Imperial College London

Facility for Imaging by Light Microscopy

Observing Life As It Happens

PROTOCOL: OPTICAL CLEARING OF SAMPLES

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Background

Biological samples, especially tissue samples, consist of a variety of components that have different refractive indices (RIs). This leads to diffraction and optical aberrations in your sample and reduces image quality. The most common way to solve this problem is optical clearing of the sample. This is done by exchanging the water in the sample, which usually has a comparably low RI, with a substance that matches the RI of the proteins and lipids. By that you get a sample with just minor local changes in RI and thus much reduced diffraction and aberration. Your image quality, especially with 3D datasets, will be much higher. Ideally, you also want to get a refractive index that matches the one of the immersion medium you use for imaging (1.33 for water, 1.43 for glycerol and 1.51 for oil).

There are numerous substances that can be used for optical clearing. Below are some of them described.

Large parts of the information provided in this document is compiled from posts at the confocal mailing list (http://lists.umn.edu/cgi-bin/wa?A0=CONFOCALMICROSCOPY) and the microscopy mailing list (http://www.microscopy.com/MicroscopyListserver). Thanks to everyone there for all the help and advice.

Xylol/ Toluol/ Methyl salicylate

Classically used for paraffin sections they can - after dehydrating the sample through a series of alcohol steps - also be used to clear a sample before mounting it in DePeX or the like. You can also mount directly in Methyl salicylate. Methyl salicylate should also work for fluorescent samples, while the others reportedly damage fluorescence. If done properly, the RI (ca. 1.50) about matches the RI of immersion oil (1.51).

BABB (aka Murray's clear)

BABB is a 1:2 or 1:1 mixture of benzyl alcohol and benzyl benzoate. By changing the ratio you can kind of adjust the RI between 1.53 and 1.57.

Involves dehydration, is hazardous and does not work with most fluorophores. BABB is frequently used for whole mount samples.

You dehydrate in a series of alcohols, from 100% ethanol go to several washes of BABB (http://www.ncbi.nlm.nih.gov/pubmed/16060974). Another possibility is to dehydrate your sample in methanol and then switch to BABB (http://www.ncbi.nlm.nih.gov/pubmed/17051584).

BABB is also the clearing agent used in so-called ultramicroscopy (see Dodt, et al., Nature Methods 4, 331 - 336 (2007).

TDE

Thiodiethanol is mixable with water and the RI can be adjusted from 1.33 (0% TDE) to 1.52 (100% TDE) via the water/ TDE ratio. It is known to quench EGFP but there is no quenching reported with other fluorophores. See Staudt et al. (Microsc. Res. Tech. 70:1–9, 2007) for further information. In our hands it worked really well but you have to plan for long penetration times if working with thick samples or arthropod/ plant specimen.

You usually start to immerse with 10% TDE in water, then go to 25%, 50%, followed by 3 times your final concentration (70% to match Glycerol, 97% to match oil). For cells you incubate for 5-10min each step. For tissue 1h or longer, for tissue with cuticular structures 1 day per step is recommended.

TDE is available from the facility for trial purposes. Please ask if interested.

Chloral Hydrate/ Lactic Acid/ Glycerol/ Gum Arabic

Several mixtures of all or some of these components in water can be found in the literature as modifications of Hoyer's mounting medium. They are most commonly used for the clearing of plants and small arthropods. Especially the mixtures containing chloral hydrate are reported to have good penetration properties. However, choral hydrate is a sedative and a hypnotic drug so its distribution is regulated under narcotics laws. The RI of the mixtures differs from 1.4 to 1.52.