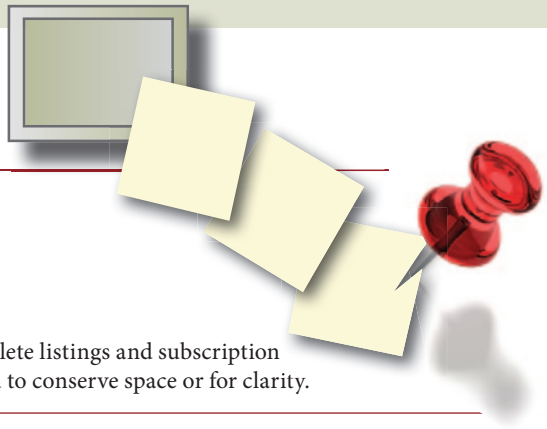


NetNotes

Edited by Thomas E. Phillips
University of Missouri

phillipst@missouri.edu

Selected postings from the Microscopy Listserver from March 1, 2011 to May 1, 2011. 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:
sputter coater target distance

I just got a new sputter coater and need help determining the sample to target distance. Instructions don't have a recommendation, just a note that says the specimen height is adjustable. Any advice? ***Jon Krupp jkrupp@deltacollege.edu Wed Mar 23***

A generic guide to setting the specimen position in a sputter coater is 5 cm target to specimen surface. This means you are in the plasma but not too close to the target. Steve Chapman protrain@emcourses.com Wed Mar 23

For your information we teach the following procedure for setting up a sputter coater. Each test, other than the first, uses a piece of normal copy paper cut down to about 2 in. × 3 in. to simulate the outgassing that would be typical of a specimen. (1) Using a piece of paper that covers the stage area, run the coater for several minutes at 20 mA. Hold the paper down with a stub placed at the centre. The coat will demonstrate the coverage of the plasma and pick out areas that are well coated or not so well coated. The coating should be pretty perfect with a new coater but patchy with a coater that has a contaminated target. If there are areas with less of a coat you need to map your stage for selecting the best position for superior coating in the future? (2) Place a test paper on the stage held down by a stub. The next step depends upon the voltage of the coater (a) using a low voltage coater (400 V to 600 V) set the current at 10 mA and coat for 30 seconds. (b) with a high voltage coater (>800 V) set the current at 20mA and run for 30 seconds. Note the current and time on the back of the test paper. (3) Repeat the experiment in steps of 30 seconds until the paper starts to exhibit a very slight gold sheen. Note the information on the back of each picture. (4) Select the condition for higher magnification work that shows the first grey coloration. (5) Low magnification operation will probably require the procedure set out in 3. Sputter coating is often treated as a no brainer, but as those attending our course in Missouri later in the year will see, we dedicate an hour to correctly judge and set up a sputter coater for the first time. It is that important if you wish to obtain information that is not influenced by the coating; just as is the selection of the correct coating material. For top class SEM results I prefer to use the full facility of the SEM, balancing specimen position, kV and probe current rather than coating. However sometimes you just have to fall back on that route, particularly for novice operators where it is so much easier to slap on a coat to keep life simple. Steve Chapman protrain@emcourses.com Thu Mar 24

Specimen Preparation:
carbon rods in evaporators

Our lab has a JEOL Vacuum Evaporator JEE-400 all a long but it's not frequently used. Recently i am trying to make use of this evaporator to establish a consistent carbon coating of certain thickness. Unfortunately there no one with expertise in our lab and I would to ask for some advice particularly on the sharpness of the carbon rod. Below is my question: How sharp must the carbon rod be for the contact? The JEOL manual illustrate a needle sharp tip but the JEOL carbon sharpener seem only able to thin the rod to a diameter of about 1 mm. Would that actually means that after using the JEOL carbon sharpener, I should find other ways to create a sharp tip? I appreciate very much for your kind advice! ***Yee Yan Tay one_twinklestar@yahoo.com.sg Mon Mar 28***

My own experience with a Cressington evaporator is that too fine a tip leads to unreliable evaporation—I spent a good deal of time getting the tip pencil sharp only to discover that it would spark up briefly then break before sufficient carbon had evaporated. A 1mm tip works well, I get control of the thickness by donning a pair of welding goggles and watching the arc light up, for general purpose grids I allow about a 2 second pulse from the point at which the arc reaches maximum brightness. Ian Portman i.j.portman@warwick.ac.uk Tue Mar 29

We used to form two wedges by moving the rod across a grinding paper. The wedge angle was approximately 30–45 degrees. We also had a rotation vacuum transfer rod, which allowed us to press one wedge against the other stationary one. Josef Zweck josef.zweck@physik.uni-regensburg.de Tue Mar 29

The ideal carbon rod combination is a spigot and a flat which is exactly what your JEOL sharpener is providing for you. The spigot system is better than a point system as it provides a possibility of prolonged coating due to the constant surface area of the contact point. A point system very soon erodes to a surface area that is too large for the evaporation to continue. Set the spigot to strike a flat surface with the sprung pressure being applied to the spigot rod. Remember the quality of a carbon coat is directly related to the vacuum level when setting up the system; give it time! Steve Chapman protrain@emcourses.com Tue Mar 29

Specimen Preparation:
membrane blebbing

I remember seeing some discussion (many years ago?), on the server about artifacts in microscopy of biological specimens in general. Specifically I think I read about membrane blebbing caused by aldehyde-based fixing. Does anyone have any actual references about this? I have seen what might be this effect, but it's hard to be sure. ***Peter Eaton petereaton@hotmail.com Sat Apr 30***

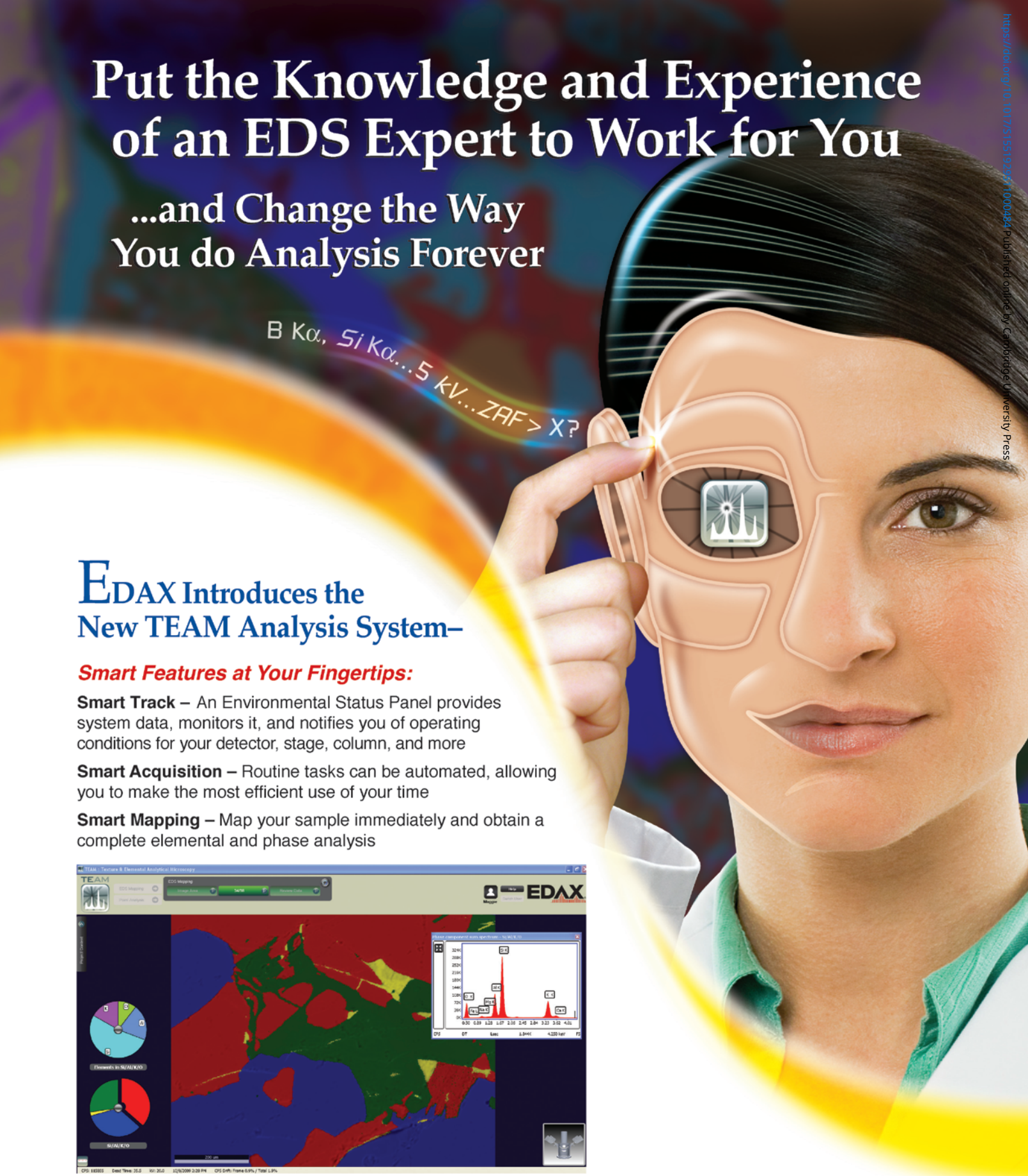
The only paper I'm readily familiar with (I'm sure there are others), is this one: G.J. Hyde, S. Lancelle, P.K. Hepler, A.R. Hardham (1991) Freeze substitution reveals a new model for sporangial cleavage in Phytophthora, a result with implications for cytokinesis in other eukaryotes. *Journal of Cell Science* **100**, 735–746; http://jcs.biologists.org/content/100/4/735.abstract. There are a number of citing articles on a similar theme. Rosemary White rosemary.white@csiro.au Mon May 2

This is a principle of science that any interaction with the specimen modifies its nature, however we all strive to limit the

Put the Knowledge and Experience of an EDS Expert to Work for You

...and Change the Way You do Analysis Forever

B Kα, Si Kα...5 kV...ZAF > X?



