

Zeiss ELYRA P.S.1 Guide

Getting Started

1. Switch on the **3 main power boards from left to right** (two are located to the left of the microscope, one near the monitor)
2. Switch on X-cite HXP Lamp.
3. Switch on the computer and log in to PPMS.
4. Start the **Zen 2012 Black** software. On the start-up window select **“Start System”**.

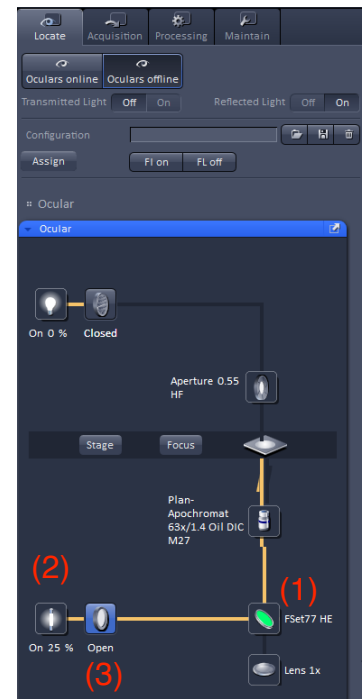


Shutting Down

1. Lower the stage and remove your sample and *gently wipe* any oil objectives you have used with *lens tissue* (**do not use kim wipes to clean objectives**).
2. Turn off the lasers within Zen.
3. Exit the software and copy your files to your *home or group network/ USB drive*.
4. Shut down the computer.
5. If you are the last user for the day switch off the power boards from Right to Left.

Visualising a Sample Through the Oculars

1. Switch on the X-cite power.
2. Use the **Locate** tab in Zen to visualise the sample.
3. Press the **Oculars Online** button to switch the microscope light-path 100% eyes.
4. Use the **Ocular** tool to configure the light-path
 - a. Choose filter set 77 for visualisation (this is green/red dual filter) **(1)**
 - b. Turn the X-Cite lamp to 'On' and **Open** shutter **(2,3)**

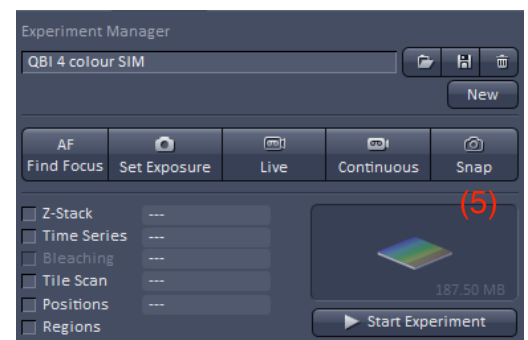
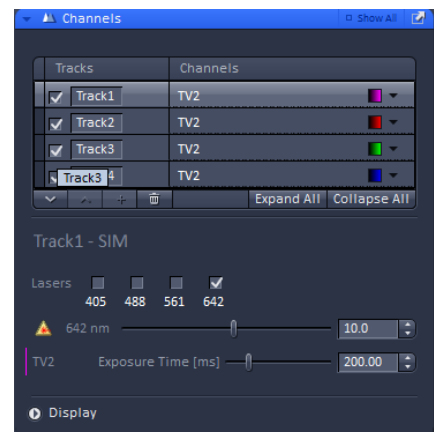
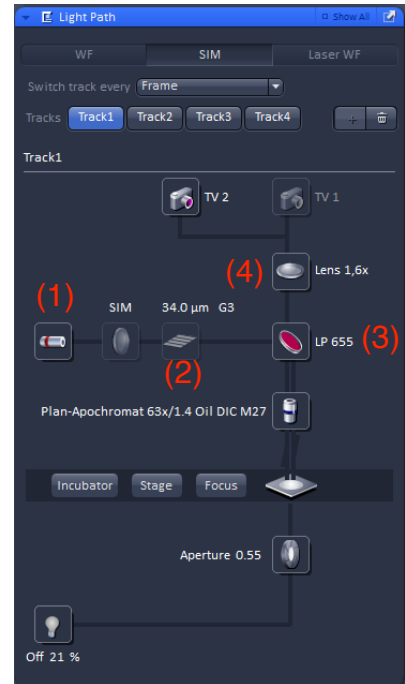


