

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
- 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

Proposals are made through the user administration system (<https://uas.diamond.ac.uk>).

Application and experiment information:

- Rapid access only
- You must be ready to carry out your session *before* you submit your proposal
- You will need at least two good grids per session
- Proposals are reviewed and scheduled by eBIC staff within two months of submission
- Grids can be supplied over multiple weeks
- Grids will be clipped into an AutoGrid ring. Ensure you have access to an autoloader enabled microscope for the imaging.
- Lamellae can be stored at eBIC
- Lamellae can be examined at eBIC using BAG time or in a rapid session (requires an additional separate proposal) or at another institution.

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"
- Request 1 shift for each session

What makes a good submission

Showing frozen grids and demonstrating grid quality in your application 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

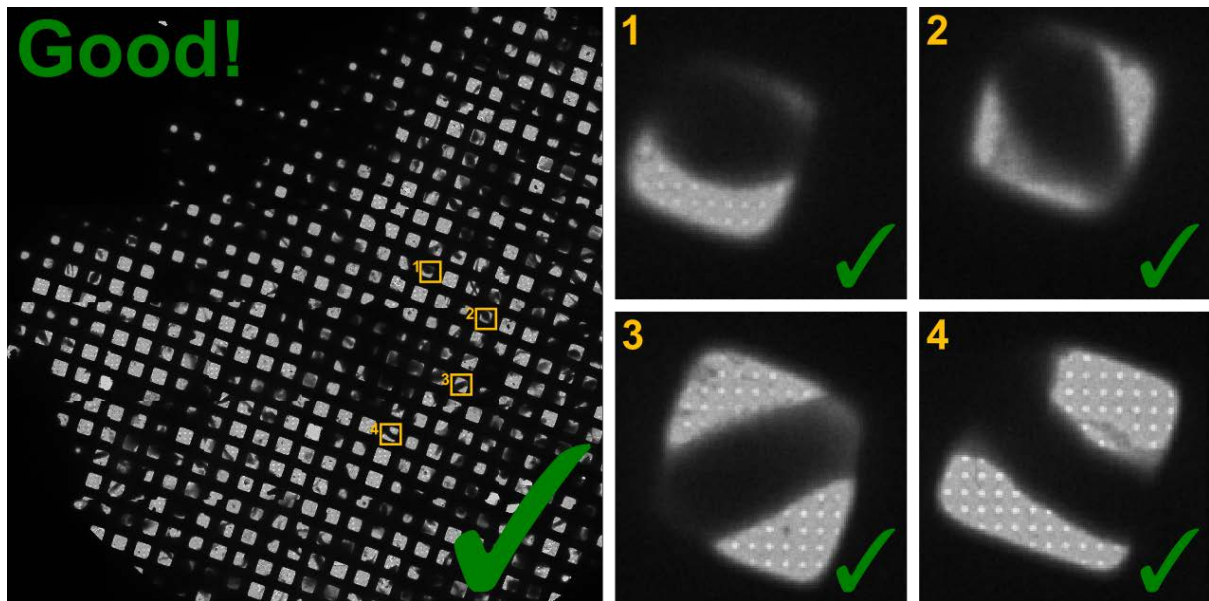


Figure 1 A TEM atlas of a grid suitable for cryoFIB milling.

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

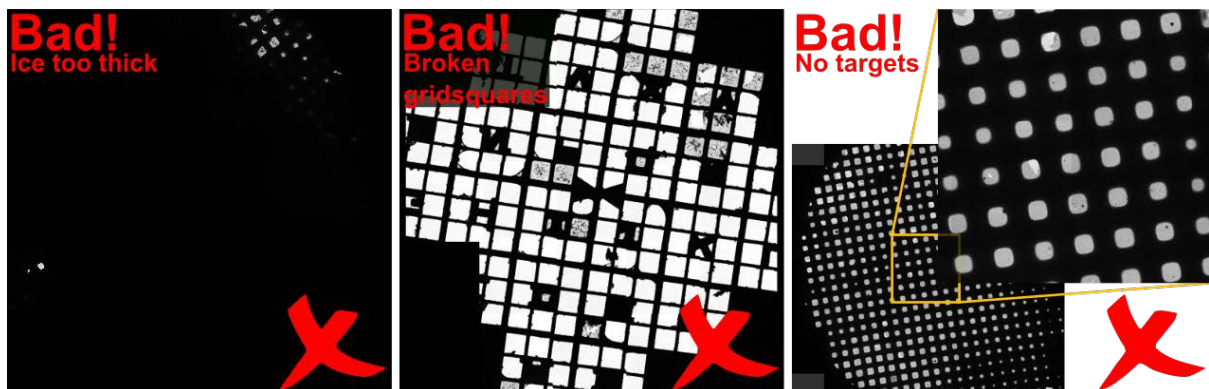


Figure 2 Examples of bad grids determined from TEM atlases