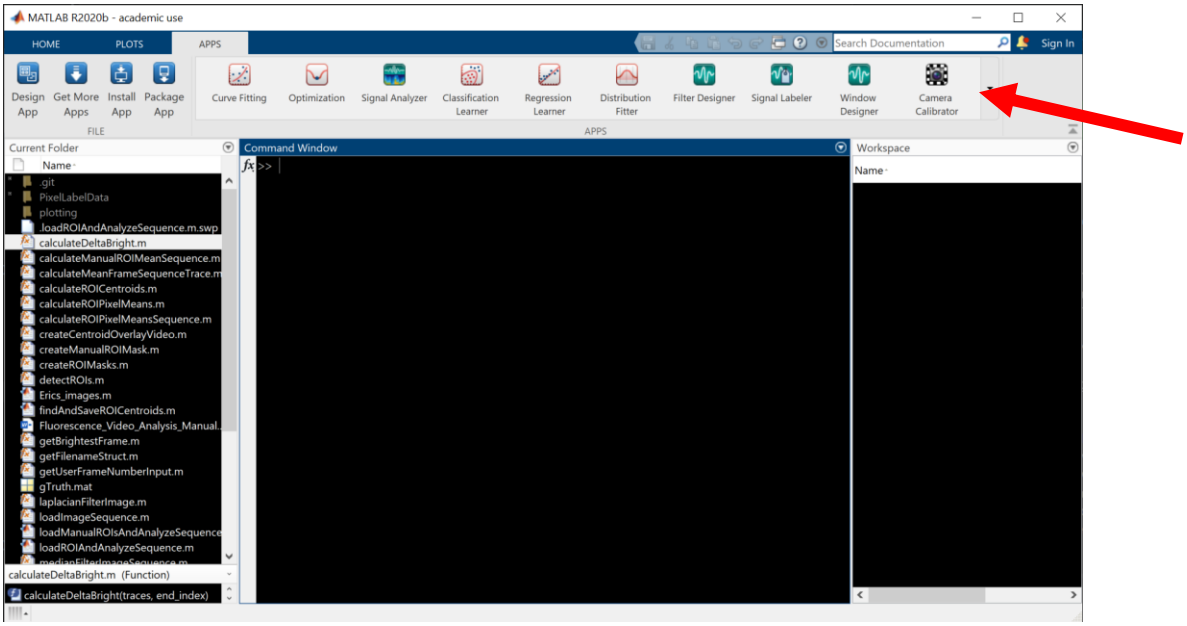
Fluorescence Video Analysis Manual

Beginning analysis

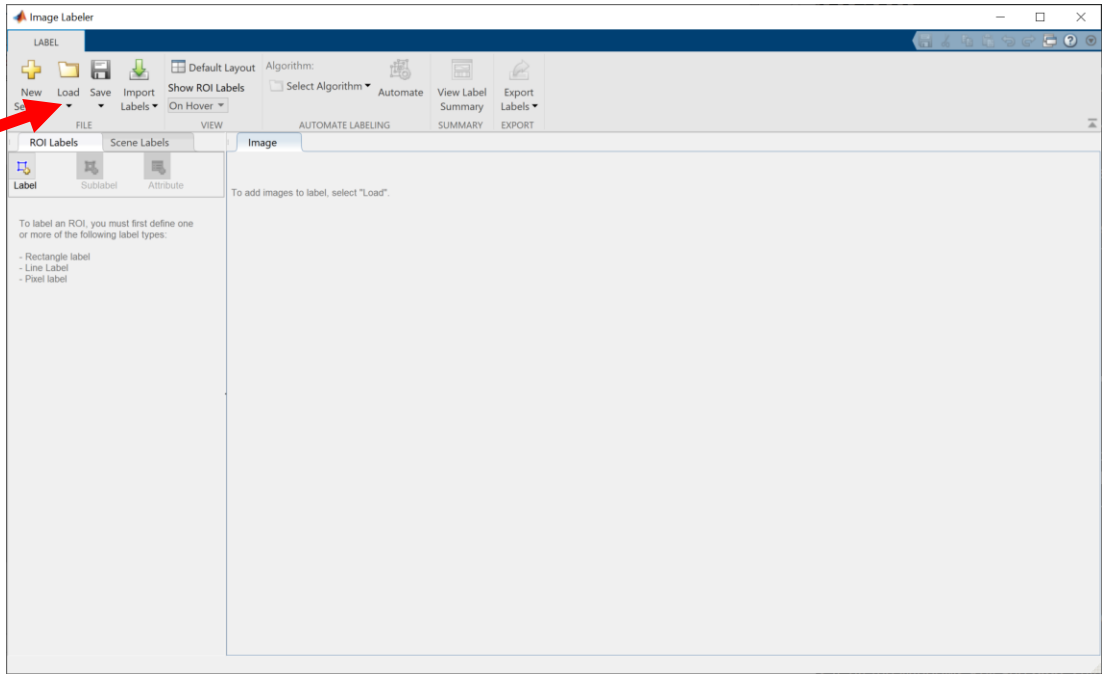
1. Open Matlab to folder containing fluorescence video analysis software by clicking “Open” and navigating to the folder where the software is saved

Manual ROI labeling

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

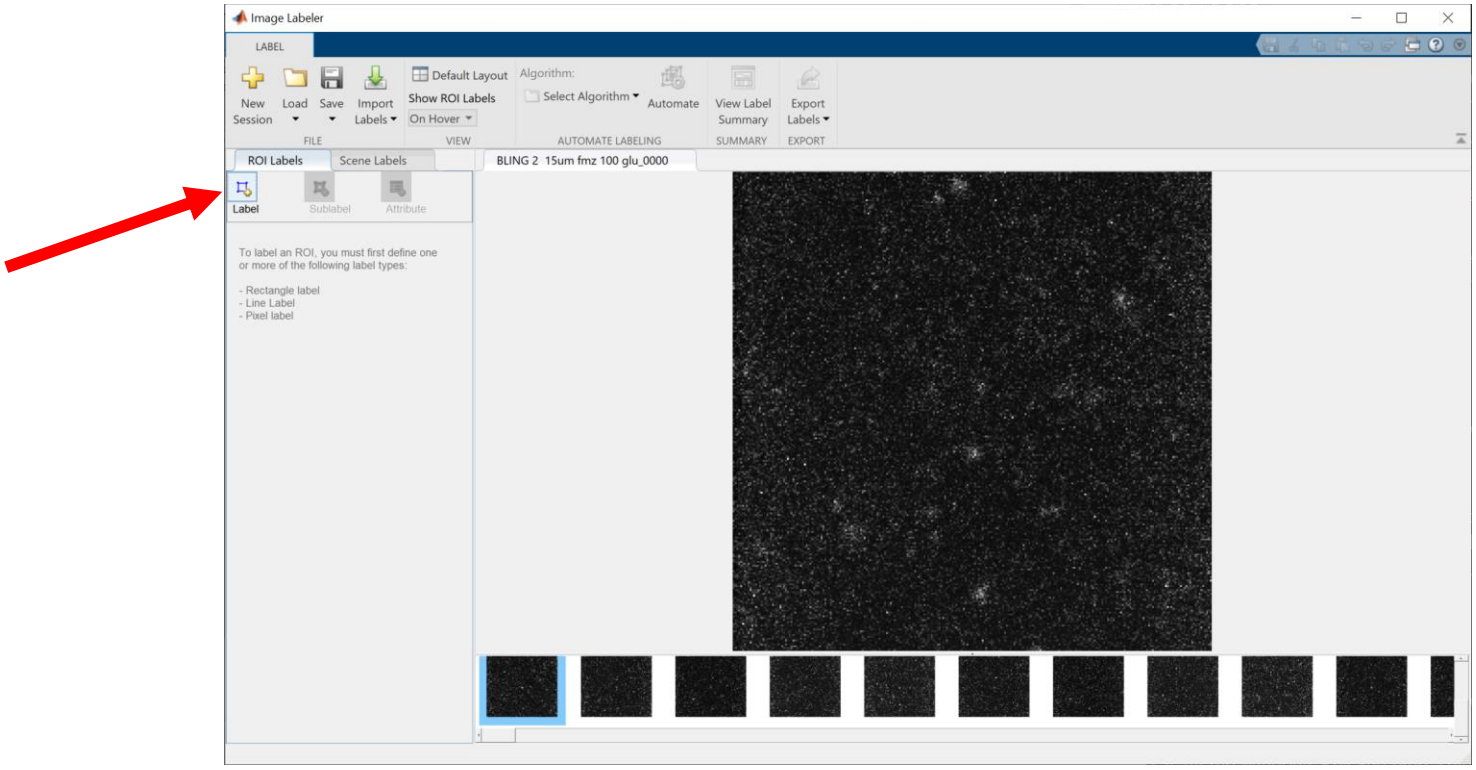


    c. Click the arrow below “Load” and select “Add images from folder”

