Bioimage Informatics

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Course

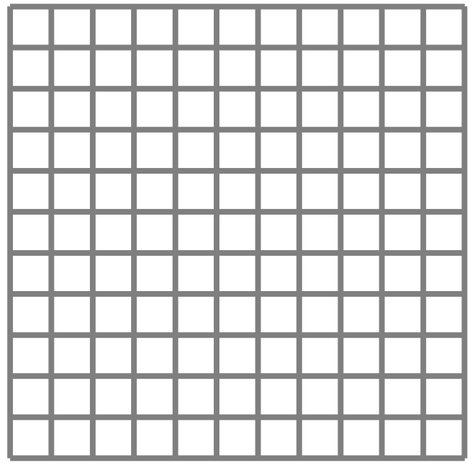
Image Statistics

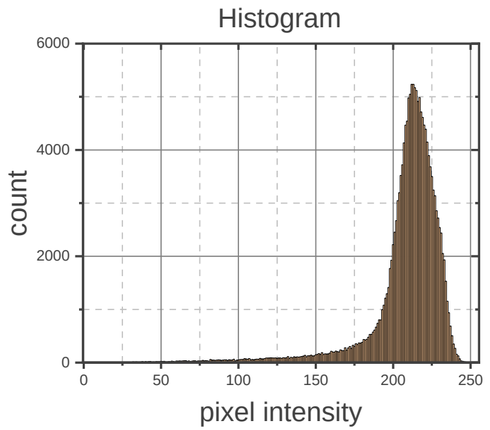
École
polytechnique
fédérale
de Lausanne

BIO-410 □ BIOIMAGE INFORMATICS

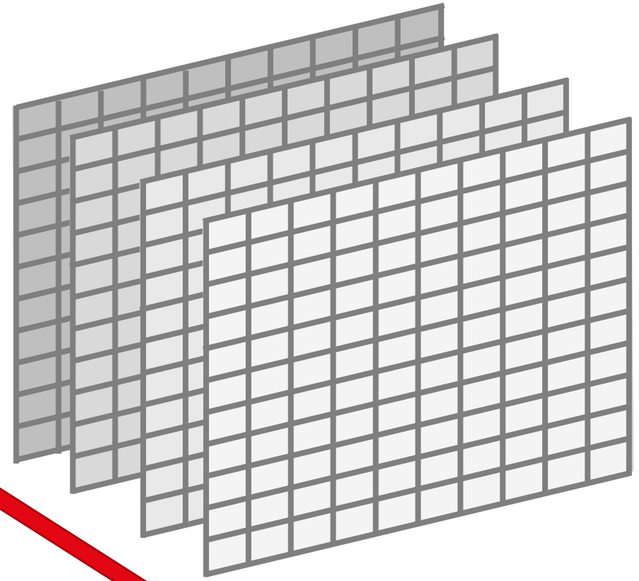
Image Statistics

2D



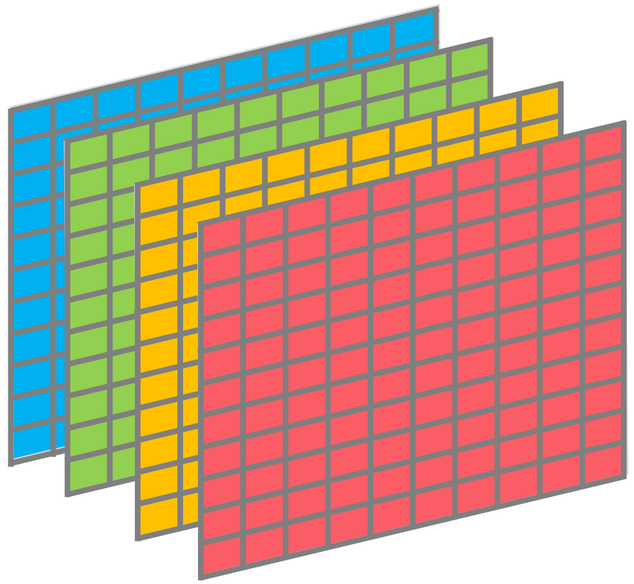


3D, 2D Time

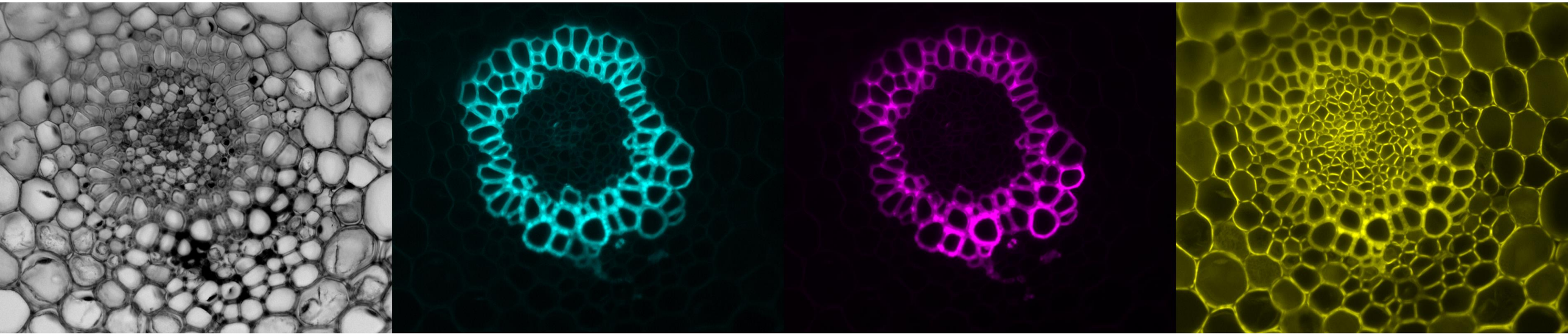


Projection
mean, min, max,…

Multi channel



Multichannel images



Transmission

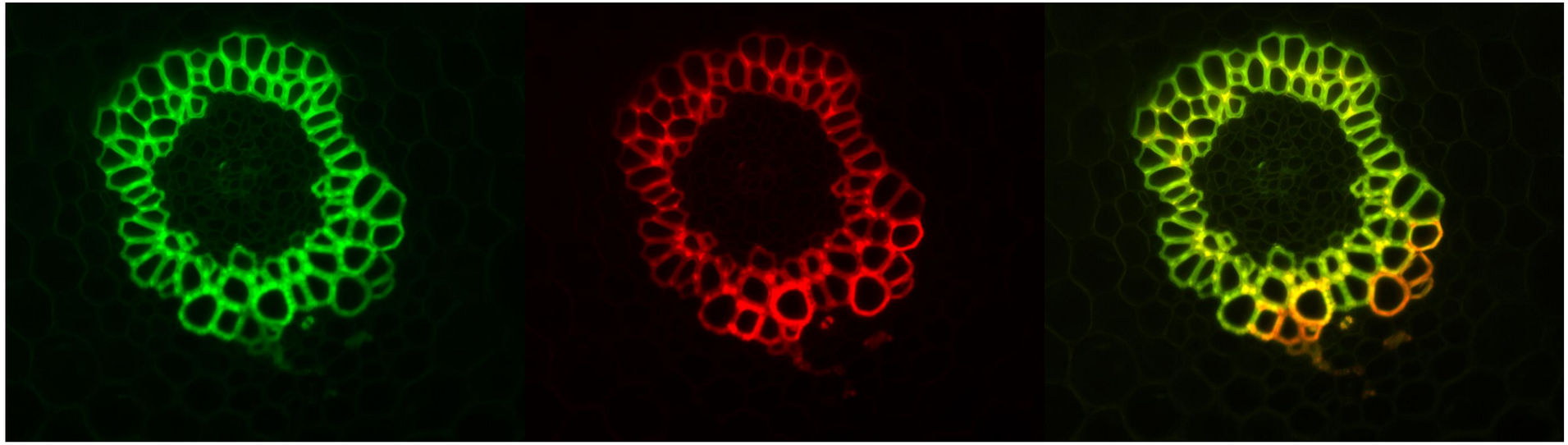
DAPI
excitation: BP 387/11
emission: BP 447/60

Green
excitation: BP 466/40
emission: BP 525/50

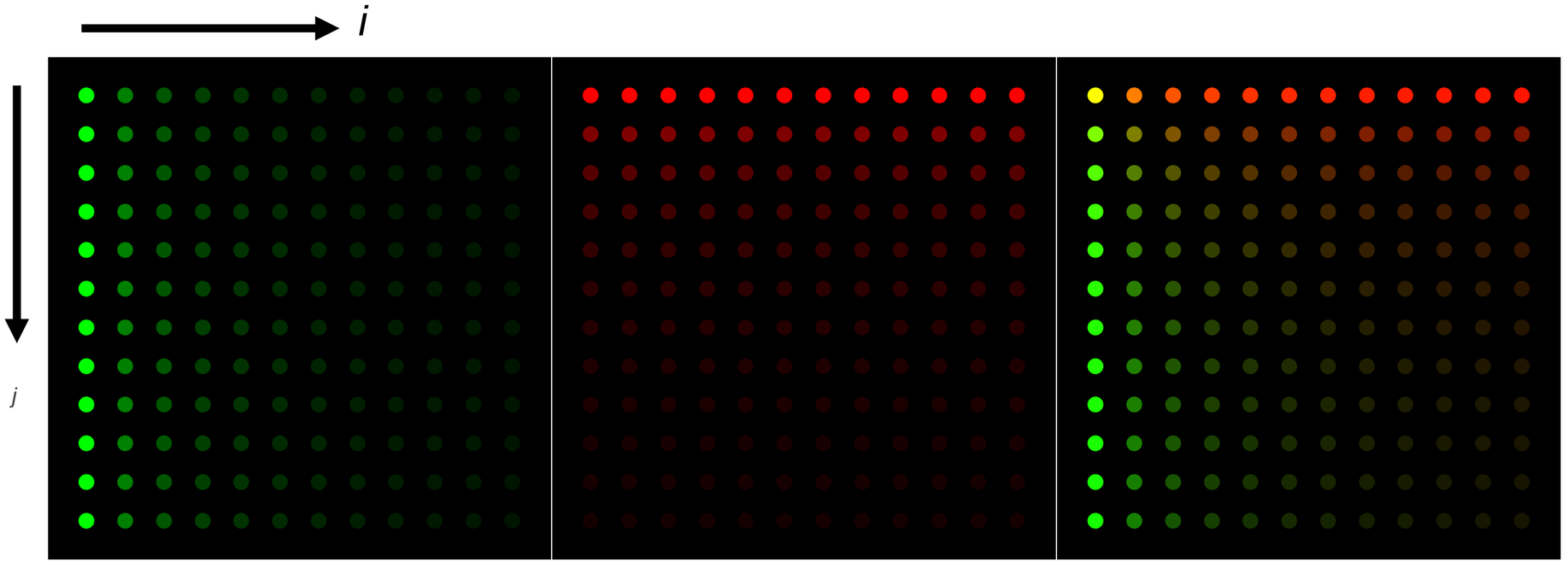
Red
excitation: BP 562/40
emission: BP 624/40

Overlap detection and quantification

Overlap detection
Color merge

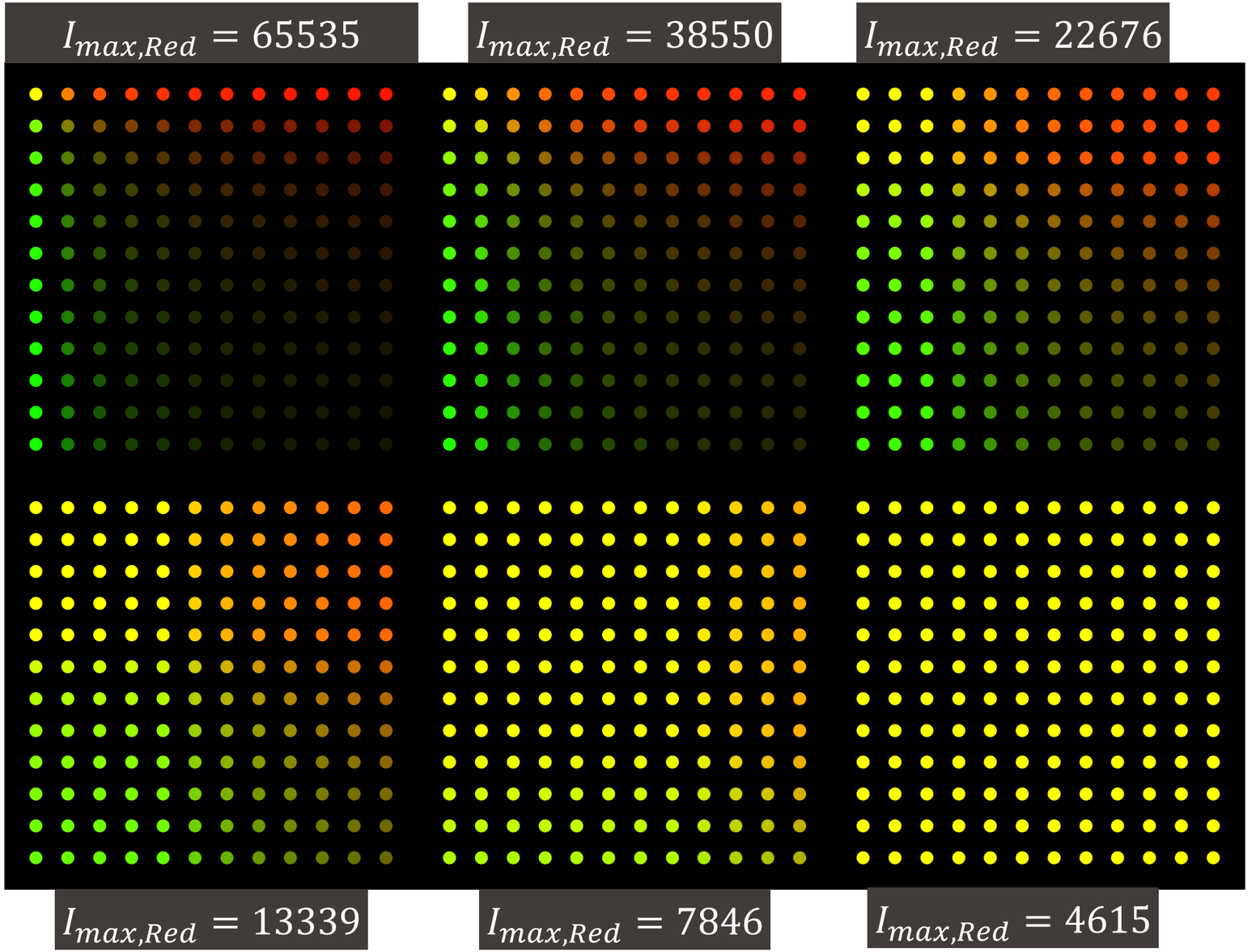


Colour is a false friend

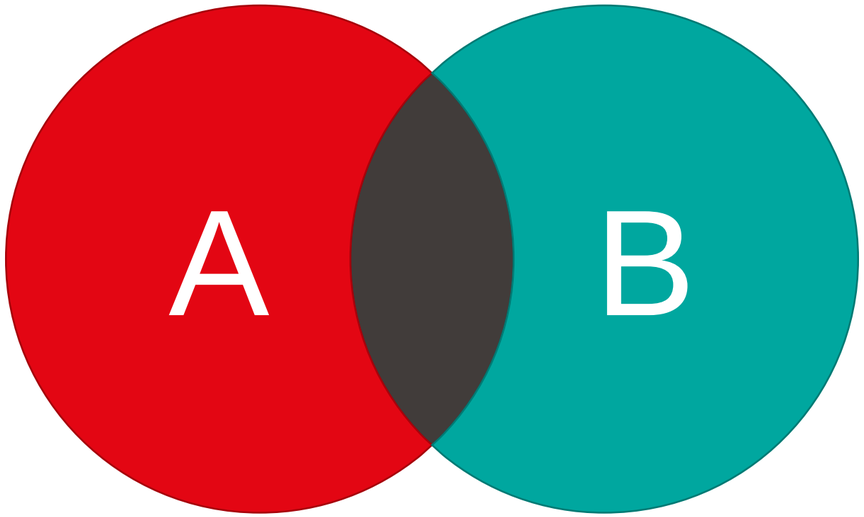


$$I_{green}(i) = \frac{1}{i} * (2^{16} - 1) \qquad I_{red}(j) = \frac{1}{j} * (2^{16} - 1)$$

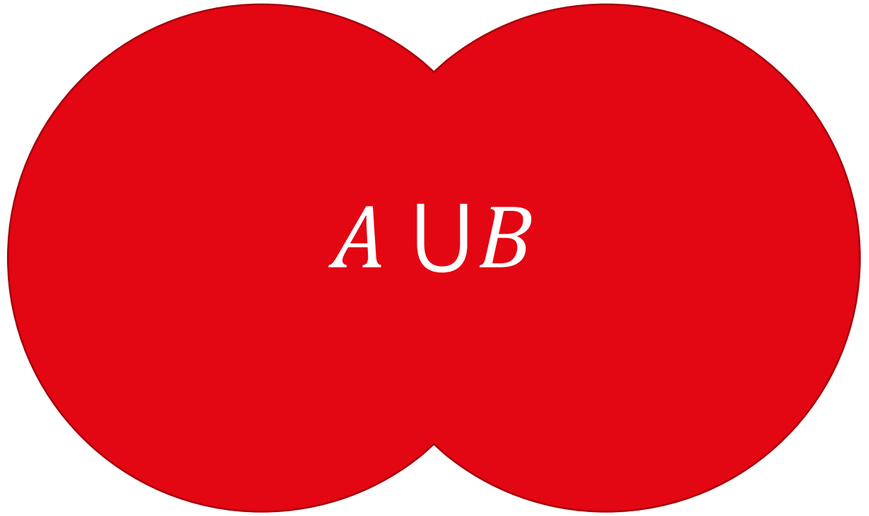
Colour is a false friend



Overlap metrics



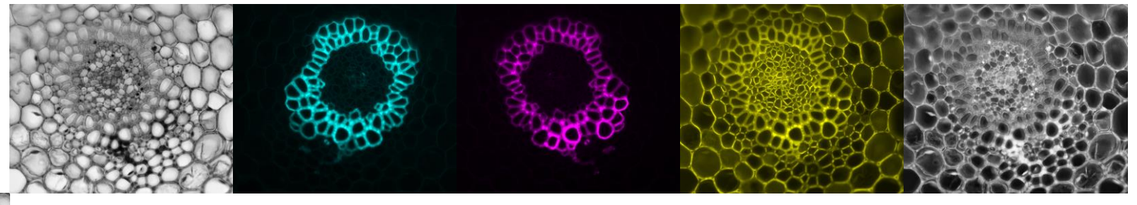
$A \cap B$

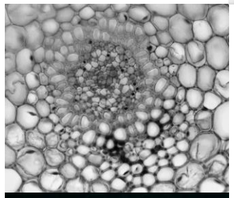


$$J(A,B) = \frac{|A \cap B|}{|A \cup B|}$$

Intersection over Union (IoU)

