# NetNotes

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Selected postings from the Microscopy Listserver from March 1, 2014 to April 30, 2014. Complete listings and subscription information can be obtained at http://www.microscopy.com. Postings may have been edited to conserve space or for clarity.

## Specimen Preparation:
### vacuum oven polymerization

*While polymerizing your resins, do you vent your oven in a fume hood or to the lab? Safety suggestions?* ***Tina Williams tina.williams@ars.usda.gov Fri Feb 28***

Thank you to all who replied to my question regarding venting in a fume hood during polymerization of resins. I especially appreciated the references and safety reminders to reduce respiratory/skin/immuno sensitivities by venting in a fume hood. I wanted to confirm, by having your collective experiences working with resins, that we continue working in harmony with these safety protocols. We have been venting the resin polymerization in the fume hood. However our current EHS officer brought up the concern regarding the oven affecting the airflow in the hood. Since we have a large/heavy oven, and the same issue may apply with a small oven (if one could be found), we have kept the oven in the hood. Your answers help us have a basis to continue with our current safety protocols, until a safer alternative is found. Tina Williams tina.williams@ars.usda.gov Mon Mar 3

## Specimen Preparation:
### lignin stain for EM

*I am searching for a method to preferentially stain lignin for SEM or TEM. The object is to be able to determine if the lignin is present in nano-size wood particles. Safranin can be used to stain lignin for LM but does not contain a heavy element that will scatter electrons so that it can be identified by EM. Any ideas would be appreciated.* ***Debra Sherman dsherman@purdue.edu Wed Apr 9***

If the lignin is at least partly exposed on the particle surface, you could use antibodies against lignin. With proper controls that will be a highly specific identification. See e.g. Visualizing Lignin Coalescence and Migration Through Maize Cell Walls Following Thermochemical Pretreatment, Bryon S. Donohoe, Stephen R. Decker, Melvin P. Tucker, Michael E. Himmel, Todd B. Vinzant (2008) Biotechnology and Bioengineering, Vol. 101(5). It does not have to be gold-labelled, but could be something like a solid phase immunoassay type of protocol: antibodies against lignin bound to a solid phase, which could be a film on a grid. Then incubate with the lignin suspension and see if you have specific binding on the film. Jan Leunissen leunissen@aurion.nl Thu Apr 10

My colleague Colleen Macmillan, who has much experience staining for lignin, suggests that the $KMnO_4$ in the Maule stain may be electron-dense enough. It certainly highlights cell membranes for TEM. I've pasted her protocol below in case attachments won't go through. Mäule reaction lignin histology of plant sections - Samples: Easiest to place the (fresh) sections in a 48-well/multi-format plate, then add and remove solutions from the sections using narrow-ended plastic transfer pipettes. Safety: Perform these reactions in a fume-hood, and wear nitrile gloves, safety glasses, lab coat, etc. Mäule reaction protocol: 1. Incubate sections (fresh is best) in $KMnO_4$ (1% aqueous) for 10 min (if very thin sections then a couple of minutes is fine). 2. Wash sections ($ddH_20$). 3. Acidify with concentrated HCl (37%) for 1 minute. 4. Wash again ($ddH_20$). 5. Incubate in $NaHCO_3$ (5% w/v; fresh) ~2-5 minutes, or until color develops. Then mount, view, photograph, etc. Indications: Red = syringyl lignin; Brown = guaiacyl lignin. Based on personal communication from Armand Seguin and reference = Sibout, R., A. Eudes, et al. (2005). "Cinnamyl alcohol dehydrogense C and D are the primary genes involved in lignin biosynthesis in the floral stem of Arabidopsis." Plant Cell 17(7): 2059-2076. Mechanism: ŒChlorination of the syringyl nucleus leads to a pink (lignifying cells) or red (lignified cells) color, whereas the guaiacyl nucleus produces a light (lignifying cells) to dark (lignified cells) brown color (Bland, 1966; Wardrop, 1981).[1] quote from US Patent Issued on October 30, 2007, Inventor(s) Laigeng Li, Vincent Lee C. Chiang. Rosemary White rosemary.white@csiro.au Thu Apr 10

## Specimen Preparation:
### LED silicone removal

*Can someone recommend a chemical recipe to remove silicone from an LED package device?* ***Marissa Libbee mlibbee@gmail.com Wed Mar 5***

Silicones used in packaging of semiconductor devices are notoriously difficult to remove, but: 1) Hot red fuming $HNO_3$ or hot 3:1 mix of red fuming $HNO_3$ with fuming $H_2SO_4$ would work very nicely, if you do not mind losing entire package and being left with a bare LED die. 2) Soaking in warm (40°C) methylene chloride overnight (under a fume hood) usually softens silicone to the point that it can be cleaned off by a Q-tip. Sonicating in methylene chloride may be even better, but I haven't tried it. When using methylene chloride make sure it is dry - if contaminated by water it becomes corrosive. 3) I had limited success with sonicating parts in warm Dynasolve 230 (under a fume hood) for a few hours. Valery Ray vray@partbeamsystech.com Wed Mar 5

