Quantitative EFTEM and STEM Tomography of Soft Materials

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Electron tomography based on the collection of elastic and inelastic scattering in the transmission electron microscope (TEM) can provide quantitative three-dimensional compositional information about soft materials. Using a focused probe in the scanning TEM (STEM) mode to acquire a series of elastic annular dark-field images from a specimen tilted successively through a range of angles enables us to reconstruct the 3D distribution of heavy-atom clusters that are bound to structures contained within a low atomic number matrix [1-3]. And using wide-field illumination in the energy filtering TEM (EFTEM) mode to acquire a tilt series of inelastic core-edge images enables us to determine the 3D distribution of specific chemical elements within the specimen [4]. Quantitative analysis is achieved by normalizing the 2D projection image intensities by the relevant elastic or inelastic scattering cross section and by using an appropriate reconstruction algorithm. This approach provides not only information about the numbers of atoms per voxel in the reconstructed volumes but also estimates of detection limits for a given electron dose.

We have used EFTEM and STEM tomography [5,6] to analyze the structure and composition of sectioned plastic embedded biological specimens. In biological materials elements of interest often occur at concentrations below one atomic percent within a pixel of a 2D projection through the specimen, which necessarily results in weak core-edge signals. Therefore to apply EFTEM tomography, it is important to model carefully the background underlying weak core edges and to consider how the background shape is influenced by plural inelastic scattering. In the technique of quantitative electron spectroscopic tomography (QuEST), we not only acquire pre-edge and post-edge images for a specific element at each tilt angle but we also obtain zero-loss and unfiltered images from which we can estimate the relative specimen thickness at each pixel [4]. Then, through modeling plural scattering effects, we can accurately subtract the background and thus determine the number of atoms of the element per pixel. Furthermore, by using a simultaneous iterative reconstruction technique (SIRT) algorithm, we can deduce the numbers of atoms of the element per voxel, as well as the theoretical uncertainty in the numbers of atoms. We have applied this approach to determine the 3D distribution of DNA within cell nuclei based on the phosphorus content of nucleic acids [7]. For total electron exposures of 5×10^7 electrons/nm^2 and a beam voltage of 300 kV, the detection limit for phosphorus in a specimen of thickness 100–150 nm is estimated as 20 atoms within a voxel of size 20 nm^3.

The high electron dose for QuEST analysis requires an assessment of beam damage, which can cause changes in elemental composition, total mass and morphology. Despite a rapid initial mass loss of around 40 percent and a concomitant reduction of specimen thickness at a dose of 10^4 electrons/nm^2, no significant loss of phosphorus or nitrogen is observed at doses as high as 10^8 electrons/nm^2 [8], although oxygen and carbon are lost as volatile molecular fragments at much lower doses. A lateral shrinkage of around ten percent occurs at doses between 10^4 to 10^8 electrons/nm^2, and at very high doses there is evidence for knock-on damage corresponding to direct ejection of light atoms from the specimen surface through rare high-angle elastic scattering events.

This process results in a gradual linear reduction in specimen mass with the loss of 10% at a dose of 10^8 electrons/nm^2. Nevertheless, it is still feasible to obtain useful 3D phosphorus and nitrogen maps, and thus to reveal quantitative information about the subcellular distributions of biological molecules.

Annular dark-field STEM tomography is the technique of choice for determining the 3D arrangement of heavy-atom clusters that are bound to structures within soft materials. For example, in biological specimens, heavy-atom clusters, such as Nanogold (67 Au atoms) or Undecagold (11 Au atoms), can be attached to specific cellular proteins of interest [1]. We have used STEM tomography at a beam voltage of 300 kV to detect Nanogold and Undecagold in a variety of specimens and imaging conditions; and we have modeled the signal from gold clusters embedded in a carbon matrix by means of the *NIST Elastic Scattering Cross Section* database [9]. Experiments and simulations show that Nanogold clusters can easily be imaged in sections of thickness 100 nm and Undecagold in samples of thickness 40 nm. Our measurements also demonstrate that it is possible to detect Nanogold in plastic sections of tissue freeze-substituted in the presence of osmium, which is a standard preparation procedure.

Recently we have also shown that STEM tomography can provide 3D structural information from one-micrometer thick sections of plastic embedded biological material that is stained with heavy atoms [10]. A narrow probe convergence angle of less than 2 mrad minimizes geometrical beam broadening while maintaining a diffraction-limited resolution of approximately 1 nm [11]. We have found that an axial bright-field detector provides improved spatial resolution throughout the specimen thickness relative to an annular dark-field detector because axial detection excludes multiply scattered electrons that have broader spatial and angular distributions at the exit surface [2].

Although we have applied these quantitative tomography techniques to biological structures in our laboratory, the same approaches should be applicable to other types of soft materials, including biomaterials and polymer systems [12].

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