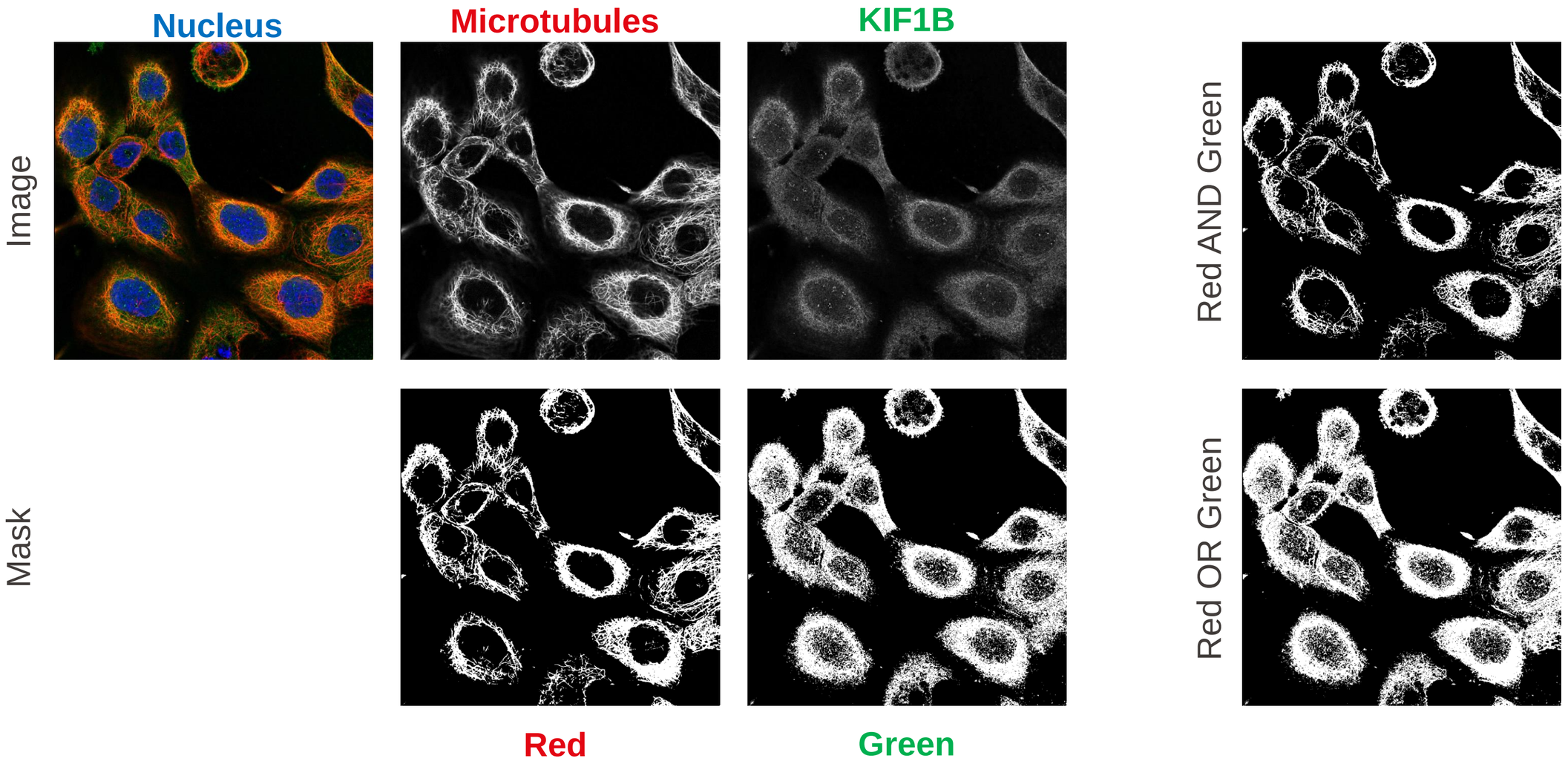
Overlap (IoU)





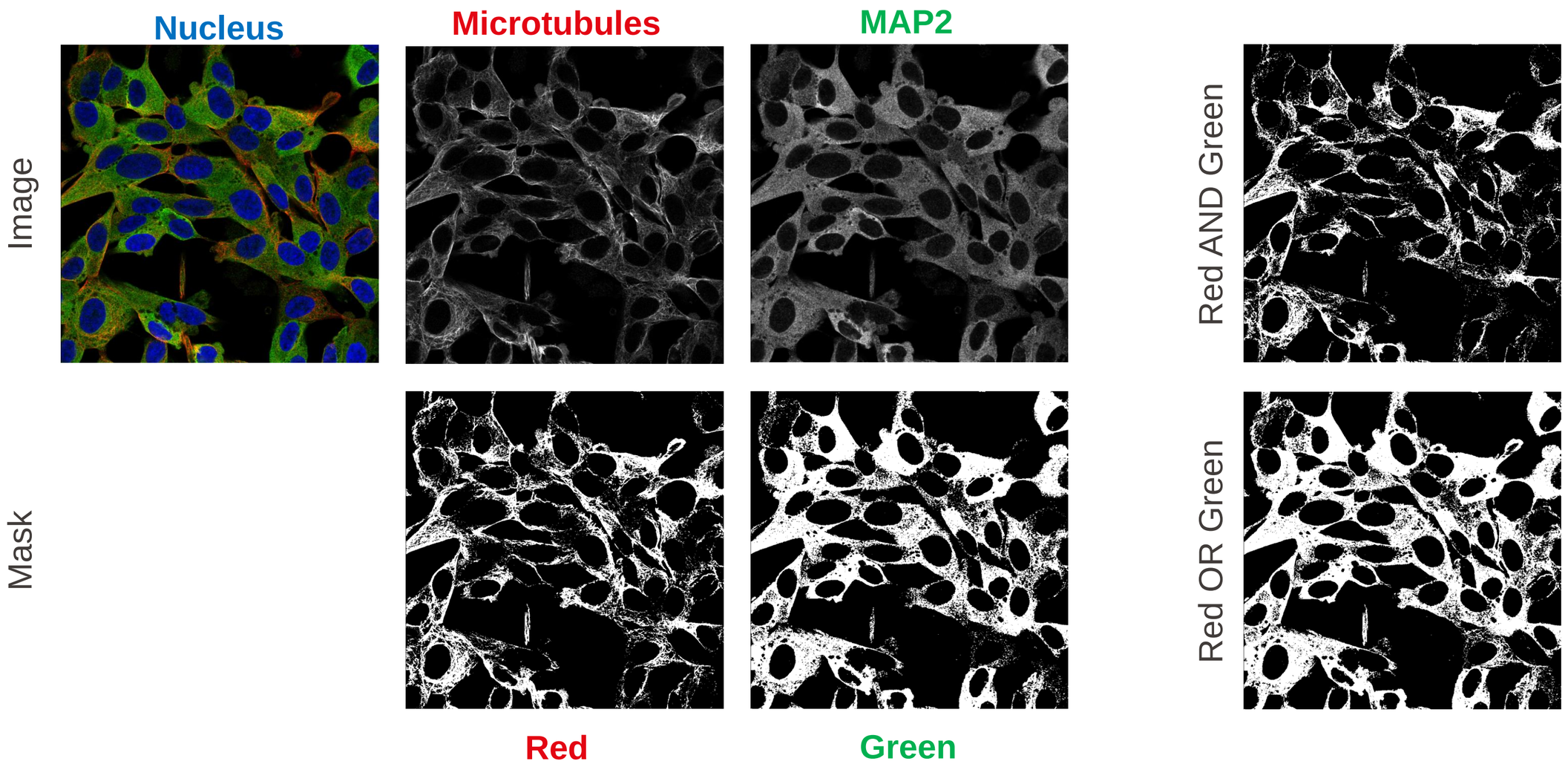
1.000	**0.010**	**0.008**	**0.018**	
0.010	**1.000**	**0.744**	**0.377**	**0.220**
0.008	**0.744**	**1.000**	**0.348**	**0.190**
0.018	**0.377**	**0.348**	**1.000**	**0.490**

Overlap in life sciences



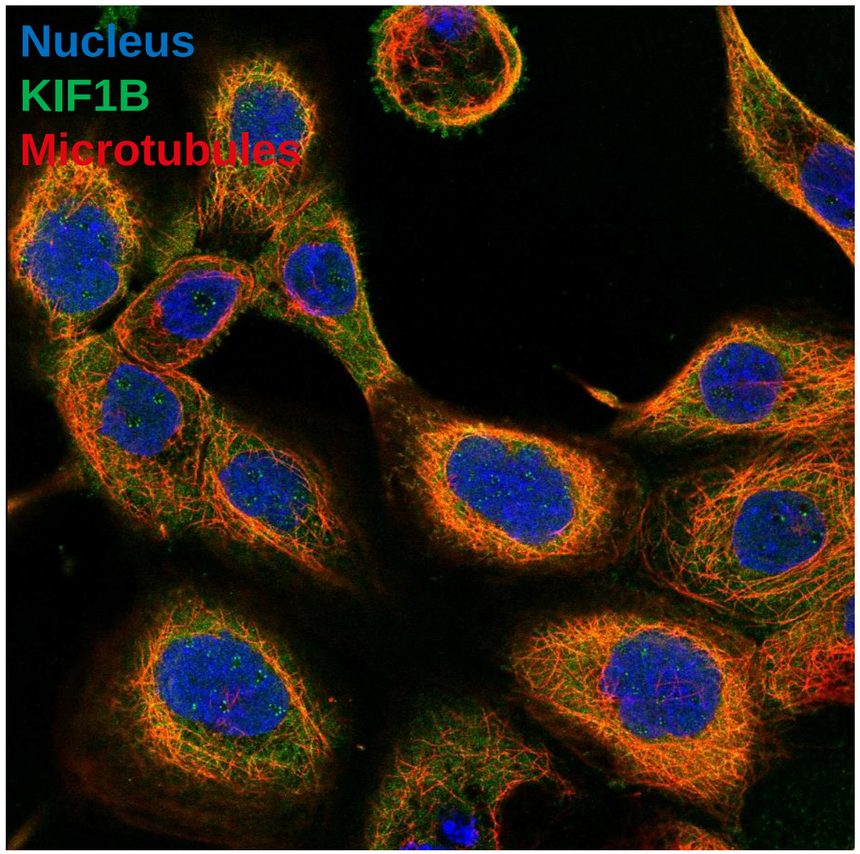
https://www.proteinatlas.org/ENSG00000054523-KIF1B/cell#img

