



NetNotes

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Selected postings from the Microscopy Listserver from September 1, 2018 to October 31, 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.

Specimen Preparation:

thin film

Hoping to get some advice on sample prep for using SEM to measure CVD film thickness via cross-section. Film is sputtered Aluminum with nominal 1-micron thickness on a fluoropolymer substrate using approximately 20-40,000× magnification. The aluminum film is too brittle for a razor cut and flakes off or doesn't present a clean edge for measurement. We have tried with some success encapsulating with epoxy support layer before cutting. Also using liquid nitrogen for freeze fracture. Any suggestions how to prepare and mount sample would be much appreciated! Mike Toalson mike.toalson@elementpi.com Fri Oct 5

Typically for SEM, it is not the section we go for, but the block itself. The ultramicrotome would be used on the embedded block to “polish the surface”. This means taking as small as 60nm slices, gently on a small surface, say 1/2 mm, using a diamond knife and sectioning out onto a water boat on the knife. Possible if the aluminum is very, very thin. If it will separate from the block at all with a razor, then using an old used diamond is worth a try first. Usually, the trim by hand step with the feel of it under my fingers is how I can tell. The flaking may be thickness dependent, does it “eat” the razor blade? If it eats the blade, it will harm the diamond. If that does not work, someone more experienced with grinding techniques may have some advice. This can be put into a holder that grabs the block, or some may epoxy to a stub. If any gluing is done, it needs to pump down in vacuum for 2-3 days before putting it to any SEM. Lou Ann Miller turtlemam@comcast.net Fri Oct 5

Two different ion beam polishing techniques should work for a sample like this, assuming you have access to such equipment. The first and easiest thing to do would be to cut a cross-section view using a dual beam (FIB). We've done samples like this before, and they turn out well, especially if you're only interested in the Al layer thickness. Another way to prepare a decent cross-section is to use a broad beam ion polisher, though you may need to use a cryo-stage to keep the fluoropolymer substrate from charring and causing the Al layer to delaminate. Both the FIB and broad beam polisher would avoid any possibility of smearing and delamination caused by microtomy and conventional polishing. If you don't have access to such equipment, then I'm sure you can locate a lab nearby that does. Christopher Winkler microwink@gmail.com Fri Oct 5

Microtomy:

difficulty obtaining Tokuyasu ultrathin sections of plant material

I am having difficulty obtaining ultrathin sections of leaves and flowers with the purpose of performing immunohistochemistry. My samples were fixed in 4% paraformaldehyde in TBS-T, infiltrated in an ascending grade of sucrose up to 2.3 M, then embedded on a sectioning pin surrounded by 2.3 M sucrose by dipping into liquid nitrogen. I am sectioning on an ultramicrotome equipped with a cryobox. I'm finding that sectioning at 1 μm thickness at -65°C has minimized the amount of sucrose flaking, and is providing me with the best sections for all conditions I have tried (from -60° to -90° at 5°C increments; 1.5 μm to 0.3 μm with 0.15 μm increments). However, I am still experiencing

several issues: The issues I'm having at 1 μm sections; -65°C: - sections are wrinkling and curling off the knife edge - tissue sections do not adhere to slides after transferring out of cryo-box - tissue is largely fragmented upon imaging with Toluidine Blue staining and transmitted light, where epidermis, mesophyll, and epidermal outgrowths are floating all over the slide My major concerns relate to the sections not adhering to my slides after transfer, which will certainly wash off my slides during the immunolabeling work up, and the fragmentation of my tissue, which removes the spatial context needed for immunolabeling. I have tried several different types of slides including uncoated, pre-cleaned slides from Fisher, uncoated and non-cleaned slides from VWR, as well as PTFE coated slides from EMS, with no apparent difference in section adherence. If you have any tips, tricks or recommendations, please let me know, as I would be very grateful for any help on this technically challenging and patience-testing method. Sam Livingston samuel.livingston@botany.ubc.ca Wed Oct 17

