

NeuronIQ (version 1.5) Manual

Installation:

NeuronIQ is running under Matlab environment, for both Windows and Linux.

Before the installation, the following should have been installed on the local machine:

1. Matlab version 7.0 or up (<http://www.mathworks.com/products/matlab/>).
 - if you use matlab in Windows, download the NeuronIQ Windows version;
 - if you use matlab in Linux, download the NeuronIQ Linux version.
2. Matlab toolbox:
 - image processing toolbox
 - bioinformatics toolbox
3. javax package, which is included in the freely available Java 2 Platform (<http://java.sun.com/products/archive/>).

Then download correspond NeuronIQ package and unpacked into one folder on the local machine. Change the current Matlab directory to the folder containing the unpacked files. To run the software, type 'NeuronIQ' in the command window of Matlab.

Team Members:

Project Leader: Dr. Stephen TC Wong, Dr. Zhong Xue

Graphic User Interface: Dr. Jie Cheng, Dr. Yong Zhang

CADMOS : Dr. Yong Zhang

MADMOS: Dr. Jie Cheng

Software Improvement & Optimization: Kun Chen, Qing Li

Software Testing: Dr. Fei Cao, Ying Zhu, Dr. Peng Shi, Vinothini Kamalesan.

Contact:

Dr. Jie Cheng

The Methodist Hospital Research Institute Radiology Department

6565 Fannin Street, B5-022

Houston, Texas 77030

Office: (713) 441-8676

Email Address: jcheng@tmhs.org

Users' Manual

Data Requirement:

NeuronIQ is designed to be used with datasets obtained by [confocal](#) or [multi-photon fluorescence](#) microscopy. The program supports files in *TIFF* (Tagged Image File Format, with suffix .tif or .tiff) or *JPEG* (.jpg or .jpeg) format, both in 3D volume data or 2D single slice. Currently the program only accepts greyscale images of 8 or 16 bit-depth. For other types of images, it should be converted to the TIFF or JPEG format before using the software.

Menu:

1. File

- Load an Image: load a single image to process
- Load Directory: load all images in a directory to process
- Save Result: Export the processing results of an image into an Excel file
- Batch Export: Export the batch processing results into an Excel file
- Save Current Image: Save the displayed image in .bmp format
- Close Current Image: Close currently displayed image
- Close Directory: Close currently opened directory
- Exit: Exit NeuronIQ

2. Analysis

- CADMOS: Curviline based spine detection and measurement
- MADMOS: Morphology based spine detection and measurement
- Single Processing: Automatic process for a single image
- Batch Processing & Save Results: Automatic process for a collection of images