Capturing SIM raw data

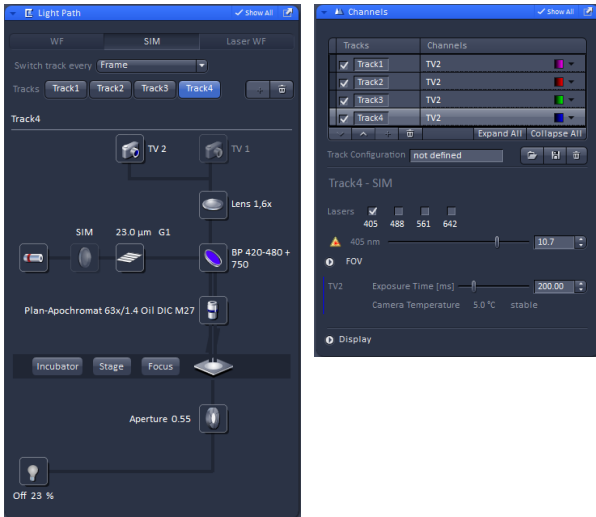
The SIM microscope is in essence a wide-field microscope with laser excitation. It is equipped with 4 laser lines (405nm, 488nm, 561nm, 642nm) and has been pre-configured with a 4-colour experiment that can be used as the basis for all SIM acquisitions: simply choose the required track for acquisition.

Tracks are easily configured as follows:

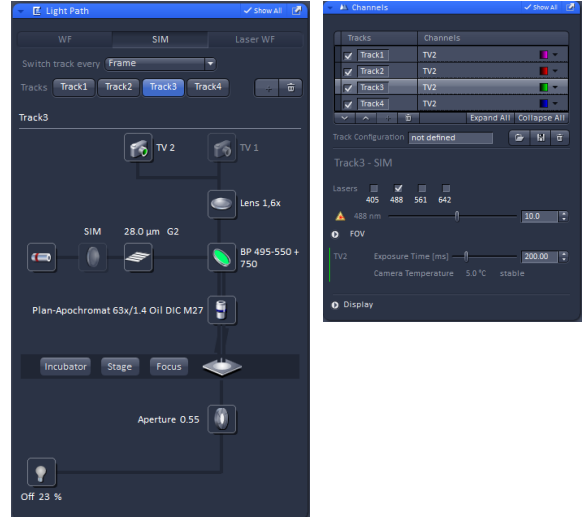
1. Turn on the required laser lines.
2. In the **Light Path** tool choose the **SIM** tab. The default camera for this acquisition mode is **TV 2** which is the sCMOS camera.
3. Select the laser line from 405nm, 488nm, 561nm and 642nm. (1)
4. Zen will recommend a grating to be used for the acquisition depending on which laser line and which objective is in use (the recommended is the **63x/1.4 Oil** lens. (2)
5. Choose a reflector appropriate for the laser line and fluorophore being imaged. (3)
6. Use **Optvar** lens **1.6x**.
7. Use **Live** or **Continuous** acquisition along with **Channels** to set a laser power and camera exposure time for each track.
8. Acquire an image in one or more tracks using **Snap** (5)



