Make Sense of .swc Files: Information Extraction via Vaa3D Platform

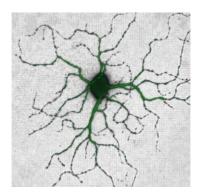


Shuxia Guo

Aug 16th, 2021

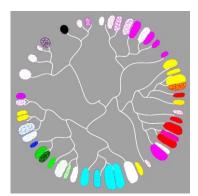


Key Questions in Neuroscience





(morphology, physiology, gene expression, locations, ...)



• Where neurons come from and how they change over time? (lineage, neuron growth/pruning/aging/plasticity,...)

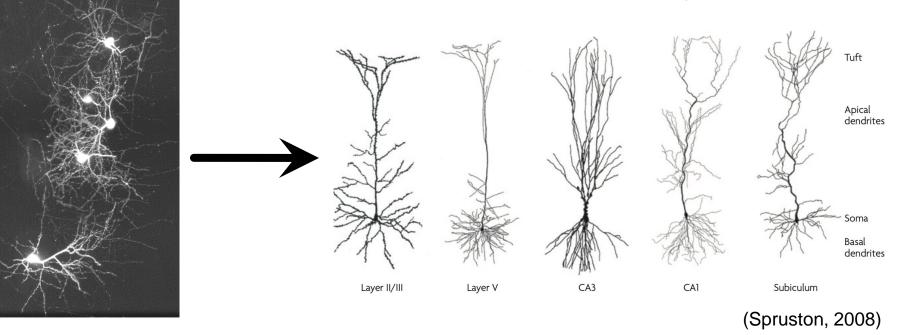


How neurons connect and work together?

(connectivity/connectome/networks, information processing, dynamics, behaviors, ...)



Key Questions in Neuroscience



Dendritic morphology



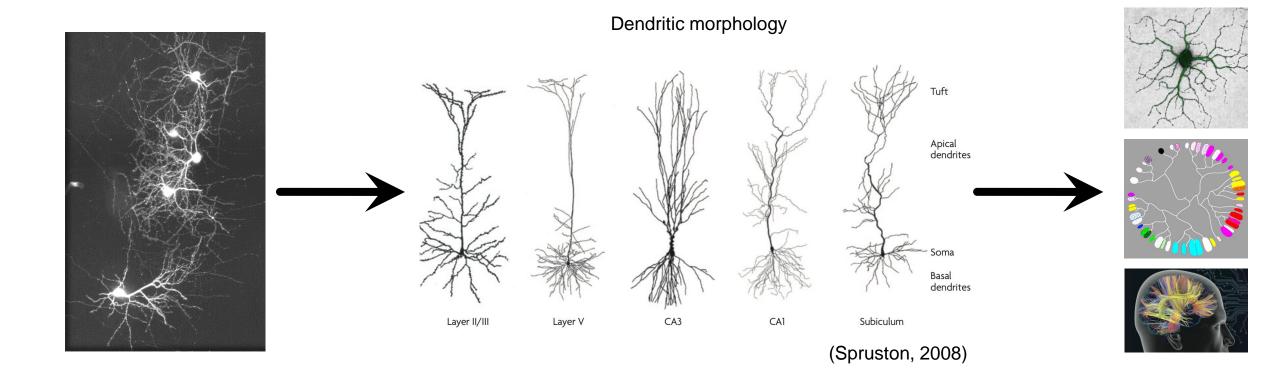
.swc files

##n type x y z r parent 1 1 513.69 514.30 258.00 1.0 -1 2 3 513.50 515.97 258.40 1.0 1 3 3 512.86 520.58 259.52 1.0 2 4 3 512.28 525.18 260.62 1.0 3 5 3 511.70 529.73 261.73 1.0 4 6 3 511.06 534.34 262.83 1.0 5 7 3 511.00 539.52 263.58 1.0 6 8 3 511.00 545.15 264.10 1.0 7 9 3 510.87 549.89 265.01 1.0 8 10 2 509.98 552.90 265.10 1.0 9 11 2 510.04 554.37 264.64 1.0 10 12 2 511.00 559.23 263.78 1.0 11 13 2 510.10 564.03 263.01 1.0 12 14 2 510.10 570.43 262.99 1.0 13 15 2 510.49 576.83 262.99 1.0 14 16 2 512.02 582.98 262.99 1.0 15 17 2 512.09 589.38 262.99 1.0 16 18 2 512.79 595.71 262.99 1.0 17 19 2 514.01 601.92 262.96 1.0 18 20 2 514.14 608.13 262.62 1.0 19 21 2 514.97 612.10 261.44 1.0 20 22 2 515.99 617.86 261.01 1.0 21 23 2 516.06 624.26 260.99 1.0 22 24 2 516.57 630.59 260.96 1.0 23 25 2 518.94 636.29 260.59 1.0 24 26 2 519.96 640.26 259.41 1.0 25 27 2 520.98 646.02 258.99 1.0 26 28 2 522.97 652.10 258.99 1.0 27 29 2 524.25 656.96 258.34 1.0 28 30 2 525.98 662.59 258.00 1.0 29 31 2 527.00 668.80 257.89 1.0 30 32 2 527.00 673.60 257.06 1.0 31 33 2 527.00 678.59 256.26 1.0 32 34 2 526.36 684.10 255.79 1.0 33 35 2 525.08 688.45 254.99 1.0 34 36 2 525.02 693.50 254.27 1.0 35 37 2 524.12 698.11 254.00 1.0 36 38 2 522.90 703.17 253.44 1.0 37 39 2 521.75 708.22 252.88 1.0 38