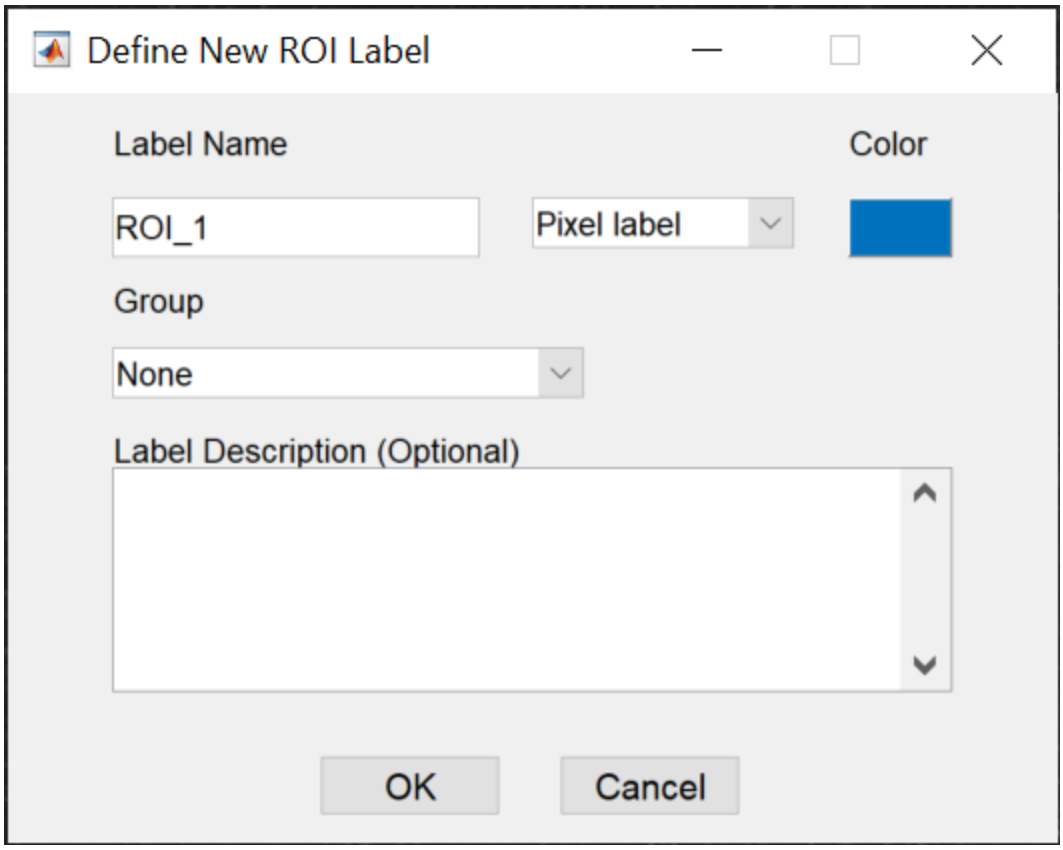


d. Select and open the .TIFF images you want to analyze

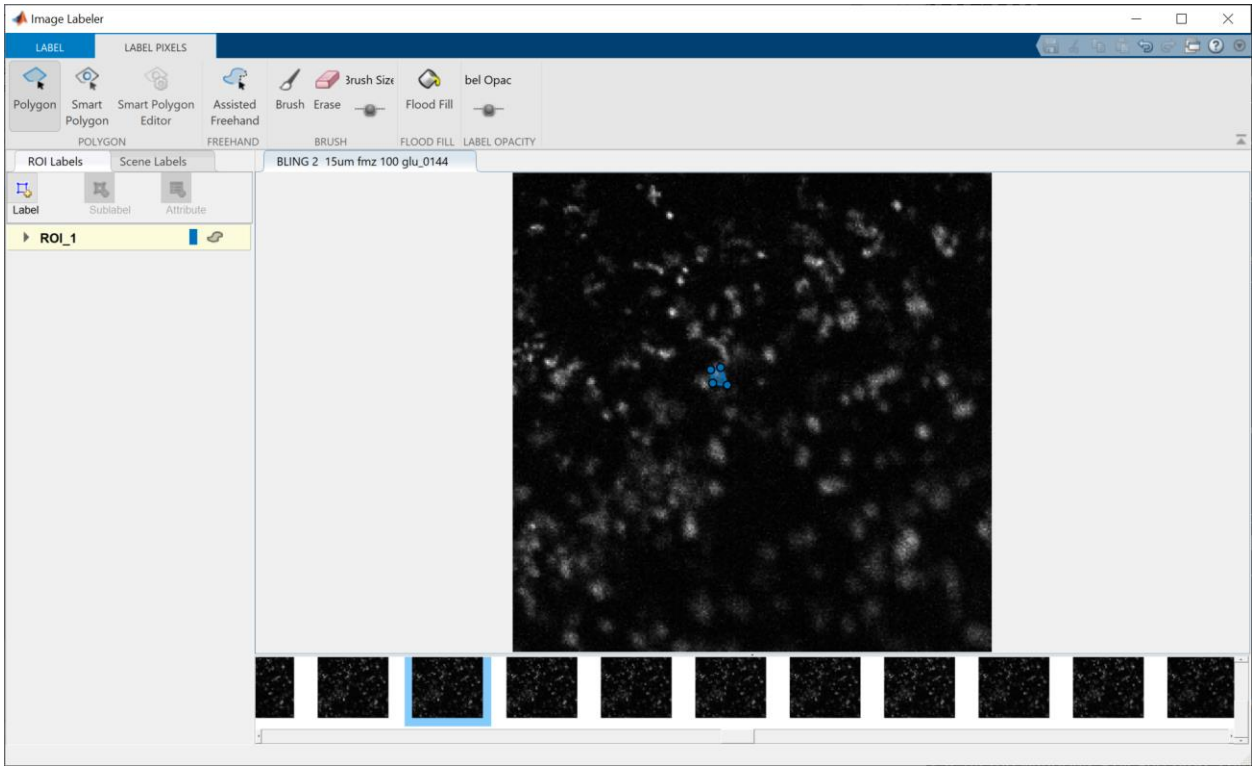
e. Click the “Label” button on the left



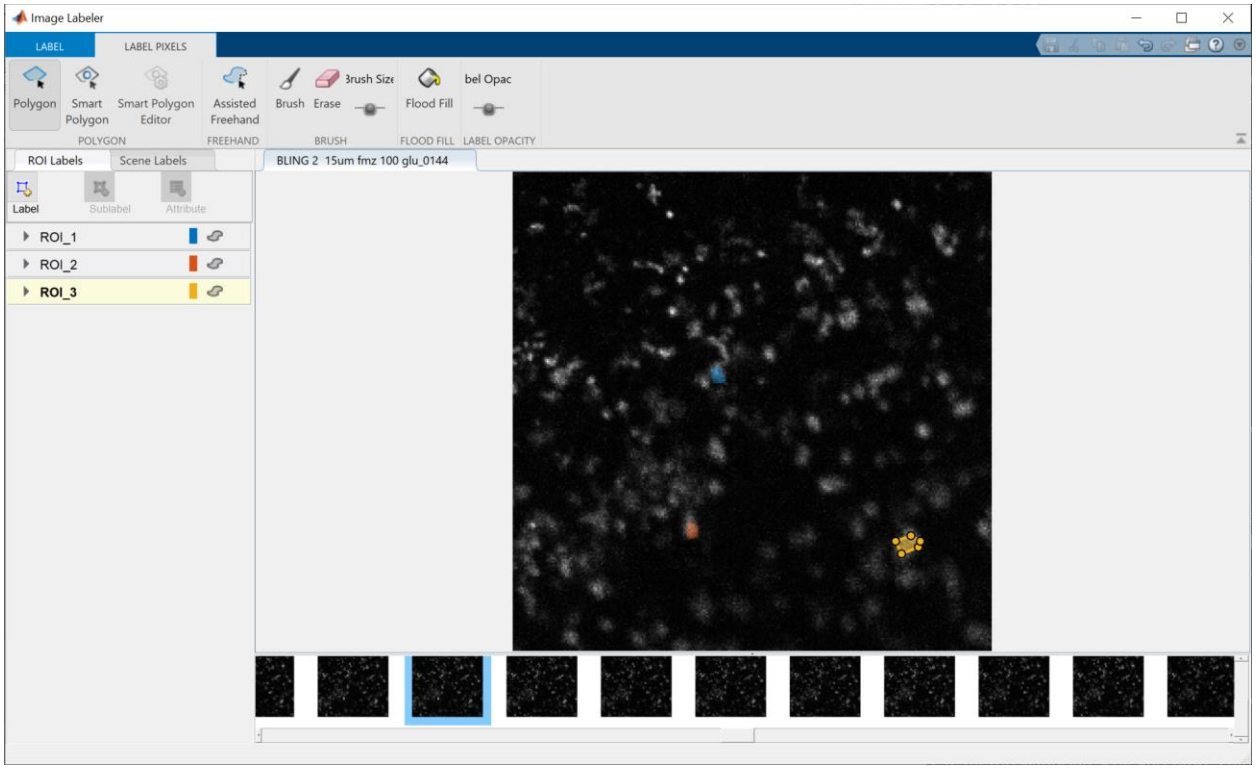
