Sholl Analysis Plugin

This is a plugin for ImageJ that automates the task of doing Sholl analysis on a neuron. Its internal algorithm is based upon how Sholl analysis is done by hand – it creates a series of concentric circles around the soma of the neuron, and counts how many times the neuron intersects with the circumference of these circles. However, since it can do this process repeatedly, and in a relatively short amount of time, it allows for new possibilities with the analysis that would be too tedious to be done manually.

Installing the Plugin

For ImageJ to be able to use the plugin, it must first be installed in the plugins folder within the ImageJ application folder. On Windows machines, the ImageJ folder is usually located in the Program Files folder, which is located in the root of the hard drive. To access it, select the "Run" item from the Windows Start Menu, then when it appears type "C:\Program Files" into the "Open" box, and click OK. On Macintosh machines, the ImageJ folder is usually located within the Applications folder. To access it, double-click on the Hard Drive icon on the Desktop, and then open the Applications folder in the resulting window. Once the plugin is placed in the plugins folder, which is inside the ImageJ application folder, the next time ImageJ starts up, an option to run the Sholl Analysis plugin will exist in the Plugins menu.

There are two files that constitute the Sholl analysis plugin: ShollAnalysis_.class and ShollAnalysis_.java. The .class file contains the actual plugin code that ImageJ runs, and as such, it is required for the plugin to work. The .java file contains the human-readable source code for the plugin, and is therefore optional. Those with a curiosity for seeing how the code works or for making changes to the plugin may be interested in obtaining and installing the .java file along with the .class file, but all that is necessary for the plugin to work is the .class file. These files belong in the aforementioned plugins folder within the ImageJ application folder.

Source Image Requirements

Since ImageJ can understand virtually any kind of image format, this plugin can be used to analyze the image content of virtually any kind of file. However, there are a few restrictions that the content of the image must adhere to, in order for the plugin to work properly. The first restriction is that the image must be an 8-bit grayscale image. If it is not already, ImageJ can convert the image to an 8-bit grayscale image by going to the "Image" menu, opening the "Type" submenu, and selecting the "8-bit" option. The second requirement is twofold: 1) That all of the pixels representing part of the neuron are of the same gray value, and 2) That all of the pixels *not* representing part of the neuron are of a different, or multiply different gray values. In other words, the neuron must all be one solid color, while the rest of the picture (noise, background objects, etc.) can be anything but that color. This can be, of course, a very restrictive condition on an actual captured image of a neuron. For some images, thresholding can be used to satisfy this condition, but for others, especially those with lots of background noise, it may be necessary to trace the neuron first.

Preparing the Image

Before using the plugin, it is a good idea to make sure that the scale information embedded in the image is correct, as the plugin uses this information to calculate and report the results of its analysis. Specifically, the scale information is the conversion factor between pixels and physical units of length (i.e. microns). This can be set by going to the "Analyze" menu and selecting the "Set Scale…" item while the image window is selected. If there is no known scale information for the image, the analysis can still be carried out using pixels as the unit of length, but the usefulness of the results, especially when comparing them results from different images, may be limited.

When the plugin displays its options dialog box, it shows the current scale information at the top, to allow the user to ensure it is correct before continuing with the analysis. If it is not, then the user must cancel the dialog box and fix the scale before continuing.

Using the Plugin

Step 1: Use the point selection tool to select a point on the image. This point will be the center of all of the analysis circles that will be sampled. Ideally, it should be placed in the center of the soma for the best results.

Step 2: Run the plugin from the "Plugins" menu.

Step 3: Set the analysis parameters in the resulting dialog box and click "OK". Note that all values are in the units of length displayed at the top (i.e. microns). Details about the meaning of each analysis parameter are described below:

Starting Radius: The radius of the smallest analysis circle, along which the # of intersections will be measured.

Ending Radius: The maximum value for the radii of the analysis circles. This does not necessarily mean there *will* be an analysis circle with this radius value. All it means is that no analysis circles will have radii *greater* than this value.

Radius Step Size: The interval between radii of consecutive analysis circles. This value may be set to zero for continuous intersections measurements.

Radius Span: The margin around each radius value in which continuous intersection measurements are made. These measurements are then combined to calculate a single value for the # of intersections at that radius. This is a break from traditional Sholl analysis, since the intersections are being counted in an area *around* the circumference of each analysis circle, instead of only along the circumference. This value may be set to zero to disable the span feature, in which case only one intersection measurement is made for each analysis circle along its circumference.

Span Type: The statistical function used to combine intersection measurements within a span to produce a single value. Options are mean or median.

