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NetNotes

Edited by Thomas E. Phillips

University of Missouri

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Selected postings from the Microscopy Listserver from May 1, 2013 to June 30, 2013. 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:
Lowicryl HM20 monostep

I was wondering if anyone has any suggestions on how to re-orient samples embedded in Lowicryl HM20 monostep resin for ultrathin sectioning. In the past, I tried using superglue, but it reacted with the Lowicryl and made the bottom of the block in contact with the glue gooey. I tried with 2 different tubes of superglue, thinking there was something wrong with the glue, but I had the same result both times. I also tried a 2-part epoxy with a quick setup time, but it didn't secure the block as well as superglue usually does with other resins. Has anyone had any similar experience? Does anyone have any tried-and-true ways that has worked for them to reorient embedded samples in Lowicryl HM20? ***Shannon Modla modla@dbi.udel.edu Mon May 6***

Like so much in science, the best way is the most tedious and time-consuming. I polymerize my tissues in flat bottom BEEM capsules, cut out the tissue as a medium size chunk using a Dremel moto-tool and re-embed in fresh Lowicryl in a new BEEM capsule. If you cut an appropriate size piece of plastic after the first embedding, it will stand up straight in the second BEEM capsule and retain its orientation during the second polymerization. I always feel like a diamond cutter when I am trimming the first block to get the shape and orientation for re-embedding. Tom Phillips phillipst@missouri.edu Mon May 6

Specimen Preparation:
bacteriophage for SEM

What is the best way to process bacteriophage for SEM? Will osmium treatment help in SEM imaging? ***ravi.thakkar369@gmail.com***

What I did was just to pretend I was doing a negative stain for TEM, without the negative stain. Deposit the viri on a coated TEM grid, remove the fluid, a quick sputter coater (Quick), or none. This was for a small dodecahedral virus that attacked blue-green bacteria—assuming you have a low-voltage FE-SEM. Fixation and a quick coat might be more necessary if you are using a tungsten filament SEM. Osmium might help, but I have not tried it. An OsO_4 vapor fix should do. It also helps if your phages are attached to their victim's flagellae or pili. Makes a neater image, anyway. Philip Oshel oshel1pe@cmich.edu Thu Apr 25

Specimen Preparation:
feathers for SEM

I am trying to image some falcon feathers in the SEM. A first look with the Stereo microscope showed a lot of dirt and maybe grease also on the feathers. Can somebody please advise me how to clean the feathers and get an as clean as possible and as stable as possible specimen for SEM? Does it make any sense to use osmium tetroxide with feathers to lower the possibility of charging under the e-beam? ***Stefan Diller stefan.diller@t-online.de Mon Jun 17***

I've looked at feathers with the SEM. No problems with dirt or grease. I just sputter coated them to reduce the possibility of charging. You can also use a lower kV. Try a little distilled water to rinse them at first to see what that will do to clean them. You could also use a dilute solution of ethanol/water. Barbara Plowman MA bplowman@pacific.edu Mon Jun 17

Specimen Preparation:
negative staining of HIV virus particles

I recently received a virus sample that had been concentrated by another lab and brought to me in 100 mM ammonium acetate. When I stained the grids there seems to be a precipitate all over the sample. I was wondering if this could be from the ammonium acetate. Most times, I receive my samples in PBS or something of that sort. Any opinions would be appreciated. ***Georgianne Ciraolo georgianne.ciraolo@cchmc.org Fri May 10***

PBS is the buffer for which I would expect a precipitate, when staining with uranyl acetate, but not with ammonium acetate buffer. Strange. The only thing that we always do, is a brief (1 to 2 sec, once) wash on bi-distilled water, before the sample is stained with uranyl acetate (after staining: only blotting, but no washing). I prefer bi-distilled (!) water, although many people use 'Millipore' water—In my opinion this is inferior to bi-distilled water, for EM. Another point: do you have a solution of 1% phosphotungstic acid, buffered to about pH 7.0 ± 0.5 (NaOH)? The staining and 'precipitation' is different, usually less precipitates are better, so worth trying. We always use carbon-coated 400 mesh Cu grids, glow-discharged (if this is of interest). Reinhard Rachel reinhard.rachel@biologie.uni-regensburg.de Fri May 10

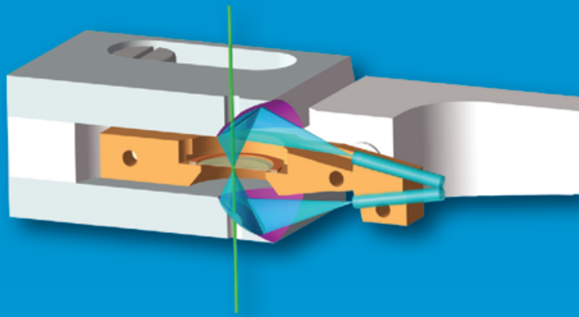
Ammonium acetate is volatile at low vacuum pressures. Researchers often use dialysis to exchange salts like NaCl with ammonium acetate so they can later evaporate off it off downstream. I use to do this with a lyophilizer but you could also use a rotary evaporator (also called a Rotavap). A Rotavap is small centrifuge that pulls a vacuum on the sample as it is being spun. Then re-suspend your sample in deionized water or PBS. Tom Phillips phillipst@missouri.edu Fri May 10

