**Guidelines for SIM - Superresolution microscopy on the OMX microscope**

**The SIM technique in brief:**

Structured illumination microscopy (SIM) is a superresolution light microscopy technique that gives improved resolution compared to conventional light microscopy (such as confocal microscopy) by illuminating the sample with structured light. The sample is illuminated with patterned light from three angles and in 5 different phases to capture 15 raw data images per z section. Then, computer algorithms extract information from the raw data and reconstruct a final high resolution image.

**Advantages of SIM**

3D-SIM can give down to approx. 100 nm lateral (xy) and 220 nm axial (z) resolution.

Conventional sample preparation techniques and common fluorophores can be used.

Image up to 4 colors simultaneously (DAPI/ green/ red/ far-red, OR cyan/yellow/red).

**Features of the Delta Vision OMX 3D-SIM Super-Resolution Microscope**:

Objective: 1.4NA Olympus 60X oil (for SIM)

Lasers lines for excitation: 405, 445, 488, 514, 568, and 642 nm.

Emission filters: 436/31, 478/35, 528/48, 541/22, 609/37, and 683/40 nm.

3 CMOS cameras for rapid multicolor imaging.

Possible color (laser) combinations when imaging: 405+488+568+642 **or** 445+514+568

**Guidelines for preparing samples for 3D-SIM on the OMX microscope**

Preparing samples for SIM is similar to preparing samples for conventional immunofluorescence /confocal microscopy, but follow the recommendations listed below to optimize your samples for SIM.

**Fixation and staining:**

Use your standard, optimized fixation and antibody staining protocol. Ultra pure (EM-grade) formaldehyde is recommended for fixation.

Choose fluorophores that match the excitation lasers and emission filters of the microscope, and choose also the most photostable fluorophores. Recommended dyes are the **Alexa Fluor series (AF488, AF568, AF647)**, DyLight series and ATTO dyes. Cy-dyes are somewhat less photo stable, but may work well. Fluorescent proteins such as EGFP and mCherry may work ok, but consider using booster/enhancer, and use mounting containing antifades.

DAPI/Hoechst staining should be carried out in a step followed by wash, since DAPI/Hoechst should not be included in the embedding medium.

**Mounting of samples.**

It is very important for the image quality that your sample is imaged through **high precision glass**, i.e. 1.5H, 170m thickness glass. Use therefore high precision glass coverslips, which should be mounted on standard object slides (76x26 mm).

Mount your specimen/coverslip close to **the centre** of the object slide, since only the central 22x22mm of the slide is accessible for imaging in the microscope (due to limited stage travel).

(An alternative to mounting the sample on an object slide is to use glass bottom (high precision glass!) cell dishes. The OMX microscope can hold 3,5 cm dishes and 8-well dishes (but here only the 4 middle wells will be accessible for imaging).)

 Mount the sample in a mounting/embedding medium with high refractive index and containing **antifade** reagents. Some recommended mounting media are **ProlongGold/Diamond/Glass** Antifade, **SlowFadeGold/Diamond** Antifade, and VectaShield H-1000.

Prolong is a hardening medium that will compress cells somewhat, while SlowFade and Vectashield are liquid media that preserves the 3D- structure of cells better.

Liquid mounting media should be sealed with nail varnish/sealant (to prevent floating and oxidation).

Do not include DAPI/Hoechst in the mounting medium!

**Imaging**

Make sure your coverslips are clean and dry before imaging. (Excess mounting may be gently wiped off with ethanol on cotton sticks.)

A range of immersion oils (Refractive Index between 1,500 and 1,534) from Applied Precision are available to match various mounting media/temperatures/wavelengths.

Your specimen should be on, or close to, the coverslip. Imaging depth is limited to approximately 16 m. Moving /floating specimens are incompatible with SIM.

The maximum field of view during previewing is 80x80 m, while for capturing images the max field of view is 40x40 m.

**Live cell 3D-SIM** is possible (but has limitations). Cells must be adherent and grown in glass bottom dishes (high precision glass, 3,5 cm or 8-well). Conditions needs to be optimized to minimize laser illumination (to reduce bleaching and cell toxicity), yet maximize signal intensity and image capture speed (to avoid image artifacts due to movement of the sample). Refractive index mismatches between glass/oil and cell medium may cause spherical aberrations and reduce the quality of the SI image reconstruction.

**Guidelines in brief:**

* Use photostable fluorophores, eg. Alexa dyes
* Use fluorophores that match the laser lines/emission filters of the microscope
* Use high precision glass (1.5H) coverslips
* Mount samples in mounting medium containing antifade
* Do not include DAPI/Hoechst in the mounting (rather, stain and wash before mounting)
* Mount coverslip(s) at the centre of the object slide (edges are inaccessible to the objective)

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