Synapse Quantification (SynQuant)

Introduction

SynQuant is a Fiji plugin that automatically quantify synapses from multi-channel fluorescence microscopy image. Both synapse and corresponding dendrite are detected. Synapses are detected on synapse channel, where they act as puncta surrounded by highly inhomogeneous interference signals. Dendrite is extracted from the reference dendrite channel.

SynQuant detect synapses through a totally unsupervised probability principled framework. In this framework, analysis is conducted on salient regions rather than pixels. All synapse candidates are scored by their own local contrast and compared fairly with each other. What's more, false discover rate (FDR) control is utilized to determine synapse selection, which not only controls the false positive rate but also provides a statistical evidence of the detected synapse. The parameter used in this framework is only the value of FDR which is easy to tune. SynQuant extract dendrite by steerable filter [1]. Extracted dendrite then are segmented into roughly homogeneous pieces by branch points and end points. Based on the dendrite pieces and synapses, linear regression is used to find the effects of dendrite's properties to the number of synapses on it.

Functions Supported

SynQuant could not only quantify synapses, but also some related tasks. Now SynQuant could totally support 6 functions including **"Pre-synaptic puncta detection", "Post-synaptic puncta detection", "Dendrite detection", "Synapse quantification", "Synaptic site detection", and "Synaptic site quantification"**. The details of these functions can be found in the "**Outputs**" section.

How to cite

The journal paper for this algorithm is in preparation. You can cite the conference paper first:

Yizhi Wang, Guilai Shi, Yinxue Wang, Lin Tian, Guoqiang Yu*, "PPSD: Probability Principled Synapse Detection," BioImage Informatics Conference 2015, October 2015.

Tutorial

Installation

Download file "SynQuant_-1.0.jar" from "https://github.com/VTcbil/SynQuant". To install the SynQuant plugin, simply save the jar file to the folder "\Fiji.app\plugins\" and call "Help=>Refresh Menus" or restart Fiji/ImageJ. The SynQuant plugin will be available in Fiji/ImageJ's "Plugins" menu.

Some functions of SynQuant plugin are based on Apache Commons Math, which is supported by latest Fiji version (1.50d). Also you can download the "commons-math*-*-bin.zip" from "address" and save the "commons-math*-*.jar" to the same file as "SynQuant_.jar".

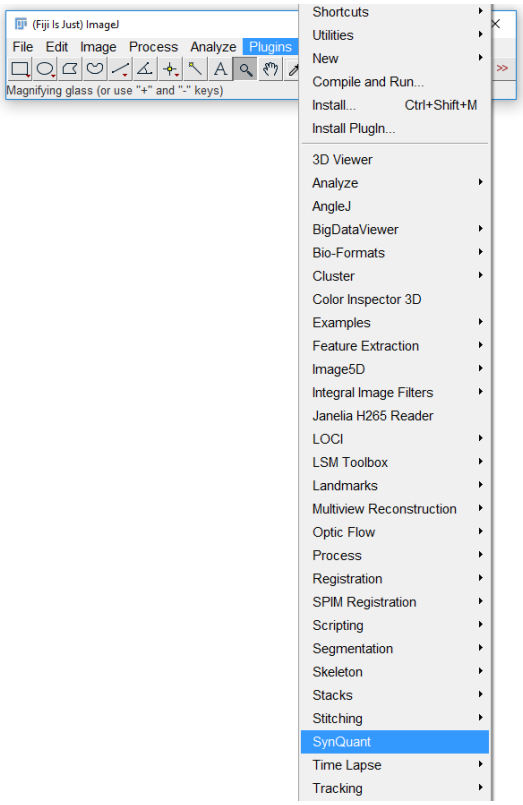

Figure 1. Start SynQuant

The plugin has been tested and successfully run on ImageJ version 1.50d. If users encounter problems using older version of ImageJ, please update your ImageJ to the newer version.

Input

SynQuant is designed to handle 8-bit or 16-bit grayscale image. User can use Fiji/ImageJ's "Image\Type\8-bit (or 16-bit)" to first change the data into these formats and then call SynQuant. If the image contains multiple channels, user need to verify the synapse channel (pre-synaptic or post-synaptic) for synapse detection and dendrite channel for dendrite extraction. It also could handle single channel 8-bit or 16-bit grayscale image for one of the two tasks.