EDAX Introduces the New TEAM Analysis System–

Smart Features at Your Fingertips:

Smart Track – An Environmental Status Panel provides system data, monitors it, and notifies you of operating conditions for your detector, stage, column, and more

Smart Acquisition – Routine tasks can be automated, allowing you to make the most efficient use of your time

Smart Mapping – Map your sample immediately and obtain a complete elemental and phase analysis

TEAM Up with EDAX for SMART EDS Analysis.
Visit our website at ***www.EDAX.com/TEAMSMART***
or call ***1-201-529-4880****.*

AMETEK®
MATERIALS ANALYSIS DIVISION

EDAX®
advanced microanalysis solutions

modifications we introduce. The formaldehyde/glutaraldehyde mix is used for decades now and frankly I won't dare stand up against the huge amount of literature produced with the classical methods and claim that they introduce systematically significant artifacts. What you can say is that you lose some structures, that you don't see everything.

This is right (and this is confirmed by the use of cryofixation). The facts are that formaldehyde is a fast-penetrating fixative, but its fixation is weak and (at least partly) reversible. This is why it is often used in association with glutaraldehyde, which is slower but stronger. Both together generally offer a fixation of quality. However I have a problem with the original message of Pete and specifically with the part "artifacts . . . in general." There is no protocol that works "in general." All protocols must be adapted to the specimen. In general, with protocols well adapted for the specimen, formaldehyde fixation does not produce membrane blebbing artifacts. However it may well be that a general protocol is not suited for Pete's specimen. Also, generally formaldehyde fixation is completed by an osmium post-fixation step but this was not stated. Not knowing with which specimen Pete is working (and how) it is hard to guess what could be wrong, but I wouldn't be surprised if it had to do with osmoticity. Stephane Nizets nizets2@yahoo.com Mon May 2

An early paper showing membrane induced artifacts resulting from aldehyde fixation: Hasty & Hay (1978) Freeze-fracture studies of the developing cell surface. II. Particle-Free Membrane Blisters on Glutaraldehyde-Fixed Corneal Fibroblasts Are Artefacts. *J. Cell Biology* **78**:756–768.

There are other papers showing the dramatic change in morphology to mitochondria following aldehyde fixation compared to those prepared by high pressure freezing or quick-freezing using the metal mirror approach. I don't have a copy to double check but I believe that is shown in Terracio L, Bankston PW and McAteer JA (1981) Ultrastructural observations on tissues processed by a quick-freezing, rapid-drying method: Comparison with conventional specimen preparation. *Cryobiology* **18**(1):55–71. Here is a study showing aldehydes cause dramatic changes in endosomes: Murk JL, Posthuma G, Koster AJ, Geuze HJ, Verkleij AJ, Kleijmeer MJ, Humbel BM. (2003) Influence of aldehyde fixation on the morphology of endosomes and lysosomes: quantitative analysis and electron tomography. *J Microsc*. **212**(Pt 1):81–90. Tom Phillips phillipst@missouri.edu Mon May 2

Microtomy:
long-term storage of blocks

Does anyone have any opinions on whether resin blocks deteriorate over time (i.e. decades), and whether some storage options (airtight, low temperature . . . ?) are more appropriate than others? This has implications for type specimens deposited in museums, where the primary description of a species is based on TEM. Single-celled eukaryotes often have no other useful type material. Anecdotally, preservation of ultrastructure in some blocks from the 1960s has deteriorated since they were looked at in the 1980s—but it's unclear if this is a general pattern. ***Giselle Walker giselle.walker@anatomy.otago.ac.nz Mon Apr 4***

Are you thinking of deterioration of resin over time? Polymerization may not be completely over and done with within a day or two, but continue more and more slowly during storage. Also UV light exposure should probably be avoided. All this would make the blocks more brittle over time, by reducing chin length or breaking bonds. But there is also the risk that any of the used chemicals (e.g. OsO_4) is not completely inactive or thoroughly washed out before embedding. And there is not much one can do afterwards. I would therefore choose conditions that reduce reaction speed (Q10) in general, i.e. relatively low temperature storage away from direct light. Jan Leunissen leunissen@aurion.nl Mon Apr 4

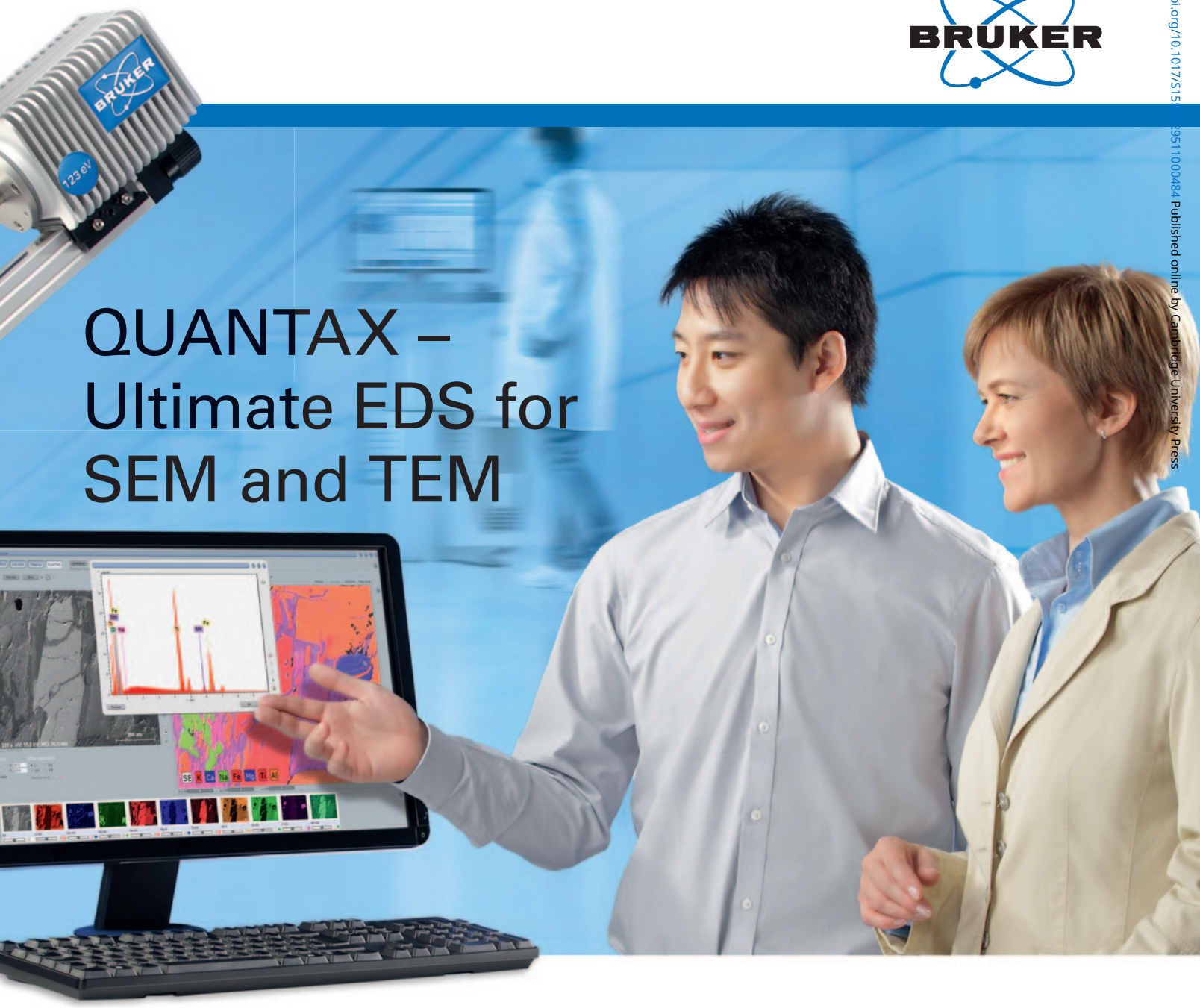
I just sectioned blocks from 1976 two weeks ago. The blocks cut fine, with the exception that some areas had not been infiltrated properly when they were initially processed. The tissue was of sea slug that was rare and irreplaceable. We got the information from the blocks that we needed. The plastic was Epon-araldite. Tissue preservation was perfect in properly embedded areas, and the plastic was stable in the beam. If the samples are prepared properly in the first place, they are permanently archived in the plastic for a long, long time. Store the blocks in a climate-controlled area. The blocks I cut were stored in someone's attic. I was surprised that they survived the harsh heat and cold in that environment! I have sections that I cut, still on grids, from 1974, that I can put in the TEM and photograph today. If the sections are stored properly, they are archival, too. They are not on carbon or Formvar films, just bare copper grids that have been kept in a low humidity environment all this time. I have re-scoped them several times before. It's been my experience that tissue deterioration in blocks comes from improper dehydration or infiltration during processing. If the samples were run up properly in the first place, the tissue is archival. It is possible, and I have seen this, to have a processing schedule that is barely usable, that just gets a person by. This produces tissue where the osmium is not fully washed out, or the tissue is not fully dehydrated, and the blocks are either soft or brittle. You may be able to cut the tissue initially, but the blocks deteriorate with time. Well processed tissue will not do this. The processing problems usually occur when trying to process tissue that is too large, or when running something up too fast, or when using old chemicals. That, at least, has been my experience. You may have come to similar conclusions. Epoxy blocks commonly used for E.M. should be archival. Lowicryls and LR White and Gold may pick up moisture with time and deteriorate. They should also be protected from UV light, since most of the work with these resins is for immunogold labeling, and antigenic preservation is the goal. I always remove my blocks from block molds. I don't know if the plastic from the mold will interact with the tissue or epoxy in the block with time. Ed Haller ehaller@health.usf.edu Tue Apr 5

