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Selected postings from the Microscopy Listserver (http://microscopy.com) from 8/14/06 to 10/14/06. Postings may have been edited to conserve space or for clarity.

SPECIMEN PREPARATION - ventricle embedding problem

I'm using glycol methacrylate to embed rat forebrains for the purpose of stereology. However, sometimes, the ventricles fail to embed properly, even though the rest of the block cures perfectly. Thus, I sometimes get holes in the resin block, but only where the ventricles are. Does anyone have a solution to this problem? The brain tissue is fixed with 10% buffered formalin and then postfixed for several weeks at room temperature in the same solution. We then dehydrate it over the course of a day, gradually infiltrate it with the embedding solution over the course of a week, and then polymerize, all at room temperature. Infiltrations take place in light-sensitive vials rotated on a shaker. John Green <john.green@uvm.edu> 26 Sep 2006

This seems like an infiltration problem. Check the block surface using a stereo microscope - if the problem area is soft (prod with the points of forceps) that pretty well confirms it. You may find that incubating the affected blocks in a 50 - 60°C oven may improve this—start with an incubation of 1 hr and let the block cool down before sectioning. If you think progress is being made, increase the time to overnight incubation and try again. To avoid this recurring you could maybe try out the following: 1) Trim the tissue to as thin a slice as possible. 2) Use increasing concentrations of ethanol/resin mixes of several hours or overnight in say, 3:1, 1:1 and 1:3 dilutions prior to going to pure resin. 3) Slow down the polymerization rate by using a crushed ice slush heat sink surrounding the molds during polymerization and/or reducing the amount of accelerator. Alastair McKinnon <a.d.mckinnon@abdn.ac.uk> 27 Sep 2006

SPECIMEN PREPARATION - sample preparation polymer blend

I want to do a TEM on a blend of 2 polymers, filled with particles. The particles that I use are carbon black particles with a diameter of 20 nm, so TEM is actually the only option to visualize them. The blend is made of poly(α-methylstyrene-co-acrylonitrile) and poly(methylacrylate-co-methyl methacrylate) (PαMSAN and PMMA), a phase separating blend. The problem is that I have no experience with this. Can somebody help me with my problem? I don't know how I have to prepare my sample. First, I want to put my sample in an oven under N_2 for about 7 hours at 220°C. Thereafter, the sample must be cooled down very quickly to freeze the microstructure. After this, I would like to take a TEM picture of the structure. How do I have to prepare my sample to do this? Steven Vandebril <steven.vandebril@cit.kuleuven.be> 07 Aug 2006 & 08 Aug 2006 (combined postings)

With a soft filler like CB, I think that ultra-microtome cutting of thin sections (at room temperature since Tg's of both polymers are > 100°C) is the method of choice, followed by a Ruthenium Tetroxide staining in order to achieve a contrast between the PαMSAN (stained by RuO_4 due to the aromatic ring in the α-methylstyrene) and the PMMA (less stained by RuO_4). If you need more detailed information on RuO_4 staining, you can contact me again. This report might be of interest for you: http://sunsite.online.globule.org/iupac/publications/pac/1998/pdf/7008x1547.pdf Petra <petra.wahlbring@goodyear.com> 08 Aug 2006

SPECIMEN PREPARATION - Staining starch in sections

Can anyone tell me a reliable method to stain starch grains in sections of plant tissue embedded in Spurr's resin? Also useful to know whether polarized light would be suitable. I hope this is easy and I get lots of replies! Tobias Baskin <baskin@bio.umass.edu> 22 Aug 2006

Have you considered the Thièry method, using periodate oxidation of vicinal hydroxyls in carbohydrates, reacting resulting aldehydes with thiocarbohydrazide or thiosemicarbazide followed by silver proteinate? It may not be the easiest method, though, but it works really well. I don't have the original reference (1964 I believe), but it is described in J. Histochem. Cytochem. 33(10):1007-1014, 1985. Jan Leunissen <leunissen@aurion.nl> 22 Aug 2006

I've never tried it on embedded and sectioned tissue, but wouldn't iodine work? It works well enough in fresh tissue, staining starch grains purple in light microscopy. I do know that we've had a lot of trouble imaging tissue with starch granules in it, as they refract light, and mess up the signal in our confocal and tomography images. So polarized light might also work very well. Again I've never tried it. Robin Young <youngre@interchange.ubc.ca> 22 Aug 2006

Iodine would surely stain starch molecules. Many years ago, I used iodine to stain blends of polypropylene and ethylene vinyl alcohol (EVOH) for analysis by SEM and TEM. The iodine did provide excellent initial selective contrast for the EVOH. Unfortunately, iodine is volatile in the vacuum of the microscope. The iodine bound by the EVOH dissipated sufficiently rapidly that I saw a significant reduction in contrast during the microscopy session. A better stain for examination of starch in electron microscopes is probably osmium tetroxide. Osmium binds the hydroxyl sites irreversibly, thus providing excellent heavy metal contrast in the preparation. Gary M. Brown <gary.m.brown@exxonmobil.com> 22 Aug 2006

