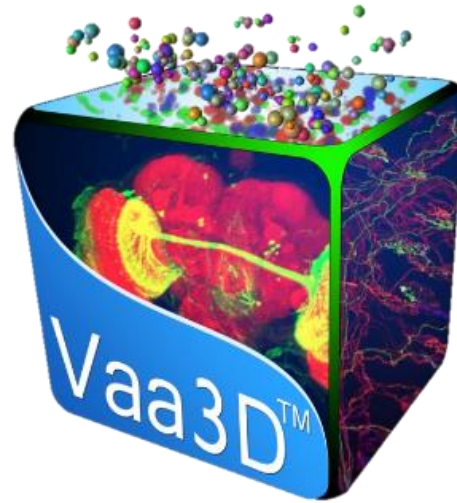


# Automated Neuron Tracing in Vaa3D

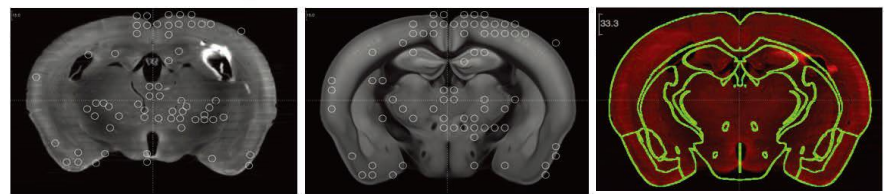
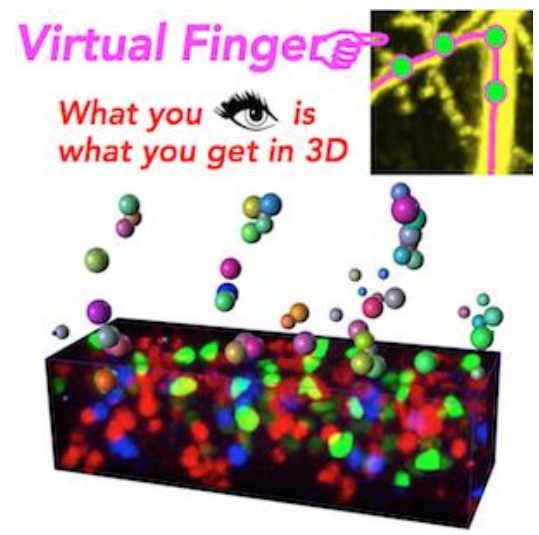
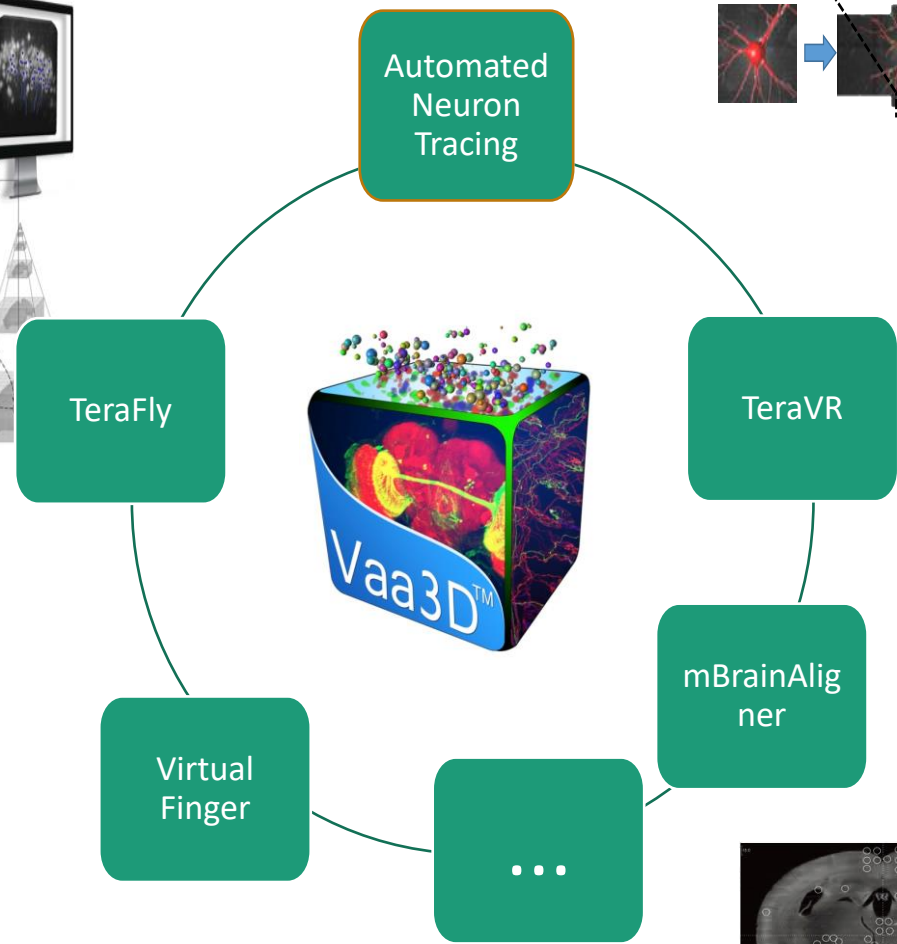
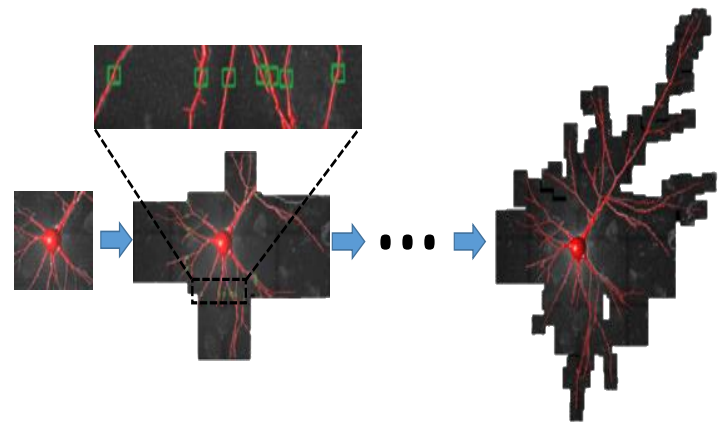
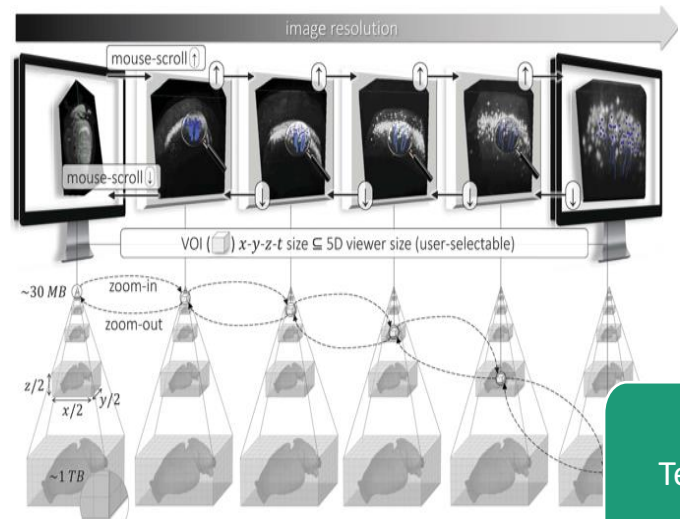


**Presenter: Liya Ding**

**Institute for Brain and Intelligence**

**Southeast University**

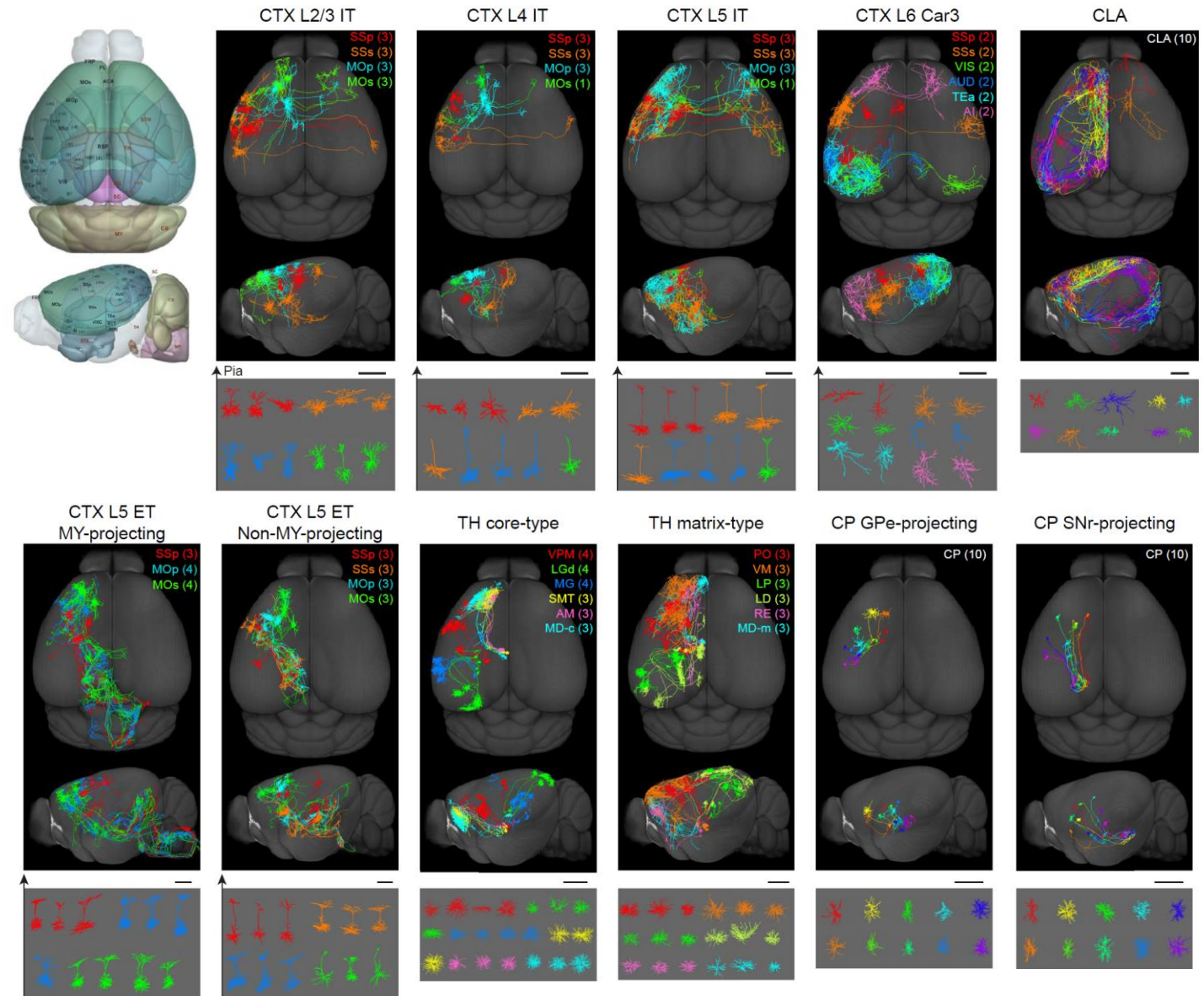
# Vaa3D platform

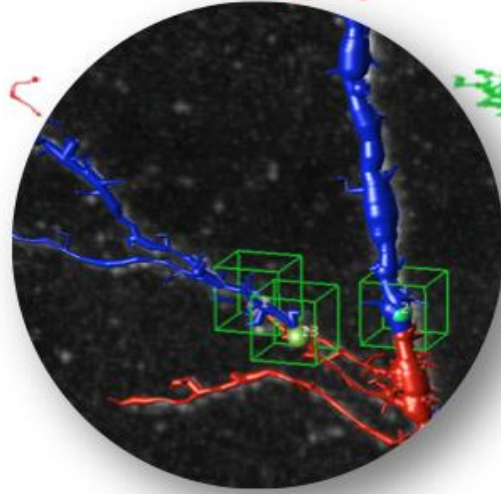
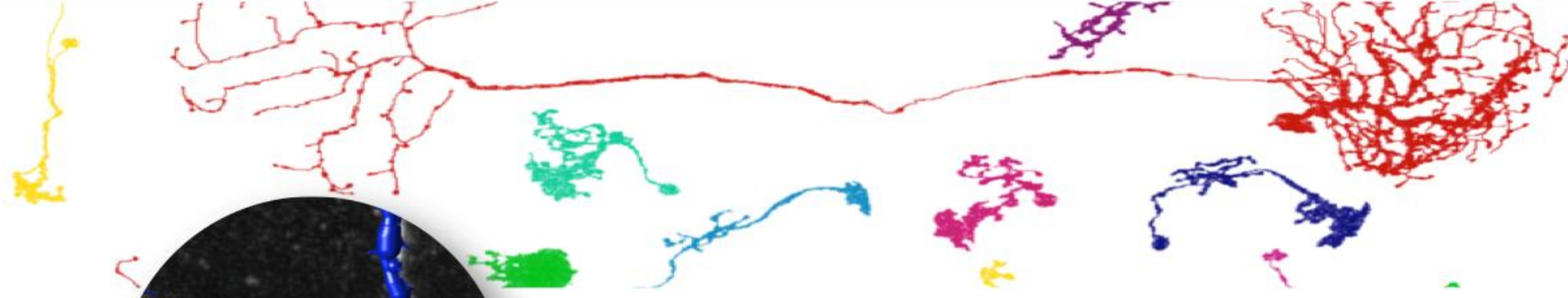


*UltraTracer: Nature Methods, 2017*  
*TeraFly: Nature Methods, 2016*  
*Virtual Finger: Nature Communications, 2019*  
*TeraVR: Nature Communications, 2019*  
*mBrainAlignner: Nature Method, 2021(accepted)*

# Neuron Morphology

- A defining feature of neuronal types
- Highly diverse and region specific
- Big number of reconstruction desired
- Automated reconstruction needed



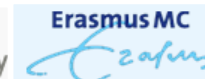


# BigNeuron

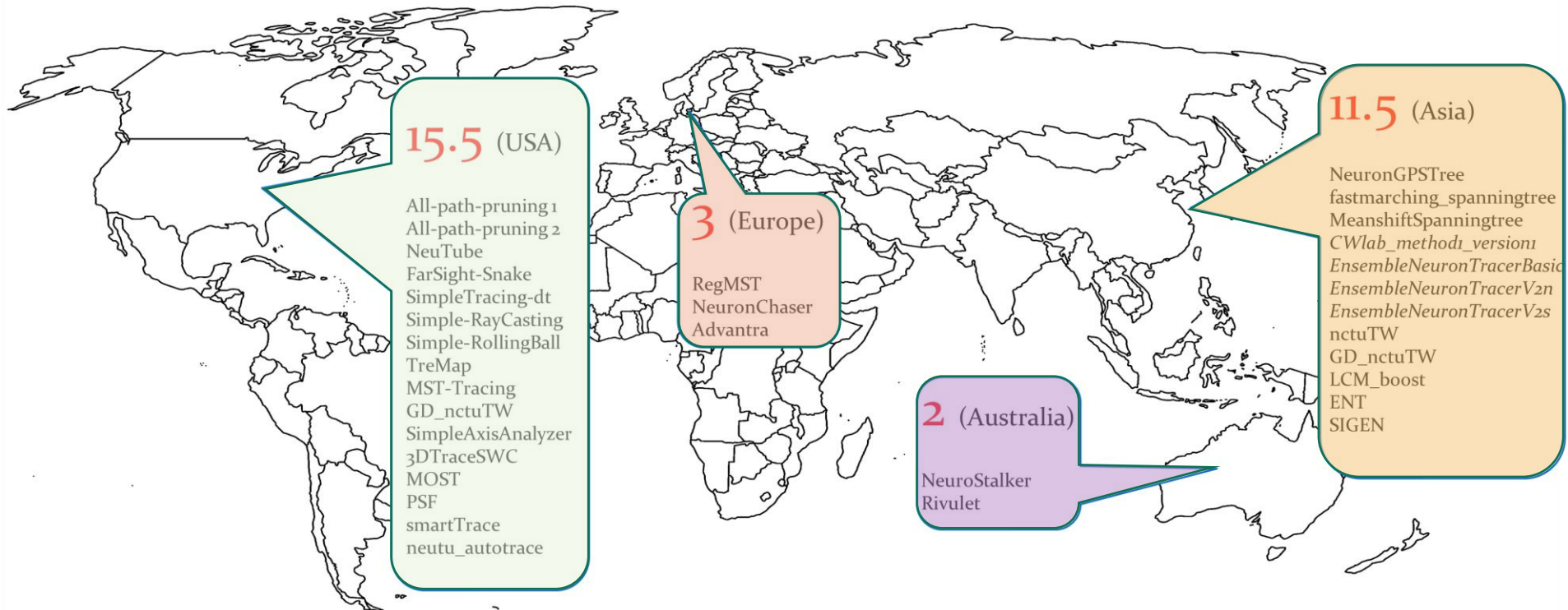
*A community effort to find out what is exactly the state-of-the-art of single neuron reconstruction, standardize the protocols, and establish a Big Data resource for neuroscience.*



