NETNOTES

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Selected postings from the Microscopy Listserver (http://microscopy.com) from 12/11/16 to 2/15/07. Postings may have been edited to conserve space or for clarity.

SAMPLE PREPARATION - frozen tissue for TEM

I have some tissue that is embedded in OCT and stored at -70°C and want to know if it can be processed for TEM. Sean Kelly <mrsean@u.washington.edu> 04 Jan 2007

Can you process it? Yes. Should you process it? Maybe. Will you get good ultrastructure? No. A lot will depend on how the tissue was treated before it was frozen: was it flash frozen without any fixation or cryo-protection? It will have a lot of freezing artifacts at the EM level—ice crystal damage to membranes, *etc.* If it was lightly fixed (*e.g.*, paraformaldehyde), it might fair a bit better. If it was fixed and cryo-protected (*e.g.*, treated with sucrose), you might get some decent structure. You'll have to cut away as much of the OCT as you can and then thaw the tissue. I would advise thawing it in a buffered glutaraldehyde solution so that it fixes as it thaws. Then process for TEM as usual and keep you fingers crossed. Over the years, I've had to process tissues that were not originally intended for EM and I've been surprised by what may be rescued. Leona Cohen-Gould <lcgould@med.cornell.edu> 04 Jan 2007

SAMPLE PREPARATION - fixation of whole mosquitoes

Does anyone have any suggestions for fixation of whole mosquitoes? We are having problems with the preservation of some external structures, particularly the antennas. Is there some trick to prevent the crumpling of the antennas and the wings? What to do to minimize the lost of limbs? We are using the following protocol: prefixing in cacodylate buffered glutaraldehyde at 4°C (no stirring) -gentle buffer washes -fixation in buffered OsO_4 -dehydration in graded Quetol 651 followed by infiltration with Quetol. Ernesto Chinea <echinea@gmx.net> 08 Feb 2007

I suggest cutting the mosquito into 2-3 pieces (in the prefixative) with a sharp razor blade (*e.g.* head, thorax and abdomen?). This should improve fixation and embedding. The cuticle is usually very hard to penetrate and cutting the insect will hopefully improve permeability of tissues to all used reagents. I have done this with leafhoppers and aphids with normally good results. El-Desouky Ammar <ammar.1@osu.edu> 08 Feb 2007 *See the article by Gorb in this issue. ...Editor*

SAMPLE PREPARATION - fixation for mitochondria

I am in need of the "best" fixation for mitochondria in mouse and rat muscle and kidney. Past experiments have yielded OK, but not great results, using 2.5% glutaraldehyde with and without paraformaldehyde in sodium cacodylate buffer followed by 1% OsO_4 and uranyl acetate block stain. Pat Connelly <connellyps@mail.nih.gov> 10 Jan 2007

If what you mean by "best fixation" is a fixation which does not allow artifacts or deformations of the structure, apart from cryo-fixation I think that your fixation is optimal (and is pretty classical). Personally I have a preference for the addition of a low concentration of formaldehyde (2%) because it penetrates faster than glutaraldehyde in the tissue. It acts as a sort of "pre-fixative" before the glutaraldehyde comes and stabilizes the structures. If you mean "increasing contrasting of the membranes", you could consider using ferrocyanide (which I tried successfully) and/or tannic acid (which I didn't try but is well documented). I found this reference for you but there are probably others: Berryman MA, et al (1992) Effects of tannic acid on antigenicity and membrane contrast in ultrastructural immunocytochemistry. *J Histochem Cytochem* 40, 6, 845-857. Stephane Nizets <nizets2@yahoo.com> 11 Jan 2007

I agree with Stephane, there is nothing wrong with the fixative you are using. If more contrast is what you seek you could also try a post-fix or mordant in potassium dichromate. Substituting Dalton's chrome-osmium fix for your regular osmium post-fix might be worth a try. Alternatively, you could try a "light" fix followed by histochemistry to demonstrate mitochondrial enzymes, they post fix with osmium. This would be more work. Geoff McAuliff <mcauliff@umdnj.edu> 11 Jan 2007

SAMPLE PREPARATION - fixation of mouse brain tissue

Does anyone have any pet techniques/fixatives for TEM processing of brain tissue? Specifically mouse brain, if it matters. We just did a run with less than stellar results—poor membranes, etc. We are now trying a couple different things, but if anyone has some tried-and-true tips, it might save us a ton of time. No immunolabeling, just ultrastructure. Some recipes call for picric acid. Can anyone tell me what purpose this serves? Randy Tindall <tindallr@missouri.edu> 24 Jan 2007

CNS tissue is best fixed by perfusion, without a doubt. Conventional procedures (excision) hardly ever give very good results with CNS. So, my first question is: did you use perfusion? John Bozzola <bozzola@siu.edu> 24 Jan 2007

I use 2-3% glutaraldehyde in 0.1M phosphate buffer at room temperature, pH 7.3-7.4, perfused via the left ventricle. While I have a small peristaltic pump, I have used gravity with excellent results. While some will argue otherwise, one does need a fancy apparatus or high pressure; I don't even measure the pressure. I use just a few ml of the same buffer (without fancy additives) as a washout and at least 50-75 ml of fix perfused in 5 minutes. In my experience most labs with less than optimal results use 1. A tiny little needle in the ventricle 2. An inadequate rate of flow and 3. Way too much washout. Use an 18-20 g needle (same inside diameter as the aorta!) inserted into the vent. Then have the assistant just nick the r. atrium, being careful not to go too deep and cut the aorta. Going too far into the ventricle can be a problem so put a piece of cork or Tygon tubing around the shaft of the needle so you don't go too deep. Make everything fresh. Fix for a few hours at room temperature, multiple rinses in buffer, osmicate (reasonably fresh osmium) thin slices/small pieces in the same buffer for several hours, multiple buffer rinses, store in buffer if needed. If you must keep orientation cut 200 micron Vibratome sections (before osmication) so you can see the anatomy after osmium turns everything black. Dehydrate rapidly in graded ethanols (too much time in ethanols will remove cytoplasm, Hyatt's book has the illustrations and references to prove this), clear, infiltrate and embed. Uranyl acetate (after multiple washes in sodium acetate to remove phosphates) will improve contrast. I have not used K-ferricyanide to improve osmium staining. Some labs use a little bit of picric acid as a protein coagulator, however. Plenty of people get good results without it. Geoff McAuliff <mcauliff@umdnj.edu> 24 Jan 2007

Geoff McCauliff's tips are my experiences too. A minute comment: use gravity perfusion; a wonderful & detailed instruction is given in: Forssmann, W.G., Ito, S., Weihe, E., Aoki, A., Dym, M. and Fawcett, D.W. (1977) Anat Rec 188, 307-14. a small but important tip: Filter all solutions used for perfusion through a 1 (or 2) micrometer filter before use! If you ever have seen a capillary in cross section and measured its diameter, you know why. Use the two respiratory tree-step technique described in the paper. In my experience, perfusion with the "rinse" solution for 20-30 seconds followed by a single or two-step perfusion (3-5 minutes) with a mixture of freshly prepared formaldehyde and glutaraldehyde (the latter prepared by the manufacturer according to the Anderson-technique) is sufficient for an adult wild type mouse. Peter Heimann <peter.heimann@uni-bielefeld.de> 25 Jan 2007

SAMPLE PREPARATION - purpose of PVP

Does anyone have any explanation for the function of PVP (polyvi-

nylpyrrodine) in the phosphate buffer for fixing perfusion? I have seen it in an article and now I am curious. Fernanda Malho <fcmalhao@icbas.up.pt> 07 Feb 2007

I suspect it is there for osmotic purposes. I believe LR Gold embedding protocols sometimes include PVP with the ethanol dehydration steps to minimize osmotic stress. Tom Phillips <phillipst@missouri.edu> 09 Feb 2007

SAMPLE PREPARATION - maleate buffers

We are trying to follow a not-very-detailed protocol for brain tissue that utilizes maleic acid buffer for wash steps before and after the "uranyl acetate colorization solution". One would presume that this refers to en bloc staining also using this buffer, but no details are given as to molarity, buffer composition, etc. We have no experience with this buffer, although I've heard of it, and are having a terrible time finding good references regarding its use and formulation. My research in our reference library and online shows many instances of en bloc staining using maleic acid buffer, Tris-maleate buffer, or simply maleic acid, so my questions are: 1) Are these terms pretty much interchangeable (i.e., is Tris-maleate buffer just maleic acid buffer with the addition of tris)? 2) Do you all have any preferred formulations for this buffer in the uranyl acetate step? Most of the references I've seen use 0.05 M to 0.2 M buffer with 0.5-1% uranyl acetate. 3) Is this buffer pretty much restricted to en bloc staining, at least for use in TEM studies? 4) What are the advantages of this buffer over aqueous en bloc staining with uranyl acetate? Randy Tindall <tindallr@missouri.edu> 29 Jan 2007

Here is what we use with success: Sodium maleate (Maleic acid, monosodium salt; molecular weight 138.1). 691 mg / 100 mls = 50 mM. Assume uranyl acetate stock is 5%. Dilute 1 part + 9 parts of 50 mM sodium maleate. *En bloc* staining protocol: After osmium, rinse tissue three times for 5 min each in deionized water, and then rinse tissue three times for 5 min each in 50 mM sodium maleate, pH 5.2. Incubate tissue for 1 hr to overnight in 0.5% to 1.0% uranyl acetate in 50 mM sodium maleate, pH 5.2 (for overnight, probably should use 0.5% UA; for 1 hr probably should use 1% UA). Rinse three times in deionized water for 10 min and then dehydrate for 10 min each in 50%, 70% ethanol, 95%, 100%, 100%, and 100% ethanol before starting resin infiltration. Tom Phillips <phillipst@missouri.edu> 29 Jan 2007

Thanks to everyone who responded to my maleic acid buffer query. We now have some things to try. By the way, the following response is irresistible, courtesy of the incomparable Snoop Leunissen (Jan, that is). Foshizzle, microscopists rock, dog. Enjoy. Randy Tindall (tindallr@microscopy.edu) 01 Feb 2007

"Buffer Rap" by Jan Leunissen

For anyone who likes to do EM
buffers at times are a hell of a wham
the chemistry lacks in every way
yet we like to have a good display

So what is this all about mall ee 8?
Does that stuff accommodate uranyl acetate?
at what pH, what ionic strength?
I better ask the LIST for some reference

With some advice here and a helping hand
I am sure I can pretend I understand
So I take some stuff from the lab supply
Mix it together and hope I will get by

Wow, man, what happens, it's workin' alright!
The negative stain is clear and bright!
No precipitate, the structures they are fine
It works! Now I can advise the next in line.