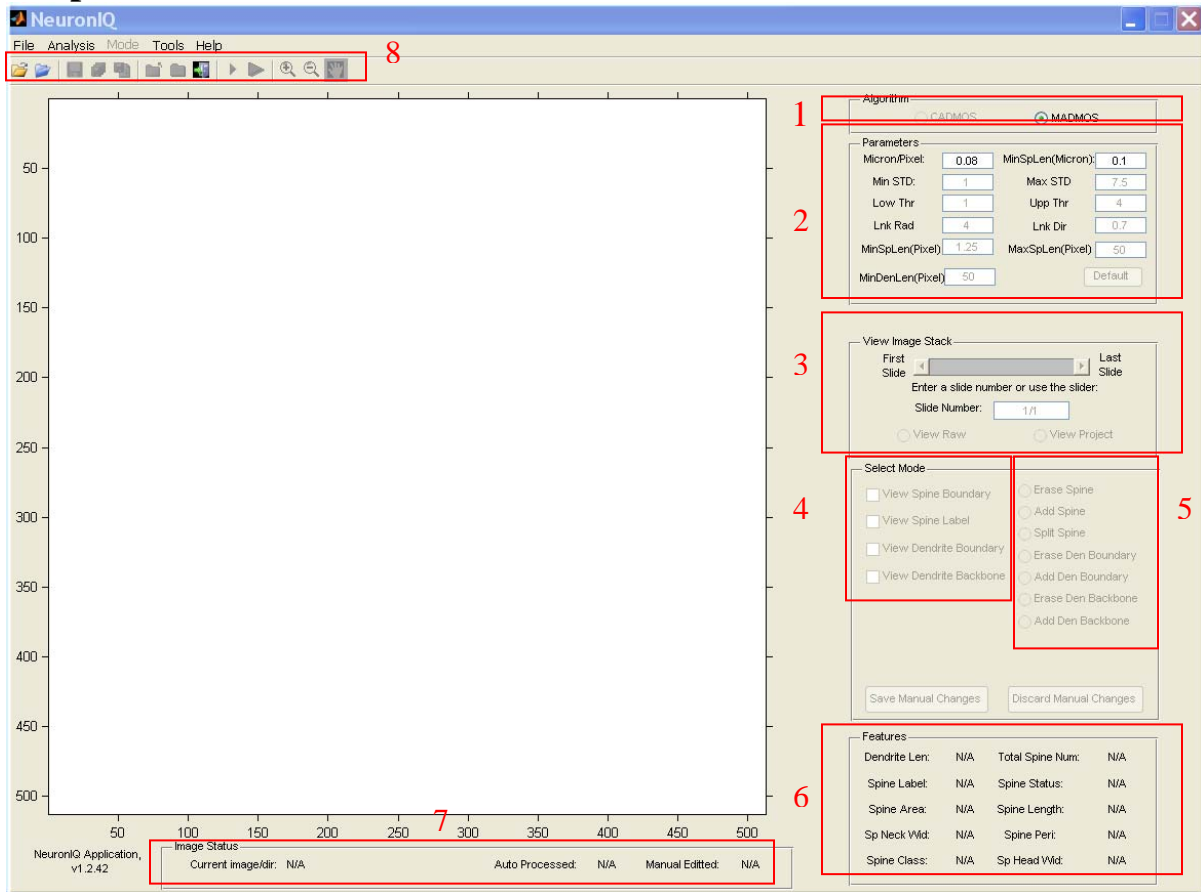
3. Tools

- Filtering: Median filter for denoising
- Interpolation: Interpolation of images with low resolution

4. Help

- NeuronIQ Help: load NeuronIQ Manual
- Online Resources:
- Contact: Contact information
- What's New: Latest changes of NeuronIQ (new functions, bug corrections, etc.)
- About NeuronIQ: Version and authorization information

Graphic User Interface



The above Graphic User Interface (GUI) is divided into 8 parts and each part is explained in the following sections.

1. Algorithm Selection

CADMOS : Curvilinear based Automatic Detection and Measure of Spines
MADMOS : Morphology based Automatic Detection and Measure of Spines

The user can select any one of them for single processing or batch processing. The MADMOS is selected by default. To learn more details about the algorithms, please refer to our published paper [1][2].

2. Parameters:

Two parameters (*Micron/Pixel*, *Min Sp Len*) are required for MADMOS, while four parameters (*Micron/Pixel*, *Min Sp Len*, *MaxSTD*, *Lnk Rad*) are for CADMOS. Details:

For both CADMOS and MADMOS:

- *Micron/Pixel*: ratio between micron & pixel; default value is 0.084
- *Min Sp Len (Micron)*: the minimal length of a spine to be counted; default value is 0.1 micron
- *MinSpLen(Pixel)*: the minimal length of a spine to be counted, equal to $MinSpLen(Micron) / (Micron/Pixel)$; This parameter can also be adjusted after the processing.

For CADMOS Only:

- *MinSTD*: minimum standard deviation. Default value is 1.
- *MaxSTD*: maximum standard deviation. Based on the width of the dendrite. $MaxSTD > MinSTD$ and it is suggested that $MaxSTD < 10$. Default value is 7.5.
- *Low Thr*: vesselness response threshold to select the linking ending point, default value is 1.
- *Upp Thr*: vesselness response threshold to select the linking starting point, default value is 4.
- *Lnk Rad*: Linking radius. It is better to set $2 \leq LnkRad \leq 5$. Default value is 4.
- *Lnk Dir*: Linking Direction. Default value is 0.7.
- *MaxSpLen(Pixel)*: the threshold of the length to distinguish spine and dendrite. Default value is 50 pixels.