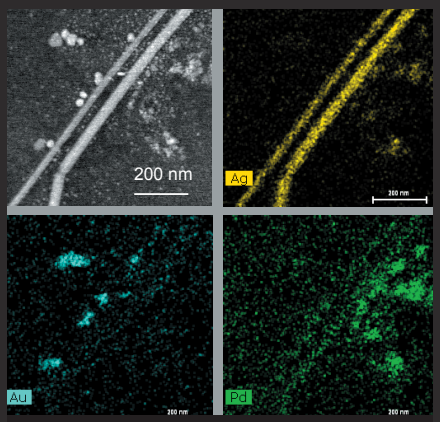
## Microtomy:
### knife choice

*I was wondering whether it is safe to section 400 nm thick section with ultra diamond knife, or whether I have to use a Histo knife.* ***John John.Kourtesis@sars.uib.no Sat Apr 26***

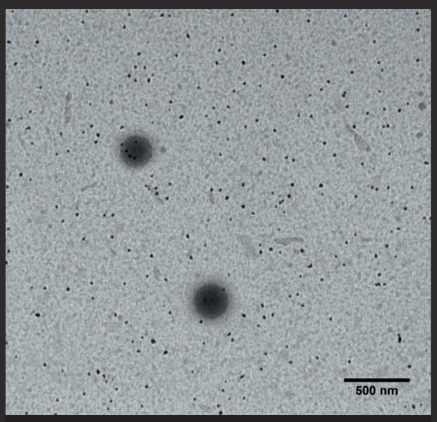
We routinely use old Ultra diamond knives to cut 1 µm thick sections. But I must say I love my Histo diamond knife. If you are cutting a lot of thick sections a Histo diamond knife equals true happiness and I encourage you to buy one if you can afford it - 6mm will rock your world;-) best, Beth Beth Richardson beth@plantbio.uga.edu Sat Apr 26

I have to agree with Beth. I used to use older ultra knives and live with some scratches, but someone brought in a 7 mm Histo knife that I made fun of... and now I wouldn't part with it for anything. I can cut

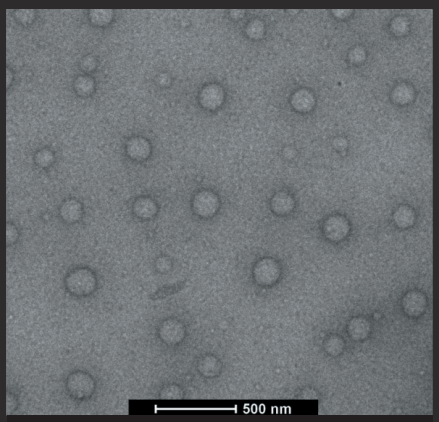
# Liquid Electron Microscopy never looked so good...



Nanoparticle EDS - Ag, Au, Pd



Liquid acrylic black paint



Oil droplets in water

# ...or generated such quality data

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1 µm sections on it and then, without moving it or changing anything else, silver sections. Tina (Weatherby) Carvalho tina@pbrc.hawaii.edu Sat Apr 26

I also agree with Tina & Beth with using "older ultra knives" prior to "resharpening." A separate Histo knife or special diamond dedicated for semithin sectioning would add some personal joy and happiness, I guess. At least one Histo knife (6-7 mm sectioning edge) should be standard in the preparation and cutting-armamentarium when doing sectioning resin blocks for LM prior to TEM. It certainly will pay off. Wolfgang Muss w.muss@salk.at Mon Apr 28

## Microtomy:
### ultramicrotome section preparation for SEM

*I am currently making ultramicrotome sections (500 nm-1 µm, cross- and longitudinal-sections) of individual hair samples that are embedded in Embed-812. I am interested in high-magnification imaging of the hair structure using SEM which should be simple enough.... however, the sections are constantly folding and wrinkling once they are transferred and dried onto a sample support for SEM examination. The wrinkling usually negates proper examination. I was wondering if there is any tricks-of-the-trade that could overcome this issue. Below is my current method: 1. Cut sections with ultramicrotome. 2. Transfer sections (using an eyelash probe) to a gold-coated microscope cover glass (functioning as a sample support) that contains a micro-drop of water. 3. While the section is still floating on the water micro-drop, it is flattened using chloroform vapor. This works very well. 4. Either allow the water micro-drop to evaporate or gently wick the water away using a pointed piece of filter paper. It is here where the sections get wrinkled! 5. Gold-coat and examine using SEM. Any suggestions on how to remove the water and have the thin section lay flat on the cover glass surface would be greatly appreciated. Perhaps a completely new substrate would work better! Is this an issue when using a TEM grid?* ***Jack Hietpas mikroskop@gmail.com Mon Apr 28***

Try without water and collect section gently with adhesive tape and good luck! Yorgos Nikas eikonika@otenet.gr Mon Apr 28

Have you tried warming the slide on a hot plate or slide warmer? One warm enough to keep the sections expanded (or to expand them with heat instead of chloroform) - what one would use to warm the sections when doing toluidine blue staining. 60 to 90°C. Phil Oshel oshel1pe@cmich.edu Tue Apr 29

