NETNOTES

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Selected postings from the MSA Microscopy Listserver (listserver@msa.microscopy.com) from 2/10/05 to 4/10/05. Postings may have been edited to conserve space or for clarity.

SEM - Particles in liquid solution

I would like to image particles (micro- or nano) in a liquid solution in an ESEM or SEM-HiVAc. What is the best preparation method? Monica Iliescu <monica.iliescu@polymtl.ca> 16 Feb 2005

One of the easiest ways is to take ordinary cover slips and cover them with large drops of 1 mg/ml poly-l-lysine. Leave the coverslips in a humidity chamber (such as a Petri dish with a rolled-up water-soaked Kimwipe or something similar) for about an hour, and then rinse them gently in distilled water. These coated coverslips will remain useful for a month or so after drying. Take some of your particles in liquid and put them on the coated coverslips and let the particles settle onto the surface, where they should adhere to the coating. After a half hour or so, gently rinse the coverslips to remove the excess solution, and proceed with your normal fixation and/or coating routine. You should be able to view your particles in high vacuum conditions with little difficulty. One problem is occasionally that the coverslips are difficult to ground and can create charging artifacts in the SEM. Usually all you need to do is be extra careful about mounting the coverslips to your SEM stub. We usually use carbon adhesive tabs, then metal tape to bridge the top of the coverslips to the stub by folding the tape over the edges in a couple of places. Following this, we sputter coat normally, starting with a light coat, then adding more as necessary until the charging is under control. Randy Tindall <tindallr@missouri.edu> 16 Feb 2005

TEM - Chilling water problem

I have a problem in that my Phillips 420 TEM is suffering from poor circulation. The recirculating cooling water comes in to the scope and is split, one part to the diffusion pump and one to the electronics. Both sides have adjustable flow meters and should have 1.5 L per min rates. Recently the electronic side dropped to about 400 ml per minute and the scope shut off. Previous experience indicated I needed to flush the cooling water with a dilute phosphoric acid based detergent. I have done this and can get the DP side up to 2.5 liters/min if I wanted, but the electronic side stays at 800 ml/min no matter how I adjust the flow meters. The cleaning solution pulled all the discoloration from the sight glass on the flow meter, so I believe I have the gunk flushed, but I know if I leave it at 800 ml/min it's only a matter of time before I have to re-flush the cooling lines. Where else could I have an obstruction and how can I get rid of it? Frank Karl <frank.karl@degussa.com> 17 Feb 2005

I've had similar problems on microscopes, usually it is because the cooling water flows through alloy heat sinks, the alloy corrodes and occludes the channel through it. On an Electroscan ESEM we had, we reamed out the cooling line through the alloy heat sink and slid copper tube through, well lubricated with vacuum grease, as a heat sink agent. John V Nailon <J.Nailon@uq.edu.au> 18 Feb 2005

I'm not sure if the EM420 used them, but it could be a problem with the "Watts" regulator for the lenses. It might need to be replaced. We've had some fail. For flushing the cooling lines, our service people have been really enthusiastic about something called "CLR" cleaner (Calcium, Lime, Rust). If I remember correctly, sulfamic, glycolic, and citric acids are the active ingredients. I picked it up at our local hardware store ("cleaners" department rather than "plumbing"). It did a good job of cleaning up the flow in our CM200 and caused no noticeable damage. You also might try running the cleaner through the system overnight and throttling back the flow through the DP to increase the pressure through the lens section. Hendrik O. Colijn <colijn.1@osu.edu> 18 Feb 2005

Have you removed and cleaned the in-line filter on the system? I've had this problem many times with 420 electronics and cleaning this usually cures the issue. On my 420, the inline filter is hidden in a small brass "Y shaped" connection on the inside of the right hand side of the electronics rack. Take off the side panel, swing open the electronics and look in the rear lower corner. Some 400 series instruments have this filter mounted on the lower back panel (much easier to get at). One leg of the Y fitting has a cap that unscrews and a metal screen filter can be withdrawn, cleaned and then re-inserted. You'll need a pan to catch the drainage, while you are cleaning the filter. What I do is put a low profile pan underneath, unscrew the cover, remove the filter and quickly screw the cover back on to stop a large loss of water. Clean the filter and then replace. Getting the cover off isn't always easy as it is in an awkward location and getting a wrench on it to get leverage is inconvenient to say the least. Nestor J. Zaluzec <zaluzec@microscopy.com> 19 Feb 2005

TEM - LaB_6 filament use

I would like to switch our JEOL 1010 microscope to LaB_6 filaments for biological applications and I was wondering what other people felt about LaB_6 filaments, and how much they liked them. Garry Burgess <GBurgess@exchange.hsc.mb.ca> 18 Feb 2005

