**Coverslips:**

Only #1.5 coverslips (170 µm) should be used.

**Mounting media:**

The choice of mounting medium can severely affect the imaging results.

* We recommend as a non-setting for of VectaShield – **VectaShield H1000**
* Avoid mounting media that solidify (e.g. hard setting Vectashield, Mowiol® 4-88 and ProLong Gold), as these media tend to shrink and flatten the sample, resulting in a loss of volume information. Also avoid Citifluor or DAKO mounting media
* Avoid mounting media that contains DAPI. This creates background in the image that can easily be avoided by staining for DAPI during the IF labeling and washing off.
* Other mounting media can be used, these include:
  + 90% glycerol with some anti-fade agent
  + Murray Antifade Mounting Media
  + nPG (n-propyl gallate) Antifade Mounting Media
  + PPD (P-phenylenediamine) Antifade Mounting Media
  + Antifade 1: 1,4-phenylene-diamine
  + DABCO (1,4 diazobizyclo[2,2,2]octane) Antifade

**The sample:**

* SIM works best close to the coverslip and best results are typically within the first 18µm. Imaging deeper is possible but after 25µm image quality deteriorites rapidly. Imaging in tissue usually gives poor results unless the tissue has been thoroughly cleared.
* Imaging plated cells and bacteria often yields great results. With good labelling and bright secondaries (e.g. Alexa488, 568, 647) you should expect excellent images.
* Imaging in transparent organisms has been succesful, but more challenging. SIM in C.elegans has given good results