Semi-automated FIB Sample Preparation Workflow for Cryo-TEM

Záchej, S.[1], Havrankova, J.[2], Rosikova, K.[2], Kostal, V.[1] and Vana, R.[1]

[1] TESCAN Brno, Czech Republic, [2] TESCAN ORSAY HOLDING, Czech Republic

Cryo-electron tomography (Cryo-ET) provides a unique insight into the structural details of macromolecular complexes, cells and tissues in a near-native state. The principal limitation of cryo-ET is size of the specimen, which needs to be thinner than 500 nm [1]. Thus, the majority of eukaryotic cells, which are much thicker, are not directly accessible for TEM imaging. Cryo-sectioning is a well-known method used for thinning such samples. However, the technique suffers from multiple artefacts, which can adversely affect data quality. The most serious defects are caused by compression, knife-marks and curtains [2].

As an alternative approach, a focused ion beam (FIB) has been employed for sample preparation under cryogenic conditions. Micromachining of a vitrified biological specimen by means of a FIB is artefact-free and can thus substitute cryo-sectioning. When done precisely, cryo-FIB thinning can deliver a thin, vitrified sample in a close-to-native state without any mechanical distortions.

Here we present the entire semiautomated workflow of site-specific FIB sample preparation for cryo-ET. We used *Saccharomyces cerevisiae* applied to a standard TEM grid to optimize the whole workflow. Yeast cells were vitrified by plunging into liquid ethane. The frozen sample was attached to a pre-tilted holder and coated with a 10-nm layer of organometallic platinum to enhance surface conductivity. The actively cooled sample was transferred to the TESCAN S8000G. The excellent accuracy of the new ORAGE FIB machining at low-currents is extremely important for the quality of the final lamella. To maintain the sample at cryogenic temperatures during milling and polishing, the instrument is equipped with a cryo-stage and a cryo-shield.

Sample processing inside the SEM chamber began by depositing a platinum protection layer above the region of interest (ROI) using a Gas Injection System and simultaneous scanning with a low-current ion beam (150 pA) [3]. This protection layer serves as a shield during the milling and polishing phases and thereby eliminates curtaining of the milled surface due to irregularities in the sample. Then the ORAGE FIB is used to mill a trench from the top and bottom of the selected plane to specifically uncover the internal structure of the cells. The maximum milling current must be kept low (usually lower than 500 pA), to avoid heat damage to the sample. The lamella is then thinned down to 300 nm and polished using low-current ion beam (10 pA). Figure 1 shows the resulting polished lamella. Once the lamella is polished, the sample holder with the sample can be transferred directly to the cryo-TEM holder for further inspection (e.g. cryo-ET or single particle analysis).

The milling and polishing process is controlled by the vector-based scanning generator DrawBeam™. The whole workflow was standardized into a single semi-automated project, where the user defines a ROI and sets up the milling process, which can then run autonomously until final polishing. The precision of the final polishing using low-current FIB is crucial for lamella quality, therefore it should be controlled by the user. The presented workflow can be used as a routine, semi-automated method to prepare frozen-hydrated cells for cryo-TEM applications.

References:

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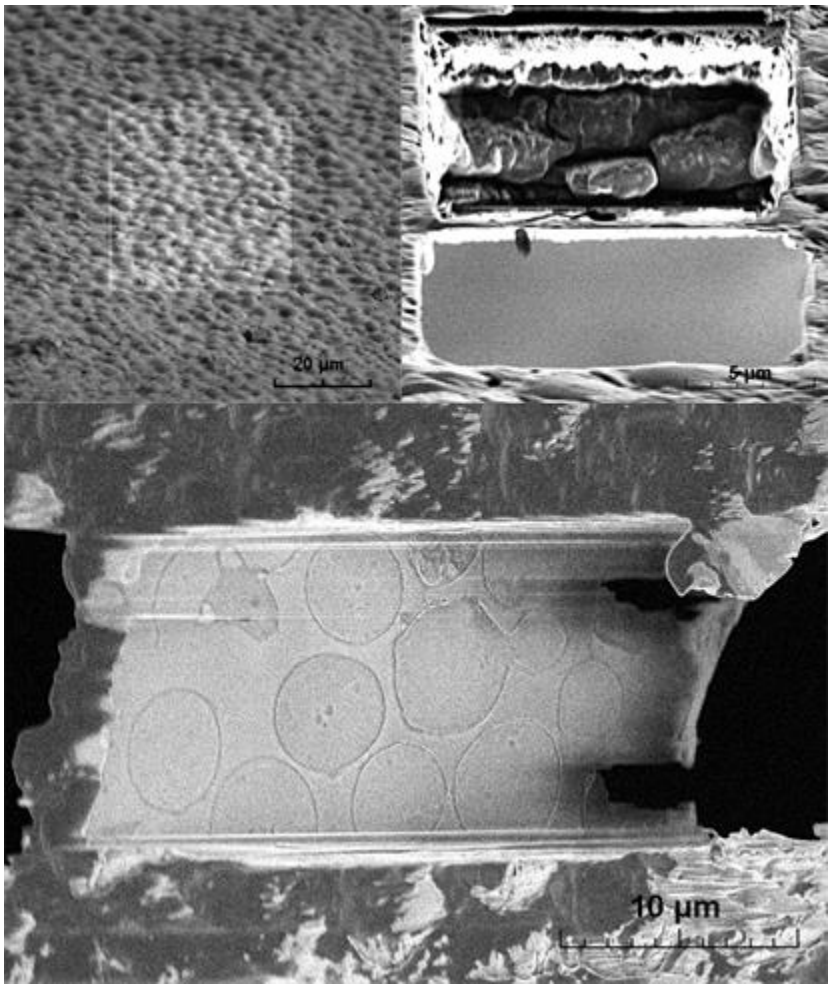

Figure 1. Top left - Sample surface after depositing the protection layer; Top right - Front view of the lamella before final polishing; Bottom - Top view of the perfectly flat sample after final polishing. Thickness of the lamella is 300 nm. Marks following heat damage caused by SEM imaging can be seen on the lamella.