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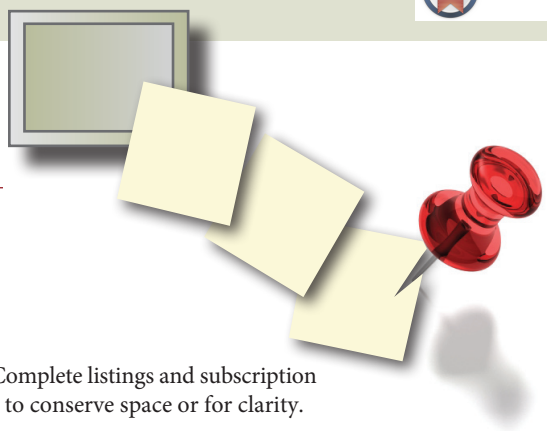

NetNotes

Edited by Thomas E. Phillips
University of Missouri

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Selected postings from the Microscopy Listserver from November 1, 2017 to January 1, 2018. Complete listings and subscription information can be obtained at http://www.microscopy.com. Postings may have been edited to conserve space or for clarity.

Microscopy Listserver Statistics:

Welcome to another year of operation of the Microscopy ListServer—a free service to the worldwide microscopy community, sponsored jointly by your Friendly Neighborhood SysOp and the Microscopy Society of America.

During 2017, the ListServer delivered your messages to more than 4,350 subscribers around the world, with minimal hassles (that I know about). For those of you who are statistics junkies, this year you generated ~250+ Gbits of email traffic, and ~2.55 million email messages were sent out by my tired and old little server. Up a bit from last year, but still a steady flow.

As usual you don't want to know how much junk mail and spam has been filtered out—far more than you might expect. Apologies to those who have problems with my filters.

The complete Microscopy ListServer Archives for (approximately) 2017–1994 are online at

http://www.microscopy.com.

A few important reminders:

- If you leave on vacation/holiday use the online form to UNSUBSCRIBE your email address from the Listserver. The out-of-office/on-vacation autoreply messages are a real nuisance to posters.
- Do not reply to messages with the return address of MicroscopyListserver-noreply-at-microscopy.com. These are messages forwarded usually from the WWW posting form. They do not go back to the poster but rather into a black hole, which I rarely check. If you see a message that has this "no-reply" return address, please post your reply/comment/answer to: microscopy@microscopy.com, or if you wish to reply privately, look at the username in the body of the message; the originator's email address is usually listed therein.
- As always if you have questions about suitability of postings or are having problems, feel free to contact me at zaluzec@microscopy.com.

Nestor Zaluzec zaluzec@microscopy.com Sun Jan 1

Specimen Preparation:

coating for Carbon evaporator bell jar

In a previous post, I asked what everyone was doing for coating the bell jar on your carbon evaporator, now that Bell Bright isn't going to be available. It seemed the consensus was to use dish soap as a replacement. Well, I've exhausted my hoard, and like a dummy, I didn't ask for how you did that. So the obvious questions, are what are you using instead of Bell Bright and how do you apply it? ***Matthew Duley duleyml@muohio.edu Fri Dec 1***

It is very simple and inexpensive; clean your bell jar, electrodes, and insulators as best you can then wipe the inside with a piece of paper towel that has had 5-10ml of dishwashing detergent applied to it. Wipe the whole of the glass, metal and insulator surfaces so that there is a very thin smear of detergent applied. Pump down the bell jar to dry the system out. The system is ready to use! Next time you need to clean the system all you need do is wash all surfaces with warm water, dry them off with a paper towel, then apply the detergent again. John Nailon jvnailon@gmail.com Sun Dec 3

I have a coater I'm refurbishing and wondering what the purpose is for this detergent coating? Sylvain Poudrette spoudrette@videotron.ca Mon Dec 4

Every time you use the evaporator to coat a sample with carbon or metal, everything gets coated. I am alluding to the inside of the glass bell jar, all of the electrodes and all of the insulated connections. If you do not clean the system periodically, then you will start to have slow pump down times, inability to see inside the bell jar and potential short circuits across the insulated connections within the bell jar. John Nailon jvnailon@gmail.com Sun Dec 10

I have the Denton floor model carbon evaporator, and I use a very small dab of metal polish on a paper towel, within a small area of the jar. I immediately chase with 100% ethanol, then move to another small region of the jar. I have never had vacuum issues cleaning the bell jar like this. Michael Delannoy mdelann1@jhmi.edu Mon Dec 4

We offer BellShine at https://www.laddresearch.com/vacuum-equipment/bell-shine that should work for you. Call and ask for Mike Bouchard if you want to discuss it. Disclaimer: Ladd research sells this product and other products for use in Microscopy Labs. JD Arnott jd@laddresearch.com Mon Dec 4