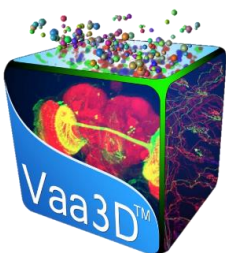
Human Brain Project

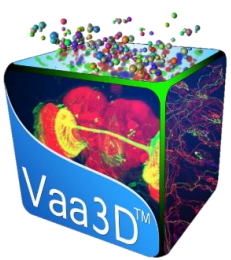
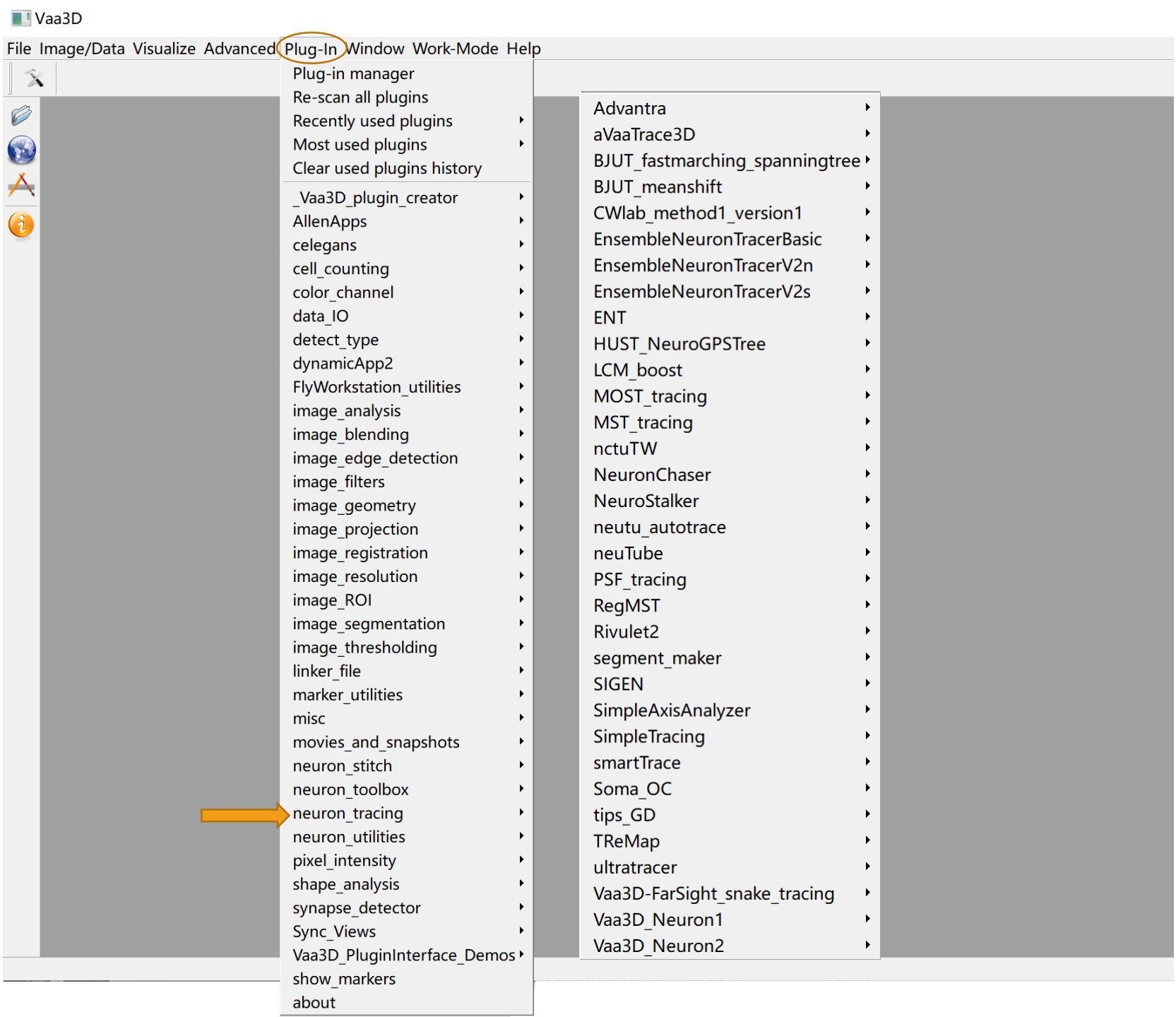


# Neuron Reconstruction Plugins(32+) in Vaa3D



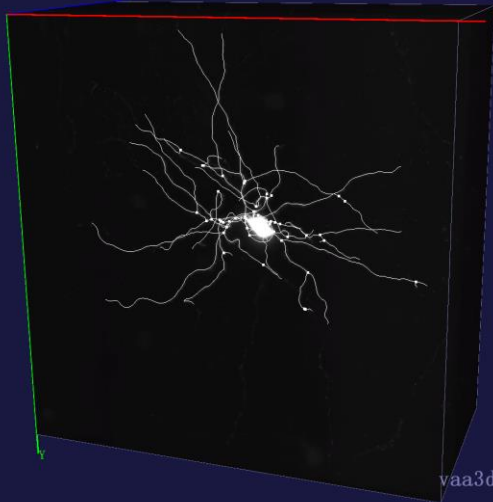
- pruning (*All-path-pruning 1, All-path-pruning 2*)
- fitting geometrical elements (*NeuTube*)
- ray casting (*Most-RayCasting, Simple-RayCasting, NeuronGPSTree*)
- spanning tree and shortest paths (*TreMap, SimpleTracing-dt, Simple-RollingBall*)
- deformable curves (*FarSight-Snake*)



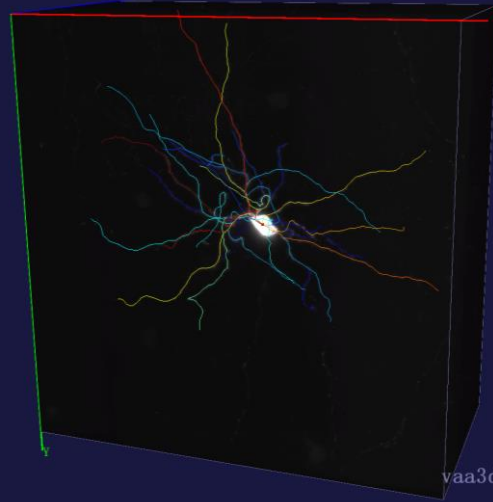


# Automatic Tracing Examples

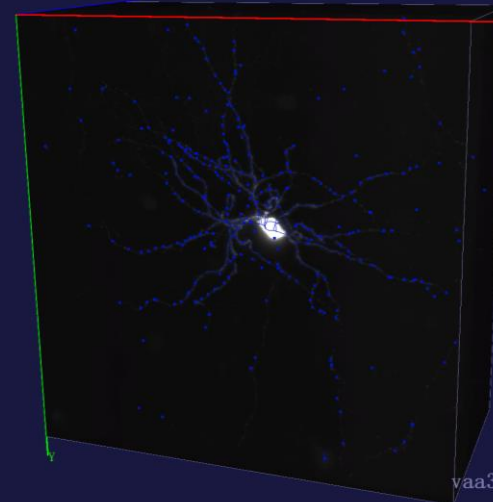
NeuroGPSTree



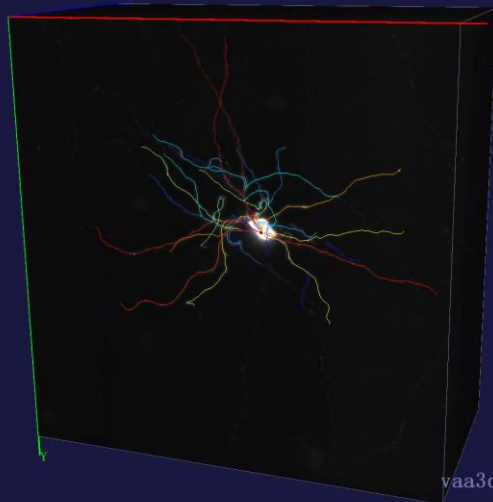
App2



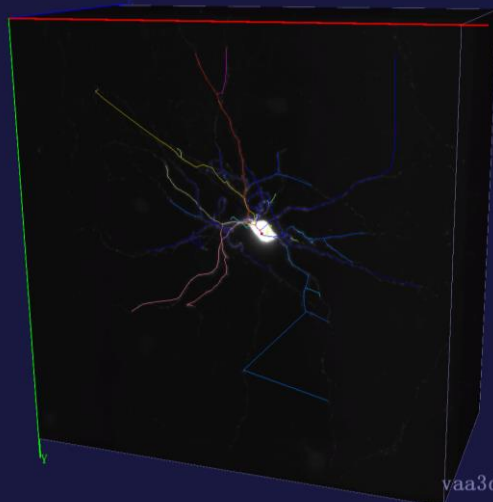
MOST



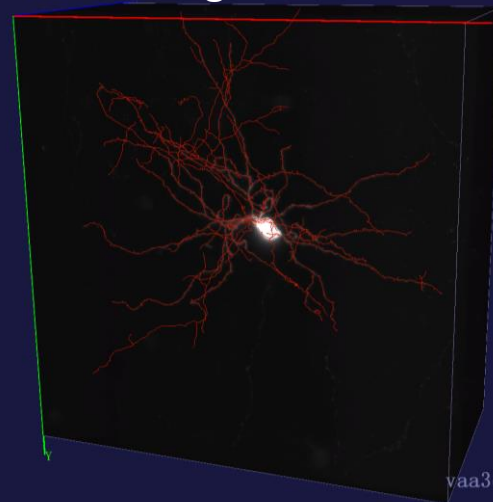
ENT



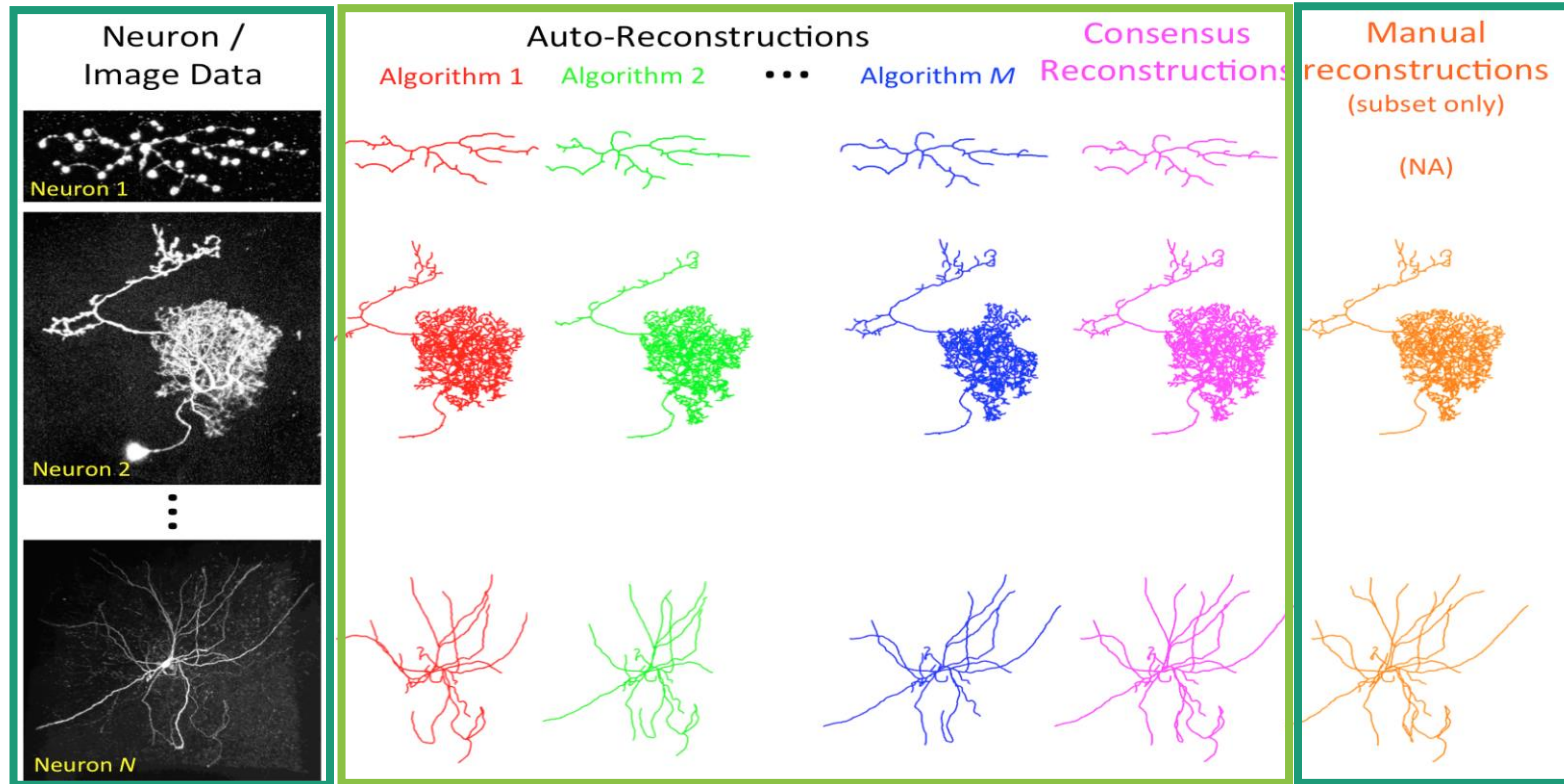
FMST



SmartTracing



# Neuron Reconstruction Results Evaluation



## Ground Truth (Ideal)

- True Morphology
- Alternative imaging modality

## Approximations

## Consensus

- Merge and Votes
- Confidence
- Automatic

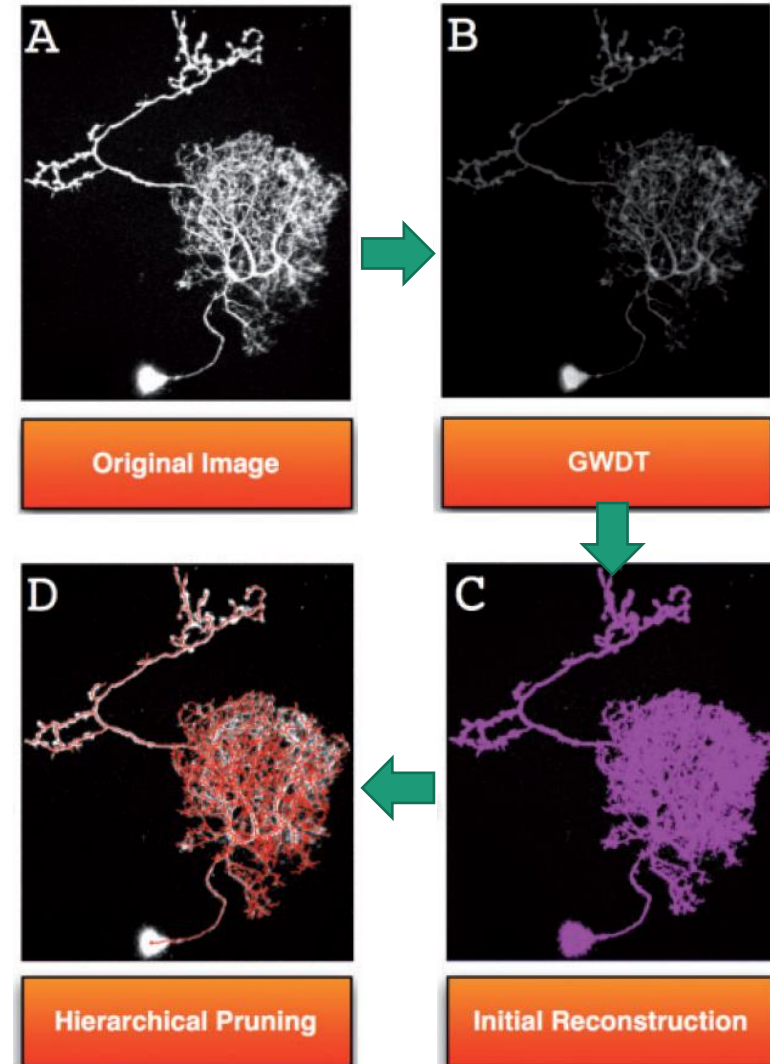
## Gold Standard

- Manual annotations
- Inspected by multiple annotators



# APP2 Intro

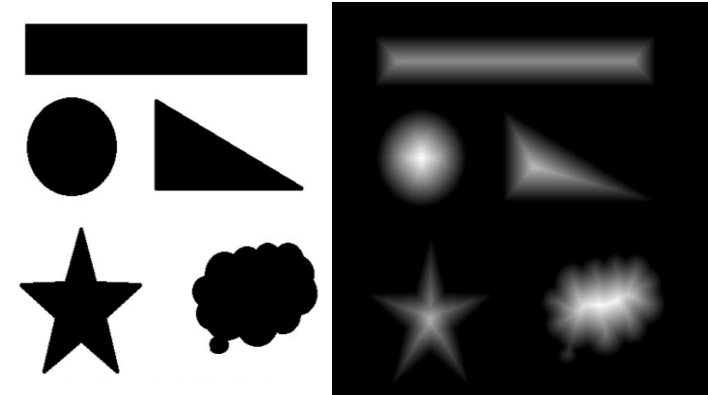
- APP2 = All Path Pruning 2.0
- Paper:  
“APP2: automatic tracing of 3D neuron morphology based on hierarchical pruning of gray-weighted image distance-trees”, Xiao, H., and Peng, H., *Bioinformatics*, 2013.
- Steps:
  1. GWDT: gray-weighted image distance transform
  2. Initial neuron reconstruction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



