

UF | ICBR Cytometry

University of Florida, Interdisciplinary Center for Biotechnology Research

<https://biotech.ufl.edu>

CTAC ICBR SOP: Conducting a Sequential Scan 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 sequential scan on a sample.

Sequential Scanning

Before conducting sequential scanning, it is recommended that testing be conducted for cross-talk. For more 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/)

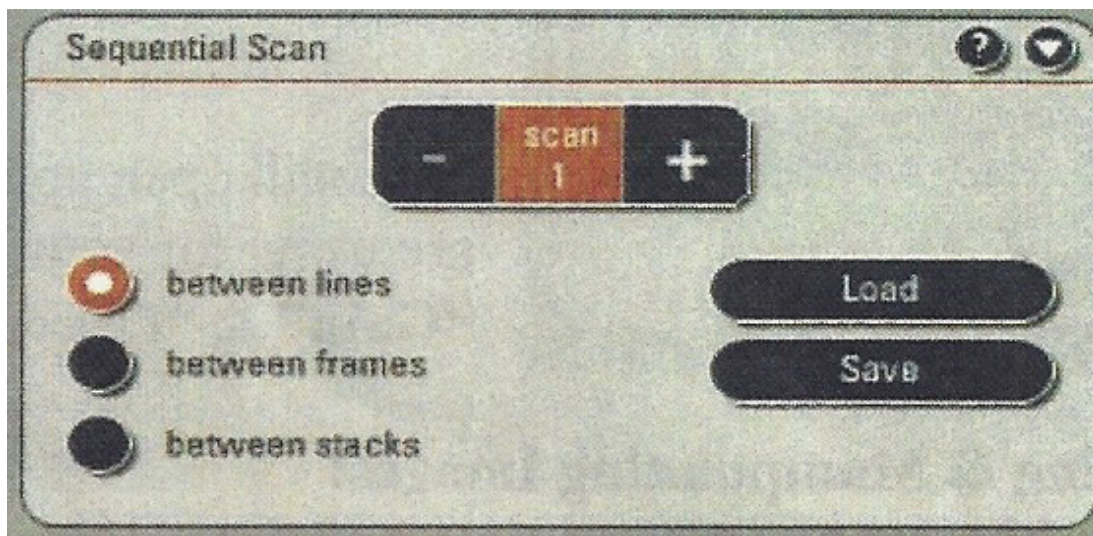
To scan sequentially (by lines)

1. Stop any current scan.
2. Beginning on the left, spread the slider underneath each PMT that you will be using to correspond to the emission spectra of the corresponding dye. You can place an emission curve on the spectrum for most fluorophores by clicking on the drop-down list beneath the PMT color bar.

IMPORTANT: Once these sliders have been set to the desired position, do not adjust them any more during the setup process!



3. Click the  button. "Scan 1" should be highlighted.
4. Select "**between lines**".



5. Set up Scan 1 by making sure that the “UV” or “Visible” button is active, the correct PMT is active and the slider for laser intensity is raised to **20%**.
6. Click the “+” button in the “**Sequential Scan**” box to move to Scan 2.

IMPORTANT: DO NOT touch any buttons after setting Scan 1 before you hit the “+” button to move to Scan 2!

7. Set up parameters for Scan 2 as in Step 5.
8. Click the “+” in the “**Sequential Scan**” box to move to Scan 3 if necessary and adjust settings in exactly the same manner as before.
9. When you have successfully made all the adjustments to do the number of scans you wish to execute, leave the “Sequential Scan” box expanded. If you need to go back to a previous scan click on the corresponding number.

IMPORTANT: Do not click the “-“(minus) button unless you wish to delete scan settings!

10. Click “**Live**”. Adjust “**Z POS**” knob to find the brightest portion of your sample.
11. As you are scanning by lines you will have all three scans on the screen. Adjust the “**Gain**” and “**Offset**” for each scan. You can switch from one scan to another by clicking on the image of the scan you wish to adjust. **NOTE:** If you need to adjust the laser intensity for a particular channel click the number of the scan to return to that scan and raise or lower the slider for laser intensity.
12. Click “**Stop**”.
13. If you wish to conduct optical sectioning, click “**Live**” and determine the beginning and end of your sample volume as described in [CTAC ICBR SOP: Conducting a Z-stack Scan Using the Leica TCS SP5 Confocal Microscope](#)
14. Choose the number of sections using either the “**System Optimized**” or “**Nr. of Steps**” buttons.
15. Click “**Start**” to begin sequential scan.



16. Click on the  button to view the images as they are scanned.

17. Scan will be finished when **Start** button reappears.

This sequential setting can be saved by clicking **Save** in the sequential scan box. When being retrieved for future use, click **Load** to recall the setting and direct the file path accordingly.

NOTE: The procedure for scanning between frames or stacks is the same except only the active channel will appear on the screen. This necessitates that you must adjust **Gain** and **Offset** individually

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