NETNOTES

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Selected postings from the MSA Microscopy Listserver (listserver@msa.microscopy.com) from 8/11/03 to 10/10/03. Postings may have been edited to conserve space or for clarity.

TEM - Formvar coated grids

I am going to make up a batch of Formvar in Ethylene Dichloride and would like some suggestions concerning safety precautions, clean up of my glassware and stir bar and the best dilution to use. Donna R. Clarkson <donna.clarkson@brooks.af.mil>

Work in a fume hood as the fumes are considered carcinogenic. Keep all wastes in a container (vented to hood and away from flames) for disposal as a volatile chemical.

To clean your glassware and stir bar, rinse them in ethylene dichloride several times and put the washes in the same disposal container. I have used 0.25% Formvar or Butvar in ethylene dichloride. My recommendation is that you purchase the solution already made up (all EM Supply houses carry it). That way you have less waste and the product is guaranteed. John J. Bozzola <bozzola@siu.edu>

TEM – Sections adhering to grids

Two individuals posted messages asking the best way to ensure sections adhere to TEM grids and one asked if these techniques would increase background binding in immunocytochemistry protocols. Another individual asked about a recipe for making "grid glue" to promote section adherence.

Poor adhesion of sections is generally due to either dirty grids or grids that are not flat (bent or wavy). Before using grid glue, try cleaning the grids by sonication in a solvent such as acetone. Alternatively, you might use an acid such as 1M HCl for 5-10 minutes. If cleaning alone does not solve the adherence problem, then place the grids onto a filter paper (with the side that will hold the sections facing up) and place droplets of 0.25% Formvar or Collodion onto the grids. This puts a plastic coating over the meshwork of the grids without obstructing the open areas. The plastic coating enhances adherence of the plastic sections significantly. Also, after the sections are put onto the grids, place them overnight in a 60°C oven prior to immuno staining. This works wonders. If all of these fail, maybe then I would consider grid glue as the last resort. Grid glue can be prepared by dissolving the adhesive from 2 inches of Scotch Brand transparent tape in 10 ml of ethylene dichloride. Please be sure to use the transparent tape (old style) rather than the Magik Tape since the adhesives are apparently different. I believe that chloroform can also be used as a solvent. It might also be possible to use the commercially prepared Grid-Stick Glue, but it would have to be diluted. However, I have not tried this. John J. Bozzola <bozzola@siu.edu>

We have used "grid pens" with glue with great success for our immunocytochemistry work. Initially, we had put up with shredded LR White and Unicryl sections in the TEM, regardless of how recently they were cleaned or flamed with an alcohol burner. Then, we moved to Formvar/carbon grids, but had severe problems with "stickiness" causing high background labeling, apparently due to both antibody binding and mechanical trapping of the gold conjugate, not to mention various other folding and layering artifacts. Finally, after a suggestion from someone on this list, we tried the pens. Now our sections remain largely intact and the background problem is under control. We do not dilute the glue in the pens, but just put one dab on grids on filter paper and let them dry a couple minutes before picking up our sections. As far as any effect on labeling, I have never done any side-by-side comparisons with and without adhesive, but I have not noticed any obvious effects on our labeling. Randy D. Tindall <tindallr@missouri.edu>

I have found that, after picking up sections, if I put the grids in the 50-60 degree oven for several hours to overnight, the sections always stick. It isn't necessary to clean them first either. I use the thin bar copper grids. Mary Gail Engle <mgengle@uky.edu>

I also use thin bar copper grids and find that 10 minutes in my 60-65 degree oven is long enough to do the trick to help in adhesion. Karen Bovard <kbovard@creighton.edu>

Another factor that affects section adhesion is the method in which the sections are picked up. If the sections are picked up by immersing the grid and coming from underneath, or picked up in a loop and placed on top of the grid, they adhere much better than if the grid is pushed down on them from above. Ralph Common <ralph.common@ht.msu.edu>