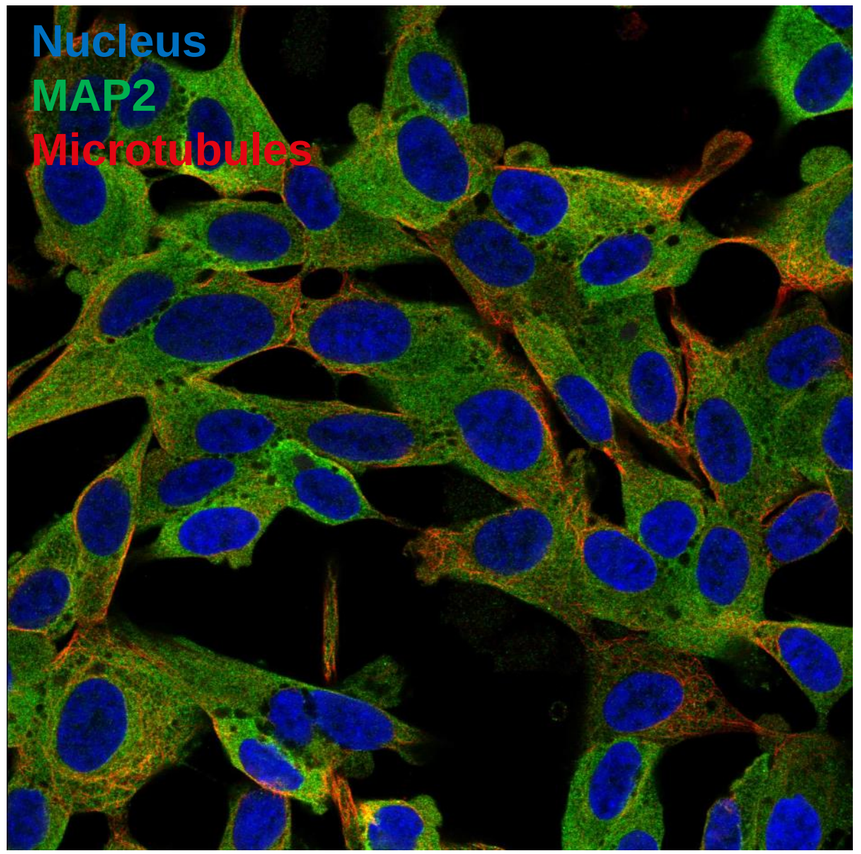
Overlap in life sciences



https://www.proteinatlas.org/ENSG00000078018-MAP2/cell#img

Overlap in life sciences

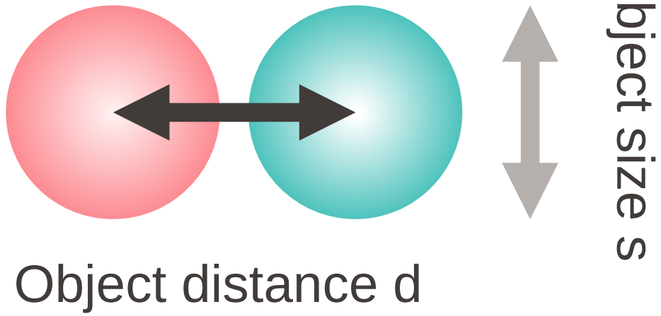


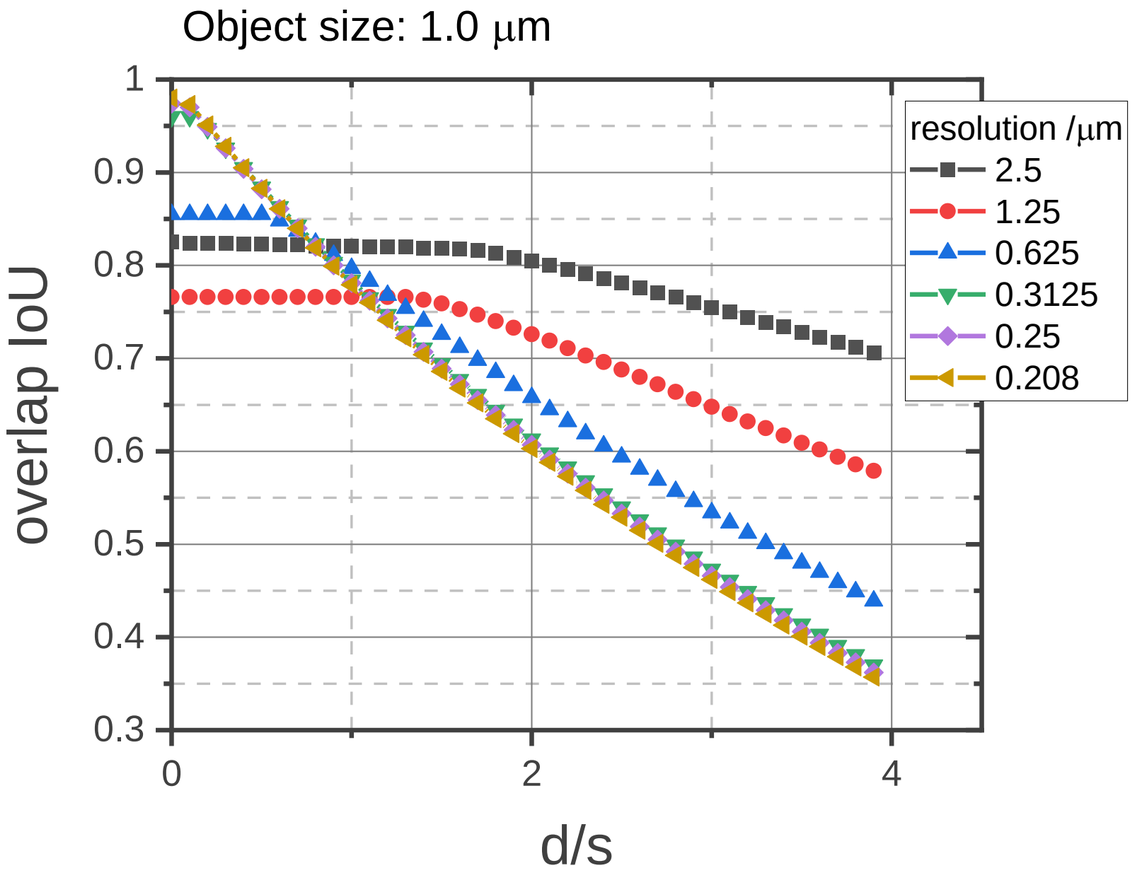


$$J(A,B)=0.498$$

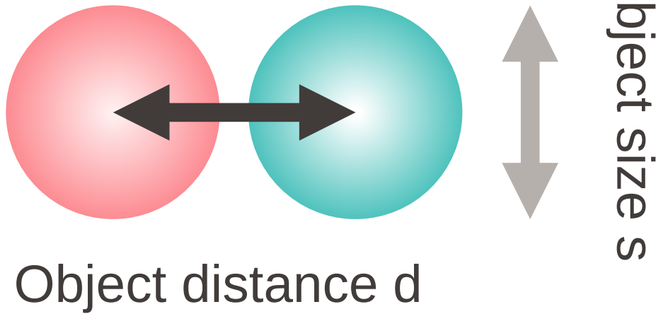
$$J(A,B)=0.437$$

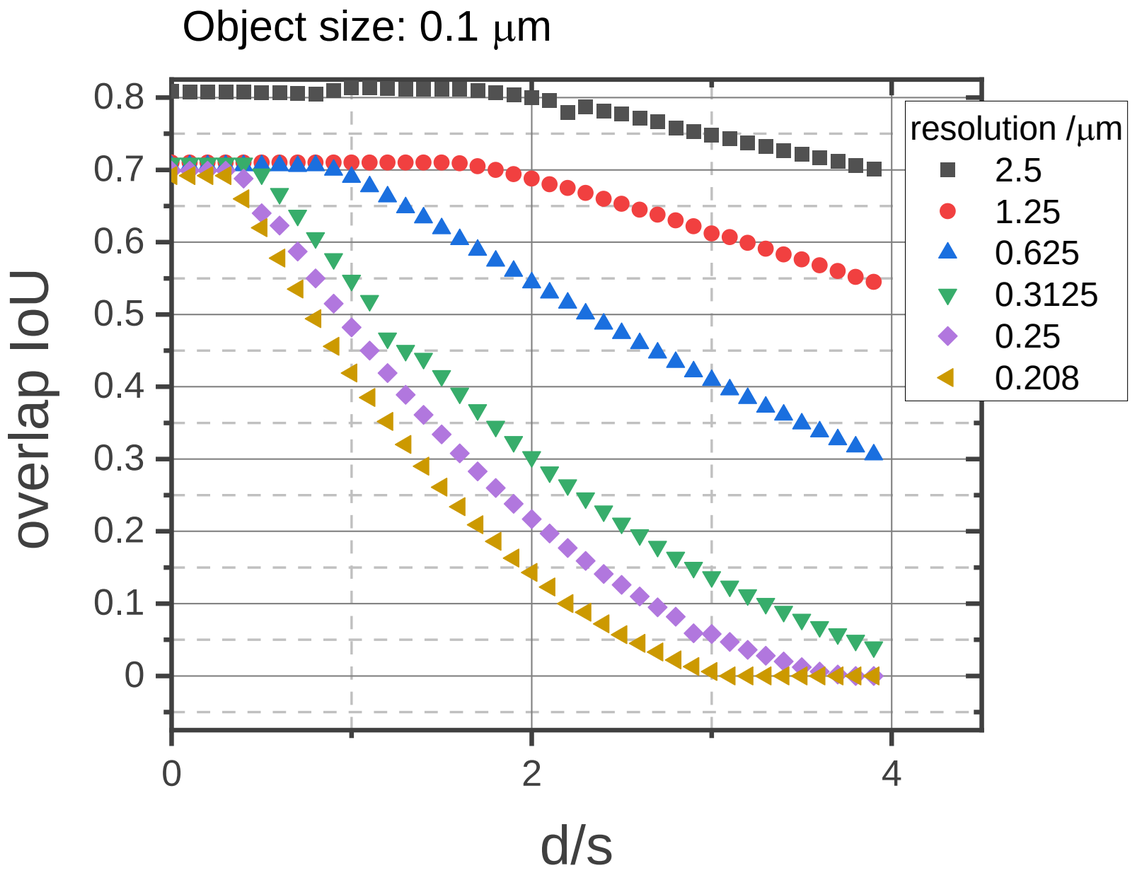
Overlap
Size dependence





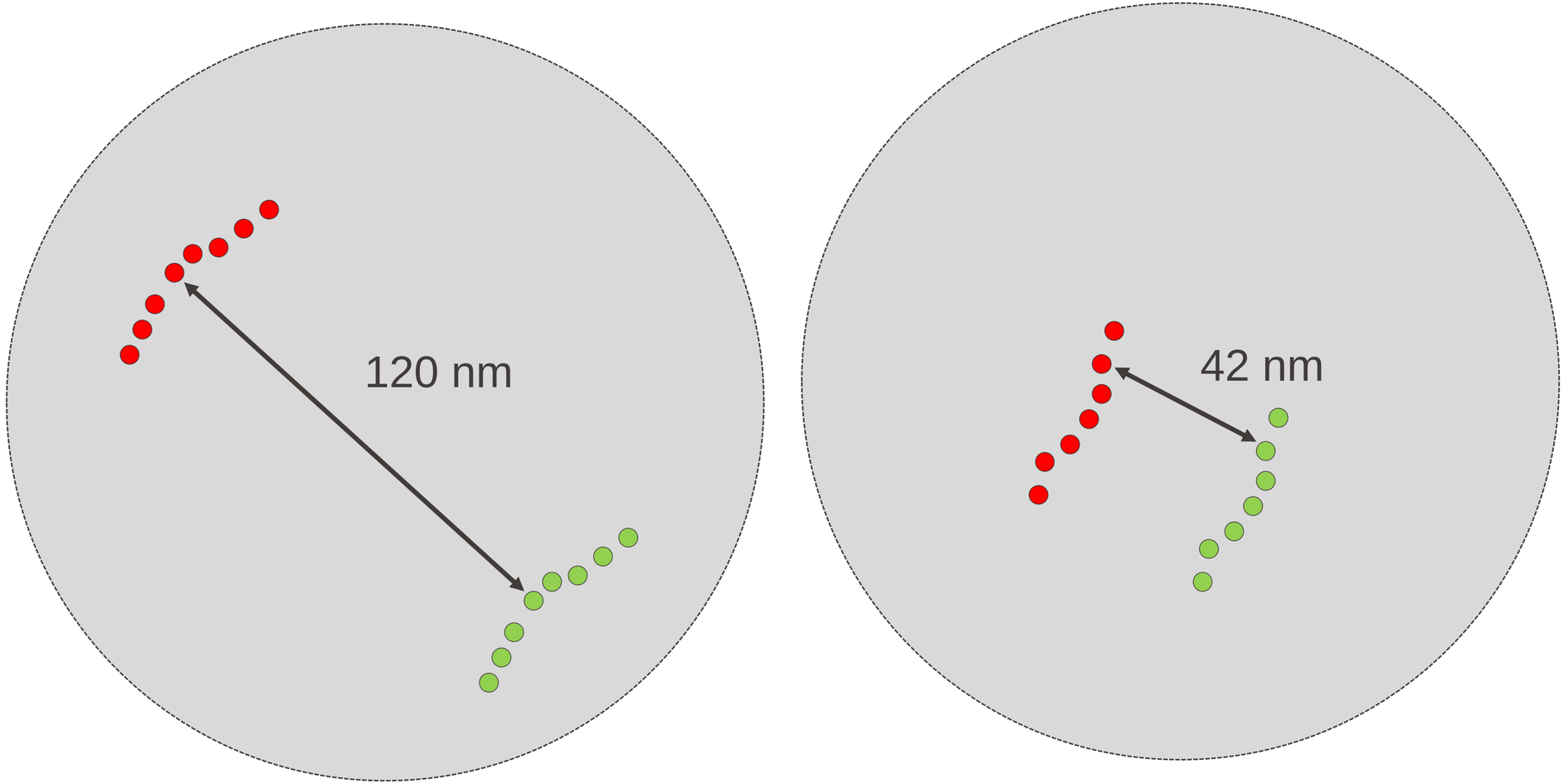
Overlap
Size dependence





Low pass filtering

Diffraction limited spot (200 nm)



= fluorescent proteins (to scale)

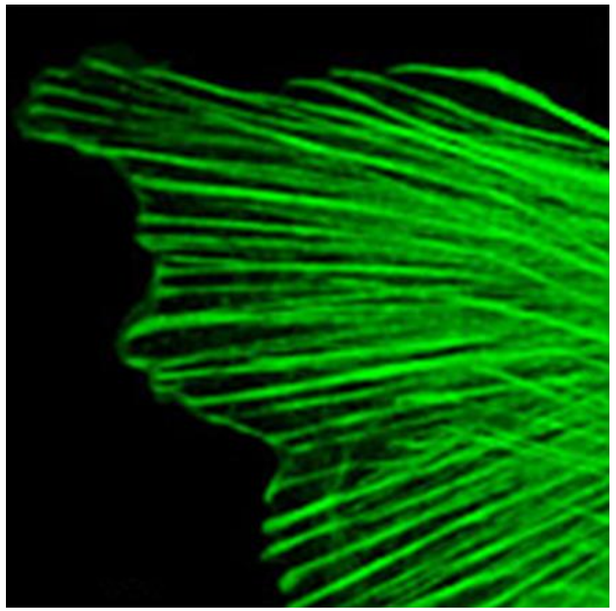
Overlap Summary

- Overlap (IoU) quantitative metric
- Requires setting thresholds for the individual channels
- Size and resolution dependence

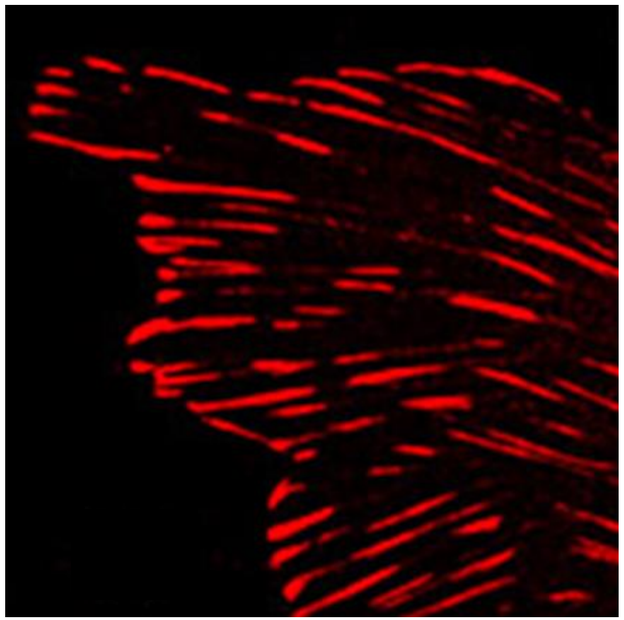
Subcellular Localization & Colocalization

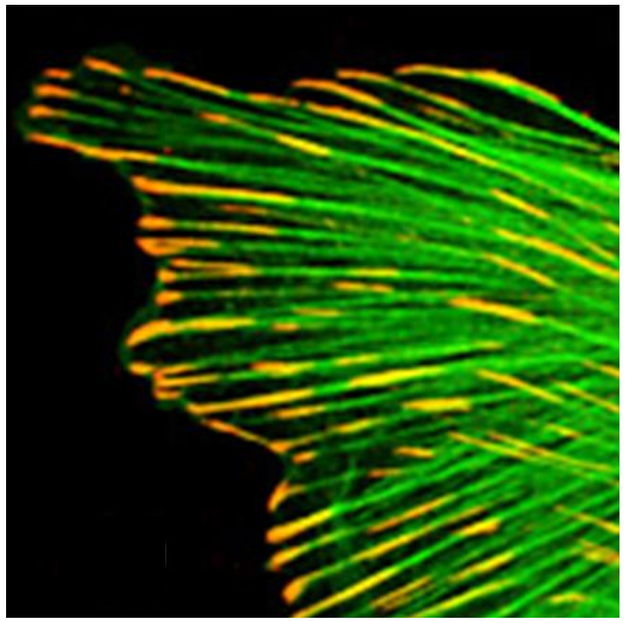
Subcellular Colocalization

Vinculin Alexa568



Actin Alexa488

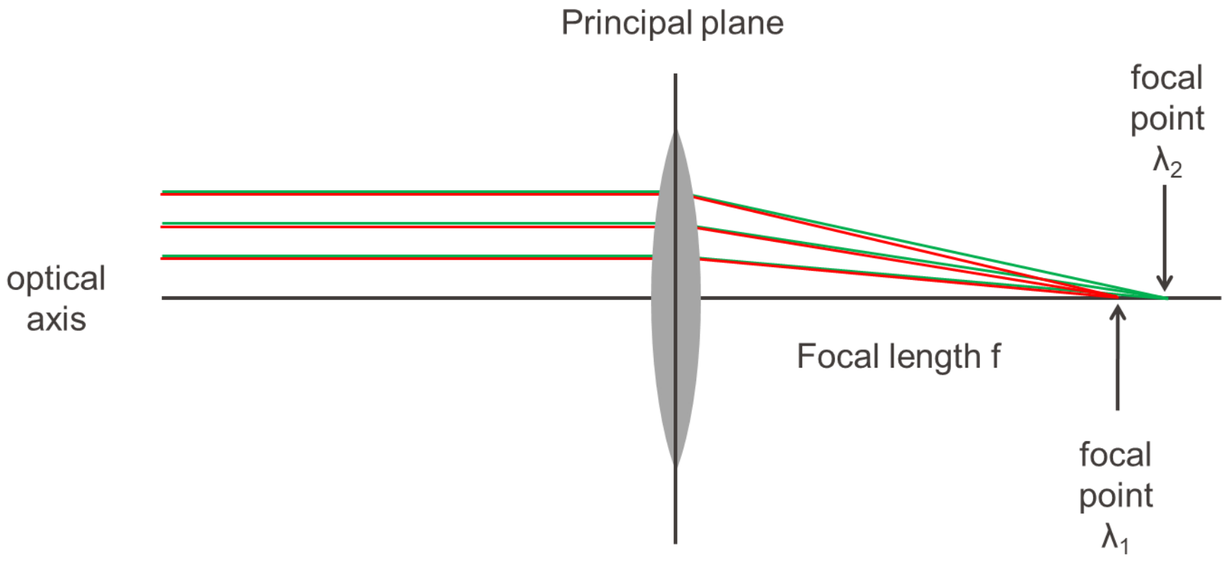


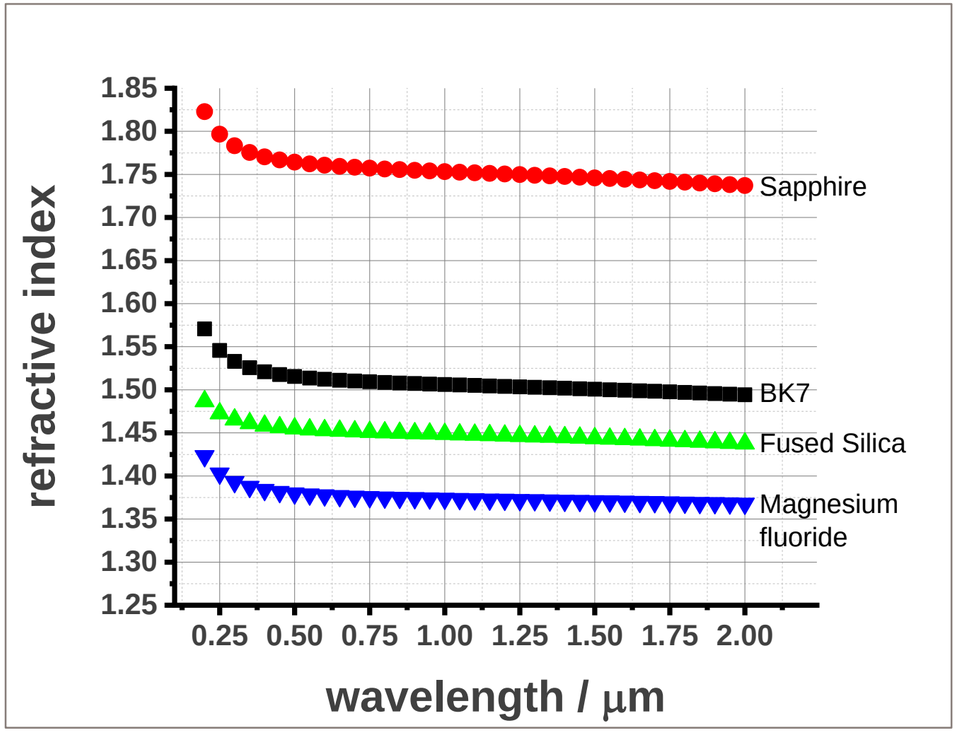


http://www.olympusconfocal.com/applications/colocalization.html

Colocalization: The presence of two or more fluorophores on the same physical structure (in a cell).

