NETNOTES

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

LM - extinction angle under polarized light

I would like to know how to determine an extinction angle under polarized light. Jeremie Compan <j.compan@fzjuelich.de> 30 Aug 2005

Between crossed polars, when the optical axis of a birefringent element (a crystal domain) in your sample is parallel to either the analyzer or the polarizer, that element will not affect the state of polarization and the field will be dark, as if there were no sample there at all. So to measure this, you need a rotatable stage and you need a way to define the axis of your sample to the axis of the analyzer and polarizer. For example, if you mount an edge of your sample parallel to the microscope slide. If both polarizer and analyzer can rotate on you microscope, then you also need to make sure that you know what direction their transmission axes are relative to the stage. With these, you can rotate the stage, say in 5 degree increments and look at (record) the intensity of your sample. When it is maximally dark, the sample is at extinction, and the angle between the reference direction on your sample and the transmission axis of the polarizer is the angle of extinction. Tobias Baskin <baskin@bio.umass.edu> 30Aug 2005

LM – *en bloc* stains for light microscopy

I was just wondering if anyone had ever happened upon a reliable method for en bloc staining insect neural tissue in araldite embedded specimens. I have tried a few things so far, including, toluidine blue, ferricyanide and p-phenylaminediamine. While these seem to help, none of them give satisfactory contrast without post-staining the sections with toluidine blue. Does anyone know of something that will stain membrane structure well and can be used en bloc? The specimens do not have to be embedded in Araldite...just something that will cut 1-2 µm sections. Richard Berry <rberry@rsbs.anu.edu.au> 07 Sep 2005

The usual *en bloc* stains used for TEM work probably won't give you enough contrast for LM unless you try phase contrast or Nomarski illumination. p-Phenylenediamine will reduce the osmium in the tissue and increase contrast, but is not a stain in the usual sense. If you used ferricyanide-osmium and *en bloc* staining with uranyl acetate you might get enough contrast for your needs. Only you can make that judgment. Toluidine blue is leached out of tissue very rapidly in the ascending ethanols used for dehydration. Try cresyl violet or thionin instead or dehydrate in acetone instead of alcohols. I don't think either will show up much in the EM though. Geoff McAuliffe <mcauliff@umdnj.edu> 07 Sep 2005

TEM - temperature of 100 kV beam

A colleague, who is experiencing specimen damage in the TEM, inquired if anyone knew the temperature generated on the specimen by the electron beam?

Unfortunately, I don't have one of my most useful references with me to get an essential parameter, but the general method of doing this calculation is to use the stopping power of the material to determine the energy deposited into the specimen, and then calculate the temperature increase from the heat capacity and account for conduction and radiation of heat. At steady state, the heat in, which is the stopping power, dE/dx, in units of joules/meter-electron times the electron beam current in electrons/sec times the specimen thickness, must equal the sum of conduction (assume a disk at one temperature surrounded by an infinite amount of the material at ambient temperature, plug in the conductivity, the temperature difference, and the area across which the heat is conducted, which is the circumference of the beam spot times the thickness of the specimen) and radiation, which is equal to T^4 (on the Kelvin scale) times the area of the beam times the Stephan-Boltzmann constant. This gives an upper limit to the heat deposited in the specimen, since not all the energy loss is converted to heat. Some is carried away by bremsstrahlung, secondary electrons, etc. The stopping power can be set equal to the sum of stopping powers for each element in the specimen times their fractions. The effect of the grid can probably be ignored (unless the illuminated part of the specimen is over a grid bar, which would greatly increase heat conduction). The parameters necessary to do the calculation are the stopping powers, the heat conductivity, and the geometry of the specimen. Then one can set heat in = heat out and solve for the temperature for which the equation holds. Bill Tivol <tivol@caltech.edu> 09 Sep 2005

The temperature in the sample due to the energy being deposited in it is very dependent on the thickness of the sample. At 120 keV, if I did not deposit a sufficient layer of carbon on glass cross sections, the glass would soften under the beam. 100 keV would be worse. When I used a 200 keV machine, the problem essentially went away. For 100 keV, to avoid the problem, the illuminated area must be very thin. One of the other things that I did that seemed to help with glass samples was to use a piece of Si as the mate to the cross section in the stack. The Si seems to take more of the heat away from the sample. Either that or it supplied a temperature insensitive portion of the total sample to prevent the sagging. Scott D. Walck <walck@southbaytech.com> 10 Sep 2005

I don't have the answer to this question but when I was renovating my TEM I was playing around with a sample of actinolite asbestos. When we increased the power of the beam we easily melted the fibers. These were thick fibers; don't think any of them were electron transparent so the maximum amount of energy was absorbed by the specimen. If my memory doesn't fail me we used 100kV and no apertures. As the TEM wasn't fully operational, I have no idea of the size of the fibers. Göran Axelsson <axelsson@acc.umu.se> 10 Sep 2005

I think Bill Tivol's outline of specimen heating is fine, and a very worthwhile exercise. One of the consequences of the T^4 power for radiated heat is that you don't get much heat loss by radiation below about 200 °C (lots of hand waving and caveats here, this is a very rough number). However, I'd like to add to the emphasis on the importance of a good heat sink. I know from experience that I can fry a liftout FIB section of InP on a holey carbon grid in a 120 kV TEM (melting point 1060 °C, but starts to decompose around 550 °C). Not very enjoyable if you just spent a few hundred £ getting the thing made. On the other hand, I never have any problems

with conventionally ion milled specimens, which have 20 μm thick InP on a Cu grid on the outside, tapering to the hole in the middle, and even materials like PbSn solders (melting point 183 °C) and Au/Ge multilayers (interdiffusion <100 °C) are fine, if there is a good thermal path to the support grid. From your description of the sample I guess it's a liftout FIB section. As others have pointed out, higher kV will help since the beam specimen interaction is less. Or you'll have no problems with a H-bar section, which has a massive heat sink all around the thin area (but you won't be able to do meaningful x-ray analysis). Or you may have to go back to the old ways of making specimens. Richard Beanland <richard.beanland@bookham.com> 12 Sep 2005

