NETNOTES

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These are selected postings from the MSA Microscopy Listserver (listserver@msa.microscopy.com) from 7/15/02 to 9/23/02. Postings may have been edited to conserve space or for clarity.

Editor's Note: NetNotes have moved from the MSA journal, Microscopy and Microanalysis, *to this MSA magazine–a more appropriate venue. Inasmuch as our traditional Microscopy-101 section is also drawn from the MSA Listserver, we will have to decide whether to combine the two features or keep them separate in the future. Reader suggestions are welcome.*

LM - Sticky Shutter

We have a shutter that has become sticky opening and closing. Does anyone have any suggestions concerning an appropriate lubricant to apply to the leaves to try and smooth its motion? David Knecht <knecht@uconn.edu>

Oils should not be used to lubricate leaf shutters or iris diaphragms. Paradoxically, instead of acting as a lubricant the surface tension of the oil film will bind the leaves together and prevent them from sliding freely. Problems with sticking are likely caused by dust or a particle of grit, a buildup of crud somewhere, excessive wear or mechanical damage. Careful cleaning of the shutter may be a better bet. Chris Jeffree <c.jeffree@ed.ac.uk>

LM - 3D Reconstruction of Fluorescent Images

Is artificial Z stretching of very small, moderately bright, fluorescent spots common when 3D movies are rotated? We are getting elongated ellipses from deconvolved images (0.2 µm stage steps, proper optical transfer function from PSF for a 100x lens), rather than spheres. Mike Delannoy <delannoy@jhmi.edu>

Artificial stretching of spherical fluorescent sources in the Z-axis can arise from a number of sources. The first thing that comes to mind is the point spread function; if the sample in question is mounted in a media with a refractive index significantly different from that which the point spread function was determined with then spherical aberration may distort the resultant image. Another source of distortion can be the fluorescent sphere itself. If the sphere is large enough, and is composed of a material of a refractive index different from the surrounding media, the fluorescent bead can act like a ball lens and image artifacts result. Hardware can also be a culprit. If the stage position feedback is encoded by the stepper motor only, then it is difficult to tell how much the stage is really moving. In other words, if the stepper receives a signal to move 2 microns, and it turns 2 microns, but the coupling to the stage Z-rack slips, then the stage just moved less than it was supposed to. On a couple of our microscopes the focus drive is actually a bearing friction coupling and it is capable of slipping. The computer, however, thinks the stage moved the correct distance, so the spacing between successive images in a stack is off. If the computer thinks it moved 8 microns, but it only really moved 6 microns, then the image will be stretched. If a linear encoder is providing feedback to the software this problem should be minimized. Lastly, the rendering software needs to have the correct information. If the spacing between individual images in a stack is somehow entered incorrectly, the voxels may be stretched. Karl Garsha <garsha@itg.uiuc.edu>

LM & TEM - Iron Detection

We are trying to localize iron in yeast with light and electron microscopy. Does anybody know of LM or EM stains for iron? Gilles Grondin <Gilles.Grondin@USherbrooke.ca>

For light microscopy, you can use either the "Prussian Blue" reaction or "Turnbull's Blue" reaction depending on whether your iron is Fe^{+3} (ferric) or Fe^{+2} (ferrous). These simple reactions, using either potassium ferrocyanide or ferricyanide in dilute hydrochloric acid, can be found in any histotechnique textbook. You should run a control for verification. At the electron microscopic level, I would think that iron deposits would be electron dense and not require any staining. In fact, they might be more visible in an unstained section. Geoff McAuliffe <mcauliff@umdnj.edu>

LM & TEM - Gloves

We are under pressure to eliminate the use of latex gloves in favor of nitrile and vinyl. Does anyone have appropriate references to support the use of these? Frida Maiers <Frida.Maiers@co.hennepin.mn.us>

There are a lot of arguments against latex; I won't recite them. I've been trying to track solid information on glove permeability to embedding resins for several years, without much success. Here are the only recent references that I know:

Tobler M, Freiburghaus AU (1990) Occupational risks of (meth)acrylate compounds in embedding media for electron microscopy. Journal of Microscopy 160 (Pt 3):291-8.

