NETNOTES

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

Selected postings from the MSA Microscopy Listserver (listserver@msa.microscopy.com) from 12/15/07 to 02/15/08. Postings may have been edited to conserve space or for clarity.

SPECIMEN PREPARATION - embedding pine needles

Has anyone embedded pine needles in JB-4 and sectioned for LM? I had problem with orientation when I used plastic BEEM capsules before. I have been trying silicon rubber molds under 10 psi. But it doesn't polymerize completely. What is the maximum psi I can go not to damage both tissue and plastic. I have been also thinking of switching to LR White. But I don't know if I will get a better result. I would appreciate any kind of advice on this. Sau Silwal <sau_silwal@yahoo.com> 2 Jan 2008

Not sure exactly if this will solve your problem, but for what it's worth, you can get capsules that are like BEEMs except that they have a completely flat bottom. They are made by TAAB but available from major supply houses. This gives a nice 7 mm (or so) flat surface. You can also get this out of a standard BEEM by flipping it upside down, although you have to mess about to keep the resin from leaking (definitely a mess, but possible to do). Tobias I. Baskin <baskin@bio.umass.edu> 2 Jan 2008

I would definitely switch to LR White. We never use methacrylate resins anymore. The flat-bottomed capsules recommended by Tobias are much better for polymerizing LR White than BEEM-type capsules. The walls seem to be thicker and are therefore less permeable to oxygen. Also, they are offered in polypropylene. The PP capsules are really a pain to get the blocks out of, but they polymerize nicely. I was told that putting LR White (and maybe methacrylate?) resins under vacuum can cause the initiator to evaporate from the resin, causing incomplete polymerization. If you need to vacuum to remove air from your needles, I would do it during fixation or during the post-fixation wash. Andy Bowling <andrew.bowling@ars.usda.gov> 5 Jan 2008

Some methycrylates have significant advantages over LR White. We routinely use a mixture of butyl and methylmethacrylate (BMMA) that we got from a paper by Tobias Baskin. This resin can be removed from sections using acetone in a manner analogous to xylene treatment of paraffin sections. This greatly increases immunostaining. Unlike JB-4, this resin easily cuts on water-filled troughs. Since it is made from generic methacrylate resins, it is less expensive than the proprietary formulations. It is no good for TEM but you cannot have everything. For TEM immunocytochemistry, I prefer LR Gold to LR White since I feel it cuts better with similar immunoreactivity. Tom Phillips <phillipst@missouri.edu> 5 Jan 2008

SPECIMEN PREPARATION – softening hard tissues

I've got a surgeon who is working with human livers that are very hard from the embalming process and he had heard that microwaving tissue may soften up the tissue and make it easier to cut. I'm going to let him use my Biowave and try different wattages. I have never heard of this effect before. Has anybody out there heard of or actually know if hardened fixed tissue can be softened by microwaves. Tom Bargar <tbargar@unmc.edu> 29 Jan 2008 Tue Jan 29

I am not aware that microwaves can soften embalmed human liver tissues. On the other hand, plant biologists often soak plant tissues in glycerol to soften them for sectioning. Check out the following article: John C. Guenther and Frances Trail. 2005. The development and differentiation of *Gibberella zeae* (anamorph: *Fusarium graminearum*) during colonization of wheat. Mycologia, 97(1):229-237. In this study, the authors did the following: After a 24 hr fixation, samples were placed in a solution of glycerol and ethanol (1:1) for an additional 24 hr to soften tissues for sectioning. They then further dehydrated the specimens in alcohol, embedded (in paraffin, in this case) and sectioned as usual. You might give it a try. Certainly, use the web to search for the use of glycerine as a softener since I know it is used successfully. Let us know what worked the best. John J. Bozzola <bozzola@siu.edu> 31 Jan 2008

I doubt if microwave exposure (cooking) will soften embalmed tissue. You might try soaking the organ in water (tap water) for a week, changing the water every day. There is good evidence that formalin-fixed tissue can be "unfixed", at least to some extent, by prolonged washing in water. Of course, it will depend on the composition of the embalming fluid, the length of the exposure, etc. Geoff McAuliffe <mcauliff@umdnj.edu> 31 Jan 2008

SPECIMEN PREPATION - SEM of wires encased in polyurethane

I have a researcher who wants to look at polyurethane coated wires with the SEM. His goal is to see any cracks, breaks or defects in the metal wire encased by the polyurethane. These are very tiny wires, he cannot remove the covering and he needs to look at the wire in sections for defects. We have a super SEM with a backscatter detector but no carbon coater. Anyone have experience with this type of sample? How can I prep the samples for the SEM?? Pat Kysar <pekysar@ucdavis.edu> 17 Jan 2008

I have several questions that may allow you to provide more specific information useful to the listserver community: Is the polyurethane cross-linked? Does he need to examine the wire in cross-section or might examination of the surface of the wire suffice? If a cross-section is required, which plane of the wire does he want to examine, i.e. the axial cross-section or the longitudinal cross-section? How do you intend to prepare the cross-sections: grinding and polishing; microtomy; FIB, etc? If the polyurethane is not cross-linked, I suggest you consider dissolving it in a good solvent, thus leaving the bare wires available for subsequent sample preparation and microscopy. Gary M. Brown <gary.m.brown@exxonmobil.com> 17 Jan 2008

I'm a little confused by "he needs to look at the wire in sections". If you mean that the wire will be cross-sectioned, then advice has already been given: mount, grind and polish. If, on the other hand, you mean that short, perhaps specific, sections will be examined for surface defects on the metal wire and "he cannot remove the covering", my question is: how thick is the polyurethane insulation? If it's only a couple of microns thick, try going to your maximum kV and see if the beam will penetrate the insulation. BSE may give the best image, but SE might also work. If the insulation is too thick for beam penetration, you're left with either stripping or sectioning. Ken Converse <kenconverse@qualityimages.biz> 17 Jan 2008

Unfortunately, many technologists are put in your position. That is, one may be asked to analyze potentially complex, multi-component materials without substantive guidance from the requestor, in areas outside one's expertise, and with little help within our immediate technical community. I encourage you to put some of the responsibility back onto the requestor. He/she should be able to obtain information on the composition of the polyurethane insulation from the manufacturer. If the requestor is not willing to spend any time or effort to work the problem, just how important can it be? The last point I would make regards the scope of your technical capabilities. Specifically, do you have the sample preparation and microscopy capabilities that will be needed in the analysis of this material? Gary M. Brown <gary.m.brown@exxonmobil.com> 17 Jan 2008

SPECIMEN PREPARATION - etchants for 6xxx aluminum

Does anyone know of any other good etchant(s) other than Keller's and Barker's reagents for 6xxx aluminum alloys? Our interest is in grain size measurement. Also, does anyone know any outlet that sells ready-made Keller's etchant? We do not want to mix the chemicals by ourselves due to some local restrictions. Ike Oguocha <oguocha@yahoo.com> 19 Dec 2007

If you have EBSD, a polished sample should produce nice grain info. Etching enhances FSD images. I do many Al films and solids without any etching other than deionized water. Gary Gaugler <gary@gaugler.com> 19 Dec 2007

If you can get hold of a copy of the 1948 edition of the Metals Handbook (published by the ASM) you can find two pages of information on

