SOFTWARE ORIGINAL ARTICLE



nGauge: Integrated and Extensible Neuron Morphology Analysis in Python

Logan A. Walker^{1,2} · Jennifer S. Williams³ · Ye Li⁴ · Douglas H. Roossien⁵ · Wei Jie Lee³ · Nigel S. Michki¹ · Dawen Cai^{1,4,6}

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Abstract

The study of neuron morphology requires robust and comprehensive methods to quantify the differences between neurons of different subtypes and animal species. Several software packages have been developed for the analysis of neuron tracing results stored in the standard SWC format. The packages, however, provide relatively simple quantifications and their non-extendable architecture prohibit their use for advanced data analysis and visualization. We developed *nGauge*, a Python toolkit to support the parsing and analysis of neuron morphology data. As an application programming interface (API), *nGauge* can be referenced by other popular open-source software to create custom informatics analysis pipelines and advanced visualizations. *nGauge* defines an extendable data structure that handles volumetric constructions (e.g. soma), in addition to the SWC linear reconstructions, while remaining lightweight. This greatly extends *nGauge*'s data compatibility.

Keywords Neuron reconstruction · Neuron morphometrics · Neuron visualization

Introduction

The comparative study of neuron morphology has been a definitive aspect of contemporary neuroscience (Ramón y Cajal, 1892). Recent technological advances have enabled huge increases in the number of neuron reconstructions that can be performed in a single study into the hundreds

Logan A. Walker and Jennifer S. Williams contributed equally on this work

Dawen Cai dwcai@umich.edu

- ¹ Biophysics Program, University of Michigan LS&A, Ann Arbor, MI, USA
- ² Department of Computational Medicine and Bioinformatics, University of Michigan Medical School, Ann Arbor, MI, USA
- ³ Electrical Engineering and Computer Science, University of Michigan Engineering, Ann Arbor, MI, USA
- ⁴ Cell and Developmental Biology, University of Michigan Medical School, Ann Arbor, MI, USA
- ⁵ Department of Biology, Ball State University, Muncie, IN, USA
- ⁶ Neuroscience Graduate Program, University of Michigan LS&A, Ann Arbor, MI, USA

(BRAIN Initiative Cell Census Network (BICCN) et al., 2020; Gouwens et al., 2019, 2020; Jiang et al., 2021). Multispectral labeling (Li et al., 2020; Shen et al., 2020) and large-volumetric electron microscopy (Motta et al., 2019; Phelps et al., 2021; Yin et al., 2020), in principle, allow reconstructing many more neurons within one brain or within a single common coordinate system (Wang et al., 2020). As a result, analysis techniques must be developed which allow these data to be integrated, with specific focuses on the ability to customize, automate and quickly expand processing workflows to handle large batches of individual neurons, including those reconstructed from various methods.

Neuron reconstructions are commonly abstracted as connected linear branches and stored using the SWC file format (Nanda et al., 2018). SWC files are light-weight and textformatted that contain tab-delimited lines. Each line represents a point in the neuronal tree structure, which contains the node ID, node type (i.e., soma, axon, dendrite, etc.), X coordinate, Y coordinate, Z coordinate, the radius of the point, and the ID of the parent node to which this node links. Previously, we have also defined a volumetric SWC format where soma records are defined as a series of X–Y contour tracings along the Z axis to allow a more precise representation of soma shape (Roossien et al., 2019). Notably, the parent–child branch linkages present in SWC files result in a data model that can be understood as a directed graph, where no cycles are allowed to form. Many traditional data analysis tools do not take advantage of the underlying tree-like structure of the data, instead, treating the data as a "point-cloud".