Tobler M, Wuthrich B, Freiburghaus, AU (1990) Contact dermatitis from acrylate and methacrylate compounds in Lowicryl embedding media for electron microscopy. Contact Dermatitis 23(2):96-102.

The authors recommend 4-H gloves, which are expensive and clumsy, but they will preserve your ability to work if you've already developed dermatitis. I've been trying to find information on nitrile, with no success. There are general glove permeability charts available, but they ignore resin monomers:

http://www.cdc.gov/od/ohs/manual/pprotect.htm
http://www.bestglove.com/. Caroline Schooley <schooley@mcn.org>

TEM - Osmium Staining Of Rubber

We need some help with a material sample. The sample is a high impact polystyrene (HIPS) that contains butadiene rubber moieties. We need to be able to identify the location of these rubber moieties and understand that they will stain upon exposure to osmium. Does anyone have any experience with this type of sample? Would it be advisable to stain by floating grids with thin sections on liquid osmium or by using osmium vapors? What type of grids would be most desirable to minimize corrosion by the osmium? Debby Sherman <dsherman@purdue.edu>

The method we use is to stain in osmium vapor for 30 minutes to an hour on ultrathin sections. We normally use copper grids without any problems. Put a couple of drops of 2% stock osmium solution in a jar, position the grids above the liquid (we use an old flexible plastic grid holder) and leave capped in fume hood. Leave the jar open to air-out after removal of the osmium, at

least overnight before discarding the jar and leave the grids open to air for awhile before putting in microscope to get rid of osmium smell. Ian Kaplin <ian@mail.emu.usyd.edu.au>

We routinely examine HIPS material in the TEM. Our method is to stain the trimmed and faced sample block in a 2% aqueous osmium solution before sectioning. Place the block in a sealed bottle with just enough liquid to cover and leave in your hood overnight. On the following morning, transfer the sample into a small bottle of tap water in the hood for approximately 5 minutes and then set the block out in the hood to air dry for 30 minutes. The block should be much darker. Section at room temperature as you normally would. Make a careful approach because the stain will only penetrate deep enough to make about ten 100 nm thick sections. 70 nm to 90 nm sections look very good. HIPS stains well with this method. Some HIPS samples will warp, making approach more difficult, but usually only to an annoying rather than catastrophic degree. Matthew Stephenson <stephenson@impactanalytical.com>

There is some literature suggesting that ruthenium tetroxide is better than osmium for this kind of thing. There is a paper by Trent, Scheinbeim, and Couchaman 1983 Macromolecules 16: 589-598 that is very informative. My understanding is that it is usual to vapor stain this kind of sample. Tobias Baskin <BaskinT@missouri.edu>

Tobias is correct that ruthenium tetroxide will stain such rubbers. Unfortunately, ruthenium tetroxide is not a selective stain for either the rubber or polystyrene components of HIPS; both will stain heavily. Osmium tetroxide, on the other hand, will stain only the unsaturated polybutadiene of HIPS. I think that you will find osmium tetroxide much more useful. Gary M. Brown <gary.m.brown@exxonmobil.com>

TEM - 3d Reconstruction

Is there a good software package that will construct 3D images from TEM serial sections? Mike Delannoy <delannoy@jhmi.edu>

The IMOD software package comes to mind (http://bio3d.colorado.edu/imod/). It is a free software package developed for SGI UNIX, which has also been ported to Linux (standard PC hardware) and probably compiles on other UNIX platforms also. A place to look for other UNIX/Linux based freeware/shareware solutions for processing multidimensional data sets is http://www.cs.ubc.ca/spider/ladic/software.html. Karl Garsha <garsha@itg.uiuc.edu>

TEM - HIV And Prion Survival

I have a student working with HIV infected tissue embedded in resin. Does anyone know whether the HIV survives through all the fixation and embedding steps including osmication, *en bloc* staining with uranyl acetate, dehydration with ethanol and acetone and embedding in Epon-Araldite? A library and Web search yielded nothing useful. Diana van Driel <dianavd@eye.usyd.edu.au>

