NETNOTES

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Selected postings from the MSA Microscopy Listserver (listserver@msa.microscopy.com) from 2/5/03 to 4/5/03. Postings may have been edited to conserve space or for clarity.

CRYOSTAT – Sucrose Protection

For work requiring sectioning on a cryostat, how do you decide whether to infiltrate specimens with sucrose? I will be fixing my tissue with 2% paraformaldehyde and some protocols call for direct freezing and others for infiltration with 30% sucrose in PBS first. I know the sucrose will cryo-protect and suspect it will also improve the plasticity but is there a disadvantage? Tom Phillips <phillipst@missouri.edu>

The preference in our facility is to cryo-protect with sucrose whenever possible. We even freeze our tissues in a 1:1 mixture of sucrose (20% in PBS) and OCT. It makes a softer block, and you may have to drop your chamber temp to -20°C, but it cuts very smoothly and yields very nice structure. The only time we don't use sucrose is when the primary antibodies to be used won't tolerate any fixation, then we snap freeze and pray. Our protocol is a modification of that published by Barthel & Raymond (1990) J Histochem Cytochem 38:1383-1388. They were looking at eyes. Leona Cohen-Gould <lcgould@med.cornell.edu>

ULTRAMICROTOMY – Dehydration with molecular sieves

I'm curious as to how many people have had problems using molecular sieves in dehydration solvents, with respect to knife damage. We seem to be going through diamond knives at an uncomfortable rate and we're wondering if this could be a contributing factor. Randy Tindall <tindallr@missouri.edu>

This is something we discovered over 20 years ago: molecular sieves work well, but it is necessary to allow the alcohols to stand untouched for one month in order for the ceramic-like "fines" to settle out. Be very careful when withdrawing sieve-dried alcohols. Do not pour the alcohols, but use a pipette, and remove it from the top of the liquid. When the level drops to less than 1 inch above the sieves, it's time to move on to the next bottle. Basically, we would prepare about 6-10 pints of ethanol at one time, allowing them to "age" for at least 30 days. I must admit that now I tend to use 100% ethanol right out of freshly opened, individually sealed pint containers except in the most critical of applications (Spurr's dehydrations, for example) and have never had a problem with water. I know of at least two investigators who have damaged diamond knives by not taking precautions with molecular sieves. Basically, once the fines get onto your specimen they are impossible to remove and will damage your diamond knife. John J. Bozzola <bozzola@siu.edu>

Try putting the molecular sieve inside some dialysis tubing and seal the ends; I just used staples. You end up with a molecular sieve 'sausage' which works quite well. Malcolm Haswell <malcolm.haswell@sunderland.ac.uk>

I believe that molecular sieves contain alumina that is very detrimental to knives. The fine powder from the sieves takes a very long time to settle and is easily stirred up. Why don't you try using sodium sulfate? We have used that for years without noticeable problems. Of course, we try not to stir up the bottles or use the last 1/3 of the bottle. Rather, we pour the remains together with about 1" of fresh sodium sulfate at the bottom of the bottle. Then let the bottle sit a day or two and you should be okay. Debby Sherman <dsherman@purdue.edu>

TEM – Carbon coating grids

I work in a lab studying the morphology of various Archaeal viruses and virus-like-particles. There seems to be some controversy in the lab as to whether or not one can carbon coat a grid without a pre-existing support film. My understanding was that one needed a support film such as Formvar or Butvar for the carbon to be deposited on. Random Diessner <random@pdx.edu>