We have a LaB_6 on our T12, and, in general, the increased brightness, coherence, and lifetime with respect to a W filament make it worth the increase in cost. We have had some uneven lifetime issues with some of the filaments we've installed, so I recommend researching which brand of filament works best in the 1010. Bill Tivol <tivol@caltech.edu> 18 Feb 2005

As long as you have got the money for the LaB_6 filament, as long as the vacuum is good in the column and in particular in the gun area (keep the oil diffusion or turbo pump and ion getter pump running all the time, overnight and over weekend; always use LN2 trap), and as long as you have well-trained users, there are a number of advantages, as Bill said: increased brightness, increased coherence, increased lifetime (in our CM12, usually around 3 years; exclusively in TEM low dose imaging modus; no EDX or EELS). 'Disadvantages' include needing a good vacuum and the need for careful, slow heating. Reinhard Rachel <reinhard.rachel@biologie.uni-regensburg.de> 19 Feb 2005

Formvar Grids

We have been using Formvar/carbon coated grids for spreading magnetic nano-particles in water and for negative staining. But as you know, an aqueous solution does not spread well on grids because of the charge characteristics on the film surface. The only way I know of for making the film surface more hydrophilic is to do a glow discharge in a sputter coater, but we do not have such a device. I heard treating coated grids with ethanol vapor works, but not for me. Does anyone have any other tricks or suggestions? Hong Yi <hyi@emory.edu> 20 Feb 2005

Do you have a vacuum evaporator? It's simple to rig it for glow discharge with an inexpensive Tesla coil. A plastic vacuum desiccator, the same Tesla coil, and a rough vacuum source will do the job also. Caroline Schooley <schooley@mcn.org> 21 Feb 2005

I don't think that a sputter coater, old or new, is going to solve your problem. I have not heard of ethanol vapors making a carbon grid more hydrophilic. Carbon coated grids lose their hydrophilic nature as they age and they become more hydrophobic. The process can be "reversed" by (a) exposure to an RF "air" plasma in a small plasma etcher (effect will last 60-90 days) or (b) a thin evaporation of Victawet® onto the grids. Our own studies would suggest that Victawet can keep the grids highly hydrophilic essentially forever (e.g. more than one year). Just remember that it is a phosphate based surfactant so if you are doing elemental analysis work,

you might not want to have P showing up in your data. But if you have an ordinary vacuum evaporator and tungsten baskets, and don't have a plasma etcher, you can solve your problem with Victawet. The best bet for having carbon coated grids with the greatest hydrophilic characteristics is to make or purchase your carbon coated grids always "fresh". If the grids are purchased, and their age is uncertain, contact the manufacturer of the carbon coated grids, give them the lot number and then you will know. Charles A. Garber <cgarber@2spi.com> 21 Feb 2005

Another method occasionally used to make C-coated grids hydrophilic is to expose them for some minutes to the direct illumination of a UV lamp. This is done at normal atmospheric pressure, so it needs no vacuum technology. James Chalcroft <jchalcro@neuro.mpg.de> 21 Feb 2005

I personally don't like glow discharge at all: it's very difficult to reproduce. It depends on the equipment and there is no way to control the "amount" of discharge. I use 0.5-1% poly-lysine from any of the EM suppliers (don't try to make the solution yourself since there is some trick required). Place the EM grid on a 10 µl poly-lysine drop for 5-10 min, wash on a few drops of deionized water, air dry - good for at least a month. Alcian Blue works in the similar way with a similar result. Of course, these methods will only work for positively charged molecules. Sergey Ryazantsev <sryazant@ucla.edu> 22 Feb 2005

TEM - Cross-section sample preparation

I have a basic question concerning sample preparation for TEM. I've recently started working in a physics and materials lab doing TEM (microscopy and sample preparation) and it's all new to me. Obviously we do a lot of cross-section samples of thin multi-layers. My colleague showed me a preparation technique that includes cleaving and polishing of the sample and finally ion milling it to transparency (making a hole in the centre with the area surrounding the whole being transparent because of the angle of milling). Does anyone have any tips or otherwise helpful "basic" information about preparing samples this way or other techniques I should consider? The sample becomes very fragile while thinning it and cleaning it is very difficult. Niko Hellsten <niko.hellsten@gmail.com> 22 Feb 2005

I do not know what you are working on, but the following might help. 1) Ion thin from both sides for a short while at a low angle to get a clean sample. 2) Carefully remove the sample and glue one side to a slotted grid. 3) Ion mill only from the top till you get a hole. This worked lovely for some samples I have done. Difficult to get it glued down right without contaminating it, but worth it in the end. S. H. Coetzees <coetzees@mopipi.ub.bw> 22 Feb 2005

