

NetNotes

Edited by Thomas E. Phillips

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Selected postings from the Microscopy Listserver from April 16, 2009 to June 15, 2009. 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: sections falling off grids

Every once and a while we run into problems with sections falling off the grids. I know this has been rehashed more than once here, but it's hard to find the answers in the archives, so I am asking for the consolidated, final analysis of the problem and solutions. I suspect it could be students using old, oxidized grids, but I'm not sure enough to just give them that one answer. I have checked on 'grid glues' and searched around, but I knew the experts and experienced would be here. So, what is your strategy? **Jonathan Krupp** jkrupp@deltacollege.edu Fri Apr 24

I have had this trouble in the past but not for a long time since I began flaming the grids. I saw this in the Bozzola and Russell book. Works better for standard grids; be very careful with thin bar grids. Use an alcohol burner with a small flame (~1cm max). Pick up a grid with forceps and sweep briefly through the tip of the flame. It is better to do too little than too much since it can be repeated until the desired effect is achieved; after a bit you get it right almost always in one or 2 passes. What you are looking for is a "scorched" look, some interference colors in the red and blue range (which must be thicknesses of surface modification (oxidation?). These grids wet beautifully and sections cling tenaciously. For the record, I always pick up sections on the shiny side; I know there are 2 teams on this topic :-). My logic is that it is like kitchen plastic film that clings better to smooth surfaces. I have had wettability issues with gold and gilded grids that can't be flamed. For these I treat 15 sec in the Harrick Plasma cleaner and they wet beautifully and sections adhere well. This probably works for the copper grids as well but I haven't tried. **Dale Callahan** dac@research.umass.edu Fri Apr 24

I routinely use a 'grid glue' as I like to immunolabel my sections immersed in the immunoreagents as it makes the whole labeling process easier and enhances the labeling, with the antibody/antibodies having access to epitopes on both section surfaces. This latter means there is a tendency to lose sections when transferring grids from one solution to the next. Anyway my trick is to just briefly immerse the grids in approximately 5ml of chloroform in which about 4-5" of Sellotape has been dissolved (remove the tape itself once the glue has dissolved off it - just shake for a few moments). This seems to work for me and hope it does for you! **Dr. Julian R. Thorpe** bafg3@sussex.ac.uk Fri Apr 24

Epon thin sections coming off naked grids during staining procedures was solved when I followed Debby Sherman's suggestion of putting the grids containing sections into my oven for at least 15 min. to overnight before staining. I only need to do this occasionally since I do not have trouble frequently (do not know why). I only use my oven for this treatment when it is empty because I do not know if the fumes that are generated by curing epoxy would have an effect on the grids even though they are either in a closed Petri Dish on filter paper or in a Grid Box. My newer grids (Electron Microscopy Sciences) do not need much cleaning, but old ones do. If you sonicate in acetone to remove oil/grease residues, I found it advisable to do a final rinse in 100% ethanol before drying since I think the acetone sometimes

leaves something behind on the grid surface. This reduced the number of sections that I lost. I have also used a very careful quick dip in HNO_3 followed by several water washes to make naked grids hydrophilic and hence make section pick-up from the boat much easier. I had good luck with sections sticking where they belonged with this technique. The problem was using the strong acid which dissolved the grid if it was not washed quickly enough. **Patricia Stranen Connelly** connellyps@nhlbi.nih.gov Fri Apr 24

You do not need to use strong acid. 1N HCl can do job just fine. You can keep a grid in this acid for a while without problems. **Vladimir Dusevich** dusevichv@umkc.edu Fri Apr 24

For basic counterstaining, a simple acid wash with proper rinsing and drying should be fine. I have experienced highly variable section adhesion when I perform immunohistochemistry with any Tween or Triton detergents, primarily when the grids sink into the reagent droplets (using square pattern, thin bar nickel grids). If this is your case, then I sympathize. **Gregg Sobocinski** gregggs@umich.edu Fri Apr 24

One thing missing from this thread is an explicit discussion of the technique for picking up sections. I have always picked up sections from below, and after 25 years and thousands of blocks, I have never had a problem with sections falling off bare copper grids without adhesive, either dull or shiny side, with Epon, Spurr's, or LR White. When you bring the grid up from below, there is water between the section and the grid, and when the water evaporates, the section becomes bonded tightly to the grid. This is not so when you come from above. I should add that I sonicate the grids in 100% ethanol then place them on filter paper in a Petri dish. If you have a problem with the sections running away when you try to pick up sections, try dipping the grid briefly in ethanol, then immediately rinse it thoroughly in distilled water, and bring the wet grid to the boat. The purpose of this is not to clean the grid, but to avoid the formation of tiny air bubbles on the grid, which tend to repel the sections. You should also rotate the grid as you remove it from the boat, so that it is vertical when it comes out of the water. This avoids bringing up a large drop of water with the grid, and having your sections shift when the water is blotted off. **Ralph Common** rcommon@msu.edu Fri Apr 24