	1	2	4	5	6	7	8
data type	Sample number	Structure Identifier	x position	y position	z position		parent sample
1	starting from '1',	<pre>Standardized swc files (www.neuromorpho.org) - 0 - undefined 1 - soma 2 - axon 3 - (basal) dendrite 4 - apical dendrite 5+ - custom A lot of data does not conform exactly to this standard however e.g. CNIC data - 0 - undefined 1 - soma 2 - axon 3 - (basal) dendrite 4 - apical dendrite 5 - fork point 6 - end point 7 - custom VNED data - seems to be standard, but uses 10 - related to soma ? Gulyas data - each number represents structure with same diameter. Other data has been observed with -1 - also possibly related to soma ?</pre>	given in 'radius' i thicknes microme the few f	microme s half the s, also give eters (note	dendrite ven in e this is or vhich use	ne of	The sample number. Connectivity is expressed with this value. Parent samples should appear before any child samples.



Key Questions in Neuroscience



• Quantify/characterize neuron morphologies



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

Plug-in manager Re-scan all pluging

Recently used plugins

calculate 2images overlap shift

Most used plugins Clear used plugins history _Vaa3D_plugin_creator adaEnhancement AllenApps app2WithPreinfo app3

celegans cell_counting color_channel compare_swc consensus data_IO data_type findCrossBranch

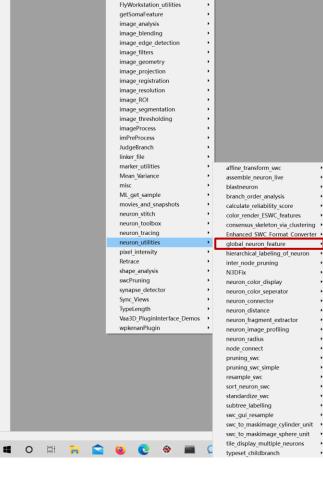
A toolkit to edit swc files

- Pre-process, e.g., sort, pruning, resampling, connector...
- Confidence score of each tracing point
- Distance between neurons
- Extract fragments from a reconstruction
- Affine transform to a reference swc

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• global_neuron_feature

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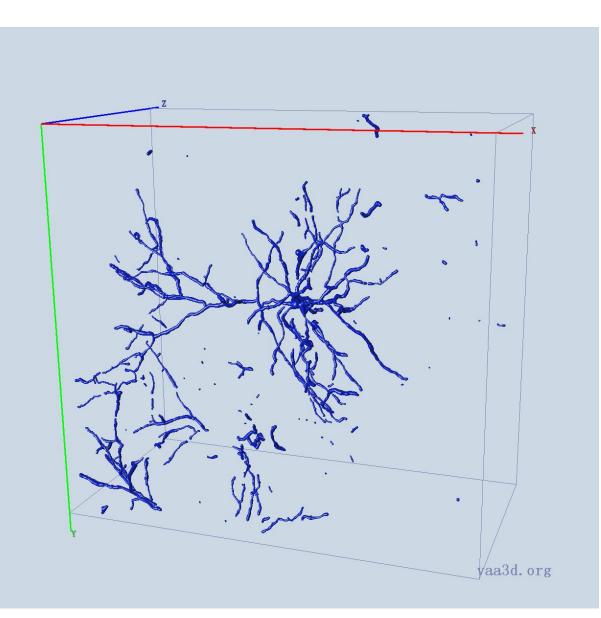
L-Measure Features

- Number of Nodes
- Soma Surface
- Number of Stems
- Number of Bifurcations
- Number of Branches
- Number of Tips
- Overall Width
- Overall Height
- Overall Depth
- Average Diameter
- Total Length

- Total Surface
- Total Volume
- Max Euclidean Distance
- Max Path Distance
- Max Branch Order
- Average Contraction
- Average Fragmentation
- Average Parent-daughter Ratio
- Average Bifurcation Angle Local
- Average Bifurcation Angle Remote
- Hausdorff Dimension



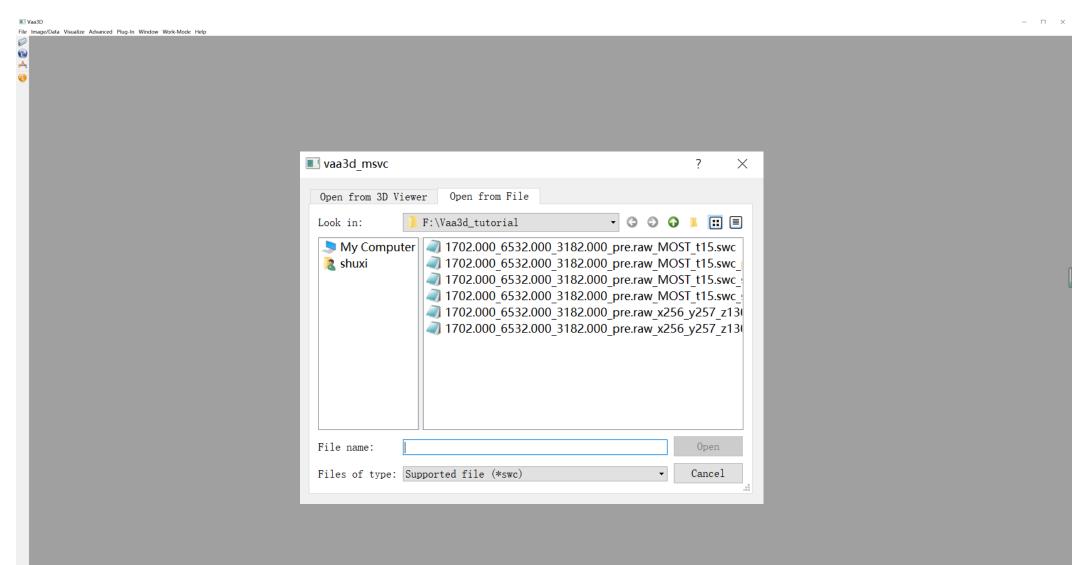
http://cng.gmu.edu:8080/Lm/help/index.htm





