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NETNOTES

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

Selected postings from the Microscopy Listserver (http://microscopy.com) from 6/14/06 to 8/14/06. Postings may have been edited to conserve space or for clarity.

IMMUNOCYTOCHEMISTRY – Colloidal gold

We are using 6 nm-10 beads conjugated to IgG (or IgM) antibodies to detect IgG (or IgM) Monoclonal antibodies bound to a viral surface antigens. Does anyone know the distance(s) from the antigens to the beads? Karen Moulton <kmoulton@usm.maine.edu> 27 Jul 2006

Your question is not so easy to answer. Probably only an approximation can be given. One has to consider at least: dimensions of antibodies (often described as approximately 10 nm max dimension for an IgG), dimensions of particles (6 or 10 nm in this case), and the orientation of antibodies on particle surfaces. If you would consider the first antibody only, sitting on the antigen, and realizing the antibody has a number of hinges in its structure, this would mean that in a worst case assumption assuming full flexibility around the antigen, the primary antibody is located within a circle with a radius of about 10 nm with the antigen binding site as the geometrical center. The radius will be bigger when working with IgM. Gold conjugates are often imagined as a particle sitting on the Fc- tail of a secondary antibody. Some secondaries may actually be oriented that way, but it is very likely that some secondaries are oriented side-on, on the gold particle surface. So there may very well be a range of conjugate sizes if you like. Again, a worst case assumption would probably bring the center of a 10 nm particle of a secondary conjugate about 15 nm away (for an IgG conjugate) from the binding site on the primary. Add all this up and the worst case assumption amounts to an area of probability with a radius of 25 nm within which the centre of the gold particle should be located. That is what the situation may be as long as the specimen is in a buffer. During drying, the dimensions of the whole structure may change while collapsing onto the specimen. Is it reasonable to assume worst case conditions? From our own work in the past, even three step labeling involving an unlabelled secondary antibody and protein A gold conjugate to label the outer segment of a membrane protein in bacteria still showed the majority of the particles located outside the cells and not projected over the periplasmic space or even the outer membrane. What has often made me wonder in working with viruses or Virus-Like Particles (VLP) and immunolabeling is that the gold label is often surrounding the virus particles rather than actually covering the structures. In practice, and theoretically for resolution reasons, it may be worthwhile to reduce the labeling to a one step—one, with the gold particle sitting directly on the primary antibody, or even on a Fab fragment of the primary. One step labeling of course also has its disadvantage in that the presence of the gold particle on the primary antibody may result in steric hindrance. Using an ultra small colloidal particle or covalently coupled particle and post-labeling enhancement would be the best choice to reduce those risks. Jan Leunissen <leunissen@aurion.nl> 27 Jul 2006

IMMUNOCYTOCHEMISTRY - Hydrofluoric acid and LR White

I am wondering if the hydrofluoric acid technique to remove glass coverslips from cultured cell monolayers is compatible with LR White resin and subsequent immunolabeling. I mostly see in the Listserv archives references to Araldite or Embed 812. Any thoughts would be appreciated. Kirk Czymmek <kirk@udel.edu> 05 Jul 2006

I would assume that over-curing or increased embedding could well interfere with the immunolabeling. So I can only suggest trying liquid nitrogen to free the coverslips or else using an alternative such as Melinex instead of glass coverslips because that can be sectioned if necessary. I cannot speak from experience about their use in immunolabeling but have used both techniques as an alternative to HF. Malcolm Haswell <malcolm.haswell@sunderland.ac.uk> 05 Jul 2006

SAMPLE PREPARATION - Flat embedding

Would anyone have any recommendations on the best way to flat embed mouse embryos (12 days old), which have been sectioned on a Vibratome? I embedded the embryos in 10% gelatin prior to sectioning and cut 50 micron sections and embedded in Epon. It was during dehydration and infiltration that my sections started to curl. After polymerizing in a flat mold, my sections looked like curled "potato chips". Would cutting the sections thicker help with this issue? Any assistance as to the best method to keep sections from doing this would be greatly appreciated. Chip Dye <dyel@mail.nih.gov> 01 Jul 2006

We routinely embed "Vibratome" sections between two pieces of ACLAR cut to fit on a standard 1" × 3" microscope slide. For convenience in handling, smaller or larger pieces can be used. This does not always result in a perfectly flat sample. If that is a problem then a small weight can be placed on top of the sample during polymerization. This may make a very fragile sample, so just carefully remove one of the ACLAR pieces (I use a razor blade to separate the two pieces of ACLAR and then gently pull them apart) and add a few drops of epoxy to the sample and polymerize. This will give a sturdy sample that is very flat on one side. If you wish to section a small part of your sample cut out the area with a razor blade and glue (with cyanoacrylate "SuperGlue") or use epoxy resin to attach to a blank specimen block. Leave a little margin around the area of interest when cutting a sample with a razor blade because the edges may curl a bit during the cutting/gluing process. Another quick solution if you do not have ACLAR on hand: Make some very thin epoxy sheets by spreading epoxy resin very thinly on your embedding molds. You can even use the backside of the molds to get larger pieces. Polymerization need not be complete but, for example, 24 hours of a 48 hour process. Cut out two pieces and embed your tissue section with a couple drops of resin between them. Polymerize. Cutting thicker sections will reduce the curling during processing but not eliminate it until you get to one millimeter or so which makes light microscopy and trimming specific areas of tissue a big problem. I have embedded many tissues, from drosophila embryos to one centimeter diameter sections of mammalian brain, with this basic technique. Larry Ackerman <larry.ackerman@ucsf.edu> 05 Jul 2006