f. Enter an ROI name, select a color (optional), and set the label type to “Pixel label”



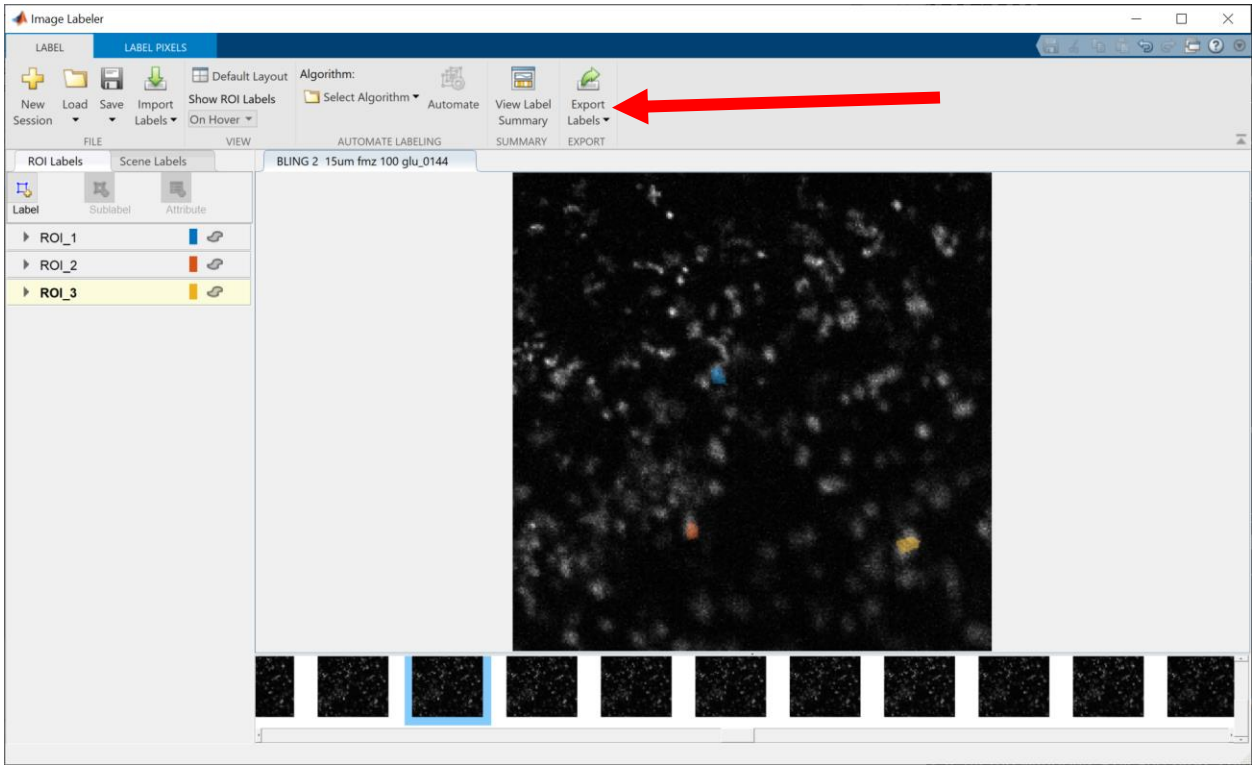
g. Create the ROI by clicking the image to form a polygon



