SynJ documentation

Overview

SynJ is an ImageJ toolbar menu, developed and maintained by Alessandro Moro, that starts from the basic idea of neurite and synapse detection in neuronal autaptic cultures. It is based on the MATLAB program SynD from Shmitz and Hjorth, 2011 "Automated analysis of neuronal morphology, synapse number and synaptic recruitment", but it aims to provide more insight into the detection to not experience users as well as being inside a native image analysis software like ImageJ.

To install the toolbar simply copy the source file into the Macro>Toolbar subfolder of your ImageJ folder. In addition, the toolbar uses two external plugins: "Ridge detection" and "MorphoLibJ" To takes most advantages of the plugins and the toolbar, it is recommended to use FIJI instead if the basic distribution of ImageJ.

Open SynJ

Once SynJ is install it can be access from the main FIJI interface by selecting the double greater sign (≫) at the far right of the interface as shown in Fig 1.

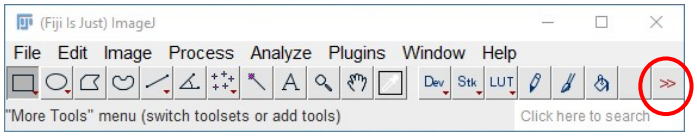

Fig 1: Opening SynJ

As result, the right side of FIJI will update to show the SynJ interface (Fig 2). From left to right now there are the buttons for "Detect Soma"; "Detect Neurite"; "Detect Synapses"; "Mark synapses"; "Save Menu" and "Options".

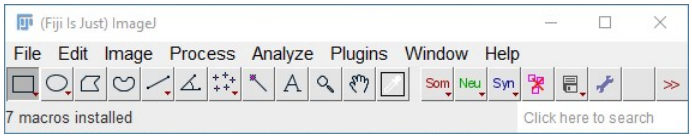

Fig 2: FIJI with SynJ opened

Basic workflow

The detection is based on intensity of different channels, Soma and Neurites are generally called "Morphology channel" and refers to a channel with a space filler, a MAP2 dendritic staining, SMI312 or tau axonal staining, or β3-tubuline microtubule staining. Synapses could be any staining the shows a punctuate pattern where the user is interested to know the intensity inside that particular regions, as well as the distribution along the neurites. These settings are changed in the Options menu.

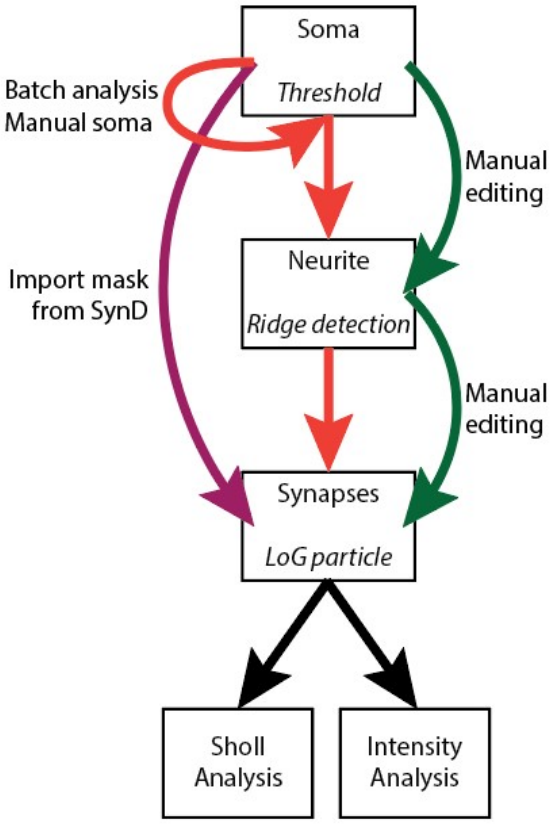

Fig 3: SynJ workflow

There are three main way to use SynJ: Semi automatically, batch processing and importing from SynD (green, orange and purple respectively in Fig 3).

The basic workflow for the semi-automatic processing consist of loading an image, detect the soma and editing the soma to have the desired mask, detect the neurites and adjust the mask to include undetected neurites or to delete background detections, detect the synapses and review the detected synapses to include or exclude particles. When the detection is done, the user can extract the intensity of the three channels in Sholl analysis or as mean intensity inside the soma, along the neurite mask or inside individual synapses.

