Simple Neurite Tracer: open source software for reconstruction, visualization and analysis of neuronal processes

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ABSTRACT

Motivation: Advances in techniques to sparsely label neurons unlock the potential to reconstruct connectivity from 3D image stacks acquired by light microscopy. We present an application for semi-automated tracing of neurons to quickly annotate noisy datasets and construct complex neuronal topologies, which we call the Simple Neurite Tracer. Availability: Simple Neurite Tracer is open source software, licensed under the GNU General Public Licence (GPL) and based on the public domain image processing software ImageJ. The software and further documentation are available via http://fiji.sc/Simple_Neurite_Tracer at the same URL. Contact: longair@ini.phys.ethz.ch

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1 INTRODUCTION

As genetic techniques for imaging neurons have improved and become more widespread, there has been an increasing demand for software to extract accurate and quantitative data on morphology, topology and connectivity patterns from these images. This process, which typically involves generating a graph of points through the midlines of neurons, is often known as ‘tracing’. Extracting path data from 3D image stacks enables researchers to publish detailed information about putative connectivity, to reduce image complexity and produce summary statistics for different neuron types. When combined with image registration, neuronal paths can be analysed for variation or used to construct connectivity maps.

In this note, we introduce the Simple Neurite Tracer, an application for precise annotation of paths from tube-like structures (such as neurons or blood vessels) in large image stacks. Unlike many packages that analyse whole images with a single set of parameters, the Simple Neurite Tracer is an exploratory tracing system. The semi-automatic approach can efficiently find the best path between points in neurons and build up complex topology. The package also provides methods for measuring neuron length and volume, an interface for Sholl analysis and interactive 3D visualization. In addition, as part of the Fiji framework, the Simple Neurite Tracer interacts with other ImageJ plugins for further analysis and visualization, as well as supporting a choice of import and export options. The application is designed for images from light microscopy techniques such as confocal, rather than electron microscopy. Any image file format understood by Bio-Formats (Lindt et al., 2010), which currently supports over 100 different formats, may be used for tracing.

Simple Neurite Tracer is one of only a few truly Open Source, cross-platform applications for semi-automated neuron tracing in 3D image stacks. One alternative is the Farsight toolkit, and its trace editor, which, while having some more sophisticated segmentation methods, is more difficult to install and use. Meijering (2010) reviews other freely available software for neuron tracing including an early version of the Simple Neurite Tracer. Our software currently supports tracing only from existing image stacks, rather than directly from the microscope as with Neurolucida. In addition, it is best suited for fast tracing of higher order neural branches, rather than precise reconstructions of smaller features like spines, such as can be done with hxskeletonize.

2 FEATURES

2.1 Tracing interface

To build a simple path, users select successive points along the midline of a neural process, and the software will find a path between them. For clear images, these points may be distinctly separated in the volume, while for noisier data users will have to select points that are closer together. This interface is inspired by that of Neurolucida. In addition, it is best suited for fast tracing of higher order neural branches, rather than precise reconstructions of smaller features like spines, such as can be done with hxskeletonize.

Finding the paths between these points in the volume is done with bidirectional A* search (Hart et al., 1968) on the reciprocal of the cost of moving to a new point (Wink et al., 2004). The cost is the distance between the points, scaled by either the intensity of the image or a measure of ‘tubeness’ at the new point.

The speed and accuracy of the tracing can be improved with a Hungarian-based analysis of image curvatures, optimized to find structures of particular sizes (Sato et al., 1998). Parameters may be set manually or picked more intuitively from a palette of previews of the preprocessed data. Alternatively, images may be preprocessed...
The Simple Neurite Tracer can apply several variants of Sholl’s method, and visualize this distribution as a graph or by colouring the neuronal processes at concentric circles about a cell’s soma. This characterizes neuronal arbors based on the distribution of crossings of neuronal processes at concentric circles about a cell’s soma. The authors would like to thank Benjamin Schmid, Albert Cardona, Stephan Preibisch, Ting Zhao and Johannes Schindelin for contributions of code and advice. The authors would like to thank Benjamin Schmid, Albert Cardona, Stephan Preibisch, Ting Zhao and Johannes Schindelin for contributions of code and advice. Conflict of Interest: none declared.

3 ANALYSIS

A common method for producing summary statistics of neuronal morphology is Sholl analysis (Sholl, 1953). This method characterizes neuronal arbors based on the distribution of crossings of neuronal processes at concentric circles about a cell’s soma. The Simple Neurite Tracer can apply several variants of Sholl’s method, and visualize this distribution as a graph or by colouring the paths. The interface can also launch the Analyze Skeleton plugin to generate further statistics that describe the morphology of the traces. Since the software can export data in CSV or the native XML-based format, creating custom analyses is a further option.

3.1 Visualization

Simple Neurite Tracer provides 2D visualization of traces and 3D rendering using the ImageJ 3D Viewer (Schmid et al., 2010). The interface by default shows a projection of all traces in the stack, but may be changed to only show elements in slices within a configurable distance. The researcher can switch between orthogonal views of the data, thus making it easier to locate the midpoints of larger neurons.

3.2 Interoperability

The open file format used by Simple Neurite Tracer (see Supplementary file 1) can be easily parsed by programmers for further analysis. However, a higher level view of the data can be obtained with the ‘Export to CSV’ option, suitable for analysis in spreadsheets or statistical software. The ‘Export to SWC’ option produces SWC files (Cannon et al., 1998), which may be analysed in software such as L-measure (Scorcioni et al., 2008), providing a greater range of analysis methods, or uploaded to databases such as NeuroMorpho.org.

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