Here's my methodology for collecting epoxy sections onto mesh grids and getting them to stay there. It works very well 99% of the time. I always clean my uncoated copper mesh grids (no support films, like Formvar) by sonication for 30-60 seconds in a dedicated, clean 25 ml glass beaker containing the following solution: 10% concentrated HCl + 20% acetone + 70% distilled water. Note: Be careful mixing this up. Add HCl slowly to water, and then add acetone and store in clean capped glass bottle. pH paper tests gives pH = 1.0. After sonication in cleaning solution, rinse 2x in 99% acetone, air dry, invert beaker over clean filter paper and they will fall down as acetone rinse dries. Always clean grids the day you section. Even after overnight, I find that some sections might tend to loosen up a bit on copper grids during staining procedures, I guess due to a new oxidation layer forming within a day or two (rust never sleeps!). I think the HCl removes oxidation layers from the grids, acetone breaks the surface tension of the aqueous solution so grids don't float, and also cleans and facilitates quick air drying. During staining, minimize the turbulence of stains and rinses over section surfaces on grids. I prefer to pick up sections from the knife's water-filled trough from above, rather than to slide the grid underneath sections (underwater) and lift up. For me, collecting from above allows me to place a group of sections right where I want them on the grid, without them sliding off to one side or wrapping around the grid edge. As I place grid over sections, I press down very gently, but not nearly enough to break the surface of water. The resultant slight water pressure seems to push the sections onto grid bars so they stay put. After lifting a grid off the water surface, I blot the edge of the grid with a filter paper point to remove that drop of water that is hanging from the grid, air dry or dry over slide warmer surface that is not too hot. Be sure to give adequate drying before you move on to the staining. I think the crucial step is the cleaning of the grids using above solution the same day as they are used to collect sections. I've been using this method for many years with consistent success. Gib Ahlstrand <ahlst007@tc.umn.edu>

With the technique that we use, no known force in the universe will be able to remove the sections from your grids. First of all, use clean grids. There are several ways to clean grids, but the way that I like, because it is fast, is to hold the grid in concentrated NaOH while I count to 6, and then briefly dip it repeatedly in water to rinse the grid. I do this just before I pick up the sections. Then, most importantly, we dry down our grids in a drying oven. The oven is just hot enough to dry glassware, but not really super hot. In other words, I can put my hand on the bottom of the oven without burning it, but I really wouldn't want to keep my hand there for more than a second or so. And the temperature isn't hot enough to warp our plastic Petri dishes. If it starts to warp the Petri dishes, it's too hot, though it still probably won't hurt your sections. You can dry your sections there for about 5-10 minutes. But, if you even forget about them overnight, it won't hurt them at all. Then, I can assure you that your sections will never wash off your grids no matter how vigorously you wash them during staining. Garry Burgess <gburgess@exchange.hsc.mb.ca>

TEM - Carbon Support Films

I have recently switched to Formvar in diethylene chloride as a base for making carbon coated grids, because I was told that I would get better continuous films. I am having a problem getting rid of the Formvar after coating. Does anyone out there have a method for making carbon coated grids that works every time? Steve Parry <sparry@cmm.uwa.edu.au>

When I want just carbon as a substrate, I will coat freshly cleaved mica with carbon in a metal evaporator. This allows me to dictate the carbon thickness and I have no trouble floating off grid-size squares (prescored) in a drop of water. Picking up the carbon with glow discharged grids (flat side) is a snap. Make sure everything is clean. This should give you thin clean substrates. Mike Delannoy <delannoy@jhmi.edu

