



## Structured Illumination Microscopy, SIM (Elyra)

Application	Sample Thickness	Objective	Camera
SIM	< 20 $\mu\text{m}$	63x/1.4 oil	sCMOS TV2
PALM/STORM/TIRF	< 4 $\mu\text{m}$	100x/1.46 oil	EMCCD TV1

### Image Acquisition

1. After locating your sample in the Locate tab, make sure all doors/windows of the black incubator box are closed and the condenser stand is in place.

2. Turn on required lasers.

3. Configure your Light Path:

a. Objective lens should be the 63x/1.4 oil.

b. Open SIM tab. TV2 is automatically set as detector.

c. Choose 1 track for each color, e.g.:

*Track 1 (SIM-642), 642 laser*

*Track 2 (SIM-561), 561 laser*

*Track 3 (SIM-488), 488 laser*

*Track 4 (SIM-405), 405 laser*

d. Click on laser graphic to select excitation laser for each track

e. As soon as a laser is selected, a SIM grid is automatically put in place.

f. Select filter cube with main beam splitter and emission filter, e.g.

*Track 1 (SIM-642), LP 655*

*Track 2 (SIM-561), BP 570-620*

*Track 3 (SIM-488), BP 495-550*



*Light Path for configuring a SIM experiment*

Track 4 (SIM-405), BP 420-480

4. Set Scanning parameters under Acquisition Mode:



*Acquisition mode parameters*

- a. Frame Size: 1024 x 1024 or 1280 x 1280
- b. Averaging: 1
- c. Bit Depth: 16 Bit
- d. Scan Area: full chip and fixed. The scan area can only be altered by Cropping and Panning.
- e. Grating: by default 3 Rotations (15 images/ plane). 5 rotations is recommended for better isotropic resolution enhancement (25 images/plane; 67% longer). 3 Rotations will have the benefit of faster image acquisition.

5. Adjust Channel settings:

- a. Set laser power via AOTF using the Laser slider (optimum between 1 to 10%; 405 nm laser may require higher power)
  - b. FOV: SIM is selected by default
  - c. Set the Exposure time. A good start value is 50 ms.
6. Set focal plane by scanning Live. Note that field of view is larger under Live scanning mode.
  7. Acquire an image by clicking on Continuous (not Live) or Snap to record an image.
  8. The grid should be visible during image acquisition (Continuous mode).
  9. For z stack acquisition: set first and last slice using one track only (uncheck the other tracks). Set interval to optimal (smallest) step size. Recheck all tracks, then, click Start Experiment to acquire the stack.

## Processing of SIM Images

1. Select the Processing tab.
2. On the Method tab, scroll to Structured Illumination (SIM) and open the tool.
3. Select Structured Illumination.
4. In the image container, click on image to be processed. On the Methods Parameter tab, click Select.
5. Select *Automatic* Processing. If you want to refine processing and want to set parameters manually, select *Manual*.
6. For z series: Expand the parameter definition field in the Methods Parameters tab and then Select First-Last and define the slices of the stack you want to process. If you want to process only one slice, select Single. It is recommended to process a stack, since this will also enhance x,y resolution.
7. Mode: 3D- automatically selected to process a stack; 2D- for processing only 1 slice.
8. As Output, select SR-SIM or SR-SIM + Widefield. If you also want to display a deconvolved widefield image (DCV), select SR-SIM + DCV + Widefield.
9. Press Apply to compute the SIM image. A new tab will open with the processed image. Save images (original and processed, if desired).



*Image Processing tab:  
Structured Illumination*

*Note: For multicolor, multi-track SIM, apply **Channel Alignment** to structured illumination output image*