NetNotes

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Specimen Preparation:
tracking samples

I'm sure this question has been asked many times before, but I wanted to see if people might share their systems for keeping track of TEM samples. In grad school it wasn't too bad, since I was focused mainly on my own samples, but now I've been deluged with samples from many different projects. I'm considering using Evernote in combination with a naming and tracking scheme, but I'm open to other options as well. Steven Spurgeon steven.spurgeon@pnnl.gov Thu Feb 12

From my experience, having good tracking, a naming method and physical storage for samples is key. I would suggest going beyond a simple text file, as this becomes unwieldy quickly. A database can be as simple as a spreadsheet, with columns for all the parameters of interest and links to image storage locations. A spreadsheet can be filtered and searched and doesn't require any programming - unless you need to do a real database for PNNL tracking or billing. Set viewing and editing permissions so that only those you trust to enter data properly can make changes. A physical organizer for grid boxes, with names that relate to your database for getting at them later if desired, is really helpful. Larry Scipioni les@zsgenetics.com Fri Feb 13

We give each project a unique project number, which is incorporated into sample labels, processing records and image records, as well as a project database (currently Filemaker Pro) so every step can be traced back to the project. Ours is prefixed by an identifying code, followed by the financial year, followed by the project number - i.e., EMLP 14-15.037 The database includes client name, department (or company), order number, brief description of work, financial details, etc. Each project has a processing protocol and job summary that is filed in a folder headed with the corresponding project number. This way, as long as you've got the project number, you can refer back to any sample. We also give each image a unique image number for the same reason. Natalie Allcock nsa2@leicester.ac.uk Fri Feb 13

Specimen Preparation:
fixation of mitochondria

I am very confused about how to go about fixing a sample of isolated mitochondria for TEM. All the procedures seem to be very vague about "washing the pellet". To me, this implies re-suspending the pellet in buffer and then spinning it back down. But the procedures I have looked at never mention the g force for this step, but do specify the time. I would assume just use the g force of the last centrifuge step performed, but this step involves addition of agarose solution to enrobe the pellet, so it seems like this would be a distinct and unrelated step. Also, the addition of agarose is never mentioned again, so would I add it to the buffer for the washing steps, assuming washing involves more centrifugation? Alternatively, does washing the pellet just mean inundating the pellet with buffer and then pouring it off? If that is the case, how do I do this for 20 minutes? Do I just let the buffer sit on the pellet for 20 minutes and then pour it off? Sam Gonzalez sameesh@uga.edu Mon Jan 26

Liquid agarose is added to make processing easier. Processing some samples such as cell suspensions and organelles can be tricky, especially when it a very small quantity, as the majority of the sample gets lost during all the washing steps—plus it can get very hard to pellet them in the thick embedding resins. Adding liquid agarose in the earlier stages—then leaving it to cool so that it sets hard - embeds the samples in a larger, gel like pellet that can then be processed through as if it were a larger sample—without the need for centrifugation at each step. When I process organelles, I usually fix in primary fixative, wash in buffer, centrifuge it down into a good pellet, remove as much supernatant as possible, then add a small drop of warm (not too hot) agarose (3%), making sure there is no air bubbles between it and the pellet, leave in a warm water bath for 10 minutes to allow the agarose to infuse with the pellet, then move to the fridge until the agarose is completely set. I then carefully remove the agarose/sample from the tube, and if necessary, use a razor blade to cut it into $1mm^2$ pieces for further processing. You should then have some nice cubes of agarose/sample (or maybe just a small smear of sample in the tip if you're processing organelles or a difficult sample) that you can process through without the need for further spinning. Sometimes, despite all my best efforts, the sample pellet gets left behind in the Eppendorf when I remove the set agarose gel. In this instance, I carefully slip the pellet onto a glass slide, and sandwich it between the agarose that way. If anyone has any helpful hints to combat this, it would be much appreciated! Natalie Allcock nsa2@leicester.ac.uk Tue Jan 27

Microscopy:
recycling old EM negatives

A colleague of mine found several boxes of old EM negatives while cleaning out her basement. She contacted me and asked if we could recycle them. It never crossed my mind that these could be recycled but apparently there are hundreds of them and it does seem kind of a waste to just through them out. Does anyone out there no if old EM negatives (Kodak 4489) can be recycled? **Greg Hendricks gregory.hendricks@ umassmed.edu Wed Jan 7**