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etching aluminum alloys (pp. 800-801). Probably similar information is contained in the more recent editions, too. Some of the simpler etchants include: 0.5 ml HF in 99.5 ml water; 1 g NaOH in 99 ml water; 10 g NaOH in 90 ml water; 20 ml concentrated sulfuric acid in 80 ml water; 25 ml concentrated nitric acid in 75 ml water. Wilbur C. Bigelow <bigelow@umich.edu> 19 Dec 2007

Better yet, try: Petzow, Gunter: Metallographic Etching, 2nd ed. ASM, Materials Park, OH. 1999. Specifically Pages 57-64. 19 Dec 2007 Tony Havics <ph2@sprynet.com>

SAMPLE PREPARATION - Ni in double-sticky carbon dots

In case some of you believe all carbon substrates are manufactured to be free of x-ray spectral peaks, I just discovered small nickel wire in a type of "ultra-smooth" double-sticky carbon dots. My impression is that it is needed to make the surface smooth. I won't reveal the manufacturer here, and I'll admit they work relatively well for background-free imaging, but if users require their carbon substrates to be contaminant-free they should check their stock or ask before purchasing. Michael Shaffer <michael@shaffer.net>

This can be an important issue. Is the wire magnetic? Or is it a non-magnetic alloy? Try putting the dots next to a strong magnet and see if they stick. I would be concerned if the wire could be magnetized and then adversely affect high resolution imaging as well as elemental microanalysis. Can I suggest that you contact the vendor and find out if this was a onetime event (i.e., the wrong wire was used in a batch) or if this is standard procedure? If it is standard, it would be appropriate for the vendor to comment on this of his/her own accord on this forum. Nestor Zaluzec <zaluzec@microscopy.com> 19 Dec 2007

MICROTOMY - problems cutting serial thick sections

I am having some problems in cutting serial thick sections (above 1-2 microns) without getting serious pitting of the block face. I heard this is not uncommon when pushing the section thickness to the extreme (such as 3-5 microns). We usually deal with the situation by re-facing the block after each thick section, but this of course would make true "serial" sectioning impossible. The blocks I am working with are either cardiac left ventricle or striatal tissue embedded in Durcupan. If anyone has tried or has a protocol or techniques, I'd be very grateful to hear about them. Thor <andrea@ncmir.ucsd.edu> 7 Jan 2008

What kind of knife are you using? I get good results cutting serial thick sections of resin embedded tissues from 1 to 5 microns with a Diatome Diamond Histo-Knife. I have never experienced "pitting" of the block face. I have my own bag of tricks for collecting sections as they sometimes curl up like a wood shaving as they come off the knife especially the thicker ones. Dean Abel <dean-abel@uiowa.edu> 7 Jan 2008

Briefly, this type of problem is down to brittle resin blocks being sectioned at too fast a cutting speed and the thicker the section, the more likely it is to occur. Try adjusting the hardener/plasticizer proportion of your embedding mix and/or reducing the polymerization time/temperature to end up with a softer block. With the blocks already processed, use the best available knife and reduce the cutting speed. If the chipping of the face recurs, your only remaining option is to reduce the section thickness. Like Dean Abel suggests, I would be using a histo-diamond (wet) with a cutting window speed of 1 mm/sec and increasing the section thickness gradually from an initial 2 microns. I would also trim off most of the surrounding resin and shape the block to a point to reduce the cutting force on the knife, particularly as you are wanting to section at up to 5 microns. Alastair McKinnon <a.d.mckinnon@abdn.ac.uk> 8 Jan 2008

MICROTOMY – sectioning seeds

Does anyone have experience thick-sectioning watermelon seeds? We're working with mature seeds - black seed coat - unfixed, not embedded. The seeds are left in a moist chamber overnight to imbibe water. Vibratome sectioning didn't work well. Would fixing and embedding make them easier to section? Beth Richardson <beth@plantbio.uga.edu> 17 Jan 2008

I only have experience in sectioning seeds of *Orobanche cumana*. I was successful doing semi- and ultrathin sections after embedding in LR White. The first preparation step was to perforate the seeds with a fine needle. Without perforating the seed coat, it was impossible to get anything into these tiny seeds. I think seeds are always difficult, because their coat is quite tight, their water content extremely low, and they store masses of starch grains or other storage material which are difficult to fix and to section. Watermelon seeds are quite large. Maybe you can cut them and try to embed the pieces? Anne Heller <heller@uni-hohenheim.de> 21 Jan 2008

MICROTOMY – multilayer film

I have a multilayer film in which one of the layers is water soluble. We want to cross section the sample for TEM to view the various layers, each of which are less than 500 nm thick. The total thickness of the plastic substrate plus multilayer film is approximately 500 microns (the substrate is approximately 300 microns of this). I've embedded the film in an epoxy resin for sectioning. I don't know how to go about capturing thin sections (<100 nm) if I can't put water in the boat. I could cut them dry, but again, I'm not sure how to transfer them to the grid. I am using a Diatome diamond knife. Any suggestions would be greatly appreciated. Another question I have regards the use of tape as a support media for cryo-sectioning. Many of our samples are films, which I typically embed in epoxy resin. We now have cryo-sectioning capabilities, I would like to sandwich my film between tape, and cryo cut thin films for TEM analyses. Is there any tape, which has good adhesion properties at -120°C and stand up well for TEM (glue does not interfere with sample), Again, any direction is appreciated! Sandra Gardner <sandra.gardner@xerox.com

I also work with water-soluble materials, and so often have to cut dry. I have found that a diamond knife is preferable to glass (less "sticky" so sections are easier to get off the knife surface), but that may be something you want to experiment with. To transfer sections from the knife to the grid, I use an eyelash probe. It takes a little practice, but that is what works best for me. I also use the probe to "flatten" gently the sections onto the grid surface. I use 300 mesh Copper grids with Formvar support film. Diatome used to make a Cryo P diamond knife (do not know if they still do) with a special platform that makes it easier to transfer sections. This has been my favorite knife for dry sectioning. Jessica Cervantes <cervantes@bendres.com> 30 Jan 2008

I agree with Jessica that you should use cryomicrotomy and collect dry sections. This is a challenging technique but with practice, you will find it to be valuable. Microtome vendors and other training facilities offer workshops on the cryomicrotomy. In our lab, we use a 'plastic chuck' for sectioning films at ambient and low temperature. The chuck consists of a piece of plastic with approximate dimensions of 4 mm x 7 mm x 3 mm (width x length x thickness). These dimensions should be set to your personal preference and to fit your sample and microtome chuck. Use a stiff plastic such as high density polyethylene or polypropylene that will hold the sample tight but is not rigid or inflexible. Using a sharp razor blade, bisect the 3 mm thickness, cutting through nearly the full length of the plastic piece and leaving a couple of millimeters of at the other end of the piece to hold the arms of the chuck together. The end result is a Y-shaped piece of plastic (see diagram below). To use the chuck, slide the film between the arms of the plastic chuck leaving less than 1 mm exposed; the more film is exposed, the more likely it is to move side-to-side during microtomy. Listers may contact me off-line for a drawing of the chuck. I recommend that you do not embed films in epoxy if possible. The embedding medium, whatever it might be, only contributes to the difficulty of cutting. The problem is that the film has several layers, each with different cutting properties at low temperature; the problem is often even worse at ambient temperature. Adding the embedding medium contributes another medium that most probably has very different cutting properties than any material in the film. Gary Brown <gary.m.brown@exxonmobil.com> 31 Jan 2008

