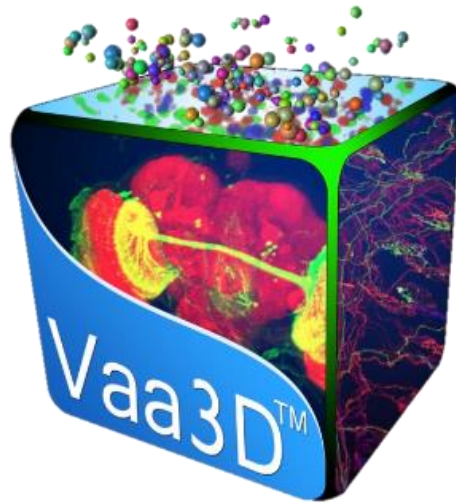


# How to apply App2 for Automated Neuron Tracing in Vaa3D?



Presenter: Liya Ding

Institute for Brain and Intelligence

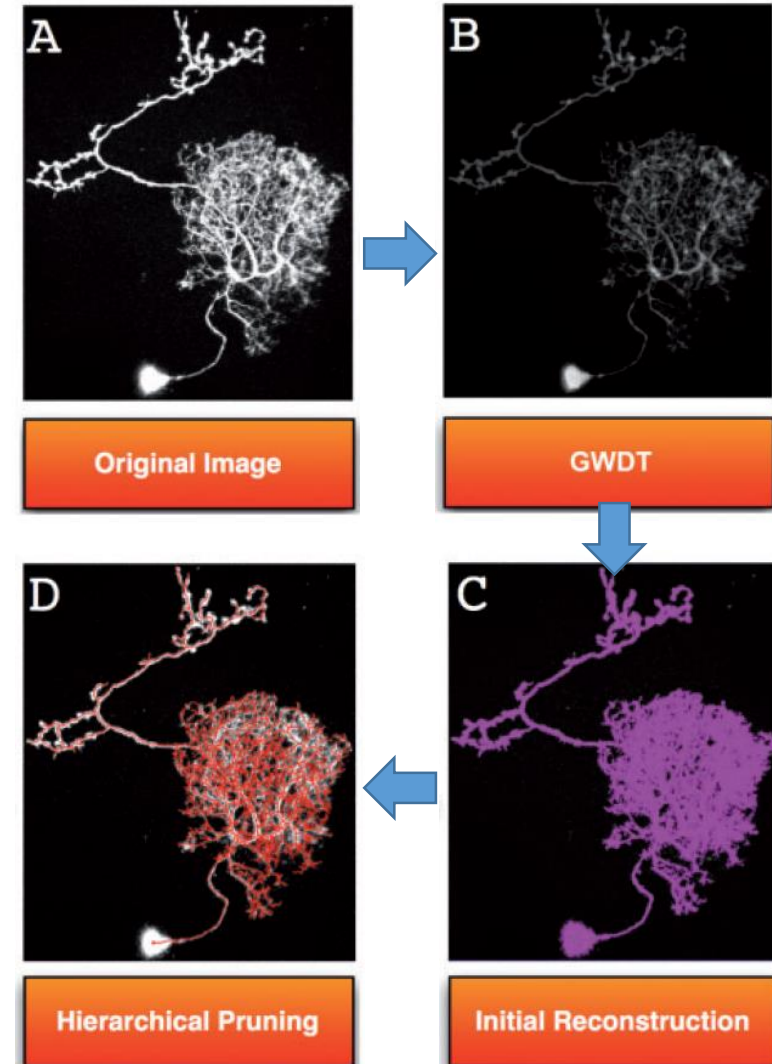
Southeast University

# Why App2?

- Manual neuron tracing is tedious
- Need for big data
- Vaa3D-App2 for automated neuron reconstruction
  - Fast
  - Easy to use
  - Superior accuracy

# APP2 Intro

- APP2 = All Path Pruning 2.0
- Paper:  
“APP2: automatic tracing of 3D neuron morphology based on hierarchical pruning of gray-weighted image distance-trees”, Xiao, H., and Peng, H., *Bioinformatics*, 2013.
- Steps:
  1. GWDT: gray-weighted image distance transform
  2. Initial neuron reconstruction
  3. Hierarchical pruning



# Get App2

- Download Vaa3D binary release

<https://github.com/Vaa3D/release/releases/tag/v3.601>

Run vaa3d\_msvc.exe in unzipped folder.

- Images used in this talk include:
  - Four FMost test image blocks from AIBS and HUST
  - Two test images courtesy of Hongwei's lab  
(not included in emails)

# Where is App2 in Vaa3D interface?

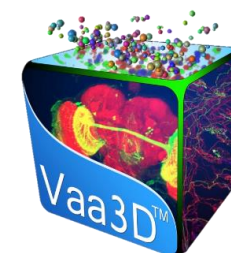
Vaa3D

File Image/Data Visualize Advanced **Plug-In** Window Work-Mode Help

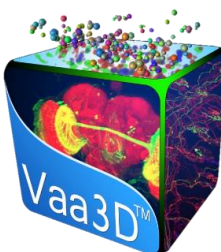
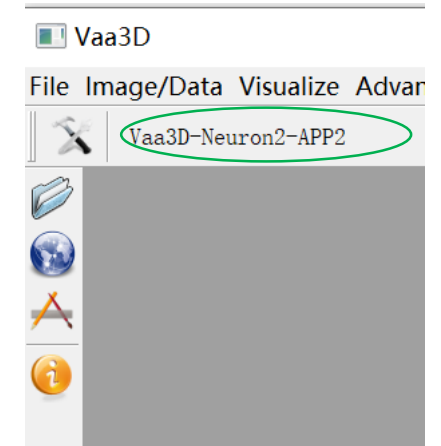
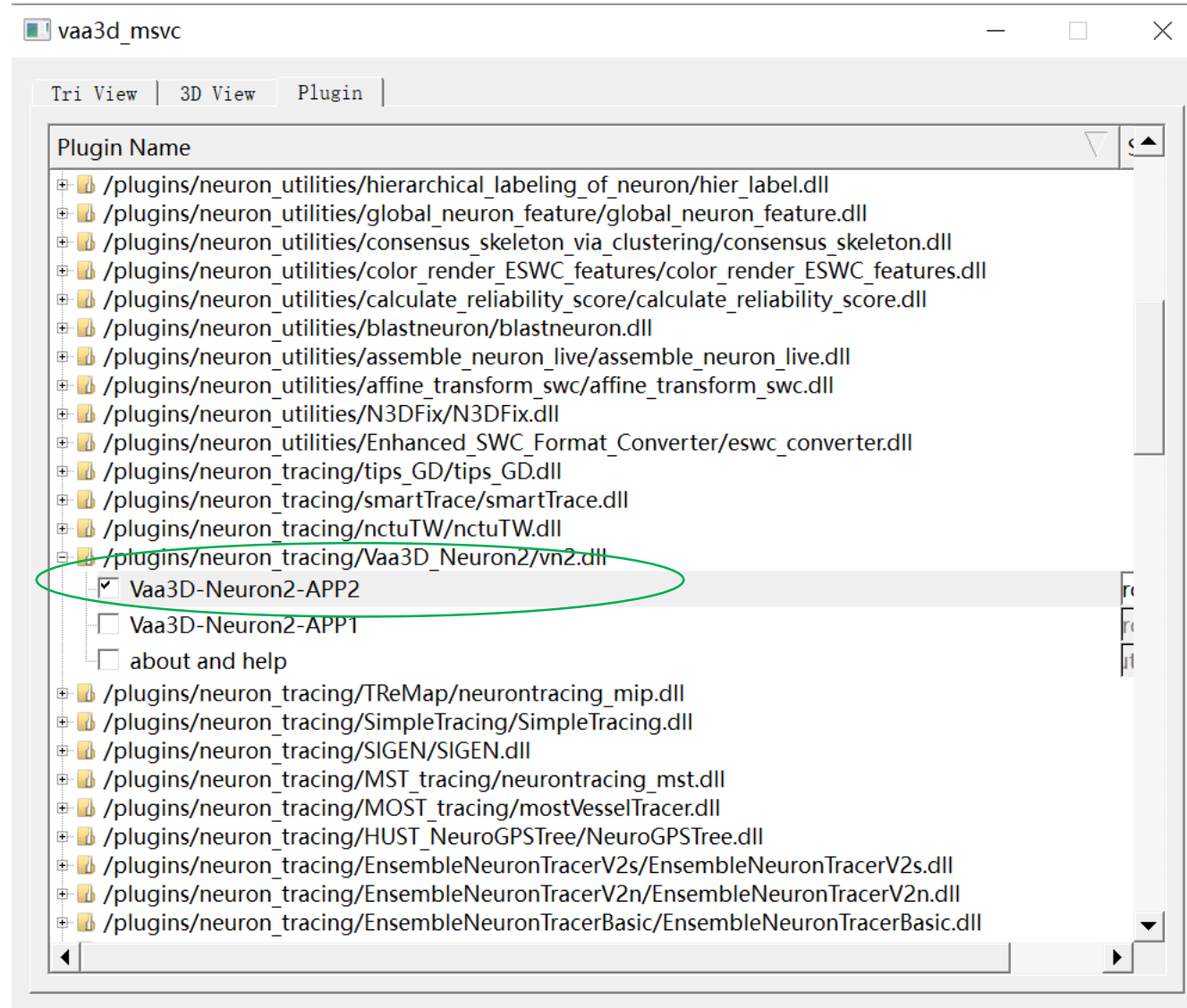
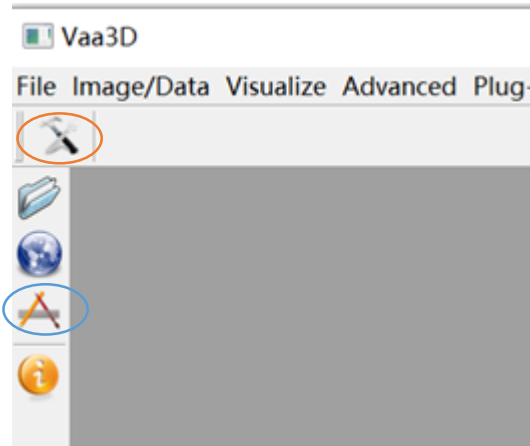
- Plug-in manager
- Re-scan all plugins
- Recently used plugins
- Most used plugins
- Clear used plugins history
- \_Vaa3D\_plugin\_creator
- AllenApps
- celegans
- cell\_counting
- color\_channel
- data\_IO
- detect\_type
- dynamicApp2
- FlyWorkstation\_utilities
- image\_analysis
- image\_blending
- image\_edge\_detection
- image\_filters
- image\_geometry
- image\_projection
- image\_registration
- image\_resolution
- image\_ROI
- image\_segmentation
- image\_thresholding
- linker\_file
- marker\_utilities
- misc
- movies\_and\_snapshots
- neuron\_stitch
- neuron\_toolbox
- neuron\_tracing
- neuron\_utilities
- pixel\_intensity
- shape\_analysis
- synapse\_detector
- Sync\_Views
- Vaa3D\_PluginInterface\_Demos
- show\_markers
- about

- Advantra
- aVaaTrace3D
- BJUT\_fastmarching\_spanningtree
- BJUT\_meanshift
- CWlab\_method1\_version1
- EnsembleNeuronTracerBasic
- EnsembleNeuronTracerV2n
- EnsembleNeuronTracerV2s
- ENT
- HUST\_NeuroGPSTree
- LCM\_boost
- MOST\_tracing
- MST\_tracing
- nctuTW
- NeuronChaser
- NeuroStalker
- neutu\_autotrace
- neuTube
- PSF\_tracing
- RegMST
- Rivulet2
- segment\_maker
- SIGEN
- SimpleAxisAnalyzer
- SimpleTracing
- smartTrace
- Soma\_OC
- tips\_GD
- TReMap
- ultratracer
- Vaa3D-FarSight\_snake\_tracing
- Vaa3D\_Neuron1
- Vaa3D\_Neuron2

Vaa3D-Neuron2-APP2  
Vaa3D-Neuron2-APP1  
about and help



# Add shortcut for App2



# Load image into Vaa3D

app2\_test\_images

名称	日期	类型
test1.tiff	2021/7/16	
test2.tiff	2021/7/16 20:30	TIFF 文件
test3.tiff	2021/7/16 20:33	TIFF 文件
test4_original.tiff	2021/7/16 17:29	TIFF 文件
test4_preprocesse...	2021/7/16 17:46	TIFF 文件

Vaa3D  
File Image/Data Visualize Advanced Plug-In Window Work-Mode Help

Vaa3D-Neuron2-APP2

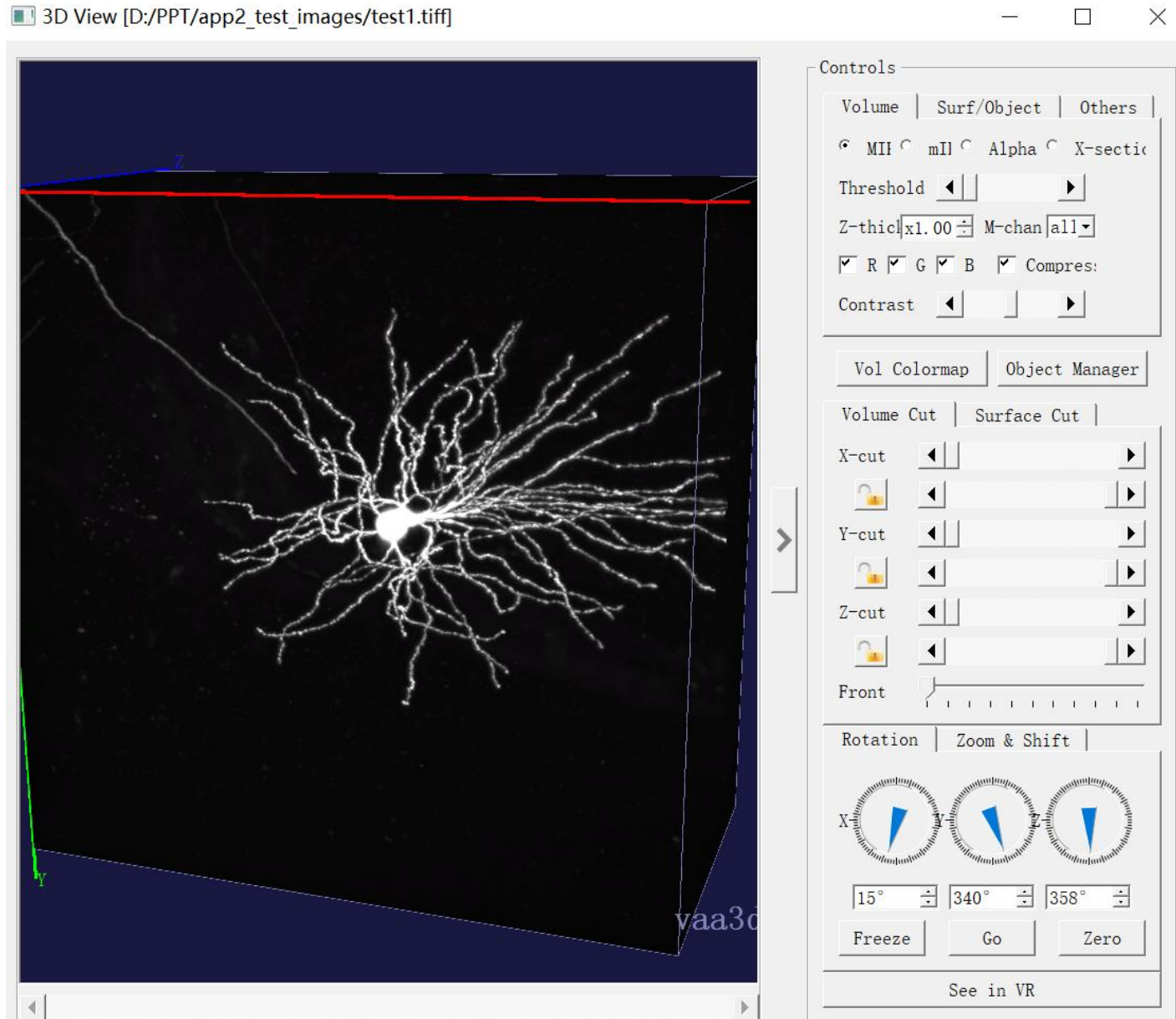
D:/PPT/app2\_test\_images/test1.tiff

Image data  
Views [XY: upper-left] [ZY: upper-right] [XZ: lower-left]

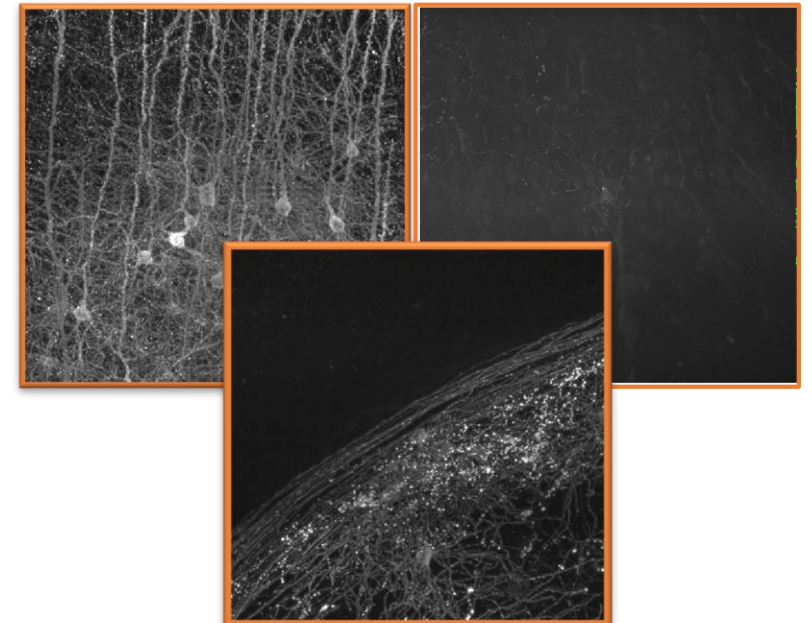
Information of your selections  
Voxel type: UINT8, Tri view zoom: 1  
Focus: (256, 256, 128) RGB = (255, 0, 0)  
Channel min/max: C1 [min=2, max=255]:  
Defined marker location.

