NetNotes

Edited by Thomas E. Phillips

University of Missouri

phillipst@missouri.edu

Selected postings from the Microscopy Listserver from July 1, 2014 to August 31, 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:
denatured ethanol

Is denatured ethanol sufficient for dehydrating samples for SEM & TEM? Or is absolute required? There is quite a price difference, as well as ordering hurdles. ***Joe Uknalis joseph.uknalis@ars.usda.gov Jul 3***

We run a large number of both TEM and SEM experiments and have always used pint sized (open just before 100% step) Ethyl Alcohol 200 proof absolute anhydrous ACS/USP grade form Pharmco-AAPER #64-17-5. We use the opened bottles to make ethanol parts and general lab use. It is crucial that the ethanol contain no water. In the old days I used to molecular sieve the ethanol, but it is no longer necessary. Michael Delannoy mdelann1@jhmi.edu Thu Jul 3

You should always dehydrate using pure ethanol, starting at 25 or 50%, and then continue with these changes: 65%, 80%, 95%, 3 × 100% then if you are using an epoxy resin transition into that. Absolute ethanol is necessary for the 100% changes. Karen Bentley karen_bentley@urmc.rochester.edu Thu Jul 3

Specimen Preparation:
organelles in whole mounts for TEM

We have isolated organelles and mounted them onto Formvar-carbon grids to prepare them for surface immunogold staining. Upon observation, we note that the organelles are quite damaged. We used a 4% paraformaldehyde + 0.05% glutaraldehyde fix in PBS, supplemented with 25 mM HEPES to resuspend them and fixed them overnight. After immunostaining, we negatively stained them with 5% aqueous uranyl acetate. We do note some immunostaining, but our major concern is the quality and integrity of the organelle. ***Vickie Kimler vakimler@med.wayne.edu Fri Aug 1***

Isolation of organelles (which one?) from any kind of tissue (which one?) is always tricky and likely to be pretty harmful. They are taken out of their native environment (usually, they are protected inside a cell, inside a tissue), and during isolation, you disrupt the tissue and the cells, inevitably—first damage—and then you centrifuge the organelles, repeatedly—second damage (most of the microorganisms we have looked at, suffer from centrifugation; some heavily, some a lot, some less). Most of the organelles (mitochondria, chloroplast) are also damaged, and you fix the isolated, i.e. damaged organelles, don't you? second, 4% formaldehyde +0.05% glutaraldehyde is not the most rigorous fixative; I understand, for immuno, you want to use 'mild' fixation conditions, but accordingly, the organelles are not rigorously fixed; best would be cryofixation + cryoTEM, or 2% glutaraldehyde (not good for immuno) plus TEM at room temperature, although this involves air-drying! Next, I understand that you use PBS, because this is the buffer in which your antibodies are in best condition (they are made in blood). But, your organelles do not like PBS: this is by no means a physiological buffer for a condition "inside the cytoplasm of the cell". Neither the pH, nor the ionic strength, nor the ionic composition does fit. To improve this, you would have to go into a deeper analysis (Literature?) of the composition of the cells of your tissue; tricky!! Finally, 5% uranyl acetate is neither necessary (far too high, in my opinion; 1 or 2% is sufficient), and by the way, why staining at all? The pH of uranyl acetate is not favorable (4.5) for organelles, and you will see the organelles without any staining, and the contrast between the gold and the organelles might even be better without uranyl acetate staining. Skip this and at the end, after application to the (plastic or carbon-coated) grid, you air-dry the organelles, after several steps which are not 'optimal'—again, this will result in 'less-than-optimal' structure preservation. A few steps can be improved—but do not expect too much. Reinhard Rachel reinhard.rachel@biologie.uni-regensburg.de Sat Aug 2

Specimen Preparation:
best grids for immunocytochemistry

What is the best Formvar-carbon grid to use for immunogold and/or Fluoronanogold? It appears that nickel, nickel-asbestos and gold seem to be the winners. It is necessary to glow discharge the grids? I am labeling thin-sections (Lowicryl and LR White hard/medium) as well as isolated organelles. ***Vickie Kimler vakimler@med.wayne.edu Mon Aug 4***

In principle nickel, gold, rhodium and even copper grids (with caution) are all suitable, as long as the films on the grids are well made without holes and the film and grid are not damaged or bent during the incubation and transfer. For that reason: consider using a loop wide enough to hold a grid instead of using sharp-edged tweezers. Transfer of incubation solutions can be minimized by blotting. Some considerations: Ni-grids are more solid than the other ones, which can be helpful when going through many steps in an incubation setup. Nickel has a disadvantage, in that it influences the electron beam and may require repeated adjustment of the astigmatism. They can sometimes magnetically stick to tweezers, which can be annoying. Gold grids are fine, especially for labelling that does not use silver enhancement, but they are soft and bend easily. If silver enhancement is used and if there is a hole in the film, you may get enhancement of your grid and debris over the sections. Many use regular copper grids. Even though copper may become oxidized over time and by chemicals in solutions, if your films are decent, those grids can be fine as well. Jan Leunissen leunissen@aurion.nl Mon Aug 4