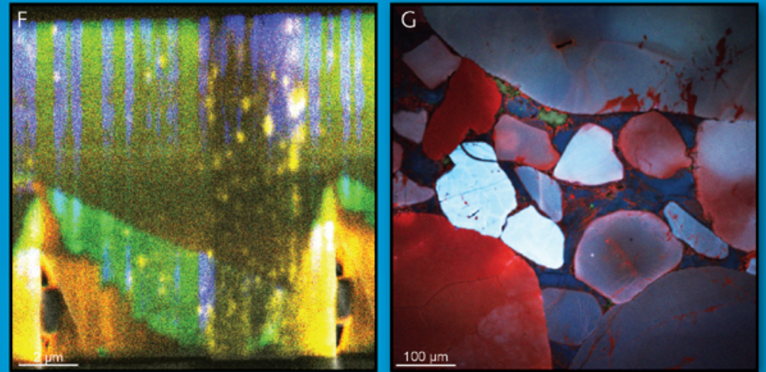
Specimen Preparation:
Formvar grids

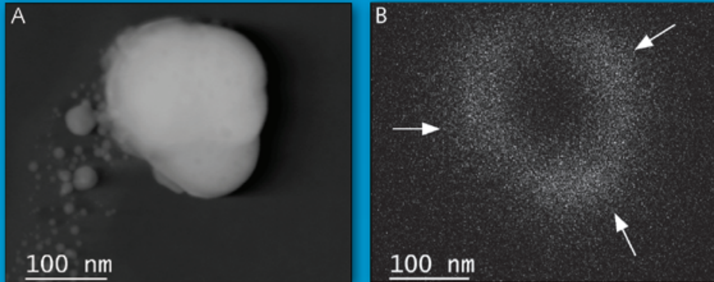
I recently used these grids to look at mice bone marrow cells, specifically megakaryocytes. In the past, I had used regular mesh grids, but found that the megakaryocytes were either partially or fully obscured by the grid bars. I had beautiful sections (and megakaryocytes), but when I took the images, they were out of focus. It seemed that the Formvar film (and section) moved under the beam. I have not used these slotted grids in a while, so I am not sure what the problem is and what trick is needed when viewing them under the scope. ***Peggy Sherwood msherwood@partners.org Thu May 23***

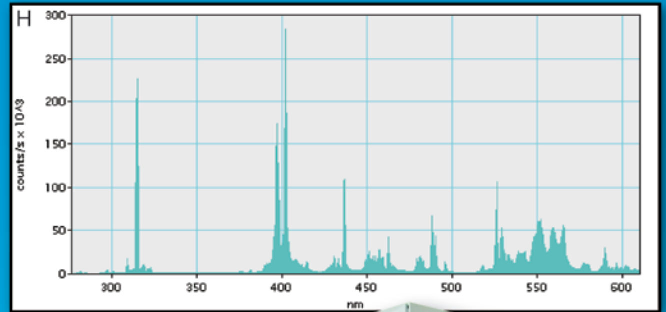
Cathodoluminescence for SEM, and now for TEM

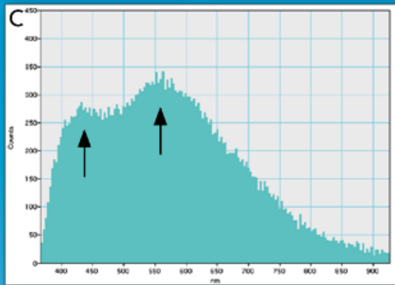
Imaging & Spectoscopy Optoelectronics Plasmonics Semiconductor Geological

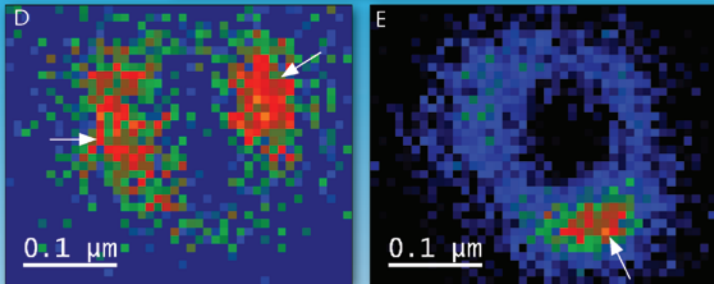












Top Image: Schematic cross section through the Vulcan™ holder showing the specimen region and top and bottom collection mirrors (mirrored surface shown in purple). An electron beam (green) stimulates the specimen to emit photons (blue) which are focussed by the collection mirrors into optical fibres situated away from the specimen region. Bottom Images A-E: CL study of colloidal silver nanoparticle; A) HAADF image; B) panchromatic CL image (acquired simultaneously to the HAADF image) displaying three 'bright' resonance nodes (indicated by arrow markers); C) cathodoluminescence spectrum with two peaks corresponding to spectrally discrete resonance modes at 430 and 510 nm; D) and E) cathodoluminescence band pass images at 430 and 550 nm ±40 nm extracted from parent spectrum-image showing resonance modes are separated spatially and spectrally.

Images F-H: F) GaN film in cross section imaged with Gatan MonoCL4™ CL imaging and spectroscopy system. Composite image of stacking fault, threading dislocation, point defect and band gap luminescence. Temperature = 6 K; G) quartz arenite polished section cathodoluminescence image prepared using the Gatan Ilion+™ and imaged with Gatan ChromaCL2™ imaging system. Image courtesy of Dr. J. Schieber, Indiana University; H) Cathodoluminescence spectrum from lanthanide doped yttrium aluminium garnet single crystal acquired at room temperature. Multiple spectral features corresponding to various Eu^{3+} d to f orbital electron transitions observed. Bottom image: MonoCL4™ *Elite* CL imaging and spectroscopy system.

