Sample preparation guide for Super resolution Microscopy SIM (ELYRA PS.1)

Coverslip:

we recommend to use **high-precision cover glasses with thickness no. 1.5** to prepare the samples. (= $0.170 \text{ mm} \pm 0.005 \text{ mm}$).

e.g.: Marienfeld Superior: www.marienfeld-superior.com

- 18x18 mm (Cat.No. 0107032).
- 22x22 mm (Cat.No. 0107052).
- round with 18 mm diameter (Cat.No. 0117580).

Zeiss 474030-9010-000 Deckgläser 1,5H hi 100002240928 QS; Thickness no 1 $\frac{1}{2}$, high-performance 18 mm x 18 mm, 0.170 +/- 0,005 mm

Fix your cells according to your preferred protocol.

Please make sure that the cover slip is centered on the glass slide, in order to fit in our samples holder

Lasers lines HR (High Resolution) solid laser

- 405 nm. 50 mW
- 488nm, 100 mW
- 561 nm, 100 mW
- 642 nm. 100 mW

Band pass and filter for emission

- BP 420-480 + LP 750
- BP 495-575 + LP 750
- BP 570-650 + LP 750
- LP 655

Fluorescent Labels for SR-SIM

For SR-SIM (superresolution - structured illumination microscopy) any type of antibody labeling or fluorescent protein expression is possible. Make sure to have a highly specific labeling **as well as a good and high signal to noise ratio.**

Mounting media:

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.

Do not use mounting media that include DAPI (too strong concentration that can not be control)

When mounting media will be dry - Seal your coverslip with a transparent nail polish-let it dry- keep at 4°C

In order to have stable imaging conditions, especially concerning the refractive index (RI) of the mounting medium we would urge you to prepare the slides at least one week before the demo.

For further reading please check:

http://www.nanoimaging.de/ (→ homepages → Sample requirements)