

ColocAnalyzer

Manual

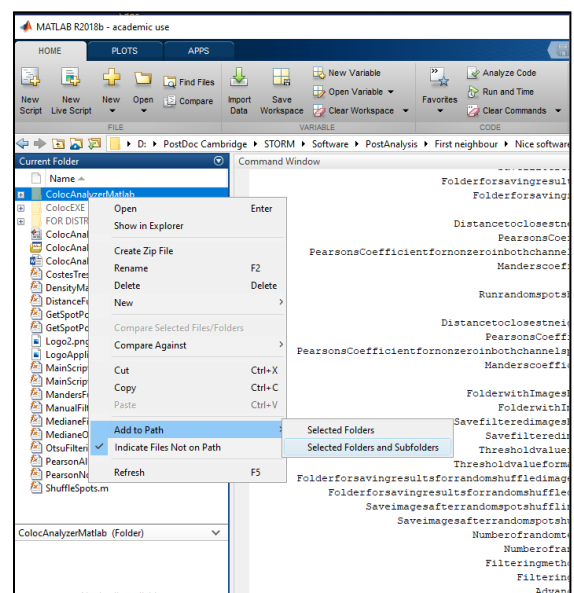
I. Installation

There are two ways of using ColocAnalyzer – either as MatLab function or as an independent .exe application. In general there are almost no differences between these ways, except the log messages that can be viewed only when program is used as MatLab function. In this case all messages will appear in a workspace.

Installation of MatLab function

For this option you must have MatLab R2018b (or higher version) installed on your PC. If it is already installed follow next steps:

- 1) Download compressed folder from LAG-MNG github page
- 2) Uncompress and save on your PC folder “ColocAnalyzer MatLab”
- 3) Open MatLab and find the path of the folder where saved folder “ColocAnalyzer MatLab” is located
- 4) With a right click on a folder “ColocAnalyzer MatLab”: Add to Path -> Selected folder and subfolders
- 5) To run a program type *ColocAnalyzer* in a workspace and press Enter.



Installation of .exe application

For this option you **don't need** to have MatLab, but you have to install MatLab Runtime, that is completely free.

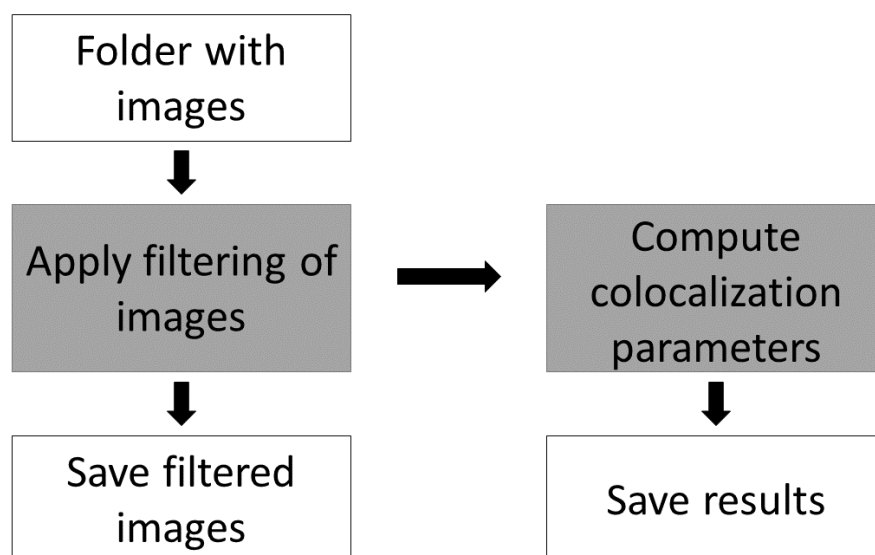
- 1) Go to <https://uk.mathworks.com/products/compiler/matlab-runtime.html>, and download MatLab Runtime with version 9.5 or higher.
- 2) Uncompress folder with “MatLab Runtime” and run “setup.exe”, follow instructions to install Runtime
- 3) Upload folder “ColocAnalyzer Exe”. Find in the folder: “colocEXE\for_redistribution” file “MyAppInstaller_web.exe” and run it
- 4) Follow installation instructions. After program is installed you can find it in “Programs” and run it.