Despite this growing need, current tools for neuron morphology calculations are largely limited to closed-form and predefined analyses, such as the popular L-Measure (Scorcioni et al., 2008) package and tools included with the various community (Cuntz et al., 2010; Peng et al., 2014; Roossien et al., 2019) or commercially-available (e.g. Neurolucida, MBF Biosciences; Imaris, Bitplane) neuron reconstruction software and plugins. Several libraries exist for the manipulation of neuron models after reconstruction, such as the TREES Toolbox (Cuntz et al., 2010) and the Neuro-Anatomy Toolbox (NAT) (Bates et al., 2020), however, their APIs preclude beginner use due to their complexity. Several analysis toolkits have been introduced, such as BTMORPH (Torben-Nielsen, 2014), PyLMeasure,¹ the NAVis² package, and python-Lmeasure,¹ in order to enable the quantification of neuron morphology inside of the Python programming language, which has rapidly emerged as the lingua franca of machine learning and data science. However, all of these tools are either limited in extensibility or simply run other binaries in the background (which lead to large software dependencies). The recent MorphoPy (Laturnus et al., 2020a, b) package solves these problems by implementing many functions in native Python code, but has only limited ability to be extended to novel metric definitions, no standardized memory structure, and no ability to produce 3D visualizations. Several software packages, such as NeuroMorphoVis (Abdellah et al., 2018) and the recent Brainrender (Claudi et al., 2020) package provide tools to prepare SWC files for complex 3D rendered figures, however, these tools are not designed to also perform quantitative analysis in the native Python environment. This data integration process for larger projects largely relies on bespoke methodologies which are limited in their reuse and accessibility.

In this report, we present *nGauge*, a software library that serves as a Python toolkit for quantifying neuron morphology. Included in the library are a collection of tools to perform standard and advanced morphometric calculations, manipulate reconstructed tree structures via SWC files, and generate visualizations within Python-native graphics libraries. We have applied nGauge to the analysis of several collections of published reconstructions, demonstrating the ability to build easily parallelizable, easily understood, and reproducible bioinformatics pipelines. nGauge exposes a well-documented API, allowing complex morphometry analyses to be programmed quickly in conjunction with other popular bioinformatics Python software, making the library extensible and customizable to new applications. nGauge also operates within the Blender 3D modeling software, allowing the creation of publication-quality animations

without the need for 3D rendering expertise. Finally, *nGauge* defines an extendable data structure to handle volumetric and linear neuronal constructions to greatly extend its data compatibility while remaining lightweight.

Materials and Methods

Library Implementation

nGauge was implemented in Anaconda Python 3.7.6 using standard object-oriented coding practices. The results presented herein are produced using the latest version of *nGauge* as of the time of writing (0.1.2). The library makes use of other numerical methods from dependencies NumPy (Harris et al., 2020) and SciPy (Virtanen et al., 2020). Additionally, the matplotlib (Hunter, 2007) library is used for library plotting functions.

We implemented 103 (at time of writing) API functions which consist of single- and multivariate morphometrics, utility functions, and data structures, as described in Results. All implemented methods were tested with the Python unittest library¹ to ensure library self-consistency. We compared the results with the output from similar functions from two previously published tools to ensure their validity (Laturnus et al., 2020; Scorcioni et al., 2008). Selected comparisons are presented in Results.

Previously Published Data Access

Previously-published neuron reconstruction data was downloaded using the bulk downloading tools on the Neuromorpho.org (Ascoli et al., 2007) website in SWC format (Nanda et al., 2018) from several previously-published articles (Fukunaga et al., 2012; Miyamae et al., 2017; Stokes et al., 2014). The standardized version of these SWC files was used to ensure format adherence. Additional SWC and image data were obtained from our previous study (Li et al., 2020).

Cell Type Clustering

To provide a use case for how nGauge would be applied in a typical experiment, cell type clustering was performed using the above-referenced released datasets with custom python scripts. For each SWC file, the following vector of morphological parameters was calculated using nGauge: number of branch points, number of branch tips, cell dimensions, number of cell stems, average branch thickness, total path lengths, neuron volume, maximum neurite length, maximum branch order, path angle statistics, branch angle statistics,

¹ https://docs.python.org/3/library/unittest.html

maximum branching degree, tortuosity statistics, and tree asymmetry. This collection of vectors was then used as input into the scikit-learn (Pedregosa et al., 2011) PCA implementation. Visual inspection of distributions was used to ensure individual clusters formed.

Cell Mask Generation

Our cell mask generation process contains two major steps. First, a minimum convex hull of all points in the SWC file is calculated using the implemented methods in SciPy (Virtanen et al., 2020), namely the quickhull algorithm (Barber et al., 1996). This hull represents a 3D polygon that includes all points in the SWC file, represented as a series of lines in 3D space. After the hull is generated, the second step runs a filling. This process is applied for each neuron with a different fill value, resulting in a single-color TIFF file that can be visualized as a segmentation mask of the same size as the original image, allowing it to easily be overlaid.