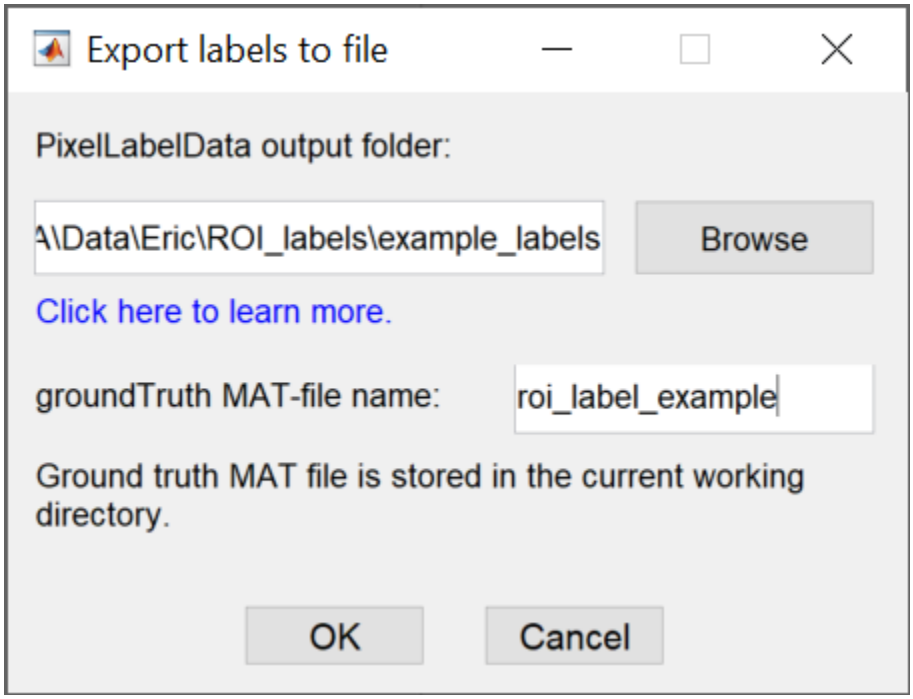
h. Repeat as desired



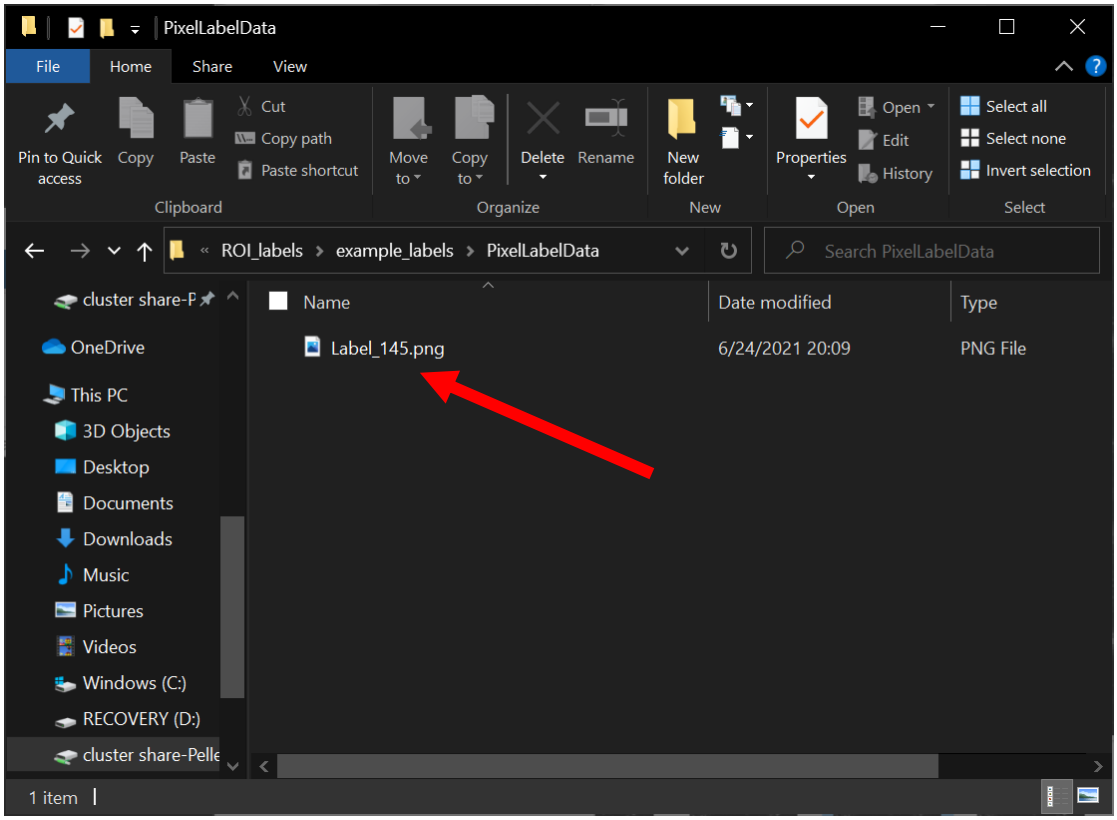
i. Under the “LABEL” tab, click “Export Labels” and select “To file”



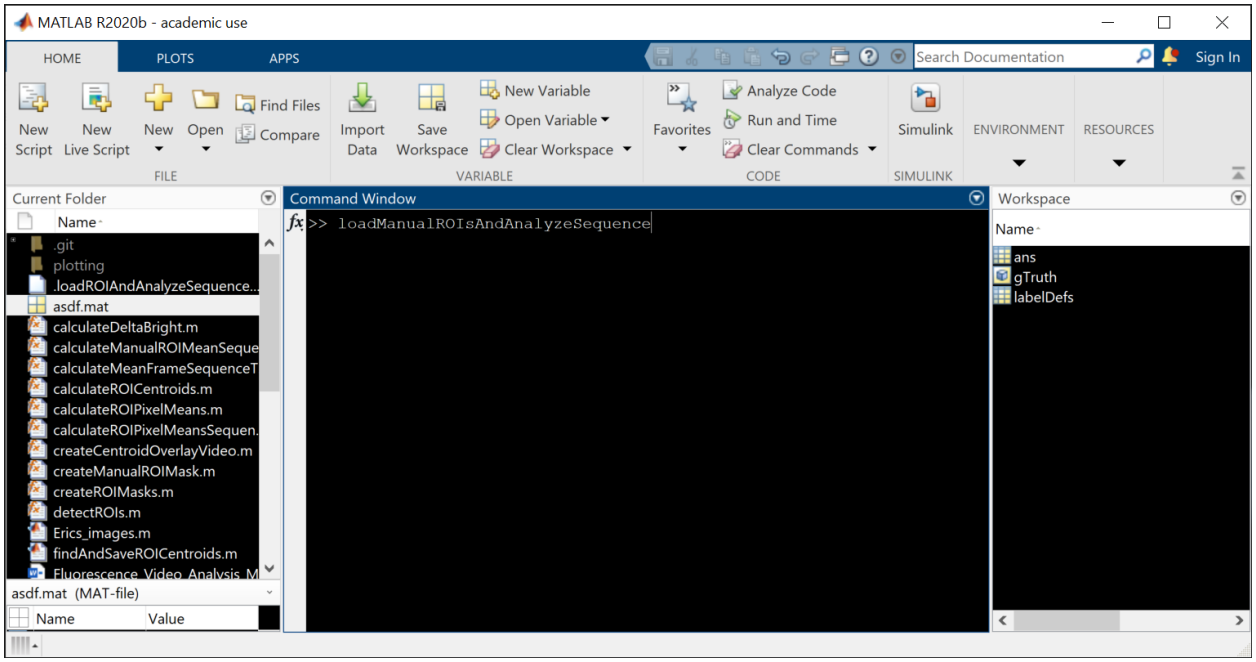
j. Select a location and name for the labels



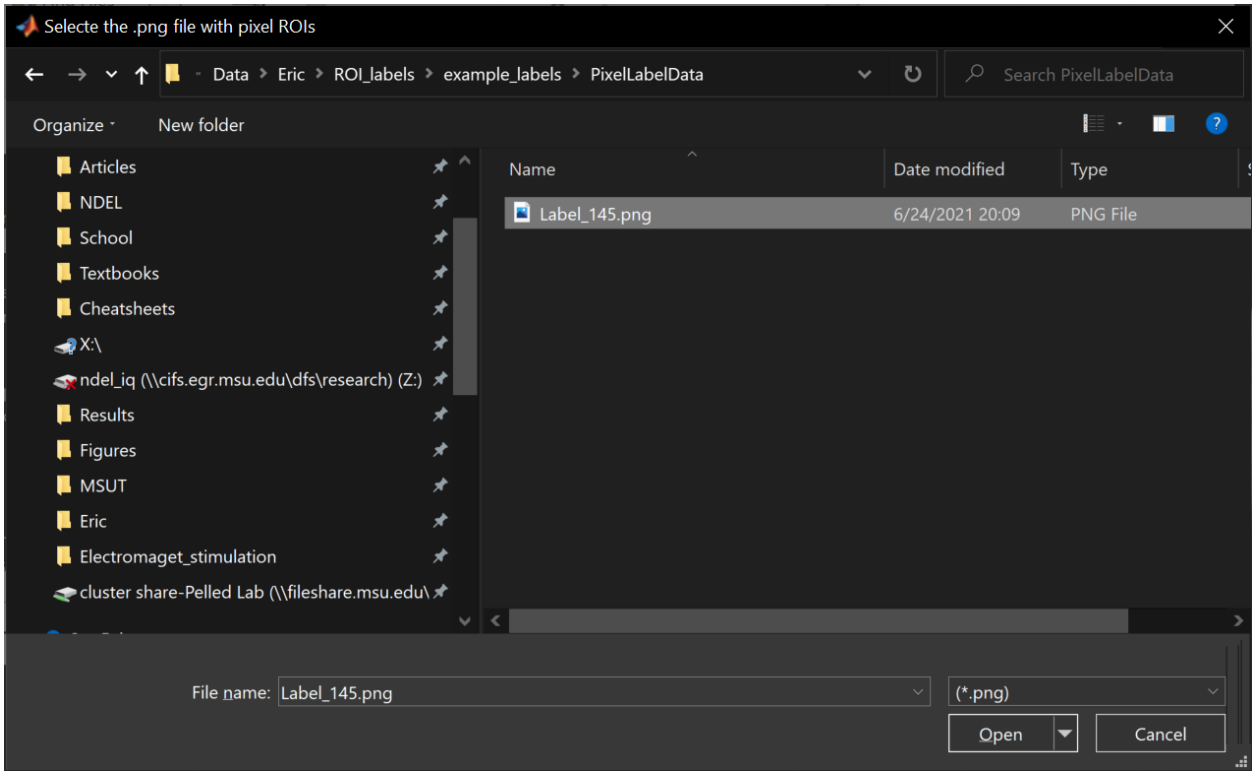
k. The Label we will use is an image in .png format located in the “PixelLabelData” folder at the location described above