# APP2 Step 1: GWDT

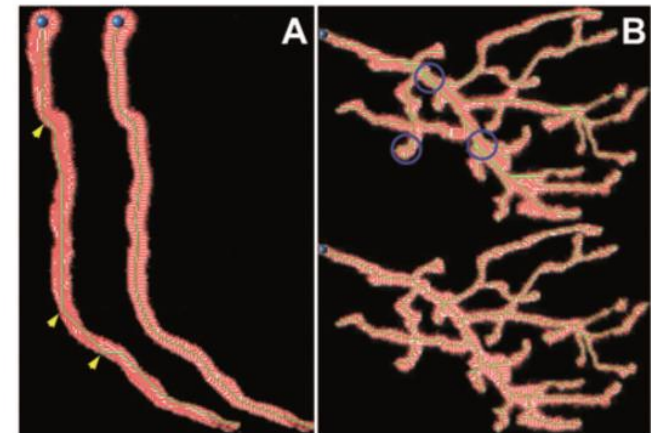
## DT: distance transform

- Create a gradient of image intensity: ICDB
  - close to center of the region, the image intensity is large;
  - close to the boundary, the intensity is small.
- DT -> a binary image (by thresholding)



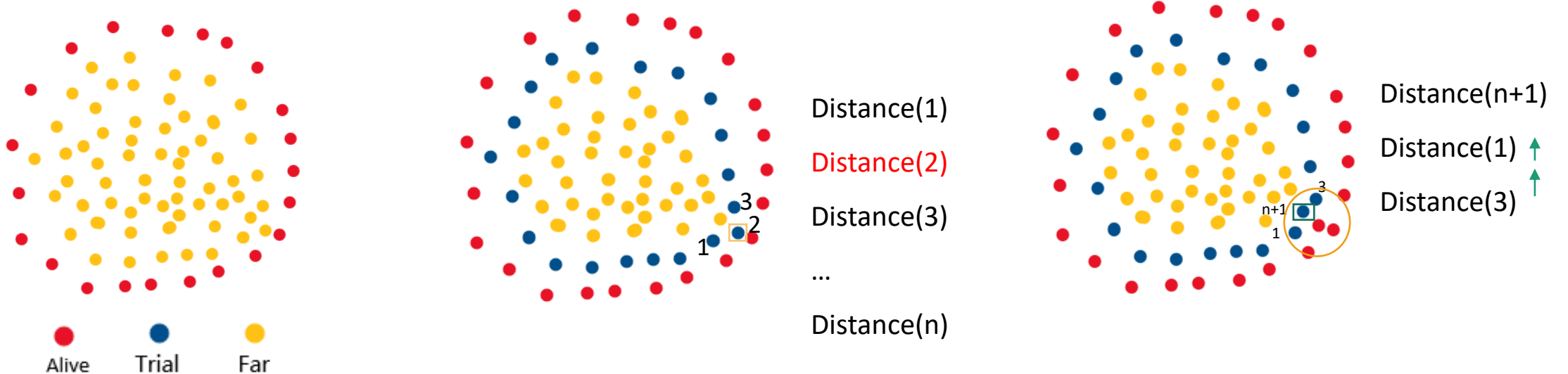
## GWDT: gray-weighted image distance transform

- sum of image pixels' intensity along the shortest path to background.
- With low threshold value
- All image background pixels as 'seeds', then compute the distances from other pixels to these seed pixels.
- Formulated within the FM framework



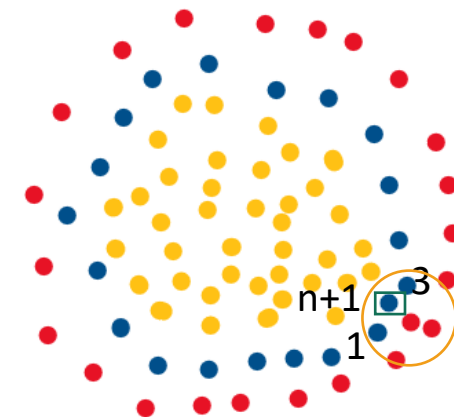
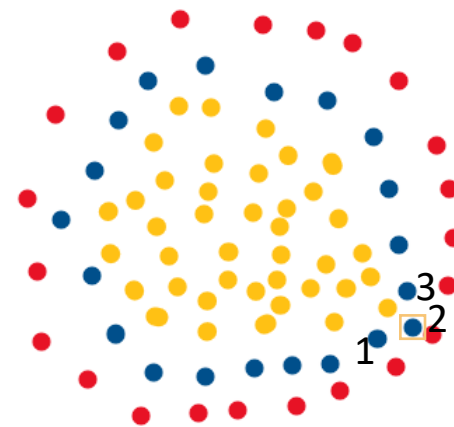
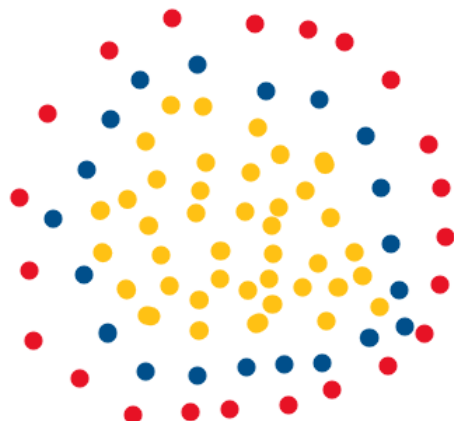
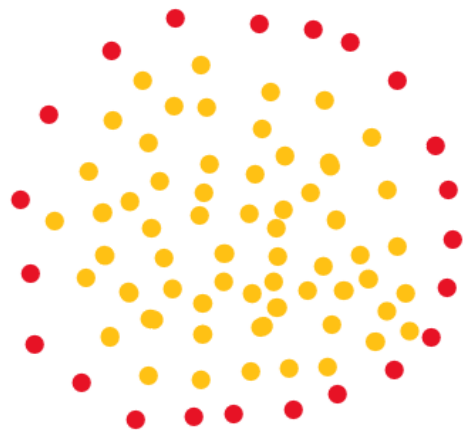
# ★ FastMarching

- A region-growing scheme, important in APP2 GWDT & Initial neuron reconstruction
  - **Step 1-initialization**
    - seed vertices - ALIVE; neighbors of seeds - TRIAL; rest – FAR
  - **Step 2-recursion**
    - from TRIAL vertices, extract one vertex  $x$ , with minimum distance value to the ALIVE set.
    - $x$  : converted from TRIAL to ALIVE,
    - Non-ALIVE neighbor  $y$  of  $x$  to TRIAL if it is FAR.
    - Distance function of  $y$  is updated



# APP2 Step 1: GWDT based on FM

GWDT based on fast marching (FM)



● Alive  
● Trial  
● Far

$$d(y) = \min\{d(y), d(x) + e(x, y)\}$$

$$e(x, y) = \|x - y\| \cdot I(y)$$

$$d(x) = \begin{cases} I(x) & x \in \{background\} \\ \infty & x \notin \{background\} \end{cases}$$

Distance(1)

Distance(2)

Distance(3)

...

Distance(n)

Distance(n+1)

Distance(1)

Distance(3)

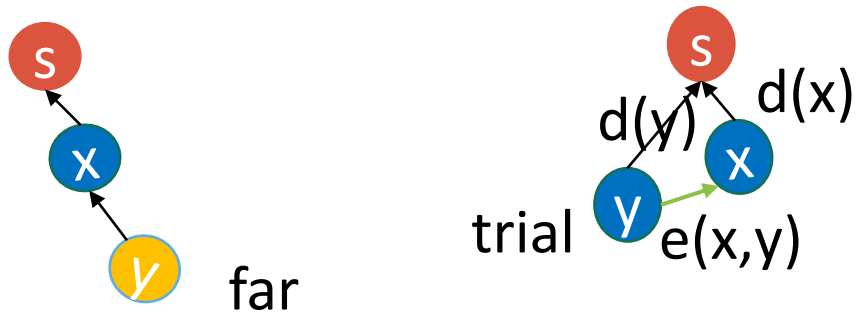
# APP2 Step 2: Initial neuron reconstruction

## FM Initialization step:

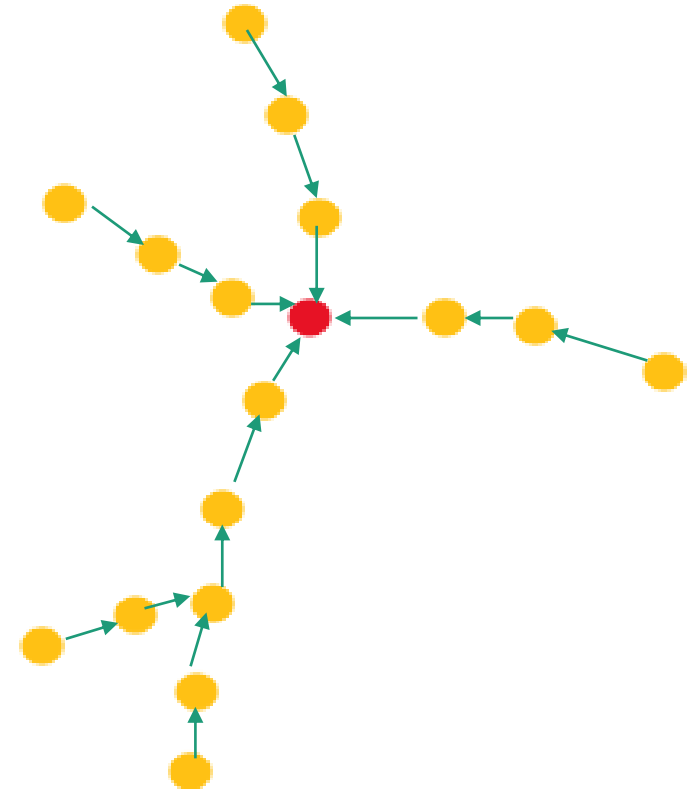
- parent of each image pixel  $x$  is set to be itself, i.e.  $\text{par}(x) = x$ .
- for each neighbor pixel  $y$  of  $s$ , we set them to have label 'TRIAL', and  $\text{par}(y) = s$

## FM recursive step:

For the minimum pixel  $x$  and each of its neighbor  $y$



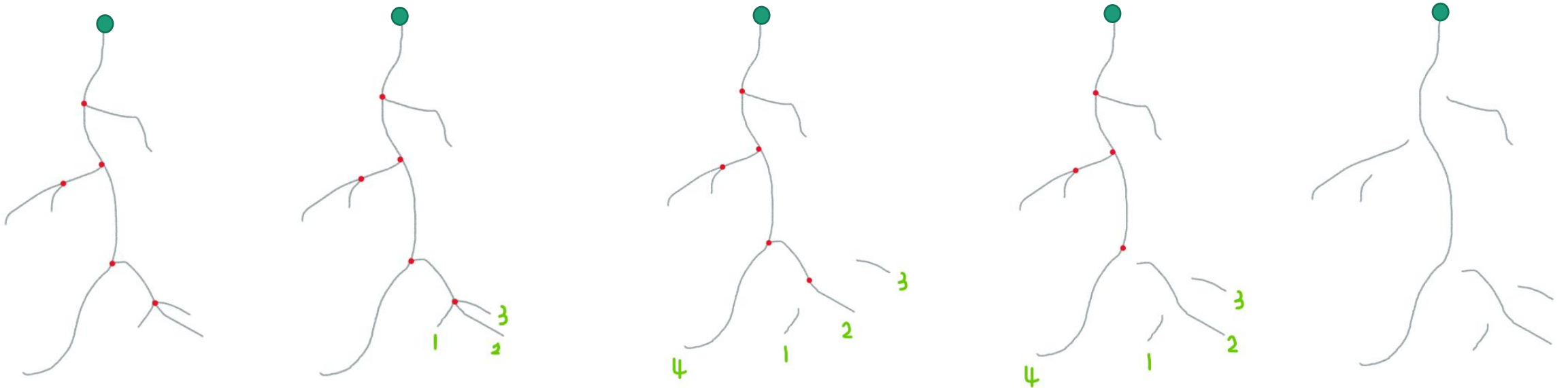
*if  $y$  is FAR, then  $\text{par}(y) = x$ ; if  $d(x) + e(x, y) < d(y)$ , then  $\text{par}(y) = x$ .*



