Global Tree Reconstruction System

Catalog

1 Introduction

1.1Summary

Global Tree Reconstruction System (GTree) software is written in C++, and freely available for academic research using. GTree is used to reconstruct neuronal population from brain-wide image stacks. In this reconstruction, individual neuronal trees with long projections can be identified and quantified. The detailed installation and usage instructions can be found in the following. For any questions, feedback and comments in the usage of GTree, please contact the authors (Hang Zhou, zhouhang@hust.edu.cn ; Shaoqun Zeng, sqzeng@mail.hust.edu.cn).

1.2 System Requirements

GTree software aims to reconstruct neuronal population from image stacks. During the reconstruction process, it is required to analyze the image stacks with large size and high spatial resolution, which involves heavy computation. Therefore, when running GTree software, we recommend high-powered computing platform. Some configurations of computing system are listed in the following table.

	Minimum settings	Recommend settings
OS	Windows vista/7/8/10 **x64**	
CPU	I5	E5
Video Card	NVidia dedicated video card	Above NVidia GTX 960
Memory	2GB	More than 4GB

The laptop with the above configurations is also suitable for running GTree.

1.3 Dataset Requirements

(1) Image read support format is TIFF (Tagged Image File Format). 8/16bit TIFF

series (Gray Image Stack) is supported.

(2) Images of other format should be converted to TIFF first by a third-part software, like free software ImageJ. For example, the image datasets collected by commercial microscopy, such as .czi (Zeiss) .lif (leica), .nd2 (Nikon) and .oif (Olympus), can be read and converted into TIFF file format by ImageJ. The detail instruction of ImageJ is shown in http://wiki.imagej.net.

(3) If the size of input image stack is larger than **3GB** or any axis length is larger than 2048 in 3D coordination, you are supposed to transform the image stack into TDat format (Li et al., Frontiers in neural circuits, 2017). TDat is a big data access format for neuron science.

(4) The recommended voxel size of the input image stack ranges from $0.5\times0.5\times0.5$ μm^3 to $1\times1\times2$ μm^3. If the z resolution is 3 times larger than x-y resolution, voxel merging procedure is recommend to carry out to drop into this range. For example, for the input image stack with voxel size of $0.21\times0.21\times1$ μm^3, it is recommend to be transformed to an image stack with voxel size of $0.63\times0.63\times1$ μm^3 for the reconstruction. Please note the reconstructed results, saved as SWC files, should be coincide with the voxel size of the input image stack, rather than the transformed image stack.

(5) Sta file store the configuration of GTree. You can load sta instead of swc file to restore last time work space completely. Default sta file will be saved automatically when you click save tree button after tracing.

Note: If you do not know the voxel size of the datasets, please check the image collection source (microscopy parameters) to find out the voxel size of the datasets.

1.4 Output Format

The output file package consists of a series of SWC files and a sta file. Sta file is a kind of configuration file to store the configuration parameter of GTree. SWC file is a text file. A single SWC file only contains the reconstructed morphology of one

neuron. Based on the assumption that the reconstructed morphology of a neuron can be described by using a series of nodes, each line of an SWC file represents the information of the corresponding node. This information consists of the node connection, node type, node scale, and node position. More details about node information can be found in http://research.mssm.edu/cnic/swc.html.

2 Installation

2.1 Double click on "*C++ runtime library(x64).exe*".

2.2 Tick "I have…", and then click "Install" button in the following interface below.

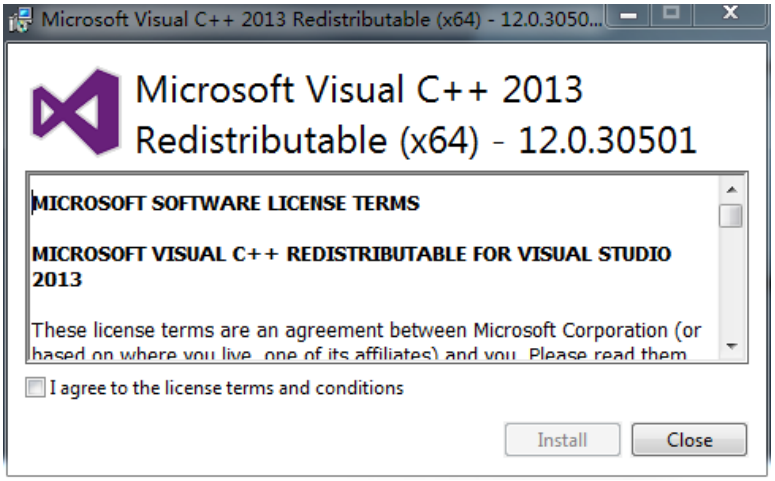

Fig.2.1

2.3 Click "Finish" button to complete the installation. This operation indicates that the installation is completed. After install the plugin, you can run "*GTree.exe*" in the installation package.

3 Quick Started

3.1 Trace local neuronal population

GTree's trace module automatically provides initial reconstruction of local neuronal population, and then the user can edit the reconstruction under edit module (see section 3.6 for editing details).

Step 3.1.1 Import image stack: Click the "open" button in the red square (Fig.3.1.1) to

import image stack (8-bit or 16-bit gray-scale, ".tif" format).

Note 1 For best visualizing, the size of the image stack should be no more than 2000 voxel in each dimension (x, y and z).

Note 2 The video memory of the user's computer determines the image size which can be import and visualized.

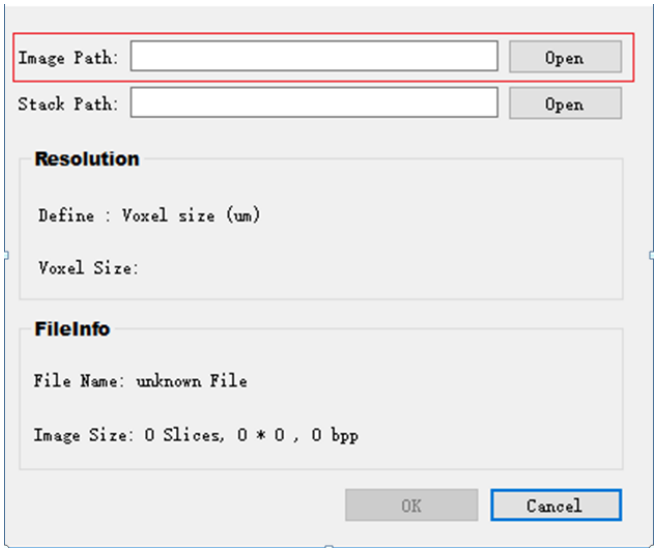

Fig.3.1.1

Step 3.1.2 Input the resolution of import image stack in red square (Fig.3.1.2), then click "accept" button.

Note the image resolution is determined by the imaging tool that user applies to acquire image stacks.

The imported image stack can be visualized in Fig.3.1.3.

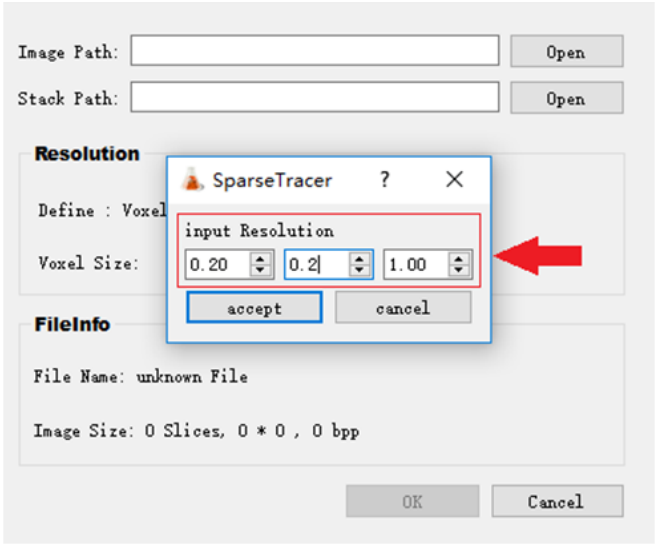

Fig.3.1.2

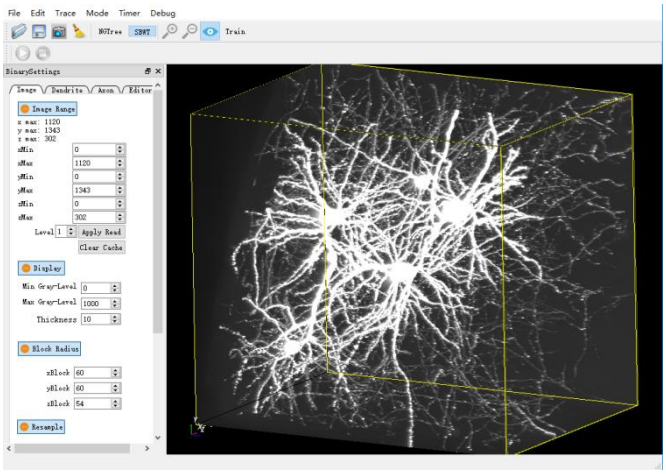

Fig.3.1.3

Step 3.1.3 Import corresponding reconstruction files: Click the "open" button in the red square to import reconstruction files(".swc" format).

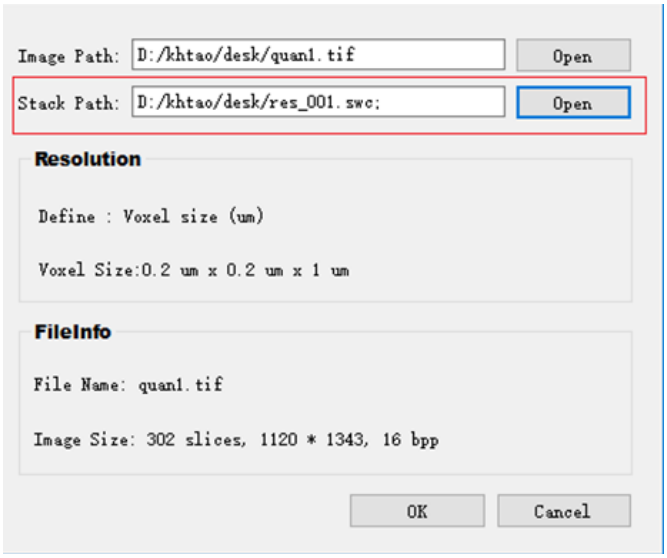

Fig.3.1.4

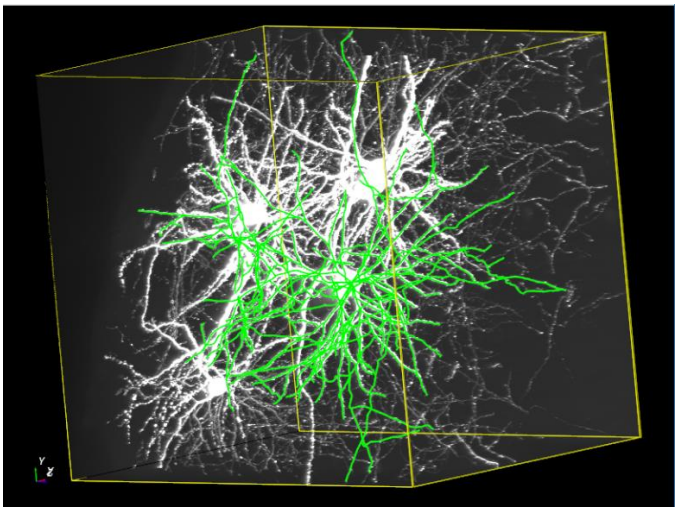

Fig.3.1.5

Note if it is the first time a user reconstructs the image, one can skip the step 3.1.3, then apply the following steps 3.1.4 and 3.1.5 to achieve corresponding reconstruction.

Step 3.1.4 Choose soma location

Manually choose soma:

a. Single click on right mouse button in Visualization Window and choose "2DView", shown in Fig. 3.1.6 (red arrow).

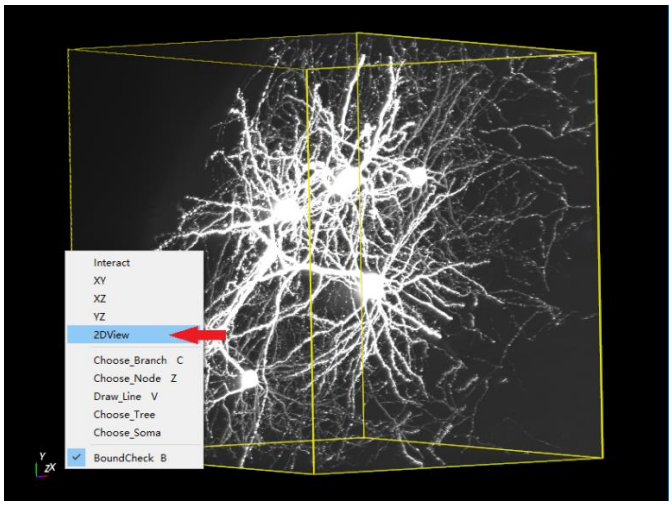

Fig.3.1.6

b. In "2DView", single click on right mouse button in Visualization Window and choose "Choose soma", shown in Fig. 3.1.7 (red arrow).

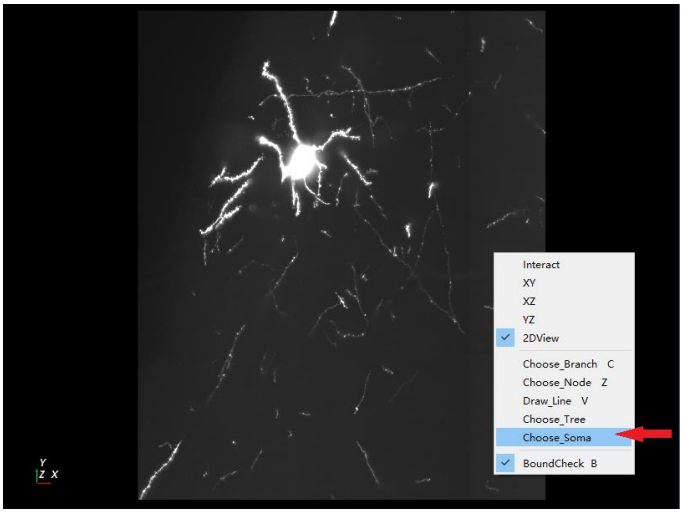

Fig.3.1.7

c. In "Choose soma" mode, scroll the middle mouse button and find soma, shown in Fig. xx (red square).Single click on left mouse button to apply soma position, shown in Fig. 3.1.8 (red dot).

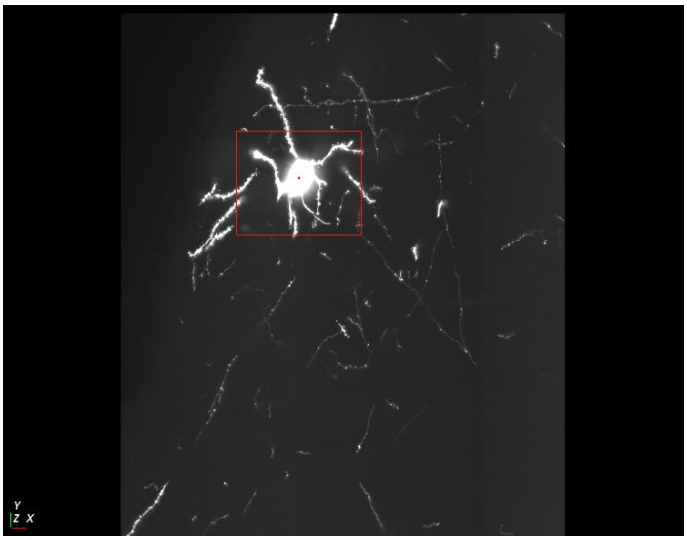

Fig.3.1.8

Note if you want to redraw the soma, select it with the left mouse button (the soma dot will be green, see Fig.3.1.9). Then click the right mouse button and choose the "Delete soma" button to delete it. Then you can draw a new one.

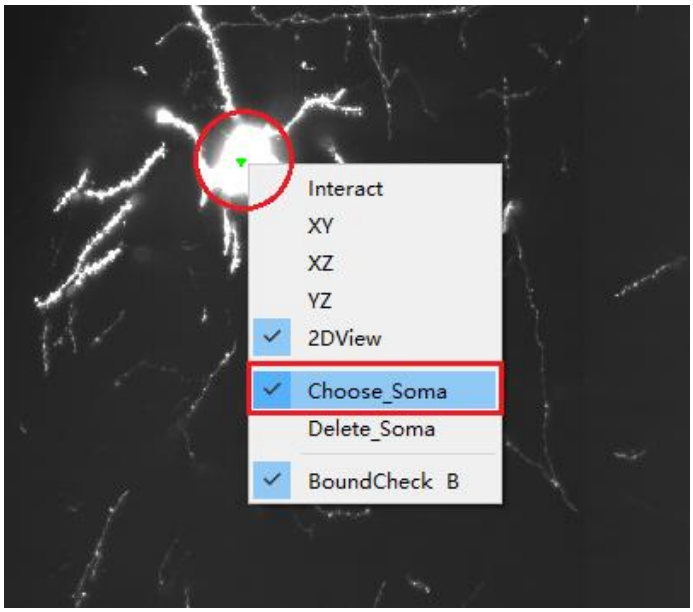

Fig.3.1.9

d. Click the "Choose soma" and "2Dview" button again to quit "Choose soma" and "2DView" mode. The soma position is shown in Fig. 3.1.10 (red arrows).

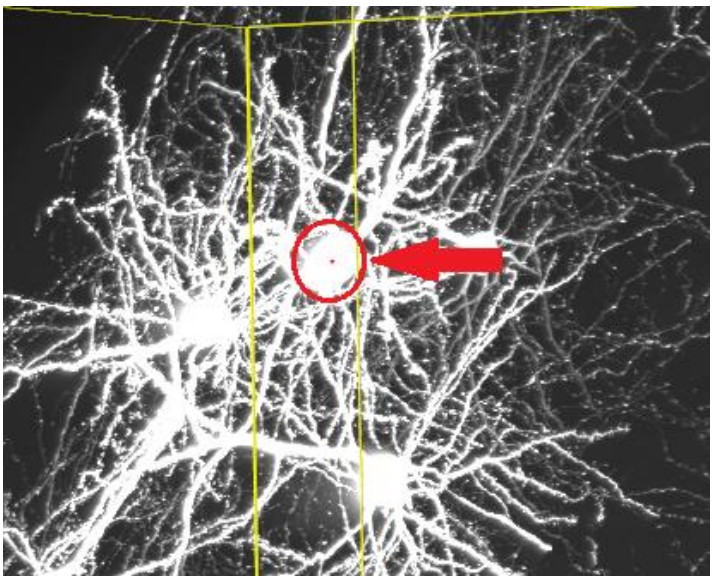

Fig.3.1.10

Automatically choose soma:

If there are multiple neurons in the image stack, it will be a difficult task to choose all the soma manually. Here, GTree implemented the soma location function in NeuroGPS (Quan et, al., Sci Rep., 2015) to locate soma automatically.

a. Choose "Binary Option" in "dendrite" Panel.

User shall input corresponding values, and click the "Preview" button, user can

view the binary result of input image stack in the right visualization window.

