

# Sample Preparation Guidelines for Superresolution Structured Illumination Microscopy (SRSIM)

## Fluorophore Selection

The SR-SIM is equipped with 405, 488, 561, and 635 laser lines. All traditional fluorophores (GFP, mCherry, Alexa dyes, Cy dyes, Mitotracker, DAPI, etc.) are compatible with the system. Care should be taken to maximize the brightness of the fluorophores, either by testing expression levels or antibody staining protocols. Selection of robust fluorophores is strongly recommended. In general, if a sample appears dim or is easily bleached by a traditional wide field or confocal system, SR-SIM will be limited in its ability to reconstruct a superresolution image.

## Sample Thickness

Thicker samples will provide more of a challenge for SR-SIM imaging. The instrument can be expected to work well with sample thicknesses of up to 5 to 10 microns. Deeper imaging may be possible, depending on the refractive properties of the sample and the intensity of the fluorophores. Please be aware that the deeper you attempt to image, the more difficult it will be to achieve a superresolution image.

## Use of Proper Coverslips

Most ZEISS oil or water lenses are designed to image through 170 micron thick coverslips. Most commercially available coverslips can range in thickness up to 12%. The upper end of this can cause a severe reduction in resolution. To avoid this, Carl Zeiss now produces high performance coverslips which vary in thickness by no more than 2.9%. Use of these coverslips is strongly recommended for preparing samples for either traditional fluorescence imaging or superresolution. You can order these coverslips from your local Sales Consultant or online at [www.micro-shop.zeiss.com](http://www.micro-shop.zeiss.com), part number 474030-9000-000.

Use at most only two coverslips per microscope slide. Do not have the coverslips flush with the edge of the microscope slide. The microscope slide needs to fit into a piezo insert and will not fit if the coverslips are fixed near the edges.

## Use of Proper Mounting Media

Mounting media should be used that has a refractive index as close as possible to the coverslip (1.52). We have successfully used the following products:

Vectashield Mounting Medium from Vector Labs (Item Number H-1000), which has a refractive index of 1.44, works well for SR-SIM. This is a non-hardening media so you must be sure to completely seal the coverslip with something like nail polish. Prolong Gold (P36930) from Invitrogen has a higher refractive index, 1.46, but this is after 160 hours of curing. It must be cured for 60 hours to reach a refractive index of 1.44. As it is a hardening media, you may also notice it can somewhat flatten samples. Fluoro-gel, with TES buffer (17985-31) from Electron Microscopy Sciences has a refractive index perfectly matched to glass at 1.52. This is also a hardening media and must be allowed to cure for the correct length of time. Although it has the highest refractive index, and makes it ideal for imaging, it will also harden even more so than the Prolong Gold. As such, it may cause flattening of samples.

In general, other mounting media can certainly be used, but take care of the refractive index. As the closer the refractive index is to glass, the more likely it is to flatten samples, it may be required to test which mounting

media is best for the samples. Also take care to not use mounting media with DAPI mixed in; this can cause general autofluorescence which will interfere with superresolution imaging.

**Common problems resulting from mounting media include:**

Not removing the wash media sufficiently prior to mounting. This can mix with the mounting media and cause changes in the refractive index or problems in hardening.

Not properly sealing the coverslip if the mounting media is non-hardening. The mounting media can leak out and mix with the oil. Nail polish can be used to completely seal the coverslip.

Not allowing a hardening mounting media to completely cure. Artifacts will occur from imaging into a sample with a lower refractive index. Refer to the manufacturer's instructions for how long a given mounting media must cure.

Trapping bubbles in the mounting media. Bubbles can refract light and cause artifacts.