Options  
Focus Coordinates  
Z 128  
X 256  
Y 256  
Zoom (Regular x1/4^x8, Lookir  
XY-plane  
ZY-plane  
XZ-plane  
Channels (1) Intensi  
#ffffff c1  
Landmark controls  
Copy Paste Load Save  
landmark/Atlas/Color Manage  
See in 3D  
Help ...

# What App2 is designed for?

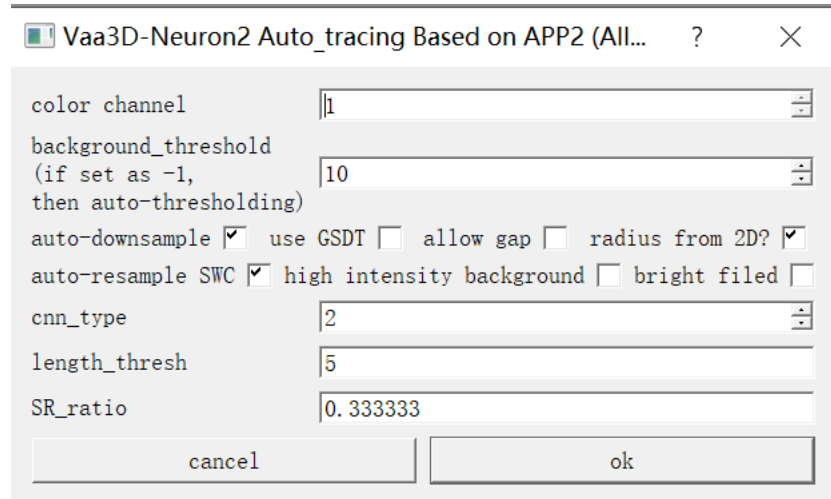


- Base tracer
  - Base tracer vs. UltraTracer
- Single and Sparse neuron
  - Not designed for densely interweaved neurons
- With reasonable SNR
  - In 8 bit, fiber visible for human eyes
- Samples not suitable for App2:

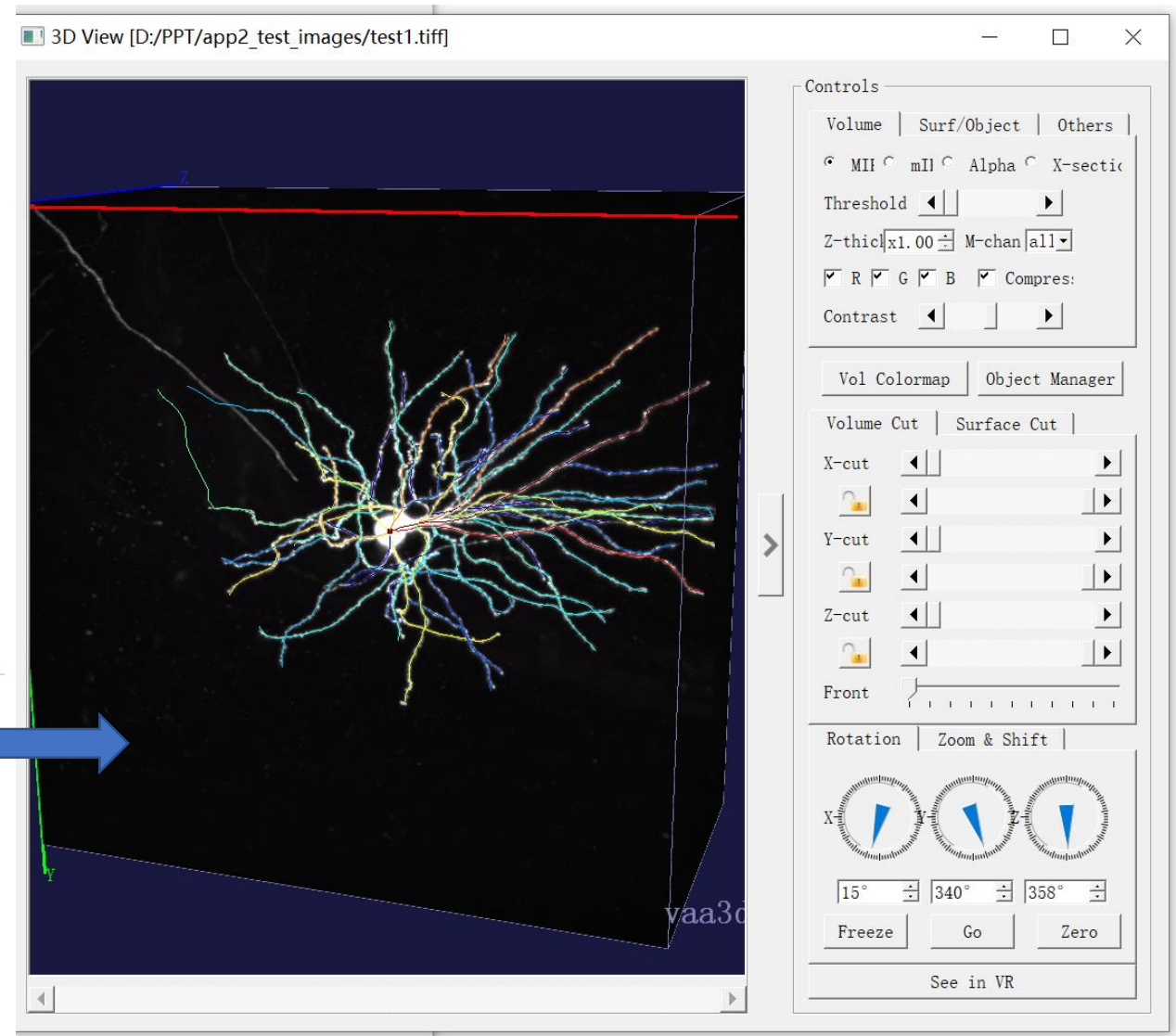




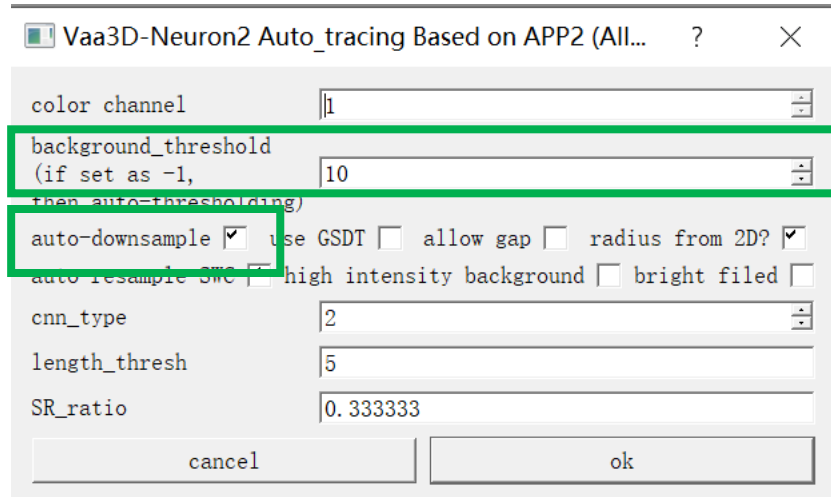
# App2 Interface with default parameters



- test1.tiff
- test1.tiff\_ini.swc
- test1.tiff\_x256\_y258\_z130\_app2.swc
- test2.tiff
- test3.tiff
- test4\_original.tiff
- test4\_preprocessed.tiff




# App2 Parameters

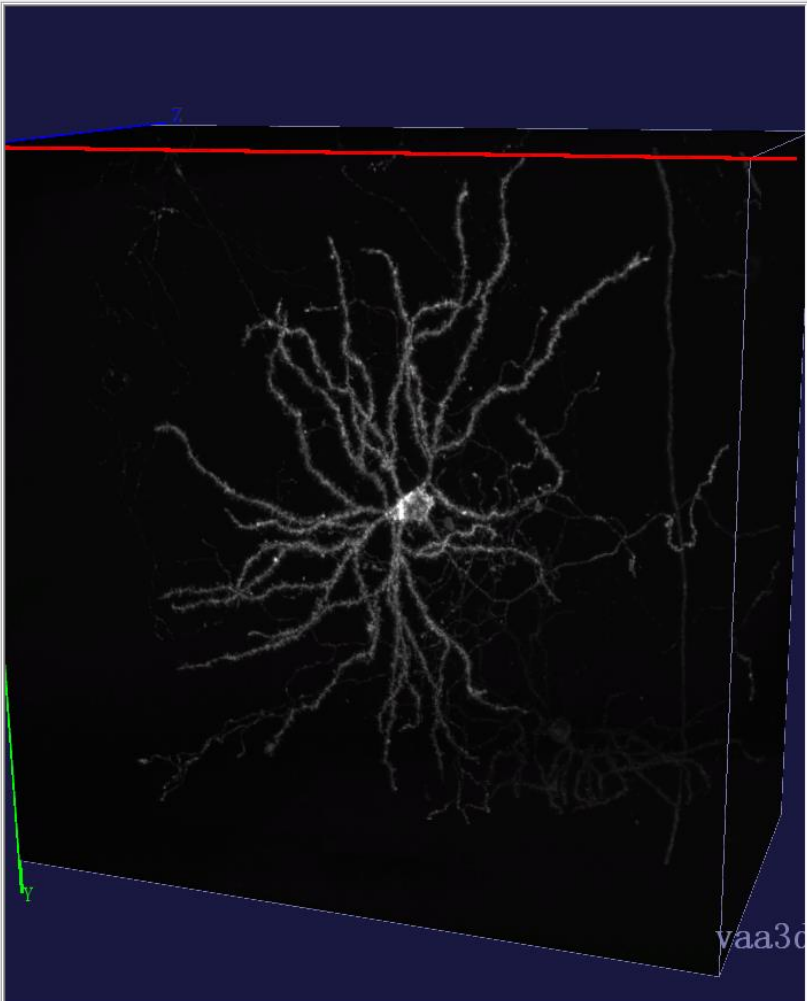


- First of all, although we allow adjustment of a few parameters, you can just use default setting and achieve reasonable results for most cases
- Two key parameters that might need to change

# Second example test2.tiff

- test1.tiff
- test1.tiff\_ini.swc
- test1.tiff\_x256\_y258\_z130\_app2.swc
- test2.tiff 
- test3.tiff
- test4\_original.tiff
- test4\_preprocessed.tiff

3D View [D:/PPT/app2\_test\_images/test2.tiff]



Controls

Volume | Surf/Object | Others

MII | mII | Alpha | X-section

Threshold

Z-thick  x1.00 M-chan  all

R  G  B  Compres:

Contrast

Vol Colormap | Object Manager

Volume Cut | Surface Cut

X-cut

Y-cut

Z-cut

Front

Rotation | Zoom & Shift

X

Y

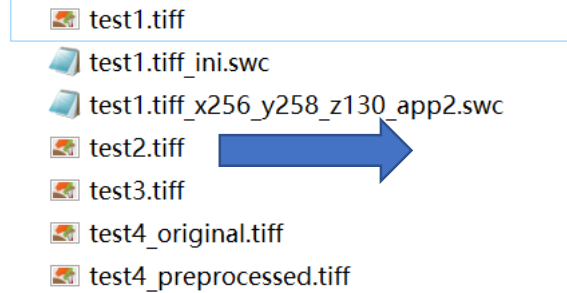
Z

15° 340° 358°

Freeze Go Zero

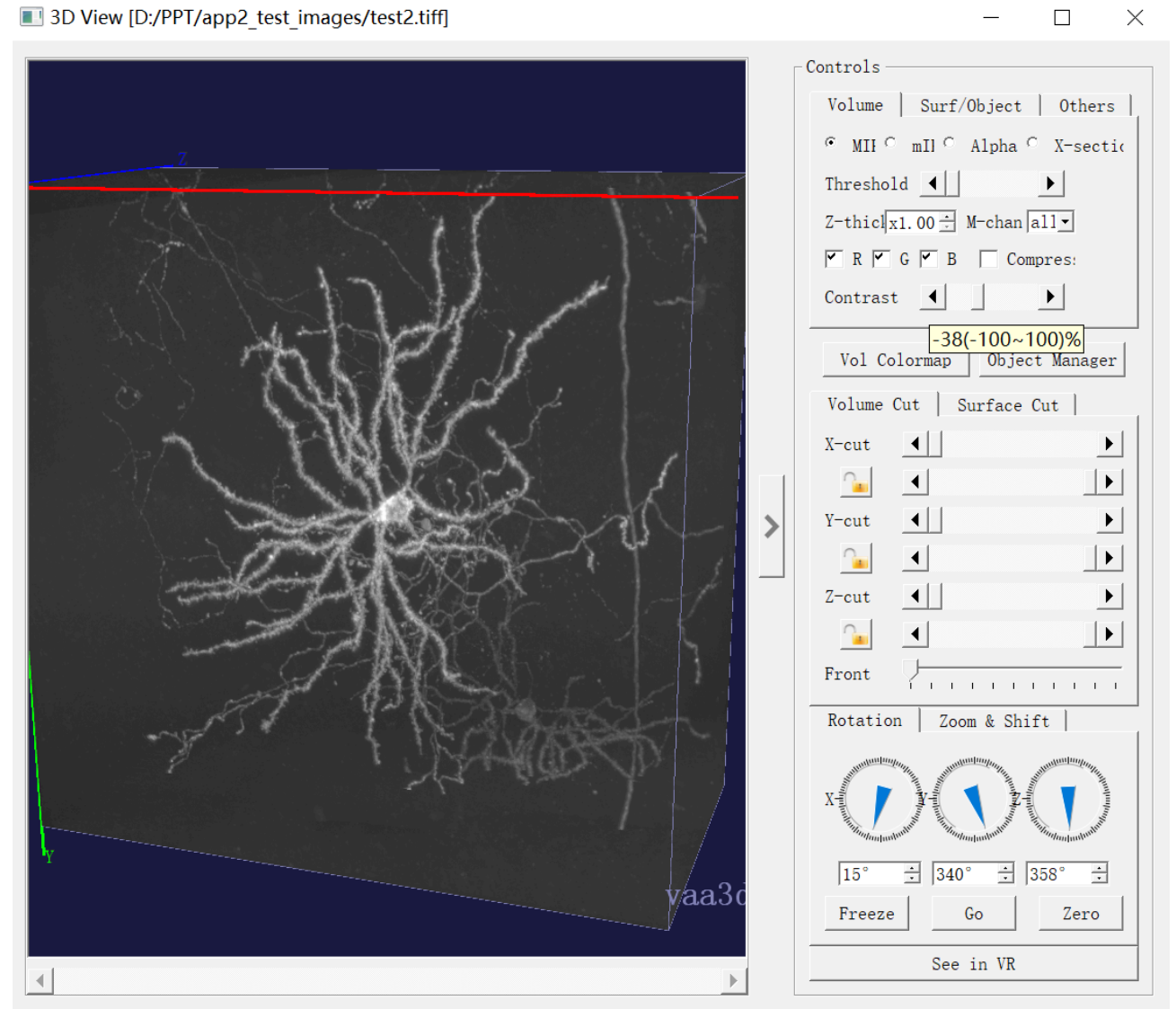
See in VR

# Second example test2.tiff

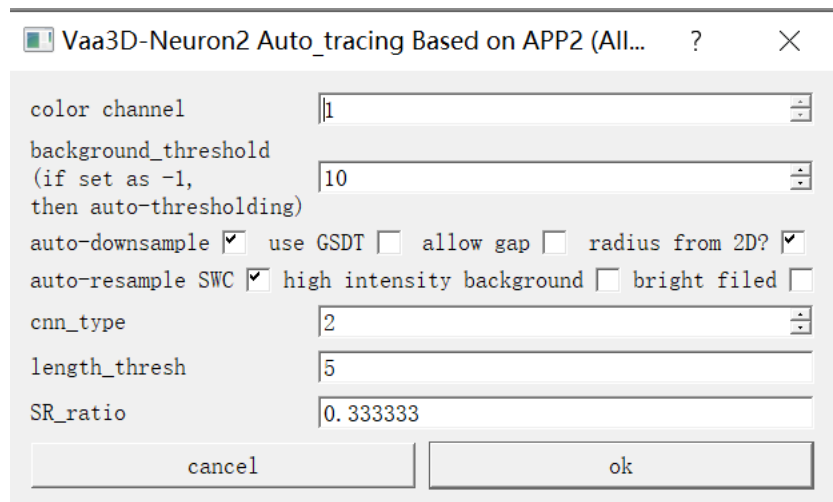


Soma location?