Have you tried using a Focused Ion Beam system in order to prepare your TEM/SEM cross-sectional samples? Looks like that would be the way to go in your case. Bobby Hooghan <hooghan@grandecom.net> 22 Feb 2005

TEM - Stability of amorphous a Ge test sample

I plan to use an amorphous Ge sample to characterize the contrast transfer function in our TEM. Does anyone have a comment about the stability of a thin amorphous Ge sample after prolonged exposure to the atmosphere? Must the sample be kept under vacuum (or in an inert atmosphere)? Joe Kulik <juk12@psu.edu> 24 Feb 2005

Ge does oxidize, and I believe the oxide is water-soluble. In my experience, Ge thin films on Cu grids also oxidize slowly, over a period of months, and crystallize when stored at room temperature in air. I don't know if crystallization can be slowed or avoided by storing samples at low temperature or in an inert atmosphere. Paul Voyles <voyles@engr.wisc.edu> 24 Feb 2005

SEM - Hollow Biological Sample

Being a novice at SEM, I'm wondering what would be the best method of sampling a hollow-centered tube of polymer with cells mixed within. I want to look at the homogeneity of the spread of cells throughout the polymer

tube (i.e. surface morphology of cross-sections). The tube is formed within a glass capillary, but it is unfortunately rather flimsy and I originally thought of backing it with a molten material, letting it set (i.e. for mechanical support) and removing the tube from the glass before slicing cross-sections, coating in gold and scanning using SEM. However, I was unaware of the water content removal needed. I am aware of alternatives including SEM with a cryostage and environmental EM. The problems include: removing of the tube from the glass capillary, and slicing the tube without smearing. What is everyone's recommended approach? Garr Chau <g.chau@ucl.ac.uk> 24 Feb 2005

Fix in glutaraldehyde and dehydrate as usual for critical point drying, but after the final 100% ethanol, throw the tubes in liquid nitrogen and snap them. The ethanol freezes vitreously, and you'll get nice breaks with no smearing. I do this for biofilm studies in catheters. Your biggest problem will be finding the bits after breaking -- capillary tubing is pretty small. Try putting black paper over the aluminum foil that you use to line the Styrofoam container holding the LN_2, and do the breaking in small Petri dishes in the LN_2 bath. Bring back into 100% ethanol and critical point dry as usual. Philip Oshel <peoshel@wisc.edu> 25 Feb 2005

TEM--Ultramicrotomy of sand

We have someone wanting to study bacterial attachment to sand grains. Does anyone have any hints on ultramicrotomy of sand? This will be a first for us. We're going to start with negative staining, hoping to avoid cutting thins altogether, but if that doesn't work we thought we'd try adhering to poly-lysine coated Thermonox cover slips or embedding in agar before resin embedding. Randy Tindall <TindallR@missouri.edu> 25 Feb 2005

If you have the equipment, the best way to do this is freeze-fixing -- plunge into slush or HPF -- then cryoSEM of the frozen sample. Next best is freeze-fixing followed by proper freeze-drying (if you don't have a real freeze-dryer, the kind that starts at -90 then goes to -60, etc., you can put the samples in a vacuum desiccator with a molecular sieve, pull a good vacuum, and stick them in a -80 freezer. The desiccant acts as a cryopump. Next best after that is routine fix/dehydration/CPD. That won't have any more artifacts than sectioning. The negative staining would be useful if you get it to work. Philip Oshel <peoshel@wisc.edu> 25 Feb 2005

This is a real nitty-gritty ultramicrotomy problem. The diamond knife people are cringing, I'm sure. How about treating the sand/bacteria with HF to soften the sand prior to sectioning? It may even be possible to totally dissolve the sand prior to embedding. Of course, then you won't be able to see how the bacteria are attached to the sand. I assume they are wanting to see the "strands" or cement that the bacteria use to adhere. Better yet, try to convince the people to culture the bacteria on something like silica gel or even a glass side which would be easier to deal with. John J. Bozzola <bozzola@siu.edu> 25 Feb 2005

Don't fight Mother Nature: Better plan is to freeze dry and look at it in the SEM. Alan Boyde made spectacular stereo images of the bacterial jungle on the surface of teeth in about 1973. If you don't have a good SEM, you might try to look at the bacterial film as a whole mount. Even at 100 kV you can see through a lot of tissue once the water is gone. Just put some sand on the grid and grow the bacteria on it, then remove, fix, CPD (perhaps a thin coating of carbon or metal, very thin) and view in the TEM. If you are careful, you should have clumps sticking off the side of the sand grains and some of these may show what you need. And if you have a TEM/SEM, you can have the best of both worlds. James Pawley <jbpawley@wisc.edu> 25 Feb & 26 Feb 2005

