HCBI Recommended Mounting Media

When imaging, it is important to match the refractive index of your mounting media as closely as possible to the refractive index of your imaging objective's immersion medium **(Water – 1.33, Glycerol – 1.47, Oil - 1.518)**. Here is a list of standard mounting media and their refractive indices:

75% Glycerol – 1.44
Vectashield – 1.457
Fluoromount G – 1.40
Mowiol – 1.49
ProLong Gold – Fresh mounting: 1.39
Cured for 1 day: 1.40
Cured for 4 days: 1.44

As you can see, none of these media are optimal for either water or oil immersion objectives (the most common objectives found in the HCBI). Therefore, mounting media should be carefully selected based on your chosen objective.

Reasons to choose a water immersion objective:

1) You are imaging live cells/tissue/organisms in an aqueous buffer on an inverted microscope (most often in chambered wells or 35mm dishes with a #1.5 coverslip bottom).
2) You are imaging thick, fixed samples that still contain, or were fixed in, aqueous buffer (often small organisms such as Zebrafish, C. Elegans, etc.) between a slide and coverslip on either an upright or inverted microscope.

Mounting medium for water immersion objectives:

1x PBS or other aqueous buffer/medium. Avoid media containing phenol red. Addition of an anti-fade agent may be helpful.

Reasons to choose an oil immersion objective:

1) You require the highest resolution possible.
2) You want to mount your sample in something more stable/solid than water for longer-term imaging/storage.

Mounting medium for oil immersion objectives:

All media listed above are usable; although none are optimal. Mowiol is the closest match to the HCBI's immersion oil (n=1.518).

TDE an adjustable refractive index mounting medium:

2,2'-Thiodiethanol (TDE) has recently been described as an excellent mounting medium, especially for high-resolution imaging. TDE has been tested and shown to work well with HCBI high NA oil immersion objectives. Please reference the following publication for a step-by-step protocol of how to prepare your samples using TDE (must be applied in a step-wise fashion!): http://goo.gl/UzUP6n

To prepare 10mL of TDE mounting Media the HCBI recommends:

9.7mL TDE
240uL DABCO (anti-fade agent)
160uL PBS

Ensure pH is ~7.5

TDE can be purchased here:
http://www.sigmaaldrich.com/catalog/product/aldrich/166782?lang=en&region=US

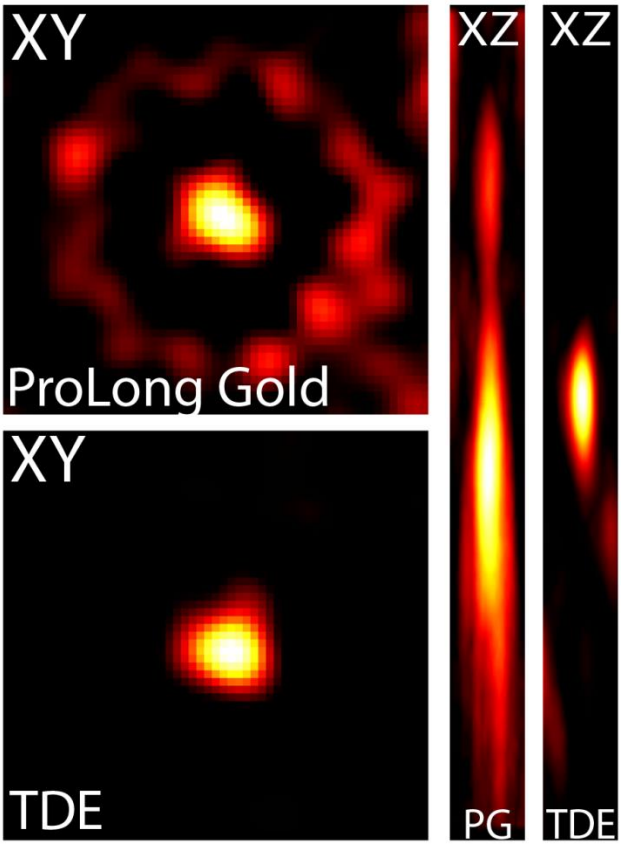
DABCO can be purchased here:
http://www.sigmaaldrich.com/catalog/product/aldrich/290734?lang=en&region=US

When not to use TDE:

As TDE is solvent based, some dyes perform better, others worse, and some are unchanged (See Table 1, Staudt et. al, linked above). TDE is not be a good choice for cells expressing GFP (however, it is fine for all red fluorescent proteins), or cells stained with Alexa 488. TDE is an excellent choice for cells stained with Cy3 or Alexa 647. TDE is a poor choice if you are performing phase contrast or DIC imaging, as the refractive index mismatch aids in producing enhanced contrast with these imaging modalities.

Sample data:

The following data were acquired on the HCBI's ELYRA super-resolution microscope in 3D-Structured Illumination (SIM) mode. Frozen sections of gastric tissue were stained for Zo-1 with Alexa 647 and mounted in ProLong Gold or 97% TDE. A number of spherical aberrations were observed around bright objects (and unfortunately, enhanced with SIM processing) when mounted in ProLong Gold. Mounting in TDE resulted in an approximately 3 fold axial resolution enhancement.



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