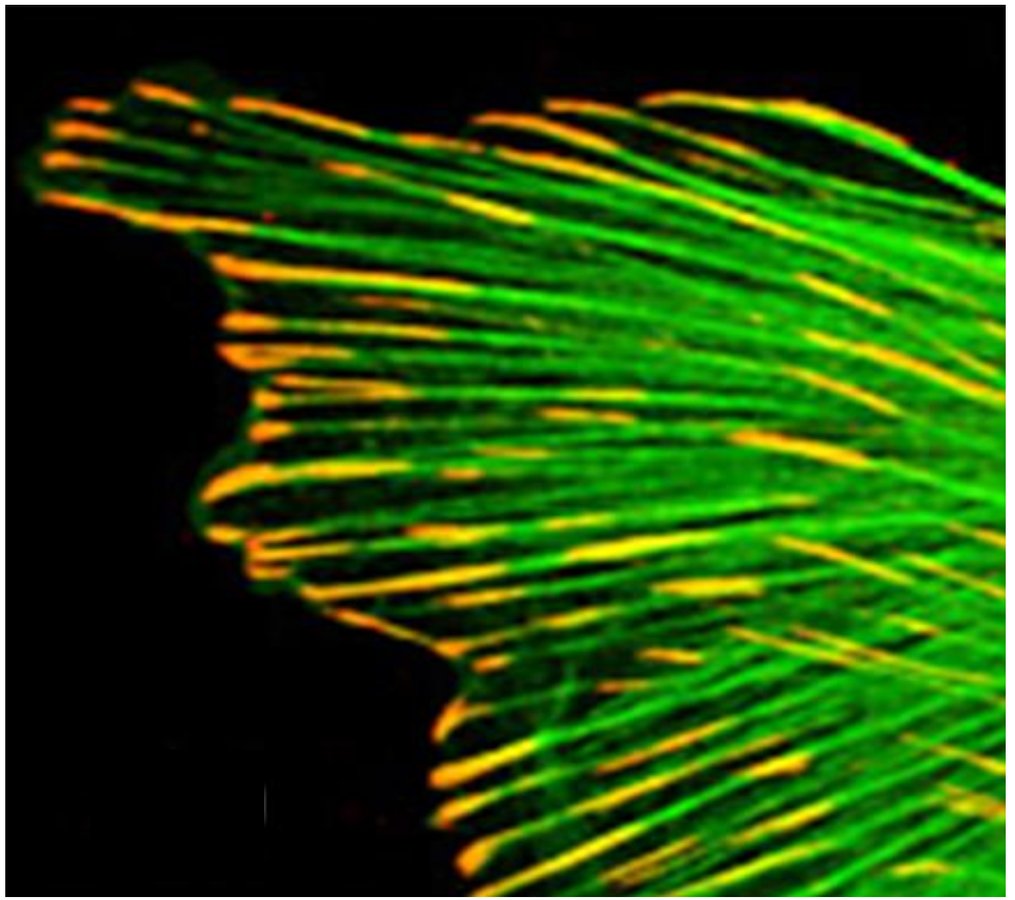
Chromatic aberrations



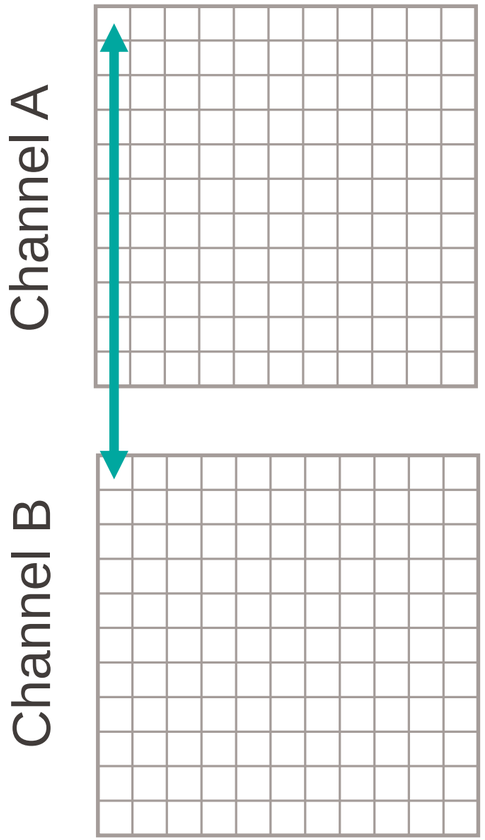


Colocalisation
Experimental requirements

- Resolution (NA of the objective lens) needs to match the structure of interest
- Avoid chromatic aberrations
  - Correct for remaining chromatic aberrations if necessary
- Avoid channel cross-talk
  - Correct for cross-talk if necessary
- Reduce the out-of-focus background
- Use preferably high SNR images

