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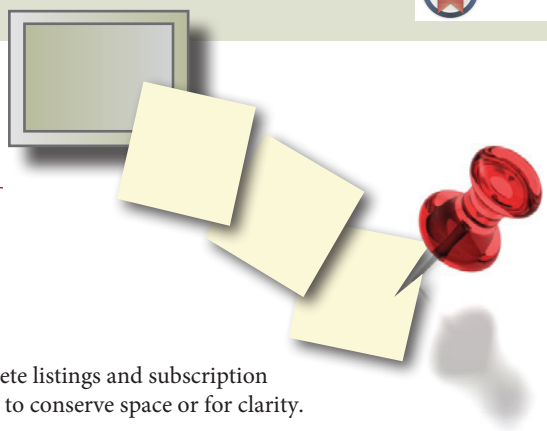

# NetNotes

**Edited by Thomas E. Phillips**
University of Missouri

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Selected postings from the Microscopy Listserver from May 1, 2016 to June 30, 2016. 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:
### mouse skin for SEM

*I've been working with a researcher that follows the following generic protocol to prep their mouse skin sample: Fixation with 2% glutaraldehyde, 4% formaldehyde in 0.1 M $PO_4$ (pH 7.3); 1% $OsO_4$ in water 60 min. Rinse in water 5 minutes. Ethanol 35% - 100% in steps. Critical point dry with liquid $CO_2$ and sputter coating. Problem: when imaging, some of their samples turn out great, a little less than half look melted and "blobby." Using the same magnification, probe current, etc. They have noticed that once out the sputter coater, all specimens appear the same. However, after sitting for 24 hours pre-imaging, the samples that appear abnormal have turned black. I'm fairly new to SEM, has anyone ever experienced a similar issue or can anyone offer a cause. We feel like its happening somewhere in the preparation, just can't track it down.* ***Adam Fries adam.fries@ucsf.edu Tue Jun 28***

Do you have an image you can share? How are you mounting the specimens? Too much adhesive (cyanoacrylate) can ride up the sides and top surface of small tissue pieces covering them to give a "melted" appearance. The only other thing I am leery about is unbuffered osmium (after primary fixation buffer rinses). Michael Delannoy mdelann1@jhmi.edu Wed Jun 29

First, how thick and consistent are the skin preps? How much of the dermis is included? Skin is mostly protein, keratin in particular in the outer layers, which does not take up much osmium. Dermis will contain more or less fat, which will take up osmium (especially if the fat has double-bonded carbon) and turn black. But, they'll be black coming out of the osmium. Maybe wash longer. Also: what $PO_4$ buffer? $Na/Na_2$ or $Na/K_2$? If you're going to use osmium, avoid K and use Sorenson's phosphate ($Na/Na_2$); you'll have less trouble with precipitate formation. Or use Na-cacodylate buffer. Also, try some samples without the osmium post-fix. It imparts some conductivity, but probably isn't needed for this study otherwise. Further, the fat in the samples may be more or less extracted by the dehydration procedure. How long in each ethanol step? What steps? Don't skip the 95% step (i.e. don't go 90 => 100%). Variations in fat content is the most likely reason for the differences in how blackened the samples are. But samples turning color in storage ... that's different. How exactly are the samples stored? When samples turn color, do all the samples in that container turn color, or just a few? I assume you're looking at the surface of the skin samples. Try taking some "good" ones and some "abnormal" ones (hey, right now, you don't really know which are the real ones and which are artefactual) - the dried samples, post storage, post "huh? They've turned black," and throw them in liquid nitrogen. Fracture them and very quickly, put them in a desiccator (maybe prefilled with dry $N_2$ from a tank). You don't want water condensing on them. Be magical - in liquid nitrogen to in desiccator without seeing the air. Look at the cross-section of the samples - what are the differences? This may help diagnose the problem. If nothing else, they'll be nifty to look at. Phil Oshel oshel1pe@cmich.edu Wed Jun 29

