Spiso-3D operation manual (US Patent 8,077,945 B2)

Section 1. Introduction :   Spiso-3D is an automated software calculating optical 3D images of neurons mathematically to analyze dendrites and spines (= post synapses). Spiso-3D software was developed by Kawato's laboratory (University of Tokyo) with a help of BioInfomatics Project of Japan Science and Technology Agency.

 We employ the Taylor expansion of the brightness function I($\mathbf{x}$) :

$$I(\mathbf{x}+\varepsilon\mathbf{u})= I(\mathbf{x})+\varepsilon I'(\mathbf{u})+\frac{1}{2}\varepsilon^2 I''(\mathbf{u})+ \quad = I(\mathbf{x})+\varepsilon\cdot gradI\cdot\mathbf{u}+\frac{1}{2}\varepsilon^2\mathbf{u^t Hu} \ +$$

$$= \ I(\mathbf{x}+\varepsilon\mathbf{u})= I(\mathbf{x})+\varepsilon(g_+ u_+ + g_- u_-)+\frac{1}{2}\varepsilon^2(\lambda_+ u_+^2 + \lambda_- u_-^2)+$$

where $\mathbf{u}$ is a unit vector of direction; and λ_+ and λ_- ($\lambda_+ > \lambda_-$) are the eigenvalues of diagonalized Hessian tensor. After smoothing dendrites using scale-space transformation, we trace the dendrite. The diameter of nodes in the dendrite is calculated from grad*I*. Next, points of the spine head are extracted as points where both λ_+ and λ_- yield negative values, since it is assumed that the spine head is an isolated closed volume with a closed surface.

The spine head diameter is determined in the digitized distance image after combining the spine center detection image with the diameter detection image as described in Mukai , Kawato et al., Cerebral Cortex (2011) 21:2704−2711 (Open Access).

First round analysis can be finished within 2 min !!
→   manual correction is necessary to obtain best head diameter values.
Spiso runs on Windows 10, 7, using tif image file (256×256 ~1024×1024).
See Sec 4. Java 6, Java 3D is required. Internal graphic card should be used, external graphic card prevents rapid expansion/reduction of image during analysis.
Play with Full Version of Spiso which can be downloaded from Kawato's laboratory Homepage http://kawato-glia.sakura.ne.jp via Google search (Publications, Mukai et al. 2011 CerCort).   Spiso results are identical to Neurolucida results within 2% error, but much better due to independence of optical intensity. Spiso analysis is objective and not subjective work.

Tabel of contents

Spiso Operation Sequence

1) Tracing of dendrite
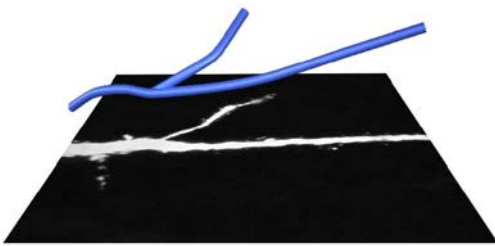
2) Determination of spine center
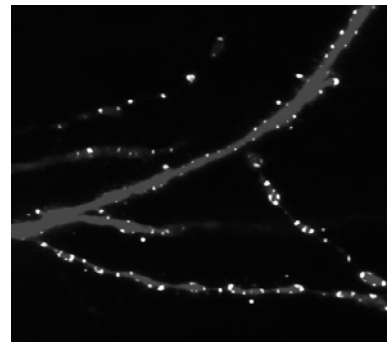
3) Determination of spine diameter + neck length

spine head

spine center

neck of spine

red circles indicate dendrite nodes

The blue circle indicates the edge

of the dendrite

Section 2. How to operate Spiso-3D

2-1 Obtaining a Password

(1) Firstly, determine the MAC address (Physical Address) of your PC by selecting

[Start] → [Program]→ [Accessories] and run [Command Prompt] → Enter 'ipconfig -all'

Your MAC address will appear, for example:

   Physical Address. . . . . : 00-24-E8-18-6C-AA

(2) To request a password email the Physical Addresses of your PC to kawato@bio.c.u-tokyo.ac.jp
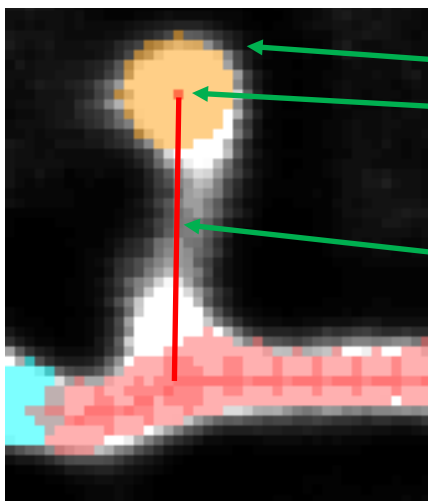
(3) Passwords (not only one) will then be issued to you via email.

(4) Double click Spiso.exe and enter your password at Password window [connect PC to internet]

→ Spiso-3D software will begin operating.

When you change Internet, then the valid Password may also be changed.

