UE : BIOMED

Colocalization

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Contents

1 Installation:

1. Colocalization Simulator
The purpose of this plugin is to generate two sequences exhibiting diverse degrees of molecule colocalization. Users have the ability to generate two synthetic sequences with specified quantities of spots and colocalization characteristics, such as percentage, mean distance, and standard deviation.
2. Colocalization Studio
This plugin incorporates a comprehensive range of colocalization techniques for both 2D and 3D fluorescence microscopy images. These methods include Pearson and Manders coefficients, Image Cross Correlation Spectroscopy (ICCS), and Object-based approaches. We first proceed to install these two plugins on Icy for further exercise:

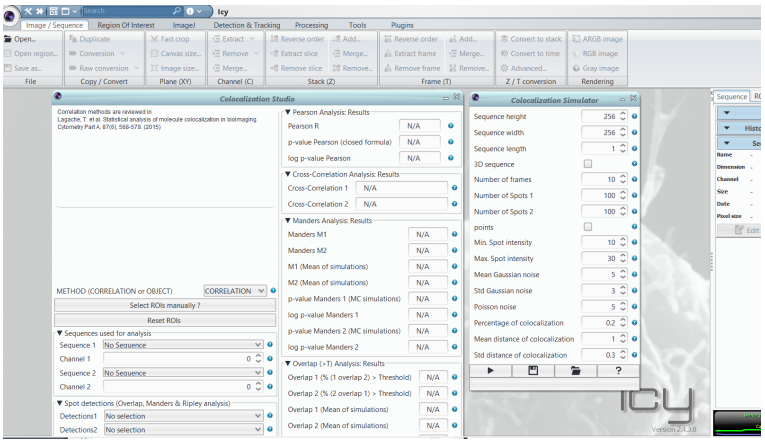
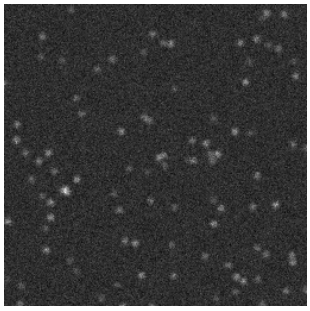


Figure 1: Colocalization Studio & Colocalization Simulator Installed Successfully

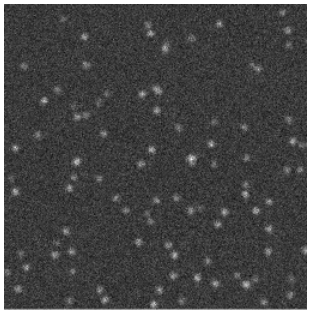
2 A. Compute the colocalisation of the sequences coloc01.tif and coloc02.tif.

1. Compute the colocalization based on correlation.
2. Compute the colocalization based on object.
a: What is the percentage of colocalization?
b: what is the mean colocalisation distance?
Discuss the results.

We first load the the .tif images:



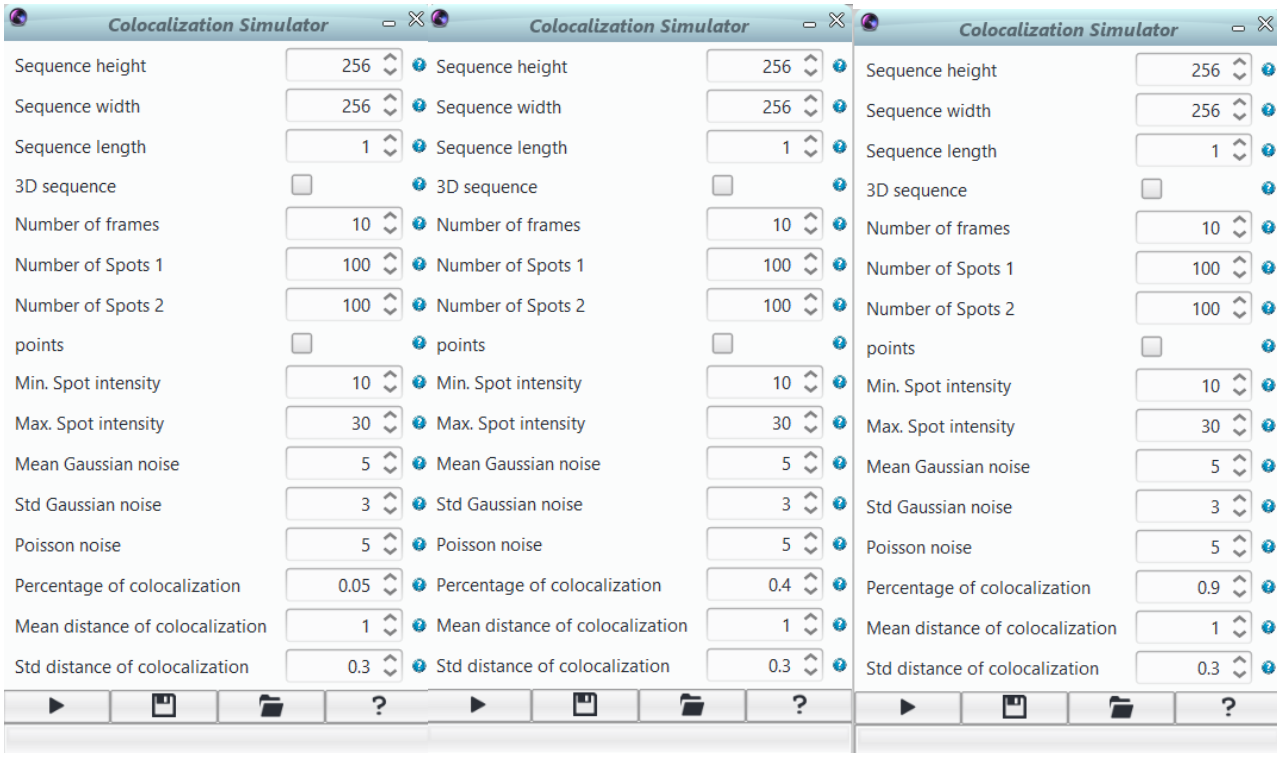
(a) Coloc01.tif



(b) Coloc02.tif

Figure 2: Our two images

Subsequently, we generate sequences employing a nearly identical default configuration, yet varying the colocalization percentage values for experimentation, exploring three distinct values: 0.05, 0.4, and 0.9



(a) 0.05 (b) 0.4 (c) 0.9

Figure 3: Almost the same configuration but with different colocalization percentage

2.1 Colocalization Percentage = 0.05

Below we can see the two sequences created as a result of setting colocalization set to 0.05:

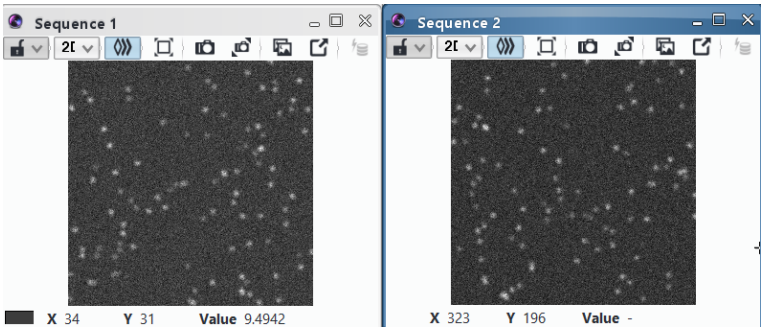


Figure 4: Sequence 1 & Sequence 2

Then we use spot detection on both images and obtain the following results. We should be careful to check the *Export to Swimmingpool* box in the Output tab of the Spot Detector plugin and also choose *Detect bright spot over darkbackground* option in the Detector tab.

