Tracking the Ultrastructure of Life: Serial block-face imaging to investigate cells and tissue in 3D

A. Elli[1], N. Braun[1]

[1] ZEISS Microscopy.

Abstract

Life is 4D. To unlock its secrets, we need to explore it in its spatial three dimensionality. Samples from different biological research areas regardless of whether the sample is cells or tissue are imaged under various imaging modalities to uncover their secrets. Each of these methods is coming with specific strengths and limitations. Light microscopy is the method of choice for live cell imaging and functional investigations but is limited by resolution. Electron microscopy (EM) can complement the picture of the sample going beyond the resolution limit of light microscopes by revealing sub-cellular structures with nanometer resolution. In comparison to transmission electron microscopes (TEM), scanning electron microscopy (SEM)-based techniques open a range of possibilities enabling scientists to overcome typical experimental limitations such as limited field-of-view and small sample volume size to better understand ultrastructural details within a larger 3D context. One prominent volume EM (vEM) method is serial block-face SEM (SBF-SEM), a technology first developed and communicated by Denk et al. in 2004 [1]. In SBF-SEM, an ultramicrotome inside the SEM chamber cuts 15 - 30 nm thick sections from a resin-embedded sample block. The exposed sample surface is imaged with an electron beam, then new sections are cut away with a diamond knife, and the newly exposed block-face surface is imaged. This cutting and imaging process is repeated until the structure of interest is completely imaged. The acquired EM images are processed and digitally aligned into a 3D data set. Cell compartments can be identified and segmented from this z-stack. The segmented 3D data set can be visualized, investigated, and statistically analyzed. The illustration in figure 1 outlines the SBF-SEM workflow. Robustness and automation of this technique even allow imaging and tracking of cells with extended protrusions over a long range, an application requirement in e.g., the investigation of neuronal network architecture as performed in Connectomics. Figure 1B and C show a typical SEM image of a mouse extraocular muscle and a reconstruction of the corresponding 3D volume stack.

In recent years a range of technical hurdles have been overcome and extending the applicability of SBF-SEM, most notably (1) a technique called Focal Charge Compensation that enables imaging of charge-prone and therefore difficult biological samples [2] and (2) workflow automation supporting unattended sectioning and imaging, which makes this technique a convenient way to acquire large, ultrastructural 3D data sets.

We will introduce to serial block-face SEM and its application benefits as well as demonstrate the technology live on site and show its robustness and ease of use. During the workshop we will also show how data sets will be processed and segmented.

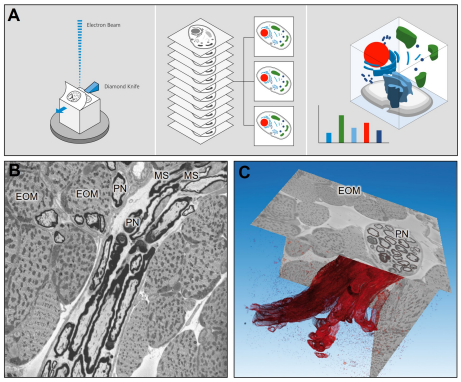

Figure 1: (A) Principle of serial block-face imaging. B) Single 2D SBF-SEM image of a sample with mouse extraocular muscle and peripheral nerves. C) Reconstruction of 3D data set (extraocular muscles (EOM), peripheral nerves (PN), myelin sheath (MS) in red. Courtesy of Dr. Peter Munro, UCL, UK.

References

1. Denk W, Horstmann H (2004) Serial Block-Face Scanning Electron Microscopy to Reconstruct Three-Dimensional Tissue Nanostructure. PLoS Biol 2(11): e329.
2. Deerinck TJ et al. (2018) High-performance serial block-face SEM of non-conductive biological samples enabled by focal gas injection-based charge compensation, J Microsc., 270(2): 142-149.