2-2 Spiso-3D Image analysis

(1) Upon start-up of Spiso-3D, the following Main Bar will appear:
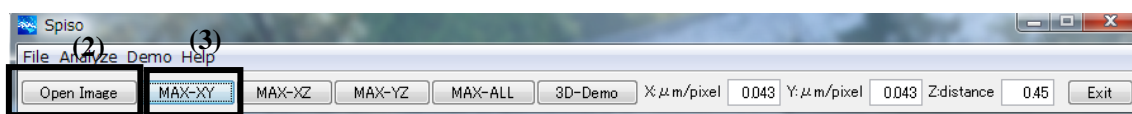
Set X, Y metrics : 0.43 μm/ pixel for Zeiss confocal, high zoom (3.0) with a 63x water immersion lens, NA 1.2 , Z metrics: 0.45 μm apart between z-axis stacks → **See 5-2 Metrics**

(2) Click on [Open Image] and select the tif image file (8 bit) to be analyzed. The image will appear in the following screen:

(Attention)   The 8 bit tif image file is obtained from confocal image file (*.slm) after the following processes. Deconvolution with AutoDeblur software (MicroCybernetics, USA) (16bit tif file) →

Using Image J, modification of <mark>contrast</mark> to obtain good S/N image, and conversion of 16 bit tif file to 8 bit tif file. <mark>In case of Zeiss LSM880, using [AiryScan mode] you can obtain deconvoluted image file (*.slm) after [Apply] processing.</mark>

(3) Click [MAX-XY], the following MAX-XY window will appear for Dendrite Detection.
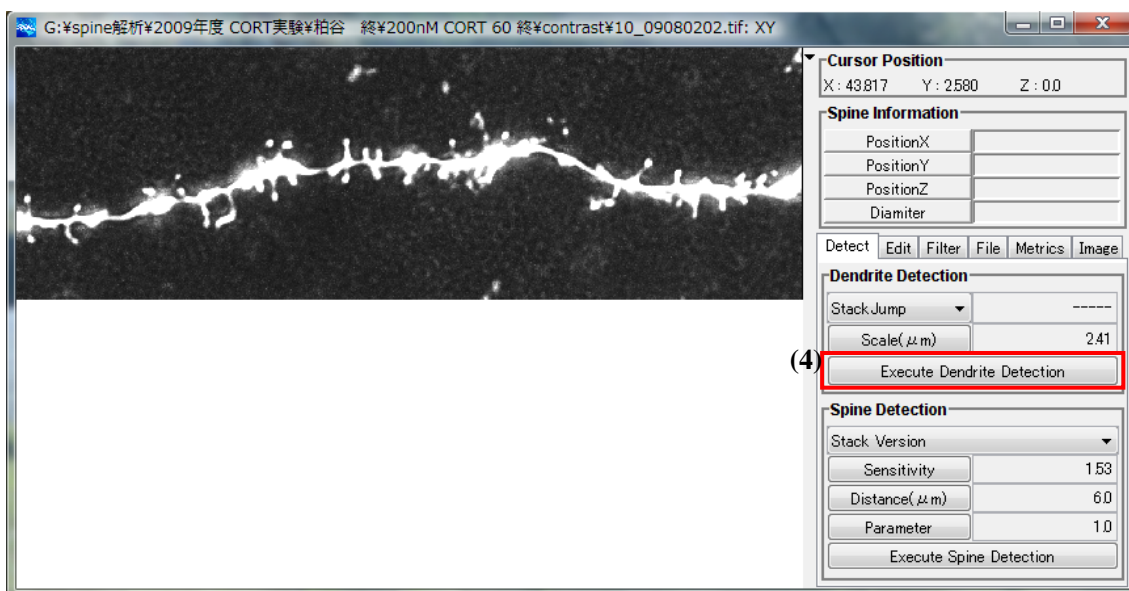
(4) <u>To trace the dendrite</u>, click [Execute Dendrite Extension]. The dendrite trace will be indicated in red, comprised of a series of dendrite nodes as in the image below:

(B) Connect dendrite trace   (A) Delete wrong dendrite nodes
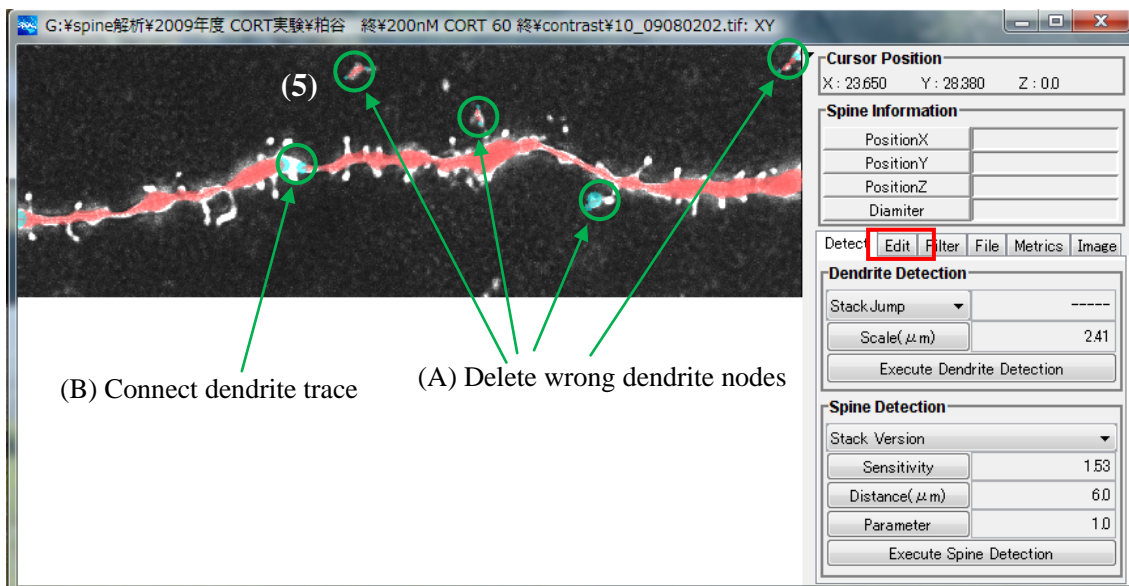
(5) During automated dendrite tracing, wrong dendrite nodes may be detected in error (A) or the dendrite trace may be disconnected (B). These errors should be corrected manually in Edit, Dendrite

