

NetNotes

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Selected postings from the Microscopy Listserver from May 1, 2018, to June 30, 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: need to re-subscribe!

A new set of legal rules, namely the General Data Protection Regulation or GDPR (<https://www.eugdpr.org>), is propagating around the world concerning internet privacy. These rules are being applied universally to any entity that communicates with groups of people using the Internet. At the moment it technically only applies to individuals in the European Union but will likely will be extended in some form to all countries worldwide in the not-to-distant future. Since information about you (that is, Name, Affiliation, Email address) is being stored in the Microscopy Listserver Database, the GDPR regulations can be interpreted to also apply to the Microscopy Listserver. These rules are detailed, and non-compliance can result in penalties of up to \$25M if not followed. Thus, in order to comply with the GDPR, which took effect on May 25, and also to err on the side of safety (I don't have \$25M, but wish I did), it will be easiest (for me) to have all individuals reconfirm their desire to be a Microscopy Listserver subscriber. So for the first time in 25 years, I must ask every active subscriber to revisit the Microscopy.Com WWW site and renew their subscription. All active subscriptions will be nullified in the existing DBase later today (May 20, 2018). My apologies to those of you who have been a long-term subscriber, as well as those of you who have recently subscribed. Because of the number of users on the Listserver, this is the simplest way to proceed. Below is the URL to the subscription page. Just copy and past the URL into your WWW browser and reenter your contact information. This (re)subscription has to be done as if you are a "new" subscriber. <http://microscopy.com/SbscrbeMicroscopy.html> Your (re)subscription will serve to indicate your consent to continue sending you email from the Microscopy Listserver as required by the GDPR. This email also reconfirms to you that the Microscopy Listserver DataBase information, as has been the policy for the past 25 years, will not be shared with any entity and will only be used to send you correspondence from

and related to the Microscopy Listserver, its subscribers, or its operations. You may, as previously, unsubscribe at any time using the online forms on the Microscopy.com WWW site (<http://microscopy.com>) by providing your active subscription email address. The FAQ page remains available for questions, or you may also send a direct email to me (zaluzec@microscopy.com). Sorry for the hassle and bureaucracy. Nestor Zaluzec zaluzec@microscopy.com Sun May 20

Microtomy: ultramicrotome arm retraction

We are experiencing an issue with our Leica EM UC7 ultramicrotome during the return stroke. The instrument does not seem to be performing the 0.2 mm retraction during the return stroke, causing the previous cut sample to be picked back up by the knife edge. Does anyone know what adjustment needs to be made and where (inside the instrument presumably)? The instrument seemed to be operating fine after the last preventative maintenance (PM) by Leica, but now it is doing the same thing as before the PM. If anyone has drawings, a procedure, or a video that shows how to make the adjustment (or tighten a component), it would be very much appreciated. Clayton Loehn cloehn@lsu.edu Mon Jun 11

This won't be of any help to you, just a commiseration. My Leica UC7 is stuck. It won't reset, arm won't retract, and knife stage won't retract by using the coarse advance knob. I have two red lamps that are winking at me, that's about it. The problem is intermittent and so far unfixable. Debra Townley debrat@bcm.edu Wed Jun 13

LM: plant tissue for science project

I am a high school biology teacher with a student question. My student has asthma, and her mother, a nurse, had a drawerful of expired inhalers that they wanted to put to use for her 9th-grade science project. Using a syringe, they injected plant seedlings with the steroid

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and monitored their growth. The growth rate of the treated plants was obviously much higher than the non-treated plants, but the student wants to extend her project to see if there are any microscopic differences that she could visualize at the tissue level of the plants using a light microscope. Aside from building stomatal peels, I don't have much experience with plant microscopy, especially not when it comes to hormone detection. Does anyone know of something she could look for?

