Structured Illumination Microscopy

Recommendations for sample preparation

SIM is a super resolution technique that improves resolution by a factor 2. SIM is well suited for samples with **well-defined structures**, and not for diffuse labeling. Sample preparation requires minor adjustments compared to confocal microscopy.

Glass coverslips and dishes

- Coverslips must be #1.5HR. High precision cover glass is recommended (0.17 ± 0.005mm). See appendix for references of coverslips and glass bottom dishes.
- Coverslips and slides must be **clean** (no dust, oil, salt residuals…). Clean for example your coverslips in water, and store them in 100% ethanol. Air dry them before use.
- Mount **only one** coverslip per slide and keep the edges free.
- Make sure the coverslip is centered on the slide. See Fig 1.
- If using slides with a frosted end, use the unfrosted side (the sample will have to be as flat as possible).

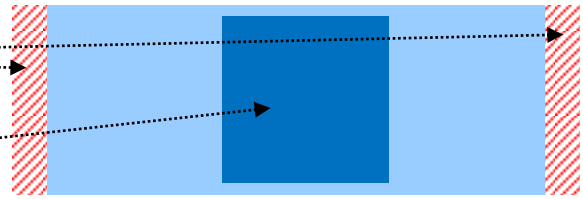

Fig 1.

Mounting

- Use a mounting medium **without DAPI**.
- The mounting medium should contain anti-fading agents
- The refractive index of the mounting medium should match the objective. For example:
  - Non-hardening VECTASHIELD Mounting Medium with RI of 1,44.
  - ProLong Gold (Life Technologies). Refractive index (RI) of the cured product:1,46.
- Be aware that hardening media might distort your samples when curing.
- In order to have stable imaging conditions, especially concerning the RI of the mounting medium, prepare the slides at least one week before use.

- Pre-incubation of your coverslips with mounting medium could prevent RI variations in your sample. Incubate your coverslip on a drop of mounting medium for 2 min. Dab the diluted mounting medium from your slide and proceed to mounting.
- Coverslips must be sealed on all sides with nail polish or another solid sealing agent.
- The sample should be as close as possible to the coverslip. SIM works best in thin samples. When working with sections, keep them thin. Consider having the section on the coverslip rather than on the slide. Cleared tissues could be an advantage for SIM, as they scatter less the light.

Staining

- Sample quality is critical for SIM. General problems are:
  - Photobleaching
  - Lack of local contrast
  - Unspecific background
  - Excess of scattering due to sample thickness
- Optimize your fixation protocol
  Check for example protocols used in Electron Microscopy as a lot of work has been made into sample preservation.
- Optimize staining procedure to get a good signal to noise ratio. Wash a lot.
  Staining protocols for dSTORM might be used as an inspiration as a lot of optimization has been made.
- Choose a primary antibody that localizes strongly to the structure you are interested in. Consider spinning your stock solution to avoid precipitates
- Consider post-fixation to prevent floating particles and diffusion of antibodies from their epitopes as they would generate artifacts.
- Choose fluorophores that can be excited by 488nm, 561nm, 642 nm (and 405nm).
- Fluorophores should be as photostable as possible.

Lasers	Example of dies and Fluorescent Proteins
488 nm	Alexa fluor 488 / GFP
561 nm	Alexa fluor 568. Red fluorescent proteins tend to bleach faster
642 nm	Alexa Fluor 647
(405 nm)	“Blue” dies bleach extremely fast. DNA staining with 2ug/mL of DAPI may work.

Appendix

Coverslips

- Marienfeld Superior: www.marienfeld-superior.com
  - 18x18 mm (Cat.No. 0107032).
  - 22x22 mm (Cat.No. 0107052).
  - 18 mm diameter (Cat.No. 0117580).
- CellPath Ltd UK High performance coverslips
  no 1.5H 18x18 mm (Cat.No SAN-5018-03A)

Glass-bottom petri dishes and multi-well plates

- Willco (http://www.willcowells.com/)
- Nunc Lab-Tek II (offered through Thermo)
- MatTek (http://glass-bottom-dishes.com)

Mouting media

- Fluoromout-G (SouthernBiotech) with RI of 1,40.
- Non-hardening VECTASHIELD Mounting Medium with RI of 1,44.
- ProLong Gold (Life Technologies). Be aware this embedding medium needs 2 day to harden (in order to reach a constant RI). In addition your sample may shrink during this process. Refractive index (RI) of the cured product: 1,46.
- SlowFade Gold (Life Technologies) with RI of 1,42.
- 2,2'-thiodiethanol (TDE) – aqueous. RI can be varied ranging from being that of water (1.33) to that of immersion oil (1.52) by appropriately diluting with water.
- 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