First of all, I would not coat the coverslip until after drying the sections. Second, you might try a technique posted by another researcher on the listserver a few years ago: "I cut 1 µm sections (and 5 µm sections) with Diatome diamond knives mostly samples embedded in Polybed 812. I would transfer them onto a glass slide with a wooden stick or a loop. I would put the slide on a hotplate 60°C and then invert a large glass Petri dish over the slide. I would place a cotton swab dipped in acetone under the dish with the slide. The acetone vapor + the heat would flatten the sections and as the drop of water evaporated the sections would anneal to the slide. It worked great for me." Good luck Dean Abel Biological Sciences University of Iowa, Iowa City IA USA I tried Dean's technique, and it works with some of my samples. What I usually do is to work with glass slides, put a 3/4 to 1 inch diameter drop of water on them with my sections on the water, and use a flame from a Bunsen burner to dry the drop of water down, passing the slide in and out of the top of the flame (I use a Touch-O-Matic burner, which gives me a small, controlled flame), and holding the slide at about a 30 degree angle so that the water dries out from under the sections. You must be careful with this technique to not get the water so hot that it boils, though! Usually, if the technique is done right, and your slides are clean and your water is clean, the sections will tend to go to the top edge of the drop of water (uphill side of the slide), and the water will dry out from under the sections, leaving the sections perfectly flat. You need to play around with the size of your flame and how hot you get your slide, but once you get your conditions right, this technique is very consistent. After all of the water is dried off the slide, I pass the slide above the flame for a few more seconds to firmly adhere the sections to the slide before allowing the slide to cool, and before staining (if doing toluidine blue staining). In your case, once the sections are flat, you can coat them, and they are ready for the SEM. Take a diamond scribe and score your slide, break it small enough for your SEM stage, coat your sample, and you're ready for viewing. Ed Haller ehaller@health.usf.edu Tue Apr 29

## Core Management:
### flash drive virus/malware protection

*I am wondering what types of things people are doing to make sure that their flash drives are virus and malware free? Our microscopes are not networked for cyber-security purposes but we do use flash drives to download data and sometimes to upload files to the microscope computers.* ***Ellen Scanley escanley@gmail.com Fri Apr 11***

I would be hesitant in using flash drives. You could set up a system to scan them in another computer before plugging them into the microscope computer. However, even if you officially have a protocol in place to scan the drives before they go into the microscope computer, it wouldn't take but a single infection to ruin your day. I think a better solution would be to attach the instrument computer to another computer that would serve as a firewall between the microscope and the broader network. Users could save their files to the intermediary and then use their method of choice to retrieve them. They could use a thumb drive or share them over the network. FEI, at least, uses such an approach for their microscopes and we have adopted the approach for other instrumentation running on old computers. Warren Straszheim wesaia@iastate.edu Sat Apr 12

In our facility, we have two dedicated, sterile flash drives to move data around within the facility, but only use a server or Google Drive for data leaving the facility. The best way to make sterile flash drives to buy new drives, and then perform a long format on a computer with a fresh OS install. In my experience, if users are bringing in their own drives to transfer data, it is inevitable that your equipment will become infected. To make our systems secure over the internet, the server is fire-walled to only allow access via the university subnet, and the routers are whitelisted to only allow access via ports 22, 80, and 443. We also have the internet browsers locked down to only allow access to Google Drive to keep people from surfing the internet on the computers. We also have a wireless guest network on the router, such that users can still get the internet on their own devices, but cannot get access to the protected NAT. We've had this system in place for over a year and have had very good success with it so far. Ben Smith benjamin.smith@ou.edu Mon Apr 14

In our facility, we have microscope PC on a local network and one other PC running Linux OS (Debian Wheezy). This PC is connected via SAMBA to the microscope PC (read-only shared folders with guest's images). Every user has a possibility to copy images from microscope PC onto her/his flash drive on Linux box. No uploads to the microscope PC is permitted for normal user (guest). Oldrich Benada benada@biomed.cas.cz Mon Apr 14zz

We have installed USB Drive AntiVirus on our computers that scan flash drives, and it supposedly prevents them from infecting the computers. David Osborn osborndc@umsl.edu Mon Apr 14

## Core Management:
### poster ideas

*I'm preparing a poster (A1 size) on transmission electron microscopy (TEM) topic to decorate our characterization center. We have two*

# *Always Ahead of the Curve*

***Dr. Eric Lifshin of the SUNY College of Nanoscale Science and Engineering is a luminary in the field of scanning electron microscopy and microanalysis. Eric's most recent collaborative research with Nanojehm Inc. applies point spread function deconvolution algorithms to electron probes to realize super resolution imaging using a TESCAN VEGA Scanning Electron Microscope.***

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*TEMs, a JEOL 2000FX, and a FEI Tecnai F20, along with typical optical microscopes and SEMs, FIB, plus surface analysis equipment. Our major interests are materials and physical sciences teaching and research. My manager suggested some entry-level information in the poster for public visitors, including 16-year-olds and their parents. I used to go to conferences with research posters. It wasn't as difficult as this. Can you give me some inspirations please? Any suggestions, text, websites, etc. are all appreciated.* ***Zhaoxia Zhou z.zhou@lboro.ac.uk Mon Apr 28***