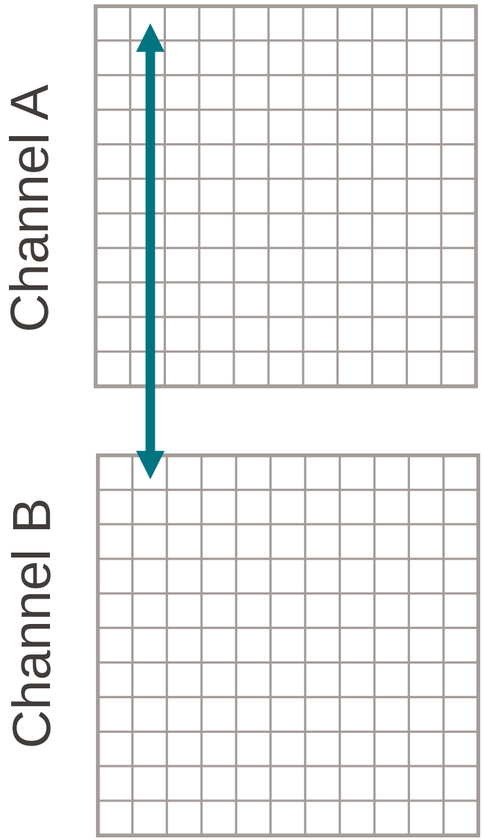


Channel Statistics



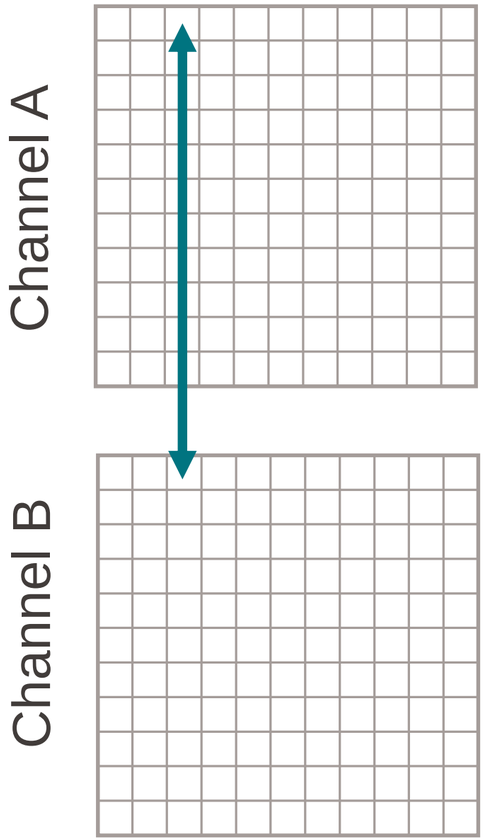
Ch A	Ch B
5	4

Channel Statistics

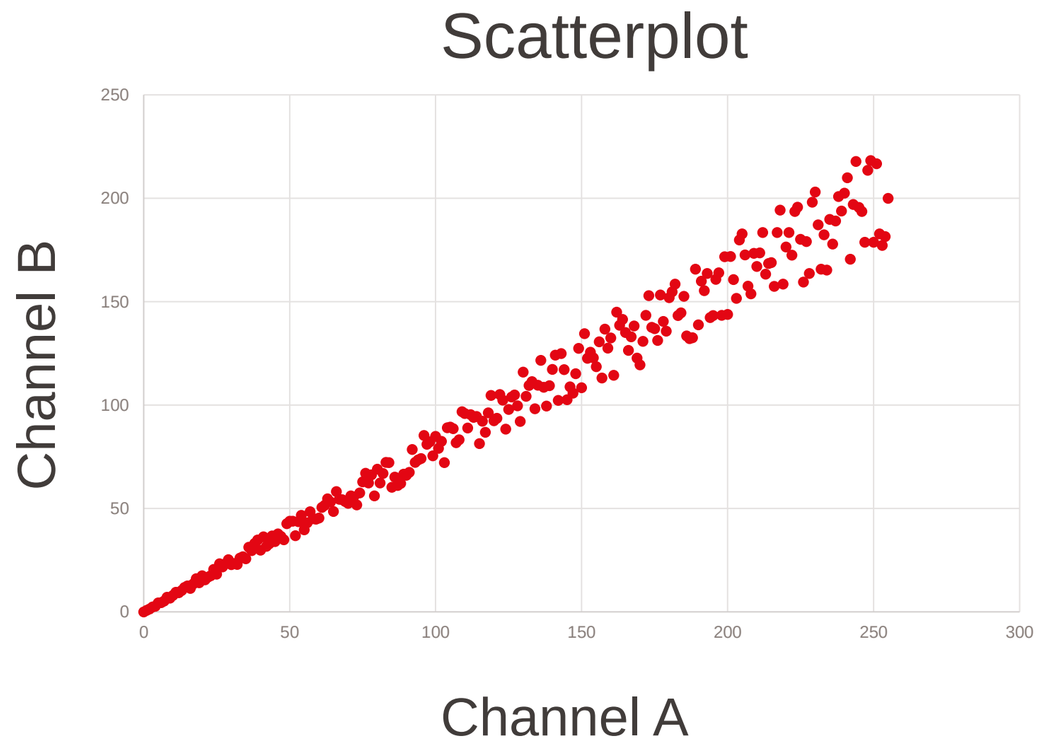


Ch A	Ch B
5	4
7	12

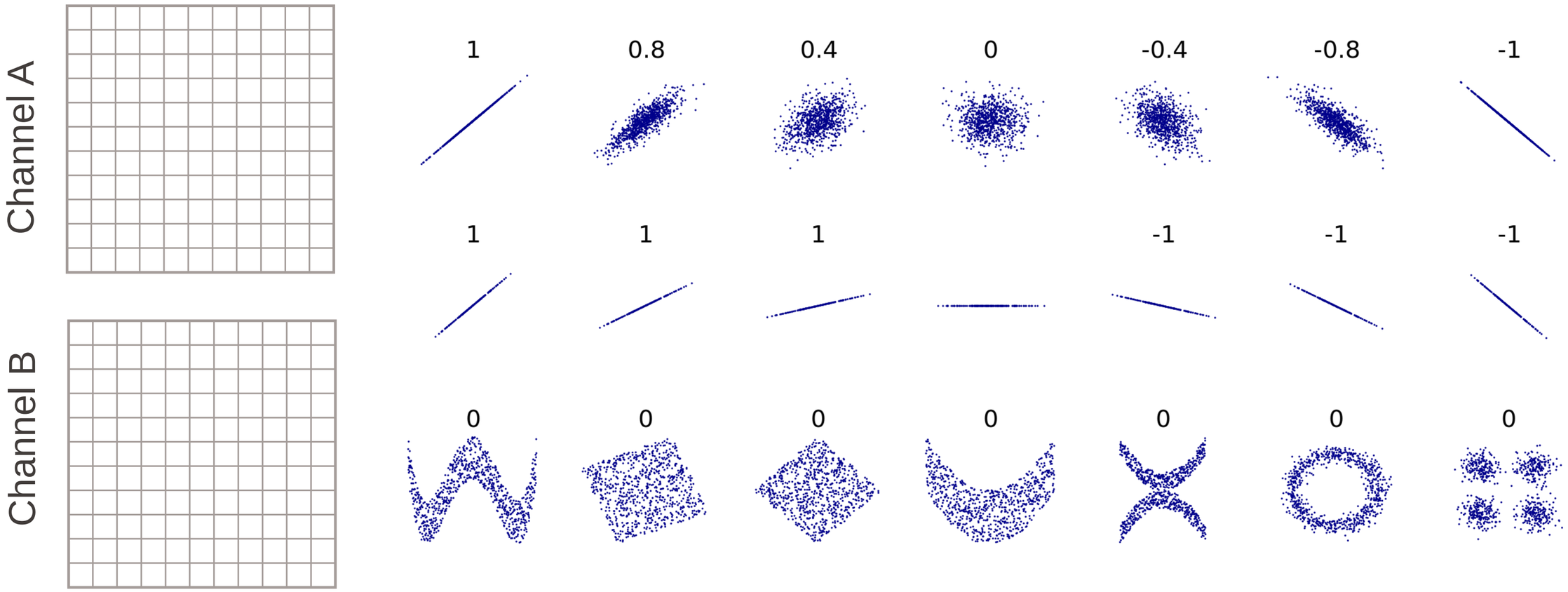
Channel Statistics



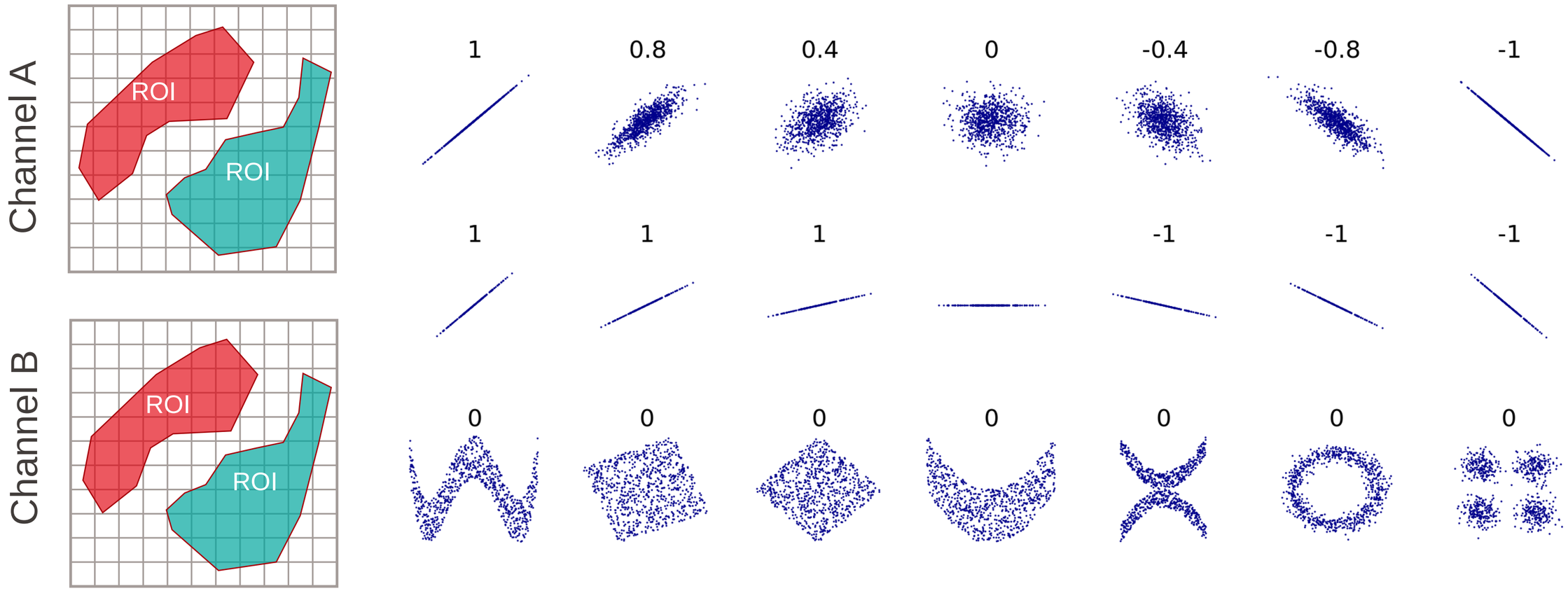
Ch A	Ch B
5	4
7	12
3	18
…	…
…	…
…	…
…	…



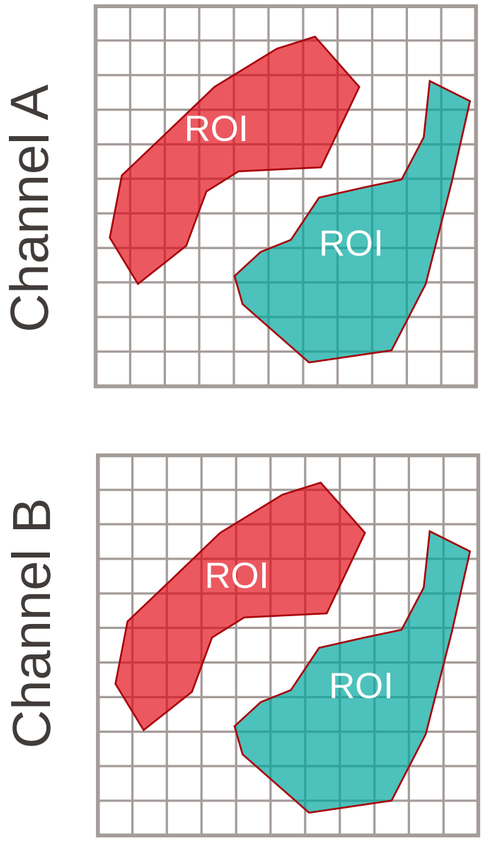
Pearson Correlation Coefficient



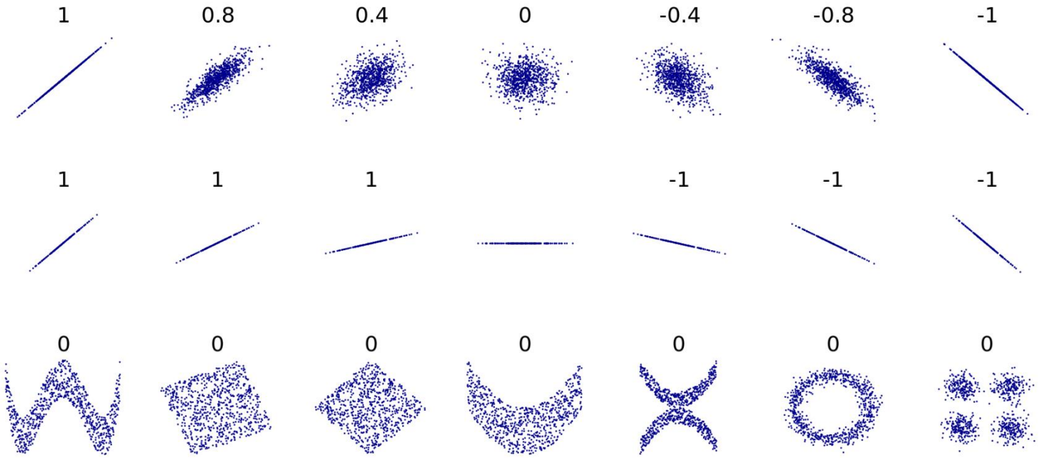
Pearson Correlation Coefficient



Pearson Correlation Coefficient



ROI	PCC
1	0.8
2	0.5
3	0.7



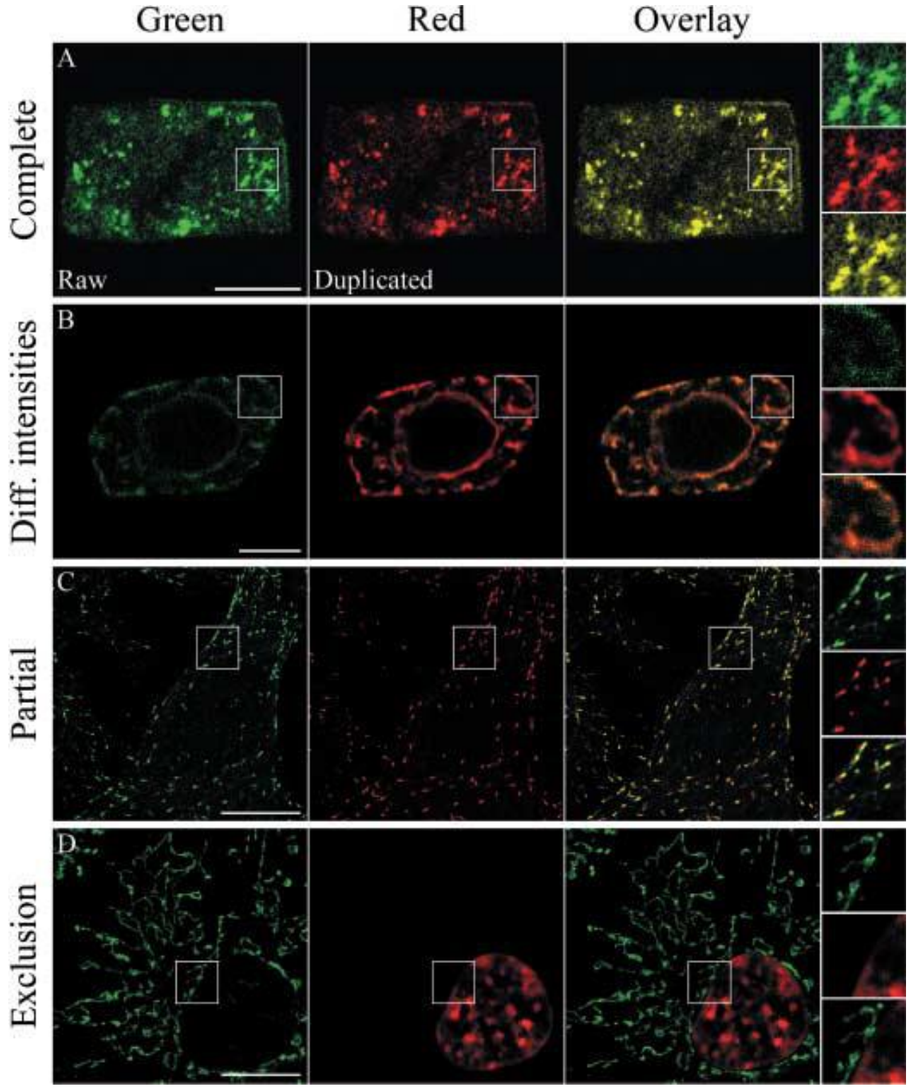
Colocalization Indicators

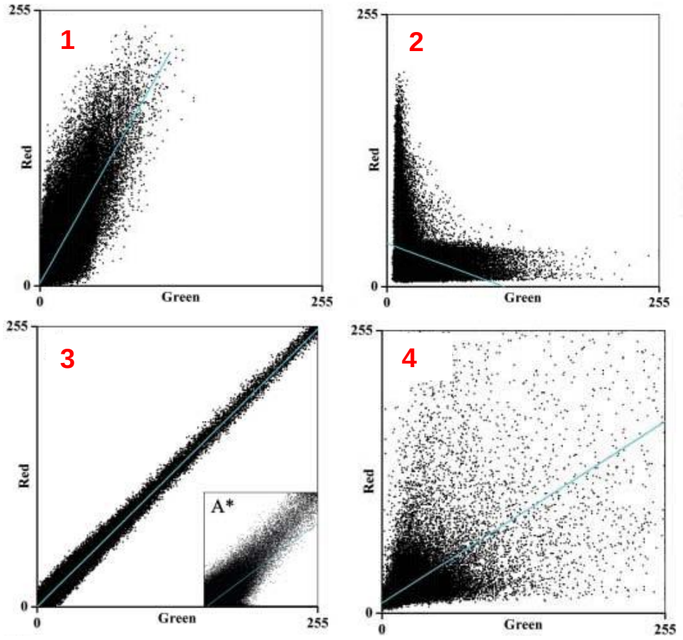
Pearson coefficient

$$PCC = \frac{\sum(R_i - \overline{R}) \times (G_i - \overline{G})}{\sqrt{\sum(R_i - \overline{R})^2 \times \sum(G_i - \overline{G})^2}}$$

Analysis

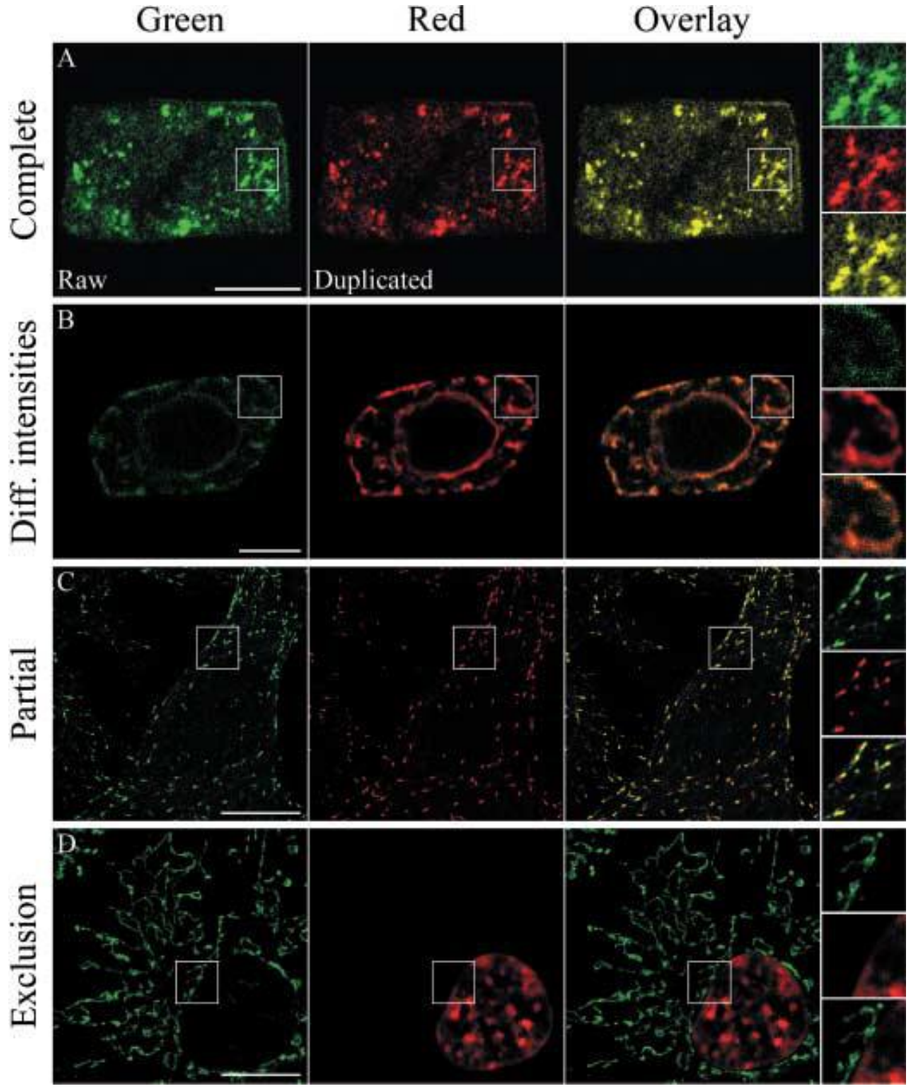
Which scatterplot belongs to which image sequence?





Analysis

Which scatterplot belongs to which image sequence?

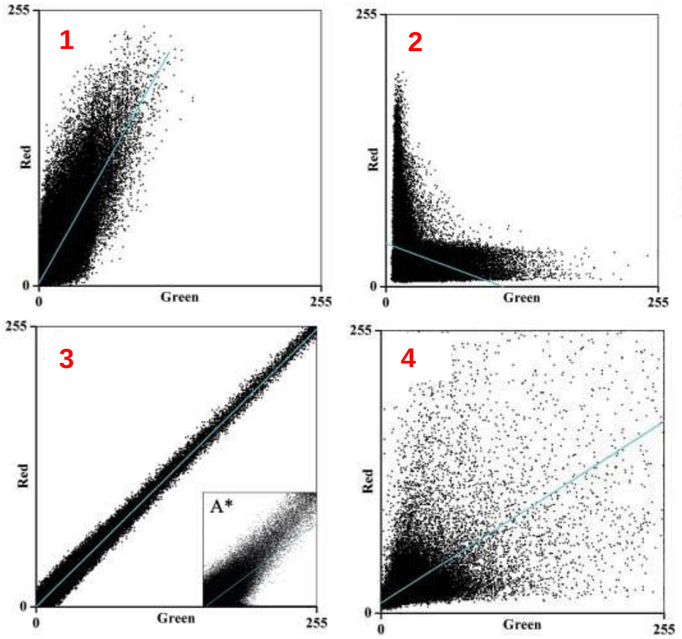


A 3

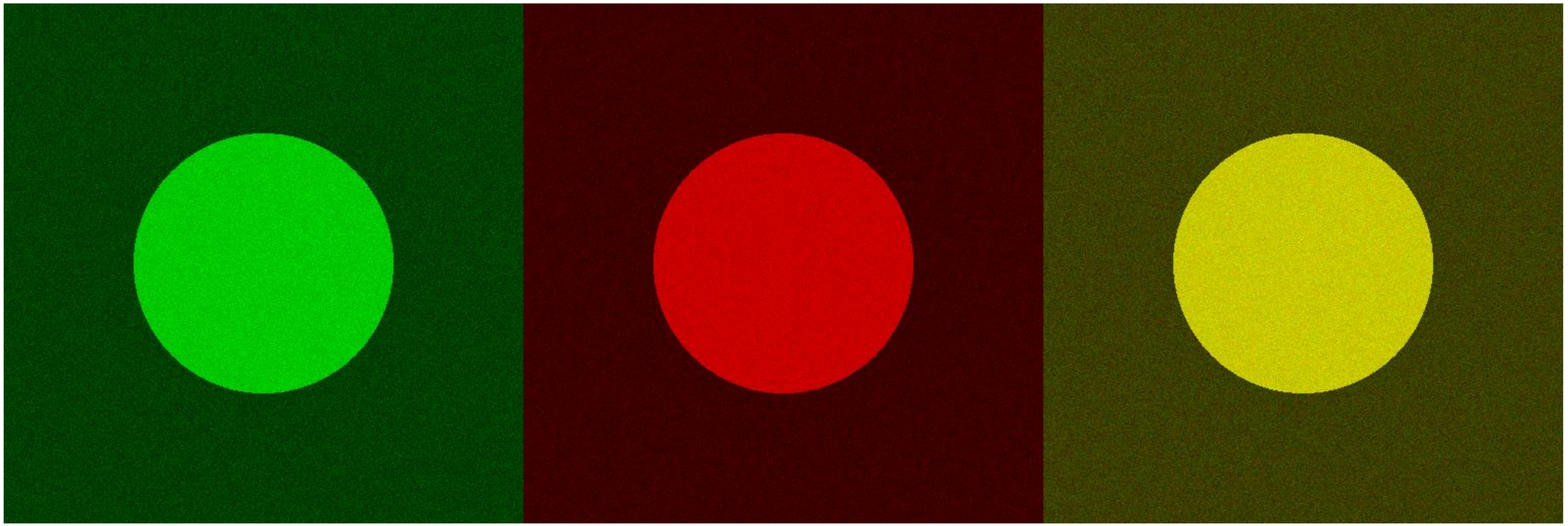
B 1

C 4

D 2

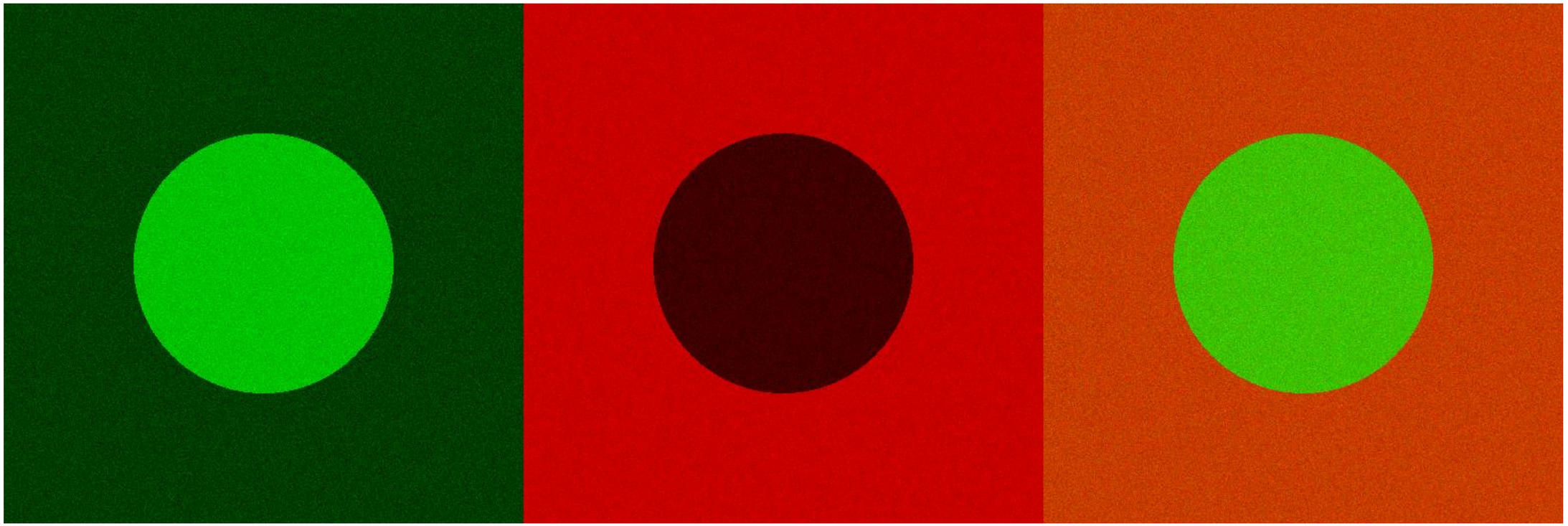


Correlation



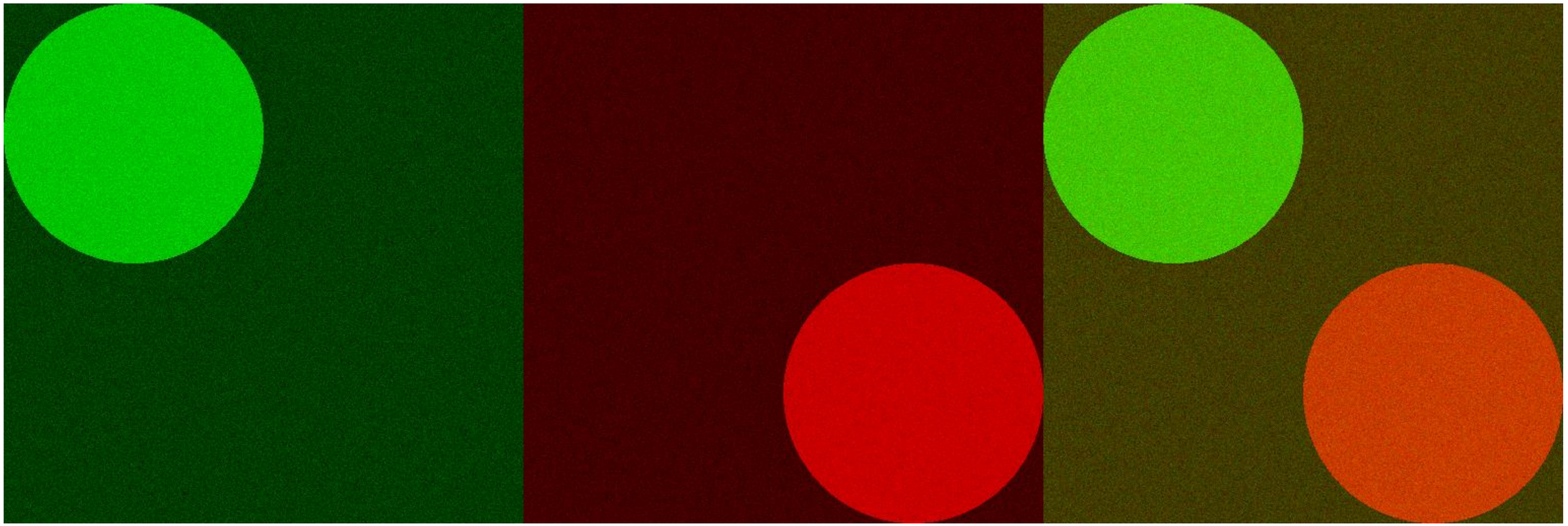
Pearson’s Correlation Coefficient: 0.94

AntiCorrelation



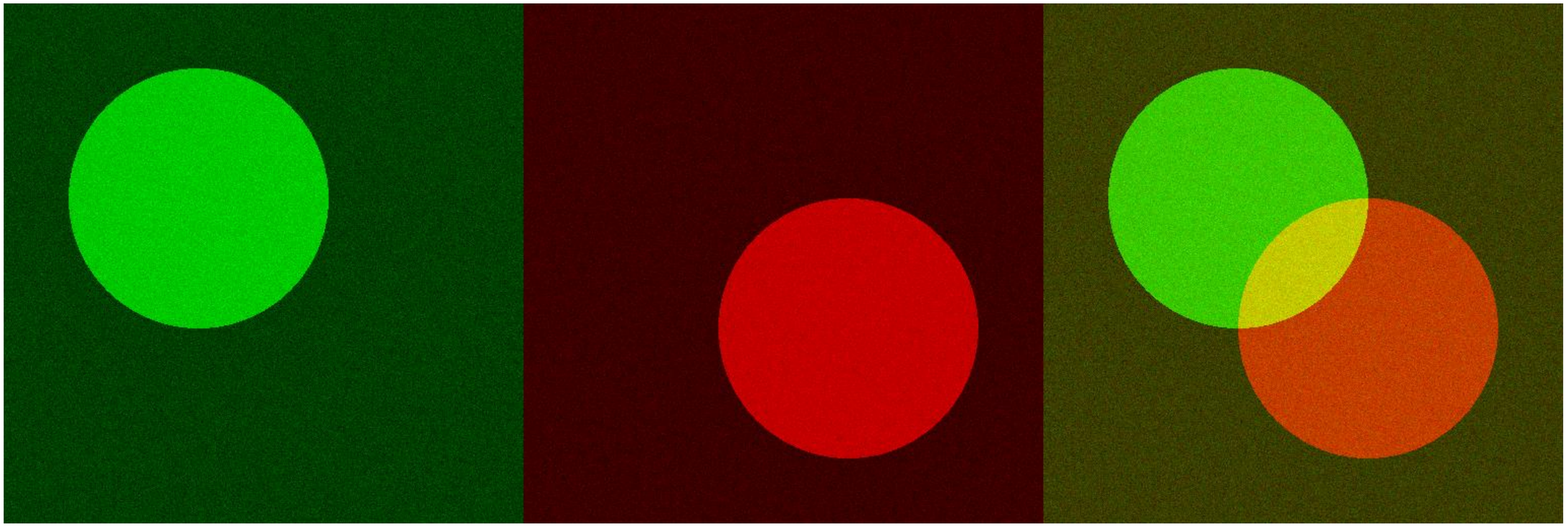
Pearson’s Correlation Coefficient: -0.94