# APP2 Step 3: Hierarchical pruning

## Hierarchical segments construction

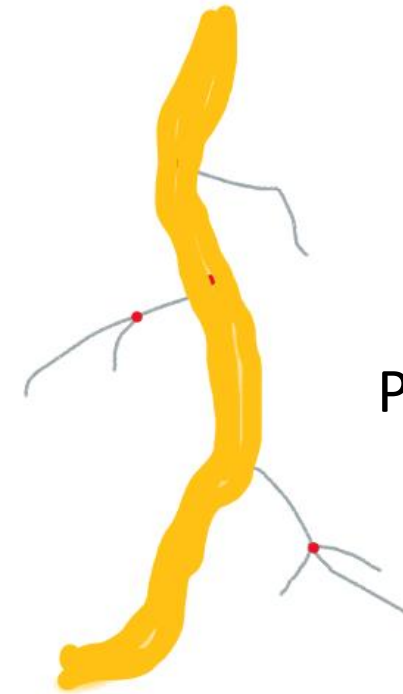
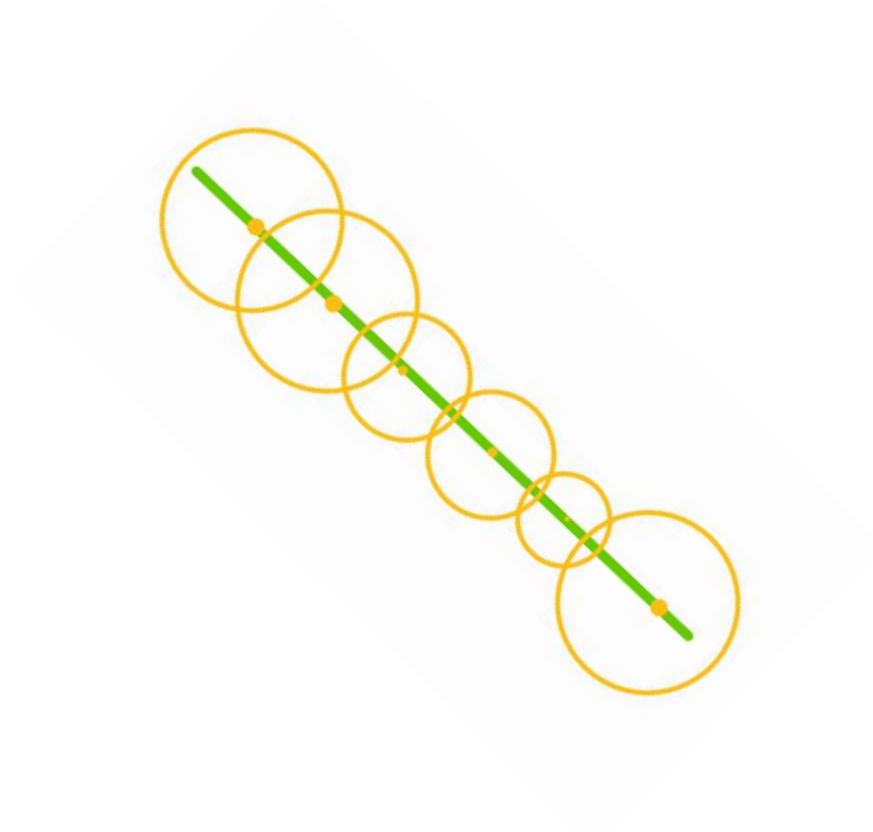
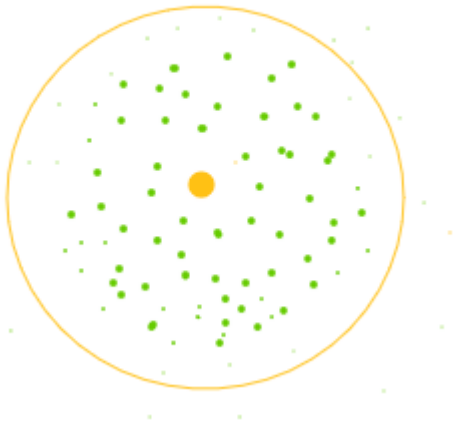
- Order segments from most important to the least important
- Generate a hierarchy of them
- The 'importance' of a segment is defined based on its length



# APP2 Step 3: Hierarchical pruning

## Recursive pruning

- coverage area of a node
- coverage area of segment
- coverage ratio of segment

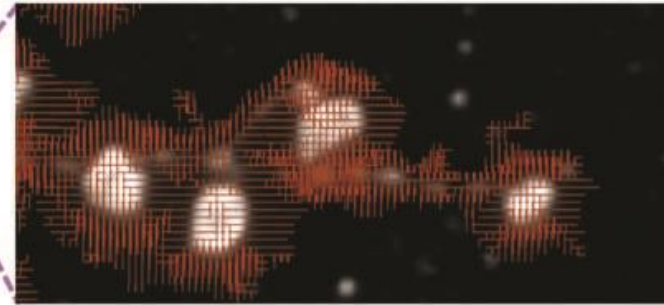
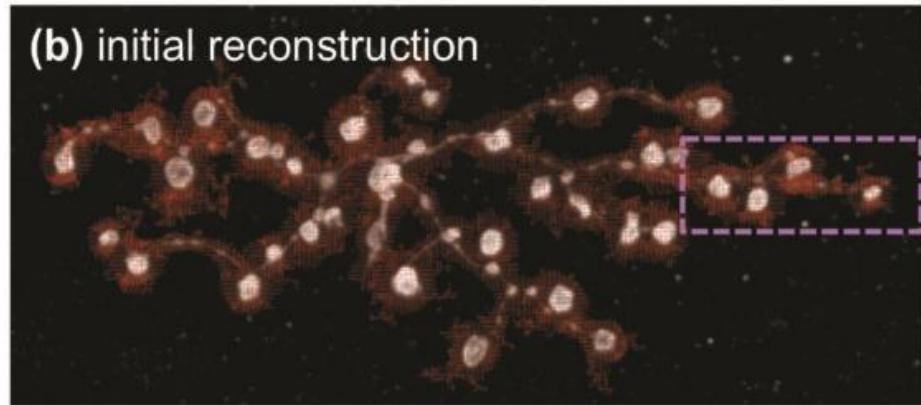


If >75%,  
Prune away

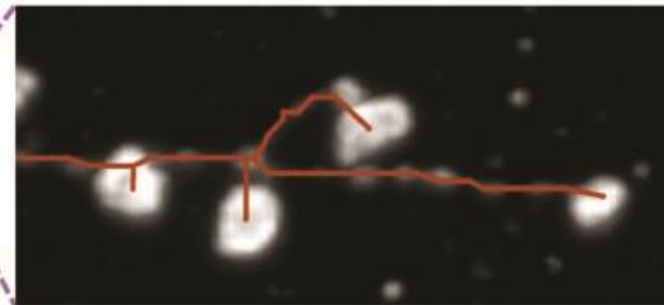
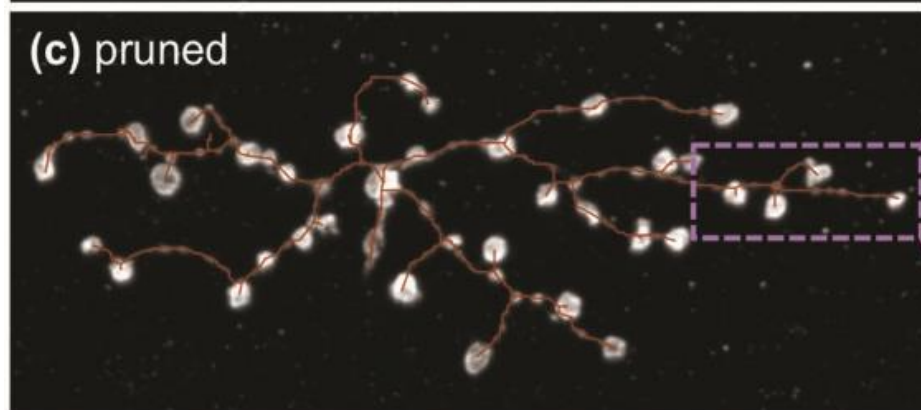
- image-pixel-intensity weighted coverage ratio

# APP2 Step 3: Hierarchical pruning

Recursive pruning -- result



Smooth

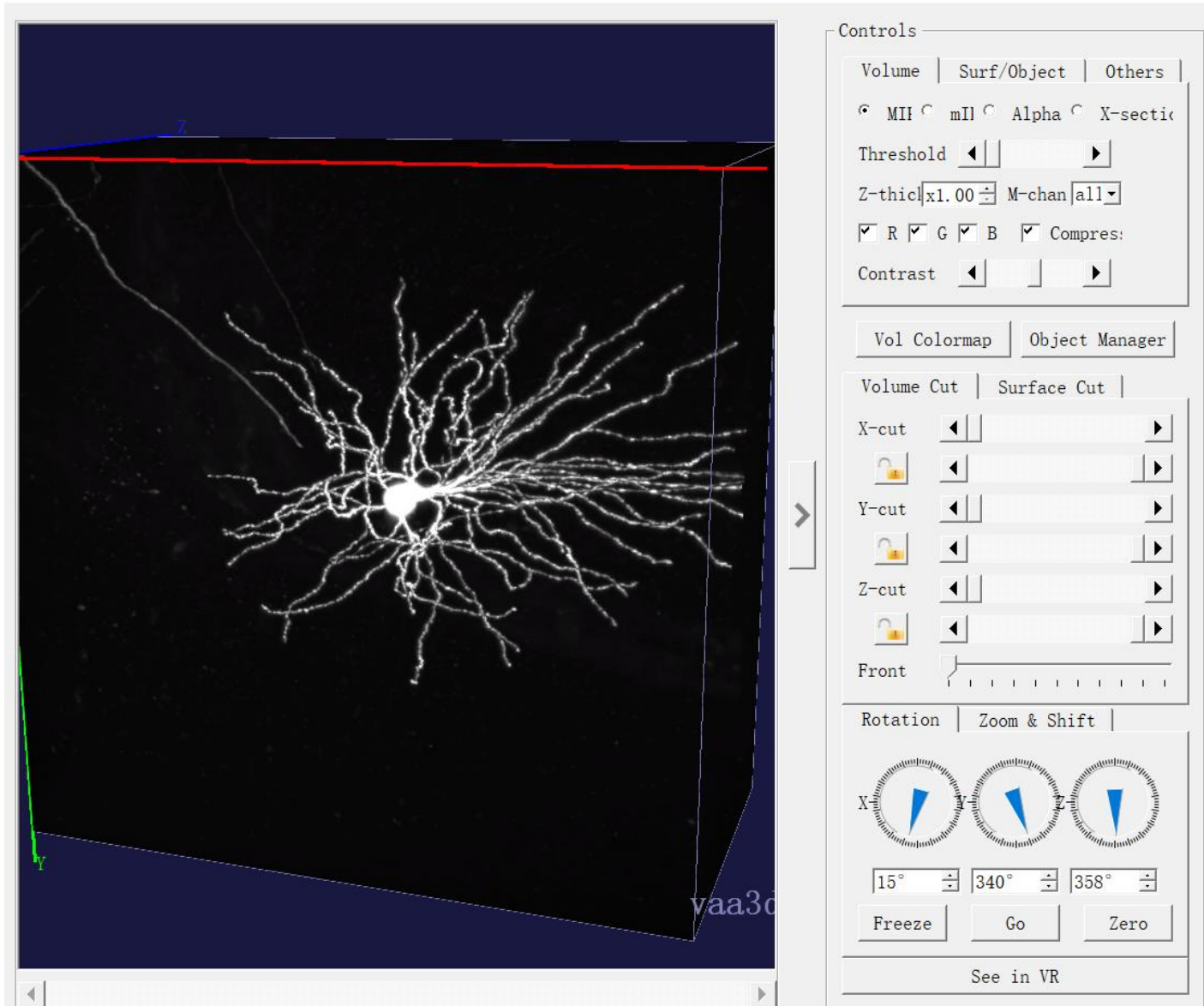


Enjoyable

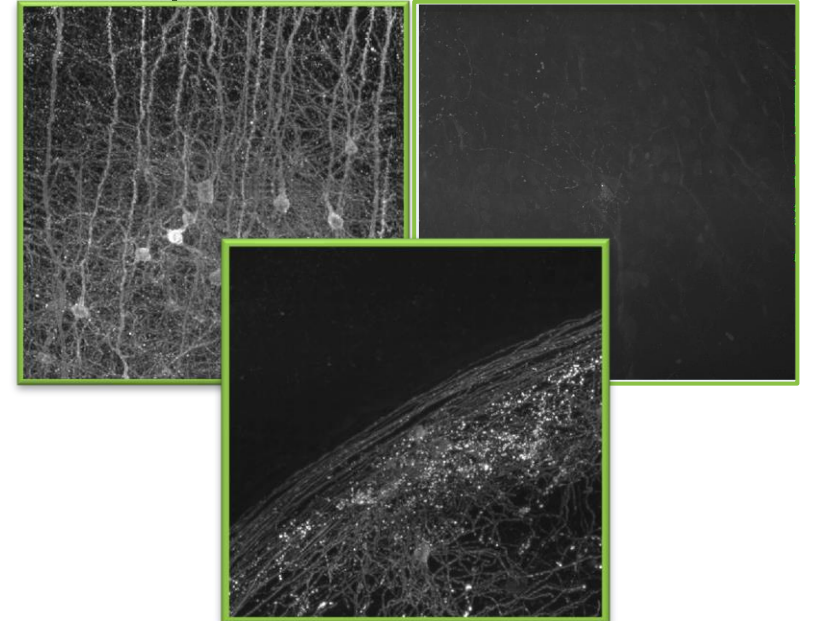


# App2 in Vaa3D

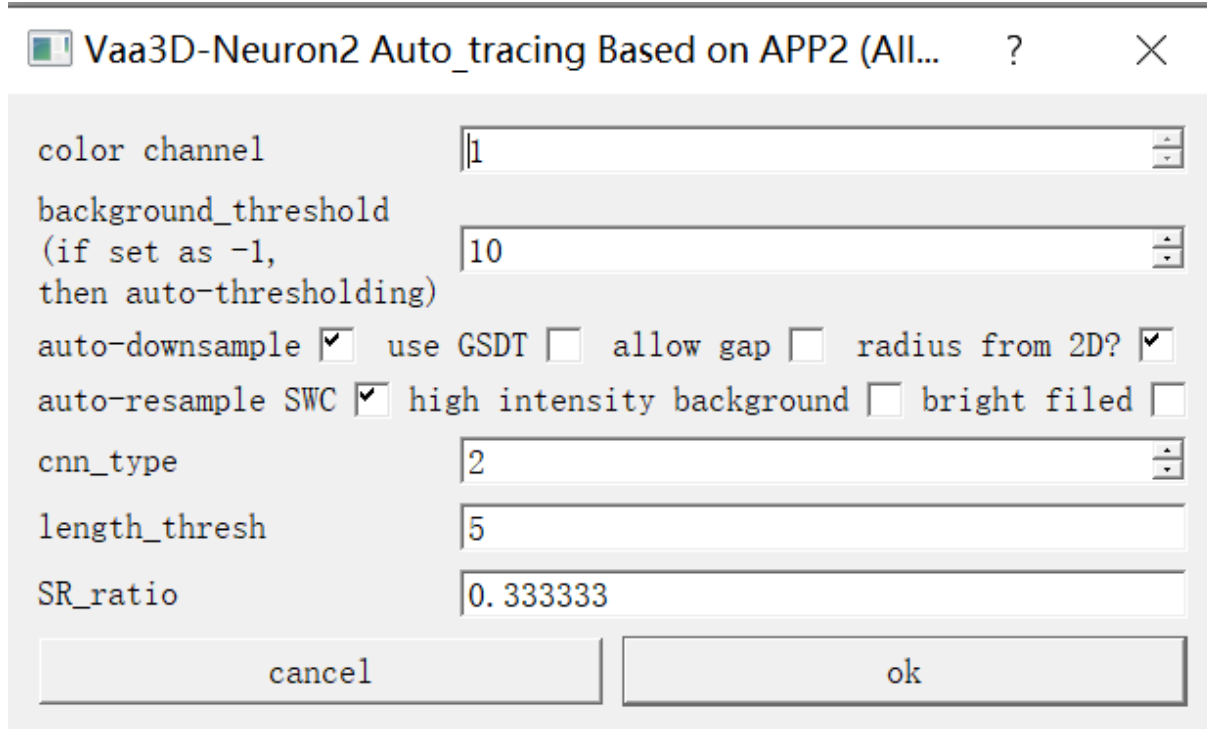
3D View [D:/PPT/app2\_test\_images/test1.tiff]