3. View Image Stack

- *Slider*: draw to the slice index which you want to observe
- *Slide Number*: input the slice index which you want to observe
- *Raw*: view the original image slide by slide
- *Project*: view the MIP (maximal intensity projection) image
- *Adjust image contrast*: the image contrast will be adjusted when the user drags rectangle in the view panel. It works when none of the manual editing functions is selected. This adjustment is only for visual purpose.

4. View Mode

- *View Spine Boundary*: view the boundaries of detected spines. Blue means valid, yellow means deleted, while cyan means this spine is clicked (cyan won't show in manual editing model).
- *View Spine Label*: view spine label information on image.
- *View Dendrite Boundary*: view the boundaries of detected dendrite. Draw with color red.
- *View Dendrite Backbone*: view the backbone of the dendrites. Draw with color magenta.

All the above modes can be viewed individually or in a combination mode, superimposed on either original image (View Raw) or projected image (View Project).

If the image has been processed by both two algorithms, different results can be viewed by selecting “CADMOS” or “MADMOS”.

5. Manual Edit

- *Erase spine:* To erase/un-erase a spine, **left-click** on that spine, to erase/un-erase all the spines attached to a dendrite piece, **left-click** on the dendrite piece.
- *Add spine:* To add a spine, **left-click** once to change the mouse cursor to cross shape; then **left-click** at a desired location as one spine-neck point; move mouse around the spine, and then **right-click** or double click to finish the boundary drawing. If the “**Left_Ctrl**” key is pressed, the nearest detected spine boundary point will be searched as the current point. User will need to confirm the adding.
- *Split spine:* To split a spine, **left-click** once to change the mouse cursor to cross shape; then **left-click** at a desired location on one spine boundary point; then **right-click** on another boundary point of the same spine to draw a line to split the spine. User will need to confirm the split after the selection.
- *Erase Dendrite Boundary:* To erase part of dendrite boundary, **left-click** once to change the mouse cursor to cross shape; then **left-click** a dendrite boundary point (note: this point does not need to be exactly the boundary point, but must be close enough to a boundary point, the program is able to automatically find the nearest boundary point around the clicked point), move mouse to select a segment, then **right-click** or double click to erase it. User need to confirm after the selection.
- *Add Dendrite Boundary:* To add a segment of dendrite boundary, **left-click** once to change the mouse cursor to cross shape; then **left-click** at a desired location and move mouse slowly (note: too fast mouse moving may result in some boundary points missing), then **right-click** the desired ending location to connect them. If the “**Left_Ctrl**” key is pressed, the program will automatically connect the nearest detected dendrite boundary points. User will need to confirm the adding after the selection.
- *Erase Dendrite Backbone:* To erase part of dendrite backbone, **left-click** once to change the mouse cursor to cross shape; then **left-click** at a location close to the dendrite backbone, move mouse to select the segment, then **right-click** or double click to select it. User will need to confirm the erasion after the selection.
- *Add Dendrite Backbone:* To add part of the dendrite backbone, **left-click** once to change the mouse cursor to cross shape; then **left-click** at a desired location as one backbone point, move mouse slowly, then **right-click** at a desired location as the other backbone point to connect them. If the “**Left_Ctrl**” key is pressed, the program will automatically connect the nearest detected dendrite backbone points. User will need to confirm the adding after the selection.
- *Save Manual Changes:* To save the manual editing results in a separate .mat file.
- *Discard Manual Changes:* To discard all manual editings.

6. Feature

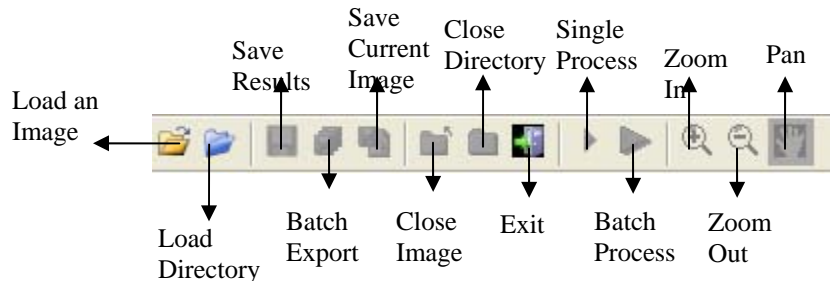
- *Dendrite Len:* total dendrite length in the image stack (micron)

