

## Sample Optimization for *In Situ* Lamella Preparation for Cryo Electron Tomography

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Cryo Electron Tomography is emerging as a technique which enables the extension of structural biology from solely purified structures, to the dynamic complexity of the cell [1]. Focused Ion Beam (FIB-SEM) erosion under cryogenic temperatures offers researchers a way of producing damage and artefact free lamella which can be utilized in high end tomography tools such as the FEI Titan Krios™. Several Cryo-FIB prototype tools exist and have been producing significant results utilizing the '*in situ*' approach [2,3,4].

Correct preparation of samples for *in situ* lamella prep is critical for making use of this emerging technique. There are many steps where the sample can be significantly contaminated or even completely damaged which would prevent from collection of high-quality cryo-electron tomography data. Standardization of the whole process of ablation of cellular material and sample handling is required to provide sufficient yield of high-quality samples for TEM imaging.

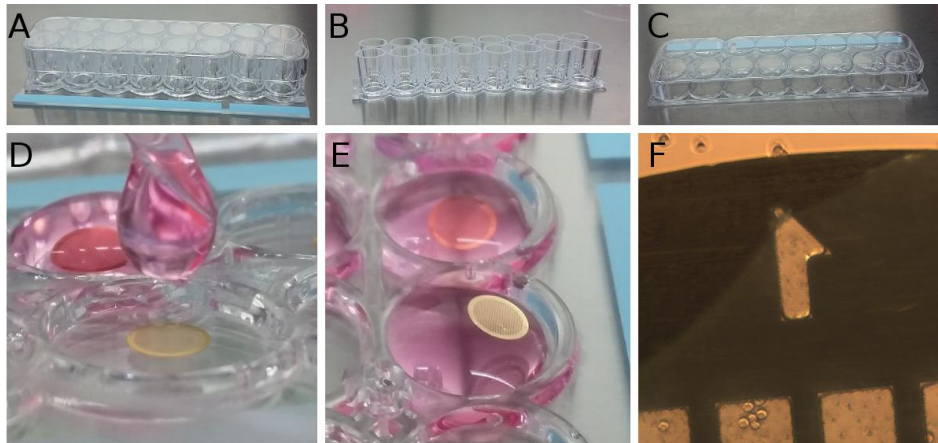
Prior to these steps, the protocols for adhesion of the cells on the TEM grids and its subsequent vitrification require standardization. Here, we provide a protocol for preparation of samples for cryo-focused ion beam micromachining (FIB-milling) which in our hands provides high reproducibility and quality of the input material for FIB-milling. An example of standardization based on *Saccharomyces cerevisiae* and A9 are presented, showing the critical steps and success indicators required to reproduce high quality samples. It is hoped that these insights will help others to quickly adapt to this new method of sample preparation.

Many existing users of cryo preparation for structural biology have developed methods which suit single particle type preparation; this can involve many grid chemistries, buffer types and preparation stages. Cells require a lengthier grid preparation stage. Here cells must be encouraged to attach with a grid surface either in the plunge freezer or under culture conditions over the course of 24 hours or so. Figure 1 shows the culture method for TEM grids. Using live cells also requires the user to work aseptically and utilize biocompatible processes; ensuring that the environmental conditions are kept controlled, the cells are in a biologically relevant condition and that the materials used in the preparation are biocompatible. Being able to control the deposition process gives better morphological presentation of the cells but also ensures more uniform freezing characteristics.

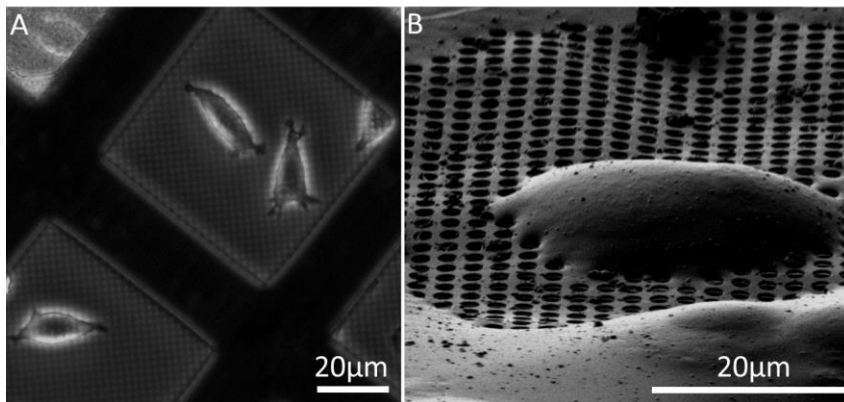
Cells can be upwards of 500nm thick. Cells of greater than 10µm will unlikely experience uniform freezing and vitrification via plunge freezing. The method of blotting, the buffer, temperature, humidity, cryogen selection and transfer methods also play important roles. All these factors can greatly affect the success rate of the method. In this presentation the approaches to sample preparation which enable successful result in the FIB-SEM and TEM are discussed. Figure 2 shows a typical view of cells before freezing and after optimum freezing on the same TEM grid.

## References:

- [1] Lučić V *et al*, J Cell Biol. **202(3)** (2013), p. 407.  
 [2] Chaikerasitak *et al*, Science. **355(6321)** (2017), p. 194.  
 [3] Mahamid *et al*, Science. **351(6276)** (2016), p. 969.  
 [4] Schaffer *et al*, J Struct Biol. **197(2)** (2017), p. 73.



**Figure 1.** Chamber slide system for adhesion of cells onto TEM grid (A). The central part of the chamber slide system which is removed before the grids are placed on the slide (B). Bottom and top part of the chamber slide system used for cultivation of cells on TEM grids (C). Correct way for pipetting the cell culture onto the TEM grid (D). Grid positioned upside down on the drop of cell culture after incorrect application of the cells onto the grid (E). Marker on the Quantifoil grids used to determine the orientation of the grid in solution (F). When “1” is mirrored (F), the grid is facing with the carbon foil up.



**Figure 2.** A9 cell which have correctly adhered onto the TEM grid viewed under growth media in a light microscope (A). Plunge frozen A9 cell viewed in a Cryo FIB-SEM ready for lamella preparation (B).