Type of presentation: Poster

**IT-13-P-2481 Cryogenic FIB Lift-out as a preparation method for damage-free soft matter TEM imaging**

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We 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 and stain artefact free imaging of soft matter samples (cells, tissues, plant samples, polymers, gels etc) preserved and maintained at cryogenic temperatures. Biological samples contain a high degree of water, which dehydrate under vacuum. Solutions are: critical point drying, resin impregnation with heavy metal stains or cryogenic fixation. Once stabilised the samples can be prepared with an ultramicrotome to yield electron transparent sections, however, they commonly suffer from compression and/ or knife artefacts. In addition, there is a desire to move away from staining or methods which can induce structural re-arrangement. 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 are currently only applied to samples at room temperature as there are a number of technological and sample handling issues for cryogenic samples. Recent efforts have demonstrated cryo lift-out is possible for materials samples[2]. This work further extends the development of cryo lift-out to allow label and damage-free imaging of soft and biological structures. To preserve the vitreous nature of the water in cryo-preserved samples the temperature should be maintained below -140°C and the probe tip held by the manipulator cooled to at least -130°C. To achieve this, an OmniProbe 100 was modified with a thermal break and cooling braid, which was attached to the cold finger of the cryo stage (Quorum PPT 2000).

Prior to lamella extraction, an alginate-collagen hydrogel, was sputter-coated with platinum and a tungsten layer from a gas injector. The gel was milled using a modified TEM lamella protocol to approximately 2µm thickness, before the lamella was attached to the cooled tip by cryo-condensation of water via a gas injector (figure 1). The lamella was subsequently secured to a TEM (lift-out) support grid (figure 2) and further thinned to electron transparency (figure 3). The sample was transferred under liquid nitrogen to a cryo-TEM holder and imaged at 200 kV in both bright and dark field imaging (figure 4).

[1] L Giannuzzi et al. in “Introduction to Focused Ion Beams: Instrumentation, Theory, Techniques and Practice”, ed. LA Giannuzzi and FA Stevie, (Springer, 2005) Chapter 10, p.201-228.

[2] N Antoniou et al, Conf. Proc. 38th Int. Symp. Testing and Failure Analysis (2012) p. 399-405.

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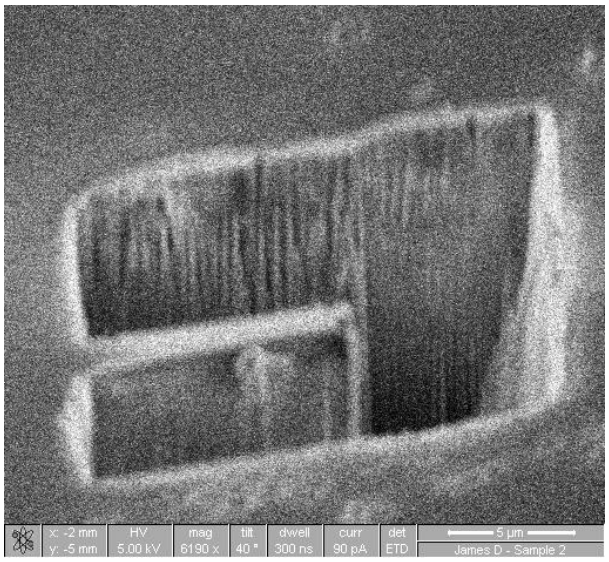


Fig. 1: Cryo-FIB milling of a bulk sample to prepare a thin lamella, scale bar 5 µm

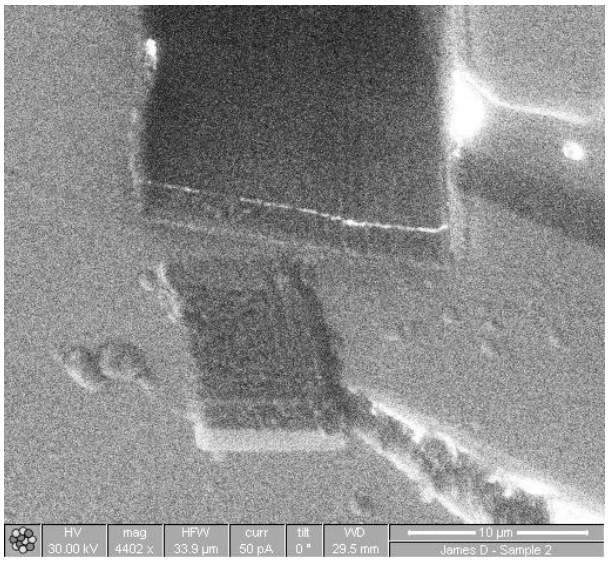


Fig. 2: Extraction of the lamella by the cooled manipulator after attachment and release of lamella, scale bar 10 µm.

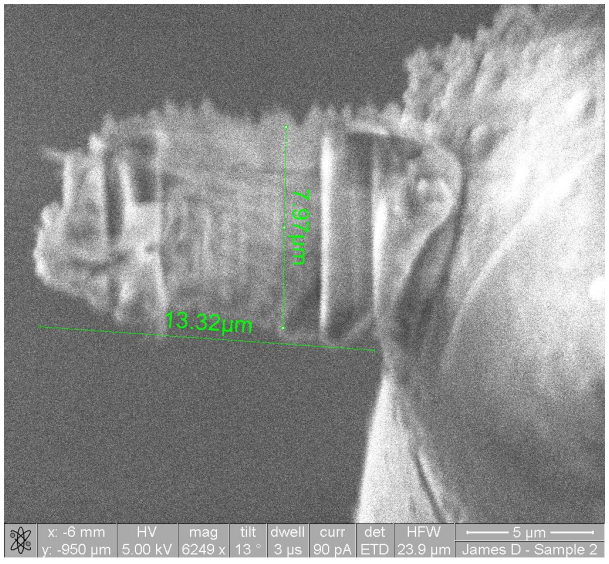


Fig. 3: Micrograph of the attached and thinned lamella, scale bar 5 µm

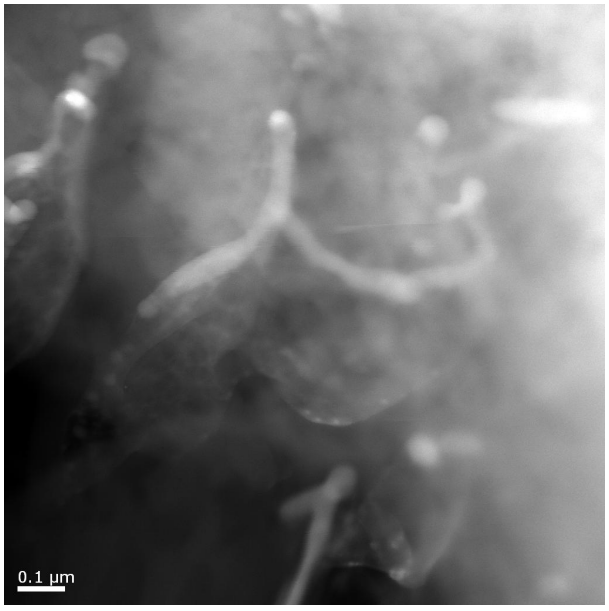


Fig. 4: Dark field TEM image of collagen fibrils in an alginate hydrogel matrix, scale bar 100 nm