- *Total Spine Num:* number of currently marked spines
- *Spine Label:* label number of a certain spine
- *Spine Status:* if the spine is removed as “Deleted”
- *Spine Area:* area of a certain spine (micron²)
- *Spine Length:* length of a certain spine (micron)
- *Sp Neck Wid:* width of the neck of a certain spine (micron); 0 for detached spines
- *Spine Peri:* perimeter of a certain spine (micron)
- *Spine Class:* class of spine based on the spine length.
- *Spine Head Wid:* the maximum width perpendicular to the length

7. Image Status

- *Current image/dir:* the name of the loaded image / the name of the loaded directory
- *Auto Processed:* Yes if the loaded image has been processed, otherwise N/A.
- *Manual Edited:* Yes if the loaded image has been manually edited, otherwise N/A.

8. Toolbar



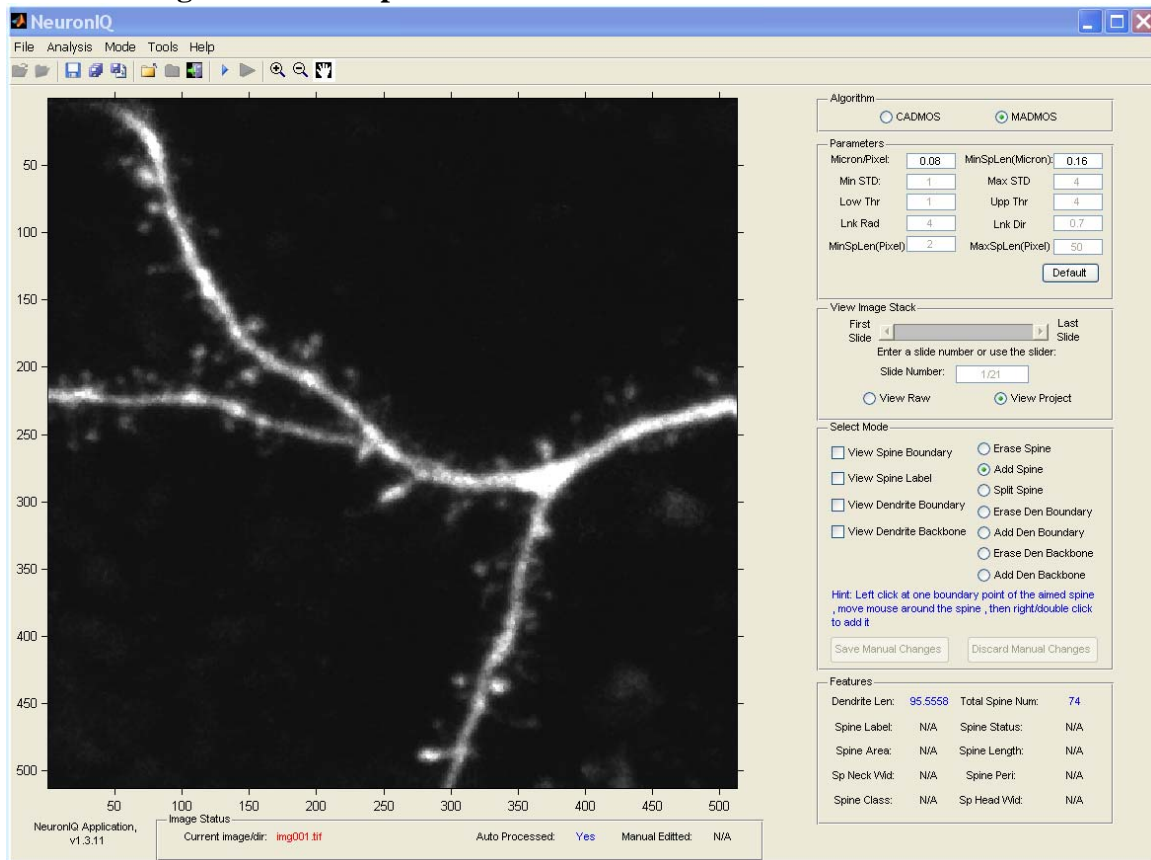
- *Load an Image:* load single 3D neuron image stack or a single 2D image to display, process, etc.
- *Load Directory:* load directory of neuron images to be batch processed
- *Save Result:* save the statistical information of the current image in excel file.
- *Batch Export:* save the statistical information of the images in current folder in two excel file. One is a single summary Excel file listing for each image, the other is an Excel file with compiled spine data from all images
- *Save Current Image:* save the current displayed figure as a bitmap image
- *Close Image Stack:* close the current image.
- *Close Directory:* close the directory.
- *Exit:* quit NeuronIQ
- *Single Processing:* processing single 3D neuron image.
- *Batch Processing:* processing all the 3D neuron images in current directory.
- *Zoom in / Zoom out / Pan*

