Advanced NeuroGPS-Tree: dense reconstruction of brain-wide neuronal population close to ground truth

Zhou Hang[1,2,#], Li Shiwei[1,2,#], Xiong Feng[1,2], Han Jiacheng[1,2], Kang Hongtao[1,2], Chen Yijun[1,2], Li Yun[1,2], Li Jing[1,2], Su Lei[1,2], Li Anan[1,2], Gong Hui[1,2], Zeng Shaoqun[1,2], Quan Tingwei[1,2,*], Luo Qingming[1,2,*]

[1] Collaborative Innovation Center for Biomedical Engineering, Wuhan National Laboratory for Optoelectronics-Huazhong University of Science and Technology, Wuhan, Hubei 430074, China

[2] Britton Chance Center and MOE Key Laboratory for Biomedical Photonics, School of Engineering Sciences, Huazhong University of Science and Technology, Wuhan, Hubei 430074, China

[3] School of Mathematics and Economics, Hubei University of Education, Wuhan, Hubei 430205, China

# Equally contributed to this work.

Email: quantingwei@hust.edu.cn and qluo@mail.hust.edu.cn

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Abstract

Neuron is the basic structure and functional unit of the brain, its projection and connections with other neurons provide a basic physical infrastructure for neural signal storage, allocation, processing, and integration. Recent technique progresses allow for labeling and imaging specific neuronal populations at single axonal level across a whole mouse brain. However, digital reconstruction of these neuron individuals needs months of human labor or sometimes is even an impossible task. Here we developed a software tool, Advanced NeuroGPS-Tree (AdTree), to solve this problem. AdTree offers a special error screening system to achieve reconstruction close to the round truth. Moreover, AdTree equips with algorithms significantly reduces intense manual interferences and achieves high-level automated reconstruction. We demonstrated using AdTree to get almost complete reconstruction from basal dendrites to far-end axonal terminals on dense datasets that challenge state-of-the-art software tools, and even the manual reconstruction with commercial software, whose accuracy is well acknowledged. We also demonstrated about 5 times speed faster over commercial software in brain-wide reconstruction. In addition, AdTree is applicable on datasets collected with different types of light microscopies such as confocal, two-photon, and light-sheet and serial sectioning wide-field tomography. This tool will provide a starting point to reconstruct neuronal population for cell type, circuit, and neurocomputing studies at single cell level.

Introduction

Neuronal circuit is responsible for processing and transmitting information(Oh et al. 2014; Bohland et al. 2009). Mapping a neuronal circuit at cellular resolution is one of the central tasks in neuroscience(Zingg et al. 2014; Bargmann 2012; Ohno et al. 2016), and essentially requires to reconstruct neuronal population at brain-wide scale, because neuronal circuit usually contains a population of pyramidal neurons whose axonal projections are across different brain areas or even the whole brain(Economon et al. 2016; Gerfen et al. 2016). The recent imaging(A. Li et al. 2010; Gong et al. 2013; Gong et al. 2016; Ragan et al. 2012; Silvestri et al. 2012; Osten and Margrie 2013; Chung and Deisseroth 2013) and molecular labeling techniques (Feng et al. 2000; Callaway 2008; Ugolini 2010; Jefferis and Livet 2012) have provided the data basis for brain-wide population reconstruction. Using these imaging techniques, the entire mouse brain images with submicron resolution can be automatically collected within a week, from which, we can observe almost complete morphology of neuronal population at single axon resolution(Osten and Margrie 2013). However, quantifying this kind of morphology, i.e., neuronal population reconstruction will cost the months of manual labor. The huge gap between ultrafast generation of imaging datasets and extremely time-consuming reconstruction hinders our understanding of neuronal circuits. Developing the corresponding reconstruction tool is essential for filling this gap.

