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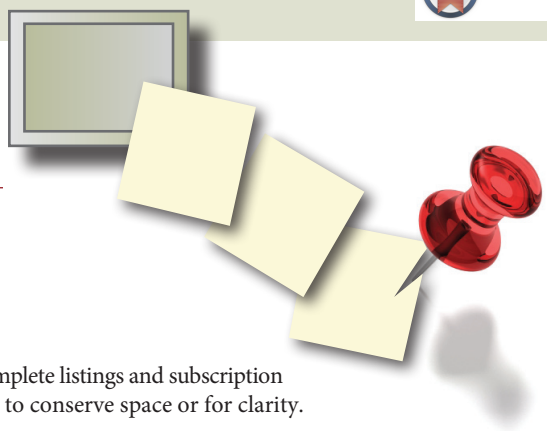

NetNotes

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Selected postings from the Microscopy Listserver from January 1, 2017 to February 28, 2017. 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:
LR White for immunogold labeling

How long, in terms of days, in advance can I cut ultrathin sections (usually 70-90 nm) in preparation for immunogold applications? The resin was medium hardness with catalyst, and I polymerized it in a 55°C oven overnight. I was wondering if the surface of the ultrathin section would be altered over a few days after cutting and antibodies would be somewhat hindered from access to epitopes. ***Vickie Kimler vakimler@oakland.edu Wed Jan 11***

I do not think there is such a thing as a maximum time you have to wait before which LR white sections would be suited for immuno. Perhaps you are wondering if there is a minimum time before sections are suited? As far as our experience goes LR White and Lowicryl sections seem to keep pretty much indefinitely. Perhaps it would be wise if they were kept in a desiccator. I do not think there is a minimum time one should consider having to wait. If things change post-sectioning I would expect epitopes to change (oxidize?) and that hardly ever is beneficial. And how would 'buffers etc.' relate to that, I do not understand? Are you getting results, or not getting results, which make you wonder whether section age plays a role? Jan Leunissen leunissen@aurion.nl Mon Jan 16

I'm sure it all depends on the epitope. Here's a story: Someone once had LR White sections that were more than two years old, from another institution. They went ahead and applied their antibody, secondary antibody and gold, and seemed to have every expectation that it would work (whether based on experience or blind faith, I do not know). However, they needed images immediately for a grant proposal, or at least they needed to know if a particular protein was on a particular organ in the squid. My TEM was down. In a panic, we stuck them in the SEM we here we could see the gold particles on the surface of the section, and we could also see outlines of the ultrastructure because parts of the tissue had bulged out of the resin enough to give some relief. In fact, it looked really cool. And they were able to state that the protein was in that organ. If you've ever sectioned a block and then let it sit for some time (hours, years?), and then sectioned again you've probably noticed that some tissues expand out further than others over time. Anyway, in this case I'm sure their antibody would have worked right away on freshly-cut sections as well as on sections that had been sitting on grids for more than two years. The resin may flow somewhat over time. Your mileage may vary. Perform a bunch of timing experiments and report back! Tina Carvalho tina@pbrc.hawaii.edu Mon Jan 16

Specimen Preparation:
negative staining

I am a relatively inexperienced TEM tech and am having problems with my phosphotungstic acid (PTA). Each time I make fresh PTA, I end up with an image full of stain blobs. Obviously this is useless. I have tried purchasing fresh PTA, but that didn't make any difference. I have also used BSA, which helps some, but not a lot. Any ideas are welcome. ***Jami Carroll jcarroll@murraystate.edu Wed Jan 25***

I do hope that you have consulted Google with "PTA negative staining" to try to find some troubleshooting advice from experts. What I am about to offer is all true and necessary for me. Thus, necessary for everyone else—if I don't think about your problem. I can state with absolutely strictly personal experience, that one—Me!—throws time away all the time if the water one starts with has not been deionized, glass distilled, filtered with the 22 µm 57 mm Millipore filter, and, if to be used in molecular biology, laced with RNAse Inhibitor [https://en.wikipedia.org/wiki/Ribonuclease_inhibitor]. Once this 'Monkish' procedure has been completed with both song and dance, I can be assured of a good experiment. If the 'blobs' are larger than 0.22 µm, then I recommend that you run your PTA through a 0.22 µm, 12 mm Millipore filter using a small Swinney holder with a 1 ml disposable syringe. You could also try a dose of high frequency sonic jewelry cleaner borrowed from your Mom or your 'gal.' I have given you both mystic and sound recommendations to attack your problem. Frederick C. Monson fmonson@wcupa.edu Wed Jan 25

