

# UF | ICBR Cytometry

University of Florida, Interdisciplinary Center for Biotechnology Research

(<https://biotech.ufl.edu>)

## CTAC ICBR SOP: Scanning with Confocal Using the Leica TCS SP5 Confocal Microscope

### Materials Required:

LCS software

Computer

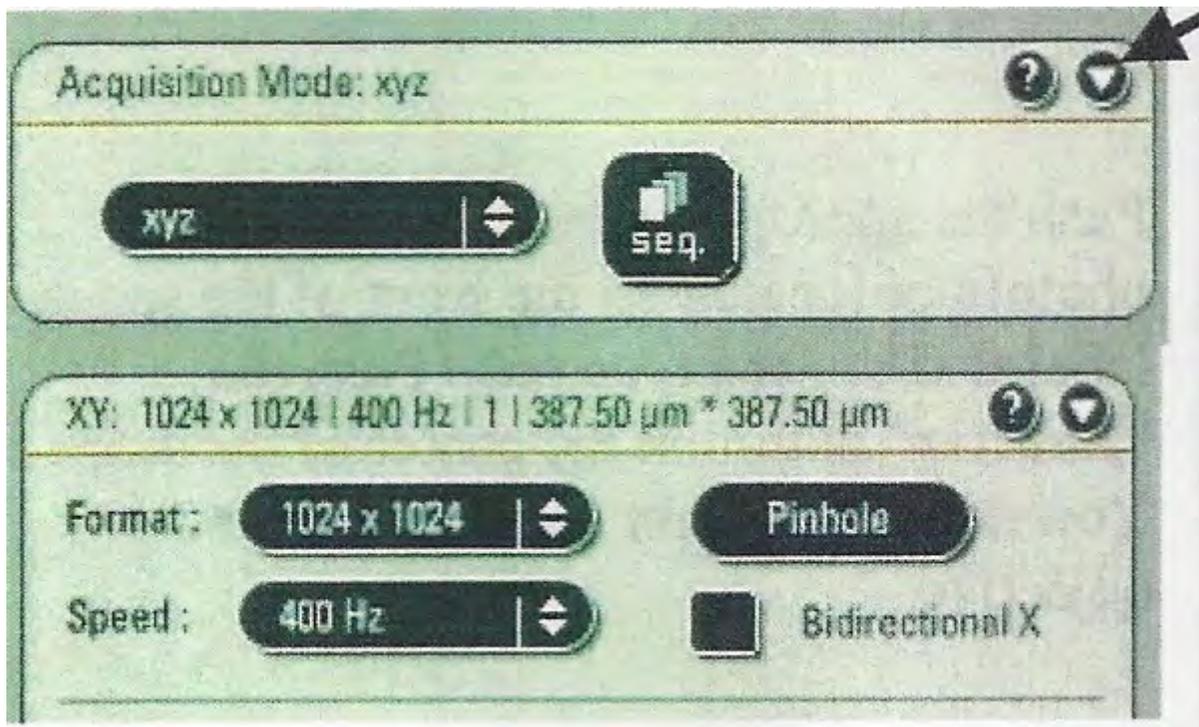
Leica TCS SP5 Confocal Microscope

### Purpose:

To facilitate operation of the Leica TCS SP5 Confocal Microscope in conducting a confocal scan of a sample.