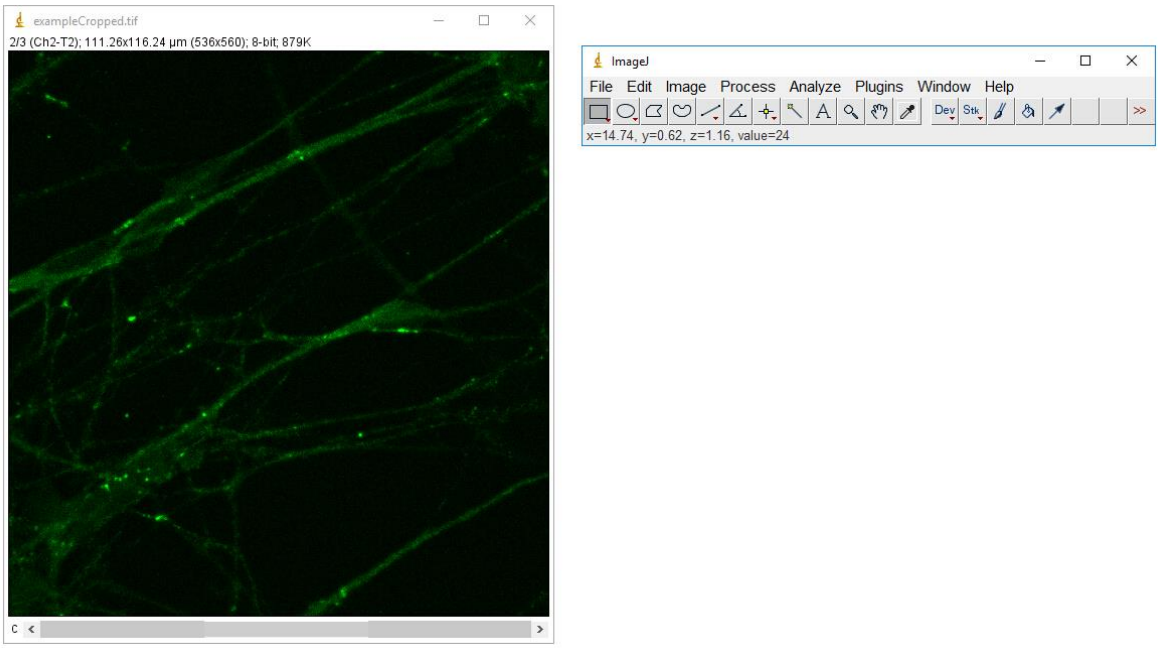

Figure 2. Open test data.

Parameter setting

We have three parameters. One is the False Discovery Rate. The other two parameters are the size interval of possible synapses. With the grayscale image, run SynQuant from the Plugins menu. A dialogue will show and ask user to input the parameters as is shown below. The first three are to specify pre-synaptic, post-synaptic and dendrite channel. If user only wants to do synapse (pre- or post-) detection or dendrite extraction, input the corresponding channel number (input 1 if only one channel is available) and set the other channel number zero. The third input is the threshold for FDR Control for synapse detection. Suggested value is given as default in the textboxes. After setting the parameters, click “OK”. The running time is decided by the image size. A 1024*1024 image typically takes 5 minute on Intel Xeon CPU E5-2630 for synapse detection and 1 minute for dendrite extraction.

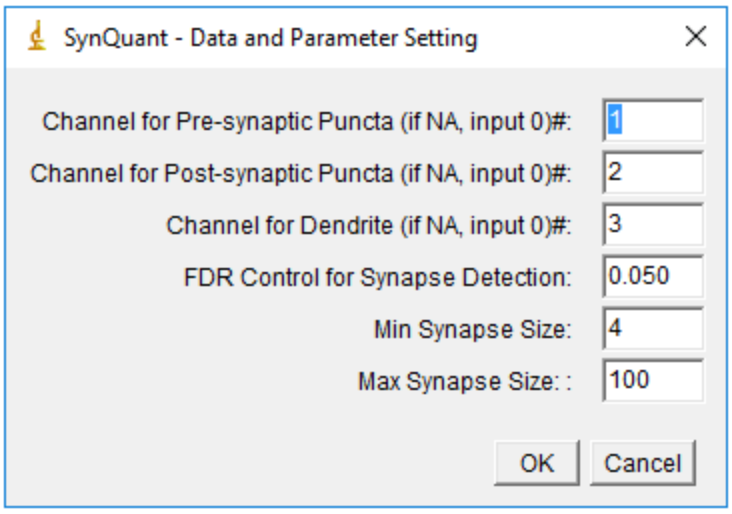

Figure 3. Parameter input

Supported functions

Based on the channels user inputs, SynQuant could fulfill totally 6 functions related to synapse quantification.

Let's assume the test data contains 4 channels. Channel 1 is pre-synaptic channel. Channel 2 is post-synaptic channel. Channel 3 is dendrite channel. Channel 4 is soma channel. Then the relationship between input channels and SynQuant functions are shown as following. For example, if user would like to do synapse quantification, she/he needs to input the channel number of post-synaptic puncta (or pre-synaptic puncta) and dendrite, and leaves the channel number of pre-synaptic puncta (or post-synaptic puncta) zero. Table I illustrates the six functions SynQuant supports.

Table I. Relationship between input channels and the function SynQuant does

Channel for Pre-synaptic Puncta	Channel for Post-synaptic Puncta	Channel for Dendrite	*Function*
1	0	0	**Pre-synaptic puncta detection**
0	2	0	**Post-synaptic puncta detection**
0	0	3	**Dendrite detection**
0	2	3	**Synapse quantification**
1	2	0	**Synaptic site detection**
1	2	3	**Synaptic site quantification**

Outputs

The outputs varies across functions. In the following paragraphs, we will illustrate the different outputs of different functions.

F1&F2. Pre-synaptic puncta detection and **Post-synaptic puncta detection**

For synapse detection, user will get a colorful output image, which is the combination of detected synaptic puncta and original synapse channel. The pre-synaptic puncta (or post-synaptic puncta) are marked out by bright color. The detected puncta are also recorded in the ROI Manager as is shown on the right side in Figure 4. User could show the labeled synaptic puncta by check the "Labels" checkbox in the ROI Manager Dialog as is shown in Figure 5. By clicking "Measure" button, all puncta's size, mean, minimize and maximize intensity values can be got and saved as is shown in Figure 6. User can also save all puncta information by clicking "More >> ➔ save…" in the ROI Manager Dialogue.

