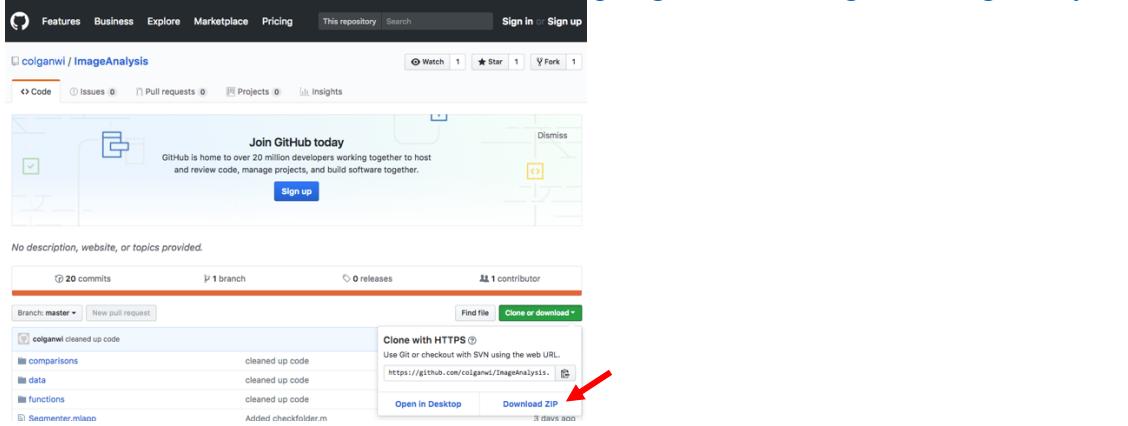


William Colgan  
2/26/2018

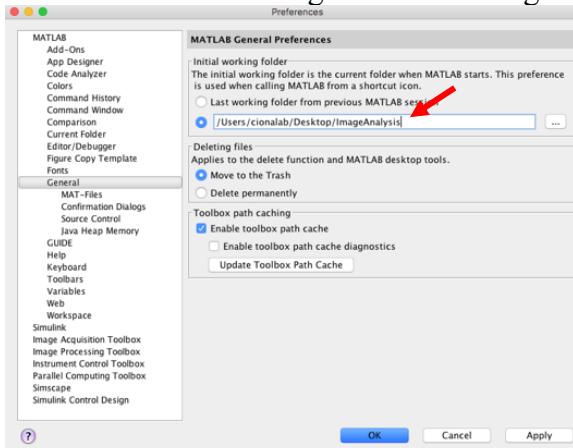
## Image Analysis Directions

### Installation:

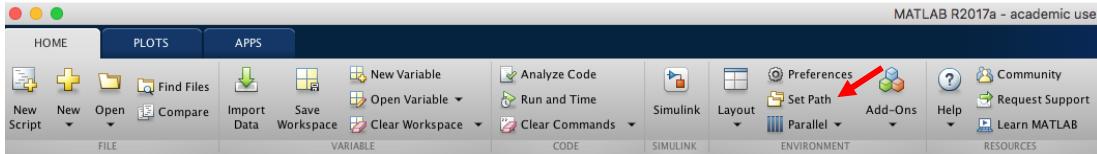
- Install MATLAB
- Open colganwi/ImageAnalysis on GitHub: <https://github.com/colganwi/ImageAnalysis>



- Click ‘Clone or download’ and then click ‘Download ZIP’
- Double click on the file to unzip it and then rename folder ‘ImageAnalysis’
- Move the ‘ImageAnalysis’ folder to where ever you would like to keep it.
- Open MATLAB
- Open MATLAB → Preferences, then click on ‘General’
- Make the initial working folder the ‘ImageAnalysis’ folder.