The batch processing workflow is useful if the user wants to get a quick overview of their data. The first step in the batch processing is to have all the images that the user wish to analyze in the same folder. After selecting that folder in the "Batch Analysis" submenu, the user is asked to draw

the soma mask per individual image, when this is done, the program will continue detecting the neurite and the synapses, save the masks and the intensity and then move on with the next image. The disadvantage of this method is that the quality of the detection is highly variable depending of the signal-to-noise ratio in the individual images, so it is recommended to empirically adjust different detection parameters to be as inclusive as possible.

In addition, SynJ gives the opportunity to convert neurites masks previously generated in SynD. This could be useful for different reasons, for example: lack of MATLAB license, meaning that the user does not have the possibility to keep on working with SynD; the user would like to have better control over the individual masks and detection output; more image analysis implementations and many other cases. To import SynD masks into SynJ, the user should indicate the folder where the images are locate and the folder where the output of SynD is located. SynJ will then load the images and try to match them automatically with their respective neurite mask, then, the program will continue with synapses detection and intensity analysis.

Option explanation

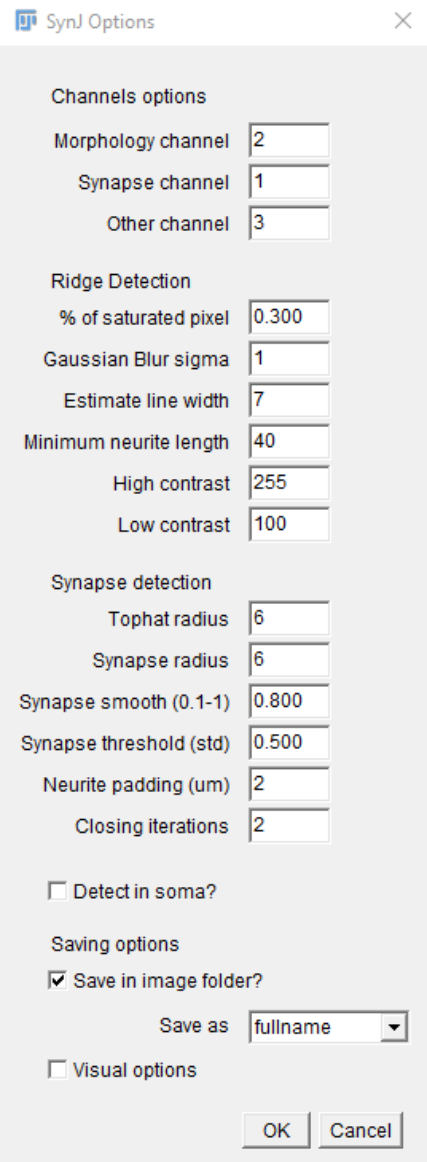

Fig 4: SynJ options

To give the user the best control over the detection depending on their need, SynJ offers different options for the different detection. The options are divided in 6 different categories: channel options; soma detection; neurite detection; synapse detection; saving options and visual options (Fig 4).

Channel options: the user will set the channel of a 3 color images to map the morphology, synapses and other channel, where "other" mean another channel that the user would like to calculate intensity but that does not contribute in the masking of the neuron.

Soma detection: allows the user to adjust the parameter for the automatic detection of the soma, discussed more in detail later. Briefly the minimum soma radius and the maximum soma radius indicate the expect size difference between neurites size and soma size.

Ridge detection: are the parameters for the neurite detections, discussed more in details in a later chapter. Here the user can adjust the parameter to remove scatter noise and to indicate the desired signal-to-noise ratio as well as the expected neurite thickness and length.

Synapse detection: the users have the possibility, as described later, to set the desired particle size, as well as the expected intensity above the noise level; in addition, synapses can be detected alongside the neurite mask with a padding option, or inside the soma.

Saving options: here the user can set the desire folder where to save the mask and the result table, as well as save it with the full name or with the coverslip and cell ID. For the full name options the result files with be save as "Result_*MyImageName*.csv", "Sholl_*MyImageName*.csv" and "RoiSet_*MyImageName*.zip". For the coverslip and cell ID the image file must have a name with the format YYMMDD_condition_csID_cellID, where YYMMDD is a date in the format "year/month/day", for example 191128 for the 28th November 2019, and csID is the coverslip ID and cellID the cell ID. The result files with be save as "Result_*csID_cellID*.csv", "Sholl_*csID_cellID*.csv" and "RoiSet_*csID_cellID*.zip". It is recommended to use the full name option.