As usual Gary Brown is dead on with his comments. I agree that

embedment is generally best avoided in these multilayer applications. We have seen swelling of the surface layer due to epoxy interaction as well, surprising some real polymer gurus around here. His specimen holder sounds elegant - I think I'll make one. We've taken a more down-and-dirty, and semi-disposable, approach here that may be useful in some of your applications. We use some 15 mil (0.37 mm - thicker wouldn't hurt) high impact polystyrene (HIPS) stock that was lying around to form a specimen support by cutting a piece approximately twice as long as Gary cited, then folding it in half to form an open envelope. The specimen is sandwiched between the folded ends with the fold near the bottom of the chuck and the specimen just protruding from the open end of the sandwich, also just above the microtome jaws. Typically I will trim away the clamped envelope to form a little truncated pyramid at the top and trim the sample to project just above, such that almost all the sample is encased in the HIPS sandwich and the clamp. For specimens that need more support, i.e. very thin films, the disposable nature of this setup comes in handy. I trim away the entire assembly with the specimen inside to form a mesa with HIPS and sample on the face (this is especially helpful when cutting thick sections of multilayer films for light microscopy). The HIPS sides of the pyramid are beveled as well to keep the HIPS sectioning to a minimum. It is generally not a major problem to winnow the sections of interest away from the sectioned support material, but make sure you take the time to get to know the support material microstructure intimately just in case! Matthew Stephenson <stephenson@impactanalytical.com> 1 Feb 2008

I learned about the plastic microtomy chuck from Lou Ban, a microscopist who worked at Exxon Chemical some time ago. I have used it for +20 years with success. Thanks, Lou. You make good points regarding the specifics of use of the chuck during microtomy. Your disposable chuck is ideal for the case in which a cross-section of a thin film is needed for SEM/EDS. After cryo-sectioning or cryo-facing (cryo-planning), the chuck/film assembly can be transferred directly to the SEM for analysis. We all have insight into what makes a better mousetrap, or in this case, a microtomy chuck. The folks in our lab over the years used the chuck with variations that fit their tastes and needs. Without folk's new ideas and modifications of old ones, man would not be where he is today. <gary.m.brown@exxonmobil.com> 1 Feb 2008

MICROTOMY - polymer films and hard particles

I was asked by a biological microscopist for information pertaining to preparation and microtomy of polymer films and materials. Like many of us, we are being asked to broaden our area of expertise into unfamiliar territory. The following suggestions are based on guidance by several other microscopists and much experience over the years. I do not claim to be an expert, only a microscopist with a few proven tricks up my sleeve. This is not a comprehensive or authoritative monograph, just friendly advice. My hope is that this will cause a flurry of responses with additional suggestions and ideas, questions (and answers) on preparation of other sample types, and correction of any wrong statements by me. Like biological microscopy, material science offers many challenges. The broad spectrum of materials often requires only several key preparation and microscopy tools and techniques. I would start with the materials that you are presented with and worry about other potential challenges when they arise. That said, there are several approaches to microtomy of materials that are worth mentioning. As in every other aspect of microscopy, sample preparation is absolutely essential. Frankly, I learned most of the methods that I used by trial and error with help from a few colleagues. I'm sure that a several if not many good review papers and books deal with preparation techniques for catalyst-related materials such as zeolites, aluminas, silicas, etc. These are exceptionally important materials used to support catalyst species. The microtome vendors can provide much insight into preparation of materials for microscopy. Alternatively, other organizations such as McCrone Associates may offer workshop. Consider polishing if larger flat surfaces of hard materials are needed for SEM analysis. Look for contract labs that will grind and polish your samples until you have a suf-

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ficiently high sample volume that you need to invest in grinding and polishing equipment. Microtomy is the key to successful analysis of polymers. Several points follow: I generally try to avoid embedment. Exceptions to this are fibers, fabrics and tiny particles that require rigid supports during microtomy. When embedment is unavoidable, try to match the hardness of the medium to the sample as best possible. Important: Do not cure any embedding medium at a temperature approaching the polymer melting point. If you don't know the melting temperature, use an ambient curing epoxy such as EpoFix. Polyolefins, a major class of polymers including polyethylene, polypropylene, etc., require special care since their melting temperatures can be as low as 40-50°C or as high as 130-170°C. Embedded or not, section the polymer at a temperature below its glass transition temperature (Tg). When uncertain, lower the cutting temperature to < -130°C. Cryo-section slowly since friction induced at higher rates can cause heating of the polymer above its Tg where it will soften and plastically deform. I use a standard approach to microtomy of hard materials like minerals and catalyst supports. Low viscosity is an important requirement for any embedding medium for small particles. I generally use LR White - hard grade - without the accelerator because it is has very low viscosity, is devoid of accelerator, is easy to use. The low viscosity allows it to readily infiltrate most catalyst support particles giving good embedment. Oven cure LR White at 80-90°C for several hours in a nitrogen-purged oven. On occasions, I will use EpoFix epoxy. Call your supplier of embedding resins to find an epoxy: accelerator mixture that will give a hard block. Cure at as high a temperature as is recommended by the manufacturer or your supplier. If the material is not in a finely powered form and grinding permits, use a garnet mortar and pestle to finely divide the material. Sprinkle the powder toward one end of a flat embedding mold. Take care to have good density of particles in the mold but avoid piles or clumps of catalyst since they may not be well infiltrated with the embedding medium. Degas the embedding medium in a vacuum to remove bubbles and absorbed air. Epoxies and other higher viscosity embedding resins will take longer to degas than lower viscosity resins like LR White. Slowly layer the degassed embedding medium into the mold. Fully cover the sample and fill the mold. Identify the sample with a small paper label printed in pencil (ink runs when exposed to epoxy or LR White) in the opposite end of the mold from the sample. Place the mold plus catalyst into a vacuum and infiltrate for half an hour or so. If the embedding resin begins to foam due to release of air from within the powder, gently break vacuum several times until the material is stable in the vacuum. Heat cure in a nitrogen-purged oven for the required time. Gary M. Brown <gary.m.brown@exxonmobil.com> 4 Feb 2008

I might add: Get a good book such as Polymer Microscopy by Sawyer. Polymer Microscopy, 2nd Edition. Linda Sawyer, David Grubb, Kluwer Academic Publishers, ISBN 0-412-60490-6, 1995. Jim Quinn <jquinn@www.matscieng.sunysb.edu> 4 Feb 2008

Lothar Engel et al., An Atlas of Polymer Damage - Surface Examination by Scanning Electron Microscope, Prentice-Hall, New Jersey (1981) has been useful to me. It is not comprehensive but helped in understanding the morphology of polyolefin failure as seen using optical, SEM and TEM. Gary M. <gary.m.brown@exxonmobil.com> 6 Feb 2008

There are a few basic FA books on polymers out there. It seems that no one book covers the topic sufficiently to be "the" reference. The book you now have and the Engel book previously recommended are good. My other favorites: Failure of Plastics and Rubber Products by David Wright; Plastics Failure Analysis and Prevention, John Moalli (Editor); Plastics Failure Guide by Myer Ezrin. Larry D. Hanke <hanke@mee-inc.com> 8 Feb 2008