TEM – polymer pre-burn

One of our TEM service reps told me that he's been told that many people who look at polymers in the TEM "pre-burn" their samples under a UV lamp before putting the grids in the column. Is this true? If so, how is it done (for how long, distance from bulb, etc.)? We have a weird "thing" with our TEM that burns a pattern into resin sections and we were discussing ways to pre-burn; chemical stretching doesn't help, and doing it in the column is too slow when there are a lot of samples. If I could do a mass burn I'd be set! Tamara Howard <thoward@unm.edu> 12 Sep 2005

Ultrathin sections of oriented polymers (stained or unstained) often deform when first exposed to the beam. This relaxation can be achieved prior to analysis by low dose exposure to the electron beam for several minutes at low magnification. The objective is to relax the sections and make them physically stable during microscopy. I prefer not to use this procedure because it can cause significant deformation of the sections. A better procedure, in my opinion, is to mount the sections on high quality continuous carbon film grids. Do not use Formvar or Formvar/carbon films since Formvar films are not very clean and can cause problems during imaging and elemental analysis. The sections adhere to the carbon film and will not deform under the beam, thus eliminating artifacts relaxation and/or orientation in images. Image quality is still very good. One must still be careful about beam damage, since this is still a real possibility, carbon film or not. Gary M. Brown <gary.m.brown@exxonmobil.com> 13 Sep 2005

Gary Brown´s advice makes sense. A UV lamp produces oxygen radicals and therefore attacks carbon compounds as a preferred chemical reaction partner. Using a UV lamp is very slow and results depend on distance to the source and radiant heat. Nevertheless, I recommend a plasma treatment before analysis. This could possibly mean cleaning, surface modification and conditioning in one step. Hopefully, you have a plasma instrument in your lab to test. Try air or bottled oxygen for a start. Jost <gala-instrumente@t-online.de> 13 Sep 2005

TEM - PT/C replicas of biological molecules.

I'm having troubles in recovering PT/C replicas on water from freshly cleaved mica. The carbon film remains stuck to the mica instead of floating on the water surface. Is there any trick to overcome

this? Daniel Thomas <daniel.thomas@univ-rennes1.fr> 07 Oct 2005

The time I had problems with removing C films from mica, the humidity was higher than usual. Bill Tivol <tivol@caltech.edu> 07 Oct 2005

We routinely remove Pt/C replicas or shadowed samples of polymers, from mica or glass cover slips, after also coating with vertically coated C, by floating on dilute (ca 1%) HF, taking all the necessary HF use precautions. The sample can be picked up on the grid, and either touched to a paper tissue or re-floated on water to remove any residual HF. If you have any questions, let me know. Phil Geil <geil@uiuc.edu> 07 Oct 2005

If you have used an electrostatic glue (*i.e.* poly-L-lysine) the replica will not separate from the mica without using HF (in my experience). If you do use HF to remove the replica, I would recommend using a glass rod (yes, glass) to remove the replica to distilled water before picking up on a grid. You just touch the rod to the surface of the liquid and "roll" the replica up onto the rod and "unroll" it onto the surface of the water. I feel this minimizes the stress to the replica. This method was mentioned in a paper by John Heuser but unfortunately I don't have the citation for you. I made a platinum/carbon replica on a poly-L-lysine-coated glass coverslip and removed the replica onto full-strength HF just a few hours ago, so I know that works! On an HF-free note, in the book "Negative Staining and Cryoelectron Microscopy", Robin Harris recommends letting carbon films evaporated onto mica sit overnight before attempting to float them off onto water. As a possible means to avoid waiting overnight, Harris suggests placing the mica into a Petri dish on some damp filter papers for a few hours. Perhaps this might be enough to get your replicas to float. Andrew Bowling <abowling@mail.utexas.edu> 08 Oct 2005

TEM - intensity of electron diffraction spots

I am working with calcium carbonate and I am trying to index a single crystal film. Should the relative intensities reported in the JCPDS match the relative intensities in the selected area diffraction pattern? Fairland Fontillas Amos <famos@ufl.edu> 13 Sep 2005

No generally not, there are dynamical diffraction effects in electron diffraction that affect the intensity of spots. This includes double diffraction effects that can allow some classes of forbidden reflections (those forbidden by glide planes and screw axis) to occur. There are innumerable other factors that make the intensities different as well. Use the d-spacings and forget the intensities. In order to index electron diffraction patterns, it really helps to have a complete list of all d-spacings and corresponding symmetrically-equivalent hkls for a given material. Such a list needs to be calculated from the cell parameters using appropriate software. I use some homegrown software to do this. I don't know if there is any commercially available software on the market right now that will do it. Calcium carbonate has the R3barC space group and will have dynamically allowed and dynamically forbidden reflections depending on whether you calculate the d-spacing based on the primitive rhomobohedral or center hexagonal cell. Roy Christoffersen <rcsaic@sbcglobal.net> 13 Sep 2005

The intensity in electron diffraction is subject to many parameters such as double diffraction and thickness of the specimen. So they very often do not match those reported in the JCPDS data base. To index, you should only consider the position of the reflexions. Francois Weill <weill@icmcbbordeaux.cnrs.fr> 14 Sep 2005