## Specimen Preparation:
### exploding plastic bottles

*We have always stored fixed specimens of various sizes in plastic Nalgene bottles, because they are so resistant to chemicals. However, we've recently realized that the bottles grow very brittle with age, and can "explode" into numerous shards when squeezed slightly. I think it is a result of age, not the stored fluids, since even bottles with 70% ethanol are brittle. Have others experienced this? Is it a common, well-known problem?* ***D Blackburn dblackburn2000@yahoo.com Wed Jun 8***

You'll receive more accurate information from a Nalgene specialist, but yes, plastic bottles do age out as the plasticizers come out. If any of your bottles start to discolor or craze, I'd start to distrust them. I believe light accelerates the degradation, and the internet reports "denatured alcohol" as another possible accelerator. I'd also wonder if the platicizers are affecting your samples. They don't "explode" as much as they simply "shatter." It is startling, though. Gregg Sobocinski greggps@umich.edu Fri Jun 10

A quick look at Thermo Fisher indicates that Nalgene is not a specific type of plastic, but a brand name. Some bottles marketed as Nalgene are flouropolymers (FEP) and very resistant to chemicals. Some are LDPE, which can be less so. I would suggest checking into exactly which type of bottles you have. A different polymer might work better. Alternatively, are the bottles exposed to strong solvents or acids? Perhaps within the same cabinet or fume hood? Acids in particular can do severe damage to plastic bottles. Jake Jokisaari joki@umich.edu Sat Jun 25

I have had several low-density polyethylene bottles (I don't know if they are Nalgene brand or not) that have become brittle and will crack when squeezed. One had had dilute nitric acid in it for a while, one had held acetone, and others were empty on the shelf, but had been previously used. I now check the flexibility of any LDPE bottles that I know are not new. Jim Murowchick murowchickj@umkc.edu Sat Jun 25

I have not experienced this exact problem, but I do know that some plastic containers, such as tissue culture water bottles, do indeed deteriorate and can crack, split or break into small shards. I'm pretty sure this happens with age regardless of the fluids stored in the bottles; nor does it seem temperature-dependent. Whether or not this is a common problem, I am not sure. I used 20ml borosilicate glass vials to store tissues. Through VWR, they are relatively inexpensive and more reliable than plastics. Debra M. Townley debrat@bcm.edu Sat Jun 25

## Microtomy:
### cryomicrotomy using glass knives

*For years we have used diamond knives to cut ultrathin sections of polymers at low temperature (around -150 °C). When cut, the sections are floating in a boat/trough integrated on the diamond knife, from where they are collected. To cut larger sections we started to make and use glass knives on which a plastic boat/trough is glued using a "Cavex Set Up Wax," a dentistry modeling wax, as suggested by the vendor. Unfortunately the boats fall off at low temperature. Can anyone suggest a solution or "glue" that would resist low temperatures?* ***Ondrej Kotecky ondrej.kotecky@gmail.com Wed May 4***

At Delta microscopy school some of us used 3M silver polyester tape, nonconductive, 850 from Ted Pella to make boats for glass knives. I think the tape is rated for -150ºC. Ted Pella also carries other type tapes that may be used for making boats with lower temperature ranges. You might check. Lita Duraine duraine@bcm.edu Thu May 5

## Image Analysis:
### counting dots

*I would like to know about the available methods and tools to count the number of dots in an optical microscope image and thus find the percentage area occupied by the dots in the image. I work with a Nikon Eclipse optical microscope and the images are captured at 1000× magnification using a Clemex Captiva image acquisition platform (Clemex CCD camera).* ***Piragash Kumar prakashkumar03@gmail.com Thu Jun 2***

