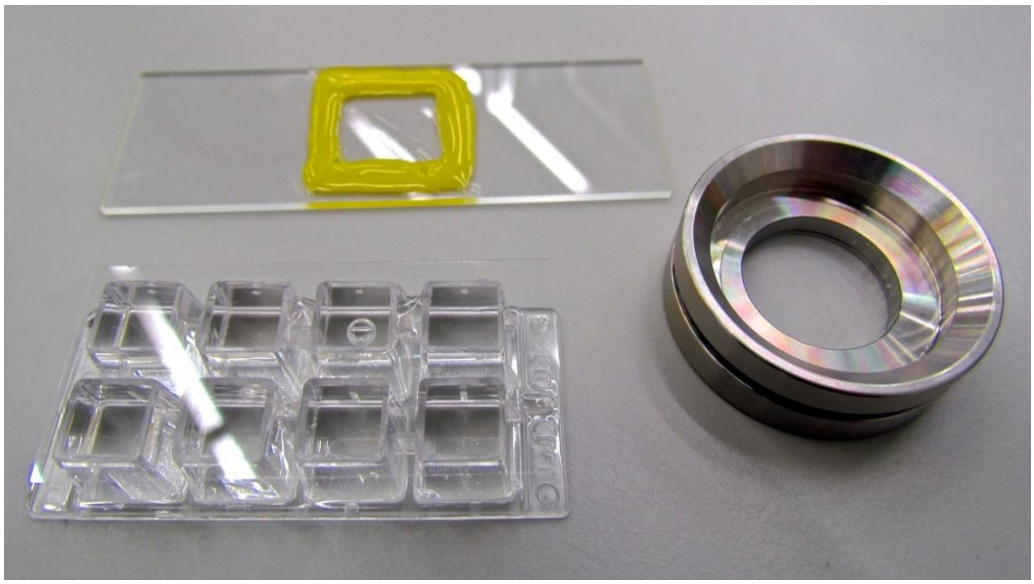
Localisation Microscopy Coverglass Guide

This guide describes 3 different sample formats suitable for (d)STORM, PALM, GSDIM and similar super-resolution microscopy techniques where sample immersion in an aqueous buffer is necessary. In particular this mounting guide is intended for use with dyes or fluorescent proteins that require the use of an oxygen scavenging switching buffer, for example Alexa 647 in glucose oxidase with MEA. Imaging should be possible for 2 to 8 hours without needing to change the switching buffer.



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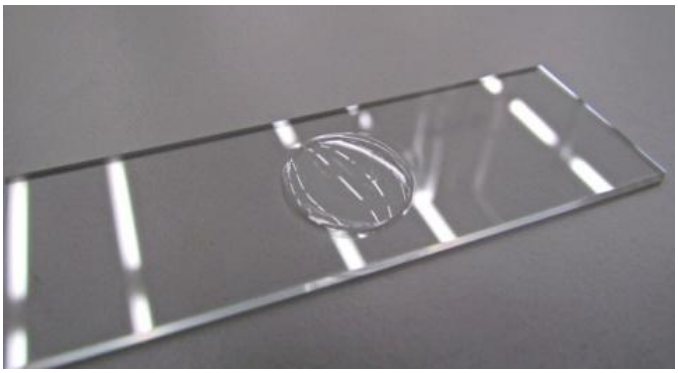
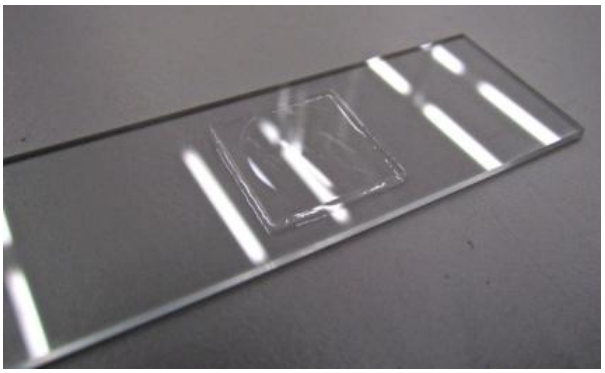
LabTek Chambered Coverglass

