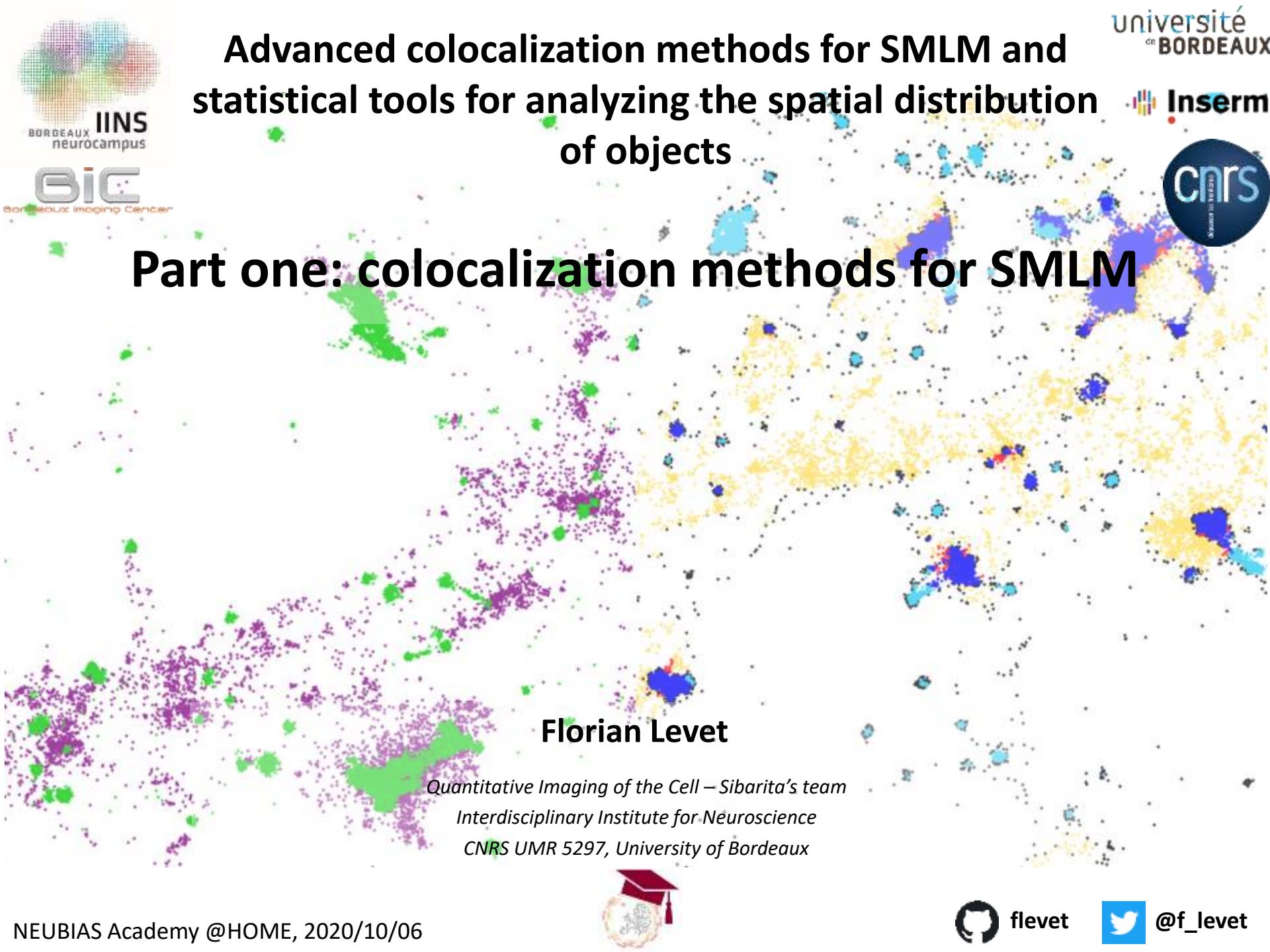


# Advanced colocalization methods for SMLM and statistical tools for analyzing the spatial distribution of objects

## Part one: colocalization methods for SMLM



Florian Levet

Quantitative Imaging of the Cell – Sibarita's team

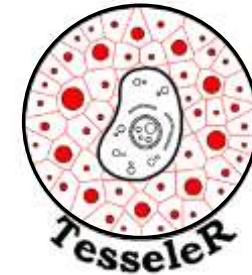
Interdisciplinary Institute for Neuroscience

CNRS UMR 5297, University of Bordeaux

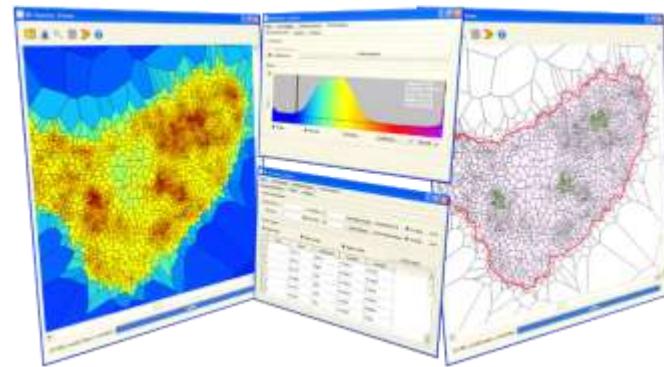


# Part one's outline

- Intro
- Unorganized neighboring methods
- Tessellation-based method
- Demos



Link to Coloc-Tesseler one-click installer (windows):  
<https://github.com/flevet/Coloc-Tesseler/releases>



Link to datasets and slides:  
[https://github.com/flevet/NEUBIAS\\_Academy](https://github.com/flevet/NEUBIAS_Academy)

## The speaker and moderator team:



Thibault Lagache



Daniel Sage



Lydia Danglot



Suvadip Mukherjee

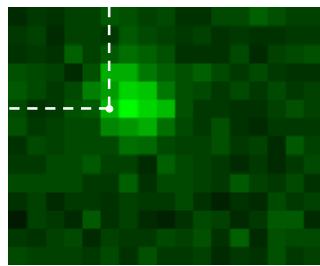
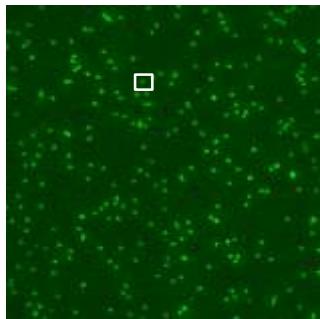
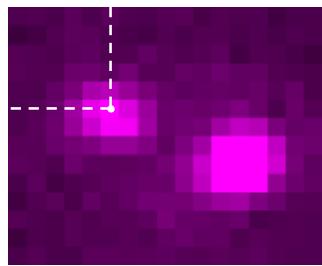
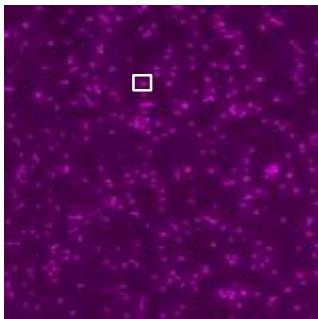


Rocco d'Antuono

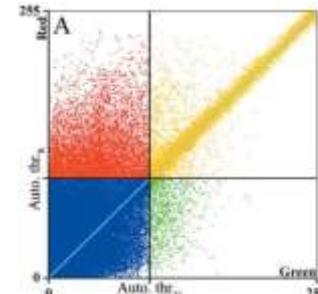
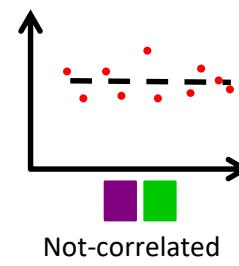
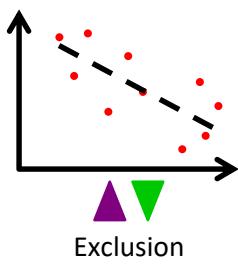
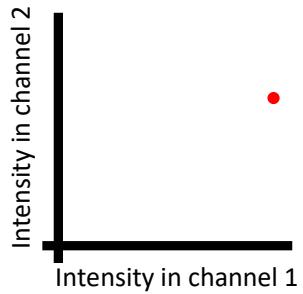


Anna Klemm

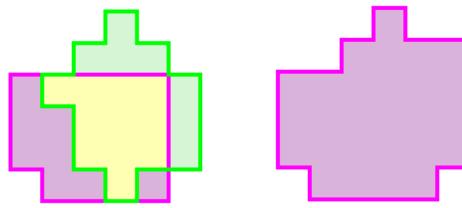
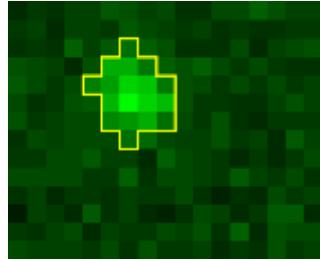
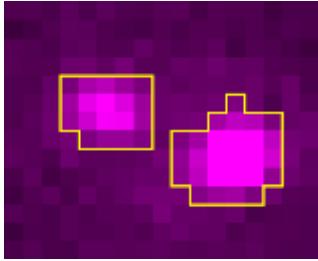
# Colocalization in fluorescence microscopy



Pearson's correlation coefficient (indicator)



Based on intensities' overlap



Manders' coefficients (quantifier)

$$M_1 = \frac{14}{46} = 0,3$$

$$M_2 = \frac{14}{18} = 0,77$$

$$M_1 = \frac{\sum_i A_{i,coloc}}{\sum_i A_i}$$

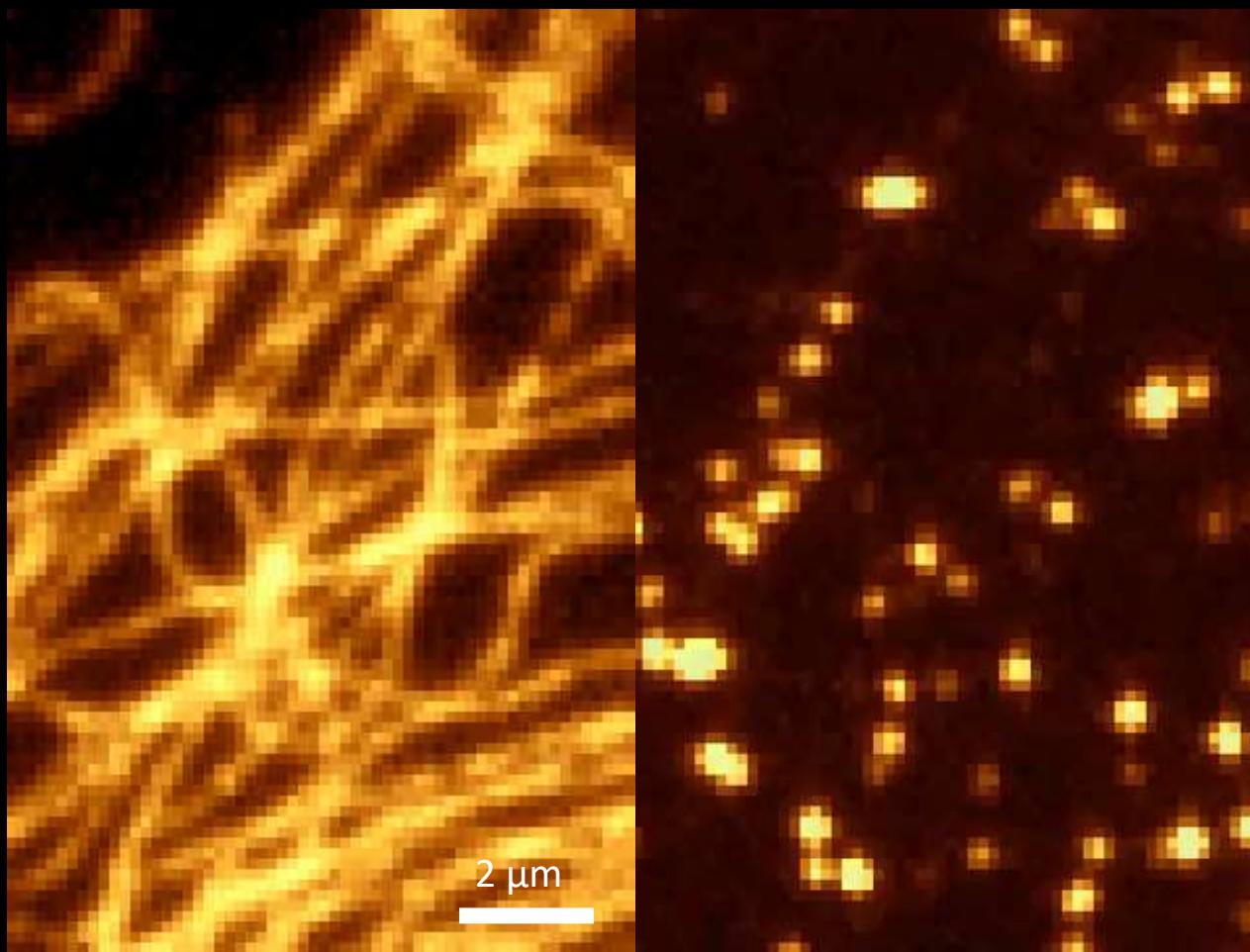
A diagram showing a yellow box (representing the area of colocalization) divided by a purple box (representing the total area of channel 1).

$$M_2 = \frac{\sum_i B_{i,coloc}}{\sum_i B_i}$$

A diagram showing a yellow box (representing the area of colocalization) divided by a green box (representing the total area of channel 2).