The reconstruction tools can be classified into two groups: the automated and semi-automated. The automated tools have been greatly developed in recent years (Peng et al. 2015; Liu 2011; Donohue and Ascoli 2011; Lu 2011; Meijering 2010; Svoboda 2011; Wang et al. 2011; Zhao et al. 2011; Turetken et al. 2011; Chothani et al. 2011; Bas and Erdogmus 2011). For example, Vaa3d employed a combination of big data technique and excellent tracing method to reconstruct a single neuron from TB or more size neuronal images (Peng et al. 2017). Our previous tool, NeuroGPS-Tree (Quan et al. 2016), aims at reconstructing a population of neurons rather than a single neuron, and achieved 960 neurons reconstruction within 3 hours from the mouse

cortex dataset. Despite these breakthroughs, it is nearly impossible for the automated tools to generate accurate reconstruction from brain-wide imaging datasets. This dataset usually contains a dense population of neurons. Dense reconstruction is an open problem and has not been well resolved up to now (Lichtman and Denk 2011; Helmstaedter 2013). Therefore, for automated methods, reconstruction errors are unavoidable in the presence of packed neurites. Furthermore, the long process of brain-wide reconstruction worsens the situation that one false reconstruction usually leads to a number of missing or miss-assigned neurites, determined by tree-like structure of neuron. In this case, reconstruction with semi-automated tools (Magliaro et al. 2017; www.amiraviz.com; www.neurolucida.com) is the only choice for trustworthy results, but it is an extremely laborious and time-consuming work. It takes about 70 hours for a neuron reconstruction with sparsely spaced neurites. When performing dense reconstruction at brain-wide scale, the time cost is sometimes beyond one's imagination.

Here, we developed a software tool, named Advanced NeruoGPS-Tree (AdTree), for brain-wide population reconstruction. AdTree offers the system to thoroughly screen the reconstruction errors. This screening system employed selective display mode to check the population reconstruction generated with NeuroGPS-Tree. In selective display mode, we can focus on checking the target reconstruction and its neighboring region. And thus can easily find errors in dense reconstruction. This screening system also provides multiple checking procedures in brain-wide reconstruction. This system can fastly locate the reconstruction errors hidden in TB size datasets. In addition, AdTree also employs the automatic algorithms for detecting the optimal skeleton of neurites and identifying neurites with weak signal, which vastly boost the automatic level of AdTree. Due to the above features, AdTree achieves brain-wide reconstruction of neuronal population with many packed neurites. The results suggest that the reconstruction is nearly equal to the ground truth, and about 5 times faster than reconstructions with commercial software. AdTree can be suitable for the brain imaging datasets collected with different optical imaging systems.

The design of AdTree

The challenges of brain-wide population reconstruction are originated from the characteristics of imaging datasets. The size of this dataset is tens of TBs, and usually contains neurites with the densely positioned, tortuous structure, and extremely weak signals etc.. These characteristics result in that reconstruction errors are unavoidable and difficult spotted. Considering the above cases, we designed AdTree for brain-wide population reconstruction. We employed the technique for multiresolution representation of dataset (Y. Li et al. 2017) to inexpensively extract a 3D region of interest (ROI) from the whole brain dataset (**Fig. 1a**). The first ROI is determined by the position of a soma which corresponds to the target neuron. Generally, the first ROI mainly contains somas and dendrites. In the first ROI, we used NeuroGPS-Tree to reconstruct the target neuron and other neurons. We revised the preliminary reconstruction of the target neuron in selective display mode (**Fig. 1b**). This mode provides an effective way to check reconstruction errors by removing unrelated signals. Meantime, AdTree can automatically record the locations where traced dendrites touch the boundary of the first ROI. These locations give cues to select the subsequent ROIs for tracing dendrites of the target neuron. After completing the reconstruction of dendrites, we located the initial part of axon for axonal tracing. Like tracing dendrites, human supervised checking procedure is implemented for the trustworthy reconstruction (**Fig. 1c**). Note that except for the first ROI, the neurites in all ROIs are traced with SparseTracer (S. Li et al. 2016). This is because SparseTracer has faster reconstruction speed compared with NeuroGPS-Tree when neurites are sparsely distributed. In a word, among population dataset, reconstructing a neuron with AdTree is required to repeat the procedure (**Movie 1**): 1) load the ROI into internal storage; 2) generate reconstructions from ROI with automatic algorithms; 3) check the reconstruction errors and edit them.

As described in the above procedures (**Figs. 1b-c**), human supervised reconstruction is performed in every sub-block. Brain-wide reconstruction of a neuron requires analyzing hundreds of sub-blocks, and reconstruction errors are still unavoidable.

