Colocalization

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FAIIA

Colocalization

- Colocalization consists in evaluating if **two fluorophores** are **spatially close**
- Colocalization **cannot** conclude about **physical interactions** (Rayleigh criterion, need to use FRET microscopy)

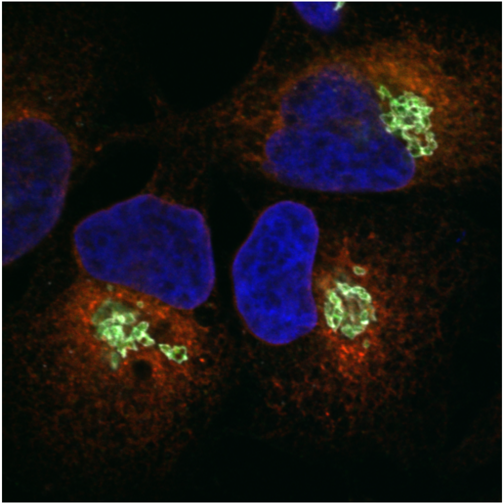
Outline

Scatterplot

Scatterplot: 2D intensity **histogram** showing the **distribution** of intensity in **both channels**

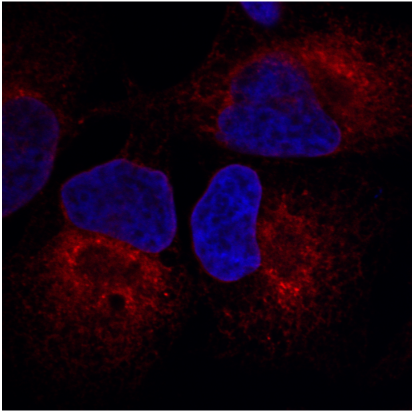
Scatterplot

Endoplasmic Reticulum (ER)
Golgi
Nucleus
Helix Pomatia Lectin (HPL)

