MICROSCOPY 101

Plunge-Freezing into Slush Nitrogen

Questions keep appearing on the various microscopy list servers about the pros and cons of different cryogens for plunge-freezing in liquid nitrogen. We at Microscopy Today have run articles on this ourselves. There are serious safety concerns about these cryogens, including disposal*. While most of us have safely frozen specimens with these cryogens, why use this method when there is a better way?

This better way is to plunge-freeze specimens into slush nitrogen. It's colder, at the freezing point of nitrogen (~ -210° C vs -196° C) there's no Leidenfrost effect, and safe. No flammable cryogens condensing oxygen out of the air, making for an even more explosive mixture.

To make slush nitrogen:

Fill a 600mL or 1000mL beaker with LN_2, place in a vacuum desiccator, and attach to a rotary pump, of the size used for most sputter coaters or larger. Pump away. In a few minutes, the surface of the LN_2 will start freezing over and breaking up as bubbles of N_2 gas surge through. Keep pumping. Soon, there will be a mix of liquid & solid N_2. Stop. Pull out, place on an insulting pad, and plunge-freeze the samples, dropping them into a container previously placed in the LN_2. Plunge-freeze until all samples are done. Time is a problem with this method, as the LN_2 is warming up, and in some minutes the nitrogen will be at the usual near-boiling-point temperature of LN_2, with the Leidenfrost problems and so on. Transfer the samples to whatever the next step is only when done freezing. Make sure the outlet in the desiccator collar is aligned with the hole in the part of the desiccator lid over which the collar fits. Otherwise you'll wonder -- as I did, one insufficiently coffee'd Monday morning -- why the lid of the desiccator keeps bumping, and the LN_2 doesn't freeze.

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*Many folks evaporate the cryogen in a fume hood when done freezing, and while this works, it must be remembered that the vapors of most organic cryogens (propane, etc.) are heavier than air. The fume hood therefore must have provisions to exhaust air from the counter top level, and not just from the top of the hood volume. Also, the blower motor must be explosion-proof (non-sparking). Since many of the chemicals we use are flammable or have heavier than air fumes, we likely have fume hoods like this in our labs. But this is not always true, especially in older buildings.

Downloadable Photoshop Convolution Plug-In

Photoshop is a widely used program for the acquisition, processing, annotation and printing of scientific images, and is frequently discussed on the microscopy listserver. It includes a custom convolution filter that allows users to enter a 5x5 array of integer weights that are multiplied by pixel values to produce smoothing, derivatives, high pass filters, and other useful effects. The interactive preview gives immediate feedback on the results, which is a great assist to learning about convolution kernels in general, and the operation can also be scripted in Actions for one-button application to images or for batch processing.

Unfortunately, the Photoshop "Custom" filter has a few significant limitations:

1. Only works with 8 bit per channel grey and RGB images.

2. Applies the kernels separately to the RGB channels.

3. Is limited to a 5x5 array of integer coefficients, with integer scaling.

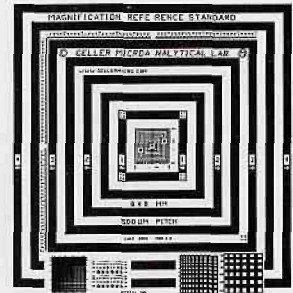4. Saves and loads files in a special binary format.

We've written and are offering for free download and use a very much enhanced version of this "custom" filter. Like the Photoshop one, it has an interactive preview, and is recordable in Actions. In addition, the plug-in:

1. Supports both 8 and 16 bit per channel grey and RGB images.

2. Works on the intensity channel leaving hue and saturation unchanged.