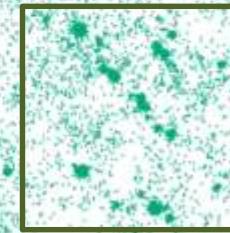
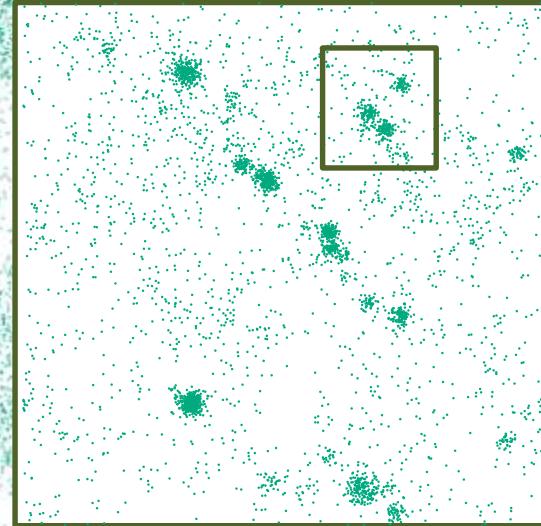
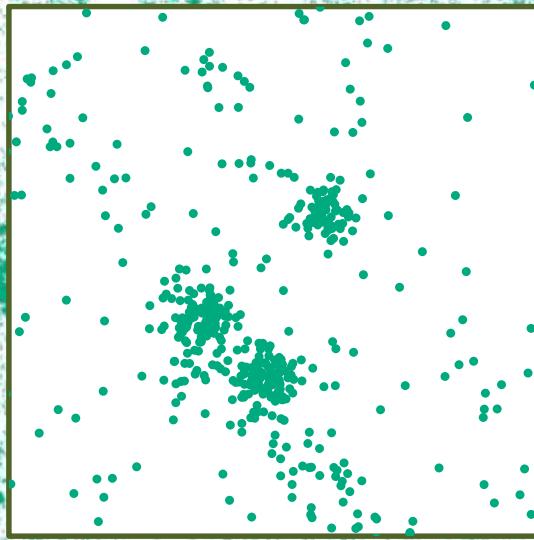
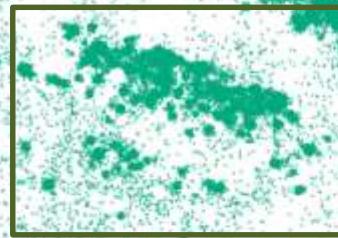
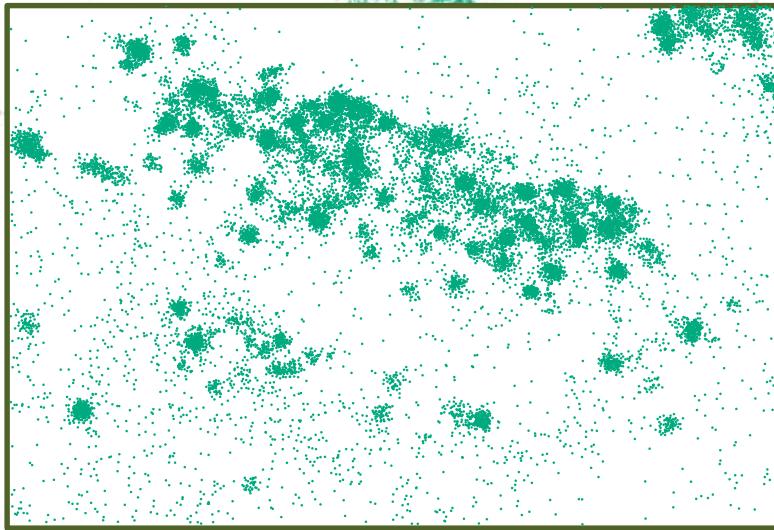
# Single-Molecule Localization Microscopy

A sparse subset of the fluorophores is switched on and localized each frame

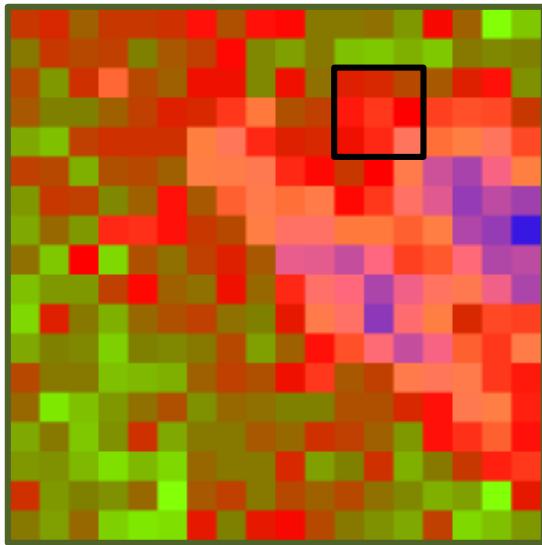


# How to use the localizations ?

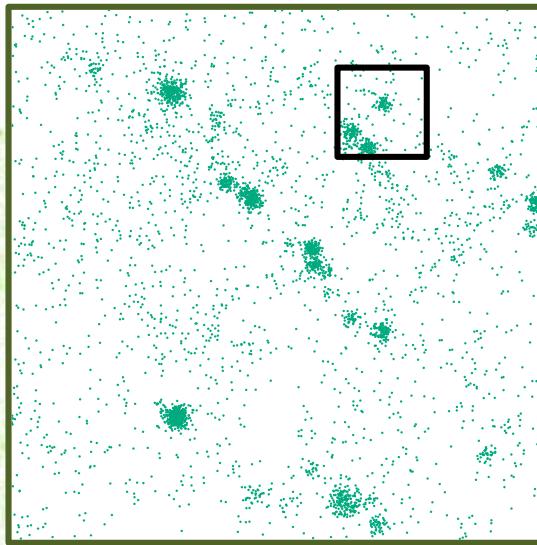
Fibroblast expressing integrin- $\beta 3$ -mEOS2



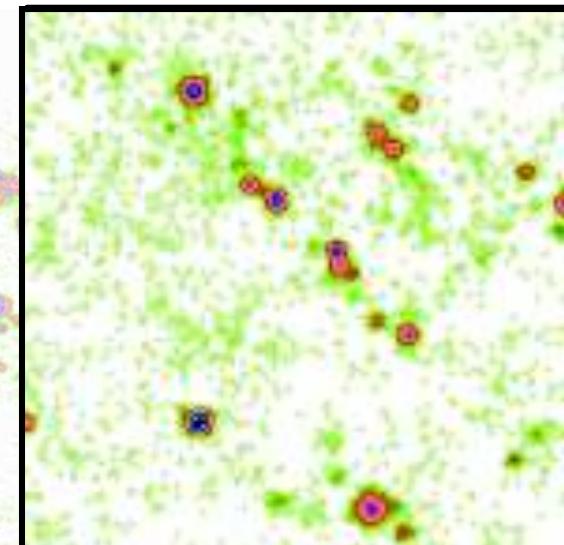
# Image reconstruction in SMLM



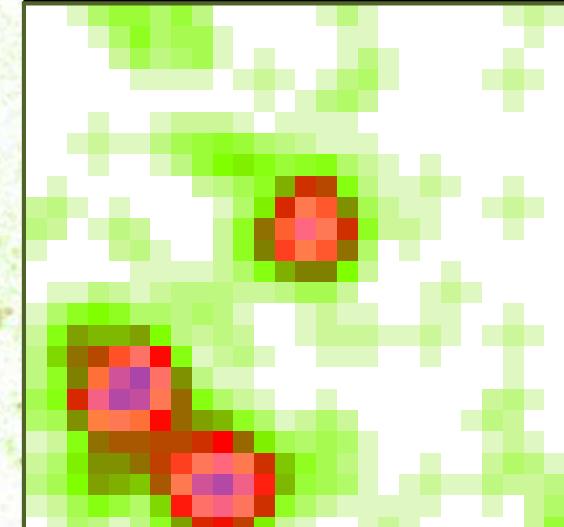
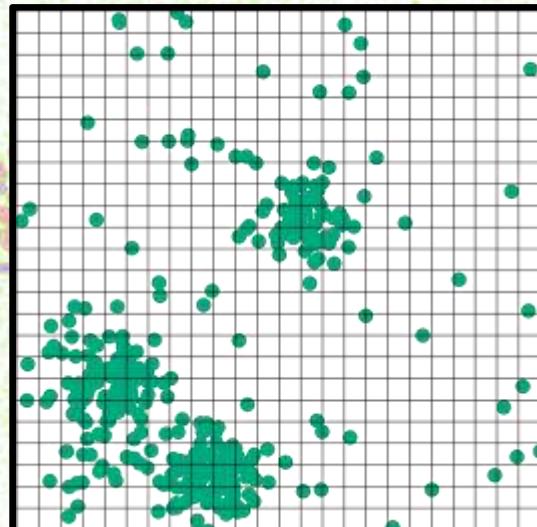
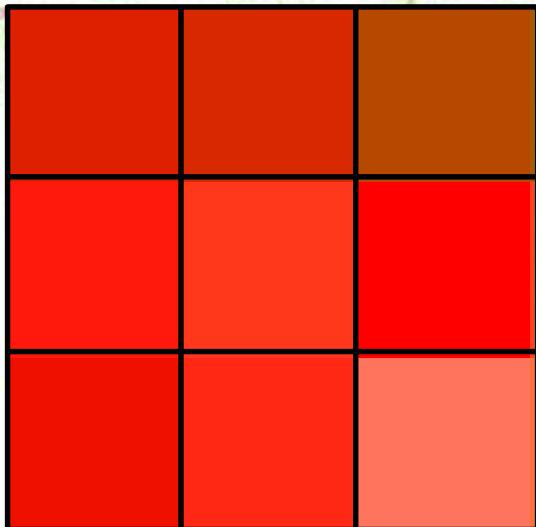
Pixel size = 160 nm



Sub-pixel localization

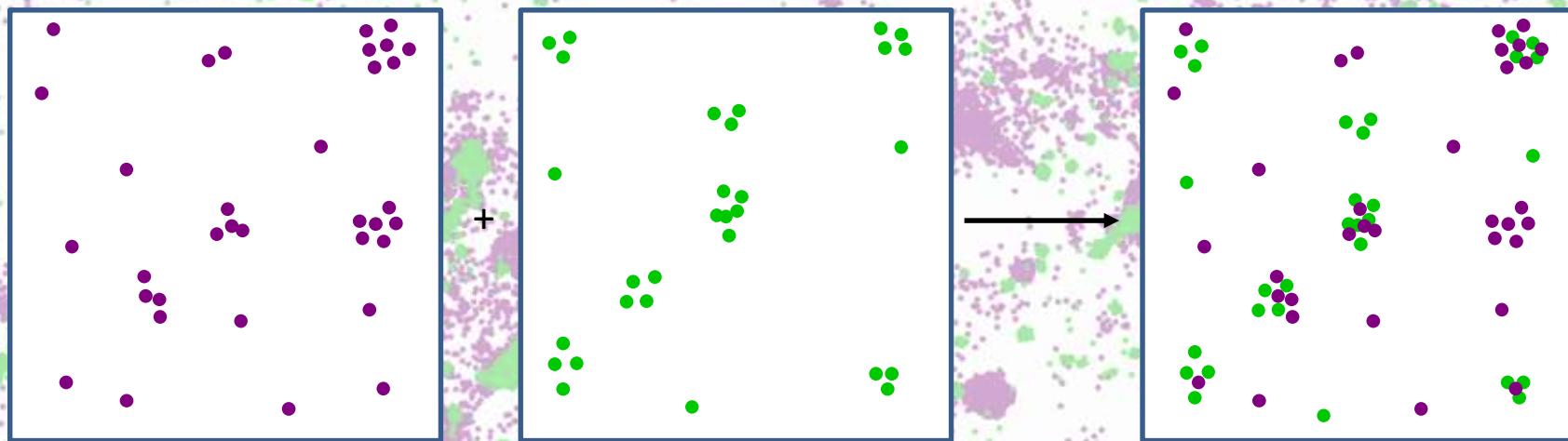


Projection



# Colocalization analysis of SMLM data

- Quantify the level of colocalization between 2 fluorescent proteins acquired by SMLM

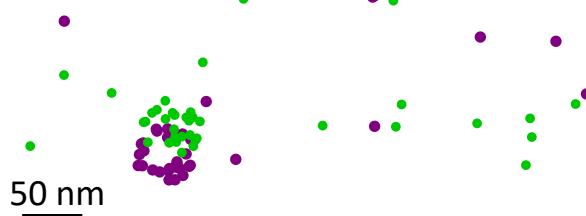
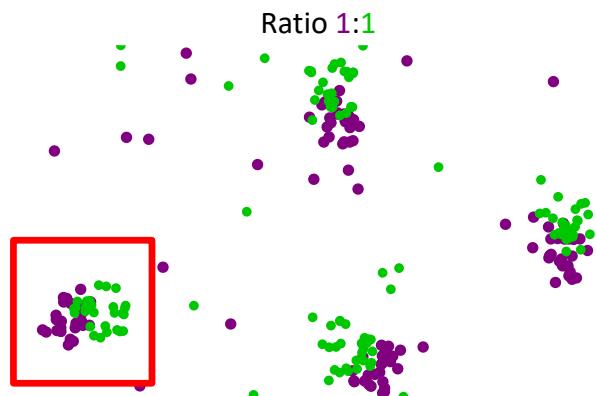


- Object-based
  - Compute statistics on segmented objects
- Unorganized neighboring techniques
  - Compare the spatial distribution of two channels in a defined vicinity, for each localization

Developed in Part 2 !!!

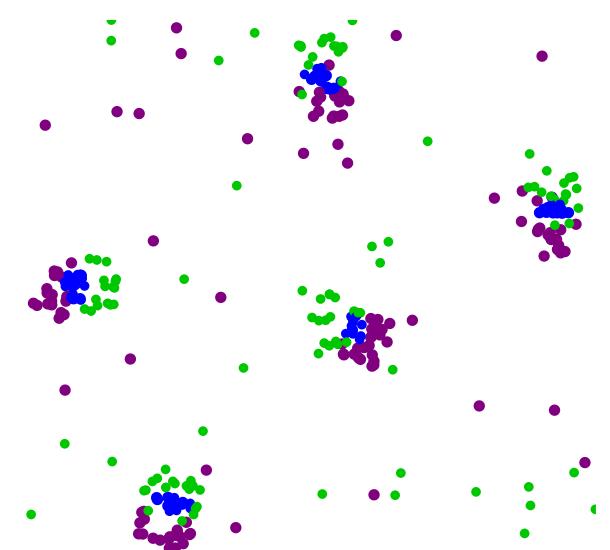
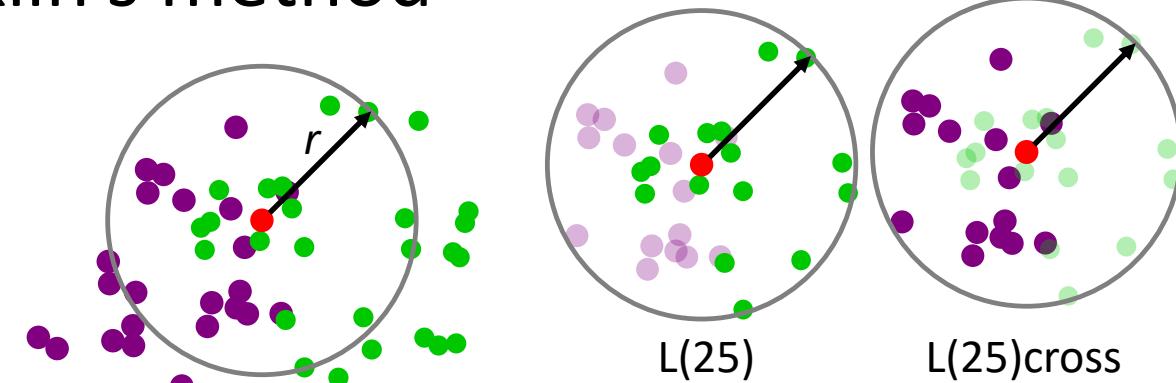
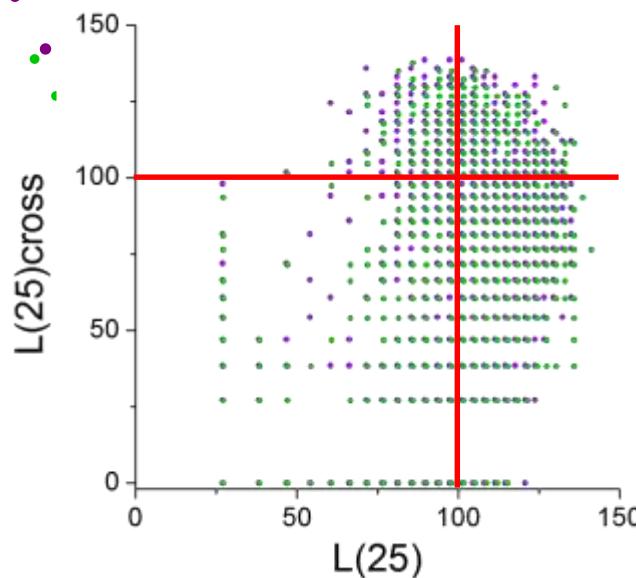
# Unorganized neighboring colocalization