Regarding staining: 1. Iodine would be useful. 1 g iodine + 2 g Potassium iodide in 100 ml distilled water. Store in colored glass as stock. Add 5 ml stock to 100 ml water for actual use. Raw amylose-containing starches—blue. Raw amylopection-containing starches—reddish. Chemically modified starches—yellow-brown. Pregelatinized/cooked—reddish and swollen. Dextrins—blue-purple. Proteins in cryosections—yellow. 2. Also Trypan blue to distinguished damaged starch: 0.25 g of dye in 100 ml distilled water. Leave on for 1-3 min, blot off excess. Damaged—blue. Bit of Lost structure—pale blue. Undamaged—unstained. Protein—lighter blue. Lignified cellulose—dark blue. Cellulose (plant cell walls)—pale blue. Mold (chitinous)—blue. 3. As far as polarization, in cross polars fresh starch will show well as a Maltese cross; if it has been cooked, it will not. Embedding mediums to one degree or another—depending on media birefringence and strain, can give a background polarization that can be countered at the right rotation. Tony Havics <ph2@sprynet.com> 23 Aug 2006

SPECIMEN PREPARATION - Thiocarbohydrazide

Does anyone have a preparation protocol for 1% thiocarbohydrazide (TCH)? I'm working with some fastidious mammal tissue for SEM and I was thinking on putting the tissue through the OTO method, but I need a preparation protocol for 1% TCH. I've been doing research on a preparation protocol with no success. I tried to mix the TCH in water to make a 1% mixture but I have difficulty getting the TCH to dissolve in water. Omayra Velez <mayas003@yahoo.com> 25 Aug 2006

I believe the original references suggested dissolving the TCH at 60°C for 1 hr with occasional vigorous agitation. I generally pre-heat some deionized H_2O to 50-60°C and then add 1% TCH and sonicate for 15 min - it almost all goes into solution - just to be sure I let it sit for another 60 min in my 60°C oven. I generally filter with a 0.1 or 0.2 µm filter before use. One other useful tip is after the first osmium, rinse well with deionized H_2O and switch the tissue to a fresh vial. This will minimize any traces of osmium on the vial or cap that cause ugly, large, precipitates of osmium when you add the TCH. When we are being super careful, we switch after the second osmium step also. I like the technique and have had success with it. Tom Phillips <phillipst@missouri.edu> 25 Aug 2006

IMMUNOCYTOCHEMISTRY – testing colloidal gold

I am having a problem with the secondary antibody – Immuno-gold conjugate (6 nm). Here is what I did - I ran a test with an LR White embedded sample following the "standard" protocol for immuno-gold labeling. I could not find any gold particles - no labeling, no background. Trying to figure out what is wrong; I tested the secondary antibody alone - put a small drop of the original antibody (no dilution) directly onto a TEM grid, air dry and viewed

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with TEM. I could not find any 6 nm gold particles. I did find a few particles ranging from 50 nm to 150 nm. I then called the company, explained my problem. I was told that that is not the way I should test the secondary antibody; instead, I should do a dot-spot test (with silver enhancement?) Has anyone had this problem before? How do you check if the secondary antibody (immuno-gold) works or not? Zhaojie Zhang <zzhang@uwyo.edu> 18 Aug 2006

There are two tests you can do when you don't get a positive result in immuno gold labeling and when you want to check whether the conjugate performs up to standards: 1. An activity test, using a dot-spot system In this test a dilution series of corresponding IgG (Rabbit IgG in the present case) is spotted on a strip of nitrocellulose, and after blocking the strip is incubated with the gold conjugate. Silver enhancement is only required if you would test an ultra small particle conjugate in which the gold does not significantly contribute to obtain colored dots. A 6 nm conjugate has 'sufficient color' and does not need enhancement. 2. A TEM test in which the gold conjugate is adsorbed (not dried from the stock solution) onto a grid that is filmed and coated with poly-L- lysine which by its positive charge will bind negatively charged gold particles. Grids are washed in distilled water after adsorption. Drying a small drop of undiluted conjugated onto a filmed grid makes it not easy, if not impossible, to see particles or to evaluate what particle sizes you have. After all, there are buffer components and protecting protein in the conjugate solution that all dry onto the grid. Also, upon drying, particles tend to aggregate and form clumps (that are usually not easy to dissolve completely again, as they are pretty solid) and which will give erroneous readings. I will be happy to help trying to establish whether antigens may have been damaged preventing positive results or whether the primary antibody or secondary antibody have lost activity. Jan Leunissen <leunissen@aurion.nl> 18 Aug 2006

