**Quick guide for GoldExt**

GoldExt was developed and tested in Windows 10 using Python 2.7 with the following dependencies: numpy, scipy, matplotlib, scikit-learn, xlsxwriter, openpyxl and PyQt4. Once these are installed, launching the Main.py file should initiate the GUI of the software.

Open an image to proceed with the analysis by clicking on ‘Open Image’ button. The software can open png, jpeg and tif formats. For proper pixel-to-micron calculations, first, the scale bar should be drawn, after clicking on the ‘Set Scale bar’ button. The next step is to draw a polygon around the structure of interest that could be accomplished by clicking on ‘Draw Synaptic Area Outline’ radio button, and simply delineate the synapse by clicks. ‘Connect Synaptic Area Endings’ radio button should be clicked to finish the polygon.

Use the ‘Mark Small Gold Particles’ button to label the gold particles. The counter next to the button displays the number of labelled localization points. ‘Delete Small’ deletes the last localization point drawn on the image. With ‘Save State’, one can save the polygon with all labelled localization points. This is particularly useful when one opens an image already analysed and aims to perform another analysis. In such case, after opening an image, by clicking on ‘Open Saved State’, the software automatically recognise the saved state of the image and displays it (the save state’s name is the same as that of the image with a .gss extension, and has an ASCII format). ‘Save Image’ enables the user to save the current state graphically into a jpeg file.

From the measure selection, one can select the type of analysis to be performed on the dataset. Distance measurements enables the user to perform all the NND, all-to-all, distance from centre of gravity and distance from nearest edge measurements with the corresponding N number of desired randomizations (default is 50). By ticking the ‘2D ACF’, the software computes spatial autocorrelation function for the generated random distributions as well. After clicking on the ‘Generate’ button, the results are saved in excel files with the original name of the analysed image and ‘\_distance\_measurements.xlsx’ and ‘\_2D\_ACF.xlsx’ suffices, containing analysis of the data and for the corresponding generated randomizations as well.

Before performing 2D ACF analysis on a particular point pattern, one should set the Rmax value (in nm), which is the maximal radius at which the software is looking for additional localization points from a given localization point. The default value is 80 nm. Ticking ‘Save’ option saves the generated *g(r)* function in an ASCII file.

The DB, AP and MS options contain the DBSCAN, affinity propagation and mean shift clustering algorithms, respectively. After setting the user-defined parameters, the clustering of the point pattern is visualized with localization points colour-coded according to cluster assignments.

For the ‘Clustering GUI’, the available analysis options are the same, but this interface lets the user to hand draw arbitrary shapes of clusters within which the localization points will be distributed with a user-defined density (AZ Density). AZ background density and background density could be also applied with a given density, the former distributing randomly localization points within the AZ (i.e. the polygon drawn by the user using the original GUI) and the latter outside the polygon area.

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