

UF | ICBR Cytometry

University of Florida, Interdisciplinary Center for Biotechnology Research

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CTAC ICBR SOP: Conducting a Z-stack Scan Using the Leica TCS SP5 Confocal Microscope

Materials Required:

LCS software

Computer

Leica TCS SP5 Confocal Microscope

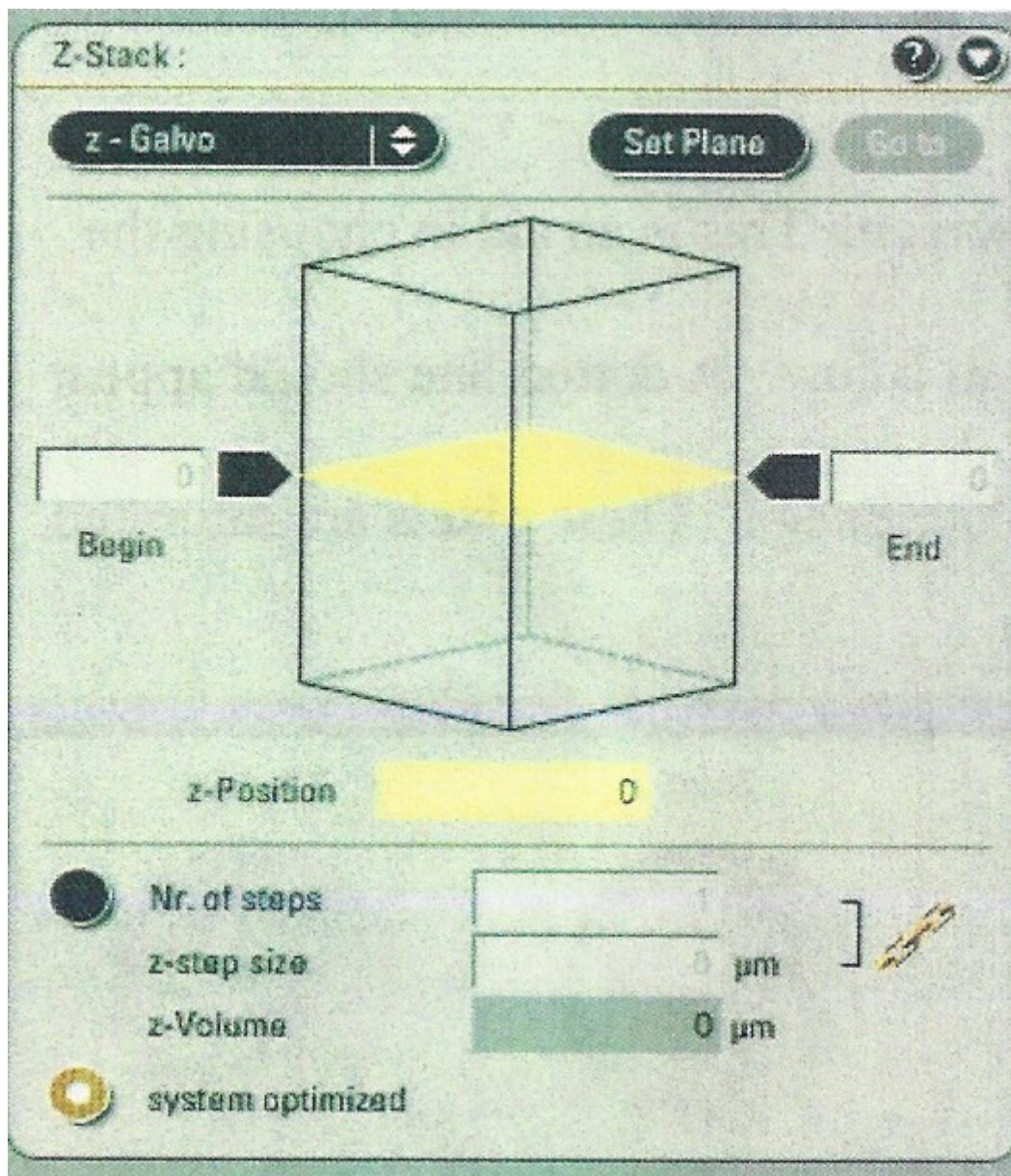
Purpose:

To facilitate operation of the Leica TCS SP5 Confocal Microscope in conducting a Z-stack operation on a sample.

To perform a Z-stack Operation

Z stack allows for multiple optical sections to be cut through the structure of interest.

Open the “**Z stack**” window on the left side of the screen. You should see a representation of a cube with two boxes labeled “**Begin**” and “**End**”.



1. Click the **"Live"** button. (It will change to stop and be highlighted). Adjust the **"Gain"** and **"Offset"** if not done previously.
2. The **"Z POS"** knob is used to set the beginning and end positions of the area to be sectioned. Turn **"Z POS"** knob to the right until the fluorescence starts to disappear. This is the part of the sample closest to the coverslip.
3. Click the black box labeled **"Begin"**. Turn **"Z POS"** knob to the left to find the end of the sample, when fluorescence begins to fade again. This is the part of the sample closest to the slide. Click the **"End"** box.
4. Click the **"Stop"** button to stop the scan. The software will suggest the optimum number of sections for your chosen volume of sample when the **"System Optimized"** button is highlighted. This will often be more sections than you want. You can click the button **"Nr. of Steps"**, and enter the number of sections that you want to scan or enter the desired section size in **"Z-step size"** and the software will calculate the number of sections needed. **Remember** to set scan for **"Line Average"**, **"Frame Average"** or **"Accumulations"** if you need these.
5. Click on the **"Start"** button to begin a Z stack series.

To view the series of sections click on the button on the right side of the working window (right monitor screen).

The Z stack scan is finished when the **"Start"** button reappears.

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