4

mode.　For magnification, move of images, see **Section 3**.

(A) To manually delete wrong dendrite nodes detected in error, select a wrong red node by left clicking on it with the mouse, then right click while holding [shift] key → Multiple nodes can be deleted at once. To delete nodes individually, select one node, by placing the pointer over the node, and right click.

  (B) To repair a disconnected dendrite trace, select the blue node, then left click with the pointer to add red nodes until the dendrite trace is connected with the other blue end. (If you fail in repair, use Ctrl+Z to go back and restart)

When connecting a dendrite trace, be careful when deciding which optical slice to place new nodes. The added nodes should be placed on the right z-optical slice where the dendrite has highest intensity and largest area. To select the desired z-optical slice, press [Alt] and rotate mouse wheel.

The image below demonstrates successful connection (in 3D) of the dendrite trace and deletion of dendrite nodes detected in error.

Select [Stack Version]
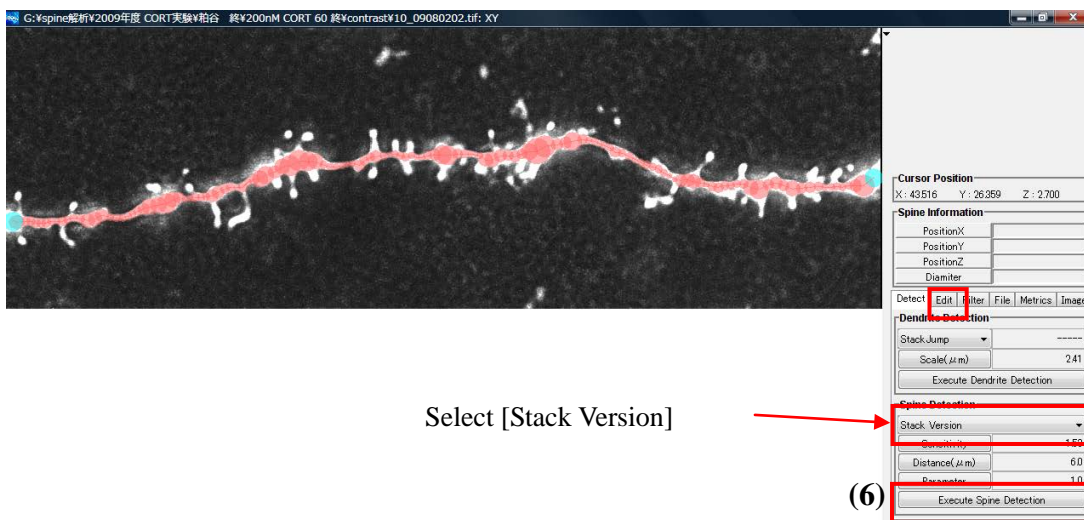
(6) To perform automated spine detection, select [Stack Version] and click [Execute Spine Detection] in Detection mode. **Orange spines** are detected after subtracting the dendrite from 3D stack of optical slices.
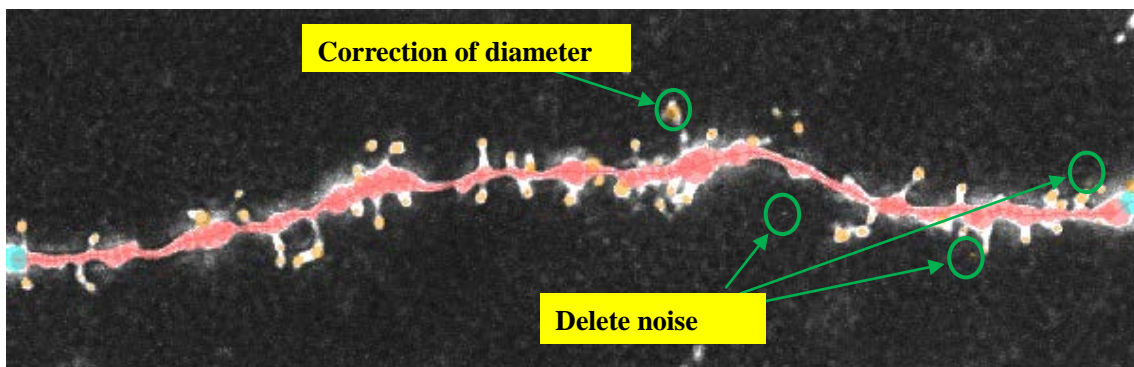
During automated spine detection, small noise may be detected as spines, or the detected spine size may be different from the true size, these errors should be corrected manually in Edit, Spine mode. For magnification, move of images, see **Section 3**.