One metric I use when communicating to laymen is "the thickness of the human hair" which is about 100 um. Everyone has a feeling for how thick a human hair is, but when you say nanometer they don't have a clue about that. So if you have a 100 nm particle, you say, "If a human hair were as thick as this room, then this object would be about 1 centimeter across!" (Well, OK, I say half an inch because the US is still in the SI backwater...) For TEMs and SEMs, I make the analogy to an optical microscope (which some people have experience with) or a magnifying glass, if that fails. The reason why we use electrons is because they are "smaller than light" and they let us see "different things inside." Finally, the most important thing: pretty pictures. Be sure to have RGB pictures of something dazzling that makes them want to pore over it and see what that is. Ideally, it should have some parallels with objects people see in the big world. For example, I once used three plasmon images of a FIB lamella to make an RGB which looked like stained glass. The scientific content was pretty low, but people loved looking at the picture and it got them interested. I also have a picture of a sample prepared for AFM that looks like the Sauron's tower from Lord of the Rings—I'll probably use that soon. I find that what you and I consider pretty pictures are not always the same as what a layman considers pretty. After all, they haven't spent the last N years looking at microscopic objects. Zack Gainsforth zackg@berkeley.edu Mon Apr 28

I did this once years ago. We decided that one point we wanted to get across was the amazing scale of magnification provided by TEM. We found that people, especially the general public (and I also) are astounded by what high magnifications actually mean in real terms. We routinely deal with numbers like 1000×, and 150,000× without thinking. Also what does nanometer mean? We simply did some back of the envelope calculations. Then we could say things like. We had a picture of the earth. If the earth was 1 mm diameter then the building we are in would be 1 nm long. If we were looking at really high magnification at some sample at 500,000× (possible with the F20?) then it is equivalent to orbiting the earth and only just being able to read the text on this poster. Our lab works on wool. So we had a micrograph of some intermediate filaments (7.6 nm diameter) and then indicated that if the filament pictured on the poster were real-size, then the wool fiber containing it would be 640 m across. I am sure there are other samples where a similar exercise can be done. Once people "get" what scale really means then it is easier to wow them with the technical aspects of how you manage this miracle of magnification. Duane Harland duane.harland@agresearch.co.nz Mon Apr 28

Having worked almost exclusively with tungsten SEMs, the example I have given for years is that taking 4×5 Polaroids at 100,000× would require a line of photos lined up for 100m (longer than a US football field) to show the area between two lines on a ruler that are 1mm apart. Even most Americans are at least somewhat familiar with a mm (about 1/32"), and if they weren't, I'd show them my ruler. The TEM (especially the new ones) are at least an order of magnitude better, so at a million ×, the mm lines are a kilometer apart (.8 miles in the US). Ken Converse kenconverse@qualityimages.biz Mon Apr 28

Years ago (1990s), I hosted a group of high school kids and showed them an image of Si displaying atomic column resolution. From the nm bar I told them the image magnifications was 16 million ×, and then, to illustrate that degree of magnification, I said "Assuming that you are two meters tall and your image was enlarged 16M× how tall would you be?" One young lady said that "If my image was blown up 16M× I would still be 2m tall." Moral: be clever if you like but be careful of your word choices! Ron Anderson, IBM retired, microscopytoday@tampabay.rr.com Mon Apr 28

## TEM:
### EDS window purpose and hole

*If there's a hole in the window of an EDS detector mounted on a TEM, what problems may arise using the detector? I realize that it's clear to me the window purpose in a SEM, where the chamber is frequently vented, but what about in a TEM?* ***Davide Cristofori dcristofori@unive.it Tue Mar 4***

The purpose is to keep the detector crystal clean. Without the window there, the detector will behave very much like a cold finger in any other high-or ultra-high vacuum system. Residual water and other volatiles in the system will condense on the crystal surface over time. The effect may not be as immediate as it would be at atmospheric pressure, but it can be just as devastating. Windowless detectors can be used if the vacuum quality is very high (ultra-high vacuum conditions with very little hydrocarbon contamination) or the detector is relatively warm as with an SDD. Even with a windowless SDD, care must be taken to ensure that the vacuum quality remains relatively high, though ultra-high vacuum conditions are not required. Jacob Kabel jkabel@mail.ubc.ca Tue Mar 4

Just another comment: Yes the window serves as a protective agent as outlined by others. Although a TEM is not leaked in the same manner as some SEM's there is still inevitably a small burst of water vapor and gas that enters the column during specimen insertion via the airlock. So there will be a gradual but slow buildup of condensate upon the cold detector, particularly if it is an older SiLi system cooled by $LN_2$. Some older SiLi systems do have small heaters/thermal cycling modes to mitigate ice buildup. If you have an SDD, which is typically turned on/off then it will warm up on power down and any ice will sublime in vacuum. What you don't want to happen is for the detector to be fully powered up and the column leaked to air. Not only will you get condensate, but more likely there will be a short circuit of the electrical connections at the back of the detector. The short circuit can destroy your detector. Qualitative analysis will not be affected by a partially broken window, unless you get ice buildup from water vapor in your column condensing on the front of the cold detector. This will decrease the detection efficiency for light elements. You will see this first on the low energy lines (< 2 keV). Please also remember since the presence or absence of a window affects the relative intensities of x-ray lines, a change in the window can impact any quantitative analyses. Literally all the quantitative analysis programs assume parameters for the initially installed window and the manufacturers will have this programmed into their analysis software. A partially broken window will be difficult to model as you don't know how much of the detector area is affected. Depending upon the x-ray lines analyzed this can change your analysis, again low energy lines will be most affected. So should you initially worry, I would not as long as the detector is still working. I have run windowless detectors (both $LN_2$ cooled SiLi and SDD's) in TEM's for years. With proper care and attention they are fine. However, if you are in a multi-user facility with variable expertise of users then I would play it safe and get the window repaired. Fixing a window is a lot cheaper than replacing the whole crystal. Nestor J. Zaluzec zaluzec@aaem.amc.anl.gov Tue Mar 4