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	cell_counting color_channel			
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	consensus			
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	findCrossBranch +			
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	image_blending			
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	movies_and_snapshots	calculate_reliability_score		
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	neuron_toolbox	consensus skeleton via clustering .		
	neuron_utilities	Enhanced_SWC_Format_Converter > global_neuron_feature >		
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	swcPruning •	neuron_color_display		
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	Sync_Views	neuron_connector +		
	TypeLength •	neuron_distance		
	Vaa3D_PluginInterface_Demos			
	wpkenanPlugin •	neuron_image_profiling >		
		neuron_radius		
		node_connect >		
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		resample_swc		
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■ Vaa3D File Image/Data Visualize Advanced Plug-In Window Work-Mode Help

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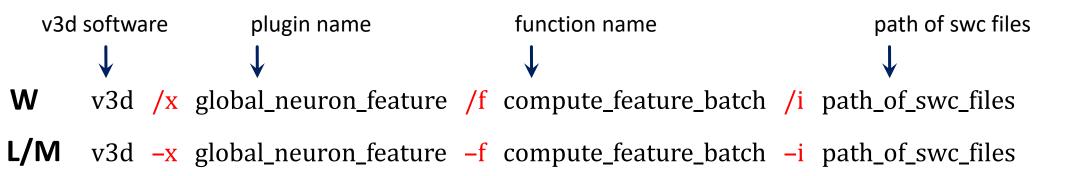
🔳 vaa3d_msvc	×
Global features of the neuron:	
number of nodes soma surface number of stems number of bifurcations number of branches number of tips overall width overall height overall depth average diameter total length total surface total volume max euclidean distance max path distance max branch order average contraction average fragmentation average parent-daughter ratio average bifurcation angle local average bifurcation angle remote Hausdorff dimension	: 59.866
	01

OK

Run Functions Via Command Line



Global Features Via Command Line



1702.000_6532.000_3182.000_pre.raw_MOST_t15.swc
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1702.000_6532.000_3182.000_pre.raw_x256_y257_z130_app2_auto.swc
1702.000_6532.000_3182.000_pre.raw_x256_y257_z130_app2_auto.swc.txt

1702.000_6532.000_3182.000_pr	e.raw_MOST_	t15.swc.txt - i	记事本	
文件(F) 编辑(E) 格式(O) 查看(V) 帮助	力(H)			
Number of Nodes: 2699				
Soma Surface:	50.2655			
Number of Stems: 1				
Number of Bifurcatons:	123			
Number of Branches:	109			
Number of Tips:	239			
Overall Width:	505.471			
Overall Height:	472.714			
Overall Depth:	254.351			
Average Diameter: 3.13028				
Total Length:	7261.26			
Total Surface:	75156			
Total Volume:	68662.5			
Max Euclidean Distance:	595.451			
Max Path Distance:		759.858		
Max Branch Order:		24		
Average Contraction:			0.92659	
Average Fragmentation:			10.7706	
Average Parent-daughter Ratio: 0.981889				
Average Bifurcation Angle Lo		59.866		
Average Bifurcation Angle R	emote:	45.6246		
Hausdorff Dimension:			1.14604	