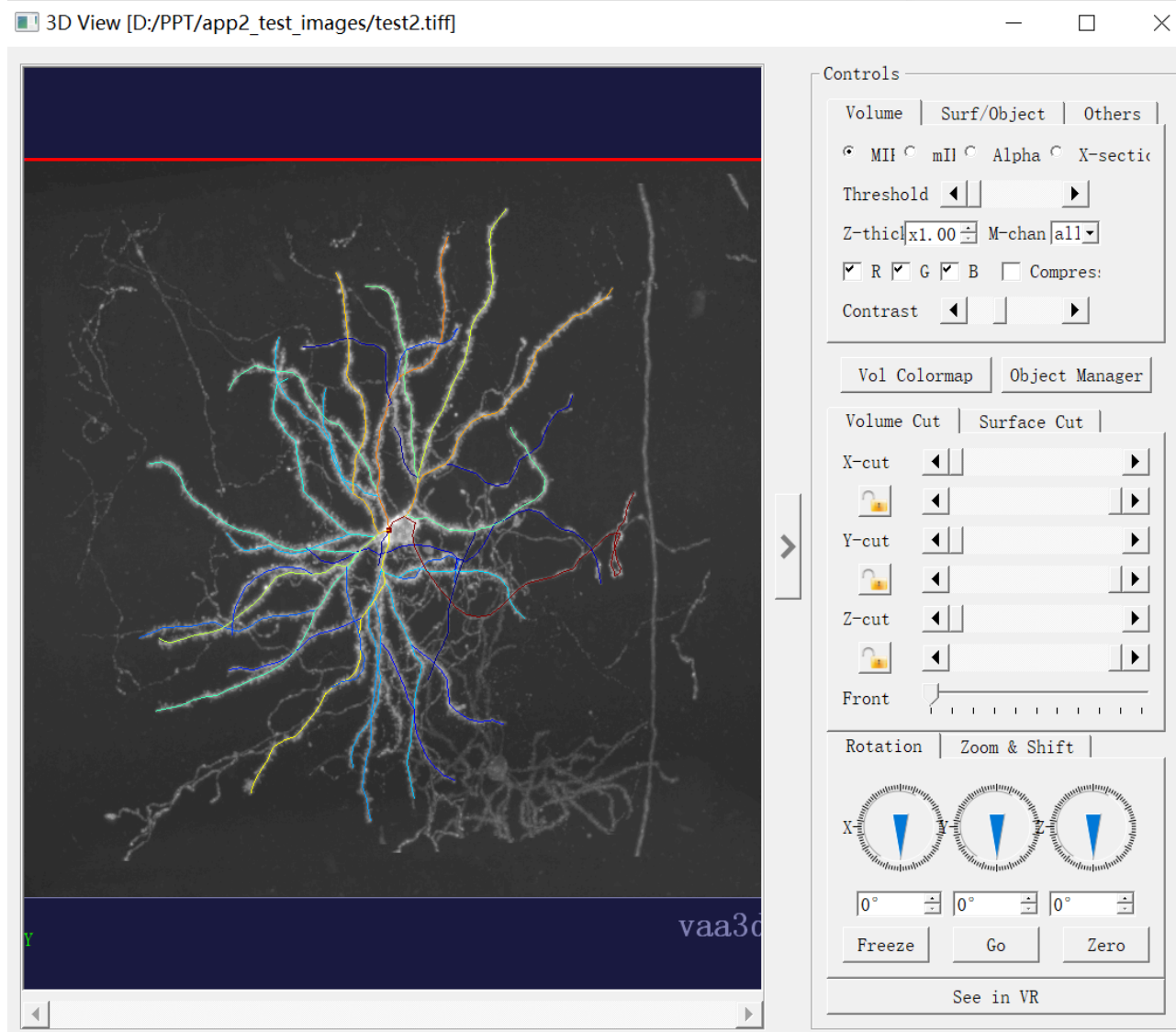
- If not defined, App2 find it automatically
- Or you can define it by adding a marker



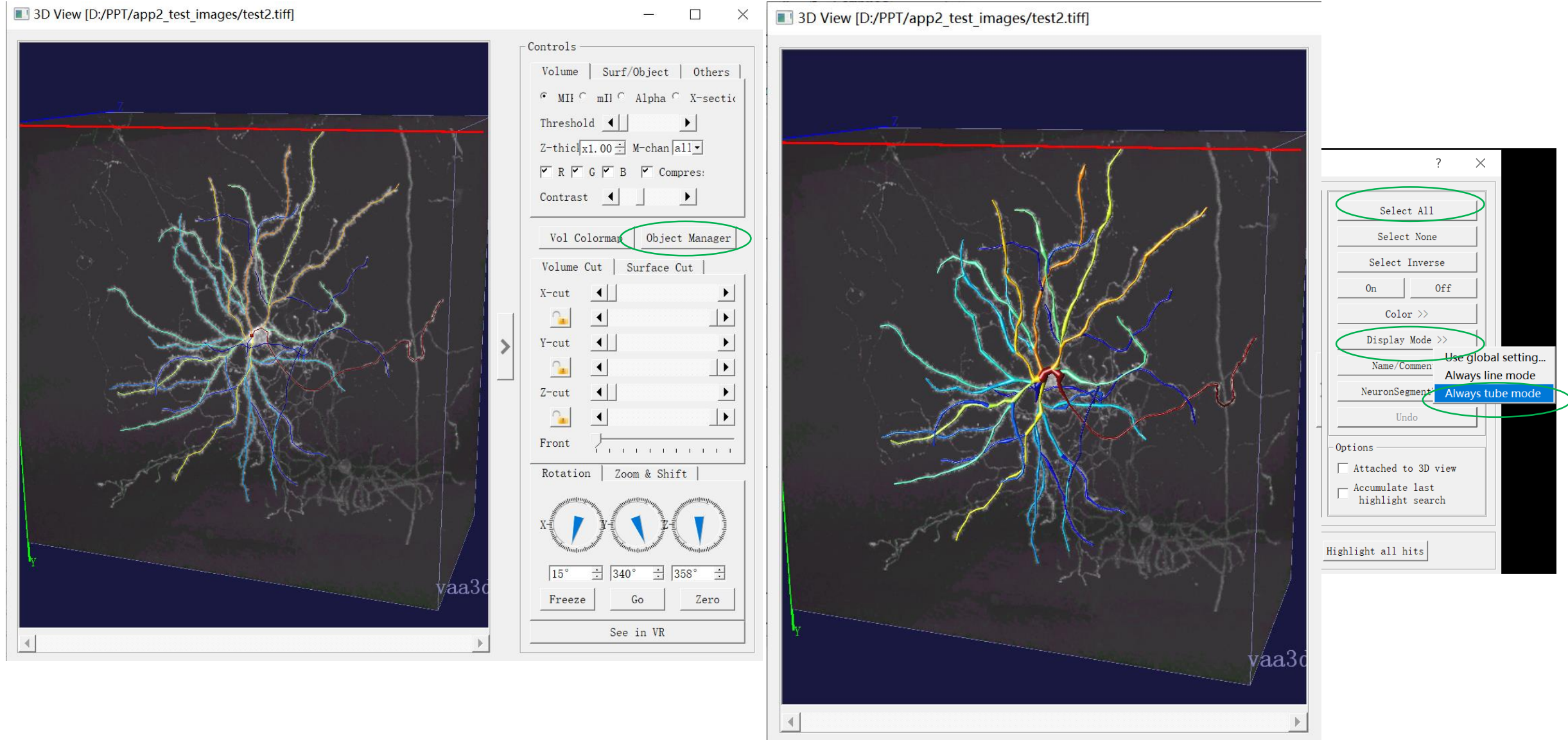
# Test2.tiff ran with App2 using default parameters



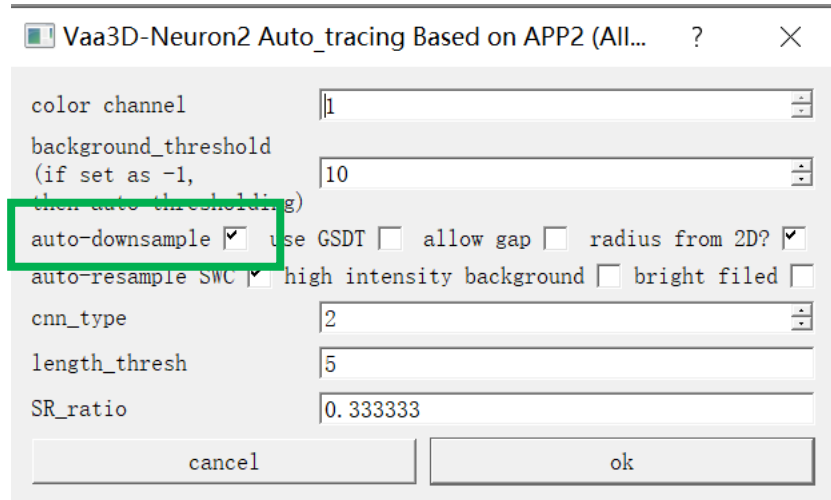
test1.tiff	2021/7/16 20:31
test1.tiff_ini.swc	2021/7/17 19:28
test1.tiff_x256_y258_z130_app2.swc	2021/7/17 19:28
test2.tiff	2021/7/16 20:30
test2.tiff_ini.swc	2021/7/17 19:44
<input checked="" type="checkbox"/> test2.tiff_x252_y256_z129_app2.swc	2021/7/17 19:44
test3.tiff	2021/7/16 20:33
test4_original.tiff	2021/7/16 17:29
test4_preprocessed.tiff	2021/7/16 17:46



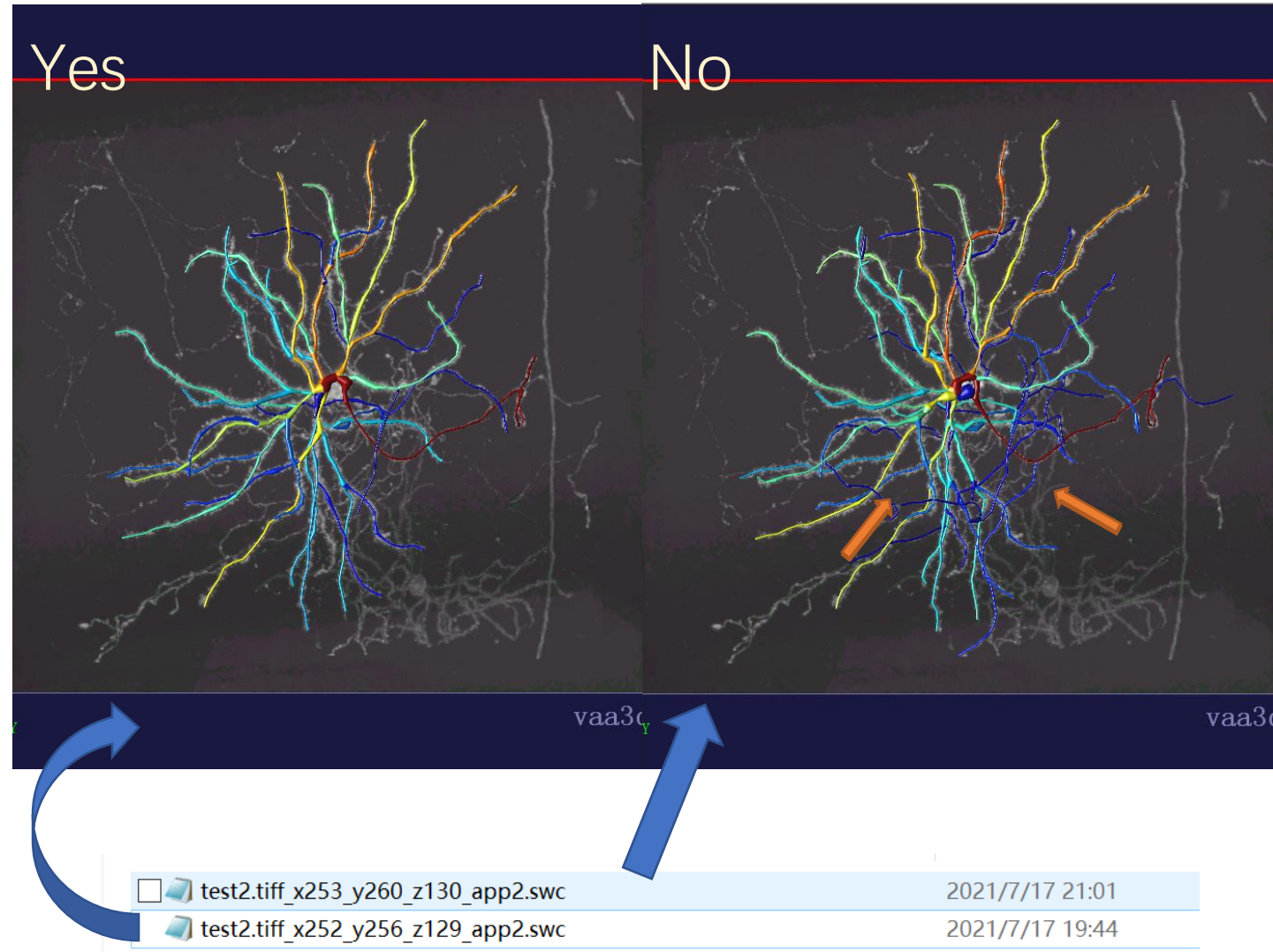
# For display: Line mode vs Tube mode



# App2 Parameters

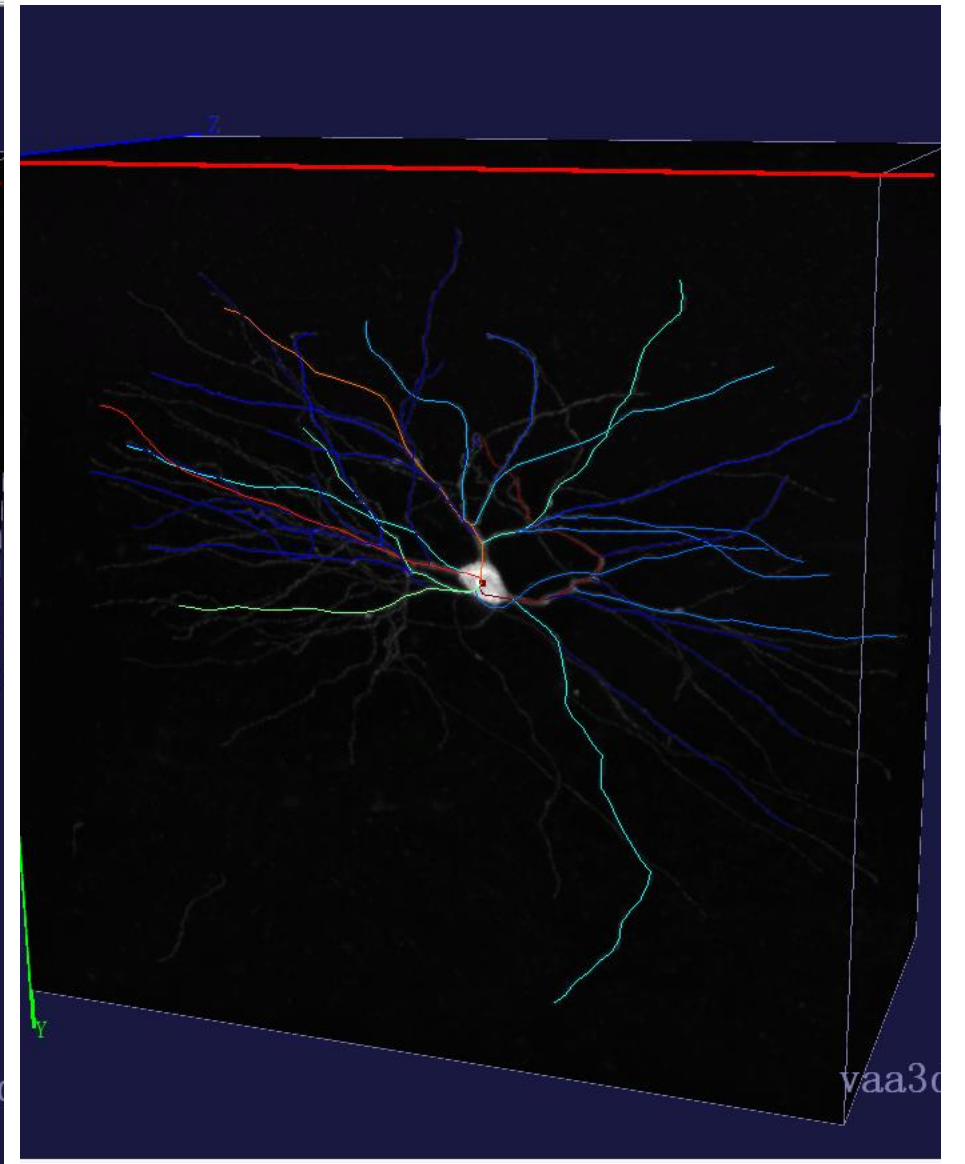
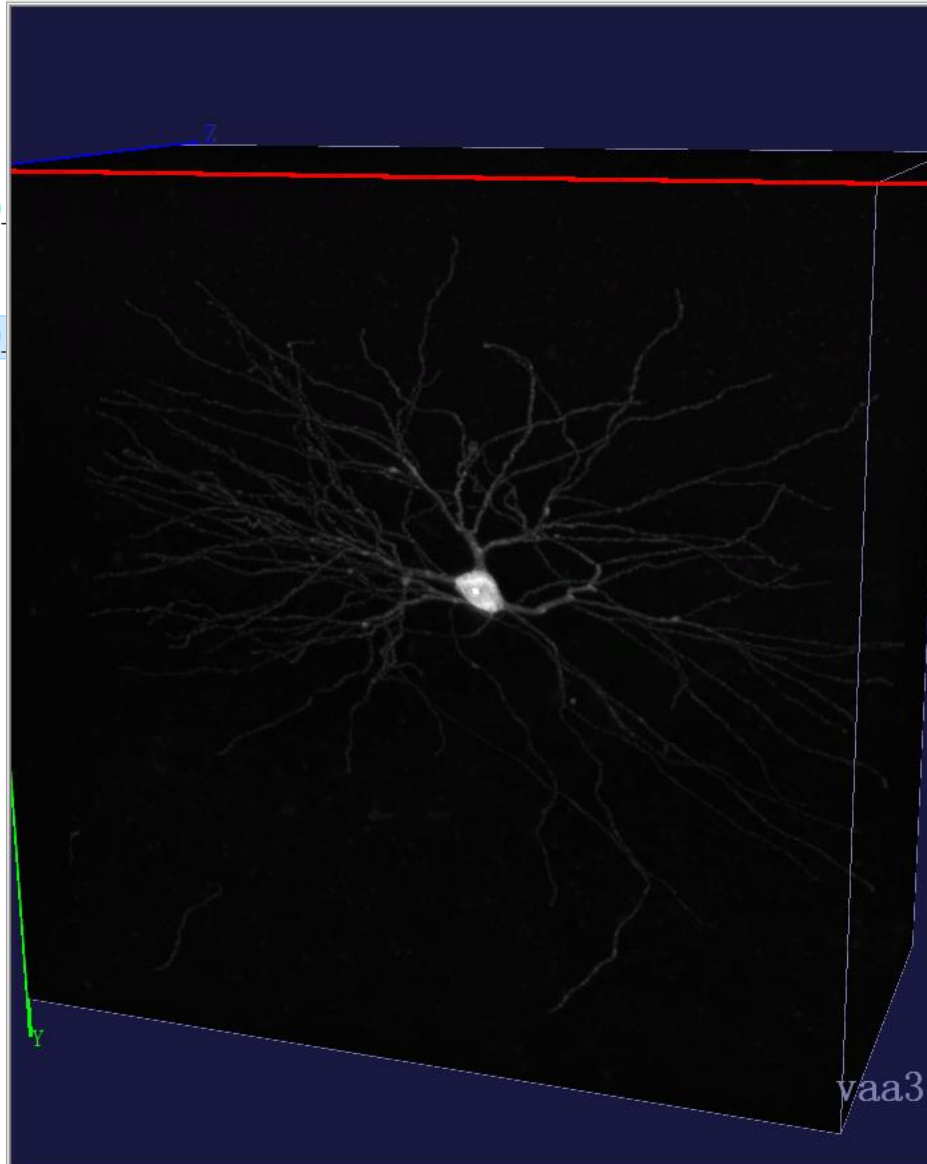


- Auto-downsample
  - Default: Yes
    - Downsample to 256\*256\*256
  - Can change it to No when image is big and detailed resolution needed