- Getis and Franklin's method

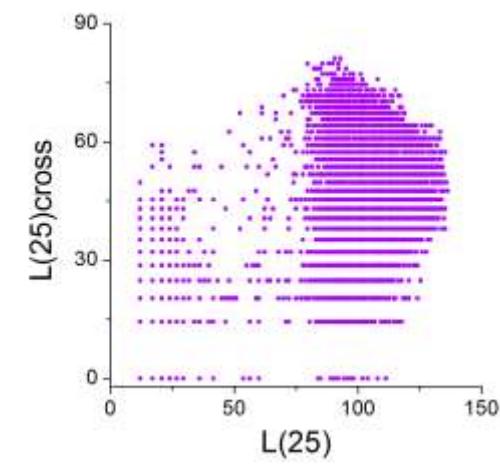
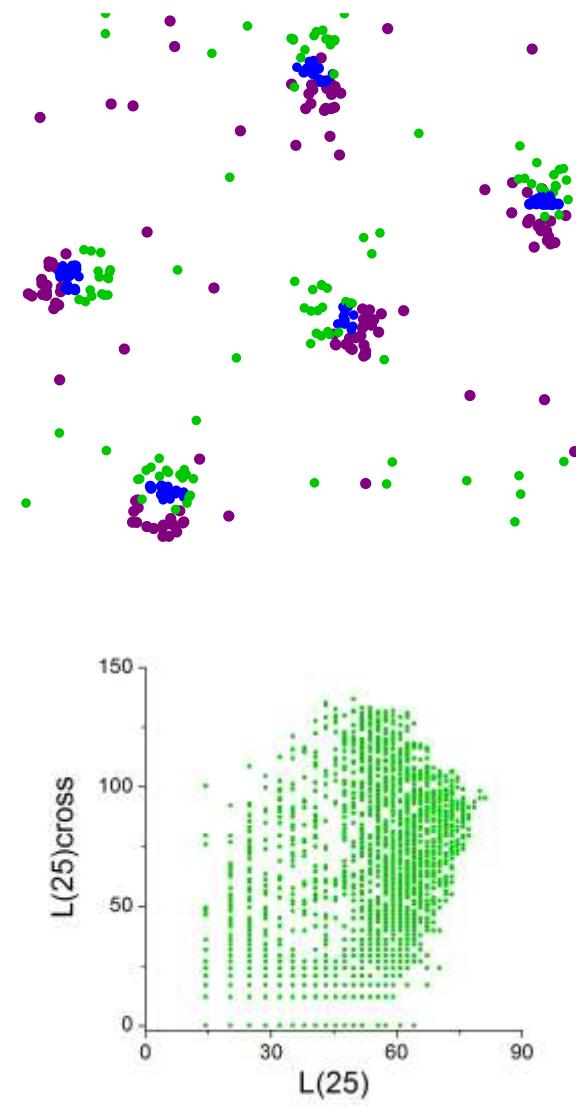
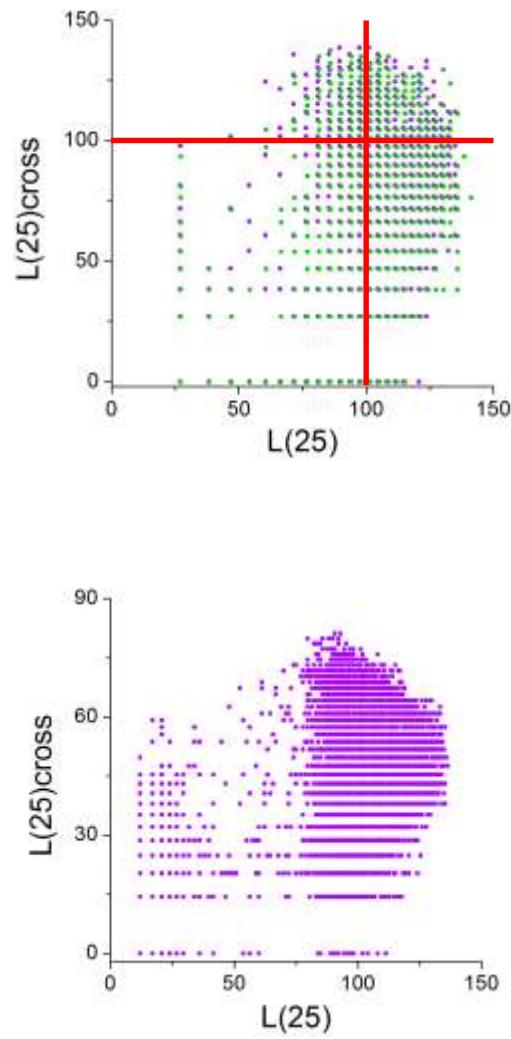
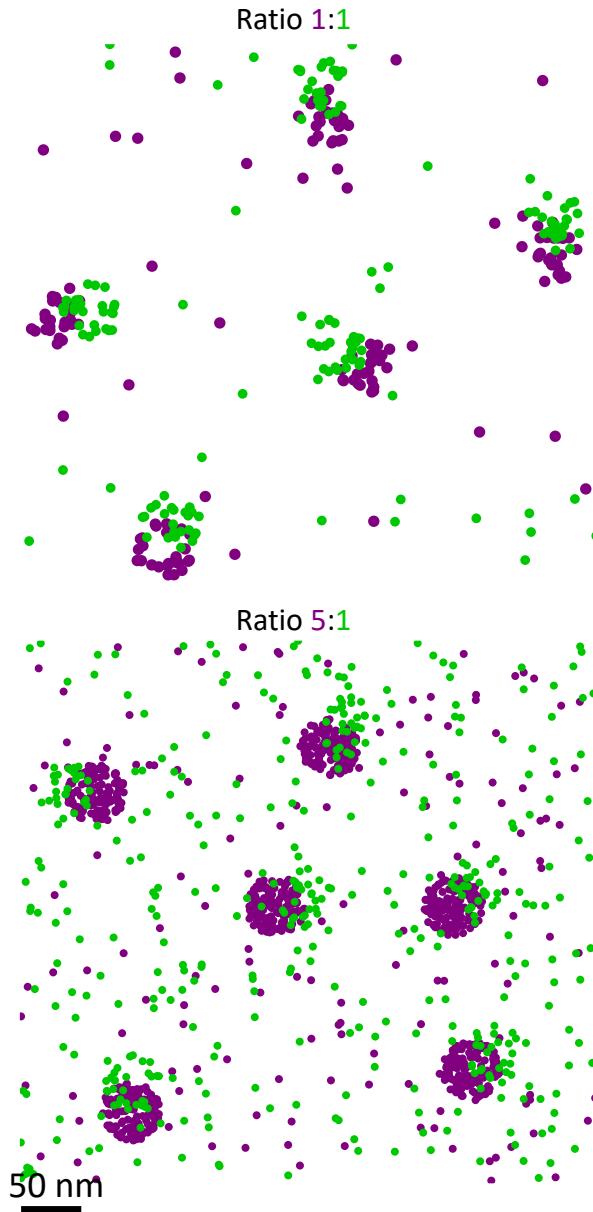


$$L(r)_j = \sqrt{A \sum_{i=1}^n \left( \frac{\delta_{ij}}{n} \right) / \pi}$$

$$\text{where } \delta_{ij} = \begin{cases} 1 & \text{if } d_{ij} < r \\ 0 & \text{else} \end{cases}$$

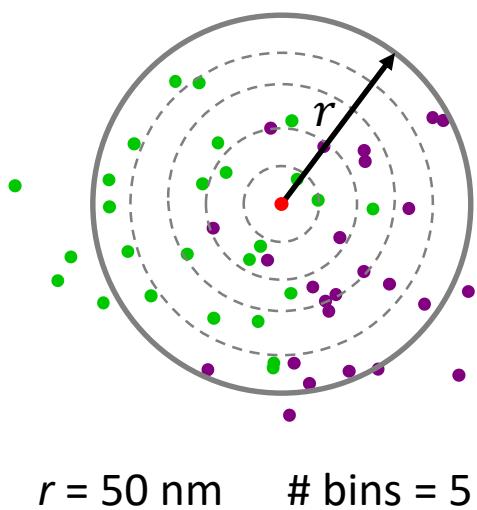


# Getis and Franklin's method

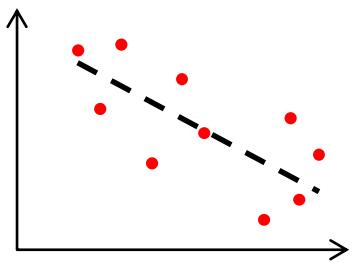


# Unorganized neighboring colocalization

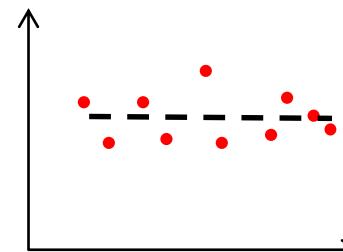
- Coordinate-Based Colocalization (CBC)



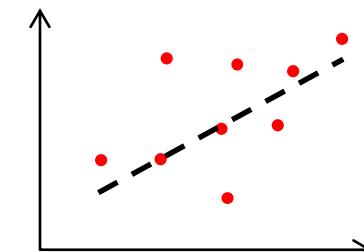
distance (nm)	10	20	30	40	50
# locs channel 1	1	4	7	3	7
# locs channel 2	2	3	6	3	8