Specimen Preparation:
annealing nanoparticles on C-film grids

Has anyone annealed nanoparticles on TEM c-film grids? If so, can you tell me the temp and time at which the carbon started to break down? And the specific grid you used for the experiment? ***Marissa Libbee mlibbee@lbl.gov Fri Jul 11***

Carbon films are stable in the TEM vacuum up to 1000°C and even far beyond. But the choice of the film supporting grid is critical. I used with success gold 200 mesh grids with thin carbon films during my PhD work on the melting temperature of

 doi:10.1017/S1551929514001114

https://doi.org/10.1017/S1551929514001114 Published online by Cambridge University Press

The single source
for all your microscopy supplies and specimen preparation equipment.

TED PELLA, INC.

Microscopy Products for Science and Industry

www.tedpella.com sales@tedpella.com 800.237.3526

https://doi.org/10.1017/S1551929514001114 Published online by Cambridge University Press

gold nanocrystals (Buffat, *Phys. Rev.* A13 (1976) 2287). *A priori* molybdenum grids should had allowed high temperatures with good mechanical stability. But it rapidly appeared that the C-film reacts with Mo to form Mo carbide and cracks (see for instance Leroy et al., *J. Appl. Phys.* 99 (2006) 063704 about Mo-C reactivity). Copper grids were excluded because of the risk of alloying with gold. Eventually gold grids were the best compromise though the largest Au crystals did melt only a few degrees before the supporting grid itself. The vacuum in the electron diffraction camera (a TEM without magnifying section) was 2×10^{-6} mbar. A slow heating cycle took about 15 minutes and I didn't observe any significant damage to the film when the sample was transferred to a TEM for crystal size observation. More experiments were performed with heating in a TEM (Philips EM430) though with less temperature measurement accuracy, but still no significant film damage due to heating. However it appeared that the carbon film may destroys under strong electron beam during HRTEM observation. At that time we still used photographic plan films and we noticed a clear correlation between the film sputtering speed and the time between loading films in the camera and HRTEM observation. This shows that despite the presence of a differential pressure aperture between the plate camera and the TEM column itself, water (or methane?) molecules were able to fly straight toward the sample and enhance sputtering (the mean free path of molecules is about meter(s) in this vacuum range). Film stability under strong electron beams depends more on the vacuum quality and residual atmosphere composition than temperature. Philippe philippe.buffat@epfl.ch Sat Jul 12

Specimen Preparation:

cellulose sample preparation

I am studying cellulose aerogel made from Nanocellulose Fibers (10-30nm diameter and several microns in length). Material: The material show highly porosity (up to 90% air) and a very intricate structure with meso and nano pores. Sample: Samples are produced via supercritical CO_2 drying after filtration and solvent exchange. The resulting sample is a ""paper-like"" fluffy material, 3 cm in diameter and a thickness of 300 micron. SEM: To study the microstructure I tried to embed a small piece of aerogel in araldite. The cross-section surface of the sample was exposed with a diamond microtome and sputtered with 20 nm of gold. In the obtained images is difficult to distinguish between the araldite matrix and the sample (a small change in contrast at low magnification). Worst is that on the surface I don't see any microstructure—only a light gray smooth surface. Questions: Is it possible in your opinion to obtain a nice cross section image of my aerogel? I am thinking to change the embedding material with a stiffer epoxy. Will this help? Do you have any others ideas? ***Ferruccio Bolla ferruccio.bolla@empa.ch Mon Jul 14***

Why embed the aerogel? I've done aerogel in the SEM, but never with embedding. Coat with something other than plain gold—Au/Pd, Pt, or something—and use low kV. "Low kV" depends on what SEM you have, but no more than 5 kV. If you can go down to 1 or 1.5 kV, that would be better. Possibly lower. If you can find the kV at which the charges on the sample balance, you may not need coating at all. Also, you can submerge the aerogel in liquid nitrogen and carefully snap it to expose the interior. I'm assuming the fibers have glass transition above the LN_2 temperature, but I'm sure it does. If you have to embed the nanocellulose fibers in Araldite, you can stain the Araldite with RuO_4: place the sectioned blocks in a sealed dish in a fume hood with a crystal or two of RuO_4, and let the vapors stain the resin. The nanocellulose should remain unstained. Or: sputter coat the aerogel with gold or any heavy metal, but use shorter coating runs, and turn over the block of aerogel between coats, so the metal gets equal access to the aerogel from all sides. Then embed. The fibers will be the bits that are surrounded by the heavy metal, which will show up in the SEM. Mind, this last is only good for sections of the fibers, and the surface will be obscured. Phil Oshel oshel1pe@cmich.edu Mon Jul 14