This is actually an interesting question, as I am curious what people generally use for image analysis. It seems like there is a bewildering array of free software, manufacturer supplied software, and third-party software out there. The methods used by a lot of these software packages seem pretty black-box. In terms of free software, I second the suggestion of ImageJ; it works well with regular optical micrographs. Another option is to look to the maker of the microscope you are using; I think that most of the makers provide at least rudimentary particle analysis software with the equipment. Nikon provides a package called 'NIS-Elements' (http://www.nikon.com/products/microscope-solutions/lineup/img_soft/), though it is not free. Apart from that, there are other programs that are optimized for EM or AFM that might work, such as gwyddion (free), SPIP (not free), or with an EM focus, Digital Micrograph (from Gatan, for EM but takes any greyscale image and I believe there is a free-as-in-beer demo version available) Other than those options, a quick google search turned up several third party commercial image analysis packages that claim to be able to do particle analysis. I have not looked at any of these in depth, so I cannot make any recommendations for these. Jake Jokisaari joki@umich.edu Thu Jun 2

Another place to look, though it's aimed more at plant biologists, is http://www.plant-image-analysis.org/, although it's assumed that most users are familiar with ImageJ, to some extent. The point-detection plugins are pretty easy in ImageJ. Is there a similar directory of image analysis packages for other applications? My feeling would be, probably not, as it'd be enormous… Rosemary White rosemary.white@csiro.au Thu Jun 2

## Image Processing:
### color scale

*Is there an easy way to add a color scale to images in Digital Micrograph (GMS 3 in particular)? I have some results from a GPA plugin represented as a color map, but no way to produce a scale bar for that color map.* ***Steven R. Spurgeon steven.spurgeon@pnnl.gov Thu Jun 30***

It is a feature that has been requested a few times and Gatan is working on it. You can create a color scale bar with the following procedure: 1) Go to menu>file>new 2) create an image of the type "ramp Y" 3) In the image option, select the same color scheme as your image 4) Copy past the new image into your original image and use the text tool to add min, max or other values. Stefano Rubino stefano@soquelec.com Thu Jun 30

## Imaging:
### photo printer

*I haven't had to buy a photo printer for micrographs for some years. What's the current best? HP doesn't make the Photosmart series anymore, and the Epson Sure color series specs look good. I need both color and greyscale. Maybe the Epson P600?* ***Phil Oshel oshel1pe@cmich.edu Fri Apr 29***

We have bought an Officejet Pro 8100 two weeks ago, and we have an Officejet Pro 8000 in use since a few years, directly at a TEM with 2k × 2k camera—to the delight of the users. Rachel Reinhard. Rachel@biologie.uni-regensburg.de Mon May 2

To recap: I placed a request for opinions on photo printers for micrographs, now that HP seems to be out of the game. I got a few responses (surprisingly few, given past interest in this topic), but those that expressed a preference all liked the Canon P600. Which does look good on paper (it's Monday morning, so that's the best pun I've got). Phil Oshel oshel1pe@cmich.edu Mon May 9

## Core Facility:
### EM rates and overhead

*My University is trying to decide whether it is appropriate to charge overhead on users of our facilities from both government agencies and other universities. I would like to know what the policy is for other universities.* ***Ken Livi klivi@jhu.edu Thu Jun 9***

I think, yes, it is entirely appropriate to charge external entities overhead for the use of your instruments. I'm surprised your university is just now trying to decide if this is proper policy. In many places it is required (such as at Central Michigan, where I am). The overhead is just recovering operating costs that the university covers for its in-house people. So it charges them to the out-house people. Phil Oshel oshel1pe@cmich.edu Thu Jun 9

## Core Facility:
### user fees for industry clients

*How do your facilities, especially for the electron microscopes, justify the industrial user rate? Do you consider the local analytical companies rate so that yours won't undercut theirs? I looked up the SEM/TEM rate across the universities nationwide and found it varies a lot, ranging from less $100/h to $300/h for industrial users.* ***Shouliang Zhang shouliang.zhang@gmail.com Thu May 5***

Like you say, we take a look at local rates for similar services and price higher so as to not undercut the local business. If anyone gives me a hard time about rates I compare it to what they would pay a professional photographer to take high school senior pictures or wedding photos. EM lab rates start to look entirely reasonable, especially given our "camera" is on the order of hundreds of thousands of dollars instead of hundreds of dollars. William Stonewall Monroe wsmonroe@uab.edu Fri May 6