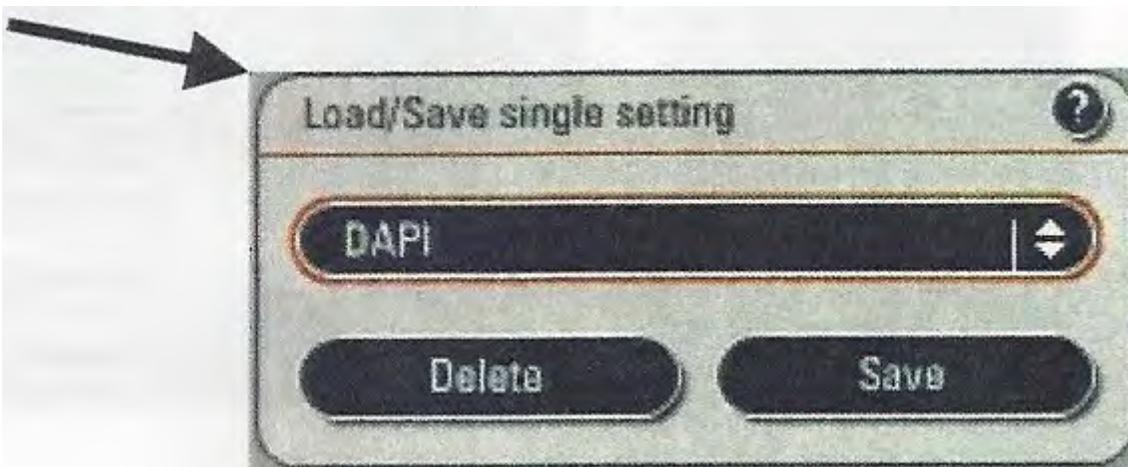
### To scan with Confocal

### Preparing to scan

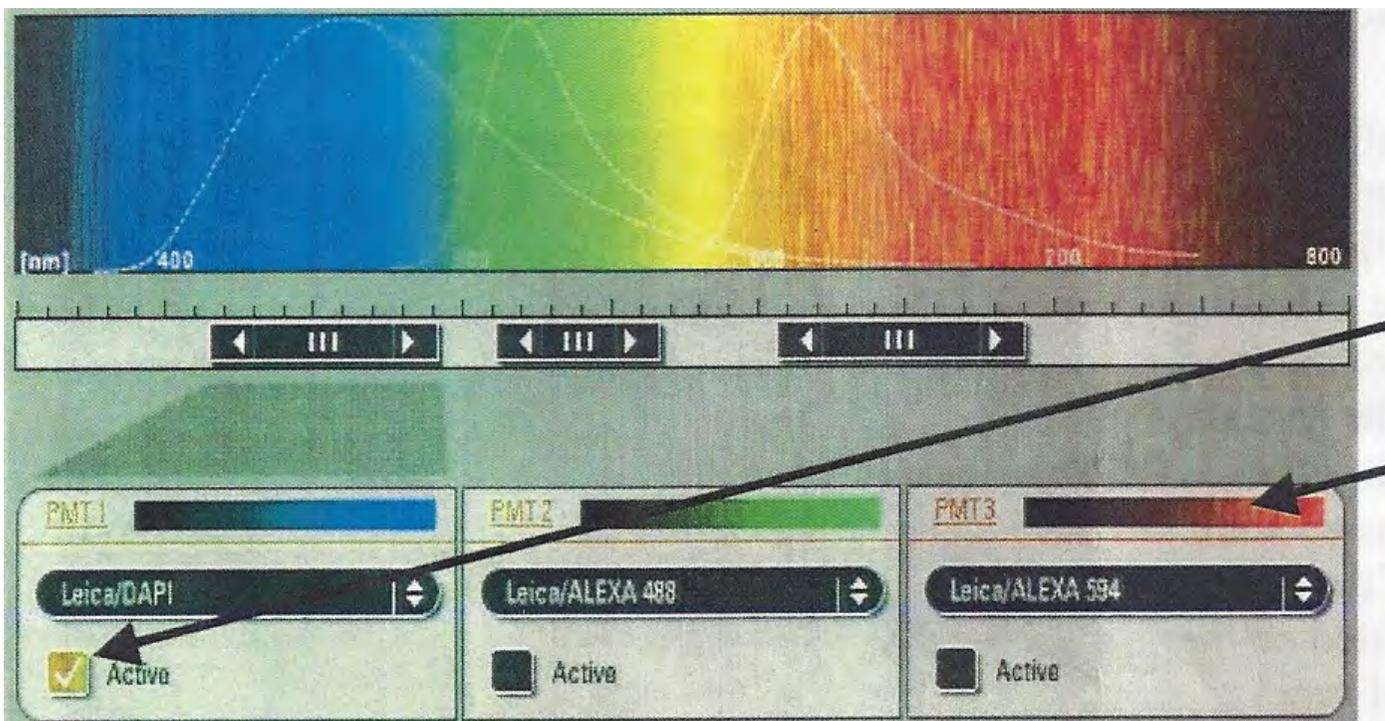


1. Under the acquisition tab, expand the window by clicking on the arrowhead (top right).
2. Make sure the “**Objective**” reading on the microscope matches the selected objective.
3. Change “**Format**” to “**1024×1024**” (this will result in bigger files but better resolution).

4. Select the proper "Mode", "xyz" is the normal mode for scanning.
5. Double click on the desired photofluor(s) at the top of the left monitor screen. If you wish to see the emission spectrum click on the drop-down menu underneath the color bar. This will place an emission spectrum curve on the spectrum in the middle of the screen.