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Some of the reasons for movement of the section on slotted grids are either a hole somewhere in the film or that the grids need to be conditioned especially if the section is a bit thick. If in fact the cause of your problem is movement and not focus it may be advisable to look at a structure that is in the corner of your viewing area both before and after taking the image. If the structure is moving during the time of capture you will see how far it has moved—proof that it is movement and not focus that is your problem. If you have a hole, you can attempt to move the grid in the opposite direction of the movement and immediately take the image. This works sometimes but not always and is dependent on the amount of movement. One can take advantage of that short interval between the grid moving in one direction while the section is moving counter to it. To counteract the change in size of the section it may be necessary to expose the whole section to a reduced beam at a lower magnification. It will be like scanning with the beam until the whole section has been exposed. If there are folds present (and there are usually some found in sections picked up on coated grids) it may be advisable to simply hit the fold with the beam until it shrinks. I have found that going from the pointed end towards the wide end works best. Then go back to the area of interest with the chosen magnification and normal beam intensity. I have also tried carbon coating the Formvar grids to give them extra support. This works if the Formvar is a bit thin. Recently I have picked up a few 50 mesh coated grids at the same time as the slotted ones in case I have a problem with the slotted ones. This gives a large area and if one square has a hole the others are usually fine hence there is usually no need to re-section. Patricia Stranen Connelly connellyps@nhlbi.nih.gov Thu May 23

I don't know what kind of camera you're using, but this happens sometimes with our Gatan digital system. It seems that often when we take a picture the beam is blanked by the shutter, then unblanked for the picture. Apparently the film/resin can react quickly to the removal of the beam (thermal expansion and contraction?), causing it to move during the exposure when the beam comes back on. We can sometimes solve this by switching the camera to use the post-, rather than pre-specimen shutter in the advanced settings for the view and acquire windows. At other times, we manually shorten the exposure time to 0.5 second or even less. We have even found that sometimes the software seems to set the acquire window for one shutter and the view window for the other, which often results in streaky images during viewing. If all else fails, we can save the viewing window directly to a file, rather than telling the system to actually acquire a final image. This is a smaller file and what you see is what you get, but way better than nothing. Randy Tindall tindallr@missouri.edu Thu May 23

Try using a carbon film. Not as susceptible to sagging and thermal stress under the beam. You will need a very high quality and fine grained film to withstand the large gap however. Alternatively try carbon coating the Formvar to stabilize. Scott Whittaker whittaks@si.edu Thu May 23

The large 1 mm × 2 mm open area of a slot grid is particularly susceptible to drift in an EM. It is usually possible to stabilize the section and support film by letting it sit in the beam for a few minutes. Sometimes I spread the beam to cover a larger area and wait longer. Sometimes I use a more focused beam and move around the section particularly covering the edges of the grid support. If there are holes or any tears in the support film stability is decreased and may not be possible. Usually if you watch the specimen with binoculars or if using a digital camera, watch the display. Any movement will blur the micrograph. Sometimes with slow drift a short exposure time will produce an acceptable micrograph. If none of the above works you can coat the sections on support film with carbon to give added strength and electrical conductivity to the sample which should eliminate or minimize drift. Larry Ackerman larry.ackerman@ucsf.edu Thu May 23

Yes, the slow heating of a large area will stop some heat/expansion problems. I've also seen a potential issue on systems with column shutters that are used with the digital camera- if the grid is shielded from the beam for a long enough time for it to cool and start to shrink, then during the exposure it will be re-expanding. On our Gatan system, I am pretty sure there is a time delay setting after the shutter is set to open (to allow for it to be fully open) which you could extend into the several second range so that things stabilize before the exposure starts, if you suspect this might be occurring. Ben Micklem ben.micklem@pharm.ox.ac.uk Thu May 23

This is a little off the subject but you might find helpful a method for picking up sections on single hole grids that minimizes folds, etc. It is great for serial sections. As more people do tomography, putting sections on single hole grids will also be very helpful in making sure the area of interest in visible even at high tilts. You can download this method from the Resource page on my website at: http://www.dsimagingllc.com/ Since this method works so well, I found that we could cut much wider sections and almost cover the Formvar film. This helped stabilize by minimizing areas of Formvar without requiring additional carbon coating. The suggestions to condition the samples by exposing them under a broad beam at low magnification also works well to minimize drift. Debby Sherman dsherman@purdue.edu Thu May 23

Immunocytochemistry:
avidin-gold

Does anybody a recipe or kit with which to couple gold to (strept-) avidin? ***Peter Heimann peter.heimann@uni-bielefeld.de Fri May 3***

From experience I recommend to choose streptavidin over avidin. The high isoelectric point (IEP) of avidin makes it hard to obtain cluster free conjugates. Coupling procedures have been documented in early immunogold conjugate literature. An important factor is the coupling pH which should be equal or preferably slightly higher than the IEP of your streptavidin. Please feel free to contact me off list if you require more information, we will be happy to help. Jan Leunissen leunissen@aurion.nl Fri May 3

Image Analysis:
software for 3D reconstruction

We are a University lab and cannot afford Amira until the next grant cycle. However we have some nice tilt series acquired on our Titan TEM. We also have some nice reconstruction files from Inspect 3d. However the inspect 3d software does a poor job visualizing the reconstruction files .rec files. I see the free reconstruction software on the Mathworks website: "CT reconstruction package". Does anybody have experience with this Matlab executable? Does anybody know of other free software? My friend told me of some free Software written by Mike Stowell of CU Boulder? ***Pete Eschbach peter.eschbach@comcast.net Wed Jun 5***

There is a free 3D reconstruction software called IMOD that was developed by David Mastronarde alongside others at the Boulder Laboratory for 3-Dimensional Electron Microscopy of Cells and the Regents of the University of Colorado. I used it during my time at the NIH and found it to work wonderfully, especially as you begin to learn to use all of its features. In addition to the program, there is a listserv

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Phase	Color	Fraction (%)	Pixel Count
1 SiAlO - Plagioclase		44.0	29,323,306
2 SiFeO - Augite		39.1	26,080,067
3 SiO - Quartz		6.1	4,048,423
4 SiKAlO - K feldsapr		4.5	3,007,415
5 FeTiO - Ilmenite		2.0	1,330,023
6 FeO - Magnetite		0.9	581,427
7 FeSiO - Pigeonite		1.9	1,262,059
8 CaPO - Apatite		0.5	352,297
9 FeSiO - Biotite		0.5	330,548
10 FeS - Pyrite		0.0	15,804
11 CaSiO - Hornblende		0.2	131,591
12 FeCuS - Chalcopyrite		0.0	14,078
13 CrSiO		0.1	34,756
14 ZrO - Badelyite		0.0	2,611

Large Area Mapping data (16K x 14K) of a granite specimen. Dataset made up of 224 individual fields.