The issue of flat embedding has been addressed by several authors, including Schwartz (1982), Schafer (1989), Nguyen and Pender (1995), Larue and Winer (1996). In my processing, I largely follow the approach established by Larue and Winer (1996) with some modifications (Larue DT, Winer JA. Postembedding immunocytochemistry of large sections of brain tissue: an improved flat embedding technique. *J Neurosci Methods*, 1996 Sep; 68(1):125132). I work with the 50 micron thick Vibratome sections of mouse brain embedded in agar which for me works better then gelatin. Normally, for flat embedding, I osmicate and/or dehydrate the Vibratome sections placed between two mem-

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brane filters disks with diameters at least two-three times exceeding the tissue size. The sections remained flat between the two membrane disks due to surface tension. The severe section distortion during dehydration is avoided or at least minimized. The main disadvantage is the increased number of fluid changes and extended dehydration times needed compared to routine dehydration protocols, which makes sample preparation relatively time consuming. Briefly: place a disk of filter paper into the wetted compartment of a polystyrene multi-dish plate, transfer fixed Vibratome sections onto the filter disk and cover with the second disk. Carefully add solution, not allowing the top filter to float. The challenge is to keep a certain minimal solution volume to maintain a surface tension between the filters and yet sufficient to avoid sample drying. I do not always use propylene oxide when working with TAAB embedding and the polystyrene plate works well with ethanol. For the last 100% change I add alcohol in excess to remove the top filter disc without breaking the section. An Aclar or Kapton films sandwich works very well for the polymerization Hope this helps. Albina Mikhaylova <amich@ufl.edu> 05 Jul 2006

SAMPLE PREPARATION - glow discharge

I am getting mixed information concerning glow discharged grids: once they are glow discharged how long does it last? and how is it accomplished? Susan Van Horn <susan.vanhorn@sunysb.edu> 28 Jul 2006

The effect of glow-discharging to make the carbon hydrophilic fades over time, and how long it lasts depends on just how hydrophilic the carbon must be to satisfy your particular need. For cryoEM, we have found that putting the material on the grid for freezing is best done within a few minutes after a 2 minute glow-discharge step. However, for other specimens a longer interval is OK, and for bacteriorhodopsin, the carbon surface should be "aged" over a weekend for best results. An additional complication is that the conversion of the surface from hydrophilic to hydrophobic depends on the environmental conditions, so it can vary from lab to lab. It is accomplished by the bombardment of the carbon by oxygen or nitrogen ions (or whatever else is in the plasma); in order to make extremely hydrophilic grids, amyl amine has been introduced into the glow-discharge volume, so in that case I think that molecular ions or fragments from the amyl amine must be particularly effective, but I don't know which component(s) is the actual active one. Bill Tivol <tivol@caltech.edu> 28 Jul 2006

SAMPLE PREPARATION - microwave processing

I am seriously considering investing in a microwave for our cell monolayers processing for TEM. I would like to have some comments from people already using this technique (advantages/disadvantages, worth the investment...) and also an idea of the money I would have to spend for it. Stéphane Nizets <nizets2@yahoo.com> 06 Jul 2006

We use microwaves as our standard processing method now, both for decreasing our processing times and for improved quality of ultrastructure. We find that microwave processing requires much less time than conventional processing and is less extractive of cell contents, as a rule. Cell layers usually require less time than more bulky samples, but even so I would expect that you will save time using MW processing. A disadvantage is that MW requires the user to be there pretty much constantly, since the steps are so short that it's difficult to wander off and do other things while your specimens sit in solutions for 15 minutes to an hour. Another interesting effect is that you may notice less apparent contrast in your stained grids, probably due to less extraction of cellular constituents leading to more even staining across structures (*i.e.*, staining still works fine, but there is more to stain, leading to less apparent difference between adjacent components.) Expense can be an issue, since a programmable laboratory microwave with variable wattage (almost a necessity these days), a vacuum chamber (highly recommended), a circulating water bath to prevent hot spots (really highly recommended) can set you back over $10,000US (about 8000 euros or so?). One could do MW processing with an ordinary kitchen microwave, using water and ice loads and neon bulbs to track hot spots, but that is, like, so last century. Not to mention far less repeatable and very inconvenient. My feeling is that once you go MW, you will not go back, at least for the majority of your samples. Hope this helps. Feel free to contact me if you have questions. Randy Tindall <tindallr@missouri.edu> 06 Jul 2006