## TEM:

### vacuum leak

*We have a JEOL ARM200F TEM in our lab. The system has two vacuum-related issues, which lead to only ~ 30% up time since we purchased it last year. The first one is that the chamber vacuum had crashed at least five times when we were filling the liquid-$N_2$ dewar. The second one is that the samples are contaminated (by carbon) very quickly when we take images and/or do EELS/EDS analysis under STEM mode. All samples are plasma-cleaned before inserting them into the ARM. And, we have not observed such high contamination rate for the same samples in other TEMs. We detected two leaking spots (by Helium leak test) on the goniometer and suspect those might be the root-cause of carbon contamination. JEOL recommends that we beam-shower the samples before STEM imaging/analysis, but that destroys some of the samples and changes the structure of the materials in some cases. I wonder if anyone had done any study on the correlation between carbon contamination (under STEM) and low level vacuum leak before. Is it a pre-requisite to beam-shower the samples to obtain atomic resolution images and high-quality EELS/EDS results?* ***Hongwen Zhou zhouhw33@gmail.com Thu Apr 17***

I think you will have realized that you have an "O" ring problem on two items that sit in the specimen area. It is extremely important to solve these two problems before trying to characterize the vacuum system and the contamination. Another point, a leak in the specimen area may also cause etching of specimens, reducing their cross section due to gas ionization. For example a holey carbon film may be reduced to the structure of a string vest in minutes due to etching. So, fix the problems that you have found, and the contamination problem may well go away. Steve Chapman protrain@emcourses.com Thu Apr 17

We have had the column vacuum crashed on only one occasion, and that was when an inexperienced user filled the anti-contamination device (ACD) to overfilling, and did not realize it was overflowing, so he poured and poured, which we believe froze the "O" ring that seals the top of the ACD. Aside from that, the column has never crashed. Contamination was a problem when we were heating the ACD every night. When we keep the ACD cold 24 hours a day, which can be easily done on the ARM, contamination from the column is eliminated. Contamination in a high-current STEM (the ARM delivers ~10× the beam current density of a 2010F) is always a bigger problem than it is in conventional microscopes. We still see specimen-borne contamination, and plasma cleaning does not seem to help as much as we would like. The best way to clean dirty specimens is baking them. We use the baking system that came with our NION UltraSTEM for samples that cannot be successfully cleaned by plasma cleaning. John Mardinly john.mardinly@asu.edu Thu Apr 17

## TEM:

### Fourier transformation

*I am a PhD student in material science from Queen's University in Canada. I am trying to use HRTEM to characterize the grain boundaries and phase interfaces and dislocations generated after deformation in Zr alloy. I have captured some HRTEM images but it is very hard to interpret them. My supervisor told me that I should be very careful when trying to explain them. As it says in the textbook written by Williams, we should use simulation software to interpret the image. However, in open literature, inverse fast Fourier transformation (IFFT) was widely used to index the dislocations and misfits between two phases. And based on my own experience, the IFFT image is related to the size and position of the mask that was applied. Could anyone give me any idea about the advantages and disadvantages of IFFT method to analyze the HRTEM data?* ***Hongbing Yu 12hy1@queensu.ca Tue Apr 22***

Your supervisor is quite right to warn you of the perils of using Fourier transforms. I've seen some terrible abuse of FFTs, even in the highest impact factor journals where the refereeing should have been better. I believe your request relates to measuring displacements and rotations of the crystal lattices either side of an interface. Probably the best article I would refer you to is Hytch, Snoeck & Kilaas, *Ultramicroscopy* 74 (1998) 131–46. They coined the term geometric phase analysis (GPA—not to be confused with geometric phases in quantum mechanics). They explain how to use the FFT of a HRTEM image to recover information about the displacement, *R(x,y)*, and rotation, omega *(x,y)*, of the lattice from each of the peaks in the FFT. The strains and shears associated with interfaces and steps can be seen as phase ramps. Differentiating these will give local strains and shears. There are certain caveats to using this technique however, which Martin Hytch & Tobias Plamann address in a later paper (*Ultramicroscopy* 87 (2001) 199–212). The problem is that, in some crystals, the peaks (and troughs) in a lattice image do not necessarily track the positions of the atomic columns. This occurs if there are a) strong thickness gradients, b) non-centrosymmetric crystals. For both, the local beam tilt (if using a large condenser aperture) and crystal tilts (buckling due to thin-film stresses) lead to the lattice fringes shifting across the unit cell leading to phantom strains (in the case of centrosymmetric crystals the lattice fringes shift by a factor of pi radians and the peaks become troughs and vice versa). From a practical point of view you need good quality HRTEM images so I would recommend using small condenser apertures and, if possible, energy filtering the image with a Gatan Imaging Filter or in-column filter. Analysis using GPA can be done either using post-analysis software, e.g. MatLab (I tend to use IDL), or by using a Digital Micrograph script that can be purchased from HREM Research (www.hremresearch.com). I hope that helps. Jon Barnard jsb43@cam.ac.uk Wed Apr 23