I, too, was plagued with sections falling off grids. Since I have been using the following, I have not lost a single section! Dip copper grid into a 0.1N solution of HCl (I usually count 10 seconds with a drop of HCL on the grid) and blot dry. Dip several times into 100% acetone to rinse and allow to dry on filter paper. I usually prepare the grids before I start sectioning so I don't have to stop each time to get my grids ready. Once my sections are collected, I put the grids into the oven for 20 minutes to dry. I can stain immediately after removing the sections from the oven or wait until later, it doesn't seem to matter. Hope this helps your students! There is nothing worse than spending your day sectioning and seeing only shreds on the TEM! **Pat Kysar** pekysar@ucdavis.edu Fri Apr 24

My technique for cleaning grids is a variation on Pat's method, by using acetone and HCl together. I mix up a 250 ml lot of cleaning

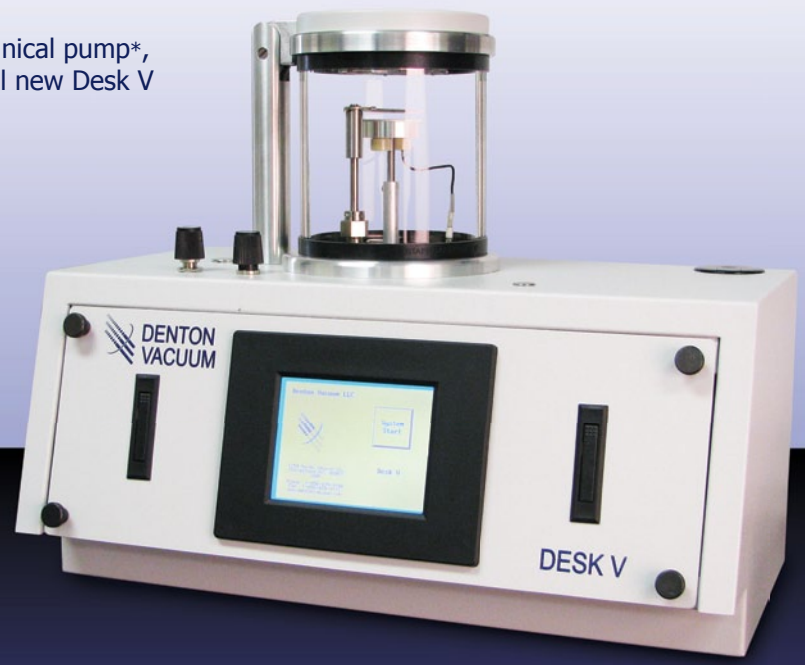
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solution as follows: 25 ml conc. HCl, 175 ml distilled water, 50 ml 99% acetone. Of course, add the acid to the water, and then add the acetone. I sonicate copper or nickel grids, mesh or slots, for about 30 seconds in a 25 ml beaker with about 10 ml solution. Then I pour that off, and sonicate once with 99% acetone as a rinse. Then invert beaker onto clean filter paper to air dry. The grids usually stick a bit to inside of beaker but will fall off when they dry. I do this each day before I begin sectioning. The copper grids get so clean - fresh copper exposed - that they may oxidize enough overnight to need cleaning again even just a day later. I also clean grids this way before any coating with films, like Formvar or Butvar. This method of cleaning has worked for me for many years and sections stick to the grid. Oh, I also pick up floating sections from above onto the dull side of the grids. Others report good results picking up from below the floating sections, or onto the shiny side of the grids. Others report good results cleaning grids by quickly flaming them in an alcohol lamp flame. In this new age of solar energy, I have not yet tried cleaning grids by concentrating the sun's rays with a 4" hand lens onto grids resting on a clean, refractory surface. But if it worked, that would be so green! Someone ought to try this. **Gib Ahlstrand** ahlst007@umn.edu Fri Apr 24

I noticed that after the advent of thin bar grids, sections didn't stick as well, probably because there was less surface area for sections to adhere to. Like Pat Common, I just put them in a 50 degree oven and never lose sections. 30 minutes works but we've left them in there inadvertently for several days, and they are fine. No pre-cleaning is necessary. An absolute ethanol dip does seem to minimize the tendency for sections to "run away" and also cuts down on what I call the Jell-O water effect. **Mary Gail Engle** mgengle@email.uky.edu Mon Apr 27

Specimen Preparation: sharpening carbon rods

File this Tip of the Week under "Okay, so why didn't I think of this before, dummy?" We have one of those hand-held carbon rod sharpeners that require you to twist the rod manually in order to get the narrow tip needed for evaporating. They are hard and uncomfortable to use, often break the tip off just as you're finally getting it to the length you need, and just generally a pain. But I could never bring myself to spring for the hundreds of bucks for a decent sharpener, especially considering the volume of evaporating we do. As I was cranking away this morning, breaking tips and saying bad words, I remembered that we had a Dremel tool in the next room. Took that carbon rod, put it in the drill bit chuck, turned the tool on at its lowest speed, inserted the rod into the sharpener and had my tip in about 17 seconds. Many of you probably had this one figured out a while ago, but if not, here it is. I now have the will to go on evaporating. **Randy Tindall** tindallr@missouri.edu Fri Apr 24

