

Transcript: Sample mounting and refreshing media on the 3i Lattice LightSheet

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This tutorial will guide you through mounting a sample and refreshing the media during your imaging session.

Before mounting, prepare your station with tweezers, a tissue, vacuum grease and a small pipette tip. Make sure your sample holder is clean and dry. If working at 37 degrees, prewarm your sample holder in an incubator. This will reduce the time needed for equilibration before imaging. It is worth practising mounting as being quick will help to keep everything warm.

Using the pipette tip, place a small amount of vacuum grease around the edge of the central hole.

Try to distribute it evenly and avoid lumps which could get on the objectives.

Using tweezers, pick up a coverslip and dab off any liquid from the bottom surface with a tissue. Place the coverslip on the holder and use the tweezers to firm down around the edges, pressing the coverslip into the grease.

If imaging cells from the incubator, be sure to promptly return them.

Lower the sample chamber by pressing 'Load' in SlideBook.

To prevent a bubble forming underneath the holder tilt it such that the long edge enters the media first. Then level the holder before carefully laying on the piezo stage. Take care not to press the piezo here.

Add screws in two diagonal corners and tighten with the allen key. These should be finger tight and not screwed down with force. If you hear a high pitched squeak back off, that is too much pressure!

Ensure that the media wets the coverslip and if not, add a drop more media. You should be able to see through to the dark aperture of the finder objective underneath. If it looks white instead, this is probably a bubble and you will need to take the sample holder out, remove the bubble and remount.

Press 'Image Home' in SlideBook to close the bath.

When you are ready to remove your sample, click 'Load'.

Undo the screws and remove them. Leave them in a safe place in a tray or dish. Remove the sample holder and close the box door. press 'Image Home' in SlideBook while you mount a new sample. This is important to keep everything warm.

Wipe your old sample off with a small piece of tissue and dispose of it in the correct biological waste stream.

Repeat mounting with fresh grease and a new coverslip.

If you feel like you need to refresh the media between samples, press 'Escape' in SlideBook.

Undo the grub screws on the bath, these only need to be backed off one quarter of a turn, do not remove them.

Carefully lift out the bath and tip the media into a beaker. Rinse the bath with deionised water if necessary. Remove any drops of water with a Kimwipe tissue taking care not to touch the glass coverslip. Only wick from the edges and corners.

Return the bath and tighten the grub screws.

Refill with 10 mil of prewarmed media.

I hope that this video has been useful, please email CAMDU@warwick.ac.uk if you have any questions or comments.

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