How to use NeuronIQ to process images:

1. Single Processing

1.1 Load image

1.2 Select algorithm & Set parameter



- 1) Algorithm Selection
CADMOS or MADMOS
- 2) Setting Parameter

If CADMOS is selected, 10 parameters in Region 2 can be adjusted:

- Micron/Pixel
- MinSpLen(Micron)
- Min STD
- Max STD $MaxSTD > MinSTD$. MaxSTD should not be too high. We advised that MaxSTD is set under the “view project” model and $MaxSTD < 10$.
- Low Thr
- Upp Thr
- Lnk Rad
- Lnk Dir

- MinSpLen(Pixel)
- MaxSpLen(Pixel)

If MADMOS is selected, 2 parameters in Region 2 can be adjusted:

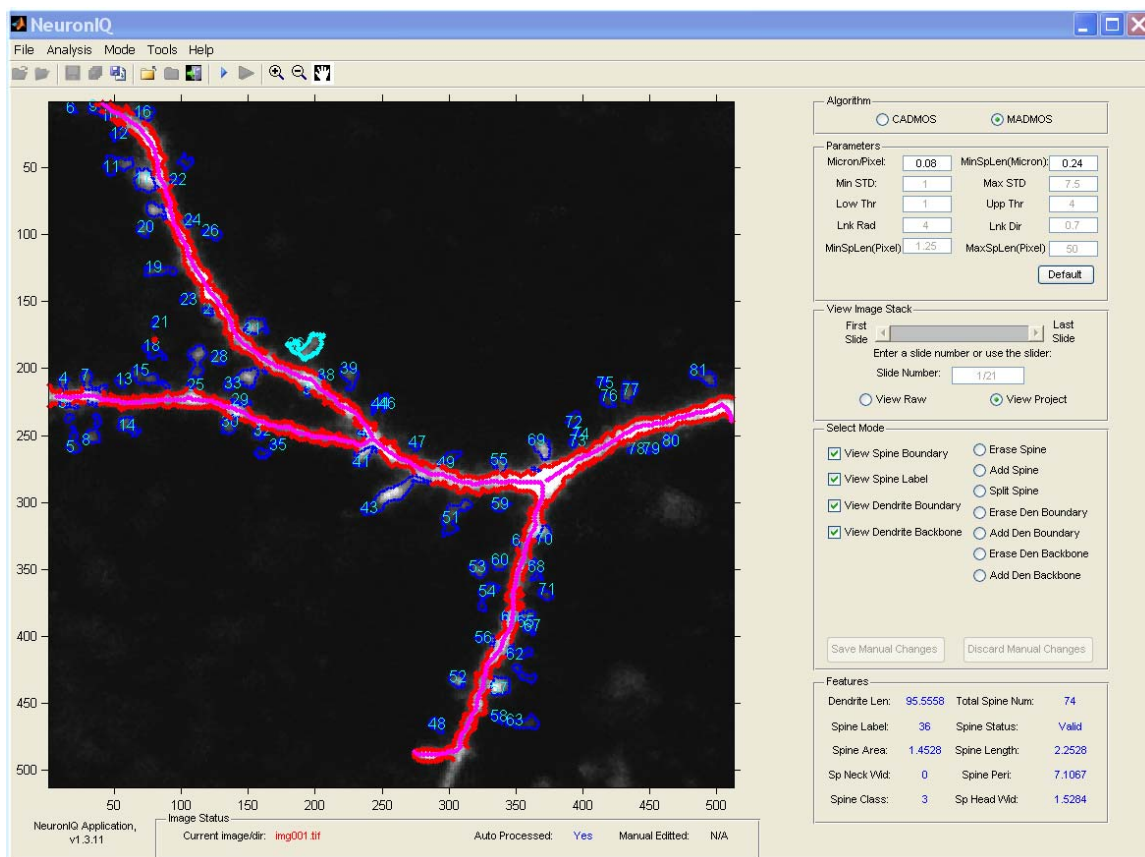
- Micron/Pixel :
- MinSpLen :

