NetNotes

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Selected postings from the Microscopy Listserver from April 30, 2014 to June 30, 2014. Complete listings and subscription information can be obtained at http://www.microscopy.com. Postings may have been edited to conserve space or for clarity.

Specimen Preparation:
annealing nanoparticles on C-film grids

Has anyone annealed nanoparticles on TEM C-film grids? If so, can you tell me the temperature and time at which the carbon started to break down? And the specific grid you used for the experiment? ***Marissa Libbee mlibbee@lbl.gov Thu Jun 5***

I have been heating C-films on gold 200 mesh grids up to 1000°C in-situ in the electron diffraction camera without damage for the film during my work on the lowering of the melting temperature of gold nanoparticles (P. Buffat, *Phys Rev* A13 (1976) 2287). The oven was designed to create a black body environment and ensure a nanoparticle temperature measurement as accurate as possible. Trials with Mo grids failed because either the grid roughness forbids any film gliding under thermal expansion or a chemical reaction occurs between Mo and C that could lead to Mo-carbide. Philippe Buffat philippe.buffat@epfl.ch Fri Jun 6

I never encountered any issues when performing annealing experiments using a conventional heating holder and a variety of support films. Silicon nitride films seem robust regardless of ultimate temperature and ramp rate. Carbon films, however, will start to show evidence of partial graphitization at just a few hundred C, and your NPs can become enveloped in sheets of graphitic-like carbon. This can be a big problem if you're studying a process which requires matter transport to or from the particle, as presumably the graphitic sheets will impede this transport. You also need to keep in mind that your metal grid can generate NPs. See: Z. Zhang and D. Su, "Behaviour of TEM metal-grids during in-situ heating experiments," *Ultramicroscopy* 109, 766 (2009). Chris Winkler microwink@gmail.com Fri Jun 6

Specimen Preparation:
negative staining nanoparticles

Researchers from another campus want to be able to prepare TEM specimens of their nanoparticles by themselves and are asking for a protocol. I want to make things a bit easier for them by substituting uranyl acetate (UA) with phosphotungstic acid (PTA) for negative staining. Can something go wrong because of this replacement? ***Vladimir Dusevich dusevichV@umkc.edu Fri Jun 20***

You did not say what kind of nanoparticles. If Metallic than they may not need any stain. In my experience UA gives a more even stain than PTA. PTA seems to have a more blotchy background. Also, do they have the capability to glow discharge? If not than any stain will be problematic and even distribution of nanoparticles on the grid may be a problem. Debby Sherman dsherman@purdue.edu Fri Jun 20

From my own experience with UA (acidic) and PTA (neutral; usually both at 2.0%) for negative staining of viruses and macromolecular complexes, I have observed differences in stain distribution, resolution and - interesting enough - also the optimum concentration of the specimen. UA typically requires a less concentrated sample for optimal results. Having said that, both stains can produce very nice results, but a switch from one to the other might require adjustment of some parameters in the protocol. Do these nanoparticles actually need to be stained? Depending on the material/size/question at hand you might try to dry them down on a C film, with or without washing. Guenter Resch lists@nexperion.net Sat Jun 21

Thank you very much for reminding me about glow discharge. Somehow it skipped away from me. Thanks to everybody for all replies. Particles are organic (pharma). Vladimir Dusevich dusevichv@umkc.edu Mon Jun 23

This conversation brought to mind a recent experience we had here with silver-based nanoparticles. We don't have a specialized instrument for glow discharge, but we use a brief low KV, low amp pulse from our sputter coater (which we normally use for SEM sample coating). For most negative staining and other dried down samples, this works well. However, for small nanoparticles (~10-20 nm diameter range) there is a problem. This procedure produces spots which are in that range and cannot be easily distinguished from nanoparticles. We know this because we always do controls. In our case we found it was not problem to examine these nanoparticles on grids that we had not treated with glow discharge. For the silver particles there was no need for negative stain of course. I guess what I am saying is with nanoparticles, make sure you do controls because there are numerous ways of particle-like artefacts of the sample preparation process. Duane Harland duane.harland@agresearch.co.nz Mon Jun 23

Specimen Preparation:
metallography release agent for epoxy pots

Our met lab produces wonderful EBSD-quality specimens using standard 32 mm epoxy pots. However, I've got some specimens of porous ceramic I want to heat-treat after polishing: breaking out the specimens from epoxy would leave residual polymer in the pores, which I don't want. (Assuming I can break them from the epoxy without shattering the specimens.) Any thoughts on how to get samples to release from epoxy pots? I'm considering embedding in superglue or wax prior to giving them to the met lab, and then using acetone to float them free from the epoxy pots later. Any thoughts on if this will work? Any better plans? The samples need to be small (10×5×0.5 mm), so I don't want to make our tech hand-polish them if I can avoid it. ***Chad Parish parishcm@ornl.gov Thu May 1***

Some time ago, as we polished ourselves some single crystal slices for surface science studies. We embedded them in a polyester resin instead of epoxy, to be able to get them free after polishing. It was a bi-component resin for metallography (Sody 33, sold by ESCIL in France), which polymerize at room temperature and is dissolved by acetone. I suppose you could get similar products in the USA by Sturers, Buhler, or others. It's less hard and has more retraction than epoxy, but it works. After polishing, one put the resin block in acetone overnight, and one would find it as a jelly the following morning. It's then easy to pick up the sample and after 1 or 2 more washings in acetone, the samples were clean enough to be mounted on the sample

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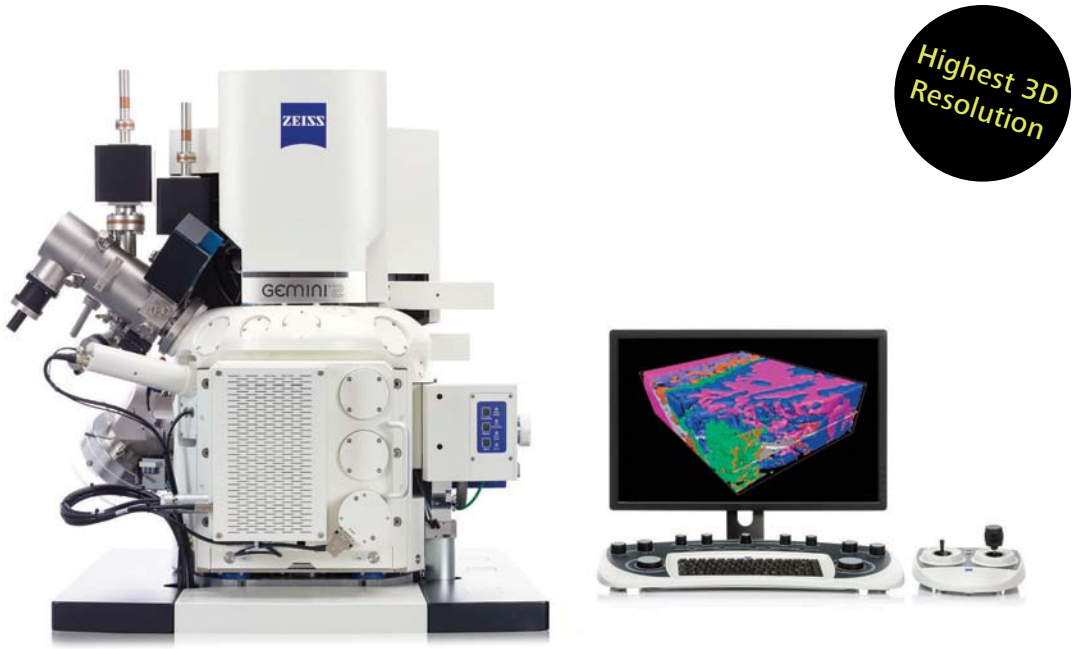

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holder for our study in an ultrahigh vacuum. They were not porous, but as far I remember, I think that washing them in acetone in a semi-closed vessel at 40°C in an ultrasonic bath should do the job for a good cleaning. Another way would be to try to dissolve your epoxy using the "Strip R" solvent mix sold by Epotech. But it's not a very nice product, toxic for the user and complicated to remove after use. Jacques Faerber jacques.faerber@ipcms.u-strasbg.fr Mon May 5