Spine nodes can be edited by the same method used to edit the dendrite trace (including the method of deleting multiple node at once). Undetected spine nodes can be added using the same method used to add dendrite nodes. (Spines more than 8 µm apart from the dendrite are automatically assigned to be spines belonging to other dendrites, therefore not detected.)

Change of spine head diameter can be performed easily by changing of spine node size via selection of spine node → use mouse wheel → increase/decrease of spine size. The addition of spines or changing spine sizes should be done on the right z-optical slice where the spine head has the largest area.

Below is an example of a dendrite trace and detected spines after manual correction.
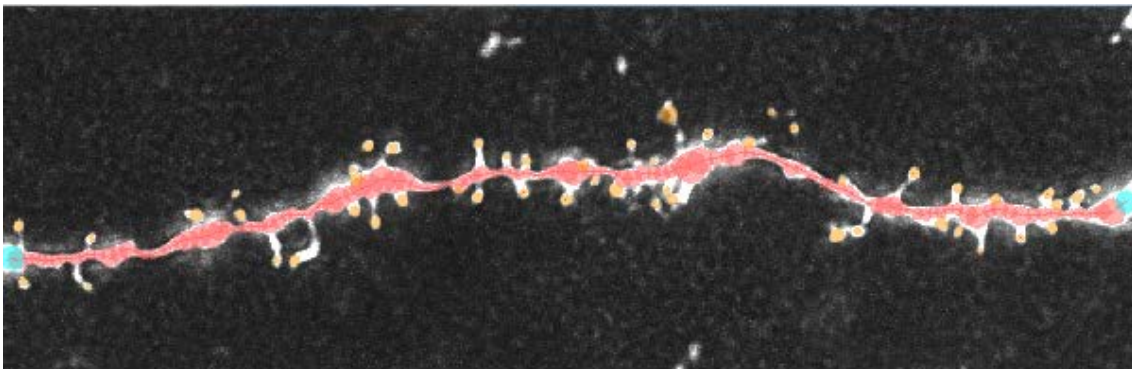
(7) To export the results of dendrite and spine analysis, select [File] , then select [Export] **(8)**.
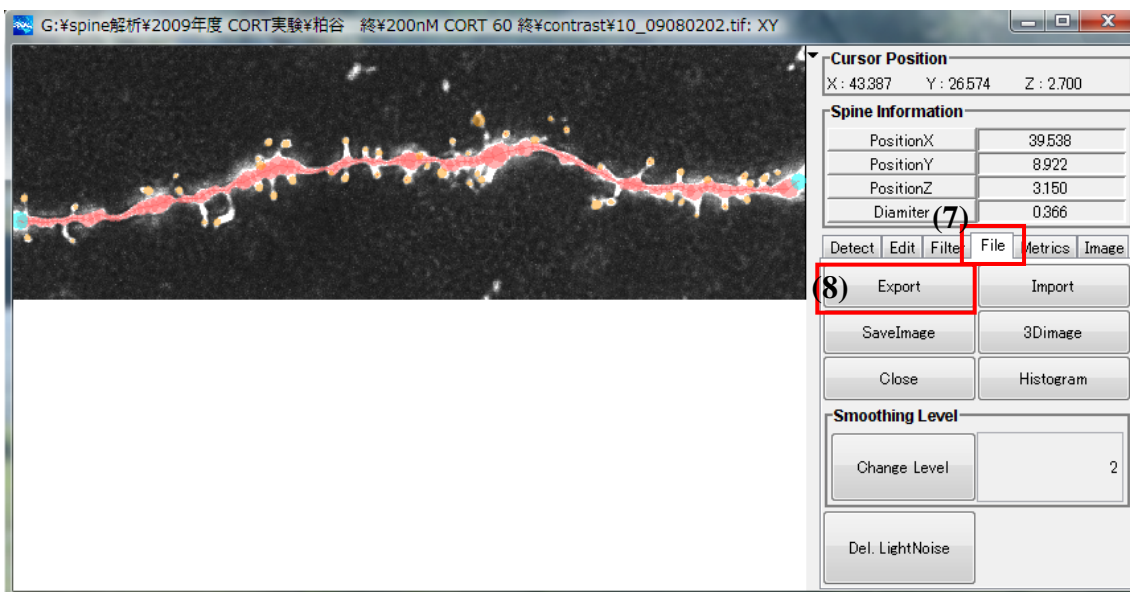
(8) The analysed digital data will be exported as an excel file. <mark>To export, set file mode to "excel", and do not use "all file mode".</mark>

The data are displayed in 3 tabs, summarised in the 'summary' tab as below.