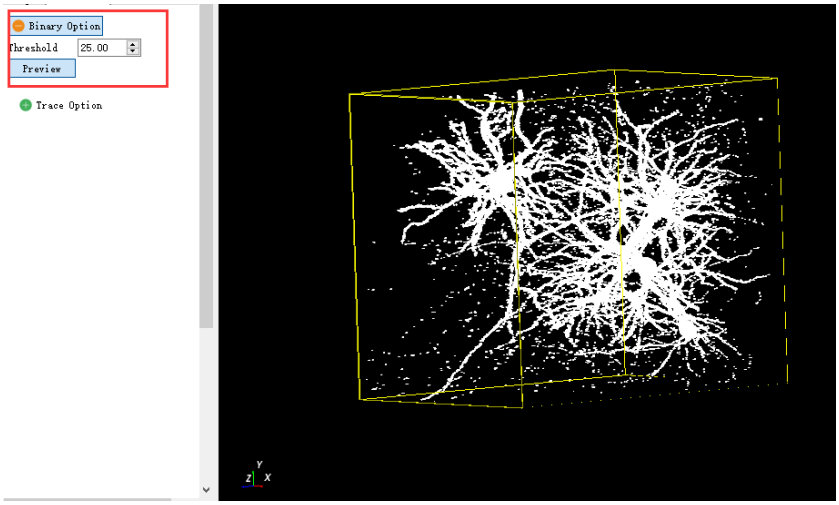

Fig.3.1.11

If the user is satisfied with the binary result, one can run "NeuroGPS" later. The binary value is settled.

Note The low threshold set will cause the binary result with many background points. When the high threshold is set, a substantial proportion of the neuronal morphologies will be disregarded.

b. Choose "NeuroGPS" (red arrow in Fig.3.1.12) in maul bar.

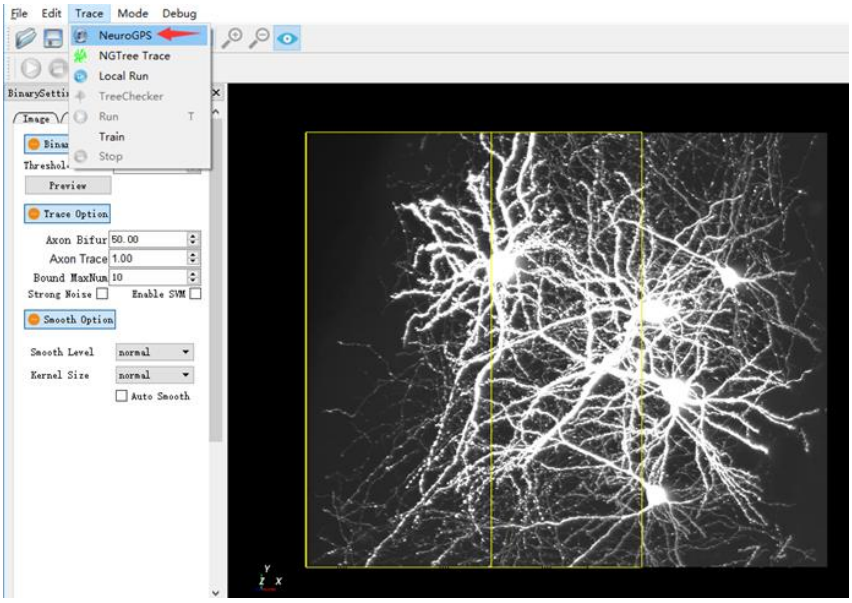

Fig.3.1.12

c. Input the parameter of "NeuroGPS"

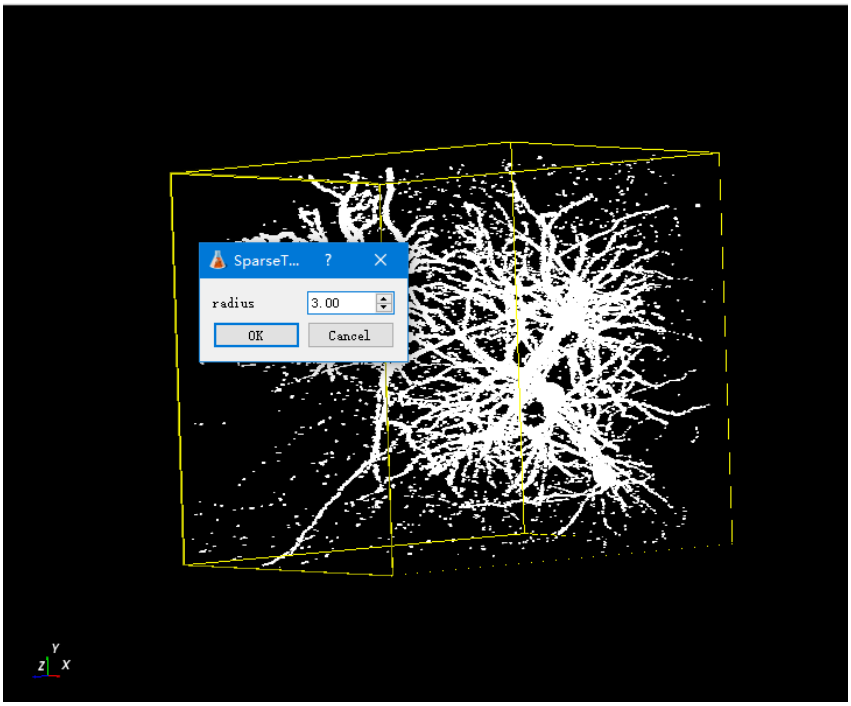

Fig.3.1.13

radius: Soma with radius under "radius" parameter will be removed. The unit of the radius is μm.

d. Run "NeuroGPS" and the soma locations are acquired (red arrows in Fig.3.1.14). User can further save the soma files (".pbo") by choosing "save soma" in manu bar.

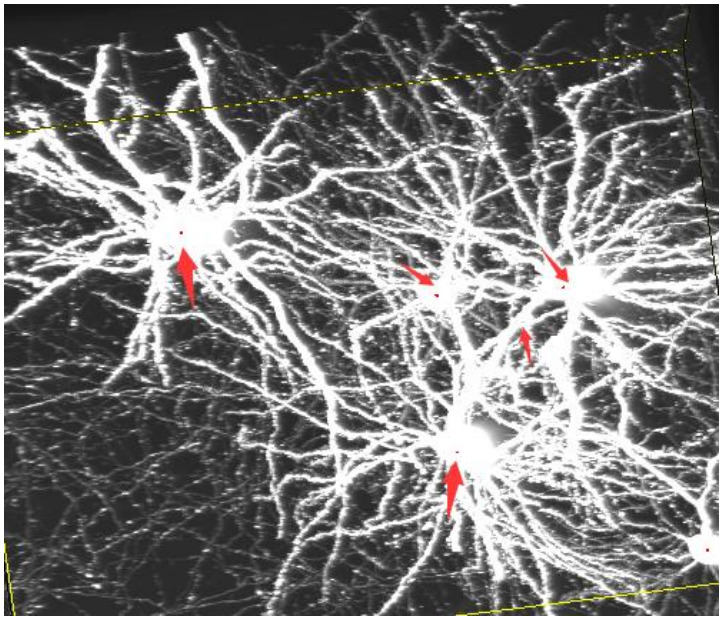

Fig.3.1.14

Step 3.1.5 Automatic reconstruction: Single click on "NGTree" button in the Tool bar,

shown in Fig. 3.1.15 (red arrow). Single click on "  " button. The automatic reconstruction results are shown in Fig. 3.1.16.

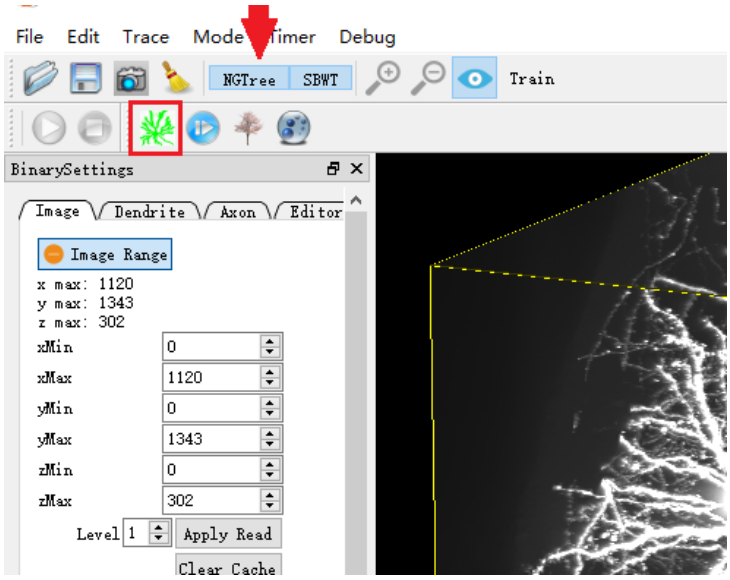

Fig.3.1.15

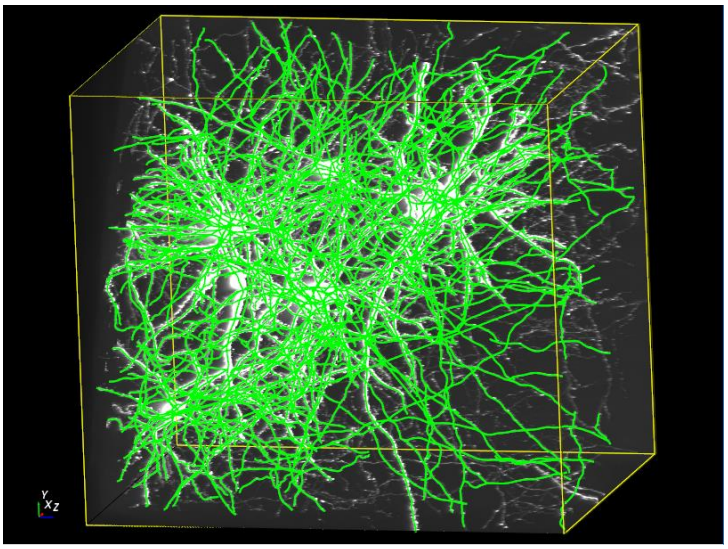

Fig.3.1.16

3.2 A brief introduction of selective display mode

Selective display mode only visualize the reconstruction of the user selected neurites and their corresponding neighborhood images. Thus it eliminates unrelated disturbance of other neuritis. As a result, we recommend using selective display mode to check reconstruction errors in densely packed neurites.

Note A neuron has the tree-like structure in which neurite can be assigned into its

corresponding layer. The neurites that directly connect to soma is called layer 1. The neurites which connect to layer 1 directly constructs the layer 2. The following neurites are divided to different layers based on the above rule. Furthermore, the neurites in a layer are considered as its branches.

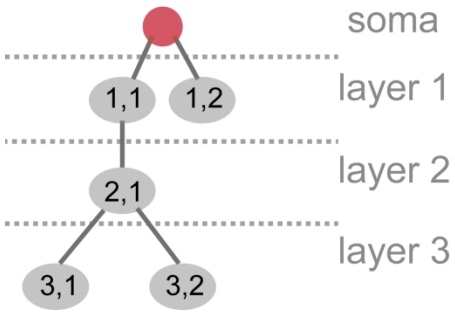

Fig.3.2.1

Take Fig. 3.2.1 as an example. The red dot represents the soma. Layer 1 includes two neurites (gray nodes “1,1” and “1,2”) which connect to soma directly. The neurite (“2,1”) connects to layer 1 consist of layer 2 . In addition, neurites at the same layer are assign with different branch ID (In layer 1, one of the nodes is branch No.1 while the other is branch No.2). For same rules, two more neurites (gray nodes “3,1” and “3,2”) belong to layer 3.

In practice, the specific layer and branch number information of every neurite can be checked through the following operations (see steps 3.6.1 a & 3.6.2 k).

Step 3.2.1 When the image and its corresponding reconstruction is imported. Single click on “NGTrace” button, then single click on “TreeChecker” button (red arrow in Fig.3.2.2), a parameter box is shown in red square in Fig.3.2.2.

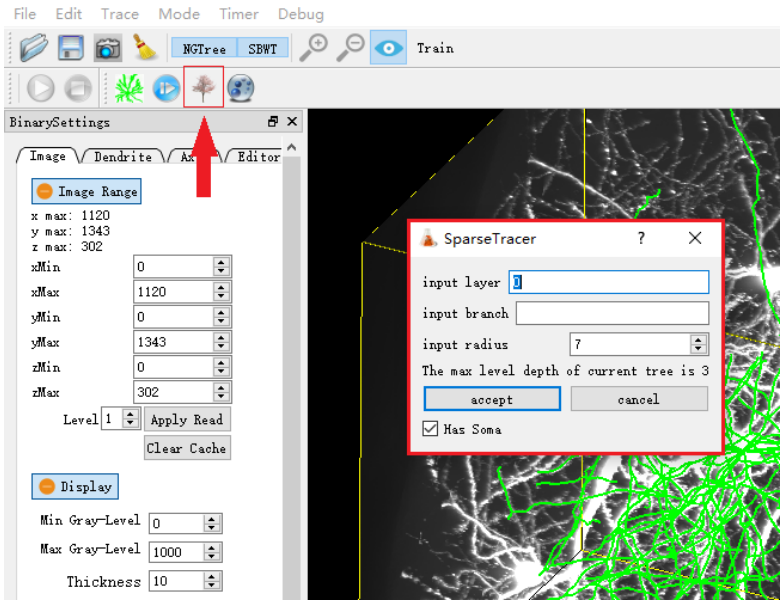

Fig.3.2.2

Note Once reconstruction of neuronal tree is imported, the details of the tree-structure are shown in the bottom of the parameter box (red square in Fig.3.2.3). The user will know the exact layer number in the structure (Max level) and by applying different layer number, the branch number of the top layer is also given (top level branch num).

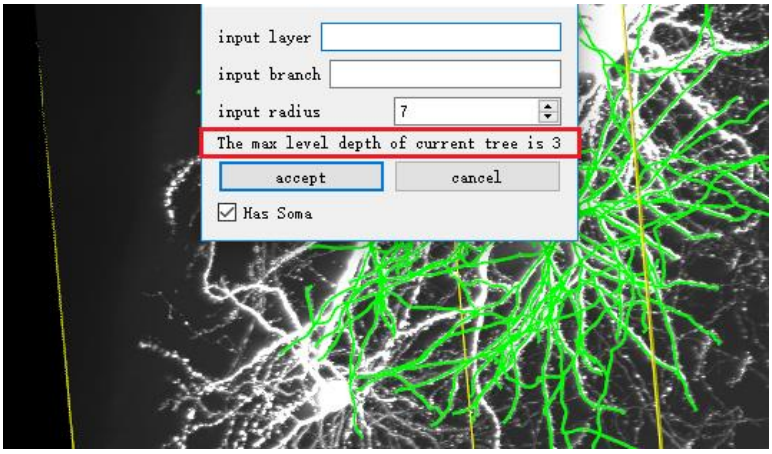

Fig.3.2.3

Step 3.2.2 Apply corresponding parameters to the number of layer, branch and radius.

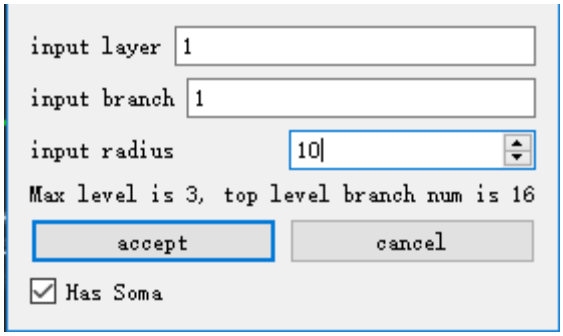

Fig.3.2.4

For example (Fig.3.2.4), "input layer: 1; input branch: 1" represents the neurite (branch No.1 in the first layer) is the target neurite. Additionally, take the target neurite as a centerline, a neighborhood region can be generated that visualize corresponding images. User should input the corresponding radius in "input radius" box.

Note The default size of the neighborhood region radius is 7 voxels. Smaller radius may not provide enough image information for user to judge the reconstruction errors, while bigger radius will introduce more irrelevant interfaces in the image. The radius may vary according to different dataset and reconstruction requirements. So we recommend our user adjust the radius of the neighborhood region according to their own dataset, and check the result in visualized window in real-time. Then the user may decide a best radius for their dataset.

Step 3.2.3 A recommend way of checking errors with selective display mode

The neurites in a neuron can be divided into corresponded layers. We strongly recommend checking the neuron by depth-first search to ensure every neurite in the neuron is checked.

Take Fig.3.2.5 as an example. User firstly checks the branch No.1 in layer 1, i.e, gray node "1,1" . Along orange path, check all the directly connected branches (gray nodes "2,1" and "3,1") to the top layer (here is layer 3). Then we check if there is any branch connected to some branch in orange path (gray node "3,2" in yellow path), check it in time to ensure that there are no omission. Complete the inspection above, the check of branch No.1 in layer 1 and all its connected branches is finished. Then follow the same procedure to check the green path (branch No.2 in layer 1). This strategy make

sure that every branch (gray node)has been gone through.

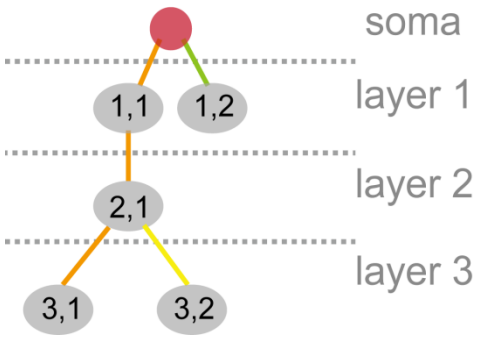

Fig.3.2.5

Step 3.2.4 An example of the recommend usage with selective display mode

a. Display branch No.1 (red curve in Fig.3.2.6) and its neighborhood images by applying corresponding parameters ("1" for layer, "1" for branch, "10"for radius).

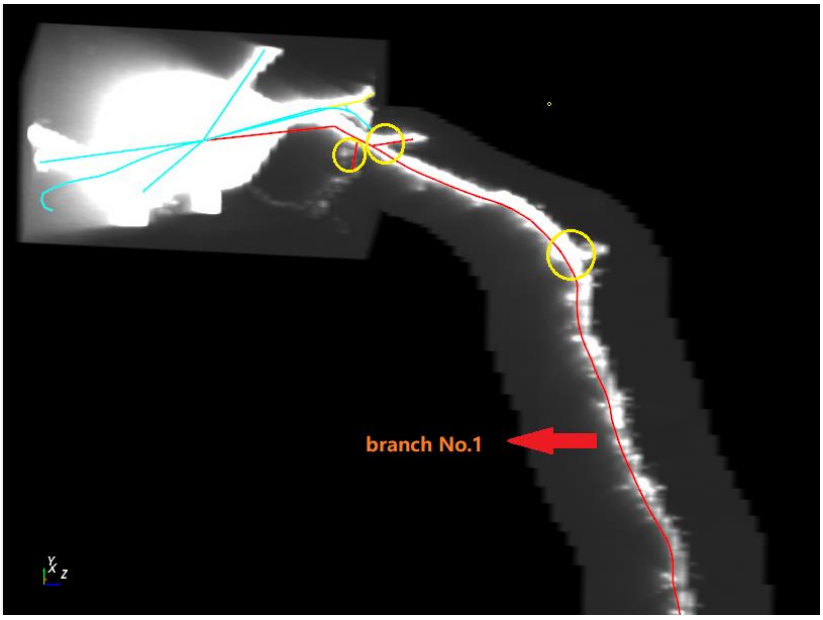

Fig.3.2.6

Note The adjacent neurites in the tree-structure have connected relations. In order to help user quickly check the errors in neuron reconstruction, besides the interested neurites, we also display patial reconstruction of directly connected parts in next layer.

b. Check whether there are missing or miss-assigned neurites in branch No.1.