ION MILLING - Conductive adhesive

I am in need of a conductive adhesive to attach glass substrate samples to a stub for ion milling in the Gatan PIPS. In a perfect world, I could buy conductive crystalbond, but I am not aware of a product like that. I have been using conductive graphite in IPA by applying 2 drops as small as possible (ideally just the edge of a 3 mm sample), and quickly placing the sample before it dries, which doesn't always work. The milling is greatly improved, but the application method and cleaning after milling leaves much to be desired. Any suggestions? Leslie Thompson <lethomps@us.ibm.com> 24 Feb 2005

Are you making cross sections or plan view samples? Regardless, I don't think that you need conductive glue for glass samples. We have been making cross sections and plan sections on glass for a number of years. Check you alignment of the guns in the PIPS. If you use float glass (normal window glass) you can see the sample fluoresce when the beam hits it. In the limited number of glass samples that I have prepared in a PIPS (about 12 total), I have found that the focus drifted more than I liked on the machine that I was using. In addition, the PIPS guns are too hot for glass and you have to de-tune them to conditions where the rates may be a little slow, i.e. Low kV and low current. It also helps to use the lowest practical milling angle to minimize heating. (However, heating helps the conductivity of glass.) It may be that the sample holder. The best results that I get for cross sections are using a blank piece of silicon on top. The Si helps several ways: it helps in the mechanical thinning process to gauge the thickness of the dimple with glass -just use the color changes that John McCaffrey described and calibrated a number of years ago; it helps dissipate the heat in the ion mill; it helps with charging in the microscope; and it helps align the cross section with the surface parallel to the beam by using the [011] zone axis of Si by tilting in the TEM. Scott D. Walck <walck@ppg.com> 25 Feb 2005

TEM – Potassium permanganate as a section stain

Has anyone ever used Potassium permanganate as a section stain? Apparently it has been shown to improve the contrast in certain types of cells in the artery wall and I wondered what might be going on in these cells that their contrast should be so improved with this stain. Has anyone a method for the staining? Ursula Potter <u.j.potter@bath.ac.uk> 28 Feb 2005

FYI, making up stains in 9% isobutanol seems to improve contrast - presumably it allows greater stain penetration. It seems to work for both light and electron microscopy. It seems also to improve immunogold label without increasing background. Roberts, IM (2002) Iso-butanol saturated water: a simple procedure for increasing staining intensity of resin sections for light and electron microscopy. J. Microscopy 207:97-107. Rosemary White <rosemary.white@csiro.au> 01 Mar 2005

Several years ago I compared potassium permanganate post-section staining with barium post-section staining, I found barium staining to be much cleaner to use and it did an excellent job (superior to potassium permanganate in my hands) on plant and fungal walls and protein/carbohydrate matrices. I can't put my hands on the procedure immediately but here is the reference: Hoch, H. C. 1977. Use of permanganate to increase the electron opacity of fungal walls. Mycologia 69:1209-2113. Richard Edelmann <edelmare@MUOhio.edu> 01 Mar 2005

When I started here in 1989 potassium permanganate was employed instead of uranyl acetate for safety reasons. I formed the impression from the literature that uranyl acetate would suit us better and discontinued use of permanganate. We used it as either: 1% (aq) or 1% in phosphate buffer pH 6.5, it is supposed to be good for membranes, but I did not do a rigorous comparison. Hayat (1989) (3rd Ed.) Principles and Techniques of Electron Microscopy. Biological Applications covers it (pp280-281). Hayat describes using it as part of a triple stain with permanganate and lead citrate. David Patton <David.Patton@uwe.ac.uk> 01 Mar 2005

I have played a lot with potassium permanganate in various conditions, and most likely when used as a stain, one is getting a precipitate of manganese dioxide ($Mn0_2$) formed around the most reactive and reducing components of the cell. I'm not a bio man myself, but I would suggest that the main thing to play around with is the pH of your staining solution, perhaps using appropriate buffers. What buffers do you normally use?