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that David oversees, answering questions that anyone may have. Through the questions and feedback he gets, he continuously updates the program and releases new versions, which is great. The website is below. IMOD http://bio3d.colorado.edu/imod/ Shervin G Esfahani shervin.esfahani@gmail.com Fri Jun 7

LM:

resolution

I had a question on an exam I gave recently and now I am trying to figure out what should be a correct answer. The question was whether you could tell an actin filament (8 nm) from a bundle of filaments by fluorescence microscopy. They were supposed to think about image resolution and brightness in their answer. Obviously, you would be unlikely to be able to resolve filaments in a bundle since they are so tightly packed. But, a bundle should be much brighter than a filament if you had both to compare. My question is, at what point would the width of the structure begin to give you information about the size of the bundle? To make things simple, let's assume the filaments were in a parallel, flat array with the 8nm filaments spaced by a cross-linking protein like alpha-actinin (about 35nm long). If your image resolution was 300nm, when would the image of a fluorescent bundle look "wider" than the image of a single filament? ***David Knecht david.knecht@uconn.edu Mon Apr 29***

Imagine your specimen is the world's best "nano-iris" surrounded by a completely dark sample. Let the diameter of the iris be anything, down all the way to zero. Start out with the iris at something absurdly tiny, say 1 nm (and pretend/assume that enough light can be transmitted through to form an image). Your imaging system will image this point as an Airy disc, whose diameter is set by the optics. Now open the iris. As you do, the Airy disk will get brighter, but its size will not change until the iris diameter becomes greater than that limiting Airy disc diameter. Then to see how this works in resolution, you need to imagine two of these gems side by side on the slide, and translocate one away from the other. Again, once the distance between them exceeds about half of the Airy disc diameter, you have a chance of seeing them as two things, not as one. Hence resolution. The various different formulae reflect the fact that there is no obvious way to define whether you have two Airy discs or just one. But they all converge to a distance that amounts to about one half a wavelength (of the incident light). That is in terms of size. You will note that having two iris together will let more light through and so the fused (unresolved spatially) pair of irises will be brighter than just one. So if you know for sure how much light one iris gets through (with a fixed diameter), you can tell by that criterion, if you have one, two or more within an Airy-disc sized spot. To the extent that each actin filament has a constant amount of fluorochrome bound, then this kind of intensity counting can in principle be used to count the number of filaments, even when they cannot be seen as objects. Practical matters like quenching and so on are a different story! Anyway this is how I have always understood it. Tobias Baskin baskin@bio.umass.edu Mon Apr 29

I think the question, or how the question is being asked, is as important as the answer. IF the question is "whether you could tell an actin filament (8 nm) from a bundle of filaments by fluorescence microscopy," the answer should be "No," regardless of the brightness, simply because of the resolution limit. If the question is "whether you could 'see' a single actin filament (8 nm), using a 200 nm resolution microscope," the answer is "Yes," as long as the filament has "enough contrast." It has nothing to do with resolution. If the question is "whether you could tell if that is a single-actin filament, or actin bundle (more than 1 filament)," the answer is "maybe," if you could provide a reference image of a single filament, and assume all filaments have the same brightness. Zhaojie Zhang zzhang@uwyo.edu Mon Apr 29

I am not getting much response, so let me try to explain my interpretation of the problem. If you consider a single filament, then you expect to image a linear extension of the diffraction pattern (airy disk) of each fluorescent point source along the filament projected onto the chip. As a diffraction pattern, the emission will decrease stochastically as you move away from the filament. In theory, if you wait long enough and acquire enough photons over a long time, the diffraction pattern spreads to decreasingly represented orders of the pattern. So what you call the "edge" of the filament as you image it is arbitrary and determined by the sensitivity of your detector and the imaging conditions. Now as you increase the number of filaments in a bundle, the total fluorescence output from the volume increases. If you were to acquire an image with the same gain and integration as you did for the single filament, you would see a "wider" structure because the higher orders of the pattern now become more readily visible due to the brighter signal and more photons coming from the same "sub-resolution" volume. If you normalize for the total fluorescence, I would expect the single filaments to look just like the bundles up to the point where you exceed optical resolution (about 8 filaments when you are nearing 300 nm wide). Of course a real bundle is much more tightly packed as a cylinder, so it would take many more filaments to look larger. But if you do not normalize, I think the bundle will look larger than a single filament before you exceed the resolution limit. My greater point is that the imaging conditions and detector will have an effect on what you call the "size" of an object and this is a different set of considerations from that we normally think about as resolving of multiple objects from each other. David Knecht david.knecht@uconn.edu Mon Apr 29

I'm not sure I can follow your argument, but I'd like to point out some things that seem a bit unclear to me. You say that the Airy disk is the diffraction pattern of a point source. As I understand it the Airy disk is the real space image of a point source. The ideal image of a point source would be a point, but due to the limited size of the lenses this point is convoluted with the airy disk, which is the diffraction pattern of the aperture (edge of the lens). The diffraction pattern of a point source should be a constant (just like the Fourier transform of a delta function). Maybe your argument is perfectly alright, if you reformulate it and clarify whether you are considering a real space image or a diffraction pattern (in Fourier space). Philip Koeck philip.koeck@ki.se Fri May 3

Another consideration is the distribution of the fluorophors along the filament(s). Variations in the number of fluorophors will fold in statistically with other variations in fluorescence, so a single filament will have variations along its length and as a function of time, so it will be more complicated than a linear superposition of Airy discs. (It is the wave amplitudes that add linearly, and the resulting intensities depend on the relative phases.) This might be indistinguishable from a filament of varying size. Of course, a sufficiently long time average will tend toward a diffraction profile with a width related to the resolution. Bill Tivol wtivol@sbcglobal.net Fri May 3