Specimen Preparation:

chick brain for Vibratome sectioning

I am doing brain electroporations with an RCASB plasmid, and I wanted to visualize how efficient my electroporations were. Currently, I am embedding my heads in 3% agarose at 37 °C between 15-20 minutes, cutting the block fairly close to the brain, and a final embedding in 6% agarose at 4°C for at least 20 minutes. The smallest sections I have been able to slice is 300 microns; however, I would like to get down to 200 microns. When I have attempted to go below 300 microns, the tissue is destroyed, which I can see that it's lacking structural support from how I am embedding. My question: What would be a more efficient way to embed my samples so I can section between 100-200 microns? ***Garrett Driscoll driscollg2@winthrop.edu Tue Dec 5***

First, are you removing the meninges before embedding the brains? They can interfere with the agarose. Second, this may/may not help — I embedded mormyrid brains, not vertebrate. Try 18% (wt:vol) 100-175 bloom gelatin instead of agarose. Liquefy the gelatin and place tissue in the gelatin in a 55-60°C oven for 1.5-2 hours. Remove from oven, blot, place in fresh gelatin & put in the oven for 15-30 minutes, remove from oven, blot, embed in fresh gelatin but do not put back

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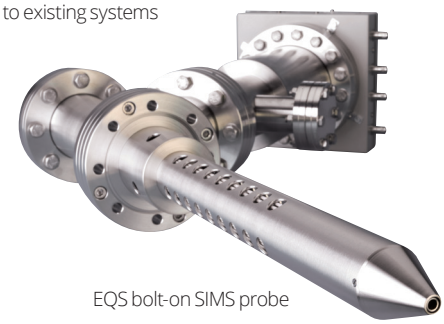

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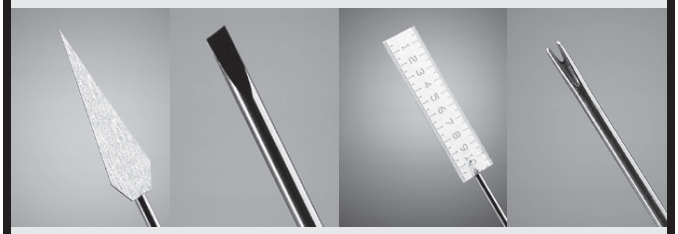

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in the oven. Let gelatin set completely. Mormyrids have a ginormous cerebellum that sits over the cerebrum and causes issues with the embedding medium getting to the rest of the brain. This gelatin method worked for them, so it should work for chick brains. I hope. Phil Oshel oshel1pe@cmich.edu Tue Dec 5

There are some missing details. What is your fixative or is this live? Are these expressing a fluorescent protein or are you planning on immunolabeling? What instrument are you using to section and how are you attaching the sample to the sample holder. I've found that embryonic avian brain requires slow advance speed and high amplitude, whether live or fixed. It is also very sensitive to knife angle. How are you attaching to the specimen holder? Not all cyanoacryates work with wet samples. I've had the best results orienting the tissue with the ventral surface facing the knife. There is a tendency for the sectioning shear force to push the brain apart when cut from the dorsal surface. As Phil mentions, surrounding membranes can cause shear forces that displace the sample. Or they drape around the razor's edge and get dragged through the tissue. I've cut live embryonic brain down to 200 µm by gluing it to the holder and just using an agarose block to "backstop" the brain. Such a backstop can also help with any fragile tissue that does not offer much internal strength. In another project, embryonic avian brain was sectioned after blocking the tissue to give a flat base and gluing to the stub. Then low melting point agarose was ladled over it and allowed to harden. Another approach for small floppy tissues, like early embryonic brain or with spinal cord, is to cut the rounded end from a gelatin capsule or BEEM capsule and use the capsule as a mold for embedding with agarose. If embedded with the cap in place, remove the cap to expose the tissue. Or trim down the upper end to expose the tissue for slicing. Glen MacDonald glenmac@uw.edu Sun Dec 10

You can try Celloidin embedding media, with that you can section as thin as 15 µm I had used it for embedding Golgi stained human and mouse brain tissue. Here is the link for detailed description hope it helps you http://stainsfile.info/StainsFile/prepare/process/celloidin.htm Arvind Singh Pundir arvind@nbrc.ac.in Sun Dec 10

Have you fixed the tissue in paraformaldehyde? Karen Bentley karen_bentley@urmc.rochester.edu Sun Dec 10

Specimen Preparation:

metal evaporation deposition units

Are there some suggestions about high vacuum evaporation deposition systems? I'm looking for something that would do high resolution deposition of Pt and Ir but does not have a large foot print like the Denton 502 units. The Denton Desk IV TSC using Ir does not produce a fine enough coating for imaging at 25K×+. I've tried 15mT, 35% current and 60 seconds time but get clumps in my images using FEGSEM. Any ideas or suggestions, please? ***Gary Gaugler gary@microtechnics.com Fri Dec 15***