Citrate, for example, would be easily oxidized by the permanganate, which would shed MnO_2 everywhere. Robert H. Olley <hinmeigeng@hotmail.com> 03 Mar 2005

TEM – Pros and cons of in-column and post-column energy filter

I would like to hear from you who have experiences with in-column and post-column energy filters in a TEM. Jian-Guo Zheng <j-zheng3@northwestern.edu> 05 Mar 2005

I'm sure there are better experts than me out there, but I just have a few comments from my own experience. Post column: Pros: can be attached to almost any TEM. Can be fairly easy to operate with proprietary software from the manufacturer. Con: Can have large post-column magnification - restricts field of view, cannot do low magnification work (but see next two points) - partially fixed in newer models - can be corrected by demagnifying image in projection lenses of microscope, when this is set up correctly, easier on some microscopes than others. Integration in/with microscope software - may vary with microscope manufacturer - relies on good relations between the two manufacturers. Cannot use with film, must use CCD. In-column Pros: Can photograph image to film or CCD as you choose. What you see is what you get from fluorescent screen to film/CCD. No extra magnification - can do high or low magnification, as you will (easier then for energy filtered diffraction). Should integrate perfectly into microscope software since microscope and filter from same manufacturer. Cons: Only available from some manufacturers. Can be rather expensive. Limited choice of high voltages. Depends if you like the manufacturers proprietary software. Ian MacLaren <i.maclaren@physics.gla.ac.uk> 07 Mar 2005

LM - Prussian Blue iron stain

Is it possible to perform a Prussian Blue iron stain on LR White sections for light microscopy. I would very much appreciate a detailed protocol. Alida Koorts akoorts@medic.up.ac.za 11 Mar 2005

Since immunostaining reagents can penetrate LR White, I would think that the reagents for Prussian blue would as well. This is a very simple "stain" (a reaction really), any histotechnique text or website will have it. It is very important that the HCl-potassium ferrocyanide solution be mixed immediately before use. Geoff McAuliffe <mcauliff@umdnj.edu> 11 Mar 2005

Has anyone observed that immunoreagents actually penetrate into LR-Qhite sections? It seems so unlikely to me. Antibodies being 8-12nm in size, not even taking into account the size increase caused by any marker attached..... Wouldn't an ultrathin section, that is anything between let's say 40 and 70 nm thick, look like a slice of Gouda Cheese instead of a smooth and even layer? Depending on fixation and maybe other treatments, penetration into even fully hydrated ultrathin cryosections is usually limited to antibodies and the very small gold particle immunoconjugates, larger ones not getting deeper than the surface. York-Dieter Stierhof from Tübingen, Germany, did some beautiful work on this topic. I am really interested in this and would appreciate to hear of anyone's experience. Jan Leunissen <leunissen@aurion.nl> 12 Mar 2005

MICROTOMY - Sections not adhering

A colleague of mine was asked to section and stain Axolotl testis (amphibian testis can be cut such that all stages of spermatogenesis are present in the section). She was given 2 sets of tissue: ones fixed in Bouin's (formalin, glacial acetic acid and picric acid), the others were fixed in Flemming's solution (chromic acid, glacial acetic acid and osmium tetroxide). The sections from the Bouin's fixed tissue cut nicely but no matter what she has tried, they slip off of the slides either during the de-paraffinization or re-hydration steps. She has tried super-frost Plus slides, gelatin-chrom alum, and even gelatin-chrome alum plus poly-l-lysine. She lets the sections dry on the slide warming tray for a minimum of 24 hours. These slides are desired for the medical students here, so she needs to cut and stain 120-150 slides. Very hard to do if you can't get the sections to stick! She is about to try the Flemming's-fixed tissue, but we just can't figure out what's going on with the Bouin's-fixed stuff. Lee Cohen-Gould<lcgould@med.cornell.edu> 16 Mar 2005

Years ago I did extensive serial sectioning of Bouin's-fixed, paraffin-embedded tissues. I had very good results (i.e., sections did not fall off slides during processing) using a light coating of albumen fixative on plain, alcohol-cleaned microslides. Jan Factor <jfactor@ns.purchase.edu> 17 Mar 2005

Bouin's fixation makes the tissue very hard, if I remember correctly. This could make a difference in the tissue's adherence to the slides. As to making the tissue stick - I have a technique that I learned many years back when I was attempting to make LM serial sections of lung tissue for a reconstruction study. I had tried every subbing solution and slide cleaning technique that I could find in all the histology books that I had. I also tried slides from 3 or 4 different companies. Many sections would disappear at different times during the staining procedure. Then I remembered Dorothy, a very experienced histology tech who worked in the same lab as I had several years before. I had never seen her lose a paraffin section! I went to her lab to observe just what she did differently than I did from the time she put her coat on the hook until she took a pinch of gelatin out of a rather large brown bottle and she just sprinkled it on top of the cold water that was in the water bath that would be used to float the sections when it had warmed to the proper temperature. That was it! A pinch of gelatin - no subbing - no washing of the slides - and I did not loose another section. I asked Dorothy where she had learned the trick, but she could not remember. She thought that everyone knew about the gelatin in the water bath. I guess that it is similar to learning electron microscopy techniques from a book without spending some

time watching or talking with someone who has things working. Hoping it works for those in need! Pat Connelly <psconnel@pop.sas.upenn.edu> 17 Mar 2005