Visual options: gives the user the possibility of changing the color of the selection for the morphology mask, soma and neurite, for the synapses that will be included in the intensity analysis, "keep synapses", and for the one that will be excluded, "delete synapses". As default the colors are white, green and red respectively.

Soma

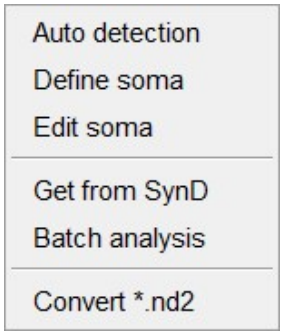

Fig 5: SynJ soma

The soma submenu (Fig 5) allows the user to select an ***Automatic detection***, where SynJ will start by detection the soma, input the user to adjust the mask manually, then it automatically moves on with the neurites and synapses detection.

If the user select ***Define soma*** SynJ will select the polygon tool of ImageJ and ask the user the manually draw the soma, when done it will ask the user to edit the soma in case the user would like to adjust the tracing. The option ***Edit soma*** will prompt the user to adjust the soma mask with the brush tool of ImageJ. The maximum soma radius parameter in the options defines the brush tool size.

The automatic detection of the soma is based on the threshold difference between the neurites and the background. First a morphological opening of the maximum soma radius will be applied to the image, and subtract to the original image. Then a morphologically closing function, with the minimum soma radius, is applied to the result image to identify the area with the biggest difference. The soma will be defined with a *Minimum error* threshold inside this area.

The ***Get from SynD*** option will prompt the user to convert the output of SynD into SynJ. The ***Batch analysis*** will prompt the user as described in the workflow chapter.

The ***Convert *.nd2*** will prompt the user for batch converting Nikon nd2 images into tiff images.

Neurites

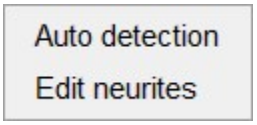

Fig 6: SynJ neurite

The neurite submenu (Fig 6) allows the user the select an ***Automatic detection*** where SynJ will use the *Ridge detection* plugin to identify the neurites. The detection will calculate two threshold based on the neurite size and the desired minimum and maximum intensity, the user should be aware that increasing the gap between minimum and maximum intensity will result in slower processing and the risk of having more detection in the background. The detection will then identify regions based on the two threshold and connect them following a line path, every path shorter than the minimum neurite length will be discarded. It is recommend setting the Gaussian blur value around one in order to have a more homogenous image, higher values might reduce the signal from thin neurites and lower value might increase the scatter noise.

The ***Edit neurites*** will prompt the user to trace manually the neuron with the brush tool of ImageJ. The brush size is set by the estimate line width parameter. To make full use of the brush tool the user can press the **SHIFT** key to add a new selection, or **ALT** (**OPTION** for Mac) to delete part of a selection.

Synapses

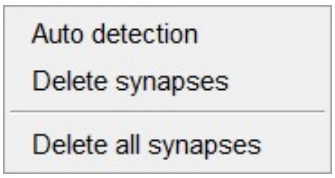

Fig 7: SynJ synapses

The synapses submenu (Fig 7) allows the user to detect puncta automatically along the neurite mask with the ***Auto detection***. SynJ will then smooth the signal from the synapse channel with a Gaussian blur filter of radius **synapse radius**, and then performs a LoG operation with the *MorphoLibJ* plugin (there called “white top hat”). The resulted image is smoothed again with a Gaussian blur filter of radius **synapse smooth sigma.** An auto local threshold is then applied to the image to identify the potential synapses. The particles that are bigger than the **synapse radius** are filtered based on their intensity. Synapses with intensity higher than the mean signal plus **Synapse threshold (std)** times the standard deviation of the intensity along the neurite mask will consider positive and marked with the “Keep synapse color”, the other will consider as negative and marked with the “Delete synapse color”.