Anyway, for mallE8 to work alright,
you see you need two solutions, mark them A and B
Empty bottle (C) in the middle, now that should do
And mixing left and right will be the clue

Solution A has sodium hydrogen maleate
23.2 grams if it's trihydrate
Dissolve in 200 ml 1M Sodium Hydroxide
And make to 1 liter with distilled water alright

Solution B is simple
just point 1 Molar NaO-age
Solution C is the trick,
Now don't get into a rage!

Chorus...
Take 25 mls out of bottle A
transfer to Bottle C without further delay
Mix in x mls of B, top up to 100 cc
Get approximate pH from the listing you will see

pH	*x ml 0.1 M NaOH*
5.2	*7.2*
5.4	*10.5*
5.6	*15.3*
5.8	*20.8*
6.0	*26.9*

For Tris maleate it is much the same
Two stocks again, it's almost lame
The first holds Tris as well as Maleic acid
24.2 and 23.2 grams, yep that's it!

And before I forget,
make a liter of that
now the other stock again is just NaO-age
a plain 0.2 Molar is all that it takes

Chorus...

pH	*x ml 0.2 M NaOH*
5.4	*5.4*
5.6	*7.75*
5.8	*10.25*
6.0	*13.0*

Apologies, the listings don't rhyme. Disclaimer: I will not be responsible for anyone getting hurt while trying to rap the numbers! X-from: "*Data for Biochemical research*", by Dawson, Elliot, Elliot and Jones Clarendon Press, Oxford, 3rd ed Recipes for maleate buffer (*J.Am.Chem.Soc* 51 (1929), 1754) and Tris/maleate buffer (*PSEBM* 68 (1948) p354 or Meth Enzym. 1 (1955) 138.

SAMPLE PREPARATION - Vibratome sections

When producing Vibratome sections (for EM or light microscopy) of insect tissue, I usually embed the tissue in 15% gelatin before sectioning, and have been doing that for years. But now I suddenly have grown tired of the sticky gelatinous mass that surrounds these fragile sections. Agarose is supposed to be a good substitute. Has anyone got experience with it or can tell me which type/temperature/concentration of agarose to test? Gerd Leitinger <gerd.leitinger@meduni-graz.at> 10 Feb 2007

Another alternative is to encapsulate in alginate, we have used this to encapsulate both individual cells and tissues. The advantage is that you work at ambient temperature (no heating), disadvantage that you

introduce calcium ions into the system which you may not want to do). One method we used was to use a 2% solution of sodium alginate and solidify by dropping into or flooding with 50 mM calcium chloride. Ian Hallett <ihallett@hortresearch.co.nz> 11 Feb 2007

For EM embedding of loose cells (in order to make them easier to handle), we have used Sigma's Type VII or Type IX agarose. I have not developed a preference, since this is not something I really do routinely. You may or may not like agarose after having used gelatin. While agarose is more clear and also less visible in the microscope and is probably better for the cryo knife, I have found it more tricky to handle - keeping it solid, *etc*. The concentrations and references are a bit further away to reach at this moment, but I'll look them up if you haven't gotten them yet from other sources. Vlad Speransky <vladislav_speransky@nih.gov> 12 Feb 2007

I have always used a simple agar embedment for sectioning various tissues with a Vibratome. I start by heating and stirring 4% agar by weight in water until it all goes into solution. Usually some water will evaporate thus the percentage is actually higher than 4% but that is not critical as long as hardens sufficiently when cooled. I store it in a

50ml tube at 4C than melt it by placing it in a beaker of water on a hot plate. To embed the tissue I put a drop of the agar on a glass microscope slide and then add the tissue and another drop or two of agar as needed to cover the tissue. Then I quickly cool the slide/agar/tissue by placing it on ice. When the agar is hardened after a few minutes I trim the excess agar away with a razor blade and glue the block to the Vibratome stage with cyanoacrylate (SuperGlue). There are many variations including the use of expensive low temperature melting point agarose or agar for temperature sensitive samples. There are a very confusing number of agar and agarose products offered for sale and I was shocked that some don't behave "correctly" for this purpose. One product would never gel. I get the most ordinary and inexpensive product. Larry Ackerman <larry.ackerman@ucsf.edu> 13 Feb 2007

SAMPLE PREPARATION - LR White polymerization problem

I am having difficulty polymerizing a sample in LR White. The cells (macrophages and dendritic cells) were grown on a piece of Aclar, fixed with formaldehyde/glutaraldehyde, post-fixed with uranyl acetate, dehydrated in a graduated series of ethanol, and infiltrated with LR White. I then sandwiched the Aclar film containing the cells between two larger pieces of Aclar (since the edges don't polymerize due to air exposure) and put the sample in the oven at 55°C. After 48 hr, the resin immediately around the piece of Aclar containing the cells appears very fragmented and cracked and is obviously unsuitable for sectioning, while the resin further from the cells appears to have polymerized properly. At first I thought it was an infiltration problem, but I did several changes of 100% resin and even left the samples overnight on a rotator at room temperature in 100% resin to ensure complete infiltration. Does anyone have any idea what they problem might be? Dennis McDaniel <dmcdaniel@usuhs.mil> 22 Jan 2007

I think that your problem is a result of incomplete dehydration. LR White is supposed to handle some water without problem, but when you sandwich the pieces, you create a limited volume for diffusion and the water becomes too great locally. Make sure your ethanol is dry (on molecular sieve or freshly opened). That's my theory. Kim Rensing <krensing@ucalgary.ca> 22 Jan 2007

I suspect that your polymerization problems arise from entrapped air. I eliminate that problem by purging my oven with nitrogen, thus alleviating other more tedious approaches. Works every time. Gary M. Brown <gary.m.brown@exxonmobil.com> 22 Jan 2007

SAMPLE PREPARATION - SEM of cells without critical point drying

I have a request for experts in SEM who have some experience with eukaryotic cells. We have a basic SEM which is normally used to study

minerals, so we are not equipped with critical point dryers and the like. Now I would like to have a look at some cells in culture with the SEM but I have no idea how I could prepare the cells without a critical point dryer. Of course if I simply fix and dehydrate in acetone, then air dry the cells, I would expect the structure to crumble. On the other hand, perhaps I could keep them in a semi-hydrated state and look at them at 10 Pa vacuum. I would be grateful if you cared to share your opinion on this subject with me. Stephane Nizets <nizets2@yahoo.com> 16 Jan 2007

I think critical point drying is best for cell preparation but we tend to use a hexamethyldisilazane (HMDS) dehydration protocol as it is much easier. I think you will find looking at wet cells problematic. With a tungsten gun XL30 ESEM I find it very difficult to get adequate resolution with cultured cells. My HMDS protocol is given below. If anyone would like to suggest improvements please fire away. Although fixing in PBS goes against EM principals the results for modest magnification SEM are often good. Preparation of dry samples for SEM using HMDS: Safety. Work in fume hood and use gloves. Retain ethanol and HMDS (hexamethyldisilazane) for disposal in non-chlorinated waste bottle. Fixation—Fix in 4% glutaraldehyde in buffer (usually 0.1M)* Leave for 1 hr at room temperature or 24 hrs in the refrigerator. Rinse in buffer x3 (total storage time should exceed fixation time by a factor of 3). SEM Dehydration—5 min in 20% ethanol; 5 min in 30% ethanol; 5 min in 50% ethanol; 5 min in 70% ethanol; 5 min in 90% ethanol; 5 min in 100% ethanol; 5 min in 100% ethanol; 5 min in 100% ethanol / HMDS (2:1); 5 min in 100% ethanol / HMDS (2:2); 5 min in 100% ethanol / HMDS (1:2); 5 min in 100% HMDS; 5 min in 100% HMDS; 5 min in 100% HMDS. Remove specimen from HMDS and place on filter paper in a Petri dish and leave in fume hood until dry. *For 4% glutaraldehyde in 0.1 M buffer take 50 mls of 0.2 M buffer, 34 ml distilled water and 16 ml of 25% glutaraldehyde. 0.1 M Phosphate buffer or sodium cacodylate are used. Fixation using PBS: 8.4 ml 0.2 M PBS plus 1.6 ml 25% glutaraldehyde gives 10 ml of a 4% glutaraldehyde fixative. <David.Patton@uwe.ac.uk> 16 Jan 2007