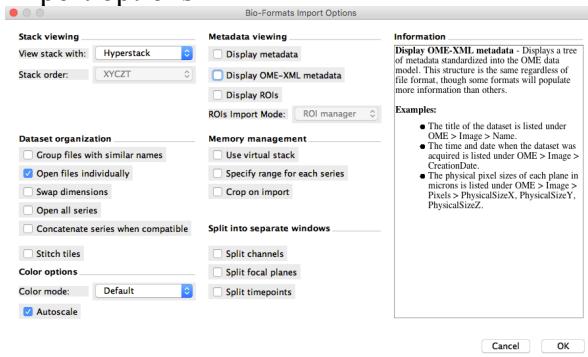
- Click ‘Ok’. Close and reopen MATLAB. It should now open the ‘ImageAnalysis’ folder by default.
- Click on ‘Set Path’ under the ‘HOME’ tab.



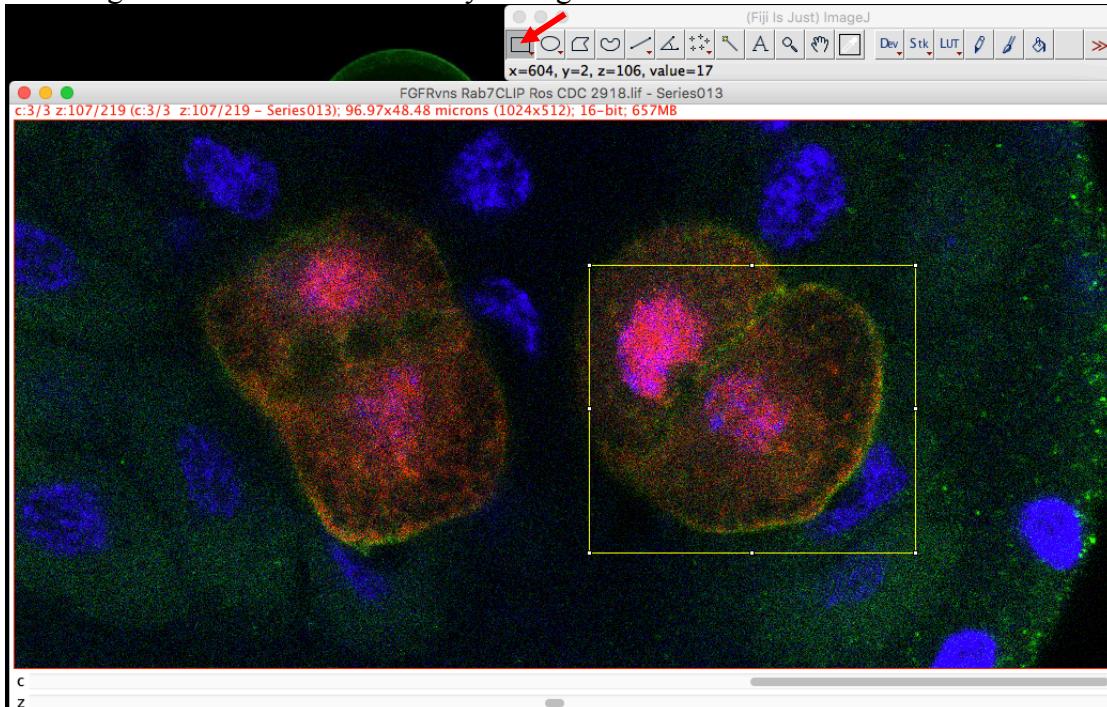
- Click ‘Add with Subfolders...’ and then select the ‘ImageAnalysis’ folder
- Click ‘Save’ and then ‘Close’

## Cropping Images:

- Open FIJI
- Select File → Open..., then select the LIF file containing your images. Use the default import options



- Select the series which you want to crop.
- Select Image → Color → Channels tools, then change Color to Composite
- Select rectangular selection and then draw a box around one of the cells. Make sure there is a margin round the cell all the way through the z dimension.

