

Technical Tip

A Fool-Proof Method for Mounting Serial Sections on Single Hole Grids

We did serial sectioning for years using large single hole grids using a very simple technique that made the potential problems of film thickness, wrinkles and section loss very minor. The method is as follows:

Supplies: 0.2-1% formvar, film caster or narrow container for formvar, glass slides for casting films, filter paper, forceps, single hole grids, plexiglass pick-up slides (new product).

1. Purchase a set of Plexiglass pick-up slides as offered through Electron Microscopy Sciences.
2. Cast the formvar films onto glass slides using your standard method. Usually a good silver film, not gray, will work fine. We formerly immersed the slide into a small container of 0.2% formvar in dichloroethane solution. We now use a film caster (EMS #71305-01) with 1% formvar solution that lets us hold the slide in the dichloroethane vapors after lowering the formvar solution level. This method tends to give you more uniform films consistently. Immersing the slide in the formvar for 3 min followed by 3 min in the vapor is a good starting point for timing. Investment in a film caster is worth it for many years use and a consistent final product.
3. Float the film off the glass slide and pick it up with the Plexiglas pick-up slide so the film covers the holes. Then draw the water out of the holes by pressing the plastic slide down onto filter paper, or using small pieces of filter paper and capillary action to draw the water out of individual holes. The films should hold nicely over the holes in the slide. Store slides in a slide box until needed.
4. Next, cut your sections using a block width that is fairly similar to the diameter of the slit in the grid. The length of the section can be narrow resulting in many sections per grid.
5. Pick up the sections on UNCOATED grids by gently lowering the grid to the surface of the knife boat. We put the dull side down on the premise that the rough surface would grab the film better during step 7. The surface tension of the water will hold the sections in the grid opening. Transfer the grid to a droplet of water until you have finished sectioning. Do not invert the grid. It is important that the top of the grid (shiny side) stay dry so that the grid will float on all subsequent solutions.
6. Transfer the grid + sections + water droplet to a drop of stain. A small amount of water will be transferred but this will not interfere with staining. If you are concerned about the dilution effect, increase your staining time slightly. Allow the section to stain and then wash by transferring through a series of droplets of clean water. Continue to post-stain if desired and wash the same way. Never let the grid dry. There is minimum problem with stain precipitation if you use very clean water and transfer the grid through a sufficient number of water droplets (6-12 recommended).
7. The final step is to transfer the grid to a film suspended over the hole in a Plexiglas pick-up slide and let it dry down. The sections will now be stuck to the film with NO wrinkles and minimum breakage. When ready to view, just punch out around the grid with the tip of your forceps, grab the grid and insert it into the microscope.

Believe me... the sections will still be there at the end!

We found that as long as the sections cover a substantial portion of the open area of the grid, carbon coating was not essential. With some practice it is possible to do 50-100 grids worth of serial sections without losing any. This method is also very helpful for preparing sections for tomography with no grid wires to interfere with tilting and imaging. The films on the plastic slides will hold for months so you can make a lot and store them until needed.

Reference:

Sherman, D.M. "A Fool-Proof Method for Mounting Serial Sections on Single Hole Grids". MSA Technologist's Forum Newsletter 16:2.1998.