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Selected postings from the MSA Microscopy Listserver (listserver@msa.microscopy.com) from 6/10/05 to 8/10/05. Postings may have been edited to conserve space or for clarity.

SAMPLE PREPARATION - Sodium azide

Does anyone know if it is o.k. to use sodium azide with TEM preps? Does it wash out o.k. or will it leave a nasty deposit? Elke Buschbeck <elke.buschbeck@uc.edu> 06 Aug 2005

We use it routinely in buffers used to make up solutions for immunolocalization on sections. I have never had a problem with precipitation from sodium azide. Debby Sherman <dsherman@purdue.edu> 06 Aug 2005

One concern I have always heard was the formation of metal azide, especially lead azide. These explosive and unstable compounds have been found in older metal plumbing. When I started as a QC chemist years ago, dilute concentrations of sodium azide was used as a preservative in some test solutions used in clinical chemistry. More than one person got an unpleasant surprise when working with the older style lead drain pipes. I later wanted to use a test solution for sulfur that contained sodium azide, my management had fits. I never did use that test. I don't want to create an electron storm of words, but I will suggest you check out disposal method for your own safety. Frank Karl <frank.karl@degussa.com> 08 Aug 2005

SAMPLE PREPARATION – low melting point agarose

Has anyone used a low-melting point (LMP) agarose to encapsulate cells for TEM? If yes, which resin is best suited for LMP agarose encapsulated cells? The samples were encapsulated after buffer wash and after osmium fixation. Claire Haueter <lukeclaire@yahoo.com> 10 Aug 2005

We use Agarose type IX (Sigma). It works well with Spurr's and Araldite. This summer I have had a problem infiltrating cells in agarose with LR White (polymerizing at 55°C) despite success in two previous years. This is probably a problem with me rather than the products. Dave Patton <david.patton@uwe.ac.uk> 10 Aug 2005

I have used LMP agarose many times for TEM. I process it just like I would a block of tissue and go into EMbed-812 or Spurs. David Elliot <Elliott@arizona.edu> 10 Aug 2005

Note that low melting point agarose (and probably all types) is nicely stained by fast green. We would make a few percent solution in 100% ethanol and add a drop to our agarose blobs. Otherwise, they can vanish in the solutions and lead to some frustrations. I suppose fast green would also dissolve in acetone, but I don't know that for a fact. However, now we trap small samples between Formvar films on wire loops and like that much better than agarose. Tobias Baskin <baskin@bio.umass.edu> 10 Aug 2005

SAMPLE PREPARATION - dehydration solvents

Does anyone have experience with acetonitrile as a substitute transition solvent for propylene oxide, or perhaps as the sole dehydration solvent in TEM prep? Randy Tindall <tindallr@missouri.edu> 28 Jul 2005

I tried dehydrating plant tissue for LM with acetonitrile instead of ethanol and the results were terrible. The tissue was preserved poorly. It was the root of Arabidopsis. Tobias Baskin <baskin@bio.umass.edu> 28 Jul 2005

I've found acetonitrile as a propylene oxide substitution, to work very well. Also, it dissolves some plastics less readily, and is better for cell cultures. The results seem just as good if not better than propylene oxide in my situations. I don't even use propylene oxide anymore. Lou Ann Miller <lamiller@uiuc.edu> 28 Jul 2005

SAMPLE PREPARATION - Sectioning stainless steel wire

We need to make 20-30 µm sections of 200 µm thick stainless steel wire coated with a polymer. The object is to be able to do some chemical analysis on the sections so as to visualize chemical distribution in the polymeric coating. The sections will be imaged using Ramon technology. We need suggestions as to embedding resin, type of microtome and type of blades that may work for sections of this thickness and samples of this type. Debby Sherman <dsherman@purdue.edu> 07 Jun 2005

I suspect that slicing through stainless steel wire without disturbing its polymer coating on a microscopic level is going to be a huge challenge. The hard wire would probably mess up knives and if you try to grind a flat on the end of the wire, the abrasive will get embedded in the interface. I would suggest simply slicing/stripping off the coating and looking at that in cross section since its surface chemistry is not going to change with the removal of the wire in contact. Roger Baker <rcbaker@eden.infohwy.com> 7 Jun 2005

It's no lie that FIB would be the best solution to this problem, but what if you don't have a FIB? It is actually easier to cut metals very thin i.e. 20-80 nm. At this thickness, they can be examined in the EFTEM, which will analyze and map the elemental composition at high resolution. Bill McManus <conniemoss@relia.net> 07 Jun 2005

I would have concerns about surface contamination from FIB which could affect the results, not to mention changes in the polymer itself from damage to the ion beam. This isn't too different from the optoelectronic packages we section on occasion, which can have everything from stainless steel through glass to adhesives. It's not clear to me why you need a thin section for Raman - can't you just pot the wire in an appropriate resin and grind/polish as for a standard metallographic section? When there are changes in material hardness it can be difficult to keep the surface flat, but a little experimentation for your application with different pads should get you there. This should give you a nice clean and flat surface for analysis, and a few polishing pads and a bit of resin is slightly cheaper than a FIB. Richard Beanland <richard.beanland@bookham.com> 08 Jun 2005