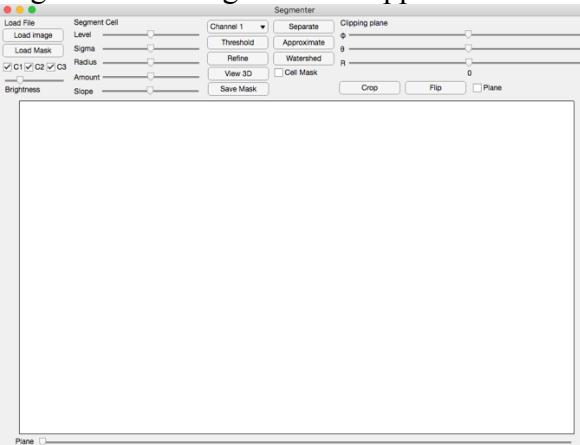


- Select Image → Duplicate. This will make a copy of the selected area.

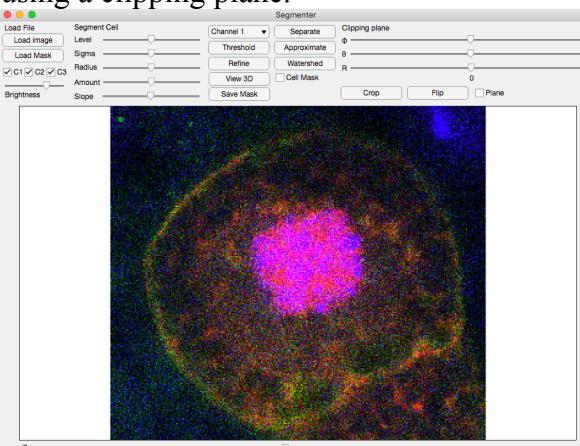
- Save this copy as a TIF by selecting File→Save As→Tif. Save it into the ‘ImageAnalysis/data’ folder and name it by appending the 1\_ to the filename.
- If there are other cell in the image select them and repeat the process. They should be named 2\_, 3\_, ect.

### Segmentation:

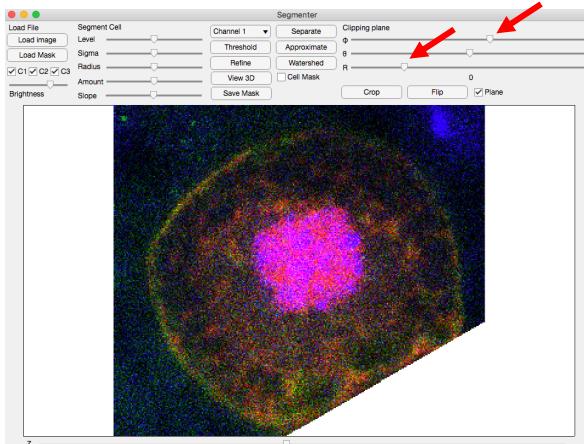
- Open MATLAB
- Right click on Segmenter.mlapp and select run. A window that looks like this will open:



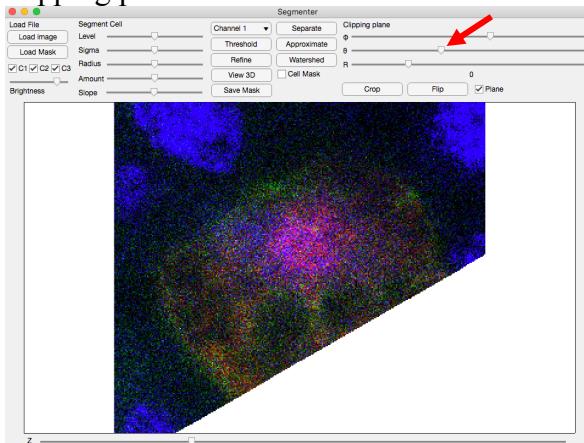
- Click ‘Load image’ and then select the image you want to segment in the ‘data’ folder.
- You can turn channels on and off using the check boxes: ‘C1’, ‘C2’, ‘C3’.
- You can adjust the brightness of the image using the ‘Brightness’ slider.
- You can view different planes of the image using the ‘Z’ slider at the bottom.
- If there is another cell next to the one you want to segment you will need to crop it out using a clipping plane.