We recently observed some polymer aerogels in a test run for the purchase of a new SEM, and with recent SEMs it is possible to do nice things at very low voltage (300 V) in deceleration mode (the sample is negatively biased to slow down the incident electrons, and the SE and BE are re-accelerated up to the detector), without any coating. It works well, with imaging up to more than 100 kx, but need some skill to find the right conditions. For the cross-section, why not try the ion beam cross-polisher. You could obtain some test run asking one of the manufacturers (Gatan, JEOL, Hitachi, Leica Technor-Linda, etc.). No embedding, possibly no metallization. Jacques Faerber jacques.faerber@ipcms.u-strasbg.fr Tue Jul 15

Can you explain why you want to embed your sample? Particularly if you can coat with something finer than gold (platinum, carbon) and if you have access to some low voltage/charge minimizing features of the recent generation of SEMs, it should work. Tobias Baskin baskin@bio.umass.edu Mon Jul 14

If you don't manage to manually open the aerogel successfully, you can try to embed it paraffin and get some thick sections, which then you treat with a warm (> 70°C) solvent compatible with your staff to get rid of the wax and then coat and view in SEM? Yorgos Nikas eikonika@otenet.gr Mon Jul 14

It might be the same problem like cutting polystyrene (Styropor). I succeeded only with a piece of polystyrene submerged in liquid nitrogen and then using a new razorblade cooled at LN_2 temp to cut the polystyrene. The cells and walls looked relatively unharmed in the SEM afterwards. See some images of it here: http://www.electronmicroscopy.info/shop_materials.htm image number 27 to 31. Stefan Diller stefan.diller@t-online.de Mon Jul 14

I have done quite a bit of imaging of SiO_2 aerogel, (not nanocellulose) but some of our imaging approaches may work for you. To image it in an SEM, I use low voltage, uncoated. Because of the low density of aerogel, the charge balance point is surprisingly high. Our aerogels are especially low density aerogels (about 20 mg/cm^3, which for SiO_2 is about 99.3% vacuum) and the charge point on most of our aerogels comes in at around 2.1 to 2.2 keV. You can get some very nice images this way. It helps to use a field emitter because of the very fine structure of the fibers, and the fact that you want to keep the current low as well as the voltage. For sectioning aerogel, we use motorized glass needle cutting: Westphal, A. J., Snead, C., Butterworth, A. L., Graham, G. A., Bradley, J. P., Bajt, S., et al. (2004). "Aerogel keystones: Extraction of complete hypervelocity impact events from aerogel collectors." *Meteoritics and Planetary Science*, 39(8), 1375–1386. This may be a lot of work to set up, but it works very, very well. One thing you can do is cut a 100 micron thick section, flip it on its side, and cut another section a few tens of microns thick. Then you can affix that to a substrate and image it uncoated with high voltage in an SEM. It works because the density is so low the electron beam just goes clean through. (Poor man's TEM.) Finally, I have gotten images in a TEM, but since I'm not usually studying the aerogel itself (but rather minerals trapped in it) I've never developed a good protocol here. However, it is my experience that epoxy embedding always alters the structure of the aerogel, so you probably will be better off going with some of the other suggestions if the above doesn't work for you. I concur with Stephan that it is possible to get a good cut with razorblades (though don't drink coffee first—it will make your hand shake!) And Phil's approach seems sharp too. Zack Gainsforth zackg@berkeley.edu Mon Jul 14

https://doi.org/10.1017/S1551929514001114 Published online by Cambridge University Press

https://doi.org/10.1017/S1551929514001114 Published online by Cambridge University Press

Specimen Preparation:

Epo-Fix epoxy as an embedding medium

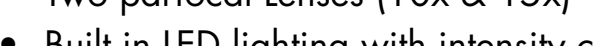
I am trying to help a user to section her sample embedded in Epo-Fix epoxy. As I am not very experienced with the ultramicrotome, I decided to section the Epo-Fix epoxy without any sample in it with a glass knife as a start. Unfortunately, I am unable to section it to a thickness of about 100 nm. What I observed is that the glass knife is only able to section at alternating intervals. I learned that this can be due to either the epoxy is soft, or the glass knife is not sharp, or the block face might be too big. The student told me that that glass transition of the epoxy is above room temperature so there shouldn't be a need to adopt a cryo-ultramicrotomy approach. I also attempted to trim the block face to about 1mm (or slightly less), but the same alternate sectioning observation is made. I have tried sectioning with a biological sample embedded in aradite previously with no problem achieving thickness of 100nm or less. My glass knife cutting angle is at 6°. May I ask if I have missed out anything? Is Epo-fix suitable to be sectioned with a glass knife to achieve a thickness of 100 nm? My second question is: does the Tg of the sample determine if one should use a room-temperature ultramicrotome or a cryo-ultramicrotome? ***Yee Yan Tay rongchigram79@yahoo.com.sg Mon Jul 14***