SPECIMEN PREPARATION – high vacuum issues with carbon evaporator

I have been using an old DV-502A for routine carbon coating for the past four years without any significant problems, but recently I have had problems getting it down to operating vacuum (~1x10^{-4}) without using liquid nitrogen in the trap. Until about a month ago I've never needed to use the trap to achieve a much stronger vacuum. The problem seems to be getting worse without any overt reason. I've changed out the mechanical pump and nearly every seal in the thing in the past few weeks with no effect. As far as I can tell there is no significant leak anywhere, it simply seems that the diffusion pump efficiency has tanked. Using nitrogen in the trap I can still pump down to well under 1x10^{-5} Torr, but without it, the diffusion pump barely works at all. Assuming that there are no leaks, that the mechanical pump is working as it should, that the diffusion oil is clean and at the appropriate level and that the tree is intact; what can effect diffusion pumping efficiency? What I am left to wonder about is the column temperature gradient. I have the model with the 3.5" column if that matters. The peak temp about one inch from the base reads around 365ºC. The temp drops quickly over the first three inches and reads around 85ºC at the first cooling coil. There is a smooth gradient up the column to the top where the temp is right at 30ºC. I never bothered to take readings when it was working so I don't know if these numbers are normal or not. We are using tap water as the coolant and draining it into the sink. With the weather as warm as it has been I wonder if the influent temp is too warm to cool it adequately. For what its worth, the influent is 25.5ºC with a flow rate of 1.65 L/min and an effluent temp of 28ºC. Any help that you guys can give would be greatly appreciated. Stanton Dowd <sandow50@yahoo.com> 25 Jul 2006

Your water temperature is good. You don't want it colder than 15ºC and hotter than 30ºC (at supply end), optimum at return end is between 30 and 35ºC. Actual numbers may depend on flow rate, but it is good to stay within the above limits. Speaking of leaks: If the TC (thermocouple) sensor reads below 20 mTorr with the main valve open, there are no leaks. A high vacuum leak always results in elevated backing pressure of the diffusion pump. No elevated back pressure means no leak. I assume that the mechanical pump works well, which is tested by pumping lines only with all the valves closed. The potential diffusion pump problems are: 1) Denton diffusion pumps have a water cooling tube coil cemented to the pump with heat conductive cement. More classy and expensive pumps such as Edwards or Varian have the cooling tubing welded to the pump column. The Denton cement eventually cracks, and stops transferring heat. You need to examine it very closely and try to move the water cooling coil to see if it is still bonded to the pump. If it isn't, then either re-cement it, or take the pump off the unit, disassemble, clean it, and braze or solder the water tubing to the pump column. 2) Depleted or lost diffusion pump fluid. Check and replace or re-fill as necessary. 3) Silicone dif-

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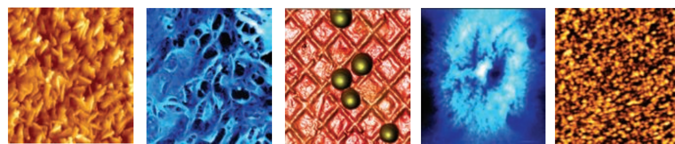
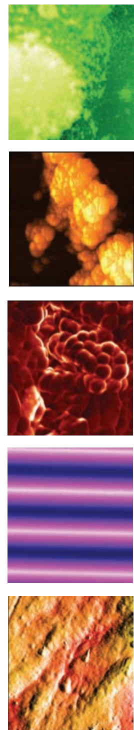
fusion pump fluid (such as Dow Corning 702 or 704 or 705) that is typically used by Denton may partially polymerize inside the pump. It will look clear and otherwise normal, with one difference. Touch the cooling tower inside the pump, and you will feel a sticky layer—feels like sticky packaging tape or 3M note stickers. The layer is clear and invisible, but easily detectable by touch. If this happened, the pump is dead, even though the fluid level is normal and all looks clean. Discard old fluid, disassemble the pump, and clean it very diligently. An acetone rub works for the sticky stuff, but solid deposits (if present) must be removed with a wire brush or sandblasting. Vitaly Feingold <vitalylazar@att.net> 25 Jul 2006

Remove the diffusion pump and check that the diffusion pump oil is good. It might be 'cracked'. Replace it if you need to do that. I would do it anyway. We had a very similar experience to yours on a JSM 35 SEM. The factory spent 3 weeks looking for a vacuum leak with a helium leak detector and examined every O-ring in the system. No good. A different serviceman came in and found the problem in one hour. The one terminal connection on a three month old diffusion pump heater was corroded. It was installed by the first serviceman to solve the same problem months ago. A new heater and terminal connection restored the pumping capacity to normal. Apparently the terminal limited the current to the diffusion pump heater, the diffusion pump got hot, but it never got hot enough to restore full vacuum pumping speed. LN_2 helped but we knew the non-LN_2 vacuum was bad. This seems to be a carbon copy of your problem and your assumptions of what is working properly. You asked, "What can affect diffusion pumping efficiency?" The diffusion pump chimney port is not lined up with the rotary pump port. The diffusion pump oil is cracked. The DP oil is less than normal. The DP heater is defective in some way. Insufficient diffusion pump heating. A bad diffusion pump heater connection. Hot or limited cooling water. The "loss of cooling water" safety switch on the diffusion pump is opening at a lower temp. A cracked or leaking O-ring at the diffusion pump interface with the LN_2 trap. Paul Beauregard <beaurega@westol.com> 25 Jul 2006

As suggested by others, Stanton's problem with his diffusion pump may very well be due to insufficient heat input from the diffusion pump's heater. If you look on the manufacturer's tag that is probably attached to the pump somewhere, you should find the value of the heater input in Watts. Contact your local electrician and he should have a gadget that he can simply clip around the wire leading into the heater and measure the current flowing through it. Current (Amps) × voltage (volts) = Watts. If the value measured doesn't equal the value on the pump's tag, you know you have a problem with the heater. Then, disconnect the heater wires and measure the resistance of the heating elements to see if one is burned out. Making a few measurements like this should give you an indication of whether or not there is a problem with the heater. If not, check the other factors mentioned. Wilbur Bigelow<bigelow@engin.umich.edu> 25 Jul 2006