## SEM:

### $LaB_6$ filament lifetime

*Is there anyone using a $LaB_6$ filament on their SEM? I would like to know what the filament lifetime is. Is it a lot shorter if we use the filament on the second saturation point? I currently using a $LaB_6$ filament on a Zeiss EVO and I have to put the Filament Intensity higher and higher every day to reach the second saturation peak. Do you think my filament is dying or it is another problem? It has 1800 hours lifetime.* ***Valerie Lecomte valerie.lecomte@usherbrooke.ca Wed Mar 26***

Kimball Physics has some useful information in their technical notes on the operation of $LaB_6$ cathodes. You can find it on the Kimball Physics web site under Cathodes/Emitters and then Technical information. The major reason for $LaB_6$ end-of-life is due to loss of brightness of the emitter. Most current emitters are [100] oriented single crystals with a 90° cone angle and a roughly 15 µm flat at the tip. As the tip is used, $LaB_6$ evaporates from it and the tip recedes. There is a faster recession on the [110] faces and as the tip recedes, there is a gradual loss of the flat, i.e. the tip gradually sharpens. Since the emitting area drops, the total current does also. It is also likely that since the cathodes are heated from the edges by the graphite support that the tip is now running cooler that previously. As an operating note, overheating $LaB_6$ cathodes significantly reduces lifetime. Don't oversaturate! Taking the temperature from the nominal 1800° K to 1850° K increases the evaporation rate by 3×. This means that the lifetime will go from ~2000 hours to less than 700! You will see deposits from the cathode on the inside of the Wehnelt. These should be a purplish

color, indicative of $LaB_6$. If the deposits are white, you have formed lanthanum oxide. Oxide formation means that the gun vacuum is not adequate. Also, the oxide is an insulator whereas the boride is a semiconductor. This means that as the oxide builds up, you will get charging effects on the inside of the Wehnelt. This will eventually result in a flickering beam intensity. Henk Colijn colijn.1@osu.edu Thu Mar 27

## SEM:
### vacuum problem

*I'm trying to diagnose a vacuum problem with the Leica S430 that was donated to the Pumping Station: One hackerspace in Chicago. We are, to our knowledge, the only hackerspace in the US with a working SEM, and we are developing programming around making it accessible to the general public. When the scope arrived, it couldn't achieve vacuum better than $6\times10^{-5}$ Torr. Over time, it did substantially better, and, after I changed the roughing pump oil, it could get down, at best, to $1.8\times10^{-5}$ Torr. Still, factory specs say it should go to $2\times10^{-6}$, and I'd be much happier if it were in the $1\times10^{-6}$ range. However, I'd noticed strange problems where, after a week of running, I'd check it and find that it seemed stuck at a rather awful vacuum of 1.2 to $1.4\times10^{-4}$ Torr. The act of venting the chamber and pumping it down again seemed to cure it, and it would pump down rather quickly to $3\times10^{-5}$ Torr and slowly get better from there. The oil change didn't solve this. I tried removing the oil mist / odor filter while the vacuum was stalled in the $1\times10^{-4}$ Torr range to see if it was creating back pressure, but that didn't do much. Since then, I've replaced the oil mist and odor filter elements. This helps somewhat, but not entirely, at removing the hot oil reek when pumping down the chamber from atmospheric pressure. Things have become substantially worse. Now, pumping down from atmospheric pressure works as expected, but within hours, pressure rises to the 1.2 to $1.4\times0^{-4}$ Torr level again. Venting and pumping fixes it temporarily until this happens again in a few hours. Thoughts: Virtual leak / sample outgassing: I doubt this. I keep the scope at vacuum 24× and haven't changed out the samples in months. I'm primarily teaching people to use the scope by looking at samples already prepared. I don't have curriculum together on sample prep, although we do have a sputter coater and critical point dryer. Physical leak: That might explain why I'm not getting below $1.8\times0^{-5}$, but I don't see it explaining why the pressure rises so much after a successful pump down. I've also wiped down the chamber and gun O-rings recently with no effect. Backing pump oil mist / odor filter back pressure: Possible, as I rebuilt the filter recently and could have caused a problem, but this didn't start immediately after I rebuild the filter, and when I tried removing the filter before, it never helped. Backing pump oil age: I changed it a little over 4 months ago. I suppose this is possible, but I'd not had this problem with this frequency when I was running oil that had been in the pump for a very long time from the former owner. Air filter desiccant: it's starting to change color in places. I know I should heat it to drive out the moisture. But even if moist air is coming into the chamber when I vent it, I'd think that would slow my pump down time, not cause a normal pump down and reappear as a problem later. I wouldn't know if this were a problem with the Penning gauge. Usually, after the Penning turns on, I see some sinusoidal oscillation in pressure during initial pump down, but then it damps out and behaves predictably otherwise. So I'm left thinking this is either a turbomolecular pump problem or a roughing pump problem. The turbopump does make a soft high-pitched whine, which sometimes changes pitch, but I've heard that is normal. The roughing pump does change its sound periodically. Especially when pumping down, it sounds like it is running rough. The scope didn't have a maintenance contract at its former home, so I question how much preventative maintenance was done. If I had to guess, I'd question if the scope needs a rebuild. I've seen kits available. (Edwards E2M12, I think.) We can't exactly afford thousands of dollars to bring in a SEM tech. Buying a rebuild kit for the roughing pump would be financially doable, but I've heard this is a lot of work. Have any of you rebuilt a multi-stage rotary vane pump? How difficult is it? I'd probably need an offsite area to do the rebuild because our "microscopy lab" is in a corner of a dusty workshop. Yes, I know this isn't ideal for a SEM. If possible, I'd really like more of an indication that the roughing pump is the problem before trying this. Have any of you seen problems like this? Any thoughts on how to proceed?* ***Ryan Pierce rdpierce@pobox.com Mon Mar 31***

