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NetNotes

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Specimen Preparation:
method for stone tools

I am an archaeologist specializing in experimental archaeology with hunting tools, and microwear on stone tools. I did an extensive project for a thesis with atlatl darts and arrows shot into a carcass, and then among other things, looked at the stone projectile points under a microscope. We used a differential-interference binocular microscope with polarized light and Nomarski optics. We took photographs at interesting locations, the most useful of which were recorded at 400×. This is not a new process, but we did find some new, previously unreported phenomena, and this can be attributed to the amount of detail recorded in the experiment. The microwear study has taken a long time, and is lacking in several aspects. Specifically, future tests would benefit heavily from having a microscopic view of the entire surface of each point both before and after the experiment for direct comparison. Using our current approach this is impossible. My question is, is there a way to scan the face of a stone projectile point at 100-400× and end up with a digital recording of the microscopic surface? I strongly suspect there is, but I wonder if the imagery would be of the type that would be useful to us. This is an exciting and very new field for me, so I'm sure my question seems very simplistic. I would benefit from a little direction. ***Devin Pettigrew dpettig08@gmail.com Fri Oct 23***

I am not sure what do you mean under "a digital recording of the microscopic surface", but if it includes 3-D reconstruction, I'd recommend Micro-CT or newer optical systems with so-called infinite focus. Some confocal microscopes could be useful also (not really sure, I do not work with them). If you need just a digital picture, the scanning electron microscope (SEM) will be of use. Vladimir Dusevich dusevichv@umkc.edu Fri Oct 23

As Vladimir said, SEM could be sufficient. If you can collect the secondary electron signal, it is sensitive to the orientation of the surface of the specimen. There are a few potential difficulties, however: I don't know what your specimen is made from, but if it's something like obsidian, there could be charging issues. There could also be difficulties with field of view, depending on the size of the specimen—a few cm times 400× mag will give an image about a meter across. This can be constructed by montaging smaller images, but there may be very many of these. Neither of these is insurmountable, so talk to your local SEM guy or find a facility that will provide the service you need—this list is a good place to start. Bill Tivol wtivol@sbcglobal.net Fri Oct 23

Why not try light microscopy? In many material labs there should be compound microscopes with epi-illumination of some sort. Also in some biomedical labs these machines are around. We have here used e.g. epi-darkfield for similar things. It will be in the magnification range you need, you can do extended depth of field projections if you're not getting the full sample focused at once. With a motorized stage it is also easy to get a tiled image so you can image a very large specimen into one dataset. I guess the sample prep for light microscopy may be easier than for SEM? But my SEM experience is from last century. Christian Liebig christian.liebig@medizin.uni-tuebingen.de Mon Oct 26

Specimen Preparation:
beach coral for SEM

I brought back a piece of coral from a beach recently. I am wondering how people would suggest drying out this sample. The sample was "found" on the beach (wet, wave-tossed). My goal is uncoated observation in SEM. I could: - bake it - leave it in a vacuum chamber - run it through a CPD. CPD seems like overkill given that this was a loose / dead sample, not a piece of living coral. ***Bryan Thompson bryan@systap.com Wed Dec 30***

You can try to treat it with bleach for 15 min to get rid of unwanted organic stuff, wash and air-dry it. I do quite often for bone and teeth. Vladimir Dusevich dusevichv@umkc.edu Wed Dec 30

Specimen Preparation:
hair sample preparation for SEM/EDS

I want to ask you about if there is a special sample preparation for a mummified hair that I need to analyze by SEM and EDS. The person who requests this analysis wants to see the surface of the hair and assess/discard the presence of heavy metals on the surface. I have some concerns about this, because I don't want to damage the sample (it seems there is only ONE hair!). We have a Zeiss EVO MA 10 with Oxford X-Max 20 EDS detector and variable pressure mode. We don't have coating or sputtering systems, and I am afraid that I could "burn" the hair when the electron beam hits the sample. ***Oscar Rivera oscar.rivera@uda.cl Wed Oct 28***

I can't say that I have ever looked at mummified hair in the SEM, but I have examined human hair. In my case, it was before and after images to compare hair care products, so I was forced to examine them uncoated. It can be done if you are careful - I did it with a tungsten filament at around 15 kV and the hair survived. One of the most important things is to make sure that each end of the hair is grounded electrically. Secondly, start with as low of a kV as possible (I realize you are doing EDS, but depending on the elements you are looking for you may be able to run your scans at lower accelerating voltages). Experimenting with the VP option may also help give you a better image. Since you only have one hair to work with, you might want to start with a piece of your own hair, and see if you can analyze it without damaging it. That should give you a fair approximation of what to expect, although I don't know what changes may have occurred to the mummified hair over time. Hopefully this at least gives you a couple of ideas. I'd be happy to try to answer any more questions if they should arise. Jeff Hall jhall@2spi.com Thu Oct 29