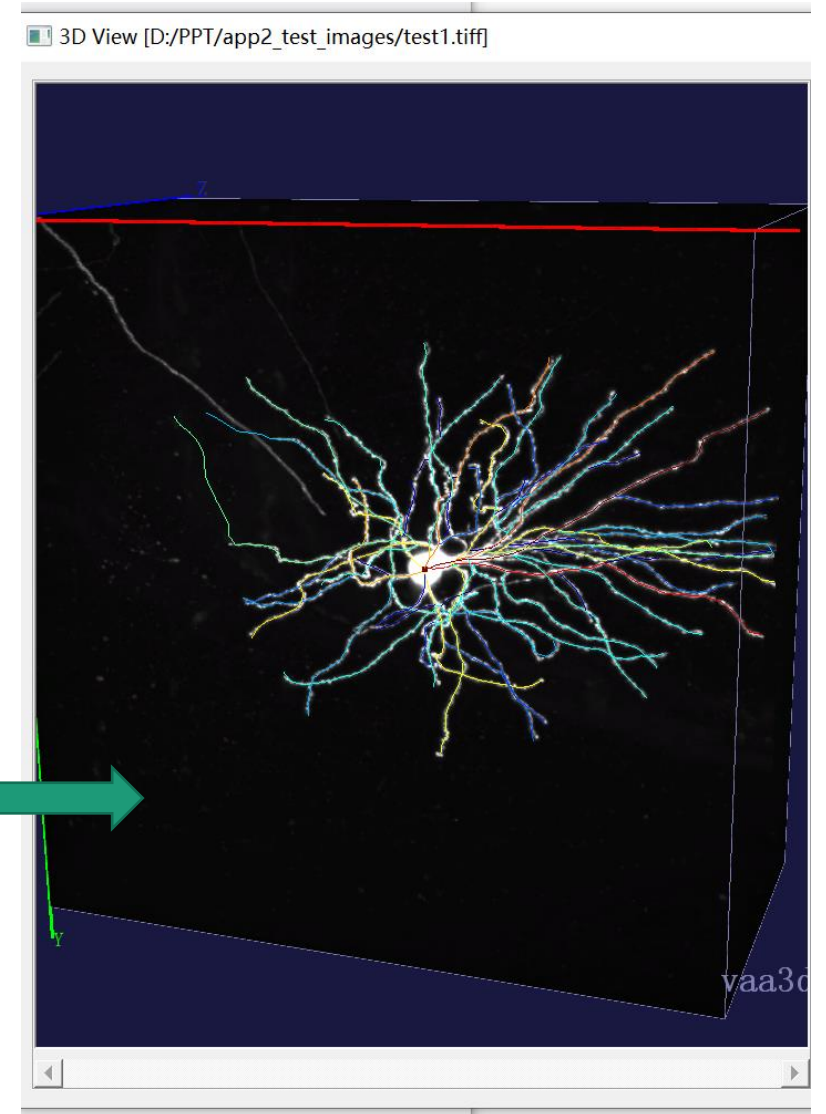
- Base tracer
  - Base tracer vs. UltraTracer
- Single and Sparse neuron
  - Not designed for densely interweaved neurons
- Samples not suitable for App2:



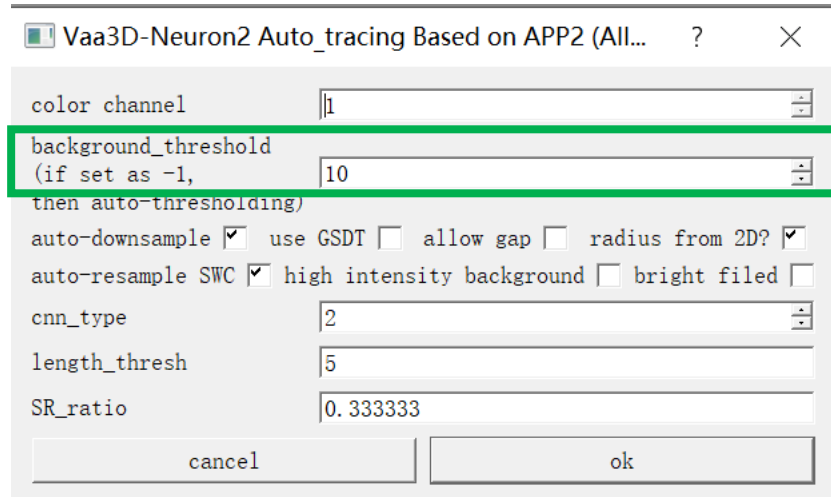
# App2 Interface with default parameters



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



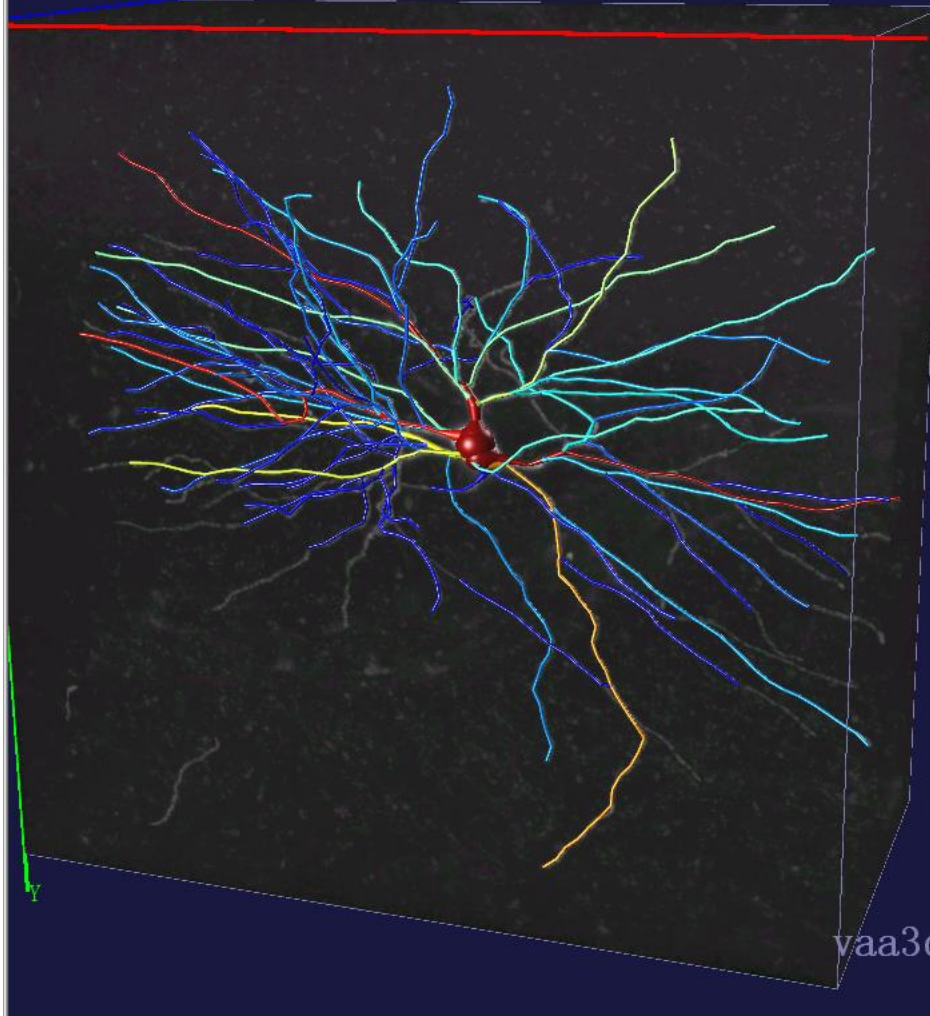
# App2 Parameters: Background threshold



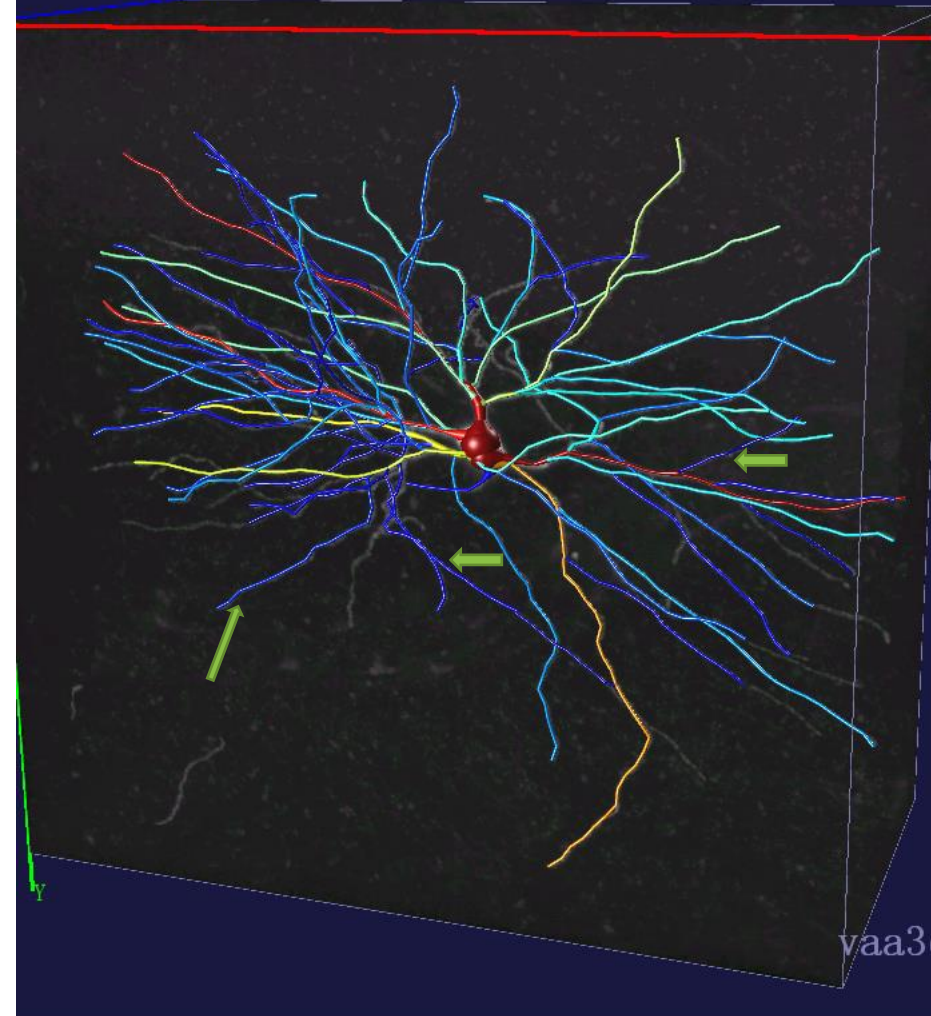
- How it works?
  - With low threshold, App2 generate initial reconstruction and prune away irrelevant segments
- Is it critical?
  - Yes and No
- Background threshold:
  - Default:10
  - Automatic determining threshold: with -1
    - $\text{Mean} + 0.5 * \text{Std}$

# Automatic background threshold

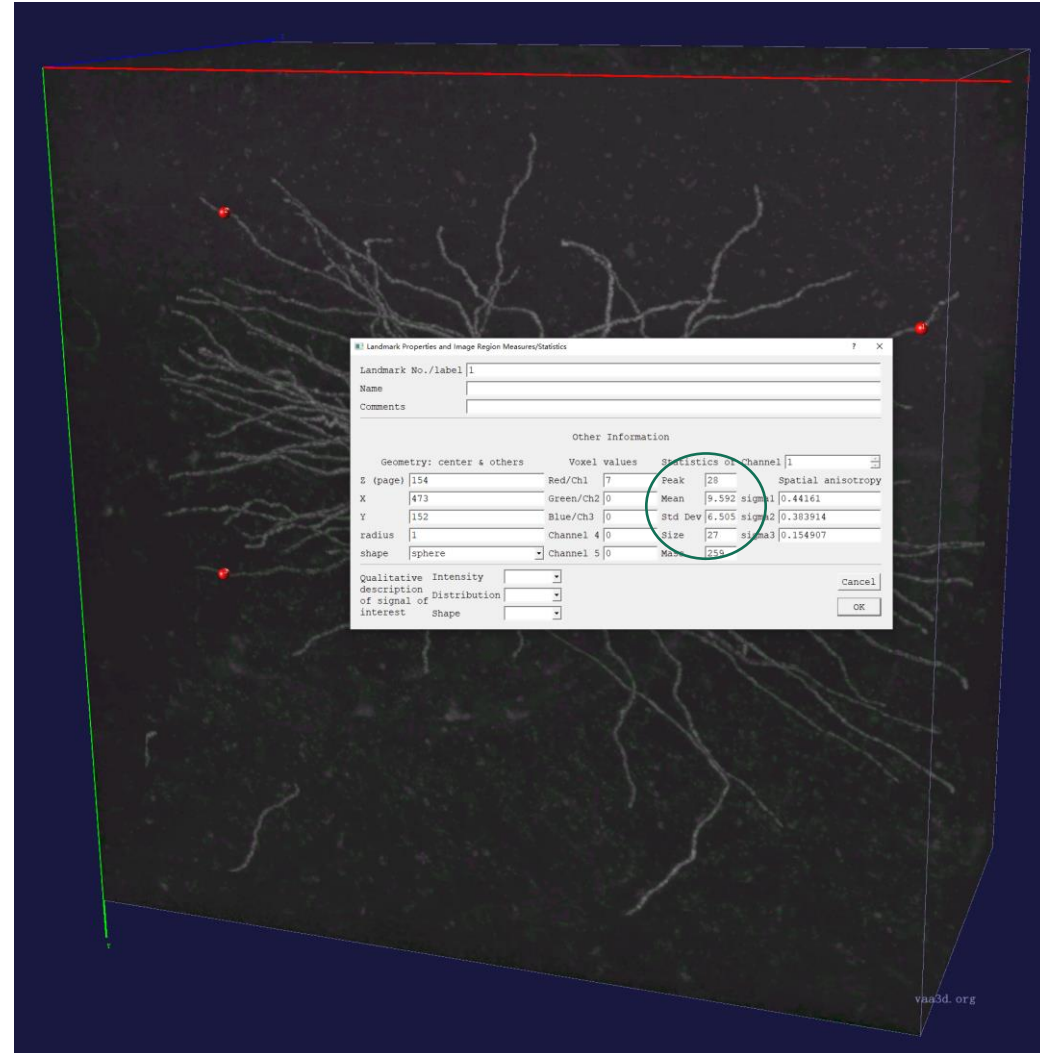
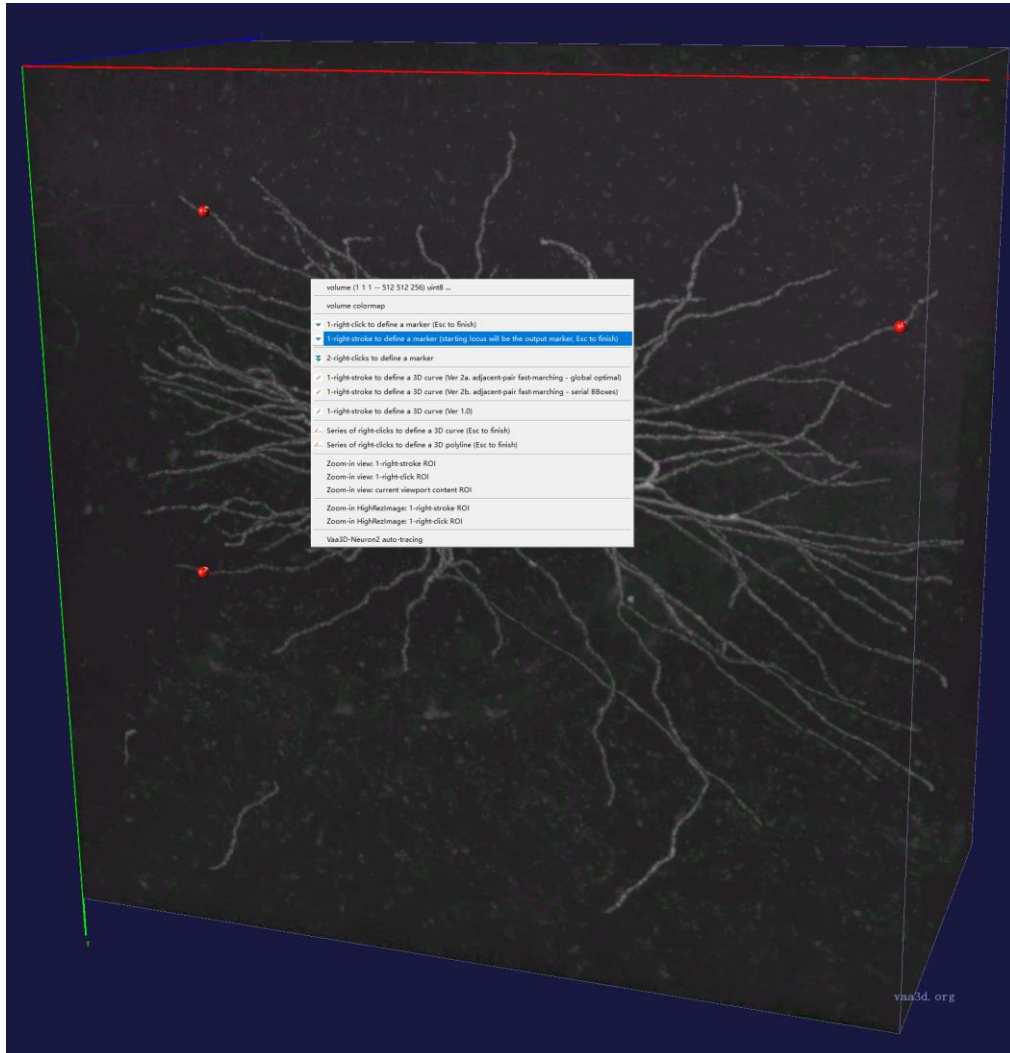
Background Thresh: 10