For miss-assigned branch, delete it or reconnect to right neurite (see section 3.6.4 b). Press key "C" to enter "Choose Branch" mode (this mode will be covered in chapter xx), then choose the extra branch (red curve in Fig.3.2.7) and

press key “D” to delete it. Press “C” again to exit this mode.

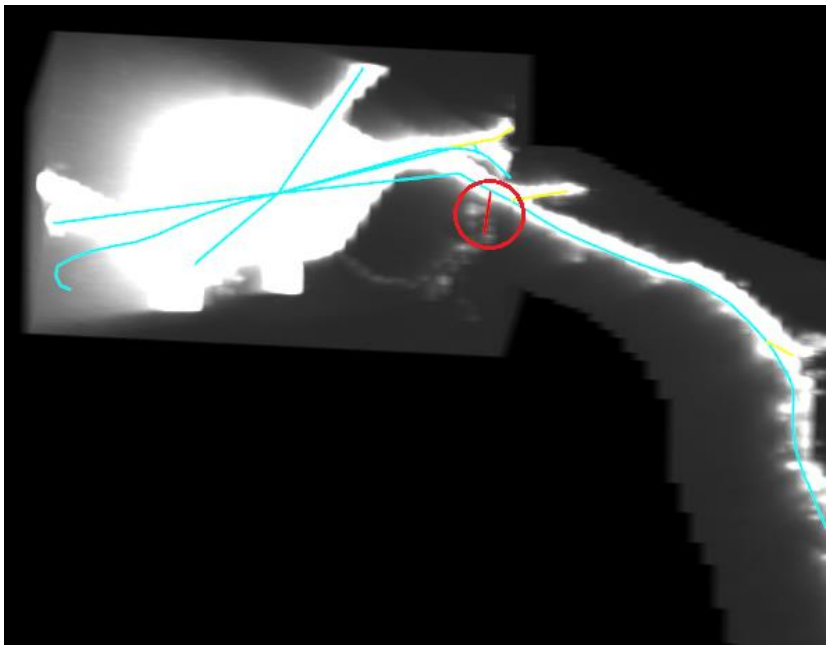

Fig.3.2.7

For missing branch, user should add it up manually (see section 3.6.4 c for more details). Press key “V” to enter “Draw Line” mode. Then double click the left mouse button at the point you want to add branch. Because it is a 3D image, there is a red line perpendicular to the screen (red dot in Fig.3.2.9).

Fig.3.2.8

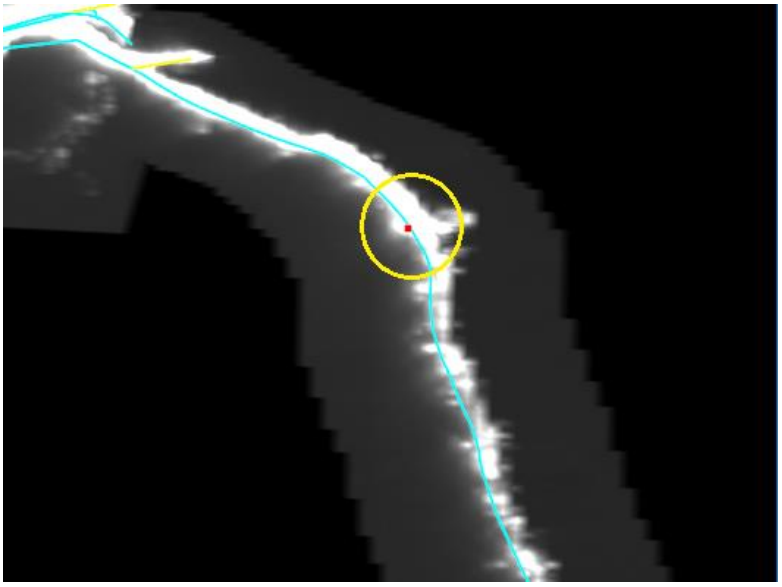

Fig.3.2.9

Press the left mouse button to rotate the image, double click the left mouse button to determine this point from another direction (Fig.3.2.10).

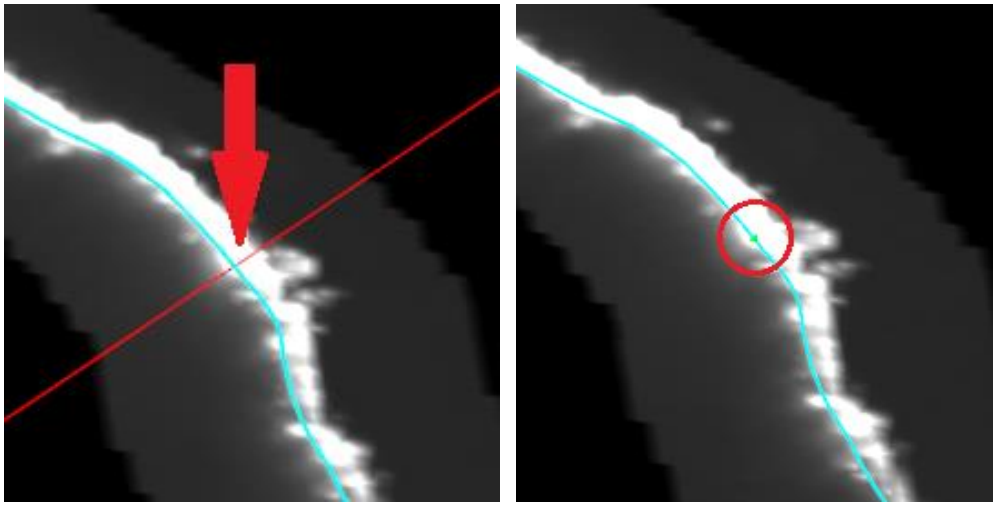

Fig.3.2.10

Similarly draw another point along the neurite. It will produce a purple line segment (user can continue to draw more lines to help him track). Press “V” again to exit “Draw Line” mode, then there will be a line with arrows which can use to trace.

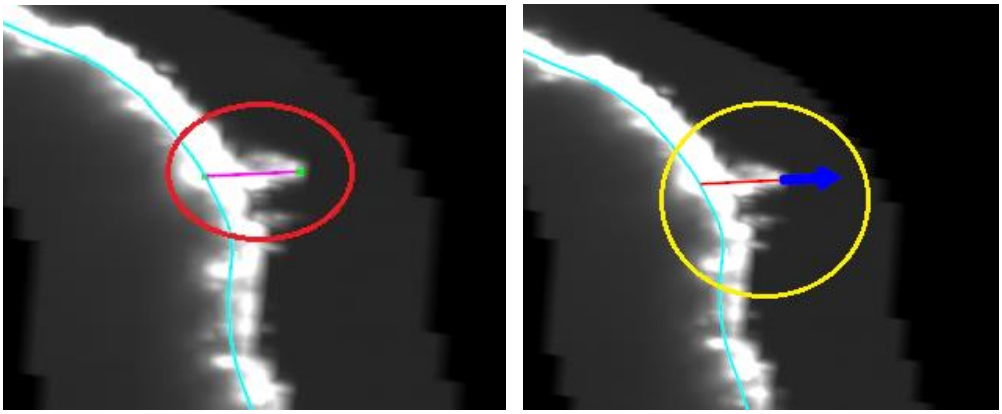

Fig.3.2.11

c. Check the branch information on the two direct connected branches on branch No.1. Press key “c” to enter “Choose Branch” mode, then choose the interested branch (red curve in Fig.3.2.12). Single click on right mouse then choose “Level Branch ID”, the corresponding branch information are shown (Fig.3.2.13).

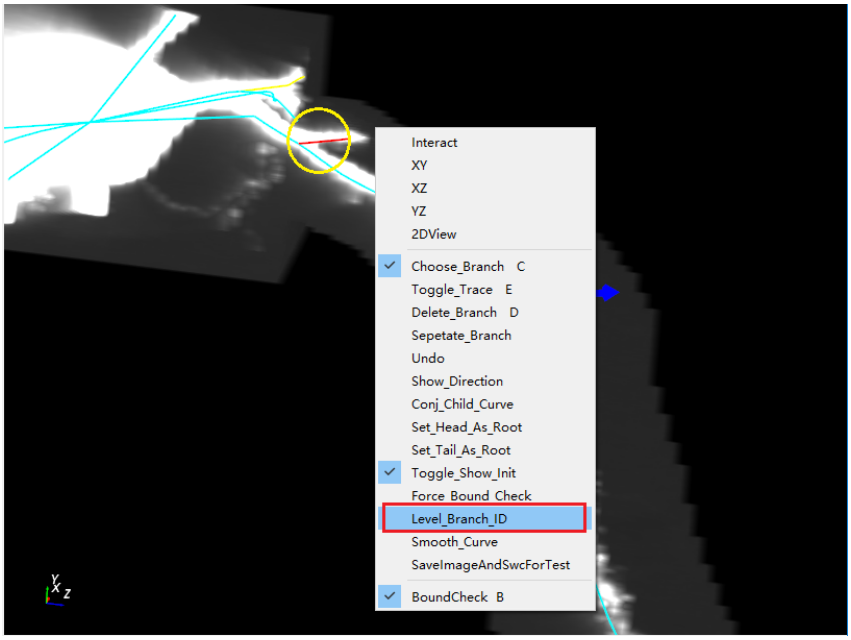

Fig.3.2.12

Fig.3.2.13

d. According to the branch information acquired in b, click “TreeChecker” button, apply the parameters (here is “2” for layer and “2” for branch) in the framework. The branch (branch No.2 in layer 2) that connected to branch No.1 in layer 1 is displayed (yellow curve in Fig.3.2.14).

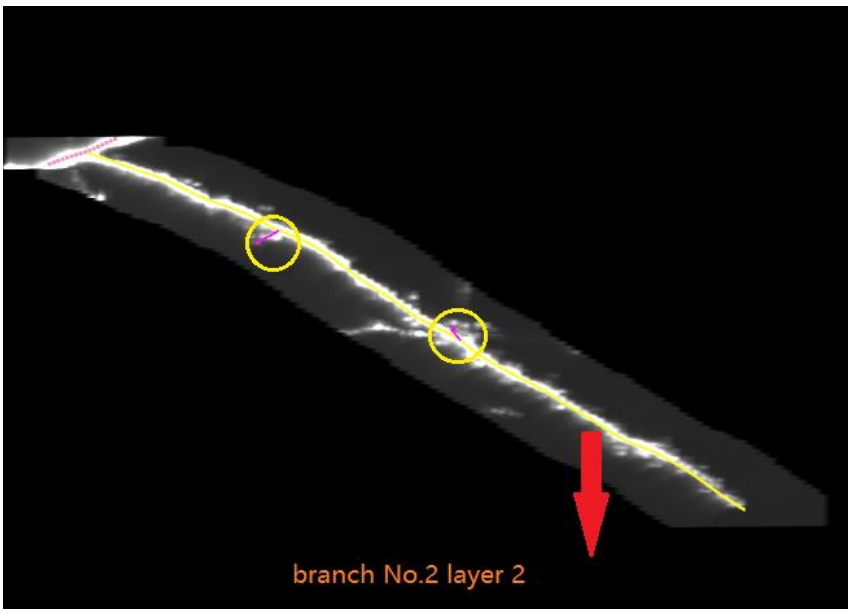

Fig.3.2.14

e. Check direct connected branches (yellow circles in Fig.3.2.14) of the current branch. Zoom (scroll up scroll wheel in the mouse), spin (hold left mouse button) and pan (click and hold the scroll wheel) the images to observe the connections

from multiple angles. Furthermore, single click on "Visible" button ( ) can hide the reconstruction and observe the neighborhood images. Here, the two branches are miss-assigned to the current branch (yellow circles in Fig.3.2.14), delete them (choose the branch by pressing key "c" and delete it by pressing key "d").

f. No more connected branches are found in current branch (yellow curve in Fig.3.2.14), back to branch No.1 (layer 1), find other branch that connect to it. Follow steps b to d to check the branch and its direct connected branches in the upper layers, till every branch that connected to branch No.1 (layer 1) is checked.

g. Follow the sub-steps (a to f) in **Step 3.2.4** to check and revise every branch in layer 1, then the reconstruction of the neuron is checked.

3.3 Trace and edit neuronal population in long range

GTree's trace module also provides the function to reconstruct long projection of a target neuron in population.

Step 3.3.1 Import image stack: Click the "open" button in the red square to import large volume image stack (mostly over 3GB, ".mostd" format).

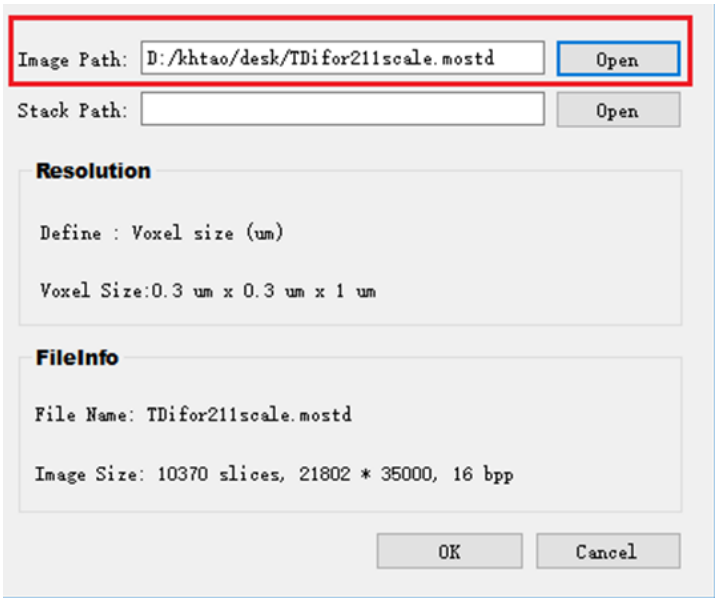

Fig.3.3.1

Step 3.3.2 Import corresponding reconstruction files: Click the "open" button in the red square (Fig.3.3.2) to import reconstruction files (".sta" format).

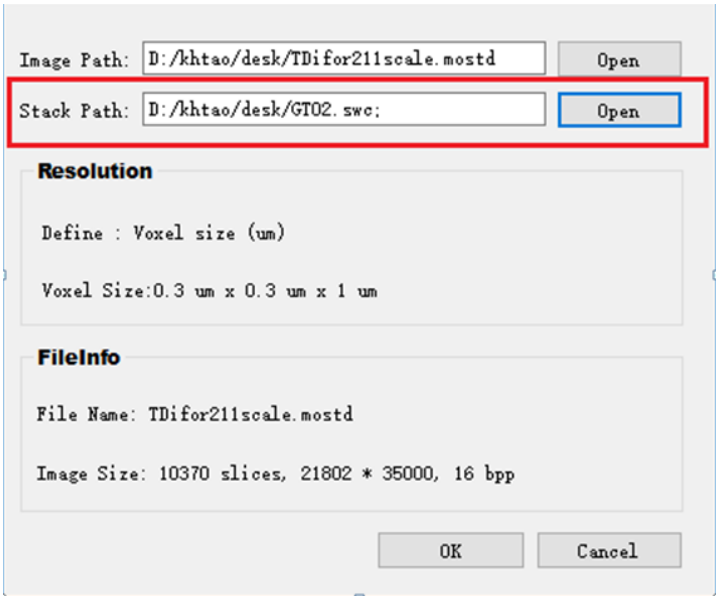

Fig.3.3.2

Note if it is the first time that user reconstructs the image, one can leave the "Stack Path" empty, then apply step 3.2.3 to achieve corresponding reconstruction.

Note the ".sta" file records the storage path of the reconstruction. If user revises the name or location of the reconstructs (".swc" file), one should revise the corresponding ".sta" file. Revise the path in red square (Fig.3.3.3) to the current path.

```
Axon_Binary_Value=3.000000
Tree_Diffuse_Value=4096.000000
Tree_Trace_Value=1.000000
Axon_Diffuse_Value=50.000000
Axon_Trace_Value=1.000000
Max_Bound_Num=10
Opacity_Low=0
Opacity_High=1000
XScale=1
YScale=1
Opacity_Adjust_Low=0
Opacity_Adjust_High=0
Opacity_Dest_Low=0
Opacity_Dest_High=0
Running_Minutes=0
[tree1]
path=Z:/TDIE1301a007/TDIE1301a0072017-12-01_20_57_000.swc
curInit=7373.260600 34600.305340 4207.039016
arrow_num=0
```

Fig.3.3.3

Step 3.3.3 Set image range and achieve corresponding reconstruction:

a. Open "Image Range" (red square in Fig.3.3.4) in the left panel of GTree. User

shall insert x, y, z coordinates of the interested region (ROI). Then single click on left mouse to import the ROI.

Note 1"Level" button applies image display in multiple resolution (from 1 to 8).

Note 2 The ROI shall include soma of the target neuron(red arrow in Fig.3.3.5).

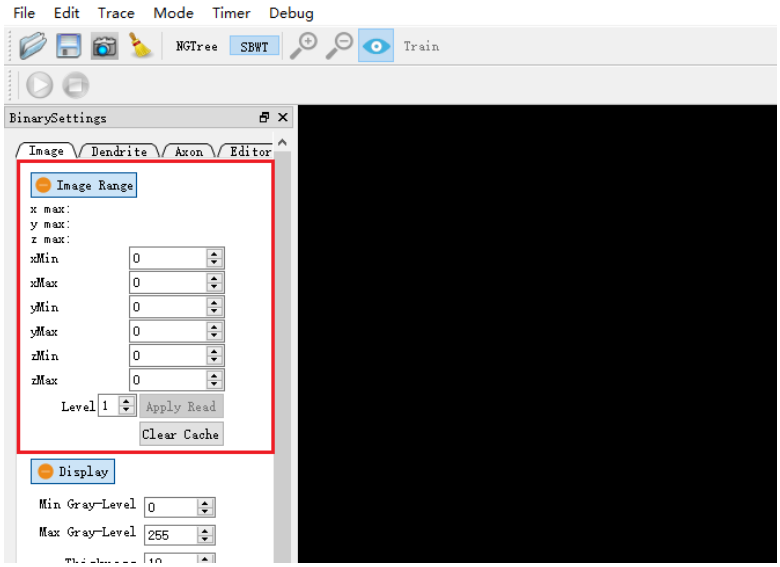

Fig.3.3.4

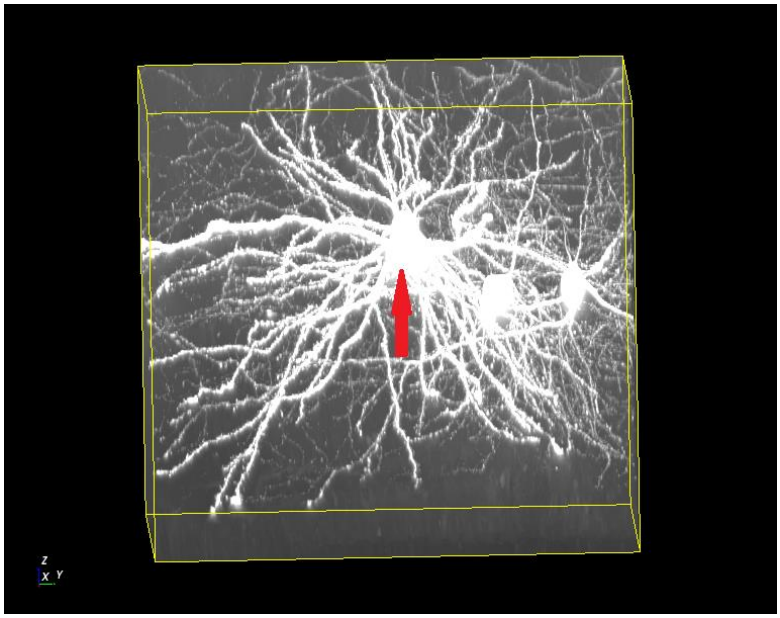

Fig.3.3.5

b. Follow steps 3.1.4, 3.1.6 and 3.2 to achieve final reconstruction of the dendrites in current ROI.