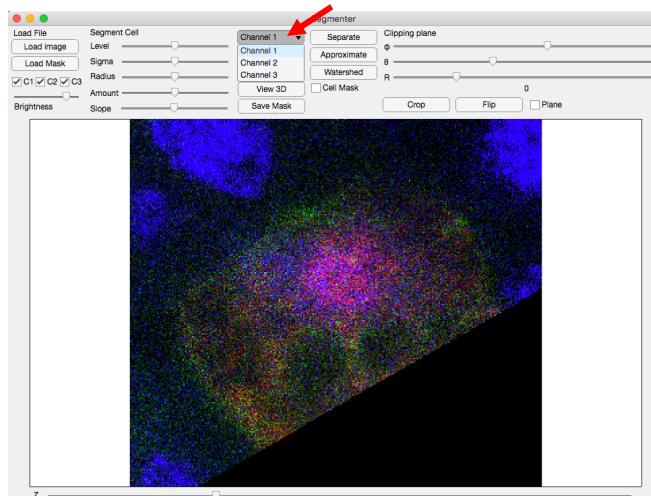
- Move the Z to where the membrane between the two cells is clearly visible.
- Click the ‘Plane’ check box.
- Adjust the ‘Φ’ and ‘R’ sliders until the clipping plane aligns with the membrane in the x-y dimension. Φ sets the angle and R sets the distance from the center of the image to the clipping plane.



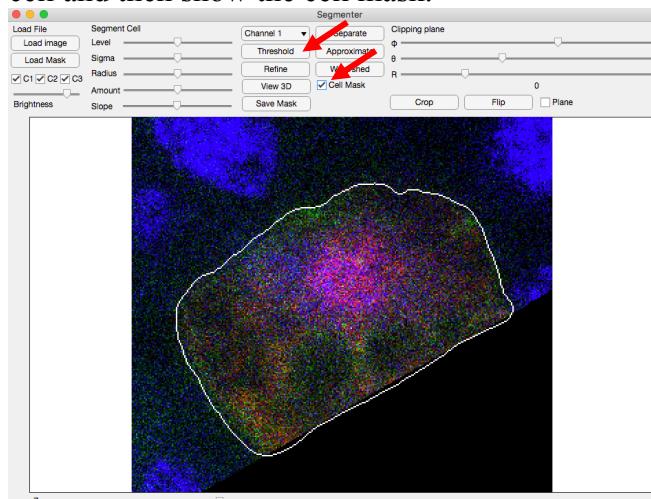
- Move to Z to another point where the membrane is clearly visible.
- Adjust the ‘Θ’ slider until the clipping plane aligns with the membrane. Θ tilts the clipping plane in the z dimension.



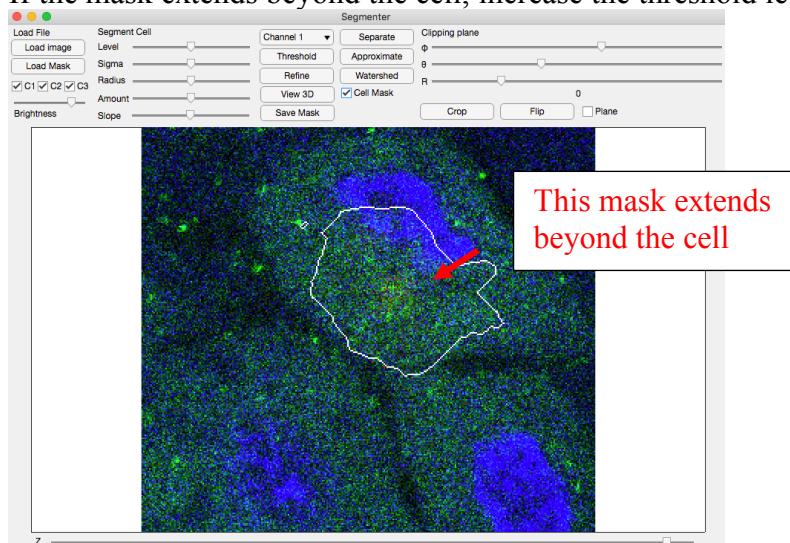
- Now move up and down in the z dimension to make sure the clipping plane aligns with the membrane. You will probably need to adjust Θ and R more. It is best to get Θ right before adjusting R. You can do this by moving up and down in the z dimension and making sure that the membrane and clipping plane move at the same rate.
- Once you have a good clipping plane, click the ‘Crop’ button. This will crop the second cell out of the image. Unclick the ‘Plane’ checkbox, so that the clipping plane is not shown.
- If you also want to segment an image with the second cell in it, do not adjust Φ and Θ. When you load the image with the second cell in it, you can just click the ‘Flip’ button and adjust the R slider, since the angle of the clipping plane is the same.
- Segmentation is done by thresholding one channel of the image. You need to select which channel this using the ‘Channel’ pulldown menu. You should select the channel which is present in the cell and not present in the background. Channel 1 is usually best.



