

Lambda Mode on LSM780

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Lambda Mode is used when doing FRET. It is good for FRET pairs to determine optimal collection ranges.

Use one 1 light path in Lambda mode.

Make note of the laser power, pinhole, gain and digital offset used in LSM channel mode so you can copy them over to Lambda mode

LSM Channel Mode

The screenshot displays the microscope control software interface. On the left, the 'Light Path' panel shows the 'LSM' tab selected, with a red box around it and a red arrow pointing to it. Below this, the 'Track 2' section shows a spectral plot and a table of dye configurations. On the right, the 'Channels' panel shows a table of tracks and channels. Below that, the 'Track 2 - LSM' settings panel is visible, with a red arrow pointing to it. This panel includes sliders for Lasers (488 nm), Pinhole, Gain (Master), Digital Offset, and Digital Gain. The 'Display' section at the bottom shows various focus and stage settings.

Use	Dye	Color	Detector	Range
<input type="checkbox"/>			Ch1	415- 735nm
<input checked="" type="checkbox"/>	Alexa Fluor 488		ChS1	490- 606nm
<input type="checkbox"/>			Ch2	415- 735nm

Tracks	Channels
<input checked="" type="checkbox"/> Track 1	Tred
<input checked="" type="checkbox"/> Track 2	A488
<input checked="" type="checkbox"/> Track 3	DAPI

