Single cell lab

NOTE-1: The **data processing section** (points (a) through (e) from pages 1 and 2) can be done by one representative student of each group, however everyone is encouraged to use the software provided its widespread use in the research community. You will need to download ImageJ and the ROI importer plugging from the following links:

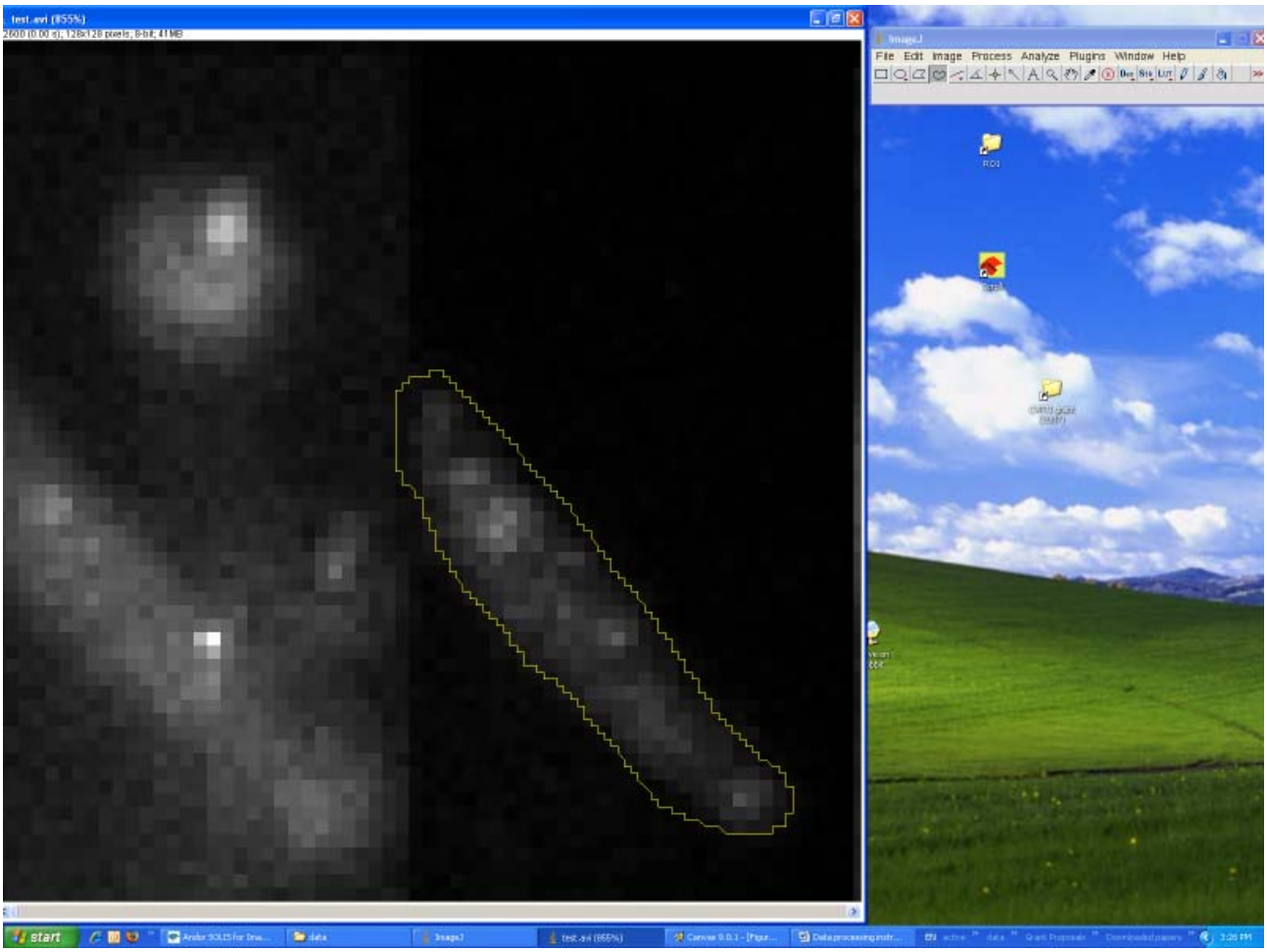
http://rsbweb.nih.gov/ij/index.html

http://rsbweb.nih.gov/ij/plugins/roi-importer.html

NOTE-2: AVI files will be provided by the instructor and are the following: two movies recorded with the infra red shifted voltage sensitive dye (IR-VSD) during perfusion with control and high potassium solution; two movies recorded with the calcium sensitive probe Fluo-4 during perfusion with control and isoproterenol containing solutions; one movie recorded in transmitted light mode. This final movie is recorded from same cell used for Fluo-4 recordings and should be used to create the mask for that cell.