There is a basic misunderstanding regarding the comparison of x-ray intensities, as tabulated by the JCPDS, with electron diffraction intensities. The data in the JCPDS (now ICDD) is "powder diffraction data" *i.e.* intensities of diffraction rings arising from randomly oriented small particles or grains where a lot of effort is made to eliminate factors like preferred orientation, etc. When those

x-ray diffraction intensities are compared with electron diffraction intensities arising from a similarly prepared, randomly oriented, non-oriented, TEM specimen, there is considerable agreement between the ICDD data and e-diff data. From nearly 40 years of working with electron and x-ray diffraction patterns, plus several years of membership in the JCPDS/ICDD, I can offer a rule of thumb regarding intensities of e-diff vs. x-ray diffraction data: At the very least, strong reflections are strong reflections and weak are weak. One cannot make an identification of an unknown phase using e-diff ring data, where very strong x-ray reflections are missing from one's pattern, without giving crystal chemical reasons to account for the missing reflections. Likewise, a 5% intensity x-ray line will not suddenly become a 100% intensity e-diff ring pattern reflection. Electron diffraction patterns will sometimes have extra and structure factor forbidden spots (& rings, as appropriate) due to double diffraction and relaxation of structure factor rules due to specimen thickness effects with thin TEM specimens, and sometimes they will exhibit altered intensities due to preferred orientation effects, etc. *Emphasis on "sometimes."* With regard to solving for unknown phases using e-diff data, the extra spots/rings, when present, are either a help or a hindrance as they are most conspicuously present at large d-values, which are the most diagnostic d-values for phase identification. In the rare instances where I had a true unknown specimen in the TEM, and I thought I had a match with a phase in the ICDD x-ray data base, except for the presence of weak, large d-

spacing reflections in the e-diff data, I could sometimes confirm my identification by computing forbidden {100}, {110}, etc. reflections and matching them to my experimental data. Should an image of your specimen show it to be loaded with twins or other features that cause extra reflections, you should make appropriate forbidden reflection calculations early on. The ICDD has products to aid electron diffractionists. The Max-d/Alphabetical Index (now called the Long-d index, I think), and products derived from the Sandia database come to mind. Check www.icdd.com. Fairland: the answer to your question is "Yes, probably." Ron Anderson <randerson20@tampabay.rr.com> 14 Sep 2005

Microscopy & Microanalysis 2006 Meeting

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We are looking for some great ideas for symposia at M&M2007! Although we have suggestions for some of the customary symposia, and have already signed on a small number of organizers, the program is largely open at this time. We would like the majority of the proposals to be submitted by the end of the year. We will start sending out acceptance letters in late December, and by mid-February we expect the program to be mostly filled.

This timetable is considerably accelerated in comparison with previous years, and we now require a description of 150-300 words for each proposed symposia. The description should take the form of those found in the Call for Papers and Expo of past years; it should be an announcement of the symposium and an invitation for contributions. The Program Committee will select symposia based on these descriptions, so that overlap will be minimized and symposia will complement each other to form a coherent overall program.

You need not be a member of MSA, MAS, or IMS to propose a symposium, although we hope that your experience with the M&M meeting will encourage you to join.

Please send your suggestion directly to the Program Chair, or to the M&M2007 Co-Chair of your Society.

Program

Mike Marko, Program Chair (marko@wadsworth.org)

John Henry Scott, Vice Program Chair (johnhenry.scott@nist.gov)

Ed Vincenzi, MAS Program Co-Chair (vicenzi@volcano.si.edu)

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Local Arrangments

Luãlle Gianuzzi, (lgiannuzzi@feico.com)

TEM - Curing epoxy in heat blocks

The question is primarily aimed at the Biological TEM crowd. Has anyone experimented with using a Heat block unit for curing plastic instead of an oven? The reasoning is that a heat block fits in the hood more easily and can be moved out when not in use. The hood space here is extremely limited and the oven typically has been curing Spurr's and Epon type Resins in the prep room, a practice I am not comfortable with (and neither are the Safety folks here). Looking at the specifications for the Dry Heat Blocks some are ± 2°C stability wise in the $200 range with the fanciest models having ± 0.5°C range stability. Thoughts? Experiences? Opinions? Geoff Williams <geoffrey_williams@brown.edu> 16 Sep 2005

Sus Ito, one of the great early TEM guys, once told me how he use to drive from Woods Hole back to Harvard in Boston and he would tape his tissue samples in liquid resin to his engine block so that he could section them upon his arrival! I just wonder how you write the Materials and Methods description of that up! Tom Phillips <phillipst@missouri.edu> 16 Sep 2005

I apologize for stating the obvious, but have you thought about purchasing a more compact oven from one of the EM suppliers. These would at least be capable of holding flat embedding molds as well as capsules at a uniform temperature. We purchased one with external dimensions of 400 mm x 330 mm x 300 mm although there was a more compact version of 335 mm x 305 mm x 230 mm in the UK. I certainly agree with your concerns about polymerizing Spurrs/any epoxides resins in the lab. I stopped doing it over 20 years ago. One other possibility would be to find a well vented outhouse/shed if your safety people would be happier with that. Malcolm Haswell <malcolm.haswell@sunderland.ac.uk> 16 Sep 2005

You ask a question which is actually quite interesting and applicable on a number of levels. First, of course, is would a block work? I will assume you mean something like the sand blocks we use in the other (one of my 2 non-EM homes) lab. As you note, there would be limitations, but the temperature control on all sand blocks I've ever worked with is a lot better than any oven I've used. You just have to take the time to set the temperature. As Tom Phillips noted, there are plenty of examples of alternative systems for providing the polymerization temperatures, so to join the chorus, I see no reason why not. And as far as using the car engine, I even remember a book about cooking while you drive which came out in the 70's, engine block pot roast and all. The interesting part of the question as I see it is: why would you want to put the block into a hood. Do you mean a fume hood, so you could use the block for an intermediate step in infiltration, with low temperature heating to assist in driving off transitional solvents? If so, it is an interesting idea, one worth some thought. Could be quite useful. Alternatively, do you mean a laminar flow hood for containment at a BSL2 level or higher? This is the most interesting potential application. Those of us who work with emerging diseases groups or with infectious pathogens at the BSL3 or BSL4 levels are confronted with a number of safety issues that this concept could address. Some of my collaborators work at higher levels. They fix with a modification of Karnovsky's fix (we use 2% paraformaldehyde/2% glutaraldehyde in cacodylate). But their safety people will not let them take the material out of containment for further processing until the samples have been in the fixative for 30 days. I feel this may lead to some deterioration

of the samples, and that there is no evidence that the pathogens are not inactivated in hours, and so do not like this. But safety people will not let them do otherwise. There are too many other things to do these days for me to give up 4-6 hour blocks to go work in containment, so I'm not too keen on taking spacesuit training it would be fun and really interesting, but there is just not enough time. Your question raises a lot of ideas which can address the problems of processing, permit good technique in processing, and meet the demands on the biosafety level. Paul R. Hazelton <paul_hazelton@umanitoba.ca> 16 Sep 2005