Track Configuration: 594

Track 2 - LSM

Lasers: 405, 458, 488, 514, 561, 633, 800

488 nm: 0.18

Pinhole: 48.4

1.00 Airy Units = 0.8 μm section

A488 Mode: Integration, Photon Counting

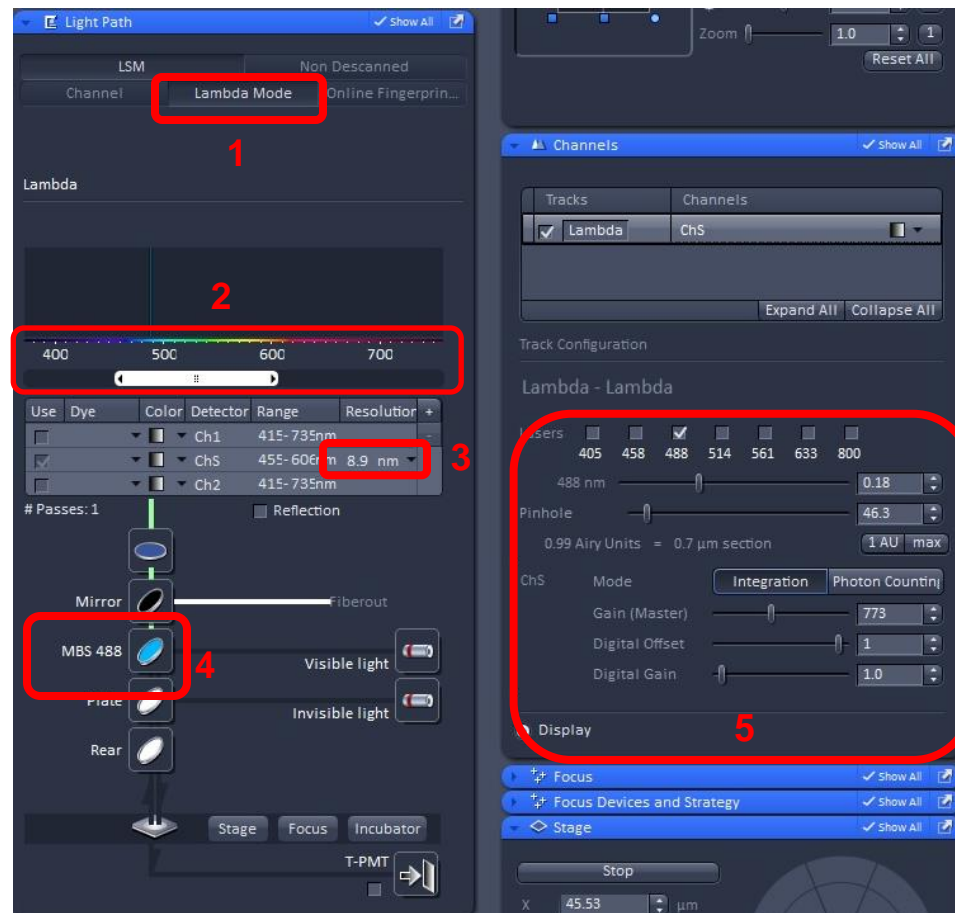
Gain (Master): 773

Digital Offset: 1

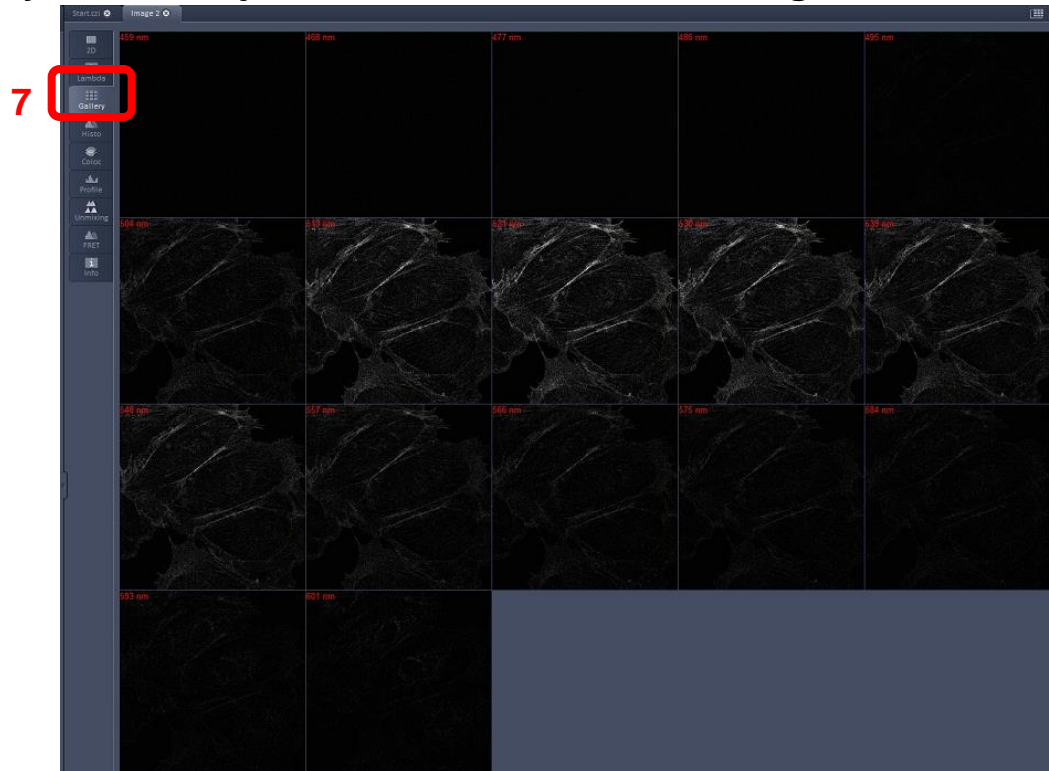
Digital Gain: 1.0

Make note of all settings

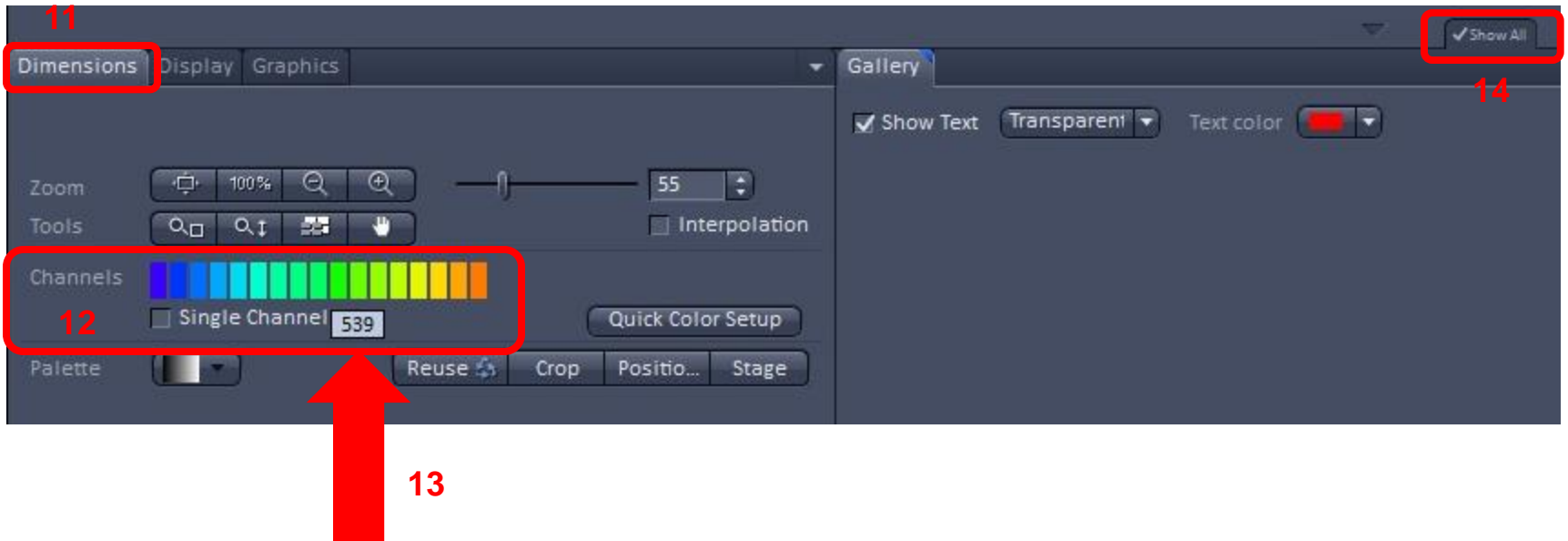
1. Select Lambda Mode.
2. Set range of emission filter. Select on both sides of the laser line in order to get an idea where brightest fluorescence is as well as range of fluorescence.
3. Select Resolution. 8.9nm means it will image every 8.9nm through the range set up in Step 2.
4. Set the MBS filter to the laser line you are using. In this case 488. Multiple lines in the MBS filter will block emitting light at those wavelengths. The images in the gallery corresponding to those wavelengths dimmer.
5. Match LSM mode settings in laser line, laser power, pinhole, gain, digital offset and digital gain.
6. Image.



7. Click Gallery to see each image
8. Make sure the Show All box is checked
9. Check the Show Text box so you will see the emission wavelength for each image.
10. You may need to adjust the contrast to see the image.



11. Click on the Dimensions Tab.
12. You will see all the channels listed.
13. Hover the mouse over one to see the emission wavelength [in nm] it corresponds to. Ex 539nm.
14. Make sure show all box is checked.



15. You can turn off any channels you want by clicking on the colored bar that represents that channel. In this example the 504 and 584 channels were turned off.