You say you've tried several adhesives but have you tried chrom-alum, which works well in many cases? It's relatively easy to make. There are many different recipes, e.g., <http://stainsfile.info/StainsFile/prepare/adhesives/chromegelatin.htm>. We resorted to this after poly-lysine-, agar-, and silane-coated slides—either purchased or prepared ourselves—proved unreliable with a particularly difficult tissue. I'm guessing the fragmentation of the tissue is because the cell walls are not sufficiently well-fixed/penetrated by the various solutions. Does happen with frozen plant tissues. For example, rapid-frozen/freeze-substituted Arabidopsis roots have fantastically-preserved cell contents, but the cells tend to separate as you section the resin blocks. Tobias Baskin comments on this in one of his earlier papers, I think. Rosemary White rosemary.white@csiro.au Sun Oct 21

STEM Image Libraries:

machine learning

My colleagues and I would like to bring to your attention the following development aimed at faster adoption of machine learning methods across electron microscopy community and enable ML/AI application in atomically resolved imaging. Modern machine learning is impossible without large volumes of labeled data. To enable faster adoption of machine learning methods in STEM, ORNL is working with Citrine Informatics to share an open library of images for the specific case of Si - vacancy complexes in graphene monolayers with plans to increase the amount of data in the library over time. The initial library is available at (<https://doi.org/10.25920/0xv3-8459>) A paper that discusses the collection, analysis, and dissemination of this data is available at <https://arxiv.org/abs/1809.04256> The notebooks for the analysis workflow will be available at PyCrosopy (on GitHub) shortly and can be requested directly from Maxim Ziatdinov (ziatdinovma@ornl.gov <<mailto:ziatdinovmax@gmail.com>>) We hope that this initiative becomes adopted by the community. Please contact Malcolm Davidson (mdavidson@citrine.io), the leader of Citrine's Open Data Initiative, for any questions about Citrine's open data repository or ML platform. We're putting the finishing touches on some tooling and will share a step-by-step guide to our workflow in the coming months if you're interested in sharing your STEM data openly on Citrine's platform.

Looking forward to the new opportunities and collaborations.
Sergei V. Kalinin sergei2@ornl.gov **Wed Sep 26**

TEM:

combined cryo and standard TEM usage

I would like to know the feasibility of having a TEM operate on a weekly basis both as a standard electron microscope and a cryo-electron microscope. Is this practical? **Michael Delannoy** mdelann1@jhmi.edu **Wed Oct 3**

In general, it is very possible to use a TEM in cryo or standard mode intermixed, if the “cryo” use involves a “cryo holder” and not a fully chilled cryo-stage. A JEOL 3200 cryo, would be a full cryo stage and not switched easily between projects. Using a cryo-holder such as the one Gatan sells, with a liquid nitrogen reservoir and cryo shields, so that the sample is protected during transfer to the column, is quite fine. UC Berkeley has been doing cryo-TEM and non-cryo in the same CM series TEM for decades. Most materials people do not fully understand polymer or biological “cryo” work, so there will be some learning involved. I’ve been lucky to have participated on the both sides of the TEM world. **Roseann Csencsits** rcsencsits@belcan.com **Wed Oct 3**

There is no general answer, honestly. It depends on the facility, it very much depends on the training of the various users you have—you have to get trained and to supervise, on the equipment, and it depends on the TEM that you have. Yes, we do it, not on weekly basis, but with daily varying schedules, and this works. Fine. Little problems, only, which are due to the limited training of the users, rather than the TEM. If you know exactly what your TEM can do and to tolerate, and what your users know, then YES, it is possible. Then, I do not see any reason why a weekly schedule would not work. **Reinhard Rachel** reinhard.rachel@biologie.uni-regensburg.de **Wed Oct 3**

FIB:

charging

I need some advice on sample prep for FIB. I have packaged parts, with gold bond wires, that goes into a FIB to do circuit edits. Due to the samples being in a package, the samples would charge. If I coat the sample, it would help the charging issue, but then I need to remove the coat after I’m done with the FIB edits. Does anyone have any suggestions on what to coat it with and how to remove the coat afterward? **Gordon weutro** gweutro@yahoo.com **Sun Oct 14**