I spent more than three years doing EM studies of several types of HIV (HIV-1, HIV-2) and SIV at the Harvard AIDS Institute, and we have no evidence that these lentiviruses can survive the harsh condition used for EM processing. Based on what we know, HIV is, in fact, very fragile and does not survive 70% ethanol and formalin fixation. It is unlikely that they will survive glutaraldehyde and osmium. I personally fixed, processed, embedded, and sectioned over a thousand HIV and SIV-infected cell samples and some autopsies, but never had any problems with HIV infection. The fact that my colleagues and I are still alive, healthy, and HIV-negative 10 years after that period is further good evidence that it is safe to do EM on HIV-infected samples. Of course, one has to be extremely careful about the potential infection when dealing with these AIDS related samples. All specimens must be properly fixed inside the hood at a P3 laboratory before being sent out for EM processing. Good practice of common sense precautions will help. However, I do not remember anyone has been able to re-infect cells with HIV isolated from glutaraldehyde-fixed and epoxy embedded samples. Qian-Chun Yu" <qcyu@mail.med.upenn.edu>

On the other hand, prions (proteinaceous filaments not known to contain nucleic acids) are not killed by routine fixation. As a matter of fact, even routine autoclaving does not inactivate them. Killing them requires high heat for a long time, or chemical treatments like hydroxide, or formic acid. Prions cause spongiform encephalopathies (e.g., Bovine Spongiform Encephalopa-

thy (BSE or mad cow disease), Creutzfeld-Jacob Disease (CJD), scrapie, chronic wasting disease of deer/elk, and others). Thus, neural tissue should be handled with care. Sara Miller <saram@duke.edu>

TEM - Low Temperature Embedding

I would appreciate some advice on low temperature embedding in the Leica EM AFS freeze substitution unit using LR Gold. My aim is to do immunogold labeling, mainly on cell walls, with sections from Arabidopsis roots. The plan is to high pressure freeze Arabidopsis roots, freeze substitute them, and embed them in LR Gold at -25˚C. I am having trouble with the LR Gold embedding. I followed the protocol for "Freeze-substitution and low temperature embedding in Leica-capsules" from the AFS handbook. I was using LR Gold + 0.5% Benzil (w/v) as the embedding medium. I started polymerization at -25˚C with the UV lamp. After 24h the resin was not yet solidified; only the bottom half of the capsules looked like it was solid. Does anybody have experience with low temperature embedding using LR Gold? Regina Himmelspach <Himmelspach@rsbs.anu.edu.au>

LR Gold won't polymerize completely if there is oxygen present. The AFS is designed to exclude oxygen by evaporating some of the liquid nitrogen, but if there is a lot of air movement around the machine (i.e., lots of people walking by, or in front of an air vent) then it doesn't work so well. Make sure the inside glass is in place during UV exposure. If the bottom of the capsule is hard, you can pour off the top liquid, cut off the soft layer and use the hard bottom layer containing your specimen. Water will also prevent polymerization. You should make sure you are not introducing water from frost or condensation during processing. You may also retain some water from the specimen if substitution is not complete. Kim Rensing <krensing@interchange.ubc.ca>

TEM - Small Sample Size

I am isolating a rare monocyte population by fluorescence-activated cell sorting (FACS) and trying to look at it by TEM. On the first go around, I isolated 8500 cells and the pathologists were unable to get images of any cells because the pellet was too small to see. Seth Ness <seth.ness@mssm.edu>

With 8500 cells, it sounds like the pathologists lost them while processing the tissue. One way to circumvent this is to "embed" the recovered cells in a pellet of albumin and crosslink the albumin with glutaraldehyde. Basically, you gently centrifuge the cells of interest (after fixation and osmication) at 600-800 x G in a microcentrifuge tube and resuspend them in a tiny volume of buffer (approximately 20 µL). At the same time, add 50 µL of 5% bovine serum albumin (BSA) and 50 µL of 2% glutaraldehyde. The glutaraldehyde fixes and crosslinks the BSA so that the cells of interest are embedded within a cocoon of BSA. This is then spun down and can then be transferred from the microcentrifuge tube, dehydrated and embedded in Epon. It will turn the 8500 cells into a tissue chunk. The BSA serves as a "carrier" that impedes the steady loss of cells after each sequential step. The BSA doesn't interfere with images because it is not osmicated. Michael C. Peters <michael.peters@stanford.edu>

