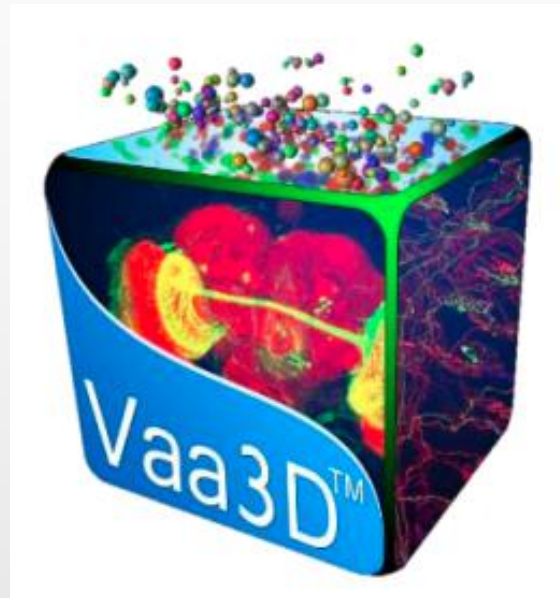# How to generate high-quality neuronal reconstructions semi-automatically through Vaa3D

Yaping Wang, 2021.11.16

Workflow

Automated Neuron Tracing in Vaa3D

Manual reconstruction of neurons in Vaa3D

Inspected and modified by multiple annotators for single neuron in Vaa3D
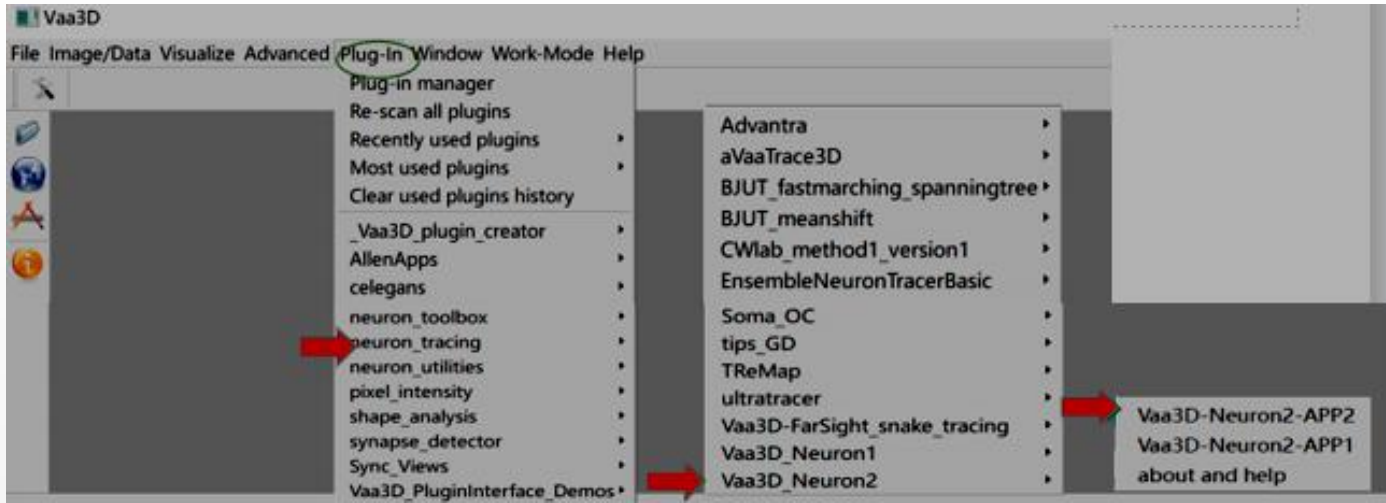
Assistance of Tera-VR annotation platform