We need also to know which ultramicrotome (thermal or mechanical feed) you are using for this approach. Epo-Fix is known to me as a specimen mountant (mounting medium) in materials sciences (cf. e.g., http://www.struers.com/resources/elements/12/255648/Cold%20Mounting%20table_Epoxies.pdf). Unfortunately I have no experience with sectioning Epo-Fix-Epoxy embedded specimens. It might be this resin too soft or too hard. It might be also the type of glass strips you use and how the quality of your knives. Trials to overcome the problem with your glass knives (including the existing angle to be 35-45-55 degrees comparable with the diamond knives 35/45/55 degrees) could be: i) Sectioning at a (narrower) cutting angle of say 4.5—5.5 degree instead of using 6 degrees. ii) Lowering the level of the water in the knife boat. iii) Playing around with section speed while ensuring that the section phase is set sufficiently long enough; i.e. start cutting at least 1 mm above block edge, end of cutting phase as short as possible below /after having sectioned through the block at its lower edge). Try to harden the block further with oven overnight at 65-80°C. v) Last but not least—only if available—use of a diamond knife. With the usual resins for TEM (e.g. Epon 812, Embed812, Glycidether 100 substitute (for Epon 812), LX-112 etc. in former times (also using glass knives)—when mixed thoroughly according to the recommendations of Luft (1961) and others I had no problems cutting also bigger specs at least up to 4×4 mm (for my thesis I had a really soft resin mixture for cutting whole rat hypothalami—1day PN to 320 days of age—including the ventricle). Wolfgang Muss w.muss@salk.at Mon Jul 14

I am using Leica UCT. I use a mechanical feed of 100 nm. At 300 nm, it seems not a problem. Yee Yan Tay rongchigram79@yahoo.com.sg Tue Jul 15

If there a mechanical problem with your Leica UCT (stepper motor, feeding)? If not then I am pretty convinced you should be able to cut at least down to 50 nm (if not 30 nm). Have you considered also the room climate as a source of failure (i.e. humidity, draft)? Perhaps some shielding around the block-face—section area could be of benefit. There exists a description of errors and trouble checking for cutting from H. Sitte (who was one of the main inventors of the classical Reichert ultramicrotome series OMU-2,OMU-3, followed by Ultracut, Ultracut E and then UCT etc.). I shall send this pdf to you, so you perhaps can find out other sources of failure in cutting serial sections with equal thickness (specified therein esp. point 4.: "irregular section thickness"). Wolfgang Muss w.muss@salk.at Wed Jul 16

Macro View **All-in-One Scope**
Compact Video Inspection System



For more info

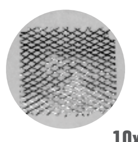
- Easy inspection on a 10" LCD screen
- Wide field of view and long working distance
- Two parfocal Lenses (10x & 15x)
- Built in LED lighting with intensity control
- 2x Digital Zoom and Mirror function built into camera
- Compact and Easy to Use
- Increases operator productivity

The Ultimate Inspection Tool

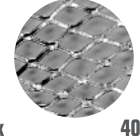
With the USB-powered **Mighty Scope**, convenience comes standard — you get built-in lighting, focus, and snapshot capabilities for one highly competitive price. It's the ultimate digital inspection tool for industrial, scientific, and research applications.

Don't worry about choosing the right model. Choose the model that has it all.

- 1.3M and 5M scopes
- 10-200x and 500x magnification
- Inspect, analyze, share, and measure with ease
- Simultaneous viewing capabilities for multiple angles
- Includes software for measurement, imaging, and documentation
- Prices start from $209



10x



40x

200x

aventools.com | 734.973.0099 | sales@aveninc.com

High Performance Precision Tools for Microscopy, Inspection & Precision Assembly

https://doi.org/10.1017/S1551929514001114 Published online by Cambridge University Press

Specimen Preparation:
flattening of thin sections

When one chloroform-flattens ultrathin sections for the TEM, is there artifactual stretching and resultant alterations in the dimensions of structures, which are critical in morphometric work? Does chloroform have a greater effect on LR White/Lowicryl versus the Embed and other harder non-immuno resins? Finally, on a serendipitous note, would chloroform have the ability, if it does indeed stretch apart structures on a nano level, open up antigenic determinants for the often difficult immunogold work? ***Vickie Kimler vakimler@med.wayne.edu Sat Aug 23***

