1. Open .oib file in FIJI (Olympus raw data)
2. In FIJI, Image>duplicate (check “Duplicate stack”)
3. F1 (*TurboReg*), select “batch” (probably already selected by default)
4. Save as “<file name>\_RGST” and close the other two .oib files (look at examples in folders if you have forgotten)
5. F2 (*Running ZProjector*), select size=3
6. F3 (*ZProjection*), select “average intensity”, start @ 1, stop @ 29 (this creates the baseline)
7. F4 (*Calculator Plus*), i1=”filename\_RunAv(3)”; i2=”AVG\_”filename\_RunAv(3)”; operation=subtract i2=(i1-i2) x k1 + k2
8. Expand window for clarity (Cntrl+)
9. F5 (*ROI Manager*), check “show all”; N.B. may need to uncheck *Associate “Show All” ROIs with slices* in ROI manager>more>options dialog box
10. Draw an ROI anywhere on “Result” as a placeholder
11. F6 (*Profile*)
12. Adjust open windows such that Profile doesn’t occult Results pane and begin moving through the stack to identify responding neurons
13. Draw ROI over responding neuron, type “t” to enter that ROI into ROI manager
14. When done identifying responding neurons, “Select All” (Cntrl A) ROIs in ROI Manager, right click, “Save As” “ROIset”
15. Go back into folder directory and rename that ROIset such that it has the same name as the corresponding “<filename>\_RGST” file. Best procedure is to Copy “<filename>\_RGST” and Paste it into the rename for the ROIset. Don’t worry, this won’t overwrite the ROIset….they are different type files. But they must have the same name for MatLab to work.
16. Open MatLab, set “Current Folder” to ► C: ► Users ► sroper ► Box Sync ► JMBreza ►
17. Double click on “df\_o\_SR.m” to open that script in the MatLab editor window
18. In the editor window, “Select All” of the script (Cntrl A) and right click > “Evaluate Selection”
19. Go to the appropriate “<filename>\_RGST” file. This should open and run the Matlab script