Is your PTA (phosphotungstic acid, 1% aqueous) adjusted for pH? After it solubilizes I use 1N NaOH to get a pH of 7.2, but it only takes a few drops (4-5). I have never had issues with fresh PTA, only with old batches would I see blotches of staining material. I also filter it (0.22 µm Millipore) as I stain. It could also be something in your buffer, try running that alone. Also try 3 quick deionized-H_2O rinses before the stain. Remember to do a complete blot and get the stain between your forceps with filter paper triangles. Check this; they talk about staining blobs: http://science.utep.edu/chemistry/cryo-em/files/PDFs/Negative_Staining.pdf. Michael Delannoy delannoy@jhmi.edu Wed Jan 25

Specimen Preparation:
carbon grains

Sometimes it is very difficult to tell between what is sample and what is carbon grain with either grids coated with compressed carbon or the carbon on Formvar grids. Can anyone shed light on this when one does protein analysis? We are working at 100 kV and 140K x magnification. ***Vickie Kimler vakimler@oakland.edu Sat Feb 25***

Carbon films come in different grades; one of which is 'ultra-thin' and has areas down to 3 nm thick according to the publicity in catalogs. I've never done protein analysis, but you need a supporting substrate and one that is amorphous, so try and look in the holes since they may be covered with thin carbon, the thinner the better. I have bought some silicon nitride supports that are thin and they can be plasma cleaned between uses, but they are a bit pricey. Rob Keyse rok210@lehigh.edu Mon Feb 27

Instrumentation:
wall-mounted fume extractor

I am considering purchasing a wall-mounted fume extractor for my microwave tissue processor, which I will use for EM and paraffin work to save space in my fume hood. I have found some filters that have been rated for use with paraformaldehyde and glutaraldehyde, but I am

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waiting to hear from the manufacturer regarding their use for OsO4, acetone, resins, and propylene oxide. I'd like to assume that the filters ("special carbon-treated") effectively filter out all of these, but I need to be as certain as possible. I will have undergraduates preparing samples and hope to use the microwave in my TEM and SEM courses, so I am very concerned about student safety. Do any of you have any experience using these systems? Are they safe? Should I stop being so worried and just buy it? ***Blanca Carbajal Gonzalez bicarbaj@mtholyoke.edu Tue Jan 17***

Why don't you run HVAC flex pipe from your wall-mounted fume extractor and plug it into the exhaust system? Failing that, modify one frame in the nearest window and exhaust filtered air outside. Decent carpenter or HVAC tech should be able to make such a setup. Be safe. Valery Ray vray@partbeamsystech.com Tue Jan 17

Instrumentation:
cathodoluminescence detector vacuum problem

We bought a GATAN cathodoluminescence detector some years ago, and had it installed on our Hitachi 2700S SEM. It breaks vacuum sometimes when it is entered into the column. We need cathodoluminescence mode for several projects. Does anybody have the same problem? If so, what did you do and did you solve it? ***Carol Heckman heckman@bgsu.edu Sat Jan 21***

Since the problem is intermittent and occurs during insertion, chances are you have either a crack in the vacuum bellows, or a worn-out O-ring somewhere in feedthrough. You would probably need to call in either OEM or third-party service, or find someone local who can bring in a leak detector and troubleshoot this on site. Valery Ray vray@partbeamsystech.com Sat Jan 21

Instrumentation:
temperature logger

I would like to ask for your recommendation for a temperature logger. Probably a reasonable sensitivity, accuracy and precision and is able to monitor live using the TEM pc. I am not sure if such a system will interfere with the performance of the TEM. If you encounter such a problem, is it possible to share your experience? I do know if some loggers have WiFi/Bluetooth capability, will this interfere with the TEM performances as well? Currently, we have a temperature logger but it is not live and we have to extract the data out from time to time which is very troublesome. ***Yee Yan Tay rongchigram79@yahoo.com.sg Thu Jan 26***

You may want to talk to Guenter Resch of Nexperion. He might be able to help. His email address: guenter.resch@nexperion.net Jan Leunissen leunissen@aurion.nl Thu Jan 26

I have used Extech RH520A temperature/humidity data logger in our FIB room and am very pleased with it in all respects. Initial setup required some programming, but features and display/download capabilities were excellent: http://www.extech.com/display/?id=14702 Valery Ray vray@partbeamsystech.com Thu Jan 26

