

Serial Block-Face Scanning Electron Microscopy to Reconstruct Three-Dimensional Tissue Nanostructure

WinfriedDenk
Max-Planck Institute for Medical Research
Heidelberg, Germany
denk@mpimf-heidelberg.mpg.de

Three-dimensional (3D) structural information on many length scales is of central importance in biological research. Excellent methods exist to obtain structures of molecules at atomic, organelles at electron microscopic, and tissue at light-microscopic resolution. A gap exists, however, when 3D tissue structure needs to be reconstructed over hundreds of micrometers with a resolution sufficient to follow the thinnest cellular processes and to identify small organelles such as synaptic vesicles. Such 3D data are, however, essential to understand cellular networks that, particularly in the nervous system, need to be completely reconstructed throughout a substantial spatial volume. Here we demonstrate that datasets meeting these requirements can be obtained by automated block-face imaging combined with serial sectioning inside the chamber of a scanning electron microscope. Backscattering contrast is used to visualize the heavy-metal staining of tissue prepared using techniques that are routine for transmission electron microscopy. Low-vacuum (20-60 Pa H₂O) conditions prevent charging of the uncoated block face. The resolution is sufficient to trace even the thinnest axons and to identify synapses. Stacks of several hundred sections, 50-70 nm thick, have been obtained at a lateral position jitter of typically under 10 nm. This opens the possibility of automatically obtaining the electron-microscope-level 3D datasets needed to completely reconstruct the connectivity of neuronal circuits.

[1] Serial block-face scanning electron microscopy to reconstruct three-dimensional tissue nanostructure. *PLoS Biol.* 2004 Nov;2(11):e329.