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 for a patient's slide showed scattered basai cells throughout the underlying fibrous. 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, for all kinds of tissue including (but not limited to, as the saying goes ... ) uterus, bone that has been previously decalcified, 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.

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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, and 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.

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