We recently purchased a Leica ACE 600 system equipped with sputter coater, carbon wire coater, and glow discharge options. We did not get a Pt target, but have Ir, Cr, Au, and AuPd. I think that it has a small foot print. It is easy to use. I use Ir. I found that the rate of deposition for a 1 nm coating was too fast, so I created a new recipe by decreasing the current by half to 40 mA. It takes about 30 sec, and I get good coatings. If you are getting clumps in your deposition, you should investigate more before investing in a new unit. Check your target for wear. Check that there is a good thermal connection. Check your gas supply for purity. You can also play around a bit with the pressure and power settings. Also, use as long a working distance as possible. Scott Walck s.walck@comcast.net Fri Dec 22

Fluorescence Microscopy:

reducing autofluorescence

I am trying to reduce the amount of autofluorescence from my embryonic chick brains (E10 and E12), which are being imaged by light-sheet microscopy. Currently, I am drop-fixing my heads in 4% PFA. The embryos hearts are too delicate for us to perfuse them, so I was hoping to have another method to remove hemoglobin from the brain, and that should reduce my autofluorescence. I have both GFP and Cholera toxin B-Alexa Fluor 555 that I am looking at with my imaging. Additionally, I am doing a BABB clearing method. ***Garrett Driscoll driscollg2@winthrop.edu Fri Dec 15***

Try 1% Sodium Borohydride in PBS for 10-15 min. Clancy and Cauller (1998). It won't kill the fluorescent proteins like some of the other treatments. Glen MacDonald glenmac@u.washington.edu Sun Dec 17

There are several other methods you can try that quench the remaining free aldehyde groups left after glutaraldehyde fixation (below). However, if you are not necessarily interested in ultrastructure, why not use paraformaldehyde? Aldehyde induced fluorescence is usually much less of a problem with the PFA. You may also want to think about whether the fluorescence you are seeing is indeed due to aldehydes - or if it could be due to some other cellular constituent like lysolipids. As an alternative, you may also want to consider using the longest wavelength your imaging and fluorophore systems will allow. Try looking at the background fluorescence above around 590 nm. It may be all you need to do.

For blocking free aldehydes remaining after fixation:

- 1% (or 150 mM) glycine with 0.1% Tween in PBS. Wash well. Glycine binds to free aldehydes.
- 50 mM NH_4Cl in PBS. Incubate for 15 min at room temperature. Wash well. (see Bendyan)
- 0.1% Na borohydride in PBS. Apply while fizzing and incubate 3 × 10 min on ice. Wash well.This reduces carbonyls.
- 0.15 M ethanolamine, pH 7.5. Incubate 30 min on ice. Wash well.
- Also from an earlier post by Scott Snyder ssnyder@niaid.nih.gov : Probably the best way I have found of getting rid of aldehyde based fluorescence is to get rid of the aldehydes. I often do this by using the bifunctional crosslinking reagent dithio bis(succinimidyl propionate), or DSP for short. This gives comparable structure retention in my hands and essentially no added fluorescence. A good reference for this is Safieko-Mroczka and Bell, *Journal of Histochemistry and Cytochemistry* 44(6) (1996) 641–56.

Michael Cammer michael.cammer@med.nyu.edu Sun Dec 17

TEM:

oil diffusion pump (ODP) water message

Today I tried to switch on our Philips CM10 TEM. After an hour the message "ODP water" appeared on the monitor. Reading the manual I figured out that the message was about high water temperature (60°C) on the ODP cooling system. There is the S30 switch that checks that temperature. Our water cooling system seems to work fine (temperature about 15°C). Any ideas how to fix the problem? ***Panagiotis Berillis pveril@uth.gr Tue Dec 5***

Check the thermal sensor on the ODP; it may need replacing. This happened on my CM200. Nestor Zaluzec anl.nestor.zaluzec@gmail.com Tue Dec 5

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I had this issue with our old CM-10 for either of two reasons: either somehow the temperature sensor on the side on the ODP was out of position, or the cooling water line was clogged, and didn't have sufficient flow. The sensor got jogged out of position once during maintenance so that it wasn't making proper contact with the pump and read wrong. This shut down the diffusion pump. The clogged cooling line was commonplace and easily fixed by running a bottle of CLR (Calcium Lime Rust), a US brand of scale remover, through the lines. The line could also be cleaned with a liter of 3% H_2O_2 or 100 mL of 30% H_2O_2. In our case, the TEM was on a Haskris chiller, so I just added the solution to the chiller's water tank and let the cooling water circulate for 4 hours - overnight (depending on how bad things were), then several changes of water until crud quit coming out of the cooling line. If you're on city water and not a cooler, try removing the in-line filter from the housing and adding the H_2O_2 to the filter housing. This worked on an old ISI EM — except at that lab, we had a problem with biofilm, not corrosion, and used bleach. Note: the cooling water coming in gets split into two lines in the CM-10, one to the ODP and one to the electronics. If there is a line clog at or after the split, the pump can overheat without affecting the electronics, and vice-versa. Phil Oshel oshel1pe@cmich.edu Tue Dec 5