I would like to add a few cents to the Mike Delannoy posting. Instead of picking up carbon depositions on individual grids in the drop of water, I use a clean glass container filled with deionized ultrapure water up to about 5 mm lower than edge. The goal is to have a relatively large flat area of water surface. Use a fluorescent lamp to illuminate the water surface similar to the way you illuminate the water surface in an ultramicrotome knife boat. You should see the reflection of the bulb on the surface. Playing with the angle and location, you need to find the orientation that gives you a well illuminated large area on the water surface. Depending on how many grids you want to make, cut the piece of mica with carbon of correspondent size. It is important that all four sides of

your mica piece be freshly cut. Slowly immerse the mica, with the carbon side up, at a 45°deg angle into the water so that the carbon film will float on the surface. It should be visible if the illumination is set right. Put the grids on the carbon film by dropping them from a few mm above the film being careful not to touch the carbon with the tweezers. Cut a piece of Parafilm slightly bigger than the carbon film. Using two tweezers, carefully put Parafilm on top of the carbon and grids. Hold one side of the Parafilm sheet with a pair of tweezers and with one smooth movement, move the Parafilm with the carbon and grids under and then out of the water. Place the Parafilm sheet with attached grids facing up on a piece of filter paper and dry at 60ºC for about 10 min. The grids are now ready for use. I prefer to use fresh carbon for every experiment, so I made about 6 grids at the time and then make more if needed. If you are using extremely thick carbon films, you may not need good illumination. I don't see any point to glow discharge the grids. If glow discharge "works" on the naked grids, it simply meant that grids are greasy and that contamination has been ionized by glow discharge. Clean metal grids will not hold a charge at all due to electro-conductivity. Covering grids with a much diluted plastic solution (e.g., 0.005-0.01% Parlodion) may help to attach carbon to the grids. Just put grids on the filter paper and put a drop of plastic on it, let it dry. A more effective way to mount carbon film on the grids is to use "holey" carbon coated grids. Carbon sticks nicely to carbon. Sergey Ryazantsev <sryazant@ucla.edu>

I second Mike Delannoy's suggestion. Mica should give you the smoothest continuous carbon films. In my experience one problem is that the C-films can wrinkle terribly if floated off too soon from the mica. This can be avoided by letting the film first "mature" overnight on the mica. James Chalcroft <jchalcro@neuro.mpg.de>

The method I use to make carbon-coated grids uses collodion in amyl acetate. A drop or two is put onto the surface of a large dish of distilled water. This dries to a circle about three to four inches in diameter on the surface of the water. I drop copper grids onto this surface, preferably before it dries too much, so the collodion is still a bit sticky. After I have about thirty grids arranged in a neat rectangle on the collodion film, I drop a filter paper on top and bring the edges of the film over the edges of the filter paper. As soon as the paper is wet, I lift it out by one edge and lay it down, grids up, on more filter paper to dry. This may take some practice to get the filter paper up out of the water with the grids still stuck on. When the paper is dry, I cut out the rectangle of paper with the grids on it with scissors and carbon coat it (grids side up) in the evaporator. The grids are then removed from the paper and put in a Jaffe washer (collodion/carbon side up) filled with chloroform for 48 hours. (A Jaffe washer is a Petri plate with a stack of four glass sides and a stack of filter paper over the glass slides. The bottom of the Petri plate is filled with solvent and the lid of the Petri plate put on. Then, another, larger dish is put over it so the solvent doesn't evaporate too quickly. The material put on the top of the filter paper stays wet with solvent and the solvent washes the material). This will dissolve the collodion and bring the carbon film down to stick it to the copper grid. These films are conductive, continuous, and strong enough for routine work at 200kV. Mary Mager <mager@interchange.ubc.ca>

TEM – glow discharge alternatives

I am attempting to do a negative stain on a sample. The problem I'm having is that according to the protocol I have to glow discharge the grid before I use it. I don't have access to a vacuum evaporator with a glow discharge unit. Any suggestions as to how to fake a glow are greatly appreciated. Paula Sicurello <patpxs@gwumc.edu>

There are several possibilities here, but none of them are as good as the real thing. 1. Put your grids in the chamber of a sputter coater but cover the grids with a "tent" of filter paper. The tent should completely cover over the grids so that few of the metal particles will strike it, but the argon ions will. Alternatively, you could turn the slide containing the grids upside down so that they face away from the target. Just be sure to leave some space underneath, so the ions can contact the grids. Activate the sputtering process so that you see the plasma for 40-50 sec. You might have to experiment here so that you minimize the presence of metal. If you have a carbon sputterer, I believe that would be the best. 2. You might try a dozen hits of the grids with one of the electrostatic guns (used by photographers). This sometimes works. 3. Finally,