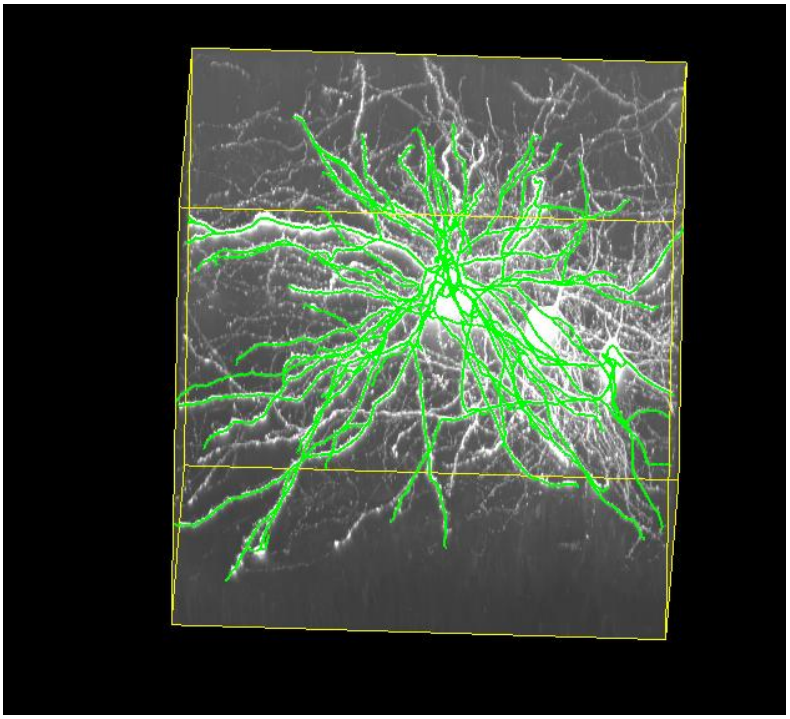

Fig.3.3.6

Step 3.3.4 Find the axon of the target neuron and add the direction on it. (red square in Fig.3.3.7)

Enter the "Choose Line" mode (shortcut is "C") and choose the axon neurite (red line in Fig.xx). Then single click on right mouse and choose "Toggle_Trace" (shortcut is "E"), the direction (denoted by blue arrow) will automatically derived (Fig.3.3.7).

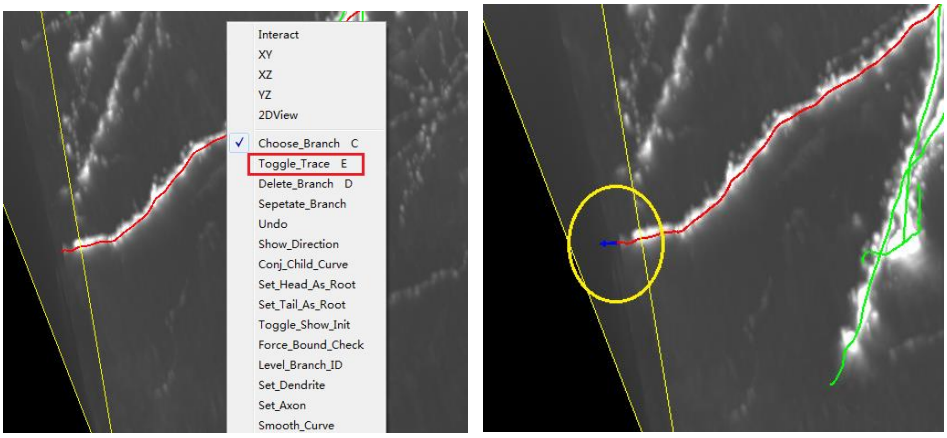

Fig.3.3.7

Step 3.3.5 Trace along the axon neurite: Single click on "Run" button (Shortcut is T) then the neurite is traced in the current ROI.

Step 3.3.6 Edit the reconstruction in current ROI (see specific details in section 3.6).

Step 3.3.7 Repeat steps 3.3.5-3.3.6 till no more neurite is needed reconstruction (Fig.3.3.8).

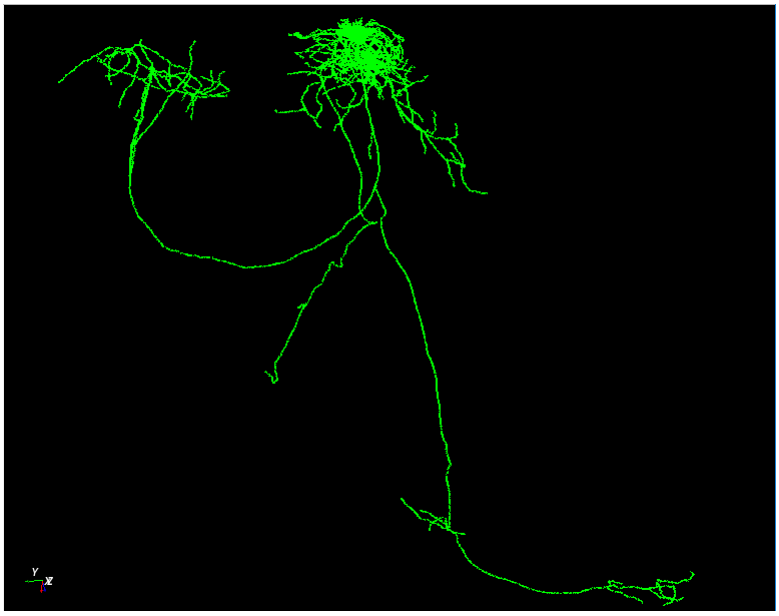

Fig.3.3.8

3.4 Brain-wide reconstruction navigation

GTree provides navigation mode to quickly screen through the brain-wide scale reconstruction. Under navigation mode, user can check and edit the errors through sub-blocks.

Step 3.4.1 Load the image stack and its corresponding reconstruction: the same operations in steps 3.3.1 and 3.3.2.

Step 3.4.2 Navigate the reconstruction:

a. Single click on right on "start traverse" button (red square in Fig.3.4.1). GTree will automatically set the start position around soma area (red arrow in Fig.3.4.2).

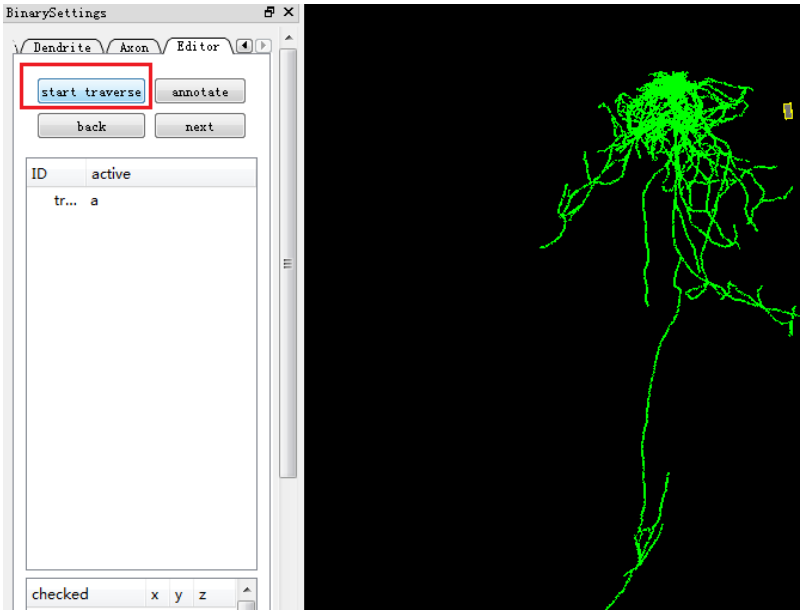

Fig.3.4.1

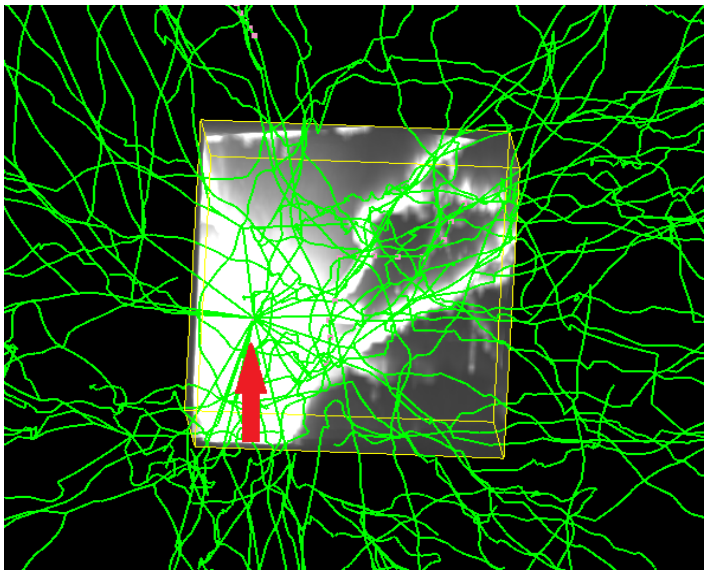

Fig.3.4.2

b. Single click on right on “next” button (red square in Fig.3.4.3). User can check along the reconstructions. The checked reconstructions are labelled in pink.(Fig.3.3.4)

Note “back” button is used for back to previous sub-block.

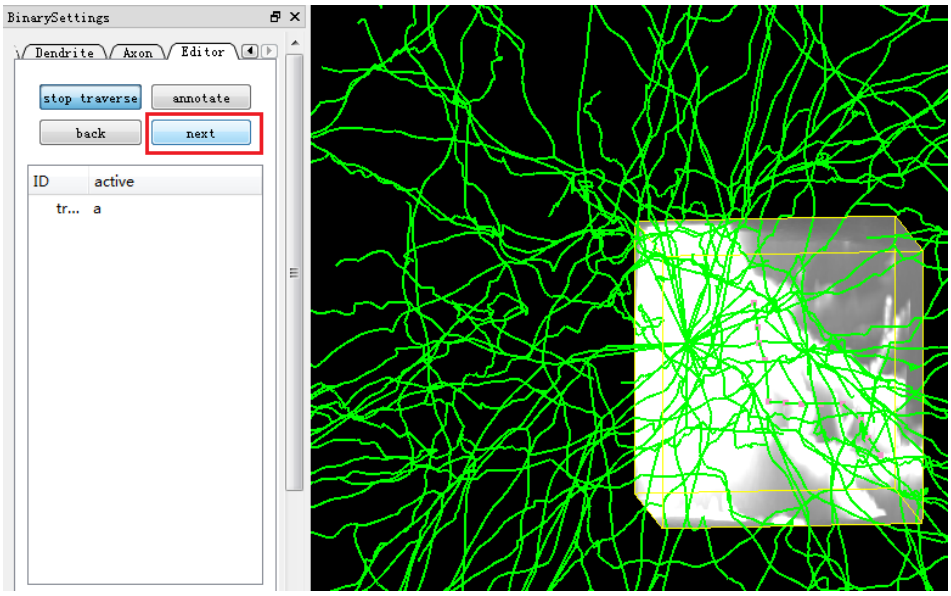

Fig.3.4.3

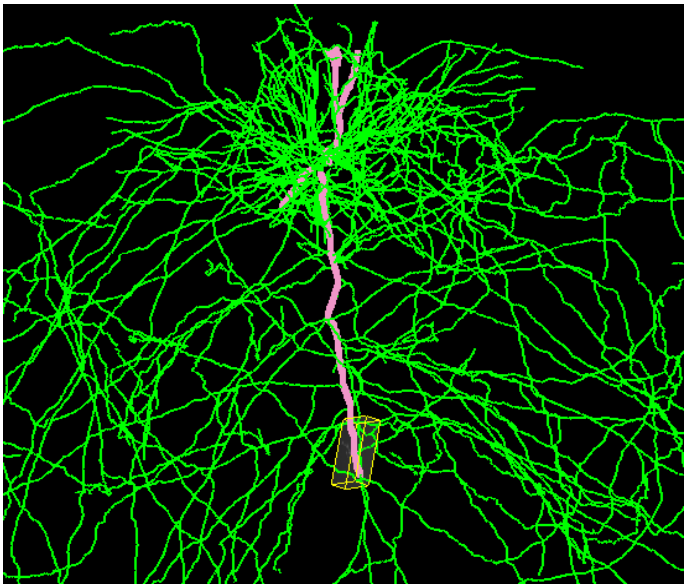

Fig.3.4.4

Step 3.4.3 Record errors through navigation: Single click on “annotate” button (red square in Fig.3.4.5) to record an error found in the navigation. All the errors will be recorded in the check list (red square in Fig.3.4.6).

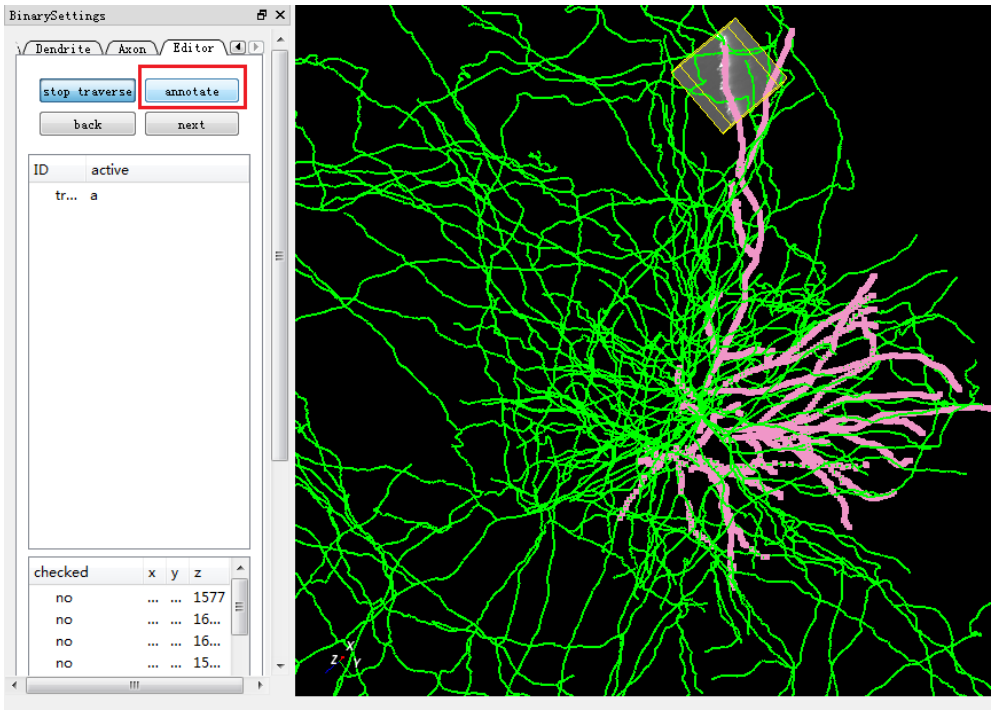

Fig.3.4.5

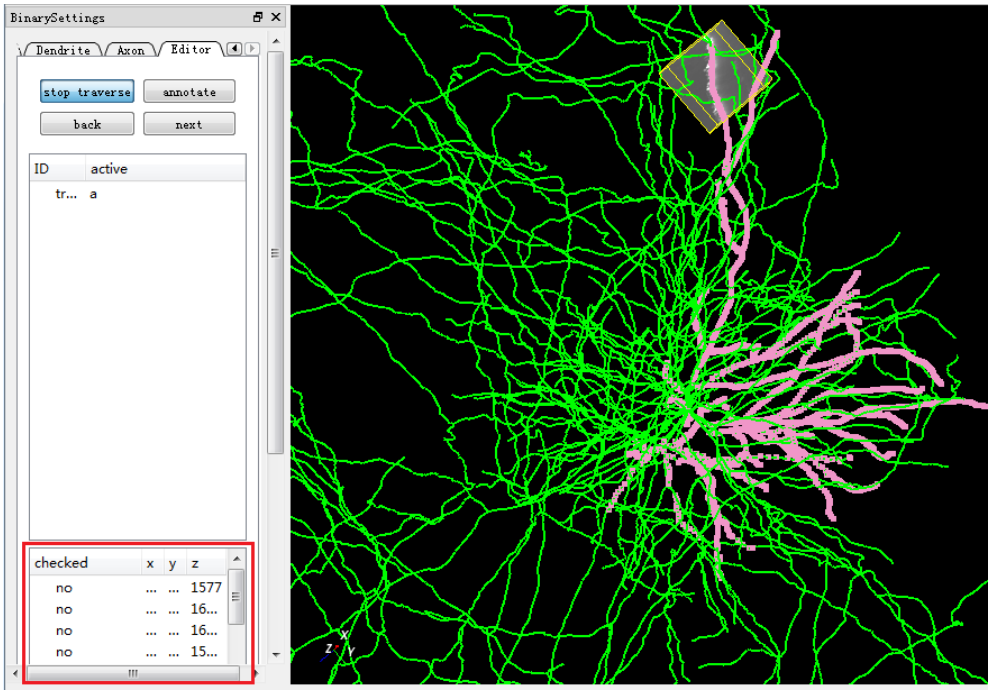

Fig.3.4.6

Step 3.4.4 Quit navigate mode: Single click on "stop traverse" button (red square in Fig.3.4.7) to quit navigate mode.

Note : the navigated reconstructions will be labelled in pink (see white arrow in Fig.3.4.7).

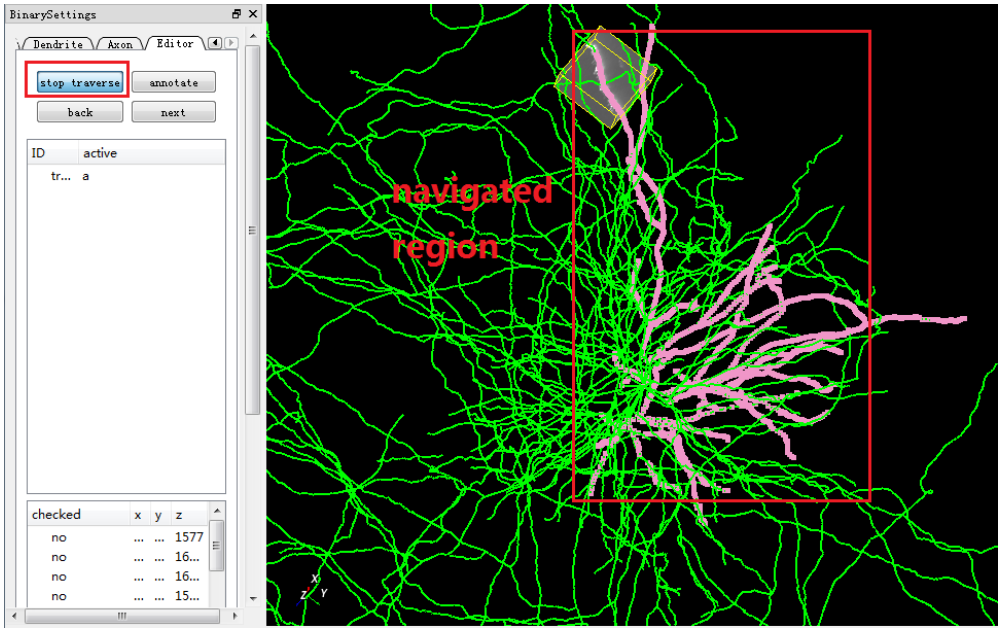

Fig.3.4.7

Step 3.4.5 Edit the record errors in the check list:

a. Choose an error in the check list. Single click on right mouse and click "Goto" (red arrow in Fig.3.4.8). GTree will show user the corresponding image sub-block and the reconstruction (Fig.3.4.9).

