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IT-4-P-2700 Volume reconstruction of biological samples by alternate physical and virtual slicing

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The ability of scanning electron microscopy (SEM) to image large volumes with high spatial resolution, throughput and reliability goes hand in hand with the development of new approaches for data acquisition. Resin embedded tissues stained with heavy metals pose typical challenges with generally low electrical conductivity and charging. One way of imaging them in SEM is based on alternate slicing and imaging of the block-face. SBFSEM (Serial Block-Face SEM) utilizes an ultramicrotome inside the SEM chamber to cut slices of defined thickness. The revealed block-face is scanned and backscattered electrons are collected [1]. The depth resolution is determined by the achievable slice thickness and imposes a limit on voxel isotropy and on the quality of the reconstructed 3D information. The acquisition parameters and hence data throughput depend not only on the sample properties but significantly on the detection part of the microscope as well. Sample charging can be suppressed by working in low vacuum mode, in-situ coating of the surface with a very thin metal layer, by the usage of the accelerating voltage in the low kV range or introducing more metals during sample preparation.

We introduce the new integrated solution for SEM volume data acquisition based on a refined SBFSEM technique. It combines physical and virtual slicing which allows for extending the current resolution limit. Virtual slicing is enabled by using the MED-SEM (multi-energy deconvolution SEM) which is a non-destructive technique capable of high-resolution reconstruction of the top layers of the sample [2]. Following each cut, the exposed block-face is imaged and not only one image but a series of images is acquired using different accelerating voltages. Collected images serve as the input for a deconvolution algorithm that computes several subsurface layers. Subsequently, a given thickness of the tissue is removed mechanically using a diamond knife, a fresh block-face is exposed and the whole process is repeated for the needed number of iterations. While in the case of physical slicing the minimal slice thickness, and thus the depth resolution, is limited; virtual slicing is capable of extending it towards nanometer range and hence high-resolution isotropic datasets can be generated. To allow automatic data acquisition the whole workflow was integrated into a hardware and software solution that combines an SEM, an in-situ microtome and a reconstruction software. Increased ease of use is further facilitated through newly developed advanced auto-functions for electron column alignment.

References

- [1] B. Titze & W. Denk, Journal of Microscopy, vol. 250(2), pp. 101-110 (2013).
- [2] F. Boughorbel et al., SEM Imaging Method, Patent US 8,232,523 B2, 31st July 2012.