AdTree uses the reconstruction navigation for finding reconstruction errors at brain-wide scale (**Fig. 1d**). With this navigation, we can fastly browse the reconstruction and its neighboring image region (**Moive2**). The browsing information is sufficient for checking reconstructions. Furthermore, for generating ground-truth reconstruction, the general strategy is that different annotators reconstruct the same neuron and regard the common consent as the ground truth (Helmstaedter et al. 2011). In this process, locating the difference between the reconstructions is an indispensable step. AdTree provides this function (**Fig. 1e & Movie3**).

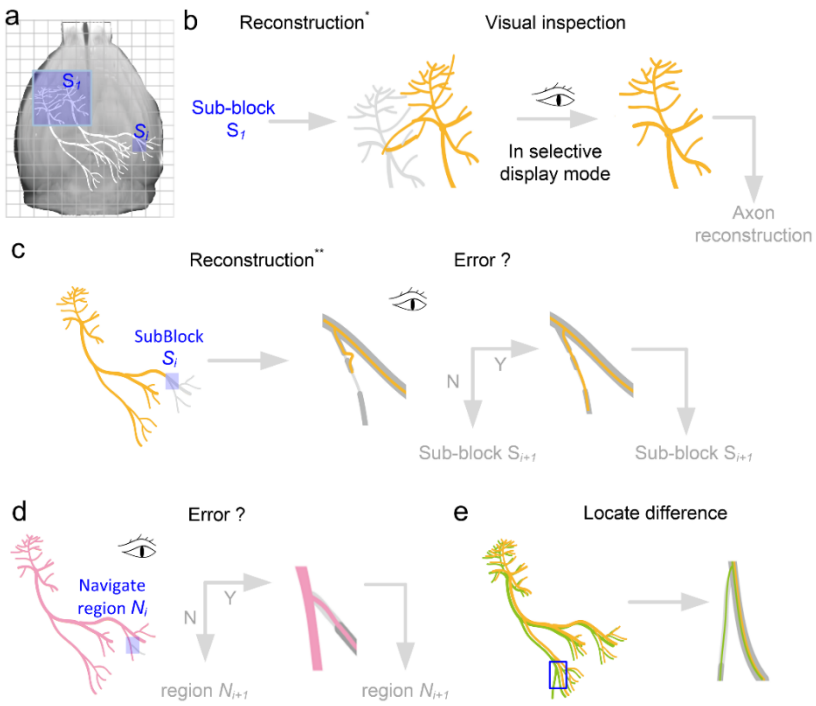

Figure 1. The pipeline of brain-wide reconstruction of neuronal population. (**a**) Divide the whole brain images into sub-blocks with the same size, and select the 3D region of interest (the 1st ROI) according to the known position of a soma that corresponds to the target neuron. ROI refers to the interested dataset including some divided sub-blocks. (**b**) Use NeuroGPS-Tree to reconstruct the target neuron and other neurons in the1st ROI. Revise the reconstruction of the target neuron in selective display mode, and find the initial part of the axon for axonal tracing. (**c**) Trace axons in the current ROI and revise the traced results; determine the new ROI that contains the locations where traced axons touch the boundary of the current ROI. (**d**) Reconstruction navigation for spotting reconstruction errors at brain-wide scale. (**e**) Online locate the differences between the reconstructions of a neuron performed by two annotators, and check the differences for trustworthy reconstruction.

From the entire pipeline of reconstruction, we find that besides selective display mode, which contributes to high accuracy of dense reconstruction, AdTree offers a system to

thoroughly screening reconstruction errors at brain-wide scale. The screening system includes human supervised reconstruction in each sub-block dataset, brain-wide reconstruction navigation, and online localization of differences between reconstructions in massive dataset. This system runs through the pipeline of reconstruction and can assure the high precision reconstruction.