This issue has been around for a long time. In general, university equipment is there to support the research efforts of their faculty and students. Commercial analytical labs exist which can serve industrial needs. University labs, though, are often tempted to sell their services to industry to make more money. A valid argument is often raised that this is unfair competition to those commercial labs because the (very expensive) university equipment most likely has been purchased with federal grants to support academic research. Some feel this means universities should not do industrial work at all, and others feel it's OK so long as they don't undercut the commercial labs, which could open you up to a lawsuit. Some of the big commercial labs are very aggressive about this! Note (for full disclosure): I am with a small commercial service lab but also work in an academic setting. Allen J. Hall ajhall@prairienanotech.com Tue May 10

There is a very good summary of many billing scenarios in the NIH FAQ issued in 2013. Well worth a read! https://grants.nih.gov/grants/guide/notice-files/NOT-OD-13-053.html Chris Gilpin gilpin@purdue.edu Tue May 10

## Instrumentation:
### pump for low vacuum

*I'm working with a wafer inspection system that uses vacuum to clamp the wafer. The manufacturer's specifications call for a modest vacuum: low vacuum: 150 Torr (-24" Hg) flow rate: < 1 cfm (28 l/m) Actual flow will be intermittent. What type of pump would be suitable for this application? I welcome suggestions for specific models and suggestions for how to configure the pump.* ***Don Chernoff donc@asmicro.com Sun May 1***

I think perhaps the Model N 64.3 ANI Mini Diaphragm Pump manufactured by KNF Neuberger (www.knf.com/usa.htm) would fill the bill. It will produce a vacuum below 10 Torr, has a speed of about 4 l/min and costs less than $600. I've used one of these on a couple of systems I've designed in the past with very satisfactory results. Wil Bigelow bigelow@umich.edu Mon May 2

I made a typo in my previous message. The correct model number for the KNF Mini Diaphragm Pump is N 84.3 ANI. If you decide to use this pump you will need a base plate to mount it on and a housing to shield the cooling fan. I can supply drawings for versions of these items that I've used previously that are easy and cheap to make. You will probably also want some vacuum valves, and for this purpose I can recommend the mini ball valves (such as Model 4114T11, T13. T64) sold by McMaster Carr (mcmastercarr.com, 562-692-5911). They also sell a full line of tubing fittings for polyethylene tubing. Wil Bigelow bigelow@umich.edu Tue May 3

I want to thank the many people who responded to my initial post about low vacuum pump. In reviewing vacuum pump specifications, I note that the flow rate chart shows a high rate at atmospheric pressure with a rapid decrease in rate as the input pressure decreases. When this type of pump is specified, is there general agreement that the flow rate is specified for free air (at 1 atmosphere pressure)? Don Chernoff donc@asmicro.com Wed May 4

Maybe I misunderstand your question, but I was long puzzled by how mechanical vacuum pumps are rated. Finally I realized that the mechanism of a vacuum pump will capture and remove a constant volume of gas regardless of internal pressure. That is, regardless of that internal pressure, the volume swept out remains the same. Clearly, that means that the volume of gas exhausted decreases proportionately as the internal pressure declines. This is why we use 'mass flow' when we want to quantify the mass of material pumped. So if you are looking at volume of gas removed from the chamber the pumping speed is (essentially) constant. But if you are looking at volume or mass of gas exhausted, the pumping speed varies proportionately to internal pressure. Does this help? Fred Schamber frederick.schamber@gmail.com Wed May 4

Thanks for your reply. You're pointing in the right direction. My question is a practical one: if someone says "you need a flow rate of 1 cfm" does that mean 1 cfm measured at P = 1 atmosphere? Don Chernoff donc@asmicro.com Wed May 4