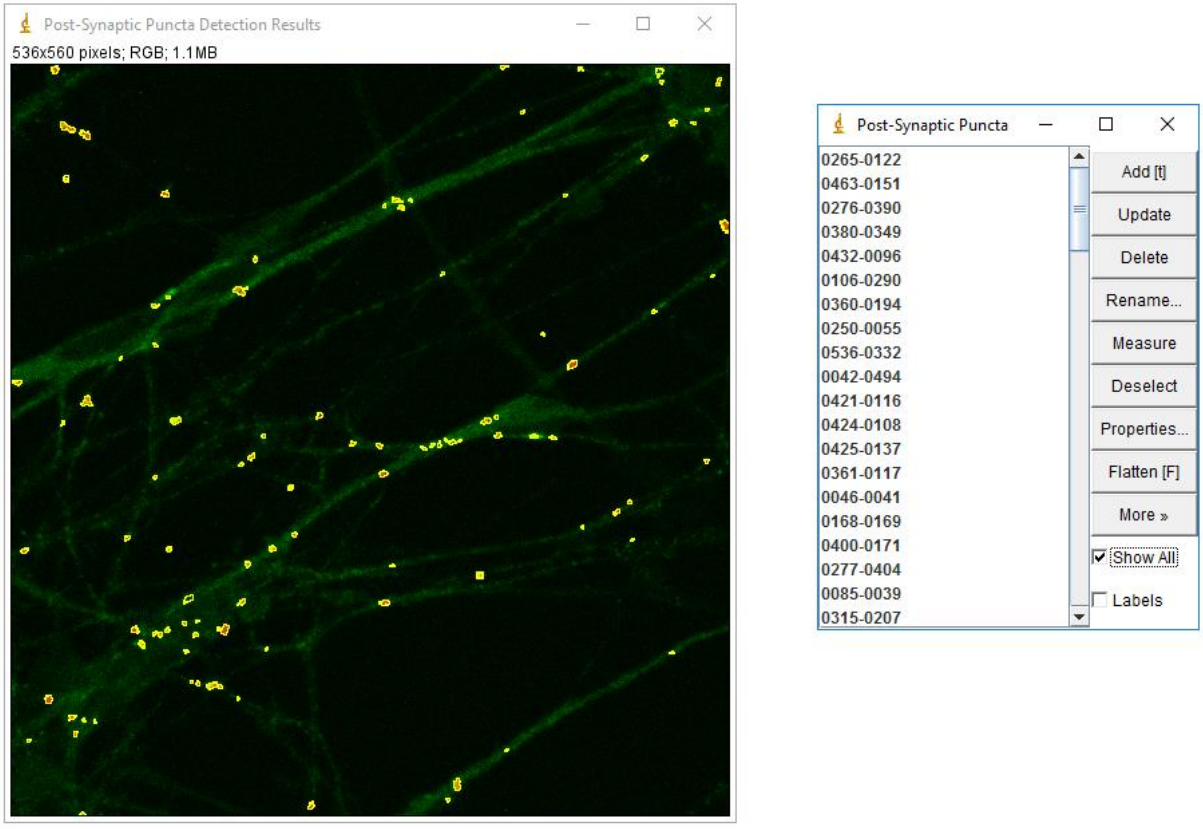

Figure 4. Synaptic puncta detection result

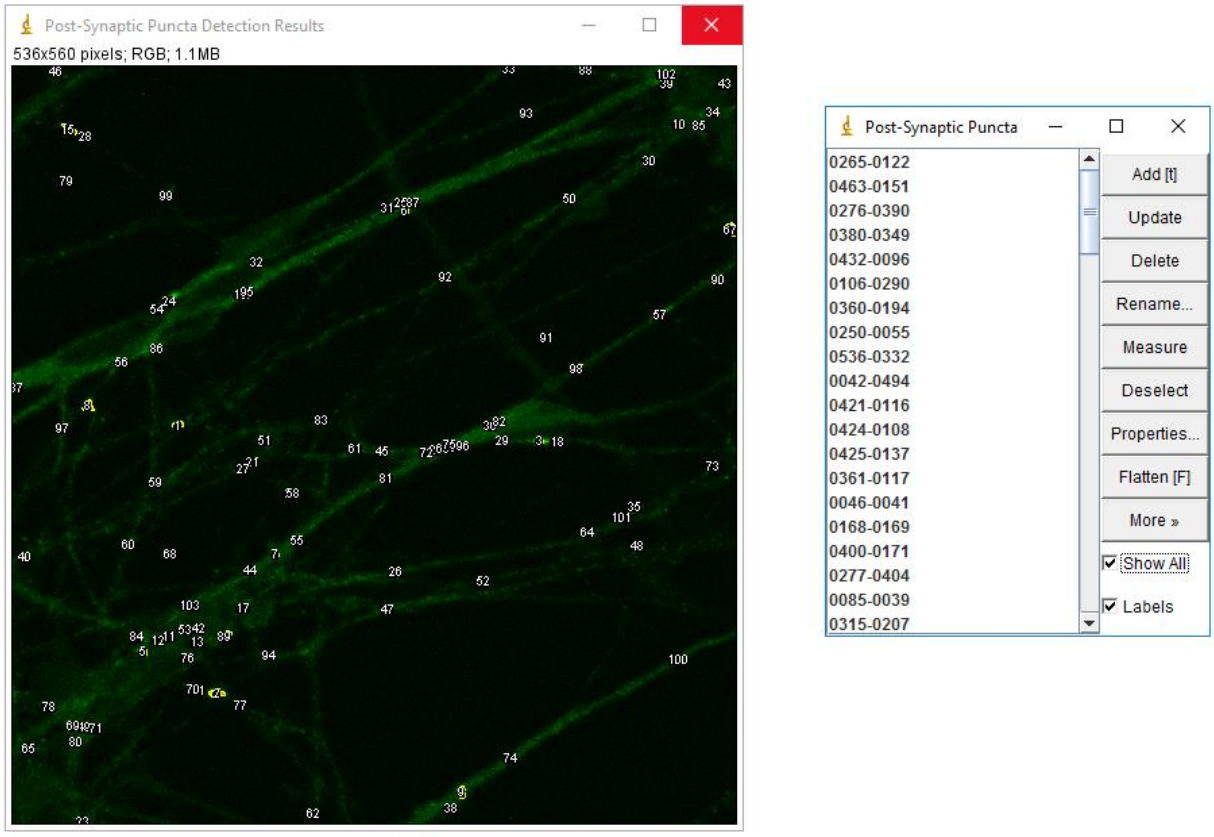

Figure 5. Synaptic puncta detection result with roi labels

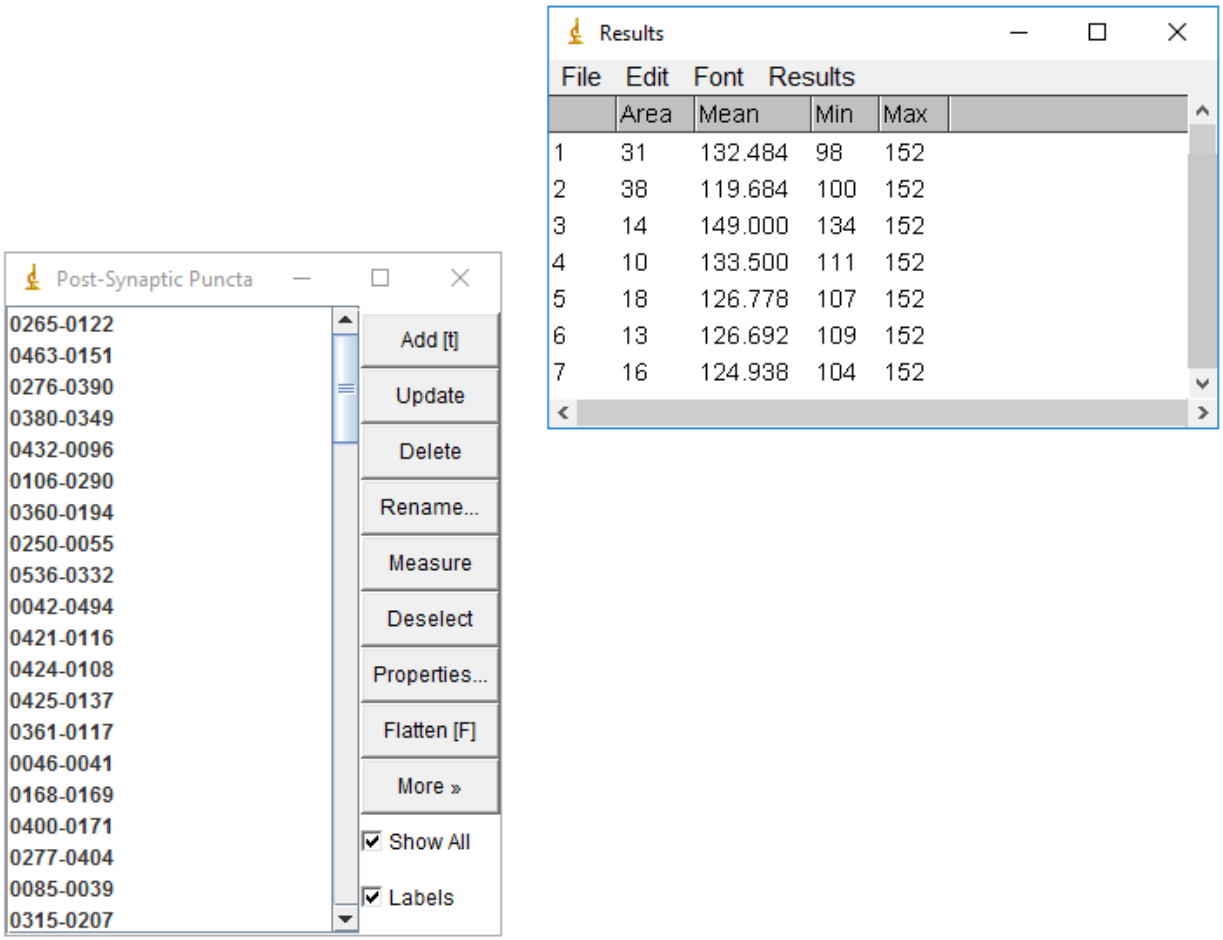

Figure 6. Puncta properties using Measure in ROI Manager.

F3. Dendrite detection

For dendrite detection, user will get a colorful output image which is combination of extracted dendrite and original dendrite channel. All extracted dendrite is marked as blue. As most dendrite are connected, it is hard to define ROI on this output. So we use a table to show user the dendrite length as Figure 8. User can use “File\save” to save the dendrite mask. If user would like to save the binary dendrite mask only, she/he could use “Image➔Color➔Split Channels” to get the three channels of the output image as is shown in Figure 9. The green and blue channel are what is needed. (The synapse mask can also be got using this way.)