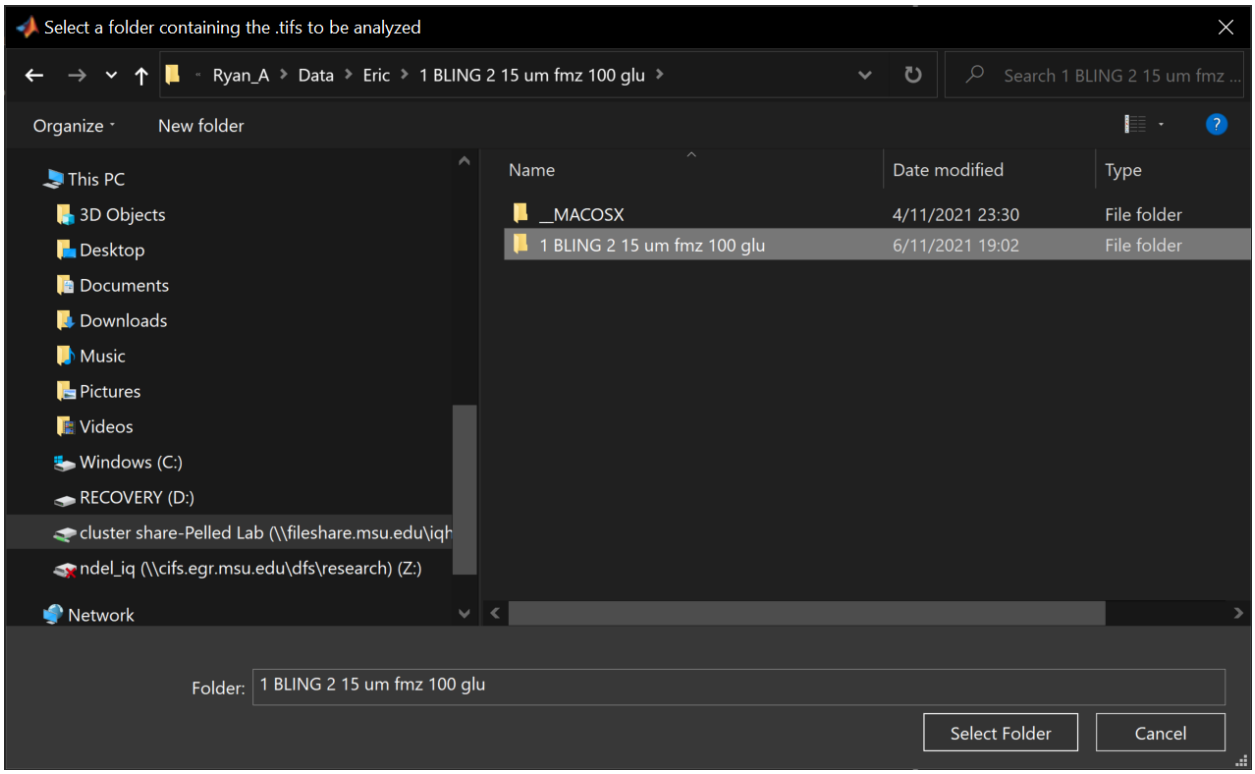
2. Analyze fluorescence changes using the manually labeled ROIs
    a. Run the file “loadManualROIsAndAnalyzeSequence” by typing the name in the command window and pressing enter, as shown below



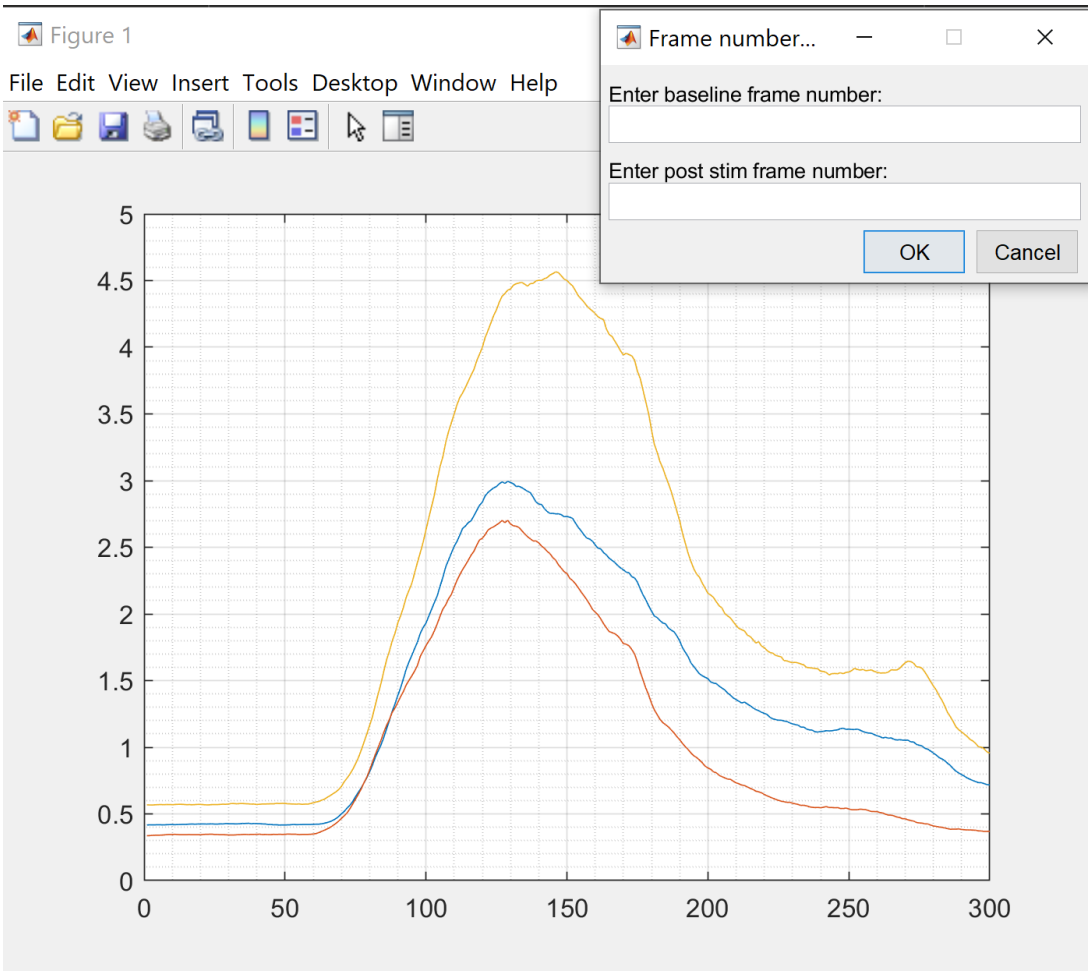
    b. Select the .png file containing the labels that was created in step 1.j



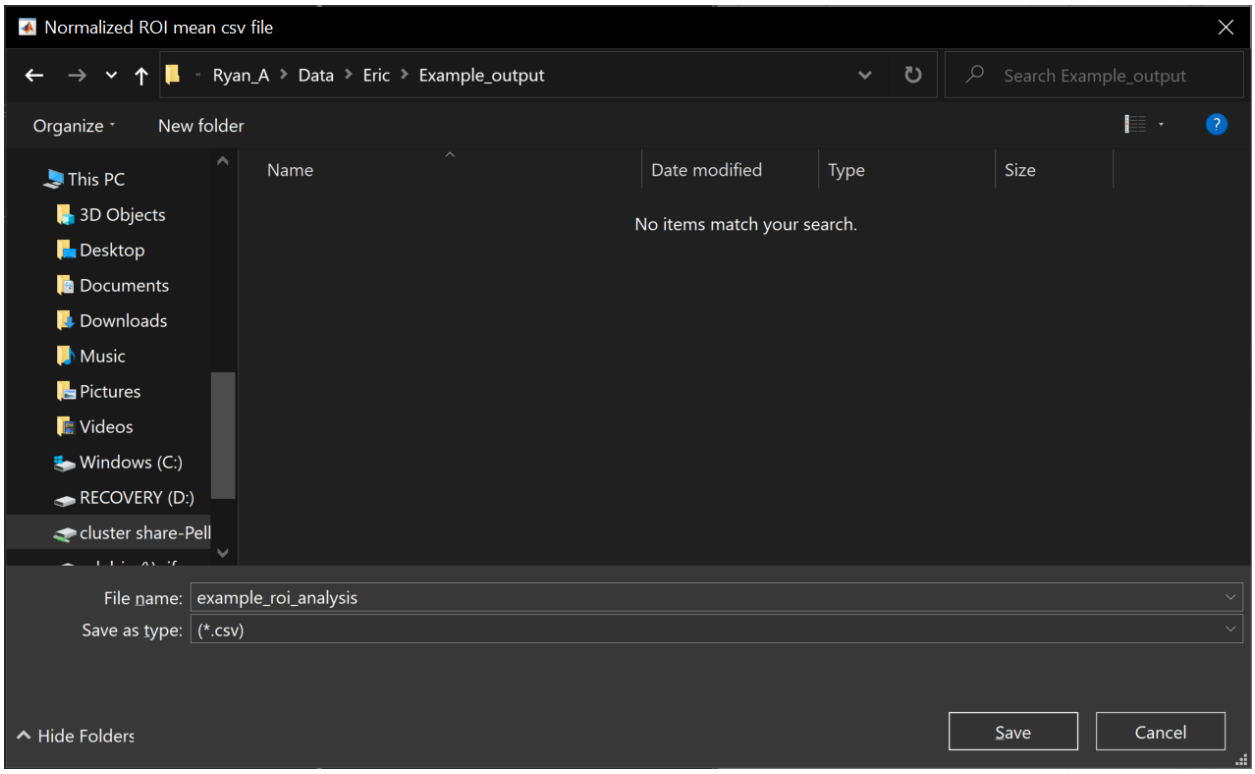
    c. Select the folder containing the .TIFF files of interest



