**Fluorescence Video Analysis Manual**

**Beginning analysis**

1. Open Matlab to folder containing fluorescence video analysis software by clicking “Open” and navigating to the folder where the software is saved

**Manual ROI labeling**

1. Label Cell ROIs using Matlab’s ROI analyzer.
   1. Open Matlab, and click the drop down button under the “APPS” tab
   2. Open the “Image Labeler” application

Graphical user interface, application, Word

Description automatically generated

* 1. Click the arrow below “Load” and select “Add images from folder”

Graphical user interface, text, application, email

Description automatically generated

* 1. Select and open the .TIFF images you want to analyze
  2. Click the “Label” button on the left

Graphical user interface, application

Description automatically generated

* 1. Enter an ROI name, select a color (optional), and set the label type to “Pixel label”

Graphical user interface, application

Description automatically generated

* 1. Create the ROI by clicking the image to form a polygon

Graphical user interface, application

Description automatically generated

* 1. Repeat as desired

Graphical user interface, application

Description automatically generated

* 1. Under the “LABEL” tab, click “Export Labels” and select “To file”

Graphical user interface, application

Description automatically generated

* 1. Select a location and name for the labels

Graphical user interface, text, application

Description automatically generated

* 1. The Label we will use is an image in .png format located in the “PixelLabelData” folder at the location described above

A screenshot of a computer

Description automatically generated with medium confidence

1. Analyze fluorescence changes using the manually labeled ROIs
   1. Run the file “loadManualROIsAndAnalyzeSequence” by typing the name in the command window and pressing enter, as shown below

Graphical user interface, application, Word

Description automatically generated

* 1. Select the .png file containing the labels that was created in step 1.j

A screenshot of a computer

Description automatically generated

* 1. Select the folder containing the .TIFF files of interest

A screenshot of a computer

Description automatically generated

* 1. Use the graph of fluorescence vs frame number to select the frame number for both the baseline measurement and the peak measurement

Chart, line chart

Description automatically generated

* 1. Save the analysis output

Graphical user interface, text

Description automatically generated

* 1. Two files will be created from the analysis

A screenshot of a computer

Description automatically generated with medium confidence

* + 1. One containing the fluorescence values from the ROI’s from each frame

Graphical user interface, application, table, Excel

Description automatically generated

* + 1. The other containing a summary of the analysis

Graphical user interface, application, table, Excel

Description automatically generated

1. To use the same labels for multiple image stacks, simply repeat step 2 and select a different folder of .TIFFs while choosing the same label .png

**Automated ROI detection**

1. Run the “findAndSaveROICentroids” file to automatically find the centers of the cell ROIs

Graphical user interface, application, Word

Description automatically generated

1. Select the folder with the desired .TIFF images

A screenshot of a computer

Description automatically generated

1. Run “loadROIAndAnalyzeSequence” to analyze the automated ROIs

Graphical user interface, text, application

Description automatically generated

1. Use the graph of fluorescence vs frame number to select the frame number for both the baseline measurement and the peak measurement

Graphical user interface, chart, histogram

Description automatically generated

1. Save the analysis output

Text

Description automatically generated

1. As with the manual selection mode, there are two output files, one with the fluorescence values having ROIs as columns and rows as frame numbers, and another with summary information