Find More from Source Code

vaa3d_tools/released_plugins/v3d_plugins/global_neuron_feature/



.swc files

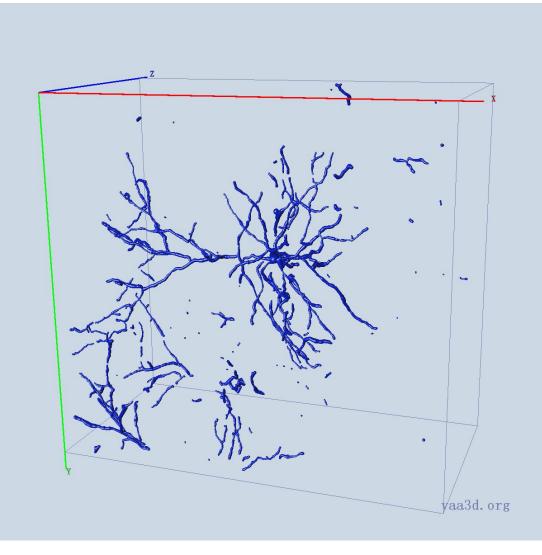
##n type x y z r parent 1 1 513.69 514.30 258.00 1.0 -1 2 3 513.50 515.97 258.40 1.0 1 3 3 512.86 520.58 259.52 1.0 2 4 3 512.28 525.18 260.62 1.0 3 5 3 511.70 529.73 261.73 1.0 4 6 3 511.06 534.34 262.83 1.0 5 7 3 511.00 539.52 263.58 1.0 6 8 3 511.00 545.15 264.10 1.0 7 9 3 510.87 549.89 265.01 1.0 8 10 2 509.98 552.90 265.10 1.0 9 11 2 510.04 554.37 264.64 1.0 10 12 2 511.00 559.23 263.78 1.0 11 13 2 510.10 564.03 263.01 1.0 12 14 2 510.10 570.43 262.99 1.0 13 15 2 510.49 576.83 262.99 1.0 14 16 2 512.02 582.98 262.99 1.0 15 17 2 512.09 589.38 262.99 1.0 16 18 2 512.79 595.71 262.99 1.0 17 19 2 514.01 601.92 262.96 1.0 18 20 2 514.14 608.13 262.62 1.0 19 21 2 514.97 612.10 261.44 1.0 20 22 2 515.99 617.86 261.01 1.0 21 23 2 516.06 624.26 260.99 1.0 22 24 2 516.57 630.59 260.96 1.0 23 25 2 518.94 636.29 260.59 1.0 24 26 2 519.96 640.26 259.41 1.0 25 27 2 520.98 646.02 258.99 1.0 26 28 2 522.97 652.10 258.99 1.0 27 29 2 524.25 656.96 258.34 1.0 28 30 2 525.98 662.59 258.00 1.0 29 31 2 527.00 668.80 257.89 1.0 30 32 2 527.00 673.60 257.06 1.0 31 33 2 527.00 678.59 256.26 1.0 32 34 2 526.36 684.10 255.79 1.0 33 35 2 525.08 688.45 254.99 1.0 34 36 2 525.02 693.50 254.27 1.0 35 37 2 524.12 698.11 254.00 1.0 36 38 2 522.90 703.17 253.44 1.0 37 39 2 521.75 708.22 252.88 1.0 38



Pre-process .swc Files

- not saved in the order of root-branches
- more than one roots
- not well-connected tree (i.e., there are gaps)
- small branches

• sort \rightarrow prune







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Plug-in manager Re-scan all plugins Recently used plugins Most used plugins Clear used plugins history Vaa3D plugin creator adaEnhancement AllenApps app2WithPreinfo app3 calculate_2images_overlap_shift + celegans cell_counting color_channel compare_swc consensus data_IO data_type findCrossBranch FlyWorkstation utilities getSomaFeature image analysis image blending image_edge_detection image_filters image_geometry image projection image_registration image_resolution image_ROI image_segmentation image_thresholding imageProcess imPreProcess JudgeBranch linker file marker utilities . affine_transform_swc Mean_Variance assemble_neuron_live misc blastneuron ML_get_sample branch order analysis movies and snapshots calculate_reliability_score neuron_stitch color render ESWC features neuron_toolbox consensus skeleton via clustering neuron tracing Enhanced SWC Format Converter neuron utilities global_neuron_feature pixel_intensity hierarchical labeling of neuron Retrace inter_node_pruning shape analysis N3DFix swcPruning neuron_color_display synapse detector neuron_color_seperator Sync Views neuron_connector TypeLength neuron_distance Vaa3D PluginInterface Demos neuron_fragment_extractor wpkenanPlugin neuron_image_profiling neuron_radius node_connect pruning_swc pruning swc simple resample_swc sort_neuron_swc standardize swc subtree_labelling swc_gui_resample swc_to_maskimage_cylinder_unit •

sort_swc

swc_to_maskimage_sphere_unit

sort_swc_batch .

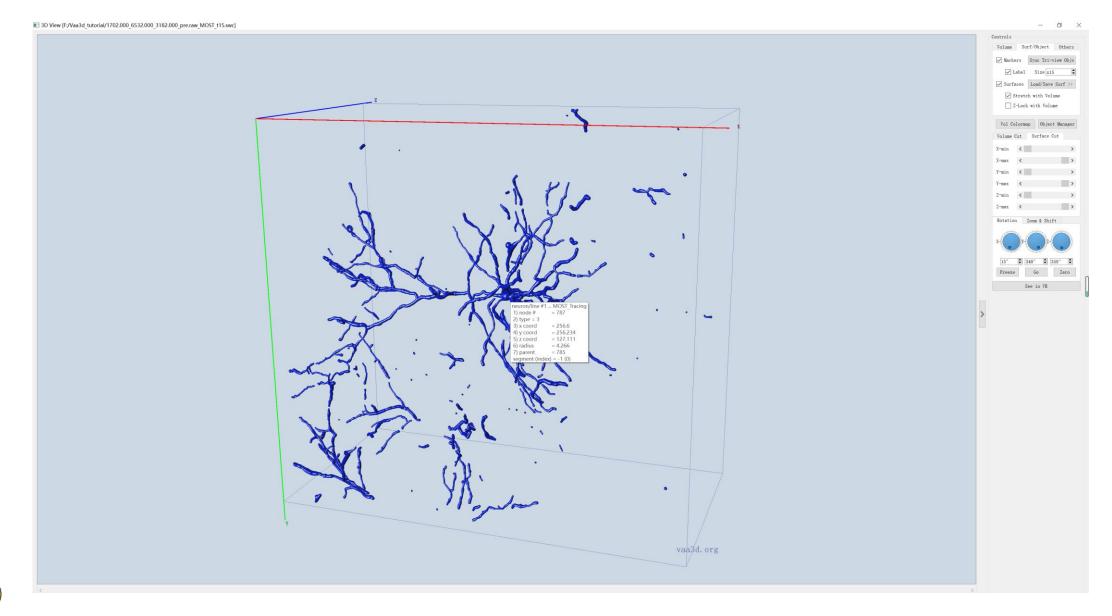
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Specify the index of the node to be used as new root.	