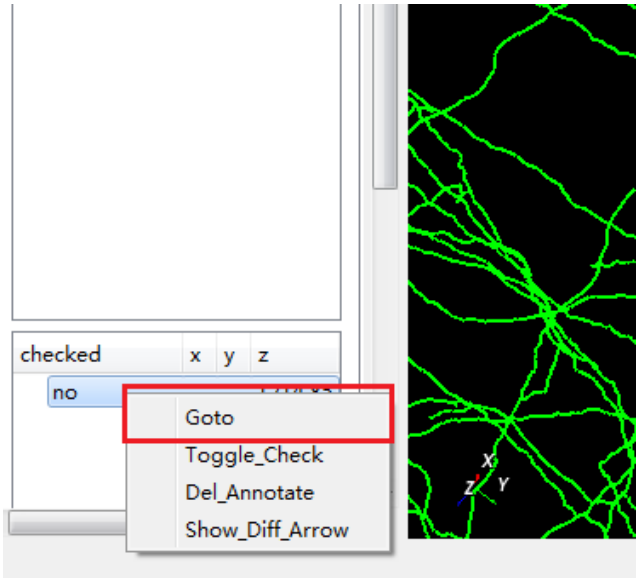

Fig.3.4.8

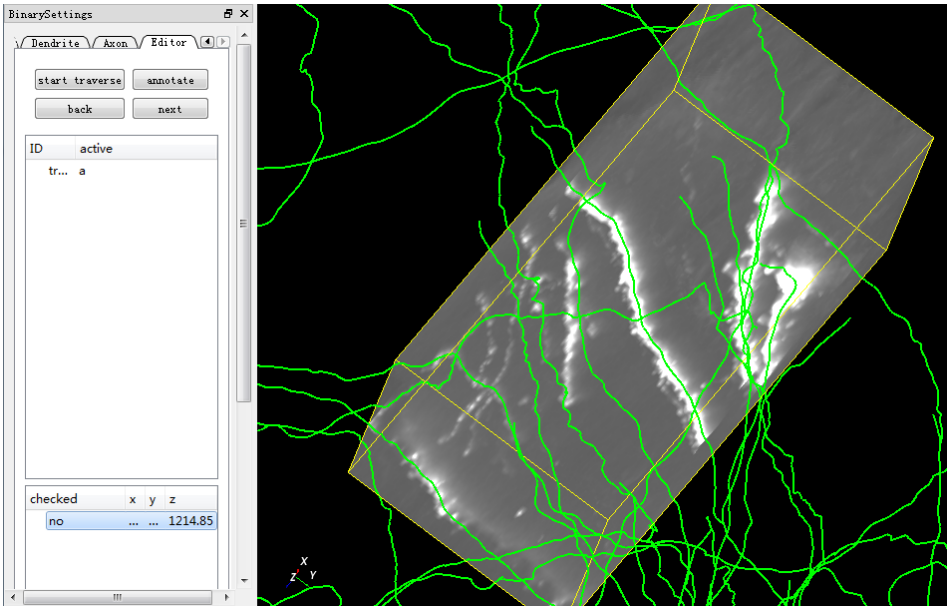

Fig.3.4.9

b. Edit the reconstruction errors in the current block. The specific edit operations can be seen in section 3.6.4.

c. Choose the revised error in the check list, Single click on right mouse and click "Toggle Check" to change the checking status. The status of the revised error is "yes" (red square in Fig.3.4.10).

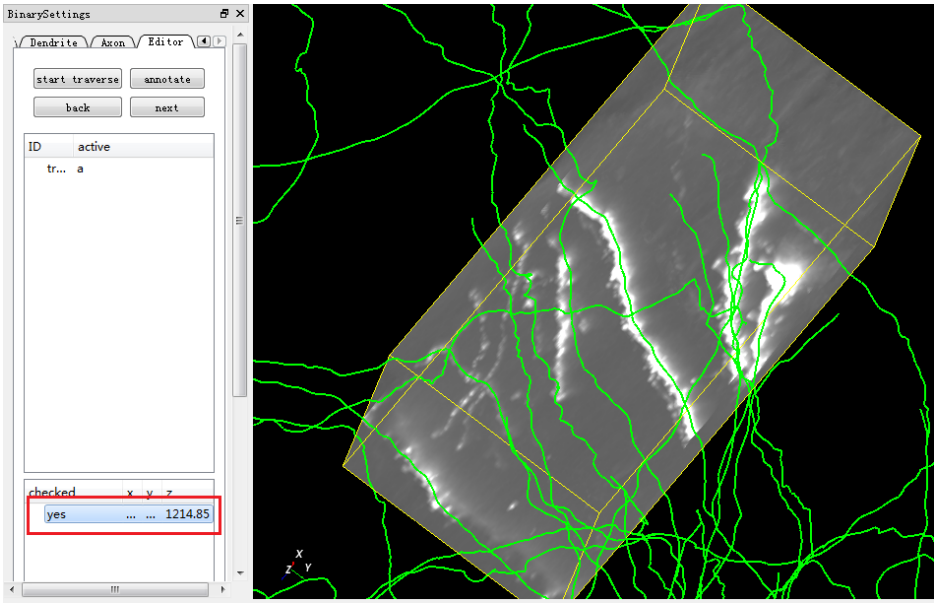

Fig.3.4.10

d. Check all the errors in the check list till the status are all turn to "yes"

3.5 On-line differences localization in reconstructions of same neuron

In order to reconstruct a brain-wide neuronal morphology with high accuracy, two or more annotators shall trace the same neuron and achieve a ground truth. GTree can fastly locate the differences among the reconstructions and provide the corresponding local images which include the discrepant reconstructions. Based on the image, user can easily compare and edit the difference among the reconstructions, then achieve a ground truth of the reconstructions.

Step 3.5.1 Import the brain dataset (".mostd") and the corresponding reconstructions (follow step 3.3.1 and 3.3.2). The brain-wide reconstructions (pink and green) are shown in the visualization window (Fig.3.5.1). In "Editor" interface (red arrow in Fig.3.5.1), the activate type of the reconstructions (tree 0 and tree 1) are shown. (red square in Fig.3.5.1)

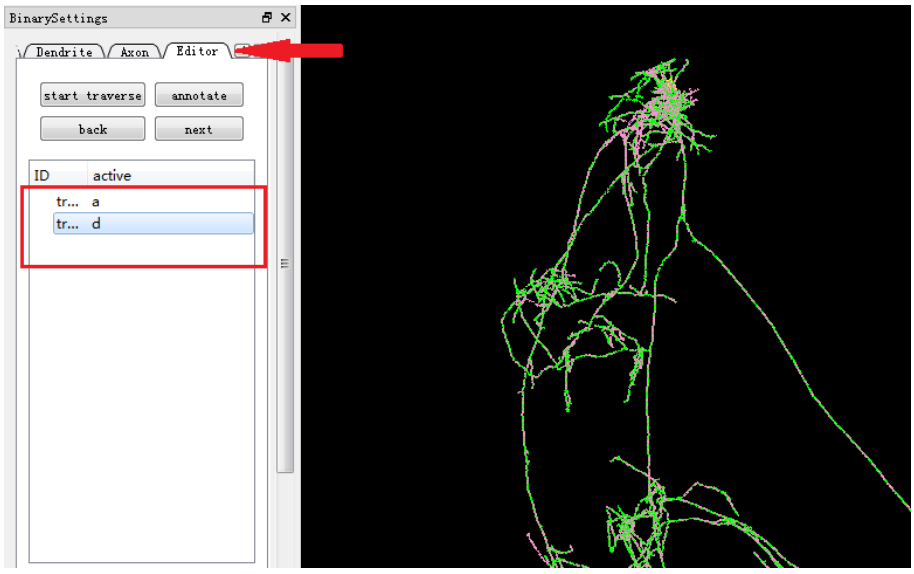

Fig.3.5.1

Note if a tree reconstruction is in "active" mode, user can edit any neurite in the reconstruction. Otherwise, the reconstruction will only be saved or deleted as a whole tree structure.

Step 3.5.2 Choose one reconstruction and single click on right mouse, choose "Toggle Visible" (red arrow in Fig.3.5.2) to display or hide the chosen reconstruction.

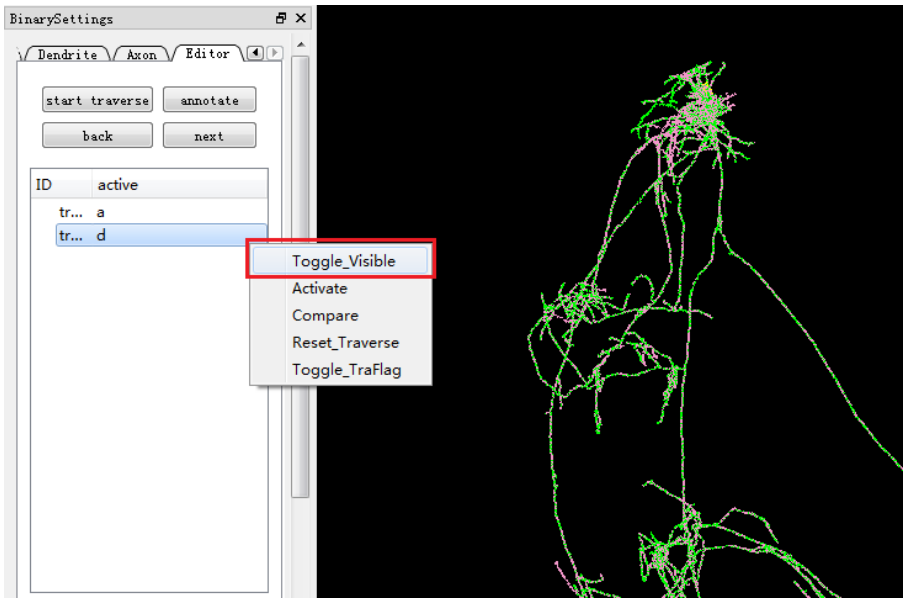

Fig.3.5.2

Step 3.5.3 Choose tree 0 in "active" status and single click on right mouse on "deactivated" tree 1, choose "Compare" (red arrow in Fig.3.5.3).

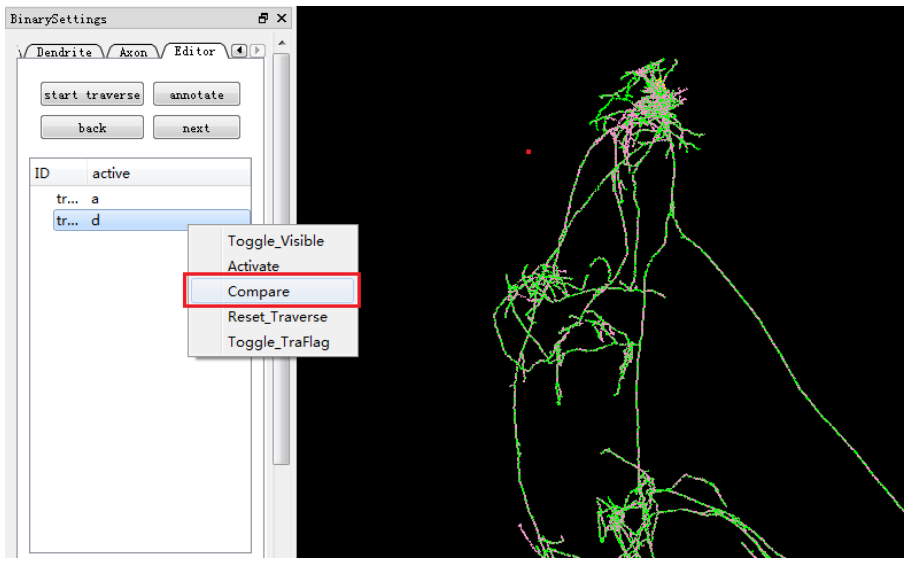

Fig.3.5.3

Step 3.5.4 Apply parameters in "Radius" and "Threshold" for comparison of the two reconstructions (red squares in Fig.3.5.4). Then press "ok" button.

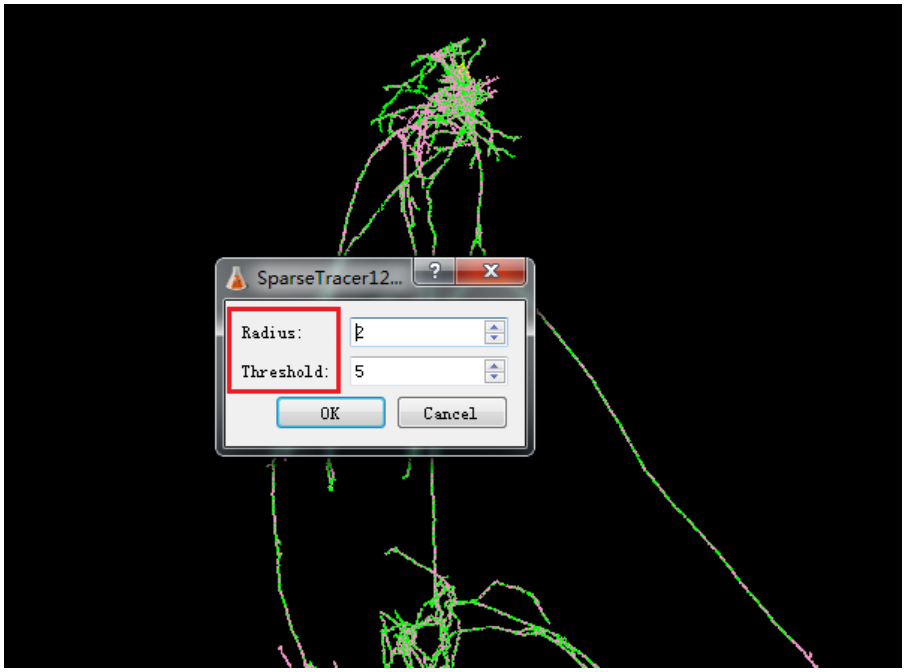

Fig.3.5.4

Note The comparison operation is based on the evaluation method in our previous article (Quan et.al. Nature Methods, 2016). "Radius" represents the distance between a point on one reconstruction and its nearest point on the other reconstruction, the unit of the "Radius" is "μm"; "Threshold" represents the number of consecutive points that are in the set radius. If the consecutive points in the radius are less than "Threshold" value, and this is one difference between two reconstructions. The default value of "Radius" and "Threshold" are "2" and "5". User can set these two parameters based on the characteristics of their data.

Step 3.5.5 The differences between two reconstructions are listed in the check list (red square in Fig.3.5.5). Furthermore, every difference can be vastly checked and edited under the same procedures in step 3.4.5.

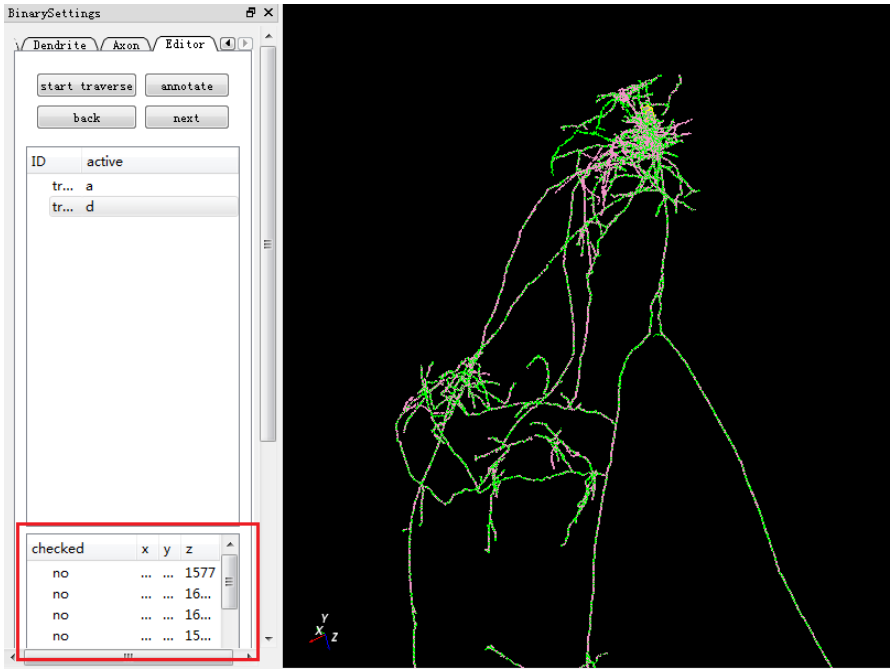

Fig.3.5.5

Step 3.5.6 After comparing the differences in the check list, user shall run step 3.5.4 again. And then, new differences will be listed in the check list. Check and revise those differences.

Step 3.5.7 Repeat **step 3. 5.6** until no more difference occur in the check list.

Note As shown in Fig. 3.2.1 the further neurites are from the soma, the higher the layer level. Here, GTree only displays the differences at the top level layer of the tree-structure, in case there might be an error involves a long-range neurites. This means, the following adjacent lower layers which start from the error maybe all wrong and the checking work do not need to be continued. Users can revise the error at the high layer and continue to check and revise neurites at the following layer. Repeat this work until there is no error at any layer.

Note GTree supports two more reconstructions' comparison. The user can compare those reconstructions in pairs following the steps 3.5.2 to 3.5.6, and then derive the ground truth.

3.6 Edit features of GTree

A neuron reconstruction can be represented by as a tree-structure. A tree structure

consists of several branches, a branch consists of several neurites and a series of nodes construct a neurite. Here, GTree can edit the neuron reconstruction under several operands, including node, branch and tree. We will introduce the specific details of the above operands.

3.6.1 Operand: Node

Single click on right mouse in visualization window, and choose "Choose_Node" (red arrow in Fig.3.6.1 left). The neurite in the reconstruction will displayed as a series of blue node, single click on left mouse to select one node, which is highlighted in yellow (yellow circle in Fig.3.6.1 right).

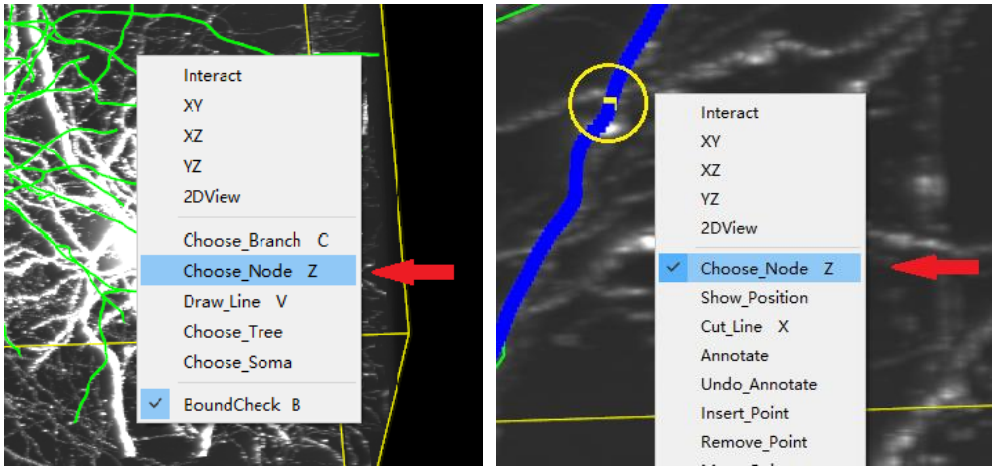

Fig.3.6.1

a. Show_position: show node's coordinate in the image (global means the coordinate in the large volume dataset, local means the coordinate in a sub-block from the large volume dataset), node's position in the tree structure (layer id and branch number), signal intensity value (see Fig.3.6.2).

