In cultured neural crest cells, Ca\textsuperscript{++} release, indicated as an increase in Fluo-4 fluorescence intensity, was observed after exposure to Ionomycin. **Left** Fluo-4 fluorescence intensity in the designated region of interest during the time course of the experiment. **Right** Individual region of interest selected for analysis. Dr. Phil Brauer, Creighton University.

**Overview of Ca\textsuperscript{++} imaging experiments.**

1. When using Fluo-4 use the standard green filter set (500 to 530nm) and excitation (488nm).
2. Select **Time Series** from the LSM 510 main menu.
   a. Select the number of images to be acquired
   b. The delay, if any between images
3. Select **Start T** from the time series window to start acquiring images.
4. Use **ROI Mean** to select individual or multiple regions of in the image window during or after the scan series is complete.
   a. Select ROI shape then apply shape to image window (can select multiple areas at one time)
   b. Display individual data points by selecting show table
   c. Copy table and paste in excel for later analysis