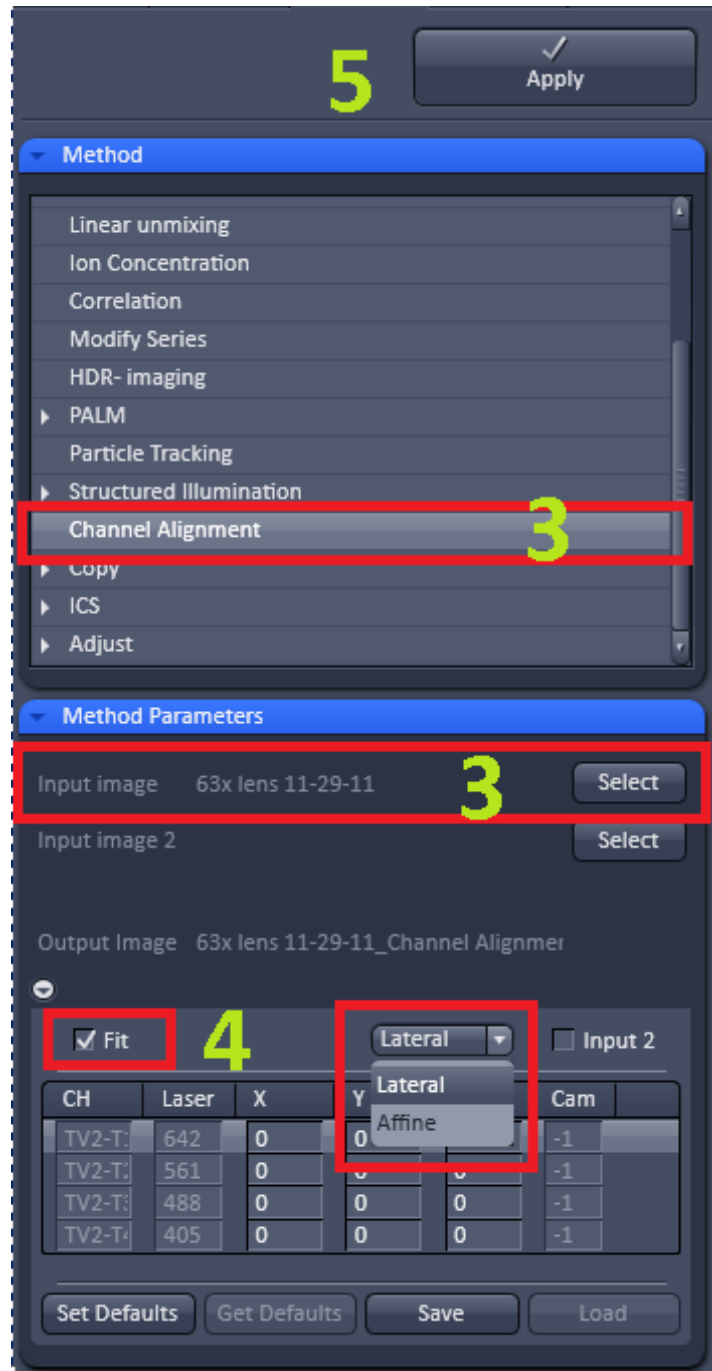


Channel Alignment Protocol

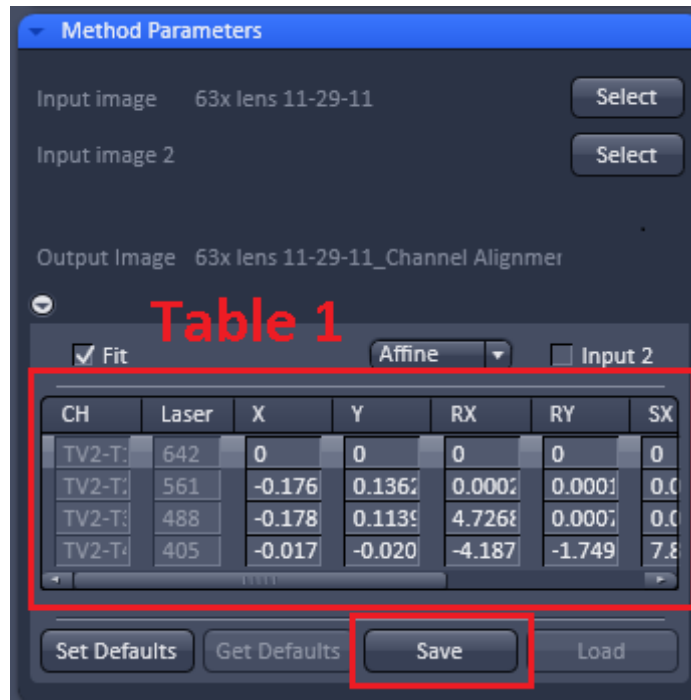
Generating a Calibration File

- 1) Collect **Dataset#1** using SIM mode and the "tool for calibration, Multi Spec" (part# 1783-455)
- 2) Reconstruct the data using automatic mode in the "**Structured Illumination**" processing function. (**Dataset#1_SIM**)
- 3) Select the "**Channel Alignment**" processing function and select **Dataset#1_SIM** into "input image".
- 4) Check the "**Fit**" box, and select "**Affine**" under the method parameters
- 5) Select "**Apply**" at the top

*Important: After selecting APPLY, you can not click any other processing methods or functions. Let it run -- go get coffee/check email while it's running. For 1024x1024, 4 color images, the alignment process can take 15-30 minutes.



6) When the process is finished, the 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.



Aligning an image using the calibration file

- 1) Select the "Channel Alignment" processing function and select your data (Dataset#2) into "input image". This dataset does not need to be any specific type (SIM, LSM, etc)
- 2) Uncheck the "Fit" box. This will open another table that represents the channels in your image (Table#2)