Sputter Coating

We are doing lots of sputter coating of various metals these days for a group of electrical engineering department clients. Up until recently, the platinum and titanium coatings that have been applied have been shiny and mirror-like, as one would expect. Just recently, however, the target has been showing a decidedly scaled, rough surface and the coating on the silicon substrate shows microcracks. We are using a target that is 0.5 mm (0.02") thick, which is thicker than the normal target for this machine, but it worked fine when installed. The coater is being run very hard----90 mA for repeated runs of 4 minutes each, often many times a day. It is Peltier-cooled, but the target area and photoresist on the sample is heating up, although the heat build-up has not been measured. The coater is turbo-pumped. My questions are: what could be causing the scaling on the target itself and the microcracks on the surface being coated, and will a sputter coating designed for general EM lab use stand up to this kind of hard use over time? Randy Tindall <TindallR@missouri.edu> 25 Mar 2005

I'm using a Denton Desk II coater with Au/Pd or Pt. This is for coating semiconductors and other specimens. I coat at 20 mA for between 30 seconds and 50 seconds depending on intended SEM KV. However, 90 mA seems rather high to me. Poor vacuum (> 125mTorr) and high current will produce a bad coating. It is best, in my opinion, to go for longer time at low current and have a good vacuum (85-95 mTorr). This works in my SEM from 100 V to 8 KV at 200 pA probe current or less without a problem. I don't see why the coater needs to run for 4 minutes. Gary Gaugler <gary@gaugler.com> 26 Mar 2005

Do you have an application for a sputter coater or a laboratory heater? I am sure you know that most SEM sputter coaters are made to run in the 5 to 30 mA range for normal coating, but for super fine grain, the Chromium coaters may use in excess of 100 mA. The crunch is that the latter technique only requires coating for just a few seconds. In the development of sputter coaters, many of us played around with trying to reduce the heating effect of the system on the specimen; we melted some plastics when trying too hard! In the time periods and currents that you that you are using there must be a good deal of heat being placed on the specimen and target heating must also be taking place. Thus, transformations related to the specimen and to the target should not be unexpected. The thicker target, provided it is making good contact, should not make any difference. Steve Chapman <protrain@emcourses.com> 28 Mar 2005

Many thanks for the replies to my original question on the scaling Pt target and microcracks in the specimen coating. The consensus seems to be that overheating is the problem. I wasn't clear in my original posting about why we are doing this. These specimens are not being coated for SEM viewing, but because we have the only sputter coater on campus and the particular lab doing all this work requires an extremely heavy layer of whatever metal they are using---which may be Ni, Pt, or Ta. They are the ones driving the 90 mA and repeated 4 minute cycles (because 4 minutes is the longest time we can program into the coater and there is no manual on/off, if we want a 16 minute coat, we have to do 4 runs). This is very heavy use for a coater designed for general EM use, rather than industrial strength use, and I am concerned that it's beyond the design parameters of the instrument and may leave us with a crippled coater (and a crippled lab). We don't have the funds readily available to replace this machine if it dies. Normally we run this instrument between 10 and 20 mA for 15 seconds to 3 minutes for SEM coating. Randy Tindall <TindallR@missouri.edu> 28 Mar 2005

SPUTTER COATING – Determination of current needed

How do you decide what sputter current is needed for a certain SEM KeV? Does it apply to an FESEM? Another question is, some recommend using carbon as a coating but when I recently used it as a coating, I found that my sample is coated with "furry stuff" which I believe is due to the carbon, under FESEM. If I really need to use carbon coating, what can I do to reduce such "furry stuff"? Tay Yee Yan <one_twinklestar@yahoo.com.sg> 26 Mar 2005

The main reasons for changing sputter coating parameters will relate to the specimen form and to the resolution required. Should the specimen surface be extremely complex (hills valleys and holes) multiple coats, and even changing the angle of the specimen in relation to the target, may be the only methods to ensure a good coating for say 15kV. However, if you are coating to obtain the highest resolution images, greater care must be taken to not overcoat, and multiple coatings should not be used. Remember multiple coating does make the metal structure more pronounced! Some of our ideas on coating will be found under Hints and Tips on www.emcourses.com. Steve Chapman <protrain@emcourses.com> 28 Mar 2005