3D Neuron Visualization

All 3D visualizations were generated using the Blender 2.82.2 (Blender Foundation; blender.org) software package, following a compositing method similar to previously described (Kent, 2014). Briefly, 3D models are exported by representing each segment in an SWC file as a series of rounded cylinders, after a percentile downsampling to reduce the total number of points rendered in the 3D mesh. A decimation filter is applied to generated models to optimize the number of rendered surface points, reducing rendering time and storage requirements significantly. Standard Blender compositing techniques are then used to apply keyframes and animate scenes, as per the software documentation.

Visualizing of raw TIFF microscopy data (Fig. 8C) was performed as follows: First, individual z-slices were exported as RGB PNG files using a script in the Fiji (Schindelin et al., 2012) image analysis software. Each slice was mapped onto the 3D model using a custom Open Shader Language (OSL) plugin (see Information Sharing Statement). This allows the rendering engine to access individual z-slices without the requirement that the entire TIFF file be stored in memory.

Performance Testing

Measurement of calculation runtimes within Python was performed with the timeit library² to run each function 4 times and automatically calculate the standard deviation using custom testing scripts. L-Measure performance was measured using the



Fig. 1 Introduction to *nGauge* **A** Individual neuron morphologies that have been reconstructed are represented by SWC files. Each SWC file consists of a tabular list of individual points that make up the neuron tree structure; **B** The *nGauge* library serves as a facilitator for a variety of common Neuroinformatic tasks

Linux time utility to time only the compiled lmeasure binary, with 4 runs manually recorded from the terminal. All tests were performed on a Ubuntu Linux 20.04 server with two AMD EPYC 7351 processors, 512 GB of RAM, and all data stored on SSDs to minimize bottlenecks.

Results

nGauge is the Center of a Complete Analysis Environment

Neuron reconstruction experiments include three primary steps (Fig. 1A). First, images are acquired containing the neurons of interest. Next, tracing software is used to reconstruct the neuron topology, and, finally, bioinformatic hypotheses can be tested from the resulting neuron reconstructions. These neuron reconstruction files are generally represented by the SWC format, which has been formally defined as a tabular linked list of the coordinates (Nanda et al., 2018). Because of the unique structure of this format, many general-purpose data science tools and data structures can not be efficiently applied for the analysis of neuron morphology. For this reason, we developed *nGauge* to simplify the wide variety of bioinformatics tasks, such as morphometry, model manipulation, visualization, as well as statistical analysis with the help of other python numerical libraries (Fig. 1B).

² https://docs.python.org/3/library/timeit.html



Fig.2 nGauge Library Schema **A** nGauge is a publicly-available python library that can be installed easily in one shell command. The library is composed of 3 separate modules: Neuron (**B**), TracingPoint (**C**), and util (**D**). These modules implement models for an entire Neuron, a single SWC datapoint, and utility/math functions, respectively. Arrows represent cross-references between the module variables. Each module is labeled with the number of functions available at time of writing (see Supplementary Table 1 for more information).

Library Structure

nGauge is implemented as 3 different modules, which can be installed in a single step from the Python package repository (Fig. 2A). Each module represents an abstraction of either a single neuron, a single line in an SWC file, or the collection of utility functions used throughout the library (Fig. 2B-D). The Neuron module (Fig. 2B) stores two primary data structures. The first is a dictionary map between all soma Z-coordinates and the points which make up that "slice" of the 3D model. This data model is adapted from (Roossien et al., 2019), where the SWC format was extended to store volumetric models of somata. The second data structure stores the locations of each branch's root node, i.e. the point at which it contacts the soma. Because each branch is a directed linked list, the only node which is needed by the Neuron module for a complete model of each of its branches is the root node of the branch. The Tracing point class (Fig. 2C) is used to represent a single SWC entry, or what would be recorded in a single line of an SWC file (Fig. 1A), including the X, Y, and Z coordinates, as well as the point radius, and links to the Tracing point's that serve as the parent and child nodes in the linked list. In effect, the Neuron and TracingPoint libraries serve as an in-computermemory representation of an SWC file, with the ability to be extended by Python object oriented programming techniques. This new memory organization allows traversal of the SWC file to be performed more effectively and efficiently than keeping data in the tabular SWC file format.

In Supplementary Table 1, we present a summarized list of 103 functions that are available in the current version of *nGauge*. Functions are located such that their use can match industry-standard object-oriented programming practices, leading to more readable and maintainable code. Some methods' scopes logically apply to both Neurons and TracingPoint structures (e.g., functions to calculate structure size) and are implemented in both classes.