I am sure overstretching is a risk but so is not stretching. To get a feel for the effects of compression on thin sections, look at the article by Studer and Gnägi (2000), "Minimal compression of ultrathin sections with use of an oscillating diamond knife." *Journal of Microscopy*, Vol. 197(1): 94–100. This article is about using a vibrating diamond knife to avoid compression but I remember it having good comparison images. Diatome's web page has an interesting discussion at http://www.diatomeknives.com/knives/pdf/ultrasonic_flyer_usa_1108.pdf. They say the compression of various resins are 10–20% for epoxy resins, 12–24% for Lowicryl K4M, 10–17% for Spurr's and 8–13% for LR White. I don't use chloroform anymore. I am a big fan of the less toxic heat pens. I feel 120 V electrical-line models are much better than battery-operated ones. Tom Phillips phillipst@missouri.edu Sun Aug 24

Specimen Preparation:
leukocytes for SEM

Can anybody give me a detailed protocol on how to grow, fix, and postfix leukocytes / T-cells on coverslips to be imaged in the SEM? Best would be to have them OsO_4-stained also at some point. Drying would be through ethanol to critical point drying. ***Stefan Diller stefan.diller@t-online.de Tue Aug 26***

What I used for FE-SEM of lymphocytes: Cultured cells on glass coverslips in their normal growth medium. Make sure they're a monolayer, non-confluent is preferred. It's best to sputter-coat the coverslips first, then sterilize with alcohol or UV. The cells like a gold or gold/palladium substrate, and they're on a conductive surface, which makes life easier. Fix 1-2 hrs at room temperature in 1.25% glutaraldehyde in appropriate buffer. The buffer weaseling is because the choice can depend on what you want to do with the cells. Sorenson's phosphate works, so does Na-cacodylate, HEPES, etc. I used 0.1 M. 0.15 M might work for yours. Add 1% monomeric tannic acid to the fix. This helps prevent holes in the cell membranes. OsO_4 wasn't needed, but if you want to use it, then 1% OsO_4 + 1% monomeric tannic acid for 1-2 hours at room temp. Overnight in the refrigerator would probably be OK, but I'd test it first. Dehydrate through ethanol—I started at 30%, then 50-70-80-90-95-3x100%, 5 minutes each. Critical point drying: 5 soaks at 3 minutes each in liquid CO_2, then do the run. 5 minute soaks can also be used. Coat with your favorite high-resolution coater. Phil Oshel oshel1pe@cmich.edu Tue Aug 26

Software:
drivers

We have two Optronics cameras on light microscopes, a MacroFire and a MagnaFire. One has a Sony chip and one a Kodak chip, and they have different software, and we like both, especially Picture Frame (MacroFire). But the MacroFire driver does not work past Windows XP service pack 3, and the MagnaFire does not work past Win XP service pack 1. Their respective computers are dying, and although we keep putting them on newer old computers, it is a losing battle. Has anyone come up with a hack to keep the older Optronics cameras going? I can sort of make them work with a generic twain driver, but we really like the capabilities of Picture Frame for mixing channels, etc. I heard a rumor they would work on Win 7 32 bit, but that didn't pan out. Any other solutions? ***Tina (Weatherby) Carvalho tina@pbrc.hawaii.edu Tue Jul 8***

Have you tried Windows XP mode in Windows 7? Odds are that the Windows 7 version you have access to allow for this possibility. Windows XP mode was designed to allow for the use of legacy software in Windows 7. I am not sure how well it handles legacy hardware. Some install info from Microsoft: http://windows.microsoft.com/en-us/windows7/install-and-use-windows-xp-mode-in-windows-7 Rafael Buono rafaelbuono@gmail.com Wed Jul 9

Core Facility:
humidity problems in cyroEM lab

Our labs are contending with high humidity problems during cryo-ultramicrotomy, cryoTEM, Vitrobot sample preparation, cryoSEM, etc. Midwestern summer weather is a challenge. Colder weather much less so. Air supply is whole-building; i.e. common source & exhaust, with some local zone control via variable air volume valves. Facilities Management has lately been able to keep temperatures in 68-73°F range; relative humidity mostly in 48%-60% range. Facilities Management requested that I seek ideas from other facilities to improve situation. I suspect we'd have to isolate the facility from rest of building; probably very expensive. Adding dehumidifiers would add heat, noise and vibrations to rooms but seem pointless if all building air is shared. Likewise we must preserve good air quality, i.e., sufficient turnover. Understood that proper sample techniques and instruments and instrument use are key factors; just looking for other ideas from those similarly challenged. ***Chris Frethem freth001@umn.edu Thu Aug 28***

I just read your message and thought about how my glass of ice water last night had so much water on the outside. What if you put several beakers of ice on the other side of the room or maybe in your sink, would enough water be removed from the air by condensation to help? Years ago I had problems with summer humidity causing too many holes in my collodion films for the grids that I was attempting to coat. I found I had to make them beside a running area heater. Hot and dry! It worked fine for me then I needed the ice water. I do question it for cryoEM however. Patricia Stranen Connelly connellyps@nhlbi.nih.gov Thu Aug 28