The reason Geoff wants to put the heat block into the hood is probably the same reason why I would never polymerize resin outside the hood: fumes released during this process must be extremely toxic. And as far as finding a very small oven that would easily fit into a hood, this is not so easy. We bought the smallest we could find, but it is still more than 1 foot wide and deep, which takes too much room. We, in fact, have been using a second, smaller oven, that is really just a box fitted on top of a hot plate! It is very similar in design to the heat block Geoff wants to use. The temperature inside is very constant, the only drawback being that you need to calibrate the temperature control button. This only has to be done once. I think the idea of using heat blocks is very elegant, and I don't see why it shouldn't work with Eppendorf tubes or even BEEM capsules. For flat embedding in molds, however, I don't see how you would manage it. Good thread, Geoff. My guess is that if nobody has tried this before, you should give it a shot and report to the list how it went! Marc Pypaert <marc.pypaert@yale.edu> 16 Sep 2005

When our current was being designed, I specified one bench with an awning hood to vent fumes from solvent dishes, paraffin baths and embedding ovens. The 30" deep bench has a 3" gap between the backsplash and wall. The ovens are beneath the bench and their fumes are pulled up behind the backsplash. The stainless steel awning is 28" above the bench with a Plexiglas skirt extending 4" below the awning to increase face flow. A heat block is likely much cheaper than remodeling one's lab. Glen MacDonald <glenmac@u.washington.edu> 16 Sep 2005

It appears to me that the problem to be solved is to find a way to polymerize resin but protect staff personnel from the fumes that are given off during heating, yet find a space saving solution. A simple approach would be to only use sealed molds such as Eppendorf tubes and BEEM capsules. The oven can thus be placed anywhere in the lab. However, I do know that many people prefer the ease and lower cost of reusable flat molds. I have been experimenting with polymerizing resins using a microwave oven with variable results. However, with some formulations of resin it is possible to polymerize in a flat mold in less than 2 hr. I have used a laboratory grade microwave connected to an exhaust duct (which is very convenient) and also with a regular kitchen microwave. The end result is, if the resin is going to polymerize, the process will work in either machine. Not all resin recipes work and even fewer of them can be polymerized in a flat mold. It may be worth giving this approach a try. The best part of using the microwave is that the exhaust duct allows us to place the machine far from the chemical extractor hoods. Connecting a regular oven to an exhaust duct may also be a reasonably effective solution. Our convection oven has a wide duct in the top to which metal ducting, similar to that connected to household clothes dryers, can be connected. Paul Webster <pwebster@hei.org> 16 Sep 2005

I am wondering reading these emails, does anyone really know how noxious the fumes are that are released from an embedding oven? We too are pressed for space in the hood so I've moved the embedding oven into a not heavily populated corner in a large lab. We polymerize LR White and epoxy resin blocks. 5-10 blocks worth, maybe once a week at most. I can't smell any fumes in there (unlike the mercaptoethanol or ETT the molecular biologists regularly use). Am I exposing a room full of people to something bad? Is it the quantity of blocks that one needs to worry about? The microwave is great for processing (hooked up to the fume hood via the duct) but I don't want to baby sit it for 2 hours to get a perfectly hardened epoxy flat block. JoAnn Buchanan <redhair@stanford.edu> 16 Sep 2005

Please be very wary of venting the oven curing the resin blocks anywhere other than in the fume hood. We have one lab technician who is unable to work with EM resins at all due to extreme sensitization several years ago. Recently, one worker decided to cure some resin blocks in another lab in an oven outside of the fume hood; within hours her eyes had

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swollen so badly that she could not see. She was only working in the vicinity, but in a big, well ventilated open plan lab area. Once sensitization has occurred, it is always potentially very serious, for anyone exposed to the fumes and not necessarily technical staff working in EM. Incidentally, resin dust makes my fingers swell within minutes of handling/sawing small blocks, even wearing gloves, so I avoid this practice now. K. Venner <K.venner@ion.ucl.ac.uk> 19 Sep 2005

Discussing the importance of safe handling of toxic vapors, liquids, solids (powders and dust), is not worth anything unless it is followed by actual safe laboratory practices and equipment; this is critical to the longevity of students and faculty. Sorry to sound like a stuck CD but toxic vapors may do more that cause rapid sensitization or neurological damage. Think long term and that what we handle as graduate students may hit us with devastating diseases 30 or 40 years later. I know from experience that vapors can do more that cause neurological damage (been there, have that) but also damage the lungs necessitating a lung transplant, if you are incredibly lucky as I have been. A fellow graduate student was not so lucky with another disease. Were these caused by EM graduate work and careers? No one knows (but it is suspected by medical experts). Do you want to take the chance? Damian Neuberger <neuberger1234@comcast.net> 19 Sep 2005

We were faced with the same type of space limitation in our hood. What we've done is vented our oven to the fume hood's duct via the thermometer hole (we removed the thermometer adaptor which left a 3" hole) on the top using 4" diameter metal ductwork. There is a damper control in the line allowing us to adjust the rate of exhaust. Voila! No new hood needed, more room in the hood, and no potentially noxious fumes in the lab. FYI, the vapors from curing epoxies are noxious, and can cause susceptible people to develop mucous membrane irritations and swelling. Richard Harris <rjharris@uwo.ca> 19 Sep 2005