## TEM:
### unknown structures after high-pressure freezing and freeze substitution

*We have some unknown structures after high-pressure freezing (HPF) and automated freeze substitution (AFS) of mast cell line RBL-2H3 (it is not fully representative for mast cell line). There is a ring of "bubbles" around each cell. However, it seems that the bubbles were filled (please use link below for downloading the pictures). As mentioned before we did high-pressure freezing and freeze substitution in a mixture of acetone (water-free) with osmium tetroxide and uranyl acetate. The samples were embedded in TAAB epoxy resin. Unfortunately the membrane contrast is very poor in these samples, though we already fixed that problem. These "bubbles" appear only close to the cells and in nearly every single bubble one could see electron dense remnants. Is it possible, that the structures are a kind of degranulated vesicles?* ***Elisabeth Pritz elisabeth.pritz@medunigraz.at Mon May 9***

These "holes" look like un-infiltrated granules. I would see this sometimes with Drosophila eyes and the pigment granules. Some would be infiltrated and others not. The fix was to do longer and more infiltration steps (resin:solvent) and/or switch to a less viscous resin. Were these cells grown on a sapphire disc or is this a suspension that was HPF/AFS? I know mast cells have a large number of secretory granules, but not outside the cells plasma membrane. Also what is your AFS cocktail? I would also introduce 5% water to your freezing cocktail for better membrane preservation, (Walther, P. & Ziegler, A. Freeze substitution of high-pressure frozen samples: the visibility of biological membranes is improved when the substitution medium contains water. *J. Microsc.* 208, 3–10). Also what was your cryo-protectant? What did you HPF the cells in? I believe the HPF images should look a lot better, it looks like there is some freezing artifacts in your cells (look for spider-web like profiles) and your mitochondria are extracted. Were these cells frozen live? If they are clinical you can use the HPF-chemical hybrid technique where you lightly fix the sample in paraformaldehyde only until you can get to the HPF. If you need a good reference for the HPF/AFS techniques, Mary Morphew at Colorado (formerly Kent McDonald's lab) has a very detailed manual online: https://mcdb.colorado.edu/facilities/ems/pdf/mmanual.pdf. Finally, all HPF experiments should be accompanied by the standard fixation counterpart for comparison. Perhaps this will give you a clue. Please keep us posted as to you results. Michael Delannoy mdelann1@jhmi.edu Mon May 9

I have very limited experience in TEM so please don't laugh: could there be air associated or originated from the cell that formed bubbles during the procedure? Yorgos Nikas eikonika@otenet.gr Mon May 9

I agree with Michael's comments, these holes are typical for uninfiltrated dense material. Try letting your specimens sit in uncatalyzed resin at room temperature a bit longer. For Epon I usually do 3 hr in 25%, overnight in 50% and 2 × 2 hr in 100% before going into catalyzed resin at 60C 24 hr. Leaving out the catalyst will also improve the viscosity. You can even heat up the uncatalyzed resin to 37°C if you want to push it. You do have quite some crystal damage from freezing, that together with a dry acetone FS will extract a considerable amount of material (see my follow up paper to Walther & Ziegler for more details, http://www.ncbi.nlm.nih.gov/pubmed/18445157). Poorly frozen material extracts a lot easier. Here are a few suggestions: 1. If you have adherent cells on sapphire discs, lift them out of the culture dish, touch a filter paper to remove excess medium, dip in hexadecene, then mount for HPF. This ensures you're only freezing the minimal layer of medium with your cells. Freezing adherent cells in pure medium and 200 um deep carriers will almost certainly fail without cryoprotection as you're essentially trying to freeze 200 µm of pure water. Safety tip: repeated skin exposure to hexadecene will give you contact dermatitis, wear nitrile gloves. 2. If you're working with suspensions, 100 um deep carriers and 20% BSA for cryoprotection works well with most cells and doesn't mess up your freeze substitution (FS) in contrast to sugars that won't come out easily during FS. 3. FS in acetone, about 1% osmium tetroxide, about 0.1% uranyl acetate, about 5% water for best contrast. To get there, dissolve $OsO_4$ in acetone, add 1:20 of your 2% aqueous uranyl acetate and that's it. Some precipitation is normal and doesn't influence the end result; it's a saturated solution particularly at cold temperatures. If it's completely snowed up, try half the amount of 2% uranyl acetate. 4. I'd advise against prefixation in aldehydes unless your cells are very sensitive (e.g. primary cells) and you can't get them to the HPF within approximately 30 minutes. Chris Buser cbuser125@gmail.com Mon May 9

