University of Antwerp
Laboratory of Cell Biology and Histology
University of Antwerp
Antwerp Centre for Advanced Microscopy

NEUROCONNECTIVITY_V1

ImageJ/Fiji macro set derived from an Acapella® (PerkinElmer) script (https://github.com/VerschuurenM/NeuronalConnectivity) to analyse images of iPSC derived- and primary neuronal cultures. Cultures are immunochemically labeled for a nuclear marker, dendrite marker and a pre- and postsynaptic marker. After maximum projection of acquired z-stacks, nuclei are detected using a manually assigned threshold or by applying the trained convolution neural network Stardist. Neurites are identified using a rough (user-defined threshold) and fine (user-defined threshold after tubeness filtering) segmentation, which is a simplified version of MorphoNeuroNet. Next, the nuclei mask is subtracted from the neurite mask after which the neurite mask is dilated to obtain a search region in which the pre- and postsynaptic spots are detected. The spots are first enhanced using a gaussian, Laplacian or multi-scale Laplacian filter with a user-defined kernel size. Next, a user-defined threshold is applied to segment the spots. The resolution of the microscope setup does not allow determining the exact location of individual markers within a synapse, but this is not the intention of the assay. Instead, the lower resolution is exploited to define synapses as those objects that demonstrate an overlap of minimum 1 pixel between the pre- and postsynaptic spots. In addition to this object-based colocalization, the Pearson correlation of the pre- and postsynaptic channel is calculated in the search region as an intensity-based colocalization metric.

Dr. Marlies Verschuuren – marlies.verschuuren@uantwerpen.be
Prof. Winnok De Vos – winnok.devos@uantwerpen.be

Requirements:

- Imagescience (FeatureJ) plugins: http://www.imagescience.org/meijering/software/featurej/
- Stardist and CSBDeep plugins: https://imagej.net/StarDist

CITATION

Please cite these papers if you are using this script in your research:

- Verschuuren M, Verstraelen P, Garcia-Diaz Barriga G, Cilissen I, Coninx E, Verslegers M, Larsen PH, Nuydens R, De Vos WH. High-throughput microscopy exposes a pharmacological window in which dual leucine zipper kinase inhibition preserves neuronal network connectivity. Acta Neuropathol Commun. 2019 Jun 4;7(1):93. doi: 10.1186/s40478-019-0741-3. Erratum in: Acta Neuropathol Commun. 2019 Aug 14;7(1):131. PMID: 31164177; PMCID: PMC6549294.
- Verstraelen P, Garcia-Diaz Barriga G, Verschuuren M, Asselbergh B, Nuydens R, Larsen PH, Timmermans JP, De Vos WH. Systematic Quantification of Synapses in Primary Neuronal Culture. iScience. 2020 Sep 7;23(9):101542. doi: 10.1016/j.isci.2020.101542. PMID: 33083769; PMCID: PMC7516133.

Neurite detection is based on:

- Pani G, De Vos WH, Samari N, de Saint-Georges L, Baatout S, Van Oostveldt P, Benotmane MA. MorphoNeuroNet: an automated method for dense neurite network analysis. Cytometry A. 2014 Feb;85(2):188-99. doi: 10.1002/cyto.a.22408. Epub 2013 Nov 12. PMID: 24222510.

If the Stardist algorithm is applied to detect nuclei, please cite:

- Uwe Schmidt, Martin Weigert, Coleman Broaddus, and Gene Myers. Cell Detection with Star-convex Polygons. International Conference on Medical Image Computing and Computer-Assisted Intervention (MICCAI), Granada, Spain, September 2018.

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INSTALL

Download ImageJ/Fiji: https://fiji.sc. And add the following plugins using: Help > update > manage update sites: ImageScience, CSBDeep and Stardist.

Download the github repository https://github.com/DeVosLab/NeuroConnectivity. Code > Download zip and extract the compressed folder.

Install the macro in Fiji using Plugins > Macro > Install…

MACRO ACTION TOOLS

Z PROJECT TOOL

A pop-up window will retrieve info about the name of the destination directory, the prefix and the image import and export format. In a next step, the directory with acquired z-stacks has to be chosen in order to make maximal projections that will be saved in a subfolder with the name given in the pop-up window.

SETTINGS

General settings include the image format, pixel size and channel number and the user can choose which objects need to be detected.

Nuclei are segmented by classic smoothing or Laplacian enhancement in combination with a user-defined threshold or by using a trained classifier (Stardist). If the latter is used, standard settings such as background subtraction, contrast enhancement or watershed separation, threshold method and threshold value are not considered. The Stardist algorithm can be tuned using two parameters: probability and overlap. High probability values lead to fewer segmented objects, but will likely avoid false positives. Higher overlap values allow segmented objects to overlap substantially. A final object filtering based on area and circularity is always applied.

Neurites are detected after applying several pre-process steps. Contrast enhancement as well as median filtering can be applied after which the neurites are enhanced in the images using the tubeness filter with specified kernel size. Final detection is based on a user defined threshold on the enhanced image (fine) in combination with another user-defined threshold on the raw image (rough). A minimal fragment area is applied to remove small fragments that are detected. In a final step, the search region for spot detection is defined by dilating the neurite mask by a specific number of pixels.

Spot detection is applied after gaussian, Laplacian or multi-scale Laplacian filtering with as specified kernel size after which a user-defined threshold is applied. Objects can be filtered based on a minimum and maximum area. If there is only one spot channel, set the second channel to 0. If both are present, colocalize allows detecting reciprocal overlap.

SEGMENT NUCLEI TOOL

Detect nuclei based on the parameters given in the settings.

SEGMENT NEURITES TOOL

Detect neurites based on the parameters given in the settings. Prior nuclei detection is automatically done.

SEGMENT SPOTS TOOL

Detect spots based on the parameters given in the settings on the channel that is given in the pop-up window. Prior nuclei and neurite detection is automatically done to obtain a search region.

ANALYSE OPEN IMAGE

Analyse the image that is open based on the parameters given in the settings. The output folder is stored in the image folder.

BATCH ANALYSIS

Analyse all images in a folder based on the parameters given in the settings. The output folder is stored in the image folder.

TOGGLE OVERLAY

Toggle the overlay on/off after nuclei, neurite or spot detection.

VERIFICATION

Create verification stacks of all images in which the created detection masks are visualized as extra channels on top of the raw images. In order to do this, the folder with maximal projections has to be selected in the pop-up window.

OUTPUT

All detected objects (e.g. nuclei, neurites, neurites_search, spot_a and spot_b) are stored as roi_set.zip files and can be opened individually in Fiji. A summary file for each image is generated in which area and intensity measurements are given for the neurite mask and neurite search mask. The nuclei and spot numbers are also given as well as the number of colocalizing spots and the pearson correlation of both spot channels in the search mask. All summary files are merged into one file: ConcatenatedResults.txt. Detected objects have also individual results file for which the naming of variables is standardized. SC refers to segmentation channel, MC refers to measurement channel (*e.g.*: Neurites_SC4_Mean_MC1 is the mean intensity of channel 1 measured in the neurite mask that was detected in channel 4).