MICROTOMY – Knife angle

I'm hoping that people here could advise me as to the best knife angle to buy on a diamond knife. All our specimens are human biopsy tissue embedded in Epon. I'd like to buy a histo-knife, and I was under the impression that 45° would be the best sort of knife angle for routine use like this. Is this correct? Garry Burgess <gburgess@exchange.hsc.mb.ca> 03 Aug 2005

I use a 45° for normal work. I find it works very well. I have a 35° that I use for special work. 35 ° has some advantages, but it is not as robust. David Elliott <Elliott@arizona.edu> 03 Aug 2005

I have had 45° diamonds for most of my career but last year bought a 35° one. I think there is a significant improvement for the lymphoid tissue we are cutting these days. I hear they wear out faster but I don't do a ton of sectioning so the tradeoff seems worth it. Tom Phillips <phillipst@missouri.edu> 03 Aug 2005

Although I am only familiar with so-called 'hard' materials microtomy (metals, alloys, minerals, ceramic fibers and the like) the interesting point about the knife angle in this area is that the 35° sections better for all of the above materials in my lab, so the 45° knives gather dust. Since wear is a function of the amount of sectioning as well as type of material, I cannot comment on that aspect, but can note that we never had any damage to the 35° except when we deliberately tried to do so on ~20 micron, extremely hard amorphous alloys particles. It was not pretty! The success of the lower angle is no fluke, as Helmut Gnaegi of Diatome has gotten good sections of 200 micron quartz particles, polysilicon/metal layers on a single crystal silicon substrate, carbon fibers (but care had to be used or nicks would occur), superconducting oxide and Ti implants in bone. Phil Swab (with a coatings company in California) has sectioned many optical glass coatings, and layers of boron nitride and artificial diamond on single crystal silicon substrate. To the best of my knowledge, both of these specialists had trouble with any other angle. My theory is that, for most of the above examples, the knife edge only initiates a crack in the fairly brittle material that is then 'wedged open' across the block face by the angle of the knife, hence the reduced damage with reduced angle (though section breakup does occur). Finally, we did a bit of fooling around with histo knives a few years back. Far from being only good for semithins, though 1 micron thick sections of aluminum were produced, they produced some of the thinnest, flattest sections we ever obtained (~10 nm for nanocrystalline metals) and was the only knife that produced decent ~20 nm thick sections of the above amorphous alloy particles. Go figure. Tom Malis <malis@nrcan.gc.ca> 04 Aug 2005

MICROTOMY – Tungsten carbide knives

I saw Tungsten Carbide knives in a catalog, and wondered if this sort of knife would be at all suitable to cut 0.7 micron sections of Epon embedding biological material. Currently we are using histo-diamond knives, but it is my dream to be able to use a more durable, cheaper knife that gives the same result. I was just wondering if anyone has tried this, and what sort of result they might get with Epon. Gary Burgess <gburgess@exchange.hsc.mb.ca> 04 Aug 2005

I have used the Tungsten Carbide knives some. They are not sharp enough to cut sub-micron thick sections as they leave a slight snowy appearance to the block face. I have been successful in cutting plastic embedded tissue sections of approximately 3-5 microns with them. The edge stays good much longer than glass does. They are great for working with harder materials. After applying Permount and coverslipping the sections, the snowiness is no longer evident. Michael D. Standing <michael_standing@byu.edu> 04 Aug 2005

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Exactly, Michael. The problem with tungsten carbide is that it is actually a microcrystalline alloy of tungsten carbide particles bonded with cobalt, and this structure interferes with very thin sections, much as with steel, but the carbide is harder and tougher, which is why it is used to machine steel. Knife facets may look quite shiny but they need to be polished much flatter than a wavelength of visible light if they are to meet at a smooth edge measured in the low nanometers, and thus be capable of cutting the sub-100 nanometer sections appropriate for EM work. The good thing about metal alloys is that the edge angle can be made more acute while retaining the desired durability, but only at the expense of minimum section thickness, so they are used for LM work. Glass does well for EM knives because it is amorphous and thus has no microstructure, while being hard enough to section. Of the hard monocrystal alternatives to glass suitable for EM knives, only sapphire, silicon carbide, and diamond seem appropriate. All these are good candidate materials for ultramicrotome knives, depending on cost and other requirements. Roger Baker <rcbaker@eden.infohwy.com> 04 Aug 2005

MICROTOMY - Water soluble crystals

I have some very tiny spheres (3-5 microns in diameter) that I need to obtain a thin section for TEM analysis. They are water soluble and crumble easily. What I have done already is to mount them on blank stubs with Extec and microtomy with limited results. Since they can't be floated onto water I have been catching them dry onto carbon coated copper TEM grids. They move quite a bit under the beam and the crystalline interior crumbles. Any thoughts would be appreciated. Al Harmon <aharmon@mvainc.com> 27 Jun 2005

