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Reporting Summary

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Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

- | | |
|-------------------------------------|--|
| n/a | Confirmed |
| ☐ | ☒ The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement |
| ☐ | ☒ A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly |
| ☐ | ☒ The statistical test(s) used AND whether they are one- or two-sided
<i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i> |
| ☐ | ☒ A description of all covariates tested |
| ☐ | ☒ A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons |
| ☐ | ☒ A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals) |
| ☐ | ☒ For null hypothesis testing, the test statistic (e.g. F , t , r) with confidence intervals, effect sizes, degrees of freedom and P value noted
<i>Give P values as exact values whenever suitable.</i> |
| ☒ | ☐ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings |
| ☒ | ☐ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes |
| ☐ | ☒ Estimates of effect sizes (e.g. Cohen's d , Pearson's r), indicating how they were calculated |

Our web collection on [statistics for biologists](#) contains articles on many of the points above.

Software and code

Policy information about [availability of computer code](#)

Data collection Gold standard neuron reconstructions were obtained using Vaa3D (RRID:SCR_002609, version 3.497, <http://vaa3d.org>).

Data analysis The source code developed is released open source and is available at <https://github.com/lmanubens/BigNeuron>. The Shiny web app can be used at https://linusmg.shinyapps.io/BigNeuron_Gold166/ and <https://neuroxiv.net/bigneuron/>. The Shiny app source code can be found at: https://github.com/lmanubens/BigNeuron/tree/main/shiny_app. Source code of automated reconstruction algorithms developed throughout the project can be found at https://github.com/Vaa3D/vaa3d_tools/tree/master/released_plugins/v3d_plugins. The source code for the consensus tree algorithm is publicly available at: https://github.com/Vaa3D/vaa3d_tools/tree/master/hackathon/xiaoxiao/consensus_skeleton_2. We developed the source code in R (version 3.4.1.) and relied in the following R packages: ggpubr (version 0.4.0), mlr3 (version 0.12.0), Shiny (<https://www.shinyapps.io/>, version 1.6.0), prcomp (version 4.0.3), ggbiplot (version 0.55), factoextra (version 1.0.7), Rtsne (version 0.15), nat (version 1.8.16), heatmaply (version 1.1.0), mclust (version 5.4.7), ggpubr (version 0.4.0). Additionally, we used the TREES Toolbox (version 2.0).

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio [guidelines for submitting code & software](#) for further information.

Data

Policy information about [availability of data](#)

All manuscripts must include a [data availability statement](#). This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our [policy](#)

3D image volumes of the Gold166 dataset and gold standard reconstructions are available at <http://web.bii.a-star.edu.sg/bigneuron/gold166.zip>. Bench-testing automated reconstructions can be downloaded from https://github.com/BigNeuron/Data/releases/tag/gold166_bt_v1.0. The fMOST showcase datasets can be found at <https://zenodo.org/record/7556104>. The complete set of image volumes gathered throughout the project, amounting to ~4TB of data, is available upon request.

Databases of the Allen Mouse and Human Cell Types projects (<http://celltypes.brain-map.org/>), Taiwan FlyCircuits (<http://www.flycircuit.tw/>), and Janelia Fly Light (<https://www.janelia.org/project-team/flylight>) can be found in the links mentioned in parentheses.

Field-specific reporting

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☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	The number of gold standard datasets used for the study was determined by the throughput of manual annotation hackathons. We did a sample size estimation based on the maximum branch order measured in two types of neurons with N=6 (m1=12.5, m2=16.7, SD=4.45). A sample size of N=18 would have a Type I error rate of 0.05 and a power of 0.80. Thus, we expected ~160 datasets would be sufficient to assess the accuracy of automated tracing algorithms.
Data exclusions	No data were excluded for the analysis.
Replication	All attempts at replicating the results were successful using the Shiny app and published code.
Randomization	Randomization was not applicable to this study because tracing algorithms were applied to all datasets. Random training and testing samples were used to train the SVM regression.
Blinding	Blinding was not applicable in this study because analysis was performed a few years after development of the algorithms by different researchers.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☒	☐ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☒	☐ Animals and other organisms
☒	☐ Human research participants
☒	☐ Clinical data
☒	☐ Dual use research of concern

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging