Colocalisation Analysis

Mandatory step: Pre-image processing – Deconvolution (Huygens):

- Go to https://huygens.bc2.unibas.ch/hrm/login.php
- Double click on the connect_hrm_data link
- Go back to the internet page, and login with your BioPhIT account
- Upload images to the server (especially if big datasets)
- Start a job and follow the instructions (create image parameters, restoration parameters, analysis parameters)
- For the output file, select **ICS** (32-bit images, for JACoP, you will have to change for 16-bit in Fiji)

Colocalisation using Fiji and the JACoP plugin:

- Open Fiji
- Open your image (already deconvolved with SoftwoRx or Huygens)
- Your images need to be 16-bit. Go to "edit", "options", "conversions" and select "scale when converting", then go to "image", "type", and select 16-bit (Needs to be done for all the windows to analyse)
- Make sure that the channels are split (using the Bio-Formats or the channel splitter)
- Go to "Plugins" and select JACoP in the list (if not in your own Fiji, you have to install it)
- Select the channels you want to compare for Image A and Image B (drop down menu)
- In the "Analysis to perform", keep all the boxes ticked, except overlap coeff. k1 and k2
- If you only want the "Intensity-based" colocalisation analysis, you can also tick off the "Object-based" colocalisation method
- Review all the tabs in red at the bottom of the window
    - Under "Thresholds", make sure that the red mask covers your signal (for each channel)
    - Under "CCF", you can keep the default value (20)
    - Under "Microscope", make sure the metadata are correct
    - Under "Costes' random°", make sure you select 200 rounds or more (the more, the longer)
- Click "Analyze"
- The calculation can take some time – use a powerful machine (in the facility for example!)
- In the log file, you will find all the statistics. Make sure you check them with the pdf of the Colocalisation talk you just attended – Check Pearson, Manders, Van Steensel's CCF, Li's ICQ, and Costes P-value results
- From the different windows, check the Cytofluogram, the Van Steensel CCF graph, and the ICAs
- You can save the log file and the values for the graphs (but not the graphs themselves) - you can take a snapshot of the windows to keep record of the results.
- The log file will be the most important file to keep – and you should use the fore mentioned values when publishing your results.

Image A: Image4_norm_540ee4452d5dd_hrm.tif - C=1
Image B: Image4_norm_540ee4452d5dd_hrm.tif - C=2

Pearson's Coefficient:
r=0.501

Overlap Coefficient:
r=0.625

Original Values

r^2=k1xk2:
k1=0.493
k2=0.792

Using thresholds (thrA=5 and thrB=5)

Overlap Coefficient:
r=0.765

Thresholded Values

r^2=k1xk2:
k1=0.737
k2=0.794

Manders' Coefficients (original):
M1=0.965 (fraction of A overlapping B)
M2=0.945 (fraction of B overlapping A)

Original Values

Manders' Coefficients (using threshold value of 5 for imgA and 5 for imgB):
M1=0.395 (fraction of A overlapping B)
M2=0.723 (fraction of B overlapping A)

Thresholded Values

Costes' automatic threshold set to 2 for imgA & 2 for imgB
Pearson's Coefficient:
r=0.417 (0.0 below thresholds)
M1=0.941 & M2=0.827

Costes Thresholded Values

Van Steensel's Cross-correlation Coefficient between Image4_norm_540ee4452d5dd_hrm.tif - C=1 and Image4_r
CCF min.: 0.244 (obtained for dx=20) CCF max.: 0.506 (obtained for dx=-1)

Results for fitting CCF on a Gaussian (CCF=a+(b-a)exp(-(xshift-c)^2/(2d^2))):
Formula: y = a + (b-a)*exp(-(x-c)*(x-c)/(2*d*d))
Status: Success
Number of completed minimizations: 2
Number of iterations: 98 (max: 6000)
Time: 5 ms
Sum of residuals squared: 0.0030441
Standard deviation: 0.0087236
R^2: 0.98887
Parameters:
a = 0.26437
b = 0.49792
c = -0.95719
d = 4.74542
FWHM=11.174 pixels

Cytofluorogram's parameters:
a: 0.419
b: 1.17
Correlation coefficient: 0.501

Li's Intensity correlation coefficient:
ICQ: 0.2054321713421342

Costes' randomization based colocalization:
Parameters: Nb of randomization rounds: 200, Resolution (bin width): 0.0010
r (original)=0.498
r (randomized)=0.0±0.0010 (calculated from the fitted data)
P-value=100.0% (calculated from the fitted data)

Results for fitting the probability density function on a Gaussian (Probability=a+(b-a)exp(-(R-c)^2/(2d^2))):
Formula: y = a + (b-a)*exp(-(x-c)*(x-c)/(2*d*d))
Status: Success
Number of completed minimizations: 2
Number of iterations: 164 (max: 6000)
Time: 0 ms
Sum of residuals squared: 5.79758E-13
Standard deviation: 4.39393E-7
R^2: 1.00000
Parameters:
a = -0.46604
b = 0.52516
c = -4.56202E-5
d = 0.0010552
FWHM=0.0020