You could try carbon coating after the particles have been picked up on the carbon-coated grids, and you could try a lower beam current. The movement under the beam indicates either charging, heating, both, or some other process--beam-induced specimen movement has not been completely characterized--and increasing electrical and thermal conductivity, or lowering the rate at which charge and/or heat accumulates on the specimen will, to some extent, ameliorate the problem. Bill Tivol <tivol@caltech.edu> 27 Jun 2005

I've had similar problems with samples only slightly soluble in water. To overcome dissolution, I saturate the water with the compound. In my case, it only takes 1-2 mg of material. Seem to work great. No more Swiss cheese! John W. Catino <john.catino@mineralstech.com> 29 Jun 2005

The simplest solution to this problem is not to keep your osmium in the refrigerator. I keep mine in a glass Schott bottle with plastic cap which is then stored within a plastic container in the fume hood. I am not sure if light affects the osmium but to be sure, I wrap the plastic container in aluminum foil. There is leakage from the glass bottle because over time, the inside of the plastic container goes black. Anything that leaks past the secondary container goes up the fume hood. Once every year or two, I splurge and replace the bottle and container. I don't experience "pre-mature" darkening of my 2% osmium stock which typically lasts about 2-3 months before I use it all up. Tom Phillips <phillipst@missouri.edu> 02 Aug 2005

I keep my working solution of osmium in a glass Wheaton or Gibco bottle with a Teflon-lined cap and wrap the cap with Parafilm and put that into the metal can that's shipped with the ampoules of osmium or a larger glass jar. I layer molecular sieves in the can/jar. Any escaping osmium vapors are indicated by blackening Parafilm... or blackening refrigerator! Our refrigerator is clean. Plastic is permeable to osmium - use glass! Winston Wiggins <winston.wiggins@cshs.or> 02 Aug 2005

We use bottles with a rubber septum to store the liquid, and keep the bottles within sealed Falcon tubes, within a sealed bottle. This keeps the vapors from leaking and discoloring the fridge. Gordon Ante Vrdoljak <gvrdolja@nature.berkeley.edu> 05 Aug 2005

LM - Congo red & protein fibrils

I am told by someone who visits my lab that protein fibrils treated with Congo red will show an apple green birefringence in crossed polars. She has brought samples for us to look at, but we cannot see the green birefringence. She 'knows' there are fibrils present by another type of assay. Has anybody heard of this technique? I think I can see the stained fibrils, but nothing shows with the crossed polars. Maybe I don't know what I should be looking for, maybe it is very subtle. Maybe it doesn't really work. If you have heard of this technique and maybe know a trick or two, let me know so I can help this person. Jonathan Krupp <jmkrupp@cats.ucsc.edu> 28 Jun 2005

The trick here is that you have to think in terms of dichroism rather than birefringence. What happens with Congo red (at least in theory) is that it binds to

the substrate in an oriented way. So, if you have a substrate that is oriented then the Congo red molecules themselves become oriented. Now Congo red happens to be good at absorbing linearly polarized light oriented along one axis of the molecule and bad along the perpendicular axis. So a Congo red stained sample should act like a polarizing filter, itself. So to see your apple green signal, what you do is remove the analyzer from the system, have just the polarizer (you can do it the other way if you want, it doesn't matter). Then rotate the sample through at least 90 degrees. You are using the sample to make "crossed polarizers". The apple green should show up when the sample fibers are at some defined orientation to the polarizer and the signal should disappear at ± 90 degrees to that orientation (no getting more specific without knowing which way the fibers go and how the Congo binds to them). You get green presumably because the Congo red absorbs red and blue better and so more green leaks through. Actually, polarizing filters themselves are often made by aligning really strong crystals that have dichroic absorption, and they too have a green cast because it is difficult to have no wavelength dependence to the dichroism. Keep in mind that Congo red stained fibers are going to be a whole heck of a lot less good at being a polarizing filter than a real polarizing filter, so the green signal may be pretty faint. But its tell tale-appearance and disappearance at ± 90 gives you a story. You can play with using green light (or other wave bands), but I think one's eyes are most sensitive to seeing differences in color rather than intensity. Tobias Baskin <baskin@bio.umass.edu> 28 Jun 2005