Several years ago I sectioned blocks that were about 20 years old and they were perfect. These were ones that had not been previously used or trimmed. They had been kept in thin cardboard pill boxes inside 8 × 10 inch Kodak photographic paper boxes in a glass door cabinet. Humidity in the summer in Philadelphia is usually very high so I'd say that the lab ran about 80% even with the air conditioning on. Temperature in the lab would range from near 65 most of the winter (trouble with heating system in our ancient building) to high-70's in the summer. To my knowledge these blocks would have been made after I had run out of Epon 812 (original formula from Shell, for the younger members of our Listserv) but they just may have been embedded with the "good stuff"toward the end of my supply. The blocks were cured at 60°C for a minimum of 48 hours if not over a weekend. You mentioned that your blocks were from the 60's. That was still a time when Epon 812 was still the embedding of choice for many labs but there were also many other embeddings being used like Araldite/Epon mixtures with which I am unfamiliar. Patricia Stranen Connelly connellyps@nhlbi.nih.gov Tue Apr 5

Microtomy:
slide labels

I was wondering what others may be using to make their labels for glass slides permanent and insoluble to ultrasonic cleaning in ethanol. I've seen some slides where someone had labeled with a Sharpie, then

BRUKER



QUANTAX – Ultimate EDS for SEM and TEM

- Data collection at the highest speed and at the best resolution you can get – first class results no matter the circumstance
- Excellent light element performance with Mn Kα ≤ 123 eV (F Kα ≤ 54 eV, C Kα ≤ 46 eV) even at 100,000 cps
- Best acquisition conditions for sensitive and rough samples through unique multi-detector systems and optimum geometry using VZ-Adapters
- Genuine standardless P/B-ZAF quantification for rough samples and VZ applications, Cliff-Lorimer quantification for TEM

www.bruker.com/microanalysis

EDS

Innovation with Integrity

applied something akin to clear nail polish; except that I'd like to use something similar for covering a tape label. Whatever your method, would it be too much to ask that it also hold up to acetone? ***Michael Shaffer michael@shaffer.net Wed Apr 27***

If you have access to a hospital histology lab, ask the histotechs what they use (or borrow one to test). My histotech uses a Statmark Pen from StatLab Medical Products. They are moderately expensive and need to be capped to ensure they don't dry out, but they work.

I should also mention that we have a printer that we purchased that prints directly onto the painted end of a microscope slide. It is completely solvent resistant. The one we have is particular about the brand of slide we use, but my understanding is that when Thermo-Fisher bought the product line from the original designers, T-F re-worked it so that it was not so fussy. This works well for the 15,000+ histology slides that my University service lab does per year. We chose this product (Accuplace PSLIM) because it was the least expensive direct slide labeling device available at the time and our tests of solvent-resistant stick-on labels left us unimpressed. I have no commercial interest in this product. Doug Cromey dcromey@email.arizona.edu Wed Apr 27

Thanks to those who have responded, but I should've also mentioned that I don't want anyone here relying on interpreting someone else's script. Students come and they go, and I cannot read half of them, so it does need to be a labeling system. We have a Brother P-Touch labeler, but I wouldn't have considered the adhesive to survive many ethanol ultrasonic baths. However, I can put it to the test. Also because it would be labeling thousands of polished acrylic blocks, I can also consider a different labeler, because I want to archive these sections and hope they last. Michael Shaffer michael@shaffer.net Wed Apr 27

Instrumentation:
cleaning bell jar

One nice thing about having interested students is that they do not hesitate to ask questions that I have never thought much about. The latest one came up while demonstrating the coaters in the lab. A student asked what to use to clean them and how often. I answered that I usually use a little water, maybe some detergent or a special solution that makes it easier to clean with water. How often was less clear, I have seen labs where bell jars etc are cleaned after each use and labs where it doesn't look like the glass has ever been cleaned. What is your advice and experience? ***Jonathan Krupp jkrupp@deltacollege.edu Wed Apr 6***

I use Bon Ami and clean when it looks like it needs it. Nice and precise, yes? If I know I'm going to need a Wonderful Perfect Must Be High Resolution coat, I'll clean at least the day before and pump awhile to make sure any residual water vapor is gone. Philip Oshel oshel1pe@cmich.edu Wed Apr 6

Bon Ami, eh? Have you had any problems with this in terms of getting carbon films to float off mica? I recall years ago trying various things to make bell jars easier to clean. Some really worked, but I often had problems getting films to float off after using them, even with long periods of time elapsed between cleaning and film preparation. Since then I've only used a tiny bit of levigated alumina, spread onto a coarse paper towel moistened with a bit of ethanol. But it takes a lot of scrubbing to get the bell jar and bottom of the evaporator clean. If Bon Ami does the job, and still lets films float off, I'd be "high vacuum happy." Jim Ehrman jehrman@mta.ca Wed Apr 6

For sputter coater with rotary pump (which I use for magnifications not higher than 100k) I coat bell jar with a layer of soap. When jar is clean, I put some liquid soap (without moisturizer) on a wet piece of paper towel and wipe a jar. Then wipe it with a dry paper towel. For a few months (3–12) afterwards, I can easily remove a layer of metal just wiping it out with a piece of the dry paper towel; easy, fast and can be done in a few seconds. Vladimir M. Dusevich dusevichv@umkc.edu Wed Apr 6

For high vacuum evaporators: A dirty bell jar slows down pumping and potentially could contaminate specimens if you are doing high resolution work. We shield our electrodes as much as possible, so non-essential coating does not end up on the entire bell jar. That way, we only have to clean the small area surrounding the electrodes. We clean the entire bell jar maybe 4 times a year. We do coat the inside of the jar with Bell Bright, a detergent, which makes cleaning a simple task. You could fashion shields from something like aluminum foil—just be very careful not to contact any of the electrical feeds—and then discard the foil. For sputter coaters: We clean the glass containment vessel after each use. It's quick and easy, compared to the high-vac system. John J. Bozzola bozzola@siu.edu Wed Apr 6

Instrumentation:
humorous operator errors

I am a relatively new user and only operator of our Hitachi-7100 TEM. I went to use the instrument for the first time in a month and was not able to find the beam. I have bright illumination but no focused beam. I have backed out the apertures and specimen holder to adjust the Gun alignment to get the brightest illumination at the largest spot size. There looks to be somewhat of a beam at the top of the screen but I am unable to get it to the centre without losing it all together. ***Tracy Lawrence tracy.lawrence@inspection.gc.ca Thu Apr 28***

Thank you all for your suggestions. I hesitate to tell you what the problem was but I guess it's a learning experience . . . the shutter was closed. ***Tracy Lawrence Tracy.Lawrence@inspection.gc.ca Thu Apr 28***

OK, show of hands; how many of you have done this, even briefly? Tracy, we all have stories like yours! Welcome to the club. Tina (Weatherby) Carvalho tina@pbrc.hawaii.edu Thu Apr 28

I should tell you what it was, so you can smile as well: I was in Vienna last year to do some tests. And you should know that I am not actively involved with an institute, out of practice somewhat (in my defense, weak . . . but still). Had only worked with the Morgagni once or twice, trying to do all well along the instructions I had received. So . . . switched on HT, started up the filament . . . nothing. Checked if I had made a mistake . . . no, didn't seem like that, HT was on, we had a filament current as well as a beam current, but the TEM chamber was dark as. I thought: I'll be b . . . d if I have to ask for help, after all those years being an electron microscopist. . . . And of course there is also that feeling of looking silly. After 10 minutes of trying, I gave up and was about to get help. Then I turned the room lights on . . . and there was the reason for not getting any beam: the cover was still on the glass pane of the viewing chamber! I sat there laughing for a few minutes and felt so silly. Decided to tell the friendly crew in Vienna anyway, but they still tease me with it, it was so funny. Jan Leunissen leunissen@aurion.nl Thu Apr 28

I may have everyone beat, so my hand is definitely up. When doing cryo-EM on a Tecnai 12 with the specimen mounted in a Gatan 626 cryostage, one closes the gun valve to load the specimen, which has a cryoshield in place to prevent ice contamination of the grid. After the specimen is in place and the temperature has stabilized, the gun valve is opened, the cryoshield is retracted, and the specimen can be observed. At one time or another I have had the experience of not seeing the beam due to forgetting each of the three items: opening the valve, retracting the shield, and, of course, failing to remove the cover. Bill Tivol william.f.tivol@aero.org Thu Apr 28

https://doi.org/10.1017/S1551929511000484 Published online by Cambridge University Press

As Tina says, welcome—you are now a full, initiated member of the club. My worst happened when I was on vacation and a tech decided that the filament was blown. Getting an enterprising group together, including several academics, including the Unit Director. Well, suffice to say the Wehnelt was so badly cross threaded when they were done that we had to replace it. While I did not laugh at the time, it proved a very valuable teaching lesson—to me and for me to use. Since then I prepared a laminated checklist titled "What to do if the screen is black." It is well used, including by me. Paul R. Hazelton paul_hazelton@umanitoba.ca Thu Apr 28

Yes indeed, we all do experience such events. When I first joined the faculty of the University of Michigan the Department had just acquired an RCA Model EML TEM. The person who had been responsible for its purchase had left, and I was put in charge of it. One day I walked into the lab and tried to turn it on, but despite all my best efforts I couldn't get the thing running. Finally, I called the local RCA Service Engineer, Tony Boulet, for help. He showed up, pushed the main power switch a couple of times, then walked around behind the instrument and said, "It helps if you plug it in." It turned out that the darned thing wasn't hard wired into the electrical system, but simply had a power line that plugged into a wall outlet. Somehow or other the plug had come out of the socket and was laying peacefully on the floor. It was so embarrassing that it was funny. I ended up taking Tony out for a steak dinner, and I enjoyed his expert assistance and his friendship for many years thereafter. Wilbur C. Bigelow bigelow@umich.edu Thu Apr 28