## TEM:
### ferromagnetic nanoparticles

*I have a user who is interested in looking at iron nanoparticles in the TEM. I would like to know what people have done to secure the particles so that they do not end up on my pole pieces. I've suggested three things, but I wanted to know if there might be better ways or which of these might be the best. 1) A long time ago, Tom Nuhfer told me that they used low magnification mode and their GIF to preserve magnetic domains in thin films, but I'm not sure that will be high enough magnification for our researcher. 2) Use two grids of thin SiN films above and below the holey carbon films containing the nanoparticles. 3) Embed the nanoparticles and microtome them.* ***Scott Walck s.walck@comcast.net Sat Jun 25***

I have had no problems containing small magnetic nanoparticles in between 2 amorphous SiN windows. You can get very thin SiN now (10 nm) from various vendors; however, if you are going

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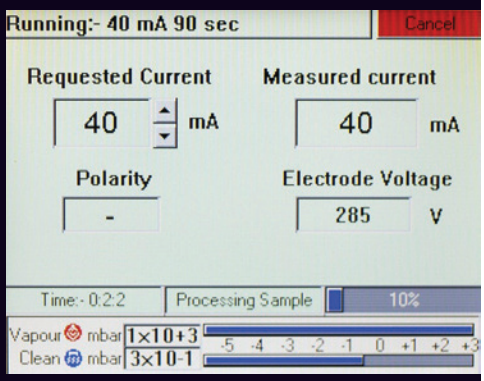


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to double them up make sure you verify the thickness of the Si support frame. I like 100 micron frames, a bit fragile but fit every holders. You can also run into 200 and 300 micron thick frames. Those may cause problems depending upon the depth of the "cup" in your specimen holder. Nestor J. Zaluzec anl.nestor.zaluzec@gmail.com Mon Jun 27

I have looked at iron nano-particles introduced into cells in culture. I processed the culture in my normal way and embedded in Spurr's Low Viscosity resin, with extra-long in filtration times. I had no trouble with the $FE^{2+}$ particles staying in place during the cutting of 60 nm sections and got good images on the TEM. I will say that cutting iron particles on a diamond knife will leave you with some new scratch marks on your knife. Debra M. Townley debrat@bcm.edu Mon Jun 27

## STEM:

### probe size

*I am a relatively new user of TEM, getting started with STEM now. As in STEM mode, the resolution depends on probe size, so I am really curious to calculate the probe size for my STEM images. I found a formula to calculate probe diameter using CBED discs in William's book but it requires Bragg angle (thetaB) in calculations. Is there any simple way to calculate the probe size?* ***Hasan Ali hasan.ali@angstrom.uu.se Tue Jun 7***

Do you want to know the probe size or the image resolution? The resolution can be measured, such as by lattice imaging or from a power spectrum. The resolution is related to—but not identical to—the probe size. The probe shape can be modeled in a program like QSTEM. Larry Scipioni les@zsgenetics.com Sat Jun 25

Thanks for your answer. I agree that the image resolution can be measured using other ways as mentioned by you, but actually I want to calculate the probe size in this case. I want to take some EELS spectra for a range of different probe sizes, say from 50 nm - 1 nm. It would be very interesting for me to exactly know which probe size I have for my current image or spectra. Can I calculate the probe size from CBED discs in a simpler way? Hasan Ali hasan.ali@angstrom.uu.se Sat Jun 25