Well, you've got most of this right, but not entirely. Your procedure is correct: use just the polarizer in a fixed position and rotate the sample. (Actually, either could be fixed, so if you don't have a rotating stage, you can leave the sample in place and rotate a polarizing filter over the light port). The sample doesn't really act as the second polarizer (analyzer). This is the classic experiment for either dichroism (materials that exhibit two different refractive indices) and pleochroism (materials that exhibit 3 different refractive indices). First, a few definitions: Refractive index is an optical property that expresses the relative interaction between the electric field in light and the electric field in matter. The stronger the interaction, the more light passing through the material will slow down and the higher the refractive index. (Mathematically, RI = velocity of light in air or vacuum/velocity of light as it interacts with a material where V air = 300,000 km/sec). If you dissect the word "birefringence", you can see that it refers to materials * having the property of ("gence") * two (bi) * refractive indices (ref in). In actuality, materials can have three different RI's, all at right angles to each other, but will only exhibit 2 at a time (think of either films, fibers, or crystal faces), so we never refer to them as "tri-refringent". Polarized light is different from ordinary light in the following way: All light has a direction of travel. You see things in the world around you because light is traveling from them to you. Light is electromagnetic radiation. As microscopists, we are most typically interested in the electrical field part (yes, I know that there have been magnetic microscopes built). The sine wave we use to describe light is actually the tracing of the tip of that electrical vector, building up then dropping off in a positive direction, then building up and dropping off in negative direction. The motion of that sine wave gives light a direction of vibration. The direction of vibration is always at right angles to the direction of travel. Ordinary light contains all directions of vibration (imagine little vectors vibrating N-S, E-W, and all angles in between; all at right angles to the direction of travel). To convert ordinary light to polarized light, you simply have to impose some sort of interaction (reflection, specific types of absorption, beam splitting) which absorbs all but one permitted direction of vibration. The result is "Plane Polarized Light", which is not necessarily always the same as linearly polarized light (tune in another time for the discussion of "States of Polarization"). However, for the sake of simplicity, plane polarized light does indeed vibrate linearly. So what happens when you rotate any birefringent material over a polarizer? I can't draw diagrams for you here, but imagine a rectangle with one RI oriented along the short edge and the other along the long edge. As you rotate the sample, the short edge will eventually align with the permitted direction of the light coming from the polarizer. In this position, the light only "sees" one refractive index (not the most scientific explanation, but accurate). Rotating 90 degrees presents the other RI. Anywhere in between, contributions from each of the RI's will be visible. In practice, we use these unique positions to isolate each of the RI's to actually measure them. And what's the story with things that are dichroic or pleochroic? In addition to being birefringent, they are also colored in normal illumination (ex: Congo red). To properly observe them, first set up Koehler illumination and just observe the normal color. Then, insert just a single polarizer and rotate. The colors that you see will not be the typical magentas, golds, and turquoises characteristic of polarized light interactions. Rather they will be absorption colors (browns, reds, yellows, etc.), derived from the unique property that, for these materials, not only does refractive index vary with direction, absorption does too. The result - changes in color and intensity. ... and yes, one of these directions can be very subtle. In normal polarized light analyses, the next step would be to insert the analyzer and rotate. It is also important to note that the normal Polarization colors may be affected by the absorption colors. (For those of you who are interested, I have a "polarized light road map" that outlines all these tests in flow-chart form). Here are some interesting examples of materials which are dichroic or pleochroic: Everyone cites tourmaline, which is dark brown in one orientation and pale yellow in another, as well as biotite (a type of mica) and cordierite. Hartshorne and Stuart (Crystals and the Polarizing Microscope, Arnold, 1970) cite magnesium platino-cyanide, which oscillates between bluish-red (parallel to "c" axis) and carmine red; hypersthene (ferro-magnesium silicate): brownish red to green; remind us that some biological materials also exhibit this property. Patzett (Polarized Light Microscopy: Principles, Instruments, Applications, Leitz - now out of print) reminds us that the phenomenon is widespread in organic materials and is responsible for the circular dichroism we chemists routinely test for in levo and dextro rotatory compounds. And finally, I still have fond memories of doing experiments in grad school from Chamot & Mason (Handbook of Chemical Microscopy, Vol. I - now available through McCrone Associates) on a slew of materials. (For those of you who are teaching, they recommend viscose rayon dyed with Congo red; crystals of o-nitrophenol, azobenzene, iodoquinine sulphate, silver chromate, copper acetate, red ammonium picrate and the magnesium platinocyanide - mentioned above). Well, we are back at Congo red, so I'll stop here. For those of you interested in the physics of this process, I encourage you to read Hartshorne and Stuart further. For the rest of you, find some dichroic or pleochroic materials and just have fun. After many years of teaching, I realized that all the beauty and complexity of polarized light boils down to one simple underlying concept: refractive index. And here it is again. Hope this is helpful. Barbara Foster <bfoster@mme1.com> 29 Jun 2005