There's also those of us (who will go unnamed) who forget to remove the digital camera from the side port of the microscope when finished using the microscope. When we fire up the scope the next day at low magnification and don't see the beam, it may take a while for the "light" to go on and remember that the camera's still blocking the beam! Ed Haller ehaller@health.usf.edu Fri Apr 29

LM:
phase contrast and refractive index

I was rereading the section of MicroscopyU on phase contrast microscopy, and one aspect of it confused me (again). I usually explain the contrast as a measure of refractive index differences between parts of the cell and the surrounding medium. The refractive index differences lead to differences in optical path length which leads to waves being out of phase. But changes in refractive index also deviates the light. MicrosopyU calls the deviated wave in phase the diffracted wave, but then proceeds to lay out the math in terms of refractive index. So what is the correct way to say this? Is it that the differences in the refractive index of the medium through which the diffracted light passes that changes the optical path length? Does deviation by refraction play any role? ***Dave Knecht david.knecht@uconn.edu Mon Apr 11***

I'm not sure about the official or definitive answer but diffraction from an aperture edge would happen in the absence of a refractive medium (such as in a vacuum). Diffraction is where a secondary wave (Huygens' secondary source) is created by scattering rather than a modification to a primary wave, which is what I think is called refraction. Diffracted waves have a stepwise difference in phase from the primary wave—often 90-degrees advanced. If the refraction of the cell and the medium differ (because of differences in refractive index and possibly thickness) then there will be optical path differences that translate directly into phase contrast, resulting from the recombination of the differently refracted waves. Diffraction is better known from the X-ray and electron study of crystals; and in the electron microscope combining diffracted with non-diffracted beams can deliver atomic resolution (lattice) images. Rob Keyse rok210@lehigh.edu Mon Apr 11

F. Zernike, *Z. Tech. Phys.*, **16** (1935), 848; *Physica*, **9**(1942), 686, 97F4. (references from Born and Wolfe, 7th ed, *Principles of Optics*) F. Zernike - Nobel Prize Talk: http://nobelprize.org/nobel_prizes/physics/laureates/1953/zernike-lecture.html. Fred Monson fmonson@wcupa.edu Mon Apr 11

The two descriptions—refractive index and diffraction—are equivalent, so it does not matter which description you use. Some people find the refractive index easier to understand, and others are more comfortable with the diffraction picture. Regarding your question about the role of deviation, remember that a lens transfers the light (or electrons in the EM) from one point in the specimen to one point in the image, regardless of the direction that the light leaves the specimen (as long as it is within the solid angle of the lens, of course). The total path length will depend on the angle of deviation, and this will affect the phase. In TEM the angles of deviation are very small, so the phase differences are correspondingly small, and the small-angle approximation is used, and the differences are ignored. In LM with a lens with high NA, some of the angles can be large, so some of the light may be sufficiently out of phase that the contrast is lowered. Bill Tivol william.f.tivol@aero.org Mon Apr 11

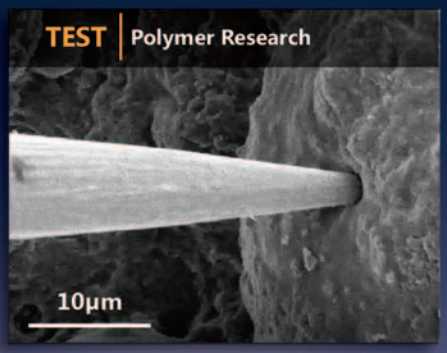
My question might be a bit basic for this list, but since we're on the subject. I've always had difficulty understanding how phase-contrast operates in terms of creating the two phases of light. (That way the two work to create contrast through interference I get.) But how does one light path for the background, and another sample/refracted light path off by 1/4 wavelength get created? I've never figured out how the condenser ring and the objective ring interact to do this. Peter Werner germpore@sonic.net Mon Apr 11

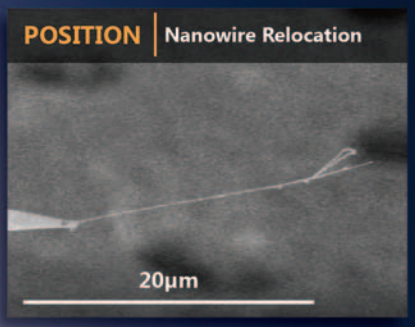
Here is the theory as I see it (correct if necessary): The phase shift is not only due to the refraction index but also to the specimen thickness. The phase shift is much more influenced by the refraction index and specimen thickness than by the difference in traveled distance due to different diffraction angles. As to the roles of the condenser annulus and phase ring: The condenser annulus produces a ring of light, such as the uninfluenced (background) light will arrive exactly on the phase ring (this must be precisely adjusted). The phase ring is represented by a special coating (or the glass is carved) in such a way that the background light is by 1/4 phase-shifted (whether positively or negatively). Because the diffracted light is naturally shifted by about 1/4 too (this is due to the refraction index and thickness of cells), the phase interference can be destructive (dark) or additive (bright) up to 180°. But even with the phase interference occurring that way, the background light would be too intense to obtain really a good contrast of small details, so the trick is to reduce the background light by up to 90° (again in the phase ring). This is an artificial (but effective way) to increase the proportion of interference light in the image creation process. In other words, without a phase ring the phase interference would still occur (although up to 90° and not 180°) but would be so small in comparison to the intense background light that it would not give a good contrast. Because the diffracted light does not follow a ring pattern like the background light but rather diffract in all directions, it will not be influenced by the phase ring and will join the background light (which passed through the phase ring) in the image plane. In other words the phase ring (along with the condenser annulus of course) allows the physical sorting of background and diffracted light. It is possible that I didn't use the very accurate terms but I think (and hope) that it makes the explanations easier to understand. Stephane Nizets nizets2@yahoo.com Wed Apr 13

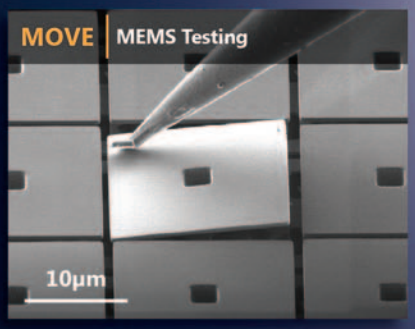
Your explanation sounds much like many other explanations I have read, and has the same problem that led to my posting the initial question. You start out talking about refractive index and then switch to talking about diffracted light. Diffraction and refraction are quite

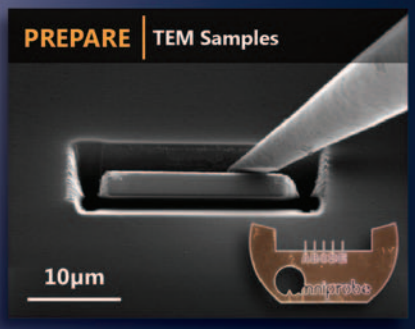
Enable Nanoscience

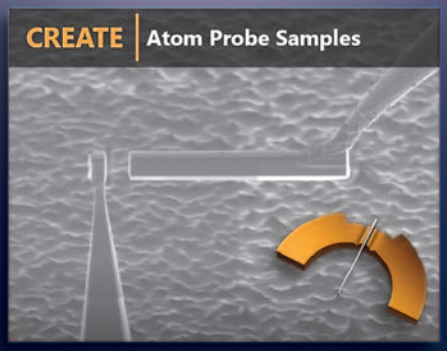
AutoProbe®
Nanomanipulators











Benefits Include: Port-Mounted, Frees Stage & Chamber Space | Move Linearly in ANY Direction with Nanometer Precision | Change Tips without Venting | Quickly Reorient Samples In Situ | Perform Electrical & Nanomechanical Testing | Easily Perform Nanomanipulation Tasks

Upcoming Conferences:

2011

M&M	Aug 7-11 - Nashville, TN
Microscopy Conference 2011	Aug 28 -Sep 2 - Kiel, Germany
AMFA	Aug 29 - Boston, MA
ISTFA	Nov 15-17 - San Jose, CA
MRS Fall	Nov 28-Dec 2 - Boston, MA

omniprobe®
EXTENDING FIB & SEM CAPABILITIES

P: 214.572.6800 | F: 214.572.6801 | http://www.omniprobe.com

different phenomenon and I can't see how they can be discussed as if they were interchangeable. The non-background light is shifted in path and phase either by refraction alone, diffraction alone or refraction of diffracted light or diffraction of refracted light and I am unclear which is the most accurate description. Dave Knecht david.knecht@uconn.edu Wed Apr 13

Abbe defined the formation of a microscope image as the result of interferences between diffracted wave fronts. But you also have refraction due to the difference in density (refractive index). Actually you get both phenomena if you observe cells. Both phenomena must be taken into account for phase contrast because they are responsible for different parts of the technique: The phase-shift, responsible for the contrast, is due to the refraction (as I said it is not enough to create a good contrast image though, you still need to phase-shift and dim the background light). The separation of background light from diffracted light is made possible by the diffraction phenomenon taking place in the specimen and by the presence of the phase ring at the objective (which specifically modifies the background light without affecting the diffracted light). In summary, both terms are definitely not interchangeable but they are still both playing a role in the formation of a phase-contrast image. Stephane Nizets nizets2@yahoo.com Thu Apr 14