1.3 Single processing

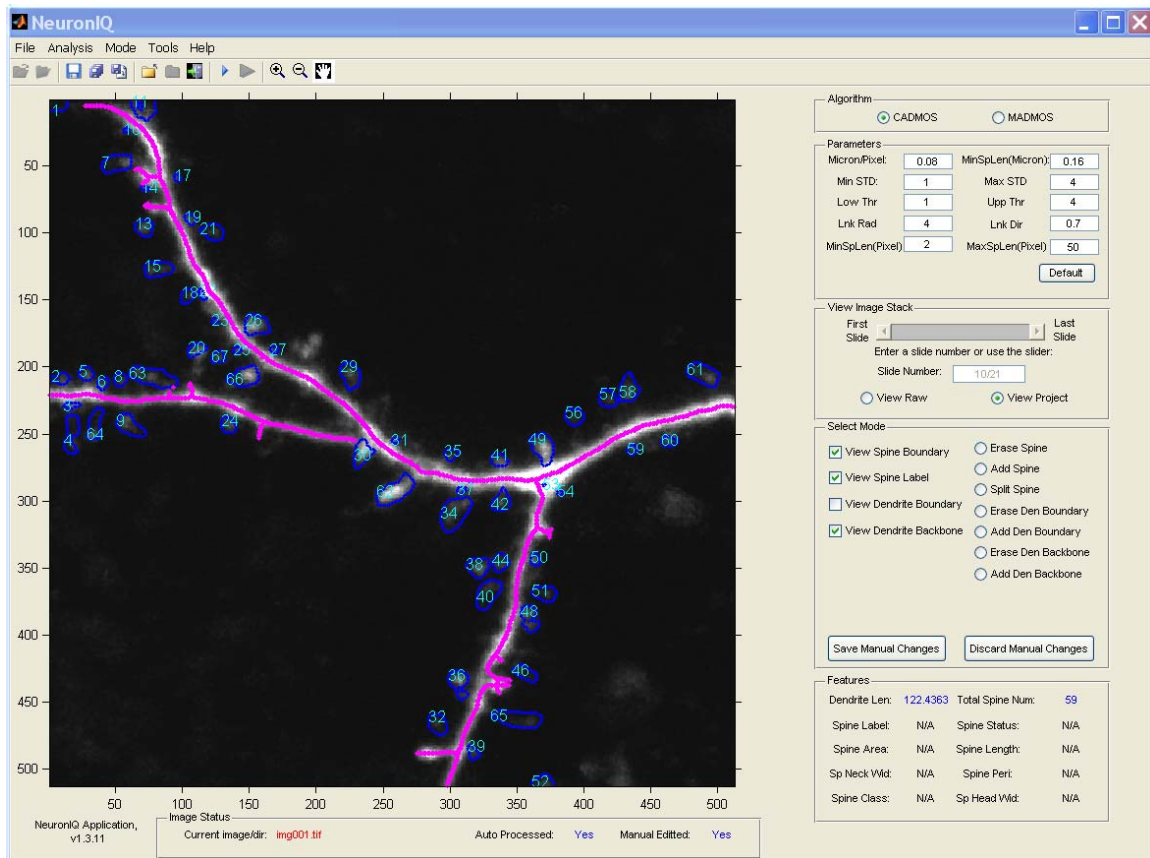
By press “single processing” button in Toolbar or “Menu→Analysis→Single Processing”

1.4 View Result

1. If this image has been processed both by CADMOS and MADMOS, different result can be viewed by changing in the Algorithm “CADMOS” or “MADMOS”.
2. The “Feature” block show the statistical information of the clicked spine with the cyan color.



