Edge Segmentation Software:

Abstract: This program takes as input 16 bit *.tif files from the lattice light sheet that contain image sections of stained cells. After running, the software will output information describing the very outer edge of the cell. With each image, the code begins with an initial kmeans clustering step, a coarse-to-fine estimation of the outer edge of the cell and a final kmeans clustering step to estimate the cell's outer edge.

Prior to Executing MatLab Code: Using ImageJ, please convert the image stacks for each of your channels to individual *.tif files.

Directions:

Part 1: Selecting Images

1) Please run the "image_segment.m" function located in the Version 4 folder. The following window appears (Fig. 1).
2) Click on "Select File" and locate the image in the stack that you would like to start with[1].
3) Click on the "Go!" button

Part 2: Enter Number of Cells per Frame

1) In the MatLab command line, a prompt will appear asking if you would like to examine 1 or 2 cells. Enter '1' or '2' and press enter

Figure 1: Edge Segmentation GUI

Part 3) Segmentation

1) A series of windows will appear requesting that you draw regions (polygons) on images using the mouse.

    a. General Rules: (1) Left click to create vertices (2) Triple left click to close polygon

2) In sequence, the 3 windows shown in Figure 2 will appear. Please draw polygons as needed to select the background, area of interest and the cell of interest.
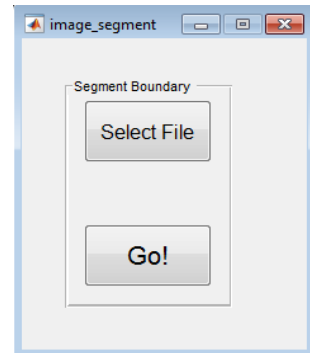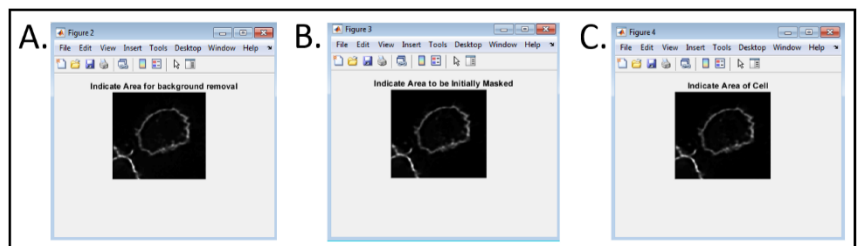

Figure 2: User-selected Regions: (A) A polygon needs to be drawn where background will be estimated. (B) A coarse polygon should be drawn to indicate which part of the image the cell is in. (C) A final polygon is then drawn coarsely around the cell of interest

Part 4) Checking Outputs

1) Once the user has selected necessary regions, the program will display the calculated boundary of the cell (Figure 3).
2) Left click anywhere on the image if you would like to save the boundary and analyze the next cell.
3) Right click if you would like to repeat the procedure to calculate the boundary of this image slice.

Part 5) Description of folders and files outputted by the program into the directory where images are contained
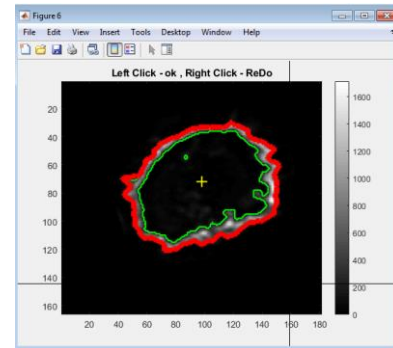
Figure 3: Checking Outputs: This image slice displays the center (yellow cross), the initially calculated boundary (green) and the final boundary to be saved (red).

1) "Images_Boundary_Masked" – 16bit *.tif images that contain intensities only from the calculated outer edge of the cell
2) "The Boundaries" – These are MatLab matrices which contain the calculated boundaries of each image.
3) "Images_w_Boundary" – These are RGB images stored which record images similar to Figure 3 for each of the images analyzed.