This is a complex area and I am far from being an optical crystallagrapher (!). But there are a couple of things I want to say, based on what I learned in school. Birefringence describes a material that has two (or three) refractive indices. In contrast, dichroism describes a material that has two (or three) different absorption behaviors. (I guess we don't call them absorption indices). It is true that the aligned Congo red molecules on the sample would probably also be birefringent, but this would presumably be so weak that unless the user has a really sensitive microscope, he or she would be unlikely to be able to detect it. In contrast, it is a lot easer to detect differences in light intensity and color, so there is a chance to pick up the dichroic absorption. And the way you do that is to illuminate the sample with linearly polarized light and rotate the sample (you are correct, you can rotate the polarizer instead) without any analyzer, and look for the changes in color as a function of the polarized light angle. Now it is true that you can also add an analyzer and with cryastals this gives you a chance to see further and different (and beautiful) colors as the analyzer is rotated. It might increase the contrast of a weak signal so it might be worth trying for the congo stained protein fibers. What you would need to do in that case would be to uncross the polars by a certain amount, say 5 degrees, rotate the sample through at least 90, then uncross by 5 more degrees, rotate the sample, and so on. Tobias Baskin <baskin@bio.umass.edu> 29 Jun 2005

To my knowledge green birefringence after Congo Red treatment appears when your protein has a lot of b-sheets in its structure. Amyloids formed from different proteins (APP, prion etc.) show such phenomenon. So, perhaps, the sample you was looking at did not have amyloid conformation. Aleksandr Mironov <aleksandr.mironov@manchester.ac.uk> 28 Jun 2005

In our lab, we currently use Congo red staining for amyloid fibers in spleen sections. We do use both a polarizer and analyzer and have a very bright specific green birefringence, provided that the intensity of light is very high. Furthermore, we have been able to see a bright red fluorescence in these areas. For the fluorescence, though, artifacts seem to be a problem. What protein are you trying to look at? Does it display some kind of alignment as beta-sheets? Regards, Marie-Claude Bélanger <mcbelanger6@hotmail.com> 30 Jun 2005

TEM: Immunocytochemistry and Cu grids

I have another one of my "back to the basics" questions that probably make people wonder how the devil I ever got into this business in the first place, but here goes anyway. We have a client who prepares his own blocks and brings them to us for sectioning, staining, viewing, etc. One day, he came into the lab with a bunch of sections on copper mesh grids which he had done immunocytochemistry on with standard gold-conjugated secondaries. After viewing them, I offered him the standard advice that next time we would mount some sections on nickel or gold grids if he let us know in advance that he would be doing immunocytochemistry. He said he always used copper and asked why he should switch. My answer was something like "Umm-uhhh.....because it's always done that way" with an additional mumble about copper interfering with the labeling reaction somehow. Now I have another client, also infinitely more savvy in chemistry that I am, asking the same question. So I checked through our

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little in-house research library and did some targeted Googling and found that some folks say that copper: 1) reacts with Tris-HCl buffer; 2) may react with PBS buffers to produce fine precipitates (but, but....doesn't that mean we should never use Cu with PBS?); 3) reacts with gold colloid (no other explanation given), 4) can alter the charge distribution of the section and cause non-specific background labeling; and 5) may be oxidized during labeling or interfere with oxidation of chemical groups in the tissue to be labeled. Mostly people just say to use Ni or Au without stating any reasons. I'd like to know the truth and pass it along to my admiring customers. Randy Tindall <tindallr@missouri.edu> 12 Jul 2005

I only ran into the "use only Ni or Au grids" rule here, in my current job, and my predecessor certainly produced some superb images showing gold labeling of TEM sections. Blissfully unaware of this rule, I had been using copper grids for all EM immunolabeling. To get good labeling of one particular structure, which is about 40 nm diameter, I used uncoated thin-bar Cu grids so I could get labelling on both sides of the section - seemed to work just fine. Rosemary White <rosemary.white@csiro.au> 12 Jul 2005

I had problems with Cu grids when incubating sections for long periods - overnight for instance. The copper would react with whatever was available and form green-blue salts. Not unexpected. We switched to nickel grids for a while but they charge, gold but they are delicate...by this time we had shortened the incubation times used, and tried copper again... And had no problem using coated grids and incubation times of an hour or so. Grids are floated on drops of media so that only the coated side is wetted - I don't know if that is important. SJ Stowe <sally.stowe@anu.edu.au> 12 Jul 2005

We used Cu grids for many years. Normally, we use Formvar and carbon coated grids for immunocytochemistry since we have a lot less loss of sections. We also tend to do long incubations ...usually overnight. This is time efficient and also lets us use highly diluted antibody to also minimize background due to cross-reactions from contaminants in polyclonal antibodies. Occasionally, we would have a reaction due to copper oxidizing with resultant green solution. I attributed this to salts or traces of Tween 20 in the buffer and breaks in the coating exposing the Cu. As far

as I can see, this is the only reason for not using Cu grids if you use coated grids. We have switched for the most part to using coated Ni grids to avoid this problem. On the other hand, occasionally we need to do a double labeling using uncoated grids and both sides of the sections. I would hesitate to use Cu for this and prefer Ni. Gold would work but is both more expensive and more delicate than Ni grids. Debby Sherman <dsherman@purdue.edu> 13 Jul 2005