EM:

vibration

We're going to install a brand new SEM with Schottky emission. We were thinking to position it on a base placed into a hole dug in the floor expressly to decouple the base from building. On the other hand we discovered that a similar base built here some 10 years ago is not isolated that much. This base was built by digging a hole in the floor, than filling the bottom part of the hole with gravel and finally putting the concrete

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base on it. I think (not sure) the gap between the sides of the base and the hole was filled with sand or gravel. Finally, the same gap at floor level was filled with a rubber expansion joint. Do you have any better recipe? Or different solutions? ***Davide Cristofori dcristofori@unive.it Wed May 15***

If a problem is seen, I suggest you look also into active vibration isolation tables. These can be provided to complement the passive system your SEM undoubtedly has. From recent research I've done, it can be quite an undertaking to do a proper foundation isolation project. For a dedicated facility it is done—although costly. A vibration consultant told me recently that he also did not measure significant improvements from homemade approaches he's inspected. Does your site meet the manufacturer's vibration specification? I have managed plenty of sub-nm resolution instruments on regular concrete slabs with no isolation other than having enough distance from neighbors. Larry Scipioni les@zsgenetics.com Wed May 15

After years of fighting with malfunctioning active systems at Intel, I would say avoid them like the plague. The one I had needed to be "tuned" periodically because it was not truly active, and had to be set close to the frequencies in the floor. Unfortunately, the tuning just would not hold and it would go into oscillation, which would shake the microscope violently. After 8 years, it failed totally, and we had to purchase a new one. To repair the old one, it would have to be removed from under the microscope, shipped to Europe, debugged for $250 per hour, repaired for labor and parts, and returned with minimal warranty. Meanwhile the microscope would have been unusable for all that time, all of which was unacceptable to Intel. We also paid for the expert to fly out from Germany to install and tune it, and it took three days to get it tuned so that it was not oscillating the next morning. He stopped by and tuned it again on the way to the airport, and fortunately that tune held for a few years, because help was far away. John Mardinly john.mardinly@asu.edu Thu May 16

On the other hand I believe we have a system from the same European manufacturer that worked well for 13 years no need for retuning. Alan W Nicholls nicholls@uic.edu Fri May 17

I've used TMC's Stacis active vibration cancelling system with excellent results on the 3rd floor of a 50-year-old building that vibrates at 15 Hz constantly. This frequency seemed to be the resonant frequency of the stage of my large chamber SEM and was causing plenty of trouble. Becky Holdford r-holdford@ti.com Fri May 17

We just built a new facility within isolated floors three years ago, but started the process before then. Yes, I had looked in to "good strategies" and had engineers designing the renovations as well. But then I found more information: (A) I found that a number of microscope manufacturers actually have folks to help with room designs and specifications—although they do not like to make recommendations lest they be held accountable. (B) I was pointed in the direction of an engineering firm who specifically designed environmental room solutions. And they first things they really pointed out was #1 each location is unique and needs a unique solution there is no one "good strategy", #2 when they found we were on ground they wanted to know what the ground was really. And that got my geologist users involved and boy we knew exactly what the ground was. Yes, we to having read lots of individual isolation slabs with rubber gaps or open gaps, sand filled, etc. had been thinking along these lines. We wound up with one big slab (14.6 ft × 139 ft × ~ 3 ft thick—13 concrete trucks) and it sits directly on un-disturbed hand dug soil, and has sloped edges connecting directly to the surrounding floors. The slopes are designed to break transmission of low frequencies. Our FESEM picked up ~5.5× improvement in resolution. Bur we also had no major electric lines overhead or around, an isolated electrical supply for the facility with lots of dedicated grounds. We used a product called "Quite Rock" which is engineered "sheet-rock" for sound studio walls (equivalent of 16-sheets of 3/8 inch drywall of sound deadening). A dedicated HVAC system with laminar flows and sound deadening in in the ducts. And a few other details. And I think one of the biggest issues is AC electrical fields. We're now in a "non-science" building, with a museum, history, and anthropology departments. By "non-science" I mean the "hard" sciences, so no incubators, no hundreds of computers, no ovens, and water circulators: no motors, no transformers, no fans, etc. All the things we lab rats usually surround ourselves with. And all the EM Fields generated by them and their acoustical noises are gone. This really cleaned things up particularly with low accelerating voltages in the SEMs. (As a test once we even shutoff the HVAC system and still picked up ~30% improvement in our highest resolution SEM or ~ 0.9 nm) Our sites were surveyed by Integrated Dynamics Engineering (IDE), and Peter has already spoken here. I have no financial ties with them, they were good folks to work with, and we've worked with them a number of times. The engineering specialist company we worked with was: Colin Gordon & Associates. Again I have no financial ties, but they were good to work with and gave us a very good solution. I am sure there are similar good companies in Europe as well. Richard E. Edelmann edelmare@miamioh.edu Thu May 23

TEM:

H7000 clogged cooling lines

Cooling lines in our H7000 are clogged. We try to flush the system with pressurized water but it helps little. The debris that comes out is green and hard like maybe copper oxide. Do you know of any treatment that would remedy his problem without damaging the scope? Here is the link to pictures depicting debris that comes out of the cooling lines: https://dl.dropboxusercontent.com/u/47838666/image001.jpg. ***Dorota Wadowska wadowska@upei.ca***

I've used both CLR cleaner (this is the brand name; for bathrooms, mostly) and hydrogen peroxide to the clean the chiller lines on our TEM—by the service engineer's recommendation. One bottle of CLR in 5 gallons of chiller water, let run for ~4 hours or overnight, then drain the tank and flush repeatedly until only clear water comes out. Or 1 liter 3% H_2O_2 or 100 mL 30% H_2O_2 and do the same. This worked when the CLR didn't. Philip Oshel oshel1pe@cmich.edu Mon May 6