# Third example test3.tiff

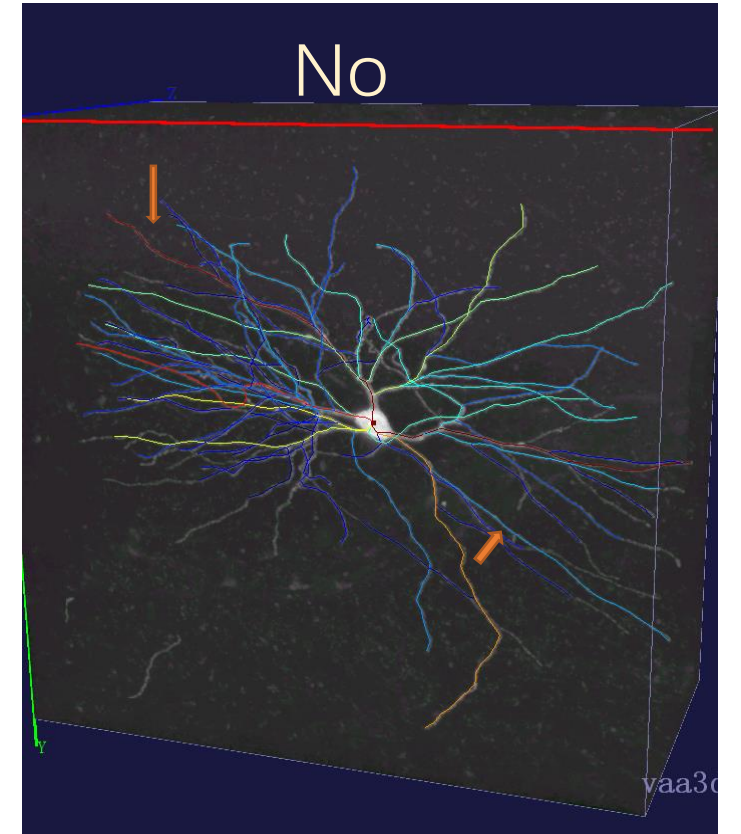
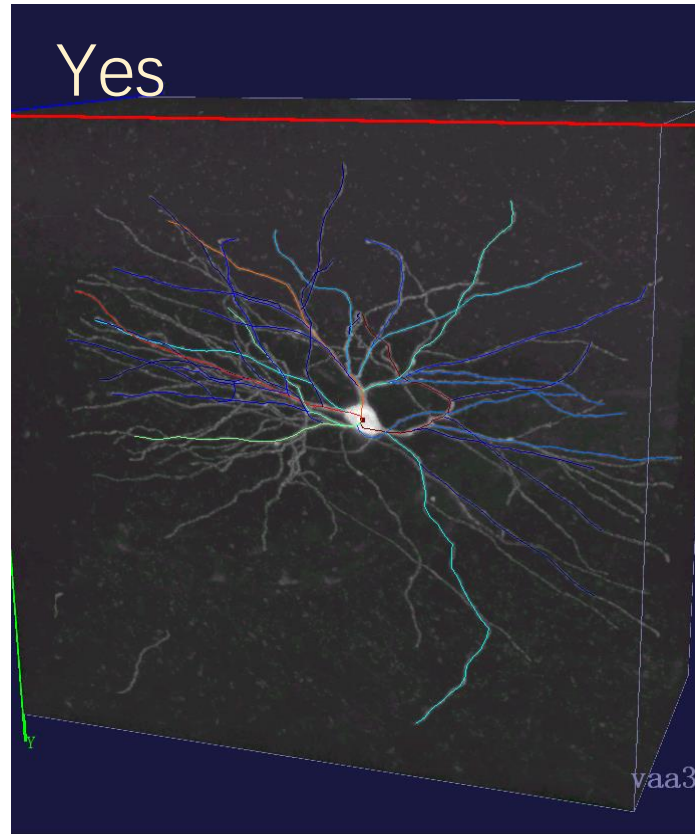
- test1.tiff
- test1.tiff\_ini.swc
- test1.tiff\_x256\_y258\_z130
- test2.tiff
- test2.tiff\_ini.swc
- test2.tiff\_x252\_y256\_z129
- test3.tiff
- test4\_original.tiff
- test4\_preprocessed.tiff



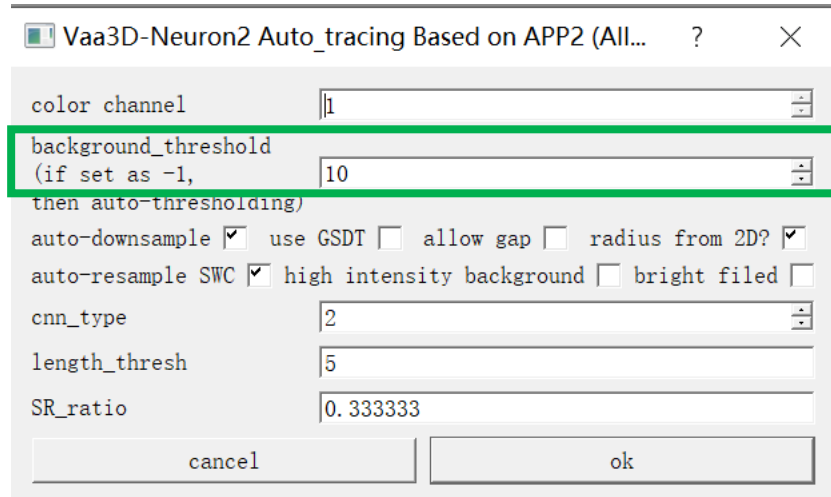


# Auto-downsample

- Auto-downsample
  - Set it to “No” whenever your computer can handle it
  - Set it to “No” for the rest of my talk



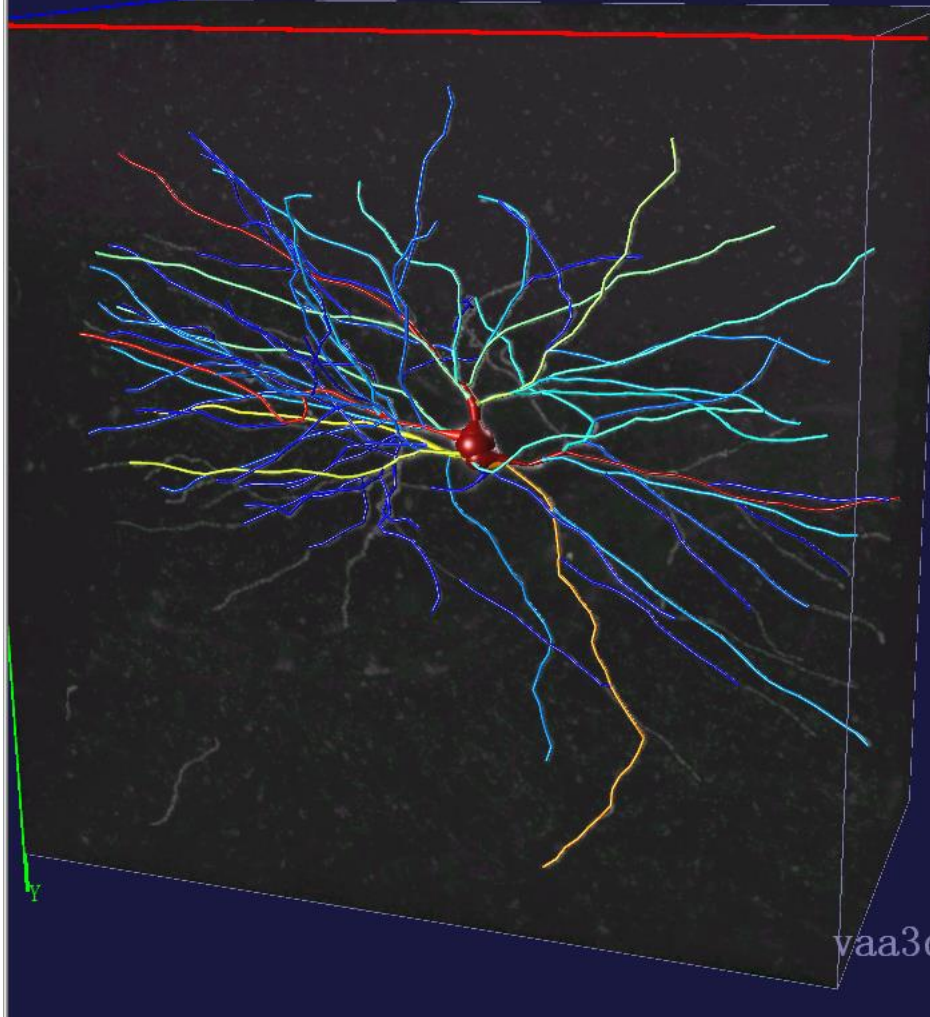
# App2 Parameters: Background threshold



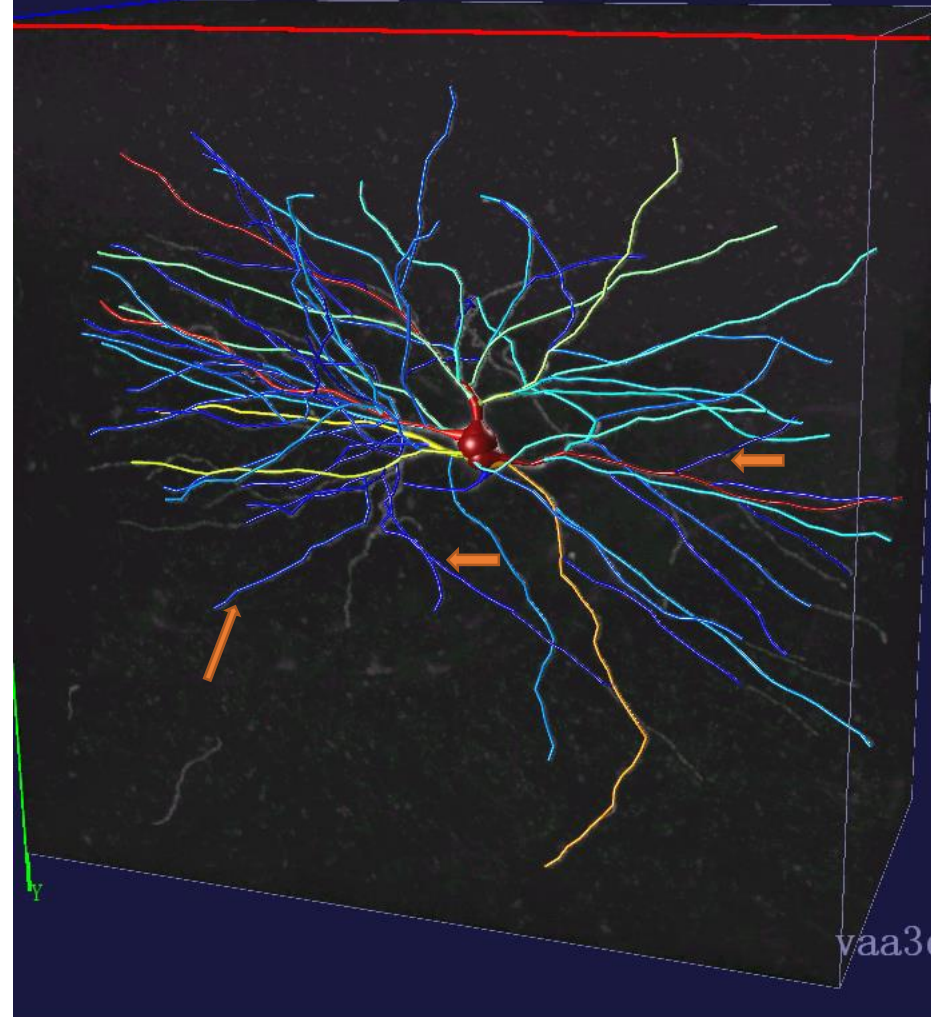
- How it works?
  - With low threshold, App2 generate initial reconstruction and prune away irrelevant segments
- Is it critical?
  - Yes and No
- Background threshold:
  - Default:10
  - Automatic determining threshold: with -1
    - $\text{Mean} + 0.5 * \text{Std}$

# Automatic background threshold

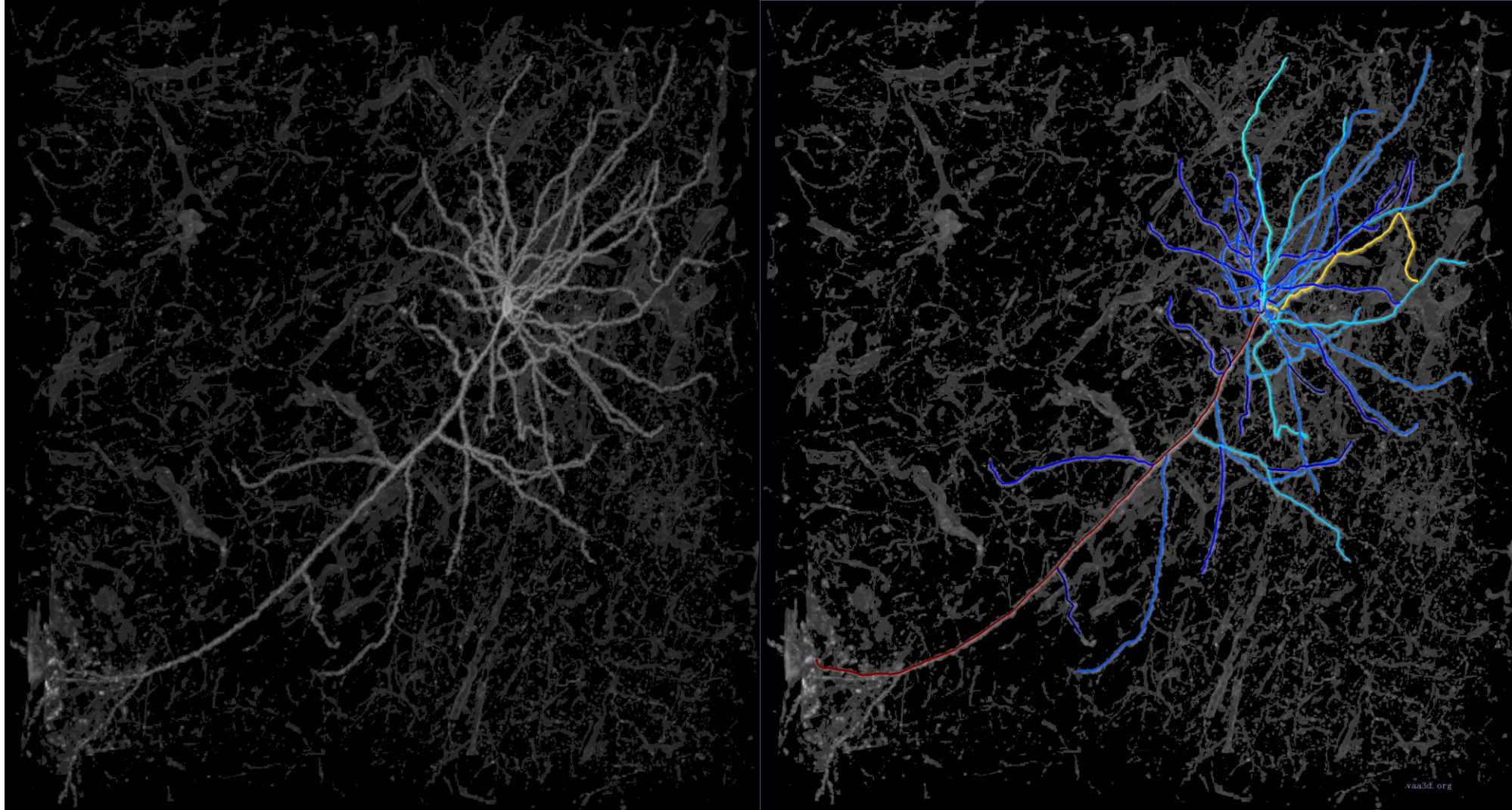
Background Thresh: 10



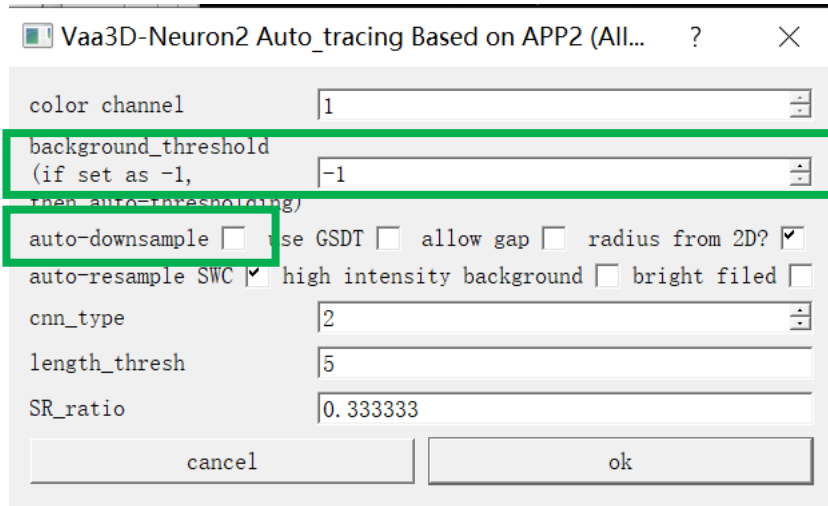
-1(Auto)