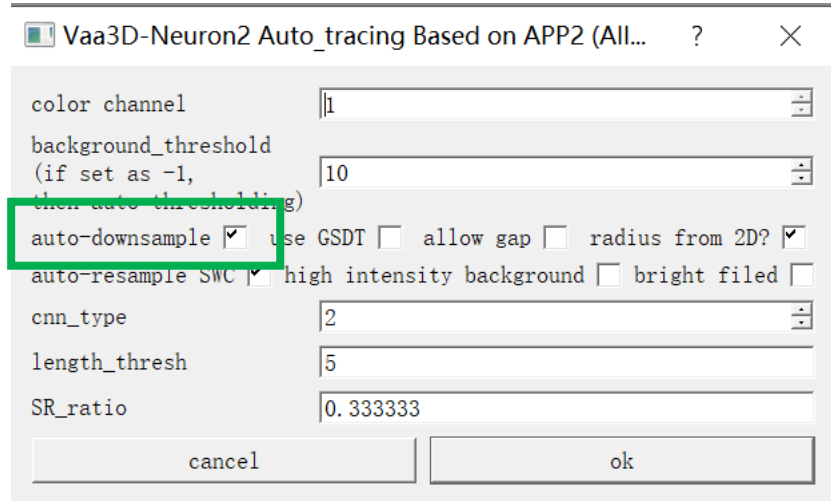
-1(Auto)



# How to estimate a good threshold?

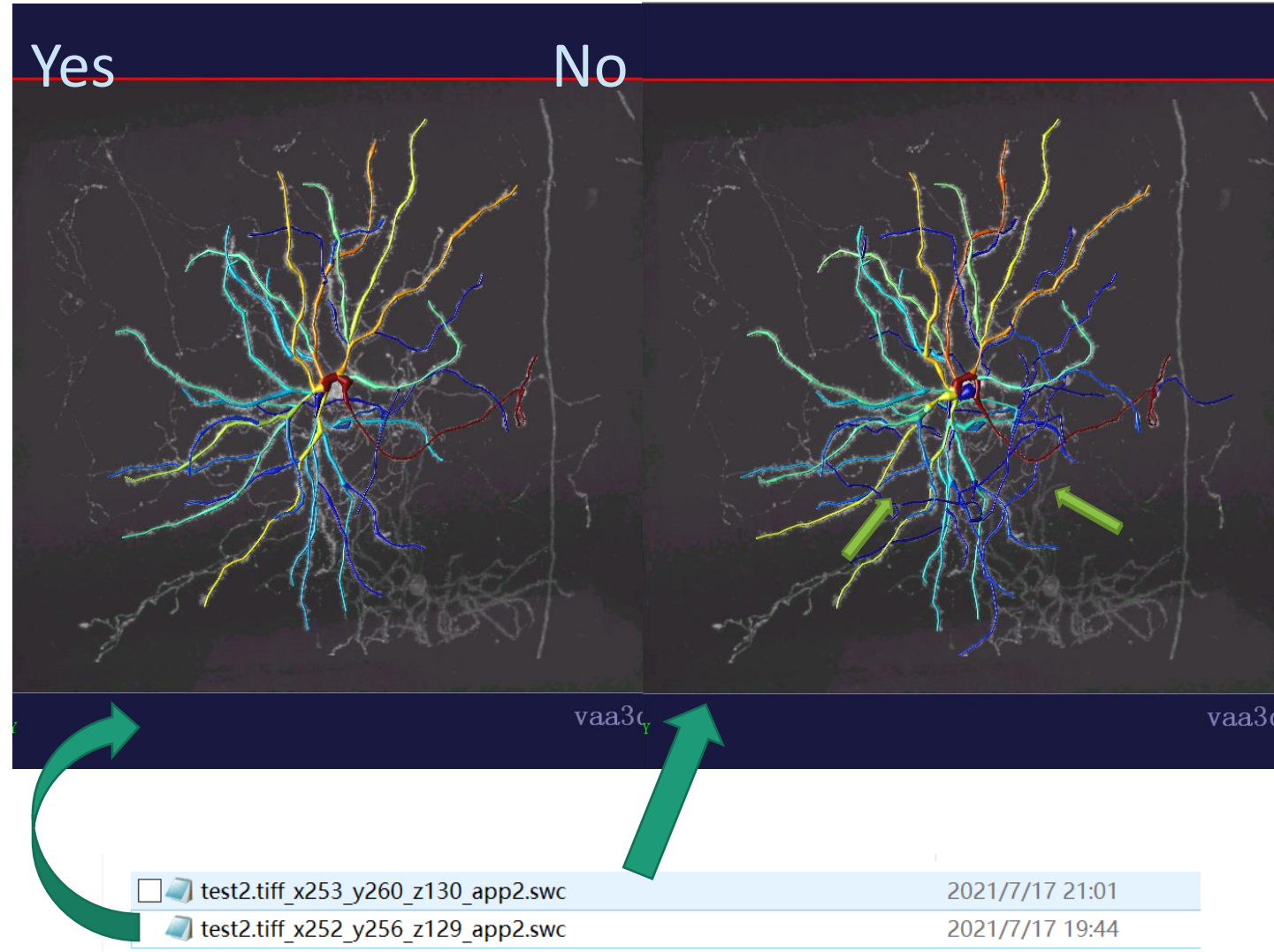


# App2 Parameters



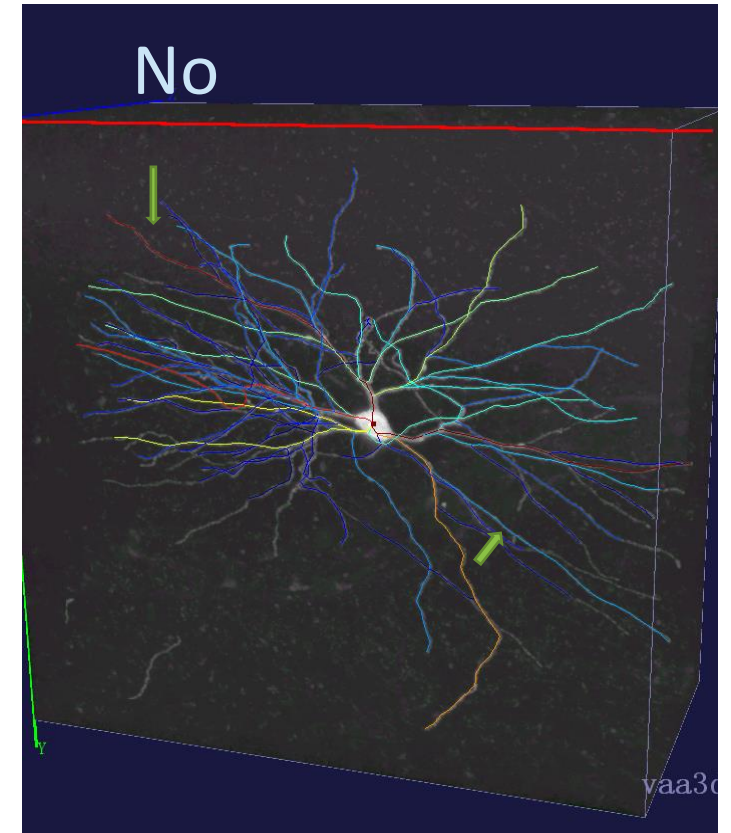
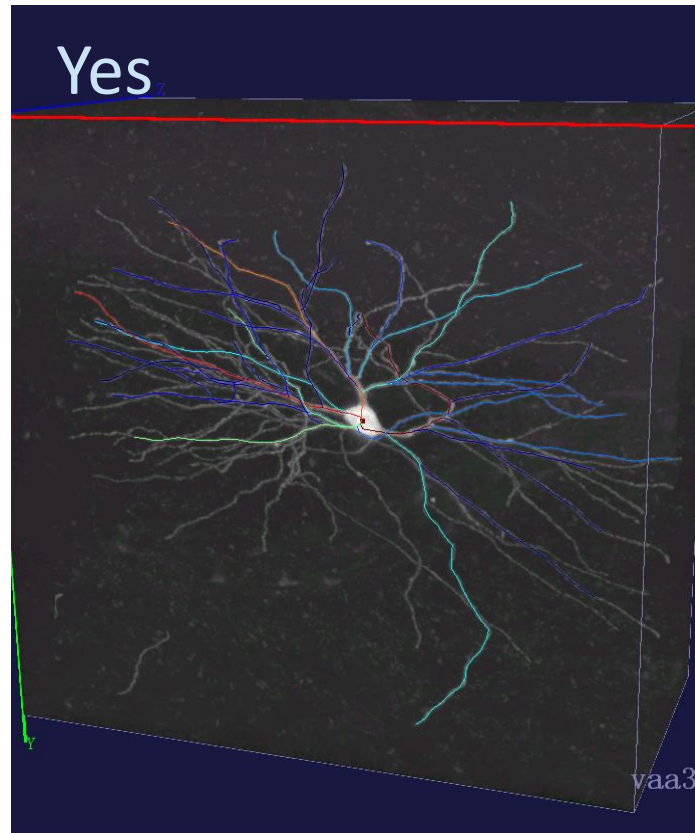
- Auto-downsample

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

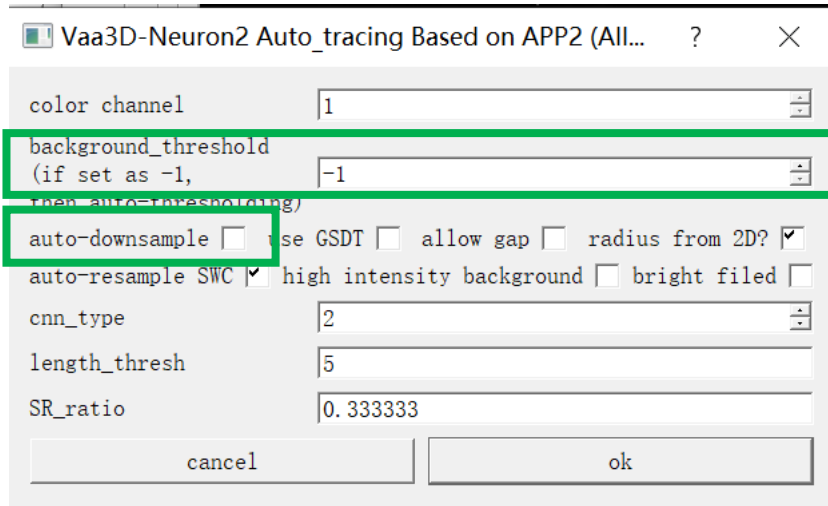


# Auto-downsample

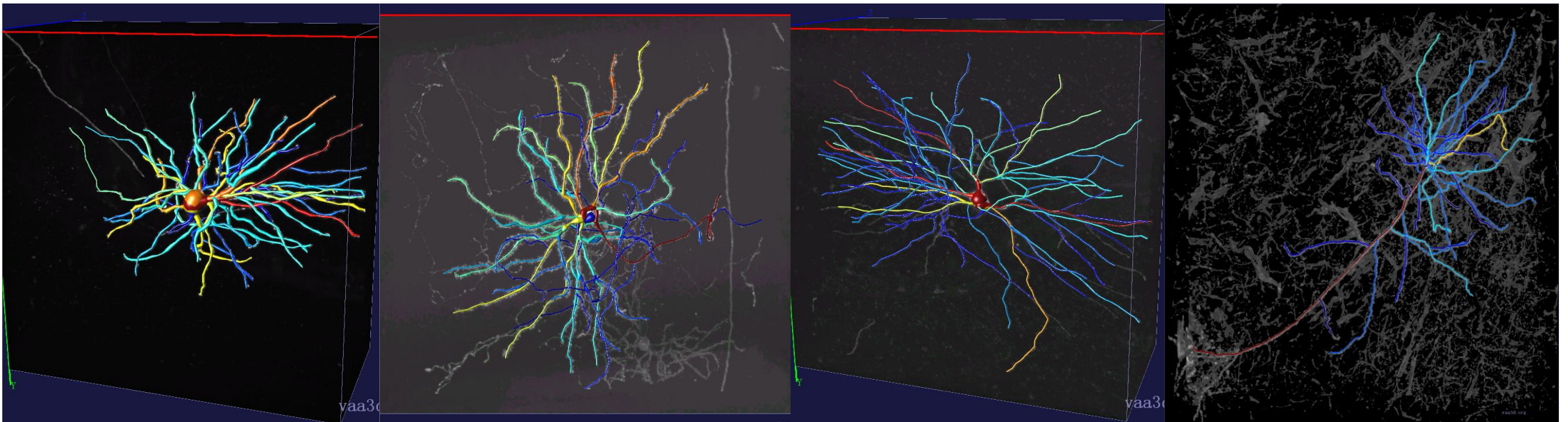
- Auto-downsample
  - Set it to “No” whenever your computer can handle it
  - Set it to “No” for the rest of my talk



# App2 Parameters that fits most cases

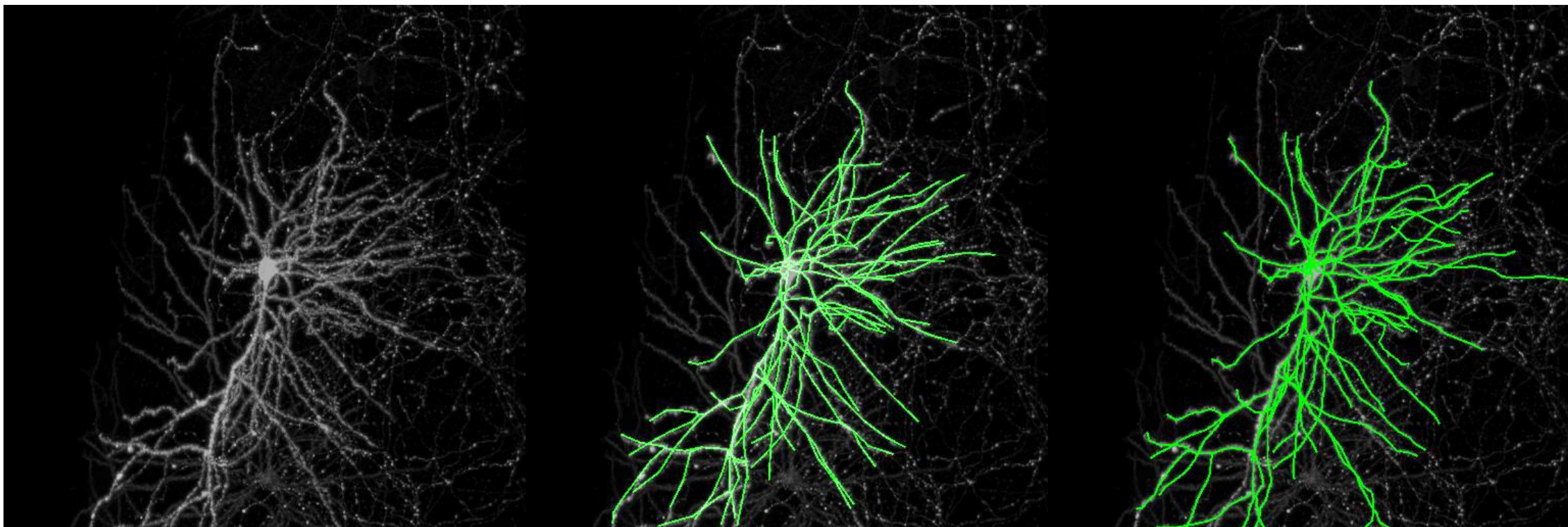


- Auto-downsample
  - No
- Background threshold:
  - Automatic determining threshold: with -1
    - Mean + 0.5 \* Std
- **Would suggest this to all cases as initial test**