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le Image/Data Visualize Advanced Plug-In Window Work-Mode Help				
	Would you like to specify new root number?	?	×	
	New root number:(If you select 'cancel', the first root in file is	set as d	efault)	
	OK	Car	ncel	

Specify the index of the node to be used as new root.



I Vaa3D				
© Vaa3D le Image/Data Visualize Advanced Plug-in Window Work-Mode Help				
	a threshold for the newly generated link? 'cancel', all the points will be connected automated; If you set		? × generated)	
<u>0. 0</u>		OK	Cancel	

The distance to be allowed for two newly connected nodes.



🔳 Vaa3D

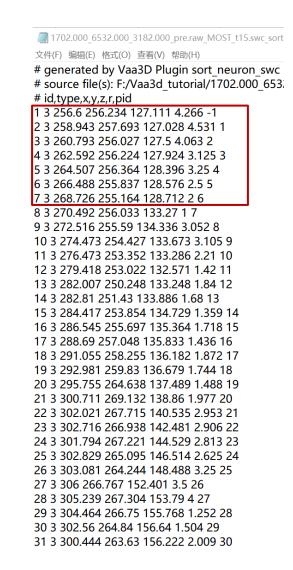
File Image/Data Visualize Advanced Plug-In Window Work-Mode Help

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3D 对象	1702.000_6532.000_3182.000_pre.raw_x256_y257_z130_app2_auto.swc	2021/3/27 20:21	SWC 文件	276 KB
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Example Result

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	Most used plugins		
	Clear used plugins history		
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	compare_swc •		
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neuron type: 3 OK Cancel		
Specify the neuron type to be pruned.		

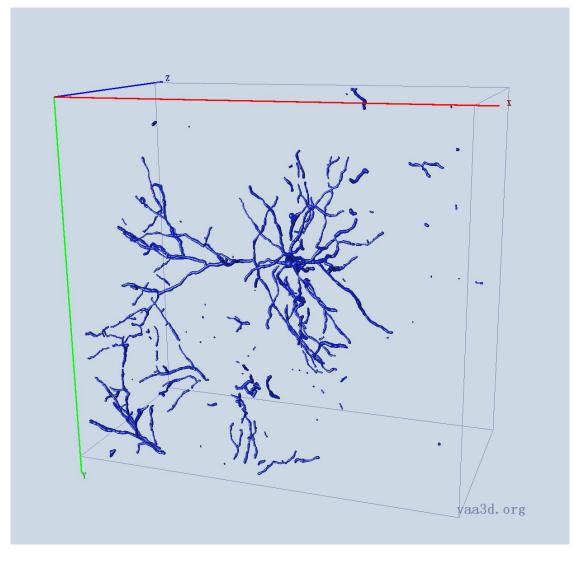
🔳 Vaa3D

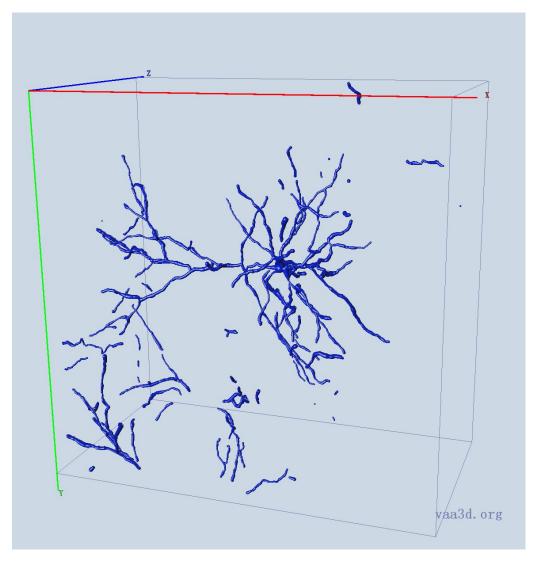
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Results







pruned

Global Features

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Global Features

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Global features of the neuron:		Global features of the neuron:	
<pre>soma surface number of stems number of bifurcations number of branches number of tips overall width overall height overall depth average diameter total length total surface total volume max euclidean distance</pre>	: 0.915022 : 15.8056 : 1.02432 : 82.9966	number of nodes soma surface number of stems number of bifurcations number of branches number of tips overall width overall height overall depth average diameter total length total surface total volume max euclidean distance max path distance max branch order average fragmentation average parent-daughter ratio average bifurcation angle local average bifurcation angle remote Hausdorff dimension	: 10.7568 : 1.00691 : 44.8316

