doi:10.1017/S1431927614007855
Microsc. Microanal. 20 (Suppl 3), 2014

Cryogenic FIB Lift-out as a Preparation Method for Damage-Free Soft Matter TEM Imaging

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The removal of a thinned lamella from a bulk sample for Transmission Electron Microscopy (TEM) analysis has been possible in the Focused Ion Beam Scanning Electron Microscope (FIB-SEM) for over 20 years either via *in situ* (by use of a micromanipulator) or *ex situ* lift-out approaches [1]. Both offer swift, site specific preparation for TEM analysis, particularly in light of advancements in corrected TEM. These techniques, however, are currently only applied to samples at room temperature, typically from the materials sector. The majority of biological samples contain a high degree of water, which will be removed under vacuum, leading to the shrinking and rearrangement of the sample. To overcome this, samples can be prepared by either critical point drying, fixation and resin impregnation (often combined with heavy metal staining) or cryogenic fixation. For both fixed and cryo-preserved samples, the preparation of thin-sections has always typically been prepared with a microtome, which yield samples of 60- 100 nm. However, these commonly suffer from compression artifacts and/ or knife marks, which distort data. There is also a desire to move away from staining and methods which dehydrate or allow / permit structural or chemical re-arrangement.

The lift-out approach for cryo samples faces a number of technological and sample handling issues. Recent efforts have demonstrated cryo lift-out is possible for materials samples [2]. This work further extends the development of cryo lift-out for access by those in pursuit of label and damage-free information, which is desired when imaging soft and biological structures. In order to preserve the vitreous / amorphous nature of the water in cryo-preserved samples the temperature should be maintained below -140°C. The probe tip held by the manipulator must be cooled to at least -130°C. To achieve this, an OmniProbe 100 was re-engineered to include a thermal break and a cooling braid, which was attached to the cold finger of the cryo stage (Quorum PPT 2000) installed in an FEI Quanta 200 3D.

This procedure was performed on an alginate-collagen hydrogel, commonly used in tissue engineering and stem cell research, which proved unsuitable for cryo-microtomy. Prior to lamella extraction, the sample was coated with platinum precursor and an organo-metallic tungsten precursor from a gas injector. The sample was milled using a modified TEM lamella protocol to approximately 2 microns thickness, before the cooled tip was used to remove the lamella (Figure 1). The lamella was attached to the manipulator tip by the cryo-condensation of water via a gas injector and was secured to a TEM (lift-out) support grid and further thinned to electron transparency (Figure 2). The sample was transferred under liquid nitrogen to a cryo-TEM holder and imaged at an accelerating voltage of 200 kV in both bright and dark field imaging (Figure 2).

Initial experiments have demonstrated that it is possible to prepare and remove a thinned lamella and transfer to the TEM, whilst maintaining cryogenic conditions. Once further refined this method offers the possibility of compression artifact free imaging of soft matter samples, including those preserved and maintained at cryogenic temperatures, including cells, tissues, plant samples, polymers, gels etc [3]

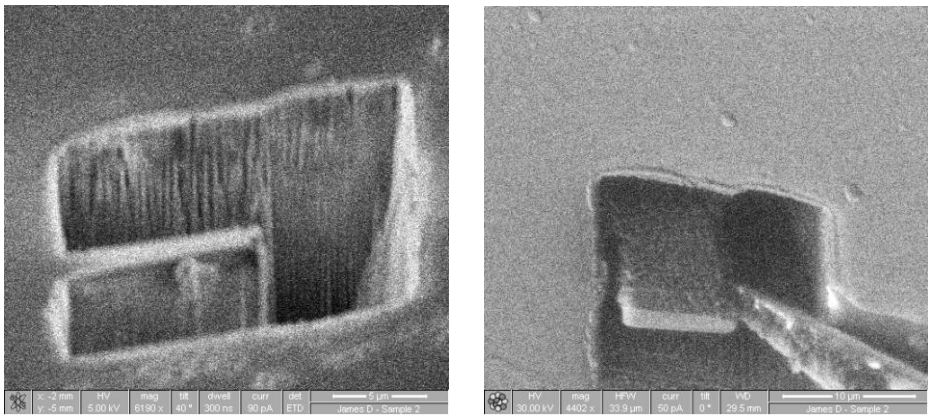

Figure 1. (Left) Cryo-FIB milling of a bulk sample to prepare a thin lamella, scale bar 5 µm. (Right) extraction of the lamella by the cooled manipulator after attachment and release of lamella, scale bar 10 µm.

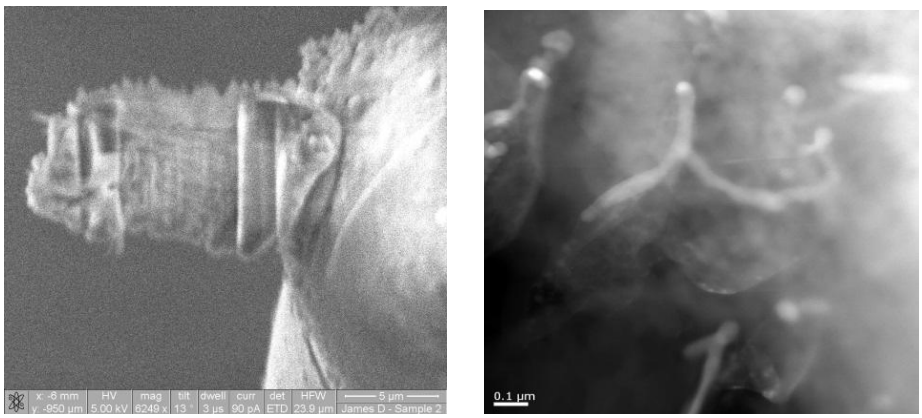

Figure 2. (Left) Micrograph of the attached and thinned lamella, scale bar 5 µm. (Right) Dark field TEM image of collagen fibrils in an alginate hydrogel matrix, scale bar 100 nm.

References:

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[2] N Antoniou *et al,* Conf. Proc. 38th Int. Symp. Testing and Failure Analysis (2012) p. 399-405.
[3] Many thanks to Dr James Dixon (University of Nottingham, CBS) for supplying the hydrogel samples.

https://doi.org/10.1017/S1431927614007855 Published online by Cambridge University Press