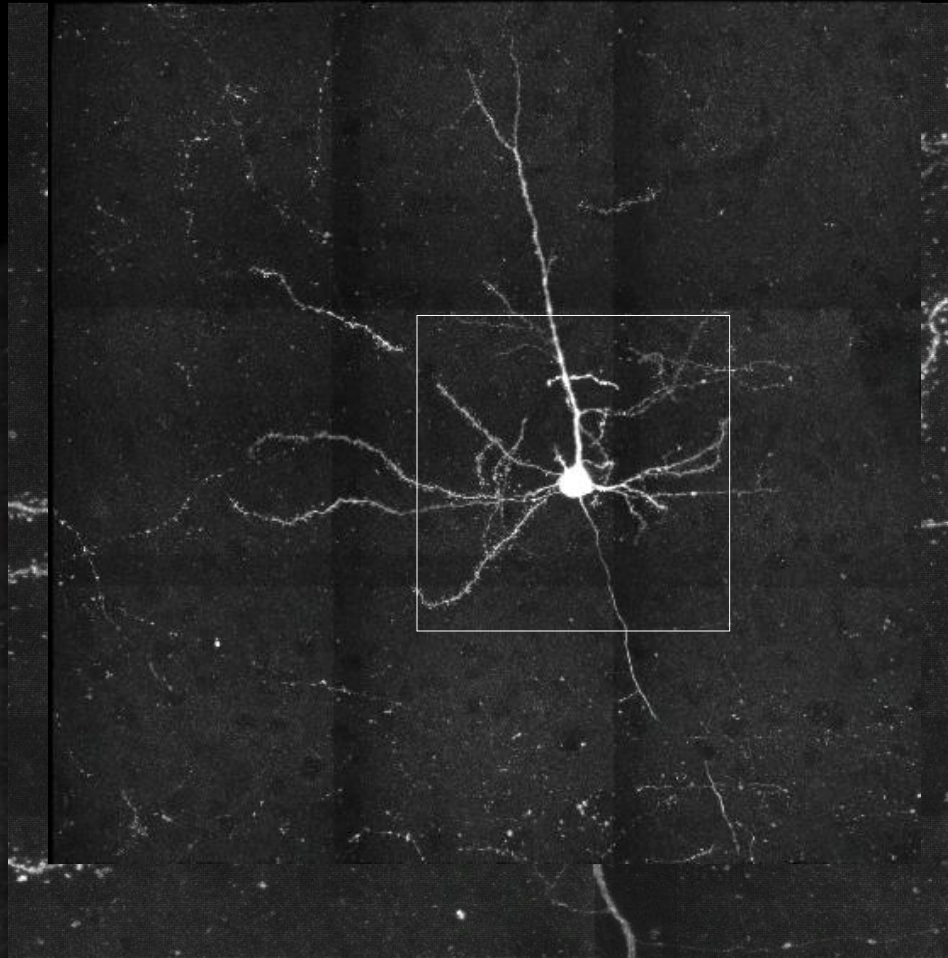


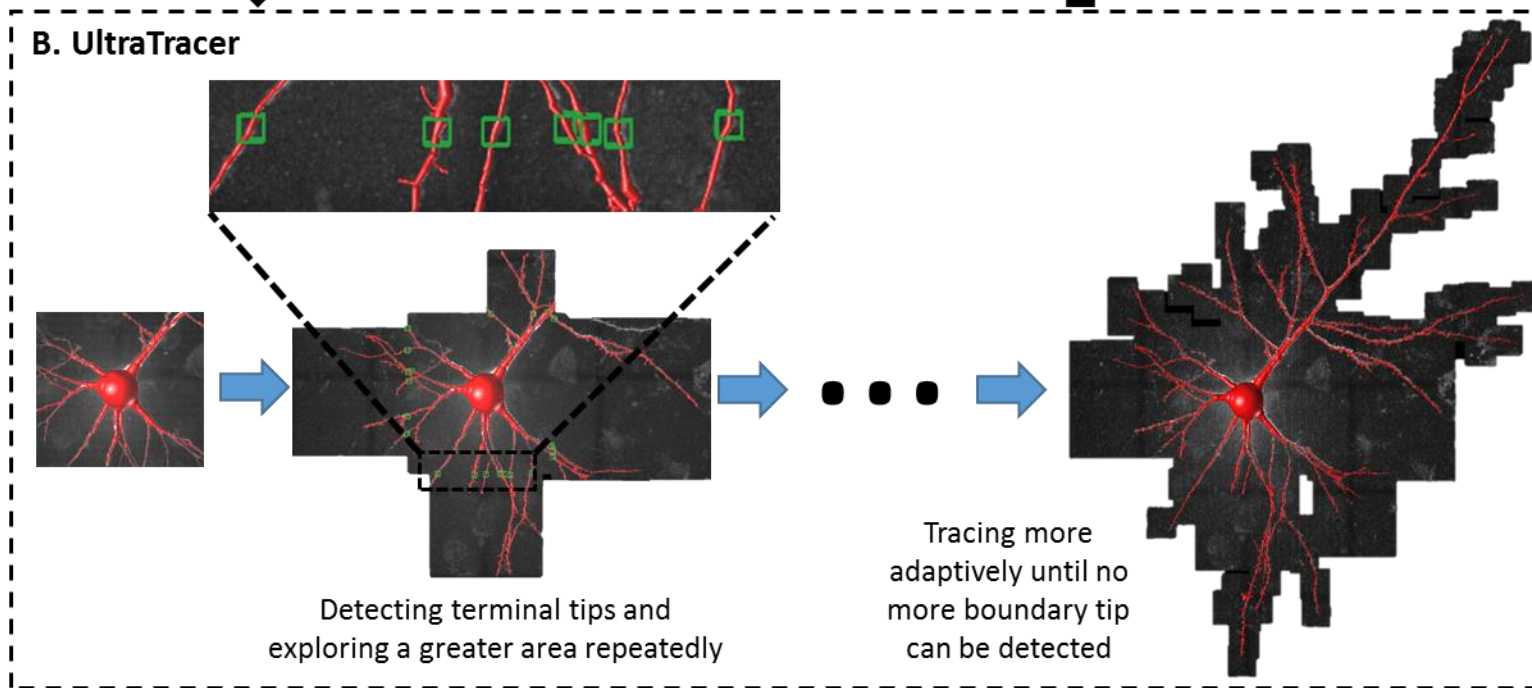
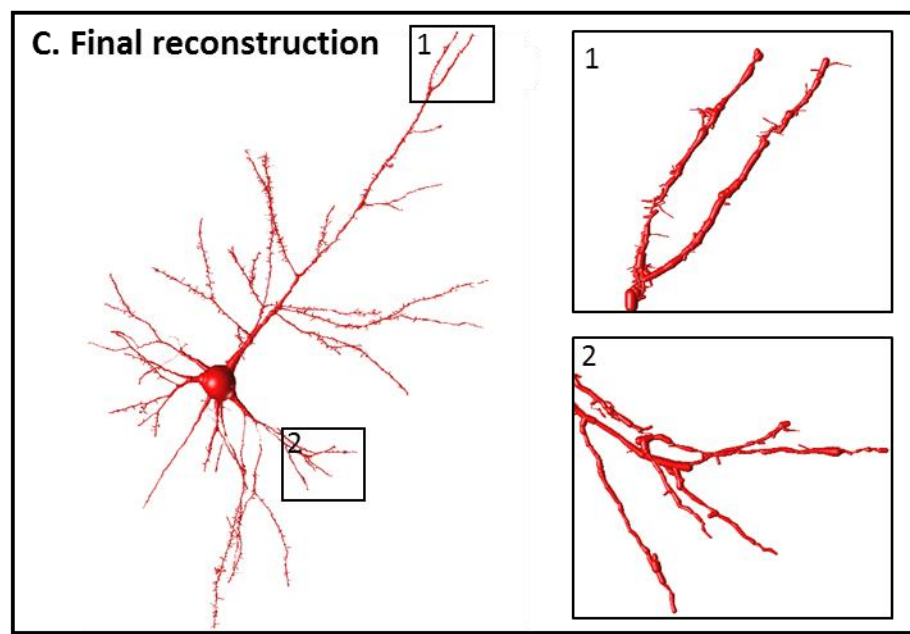
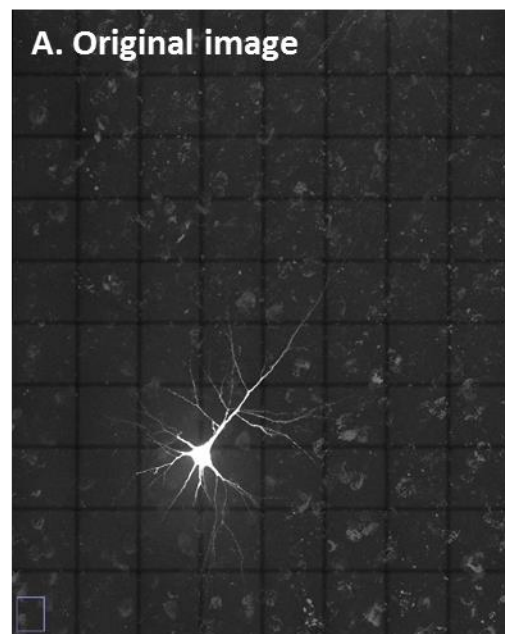
# Automatic Tracing Examples

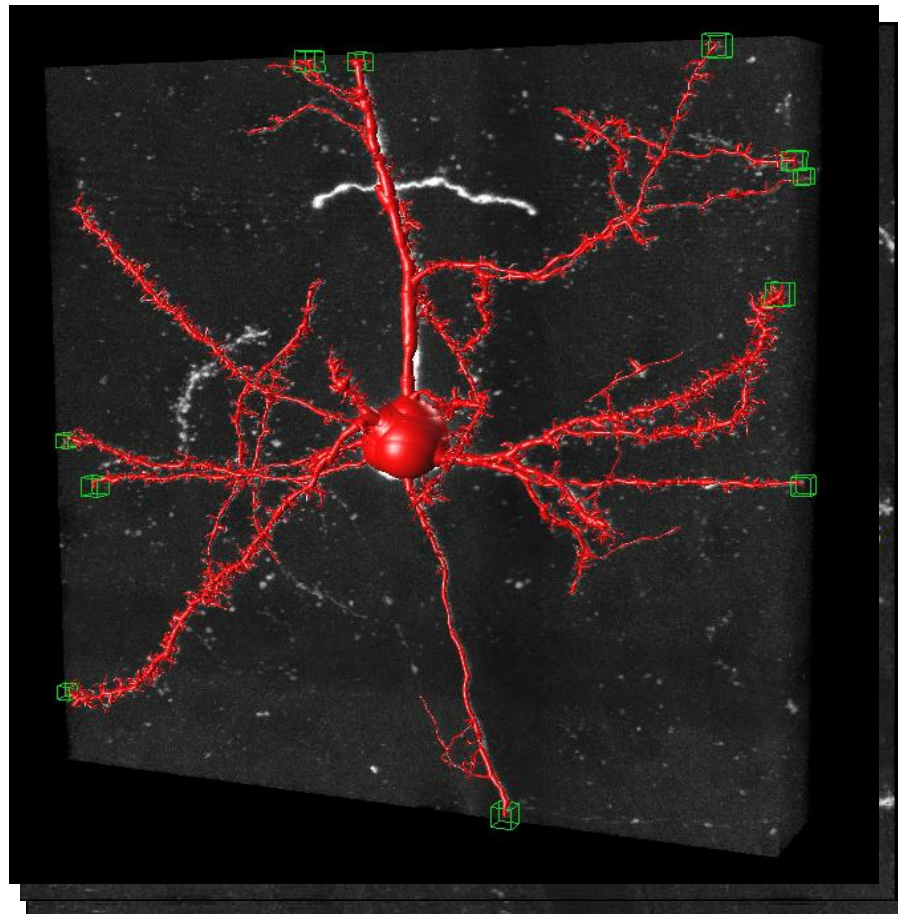
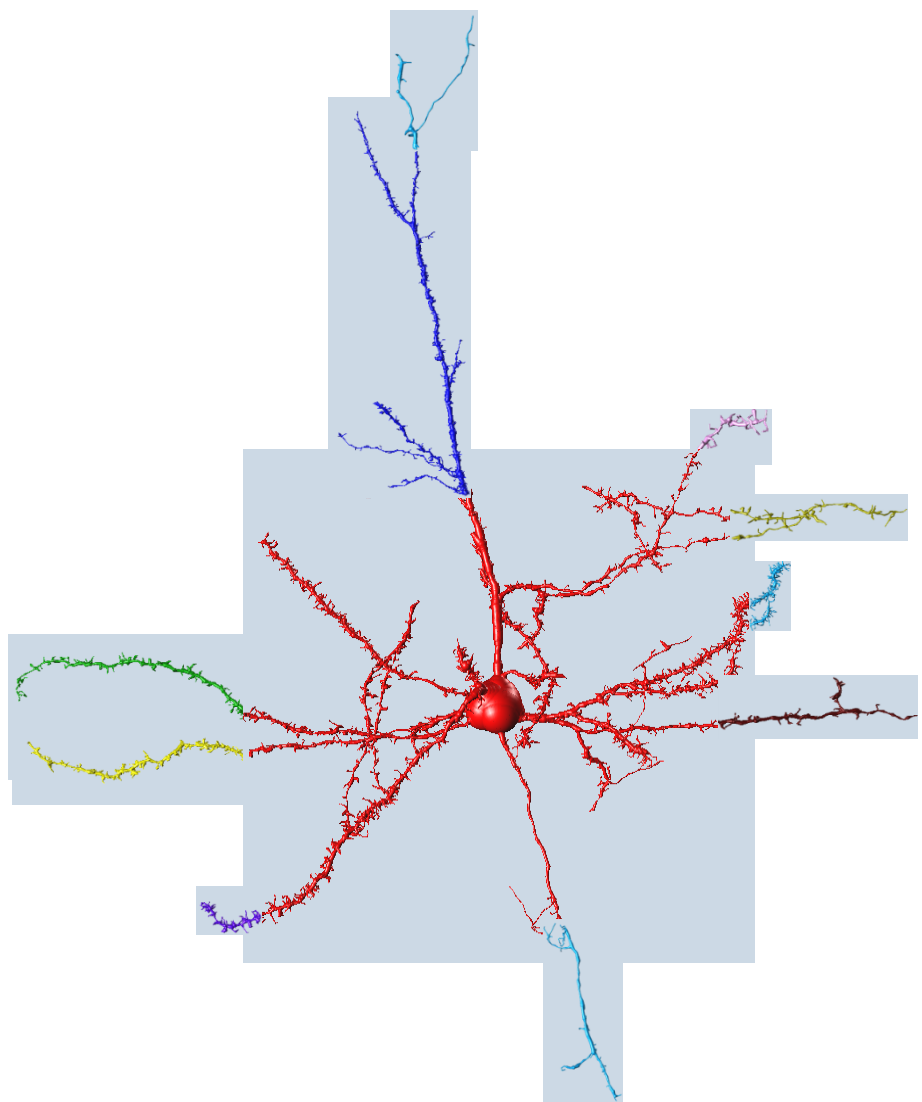


UltraTracer

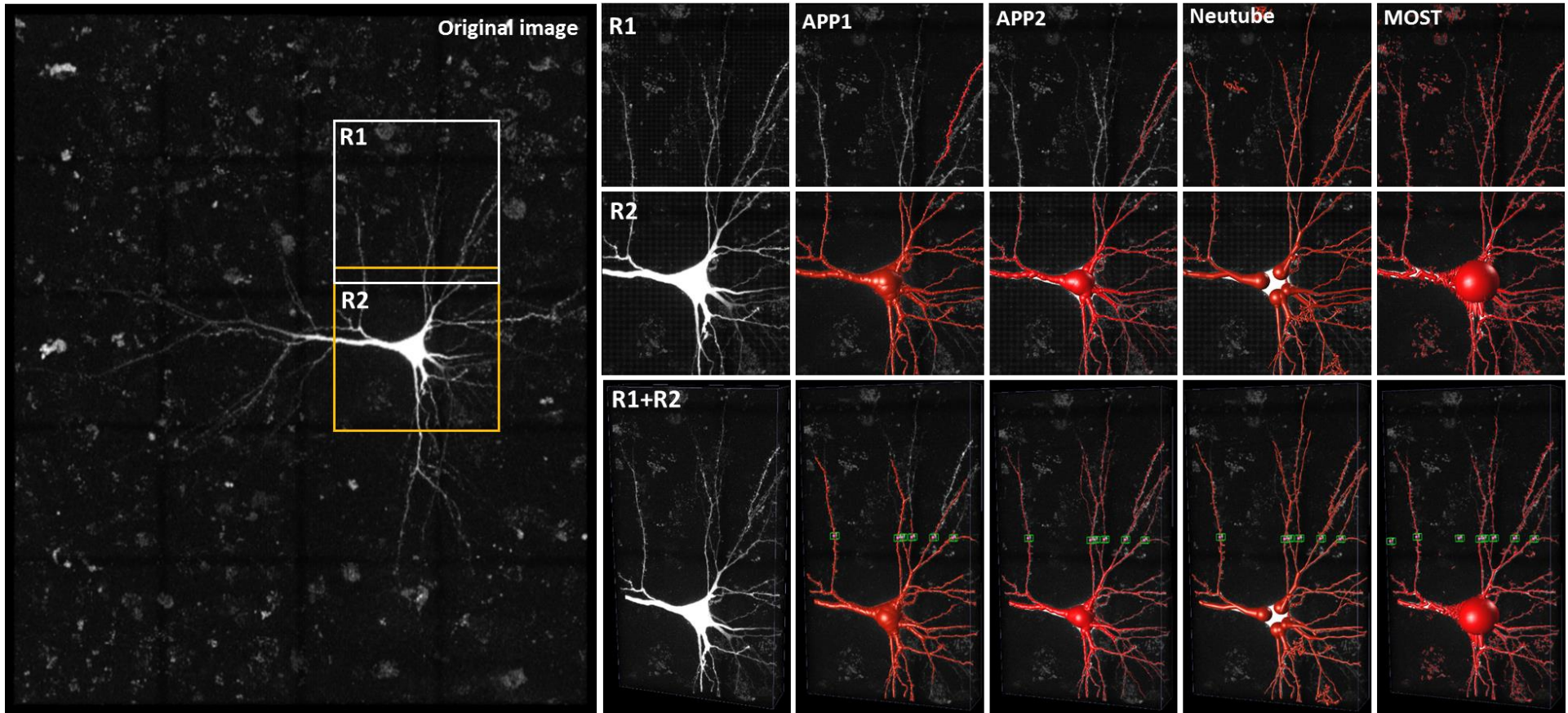
- Mouse RV-GFP-layer4\_Slide1.3, Imaging with a 63x, 1.4 NA objective.
- Voxel size: 143 nm x 143 nm x 280 nm.
- 126 tiles: 1024 x 1024 x 159.
- 29.9 GB.



