

## OMX 3D-SIM SAMPLE PREPARATION

(adapted from [http://www.imb.a-star.edu.sg/imu/instructions/OMX\\_sample\\_prep.pdf](http://www.imb.a-star.edu.sg/imu/instructions/OMX_sample_prep.pdf))

- The **coverslips** must be **type #1.5**, which is 170  $\mu\text{m}$  thick. If you are not sure what you have - do not use them! We can give you some of the correct type to get you started.
- The slides and coverslips must be very **clean** (no dust, residual oil, salt, etc).
- The coverslip must be mounted in the **exact centre of the slide** (the distance from the center of the coverslip to both short ends of the slide must be the same).
- Only **one coverslip** per slide
- Coverslips must be **sealed on all sides** (no leakage) with nail polish or another solid sealing agent.
- The sample has to be mounted **on the coverslip surface** (or within 16  $\mu\text{m}$  of it) - not the slide surface.
- You should use a **mounting medium** with an antifade agent, but without DAPI. Examples that have worked well for us or others on the OMX:
  - Prolong Gold (Molecular Probes/Life Technologies), hardening
  - Vectashield H-1000 (without DAPI), non-hardening
  - You can make your own mounting media, a suggested recipe can be found at <http://mitchison.med.harvard.edu/protocols/gen1.html>.
- For SIM on our OMX you can use **fluorophores that excite with 405nm, 488nm, 568nm, or 642nm**. The fluorophore must be bright and photostable.
- FITC, TRITC, Cy3, Cy5 etc do not work well as they bleach too fast.
- Fluorescent proteins can be used, but if the sample is fixed, better results can be achieved by **staining the FP with an anti-FP antibody** and labeling with an Alexa488 conjugated secondary antibody.
- Bring **freshly prepared samples** and, if possible avoid freeze-thaw cycles.
- In terms of dyes/fluorophores, **good signal and low background** is essential. Especially background speckles can make the data processing difficult.
- **Objects with structure** (filaments or concentrated spots) work well in the OMX, objects with diffuse labeling generally do not.