If your cells are in suspension I would coat cover slips with poly-l-lysine (1 mg/ml in distilled water), place a drop of the suspension onto the cover slips and allow to settle for up to an hour in a humidity chamber. Then, after the cells attach, just gently rinse the cover slips, fix, and dehydrate using David's HMDS protocol. I would suggest 2% glutaraldehyde in buffer (0.1 M phosphate, cacodylate, or common buffer for biological EM) for 10-15 minutes, followed by three or more buffer rinses, post-fixation in 1% osmium tetroxide (buffered or aqueous) for the same time, followed by water rinses for 5-10 minutes each, then into your dehydration schedule. Sputter coat and mount. Randy Tindall <tindallr@missouri.edu> 16 Jan 2007

When I was at the high-voltage TEM, I designed a hydration stage, which allowed me to examine cells that were fully hydrated as determined by the fact that upon removal from the scope, at least some of the cells were still viable. These were prepared by placing Formvar/carbon coated gold grids in the culture dish, so the cells would grow on the grids, then just removing the grids from the dish, placing them in a 100% humidity chamber, blotting off the excess fluid, and transferring the grids to the hydration stage maintaining the humidity as close to 100% as possible. If you can operate your VPSEM at the vapor pressure of water for the temperature of the specimen chamber (~25 torr), then you can look at them fully hydrated. I do not know, however, whether you will see anything of interest, or just the surface of the thin film of water that will still coat the cells. Bill Tivol <tivol@caltech.edu> 17 Jan 2007

SAMPLE PREPARATION - SEM of cells in a monolayer

A colleague needs to SEM image cells growing in a monolayer on a coverslip in a thin layer of collagen matrix. I tried to prepare it more or less the usual way—aldehyde fixation in cacodylate (2% formaldehyde/2% glutaraldehyde for about 90 min) followed by osmium, dehydration in increasing concentration of ethanol, replacing ethanol with amyl acetate and eventually CPD from carbon dioxide. The results are suboptimal at best—cells seem to shrink and get extracted. Does anybody have a good idea they would like to share? Michal Jarnik <michal.jarnik@fccc.edu> 02 Feb 2007

The protocol sounds very "usual" as you say, and I wonder if something just went wrong along the way? You didn't mention any osmotic agents in the fixative, sometimes beneficial for cultured cells; various things and concentrations are used. I just did some samples that used 7.5% sucrose in the fixatives and buffer washes until the OsO_4 was rinsed out. A somewhat longer time in the glutaraldehyde for SEM is sometimes beneficial; the cells toughen a bit—tip from our old Polaron CPD manual. Also, while I don't think it is the problem, it is not necessary to use amyl acetate: using 100% ethanol works just fine for the CPD process. Make sure the ethanol is really dry—store ethanol for final changes over good molecular sieves - Type 3A, recently baked, ~5% of ethanol volume, let is stand some days well sealed, don't stir up fines. The CO_2 also needs to be a dry grade with a good molecular sieve trap on the line. Make sure to exchange well to get all the ethanol out. The cells on coverglass should exchange quickly, but were they in some "capsule" that limited exchange? Was the vapor phase clear when it went supercritical, or hazy? Was there any smell of amyl acetate when you opened the chamber? Could the cover glasses have gone dry at some point—even in the CPD process? Did you do this job yourself, or did you have an underpaid, un-benefited work-study student do the work? Sorry, that's not fair; I used to be one; they aren't ALL inattentive.... The liquid drops as gas phase pressure increases; maybe they got above the liquid surface? The instructions printed on our Balzers CPD-030 unit say to "drain and refill several times" but the sample should never really be drained from liquid during the flushing exchanges; always do partial changes to keep the sample submerged. Hope this helps. Dale Callaham <dac@research.umass.edu> 02 Feb 2007

First, skip the amyl acetate, it's not needed. Second, how many soak-purge cycles did you do in the CPD? Fill the chamber, flush with liquid CO_2 until the ethanol is gone, let cells soak X minutes, then purge with liquid CO_2, and repeat N times. I found a monolayer on coverslips usually only needed 3 soaks for 5 minutes each, but sometimes could require 4 or 5 soaks. If all of the ethanol (or amyl acetate) is not removed, you will get serious shrinkage. I also didn't bother with formaldehyde or osmium, just 1.25% glutaraldehyde (2% is pretty strong). Depending on the kV you're using, 1% OsO_4 may be helpful, though. Fixation in OsO_4 should only need an hour. Glutaraldehyde can go 1 to 2 hours. What % ethanol did you start the dehydration at? I've used 30% and 50% successfully; any higher is too high, and sometimes one needs to start at 15%. How long in each ethanol step? 5 minutes should be enough. And then, you will get some shrinkage no matter what you do. Other things to try: Hexamethyldisilizane (HMDS). After ethanol dehydration, go through a 2:1, 1:2 ethanol:HMDS, 3 x 100% HMDS series and then air-dry for 1 to 2 hours. Room temperature or at 60°C; sometimes one works better than the other. Do in a hood! If you have the proper equipment, freeze-drying can work very well, and since there are no chemical fixatives or dehydrating agents involved the cells may be "more real". Phil Oshel <oshel1pe@cmich.edu> 02 Feb 2007

I would differ only slightly with Phil's suggestions: since your cells are on, or in, collagen, you will probably need to extend your dehydrations: perhaps 2 changes at each concentration, starting at 50% or even 30% as Phil suggested. Collagen is an incredible sponge and I've had a miserable time with it over the years. Leona Cohen-Gould, <lcgould@med.cornell.edu> 05 Feb 2007

We often run into the same problem. I think some occurs when cells are left with minimal fluid for even very short times while dehydrating. Surface tension is a real problem with very little fluid coverage when changes are being made. It is best to leave a little fluid in the culture dish

HUMOR

Dear Abbé

Dear Abbé,

We have a professor in our microscopy facility that claims a "watched ultramicrotome never sections." If that isn't enough, he only casts formvar films when there is a full moon the night before and spins around three times in the lab before starting. I believe he is just a superstitious old goat, but my lab mate thinks he is an EM Shaman. What is your opinion?

Skeptic in Syracuse, NY

Herr Skeptic,

Ach! The pitiful whining of the unversed and unwashed few. Obviously, you are unaware of the presence of EM mystical powers. These mystical forces were originally recognized by the Blessed Hildegard of Bingen, but have been acknowledged by other cultures as well. Most uninitiated scientists believe that these mystical musings involve spiritual explanations. In reality, they enlighten us about the metaphysical aspect of Electron Microscopy and related disciplines. Call it what you will – EM shamanism, mojo, voodoo, tao, druidism, Republican policy – it all adds up to unexplained forces at work that are ignored at your peril.

Dear Abbé

So, like, several of us musically inclined microscopists get together and play passable, but no where near perfect, versions of familiar songs, and our front man is a little guy who is rather adept at slide guitar. Our hit single, *It's my sample and I'll cry if I want to,* is about to go copper. If we were to call our group 'Microslide and the Coverslips' do you think that anybody would, like, you know, get it?

Anxious in Athens, GA

Dear Anxious,

Quit your whining. Of course they will get it and if they don't then they are not worthy to listen to your music in the first place. At the beginning of the set you should require everyone in the audience to write down Bragg's equation, anyone who cannot do so should immediately be asked to leave with no refund. Fine music and microscopy have a long history together. My good friend Richard Wagner was an accomplished microscopist. In fact his original name for *Tannhäuser* was "*Ich liebe Fresnel Franse*" but at the insistence of his publisher he capitulated and changed it. Don't make the same mistake!

Got a question for Herr Abbé? Please write to his personal assistant at jshields@cb.uga.edu. Abbé may not answer all the questions, only those he deems worthy.

and do a few more changes rather than risk the problems of excess shrinkage. Even under the best of conditions, some shrinkage is inevitable. Years ago I did a large number of cell cultures using Ducupan resin to infiltrate the cells. Cells were fixed and then infiltrated with successive mixtures of Ducupan:H_2O. The excess 100% resin was washed away using propylene oxide and then cultures polymerized. This minimized shrinkage but also you should expect that surface detail could also be obscured if any resin remained on the outside of the cells. This method did minimize breakage of long processes from axonal and dendritic cells. Debby Sherman <dsherman@purdue.edu> 02 Feb 2007

When I was working with cultured fibroblast monolayers for SEM back in the 70's, I used aldehydes and osmium, as most do, and experienced ripped cells and ones that appeared to 'crack' along the surface, particularly where long filopodia extended from the cell bodies. But, my TEM looked fine. So for kicks, I tried my usual TEM protocol for the SEM samples, which used the same aldehydes and osmium but also used uranyl acetate as a post-fix. I had excellent results. No cracks or tears at all. Something you might wish to try. Ann Hein Lehman <ann.lehman@trincoll.edu> 02 Feb 2007