-1



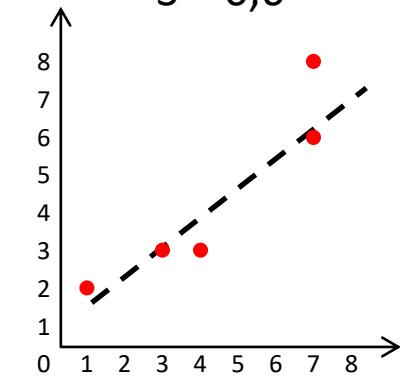
0



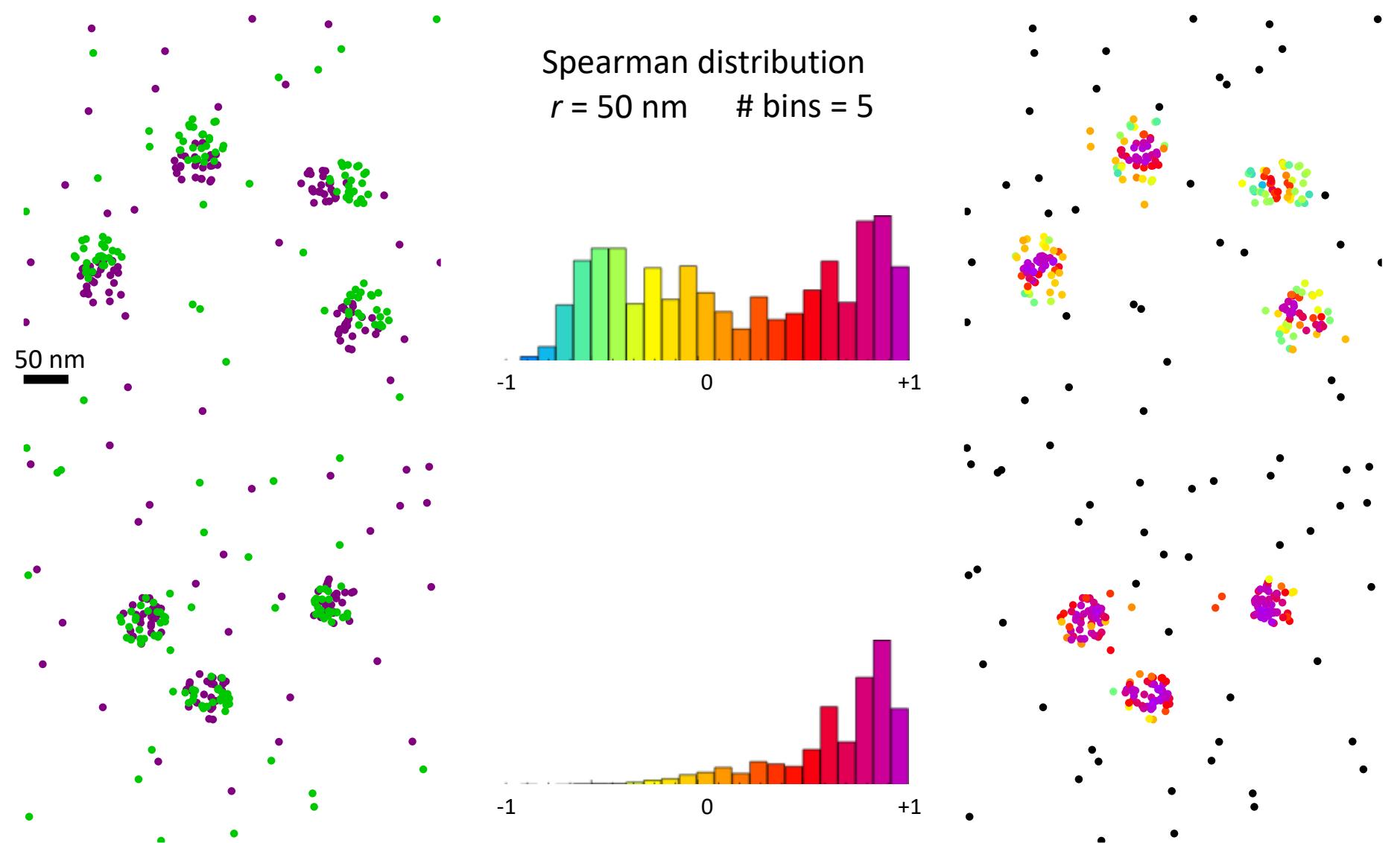
+1

Spearmann rank correlation

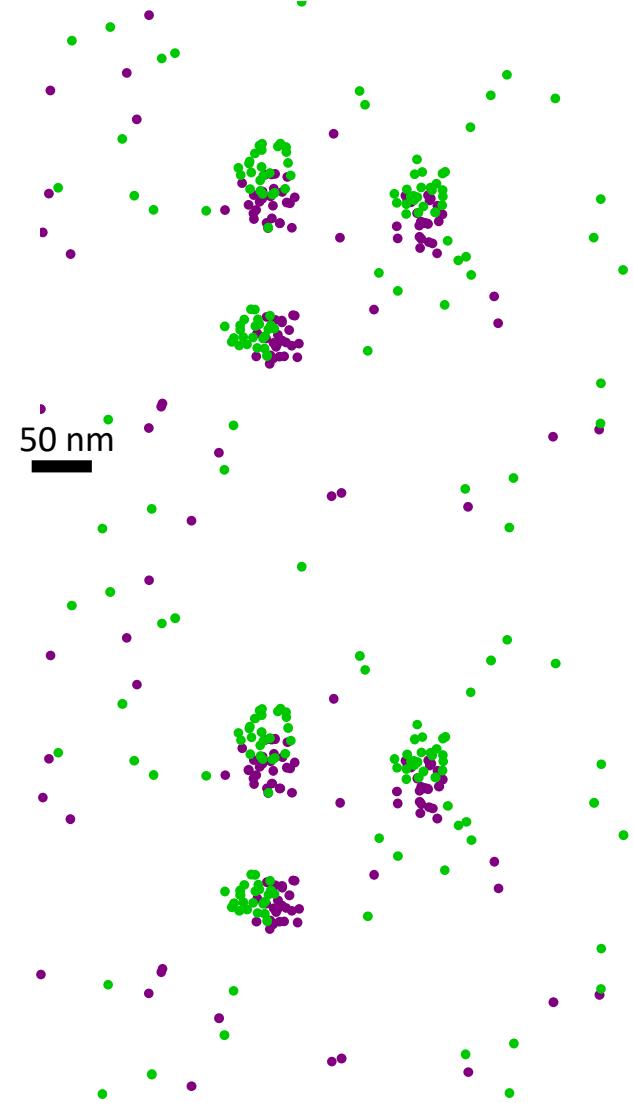
$S = 0,6$



# Coordinate-Based Colocalization (CBC)

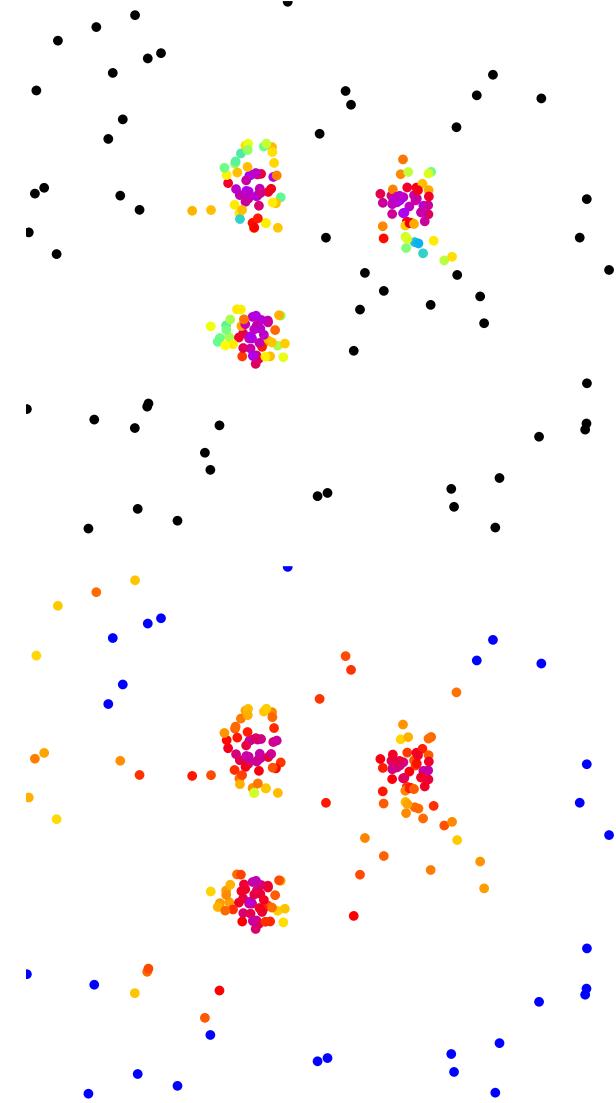


# Coordinate-Based Colocalization (CBC)



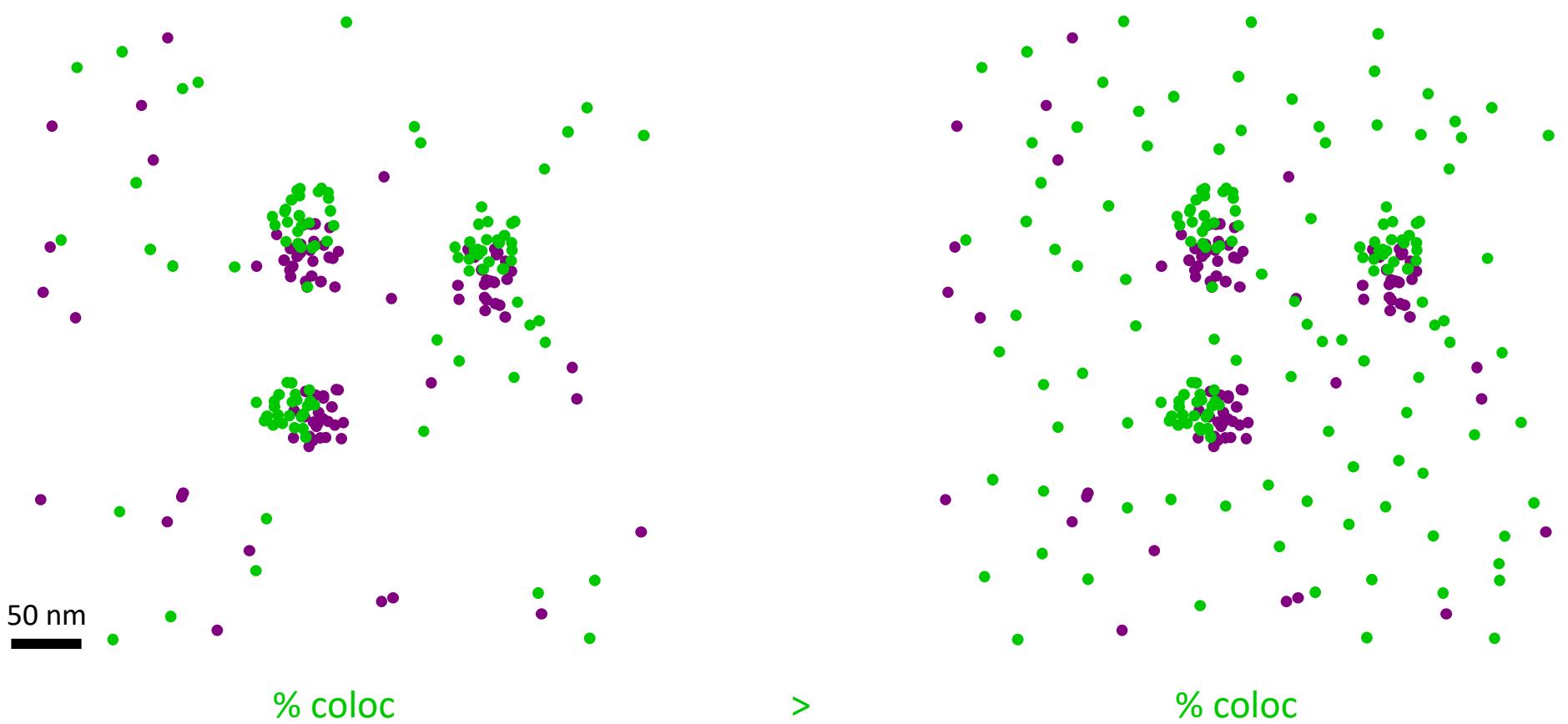
$r = 50 \text{ nm}$    # bins = 5

$r = 100 \text{ nm}$    # bins = 20



# Coordinate-Based Colocalization (CBC)

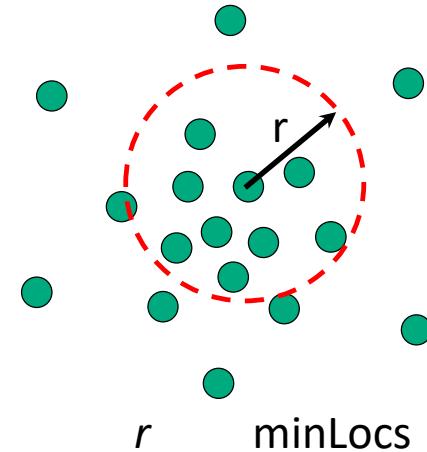
- $\% \text{ coloc} = (\# \text{ loc} > T) / \# \text{ loc}$



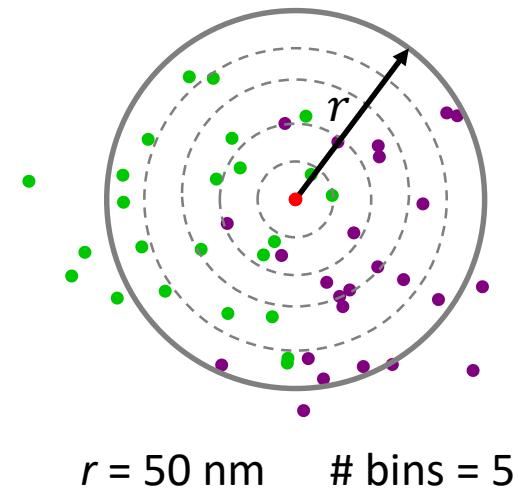
# Unorganized neighboring colocalization

- Clus-DoC (DBSCAN + CBC)

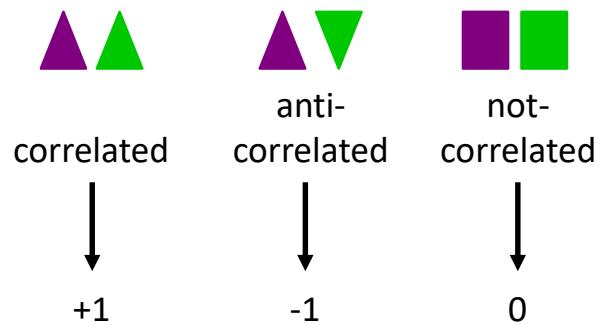
Density-based spatial clustering analysis with noise



Coordinate-Based Colocalization



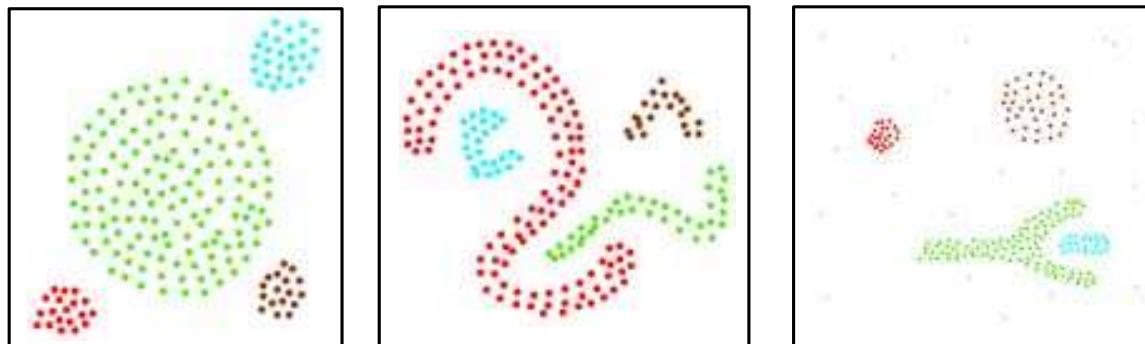
Spearmann rank correlation



# DBSCAN

Density-Based Spatial Clustering Analysis with Noise

- Segmentation technique
    - Suitable for objects and clusters
  - Organizes the localizations wrt density in 3 classes
    - **Core**, density-reachable or **outlier** points
  - Two parameters
    - Radius  $r \rightarrow$  neighborhood size
    - $MinPts \rightarrow$  min nb of points in  $r$  to be a core point
- with  $MinPts \neq$  min number of points of a cluster



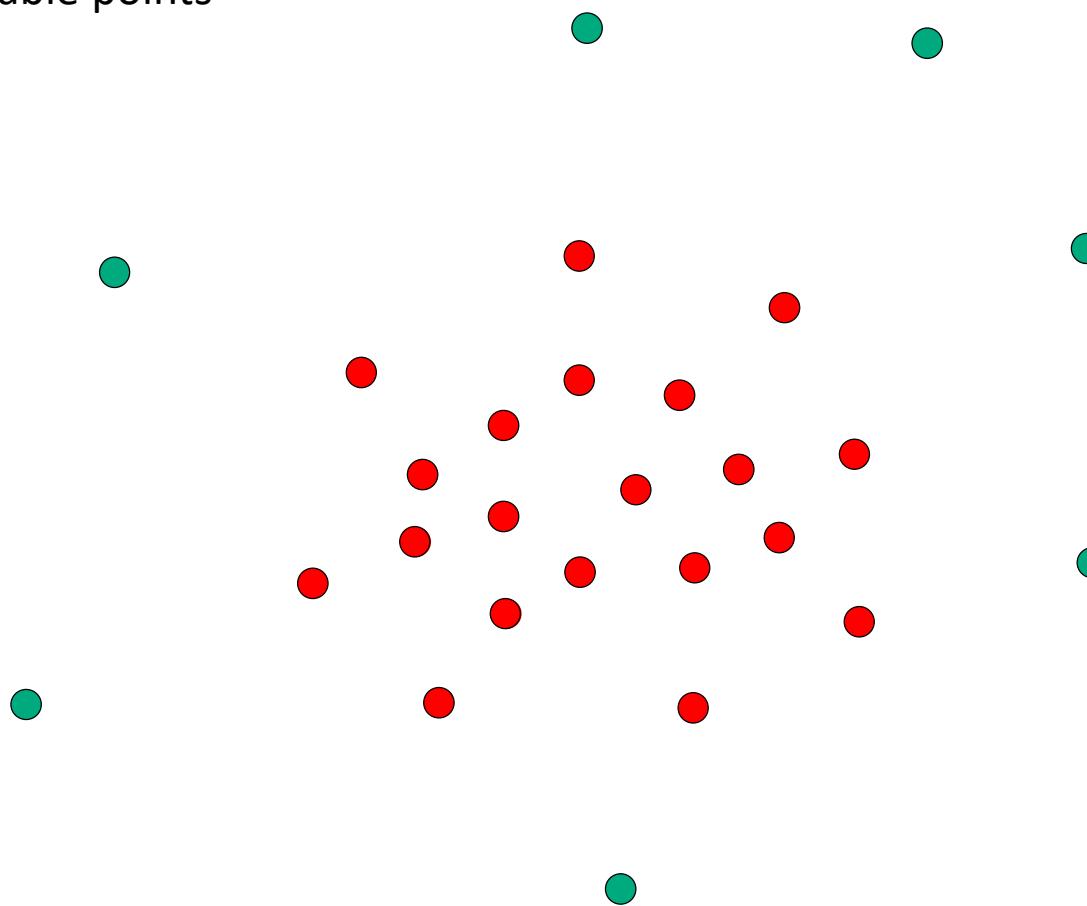
# DBSCAN

*MinPts = 4 (including current)*

■ Core points

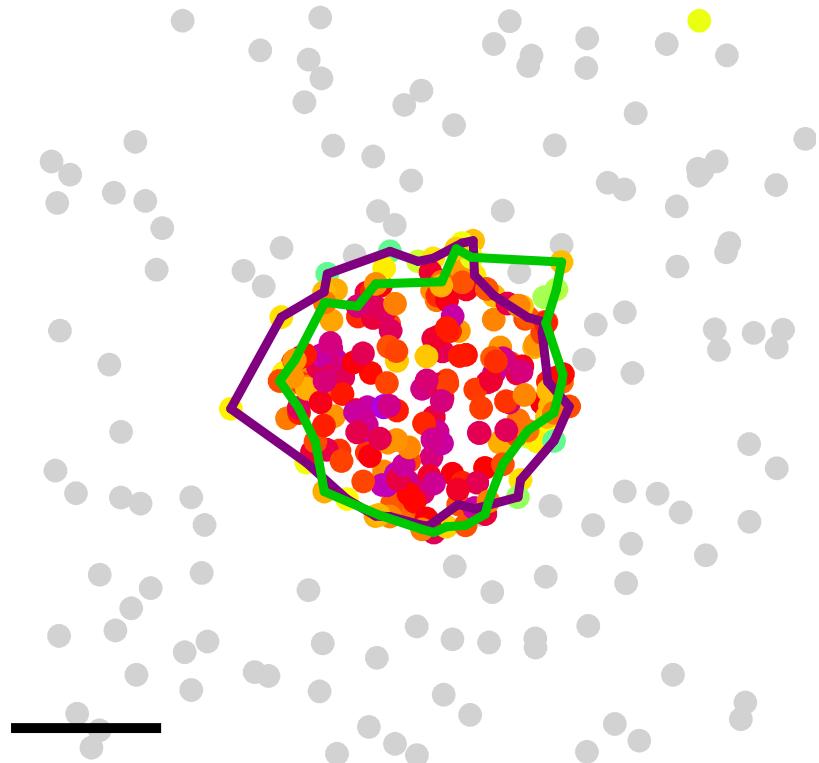
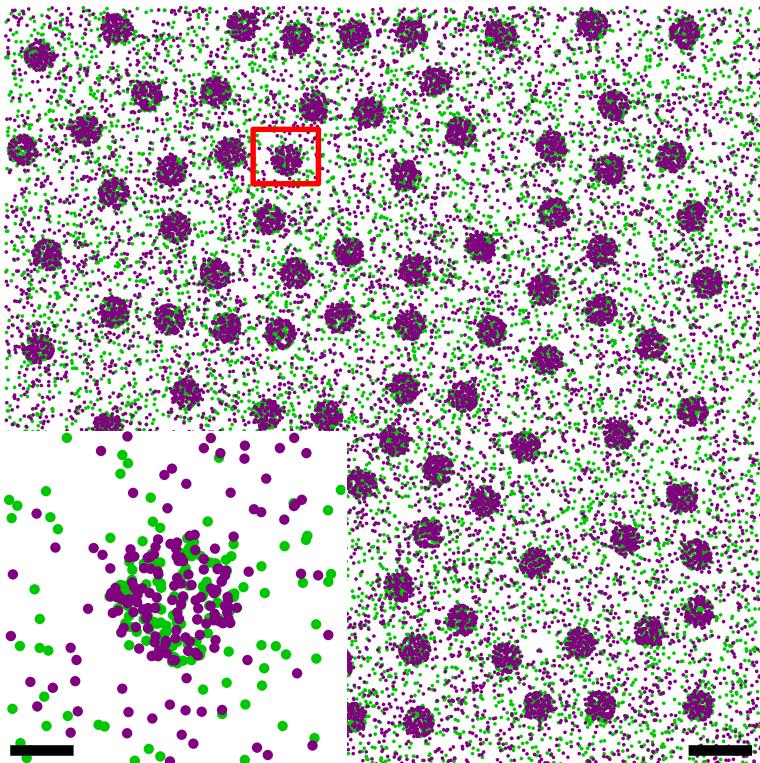
■ Density-reachable points