A Sears Craftsman variable speed 3/8th inch drill works just as good, has more low speed range, and takes any size carbon or graphite rod (1/8" to 1/4" or more). I made a sharpened rod one inch long that way with just a Sears drill and a manual sharpener. I also used a fast turning lab scale miniature lathe years ago but the drill works better and is smaller but heavier than a Dremel tool. Kiss sore and black fingers goodbye! **Paul Beauregard** beaurega@westol.com Fri Apr 24

Image Processing: image stitching

Photoshop CS2's Photomerge function works well on my LM flatfield images, but with TEM images sometimes there are misalignments in some areas. I assume this is due to spherical aberration in the TEM images. Can any of the other software options out there detect and correct for spherical aberration? **Ralph Common** rcommon@msu.edu Fri Jun 5

I've been experimenting with Microsoft's Image Composite Editor ("ICE") to make mosaics of microscopy images, and it seems that it

works well. Though most programs such as this are meant to stitch together consumer-level JPEG photos into a panorama, this particular one can use planar / flat field TIFFs - which is great for microscopy. ICE is free of charge but is officially unsupported by Microsoft. It appears to be stable in my limited testing. It is available for 32- and 64-bit Windows systems from: <http://research.microsoft.com/en-us/um/redmond/groups/ivm/ICE/> There is an Image Composite Editor forum that has a few support notes describing the various functions and settings of the program (link at the bottom of the ICE page): <http://community.research.microsoft.com/forums/112.aspx> <http://community.research.microsoft.com/forums/t/2002.aspx> ICE appears to be faster than the Mosaic] plug-in for Image], though the latter gives you more flexibility and options in layout and is documented in the scientific literature. I'm not sure which algorithms ICE uses. <http://bigwww.epfl.ch/thevenaz/mosaic/> **Marc Takeno** takenomm@u.washington.edu Fri Jun 5

In our lab, people now use PS CS4, with - I assume - further improved function(s) for image stitching / merging. At least, the results are very good. Photomerge. TEM: this very much depends on the magnification. It works +/- easily and automatically (Photomerge) with images taken in medium / high magnification mode (above 3000x). In low magnification mode on our CM12, images contain obvious distortions, preventing perfect alignments. People who are well trained in PS CS4 can use built-in functions for correcting these distortions, before the images are merged. Not easy, but works satisfactorily. I just checked PS CS4 again: "Photomerge" even includes filters ("layout") offering some preliminary correction of distortions. **Reinhard Rachel** reinhard.rachel@biologie.uni-regensburg.de Fri Jun 5

Gatan include 'Digital Montage' in the software available for their cameras that seemed to work well stitching together the TEM images [it was only used with their camera, albeit with an offline workstation]. But it was fantastically expensive [well the camera was and it came as a package]. See: http://ftp.gatan.com/products/digital_imaging/products/Digital_Montage.php. Not used it since I left UCL a couple of years ago though, but it went down well with the TEM users. The software balances intensities between images as well. The Montage image files were large though. I always found Photoshop CS2/CS3 Photomerge to be pretty awful at photo-stitching photographs and I've long abandoned it. I'll try the CS4 version next time to see if it has improved any. In fact the software that came with my camera 'Olympus Master,' after you paid for the Pro upgrade, worked far better for camera photos so I still stick with that. It can't get it right every time and so a manual edit to taste is often required. Camera lens distortions and positional errors are presumably worse than that found in 'flat' 2D TEM images. Try things like: copy & paste badly affected areas, and Free Transform, and Transform 'Distort' etc. to overcome poor stitching at the overlap [plus see the link below]. Image analysis packages like MetaMorph & AxioVision seem to go for the more conservative Montage, where the 'overlap' edge is left as is, but I don't see much problem ethically with very minor photo editing to get it to look nice assuming nothing of importance is edited away or added. Generally though 'looking nice' is just for the web or presentations, being aesthetic, and is often of no particular scientific importance. You can buy various similar photo-stitching panorama software, all largely aimed at photographers though: <http://www.cs.ubc.ca/~mbrown/autostitch/autostitch.html> [seems well regarded] http://www.ptgui.com/info/photo_stitching.html <http://www.vrtoolbox.com> <http://www.easypano.com/photo-stitch-software.html> <http://www.panobuilder.com/index.asp> <http://www.vextrasoftware.com/rasterstitch.htm> This is just a selection, prices vary, and all can't perform miracles, although the results can be very impressive after a minor edit or smudge here and there. I tried a few on demo, but my Olympus E500 SLR camera's Olympus Pro* software did as well so I never bothered 'upgrading'. Often it's brightness differences