Dense reconstruction

We demonstrated the feature of AdTree in dense reconstruction using a typical dataset from neocortex. The dataset contains the packed neurites with extremely large range of signal intensity (**Fig. 2a**), and thus challenges the current automatic reconstruction methods. For dense reconstruction, we designed the selective display mode in AdTree. This mode enables us to focus on checking the target reconstructions and their related signals. The design of selective display mode is dependent on tree-like structure of a neuron. In fact, the reconstructed neuron can be mapped as a tree graph in which a node represents a traced neurite (Quan et al. 2016). Due to this mapping, when selecting the sub-structure of the tree graph, the corresponding reconstructions and their neighboring image region can be visualized (**Figs. 2b&c, Movie 4**). Obviously, without the selective display mode, it is a time consuming and laborious task for a skilled annotator to find the reconstruction errors because of the interferences of other signals (bottom panels in **Fig. 2d**). In contrast, the selective display mode can eliminate this kind of interferences and thus check reconstruction errors effectively (**Fig. 2d & Movie 5**). Note that in dense reconstruction, AdTree can automatically reconstruct neuronal population with our previous tool, NeuroGS-Tree, and check the automatic reconstruction in selective display mode.

To quantify the performance reconstructions driven from AdTree, we designed the procedure for reducing the reconstruction variances among different annotators. Two annotators are classified into one group. The one presents the reconstructions and the other checks the presented reconstructions. The total time is set to 4 hours a neuron for AdTree. Checking reconstructions occupies a third of time consumption. Using this procedure, AdTree produced two reconstructions for the same neuron (AdTree-a

and b). One of two reconstructions (AdTree-a) presented has the obvious differences from the reconstructions provided by NeuroGPS-Tree (**Fig. 2e**).

We quantified the reconstructions from population dataset (**Fig. 2a**) including 5 neurons with recall and precision rates (**Fig. 2f**). To calculate these two evaluation indexes, ground truth reconstruction is necessary and can be obtained by the strategy that uses the vote to reach a consensus on the discrepancies among the reconstructions performed by three annotators (Helmstaedter et al. 2011).

We found that AdTree can provide more reconstruction accuracy than NeuroGPS-Tree (**Fig. 2f**). The recall and precision rates are 96.70% and 95.85% respectively for AdTree (AdTree-a) versus 71.99% and 69.45% for our previous software NeuroGPS-Tree. This reconstruction difference is significant. We also found that there are no significant differences between two groups of reconstructions with AdTree (Kolmogorov–Smirnov test, Precision: $p > 0.2$; Recall: $p > 0.99$), indicating that AdTree can provide the robust reconstructions. Furthermore, the reconstructions provided by Ad-Tree are better than manual reconstruction (recall and precision rates : 89.32% and 87.56%, the reconstruction time is set to 12 hours).

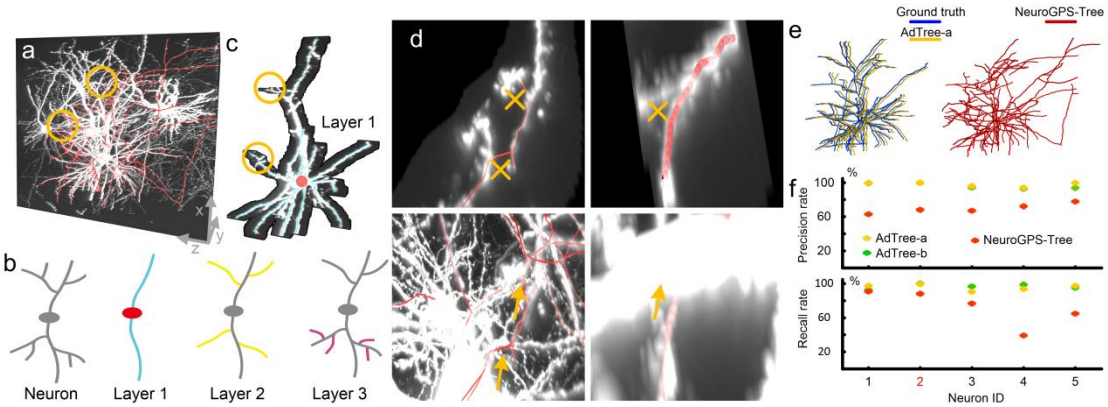

Figure 2. AdTree achieves dense reconstruction with high precision. (**a**) The imaging dataset from neocortex has the size of 1343 × 1120 × 302 voxel and contains packed dendrites and axons. The red curve represents the reconstruction of a neuron generated with automatic method. The circle represents the reconstruction errors; (**b**) A neuron has the tree-like structure in which neurite can be assigned into its corresponding layer. (**c**) For the reconstructed neuron (red in **a**), its reconstruction of neurites in layer 1 (blue curves) and its neighboring image region are displayed by selective display mode in AdTree, red dot represents the soma. (**d**) Reconstruction errors shown with (upper panels) and without (bottom panels) selective display mode, and labeled by crosses and arrows respectively. (**e**) Reconstructions of a neuron (red in **a**) with AdTree and NeuroGPS-Tree; (**f**) Comparisons of reconstruction performance from AdTree and NeuroGPS-Tree.