I remember a similar problem, which could have well been the heater output ... But in this case it was because I had changed the

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diffusion pump fluid to another that had a higher boiling point. The heater did work. But it sure was sluggish in the beginning. Michael Shaffer <michael@shaffer.net> 25 Jul 2006

Michael has made an important point, the type of diffusion pump heater is matched to the original fluid in the DP, you cannot just change to another fluid; the fluid has to match the original fluid characteristics, boiling point for instance! Steve Chapman <protrain@emcourses.com> 26 Jul 2006

The matter of changing from one type of diffusion pump fluid to another is a rather complicated one, which is discussed in some detail in Section 5.4.6 (p. 188) of my book 'Vacuum Methods in Electron Microscopy'. Theoretically, fluids that have similar slopes for their vapor pressure vs. temperature curves, shown in Fig. 5.6 (p. 182), should have similar heats of vaporization, and therefore should require similar heat input from the DP heater, and so should be interchangeable. Note, however, that this plot involves logarithmic scales, so that small differences in slope may involve large differences in actual values. Therefore, just to be on the safe side, I would recommend checking with the pump's manufacturer before making a change. Wilbur Bigelow <bigelow@engin.umich.edu> 27 Jul 2006

I do believe that the comparatively warm water you are using in the cooling system would also be a factor. 25 degrees is fairly warm. I run my Denton cooling water at 15 degrees. Ted Dunn <drteddunne@yahoo.com> 27 Jul 2006

TEM - image diffraction pattern rotation

I have a question regarding the image diffraction pattern rotation in a TEM. While, in some TEMs, this rotation is compensated for, it is still possible to have a 180° rotation between a TEM image and the corresponding diffraction pattern. What are the procedures to measure this rotation with or without special calibration samples? Silvija <gradecak@fas.harvard.edu> 07 Jul 2006

Go to convergent beam mode and go to cross over with the condenser lens. Then decrease the lens strength on the condenser (CCW) until you see the shadow image of the sample in the bright field disk. It should agree with the image orientation in the mag mode. I think all microscopes have the knobs go clockwise to increase lens strength and CCW to decrease lens strength on the lenses. Scott Walck <walck@southbaytech.com> 07 Jul 2006

I may be wrong, but what I learned for 20 years in TEM: rotating the "Intensity" knob CW makes a beam parallel (*i.e.* decreases the strength of the C2 lens). As you understand, this uncertainty will make a conclusion regarding a 180° rotation uncertain. Andrey (sent directly to Scott Walck in response to the above posting – Scott's reply is shown below).

Cross over, under or over-focusing the condenser lens will both make the electron beam parallel. In the analytical machines that have more than two condenser lenses (as in the last 20+) years, you obtain the parallel condition faster (less turns of the knob) by over-focusing the C2 lens than by going underfocus. In machines that only have the two condenser system, you under focus the condenser to go to parallel condition faster. In either case, you never get to a parallel condition; you get to a very small convergence angle. With respect to my previous answer, I believe that you can check the technique that I gave with a GaAs [011] CBED image since it is a noncentrosymmetric crystal. As far as I know, TEM's still have the condition that CW and CCW still relate to the strength in the lens. You can watch the current or voltage monitor of the lenses when you turn the knob. Some manufacturers of SEM's have changed that, especially with the objective lens. Now they relate the CW turn of the focus now with increasing the working distance, which of course, is decreasing the strength of the objective lens. Scott Walck <walck@southbaytech.com> 09 Jul 2006

TEM - Darkfield imaging

I have a question involving darkfield imaging. We are using a Philips CM10 and the imaging seems to be fine with one important exception. We have been capturing the area on film in standard brightfield mode and the images come out fine. Then we switch to darkfield by just pressing the DF button. The captured DF image is often strongly directionally blurred, as though there is a lot of movement during capture. When the image is again checked in brightfield it looks fine. Is it possible that there is charging in DF mode that could cause this but not in BF mode? Any other thoughts? Debby Sherman <dsherman@purdue.edu> 23 Jun 2006

Sounds like you either don't have the DF tilt alignments done properly, or are running in tilted DarkField mode. You should be tilting the illumination to bring the post specimen diffracted beam down the optic axis so that there are minimal aberrations as well as have the Objective aperture centered. This is generally called centered Dark Field Mode. Try to get hold of Eric Stach at Purdue (Engineering College) and have him come over to your lab and have a look. He should be able to sort out what is wrong reasonably quickly. Nestor Zaluzec < zaluzec@aaem.amc.anl.gov> 23 Jun 2006

TEM - Wehnelt assembly

I'm in the lab on Saturday cleaning the SEM Wehnelt assembly (I don't have a spare). Scrubbing, scrubbing, scrubbing to get that tough tungsten oxide coating off the business end. My mind begins to wander, and I recall showing a student the day before how thin the gold coating is on SEM specimens by wiping it off the bell jar of the sputter coater with a swipe of a lens tissue. Wouldn't it be nice if the junk on the Wehnelt came off as easily? So, has anybody tried this, i.e., sputter coating the Wehnelt orifice, with, say, gold? Would it help, hurt, make no difference, or be disastrous? Maybe someone out there has already tried this, and lived to tell about it. Jim Ehrman <jehrman@mta.ca> 24 Jun 2006