I had a bad experience with Formvar-copper grids in immunogold experiments many years ago when incubating primary antibody overnight and the rest done on the following morning. I am using Nickel grids now -- no more problems. Ann Fook Yang <yanga@agr.gc.ca> 13 Jul 2005

We have had problems with copper grids, even Formvar coated ones. I have pre-coated copper grids with Parlodion by dipping, blotting and drying before use, with or with out a Formvar coating. That method seems to protect the copper from reacting with PBS or whatever. Greg Erdos <gwe@ufl.edu> 13 Jul 2005

The main problem encountered with copper grids used for immunocytochemistry or immunogold labeling is the reaction of the copper with salts in the buffers used during labeling. This is a time-related reaction, and can usually be avoided by having short labeling runs and using grids with films on them. Gilder grids, available from major microscope supply vendors, are gold-coated copper grids, and are not that much more expensive than regular copper grids. These grids may be the variety used by researchers who have reported having no problems with their grids during their immuno runs. I usually use nickel grids for my work, but have used Formvar-coated copper grids without problems for 1 day immuno runs so long as the film is intact and as long as I don't get clumsy and end up sinking my grids in my solutions (if I do sink them, I do a quick distilled water rinse, blot the back of the grids with filter paper until almost dry, then re-float them where I left off). As others have shared, the nickel grids are much sturdier, not too expensive, and are not a problem to handle as long as you use antimagnetic forceps. With the nickel grids, remember to correct for astigmatism in the TEM by using a hole in your sample on the nickel grid. This will correct for any inherent residual magnetic field in the grid itself. Edward Haller <ehaller@hsc.usf.edu> 13 Jul 2005

Over the years I have frequently seen cases where the samples themselves have reacted with the copper grid. Two examples: 1) Looking at Fe-S precipitates in magnetotactic bacteria, when the precipitates were ugly and analyzed as copper sulphide. Our surmise was that the iron and copper had undergone ion exchange while the grid (and carbon film) was wet with the culture medium. When we used nickel grids, the precipitates were well formed and composed of iron and sulphur. 2) Looking at Si precipitates in Al-Si electronic bond wire. The specimens were prepared by embedding and microtoming, floating in deionized water. The Si precipitates were surrounded by an ugly mess containing masses of copper. Again, presumed by me to be an electrochemical reaction between the Al and the Cu grid, through the deionized water medium preferentially at the Si precipitates. Again, the use of Ni grids produced good pictures allowing us to characterize the precipitation. Having said all that, we continue to default to use copper grids! Anthony J. Garratt-Reed <tonygr@mit.edu> 13 Jul 2005

I use titanium tweezers with nickel grids - they are cheap nowadays and I like them (tweezers) for light weight and soft action. I use carbon coating on top of all my plastic films (copper or nickel grids) and have no problems with charge/astigmatism. I suspect charging problems happen when grids are quite old and/or dirty. Sergey Ryazantsev <sryazant@ucla.edu> 13 Jul 2005

TEM - Stain solutions

I have a small lab, we do mostly basic EM type work. Nowadays, we don't do much sectioning for the TEM. Once in a while someone wants me to do some sectioning, but many weeks (months) may pass between requests. I am trying to figure out a way to manage the post staining solutions so I don't have to mix them up every time and/or how to make up just a little for the occasional job. Any neat tricks or systems for storing post stains like lead citrate and uranyl acetate out there? How about storage life time and/or ideas about mixing minimum quantities? Jonathan Krupp <jmkrupp@cats.ucsc.edu> 27 Jul 2005

One of the best tricks I have found over the years is to weigh out small amounts of lead citrate (0.1 to 0.4 grams) into 15 ml centrifuge tubes. When you need to make stain, add 1 ml of carbonate free 1 N NaOH to the tube to dissolve the lead (solution should be clear). Then add 9 mls of autoclaved distilled and deionized water and shake well. Put solution through a syringe filter and it is ready for use. I stain 30 seconds to 1 minute. Never have a problem with precipitate and stain is fresh every time. JoAnn Buchanan <redhair@stanford.edu> 27 Jul 2005

I make up my lead citrate using the Venable & Coggeshall method (0.01% Pb citrate in 10 ml of water + 1 drop 10 N NaOH) and store it in a syringe that is wrapped in foil. If I keep the syringe wrapped and its tip capped, the stain is quite stable for a long time. I use a 0.2 micron filter on the syringe to dispense the stain. I usually do en bloc staining with uranyl acetate in water just before I begin my dehydration steps, and so I can usually avoid staining the sections with more uranyl acetate. That solution is pretty stable if kept in a foil-wrapped bottle. I've been using a 1.5% solution, but there was a recent thread on that topic where the consensus was that newer bottles of "depleted" uranyl acetate necessitated higher concentrations (up to 8%, if I remember correctly). Leona Cohen-Gould <lcgould@med.cornell.edu> 27 Jul 2005