High precision reconstruction at brain-wide scale

We quantified the brain-wide reconstructions of AdTree with the dataset collected with Florence Mircro-Optical Sectioning Tomography (fMOST)(Gong et al. 2013). We randomly selected five neurons in the dataset, and reconstructed them. For each neuron, three annotators performed the reconstruction. They located and checked the differences between reconstructions of the same neuron with AdTree, and generated the consensus reconstruction, i.e., the ground-truth reconstruction. We presented the ground-truth reconstructions of these five neurons (**Fig. 3a**). The results indicate that these neurons span the different brain regions. We also used commercial software to manually reconstruct these five neurons. We compared the manual reconstruction of a neuron with its ground-truth, and find that the differences between these two reconstructions are small (**Fig. 3b**). We labeled the two locations with different reconstructions and enlarged them (**Fig. 3c**). The results indicate that carefully checking the reconstructions in some locations is necessary for trustworthy reconstruction. AdTree provided multiple procedures to check reconstructions and thus has more chance to find reconstruction errors. We quantified the reconstructions of these five neurons, performed by three annotators with AdTree (**Fig. 3d**). The results indicate that the reconstructions are close to the ground-truth. For reconstructions from each annotator, the average recall and precision rates are over 94% and 97%. We also find that there is no significant difference between the reconstructions performed by different annotators (**Fig. 3d**). The p values in Kolmogorov–Smirnov test are all more than 0.2. This indicates that despite human supervised reconstruction in AdTree, the output reconstructions become impersonal to some extent. In addition, we counted the time cost of AdTree in the reconstruction of these five neurons. It ranges from 9 hours to 20 hours per neuron, about five times speed gain over manual reconstruction with commercial software.

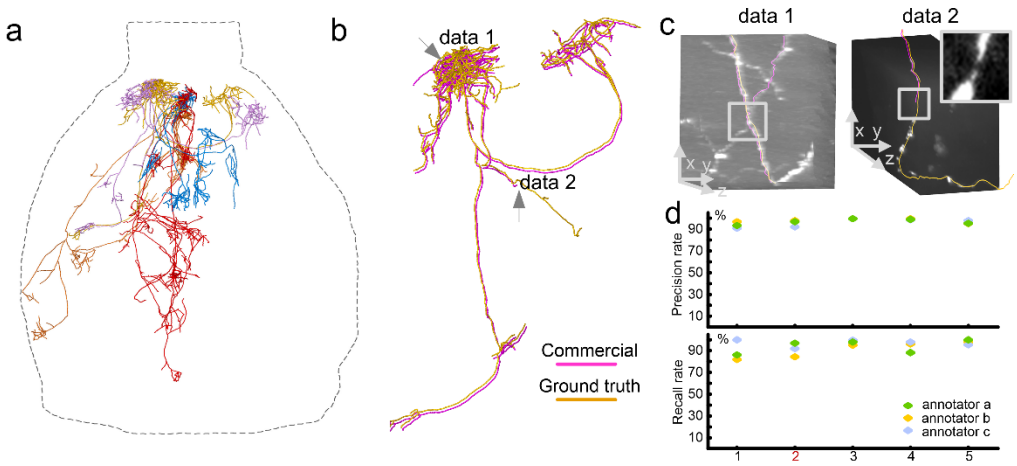

Figure 3. High precision reconstruction at brain-wide scale with AdTree. (**a**) Ground-truth reconstructions of five neurons for quantifying reconstruction performances. (**b**) Comparisons of ground-truth and the reconstructions derived from commercial software. Two reconstruction discrepancies are labeled with arrow. (**c**) The differences between the ground-truth (yellow) and the reconstructions with commercial software (red) are exampled. (**d**) The reconstructions derived from AdTree close to the ground-truth.