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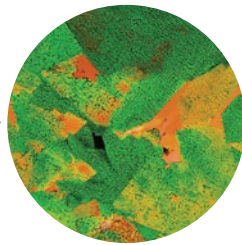
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rather than stitching that really ruins things, so camera lock on the same area of the view and move to the next one in sequence - likewise on the microscope: keep exposure times as identical as possible. I'm an optical microscope guy. Better photo-stitching software should attempt to correct uneven lighting. If you have a decent digital camera, though do try panoramas as fantastic ones are really easy to create these days. Plus the software to do it should have come free with the camera, e.g., typical photo stitch help guide: <http://www.phong.com/tutorials/photostitch>. Canon cameras offer similar free photo-stitch software that is highly regarded. **Keith J. Morris** kjmorris@well.ox.ac.uk **Wed Jun 10**

I once saw an impressive demo of this freeware software, with fluorescence data: <http://www.xuvtools.org/>. You may want to give it a try! **Jose jose.vr@gmail.com** **Wed Jun 10**

On the subject of image stitching, I'll admit I've never used it with any EM work, but I have had tremendous success with camera panoramas using hugin: <http://hugin.sourceforge.net/> Not only does it do the best job of things I've used, the best part is it's Free Software. Lots of power over the stitching and final image projection. Some folks on flickr tag their photos with it: <http://www.flickr.com/search/?q=hugin> although not everything is hugin related. I have used my old Canon Powershot's Photostitch software for stitching TEM images together, which was pretty successful. If I were to do it again, I'd definitely be trying Hugin. **Steven Cogswell** cogswell@nbn.net.nb.ca

I have had a go with Photoshop CS4's PhotoMerge*, and yes it is doing a far better job than the older Photoshop version I last used [probably CS2]—and it's now producing better output than my Olympus Master Pro in manual mode [although the Olympus cameras have a hardware panorama mode of sequential capture that works far more reliably]. The SLR barrel lens distortion effects remained, but the stitching was pretty perfect. I'll give it five, although it was slow at the stitching [presumably because it's thinking hard]. I expect dedicated commercial stitching software has likewise improved over the last couple of years - I'll check them out as demos, but I can live with CS4's output. I've never had a problem with slightly varied light levels on a scientific montage of say a completely scanned section using Zeiss Axiovision and a motorized stage - it kind emphasizes that it is a montage and easily identifies each specific image within the entire raster scan. For a scene of Tenby harbor in the real world though, it does look appalling. **Keith J. Morris** kjmorris@well.ox.ac.uk **Thu Jun 11**

Photoshop has the ability to correct for barrel (or pincushion) lens distortion. I think it is under Filter -> Distort -> Lens Correction. The process can be automated with a Photoshop 'Action'. If you are using a standard camera (SLR or P&S), there is a great Photoshop plugin called LensFix that is very capable at fixing geometric distortion. <http://www.kekus.com/software/plugin.html> **David Elliot** elliott@arizona.edu **Thu Jun 11**

Image Processing:

PowerPoint poster

I'm getting ready to "teach" my undergraduate EM class how to create a poster presentation of their work, and I need a little educating as well. Could you please send me your sage advice on creating a poster in PowerPoint (that's what we are required to use) regarding the resolution of the electron micrographs? As background, we are developing TEM film, scanning the negatives, and manipulating them with Adobe Photoshop.

Kristen A. Lennon kalennon@frostburg.edu **Mon Apr 13**

You and your students will be fine with PowerPoint. For posters larger than what PowerPoint's setup allows, we scale a little down, keeping the desired proportion, then we increase the size (e.g., 123%) during printing. I have been using PowerPoint for posters since 2000, with different printers. Until 2-3 years ago, we would have to go with

our files to a centralized printing facility, but now we have our own large HP printer, and even new users have little problem using it from their desktops. I've been meaning to switch to Adobe InDesign for posters for years, but guess what, whenever the time comes to make a new poster, there is always a hurry, and never enough time to learn new software! I am neither a lover or hater of Microsoft products, use them as a tool, and PowerPoint has been very convenient for us. I recommend making all adjustments to your photos while still in Photoshop, and all labels, lettering, and scale in PowerPoint. This way it'll be easier to do late adjustments like font size. We switch PS color management off and try to achieve the desired gray levels on all images. Once everything is to our liking, we reduce the pixel size and save under different name. Now, to your particular question - we aim for 150-200 pixels per inch in the resulting full-size poster print. That's from some early testing we did. We set picture size in PS, in inches, to what it approximately is going to be in the final print and then set resolution to 150-200 ppi. Then save the new image separately as TIFF, that's the best and should not cause any file size issue these days. Then we insert (or drag-n-drop) those saved images onto the poster in PowerPoint. It is very common to have to resize the image a bit this or that way before the poster is finished, that's why the ppi range. 175 ppi is a good starting number. You'll get a feeling after a few posters. Don't be afraid to explore lower ppi - as long as you see all relevant features, of course, images printed from lower ppi tend to look better than oversampled (too high ppi) images on a poster. Below 150 ppi in the final print, you may notice the reduced resolution, but it won't look bad. As long as you use TIFFs, PowerPoint will do nice smoothing, and there'll be no pixelation. **Vlad Speransky** vladislav_speransky@nih.gov **Tue Apr 14**

