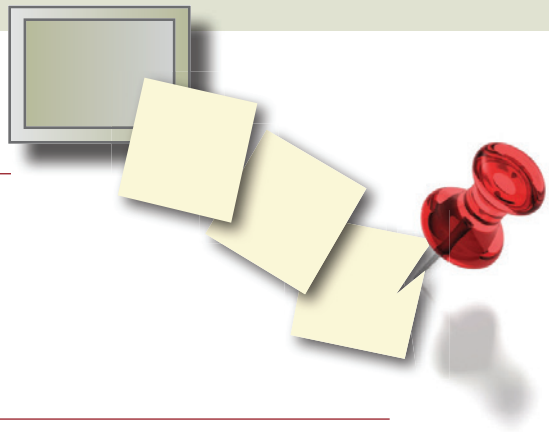
NetNotes



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Editor's note: *With this issue we welcome the addition of material from the Confocal Listserver (confocalmicroscopy@lists.umn.edu) to NetNotes. The Confocal Listserver, much like the Microscopy Listerver, provides a forum for asking questions about specimen preparation, instrumentation, and image processing. While NetNotes will focus on current topics of interest posted on the Confocal Listerver, the archives dating back to November of 1991 are available by creating an account. We thank Dr. Martin Wessendorf, moderator of the Confocal Listserver, and members of the list for allowing* Microscopy Today *and NetNotes to publish posted material.*

Selected postings are from January 1, 2019 to February 28, 2019. Postings may have been edited to conserve space or for clarity. Complete listings and subscription information can be obtained at http://www.microscopy.com and at https://lists.umn.edu/cgi-bin/wa?A0=confocalmicroscopy.

Confocal Microscopy Listserver

cell culture and hanks balanced salt solution autofluorescence

We are trying to image live cultured cells in Hanks Balanced Salt solution without phenol red. It's widefield imaging with a standard Chroma GFP filter set, and I'm seeing something I've never observed before. There's very high background all over the field that quickly fades within a few seconds. If I stop the acquisition briefly and resume, the background goes right back up by about 25%. I've never seen this before. What could be the source of this in HBSS, or is it something else? ***Chris O'Connell coconnell@uconn.edu January 15, 2019***

Hi. We've seen this a lot and have come to the conclusion it's riboflavin. I think it sticks to the glass/plastic on which the cells are growing and briefly fluoresces before bleaching. It's replenished from the solution, so once the imaging has stopped, the signal recovers. Using low-riboflavin medium should resolve the issue. Simon Walker simon.walker@babraham.ac.uk January 15, 2019

Vendor reply: We've seen the autofluorescence from riboflavin as well. It's part of what prompted us at Thermo Fisher to release our FluoroBrite DMEM and our Live Cell Imaging Solution (a HEPES derivative), which lack the autofluorescent components as well as phenol red (which can partially quench visible wavelength dyes). Jason Kilgore jason.kilgore@thermofisher.com January 15, 2019

Vendor comment: The link below, from 2014, supports Simon's riboflavin conclusion and how phenol red serves to quench it. Mistakenly people have felt that phenol itself was autofluorescent, but instead it is a quencher and thus can disturb overall fluorescence as well. And, though not written by us, the link does reference a product our company, Marker Gene, has been offering for over five years to positive reviews: Opti-Klear—no riboflavin or phenol red, and some of what cells need to thrive for imaging sessions as long as 4 hours, without the need for CO2. https://www.tebu-bio.com/blog/2014/03/28/lower-background-fluorescence-in-live-cell-imaging/. Mike Ignatius mjignatius72@gmail.com January 16, 2019

Thanks for all the input, everyone, regarding riboflavin. I didn't prep the samples, but there may be trace amounts of media left after they added HBSS. Maybe enough to cause what we are seeing so we can troubleshoot it. Chris O'Connell coconnell@uconn.edu January 17, 2019

Confocal Microscopy Listserver

appropriate lubricants for optical components

I want to change grease in condenser and binocular tubes of a Leica DM IRE2 microscope. The old grease solidified and made those parts stuck. Can anyone recommend a type and/or supplier of grease for such purpose? Thank you. ***Petro x.piter@gmail.com February 3, 2019***

I use white lithium grease for this. Available in hardware and auto parts stores. Phil Oshel oshel1pe@cmich.edu February 4, 2019

Dear Phil, Lithium grease was the first thing that came into my mind. I used to have a big can of Litol for my bike. But then I have read some scary info that microscope grease has to be very special or it will evaporate, leak, and destroy your equipment, so that kind of stopped me from using it. Have you used it for a long time? My guess is you had no problems with it :) My intention was to put some dumping grease there, like Nye A975. This is recommended for focusing mechanisms. Maybe it is overkill. Thanks. Petro x.piter@gmail.com February 4, 2019

There is grease available for vacuum applications that are very low off-gassing. If you use that stuff you will probably be fine. It is typically used for moving parts inside vacuum chambers or other low-pressure environments. Craig Brideau craig.brideau@gmail.com February 4, 2019

