This document describes the steps to run conditional colocalization analysis (described in *Analysis of conditional colocalization relationships and hierarchies in three-color microscopy images,* Vega-Lugo et al. J Cell Biol 2022) using "condColocGUI". This interface runs conditional colocalization analysis functions (Pt2Pt2Pt, Pt2Pt2Blob, and General). The GUI allows for loading of MovieData or MovieList formatted data, and sets relevant parameters for each type of analysis, all on the same interface. Please see "HowTo_conditionalColoc" (at https://github.com/kjaqaman/conditionalColoc) to see more information on MovieData files and how to run detection and segmentation.

To open the GUI type `condColocGUI` in a MATLAB console window.

Movies Panel:

This panel loads and displays movies to be analyzed using conditional colocalization analysis.

Load Movie or Movie List:

This button loads in an existing movieData or movieList. A dialog box will open to select the desired file.
NOTE: The "Load Movie" button will only load movieData files and the "Load List" button will only load movieList files.
If the movieData file has been relocated on the disk, the software will ask for relocation of all its components by comparing the new path of the movieData file to the old path. Select "Yes to All" to allow for relocation.

Clear:

This button removes all the current movies from the list of movies to analyze, as well as the current path for the Statistics/P-Value panel.

Save as List:

This button saves the current list of movies as a MovieList that can later be reopened using the "Load List" button.

Colocalization Panel:

This panel displays different options that determine what analysis type will be run and the parameters needed to run it. To start the analysis, make sure to fill out all the necessary parameters. When ready, click the "Run" button to analyze the list of movies using the selected function. A movie or movie list must be loaded to run analysis.

The different input parameters are as follows:

Channel 1/2/3:

The channel options are buttons that determine whether the specific channel is either Punctate or Non-Punctate.

NOTE: The GUI does NOT automatically identify which type of object is in each channel. The user must select the appropriate option for the analysis to run. The default selection is Punctate for all three channels.

Detection Process:

These drop down menus contain two options, PointSource or SubRes, that specify the detection process for punctate channels. The default selection is PointSource for all three channels.

If Non-Punctate is selected, the drop down menu below the corresponding button group will gray out and not allow for any selections.

Coloc Radius (Pixels):

Three input boxes that can only accept numeric values that represent the colocalization radius (distance threshold) between each pair of channels (Channel 1 to Channel 2, Channel 1 to Channel 3, and Channel 2 to Channel 3). The default threshold for all three pairs is 3 pixels.

Randomizations:

This subpanel contains two input boxes that can only accept numeric values. The Condition input box represents the desired number of condition randomizations. By default, the number of condition randomizations is 100.

If running General Analysis function,

- The Tar/Ref input box represents the desired number of target/reference randomizations. The default number of tar/ref randomizations is 10. Recommended number is > 50.

Analysis Function:

This drop-down menu determines the analysis type that will be run on the loaded movie(s). The drop-down menu contains selections that include Pt2Pt2Pt, Pt2Pt2Blob, and General. The options available will change based on the selections made in the Channels button subpanels. *Choosing the combination of three Punctate channels will allow you to select either Pt2Pt2Pt or General as the analysis function to run. Choosing the combination of two Punctate channels and one Non-Punctate channel will allow you to select either Pt2Pt2Blob or General. Any other combination will result in the General analysis function to be chosen.*

Run:

This button sets all input parameters and runs the selected analysis on the loaded movie(s).

Results Panel:

This panel contains two buttons, Results Options and View Results. To be able to view the results, ensure that a movie or movie list that has stored analysis data is loaded. Set the "Result Options" first, and then press on "View Results".

Results Options:

This button launches a separate window containing options on how the results will be saved and/or displayed. The separate window contains a drop down menu, two checkboxes, and a continue button.
NOTE: The channel types AND analysis function must be set correctly in the Colocalization Panel for the Results Panel to function correctly.

Analysis Function:

This drop down determines the analysis type used for the loaded movie(s) and thus where to find the data needed to display the results. The available options in the drop down include Pt2Pt2Pt, Pt2Pt2Blob, and General. By default, the value will be the same as in the main window.

Plot Checkbox:

This checkbox determines whether you would like to plot and display the results. Check box for plotting, keep unchecked otherwise.
NOTE: If plot checkbox is not selected, but save checkbox is, the compiled results will be saved as .mat files, without any figures.

Save Checkbox:

This checkbox determines whether you would like to save the results. If the checkbox is selected, a folder selection dialog box will open and ask for the folder where the results should be stored.
NOTE: If running multiple analyses with different colocalization radii, one folder should be used to save all results. The software will create separate subfolders for the different colocalization radii.