IMAGE ANALYSIS – object size

Recently I been in a discussion about how many pixels a feature should contain to provide meaningful results from image analysis. For example, if I threshold an image or measure a perimeter, how many pixels do I need a feature to have as to insure I have "statistical meaningful" data. It seem intuitive that I should have as many as possible, but what about a particle or feature that has only 12 pixels maximum in one direction (say a fiber)? I realize I could have a rectangle 9 by 7 pixels which would give me a diagonal of 11 pixels, but if I could only measure features that had at least 10 pixels would this feature have meaning? Frank Karl <frank.karl@degussa.com> 30 Aug 2006

I too have given this much thought, without finding any reference to any work regarding an error analysis for pixel segmentation. To a 1st approximation, significance (or confidence) is probably tied to counting error. This would make a single pixel insignificant, and imply a 10% error for a feature with 100 pixels. However, I've often thought that 2 features, both with same number of pixels but both with different perimeters, must have different error. Or, at least it cannot be as simple as counting error. Error analysis is also complicated by the process by which you segment features. For example, I might segment for features with 2 algorithms, and both seemingly and visually provide good results. However, because of the precision associated with 8 bits (as well as where in the histogram that value might be), the 2 resulting values can differ as much as 10%, and have nothing to do with "number of pixels"!

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Michael Shaffer <michael@shaffer.net> 30 Aug 2006

Nyquist's Theorem is the general sampling theorem used in microscopy. While the exact interpretation is that you need 2.3 pixels/object (assumedly, per dimension, if you are doing sizing), most of us use a practical limit somewhere between 3 and 10. Hope that is helpful. Barbara Foster <bfoster@mme1.com> 30 Aug 2006

The highest spatial frequency at which one can hope to collect information, called the Nyquist frequency, is such that the wavelength is 2 pixels. In practice one would have difficulty collecting information from greater than 2/3 Nyquist, corresponding to a feature comparable to 3 pixels. Of course, such considerations as noise and contrast limit the significance of the information collected, but if one can combine images of identical particles, these limitations can be overcome to an extent, and one can demonstrate that the average images do, indeed, contain information at the 2 to 3 pixel level. Microscope performance, as measured by the contrast transfer function, will also limit the information available, but for larger objects at lower magnifications the CTF should be nearly 1. Thus, there are different considerations for cryoEM of radio-labile, low-contrast specimens *vs.* radiation-resistant, higher-*Z* specimens. For specimens that are thin in one direction, such as the fiber you mentioned, the point-spread function of the detector must also be considered (this is also true for specimens that are small in both directions). The PSF will smear out an edge over several pixels, and it will also lower the contrast of your feature, since the same signal will now be spread over a larger range. A fiber that has the same width as the PSF and a signal that is twice the background noise will now appear to be anywhere from much smaller than the PSF to about twice as wide as the PSF with a signal about 50% larger than the background. In this case, measuring the diameter at many points along its length and deconvoluting the PSF could allow one to recover the width accurately. The bottom line is that 'to insure I have "statistical meaningful" data' is a complicated issue. Bill Tivol <tivol@caltech.edu> 30 Aug 2006

Here's my two-pixels-worth on this question: I want to ignore the question of statistics since you seem to have a SNR high enough to do thresholding and segmentation. The sampling theorem is only valid if the scene you digitize contains no spatial frequencies beyond some maximum value. In that case, the digital image is completely equivalent to the original scene (in the sense that you can recreate the original scene with arbitrary sampling) if the highest frequency wave is represented by at least two pixels per wavelength. The Nyquist frequency is at least as high as the highest frequency contained in the scene. In this case there is no point in reducing the pixel size. The situation is different if a scene contains higher frequencies, no matter whether these are noise or signal (assuming you have some way of telling the difference). Frequencies in the scene lying above Nyquist will appear in the digital image as aliases, that are frequencies below Nyquist that don't actually exist in the scene. I believe one reason why 1.5-fold oversampling is often recommended is to reduce the effects of noise-aliasing (noise decreases with frequency). If the scene contains higher frequencies other than noise, the digitized image will show features that are not present in the scene and any segmentation will produce meaningless results. For example try taking a digital photograph of a fence from a distance such that the pixel size becomes larger than the width of one board in the fence. In your picture you'll see something resembling a fence, but the spacings will be completely wrong (much too big). In summary: I don't think more pixels is better, but it might be worse. Choose the pixel size based on the contents of the scene you want to digitize. If somebody can explain the merits of oversampling 1.5-fold to me (beyond the simple explanation I've offered) I would be grateful. Philip Koeck <philip.koeck@biosci.ki.se> 31 Aug 2006

