This documentation describes the steps to run colocalization analysis for objects detected/segmented in two separate channels (using metrics similar to those described in *Analysis of conditional colocalization relationships and hierarchies in three-color microscopy images,* Vega-Lugo et al. J Cell Biol 2022) using "colocGUI." This interface runs colocalization analysis functions (Pt2Pt, Pt2Blob, and Blob2Blob). 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 colocGUI in a MATLAB console window.

Movies Panel:

This panel loads and displays movies to be analyzed using 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 for 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 current movies from the list of movies to analyze, as well as the current path for the Statistics 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:

Select Channel Drop-Down Section:
These drop-downs allow you to select which channels you would like to analyze. It is grayed out until a MovieData or MovieList is loaded. The available selections are automatically detected when a MovieData or MovieList is loaded. Also, specify whether the specific channels are 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 is Punctate for both 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 both 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):
An input box that can only accept numeric values that represent the colocalization radius (distance threshold) between the two channels. The default threshold is 3 pixels.

Randomizations:
This subpanel contains an input box that can only accept numeric values. It is only needed for Blob2Blob analysis. By default, the number of randomizations is 1. 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 may include Pt2Pt, Pt2Blob, and Blob2Blob. The options available will change based on the selections made in the Select Channels subpanels.

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 which results (which channel combination) will be saved and how the results will be saved and/or displayed. The separate window contains another channel drop-down menu, an analysis function drop-down menu, two checkboxes, and a continue button.

Select Channels To Analyze Drop-Downs:
These drop-down menus determine the channels that whose results will be viewed/saved. By default, the selected channels will be the same as in the main window. If the channels selected are different, the analysis will run on the channel selection here rather than what is shown in the main window.
Ensure that the selected channels have stored colocalization analysis results.

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 Pt2Pt, Pt2Blob, and Blob2Blob. 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, only the compiled results will be saved.

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 saved.
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 Results 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 Radius used when analyzing the movie list. The path in the Statistics panel will also be updated to this new folder if saving was selected.
Ex. If the coloc radius used to analyze the movie list was 3, the created folder would be called Threshold_3.
NOTE: View Results does not need the Analysis Function to have run in the same instance in order to work. However, the correct settings for the channels must be selected for View Results to run properly, meaning Punctate or Non Punctate.

Statistics Panel:

This panel contains a path text field, two checkboxes – Run for All and Save Tests – and two buttons – triple dots (…) and Statistical Test. The path text box specifies where the statistical test will take data, run and save. Select the triple dots button next to the text box to change this path. Select the Statistical Test button to perform a statistical test on results inside the current folder specified by the path text box.

Run for All:
This checkbox determines whether to run Statistical Test on all files located in the directory the path specified. If it is not checked, the test will ask you to select one file to run on.
Statistical Test will run for all files with 'compiledColoc' in its 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 compiled 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.

Example – Pt2Pt:

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 List box.

Setting Parameters

2. In the channel drop-downs, select the channels you want to analyze and select the options that match the channels of the selected movieData or movieList. Select Punctate for both channels. The user should then select the Detection Process for each **Punctate** channel, either PointSource or SubRes.
3. In the Coloc Radius panel, enter the desired radius in pixels for colocalization analysis.
4. Select what analysis function should be used to analyze the movieData or movieList.
    a. *The corresponding correct option should have already been selected automatically.

Running Analysis

5. 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 is completed.
6. 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

7. To allow for View Results, click on **Result Options**. This button will launch a separate window with options on how to view and save the results.
    a. Select the desired channels (can be different from channels selected in the main window).
    b. Select the proper analysis function (this function drop-down does not automatically select the correct one unlike the one in the main window).
    c. 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.
    d. Click on the **Plot Compiled Results** checkbox if you wish to plot the results.
    e. Click the **Continue** button to update the settings and return to the main GUI window. The **View Results** button should now be enabled.
8. Click **View Results** to compile all the colocalization data obtained from the movieList. A pop up will appear after to notify that the result compilation is complete, and the statistical test path is updated to the new folder.

Statistical Test

9. If you would like to change the current path where the statistical test will run and save, press the triple dot button next to the text box. A folder dialog box will pop up asking where you would like to do your statistical test.
10. Select whether you would like to run a statistical test for all relevant files in the directory by checking the **Run for All** checkbox. If it is left unchecked, running the statistical test will result in a file dialog box asking which file you would like to use.
11. Select whether you would like to save the statistical test by checking the **Save Tests** checkbox. If it is left unchecked, running the statistical test will result in displaying the results in a table instead.
12. Click the **Statistical Test** button to run the test.