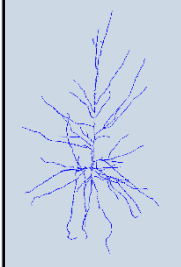
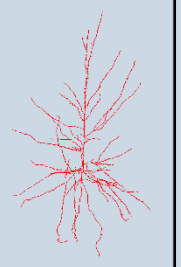
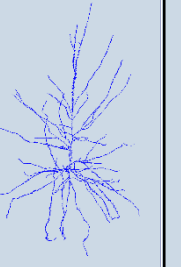
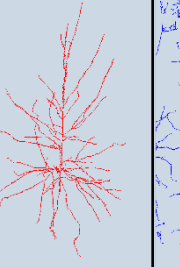
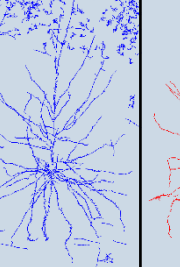
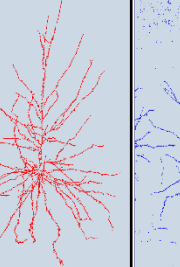
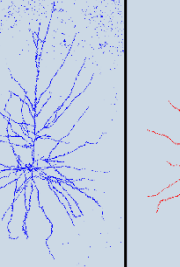
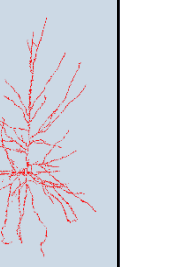




# Tip-queue based Neuron Growth Algorithm



# Comparison Results

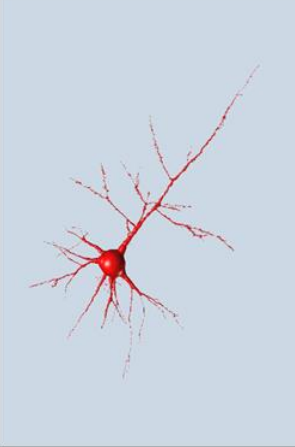
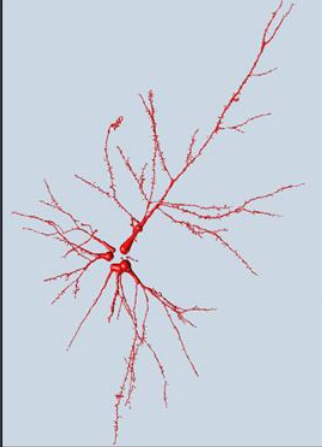
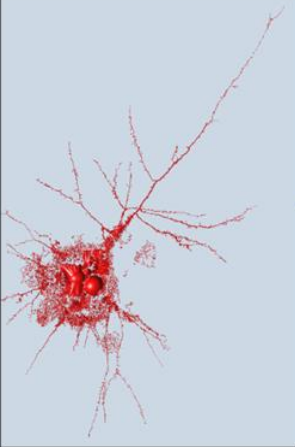
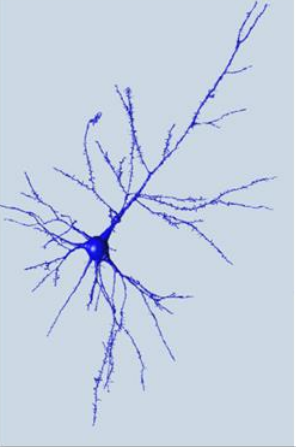
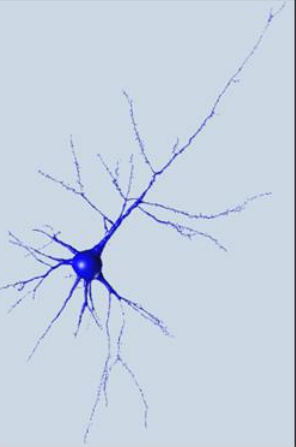
Algorithm	APP1		APP2		Neutube		MOST	
Tracing Method	TR	UT	TR	UT	TR	UT	TR	UT
Morphology Reconstruction								
BASDM (voxels) (against 2 ind. manual tracings)	1.83, 2.35	1.99, 2.50	2.30, 3.17	2.78, 3.57	179.72, 173.71	5.17, 5.42	39.83, 38.83	3.01, 3.42
Peak Memory (GB)	77.18	14.21	31.23	2.82	27.9	1.92	4.61	0.64
Tracing Time (s)	956.94	1132.82	69.95	115.67	1322.50	3317.86	22.43	29.65
Total Cost (PM x TT)	73856.63	16097.37	2184.54	326.19	36897.75	6370.29	103.40	18.98
Performance Gain (UT/TR)	3.59		5.70		4.79		4.45	

BASDM (BASD score compared to a pure human manual reconstruction);

BASD: Best Average Spatial Distance.

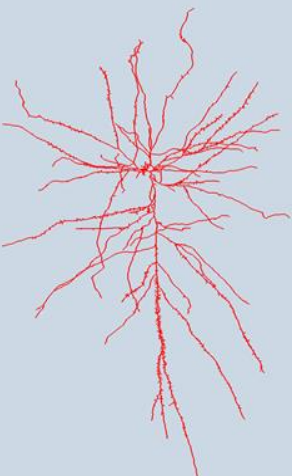
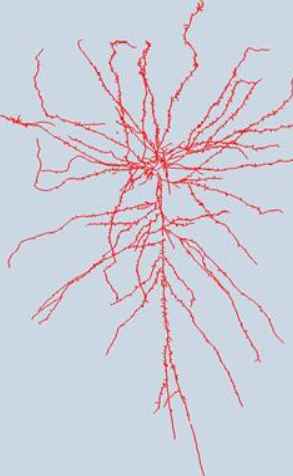
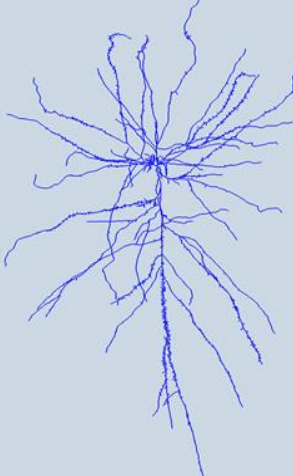
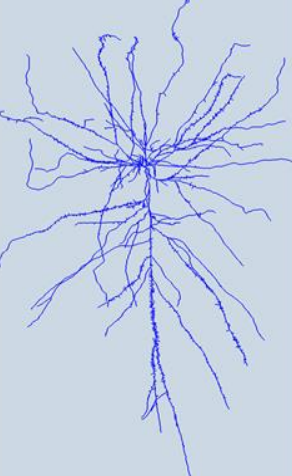
**BASD score between two pure human manual reconstructions is 3.56.**

# Combination Scheme 1

Algorithm	APP2	Neutube	MOST	APP2+Neutube	APP2+MOST
Morphology Reconstruction					
Total Scanned Areas (billion voxels)	0.94	2.80	1.90	2.80	1.90
Tracing time (s)	40.63	2250.73	408.91	2158.69	89.18





# Combination Scheme 2

Algorithm	APP2	Neutube	Real-time selection	
			Best candidate	Consensus
Morphology Reconstruction				
BASDM (voxels) (against 2 ind. Manual tracings)	2.78, 3.57	5.17, 5.42	2.62, 3.42	3.73, 4.18
Tracing time (s)	115.67	3317.86	3565.69	3204.97

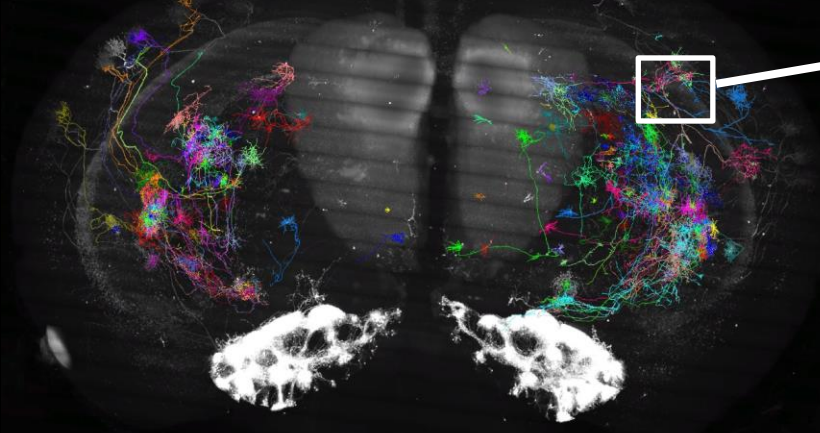
# Availability

**Software availability:** UltraTracer is open source and available in Vaa3D software ([vaa3d.org](http://vaa3d.org)). Currently Linux only.

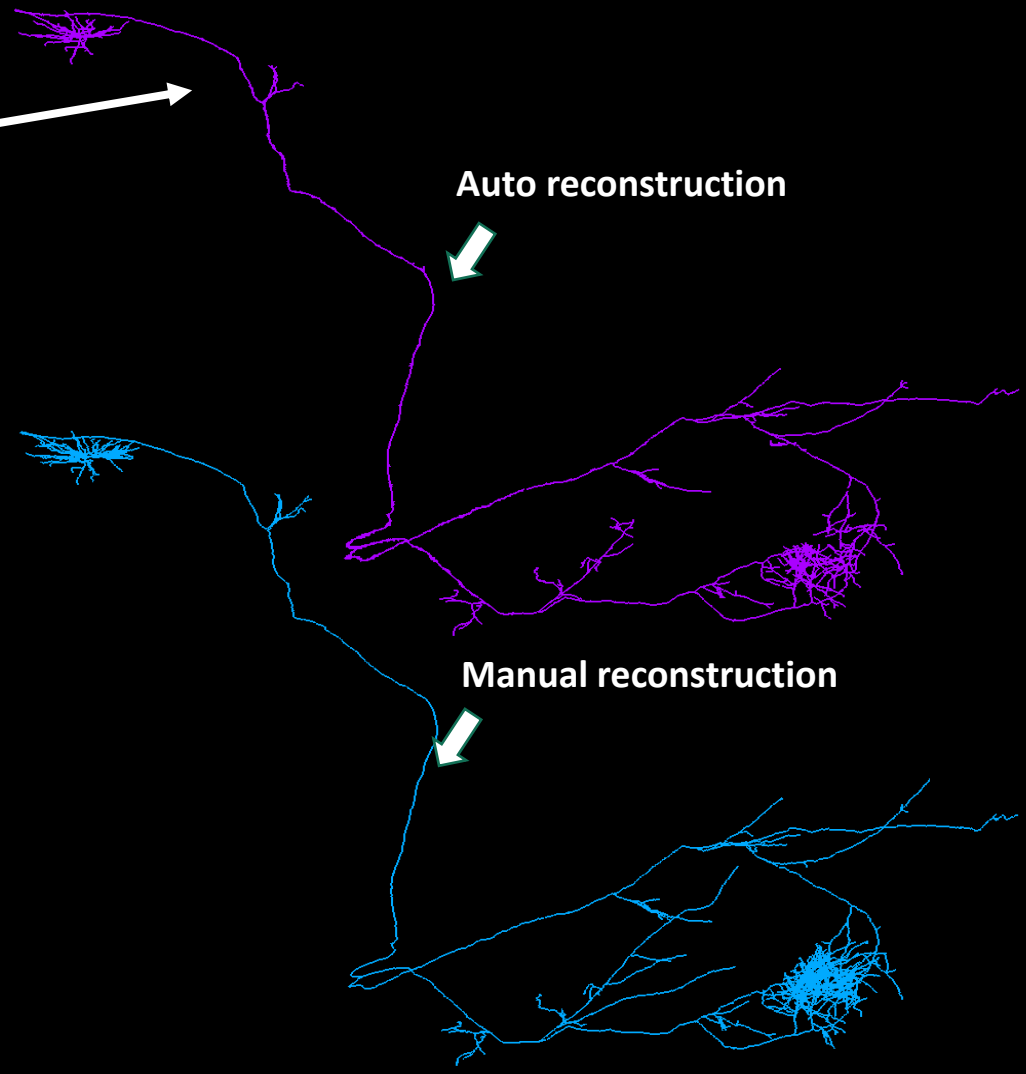
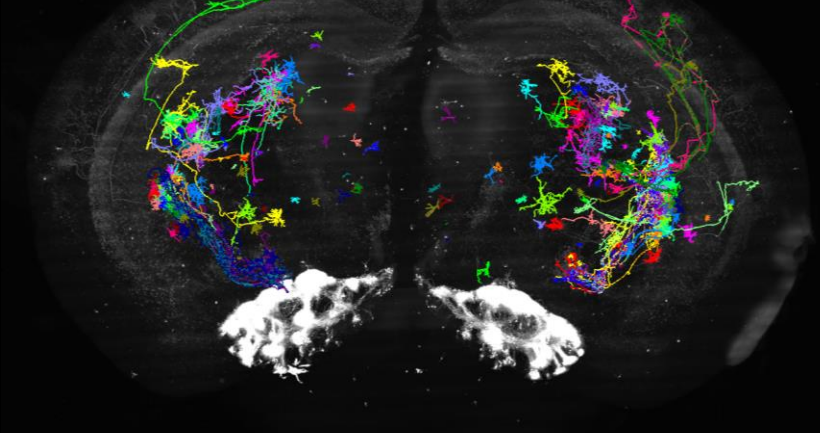
**Data availability:** The sample data are publicly available and can be downloaded from GitHub ([https://github.com/Vaa3D/Vaa3D\\_Data/releases/download/v0.9/ultratracer\\_testing\\_data.zip](https://github.com/Vaa3D/Vaa3D_Data/releases/download/v0.9/ultratracer_testing_data.zip)).

-  m16\_cing\_1\_9\_cropped\_neurona.v3dpbd.marker
-  m16\_cing\_1\_9\_cropped\_neurona.v3draw

124 Auto reconstructions on brain 17302



174 Auto reconstructions on brain 17545



Auto reconstruction

Manual reconstruction

***Thank you***