When I did cultured cells on coverslips I found problems like you were experiencing. 1. We changed to Parducz fixative instead of Osmium which is a wonderful hardener for filipodia, cilia, *etc.* and was used mainly in the LM until SEM preps came along. Parducz is 6 parts of 2% osmium(aqueous) to 1 part saturated $HgCl_2$. ($HgCl_2$ can be made by merely dumping in some $HgCl_2$ into distilled water until it doesn't dissolve and go a bit further until there is a small amount of precipitate on the bottom. We kept it in a brown bottle and kept it at room temperature for many months. Take only from the top of the bottle.) Mix it together just before use. Thus mix for instance 12 ml. of 2% aqueous osmium and 2 ml $HgCl_2$. We only used the mixture once and then properly disposed of it. We often didn't bother with the glutaraldehyde as the Parducz did the trick, but if we had to keep the cells before they were prepared then we kept them in glutaraldehyde. (Something like a 3% glutaraldehyde in a non-phosphate buffer. Phosphates precipitate for SEM.) Time: 1-2 hrs in glutaraldehyde (unless stored) and then 1 hr in Parducz then either freeze drying or CPD after proper dehydration in ethanol. We never went through amyl acetate. OR we fixed directly into Parducz for 1 hr., then dehydration and CPD or freeze drying. Freeze drying doesn't require dehydration and we went directly from the Parducz - quickly froze it, then Pearse Tissue Dryer. 2. Also check how quickly your CPD is releasing pressure. If it is more than 100 psi per minute then it is likely doing the damage. 3. I Agree with the other dehydration comments. Judy Murphy <murphyjudy@comcast.net> 03 Feb 2007

SAMPLE PREPARATION - SEM of Zebra fish

I am about to prepare some zebra fish heads for SEM - to look at morphology & take some measurements. What do you think are the pros and cons of critical point drying versus HMDS drying after fixation in glutaraldehyde plus PVP in sodium cacodylate buffer followed by osmium plus potassium ferrocyanide followed by staining in aqueous uranyl acetate before dehydration for small fish heads? I have had success with the HMDS drying using cultured cells and insect tissue. The thermocirculator attached to my CPD has problems—it is difficult to heat slowly enough so until I can replace it I thought HMDS might be an alternative method. Ursula J. Potter <u.j.potter@bath.ac.uk> 02 Feb 2007

If you want to make dimensional measurements on dried tissue, I strongly recommend that you take the time to read Boyde, A and Maconnachie, E (1979) Volume changes during preparation of mouse embryonic tissue for scanning electron microscopy, *Scanning* 2:149-163 Boyde, A and Maconnachie, E (1981) Morphological correlations with dimensional change during SEM specimen preparation, *Scanning Electron Microsc.* 1981, IV:27-34 These are the only two publications of which I am aware where the authors actually made dimensional measurements of soft tissue (embryos) as they proceeded through all the stages of fixation, dehydration, and CPD or other drying. The best they got was only 60% volume shrinkage (*i.e.*, the final volume was 40% of the live volume. This works out to be about 25% linear shrinkage.). This dirty secret often goes unremarked because, as tissue-culture cells are tacked down to glass slides, the *x-y* dimensional are stabilized by the glass. The thickness shrinks but that is harder to measure. Of course, I am sure that some tissues may be more robust but I can also assure you that many are much more sensitive. And as I noted, 60% was the best they got. There were many ways to make it worse. Freeze drying was better, especially if you kept the tissue frozen to about -100°C while you looked at it. But this has its own problems, particularly those to do with ice crystals. As many readers will likely find my claims "ridiculous," I do encourage you to read the papers and then do you own tests. James B. Pawley <jbpawley@wisc.edu> 04 Feb 2007

Let me chime in here and completely agree with Jim. I have measured shrinkage on the order of 50% to 60% in soft tissues, in this case neuromast end organs from fish lateral lines. The original sizes were measured both by simple measuring of the intact end organs in a light microscope, and measuring the impressions the end organs made in silicone casts of the lateral lines. These two measurements were in almost exact agreement. The end organs as seen in the SEM were at best 1/2 the size of the living and the "fixed-only", *i.e.*, not dehydrated, organs. Further the shrinkage was *not* isometric. That is, the shrinkages along *X* and *Y* axes imposed onto the end organs were different. Keep in mind that tissues have mechanical properties, and these properties depend on the histology of the tissue. *E.g.*, what kind of collagen does the tissue have? In what directions do the fibers run? Shrinkage in the direction of the fibers will be different than shrinkage in the direction orthogonal to the fiber direction. And that's just the beginning. I don't know of any studies on such tissue properties and how they affect dimensional changes. If there are such references, I would very much appreciate it if they were posted to the list. Best answer to measuring zebra heads: do all your measurements with a light microscope. Make yourself a little jig, so the heads can all be held in exactly the same set of positions so all the measurements will be correct. Otherwise you'll have measurement errors caused by slightly different tilts of the heads. Do this before fixation. A rule of thumb used by fish ecologists, back when I was doing marine ecology, was that all formalin-fixed fish lengths were 10% too short. That might not be really true, but the shrinkage was. Phil Oshel <oshel1pe@cmich.edu> 05 Feb 2007

Anyone seriously interested in CPD should read Hans Ris' paper. Anderson used amyl acetate after ethanol in the first paper in the 1950s, mostly so he could be sure that all of it had been replaced by CO_2 before he went through the critical point. Using his original equipment, I can attest that this could take a long time. The amyl acetate used to get forced up into the stem of the pressure gauge and took forever to be washed out. Ethanol and acetone work fine (if you ignore the unavoidable shrinkage mentioned in a previous post on dimensional measurements). The main difference is how their slightly different densities (with respect to liquid CO_2), affects mixing. The bottom line is to use agitation (either by using a magnetic stirrer or by shaking the entire drier) as described by Hans Ris many years ago. Ris, H, (1985) The cytoplasmic filament system in critical point dried whole mounts and plastic-embedded sections, *J. Cell Biol.* 100:1474-1487 His other main point was the absolute necessity of making sure that no water remained in the final CO_2 transition liquid. As both ethanol and acetone love to pick up water from the air and, as biological tissue loves water even more, you must use molecular sieve to dry both the final ethanol and the liquid CO_2 (with a high-pressure filter sold by several manufacturers). Then put the resulting specimen in a desiccator over phosphorous pentoxide. If you think that parts/million CO_2 is dry enough, I suggest that you work out what "concentration" your cells are in

the bomb (volume of dry cellular material/volume of bomb). Admittedly, this is more a factor when drying a monolayer on a few EM grids, as he was. Ris was able to show that, on a number of purified fibrous structures (microtubules, chromosomes, intermediate and actin filaments) failing to use such a filter destroyed their (known) shape. By viewing whole-mount cells in the HVEM he could show that the cytoskeleton was converted into formless "trabeculae". This paper should be read by anyone planning to use CPD for high-resolution SEM or TEM studies. Jim Pawley <jbpawley@wisc.edu> 13 Feb 2007

SAMPLE PREPARATION - critical point drying

Is ethanol miscible in liquid CO2? I can't seem to find anywhere this is explicitly stated. I have seen SEM protocols that imply they use ethanol as dehydrating agent and then directly CPD with CO2. We have an older Balzers CPD020 unit and the manual has a flow-chart which shows only acetone or amyl acetate as dehydrants prior to CO2 transition. It is my understanding that ethanol is a weaker organic solvent than acetone, and if ethanol mixes with CO2, this may be useful for certain CPD applications. Thanks David Lowry <dlowry@asu.edu> 07 Feb 2007

We have used ethanol followed by liquid carbon dioxide for years in our Tousimis Samdri-780 CPD. I switched from acetone, which I have been told mixes better than ethanol with liquid carbon dioxide, to reduce extraction from samples. David Patton <david.patton@uwe.ac.uk> 07 Feb 2007

I routinely use acetone in a Balzers critical point drier but sometimes want to dry samples that may be damaged by the acetone. In these cases (for example, preserving the outer surfaces of insect eggs prior to SEM examination) I use ethanol. From my experience, the ethanol is nowhere near as miscible as acetone in liquid CO_2 but still works perfectly well. You need to use more exchanges of the liquid CO_2 in order to replace the ethanol, leaving to stand for a few minutes between each exchange. If your CPD system has a stirrer, even better! I find that the drying process in the CPD with ethanol can take around four times longer than the conventional technique using acetone. However as long as it achieves the result, then the additional time has been worthwhile. Chris Jones <chrisj@hitachi-hitec-uk.com> 07 Feb 2007

I've also used ethanol as the transfer fluid with liquid CO_2 for many years. Both Polaron and Ladd CPD's have been used without any problems. I tried amyl acetate for awhile but wasn't too fond of it. Its only advantage was being able to smell minute residues of it during the CPD flushing process. However, the number of flushes of liquid CO_2 and length of time between flushes depends on the size or thickness of your sample. Most of the ethanol is removed in the first few flushes. You really have to become familiar with your samples to determine the time required to remove all of the ethanol. And as usual add a few flushes for good measure. I've found certain arthropods/insects etc. to be most difficult using CPD. The hard exoskeleton or cuticle allows ethanol to enter the body of the organism but doesn't easily allow the exchange of ethanol with liquid CO_2. Tardigrades were especially aggravating. Alternative drying techniques than CPD with arthropods and insects are welcome. Bruce F. Ingber <bingber@srrc.ars.usda.gov> 07 Feb 2007

MICROTOMY - cryo-ultramicrotomy

I have just recently begun using the Leica EM FCS cryo set-up for our ultramicrotome. I'm having a little difficulty and was hoping that members of this list could clue me into some tricks of the trade. I am presently trying to section polymer samples and I need them to be around 50nm in thickness. During cutting, the sections often curl or fold like an accordion. So I pick them up with an eyelash tool and try (with much difficulty and cold fingers) to unfold and stretch them out onto a copper grid. The sections have no affinity for the Cu grid and are much happier adhering to the eyelash or wadding up into an unusable lump. Short sections don't work any better, since these just tend to flip away. I'm using an anti-static device but it's difficult to place it into the correct position since I'm using it's holder to hold the copper grids close to the diamond. Thank you for any advice! Shawn 08 Feb 2007