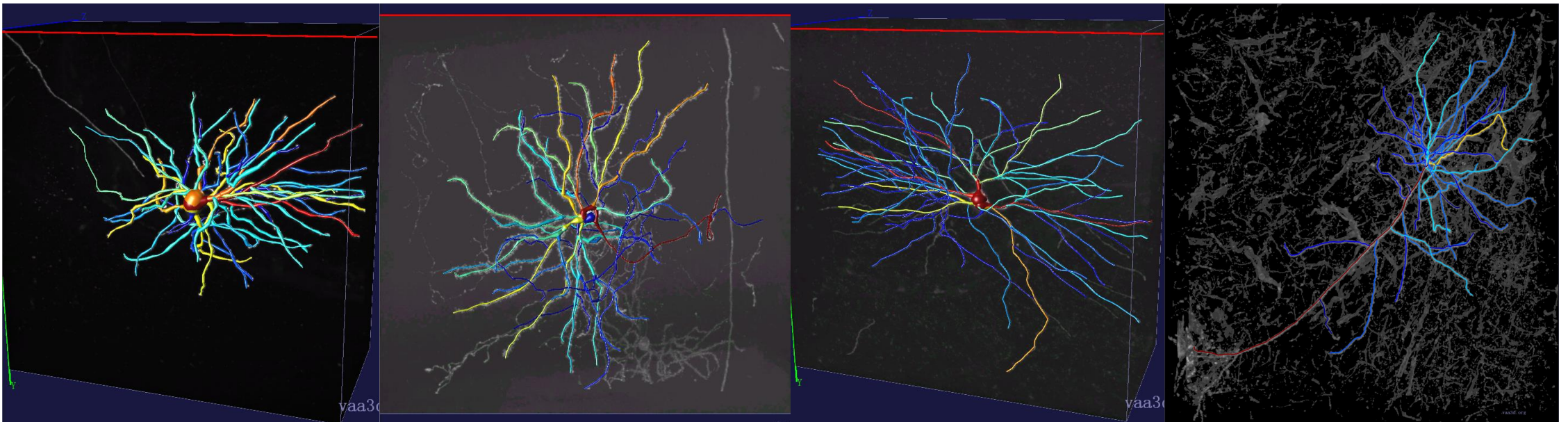
# Another test image



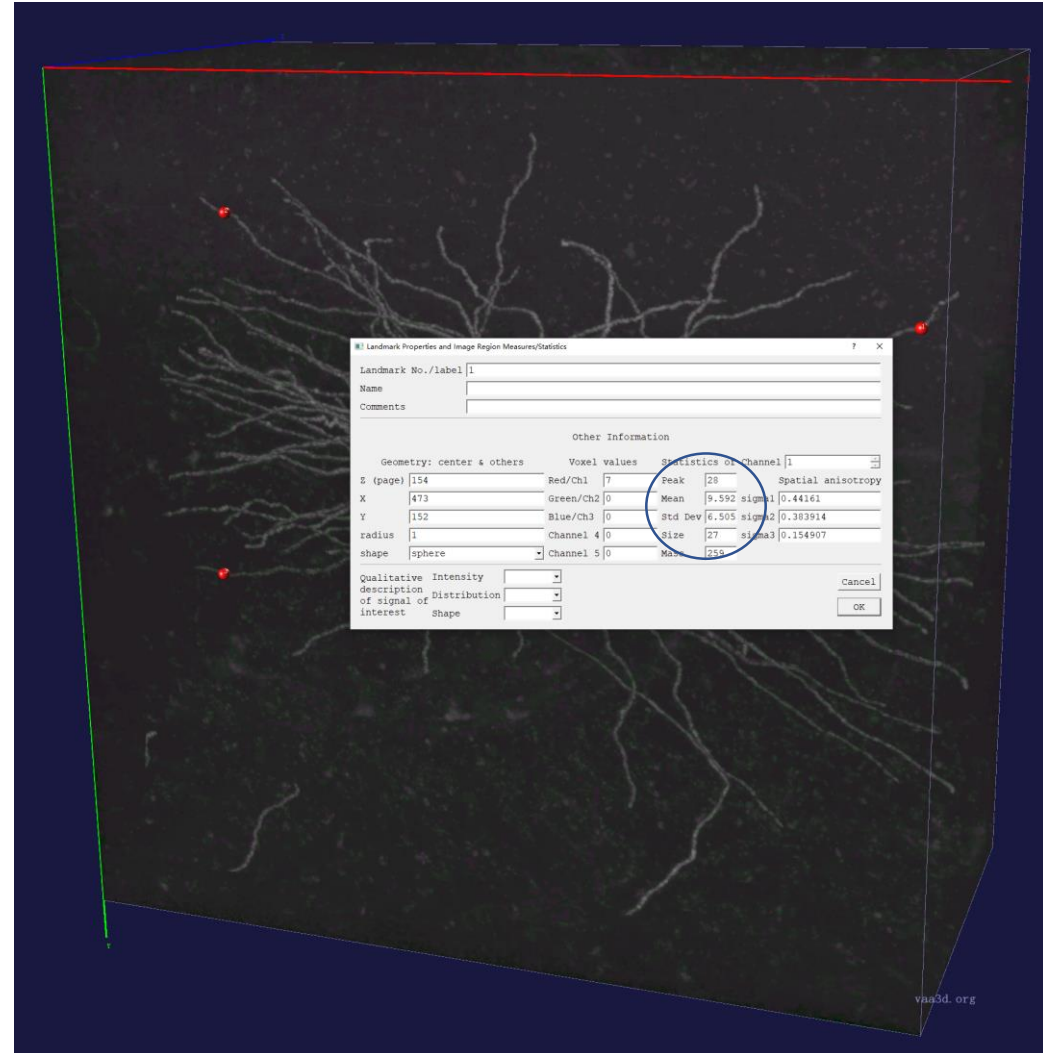
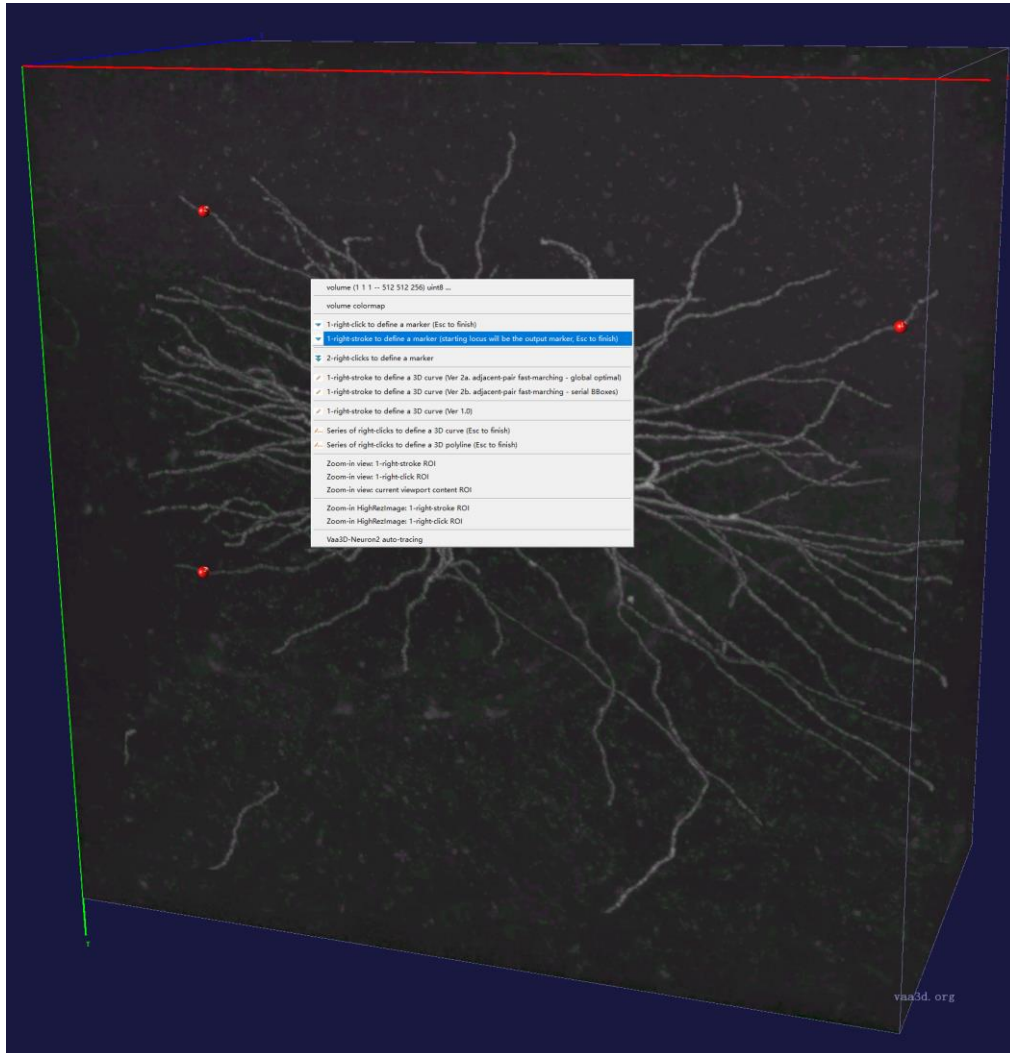
# App2 Parameters that fits most cases



- Auto-downsample
  - No
- Background threshold:
  - Automatic determining threshold: with -1
    - Mean + 0.5 \* Std
- **Would suggest this to all cases as initial test**

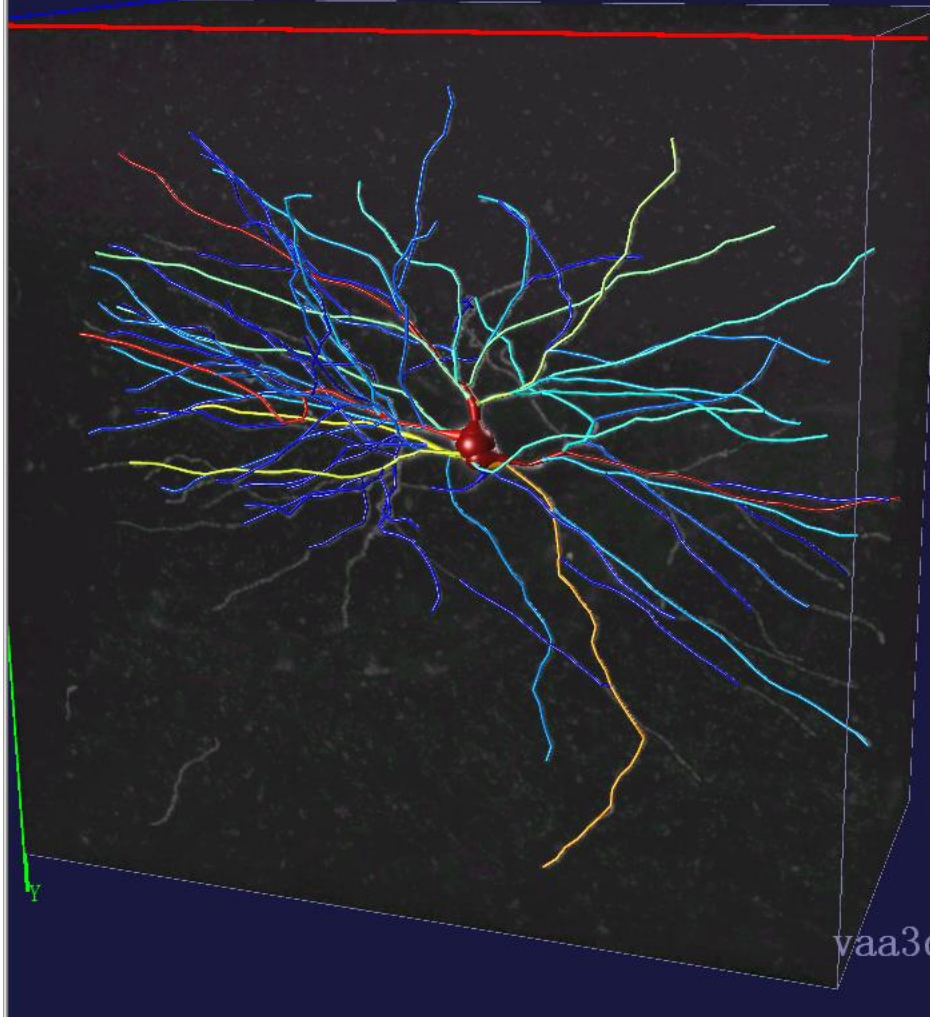


# How to estimate a good threshold?

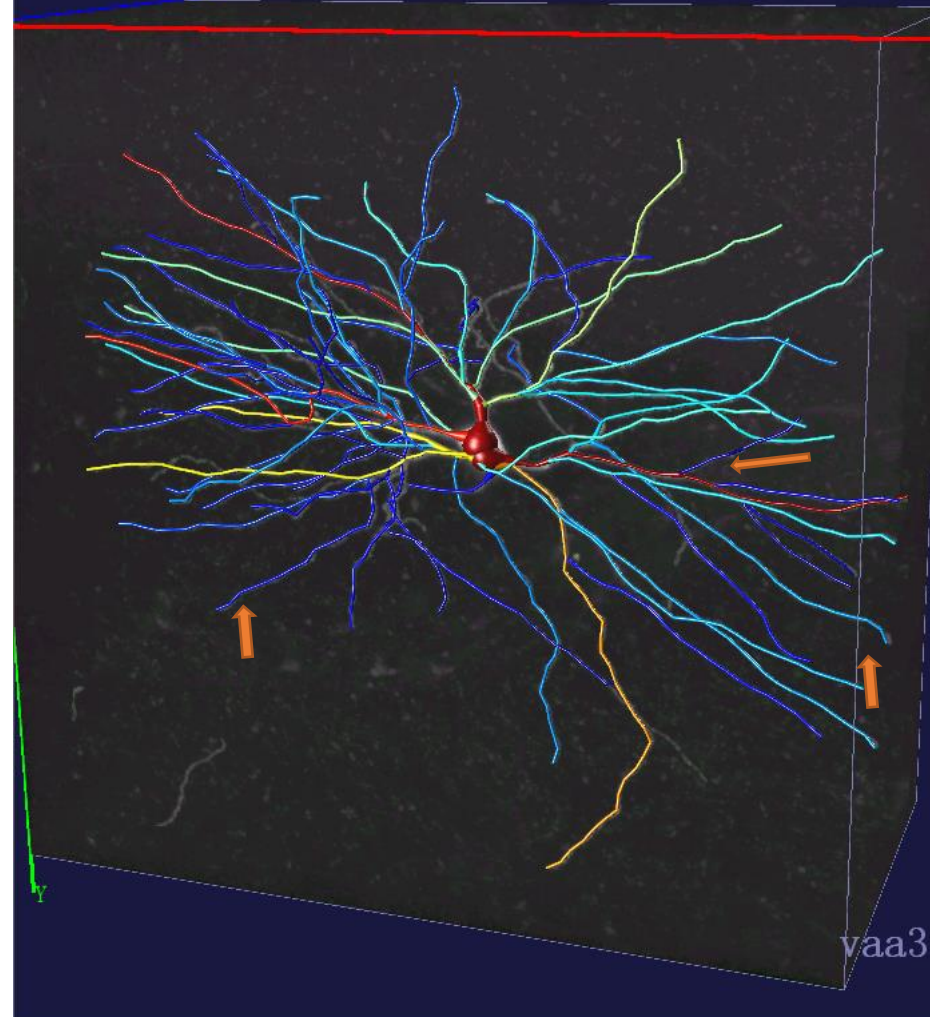


# Self selected threshold

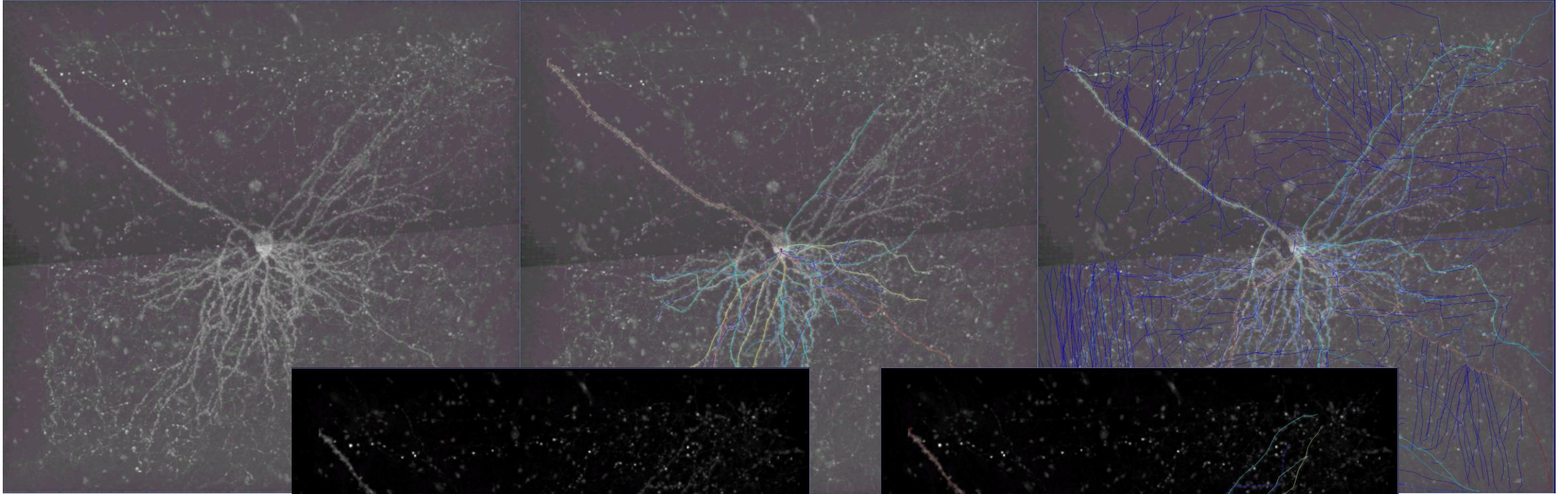
Background Thresh: 10



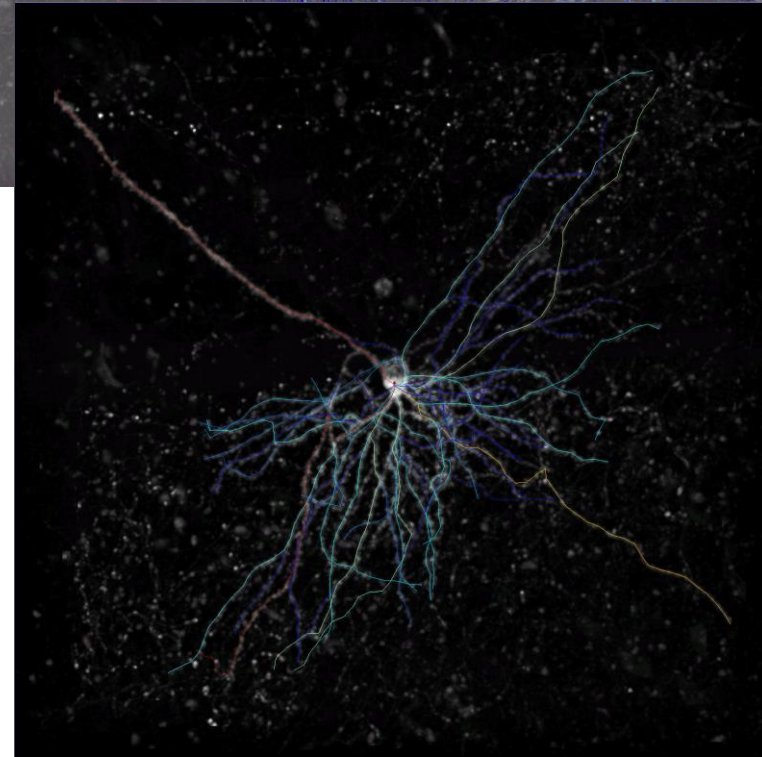
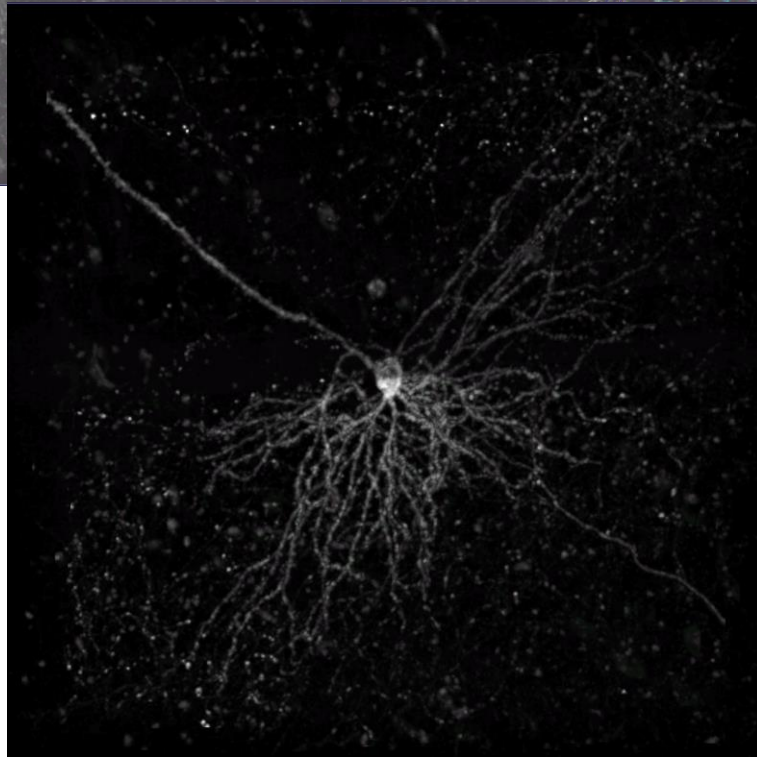
6



