

# Colocalisation and Deformable Models

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## 1 Colocalisation

First of all we have to install:

- Colocalization simulator
- Colocalization studio

### 1.1 Point A

We start with the point **A**, first of all we have to create two images :

- We have to use "Colocalization Simulator" that creates automatically one sequence formed by two images, I used the default parameters as shown in Fig 1.

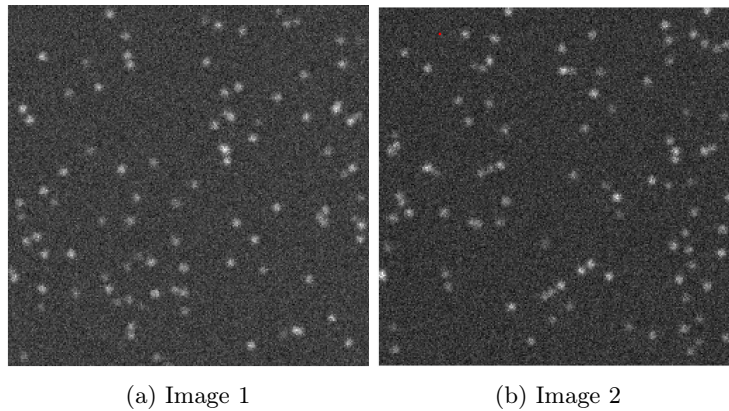
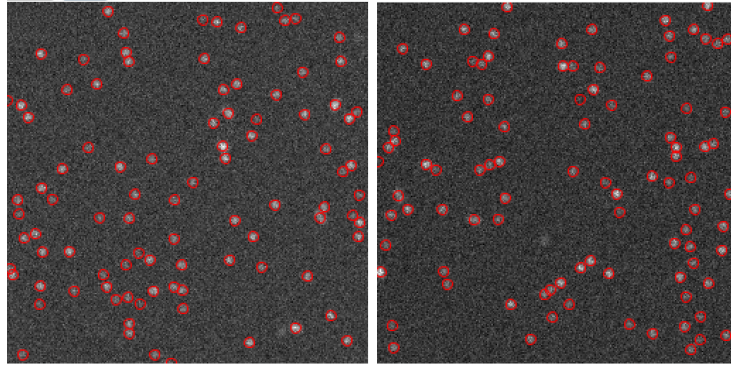


Figure 1: Sequence creation

- Use the "Spot Detector" in both images as shown in Fig. 2



(a) Detection of Spots in Image 1 (b) Detection of Spots in Image 2

Figure 2: Detection of Spots in Sequence

- Use the "Colocalization Studio" with both the two images and the images with the Detection of the spots.

1. Compute the colocalisation based on correlation, result are shown in Fig. 3

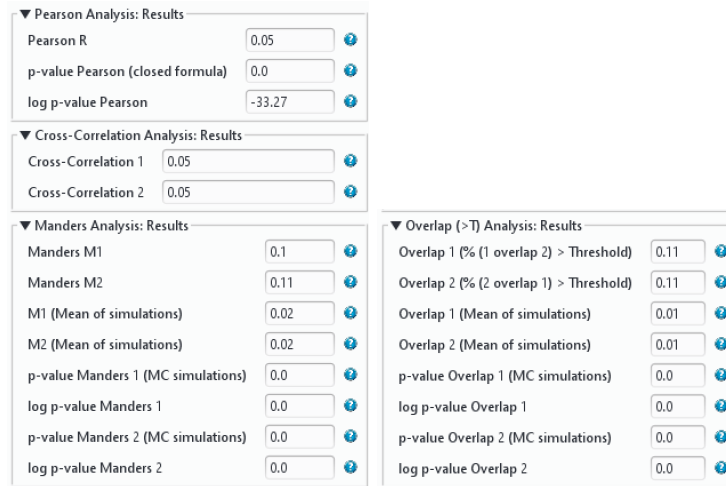


Figure 3: Correlation based Results

2. Compute the colocalisation based on object, result are shown in Fig. 4

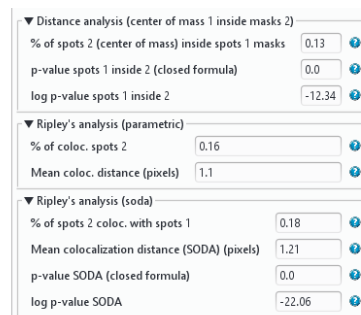


Figure 4: Object based Results

3. The results show that the Pearson's constant R is 0.05.

We know that Pearson's coefficient evaluate the linearity of relationship, it is not sensitive to differences in mean signal intensities or range, or a zero offset between the two components. The result is +1 for perfect correlation, 0 for no correlation, and -1 for perfect anti-correlation. Noise makes the value closer to 0 than it should be. Since our result is close to zero, we can conclude that this sequence has low correlation.