You can get folding grids that look like two regular mesh grids attached along one side, which is the "hinge". You collect a section on one side, then fold other side over on top so section is clamped in place mechanically, so no adhesion of section to grid takes place. This would also tend to flatten the section to some extent. Most EM vendors that sell grids would have them. Whether these folding grids are compatible with requirements for collecting and observing cryosections, I don't know, but it might be worth a try. Just my 2.5 cents worth. Gilbert Ahlstrand <ahlst007@umn.edu> 08 Feb 2007

Are you cutting dry or do you float the sections? In case you are cutting wet, it helps to use a pipette filled with the water/DMSO mixture at room temperature and bring some of the "hot" liquid under the folded sections. The fluctuations in the fluid caused by the temperature difference will unfold the cuts very nicely. Petra Wahlbring <petra.wahlbring@goodyear.com> 09 Feb 2007

If you are cutting dry, maybe using the technique we use for biological samples (Tokuyasu wet retrieval method). Pick your section up using a drop of 2.3 M sucrose in phosphate buffer on a small 2 mm loop. Dip the loop in the sucrose and then bring the drop over the section and lower it until the section "sticks" to the bottom of the drop. The sucrose will freeze quickly, so be fast. Remove the loop to room temp and wait a couple seconds for the droplet to melt and then touch it to your prepared grid. The section will attach to the grid and hopefully be flat again. Again, the temperature difference may help straighten them out. Jo Dee Fish <jfish@gladstone.ucsf.edu> 09 Feb 2007

I have extended experience in polymer samples and here are some suggestions: Use a wire loop attached to a wood BBQ stick to collect the sections from the cryo-chamber. The wire loop could be about 3 mm in

diameter. Dip the loop in 1-2% sucrose solution and insert it into the chamber. Wait until you see the solution in the loop start freezing and then approach the loop to the section to collect them. Remove the loop from the chamber and allow the solution to un-freeze. While looking through a stereo scope, place the section on the grid which is held with tweezers. Remove the excess solution from the grid with a small piece of filter paper through the place between the tweezers' fingers. Place the grid on deionized water in a beaker with the section facing the water until you are done sectioning, to remove the sucrose solution. Remove the grids and dry them as mentioned above. Ani Issaian <ani.issaian@csun.edu> 09 Feb 2007

MICROTOMY - specimen advance

I have a pretty basic, non-life-threatening question about ultramicrotomy (with a Leica EM UC6 in my case). When I am cutting ultrathin sections it may happen I have to pause for a while (because I have to sign autographs or to pick up sections from water). After the pause when I start the cutting cycle again, it very often does not cut immediately but I have to cut 500 nm or more to touch the block again. I wondered if this was a sort of automatic protection from the machine, which retracts a little after some time of inactivity to avoid damage to the knife, or if this a the normal consequence of an effect of physics. Any comment on this? Stephane Nizets <nizets2@yahoo.com> 30 Jan 2007

You are lucky - no accidental 1000 nm sections! When I stop to sign autographs or explain to new users what is going on, the block in my Ultracut E sneaks forward and I demonstrate how to ruin the knife with a thick section. I accept this is probably due to operator error although an expert suggested that the block is compressed during sectioning and if left for a while will expand forward. Dave Patton <david.patton@uwe.ac.uk> 30 Jan 2007

I have the opposite problem, maybe because I'm usually asking for autographs, instead of signing them. If I take a break, I learned the hard way that the first swipe of the block over the knife will usually cut off a section about as thick as a slice of bread. I've often wondered why especially after one time losing a single layer of cells which floated off into the sunset. Randy Tindall <tindallr@missouri.edu> 30 Jan 2007

I don't really think this is an operator error, it does happen all the time, standard or cryo, I am not sure why. After breaking a couple of cryoblocks (not ruining any diamond on plastic, so far), I always go 200-400 nm back (using coarse advance) when pausing for section retrieval. Usually I have to wait for 3-4 cycles for the microtome to start cutting again, but it solves the problem. Michal Jarnik <michal.jarnik@fccc.edu> 30 Jan 2007

I must be doing something very wrong - I'm only called away from the microtome to be informed of malfunctioning microscopes/gel boxes/processors/etc. - never to sign autographs. When I return from the crisis, sometimes the block is further forward, sometimes back - I always retract the knife out of paranoia. I think the block moving away from the knife is due to thermal effects; you may generate enough friction during cutting to cause thermal expansion of the block, which then shrinks back when you stop cutting. At least, that is what I was told when I was learning to cut. I think it moves forward out of pure contrariness. I know on the old thermal advance microtomes you were pretty much guaranteed a trashed block/knife if you didn't retract when re-starting...there were all kinds of odd behaviors associated with those machines. Tamara Howard <thoward@unm.edu> 30 Jan 2007

The chucked block in the unattended ultramicrotome obeys the Hehesenberg Uncertainty Principle. As I trust you remember the position of a chucked block whose momentary momentum is zero will come to rest at a less accurate location. When the circular motion of the microtome wheel approaches zero then the product of the position and momentum approaches the Kerplank's constant. Thus using more mathematical rigor the conjugate quantity of the sections becomes the standard deviation from the property of being silver. Associated with this pause in the angular momentum of the microtome will be section chatter due to wave-particle duality. Ultramicrotome manufactures have tried to address this problem with an adaptor known as the von Neumann measurement, which according to some is better than the Landau calculation. I hope recalling this information lays to rest in no uncertain terms the phenomenon of zero momentum block creep or in some cases inverse block creep. Bob Blystone <rblyston@trinity.edu> 30 Jan 2007

During the cutting process, the blade is compressing the remainder of the block, which then expands afterwards. My mentors led me to believe the overlap with the knife was due to expansion of the block whenever it was allowed to 'rest'. The forces equalize when sectioning steadily. This is one reason microtomes for ultra-thin or semi-thin (<3 μm) sections retract during the return stroke. I don't know if this is really the case, but it does seem to account for the fact that we can break knives and blocks if not separated before resuming sectioning. Glen <glenmac@u.washington.edu> 30 Jan 2007

Your question has three answers. The block can move away from the knife edge, towards it, or stay at the same distance. The main governing factors are the mechanical advance setting, temperature, and other heating (or cooling) affects. Most microtomists would like to believe that a mechanical advance setting on a microtome determines the final thickness of sections. The mechanical advance is a guideline or course adjustment of how thick your sections are. The final thickness is the sum of the positive mechanical advance and the ± thermal advance. The section's interference color tells you how thick the section really is as it floats on water. This assumes you are not cutting something like polyurethanes or high index eyewear lenses which "mess up" or shift the interference colors on a thickness chart. Your gap is caused by a discontinuous use of the mechanical advance and block cutting while thermal affects continue to operate and you sign your notebook. If the block, arm or stage are warm and cooling, the gap will be seen as you described. If the block is cold and warming up, then the thin sections will be too thick. If all the temperatures of all the equipment, the air, and your body & fingers are the same; then the mechanical advance setting will probably be real. It was not unusual for me to see the block retreat from the knife edge at first, later stabilize, and finally advance towards the knife edge during a thin sectioning session on one block. You have to remember that your body is giving off heat, you are touching adjustments on the chuck or arc segment holder, the air temp might be varying, you picked up the diamond knife and mounted it, you put cooler water in the knife boat, the internal electronics are heating the cutting arm, water from the boat cools the knife edge and block face, or maybe you just took off a cryo unit and the arm and stage are still warming up. These thermal conditions have affects on thickness (and your gap). If you are going to stop cutting for a few minutes, you should always back up the knife about 5-10 microns and place the arm in the recommended position. The knife should never be parked in front of the block face. Failure to do so can result in a block of resin being shattered and/or a knife being ruined. One time I was cutting a very long and 0.25 mm wide block of an automotive coating in cross section. My lab was located on bedrock and the microtome was on an isolation table. My sections were being cut and I was getting bright gold sections. I started the next section and it was gold. A guy walked up to me and stood next to the microtome table but off to the side. He was three feet away. The middle of that section changed to another interference color. Just his standing there made a difference in the interference color on the rest of that cut. I usually told these people to not move and finished the next two sections and stopped. Once I had stopped cutting a block and backed up the knife. When I returned, the block face did not face off uniformly. It would cut a wedge shape from right to left. That meant the block was deforming under just the pressure of

the clamping and I was using a "hard" Epon 812 clone epoxy formulation. The number of variables in use during microtoming of blocks for TEM quality thin sections is high and sometimes subtle. AO Reichert sold or gave away at their convention booths, a nice booklet on microtoming. I think it was called "Ultramicrotomy-Faults and Problems". It was a blue color and old. Maybe someone on the list can tell you how to get a copy. It's worth getting but it is not a textbook. It was more like a reprint of an article. Microtomy is a learned skill just like riding a bicycle, only harder. Some people just can't do it very well. Others could and we said they had "the touch". Paul Beauregard <beaurega@westol.com> 30 Jan 2007

I've had it both ways; block retracts and when I restart it misses, or block expands and when I restart it whacks the knife. Depends on the type of resin, how hard it is, the sample in the resin, temperature, humidity, all kinds of things. Some of my stuff compresses with lengthy sectioning and the "relaxes" forward when I stop. Other times the block seems to heat up and be expanding during sectioning, then cools off and retracts when I stop. In all cases I have learned to retract the knife before starting again, just in case. Annoying, yes, but better safe than sorry. I had one customer who had lots of material embedded in LR White many, many years ago, and brought them in whenever she wanted to try a new antibody. I had become familiar with them and knew that over time the tissue (squid parts) expanded out of the face of the blocks. I guess the tissue was softer than the surrounding resin. I used this "feature" once when all the TEMs in the state were down and they were desperate to see if they had immunogold staining. I put the labeled grids in the SEM and was able to see the features of the tissue by topography, and the colloidal gold sitting on top. Please; no autographs! <tina@pbrc.hawaii.edu> 30 Jan 2007