Specimen Preparation:
cryo glue options for SEM

I'm interested in hearing about your experiences with various cryoSEM "glues" for sticking down samples to holders prior to freezing, including pitfalls, advantages, disadvantages, etc. Also, for live insect or other invertebrate samples, what are your suggestions for immobilizing them so they can be stuck to the glue prior to freezing? ***Marie Cantino marie.cantino@uconn.edu Mon May 19***

I was involved with the development of one of the first SEM cryo systems and since that time have always used Tissue Tec as my adhesive of choice. Steve Chapman protrain@emcourses.com Mon May 19

We used to use TissueTek but switched to Instrumedics Cryo-Gel because it's so much easier to handle. It's a gel, so doesn't run all over the place before being frozen. We use it a lot to arrange our samples precisely before cryostat sectioning. If there's a gel form of TissueTek I'm sure that'd be fine too. Rosemary White rosemary.white@csiro.au Mon May 19

Specimen Preparation:
cleaning an evaporator

Does anyone have an effective method for cleaning metal deposition from the inside of an evaporator bell-jar? ***Roger Ristau roger.ristau@uconn.edu Thu May 8***

I use Bon Ami, a non-abrasive kitchen cleaner, a damp paper towel, and scrub. Nothing else. Phil Oshel oshel1pe@cmich.edu Thu May 8

I have used a number of solvents to little effect. I have also used mild abrasives (Softscrub or polishing abrasive followed by thorough rinsing and drying) to some better result. However, the most effective cleaning I have done was using 2" wide, clear packing tape. Simply stick it on, rub it a little, and the residue comes right off with the tape when you pull it off. It works with both metal and carbon. You can even recover the gold if you want, however, there is usually very little. This works on the evaporators and sputter coaters with plastic chambers which would not fare well with either solvent or abrasive cleaning. This does not work very well on the metal parts inside the evaporator. Elbow grease and abrasive cleaning (and patience) is required there. On a side note, given enough use of the evaporator, the metal or carbon will coat the vacuum feed-through, ceramic insulators, and O-rings so that they are no longer insulating. This will cause the evaporator to use too much current during evaporation and the unit will begin to blow fuses or cause other malfunctions. It is necessary to disassemble and clean those from time to time. For the ceramics, it is necessary to use an abrasive cleaner such as Ajax, Bar Keepers Friend, etc. Products with finer alumina such as Softscrub (or polishing compound from your lab) are not as effective on the unpolished ceramics. O-rings can be cleaned with lint-free cloths, re-greased and replaced. Better yet, replace the O-rings with new ones. Phil's message suggesting using Bon Ami, damp paper towels and scrubbing is in line with my experience. I know it is obvious, but do not leave water or cleaner residue in the chamber. Dan Crane crane.dan@dol.gov Thu May 8

This may not help you with current cleaning but will with subsequent ones. After the bell jar has been cleaned, we have had good luck using a thin layer of dish soap on the inside of the bell jar which allows the next cleaning to relatively easy as the metal coating will release much easier with warm water. The procedure we use is to clean the bell jar with warm water to remove the prior coating, then using a small drop of dish soap and rubber gloves, smooth it around the inside of the bell jar until it is a white film and allow to dry for a little while. Then replace the bell jar and pump down to outgas. I usually run a "dummy" coating to make sure all is well. Scott Payne scott.payne@ndsu.edu Thu May 8

Fullam used to sell a product called Victawet that you evaporated onto the surface to make cleaning easier. Dan Crane crane.dan@dol.gov Thu May 8

I agree that a thin layer of detergent is good, but my experience is that dishwashing detergent is better than dish soap. And this gives me the opportunity to get off track for a moment. The main purpose of shaving foam is to make the skin wet. So I shave after showering with no shaving soap - it works just fine. However the mirror tends to get steamed up during the shower. The solution to this problem is to apply a little dish-washing fluid to the mirror. Then it does not steam up. Alwyn Eades jae5@lehigh.edu Thu May 8

I spray the inside of the bell jar or cylinder with unscented White Rain hair spray. Warm water will dissolve the hairspray and release the metal, whether evaporated or sputtered. Using unscented avoids unneeded volatiles in the vacuum system. Ken Converse kenconverse@qualityimages.biz Thu May 8

We use Jif (known as Cif in some places). It is a non-abrasive cleaner probably exactly like Bon Ami. After years (decades in my lab's history) of using Wenol to clean parts of our TEM gun etc., we were first surprised then tried it on the advice of a senior FEI engineer. The main ingredients are calcium carbonate particles and a couple of surfactants. As long as the scented versions are avoided, it leaves no residue if rinsed properly since all ingredients are very water soluble. Duane Harland duane.harland@agresearch.co.nz Thu May 8

It has been a long time since I cleaned a bell jar, but I seem to recall using Bon Ami, as others suggest. However, to facilitate removal of subsequently deposited materials I seem to remember evaporating a fair size piece of rock salt onto the inside surface right after cleaning. This is a non-organic material with low vapor pressure, and, as I recall, did a pretty good job as a release agent for the next cleaning, Wilbur Bigelow bigelow@umich.edu Thu May 8

Who would have thought that such an innocent question would bring so many interesting solutions? Thanks to all for great ideas. If the improvement in my results is as good as suggested, I may quit my day job and go into the "window washing" business! Roger Ristau Thu May 8

Specimen Preparation:
non-destructive viewing of insects

I am an undergraduate student at the University of Manitoba working on a revision of a genus of parasitic wasps. I have three new species that I would like to take images of in order to see their sculpturing, and I'm trying to figure out which SEM I should use. The specimens are about 3mm in length, and because they are not mine, I need them to remain as is (they have to be returned in the same condition in which I received them). The insects are point mounted, which means they are glued to the end of a small, pointed piece of paper which then has a pin stuck through it. The glue used likely dissolves in ethanol. The two scopes we have here at the university are: Philips XL 30 and JEOL JSM-5900LV. I'm hoping someone could provide me with a recommendation on which to use, and advise me on what I will need to do if one of these scopes is suitable. Any information would be of great help! ***Melanie Scallion scalliom@myumanitoba.ca Sat Jun 28***

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I think you are going to have a tough time viewing these in an SEM in a way that leaves them in an untouched condition. An alternative would be to see if you can get access to a high quality stereomicroscope - ideally one with software that allows extended depth of focus (EDF) images to be taken. One problem with a conventional stereoscope is that you can see the top of the insect in focus but not the bottom or vice versa. With EDF software, one can grab a digital image of each focal plane and the software reconstructs an image that is in good focus for the entire depth of the specimen. We have a motorized Leica MTOF FA stereomicroscope in our core facility and clients working with insects love it. You can see some examples of images taken with and without EDF on this microscope at our website http://www.biotech.missouri.edu/mcc/Stereoscope_M205.html. Tom Phillips phillipst@missouri.edu Sat Jun 28

Tom's suggestion to use EDF is a good one. Those motorized Leica systems are excellent and pricey. Good manual alternatives are available at much lower cost. A good DSLR kit on a stereo with Helicon focus or other free or inexpensive software packages do a good job.

Just ensure the software has a stereo microscope plugin to account for the lateral image shift as the stereo is focused. Jim Schulte jim@ms-imaging.com Sat Jun 28

I agree that the light microscope and EDF software would produce some great images. I have produced some fun images of bees, flies, and mosquitos with a stereo microscope and free edf software called Combined ZM. The natural color of the light microscope images adds to the image compared to gray-scale SEM images for low-mag work.

