NetNotes

Edited by Thomas E. Phillips

University of Missouri

phillipst@missouri.edu

Selected postings from the Microscopy Listserver from November 1, 2018 to December 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:
fluorescent beads surviving histological treatment

I am doing a difficult search and Googling leads me to directions I don't want. I hope you can help me with this. I want to use fluorescent microparticles as positive controls for an injection in skin samples. The skin samples are injected, and then they are fixed, dehydrated, and embedded in paraffin (using Histoclear instead of xylol), using classical histological methodology. I want to be able to detect the fluorescence in paraffin sections without any further treatment. I don't want to detect the beads by immune detection or any labeling method on the paraffin sections. Optimally, the beads are 3-5 µm in size and have a fluorescence spectrum similar to TRITC. Alternatively, I am looking for metallic beads (like gold or silver) with a diameter of several micrometers (unusual), which I can probably detect in bright field. A colored solution is no alternative; I want to detect particles. **Stephane Nizets nizets2@yahoo.com Tue Dec 18**

What are your microparticles composed of? Make sure that they are not soluble in alcohols or Histoclear before your process your tissues. If you have any doubts, I would suggest freezing the samples and cutting cryosections. Tissues are fixed, cryo-protected with sucrose, and then frozen, so there are no solvents involved. I can send you an excellent protocol that gives very good histology (off-list, since Nestor's filters will block attachments). Please let me know if you would like to try that. Lee Cohen-Gould lcgould@med.cornell.edu Tue Dec 18

You could use gold nanoparticles, for instance a BSA stabilized gold tracer, of any size and use silver enhancement on the section to enlarge their size for bright field observation. Very easy. Feel free to contact me off-list. I will be happy to help. Jan Leunissen leunissen@aurion.nl Tue Dec 18

Have you considered using ceramic microbeads instead of the metallic ones? I've never used them, but they should come in the size range you desire. Christian Feldhaus christian. feldhaus@tuebingen.mpg.de Thu Dec 20

Ultramicrotomy:
alternatives to water

I have a user cutting thin sections of a polymer that is soluble in water, methanol, ethanol, and other organic solvents. Any thoughts about alternative liquids? **Chris Gilpin gilpin@purdue.edu Wed Nov 28**

How about fully fluorinated liquids, such as Galden by Inland Vacuum? Another one of chemically inert variants, such as Perfluorodecalin maybe? Valery Ray vray@partbeamsystech. com Wed Nov 28

Not so sure these would be compatible with Diamond knives and their mounting cements. Best to ask Helmut Gnaegi (helmut.gnaegi@diatome.ch) of Diatome for ideas how to do this. Al Coritz acoritz@emsdiasum.com Wed Nov 28

TEM:
screen lifter problem

My Philips CM12 TEM has a tendency to shut off if I lift the focus screen too fast (too hard?) to expose the bottom mount camera. This is relatively new to me, as I was using a side mount camera and never had to lift the plate. Is this normal? If it's not, other than being slow and delicate, what is the cure? **Frank Karl frank_karl@ardl.com Wed Dec 5**

Disassemble feed-through shaft assembly, clean it, and replace O-rings. Viewing windows O-rings may need replacement. Ditto large screen assembly. Most O-rings around projection chamber are Buna-N rubber. Install Viton O-rings instead. These last forever, more or less. Vitaly Feingold vitaly@sia-cam.com Wed Dec 5

I have had a CM12 since 1990 (oh yes, I remember it very well—the boxes were delivered on Dec 4th, 1990, two days before St. Nicklaus day), and it is still in use. It had quite a few peculiarities, but never heard of this one. No, this is not normal. We have a bottom-mount camera in operation (TVIPS) since 1998, daily, heavily used—no problems with any of the phosphor screens, and I keep my fingers crossed that we will never have a problem like this. Reinhard Rachel reinhard. rachel@biologie.uni-regensburg.de Wed Dec 5

It is really strange as Reinhard wrote. We have been running CM12 since 1985 up to 2014 when HT tank has gone. We had some problems with leaks in the viewing chamber (O-ring of diffraction beam stopper assembly and sealing of 35 mm camera) during those years. But those leaks never led to the microscope complete switch-off. Usually HT was switched off and IGP pump, too. However, we observed that after strong discharge the CM12 was sometimes switched off, and this also restarted the EDAX system connected to the CM12. What tells you the plate exposure meter when you change the intensity on the main screen? Is the exposure time changing? I hope that you solve the problem soon. Oldrich Benada benada@biomed.cas.cz Wed Dec 5

It is a little unclear what you mean by the Microscope "shuts off." Is it just the HT, the vacuum system, or all the electronics? Assuming that it isn't all the electronics, it sounds like there is a vacuum leak on the screen lift lever. If you are operating, the loss of vacuum will shut off the emitter and the HT and (quite likely) the IGPs as well. The vacuum page will then show the IGP shut off and V6 open so that the diffusion pump is pumping on the column and gun. The fix would be to disassemble the screen lift feedthrough and replace the O-rings. Henk Colijn colijn.1@osu.edu Wed Dec 5

EDS:
spectral imaging

My lab switched to ThermoFisher Pathfinder software from an older NSS. Still on the "uphill" part of the learning curve. My question concerns accessing the EDS map images in Spectral

Imaging mode. How can I access the individual map images in a format that can be read into ImageJ? Can I get a TIFF image, for example? Perhaps a silly question, but right now I'm unable to solve this nut! **Tom Williams tomw@uidaho.edu Mon Dec 17**

Are you sure you want to? Why? I manage a lab and have students inquire about that from our Oxford Aztec system now and then. I tried to persuade them that the vendors' software can do a lot. They may not want to reinvent the wheel. If they insist, it is a fairly easy manner to export the Oxford maps into TIF format, although I think it a poor choice. (I don't know what PathFinder offers.) It does not retain the scaling of the data but contains 8-bit image values from 0–255 where the data has been stretched to fit. Many of my good maps do not have 100 counts per pixel. Most have far less. That pixel data was integrated over many channels, so you are giving up a lot of potential information going to one, rescaled number. If you are still interested in processing the data on your own, I suggest you look into what others have already done, for example, Lispix. It was developed at NIST for working with data cubes of spectral data. https://www.nist.gov/services-resources/software/lispix Warren Straszheim wesaia@iastate.edu Mon Dec 17

— Mт