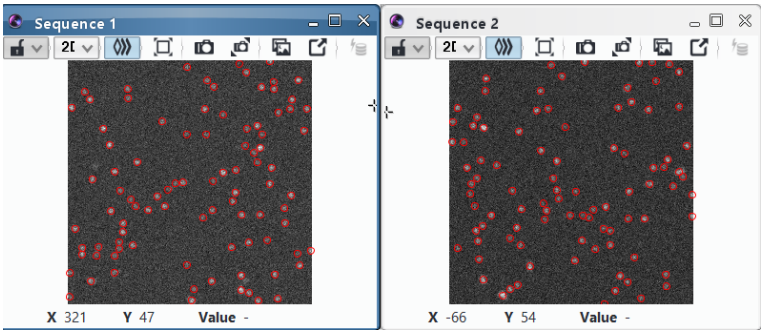


Figure 5: Sequence 1 & Sequence 2 after spot detection

2.1.1 Pixel-Based Correlation

We now can make use of the colocalization studio to compute colocalization based on pixel using the two sequence images and the results obtained from the spot detector. We can see the result in the image below:

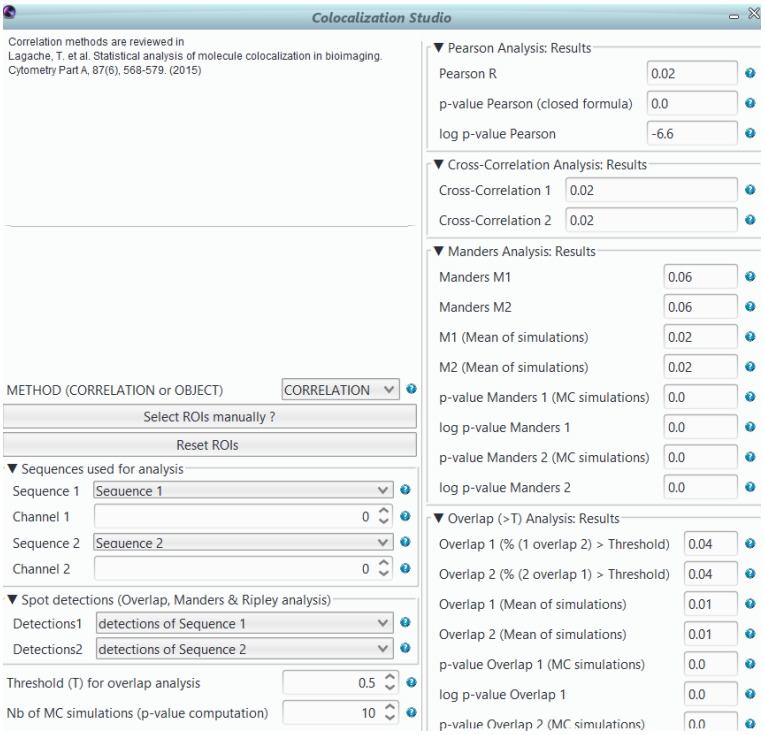


Figure 6: Correlation based on pixel results with colocalization percentage of 0.05

2.1.2 Object-Based Correlation

We use the colocalization studio to compute colocalization based on object using the two sequence images and the results obtained from the spot detector. We can see the result in the image below:

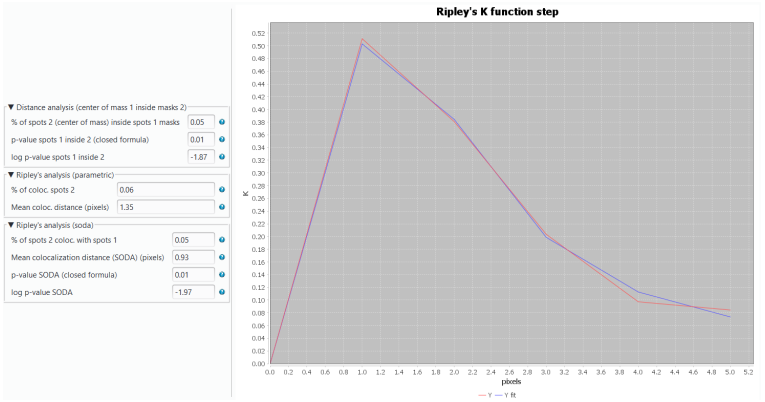


Figure 7: Correlation based on object results with colocalization percentage of 0.05