For a large number of neuron data generated, there are batch inspection and screening of automatic post-processing.
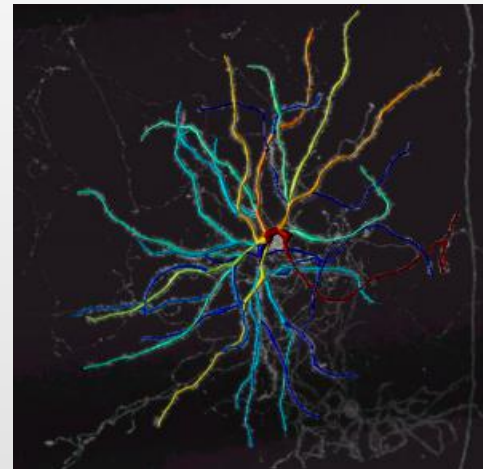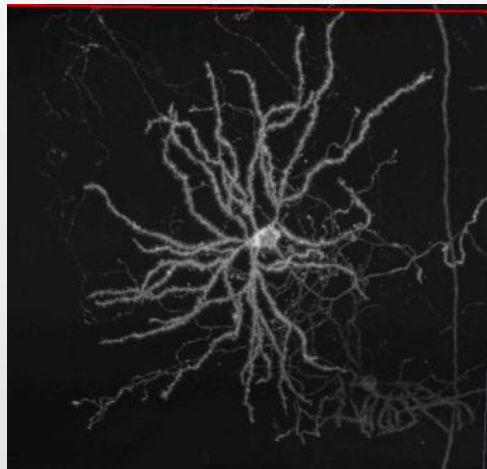
The high-quality neuronal reconstructions
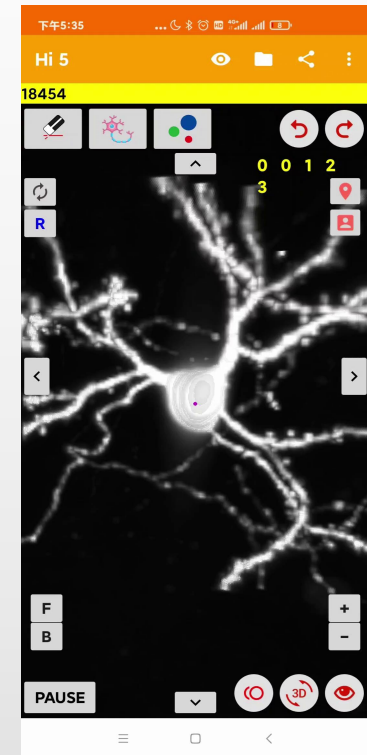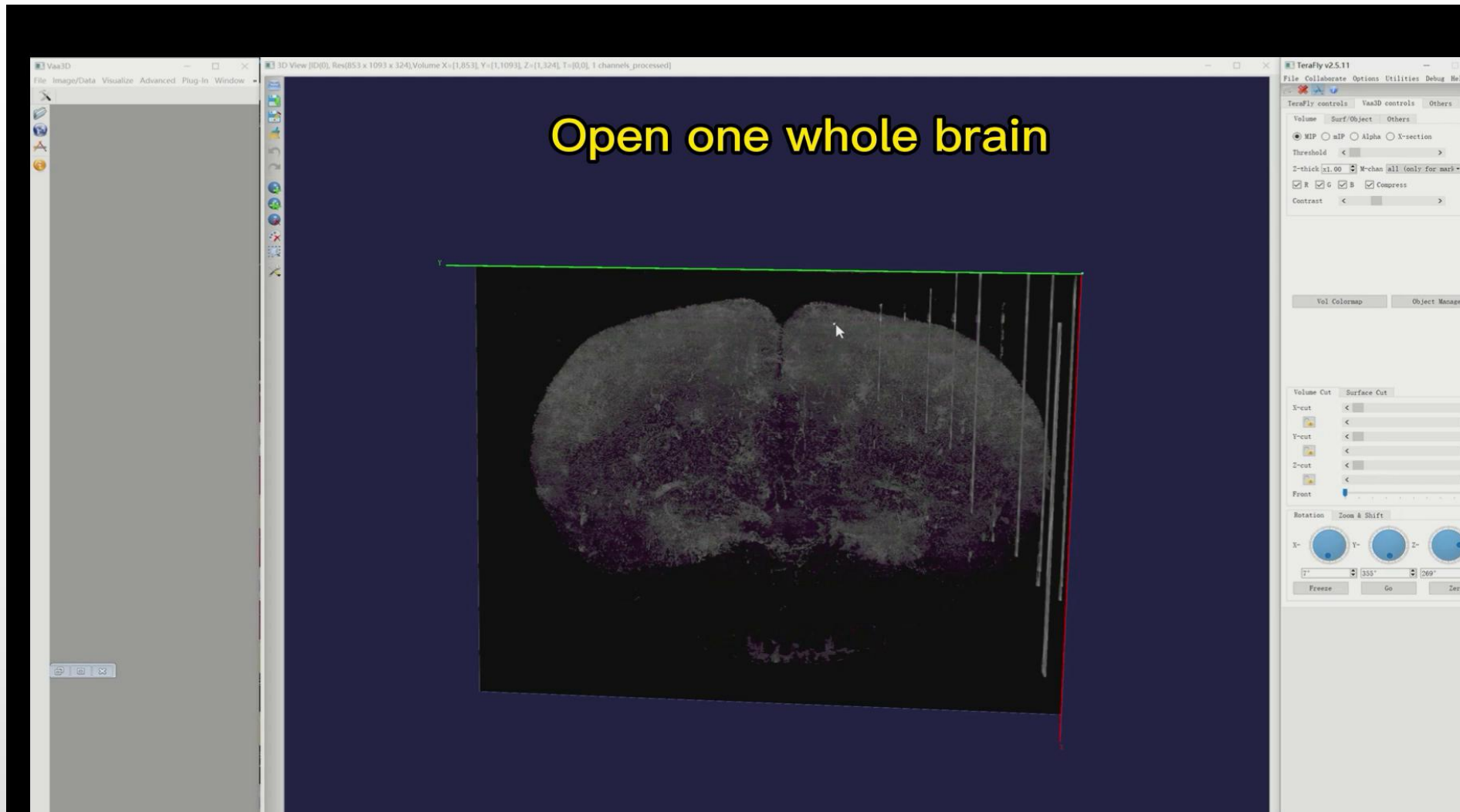
Automated Neuron Tracing in Vaa3D