OK



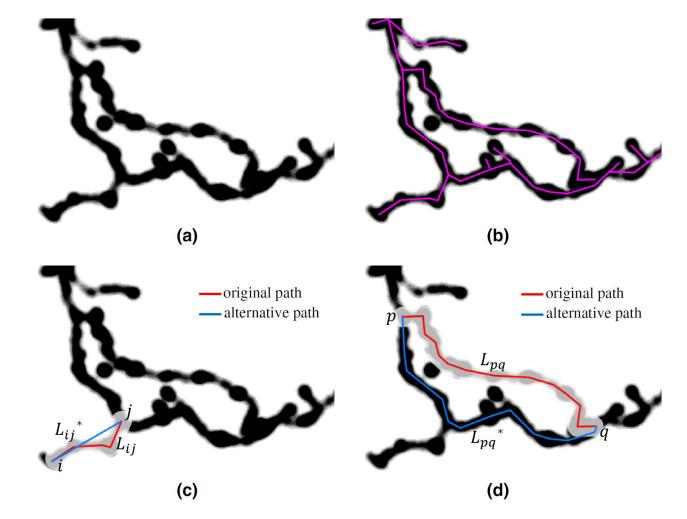
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Confidence (Reliability) Score

Fig. 2 Illustration of alternative path. For each segment in the reconstructions, after masking the image along the segment, the alternative path will be searched by fast marching from one end to the other end of the segment based on intensity. **a** neuron to reconstruct, **b** initial reconstructions, **c** alternative path of L_{ij} , **d** alternative path of L_{pq}

- To what extend can I trust the reconstruction
- Whether or not a segment is trustworthy can be tested by checking if there is an alternative path connecting the two ends of the segment.
- A segment with no better alternative pathway is more reliable in comparison with a segment with alternative pathway.





Confidence Score

Steps to calculate the score:

- 1. Find the segments in reconstructions;
- For each segment, mask the image by radius*radius_factor and redo the tracing using fast marching;
- 3. The average intensity along original segment (x) and the new segment (y) will be calculated;
- 4. For segment wise comparison, the score will be y/x; For node wise comparison, the score will be y/node_intensity.



Calculate Confidence Score in Vaa3D

Vaa3D File

Ø

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vaa3d msvc Input Image: F:/Vaa3d_tutoria1/1702.000_6532.000_3182.000_pre.raw Input SWC File: F:/Vaa3d_tutoria1/1702.000_6532.000_3182.000_pre.raw_x256_y257_z130_app2_auto.swc F:/Vaa3d_tutoria1/1702.000_6532.000_3182.000_pre.raw_x256_y257_z130_app2_auto1.swc Output Prefix: topology segment, segment wise scale radius: 2.00 OK Cancel The scores are calculated as follows: 1) Find the segments in reconstructions;

- 2) For each segment, mask the image by radius*radius_factor and redo the tracing using fast martching;
- 3) The average intensity along original segment (x) and the new segment (y) will be calculated;
- 4) For segment wise comparison, the score will be y/x. For node wise comparison, the score will be y/node_intensity.

Calculate Confidence Score in Vaa3D

rafly

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

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2) For each segment, mask the image by radius*radius_factor and redo the tracing using fast martching;

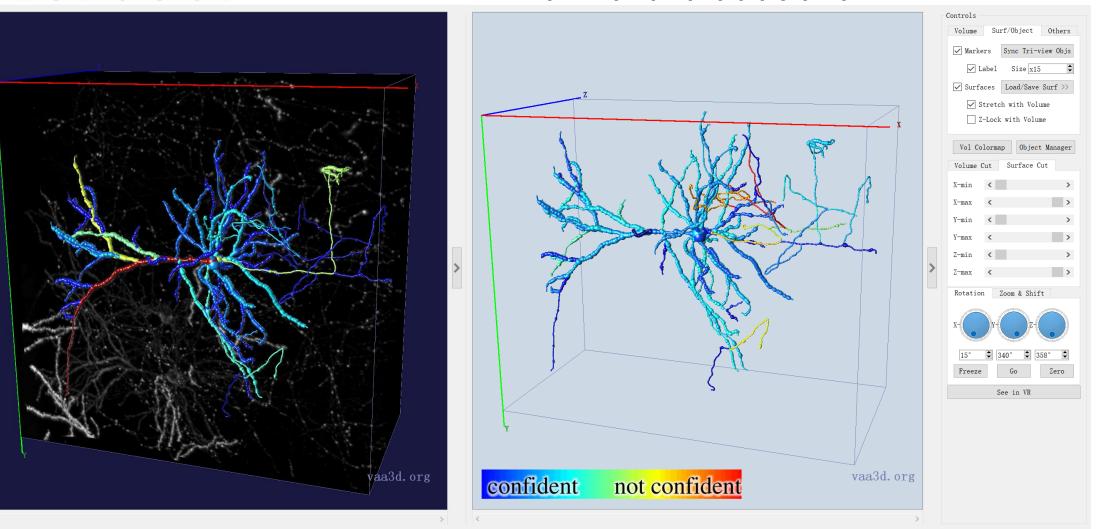
3) The average intensity along original segment (x) and the new segment (y) will be calculated;

4) For segment wise comparison, the score will be y/x. For node wise comparison, the score will be y/node_intensity.