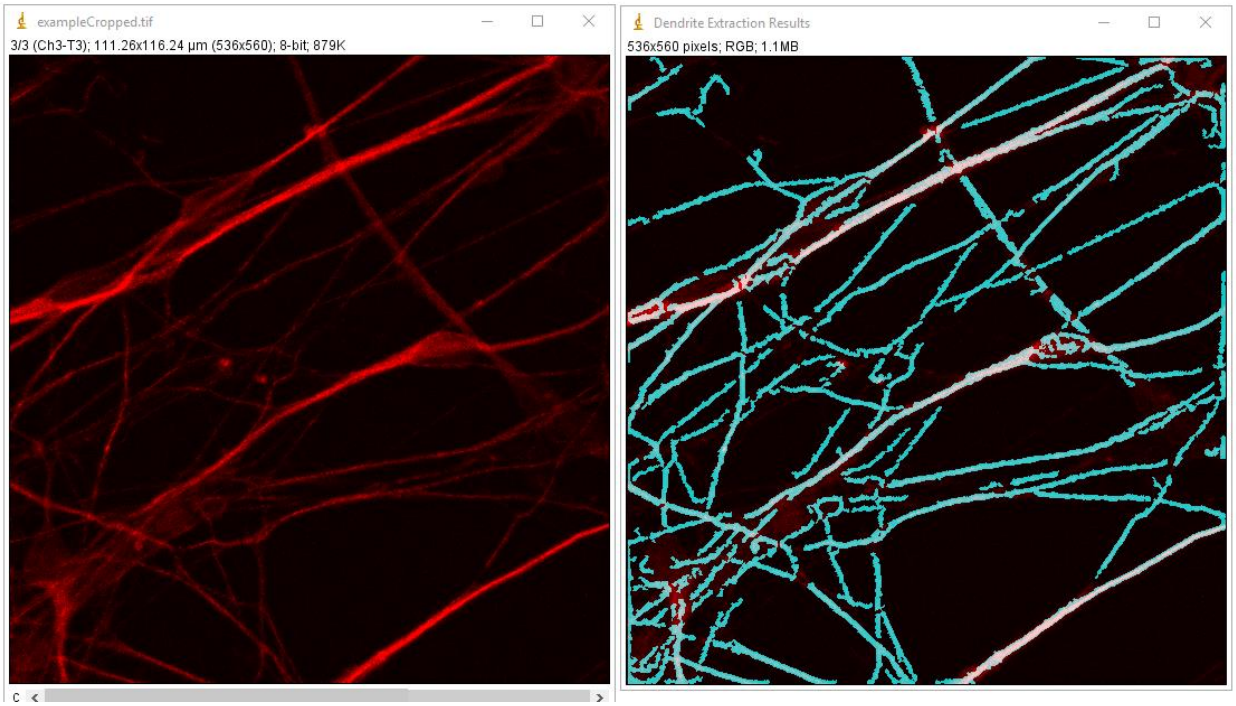

Figure 7. Dendrite detection result

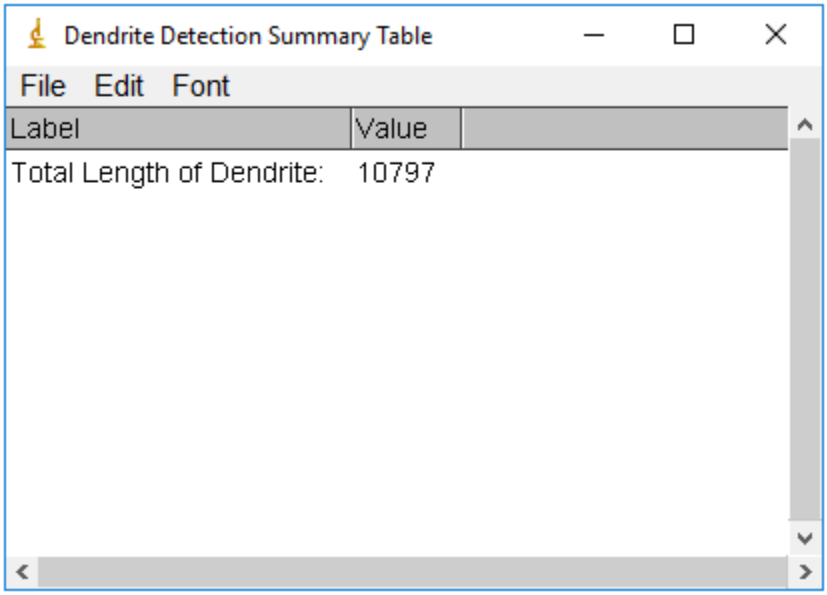

Figure 8. Dendrite length table

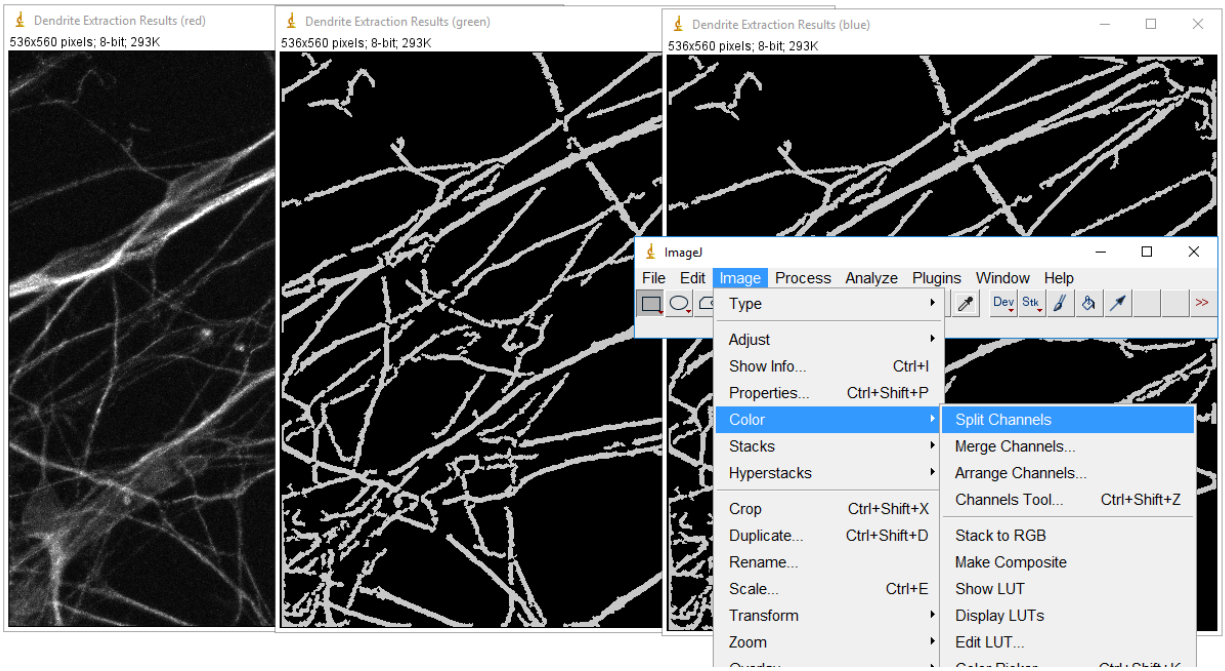

Figure 9. Channel split to get dendrite mask

F4. Synapse quantification

For synapse quantification, all the outputs in F1, F2 and F3 will also be shown. Besides, the quantification step will output three more tables.

The first one is “Feature Table” as is shown in Figure 10. All dendrites are segmented into small homogeneous pieces and for each piece, we collect its length, scale and mean intensity as its properties. Because each detected synapse is assigned to its nearest dendrite piece, each dendrite piece also have a property that indicates how many synapses related to it.

Synapse Quantification Feature Table

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Label	Number of synapse on it	Dendrite length	Dendrite scale	Dendrite intensity