Manders coefficients are proportional to the amount of fluorescence of the colocalizing pixels or voxels in each colour channel. It's values range from 0 to 1, expressing the fraction of intensity in a channel that is located in pixels where there is above zero (or threshold) intensity in the other colour channel, are  $M1=0.1$  and  $M2=0.11$ , expected result since there is no fluorescence in the sequence.

The overlap of the cells is quite small, only 0.11, both cross-correlation are 0.05.

Since it's difficult to relate R or M to real percentage of interacting molecules we can also consider the percentage of colocalisation. The percentage of colocalisation spot 2 is 0.16, the percentage of spots 2 colocalisation with spot 1 is 0.18.

The mean colocalisation distance is 1.1 using the parametric method and 1.21 using the soda method, as we can see the two values are close, but not as much as in the next example.

## 1.2 Point B

For the point **B** we have to compute the colocalisation of the sequences `sequence01_coloc.tif` and `sequence02_coloc.tif`. To do that we have to:

- Upload the two images in icy, as shown in Fig. 5

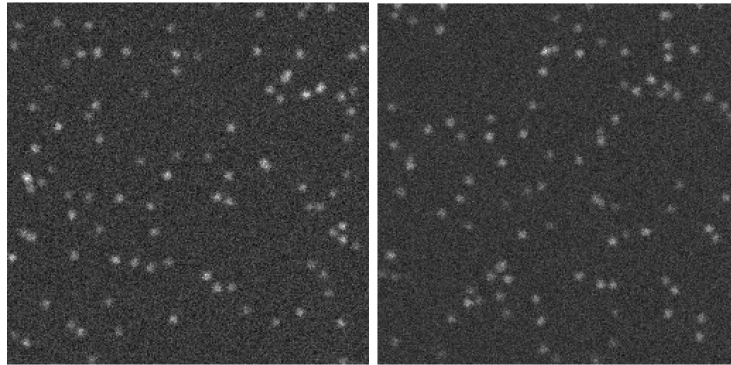


Figure 5: Sequence uploading

- Use the "Spot Detector" in both images, as shown in Fig. 6

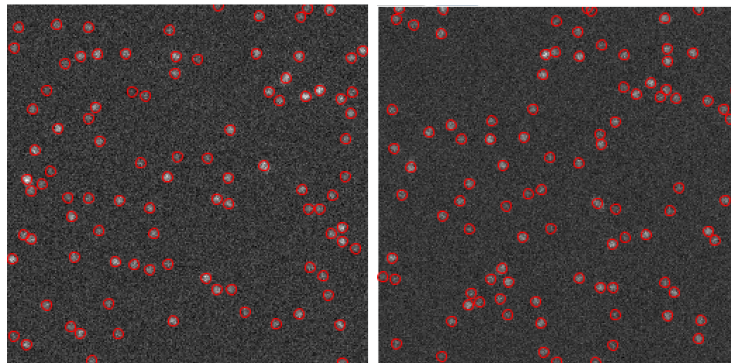


Figure 6: Detection of Spots in the upload Sequence

- Use the "Colocalization Studio" with both the two images and the images with the Detection of the spots.
1. Compute the colocalisation based on correlation, result are shown in Fig. 7

