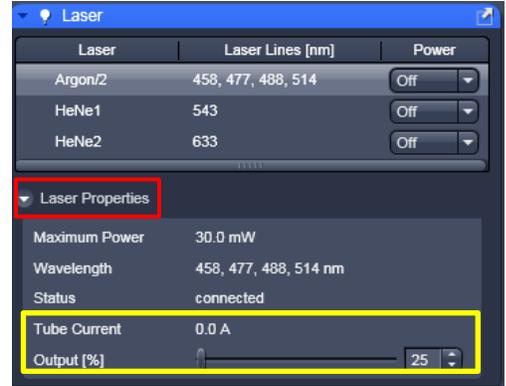


Setting up a Single Track Configuration

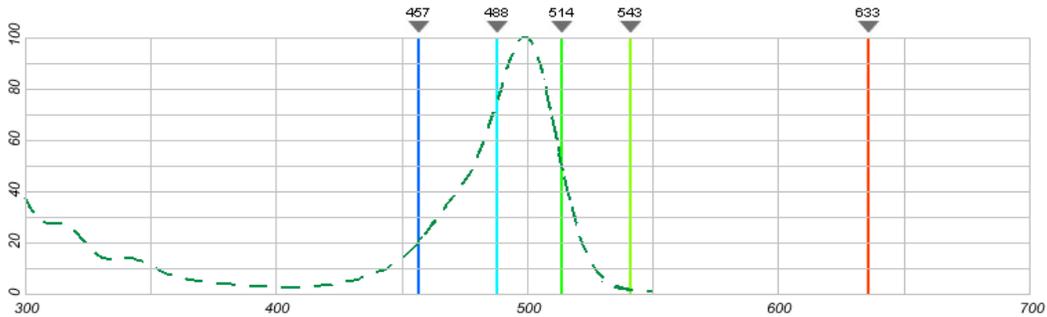
You will need to know the excitation and emission spectrums for each dye you are setting up.

- Turn on the laser you need by clicking the  tab and opening the  toolbar from the Setup Manager.

- You want to be as close to the Excitation spectrum peak as possible. If the exact number isn't available go with the closest wavelength below the peak.
- If using the Argon/2 laser it must go to Standby first. Once you can turn it on you need to open the Laser Properties drop menu and increase the Tube Current to 6.1 A.



Example: Alexa 488; Excitation peak is listed at 498nm. There isn't a 498 laser available so you'd choose the 488 laser.



- Open the  and  toolbars from the Setup Manager

- Select the laser (1) that will be utilized based off your dye's EXCITATION spectrum

- Select the dichroic (2) that will reflect the laser line you chose. The numbers associated with the dichroic represents the wavelengths reflected by that dichroic.

Examples:

HFT 488- Reflects the 488 laser line ONLY

HFT 488/543-Reflects the 488 AND 543 laser lines

- Select the emission filter (3) that will transmit the wavelengths you want to reach the detector (4); based off your dye's EMISSION spectrum. Capture as much of the emission spectrum peak as possible by selecting a filter that encompasses both sides of the emission peak.

- Each channel has multiple filter options. If you don't see a filter that fits what you're looking for in one channel, try the other channel.
 - LP ### - Long Pass filter - transmits all wavelengths greater than the indicated number
 - BP ###-### - Band Pass filter – transmits only the wavelengths that are between the two numbers.

- Select the color you want this dye to show on the screen by clicking on the Channel box (4) (Ch2, Ch3, or Ch5) you've selected to use.

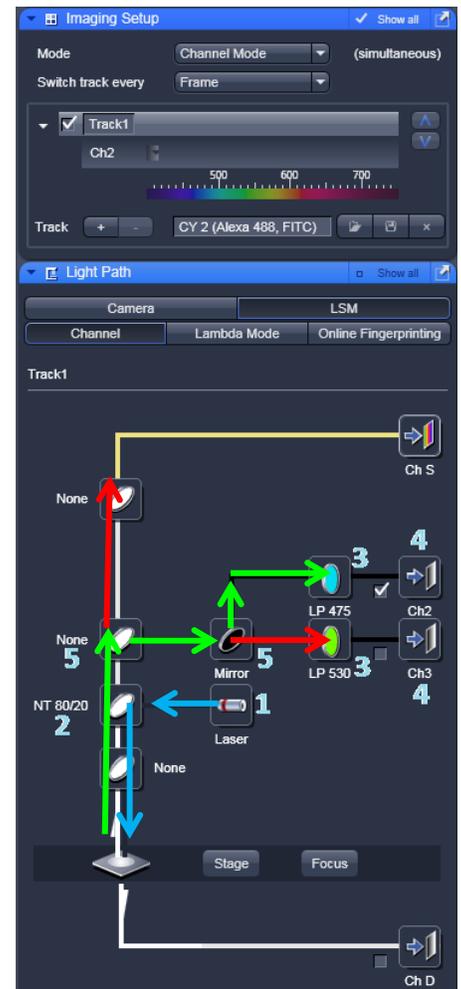
- Select the dichroics (5) that will allow the emission wavelengths you want to reach the appropriate detector.