Note that for-real vacuum grease is NOT silicone vacuum grease. The silicone grease is only suitable for sealing desiccators. Fomblin is probably the best choice of vacuum grease for moving parts. Be aware that any high-vacuum grease is high-viscosity and can be "sticky." Use a very small amount and apply as a *thin* layer. It's also expensive, but all true high-vacuum greases are. Also, if you're applying the grease to any polymer or rubber parts, first check it on a bit that doesn't matter. The grease may goo up the polymer over time, especially if the part is warmed or heated. Phil Oshel oshel1pe@cmich.edu February 5, 2019

Seems to me that Dow silicone high-vacuum grease is a good choice; they recommend it for optical components,

and silicone protects o-rings well. It's not so sticky. No need to worry about super-high vacuum in this application? Mark Cannell Mark.Cannell@bristol.ac.uk February 5, 2019

Correct, the vacuum is not the issue, but Fomblin and the like are still good greases, just pricey. They don't outgas, and so won't get gunk on the optics or otherwise contaminate the optics. I don't think the silicone high-vacuum greases are good for lubricating. First, they give off acetic acid vapor; I don't see why Dow would recommend it for optical components. Second, the silicone greases are sticky. Not good for moving parts. It does protect o-rings, but watch the acetic acid and o-ring composition. Silicone grease seals coverslips well, though. Phil Oshel oshel1pe@cmich.edu February 5, 2019

No acetic acid from the Dow corning stuff I've used—it's a specialty silicon polymer plus thickener, not polymerized "bathroom caulk," as far as I know. It's more like low-MW Sylgard, I think. While its exact composition may be a trade secret, its vapor pressure is very low at 100°C, so I doubt it could be outgassing much, if any, acetic acid. I've not seen it corroding brass parts. Mark Cannell Mark.Cannell@bristol.ac.uk February 5, 2019

I'm guessing you DON'T mean Dow Corning 732, as that is the one that has acetic acid. As Phil clarified, there is "vacuum grease," and then there is "grease for use in a vacuum." They are not quite the same thing. Vacuum application servo lubricant grease is safe (grease for use in a vacuum), whereas the Dow Corning 732 is actually meant for creating a seal in a vacuum chamber, etc. and can off-gas. Craig Brideau craig.brideau@gmail.com February 5, 2019

732 is certainly not high-vacuum grease! Here is a spec sheet for Dow Corning high-vacuum grease: https://www.emsdiasum.com/microscopy/technical/datasheet/60705.aspx Mark Cannell Mark.Cannell@bristol.ac.uk February 5, 2019

Microscopy Listerver

preparation of cross-sectioned TEM samples of metal

I'm working with a student who has metal samples with an ~1 µm thick layer of amorphous silicon oxynitride on the surface, and they want to prepare cross-section TEM samples. We have tried gluing the sample sandwich together with Epo-Tek 353ND (also known as Gatan G1) and MBond 610, and both stacks fell apart on cutting. I assumed that the SiON layers were delaminating, but a visual inspection using the optical microscope convinced me this was not likely the underlying cause of the problem. Both epoxies used were not expired and were cured according to the manufacturer's cure schedule. I have had no issues with cross sections of other sample systems and these exact same epoxies, so I wonder if we need to use a different epoxy chemistry than these standards. Does anyone have experience with the SiON sample system? Is there a better epoxy to use, like Araldite? We would like to try to create a sample stack before giving up and switching to attempting tripod/wedge polishing. ***Christopher Winkler microwink@gmail.com January 23, 2019***

I have summarized the replies I received regarding this issue below:

1. "Since the SiON is ~1 µm thick, top monolayers probably do not matter. I can suggest trying the following: 1. Plasma clean the sample just before applying epoxy. We are using 4–5 min. of pure Ar at 50W forward RF with range 5W in our Gatan Solarus plasma cleaner. Or, 2. Sputter ~1–2 nm of Cr or Fe on the top surface before applying epoxy. We use Gatan PECS to do that. If Fe or Cr targets are not available in your lab, sputtering with any reactive metal available should also work."
2. Many are concerned the film is delaminating from the metal substrate. We should probably check this using the SEM, but we don't see any evidence of delamination in the optical microscope (when comparing the failed glue specimens with pristine specimens).
3. Many suggested FIB. The student prefers a conventional TEM sample preparation to generate a larger electron transparent region than the FIB would provide, but if all else fails then we can fall back to the FIB.
4. "A simple suggestion that, perhaps, might work (this is how we work):

   Why don't you inverse the order of operations? I mean: first, cut the small pieces and then glue them as a sandwich. Hopefully, the glued pieces will hold together during the grinding steps. Good luck with the ion milling afterwards."
5. "I have used Devcon 5-minute epoxy for many years on all sorts of samples with success. It is much more viscous than the other usual TEM epoxies, but you know the sample is ready for the next step in 5 minutes." Christopher Winkler microwink@gmail.com January 25, 2019

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