For SEM, the JEOL 5900LV will be your best bet for examination in an undisturbed state. In the LV mode, you can observe the wasps without coating and should be able to get some good images of general physical characteristics. You will not be able to get real high magnification images since you will be limited to backscattered imaging you would have to sputter coat the samples to image secondary electrons in the 5900LV or the Philips XL 30 for higher magnifications. If you can sputter coat a specimen, you should be able to get great images of the fine details with the Philips. Larry Hanke hanke@mee-inc.com Sun Jun 29

I would recommend that you contact Sam Droeg. He works for the U. S. Geological Survey Department, and has developed a technique and hardware system for macrophotography of insects. He has posted remarkable insect photos on the web, and can talk you through your macrophotography need with a digital camera and macro lens. His contact information is: sdroege@usgs.gov or 301-497-5840. Ed Haller ehaller@health.usf.edu Mon Jun 30

Software:

Windows XP replacement

I am required to eliminate all Windows XP systems and replace them with Windows 7 systems. I have been unable to determine how this may affect a JEOL1400 or if new software and drivers are required. Has anyone completed this OS upgrade on a JEOL1400? ***Larry Ackerman larry.ackerman@ucsf.edu Wed May 7***

We got around this by getting the IT guys to set up a separate VLAN for our old XP systems. We have at least 3 systems that cannot be upgraded. This seems to work fine - data can be uploaded to the stand-alone server and people can then download it to their computers. This could be an interim solution even if you do eventually convert all to 7. Rosemary White rosemary.white@csiro.au Wed May 7

Image Processing:

Digital Micrograph and Windows 8.1

Has anybody tried Digital Micrograph (DM) in a Windows 8.1 environment? I have heard mixed stories, and the agent in the Microsoft store insisted that anything that runs in Windows 7 will run in 8.1. ***John Mardinly john.mardinly@asu.edu Mon Jun 9***

I'm running DM 2.11 (actually Gatan Microscopy Suite) under Win8.1. I'm running the off-line version of DM. Note that the device drivers that talk to the hardware may or may not work under Win8. Henk Colijn colijn.1@osu.edu Mon Jun 9

Instrumentation:

sprinkler systems

I know this has been discussed before, just wondered if there were any new thoughts. We're moving twice, first into a refurbished basement, second time into a new building. The basement has a sprinkler system in case of fire, unlike our current building which just has alarms. It's a single story brick building. I imagine the new building will have some sort of sprinkler system too. What precautions, if any, do people take to protect against the unlikely showering of your confocal or EM? Are there any relatively simple alternatives? One alternative is to have fire-doors and walls for each room but that is prohibitive. ***Rosemary White rosemary.white@csiro.au Thu Jun 26***

Out of interest I have dealt with water cascading over microscopes, yes it has happened twice in my 49 electron microscopy years. As a service technician the main problem is not so much the water, but the debris that the water beings down onto the electrical circuits. Both of the instruments in question were running on vacuum only and managed to keep going overnight until security found the water running through the particular laboratories. One was a tap exploding, shooting a fountain of water into the EM room, the other the result of putting out a fire three levels above. A third situation occurred when a water pipe came off one instrument in a basement EM unit, flooding the complete area to about 6 inches. Every one of the instruments kept going, but as I arrived a technician with rubber boots and a long wooden pole was taking care of switching the most endangered instruments off. However this was in the 1980s when most EM Units were underground; the one I mentioned was also used as a Tornado shelter! Steve Chapman protrain@emcourses.com Fri Jun 27

We had Perspex canopies made up for our SEMs, Auger system and SIMS machines to protect from direct sprinkler spray. Barry Lamb barrylamb@meadtest.com Fri Jun 27

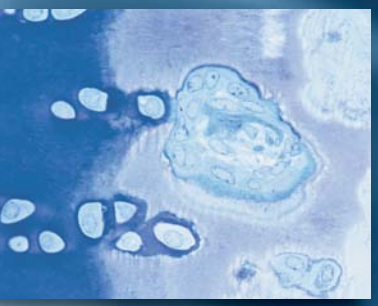
My question is: "How many people have experienced sprinkler systems go off in their microscope rooms?" Anyone want to jump in a relate experiences? John Mansfield jfmjfm@umich.edu Fri Jun 27

As hinted at from my post on the confocal listserver, I worked in an E. M. lab years ago in a hospital with a sprinkler head that developed a drip. Fortunately, the head wasn't over the microscope, and was swapped out. In my new lab, the engineers and construction crew put a sprinkler head directly above my TEM column, which I made them move to the left wall of my microscope suite. Ed Haller ehaller@health.usf.edu Fri Jun 27

At a previous job of mine, we had a sprinkler head spontaneously fail catastrophically. The entire contents of the rooftop reservoir emptied into the space, which had offices and labs (fortunately "just" test equipment). Lots of water, and had a dispersion of fine black particulates in it. Apparently this built up from reactions of water with the pipes over long times. Very destructive. Larry Scipioni les@zsgenetics.com Fri Jun 27

I am willing to bet that no one has experienced an accidental release of water from a sprinkler system. Flooding from other labs or burst pipes, yes, but sprinklers I think not. John Mansfield jfmjfm@umich.edu Fri Jun 27

Here is a situation that I bet not many have encountered: We had a TEM that developed a leak in lens cooling with the result that the inside of the TEM filled with water. (I was not here but was told the



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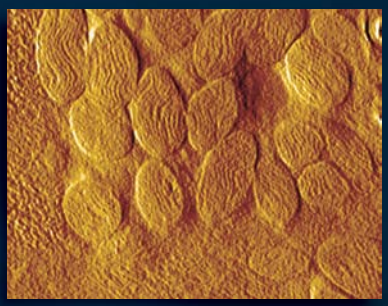

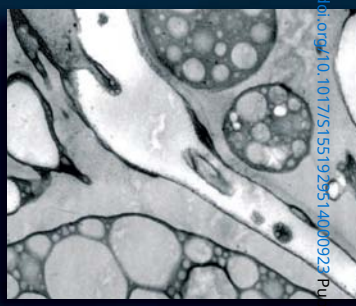

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camera/viewing chamber looked like "a fish bowl.") My predecessor, Larry McCurdy, courageously attacked the mess and restored the TEM to service! It soldiered on for many years. Point of story: Flooding the outside of the TEM may actually be more harmful (electrical shorts, etc.) than flooding the inside! Roger Ristau roger.ristau@uconn.edu Sat Jun 28

I would like to inform that the same thing (micro-leak in the cooling line at the junction plastic hose to copper-fitting of the projective lens of my Zeiss EM109) happened in our EM-Lab (it was 18th April 2000 after nearly 20 years of operation). It took some hours to get rid of all the water within the viewing chamber and the 6×9 camera beneath the viewing chamber. It needed a new projective lens, which is still functioning. I don't remember what the cost for repair was. Wolfgang Muss w.muss@salk.at Mon Jun 30

For your information - a summary of responses. Thanks much to all, very useful. 1. Alternatives are available, but expensive. These include: firewalls around each room lacking sprinklers, and fire door entryway - rather expensive. The walls and door have to be able to resist fire for 1 hour. Gas systems - also expensive, and in Australia these are apparently only allowed in non-occupied rooms (I am told, not going to fight it, save fight for other stuff). 2. Accept the inevitability of sprinklers, and insist on the following: Sprinkler head(s) as far from instrument as possible, esp. from main power supplies. Sprinkler heads enclosed in wire cages to prevent accidental knocking when moving large items in the room. 3. Realize that floods from pipes/sinks/rain events are much more likely than sprinkler activation, so: Install drip pans above the sprinklers, either below the ceiling or within it, to deflect potential floods from above away from the instrument. Raise important components at least several cm above floor level. Have a system that only fills the sprinkler pipes when the sprinkler(s) are activated to avoid slow leaks (drips). 4. Also realize that sprinkler pipes may be sources of conductive interference, so: Ask that pipes are empty unless sprinkler(s) activated. Insert a rubber or other non-metallic connection between the main sprinkler supply system and the sprinklers in instrument rooms. And also, check your insurance policy! Like others, the only floods I've experienced were from other sources - in a previous institution on one torrential weekend several staff came in to check for leaks and ended up doing a bucket brigade from the EM unit in the basement. All fine, except for the piles of paperwork around desks. And Roger Ristau's email reminded me that about 15 years ago the in lens cooling system in our JEOL 6400 developed a leak and flooded the lens, detected by water dripping down into the chamber - messy! The old girl is still chugging along, though not for much longer - the old electronic boards are cracking and won't survive the move, so turning off for good in October. Rosemary White rosemary.white@csiro.au Sun Jun 29

Instrumentation:

floating floor

As mentioned, we're eventually moving into a new building, and if possible, we'll incorporate all the best building recommendations. I remember when all TEM and SEM rooms had floating floors to eliminate or at least substantially reduce vibrational noise. Is this still the best option? It's rather expensive and if not really needed we could divert the saved funds elsewhere. We are planning to have a plant room in which all the noisy components - pumps and fans, mainly, are isolated from the main instruments. We have a VP-SEM and have a FESEM on the to-buy list for 2-3 years' time. No TEM any more. We'll be on the ground floor and it's a fairly vibration-free site - no main roads nearby, etc. Just need to make sure the threshing and grinding equipment isn't installed next door. ***Rosemary White rosemary.white@csiro.au Mon Jun 30***

We moved the department wholesale into a new building last August. During the design stage I was heavily involved in benchmarking good laboratories with the structural engineers (Ramboll, www.ranboll.co.uk) and our vibration/noise consultant, Michael Gendreau of Colin Gordons & Associates (www.colingordon.com).