Venting the oven to the current hood was the first consideration. We do have a very nice oven, it works well. But there is little to no counter space near the hood and to tie the oven exhaust into the existing hood in the lab would first require a Facilities Management Feasibility study, and more than likely a few thousand dollars in modification, mostly because it has to be certified to draw a specific amount of air and also to not affect the functioning of the rest of the hood. I want to try either one of the economical units on the market or ideally borrow one from a lab for the trial run. And sealed capsules are not vapor free. For a brief idea on the toxicity of the chemicals in any of the most common epoxy/resins read the warnings on the bottles. It is how I always started the lab portion in TEM when we got to mixing the Spurr's. Nothing like getting the attention of students by talking about central nervous system toxins. Geoff Williams <Geoffrey_Williams@brown.edu> 19 Sep 2005 16:22:50 0500

TEM - preparing coated grids

We have been preparing coated grids for TEM for a long time, with relatively little trouble. We follow the protocol for preparation of Formvar coated grids from the Bozzola and Russel text, Electron Microscopy. I recently tried to prepare grids with very little success. I was using fresh Formvar solution thinking that our old solution might be the problem. I tried several different brands of slides as well, all leading to no success. Have any of you out there experienced similar problems? Dave Fulton <fulton.2@osu.edu> 30 Sep 2005

We use 0.81% Formvar in chloroform with good success. We dissolve the Formvar directly into a new chloroform bottle. This avoids any contamination from lab glassware and humidity in the air. Glass slides are washed in acetone before use. They are coated with Formvar using a film casting device. Before dipping the slides into a water tank to detach the film, my technician scores the film at the edges of the slide with a razor blade and then breathes gently on the slide. This apparently helps the film to come off in the water. One problem we have sometimes had is the film coming off the grids later on. My technicians have recently found out that this only happens when they use grids that have been stored for various periods of time after washing and drying. So we recommend washing the grids directly before use (at least for nickel). Marc Pypaert <marc.pypaert@yale.edu> 30 Sep 2005

There are some subtle environmental conditions that can affect your success rate. You don't say which step is failing, so I can't be too specific, but the following have adversely affected me: humidity, cleanliness of the slides (more is not necessarily better), temperature of the water bath used to float off the Formvar, thickness of the Formvar, freshness of the solvents especially $CHCl_3$, quality of the razor blade used to scrape the edges of the slide, and the type of grease used to facilitate separation of Formvar from the slide. I have even found that nose grease from different people can have different properties, so if there are new people in your lab, and they are using nose grease, have them let others donate to see if that changes things. I have found that Apiezon L makes a suitable grease, and I have floated films off using 0.25 g of Alconox in 1 L H_2O when I have used Apiezon. Bill Tivol <tivol@caltech.edu> 30 Sep 2005

I've always used dichloroethane for Formvar. What advantage would there be to chloroform? How well does it work? But in 35 years I've never heard of using Apiezon to help get the Formvar off the slide for coated grids. Enlighten me. It could be a good trick to know. Paul Hazelton <paul_hazelton@umanitoba.ca> 30 Sep 2005

Our lab in Albany had a recipe that called for dissolving Formvar in equal parts chloroform and acetone. I have also used the pre-made solution of Formvar in dichloroethane, and I haven't found any particular difference. I don't know which of the two chloro-carbons is more stable. I suspect DCE but that would be the preferred solvent. I tried two methods with Apiezon, and had about equal success. I should say that I was making holey Formvar to end up with holey carbon films, and I was having absolutely no luck getting the films to separate from the glass slides until I tried the Apiezon. The lab's procedure called for taking pre-cleaned slides and rinsing them in ethanol, then wiping them dry. They were then made more or less controllably less clean by applying a thin coat of grease, but when the oil from my skin proved to be deficient, and that from other members of the lab worked, I decided to try something more well-defined. One trick for aligning the holes in holey carbon is to apply the grease to the slide, then rub with one's finger and thumb along the long direction of the slide. The ridged residual grease will cause the holes (made by glycerol droplets) to line up along the ridges. When I first tried Apiezon, I just used

the same technique as with skin oil, using as small an amount of Apiezon as I could. Later I thought to make the process even more well defined by dissolving the Apiezon, and a relatively high boiling point petroleum ether (hexanes or heptanes most likely) proved to be a good solvent. For this procedure, I dissolved a measured amount of Apiezon in 50 ml of solvent, then dipped the slide and let it drain in the same manner as used for dipping in Formvar solution. In order to try to avoid any residual grease remaining on the underside of the Formvar, I floated the film off with a dilute, warm detergent solution 0.25 g Alconox in 1 L distilled, deionized water. Bill Tivol <tivol@caltech.edu> 03 Oct 2005

SEM - shelf life of the conductive paint

May I know the shelf life of the conductive silver paint and carbon paint? Phay Fang Gan <pgan@ap.ansell.com> 08 Sep 2005

I subdivide a new bottle of colloidal graphite into several small vials (usually liquid scintillation vials) and cap them tightly. This way, as the vial in use dries out (or gets left uncapped), I don't lose the entire stock. Jan Factor < jfactor@ns.purchase.edu> 09 Sep 2005

One thing that seems to help prolong the usability of carbon paint is to shake the bottle *after* you're through using it. This helps wash the semi-dried material at the neck back into solution, so you don't get as much dried up gunk in the neck of the bottle after repeated use. This is especially true if the cap has a brush built in, and you use the edge of the bottle to slurp off the excess before applying to stubs. Just make sure the bottle is tightly closed. Jim Ehrman <jehrman@mta.ca> 09 Sep 2005

