Code used for 3D Sholl analysis as in Madry, Kyrargyri, Arancibia-Cárcamo, Jolivet, Kohsaka, Bryan & Attwell (2017) Neuron

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This code requires the following files (all found in this repository)

* MicronConvert.m
* PoolData.m
* ShollAnalysis.m
* ShollGUI.m
* ShollGUI.fig

All cells need to have been previously traced (e.g. in Vaa3D (<http://www.alleninstitute.org/what-we-do/brain-science/research/products-tools/vaa3d/>) or in FIJI using Simple Neurite Tracer plugin (<https://imagej.net/Simple_Neurite_Tracer>) and traces saved as swc files.

It is important to know whether the swc files have the coordinates saved in pixels or microns, as well as how many header lines (lines of text) the swc files contain.

Data should be saved in folders depending on how the data should be pooled - e.g. one folder for WT and one for KO. Each genotype/treatment will need to be analysed separately. Data will be saved in a subfolder titled ‘SR\_n’ where n represents the radius increment of the Sholl spheres.

A ‘.dat’ file, which can be opened in Microsoft Excel, will be generated for each trace indicating the values of intersections, branches and tips for each Sholl sphere.

In addition, three other ‘.dat’ files (*name\_*Intersections.dat, *name\_*Branches.dat, *name\_*Tips.dat) will be generated pooling the data from all cells in the folder being analysed.

### Installing

Clone or copy the directory to any location. Add the directory to the MATLAB path.

### Run

From MATLAB right click on ShollGUI.m and select Run

A graphical user interface should open.

*Are swc files in microns?* – Select YES if swc coordinates in microns

Select NO if swc coordinates in pixels

*XY Resolution-* If swc in microns ignore this

If swc in pixels, input resolution in microns/pixel

*Z Resolution-* If swc in microns ignore this

If swc in pixels, input resolution in microns/pixel

*No. Header Lines* Input number of text lines in swc file

*Soma radius* Enter the radius of the soma (intersections/branches within this radius of the cell centre will be ignored)

*Sholl radius* Enter the radius increment of the Sholl spheres

*Maximum radius* Enter a maximum radius beyond which measurements will be ignored

*Plot Graph* Select this if you wish to display an intersections graph of the pooled data

*Save parameters* All inputted values may be saved to re-use with different data sets

*Load parameters* Load previously saved parameters

*GO* Run the program

### How to run the Example.

There are three example traces (in .swc format) which are all from the same genotype. The swc files have the coordinates in pixels and 24 header lines. The X, Y and Z resolution for the images from which these traces came from are: X and Y 0.198m/pixel; Z 0.335m/pixel. These are microglial cells with an estimated soma radius of 5m. In this example we will look at the number of intersections every micron up to 60m away from the cell soma. A copy of the expected results and graph is found in the Example Traces folder.

Clone or copy the directory “Example Traces” from GitHub to any location.

From MATLAB right click on ShollGUI.m and select Run

A graphical user interface will open. Input the following data.

*Are swc files in microns?* – Select NO (as swc coordinates in pixels)

*XY Resolution-* 0.198

*Z Resolution-* 0.335

*No. Header Lines* 24

*Soma radius* 5

*Sholl radius* 1

*Maximum radius* 60

Click on GO

A pop up window will appear asking for the directory to analyse

Select the folder called “Example Traces” and click Open

A graph showing the mean number of intersections at each Sholl sphere will appear.

The “Example Traces” folder will now have the following subfolders-

Example Traces/Micron SWCs

This will contain the traces with the coordinates saved in microns

Example Traces/Micron SWCs/SR\_1

This will contain the results per cell (in .dat format which can be opened in Excel)

and in addition:

The pooled data for Intersections

The pooled data for Branches

The pooled data for Tips (number of processes)