Quick tutorial on using the pipeline:

1. Start by opening Master\_file
2. Make sure all the initial parameters are correct – folders, file names, etc (up to line 20)
3. Enter the number of samples to run on line 24 – if you have a set of 10 images and they are saved with numbers in the file name, then you can do position = 1:10 to run through all of the files
4. Click Run on the first section and it will start running through the pipeline.
5. The first manual action is to draw the pouch boundary but clicking along where the pouch is. Double click on the last point to finish:

A close-up of a brain

Description automatically generated

1. Next the pipeline will ask you to define the AP axis, which is stained by Wingless. You will do the same as before, start on either end, in the back region outside of the defined pouch, click along the staining and finish by double clicking outside the pouch.
   1. If the boundary is ambiguous, consult the image on the right where the pipeline tried to segment out the boundaries for you.

A close up of a black and white image

Description automatically generated

1. Repeat the same thing for the DV axis which is stained by Engrailed

A close-up of a grey object

Description automatically generated

1. Next, the pipeline will show you what you drew, make sure it looks correct before moving on.

A close-up of a black and white image

Description automatically generatedA black object with a blue line

Description automatically generated

1. Next, the pipeline will plot the first half of the defined AP axis, make sure it starts in the anterior side, left click on the mouse if it is the correct side. Hit Enter on the keyboard if its on the wrong side.

A green object with a black background

Description automatically generated

1. Repeat for the DV axis. Make sure it’s starting from the dorsal side.

A green object with a black background

Description automatically generated

1. After this, the pipeline will save a new file ready to be ImSAnE’d and go to the next position if you have multiple images. If you are only measuring fluorescent intensity in one channel, open “draw\_pouch\_axis” and change lines 271 and 272.
2. After you’ve done all the manual defining of the pouch and axis, the pipeline will start running imsane in the next section. Make sure you’ve run the setup file in the ImSAnE folder
3. This section takes a while to run depending on you machine, if it crashes, set parallel pool to lower workers may prevent freezing you machine
4. After ImSAnE is done, it’ll save a new file for each image, named “\_\_\_ imsaned.tif” which you will open that file in FIJI and manually mask out any peripodial signal using the pen tool and save the image.
5. You will run the next section to measure the axis, the supplied example will measure both Fat-GFP channel and anti-Ds channel. If you only have one channel, just delete code for the other one (line 111) This section also takes a while to run
6. Lastly, the graphing and output section will plot the measured intensities in Matlab. As well as output CSV files for plotting elsewhere. If you have edge effects – which is common because the edges of the pouch are difficult for ImSAnE to figure out, you might need to delete some values that are either too high or too low. And re-normalize, plot, and output.