EM:
chamber vacuum problems

We have EPMA SX100, in which there is problem in its chamber. After opening the chamber because of sample trapping (I opened the bottom of chamber and took out the samples), the vacuum isn't completed. Does anybody have an idea to help me to solve this problem. ***Kazem artur.irani@yahoo.com Tue Apr 19***

First action is to wipe your finger all of the way round the "O" ring surface; do not clean with a solvent. Then clean the flat plate surface in the area where the "O" ring would sit; here you may use a solvent. Do not grease the "O" ring. Steve Chapman protrain@emcourses.com Tue Apr 19

EM:
poor vacuum

We are trying to figure out what is wrong with our Zeiss TEM. The vacuum reads 5×10^{-3} milliBar and goes no further down on the gauge on the console. We have checked the seals on the gun chamber and camera chamber and they seem to be o.k. We checked the forepumps by disconnecting the vacuum lines from the column, and both are pulling over 30 lbs/sq in. Diffusion pump heater is going on, and the cooling water is cooling the outside. The Penning gauge was cleaned and that didn't change the reading. We think the main valve is opening because the gauge on the console only reads the column vacuum. We are running out of ideas. Perhaps there is something obvious that is preventing the column from pumping down. Ideas, anyone? ***Carol Heckman heckman@bgsu.edu Fri Mar 11***

I once had a leaky aperture changer, but only leaking in one of the three possible positions. Found it only by chance. Did you check if the pressure gets down when you are changing positions on one of the three aperture changers? Other possible points of interest: —all movable parts (plate camera, viewing screen, specimen movements)—what happens when you set the plate camera to atmospheric pressure? Fastest solution for a fix: get a leak tester. Stefan Diller stefan.diller@t-online.de Fri Mar 11

EM:
asynchronous electromagnetic interference

We're getting a new FEI650FEG SEM and just had the site survey. We've been running an ancient SEM in the room for many years. They have informed us that our room failed the asynchronous electromagnetic interference (ASYNC EMI) requirements. We can go ahead and get it installed with a waiver without fixing the ASYNC EMI issues and then try to fix them later if they are a problem. Alternatively, we can try and get it fixed now. Do any of you have experience with this sort of problem? Is it really a big problem or a little one? If it is a big problem that needs fixing, do you have recommendations on how to fix it? ***Robin Foley rfoley@uab.edu Wed Apr 20***

About a year ago we had faced the same problem before installation of FEI Magellan in a newly prepared room. The room has ASYNC EMI a bit higher than the pre-installation requirement states. We made several efforts with more than one EM expert, failed to identify the source of this EMI, but we mapped all the EMI sources neighboring the room and found that we can decrease their effect by installing EM-dissipating metal plates over surrounding walls. Then we installed the instrument and found no problem of interference at any scan condition, and the specs. were OK as well. Before the installation we asked FEI about possible effects of this over-spec ASYNC EMI. According to FEI, there could be flagging at specific scan condition set. Our ASYNC EMI was about 10% higher than specs. in only one direction. We decided to proceed with installation first, because the EMI was not too large and because FEI Magellan has extremely wide set of scan conditions at which we hoped to escaped flagging even if it will occur. Hope this will help you to make you decision. Inna Popov innap@savion.huji.ac.il Thu Apr 21

TEM:
beam stability

What is a reasonable length of time to wait for a beam to stabilize? I take a series of 256 images with a 120 kV TEM and have found that I have to wait one to two hours for the beam current to stabilize sufficiently to provide equidense images for montaging or analysis. The beam instability occurs even without apertures or a specimen inserted and with both tungsten and LaB_6 filaments—the LaB_6 does seem to take longer to stabilize. ***Larry Ackerman larry.ackerman@ucsf.edu Mon Mar 21***

There are two areas that need to stabilize, both thermally. One is the high voltage power supply. The components change value slightly with temperature so the output (actual kV and filament current) may drift slightly until everything equilibrates. The other area is the gun. The cathode to Wehnelt distance changes as the gun changes temperature (so does the Wehnelt to anode distance). That spacing is far more critical for LaB_6, hence the longer time to stabilize. My SEM customers who do e-beam lithography generally don't even reduce their LaB_6 cathodes to the standby temperature. They leave the gun fully at temperature and saturated. The cost is that the cathodes get replaced every 6 months. Since they often operate at 40 kV (high for an SEM), I always had them reduce the kV to 20 when they weren't using the system. Aside from protecting the high voltage power supply and some of the gun components, this also keeps the lenses warm. Yes, that's a third area that needs to stabilize thermally, but it doesn't seem to be as critical as the first two areas. If your TEM is stabilizing in 1–2 hours from a cold start, I'm stunned! The thermal masses involved in both the power supply and the gun are probably far greater than most SEMs. If it takes that long from a semi-warmed up standby situation, then there might possibly be some kind of issue, but maybe not. 1–2 hours for high stability doesn't strike me as at all unreasonable. Ken Converse kenconverse@qualityimages.biz Mon Mar 21

The time to stabilize that I mentioned starts when the filament is heated after a sample change. The high voltage is never off—for

months and the lenses at 40,000× when not doing microscopy which is typically 1000–10,000×. Larry Ackerman larry.ackerman@ucsf.edu Mon Mar 21

To this point: in our faculty-EM-facility, we are using a 120 keV TEM, vintage 1990 - daily, with (regularly) 10+ different users. Standard voltage is 120 keV, always, for more than 18 years. LaB_6, always; lifetime; about 3 or up to 3.5 years. Vacuum excellent and stable: Rot, ODP plus IGP, plus always LN_2; cryo cycle (IGP off) every evening for 4 hrs; computer-controlled HT and filament start every morning, with wobbling the HT at 120 keV. — LaB_6 is cold for specimen change, always; re-heated for 19 × 5 sec, each time. After this, we can start recording "equidense" images almost immediately (after 3 to 5 min "searching"). —Yes, some Master or even PhD students don't see or experience a change of a filament. (I have an old one, on the bench, for demo). We take digital micrographs on a 12 bit 1k camera for the last 12 years, with lots of stitching, daily, and never experience problems with instability or uneven or varying density (at least not observed / observable!). We do take montages with usually 4 × 4, up to 8 × 8 (or more) frames. —If there are problems, then they are related to "too old" dark and flat fields, which are used for correcting the digital images. Dark/Flat fields are needed about once a week, but sometimes only every 2 or 3 weeks. (no cryo work at very low dose!) —Only problems are when working with new or old filament. Reinhard Rachel reinhard.rachel@biologie.uni-regensburg.de Mon Mar 21

After many years of taking commissioning test pictures to prove the performance of TEM and SEM it is clear there are a number of features that need to be taken into account. In my early days as a service technician (probably 1965 to 1970) I was amazed how it always took me about three hours to "get in the groove" when I was taking pictures for final commissioning. Then I noticed if I simply switched the high voltage on, with the specimen in the instrument, and to return about two to three hours later, I had a test picture within half an hour. Eureka, when I then realized about high voltage stability—heat gained must equal heat lost. Having a very nice meal with a client one evening he suggested that we go back to the lab and I take a test picture on the TEM that they have just moved from one lab to another. With the same simplistic reasoning I thought "Oh no three hours!" But I had forgotten the instrument had a gas filled tank and was thrilled to within forty five minutes have the test resolution actually visible on the screen; no picture required! Silly me, I always thought they gave us a gas filled tank so it was less of a mess going in to change components, not that it cut down mass! Working with automated analytical SEM we found that the backscattered electron results and x-ray emission did not become stable for some hours after specimen exchange. Investigating the problem, colleagues found that when the vacuum in the gun became absolutely stable so did the results! Outgassing the samples prior to placing them in the microscope halved the time for stability to be reached. Of course a cathode assembly will need to become thermally stable, but it is the other areas that take the longest time. So putting all the knowledge together here are the results—Transmission Instruments: (1) High voltage generator—the critical period is the time taken for heat gained to equal heat lost once the high voltage has been switched on. This relates to the type of high voltage tank (generator box if you like). (a) Oil filled tanks take up to three hours to stabilize depending on how large the tank is. (b) Gas filled tanks generally take about an hour or so to reach stability. (2) The vacuum system in the gun also has to be absolutely stable, the speed of pumping matching the leakage. This will relate to when the gun chamber was last opened. Scanning Instruments: (1) The high voltage tank is very much smaller and in general stability is reached within a hour. (2) If the electron gun is brought to atmosphere each time a specimen is exchanged, depending how gassy the routine specimens are, it may take over two hours for the gun vacuum to stabilize. Both of these sets of comments assume that there are no electronic problems with the instruments high voltage circuitry. Steve Chapman protrain@emcourses.com Tue Mar 22

TEM:
diffraction pattern

I am looking for some tips to collect a diffraction pattern of nanoparticles using a TEM JEM2100 with a high resolution camera Gatan Orius SC600, without damaging the sensitive CCD. I appreciate any help. ***Marcela Redigolo marcela.redigolo@mail.wvu.edu Thu Mar 31***