Kodak has a document called KES-60, which has a list of scrap film buyers. The most recent version I could find in Google was this: http://www.kodak.co.uk/ek/uploadedFiles/Content/About_Kodak/Global_Sustainability/Health,_Safety_and_Environment/HSE_Support_Center/Product_End_of_Life_Management/KES-60_Scrap_Film_Buyers.pdf. Ben Micklem ben.micklem@pharm.ox.ac.uk Wed Jan 7

There are people around the world that recover silver from both unexposed film and negatives. Many have gathered together on the http://goldrefiningforum.com/ forum. There you could probably find someone close-by that can recover the silver from the films. The process is also described in several places on the forum for anyone interested. Göran Axelsson axelsson@acc.umu.se Wed Jan 7

Image Processing:
offline TIA viewer

How can I view.emi and.ser files on Windows? Is there any offline TIA viewer for Windows available? **Ravi Thakkar ravi.thakkar369@ gmail.com Fri Jan 2**

This won't help entirely, but ImageJ has a plugin that can open the.SER files. You need to be sure that you point to the correct file that contains the image. Most of the experimental information is stored in the.EMI file. The FEI website has a link to an off-line version of ESVision (http://www.fei.com/service-support/es-vision/) which is the original version of TIA. You may be able to run it and analyze the data directly. I've not tested it. Henk Colijn colijn.1@osu.edu Fri Jan 2

EM:
hardware and software for remote viewing

As part of a pitch to install remote viewing / webinar capabilities in our microscopy lab, my administration as asked me to find example systems and collect information to help us optimize and balance our wants, needs, and costs. If you have an electron microscope that you can remotely view, remotely operate, send the display output to a classroom, send the display outside your institution's network for a webinar, etc. and are willing to answer some questions about components and costs would you please contact me. **Stefanie Brachfelds brachfelds@mail. montclair.edu Thu Feb 26**

You can also look at this site. http://telepresencecollaboratory. org, Telepresence Collaboration, which is fairly old. We started doing it at ANL when the Mosaic WWW browser first appeared ~ 1994. There are also a few old PDF's of Lectures here: http://tpm.amc.anl. gov/Lectures/ Today, there are numerous solutions that update these older protocols. To be honest, because of network safety (i.e. hacker) issues, I no longer allow remote control, only passive observation. I would caution you that you will need to do the same. All publically accessible sites get attacked. You don't want to know how many hackers try to break into the Microscopy Listserver every day. Nestor Zaluzec anl.nestor.zaluzec@gmail.com Fri Feb 27

TEM:
preventing charging

Does anybody use a coater to coat ceramic TEM samples with a very thin layer to mitigate charging? I would also be happy to know if there are any other ways to stop charging of samples. I am looking at manually polished complex oxide samples. **Debangshu Mukherjee debangshu@psu.edu Sat Feb 28**

We've had good luck putting down a few nm (usually 3 nm on our system) of carbon to mitigate charging and to stabilize the Formvar films our bio friends make. If you are doing atomic resolution STEM, make sure the C is on the bottom side. Henk Colijn colijn.1@osu.edu Sat Feb 28

TEM:
holder insertion/extraction problems

We have a custom holder for the JEOL 2100 but have been having problems with insertion and extraction. The holder does not get "sucked in" by the vacuum after the first and second rotations. It actually requires a little pushing, and sometimes extraction is very difficult, requiring more force than a standard holder. The puzzling thing is that the holder works perfectly in a JEOL 2010F. The holder manufacturer has rechecked all physical dimensions of the holder and the o-rings, finding them within tolerance. JEOL also has taken back the goniometer on repeated occasions but has not found a solution. Oddly, a loaner goniometer JEOL installs when they take ours away, happens to work without problems. Last incident we had, the vacuum was breached upon holder extraction. While pulling the holder, the outer gasket on the goniometer was extracted too. What is the most likely cause for this? We see wear marks on the alignment pin of the holder. **Rodrigo Bernal robernal@u.northwestern.edu Mon Jan 26**

