A novel cryo-FIB lift-out procedure for cryo-TEM sample preparation

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The focused-ion-beam (FIB) is the method of choice for site-specific sample preparation for Transmission Electron Microscopy (TEM) in material sciences. A lamella can be physically lifted out from a specific region of a bulk specimen with submicrometer precision and thinned to electron transparency for high-resolution imaging in the TEM. The possibility to use this tool in life sciences applications has been limited by the lack of lift-out capabilities at the cryogenic temperatures often needed for biological samples. Conventional cryo-TEM sample preparation is mostly based on ultramicrotomy, a procedure that is not site-specific and known to produce artifacts. Here we demonstrate how a cooled nanomanipulator and a custom-built transfer station can be used to achieve cryo-preparation of TEM samples with the FIB, enabling high-resolution investigation of frozen-hydrated specimens in the TEM.

FIB milling of frozen biological specimens has been reported, but only in two instances in connection with a transfer to a TEM for further higher resolution studies [1,2]. In both cases the entire sample (rather than just the thin area of interest) was transferred and traditional lift-out had been deemed impossible because of the difficulty in cooling down the nanomanipulator and in obtaining Pt deposition at cryogenic temperatures. Cryogenic Pt deposition has been recently demonstrated as a method of improving the quality of FIB cross-sections of frozen-hydrated samples [3].

We were able to obtain a cryogenic lift-out by modifying the nanomanipulator so that it could be cooled. We demonstrate the technique on *Aspergillus niger* spores stained with osmium tetroxide and potassium permanganate. A cryopreparation system (Gatan Alto 2500) was modified for this purpose. In this system, the tip of the manipulator can be kept at about 100 K. With this modification, common FIB/TEM sample preparation procedures can be adapted in a straightforward manner to frozen hydrated samples, with the entire preparation carried out at cryogenic temperatures (fig. 1). A cryo-transfer station has been developed to transfer the thinned lamella from the FIB/SEM to a cryo-TEM holder. Once in the TEM, high resolution images can be obtained at cryogenic temperatures with atomic resolution. It is also possible to perform elemental analysis (fig. 2) [4].

References

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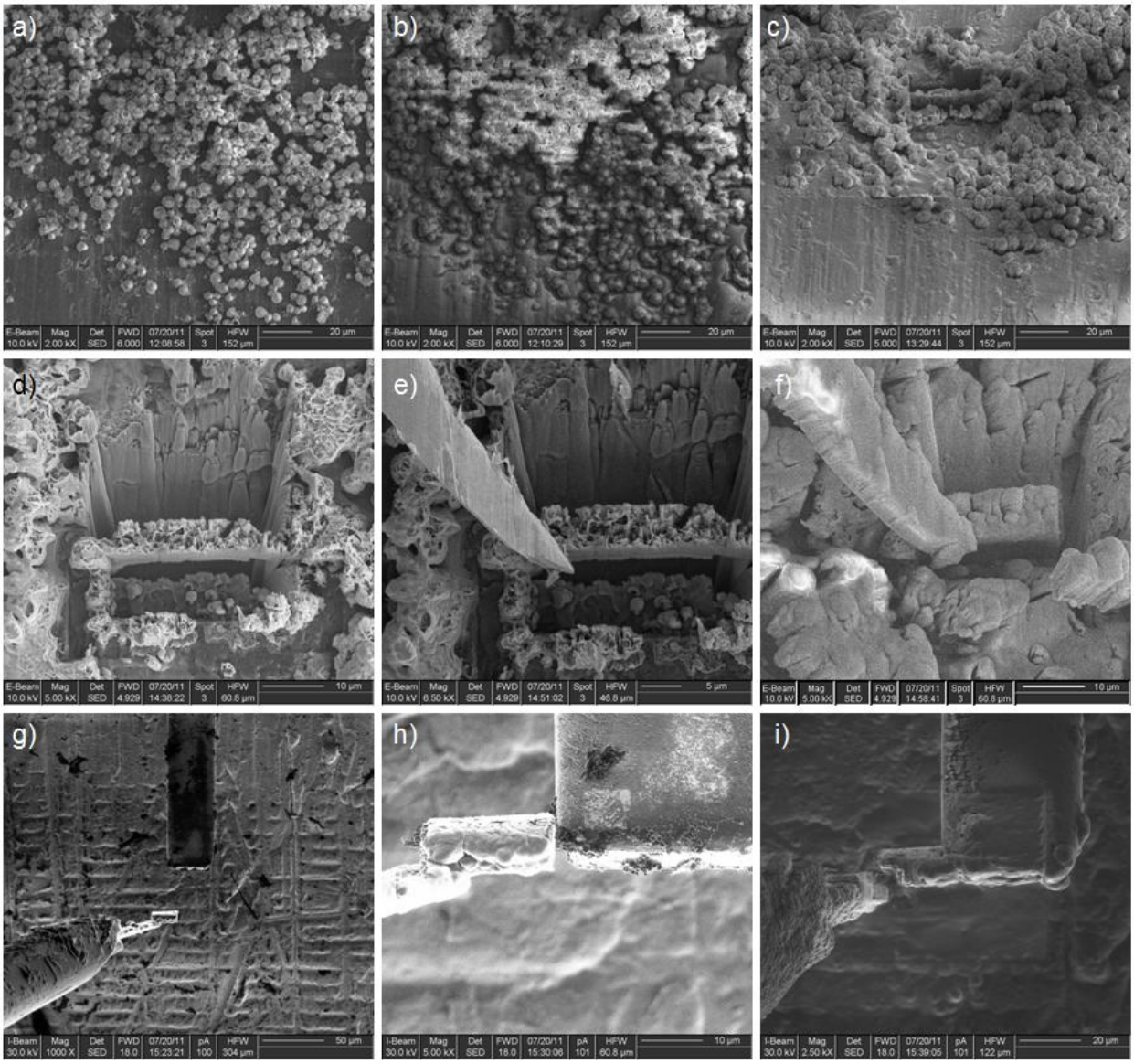

