Serial block-face electron microscopy of neuronal circuits in the retina

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The computations performed by neural circuits are defined by the physiological properties of individual neurons and the synaptic connectivity amongst them. While ever more sophisticated methods to record and manipulate the physiology of neurons have appeared over the last few decades, methods for the anatomical reconstruction of synaptic connectivity have lagged behind.

This is in large part due to the disparate length scales involved; the processes (including dendrites and axons) of neurons can be as thin as 50 nm and yet extend over many millimeters. The tortuous trajectories of these processes in the brain necessitate the ability to acquire large volumes at high resolution in all 3 spatial dimensions. High lateral (x-y) resolutions of 5-10 nm are easily achieved in scanning electron microscopes, but the major challenge has been to repeatedly cut tens of thousands of thin sections (each at least <50 nm) from a block of tissue. Historically, tissue sectioning is performed manually using an ultramicrotome which is tedious, error-prone and is limited to, at best, 50 nm sections. We have therefore automated both image acquisition and sectioning by developing a serial block-face scanning electron microscopy (SBEM) technique. This method allows us to image large 3-dimensional EM datasets, typically hundreds of microns on a side, at nearly isotropic voxel resolutions of 12 x 12 x 25 nm^3.

I will discuss the technical aspects of SBEM in relation to other current volume electron microscopy techniques and present some of the first neurobiological results using this technique. In particular, I will stress the power of combining functional recordings of neurons, such as two-photon excited fluorescence calcium imaging, with subsequent anatomical reconstruction of neuronal circuits in the mouse retina. I will also briefly discuss the next major challenge of how to efficiently analyze large (terabyte-sized) SBEM datasets.