Observations for pixel-based approach:
The Pearson R value of 0.02 denotes a very low positive correlation between the intensities of the two channels, indicating a lack of direct proportional relationship as anticipated. The logarithmic transformation of the p-value yields -6.6, signaling a minuscule p-value. Cross-Correlation values of 0.02 for both channels signify extremely low correlation, useful for identifying shifted versions of signals that might hint at colocalization patterns. Manders

coefficients M1 and M2, at 0.06 each, imply a low degree of signal overlap without considering intensity. Similarly, overlap percentages of 0.04 for both channels at a threshold of 0.5 suggest minimal overlap, with only 4% of one channel's signal overlapping with intensities above the threshold in the other. Mean overlap values from simulations stand at 0.01, utilized for statistical comparison. In sum, the findings point to minimal colocalization between the analyzed channels.

Observations for object-based approach:

Ripley K's function, a spatial statistics tool, is utilized to analyze spatial point distributions, aiding in discerning whether objects such as cells or particles labeled with fluorescent markers exhibit random, clustered, or regular patterns within a specified area. The % of spots 2 (center of mass) inside spots 1 masks reveals a minimal proportion (0.05) of the center of mass from channel 2 spots located within channel 1 spots, while the p-value for spots 1 inside 2 is 0.01, indicating statistical significance with only a 1% likelihood of random occurrence. Ripley's Analysis highlights that 6% of channel 2 spots colocalize with channel 1 spots, with an average colocalization distance of 1.35 pixels. The "Ripley's K function step" graph displays a curve illustrating the K function against various spatial scales in pixels, exhibiting peaks followed by declines, typical of such analyses and suggestive of clustering at certain scales followed by dispersion or regular spacing at larger scales. In conclusion, the analysis suggests a minimal degree of colocalization between the two types of spots under scrutiny.

2.2 Colocalization Percentage = 0.4

Below we can see the two sequences created as a result of setting colocalization set to 0.4:

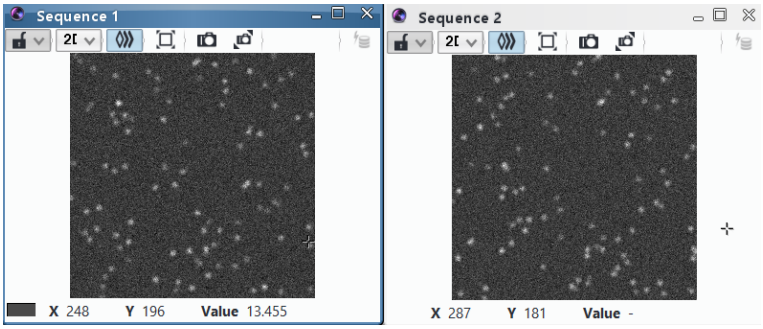


Figure 8: Sequence 1 & Sequence 2

Then we use spot detection on both images and obtain the following results.

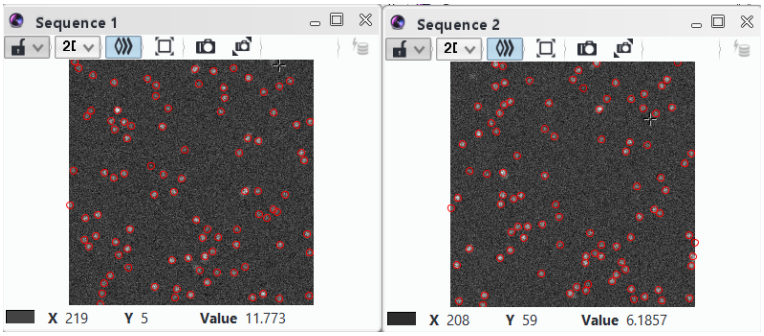


Figure 9: Sequence 1 & Sequence 2 after spot detection

2.2.1 Pixel-Based Correlation

We now compute colocalization based on pixel using the two sequence images and the results obtained from the spot detector. We can see the result in the image below:

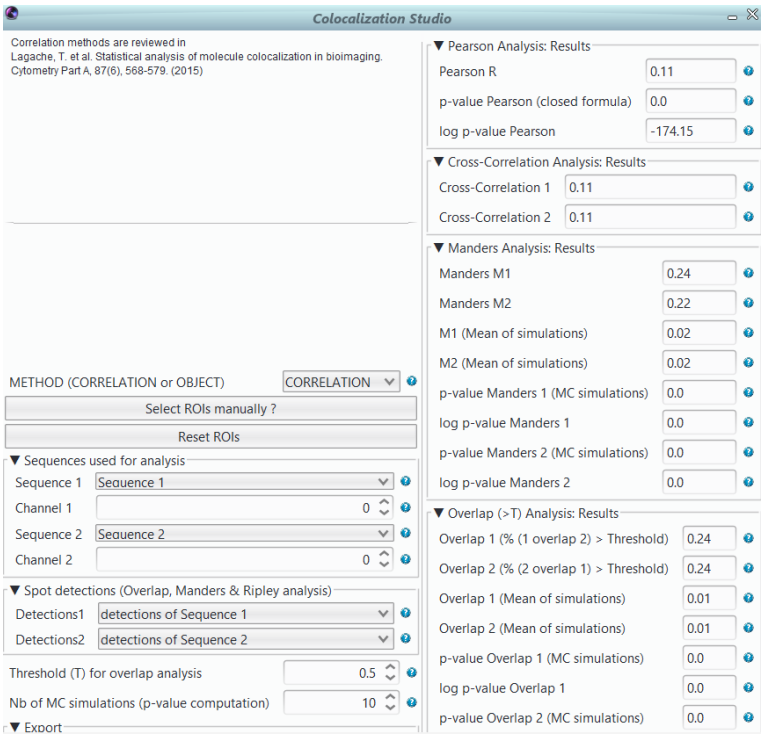


Figure 10: Correlation based on pixel results with colocalization percentage of 0.4

2.2.2 Object-Based Correlation

We compute colocalization based on object using the two sequence images and the results obtained from the spot detector. We can see the result in the image below:

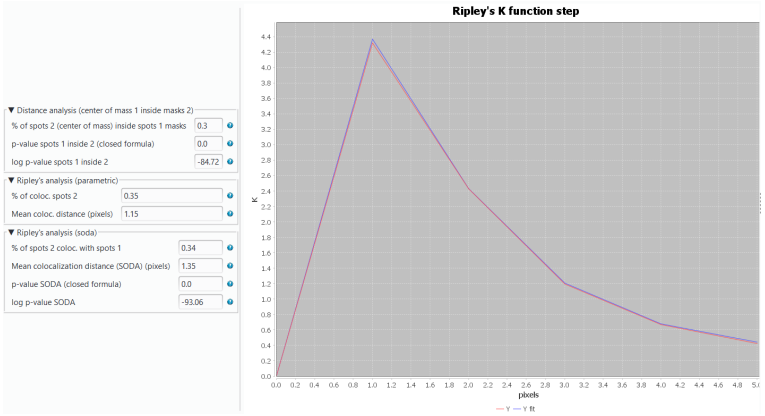


Figure 11: Correlation based on object results with colocalization percentage of 0.4

Observations for pixel-based approach:

The analysis reveals several key findings. Firstly, the Pearson R value of 0.11 signifies a weak positive correlation between the fluorescent intensities of the two channels, indicating a slight linear relationship. Similarly, the Cross-Correlation values of 0.11 mirror the Pearson R value, suggesting a weak correlation without significant temporal displacement between the signals. The Manders coefficients M1 and M2, at 0.24 and 0.22 respectively, imply a low to moderate overlap between the channels, while their mean values, along with the overlap percentages of 0.24 at a threshold of 0.5, indicate a moderate level of overlap. Moreover, both the mean Manders and overlap values from simulations are higher than expected by chance, suggesting a degree of colocalization between the represented molecules or structures across the channels.
Observations for object-based approach:
The analysis indicates that there is a low percentage of overlap between the spots of the two channels, with only a small fraction of one channel's spots falling within the area of the other channel's spots. This is complemented by Ripley's K function analysis, which suggests a moderate degree of colocalization between the channels, as the average distances between colocalized spots are fairly small. However, the p-values, which are important for determining the statistical significance of the results, are shown as zero. This is likely due to the values being very small and the software rounding them down for display purposes. The accompanying graph visualizes the Ripley's K function, showing the degree of clustering at various spatial scales. The peak of the curve indicates the spatial scale at which clustering is most evident, beyond which the clustering effect diminishes.

2.3 Colocalization Percentage = 0.9

Below we can see the two sequences created as a result of setting colocalization set to 0.9:

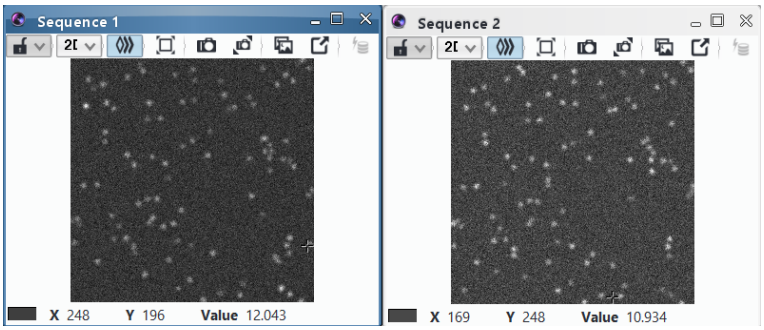


Figure 12: Sequence 1 & Sequence 2

Then we use spot detection on both images and obtain the following results.

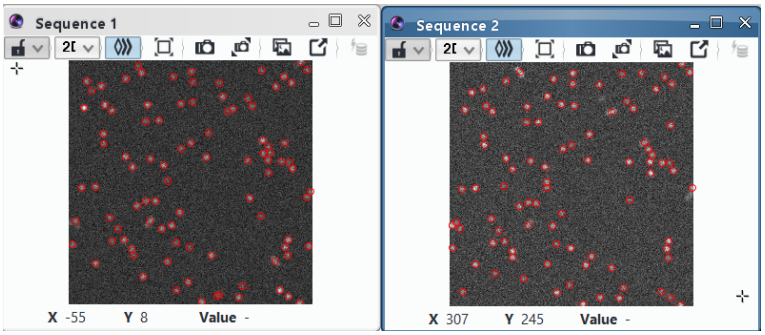


Figure 13: Sequence 1 & Sequence 2 after spot detection

2.3.1 Pixel-Based Correlation

We now compute colocalization based on pixel using the two sequence images and the results obtained from the spot detector. We can see the result in the image below:

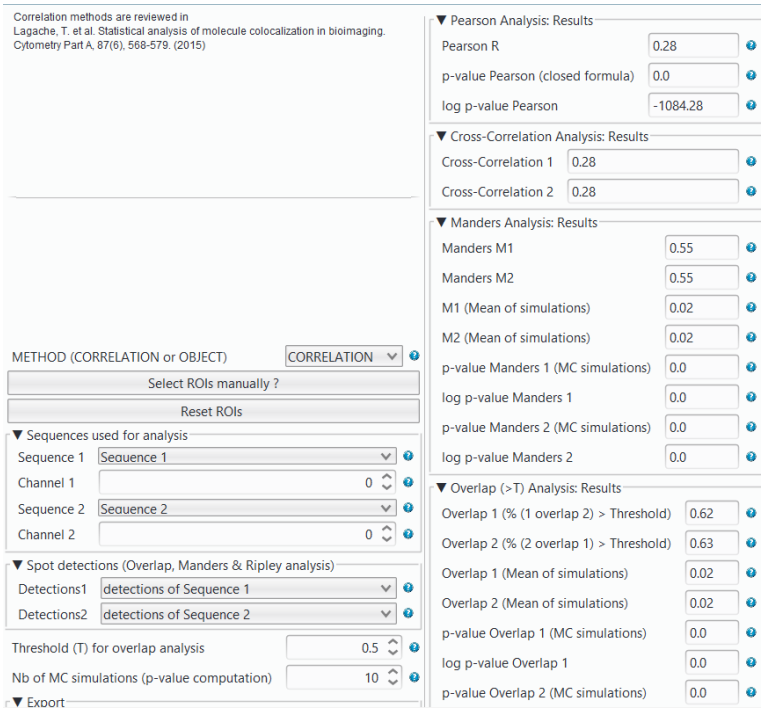


Figure 14: Correlation based on pixel results with colocalization percentage of 0.9

2.3.2 Object-Based Correlation

We compute colocalization based on object using the two sequence images and the results obtained from the spot detector. We can see the result in the image below:

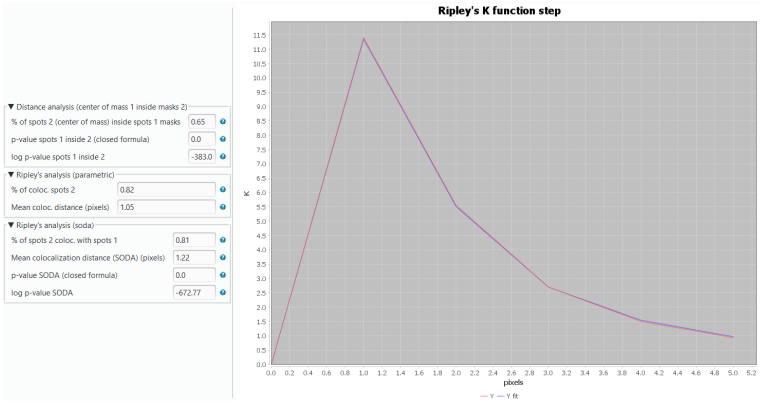


Figure 15: Correlation based on object results with colocalization percentage of 0.9

Observations for pixel-based approach:
The Pearson correlation coefficient is moderate at 0.28, suggesting some degree of linear correlation between the channels. The Manders coefficients are higher, at 0.55 for both M1 and M2, indicating a stronger colocalization or overlap in the distribution of signals from both channels. The overlap analysis supports this, showing that about 62-63% of the signal in one channel overlaps with the signal in the other channel above a set threshold.
Overall, these metrics suggest that there is a moderate to high degree of colocalization between the signals from the two channels, which could indicate that the molecules or structures labeled by these channels are located in proximity or have a functional relationship within the spatial resolution of the imaging method used.
Observations for object-based approach:
The statistics indicate a high degree of colocalization between two sets of points, with over 80% of spots from one channel colocalizing with those from another channel, as shown in both the parametric and SODA versions of Ripley's analysis. The mean colocalization distances are close, at just over 1 pixel, suggesting that the colocalized spots are nearly adjacent to each other.
The graph is a plot of Ripley's K function, which measures spatial clustering. A peak in the graph suggests the scale at which the most significant clustering occurs, and in this case, it shows a sharp peak followed by a decline, which is characteristic of a clustered pattern at a specific scale.
Overall, the statistical results and the graph together imply a strong colocalization or clustering effect at a particular spatial scale, which was expected since we sat the colocalization percentage very high.

3 A. Compute the colocalisation of the sequences particuleTracking01.tif and cparticuleTracking02.tif.

1. Compute the colocalization based on object.
2. Discuss the results.
Below we can see the pipeline of the protocol.
And here are the final results:

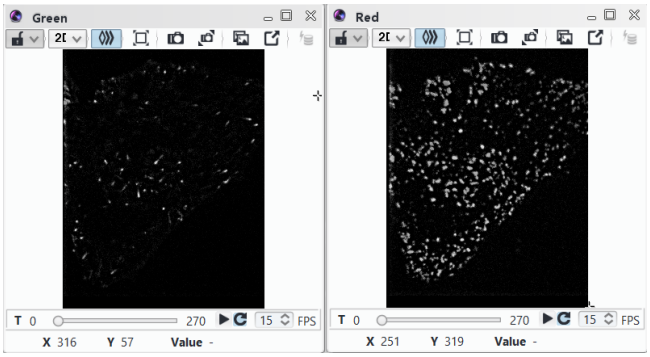


Figure 16: Sample Image

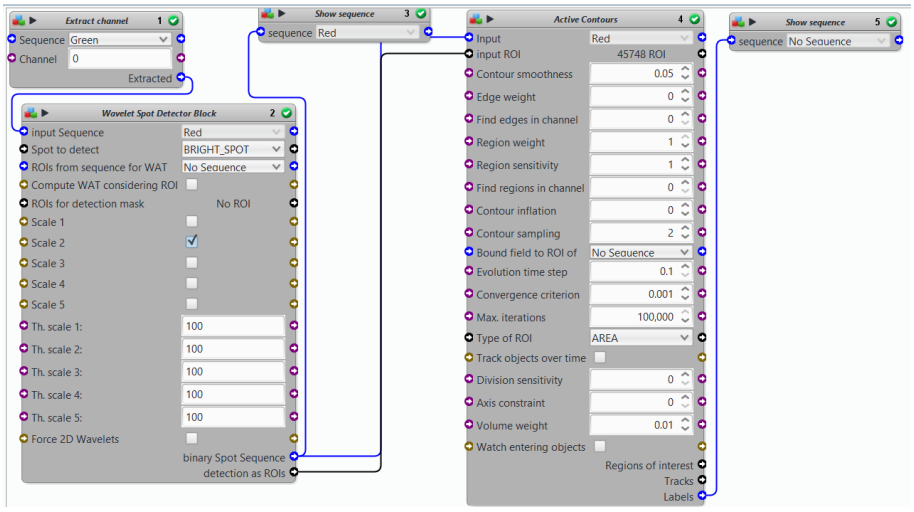
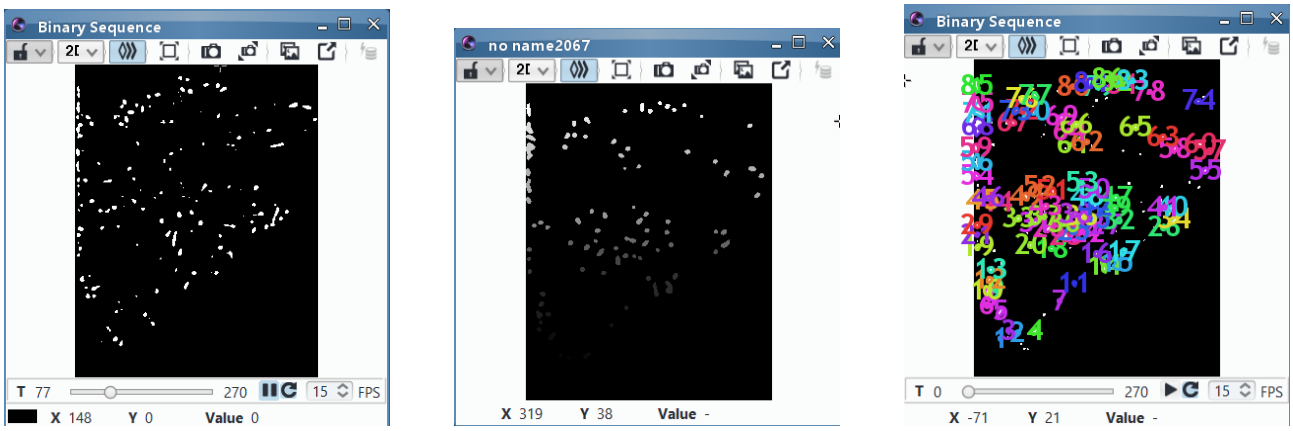


Figure 17: Protocol



(a) Image 1 (b) Image 2 (c) Image 3

Figure 18: Results