The ***Delete synapses*** option will delete all the synapses marked with the “Delete synapse color”, while the ***Delete all synapses*** will delete all synapses regardless of how they are marked, resulting with only the soma and neurite mask.

Fig 8: Soma mask

Fig 9: Neurite mask

Fig 10: All synapses marked

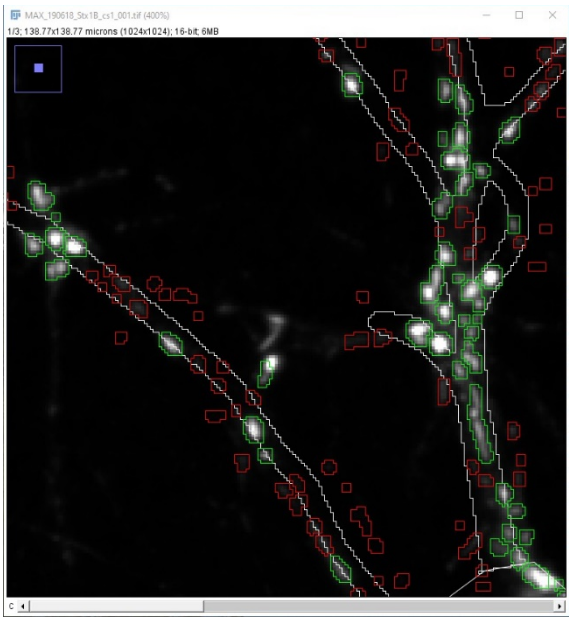

Fig 11: Zoom all synapses marked

Fig 12: Keep synapse mask

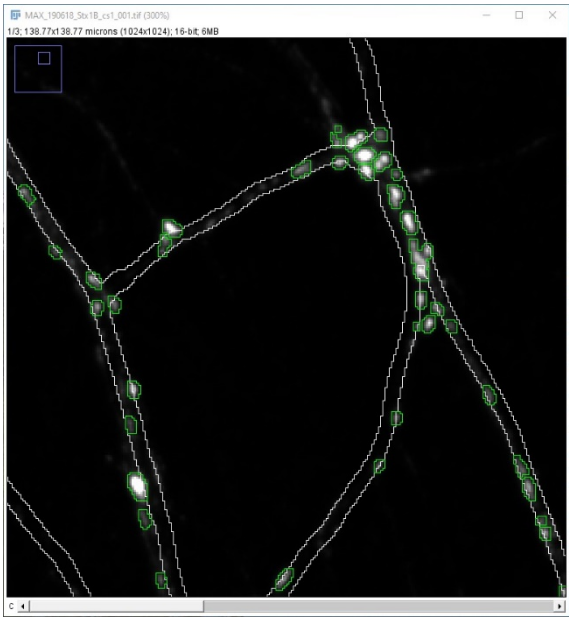

Fig 13: Zoom keep synapses

Analysis

Fig 14: SynJ Saving

Once the tracing per cell is complete, the user can save the ***Roi Manager*** and have two options for analysis (Fig 14). The ***Intensity*** analysis will measure the area of the different masks, soma neurite and synapses individually, as well as the intensity per channel. The measure are then reported in a Result table. The ***Save all*** option will save the Roi Manager and the result table automatically.

The ***Adjust table*** option will either add new feature for different version of SynJ, neurite ends for example, or recalculate the intensity value if the channel map is changed. The ***Collapse data*** option will calculate the average intensity and area per synapse per cell and then store the new values in a new table together with the values for other images. To do so, the user have to select a folder that contain more than one "Result_*MyImageName*.csv" file, it is recommended to have the entire dataset in such folder to make use also of the condition label automatically generated by SynJ.