- Click the ‘Threshold’ button and then the ‘Cell Mask’ checkbox. This will threshold the cell and then show the cell mask.



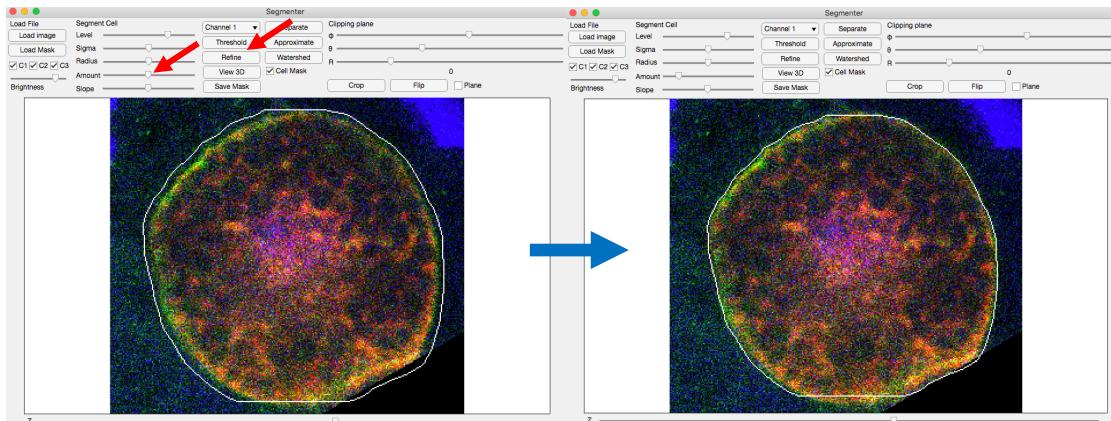
- Move the ‘Z’ slider to look through the z dimension. You can also click on the ‘View 3D’ button which will display the mask in 3D.
- If the mask extends beyond the cell, increase the threshold level using the ‘Level’ slider.



