A Universal Method of *In Situ* FIB Lift-Out for Cryogenic Samples

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Introduction

Cryogenic electron-imaging (Cryo-EM) of biological materials enables the observation of hydrated cells un-tainted by sample preparation. Cryo-EM is not limited to biological materials but is beneficial for a range of beam sensitive materials (i.e. polymers and III-V semiconductors) and vacuum incompatible samples (solid-liquid interfaces, i.e. in hydro gels and batteries). However, cryogenic conditions restrict the ability to produce specimens for transmission electron microscopy (TEM)/ tomography.

Established methods of producing Cryo-TEM samples include cryo-ultramicrotome [1] and on grid cryo-FIB thinning [2, 3]. Cryo-ultramicrotomy introduces significant deformation and has a limited capability to prepare site specific samples. On grid thinning is an improvement, but requires the feature of interest to be positioned in the central region of a TEM grid before freezing. This method also requires the full thickness of the sample and grid to be milled, which is restrictive.

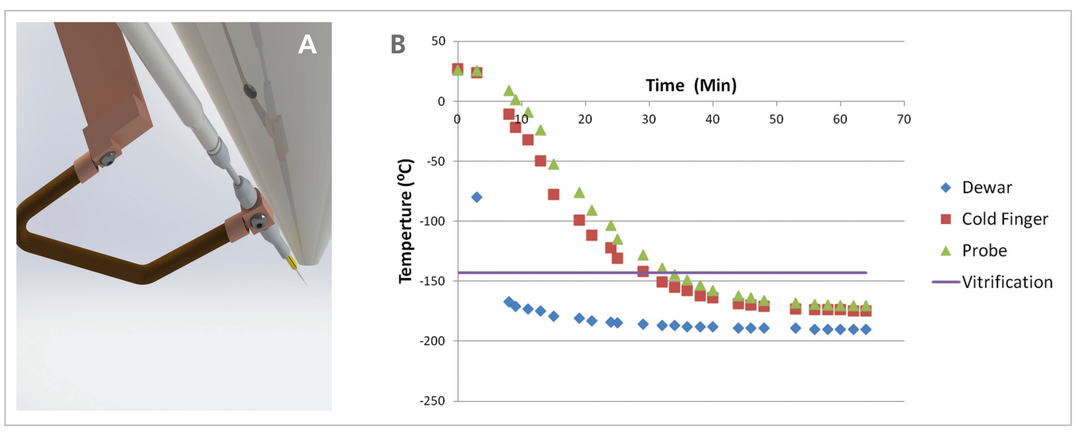

Fig 1. (A) Schematic of the OmniProbe 200, CryoProbe attached to cold finger and (B) plot of probe, cold finger and external dewar (for cooling the cold finger) temperatures against time achieved under vacuum.

Technical Challenges of Cryo Lift-Out

FIB-TEM lamella preparation is a well established method for preparing site specific samples but is not suitable for cryogenic samples. The manipulator will induce thermal damage and the cryogenic conditions prevent conventional attachment methods.

To solve this, a OmniProbe 200 nanomanipulator was coupled with a custom shaft including a oxygen free copper cooling piece (CryoProbe). This can cool the OmniProbe tip to less than -170 °C by use of a dewar-cooled cold finger, as shown in Fig.1 (above).

To attach the probe to the sample, low levels of moisture in a carbon selective etch GIS were used. This forms a thin layer which can attach a lamella to the probe, but does obscure the sample or fill in the FIB milling, as shown in Fig 2.