Generally I use PowerPoint to produce my posters, which is fine as my predecessor created our Core poster in PowerPoint and we never go above poster size A0 [paper size 3 feet x 4 feet]. I have converted a few to MS Publisher though. Other applications can be used but it should be noted that Word will not accept paper size as big as A0. Our University printers do the printing, we just provide the Excel, ppt, or Publisher pub file. Image size is kind of irrelevant provided your image resolution is at least 600 pixels per inch on the paper [it can be higher res in the document]. You can often get away with less if that's all you have [upscaling in Photoshop can help], as often it's only the University/Centre logos that look really naff and pixelated at low-resolution. Generally it's useful to make all the images quite high resolution, say 1000 pixels per inch, as I quite often nick images from our poster, say for the website, and it's a pain having to track down the original. You do need Photoshop or similar [Elements or Serif PhotoPlus] to edit/crop/enhance images. Large PowerPoint size [Mb file size] due to high resolution graphics isn't a problem for a modern PC [well for mine anyway as it is an imaging workstation and gaming powerhouse], plus it simply goes off to the printers on a CD or RAM drive. Don't use 6,400 dpi scanned TEM ones though unless you are a patient sort on the PC, downscale to or scanning at 1,200 dpi seems fine for most applications [unless of course you are printing that single TEM film image at size A0]. Going below 300 dpi on the printed page might look bad, but it depends a lot on the image itself. For pdf's use Adobe's Acrobat Pro's pdf optimizer and generally output for the latest acrobat version compatibility. Our OUCS printers seem to prefer output from ubiquitous PowerPoint or Publisher [word is limited to 22 inches size I believe]. The advantage of PowerPoint is that everyone has it, and it works well at least up to the standard A0 poster size. Microsoft Publisher is probably a better option, but it's less common and not MS Office's greatest app [Serif's PagePlus is better and a third of the price]. Plus there's Adobe's InDesign and Quark express for those with deep pockets [probably not students]. **Keith J. Morris** kjmorris@well.ox.ac.uk **Wed Apr 15**

Microscopy Society of America Awards

Nominations are now open for the Microscopy Society of America Annual Awards. The awards process is one way in which the Microscopy Society of America recognizes the significant and diverse contributions that individuals make to our field. Deserving nominations for consideration should be submitted online no later than December 15th, 2009 to:

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This Award was initiated to recognize outstanding volunteer service to the Society as exemplified by Mort Maser, who served the Society for many years with great dedication. This award is made to honor an MSA member who has provided significant volunteer service to the Society over a period of years.

**Further details of the nomination process
can be found on the society webpage at:**

www.microscopy.org



As we also produce our posters for pdf [where the viewer can zoom] we aim for higher dpi in the original ppt file [doesn't do any harm] - plus I do like to nick them for other uses. I guess many of our images are generally lower than 600 dpi on the actual page, typically 300 dpi, and as you say they still look fine. There is nothing wrong with higher resolution though. Once scaled down on-screen, PowerPoint doesn't seem to mind our 'hi-res' images though [at 600 dpi], in terms of PC response when moving around the presentation. With any image editing/graphics design more PC memory helps a lot [my laptop, 2.25GHz Pentium M] was sluggish with Photoshop CS2 at 512 and 1 GB RAM, but when upgraded to 2 GB [motherboard max] it noticeably improved. Windows 32-bit has the 3-4 GB OS max though [including graphics card memory], and only 64-bit OS goes beyond the 4 GB max [motherboard dependent]. **Keith J. Morris** kjmorris@well.ox.ac.uk Wed Apr 15

Our school has been using PowerPoint as the default software for creating posters for many years. While 300 ppi is a "golden standard" for printers (glossy magazines), it is surely overkill for posters printed with inkjet printers. From my experience printing images from the XL30 SEM (about 1400*1000 pixels) at 11" image size (i.e. 127 ppi) produces good results. Even 100 ppi on a poster could be good enough. But for line drawings you need higher resolution. **Vladimir M. Dusevich** dusevich@umkc.edu Thu Apr 16

