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Volume imaging of cellular ultrastructure in native frozen specimens using focused ion beam scanning electron microscopy at cryo conditions

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To understand cellular functions in the context of three-dimensional assemblies volume microscopy at high resolution is required. Focused ion beam (FIB) milling combined with serial block face imaging in the scanning electron microscope (SEM) is an efficient and fast method to generate such volume data for 3D analysis. In this study, we apply this technique in a cryo FIB-SEM to image fully hydrated frozen specimen of mouse optic nerves and *Bacillus subtilis* spores prepared by high-pressure freezing (HPF). We were able to establish imaging conditions for the direct visualization of the ultrastructure in the block face at −150°C by using an in-lens secondary electron (SE) detector. By serial sectioning with a focused ion beam and block face imaging in the optic nerve we obtained a volume of X=7.72 µm, Y=5.79 µm and Z=3.81 µm with a lateral resolution of 7.5 nm and a Z-resolution of 30 nm. The intrinsic contrast of membranes was sufficient enough to distinguish subcellular structures like Golgi cisternae, vesicles, endoplasmic reticulum and cristae within mitochondria. Serial images could be used for a three-dimensional reconstruction of different types of mitochondria within an oligodendrocyte and an astrocytic process. Applying this technique to dormant *Bacillus subtilis* spores we obtained volumes containing several spores and discovered a novel core structure, which was not visualized before by any other imaging technique. In summary, we describe here the use of cryo FIB-SEM as a tool for direct and fast 3D cryo-imaging of large native frozen samples.