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FIB Micromachining of Frozen Systems for TEM

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Focused ion beam (FIB) milling as a means to prepare electron-transparent specimens for transmission electron microscopy (TEM) is a well-established technique in materials science. Using a cryo-transfer stage, FIB micromachining can be extended to frozen specimens [1], opening the door to a range of exciting opportunities. We report on experiments using a new cryo-transfer system from Hummingbird Scientific installed on an FEI Quanta Dual-Beam FIB and discuss future prospects. This system allows for the transfer of a frozen specimen into and out of the FIB. The sample can be sectioned in the FIB to electron transparency, removed and then transferred to a cryo TEM stage for imaging in a TEM, all while maintaining the frozen state.

In the life sciences, rapid freezing of specimens in vitreous ice is routinely used to preserve structures in their native hydrated state for analysis by cryo TEM. Small isolated particles such as viruses can be conveniently prepared directly on TEM substrates by plunge freezing and then imaged at high resolution [2]. However, in the case of larger objects such as eukaryotic and most prokaryotic cells, the sample thickness generally prevents one from resolving inner architectures in sufficient detail. Figure 1a demonstrates this point, showing a cryo TEM image of a frozen hydrated *Plantomycete* cell prepared on a lacey carbon support by plunge freezing. The cell diameter is approximately 1μm, hence the cellular compartments cannot be clearly resolved. Upon tilting the sample to acquire a tilt series for 3D reconstruction, the projected sample thickness becomes ever greater, decreasing the attainable spatial resolution even further. As an alternative, cryo FIB milling can be employed to prepare sections through the cells to enable closer scrutiny of their internal structures by TEM tomography. We prepare samples for cryo FIB milling by plunge-freezing cell suspensions onto lacey carbon grids that have been clipped to facilitate milling parallel to the ice surface, as shown in Figure 1b. The FIB-based method presents significant advantages compared with conventional mechanical sectioning of bulk samples by cryo ultramicrotomy, which typically results in artifacts such as knife marks and cell compression.

Using the versatility of FIB micromachining, alternative sample geometries can also be prepared. For example, cylindrical or needle-like shapes can be milled into the ice with a region of interest embedded inside. Figure 1c shows an example of needle-like structures milled into vitreous ice using the cryo FIB technique. Decreasing the needle diameter will enable cryo TEM imaging of embedded structures, and by tilting the sample about the needle's longitudinal axis, a constant projected sample thickness at all angles will enable 3D reconstructions with superior spatial resolution. A further potentially groundbreaking opportunity is to use the cryogenic approach to literally freeze interactions at liquid-solid interfaces, which can then be isolated in a FIB-prepared specimen. Upon transferring the frozen section to a cryo TEM stage, characterization of liquid-solid interfaces with the full capabilities of modern TEMs becomes possible.

References:

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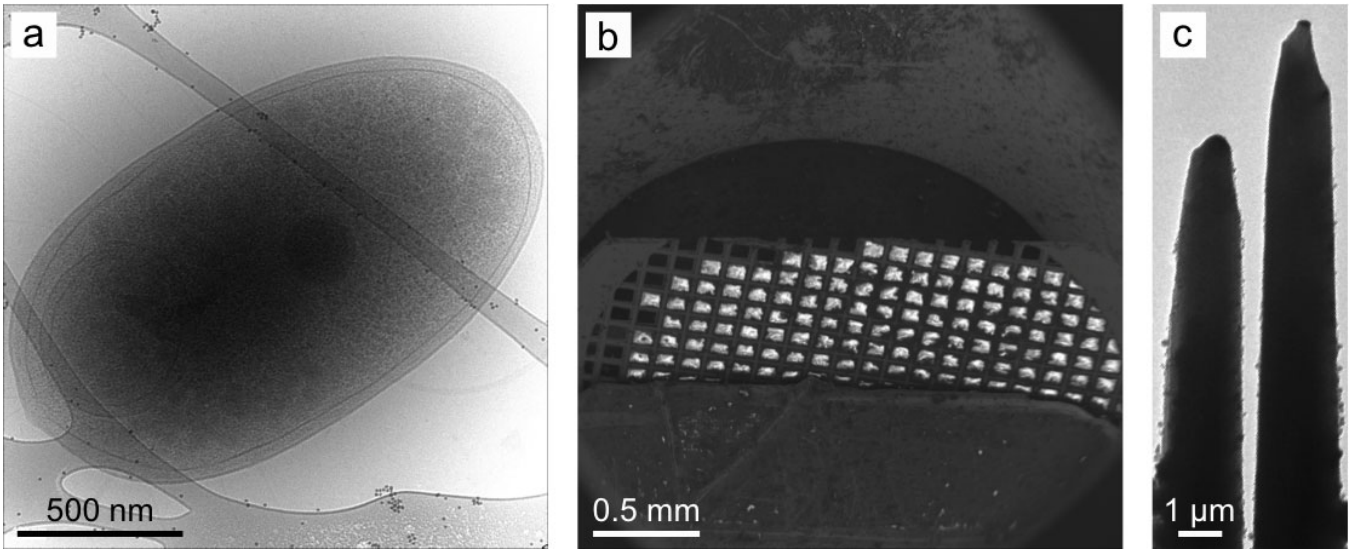

Figure 1a Cryo TEM of frozen hydrated *Plantomycete* cell, **b** Cryo SEM of vitrified ice on a TEM copper mesh grid prior to FIB milling, **c** Cryo TEM of ice needles prepared by FIB milling.

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