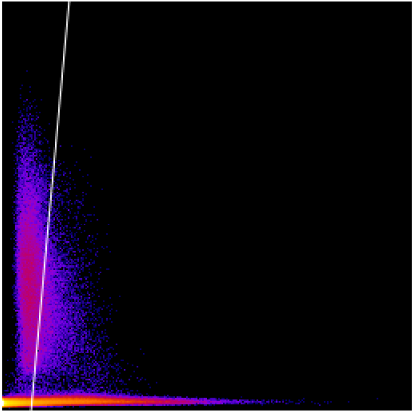


Scatterplot

ER - Nucleus

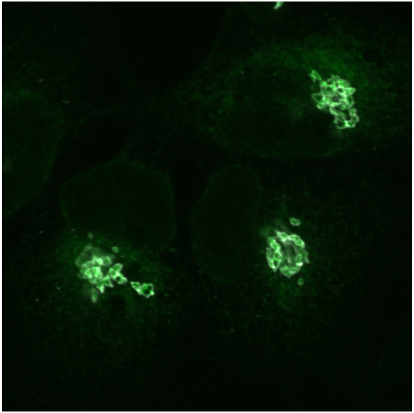


Scatter plot

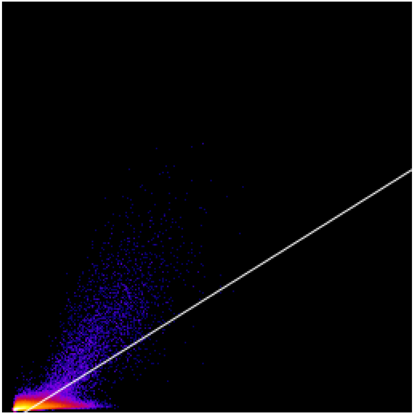


Scatterplot

Golgi - HPL

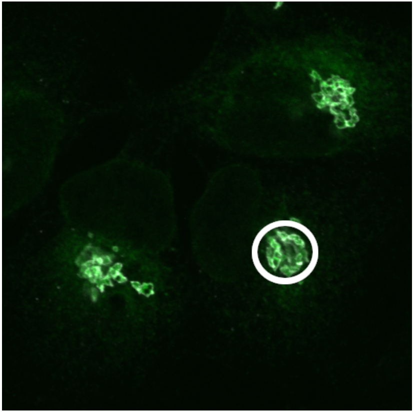


Scatter plot

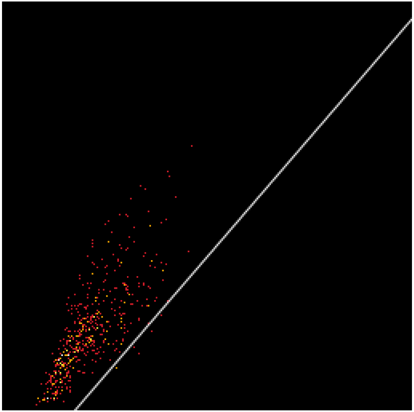


Scatterplot

Golgi - HPL + ROI



Scatter plot



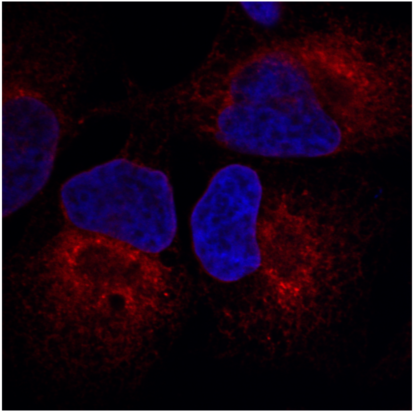
Pearson's correlation coefficient

$$PCC = \frac{\sum_i (I_i^0 - \overline{I^0}) \times (I_i^1 - \overline{I^1})}{\sqrt{\sum_i (I_i^0 - \overline{I^0})^2 \times \sum_i (I_i^1 - \overline{I^1})^2}}, \tag{1}$$

where I_i^c is the **intensity** observed at pixel i for channel c and $\overline{I^c}$ is the **average intensity** observed in channel c

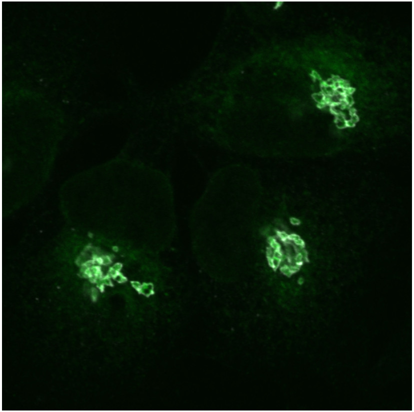
Pearson's correlation coefficient

ER - Nucleus



PCC = 0.07

Golgi - HPL



PCC = 0.8

Pearson's correlation coefficient

Advantages:

- **No need** for segmentation
- **Simple** and **fast** approach

Drawbacks:

- **No statistics** for decision

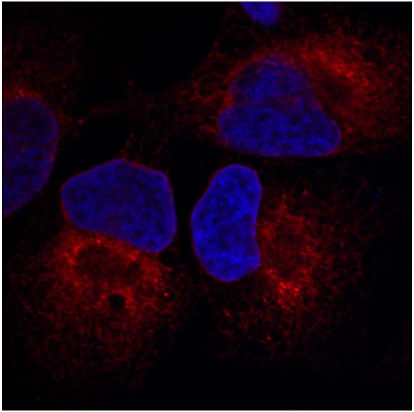
Costes' automatic threshold

Costes *et al.* have proposed to **automatically** compute a **threshold** in each channel:

- thresholds are **initialized** to the **maximum intensity**
- thresholds are **progressively decreased**
- **Pearson's correlation coefficient** is computed for **each value**
- final thresholds **minimize** the contribution of **noise**

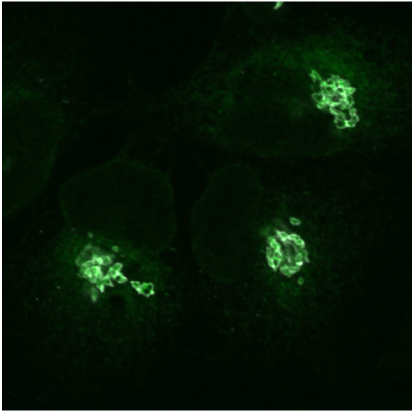
Costes' automatic threshold

ER - Nucleus



PCC = -0.53

Golgi - HPL



PCC = 0.66

Costes' automatic threshold

Advantages:

- **No need** for segmentation
- **Simple** and **fast** approach
- Only **informative regions** of the image are taken into account

Drawbacks:

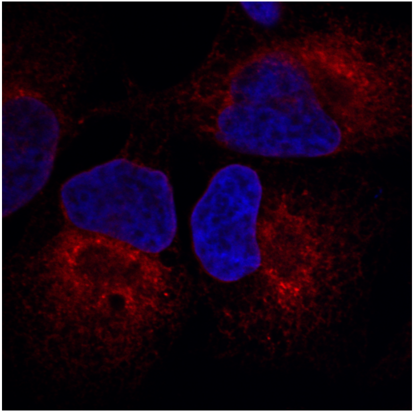
- **No statistics** for decision

Pearson's correlation coefficient with block resampling

Costes *et al.* have proposed to first compute the PCC and then **randomly sampling** the images using blocks to compute an **adjusted p-value**.