This figure shows the result of MADMOS in combined mode under “View Project”.



This figure shows the result of CADMOS in combined mode under “View Project”.

1.5 Manual Change

Including:

- **Erase Spine**
- **Add Spine**
- **Split Spine**
- **Erase Den Boundary**
- **Add Den Boundary**
- **Erase Den Backbone**
- **Add Den Backbone**

Enter manual editing mode by clicking the button. Click the selected manual editing button again if you want to quit manual editing mode.

1.6 Save Result

Press the “save result” button in the Toolbar, an excel file with the statistical information will be saved.

1.7 Close Image Stack

2. Batch Processing

2.1 Load Directory

2.2 Algorithm Selection & Parameter

Same with Single Processing

2.3 Batch Processing & batch export

Two excel file will be generated:

Excel file 1: Statistical information of spines for all the images in current folder.

1	File Name	Spine Ids	Label Num	Length	Area	Peri	NeckWid	HeadWidth	Spine Class	J	K	L	M	N
2														
3	Cloz1bN1D1.ism.tif	1	1	1.21062	1.2864	4.30648	0.56	1.28996124	2					
4	Cloz1bN1D1.ism.tif	2	2	2.397332	1.2608	5.67488	0.16	0.536656315	3					
5	Cloz1bN1D1.ism.tif	3	3	1.517893	0.7552	4.14368	0	0.6835203	3					
6	Cloz1bN1D1.ism.tif	4	4	0.576888	0.4928	3.00432	0.48	0.576888204	2					
7	Cloz1bN1D1.ism.tif	5	5	1.975854	1.1328	5.7852	0.78624	0.6835203	3					
8	Cloz1bN1D1.ism.tif	6	6	1.067333	0.7104	3.71056	0	0.91214034	2					
9	Cloz1bN1D1.ism.tif	7	7	1.114271	2.0096	6.73704	1.3656	1.1892855	2					
10	Cloz1bN1D1.ism.tif	8	8	1.131371	0.7808	3.72992	0.57936	0.724430811	2					
11	Cloz1bN1D1.ism.tif	9	9	1.153776	0.6016	3.10368	0.46624	1.153776408	2					
12	Cloz1bN1D1.ism.tif	10	10	0.905097	0.96	4.08304	0.8828	1.35764502	2					
13	Cloz1bN1D1.ism.tif	11	11	0.339411	0.2304	1.75184	0.43312	0.339411255	1					
14	Cloz1bN1D1.ism.tif	12	12	1.369379	2.1824	5.75208	1.05936	1.776288265	3					
15	Cloz1bN1D1.ism.tif	13	13	1.647301	1.9456	5.73832	0.48	2.004794254	3					
16	Cloz1bN1D1.ism.tif	14	14	1.933287	0.7872	4.91896	0.24	0.609261648	3					
17	Cloz1bN1D1.ism.tif	15	15	1.641463	1.824	5.7052	1.30216	1.641463752	3					
18	Cloz1bN1D1.ism.tif	16	16	1.926655	2.3872	6.07488	0.9656	1.292497563	3					
19	Cloz1bN1D1.ism.tif	17	17	2.183575	1.8112	9.5592	0.73936	1.052235715	3					
20	Cloz1bN1D1.ism.tif	18	18	1.681904	3.4688	9.53168	1.31692	2.001599361	3					
21	Cloz1bN1D1.ism.tif	19	19	1.76	2.272	10.56072	1.46216	1.04	3					
22	Cloz1bN1D1.ism.tif	20	20	1.111396	0.64	4.48584	0.75312	1.111396519	2					
23	Cloz1bN1D1.ism.tif	21	21	1.697056	2.5984	11.0076	1.4456	2.828427125	3					
24	Cloz1bN1D1.ism.tif	22	22	1.36	3.296	8.1936	1.69936	2.64	3					
25	Cloz1bN1D1.ism.tif	23	23	0.452548	0.704	5.12864	0.9628	0.4	1					
26	Cloz1bN1D1.ism.tif	24	24	0.288444	0.1216	1.2056	0.60968	0.24	1					
27	Cloz1bN1D1.ism.tif	25	25	1.202664	2.2336	8.65704	1.77936	2.405327421	2					
28	Cloz1bN1D1.ism.tif	26	26	0.609262	0.5504	3.6168	0.51312	1.218523697	2					
29	Cloz1bN1D1.ism.tif	27	27	0.48	0.4864	3.00432	0.83312	0.96	1					
30	Cloz1bN1D1.ism.tif	28	28	0.737564	0.6336	2.94088	0.54624	0.98954535	2					
31														
32														
33	fCloz1bN1D1.ism.tif	1	1	1.442221	1.152	4.3368	0.48	1.043072385	3					
34	fCloz1bN1D1.ism.tif	2	2	2.406657	0.9408	5.73832	0.24	0.6835203	3					
35	fCloz1bN1D1.ism.tif	3	3	1.302306	0.1792	1.51184	0	0.407921561	1					
36	fCloz1bN1D1.ism.tif	4	4	0.252982	0.16	2.05808	0.16	0.505964426	3					
37	fCloz1bN1D1.ism.tif	5	5	1.609969	0.6976	4.30648	0.16	0.609261648	3					
38	fCloz1bN1D1.ism.tif	6	6	0.976524	0.2496	2.05808	0	0.4	2					
39	fCloz1bN1D1.ism.tif	7	7	1.07629	0.5568	3.52024	0.57936	0.850411665	2					
40	fCloz1bN1D1.ism.tif	8	8	0.91214	0.2944	2.19776	0.16	0.592408791	2					
41	fCloz1bN1D1.ism.tif	9	9	1.24964	1.152	5.15986	0.6828	1.249639948	2					
42	fCloz1bN1D1.ism.tif	10	10	1.264911	2.0352	5.78864	1.15592	1.553319027	3					
43	fCloz1bN1D1.ism.tif	11	11	1.68	0.6976	4.14928	0.16	0.64	3					
44	fCloz1bN1D1.ism.tif	12	12	0.976524	0.224	1.6884	0	0.512249939	2					
45	fCloz1bN1D1.ism.tif	13	13	0.576888	0.1344	1.47872	0.08	0.288444102	2					
46	fCloz1bN1D1.ism.tif	14	14	1.770875	0.1088	1.2856	0	1.078885438	3					
47	fCloz1bN1D1.ism.tif	15	15	1.53675	1.5168	5.37144	1.49528	1.424359505	3					
48	fCloz1bN1D1.ism.tif	16	16	1.936595	2.0288	5.7852	0.94904	1.82428068	3					