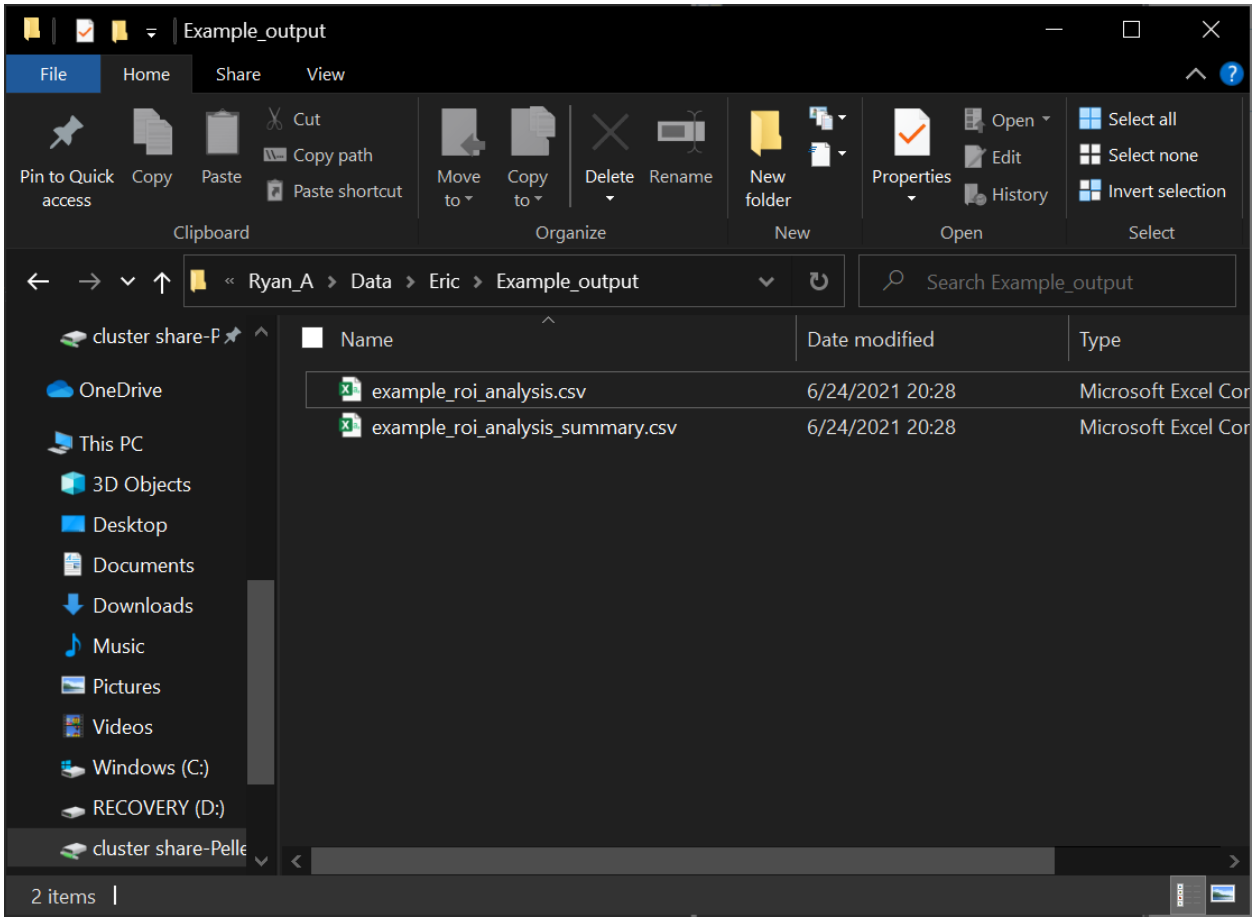
d. Use the graph of fluorescence vs frame number to select the frame number for both the baseline measurement and the peak measurement