# Need for pre-processing

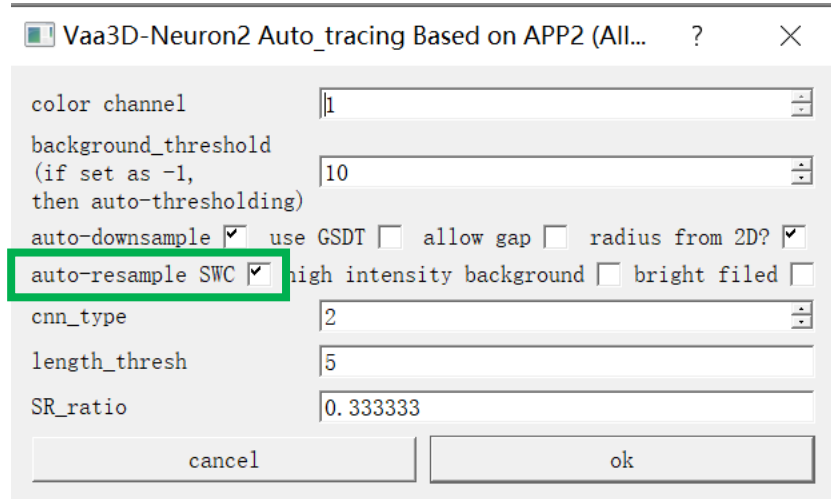


Plugin:  
imPreProcess

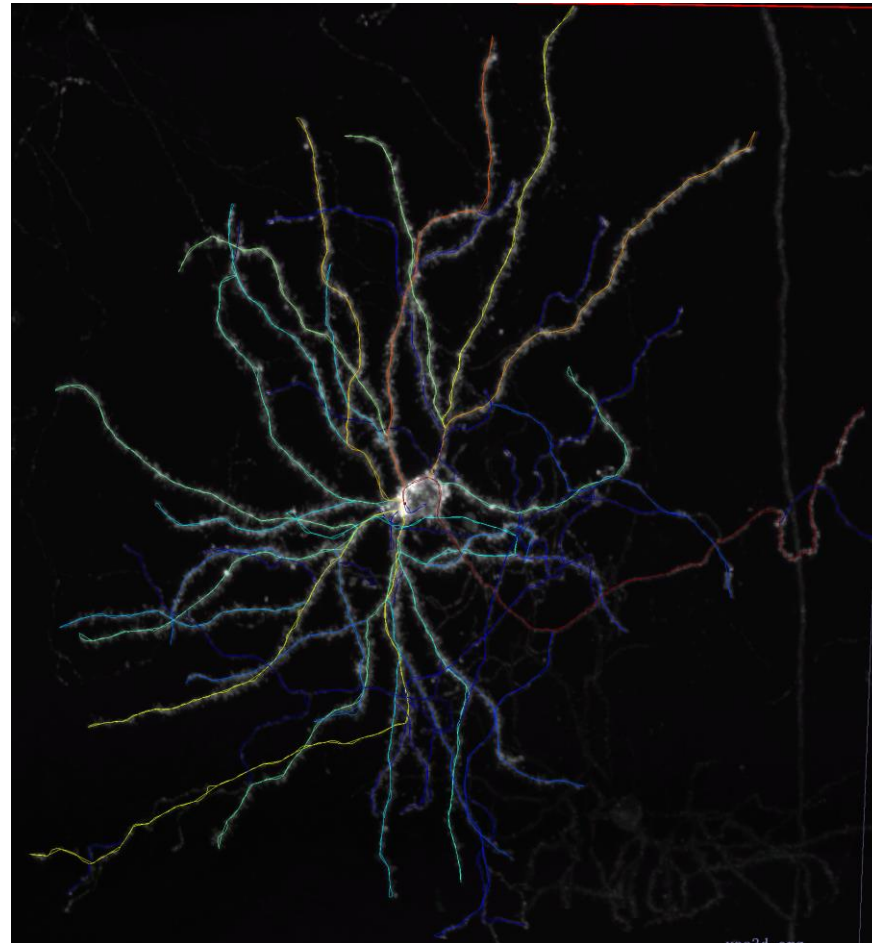




# App2 Parameters: auto-resample SWC



- Auto-resample SWC
  - Default: Yes



With resample

Without resample

Object Manager

Marker (0) | Label Surface (0) | Neuron Segment (0) | Neuron/line Structure (2) | Point

	on/off	color	count	display mode	editing	name	comment
1	<input checked="" type="checkbox"/>	#000000	870	global		APP2_Tracing	D:/PPT/app2_test_i
2	<input checked="" type="checkbox"/>	#000000	7474	global		APP2_Tracing	D:/PPT/app2_test_i

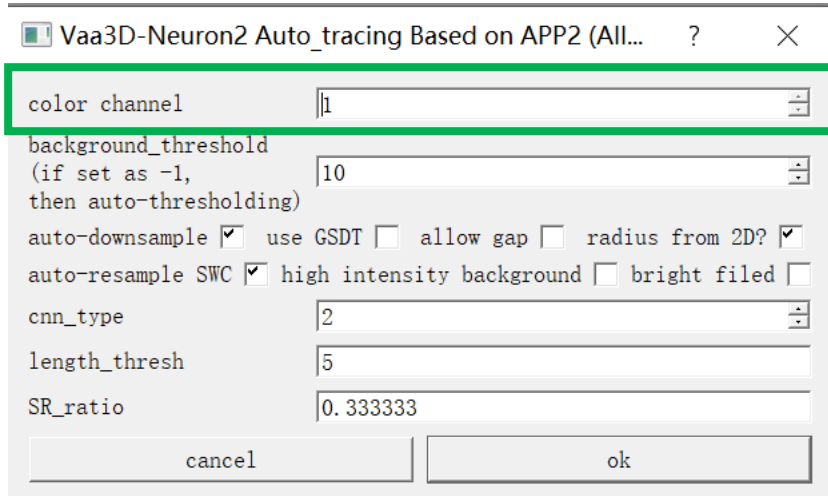
Select All  
Select None  
Select Inverse  
On Off  
Color  
Display  
Name/Cc  
NeuronSeg  
Undo

Single Color...  
Hanchuan's Color Mapping  
Random Color Mapping  
Neuron Segment Colorful  
Multi-neuron Colorful

Options  
 Attached to 3D view  
 Accumulate last highlight search

Find in name/comment:  Next Previous Highlight all hits

# App2 Parameters



Vaa3D-Neuron2 Auto\_tracing Based on APP2 (All... ? X

color channel |1

background\_threshold  
(if set as -1,  
then auto-thresholding) |10

auto-downsample  use GSDT  allow gap  radius from 2D?

auto-resample SWC  high intensity background  bright filed

cnn\_type |2

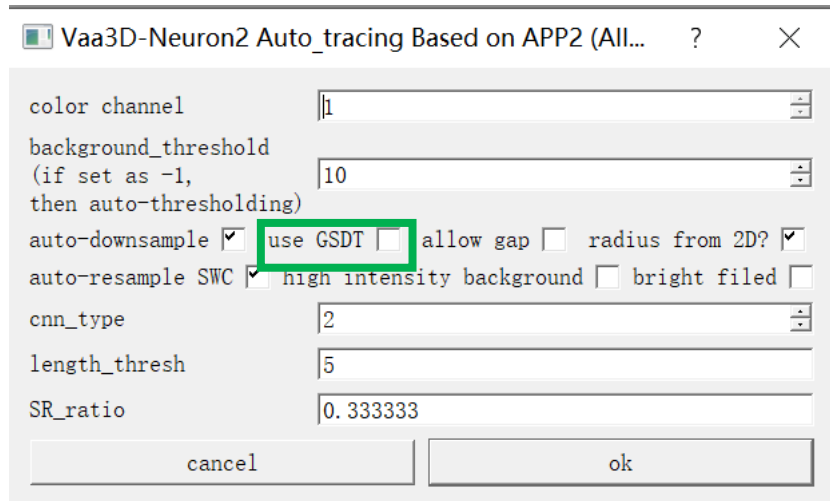
length\_thresh |5

SR\_ratio |0.333333

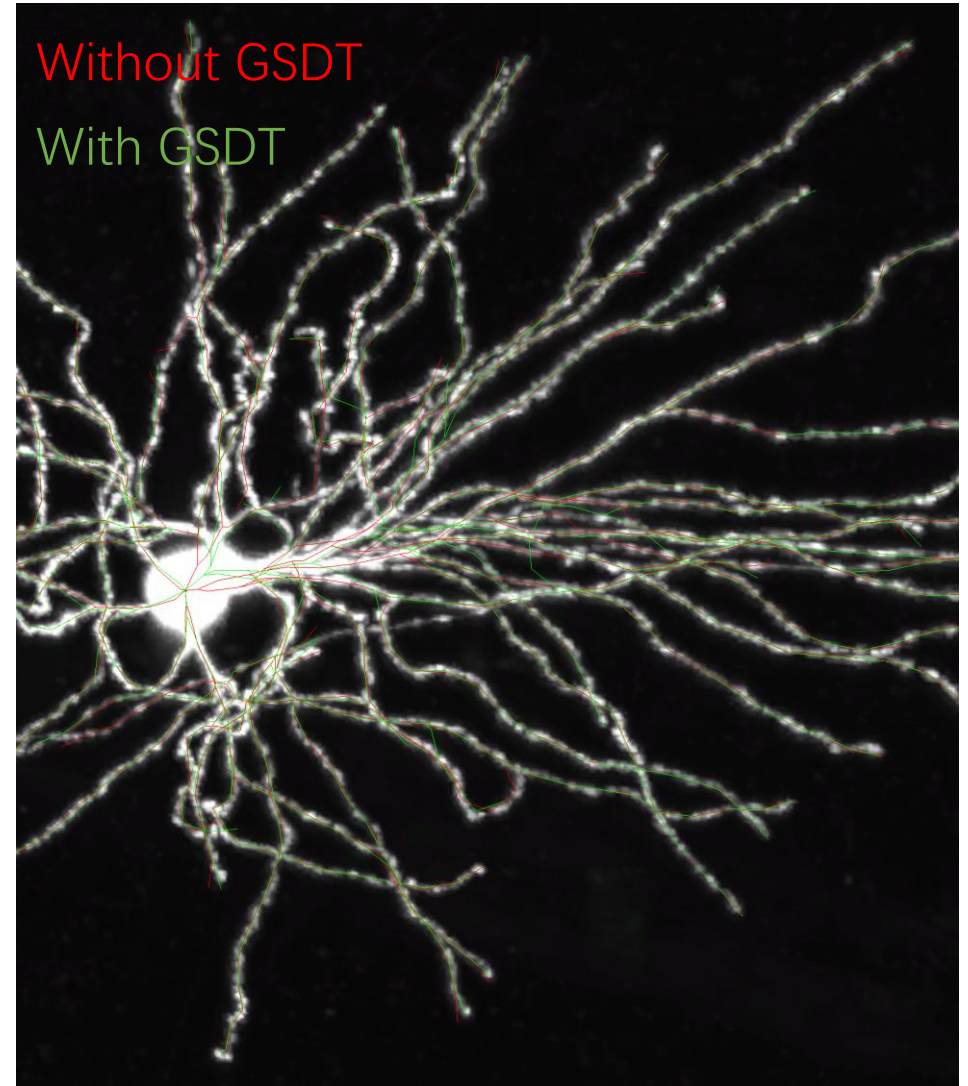
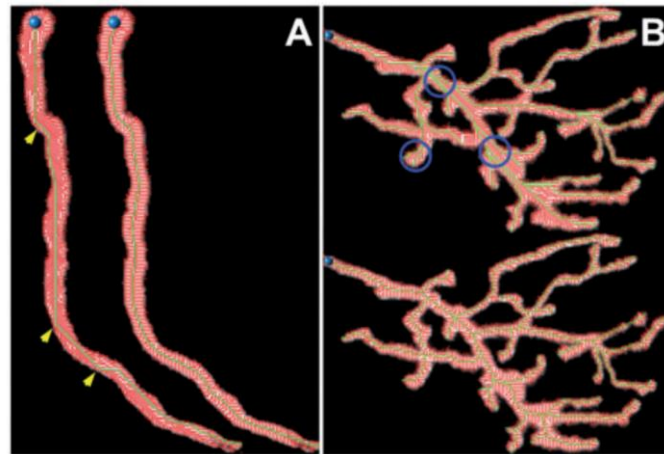
cancel ok

- Color channel:
  - Default:1
  - If you have more than 1 channel, and need to trace channel other than ch1, then specify.
  - In most single channel images, **no need** to adjust

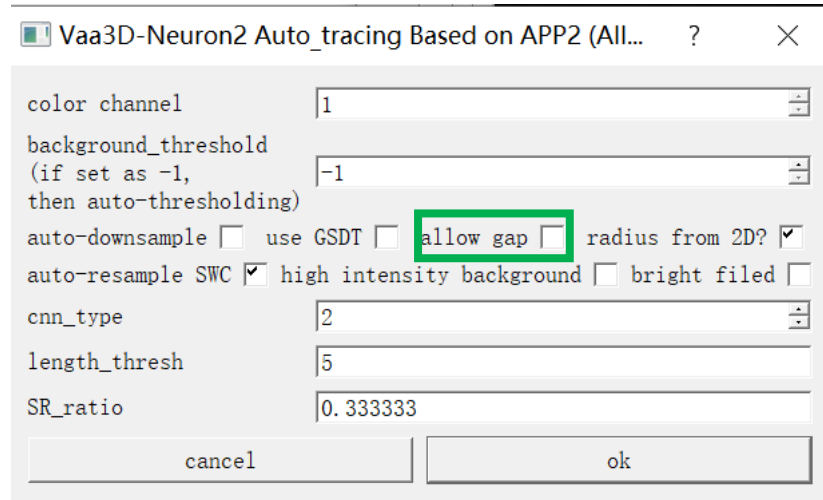
# App2 Parameters: use GSDT



- GSDT:
  - grayscale-weighted image distance transform
- Use GSDT
  - Default: No
    - Not using GSDT but directly use intensity

