



MICROSCOPY 101

We appreciate the response to this publication feature - and welcome all contributions. Contributions may be sent to Phil Oshel, our Technical Editor at:

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Plunge-Freezing into Slush Nitrogen

Questions keep appearing on the various microscopy list servers about the pros and cons of different cryogenics for plunge-freezing in liquid nitrogen. We at Microscopy Today have run articles on this ourselves. There are serious safety concerns about these cryogenics, including disposal*. While most of us have safely frozen specimens with these cryogenics, 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 cryogenics condensing oxygen out of the air, making for an even more explosive mixture.

To make slush nitrogen:

Fill a 600mL or 1000mL beaker with LN₂, 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 LN₂ will start freezing over and breaking up as bubbles of N₂ gas surge through. Keep pumping. Soon, there will be a mix of liquid & solid N₂. Stop. Pull out, place on an insulating pad, and plunge-freeze the samples, dropping them into a container previously placed in the LN₂. Plunge-freeze until all samples are done. Time is a problem with this method, as the LN₂ is warming up, and in some minutes the nitrogen will be at the usual near-boiling-point temperature of LN₂, 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 LN₂ 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 cryogenics (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.

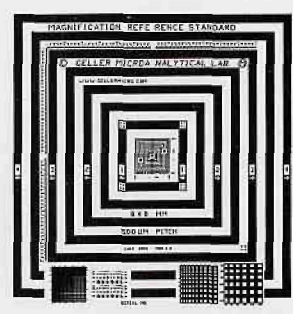
We've written and are offering for free download and use a very much enhanced version of this "custom" filter. Like the Photoshop one, it has an interactive preview, and is recordable in Actions. In addition, the plug-in:

1. Supports both 8 and 16 bit per channel grey and RGB images.
2. Works on the intensity channel leaving hue and saturation unchanged.

MRS-4

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- 3. Accepts up to a 7x7 array of floating point (real number) values, for much greater precision.
- 4. Can scale the results with a floating point number, or automatically for maximum unclipped contrast.
- 5. Reads Photoshop format files AND ALSO plain ascii text files.
- 6. Works in Photoshop and all compatible programs on both Mac and Windows computers.

Encouraged by the recent response by readers of the microscopy listserver to our offering of a free plug-in to draw magnification bars on micrographs, we are offering this plug-in for free download and use. It is one of nearly 200 plug-ins in the widely used Fovea Pro package, but can be used without installing or owning that package.

The plug-in, along with instructions and some example filter files, can be downloaded as a .sit file for Macintosh or a zip file for Windows from:

<<http://www.reindeergraphics.com/free.html#custom>>.

While you are there, please take a look at the full range of Fovea Pro capabilities, which include comprehensive tools for image processing and analysis. Also, check out some of the other free downloads that are available.

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A Home-made Antifade Medium for Fluorescent Dyes

Most antibody companies (*i.e.*, Dako, Vector, Molecular Probes) sell a medium which contains an antifade medium. This can also be easily prepared in one's own lab. Here is my favorite formulation, which also contains DAPI (Blue fluorescent nuclear counterstain), but it can be left out.

25 mg/mL 1,4-diazabicyclo[2.2.2]octane (DABCO) with 0.5mg/mL 4,6-diamidino-2-phenylindole (DAPI), in 50% glycerol/phosphate buffered saline, pH 8.6.

DABCO is dissolved in 100% glycerol then double-strength phosphate-buffered saline at pH 8.6 is added in equal volume to the glycerol. The DAPI can be added from a higher concentration solution stock which can be stored frozen. All reagents can be purchased from Sigma Chemical. pH is very important.

(Note: mention of a vendor does not constitute endorsement, I just use these particular products.)

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