

Elyra 7: Acquiring and Processing a Lattice SIM Image

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 **UT HSC** RESEARCH

LATTICE SIM INTRO

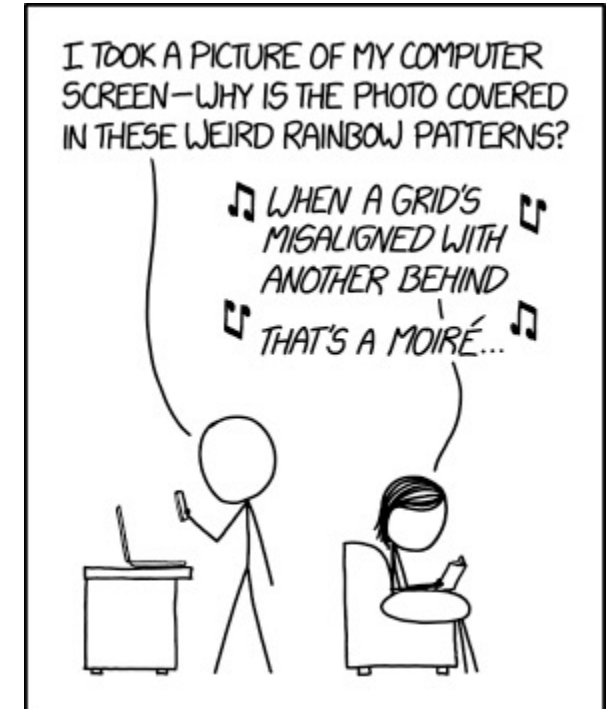
LATTICE SIM

Structured Illumination Microscopy (SIM) imaging introduces a diffraction grating into the light path, so that the sample is illuminated in a known pattern (in our case, a lattice pattern).

- ▶ When two grids of different spatial frequencies are aligned to each other, moiré fringes will appear.
- ▶ If you have one grid of a known spatial frequency (the grating pattern we use when imaging), you can use the information from the moiré fringes to obtain information on spatial frequencies of the second grid (fluorescence from our sample).

With the Elyra 7:

- ▶ Each image is technically made up of 13 individual images (or phases) as the grating moves the lattice grid illumination across the sample.
- ▶ The software will automatically select the appropriate size grating based on the light path settings.



