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A Simple Vacuum for Controlling Resin Dust When Trimming Embedding Blocks

I made a vacuum set up with a bong-like flask arrangement in between the suction hose and the vacuum to collect the dust in water, then replaced the water regularly. The tube from the suction hose does not actually touch the water I know by experience that if the hose touches the water, the end gets clogged with wet GMMA. Then at the top of the flask, there is an outlet connection to the vacuum, well away from the water-hose interface. Useful for cleaning the work area too.

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TWO METHODS FOR USING FLUORESCENT PROPERTIES OF EOSIN, A ROUTINE TISSUE STAIN FOR BRIGHTFIELD LIGHT

Finding Extravascular Red Blood Cells (RBC's) in Hematoxylin and Eosin Sections

For many years, I have used a simple method for finding accumulations of RBC's that lie outside of normal vascular channels. For example, in the urinary bladder of the mammal, the urothelium lies over a very rich capillary network. Overdistension of the bladder (100mL in a rabbit in which normal micturition occurs when the bladder volume reaches around 20mL) often causes petechial (punctate) hemorrhagic damage. Sites of this damage are difficult to find once one has fixed, embedded, sectioned and stained the tissue.

One of the most common dye combinations is hematoxylin and eosin (H&E), and a visible characteristic of fresh, *alcoholic* eosin (0.7g Eosin Y in 100mL 95% ethanol) is a "green sheen" visible through the clear glass bottle when it is viewed in daylight. If one has H&E-dyed sections and wants to find sites of extravasated blood or petechial hemorrhages, one can do so easily by using a fluorescence microscope with filters for FITC (Fluorescein IsoThioCyanate) to find small accumulations of RBC's in the tissue even at low power.

Normally, I insert a neutral density filter in the path of the fluorescent light to prevent the massive photobleaching of eosin during the search at low power. Once I find a site of petechial hemorrhage, I change to higher power and briefly remove the neutral density filter. When I am finished finding all of the hemorrhagic sites, I can switch to bright field and merely look for (and count?) the little circular dark holes in the pink eosinophilic background of the slide.

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Locating and Quantifying Osteoid Using Eosin Fluorescence

In our Metabolic Bone Disease Biopsy Lab at UCHSC, we routinely use the fluorescent property of fresh *aqueous* eosin to measure osteoid seams on bone biopsies embedded in Glycol Methacrylate without decalcification, using the following method:

1) We first stain the 5 µm sections with 5% silver nitrate, exposed to UV light for 5 minutes (Von Kossa).

2) Then the silver is poured off and the sections washed in running, deionized water for 5 minutes.

3) The section is then stained with Gills 3 hematoxylin for 5 minutes, rinsed, blued in ammonia and rinsed again.

4) The sections are then stained in freshly prepared aqueous 0.2% eosin for 10 minutes.

5) The eosin is quickly rinsed through 2 changes of 95% ethyl alcohol (EtOH), 3 changes of 100% EtOH, 3 changes of xylene and the slide mounted in Permount.

Results of this stain with the light microscope are: calcified bone-black silver stained, cell nuclei-blue hematoxylin stained, cell cytoplasm and osteoid-pink eosin stained.

With fluorescent illumination, the only tissue component visible was the eosin-fluorescent osteoid. Take separate digital images using bright field and fluorescence illumination and then use a computerized image analysis system (Universal Imaging by Image One) to measure the area and lengths of both fluorescent osteoid and the silver-blackened, calcified layers of bone.

Note: If there is a difference between these two procedures, it likely lies in the degree to which we permit the eosin to remain in 95% ethanol after staining. FCM tends to short staining times and brief rinses in 95% ethanol while PR uses a long staining time and a long rinse. While both use mercury arc excitation, PR does not notice the 'holes' in the eosin background after viewing.

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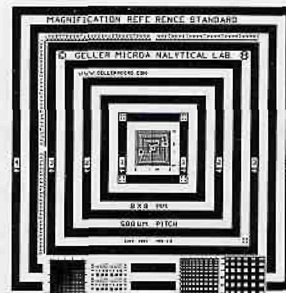

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