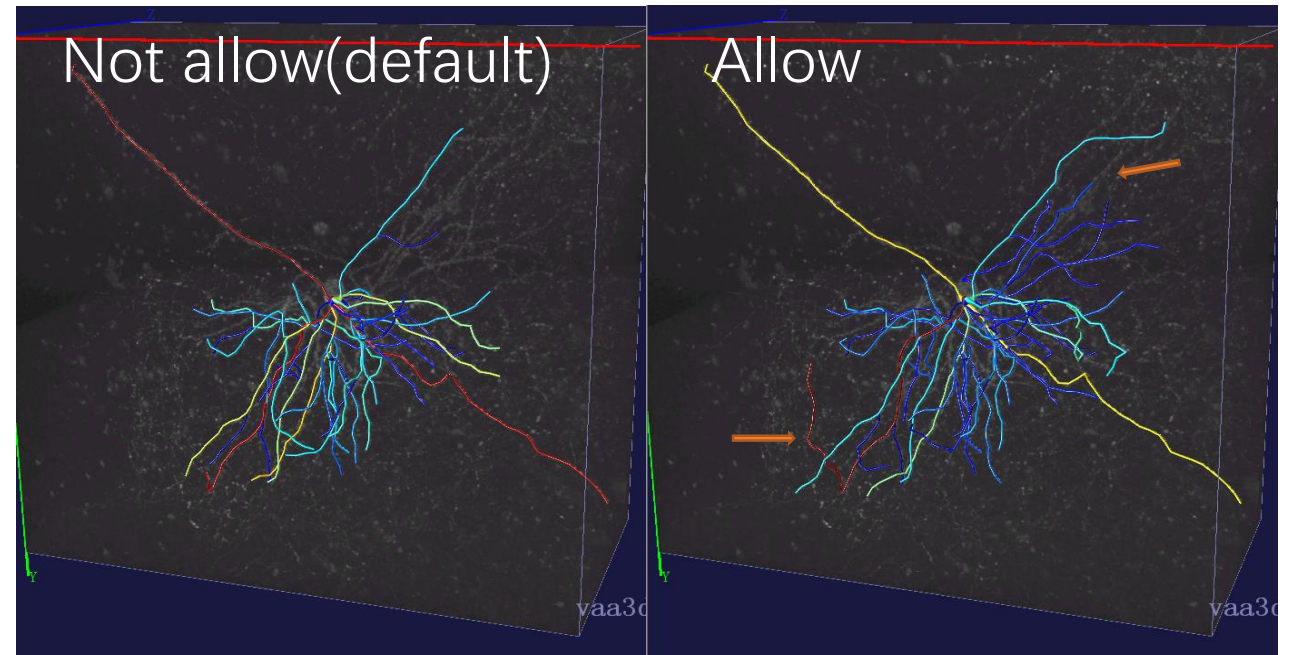


# App2 Parameters: allow gap

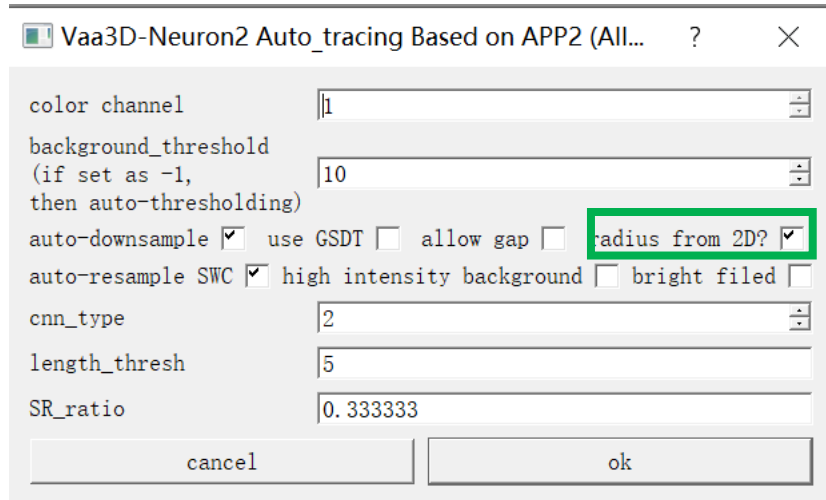


- Allow gap

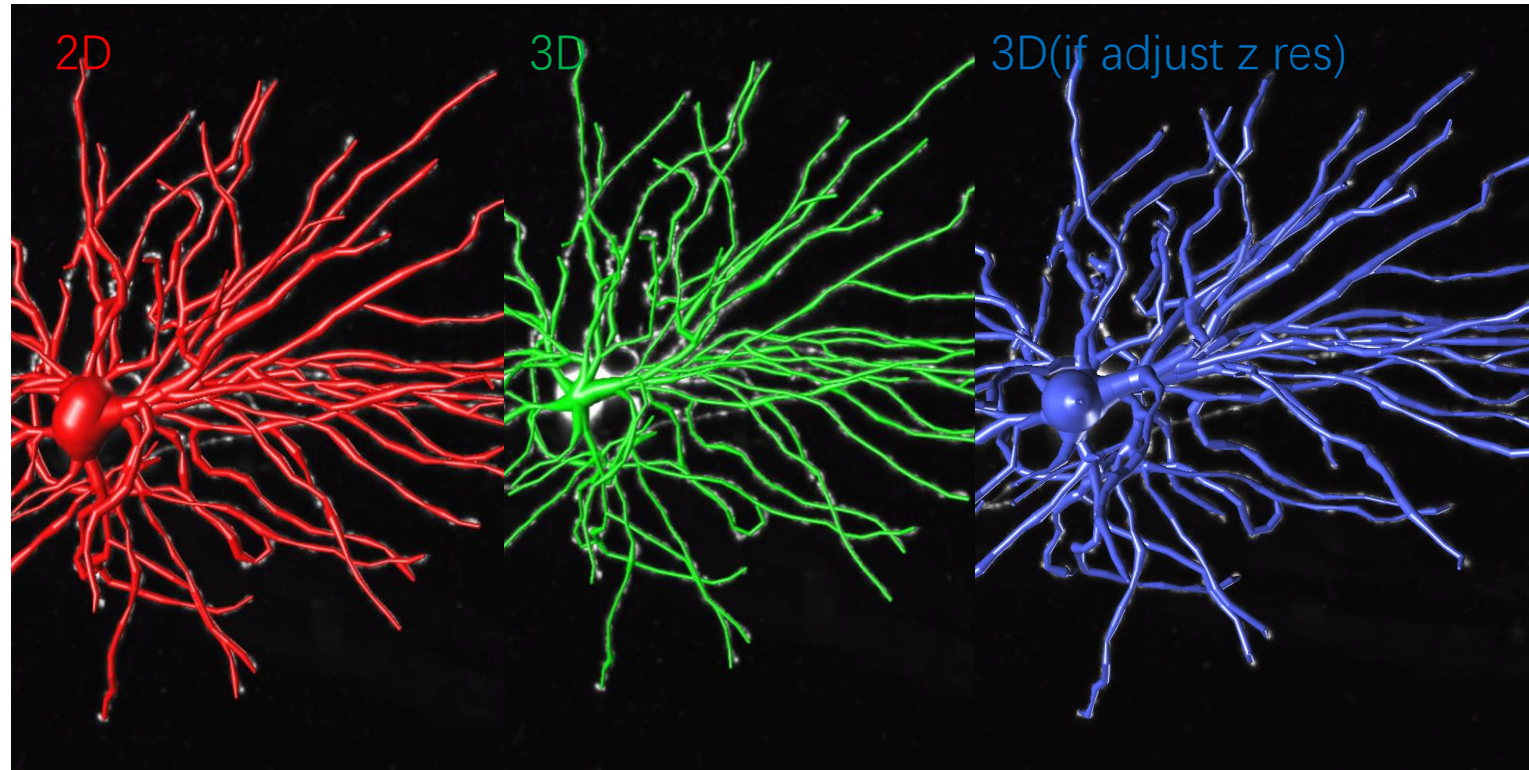
- For punctuated fiber lines
- Allow 1 pixel gap in fastmarching
- Could help to link lines



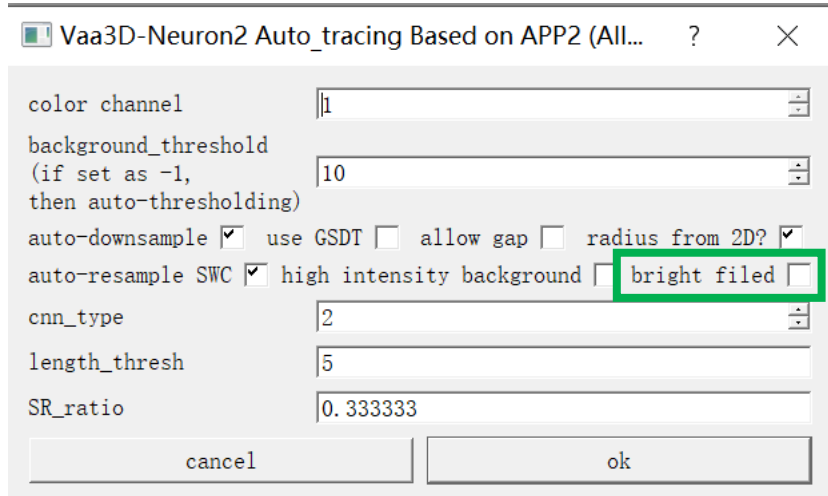
# App2 Parameters: Radius from 2D?



- Radius from 2D
  - Default: Yes
    - Dealing with Anisotropy

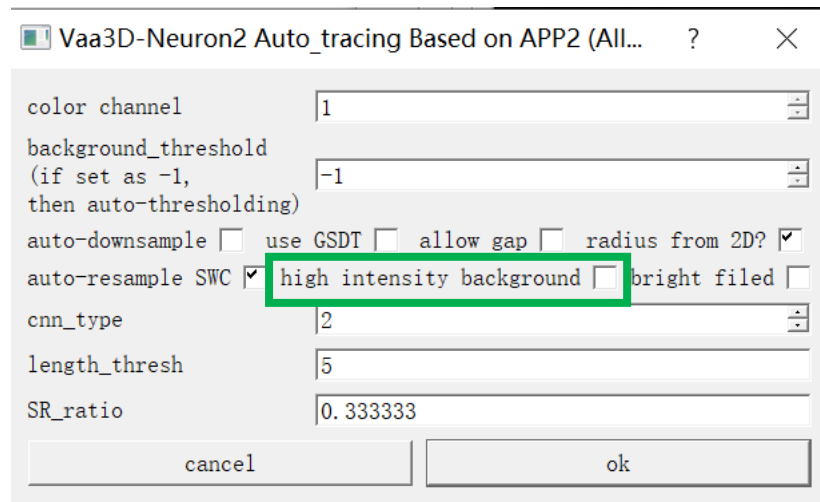


# App2 Parameters: bright filed

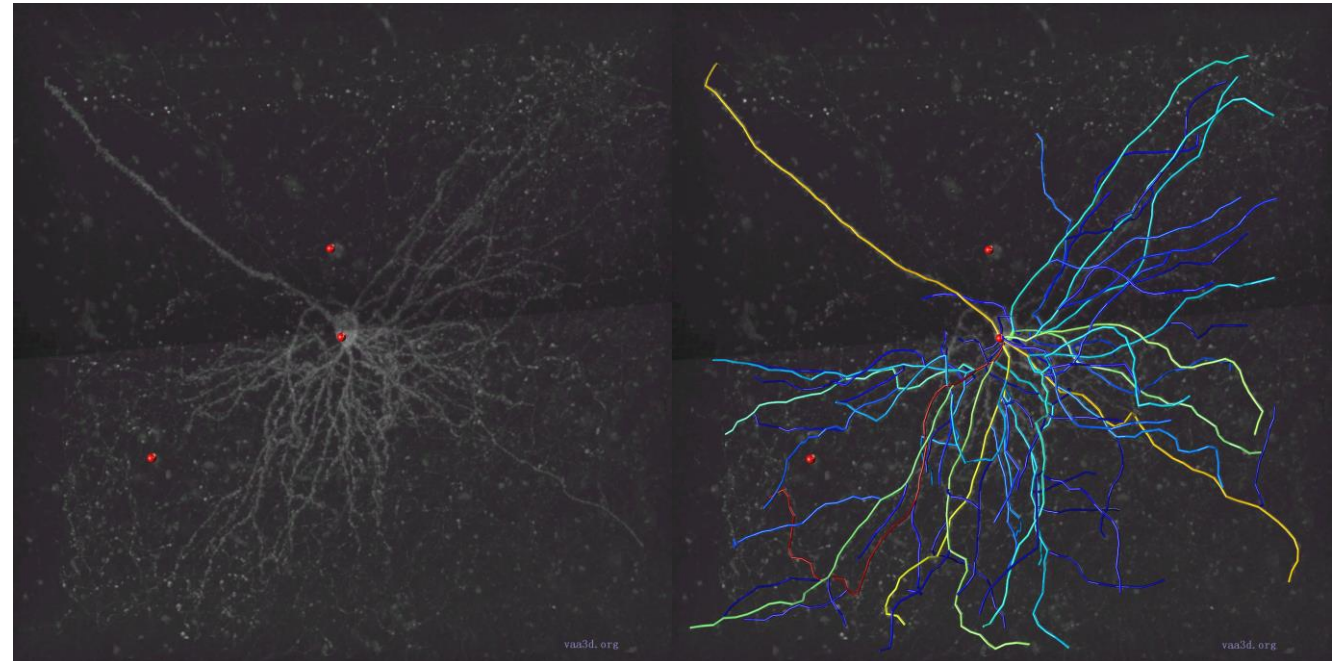


- Bright filed:
  - Check this if the image is inverted
    - Signal-dark
    - Background-bright

# App2 Parameters: high intensity background

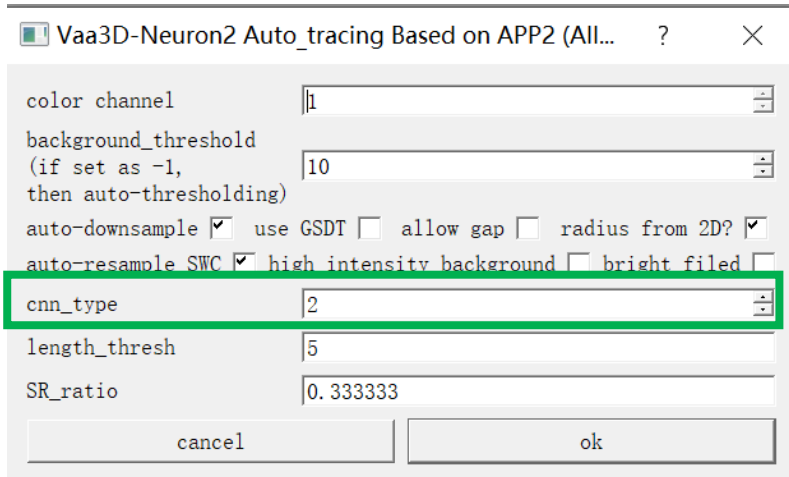


- High intensity background
  - Default: No
  - If yes, input a set of markers and use min intensity -10 as background threshold

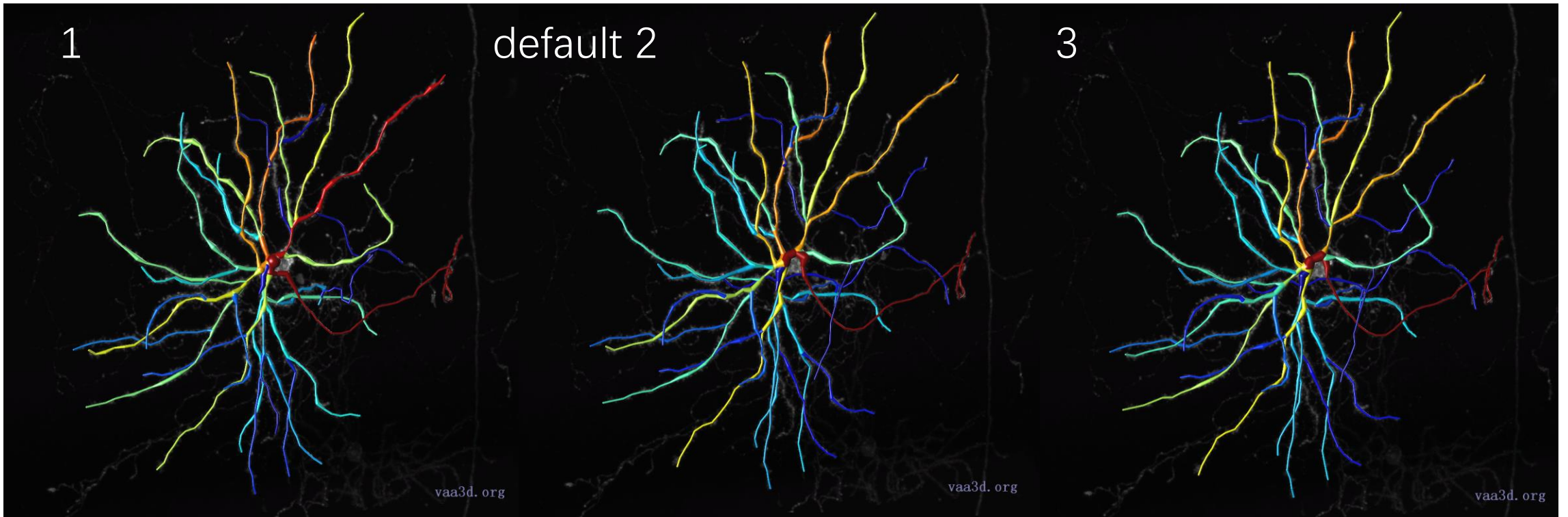




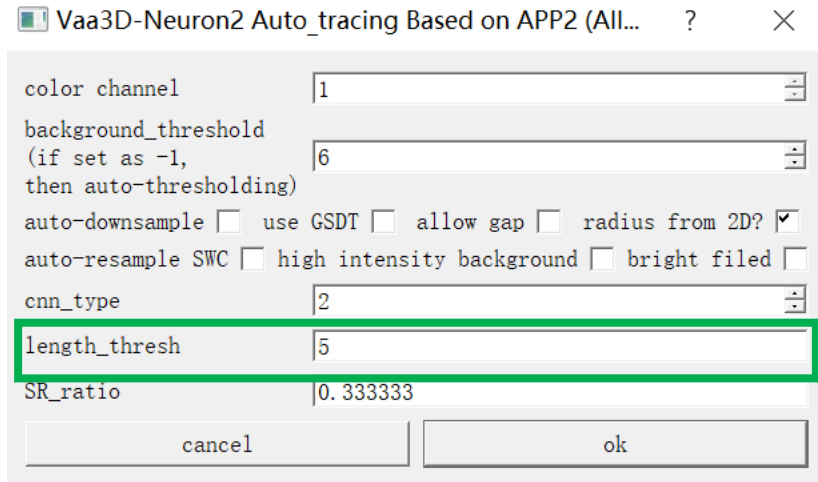
# App2 Parameters: cnn\_type



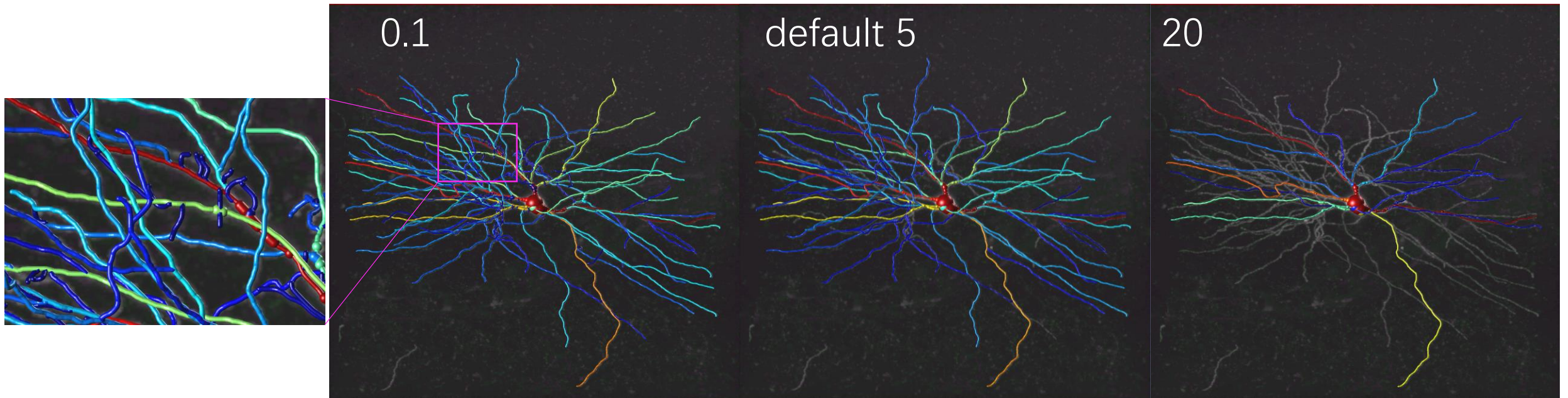
- Connect component type:
  - 1: 6 neighbors only in x,y,z direct neigh
  - 2: 14 above + 8 in 2D such as (x-1,y-1,z)
  - 3: 26 neighbors in 3\*3\*3 block



