Specimen Quality

Slides/fixed specimens

In Bio-imaging most of the objectives have Korr=170um. Please use coverglasses number '1.5' as they actually have a thickness of 170um. However, we do have a few objectives which have adjustable collars for coverglasses with different thickness.

Use coverglasses at least 18 or 22mm size because they are big enough to accommodate the drop of oil without it spreading and mixing with nail polish sealant.

The optics are designed to focus just below the coverglass, so you should mount your specimens on the coverglass rather than the slide. However, if this is impossible, then use as little mounting medium as possible, to minimise the distance between the specimen and the coverglass.

If your specimens are mounted in buffer, you should seal the edge of coverslip with nail polish to prevent liquid spilling onto microscope. Do not get any nail polish on the centre of the coverslip, or any immersion oil on the edge, or the two will combine to make a nasty emulsion which is bad for lens and image quality. Note: slides prepared this way are *temporary* and should really be thrown away as soon as you have collected your images. If you must re-use them, then please do NOT bring them back covered in old immersion oil. Use a jet of 100% ethanol to clean the oil and dirt off the back and front of slide then air dry.

A better solution (especially if doing oil immersion) is to mount specimens PVA-based liquid mounting medium which will set solid.

Vectashield hard set mounting medium H 1400 vector laboratories

This will prevent the objective dragging the coverslip around by mistake. In addition, if cells are mounted this way, you can immerse the entire slide into 100% ethanol to clean off oil or dirt before storing for future sessions, so slides are more *permanent* and *re-usable.* Do NOT use slides covered in old immersion oil.

If you are viewing thick specimens, you may like to use a *spacer* to prevent squashing, either commercially available or home-made from electrical tape.

If you *are* using oil immersion, then the safe way to apply the oil is to take the stage down, unscrew dropper and remove top, allow first drop of oil to drip back into bottle, then drip second drop of oil centre of field (use transmitted light beam to work out centre of field). Try not to touch the coverslip with the dropper. Finally bring stage back up again.

Hi,

I would like to remind all users that the best results will be obtained on all our light microscopes (including LSMs) by the use of No. 1.5 coverglasses (170um thick). This is particularly important for lenses with high numerical aperture (air and immersion). It is sometimes tempting to use a thinner (e.g. No. 1) coverglass when the sample is mounted directly onto a slide, with a significant amount of mounting medium between sample and coverglass. This is not recommended where, with additional care, a thinner mount can be prepared resulting in the sample being close to the coverglass - or ideally just touching it. Cover glasses other than No. 1.5 should not be used with aqueous mounted specimens.

The only situation where the coverglass thickness can be relaxed with no subsequent aberrations is when using an oil immersion lens and mounting the sample in a medium with a refractive index around 1.52. Glycerol-based mountants don't really meet this criterion, but compounds such as DPX (RI
1.523) will be much better.

Specimen Quality

Live cells

We prefer cells to be grown on glass than plastic, because most of the objectives have Korr=170um which assumes a coverglass 170um thickness. Use glass bottom dishes (e.g. WillCo-dish or MatTek microwell dishes) either uncoated or coated with collagen or poly d-lysine.