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IT-10-O-2543 On-axis electron tomography of needle-shaped biological samples

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A key challenge in structural biology is to image large volumes while maintaining sufficient resolution to identify small features in their original cellular context [1]. Electron tomography (ET) has contributed greatly to this field, but imaging sections thicker than a few hundred nanometers is difficult because of the sample geometry and microscope configuration: as the specimen is tilted to high angles, the thickness increases and the quality of the images deteriorates. Moreover, for geometric constraints, the tilt range rarely exceeds ±70°, leading to elongation and blurring of features, and an overall challenging volume to segment. Here, we show that preparing a needle-shaped sample rather than a flat section can alleviate many of the limitations encountered in biological ET. The technique is illustrated on a 500nm diameter needle extracted from an epoxy-embedded portion of the nucleus accumbens shell from a rat brain. The sample was prepared in a Helios NanoLab focused ion beam (FIB) machine and transferred to an on-axis tomography holder. An HAADF-STEM tilt series was then acquired from -90° to +90° with 1° increment, using an FEI TITAN microscope operating at 300kV, and Inspect3D was used for the alignment and reconstruction by weighted backprojection. Figure 1(a) shows a low magnification view of the needle and the region selected for tomography. A voxel projection and slice through the reconstructed needle (b,c) provide highly detailed structural information. In Figure 2, we compare the quality of ET results from -90°:2°:+90° and -60°:2°:60° acquisitions. Cross-sections through a portion of exitatory synapse and mitochondrion (positions 1 and 2 in Figure 1(c)) illustrate the advantages of a full tilt range on-axis ET experiment with enhanced signal-to-background ratio and isotropic sharpness of features. Note that the ±60° cylindrical volume shown here is still of better quality than a reconstructed section from similar tilt range, since the thickness remains constant throughout the tilt series.
Combining this novel sample preparation technique with advanced imaging modes (BF-STEM for example [2]) and sophisticated reconstruction algorithms such as compressed sensing [3], we anticipate that ET will provide a complementary method to serial sectioning and FIB-SEM slice-and-view techniques [4].

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[2] A.A. Sousa et al., Journal of Structural Biology 2011, 174(1): 107.
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[4] Samples provided by Andrea Falqui and Roberto Marotta, IIT, Genova, Italy.

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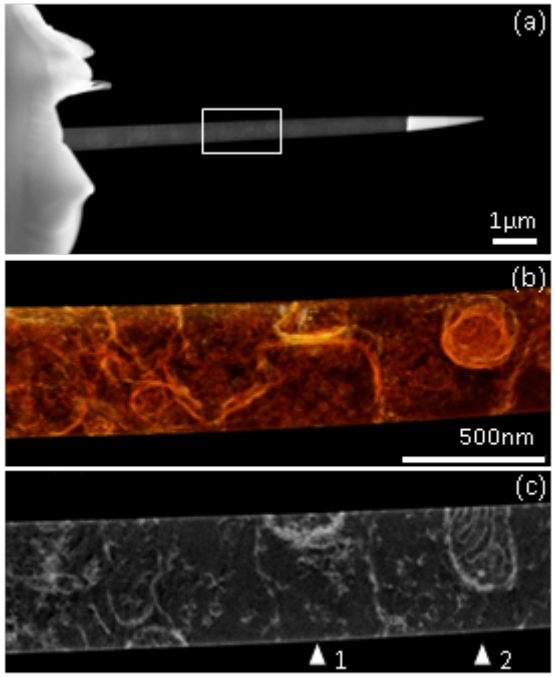

Fig. 1: (a) Needle-shaped biological sample, prepared by FIB. The rectangle indicates the area selected for electron tomography. (b) Voxel projection of the reconstruction from a full range acquistion. (c) A slice through the volume showing a detailed view of an exitatory synapse (1) and a mitochondrion (2).

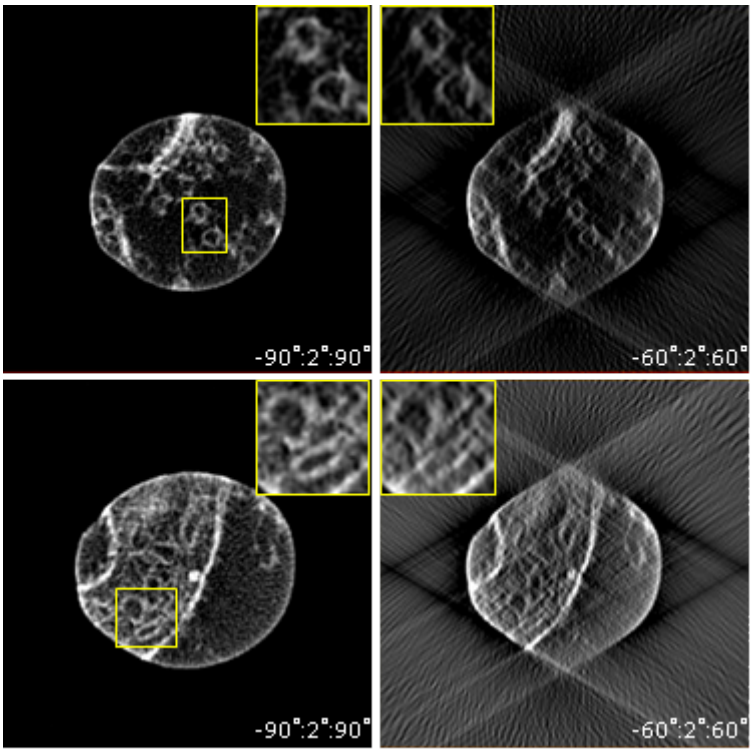

Fig. 2: Cross-sectional slices through the synapse (a,b) and mitochondrion (c,d) with different tilt ranges. Isotropic resolution is observed for full tilt range on-axis tomography (left), as illustrated in the insets.