It could be the sensor itself problem, but mostly it can come from the water flow or too low of pressure. You need to get the necessary flow rate. Michel Ribardière m.ribardiere@jeol.fr Sun Dec 10

Is there adequate water flow in the DP site glass? Are the tops of the cooling coils on the DP cool to the touch? Occasionally the reducer in the water line gets clogged with debris. Mike Calabrese michael.calabrese@teledyne.com Sun Dec 10

SEM:
inert sample transfer

I would like to get some advice/input from folks that have worked with air sensitive and moisture sensitive samples for SEM-EDS. What are some of the methods and best practices to get the sample from the glovebox into the SEM without exposing, and thus modifying, the sample? Thanks in advance! ***David Reuschle*** dreuschle@dow.com ***Sun Dec 10***

We do some work with Li-ion batteries. I built an airlock chamber for our SEM that I would purge with N_2 or Ar, and then we used the South Bay Technologies Sample Savers to transfer the specimen from the glove box to the airlock chamber on the SEM. It was adequate. Brad Johnson bradley.johnson@pnnl.gov Mon Dec 11

Quantomix capsules from the EM suppliers might work. You can seal them in the glovebox and easily transport them to the SEM. Rich Hailstone hailstone@cis.rit.edu Mon Dec 11

I have designed specimen rods that protect specimens from air during transfer between a glove box and the microscope. If I can be of help, let me know. Wil Bigelow bigelow@umich.edu Sun Dec 10

I would be interested in hearing or seeing more about some of these designs. We had a chamber built for our JEOL 840A that mated with its load-lock. There was always some question about the atmosphere in the load-lock that we were exposing the sample to during pump-down. It certainly limited exposure to air. There are at least two commercial products that might be suitable. I have no vested interest in them.

https://www.quorumtech.com/quorum-product/pp3004-quicklok-ambient-temperature-airlock

https://www.kammrath-weiss.com/en/products/materials/transfer-module.html

Currently, we are running a Quant 250. We do not have a means to positively control the opening or a door or hatch. We made a small chamber with a swing-away door on the top. We load the chamber and close the door in a glove box and pump down the chamber. We mount the chamber in the SEM, and a torsion spring swings the door open when chamber pressure gets low enough in the SEM. It does pretty well and would do even better if we could guarantee that the SEM was pumping down from an inert atmosphere. Warren Straszheim wesaia@iastate.edu Mon Dec 11

SEM:
filaments burn out immediately

Our SEM JSM5600LV burns filaments immediately upon turning high tension ON. There was a thunderstorm here, and after a big thunder, the microscope went completely OFF while there was no power cut. Very soon the microscope's power came back and worked normally, but I noticed the image was bit noisy and definition less high. Then I switched it off overnight, and today I started and realized the problem (after I burned a couple of filaments). Any comment or suggestion will be greatly appreciated. ***Yorgos Nikas eikonika@otenet.gr Fri Nov 17***

Thanks to everybody provided ideas about the problem. We found a faulty voltage regulator in the HT I/O board. This regulator provides a 39V power rail to the HT Drive board, used by the filament current generating circuit. The regulator had failed, resulting in an overvoltage of some 70V going to the filament circuitry, probably causing more damage there (we replaced the regulator, but it was destroyed instantly). Finally, we replaced the faulty voltage regulator, the HT drive board and the HV power supply in the oil tank. I was lucky to have spares for the last two. The microscope now works fine. The only thing that we still try to solve is that the readings on the SEM menu regarding gun alignment values seem to be incorrect. Also, when we turn HT on, the value of the current fluctuates for several seconds before stabilizing to the expected value. Since the HT board and power supply are coming from another microscope, it may need some sort of reset that we haven't found yet. Has anybody any clue? Yorgos Nikas eikonika@otenet.gr Wed Nov 29