Fig.3.6.2

b. Cut_line : separate the branch apart at the selected node.

c. Annotate: record the position information of the selected node and visualize a marker in the reconstruction (a marker is the yellow circle in Fig.3.6.3).

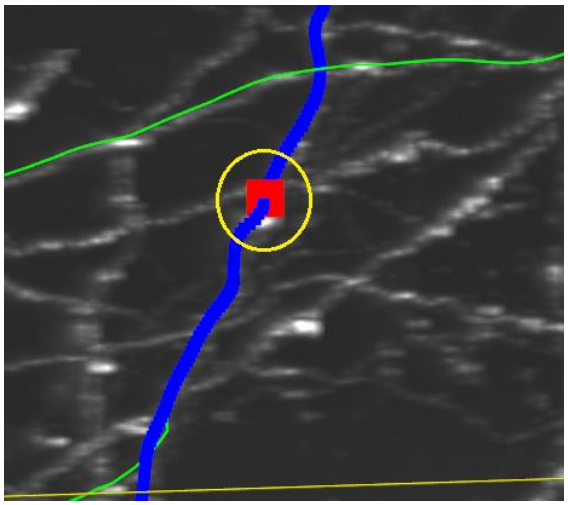

Fig.3.6.3

d. Undo_Annotate: Cancel the recordation of the selected node.

e. Insert_point: Insert a new node between the selected node and its parent node.

f. Remove_point: delete the selected node.

g. Move_point: pin the middle mouse and move the selected node (yellow square in Fig.3.6.4) to a new position (red square in Fig.3.6.4). After the operation, quit "Move_point" with shortcut key M.

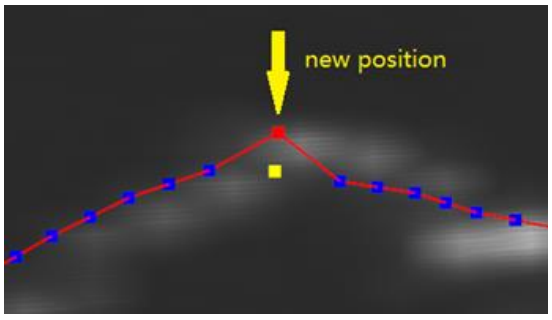

Fig.3.6.4

h. Recon_parent: reconnect the selected node from its former parent node to a new parent node.

Note the selected node should not be the head node of a branch.

3.6.2 Operand: Branch

Single click on right mouse in visualization window, and choose “Choose_Line” (red arrow in Fig.3.6.5). Single click on left mouse on the selected neurite (branch), the neurite is highlighted in red.

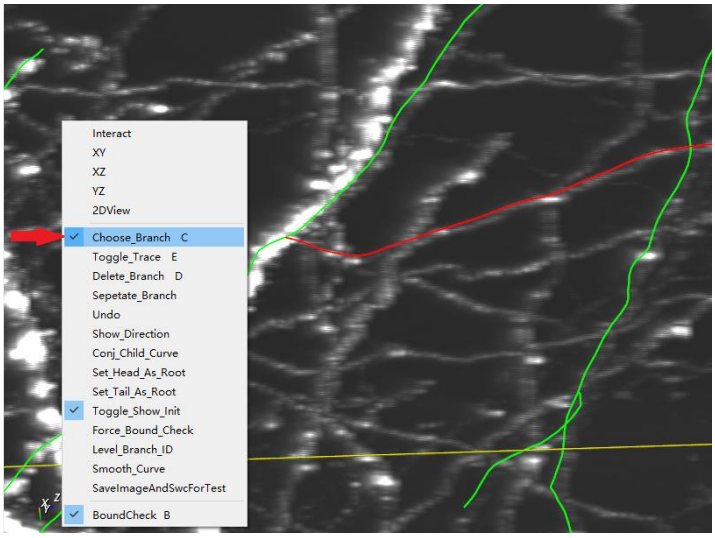

Fig.3.6.5

a. Toggle_Trace

User can decide whether to trace the selected branch or not. If the current branch is selected to be traced, a blue arrow is shown (yellow circle in Fig.3.6.6) by choosing “Toggle_Trace”. Otherwise, if user decide not to trace the branch with blue arrow, then choose “Toggle_Trace” to cancel the procedure.

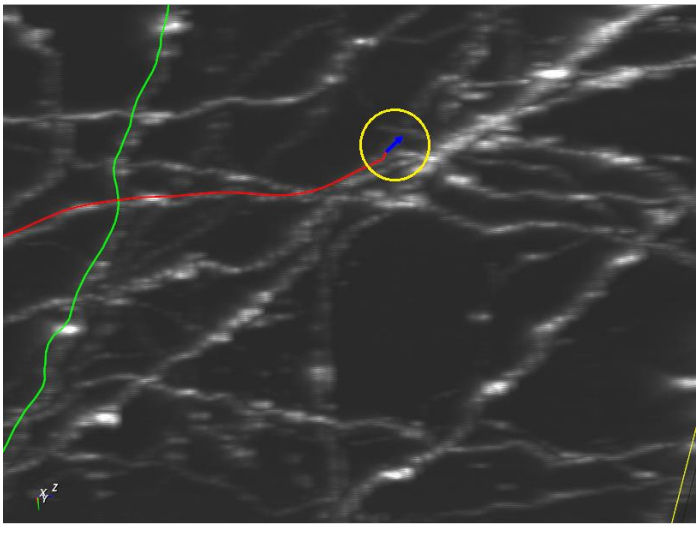

Fig.3.6.6

b. Delete_Branch

Delete the selected neurite. Shortcut Key : “D”

c. Sperate_Branch

The operating type of the selected neurite will be changed to “Tree” type.

d. Undo

Withdraw the last operation.

Note The operation can only be withdrawn once.

e. Show_direction

Display the forward direction of the selected neurite with blue arrow.

f. Conj_Child_Curve

Connect two separate neurites (end to end) to a single neurite.

g. Set_Head_As_Root

Set the head node of the selected neurite’s head as the root of the current tree.

h. Set_Tail_As_Root

Set the tail node of the selected neurite’s tail as the root of the current tree.

i. Toggle_Show_Init

During tracing, a light blue ball will display the position where the next turn of automatic tracing starts (yellow circle in Fig.3.6.7). The operation “Toggle_Show_Init” can display or hide of the ball.

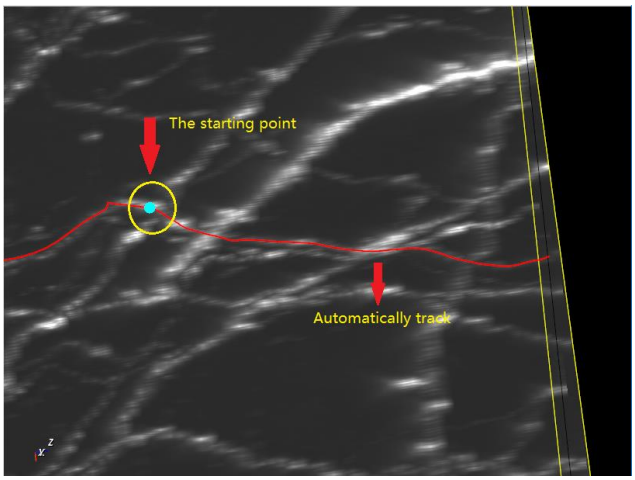

Fig.3.6.7

j. Force_Bound_Check

When tracing large area image stack through multiple sub-blocks. The “Force_ Bound_Check” helps the user check whether the automatic or manual traced neurites touch the boundary of the sub-block.

If a neurite does touch the boundary, the next sub-block will be generated based on the current tracing neurite's tail position and its forward direction. Otherwise, GTree will not generate the next sub-block which included the current neurite.

Note "Bound_Check" only checks whether the automatic traced neurites touching the boundary in the sub-block.

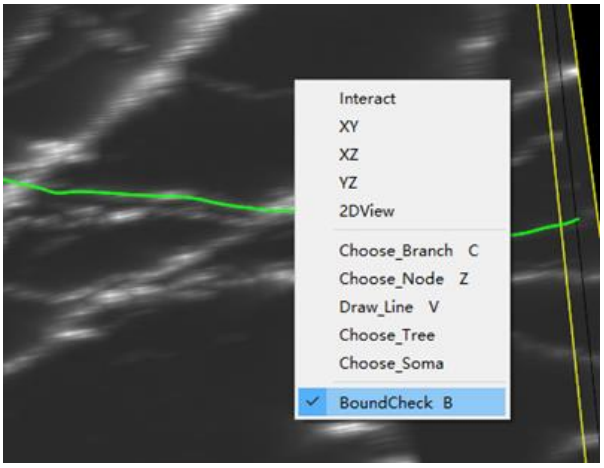

Fig.3.6.8

k. Level_Branch_ID

Show the level id and branch number of the selected branch.

l. Set_Dendrite

User can assign the selected branch to a dendrite type. Three dendrite types are included: common dendrite, apical dendrite, basel dendrite.

m. Set_Axon

User can assign the selected branch as an axon.

n. Smooth_Curve

GTree automatically smoothed the selected branch, which adjusted the branch to the optimal position in the image.

o. Build_Caliber

Build the shape of the selected branch automatically.

3.6.3 Operand: Tree

Single click on right mouse in visualization window, and choose "Choose_Tree" (red arrow in Fig.3.6.9). Single click on left mouse on the selected tree, the neurite

is highlighted in green.

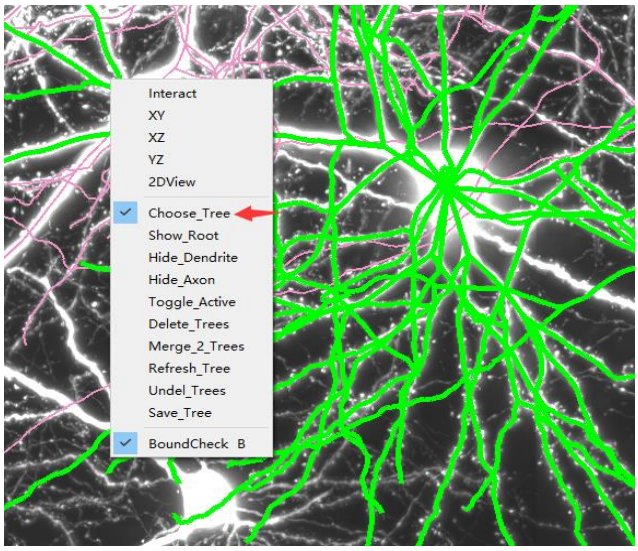

Fig.3.6.9

a. Show_Root

Display the root node of the current tree (red arrow in Fig.3.6.10).

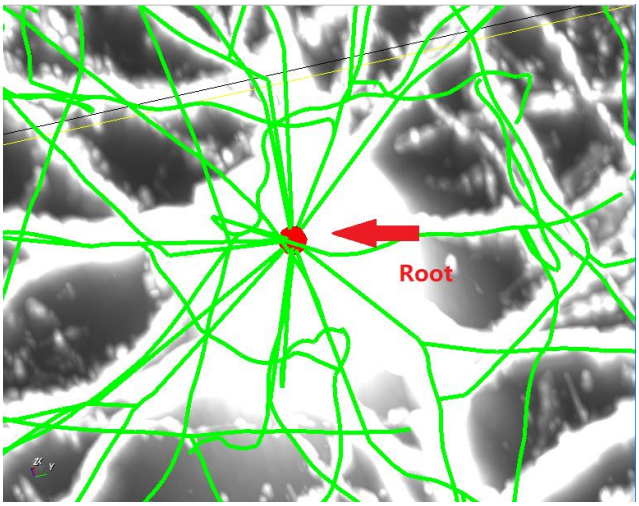

Fig.3.6.10

b. Hide_dendrite

After this operation, if any neurite in the current tree has been assigned to a specific dendrite type (see 3.6.2 step l). This neurite will be invisible in the visualization window.

c. Hide_axon

After select this operation, if any neurite in the current tree has been assigned as an axon (see 3.6.2 step m). This neurite will be hide in the visualization window.

d. Toogle_active

Set the chosen tree to “active” or “deactivate”. If the current tree is “active”, user can edit the any node or branch of it. Otherwise, the deactivate tree can only be operate as “tree”.

e. Delete_Trees

Delete the chosen tree.

f. Merge_2_Trees

If a distance between the root node of a tree and a branch of another tree is less than 2-3 voxels. The branch can be assigned to the former tree. As a result, two trees are merged as a single tree (see section 3.6.4 b).

g. Refresh_Tree

Automatically search the entire tree. Delete the redundant nodes (two more nodes in one voxel distance) and connect the branches (the distance from one’s tail node to another’s head node is less than one voxel).

h. Undel_Trees

Undo the delete operation in 3.6.3 step e.

i. Save_Tree

Save the current tree in “.swc” format.

3.6.4 Examples of edit operations with GTree

a. Smooth the reconstruction of the neurite

The traced reconstruction of the neurite may not fit to the current image and has some non-smooth parts (yellow circles in Fig.3.6.11).

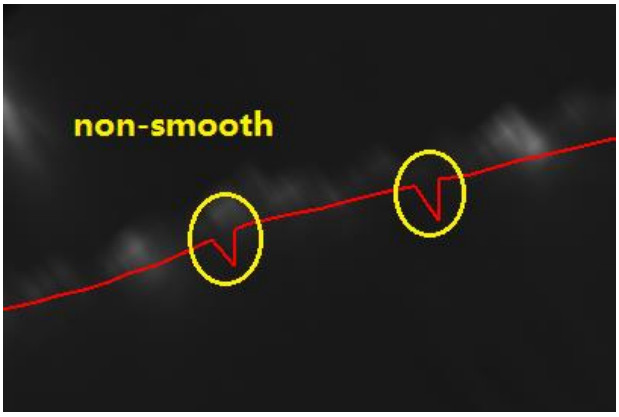

Fig.3.6.11

GTree can automatically smooth the reconstruction:

Choose the current neurite in "branch" operation mode→ Choose "Smooth_Curve" (3.6.3 step m) → smoothed reconstruction (see Fig.3.6.12)

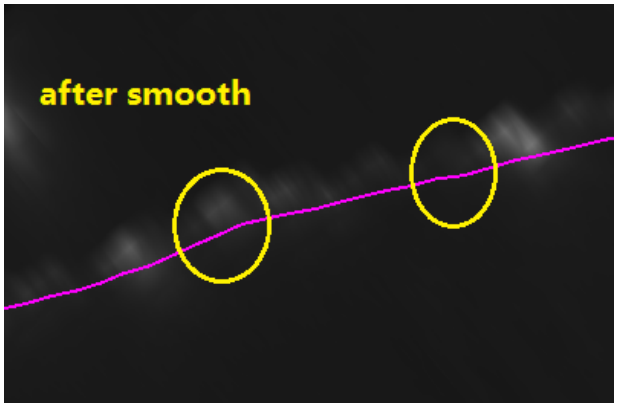

Fig.3.6.12

Note if the user is unsatisfied with the smooth reconstruction, one can take the following steps to manually smooth some difficult parts.

Choose the current neurite in "node" operation mode→ take several nodes operation to smooth the reconstruction (3.6.2 steps e, f, g and h) → smoothed reconstruction

b. Change the connection between neurites

GTree can change the neurite connections both in a single tree and between two trees.

Change neurite connection in a single tree: In Fig.3.6.13, the red curve should connect to the node in red circle but it is wrongly connected to the node in the yellow circle.

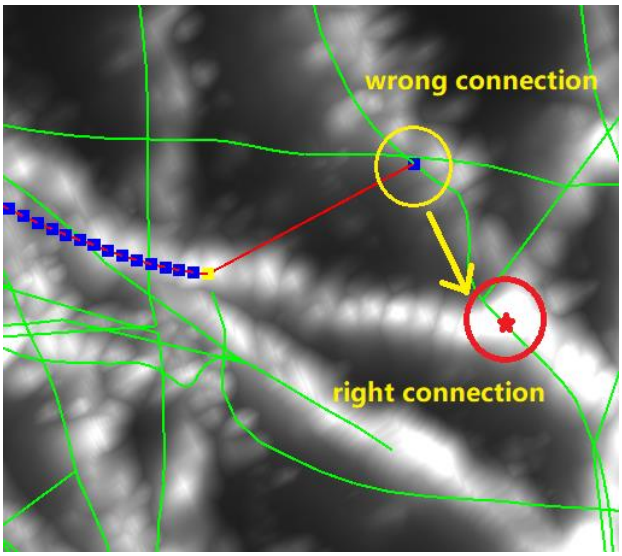

Fig.3.6.13

Take following steps:

Choose the neurite (green) in "node" operation mode → select the node (red circle in Fig.3.6.14 left) and change its parent node (3.6.1 step h) → new parent node (red circle in Fig.3.6.14 right) is connected and right neurite connection is achieved.