LM - S waves in phase contrast optics

Here is one of those topics I thought I understood but now that I am teaching it I find myself confused. How can an S wave pass through a phase specimen and NOT interact with it? I'm using Douglas Murphy's excellent text on light microscopy and digital imaging. In introducing PC optics he states (pg 99): "Upon transit through a phase object, and incident wave of an illuminating beam becomes divided into two components: (1) an undeviated (0th order) waved or surround wave (S wave) that passes through the specimen but does not interact with it, and (2) a deviated or diffracted wave (D wave) that becomes scattered in many directions." . At the (also excellent) Molecular Expression web site, Murphy and colleagues say it slightly differently: "The primary component is an undeviated (or undiffracted; zeroth- order) planar wavefront, commonly referred to as the surround (S) wave, which passes through and around the specimen, but does not interact with it." [http://www.microscopyu.com/articles/phasecontrast/phasemicroscopy.html] How is it that a light wave can pass through a phase object yet not interact with the object and not have its phase changed, while the diffracted light does have its phase changed? Gary Radice <gradice@richmond.edu> 11 Feb 2007

It is not that the S wave does not have its phase changed (it does have it's phase changed), but that the D wave is diffracted and the S wave is not diffracted. The diffracted light is also changed in phase by the fact that it is diffracted. So, both change phase (with respect to light that does not pass through the object) due to passing through the object and the D wave has it's phase changed also by being diffracted. The phase plate then puts the D wave and the S wave about 180° out of phase (if it is a positive phase plate) and decreases the intensity of the S wave. Make sense? David Elliot <Elliott@arizona.edu> 12 Feb 2007

LM - 3-D reconstruction from serial paraffin sections

I am attempting to follow the path of cotton fibers around a seed in an attempt to see how they pack as they develop. I have embedded the samples in paraffin and serially sectioned through the bundle in 12 micron sections. As such, I have almost 900 sections. Is there a program or method that can generate a 3-D reconstruction from some or all of these 2-D sections via light microscopy with a digital camera? Mark Grimson <mark.grimson@ttu.edu> 27 Dec 2006

You might look at Reconstruct, http://synapses.bu.edu/tools/index.htm It focused on reconstruction of serial TEM images but would probably work well for this application. –Davi Bock <dbock@hms.harvard.edu> 28 Dec 2006

MICROSCOPY - LASIK, floaters, and posterior vitreous detachment

I have a question that's just for fun. I'm using a Nikon TE-2000 with all 4 ports and a beam splitter that can direct light 50/50 split between two ports. Is there a way to set up a camera to image not a sample but specifically the image that I'm seeing? I have a minor defect in my eye, a wrinkle caused by slight detachment of the vitreous (I think due to having LASIK done). When I look through the scope at a bright field, I can see an image of the wrinkle. I'm curious to know if I can capture it, but my guess is that the image only exists in the oculars. Maybe I could somehow use a dichroic mirror? Jessica Wagner <jessica.wagner@childrens.harvard.edu> 26 Jan 2007

I have experienced this same phenomenon with "floaters" in my eyes. Under the right conditions, I can see the floaters beautifully and sometimes even the corneal surface. As explained to me by an ophthalmologist, this is due to the projection of a shadow (of the objects) onto the retina. Therefore, you would not be able to "go the other way" and see the image in a viewing port. I believe that the only way to image this would be with a slit lamp (as in an ophthalmological examination). Maybe others on the list would care to comment since I am certainly no expert but speaking only from personal experience. John Bozzola <bozzola@siu.edu> 26 Jan 2007

The image "that I'm seeing" can be thought of as the image that a user with normal sight would see transformed by the effect of the defect, so although it is easy to see the "normal" image, and as John suggests, you could see the defect with a slit lamp, you could not see the perturbation caused by the defect (although there are ways in theory to calculate it

from the structure of the defect). Of course, you could look at a grid or some such test object and draw what you see, so the distortions noted in the grid will tell you what distortions to expect in a more complex image. You could even look at the grid separately with each eye and compare the image from the undamaged eye to that from the eye with the defect, and, if your brain has not already adapted to seeing the distorted image, looking at a grid that has been distorted in one eye could be interpreted by your brain as a 3D image of the grid that appears to be closer to you in some places than in others, like the effect of viewing stereo images, but much more subtle. Bill Tivol <tivol@caltech.edu> 26 Jan 2007

Some time ago, there was a thread about LASIK and microscopists. I do not recall any of the reports mentioning this sort of post-surgical vision difficulty. Is this perhaps a subject that should be re-visited? John Mardinly <john.mardinly@intel.com> 29 Jan 2007

This isn't about LASIK, but I caught the bit about floaters and just want to relay my experience, if only to prevent others from going through the ordeal I did last summer. Don't ignore floaters, even if they've been noticeable for a long time. My right eye always had a significant number of them, and my optometrist told me to come back if I noticed an increased number of them. That's not very easy to quantify over time, but in hindsight the number probably did increase in the months before I had a partial detachment of the retina. No other symptoms until a ominous black spot appeared in the corner of my vision. The surgeon said that the whole thing could have fallen off at any time, probably resulting in total blindness in that eye. Fortunately surgery corrected everything, and six months later I have nearly perfect (well, as perfect as it was before) vision again in that eye. Get your eyes dilated and checked for retinal tears *every* time you have an eye exam, especially if you're over 40. I know, it's a pain, but losing binocular vision is a much bigger pain! Retinal detachment is *not* most common in boxers, drag racers, sky divers - people who get their heads banged around a lot. It happens most often to people who are strongly nearsighted. I know quite a few of those in the microscopy world and I don't think any of them would be keen on saving operating expenses by only buying monocular scopes. **James M. Ehrman** <jehrman@mta.ca> 01 Feb 2007

I missed that thread, but I'll be happy to add my two cents now. Assuming the PVD (posterior vitreous detachment) doesn't get any worse, for me the benefits of LASIK still outweigh this minor negative. The floater that I see is an annoyance, but it doesn't truly inhibit my vision through the scope (or outside the scope for that matter). But I had LASIK only a year ago, and was never warned PVD could be a complication, so this says to me that there is still much unknown about the procedure and its results. Unfortunately, complications of laser eye surgeries really aren't tracked all that well; doctors are not required to report them, unless they are related to a device, but even then, many doctors are not aware of FDA regulations about device event reporting or how/to whom they should report adverse events. Here is an article about PVD's and LASIK: http://www.springerlink.com/content/j3100858467pu5k1/ And thanks to everyone who offered imaging advice! Jessica Wagner <jessica.wagner@childrens.harvard.edu>

Since the list played a big part in my decision to go through with LASIK 2.5 years ago, I'll put in 2 cents on this topic as well. My doctor did warn about floaters, halos, possible mis-correction as side effects and then gave me a realistic assessment of what it would mean should I suffer these side effects. He spent a great deal of time addressing my concerns and assured me that such problems were quite rare and often very minor. In the 2 months following the surgery, I experienced halos and starburst patterns around streetlights and headlights while driving at night. Eventually these symptoms subsided and I see less starburst-type patterns now than I ever did before the surgery. At 2.5 years post surgery, I now have a slight floater in my right eye which I rarely notice unless conditions are just so. I never noticed it at all until 4-5 months ago. That said, I know several friends and family members who have floaters that have never had LASIK surgery, so I do not feel confident that LASIK was the cause. All in all, the improvement in my vision from about 20:250 to 20:20 has been an immensely positive development. I would recommend LASIK to anyone who has been declared a good candidate by a reputable eye surgeon. Do your research on the physician. I had no less than 4 recommendations from optometrists with no connection to each other. I also spoke with 3 patients he had treated in the past, so I felt pretty comfortable that he was competent. For anyone, especially a microscopist, your vision isn't something to bargain shop for. One place in town offers LASIK "as low as $599 per eye". It didn't take long to find out that they have higher complication rates. Ultimately, I paid close to $3,000 for both eyes and was confident that I was getting the best treatment available in the area. Jay Campbell <microtomy@gmail.com> 01 Feb 2007

I can also add that the number of eye 'floaters' I have is increasing with age (I'm 43), and I have never had eye surgery. The thought of trying to zap them, and nothing else is important, with a laser is terrifying, but it is getting more difficult to use my light microscope. I am also very nearsighted and wear contact lenses; does anyone know if there's a correlation? Jane L. LaGoy <jane.lagoy@bodycote.com> 01 Feb 2007

I'm also extremely nearsighted and my floaters have increased with age. Sometimes when I'm reading, I have to move my head to get a pesky one out of the way. I had them before wearing hard contacts for 12 years and I still have them. So I don't think there's a correlation but I realize my opinion is not a scientific study. My optometrist once explained to me why very near-sighted people should have their retinas examined frequently for tears/detachments. Near-sighted eyeballs are longer front to back than normal eyeballs. He said one can be born with normal-sized retinas stretched to fit the larger eyeballs. (My father is near-sighted; my mother is not.) These 'stretched' retinas are more prone to tears/damage/detachments than normal ones. I have no training in eye physiology so I was taking him at his word. As microscopists, our eyes are more valuable than our hands, which are pretty darn valuable. Take care of them. Becky Holdford <r-holdford@ti.com> 01 Feb 2007

