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The Nuts and Bolts of Electron Tomography: Imaging of Big and Messy Biological Structures

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Proteomics has been described as the attempt to capture the dynamics of a living system from the collection of proteins that are up and down regulated during a cell's lifetime [1]. Protein structures once thought of as complex are being determined to atomic resolution. Another approach has been to integrate protein structures at the atomic level into lower resolution larger macromolecular complexes obtained by molecular microscopy [2, 3]. However, a challenge for structural biology is recognizing, analyzing, and integrating these protein structures into macromolecular assemblies *in situ* at complexity levels ranging from organelles to tissues.

Tomography is a generalized method for reconstructing in 3D the interior of an object from independently acquired 2D views or projections. Examples of tomography applications include not only those for electron microscopy (called electron tomography) but also computerized axial tomography (CAT)-scan imaging for medical imaging. The principles of the data collection and reconstruction are the same in spite of the different resolution scales and specimen preparation methods. Electron tomography involves the tilting of the specimen holder about a common axis and recording images in a broad angular range so that the 3D structure will have adequate resolution along the three axes. The specimen is tilted usually along a single axis from –60° to 60° with images (projections) recorded at equal 1 or 2° increments. If recorded on film, the micrographs are digitized. The data are aligned to a common origin a fiducial alignment algorithm where the markers are 10 or 20 nm gold particles put on top of the sections. Computer programs track the fiducials and calculate a least squares fit of the fiducial markers. A volume is calculated using standard reconstruction algorithms. The whole volume is visualized by rendering methods or specific features in it or can be analyzed by segmentation. A 3D structure is obtained containing all internal substructures with the potential of mapping the location and segregation of functionally important macromolecules in 3D [4, 5].

Using electron tomography, new information about the 3-dimensional ultrastructure of tissues, cells and macromolecular complexes can be accurately and easily obtained and studied (as reviewed in [6, 7]; see examples in Fig. 1). Improvements and the availability of higher voltage (> 200 kV) microscopes with computer control of specimen stages, digital image recording and brighter electron sources have moved electron tomography from a specialized technique of a handful of laboratories to one that is becoming an essential tool of biologists interested in structure . Electron tomography is fast becoming as important a structural technique as cryo-EM/three-dimensional reconstruction of isolated macromolecular components. In addition, the increased computational power, software design, automation of data collection and processing and decreased costs of modern computing associated with new workstations and clusters have changed the time course of obtaining tomography structures to ~1-3 days once the data is collected. The increased computing power has also facilitated the determination of larger scale structures over microns but at sampling sizes sufficient to identify cellular components such as microtubules and vesicles. Interpretation and

segmentation still remains the most time consuming step for large and complex structures such as tissues, however, new algorithms and approaches are being developed to facilitate automation or semi-automation. This tutorial outlines the basic steps for obtaining and reconstructing tomograms from a tilt series of electron micrographs. This includes optimal conditions for data collection, alignment of the series and diagnostics of good alignment, a brief discussion of reconstruction algorithms and segmentation or redaction of the volume. Also included in this tutorial will be a discussion of recent technological developments that has advanced this field (such as those described above). Finally, I will discuss the reconstruction of larger scale objects either in the x-y plane using image montaging or in the z-direction that involves the use of serial section electron tomography that allow for imaging of multicellular and multicomponent structures.

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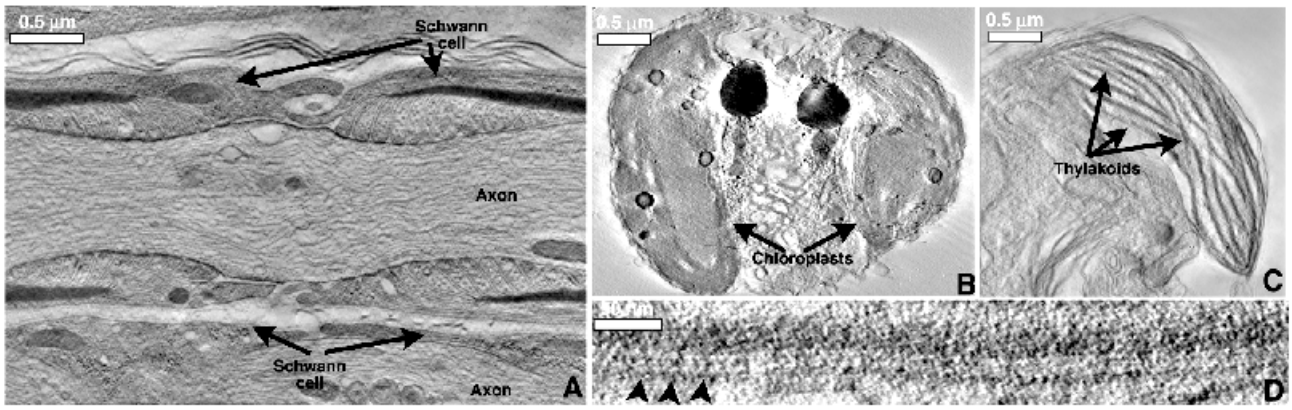

FIG. 1 Examples of a slice from 3D volumes of large scale structures that have (**A**) different cell types such as dorsal spinal roots (neurons, Schwann cells); (**B**) an entire unicellular organisms (the alga *Phaeocystis antarctia*); (**C**) an organelle (chloroplast from a *Phaeocystis* alga showing stacking and continuity of thylakoids); and (**D**) an *in situ* selectively enhanced gap junction between two tissue culture cells showing striations from the side views of the intercellular channels. Note the clarity of features.

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