This seemingly simple question has a complicated answer. First, despite "conventional wisdom", the silver paint used in EM labs is not "all the same". In addition to the obvious difference in silver solids between products, and variations in silver colloid size, some paints contain a small amount of an "amyloid" polymer, not enough to affect negatively its conductivity, but enough to greatly enhance its adhesive characteristics. But this is not the only function of the presence of the amyloid polymer: If one should forget to screw on the cap to their silver paint bottle, the addition of the recommended thinner and a few minutes in a laboratory ultrasonic shaker will quickly "rejuvenated" it and bring it back to life. But those silver paints without the amyloid polymer or perhaps some other polymer that is not so readily dissolved will either be rejuvenated much more slowly or as we have seen, in some cases, not at all. So if you are using at least certain silver paints, since the life time of the silver colloid is essentially infinite, and solvent that evaporates can be replaced with the right thinner (even to the point of its having dried out into a brick), there is no real lifetime limit. There are legal and other reasons why manufacturers might publish some "expiration" date for such products but from a practical stand point, at least for some brands of silver paint, the life time is essentially infinite. But if your question had to do more with the lifetime of the silver paint product unopened, and sitting on the shelf, then this has more to do with the closure system, including the heat seal. Again, not all closure systems are the same. I have seen some silver paint products on the shelf of certain distributors in foreign countries where the paint was as it was delivered ten or more years prior. And I have also seen paints of other brands that had dried out into bricks after only a few years on the shelf. When discussing the shelf lives of carbon paints, you could almost substitute "carbon" for "silver" above (except that for those carbon paints that do contain a polymer, it is not (to my knowledge an amyloid type). The shelf life of at least some carbon paints is indeed just as infinite as their silver paint counterparts. Charles A. Garber <cgarber@2spi.com> 09 Sep 2005

SEM – critical point drying

I have a question about one of the steps in critical point drying (CPD) using ethanol and CO_2. I was taught that I should never let the sample exposed to air after passing the higher alcohol concentrations e.g., 75% and above. When transferring the dehydrated sample from the ethanol into the CPD chamber, I will fill the chamber with enough ethanol to submerge the prep and then transfer the sample basket/container quickly into the CPD chamber. However, I have come across quite a few protocols that either do not specify this or simply fill the chamber with CO_2 "snow". My question is, should I fill the chamber with ethanol? Am I being overly cautious or have I missed anything critical (no puns intended!)? Can this be sample specific (e.g., smaller or delicate samples may be more prone to surface tension disruption)? Wai Pang Chan <wpchan@u.washington.edu> 03 Oct 2005

I think your being a little overly cautious which should not hurt, but may take longer in CO_2 flushing to remove (plus sends a lot of ethanol out the CPD exhaust to evaporate which generally appears be against most chemical waste protocols, which I will not argue either way). In my experience the rule is: Always keep the samples wetted, prevent "air drying". We usually process CPD samples inside of little containers (metal baskets or scintered Teflon, i.e. marshmallow baskets, or coverslip racks). We load the samples into the containers under 100% ethanol, and then transfer the containers to the CPD chamber. A lot of ethanol gets transferred with the samples into the CPD chamber. (To test this place a basket into ethanol, carefully remove it from the ethanol with forceps, and then give it a hard shake over a counter top. You will see a lot of ethanol coming from the basket). This ethanol carries over long enough for us to replace the CPD chamber lid, tighten down the retaining bolts, and crack open the CO2 fill valve. As soon as some CO_2 hits the chamber: (1) the expanding gas cools the chamber atmosphere, (2) rapidly increases the chamber pressure, and (3) starts wetting the samples with CO_2. The first two conditions slow and stop the vaporization of the ethanol, and the third wets the sample with a new solution. As I tell my students, the only "rush" period in CPD preparation is from the moment the sample baskets are removed from the 100% solvent and the CO_2 fill valve is cracked open (cracked open slowly to prevent throwing the sample basket(s) around inside the CPD chamber as the CO_2 rushes into fill the chamber. And as a follow up to this: Never allow the CO_2 level to fall below the sample height during the CO2 flushes. Until you are transitioning to the critical point (in which case you are also dealing with a saturated liquid CO_2 environment anyway). Richard Edelmann <edelmare@muohio.edu> 04 Oct 2005

We typically dehydrate our samples (e.g. mouse embryos) in increasing concentrations of ethanol and then change it to amyl acetate. This has three advantages a) amyl acetate is less volatile than ethanol - we do not have problems with air drying samples during transfer; b) supposedly, amyl acetate mixes better with liquid CO_2; c) amyl acetate has a specific aroma - it's absence is a good indicator the sample is ready for CPD. Michal Jarnik <m_jarnik@fccc.edu> 05 Oct 2005

SEM - sample preparation for cross section

I am trying to investigate the cross-section of a thick (~1 mm) metal substrate coated with a very thin (~1 μm) silane film. My attempts to prepare a clean cross-section have been unsuccessful and I have not been

able to distinguish the thin film from the substrate. I am wondering if anyone has advice on the best way to prepare a cross-section of this type of sample. Olivier Guise <olivier.guise@ge.com> 29 Aug 2005

Sample preparation for polymeric films on metallic substrates takes some practice and patience. If you can prepare good samples of polymer and metals separately, you should be able to work a procedure to get this sample adequately prepared. Coatings at 1 μm and below are much harder to get consistent reliable results than for thicker coatings. One possibility to explain your difficulties is that the coating is thinner than you expect. If you have good confidence in your sample preparation, you might want to use another method to check the approximate thickness. We have struggled to prepare cross sections of samples only to find that the coating thickness was really less than 100 angstroms. If the sample will tolerate compression mounting, you can get good contrast between the coating and mounting material by wrapping the sample with aluminum foil. The compression during molding will press the foil tight against the samples surface and retain the coating during polishing. Clamping the sample against a flat, conforming backing will achieve similar but sometimes less satisfactory results if you are using castable mounting. Use low nap polishing cloths to avoid rounding during polishing. Beyond that it may require trial and error of the process to find what works for this particular substrate and coating material combination. Larry D. Hanke <hanke@meeinc.com> 31 Aug 2005

Cryo SEM - Freeze drying question

I have occasionally used the freeze drying effect of our FESEM's cryostage to sublime ice off of liquid nitrogen plunge frozen samples. Now I have someone who would like to try this with liquids such as benzene and other solvents. I'm willing to give it a shot, but have no clue how such liquids behave at low temperatures. Our client says the solvents will freeze at the temperatures we operate our stage at, but she also has no idea whether "freeze drying" will occur with them. Has anyone tried this? As usual, I'll be checking the literature, but personal experiences are always the most useful, and I figure you folks have collectively done it all at some point. Randy Tindall <tindallr@missouri.edu> 02 Sep 2005