High-level automatic reconstruction

We demonstrated that AdTree can achieve high-level automatic reconstruction at brain-wide scale. We developed a series of algorithms for those reconstruction steps that require intensive manual labors. Two algorithms are here exampled. One is used for detecting the optimal skeleton of neurite, and the other is used for identifying the neurites with weak signals.

Due to the tortuous structure of neurites and the imaging artifacts, there are some reconstructions that obviously deviate from the centerline of neurites or the true bifurcation point. In this case, human editing is necessary, which reduces the reconstruction efficiency. We developed a method based on Lasso model for detecting the optimal skeletons of neurites. The optimal skeleton refers to the centerline of this neurite, which has the maximum signal intensity in general. We evaluated the Lasso model using the image dataset of axons (**Fig. 4a**). In the reconstruction process, there are nineteen sub-blocks in which the reconstruction skeleton of the tortuous neurites is required to revise. With the Lasso model, the reconstruction skeletons in seventeen sub-blocks were successfully corrected. We also presented two typical correcting results (**Figs. 4b-e**). The results show that the Lasso model can identify the skeleton of

the tortuous structure.

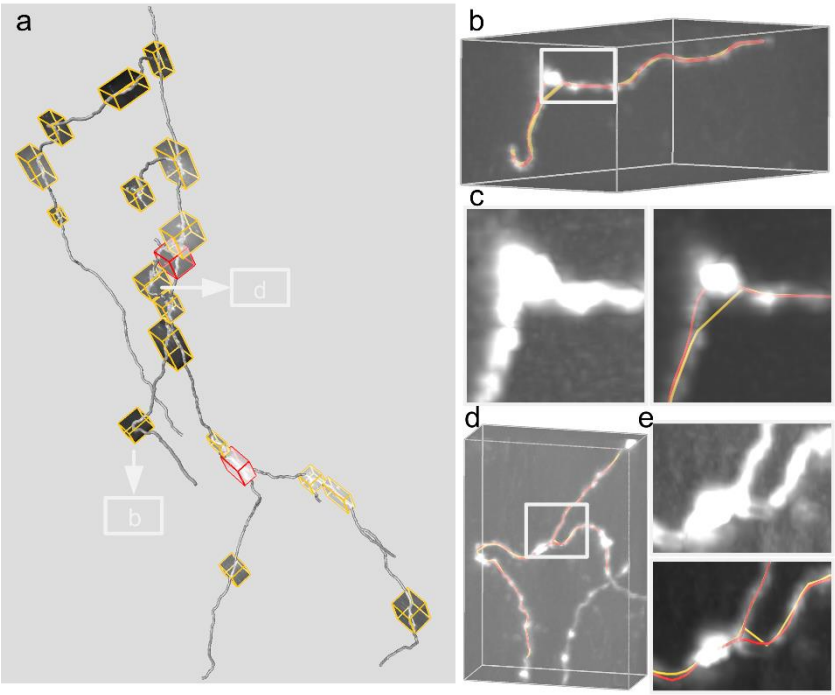

Figure 4. The Lasso model to detect optimal skeleton of neurites. (**a**) Reconstruction of large-scale neurites. The neurites have many locations where tortures structure appears. These locations hidden in sub-blocks labeled by cuboid. The yellow cuboids represent the sub blocks where the skeleton of neurite can be corrected to its centerline with the Lasso model. The red represents the case that the Lasso model fails to deal with. (**b**) The traced skeleton (yellow) deviated from the centerline of neurite and was corrected (red). The white rectangle labels the tortuous structure of neurites. (**c**) The labeled with rectangle in (b) is enlarged. Left panel shows imaging artifacts and right panel shows the correcting results. (**d**) The bifurcation point located by tracing method deviated from its true position, labeled by rectangle. The labeled region is enlarged in (**e**).

In general, the characteristics of brain imaging dataset are closely bound up with neuronal morphology. Small-radius neurites have a few chances of being labeled with fluorescent molecules which results in low signal intensity. AdTree employed our method for identifying the neurites with weak signal. The identifying method has been built on the characteristics of neuronal images, along with machine learning algorithm. The details of this method and the related results can be seen (S. Li et al. 2017). We demonstrated that the weak signal identification method can vastly reduce human editing on the reconstructions using three sub-blocks of brain imaging datasets. Each sub-block contains the axons with weak signals (**Fig. 5a**), and we reconstructed these axons with and without the identifying method (**Figs. 5b-d**). The identifying

method effectively detects weak signals and thus vastly reduces human interferences. The total number of human editing for three sub-blocks is 18 versus 136. In addition, we calculated signal-to-backgrounds of the points on the reconstructed skeletons (**Fig. 5e**). The points with SBR<1.3 occupy 80% percent of the total number of skeleton points. This can explain why a large number of human editing is required without the identifying method.