I agree that 100 dpi is perfectly fine for poster printing of most images where detail is difficult to discern anyway, say fluorescence in cells or tissues. You do notice low-resolution jaggies in the text and in things we are good at discerning, say a microscope image, illustrator image, or lab view [when we know what they should look like]. Blurred printing on cheap paper [the standard for most poster printing] means that the 600dpi of a modern printer is unlikely ever to be realized. So although we easily spend £2,000+ in man hours producing a poster, we rarely spend more than £40 printing it [and for most purposes this poster resolution is perfectly adequate anyway]. Plus the inks used may be rubbish; our University-printed posters have all faded in a month or two after being left up in a windowless lab [obviously not HP inks and HP photographic paper where the inks are guaranteed fast for 100 years]. Fine for posters you use once and bin, but a pain for our constantly re-used & recycled Core Facility poster. However, I would still advise against going to lower than say 1,000x750 for an image whatever size it's going to be printed, if you think you might possibly use it again. Our Core poster has 40+ images going back 5 years, and the 'master' hi-res images are simply lost to history. Probably they were deleted from my processors personal hard drive space when he left, and probably they weren't all ours, but from our collaborators/users. So it is an incredible pain to find that all our images on our main poster ppt files are about 250x300 max pixel size - with multiple backups at different locations, it's often only the poster master ppt file itself that survives. If, at a later date, I want to increase the printed image size from 2x1.5" to say 6x4.5" the pixilation is very noticeable. Plus they look bad in Core PowerPoint and pdf presentations - and we are supposed to be the kings of imaging. You can get by, say by upscaling and re-adding text at higher res - but what a pain, more hours in Photoshop, and with typical day rates of about £400+. And the extra file size of the larger images in the original ppt poster file would have been insignificant on a modern PC. Keeping track of digital images for the next 30 years is just about impossible, if I want an image from one of my old papers these days I have scan the printed copy. I guess I produce 10,000+ digital images a year at home and at work these days. Backup is one thing, finding a particular image again quickly years on is another matter. **Keith J. Morris** kjmorris@well.ox.ac.uk Tue Apr 21

I'm sorry if this sounds harsh Keith, but it seems a little odd to me that you use PowerPoint, designed for on-screen presentations, to produce posters. It is under £120 for you to buy InDesign CS4 (OUCS

shop- license and media), and it will save you time as you never have to re-make posters for different sizes, and the process itself is a lot faster. You can probably justify the cost in time savings after only one or two posters. I know it doesn't address Kristen's question, as she is required to use PowerPoint. For importing into PowerPoint (or any Office application), PNG is the best file type, as it is the native compression for Office. I would tend to go for 300ppi, just because computers can cope with large files, but realistically 210ppi is indistinguishable. Don't be confused with printers' dpi (typically inkjet for this application), and pixels per inch. Inkjets cannot print continuous tone; they have to place many dots to achieve the color information contained in a single pixel. Now to preach on the alternative: InDesign links to the original full-resolution version of all images, via the 'place' command, and you can work with thousands of images of very large file sizes without it affecting the speed or reliability of the program (it is designed for typesetting whole books after all) - this is because it only works with a low-resolution preview of the original file. The InDesign files themselves are never over a few megabytes. You can 'package' all the linked images for a file together in the same place (this duplicates all the linked files and places them together- useful if you have linked to originals in multiple folders spread around your computer). When you want to have the poster printed, you export as a PDF, down-sampling all the images to the desired resolution and compression in one simple step. The result is a very robust file, with all fonts embedded and almost fool-proof for printing, that is never too large or too small for the job. If you want to edit an image in the poster, you don't have to find your original high resolution file, and make changes, down-sample and re-import, as you would have to do in PowerPoint. Instead, you just right-click, and select 'edit original' from the contextual menu, and it opens the original in Photoshop, and after making changes you close the document, and the preview in InDesign will automatically update. If you ever use vector files, PowerPoint is a nightmare; whereas InDesign links directly to Illustrator files, and changes are very quick. And the PDFs created by InDesign will obviously contain perfect copies of the vector information, so even if you do lose all versions of a diagram, except a low-resolution PDF of a A0 poster designed for printing A4 as hand-outs, you can open the PDF in Illustrator and copy the perfect diagram out of the file. These are all in addition to the much more sophisticated text tools (justified text with complete control over the balance between enlarged spaces between words or letters and hyphenation), automatic alignment tools, and the ability to instantly spread items at equal spacing, or a pre-set spacing. **Ben Micklem** ben.micklem@pharm.ox.ac.uk Tue Apr 21

As Vlad, Ben and others have commented, the best program is not always the one being used to make posters. In our central facility, we produce many hundreds of posters a year. The vast majority are produced using PowerPoint, or, as our graphics person calls it, ProblemPoint. If done properly (a rare event), the results can be as good as those obtained using InDesign. However, when problems are encountered, it takes a long time to figure out why PP is not producing posters that print properly. We only rarely have this problem with InDesign. We have been using InDesign (and previously PageMaker, Quark and Illustrator) to produce posters over the years, but when researchers bring in "pre-made" posters, it is nearly always a PP job. When problems are encountered (usually due to the Postscript printer balking at the PP hack job), the harried researchers want to know "the best" program to use. We always recommend they purchase the Adobe Design Suite CS4. More and more researchers are doing this and the word is slowly getting around. It will take time and patience. This summer we will be giving a series of training sessions on how to produce high quality posters using the CS4 Design Suite (Photoshop, Illustrator, InDesign and Acrobat). We'll see if this improves the situation. I believe it will. **John J. Bozzola** bozzola@siu.edu Tue Apr 21

