so fixatives like osmium tetroxide and stains like uranyl acetate must be avoided. On the other hand, the ultrastructure must be maintained without the heavy metals that, in osmicated and stained preparations, help to preserve the morphology. Therefore specimens were prepared by high-pressure freezing at liquid nitrogen temperature in a Baltec HPM 010 machine, and they were subsequently freeze-substituted, embedded in epon-araldite, and sectioned to a thickness of approximately 70 nm. The unstained sections were analyzed in a Philips/FEI CM120 transmission electron microscope interfaced to a Gatan GIF100 imaging filter that was equipped with a cooled 1024x1024 pixel CCD camera [7]. Phosphorus maps were acquired at a beam voltage of 120 kV using the Gatan Digital Micrograph program. Specimen were tilted through a range of $\pm 60^\circ$ at increments of 5° to obtain the projected phosphorus distributions in tomographic series. A phosphorus map was acquired at each tilt-angle using the three-window method, in which two pre-edge energy-selected images are recorded (92 ± 5 eV and 127 ± 5 eV) to obtain a background fit, and one image acquired above the edge (157 ± 5 eV), from which the extrapolated background image is subtracted.

A zero-loss, bright-field, micrograph from a region of a *C. elegans* cell in Fig. 1 shows an almost complete lack of contrast in the unstained specimen. Fig. 2 shows phosphorus maps recorded at tilt angles of (a) 0° and (b) 50° . Bright features in the maps are attributed to the presence of ribosomes that contain phosphorus in their RNA. Despite the high electron dose required for mapping phosphorus with a sufficient signal-to-noise ratio, specimens appeared to be stable during tilt series.

Efforts are now in progress to determine the three-dimensional distribution of phosphorus, by using the IMOD program developed by the University of Colorado [8]. Preliminary results indicate that plural inelastic scattering does not compromise collection of phosphorus maps at a beam voltage of 120 kV in specimens with a nominal section thickness of 70 nm that are tilted to angles as high as 60° . This rather surprising finding, that elemental mapping is possible for effective thicknesses along the beam direction of 140 nm, may be explained by the low density of the plastic embedding resin and to significant mass loss during the initial exposure.

The effective resolution of elemental tomography in biology is limited by radiation damage since the electron dose must be increased by a factor of $\sim 10^3$ relative to that required for standard tomography. Nevertheless, our preliminary results indicate that plastic sections become quite stable after an initial expose to the electron beam. Potential applications for phosphorus tomography could include mapping the distribution of DNA in the nucleus. Furthermore, the distribution of phosphorus within the volume of the specimen can be made quantitatively. Finally, it is worth noting that at higher beam energies, elemental tomography could be performed on even thicker specimens.

References

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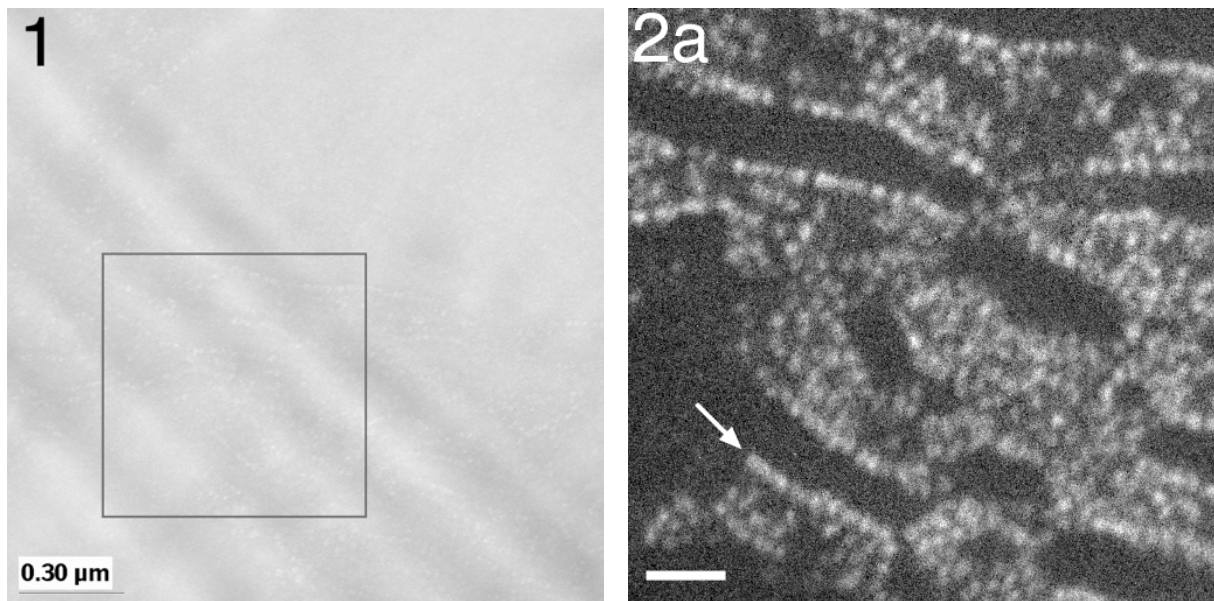


FIG. 1. Zero-loss image of region of cell in unstained, unosmicated plastic section of high-pressure frozen and freeze-substituted *Caenorhabditis elegans*, showing the almost complete lack of contrast.

FIG. 2. EFTEM phosphorus maps from region of cytoplasm indicated by box in Fig. 1. (a) 0° specimen tilt and (b) 50° specimen tilt. Bright features are ribosomes associated with endoplasmic reticulum and cytoplasm. Distributions were generated using three-window technique with acquisition times of approximately 30 s per elemental map. Arrows indicate row of ribosomes at the two tilt angles. Recording such tilt series from -60° to +60° should enable 3-D phosphorus distributions to be obtained. Bars = 100 nm.