SEM-CL:
missing red wavelengths

Does anybody know why an SEM-CL instrument could be missing the red-to-orange wavelengths of the cathodoluminescence spectrum? I run a JEOL 6010LA benchtop SEM with a recently acquired Gatan MonoCL4 cathodoluminescence spectrometer. It works great for zircons, quartz, etc., but picks up little to no signal in the red to orange part of the spectrum even on samples that glow brilliant red under our ancient optical cold-cathode Luminoscope. I checked the same samples out on a ChromaCL hooked up to a Hitachi S3400 in another lab, and there is a similar lack of red signal. I've checked, and the accelerating voltages on the Luminoscope are about the same as on the SEM, although the Luminoscope has higher beam current, so I'm not sure why the SEM-CL instruments doesn't pick up the red luminescence. I've read that filters placed in front of a detector can eliminate red-orange luminescence and phosphorescence (Reed and Milliken, Journal of Sedimentary Research 73.2: 328-332). There was no mention of such a filter in the Gatan datasheets for either the ChromaCL or the MonoCL4, but that seems to me to be one possible explanation for the lack of red luminescence in SEM-CL compared to optical cold-cathode CL. I've left a message with Gatan but haven't heard back yet. Has anybody else with SEM-CL encountered a similar problem, or know of any fixes? We got the MonoCL4 partly to look at carbonate cements in rocks, so it's pretty important that we figure out how to get good spectra and images in the red and orange zones. Any help would be appreciated. ***Isabel Barton fay1@email.arizona.edu Fri Nov 17***

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The Gatan MonoCL uses a mirror arrangement to direct the light to the optics. It may be that the longer wavelengths (red-orange) need a different geometry for optimization of the collection. I would try and move the sample height and see if the spectra sensitivity changes with respect to the red end of the spectrum. The MonoCl systems typically have a PMT detector to detect the light, and these usually have adjustable gain. Since the longer wavelengths are energetically weak, you may have to increase the gain to detect these less energetic wavelengths. Colin MacRae colin.macrae@csiro.au Mon Nov 20

I believe Colin is correct about finding the right focal point with the GatanCL. We also have this detector on a Hitachi 3400 and have no problems with the red signal. However, finding the right focal point can be essential. 0.5 mm adjustment in the Z axis or less can make a big difference in signal strength. Phil Oshel oshel1pe@cmich.edu Mon Nov 20

EDS and SEM:
project for outreach

My company runs a community outreach program for local elementary school students (8-10 years old). They tour our facility and see quick demonstrations/lectures on a variety of topics. We have had an SEM demo for years, and I would like to incorporate our EDS, but I'm having trouble coming up with samples that kids that age understand, especially since I only have 15 minutes per group and we try to go through at least four samples. I have generated a map of different elements in a vitamin tablet which went over well and gives them a good understanding of what the system is doing. Any recommendations for samples that generate interesting maps or have unexpected elements? I'll be doing the prep/high quality maps ahead of time so that doesn't need to be done in the 15 minutes. I'd also like to get a wireless projector to use with our Hitachi SU3500 to project a live image of the screen onto the wall. Our service rep said some models could cause driver issues so if anyone here has a model that works, please let me know. We need to stay under US$500, but under $300 would be ideal. ***Annie Muske-Dukes annie.muske-dukes-driggs@lonza.com Fri Nov 17***

I've had good results showing young people fossils, shells or dinosaur bone fragments. Kids love those! Also, teeth and hair can be interesting. Hair is especially captivating because they can donate it from their head and it becomes personal. It also contains S which is a surprise to most people. Zack Gainsforth zackg@berkeley.edu Sat Nov 18

It is always fun to present science to young minds! I would suggest table salt. Nice crystal structure for morphology and an easy spectrum. I've also done a comparison with table salt and natural salts (Sea or Himalayan) which can show differences in both morphology and spectra. Gene Rodek erodek@2spi.com Mon Nov 20

I once used a nice paper towel with Christmas decorations, gold, silver and black patterns on the blue towel. It is particularly stiff and looks very close to a real woven towel. So I kept one for SEM practicals with students. You should be able to find something similar. I didn't try it with young children, but I imagine it could do the job. They can have the towel in their hands, and see in Se/BSe a synthetic/cellulose fiber network, in BSe a compositional contrast image, and by EDS in spectrum mode showing that the "gold" is brass, the "silver" is aluminum, and the black is carb. All that shines is not gold. Jacques Faerber jacques.faerber@ipcms.unistra.fr Mon Nov 20

Teeth, rocks (granite). Kleo Pullin kleopullin@email.arizona.edu Mon Nov 20

I usually show coins and matches (new and burnt) for similar purpose. Coins for oxidation (Copper coin) and metal allergies (Ni alloy). Matches produce nice elemental maps of Cl, K, Na. Elio sem.nattabg@gmail.com Mon Nov 20

I usually deal with kids in the 12 - 18 YO range for outreach. In the past, we offered a week-long half-day experience in the lab. In my experience, it is critical to find samples that kids can relate to. Insects can be a good for imaging. My favorites are honey bees, house flies and fruit flies. Kids like the structure of the eye. Epsom salt has a nice structure and is useful for EDX; however, it can take a long time to get vacuum since it is usually hydrated. Best to only use a few crystals or pre-pump. Geological samples. I prefer a thin section to reduce pump time, but bulk sections work too. If you need a thin section, I suspect Spectrum Petrographics could help. I have no financial connection to them, but I know several of our geology Professors have Spectrum do their mounts. I had one student bring hair and feathers from the farm she lives on. There is a significant difference between animals. House hold dust can be a good sample. Just pick a spot like a window sill and don't clean it for a week or two. Collect the sample with C tape on a stub. I have seen pollen, insect parts and silicate minerals. Greg Baty gbaty@pdx.edu Mon Nov 20

