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Selected postings from the Microscopy Listserver from August 15, 2008 to October 15, 2008 plus some older items. 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 - Negative staining cyanobacteria

I have a person who wants to negative stain marine cyanobacteria. Some are grown on agar plates and scraped off and others are grown in media. What is the best way to negative stain these bacteria? We have used 2% aqueous uranyl acetate and 1% phosphotungstic acid. The stains work but not as well as we would like. We let the bugs sit for 5 minutes, wick off the excess and then have applied the stain from anywhere from 15 seconds to 1 minute. We are definitely seeing the bacteria and they appear collapsed. Paula Sicurello vapatpxs@yahoo.com Thu Aug 21

I learned to use the stain to wash off the unattached sample and carrier fluid rather than wicking it off. Add the staining solution drop-wise (3-4 drops) to the grid with sample holding it at a slight angle with forceps over a dish and let the overflowing drop slip off. Hold the grid flat and let the last drop of stain sit for about a minute on the grid to accomplish the staining then wick the excess stain off slowly. This eliminated a premature drying of the sample. Patricia Stranen Connelly connellyps@nhlbi.nih.gov Thu Aug 21

I experienced a similar situation with negatively staining some fimbriae-producing E.coli. The pili and fimbriae were negatively stained beautifully, but the bacterial cells themselves were crushed, just as you described for your cyanobacteria. I'm sure that this collapse is due to air-drying, which is unavoidable when negative staining. Fixation in a (relatively) high concentration of gluteraldehyde might stabilize your cyanobacteria enough to be photogenic - I would try something like 3-6% glutaraldehyde - followed by conventional negative staining. You could also try positive staining followed by CPD or HMDS. The route I took was to CPD/HMDS and then platinum/carbon shadow. However, working with replicas is a lot more involved and tedious than a simple negative stain! Andy Bowling andrew.bowling@ars.usda.gov Fri Aug 22

SPECIMEN PREPARTION - Aclar film

I am about to use Aclar film to grow some cells which will eventually be embedded while still attached to the film (I can't use glass or plastic coverslips with the particular resin I intend to use). I can't remember what I did to sterilize the Aclar film in the old days when I last used it. I assumed we just autoclaved it but I found a dish labeled sterile with some pieces in it and they were all curled (presumably from autoclaving). Is there a way to avoid this? Any tips will be much appreciated. Thomas E. Phillips phillipst@missouri.edu Mon Oct 6

We recommend exposing the Aclar to UV light in the cell culture hood. I think it is a matter of seconds and it is sterile. Jill Verlander Reed jill.verlander@medicine.ufl.edu Mon Oct 6

A few grad students here have grown culture cells on Aclar and our method was to incubate films in 70% ethanol for approximately 30 min, rinse well with water, then put under UV irradiation for approx 30 min in the dish to be used for culturing. Our laminar-flow benches have a UV source so we could irradiate and then seed cells without moving from flow bench. This worked well for us. David Lowry dlowry@asu.edu Mon Oct 6

There are several ways to sterilize plastics. The best way (if you have the capability) is by ethylene oxide gas sterilization. Most hospitals have (or had) this capability. Next best is immersion in 70% ethanol for several hours, then air drying in a sterile Petri dish. Third way: if you have a fume hood, generate some formaldehyde gas by heating paraformaldehyde on a hotplate and flowing it into a chamber (beaker) containing the Aclar strips. Stand up the Aclar (or put it on cotton) so that the gas contacts all surfaces. Takes about an hour. 4th way: germicidal UV lamp, 3-6 inches away for 30 minutes each side. 5th way: although I have not done it, put the strips in acidified 2% glutaraldehyde, 30 min at RT, rinse in sterile distilled water, air dry in sterile Petri dish. 6th way: Immerse in Clorox bleach for 15-20 min at room temperature, rinse in deionized water, dry in sterile Petri. John J. Bozzola bozzola@siu.edu Mon Oct 6

Can you sterilize it with 100% ethanol washes? I do Thermanox that way - dip in ethanol, air dry, repeat twice. I'm not sure where we came up with 3 dip/dry cycles as being magical, but it works! Tamara Howard thoward@unm.edu Mon Oct 6

Is it possible to put the Aclar pieces into a (glass, plastic) Petri dish and to place them for a minute or so into a glow discharge unit (= sterilizer), which is mainly used for rendering grids or carbon films hydrophilic? I think this treatment should also be sufficient to sterilize surfaces, i.e. kill bugs (if not present in huge amounts, in liquids etc.). At least, it is worth a try. - let me know if it works. Reinhard Rachel Mon Oct 6

Some of my students have been using it to grow their cells. I believe they clean them with water and then UV sterilize them. Margaret E. Bisher mbisher@princeton.edu Mon Oct 6

I use 70% ethanol and then let the Aclar air dry. Very easy. David Elliott@arizona.edu Mon Oct 6

Another easy sterilization method is to place items to be sterilized in a container - an old desiccator works fine, add a beaker containing 100 ml standard bleach (from the shop), add 3 ml concentrated HCl to the bleach, quickly place lid on desiccator. This will sterilize seeds in 2-3 hours, should sterilize something like film in a shorter time. Rosemary White rosemary.white@csiro.au Mon Oct 6

Please let me apologize for not responding more rapidly to the suggestion by Rosemary that we mix household bleach (5-6% sodium hypochlorite) with concentrated Hydrochloric Acid. Been a bit busy here and a long time since basic chemistry (we'll not say how many years). While it has been long since basic chemistry, as a virologist (my non EM side of life) who deals with disinfectants regularly, this mixture was intuitively not good (to understate the non chemical reaction). The problem with bleach and HCl is the products of the chemical reaction. First, in the aqueous state, as bleach, the sodium hypochlorite reacts with the water to give hypochlorous acid. (For the sake of the chemists it looks like this)

1. $NaOCl + H_2O <-> HOCl + Na^+ + OH^-$

Hypochlorous acid is the mechanism of both bleaching and disinfection. It you want to kill a virus or other living organism, and do not care about structural impact - douse it with bleach.