Just a word of caution when trying to calculate spot size on a scanning probe system! The resolution should not be taken as a spot size measurement. In order to resolve a structure a number of "spots" are required; a typical figure banded around is 4 spots. Steve Chapman protrain@emcourses.com Sun Jun 26

The "probe size" in your instrument will depend on a number of factors. At the nm level (not valid for aberration corrected, or even particularly good for high end FEGTEM's) these can be summarized by the effect of diffraction (convergence angle), spherical aberration (from your lens) and the source limit (gun). For a simple look at all these approximations I suggest checking out Michael and Williams, Journal of Microscopy 147 (3), p289 (1987). As other responders have suggested, this takes no account of your sample and the broadening of the probe. So this is not the resolution. In addition, once you get into crystals and aberration corrected instruments this is not any use. If the concept of measuring your convergence angle via diffraction causes you trouble; it is very simple, just use a standard gold specimen. I would suggest you approach STEM on any quantitative level with caution! Matthew Weyland matthew.weyland@monash.edu Mon Jun 27

## ESEM:

### electrical pass

*I would like to do voltage contrast and active EBIC on our FEI Quanta ESEM and Helios FIB. I am interested in knowing what folks have done to make up pass through plates (and what connectors (BNC, DB9 or DB25) would be the most useful for this for the 4 or 2 3/8 inch ports.* ***Wallace Ambrose wambrose@unc.edu Thu May 5***

My friend recently made a feedthrough using stiff piano wire, a rubber stopper from home depot, and super glue (I think, or maybe it was Torr-seal type epoxy). He used a DB9 port to mark the pin locations, then drilled pilot holes (which I don't think were fully drilled through the rubber) and I believe then he pounded the wire through the remainder of the rubber. This stopper was jammed into a tapered hole in a blank/unused blocking plate that came with his machine. It holds working pressure as good as before the feedthrough was installed. If you want more info, I can probably pass you his direct email to ask for more details. Nathan McCorkle nmz787@gmail.com Thu May 5

If you are not fussy or fancy just drive some sharpened 1/8" diameter stainless steel rods through a clean rubber stopper. Will work OK through the HV range ($10^{-6}$ Torr), but probably not into the UHV range. Wil Bigelow bigelow@umich.edu Thu May 5

## EDS:

### table of ionization cross sections for different elements

*I'm looking for a table of ionization cross sections for different elements to use in some EDS calculations. Can anyone recommend a good book or paper that I might refer to?* ***Steven Spurgeon steven.spurgeon@pnnl.gov Mon Jun 27***

You did not mention the electron beam energy. However, Cedric Powell's most recent review paper is very good as are most of his papers on the topic: Use of the Bethe equation for inner-shell ionization by electron impact (May 14, 2016) Cedric J. Powell, Xavier Llovet and Francesc Salvat American Institute of Physics (AIP) *Journal of Applied Physics,* Description: We analyzed calculated cross sections for K-, L-, and M-shell ionization by electron impact to determine the energy ranges over which these cross sections are consistent with the Bethe equation for inner-shell ionization. Our analysis was performed with K-shell ionization cross sections for 26 elements, with L-shell ionization cross sections for seven elements, L3-subshell ionization cross sections for Xe, and M-shell ionization cross sections for three elements. The validity (or otherwise) of the Bethe equation could be checked with Fano plots based on a linearized form of the Bethe equation. Our Fano plots, which display theoretical cross sections and available measured cross sections, reveal two linear regions as predicted by de Heer and Inokuti [in *Electron Impact Ionization*, edited by T. D. Märk and G. H. Dunn, (Springer-Verlag, Vienna, 1985), Chap. 7, pp. 232–276]. For each region, we made linear fits and determined values of the two element-specific Bethe parameters. We found systematic variations of these parameters with atomic number for both the low- and the high-energy linear regions of the Fano plots. We also determined the energy ranges over which the Bethe equation can be used. Print ISSN: 0021-8979 Electronic ISSN: 1089-7550 Published by American Institute of Physics (AIP). Nestor J. Zaluzec anl.nestor.zaluzec@gmail.com Mon Jun 27

