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Tools for 3D Electron in Life Sciences – Generate meaningful statistics from 3DEM Data Microscopy

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Scanning electron microscopy (SEM) is a versatile method to obtain high-resolution information on the nanometer scale. While traditionally used for topography measurements, modern SEMs in biomedical research are utilized increasingly to obtain large volume data of biological samples. New developments in hard- and software as well as in the electron optics enable an ever-larger range of applications. With an SEM you are not limited to grid-mounted samples and today it is possible to produce hundreds of serial sections automatically, placed them on large solid supports and investigated automatically in the SEM. Furthermore, blocks of cells or tissue can be placed and processed directly in the microscope to produce large 3D volumes of pre-selected target areas. In this talk various 3D methods will be presented and discussed. The advantages of every method will be shown elucidating the broad range of possibilities every single method will bring along. Special focus will be set on Focal Charge Compensation – a tool which extend the usability of serial block-face imaging.

Single images are snapshots of a complex three-dimensional situation reduced to a 2D image. To fully understand the sample, volume data is necessarily acquired. Different techniques like serial section tomography (Array Tomography), block-face imaging (3View) and focus ion beam scanning electron microscopy (FIB-SEM) gives access into the world in 3D.

For serial section tomography, a resin-embedded sample is cut into ultrathin serial sections where, the sequence of the sections defines the z-information of a subsequently computationally reconstructed 3D data set and the thickness of the sections determines the z-resolution of this z-stack [1]. The thickness of the sections is typically between 40-100 nm. A tape collecting ultramicrotome (ATUMtome) can be used to produce and collect the sections automatically on a continuous tape [2]. The acquired 2D images are then computed into a 3D model. The serial sections can be stored and used for further imaging experiments.

As an alternative to sequentially cutting a block tissue into serial sections and subsequently imaging the sections, resin-embedded cells or tissues may be imaged in 3D directly within the SEM chamber in a fully automated workflow: the surface of a specimen block is repetitively cut away with images of the exposed block surface taken after each sectioning event (block-face imaging). Two solutions to cut the sample within the scanning electron microscope are available: either an ultramicrotome inside the SEM chamber (3View) or a focused ion beam FIB-SEM that combines a FE-SEM with a focused ion beam for milling can be used. While 3View provides fastest imaging of 3D volumes with z-resolutions down to 15 nm, FIB-SEM is the best choice for higher z-resolutions down to 3-4 nm. Together with NCMIR, ZEISS developed the Focal Charge Compensation, an accessory of the 3View system which eliminates specimen charging making the 3View universally usable.

In this talk, different applications will demonstrate the strength of the individual 3D methods and introduce mining the 3D images to generate statistical data. Results from cell biology, developmental biology and plant research are shown. Figure 1.1 shows the 3D reconstruction from serial sections of root nodules at ultrastructural resolution. The alignment, processing, segmentation and visualization of the 2D images into a 3D data set was done by using Dragonfly Pro. In Cell Biology, high resolution imaging of cultured monolayers of cells forms the basis of much fundamental research into cellular structure, function and phenotype. A high ratio of resin to sample means that cell culture monolayers are extremely difficult to image with block-face SEM due to charging effects. Focal Charge Compensation eliminates the charging and thus enables high signal-to-noise 3D imaging of cellular ultrastructure. Figure 1.2 shows single mitotic HeLa cell with the DNA stained and embedded in Durcupan resin. In figure 1.3 an entire nematode acquired with a FIB/SEM is presented. *C. elegans* was high pressure frozen and freeze substituted in EPON. The 3D data set consists of 10.080 z-sections with 5x5x8 nm pixel size. In this talk you will see a movie of this reconstruction revealing the innermost of the worm.

References:

[1] Collman F, et al. J Neurosci. **35**(14) (2015) p. 5792-807.
[2] Micheva KD, Smith SJ., Neuron. **55**(1) (2007) p.25-36.

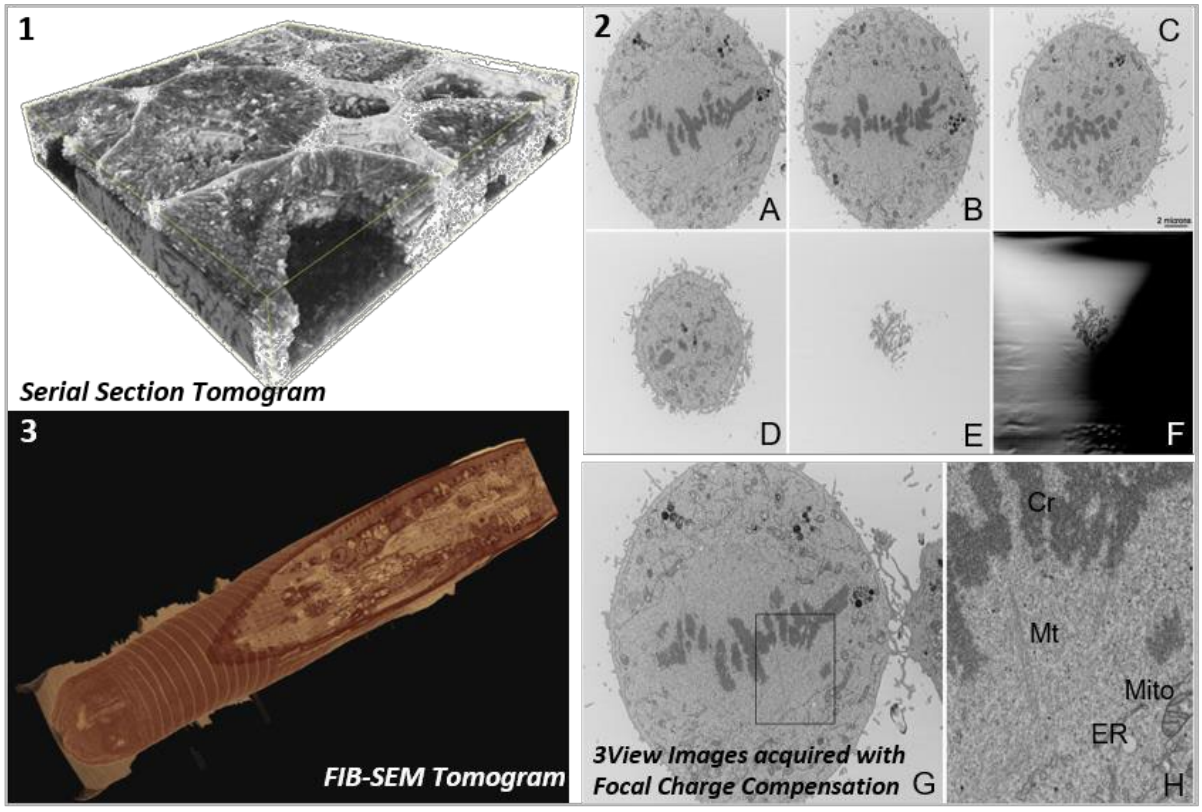

Figure1. Collection of results created with different 3D methods. (1) Root nodules: Samples provided courtesy of Dr. Janine Sherrier, Dr. Jeff Caplan and Ms. Shannon Modla, University of Delaware, US. (2) Single mitotic HeLa cell: Images courtesy NCMIR, San Diego, USA (3) C. elegans: Courtesy: Anna Steyer, Yannick Schwab (EMBL, Heidelberg, Germany) Sebastian Markert and Christian Stigloher (University Würzburg, Germany).

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