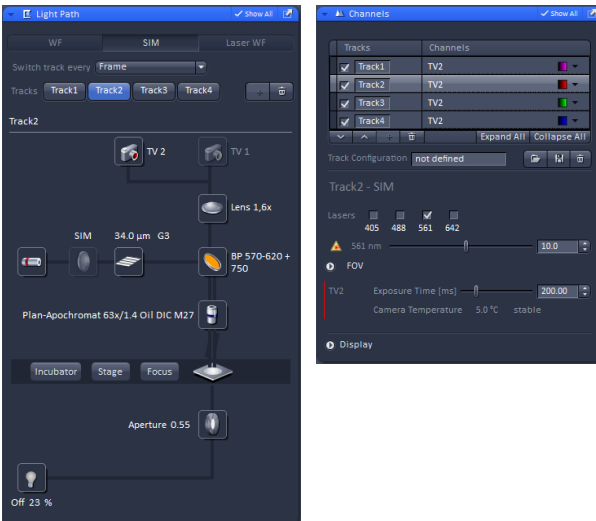
405nm track



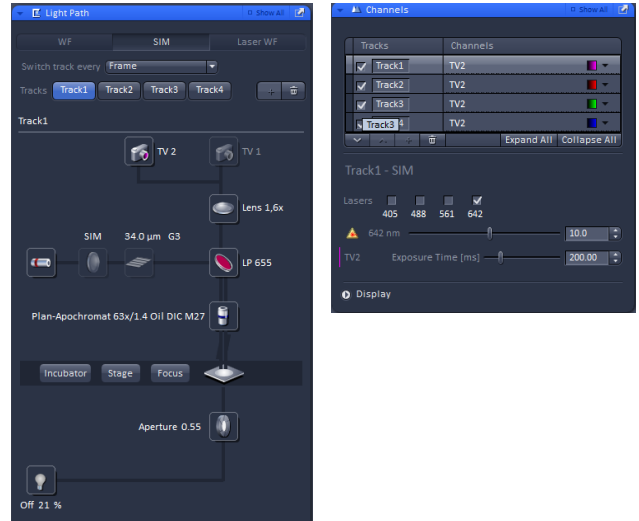
488nm track



561nm track



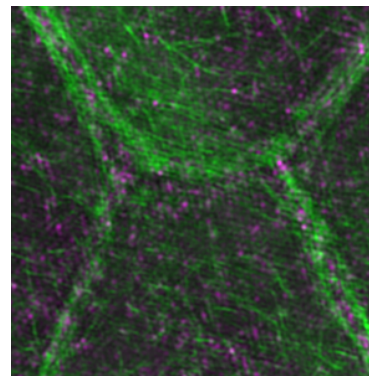
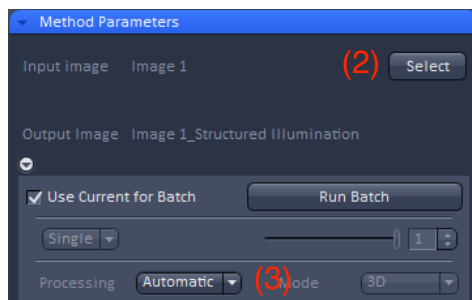
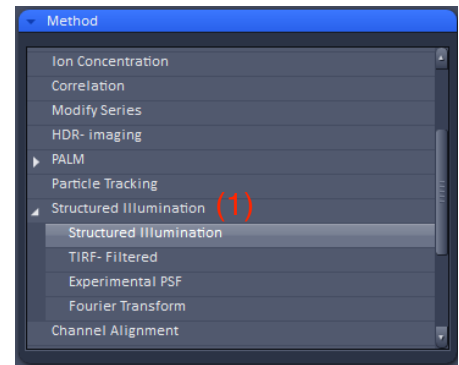
642nm track



Processing SIM data

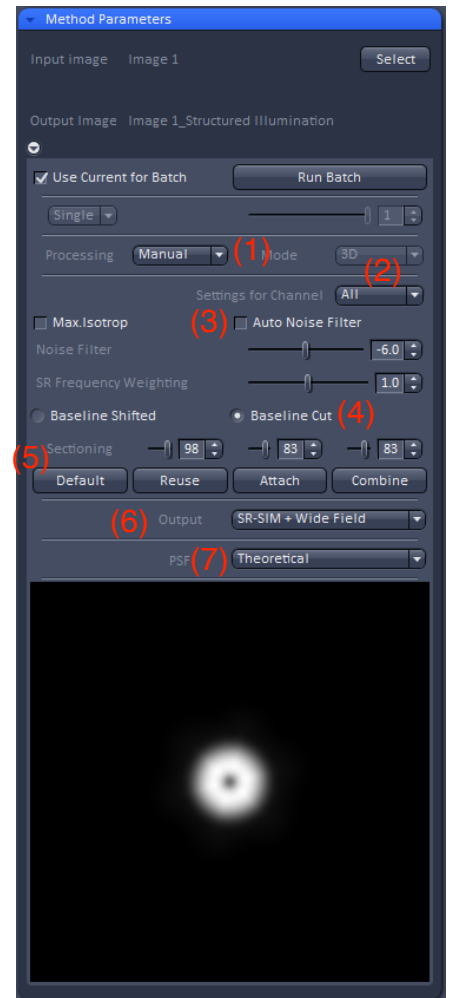
SIM images are reconstructed under the **Processing** tab:

1. Open **Structured Illumination** in **Methods** and select **Structured Illumination** from the list. (1)
2. Under **Method Parameters** press **Select** to analyse the currently displayed image. (2)
3. Processing can be performed in two ways: select between **Automatic** or **Manual** (3). It is recommended that **Manual** is used since the automated routine does not necessarily choose the optimum parameters. Typically it will choose too low a value for the noise filtering step (around -7) which can produce image artifacts.
4. Press **Apply** to process the raw data.