II. Description of software

This software uses microscopy **tif** images to perform a filtering and then **compute colocalization parameters** and/or run a **random shuffling test** for existing microscopy images.

Computing colocalization parameters

Software uses the folder with **tif** microscopy images, apply one of the chosen filters to remove low intensity spots and then compute chosen colocalization coefficients. The diagram of computing colocalization parameters part of ColocAnalyzer software:



Colocalization parameters and filtering methods are well described in *Dunn et al. A practical guide to evaluating colocalization in biological microscopy, Am J Physiol Cell Physiol 300, 2011*. Short description of parameters is below:

$$PCC = \frac{\sum_i (R_i - \bar{R}) \times (G_i - \bar{G})}{\sqrt{\sum_i (R_i - \bar{R})^2 \times \sum_i (G_i - \bar{G})^2}}$$

- 1) Pearson's coefficient:

Where R_i , G_i – pixel values in [Channel 1](#) and [Channel 2](#) (*) respectively, \bar{R} , \bar{G} – are averaged values in each channel respectively.

- 2) Pearson's coefficient for “non-zero” in both channels pixels:

computed with the formula above but only for those pixels which has intensity above zero in both channels.

- 3) Distance to closest neighbour: computes distance from every spot in [Channel 1](#) to the closest spot from [Channel 2](#)

- 4) Manders coefficients:

$$MOC = \frac{\sum_i (R_i \times G_i)}{\sqrt{\sum_i R_i^2 \times \sum_i G_i^2}} \quad M_1 = \frac{\sum_i R_{i,colocal}}{\sum_i R_i} \quad M_2 = \frac{\sum_i G_{i,colocal}}{\sum_i G_i}$$

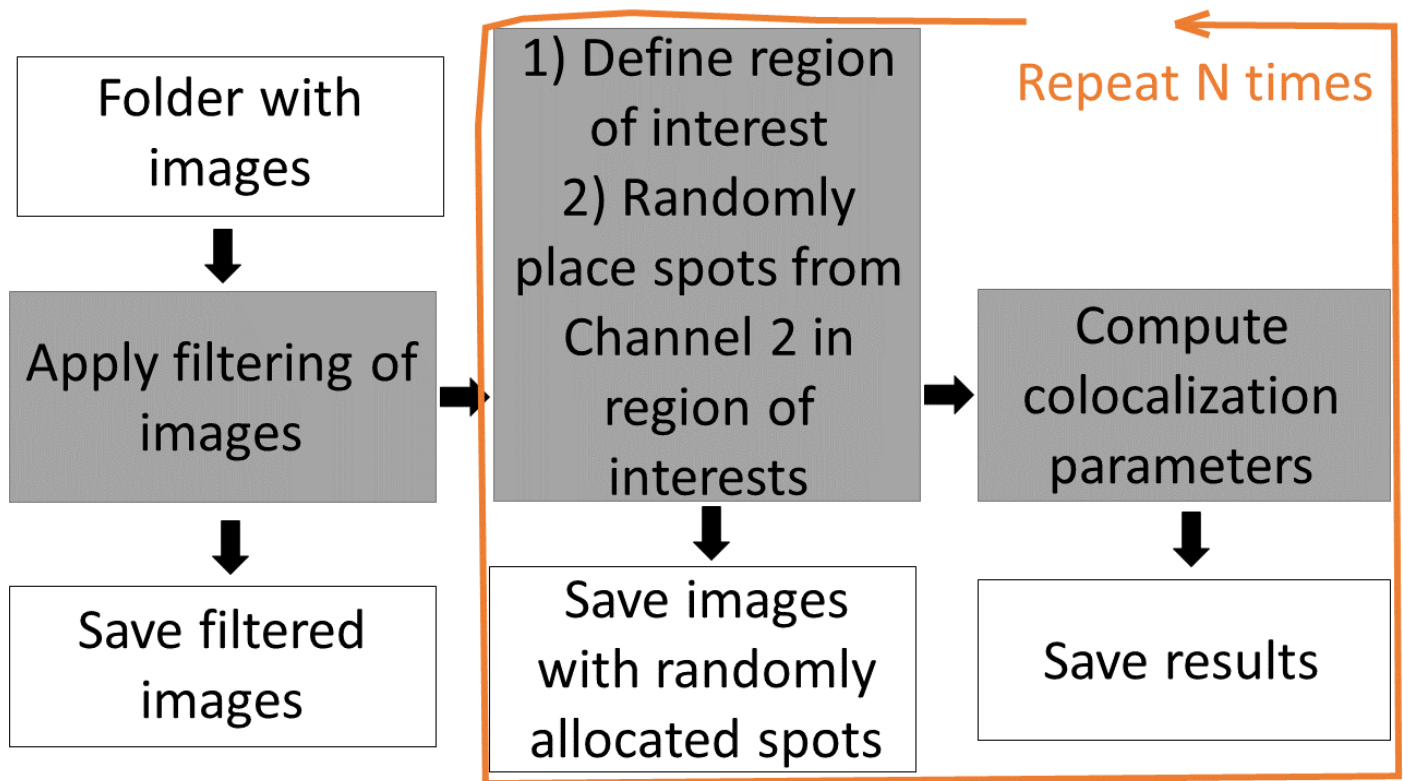
Where $R_{i,colocal}$, $G_{i,colocal}$ values of pixels in [Channel 1](#) and [Channel 2](#) respectively, where both channels has values above zero.