■ Outlier points



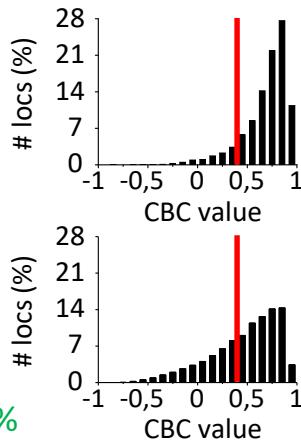
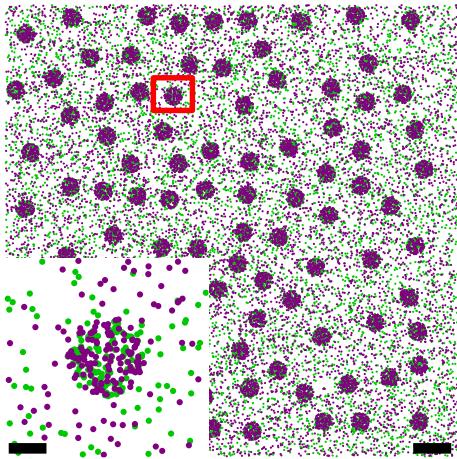
# Clus-Doc: DBSCAN + CBC

- Limit colocalization analysis to clusters

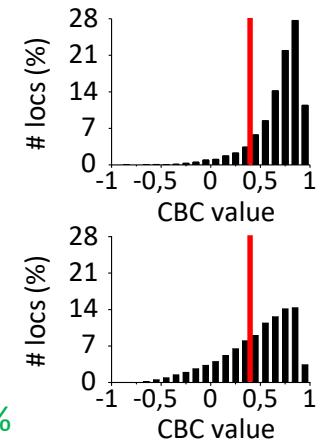
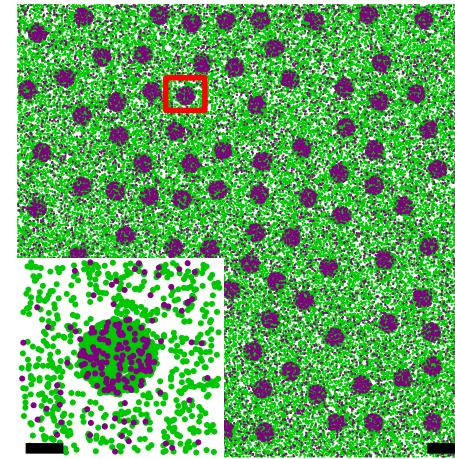


# Clus-Doc: DBSCAN + CBC

- 5 parameters (DBSCAN 2, CBC 2, threshold)



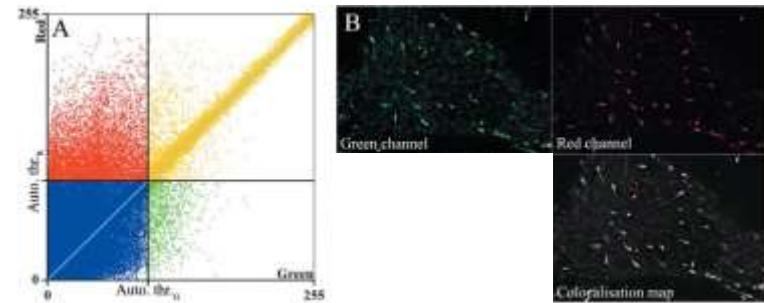
coloc = 68%    coloc = 68%



coloc = 89%    coloc = 65%

# Colocalization analysis

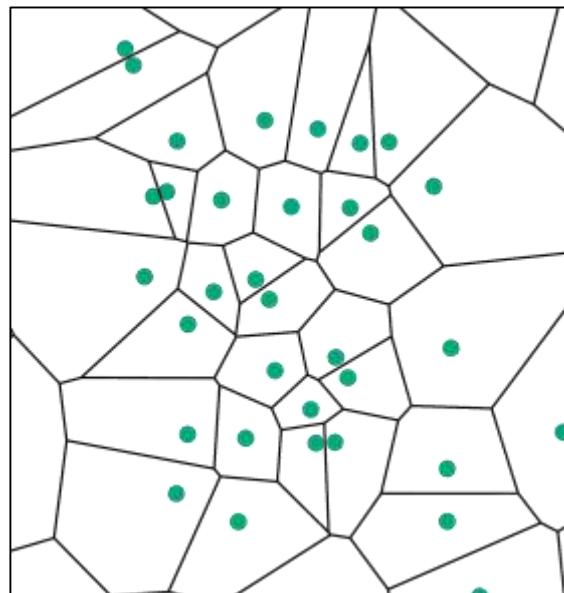
- Gold standard in fluorescence microscopy
  - Pearson coefficient
  - Manders coefficients
  - Same image dimension



- Colocalization of  $\lambda$ SMLM can be difficult to generalize to all data shapes and densities
- Adapt Pearson and Manders coeffs to  $\lambda$ SMLM
  - Robust to variabilities

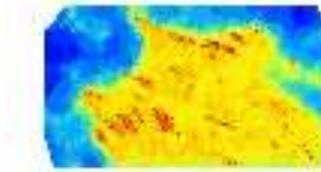
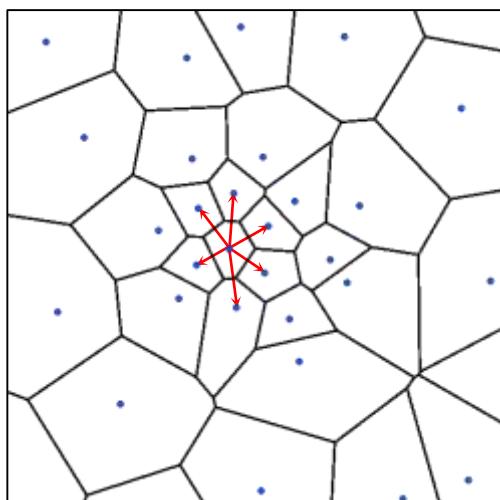
# Voronoi diagram

- Space-subdividing technique
- Anisotropic by nature
- One loc/polygon → bijective representation



# Voronoi diagram

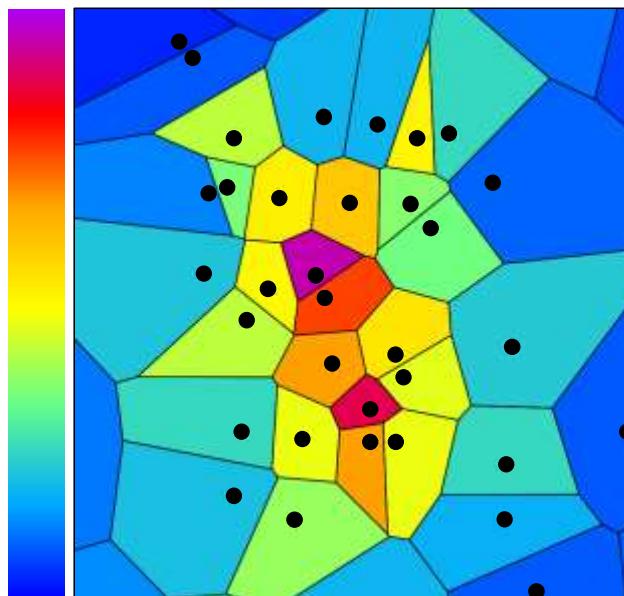
- Connectivity
  - Direct neighbors are known
- Scalability
  - The denser the locs, the smaller the polygons are



# Voronoi diagram

- Space-subdividing technique
- One loc/polygon → bijective representation
- Statistics computed on the polygons (area, density...)

Polygon P  
# of neighbors n  
Polygon area A  
Local density  $\delta$

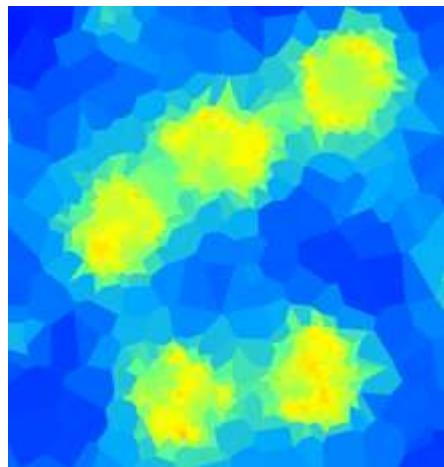
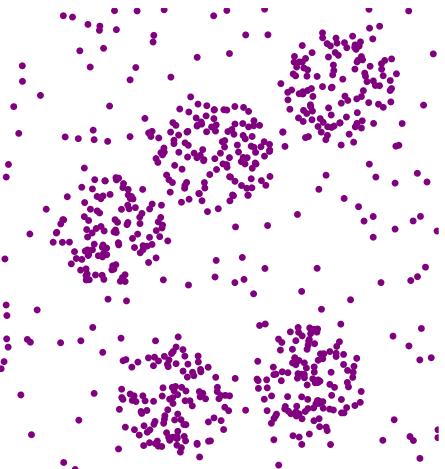


Rank 0  
 $n_0 = \# P_0 + n_{n+1}$   
 $A_0 = \sum(P_n) \square A_{n+1} \square$   
 $\delta_0 = n_0 / A_0$

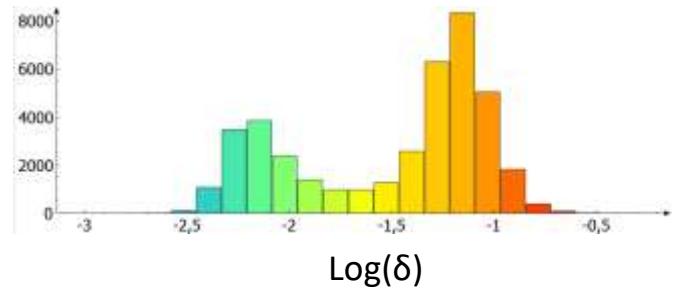
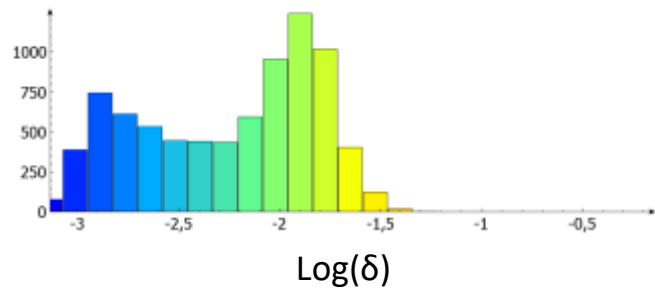
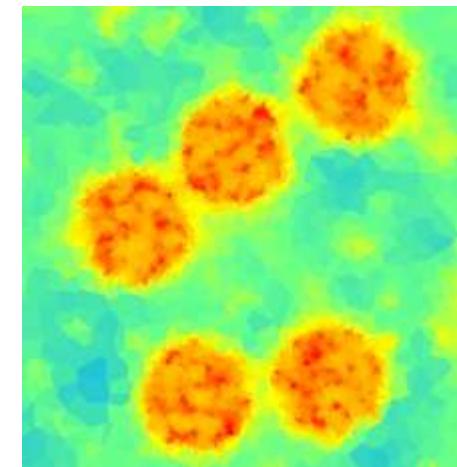
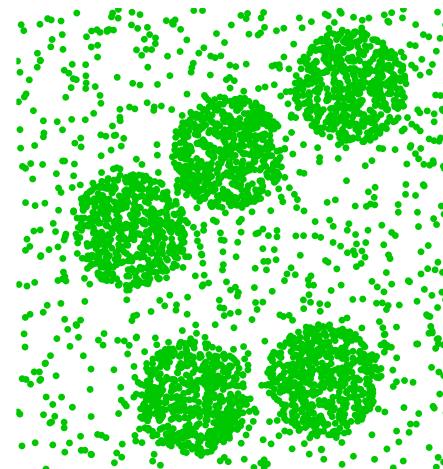
# Voronoi normalization