Apply APP2

Single and Sparse neuron
Not designed for densely
interweaved neurons

**Pay attention
to the meaning
and setting of
various
parameters**

Peng, H. et al. Bioinformatics, 2013.

# Trace the dendrite of neurons in whole brain mode and in real time using App2_terafly

The extension of manual reconstruction of neurons with Vaa3D

- The single and sparse neuron: App2 is preferred

- Complex and fine structures: need to reconstruct manually.

- Vaa3D can not only provide many shortcut function keys for labeling, but also help us achieve a high standard of neuron reconstruction.

These are the useful function keys in the process of recongstrcti on.

| Shortcut function keys	Function name	Function key	Function name
Alt-B	Line segment drawing	Shift	Area segment deletion
Alt-D	Segment deletion		Region segment color transformation
Alt-S	Segment cutting	Q	Maker shortcut tag
Alt-T	Segment color transformation	G	Automatic drawing of line segments(GD)
Alt-G	Global segment drawing	S	Hide line segments
Alt-C	Multi segment direct connection	…….	
Alt-N	All connected segments are highlighted		
Ctrl-Q	Automatic tracking marker		

How to use Vaa3D-TeraFly platform to reconstruct neurons manually .
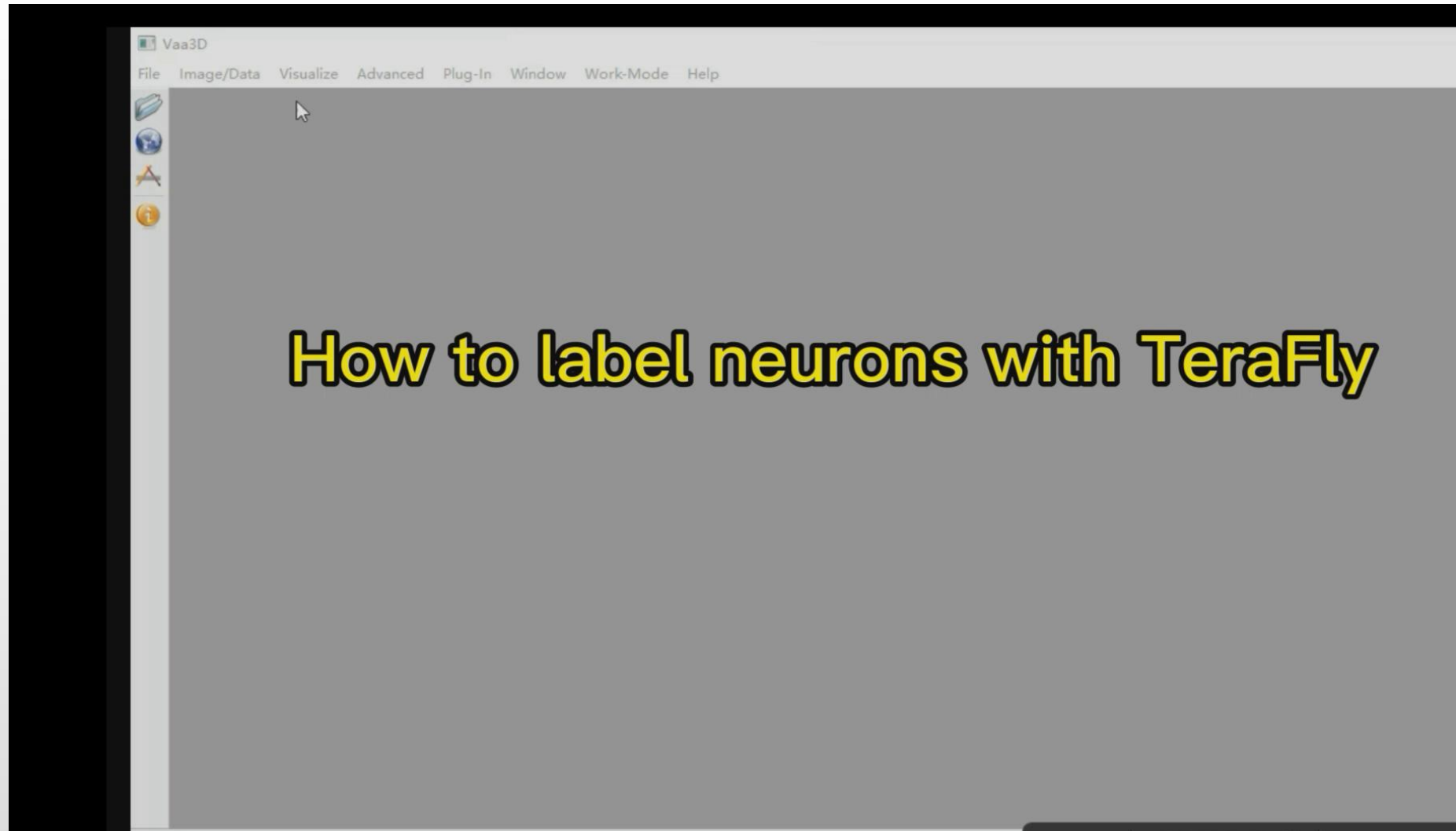
Inspected and modified by multiple annotators