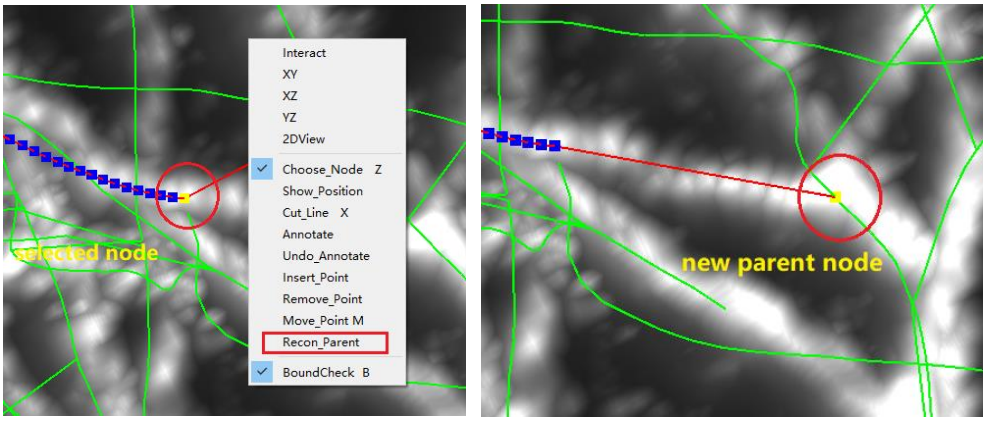

Fig.3.6.14

Change neurite connections between two trees:

If the user wants connect the branch (pink tree 2) to the former tree (green tree 1). (Fig.3.6.15)

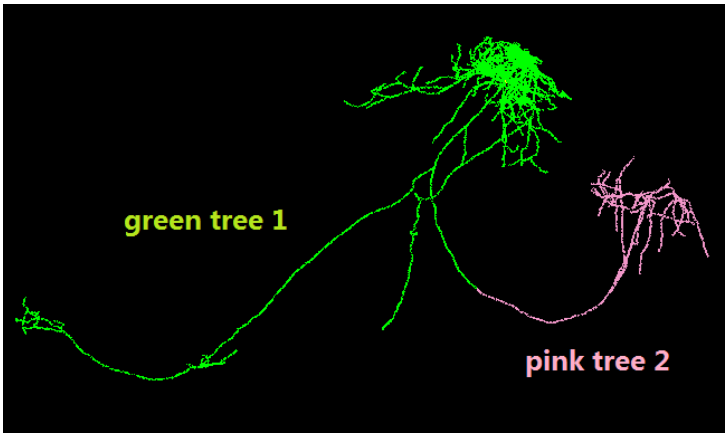

Fig.3.6.15

One shall take the following steps:

In “tree” operation mode (Fig.3.6.16) →Set “tree 2” as active status (3.6.3 step d) → choose activated “tree 2” first → press “shift” key and choose deactivated “tree 1” → select “Merge_2_trees” (3.6.3 step f, Fig.3.6.17) → “tree 2” is connected to “tree 1”

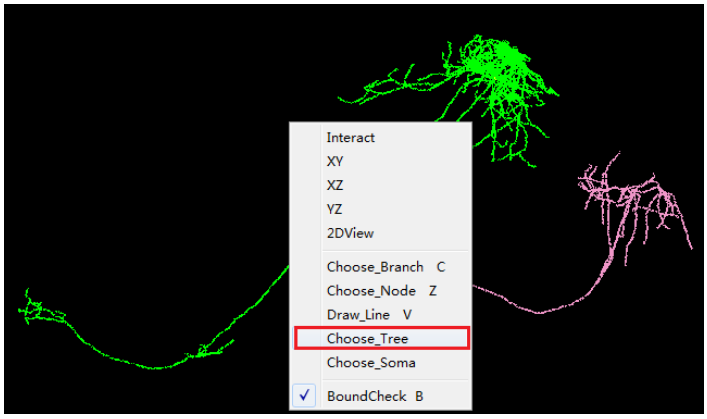

Fig.3.6.16

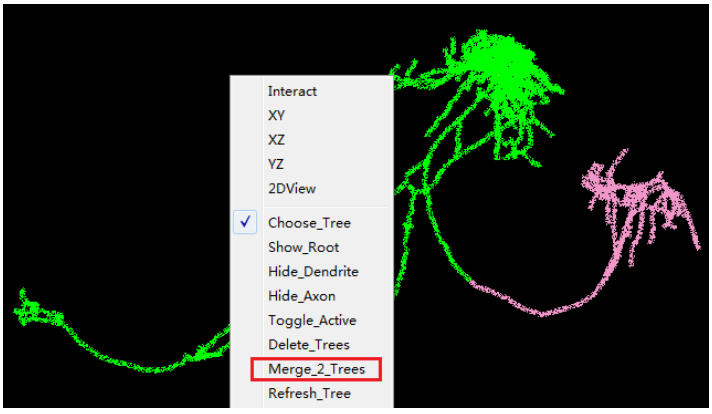

Fig.3.6.17

c. Draw a line in 2D/3D

Draw a line in 2D:

Single click on right mouse and choose “2DView” → Single click on right mouse and choose “Draw_Line” → Single click on left mouse and trace along the image (traced result is shown in Fig. 3.6.18 left) → Quit “Draw_Line” and the drawed lines is generated (Fig.3.6.18 right)

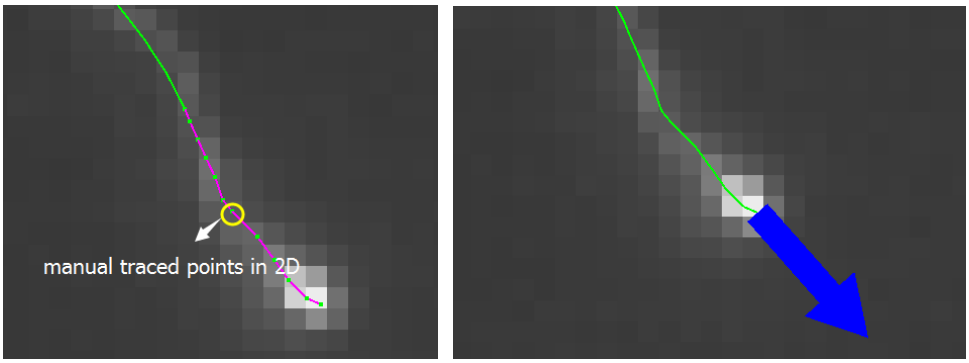

Fig.3.6.18

Draw a line in 3D:

Single click on right mouse and choose “Draw_Line” → Double click on right mouse near the end of the traced reconstruction (Fig.3.6.19) → rotate the image and double click on right mouse near the red line in Fig.3.6.19 → Repeat the last two steps to generated traced point in 3D → Quit “Draw_Line” and the drawed lines is generated

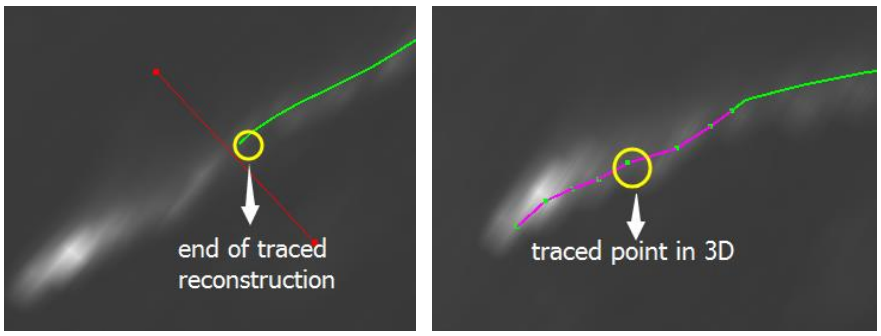

Fig.3.6.19

Note When draw line, the first traced point should be close to the end of traced reconstruction. If user wants to undo his tracing, press key “U”.

d. Change the root of a tree

If the root position of the tree is not soma (Fig.3.6.20).

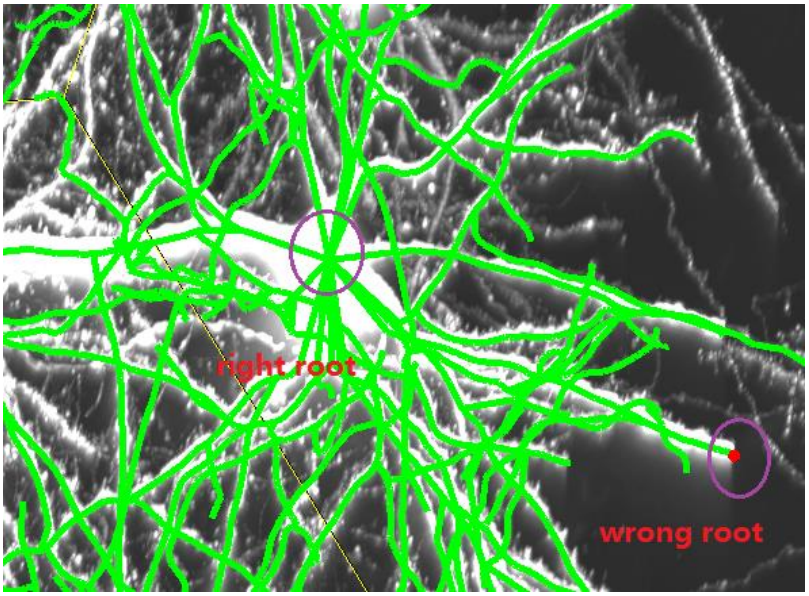

Fig.3.6.20

Take following steps to change the root position:

Choose the "branch" operation mode→ Choose one neurite which connected to the soma directly → Choose "show_direction" in "branch" operation mode (3.6.2 step e) , to check the head and tail of the current neurite (direction is shown as blue arrow in the yellow circle of Fig.3.6.21)→ Set the neurite part which connect to soma as the root (3.6.2 step g or h) → The root is refreshed to soma position (Fig.3.6.22)

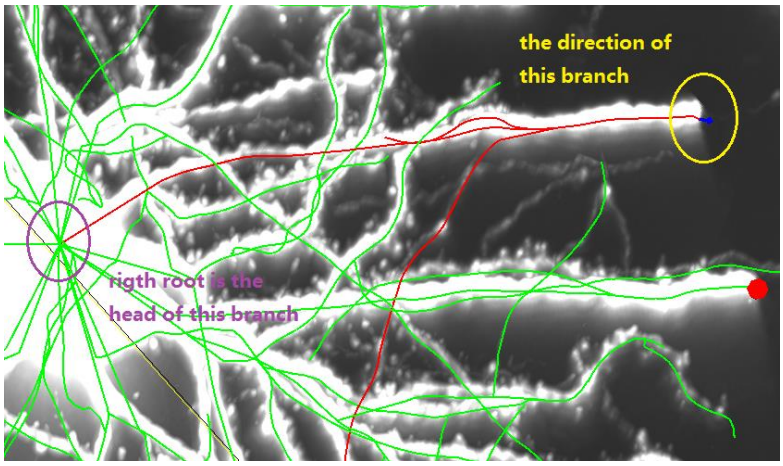

Fig.3.6.21

Fig.3.6.22

e. Activate or deactivate a tree

Activate a tree

GTree can change the status of the current tree, to make the tree activated or deactivate (Fig.3.6.23). Once the tree is in the "activated" mode, user can edit any branch or node of the tree and apply corresponding tracing operations.

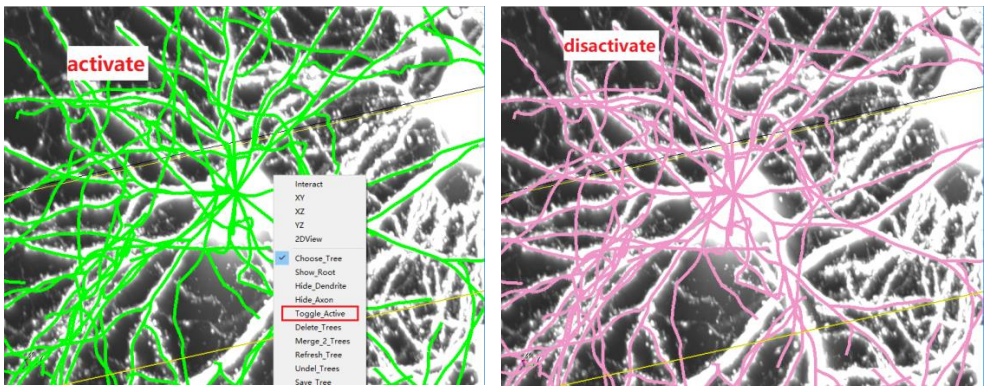

Fig.3.6.23

Take following steps to change the tree status :

Single click on right mouse and choose "Choose_Tree" to enter the "tree" operation mode → Choose the tree you want to activate (or deactivate)→ Single click on right mouse and choose "Toogle_active" (3.6.3 step d)

Note Only one tree is activated at one time.

f. Visible a tree

You can hide or display the tree by "visible" button. ( in red circle of Fig.3.6.24 and the shortcut key is "A")

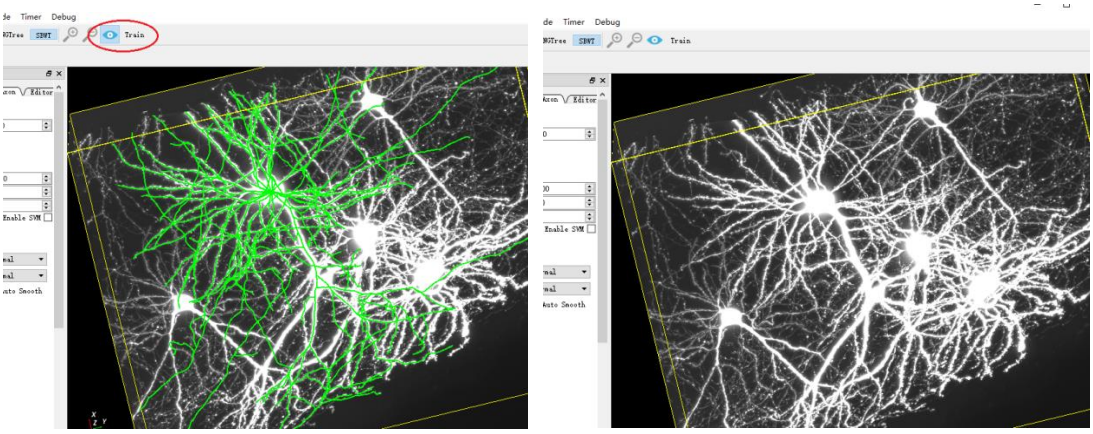

Fig.3.6.24

g. Display the dendrites and axons separately

If the user assign the branches as dendrites (step 3.6.2 l) or axons (step 3.6.2 m) in advance. One can hide those branches by choosing the step 3.6.3 b & c.

4 Interfaces of GTree

4.1 Menu bar of GTree

GTree's menu bar can be divided into "File", "Edit", "Trace", "Mode" and "Timer". We will introduce the specific details of the above menus.

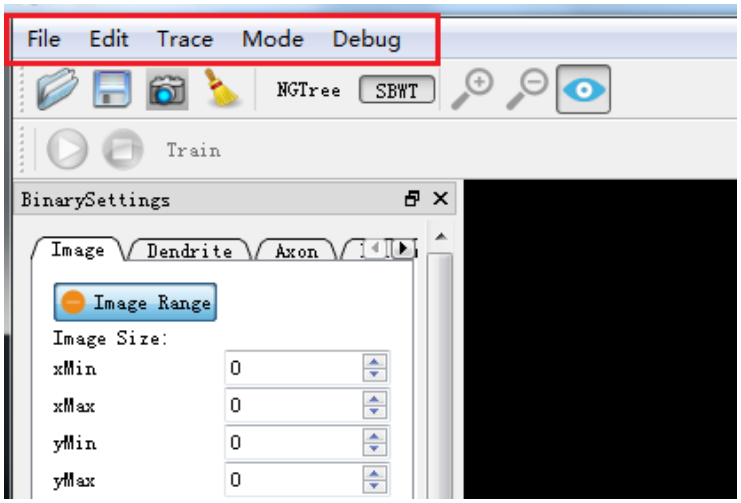

Fig.4.1.1

4.1.1 File

The "File" menu includes operations on files such as "open", "save" and so on.

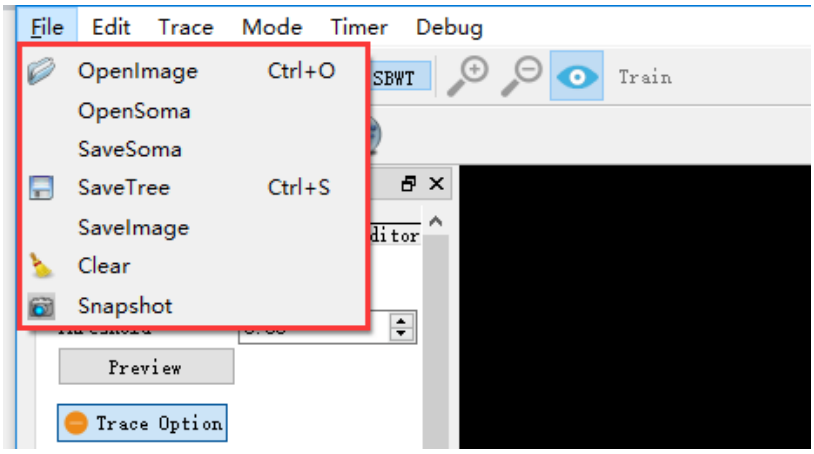

Fig.4.1.2

a. OpenImage

Open the image stacks and corresponding reconstruction files (".swc" or ".sta" format). (Specific operating steps can be seen in 3.1.1-3.1.3)

b. OpenSoma

Open files (".pbo") that include the soma location.

c. SaveSoma

Save files (".pbo") with soma locations to the computer disk.

d. SaveTree

Save the reconstruction (in ".swc" format) derived from GTree.

Note The format (". swc") of the reconstruction coincide with the published standard in NeuroMorph database (www.neuromorph.org).

e. SaveImage

Save the image in current sub-block (red circle in Fig.4.1.3).

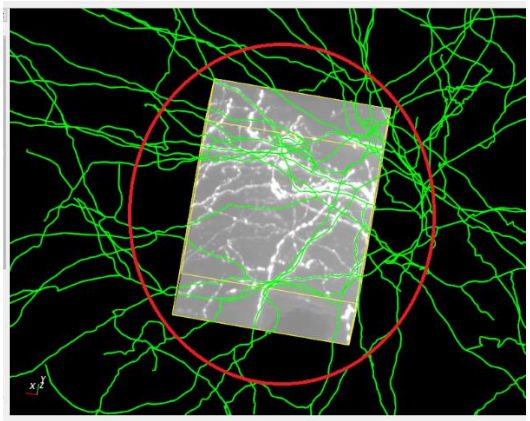

Fig.4.1.3

f. Clear

Clear all data (image stack and the reconstructions).

g. Snapshot

The “Snapshot” provides the user interface of the viewer's snapshot facility.

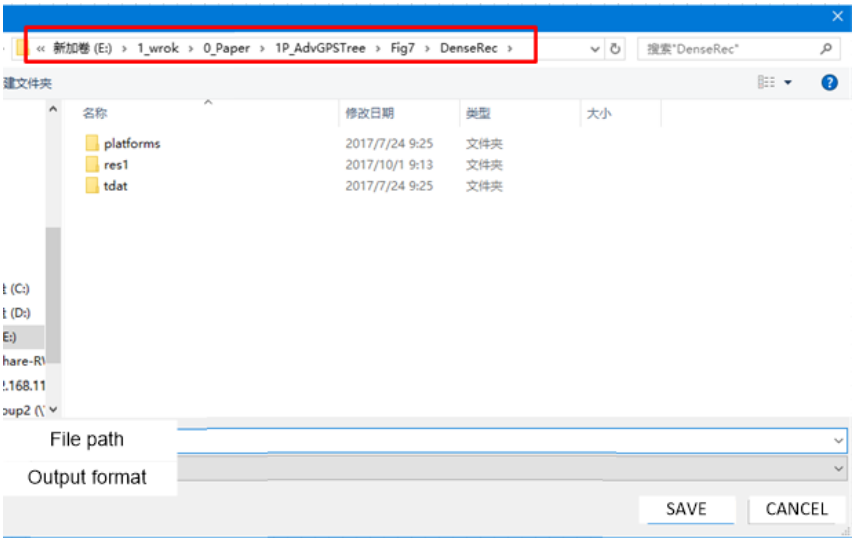

Fig.4.1.4

File path: Lets user specify the filename and a desired location within the file system.

Output format: the file format to be produced for file output is PNG (“.png”).

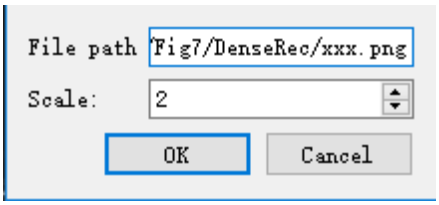

Fig.4.1.5

Scale: Lets the grab images larger than the actual screen size. When the scale is “1”, it grabs the original dimensions of the screen, otherwise the width and height of the grab image will multiple by the selected scale.

4.1.2 Edit

The “Edit” menu includes the following operations (red square in Fig.4.1.6).

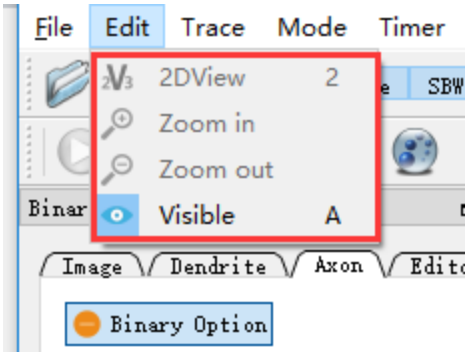

Fig.4.1.6

a. 2DView

Enter the "2DView" mode. (shortcut is "2")

b. Zoom in

Zoom in the image in "2DView" mode.

c. Zoom out

Zoom out the image in "2DView" mode.

d. Visible

Display or hide the reconstruction in visualization window. (shortcut is "A")

4.1.3 Trace

The "Trace" menu includes the following operations (red square in Fig.4.1.7).