Our core poster was originally created in Powerpoint [not by me I might add]. I had a go at converting it to InDesign CS3, but after an hour or so I lost the will to live. Not helped by Adobe's obtuse help. Adobe's stuff is buggy as well on first release. With our Core poster, we only have to change the odd staff photo mainly, as predecessor's relocate to the Solyent Green factory. Plus, of course, all the images are mostly stuck at 250x300 resolution anyway and our ppt file always prints OK [so if it's not broke...]. InDesign is much easier if you use it to create the poster from scratch. Having spent the last year wrestling with *Flash CS3's incomprehensible help [often not even giving info on the right program], it doesn't make InDesign that attractive for very occasional use - if you want to see how easy to use a DTP program can be, try Serif's PagePlus X3, it makes Publisher look pants [well I suppose Publisher is pants]. Trouble is the learning curve on Adobe software is so steep you really have to use it every day to become comfortable, not once a year. Quark Express is no better in terms of intuitive use either. I did get to grips with GoLive, then Adobe killed it off after buying Macromedia. I use Illustrator occasionally, but mostly it's Photoshop, Flash, Acrobat Pro, Dreamweaver, and PhotoShop [again & again]. I know Photoshop so well now I have fooled myself into thinking it's easy to use. Besides it's Mat Lab and Dragon Naturally Speaking 10 that are on my to-do list for this month - plus of course Microsoft's 'intuitive' new ribbon interface [I have kept Office 2003 installed with Office 2007 - easy to do, with a few modifications]. That said Adobe on-line video help is very useful [but it's only available for the latest products]. Try: http://www.adobe.com/designcenter/video_workshop/?id=vid0118 Adobe Video Workshop. Plus we have our local OUCS courses, and there's Amazon for a selection of books. **Keith J. Morris** kjmorris@well.ox.ac.uk Wed Apr 22

EM: insurance-based contracts

It looks like we are pushed (gently, for now) to consider possibility of switching our service contracts to insurance-based contracts. So, now I am very interested in opinions on this type of servicing microscopes: SEM and TEM. **Vladimir M. Dusevich** dusevichv@umkc.edu Thu Apr 16

Push back---and don't be gentle. We tried this a number of years ago, and it was a disaster. It once took us nine months to schedule preventive maintenance, since an insurance provider wasn't paying its bills and the service provider understandably went on strike. Despite what they told us when they were selling us these contracts, the procedure to get service was much more complicated and time-consuming, and we were given a much lower priority by the vendors than their service contract holders were. You may have to justify service and parts before they will authorize it. Maybe we just had a poor experience and things are better now, but the day we went back on vendor service contracts for our scopes was one of the happiest days of my life. Have you considered third-party service provider contracts? Some people say they are very happy with these. I have no personal experience with them, however. **Randy Tindall** tindallr@missouri.edu Thu Apr 16

I used one in a previous incarnation (job elsewhere). It was fine, saved money, got the microscope company to service. Then we had a big claim and a diagnostic procedure -- looking for stray magnetic fields. Suddenly the contract was worthless. The insurance company refused to pay the claim (contrary to their salesman's statements) because of the magnetic field survey. When I explained why the survey and what it was, they (supposedly) referred the claim to their engineers, who may be great on elevators and escalators, but know nothing of EMs, who denied the claim. They had paid the microscope company, but they were trying to get me to reimburse them. I referred them to the University lawyers (said University having several contracts with this company for various equipment). I don't know how it finally ended,

but I didn't give the insurance company any money, and as soon as the contract was up, went to the microscope company for a contract. Where I am now we have company contracts and no hint of insurance contracts, and I'd fight them pump and electron to avoid changing. **Philip Oshel** oshel1pe@cmich.edu Thu Apr 16

I've always had contracts, but my colleague at a nearby institution was forced to go with insurance. They have an older TEM and at one point needed a major repair that took over 2 weeks....charged hourly. The end result was a bill close to if not more than the vendor's contract would have been. They also have trouble getting their preventive maintenance visits scheduled, since labs under contract do get priority. **Lee Cohen-Gould** lcgould@med.cornell.edu Thu Apr 16

Don't do this, you will be sorry. I serviced TEM/SEM tools first working for Philips, then as an independent, servicing and moving all brands plus associated instruments and equipment. You may get lucky for a while and save some money, until a major problem happens, such as a defective HT tank for example. Insurance will try to locate a used one, or an independent service provider who will repair it. I did that. But insurance is unlikely to pay tens of thousands of \$\$ when all else fails. There is no miracle. This kind of insurance does not have enough of a base to be a reliable one, even if they try hard, this will be mathematically impossible in the end. It is difficult enough for an OEM to make service and support business profitable while dealing directly with end users. How can you add a middleman in-between and expect profit? Please don't do this. If life comes to the point when your employer can't afford an OEM or a 3rd party contract - do you own PMs and pay directly to service providers when their help needed. And keep your fingers crossed that nothing major will go. It sounds scary, but works better than insurance based service. Common sense versus peace of mind. **Vitaly Feingold** vitalylazar@att.net Fri Apr 17