Beth Dixon bdixon@rafos.org Thu May 10

This sounds like fun. You can cut hand sections with a razor blade and look at them in brightfield (or fluorescence if you have that ability). Many plant tissues are auto-fluorescent. If you have access to a drawer with stains, you can try staining. Lots of these dyes stain plant tissues differentially, and this can simply provide more contrast for you (in either brightfield or fluorescence). It doesn't matter really what exactly they stain (and in many cases that won't be well known). I am a little curious about the control your student used? Plants do have steroid-type hormones, but they are not exactly like those of animals. And certainly some steroid hormones don't do anything to plants. I wonder if there are other materials in the inhaler "juice"? I would be happy to correspond with you offline about that if you like. **Tobias Baskin** baskin@bio.umass.edu Thu May 10

Specimen Preparation:

TEM artifact

We are embedding kidney samples that have been stored in paraformaldehyde/glutaraldehyde/phosphate buffer fix for a day to weeks using immersion fixation. I am seeing what looks like classic lead precipitate—dense round spheres over the tissue after staining with uranyl acetate/lead citrate. However, when we look at unstained sections, the artifact is also present everywhere on the tissue, not on the resin area of the section, only the tissue: mitochondria, nuclei, etc. Please comment on what is causing this. **Sue Van Horn** susan.vanhorn@stonybrook.edu Sat May 19

This reminds me of "salt and pepper" precipitates due to your fixation—generally, processing of the tissue. NB: phosphate buffer (PB) and molarity of working solution? Too rapid or less careful dehydration (i.e., for example, dehydration out of buffer washes post-paraformaldehyde or paraformaldehyde/glutaraldehyde or glutaraldehyde with 70% ethanol (for sure 76% ethanol) may cause (sometimes huge) PO₄-precipitation within tissue. Not knowing about your using OsO₄ [if yes: in PB too?] as secondary fixative and afterward. It would be interesting to see your standard processing protocol just to follow all the steps done until examination of the grids. Such precipitates also would also happen after processing as before + uranyl acetate *en bloc* tertiary fixation (= a pre-staining option) without applying rigorous washing tissue specimens before with the maleate buffer method/sequence to get rid of the whole phosphates. Worst case would be (but for sure you can discriminate between microorganisms like bacilli or bacteria from long-storage PO₄ or other "ion" precipitation in specimen in primary fixative solutions) if some detrimental alteration of your kidney specs happened during storage. Naturally, it would be of benefit to see a typical micrograph/digital image of the precipitates (i) after conventional double staining (uranyl acetate-lead citrate) as compared to (ii) unstained ultrathin sections from same specimen. **Wolfgang Muss** wij.muss@aon.at Sat May 19

I ran into this problem several years back. I changed almost every variable that I could: water source, filtration of buffers and fixes, new vendors for chemicals, etc. After an exhaustive search for the culprit, I found (through EDS on a new Hitachi TEM) that osmium was the major offender! Do you use a post-fix in OsO₄? If so, you will find that adding 0.8–1% potassium ferricyanide as a chelating agent may solve your problem. The solution will be bright yellow, like uranyl acetate; it will still act as an oxidizer (tissue will be black at the end of

an hour); you may process as usual. It took me forever to figure it out (it seemed like forever anyway...), and I'm hoping to save you time and aggravation. **Debra Townley** debrat@bcm.edu Fri May 25

I had a similar problem a few years ago. I contacted the listserver for suggestions and got the following from Ann Ellis. She was a wonderful source of help and information. It worked for me so give it a try. Ann Ellis email from archive: *The pepper sounds like the same old thing we have had from time to time over the years with glut and osmium fixation. Traditional buffer washes will not solve the problem. I published a paper way back in Stain Technology (1979) 54:282–85. We ran out of reprints twice since every pathologist and his brother wanted one. I don't have any more, or I would send you one. The salvage method is simple. Cut new sections and pick up on nickel grids. Oxidize the sections with 1–2% (wt/vol) freshly prepared periodic acid for 5–10 minutes. Wash the grids several times with deionized water. [With nickel grids you can make the grids wash themselves by setting them on a magnetic stirrer at low speed.] Post stain as usual with uranium and lead. More importantly, I do have some ideas about how to prevent the problem from happening again. In my many years of doing cytochemical localization, I washed the tissue in buffer wash, which contained 0.5–1.0% (vol/vol) DMSO. This removed the aldehyde and protected the enzymatic activity. In the last several buffer washes before localization procedures, I added 0.1 M glycine to the buffer wash. This has been recommended for years for removing unbound aldehydes to improve immunolabel. I have never seen the osmium pepper in any of those preps. A while back I was going through the list server archives, and Randy Tindall had a post about putting a small amount of beta-mercaptoethanol in the buffer washes to prevent this problem. It probably works similar to the DMSO and glycine.* **Ann Debby Sherman** dsherman@purdue.edu Fri May 25