Random spot shuffling tests

Every image have random noise and artefacts, that may influence values of computed colocalizations. Random spot shuffling test is used in order to check “randomness” of obtained colocalizations. The scheme of the test is similar to **computing colocalization parameters** part (see below) with one additional step. After images are filtered, before computing colocalization parameters program performs next steps:

- 1) Define regions of interest (usually approximate area occupied by the cell) by defining regions with high densities of the [Channel to determine cell boundary](#)
- 2) Random positioning of spots from [Channel 2](#) in the regions of interest while leaving unchanged [Channel 1](#) in regions of interest. Concentration (number) of randomly allocated spots from Channel 2 is regulated by [Density correction factor](#)

(*) – here and below values marked with [blue color](#) are the parameters that can be adjusted in section **Advanced parameters**



Random shuffling tests can be run N times, that is defined with [Number of random tests](#). Then, program will produce N random versions of every image with randomly distributed spots from [Channel 2](#) and compute chosen colocalization parameters for every image.

III. How to use software

Computing colocalization parameters

- 1) Put **tif** microscopy images for analyzing in one folder. Provide path to this folder in [Folder with Images](#) filed
- 2) Choose a filtering method. If you have chosen [Manual](#) filtering you will need to specify relative threshold value from 0 to 1 at [Threshold value for manual](#).
- 3) (Optional) Specify the path where images after filtering would be saved in the field [Save filtered images](#). If this field is empty, filtered images will not be saved but program will be still running.

The screenshot shows the 'Compute colocalization parameters' tab of the software. It features several input fields and checkboxes:

- Folder with Images:** A text input field for the source image folder.
- Filtering method:** A dropdown menu with options: Manual, Otsu's, Costes, Median, Median + Otsu's, and No filtering. 'Manual' is currently selected.
- Threshold value for manual [0-1]:** A slider control set to 0.
- Save filtered images:** A text input field for the destination folder.
- Folder for saving results:** A text input field.
- Checkboxes:**
 - ☐ Pearson's Coefficient
 - ☐ Pearson's Coefficient for "non-zero" in both channels pixels
 - ☐ Distance to closest neighbour
 - ☐ Manders coefficients
- Run! Button:** A large button at the bottom right to execute the process.
- mng molecular neuroscience group logo:** Located at the bottom left.

- 4) Specify path for the folder where results will be saved in the field [Folder for saving results](#). If the path field will be empty or invalid program will not compute the results and will only perform image filtering. Program saves all data together in .mat file; each parameter results in a separate .csv file and plots (mostly for
- 5) Choose by clicking on a checkbox which parameters you would like to be computed.
- 6) Go to **Advanced parameters** tab to set 4 top parameters.

[Channel 1](#) and [Channel 2](#) defines pair of colors that will be used to compute colocalization parameters. Please note, that only three colors can be used: red (value 1), green (value 2) and blue (3).

[Size of the averaging area median filtering](#) is used only when [Median](#) or [Median+Otsu's](#) filtering was chosen. This is a length of the box side around chosen pixel over which median value of intensity is computed. Then, this median value is subtracted from pixel intensity, so usually larger than one spot, but smaller than the distance between two spots.

