

Protocol for Making EM Grids

For Flat Carbon

- Try Hitachi carbon (if we can get it to fit the Cressington evaporator)
- Multiple, short evaporation steps
- Avoid sparking- you may have to experiment on this one
- For room temperature work, you can use copper grids. Float the carbon onto the dull side of the grid.
- For cryoEM, use Pacific GridTech molybdenum grids.
- Check molybdenum grids under a light microscope and discard any with large holes (about 10% of grids)
- Deposit sample on mica side of carbon film

For Adherence

This will depend on the specimen and there seem to be few rules. However, the surface properties of the carbon can be important factors. The **back injection method** seems to work for many specimens (Vonck, J. Ultramicroscopy 85 (2000) 123-129).

For Hydrophobic Grids

- Try ageing the grids
- Try baking the grids at 80 °C for 1 hour

For Hydrophilic Grids

- Glow discharge in air or water vapour gives negatively charged carbon
- Glow discharge in amylamine gives positively charged carbon

Purple Membrane as an Example

- Age the carbon in air at room temperature for 4 to 8 days.
- One day old carbon is more adherent but disorders the crystals
- One to two month old carbon is not adherent at all
- Good spread is achieved with a small amount of detergent (below CMC)
- Glucose embedded grids could be stored in grid boxes on dry ice