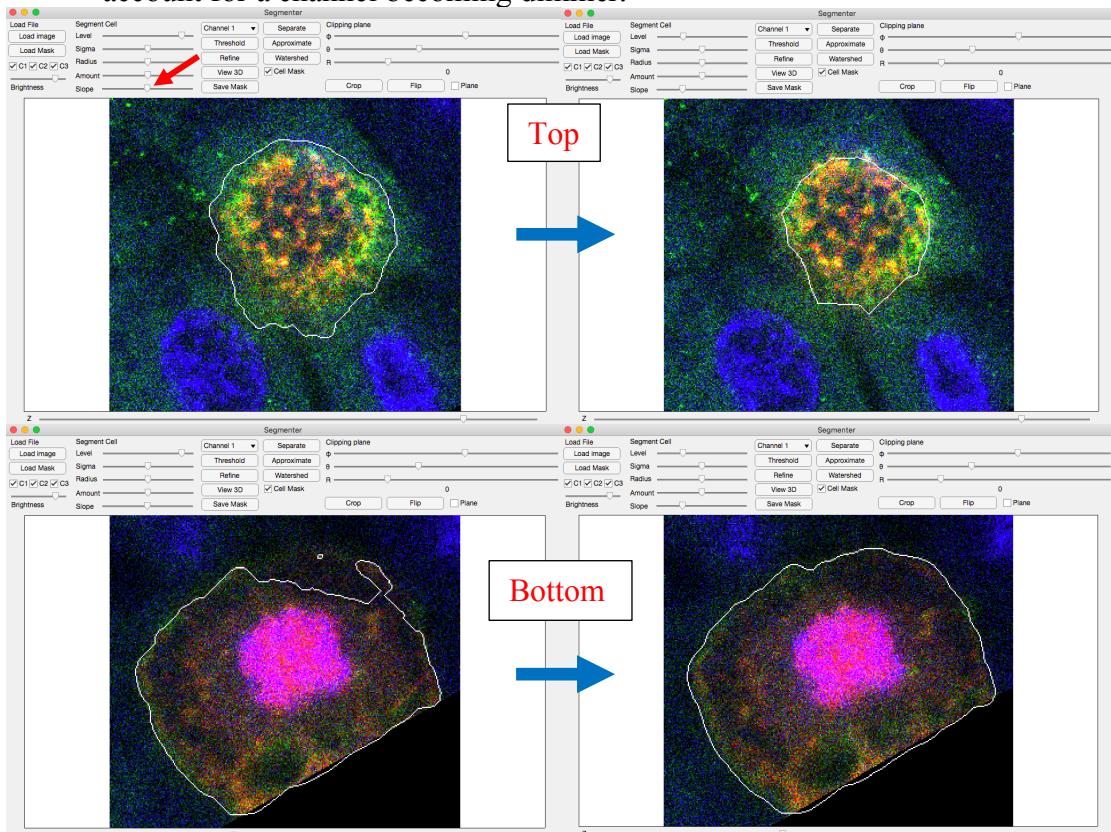
If the mask is missing part of the cell, decrease the threshold level.

- If adjusting the threshold level is not enough to get a good cell mask, there are a couple of things you can do.

- If the mask is slightly larger than the cell in all the planes, move the ‘Amount’ slider to the left and then click the ‘Refine’ button. Amount specifies how much size of the cell mask will increase or decrease.

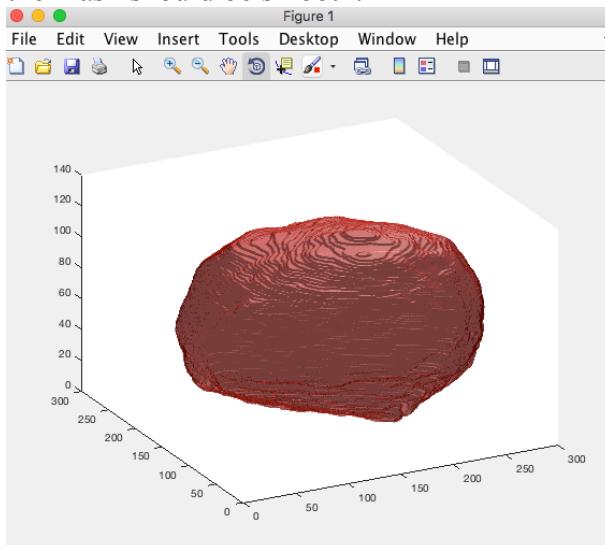


- If the cell mask extends beyond the cell at the top and is missing part of the cell at the bottom because the channel gets dimmer deeper in the embryo, move the ‘Slope’ slider to the left. Slope sets the slope of the threshold in the z-plane to account for a channel becoming dimmer.



- If the channel is really punctate, move the ‘Sigma’ slider to the right. Sigma sets the standard deviation of the Gaussian filter applied before thresholding.