Ratio 1:5

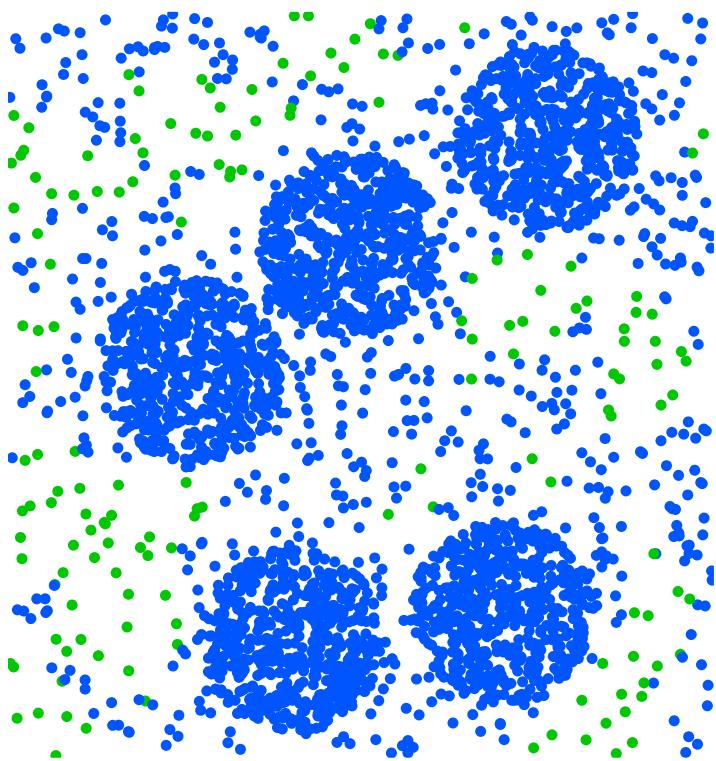
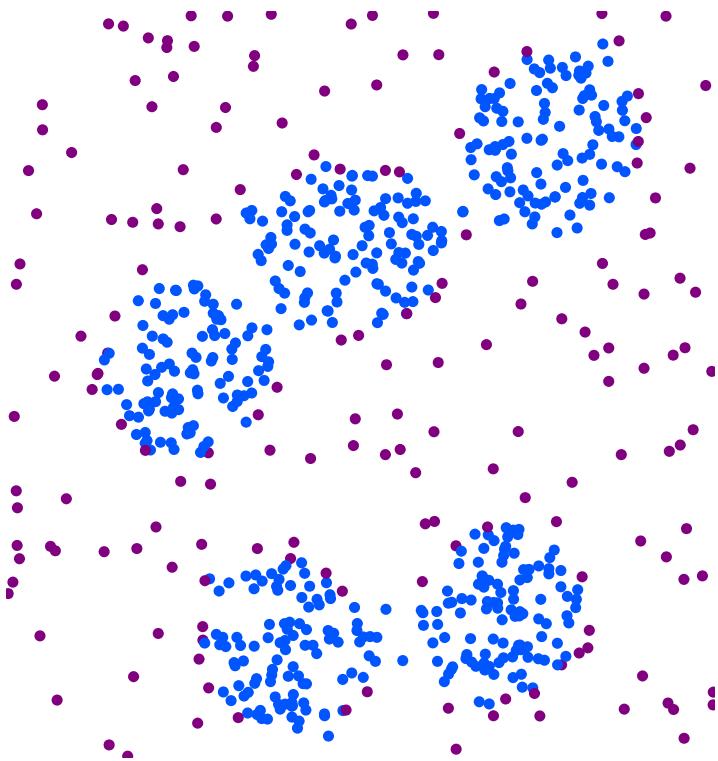
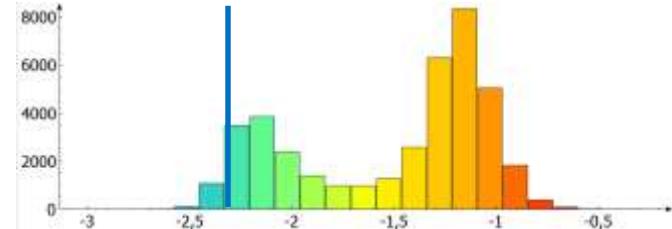
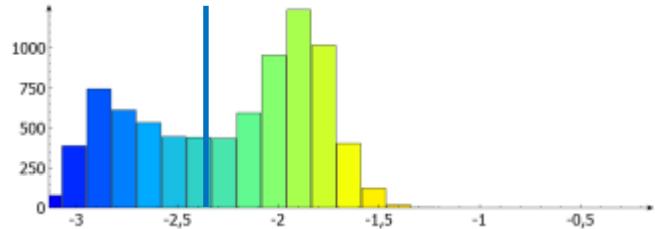
0.013 mol/nm<sup>2</sup>



0.065 mol/nm<sup>2</sup>



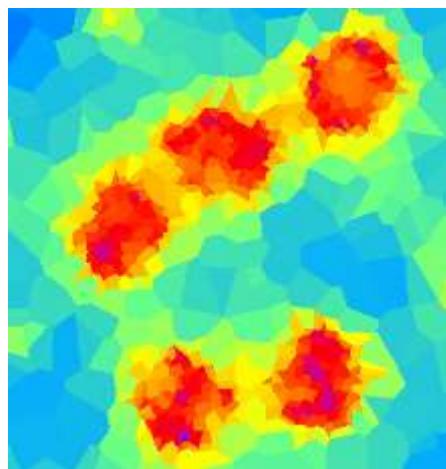
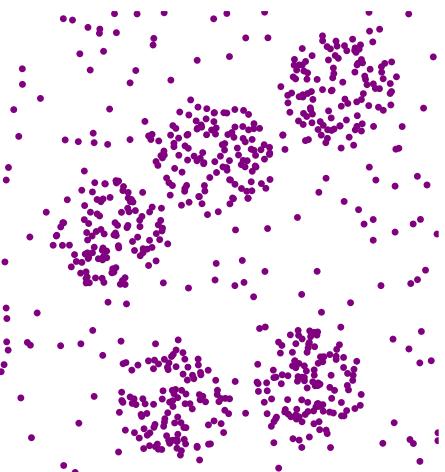
# Voronoi normalization



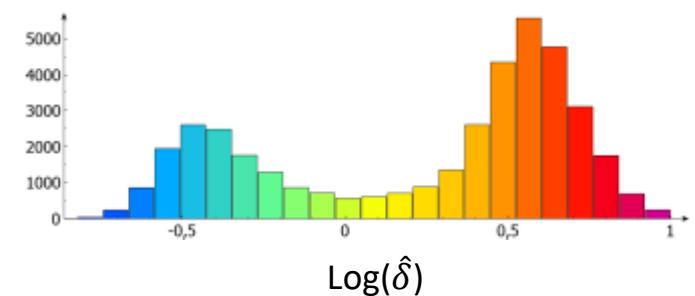
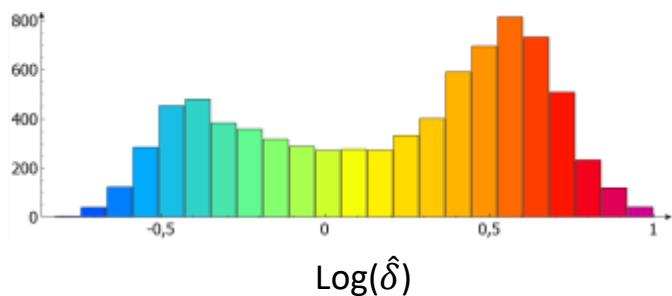
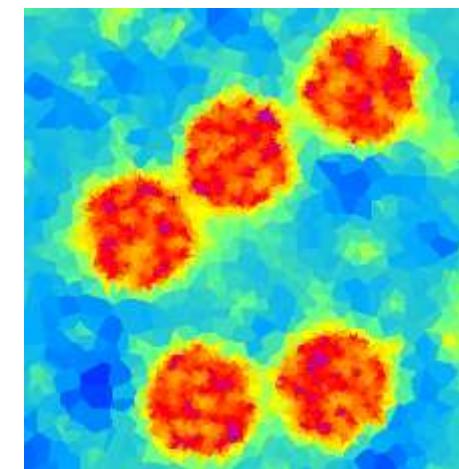
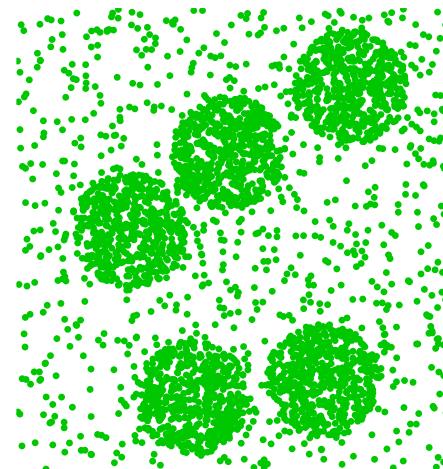
# Voronoi normalization

Ratio 1:5

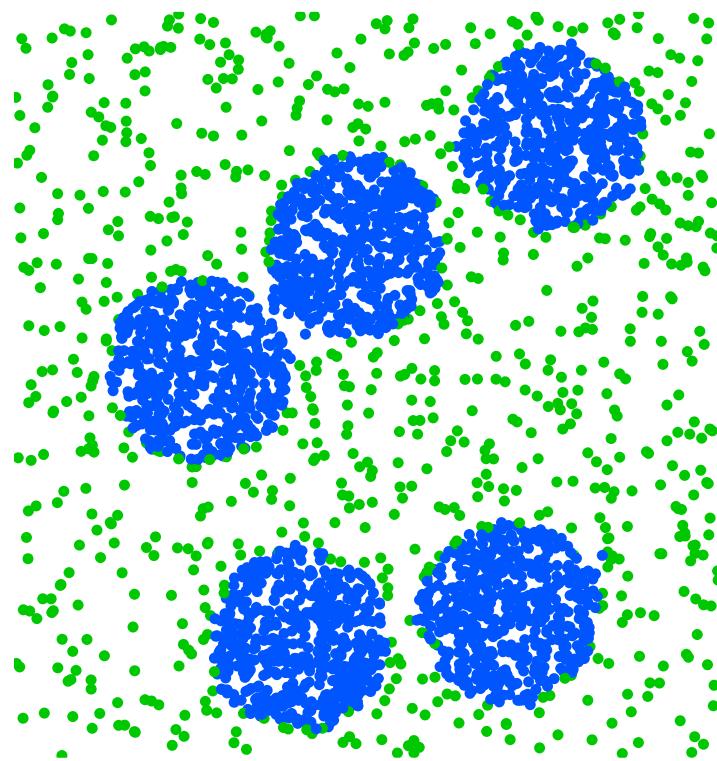
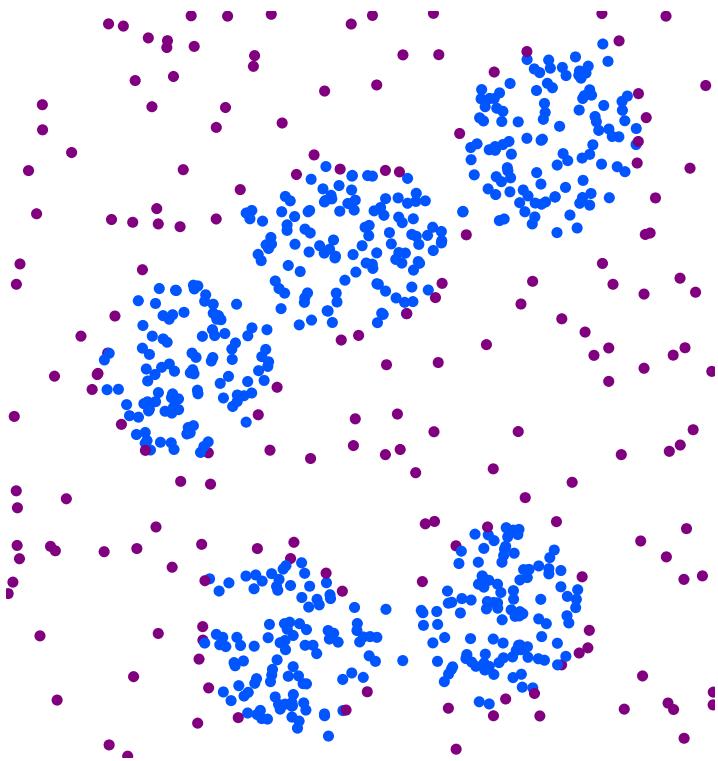
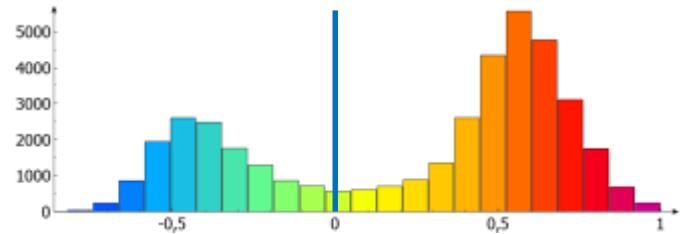
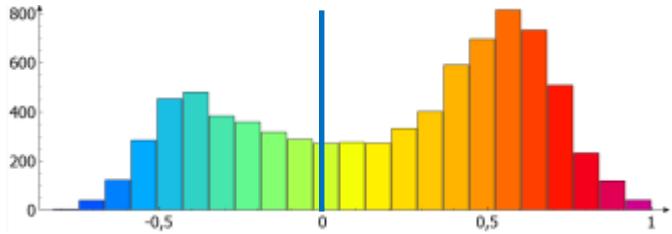
0.013 mol/nm<sup>2</sup>



0.065 mol/nm<sup>2</sup>

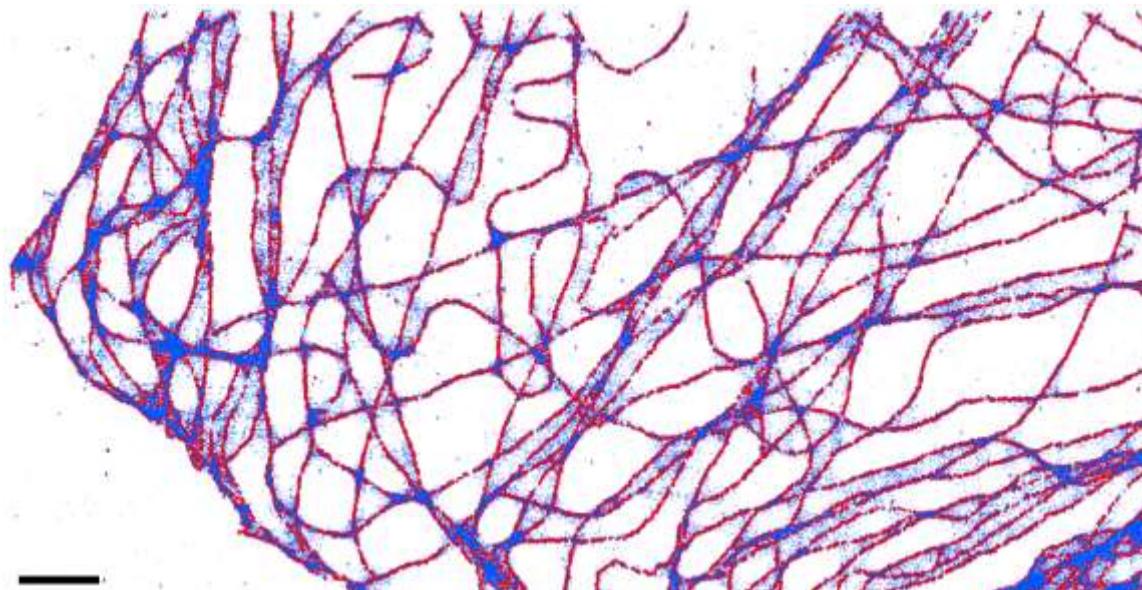
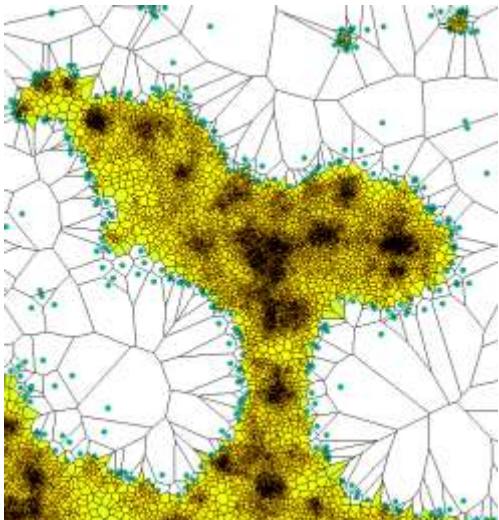


