

MICROSCOPY 101

mirrors were typically glued into a cube/holder device, and blanks were used so that the light from the mercury or xenon lamp source could not get to any detector (including the eye) without at least going through the emission filter. Mercury and xenon lamps create huge amounts of energy, including the 'invisible' UV, which can destroy detectors in very short order. Keep in mind that the transmitted illumination for a microscope is fairly weak, and has virtually no UV emissions, which makes it safe for virtually all detectors.

In modern microscope design, the filters for doing epi-fluorescence illumination can be easily moved or exchanged. This presents the possibility of someone accidentally removing a protective filter, or not matching the exciter/emitter combination correctly. There is also a trend to use emission filter wheels in microscopy. This adds a great deal of flexibility to the system, but it also removes the emission filter from the beampath that goes to the eyes/oculars. This means that a great deal of the excitation light can reach the most important detector of all, your eyes. If this light happens to be either UV or NIR, it can destroy the retina without you actually 'seeing' it. It was thought for many years that the human eye was only damaged with UV light. We now know that violet and blue light may be just as harmful, and that green light, if strong enough, can also blind you. Red may be the safest in small doses, but near infra-red (nir) becomes dangerous again. (use bgg22 from Phila Optics Inc; or KG1/KG5 from Schott Glass).

Know the beampath of your microscope. Do not trust to chance or to others in the lab. If you have emission filter wheels, and therefore no emission filter in your cube/mount, ask that someone put UV-blocking optics in the oculars/eyepieces. There is usually room in the reticle shelf of the ocular. A 420 nm long-pass filter would block deep UV to 410 nm or so (L42 from Hoya Corp.; YG11 from Phila Optics Inc.; or gg420 from Schott). You still have to be concerned with very bright violet/blue/green light, but this light is 'visible' to you. Never look through the microscope while switching to a new/different cube. Move the filters/set, and then begin by looking several centimeters away from the oculars/eyepieces. Move your eyes closer to the focal plane of the oculars slowly. If you see a bright light, of any color, do not stare and make changes in the illumination system.

Dissolving Osmium Tetroxide the Easy Way

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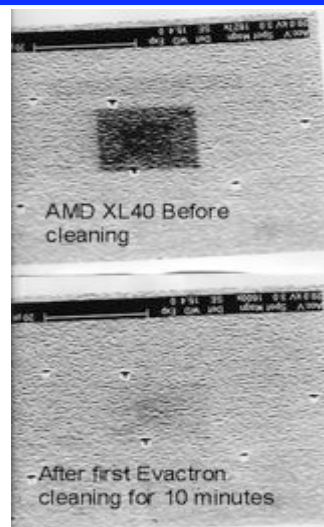
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Osmium crystals tend to cling to the glass ampoule walls making dissolving them difficult. Smashing the vials results in glass shards that may get into sample vials and can be dangerous, or carry into embedding media and damage diamond knives.

A simple trick to eliminate smashing osmium vials is to dip the sealed ampoule into liquid nitrogen. This releases the osmium crystals from the glass walls. Then simply break the vial using an ampoule cracker (available through EM supply houses) and pour the osmium crystals into a bottle containing the water for the OsO₄ solution. Wait a few minutes to let the ampoule warm-up, and it is easy to see if all of the crystals have been dumped out of the ampoule. Let the solution sit overnight at room temperature in a hood and the next day the crystals will be totally dissolved.

Another benefit is that if the solution is needed in a hurry, the bottle of water plus OsO₄ can be sonicated, and it is easily seen when the crystals are all dissolved. Just make sure the sonicator is in a hood and the bottle is well sealed.

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