I doubt that your Rotary pump (RP) is the problem. I'm not familiar with the S430 but have some familiarity with the 1430 and 1430VP. First, check and see if the turbopump is going into a "standby mode" at a reduced RPM. In conjunction with a leak, this could cause the vacuum deterioration that you are seeing. More likely, the Penning gauge needs to be cleaned.

Determine the model and you should be able to find a user's manual on line with instructions on how to clean it. I have seen systems behave in a somewhat similar manner with oil diffusion pumps due to either a lack of diffusion pump oil or because of issues in systems that change the power settings to the diffusion pump in different pumping stages. This is not generally the case with turbomolecular pumps, so clean the Penning Gauge and see what happens. Ken Converse kenconverse@qualityimages.biz Tue Apr 1

## SEM:
### gunpowder

*Anyone ever examine/analyze gunpowder or primer samples by SEM? Any hints or cautions?* ***Larry Hanke hanke@mee-inc.com Tue Apr 22***

Not gunpowder, but all kinds of explosives powders - RDX, HMX, HNS, PETN and so forth. We make shaped charges for perforating oil and gas wells. I usually gold coat to make the powders conductive and improve secondary electron yield, and use an aluminum stub for best thermal conductivity. I have a Lexan shield between me and the coater but have never, in the 20+ years I've been doing this, had any explosive decompose or deflagrate in the coater. Could be wrong but I think most smokeless powders have a graphite coating, so they may already be conductive. Andrew Werner werner1@slb.com Tue Apr 22

I have not worked with what the customer called gun powder, but I guess the material had similar properties. I carried out a good deal of consultancy work with the then Nobel Explosives Division of ICI (they have made explosives since the days of galleons!). The specimens were mounted on a conventional stub using the smallest possible quantity. There was no reason to coat the specimens. I used basic specimen safety measures on a tungsten hairpin sourced instrument- low kV (<2) lower emission current (30 mA) and lower than normal spot sizes (i.e. a spot size suitable for 40,000× when I was working below 10,000×). My safety argument was that the amount of material was very small, and being contained in a vacuum, I thought I was pretty safe. The client was frightened to carry out the work themselves but were happy with the results I provided for then on several 3 day visits. Steve Chapman protrain@emcourses.com Tue Apr 22

I would be happy to be corrected if I was wrong but I always thought that an explosion was a very fast combustion. And that combustion needs oxygen, which is by definition absent from a high-vacuum environment. Can I convince somebody with my argument? Stephane Nizets nizets2@yahoo.com Thu Apr 24

Yes, I 100% agree and you will see that I took this into account in my email. I see no reason to go into fancy techniques when the simplest of procedures will do the job. I did outline those that I had used without a problem during my 6 days of investigations. I did not carry out EDX and understand that if I had needed to do so I may have required higher than the 2 kV that I had used. Steve Chapman protrain@emcourses.com Thu Apr 24

I believe that most explosives contain their own oxidizers to enable the rapid reaction. Hence the presence of peroxide in many of the homemade concoctions. Anyone want to clarify the issue? Henk Colijn colijn.1@osu.edu Thu Apr 24

That is my understanding as well. In an explosion, there is not enough time to rely upon mass transfer of oxygen to fuel the reaction. The reaction is a rearrangement of atoms already present. The question will be what energy threshold needs to be exceeded to start the reaction. Low voltages and beam currents are a good approach. High explosives may be safer because they need quite a kick from a detonator to get started. Of course, it is also helpful to limit the material examined so if something goes off it won't damage the instrument. I'd be interested if someone could address those issues. Warren wesaia@iastate.edu Thu Apr 24

Combustion does require fuel and oxidizer. However, explosives and propellants such as smokeless powder are molecules containing hydrogen, carbon, nitrogen, and oxygen in a relatively stable configuration, that can be disturbed by energy input (shock, heat, etc.) an decompose into carbon dioxide / monoxide, water vapor, and nitrogen. That is, these compounds contain both fuel and oxidizer, and the explosion is essentially the reconfiguration of the components into more stable compounds, with a concomitant release of energy (heat and noise). If you Google TNT you will find that it is a 6 carbon benzene ring with one hydrogen, one $CH_3$ group, and three $NO_2$ groups. The reaction can be written: $C_7H_5N_2O_6$ ---> 1.5 $N_2$ + 2.5 $H_2O$ + 3.5 CO + 3.5 C. If it had a little more oxygen the last CO and C would be fully oxidized to $CO_2$. Anyway, it takes a "spark"—some destabilizing input—to kick off the reaction. And since this has little to do with microscopy I'll shut up now. Andrew Werner werner1@slb.com Thu Apr 24