Two replies to different aspects of this question: (1) Well, the reason for oversampling above Nyquist is actually fairly simple. Think of the spatial frequencies (edges) as sine waves. Sampling at the Nyquist level of 2× the highest frequency allows you to sample the highest frequency incoming sine wave at peak and trough. Anything less and you will instead get a beat frequency from your samples. This is the "minimum" sampling to get that high frequency. Here's where I start reaching for the white-board pens... However, if the high frequency is "out of phase" with your samples, you might be perfectly sampling exactly where the input is crossing zero, resulting in no output data. Sampling right in phase gives maximum response, 90 degrees out of phase gives zero response. Hence, the idea of sampling a little faster, as in 1.5× Nyquist, so that you get some peaks, some troughs, and can estimate the high frequency better. (2) How many pixels? Depends on what you're measuring. You've got intensity variations, camera variations, illumination artifacts, segmentation bias, on and on and on. I would suggest taking your individual measurement(s) and looking at what happens statistically when you add/subtract some pixels. Diameter, area, shape, and integrated optical density will have different responses, linear, a^2 relationship, or something else, based on how it's calculated. Keep in mind that you're often polishing a pig! I have talked to people complaining about their 7th-8th decimal place of measurements varying (due to round-off of single precision floating point values), when uncorrected distortions of their images make their data accurate to maybe ±15 percent

HUMOR

Dear Abbé

Dear Abbé,

Lately my wife has been reducing her time assisting me with fixations, and the other day I caught her aligning another man's scope. I still care for her technique but am afraid that if I confront her about her dalliances, she will leave me for another lab. Do you think I am overreacting?

Misaligned and Miserable at Oak Ridge

Dear Misaligned,

Quit your whining! In my day we were just happy to have a woman in the lab! Remember what my friend Freud once noted, "Sometimes a scope is just a scope."

Dear Abbé,

We have a coworker, "Carl", who insists that the "shiny" side of the grids is the best side for picking up sections. I am positive that the "dull" side of the grids is correct. It has become a serious disagreement, almost coming to blows. Who is correct, Carl or me?

Picking Sides in Athens

Dear Dull Boy,

Quit your whining! In my day we were not allowed to use either the dull OR the shiny side. Instead we had to pick up the sections on the edge of the grid and then carefully roll them off onto a support film that we made by digesting our own fingernails in boiling ether. You guys in Athens find the silliest things to argue about.

Please post your questions for Dear Abbé to his personal secretary, jshields@cb.uga.edu. Although it will be impossible to post all queries to Dear Abbé, he will make sure that all readers will feel special about their particular problems.

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if they're lucky. Minor shameless self-promotion here - I did a poster on imaging artifacts in microarrays a few years ago, including in particular segmentation errors. Take a look; I don't promise rocket science, but some of it might be useful: http://www.mediacy.com/pdfs/ArtifactsMicroarray.pdf Kevin Ryan <kevin@mediacy.com>31 Aug 2006

I respectfully refuse to buy what you say in point 1. I agree that a wave at Nyquist frequency, which is aligned with the rows of pixels will in general be downweighted (and in the worst case deleted completely) due to the phase-mismatch you describe. However, this applies to exactly two plane waves in the entire spectrum, assuming that the scene to be digitized has a maximum frequency equal to Nyquist. Waves that run at an angle to the rows of pixels will be perfectly reconstructable (given all the relevant transfer functions). This is a minor problem, in my opinion, and doesn't require 1.5-fold oversampling. (1.1-fold would be plenty if you insist on fixing it.) Any other ideas? Philip Koeck <philip.koeck@biosci.ki.se> 01 Sep 2006

In order to get measure of perimeter the image analyzer software must be able to recognize a corner as distinct from a horizontal or vertical row of pixels, and apply a weighting factor when it encounters a corner (otherwise a D shape would give the same perimeter as an O shape if perimeter is calculated merely by pixel count). However, even more importantly, perimeter is a measurement that truly varies with magnification, and hence resolution, of the measuring conditions. If you measure lung tissue alveolar wall area & perimeter by light microscopy and then by TEM, you will get roughly the same area measurement for both, but the perimeter value will be far higher. This is because under very high magnification you will encounter further tiny small scale convolutions that mirror the larger ones seen under light microscopy. Likewise, if you measure the perimeter of UK on a map, you will naturally get an incredibly small perimeter value compared the very large value you would measure if you actually walked

Electron/Ion Beam Instrument Engineer

The University of Oregon's Center for Advanced Materials Characterization in Oregon (CAMCOR) is seeking applications for a full time staff position to begin January 2007. A strong background in maintaining, trouble shooting and upgrading electron/ion beam instruments and associated high voltage, vacuum, mechanical and electrical systems is required. Experience in x-ray diffraction instrumentation is also desirable.