I recommend a temperature sensor from Vernier.com. Their line of Go Direct sensors connect to a laptop by USB, are dirt cheap ($39 for the one I have), and come with a very nice free software package (I have never seen it crash!) for recording your measurements. I see now they have options for wireless with iOS devices. John Mardinly john.mardinly@asu.edu Fri Jan 27

I have several units from ITWatchdogs. Never had any problem and they email and text me and the building managers when there is a thermal issue out of range. Interface is a standard web browser. Scott Whittaker whittaks@si.edu Fri Jan 27

http://www.practicaldesign.com/THUM/thum.html I have used two of these for over a decade to monitor temperature and humidity in microscope rooms. USB interface, so PC should be on, and data stored in a database. Fred Monson fmonson@wcupa.edu Mon Feb 6

FE-SEM:
constant beam drift

A quick question that I hope someone can help me with. In our daily routine analyses with our Hitachi SU6600 FE-SEM, we are getting a large amount of beam drift. As in, the image seems to migrate while doing an analysis, leading to blurry images and maps. When the SEM initially turns out, we can correct for this drift with the stigmators, but eventually, the range for that tops out, and we cannot get crisp images. This occurs with well-grounded samples, while doing routine imaging or EDS analyses. We typically analyze geological thin sections, but we coat with carbon and ground with either carbon or silver paint, or copper tape. I originally thought it was due to my grounding, but it used to be fine, and no matter what I do the images still drift. However, if I put things into variable pressure mode it is fine. It has been a while since we've done a "bakeout," so I'm just wondering if it could be something related to tip alignment, or if there are particles in the column. What can I do to check the instrument? I just worry there's something going on with the tip. How can I check that? ***Ivan Barker ibarke2@uwo.ca Tue Feb 21***

Although it is hard to diagnose remotely, chances are that the problem is not with the gun. If drift was related to the electron source, then most likely it would be appearing similarly in both HV and variable pressure modes. There is a good chance that there is some kind of dielectric contamination, particle, or a piece of fiber stuck at the bottom of objective lens. It is getting charged during high-vacuum operation and deflects/astigmates the beam. When you switch to variable pressure mode then ADAPT is inserted, shielding contamination from the beam, thus preventing the drift. You would need to open the specimen chamber and carefully inspect the bottom of the column and opening in the polepiece with mirror and a good flashlight, and try to look inside of the polepiece opening. Also take a look on outside of the insertable variable aperture for any traces of contamination or particles. Make sure that in high-vacuum mode the ADAPT is fully retracted. Check resistance from polepiece to ground with ohmmeter, however strange this sounds. Also check grounding of your stage - there is some possibility that the stage is electrically floating and thus getting charged in high vacuum mode. Valery Ray vray@partbeamsystech.com Wed Feb 22

We had a similar problem with a Hitachi S-2460N many years ago. I don't know how it compares to the 6600. We had let the PM schedule lapse and got similar drifting. The engineer came in and cleaned out the liner tube, as I recall. It had built up a deposit on one side that took on a charge in high vacuum mode. Once it was cleaned, things were back to normal with no drift in high vacuum or low vacuum mode. Warren Straszheim wesaia@iastate.edu Wed Feb 22

EDAX:
format batch conversion

I'm looking if someone could have written a bash script (or else working on Linux system) to convert in batch mode EDAX spectra from the .spc file format to .emsa/msa. The "Export" function of the TEAM software has only the .spc format as output, and the "Send_To_Folder" function is far too time consuming for saving a project part with tenth of spectra, in particular as it doesn't keep the original names of the data. I tried Spectrum Viewer but I didn't bring it to work on Linux (via Wine, but I'm very binary with Wine; it works or doesn't work... I've no time to learn to configure it!). Fortunately, DTSA-II reads the .spc format and can save in emsa, which allows me to work. But DTSA has not the ability to do batch format conversions! ***Jacques Faerber jacques.faerber@ipcms.unistra.fr Wed Feb 1***

The open-source HyperSpy software (www.hyperspy.org) can do what you are looking for. EDAX file reading (both .spd and .spc) has been added in the latest development builds (and should be included

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in the next minor release -- v1.2). If you cannot wait for that, setting up a copy of the development version isn't too difficult if you have some proficiency with Python and git (http://hyperspy.org/hyperspy-doc/current/user_guide/install.html#development-version). NB: Any mention of commercial products or software is for information only; it does not imply recommendation or endorsement by NIST. Josh Taillon joshua.taillon@nist.gov Wed Feb 1