For a much quicker method for cleaning cathodes take a look at Hits and Tips on our web site? Steve Chapman <protrain@emcourses.com> 24 Jun 2006

The gold coating likely came off easily because it is thin. Plus, it is not homogeneous. If you look at gold sputter coatings they look like spider webs. For low magnification work, this is fine. Plus, they will change depending on your coating equipment. Hence, high resolution FE work is done with Au/Pd, Ir, Pt or Pd at low vacuum. The gold coating on apertures is most likely done by plating, plasma deposition, evaporation or some other method that puts down a really nice even thin film. Therein lies your culprit and vulnerability. The thin film can be easily removed via mechanical means. So trying to clean it would more than likely remove it. I think you are stuck with what you have. The blue plague from the W filament is tough to remove. Pol works but takes time. Gary Gaugler <gary@gaugler.com> 25 Jun 2006

Sonicate in diluted ammonia, sudsy ammonia if possible, and the tungsten residue will come off easily. I usually dilute 2 ammonia:1 water. This hint originally came from the listserve but I have forgotten who provided it. Debby Sherman <dsherman@purdue.edu> 25 Jun 2006

Let me take a stab at what I think would happen. First, you wouldn't want to sputter the gold you would want to evaporate it. Sputter films are usually more adherent than evaporated films. Secondly, you are putting a material that is easily evaporated into a situation where it could be evaporated onto the wrong components, namely the

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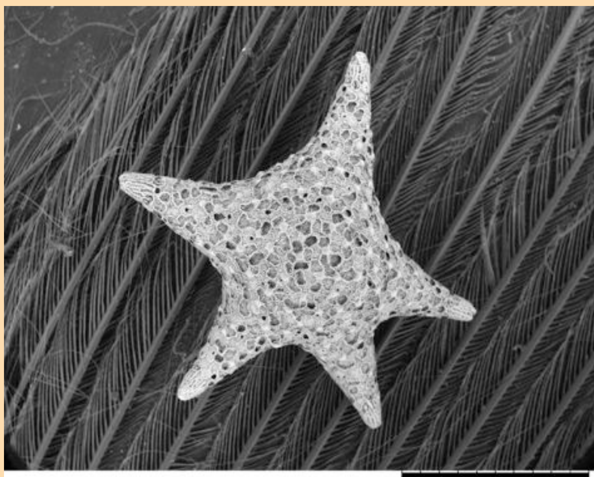

Hitachi High Technologies America held a photo contest during the week at M&M 2006 with the new TM-1000 tabletop microscope. The purpose of the contest was to demonstrate the ease of use and quality imaging of the new microscope.

The overall winner was Monica Vargas, Hitachi Global Storage Technologies as judged by Dr. Larry Allard of Oak Ridge National Laboratories. Ms. Vargas chose a sample of star sand on a feather.

Other winners during the week included Tim Maugel of the University of Maryland; Caroline Miller, Indiana University School of Medicine and Laurie Wallace, Intel Corporation.

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insulator of the filament. In the gun, you typically have a very good pressure and high temperature and you are hitting the filament side of the Wehnelt with electrons which would heat it up further. Keep scrubbing the W off or go to a LaB_6 which seems to come off a little easier. I also recommend plasma cleaning the Wehnelt after solvent cleaning. Scott Walck <walck@southbaytech.com> 26 Jun 2006

I've never had a problem getting deposited tungsten off using a diluted ammonia solution in a sonicator (use in hood!). The only warning would be not to put the brass bits in the strong ammonia solution, MicroQ does a good enough job discoloring them; although generally nothing a nice relaxing afternoon with some cotton cloths, cotton applicators and a tube of Pol won't cure. I would sonicate in the ammonia for a while (till the W was gone), then in warm water, then a polish with some POL, followed by some MicroQ sonication, hot water, and then 95% ethanol and blown dry with canned air. The yellow metals generally start with the POL steps. But as always, your experiences may vary; in other words I see no need to put gold on the Wehnelt. Geoff Williams <geoffrey_williams@brown.edu> 26 Jun 2006

It gets pretty hot near the filament. I suspect that the sputtered on coating would flake off due to the differential thermal expansion of the two metals since conventionally sputtered metals are sitting lightly on the surface. On the other hand, electroplated metals would be more stable. For example, we have an ancient Wehnelt from an RCA that is gold plated. It was stable for 30 or more years. But, then, apparently, someone tried to clean it too vigorously and removed some of the gold. Now the cleaned area is tarnished. Perhaps Steve Chapman and Chuck Garber might want to chime in here as they are experienced in scope maintenance and metallurgy, respectively. John Bozzola <bozzola@siu.edu> 07 Jul 2006