An iterative reconstruction workflow

●The manual reconstruction process is divided into Level-1and Level-2.

●Multiple collaborative 'reconstruction-checking-correction' cycles workflow.
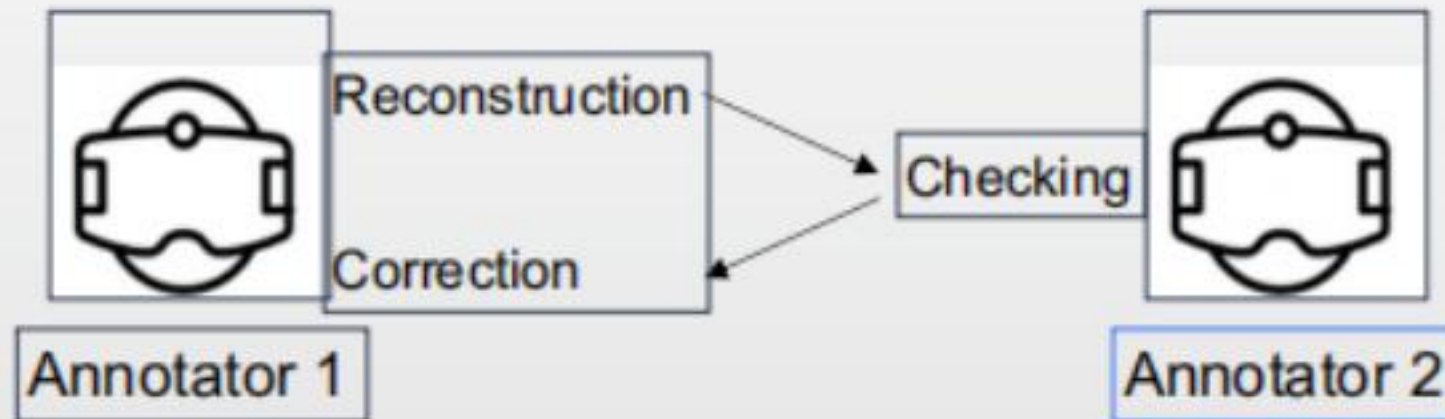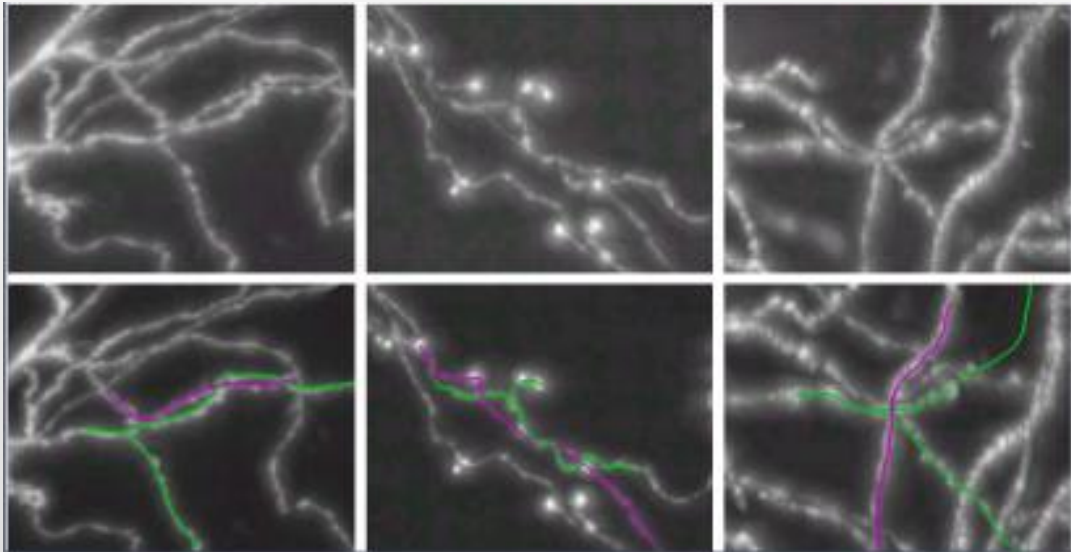

