Focused Ion Beam – Scanning Electron Microscopy (FIB-SEM) volume imaging closes the gap between electron and X-ray tomography. 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. The 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 conventional resin-embedding preparation technique involves dehydration and impregnation with heavy metals by freeze substitution or chemical fixation followed by resin-embedding. In contrast, biologists aim to visualize cellular ultrastructure of specimens as close as possible to their native or living state.

A new and very exciting approach for FIB-SEM Microscopy is block face imaging of native biological samples in the high pressure frozen state omitting any staining, chemical fixation or dehydration. In case of the high pressure frozen mouse optic nerve a data cube with a volume of 7.7 μm x 5.8 μm x 3.8 μm was obtained within three hours by serial FIB milling and subsequent block face imaging. The image pixel size was 7.5 nm and FIB slice thickness was set to 30 nm. The observed contrast between lipid-rich membranes and water-rich areas allowed differentiating subcellular structures like Golgi apparatus, nuclear envelope, vesicles, endoplasmic reticulum and cristae within mitochondria.