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HOCl does all sorts of nasty things. Used appropriately bleach is fine. I also buy it by the case. The real problem occurs when you mix in an acid, as with the HCl in this case. For the chemists, the reaction looks like:

2. HOCl + HCl <-> H2O + Cl_2 (gas)

That yellowish vapour that comes off is the Cl_2, or in the vernacular, chlorine gas. In my humble opinion, this is not a good idea in the lab. If you really have to try it, at least use a chemical hood (not a BSL containment hood, below BSL3 they exhaust directly back to the room). A desiccator would not be considered acceptable containment. Paul R. Hazelton paul_hazelton@umanitoba.ca Wed Oct 8

Yes, we do this in the fume hood, of course! I guess I should have said this. Rosemary White rosemary.white@csiro.au Wed Oct 8

If you happen to have access to a "Plasma Cleaner" (often used for making carbon films wettable) they can be used for sterilization. Just put the films in Petri dishes - the field and plasma penetrate inside the containers (see references). http://www.harrickplasma.com/applications_cleaning.php http://www.harrickplasma.com/articles/category-67/. I have no connection with Harrick except being a happy user and found their info easily accessible. Dale Callaham dac@research.umass.edu Tue Oct 7

FREEZE-SUBSTITION - water in acetone

I experienced that 5% water in acetone, once placed at -80°C, will freeze at the bottom of the tube. I am very inexperienced in high-pressure freezing and freeze substitution, but I know that Paul Walther proposed to mix water with acetone to improve membrane visualization. Well I suppose that the mix is first cooled down to temperature, so this means that water separates from acetone in the mix! Something must be wrong with my argumentation, but I don't see what/where. Stephane Nizets nizets2@yahoo.com Tue Sep 2

When we were setting up to do low-temperature substitution (mid-80s) I found some info detailing that water is only sparingly soluble in acetone at -80°C (fraction of a percent or so). So we used molecular sieves in the bottom of the sample vials to constantly keep the acetone dry and able to dissolve more water from the sample; also don't overload the sample volume relative to acetone volume, and use properly dehydrated acetone for the substitution fluid whether or not there is molecular sieve used. What you experience must be the proof of the low solubility. We did have very low tissue contrast with the dry acetone. Methanol is commonly used if higher concentrations of water are to be present for low-temperature substitution or subsequent uranyl acetate staining at low temps. Dale Callaham dac@research.umass.edu Tue Sep 2

If one looks at a phase diagram of a two-component mixture or solution, one sees that the line separating the liquid and solid phases is at a higher temperature for one of the pure components than for an admixture of the second component, and this is true for the second component if both components are liquid. (I know that even solid solutes will liquify if the temperature is raised enough, but the statement is true for the water-acetone system.) The liquid-solid line goes to lower temperatures as the amount of the second component increases until it reaches a minimum for a particular ratio of components called the eutectic. If one takes a mixture of the two components and lowers the temperature, the composition of the liquid phase of the mixture is constant until the temperature intersects the liquid-solid line. Then if the temperature is lowered further, the component in excess over the eutectic ratio will freeze out of the mixture until the temperature is that at which the eutectic mixture freezes, at which point the remaining liquid is the eutectic mixture. In a diagram with component ratio along the x-axis and temperature along the y-axis, the liquid-solid line generally looks like two concave-downward curves extending from the freezing points of the two components at the lowest and highest fractions of a component (0 and 100%) and intersecting at the eutectic ratio and its freezing point. The starting ratio and temperature define a point in the liquid region. The line that represents what happens drops straight down until it hits the liquid-solid line, then travels along that line until it reaches the eutectic point, then drops straight down again. This line defines the ratio of components in the liquid as the temperature is lowered, then represents the eutectic ratio as the whole mixture freezes. In your case, the 5% water in acetone would seem to be more water than the eutectic, since acetone freezes at -95°C. Did you put the pure acetone-water mixture in a -80°C freezer, or did you first add your specimen? In the latter case, there may have been water in your specimen or some other component may be present, which could complicate the situation. Bill Tivol tivol@caltech.edu Tue Sep 2

Bruno Humbel and Martin Müller contributed a paper in 1984 at the Budapest meeting regarding acetone and the temperature related uptake of tritiated water. Tritiated water may not have the same hydrogen bonding characteristics as the'normal' isotope and therefore differences may exist. But nevertheless, very useful! Reference: Humbel, B and Müller, M. Freeze substitution and low-temperature embedding Proc. 8th Eur. Congr. Electron Microscopy, Budapest 1984 (Csanady, A Röhlich, P Szabo, D eds) p 1798-1798. Jan Leunissen leunissen@aurion.nl Tue Sep 2

MICROTOMY- vibration dampening

I am looking into installing a cryo-ultramicrotome in a room that has some vibration issues. Our testing shows that the building itself is decent, but movement of people in the lab leads to significant vibrations (apparently the floor is wood). I'm interested to hear if anybody has experience with vibration dampening in this kind of situation, especially for cryo work. In particular, we're considering an active vibration dampening plate (The TS150 from HWL) or an air table, so it would be useful to hear if anybody has experience with these or other solutions. Eli Sone eli.sone@utoronto.ca Wed Aug 27

If there is no one on the floor above you, hanging the support for the cryo-ultramicrotome from the ceiling may help. Engineering the members the support hangs from is a complex endeavor and the next floor if there is one has to be noise free. It may be an inexpensive solution if you have the right conditions. The other way to isolate something from the floor is to bore holes in it for supports that go to the basement. Occasionally it might be a solution for some labs on first floor. But it would be too expensive for most. Gordon Couger gcouger@science-info.net Thu Aug 28

TEM - high resolution mode

I have a user who asked that her samples be scoped in high resolution mode at 200K. Is there a special alignment? Dorota Wadowska wadowska@upei.ca Thu Aug 21

I have not seen an answer to your problem, so I will tell you

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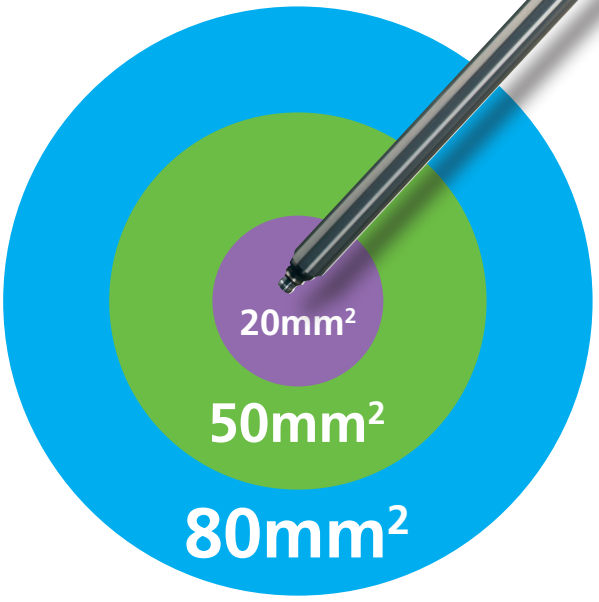