LM – arc lamps and electromagnetic fields

I've been searching for actual published material to support the industry standard that requires igniting (turning on) mercury or xenon arc lamps prior to turning on electronic microscopes or computer systems. Highly respectable microscopists have informed me that the electromagnetic fields (EMFs) produced by the intense 'welder's arc' spark can damage microchips and computer monitors nearby. Does anyone have hard, fast evidence or direct experience to support this behavior? When consulting an information technology (IT) specialist about EMFs and computers, he says it will occasionally create problems on a computer monitor that is easily fixed by degaussing, and it can wipe temporary memory such as flash drives. Again, no permanent damage, but something to consider. If so, how far would one need to keep the electronics from the arc lamp? I suspect that behavior is being dictated by a fear of the high expense in case there truly is a risk, but I do not want to perpetuate paranoia and extra lamp hours when not necessary. For example, my previous job did not encourage the arc lamp being turned on first, and I don't recall any electronics issues attributed to such 'reckless behavior'. Gregg Sobocinski <greggps@umich.edu> 19 Dec 2007

It seems that the EMF from starting the lamp is one possible reason for computer problems (if, in fact, there are computer problems). Another problem could be in the power fluctuations that are propagated back into the house wiring. If this is the case, then the distance of lamp to computer would be less important that the shielding of the computer to bad power. David Elliott <elliott@arizona.edu> 19 Dec 2007

I've got a UV fluorescence microscope (w/ Hg vapor lamp) in the same (small) room with both my SEMs and the flat panel monitor of the imaging computer is 6 inches away from the lamp housing; I've never noticed any interference or had any damage that I could track to the lamp arc. We have the lamp on a timer that keeps it on about 10 hours a day, which is when most people here are likely to use the scope. I've been running the SEMs when it turns on and when it turns off and wouldn't have noticed if the timer hadn't clicked. Becky Holdford <r-holdford@ti.com> 19 Dec 2007

I worked in a facility that used a xenon flash tube. The electrical pulse to trigger the bright light was strong enough to put out a pulse that occasionally tripped some of the encoders on the system. During a typical manufacturing run, the light flashed millions of time to draw a pattern on a photographic glass plate. Over a long period of time the position of devices was incorrect due to these EMP induced counts. The same may be true of laboratory equipment. In our case, the absolute position values drifted. This caused a failure in the manufacturing procedure, which was detected by a visual inspection system. Shielding and grounding of the electrical system that powered the bulb was sufficient to prevent these events from interfering with the rest of the equipment. Now that 25 years has passed, chip sets have smaller geometries that are packed tighter. This means less insulation between components and components less able to weather damage. A fatal 'nick' in the chip wiring is substantially smaller than it was 25 years ago. I have no hard evidence of actual damage done to chips, but I do have firsthand experience at seeing the effect of EMP on high precision equipment. Robert Boehringer <rboehrin@vt.edu> 19 Dec 2007

Video microscopy got going in the early 1980's with the inclusion of computers not far behind. In those days many people were using 200W lamps with massive (often old) power supplies. These things hummed, banged, and sometimes dimmed the room lights when fired. I suspect the newer, slimmer units, 50 and 100W, have better behaved electronics, and cause less electrical stress. They certainly run cooler and quieter. There is also probably a wider use of surge protectors and related circuitry in computers. So I guess in the early days there may have been problems but I think now could be folklore. I have heard mercury arc explosion stories and even witnessed one, but never heard of, let alone seen an example of, a story where firing an arc lamp EMF'd a neighboring PC. Tobias Baskin <baskin@bio.umass.edu> 19 Dec 2007

This is not hard evidence about EMP damage, but the type of lamp, type of housing (internal/external igniter?) and type of ignition certainly affect the potential for problems. First, let's assume that no one is still using the old units with spark-gaps. Second, Hg and Xe(Hg) lamps breakover at about 4 kV and I have seen some mention of "soft start" but don't know about that. The current surge through the cable following breakover is what is most likely to radiate a pulse. Xenon lamps with no mercury in-

cluded break over at a much higher voltage (igniters for Xe typically put out 20 kV) and the start pulse typically has more energy as well. I have run a confocal system in close proximity to HBO-100 Hg lamps (Nikon supplied) for years and saw no effects; keeping the arc lamp cable well away from other cables to be safe. We also had an SGI server in the room that ran 24 hr and it never had a problem. Dale Callaham <dac@research.umass.edu> 20 Dec 2007

I suppose Nikon Microscopes can be trusted when they say: "As digital imaging workstations become more popular and microscopes equipped with high-end camera systems grow more complex, it is important to remember how dangerous the arc lamp power supply can be to electronic equipment. Always turn the arc lamp on before powering on auxiliary computer or camera equipment that is in close proximity to the power supply, and always turn this equipment off before turning off the arc lamp. The cable supplying current to the lamp from the power supply is generally quite well shielded, but a momentary 20,000 to 50,000 volt surge is possible when the lamp is fired. This large voltage can generate a magnetic field strong enough to damage sensitive integrated circuits that are nearby." (http://www.microscopyu.com/tutorials/java/arclamp/index.html). I had always assumed that the Faraday cage (metal box) around the mercury lamp and power supply shielded other equipment quite well from electro-magnetic pulses and that mains spikes from the power surge as the lamp starts up were the main problem*. That said I've always followed the microscopists' aural tradition by intoning the dogma to users that "the mercury lamp goes on first and off last", although what about the other two imaging microscopes in the same room that are already in use? - I just hope that they aren't 'nearby' enough to suffer. Ironically, I have always found the PCs and cameras on microscopes far more reliable than their cousins in the office and home. I do notice that often lights dim, TV screens fluctuate, etc. when you switch on high current devices (e.g., 8 kW showers at home), but that's obviously not due to EMP. At UCL our 18 W Spectra Physics laser draws 53 kW from the mains but has no discernable effect at all on lighting, PC screens, etc. when we switch it on, probably as it's isolated behind its own personal three phase power supply. Plus, of course, there is RF interference** that plagues TV, CRT and radio reception, that can be caused by switches, motors, etc. but that is also rather unlikely to kill off a PC or TV, although it isn't CRT and loudspeaker friendly (indeed audio noise from a weak signal to a stereo TV/radio decoder can often sound worse as it switches repeatedly in and out). In comparison I've never noticed a mercury lamp EMP produce any effect whatsoever on nearby equipment. *I suppose I am more concerned about EMP from a air-burst atomic bomb explosion, if our lab is unfortunate enough to be less than 10k m from it (or as Edward G Robinson put it "Sweet Mother of Mercy, is this the end of our confocal?"). **http://www.scribd.com/doc/267489/Tracing-And-Eliminating-Power-Line-Interference. Keith J Morris <kjmorris@well.ox.ac.uk> 21 Dec 2007

INSTRUMENTATION – plasma cleaners

Our lab is going to buy a plasma cleaner for our Tecnai F20 TEM. The price for commercial products from SPI, SBT, Fischione, and Gatan, can be from $12K to over $50K. Is the technology really so superior for the higher priced products? Could someone give some guidance for selecting a plasma cleaner? Yanling Ge <yanling.ge@tkk.fi> 19 Dec 2007