a. To read the AVI file (provided by instructor) in ImageJ, select menu item File/Open in ImageJ. "Open" dialog window will appear. Select the AVI file of interest. The "AVI Reader" dialog window will open. Check "Convert to Grayscale" checkbox. Click "OK" button. ImageJ will open the AVI file in a separate window:

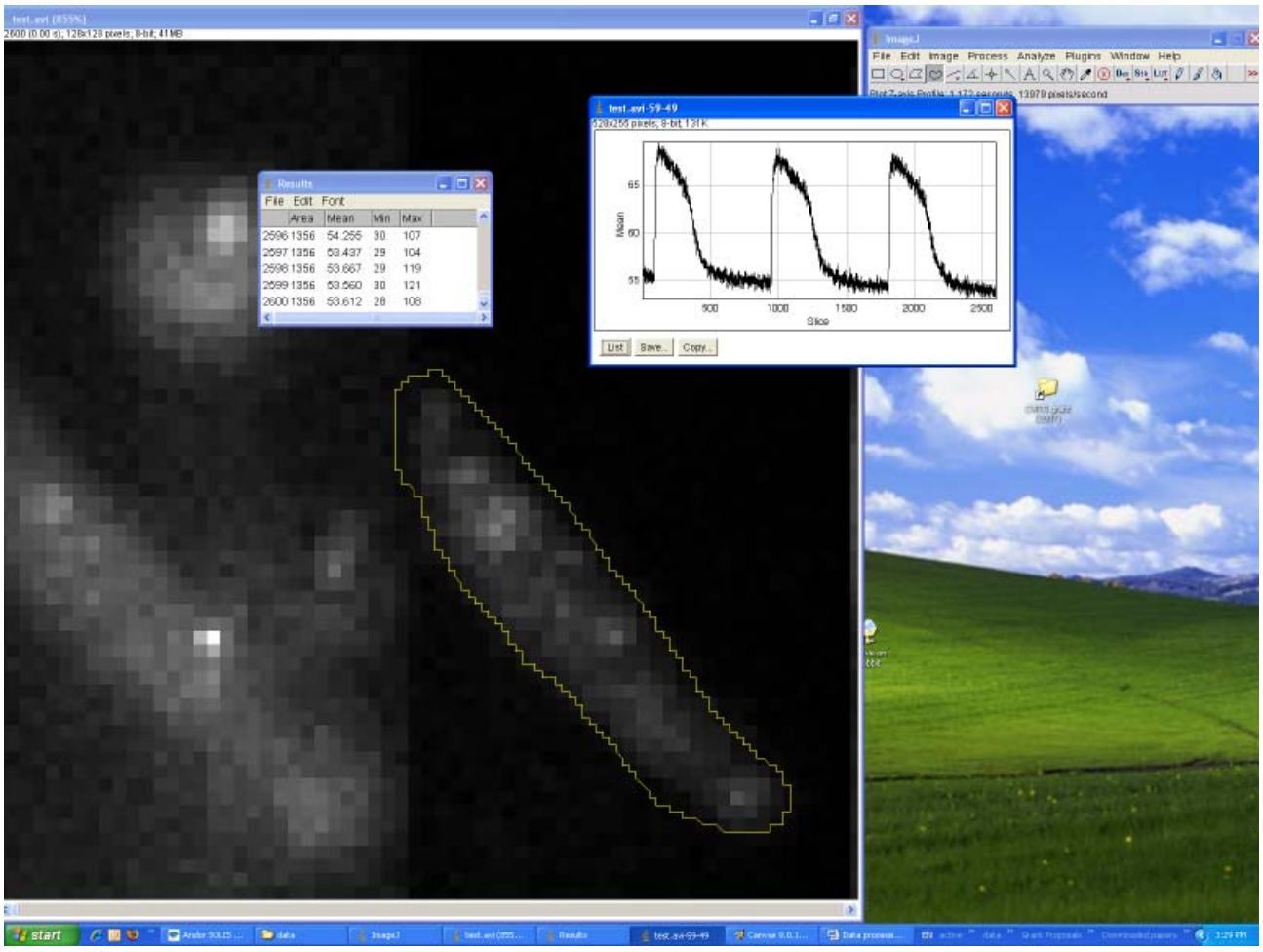
b. To create a mask (only one mask for each cell is needed), pick the "Freehand selections" tool from the ImageJ toolbar and select the area containing the cell image for a particular AVI movie (for example: cell#1-MOVIE.avi). Note that the movie can have more than one cell image. Also, there can be cell debris or other bright objects which are basically garbage. Ask the instructor if you are not sure how to select the cell. Once the cell area has been outlined with the freehand selection tool go to "Save as" and select "XY coordinates". This will create a text file (for example: cell#1-MASK.txt) containing the coordinates of the polygon enclosing the cell.