Starting Radius

Ending Radius

Step Size

Radius Span
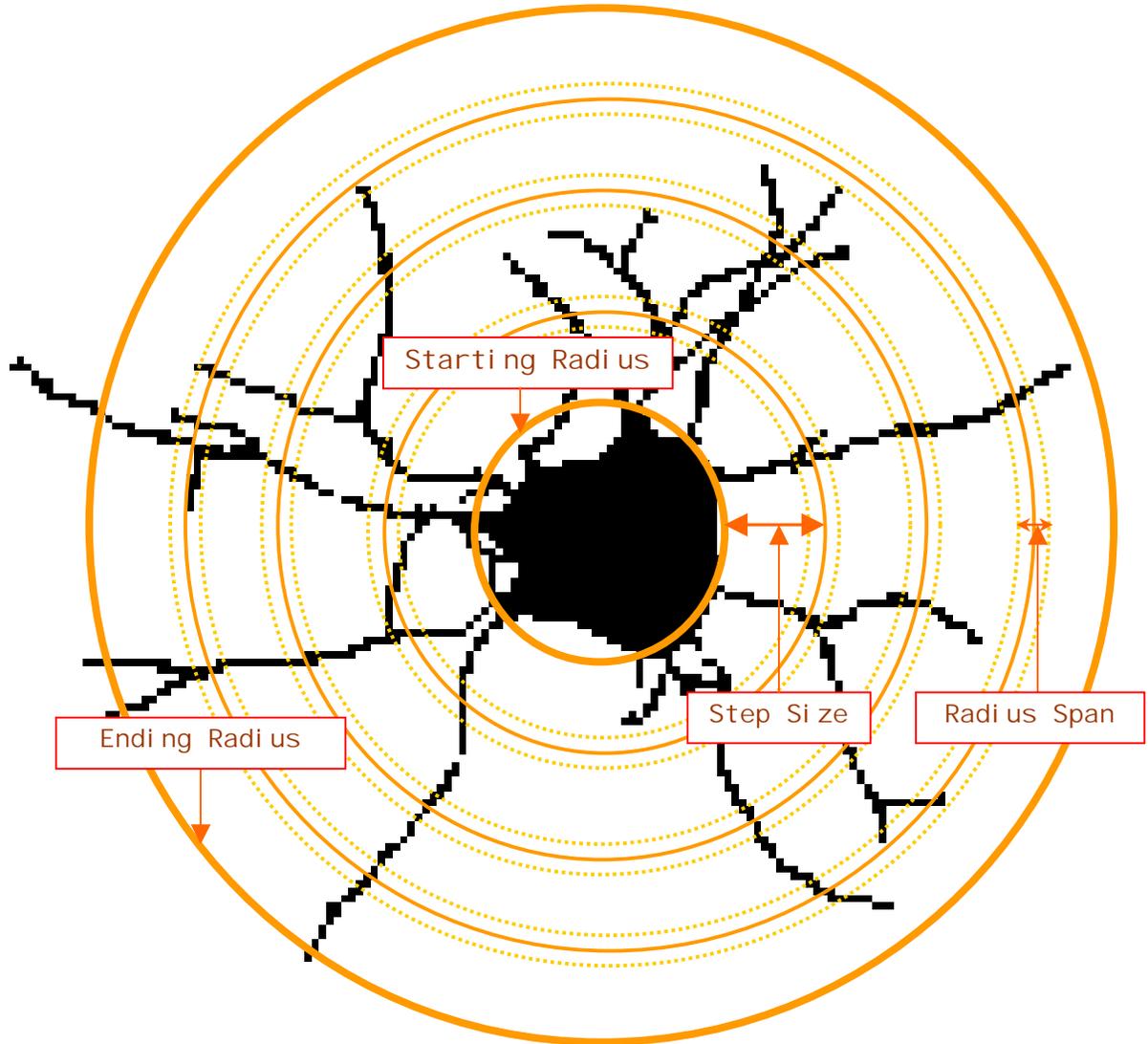
Step 4: Save the results, which can be done in many ways. The user can chose to save the resulting graph as a picture file by going to the "File" menu and selecting the "Save" option while the graph window is selected. Or the raw data from the analysis can be saved to a text file by clicking the "Save…" button on the graph window. The raw data can also be copied to the system clipboard by clicking the "Copy…" button on the graph window. Common spreadsheet programs can import the raw data directly from the saved text file or from the clipboard, to be used in further analysis.

Release Information

v1.0:   Initial release of the plugin.