Exclusion



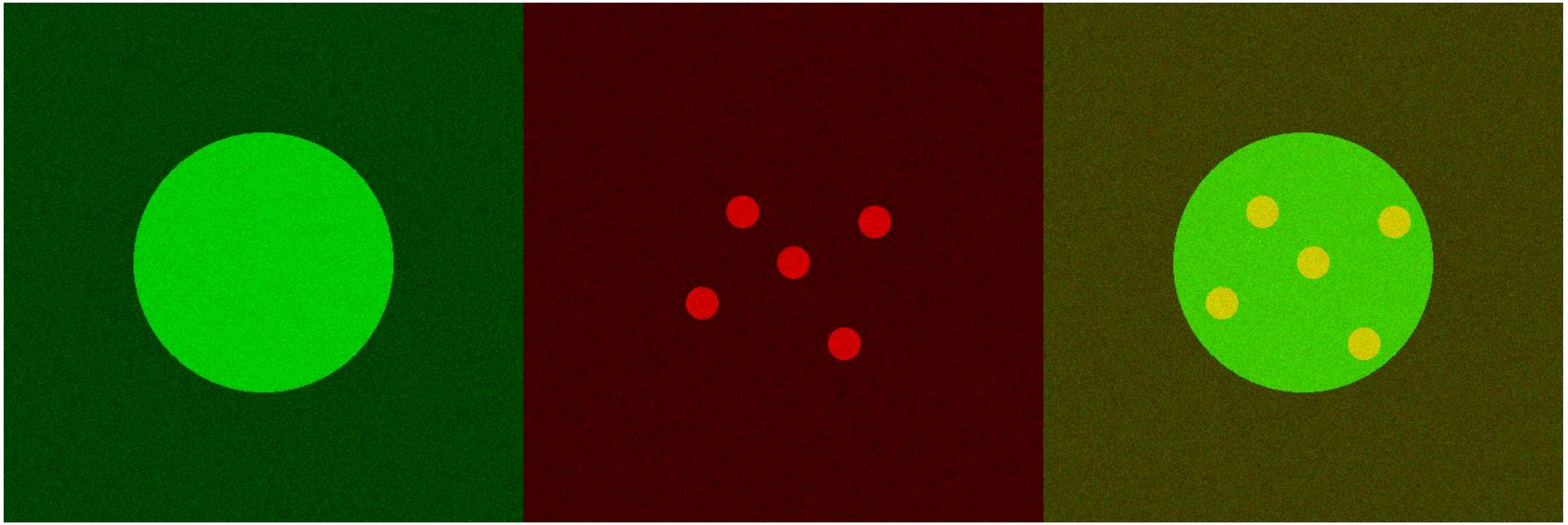
Pearson’s Correlation Coefficient: -0.29

Partial Overlap



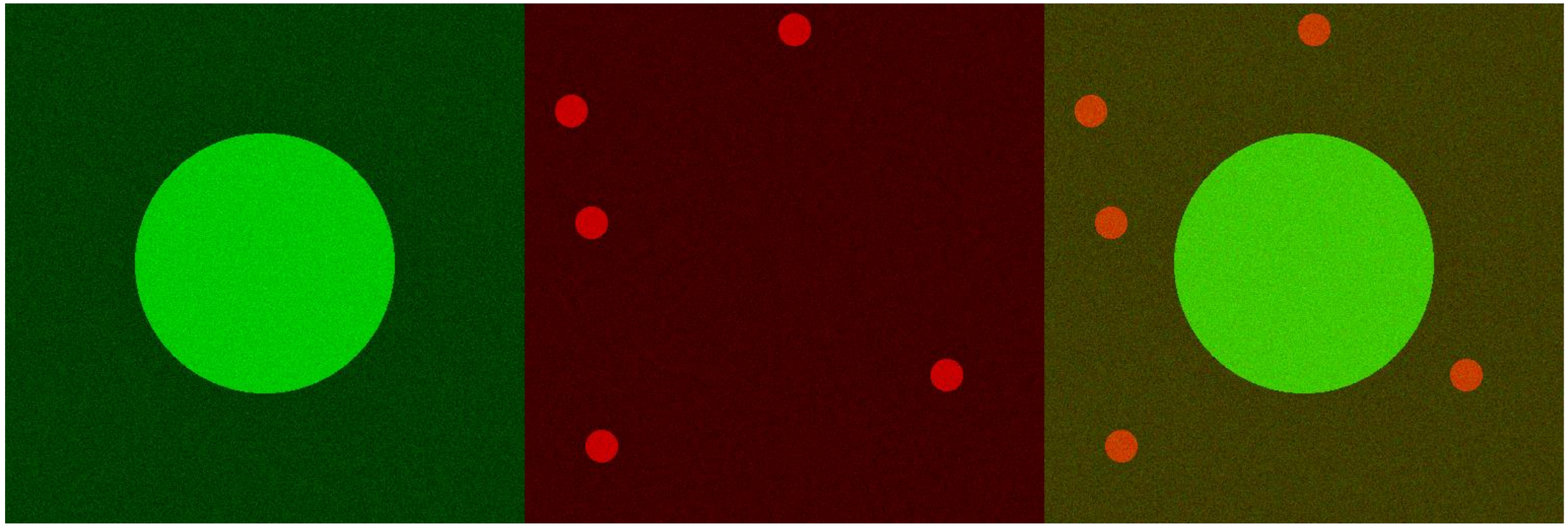
Pearson’s Correlation Coefficient: -0.016

Big/Small Colocalization



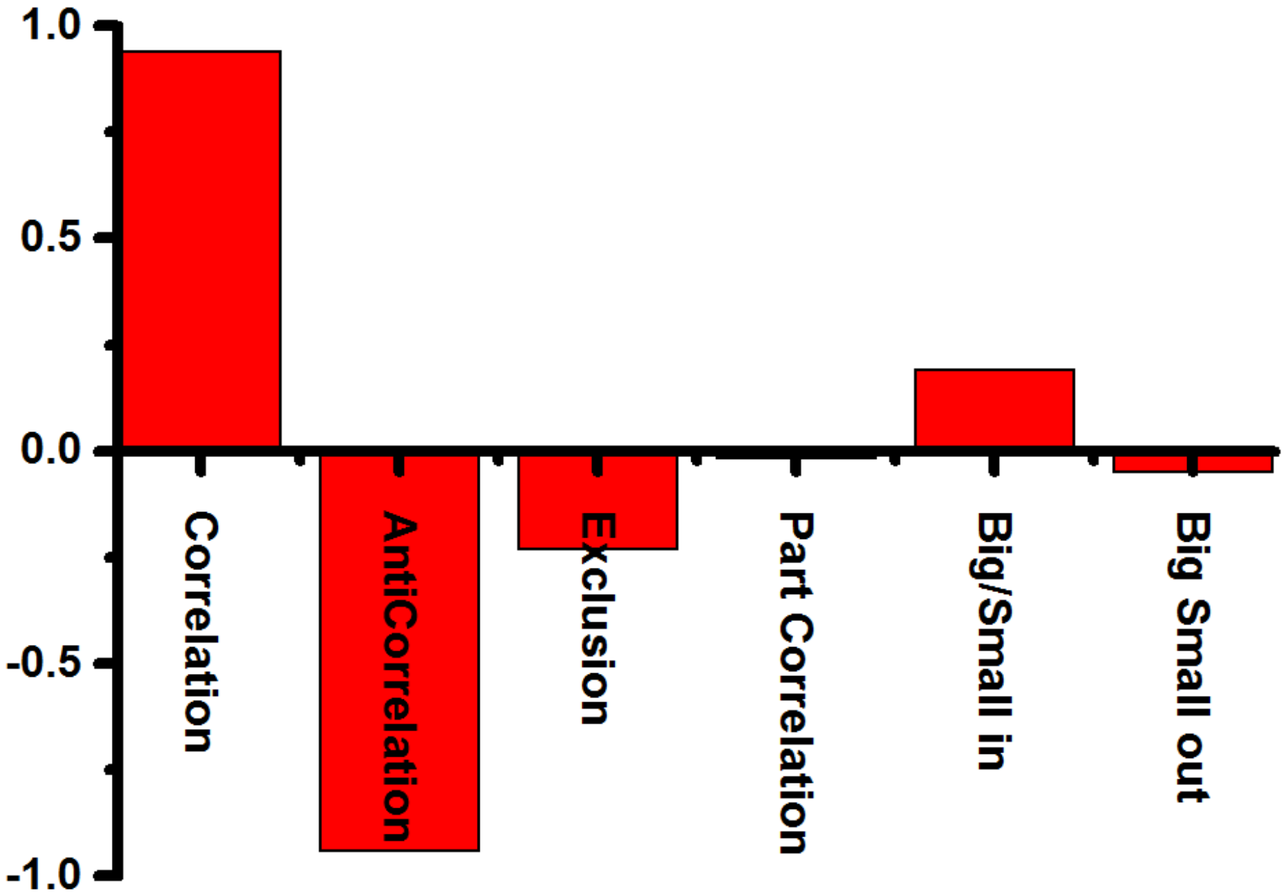
Pearson’s Correlation Coefficient: 0.19

Big/Small Exclusion

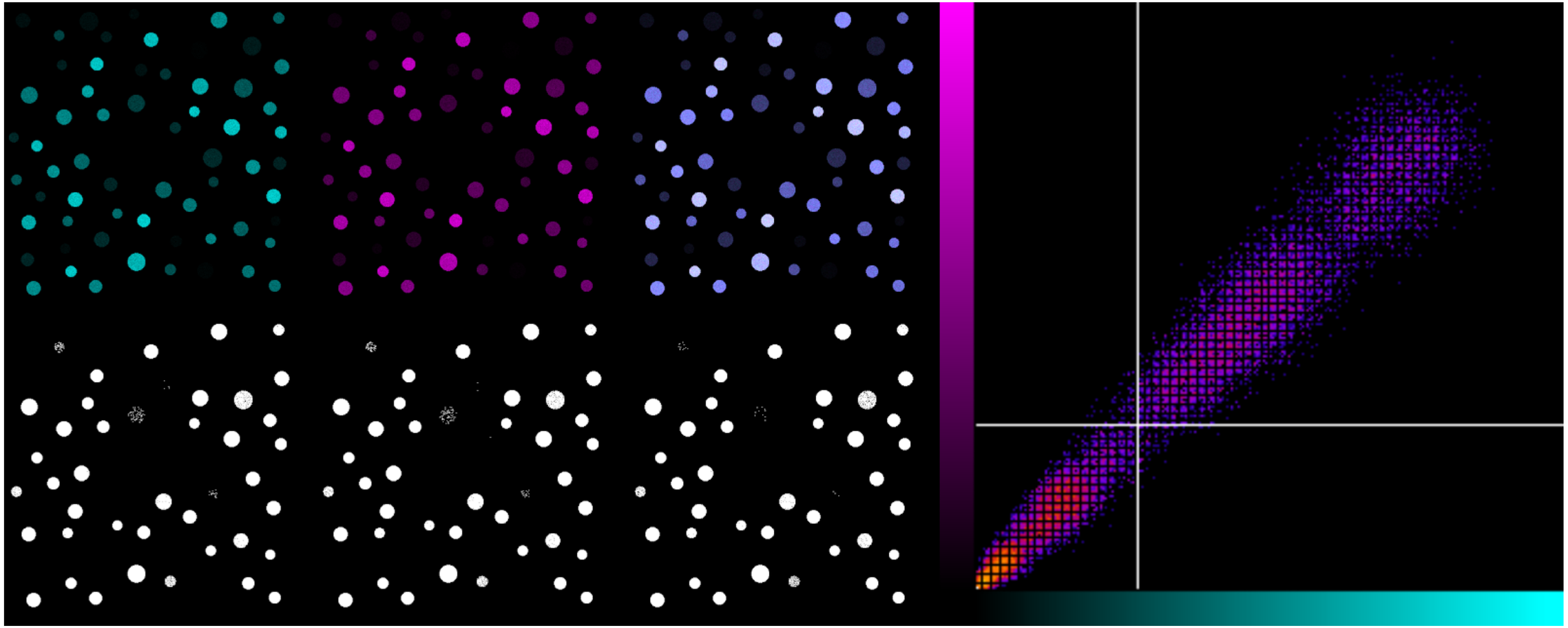


Pearson’s Correlation Coefficient: -0.047

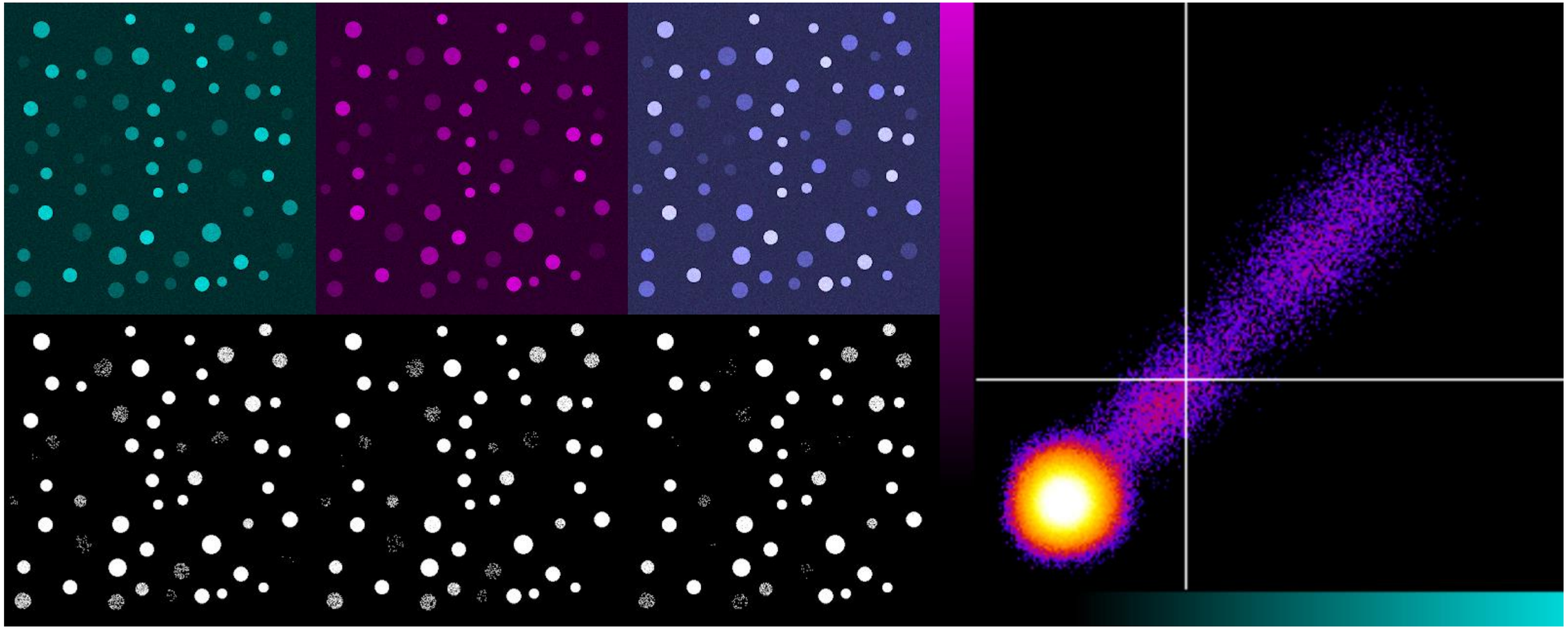
Pearson coefficient



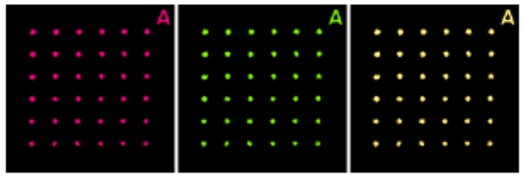
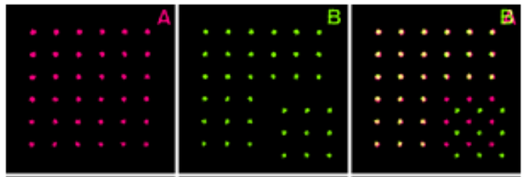
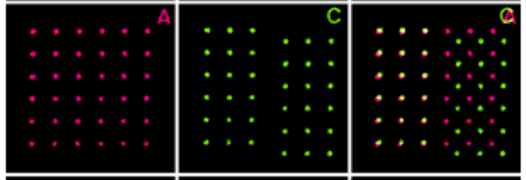
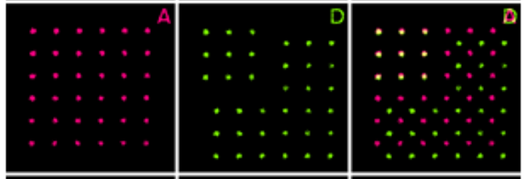
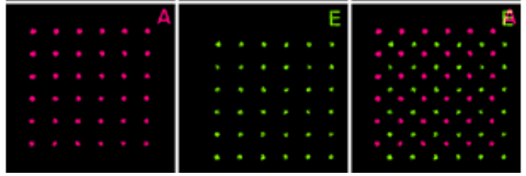
PCC limitation