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and don't ask why this works, just leaving the grids for several days inside of a refrigerator will cause them to become hydrophilic. I am guessing that the trapped organics (odors, fumes, etc.) are depositing onto the active surface of the carbon. This is what happens when you place sodium bicarbonate in the refrigerator to trap odors. John J. Bozzola <bozzola@siu.edu>

First, have you tried a standard negative stain preparation? A lot of the time there is enough protein present in the sample to overcome the hydrophobicity. Just leave the sample on the grid for about a minute. Second, try an Airfuge (EM-90 rotor) preparation. The combination of centrifugal force for 1/2 hr plus protein present in the preparation usually overcomes the need for glow discharge. Third, try pre-treating the grids with either poly-L-lysine or Alcian blue. Use a 1% solution, float the grids on the solution for 1-2 minutes, wick off the excess, and then let dry. Paul R Hazelton <paul_hazelton@umanitoba.ca>

It came to my mind, that exposure of the grids to the strong UV (real UV) light does the trick also. The effect will vary depending on the UV source and intensity. As I remember, grids are good for about 40 min. afterwards. Personally, I prefer to use poly-lysine treatment. In my hands it works nearly the same as glow discharge, but it is even better because it does not make the surface rough. Sergey Ryazantsev <sryazant@ucla.edu>

SEM – Magnetic particles

I have a student who wants to image magnetic particles with SEM. The particles are in the 400 nm to 1μm size range. I had planned to stick them to double stick copper tape prior to imaging. I am concerned about their adversely affecting the microscope. Are there any suggestions as to mounting the particles and whether a short working distance may magnify potential problems if some particles break loose and get into the lens? If there is a concern then I guess it would be possible to mount on sticky carbon tape, overlay the sample with a Formvar film, and then use backscattering to image the particles. Is this necessary? Debby Sherman <dsherman@purdue.edu>

Is it possible to simply degauss the samples before putting them in the SEM? There is a border, I believe, between too much degaussing and too little. Looking at hard drive tracks is a case in point. Depending on your ultimate resolution, the working distance may facilitate your specimens by keeping them away from the final lens. 1 μm specimens should not require a short working distance. Your BSE ought to be a separate issue depending on what type it is and what KV it works at. Gary Gaugler <gary@gaugler.com>

We have routinely examined (gamma-) ferric oxide particles in the SEM by dusting the particles onto double-sided sticky carbon tape. Or you can paint a small dab of carbon paint onto your stub and dust the particles onto the paint while it is still wet. Then tap the side of the stub firmly on a hard surface to dislodge any loose particles. If you are really paranoid you can use a low-pressure gas stream (like a "Micro Duster") to make sure there are no loose particles present. However, the more aggressively you remove looser particles, the less you are likely to see larger particles that may exist in the specimen. This may be important if you are trying to do particle size distribution measurements.

Particles like Fe_2O_3 are weakly magnetic, so there is not much danger of them being sucked up into your lenses by the magnetic field. You will probably want to keep the working distance fairly short (<10 mm) to achieve acceptable image quality. Ken Gaugler <ken@gaugler.com>

Wouldn't it also be dependent on the type of SEM used (*i.e.* HRSEM)? If the final lens (pole piece) is not energized for through-the-lens detection, the sticky Cu tape should hold them down. Disperse the particles then blow off the weakly held particles. Now lower a ferro magnetic material over the samples and see if any pop off. If particles do "pop" off then use Formvar and a high kV. The magnetism of the particles may play a role in their physical construct so degauss with caution. I believe imaging aberrations will most likely be the issue over that of tool contamination. I have successfully imaged toner particles in a relatively low resolution SEM. It should be noted that this tool has been classified as a "dirty" tool. The toner particles were evenly dispersed on the "new" C sticky tabs that are rock hard with barely any adhesive. Krisopher Springsteed <kristopher.springsteed@hp.com>