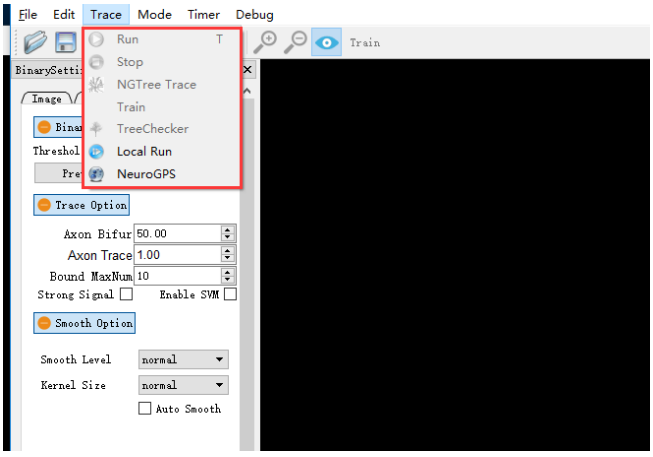

Fig.4.1.7

a. Run

Trace the neurite in current image sub-block (shortcut : "T") and automatically update the next sub-block for tracing. This function key is special for long-range neurite tracing (see section 3.3).

b. Train

In case user is not satisfied with the reconstruction of current image sub-block, user can firstly choose “Train” key to acquire more prior information, and then “Run” tracing procedure again.

c. Stop

Terminate the tracing procedure. This function key is special for long-range neurite tracing (see section 3.3).

d. NGTree Trace

Trace the dendrites of the current tree (see section 3.1 for details).

e. TreeChecker

Insert corresponding parameters to enter “select display mode” (see section 3.2 for parameter selections).

f. Local Run

Only trace the neurite in the current image sub-block.

g. NeuroGPS

Automatically acquire the soma positions.

4.1.4 Mode

User can select the two modes in “Mode” menu (red square in Fig.4.1.8). This operation is equal to click the corresponding button on tool bar with left mouse click (see section 4.2 for details).

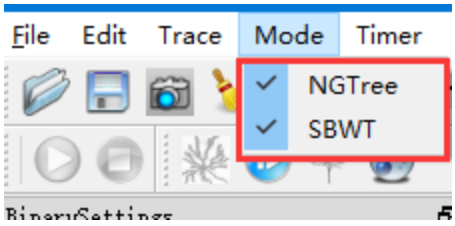

Fig.4.1.8

a. NGTree

“NGTree Trace”, “Local Run”, “TreeChecker” and “NeuroGPS” are included. See section 4.1.3 d-g for function details.

b. SBWT

“Run” and “Stop” are included. See section 4.1.3 a & c for function details.

4.2 Tool bar of GTree

Tool bar (red square of Fig.4.2.1) is a shortcut for the functions in the menu bar. The corresponding function of the icons in Tool bar is list below.

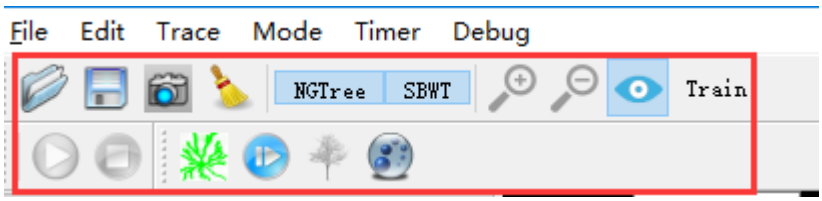

Fig.4.2.1

a. OpenImage

Same function in section 4.1.1 a.

b. SaveTree

Same function in section 4.1.1 b.

c. Snapshot

Same function in section 4.1.1 g.

d. Clear

Same function in section 4.1.1 f.

e. Zoom in

Same function in section 4.1.2 b.

f. Zoom out

Same function in section 4.1.2 c.

g. Visible

Same function in section 4.1.2 d.

h. Train

Same function in section 4.1.3 b.

i. Run

Same function in section 4.1.3 a.

j. Stop

Same function in section 4.1.3 c.

k. NGTree Trace

Same function in section 4.1.3 d.

l. Local Run

Same function in section 4.1.3 f.

m. TreeChecker

Same function in section 4.1.3 e.

n. NeuroGPS

Same function in section 4.1.3 g.

4.3 Panels of GTree

GTree has four panels for more specific parameters. The four panels are “Image”, “Dendrire”, “Axon” and “Editor”, respectively.

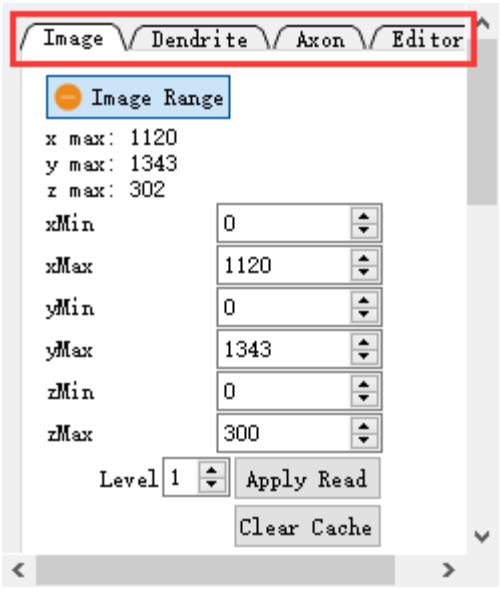

Fig.4.3.1

4.3.1 Image Panel

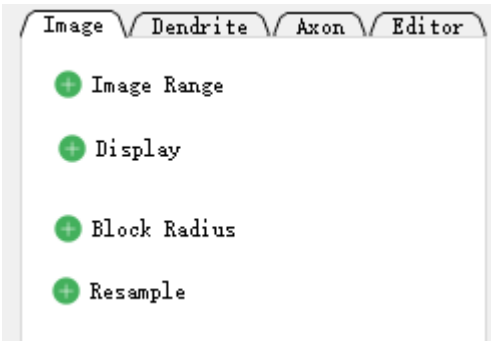

Fig.4.3.2

a. Image Range

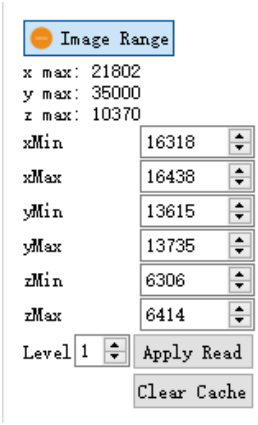

Fig.4.3.3

x max, y max and z max : The image size of the imported image stack.

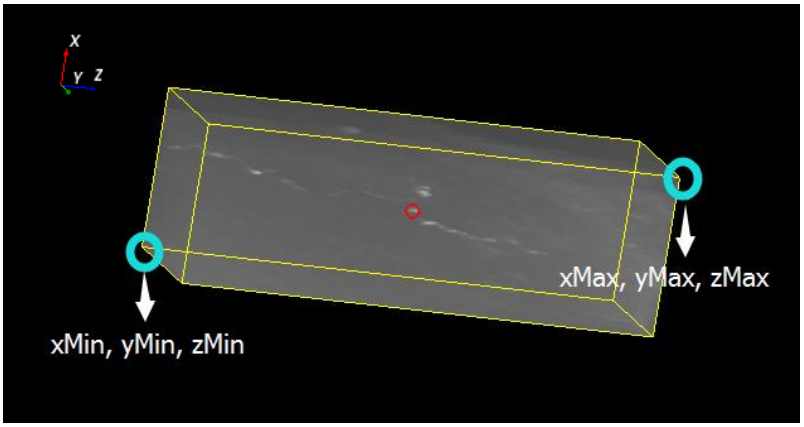

Fig.4.3.4

According to the global axis in the left corner of Fig.xx, the user can import a interested sub-block by inputting the coordinates (xMin, yMin, zMin) and (xMax, yMax, zMax).

Level: Apply image display in multiple resolutions (from 1 to 8). Choose Level "1" means display a full-resolution image.

Apply Read: Ensure the input coordinate and display resolution, import the image stack.

Clear Cache: Release the image cache in computer memory.

b. Display

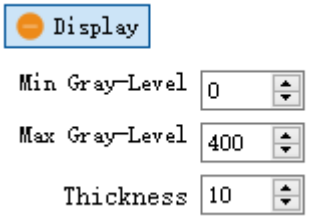

Fig.4.3.5

Min/Max Gray-Level: Used for adjusting the display gray value of the image.

Thickness: In "2D view", the thickness value represents the projection thickness of the image stack in z dimension.

c. Block Radius

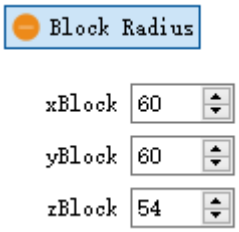

Fig.4.3.6

Size of the current sub-block in x, y, z dimensions.

d. Resample

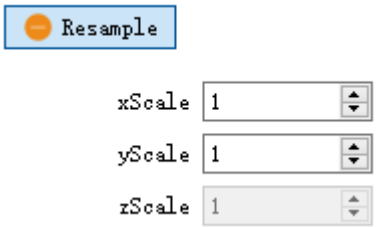

Fig.4.3.7

User can choose different Scale value on x and y dimensions. The scaling result will not be displayed to the user.

Note We recommend using the "Resample" operation if the voxel size of the input image stack in three dimensions are anisotropy. For example, for the input image stack with voxel size of $0.21 \times 0.21 \times 0.39\ \mu m^3$, it was recommended to scale to an image stack with voxel size of $0.63 \times 0.63 \times 0.78\ \mu m^3$ for the reconstruction. Pleases note the reconstructed results, saved as SWC files, still coincide with the voxel

size of the input image stack, rather than the scaled image stack.

Attention: If you do not know the voxel size of the datasets, please check the image collection source (microscopy parameters) to find out the voxel size of the datasets.

e. Illuminate

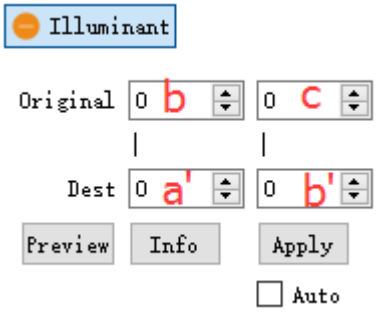

Fig.4.3.8

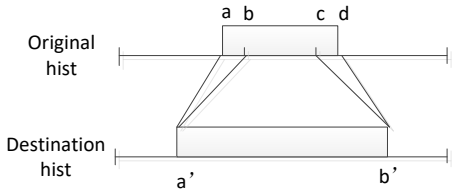

Fig.4.3.9

We set 4 parameter to adjust the gray scale for varies histogram. In Fig.4.3.8, point a represents the minimum value of original image, point d represents the maximum value of original image. Point b and c represent the gray scale range we are interested in. Point a' and b' represent the expected minimum and maximum value of destination image. The voxel values which are lower than b will be set to a'. The voxel values which are higher than c will be set to b'. The voxel values fall into [b, c] (red in Original in Fig.xx) will be scale to range [a', b'] (red in Dest in Fig.4.3.9) linearly.

Preview: User can observe the result of the linear transformation of intensity value in visualization window.

Info: Automatically input the maximum and minimum voxel value of current image in "Original" boxes.

Apply: Run intensity linear transformation.

Auto: Automatically run linear transformation on upcoming sub-blocks with same

parameters.

4.3.2 Dendrite Panel

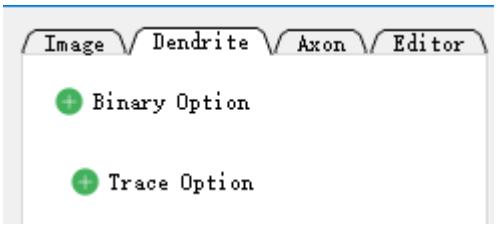

Fig.4.3.10

The dendrite panel is designed for reconstruction of dendrites. In addition, it can be used for reconstructing local image stack.

a. Binary Option

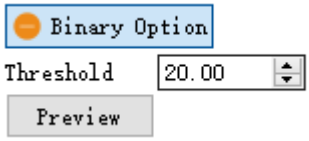

Fig.4.3.11

Binary: This threshold is used to roughly discriminate between the foreground and background.

Preview: After setting the "Threshold", the user can observe the binary result in visualization window immediately. And then, according to the result, the user can adjust the "Threshold" to achieve a satisfying binary result.

Note If the binary threshold parameter is set too large, only a small number of seeds pints will be detected which will end in a miss of some dendrites during tracing. If the binary threshold parameter is set too small, a large number of redundant seeds points will be detected which will interfere with the dendrites tracing. The suitable Binary Threshold should assure that most of neuronal morphologies of neuronal population can be identified from binary image stacks. It is a key parameter to the number of seeds points.

b. Trace Option

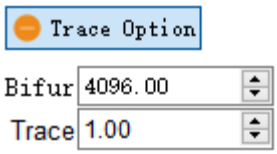

Fig.4.3.12

Bifur: GTree implements automatic bifurcation detection algorithms. This threshold is used when the automatic detection fails, when encounter bifurcations with weak signal. Generally, a small threshold corresponds to more detected bifurcations. However, user may careful with too small threshold in case some burrs will be mistaken for bifurcations.

Trace: This is used to terminate neurite tracing. Its recommended value ranges from 0 to 30. Generally, a small trace threshold corresponds to the strong background, and a big trace threshold corresponds to the weak background. The background can be roughly estimated by modifying the two parameters (Min Gray-Level, Max Gray-Level) of data visualization.

4.3.3 Axon Panel

The dendrite panel is designed for reconstruction of long-range axons.

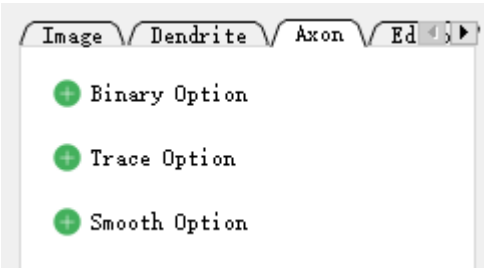

Fig.4.3.13

a. Binary Option

Same as 4.3.2 a.

b. Trace Option

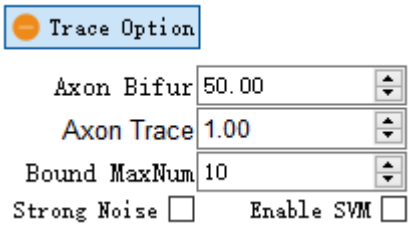

Fig.4.3.14

Axon Bifur: same explanation as parameter "**Bifur**" in 4.3.2 b.

Axon Trace: same explanation as parameter "**Trace**" in 4.3.2 b.

Bound MaxNum: The number of the intersections where newly reconstructed neurites touch the sub-block boundary. If the intersections are more than the set "Bound MaxNum", GTree will not provide initial tracing information of the adjacent sub-blocks in case the reconstruction errors may occur. Too many intersections indicates a noisy environment and the reconstructions of current sub-block needs to revise.

Strong Noise: If selected, GTree will strongly suppress the noise.

Enable SVM: This shall be selected when there are lots of weak signals occur in the image. An SVM model will be enabled to boost the automatic level of weak signal identification.

c. Smooth Option

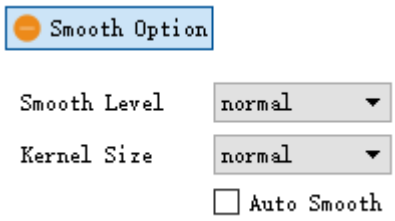

Fig.4.3.15

Smooth Level: The "smooth level" determines the smoothness of the current chosen reconstruction.

Kernel Size: This determines the local neighborhood image sizes which are considered for reconstruction smoothness. If the kernel size is large, it indicates that larger images are taken into account, and the computing time for smoothness will also arise.

4.3.4 Editor Panel

The Editor Panel is mainly used for fastly navigation and differences localization. (Fig.4.3.16-4.3.18). The specific details of the function keys can be seen in section 3.4 & 3.5.

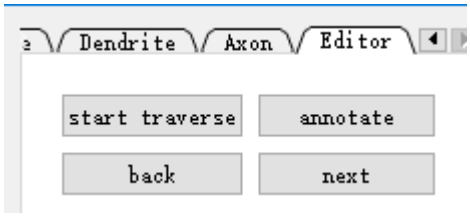

Fig.4.3.16

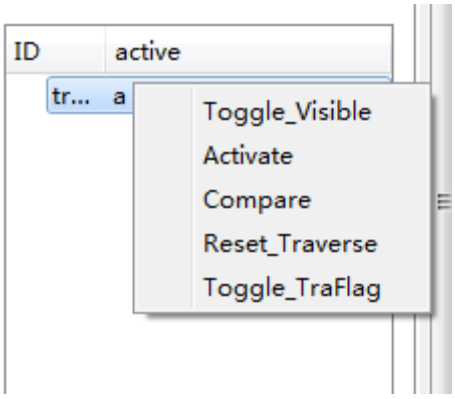

Fig.4.3.17

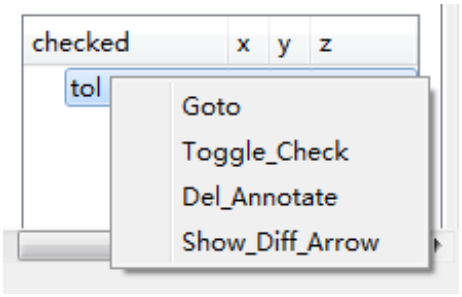

Fig.4.3.18

a. start traverse

   See section 3.4 step 3.4.2 a

b. annotate

   Annotate the location you want to check (see step 3.4.3)

c. back

   Go back to the previous sub-block.

d. next

   Go to the next sub-block.

e. Toggle_Visible

   Same function to “Visible”, hide or visible the tree

f. Activate

Activate or deactivate the tree

g. Compare

   Compare two trees (see step 3.5.3)

h. Reset_Traverse

   Reset the travers

i. Toggle_TraFlag

   Show the pink neurite which have been browsed

j. Goto

   Goto the coordinate you want to check

k. Toggle_Check

   Change the state (yes or no) of sign (see step 3.4.5 c)

l. Del_Annotate

   Delete this annotate

m. Show_Diff_Arrow

   Show the different arrow

Appendix

A.1 Mouse Operation

Click the left mouse button: select items

Click the right mouse button on tool bar: select toolbars display

Click the right mouse button on Graphic window: open the drawing menu bar

Rolling mouse wheel forward: zoom in the image

Rolling mouse wheel back: zoom out the image

Rolling mouse wheel forward in 2DView: turn to next slice

Rolling mouse wheel back in 2DView: turn back to last slice

Pin the left button to move the mouse: rotate the image

Pin the roller to move the mouse: translation image

Pin the roller to move the mouse in Move_point mode: move point

A.2 Shortcut Key

OpenImage: **“Ctrl+O”**

SaveTree: **“Ctrl+S”**

Interact: **“Q”**

2Dview: **“2”**

Visible: **“A”**

Run: **“T”**

Choose_Branch C: **“C”**

Toggle_Trace: **“E”**

Delete_Branch: **“D”**

Choose_Node: **“Z”**

Cut_Line: **“X”**

Insert_Point: “I”

Move_Point: **“M”**

Draw_Line: **“V”**

Undo_Draw: **“U”**

BoundCheck: **B**