Number of spines with each head diameter (µm)

1. Total spine number
2. Dendrite length
3. Total spine density

Spine neck length distribution

Number of Spine	Dendrite Length	Density	-0.2	0.2-0.3	0.3-0.4	0.4-0.5	0.5-0.6	0.6-0.7	0.7-0.8	0.8-0.9	0.9-1.0	1.0-
51	48.14846276	1.059223848	0	5	21	20	2	2	1	0	0	0

Scale	Sensitivity	Distance
2.41	1.53	

neck_length	count
0.0-0.2	0
0.2-0.4	1
0.4-0.6	1
0.6-0.8	8
0.8-1.0	12
1.0-1.2	8
1.2-1.4	9
1.4-1.6	5
1.6-1.8	4
1.8-2.0	0
2.0-2.2	0
2.2-2.4	1
2.4-2.6	2
2.6-2.8	0
2.8-3.0	0
3.0-3.2	0
3.2-3.4	0
3.4-3.6	0
3.6-3.8	0
3.8-4.0	0
4.0-4.2	0
4.2-4.4	0
4.4-4.6	0
4.6-4.8	0
4.8-5.0	0
5.0-5.2	0
5.2-5.4	0
5.4-5.6	0
5.6-5.8	0
5.8-6.0	0
6.0-6.2	0

real / pixel / summary

Using these data we analyze:

1. Total spine density

2. Spine head diameter distribution

3. Spine subclass density (small, middle, large)

4. Spine neck length distribution

(9) Three dimensional model   (See next page).

Demonstration of a model 3D image can be performed by File → Import excel file → Select 3D image.   While rotating 3D image, if you find wrong spines or abnormal dendrite nodes (abnormal kinks) →   correct them with Edit mode.
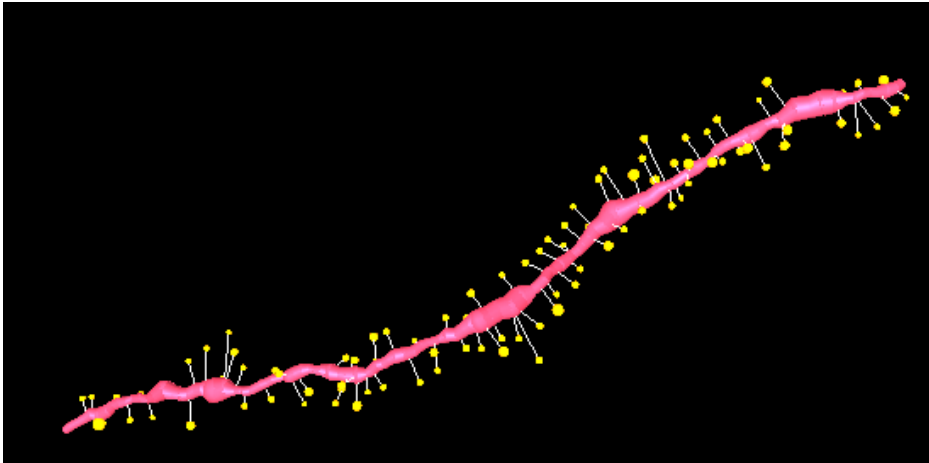
Section 3. Table of Spiso handling keys and their functions

3-1. Common keys
- Alt + mouse wheel → move image in z direction
- Ctrl + mouse wheel → magnify image
- Ctrl + cursor movement → move image

3-2. Dendrite keys
- Left click on node → select node
- selection + left click outside of the selected node → addition of node connected to the selected node

  (after addition, the new node is automatically selected)
- selection + left click within the selected node (keep pressing) + cursor movement → movement of the selected node
- selection + left click on an unselected node → connection between the clicked node and the selected node
- selection + right click on the selected node → deletion of node
- Selection + right click on an unselected node →deletion of the connection of the two nodes
- Selection + right click outside of node → unselection of node
- Selection + wheel → change the size of node

3-3. Spine keys
Left click within the spine → spine selection

Left click outside of the spine → addition of spine (after addition, the new spine is selected)

Left click on the selected spine (keep pressing) + cursor movement → move spine

Selection + right click on the selected spine → spine deletion

Selection + right click outside of the selected spine → spine deselected

Right click (keep pressing) → spine deletion (eraser function)

Selection + wheel → change spine size

Section 4. Image and System requirements for Spiso analysis

4-1. Recommended computer conditions

CPU: Pentium 4, 3GHz or better

Memory : more than 2GB

OS: Windows XP, Vista, 7 (32bit, 64bit)

Java version 6.1, Java3D*

Software Set [Spiso.exe, lib(library), four of j3dcore-*.dll, innifiles]

4-2. Image requirements for analysis

a) size of image   XY-axis: between 256×256 and 1024×1024 , Z-axis 16-20 stacks

b) Rectangular image can be analyzed

c) type of image file: tif

4-3. Consumption of CPU time for analysis

It takes about 120 sec for a 1024×250（20 stacks）image

4-4   Internal graphic card should be used, external graphic card prevents rapid expansion/reduction of image during analysis. Image resolution can be improved by setting desktop image resolution to "high"

Section 5. Important buttons on Main Bar and Windows for analysis
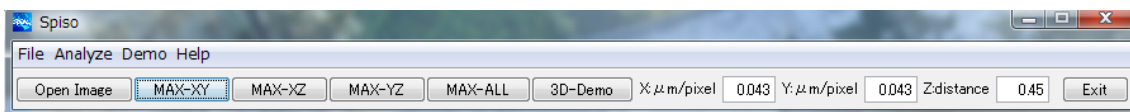
5-1. MAX projection buttons

MAX-XY Button → open Max-XY Projection Window

MAX-XZ/YZ Button → open Max-XZ,   Max-XZ Projection Window

5-2. Metrics

Ratios of X,Y, Z images to true scale should be set in MAX-XY Projection Window.