Unless someone has already written and would share the script you need for converting .spc to .emsa, it should be possible to automate sequential opening of files within a folder by DTSA-II, saving data in another format, and repeating the operation until all files are processed with one of GUI scripting tools. I am using WinBatch (US$100 from http://www.windowware.com/, no connection just a happy user) for file processing purposes, although different than your application. There is also AutoIt which is free from www.autoitscript.com, but I did not try it. Not aware of similar GUI scripting tools for Linux, but would be surprised if they didn't exist as well. Valery Ray vray@partbeamsystech.com Wed Feb 1

Jacques Faerber asked about a script for the conversion of EDAX .spc to msa format. DTSA-II has a nice scripting language (jython) that permits this. One can also apply a DTSA calibrated detector instance to the spectrum. I sent Jacques an earlier version of this this morning. I want to make certain that the formatting stays correct, so I created a public gist on GitHub. You can obtain the script at: https://gist.github.com/jrminter/a697f83bec3f37dfb824fb7126542c41 John Minter jrminter@gmail.com Wed Feb 1

FIB STEM EDX:

spurious Al peak

A question for anyone with FIB-STEM-EDX system: Our FEI Helios 460F1 has single-sample Flip Stage (the 'turret' type Flip Stage with 180° rotation) and STEM detector for imaging TEM liftouts in situ. When attempting to acquire EDX of the liftout, mounted on the Flip Stage, using EDAX Octane Plus SDD EDX, the spectrum is overwhelmed by the Si peak from the STEM detector itself. If the STEM detector is retracted, the Si peak is replaced by an Al peak from the stage area situated below the STEM position. Obviously the EDX detector is 'seeing' the X-rays generated by the e-beam that passes through the thin liftout sample. However, this problem did not occur in our older Strata FIB with a SiLi detector. I am presuming it may have something to do with the larger collection angle of the SDD. I have confirmed with EDAX that the detector is installed correctly, the collimator is in position and the system is operating normally. There are no problems doing EDX with the sample in the 'bulk stage' position. My temporary solution is to affix a strip of carbon tape to the 'underside' of the Flip Stage to absorb the transmitted e-beam, eliminating the spurious Al peak, but this is obviously undesirable as that makes STEM imaging impossible. Has anyone else encountered this with a similar system? ***Roger Ristau roger.ristau@uconn.edu Sat Feb 18***

For a quick fix, get Beryllium foil of adequate thickness (0.5 mm is widely available) and cover areas "behind" the retractable STEM detector exposed to e-beam transmitted through the sample. You could get STEM image with extended detector and EDS+SE image when detector is retracted. It is possible that once main Al peak is dealt with, some additional stray signal generated by electrons scattered from/through the sample could become detectable - find out where they come from and shield with Be foil. Be is considered "hazmat," but that is when it is in dust/powder/fume/solution form which can be ingested, inhaled, or absorbed into the body. If you do not drill/sand/machine Be foil but only attach it by conductive vacuum epoxy it should be safe enough to stay there. Lots of Be windows were (and still are) out there installed in SEMs. If Beryllium is too much of a scare, use Aquadag paint of adequate thickness to cover area behind STEM detector. Best would be to manufacture a shield from some kind of metal, coat it with Aquadag, anneal in vacuum oven at 300°, and then mount into SEM chamber. Valery Ray vray@partbeamsystech.com Sat Feb 18

EDS:

detector dead time

We are having issues with our Oxford EDS detector on our F30. With no electron beam on, the dead time stayed at 100% no matter which dispersion rate we choose. Initially we thought the misconnection between Oxford and TEM may be responsible. But the issue persists even after we reboot Microscope PC, Oxford x-stream processor, software, etc. Any inputs are greatly appreciated! ***Xiaoqing He hexi@missouri.edu Fri Feb 3***

It could be that you have different grounding points in use, and an electric potential between them causing a ground current being the issue. You might want to check that your ground lines are correctly wired. The general recommendation is to use the frame of the TEM as the only grounding point for your TEM connected accessories, arranging a grounding bus at the frame and avoiding any ground loops between the accessories. For instance, do not use the house grounding line feeding to the power socket which powers the EDX equipment and EDX computer. The house grounding line stays disconnected from this power socket. This socket's grounding (not the house grounding line) becomes linked to the frame of the TEM, instead. If I remember correctly, then Oxford ships an accordingly manipulated distributor with their equipment for easing such wiring. Don't forget to also connect the chassis of the EDX computer to your grounding point at the frame of the TEM. Consult a certified electrician for assuring electrics security in your setup! Marco Möller mmoller@cicbiomagune.es Thu Feb 9

MT