It sounds like a goniometer problem. Wear marks on the alignment pin are not good because the inner tube of the goniometer can be scratched. This tube is a rotating part that moves with the holder and scuffing is possible between this tube and the internal wall of the goniometer. Scratches on the inner tube can weaken the vacuum sealing during the movement of the holder. If this hypothesis is correct, it's necessary to remove this tube to get it into the shape of the goniometer wall (by soft abrasion with Pikal for example). This is a job for a JEOL engineer. If there are scratches this inner tube must be replaced. This is a tricky job and expensive part. Each vacuum leak during holder push/pull movement is bad experience for the ion pump. Nicolas Stephant nicolas.stephant@univ-nantes.fr Tue Jan 27

TEM:
monitor turned black

When coming back from holidays, I found our CM10's monitor display was completely dark—A small part of the data display on a corner of the screen has disappeared or faded for a while but the machine was operational without any problem. Now the whole screen has blacked out. The Data Dim and Panel Dim knobs were usually set at low or off during non-usage times. Other panel lights are all normal. It seems like a small part (bulb or circuit board?) wearing out. Is there any simple way to check or order/replace the part? **Guosheng Liu guosheng.liu@usask.ca Mon Jan 5**

One thing you can try is to shine a bright light up in the upper left corner of the Data Monitor and see if the characters show up. The Data monitor intensity circuitry is a little strange. When you adjust the Data Dim knob it controls the intensity of a bulb on a circuit board behind the panel. There is a light absorbing diode that is next to the bulb that measures the intensity and adjusts the circuitry that controls the Data brightness. If this bulb is burned out, then the monitor goes black. John Schreiber js51@princeton.edu Mon Jan 5

SEM:
vacuum hose source

First, thanks to everyone for all the excellent advice I've received in the past on this list. I've got the rather unique task of running an SEM program accessible to the general public at a makerspace in Chicago, as a volunteer with no formal training and a shoestring budget. As far as I know, our circumstance is rather unique. There's no way I could have managed this long without all the help I've received here. I managed to track down and repair the problem with wildly fluctuating vacuum readings (an extremely dirty Penning gauge) and the reason the vacuum was really bad after I fixed it (a leaking air admit solenoid.) But a bad shaft coupling on our Edwards E2M12 rotary pump was causing it to overheat and burn its oil, and, unfortunately, splattering said burnt oil up the rubber vacuum hose. I secured funding to get the pump overhauled, but now I need to know what to do with the hose. I figure it needs to be replaced, seeing as it reeks of burnt oil. What I'm measuring seems to be about 7/8 I.D. and 1/4" wall thickness, so about 1 3/8" O.D. It's difficult to get a good reading as the hose ends were either clamped around something or stretched. Looking at http://www.pchemlabs.com/subcatagoryb.asp?pid=Pure-Gum-Rubber it looks like the gum rubber vacuum hose they sell jumps from 13/16" I.D. with 3/8" walls (listed as used for KF16) 1" with 1/2" walls (listed as used for KF25). The pump has a KF25 fitting, and the hose slips over a copper pipe in a concrete block as a vibration damper. But that size seems like it would be too large. McMaster-Carr is local for us, so I checked http://www.mcmaster.com/#abrasion-resistant-gum-rubber-tubing/

and see vacuum rated red and tan tubing. (I have no idea what the color means. For reference, the tubing that came with the SEM was black.) The tan jumps from 3/4″ with 5/8″ walls to 1″ with 1/2″ walls. The red does come in 7/8″ but it has 1/2″ walls too. I'm not even sure if that would fit out the opening in the back of the SEM. There's a good chance the vacuum hose is original to the scope, which is a Leica S430. Any help would be appreciated. Am I correct in assuming the hose should be replaced? Ryan rdpierce@pobox.com Tue Jan 27