TEM: books related to materials

We have traditionally been a Life Sciences Imaging core facility on campus (SEM, TEM, STEM, confocal, fluorescence, etc.), but we are finding ourselves leaning more towards the material sciences. As such, we would like to augment our Center's reference library with books relating to the imaging and analytical side of microscopy. I was wondering if I could get ideas on basic book requirements that material scientists would look at as basic references and might be considered indispensable in a materials-oriented research center. **Mark Grimson** mark.grimson@ttu.edu Fri Apr 24

You should start with some books from ASM (American Society of Materials). Two basic books would be Volume 9 of the ASM Handbook, Metallography and Microstructures (LM of Metals) and Volume 11 of the ASM Handbook Failure Analysis and Prevention (SEM). It depends on how extensive you want to be in your library. There are many other good books, but those are two good books to start with. **Gerald Shulke** gas19@chrysler.com Fri Apr 24

TEM: Willilams & Carter, "Transmission Electron Microscopy"; new edition due this June. Reimer & Kohl, "Transmission Electron Microscopy: Physics of Image Formation (Springer Series in Optical Sciences)". SEM: Goldstein et al., "Scanning Electron Microscopy and X-Ray Microanalysis" get the latest edition -- 3rd, I think. Reimer, "Scanning Electron Microscopy: Physics of Image Formation and Microanalysis (Springer Series in Optical Sciences" 1998). General: Sawyer & Grubb, "Polymer Microscopy" 3rd edition. What I have a hard time finding is a reference on specimen preparation for materials science. Be interesting to see if anyone posts such a book. **Philip Oshel** oshel1pe@cmich.edu Fri Apr 24

I'm looking for a general sample prep book that discusses the "how to", "why to", and the wherefores of the various methods -- ion-milling, etching, tripod polishing, small-angle cleavage, etc. -- for a

variety of different kinds of materials. Naturally, all this varies with the sample type and microscopy, as well as with the questions asked. So, not a specific, say, fractography book, or thin-film book, or even more-general-but-still-specific book such as for metallurgy, but a text like one would use in a materials EM course, but concentrated on sample prep, as opposed to a chapter on sample prep in a more general EM text. Which would also be handy to have hanging around the facility when whoever walks in with whatever kind of sample they have. Philip Oshel oshel1pe@cmich.edu Fri Apr 24

Sample prep, well, I can say it depends.... What type of samples are you interested in? Metals, plastics, or ceramics? Are you more interested in light microscopy or electron microscopy? Basically we use microscopy for two things: materials characterization and failure analysis. For materials characterization there are a number of protocols depending on the material and what you are looking for in that material. For metals, let's say, it depends on the alloy, how it was processed, and what phases you are looking for. Most samples are mounted in Bakelite or epoxy, ground, polished, and etched to reveal the microstructure. For failure analysis, there is not much sample prep. Usually you are looking for contaminants, deposits, etc. so you look at the sample in the as-received condition first. After you have collected what information you can, then you clean the sample to see the fracture surface topography. For metals, it may be acetone or hexanes to remove oil and dirt. There are more aggressive approaches, but the idea is to remove most of the surface contamination without damaging the underlying material. It depends if the sample was corroded or not. For polymers, the surface may be cleaned with soapy water. Anything harsher can destroy the surface. If the material is not very conductive for SEM, then we gold sputter coat the sample. Usually this is a last resort, because you can't

take it back off. You can do a lot with low-voltage or in an ESEM. I can go into more detail if you want. I can post to the server to share with everyone if you could tell me more specifically what you are after. There are numerous books, especially for metals. Gerald Shulke gas19@chrysler.com Fri Apr 24

I'll refer to my other post for good general books for material science for both LM and SEM. I do not know of a specific book for sample prep for SEM in the materials field. There is an older book "SEM: A User's Manual for Material Science" by Gabriel, also published by ASM, but it is from the 80's and somewhat outdated. Sample prep techniques for SEM is usually covered in a chapter or a general discussion in several books based on failure analysis or specific for a type of material. For general sample prep of metals for LM, I would add "Metallography Principles and Practice" by George Vander Voort. Gerald Shulke gas19@chrysler.com Fri Apr 24

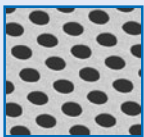
These are two good refs for metals: ASM Metals Handbook Volume 9 Metallography and Microstructures and Metallography Principles and Practice by George Vander Voort. Jeff Stewart jeff@metallography.com Sat Apr 25

Not a book (the book is in French to my knowledge but the English version is expected in august 2009) but a good web site for the preparation of TEM samples: <http://temsamprep.in2p3.fr/techniques.php?lang=eng> Patrick Weisbecker weis183@yahoo.fr Mon May 4

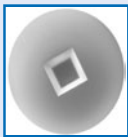
Editor: A general book that fits your description has just been published: Patrick Echlin, Handbook of Sample Preparation for Scanning Electron Microscopy and X-Ray Microanalysis, Springer, New York, 2009. MT

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