Introduction to nGauge Usage

Care has been taken to make the use of *nGauge* as beginnerfriendly as possible. To demonstrate this, we analyzed a collection of neurons from (Li et al., 2020) with our library (Fig. 3). First, the library and data are loaded (Fig. 3A, B). Single-named morphometrics can be calculated easily by calling the methods associated with the Neuron class-in this case, the width and height of the loaded neuron (Fig. 3C). Creating a plot of the neuron is also a single command (Fig. 3D). While it is not shown here, plot axes and appearance parameters can be modified to get different views of the same data. Upon execution, matplotlib (Hunter, 2007) is loaded, allowing plots to be customized. When analyzing entire experiments or sample groups, Python list comprehension can be used to generate whole figures quickly (Fig. 3E, F).

Comparison with L-measure

We chose to first compare our tool with L-Measure (Scorcioni et al., 2008) because it is one of the most widelyadopted and established tools for neuron morphology analysis (Fig. 4). Additionally, several existing Python tools, such as PyLMeasure and python-Lmeasure run L-measure binaries to perform calculations in the software backend. For this comparison, we downloaded the SWC files of 42 neurons from (Stokes et al., 2014) and (Fukunaga et al., 2012), which are curated on Neuromorpho.org (Ascoli et al., 2007) (Fig. 4A, Methods). Three representative metrics were selected to compare the tools: the number of neurite tips in the entire neuron (Fig. 4B), the path distance of all segments of the neuron (Fig. 4C), and the total neuron width (Fig. 4D). As expected, the number of neurite tips and path distances are the same as calculated between the two tools (Fig. 4B, C). The result for the total neuron width (Fig. 4D) is more nuanced, however. The L-Measure width function is defined as the width after "eliminating the very outer points on the either ends by using the 95% approximation", to prevent small structures from interfering with quantification. In nGauge, this is implemented as an explicit percentile calculation using an optional parameter that ranges from 0 to 100%. Finally, comparable methods between the two software packages perform faster in the

Fig. 3 nGauge Usage Examples Several code examples are included to demonstrate the processes of using nGauge. A Importing the library is a single command; B SWC files can be directly imported as Neuron objects; C morphometrics can be easily calculated, in this case, neuron width and height (including 100% of neuron points) are calculated as members of the Neuron class; D Interaction with Python graphical libraries such as Matplotlib allows the generation of publication-quality figures; E, F Entire lists of files can be processed at once to run statistical analyses using python list comprehensions.



nGauge implementation, although the speed difference varies (Fig. 4 Insets). A more completed comparison analysis included 13 metrics calculated with both nGauge and LMeasure is shown in Supplementary Fig. 1.

Performing Advanced analysis With nGauge

In addition to quantifying basic morphometric parameters, *nGauge* includes comprehensive utility



Fig. 4 Comparing *nGauge* with L-Measure **A** An overview of the comparison study; **B**, **C** Two example functions (tip node count and maximum path distance) produce identical output between *nGauge* and L-Measure; **D** Another example function (neuron width) produces similar output between *nGauge* and L-Measure, however, a dif-

ference of definitions produces a slight bias. Two parameter choices are shown for the nGauge result, as indicated by marker style; Inset for each plot *nGauge* scripts complete faster than their L-Measure equivalent (avg. \pm std., n=4 per script)

functions for advanced neuroinformatics analysis (Supplementary Table 1). For instance, *nGauge* implements the widely used Principal Component Analysis (PCA) to identify the differences between vectors of morphometrics, which allows the classification of neuron morphological "subtypes" (Gouwens et al., 2019; Laturnus et al., 2020a, b). We performed PCA on a collection of pyramidal cells and basket cells (Miyamae et al., 2017) (Fig. 5A) and a collection of tufted cells and mitral cells (Fukunaga et al., 2012) (Fig. 5B). We find that both comparisons yield group separation along "principal component 1", indicating that neuronal subtypes are divisible by their morphological differences, matching what has been reported in the previous literature.