c. To create a time sequence of the signal recorded in a particular movie, open the corresponding AVI file with ImageJ as previously described. Load the mask by going to the "Plugins" tab and select "ROI importer". Use the dialogue box to select the mask file (for example: cell#1-MASK.txt). This will automatically superimpose the mask over the movie. To average the signal over the cell area, go to menu item Image/ Stacks/Plot Z-axis profile. ImageJ will create a stack of statistical values calculated for the selected area in all frames of the movie:

d. We are interested only in the average value versus time (time series). In order to save the time series, click "Save" button in the window displaying the time series. A "Save As text" dialog box will appear. Type in the file name, add extension ".txt" and save the text file for subsequent analysis at home. The file contains two columns (frame number and the average level of signal) separated by TAB character. You can use software of your choice (e.g., Matlab, Excel, etc.) to load this text file and analyze it at home.

e. Repeat the steps above for as many original data files and cell images as needed.



Homework assignment

Note: A simultaneous electrical and optical AP recording will be provided to the students for the purpose of validating the optical method of AP recording as a part of their homework assignment). The electrical recording of the AP is obtained using whole cell current clamp protocol under experimental conditions identical to those used in this lab. Given the time and space constraints, it would not be practical to require the students to obtain their own patch clamp recordings of the AP in cardiac myocytes during this lab.

Once data files have been created by one representative of each group, each student will please do the following:

1) Describe briefly the experimental setup, the experimental object, the main advantages and limitations of the optical method in case of whole heart and single cell. The description should be brief and minimally sufficient to cover all aspects of the labs.
2) Please follow instructions:
    a. For the optical AP recording, measure and tabulate the following parameters for baseline conditions and in the presence of high K^+:
        i. the resting level of florescence (F_0)
        ii. systolic level (F),
        iii. $\Delta F=F-F0$
        iv. $\Delta F/F0$
        v. AP rise time (time between 10 and 90% of the AP upstroke),
        vi. APD at the level of 90% of repolarization (APD_{90}). (Due to a large drift of the VSD signal in this particular recording it may be difficult to accurately calculate APD_{90}. Please be creative and try to find a solution for the accurate calculation APD_{90}. Explain what you think is the origin of this drift and why it may be more prominent than expected in this particular recording).
        vii. Measure RMS noise and estimate signal-to-noise ratio.
    b. Summarize and explain (very briefly) main effects of high $[K^+]_o$.
    c. Compare the AP rise time and APD_{90} in simultaneous electrical and optical recordings (*provided by the instructor*). Make a conclusion with regard to validity of optical AP as a surrogate of electrical AP.
    d. For the optical Ca transient, measure and tabulate the following raw fluorescence parameters for baseline conditions and in the presence of norepinephrine:
        i. the diastolic level of florescence (F_{rest})
        ii. systolic level (F),
        iii. $\Delta F=F-F_{rest}$
        iv. Measure RMS noise and estimate signal-to-noise ratio.
    e. Convert fluorescence into $[Ca^{2+}]_i$ using equation

$$[Ca^{2+}] = \frac{K_d R}{K_d / [Ca^{2+}]_{rest} + 1 - R}$$

where R is the normalized fluorescence (F/F_{rest}) and K_d is the dissociation constant for the Ca^{2+}-

fluo-4 complex.
Assume $[Ca^{2+}]_{rest}$ = 100 nM and K_d = 840 nM.
See file **"Fluorescence primer.pdf"** for more detail.

f. Measure and tabulate the following parameters of calibrated Ca transient:
   i. Ca transient amplitude ($[Ca^{2+}]_{max} - [Ca2+]_{rest}$)
   ii. Ca transient rise time (time between 10 and 90% of the Ca transient upstroke),
   iii. Ca transient relaxation half-time (time between the peak value and the time when Ca transient declines to 50% of its amplitude).
   iv. Ca transient duration (at the level of 50% of the amplitude)

Summarize and explain (very briefly) the main effects of norepinephrine on Ca transient.