

A Fool-Proof Method For Mounting Serial Sections On Single Hole Grids

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I did serial sectioning for years on large single hole grids using a very simple technique that made the potential problems of film thickness, wrinkles and section loss very minor. I was not the original developer of the method and do not remember who originally gave it to me. It goes as follows:

- 1) Have your machine shop cut some thin pieces of Plexiglas into the size of glass slides. At one end, drill about a dozen holes, roughly 5 mm in diameter, in an area about the size of a formvar film cast on glass slides. These slides will serve as your template for holding your films.
- 2) Cast the formvar films onto glass slides using your normal method. Usually a good silver film, not gray, will work fine. I routinely used 0.2% formvar in dichloroethane when casting by

Cure at 60°C or with UV for 1-2 days.

Once you've removed the resin block from the mold (you should be able to pop them out of the PE molds like ice cubes; Al molds have to be peeled), wipe away any uncured resin. Use a razorblade to remove any resin from the back of the coverslip, then peel away the coverslip (Thermanox) or use a heat/cold regimen to release glass coverslips (wear safety glasses!). The areas of interest can then be cut out with a jeweler's saw and mounted on blanks for sectioning. I also cut down the resin backs of the cell blocks to ~2 mm before mounting to avoid skyscraper blocks.

immersing the slide into the solution in a small jar, etc. We now use a film caster that lets us hold the slide in the dichloroethane vapors after lowering the formvar solution level. This method tends to give you thinner films consistently so the correct solution percentage and timing would have to be redetermined.

- 3) Float the film off the glass slide and pick it up with the Plexiglas 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 until needed.

- 4) Next, cut your sections using a block diameter that is fairly similar to the size of the slit in the grid. Pick up the sections on UNCOATED grids by gently lowering the grid to the surface of the knife boat. I put the dull side down on the premise that the rough surface would grab the film better during step 6. 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.

- 5) 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 stain ing time slightly. Allow the section to stain, 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

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Odd Vibration Sources

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Chasing sources of vibration seems to be a never-ending problem in microscopy. Here are the approaches we've taken to two different sources of vibration affecting our Hitachi S-800 FESEM. One of the first problems was with a window air conditioning unit in the room that was needed to augment our so-

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with stain precipitation if you use very clean water and transfer the grid through a sufficient number of water droplets (6-12 recommended).

6) The final step is to transfer the grid to a film suspended over the hole in a Plexiglas 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 into the microscope.

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

I found that as long as the sections cover a substantial portion of the open area of the grid, carbon coating was not essential. I used to do 50-100 grids worth of serial sections without losing any. The films on the plastic slides would hold for months so I could make a lot and store until needed. The method really works...do give it a try.

called central system. The existing window unit vibrated the window, walls, and floor. One solution may have been to buy a split unit so that the compressor was outdoors, but there was a moratorium on buying new air conditioning units. An additional problem is that the split units do not dehumidify well enough for the tropics. Our physical plant people came up with a design that worked with the existing unit. The air conditioner was mounted on a welded steel frame set into concrete in the ground outside the window. Where it would have been mounted in the window in a wooden frame, the wood was instead replaced with a heavy rubber bellows. For magnifications of up to about 80,000x we can leave the air conditioner running and we do not see any changes as the compressor cycles on and off, but we still turn off the unit at greater magnifications. Our only problem now is that people often use the steel frame outside as a bicycle rack, resulting in more activity under that window and against the wall than we would prefer!

A second source of vibration turned out to be the metal shrouding over the ion getter pumps mounted behind the column. Although the column has its own, isolated suspension, we still noticed jaggies on the screen at high magnifications when people spoke in the room. Further investigation (well, OK, it was an accident) proved that when the metal shrouding around the pump was removed that particular source of vibration was eliminated. It turns out that the pump shrouding was acting like a drum and transmitting audio noise to the column. Interestingly, it did not seem to transmit the noise of the rotary pumps. I simply leave the shrouding off these days, and Hitachi has subsequently redesigned the shrouding to be perforated to minimize the drum effect.

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