I have kept out of the gold coated cathode debate for those that know me will already have guessed my reply! However, I too know of the RCA gold plated cathode and 100% agree with John Bozzola in that I believe sputtering of gold would not be efficient in this case. The answer my clients would expect is "why go to such lengths when cleaning a cathode assembly should be simple?" This is what we use "The cathode assembly is placed, aperture face upwards, in a beaker of stock ammonia solution diluted 3 parts ammonia to one part water. The stock solution is thought to be about 40% ammonia. After 15 minutes in the ultrasonic cleaner the beaker is placed under running water and thoroughly flushed through. Care is taken to ensure that none of the clamping or alignment screws had fallen out of the cathode assembly and could be flushed away! The cathode is then washed with alcohol before being dried with a hair drier. A new filament is fitted and centered. The assembly is checked for cleanliness by observing with a 20× lens prior to re installation in the microscope. Total time for this procedure should be less than 25 minutes break to pump down." If the contamination in the nose of the cathode is obstinate, use a little metal polish but only a polish soluble in ammonia. And follow the above procedure again but for only two or three minutes in ammonia solution with agitation. The full story is available on our web site. Steve Chapman <protrain@emcourses.com> 12 Jul 2006

TEM – powder sample preparation

In the past, I have suspended fine particles in methanol when preparing TEM grids of powders. This seemed to work well with the crushed minerals, ceramics, etc that I generally examine. However, questions have been raised about possible problems with particulate emissions from coal fired power stations. In particular: 1) Does methanol evaporation cause particle agglomeration on the grid? 2) Does methanol use cause morphological changes of the particles? 3) Are particles size-fractionated on the TEM grid during the evaporation process? 4) Does methanol dissolve some phases? My question to the Listserver is: Should I avoid methanol and if so which solvents should I use and why? I would appreciate your advice and particularly if references can be provided. Mark Blackford <mgb@ansto.gov.au> 26 Jun 2006

I have worked with coal and coal-derived particulates although I have not worked with TEM preparations. I would take those questions as standard ones that should be asked of any liquid used for dispersion of any material. I don't think methanol deserves any special attention with regard to coal ash, but you should always be on the lookout for these possible interactions. I would not expect any morphological changes in the particles unless you were dissolving a phase. I expect the minerals, oxides, and any glasses formed from them during combustion would be inert to methanol and most solvents. I think the carbon would be charred and also rather inert. Agglomeration would be a surface chemistry question and is beyond me, but I would not expect any special problems with methanol. I would be on the lookout for size segregation with most solvents depending on many factors. I suppose it would be worse for slower evaporation processes, but a lot probably depends on an individual's technique as much as the choice of liquid. Bottom line: I would probably try methanol and if you verify that there is no obvious agglomeration or segregation, then call it good and don't argue with success. Warren Straszheim <wesaia@iastate.edu> 26 Jun 2006

I am a bit puzzled by your questions because you used methanol on other materials and it appeared to work for you. Anyway, the questions you asked are probably of general use. You asked: 1) Does methanol evaporation cause particle agglomeration on the grid? I think you mean flocculation and not agglomeration. Yes, pure methanol does cause this problem and so do other solvents. It depends on the nature of your particles as far as the materials they are made up of and whether they are hydrophobic or hydrophilic as well. These are not the only variables by a long shot. 2) Does methanol use cause morphological changes of the particles? Not to the primary particles or primary structural units, unless they dissolve in methanol. 3) Are particles size-fractionated on the TEM grid during the evaporation process? Absolutely. Particles do not 'drop out' of solution by gravity onto a grid. The particles move around in a dynamic way. So they can be segregated. Some of this can be stopped to get good representative dispersions. Furthermore, dropping the particle suspension onto a grid on a piece of filter paper should be avoided unless you want to skew the size results to get a biased result. This is done in some patents and is a 'hidden' bias in one ASTM TEM method on carbon blacks. Flocculation on a grid can also combine aggregate structural units to make you think the 'particle sizes' are larger. 4) Does methanol dissolve some phases? I guess the technical answer is yes, if the phase is even slightly soluble in methanol. I would not think this applies to your 'flyash' samples that should have things like glassy particles and unburned coal or carbon in them. 5) My question to the Listserver is should I avoid methanol? Not if you need a slightly polar solvent to disperse the particles of interest. 6) Which solvents should I use and why? Try different solvent systems. Use the one you think works the best for your sample situation. General comments: Don't get tunnel vision when dispersing powders, aggregates, or primary particles. Try different solvents and look at them in a TEM. Look at how they behave. Examine the whole grid to see the different patterns you see as a dried dispersion. There's no magic bullet single dispersing sys-

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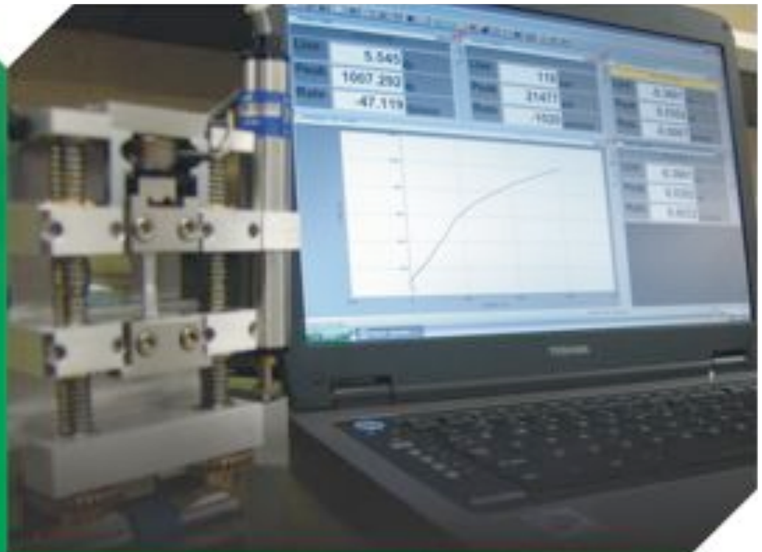

