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Biologically Accurate Modeling of Mouse Brain Requires Biologically Accurate Networks

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Biologically accurate brain networks are made possible by a combination of new methods in electron and light microscopy. Two new instruments, both using physical sectioning, make this possible: the *Serial Block Face Scanning Electron Microscope (SBF-SEM*) [1], invented by Winfried Denk, Max Planck Institute, Heidelberg, and the *Knife-Edge Scanning Microscope (KESM)* [2], a light microscope invented by Bruce McCormick, Texas A&M University. The SBF-SEM necessarily relies on heavy element staining of tissue to provide image contrast. The KESM light microscope is unique in its ability to acquire 3D brain microstructure from tissue stained in common with identical heavy element stains.

Shown here are exploratory tracings and reconstructions of mouse brain tissue stained with osmium tetroxide and scanned with the KESM. Segmenting the osmium datasets poses a unique challenge because, unlike Nissl or Golgi stains, osmium staining gives a rich axonal detail, throughout the tissue. The osmium images, at lower resolution, look not unlike those seen at higher resolution in the SBF-SEM. Also shown are exploratory tracing and reconstruction from an SBF-SEM dataset. Our exploratory image analysis of brain tissue stained with heavy elements, both with light microscopy and with electron microscopy, has forced us to adopt a new image analysis paradigm: *Stain all neurons; reconstruct selectively*. This reverses the conventional wisdom held previously: *Stain sparsely, reconstruct exhaustively.* In the new strategy we can return to the original data set for additional data, if required; in the old strategy, there is no additional data to return to, at least not in the same mouse.

The Stanford group is developing high-contrast heavy-element staining methods to be used with a new, automated Serial Block Face Scanning Electron Microscope (SBF-SEM) for tracing neural circuit wiring diagrams [3]. The SBF-SEM provides, for the first time, both the high resolution and the large, three-dimensional field of view needed to track small and tightly packed axons and dendrites over the entire volume of a functional microcircuit. The SBF-SEM enables tracking of even the finest (< 150nm in diameter) axons and dendrites through complex synaptic neuropil (sampled with a voxel size of 12.5nm × 12.5nm × 50nm) [1].

Sectioning and imaging for the nanoscale (SBF-SEM) was done in Heidelberg by Winfried Denk's group, and for the microscale (KESM), at Texas A&M. Selected regions in the cortex (e.g., barrels in the somatosensory cortex) were stained, sectioned, and imaged. Use of common staining methods in the two modalities has allowed us to directly compare and register data from the two levels of special resolution.

Initial data compression and segmentation of the observed volume datasets uses our polymerization algorithms, developed earlier [4,5,6]. The strategy is based on *L-blocks*, which are *k*-dimensional axis-aligned iso-rectangular block of voxels (vertices) with associated connectivity (edges). This segmentation is also used to find seed points for the initiation of tracing, using our modification of the vector tracing algorithm of Al-Kofahi et al [7]. Once fiber trajectories and their branch points have been found, the generalized cylinders surrounding these trajectories are geometrically modeled, and then visualized.

The electron and light microscopy data closely mirror each other in almost all aspects, from the heavy element staining, automated sectioning, imaging, image processing, all the way to 3D volume reconstruction. We are developing algorithms for SBF-SEM image analysis and 3D reconstruction, to automate reliable and exhaustive recognition and tracking of circuit elements through a volume comparable to a full mammalian cortical microcolumn. These same methods, with minor modifications, suffice to process heavy element stained brain tissue volume datasets acquired by the KESM. Thus we have developed a common set of methods and software tools to simultaneously address the needs of brain image analysis at these two scales, nano- and micro-scale.

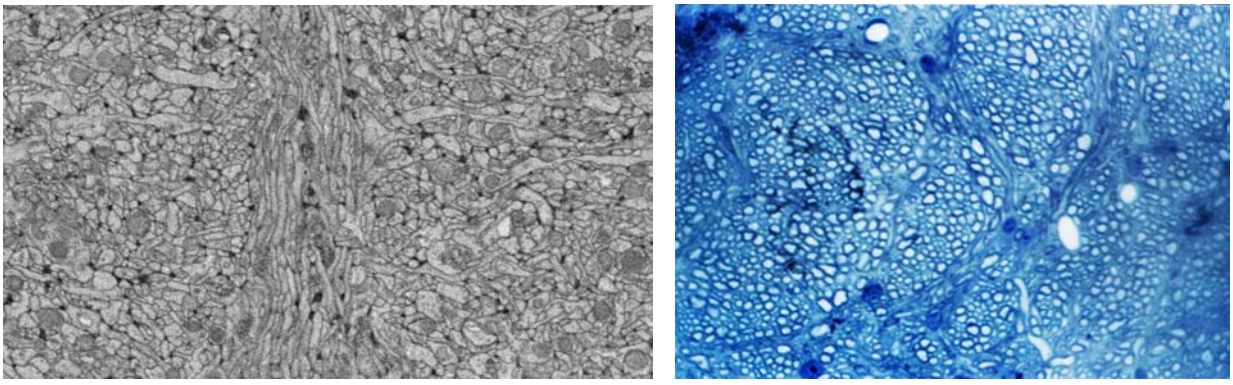

Fig. 1. SBF-SEM scanned section (left) and osmium section 40X light microscopy (right).

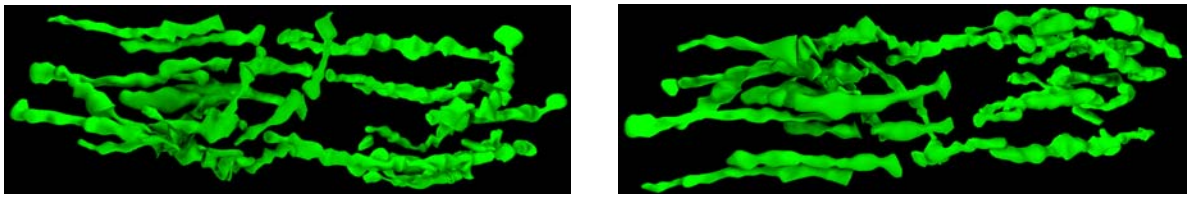

Fig. 2. Selective isosurface reconstructions of SBF-SEM data.

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