6. Alternatively, you may manually select the desired PMT by clicking on the "Active" box. The color of your image can be changed by double clicking on the PMT color bar for each PMT.

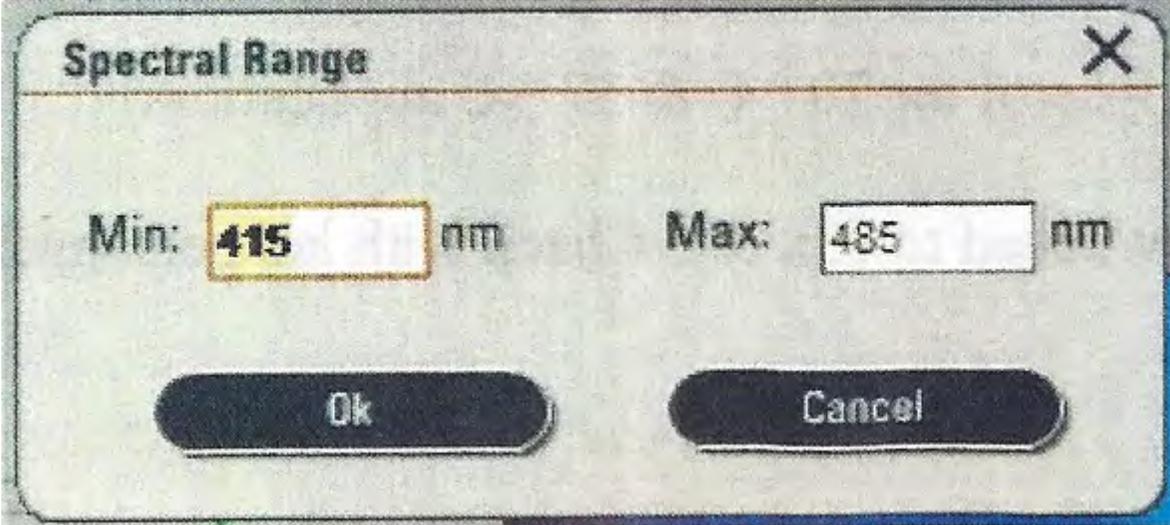


7. The emission wavelength can be adjusted in two ways:

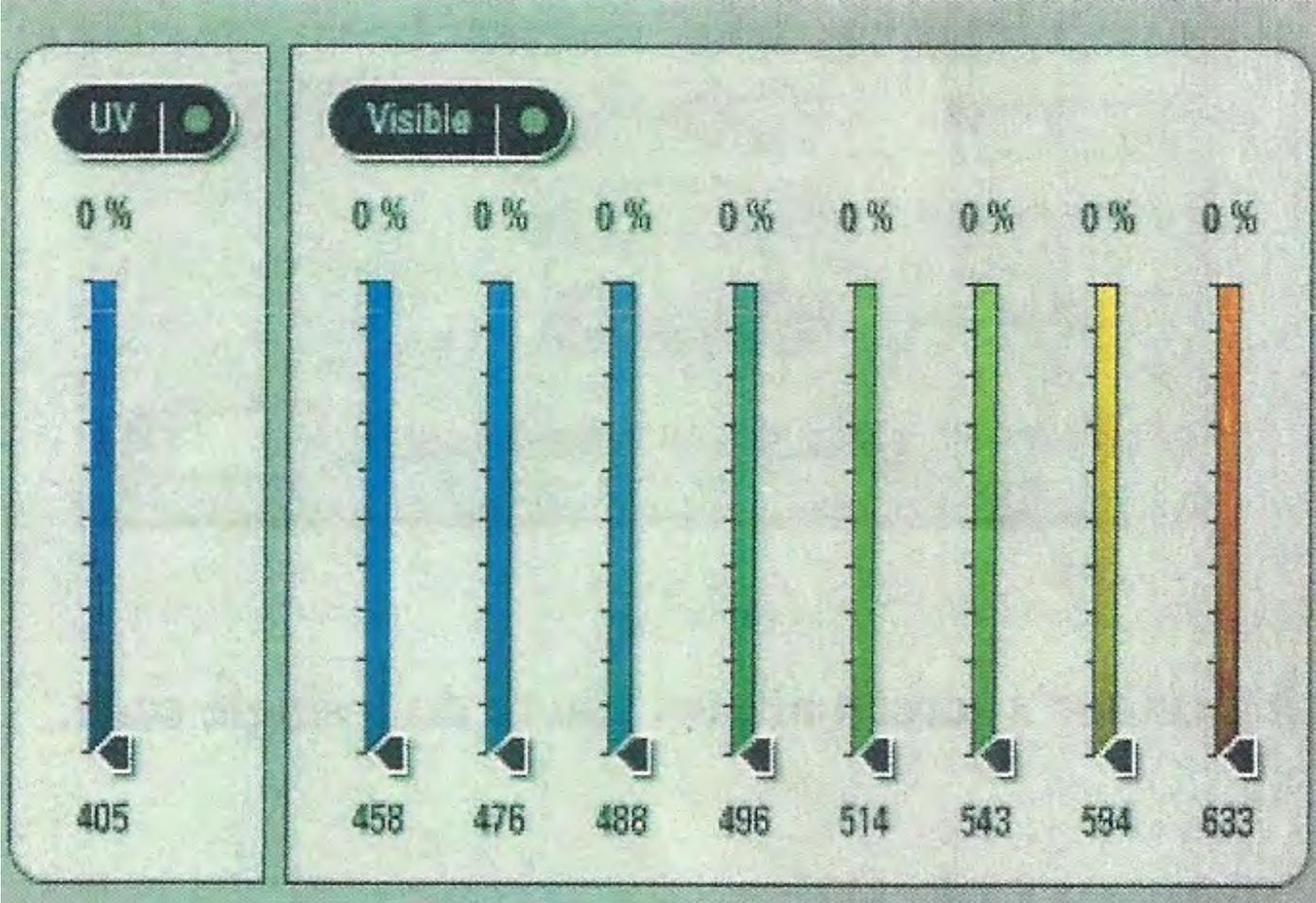
Move the mouse arrow over one edge of the horizontal black boxes until it becomes a double arrow, then drag the edge in the appropriate direction.