Michael was instrumental in our decision to do without isolation plinths when he demonstrated that, in the low frequency regime, i.e. below the resonant frequency of the plinth, the amplitude of the vibration was worse than the outside structure, i.e. it was an amplifier of noise. Above the resonant frequency, the plinth attenuated it, as expected (referring to a damped harmonic oscillator). The Q-factor of each plinth was determined by the damping material underneath the plinth. In some cases the isolation block was moving 100 times faster (at resonance) than the surrounding building (in one lab we tested).

The money was spent on making the single raft slab thicker - we doubled it from the early design thickness of 1 m to 2 m thick and that is what we have now (the main department building is 0.5 m thick). Further, we have separate room-within-a-room build with each microscope having a cross-laminate timber walls and ceiling that are 20 cm thick. The rooms were finished with acoustic panels on the ceiling and 2 walls to reduce noise. Room cooling was with a chilled ceiling (95% cooling) with two plenums for a little cool fresh air. For a view of the room, see here: http://www-hrem.msm.cam.ac.uk/facilities/cm30/index.shtml. We have found that this works really well for us. For example, we were getting information out to 0.9 Angstrom with an FEI Titan TEM in the old building, but now, in the new, heavy, foundation we get information down to 0.6 to 0.7 Angstrom. In summary, the foundation of the building is as big and heavy as we could make it and we avoided floating tables/isolation plinths. Michael acknowledged that this was what they were recommending ten-to-fifteen years ago, but have decided against it since when evidence accumulated that they only work in certain circumstances. Jon Barnard jsb43@cam.ac.uk Mon Jun 30

Instrumentation:

chiller problem

The chiller of our FEI HRTEM F30 is consistently at 10°C. Even after increasing the temperature, it is not increasing. I would like to know the possible reasons: is it due to the faulty temperature controller or the sensor? ***Rashmi Mehata rashmi_mehata@yahoo.com Wed Jun 25***

Did you measure the 10°C yourself or is this what is displayed on the cooling unit? This might help to identify if the sensor is broken or not. Depending on manufacturer, another culprit might be the valve that controls the flow of water of the secondary cooling circuit through either the heat exchanger or the bypass - if that is stuck, you are constantly cooling. Did you already try to contact the manufacturer of the cooling unit? Guenter Resch lists@nexperion.net Thu Jun 26

If your chiller is from Haskris, you can call them for "over the phone diagnostics". I have done it already twice; bad parts were correctly identified, ordered, and replaced by myself. Vladimir Dusevich dusevichv@umkc.edu Thu Jun 26

EM:

disposal of old negatives

I have scanned in all the old images that I thought were good and now want to get rid of the TEM and SEM negatives I have had for many years. Does anyone know if they are recyclable? ***JoAnn Buchanan buchsmith@gmail.com Wed Jun 4***

Yes, your TEM and SEM negatives are recyclable. Rochester Silver Works (http://www.rochestersilverworks.com/) does this. For many years they were part of Eastman Kodak and they recycled over 50 years of negatives from our lab. I suspect that there are other recyclers who do as well. John Minter jrminter@gmail.com Wed Jun 4

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TEM:
diffraction pattern calibration

We have a new Tecnai Osiris TEM in our lab. We tried to calibrate the diffraction pattern with Standard MoO_3 specimen. It was found that the long edge corresponds to the long g vector(001). According to TEM textbook edited by William, there is no rotation between diffraction pattern and image. However when we go to HRTEM, we just found that the FFT for HRTEM rotates 90° relative to the diffraction pattern (the sample we use is a HCP structure metal). Could anyone give me some idea to figure out the 'conflict'? ***Hongbing Yu 12hy1@queensu.ca Tue May 6***

Most scopes arrange the lens currents so that the image and diffraction modes have no rotation for as much of the magnification/camera length range as practical. For the magnifications at the very low and very high ends, the manual should list the rotations for both image and diffraction, from which it is easy to determine the rotation between the two modes. If that gives 90°, there is no 'conflict'. Bill Tivol wtivol@sbcglobal.net Tue May 6

TEM:
LaB_6 on Tecnai BioTwin

We are in the process of switching for the first time from a tungsten to a LaB_6 emitter on our Tecnai BioTwin 12, circa 2006. I have gotten varying opinions about whether we should shut off the emitter every time we change samples. On our BioTwin (perhaps on all Tecnai 12's?) the gun does not seem to be isolated from the column during specimen exchange (V5 stays open to maintain pumping by the single ion pump), so we are concerned that blowing vacuum during sample exchange (as happens from time to time) could destroy our expensive filament. On the other hand, I have been told that it's not good to turn the LaB_6 on and off frequently, as would be necessary to load multiple samples during a session. I am interested in hearing your experience running a LaB_6 on this instrument: do you shut off the beam each time you change samples, and has the emitter been damaged when you have blown vacuum on the column? Also, how long have your emitters been lasting? ***Marie Cantino marie.cantino@uconn.edu Thu May 29***

We have been running a LaB_6 emitter in our Philips CM12 for over 20 years. When changing samples we leave the beam saturated. Even the most experienced users and well-trained newer users will, on infrequent occasions, encounter a situation when enough air enters the column after pre-pumping to cause the high tension to go off and on rare occasions to cause vacuum valves to close as part of the fail/safe system. We believe the impact on the life of the LaB_6 emitter is minimal compared to what daily multiple saturation and desaturation would cause. Typically, our emitters last more than 2000 hours of saturated beam time. Incidentally, it was recommended to us by our service engineer that we leave the high tension on and the beam desaturated at all times when not in use. Donald Gantz gantz@bu.edu Thu May 29

We run one or two instrument on LaB_6 emitters - a Philips CM30 (300 kV) and a JEOL 200CX (200 kV). We always turn the heating current and high tension off for every sample exchange for two reasons. First, there is a substantial danger that you will get a beam of X-rays emerging from the goniometer, especially if the beam is on and the HT is active. We have measured X-ray flux of several hundred micro-Sieverts per hour coming from X-rays emerging from the (empty) goniometer with the beam on, several micro-amps of beam current and the objective aperture left in. With older instruments this is a significant problem (and enough to warrant inspection from the Radiation Safety Officer). If the HT and beam is off, there is no risk at all. Yes, it is a mild inconvenience, but it beats the risk of inadvertently radiating our users. Second, LaB_6 filaments tend to oxidize over time so having a puff of air go up into the gun with a hot filament is unwise. We have about 30-40 users on the instrument and everyone has, at one time, vented the vacuum system during a sample extract. It is a matter of when not if. The last LaB_6 filament we had lasted 3600 hours or about over a year and a half of use. Jon Barnard jsb43@cam.ac.uk Thu May 29

This topic has been discussed a few times, I guess, but I am happy to forward our (overall very positive) experience with LaB_6 on FEI 120 kV TEM's. Our CM12 is operated with a LaB_6 of one manufacturer, at 120 kV, always, exclusively, without any problems. Contrast at 120 kV is fine and depends very much on the sample preparation and the specimen quality and (also on the voltage, I know, but...), and also on the quality of the CCD camera and its settings. Since >20 yrs, we decrease the Filament (=emission) from 18 to 20 down to Zero, during sample exchange, and raising back to 18 to 20, after inserting the holder (and IGP below around 20 or so); that's all. High voltage is left on at 120 kV, always, during day time, even when inserting a cryo holder. Really no problems at all, here, on a TEM CM12 used by >20 users over the years. Our LaB_6 filaments (manufacturer on request only) last for >3 years, the present one for almost four years, with 5 days-a-week-use, 8-12 hours per day. Vacuum on our TEM is really good, with IGP at 5 to 10 (liquid nitrogen is used every day, every hour). We do not open the column... as we record images fully digitally (no films, since 1998), and the LaB_6 stays in the column for 3 to 4 years. This is advantageous... only positive experience with LaB_6 on a TEM of this kind, for bio-samples of all kinds. It also depends on the training of the users, what 'high vacuum' really means, how to achieve it, proper starting of the TEM in the morning, cryo-cycle every night (=IGP off for 4 hours), and so on. Reinhard Rachel reinhard.rachel@biologie.uni-regensburg.de Fri May 30

