NETNOTES

Thomas E. Phillips, Ph.D.
University of Missouri
phillipst@missouri.edu

Selected postings from the MSA Microscopy Listserver (listserver@msa.microscopy.com) from 4/1/03 to 6/10/03. Postings may have been edited to conserve space or for clarity.

TEM – Ultramicrotomy problems

Is anyone experiencing what we call "concrete water" in the diamond knife boat? The symptoms of "concrete water" are that the initial section cuts ok, but subsequent sections pile up because they will not move out across the water surface. Even when you manage to separate one section, it is almost impossible to move it about on the surface of the water with a one-hair brush. Note: this is not like the problem of an unclean knife edge when sections pile up for that reason. It is something entirely different. Jan Redick (posted by Bonnie Sheppard) <bls4u@cstone.net>

I know exactly what you mean: the water behaves like molasses so that the sections are nearly impossible to move. In our experience, we found the cause was a thin film of either protein or oil on the water surface. Cleaning the water surface with lintless lens paper will get rid of the phenomenon temporarily. You need to eliminate the source of the contamination. This could be traced to either: a dirty knife trough (clean with a gentle detergent), a dirty eyelash probe (caused by cleaning the eyelash by pinching between fingers), dirty glassware that contains the distilled water, contaminated micropore filtration apparatus (change micropore filter and rinse entire apparatus, especially the new micropore filter, with sterile, clean distilled water). Finally, water purification systems (like reverse osmosis or deionization) that become overloaded (or have been recently "recharged") are more likely to cause this than traditionally prepared distilled water. Clean everything mentioned. If the problem persists, borrow or purchase some distilled water from a different source and check it out. John J. Bozzola <bozzola@siu.edu>

Microtomy – polymer films

I'm looking for tips on sectioning 5 to 50 micron thick polymer films. Typically samples would be polyolefins, but sometimes they are nylons. I have a room temp microtome available, and have had limited success sectioning some nylons to around 2 microns, but sometimes need to go thinner. Jim Passmore <james.passmore@sealedair.com>

To keep the resin from splitting away from the film, you will need to section a small piece of the film that is surrounded by the resin. To do this, cut your polymer film into small triangles and then glue a thin wire with super glue to back end of the triangle. Put the pointed end into a beam capsule and wrap the end of the wire around the little piece of plastic that holds the cap on and try to wrap it tight enough to hold securely. You may have to bend the wire so that the pointed end is positioned properly for sectioning. Add some medium grade LR White and polymerize. We have cut both thick and thin sections from these blocks. Use Formvar coated grids for thin sections. Phoebe J Doss <pjdoss@cvm.okstate.edu>

Ambient temperature microtomy of polyolefins is a difficult task and one that gives generally poor results at best. Although apparently acceptable sections may be obtained for optical microscopy, one should still be very conservative in the interpretation of morphology. I recommend that you spring for a cryogenic ultramicrotome if you plan to continue such work. This is the only way to get good quality sections from polyolefins. When sectioning polymers, the temperatures of the sample and knife need to be below the glass transition temperature of all components in the film to avoid deformation during cutting. Polypropylene should be cut below -10°C; polyethylenes of any type below -120°C. <gary.m.brown@exxonmobil.com>

I've had good success using a room temperature microtome, by freezing the specimen before cutting sections. The results are not as consistent as one would expect with a cryomicrotome, but might provide suitable results, especially if a cryomicrotome is not available. I freeze the sample while it is mounted in the chuck by holding a cotton swab saturated with liquid nitrogen close to, but not touching, its surface. James Martin <james.s.martin@att.net>

SEM – Cross section preparation Al/polymer/paper composite

We have an Aluminum sheet composite material that consists of Aluminum sheet/ polymer layer/ paper backing. The Aluminum sheet corroded through the paper and polymer layers in some areas. The Al corroded to the point that it contains large holes. We would like to cross section these samples for SEM keeping the paper layer intact. We are looking for ideas on how to cross section the samples for SEM so all three layers can be imaged. Kerry N. Siebein <ksiebein@erc.ufl.edu>

In our lab we routinely and effectively cryo-slice or cryo-cut papers/films. Freeze your material, and tweezers using liquid Nitrogen in a mini Dewar. To slice we use a variety of tools depending on what works best with the material in question. The tools can be micro scissors or straight blades. Be careful when using the blades, though! Be sure to freeze whatever slicing tool you choose. Jane Dowell <jane.e.dowell@grace.com>

