Volume Imaging of Cellular Ultrastructure in Vitrified Biological Samples using Cryo FIB/SEM and Light Microscopy.

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Focused Ion Beam – Scanning Electron Microscopy (FIB/SEM) volume imaging of heavy-metal-stained biological specimens embedded in resin is a well-established technique to reconstruct and to analyse subcellular structures in all three dimensions, e.g. brain mapping. Cellular ultrastructure is visualized by detecting the low loss backscattered electrons generated by the interaction of the primary electrons with the stained resin-embedded tissue. The z-limitations given by conventional serial sectioning using an ultra-microtome are overcome by FIB milling (FIB slices ≥ 5 nm and below).

Classical resin embedding preparation technique involves dehydration and impregnation with heavy metals by either freeze substitution or chemical fixation. Any step of these preparation protocols involves the potential risk of introducing structural modifications or artefacts. In contrast vitrification preserves the cellular ultrastructure of specimens as close as possible to their hydrated, living state. Here the water is frozen quickly enough to prevent the formation of ice crystals resulting in a glass-like state.

A novel approach for FIB/SEM is block face imaging of vitrified biological samples omitting any staining, chemical fixation or dehydration. In our recent work, we applied serial FIB milling and block face imaging to acquire 3D data of high pressure frozen mouse optic nerves and bacillus subtilis spores under cryo conditions [1]. By using Inlens secondary electron detection we succeeded to directly visualize the cellular ultrastructure in the freshly exposed serial FIB cross-sections. The observed contrast between lipid-rich membranes and water-rich areas allowed differentiating subcellular structures like the Golgi apparatus, nuclear envelope, vesicles, endoplasmic reticulum and cristae within the mitochondria (Fig.1). The new method is comparatively easy and fast. Only one step – the cryo-immobilization – is required for preparing the specimens for cryo FIB/SEM. Here recent cryo FIB/SEM results on high pressure frozen HeLa cells, yeast cells and Algae Emiliania huxleyi (EHUX) are presented. The Cryo/FIB-SEM data of vitrified yeast and EHUX contain one complete cell each.

In addition, this novel technique offers extended possibilities for correlative workflows between light, electron and x-ray microscopy. Especially investigating frozen hydrated sample with the new ZEISS LSM 880 Airyscan offer significant improvements in image quality under cryo conditions. During the talk we will present an outlook on the combination between cryo Airyscan and FIB/SEM.

[1] A. Schertel , N.Snaidero, H. M. Han, T. Ruhwedel, M. Laue, M. Grabenbauer, W. Möbius: “Cryo FIB SEM: volume imaging of cellular ultrastructure in native frozen specimens.” J Struct Biol. 2013 Nov; 184(2):355-360. DOI: 10.1016/j.jsb.2013.09.024.

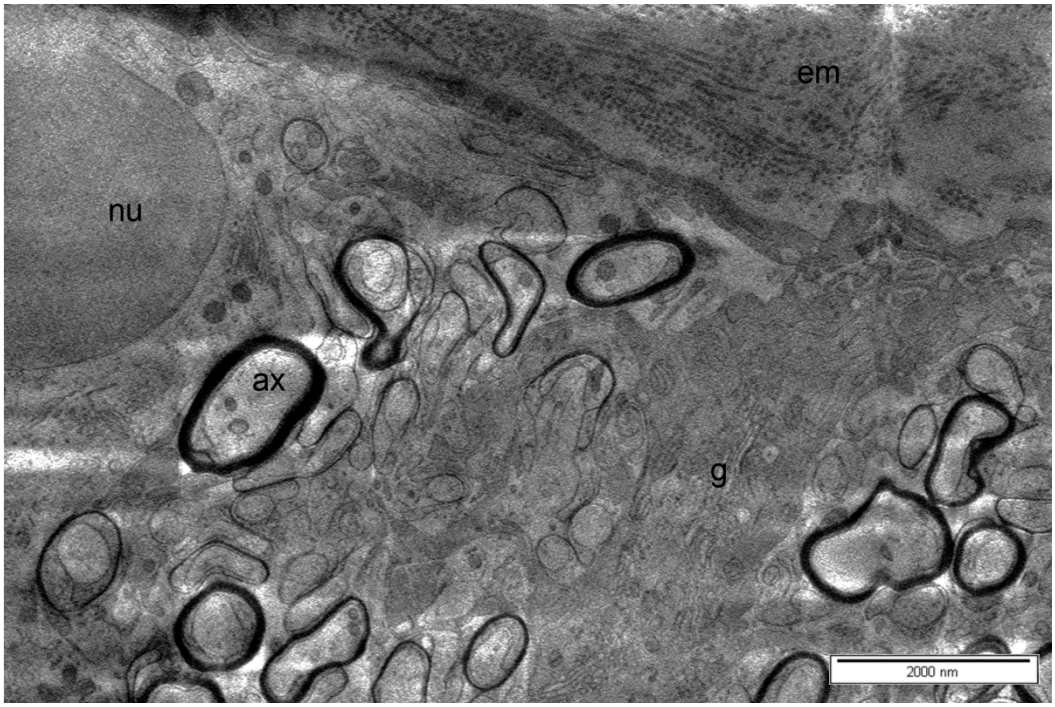

Figure 1. Cryo FIB/SEM block face imaging of high-pressure frozen mouse optic nerve.