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what I do. For 200,000 times magnification, I would use condenser aperture #2 and a spot size of 2 or 1 micron. Set those and then align. As you reduce your condenser aperture size and spot size, your beam will be more parallel, but less bright, so you have to balance resolution and contrast. Mary Fletcher maryflet@interchange.ubc.ca Thu Aug 21

I have seen your problem and hope that I am able to help. A procedure is set out below that is taken from my web site hits and tips pages. A number of controls are used during normal operation: (1) Focus (objective lens) controls, enable the beam to be adjusted to place the first image in the plain of the diffraction lens "in focus". This action should be carried out at double the photographic magnification to be used. (2) Brightness or Second Condenser adjusts the intensity of the illumination on the specimen. Its alignment is corrected during operation with the Brightness or Illumination centering controls. Always reduce the illumination level by turning the control clockwise from focus. In this way the best beam coherence is achieved. (3) Spot size, should be adjusted to attain a satisfactory image quality, in relation to the magnification, i.e. high resolution requires a spot size of <2 μm; normal operation 1-5 μm. (4) Objective Stigmators should be used like fine focus controls, to adjust the image sharpness, again use at double the photographic magnification. (5) Objective Aperture is used to set the image contrast; it should be inserted and aligned in the diffraction mode. (6) Magnification varies the strength of the imaging lenses which are automatically adjusted for minimum distortion. (7) Illumination alignment should be corrected, to the screen centre, prior to focus at each new magnification (8) Emission current should be adjusted to enable the illumination to be used in the over focus mode (high coherence) at the maximum magnification to be used. Insufficient emission will limit performance. Emission is controlled by the bias or by moving the filament forward (higher current) or backwards (lower current). To emphasize a point, parallel beams are very important if you are chasing the best image quality, biology or materials, most times we need high coherence. There are misunderstandings on how to obtain a parallel beam or high coherence, setting the final condenser under focus is incorrect. The procedure for high coherence would be to use the smallest spot size you can tolerate (this probably means you must up the emission current). Once in this condition over focus the final condenser (clockwise from crossover) the spot, whatever size it is now, becomes your virtual source. The further over focus you go the greater the beam coherence and that is what we are after. You will deduce the smaller the condenser aperture the sharper the spot and the smaller the spot the greater the coherence for a given degree of over focus. Work with a design team and they expect everyone to over focus, they expect everyone to use small condenser apertures and they expect everyone to use small spot sizes. They do not expect everyone to use too low an emission current (almost everyone does, we have talked before about filament life being the most important feature of many laboratories) because this makes the task too difficult! Steve Chapman protrain@emcourses.com Fri Aug 22

I use a Philips CM30ST for HRTEM however the procedure should be quite similar for a Hitachi: (1) Standard alignment with a small spot and a small condenser (gun tilt, gun shift, condenser alignment, astigmatism cond, specimen height, focus, pivot point, voltage modulation (rotcenter volt). (2) Coma free alignment (for the best resolution, not compulsory for standard HRTEM). (3) Choose an appropriate objective aperture, a larger aperture give higher resolution but less contrast. If you want to obtain lattice fringes images the aperture must be larger than the diffraction ring (or spot) you want to observe, (4) Center carefully the objective aperture. (5) Check astigmatism of the objective lens (use the FFT device of your camera on an amorphous area). (6) Make a hysteresis loop of the lenses (go from lower magnification to higher magnification and come back). (7) Adjust the focus of the objective lens and slightly underfocus (Scherzer defocus usually around -50nm). (8) Spread the illumination to have parallel beam geometry. If there is a beam drift or a specimen drift, it can be useful to reduce the acquisition time by using a larger spot size, condenser or increase the illumination/emission. Patrick Weisbecker weis183@yahoo.fr Fri Aug 22

TEM - Increasing contrast

I'm having trouble clearly visualizing synaptic vesicles from rat brain staining. I need to count synaptic vesicles so it is critical to get clear staining. If anyone can share any ideas on increasing contrast on EM images, I would appreciate it. 1) Sample: rat brain, fixed with 4% paraformaldehyde, 0.5% glutaraldehyde, section vibrosliced. 2) Staining: Nanogold, HQ silver, 1% osmium 30 min in sodium cacodylate buffer+ 0.13 M sucrose. 3) aqueous uranyl acetate staining 5 min; Reynold's lead citrate 8 min. We experienced precipitates when we increased osmium incubation time, so we are limited to 30 min incubation time. We are trying now to vary uranyl and lead staining time but it doesn't seem to make much difference. Chee-Yeun cychung@mclean.harvard.edu Tue Sep 2

Not all contrast problems can, or need to be solved by changing the stain. It's a bit of an old solution, but try using a smaller objective aperture (OA) - better contrast, better resolution - not that the size of OA is as important for resolution with thin sections as it is with negative staining. You could also change your acceleration voltage. Paul R. Hazelton paul_hazelton@umanitoba.ca Tue Sep 2

One comment about the precipitates: you may be able to get this under control by using 2-mercaptoethanol in your processing steps. If you're interested, you can find protocols and formulas on our website at http://www.emc.missouri.edu/Pdfs/General%202-ME%20Microwave%20Processing%20Protocol.pdf\ and http://www.emc.missouri.edu/Pdfs/Common%20Buffer%20and%20Fixatives%20for%20Electron%20Microscopy.pdf. We have never done it in a procedure exactly like you describe, but it may be worth a try. Also, you can try "double staining", which involves starting with 2-3 minutes of lead stain, followed by uranyl acetate, and ending with a second lead stain for 8-10 minutes. This can provide a dramatic increase in specimen contrast. Randy Tindall TindallR@missouri.edu Wed Sep 3

In addition to the excellent ideas already offered (smaller objective aperature, reduced accelerating voltage, what we used to call PUP stain, lead uranyl lead). Cut thicker sections. More tissue will pick up more stain and give more contrast. Geoff McAuliffe mcauliff@umdnj.edu Wed Sep 3

I have used protocols similar to yours but usually included one hour in 2% uranyl acetate just prior to dehydration. My epoxy is an Epon equivalent. I cut pale gold sections and use a support film.