This position will be located in the new Lorry Lokey Integrated Science Laboratory, a state of the art nano and micro science analytical instrument facility designed specifically for exceptional nano-science performance. It will house the latest electron, ion and x-ray beam instrumentation available, including a Zeiss Ultra TFEM, FEI Quanta 200 E-SEM, Cameca SX50 and SX100 microprobes, Ion-TOF SIMS, Philips Auger Spectrometer, Philips TEM, Kratos XPS and various assorted coaters, etchers, and other vacuum deposition systems.

The successful candidate will have a BS in a beam microscopy related field and an extensive background in instrument field service with significant practical experience troubleshooting high vacuum electron and ion beam instrumentation at both the system and PC board levels. Must be able to read and understand schematics for electronic circuits and systems. The successful applicant will be involved in modifying/improving instrumentation capabilities to enable the equipment to more fully support unique research needs and will be expected to work intimately with the scientific staff and research faculty. We seek candidates with a demonstrated commitment to working effectively with students, faculty and staff from diverse backgrounds.

Interested persons should send a resume with a detailed description of work experience and skills, and arrange for two letters of recommendation to be sent to: CAMCOR Instrument Engineer Search Committee, 1253 University of Oregon, Eugene, OR, 97403-1253. To be assured of full consideration, application materials must be received by November 1, 2006, but the search will remain open until the position is filled.

For further information, contact:
John Donovan (donovan@uoregon.edu).

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round all of the coastline with a treadmill. Object area can vary also with magnification as well, if the lower magnification fails to resolve many small objects amongst larger ones, but even in this case the area differences due to magnification would still be relatively small. Keith Morris <keith.morris@ucl.ac.uk> 01 Sep 2006

The fact that the length of, say the coast of the UK, depends on the scale you are using is known as "fractal dimension". A lot of work has been done on fractal dimensions, and it is not limited to digital images. Regarding the original question about errors and reliability of measurements of digital images, I think that the answer is determined by applying the Nyquist-Shannon theorem to digital images. There is actually a very good explanation of this in Wikipedia (for the mathematically inclined): http://en.wikipedia.org/wiki/Nyquist-Shannon_sampling_theorem. If you read the article, it says that this theorem is valid for certain types of signals (band limited, "infinite sample", *etc.*) and that there are several practical considerations that need to be taken into account for real world signals and may require some oversampling: "The sampling theorem does not say what happens when the conditions and procedures are not exactly met, but its proof suggests an analytical framework in which the non-ideality can be studied. A designer of a system that deals with sampling and reconstruction processes needs a thorough understanding of the signal to be sampled, in particular its frequency content, the sampling frequency, how the signal is reconstructed in terms of interpolation, and the requirement for the total reconstruction error, including aliasing and interpolation error. These properties and parameters may need to be carefully tuned in order to obtain a useful system." Michael Bode <mike.bode@olympus-sis.com> 01 Sep 2006

EM - Venting EM Chambers

I was wondering if anyone had any advice for venting electron microscope columns/sample chambers. In particular, we want a system that allows the column or sample chamber to reach atmospheric pressure by venting with dry N2 but does not allow the chamber to over pressurize above atmosphere to protect seals, thin window on EDS detectors, etc. I'm aware that there are on-demand gas regulators that can control this sort of thing. Does anyone have any suggestions for a good vendor or model number for this sort of regulator? Alternatively, I've also heard that demand valves that divers use are good for this sort of application as it only supplies gas when the diver breathes or sucks through the mouthpiece (or in this case when the chamber is still under vacuum). Does anyone know a particular brand or type of diver demand valve that works well for this application and is easy to modify (i.e. relatively easy to install standard fittings on the ends like NPT, etc.)? Any comments or suggestions would be greatly appreciated. Preston Larson <plarson@ou.edu> 27 Sep 2006

I like simple (and cheap) solutions. We use helium with our Hitachi VP-SEM for both the residual atmosphere and venting. We set the regulator to less that 1 psi, which is enough to vent the chamber in about a minute. Of course, someone could inadvertently change the regulator setting. Therefore, we also have a commercial (Circle Seal), spring-loaded pop-off valve in the line from the tank. It releases at 2 psi, if I recall correctly. It effectively guarantees we won't over-pressure any more than that. We have a similar valve tapped into the EDS mounting plate on our JEOL. I don't remember if that was installed with our EDS system by the EDS people or if it was an afterthought. The pop-off valves look like they should cost only about $5. They probably cost more, but they have got to be a lot cheaper than an on-demand regulator. Also, we have unbolted the front of both microscope chambers. The Hitachi only loads through the front of the chamber. The JEOL has a load-lock, but we sometimes have to open the chamber for big samples. That way, any extra pressure simply leaks out through the front of the chamber. Warren Straszheim <wesaia@iastate.edu> 27 Sep 2006