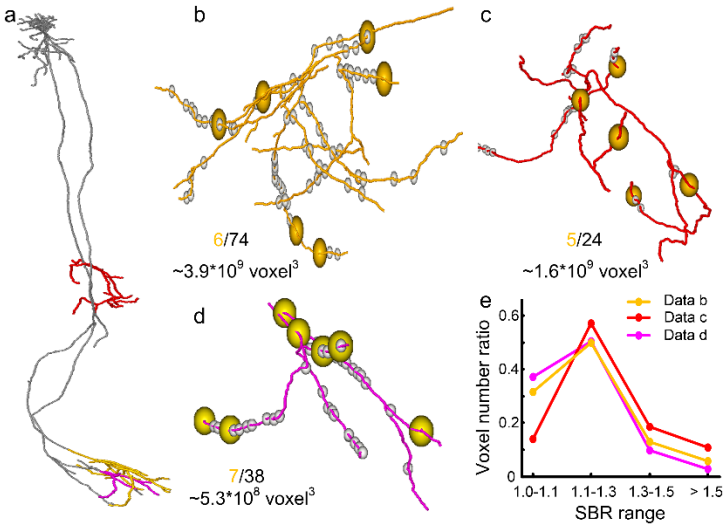

Figure 5. The machine learning method to identify neurites with weak signals. (**a**) The reconstructed neuron, and its three sub-structures labeled with different colors. (**b**) The performance of the proposed method in identifying weak signal. The locations where the tracing fail to identify the signal of neurites with (yellow balls) and without (white balls) the identifying method. (c) &(d) has the similar meanings to (**b**). (**e**) The distribution of signal-to-background of the points in the reconstructed skeletons shown in (**b**), (**c**) and (**d**).

Brain-wide population reconstruction

We used AdTree to reconstruct a population of neurons from the whole mouse brain imaging dataset. The imaging dataset has the size of 10 TBs and contains specially labeled neurons whose morphologies span different brain regions or even the whole brain. Despite the specially labeling, the packed neurites are still commonly found in the imaging dataset. AdTree resolved these packed neurites, which contributed to high precision population reconstruction. Using AdTree, all neurons in this imaging dataset can be thoroughly reconstructed (**Fig. 6a & Movie 6**). According to our rough estimation, the time cost is about 15 hours per neuron for AdTree. A

typical neuron is nearly completely reconstructed in which dendrites, local axons, distal axons can be easily identified (**Fig. 6b**). The reconstructions allowed for the morphological quantitative measure such as the total length of neurites per neuron and Sholl analysis (Sholl 1953). In word, from our results, we conclude that AdTree can reconstruct the detailed structure of a population of neurons at brain-wide scale.

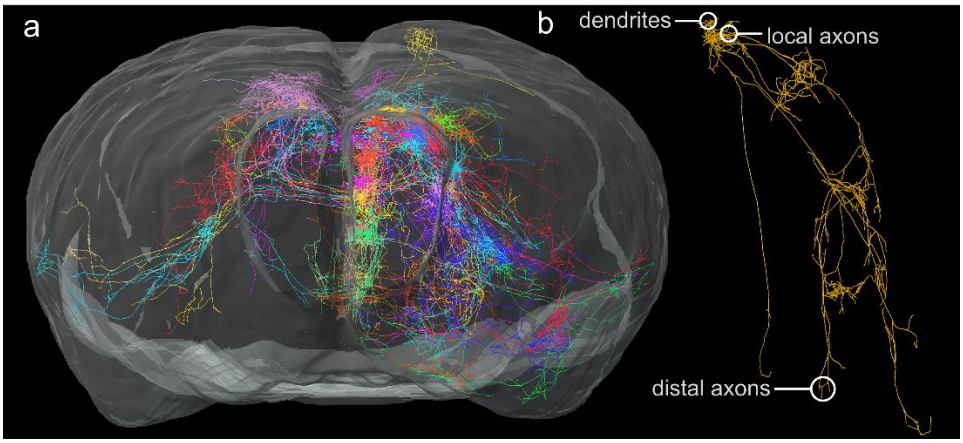

Figure 6. Reconstruction of neuronal population at brain-wide scale. (**a**) A neuronal population across the whole mouse brain including 30 neurons was reconstructed. The imaging dataset is 10 TBs size. (**b**)A reconstructed neuron displayed in which dendrites, local axons and distal axons are included.