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I stain with 2% aqueous uranyl acetate for one hour and then for twenty minutes in Reynolds lead citrate (this stain will not perform after several months of storage--try some freshly made stain). I typically use 80 kV and a medium size objective aperture. Synapses are very apparent when the primary fixative perfusion is good. The suggestions by other Listers are also appropriate. Larry Ackerman Larry.Ackerman@ucsf.edu Wed Sep 3

EM – water chillers

We are in the process of building a new microscopy facility and the question becomes air-cooled or water-cooled chillers - which way to go. Now, I am not interested in running the scopes themselves off of a chilled building water loop, there will be dedicated water chillers for the scopes, it is cooling the refrigerant systems of the scope chillers, i.e. water or air cooled condensers on the water chillers. For years, I worked with air-cooled chillers maintenance consisted of replacing pumps, pump couplings, and pump motors occasionally. Moreover, cleaning the condenser coils. I never had a noticeable problem with air temperature effecting scope water loop. For the last 14 years, I've been working with water cooled condensers still replacing pumps & motors, but also adding significant repairs due to rotting out condensers which usually causes the compressors to go as well. However, these have been chilled with city water not a building closed water loop (i.e., non-treated corrosive city water). Is this a significant issue with closed building chiller loops? Secondly, in general do folks using building water-cooled condensers have issues with fluctuation or loss of the building water loop? Yes, I realize these issues may depend on how well the building system is maintained but in general. Air-cooled would have to deal with noise and ventilation of the utility space the chillers sit in. Moreover, we have water-chilled condensers presently and would have to replace the four chillers. In addition, in order to deal with noise the air-cooled chillers may have to be placed further away and then have longer runs to the scopes. But this may really help with any noise issues. Water-cooled are quieter and would not need significant ventilation. We currently have water chilled condenser systems. But the building that will house the new facility does not have it own building chilled water loop, so the cost of bringing the chilled water (for the condensers not the scopes) from the next door building (70 meters) does play into it. Therefore, cost is: new chillers, ventilation, and noise abatement, vs piping water from next door building. All of which may balance out. In which case, I'm back to the best solution in terms of the scopes and scope environment. Richard E. Edelmann edelmare@muohio.edu Mon Sep 29

If your options include water-cooled and air-cooled chillers, then the heat load must not be too great. If it were, water-cooled would be the only option. Otherwise, I'd suggest air cooled. Simple, effective, easy to maintain. Haskris chillers that are air-cooled don't make a lot of noise. The Merlins also are quiet. A big issue is the reservoir volume and the amount of water turnover per hour--flow rate. Haskris is good since when you need parts, they come from IL rather than EU or another laborious route. Gary Gaugler gary@gaugler.com Mon Sep 29

My experience has been that building services have been quite good for water in terms of water temperature and pressure, and that corrosion in a closed system is minimal. However, they occasionally have emergency shutdowns due to leaks and shutdowns for preventive maintenance. Air conditioning, on the other hand, is just terrible in terms of reliability, but there is at least usually some air. I would worry about the air temperature range that air cooled chillers can accommodate. At worst, you could have a chiller room that was very uncomfortably hot. John Mardinly a.mardinly@numonyx.com Mon Sep 29

I'd like to add my observations. Air-cooled chillers work very well and Haskris chillers are great. We have one on both our SEM and our TEM. We designed a new facility a few years back and had purchased a new SEM that had an air-cooled Haskris chiller. While the performance of the chiller has been reliable and excellent, as most Haskris chillers typically perform, it is noisy and it does generate heat. We unfortunately allowed the in-house engineer who designed the room to persuade us to place the chiller in a closet within the SEM room. It seemed like a good idea at the time and we thought we would be able to close the closet door on it. Because of the heat the chiller generates, we cannot close the door, so we're dealing with the noise and heat. We'd like to move the chiller out but it will cost us several thousand dollars to relocate the chiller in a machine room down the hall. Lesley S. Bechtold lesley.bechtold@jax.org Mon Sep 29

For more than 20 years the HVEM at Albany has had air-cooled chillers with no problems. In addition to replacing the occasional motor or compressor, we checked the pH and concentration of an anti-corrosion treatment each month. Not only did this prevent the water from becoming too acid, it also gave us a chance to see the units, check the in-line filters for accumulated crud, etc. The Tecnai EMs in the cryoEM lab here were installed about six years ago with chillers that are cooled by a building chilled-water loop, and they also have performed well--in any case, the water cooling was not at fault in any chiller repair. On the one or two occasions when the building water failed, the chillers and microscopes shut down safely, and we had to put the scopes in a safe condition when we were notified of a planned chilled-water shutdown. All in all, I'd say that the two systems were both pretty reliable, and a one-time expense of bringing a chilled-water line into your facility would probably be cheaper than the alternative. Another consideration is that the Albany system had the chillers in a large open area, whereas the chillers here are in a small room, which makes air cooling less practical. Bill Tivol tivol@caltech.edu Mon Sep 29

My first question would be, "Have you looked at the water in the closed loop?" Some in-house cooled water loops are quite good and others, well, I wouldn't put that water in my car's cooling system. I've seen the exchangers get plugged and it took a while to diagnose the problem. If the water looks good and tests good chemically (Ph minerals, etc.), it may be a good alternative. In the winter, the added heat load from air-cooled chiller-circulators can actually be a plus, although in the summer it can be an expensive drawback. Ken Converse kenconverse@qualityimages.biz Mon Sep 29

Haskris used to make water-cooled heat exchanger for use with the building water. It has no refrigerator - but only a valve regulating cold water flow (building water) through the heat exchanger coil submerged in the closed loop water reservoir. And of course a pump for closed loop water. Could be most reliable and least expensive solution. Plus most quiet and most compact. Of course building water has to be colder than the closed loop water for this to work. Vitaly Feingold vitalylazar@att.net Mon Sep 29

NETNOTES

EM - pH in recirculators

What pH should the coolant be in an EM chillers/water recirculator? Beth Richardson beth@plantbio.uga.edu Wed Sep 10

Slightly basic, say ~8.5, so as not to dissolve the copper in the fittings. The water will dissolve CO_2 from the air over time, so if you adjust the pH to 8.5, it should be sufficiently basic for > 1 month. I suggest checking every month and adjusting pH, anti-corrosion agent (if any), and removing any crud in the chiller tank. Bill Tivol tivol@caltech.edu Wed Sep 10

ESEM validation

I am using an ESEM Quanta 200 FEG miscroscope from FEI for analyzing samples for industry under good laboratory practice (GLP) regulations. Our industrial partner asked us to perform an instrument validation action. Does somebody know how to perform the validation of an SEM? What does "validation" mean? Monica Nelea monica.nelea@polymtl.ca Wed Sep 10

When the laboratory faces the type of question you have, it usually means the following: 1. Do you follow recognized operating procedures, those that are set down in writing? 2. Do you calibrate your machine regularly with a recognized standard? Do you follow recognized operating procedures, those that are set down in writing? 3. If you are providing analytical information do you run analytical standards to check your accuracy? Do you follow recognized operating procedures, those that are set down in writing? 4. What are the qualifications of those carrying out the consultancy and are they to a standard that is adequate to carry out the investigations? In short they are asking to what level you are competent to operate and do you have accreditation from a recognized body? Check out the body or bodies in your country that certify competence? There is much to explain so check out our web site for more on Quality in Electron Microscopy. Steve Chapman protrain@emcourses.com Wed Sep 10

EDX – how does it work?

I use Horiba supplied EDX system with an atmospheric window equipped with lithium drifted silicon detector. I understand the generation of X-rays and detection of these X-rays by detector in the liquid nitrogen cooled EDX detector. I use software supplied with the instrument called EMAX. From the point of generation of the x-ray signal, how does the data gets analyzed and displayed, There are terms like fit index and sometimes though the spectra shows some peaks but the relative weight percentages are in negative values! I will be happy if people who have a detailed understanding of these phenomena could comment on the entire process of translation of the electronic signal to the data display by the software. Johnson reganhll@gmail.com Wed Sep 24

I have found that the best teaching materials in EDX are from the Oxford web site. (http://www.x-raymicroanalysis.com/pages/main/main.htm). They have a very complete explanation of how the EDX hardware and software works. If you are getting nonsense from your EDX results, the first thing to determine is if your data is what the software is expecting. It sounds like a software problem, but the software can only work with the data it is given. Is your spectrum the right shape? Mary Fletcher maryflet@interchange.ubc.ca Thu Sep 25

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EM - Imaging Tantalum particles

I'm wondering if anyone has had any experience imaging Tantalum particles about 1-2 nm in size. For whatever reason, I seem to be having trouble visualizing them. Are these generally less electron dense than gold particles, making them less visible to an EM (Ta does have a slightly lower atomic number) and should I therefore try either negative staining or some other course of action? David Parmiter parmiterd@ncifcrf.gov Mon Sep 29

The Ta particles you're looking for are pretty small. Gold beads of that size often have to be enhanced in order to see them, but this depends on the context. Are you looking for isolated particles on a low-Z substrate, or are they used to label a specimen? I would not think that negative staining would make the particles more visible-- there is not as much difference between Z = 74 (Ta) and Z = 92 (U) as between Z = 74 and Z = 6. I do not know whether there is a chemical process that can be used to enhance the Ta, but if there is, I'd suggest that. Bill Tivol tivol@caltech.edu Mon Sep 29

We look at 10 nm tantalum films regularly, and the contrast is extremely strong. John Mardinly a.mardinly@numonyx.com Mon Sep 29

I only have a little experience with this type of imaging. I assume that this is particles on a carbon film. Even for gold the mass of a 1 nm particle makes it much harder to see than a 4 nm particle (easy). 2 nm is not even so bad, but with 1nm gold, even on a very thin pure carbon film (<3 nm), the granularity of the carbon film seems to be on the same order as the particles and with the random variation in recorded density it leaves one wondering if you are really seeing the target particles. There must be some more sophisticated methods, but regarding simple visual or photographic visualization, and with a lower atomic number, I think it is not so easy. Dale Callaham dac@research.umass.edu Mon Sep 29

I forgot to include the possibility of dark-field imaging in my previous message. If you have Ta on a low-Z substrate, dark field will show up the particles as bright spots, which are more easily distinguished than the small dark spots you'd observe in bright field. The down side is that the total number of counts in a dark field image is smaller than in bright field, but the Ta particles should not be too sensitive to a high dose, so that might not be a problem. Bill Tivol tivol@caltech.edu Mon Sep 29

One of the most important things I would suggest is make sure your alignment of the machine is done correctly, (yes it sounds obvious, but often people will just give a rough alignment as they want to get on with their sample). Especially, if your NPs (nanoparticles) are on carbon film, the objective astigmatism, as it can make it very difficult to see particles if it is out. I did some analysis on gold NPs that were sub 1.5nm and whose contrast was very low. I found that they were best viewed with close to in focus conditions when the carbon background was at its minimum. I did not have any objective aperture in and was using the largest condenser aperture. You might get better contrast by selecting smaller aperture sizes. I am assuming it is a TEM we are talking about here. Calum Dickinson calum.dickinson@staffmail.ul.ie Mon Sep 29

I've observed some Pd nanoparticles of 1-2 nm and had also great difficulties to see them particularly because they were amorphous or poorly crystallized. The carbon film must be very thin (holey carbon with another thinner film in the holes). The imaging condition the best suited to see them was dark field imaging. Bright field with strong underfocus can also allow to see them but you can obtain only few information from this kind of imaging. Patrick Weisbecker weis183@yahoo.fr Tue Sep 30

MICROSCOPY – resolution limits

I am basically wondering what kind of resolution can be obtained with various microscopies (TEM, SEM, AFM, STM) with regard to noting changes in the small (5-10 Angstrom) and large (up to 500 Angstrom) ranges. I believe that our samples, which consist of ~45A thick "purple" membrane - which is tightly packed (~50% of membrane surface area) with the purple protein bacteriorhodopsin (BR; 26 kDa, comprised of 7 alpha-helices arranged in a circle with the long axes of the helices perpendicular to the plane of the membrane). The BR molecules are organized into a hexagonal lattice of trimers, with distances of ~60A separating the center of adjacent pairs of trimers (see attached "Protein Membranes..." figure). We believe that when we induce a purple-to-blue color change with green (532 nm) laser pulses, that ~1/3 of the molecules (~1 molecule per trimer) are converted to a colorless species, and that this damaged molecule induces conformational changes in BR molecules in its own trimer or nearby trimers which lead to the color change. We are interested in knowing if we would be able to detect such conformational changes via any of the microscopies listed above. We would need to be able to look at the membrane at both the large and small scales listed above to determine if the membrane becomes distorted in and/or out of the membrane plane. Scott Streiker scott.streiker@udri.udayton.edu Mon Oct 6

Since no one else has replied, I thought I'd have a go. I can say a little about TEM, but can only give wild guesses about the other techniques. TEM is inherently capable of collecting images in the resolution ranges you want, but there is a very big caveat: biological specimens are very radiation sensitive, and even more so when in an aqueous environment, so if your membrane is wet--for example, enclosed in an environmental chamber--the dose it can tolerate will be so low that the noise in the image will smear out detail at least in the 0.5-1 nm range. On the plus side, you could either take diffraction patterns or FFTs of your images (or both) and use the idea that each molecule that changes will do so in the same way to get an averaged image, which will have the information about the laser-induced changes. It will require a fair amount of image processing to tease apart the unchanged trimers from the changed ones, and if the change is not into one or a few distinct states, you won't be able to characterize it (but you may be able to locate the altered trimers). You could tilt the specimen to observe out-of-plane changes, but you will need to have very flat membranes so that each part of the image has the same orientation. If you can cause the changes in a membrane that is embedded in a thin film of water, then plunge-freeze the grid while maintaining the changes, you will get a considerable improvement in radiation sensitivity by using cryoTEM, but you don't say whether the laser induces transient or permanent changes. In vivo, BR is supposed to absorb light, change conformation to pump a proton across the membrane, then return to its initial state, but I don't know whether this is true in your preparation. I do not think that SEM will be useful, since you will not likely be able to coat your specimen, and the thin, low Z specimen will produce very little detectable signal, especially compared to the substrate. AFM and STM can be used

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in an aqueous environment, and it is likely that the membranes can be placed on a suitable substrate and imaged. I do not know what the resolution limits of these techniques are, and they only image surfaces, so if the changes do not alter the topography, they will not be observed (unless there is some change in the stiffness of the membrane, which could be detected in some AFM modes). Any real experts in these techniques will be able to give you much more authoritative information. Bill Tivol tivol@caltech.edu Tue Oct 7

INSTRUMENTATION – sputter coater troubleshooting

I'm hoping maybe someone can offer a quick "I know that problem exactly and here is what to do" to get our sputter coater back up and running. As the subject says it is an Emitech K-550 (old model). Problem: it isn't sputtering, voltage is under 5mA, but it jumps and spikes all over the place with arcs. The 4-5mA is obtained with the same setting we used to generate 20mA. I first noticed exposed copper on the green wire going to the head (through the support arm). Took that apart, soldered it back together. Same problem. I happened to wiggle the screw on BNC connector at the top of the sputter head and got a dramatic jump in voltage and found the connection to be failing. Although re-working that connector hasn't improved anything. (taking it off and testing it I would get no-connectivity, fixing it and the meter says it is fine). That back on and it still isn't sputtering properly? Could the target be causing these problems? Anyone have any further suggestions? It was one of those 'it was working great' then the next user 'it is broken' kind of situations. geoffrey_williams@brown.edu Mon Oct 6

Have you tried cleaning the insulator around the target head? It's made of Teflon, I believe, and should be mostly white. After a while it gets coated with whatever you're sputtering with and no longer insulates effectively. It is held on with screws and can be removed cleaned with acetone or ethanol and a good polishing, or just flipped upside down to use the other side. We had some extras made by our local instrument shop for when it becomes too coated to clean thoroughly, which entailed a set-up fee, but was much cheaper than ordering them from Emitech. I don't know if this would greatly affect the mA value on the readout, but I do know that it can have a great effect on sputtering efficiency. Randy Tindall tindallr@missouri.edu Mon Oct 6

I've encountered similar issues with other brand coaters. First, clean the head and target holder/support well. Deposition of sputtered metal there over time will cause shorts. Second, check the feed-throughs in the top of the coater. There likely is high-vacuum epoxy there, and it can break down over time. This can be resealed with more high-vacuum epoxy, but be careful not to make things worse. Frustrating, that is. If it's not either of these, it's most likely one or more of the wires or connections. Phil Oshel oshel1pe@cmich.edu Mon Oct 6

In response to the suggestions: When I had it all apart I cleaned the Teflon insulator as best as I could (nearly white now, instead of well coated), the ohm meter is showing the target shield to be completely insulated from the lid. Short version of what follows: I think it is fixed. Long version: Between sending the email out and now, the one thing that was bothering me was the lack of connectivity to the Au/Pd target. So I checked it again and noticed a gap or two around the edge. Next thing I know the film detaches from the backing. It seems to have been held in with a clear looking 'glue.' I put a bit of carbon adhesive on there and seemed to get not quite zero Ohms resistance but pretty close. And after letting it pump for a good 30-40 minute (on hold), it started sputtering perfectly. This led me to guess that the wires and the sketchy connections might have had nothing to do with the "problem" in the first place. Geoff Williams Geoffrey_Williams@brown.edu Mon Oct 6

SPECIMEN PREPARATION – room temperature embedding

We are looking for a media to embed polymeric (PVP) nanofibers in at room temperature. We have tried Araldite and Spurr's resin, but the metallic (titanium) precursor in the nanofibers begins to nucleate grains during curing (around 60°C), whereas we want to section the pristine material. So we would like to try an embedding media that cures without heating, maybe using UV. Could anyone suggest something for this? It might spare us some trial-and-error. Phil Ahrenkiel phil.ahrenkiel@sdsmt.edu Tue Jul 22

Epon and Araldite, or their mixtures, do polymerize at temperatures lower than 60°C, and as far as I know, that polymerization reaction is not exothermic (am I wrong?). Needless to say, such polymerization is usually avoided, because blocks tend to come out soft. Many prefer 65°C. For your situation, however, it seems reasonable to try, say 45°C, having mixed the resin according to the "hard" recipe. I would still stay away from Spurr's. Acrylics, on the other hand, do generate a lot of heat during polymerization. Some are formulated, though, to polymerize at low temperatures - as low as minus 60°C. The most established, "respected" one is Lowicryl HM-20. It is relatively easy to make a do it yourself setup, with a box of dry ice and some kind of ethanol-filled heat sink to put your capsules in. It is important to get the UV light of right wavelength and intensity. EM suppliers offer ready setups for ~$900, I think. Another potential choice I just remembered - LR Gold, if they still sell it (not LR White). LR Gold was developed for LM but has proven useful for immunoEM as well. It cuts very nicely. Both Lowicryl HM20 and LR Gold are well covered in literature, but please don't hesitate to ask more questions if you get confused. Vlad Speransky vladislav_speransky@nih.gov Wed Jul 23

SPECIMEN PREPARATION – mineralized bone

Does anyone have a protocol for prep of mineralized bone? We want to preserve the minerals within the bone so decalcification is not an option. Debby Sherman dsherman@purdue.edu Wed Jul 30

No prep is needed per se. The reference below shows direct FIB/SEM/TEM of bone on a dental implant. We just made sure it was "dry" enough to be put into the vacuum chamber of a DualBeam prior to analysis. L . Giannuzzi , D . Phifer , N . Giannuzzi , M . Capuano, "Two-Dimensional and 3-Dimensional Analysis of Bone/Dental Implant Interfaces With the Use of Focused Ion Beam and Electron Microscopy," Journal of Oral and Maxillofacial Surgery , Volume 65 , Issue 4 , Pages 737-747. Lucille Giannuzzi lgiannuzzi@comcast.net Sat Aug 9

SPECIMEN PREPARTION – tray for small items

Looking for some input for a small tray with sidewalls to catch small specimens dropped when observing under optical microscopes. I can make something for this application and got a good idea from a local jeweler but hope there may be someone who has bought something commercially. This is in response to a student that lost a key thesis tooth fragment when it popped out of her tweezers while trying

to place it under a microscope. Any ideas welcomed. Roy Beavers rbeavers@mail.smu.edu Thu Jun 26

I've found tiny pieces can jump feet away from microscopes. Some I have yet to find! How far something goes depends on the pressure on the forceps and the angle of the hand holding them. I doubt that a small tray would catch most fragments. I would think a curtain that surrounds the sides and back of the scope would stop the object from going further then add a large ridged or slightly sticky rubber mat on the counter to catch the piece before it rolled further. That should catch about 75%. How about prevention? Could you use a single chambered slide to capture the piece before putting it on the scope stage? That way it would not be lost if the slide jumped a bit as it was put on the scope stage. Borrow an inverted microscope from the Life Sciences Department if the magnification requirement is equal. Working from the top is much easier to do. Another thought...a small piece of double sticky tape on the slide with the object pushed up against the side of the tape. Not good if using high power since the objective would also stick when it got close to the piece of interest. Just about anything that avoids using the forceps at such an angle as is necessary to get something under an objective would be helpful. All sorts of things are going through my brain like a thin coating of honey on the slide so the tooth could be put on easily, stick during observation, easily washed clean with water... "Small tray with side walls" - a square Petri dish? What size are you thinking about? Patricia Stranen Connelly connellyps@nhlbi.nih.gov Thu Jun 26

Why not go to your local photography dealer and get an appropriate size of photo paper processing tray. These have many non-photo uses in our lab at Kodak. The largest ones (or 'liberated' cafeteria trays) are great to hold rotary vacuum pumps that always choose the most inopportune moment to dump oil all over the lab. J.R. Minter jrminter@rochester.rr.com Thu Jun 26

Another alternative might be to get a small pump, which not only blows but sucks. You can get a variety of attachments to hold tiny things, or make them yourself. We use ours mainly as an air jet for cleaning things, but we can attach the hose to the other end and get suction. Rosemary White rosemary.white@csiro.au Thu Jun 26

Handling TEM specimens with tweezers is not advisable. I also have had similar bad experiences in the past. The best way is to handle them vacuum pipette. You can buy 18" needles from SPI as

The Ghost of the IEL: A Halloween Photoshop Exercise

Emily Bradford,* Gary Shull,* and MarianMiller**

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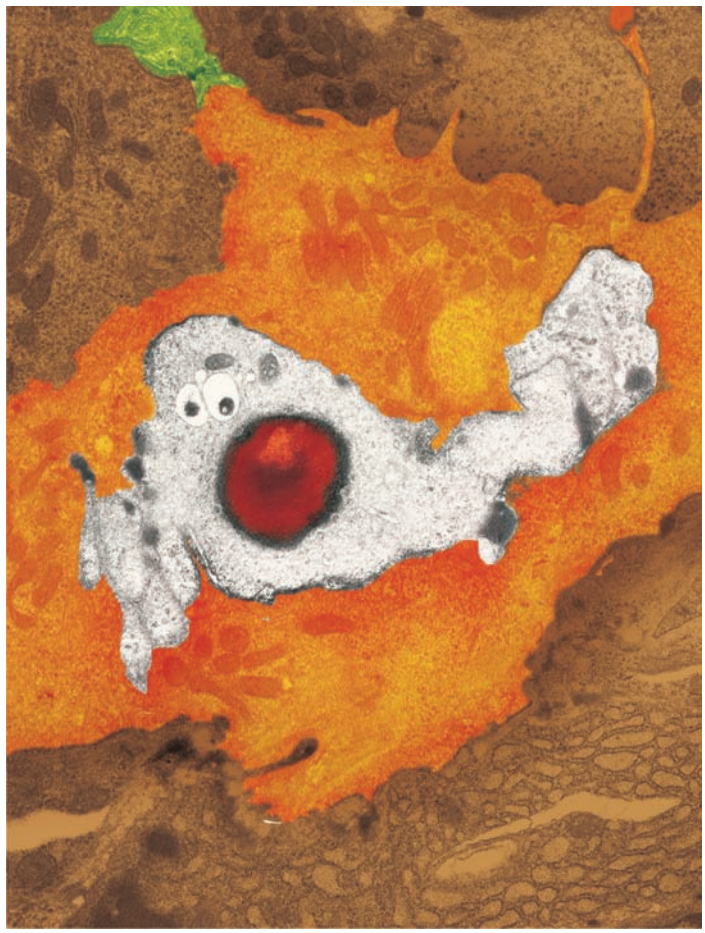
Image of an intraepithelial lymphocyte (IEL) from a CLIC5 mutant mouse small intestine. The CLIC (Chloride Intracellular Channel) family of proteins is expressed in a wide variety of cell types, and several isoforms are known to cycle between soluble and membrane-bound forms (Cromer *et al.* 2002). As well as being widely expressed, the CLICs are involved in diverse functions, including tubulogenesis (Berry *et al.*2003), immune cell activation, apoptosis (Suh *et al.* 2005) and calcium handling (Board *et al.* 2004). CLIC5 has been shown to



associate with cytoskeletal proteins in placental microvilli and inner ear cells, and is required for proper maintenence of hair cell steriocilia (Berryman *et al.* 2000; Gagnon *et al.* 2006). It has also been localized to the cytosol of human intestinal epithelial cells, though its function there remains unclear. The study in which this particular "IEL" was found, involved a search to see what function CLIC5 played in the modulation of tubulovesicles and microvillar apical membranes in the process of acid secretion. In particular, the relative amounts and structural characteristics of these two membrane types was quantified in parietal cells.