# Voronoi normalization

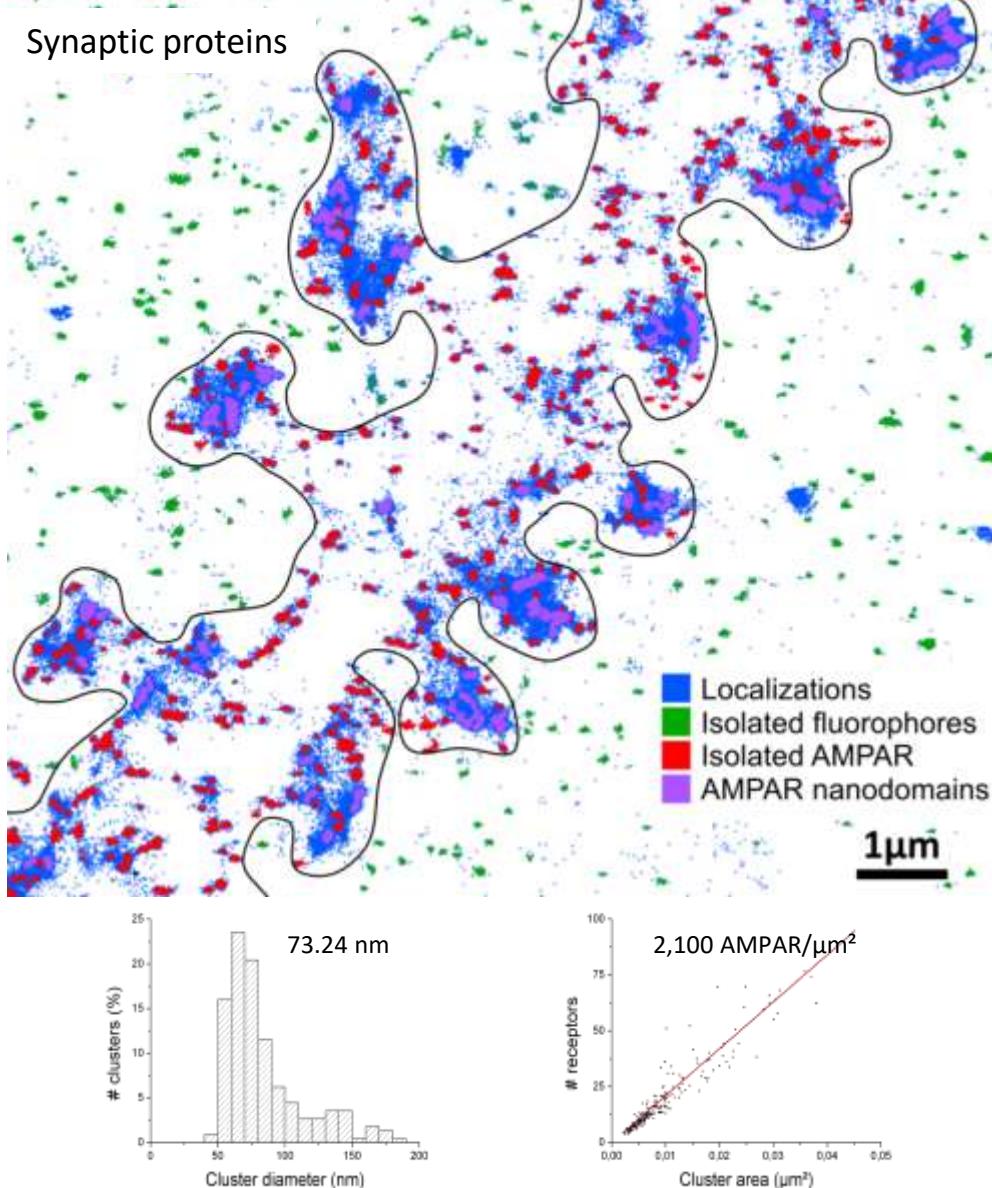


# Voronoi segmentation (1/2)

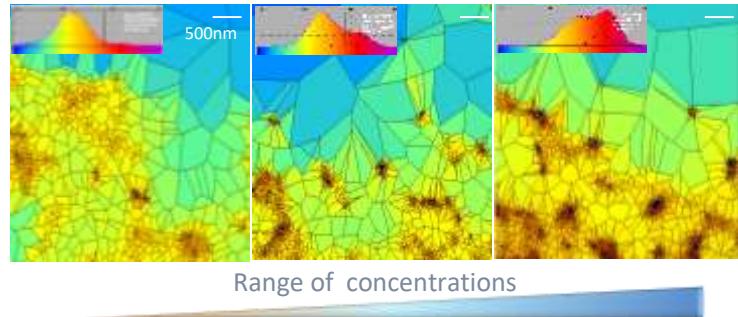
- Voronoï diagram is an intrinsic multi-resolution structure that can be normalized
- Compare the local density to the average density  $\delta$



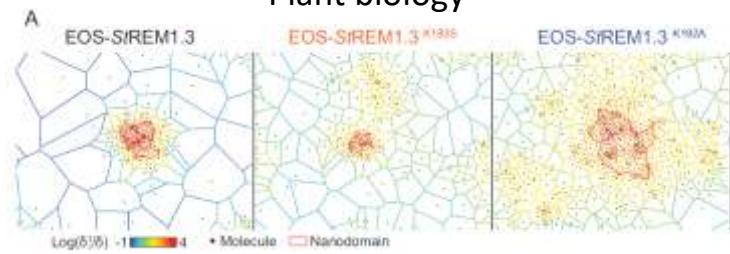
# Voronoi segmentation (2/2)



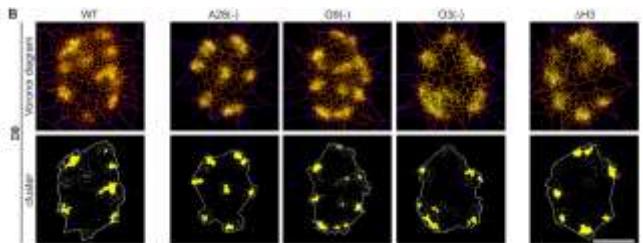
Automatic segmentation of 96-wells plates



Plant biology

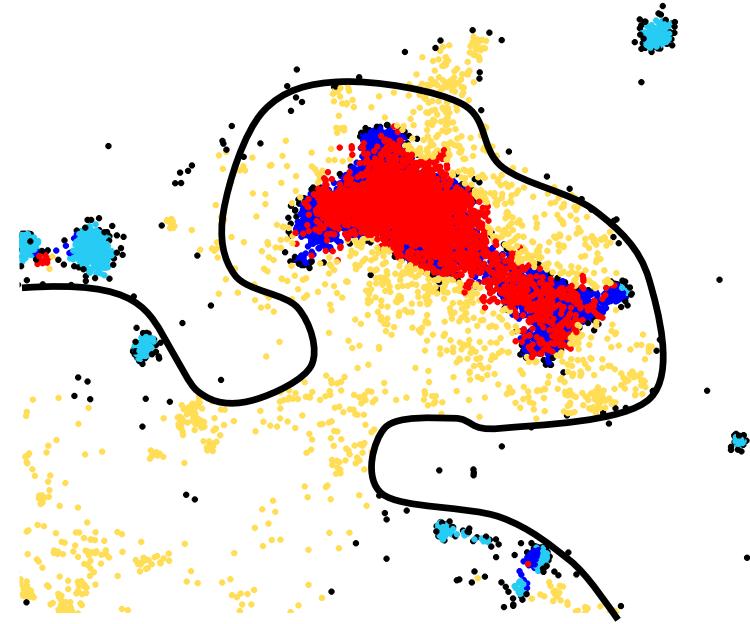
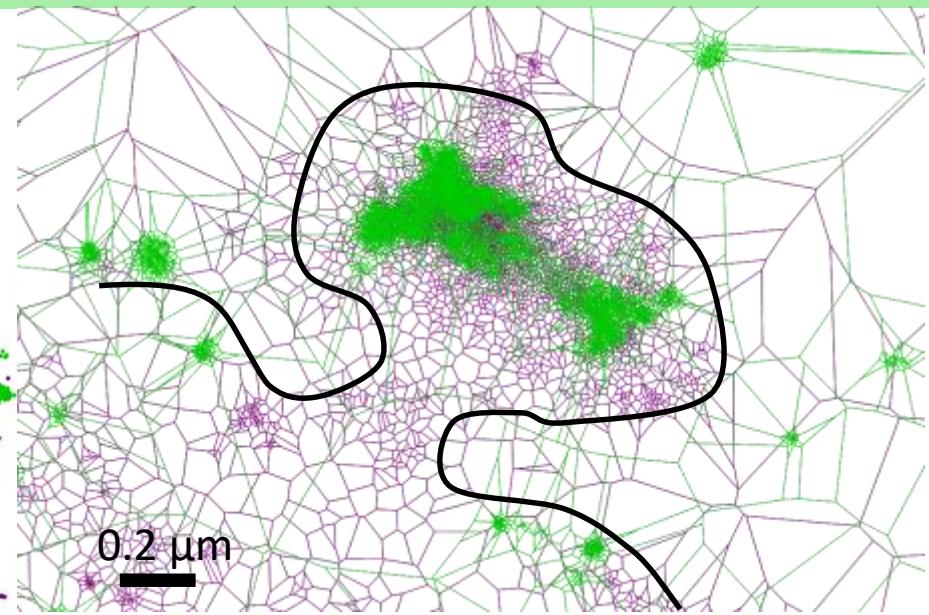
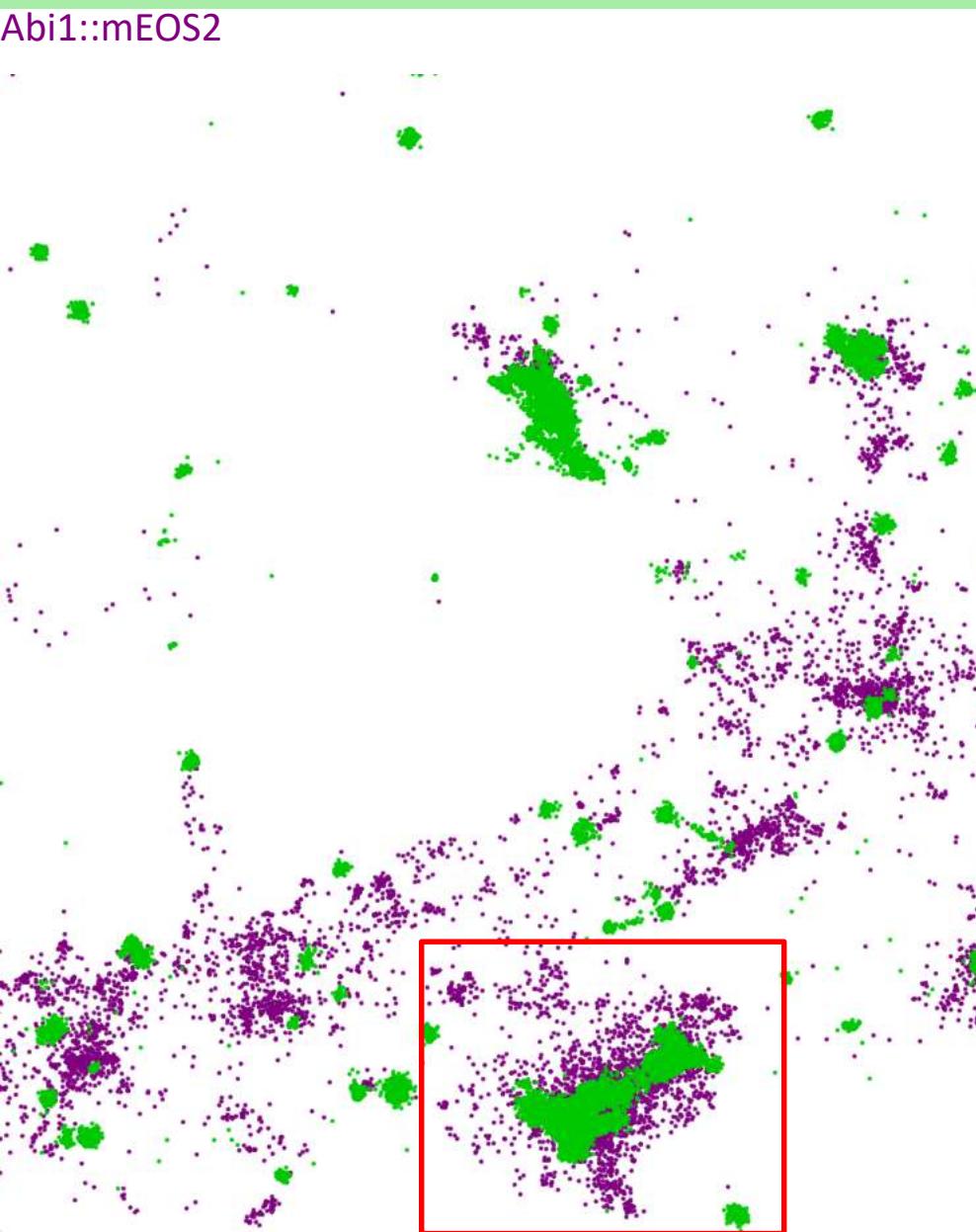