help to ensure the
accuracy of the results

Assistance of VR annotation platform

In case of complex signals, the following tools are more helpful:

● Tera_VR:can be used in visualization and annotation

● VR_Farm:can be used in collaborative annotation and editing

Complex signals

VR_Farm platform

TeraVR: Nature Communications, 2019

A pipeline for automatic post-processing

- 1.Soma correction

- 2.Duplicates removal

- 3.Tiny branch pruning

- 4.Scaling

- 5.Resampling

- 6.Sorting

- 7.Multifurcation correction

- 8.Retyping

Choose the node with the highest degree and set as soma.
Its node type=1 and degree>1

Extra tiny branches can be created when connecting segments.
We found branches with length<10(voxelsize) are mostly artificial.

After the reconstruction steps above are completed,
there are batch inspection and screening of automatic post-processing.

A pipe line for automatic post-processing

- 1.Soma correction

- 2.Duplicates removal

- 3.Tiny branch pruning

- 4.Scaling

- 5.Resampling

- 6.Sorting

- 7.Multifurcation correction

- 8.Retyping

SWC nodes are resampled at a step size of ~2 micron. We found this step size can retain the curvature of branches.

The following errors should be identified:
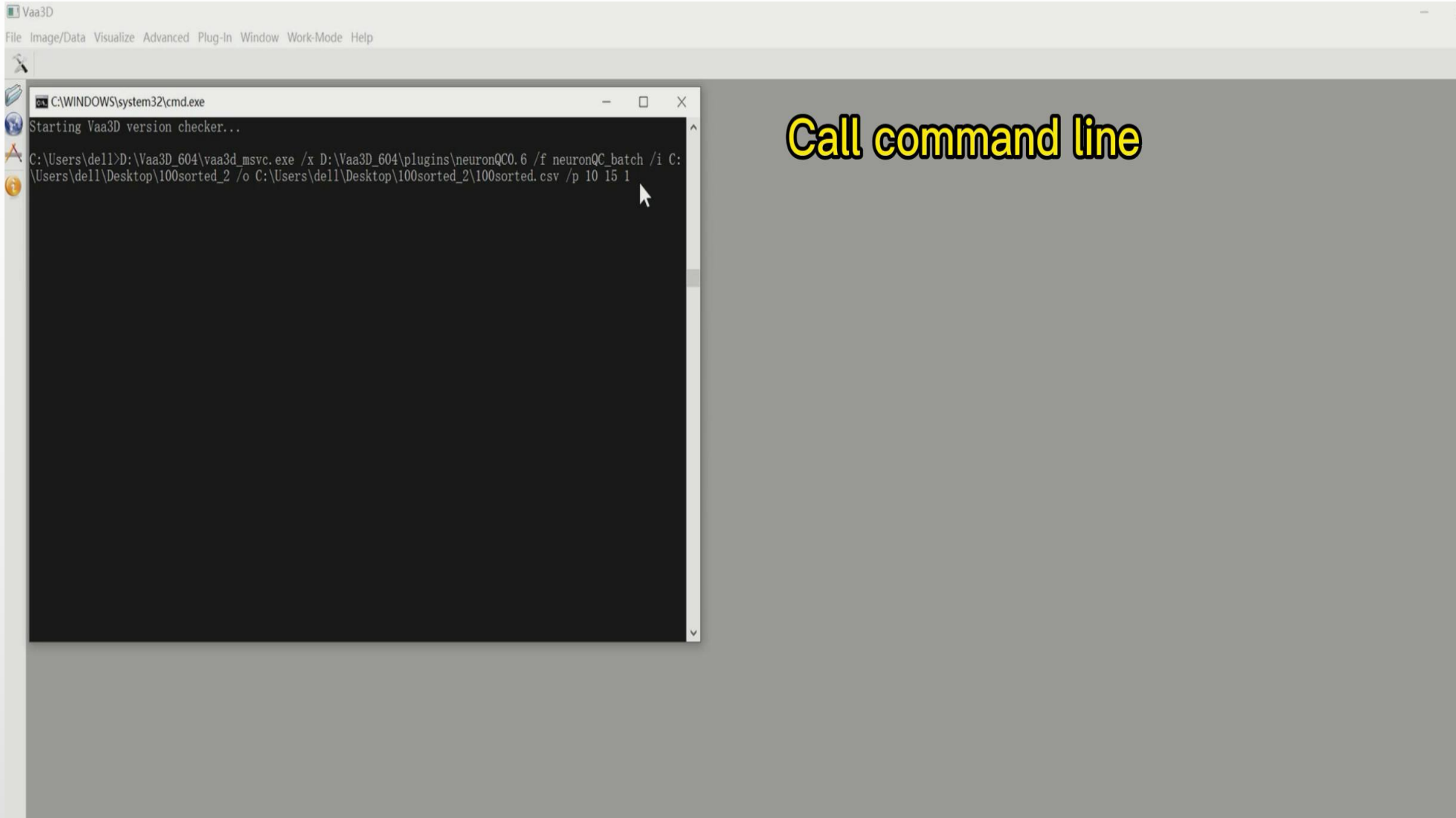More than one node with type=1
There are nodes with types other than 1/2/3/4