While it is reasonably straightforward to generate a plasma, there are important factors that can affect TEM samples. The first is gas chemistry. A plasma creates a wide variety of ions and radicals. Most of the important chemistry is the result of radicals. While oxygen radicals are the most important species involved in removing hydrocarbons from sample surfaces, other species and ions can be significant. Many vendors recommend high purity gases rather than air so that the gas chemistry can be controlled. A common gas chemistry is Ar-O_2. Argon is not reactive and can affect the sample only by ballistic impact. In a poorly tuned or designed system positively charged Ar ions can damage thin specimens and sometimes sputter material from holders. Replacing Ar with H_2, for instance, results in much lighter positive ions that do not damage specimens or holders. In addition, the design of the system affects how much energy the ions have, and thus how likely they are to damage your specimen. The second major factor is tool automation. More automated systems, if well designed, can result in more consistent results. For instance, the output impedance of the RF power source should be matched to the impedance of the chamber/plasma. Some systems have an auto-matching network that insures that power is delivered efficiently to the plasma, rather than being reflected back due to an impedance mismatch. Systems that rely on manually matching the impedance are prone to user errors that can dramatically affect cleaning efficiency. Likewise, if the gas flow is set manually, there is a danger that untrained users can get poor results. For example, if the gas flow (thus pressure) is too low then the mean free path of positive ions is increased. This may result in increased sputtering of the specimen. I think a sophisticated user can get good results from an inexpensive system. What you should get from a more expensive system is more flexibility and results that are more consistent. TEM specimens can be quite precious. Every facility has to weigh the costs/benefits against the risk of damaging specimens. Steven T. Coyle <scoyle@gatan.com> 19 Dec 2007

INSTRUMENTATION - sputter coater maintenance

I want to clean up the Denton Desk II sputter coater (equipped with AuPd target) in my lab facility. Does anyone have any suggestions as to how I can clean the plastic casing? Are there any solvents safe to use for this purpose? Marissa <mlibbee@gmail.com> 3 Jan 2008

Plastic encasing? Are you sure? Our Denton Desk II has a glass cylinder, which we regularly clean the inside by simply but carefully running a new (cleaned) razor blade around. It slides on the glass and peels of the metal. We remove the L-seals, and then we use an ethanol soaked cotton cloth to remove any remaining bits and fingerprints. The aluminum bits we polish with a "Green-Scratchey-thing" (3M) and more ethanol, then wiped down with toweling. The only "plastic" is the Teflon bits inside, and again we use ethanol and paper towels or cotton cloth to polish them up. The case work is painted metals and we use general purpose glass/surface cleaner on it. Richard E. Edelmann <edelmare@muohio.edu> 4 Jan 2008

If it turns out that your chamber is glass (which I suspect), after you get it clean, buy yourself a can of unscented White Rain hairspray and spray the inside surface of the glass tube. I would hesitate to do this to plastic because of the possibility of incompatible solvents. The next time you want to clean, just place the glass in hot soapy water. When the hairspray dissolves, your Au/Pd will also depart with little or no effort, and no scratches or cut fingers. Ken Converse <kenconverse@qualityimages.biz> 5 Jan 2008

INSTRUMENTATION – large capacity tap filters

We have been having major problems with our in-line tap water filters becoming clogged (sometimes on a bi-weekly basis). These string-filters remove sediment from the tap water that is used to remove heat from our closed-loop water chillers. Our Physical Plant has not been able to locate the source of the sludge and we end up paying them several hundred dollars to come and change two sets of filters. Does anyone know of a large capacity (self-cleaning) filtration system that could be used on the water coming into our small, microscopy building? Since our major use of water is probably the water chillers, it may be more advantageous to go this route rather than relying on the small, under-sink string filters. John Bozzola <bozzola@siu.edu> 28 Jan 2008

Our water line currently has one filter and no bypass; on days when I've had enough coffee to be especially lucid I can change a filter without the chillers noticing. However it is directly over a 208 3-phase outlet so while rushing to do the job fast, water drips and I end up standing in a pool of water while watching it drip into the outlet - not great. I sketched out a plan (as yet unimplemented) for a system with 2 alternate branches so that the flow can be switched to an unused filter unit while the loop with the used filter is isolated and the filter replaced. I have planned a drain/

flush spigot on the inlet side so I can flush the line upstream of the filters from time to time, or before a filter change. The standard filters are really cheap at McMaster-Carr (a couple of dollars, I think; I get a case at a time, we also have 'enriched' water). I can send the Prod# - we use a spun plastic (5 µm I think) instead of the string filters. It is going to be hard to beat the economy of standard mass-produced filter units for a low volume application supporting some chillers, etc. If you have the usual 'before and after' pressure gauges you can see the pressure drop developing across the active filter and change filters before trouble comes knocking. Dale Callaham <dac@research.umass.edu> 28 Jan 2008

I agree with Dale. You should change your own filters, if possible. I have seen the dual in-line filter and switching setups used in plants that he described. Get filters with a pressure relief button to make changing cartridges easier. The real questions are, "What is plugging the (cartridge?) filter and what is the size of the 'dirt'? Once you know the ID, from TEM for example, then you might be able to find the source or cause. McMaster-Carr shows a fiberglass unit very similar to a water softener. It is a 'sediment and dirt' filter unit with a back wash timer. It is item 9843K11, ~$400. It says, "Rated at 50 microns." You could try to rent one from a water softener company with the option to buy. If you are lucky, a large dealer will have softener units used in apartments for a short periods of time that are quite new and half the price. I did that to get a year old iron filter that used $KMnO_4$. I also bought a used water softener that had the resin and sand changed out by the dealer. Both worked just fine. Paul Beauregard <beaurega@westol.com> 28 Jan 2008

INSTRUMENTATION - chiller pump and lifting power

I finally got sick of the noise and heat being thrown off by my water chiller system for the SEM, so I decided to move it to another room. Before I do this, though, the water lines have to be run up into the ceiling, over about 20 feet, then down to the microscope again. I've taken into consideration the connection end and plumbed in a valve system so I have a cut-off to run water through the chiller and piping without circulating it through the SEM, but I'm still wondering about the lifting power of the pump. Does the power required to lift the water lower the flow rate, or does the drop on the other end create enough of a siphon effect to cancel out any effects of the lift? Justin A. Kraft <kraftpiano@gmail.com> 28 Jan 2008

I am sure the chiller manufacturer can answer this question for you. Our own experience in doing exactly the same thing was that we were able to run our (Haskris) chiller lines through the ceiling and move the chiller two rooms away----about 20 feet as the crow walks---with no problems whatsoever. However, when we needed that room for a new scope and moved the chiller again, we went a room too far with that extra 10-odd feet. The TEM began shutting down its lenses at frequent intervals and our JEOL engineer quickly figured out that the water flow was reduced to the point that we were running right at the edge of the water's ability to cool the lenses. A few degrees and the scope would shut them down. We were due to install a new TEM anyway, so he tweaked the temperature settings to allow a slight extra increase in temperature before it shut down, and we limped over the finish line until the new scope came. Another caution---when the lines run overhead be very careful to check for the development of leaks. Water spraying down on equipment can be fundamentally different that water puddling on the floor (we've had both happen and we've been lucky each time). Copper lines have the nasty tendency to occasionally develop pinhole leaks from being etched internally by distilled water. So, yes, problems are possible, but it will be a function of chiller pumping capacity and distance from the scope. Again, check with the manufacturer. Randy Tindall <tindallr@missouri.edu> 28 Jan 2008

From a plumbing point of view, your latter comment is correct: if the inlet and outlet are at the same height as before, there is no extra consideration from running the hose up and down. If the piping system were open, the height of the loop would be a factor. If there is a difference in height between inlet and outlet, that must be taken into account. However, the