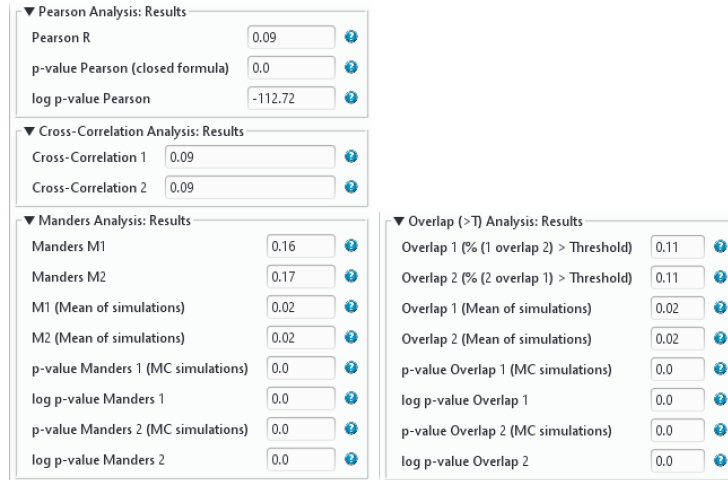


Figure 7: Correlation based Results

2. Compute the colocalisation based on object, result are shown in Fig. 8

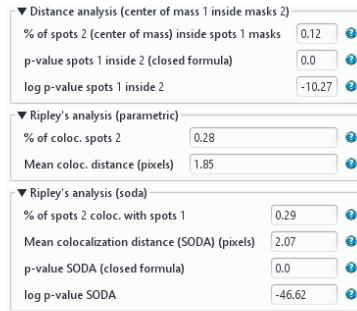


Figure 8: Object based Results

1. The percentage of colocalisation spot 2 is 0.28, the percentage of spots 2 colocalisation with spot 1 is 0.29.
2. The mean colocalization distance is 1.85 using the parametric method and 2.07 using the soda method, as we can see the two values are very close.

## 2 Deformable Models

Now we have to create a protocol to extract the red spots in the Hela cells:

- Open Protocols editor
- Adding blocks, as shown in Fig. 9, precisely:
  1. Extract channel
  2. Wavelet spot detector block
  3. Show the result
  4. We use the Image as input for Active Contours but also as a ROI