How to correct:
Trace back from nodes with errors and determine the type of the branch.
Correct the node types.

Batch inspection and screening of automatic post-processing

Call command line

Call plug in "neuron QC batch"

Get batch results quickly

According to the result,
we need to check and modify the reconstruction again until all results are correct.

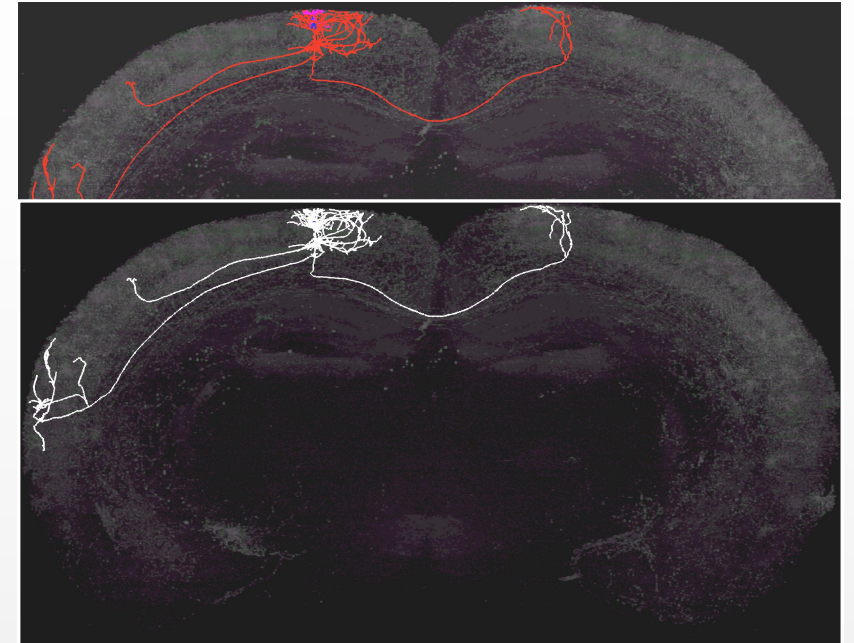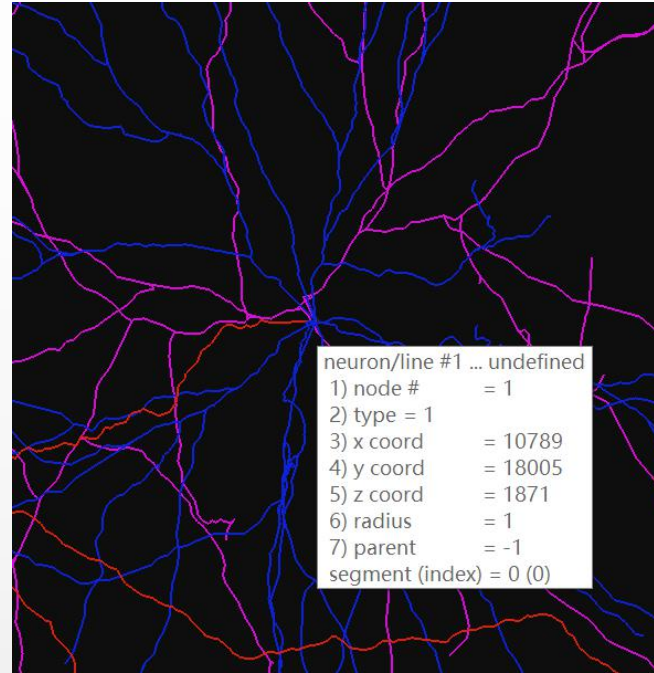# The quality standards of neuronal reconstructions

