

Automated Serial Section Large-field Transmission-Mode Scanning Electron Microscopy (tSEM) for Volume Analysis of Hippocampus Ultrastructure

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Serial thin sections of ~50 nm thickness have been used to visualize and reconstruct cellular and subcellular structures in a three-dimensional context from a wide variety of biological systems. In the central nervous system, serial section (ss) EM (traditionally on a Transmission Electron Microscope platform) provides sufficient resolution and extent to reveal cellular and subcellular structures and normal and pathological changes in neuronal morphology within the context of the surrounding neuropil, including dendrites, axons, and astroglial processes [1]. Renewed interest in EM as a high-resolution 3D tool for neuroscience has led to improvements over the last decade in this time- and skill-intensive method. Much of the innovation has occurred without the transmission signal using serial block-face or serial section array imaging on an SEM platform [2] [3] [4] [5].

Large-field scanning electron microscopy in the transmission mode (tSEM) on a field emission SEM platform was developed for efficient and cost-effective imaging of circuit-scale volumes from serial ultrasections of hippocampus. Individual scanned image area is maximized and STEM detection is used to optimize the XY resolution and the dynamic range necessary for discriminating particularly small but key subcellular structures (vesicles, microtubules, ribosomes). Image fields from the tSEM system were up to 65 μm per side at 2 nm pixel size, contrasting with image fields from a modern TEM system at about 8 μm per side at the same pixel size. The tSEM produced quality images with negligible scan or beam-caused distortion, no apparent charging effects, and with reduced drift relative to TEM [6]. Automated stage and scan control in this tSEM system and additional software provided unattended and stable serial section imaging and montaging across multiple grids on overnight runs. Images were stacked and registered across serial sections with elastic alignment tools in Fiji/TrakEM2 software and exported for tracing and volume analysis in RECONSTRUCTTM [7] [8].

Axial resolution dependence on section thickness limits segmentation accuracy and the analysis of small structures or for very oblique membranes contained within the thickness of a section. Current research pursues improvement in both axial resolution and the extent of the volume along this dimension, within the context of serial cutting and large-field tSEM at various accelerating voltages. *En bloc*-only tissue staining methods now have the quality and intensity at the EM level integral to these pursuits. Simultaneous multi-channel acquisition at greater bit depth and tilt tomographic strategies are being investigated. Further progress will be made with automated stage movement, transmitted detector modifications and signal partitioning, and flat and robust electron-transparent section supports [9].

References:

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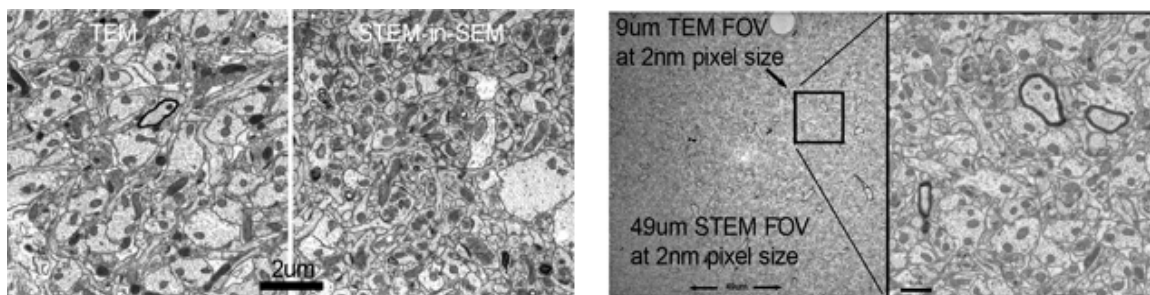


Figure 1. TEM and tSEM quality comparable on same ultrasection preparation. Image scan dimensions comparing 24k x 24k pixels tSEM to 4k x 4k TEM CCD field at 2nm pixel size sufficient for brain ultrastructure.

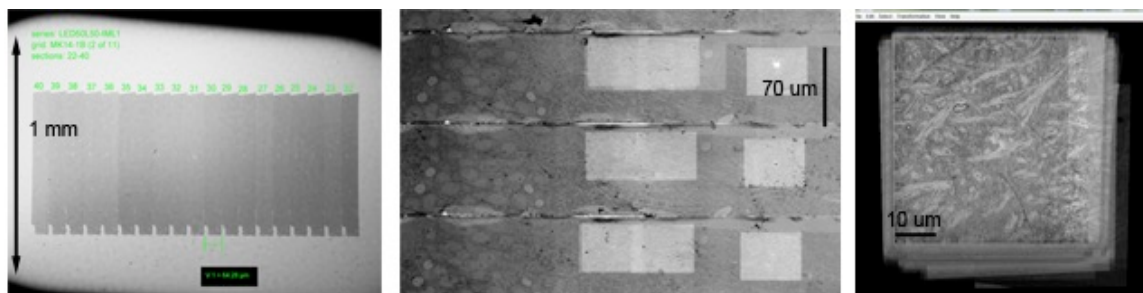


Figure 2. TEM grid with polyimide support and ultrasection series. Several sections of hippocampal arbor showing scan areas (light squares). Stack of a series of ~200 images with TrakEM elastic alignment.