TEM:
beam fluctuation

A variation in beam intensity is observed with a LaB_6 filament. But the LaB_6 filament is showing the connectivity. What could be the possible problem? ***Rashmi Mehata rashmi_mehata@yahoo.com Sat Jun 2***

There are many reasons why a tip may become unstable but one of the most common is a buildup of material inside the cathode aperture. Steve Chapman protrain@emcourses.com Mon Jun 30

TEM:
cryo-safety/risk assessment and other questions

I am helping to start out the cryo-TEM in my lab and is planning to train new users who need to use this technique. While generally I am familiar with the procedures in cryo-plunging, sample transfer from TEM-box to Gatan cryo-holder in the cryo-workstation, I cannot perform such procedure if I am wearing a pair of cryo-gloves because the gloves are thick and it is impossible to use them to hold the tweezers and to pick up the copper grid in the pool of liquid nitrogen/liquid ethane. That would mean that my hands are not protected from the liquid nitrogen and this can be risky. My institute is very particular on safety and I need to conduct a Risk Assessment on this procedures. May I ask how safety is implemented during these procedures? Another enquiry would be on the technical aspect during sample transferring. I notice that if I have to transfer the grid box from the Dewar (during storage) to the Gatan workstation, the grid box will be exposed to the atmosphere where a rise in temperature and icing are expected. This can be minimized when I quickly move the grid box into the workstation.

However, I find that it takes a longer time to transfer the grid box from the workstation back to the conical tube or into the Dewar. Will there a better solution to reduce the time for the grid box to expose to the atmosphere and humidity? There is a cryo grid box handling tool by Gatan. Is it very useful in minimizing the temperature rise and humidity when transferring the grid box from the Gatan workstation to the storage Dewar/conical tube? ***Tay Yee Yan rongchigram79@yahoo.com.sg Sun May 18***

Here in the UK we have a health and safety clause called "best practicable means", this basically says if you cannot perform the action you may take another route. To handle cryo-related components with sufficient dexterity is often made impossible using what would be called suitable gloves. In which case we would fall back on the clause, what happens in other countries? I am responsible for making health and safety checks on car race tracks, so you may imagine the complex areas that have to be covered by the same clause. Steve Chapman protrain@emcourses.com Mon May 19

Thank you very much for your suggestions and recommendations. A consensus would be to avoid wearing gloves which does makes sense because it would be extremely dangerous if liquid nitrogen is trapped. On the other hand it would be interesting for me to check with my safety office if 'the best practicable means' exist in my country. Guenter and Jen, if you didn't mind, could you send me the link to access the safety presentation as well as the picture for the modified Nalgene bottle? Tay Yee Yan rongchigram79@yahoo.com.sg Tue May 20

As others have said, trying to use cryo-gloves is bad practice. In fact, it is much more likely that you could be harmed trying to do the fine manipulations required while wearing these than without gloves. Liquid nitrogen (LN_2) will not harm the skin unless it stays in contact for an extended time, but the same is not true of liquid ethane or propane. Occasionally small drops of this cryogen can be produced during the plunging process, and if they contact your skin you will see initially a white patch, typically ~1 mm in diameter, on the skin, which becomes a small blister. This is generally only an annoyance. A certain level of skill is required to manipulate the grids with cooled tweezers. If one leaves them cooling too long, the upper part can get too cold, but too little cooling of the tips can heat the grid. I have taught people to move quickly, but not to rush, when performing the necessary actions. When transferring a grid box, keep it upright so that some LN_2 is present in the slots holding the grids. I usually use a large tweezers, ~20 cm long with ~2 mm wide tips, to grab the box either from the tube or station. Once again move quickly, but don't rush, and drop the box into the station or tube. If you want to remove a box from a tube containing other boxes, I use an intermediate holder. A Styrofoam box ~10 cm×10 cm×3 cm deep is good for this. Dump out all the boxes from the tube, find the one you want, then put the others back. I have not had any problems with ice on either the grid boxes or the grids themselves using these procedures. Bear in mind that there is cold N_2 vapor above the LN_2, so anything in that layer will stay cold, but too rapid movement can mix the cold layer with room air; another reason not to rush your movements. I have found the old style covers, disks with a slot that can be rotated to uncover a slot or to keep them all covered, work better than the newer style "witches hat" covers. The newer covers often drag a grid out of its slot as they are unscrewed, and this is particularly problematic if the cover is opaque. It is also the case that the boxes with the disk covers are more compact, so more of them will fit into a tube. Our lab had a 13 mm punch, and we bought 30 cm × 30 cm sheets of an appropriate plastic—it has to be OK at LN_2 temperature, and it is best if it is transparent. We punched out as many disks as we would need, drilled a central hole for a screw and another for the inner end of the slot, then made the rest of the slot with a Dremel tool. I prefer using steel screws to plastic ones, since I have broken a few plastic ones trying to loosen them. Bill Tivol wtivol@sbcglobal.net Tue May 20

TEM:

selected area electron diffraction software

We are looking for suitable software allowing calculation of selected area electron diffraction (SAED) spot patterns. The aim of our investigation is to determine the zone axis of minerals. Using an experimental SAED pattern three diffraction vectors and angles between them are measured. These parameters should be compared with the calculated one by taking into account the lattice constants, space group and extinction conditions of the studied crystal. Do you know any software allowing this task? ***Boris Reznik boris.reznik@kit.edu Wed May 7***

I use Single Crystal. It works very well for many cases. It is not really the tool for indexing a completely unknown crystal. It has some zone searching features, but that can still require elbow grease. Pros: If you have a pretty good idea what the crystal is, or you know it is one of a few. (I usually do EDS first so I do have a decent idea what I'm looking at) then this is hard to beat because you can rotate the crystal live. (Also great for getting a feel of the reciprocal space of a given crystal before your TEM session so you go quickly to a good zone.) You can easily see relative goniometer positions. If you know what a specific reflection is, you can tilt the crystal orthogonal to it to find a specific zone axis, so it has been useful in recognizing that sometimes there are similar looking zones and with other software you may get fooled. For ALCHEMI, you can see when you are aligned along a certain column of atoms easily. You can adjust the unit cell parameters easily for comparison between a couple phases. Cons: Kinematic only. Searching for complete unknown zones is a bit tricky. I recommend you get on a good zone axis when taking your patterns, don't just try to index a [15,3,7] zone (although, I have managed it in the past). http://www.crystalmaker.com/singlecrystal/ Zack Gainsforth zackg@berkeley.edu Wed May 7

For this, I use CaRIne crystallography software http://carine.crystallography.pagespro-orange.fr/ Elena Belluso elena.belluso@unito.it Thu May 8

TEM-EDS:

spatial resolution of Zn

I was asked if it would be possible to use TEM-EDS to map locations of zinc ions incorporated into cellulose nanofibers (CNF). These fibers can be quite long but are often less than 20 nm in width. At this point I have no idea as to the amount of zinc that could be incorporated into the fibers or the distribution. Does anyone have an idea as to the spatial resolution limits of mapping nano-scale ZnO on CNFs? I am not sure that TEM-EDS is the way to go or if another technique, such as AFM, would work better to confirm the presence and distribution of the Zn on the CNFs. ***Debby Sherman dsherman@purdue.edu Fri Jun 13***

My initial thoughts are that the first back-of-the-envelope calculation I would look at is minimum mass fraction and minimum detectable mass - see Carter & Williams, 2nd ed., chapter 36. If the Zn loading is low and they are not agglomerated into dense clusters, the whole thing might be undetectable before spatial resolution

ever comes into it. However, if you do get good ZnO clusters (~ nms), the carbonaceous nanofibers are probably small enough and poorly scattering enough that spatial resolution will be ~ probe size, I would guess. (But run the calculation -- also Chap. 36 in CW2) A FEG with a big X-ray detector is indicated. HAADF might do the job, too, because Zn will scatter much better than carbon. As an example, I've mapped ~hundreds of PPM aluminum in a steel, but only because it was agglomerated into aluminum oxide precipitates, and because I used a Titan w/ChemiSTEM instrument (Parish et al., *J. Nucl. Mater.,* V445, P. 251, 2014). Chad Parish parishcm@ornl.gov Mon Jun 16

