

UF | ICBR Cytometry

University of Florida, Interdisciplinary Center for Biotechnology Research

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CTAC ICBR SOP: Conducting a Cross–Talk Test 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 cross-talk test for two dyes intended for use.

Testing for cross-talk

1. Load the two dye parameters you are interested in simultaneously and adjust the “**Gain**” and “**Offset**” for each.
2. Click “**Live**” to start the scan.
3. In the beam path setting box, pull down the slider for the first dye to **0%**. The screen should go to black. If you are still seeing color there is cross-talk. Conversely, raise the slider for dye number one back up and lower the slider for dye number two. The signal for dye number two should disappear. If it does not, then again there is cross-talk.

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