I was once facing a similar problem, aggravated by the fact that the health and safety inspector had demanded a humidifier for the building. The high rate of air exchange (8 times per hour, as far as I remember) in the lab precluded the use of mobile dehumidifier units and any other provisional solutions. In the end, a large, fixed dehumidifier was installed on the corridor in the incoming air supply of three rooms—cryo-preparation, Polara, and another microscope. Yes, this unit is noisy (hence on the corridor) and using a lot of energy, but it is worth it! Guenter Resch lists@nexperion.net Fri Aug 29

Our solution to temperature and humidity control for an aberration corrected STEM (though not cryo) is a dedicated, semiconductor industry type A/C unit. They can be small enough for just a room—though they do need their own closed cycle duct work. Temperature is very precise and maximum allowable humidity and air flow rate are set independently. Totally quiet and stable in the room, as they are mounted remotely. Does not need venting to the outside. It is a more expensive solution, perhaps, but not compared to the price of the microscope or your lost

productivity. I can pass along more details if you are interested. Larry Scipioni les@zsgenetics.com Fri Aug 29

Core Facility:
EM relocation

Any hints as to how to handle relocation into a new building, in a smallish area between the four main lift (elevator) shafts, and next to the heaviest traffic corridor from the main loading dock. Building will house 600+ scientists, etc. which gives an idea of the traffic. The architects, and presumably our corporate overlords, appear to have locked into the building design, and say the microscopes just have to go in this place. My preferred option is to insist on external specialist consultants to design something that will eliminate or at least minimize any vibration and electro-magnetic interference. Any other suggestions? ***Rosemary White rosemary.white@csiro.au Fri Aug 1***

I'm dealing with a move myself, although not like this. Assuming you have to move, and can't stay where you are, then first, have the EM companies whose 'scope you have come in and do site surveys. When the site fails for both vibration and EMF, then insist on getting the antivibration and EMF shielding before the move. Might even be cheaper to build a ground-level addition to the building with a proper, isolated foundation. Since this is an "addition" and not a change to the building design, that might be approved. Especially after the survey failures. Phil Oshel oshel1pe@cmich.edu Fri Aug 1

Putting microscopes near an elevator would be a disaster. The room may pass EMI specs, but the large ferromagnetic mass alters the earth's magnetic field for quite a distance, so when the cars go up and down, the alignment and focus of the microscope will change and the image may move. John Mardinly john.mardinly@asu.edu Fri Aug 1

I could also mention that the substation serving the building, plus the main switchroom and distributor room are all within 10 meters of the microscopes in the current building plan! The main electrical (and everything else) risers for this 4 (or 5, not sure, haven't seen final plans) story building are 2 and 4 meters away. The elevators are closer at 2 and 3 meters away. Rosemary White rosemary.white@csiro.au Sun Aug 3

TEM:
three-way valve and filament

Our 'FEI' HRTEM F-30 is down over a month. There is a problem in 3-way valve in Zephyr Chiller. We would like hear from supplier who can supply us on urgent basis. FEI TEM T-20 is down as the filament is blown off. Can we get the supplier in India who can supply the tungsten filament on urgent basis? ***Rashmi Mehta rashmi_mehata@yahoo.com Thu Jul 10***

Sometimes a good hit with a hammer works on the three-way valve. It can also be opened and cleaned. Filaments can be ordered directly online at kymbalphysics.com. Wim Hagen wim.hagen@me.com Thu Jul 10

In addition to the option of purchasing electron sources from either Kimball Physics or Denka, you may consider refurbishing the old source by replacing filament and the tip. You can contact York Probe Sources in UK for tip rebuilding service. Valery Ray vray@partbeamsystech.com Thu Jul 10

TEM:
sticky specimen control knob

The specimen control knob that moves the specimen in the y-plane has gradually become unresponsive and now will not move the specimen to the right at all. I have a Hitachi H-7100 TEM. Can anyone give me some ideas how to fix it? ***Tracy Lawrence tracy.lawrence@inspection.gc.ca Mon Aug 11***

It is a long time since I operated a Hitachi 7000 series but your problem could be traced to what is a generic TEM problem? The "O" ring on the specimen rod becomes dry over time, and, as this is the bearing on which the stage movement runs, the stage in that direction will become erratic. The control being used here is the right hand stage drive. If this is your problem, running a finger lightly coated in a good quality vacuum grease round the "O" ring will ease its movement. Once free the specimen rod will move into the microscope more quickly on specimen exchange, so take extra care! If the problem is on the left hand stage drive take a close look at where the vertical shaft enters the pivot joint; does this move easily? Steve Chapman protrain@emcourses.com Tue Aug 12