This looks to be an ideal application for the atom probe tomography. Samples are already forming sharp tips so not much sample preparation would be necessary - just their lift-out (which could be still difficult). But it might be more difficult to access such an instrument and it is a question how it would be able to analyze this heterogeneous sample, a combination of metal and organic material. Tomas Hrncir tomas.hrncir@tescan.cz Mon Jun 16

You have an additional problem with ZnO. In addition to what others have said, ZnO nanoparticles are very electron beam sensitive. My experience is that this material damages and sputters quickly over the entire range of 80-300 kV. You will likely need low dose techniques which are not really useful during XEDS analysis. Nestor Zaluzec zaluzec@aaem.amc.anl.gov Mon Jun 16

SEM:
no filament current

Anyone have suggestions on how to diagnose 'no filament current' on my vintage JEOL 6100. Recently I relocated the SEM to a new facility and now after replacing the tungsten filament twice I still get no current on the filament current meter? I'm just looking for a simple set of trouble shooting steps I can try. ***Rod Rowland vwporscherxr@gmail.com Fri May 23***

If you already have checked for broken filaments without improvement, it may be tricky and possibly expensive. 1. Check all fuses, 2. Check the HV cable using an insulation meter (megaohm-meter capable of 1000 V) between both ends of the cable. It should read values below 1 Ohm. 3. If the cable is ok, the HV-tank may be defective. It happened to me after moving a JSM35c 100 m. Or the load current control unit if you are lucky. 1&2 you can do yourself, but you will at least need telephone advice or email advice for 3 & 4 from a service technician. Erik Ordell erikordell@icloud.com Fri May 23

SEM:
asphalt samples

I have a request to run natural asphalt samples on the SEM for imaging and EDS microanalysis. The customer, an astrobiologist, is interested framboidal pyrite as a biomarker in samples from an asphalt lake. He describes samples as "gooey" which gives me pause. I am in need of guidance from anyone who has experience with asphalt or tar samples on the SEM? Any precautions, caveats, or sample prep tips. Any assistance or recommended references would be greatly appreciated! ***Tom Williams tomw@uidaho.edu Mon Jun 9***

If it is gooey, expect a lot of outgassing and possible -- even probable -- contamination. Can you get him to run a specimen through a GCMS and determine what volatiles are present in what concentrations? Mike Andrews udsd007@gmail.com Mon Jun 9

Can't claim having actually done imaging of tar in SEM, but I wouldn't dare to put asphalt into an otherwise clean instrument unless there was a way to cool it prior to pumping and keep cooled during imaging (i.e. cryo-stage) to prevent evaporation of (and coating insides of chamber by) heavy volatiles. Valery Ray vray@partbeamsystech.com Tue Jun 10

SEM:
variable pressure control

While operating in variable pressure mode, our system will not control below 100 Pa. I normally operate at 30 Pa for non-conductive samples. It is an older system and the air supply lines are starting to fail. But I don't hear any air leaks when I remove the front of the cabinet. We did verify the fluid levels in our roughing pumps and topped them both. We do have a call in to Hitachi service, but they can't get to us until July. Does anyone have experience with this problem? Are there some suggestions you can make to diagnose the problem? ***Dan Fairweather dan.fairweather@delphi.com Wed Jun 25***

We had a S-2460N for many years. In fact, we just shipped it off working a week ago. I think it may have had a similar problem. About every six months to a year, the system would stop controlling and we would find that the needle valve that determines the chamber pressure had bound up. Under the original setup, the valve would never really settle down. It remained in an oscillating mode turning a half turn this way then a half turns back. The valve was always in motion. Eventually the threads galled and it became hard to turn. You could still turn it by hand - with difficulty, but the motor was not up to the task. Hitachi eventually got it figured out and changed some components in the control board so that it did settle in and the valve turned much less and lasted much longer. Our valve was located in the right rear of the column cabinet near the top. We could remove the back panel and watch the valve in action. As I recall, when we had problems, we would unplug the motor cable and set the valve by hand for the desired pressure. I think there was another line used to vent the chamber so we could just about "set it and forget it". Of course, Hitachi then came in, replaced the valve, and we were back on our regular routine. Warren Straszheim wesaia@iastate.edu Wed Jun 25

Specimen Preparation:
vital DNA stain

I am trying to correlate super resolution imaging with TEM on Tokuyasu frozen sections. As an internal marker in fluorescence, we would like to stain the nuclei before cryoprotection and freezing. I would appreciate any recommendations for a rather robust vital nuclear stain. The STED imaging is done in green channel. ***Michal Jarnik michal.jarnik@nih.gov Wed Jun 4***

Have you tried DRAQ5? You could excite the dye with a 488 nm laser - Is that what you mean "green channel"? Zhaojie Zhang zzhang@uwyo.edu Wed Jun 4

DRAQ5 is far red, so 488 nm excitation would be far from optimal. What about SYBR Green? Jill Pflugheber jpflugheber@stlawu.edu Wed Jun 4

Fluorescent dyes which stain nucleic acids are intercalating agents, don't expect them to be "vital" (well it depends on how long you want to observe the living cells). Vital stains also have to permeate live cells and even permeate them 2× for the nucleus, one through the cell membrane and one for the nuclear envelope, which is not so easy. I don't mean to be deceptive but as far as I know all nuclear labels are based on nucleic acid stain, meaning they are not vital. That said, it is seldom necessary to stain the nucleus both in LM and EM in single cell experiments because it is such a big structure with high contrast. I hope someone proves me wrote and offers you a solution. Stephane Nizets nizets2@yahoo.com Thu Jun 5

Specimen Preparation:
re-sputtering samples for SEM

Need some advice about the effect of sputtering twice the same sample for SEM. I had a delicate preparation (pre-embryo hatching from the zona pellucida) showing fine details in SEM. Then I removed

some rubbish from the surface of the sample and gave it a second gold sputtering with exactly same settings but half the time of the first one. In the second observation the surface was like "fried" and fine details were lost. Please click here: http://www.eikonika.net/v2/download4.php (upper photo is the first sputtering, lower is the second). Looks like the surface was either blebbing or it got a heavy and uneven disposition of gold particles. I was surprised by such a strong detrimental effect from re-sputtering and wonder if anybody knows more about this phenomenon and how can be avoided or minimized. ***Yorgos Nikas eikonika@otenet.gr Sun Jun 8***

I am not a biologist, but I have worked with all sorts of sputtering systems, and what you see in your second picture has nothing to do with sputter coating. Your second picture would suggest to me that you have removed a surface layer and you are seeing underlying structures; which is quite fascinating but quite typical with SEM studies. Steve Chapman protrain@emcourses.com Sun Jun 8

Thanks for your input. The problem occurs only after such delicate biological specimens pass again from the sputter coater. Of course "after" does not mean "because", and I keep you comment that it is not looking like a metal disposition. Rather the specimen surface is affected and it needs a second passage from the sputter coater for this to happen. Maybe the first observation in SEM renders the surface susceptible to subsequent metallization? I use 10 KV and a current beam ca 20ua that is 2-3 times lower than the factory recommended value, because I found that these settings prolong 4-5 times the filament's life. Also my sputter coating is not optimal because I don't use argon. Could these aberrations be involved in the problem? I think this is possible, especially since I had no comments from people using more orthodox settings (somebody must have tried second sputtering). Probably the best way to tackle the issue is to use very short times of re-sputtering and see. Yorgos Nikas eikonika@otenet.gr Mon Jun 9

It seems to me, that your samples were stored in the high humidity before second sputter-coater run. In such conditions the biological tissues might be rehydrated and then their surface might collapse in the sputter-coated vacuum. This might result in structures you have seen after second sputter-coating. Oldrich Benada benada@biomed.cas.cz Mon Jun 9