I did a CSI type demo using light bulb filaments. The scenario was that there was a traffic accident in the dark and each driver claimed the others headlights were not on. The headlamps in both cars were broken. The 2nd driver claims that the 1st driver turned on his headlights after the bulbs were broken in order to cover his fault. The officer collected the light bulb filaments from the cars, and you are comparing them in the SEM. (You can spin out the story as much as you want!) I took 2 small incandescent bulbs (15 watt if I remember), broke the glass on one and then applied power so that it burned out. I took the 2nd and broke the glass while it was running. I then mounted the 2 filaments on an SEM sticky tab and put them in the scope. Note: be careful about breaking the glass. Do it safely! I wore safety glasses and leather gloves and used a pair of channel pliers to hold the bulb inside a mostly closed cardboard box to contain the glass fragments. On examination, the student will find small spherical globs on one filament and none on the other. Doing EDX shows that the spherical globs are Si and O (i.e. glass that melted). The inference is that the filament was hot when the glass was broken, hence the light bulb was on. The other bulb is just oxidized and doesn't show any melted glass. Hence it was not running when the glass broke and was burned out afterwards. They seemed to enjoy the "real life" problem solving. Henk Colijn colijn.1@osu.edu Tue Nov 21

Thank you so much for this question. I am always in need of good ideas. We usually have a Solve the Mystery in the Family Affair, which is changing its name this year to Microscopic Explorations, for the delegates' families and friends at the M&M conference. The pattern has developed whereby in a session on the Wednesday afternoon, it typically starts with an introduction and then a lot of hands on activities using loupes and light microscopes adapted from the GEMS Microscopical Explorations activities. At least three of the stations have a "solve the mystery." At the next meeting in Baltimore, we are developing one step further, and we hope to have Fold scopes and micro meteorites. Then the participants go to the vendor hall where we usually have access to four SEMs to "Solve the Mystery". I have tried to correlate the mystery with the place and change it each year. For instance, the CSI Albuquerque story was: The Governor of New Mexico is coming to town. He is a baseball fan, and his favorite team is the Albuquerque Isotopes. He was going to have his photograph taken with the cup the Isotopes just won! But disaster struck! Someone stole the cup! It is very important we get the cup back. We have to find out who did it. The thief left several clues at the scene of the crime:

- Some pollen
- Some hair
- Some sand
- Some fibers

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He/she wasn't a very good thief! There were the six suspects. We decided we could not use real people as suspects so we chose to go with Harry Potter villains.

- Delores Umbridge - When she was interviewed Delores Umbridge was wearing a red flower. She has brown straight hair; She had sandy shoes because she played golf and her golf ball went into a sandy bunker; She was dressed in pink clothes.
- Bellatrix Lestrange - When she was interviewed: Bellatrix Lestrange was wearing a pink flower; she has brown straight hair; she had sandy shoes because she was in the garden; she was wearing blue clothes.
- Draco Malfoy - When he was interviewed Draco Malfoy was wearing a lily flower; he has blond straight hair; he had sandy shoes because he took a short cut through the sand box in the garden; he was wearing grey clothes.
- Voldemort - When he was interviewed: Voldemort was wearing a red flower; no hair; he had sandy shoes because he went hiking along a trail in California; he was wearing grey clothes.
- Lucius Malfoy - When he was interviewed: Lucius Malfoy was wearing a pink flower; he has blond straight hair; he had sandy shoes because he went hiking along a trail in California; he was wearing black clothes.
- Severus Snape - When he was interviewed: Severus Snape was wearing a lily flower; he has straight grey hair; he had sandy shoes because he played golf and his golf ball went into a sandy bunker; he was wearing black clothes

Mostly the clues fit whatever materials we had, such as what three flowers with lots of pollen were available to us that day. Two suspects for each clue. We didn't want one clue to give the suspect away. It had to be two clues. Each group took on one type of material. On the stub there were three possibles and the actual to compare. So we had a flipchart at headquarters (the Outreach Booth) with the suspects down one side .and clues along the top. As each of the four groups got their answer they put a tick in the right box. It worked just fine.

In Portland, we had access to an X-ray analysis SEM. The story was: Someone came to the desk in the Museum and handed an envelope over to the cashier. Written on the envelope was "Put all the cash in the envelope and don't say anything. I've got a gun!" She complied but followed him to see where he went. He took a bicycle and went through a construction site (sand), scraped the bike along a wall (metal for X-ray analysis), over a flower bed (pollen) which had thorny rose bushes (fibers). By that evening the police had four suspects each with sandy shoes and bike wheels, different materials making up the bikes etc.