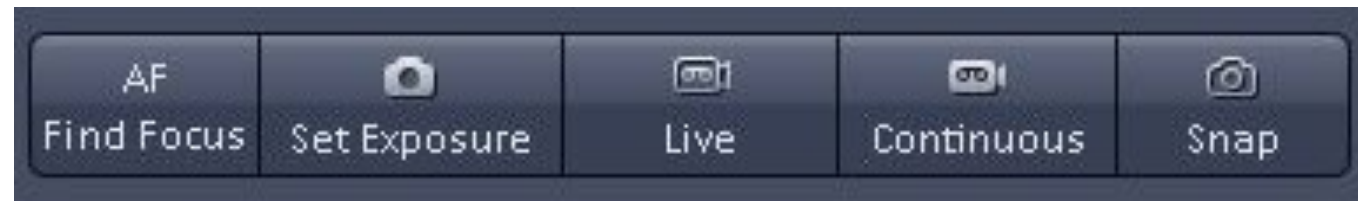
LATTICE SIM

**Prior to the steps in this tutorial, you should have already:

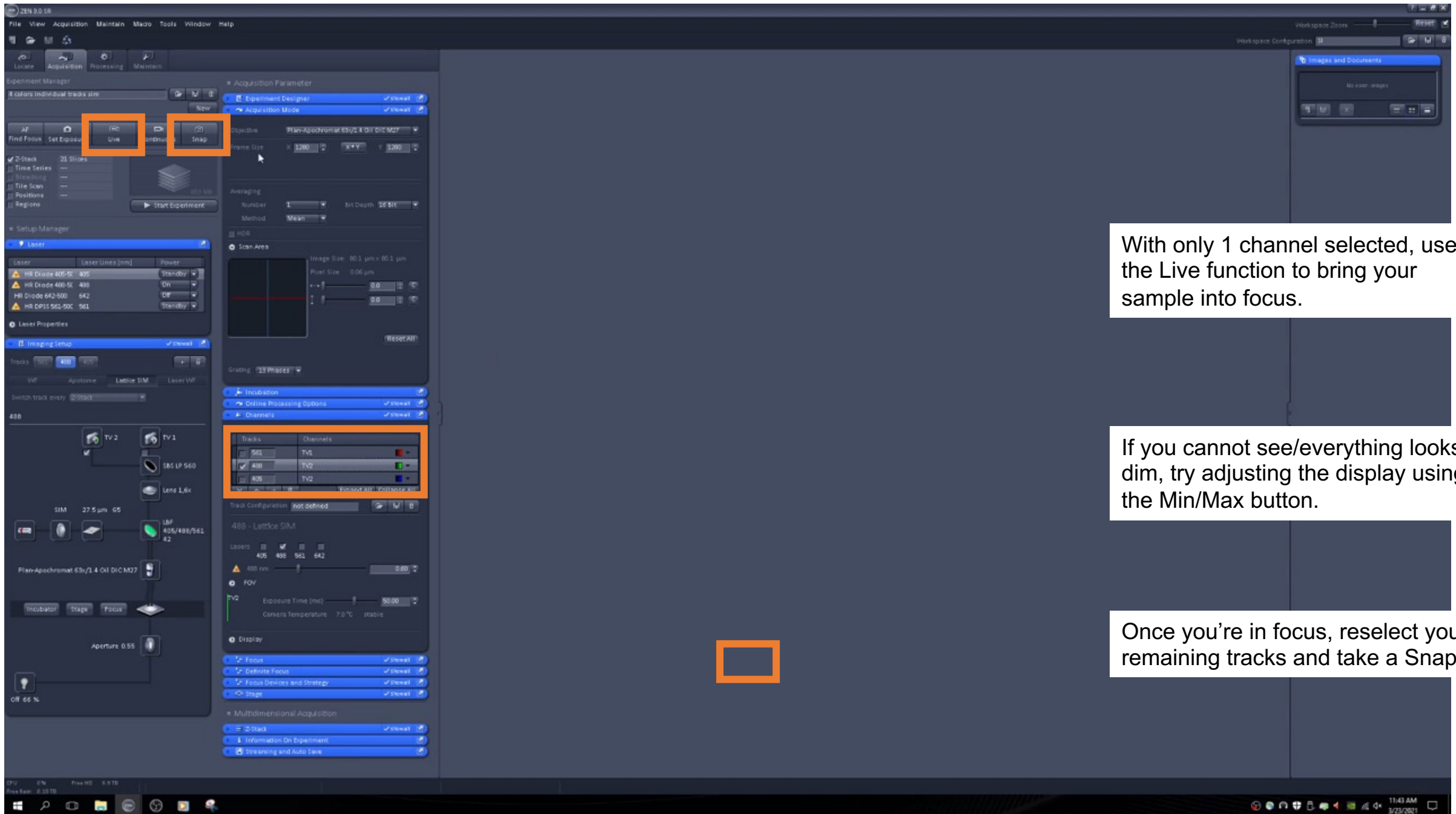
- Secured the sample in a holder, and placed it at the stage
- Found an area (using the eye piece) and gotten it in focus as much as possible
- Selected the light path settings for your fluorophores

This guide contains videos with instructions, followed by a slide of just the instructions and added details.

BEGIN: With only 1 track selected (via checkbox) press the LIVE button in the top left of the Acquisition tab.



