Lab 5. Live Cell Imaging – Fluo-4

Objective: Learn the hardware and software required for efficient live cell imaging.

Samples: βTC-3 cells   Dye: Fluo-4 for $[Ca^{2+}]_i$ (cell permeant AM ester)

Background: Fluo-4 has an absorption spectrum compatible with excitation at 488 nm (blue excitation/green emission) and a very large fluorescence intensity increase in response to Ca^{2+} binding. The demonstration involves the pancreatic β beta cell line, βTC-3, which responds to glucose stimulation by fluxing calcium.

Procedure: 1. Select the 20X objective lens.

2. Find the focal plane of the cells using transmitted light. It is preferable to image a clump of these cells.

3. Select the appropriate filter configuration for collection of green fluorescent signal.

4. Acquire an image. It is appropriate to use binning (4) for this experiment. You may also want to assign a pseudocolor LUT.

5. Determine exposure time using the autoscale function. Base signal should be 25% maximum allowable signal. Turn autoscale off once exposure time is determined.

6. Set up a time series experiment using multi-dimensional acquisition. Acquire a short series of images at 5 sec intervals to familiarize yourself with the system and software. Measure the baseline signal.

7. **The experiment**: set up a time series with acquisition at 2 sec intervals (106 images in total = 3.5 min).

8. Acquire the time series. At the 16th interval, add 0.5 ml of Buffer 1 (low glucose). **What is happening?**

9. At the 46th interval, add 0.5 ml of Buffer 2 (high glucose). **What happens now?**

10. At the 76th interval, add 0.5 ml of Buffer 3 (KCl) to completely depolarize the cells. **What happens this time?**

11. Create a stack of images for viewing and analysis.

12. Create regions of interest around different cells. Use the software to plot the individual traces as a function of time for those cells.

FYI: FLUO-4 LABELING

1) Cells grown in glass-bottom dishes (MatTek).
2) Wash 2x with buffer (PBS containing Ca^{2+}/10mM HEPES, pH 7.4).
3) Incubate cells with 1 ml of buffer containing 1:500 Fluo-4 (1 μM final) for 30 min at RT (dark).
4) Wash 2x with buffer.
5) Leave a final volume of 1.5 ml buffer in dishes for imaging.