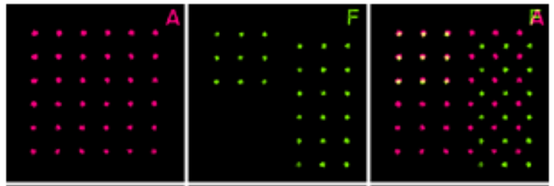
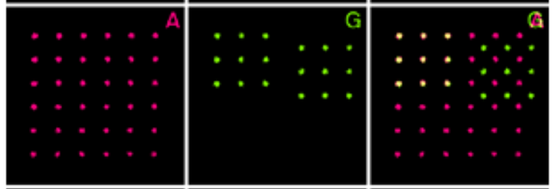
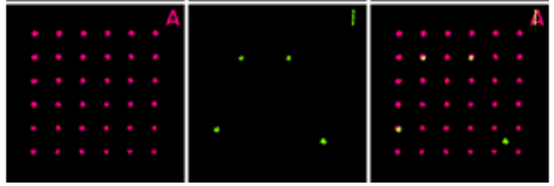
PCC limitation



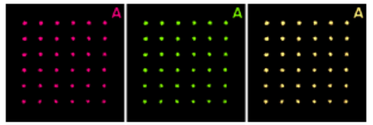
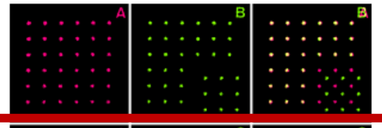
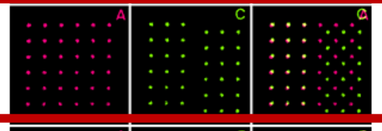
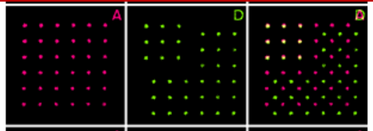
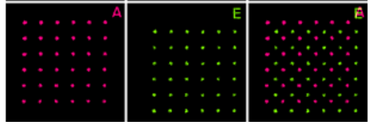
PCC limitation

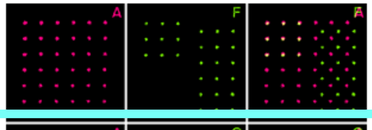
Case	Ch. 1	Ch.2	Merge	Spots Number			Pearson
				Ch. 1	Ch. 2	Co-loc.	
AA				36	36	36	1
AB				36	36	27	0.72
AC				36	36	18	0.44
AD				36	36	9	0.16
AE				36	36	0	-0.12

PCC limitation

Case	Ch. 1	Ch.2	Merge	Spots Number			Pearson
				Ch. 1	Ch. 2	Co-loc.	
AF				36	27	9	0.22
AG				36	18	9	0.30
AH				36	9	9	0.48
AI				36	4	3	0.23

PCC limitation

Case	Ch. 1	Ch.2	Merge	Spots Number Ch. 1	Spots Number Ch. 2	Spots Number Co-loc.	Pearson
AA				36	36	36	1
AB				36	36	27	0.72
AC				36	36	18	0.44
AD				36	36	9	0.16
AE				36	36	0	-0.12

Case	Ch. 1	Ch.2	Merge	Spots Number Ch. 1	Spots Number Ch. 2	Spots Number Co-loc.	Pearson
AF				36	27	9	0.22
AG				36	18	9	0.30
AH				36	9	9	0.48
AI				36	4	3	0.23

Colocalization Indicators

$$PCC = \frac{\sum(R_i - \overline{R}) \times (G_i - \overline{G})}{\sqrt{\sum(R_i - \overline{R})^2 \times \sum(G_i - \overline{G})^2}}$$

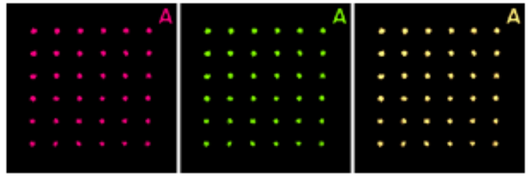
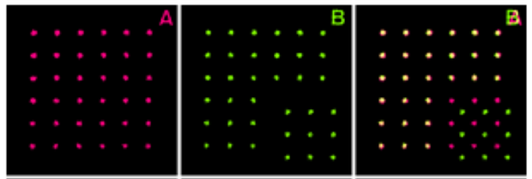
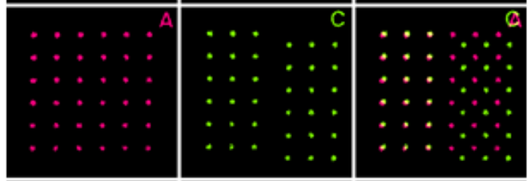
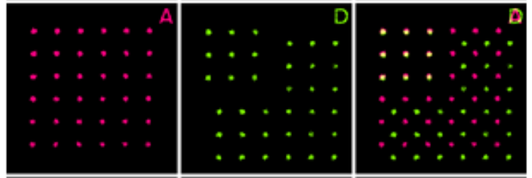
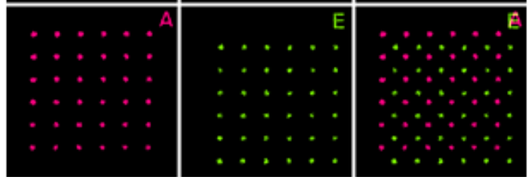
$$overlap = \frac{\sum R_i \times G_i}{\sqrt{\sum {R_i}^2 \times \sum {G_i}^2}} \qquad k_1 = \frac{\sum R_i \times G_i}{\sqrt{\sum {R_i}^2}}; k_2 = \frac{\sum R_i \times G_i}{\sqrt{\sum {G_i}^2}}$$

R_i, G_i: *intensity of the pixel i in image R and G respectively*
$\overline{R}, \overline{G}$: *average intensity of the image R and G respectively*

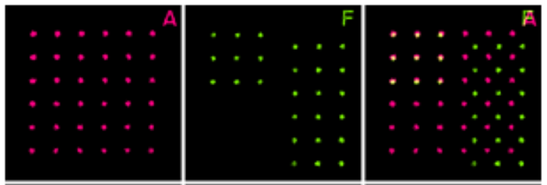
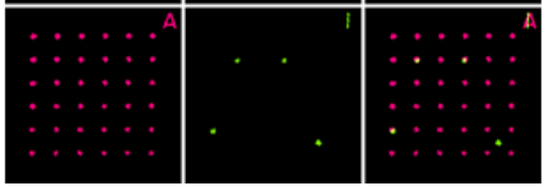
$$M_1 = \frac{\sum R_{i,coloc}}{\sum R_i}; M_2 = \frac{\sum G_{i,coloc}}{\sum G_i}$$

$$R_{i,coloc} = R_i \quad if\ G_i > 0$$
$$G_{i,coloc} = G_i \quad if\ R_i > 0$$

Mander's Coeeficient

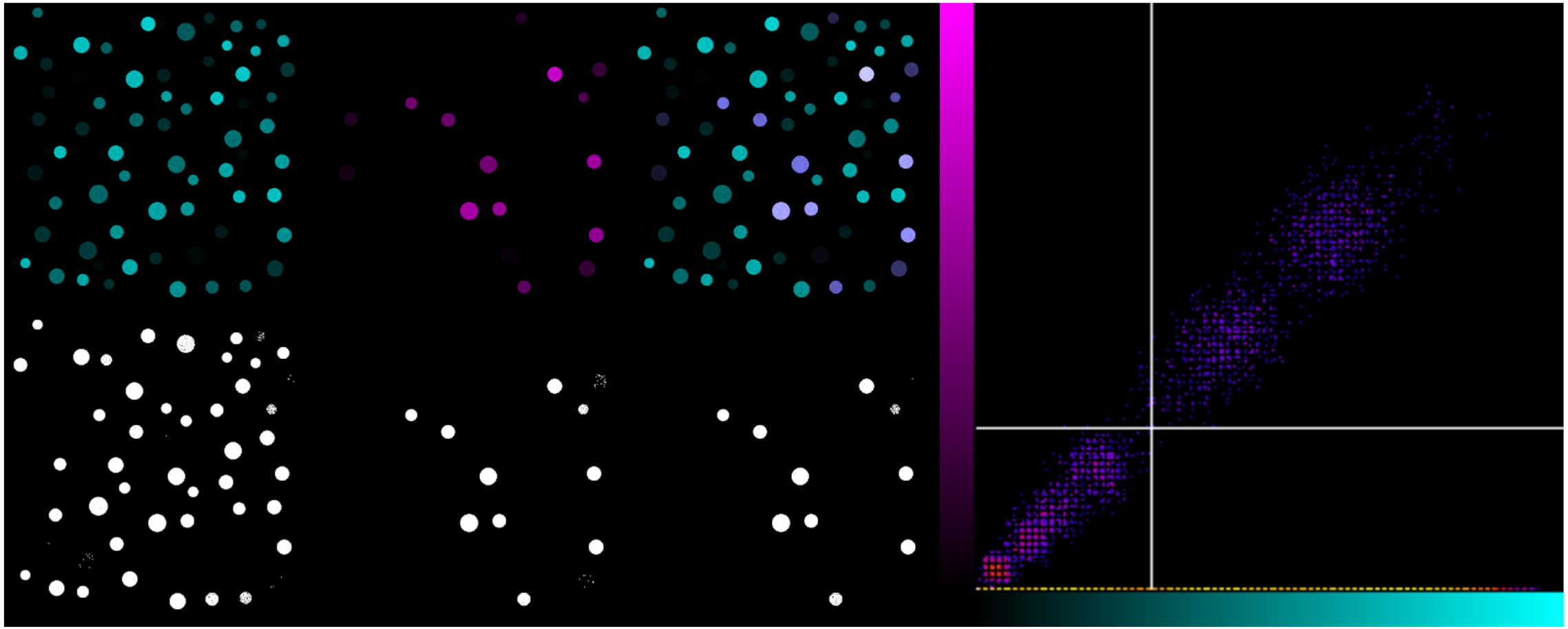
Case	Ch. 1	Ch.2	Merge	Spots Number			Pearson	Mander	
				Ch. 1	Ch. 2	Co-loc.		Ch. 1 = M1	Ch. 2 = M2
AA				36	36	36	1	1	1
AB				36	36	27	0.72	0.75	0.75
AC				36	36	18	0.44	0.5	0.5
AD				36	36	9	0.16	0.25	0.25
AE				36	36	0	-0.12	0	0

Mander's Coeeficient

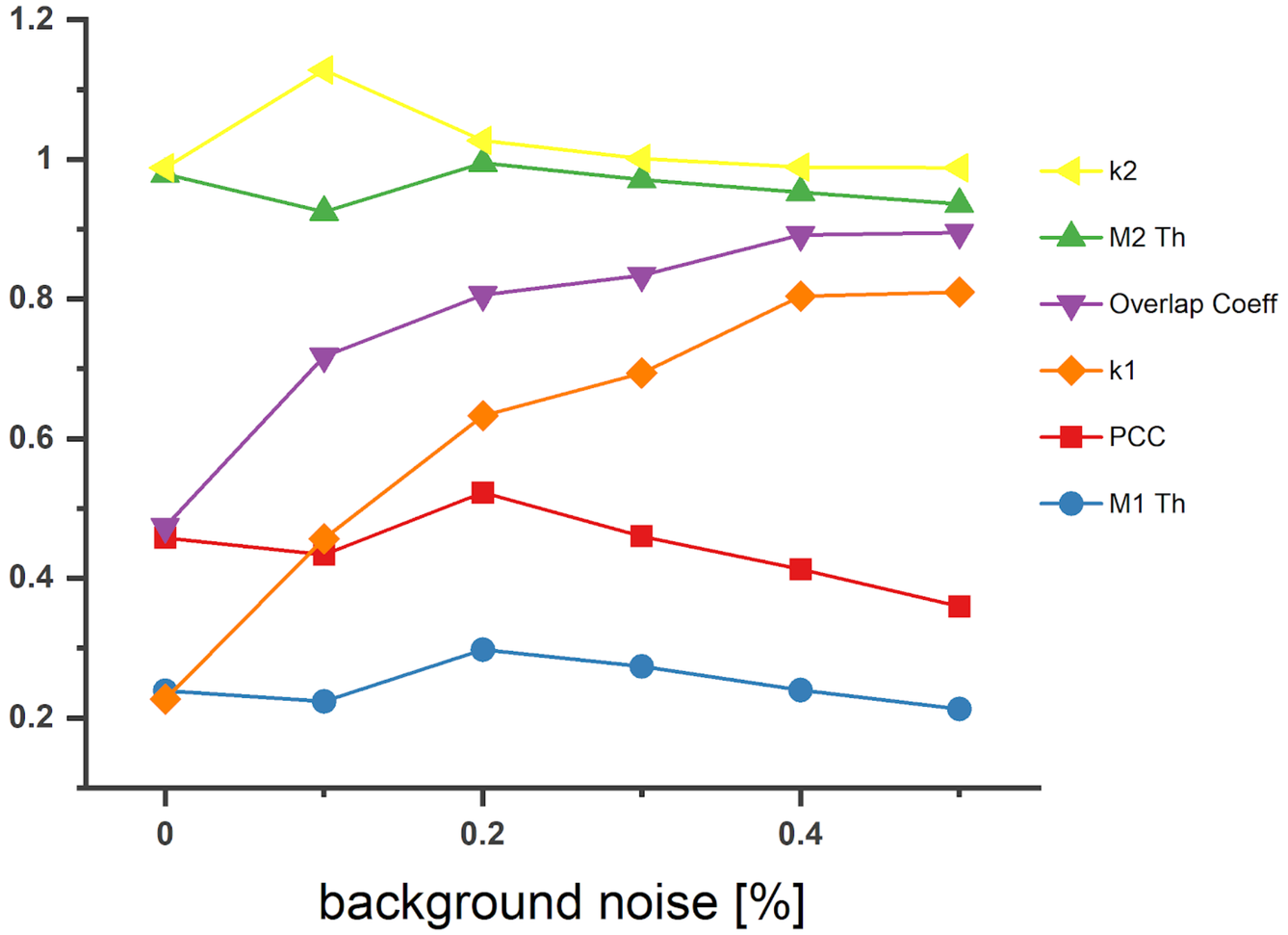
Case	Ch. 1	Ch.2	Merge	Spots Number			Pearson	Mander	
				Ch. 1	Ch. 2	Co-loc.		Ch. 1 = M1	Ch. 2 = M2
AF				36	27	9	0.22	0.25	0.33
AG				36	18	9	0.30	0.25	0.50
AH				36	9	9	0.48	0.25	1.0
AI				36	4	3	0.23	0.08	0.75

Colocalization and noise

PCC: 0.49 M_1:0.239 M_2:0.979



Colocalization and noise

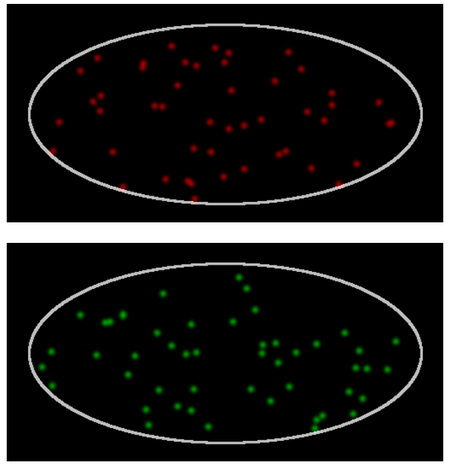


Pixel based Colocalization

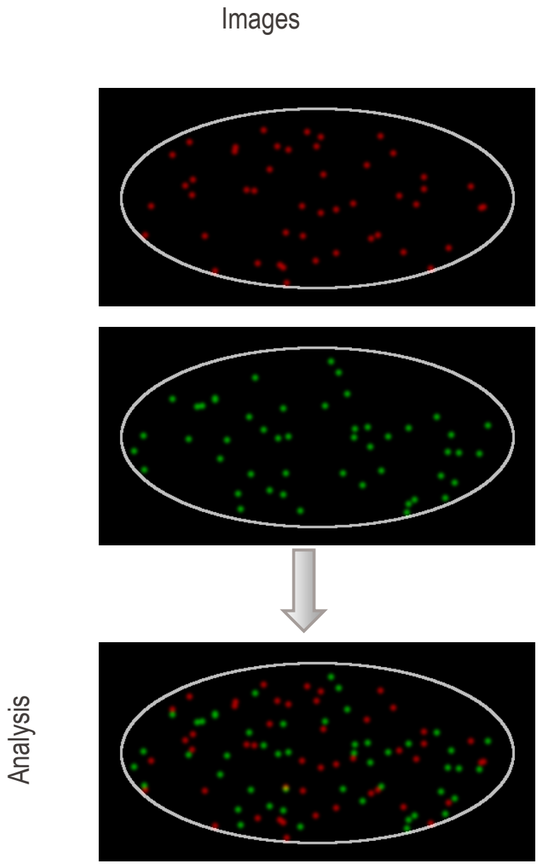
- Two different molecules can never be at the same physical spot at the same time.
- Colocalization seen in images is coming from the low-pass filtering of the image formation in light microscopy.
- Colocalization is an artificial phenomenon and therefore always relative (e.g. it scales with noise, resolution, object size).
- The coefficient used needs to fit your experiment.
- Coefficients are well suited for cell-based analyses.