" Click Metrics → click Change → Insert numbers in the Change Metrics Window → Apply "

X, Y metrics : μm/pixel (1 pixel = 0.43 μm for Zeiss confocal microscopy LSM PASCAL,

at high zoom (3.0) with a 63x water immersion lens, NA 1.2

Z metrics 0.45 = 0.45 μm apart between z-axis stacks

These ratio values are used in display of MAX-XY/XZ/YZ Projection Windows

5-3. Max-XY Projection Window

Big jump of dendrite nodes in Z stacks can be limited by setting threshold (no limitation now)

Scale σ setting for dendrite detection

Automatic tracing of dendrite

Selection of algorithm for spine detection

Setting of sensitivity of spine detection

Automatic detection of spines
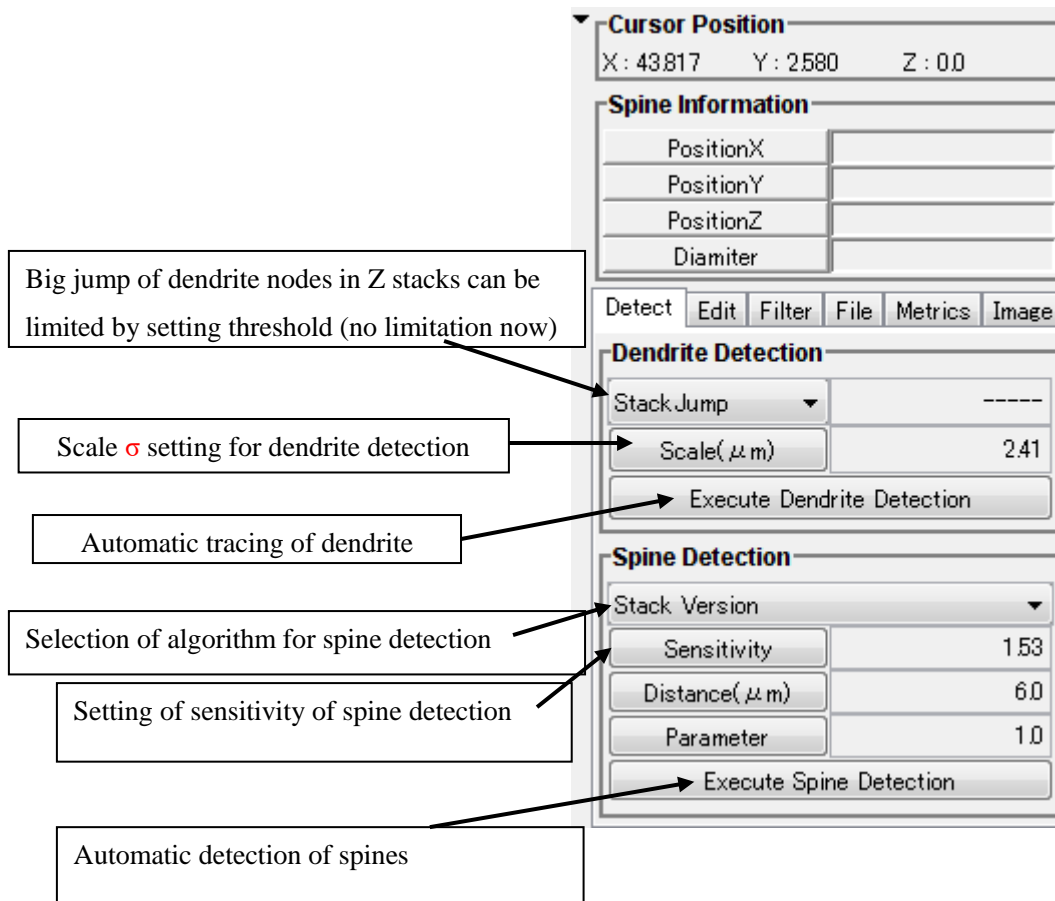
5-4. Edit tab

    1. Mode select: select the desired mode for manual manipulation (dendrite, spine or classify)

    2. Undo button: up to 100 previous changes can be reversed using the undo button

    3. Redo button: restores changes made with undo button

    4. Adjust button: expands image size to full canvas size

    5. Background button: changes background between stack image and Max XY Projection image

     6. Hide Spine button: hides detected spines

    7. Hide dendrite button: hides the dendrite trace

    8. Classify button (not useful now): automatically classifies spines into stubby, mushroom or thin class based on spine size

    9. Edit Scale : Default setting is 0.5 pixels = 0.0215 µm. This can be adjusted to your specific experimental parameters.

5-5.　Filter tab

　1. Spine Diameter Filter

　　（A）Change Range button: Set minimum and maximum spine diameter filter parameters.

　　（B）Filter button: Selects spines with diameter between specified minimum and maximum range.

In updated version this function is disabled.

2. Dendrite Width Filter

　　（A）Change Range button: Set minimum (lower) and maximum (upper) node diameter filter parameters.

　　（B）Filter button: Selects dendrite nodes within specified minimum and maximum range.