I've often prepared similar samples for study. If the sample is large, I would start by buttering the porous area with a prepared mixture of two-part epoxy. This epoxy is available from any metallographic supply house, has excellent wetting properties, low viscosity for sample impregnation, and should be allowed to cure overnight. This epoxy will "fix" the porous and loose material in place for subsequent sectioning. After the buttered area is hard, section slightly to the side of the area of interest. Rough cut the rest of the sample to fit in a metallographic mount. Mount the area - again using epoxy. After the mount has cured, rough polish the area of interest to remove cutting damage. At this point, the sample should be "frozen" in place with a bit of porosity at the cut surface. Again, put some epoxy on the cut and rough polished surface. Use vacuum techniques to draw air out of the porosity and drive epoxy into the sample. After curing, prepare the sample metallographically for an optically flat surface. After sputter coating to make the sample conductive, it is ready for viewing in the SEM. It takes a couple of days to prepare the sample, but this is the routine I use for samples of this type. If imaging is all you need and don't need EDS information, you may forego sputter coating and SEM examination and simply use a metallograph for your study. Stu Smalinskas <smalinskas@yahoo.com>

You do not say how thick the material is, but I have had success with a "cryo" method on similar materials. The SEM is a clever tool, cut a material and it sees the cut, not the material interfaces! The only alternatives are embedding + mechanical sectioning + polishing, or cryo. For cryo: cut a piece of the material into an egg timer (hour glass) shape being ½ inch wide at top and bottom, but only 1/4 inch wide in the neck. Lower this into liquid nitrogen and wait for the bubbling to subside. Remove with light weight pliers and, holding each end with the pliers, crack the material by bending it slightly. If the material does not crack, do not repeat the bending, but try again with another piece of material–this time make the neck

1/8 inch wide. Steve Chapman <protrain@emcourses.com>

TEM – Bacterial immunocytochemistry

I am interested in labeling bacterial surfaces with colloidal gold for negative stain and TEM. So far negative stain works OK, also indirect immunofluorescence with a primary mouse monoclonal. However, secondary antibodies conjugated with 10-15 nm gold do not work at all. We have adhered fimbriated E. coli *to Formvar-coated gold grids with or without fixation in various concentrations of formaldehyde, labeled them and fixed with glutaraldehyde. Any tips or tricks?* Margaretha Lindroth <margaretha.lindroth@ibk.liu.se>

We've observed similar behavior in gold labeling *E. coli* outer membrane proteins quite some time back. We were not successful in trying to label even unfixed intact cells, whereas labeling was not a problem at all on ultrathin cryosections of aldehyde fixed cells. A small percentage of the intact cells would label however, being about the same number as the number of mutant cells with shorter LPS molecules. That was the basis of our conclusion that long carbohydrate chains of LPS caused steric hindrance in intact cells, whereas an approach 'from the side' on sections was just working fine. You mention using 10-15 nm particles; I am not sure whether ultra small particles would have the same limitations if LPS carbohydrates are causing this phenomenon. The reference to this is: Voorhout WF et al. (1986) Steric hindrance in immunolabelling. J. Microscopy 141: 303-310. Jan Leunissen <leunissen@aurion.nl>

SEM – Immunocytochemistry

I need to do some experiments using a FEG SEM. I am using infected cells that I am planning to immunolabel with antibodies coupled to colloidal gold. Is immunolabeling carried out in the same way as for labeling on grids? Should I use critical point drying or not? Should I use a metal or carbon coating? Daniele Spehner <daniele.spehner@efs-alsace.fr>

The cells can be labeled in their incubation medium in the wells or wherever they're grown, including in suspension. After labeling, fix with 1 or 1.25% glutaraldehyde + 1% tannic acid (helps preserve the cell membrane -- we prefer the Mallinckrodt cat. # 1764), in whatever buffer is appropriate for the cells. Osmium tetroxide secondary fixation is not needed. They definitely must be critical point dried. I recommend using a high-resolution platinum coating, 2 to 4 nm thick. We use an ion-beam coater for this. Use a thinner coating if the cells and the mounting method allow it. Image the sample at 5kV; gold lights up nicely in the BSE at this accelerating voltage. The BSE detector will likely control what minimum kV must be used, though, but a FEG SEM should have a high-resolution BSE detector anyway. Philip Oshel <peoshel@wisc.edu>

As Philip suggested a 2–4 nm coating of platinum will allow you to examine the sample at 5Kv using the backscatter detector. In addition, it has been my experience that under those conditions the lower SE detector provides some atomic number imaging, at least that's the case in my instrument. While I have not tried this technique on gold labeled cells, I have had success looking for metal contaminants in organic crystal samples. The advantages offered by using the SE detector certainly make it worth a try with your equipment/samples. John A. Robson <jrobson@rdg.boehringer-ingelheim.com>

The secondary electron detector does show the gold nicely, also. The gold particles don't light up with the SE like they do with the BSE, but they do stand out from the background of biological samples. I don't think this is atomic number imaging, though, as much as it is just that the gold particles are very good sources of secondary electrons, and much denser than the rest of the sample. Recall that biological and other low molecular weight specimens are sputter-coated not just to reduce charging or beam damage, but because "...high molecular weight materials yield stronger secondary electron signals, coating a predominantly low molecular weight specimen with a conductive metal such as gold or gold-palladium will increase the yield of secondary electrons ..." (Postek, 1980, Scanning Electron Microscopy). If the instrument allows it, the best way to go is simultaneous acquisition of SE and BSE images. Philip Oshel <peoshel@wisc.edu>