1. Fill the chambers to the top with switching buffer (approximately 850 µl).

2. Place a coverslip on top of the chambers ensuring that there are no bubbles inside.

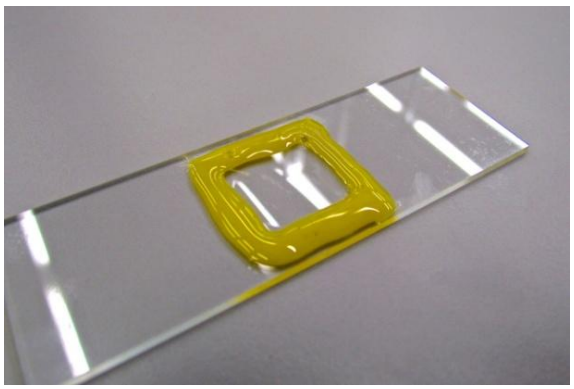
Coverslips with Cavity Slides

1. Add approximately 150 microlitres of switching buffer to the cavity and place inverted coverslip (18 – 24 mm diameter circular or square) onto it, with sample side facing down. Push lightly down into place with a pair of forceps or a cocktail stick. Carefully wipe away excess buffer.

2. Mix silicone adhesive components A and B together in a 1:1 ratio and then carefully apply with a cocktail stick around the edges of the coverslip. Wait 15-30 minutes for it to set firmly before imaging.





Tip: Try and keep the amount of silicone adhesive to a minimum. This results in quicker setting & reduces the chances of the objective lens pushing against the silicone when imaging.

Coverslips with Attofluor Cell Chamber

1. Insert 24 mm diameter circular coverslip into cell chamber with sample side facing up.

2. Screw top section down onto coverslip gently to form a seal with red O-ring.

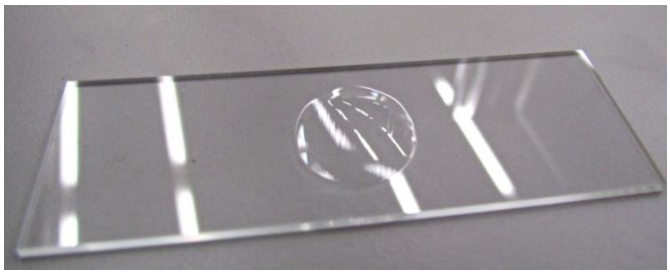
3. Fill with switching buffer, approximately 1.5 ml, and place another 24 mm diameter circular coverslip over the top. Press gently down with forceps or a cocktail stick ensuring that there are no bubbles. Carefully wipe away excess buffer.

Product References

LabTek chambered coverglass 8-well (#1 thickness)
Fisher – 11377694*

Attofluor Cell Chambers
Invitrogen – A-7816

Microscope slides with single cavity
Fisher – 12312158



Silicon adhesive (Exaxtosil N21)
Bredent UK, 54001147

Certain commercial materials, instruments and equipment are identified in this document in order to specify the experimental procedure as completely as possible. In no case does such identification imply recommendation or endorsement by the National Physical Laboratory, nor does it imply that the material, instrument or equipment identified is necessarily the best available for the purpose.

*There is a similar product with #1.5 thickness glass available (Fisher – 12812794). Be aware this has a plastic tab on the end of the chamber which is incompatible with some sample holders. It can be cut off with sharp scissors or a scalpel if careful.

Glass thickness

In most cases coverglass thicknesses of #1 or #1.5 will be suitable for use with high NA oil immersion objective lenses. Match your objective lens, glass thickness and oil refractive index!

Coverglass thickness can vary. Correction collar adjustment is recommended. If there is a mismatch localisation algorithms will not be able to localise molecules as accurately due to spherical aberration. This results in super-resolution images with worse resolution and fewer localisations in the final image.

Number	Thickness (mm)
0	0.08 – 0.13
1	0.13-0.16
1.5	0.16-0.19
2	0.19-0.25