Sectioning Fat

I have worked for many years cutting frozen sections on skin specimens (for light microscopy) in a dermatopathology laboratory. In order to accomplish our goal of demonstrating a complete margin which includes the fat, my specimen must be frozen to a colder temperature than most tissues. My cryostat is set at -28 degrees C. However, this is not cold enough to demonstrate fat. Fat has to be chilled to around -50 degrees C. Remember, the knife blade and anti-roll plate must be at the right temperature as well. I use liquid nitrogen sprayed directly on to the block. I am able to do this because we keep the cryostat chamber free of debris and do not handle infectious cases. In a hospital setting, immerse a swab in liquid nitrogen, then place the swab onto the fat. When the fat turns white, section it. Sometimes, it also requires a faster rotation of the fly wheel to get a section.

There are many different techniques for sectioning fat, some as simple as giving the fat extra time to freeze. In most labs time is of the essence and techs are constantly seeking that one tip which will make their job easier while producing excellent sections. So even though the liquid nitrogen can be difficult to obtain it is well worth the trouble. Just recently, I saw first hand the need to demonstrate the fat with a complete intact section when a patient's slide showed scattered basal cells throughout the underlying fat in the margin. Give this technique a try. The doctors will be singing your praises when they see your fat sections.

Mequita Praet, Dermatology Associates
email: mdpraet@bellsouth.net

Caveat on Time Differences Between Manual Staining and Machine Staining.

One of the biggest differences between hand and machine staining is how the surface tension of the reagent currently on the slide is broken and then replaced by the next reagent. When we stain by hand we exert much more and varied force than a machine does when plunging the slides into the reagent.

We also knock off more reagent when time is done so less reagent clings to the slide. A stainer (machine, not human) simply lowers the slides slowly, in a single plane, into the reagent. Even the agitation of machine staining is in a careful, single plane (up and down) movement. When we stain by hand we cause the reagent in the dish to bombard the slide from several angles and with greater force that breaks the surface tension in less time than a machine can accomplish. Therefore, a longer exposure time (of tissues to stain) may be required on a machine to yield the same results as hand staining.

When programming the machines I find it necessary to watch the hand staining carefully in order to make an accurate translation of a "dip" to a time value that the machine could reproduce. A "dip" in acid-ethanol in manual staining may not be reproducible by a machine. I may be able to use 1% acid-ethanol in hand-staining but have to use 0.5% acid-ethanol on the staining machine with a 2-second timing value to get the same results. Ten "dips" in a manual stain may require 30 seconds on a machine. Ten "dips" in a manual alcohol step may require 1 minute on a machine for the same results.

One of the things we need to remember is that the machine will move the slides exactly the same way for the programmed time. We humans (consciously or unconsciously) adjust our handling of the slides based on how the tissues or even the reagent looks.

Nancy Klemme, Sakura Finetek USA, Inc.
nancy.klemme@sakuraus.com

A Tip on Reducing Outgassing of Specimens in the SEM

The problem of dealing with the evolution of contaminating materials from plastics used to mount metallurgical, ceramic and mineralogical specimens is discussed on pages 75 & 76 of my book, 'Vacuum Methods in Electron Microscopy' (see http://www.2spi.com/catalog/books/book48.html for a description). Related topics also discussed are gas evolution from leaks, construction materials, specimen materials, and from cleaning reagents and procedures.

Contamination from mounting polymers can indeed be a very vexing problem, especially for SEMs that have field emission guns (FEG) and must operate with a relatively good vacuum in the specimen chamber.

Basically, what we found after a number of episodes of very serious contamination, was that it is necessary to be sure that the mounting polymers are mixed carefully and thoroughly, so that the correct relative amounts of polymer and hardener are used, and so that these components are thoroughly intermingled.

Then we found it to be necessary to be sure that after they are mounted the specimens are allowed to stand for a long enough period (at least 24 to 48 hours) to ensure that the mounting polymer is completely polymerized (moderate heating can sometimes be used to accelerate the polymerization reaction - even a 15 or 20 degree increase can have a significant effect).

Finally we ended up requiring that after curing all such samples had to be pumped overnight in a chamber of the type that is used to evacuate photographic film before it is placed into an electron microscope.

Such procedures did not totally eliminate the problem, but reduced it to a level where we could operate for a month or more before contamination built up to the point where cleaning of the chamber and apertures became necessary.

Wilbur C. Bigelow, University of Michigan
bigelow@engin.umich.edu

Softening Tissue Blocks with Nair

Nair works well for softening paraffin embedded tissue blocks, especially on keratotic skin lesions. I just put enough Nair in half of a petri dish, or an empty plastic slide box. Set this on ice or a cold plate, put the block in the Nair face down, let it set for 3 to 5 minutes, then cut. With some tissues, plain old water works just as well with the same technique.

Many people who work only with paraffin swear by this for all kinds of tissue including (but not limited to, as the saying goes ...) uterus, bone that has been previously decalcified, but is still hard to cut, finger and toe nails and nail beds, and basically anything difficult to cut because it is too hard or fibrous.

Wanda Shotsberger, Harris Methodist Hospital
WandaShotsberger-Gray@hmhs.com