Check the paper below, in which SEM and immunogold are used to localize myosin on sperm cell surface. Z Zhang, HQ Tian and SD Russell (1999) Localization of myosin on sperm-cell-associated membranes of tobacco. Protoplasma 208:123-128. Zhaojie Zhang <zzhang@uwyo.edu>

EM- cooling systems

In response to a question about leaks in cooling system lines, the following replies were posted:

We have had a rash of cooling leaks in our facility in copper lines running to and from our refrigerated water recirculators and in the brass plumbing connectors. The pipes and fittings are being eaten out from the inside, resulting in pinhole leaks at unpredictable times. Fortunately, the leaks usually start small and so far we've caught them before a deluge causes major damage. There have been two causes for these leaks. One is that, until recently, I was unaware of the presence of a shut-off valve inside our water chillers, which turns the cooling water to the chiller on and off as the compressor turns on and off. The water cooling the coils should only be running when the compressor is delivering a heat load to dissipate. The water from the tank to the scope runs constantly, of course. These shut-off valves wear out routinely every couple of years and should be checked once a year, at least, I now know. If they wear out, the water runs constantly and can cause major internal "etching" of the metal cooling lines. Also, one of our chillers had the flow rate to the coils set way too high, with the same effect. Moral: have a refrigeration person check your shut-off valves and flow rates. The other reason is that some water supplies are simply more corrosive than others (our refrigeration guy used the term "hungry" water) and will eat your copper lines and metal fittings from the inside out over varying periods of time, regardless of flow rates. Have your water checked for hardness and other parameters by some qualified person. Regular copper lines can be replaced by hardened copper (I think it's called K-copper) or stainless steel lines, but there is an expense factor involved. Randy D. Tindall <tindallr@missouri.edu>

Where in the cooling system did the leak occur? I assume it wasn't a coupling or someplace obvious. If it wasn't, I would look into two possible causes: (1) You have electrolysis from improper grounding between the cooling system and/or your TEM.

(2) Turbulence from air in the water lines is eroding the copper cooling lines. We have had the latter happen to us this past year. Bill Carmichael <wcarmichael@charter.net>

We too have had these internal leaks in copper cooling lines. We tracked the source to impingement corrosion, particulate laden water moved through small diameter tubing at high speed. We have (no, had) street water cooled condensers on our refrigeration chillers. As Randy Tindall said, without careful monitoring and adjustment, the valves controlling the required amount of street water necessary to remove the heat load stay open all the time allowing tremendous amounts of water to flow through that circuit. At high

pressure (our street water is 85 psi) with some micron size particles in it, will eat copper tubing at any bends in the tubing. We've also seen the problem on street water cooled diffusion pumps, where the coiled copper tubing soldered to the side wall of the diffusion stack will perforate. Yes, this tubing can be replaced in the field in a few hours with common plumbing tools. So, the result is we no longer have refrigerant cooled chillers. I converted all to water-water cooled systems with the new "braze-pak" style heat exchangers that are resistant to this corrosion. No problems since. Owen P. Mills <opmills@mtu.edu>

FIXATION – tissue storage

We have a set of samples from an experiment on rats performed on Day 1. The lung and aorta samples were divided into two parts each: one, for embedding in EMbed 12, was brought to the step of washing after osmium tetroxide staining and the other, for cryosectioning, was brought to the step of infiltration with PVP and sucrose. The first set of samples is currently in 0.1 M sodium cacodylate buffer, pH 7.4, and the other in the PVP with sucrose in phosphate buffer pH 7.0. However, there is a delay in delivery of the embedding kit and aluminum specimen pins. Most likely, both will arrive here on Day 7. Is it safe to keep the samples at this stage of preparation for 7 days? If yes, at what temperature? Halina Witkiewicz <hwitkiewicz@skcc.org>

I don't see a big problem. Keep the tissue refrigerated at +4°C. An extended wash in cacodylate buffer may wash out some components of the cell. You may expect some artifacts from that. If your samples for cryo-EM were fixed with formaldehyde, be aware that formaldehyde fixation is reversible and an extended wash after formaldehyde may result in "less" fixation. Sergey Ryazantsev <sryazant@ucla.edu>

If you're forced to store half-processed tissue for TEM, it's better to keep it at the buffer stage after glutaraldehyde and before OsO_4. Since it's too late for this, you might find it a good idea to repeat the osmium step when your materials arrive and you can continue. Meanwhile, keep it at +4°C. Lesley Weston <lesley@vancouverbc.net>