ACQUIRING AN IMAGE

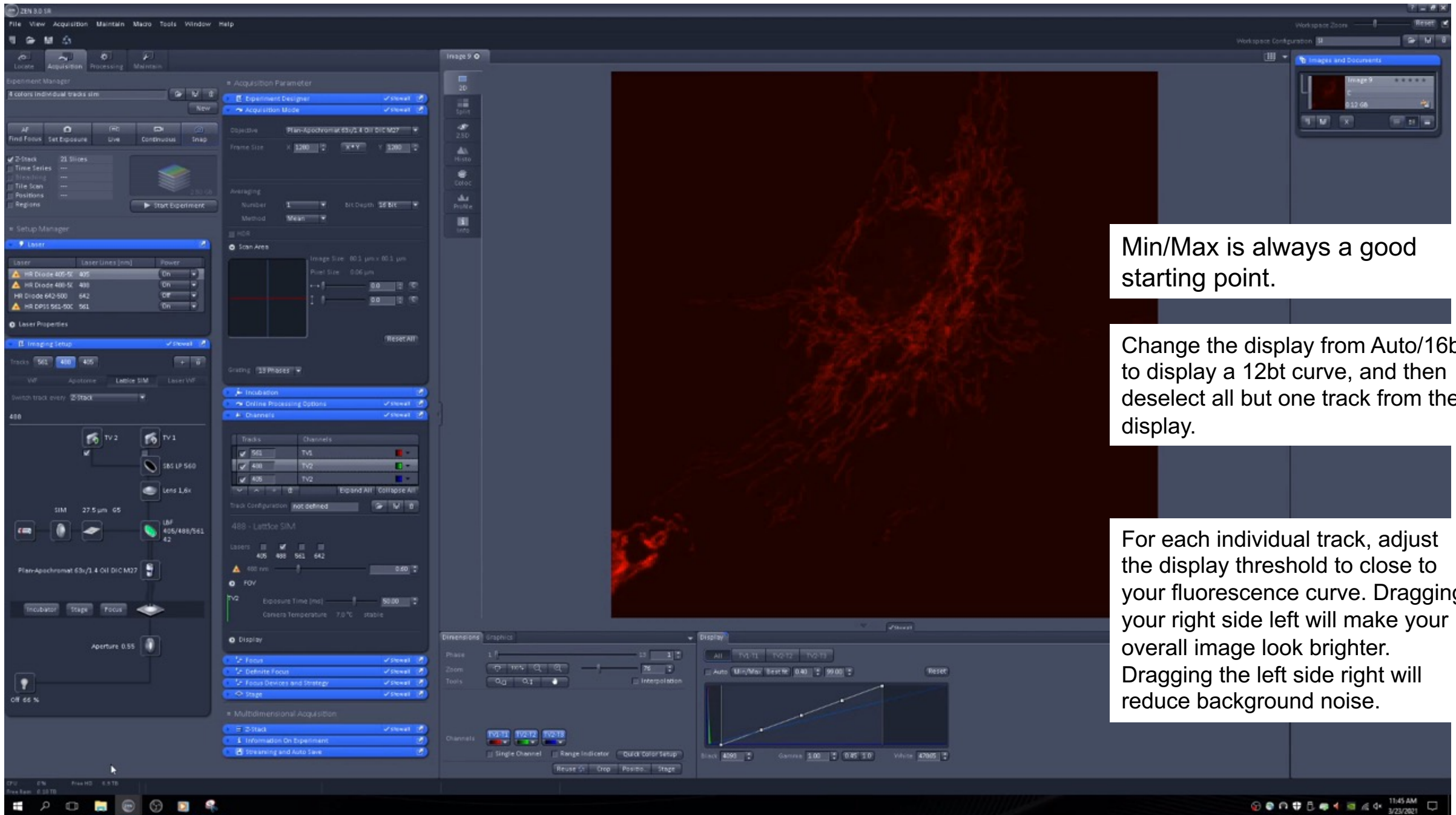


ACQUIRING AN IMAGE

1. With only 1 channel selected, use the LIVE function to bring your sample into focus.
 - This will be where the focus knobs on the auxiliary screen come in handy!
 - The LIVE and CONTINUOUS functions are nearly interchangeable. But with the CONTINUOUS function in Lattice SIM mode, you will be able to see the SIM grating structure on the screen.
 - More than one channel can be used in LIVE and CONTINUOUS, however the Elyra cycles between these channels fairly slowly. For finding the right FOV and focal plane, this will not be very useful.
2. If you cannot see/everything looks dim, adjust the display using the MIN/MAX button.
 - This is normal and not necessarily indicative of a bad fluorescence signal.
 - Adjusting the display only adjusts how your image is displayed and not any properties of the image itself.
3. Once you're in focus, reselect your remaining tracks and take a Snap.

Most likely, even if all your light path/laser settings are appropriate, you will need to adjust the display settings to best view your image.

ADJUSTING THE IMAGE DISPLAY



Min/Max is always a good starting point.

Change the display from Auto/16bt to display a 12bt curve, and then deselect all but one track from the display.

For each individual track, adjust the display threshold to close to your fluorescence curve. Dragging your right side left will make your overall image look brighter. Dragging the left side right will reduce background noise.

ADJUSTING THE IMAGE DISPLAY

MIN/MAX is always a good starting point.

- BEST FIT does not seem to give the optimal image on this system.

