

# Focussed ion beam scanning electron microscopy in biology

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Focussed ion beam applications were, for many years, only in the domain of semiconductor industry. One of the first applications in biology was making cross-sections to image the eye of a housefly [1]. This application was further extended by biologists [2] to analyse internal structures of *Porcellio scaber*. Later the focussed ion beam was used to analyse difficult to cut material such as bone and dental implant [3].

In biology the three-dimensional organisation of cells, but also subcellular organelles, becomes more and more important. For instance the contact between cells, e.g., how do endothelial cells seal between exterior and interior but are still permeable for needed components, or describing the three-dimensional organisation of synapses in the brain. To gain this volume information serial resin sections are made and imaged by TEM, which is a very time consuming and technically challenging.

TEM tomography [7-9] is a first step into easier acquiring 3D data. Instead of ultrathin sections (60 – 100 nm), semithin sections (200 – 500 nm) are used to retrieve volume information. TEM tomography is considered to provide a resolution of ~1 nm in x / y and 2 nm in z and the analysed area is in the range of 2 - 10  $\mu\text{m}^2$ . This volume is often not sufficient especially considering cell-cell interactions.

More recently the FIB-SEM instruments became more and more important to extend the three-dimensional information. This instrument uses a  $\text{Ga}^+$ -ion beam to mill off the surface of a resin embedded biological sample and the new surface is imaged with an electron beam. The FIB-SEM approach has presently reached a resolution of about 10 nm with  $5 \times 5 \times 5 \text{ nm}^3$  voxel size, reasonable volumes that can be imaged are around  $60 \times 60 \times 100 \mu\text{m}^3$ . To analyse even larger volumes, comparable to the serial resin sectioning, Denk and Horstmann constructed a microtome in a scanning electron microscope [10]. This microtome (3View, Gatan) can cut much larger surfaces than the FIB-SEM but the section thickness is limited to about 50 nm. In contrast to the general opinion the x-y resolution is not limited by the microtome but only restricted by the quality of the SEM column. Scientifically the two approaches are complementary and the 3View is the method of choice to automate serial sectioning. Both FIB-SEM tomography and 3View serial sectioning have a great success in neurobiology [10, 11].

One of the limiting factors of block-face imaging is the sample preparation. Usually biological surfaces are covered with a thin metal deposition to avoid charging by the electrons and to restrict penetration of the electron for high resolution imaging. In the process of cutting and imaging, however, the metal layer is removed and the organic surface, biology embedded in resin, is exposed to the beam. In a modern FIB-SEM, e.g., the Helios (FEI Company), charging can easily be overcome by adjusting the relative bias voltage of the detectors. The imaging electron beam, however, may cause considerable damage on the resin surface and changing the cutting properties of the block. This is one of the reasons why the 3View cannot cut as thin as the FIB instrument. The electron beam irradiation can induce surface damage during imaging, resulting in bad images and loss of resolution. To reduce the damage and decrease acquisition time, the biological structures need a high contrast. Present high contrast preparation techniques [12], however, are rather damaging for the cellular ultrastructure. The best compromise needs yet to be found.

The FIB-SEM can also be used as a machining tool for biology. The most successful applications have been shown in cryo-electron microscopy. Cells cryofixed on an electron microscopy grid were thinned in the electron beam that they could be analysed by cryo-electron tomography [13, 14]. Recently, Rigort et al. published a method how to make a lamella perpendicular to the cells grown on a grid to analyse the cross-section by cryo-tomography [15]. The FIB was also used to prepare cryo-sections, i.e., 200 nm to 300 nm thick lamella for cryo-TEM [16] from high-pressure frozen yeast cells.

In this presentation I will give an overview of the problems discussed, and where possible, suggest solutions.

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