Calculate Confidence Score in Vaa3D

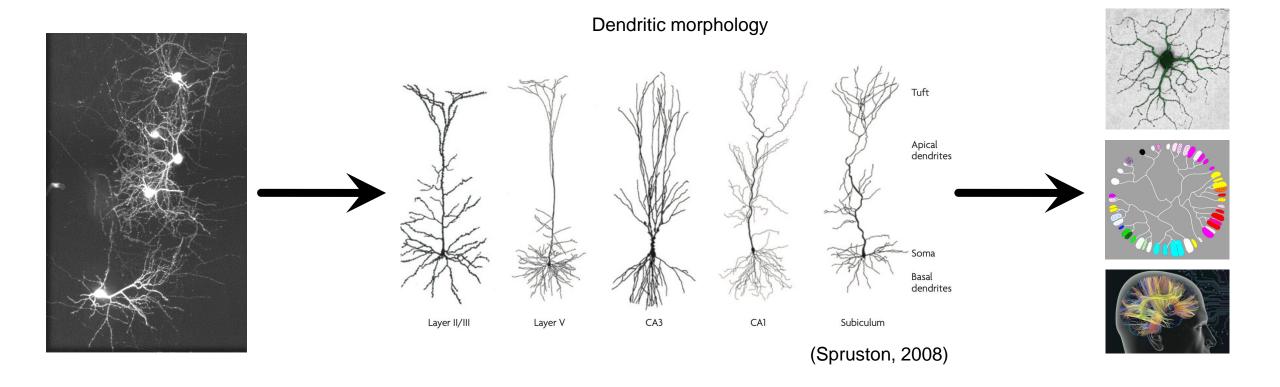
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I 3D View [F:/Vaa3d tutorial/1702.000 6532.000 3182.000 pre.raw x256 y257 z130 app2 auto1.swc scored.swc]