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tem here. That's certainly true of colored ink jet pigment dispersions. The number of factors that come into play in dispersing powders is not a single dimensional variable called a solvent. Here's a pointed example of tunnel vision. I was told that a sample of some plasma generated nanoparticles was hydrophobic. The newly hired chemist's sonicated settling tests showed it would not disperse in water based systems. What would you use? I was told which organic solvent to use and I used that one. Then I dispersed the hydrophobic sample in a water based system. Both preps gave equivalent flocculation free dispersions on TEM grids. This was done by manipulating the other factors important in dispersion besides the type of solvent system. In a previous posting from Leslie at IBM the following summary item was detailed: "There were two (who) mentioned dry loading the grid, which is dumping some of the powder on the grid and tapping off the excess. This is the method I tried first as it was the easiest, but I have not looked at the grid yet to see if there is enough material to do EELS on." This (DeGussa Germany?) method relies on the 'adhesion' of the finest aggregates to a grid film. They are attached simply by static charge. My experience with silica and plasma nanoparticles was that the populations were usually too light for me to use. The aggregate size distribution was skewed towards smaller sizes. Another problem is that the powder aggregates get on both sides of the grid without special handling precautions. This causes some aggregates on the wrong side of the grid, to be over focused. Yet, dry preps are faster to perform than solvent preps or mulling. I used dry preps when speed was critical, and the structure or size was not of interest. Recently, the change in the size of precipitated silica particles was blamed on a certain dispersing solvent. A dry prep would have shown that the size increased without that solvent ever being used. A dry prep is a good referee technique. For me, high speed high resolution imaging of silica dry preps showed the presence of single digit nanometer scale salt on the surfaces of primary particles that accelerates this above mentioned "poached egg" look but it would happen even without the trace salts, given enough beam time. So dry preps have a place in a TEM lab and are useful. Disclaimers: I performed dispersions on all types of powders and examined thin sections of the interiors of agglomerates, large aggregates, coatings of all types, and silica tire cleats for over 20 years. Your samples might perform differently than stated here. There are at least 10 factors or variables that apply to dispersions of powders and aggregates. That's too many to discuss here. Obtaining a good dispersion is a learned skill and takes practice. Paul Beauregard <beaurega@westol.com> 10 Jul 2006

SEM - critical point drying insects

In order to prepare very small (less than or equal to 5mm) insects for electron microscopy, is it necessary to chemically dry them with acetone and amyl acetate in addition to CPD, if they have already been prepared in 100% ethanol? Also, I wonder if the above chemical damage DNA? Joseph Fortier <fortier@slu.edu> 17 Jul 2006

If the samples are currently in 100% ethanol, you can go directly into the CPD device. Passage through amyl acetate is not necessary since liquid CO_2 is miscible with absolute ethanol. Simply, place 5-10 ml of abs ethanol in the CPD chamber to maintain a saturated ethanol atmosphere (thereby preventing evaporation and drying of the insects), transfer the insects into the chamber and CPD as usual. Since the specimens are so small, I assume that they will be placed into appropriate carriers, otherwise they will be lost in the exchange of CO_2. I do not believe that liquid CO_2 will extract DNA, but I would defer to a molecular biologist if they know otherwise. If you need more info, please do not hesitate to ask. John Bozzola <bozzola@siu.edu> 17 Jul 2006

Go from 100% ethanol directly to the CPD using ethanol as your transition solvent. The other solvents are not necessary. I have also just recently run across a paper that uses the CPD to reverse the effects of chemical fixation with formalin allowing sequencing so I would surmise damage if any is negligible. See: Formalin Removal from Archival Tissue by Critical Point Drying. *Biotechniques* 33:604-611 September (2002). Scott Whittaker <whittaks@si.edu> 17 Jul 2006 & 18 Jul 2006

I just returned from Woods Hole, Massachusetts where we did some SEM on some small ants, aphids and spiders. An entomologist there gave us a protocol where you rinse the bugs in 70% ethanol and directly sputter coat. I am not sure whether the ethanol killed them completely. We carefully mounted them on double stick tape on stubs and sputter coated them. The structure was very nice and the preparation extremely easy. JoAnn Buchanan <redhair@stanford.edu> 17 Jul 2006

SEM - Observation of polymers morphology

I would like to know if it is possible to observe with a SEM the morphology (spherulites, crystals) of a polymer; for my current study it is a PEEK. Which conditions (HV or LV, pressure etc.) should I work in? Laure Gauthier <lgauthier@victrex.com> 19 Jul 2006

Many years ago, we developed a technique for etching PEEK for observation under TEM. But it would also work under a good modern SEM. Here are the two papers. If you want any more details, please contact me. Permanganic Etching of PEEK Olley,R.H., Bassett,D.C., Blundell,D.J. *Polymer*, 1986, vol.27, pp.344-348; On Crystallization Phenomena in PEEK Bassett,D.C., Olley,R.H., Al Raheil,I.A.M. *Polymer*, 1988, vol.29, pp.1745-1754. Robert Olley <r.h.olley@reading.ac.uk> 19 Jul 2006

Best guess would be using confocal microscopy. Andrew Anthony "Tony" Havics <ph2@sprynet.com> 19 Jul 2006

