Sample Preparation Guide

Nikon's N-SIM system can provides a 2x increase in the resolving power of a diffraction limited fluorescence microscope. It can provide up to 85 - 120nm lateral resolution and 300 – 400nm axial resolution.

It does not require z stacks, and can get rid of out of focus light with one single image when doing 3D SIM. The fastest acquisition times are .6 sec for 2D/TIRF SIM and 1.0 sec for 3D SIM.

It has 6 lasers and emission channels: **405nm**, **445nm**, **488nm**, **515nm**, **561nm** and **647nm** and will work with all fluorophores that can be excited with these lasers as long as they are bright enough.

Cover Glass

For the optimal results samples must be prepared correctly. All samples must be mounted on #1.5 coverslips (170um thickness). Maximum sample thickness recommendations: 60x 3D SIM < 20um, 100x 3D SIM < 7um, 100x 2D SIM < 3um.

Slides & Dishes

We recommend that samples are mounted on regular sized slides for fixed tissue and 35mm dishes with cover slip bottoms or Nunc LabTek II Chambered Cover glass with #1.5 cover glass.

Mounting Media

For the 100x/1.49 NA oil lens we recommend that fixed slides are mounted in Prolong Gold[1], TDE[2] (2,2'-Thiodiethanol) or glycerol (not Vectashield!). Prolong gold takes 48 hours – 1 week to fully cure and increase to the correct refractive index so best results will be obtained after 48 hours. TDE can be mixed with water to match oil exactly.

For the 60x/1.27 NA water lens we recommend water or agarose.

Do not hesitate to ask if you have any questions!

[1] http://tools.lifetechnologies.com/content/sfs/manuals/mp36930.pdf

[2] Staudt T, Lang MC, Medda R, Engelhardt J, Hell SW. 2,2'-Thiodiethanol: A New Water Soluble Mouting Medium for High Resolution Optical Microscopy. *Microsc. Res. Tech.* (2007) 70:1-9 PMID: 17131355