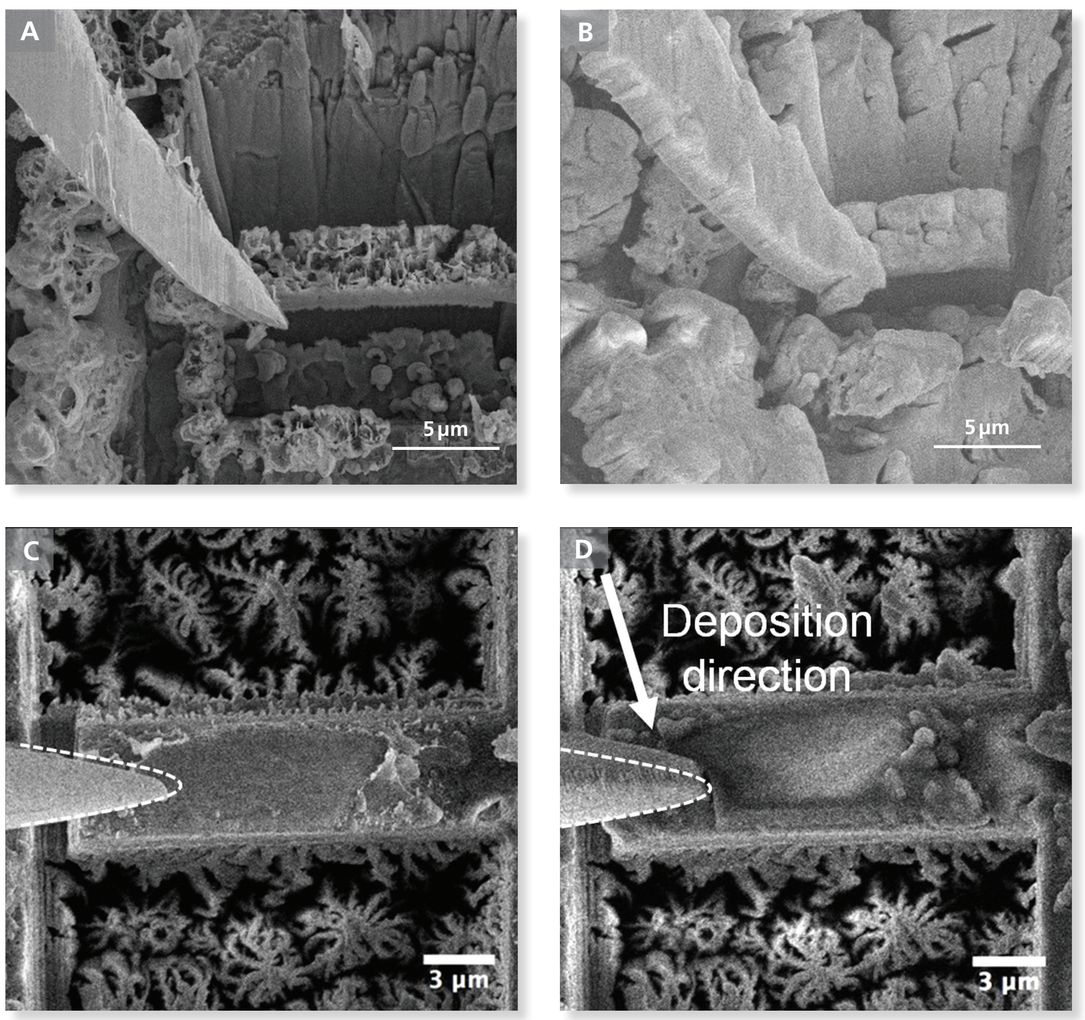

Fig 2. Cryogenic lift-out using Pt from [4], before (A) and after (B), Pt deposition. It is seen that the Pt coats the sample with a thick layer and inhibits lift-out. Cryogenic lift-out using carbon etch GIS to attach lamella to the CryoProbe, before (C) and after (D), the GIS valve is opened. The milling is preserved after the deposition and sample is not obstructed.

Room Temperature Lift-Out

In situ FIB lamella lift–out is a common method of preparing TEM samples (Fig. 3). There are multiple method of making a FIB lamella, the common steps are:

- Select site of interest
- Deposit surface protective layer
- Mill lamella in a bulk sample
- Attach the lamella to a manipulator
- Reposition the lamella to TEM grid
- Attach to grid
- Thin the sample
- Sample transfer to the TEM