Normally, according to my information, the lower the coating current, the finer-grained the coating should be. If this is correct, then if you need a rather thick, but fine-grained coating for high-magnification, you should use a low current (5-10 mA) for a long time (several minutes?). If you are only looking at low magnification, than a higher current and shorter time should work. Generally speaking, a lower SEM accelerating voltage should theoretically require a thinner coating, since it is less likely to cause charging. In my experience, however, this is not always as straightforward as one would expect and requires experimentation with each sample. For critical samples, I always start with the thinnest coating I think might do the job, and then add more coating as necessary. You can always add more, but removing it once it is applied is another story. Coating current is also determined by the metal being used for coating, since each one has different properties relative to the sputtering process. Unfortunately, I don't know of a good source where you can fine this information in one place, but it must exist somewhere. Regarding the "furry" carbon coating, this sounds like something is going wrong. I have never seen this, although sometimes when evaporating carbon with the current too high you can get "chunks" of carbon flying off the braid or rod and showing up on the sample. The "furriness" is something else, though. Not sure about that one. Randy Tindall <tindallt@missouri.edu> 28 Mar 2005

EDS – re-pump protocol

Having just found out the hard way that my EDS detector wouldn't make it through a 3- day weekend without a LN_2 top up, I guess it's now my turn to learn how to re-pump a detector. Electrically it's still fine, resolution is what it was last week, but the LN_2 now is boiling gently but continuously, so I suppose the getter has released whatever it had collected, further degrading the Dewar vacuum, which had been on a downhill path since it's last factory pump 'n' bake a couple of years ago. As my dealings with the factory have always been pretty labored and unsatisfactory (it should have lasted more than a couple of years after a factory pump 'n' bake, in my opinion), this seems like the ideal time to use the vacuum port adaptor which I bought from them. Does anyone know what I need to get the Dewar vacuum down (or up) to for adequate performance? I plan to use the SEM vacuum, via an adaptor I've had made for the sample insertion port, but of course the adaptors, valves and tubing do compromise the vacuum somewhat. I am toying with the idea of putting a high-quality shutoff valve at the detector instead of the factory port, with a tube permanently leading to the SEM chamber, so that I can re- evacuate the Dewar at will by simply opening the valve. Any comments on that idea? For you lucky people in Europe or the USA it's probably no big deal to send an EDS detector off to a factory

service center for a quick pump 'n' bake, but if you look at a map or globe, you can see what a major logistical exercise it is for me. Ritchie Sims <r.sims@auckland.ac.nz> 29 Mar 2005

When I re-pumped the detector on a TEM, I replaced the back panel that came with the detector with one that had a high quality shut-off valve, and I created a branch line off the column vacuum line. The column vacuum was ~10^{-6} torr at the ion gauge, but I didn't measure it nearer the detector. This worked for many years to restore the resolution of the detector to its specified value. I had to remove the LN_2 from the Dewar, and, of course, make sure that the bias stayed off while the detector was warm. For a more complete regeneration, I might have tried heating the Dewar by filling it with boiling water, but this would have been awkward, and, since I got good resolution without it, I never heated the Dewar beyond room temperature. I should also say that this was a SiLi with 148 eV specified resolution, so my method might not be satisfactory for a newer type of detector. BTW, even in the USA it was a big deal to dismount and ship the detector for service, and one time the shipping company damaged the detector on its return voyage. Bill Tivol <tivol@caltech.edu> 29 Mar 2005

I have done this on two detectors. I used a small vacuum system with an air cooled diffusion pump and a simple LN_2 cold trap. The desiccant in the Dewar is usually located around the neck, so I applied a heat gun to that area while I was pumping. Be sure the Dewar is empty and has warmed thoroughly before you begin, or you will be fighting a losing battle. Also, be sure that the outside of the detector window is at atmospheric pressure before you start the warm up or there is a danger of rupturing the window as the condensed matter in the Dewar vaporizes; the windows can't take much reverse pressure. (UTW'S are probably the only concern here, Be-windows are probably not going to be ruptured by any internal pressure likely to be attained during warm up even if looking at column vacuum.) Your plan to use the SEM vacuum, via an adaptor you have made for the sample insertion port should work, but remember that whatever you pull out of the detector Dewar (mostly water) is going to wind up in your microscope vacuum system. Putting a cold trap in your pumping line would take care of that, and improve the pumping. Your idea of putting a high-quality shutoff valve at the detector instead of the factory port should work, but note the caveats above. Andy Buechele <andrewb@vsl.cua.edu> 30 Mar 2005

Microtomy - Picking up ultrathin cryosections

I have a question about picking up ultrathin cryosections. I have noticed that when I am using very lightly fixed tissue, many of the sections look like the tissue is stretching apart, leaving small holes throughout. I thought at first that this was just due to lack of fixation; however, I could find an occasional section/grid that was amazingly better. This indicated that the pickup is a major factor. I have tried sucrose vs. methylcellulose/sucrose vs. methylcellulose/sucrose/UA and find that it is still just in the pickup. Do you have some advice on how to pickup the sections to avoid this type of artifact. Robert Underwood <underwoo@u.washington.edu> 07 Apr 2005