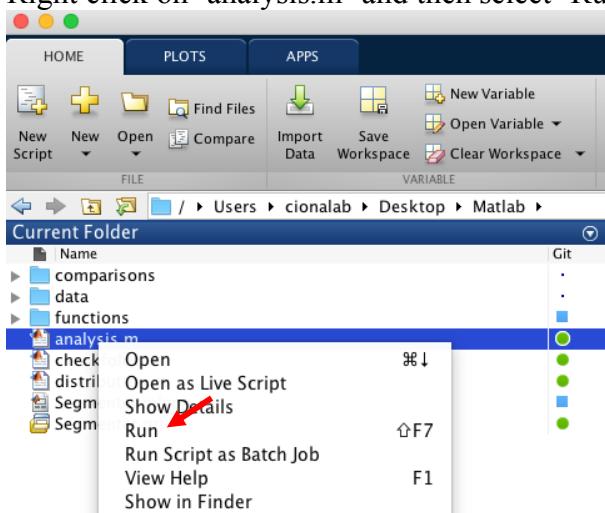
- Once you have a good mask, check it by clicking the ‘View 3D’ button. The surface of the mask should be smooth.



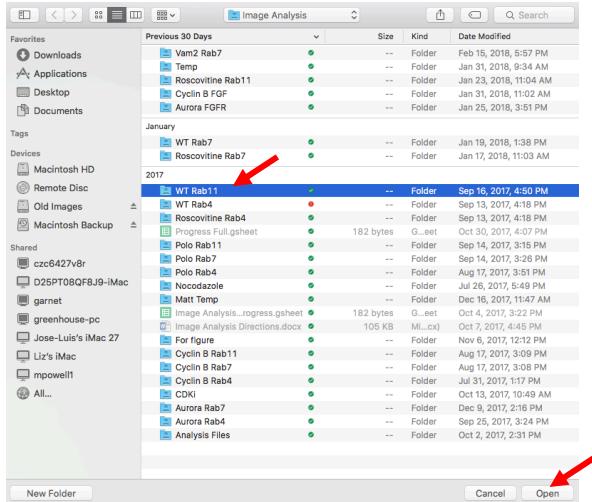
- Save the mask by clicking the ‘Save Mask’ button. This will save the mask in the data folder as a TIF image.
- Once you are done segmenting a set of images, move all the images into a new folder and out of the ‘data’ folder.

### Analysis:

- Open MATLAB.
- Right click on ‘analysis.m’ and then select ‘Run’.



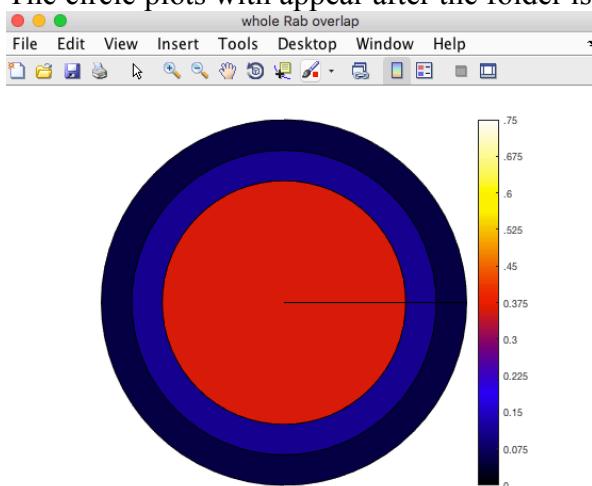
- Select the folder containing image and masks which you want to analyze, then click ‘Open’.



- Select ‘whole’, ‘ventral’, or ‘dorsal’ to set which region will be analyzed.
- The script will analyze all the images in the folder and save an excel sheet in the folder which was analyzed.
- If the folder does not work, right click on ‘checkfolder.m’ and then select ‘Run’. This will tell you what is wrong with the folder. The most common issue is not having a mask file for one of the image in the folder.

### Making figures:

- Open MATLAB.
- Right click on ‘distribution.m’ and then select ‘Run’.
- Select the folder containing image and masks which you want to analyze, then click ‘Open’.
- Select ‘whole’, ‘ventral’, or ‘dorsal’ to set which region will be analyzed.
- Select the color map for the FGFR distribution and Rab co-localizaiton.
- The circle plots with appear after the folder is analyzed.



- Select File→Save as, and then save the circle plot as a TIF image.

- If the folder does not work, right click on ‘checkfolder.m’ and then select ‘Run’. This will tell you what is wrong with the folder. The most common issue is not having a mask file for one of the image in the folder.