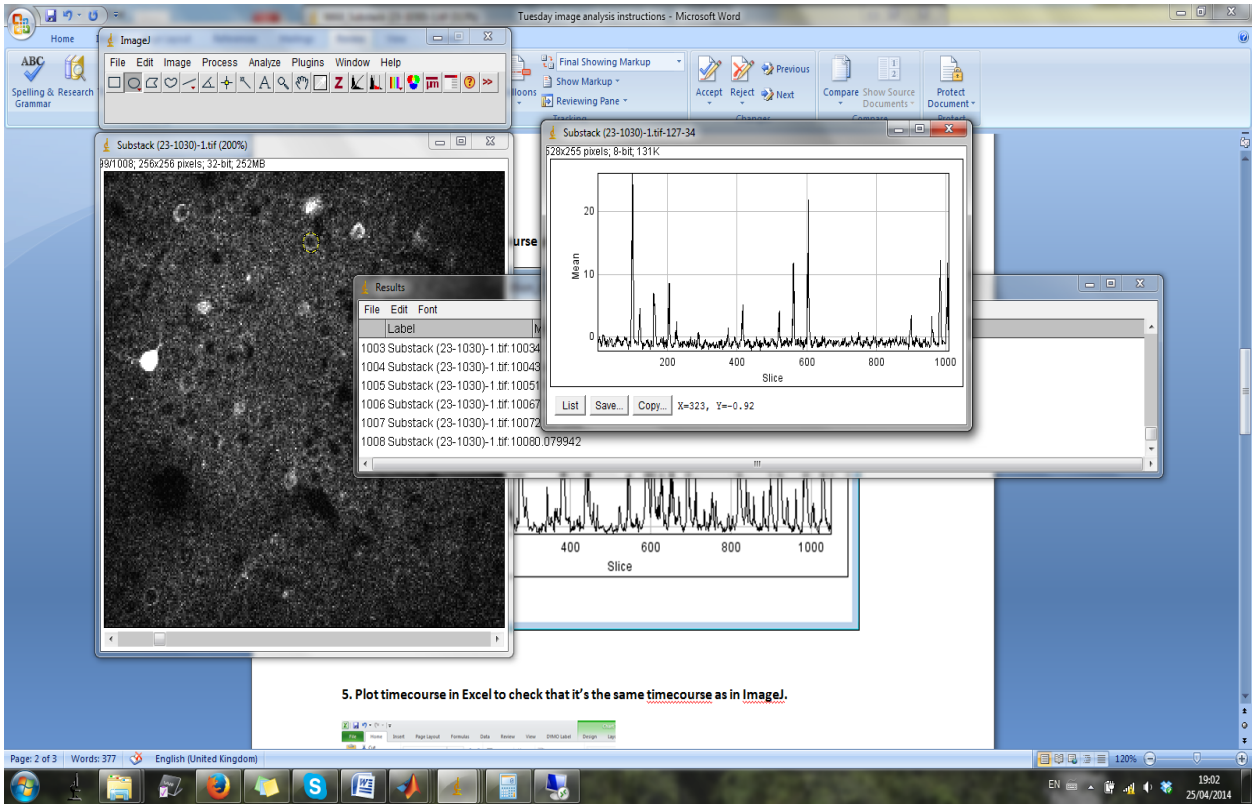
Wednesday 27th May 2015

Analysis of calcium imaging data

Protocol

1. Open ImageJ. In ImageJ open filename ‘Calcium_imaging_data’
2. You might need to adjust the brightness and contrast by selecting: Image-> Adjust -> Brightness\contrast -> Auto
3. By pressing '\' on your keyboard you can follow the activity of the neurons over time.
4. Create an average Image and a maximum intensity projection by selecting: Image-> Stack -> Z Project.
   What does this projection tell you about the activity of different neurons?
5. Select a responsive region, such as a cell, using the freehand select tool.
6. Plot time course of the region by selecting: Image-> Stacks -> Plot Z-axis Profile



7. Select ‘Copy’ on the timecourse plot, open Excel file ‘Analysis of calcium imaging data’ and paste into the third column:

8. Plot timecourse in Excel to check that it's the same timecourse as in ImageJ.
9. Compute change in fluorescence over time (F-Fo/Fo):
    a. Determine Fo as the median of the fluorescence (F) distribution (values in the column)
    b. Subtract Fo value from each fluorescence (F) value, and then divide the resulting value by Fo.
    c. Plot the (F-Fo)/Fo

10. Compute the average response timecourse over multiple repetitions of the same stimulus. In this dataset, each of the 16 stimulus orientations was presented 3 times (The drifting grating was presented for 1.5 seconds preceded by a gray screen presented for 3.5 seconds). The stimulus orientation is indicated by the number in the first column.

    a. Draw on piece of paper how this stimulation paradigm looks like by indicating the time in seconds for baseline and for stimulus presentation for an example orientation presentation (one repetition).
    b. Using the number of seconds for one repetition and the number of frames per repetition (see spreadsheet), Estimate the imaging frame rate. Add the number of frames on your drawing in Q. 10a.
    c. Average the three traces for each orientation and plot these average traces for each of the 16 stimulus orientations on the same plot.
    d. Plot a single average trace for all orientations. Where does the peak fall with respect to the stimulus start time? Refer to your diagram from Q. 10a.

11. Find the mean response to each of the 16 stimulus orientations:

    a. Average the 5 time points around the peak response (during the stimulus period) from the average timecourse of each stimulus orientation.
    b. Plot the mean responses on a polar plot, which will reveal the orientation tuning of the cell. Why is a polar plot a better choice than a conventional x/y line or scatter plot?

12. Compute orientation selectivity index. Find the stimulus orientation that caused the biggest mean response ('preferred' stimulus). Find the mean response of the two stimuli that are 90 degree away from the preferred stimulus ('orthogonal' stimulus), and average the two values. Orientation selectivity index (OSI) is calculated as the: OSI = (preferred - orthogonal) / (preferred + orthogonal)
13. Repeat steps 5.-12. for 1-2 other cells, and compare the differences in their orientation preference and orientation selectivity.
14. The goal is to obtain the orientation selectivity (OSI and polar plots) for at least 5 cells. You can choose to do that in Excel or you can ask us to show you a more automated approach using the programming language, Matlab.