Continue:

This button takes the options selected from the Result Options window and updates the variables in the main window.

View Results:

This button runs the function that can save and/or plot the results. If saving, it will automatically create a new folder called Threshold followed by the Colocalization Radii used when analyzing the movie list. The path in statistics/P-Value panel will also be updated to this new folder.
Ex. If the coloc Radii used to analyze the movie list were [1 2 3], the created folder would be called Threshold_123.

Statistics/P-Value Panel:

This panel contains a path text field, three buttons – three dots (...), Statistical Test, and View Stat Test – and three checkboxes - Run for All, Save Tests and Save Figures. Path specifies where the statistical test will take data, run and save. Select the triple dots button to change this path. Select the "Statistical Test" button to perform a statistical test on results <u>saved</u> from the "View Results" button. Select the "View Stat Test" button to view the results of the Stat Test.
NOTE: For saving tests and/or figures, check the save buttons first, and then click on the corresponding execution button.

Run for All:

This checkbox determines whether to run Statistical Test and View Stat Test on all files located in the path specified. If it is not checked, the test will ask for one file to run on.
Statistical Test will run for all files with 'colocalMeasure' in their name, and View Stat Test will run for all files with 'statTest' in their name.

Save Tests:

This checkbox allows you to determine whether you would like to save the statistical test MAT file.
This will save in the directory specified by the value in the path text field.

Statistical Test:

This button runs a statistical test on the saved conditional colocalization results. When the "Save Tests" checkbox is selected, the statistical test result is saved in the directory specified by the value in the path text field. If the "Save Tests" checkbox is not selected, the statistical test will be displayed as a table.
By default, the path is in the same folder as where the results are saved if View Results was run.

Save Figures:

This checkbox allows you to determine whether you would like to save the Statistical Test/P-Value figures. For tests that run without saving, the results will be displayed in a figure.
This will save in the directory specified by the value in the path text field.

View Stat Test:

This button runs the function that takes the saved statistical test and plots the values as a table. When the "Save Figures" checkbox is selected, the table is saved as a figure in the same folder as the statistical test.
NOTE: This button will only show the result for a <u>single</u> colocalization radius.

Example - Pt2Pt2Pt:

Loading Data

1. Load in an already made movieData or movieList by clicking on the "Load Movie" or "Load List" button. The path of the movieData and/or movieList should be displayed in the Listbox.

Setting Parameters

2. In the Channel 1/2/3 boxes and Detection Process box of the Colocalization Panel, select the options that match the channels of the selected movieData or movieList. Select Punctate for all channels. The user should then select the Detection Process of each **Punctate** channel, either PointSource or SubRes.

3. In the Coloc Radius Panel, enter the desired radius in pixels between each pair of channels in the corresponding numeric textbox.

4. In the Randomizations Panel, enter the number of randomizations to be used to calculate the randC value in the numeric textbox labeled Condition

   a. The Tar/Ref numeric textbox is grayed out because the selected analysis function is Pt2Pt2Pt. If General was selected, the number of randomizations for the target/reference must be entered.

5. Select what analysis function should be used to analyze the movieData or movieList.

   a. When running analysis on a Pt2Pt2Pt movieList, the user has the option to pick either Pt2Pt2Pt or General.

Running Analysis

6. Click the **Run** button and analysis will begin. In the MATLAB console, the GUI will state that analysis is running, and the function that is being used is displayed. The console displays when the analysis on each individual movieData has completed.

7. A dialog box will pop up stating that analysis has completed on the entire movieList. Click **OK** on the pop up to continue.

Plotting and Saving

8. To view and/or save the results of the analysis click the **Result Options** button. This button will launch a window with options on how to view and save the results.

   a. Select the proper analysis function.

   b. Click on the **Save Compiled Results** check box if you wish to save the results. A folder dialog box will pop up asking where you would like to save the Results.
   If multiple analysis runs are going to be performed on one list of movieData with different thresholds, the user should create a single folder where all the results will be stored.

   c. Select the folder where you would like the results to be saved.

   d. Click on the **Plot Compiled Results** check box if you wish to plot the results.

   e. Click the **Continue** button to return to the main GUI window, and the **View Results** button should be enabled.

Statistical Test

9. Select whether you would like to save the Statistical Test by checking the Save Tests checkbox.
10. Click the **Statistical Test** button to perform a statistical test, and a dialog box will pop up stating that the statistical test has been completed.
11. Select whether you would like to save the figures by checking the Save Figures checkbox.
12. Click the **View Stat Test** button and a file selection dialog box will open up asking for the user to select a "statTest" file. A figure containing the P-Values of the selected statistical test will pop up.