I make up 10 ml of each of my stains and store them in syringes fitted with 0.2 micron filters, excluding air. They keep pretty well this way - weeks to months at a time. I wrap the uranyl acetate syringe with aluminum foil. I inspect for obvious precipitates before use. Expel several drops through the filter before use. Tina (Weatherby) Carvalho <tina@pbrc.hawaii.edu> 27 Jul 2005

TEM - 200kv TEM and field emission

I've been normally using a 120kV TEM with a LaB_6 filament to image biological materials. I was using a 200kV TEM with a field emission gun recently and had problems with damage to the samples embedded in Epon Araldite and Formvar-C TEM grids. Is it necessary to use a different embedding media with higher emission microscopes? Gordon Vrdoljak <gvrdolja@nature.berkeley.edu> 28 Jul 2005

Unless you were operating the 200 kV FEG at a much higher intensity than the 120 kV LaB_6, you shouldn't experience damage problems. One can always use a lower-intensity beam (larger spot size number) and spread the beam over a wide area so that a FEG will deliver a low dose rate to the specimen. If you have an intense beam and a correspondingly short exposure for your image, the beam can do damage during the time that it is on the specimen but the image is not being exposed. To clarify that last sentence, if you're scanning the grid with the LaB_6 beam, imaging with a 1 sec exposure, and you experience no significant damage in the (say) 10-20 sec during which you're focusing, framing the image, etc., then you go to a FEG beam with a 0.1 sec exposure, taking the same length of time to focus, etc., you will have exposed your specimen to 10 times the dose, so there may well be unacceptable damage. Otherwise, the 200 kV beam does less damage per unit dose than the 120 kV beam, and there is nothing inherent in the FEG that will increase the damage. Standard techniques for producing plastic-embedded specimens should work as well for the 200 kV FEG as for the 120 kV LaB_6. Bill Tivol <tivol@caltech.edu> 28 Jul 2005

TEM - Cone angle of LaB6 emitter

We use a LaB_6 emitter with a 90 degree cone and 15 micron tip. We are considering using a smaller cone angle and/or smaller tip radius. Does anyone have any comments concerning whether the supposed improvement in brightness and coherence is worth the shortened lifetime? Joe Kulik <juk12@psu.edu>

I have used 60 degree cone emitters with great success on a 2000FX (don't recall the tip radius) and 60 degree cones with 5 micron radius on a Topcon 002B. They add a nice bit of crispness to the high resolution image, although they are no substitute for field emission. As for brightness; no problem. As for lifetime, they don't last quite as long at a 90 degree, so if you are budget constrained that might be an issue, but for us it was well worth the minor extra operational cost. Six months was typical, for a TEM used a lot every day. Usually, the Wehnelt needed cleaning once or twice during the life of the tip so maintenance effort was about the same as a 90 degree. John Mardinly <john.mardinly@intel.com> 22 Jun 2005

SEM - Stray x-rays

We have noticed artifact elemental peaks in EDS spectra obtained with our Oxford Inca system on a Hitachi S-4700 FESEM. For example, an analysis in the center of a strip of 6 mm wide clean copper tape on an aluminum stub generates a spectrum with large peaks for copper (this is good) and a small aluminum peak (not good). Repeat the experiment with a carbon stub, and the small aluminum peak is replaced by a carbon peak. Oddly, the phenomenon is observed only with the microscope operating in High Magnification mode. The same analysis (same magnification, count rate, etc.) on the copper tape in Low Magnification mode detects only copper. This result is similar to what we might expect from beam scatter in a variable pressure SEM. Anyone one out there experienced this problem? Anyone with a similar microscope willing to try the copper tape experiment to let us know if this is unique to our instrument or fundamental to the S-4700? Larry D. Hanke <hanke@mee-inc.com> 12 Jul 2005

I'm not overly familiar with the Hitachi SEMs, but is the "hi-mag" mode on your SEM an immersion lens mode? This is most likely the case if you are using an "in-lens" or "in-column" detector. If so, it is possible that the BSE are being bent around by the immersion field and striking back on the sample. Depending on the immersion field, this could be close or far from the beam. These will probably still be within the viewing angle of your EDS collimator. In their immersion lens SEMs, FEI provides an EDS mode with a weakened immersion lens field so that the BSE are bent somewhat less and strike outside the field of view of the EDS collimator.