A simple single stage regulator will do the job. I use the cylinder N_2 regulator to drop tank pressure from 1800 psi to 8 psi. This then goes through a molecular sieve to dry the N_2, then into the SEM chamber through the vent valve. Depending on the size of your chamber, just wait until the door slides open. If the SEM is set up correctly, it takes very little force to move the door open when completely vented. If you have any ion pumps, be sure to only use N_2, or air if no N_2. Gary Gaugler <gary@gaugler.com> 27 Sep 2006

I made a demand regulator from a scuba regulator and it works well. On the high pressure side of the scuba regulator, I cut the pressure hose and used a ¼ MPT - ¼ brass hose barb adapter to attach the scuba regulator to the standard gas regulator on the nitrogen cylinder. I got some relatively thick wall PVC tubing and slipped it over the mouthpiece using a hose clamp as a retainer. To reduce the tube diameter for the microscope, I went to my local hardware store and got copper plumbing fittings. I just soldered together a number of pieces of copper pipe and reducing unions until I could switch to a brass pipe thread. The largest copper pipe fits snugly in the PVC tubing and I again used a hose clamp as a retainer. I've been using several of these for backfilling TEM and SEM chambers for over 10 years. No launching TEM windows into the users' laps, no blown EDS detectors, *etc.* Henk Colijn <colijn.1@osu.edu> 27 Sep 2006

Perhaps I didn't note in my initial post about simple pop-off valves that both of our SEMs have light-element EDS detectors. They have been mounted for 10 years or more without any over-pressure incidents. I think a stray, flyaway particle may have punctured one of the panes on one detector necessitating a repair. However, it was a pinhole defect, not a big rip. Detectors are a big investment and you want to make sure some protection system is in place and that it is not going to fail. Still, cheap solutions are available and might be more fail-safe than more expensive and complicated

systems. Warren Straszheim <wesaia@iastate.edu> 28 Sep 2006

TEM – electron diffraction

Although I understand the principle behind electron diffraction in TEM, I have no idea about the kind of information this technique gives and how to interpret the diffraction pattern. If you could give me a www address which explains that I would be much grateful. Now a very practical problem could be perhaps used as an example: we have a mixture of aluminosilicate mineral (I have cut 70 nm sections) with approx. 90% of mordenite and 10% quartz. 1) Can I use electron diffraction to distinguish both types of particles to verify the purity of the powder? 2) What kind of information about the crystal structure can electron diffraction give me in this case? 3) Can I detect a change -and which change- to the crystal structure using this technique if the mineral is heated and treated with strong acids, which actually modifies the structure? We have a tilt stage for tomography. Can this bring further information? Stephane Nizets <nizets2@yahoo.com> 14 Aug 2006

The example you give, distinguishing between two phases of different structure in a single sample, is the most common use of electron diffraction. This is essentially a fingerprinting of a structure using a measure of angles and spacings provided by the pattern and fitting these to the geometry of a known unit cell. Strictly speaking, this can only reject a candidate phase by failure to fit, because you aren't really proving the presence of a particular structure, just showing that it's a plausible match to the data. This is nicely suited to your case of only having two structures to choose from. If you've never done this before, you should try it first on a single-phase material (for example a finely ground silicon). This will give you some practice at orienting a zone axis (you will need to use a double-tilt holder, so this will eliminate the tomography holder unless yours can tilt on two axes). Once you have done this you will also know the camera constant of the microscope for a given camera length, which will provide essential information for distinguishing phases (relates the spacing in Å to the distance of a reflection from the pattern origin). One thing working to your advantage will be the large spacings present in mordenite and the absence of any very large spacings in (alpha) quartz. Because of this, any time you see a reflection indicating a spacing greater than about 4.5 Å, it must be the mordenite. Structural changes may be difficult to detect depending on what they are. They would have to involve a fairly large change in the size and shape of the unit cell in order to detect with 'spot pattern' diffraction. If you have a material amenable to convergent beam diffraction you can have a lot more sensitivity to structural changes but your sample must be quite beam stable and have relatively small unit cell (neither is likely to be the case for your mordenite). As far as references go, the old standard Hirsch *et al* (Electron Microscopy of Thin Crystals) is pretty good on basic spot pattern indexing - see chapter 5 and appendices 5 and 6 which show some worked examples. In fact, any TEM textbook should have at least some discussion of how to acquire and index spot patterns. Wharton Sinkler <wharton.sinkler@uop.com> 14 Aug 2006