Colocalisation Workflow

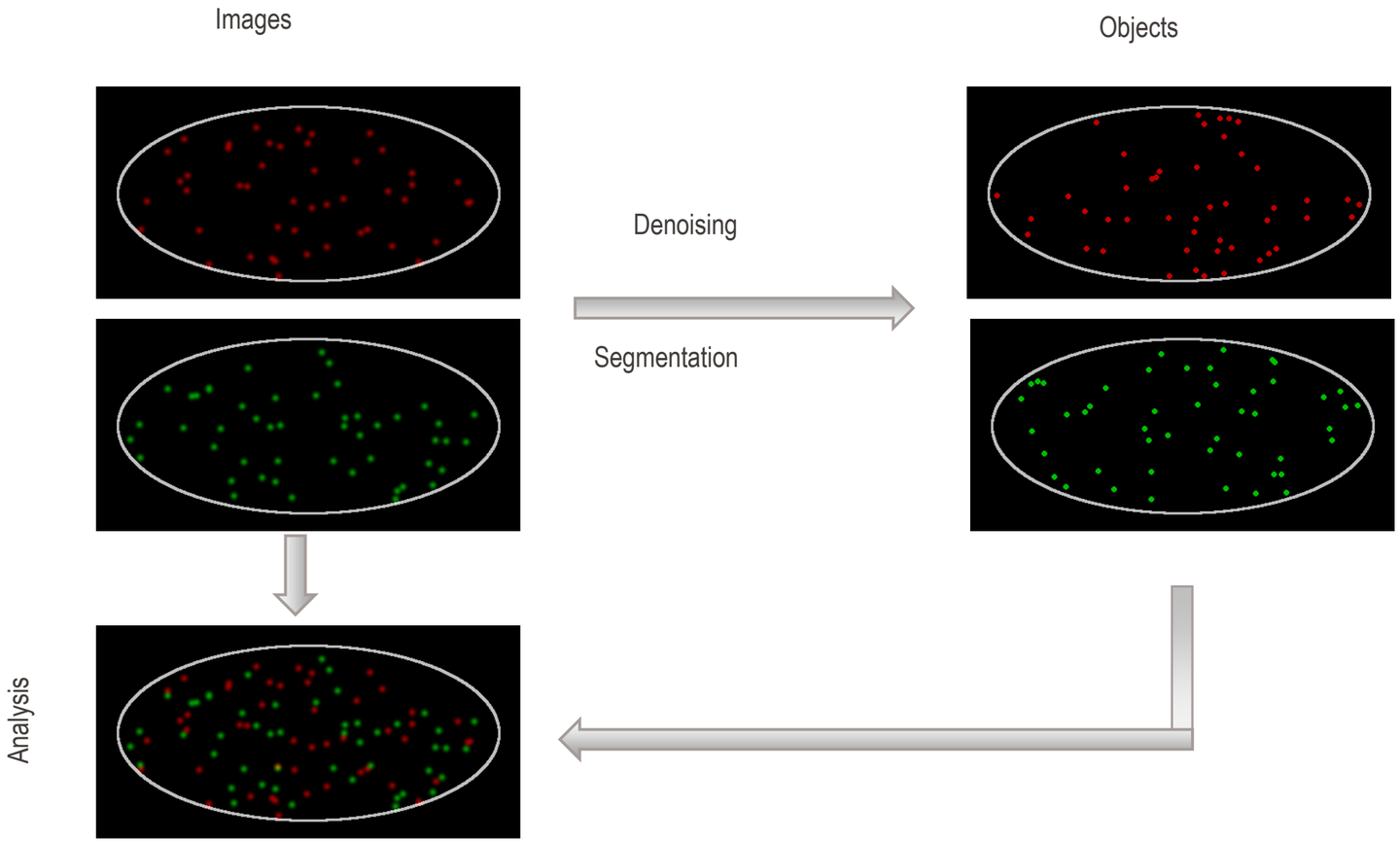
Images



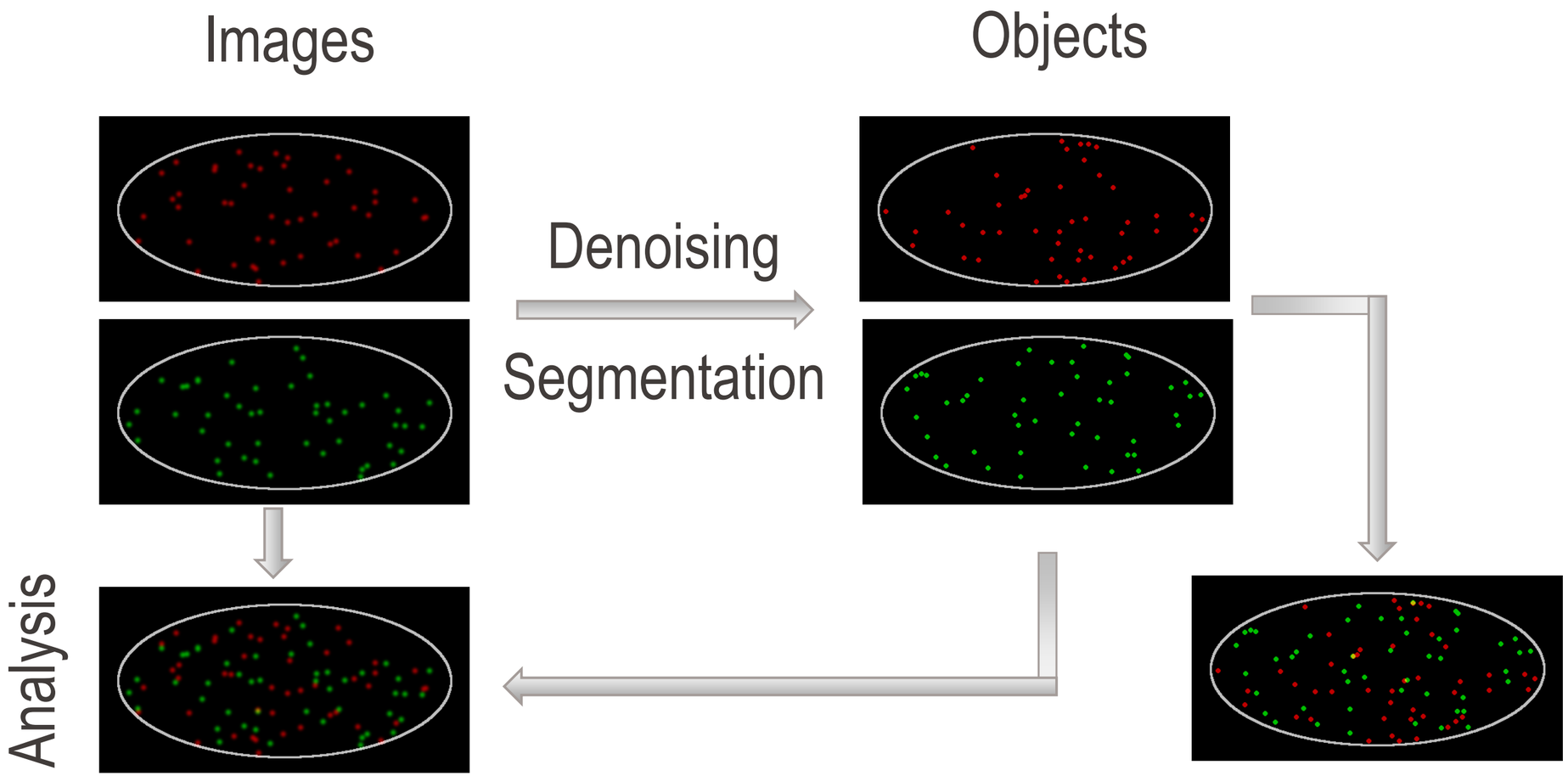
Colocalisation Workflow



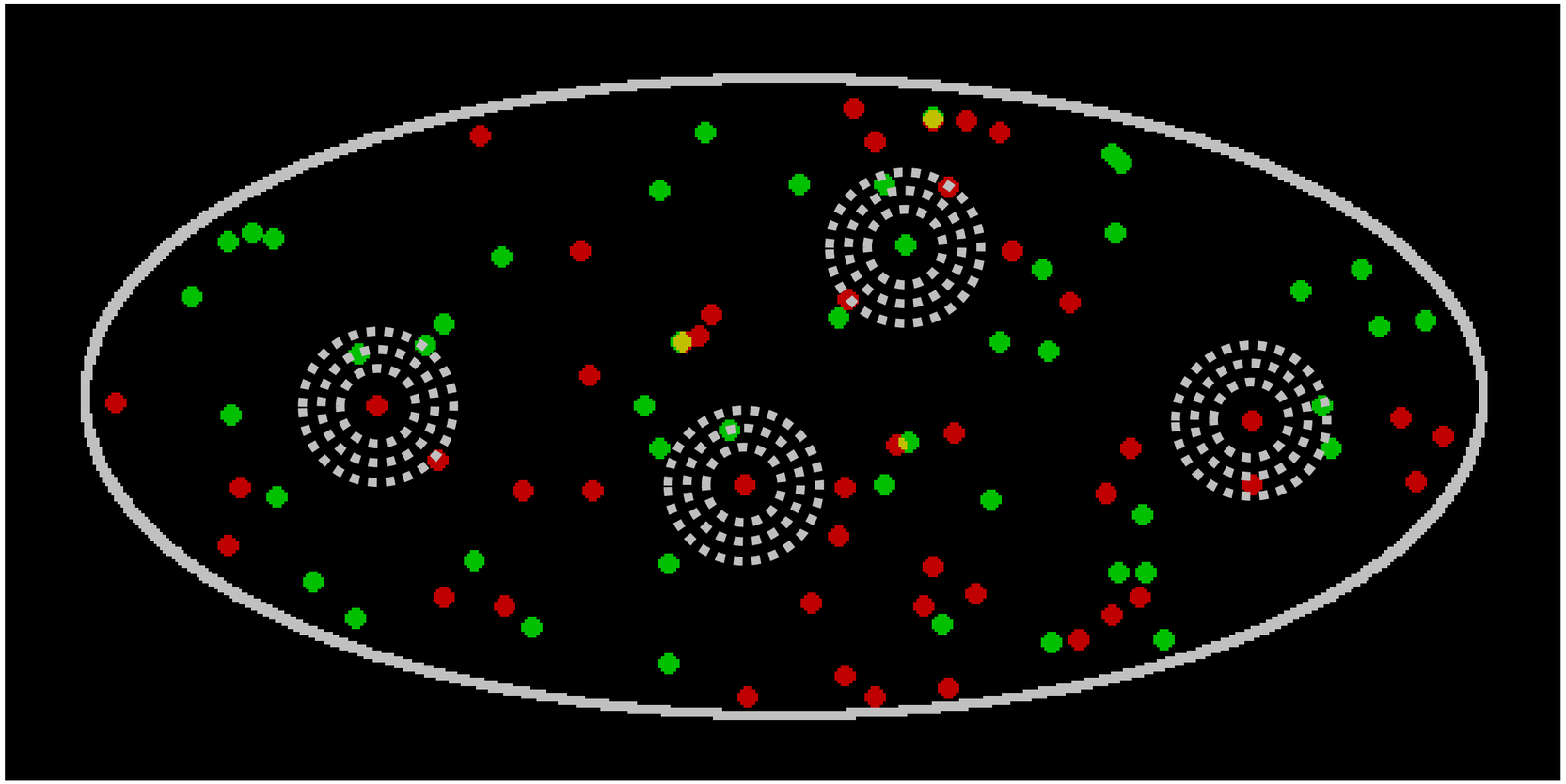
Colocalisation Workflow



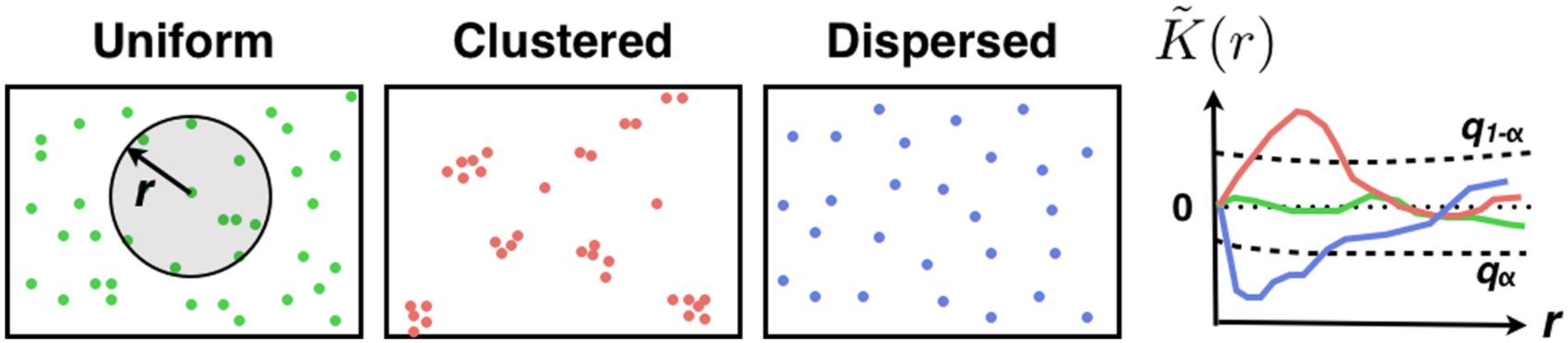
Object Based Colocalisation



Neighbourhood analysis



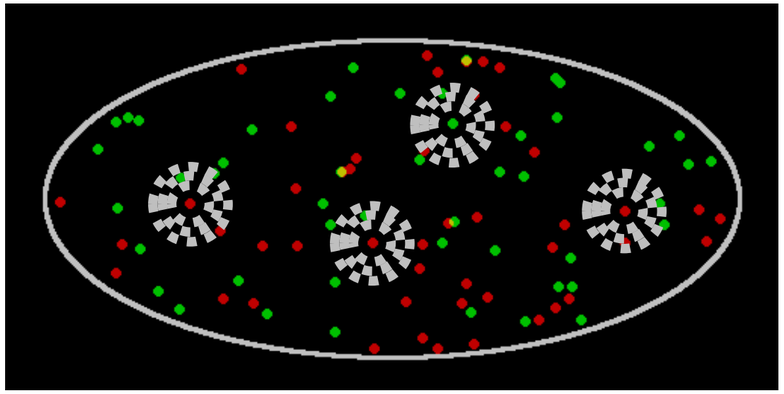
Ripley's K-function

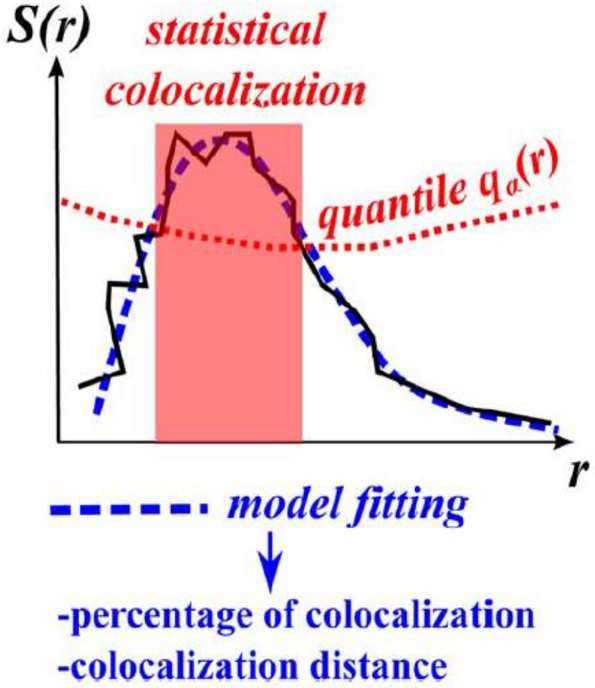


Lagache T, Lang G, Sauvonnet N, Olivo-Marin JC.
PLoS One. 2013 Dec 4;8(12):e80914. doi: 10.1371/journal.pone.0080914. eCollection 2013.

Neighbourhood analysis

- Nearest Neighbour
- Ripley's K-function

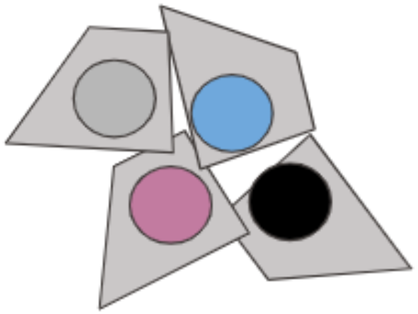




Lagache T, Sauvonnet N, Danglot L, Olivo-Marin JC.
Cytometry A. 2015 Jun;87(6):568-79. doi: 10.1002/cyto.a.22629.

Lagache T, Grassart A, Dallongeville S, Faklaris O, Sauvonnet N, Dufour A, Danglot L, Olivo-Marin JC.
Nat Commun. 2018 Feb 15;9(1):698. doi: 10.1038/s41467-018-03053-x.

Co-Localization Summary

Pixels	Object
Image Coefficient(s)	Features
	 
Pearson Correlation, Manders', ...	Intensities / Distances
Co-Occurrence Correlation	Co-Occurrence Co-Expression / Co-Distribution Pattern analysis

Colocalization Summary