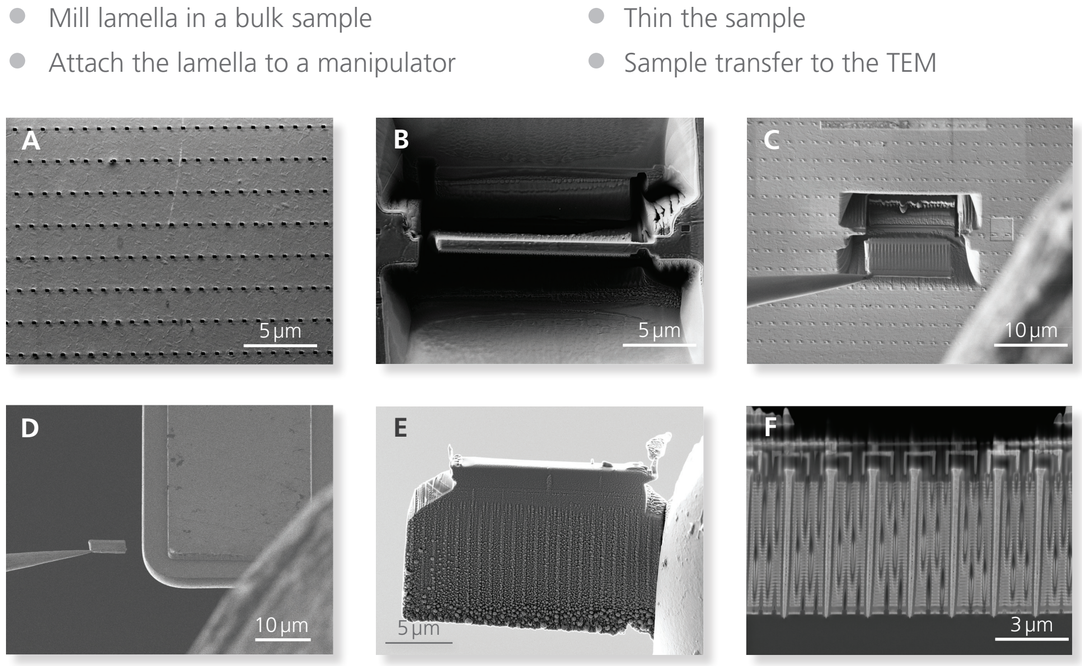

Fig 3. Typical steps for the preparation of a TEM lamella via FIB-SEM lift-out: (A) select the site, (B) deposit and mill, (C) attach and lift-out, (D) relocate to grid, (E) attach to grid, (F) thin the sample.

Cryogenic Lift-Out

The method of cryogenic lift-out was developed to follow the same process as a room temperature (RT) lift-out, with only slight variation. The process is shown in Fig. 4 and can be summarised as:

- Select site of interest: Same as RT
- Deposit protective layer: layer condenses on the surface, not beam assisted deposition used at RT
- Mill the lamella in a bulk sample: milling is the same as RT
- Attach the lamella to a manipulator: use a Carbon etch GIS to attach
- Reposition the lamella to TEM grid: Same as RT but with a CryoProbe
- Attach to grid: Use Carbon etch GIS to attach
- Thin the sample: Same as RT
- Sample transfer to the TEM: Challenging as atmospheric exposure damages the sample

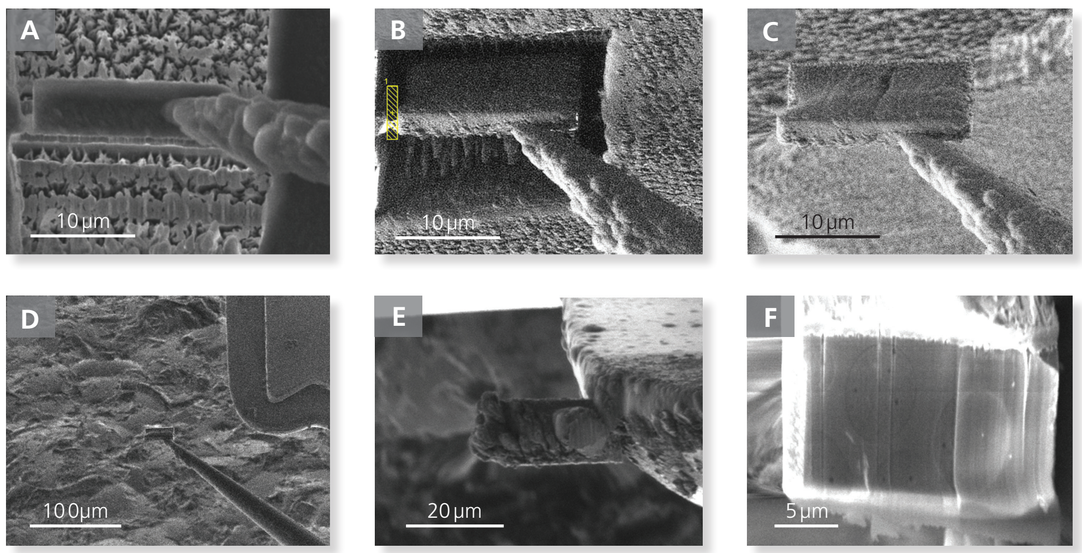

Fig 4. A Cryo-TEM lamella containing yeast cells prepared using the CryoProbe. The steps closely resemble that of the room temperature process. (A) prepare sample, (B) attach lamella, (C), lift-out, (D) reposition to grid, (E) attach to grid, (F) thin the lamella.

Conclusions

Using a FIB-SEM with Oxford Instruments CryoProbe site specific Cryo-TEM sample preparation has been demonstrated. The technique is suitable for both biological and non-biological samples allowing unprecedented access to a wide variety of materials. Use of a standard port-mounted accessories already present on most FIB-SEM and conventional lamella lift-out technique means the requirements on sample geometry/thickness are significantly relaxed and Cryo-TEM preparation is within reach of most FIB-SEM users.

References

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