Cryo-FIB-Lift-out for Biological imaging - The impossible made 'merely difficult'

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The preparation of soft matter samples for electron microscopy (EM) has been revolutionized in recent years by the development of cryogenic transmission electron microscopy (TEM). Cryogenic fixation preserves high water content samples (cells, tissues, plant samples, suspensions, gels and food products) in their native states. In the case of all but plunge freezing of small objects in thin vitreous layers, some sort of microsampling must be conducted to isolate the region of interest for TEM analysis.

Traditionally Cryo-ultramicrotomy is a route to cryogenically preserved electron transparent sections of sample. More recently cryo-Focused Ion Beam Scanning Electron Microscopy (FIBSEM) has been used to prepare thinned sections of sample that are grown on a TEM grid. For over 20 years thin sections or lamellae have been prepared from bulk samples via the *in situ* lift-out method. Lift-out offers a site specific preparation method for TEM analysis, however, this was typically in the field of materials science.

To enable lift-out under cryogenic conditions, a number of technological and sample handling issues had to be overcome for application to cryo-preserved samples. In 2014 we presented our first results applied to hydrogel samples in pursuit of label- and damage-free information of soft matter samples. Subsequently strategies have been explored for milling parameters, grid attachment using cryo-condensation of water, maintaining cryogenic conditions and protection of the lamella during transfer to the TEM to permit transfer of cellular materials.

Attention has turned to the preparation of cryo lamellae from cells grown on TEM grids and yeast, a well-recognized test specimen. Images from the extraction of yeast (Figure 1) and images of nuclear membrane (Figure 2) have been presented. These images demonstrate that this approach is capable of delivering electron transparent lamellae / sections for damage free imaging of soft-matter and biological samples.

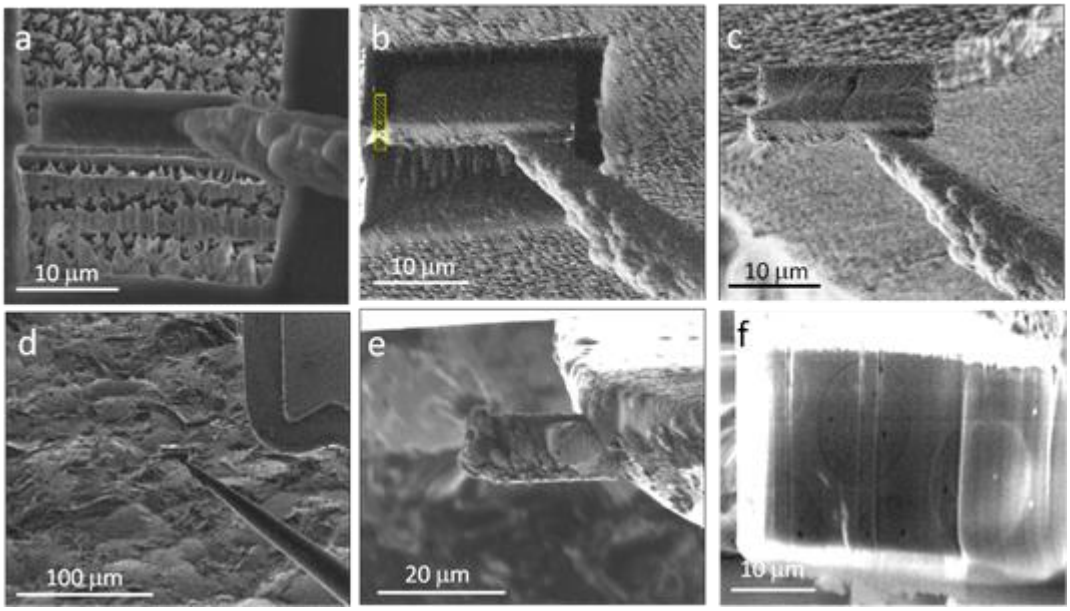

Figure 1 Cryogenic lift out of yeast cells. The sample was prepared by the modified total release methodology and attached to the Cryoprobe with vitreous ice (a, b electron/ion image of attachment respectively). The lamella was extracted (c) and repositioned to a TEM grid (d). The sample was attached to the grid using vitreous ice deposition (e). Excess ice was removed (f) to prepare the sample for conventional FIB thinning.

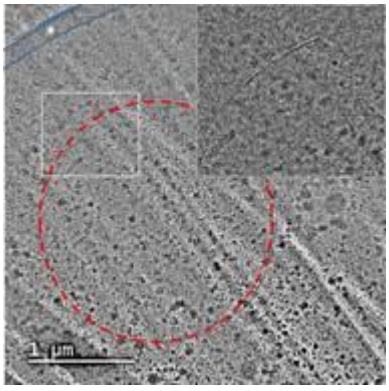

Figure 2 Cryo-TEM image of a yeast cell prepared by Cryo-FIB-LO. The cell wall (top left of image, denoted with a star) and nuclear membrane (red lines) are visible. The inset show higher magnification of the nuclear membrane. Contamination from frost is visible and protocols are being developed to limit this.