5-6.　File tab

1. Export tab: exports analysed digital values (dendrite, spine) to excel file

2. Import tab: imports data from excel file to the canvas of Spiso to reconstruct 3D model of spines and dendrite

3. SaveImage button: saves canvas data as image file

4. 3Dimage button: reconstructs 3D model image from digital values

5. Histogram tab: creates histogram of intensity profile.

6. Smoothing level : Dendrite smoothing is set to 2 nodes in the default setting, this can be manually adjusted.

5-7.　Metrics tab (ratio between the image and real scale)

1. To change the ratio between the screen image and true scale, press the change button

5-8.　Image tab

1. Information of image size (vertical and horizontal pixel and stack numbers)

Section 6.   Results of analysis exported to an excel file

(1) dendrite information

number of dendrite nodes

Parent node numbers

Node x,y, z position

Node diameter

x, y, z position after smoothing

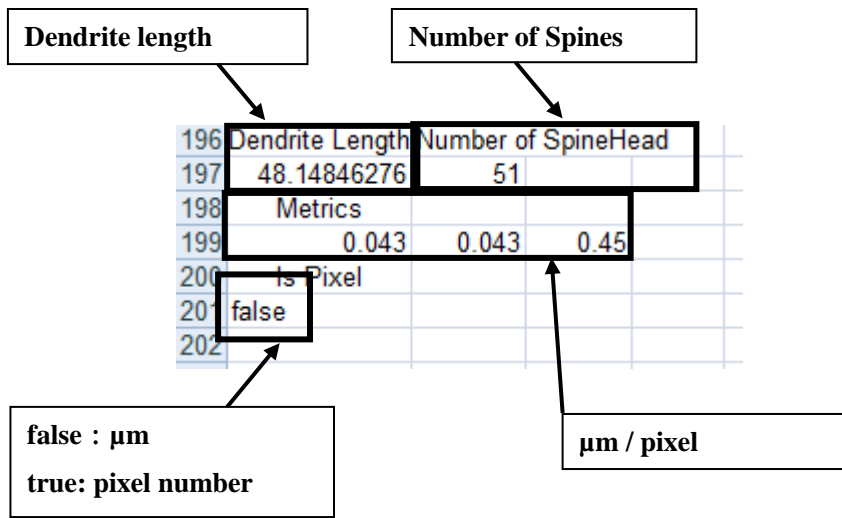|	A	B	C	D	E	F	G	H	I
1	CenterLine						smoothed value		
2	number	parent_numeber	x	y	z	diameter	x	y	z
3	0	-	0	0	0	0	0	0	0
4	1	-	0	0	0	0	0	0	0
5	2	130	15.566	6.708	4.5	0.802	15.566	6.708	4.32
6	3		15.953	6.88	4.5	0.456	15.953	6.88	4.41
7	4		16.297	6.966	4.5	0.396	16.297	6.966	4.5
8	5		16.598	6.966	4.5	0.383	16.598	6.966	4.41
9	6		16.899	6.923	4.5	0.359	16.899	6.923	4.41
10	7	6	17.157	6.837	4.05	0.339	17.157	6.837	4.41
11	8		17.415	6.794	4.5	0.343	17.415	6.794	4.41
12	9		17.759	6.88	4.5	0.603	17.759	6.88	4.41
13	10		18.017	6.665	4.5	0.504	18.017	6.665	4.5
14	11	10	18.318	6.493	4.5	0.495	18.318	6.493	4.5
15	12	1	18.576	6.45	4.5	0.654	18.576	6.45	4.5
16	13	12	19.006	6.407	4.5	0.422	19.006	6.407	4.5
17	14	13	19.307	6.364	4.5	0.327	19.307	6.364	4.5
18	15	14	19.565	6.321	4.5	0.379	19.565	6.321	4.5
19	16	19	19.823	6.407	4.5	0.435	19.823	6.407	4.59
20	17	16	20.081	6.493	4.5	0.454	20.081	6.493	4.77
21	18	1	20.382	6.407	4.95	0.399	20.382	6.407	4.95
22	19	1	20.64	6.493	5.4	0.31	20.64	6.493	5.22
23	20	19	20.898	6.493	5.4	0.372	20.898	6.493	5.49
24	21	20	21.199	6.407	5.85	0.47	21.199	6.407	5.67
25	22	2	21.543	6.364	5.85	0.611	21.543	6.364	5.76
26	23	2	21.93	6.364	5.85	0.952	21.93	6.364	5.85
27	24	2	22.188	6.407	5.85	1.003	22.188	6.407	5.76
28	25	24	22.704	6.321	5.85	0.546	22.704	6.321	5.67
29	26	25	23.048	6.321	5.4	0.473	23.048	6.321	5.58
30	27	26	23.349	6.321	5.4	0.356	23.349	6.321	5.49
31	28	27	23.607	6.364	5.4	0.284	23.607	6.364	5.49
32	29	28	23.822	6.407	5.4	0.375	23.822	6.407	5.58

