User's Guide to SynapseJ

Citation:

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SynapseJ: an automated, synapse identification macro for ImageJ
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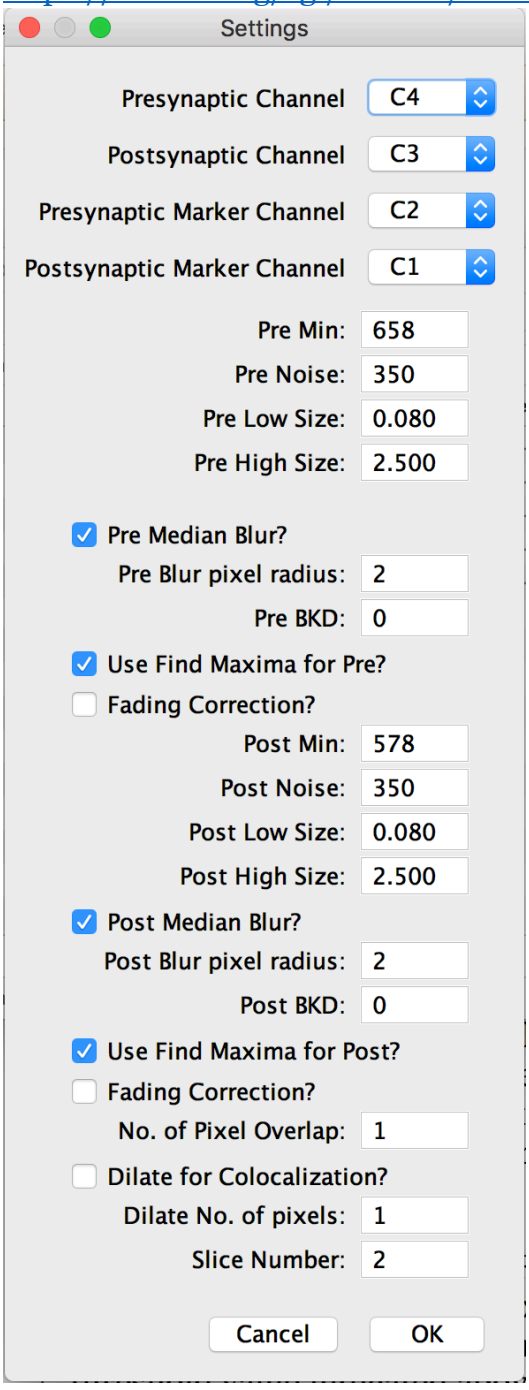

Preparation

Start by creating a folder containing only images to be analyzed. Install the macro anywhere in the plugins folder for your installation of ImageJ. You may also need to install the 'Close_All_Without_Saving.ijm' and 'Find_Stack_MaximaGB.ijm' into the plugins folder of your ImageJ installation.

Now open ImageJ. You will be asked for the folder containing your images, select the appropriate folder. Next, you will be asked for a place to put the files that are produced by the macro. I suggest creating a new folder separate from the folder that contains your images. The third window to pop-up will ask you for the settings to properly analyze your image.

In general, the default values may be close to the settings you need. The first four settings instruct the macro which color channel is used for the presynaptic marker, and the postsynaptic marker, gephyrin in the green channel. If your image has staining for a cell type, select those color channels for the marker channels.

The next several blocks are used to analyze the pre- and post-synaptic channels. Pre Min or Post Min indicates the threshold intensity value. Pre Noise or Post Noise is the value used for the Find Maxima filter. The size of puncta considered real staining is indicated by the size range Pre Low Size and Pre High Size or Post Low Size and Post High Size in square microns. The Median blur can make the image a lot easier to analyze. This requires checking the box and filling in a pixel

radius. Pre BKD and Post BKD is an optional filter used to remove excessive background from an image with the indicated pixel radius. Check the box to Use Find Maxima for Pre? Or Use Find Maxima for Post? if your image has adjacent puncta not clearly defined by only the threshold. If your images show systematic changes in intensity through the Z-stack, the Fading Correction may be selected. The 'Slice Number' field below is used to enter in correction values for each slice in the Z-stack.

Two additional features that are the 'No. of Pixel Overlap' and the 'Dilate for Colocalization'. No. of Pixel Overlap is the minimum number of pixels that must overlap between pre- and post-synaptic puncta to be considered synaptic. Dilate for Colocalization is used to look for puncta that are further apart from each other. This feature may be used for super-resolution images.

Once you click OK the macro will start to run. It will briefly pause for you to accept the default image settings for opening the image, but then the macro will start to run. Plan for 0.5-1 hours for the macro to run for a 1024x1024x5 Z-stack, and possibly as much as 12 hours for larger images.

This macro proceeds the fastest in batch mode, which means that images are stored internally and not displayed to the screen. The task bar for ImageJ will sometimes list a current activity but at times this may seem stuck. It is not stuck! Until an error occurs or the log displays "All finished…Next!" then ImageJ is still churning through your image. The macro is experimental with some features not fully implemented, but still listed so you may try it if you like.

Output from the macro:

Area Z100Post.txt
Area Z100PostALLRoiSet.zip
Area Z100PostF.tif
Area Z100PostSYNRoiSet.zip

Area Z100Pre.txt
Area Z100PreALLRoiSet.zip
Area Z100PreF.tif
Area Z100PreSYNRoiSet.zip

All Post Results.txt
All Pre Results.txt

NP100Post.txt
NP100PostALLRoiSet.zip
NP100PostF.tif
NP100PostSYNRoiSet.zip

NP100Pre.txt
NP100PreALLRoiSet.zip
NP100PreF.tif
NP100PreSYNRoiSet.zip

Collated ResultsIF.txt

CorrResults.txt
CorrResults2.txt

IFALog.txt

Syn Post Results.txt
Syn Pre Results.txt

Excel folder:
Area Z100PostResults.txt
Area Z100PreResults.txt
NP100PostResults.txt
NP100PreResults.txt
Merge folder:
Area Z100PrePost.tif

All files are marked with the name of the original file and whether it is the pre- or post-synaptic marker.

Anything marked ALL or All means it is the unfiltered results for either synaptic marker.

The Files marked SYN or Syn are the filtered results that are selective for the puncta that overlap with the opposing synaptic channel.

The .txt files are the tab-delimited results of the puncta measurements. There are individual text documents for each image, with the unfiltered puncta measurements listed in the enclosing folder and the filtered for overlap puncta measurements in the Excel Folder.

The puncta measurements for all of the image files are also combined in appropriately marked .txt files.

The IFALog.txt file contains the log readout which states all of the user-selected options for the macro.

Collated ResultsIF.txt contains the puncta numbers for each channel and all images.

Finally, the CorrResults.txt and CorrResults2.txt are the correlative results listing either the pre synaptic puncta along with the associated post-synaptic puncta, or the reverse post-synaptic puncta correlated with the pre-synaptic puncta.

The .tif files are images of either the unfiltered puncta for each channel, or there is a single overlay image in the Merge folder that has only the puncta that overlap between the two channels.

The RoiSet.zip files are the region of interests for each puncta analyzed.