Beyond single-value morphometrics, many tools have been integrated into nGauge for performing advanced analysis techniques. Influenced by recent work (Laturnus et al., 2020a, b), nGauge includes tools to calculate 2D morphometric histograms. Two example cells from (Miyamae et al., 2017) are shown to compare the location (distance from the soma) of bifurcation and the shape (branch angle) of bifurcation (Fig. 6). We can see that the mouse chandelier cell on the top (NeuroMorpho ID NMO 104470) has a much higher density of bifurcation points and its bifurcation points locate farther from the soma (Fig. 6, red dots) than the Basket cell at the bottom (NeuroMorpho ID NMO_104476). Plots like these can serve as "fingerprints" for any morphological properties of individual neurons, and allow comparison between cells for similarity in correlation between metrics.

Finally, we show an example to demonstrate that nGauge can be extended to work with other Python-based scientific computation packages to create complex statistics. Using the SciPy library combined with a simple nGauge script, we created a unique tool for the generation of TIFF 3D masks to represent the convex hulls that enclose the extent of individual neurons. This function enables the visualization and quantification of the spatial "coverage" of each significant neuronal arbors (such as dendrites and axons). In Fig. 7, the tool is applied to identify the neurite fields of individual Drosophila ventral nerve cord serotonergic neurons reconstructed from (Li et al., 2020). This is of biological interest because, as serotonin can act as a diffusive volume transmitter (Quentin et al., 2018), each neurite field may be used to estimate the range of that serotonergic neuron's modulation. These TIFF masks can be used to directly quantify this range. The TIFF masks can also be used to quantify more complex geometric properties. For instance, the intersection volume between two neurons' projection fields can be calculated using NumPy (Harris et al., 2020) as 'np.sum(np.and(a, b))', or using Fiji's Image Calculator



Fig. 5 *nGauge* for Cell Type Discrimination *nGauge* can be used to perform unbiased exploratory data analysis based on morphological parameters; **A** Pyramidal Cells are compared against Basket Cells (Miyamae et al., 2017); **B** Tufted Cells are compared against Mitral Cells (Fukunaga et al., 2012). We note that in both of these comparisons, groups form along PC1 based on cell type. Each comparison is displayed as a principal component scatter plot and a projection of each SWC file is shown adjacent to each datapoint.

library (Schindelin et al., 2012). Figure 7E plots the total arbori-zation volume of each neuron as a bar chart of total voxels(bottom) and displays this intersection volume as a heatmap between each pair of cells (top). Together, this demonstrates the utility of *nGauge* as a data structure API. **Fig. 6** 2D Histograms of Cell Morphology Two cells (see **Results** for descriptions) are plotted as 2D histograms comparing the path distance from the soma to the branch angle for each bifurcation point in the neuron. Colorbar indicates the count for each square in the grid. To the left of each plot is a projection of the source SWC file, with the soma point highlighted in red



Blender and nGauge Enable Advanced Visualization

Visualizing neuron reconstructions in their physical context is highly valuable as it can create a direct perspective of how these neurons interact with each other and with other unreconstructed objects in the brain. We used *nGauge*'s API to create a script that renders publication-quality images and movies in the Blender 3D modeling software, which is an industry-standard open-source tool for 3D animation and visualization. We rendered the full tracing results of



Fig. 7 Projection Field Mapping of Multiple Neurons We developed a novel tool for rendering the projection field volume of a specific SWC file. **A** A maximum projection of an example image from (Li et al., 2020); **B** An overview of neuron tracing reconstructed in (Li et al., 2020); **C** 4 randomly-chosen neuron reconstructions; **D** *nGauge*'s domain mapping tool was used to identify volumes corresponding to

each SWC file in **C**; **E** A heatmap of the volume overlap percentage between each pair of samples in the experiment. Nonnegative matrix values identify cells which have overlapping domains. Each square is normalized to the volume of the cell identified in the X-axis, which is depicted in the bar plot to the bottom of the figure



Fig. 8 3D Modeling with *nGauge* and Blender *nGauge* includes utilities to render publication-quality images and movies in the Blender 3D modeling software; **A**, **B** All somas and neurites (n = 182) reconstructed in Roossien et al., 2019 are modeled. Each panel displays a

182 Brainbow-labeled neurites from the CA1 region of the mouse hippocampus (Roossien et al., 2019) in two different projections (Fig. 8A, B). These renderings, generated by only a few lines of code (available in the source repository, Methods), visualize the density of the reconstruction, as well as how different somata in the reconstructed volume are positioned relative to each other. More advanced rendering techniques were used (Li et al., 2020) to visualize reconstructed serotonergic neurons of the *Drosophila* ventral nerve cord (VNC) in the context of the Bitbow fluorescence microscopy data (Fig. 8C). Because Blender is designed for rendering still images and animations, it was possible

separate view of the same data; C Data from (Li et al., 2020) is plotted atop the raw data. A full animation of this figure is available as Movie S2 in (Li et al., 2020)

to create a movie to display multiple angles of the neuron models (see Movie S2 in (Li et al., 2020) for example).