Just an addition to Wharton's excellent response. If your specimen consists of crystals too small to isolate one or two, you will likely have a ring pattern either a solid ring if the selected area contains very many crystals or a series of spots arranged on a ring if there are fewer crystals. Measuring the diameter of the ring and comparing it to that for a known substance will give you spacings, which you can fit to those in model structures, and changes in the relative intensities of the rings can give structural information also. This could be due to slight atomic displacements that do not affect the unit cell, but change the relationships of the scattering from the atoms in the crystal. One of the recent publications from our lab (Wright, E. R., Iancu, C. V., Tivol, W. F., and Jensen, G. J., Observations on the Behavior of Vitreous Ice at ~82 and ~12 K. Journal of Structural Biology, (2006) in press.) used electron diffraction to determine the dose at which low density amorphous ice underwent a transition to high density amorphous ice, so you could expect to see similar changes. Bill Tivol <tivol@caltech.edu> 15 Aug 2006

And I would go one step further and suggest expanding on Bill's comments about finding many crystals that will produce ring-type diffraction

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pattern with solid (or near-solid) rings. It is FAR easier to search/match (solve by comparing unknown d-spacings to those of standards) ring patterns vs. single crystal spot patterns! Besides the classic text books mentioned, one needs access to standard diffraction pattern datasets—the International Centre for Diffraction Data is the gold standard here: Google "ICDD". Check their educational resources for more "how-to" help. Ron Anderson <randerson20@tampabay.rr.com> Editor's note.

TEM – Nickel grids & EDX

I have some basic questions about the observation of minerals on nickel grids in TEM. I use Formvar-carbon-coated nickel grids and deposit a fine powder of crystal mineral onto them. I would have preferred copper but we have nickel grids in stock. When I observe the particles, even with the diffraction aperture inserted, the surface illuminated by the beam darkens pretty quickly. Sometimes I even have dark rings imprinted on the coating film (if I leave the beam for some minutes). If I take the diffraction aperture out of the way for EDX analysis the surface becomes quickly completely black in a matter of minutes. 1) What is the black? Does the mineral melt? 2) If I heat the mineral to make it amorphous before the observation I sometimes still see dark rings. Are they diffraction rings? (in normal mode, not diffraction mode). Is there any diffraction in amorphous material? 3) In EDX I see a peak for copper, just after the nickel peak (at 8,070 keV) and I don't expect copper in my material. Is there copper in the nickel grids? Stephane Nizets <nizets2@yahoo.com> 04 Sep 2006

1) The black is most likely carbonaceous contamination. Volatile organic molecules diffuse across the sample surface under the influence of the beam's electric field. When they hit the beam they "polymerize" and form a thick layer which appears dark in the image. In TEM, the contamination layer usually appears as a ring around the perimeter of the beam. As you decrease the size of the beam (*e.g.* STEM), the contamination problem increases. 2) You will see diffraction rings from amorphous material. They will be diffuse, not sharp like the rings from most crystalline materials. The carbonaceous contamination will also give rise to diffuse diffraction rings. 3) My suspicion is that you are seeing the Ni *K*-beta peak, although the Ni *K*-Beta is at 8.265keV. The *K*-Beta is ~20% of the intensity of the *K*-Alpha peak. The 1st row transition metal *K*-lines have the characteristic that an element *Z*'s *K*-Beta falls under the (*Z+1*) *K*-alpha. Henk Colijn <colijn.1@osu.edu> 04 Sep 2006

I agree with Henk that it is probably contamination on your samples. To make sure, if you tilt the samples at a high angle, you should see a split of the rings where you are seeing the top and bottom surfaces. This used to be a way of measuring the thickness of a TEM sample. You can download a copy of the paper from our website on contamination of samples. The URL is http://www.southbaytech.com/app_index.cfm?main_action=tech_papers" and the title is "Surface Science Aspects of Contamination in TEM Sample Prep" by John Grant *et al.* and it is paper number 225. I am one of the authors. In that paper, you will see a tilted sample with heavy contamination. To avoid contamination like this, you should plasma clean your sample prior to putting it into the TEM. Scott D. Walck <walck@southbaytech.com> 04 Sep 2006

SEM – Back scattered electrons and edge effects

Edge effects are common in secondary electron images of samples having the appropriate topography. However, on a flat sample, do you think there may be something equivalent to edge effects in a back scattered electron image? In this case there would be no topography effects but could areas of concentrated mineral give an enhanced signal due to not just the additional high atomic number atoms but also due to the particle distribution? Debby Sherman <dsherman@purdue.edu> 14 Sep 2006

BSE generation is a bulk property of the atoms within the interaction volume, therefore variations in local element distribution will cause variation in BSE. I have seen this for instance in crustacean cuticle, where the edges of pores were brighter in BSE imaging because of an increase in Ca concentration at the pore edges. One way to detect this is to compare compositional and topographic images. This can be confusing if there is both a topographic feature (like, say, a pore) and a compositional feature (like, say, an increase in Ca concentration at the edge of the pore). Then EDS mapping comes in handy. Not to mention changes in BSE images caused by elements migrating due to beam-specimen interactions . "BSE generation is a bulk property of the atoms within the interaction volume." Makes for a great exam how can it be truthful to say that a BSE detector can resolve 0.1 *Z* (atomic number), if *Z* is always an integer? Phil Oshel <oshel1pe@cmich.edu> 14 Sep 2006

