Enhanced imaging for serial Cryo-FIB-SEM microscopy of biological samples with fluorescence navigation

Pavel Krepelka[1], Jana Moravcova[1], Jiri Novacek[1]
[1]Central European Institute of Technology, Masaryk University, Brno, Czech Republic

Traditional methods for studying biological specimens with volume electron microscopy face two significant hurdles: the complex process of sample preparation, which includes drying and staining with heavy metals and can introduce artifacts, and the difficulty in maintaining fluorescence signals in samples set in resin. In contrast, cryo-FIB-SEM keeps specimens nearly unchanged in a frozen state, streamlining the preparation process, reducing structural distortions, and allowing easier observation of fluorescence. Yet, imaging non-contrasted biological specimens in cryo-FIB-SEM can be challenging due to low signal-to-noise ratios and charging artifacts.

Our research shows that by fine-tuning the imaging conditions, electron exposure, plasma milling parameters and the workflow management, we can greatly improve the quality of the data, speed up the rate of data collection, and capture large volumes of samples with outstanding ultrastructural clarity. This allows us to view all cellular organelles in detail, achieving resolutions as fine as 16nm. An additional advantage of cryogenic imaging is the preservation of the fluorescence signal, which helps in locating cells and guiding the initial trenches in the sample.

With the mentioned improvements, we acquired high-quality cryo-images. We demonstrate the effectiveness of our cryo-FIB/SEM method by reconstructing a segment of a pancreatic INS-1E beta cell, showcasing the workflow's capability to produce high-quality imaging of biological samples.

Keywords:

FIB-SEM, volumeEM, cryo, native, cell