NETNOTES

Henk Colijn <colijn.1@osu.edu> 13 Jul 2005

Henk: I believe you are correct. The "hi-mag" mode does use an in-lens detector and you will get x-ray peaks you are not wanting due to the way the magnetic lens acts. Larry: using "Analysis" mode will correct this problem. The WD for this scope with an INCA detector should be about 12 mm +/- 1 mm. Using the "hi-mag" mode for EDX work is not recommended; you won't get good resolution at this working distance anyway and the in-lens detector won't give a very good image at this distance, besides being very sensitive to charging. Becky Holdford <r-holdford@ti.com> 13 Jul 2005

With magnetic field active, test stray signal as a function of WD. Same experiment with a charge sensitive sample should also be interesting, not for x-ray, but SE response. Edward Principe <eprincipe01@hotmail.com> 13 Jul 2005

Your problem is simply one of backscattered electrons bouncing off the final lens and irradiating areas off axis, generating x-rays. This is not just a problem with the SEM you mention, it is a general problem of which many people are unaware. The worst case I have ever seen was obtaining x-ray information from 0.75cm (~1/4inch) from the point of initial beam impact! Modern detectors and collimators do help but scatter is always a problem. I believe that is why SEM manufacturer's main holder is often one which places the specimen surface at a higher level well away from the holder/stage interface. A specimen "in space" will always offer a cleaner x-ray signal. It is a personal suggestion to clients that when trying hard with an analysis they DO NOT use a multi specimen holder! Those who wish to know more should read texts relating to the production of SE in relation to those produced by "bounce off" backscatter. We have a short piece in the "Hints and Tips" section of our web site. Steve Chapman <protrain@emcourses.com> 14 Jul 2005

SEM - Sample prep question

I need to prep some samples for SEM, but I'm not sure of the best way to go about it. We have some bacteria that normally grow in a type of liquid nutrient medium as free, discrete and separate motile cells. However, we have come across a strain that forms into 'mats' on the bottom of the culture vessel. It is these that we would like to examine by SEM. The problem is that the 'mats' are not like a normal biofilm in that they are not strongly adherent to a solid surface, and the mat is not very robust. The mats will 'float' off the bottom of the culture vessel if they are disturbed and they will fragment into small flakes if the vessel is shaken or swirled. The fragments are usually a millimeter or so square. Of course, the 'flakes' need to be flat in order to view them properly. I thought I might be able to pipette them, in a drop of the media they're already in, onto Nucleopore membranes and allow them to settle, and then try to remove the rest of the media and hope that they stick through the dehydration process... but I'm not sure they will. I'm planning on using HMDS. I'm going to try it just to see what happens... but has anyone prepared a sample like this that could suggest something that is know to work well? Scott J. Coutts <scott.coutts@med.monash.edu.au> 19 Jul 2005

Interesting problem. The "mats" you refer to are likely to fragment or worse if you use the usual filter-based preparation methods. First question: do you have access to cryoSEM? If yes, this would be the best way to do your samples. Freeze in a high-pressure freezer, if available, or by plunging into slush nitrogen, and then do the cryoSEM. This is the method most likely to give you the unaltered structure of the mats and the critters therein. If you don't have access to cryoSEM, then the best way is to use minifuge tubes or the like. Let the samples sit in the tube in fix, then the ethanol dehydration steps, being very careful when you withdraw the fluid to change it. Add the fluid for the next step as you withdraw the old fluid to minimize disturbance of the mats, and maybe add the fluid for each succeeding step down the side of the tube. I suggest a 2:1 1:1 1:2 ethanol:HMDS series before the 3 changes in 100% HMDS. I've found it helpful to deposit the last HMDS + sample drops on a sputter-coated membrane filter* stuck to a SEM stub for drying. This further minimizes handling. * 0.22 micron "Nucleopore" type -- the ones with the nice, round holes, not the torturous-path type of filter. Sputter coat both sides of the filter before sticking to the stub, best, or stick to the stub with conductive carbon tabs, then sputter coat. Then drop on the samples in HMDS. And yes, sputter coat for viewing in the SEM. Phil Oshel <peoshel@wisc.edu> 19 Jul 2005

This sounds like a job for ESEM. How much magnification do you need? You can work with fully hydrated samples. You can't get into the ultrastructural range of magnifications but excellent imaging in the classic SEM magnification range, particularly with the newer line of ESEM's. Tobias Baskin <baskin@bio.umass.edu> 19 Jul 2005

How about using a wire loop to place a mat of cells on a poly-L-lysine coated cover slip and then running it through fix, dehydration, etc.... Maybe you don't need the loop at all. Just slide the cover slip under a mat and lift out of the solution. I've never done a mat of cells but I've had success with SEM of cells from suspension on coated cover slips or pieces of Fisher Brand Plus Slides. Andy Carol <acarol1@uic.edu> 19 Jul 2005

Microscopy Today Art Director Awarded MSA Citation

Dale C. Anderson, MT Art Director, was presented with an "MSA Citation for Meritorious Service to the Society" at the recent M&M-2005 meeting in Honolulu by MSA President M. Grace Burke.

Dale is a graduate of the prestigious Rhode Island School of Design, with a B.S. in Graphic Design, and went on to an M.S. degree in Art Education from the State University of New York. She spent her working career in an elementary school environment as an art and 2nd grade teacher–which is rewarding in its own right, but is not "design." When her husband, Ron Anderson (MSA Past-President), was appointed editor of Microscopy Today in 2002, Dale pitched-in with enthusiasm. She redesigned the look and layout of the magazine, selects and composes each cover, supervises the layout and format of articles, and proof reads each entire issue.