A good "standard" supplier is http://duniway.com/catalog/su-tubing-clamps.php (many components, not just the hose). A cheap option is more work but you can make whatever diameter hose plus it can be easily compressed around a (much) smaller diameter tube since the support spring is separate. Components: a) braided PVC of suitable diameter, either Home Depot etc. or McMaster Carr; b) continuous spring for supporting hose from collapsing, Again McMaster Carr. Tubing: http://www.mcmaster.com/#standard-high-pressure-pvc-tubing/=vnotog then select "clear" or from home page enter "High-Pressure PVC Tubing" in search line, then select "clear" spring: http://www.mcmaster.com/#cut-to-length-springs/=vnp0wo then select "extension spring" or from home page enter "Cut-to-Length Extension Springs" stainless springs softer and won't rust. They are sold in 20″ pieces and will easily extend 10+ times. Springs made with wire 0.04″ to 0.08″ thick are best for diameters around 1″. 3/4″ to 1″ ID vac. hose made in this fashion costs between $2 and $5 per foot depending on source of materials. It is transparent so oil accumulation can be seen before problems begin, plus PVC will outlast rubber hands down. The oldest still in use I installed in 1998. Use standard common hose-clams as long as they flat and at least close to 1/2″ wide. Wire clamps not recommended. Thin-wall-soft-not-braided low pressure PVC is even cheaper but less durable and unforgiving with regard to vacuum leaks at installation. Same longevity unless stressed. For long straight connections use large diameter thin-wall copper tube, again from Home Depot, just ensure adequate cross-section for very long connections. Vitaly Feingold vitalylazar@att.net Wed Jan 28

If you make a cut through the tubing, you should be able to measure ID and OD accurately. Bill Tivol wtivol@sbcglobal.net Wed Jan 28

SEM:
power supply board failure

We have a JEOL 6100 that needs its magnification power supply board replaced. Our chiller stopped working the other day for about an hour and the mag. power supply got so hot half of the wires melted. We are trying to repair it in house but it's not looking good. It would be greatly appreciated if anyone can suggest a source for either repairing or replacing it. The JEOL 6100 model is SM111040-154. The power supply model is SM111040-7A. **Rod Rowland rowland@matsys.com Fri Jan 16**

According with my experience, you'll have to change this power supply because it's hard to repair when such problem occurs. This part contains many power transistors and, for this reason, is water cooled. The most common accident occurs when water continues to flow in the hose when power is switched off—the gradient between room temperature and the cooling water temperature can cause condensation of water from air humidity on the surface of the power supply components and circuitry, which then short-circuits when the power supply is switched back on. Week-end and holidays are dangerous periods where the SEM is shut down but not the water cooling supply. Normally there is a small detector that shut off the board when temperatures exceed 70°; it seems this security has not worked for your SEM or this was shorted in the past. It's very easy to do from outside of the board and very easy to forget later. Probably JEOL has no spare parts because the JSM 6100 is rather old. Another solution is to find this part from a scrapped 6100. There is currently a 6100 on EBay for 12000 dollars. Last solution is to change

all the broken parts of the board. This is possible for a good electronics engineer if you can supply him the diagrams. Nicolas Stephant nicolas.stephant@univ-nantes.fr Tue Jan 20

SEM:
beam alignment problem

We cannot align the aperture in our JSM5600LV SEM. Using the wobbler tool we cannot correct horizontal movement, in all 3 aperture positions. It looks like the alignment point lies outside the aperture openings. The problem worsens with increasing tilt angle and affects the resolution; 5K looks like 15K. I cleaned the upper column up to the aperture level and the aperture, but didn't help. It was not dirty anyway. However, last time I changed filament I noticed some little white crisps around the Wehnelt orifice. Is it likely to have contamination of the lower parts of the column or something else? **Yorgos Nikas eikonika@otenet.gr Thu Jan 22**

I don't expect the dirt on the Wehnelt aperture to be much of a problem, but better clean it away. Are you sure your final aperture strip (or do you have single apertures?) are not moving in the aperture strip-holder? Did you check that the holder is perfectly clean? Since you say you have the same problem with all three aperture openings it might also be a problem with the astigmatism correction voltage going to the coils. If you have the schematics, you can measure this at the plug. Stefan Diller stefan.diller@t-online.de Thu Jan 22

May I suggest you take out the aperture strip and see if you can align the instrument to your satisfaction? The problem may be in another part of the column resulting in an apparent aperture problem. I too do not expect the dirt on the Wehnelt to be a real problem. Try this and let us know what happens. You will need to use small spot sizes to obtain a reasonable image quality in the test. Steve Chapman protrain@emcourses.com Thu Jan 22

SEM:
Wehnelt aperture sizes

Has anyone with a LEO/Zeiss 1455VP LaB_6 SEM compared the Wehnelt aperture sizes? They are 1mm and 500um. Zeiss seems to have settled on the 500um Wehnelt aperture size for LaB_6 and W emitters. Is there any feedback from users about which aperture size produces the best results? **Gary Gaugler gary@gaugler.com Fri Feb 20**