This is indeed possible, but you should also be careful that your BEI detector isn't also the problem. Both (or all 4) BSED segments need to be perfectly balanced, and I have often observed that putting the sample too near the BSED (short WD) can also enhance edges. In this same regard, I believe that the scintillator type BSED can have a large enough acceptance angle to enhance edges at short working distances. HTH, Michael Shaffer <michael@shaffer.net> 15 Sep 2006

If there is a significant difference in the hardness of phases of a specimen, then a "flat" specimen may not really be flat, especially if it is prepared by polishing. In cases like this, I did observe some "edge effect" in BSE, which is due to the curvature of the edges of phase boundaries. Also I have observed strong edge effect on bone specimens embedded in a resin (as for TEM) and cut with diamond knife. Vladimir M. Dusevich <dusevichv@umkc.edu> 15 Sep 2006

Personally, I would regard an 'edge effect' as a change in contrast due to sample topography which I don't think occurs in BSE imaging. However, in a multi-element composite sample, 'diffusion' of elements

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towards morphological and topographical features - such as edges, grain boundaries, *etc.* could occur. Such segregation is 'real' and is revealed by BSE. The 'edge effects' observed in SE imaging are purely a consequence of sample topography on the physics of the imaging method. As has already been mentioned, preparing a truly flat sample is difficult. In this case, SE imaging can reveal differences in sample height but BSE imaging will tend to indicate compositional variations. You should also keep in mind channeling effects, arising from sample crystallography, which give rise to contrast variations unrelated to composition or topography. And while these are generally 'bulk', that is the whole grain has a contrast determined by orientation and crystallography, it is possible for crystal orientation to be distorted at grain boundaries, leading to contrast changes which could be interpreted as elemental segregation. To separate such effect, you need BSE images plus EDS mapping. Larry Stoter <larry@cymru.freewire.co.uk> 15 Sep 2006

SEM – Backscattered electron images

I am trying to understand what is happening with a set of BSE images. Your comments will be welcome! Below are links to two images. The first (1.5 Mb) shows two BSE images of a nickel based super alloy (Ni-Cr-Fe-Ti). Both were acquired using a 4-diode detector, 5 kV. beam, and as close to zero degrees tilt as I could set the stage. The top of the first image is in the "as polished" condition, the lower portion of the image is after a very light electro-etch. Notice the difference in channeling contrast. Z-contrast seems largely unaffected (e.g. Ti and Cr carbide inclusions). Perhaps the difference is from my inability to set exactly the same tilt, but they should be within a few degrees (or better) of the same value. Why the dramatic reversal of contrast for some grains? The second image is simply a 60 degree tilt SE image of the same general area to show relief of the carbides due to both polishing and the etch. Not much. http://www.bwxt.com/operations/images/sem/126867_859.jpg and http://www.bwxt.com/operations/images/sem/126866.jpg. Woody White <nwwhite@bwxt.com> 19 Sep 2006

What a great puzzler. Have you tried tilting on purpose? Perhaps going through a tilt series would be informative. One degree increments or even half a degree could show significant changes in grey level of some grains. John Chandler <jpchandl@mines.edu> 18 Sep 2006

It looks as if the crystallographic contrast would dominate on chemical contrast. As John proposed, try with tilting. Channeling is very sensitive to small angle tilting, half a degree to a few degrees. If the contrast changes with so small angles, it's channeling; then try with higher energy. And another question: I've never worked with a 4 sector BSE detector, but people from FEI talked me from artifacts arising on these. Can you work in two sector mode, combining the four sectors in two pairs? Try with different pairs. Maybe it helps to understand what happens. J. Faerber <jacques.faerber@ipcms.u-strasbg.fr> 19 Sep 2006

Can you repeat these 2 images? If so, I'd suggest duplicating this, while being particularly careful of the conditions. That is, I have seen a BSED flip its BEI contrast for different beam currents. Which is still a question in my mind why it happened, but it did happen with a Cameca multichannel (5-pair) BSED, and I watched the BEI response flip in going from 15 to ~20 nA. I thought at the time it must have been a fluke with the BEI video amplifier. On another note, can you play with the effect of tilt by rotating the stage? Michael Shaffer <michael@shaffer.net> 19 Sep 2006

I would suspect that the reason for the difference has more to do with the removal of the thin, amorphous layer left on the as-polished sample, but I must admit that the contrast reversal is dramatic. BSE can be very strange that way and I never get the same image contrast twice on the same sample. Try tilting slightly and watch it change, particularly when you are viewing channeling contrast on a homogenous, single-phase sample. Mary Mager <mager@interchange.ubc.ca> 19 Sep 2006