SEM:

rotary pump's gas ballast

I was replacing mist filters for our JEOL SEM's rotary pumps recently when it occurred to me that I never ballasted the pumps. In my previous job, I used a lot of mass spectrometers, and we ballasted the rotary pumps once a week. Mass spectrometers handle a lot of solvents, while SEMs don't. So I'm guessing that we almost never have to ballast rotary pumps for SEM. Am I correct? Our SEM has Variable Pressure capability, but we don't analyze wet or moist samples frequently. If anybody has any thought on this subject, I would appreciate it. **Tsutomu "Shimo" Shimotori** shim0102@umn.edu Wed May 9

We always ballasted TEM rotary pumps once a week, back in the day when they used film. Wouldn't seem to be necessary with an SEM, unless you use LV mode a lot with dirty specimens. I'm not sure that it would hurt, but in changing RP oil in our SEM once a year, I don't see any problems with the used oil coming out of the pump. We use LV mode occasionally as well, but common sense regarding sample size and amount of outgassing materials shouldn't make this an issue. **Jim Ehrman** jehрман@mta.ca Wed May 9

SEM:

vacuum problem and desiccant replacement

We have a Zeiss 1450EP SEM that is having an issue with the vacuum. It gives a vacuum error message; it doesn't want to recognize the vacuum hardware. Has anyone else experienced this problem with their scope? Any advice would be greatly appreciated. If we should just order a black wreath, please let us know that, too. We took the panels off the scope and noticed a filter on the side of the scope. It contains an amber-colored substance that we're assuming is similar to Drierite. Does anyone know if we can recharge this filter by drying it in an oven?

Can these still be ordered? **Beth Richardson** bethrichardson@uga.edu **Fri Jun 8**

After a fresh reboot of the computer, start your LEO application. Screen print "Shift Print Scrn" the LEO-SRV. Post that image to a public webpage. Then repost your query with a link to the screen-dump. I am looking to see if you get the "L-REM is not responding" or "Vac firmware" message. However, anyone helping you (including Zeiss service) would want to see all the messages. As for the desiccant on the air line, just ask Zeiss service for the part number. Mine is blue, but we use xdry-N₂ for the pneumatics. **Jim Quinn** jquinn11733@gmail.com **Fri Jun 8**

Data Management:

laboratory information management system (LIMS)

I'm looking for a laboratory information management system (LIMS) for microscopy. I don't need all the online analysis provided by OMERO, and I'm looking for something simpler. Many of our students just save their data on our server with stupid names like test123. I'm looking for a way to stick a protocol next to every image taken in a clean searchable way. For example, if the image is immunofluorescence of a bovine embryo, I would want the user to select from a list of protocols, then enter species, cell type, antibody, wavelength, etc. The actual microscope settings are not so important because they are already stored in the metadata. The best solution would be something open source that we could run locally on our servers. **Alexandre Bastien** alexandre.bastien@fsaa.ulaval.ca **Sat May 12**

At University of Victoria we used this one: http://www.fomnet-works.com/about_us.html. You can also get a free trial. **Stefano Rubino** stefano@soquelec.com **Sat May 19**

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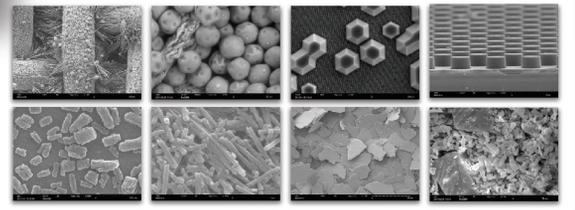


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