I can't provide any guarantees in regard to potential possibility of "damaging the scope," therefore "use at your own risk, yada, yada, yada," but over past two years I've applied following technique to two instruments. One is to clean clogged cooling line in the objective lens (FEI) another is to remove deposits from cooling lines and chiller tank (Hitachi), and so far—so good. 1. Pour liberal amount of "Micro-90" (available from both Cole-Parmer and Sigma-Aldrich) into the tank of recirculating chiller. I've used about 200cc for a small Neslab chiller, about 0.5L for dual-loop Haskris. Let it run overnight—by morning the water will be green and all (or nearly all) of the deposits from the tubing and the walls of the tank will be dissolved. 2. Drain (now green) water from the cooling system, purge with compressed air, refill with DI, and run chiller for a 15min. or so. If any visible deposits come out during air purge or accumulate in the tank after the refill, then repeat the Micro-90 treatment. 3. Drain the cooling system, purge with compressed air, and flush with regular and then DI water. I've done at least 3 full refills with tap water, followed by at least two refills with DI, in each case. Fill with DI and run at least overnight, or for a day or two. 4. Drain the system, purge with compressed air, and refill with DI one last time—maybe overkill, but I did not want to have any traces of Micro-90 remain in the system.

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Faculty Position in Electron Microscopy
Department of Materials Science and Engineering, The Ohio State University

The Department of Materials Science and Engineering at The Ohio State University (mse.osu.edu) invites applications for a tenure-track position in electron microscopy. This position is anticipated to be the first of several associated with the newly founded Center for Electron Microscopy and Analysis (CEMAS cemas.osu.edu). CEMAS is a multi-million dollar investment in advanced characterization equipment and infrastructure bringing together multidisciplinary expertise to drive synergy and amplify our characterization capabilities in engineering, medicine and the physical and biological sciences.

We welcome applicants with expertise in: (a) electron microscopy of biomaterials and biopolymers, including cryogenic-TEM, electron tomography for 3-D imaging and reconstruction, imaging and analysis of cellular structures, in-situ methods for investigation of live cells and analytical microscopy, and/or (b) 3-D imaging of structural and functional materials with emphasis on in-situ characterization.

The faculty appointed through this hire are expected to complement existing expertise in CEMAS and the wider OSU materials community to dramatically enhance and sustain federal funding and industrial partnerships for materials characterization. In view of our aspirations and the nature of this opportunity, we seek candidates who are ardent discoverers, passionate teachers and mentors, committed stewards to our discipline and proven collaborators. For the successful candidate, we offer a vibrant research environment at one of the largest, best equipped and most-highly connected electron microscopy facilities in the world.

The Strategic Plan for the College of Engineering at Ohio State outlines ambitious teaching and learning objectives to enhance overall research and discovery goals and align with major national initiatives such as the Materials Genome Initiative (http://engineering.osu.edu/strategic-plan). The ideal candidate will possess the ability to work with internal and external groups to develop significant new activities.

We seek a person with a demonstrated track record of leadership and collaboration in an academic and/or R&D environment with an appointment anticipated at the Assistant or Associate Professor level. Candidates must have established a record of accomplishment in electron microscopy research and earned a doctoral degree in materials science and engineering or in a closely related field. The successful candidate will be expected to develop and sustain active sponsored research programs, teach core undergraduate and/or graduate courses, and develop new graduate courses related to their research expertise. The anticipated start will be in the first half of 2014. Screening of applicants will begin immediately and will continue until the position is filled. Interested candidates should submit a complete curriculum vitae, separate 2-3 page statements of research and teaching goals, and the names, addresses, and e-mail addresses of four references electronically to the following email address:
cemas@osu.edu

The Ohio State University is an affirmative/equal opportunity employer. Women, minorities, and people with disabilities are encouraged to apply and build a diverse workplace. Columbus is a thriving metropolitan community, and the University is responsive to the needs of dual career couples.

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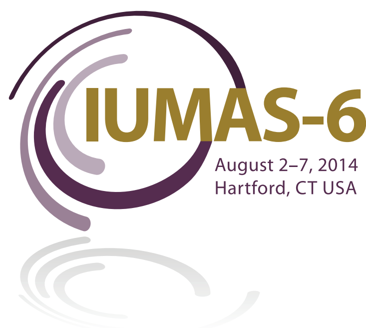

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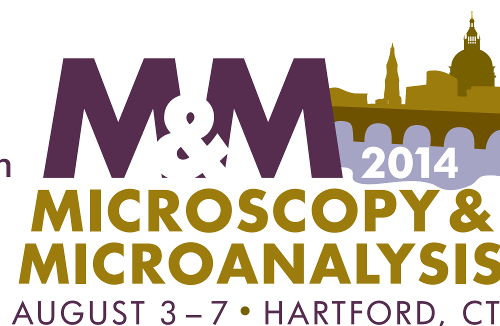

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One of the tools treated in this way is running over a year without visible signs of corrosion in the cooling system (no green particles coming out, or accumulating in the chiller tank), another one was treated just a couple of months ago. Again—I make no guarantees for the safety of such a treatment; and realize that the absence of visible signs of corrosion does not mean that nothing is happening inside of the cooling lines, etc... so, use (or don't) at your own risk. Valery Ray vray@partbeamsystech.com Mon May 6

One possible problem is if the corrosion in the cooling lines, which caused the clogging, has proceeded to the point that the tubing in the lenses is leaky or weak enough that removing the deposits will allow leaks to develop. When you clean the lines, monitor for these possible leaks. If everything is OK, maintain the cooling water at a slightly basic pH (7.5 to 8.0) to prevent further corrosion, and, if warranty or service contract permits, add a corrosion inhibitor to the water. When I was in NY State, I used a product called AquaTreet 42 from Aqua Labs, and in California I used an equivalent Skasol product. Check the pH and inhibitor levels—the products come with test kits—every month and adjust levels appropriately. Check with the manufacturer or service person to be sure that these additions will not cause any problems with either the scope or your contract, but they have worked on all the scopes I've been in charge of. Bill Tivol wtivol@sbcglobal.net Mon May 6