5. Show the result again

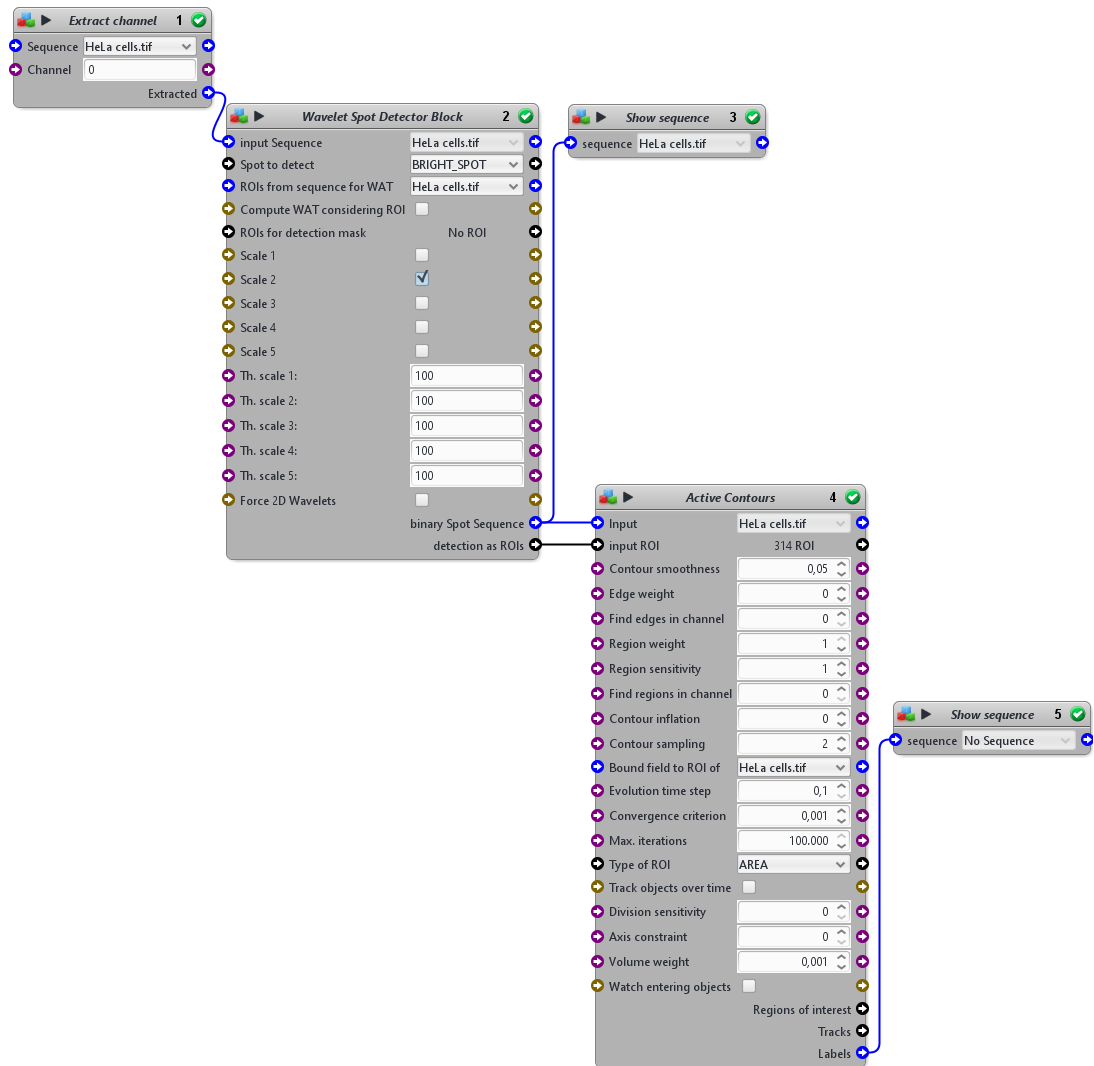
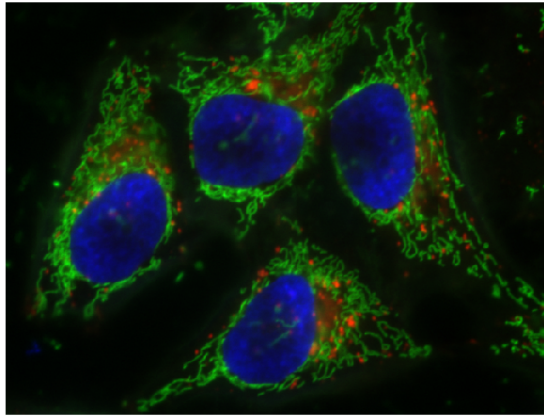
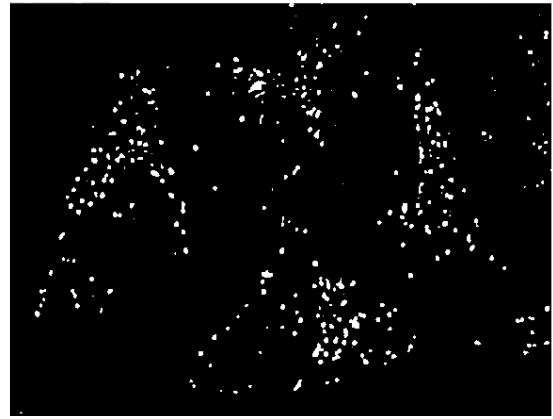


Figure 9: Protocol to extract the red spots

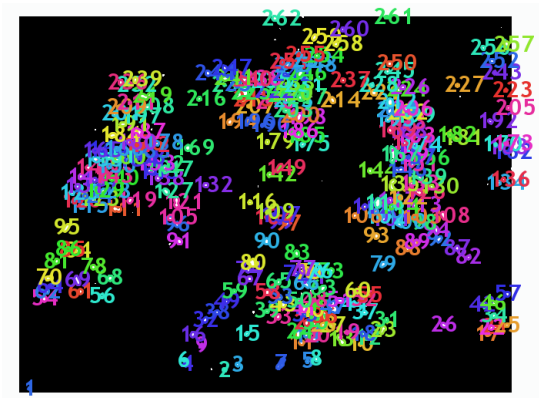
Final result is shown in Fig. 10.



(a) HeLa Cells



(b) Red cells



(c) Labels



(d) After Active Contours Application

Figure 10: Results

We can do the same also for the blue and green cells, just changing the channel in "Extract channel", for we used channel 0 that corresponds to red.

I also tried to do it using "Thresholder" and "K-Means" plug-in, but the results are strongly worse!