Key Questions in Neuroscience

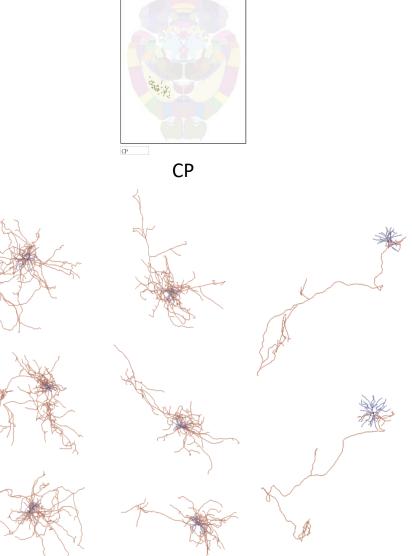


• Quantify/characterize neuron morphologies



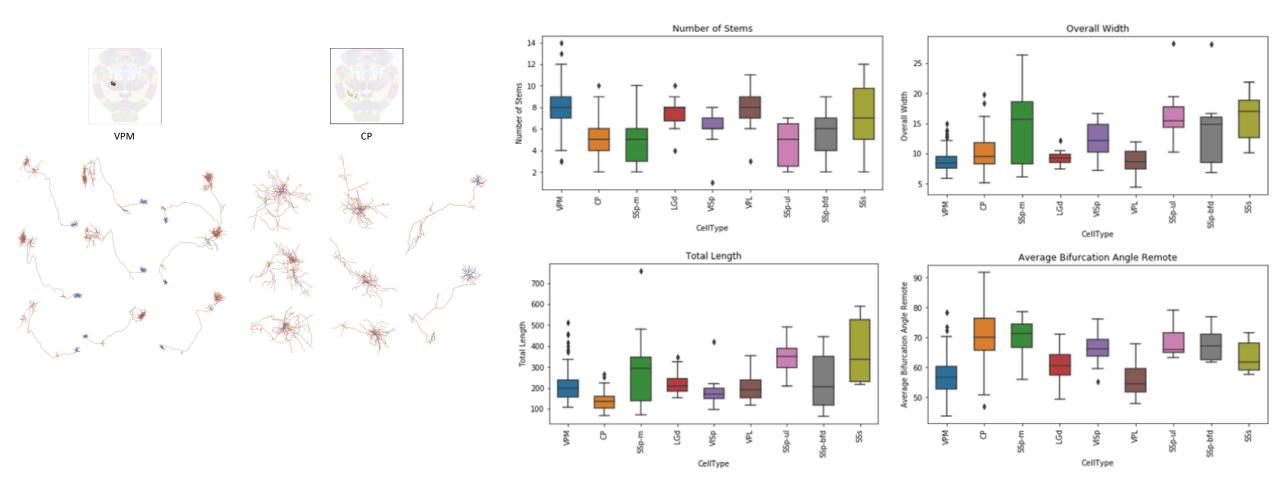
Single Neurons

VPM VPM

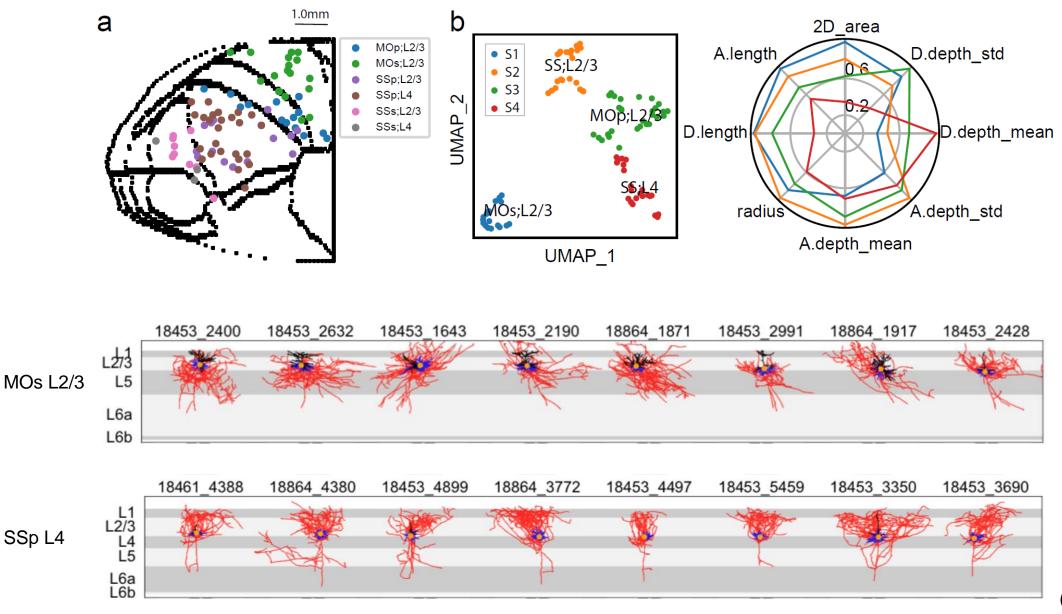




Morphological Features



Local Arborization Patterns of Cortical L2/3/4 IT Neurons



10 × 1

(Peng et al, 2020)

Automatic Quality Control

features after initial screening Number of Tips Overall Width **Overall Height Overall Depth** Total Length Max Branch Order Number of Stems Number of Bifurcatons Number of Branches Max Euclidean Distance Max Path Distance Average Contraction

Tolerance Interval Plot for Number of Tips

95% Tolerance Interval At Least 95% of Population Covered

Statistics

Normal

Nonparametric

Achieved Confidence

Normality Test

237

53.401

15.141

21.207

85.595

29.000 93.000

95.4%

3.688

0.005

Ν

Mean

StDev

Lower

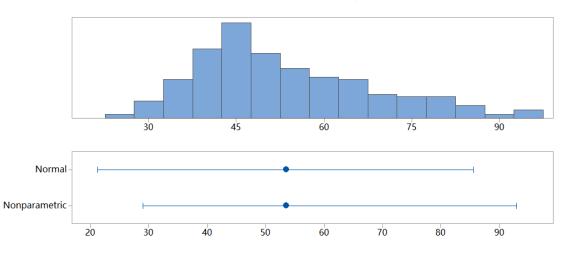
Upper

Lower

Upper

AD

P-Value <



Normal Probability Plot



Summary

- To calculate morphological features of a reconstruction
- To sort and prune .swc files as preprocessing steps
- To check the reliability of reconstructed segments/nodes

• Brief showcase where we can make use of the morphological features



Reference list

- L-Measure: http://cng.gmu.edu:8080/Lm/help/index.htm
- H. Chen *et al*, SmartTracing: self-learning-based Neuron reconstruction, *Brain Informatics*, 2015, 2:135–144, DOI: 10.1007/s40708-015-0018-y
- H. Peng *et al*, Brain-wide single neuron reconstruction reveals morphological diversity in molecularly defined striatal, thalamic, cortical and claustral neuron types, 2020, DOI: 10.1101/675280



Find More from Source Code

- vaa3d_tools/released_plugins/v3d_plugins/global_neuron_feature/
- vaa3d_tools/released_plugins/v3d_plugins/sort_neuron_swc/
- vaa3d_tools/released_plugins/v3d_plugins/pruning_swc/
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Plug-in manager Re-scan all pluging

Recently used plugins

calculate_2images_overlap_shift

FlyWorkstation utilities getSomaFeature image_analysis image_blending image edge detection image_filters

image_geometry

image_projection image registration image_resolution

image_ROI image_segmentation image thresholding imageProcess

imPreProcess JudgeBranch linker file marker utilities

Mean Variance

ML get sample

neuron stitch

neuron toolbox

neuron_tracing

neuron utilities

pixel intensity

shape_analysis

synapse detector

Vaa3D_PluginInterface_Demos

swcPruning

Sync Views

TypeLength

wpkenanPlugin

Retrace

movies and snapshots

misc

Most used plugins Clear used plugins history _Vaa3D_plugin_creator adaEnhancement AllenApps app2WithPreinfo app3

celegans cell counting color channel compare swe consensus data IO data_type findCrossBranch

A toolkit to edit swc files

- Pre-process, e.g., sort, pruning, resampling, connector...
- Confidence score of each tracing point •
- Distance between neurons

....

- Extract fragments from a reconstruction
- Affine transform to a reference swc

	pruning_swc_simple
	resample_swc
	sort_neuron_swc
	standardize_swc
	subtree_labelling
	swc_gui_resample
	swc_to_maskimage_cylinder_unit
	swc_to_maskimage_sphere_unit
	tile_display_multiple_neurons
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affine transform swc

assemble_neuron_live

branch_order_analysis

global neuron feature

inter node pruning

neuron color display

neuron connector

neuron distance

neuron_color_seperator

neuron fragment extractor

typeset childbranch

neuron image profiling neuron radius node connect pruning swc

N3DFix

calculate reliability score

color render ESWC features

consensus skeleton via clustering >

Enhanced_SWC_Format_Converter

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