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bigger concern in this case is the pressure drop due to the sheer length of tubing involved. Moving the chiller to another room and adding 15 to 20 feet just to loop over the wall adds a lot of extra flow restriction. Extra fittings may also be significant. Chemical engineers have tables of equivalent tube lengths for the many kinds of fittings. I have forgotten the details, but each fitting adds a substantial length (maybe up to a foot) to the effective length of the loop. It is good to keep them to a minimum. The pressure drop can be handled by switching to a larger pump or by switching to a larger size hose. However, neither option is cheap. Consider it as you make your plans. Warren Straszheim <wesaia@iastate.edu> 28 Jan 2008

The short answer is that it takes only 5 PSI of pressure for every 10 feet (one story tall) of vertical head or drop. So you only need 5 PSI extra to push "up hill". I am sure you have that much pressure. Suppose the chiller output is 50 PSI. The pressure at the top of the pipe would be the output of the chiller under normal SEM conditions minus 5 PSI. You will gain that 5 PSI back on the drop side of the inverted U tube. So the pressure at the SEM input should still be 50 PSI. If it's lower than 50, you could still have an air lock (see below). If you keep your bypass open, then the pressure will drop. Keep it closed and the SEM on-line during pressure measurements. The pressure losses from the delivery lines should be minor unless you use really small diameter pipes or have a lot of bends. Stick with 1/2" ID plastic, not copper. Use pipe rated for at least 100 PSI. Your problem might be in eliminating the initial inverted U loop's air lock at the top of the loop. You might consider installing a tee and globe valve at the top of the loop to bleed off *most* of the tapped air. Paul Beauregard <beaurega@westol.com> 28 Jan 2008

INSTRUMENTATION – ion getter pump longevity

just changed the ion getter pump (IGP) of the gun chamber only 4 years after the installation of our Tecnai G20. This change was advised by a FEI engineer. We do not even have 400 working hours on this machine and I wondered if it was normal that we already have to change the pump. We work mainly with ultrathin sections of biological probes embedded in Epon. They are pretty contaminated with carbon, as evidenced by light circles remaining on the sections after even a very short illumination time (I am usually working at 120 kV, LaB_6). I am wondering if this carbon contamination, evaporated by the electron beam, is not at least partly responsible for the dirtiness of the pumps (the pump of the column is bigger than the one for the gun, which would explain why it was not so dirty). If this is the case, perhaps a plasma cleaner would not only be a convenience for me but could bring a financial advantage for my boss). What would be the cost of a plasma cleaner? What is your opinion on the question? Do you think that a plasma cleaner would increase the lifetime of the IGPs? Stephane Nizets <nizets2@yahoo.com> 20 Jan 2008

The IGP is on full time, so even though there have been few hours that the instrument was in use, the pump has been working for the entire 4 years. This is still a short period, especially since there are relatively few contaminants in the gun volume (or should be). The mechanism of ion pumps is that residual gas is ionized and the ions are accelerated by an electric field and become embedded in the getter, which can get filled to the extent that any additional ion incident on the getter will dislodge an ion that is already there. The lifetime, therefore, depends on how good the vacuum is and how large the surface of the getter is. Other things that can shorten pump lifetime are the presence of water in the column--which is not likely to be an issue for your usage and is more of a problem in cryoEM and the formation of spurs on the getter surface. The indication for pump replacement is loss of performance, so if your vacuum is poor, where it had been good, then the pump should be replaced (assuming that a vacuum leak has not developed). I doubt that carbon is the problem, but I could be wrong about that. A plasma cleaner can remove contamination from the column, which will give you a cleaner instrument and a better vacuum, which, in turn, will increase pump lifetime, but I don't know whether that will be enough to cover the cost--I'll let the manufacturers of these systems answer that question. Bill Tivol <tivol@caltech.edu> 30 Jan 2008

I've been following this discussion with interest since I have never had an instrument with IGP's. I'm surprised to find that IGP's have a (sometimes rather) limited lifetime. When you say they need to be replaced, do you mean just the adsorptive surfaces or the entire sealed system? How much does this cost? A ballpark figure is fine. I need this information for planning and teaching purposes. John Bozzola <bozzola@siu.edu> 30 Jan 2008

Not seeing a discussion of bakeout, I'd bring that up. New generation pumps have built-in bakeout coils and instrument vacuum connections also have a bakeout coil surrounding each one. These pumps are essential for high vacuum that is highly beneficial for LaB_6 cathodes and especially critical for FE systems. For W systems, I have never seen an IGP unless it was set up to handle both W and LaB_6. Depending on the use of the system, eventually the gun chamber will become contaminated with gas and always does when the cathode or FE tip is changed. Part of the change out process is to bake out the gun chamber, IGPs and vacuum connections (the connections with a gazillion bolts like between IGP and column/chamber). During this time, isolation valves that seal off the column and the gun chamber are opened while baking so the main vacuum system pulls the junk out. When done, the valves are closed and IGPs start pulling the vacuum down further. During the bake out process, the junk in the Ta and Ti parts of the IGP also get dislodged and dumped. This can be a separate part of "recycling" an IGP that is saturated but not requiring repair. When the IGP needs repair/rebuild, the whole thing (minus magnet) is sent off for rebuild or exchange. The following list from Duniway gives some typical costs for this. http://www.duniway.com/images/pdf/pg/p-42-var-style-rebuilt.pdf I have not seen prices or services for the newer IGPs. Older tools that do not have bakeout coils built-in can still be baked using off the shelf heating coil tape. They are rated in watts, width and length and are controlled by a simple thermal feedback box. These units are typically around $500 or less and can save lots of bucks if a simple bakeout is all that is needed from time to time--and with cathode/filament change. With bakeout when the gun is changed and with reasonable care, my experience is that IGPs last for many years...perhaps up to ten years of continuous service. But of course, there is always the flakey one that does develop a flake and trips the IGP power supply and must be replaced. Gary Gaugler <gary@gaugler.com> 30 Jan 2008

For the ion pumps I've dealt with, the getter assembly is replaced. This is the plates and whatever holds them at the proper separation. I've sent the pump back to the EM manufacturer and gotten a refurbished pump back. I have had only very limited experience with this, however, because the ion pumps on the HVEM were very large with correspondingly large capacity and didn't need replacing for the entire 20+ years I was in Albany, and the pump in our FEG here at Caltech was covered by the service contract. Apparently it developed a whisker on the getter, the symptoms of which were that the vacuum would get bad fairly quickly and shut the FEG off. This happened every few months until the problem was diagnosed. Bill Tivol <tivol@caltech.edu> 30 Jan 2008

My IGP on my CM series TEM lasted 11-13 years. 4 years is quite short. If you think you have carbon or hydrocarbon issues, have your serviceman check the long column tubing liner just under the anode cap for a black coating of residue that builds up on the liner's inner wall. There should be a tool in the factory tool kit to remove this tubing liner. Look at the amount of the deposit and the color. Ask the serviceman if that amount of deposit seems normal or excessive. I would have a supply of 6-8 inch long wooden "Q-tip swabs" and Pol polish handy for him. Paul Beauregard <beaurega@westol.com> 31 Jan 2008

There are three little tricks for extending the life of ion pumps that have not been mentioned yet that I thought I could contribute based on years of experience with JEOL and Topcon TEM's: 1) The hammer. Yes, just smack the dickins out of the ion pump body (not the magnet) with a hammer. The shock knocks most of the whiskers and flakes loose. Do it