Change the display from Auto/16bt to a 12bt curve (right click inside graph), and then deselect all but one track from the display.

- This makes it easier to see what you're doing with the histogram on the next step.

For each individual track, adjust the display threshold to close to your fluorescence curve. Dragging your right side left will make your overall image look brighter. Dragging the left side right will reduce background noise.

- Make sure you also select the individual curve for each channel and are not universally changing the settings for all channels! (ALL vs TV1-T1, TV2-T2...)
- Assuming you have SHOW ALL selected, you can also type threshold values directly into the boxes at the bottom left and right of the graph.



ADJUSTING THE IMAGE DISPLAY

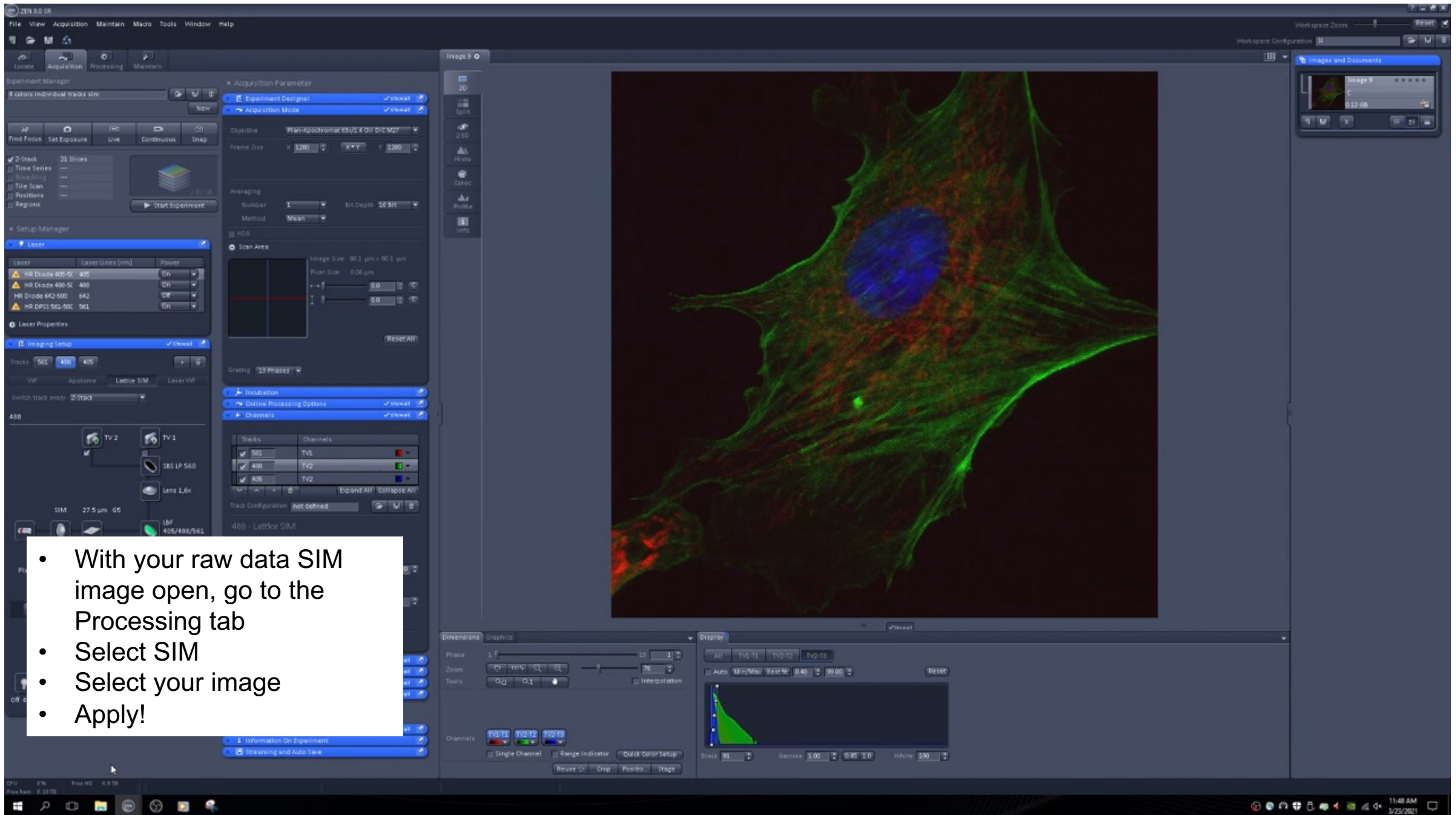
Note: There is a scroll bar directly under the image that allows you to move through each of the 13 phase images