Dendrite piece #1	1	12	36	27
Dendrite piece #2	0	34	180	46
Dendrite piece #3	1	104	605	137
Dendrite piece #4	0	47	145	35
Dendrite piece #5	0	26	102	88
Dendrite piece #6	0	23	80	102
Dendrite piece #7	1	10	26	39
Dendrite piece #8	0	12	36	49
Dendrite piece #9	0	8	35	56
Dendrite piece #10	0	7	31	56

Figure 10. Synapse Quantification Feature Table

The second table is to illustrate the effects of the three dendrite features to the number of synapses grow on this dendrite piece. The values are the corresponding coefficients got through generalized linear model (Poisson regression).

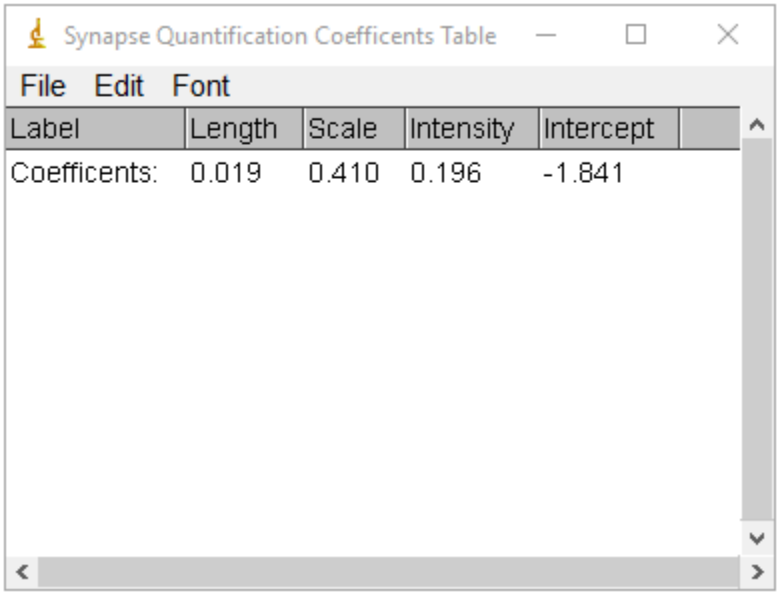

Figure 11. Relationships between synapse number and dendrite features

The third table is a summery table including the total number of detected synapse puncta, total length of dendrite, and puncta density per unit length as is shown in Figure 12.