I have sectioned cell fractions by putting the sample into a clean polypropylene microcentrifuge tube, centrifuging it well and doing the total fixation through embedding process without disturbing the pellet. Care must be taken to totally exchange the solutions. Repeat a step if there is a question. I usually fill 1/4 of the tube with the final Epon resin mixture. After the resin is hard, pop it out of the tube. The pellet should be visible on the side of the cone at the bottom - try using a dissecting microscope with the light under the block. It is usually necessary to cut the cone to a flat side and glue it onto a blank so that it can be sectioned. Pat Connelly <psconnel@sas.upenn.edu>

TEM - Cell Membranes

In my first attempt at TEM, I lost all the membranes on cultured primary hippocampal neurons. All cellular organelles were intact and had no apparent distortion. Has anyone else experienced this and if so, do you know what causes the membranes to vanish? Alternately, I'd be very interested in any fixation protocols anyone has for primary hippocampal neurons that have worked for them in the past. I am particularly interested in observing synapses. Stephanie Tyndall <stephanie.tyndall@uconn.edu>

I, too, have had trouble in the past with vanishing membranes in cell monolayers, though not hippocampal cells. The remedy was to en bloc stain in aqueous 3% uranyl acetate for 15 minutes prior to dehydration and then

do a rapid dehydration in a graded series of ethanol beginning with 50 % ethanol. End the dehydration with just one treatment of 100 % ethanol and then go right into the infiltration with increasing concentrations of Epon mixed in ethanol. Try to minimize the cells' exposure to that ethanol that tends to leach out membranes. Dorothy Roak Sorenson <dsoren@umich.edu>

TEM - Rough Edge Of FEG Beam

On our recently installed FETEM, I have noticed that when the magnification is x800K-1.5M, the edge of the beam crossover loses its smoothness. The shape also becomes non-circular. The alignment, as checked by both the service engineer and myself, doesn't seem to be an issue. My question is whether this is normal or acceptable? Chaoying Ni <cni@udel.edu>

Such distortion of the beam is usually caused by charging of contaminants on the surface of the condenser aperture. Changing or cleaning the aperture may help. When the beam diameter is very small, like the one needed for the magnification range you mentioned, such roughness of the beam edge is considered normal. Try switching the condenser apertures and see if there is big difference in the beam shape between different apertures. If one of the apertures is dirty, there will be big difference in distortion between it and the other apertures. If all the apertures show comparable roughness, don't bother to change or clean them since the situation may worsen. The problem of condenser aperture charging is much more severe for FEG than LaB6 guns. The FEG is much brighter source producing very small crossover (high coherency). If you were using LaB_6 in the magnification range you mention, you'll usually work with the beam in crossover. Radostin Danev <rado@nips.ac.jp>

TEM - Dirty Condenser Apertures

The C2 apertures in our CM12 are heavily contaminated. Can anybody suggest how to clean them? Heating in a molybdenum boat does not work. Oldrich Benada <benada@biomed.cas.cz>

Plasma cleaning is another solution that I have used on condenser apertures. Make sure you use the most reactive species possible, i.e. an oxygen-rich plasma. If they are badly contaminated you may have to go to the high power plasma ashing regime. However, if they are very heavily contaminated, even that may not work since some residue may remain. In this case, you will just have to replace them. Nestor Zaluzec <zaluzec@ultra5.microscopy.com>

I just cleaned my condenser aperture using a cotton swab with the same metal polish I use to clean the Wehnelt. It took care of the very heavy contamination. I sonicated it in two changes of acetone for 5 minutes each and it is now working fine. You cannot do this with thin foil apertures (i.e., objective apertures). William Oxberry <William_Oxberry@downstate.edu>

