First steps of analysis in Image J.

1. Bring the individual images in the stack in better alignment with turboreg.
2. Open your movie file in ImageJ
3. Duplicate the file.
4. Next you need an Addin called TurboReg (set it to your functions so you can select it using the F1 function key). The source is your original stack file the target is your duplicate file. Select rigid body and accurate and then batch.

(It may be possible to run this more than once, or to use slices rather than a duplicate file to get the best registration)

This registered file is what you analyze, so be sure to save it as a tif file.

If the full file does not come into registration because it fades out and parts can not be analyzed note this in the excel sheet and don’t analyze any of these data.

1. Your next goal is to set the ROIs. To accomplish this you will alter you image file so responding neurons are more easily seen
2. calculate a running average of the movie (every two slices will be averaged) to do this you will need plugin called “running Z projector” (you can also set this to a function key.
3. Next compute a z-projection (average) of baseline (when you start a recording you should have a set number of baseline frames – at least 10 seconds. To do this select stack (Stk) then Z-projector then put in the start and end frame of baseline (i.e. 1-40), use average intensity.
4. Subtract the baseline from your running average, this a the “results” file. To do this look under process and choose calculator plus, I1 is the stack and I2 the baseline, then choose subtract as the operation. Running this will create an image file that has only the responses.
5. Next you need to bring up the ROI manager, to do that go to analyze, select tools and then ROI manager. Scroll through your file and draw around the parameter of each location where the cells appear bright in any frame. Try to make the borders accurate as we can use these ROIs to measure the cell diameters of the responding cells. Each time you draw a ROI, his add in the ROI manager. Select the button “show all” to see the ROI’s that you have already drawn, continue through the file until you have drawn all ROIs. Save the ROIs in the ROI manager by selecting “more” and then “save” If you have more than one file then you need to use the same ROIs for all the files. To do that just open the new file with your ROIs still open and keep adding to them. THIS IS IMPOTANT: You can only do this if all your movie files are cropped exactly the same way and are the same size and so the cells are in the exact same place. This is why it is important not to mess with these parameters during an experiment. You need a minimum of two responses for each stimulus. They don’t have to be in the same file, our Matlab program will not analyze the files in they are too large. Most of your somatosensory ones have this issue.
6. Once you are done setting all of your ROIs same the ROI file. Then project a maximum intensity z-projection, and make sure that all white spots have a ROI – use his view to add ROIs do not subtract them using this view. However, check for overlap of cells. If an ROI of one cell overlaps another cell redraw the ROIs so the area of overlap is not analyzed for either cell. If this occurs frequently, we need to use a lower dose of tamoxifen.
7. Save the ROIs in the ROI manager by going to more and hitting save. Do not save from the main ImageJ program.

For analysis we move to Matlab. However, before going to Matlab, make sure your registered file and the file with your ROIs have the same name. Also you need to have a file open that has the start and stop frames for each stimulus in order to answer the questions in Matlab

1. Open Matlab
2. Go to the data analysis test, JMBreza file folder
3. In the left window where you select the Matlab program choose “New\_no Drift\_part A”.
4. Once this is selected you should have a green arrow that you can select to run the Matlab program and select the file you want to analyze.
5. It takes a long time for Matlab to read the file be patient
6. Once the file is open in Matlab, you will receive a series of questions. Use 5 deviations from baseline and answer the last one with 1.25. The rest are for the start and stop for each stimulus.
7. When analysis is complete, save the heatmap
8. Open “Original and Corrected data” outside of Matlab. Outside of Matlab is required to get it in an Excel format.
9. Save the excel sheet.
10. Delete the “Original and Corrected data” file from Matlab before analyzing the next image file.