PROCESSING A SIM IMAGE

PROCESSING A SIM IMAGE

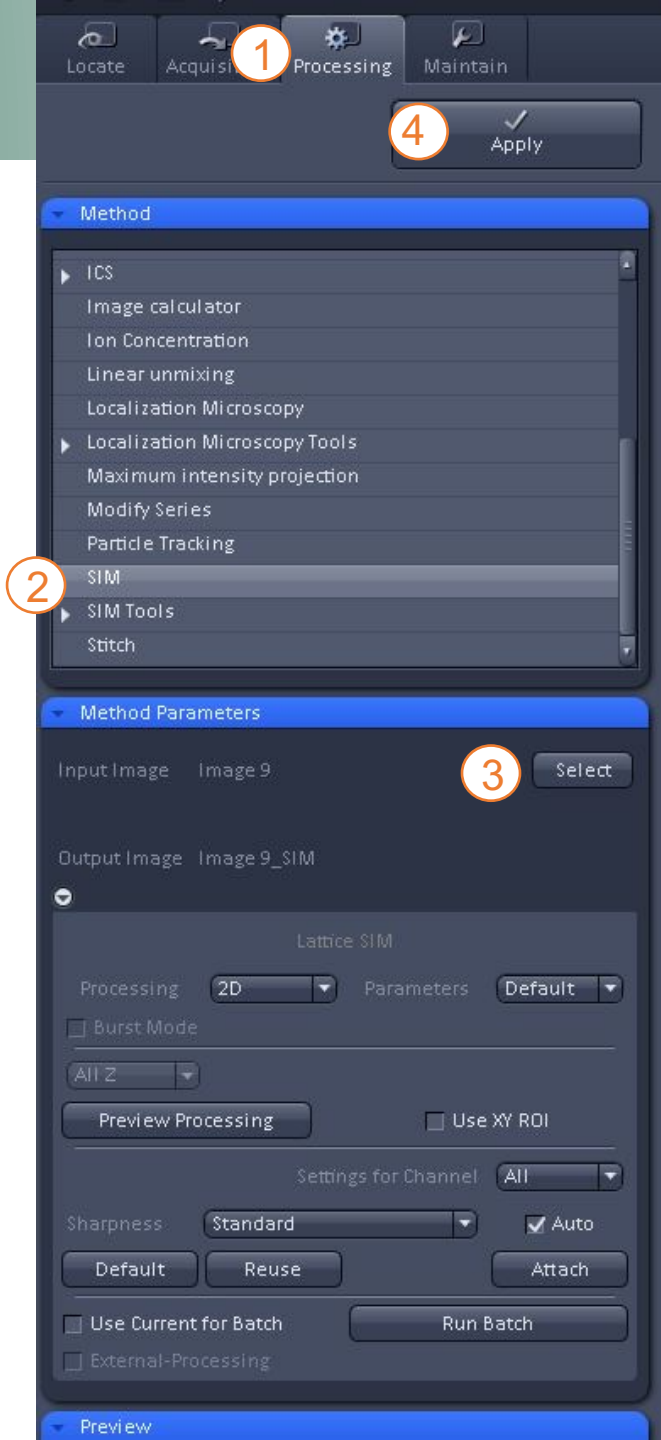
- ▶ All Lattice SIM images will require processing, as each image is actually 13 images.
- ▶ While you may want to process each image immediately after acquisition (as processing is pretty quick), you can also run Batch Processing at the end of your experiment.