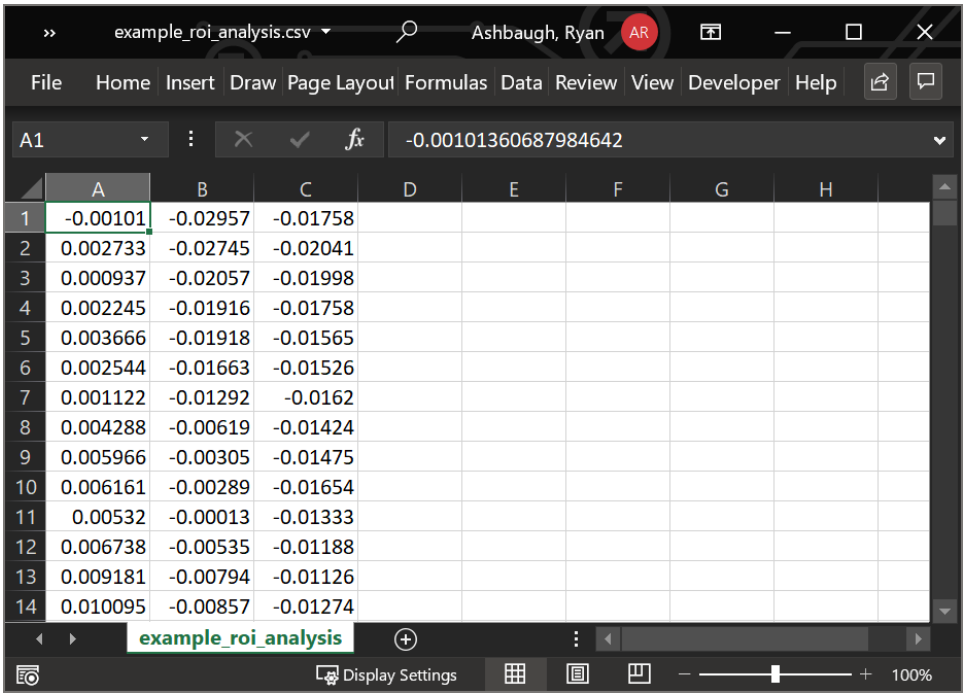
e. Save the analysis output



f. Two files will be created from the analysis

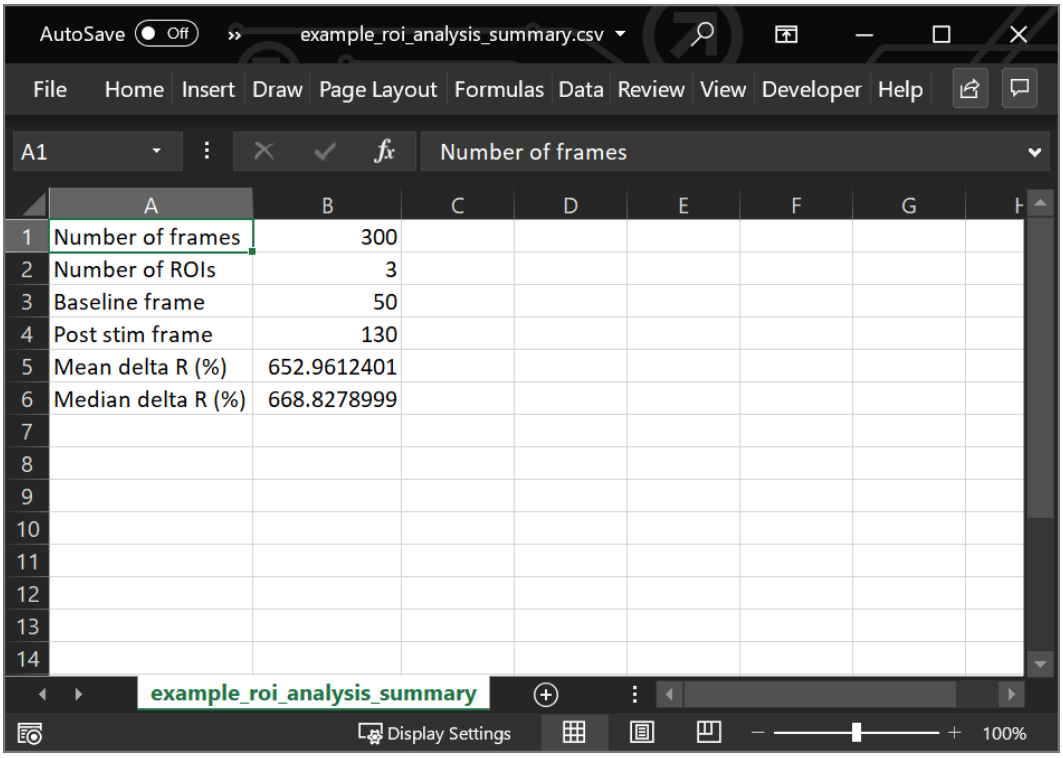


i. One containing the fluorescence values from the ROI's from each frame



	A	B	C	D	E	F	G	H
1	-0.00101	-0.02957	-0.01758					
2	0.002733	-0.02745	-0.02041					
3	0.000937	-0.02057	-0.01998					
4	0.002245	-0.01916	-0.01758					
5	0.003666	-0.01918	-0.01565					
6	0.002544	-0.01663	-0.01526					
7	0.001122	-0.01292	-0.0162					
8	0.004288	-0.00619	-0.01424					
9	0.005966	-0.00305	-0.01475					
10	0.006161	-0.00289	-0.01654					
11	0.00532	-0.00013	-0.01333					
12	0.006738	-0.00535	-0.01188					
13	0.009181	-0.00794	-0.01126					
14	0.010095	-0.00857	-0.01274					

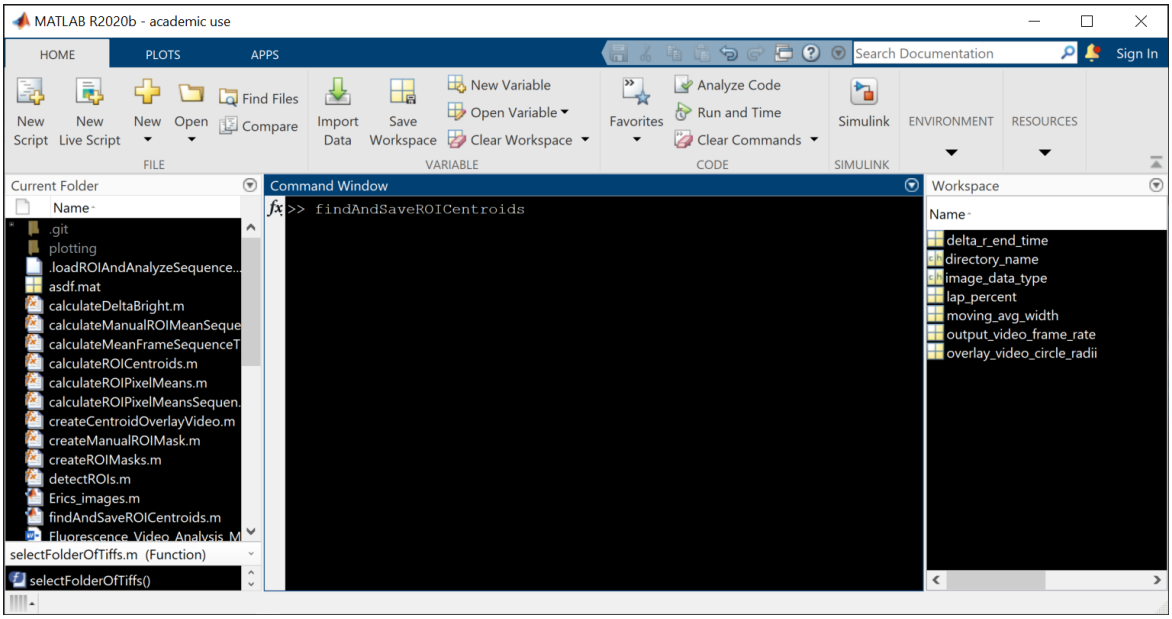
ii. The other containing a summary of the analysis



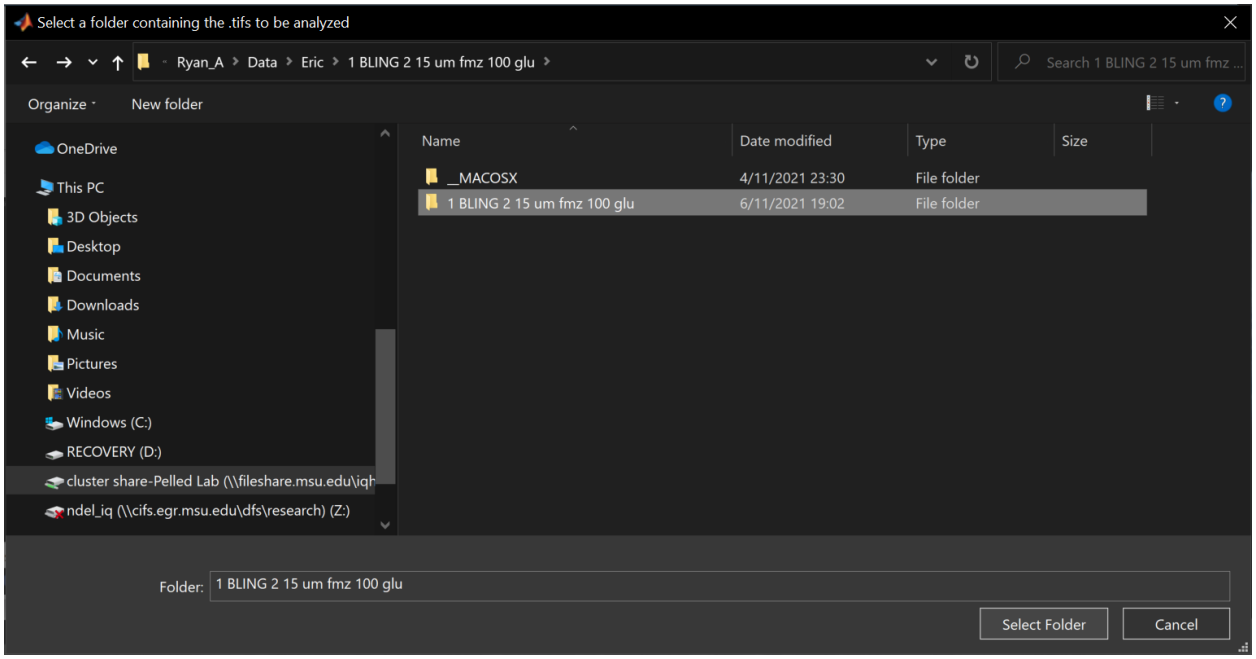
3. To use the same labels for multiple image stacks, simply repeat step 2 and select a different folder of .TIFFs while choosing the same label .png