I have looked at fresh hair in VP mode without any special preparation. It is definitely a subject to beam damage, do not remember exactly at what magnification, but it should be something in between ×1000 and ×5000. Just a reminder: when doing EDS in VP mode be sure to place hair on wide enough carbon substrate (could be carbon tape);

LUMENCOR
CUSTOMER FOCUS

Kurt Thorn
UNIVERSITY OF CALIFORNIA, SF

UCSF

THE NEED for SPEED

What is the Nikon Imaging Center @ UCSF and what is your position in it?

The Nikon Imaging Center is a core microscopy facility established to provide access to cutting edge light microscopy equipment to the University of California, San Francisco community and to outside researchers. I am the director of the center.

What are some of the challenges that you face in fulfilling your mandate to provide cutting edge light microscopy access?

One of our major interests is in high-speed acquisition.

"With the advent of sCMOS cameras, acquiring data at 100 frames per second is now routinely possible, but the rest of the microscope, including the light source, also needs to run at that speed."

How has Lumencor's SPECTRA X light engine helped you to achieve this objective?

The Lumencor SPECTRA X features fast switching times between excitation wavelengths, which can be triggered from the camera. In this way, we can acquire multicolor images at 100 frames per second. Combined with a triggered piezoelectric Z-stage, **we can now acquire multicolor Z-stacks at 100 frames per second. This is not possible with a conventional arc lamp source.**

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electron skirt may spread as far as a millimeter or two, and EDS can pick up elements from a specimen stub. Vladimir Dusevich dusevichv@umkc.edu Thu Oct 29

Specimen Preparation:
removing Canada Balsam on old glass slide

Here's a "blast from the past" question. I have a set of 100+ year old petrographic glass, thin section slides [sections are from the Vermont talc district...fascinating]. Sample and coverslip mounted with Canada Balsam. We need to remove the coverslip for SEM and EPMA without also destroying the sample. Tried gentle heating of the slide, which softened the slip and dislodged the sample. No joy. Wondering if anyone had experience or helpful suggestions?? ***Tom Williams tomw@uidaho.edu Fri Nov 13***

Just immerse the slides in xylene. It will take some time until the balsam dissolves. Maria Castello maritacastello@gmail.com Fri Nov 13

You may find that the dissolution is more thorough and faster to do this in two stages, where the first involves some fresh Canadian balsam dissolved in solvent and the second employs fresh solvent alone. Although counter-intuitive, this is often the fastest way to remove aged or thermally degraded resins by pulling the solubility parameters of the solvent closer to those of the target material. This also might allow you to get effective cleaning without recourse to xylene, using a solvent such as ethanol or isopropanol with fewer health issues. John Twilley jtwilley@sprynet.com Mon Nov 16

Specimen Preparation:
collagen

One of our users is planning to look at cross-section diameter of collagen fibrils in a human periodontal ligament. However, he cannot put the tissue into the usual mix aldehyde EM fixative right away. There is most likely a few hours in transit between tissue harvest and arrival at the lab. What would be a good compromise procedure? I can think of the following but your advice will be much appreciated. 1. Will ice cold buffered saline during transit slow down autolysis enough to get reasonable ultrastructure? 2. I found a Nature Protocols paper on the use of buffered formalin when EM fix is not available and it also recommend to transfer to glutaraldehyde as soon as possible. However, will the small amount of methanol in formalin affect the size of collagen fibrils? Graham, Lesley, and Jan Marc Orenstein. "Processing Tissue and Cells for Transmission Electron Microscopy in Diagnostic Pathology and Research." Nature Protocols 2, no. 10 (October 2007): 2439–50. doi:10.1038/nprot.2007.304. 3. Freezing is probably a bad idea for ultrastructure preservation but will it affect the size of collagen fibrils? ***Wai Pang Chan wpchan@uw.edu Mon Nov 16***

I have worked with collagen (type 1) in teeth stored for months in a buffer or just saline before fixation, have not seen visible changes; banding pattern was good. But I have not measured diameter of fibers. Collagen is a tough thing, I believe (just believe...) delay of a few hours with fixation will not harm it. Vladimir Dusevich dusevichv@umkc.edu Mon Nov 16

Image Processing:
removing knife marks

Could anyone guide me how to remove the knife marks from an image using Gatan Digital Micrograph? Images are taken with a Gatan TEM camera. ***Ravi Thakkar ravi.thakkar369@gmail.com Thu Oct 29***