The pseudo-colored image was created as follows: The original electron micrograph was converted to RGB. The image of "Casper, the friendly ghost" was prepared in Photoshop. Separate layers of electron micrograph were cut with the lasso tool, to include the ghost, the pumpkin the stem and the background. For each layer, a foreground color was chosen, then the layer was selected (AltS>A), and a gradient map was applied (Image>Ajustments>Gradient map. Double clicking on the gradient map image allowed for selecting additional colors and editing the gradient. The bandaid tool was used to remove dust and scratches, the burn and dodge tools were used to create the drop shadow effect and give a 3-D effect to the objects. The photoshop image was imported into Corel Draw x3, and power clipped into the appropriate sized rectangle and exported as a tiff (CMYK at 300 dpi).

Berry et al. Science. 2003 Dec 19;302(5653):2077-8.
Berryman et al. Mol Biol Cell. 2000 May;11(5):1509-21.
Board et al. Int J Biochem Cell Biol. 2004 Aug;36(8):1599-612.
Cromer et al. Eur Biophys J. 2002 Sep;31(5):356-64
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Suh et al. J Investig Dermatol Symp Proc. 2005 Nov;10(2):105-9.

well as tubing. Moreover, this is very economical too. Rao Karavadi rao@lehigh.edu Thu Jun 26

I don't have the answer for the tray, but I would like to make some comments about handling small samples with tweezers. I have taught the MicroCleave™ technique to a lot of students at a bunch of different universities over the years. These samples have at different stages of the process very thin, fragile samples and then ultimately very, very small samples that must be handled with the sharpest tweezers available under the stereomicroscope. There are two common bad habits that students have with respect to handling samples with tweezers. One is that they try to move the sample large distances. Often they do this without a catch basin such as their cupped hand. The other bad habit that they have is not to anchor their hand while they are manipulating the tweezers to position the sample. They will have their hand in the air trying to keep their hands steady. The following are points that I always seem to have to train students to do when handling very small and delicate samples. 1. If you have to move your samples in a tweezers a long distance, then use a self-closing or one that has a sliding O-ring to keep them closed while you move them and to cup one hand under the tweezers while doing so. Better yet, put the tweezers, with the sample held clamped in the tweezers, on a tray and then move the tray. 2. When positioning the sample, anchor the heel of your hand firmly and then just use small movements of your fingers to manipulate the tweezers. 3. If you are transferring the sample from one holder to another under a stereomicroscope, again, anchor your hand and don't move it. Lift the sample out of the holder and then move that holder out of place while holding the sample stationary and then move the second holder under the microscope under the sample. Then lower the sample into that holder. This way, the sample only makes a small distance move up and down while the hand is stabilized with the heel of your hand anchored. 4. If you can't anchor your hand on the desktop because you have to have the tweezers high off the desktop, then get a block, brick, or box that is rigid so that you can raise your hands to the correct height and still anchor the heel of your hand. 5. If you have delicate samples, then don't drink coffee or be hungry while you have to do the work, otherwise your hands will shake too much. 6. Never hold your samples where there is nothing to catch them other than the floor. If your hands aren't far from the table top, then they won't go far when you drop them. (Notice I said "when" and not "if".) 7. If you get a choice of colors for bench tops and counters, go with a single color, never go with a pattern, especially patterns that look like granite! Otherwise, you won't even find a screw that falls on the tabletop, let alone a sample. Scott D. Walck walck@southbaytech.com Thu Jun 26

You might try a surplus outlet for electronics manufacturing equipment to obtain an inexpensive "vacuum tweezers" or die pick-and-place device. This consists of a tiny vacuum wand (shaped a bit like an airbrush sprayer) with a soft tip and a finger-controlled push button valve to release the vacuum. In its intended use, it is used to pick up a bare silicon chip (integrated circuit or "die") and set it down where it is to be bonded to a substrate. Tips are available that are small enough to handle a chip that is 20 thousandths of an inch square, or so. The vacuum can be provided by something as simple as an aquarium pump since the airflow is minimal. John Twilley jtwilley@sprynet.com Thu Jun 26

Microscopy Today Gets a New Editor

Ron Anderson

Microscopy Today Editor

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Nothing lasts forever. After retiring from IBM after a 42-year career in 2001 I was honored by the Microscopy Society of America with their trust when they appointed me Editor of *Microscopy Today*. MSA bought MT from its founding editor, Don Grimes, in 2002 and I assumed the editorship in April of 2002. The May 2002 issue was my first. My use of the singular "I" and "my" is not correct. From the beginning, my wife, Dale Anderson, was the other half of the MT team as Art Director and proof reader. She was responsible for the redesign of the magazine that was introduced with the July 2002 issue and selects and creates the cover eye-candy.

As much as we enjoy producing MT, we have to recognize that we are both into our seventies and it is time to retire for good.

MSA has appointed Charlie Lyman, Lehigh University, as the new editor effective January 1, 2009. Charlie is coming off of nearly a decade as editor of MSA's journal, *Microscopy and Microanalysis*. I have told Charlie that I will stay on to help him where ever and for as long as he needs me. Dale and I plan to attend M&M meetings into the future and look forward to seeing many of you there.

Dale and I would be remiss if we did not thank the many people who have made MT what it is today.

- Phil Oshel is Technical Editor. He has procured many articles for MT and has been a resource for MT with regard to biological science issues. Phil performed the same function for Don Grimes.
- Tom Phillips, Contributing Editor, provides us with NetNotes—one of our most popular features.
- Renée Stratmoen, Advertising Director, helps provide the advertisements.
- Jon Shields, AKA Dear Abbe, brought humor to the pages of MT.
- Stephen Carmichael whose one-hundred and fifth (105!) consecutive "Carmichael Article" is in this issue!
- Nestor Zaluzec designed our website, automated the subscription process and saw to the creation of a downloadable archive of all past MT issues.
- The authors of all of the articles we published. The authors range from high school kids to all level of amateur and professional microscopists to retirees. Some authors have submitted more than five articles. You have made MT the respected publication it is today!
- All of us are grateful to the advertisers who have made MT financially viable. Without them there would be no MT. Their advertisements are technically and artistically superb. On a personal level, the many warm friends that we have made with the fine people who work for the advertisers and who place the ads is a treasure.

We look forward to enjoying our children and grandchildren, staying active as volunteers for our church and local symphony orchestra, staying in touch with microscopy one way or another, and living the Florida retiree lifestyle (whatever that is). Please feel free to e-mail us at the address above from time to time.

Ron and Dale Anderson