Wehnelt apertures are usually selected depending upon the application. High magnification operations are best made with a small aperture, with the filament closer to the front of the Wehnelt, whereas for low magnification work, moving the filament back, and using a larger aperture, provides sufficient performance with longer filament life. Those carrying out a large amount of BSE investigations will also be better served using a larger aperture as bigger sources provide higher signals. Most manufacturers use a smaller Wehnelt aperture than for tungsten, when LaB_6 filaments are fitted, as stronger bias fields are required to control the source. Steve Chapman protrain@emcourses.com Tue Feb 24

SEM-EDS:
analysis of Pb

I have several copper-tin-lead alloy standards with varying Pb contents (5, 10, 15, 20). I analyzed them by the SEM-EDS, but it keeps giving me lower Pb concentrations (around 2, 5, 7, 10 individually). The totals are good around 100 percent, but just inaccurate (tin is the same, but Pb is lower and Cu is higher than their real values. I use the default internal calibration (leaded glass) to calibrate Pb element. It is known that Pb in the copper alloy will form a separate phase. Has anyone encountered this problem and how did you solve it? **Gary sniper711220@hotmail.com Fri Jan 30**

How flat is the surface? What is the phase distribution on a micro-scale? If the Pb is present as a second phase it might well be preferentially polished away changing the volume (and mass) fraction and skewing the results. Also, EDS correction routines are written to process intensities from homogeneous analysis volumes. If you are trying to lower the magnification and scan an area, you will present the sum of the phase intensities to the analyzer. However, Pb probably had nothing to do in determining the interaction volume in the regions where the Cu and Sn are, nor did the Cu and Sn x-rays undergo absorption by Pb atoms. Once in a while, EDS might give the right answer in such cases, but I would not count on it being the right. BTW, the same issue would be true of WDS too. The matrix corrections are much the same. They assume a homogenous sample volume. Warren Straszheim wesaia@iastate.edu Fri Jan 30

I would not use the Pb in glass standard for this analysis. You should try to use a standard as close to your unknown composition. Pb in SiO_2 is not close. I would try using one of your CuSnPb standards as the standard and see how the results turn out. You say you "use the default internal calibration (leaded glass) to calibrate Pb element." Did you use the same operating conditions? kV, take-off angle? Also which lines for each element did you use? K L M? Be careful of background too. David Hull drhull@zoominternet.net Fri Jan 30

EDS:
calibration

I have performed EDS analysis of Au Nanoparticles on Copper TEM grids using an Oxford EDS. I am getting a lot of Cu peaks instead of an Au peak. I am using INCA microanalysis suite version 4.15.

Could anybody suggest to me how to do a calibration and what kind of standard reference sample should be used for calibration? **Ravi Thakkar ravi.thakkar369@gmail.com Wed Feb 4**

You are always going to see Cu under those circumstances. The amount of Cu metal in the grid is vast compared to the size of an Au nanoparticle. As your electron beam passes through the Au nanoparticle, some electrons will scatter and hit the grid producing fluorescence. There can also be electrons scattered from electron microscope components such as apertures as well — though this is less a problem these days than it used to be (depends on the age of your microscope). If you aren't interested in Cu, I suggest you just ignore the peak. If you are interested in Cu in your nanoparticle, then producers such as EMS, Ted Pella and Ladd make a variety of grids out of a variety of elements and compounds so that you can almost certainly find some element you don't care about. When avoiding Cu, I've had luck using beryllium, and silicon nitride. For general calibration, a simple and very common approach is to use the Cliff-Lorimer method of k-factors: Cliff, G. & Lorimer, G. W. (1975). "The Quantitative Analysis of Thin Specimens," *Journal of Microscopy* 103(2) 203–07. The Oxford software has this method built in and allows you to define those k-factors once you measure them (using the method from the paper above). The Oxford software also has some good guesses built in if you don't need very much accuracy. I have also found it useful to extract peak areas from TEM/EDS spectra and process them myself. I first use fityk, Python, or the Oxford/Bruker software to fit peak areas. Then I use k-factors and apply a thickness correction using some software I wrote for myself (https://github.com/ZGainsforth/StoichiometryFitter/blob/master/Stoichiometry%20Fitter%20Screenshot.png). You might like a solution like this if you want to control every aspect of the analysis. Zack Gainsforth zackg@berkeley.edu Wed Feb 4

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