*Note: 1. Resolution of images must be the same.*

*2. All operations can be done in the same folder (each code generates processed files with different names).*

1. Color\_normalization.m

Normalizes images

Prepare an image where the background signal is removed (on Photoshop, ImageJ, etc…) as a reference.

ref = imread('reference image here.jpg');

Run with folder containing all ‘###.jpg’.

You may need to delete the normalized image generated from the reference image.

The output is ‘normalized\_###.jpg’

2. Padding\_batch.m

Normalizes image size

Run with folder containing all ‘normalized\_###.jgp’ produced from Color\_normalization.m

The output is ‘padded\_###.jpg’

3. Axon\_detection\_CannyEdge\_activecontour\_ver2.m

Detects axons and makes binary images

Run with folder containing all ‘padded\_###.jgp’ produced from Padding\_batch.m

Note: If the signal is Red, change line 14 from green: I = I(:, :, 2); to red: I = I(:, :, 1);

You may need to adjust parameters accordingly.

The output is ‘BW\_###.jpg’

4. Merge\_nissl\_ver2.m

Merges the binary images with DAPI/Nissl images to facilitate manual ROI detection.

Run with folder containing all ‘padded\_###.jgp’ and ‘BW\_###.jpg’ produced from Axon\_detection\_CannyEdge\_activecontour\_ver2.m.

The output is ‘merged\_###.jpg’

5. Outside of Matlab

ROI selection

You must manually select an ROI and fill outside of the area with red.

Run with folder containing all ‘merged\_###.jpg’ produced from Merge\_nissl\_ver2.m.

6. Get\_Values\_from\_ROIs\_ver2\_PC.m

Counts the number of positive pixels and total pixels in ROIs.

The output is Excel file.