TEM:
angle correction

We are using a JEOL 2100F TEM. I have 3 questions! 1. What is "Angle" correction? (It is shown on "Alignment" options and grouped along "Tilt" and "Shift", when "Angle is wrong my tilt wobbler will wobble in oval trajectory rather than on single line) 2. How badly can it affect resolution of STEM? In other words, how important is it? 3. Shall I align it in Tilt or Shift mode? As when I align it in one of modes, it gets disoriented in other mode. ***Amit Gupta amit.welcomes.u@gmail.com Thu Aug 14***

1) The tilt and shift alignments are making sure that you get pure tilt when you tilt and no beam shift and pure shift when you shift and no tilt. The reason that you have to do this is because they use the same sets of deflection coils to do both. For the 2100F, when correcting tilt in TEM image mode with the beam at crossover, you do X only with X-tilt wobbler and then Y only with Y-tilt wobbler. When you use the each deflector, it will move the two beams across the screen and you can bring them back to the center using the corresponding X or Y beam shift control. Of course, you stop when they are on top of each other. You do the Shift correction in diffraction mode with the condenser fully counter clockwise and the diffraction focused to a caustic spot and brought to the center of the screen with PLA control. Again, shift X wobbler with X only and shift Y wobbler with Y only. If you are doing the Tilt correction, X or Y, and the two spots are offset and go past each other, i.e. you can't get them to coincide, and then you have to make the Angle correction. Adjust the two spots so that they are perpendicular to the direction of travel during the adjustment. This should be the closest approach to each other. Then switch to Angle with the X or Y wobbler still on and correct this offset, again, only using the same X or Y deflector knob. Once you get that set, it just doesn't change over months. The shift corrections on my instrument never seem to wander very much at all and they line up. I don't know whether the Angle should be used in this mode or not. I've never had to do it. 2) Before aligning the STEM, you have to align the TEM. I assume that not having the TEM properly aligned would be bad for STEM. You are using beam deflection coils in STEM and you have to have the tilt and shift compensated. 3) As I said above, I've only seen it occur in Tilt correction and not the Shift correction procedure on my system. I will send you my alignment procedure in a separate email from work. If they are split when doing the shift correction, you might consider asking your JEOL service engineer about it. We've had the preventative maintenance done several times since I've taken over the instrument and it is always perfect. Scott Walck s.walck@comcast.net Thu Aug 14

https://doi.org/10.1017/S1551929514001114 Published online by Cambridge University Press

SEM:

magnification calibration variation

I recently got back from MMC 2014 in the UK (microscopy convention) and I was truly surprised that all the SEM manufactures still do not allow users to calibrate the scale bar on the new SEMS. The responses were all the same: "Our product IS-calibrated in the factory, there is no need to mess with it." Or: "If you want to calibrate, then why don't you run a standard in parallel?" When I push the reps, most confess that the calibration is done at best on a ~2000l/mm grating, which on our SEM does not equal good high-mag measurements 400 kX+. I am also amazed that most companies also do not correct for slow scan drift in the pixel so seeing as there is never a vertical scale bar in the images (well not ours) how can you believe the measurements? I am not sure if it just me or my experience with older SEMs, but I am sure there are many fields where a user might want to have independent calibration? I know this has been much discussed in the past but with digital recording we seem to have lost respect for calibration (perhaps this is just my jaded view?) In our center we use a third party imaging system which is calibrated by ourselves against standards of our own making and verified with commercial standards and we get very good accuracy and reproducibility (we think). We account for all changes in beam and vacuum state. It certainly agrees with the TEM, XRD and other techniques we might use. The same cannot be said for trusting the machine scale bars. We have very old machines. I am right in not trusting new machines as much as the old ones? I would be interested to hear what people's experiences are with this mag calibration issue especially in high mag. Has anyone found a way of doing this within a modern SEM? What standards do you use? Do you think the standards are good enough? I also wanted to see if anyone would be interested in a little global test. We make LED devices in house so measurement for us is critical. I would like to propose that I send out to anyone interested a small piece of a bulk wafer (the same one!) with a pin array on it with a set size, shape, and spacing. The aim would be to take pictures at set magnifications e.g., 10, 100, 250, 500kx and then make measurements based on your own lab method. You could then return the data to me along with the make and model of the SEM and your method of imaging/calibration. I would then collate and circulate the anonymized data to the list. This is not about the age of the SEM, the maximum magnification, the type of source or even the lab budget I am just really interested in seeing what the real variation is out there. If you have an interest in this and perhaps would like to participate in my little test, contact me. I would welcome instrument manufacturers to take part. ***John Mitchels john.mitchels@gmail.com Sat Jul 19***