I have tried two variations on the theme. I have used a paper punch to punch out rounds of freshly cleaved mica, stuck one edge of each round onto a clean glass slide with double stick tape, and evaporated carbon onto the slide. Score around the edges of the coated mica, or make a tic-tac-toe grid on each with a needle, leaving the center square large enough for a grid, then float the films off the mica onto water. Place a grid on the film and pick up. My favorite way to pick them up, which I also use for making Formvar-coated grids, is to come down on top of them with a piece of Parafilm, then lift the Parafilm off. The films seem to float off the mica pieces easier than off a slide, at least in my hands. I've made some pretty sturdy and thin films this way – mostly to image nanoparticles. Alternatively, I have evaporated carbon onto Formvar-coated grids, stuck them onto a slide as above, and then dissolved away the plastic film. With uneven success, I must admit. Right now I can't remember what solvent(s) worked the best, and I often ended up with shreds of Formvar remaining on the grid. However, in these cases I still had enough pure carbon areas that I could easily image proteins and particles. The pure carbon films do allow much better resolution and contrast than the Formvar or Butvar, but are certainly more hassle! Tina Carvalho <tina@pbrc.hawaii.edu>

I prepare carbon films by evaporating carbon onto a collodion-covered grid. The collodion is dissolved by putting the grid on a chloroform-soaked filter paper stack for 48 hours ("Jaffe washer"). This leaves an amorphous, 20 to 30 nanometer-thick carbon film adhered to the grid. I find these films more robust than the Formvar films in my 200kV TEM because they are conductive. I use them to study small particles. Mary Mager <mager@interchange.ubc.ca>

SEM – mounting delicate samples

In response to a question concerning mounting delicate insect and mouse embryo samples on SEM stubs, the following replies were posted:

What I've done for such delicate samples as you describe and with some success is to take a pin (like an insect mounting pin, or push pin) cut to about 1/4 inch in length, file a flat end, dip end in colloidal carbon paint, gently touch that end to "underside" (or side opposite the one you want to look at) of insect or embryo. The sample will stick to the paint so then you can lift it up. Hold the pin by its side with a clamping type fine tipped forceps. The next step is to mount it onto an SEM sample stub. You can drill a small hole into the stub surface that will take the diameter of the pin, or use a clamping type SEM stub. After mounting or clamping the pin onto the stub, paint carbon paint over the stub surface, then you will have a nice dark background behind the sample, which may also be conveniently out of focus due to height of sample above stub surface, which helps to give dark featureless background for those full body glamour shots! Gib Ahlstrand <ahlst007@tc.umn.edu>

I use a 5/0 Red Sable artist's brush. You can buy these from EM suppliers. I use them on embryos and insects. Just gently touch the sample and it will usually stick to the hairs. Then touch it down to your adhesive substrate. If the sample won't stick to the brush, touch the brush to your tongue (or touch your finger to your tongue, then touch the brush to your finger). Saliva is a wonderful thing. Rick Harris <raharris@ucdavis.edu>

Already dried insect specimens may be placed in a steam bath to absorb moisture and then carefully arranged. Some researchers place the insects in warm water overnight with a little surfactant. I prefer the steam bath because it doesn't require critical point drying or hexamethyldisilazane so long as you control it. If samples are in alcohol or fixative, you may pin mount them, but not through the body. Splay out the insect, and place a pin to either side of the body, angled so that they cross just over the structure. It takes time and lots of practice to pin down all the legs, antenna, wings, and body, but the results are well worth it. I use silicone mats or filter paper underneath. If you don't have the miniature pins, a cactus works really well (my preference). From this point dry in whatever manner suits you. Fresh samples are most easily done by getting them to land on a damp piece of filter paper. When happy, set on liquid nitrogen chilled block to snap freeze. I have also had success getting creepers to walk across tape and then freezing in a lab freezer to kill. Freeze drying would be the preferred method from this point. A third method would be to use an ESEM or cryo-stage equipped instrument. Getting insects to sit still under the beam is the major drawback with ESEM. Scott Whittaker <whittaker.scott@nmnh.si.edu>

Besides the replies already given, I have used "live insect handling forceps" for both types of samples mentioned and many other fragile, dried samples. These are blunt or fine-pointed forceps made of thin, flexible stainless steel that allows a sure grip with minimal force. As the name implies, they were originally developed by entomologists, but I believe not for live insects, but minute, dried insects in collections. The other trick is to put a pointy bit of latex rubber on the end of a dissecting needle and rub it on silk or wool or something to give it a charge. The static electricity can then be used to pick up and move fragile specimens. Philip Oshel <peoshel@facstaff.wisc.edu>