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with the emitter cold, because it will shock the whole TEM. Put emphasis on a large number of mild whacks in every direction of the pump housing. Keep the pump HV on, reading pump current and you will see lots of little spikes as the whiskers and flakes get dislodged. Wear hearing protection because you will need to do this for about 20 minutes. When you stop seeing any spike in ion current with hammer whacks, you are done. 2) Vent the system, pump it down, and restart the ion pump prematurely with over current protection turned off. The pump should get hot. That's basically a poor man's bake. It works. John Mardinly <john.mardinly@intel.com> 1 Feb 2008

For the ion pumps I've dealt with, the getter assembly is replaced. These are the plates and whatever holds them at the proper separation. I've sent the pump back to the EM manufacturer and gotten a refurbished pump back. I have had only very limited experience with this, however, because the ion pumps on the HVEM were very large with correspondingly large capacity and didn't need replacing for the entire 20+ years I was in Albany, and the pump in our FEG here at Caltech was covered by the service contract. Apparently it developed a whisker on the getter, the symptoms of which were that the vacuum would get bad fairly quickly and shut the FEG off. This happened every few months until the problem was diagnosed. Bill Tivol <tivol@caltech.edu> 30 Jan 2008

As the topic is still discussed, here are a few sentences on our experience: For an IGP pump, four years is short. 400 working hours far too short. On our FEI CM12, after 17 years of use, always at 120 keV, about 48 weeks each year and > 40 hours each week, at least 10 or sometimes 15 different users per year, we only have the second IGP now in place, since about 3 or 4 years. We have a TEM with a good vacuum, indeed. Since end 1999, we have a slow-scan CCD - so we do not use film any more. So the TEM is always on and under vacuum, at least 360 days per year. LaB_6 filament: changed once in 3 years, only, although frequently used. What we do: we always use LN_2, every day. Every evening, we shut down the IGP, for four hours, during the time when the anti-contaminator is warming up. This is part of the software package we have (v.12.5). This extends the lifetime of the IGP, we were told, by preventing the water, which was trapped at the anti-contaminator, from reaching the IGP. - This apparently helps to keep a good vacuum status, in general. Reinhard Rachel <reinhard.rachel@biologie.uni-r.de> 5 Feb 2008

Basically, the lifetime of ion getter pumps is determined by the amount of titanium available in their cathodes for producing the gettering action that is the basis of the pumps' operation, and also by the pressure range at which the pumps are operated. This matter is discussed in some detail in Sect. 7.1.8 (p 294) of my book Vacuum Methods in Electron Microscopy. As noted there, most manufacturers rate pump life on the basis of continuous operation at a pressure of 10^{-6} Torr. Typical values are 45,000 to 50,000 hours (5 to 6 years). However, if the pump operates at a pressure of 10^{-7} Torr (not unusual for pumps on the electron guns of modern instruments) then the operating life would be ten times as long, or of the order of 50 years. The development of "flakes", whiskers, and contamination on the insulators can shorten pump life, as mentioned by others. All of these factors are discussed in the above reference. Wilbur C. Bigelow <bigelow@umich.edu> 12 Feb 2008

LM - infinity corrected objectives

I have a user who was trying to use a camera without microscope to image something that he could not get on the stage of the microscope. In playing around, he discovered that Nikon objectives actually have a C-mount compatible thread. When he screwed a 2 cm extender and then a 20x objective onto the CCD camera, he was able to image a specimen at a distance of about 2 cm. Naively, I would have said that an infinity corrected lens should not form an image in the plane of the CCD chip, but obviously, the specimen is not at the appropriate focal point of the lens (~2 mm). Can someone explain why this works? David Knecht <david.knecht@uconn.edu> 24 Jan 2008

Clever set up! The answer is quite simple. For the sake of simplicity, a compound microscope has two key "imaging" lenses: the objective and the eyepiece. Optically, the job of the eyepiece is to create a (usually) magnified image at the appropriate location to act as a "specimen" for the eyepiece. In order for there to be an image, the light carrying the original specimen information must converge to a point of focus. There are two ways to achieve that result: a. To place the object just beyond the front focal plane of the objective (FFPo), resulting in a real image at some fixed distance b. To place the object exactly at the FFPo, sending the imaging information up through the optical train in a bundle of rays which is either parallel to the optic axis (on-axis info) or some principle ray at some angle to that axis (off-axis info). Note that if these rays are parallel, they cannot converge to form an image. We say that the information "goes to infinity", hence never forms an image. In this case, you need a second lens (the Telan or tube lens) to create the necessary convergence at the right location for the eyepiece. The space between the back focal plane of the objective and the tube lens is what is known as infinity space, a design which gives considerable freedom to microscope designers. So, if you take a lens that was meant to work in Condition b and move the object slightly further away from the front of the lens, you will change the optics to Condition a. This is another one of those cases, like NA, where what is written on the objective is only true when the microscope is properly set up and aligned for Koehler illumination. Barbara Foster <bfostermme@sbcglobal.net> 24 Jan 2008

David sent me a follow-up to this posting, asking how to incorporate all of this info into teaching microscopy. I know that a number of you teach and might find the information useful, so here's a copy of my answer to him. There is a really neat way to do all of this. It starts with a simple experiment with a hand lens. a. Difference between object and image: Using a simple hand lens, have the students look through the lens at their finger nails (be prepared for lots of silly groans!). First, have them put their finger close to the lens, and then have them slowly move it back. As they do, the image of their finger will become larger and larger. At some point it will disappear. Then, if they keep watching carefully, the image will reappear, inverted. Then, have them remove the lens and look directly at their finger. At this point I tell them, "Notice that at no point did your finger leave your hand." This really solidifies the concept of object (their finger) and image (what they saw through the lens, after the lens has operated on that information. Take home message: our job as microscopists is to capture in the image, with as much fidelity as possible, the information from the object. Second take home messages: Lenses can lie. b. Find the focal length of the lens: Using a simple, single hand lens, have the students find the focus the image of the overhead lights on the table in front of them. The distance from the physical center of the lens to the table top is the focal length. This set up the following concepts: focal length, focal plane, and front focal plane. c. Four cases of lens: Now that they understand the concept of object/image and focal length, you can repeat Experiment A to illustrate the case of the object. (1) Inside the focal length (forms virtual, upright image on same side of the lens as the object. (2) At the focal length (information goes to infinity: no convergence of date; No image). (3) Slightly beyond the focal length (real image, on other side of the lens; magnification determined by distance of object from lens). (4) a great distance beyond the focal length (light coming from "infinity"; rays form bundle which is parallel to either optic axis or principle ray through optical axis). You can reinforce all of these using simple ray diagrams found in any high school physics book. d. All of this sets up the discussion for: (1) Infinity vs. fixed tube length optics and why you just can't willy nilly change objectives from stands of one design to stands of the other. (2) Spherical and chromatic aberration. (3) Which then leads to discussions of different types of glassware on the microscope and how to make educated buying decisions based on corrections, working distances. It's a great set of lecture/demonstrations that really carries through to discussions of NA, resolution versus detection, and contrast techniques...a little bit of physics that goes a long way. And because they are doing demonstrations throughout the lecture, they stay involved and tend to remember it all (every teachers' dream). Barbara

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Foster <bfostermme@sbcglobal.net> 24 Jan 2008

