pti ion imaging system

ACQUISITION

1. Depress the power switch of the LPS-220 Lamp Power Supply, wait 2-3 seconds, and depress and hold the ignite button. The LED readout should be somewhere between 65-75 watts.
2. Depress the power switch of DeltaRAM power module.
3. Depress power switch of SC500 Shutter Controller.
4. Locate camera controller box and make sure all knobs are at their minimum settings (fully CCW).
5. Make sure the light path selector (on the right side of the microscope body, near the front) is in the A position.
6. Turn on the power switch of camera controller (located on back of unit).
7. Turn on computer, logging on to your College of Medicine if desired
8. Double click on IM icon (Image Master New Version) to load the software.
9. In the Project Organizer window click on Acquisition suing the Fura-2 or Tom1 setting.
10. In the Acquisition menu select Time-based and then Setup
    a. Set the save interval to Variable and the desired time between ratios
    b. Select Automatic Shutter Control
11. Under the file menu select Setup Default Path File
    a. Set the Data, Image and Region of Interest to the desired directory. Do not change the directory for the other selections.
12. Enter desired excitation wavelengths for DeltaRAM (340nm and 380nm for Fura-2)
13. Locate sample using Phase Contrast imaging
    a. Load 25mm round cover slip into holder and place holder into temperature-controlled stage
    b. Completely lower the objectives using the focus knob (rotate top away from you)
    c. Rotate the 40x oil lens into position and adjust the stage position so that the lens is centered in the stage opening.
    d. Carefully place a drop of immersion oil onto the lens
    e. Place the temperature-controlled stage onto the microscope stage, making sure the temp stage drops into place.
    f. Turn on the transmitted light
    g. While watching from above bring the objectives up until the oil contacts the bottom of the coverslip.
    h. Looking through the eyepieces continue to raise the lenses until the cells come into focus.
    i. Turn off the transmitted light
14. Visually check the Fura-2 fluorescence
    a. Make sure the lever on the right side of the microscope is set to Fura-2/BCECF and that the light path selector is set to A
    b. Turn off room light
    c. Select Fast Focus in the Acquisition Control Menu and select 380nm
    d. Observe cells trough eyepieces
15. Camera Setup:
    a. All settings of camera controller are at zero settings.
    b. Light path selector is set to the A position
    c. Turn transmitted light off!!!
    d. Turn off room lights.
16. Adjusting sensitivity of camera to cells:
    a. Click on the hand icon (fast focus) next to the clock on the desktop.
    b. Slowly rotate light path knob to C position, returning to the B position if image is red.
    c. If no red is visible and the image is still too dim, increase Gain on the camera controller to achieve desired intensity.
    d. If the Gain is at maximum and the image is still to dim, increase the Voltage control to desired image intensity. (When the fast focus is turned off and the pseudo color returns you should see little if no red.)
    e. Click done.

f. Close light path (go back to A).

17 Adjust Black Level setting
   a. In the Acquisition Control window, select Black Level
   b. Adjust the Black level knob on the camera controller until most of the histogram is to the left of the window and a value of .55 to .65 is displayed
   c. Select done

18 In the Acquisition Control window enter a root name and starting number. (The root name and the number can have a combined total of 8 characters so keep the names short.

19 Enter other information in the Acquisition Control window as needed.

20 To acquire a time series
   a. Set the light path knob to C
   b. Select Snapshot or Test run to see that things are working properly.
   c. Reset image number to 1
   d. Select Save run to start the experiment

ANALYSIS

1 Under Mode select Analysis

2 Load an image from the time series, preferable a 380nm image.

3 Prepare a Region of Interest file (ROI)
   a. Place ellipses or squares on the cells/areas of interest
   b. Save the ROI file

4 Under Measurement select Make Mask
   a. Set the threshold to the desired level and save

5 Under Macro select Load Macro and choose the ROI.mac setting.
   a. Read Root-Insert the root name and starting number
   b. ROI Setup-Select your ROI file and set to average and time-based
   c. Mask-Select mask file
   d. Repeat loop-Set number of times to repeat (number of ratios acquired)

6 Select Run

7 Save resulting text file for further analysis or graphing