I have the luxury of a metallographic lab available where I work. To clean apertures, I simply use diamond polishing compound and napped cloth. I use the tip of my finger to move the aperture in the diamond compound around on the cloth until the contaminants are gone. It usually takes just a few seconds. I follow this up with ultrasonic cleaning and rinsing. I believe this method is far superior and easier than baking in removing contaminants. Keep in mind there is always the risk of catching a corner and folding the aperture, rendering it useless, but this hasn't happened to me yet. Stu Smalinskas <smalinskas@yahoo.com>

The standard way to clean molybdenum apertures is in a high vacuum coating unit, heating the apertures to orange-red and holding them at that temperature until the deposit(s) evaporate. Heating in a sputter coater or in a flame does not work with molybdenum. Heating to white heat is not a good idea, as this often distorts the aperture. If you use the cleaned apertures and try to re-clean them again, grain growth also tends to spoil the aperture shape. If you are unable to clean the apertures by the first method described, you are better off discarding them; dirty apertures are not a good idea! Platinum apertures may also be cleaned using a Bunsen flame, heating to orange-red whilst holding the apertures on a platinum boat or with platinum tipped tweezers. Platinum suffers from grain growth at a far greater rate than molybdenum; that is why some manufacturers prefer the molybdenum when it comes to cleaning. Every "clean" aperture should be checked with a light microscope for cleanliness and aperture shape; if it is not round you should reject it! Steve Chapman <protrain@emcourses.com>

If the apertures are from a Philips TEM, they are most likely made of platinum. You don't heat these in vacuum as you do with molybdenum apertures. These can be cleaned by heating them in a platinum basket over a Bunsen burner in air. The oxygen in air won't oxidize the platinum, but will remove the hydrocarbon contamination quite effectively. If you look in your CM12 owner's book, Philips should have a section on maintenance where they describe the procedure. Hendrik O. Colijn <colijn.1@osu.edu>

SEM - Critical Point Drying

I would like to use HMDS instead of a critical point dryer for an SEM course I will be teaching. The HMDS is to be used with plant parts, yeast, bacteria and animal tissue (mouse jejunum). Are there simple protocols for HMDS for each of these specimens? L. Domenico <lillianft@aol.com>

The simplest starting point is to dehydrate to 100% ethanol as usual, keeping to the normal times. Then we use: (1) ethanol:HMDS 2:1; (2) ethanol:HMDS 1:2; (3) three washes in 100% HMDS. Remove from HMDS and leave to dry in fume cupboard. If the sample is too small (e.g., bacteria), let a drop dry on a coverslip. David Patton <David.Patton@uwe.ac.uk>

SEM - Eye Protection During Evaporation

For many years, I've used nothing but self-discipline to avoid direct eye contact with the white-hot electrodes resulting during a carbon evaporation run. However, times have changed and now I am responsible for the safety of student users. Does anyone know whether an arc-welder's face-shield protects the eyes from this type of intense light? Ann Lehman <Ann.Lehman@trincoll.edu>

For years I have used a pair of welder's goggles with my vacuum evaporator. Since there is no danger of sparks there is no need for full head protection and they are a little easier to wear than the full welder's helmet, so students are more likely to put them on. You are correct in assuming that the UV from the arc is dangerous to the eyes and may contribute to cataract formation. Mary Mager <mager@interchange.ubc.ca>

SEM - Electroplating And Electrolysis

I am looking for a reference that documents various anomalies associated with electroplating and electrolysis plating of metals such as gold, copper, nickel, zinc, etc., as seen through the SEM. Jeff Oakley <oakleyj@rayovac.com>

You may want to check publications from ASM international (www.asminternational.org). Start with Volume 5 (10th edition) of the Metals Handbooks-Surface Engineering. Volume 11 deals with Failure Analysis and Prevention and may have some examples. Joseph Oparowski <Joseph_Oparowski@bose.com>

You may want to try "The Electrodeposition of Tin and its Alloys" by Dr. Manfred Jordan (ISBN 3-87480-118-7). This is an excellent reference with lots of SEM pictures. Robert Fowler <robert.fowler@tdktca.com> ■