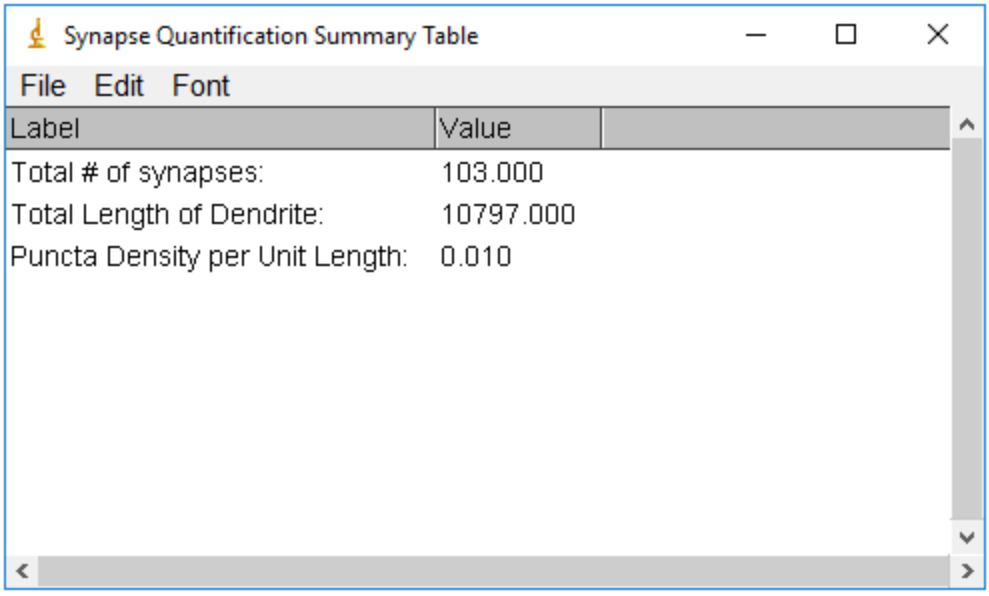

Figure 12. Summery table for synapse quantification

F5. Synaptic site detection

Often, researchers want to compare locations of pre- and post-synaptic puncta (i.e., indicating the position of true synaptic sites). Therefore, we allow user to detect pre- and post- synaptic puncta at the same time and based on the detection results, generate the overlap of pre- and post-synaptic puncta. The pre- and post-synaptic puncta detection results are shown in Figure 13 (left for pre- and right for post-).

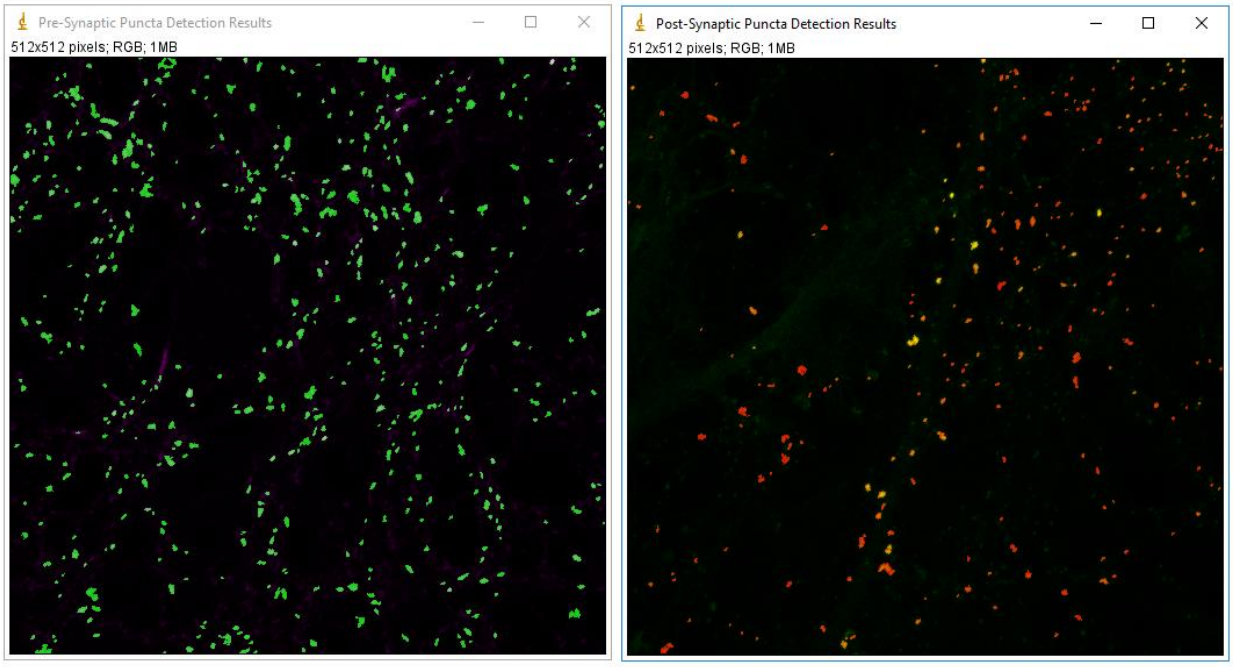

Figure 13. Pre- and post-synaptic puncta detection results

The overlapped regions are shown in Figure 14. This output is just like that in F1 and F2. It includes pre-synaptic channel (blue), post-synaptic channel (green) and overlapped puncta (bright orange). User can output the ROIs using ROI Manager.

We also output a table to show the number of detected puncta on these two channels as well as the number of overlapped puncta as is shown in Figure 15.