EELS - carbon contamination

I found bad carbon contamination when I put Cr_3C_2 samples in a 2010 FEGTEM for EELS analysis. They were thin film cross sections made by conventional sandwich method by Epoxy glue, and Gatan PIPS ion milling. I have examined three samples made by the same route, two turned out contaminated easily, but one seemed not much affected. The samples were subjected to selected area EELS in diffraction mode and STEM ADF imaging. Carbon accumulated at the edge of the selected area circle. Once the beam was focused, carbon K-edge was enhanced in the EELS acquired. Amorphous carbon in EEL spectra stops me from studying the real carbon bonding from the Cr_3C_2 phase. My questions are: (1) Why were some samples contaminated, and others less so? (2) What could be the most possible contamination sources? (3) How can I minimize the contamination both during sample preparation and during TEM examination? (3) How reliable is the carbon data acquired? Zoe Zhou <z.zhou@sheffield.ac.uk> 12 Feb 2008

Let me hazard a guess or two. I suspect that may be breaking down your carbide to form your amorphous carbon when you are focusing the beam onto the sample. I had a sample of a pulsed laser deposited diamond-like carbon film. You can see an image of this film on our website in the application note #59 (http://www.southbaytech.com/appnotes/59%20EELS%20of%20PLD%20DLC.pdf) that was prepared by MicroCleaving(TM). The film was amorphous, but there are alternating light and dark bands in the DLC film. EELS showed that the darker bands were SP3 bonding while the light bands were SP2/SP3 or more graphitic. We only had a parallel EELS on a CM200FEG. When the spot was focused down in order to isolate a dark band, the earliest EELS spectra showed no Pi-star peak, but almost instantly, started growing one and a spot formed. If the beam was spread out, no contamination was seen. It was only in the highly focused beam that we got it and in the first instant, you could clearly see that the dark bands had no SP2 bonding. I always wanted to revisit these samples with a GIF to examine the sample without the conversion, similar to what Jim Bentley did with his GIF and carbon films. Another question for you if you think that it is actual contamination is have you plasma cleaned your sample? If you always plasma clean your samples before putting them in the microscope and other samples do not contaminate in your microscope, then the source is not your sample and not your microscope, but is probably the mechanism that I described above. For a source of contamination, check the O-rings and lubrication on your whisper lock on the PIPS. The same Ar gas that is used for the guns is used for the lift assembly. A colleague of mine has demonstrated conclusively that the Ar gas was contaminated by the lubrication from the lift mechanism in the PIPS. We strongly advise our customers who have either a Gentle Mill(TM) or an IV3/4 equipped with a low energy gun to not share the gas line with a PIPS instrument for that reason. Your difference in contamination could be the amount of time the samples were left in the PIPS. However, if you plasma clean your samples, you will eliminate this source or any other external source of contamination on your samples. Scott D. Walck <walck@southbaytech.com> 12 Feb 2008

I have had the same experience with many different samples. The contamination is visible on the sample and why or how some are cleaner than others seems to be a mystery. However, it must be correlated to cleanliness of glues, acetone, and alcohols used during preparation. Try to keep them clean, generally not sharing with others helps. After a soak, follow with a rinse of fresh liquid before drying with clean air. If possible plasma clean your specimens before putting them in the scope. Any plasma cleaning will help, but I have found the H_2/O_2 recipes superior to Ar or Ar/O_2. I am assuming that you are using the liquid N_2 cold trap on the 2010. Have is cold before inserting the sample. If you have some old liquid Freon in the lab, you can use it to rinse the sample before inserting in the TEM. This is a great help, but not for the environment. If you do not have Freon, or access to a plasma cleaner, and must work with the samples as they are, then you could try "locking the carbon in place on the surface of the sample". This might be done by using a large spot size beam spread over a relatively large area, to bake the carbon in place. I am not sure how well this works or for how long you have to let the beam set to be useful, but others will probably offer advice on this approach. Roseann Csencsits <rcsencsits@lbl.gov> 12 Feb 2008

RUTHERFORD BACKSCATTERING SPECTROSCOPY - elemental composition

I was wondering if anyone can give a comment on which analytical tool can give the best composition ratio of the solid materials. As far as I know, Rutherford backscattering spectroscopy (RBS) can determine the composition of bulk sample. X-ray photoelectron spectroscopy (XPS) can determine the composition of the surface. Does anyone know which one can give a more accurate composition? By the way, is RBS a non-destructive tool or a destructive tool? Jean Chen <andreana782@yahoo.com.tw> 19 Dec 2007

It will be difficult to find a direct comparison between the two methods. The dependant scales are too different. To make a comparison, one should make XPS depth profiling, supposing that the ion gun etching will not modify the composition of the sample. We have tried a lot, between XPS, AES, RBS and X ray reflectometry, and we never get a direct accord. One gets different sets of results, and one has to weight them with all one knows about the samples, and decided where is the right (or the less wrong) line between them. About the RBS, it should not be destructive, but as someone has soon said, it depends on your samples. The destructive effect may be surprising! I had once had a set of samples to observe with the SEM, after they were "treated" by RBS. They were covered with a very thick layer of carbon (tens of nm) due to contamination by the poor vacuum of the RBS vessel, and induced by the He^+ beam! For our purpose, it was "destructive"! Jacques Faerber <jacques.faerber@ipcms.u-strasbg.fr> 20 Dec 2007

CATHODOLUMINESCENCE DETECTOR - magnification

We have a JEOL 8600 Superprobe with what has been described as the "poor man's" cathodoluminescence (CL) detector, which is simply a PMT inserted in the optical path of the probe's integrated light microscope. I have a very basic question concerning the system: where does the magnification of CL image come from? In addition why is the field of view the exact same as that in BSE or SEI? I find it hard to believe (perhaps incorrectly) that the magnification comes from the light microscope: the magnification is continuous as opposed to the fixed magnification (focal length) of the light microscope and the magnification range of the CL images is beyond the useful magnification of the microscope. What am I missing? Jack Hietpas <mikroskop@gmail.com> 31 Jan 2008

I don't know your exact setup, but my guess is that the signal from the PMT is synchronized with the beam scanning on the sample. For each position on the sample (pixel) the magnitude of the PMT is recorded as the intensity on the image. Just like a SE or BSE images. Hendrix <drix00@gmail.com> 31 Jan 2008

Project MICRO: New Sandbox Contact

We are pleased to announce that the Microscopy Society of America's (MSA) collection of sand for use with Project MICRO Microscopic Explorations activity 6 is now housed at McCrone Associates, Inc. in Westmont , Illinois; and is under the direction of Heidi Ullberg. Mr. Joe Neilly has faithfully dispatched sand samples to educators all over the country for many years. Joe has enjoyed his duties as "Keeper of the Sandbox", and as he passes the shovel to Heidi says that he will miss visiting the far reaches of the world - one sand sample at a time. To view an inventory of the collection visit MSAís website at: http://microscopy.org/ProjectMicro/Sand/SandCollection.html. To request sand samples, for educational purposes only, please email your request to hullberg@mccrone.com . Please consider donating sand samples to help keep the SANDBOX full. Donations can be mailed to: SANDBOX, Heidi Ullberg, McCrone Associates, Inc., 850 Pasquinelli Drive, Westmont, Illinois 60559-5539. Heidi Ullberg <hullberg@mccrone.com> 21 Jan 2008.