I assume that in checking the literature you will be determining the temperature and pressure at which each organic will sublime and the possibility of contaminating the chamber and other lower parts of the column. I assume that you are using an anti-contaminator around/above the specimen holder; have you ever lost control of the temperature or otherwise had the specimen "warm" up to room temperature under the beam? Working with frozen organic solvents is something that I would contact your SEM manufacturer about and get their input. I also wonder why you are getting ice on your samples. Are you using a device and method of avoiding ice contamination? Do you use liquid nitrogen slush? Having worked also with liquid helium 4 to freeze biological specimens, ice contamination was not an option so it can be done. Damian Neuberger <neuberger1234@comcast.net> 02 Sep 2005

One question I would have is where the benzene and other solvents are being vented? Also, what is it going to do to your seals? The National Institute for Occupational Safety and Health (NIOSH) recommends an exposure limit of 0.1 ppm as a 10 hour TWA (time-weighted average). NIOSH also recommends that benzene be handled in the workplace as a human carcinogen. In 1997, the American Conference of Governmental Industrial Hygienists lowered its TWA

threshold limit value to 0.5 ppm to reflect the change in cancer classification to A1 (i.e., confirmed human carcinogen). Tom Phillips <phillipst@missouri.edu) 02 Sep 2005

A follow up to my first question: I've just been reminded of the obvious hazard of solvent fumes, although the amounts involved would be quite small. Does anyone know if the mist traps on rotary pumps would be effective at trapping these? Our rotary pumps are, unfortunately, in the same room as the scope. Randy Tindall <tindallr@missouri.edu> 02 Sep 2005

Have a look at http://www.sisweb.com/referenc/applnote/app84.htm. I found this application note related to your follow up question while looking for a new mist filter for our mechanical pump. It seems that normal oil mist filters are only effective at trapping the high molecular weight hydrocarbons from pump oil. Steve Szewczyk <sszewczyk@arl.army.mil> 02 Sep 2005

In our group (polymer physics) a long time ago, freeze drying of para-xylene (m.p. 19°C, b.p. ~ 144°C) was used. There are quite a few organic solvents freezing around 0°C, and many of them should respond to this technique. What is important is the vapor pressure, so things with too high a boiling point wouldn't work. However, as the other replies state, it's the pumping system one should worry about. Robert H. Olley <r.h.olley@reading.ac.uk> 06 Sep 2005

SEM - Chromium sputter coating

We have just taken delivery of a chromium sputter coating unit and I am attempting to do a risk assessment and having read some of the MSDS on Chromium am now very apprehensive about the toxicity of the fumes and flakes produced by the target. As I am not a chemist, I am unsure what I am dealing with. If anyone out there has any advice they would be willing to share, including things like should it be used in a fume hood and how to dispose of the 'flakes' of chromium, it would be greatly appreciated. Christine Richardson <a.c.richardson@durham.ac.uk> 05 Oct 2005

There is little to worry about. The nasty Cr is Cr in the hexavalent state (+6). The Cr of your target is metallic with a thin oxide on it. The natural oxide of Cr is Cr_2O_3 which means that the Cr is in the +3 state and is bound up with the oxide. The process of sputtering is physical bombardment of the target with Ar ions that removes the atoms from the near surface of the target. The process is line of sight deposition onto your substrate and metallic Cr is being deposited (valence state is 0). That is why you need a rotating and tilting sample to get a uniform and continuous coating on your sample for high resolution imaging in the SEM. I assume that the flakes that you are talking about are flakes in the deposition chamber. If you are getting flakes of material on your chamber walls, you must be putting down very thick coatings or very many coatings. Our IBS/e sputter coater can put down a uniform, continuous coating that is less than 10 Angstroms. Your coater should be capable of doing the same. If you deposit these types of films, it should take a long time before you start to get flakes in your system which is thick coatings that peel from the walls because of high stresses in the films. Regardless, in the vacuum system, what is deposited is the metal Cr and it oxides to Cr_2O_3 when exposed to air when you open the chamber. When you clean your chamber and there is dust or flakes, wear a mask and discard the cleaned material as you would a heavy metal. There will be no Cr fumes anywhere. Cr will not be present in the pump exhaust. As Gary Gaughler said in his reply, Cr does oxidize fairly rapidly, so samples coated with it must be run soon after. The natural protective oxide that forms on Cr is about the thickness of the coating that you want on your sample. When you consider how thin the thickness of the coating is and how long they can be exposed to atmosphere the actual oxidation rate is not all that high. Storage containers to help prevent this and maintain sample in an inert atmosphere are commercially available. It is generally accepted that Cr coatings give the best results, but Ir and W coatings approach the quality of Cr and are less susceptible to complete film oxidation. Pt, Au, and Au-Pd coatings do not give as fine a grain size as the other coatings. Scott D. Walck <walck@southbaytech.com> 05 Oct 2005

SEM - blood cells

We used to prepare blood cells using standard fixation and dehydration but then transfer into a Freon. Once in the Freon you could just put a droplet of the sample solution on a Nucleopore filter. The Freon would evaporate instantly leaving lovely dried cells. We tried this recently with the remains of Freon we have had around for years. It did not work well, so I am assuming that the Freon absorbed water over the years and is not longer usable. I do not know what type of

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Freon this was, as I inherited it. Most types are no longer available. Does anyone know of a type that is still available and can be used for this purpose? Any other hints for processing blood cells? We can do standard CPD, but this other method was so nice and easy with such good results when it worked. Debby Sherman <dsherman@purdue.edu> 21 Sep 2005

Try Vertrel. This is an 'environmentally friendly Freon replacement brought to us by Dow, the same people who gave us Freon. All considered, we can only assume it is environmentally friendly in that there is currently no evidence of damage which it can cause. Having said that last sentence, I use it in place of Freon for cleaning scope parts and for purifying virus for different uses. It is almost as expensive as Freon, but I think you can get it in 1L bottles (we use enough that we usually buy 4 L bottles). See: Mendez, Hermann, Hazelton and Coombs. 2000. A comparative analysis of Freon substitutes in the purification of reovirus and calicivirus. Journal of Virological Methods, 90:5967. It is available as a free paper in .pdf form from the journals division of Virus International, a section Elsevier has. Paul R. Hazelton <paul_hazelton@umanitoba.ca> 21 Sep 2005