For some difficult samples I often have to use multiple coatings, with different tilt angles, but only for short periods, say 30 seconds per coat. Multiple coating may build structure on a specimen, but that is usually only visible above 15,000× if carried out with care. In some laboratories the operators forget that even when coated specimens may charge, and the best solution is to lower the accelerating voltage as well as to coat. Modern instruments work very well at 5 kV or lower, and if you continue to use the low emission currents that you mention that too helps reduce charge. The third route to operation at a lower charge rate is to use a spot size/probe current that is far smaller/lower than normal. For example, working at 5,000× use a probe current that would be more suitable for 30,000×; all part of the small steps that you need to make to operate without the complexities of too much coating. If you have a TTL instrument move away from the upper detector to introduce more of the converted backscatter (SE3) to subdue charge. Using air for sputtering does not make that much difference at low magnifications, it just means you will have a different coating on a dry day compared with a wet day. Long coating periods, particularly using old style high voltage sputter coaters, may cook the specimen! However the modern low voltage coaters should never reach anything like the same heat generation. After all that I have said there is one sure route to determining the true "surface" structure of your specimen, and that is to use all of the tricks I have mentioned above and very low accelerating voltage; i.e. 1 or 2 kV. Even if you are only able to look at the specimen for just a short time, it will give you an idea of what the true structure is. But remember you are now penetrating far less into the material so the structure may be totally different from your 10 kV! I never look at a specimen with a sputter coating until I have tried to see what it looks like and how it behaves using all of the low dose methods mentioned above. May I say I love the grey scale that you have produced with your SEM pictures, for me the levels are very good. Keep trying and I am sure you will see that sputter coating may not be your problem? Steve Chapman protrain@emcourses.com Mon Jun 9

Thank you for your comments. As Oldrich says, re-hydration may be an issue, however specimens more than one year old have no signs of deterioration and humidity is generally low in Athens. But maybe the combination of some humidity and repeat sputtering changed things. I have an old style coater (Hummer V) using high voltage and the specimen looked rather cooked (to use Steve's word) than anything else. Also there were two off line comments, one from a plant morphologists saying that some changes after re-coating occur even in the relatively hard plant surfaces. And a material scientist says that using gold without argon may etch the membrane. Since no problem occurs in the first coating even if it is a very long one, I feel that the combination of second sputtering plus an unknown factor are holding the answer. If any of you has soft animal tissue specimens, a gold target, and can take the trouble to re-sputter a spare specimen, this will be of great help to understand what is going on. Steve, thank you for praising my gray scales. It took me almost two decades to understand that a low contrast micrograph contains much more information than a high contrast one, and if you wish, this information is easy to enhance later with a computer program Yorgos Nikas eikonika@otenet.gr Tue Jun 10

Specimen Preparation:
cornea for SEM

Someone is asking me to do SEM on his samples of cornea. I do SEM on metals, semiconductors and so on normally. I do not have much experience on this kind of biological samples. If someone on this list server knows how to do it, please tell me. I have a SEM of Fei XL-30 available for this sample. ***Zhenquan Liu zhenquan.liu@asu.edu Wed Jun 4***

The preparation of biological material for SEM depends very much of the instruments you have at your disposal. Unlike metals and semiconductors, biological material contains a lot of water and, because you have to remove it for SEM, the material loses its structure and tends to be very unstable under high vacuum. For this reason the biologists need critical point dryers for example. Hopefully you have access to such instruments as well as to the person with the expertise to use it. By googling with "preparation cornea for EM", I found the first link to be interesting: http://www.optics.rochester.edu/workgroups/cml/opt307/spr05/anant/, Stephane Nizets nizets2@yahoo.com Wed Jun 4

Thanks a lot for replying from several people for my previous email which asking for a way to do SEM on cornea. I have learned a lot form these replies. There are so many things I have to go through sample preparation and actually we do not have most of the instruments in our lab, but there are available in School of Biology. I read these replies carefully and I did not see anyone who mentioned wet method on environmental SEM to get images from cornea. We have Fei XL-30 Environmental SEM. We can do wet method. A wet method is to introduce some water vapor near the sample to keep sample wet. The humidity can be controlled to 100%. I think that it might be difficult to do so. On this list server there are many experts in biology field, but none of them mentioned wet method. I think if it were a

good way to work on cornea, people would have told me. Many years ago I tried wet method on the surface of the samples in water. I did not get much useful information. I might miss something when I did it. Therefore if someone has some experiences on wet method, even better on cornea, please tell me. It will be great. Thanks to Stephane, Lee Cohen-Gould, Debra Sherman and Ed Haller and more people who replied to my previous email. Zhenquan Liu zhenquan.liu@asu.edu Wed Jun 4

We have an FEI Quanta and could probably work in the same regime as you can. Also like you, I am a materials scientist, not a biologist, but I get the occasional question to look at biological material. Most recently, it was an aggregate of bacteria. The request came from a Civil/Environmental Engineer so I don't think they understood what they were asking nor would they quite know how to interpret what I found, if I had found something. That should be the first question - What are they looking for and what do they expect it to look like? A lot of biological material will not be that interesting given all the water present and the organic membranes. In my little experience, the unprepared samples just don't have much contrast when you are looking at the outside. It is cell wall and maybe a film of water. I would be interested in hearing what the biologists have to say about imparting contrast. Of course, the structure should be much more interesting on the inside but I suppose it needs enhancement, even then. I understand it is technically possible to keep a sample from dehydrating. The triple point of water is at 0.006 atm or 600 Pa at near 0°C, so it should be possible to maintain a saturated atmosphere. Of course, that requires a cooling stage. If you are working at room temperature, that number rises to about 2300 Pa. That presents more of a challenge. I was not able to keep my bacteria granule from drying out in the SEM. I'll be interested in hearing any recipes that prove helpful. Warren Straszheim wesaia@iastate.edu Wed Jun 4

I have worked with XL30 ESEM-FEG and with Quantas. For biological samples there are some realities often poorly understood until you starting trying things yourself: 1) You can't really see much on samples that are truly "wet". You will see a flat boring film of water. 2) There is a point during the pumping process, before the ESEM valve starts cycling, where you actually have no control over the chamber environment. Some dehydration WILL occur during this stage. 3) If samples are in solution, any dehydration will cause salts to precipitate and obscure what you want to see. Assuming you have the cooling stage, these problems can be (mostly) worked around. Placing a few drops of water around the sample, on the Peltier stage, can help keep the local environment "more humid" during pumpdown. Pre-chilling the sample helps for the same reason. Using too much water can lead to other problems, such as rapid bubbling which can dislodge a loose sample or splatter on the final lens/detector/etc. Keep a long working distance during pump down. As Warren mentioned, make sure you really understand what they/you are looking for. Growth or debris on the surface? These should be possible. Shape distortions or internal structure? Maybe not so much. I did actually look at corneas and other eye parts some years ago. The truth is that we got the best results, given what the customer was looking for, with a 'gentle' fixative followed by a few rinses and then imaging in ESEM. Sorry that I can't remember the fix or solution, a med student came up with those. You are still getting some advantage by not coating the samples, and you have several minutes to an hour or more in which to study the surface...if your humidity control is good. This is certainly not to say that ESEM doesn't work, it's great for many things but you will have trouble with the most delicate, high moisture content samples. In reality you are trying to keep the humidity as close to 100% as you can, and work reasonably quickly. Once water droplets actually form on your sample, imaging is usually poor. Make small adjustments with ESEM pressure and stage temperature, wait for the system to stabilize a bit and go again. Also note that the Peltier sample holders cannot be too large. We had one made to keep the entire cornea or lens in contact with cold metal. Even then, the top surface may be wetter OR dryer than the rest. Make sure you watch some samples drying out so that you really understand what the initial dehydration artifacts will look like. Good luck! Matt Lynn mlynn@falaboratories.com Thu Jun 5

Specimen Preparation:

Buehler Vibromet polisher

I have resurrected an ancient Buehler Vibromet polisher (model 67-1517) and would like to use it to put a final polish on probe samples. I have been unable to find cloths or abrasive pads for it (12-inch model) and wonder if anyone has experience with this polisher. ***Nick Foster nfoster1@uncfsu.edu Tue May 27***

We get ours from Buehler, Part Number: 40-7222, 10 pack of 12" Microcloth with adhesive on the back. John Chandler jpchandl@mines.edu Tue May 27

Are you sure you want to do that? What kind of samples do you process? My experience is that vibratory polishing with colloidal silica can lead to topography that causes problems rather than resolving problems. Differences in hardness can lead to polishing relief. It can also chemically etch some samples. And there can also be a problem of silica residue on the sample after polishing if the cleaning protocol is not thorough enough. It is quite difficult to remove those deposits once they form. Warren Straszheim wesaia@iastate.edu Tue May 27

When I worked at NASA GRC we used the vibratory polishers for final polishing of microprobe samples. Instead of colloidal silica however we used something we called 'Magic Dust'. It is 0.5-micron diamond powder available from Kay Diamond Products. Unlike colloidal silica it would keep the samples flat with little contamination. After installing a cloth we would use a diamond extender lubricant from Metlab Corp then sprinkle the Magic Dust on the cloth and put the samples on using the weighted holders for 1 to 2 hours. David Hull drhull@zoominternet.net Wed May 28

