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# Guidance for cryoFIB proposals

Find the latest document here:
https://www.diamond.ac.uk/Instruments/Biological-Cryo-Imaging/eBIC/Current_calls.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 automated, on-grid thinning is performed
- TEM grid atlases are required for a rapid proposal
- The number of lamellae prepared will vary depending on the size, density and location of milling targets as well as ice thickness
- Instrument cooling starts at the beginning of the session. Onsite LC support ends 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:

- For rapid access:
    - 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
- For BAG proposals:
    - Sessions can be scheduled any time within the AP you apply for
- For either rapid or BAG routes, grids can be supplied over multiple weeks. Grids will be clipped into an AutoGrid ring. A maximum of two grids are loaded for a 48-hour session.
- Investigators can attend remotely
- Lamellae can be stored at eBIC
- Lamellae can be examined on an eBIC Krios using BAG time or a rapid session 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 “Aquilos”
- For each cryoFIB session you want, choose 6 shifts *i.e.* 48 hours

## 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

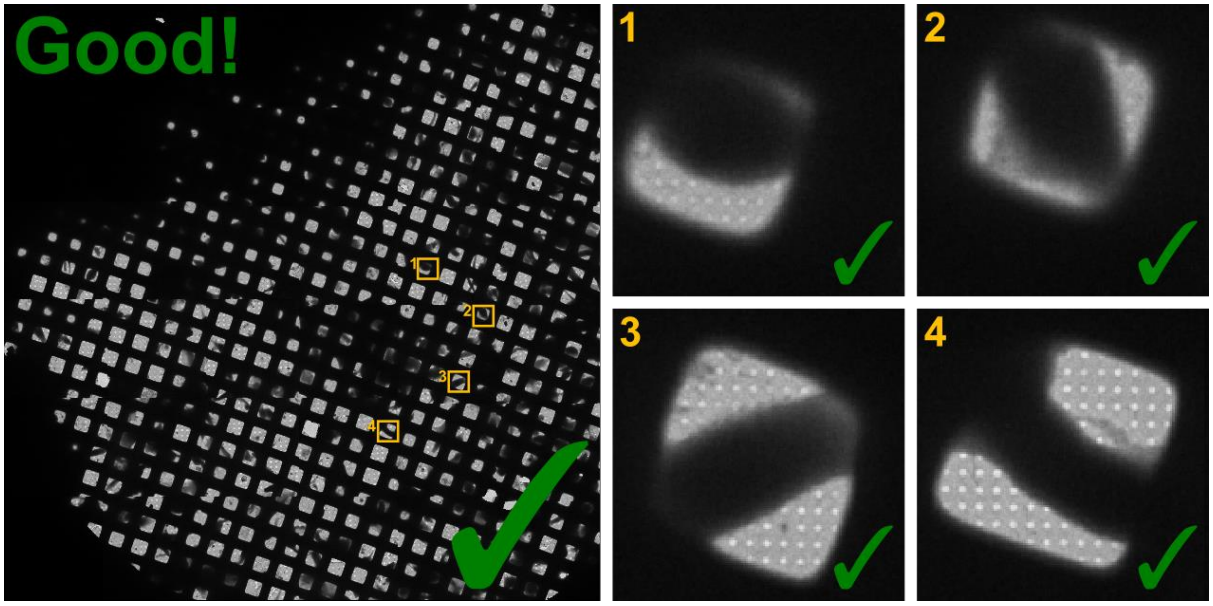


*Figure 1 A TEM atlas of a grid suitable for cryoFIB milling.*

### 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*