(2) spine information

spine center x,y,z position

spine diameter

not used

spine umber not used

spine neck length

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
143	SpineHead															
144	number	x	y	z	diameter	paxis_length	paxis_x	paxis_y	paxis_z	type	light	light_volume	topz	peakz	bottomz	neck_length
145		25.821	4.106	6.3	0.7	0.731	0	0	1	Stubby	0	0	0	0		2.234
146		29.864	4.063	6.75	0.322	0.322	0	0	1	Stubby	0	0	0	0		2.452
147		30.723	4.666	6.75	0.387	0.387	0	0	1	Stubby	0	0	0	0		2.5
148		27.37	4.88	4.5	0.387	0.387	0	0	1	Stubby	0	0	0	0		1.302
149		28.96	5.224	6.3	0.473	0.473	0	0	1	Stubby	0	0	0	0		1.26
150		18.468	5.267	4.5	0.408	0.408	0	0	1	Stubby	0	0	0	0		1.187
151		24.402	5.268	6.3	0.344	0.344	0	0	1	Stubby	0	0	0	0		1.306
152		14.212	5.353	3.6	0.43	0.43	0	0	1	Stubby	0	0	0	0		1.51
153		15.157	5.439	4.05	0.43	0.43	0	0	1	Stubby	0	0	0	0		1.281
154		19.586	5.74	3.6	0.344	0.344	0	0	1	Stu				0		1.071
155		22.554	6.127	6.3	0.43	0.43	0	0	1	Stu				0		0.676
156		25.262	5.956	6.75	0.258	0.258	0	0	1	Stu				0		1.007
157		13.609	6.256	4.05	0.258	0.258	0	0	1	Stu				0		0.834
158		35.368	6.428	3.6	0.43	0.43	0	0	1	Stubby	0	0	0	0		1.437
159		13.738	6.643	4.95	0.537	0.537	0	0	1	Stubby	0	0	0	0		0.661
160		23.8	7.073	4.95	0.602	0.602	0	0	1	Stubby	0	0	0	0		0.909
161		37.088	6.988	3.15	0.387	0.387	0	0	1	Stubby	0	0	0	0		0.998
162		42.462	7.073	2.25	0.344	0.344	0	0	1	Stubby	0	0	0	0		1.075
163		25.778	7.116	6.3	0.344	0.344	0	0	1	Stubby	0	0	0	0		0.827
164		20.231	7.159	5.4	0.344	0.344	0	0	1	Stubby	0	0	0	0		0.802
165		18.426	7.331	4.05	0.451	0.451	0	0	1	Stubby	0	0	0	0		0.873
166		40.57	7.246	1.35	0.43	0.43	0	0	1	Stubby	0	0	0	0		1.372
167		41.731	7.331	1.35	0.43	0.43	0	0	1	Stubby	0	0	0	0		0.812
		.202	4.95	0.258	0.258	0	0	1	Stubby	0	0	0	0	0		1.371
		.632	6.75	0.344	0.344	0	0	1	Stubby	0	0	0	0	0		1.505
		7.46	4.95	0.43	0.43	0	0	1	Stubby	0	0	0	0		1.367
		7.46	4.05	0.494	0.494	0	0	1	Stubby	0	0	0	0		0.784
		.847	4.05	0.451	0.451	0	0	1	Stubby	0	0	0	0		0.784
		.019	5.85	0.516	0.516	0	0	1	Stubby	0	0	0	0		1.045

(3) Other information

Dendrite length

Number of Spines

196	Dendrite Length	Number of SpineHead
197	48.14846276	51
198	Metrics	
199	0.043	0.043	0.45
200	Is Pixel	
201	false	
202		

false : μm

true: pixel number

μm / pixel

Summary of analysis

	A	B	C	D	E	F	G	H	I	J	K	L	M	N
1	Number of Spine	Dendrite Length	Density	-0.2	0.2-0.3	0.3-0.4	0.4-0.5	0.5-0.6	0.6-0.7	0.7-0.8	0.8-0.9	0.9-1.0	1.0-	
2	51	48.14846276	1.059223848	0	5	21	20	2	2	1	0	0	0	
3														
4	Scale	Sensitivity	Distance											
5	2.41	1.53												
6														
7														
8														
9	neck_length	count												
10	0.0-0.2	0												
11	0.2-0.4	1												
12	0.4-0.6	1												
13	0.6-0.8	8												
14	0.8-1.0	12												
15	1.0-1.2	8												
16	1.2-1.4	9												
17	1.4-1.6	5												
18	1.6-1.8	4												
19	1.8-2.0	0												
20	2.0-2.2	0												
21	2.2-2.4	1												
22	2.4-2.6	2												
23	2.6-2.8	0												
24	2.8-3.0	0												
25	3.0-3.2	0												
26	3.2-3.4	0												
27	3.4-3.6	0												
28	3.6-3.8	0												
29	3.8-4.0	0												
30	4.0-4.2	0												
31	4.2-4.4	0												
32	4.4-4.6	0												
33	4.6-4.8	0												
34	4.8-5.0	0												
35	5.0-5.2	0												
36	5.2-5.4	0												
37	5.4-5.6	0												
38	5.6-5.8	0												
39	5.8-6.0	0												
40	6.0-6.2	0												

Head diameter distribution of spines

1. Total spine

2. Dendrite length

3. Density of spines

Neck length distribution of spines

real / pixel / summary

Neck length is the shortest distance between the center of spine and the center of dendrite node.

・Therefore, true neck length = neck length (excel file) - dendrite node radius - spine radius

Section 7, Questions and Answers

Answers to Frequent Questions are indicated in red letters or painted yellow in the text.