For quantifying fibrousness:

1. **\*\*DO THIS AGAIN (SORRY)\*\*** Run actin\_batch\_analyze.m for each folder. This will apply actin\_extravaganza.m for each image.
2. This script will generate .mat files and .fig files (.figs will be images showing outline and directors indicating detected actin alignment)
3. For each genotype, generate a folder. Each folder needs to contain the .mat files just created.
4. Run actin\_evaluator and select one of the genotype folders with .mat files. Also change the scale at the top of the script where indicated.
5. **Before running, choose your cut off value for aspect ratio, and change the value of “ARcutoff” (currently set to 4 – also probably should use this when you name the files so that you don’t overwrite previous version if you run again with a different cut off value).**
6. **Also before running, choose a thickness (“th”) – this is how many pixels wide the “outer” region will be. The inner region is just everything within the cell that is inside the outer region.**
7. This will generate .mat files (which I can use later) and .xls files with data for each image. The parameters right now are:
   1. Cell area (in microns squared)
   2. Cell aspect ratio
   3. Cell circularity
   4. Cell irregularity (perimeter divided by convex hull perimeter – i.e. the outline if you ignore all the squiggly bits and draw a smooth line outlining the whole cell)
   5. ~~Cell angle~~
   6. **Percent fibrousness – the percentage of windows with fibers, i.e. with FFT aspect ratio above the chosen cut-off**
   7. **Percent fibrousness inner**
   8. **Percent fibrousness outer**
   9. Mean “actin aspect ratio” – this is the aspect ratio of the 2D Fourier transform, if it’s larger, the window contained fibrous structures and if it’s closer to 1, no fibers were detected. This number is the mean aspect ratio for all windows in the cell
   10. **Mean actin aspect ratio inner**
   11. **Mean actin aspect ratio outer**
   12. ~~Mean actin angle~~
   13. Mean adjusted actin angle – this is just adjusted to the cell angle, i.e. angle of fibres detected minus cell angle – **this is now subject to your aspect ratio cut off**
   14. Order parameter (currently a wild card idea, if it shows a difference then we’ll look into it some more :P) – **this is now subject to your aspect ratio cut off**

For quantifying protrusions:

1. Run batch\_protrusions. This will call the actin\_protrusions.m function to automatically identify and measure protrusions. The outputs are 2 .xls files, one ending in “\_means.xls” and one ending in “\_raw.xls”. Means will have the mean of each protrusion parameter for each cell, as well as the number. Raw will just have giant ass columns of parameters for each protrusion found in all cells. The outputs right now are:
   1. Height – measured as the distance between the protrusion peak and the average of the two adjacent troughs
   2. Width – measured as the distance between the two adjacent troughs
   3. AR – aspect ratio, or ratio of height to width (AR>1 if it’s a tall one, or AR<1 if its wide)
   4. Height 2 – from the best fit ellipse – chosen between the major or minor axis length based on the results for height and width
   5. Width 2 – same as height 2
   6. AR 2 – same as height 2
2. NOTE: the measurements with 2 on the end are kind of alternates to measurements without – they’re measured in slightly different ways and they *might* be better but I’m not totally sure. See what the data looks like then we’ll talk.