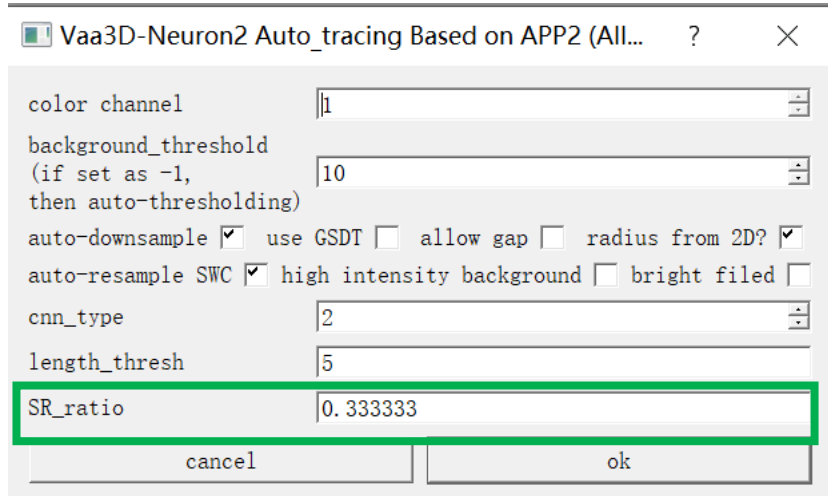
# App2 Parameters: length\_thresh



- Threshold for integrated length in hierarchical pruning
  - Lower: shorter/dimmer segments be kept
  - Higher: shorter/dimmer segments be pruned



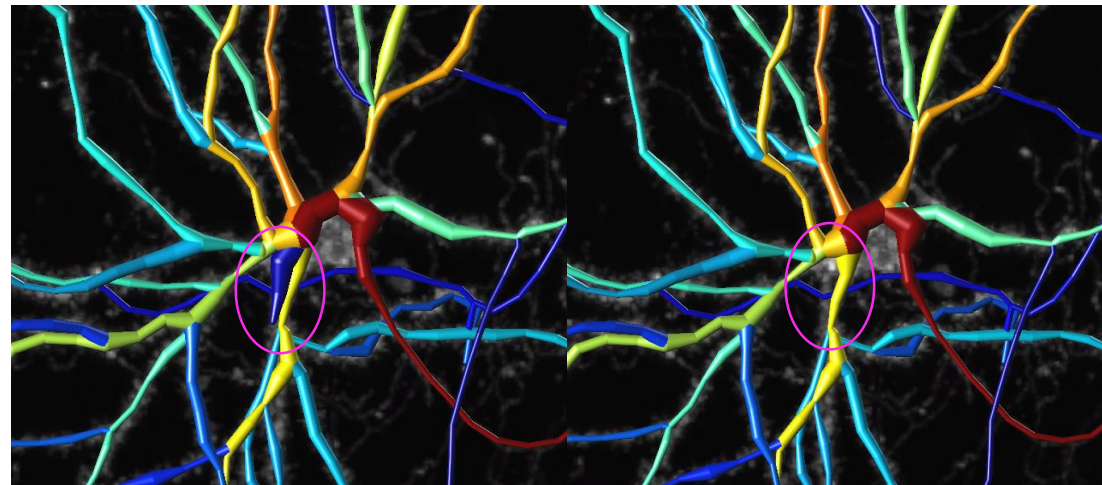
# App2 Parameters: SR\_ratio



- A metric in hierarchical pruning
  - To remove non-significant short segments
  - Definition:
    - $\text{Sum of signal} / \text{sum of residue}$

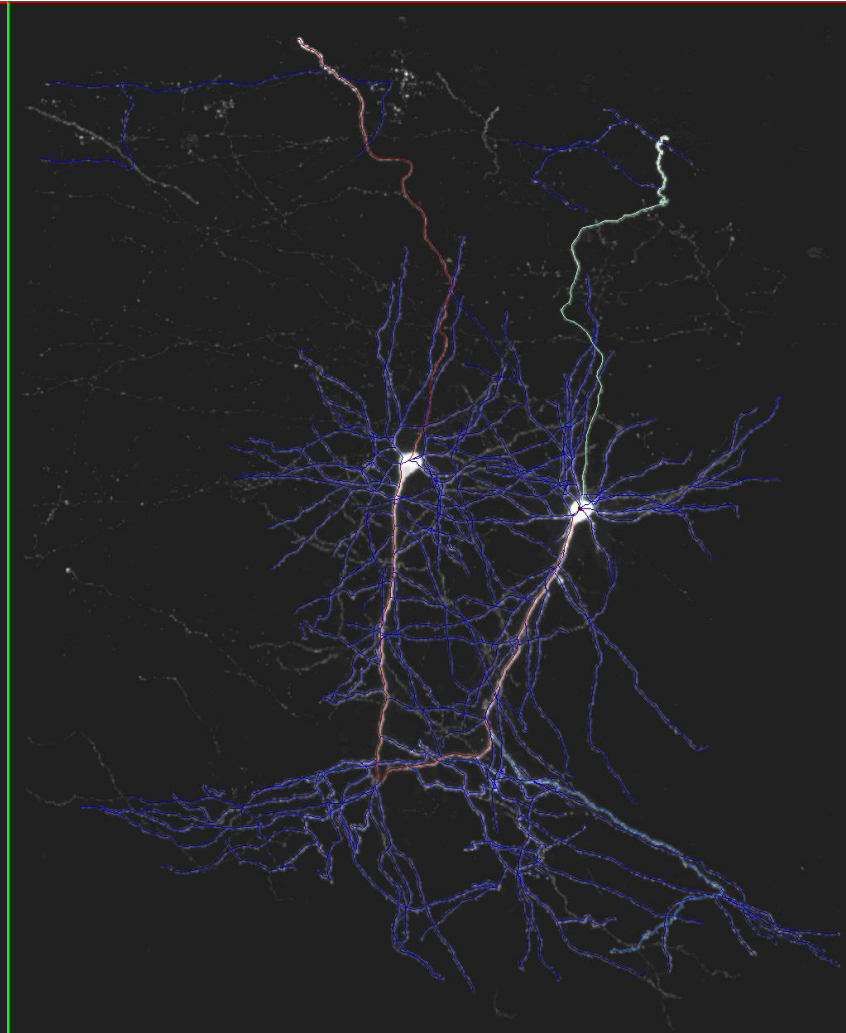
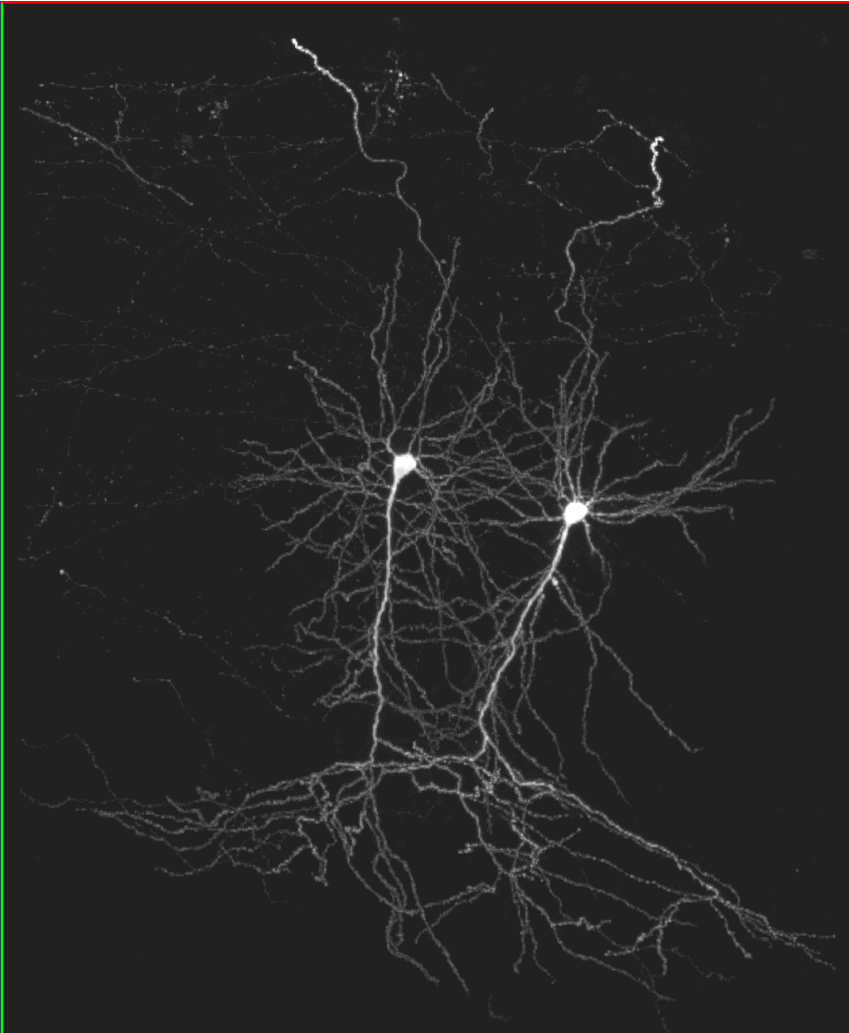
Default

SR\_ratio=5



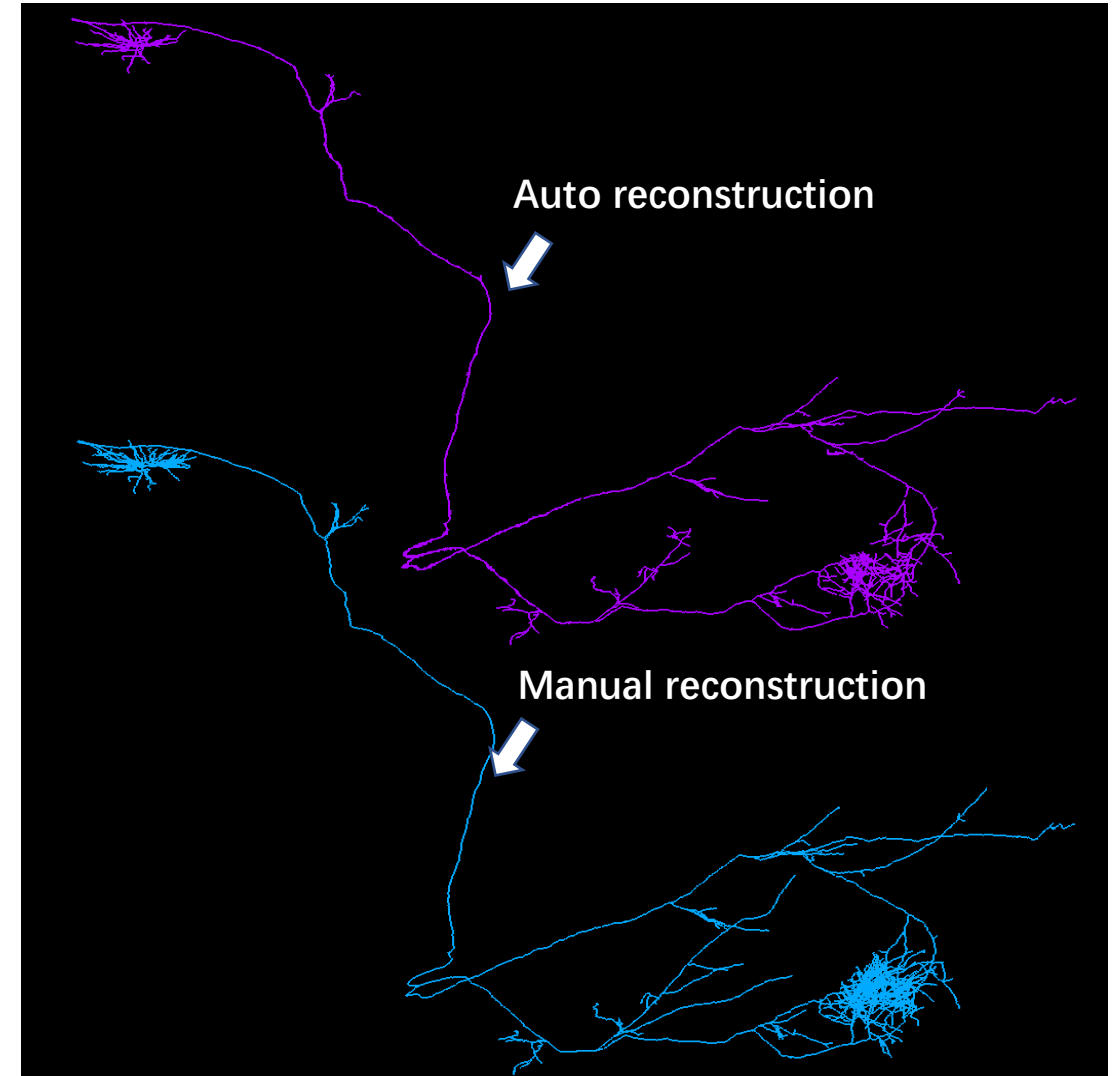
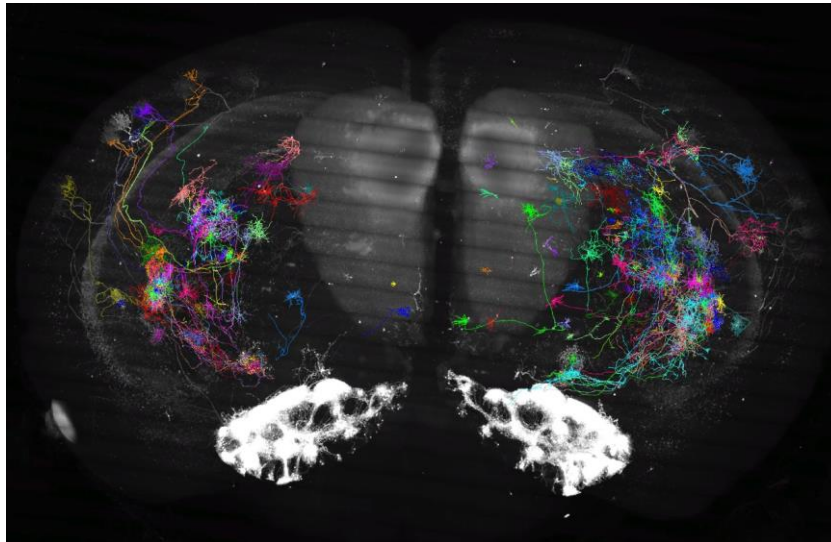
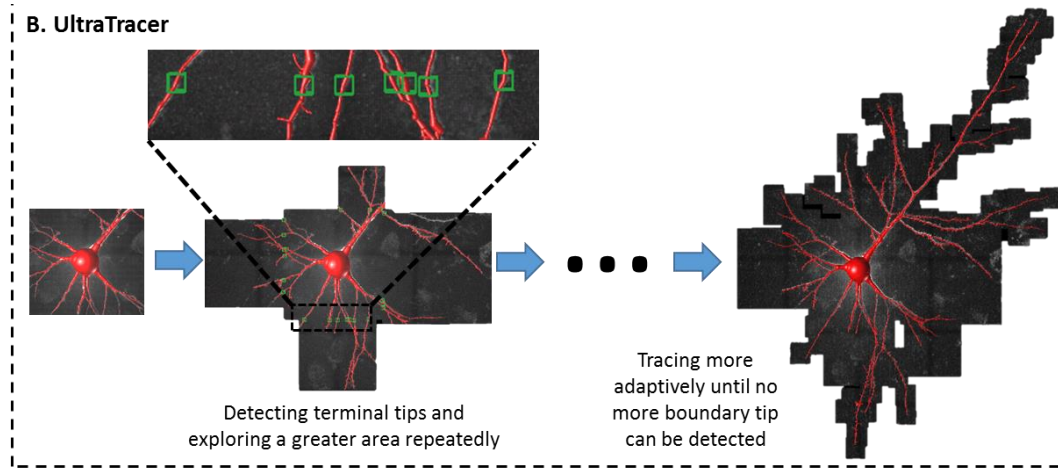
# For larger field of view

Plugin: SNAP



# For whole neuron morphology or bigger field of view

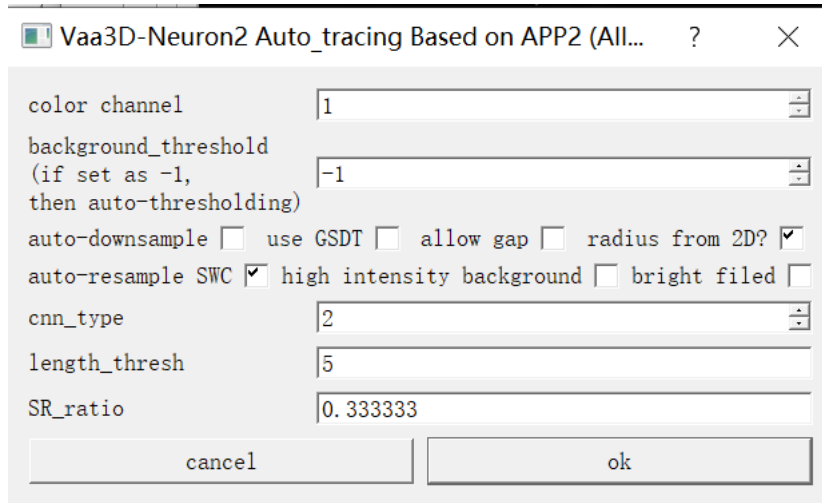
- UltraTracer





# Take Home messages for App2 application

- 1.App2 with this set as default works for most cases:



- Auto-downsample
  - No
- Background threshold:
  - Automatic determining threshold: with -1
    - Mean + 0.5 \* Std
- Would suggest this to all cases as initial test

- 2. Command line recommended:

```
vaa3d -x vn2 -f app2 -i input.tiff -o output.swc -p NULL 1 AUTO 0
```

***Thank you***