Microtomy:

hand microtome

Do any of you have experience using a hand-held microtome? I have a colleague who is interested in purchasing one to use as part of an introduction to microscopy class for our undergraduates. Hand-held is not something I've ever used, and I'm a bit skeptical. I'd love to get her at least a Vibratome, but I'd bet they are out of our current price range. Any advice? ***Kristen Lennon kalennon@hagerstowncc.edu Wed May 14***

For undergrad classes, we've always just used double-edged razor blades, snapped in half. Students can either hold the tissue down with their finger and slice across (like slicing an onion), or put the tissue between pieces of potato or carrot to support it while cutting. Students learn this easily, just need to start them on some big, easy to cut tissue, e.g. Coleus stem or similar. Our 2nd year students learned all their plant anatomy by doing a project sectioning different parts of a native seedling, some of these were quite woody! Reason for selecting these plants is that there are no diagrams to copy from books, so they have to look at their own tissue. (Cynical from experience.) We have a couple of hand microtomes, they don't get used much. You need a good old-fashioned large (beard) shaving blade to get good sections from this, which is more dangerous than razor blades in my opinion. A simple one is described here: http://

www.microscope-microscope.org/activities/school/microtome.htm But see http://prometheuswiki.publish.csiro.au/tiki-index.php?page=Making+hand+sections+without+support+material for hand sectioning without support material, and in the grey box on that page are some alternatives using various support materials. We still do most of our sectioning by hand. Rosemary White rosemary.white@csiro.au Wed May 14

Perhaps the pdf via link http://www.modernmicroscopy.com/article_pix/040810_quarterblades/quarterblades.pdf will (Anna Teetsov: "Quarter-Razor blade for hand-sectioning") will contribute a little bit. I recall a very old publication (technical note, 1960/70ies?) on a hand-made serial cutting instrument, where, several full-sized razor-blades were mounted into a "holder construction" with at least two leading pins (as in wet shavers for men), up to 5 or 6 razor blades slipped over using kind of (really thin, according to the thickness of slices wanted) spacer/distance rings in between each razor blade, fixed at both ends and equipped with a handle. The "instrument" was fabricated either in a shop or was built as handicraft work. This way one would be able to "section" slices/slabs (like "micro-grossing" with a defined thickness equidistant from the original big specimen. Unfortunately I can't find the article/sketch of that instrument; nevertheless I think this is worth to be mentioned. Wolfgang Muss w.muss@salk.at Fri May 16

Image Processing:

opening Bruker Esprit EDX HyperMaps in Matlab

I would like to take STEM-EDX maps acquired using Bruker Esprit and open the raw spectral data in Matlab for more hands-on processing of the spectrum images. Has anyone had experience converting the BCF file to another format or importing directly into Matlab? Ideally I would like to convert the product back to BCF as well to make use of what the Esprit software does well. ***Andrew Wagner awagner@umn.edu Fri May 30***

I work with an Oxford Aztec that also takes spectral maps. I've also had users ask about taking the data in a portable format that they can work with it on their own. I generally discourage them. At a minimum, I try to discuss with them at length what they have in mind for the data. Many times, it seems that they don't know what they want to do with the data, but they'd like to have it anyway. Most do not seem to appreciate what would be involved in processing the data. In fact, I had one Chinese student from Civil Engineering ask yesterday if he could get text versions of all his spectra. There are only about 30 spectra, so that isn't too nasty a request. I took one spectrum, exported it in MSA format, imported into Excel, and made a chart. He saw the shape as before but then asked which peaks were which. I showed him where in the MSA file he could find the peak labels and locations including the 4 different peaks for Ca. Then the question came up about how to quantify the data into weight fractions. I did not cover background modeling and removal, nor did I discuss peak deconvolution or integration. I did explain that he would have to have a standard for each of the elements so he could compare his sample intensity to the standard intensity. I did not launch into the necessary matrix corrections. I did tell him that his sample violated many of the assumptions necessary for quant analysis. What I did tell him was enough to dissuade him for following through on his request. I would go through the same exercise with spectral images. The vendors do a lot in their software and I am hesitant to try to reproduce it. Maybe I am too old and lacking creative juices, but I find it hard to believe that I could seriously improve on what is available. I could see exporting the map images and doing some math on them to try to tease out phases. That would be a much simpler data set to work with. If you are still set on doing your own processing, I recall NIST was promoting a format for spectral images. They also had developed some routines for basic processes. I have not followed it in recent years, so I cannot tell you where things stand. Maybe someone else can chime in. Warren Straszheim wesaia@iastate.edu Fri May 30

Bruker have a built in facility to output the data in portable form. In Esprit you can save out data as a "Raw database file" from the File type dropdown menu. This will then save the dataset into a raw binary datacube x,y,energy, that can be imported into Matlab/IDL/whatever... You will need to be familiar with how to import raw binary files. I've done this for Bruker files in the past but of the top of my head I can't remember the order (x,y or energy first?) or the bit depth used, but a bit of playing around should find this out for you (as you already know the data dimensions). A word of warning however, Bruker saves their data in countwise, i.e. for each X-ray count they place 'energy,x,y' and entry into their datafile. This results in very memory-efficient storage. A datacube (such as the "Raw database file") has simply the dimensions of the datacube and as such can be very space-inefficient, especially for sparsely sampled data. For example one of my 6Mb Bruker files expanded to 1GB in raw form! I can't help you with loading the data back in to Esprit after that however (as you cannot import Raw Database files back into the software) this "one-way" nature is not uncommon as manufacturers formats contains much information, and simulating/re-generating it would be very difficult! Matthew Weyland matthew.weyland@monash.edu Sun Jun 1

Thanks Matthew! I actually figured it out yesterday with some helpful comments from another listserv member and it's just as you said. The format is Lispix: http://www.nist.gov/lispix/. And for those who use EDX but might not be aware, see also HyperSpy: http://hyperspy.org/ and DTSA-II, http://www.cstl.nist.gov/div837/837.02/epq/dtsa2/. It is a raw binary format with each bit of data in a continuous stream, i.e. no delimiter. Each channel of the first pixel is followed by each channel of the second pixel (left to right, top to bottom). An associated "rpl" file tells you the channel depth per pixel and the height and width of the spectrum image. It is uncalibrated (in space and energy) so that must be taken care of by hand. Bruker opts for 8-bit data, so if you happen to have more than 255 counts in any channel you will have trouble. Esprit appears to truncate the data at the last channel with any counts for sake of file size, so the channel depth is important and potentially variable. Lispix works great for spectra analysis and moving this data into DTSA. I have not played much with HyperSpy yet. On the off chance anyone else is interested in getting the data into Matlab, feel free to contact me. I put together a Matlab script to read the file into a datacube and also perform some basic processing. Simple, but if it saves someone some time in Matlab, great. I am looking at some exceptionally beam sensitive specimens, so I am playing with probe size, step size, dwell time, and probe current to see what compromises I can make while being able to spatially resolve changes in relative composition. That's where Matlab comes in as I'd like to do running sums of the spectra while also doing 2-D running pixel sums as opposed to just binning or increasing probe size or step size. Some nice discussion of EDX sensitivity, SDD detectors, and approaches to analysis can be found in this PowerPoint: http://epmalab.uoregon.edu/Workshop2/DonovanWorkshop07_SDD_Newbury.pdf and this associated paper: http://www.geology.wisc.edu/~johnf/g777/Scanning/NewburyBright-2005.pdf Andrew Wagner awagner@umn.edu Sun Jun 1

Last year, I made a video and an ImageJ plug-in for doing this for stacks acquired from the Bruker EDS on the Titan TEM at NCEM. Â So here is about a 15 minute video where I import the data into ImageJ, make maps, make an RGB, export a spectrum from ImageJ and load it in a spectrum processing software: https://berkeley.box.com/s/bya3oaiqat25hxwrsate And here are the links to the source code for the ImageJ plugin which you can use as a starting point for whatever you want to do: https://berkeley.box.com/s/bd74bnmg405lppfmfakd and https://berkeley.box.com/s/brtal2oz4hztj8wfws3y I hope you find it helpful. Zack Gainsforth zackg@berkeley.edu Mon Jun 2

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