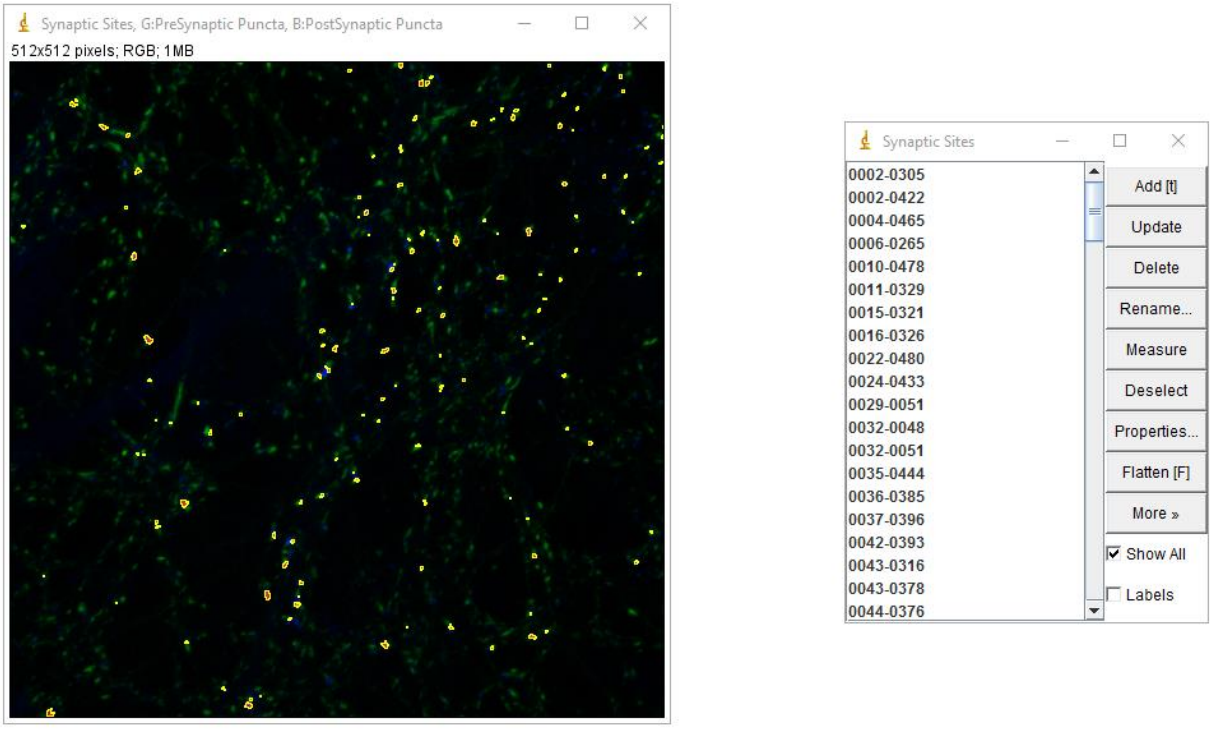

Figure 14. Overlapped puncta detection result

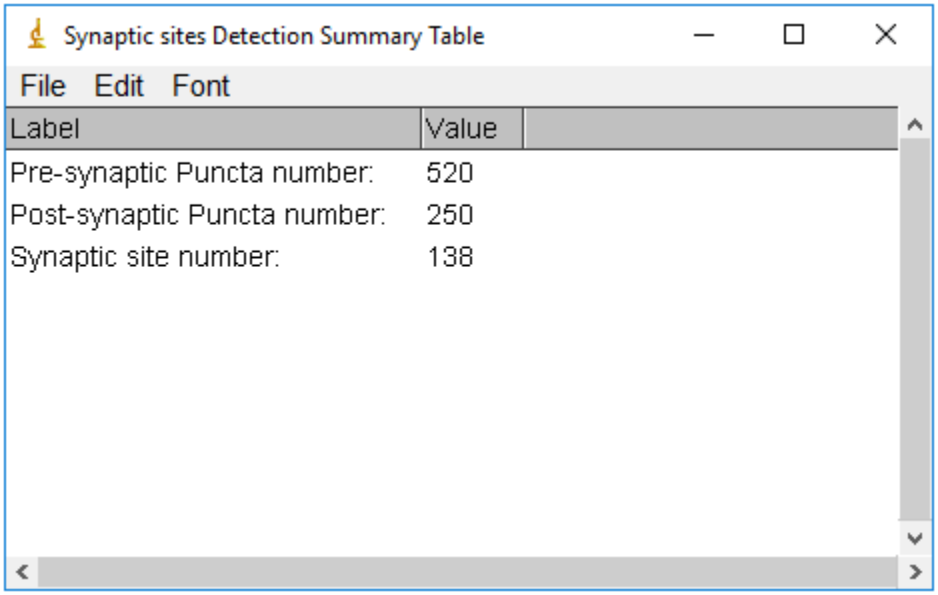

Figure 15. Summary of detected puncta

F6. Synaptic site quantification

Synaptic site quantification is similar with synapse quantification which change the dependent variable from number of synapses on one dendrite piece to the number of synaptic sites on one dendrite piece. The outputs of F6 include those of both F4 (synapse quantification) and F5 (synaptic site detection).

If you have any question, please contact ccwang@vt.edu.

[1]. Meijering E, Jacob M, Sarria J, Steiner P, Hirling H, Unser M. Design and valida-tion of a tool for neurite tracing and analysis in fluorescence microscopy im-ages. Cytometry A 2004;58:167–76.