Brightfield image analysis v2.3:

1. Put all of the images to be analyzed together in a single folder.
   1. You want the files that are raw data indicated by “\_R\_” in the name.
   2. This pipeline only works with the “TIF” image format.
2. Open FIJI.
3. Go to plugins->Macros->Edit… (this will be in the menu bar at the top of the screen).
4. Open “Batch\_cellpose\_v2.ijm” in the BF\_pipeline\_v2 folder on the desktop.
5. Click “run” at the bottom of the window.
6. A new window will pop-up. The first field is for the location of your files. The second field is for the location you would like to save your images. Click “Ok” when finished.
   1. The pipeline will now analyze all images in the selected folder. This will take about ~3.5 minutes per image.
7. When FIJI is finished, open “BF\_cellpose\_data\_processing\_v2.3.Rmd” in the BF\_pipeline\_v2 folder on the desktop.
   1. We will format the data into a single file in R and convert the measurements to microns using 2.2 pixels/micron.
8. Press “Option”+”command”+”R” on the keyboard at the same time to run the R script.
9. A popup window will open. Select the folder that contains the results from Batch\_cellpose\_v2.
   1. This pipeline will have troubles if there are other files in the folder besides the files that were just created in step 6.
   2. This pipeline requires all of the files (both jpeg and csv) from step 6 to be in the same folder.
   3. This pipeline currently only works with 1 experiment at a time.
10. The results will be saved in the BF\_pipeline\_v2 folder on the desktop. Move the results to a long term storage location and upload them to google drive (lab resources->experiment tracking->quantification->*your experiment name*)