➤ A connected single tree:

● Soma defined as root of the tree

● No floating nodes or segments

● No loop

➤ Accuracy of reconstruction:

● No missing branches

● No mis-connections

● No extra segments

➤ Data formatting:

● No redundant/ duplicated nodes

● Evenly spaced child-parent nodes

● Correct node type assignment

neuron/line #1 ... undefined
1) node #   = 1
2) type = 1
3) x coord   = 10789
4) y coord   = 18005
5) z coord   = 1871
6) radius   = 1
7) parent   = -1
segment (index) = 0 (0)

redundant branch

triple bifurcation

With the helps of Automated Neuron Tracer:

APP2

Neural reconstruction platforms based on Vaa3D

TeraFly

TeraVR

Hi5

VR_Farm

.....
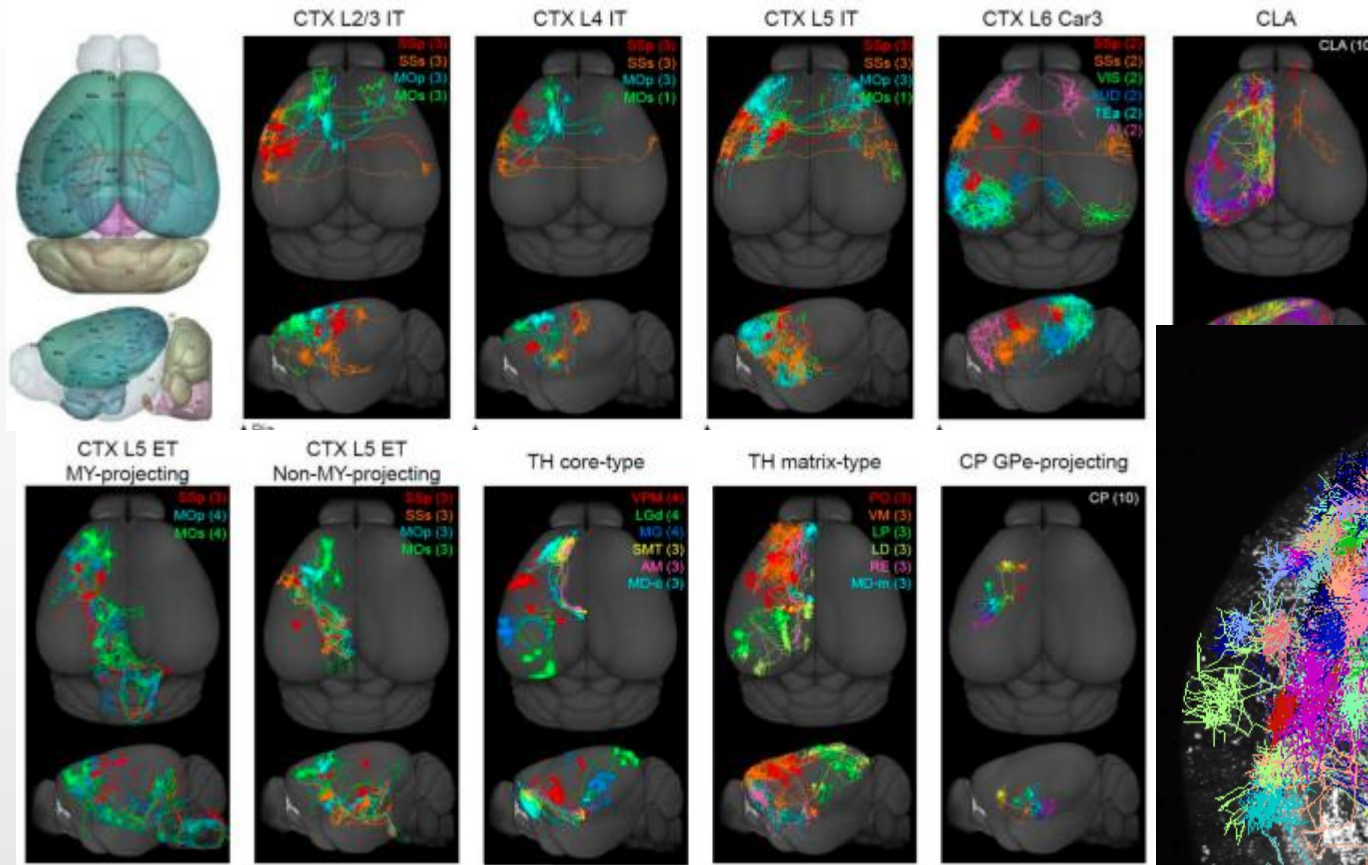
Inspected and modified by multiple annotators
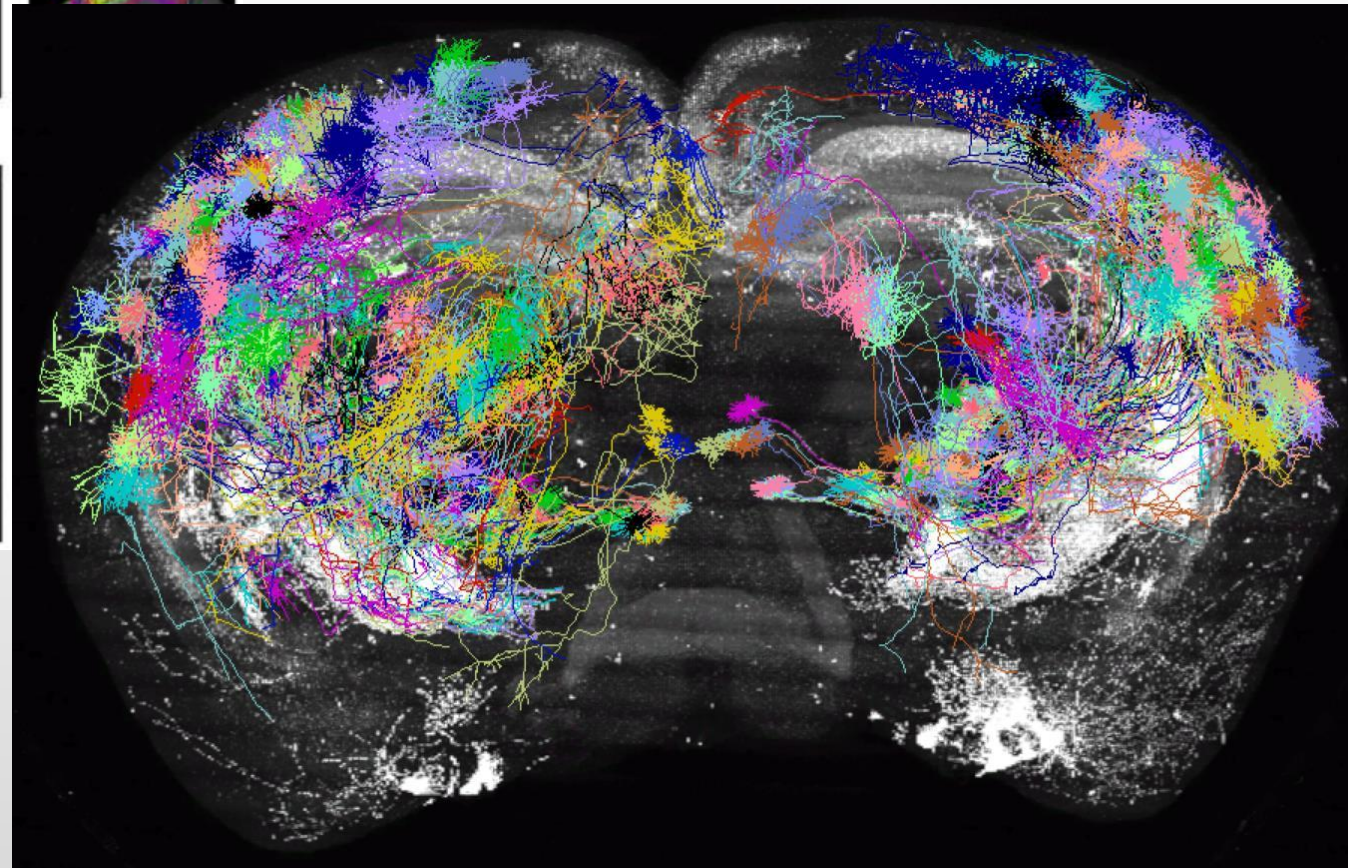
Automatic post-processing

Efficient whole-brain morphology reconstruction utilizing Vaa3D

All individual neuron reconstructions were registered to CCFv3

H. Peng, P. Xie, L. Liu, et al. Nature, 2021,

Video demo summary

1. App2_terafly track the dendrites of neurons in whole brain mode and in real time.
2. How to use Vaa3D-TeraFly platform to reconstruct neurons manually.
3. Batch inspection and screening of automatic post-processing.

Thank you very much!