In Nashville: Someone stole a very valuable Elvis guitar and put it up for sale clandestinely. One of the potential buyers wanted it authenticated by identifying the strings by X-ray analysis. I went to our local music store and asked if they had any strings from 1960s. They gave me one and I bought a new one. The composition is very different and easy to identify by X-ray analysis.

Now I need a story for Baltimore. I've never been to Baltimore. Is there something special we can use in a story? Elaine Humphrey ech@uvic.ca Thu Nov 30

Edgar Allan Poe theme? The Murders in the Rue Morgue? Probably not "the Pit and the Pendulum" or "The Cask of Amontillado" Not sure what you could do with crab cakes! I believe that McCormick's has a large spice facility near the harbor front. We could smell it the last time we were there. Henk Colijn colijn.1@osu.edu Fri Dec 1

Michael Phelps, the American sports champion, was born in Baltimore. You can make some plans related to his medals! A number of metallic medals with surface patterns can be designed, cut, and painted in yellow or silver or bronze colors, similar to those awarded in sports competitions. The question can be "Which medal belongs to Michael Phelps (if any) and which one is fake?" Details can be designed in a way that participants need to do microscopy work and chemical analysis to find out the answer! Nima Nikpoor Badr 14nnb1@queensu.ca Fri Dec 1

Unfortunately the McCormick warehouses moved up to the north side of the city many years ago. I miss the smells that were there by the Inner Harbor. There is also a railroad theme in that the first railroad station (B&O) is situated just west of Baltimore. There could be steel, coal, and slag involved. There is the Johns Hopkins Hospital with a plethora of medical samples (blood?). The Chesapeake Bay has lots of biota. There is the infamous Chromium smelting talus that the Inner Harbor is partially built on (maybe you don't want to have samples of Hexavalent Chromium around). Also, the Babe Ruth Museum for a baseball theme (including the Orioles). The Poe theme can have both raven's feather and Ravens leather. To add a twist to that John Astin (Gomez Addams from the Addam Family) was born here and teaches at Johns Hopkins. Then there is history of Frederick Douglas and the Underground Railroad. Fort McHenry where the Star-Spangled Banner was penned by Francis Scott Key and the flag sewn by Mary Pickersgill in 1813 that resides in the Smithsonian. There are cloth fibers (red and blue wool and white cotton) and gun powder themes here. Enoch Pratt Free Library — one of the oldest libraries in the US, Peabody Library focused on music (paper, vinyl, tape). There's much more but I have to do some work today. Ken Livi klivi@jhu.edu Fri Dec 1

Baltimore is a great city, some faults notwithstanding. It has a wealth of old buildings and structures that have built up many layers of paint. I collected a nice thick paint sample (many layers) from a lamp post near Fell's Point to compare with some samples I collected on our campus. This was used in the SEM/EDS section of a graduate instrumentation class. I called it " The Doorway, The Downspout and the Lamp Post". It was a great introduction to examining various colors and constituents in paint layers, as well as to restoration processes. Lead paints could be dated as pre-the late 1950s. Wallace Ambrose wallace_ambrose@unc.edu Mon Dec 4

EDS:
high intensity low energy peak

Our EDS is showing a high intensify peak at about 0.2 keV even with beam-on vacuum. We're not getting C signal anymore, and the energy resolution is getting worse. There has been an energy shift also. The issue remains after run an automatic calibration. Does anybody know what more could be done? Or is it a sign that our detector is almost dead? ***Erico Freitas freitas.erico@gmail.com Sun Nov 19***

This sounds to me as there is an issue with an additional light in the chamber. Make sure that all light sources—especially chamber scope—are turned off. A little bit tricky might be a touch alarm: on some SEM (do we talk about SEM?) the stage alarm triggers some safety light barrier to prevent another collision. To turn these lights off, you have to initialize the stage. If you can exclude additional light in the chamber, there is maybe an issue with the detector: it can be the cooling system or some issue with the preamp of the SDD/SiLi. I hope that helps. In general, EDS detectors are very sensible to all kind of light, not only the X-ray you want to see. Ferenc Molnar erenc.l.molnar@googlemail.com Mon Nov 20

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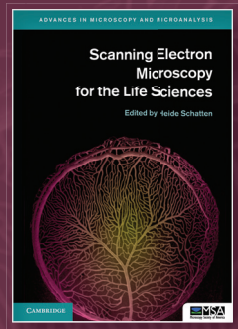

Recent developments in scanning electron microscopy (SEM) have resulted in a wealth of new applications for cell and molecular biology, as well as related biological disciplines. It is now possible to analyze macromolecular complexes within their three-dimensional cellular microenvironment in near native states at high resolution, and to identify specific molecu les and their structural and molecular interactions. New approaches include cryo-SEM applications and environmental SEM (ESEM), staining techniques and processing applications combining embedding and resin-extraction for imaging with high resolution SEM, and advances in immuno-labeling. With chapters written by experts, this guide gives an overview of SEM and sample processing for SEM, and highlights several advances in cell and molecular biology that greatly benefited from using conventional, cryo, immuno, and high-resolution SEM.