Use your micro manipulator to earth the local region. But be careful not to blow any sensitive circuits. **Richard contact** richard.contact@integrityscientific.com **Sun Oct 14**

If you set proper GAE processes and use primary ion beam currents appropriate for edits, then there wouldn’t be any problems with surface charging or ESD damage. If there is no time/bandwidth/money/expertise to accomplish the above, then there are two surface coating options suitable for brute-force elimination of surface charging during FIB circuit edit: (a) carbon, either evaporated or PIPS, or conductive polymers, either spin-coated or ultrasonic-nozzle dispensed. Carbon deposited with thickness of about 20 nm provides sufficient charge dissipation for CE-appropriate beam currents while having little to no influence on regular operation of most ICs so that it can be left on in most cases. If removal is required, then O₂ plasma cleaner (<20W), or ozone asher, or UV cleaner would work (from fastest to slowest) depending on sensitivity of the device. Conductive polymers simply washed away after the edit site has been capped and sealed. Google for “Free of charge FIB circuit edit of ICs” and “New FIB tricks with old conductive polymers” I am assuming that all pins of device under edit already have solid connection to the stage of your CE FIB. **Valery Ray** vray@partbeamsystem.com **Mon Oct 15**

Sorry for the shameless self-promotion, but we published a paper a little while back about using conductive polymers (used for e-beam lithography) in the FIB. We were using it on flat wafer samples, but you might be able to adapt the technique we described for your needs. Most of the polymers are water soluble and can be removed with a rinse afterward. Please let me know if you need a PDF copy of the article. <https://www.cambridge.org/core/journals/microscopy-and-microanalysis/article/teaching-an-old-material-new-tricks-easy-and-inexpensive-focused-ion-beam-fib-sample-protection-using-conductive-polymers/7E9EAE6546D968E9537A56CFDB70D6AA>. **Josh Taillon** joshua.taillon@nist.gov **Mon Oct 15**

FE-SEM:

gun lifetime

I’ve sort of inherited oversight of a shared EM facility that includes a 2-year old FE-SEM. Unfortunately, the system does not get much use, so I’d like to be able to shut down the gun when there are no projects anticipated - it just costs too much for us to be able to replace the tip every 12-16 months. The original one was swapped out at just under 24 months of age with less than 200 hours of actual use. It was still working & seemed to be stable but was well over the suggested run time. I’ve been told by various people that there are facilities that routinely turn their FE-gun off when not in use, but they did not know if anyone had standardized a re-start procedure. So, my question is: would anyone who does this mind sharing how they manage this? I will see replies to the ListServer; I’m having some issues with Outlook and could not post directly. Thanks for your help! **Tamara Howard** thoward@unm.edu **Sat Oct 27**

Inheriting other microscopes can be a gift or a burden - who knows. But in this case, a 2-yr old FE-SEM is usually a wonderful gift. The original gun was swapped out at just under 24 months of age? Strange. If it is an FE-gun, it can live ‘forever’, if not heavily used. In our FE-SEM (from 1999), we had one for far more than five years with little/occasional use (before I got it). What a shame for such an instrument. The gun was fine. But, after 200 hrs, an FE-gun is not “well over the suggested run time.” No, never. A tungsten filament may be. But even then: if it still gives nice images, it was treated well, and it can stay there. You write: “it was still working & seemed stable” - then, why changing? Although, exchanging a W filament is not too tricky, and from the budget, not too heavy. Basically, for all types of filaments, it is the usage and the vacuum, which limits the lifetime. The level of vacuum which is required, is very different, for the different guns (W, LaB₆, FE). If you have an FE-SEM, then you may ask the manufacturer for an appropriate treatment during extended periods of “no use.” And they also should have a procedure for a proper re-start. Our FE-SEM and also our FE-TEM, both have one. For extended periods, I use the shut-down / re-start of the manufacturer). Works fine. - In our W-SEM and W-TEM, filaments are used until burnt. **Reinhard Rachel** reinhard.rachel@biologie.uni-regensburg.de **Sun Oct 28**