TEM - defect density threshold

What is the approximate defect density in silicon, one can easily visualize using a 120-200 KV TEM (plane view and cross-sectional view)? Sandra Keller <swtkeller@yahoo.com> 30 Jan 2007

It's an easy calculation to do if you make some rough assumptions about the amount of thin material in your TEM specimen and the visibility of defects. For a standard cross section which has roughly 100 μm of electron transparent material away from the edges of the hole and is on average 1μm thick, you have 0.01 x 0.0001 = 0.000001 cm^2 of the original wafer surface in your cross-section specimen. So if you see just one defect you have a density of 10^6 defects cm^{-2}. If your defects are only visible in thin material then revise the number upwards. If you have a FIB section (say 30 μm wide and 0.3 μm thick) you need over 10^8 defects cm^{-2} to catch one in your specimen. For a plan view specimen which has the same 100 μm electron transparent area and average thickness of 1 μm, say around a hole 50 μm wide then the amount of the original wafer surface you can see is pi*(150 μm)^2-pi*(50 μm)^2 = 0.0006 cm^2. So if you see one defect you have a density of about 1600 defects cm^{-2}. Having said that I know from experience it can take some time to find defects in plan view specimens even when the density as high as 10^5 defects cm^{-2}, simply because the specimen is always bent and you have to tilt the specimen through a diffraction condition to see them. Defect etching is very useful for seeing low densities of defects if processing allows. You can even do a light defect etch and then make a TEM specimen - in which case you will find that not all of the defects actually produce an etch pit, which just goes to show that there is no perfect technique for measuring defect densities. Richard Beanland <richard.beanland@bookham.com> 30 Jan 2007

TEM – effect of high temperature on grids

Can carbon TEM grids withstand 450°C in a furnace? Dorothee <almecid@tcd.ie> 12 Jan 2007

Carbon can certainly withstand 450°C, so if the grid itself is made of carbon, the answer should be "yes", assuming that there are no problems with heating or cooling at a rate such that thermal stresses are not introduced. Carbon films on metal grids could be a different story, however. The large difference between the coefficients of expansion of metal and carbon will very likely cause problems. Bill Tivol <tivol@caltech.edu> 12 Jan 2007

TEM - cleaning a Lab_6 emitter

I am running a Denka Lab_6 on an AMRAY 1830. We view a lot of samples mounted in epoxy and the LaB_6 now has a bad coating of what I think is epoxy residue (visible under a binocular). Does anyone have a suggestion on how to clean this safely? Tom Williams <tomw@uidaho.edu> 14 Dec 2006

You may use any organic solvent that is not corrosive to metals, Freon-113 or chloroform are very good, tetrachloromethane is good, or any other chlorinated hydrocarbon if you can get any- these are usually restricted. Just soak the emitter, do not sonicate! I dealt with a number oxidized LaB_6 cathodes, when for example a user accidentally inserted a specimen holder in the TEM without pre-pumping, while LaB_6 was hot (some did so many times in row. Then the vacuum system shuts down, *etc.*, and the LaB_6 catches some O_2 from the air. All I do to cure that condition is to run LaB_6 (for a day or so) at a slightly higher temperature at low KV and low emission to let poisoned layer of the crystal evaporate. It usually works, but then there is a limit of how much abuse LaB_6 can take. However, I have doubts about the very cause of your LaB_6 problem. What is the history of symptoms? First, how did contamination got in the gun area? An SEM equipped for LaB_6 use must be pumped differentially—the gun and column have dedicated IGP and are separated by differential aperture from the specimen chamber. Second, I can hardly imagine organics condensing on an object heated to about 2000 °F. Vitaly Feingold <vitalylazar@att.net> 14 Dec 2006

I'm not sure about how to clean the LaB_6 from Denka. But I have done this once with FEI cathodes. What is puzzling is why you have to do this at all. The 1830 if set up for LaB_6 has a 30L/m ion pump for the gun chamber. This should and does keep gun chamber vacuum really good. What IPG value are you currently reading? If you do not have a gun chamber ion pump, you cannot run LaB_6. If you do have it, there is no obvious reason why the LaB_6 would be contaminated. It makes no sense to me. I never had this happen. IPG was typically 40 μA. Hopefully, your stand pipe valve is closed. Gary Gaugler <gary@gaugler.com> 14 Dec 2006

Are you referring to a residue on the Wehnelt or emitter? I have seen non-conductive residue deposited onto the Wehnelt by a LaB_6 (as if LaB_6 itself). It was relatively difficult to remove, but a weak acid solution helped (*e.g.*, 10% HCl). Michael Shaffer

I would not suggest using 10% HCl for cleaning Wehnelt assembly. 10% HCl solution might be too aggressive for highly polished stainless steel surface. In our lab, we routinely use ammonia solution. Is it also possible to use alcoholic solution of KOH. However, you can clean the stainless parts of Wehnelt, only. All brass components should be dismounted prior cleaning in ammonia or KOH solutions. Please, see the users guide for proper cleaning of your Wehnelt. Oldrich Benada <benada@biomed.cas.cz> 15 Dec 2006

I routinely clean my stainless steel Wehnelt with a little dilute NH_3OH, but I'm removing tungsten. I'm not sure what you're cleaning off with a LaB_6 filament. But I would never use NH_3OH or NaOH to clean brass parts. Most commercial brass and copper cleaners proudly exclaim "Does not contain ammonia." There is a reason for that. I sometime use an ammonia solution to strip copper fouling off steel, but I'm interested in cleaning the steel and not preserving the copper. I'm not saying you're wrong, but I would be very hesitant to clean brass in a basic solution. Frank Karl <frank.karl@degussa.com> 15 Dec 2006

The gun for the 1830 and associated components are compatible with Pol for cleaning and polishing. I used it for years on the gun, final apertures holder, anode aperture holder, etc. Not sure about the LaB_6 cathode however. Gary Gaugler <gary@gaugler.com> 15 Dec 2006

There are several suggestions for cleaning the interior parts of electron microscopes in Section 2.10.4c (pp. 71-74) of my book Vacuum Methods in Electron Microscopy that might be useful in solving the present problem. Specifically, mentioned there is a recommendation from Peter B. Sewell of LaB_6 Inc. for removing LaB_6 deposits by soaking for about a minute in a solution consisting of one part concentrated hydrochloric acid and 4 parts water. Wil Bigelow <bigelow@engin.umich.edu> 15 Dec 2006

TEM - power line and stray field shielding

As we'll welcome next year a new TEM, we are studying the question to move all our microscopes in another part of the building. But, if the place seems to have interesting side, obviously something must be wrong! There is a power line which runs in a technical corridor in the underground, carrying some 800-1000 Amps for the supply of the whole building, and generating something like 50 mG in one of the possible room. Is it possible to shield such a power line, to lower the field at it source (1" thick aluminum or copper, or so)? Is such a shielding technically possible, and not too expensive? I think it would be better to try first to limit the perturbation at it source! Secondly, what are the feedback from people working with dynamic magnetic field compensation? How much do you have without it, and how does it work, with such a big field. Does it react fast enough, when the stray field changes. J. Faerber <jacques.faerber@ipcms.u-strasbg.fr> 15 Dec 2006

50 mG tells me that you have a mis-wiring issue or a grounding of the neutral, or an unintentional grounding to metal to begin with. You should do an EMF survey (grid style) then hunt down the wiring problems. This should drop it to under 5 mG, likely under 3 mG. Tony Havics <ph2@sprynet.com> 17 Dec 2006

I can only address the shielding. You can employ shielding if physically practical, but do not use Al or Cu. The conductors must be totally enclosed for best result. Annealed iron/steel is good. It does not have to be extremely thick. Best is a material called Mu-metal (a hydrogen annealed Ni/Fe alloy if memory serves. Mu-metal is a bit pricy, however, and for best results work-hardening during fabrication should be minimized. Woody White <NWWhite@bwxt.com> 18 Dec 2006

SEM - solid state versus a scintillator

What is the difference in performance between a solid state and a scintillator (Robinson) backscatter detector? I am very interested in a side by side comparison. Willem Wennekes <wawennekes@woh.rr.com> 30 Dec 2006

We have both types of detector on one of our SEMs. While I can offer you some distinctions, others may have newer models and care to join the discussion. Our SEM was originally supplied with a Robinson scintillator style detector. Its strengths were fast response for rapid scan rates up to and including TV-rate. It is good for general imaging and gives a nice topographic effect. We later purchased a solid-sate detector for particular use for image analysis. Our application required lower voltage (6kV) than normal. We also required an even response across the field of view. For that, the solid-state detector was considerably better, but it has a slower response than the Robinson detector. So the better detector will depend on your requirements. As it is, we run the solid state most of the time. Warren Straszheim <wesaia@iastate.edu> 02 Jan 2007

SEM - low vs. high vacuum mode

Back again with my basic questions about SEM. I took pictures using both low vacuum mode (1 Pa) and high vacuum mode (8x10-3 Pa) at

magnifications from 3 kX up to 25 kX with my SEM (at 10kV with quartz). I can't see the slightest improvement in image quality! The only difference is a slight increase in contrast at higher vacuum (which is not necessarily better because low-contrasted parts are not visible). Is it normal? What would be the use of high vacuum? Is it useful for EDX analysis but not normal scanning? Stephane Nizets <nizets2@yahoo.com> 05 Jan 2007