SEM:

astigimatism at low accelerating voltage

I'm experiencing very large astigmatism at lower accelerating voltages in our JEOL SEM. The only voltage at which I can compensate for and obtain a decent quality picture is 25 kV. Any ideas why? ***Laci jakabfarkaslaszlo@gmail.com Tue May 21***

Any chance your sample is magnetic? That would likely have a greater effect at lower kV's. Randy Tindall tindallr@missouri.edu Tue May 21

What you mention is a common problem; there is a high degree of charge on the components requiring an excessive level of astigmatism correction. Basically the column is contaminated and by moving to a higher accelerating voltage you enable the contamination to be penetrated enabling an earth to be achieved and an astigmatism correction to be made. At lower accelerating voltages the beam does not penetrate the contamination thus there is a high degree of astigmatism, too high for the stigmator coils to compensate. Clean the column and if you have a service technician who maintains the instrument make sure they cover all aspects of operation during a maintenance procedure. Running the instrument at a high accelerating voltage checks out the gun; fine. Running the instrument at a very low accelerating voltage checks out the column and this is the more important check. Thus a 30 kV test picture for the gun stability and as a resolution check, but a 2 kV test picture for a real check of column cleanliness! Steve Chapman protrain@emcourses.com Tue May 21

On my FEI Quanta ESEM, that kind of trouble tells me to clean the last PLA first. Hope it's that simple. Fred Monson fmonson@wcupa.edu Tue May 21

I agree. But many use an alternative, much easier method to solve the problem—an in-chamber downstream plasma cleaner. To be sure this does not clean up right inside the column, but most of the problem is at the sharp end, where the plasma can reach, for two reasons: (1) that is mostly where the contamination condenses (2) that is where the beam energy is low. (The method is a high energy flight tube followed by beam-deceleration.) So a method that I know some have found to be effective is to mount an in-SEM-chamber downstream plasma (such as that from XEI) and run this for a few minutes a week, restoring much low-energy performance. Ian Holton ian@acutance.co.uk Wed May 22

We agree with Ian Holton's possible solution to the problem. Removal of carbon contamination from the chamber will minimize astigmatism and improve low kV image quality. Plasma cleaning solutions other than those offered by XEI exist. Sample cleaning before examination removes the carbon prior to polymerization by the electron beam. ibss Group offers an *in-situ* plasma cleaner that runs at pressures compatible with TMP operation. This provides more thorough chamber and column cleaning with fast turnaround time. Disclaimer: the sender is President of ibss Group, Inc. Vincent vcrvince@comcast.net Thu May 23

Thank you all for the valuable input; the problem has been solved through a full cleanup of the lower column, where impurities have been found, especially cracked oil. So it seems to me that I do now really have to mount a high vacuum gauge on the scope. What would be an acceptable level of vacuum for a 30 year old SEM, supposing we do not want to contaminate the column when working? Laci jakabfarkaslaszlo@gmail.com Thu May 23

A high-vacuum gauge is helpful and vacuum levels somewhere from low 10^{-5} range down to 10^{-7} may be reasonable, depending on the design of your vacuum system. But to prevent contamination of the column, content of your "vacuum" (i.e., partial pressure of oils) is way more important than ultimate pressure. It may seem counter-intuitive, but there will be way more contamination from cracked oils in an SEM chamber with an ultimate pressure 10^{-8} Torr (very good vacuum) and the main component of the residual gas mixture being mineral oil from the roughing pump, then in the SEM with an ultimate chamber pressure in low 10^{-5} Torr range (not so great vacuum) but the main component of gas mixture being atmospheric gases. What I am trying to say is that it is more important to keep your chamber "dry", i.e., free of crackable hydrocarbons, and then pump it down to lowest possible vacuum level. Plasma cleaners do help here, but old instrument would typically need a thorough cleaning of the entire vacuum system. Cleaning is not difficult to do, if you have couple of days of time and either a good on-site technician or just someone who is handy with wrenches and vacuum flanges. There are lots of people on this list with good vacuum expertise, so if you describe what kind of pumping system you have in the SEM I am sure you could get all the advice needed to dry your instrument. Valery Ray vray@partbeamsystech.com Thu May 23

FIB:

copper surface migration

Like many other semiconductor labs, we are currently struggling with Cu surface migration on freshly FIB'ed TEM cross sections. Of course, these samples are rush-rush. Has anyone come up with a robust solution? I suspect part of the problem is that the Cu is like six nines pure. One idea I had was to do an oxygen plasma chamber clean when the sample is still inside the FIB to stabilize/oxidize the Cu surfaces. Any speculation / tips much appreciated. ***Bryan Tracy bryan.tracy@spansion.com Thu May 30***

I don't know much about Cu migration across the surface of a FIB prepared sample, but I'm interested in hearing more about it. I was experimenting with sample preparation of copper for EBSD analysis. I can tell you that I took a polished, OFHC copper sample, ion polished it with low-angle, low-energy Ar ions, and then plasma cleaned it with 100% O_2, 5 W for 5 minutes, and you could see that it had oxidized the surface. You will need to control that process to limit the thickness of any oxide layer that you grow on the sample. Scott Walck s.walck@comcast.net Fri May 31