- 4) Select "Load" to load the .bin calibration file that you created previously. This will populate Table#1.

Method Parameters

Input image 63x lens 11-29-11 Select

Input image 2 Select

Output Image 63x lens 11-29-11_Channel Alignmer

Fit **Table#1** Affine Input 2

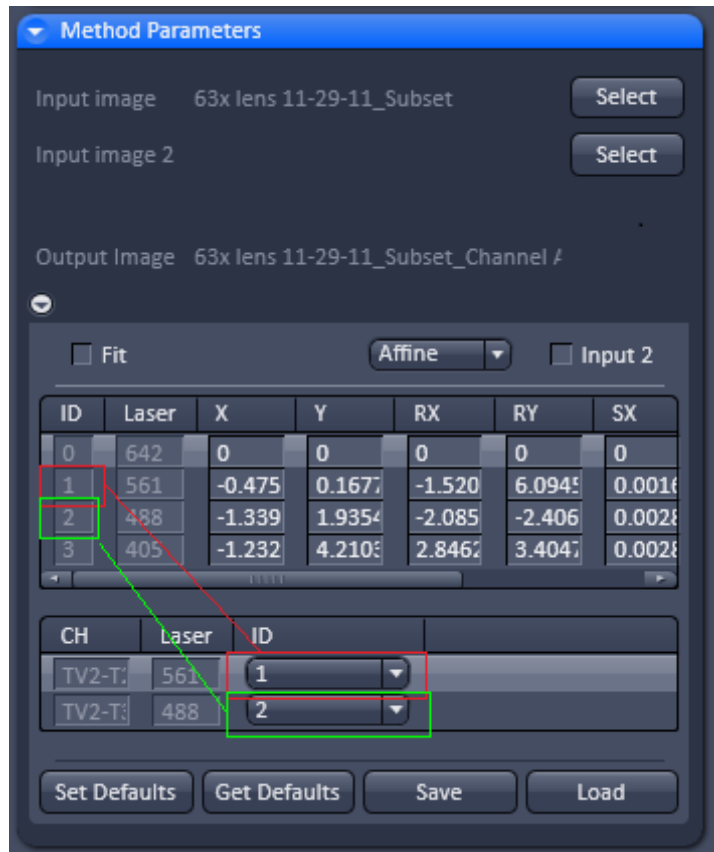
ID	Laser	X	Y	RX	RY	SX
0	642	0	0	0	0	0
1	561	-0.475	0.167	-1.520	6.094	0.0016
2	488	-1.339	1.935	-2.085	-2.406	0.0028
3	405	-1.232	4.210	2.846	3.404	0.0028

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

Table#2

Set Defaults Get Defaults Save Load

5) Channels in **Table#1** are assigned an ID (these are channels in your calibration file). Channels in **Table#2** represent the channels in **Dataset#2** that you inputted above. You should match the channels in **Table#2** to the IDs of the appropriate channels in **Table#1**.



5) Select "Apply" at the top.

*Important: Sometimes the "laser" column in the **Table#2** region does not report the correct lasers. You must depend on your knowledge of the channels in your data as you collected it, rather than what is reported in this table. For example, if you acquired data as "Red-Green", but **Table#2** displays "Green-Blue", you should select the ID as if it is "Red-Green".