Figure 1. *SEM micrograph detailing the lift-out procedure. a) Spores of A. Niger are plunge frozen and coated with a few nm of Au/Pd. b) Cold deposition of a Pt cap layer to protect the spores from ion beam damage and to create a smooth surface to reduce curtaining during milling. c) Milling of two side trenches. d) Milling the side and bottom of the lamella, leaving only two small connections. e) Contacting the lamella with the cold nanomanipulator. f) Welding the nanomanipulator to the lamella with another cold Pt deposition and subsequent cut-free. g) Lift out and transfer to a Cu TEM grid. h) Contacting the lamella with a sample post on the Cu TEM grid. i) Cut-free of the needle, leaving the lamella on the Cu grid for further thinning.*

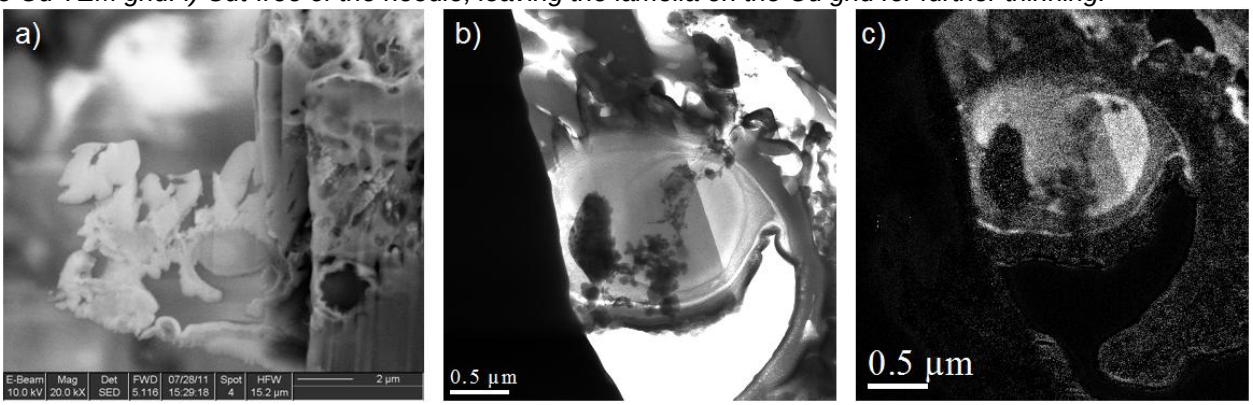

Figure 2. *a) SEM micrograph of a thinned A. Niger spore; b) BF TEM image of the same spore; c) EFTEM Carbon map revealing details in the cytoplasm and cell walls.*