I will give you a few factors we have observed and implemented in our lab. 1. FIB preparation is way better than polishing and ion milling. It can be done, but you have extreme challenges. With FIB prep., you cannot do low KV final thinning, which we have started doing to minimize amorphization of the Si. We have found our cutoff to be ~8 KV; if we use that, we have not observed extensive Cu corrosion problems. The Ga beam appears to induce a small amount of surface mixing which makes the structure more robust to corrosion, and more so at higher KV. This probably also explains why polishing plus Ar milling has so many problems; the KV is generally quite low (<3 KV) and the surfaces are too pristine to avoid corrosion. 2. The samples should be stored under vacuum, with a nitrogen purge when venting. This keeps moisture away from the sample, and moisture is the key problem. (Plus whatever contaminants your local power generating station is pumping into the air!!) We tried a flowing dry nitrogen cabinet, but vacuum has worked much better, probably because the pump-down removes the air around the sample, and the next purge surrounds it with nitrogen. 3. Do not plasma clean unless absolutely necessary. With vacuum storage, we generally find that it is not needed. Some samples, however, do have severe contamination, usually from a dirty grid. These will have to be plasma cleaned for STEM or analysis, but you will likely find corrosion of the Cu if you go back to the sample again. 4. If you have used Enhanced Etch (iodine) in your FIB, you are really stuck. The iodine will end up accumulating on the interior surfaces, and will spontaneously react with Cu to form CuI_2. A chamber clean plus a bake might clear things up if the GIS has been removed. Let me know if any of this helps. Since we implemented vacuum storage and >8 kV FIB beam, we have had virtually no problem with Cu corrosion, and have been able to revisit stored samples for up to a few weeks. Philip L. Flaitz flaitz@us.ibm.com Fri May 31

When Intel first started copper, the TEM labs were borrowing time on FIBs that were used for F/A, all with iodine or xenon difluoride. The samples that came out of these FIBs tended to be covered with "fur" by the time they got to the FIB. What we found was that whenever a FIB had ever been used for enhanced etch, it was never again suitable for use as a TEM prep tool for copper. This required purchasing new FIBs dedicated to TEM prep, something that did not go down well with management! However, it did solve the problem. The other approach that helped a lot was vacuum storage of the specimens. We purchased a Gatan unit, specially designed for specimen storage. It used the vacuum system of the model 655 specimen holder. You can see the design on their web page. This worked well for some time. However, a word of caution: You must change the rubber diaphragms annually, *without fail*. If the diaphragms develop cracks, it will back stream sulphur containing motor oil into the system, contaminating everything and corroding the copper. Our $17,000 system had to be scrapped because the molecular drag pump was contaminated, and it could not be successfully cleaned. My experience with the nightmare of copper returned after Intel closed in Santa Clara, and I moved to Western Digital Media R&D. Some experimental disks contained a layer of copper. Before we had our own FIB, we had all of our samples prepared by a local vendor of TEM services. On a few occasions, their weekend shift used an 855 (with enhanced etch installed but not used) just to do the lift out and glue the 1 micron lamellae to the copper grids. They were then finished in a new Helios. By the time they got to the TEM, they were destroyed. I knew what the problem was right away, but nobody believed me. Eventually, they came to accept the idea that copper could never go into a FIB with enhanced etch installed, whether it was being used or not. Immeasurable traces of that stuff just destroy copper TEM samples. John Mardinly john.mardinly@asu.edu Sun Jun 2

One other thought came to mind a few hours after I first replied, and that was how we dealt with wedge or dimpled and ion milled samples. As I recall, and it has been a few years, we typically had corrosion problems if the sample was not ion milled immediately after dimple or wedge polish. This idea popped up after one technician polished a batch of samples on a Friday, cleaned and dried them, but then when they were milled on Monday, they all corroded instantly after milling. One thought was that something in the Syton was diffusing into the samples, perhaps into the epoxy. One suggestion was that it was the phosphoric acid in the Syton. In any event, when we adopted the procedure that ion milling was done immediately after polishing, the problem of copper corrosion on argon milled samples faded into the background . . . until of course, the rubber diaphragms on our vacuum storage system cracked. Remember, miniscule amounts of the wrong materials seem to be able to have a catalytic effect on the corrosion rate of copper. John Mardinly john.mardinly@asu.edu Sun Jun 2

As I mentioned, Intel had to purchase at least one dedicated small chamber FIB at each of their sites that did TEM, and let me tell you, that did not go over well because Intel is extremely cost conscious about lab equipment. Of course FEI knew about it—they were selling us the FIBs! However, FEI is a big company, and not everyone knows everything. BTW, I am not familiar with the abbreviation IEE. Our experience was with iodine and xenon difluoride, and we never found any success with wiping down the walls of the big FIBs. Besides, the yield and F/A guys still had an ongoing need for enhanced etch, so we had to have dedicated clean systems for doing copper. As for images, sorry, we tended to not waste time taking pictures of ruined samples. John Mardinly john.mardinly@asu.edu Mon Jun 3

Scanning Tunneling Microscopy:
movie

If you haven't seen IBM's 60-second movie "A boy and his atom" made using a scanning tunneling microscope, Google the title and there are a lot of links out there. I didn't paste one here because I suspected the listserver's filters would have blocked it. They used 242 frames of stop motion photography at 100 million × magnification to create this cute movie. Enjoy. ***Tom Phillips phillipst@missouri.edu Wed May 1***

It's at http://www.guardian.co.uk/technology/video/2013/may/01/ibm-smallest-ever-animation-molecular-video but why are there always two dots moving around when it should be only one atom? Without a good explanation for that I am reluctant to show it in my introductory nano-materials science and engineering class kind regards. Peter Moeck pmoeck@pdx.edu Thu May 2

The version on Youtube has a link to a "behind the scenes" movie. http://www.youtube.com/watch?v=xA4QWwaweWA&feature=youtu.be. About 1:41 in they mention that the man is built with carbon monoxide molecules, which would explain the two atoms. So the title should really be "a boy and his molecule" Tobias Starborg tobias.starborg@manchester.ac.uk Thu May 2

Yes, there are two spots. Yes, it is a molecule, not an atom. But why let that take away from the beauty of what is shown, or recognizing the tremendous technological advances in imaging over the past 10 years. It is a Boy and his Atom, and I watch it with overwhelming awe and thanks that after 44 years in electron microscopy I am still privileged enough to see such things. Paul Hazelton paul_hazelton@umanitoba.ca Thu May 2

MT

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