



## Channel Alignment (Elyra)

If you use the default filter cube set (FS1) for SIM with 2 or more channels, you will need to do the following procedure on your processed Structured Illumination images in order to correct for spatial misalignment between different channels.

### Generating a Calibration File

1. Use 0.1- 0.2 um TetraSpeck beads (drawer). Since the beads are very small, you may need to focus on the beads by Live acquisition. Using the eyepieces via the Locate tab may not be very helpful.
2. Collect data using SIM mode with the same imaging parameters as your sample.
3. Reconstruct the data with the "Structured Illumination" processing function.
4. Select the "Channel Alignment" processing function (1) and select the processed SIM image into "Input Image" (2).
5. Check the "Fit" box (3), and select "Affine" under the method parameters (4).
6. Select "Apply" at the top (5).



Image processing: Channel Alignment

- When the process is finished, the top table (Table 1) will populate with values. Save these values as a .bin file. This is your calibration file that you will use to align data in the future.



*Image processing: Calibration values obtained after application of Affine (or Lateral) fit*

## Aligning an Image using the Calibration File

- Select the "Channel Alignment" processing function and select your sample data (processed SIM Image) into "input image".
- Uncheck the "Fit" box. This will open another table that represents the channels in your image (Table 2).
- Select "Load" to load the .bin calibration file that you created previously. This will populate Table 1.
- Channels in Table 1 are assigned an ID (these are channels in your calibration file).

Channels in Table 2 represent the channels in your dataset that you inputted above. You should match the channels in Table 2 to the IDs of the appropriate channels in Table 1.

- Select "Apply" at the top.

*You can also load the latest .bin calibration file under **Users > SIM Calibrations > Transformation Tables** if you have not generated your own calibration file. Note, however, that the imaging parameters may be different from what you use for your images.*

Method Parameters

Input image 63x lens 11-29-11 Select

Input image 2 Select

Output Image 63x lens 11-29-11\_Channel Aligner

Fit **Table 1** Affine  Input 2

ID	Laser	X	Y	RX	RY	SX
0	642	0	0	0	0	0
1	561	-0.475	0.167	-1.520	6.094	0.001
2	488	-1.339	1.935	-2.085	-2.406	0.002
3	405	-1.232	4.210	2.846	3.404	0.002

CH	Laser	ID
TV2-T	642	0
TV2-T	561	1
TV2-T	488	2
TV2-T	405	3

Set Defaults Get Defaults Save Load

Each channel is assigned an image ID. The IDs should match in both Tables 1 and 2.

Method Parameters

Input image 63x lens 11-29-11\_Subset Select

Input image 2 Select

Output Image 63x lens 11-29-11\_Subset\_Channel /

Fit Affine  Input 2

ID	Laser	X	Y	RX	RY	SX
0	642	0	0	0	0	0
1	561	-0.475	0.167	-1.520	6.094	0.001
2	488	-1.339	1.935	-2.085	-2.406	0.002
3	405	-1.232	4.210	2.846	3.404	0.002

CH	Laser	ID
TV2-T	561	1
TV2-T	488	2

Set Defaults Get Defaults Save Load

When only two channels are selected for alignment, these should match assigned ID in calibration file.