OR

Double click on the appropriate black slide bar – a dialogue box will appear and you can type in specific limits.



- 8. Click the "UV" button for the ultraviolet laser
- 9. Click the "Visible" button for all other lasers (or both if you want both UV and visible light).



**Important: If using more than one dye, see “[CTAC ICBR SOP: Conducting a Cross-Talk Test Using the Leica TCS SP5 Confocal Microscope \(https://ctac.mbi.ufl.edu/ctac-icbr-sop-cross-talk-test-on-the-tcs-sp5/\)](https://ctac.mbi.ufl.edu/ctac-icbr-sop-cross-talk-test-on-the-tcs-sp5/)” before continuing, in order to test for crosstalk.**

The strength of the individual lasers can be adjusted by moving the appropriate vertical slider arrow from 0 to 100%. A good starting point for this setting is **15-20%**.

**Important: Settings that are much higher than this can cause photobleaching of your samples!**

### **Beginning the scan**

1. Click on  button at the bottom of the left monitor's screen to begin scan. It will change to “**Stop**” and be highlighted.
2. On the right side of the control box, turn the “**Z POS**” knob slightly in both directions to choose the brightest portion of the sample.
3. Click  button located on the left side of the right monitor's screen. This is an aid in choosing the proper PMT settings for gain (brightness) and offset (contrast).
4. If you have more than one color, click on the image that you want to adjust. A dotted line should appear around that box.
5. Turn the “**Gain**” knob until bright blue pixels just begin to appear in the image. These pixels are saturated. Next, back the “**Gain**” knob off until the blue disappears.

6. Turn the “**Offset**” knob until green pixels begin to appear in areas where there should be no fluorescence or tissue.  
**NOTE:** The “**Offset**” knob operates in **REVERSE** as compared to most controls. Turning it to the right will give you less green, and turning it to the left will give you more green in the background.

7. Click \_\_\_\_\_ button again to get a greyscale image. Click \_\_\_\_\_ button one more time to get back to the original color image.

8. Click “**Stop**” to stop the scan when all the adjustments have been made.

9. Click on “**Line Average**” or “**Frame Average**” buttons to choose the number of sweeps by the laser to average out background noise. The greater the background, the more averaging should be done.

10. Clicking on “**Accumulation**” button allows you to stack scans on top of each other in order to boost a very weak signal.

11. Clicking on \_\_\_\_\_ button at bottom of left monitor’s screen allows you to do a single scan.

**IMPORTANT: When you are finished return the Argon laser power to 0% and clean the objectives used with clean lens tissue paper.**