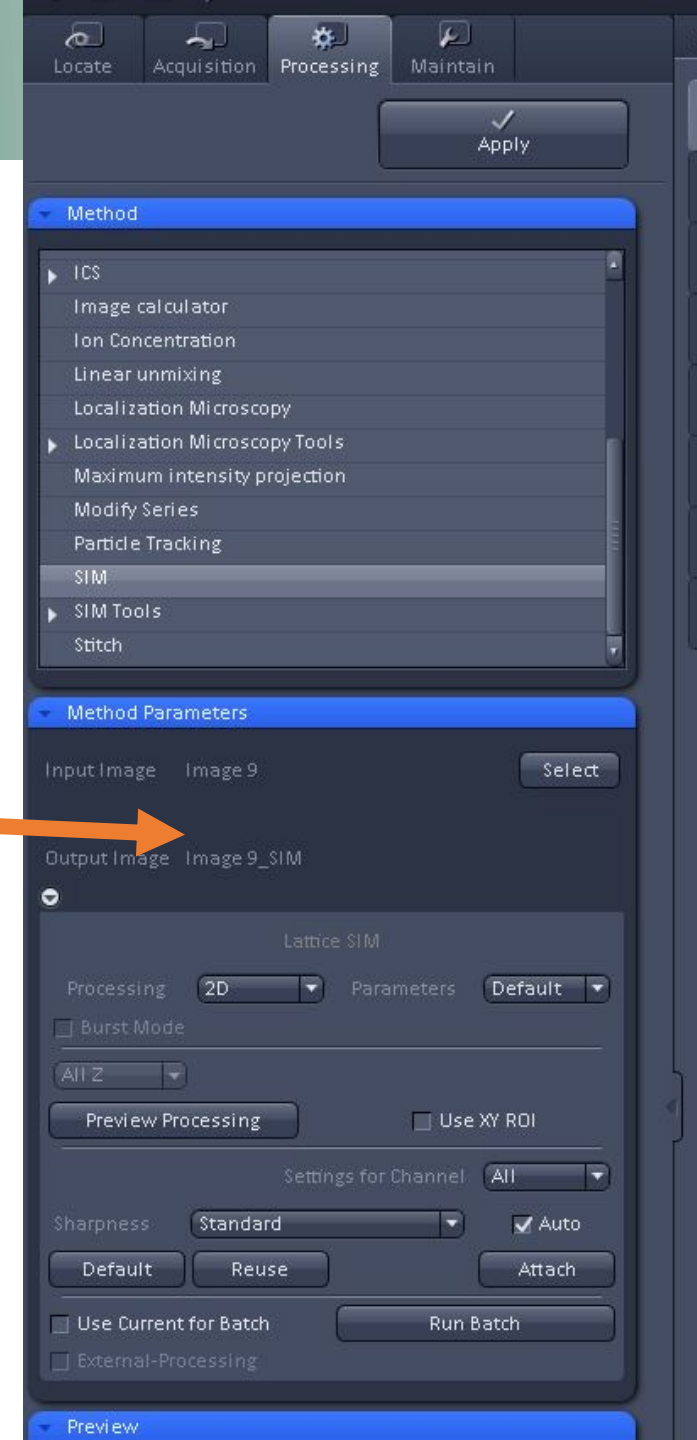
- With your raw data SIM image open, go to the Processing tab
- Select SIM
- Select your image
- Apply!

PROCESSING A SIM IMAGE

1. With your raw data image open, select the Processing tab
2. From the list, select SIM.
3. Select your image.
 - Whatever image you have open when you press “Select” will be used.
4. Click the APPLY button located at the top.
 - The APPLY button is always the last step in any processing and is always located at the top of the panel (as opposed to the settings shown for each individual function).



- ▶ The default settings for the SIM processing are usually appropriate, as for most images there is no significant visual difference when selecting different levels of sharpness.
- ▶ NOTE: For all processing functions, the newly processed image will open in its own window. It will also automatically generate an image name using the name from the original image and appending the name of the process applied.
 - Example: If you process an image with the name “Image 1”, you will get a new, processed image with the name “Image 1_SIM”.



- ▶ For processing a batch of raw data SIM images, all the images must already be saved in your folder.
- ▶ To start, you can select any of the images you wish to process (you will select the rest after).
- ▶ Check the box next to “Use settings for batch”.
- ▶ Click the RUN BATCH button.
 - ▶ A separate File Explorer window will pop up for you to select all of your raw data images.



NOTES:

- When running batch processing, the new images will not automatically open in Zen Black, but will automatically be saved in the folder with the original images.
- You will see a progress window in the bottom panel showing the processing status of your files.
- Files will simply be appended with “Out” as opposed to the name of the processing function.

ACQUIRING AND PROCESSING A LATTICE SIM IMAGE

You should now be comfortable taking and processing SIM images.

The next guide will cover multi-step experiments, including time series, Z-stacks, and Tiles.