Automated ROI detection

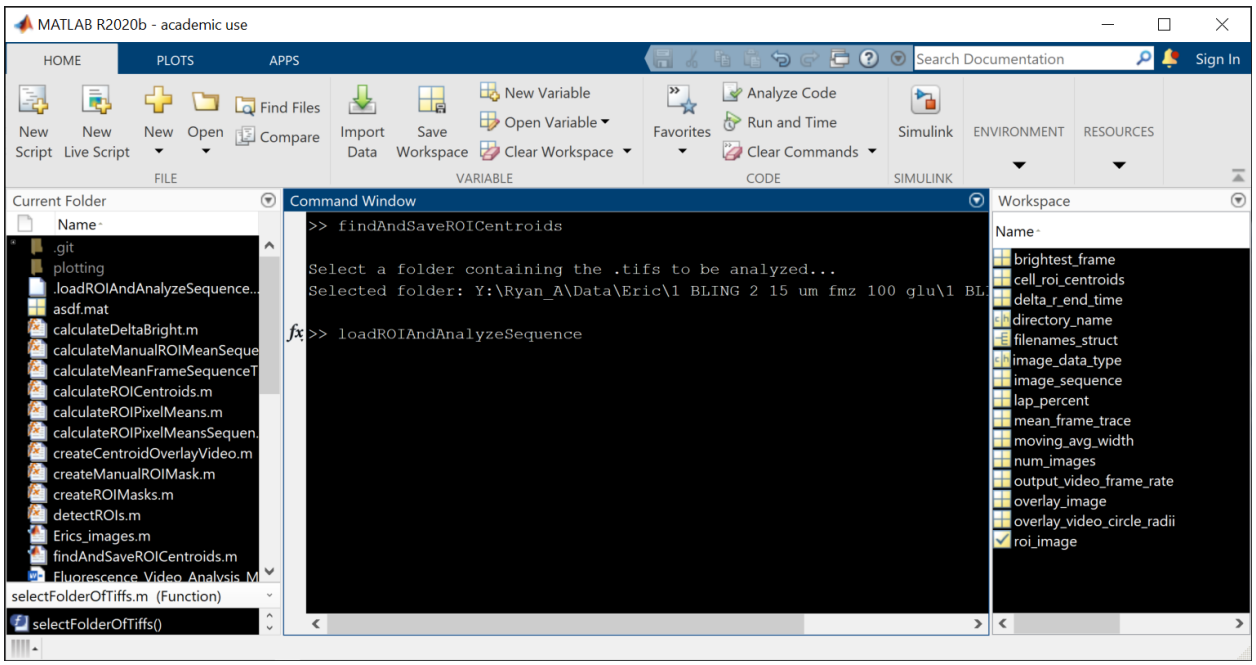
1. Run the "findAndSaveROICentroids" file to automatically find the centers of the cell ROIs



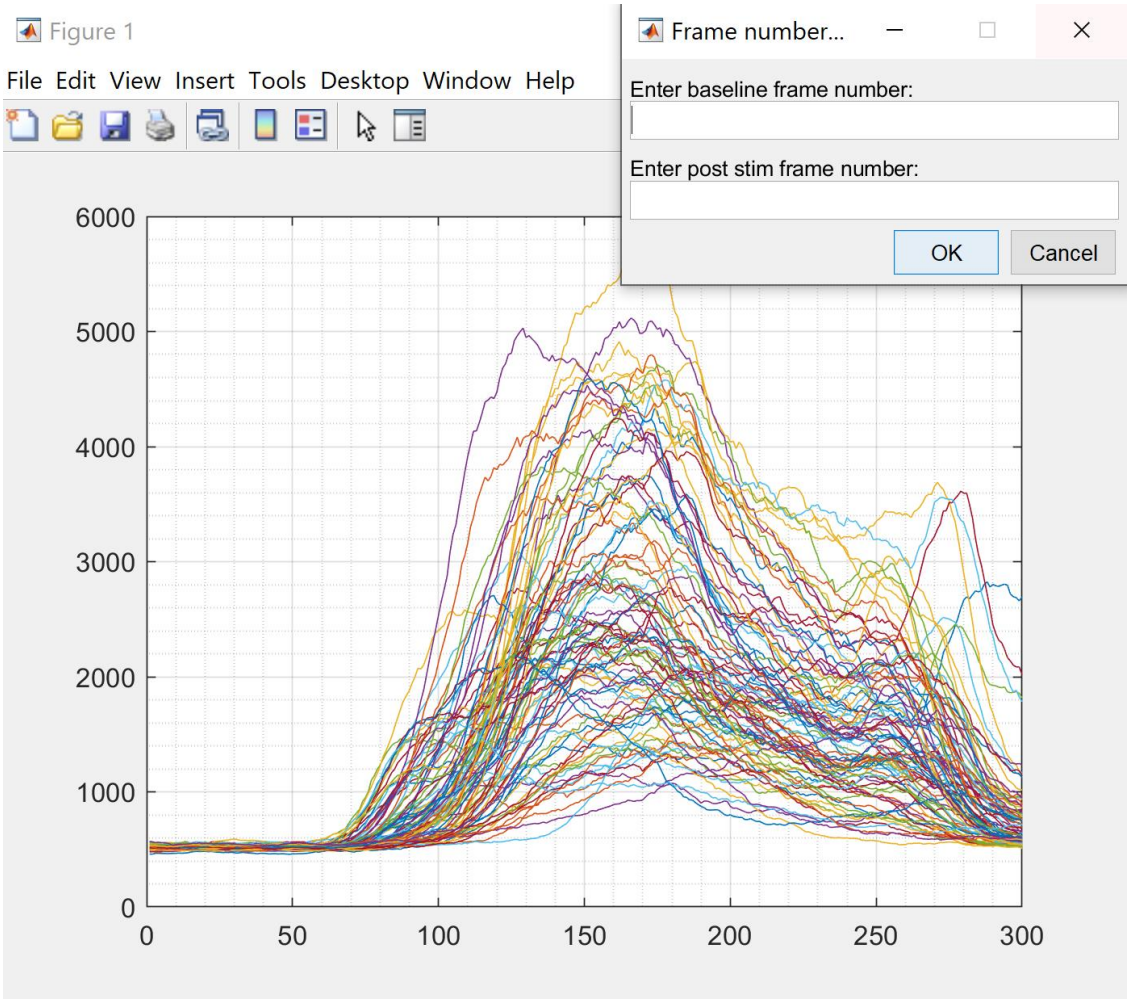
2. Select the folder with the desired .TIFF images



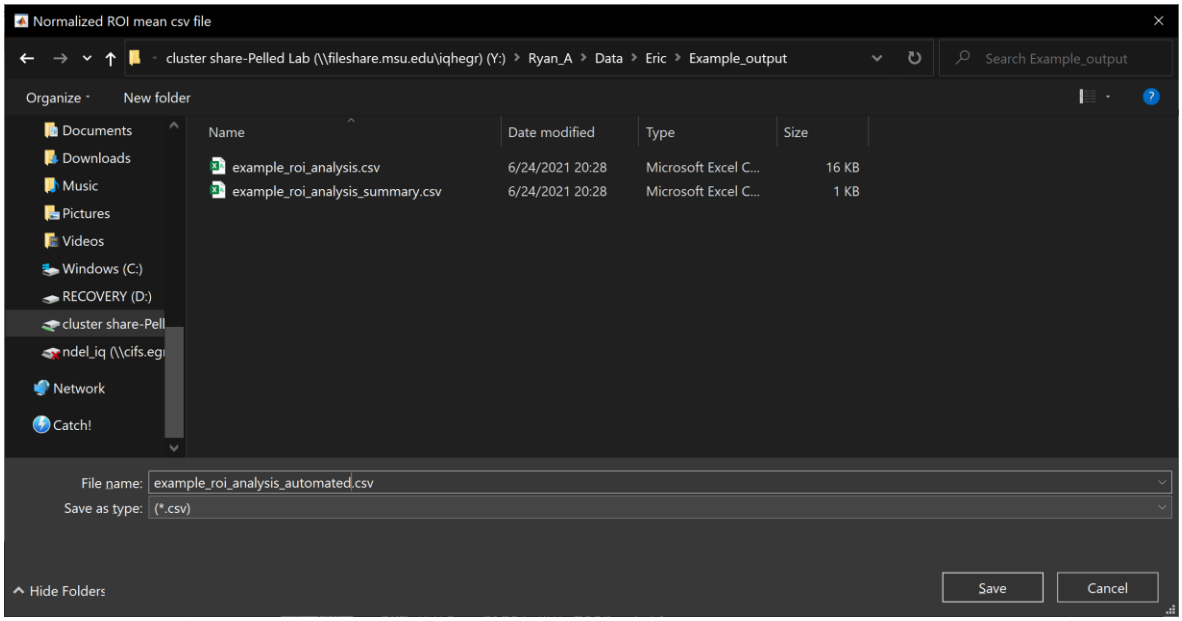
3. Run “loadROIAndAnalyzeSequence” to analyze the automated ROIs



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



5. Save the analysis output



6. 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