Intensity

Results_MAX_190618_Stx1B_cs1_001.csv

File Edit Font

	Region	Area	Morphology	Synapses	Other
1	Soma	246.001	1977.392	295.436	167.471
2	Neurite (42)	6130.009	879.996	341.159	185.684
3	Synapse_1	1.010	678.618	1542.364	204.582
4	Synapse_2	0.533	650.069	669.655	174.103
5	Synapse_3	0.569	700.387	1115.484	246.806
6	Synapse_4	0.422	829.043	990.043	181.348
7	Synapse_5	0.496	58.778	660.926	125.630
8	Synapse_6	0.588	282.750	717.188	224.719
9	Synapse_7	0.569	994.484	873.355	212.806
10	Synapse_8	0.294	484.188	669.750	161.438
11	Synapse_9	0.514	688.643	812.214	225.179
12	Synapse_10	0.514	61.929	1075.857	169.500
13	Synapse_11	1.065	432.448	1829.345	202.379
14	Synapse_12	0.661	231.889	1012.111	128.194
15	Synapse_13	0.679	109.730	1581.000	157.243
16	Synapse_14	0.643	107.571	1381.171	207.914
17	Synapse_15	0.294	72.688	653.438	170.688
18	Synapse_16	0.349	113.316	634.526	200.000
19	Synapse_17	1.102	268.867	1696.467	172.050

Fig 15: SynJ intensity result table

The result table consist of five columns and the number of synapses plus two rows (Fig 15).

The columns are: **Region** indicating the soma, neurite with neurite ends in brackets, and synapse number; **Area** of the region, in case of the neurites this measure is the total neurite length; **Morphology, Synapses** and **Other** are the intensity of the morphological, synaptic and last channel the user specify per region.

Sholl

Results

File Edit Font Results

	Distance	Morphology(N)	Synapses(N)	Other(N)	Morphology(S)	Synapses(S)	Other(S)
1	Soma	1977.392	295.436	167.471	0.000	0.000	0.000
2	11.044	688.783	461.176	239.776	551.636	1268.436	330.192
3	16.058	848.730	473.201	242.105	1034.916	1504.406	340.204
4	21.072	757.757	464.658	195.816	679.628	1524.038	363.211
5	26.086	960.318	304.459	204.287	662.595	1561.811	509.671
6	31.100	1110.168	345.423	189.323	793.372	1483.500	368.761
7	36.114	1343.355	245.481	203.450	927.272	1072.863	395.503
8	41.128	1385.280	415.418	296.619	618.175	1837.392	585.449
9	46.142	1397.375	323.264	276.569	861.301	978.374	470.232
10	51.156	1321.611	302.643	225.583	891.477	1080.063	343.357
11	56.170	1027.217	440.409	247.231	789.300	1471.899	357.471
12	61.184	906.676	614.659	238.192	654.475	1464.243	333.730
13	66.198	958.232	240.665	140.052	590.856	1548.247	360.013
14	71.212	792.641	322.074	136.683	412.559	1303.555	243.156
15	76.226	619.289	138.215	90.815	286.343	1014.283	179.040
16	81.240	644.018	170.215	81.694	468.907	1491.782	192.788
17	86.254	540.341	292.060	105.149	307.449	1244.227	180.990
18	91.268	352.157	287.791	98.370	395.075	1340.023	178.085
19	96.282	308.201	96.658	44.439	61.929	1075.857	169.500

Fig 16: SynJ Sholl result table

The Sholl analysis table (Fig 16) is divided in four or seven columns depending if the user decided to calculate the intensity only in neurite or also in synapses.

The **Distance** represent the Sholl radius from the soma (first row) to the next radius as defined by the user at the beginning of the Sholl analysis. The **Morphology(N)**, **Synapses(N)** and **Other(N)** are the intensity of the three channels in the neurite mask in that particular Sholl radius. The **Morphology(S)**, **Synapses(S)** and **Other(S)** are the intensity of the three channels in the synapse mask in that particular Sholl radius, the **Soma** intensity is set to 0 by default, and in the case that there are no synapses in that particular Sholl radius the value of the intensity is set to NaN.

Summary

In summary SynJ is a ImageJ toolbar that is designed for the deep intensity analysis of single neuron, giving information on the soma, neurite and synapses. It is flexible and it can be used for different cell types that present complex morphology, like astrocytes or microglia, and that are stained for punctuate structures. Moreover, SynJ takes advantages of the ImageJ platform, first being free and open source, and implemented periodically by the image analysis community.

SynJ was developed by

Alessandro Moro

Functional genomic department (FGA)

Center of neuroscience and cognitive research (CNCR)

Vrij Universiteit (VU) Amsterdam

The Netherlands

For questions, suggestions and general feedback

e-mail: a.moro@vu.nl al.moro@outlook.com

Github: https://github.com/alemoro

For more check Github page or iamoro.me