My mentor at HP, Nancy Phillips always had us check the Mag Cal on our many SEMs every quarter. We used an MRS 5 at that time, NIST traceable. If we found a measurement of the MRS 5 that deviated more than 5% we let FEI know and they (Jeff Cohen) would come and change the calibration for us! It was nice, as we had an FEI service engineer, Jeff, onsite. We really never saw deviations above 5% ever, a testament to Jeff's ability. Now that I am at Oregon State, I have implemented a similar policy. I purchased an MRS 6, at $5k they are not cheap, and then I check all our SEMs at a random selection of Magnifications, voltages, detectors, we then update an excel spreadsheet that is shared between my coworker and I on a Google drive. I have not seen deviation above 5%. Again, testament to our FEI guys: Bob Johnson and Frank. Just today I checked our Nova NanoSEM at 5 kx, 10 kx using the ETD and 100 kx with the TLD in immersion. Deviations of the MRS 6 pitch were well below 5%. I would certainly participate in an inter lab comparison with your LED. I did one of these with the ANSI TAG group on gold nanoparticles a few years back and it was fun and educational. Pete Eschbach peter.eschbach@oregonstate.edu Mon Jul 21

Happy to participate in your test. My impression from my JEOL JSM5600LV is that a variation of at least 5% in magnification scale may occur within two measurements on the same sample with the same microscope settings, provided that the microscope was shut down and restarted between these two measurements. Also, I have an external scan generation device (DISS5 from "point electronic") that is also calibrated and can be tested for accuracy. Yorgos Nikas eikonika@otenet.gr Mon Jul 21

STEM:

optimizing angles for high angle annular dark field microscopy (HAADF)

We recently had the Gatan HAADF detector off our JEOL 2100F. I measured inner and outer diameters and found that the angles in the manual at the specified camera length were not correct. So, in addition to going through the exercise of measuring the collection angles at different camera lengths for my GIF, I carefully measured the camera lengths at the HAADF position and calculated the inner and outer collection angles for the HAADF detector. I work with a lot of different materials, from polymers and light element ceramics to heavy element alloys. My question is there a method to optimize the camera length, i.e., collection angles for a particular material system when you know the elements? Should I base it on the EELS characteristic angles for the elements? If I do, use the K shells? If I want to maximize the contrast, should I try to exclude the lower Z characteristic angle from the detector? ***Scott Walck s.walck@comcast.net Thu Jul 24***

I did this task using NIST's Elastic32 program. This calculates the differential electron scattering cross section for elements; combined with a quick integration over the correct angles, relative counts can be determined. Larry Scipioni les@zsgenetics.com Thu Jul 24

EDS:

elements associated with people

Besides carbon and oxygen what elements would you expect to find in a fingerprint, dander, or hair? When doing failure analysis I would like to know if there are elements that I can look for and combine with other information that would point to poor handling or cleanliness vs some other cause. ***Jonathan Abbott jabbott@moxtek.com Fri Aug 8***

Besides C and O, Cl, Na, and K are common. N and P are not uncommon but generally at low levels. Warren Straszheim wesaia@iastate.edu Sat Aug 9

My high school chem teacher had an acronym for that: C HOPKINS CaFe. I'm not sure what the best of these—highest concentration in finger print and lowest in the environment—would be. I'd guess P or S. Bill Tivol wtivol@sbcglobal.net Tue Aug 12

Hair and (probably nails) will be unusually rich in sulfur compared to other tissues including skin. Hair proteins, keratins and keratin-associated proteins (or KAPs) contain a disproportionately high level of cysteine amino acid which has sulfur. As I understand it (getting into less certain territory, but something you could follow up) hair also absorbs elevated levels of copper and possibly other heavy metals. Copper is the main one. Other than that it would just be the same crew as for all biologically based material that Bill mentioned above. Also, some of these samples, especially skin and fingerprints, probably have high levels of lipids that (given enough fingerprints) will be something you don't want inside a high vacuum system I would imagine. Duane Harland duane.harland@agresearch.co.nz Tue Aug 12

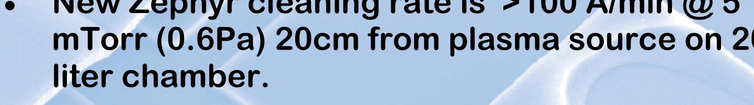
MT

https://doi.org/10.1017/S1551929514001114 Published online by Cambridge University Press

Evactron® De-Contaminators

Fastest Removal of Performance Degrading Hydrocarbons

Evactron® *Zephyr* ™ remote plasma source



- New Zephyr cleaning rate is >100 Å/min @ 5 mTorr (0.6Pa) 20cm from plasma source on 20 liter chamber.
- Easy operation with turbo molecular pumps
- Interlock protects E-gun and detectors
- Proven safe for EDS windows –warranted.
- 2000 units sold, 5 year warranty
- Fully compliant: CE, SEMI S2, and NRTL standards
- Front panel control or remote computer commands

By XEI Scientific

www.evactron.com

https://doi.org/10.1017/S1551929514001114 Published online by Cambridge University Press