Manual Processing

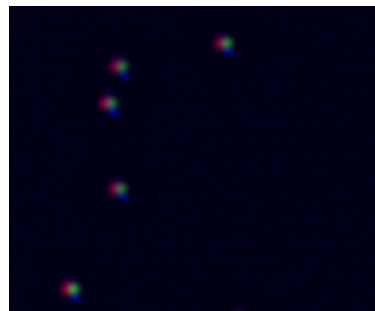
1. Choose **Manual** from the **Processing** list. (1)
2. The settings for processing can be applied to all channels or they can be defined for each channel in the image separately. (2)
3. Deselect the **Auto Noise Filter** checkbox to allow setting the (Wiener) filter level (3). Typically this will range between -5 and -6 and will be sample dependent (due to signal-to-noise ratio considerations). It usually necessary to test this parameter in order to avoid image artifacts such as those produced when using automated processing.
4. Keep **SR Frequency Weighting** at **1.0**.
5. Choose between **Baseline Shifted** or **Baseline Cut** (the default option being the latter). Noise filtering often introduces negative values in the image. The image can either be positively shifted (**Baseline Shifted**) so that minimal (negative) intensity values become zero or the image can be clipped (**Baseline Cut**) by setting negative values to zero. (4)
6. In addition to noise filtering the **Sectioning** parameters may need to be optimised depending on the sample being imaged. The three controls determine the strength of filtering applied for the 0-frequency components of the (non-frequency-shifted) 0th, 1st and 2nd orders (both +ve and -ve components) of the grating. The default values are **98**, **83** and **83** (for 0th, 1st and 2nd respectively). It is important to note that in addition to removing the effect of the grating from the final image these parameters also remove the effect of out-of-focus light (which appears at 0-frequency). **A 0th order filter strength of 100 can be particularly useful when imaging tissue.** (5)
7. Choose **SR-SIM+Wide Field** from the **Output** drop-down menu to generate a reconstructed wide field image to accompany the super-resolved data (6). This is important to demonstrate the resolution enhancement in the SIM data.
8. Choose **Theoretical** from the **PSF** drop-down menu (default and preferred). This uses a calculated point-spread-function for processing as opposed to one determined experimentally (note that the Fourier transform of the PSF is shown in the method parameters where the central dark spot is the filter defined in Sectioning). (7)
9. Press **Apply** to process the raw data.



Channel alignment

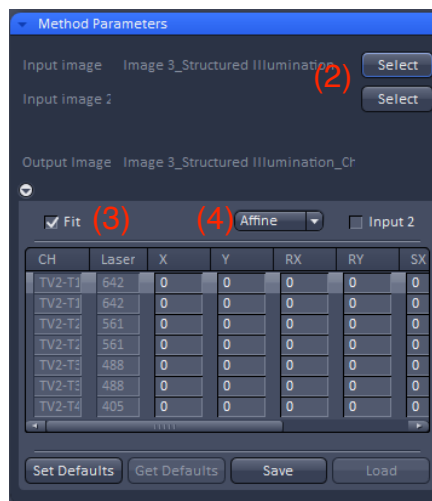
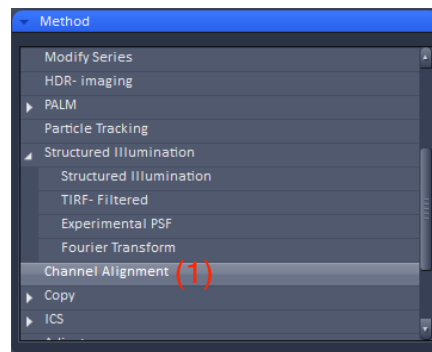
Since we are performing wide-field microscopy with laser illumination we need to be aware of spatial misalignment between spectrally distinct channels resulting from imaging with different sets of filter cubes (variations in the dichroic mirrors can cause this misalignment). This is particularly important when producing super-resolved images.

We can adjust the images for spatial misalignment using a calibration sample consisting of polystyrene beads coated with multiple fluorophores (so that the same object appears in all channels).



Alignment calculation

1. In the **Processing** tab choose **Channel Alignment** under **Method**. (1)
2. Under method parameters use **Select** to choose an image. Typically this will be an bead-image recorded in multiple channels but **Input Image 2** can also be used if the channels have been recorded in separate images. (2)
3. Make sure the **Fit** box is checked. (3)
4. Choose **Affine** from the list of possible transformations and check the **Input 2** box to align to the second image. (4)
5. Press **Apply** to perform the alignment.
6. After the alignment has finished the data table under the processing options will be populated and can be saved for later use.



Alignment application

1. Deselect the Fit checkbox. (3)
2. Choose the type of alignment from the dropdown list. Make sure to use the same type of alignment as used when determining the calibration parameters. (4)
3. Match the **ID** for each channel being aligned to that from the first column of the alignment parameters (note that there are two images for each channel: a super-resolved and a wide field). (5)
4. Press **Apply** to perform the alignment (this will generate a new dataset appended with "ChannelAlignment").