This file would report for each spine: spine length, spine head width, spine neck width, spine head area, spine head perimeter and spine classes.

Excel file 2: Summary for all the images in current folder

1	File Name	Dendrite L	Spines Nu	Spines Nu	Spines Nu	Spines Nu	Total Spine	Spine Den	Spine Den	Spine Den	Spine Den	Total Spine Density
3	Cloz1bN1C	42.42504	4	11	13	0	28	0.094284	0.259281	0.306423	0	0.659988
6	fCloz1bN1I	42.53536	2	13	14	0	29	0.04702	0.305628	0.329138	0	0.681786
9	fcore-slice	53.16512	9	5	22	0	39	0.169284	0.094047	0.413805	0	0.733564
12	fdorsal-slic	80.79944	17	23	17	0	58	0.210397	0.284655	0.210397	0	0.717827

This file listing for each image: dendritic backbone length, spine count in each class, total spine count, spine density by class (=spine count in spine class N / dendritic backbone length), total spine density (=total spine count / dendritic backbone length).

2.4 Close Directory

Press the “Close Directory” button in the Toolbar.

Note: During the batch processing, if some images can’t be processed by NeuronIQ, the file names will be stored in “Error.txt”.

Reference

[1] J. Cheng, X. Zhou, E. Miller, R. M. Witt, J. Zhu, B. L. Sabatini, and S. T. Wong, A novel computational approach for automatic dendrite spines detection in two-photon laser scanning microscopy. *J. of Neurosci. Methods* 165: 122-134 (2007)

[2] Y. Zhang, X. Zhou, R. M. Witt, B. L. Sabatini, D. Adjero, and S. T. Wong, Dendritic spine detection using curvilinear structure detector and LDA classifier. *Neuroimag.* 36: 346–360 (2007)