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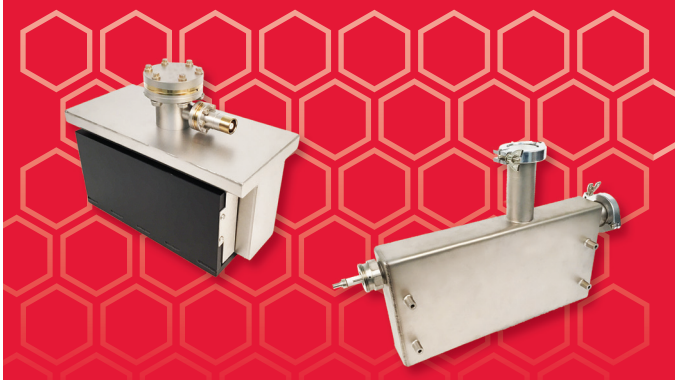

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We got our EDAX system fixed. At the end, we needed to replace the data processing board. Fortunately, we had one available in out lab from another EDAX system. Thanks for the suggestions anyway Erico Freitas freitas.erico@gmail.com Sun Dec 10

Image Anaysis:

pycroscopy

We would like to bring to your attention the GitHub repository for the community-wide development of codes for image analytics (cleaning, feature extraction, analysis of atomic positions), hyperspectral unmixing of high-dimensional data (STEM-EELS, ptychography, SPM spectroscopies), and supporting universal file format.

The codes are realized via Jupyter notebooks that are straightforward to use. The site is:

https:pycroscopy.github.iopycroscopyabout.html. Additional description is provided below. Join the open-code movement!

Pycroscopy is a free and community-driven python http:www.python.org package for scientific analysis of imaging modalities such as scanning transmission electron microscopy, scanning probe microscopy, scanning tunneling spectroscopy, and X-ray diffraction microscopy.

Pycroscopy uses a data-centric model wherein the raw data collected from the microscope, results from analysis and processing routines are all written to standardized hierarchical data format (HDF5) files for traceability, reproducibility, and provenance.

Pycroscopy uses an instrument-independent universal data format that facilitates the generalized representation of data from any instrument

The instrument-independent data format facilitates the development of a single and generalized version of analysis code to be reused for data from a variety of sources.

The universal data format and generalized codebase enables Pycroscopy to serve as the hub for community-driven imaging and microscopy research.

Motivation:

1. Growing data sizes
- Cannot use desktop computers for analysis
- Need: High performance computing, storage resources and compatible, scalable file structures
2. Increasing data complexity
- Sophisticated imaging and spectroscopy modes resulting in 5,6,7…dimensional data
- Need: Robust software and generalized data formatting
3. Multiple file formats
- Different formats from each instrument. Proprietary in most cases
- Incompatible for correlation
- Need: Open, instrument independent data format
4. Disjoint communities
- Similar analysis routines were written by each community (SPM, STEM, TOF SIMs, XRD…) independently!
- Need: Centralized repository, instrument agnostic analysis routines that bring communities together
5. Expensive analysis software
- Software supplied with instruments often insufficient incapable of custom analysis routines
- Commercial software (e.g., Matlab, Origin) are often prohibitively expensive.

Need: Free, powerful, open source, user-friendly software

Available tools in pycroscopy:

Analysis:

- Finding and refining atomic positions in atomically resolved images
- Reconstruction of current in ultra-fast current-voltage spectroscopy via Bayesian inference
- Fitting peaks to models in hyperspectral data o Fitting piezoresponse hysteresis loops to models Data processing:
- Statistical de-noising of images
- Utilities to simplify clustering and decomposition of data
- Feature extraction from images
- Image transformations
- FFT signal filtering File and data handling:
- Utilities for advanced and file-safe writing
- Ability to read, write, manipulate multi-dimensional hyperspectral datasets (1D – 8D and beyond)Translating from proprietary data formats:
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- Tools for interactive visualization of complex multi-dimensional datasets
- Utilities for generating publication-ready plots Journal papers published using pycroscopy:
- "Big Data Analytics for Scanning Transmission Electron Microscopy Ptychography," https:www.nature.comarticlessrep26348, Jesse et al., *Scientific Reports* (2015)
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- "Feature extraction via similarity search: application to atom finding and de-noising in electron and scanning probe microscopy imaging," Somnath et al.; under review at *Advanced Structural and Chemical Imaging* (2017)

Sergei Kalinin sergei2@ornl.gov Thu Dec 21