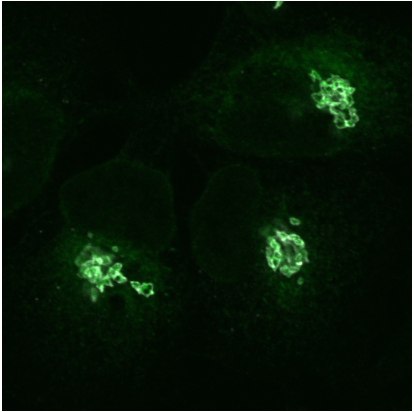
Pearson's correlation coefficient with block resampling

ER - Nucleus



p-value $= 0$

Golgi - HPL



p-value $= 0$

Pearson's correlation coefficient with block resampling

Advantages:

- **No need** for segmentation
- **p-value** from the resampling procedure

Drawbacks:

- **Slow**
- **Arbitrary** resampling with **blocks**

Outline

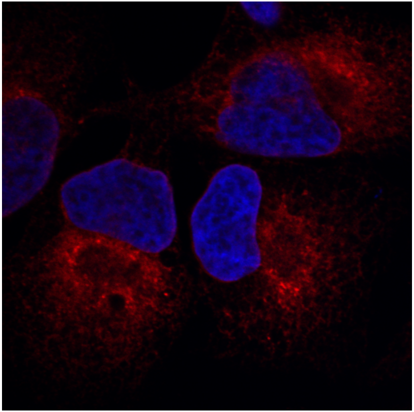
Manders' colocalization coefficients

$$M_1 = \frac{\sum_i I^0_{i,coloc}}{\sum_i I^0_i}, \text{ where } I^0_{i,coloc} = \begin{cases} I^0_i & \text{if } I^1_i > Th_1 \\ 0 & \text{otherwise} \end{cases} \tag{2}$$

$$M_2 = \frac{\sum_i I^1_{i,coloc}}{\sum_i I^1_i}, \text{ where } I^1_{i,coloc} = \begin{cases} I^1_i & \text{if } I^0_i > Th_0 \\ 0 & \text{otherwise} \end{cases} \tag{3}$$

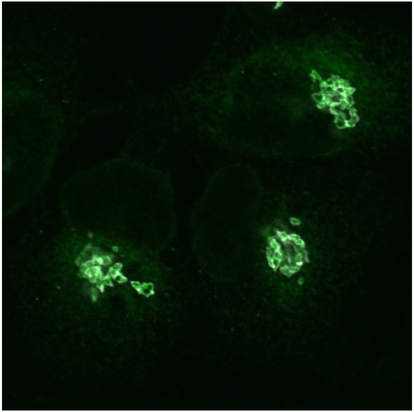
Manders' colocalization coefficients

ER - Nucleus



$M1 = 0.095$ $M2 = 0.091$

Golgi - HPL



$M1 = 0.657$ $M2 = 0.98$

Manders' colocalization coefficients

Advantages:

- **Easy** interpretation

Drawbacks:

- Need for **segmentation**
- **No statistics** for decision

Ripley's analysis

- Segmented objects are reduced to **points** (their centers)
- These points provide a **point pattern for each channel**
- The **cross Ripley's K-function** is analyzed between the two point patterns
- Assuming stationarity of the two point patterns, a **statistical procedure** is defined

Ripley's analysis

Advantages:

- **p-value** for decision

Drawbacks:

- Need for **segmentation**
- **Reduction to points** is not always suited (large and/or elongated objects)

GcoPS

- Segmented objects are considered as **random sets of spots**
- Testing relies on a closed formula of the **Pearson correlation between binary images**
- Robustness to object size allows to apply GcoPS to small windows and then **localize colocalization**

GcoPS

Advantages:

- **p-value** for decision
- **Robust** to **noise**, object **shapes** and **sizes**
- **Localizing** colocalization

Drawbacks:

- Need for **segmentation**

Practice

https://youtu.be/ruF4qg5nTcY
https://youtu.be/SE8BQwRLakc