If it makes you feel better, most of us are struggling with the very same problem! So I am very interested in the responses you are going to receive to your question. I used to think that when picking up, either with sucrose alone or sucrose/methyl cellulose, one had to be extremely quick, and the sections had to magically lift up onto the still liquid drop in which it would disappear as if they were dissolving in the sucrose. But talking to others since then, I have come to realize that being too fast can actually harm the sections, by over-stretching them and making holes and tears appear. One person told me that she waits until the rim of the loop of sucrose/methylcellulose just starts freezing (a white ring appearing at the periphery of the droplet) before picking up the sections. Given the speed at which sucrose/methylcellulose freezes, this often means that by the time the sections have been picked up and the loop retrieved from the chamber, the whole drop is now frozen. You just have to wait for it to thaw again, and then transfer the sections on the grid. But this technique hasn't worked too well in my hands so far–I get too many folds. There are too many factors that are involved here: size of the loop (we use a very small one - smaller diameter than a grid), percentage of sucrose and methyl cellulose (I use 50/50), temperature of the chamber (-108°C as opposed to -120°C), the use of gelatin to embed samples, and. of course: the fixation protocol. But anyway, you should maybe give this a try and see if waiting a few more sections during pick up, so that the surface of your drop is close to freezing point, might help prevent some of the tearing. Marc Pypaert <marc.pypaert@yale.edu> 07 Apr 2005

1) It would be better to use tissues fixed with a little percentage of glutaraldehyde (0.1-0.2%). This will not kill much of antigenic epitopes but will greatly improve the ultrastructural stability of the sample. You can find an exact reference in the papers of P. J. Peters' group in Amsterdam or H. Geuze's group in Utrecht. 2) In my hands, the best solution for picking up is methylcellulose/sucrose. Sucrose alone has a very big surface tension, which will eventually destroy sensitive structures such as the Golgi complex. Addition of uranyl acetate to the pick up solution can improve the ultrastructure but can reduce labeling. You can try to add low concentration of tannic acid (less than 0.1%) in solution. Sometimes it helps but be aware of the effects on the epitopes for labeling. 3) The other thing to consider is the timing of your pick up. It is very critical to pick up at the moment when the solution is just on the verge of freezing. If you pick up sections sooner or later then you will probably end up with a horrible ultrastructure. It is very hard to explain how to determine the right moment. I think it will be better that you just try several settings because our instruments for pick up could be different from yours (loop diameter, wire thickness etc.) and it will affect the timing. Maybe this movie from P. Peters' lab will help: http://129.112.18.40/cryomovies/ Aleksandr Mironov <aleksandr.mironov@manchester.ac.uk> 08 Apr 2005

You have come across an interesting observation with cryosections. As you know cryosections are not embedded in a continuous, polymerized plastic sheet, as are resin-embedded sections. The only physical bonds holding the structure together are those protein interactions that may remain after the tissue has been treated, and cross-linking bonds formed by the aldehyde fixation. Of course, less fixation will mean fewer cross-linking bonds to hold the sections together. To cut the story short, the variability you are seeing in the section morphology is most probably due to surface tension effects on the surface of the pick-up drop. Reduce the surface tension and you will improve the section morphology. Warm pick-up solutions will spread the sections more than frozen drops of pick-up solution. More methyl cellulose in the sucrose appears also to reduce the surface tension. You could also try adding gelatin to the sucrose to lower the surface tension. Or, you could also experiment with the time taken to collect the sections from the knife. You may find that as you get better at picking them up, the morphology will get worse. If this is so, slow down the process and give the pick-up solution time to cool down. Once you get a good result, stick with the protocol. Alternatively, you could add a very small amount of glutaraldehyde to your fixative. This may toughen up the sections enough to improve the morphology. Finally, make sure that you are not drying the sections during the labeling process. If you are in the habit of taking off excess buffer before you float grids on antibody you may be drying them enough to cause morphology changes. Thin sections are very sensitive to drying. I am sure that you are aware that you can take your frozen, cryoprotected blocks and warm them up again for embedding in epoxy resin. This has always been a good way to convince people that poor fixation or freezing is not the reason for poor morphology in cryoprotected frozen material. You can also take the frozen blocks of sucrose-infiltrated material and freeze substitute in cold methanol. This is also a good way of checking the morphology if the material is then embedded in epoxy resin. Paul Webster <pwebster@hei.org> 07 Apr 2005