READ ME

1. **System requirement**
   The Matlab code runs on Linux, PC and mac computers and requires MATLAB R2018a or more recent (through the App Designer Matlab interface)

2. **Installation Guide**

- launch MATLAB
- browse for folder until you locate the Colocalization_analyzer.mlapp file in your current folder. The segment_local_threshold file should also be located in the same folder
- right click on the Colocalization_analyzer.mlapp file and press Run
- Typical launch time should be between a few seconds and one minute on a "normal" desktop computer

3. **Demo**

- In section Image 1, Import Image 1: GFP, choose Automatic Segmentation: Midgrey, Press Segment, move the slider Remove Noise to 3
- In section Image 1, Import Image 1: Cy3, choose Automatic Segmentation: Midgrey, Press Segment, move the slider Remove Noise to 6
- In section Image 1, Import Image 1: Cy5, choose Automatic Segmentation: Midgrey, Press Segment, move the slider Remove Noise to 3
- In section Analyze, choose Mask 1 = Image 1, Mask 2 = Image 2, Signal 1 = Image 1, Signal 2 = Image 2. Press Analyze. The results should be:
  - Pearson : 0.69, Spearman = 0.64, M1 = 0.58, M2 = 0.54
- In section Analyze, choose Mask 1 = Image 2, Mask 2 = Image 3, Signal 1 = Image 2, Signal 2 = Image 3. Press Analyze. The results should be:
  - Pearson : 0.32, Spearman = 0.40, M1 = 0.31, M2 = 0.47
- The run time for each operation should be a few seconds on a “normal” computer

4. **Instructions for use on your data**

- Launch the Colocalization Analyzer interface
- In the **Image 1** section, click on the import image **import Image 1** button. Supported formats are TIFF files.
- The image can be cropped : click on the **Crop** button, a window should pop up. Draw a rectangle of the desired size. It can be cancelled if the size is not good.
- Segmentation is required: choose an **automatic segmentation method** (**Midgrey** or **Phansalkar**) and press segment. You can then remove noisy pixels by moving the **Remove Noise** slider. You can change the segmentation method at any moment. If no automatic segmentation method is satisfying, it is also possible to draw the Regions Of Interest (ROI) to segment manually: press **Freehand** and then draw one region on the image window. You can add other ROI by pressing the **+** button , or removing some with the **–** button. Careful Segmentation is crucial as the analysis will depend on it.

Commenté [s1]: + d’autres ?

- Proceed similarly with at least one other image that you want to compare to image 1: click on tab **Image 2** and follow the same steps (cropping is automatically done on the same region as for Image 1). You can compare up to 4 images, 2 are required.
- Once you have segmented all the images to be compared, click on the Analyze Tab.
- Choose the Regions of Interest in which intensity levels will be compared. If you click on **Mask 1:Image 1** and **Mask 2:Image 2**, the signals in all pixels from the ROI obtained from segmentation of image 1 and Image 2 will be compared. You can also select more complex options: for instance, for **Mask 1** you can select ROI from 2 images (by pressing Ctrl + click or cmd + click): the resulting ROI is the intersection of both ROI (pixels belonging to the ROI of Image 1 AND Image 2).
- Choose the signals to compare. In principle, if you have chosen **Mask 1:Image 1** and **Mask 2:Image 2,** you should choose **Signal 1:Image 1** and **Signal 1:Image 2,** but different options can also be informative.
- Press the **Analyze** button. A cytofluorogram, colocalization map as well as Pearson, Spearman and Manders coefficients will be displayed. Press the **Save** button to export them.