I would use caution on doing that. It would probably invalidate the use of the photo in a scientific publication since you would be seriously modifying the pixels. One acceptable solution for small defects is to make sure you overlay the defect with an arrow or something as you label the images. As long as you are not intentionally hiding actual data (e.g., an organelle that you say are absent in the cell), I see this as a valid strategy. This seems "fair" since any viewer can deduce that what is under the arrow is not visible whereas a good image processing manipulation to "erase" a knife mark would "fool" many viewers and therefore be unethical. Thomas E. Phillips phillipst@missouri.edu Thu Oct 29

The issue of digital manipulations was discussed many years ago, and, at that time, it was agreed that such manipulations were deemed OK if a full explanation of what was done was included in the figure caption and the original image was made available. If the caption contained the words, "A knife mark was removed from this figure using [insert process name here]." I as a reviewer would be OK with it; however, I might ask the author to see the original image if there were any indications that such processing could change the information the author intended to present. One tedious way to remove the knife mark would be to copy nearby pixels and paste them over the mark. I'm not sure if this can be done in DM, but ImageJ can do it. Bill Tivol wtivol@sbcglobal.net Fri Oct 30

Image Analysis:
linear measurement significant figures

I have a very fundamental micrometry question: What is the valid number of significant figures that can be reported for linear measurements made with a light microscope coupled with a digital camera? This is an incredibly basic question, I feel a bit naive/ sheepish for asking it but I have not been able to find suitable references that address this issue. Here are the experimental parameters/ conditions under which I am making measurements: Nikon PLM with a 20×, 0.45 NA Plan Apo objective The theoretical resolution of the objective is: (0.61×λ)/NA: (.61×0.550 µm)/0.45 = 0.746 µm The CCD camera attached to the microscope has a chip size that measures 2560 × 1920 pixels; each pixel is 3.4 µm (according to manufacturer's specifications). Using an Edmund Optics Certified (NIST-traceable) stage micrometer (typical 10 µm divisions) for calibration: Draw line that starts at 0 and ends at 400 µm. This equates to 2350 pixels, thus the calibration is 0.1702 µm/px or 5.8750 px/µm. Linear measurements and other shape parameters are calculated using typical image analysis software return values with a huge number of decimal places (7+)... these all can't be valid! Thank you very much for any information/ reference you can provide. ***Jack Hietpas mikroskop@gmail.com Fri Dec 18***

Of course, most of the digits generated are going to be nonsense. It is good that you asked. Certainly you should not quote values beyond the resolution of your system. That will be a convolution of your microscope resolution and your camera resolution. For most current cameras, I would suppose that the microscope is the largest portion. That seems to be your case given your figures. There is also the bigger, practical question of operator reproducibility. How consistently can you measure your 400 µm micrometer? If you get a standard deviation of 4 um on your micrometer, then you should limit yourself to three digits. Repeat the experiment at various magnifications and see what you get. I expect you will find the operator is the limiting factor. Warren Straszheim wesaia@iastate.edu Fri Dec 18

LM:
folded paper microscope

An interesting article in the Dec. 2015 New Yorker about the folded paper microscope. Apologies if this is a repeat. http://www.newyorker.com/magazine/2015/12/21/through-the-looking-glass-annals-of-science-carolyn-kormannw. ***John Shields johnshields59@gmail.com Thu Dec 17***

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EM:

humidity

Have you designed a facility in a humid city? If yes, can you please help me? We are currently planning an expanded EM Facility - the sticking point is humidity control. Our building maintenance / construction team want to know how other EM facilities regulate humidity in rooms with fume hoods. They have the logical comment that it is energy expensive to dehumidify air that is then pumped into a room with fume hoods because the fume hoods just pump the air out. I understand that - however doing sample prep in a space with 80% humidity is causing us a lot of problems. It is virtually impossible to keep our liquid nitrogen ice-free and despite our best efforts we have a horrendously high sample mortality rate. We need to reduce the humidity. And so we are looking for guidance on innovative solutions others have found. At this point any ideas or comments are most welcome! ***Erin Tranfield etranfield@igc.gulbenkian.pt Tue Nov 10***

Fume hoods should be closed when not in use, i.e. most of the time so they do not pump air out. Vladimir Dusevich dusevichv@umkc.edu Thu Nov 12

