**FISHing** (written by Michael Priest, with modifications by Lei Xiao, in Yevgenia Kozorovitskiy's lab at Northwestern University)

**Instructions**

**Setup:**

In addition to the included scripts, if you are using .lif files, you will need to download bfmatlab from <https://docs.openmicroscopy.org/bio-formats/5.3.4/users/matlab/index.html> and add it to your Matlab path.

The software is going to ask you to input some information. You should have an idea of the following ahead of time. 1) What confocal stacks within each .lif file you wish to analyze. 2) The number of channels you have imaged. 3) Which channels you want to analyze, 4) Whether you want to define cell bodies off of the soma or the nucleus, 5) The number of z planes you wish to include for analysis, and 6) The first z plane you want to start at.

If you define cell bodies off of the soma (as done in this paper) it should work with Matlab versions 2009 and onwards. If you define cell bodies off of the nucleus (as done in Xiao et al., Neuron, 2017)) it should work with Matlab versions 2015b and onwards.

There are two times when you need to define a threshold for each channel. The first is used to remove background fluorescence from your quantification. The second is to determine what quantity of puncta within a cell constitute positive expression. These should be kept consistent within a channel, between images, especially the second.

Make sure your scripts (and bfmatlab) are all in your Matlab path.

**Run:**

Make sure you have the information discussed in ‘Setup’ above.

Run FISHing. Follow the prompts.

After selecting threshold values, you will be given an image of cell bodies. Please draw lines on the mask to separate any nuclei that are improperly connected. When finished, click anywhere on the mask.

You may find that you wish to (or need to) change the parameters for defining cell body regions. These can be found in the first lines of the m files ‘CellSegmenterNucleus’ and ‘CellSegmenterSoma’. On the other hand, once everything is working well, you can comment out some lines towards the bottom of these same m files to improve analysis times.