We used HMDS recently. Lots of echinocytes but that is probably unrelated. Dave Patton <david.patton@uwe.ac.uk> 21 Sep 2005

SEM – Coating effect on morphology

I was once asked by a consultant whether gold/palladium or gold coating would affect the surface morphology of a specimen coated. I would appreciate your professional advice. Phay Fang Gan <pgan@ap.ansell.com> 11 Oct 2005

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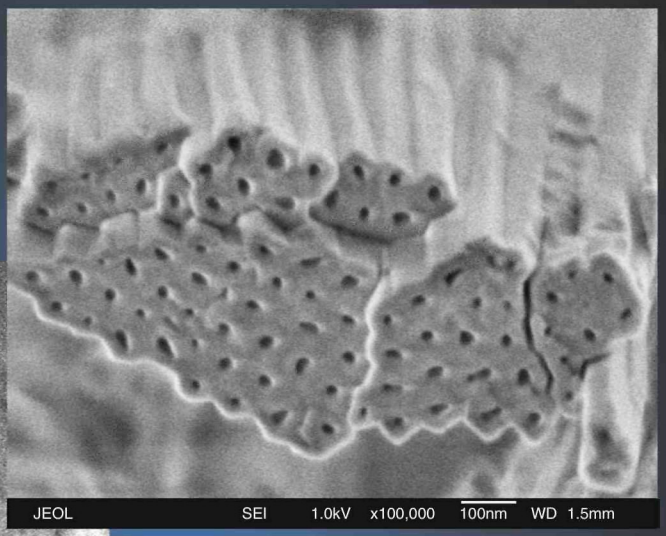
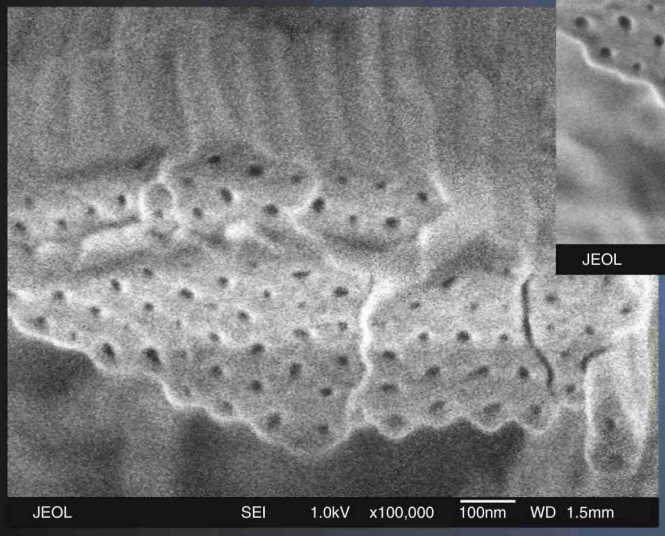


Anodized aluminum.
Normal SEM mode.



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The coating itself won't affect the surface morphology, although the coating process might. Sputter coating can heat specimens. Normally, this isn't a problem, since sputter coaters generally are designed to keep the electrons, which do most of the heating, away from the samples. But for low melting-point samples, such as bloom on chocolate, even short bursts of coating with melt the surface. As I found out from experience. But, at high magnification, say 30,000X and above, the "surface morphology" of the sample is modified in the sense that the structure of the coating becomes visible. Pure gold produces a lumpier coat than say 60/40 gold/palladium, and since Au/Pd is cheaper than pure Au, I find it best to just use Au/Pd targets. Phil Oshel <oshel1pe@cmich.edu> 12 Oct 2005

Is there any disadvantage in moving from Au to Au/Pd targets, e.g. vacuum requirements or quality/quantity of coating for low magnification applications? Dave Patton <david.patton@uwe.ac.uk> 12 Oct 2005

No. The Au/Pd targets work the same as pure Au targets, and I use the same coating parameters. If you use a coating thickness monitor, like a quartz-crystal instrument, you have to change the work function and mass in the programming. If a thickness monitor isn't used, then I don't find any need for changes. The Au/Pd coating at low magnifications is as good as gold. Phil Oshel <oshel1pe@cmich.edu> 12 Oct 2005

STEM - EDX analysis

I have recently carried out some EDX analysis on a Ga-Au alloy for which I would like to a have rough idea of composition. The analysis was made for an object which is about 40 nm thick with a STEM probe in a FEG 200 kV instrument. Since the Au K signals come at too high energy for our detector, my question is if it is possible to compare the K-shell band of Ga with the L-shell band of Au. Or would it be more correct to do that analysis comparing the two L-shell bands of Ga and Au? Pedro Costa <pcosta33@hotmail.com> 12 Sep 2005

Since all the Au-Ga compounds are known, all you need is a rough value of composition to say which one it is. I usually take a diffraction pattern or two and compare measured d-values with the international crystallographic database if there's any uncertainty. You may have to do low angle convergent beam (using a tiny condenser aperture) rather than selected area diffraction if the grains are small in a multiphase compound. The nice thing about TEM is that you can get EDX and diffraction analysis from the same grain. As for the EDX analysis, using different lines (K,L,M) shouldn't be a problem anyway if you had to do a proper job, you would be comparing it with a known standard and you can use whichever lines you like as long as there's no strong overlaps. Richard Beanland <richard.beanland@bookham.com> 12 Sep 2005

You should use Ga-Ka and Au-La because of the comparable excitation and absorption conditions with energy of 9..10 keV. This is the best choice, even if your detector would be able to detect Au-K. But bear in mind for (only rough) concentration determinations, if the Ga/Au-concentration ratio is expected with 1/1, then the peak heights or pak-net counts are about 6/10. Frank Eggert <eggert@mikroanalytik.de> 12 Sep 2005