Well, I said I'd shut up but a couple of additional points: You are right, no time for mass transfer, the explosive molecule is broken down and rearranged into smaller, simpler molecules with the release of heat. The energy threshold is sticky - it is why we have rules about cutting detonating cord with a very sharp blade and not "sawing" on it - friction can set it off, but it has to be enough, and concentrated/localized. High explosives can go "low order" - they can deflagrate instead of detonate. High-order detonation means the reaction front moves faster than the sound speed in the material; low order means the shock wave (sound) can outrun the reaction. High order puts the energy out faster/sooner. But high explosives aren't necessarily harder to initiate than low. You are absolutely right about limiting the amount of energetic material, for a couple of reasons. One it total energy involved - deflagration of a few milligrams will contaminate the vacuum system but not damage the microscope chamber. The other is what is called "run up to detonation" and (roughly), works like - starting one spot burning, the reaction is relatively slow, but picks up speed as heat accumulates and finally gets fast enough to outrun the sound wave - it goes high order. Limiting the amount of material obviates this possibility. Look, I'm a Metallurgist who works with explosives people and happens to use microscopes - not really a Microscopist or Shock Physicist or Chemical Engineer. But explosives are fascinating and useful - we use a lot more in mining and oilfield than they do in military applications every year. So, if anyone is interested in them, get Paul Cooper's book (with Stan Kurowski) Introduction to the Technology of Explosives - ISBN 0-471-18635-X - it is very accessible and fascinating. Andrew Werner werner1@slb.com Thu Apr 24

A gunpowder explosion is a deflagration (rapid burning, yes, but with the oxidizer in the composition) instead of a detonation, which is what defines "high explosives". If you google those two terms, you'll get a complete explanation. Rick Mott rmott@pulsetor.com Thu Apr 24

Exactly! This is what is known these days as "black powder" and was originally known as gunpowder before the advent of smokeless powder. Black powder is an intimate mixture of the three components, mixed, dampened, granulated, and dried. Modern small arms propellants (smokeless powder) are generally nitrocellulose based (there are single base and double base powders, the double base contain some nitroglycerine), granulated, and coated with deterrent coatings that encourage progressive burning - controlled deflagration - to tailor the pressure-time curve to the projectile/barrel length combination at hand. You can make nitrocellulose easily by nitrating cotton or paper with nitric and sulfuric acids. Note that this is something to do in controlled conditions, with proper cooling and safeguards - but my 7th grade Science Teacher, Miss Wyle, showed us and actually may be the person ultimately responsible for the course my life has taken (she was great). Andrew Werner werner1@slb.com Thu Apr 24

Right, sorry, I was thinking about black powder, not modern smokeless powder, which is what "gunpowder" currently means. Andrew was typing his more detailed explanations while I was still sending mine. This is a bit off-topic, but: http://explosives.mst.edu/media/academic/explosives/documents/camp/2014ExplosivesCamp.pdf if you have a kid 16 or older (or if you're young enough to pass for a high-school kid, which definitely excludes me), check this out! Rick Mott rmott@pulsetor.com Thu Apr 24

In relation to the comments about column contamination, the fumes from the "explosive" instruments pump were disgusting. A column clean, new filters, and fresh fluids in the pumps, as expected, made no difference. This column was in real trouble after years of working with explosives. The best solution we could use to reduce "operator" contamination was to dump the rotary pump outlet to the outside world; sorry greens! Steve Chapman protrain@emcourses.com Thu Apr 24

So is the fear that the SEM Vacuum chamber can blow up since the gunpowder can fuel its own combustion? So does this mean current guns and ammo technology will still be good weapons to use in outer space? Mike Milewski mmilewski@comcast.net Thu Apr 24

I think the concern is that the energetic material could potentially decompose into its reaction products, liberating some heat and gas - $CO_2$, $H_2O$, $N_2$, CO - that might be a pain for the vacuum system to deal with. But yes, current guns and ammunition will work in vacuum. You would need less volatile lubricants on moving parts, or solid-state anti-friction coatings (hard chrome or spray moly might work), and the barrel might be subject to excessive copper fouling - the bullet jacket riding directly on the lands might gall - but we moly coat match bullets now, so I can't immediately think of any insurmountable difficulties. Except - you need a good backstop (here on Earth a substantial dirt berm works well), and in the absence of a significant gravitational field, recoil will send the shooter tumbling / spinning. And - with no wind to read and compensate, where is the challenge? Andrew Werner werner1@slb.com Thu Apr 24

I would say that the reactions (deflagration?) will still occur, so ammo should work in outer space. However, you may want to remember Newton's 3rd Law! Also the combustion products will significantly affect the local vacuum. Henk Colijn colijn.1@osu.edu Thu Apr 24

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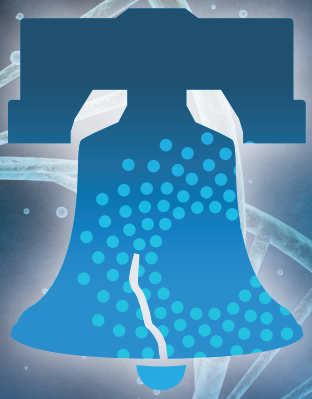

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