This is based on which filter and channel you've chosen in step 5

What each dichroic does:

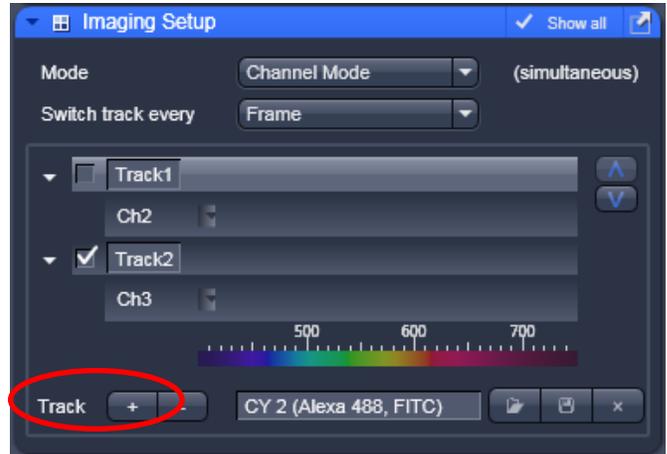
- None- allows everything to pass through (no mirrors)
- Plate- **TRANSMITS** all wavelengths
- Mirror- **REFLECTS** all wavelengths
- NFT ###- **REFLECTS** wavelengths BELOW the listed number; **TRANSMITS** wavelengths ABOVE the listed number.

- Double check the path you've set is correct.



Setting up a Multiple Track Configuration

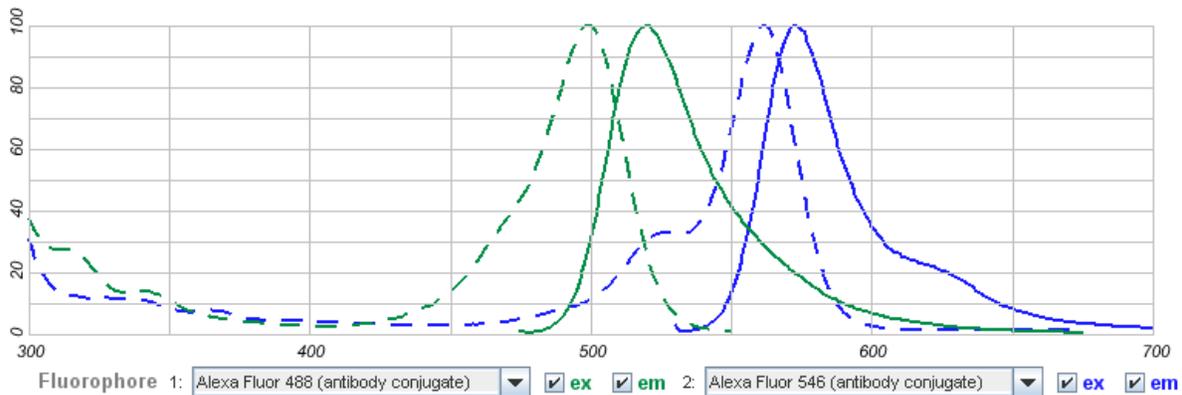
1. Select the Track + button from the Imaging Setup toolbar from the Setup Manager to input another track.
2. Add as many tracks as necessary using the Add Track button.
 - You can rename any track by typing where it says "Track"
 - Checking the boxes tells the software that it is the track you're taking a picture of. HIGHLIGHTING the track tells the software you're changing the configuration for that specific track.
 - Ex: The bottom track is checked, but the top track is highlighted. If I change any dichroics in the light path set-up it will change them for the top track ONLY.



3. Select a track and then set up the filter and dichroic configurations as described above for the single track configuration. Do this for each track. In some cases multiple tracks will have to share Channels. This is OK!!

When selecting filters, it will frequently be necessary to use band pass filters for the dyes with shorter emission wavelengths so that you are not picking up the wavelengths from the dyes with longer emission wavelengths. Be aware that emission spectra (solid lines) may also overlap and require the use of band pass filters to separate out the separate fluorochrome signals.

Also note that synchronous scanning (Switch track every "Line") of multiple fluorochromes requires that the same dichroic mirrors and filters be used for all track configurations. If this is not possible, then scanning is done sequentially (Switch track every "Frame").



Things to keep in mind for ANY Configuration

1. Only turn on the lasers you are using. If you're using the Argon (458, 488, and 514) and HeNe1 (543), you do NOT need to turn on the HeNe2 (633).
 - Please record the lasers you've used by checking the appropriate boxes on the sign-up sheet. This will allow us to accurately record usage hours of the lasers.
2. "Far red" dyes (typically dyes excited by the 633 laser) may not be visible through the oculars.
3. We do NOT have the necessary laser line to take a DAPI confocal image.