## EDS:

### detector resolution

*Have any of you checked the resolution of your EDS detector to compare its resolution with what you bought versus what you have? I discovered that the Mn FWHM is a known standard method, but the EDS makers don't necessarily actually use this. It seems that they use a radioactive Fe specimen and then correlate that to Mn. I'm wondering if the correlation/translation from Fe to Mn is valid. My current system is not. The situation could be that you bought a 123 eV detector but*

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*wound up with one that is greater than 123 eV. The delivered spec might say ≤ 123 eV yet the resolution is actually worse than this. Any feedback or comments?* ***Gary Gaugler gary@gaugler.com Thu May 12***

I bought a small standard block from Ted Pella that has Mn as one of the standards. It also has a BN standard useful for checking low end performance. I record a spectrum from the standard using my standard conditions, save it in MSA format, and then use the NIST-DTSA-II to process the spectrum. One first needs to configure a DTSA detector and then run the detector Calibration alien (i.e. tool) from the "Tools" menu. It will calibrate from the Mn. The routine automatically computes the resolution. It turns out that one can do a pretty good job with a piece of Cu tape or a Cu TEM grid mounted on an SEM stub. If one measures the Cu spectrum at 25 kV the intensity of the Cu K and L lines are pretty close. One can then run the detector calibration from the Cu spectrum. The results on our system are within experimental error (which DTSA automatically computes!) of the results from Mn. What else would you expect from Nicholas Ritchie and his colleagues at NIST! I have no commercial interest in Ted Pella; just a long time satisfied customer. Also a grateful user of DTSA-II. John Minter jrminter@gmail.com Fri May 13

I'm one of the guilty parties, going back about 4 decades. First, the emission of an Fe55 radioactive source really is the Mn K lines. It is identical to the K emission of Mn under the beam, except there is no Bremsstrahlung background. Instead of ionization by an electron beam, the excited Mn is created by electron capture from an Fe55 nucleus (nuclear proton grabs an electron and becomes a neutron, reducing the atomic number by 1). It is convenient and very safe, because no other radiation is emitted (*). Sources up to 100 μCuries can be sent by regular mail with no special packaging. Detectors are normally tested in a controlled environment "on the bench" with a source. There are many reasons a detector might not meet spec on a column in the real world besides the detector itself. For example, CE marking be damned, we've discovered that one of our Tektronix digital oscilloscopes costs about 1.5 eV if it's anywhere near the BNC cables from the detector to the pulse processor. X-ray detectors are exquisitely sensitive EMI detectors too. You might find for that resolution varies slightly with the time of day, depending on what other equipment in the area is operating. I've spent many happy (?) hours at various installations trying to figure out what was causing resolution degradation. 123 eV is close to the absolute limit for a silicon-based detector, and it only takes microvolts of noise to kick that up an eV or so. Anybody else have enough gray in their hair to remember when anything better than 150 eV was a pretty decent detector? I second the kudos to Nicholas Ritchie! DTSA-II is an extraordinarily useful tool for all aspects of EDS analysis. Good luck figuring out what's going on. (*) Ok, all you picky nuclear physicists out there. There are extremely low probability gammas up to 231 KeV, 5 to 7 orders of magnitude lower in intensity than the Mn K lines. Not worth losing sleep over. Rick Mott rmott@pulsetor.com Sat May 14

## EELS and XAS:
### announcement

*I would like to inform you that the EELS and XAS database website has been completely rewritten and is now accessible at https://eelsdb.eu/ and can be used without registration. We have performed many changes, in particular an improved design, a streamlined submission process and an online plotting function. An application-programming interface (API) has also been created, allowing external tools and software (such as hyperspy) to easily access the information held within the database. I hope you will like this new version of the website and I encourage everyone to submit their spectra to the website!* ***Luc Lajaunie luc.lajaunie@cnrs-imn.fr Fri Jun 10***

MT