



# MICROSCOPY

## 101

We appreciate the response to this publication feature - and welcome all contributions. Contributions may be sent to Phil Oshel, our Technical Editor at:

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### On Osmium Tetroxide Staining of Polymers for SEM

We have found over the years that in general, TEM is light years better than the best SEMs for the characterization of the multiphase structure of modified polymer systems.

When staining polymers, remember that just about any polymer, if left in the presence of osmium tetroxide, will eventually turn black. What makes the osmium approach work is that some phases stain far faster than other phases. A good rule of thumb is that if the contrast between two phases needs to be increased, slow down the staining, either by reducing the concentration (if vapor phase, then the partial pressure) of the osmium tetroxide or by lowering the staining temperature.

The staining is usually done with the vapors that come off of a standard 4% water solution. Vary the effective concentration by

varying the distance from the water surface to the grid or sample being stained. The staining times, etc., all will be dependent on the nature of the polymer and the amount of unsaturation present. HIPS or ABS, for example, with lots of unsaturation, generally stain quickly and might take only about a minute or so. EPDM, however, which has very low levels of unsaturation could take tens of minutes.

If one must use only an SEM, then we first make a "faced off piece" of the sample with a diamond knife in a cryostage ultramicrotome, thereby making the smoothest possible surface, then vapor-stain the system with osmium tetroxide (or ruthenium tetroxide depending on the chemistry of the sample). However, if it is looked at by SEM at this point, there can be background staining of the unstained portions of the sample. The next step then is to oxygen plasma etch the sample in a simple RF plasma etcher. The oxygen will etch down the unstained portion of the sample and will etch far more slowly the osmium (or ruthenium) stained portions of the sample. One need not etch very long but this does produce enough additional topography to result in acceptable contrast when the unetched sample has insufficient contrast.

We think that coating with osmium metal (in the OPC osmium coater), instead of gold or carbon has some advantages. One might normally think that a heavy metal is not the way to go, but the layer is much thinner and for backscattered electron imaging, it can have some advantages. This is speculation on my part, but it is based on experience with osmium coating of immunogold tagged cells.

The main problem with SEM approaches is that if matrix inclusions are present, they tend to be small, and are often times missed, whereas by TEM, they would be seen readily.

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### A Freeware Photoshop Plug-In For Putting Scale Bars On Micrographs

There has been a recent thread discussing methods for using Photoshop to place micrometer markers on micrographs. We need a method to directly use the (presumably known) image magnification to create the final result. In response, I've created a plug-in for Photoshop and compatible programs that does this. Enter the magnification of the image (e.g., 1000X), the dpi with which it was acquired (e.g., 300 dpi for a scanner, or the corresponding pixel spacing for a digital camera), and the length of the bar desired in micrometers, and the program draws and labels the bar in the lower right corner of the image using the selected foreground and background colors. This procedure can easily be applied to an entire folder of images using a Photoshop action.

This plug-in is freely downloadable for use with Mac or Windows computers at <http://www.reindeergraphics.com/free.html#entermag>. It can be used on 8 and 16 bit grey scale images or 24 or 48 bit RGB color images. While it is compatible with the Fovea Pro and Image Processing Tool Kit software, it does not require them. If you have comments on the plug-in, or urgent needs for other features or capabilities, please let me know.

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