My experience is primarily with cleanrooms. They require more control than you likely need - but this may help. Generally, dehumidifiers in conjunction with humidifiers are used to adjust relative humidity in controlled environments. Despite inefficiencies, relative humidity in cleanrooms I "built" with several fume hoods can be controlled to say 40.0 +/- 0.5 % - that's a commonly used range for cleanrooms. Not too low for breathing and electrostatic discharge and not too high for condensation related issues like you are referring to (ice in liquid N_2). Changing relative humidity during the 24 hour day and seasonal variation can really cause technical issues (variation in sample oxidation, condensation, evaporation rates, optics, etc.) without proper control. Depending on the size of the controlled area there are a variety of appropriate solutions. Examples, not endorsements, of equipment manufacturers are Munters for dehumidifiers, Condair for humidifiers. If control is what you need, equipment like this needs to be used together and integrated with very good room temperature control schemes - or it could rain inside the lab! John Elzey john_elzey@hotmail.com Sat Nov 14

TEM:

benchtop microscopes

Does anyone in the life science and / or diagnostic pathology EM microscopy community have experience with Benchtop TEMs (e.g., LVEM25)? I have been asked to consider how appropriate a benchtop TEM would be to diagnostic pathology. All thoughts and experiences are welcome. ***Levina Dear levina.dear@health.nsw.gov.au Wed Oct 14***

I worked in biological microscopy many years ago and in polymer microscopy for the last 33 years. TEM of conventional biological and polymer microscopy use heavy metal staining, i.e. osmium and ruthenium tetroxides in polymer microscopy and osmium tetroxide, uranyl acetate and lead citrate in biological microscopy. In all heavy metal stained samples (polymer and biological), amplitude scattering is the mechanism of imaging. I have used TEM at 100-200 kV in both disciplines with excellent results. More pertinent to your question, I have had good results using brightfield STEM imaging (STEM) at 30 kV in our field emission SEM (FESEM) using a brightfield transmitted electron detector located below the sample stage. The ~1.5 nm at 15 kV resolution of our Hitachi S-4300 FESEM/STEM is lower than is seen in conventional TEM's, and modern FESEM's and TEM/STEM's. As one would expect, the contrast using FE-SEM/STEM is appreciably higher at 25 kV than at 100-200 kV. We found this an advantage in polymer microscopy. The lower spatial resolution was an issue at magnifications above ~50k×. You would need to use fixed beam TEM, not STEM. The limiting resolution of STEM in a bench-top instrument will undoubtedly be poor compared to a much more sophisticated high voltage TEM/STEM. Assuming that the spatial resolution of the bench-top TEM is adequate, my biggest concern is vibration. A good quality table with active an anti-vibration isolation will almost certainly be essential. Under no circumstances should you place this bench-top TEM on a standard lab bench that is shared with other folks in the lab. These benches make poor microscope tables under any circumstances. When shared with people doing other tasks, or even leaning against the bench discussing the latest football match, the vibration becomes prohibitive. Finally, I would send several vendors a couple of samples of differing section thickness, possibly 70 nm and 100 nm (or thicker if you work in that range), for analysis useful magnifications. Have them send you TIF files of each sample; don't accept JPG or other compressed formats. Gary Brown microscopy.gmb@gmail.com Fri Oct 16

SEM:

unidentified object

I would like to ask this wise list to help me identifying an object found in an animal tissue biopsy - please click http://www.eikonika.net/v2/downloads/stranger.jpg The object is apparently biological, sized 20 µm and resembles small pollen. The way it is mixed with blood and mucus makes difficult to imagine that this object came as a contaminant after fixation. ***Yorgos Nikas eikonika@otenet.gr Fri Dec 18***

It's a diatom. Lot similar to this, and many others, on our website: http://www.mta.ca/dmf. This could have entered your specimen processing stream in a variety of ways - water, some filter device, random dust, even airborne. They're really everywhere. James Ehrman jehrman@mta.ca Fri Dec 18

This looks like either a diatom or dinoflagellate. What is the tissue? I see RBC's and microvilli or cilia. Was this animal underwater? Let us know when you find out. Michael Delannoy mdelann1@jhmi.edu Fri Dec 18

Thank you for your replies, all agreeing that the stranger is a small diatom present in the water. So I can imagine how this was found mixed with blood and mucus in my specimen. It is an endometrial biopsy from a lady's womb with endometritis - ciliated and secretory cells and RBC are in the scene: Small diatom → penetrates water filters → arrives into my fixative → binds to the tissue upon fixation → visible on the sample. Yorgos Nikas eikonika@otenet.gr Fri Dec 18

If you have any filters in your water lines for the water you or the sample provider use to make solutions, check them for diatomaceous earth filters. Fairly common, and another source of diatoms as contaminants. Philip Oshel oshel1pe@cmich.edu Sat Dec 19

MT

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