There is a variety of tools that may be used to study the crystalline morphology of polymers. Confocal microscopy, as suggested by Tony, may be of use. The choice of tool will depend on several factors including the form of the polymer (reactor particles, molded parts, blown/cast films, etc.), the scale of the structures to be analyzed (spherulites, crystallites/lamellae), the tools available for sample preparation, and the type of SEM you use (conventional, variable pressure, or field emission SEM). Suggest you check out the latest edition of Sawyer and Grubbs' Polymer Morphology. This book is a great place to start and should have a place on every lab's book shelf. Gary Brown <gary.m.brown@exxonmobil.com> 19 Jul 2006

SEM - electromagnetic fields

Our Hitachi S4700 is suffering from 60 Hz EMF's in our lab. We see jagged lines in the horizontal direction at magnifications ≥ 150kX. I just had a vibration survey done and the results are within Hitachi specifications. Do any of you pay particular attention to movement (of people, people in chairs, talking, etc.) when you are operating the SEM >200kX? We pay particular attention to this in our HRTEM lab. Just wondering if it is an issue in the FESEM lab too. I'm looking for solutions. Any ideas? Thanks! Owen Mills <opmills@mtu.edu> 19 Jun 2006

I am sorry to say but round the world one of the problems that we all have is electromagnetic fields. To prove your problem, compare working at a long working distance (WD) with working at a very short WD. If the problem changes for the better at short WD, it is a field

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problem. If it stays the same its most probably vibration or an instrument fault. Check laboratories above, below and on all sides to track down the equipment that may be causing the problem, try switching equipment off. As a warning to all, magnetic fields now prove to be the biggest FEG installation problem, so always have a professional check your site. Remember the field will change by the inverse square so in some cases to move the instrument in the room may help. Field reduction systems work if a field is not over strong but the best route is to find a room which is truly field free. Good luck Steve Chapman <protrain@emcourses.com> 19 Jun 2006

SEM - Critical Point Drier solvents

We have always used ethanol as the dehydration solvent with our Balzers CPD 020. Does anyone know whether the seals and other components of this instrument are compatible with methanol? It is used as a primary fixative for plant tissue in a publication, but it is unclear from the methods section whether the tissue is changed to ethanol before critical point drying. We have sent emails to the author and US supplier of Balzers equipment, but not yet received any replies. Also, does anyone know whether absolute ethanol can be directly changed for anhydrous methanol without damaging plant tissue? Heather Owen <owenha@csd.uwm.edu> 22 Jun 2006

I have used the methanol fixation for plants and dried using the BalTec CPD030 and have had beautiful results. No problems with the seals. <klk@biotech.ufl.edu>

MICROANALYSIS - X-ray emission table

Does anyone know where I can find a spreadsheet or text version of x-ray emission lines? Thanks, Owen Mills <opmills@mtu.edu> 28 Jun 2006

Here is the link to the table of x-ray energies from J. A. Bearden, in "X-Ray Wavelengths," Rev. Mod. Phys. 39, (1967) p.78. http://xray.uu.se/hypertext/XREmission.html. Henk Colijn <colijn.1@osu.edu> 28 Jun 2006

You can also try http://tpm.amc.anl.gov/NJZTools/XEDSTabulateParam.html Nestor Zaluzec <zaluzec@aaem.amc.anl.gov> 28 Jun 2006

One of my favorites is EM Periodic Table, a program authored by Scott Walck many years ago. It has a periodic chart layout and a table layout that can be translated to Excel. I got it at a Lehigh short course and I find it very handy. Here's a link to download the files: http://www.amc.anl.gov/ANLSoftwareLibrary/EMMPDL(old)/EELS/EMPeriodicTable/. Scott calls this "beerware" meaning when we meet him at conferences, we owe him a beer if we use it. Scott: you can claim your pitcher at M&M in Chicago. This program would be cheap at twice the price. Becky Holdford <rholdford@ti.com> 05 Jul 2006

Thanks for the comment about the program. I was surprised anyone was using it except for me. I use it to look up potential overlaps for X-ray and EELS peaks. I used several tables to try to get the best possible data available and crosschecked the values. For some lines that were not available, formulas were used to calculate them. EMPeridodicTable is available on the EMMPDL. It is also incorporated in the program, EELS_Plot. Both are Visual Basic programs that you have to install in Windows. I believe that the table is in tab delimitated form or CSV form on one of the data files that are loaded into the program directory and that it can be brought into Excel without problems. You would have to extract the files and look at the data files or extract it out of the CAB file for the program. Scott Walck <walck@southbaytech.com> 29 Jun 2006

This is a very useful little program. But more useful if it also included absorption edges. Michael Shaffer <michael@shaffer.net> 06 Jul 2006

The program does have the absorption edges in it. That's what an EELS line corresponds to. Check out Si for example. You will find the *K*α line for Si at 1.74 in the XEDS mode. Then go to EELS mode and click on Si again and you will see that the Si *K* edge is 1839 eV. BTW, to address the original question, the data files that come with the program are CSV (comma separated values) files and will open directly into Excel or another spreadsheet program. Two are XEDS data, one in keV and one it eV. The other file is the EELS data only and is only in eV. Before you copy it to use it, please look at the credits to Nestor, Noran, and EmiSpec for the data in the help menu of the program. Scott Walck <walck@southbaytech.com> 06 Jul 2006

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