Discussion

In biomedical image processing, the Fiji package (Schindelin et al., 2012) has simplified creating reproducible image processing protocols through an open environment of plugins and tools which use the Fiji data models to perform novel analyses. The development of extensible libraries such as *nGauge* are an important

step to produce the same standardization in the pipelines used to analyze neuron reconstruction experiments. In its current form, nGauge's library implements more than 100 morphometric calculation functions as well as provides APIs for developing new informatics tools. Notably, this simplifies the number of software tools that need to be managed and connected together to complete morphometry analysis, which lowers the learning barrier and saves time for non-informatics specialists. Combined with visualization tools, nGauge empowers the creation of publication-quality figures with ease. In fact, during the development of the *nGauge* project, we have already applied all of the individual modules to produce results both in publication and in preparation, finding it to be a very effective toolkit for efficient data science (Dizaji et al., 2020; Duan et al., 2020; Li et al., 2020; Shen et al., 2020).

We have demonstrated that *nGauge* is a powerful tool for neuroinformatics, however, it is not without limitations. First, some tools, including L-Measure and TREEs toolbox, include graphical user interface (GUI) programs that make it possible to do analysis without ever touching code at all. While this is not the goal of nGauge, it is important to note that we do not include a GUI component. Furthermore, there are some neuron reconstructions that are performed using volumetric reconstruction or mesh reconstruction. nGauge does not allow analysis of these data directly, because they are not describable as a SWC file.

Large-scale programs such as the NIH BRAIN Initiative Cell Census Network (BICCN) are providing the neuroscience community with ever-expanding collections of reconstructed neuron morphology, like many other data types and modalities. Making use of this data will require a new generation of neuroinformatic data science tools that are optimized for contemporary programming techniques and are easily extensible. We believe that *nGauge* represents a significant step toward this goal, by both providing an easy way to run a large collection of "canned" analyses and by providing a platform for the experimentation and development of new metrics through a welldocumented data API. As a Python library, *nGauge* can be seamlessly integrated into the most popular machine learning and data science pipelines.

Conclusion

We have presented nGauge, a Python package for performing neuron morphology calculations. To demonstrate its utility, we performed various visualization and analysis experiments, including performing cell type clustering and volumetric analysis of dendritic fields. nGauge produces equivalent results to L-measure in a collection of morphometrics. Finally, nGauge can be used as a data structure backend for developing, which can be used to integrate nGauge with Blender for 3D rendering.

In the future, we plan to continue the development of additional features for *nGauge*, such as adding tools for identifying synapse locations and performing connectivity analyses. Due to the lightweight data structure definition described here, it is straightforward to include new annotation types, such as volumetric segmentation (used in the soma here) or connectivity between tracing points in *nGauge*. We envision that *nGauge*'s open-source and expandability nature will attract contributions from the community to its public repository to make it an important toolkit of neuroscience research. Finally, we envision that *nGauge* can be used in future educational applications, such as building 3D print models for pedagogical purposes.

Information Sharing Statement

nGauge is developed for Python 3.7 and has been tested for compatibility on the most recent version of Python at the time of writing (Python 3.9). The library is available from the Python pip package manager by executing the following command in a terminal: pip install ngauge. The source code, documentation which is automatically built with Sphinx,³ and issue tracker are also available from the following Github repo: https://github. com/Cai-Lab-at-University-of-Michigan/nGauge. *nGauge* usage tutorials are provided in this repository. The provided Blender rendering tools are compatible with any version of Blender which uses a Python 3.8+ scripting interface. Installation instructions are included within the above-referenced GitHub repo. All data is available through the GitHub repository above or from the corresponding author upon reasonable request.

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Author Contributions LAW and DC conceptualized the *nGauge* library, which was then implemented by LAW and JSW. YL and DR provided imaging and neuron reconstruction datasets which were used in library testing. LAW, JSW, YL, WJL, and NM contributed to beta testing of early versions of *nGauge* and provided comments on the library design. LAW, JSW, and DC wrote the manuscript, which was edited and approved by all authors.

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