Discussion

In this study, we build the software tool, AdTree, to achieve brain-wide reconstructions of neuronal populations with high precision. Compared with the commercial software for this reconstruction, AdTree has about 5 times speed gain. A suite of algorithms in AdTree were designed to overcome a series of challenges in the brain-wide reconstructions, including dense reconstruction, checking reconstruction in TB size dataset, boosting the automatic level of brain-wide reconstruction. AdTree also integrated our established works (Y. Li et al. 2017) that includes loading sub-blocks of TB size brain images(Y. Li et al. 2017), neurites tracing(S. Li et al. 2016), automatically reconstructing local neuronal population(Quan et al. 2016), identifying weak signals(S. Li et al. 2017), detect the optimal skeleton (paper in preparation) and so on. Furthermore, AdTree offers a system to check the reconstructions at different scales from local neurite to a single entire neuron. Due to

these multitudinous and indispensable function modulates, AdTree is an effective tool for brain-wide population reconstructions, and may be helpful to identify the type of a neuron, investigate the projection pattern of neurons, map the neuronal circuit and many others.

Dense reconstructions essentially need to resolve the closely spaced neurites, and is one of the most challengeable problems in neuronal population reconstruction(Lichtman and Denk 2011; Helmstaedter 2013). Sparse neuronal labeling technique can specifically lighten up a few neurons whose soma can be well separated. This technique mitigates this challenge, but cannot solve it entirely. In addition, reconstructing neuronal population that contains more neurons can provide ways to better understand its topological organization, the spatial distribution pattern of neurites, the connecting pattern between neurons. Therefore, dense reconstructions are meaningful and unavoidable in population reconstruction. To overcome this challenge, AdTree integrated NeruoGPS-Tree and selective display mode, i.e., effectively checking reconstructions provided by NeuroGPS-Tree. AdTree boosted the capability of NeuroGPS-Tree in dense reconstruction, because AdTree can make the dense reconstructions close to the ground truth (**Figs. 2e&f**), and thus have more applications.

Brain-wide reconstruction of neuronal populations requires a series of image processing technologies, which can be integrated into a target software tool. As described previously, AdTree has this characteristic and thus can achieve brain-wide reconstruction with high-precision and relatively high-throughput. The high- precision can be attributed to the following works. Firstly, selected display mode of dense reconstructions vastly eliminates the interferences of other reconstructions and thus can effectively check the reconstructions. Secondly, online feedback of reconstructions from sub-blocks of brain images and editing function modulates are combined to achieve supervised reconstructions; Thirdly, navigating the brain-wide reconstructions enable us to check the reconstructions at the global scale. There are two primary reasons for relatively high-throughput reconstructions. One is because of the contributions of many automatic algorithms including neurites tracing, spurious

links between neurites identification, weak signals identification, optimal skeleton detection and many others. The other is because of user-friendly visualizing and editing functions, which allows for fastly revising the reconstruction errors.

The tree-like structure of neuron determines that reconstruction error has highly correlated effect on the subsequent reconstructions. Without human supervision, one false reconstruction will lead to missing neurites or miss-assigned neurites. This can explain why manual reconstruction is still the primary way to quantify neurons in the presence of a mass of automatic algorithms. The whole brain images are more challengeable and thus human supervision is necessary for trustworthy results. However, the manual reconstruction software lacks of some key algorithms and editing functions to resolve the challenges in the brain-wide reconstructions. Therefore, manual reconstruction becomes time-consuming and laborious, difficult to match the development of neuronal images. AdTree, to a certain extent, fills this gap. It is noted that automatic algorithms are still required to be integrated into AdTree for high-throughput reconstructions.

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