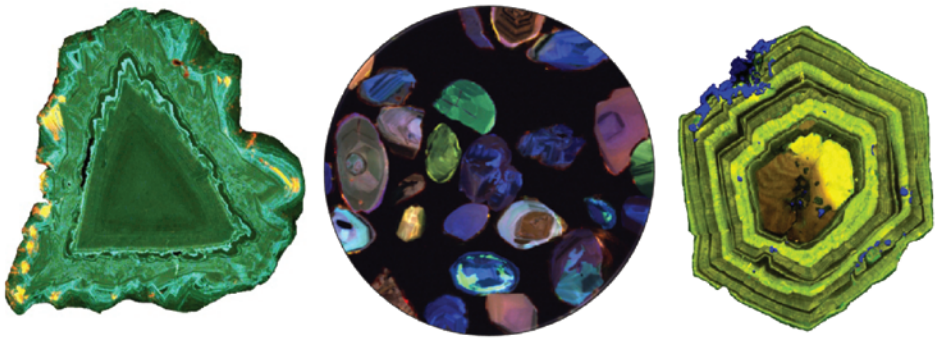
Your TEM needs to have a beam stop above the camera to capture diffraction patterns. Typically this is inserted and adjusted with a small knob on the side of the column just above the viewing chamber. Normally you would get the diffraction pattern you want to record on the fluorescent viewing screen, then insert the beam stop to a point that covers the bright central incident spot of the diffraction pattern, and then reduce the brightness of the pattern as much as possible with the brightness/intensity/C2 knob to reduce the possibility of damage to the CCD. After this is done it should be ok to lift the fluorescent screen and acquire an image with the camera. Once you have acquired the image, immediately lower the fluorescent screen again to protect the CCD. If you need to change camera lengths, do not do this while the pattern is being viewed/recorded on the camera because the pattern may change position and the central spot could move away from the beam stop and damage the CCD. Bradford Ross bnross@interchange.ubc.ca Thu Mar 31

Thanks for your reply! This is the process I am used to when working with other TEMs. This TEM we have has two cameras. The upper one used for great part of the measurements, and the SC600 that was installed for high resolution imaging for certain interface analyses. I was wondering if, different from the usual process, is there any other settings to use with this camera without causing damage. I am new to this microscope and apparently, in the past someone caused a damage to the camera, because of the diffraction pattern. It's my belief that something was done wrong and the usual process would work just fine. But I decided to check just to be sure I'm not missing anything. Marcela Redigolo marcela.redigolo@mail.wvu.edu Thu Mar 31

If you have an Orius camera you are well set up for diffraction. They are almost bullet proof and have anti-blooming, so spots stay sharp. If you are sensible and careful there is no need for a beam stop. Use small condenser apertures and parallel illumination with a selected area aperture and well-focused SADP, you can have the pattern on the screen for minutes with no obvious damage. Our camera was damaged by a user who had a large condenser aperture, no SA aperture, and an unfocused diffraction pattern giving a very high intensity caustic. That user didn't really know what they were doing; before it would only been a sheet of film but getting a digital camera fixed is expensive. Richard Beanland contact@integrityscientific.com Fri Apr 1

Occasionally we have requests for diffraction patterns on the slow scan CCD camera on our FEGTEMs. Two tips I can offer are these: (1) Start with high spot sizes (high excitations of the first condenser lens). This will reduce the beam current, but improves

Cathodoluminescence
Topical Conference



October 24 - 28, 2011, NIST Gaithersburg, MD

http://www.microbeamanalysis.org/topical-conferences/cl-2011/index.htm

The CL TC is limited to 80 participants. Early registration is essential.

Cathodoluminescence provides high sensitivity spectra and high resolution images associated with impurity and structural defects in ***nanoscale and bulk luminescent materials*** including minerals, semiconductor, and dielectric/ insulating materials.

The CL TC will include demonstrations of advanced CL instrumentation, CL analysis techniques, and software. There will be sessions on CL theory, CL applications and experimental best practice, and CL combined with other complementary techniques etc., as well as presentations by professionals and students. The CL TC is recommended for experts and novices alike, and student support is available.

Sponsored by ***GATAN*** with additional generous support from ***JEOL, Attolight, Horiba, and Leica***.

CL TC 2011 NIST GAITHERSBURG MD USA

MAS MICROANALYSIS Society

AMAS AUSTRALIA

Images courtesy of CSIRO, Australia

the sharpness of the diffraction spots (all other things being equal). (2) Keep the exposure times reasonably short (<0.1 s) in the first instance. Check the maximum intensity against the dynamic range of the camera (your 14-bit camera may have a realistic maximum intensity of 14000 counts per pixel). Find the exposure time that will give get about 10000 counts maximum and then use multiple exposures to boost the total acquisition time. In Digital Micrograph this will be via "Camera>Acquisition Parameters>Acquire" scroll down menus. On the "GIF CCD Setup" tab you will see a panel labeled "Processing" and "Frame Sum(# frames)". This is usually set to "1" for a single shot image. Set this to 10 or more for a high signal diffraction pattern. I've used this method a number of times to get reasonably sharp diffraction patterns with good success. Jon Barnard jsb43@hermes.cam.ac.uk Fri Apr 1

TEM:
viewing $CdHgTe_2$

Has anyone had experience looking at $CdHgTe_2$ on a TEM? I expect that using a cryo stage would be the best way to inhibit contamination of the scope from the volatile components. Is this the case, and are there other methods that would allow this compound to be imaged without damage to the scope? ***Bill Tivol william.f.tivol@aero.org Fri Mar 25***

I'm not familiar with the compound, but have heard wonderful (terrible?) stories about what can happen to a high voltage power supply when Hg becomes Hg vapor and rather conductive. I would think that if the Hg is tightly bound chemically, then it shouldn't be an issue, but if you have any doubts, I think cryo might be a good bet, or at least a hedge. The biggest problem seems to be its migration after being vaporized. If you don't get rid of all of it, you'll blow up your HVPS again. Ken Converse kenconverse@qualityimages.biz Fri Mar 25

Before we have too much panic, it might help to remember that for many years, JEOL used a mercury diffusion pump in their tandem pumping system for their best 100 and 200 keV TEMs. Such devices intentionally produce massive amounts of mercury vapor. Jim Pawley jbpawley@wisc.edu Fri Mar 25

Massive amount of Hg vapor in the room? nizets2@yahoo.com Mon Mar 28

Your big problem will be making specimens that do not have point defects galore all over the surface. I believe Tony Cullis did a lot of $CdHgTe_2$ work back in the 80's, and that was one of the motivators to develop iodine milling. I don't recall any problems with the compound volatilizing. John Mardinly john.mardinly@asu.edu Fri Mar 25

TEM:
magnetic particles

I have a request for imaging magnetic nanoparticles in TEM. The particles are currently in dry powder form. They are easily attracted to a small permanent magnet. My initial reaction is to refuse the request for fear of coating the objective lens with magnetic particles. However, perhaps someone has successfully imaged such and can advise a safe method for introducing them into the TEM. ***Roger A. Ristau raristau@ims.uconn.edu Tue Apr 26***

No problem with magnetic nanoparticles—this is one of my specialties, and I do it all the time with no problem at all. Just take care that they are dispersed, by creating a suspension in water, shaking all particles apart by a supersonic bath and then drip a droplet on a holey carbon grid, before they re-coagulate. The smaller the individual particle, the less the magnetic force acting on it. The force is proportional to the integral over the particle's volume taken over its magnetization times the gradient of the magnetic field. (You may want to check http://en.wikipedia.org/wiki/Magnetic_force_microscope). The gradient is usually small, although the total field is around 1–2 Tesla for a 300 kV microscope, as is the volume of the specimen. Thus the total force is small. Care should be taken for extremely high-magnetic moment particles above a certain size. I also recommend to switch off the lenses (objective is most important) during specimen insertion, as the stray fields at the vacuum transfer port may be rather large. Joe Zweck Josef.Zweck@physik.uni-regensburg.de Tue Apr 26

Should you find a way of mounting the particles in safety may I help on the observation side? With any magnetic material it is useful to reduce the level of the magnetic field in the objective lens. This is easily done using the eucentric stage to raise the specimen within the pole piece. With a "normal" specimen adjust the eucentric system so that as you change the Z prime you need to turn the objective lens anti clockwise. There are a few millimeters available to you and this will have quite an effect upon the lens field. Remember your magnifications will be lower and your contrast will be higher. The magnification change due to the weaker lens and the contrast due to increasing the specimen to aperture distance and increasing aberrations. Whilst I rarely carry out this action in this direction but often obtain more than the manufacturer claims in resolution by working the Z prime to lower the specimen within the lens, increasing the lens field and reducing its aberrations. Steve Chapman protrain@emcourses.com Tue Apr 26

When I was at Western Digital, the SEMs would periodically need to have the final pole piece cleaned of magnetic particles, and these samples were solid sputtered films with obvious no loose particles and sub 10 nm grain size, not powders. Be very careful with loose particles. A. John Mardinly john.mardinly@asu.edu Tue Apr 26

In order to avoid any problem with deposition of nanoparticles on OL pole piece you can adopt the extreme solution of embedding the particles in a resin and afterwards processing the sample for TEM as if it were a solid sample. It is time consuming, but is the safest. I use that procedure when I do TEM on small grains of magnetic alloys. It takes time and effort to prepare the sample, but it guarantees no deposition of particles on OL pole piece will occur. Corneliu Sarbu crnl_srbu@yahoo.com Sat Apr 30

TEM:
compressed air

We have a major problem with compressed air for our Tecnai G20 TEM. I'll try to be as precise as possible but in the end I think that the answer should be quite standard. I can remember that in the end of last year we had a discussion on the list about how important it was to make sure that the compressed air arriving at the TEM is dry to avoid wasting the valves. I took this message seriously and reviewed our own system but I found it to be well conceived since we had (1) a large pressure container which can collect a large volume of water between the compressor and the pipes. (2) a kind of filter after the container, although I admit I don't really know its function. I guessed it was there to retain water. The problem itself: about 2 weeks ago during a routine check we noticed that the small container located in the flow meter rack, which purpose is to collect the rest of condensed water from the pipes and which remained completely dry for 5 years was completely full with water. At that time it seemed that we came in time and the water did not overflow in the tubing to the microscope parts. The container

Expand your Knowledge of Microscopy with MSA Membership!

Whether your primary focus is in light, electron, or scanning probe microscopy, or the biological or the physical sciences, MSA takes your knowledge to the next level!

