doi:10.1017/S1431927613004789
Microsc. Microanal. 19 (Suppl 2), 2013

Three-Dimensional Reconstruction of Whole Synapses by STEM Tomography

A.A. Sousa[1], J. Zhang[2], X. Chen[3], J.S. Diamond[2], T.S. Reese[3], R.D. Leapman[1]

[1]Laboratory of Cellular Imaging & Macromolecular Biophysics, NIBIB, NIH, Bethesda, MD 20892
[2]Synaptic Physiology Section, NINDS, NIH, Bethesda, MD 20892
[3]Laboratory of Neurobiology, NINDS, NIH, Bethesda, MD 20892

Scanning transmission electron microscope (STEM) tomography [1-3] enables determination of 3D ultrastructure of embedded cells sectioned to a thickness of 1 to 2 μm [4-7]. Such specimens are considerably thicker than can be analyzed by conventional TEM tomography, for which resolution is limited by chromatic aberration due to multiple inelastic scattering. We utilize a probe of small angular convergence (~ 1.6 mrad) to give a diffraction-limited probe diameter of ~1nm. The small angular convergence also results in a large depth of field throughout the specimen thickness since geometrical spreading of the probe is less than ~3nm [5,6]. Moreover, the use of an on-axis bright-field detector reduces effects of beam broadening by multiple elastic scattering in the lower part of thick specimens, thereby improving the spatial resolution of STEM images compared to those acquired with an annular dark-field detector.

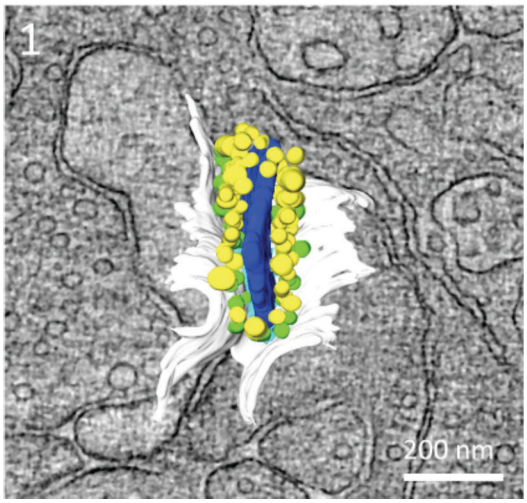
We have recorded dual-axis bright-field STEM tomograms using an FEI Tecnai TF30 transmission electron microscope equipped with a Shottky field emission gun operating at an acceleration voltage of 300 kV. Specimens were prepared by fixation, dehydration, embedding, ultramicrotomy and post-staining, with or without rapid freezing and freeze-substitution. Gold nanoparticles were deposited on the top and bottom surfaces of the sections to aid in alignment of the tilt series. Images were acquired over an angular range of ±54° after pre-irradiation with a broad beam in TEM mode to stabilize ultrastructure. Tomograms were reconstructed by means of the IMOD program (University of Colorado) [8], and visualized with the Amira software package.

The STEM tomography approach is ideally suited to visualizing whole neuronal synapses, which have dimensions of order 0.5 to 1 μm, and for making quantitative measurements on the numbers, sizes and shapes of synaptic components. For example, we have applied the technique to study the architecture of two types of retinal ribbon synapses, which are specialized structures at presynaptic active zones encoding a wide dynamic range of sensory signals through continuous vesicle release [9]. We have thus determined a full 3D architecture of ribbon synapses in mammalian (rat) cone photoreceptor cells and rod bipolar cells [10]. The reconstructions showed regular docked and tethered vesicles (Fig. 1).

In another application of STEM tomography, we have reconstructed entire spine postsynaptic densities (PSDs) in cortical regions of mouse brain (Fig. 2). It was possible to determine the thickness, shape and area of PSDs for every synapse within defined (e.g., 8 μm x 8 μm x 1 μm) volumes of neuropil [11]. We have also used the technique to determine structural changes after RNAi knockdown of specific PSD95 scaffolding proteins in cultured hippocampal cultures [12].

STEM tomography of thick sections provides a useful approach for determining the nanoscale structure of entire synapses in different types of neuronal tissues, which can help to gain insight into specialized synaptic function [13].

References
[1] P.A. Midgley et al., *Ultramicroscopy* 96 (2003) 413.
[2] I. Arslan, J.R. Tong, P.A. Midgley, *Ultramicroscopy* 106 (2006) 994.
[3] A.E. Porter et al., *Nature Nanotech.* 2 (2007) 713.
[4] A.E. Yakushevska et al., *J. Struct. Biol.* 159 (2007) 381.
[5] M.F. Hohmann-Marriott, A.A. Sousa et al., *Nature Methods* 6 (2009) 729.
[6] A.A. Sousa et al., *Ultramicroscopy* 109 (2009) 213.
[7] A.A. Sousa et al., *J. Struct. Biol.* 174 (2011) 107.
[8] J.R. Kremer, D.N. Mastronarde, J.R. McIntosh, *J. Struct. Biol.* 116 (1996) 71.
[9] J.S. Diamond, *Nature Neuroscience* 14 (2011) 1097.
[10] J. Zhang et al *Proc. ARVO* (2012) 4318.
[11] X. Chen et al., *Springer Protocols Neuromethods Series: Imaging Nanoscale Dynamics at Synapses* (2012) in press.
[12] X. Chen et al. *J Neurosci.* 31 (2011) 6329.
[13] This research was supported by the intramural programs of NIBIB and NINDS, NIH.



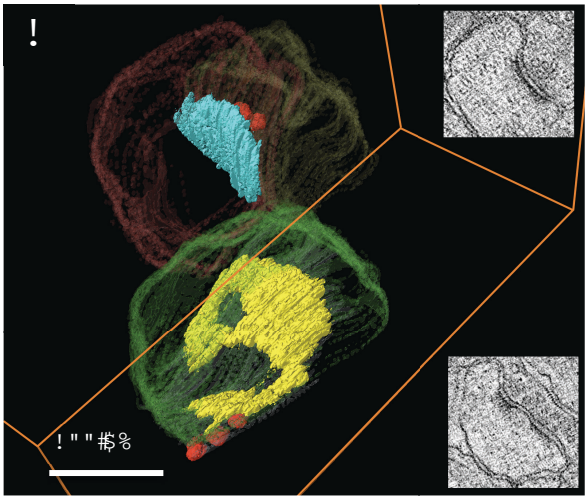


FIG. 1. STEM tomography of 1.2-µm thick epon section of rat retina showing entire ribbon synapse in cone photoreceptor cell, superimposed on an orthoslice through 3D reconstruction. Surface rendering shows plate-like rectangular morphology of central ribbon surrounded by synaptic vesicles, some of which are tethered to the pre-synaptic membrane.

FIG. 2. STEM tomography of 1-µm thick freeze-substituted preparation of mouse cortical brain region showing surface rendering of two adjacent synapses whose entire postsynaptic densities (PSDs) are reconstructed. The upper PSD has a continuous disk shape, whereas the lower PSD is perforated. Insets show orthoslices through the reconstructed synapses.

https://doi.org/10.1017/S1431927613004789 Published online by Cambridge University Press