In practice this value usually kept constant and doesn't have a huge influence on final colocalization parameters computed.

[Pixel size](#) – length of one pixel side in nanometers.

- 7) Press Run! Button to start the program

Random spot shuffling tests

- 1) Put **tif** microscopy images for analyzing in one folder. Provide path to this folder in [Folder with Images](#) field
- 2) Choose a filtering method. If you have chosen [Manual](#) filtering you will need to specify relative threshold value from 0 to 1 at [Threshold value for manual](#).
- 3) (Optional) Specify the path where images after filtering would be saved in the field [Save filtered images](#). If this field is empty, filtered images will not be saved but program will be still running.
- 4) Choose [Number of random tests](#) as any integer number above 0

- 5) (Optional) Specify the path where images after filtering and random shuffling of the spots would be saved in the field [Save images after random spot shuffling](#). If this field is empty, filtered and shuffled images will not be saved but program will be still running.

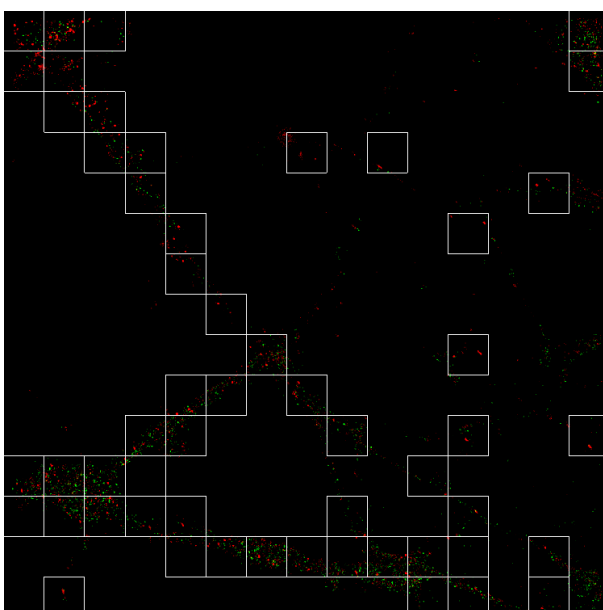
- 6) Specify path for the folder where results will be saved in the field [Folder for saving results](#). If the path field will be empty or invalid

program will not compute the results and will only perform image filtering and spot shuffling. Program saves all data together in .mat file; each parameter results in a separate .csv file and plots (mostly for

- 7) Choose by clicking on a checkbox which parameters you would like to be computed.
8) Go to **Advanced parameters** tab to set parameters. Top 4 parameters described above and bottom 4 parameters are used only in the spot shuffling:

[Channel to determine cell boundary](#) – the channel that used in order to measure the spot density and determine the regions with high density of the spots as region of interest (see image below – regions inside white squares). It can be one of the channels that are used in analysis or different one. As for [Channel 1](#) and [Channel 2](#) it should be one from the next colors – red (1), green (2) or blue (3).

[Length of the box side for determining cell boundary](#) – length of the side of one box used to



determine field of interest. It shouldn't be neither too big (that may cause low resolution in the boundary defining) neither too small (may result in missing parts inside the cell). Playing with this parameter and [Threshold for the density of the spots](#) is an essential in defining approximate area covered by the cell as accurate as possible.

[Threshold for the density of the spots to determine cell boundary](#) defines relative value (from 0 to 1) for the spot density threshold inside a box. If value is lower – program will take to account larger area of interest

Density correction factor – user can change number of spots (so density of spots) in **Channel 2** which will be randomly located from original one. Density correction factor then is defined as the ratio between the desired number of the shuffled spots to original amount of the spots in **Channel 2**: $DensFactor = N_{shuffled}(Channel2)/N_0(Channel2)$

So if you want to place randomly only half of all spots from **Channel 2** you have to set this value to 0.5, if you want to have twice more spots - to 2. *DensFactor* could be any number from 0 to 2. Default value is 1 that corresponds to exactly the same number of shuffled spots as in original image in **Channel 2**

9) Press Run! Button

Please note that two parts of software - **Computing colocalization parameters** and **Random spot shuffling tests** should be runned independently, eventhough they are mostly identical. Also top 4 parameters in **Advanced parameters** tab works simultaneously for both of this parts, so it worth to check them every time before running program.