There are two types of field emission guns; both have significantly different lifetimes. One is a thermal, or Schottky field emission source, and the other is a cold field emission source. I have seen cold field emission sources last for ten years under the right conditions. Schottky field emission sources do have a finite lifetime, as the ZrO₂ reservoir is depleted, but this depends on how long it is on. Typically a thermal field emission source is ramped up over a 45 minute period, then stays in an emission state for prolonged periods of time, as with the Zeiss/Leo field emission systems. Which microscope do you have? **Justin A. Kraft** kraftpiano@gmail.com **Sun Oct 28**

A rep in your area for the original equipment manufacturer should be willing to help you with this even if the instrument is not under warranty and rather old. Probably someone on the list will have an answer but connect with the OEM rep, too. **Kleo Pullin** kleopullin@email.arizona.edu **Sun Oct 28**

As Reinhard stated, an FE gun should last much longer. However, we had one of those rare instruments in which our gun went out after one year. It is not common, but it happens. **R. Steven Pappas** rlp6@cdc.gov **Mon Oct 29**

I agree with Reinhard. The specifics depend on the type of FEG that you have. In general FEG microscopes require a high vacuum. The SEM and FIB in my lab had Schottky Field Emission guns. Like all FEGs, they required very high vacuums to work. The tip was always on. If your microscope is like this, you may use the microscope for only a few hours per month, but the tip is running all the time. A complete shutdown of our FEG SEM and FIB for more than a day or so required a bake-out procedure to bring it back up. This could take a day or two to get the microscope back to working order. Like Reinhard's microscope, our microscopes had a "standby" mode where the gun was maintained under vacuum with the ion getters on and could be restarted easily and the tip turned back on in a few hours. I never tried using this mode for longer than a week or two. We typically needed to replace the tip every couple of years, but our microscopes were used daily. I recommend you contact the manufacturer and ask for their best practices for your microscope and use case. **John Minter** jrminter@gmail.com **Mon Oct 29**

EDS:

new video tutorials for software

Over the years, many people have asked about video tutorials for NIST DTSA-II. NIST DTSA-II provides software for quantification and simulation of electron-generated energy dispersive x-ray spectra. It is used all over the world in both the laboratory and class-room environments. Recently, in response to the requests, we've created half-a-dozen videos and published them on YouTube. The YouTube channel is here: <https://www.youtube.com/channel/UCt4nKyhfFQ8xecHyuTnCvIA> or search for DTSA-II on YouTube. Currently, the videos cover an introduction to DTSA-II, quantification, simulation and a couple of other topics. Subscribe to the channel, and you will be notified when new videos are published. Hopefully, you find the videos helpful. **Nicholas Ritchie** nicholas.ritchie@nist.gov **Fri Oct 5**

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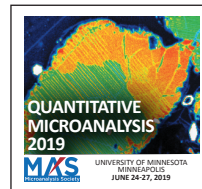
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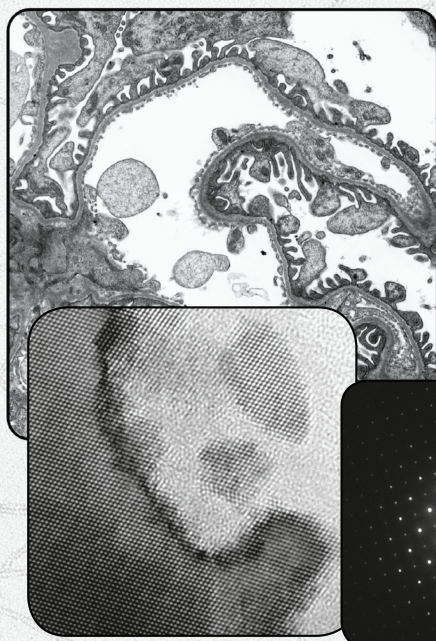
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