Members Receive:

- A personal subscription to MSA's official journal, *Microscopy and Microanalysis*, and MSA's popular bi-monthly magazine, *Microscopy Today*.
- Peer Networking through the Society's Focused Interest Groups and Local Affiliated Societies.
- Discounts on books, journals and other educational materials.
- MSA Awards Programs, Scholarships, Speaker Opportunities, and more!

MSA
Microscopy Society of America

Join MSA Today!
Visit: www.microscopy.org or call 1-800-538-3672

was purged. Incidentally, several days later, we had a planned power cutoff because of works on the main line and I decided to switch off the microscope completely. I was confident that with the power off there was no risk for the microscope until the problem of condensed water was solved by the engineers. However, several days later, with the system still switched off, we noticed again that the container in the flow meter rack was completely full, this time we could also see water in the transparent tubing going to the microscope. Some tubes are blue so we can't see through. Now we blocked the compressed air entry to the flow meter rack with a valve but I need advice for the cleaning steps. The main question is: may I clean the tubes myself or is there a risk that I make a major mistake? And then, if I can do it myself, how should I proceed? What should I be aware of? I have nitrogen and SF_6 tanks at my disposal to dry the tubes if it is necessary. ***Stephane Nizets nizets2@yahoo.com Fri Apr 22***

Firstly I feel there is a misunderstanding in relation to the compressed air and moisture. Moisture, anywhere in the system, is a big problem. The compressed air when generated or contained in a moist zone will carry the moisture to the control valves which usually have aluminum bases. When an aluminum control valve becomes moist it will oxidize, which ultimately damages the valve and causes an air leak; hissing! How to minimize the damage that your problem may cause? Firstly drain all of the compressed air containers; there should be a tap at the base of each. Now it is down to ideas on cleaning the lines. If it was me I would disconnect the input line to the microscope and blow through the pipes with compressed air from another "dry" source or from as gas bottle (nitrogen would be fine). Cleaning the actuation valves without taking them all apart and blowing though is I think an impossible task. Steve Chapman protrain@emcourses.com Fri Apr 22

Thanks to all who offered their advice. I have unplugged all the lines and they all contained water, but I am quite sure that the water was lying in the bottom because the system was off so there was no air movement. We'll probably replace the lines because we are almost sure that the water contained some oil. It is still a mystery why this happened but I don't want to care about it anymore. In the future we'll connect an N_2 bottle in place of compressed air for the valves. No way that I worry again to waste 600,000 euros worth of material for some stupid moisture (you may replace "stupid" by other not-politically-correct words here, to better reflect my thoughts). Stephane Nizets nizets2@yahoo.com Tue Apr 26

I didn't chime in on the original question, but I would like to add my "2 cents" as they say on the general issue of compressed gases. When we designed our new microscopy facility we found a large number of instruments using a large number of compressed air cylinders for valve control and N_2 cylinders for vent gas. We then did a cost analysis and found that we could replace all this cylinder usage by a cheaper and easier means. For the compressed air cylinders we designed an air drying system which utilized already clean and compressed dry air from the chemistry building to which we added two large capacity moisture dryers in parallel (so one could be changed while using the other) which happens about every three years or so and also a final activated carbon filter to remove hydrocarbons and a particle filter which gives us extremely dry, HC free compressed air at 110 psi. This compressed air is supplied to each instrument's gallery section to control instrument valves and the air moisture is monitored and connected to an alarm system. For N_2 use, this dry clean air is supplied by an analytical grade N_2 generation system (from Perkin Elmer) designed for ICP-MS systems so the purity is 99.996% or better with an N_2 generation rate of 12 liters per minute. The unit cost around $15K and uses a membrane filter that costs about $100 every 3 years to replace. This N_2 gas is also supplied to each instrument's gallery section for use in venting. With these two systems we can supply around 16 different analytical instruments and never buy/rent/haul or connect an air or N_2 compressed gas cylinder again. I highly recommend this type of system for both low cost and ease of use. I have to admit, I never liked hauling those cylinders around very much. The only compressed gases we still buy are a few P-10 and Ar cylinders which are used at very low rates and changed every 6 months to one year or so. The only caveat I would add is that if you hire contractors to plumb such a system in your lab you should specify "medical grade" plumbing which means they don't use any fluxes which could contaminate the system. One can also use Silphos solder while flowing N_2 gas through the copper (used in refrigeration systems) which is also a fluxless method to prevent flux or scale formation inside the tubing. John Donovan donovan@uoregon.edu Tue Apr 26

I had an IDE air system on a 2010F fill up with water once when we went too long without purging the compressor. The replacement system had an automatic purge and an air dryer in series. Be advised that using nitrogen cylinders for valves is ill advised: valve actuators tend to develop leaks over time, and a catastrophic leak could not only deplete your cylinder rapidly, it could fill the room with asphyxiant, which is an unexpected but lethal consequence. There were multiple fatalities from nitrogen use when I was at Intel. John Mardinly John.Mardinly@asu.edu Tue Apr 26

My old advisor at Michigan, Wil Bigelow, had a unique and cost effective method of capturing ultra high purity nitrogen gas for venting microscopes. There was an added bonus in that no regulator was required, and it could never overpressurize the chamber and damage ultrathin detector windows. The cost? Under $20! How? He would put a rubber stopper in the EDX Dewar with a tube in it for attaching a surgical rubber hose that ran to an extra large beach ball. That hose was teed to another hose that ran to the microscope vent inlet. A small slit in the surgical rubber hose provided over-pressure protection. Boil-off from the EDX Dewar would fill the beach ball, and that nitrogen would be used to vent the microscope. The only disadvantage is that some EDX systems have an alarm for sensing low LN_2 levels that goes through the fill opening, and that would be difficult to accommodate. I might add that it made the lab look a LOT more cheery and colorful, which was a nice thing to have during the dreary Michigan winters. John Mardinly john.mardinly@asu.edu Tue Apr 26

I have always been cautious with my liquid N_2 for the reasons John raises but have never thought about compressed N_2 tanks. In my microtome room, I run 3 N_2 tanks to blow off debris from my ultramicrotome knives. It is a lot cheaper and environmentally nicer than using those old compressed Freon cans of gas. Am I really running a serious risk here? It is a relatively big room with an open door into the main lab. Comments? Tom Phillips PhillipsT@missouri.edu Tue Apr 26

John's post got me thinking too. I am running some valves with N_2 because our CDA (compressed dry Air) supply died three times which crashed the vacuum logic of our 300 mm FIB (bad!). Almost killed a Seiko turbo! Now I am rethinking and maybe will go back to CDA—or install a nitrogen alarm—the problem is the unending supply of nitrogen. Bryan Tracy bryan.tracy@spansion.com Tue Apr 26

In any case, a single bottle is not nearly the hazard that plumbed in nitrogen is. A. John Mardinly john.mardinly@asu.edu Tue Apr 26

Just a word of caution about using gases in cylinders. We used to use 99.999% nitrogen on our microscopes but discovered water

https://doi.org/10.1017/S1551929511000484 Published online by Cambridge University Press

and other contaminants in the gas lines. We asked the gas supplier and they checked a number of the "high purity" cylinders of different gases only to discover water, rust and other "crud" in them. We now use the nitrogen gas blow off from a 3000 liter liquid nitrogen vessel with multiple fail-safes in the line and multiple oxygen level monitors (and safety lock-outs) in all the labs that utilize this source. We have a single cylinder as a back up if the main vessel has to be disconnected. Colin Veitch colin.veitch@csiro.au Tue Apr 26

I just learned a few things in a compressed gas course I took recently, and that is that tanks should not ever be fully emptied because then they need to be purged and cleaned before being refilled so that they do not get contaminants in them. Trouble is 99% of gas users do not know this, and run tanks until nothing comes out. Then since they did not notify the gas vendor, they did not clean or purge the cylinder. Another thing is that the instructor said tanks are supposed to be immersed in water to cool them while being refilled. Perhaps that is one way they can pick up water. John Mardinly john.mardinly@asu.edu Tue Apr 26

John makes a good point about plumbed in N_2 (or should we be saying "coppered in" nowadays?). I think this is a real hazard, which is why all our N_2 lines terminate in a separately ventilated equipment gallery that surrounds our lab complex, not to mention that we have O_2 monitors as well. All the fume hoods and room ventilation are also on emergency power in addition to the instruments. Another point worth raising is that each instrument has different requirements for psi delivery so at each instrument station in the equipment gallery we provide a two stage regulator to take it from 110 psi to a more moderate pressure, say 10 to 60 psi and then for those instruments that require further stepping down, an additional precision low pressure regulator for regulating from zero to 2 or 3 psi. John Donovan donovan@uoregon.edu Tue Apr 26

We have two 160 liter LN_2 Dewars for general usage in our lab. We tap off the head pressure with standard compressed air connections typical of what is used in auto repair shops connected to a 3/8" OD Dekoron tubing manifold to all our labs. The head pressure is normally about 20 psi. We use this free, dry and very pure nitrogen gas to vent, purge and backfill all our instruments with a low pressure regulator at the instrument. It has worked well without any problems for quite some time. With two supply Dewars we can switch from one source to the other when either supply is empty resulting in a constant nitrogen source. 20 psi may not be enough for some applications but for most of our uses it is adequate. Fran Laabs fclaabs@iastate.edu Wed Apr 27

SEM:
magnification

I am curious about the distribution of magnification in the images we've acquired with our (tungsten-based) SEM. Since we've logged all the images it wasn't hard to find out: http://www.mta.ca/dmf/sem_mag_dist.html. We do SEM for many different departments, but the bulk of our work is biological, with heavy emphasis on microorganisms, particularly diatoms. My questions to others out there that might have been in similar circumstances are: (1) Do you feel that this sort of distribution is determined more by the instrument capabilities, or the inherent properties of the specimens observed? (2) If we were go for a ~5–10× $$$$ investment in a field emission scope (a) would this distribution of magnifications change significantly and (b) would the upgrade be demonstrably worthwhile? I realize there is a big pile of ifs/ands/buts in these questions—I'm just looking for people who are willing to share thoughts, particularly if they have experience in taking this route. ***Jim Ehrman jehrman@mta.ca Thu Mar 3***

