Read through and understand this document before contacting james.gilchrist@diamond.ac.uk

Guidance for cryoFIB proposals

https://www.diamond.ac.uk/Instruments/Biological-Cryo-Imaging/eBIC.html

Who should apply

Researchers who want to examine structures within the interior of cells at high resolution using tomography or diffraction of crystals in a cryogenic TEM.

Important points

- Only on-grid thinning is performed
- For crystal lamella apply *via* rapid access (see below)
- TEM grid atlases are required for a proposal
- The number of lamellae prepared will vary depending on the size, density and location of milling targets as well as ice thickness
- Instrument preparation will start at 9 am and sessions are over at 5 pm
- Samples entering eBIC must be biosafety level 2 or lower and be deactivated by 70% ethanol

How to apply

Applications are made through the user administration system (UAS, https://uas.diamond.ac.uk).

There are two access routes: Standard and Rapid

Rapid access:

- Single day cryoFIB sessions with lamellae imaged at another institution
- Grids will be clipped into an AutoGrid ring. Please ensure you have access to an autoloader enabled microscope for the imaging.
- CryoFIB Rapids may be combined with Krios BAG sessions if eBIC are given enough notice
- Proposals are reviewed and scheduled by eBIC staff within two months of submission
- You will need at least two good grids per session
- You must be ready to carry out your rapid session *before* you submit your proposal

Standard access:

- Three days of cryoFIB milling and two days of Krios tomography (for a five day eBIC visit)
- Standard proposals are assessed for feasibility by eBIC staff before being peer-reviewed
- You will need at least six good grids

In the UAS:

- For uploading the Science Case there is a two A4 page and a 2 MB limit. Links to file hosting services can be within this document.
- Under Instruments, select “Scios - Scios at Diamond”
- For Rapid access request 1 shift
- For Standard access request 3 shifts

What makes a good submission

Showing frozen grids and demonstrating grid quality in your submission is essential. Screen your grids in a TEM and show the TEM atlas.

Good grids (Figure 1) will have:

- Thin vitreous ice
- Enough milling targets close to the centre of grid squares
- An intact support film

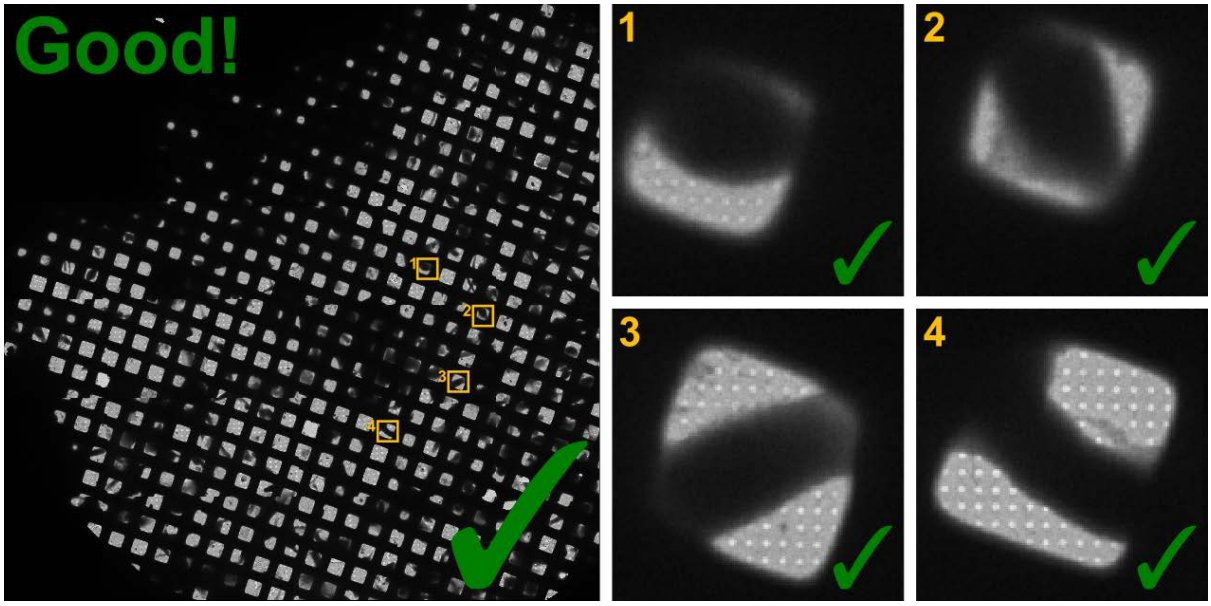

Figure 1 A TEM atlas of a grid suitable for cryoFIB milling.

Bad grids (Figure 2) will have:

- Ice that is too thick
- Extensive support film damage
- Few milling targets or targets on or adjacent to grid bars

Figure 2 Examples of bad grids determined from TEM atlases