One of the problems with assessing SEM performance is often the specimen you select. I travel around the world looking at customers' instruments training the people to obtain more from their instrument. In order to judge the performance of the instruments I spent a number of months experimenting with test specimens until a came up with one where the image always changed in some way when I changed parameters. I remember a ListServer posting some time ago when a scientist commented that the new FEG instruments are amazing as they are just as good (with their specimen) at 1kV as 30kV! In my mind the test specimen was not testing and I feel this could be the reason that you do not see changes when you vary the parameters in your instrument. A poor quality vacuum is great to reduce charge. But a poor vacuum causes beam spread, a problem that will increase the spot size and, if sufficient performance is demanded, ultimately degrade the image quality. Steve Chapman <protrain@emcourses.com> 05 Jan 2007

There are several fundamental differences between high vacuum and low vacuum that make each unique in application. 1. Low vacuum can only use BSE or special low vacuum SE-type detectors that make traditional, high-resolution SE images impossible. Low vacuum is usually 10 to 200 Pa. Usually these detectors require higher beam voltage (10 to 20 kV) and beam current than high-vacuum SE imaging. 2. High vacuum requires that a sample be conductive. Try your experiment with a freshly-plucked flower, looking at the stamen and pollen grains and you will see that it is impossible in high vacuum mode but quite possible in low vacuum. You may have to increase the gas pressure to 20 to 100 Pa to eliminate charging. If you need to do high resolution imaging of the small features on a sample surface, only high vacuum will allow you to use <5 kV, small aperture, short working distance and low beam current to get detailed SE images of the sample. 3. The scattering of the electron beam in low vacuum mode gives EDS contributions from areas away from where you may want to analyze. It is nice to be able to do EDS on anything without coating, but if you need to analyze one small spot without contribution from neighboring areas, you need high vacuum. Both modes have their place and strengths and it is nice to have the choice. Mary Mager <mager@interchange.ubc.ca> 05 Jan 2007

You do not give any indication of the beam current you are using (possibly the 'spot size' adjustment for your SEM). I suspect you will not realize the real advantages of employing high vacuum unless the beam current (and spot size) is small. Better vacuum will also provide better contrast. But exactly what you see is specimen and preparation dependent. Michael Shaffer <michael@shaffer.net> 05 Jan 2007

SEM - beam penetration

I'm an undergraduate working in a lab and I would like to use SEM to view gold nanoparticles embedded in agarose gel. About how far could the SEM beam penetrate into 1% agarose (I'm guessing I would have to let the water in the gel evaporate)? Would it be better to use SE or BSE? What kV should I try? Thanks for any help you can give me! Michael <mconstan@princeton.edu> 29 Jan 2007

What kind of detail do you need? That will probably determine your conditions. Understand that the beam will start scattering and interacting once it encounters the first surface which will be agarose. Secondary electrons are limited to escaping from the first few nm of surface, so even if they are generated at some depth, they will not escape to be detected. I expect you would see primarily the agarose surface. There is also the question of imaging the agarose at all. I presume it would charge without coating and would need to be examined in variable pressure or environmental mode. That would eliminate the choice of SE unless you have a gaseous SE detector, in which case the previous comments still apply. BSE could give you some information from some depth below the surface. It will still be difficult and there will be some scattering. Also, what happens to the structure of the agarose under vacuum? I suppose there will be appreciable loss of moisture, so what you see may not be anything like what you had. I would check the mass loss as a result of exposure to vacuum. It might be too extreme. I would probably start at 20 kV for voltage and set the current to give a decent BSE image. You will probably have to experiment from there. Warren Straszheim <wesaia@iastate.edu> 29 Jan 2007 <protrain@emcourses.com>

The simplest solution to your problem is to down load one of the basic Monte Carlo simulations and consider your material to be Carbon. The Joy-Nockolds version is on our web site under hints and tips. Depending upon the microscope that you are using, the magnification, the kV and the working distance, you could find the so called "secondary electron signal" (Everhart-Thornley detector) carries sufficient backscattered information to provide the data that you require. Be aware of the fall off in resolution when using dedicated backscatter as the performance may fall below that required to visualize your particles. You should also consider that depending on the microscope and your own skill the size of particles may be too small to visualize? You should try the complete range of kV as the information will always be better at one particular kV. It is impossible perhaps foolish to try and guess what that may be. You may need higher kVs for penetration but too much will fog the image with unimportant sub surface data, whilst too low a kV may not provide the resolution that you require. Microscopists are scientists we should experiment! Steve Chapman <protrain@emcourses.com> 30 Jan 2007

EBL/ PMMA Mask

I'm looking for a gold etching recipe that will allow me to use PMMA as a mask. KCN looks promising, but is highly toxic. Does anyone have any better ideas? Thanks, Kurt Langworthy <klangwor@uoregon.edu> 18 Jan 2007

In an ancient edition of The Metals Handbook (American Society for Materials, ASM) I found the following: "The most widely used nonelectrolytic etch is a mixture of equal volumes of 10% ammonium persulfate in water with 10% KCN in water. Although the separate solutions are stable in air, the mixture must be used within a few minutes of mixing. Since this solution and the fumes from it are poisonous, it should be used under a hood (and with other relevant precautions), When swabbed on specimens of the usual gold alloys and palladium alloys the mixture acts rapidly and smoothly. It may also be used on silver and certain nickel alloys. For more resistant alloys, 20% solutions may be employed" "The same metals can be etched when made the anode in a 5% KCN solution" (I'd suggest a voltage of about 5 V). Also mentioned is electrolytic etching using an AC voltage (you can probably use a Variac as a source for this, again about 5 volts) with a 20% solution of hydrochloric acid saturated with sodium chloride. The great advantage of gold and the other noble metals is their chemical stability, and so your task is a non-trivial one. Wilbur C. Bigelow <bigelow@engin.umich.edu> 18 Jan 2007

Since my company banned cyanide-based etches some years back. one of my favorite gold etches is as follows: 4.6 grams potassium iodide + 1.3 grams iodine in 100 milliliters of deionized water (ratios can be increased/decreased as needed). Use at room temperature. This removes 2-4 microns of Au in around 5 minutes, according to my notes. You might want to test it on something expendable before you use it on the real deal. It will store a long time. <r-holdford@ti.com> 19 Jan 2007

Local Affiliate Societies (LAS) News and Views

Lou Ross
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I want to thank everyone who contacted me about starting this column in Microscopy Today and hope this becomes a permanent feature in MT well into the future. Again my extended thanks to Ron Anderson for his support in promoting the LAS News and Views to encourage readers of MT to join and participate in the LAS in their area.

New speakers for the 2007 Tour Speaker list are still being finalized based on the suggestions from last summer's LAS breakfast. One new speaker from that list is Kent McDonald from UC-Berkeley who many of you already know as a recognized leader in the area of cryomicroscopy. Kent will offer two presentations, "Biological EM in the 21st Century: Cryotechniques, Correlative LM/EM, and Cellular Tomography," along with "High Pressure Freezing and 3-D Analysis of Mitotic Spindle Ultrastructures." Another choice from this summer, Frank Platek from the US FDA in Cincinnati, OH will present "Applications of Light and Electron Microscopy at the US FDA's Forensic Chemistry Center." Frank has been teaching the short course at Scanning in the always interesting and entertaining area of forensic science.

MSA Director Robert Simmons, MSNO President and MSA Membership Chair Jeanette Killius, and MSA Past-President Jay Jerome receive their complimentary MSNO mugs for speaking at the 40th anniversary kick-off meeting.

I would also like to congratulate and welcome Bill Gunning, MSA President-Elect, as a Presidential Speaker. Bill is the Director of the Electron Microscopy Core and a faculty member in Biochemistry and Cancer Biology at the University of Toledo. Congratulations also go out to the new MSA Directors, Paul Fischione of E. A. Fischione Instruments, Inc and Heide Schatten of the University of Missouri-Columbia.

This spring appears to be quite active for many LAS for workshops and meetings. As mentioned in the last column, the Indiana MS is hosting a 5 society joint meeting (see announcement in this issue) with the Central States, Iowa, Michigan and Midwest societies April 20-21 , and the Oklahoma MS and Texas SM societies will hold a joint workshop April 20-22. Other upcoming LAS meetings include the Florida SM (jointly with FLAVS) March 11-15, Midwest MMS March 22-23 and May 17, Minnesota MS March 15 and April 20, New England SM May 3-5, Southeastern MS April 11-13, and the Texas SM April 13-14. The New York MS will hold its annual Polarized LM short course each Friday in May. Also, the Connecticut MS celebrated its 25th anniversary last spring, and both the Midwest MMS will be celebrating 50 years and the Minnesota MS 40 years this fall. More information on the above upcoming meetings and other LAS contacts and events can be found at the MSA homepage, www.microscopy.org.

MSNO student poster winners, Sarah Smith, Parth Shah, Lisa Cooper, and Emily Njus pose after the awards ceremony.

If you haven't had the opportunity to look at the LAS home pages, I encourage you to do so. Not only is the amount of activity and involvement quite impressive, it can also serve to stimulate your own LAS with new ideas. And if you don't have a homepage, our webmaster extraordinaire Nestor Zaluzec has provided simple and easy guidelines for content submission so he can build one for your LAS. Nestor also has a simple electronic form to update your LAS listing information even if you do not have a webpage. Please keep your LAS listing up-to-date. As we all know there is nothing more discouraging than to try to make an electronic contact and it is either non-existent or wrong.

Within the next few weeks, the MSA-LAS Tour Speaker web site will be updated for 2007 along with a better description and procedures of the financial assistance programs available to each LAS. If you have any suggestions or ideas on this, or if your society has an upcoming meeting, news or photos to submit, please contact me.