Preparation of general use bead slides

- Use good quality, clean slides and no. 1.5, 22x22 mm coverslips with a nominal thickness of 170 μm (160 to 190 μm).
- Treat coverslip with poly L-lysine solution by applying 200 μL to coverslip and spreading it with pipette tip. Leave for 10 s and remove by washing with distilled water, tilt coverslip and leave to dry.
- Use NileRed beads 0.2 μm for assessing microscope alignment or Red/Green channel registration.
- Vortex beads and make solutions of 200 μl at 1:500 to 1:1000 for NileRed alignment test slides.
- When preparing beads you can avoid clumping by sonicating. Sonicate a sample of stock for 3 min, vortex and dilute and vortex again.
- Apply 100μL per coverslip (or coverlip and slide for a two layer preparation) spreading the beads as much as possible. Leave to settle for 30 min to adhere to the polylysine and then remove as much liquid as possible with a pipette. Leave another 5 min when a dry film should have formed around the edge and then remove remaining liquid by tilting the coverslip and sucking the solution off with tissue paper.
- Leave to dry completely in a tilted position.
- Mount on glass slide with approx. 11-15 μl of mountant e.g. Vectashield, glycerol or Prolong Gold. Formation of air bubbles as you place the coverslip are best prevented by placing a needle under one side of the coverslip and slowly withdrawing it as you lower into position.
- Double seal the slide with nail varnish. For Prolong Gold, cure as appropriate, usually overnight at room temp. before sealing.

Preparation of PSF bead slides

- Use good quality, clean slides and no. 1.5, 22x22 mm coverslips with a nominal thickness of 170 μm (160 to 190 μm).
- Wash the coverslips thoroughly in ethanol and then in MilliQ water in a clean plastic Petri dish. Handle only with clean tweezers.
- Use red beads 0.1 μm (0.11 μm) and yellow green beads 0.1 μm (0.1 μm) for PSF measurements at dilutions where single bead can be found in a field of view.
- Vortex beads and make dilute solutions of 200 μl in MilliQ H_2O: 1:10,000 or below will be good for PSF measurements. Apply up to 100ul, spread with the Gilson tip and allow to air dry in a clean dust free environment (e.g. a cupboard)
- Alternatively, Spread 1 μL of neat 100 or 170 nm PSF bead solution as thinly as possible on the coverslip using the side of a 200 ul Gilson tip, and allow it to dry in a clean dust free environment. This gives a mixture of sparse spread beads and bright puddles of dense beads which can assist in finding the focal plane.
- Mount on glass slide with approx. 13 μl of mountant e.g. Vectashield, glycerol or Prolong Gold. Formation of air bubbles as you place the coverslip are best prevented by placing a needle under one side of the coverslip and slowly withdrawing it as you lower into position.
- Double seal the slide with nail varnish. For Prolong Gold, cure as appropriate, usually overnight at room temp. before sealing.
- Ideally for PSF measurements there should be single isolated beads with a field of at least 256x256 pixels. To measure a PSF there should be no contamination of the single bead image with Airy rings from nearby beads.