I read with interest your recent posting on magnifications people use in your SEM lab, as well as the comments of other colleagues, and I would like to add a few words: To your first question if magnification is determined more by the instrument capabilities or the inherent properties of the specimens observed, I would say frankly: It's the specimens! I went through your site and saw your very decent micrographs that combine a fine resolution with a nice depth of field. And saw that your JEOL instrument has a 3–4 nm resolution limit that is not so far from Tina's Hitachi with 1nm resolution. Do you think this can make a great difference in your work? We all know that to reach these low numbers we need to have a perfectly working source of electrons, a perfectly aligned scope, a perfectly attached and coated specimen (like the ones for resolution assessment) and a perfect focus and stigma etc. Obviously imperfections on these parameters will stop us well before the ultimate resolution, and usually at around 10 nm. Biology microscopists are like detectives, probably more than colleagues from other fields of microscopy. We start from a live organism where the cellular functions including plasma membrane morphology are changing every little fraction of a second. We have to kill this organism as soon as possible and fix it, then to take out the 70 or 80% of water and liquid from around and inside it, and then coat it with metal (if we want to enjoy the clear high resolution images of coated specimens as opposed to native state cells viewed in low vacuum instruments). Then we take images and we try to guess what the morphology can tell as in relation to the cell's function. To me sounds impossible that after all these torments the morphology of the cell will stay intact at the level of 1 or 2 nm that is really the size of big biomolecules. And I think that if I could go so small in cell specimens I would feel really embarrassed: For I would have to describe structures appearing at this level that could be mostly artifacts. For instance the cover on *Science* magazine that Tina showed us is a microbe with its surface having thousands of tiny grains at the size of a few nanometers. Do these grains represent any real structures or they are artifacts? And if the micrograph is only for decoration, that's fine. But if it is part of the research findings, it could be a trouble for the researcher to comment on it. Nevertheless here I have to admit that my SEM experience is limited to eukaryotic cells. It's amazing how we tend to get crazy with numbers and magnifications. It happened to me several times to take a high magnification picture on a biological specimen (high for me is like 30K) only to find out afterwards that this picture was not any better from a 5K one. For obtaining a nice clear micrograph there are other parameters more important than the instrument's ultimate resolution. Apart from those already mentioned it is also the averaging and oversampling parameters—in other words, how much time the electron beam scans the specimen. And of course the depth of field is critical for the uneven surfaces found in biological samples. For me a real improvement in biology SEM would be a substantial increase in the depth of field, so that more of the specimen can be in focus. Yorgos Nikas eikonika@otenet.gr Fri Mar 4

SEM:
venting problem

Our JEOL JSM-5600LV has a problem with the vacuum system. In particular, it doesn't vent the chamber. Maybe it's a problem of some valves, but before I dismantle and check them I'd prefer to vent the system in a way safe for the EDS window. Does anyone know a safe way to vent manually? ***Davide Cristofori dcristofori@unive.it Mon Mar 7***

On my old JEOL 840, this happens when the compressed nitrogen tank that provides the gas to vent the system runs out.

https://doi.org/10.1017/S1551929511000484 Published online by Cambridge University Press

Since a cylinder lasts for years, it is easy to forget about. Dave Wilbur Headers David.Wilbur@tufts.edu Tue Mar 8

I do not know the type of valve that you are discussing but may I offer some general advice on trying to fix it? (1) Switch off the microscope at the wall. (2) Work with the idea that someone had to build it therefore there must be a way of taking it apart. (3) Have someone with you so that you can dictate what you did to take the unit apart, it will help when reversing the process. (4) Expect a spring under tension to be inside the unit so take great care when finally opening it. (5) A standard reason for such an item to jam is the "O" ring has dried out and it is sticking to the degraded grease within the chamber. (6) Give the chamber and the plunger a good clean and use a good quality vacuum grease on the "O" ring. Steve Chapman protrain@emcourses.com Wed Mar 9

I found the problem was that the piston of a mechanical valve is slowed down, which prevented a complete the venting sequence. I helped the piston movement by pushing it gently, and the sequence could be completed. Now I hope it's enough to clean the valve, though the dismantling won't be that easy. Davide Cristofori dcristofori@unive.it Wed Mar 9

EDS:
unexpected Si peak

I use a 2000FX TEM at 200kV equipped with an Oxford Inca EDS, ultra-thin window Si-Li detector. Sometimes I get Si K line from a sample which should have no Si at all. Can anybody please explain where the Si peak comes from? ***Zhou z.zhou@lboro.ac.uk Wed Mar 30***

I can think of two reasons for seeing the Si K peak. First is the silicon internal fluorescence peak, that is, the SiLi detector itself emits Si K x-rays from the "dead layer," which are then detected by the "active layer" of the detector. I have experienced this in some detectors more than in others. Second may be contamination from Si-based vacuum grease, transferred from o-ring to loadlock wall to sample holder tip, during sample holder insertion and retraction. Of course, it could also be from sample contamination: We had an associate that habitually secured the TEM sample to the ion-milling stub with Si-grease. This got transferred to the TEM holder and appeared in all EDX spectra on that instrument. A thorough cleaning of the TEM sample holder eliminated the Si K peak due to this problem. Roger A. Ristau raristau@ims.uconn.edu Thu Mar 31

I ran into one additional source of Si. A specimen had been placed on a glass slide and subjected to evaporation, then embedded and sectioned. Sure enough, there was a prominent Si peak. Bill Tivol william.f.tivol@aero.org Thu Mar 31

Most modern detectors have much better resolution that in the past, but you may want to see if you have an overlap with a heavier element. If memory serves me Ta or W had m lines close to the Si K line. If your Si line is very high then this will be less likely. Your settings on the Oxford may be such that if you are using auto ID the software is identifying it as Si to be sure you look for it. Tommy Derflinger gtuser@comcast.net Thu Mar 31

This is a good subject for discussion. A way to identify the Si source is to collect the spectra at difference spots. If the Si is seen on the particle (or phase A), but not on the support film background (or phase B), then we can say the Si is from the sample itself. In the case Si is seen everywhere even on the hole without specimen, then it is from the TEM system—service is needed! Considering the spurious signals in the TEM, the different spots tested should be far away enough. Occasionally I receive the complaints about the Si that is not supposed to have, mostly from the chemistry users. I prove to them that it is the sample contaminated with Si, rather than the TEM. If the TEM is well aligned, the EDS is quite reliable. Zhiping Luo luo@mic.tamu.edu Thu Mar 31

EDS:
ZnO

A recent paper in JACS (do not want to provide citation intentionally) claims to have used EDS analysis in the TEM to determine presence of about 1% of Na in ZnO single crystals. Any comments on validity and prudence of such a claim? ***Krassimir Bozhilov bozhilov@ucr.edu Wed Apr 20***

1% is possible, but close to the limit of detectability. I presume that the EDS system has an ultrathin window or no window. As to the prudence, if the spectra show a bump at the right position, and there is no possibility of interference from another element, (I don't have access to a table of x-ray lines, but I know that Cu-L is somewhere near Na-K.) I see nothing wrong with reporting it as Na. At one time I was looking for chlorinated hydrocarbons in clay samples, and I could see a small bump where the Cl should be in some of the grains. I also saw the Ti-L alpha clearly and the Ti-L beta was the same size as the Cl and obviously must be there. This gave me confidence that the Cl bump was also real. Bill Tivol william.f.tivol@aero.org Thu Apr 21

I have been posting replies privately. I suppose I should summarize to the list. There should be no problem detecting Na with even a Be window detector, but I would assume this is a thin-window detector. The big problem is an overlap with the Zn-L line, and they are measuring Na in ZnO. That will be difficult. It might be possible if they are using their own profiles for the elemental peaks under their exact conditions and calibration. If they are using the factory profiles, it is probably not feasible. We collect our spectra a little faster than the factory used for their profiles, so the peaks are a bit wider. That may be fine in the case of widely separated elements. It breaks down in cases of peak overlaps. A follow-on post indicated that this researcher had tried mapping for Na and found it uniformly distributed. It was not at all clear if the researcher considered the Zn-L overlap with Na-K. I know our system does not deconvolve peak overlaps for mapping. I don't think it even does background corrections. Maybe new systems do with their spectral imaging. I make sure to label the Na map as Na/Zn if there is any chance of the overlap. Warren Straszheim wesaia@iastate.edu Thu Apr 21

Except of course for the possibility of the doublet of Oxygen K ($0.523 \times 2 = 1.046$ keV). The sample is ZnO! Peter Ingram p.ingram@cellbio.duke.edu Thu Apr 21

It is pretty obvious from the paper's supporting documents that the EDS/EDX spectrum is misidentified: http://pubs.acs.org/doi/suppl/10.1021/ja908521s/suppl_file/ja908521s_si_001.pdf

NaK 1.04KeV

ZnL 1.01KeV

This is a common overlap, that like many people, I experience several times a week. I hope the authors print a retraction. Jim Quinn jquinn@ms.cc.sunysb.edu Thu Apr 21

Follow-up questions to Peter and Bill: (1) Would WDS give a definitive identification? (2) Would using a variety of kV's using EDS give a better identification? John Bozzola bozzola@siu.edu Thu Apr 21

Except of course for the possibility of the doublet of Oxygen K ($0.523 \times 2 = 1.046$ keV). The sample is ZnO! Peter Ingram p.ingram@cellbio.duke.edu Thu Apr 21

MT

https://doi.org/10.1017/S1551929511000484 Published online by Cambridge University Press