Polarization of Vaccinia virus

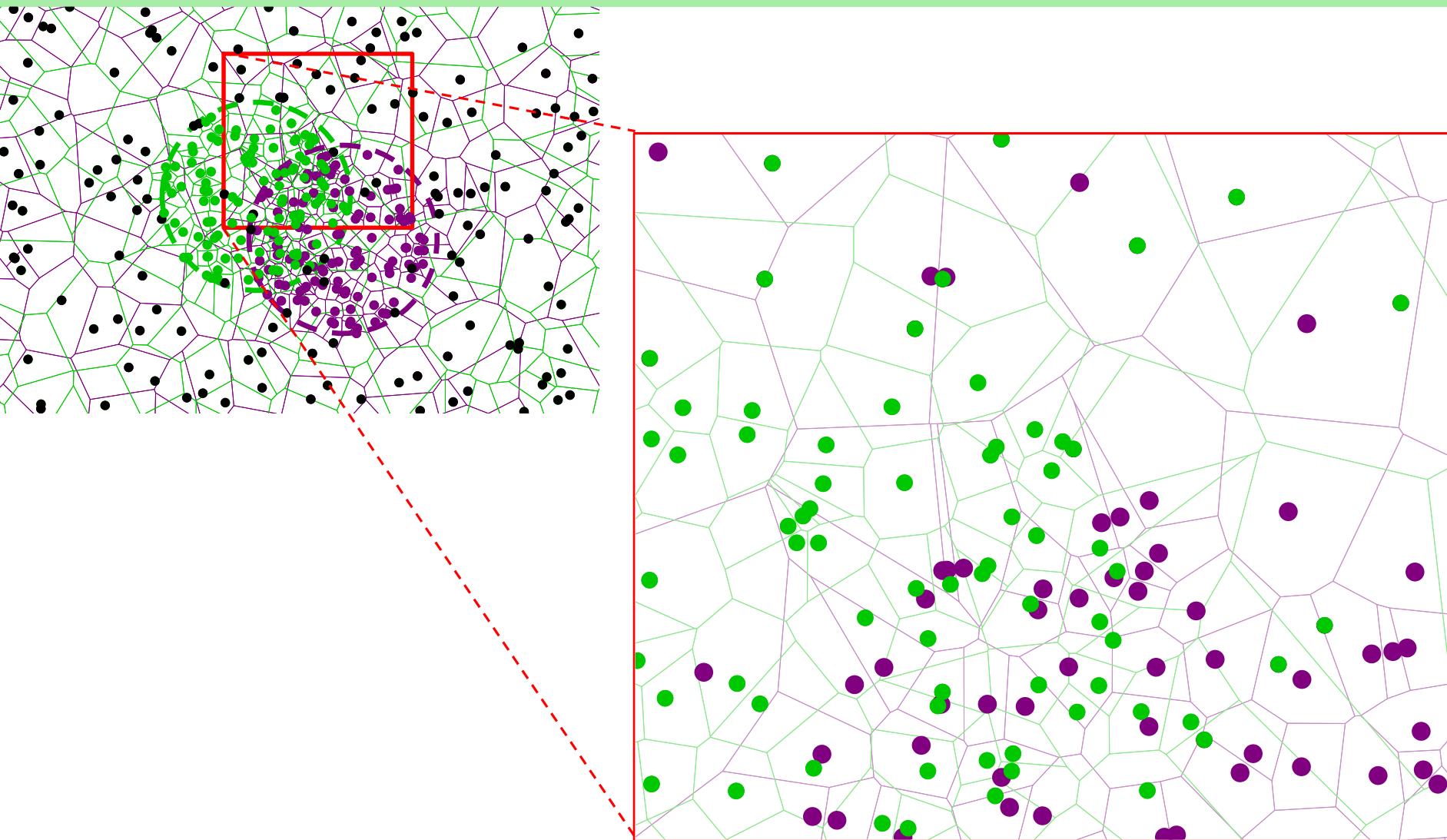


# Colocalisation analysis of λSMLM

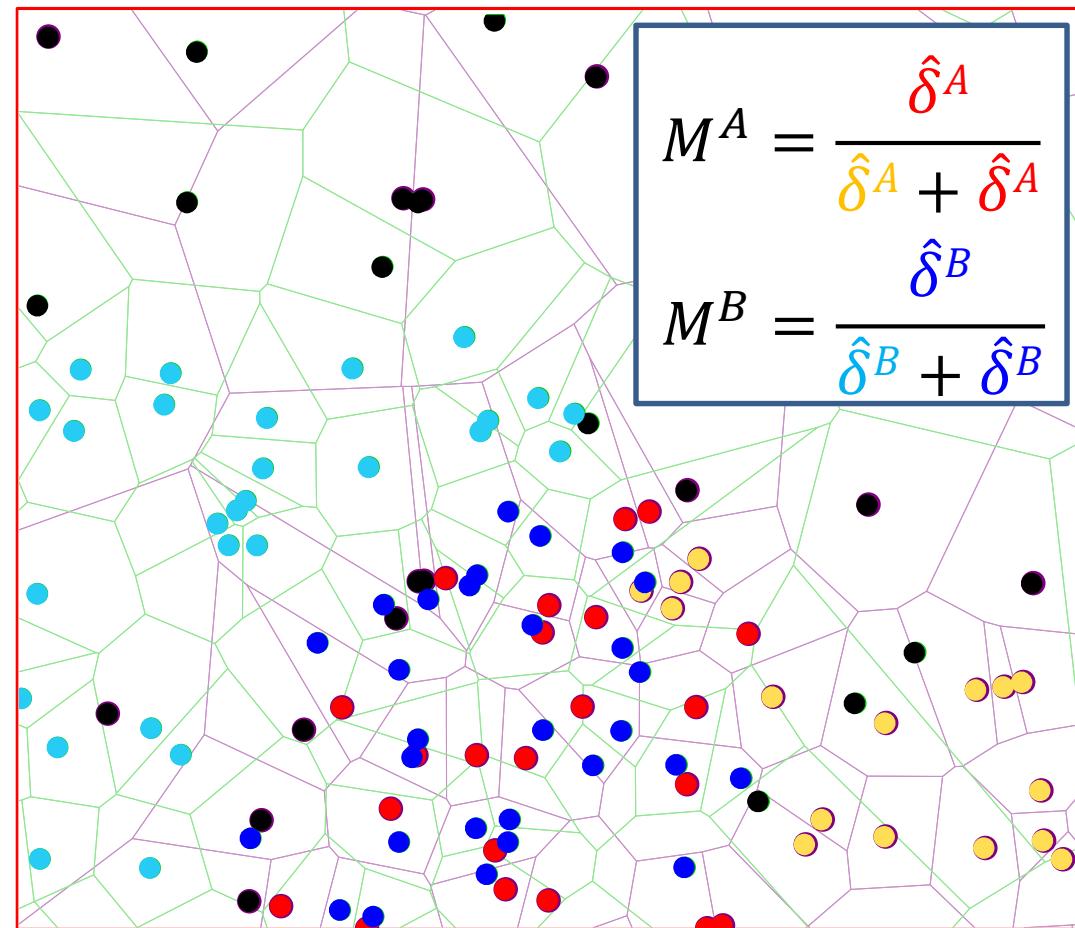
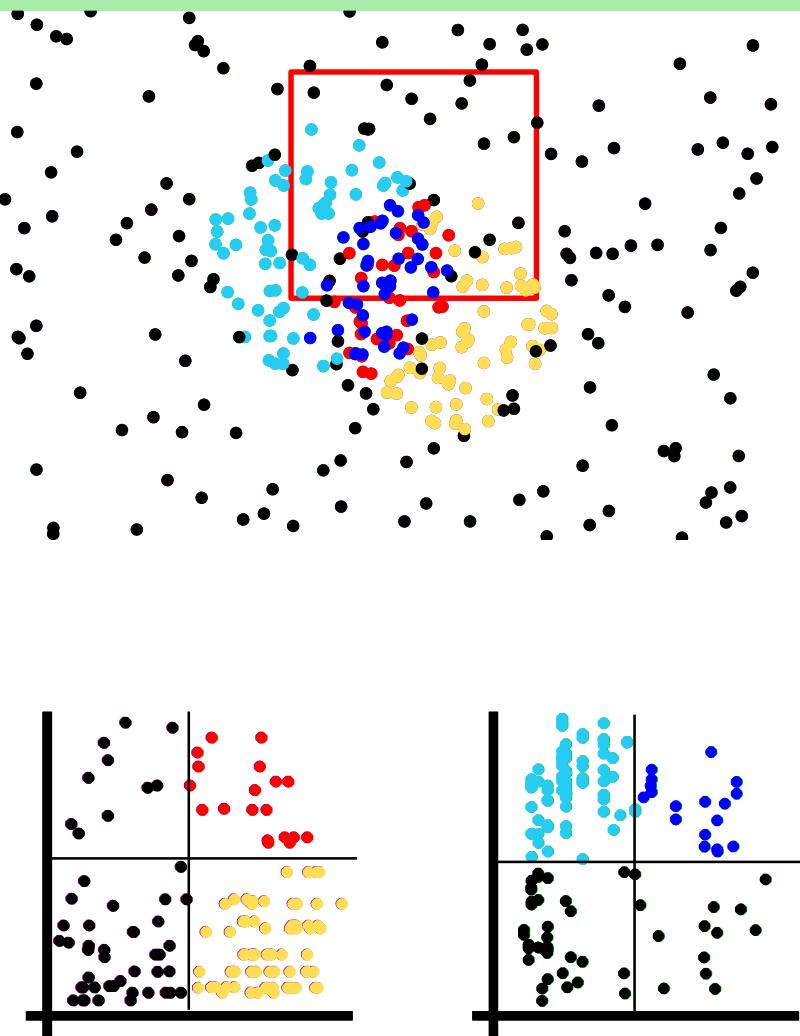
Abi1::mEOS2



# Colocalisation analysis of $\lambda$ SMLM



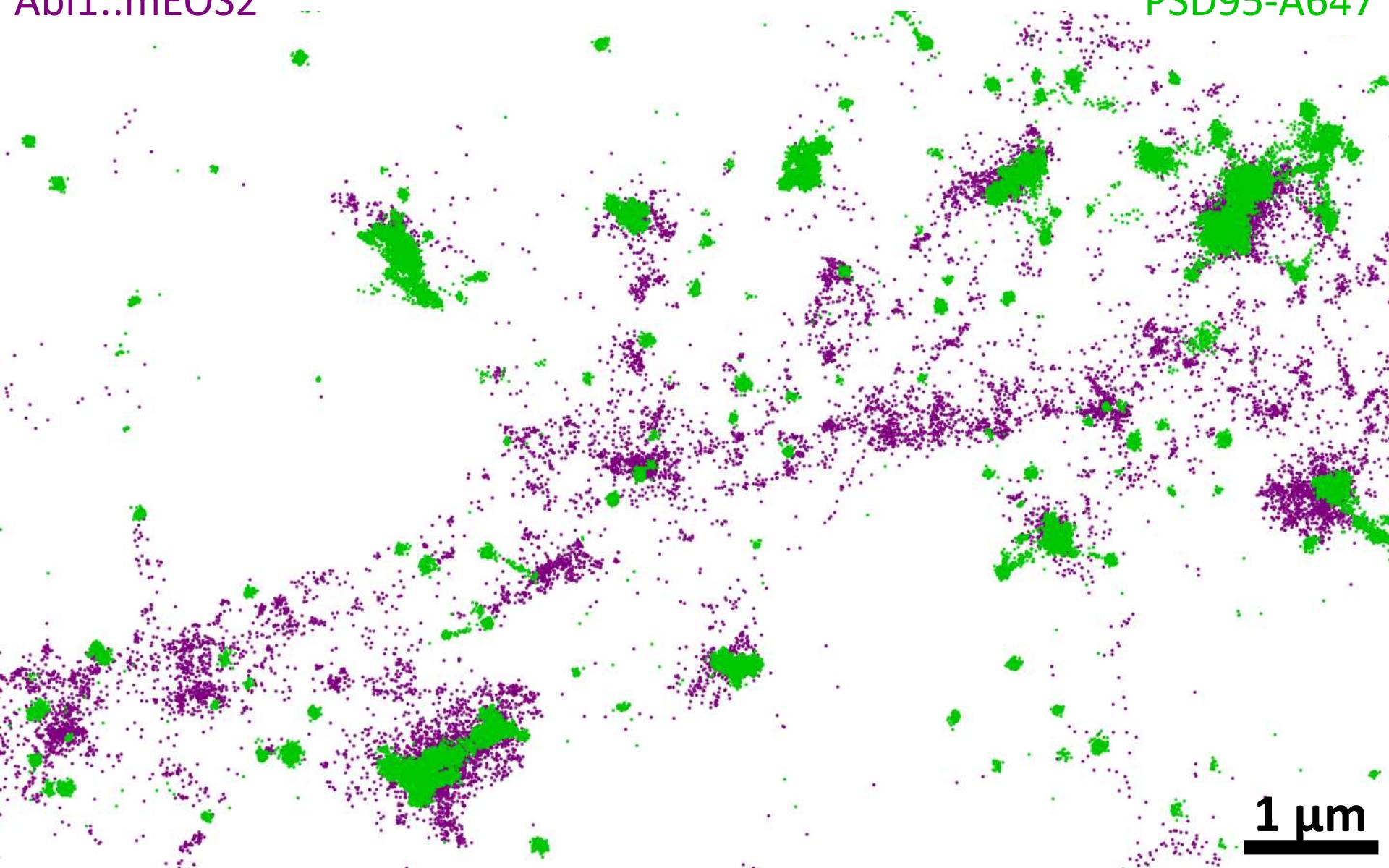
# Colocalisation analysis of λSMLM



# Colocalisation analysis of λSMLM

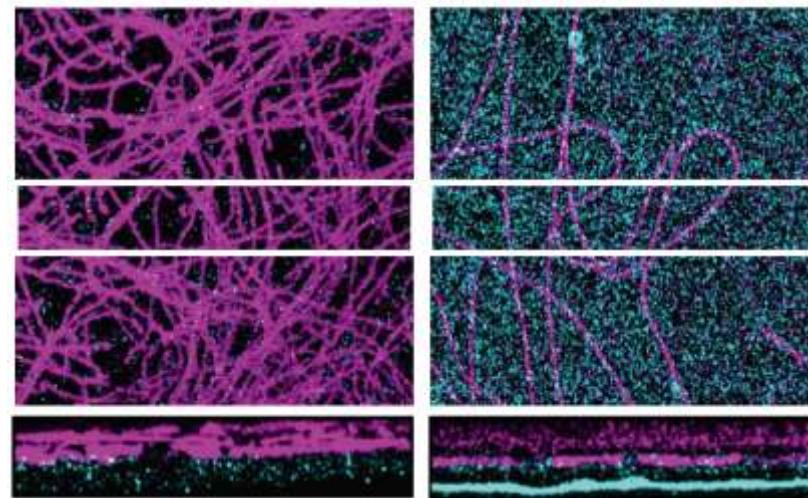
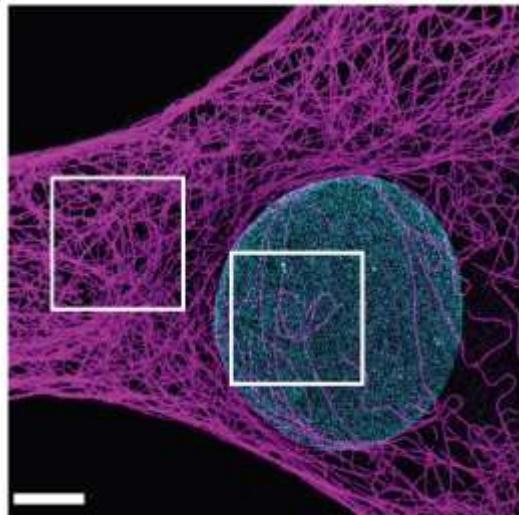
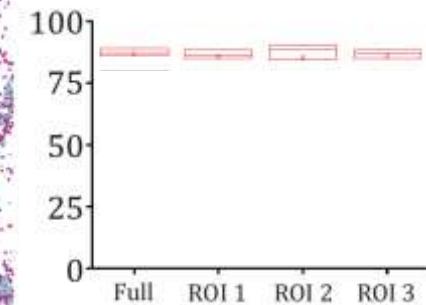
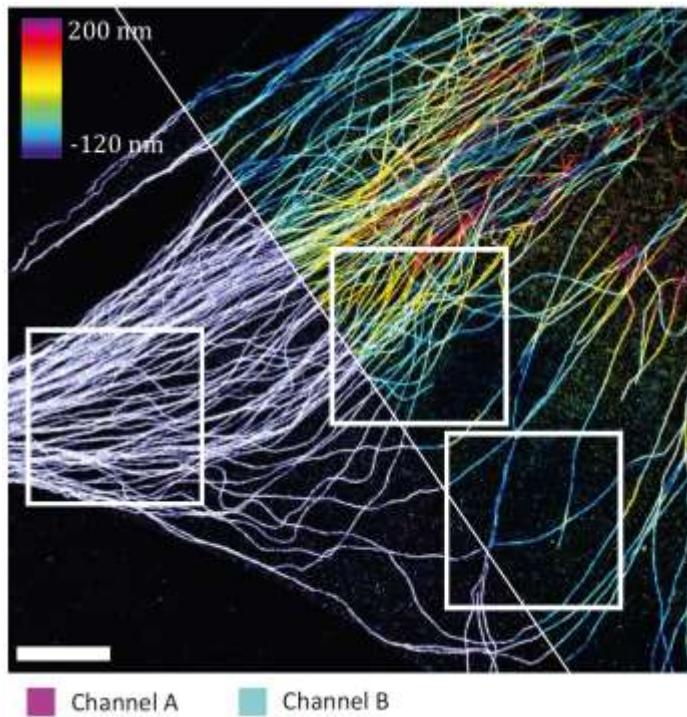
Abi1::mEOS2

PSD95-A647



1  $\mu\text{m}$

# Experimental controls



$M < 0.2\%$

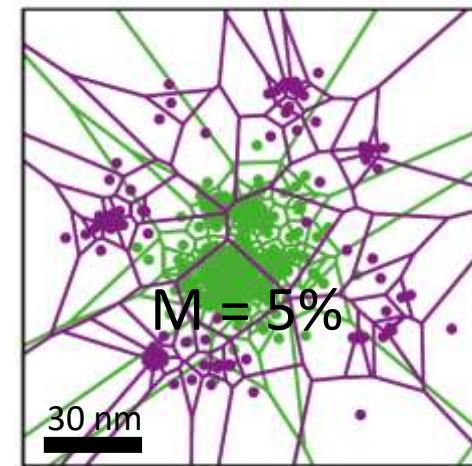
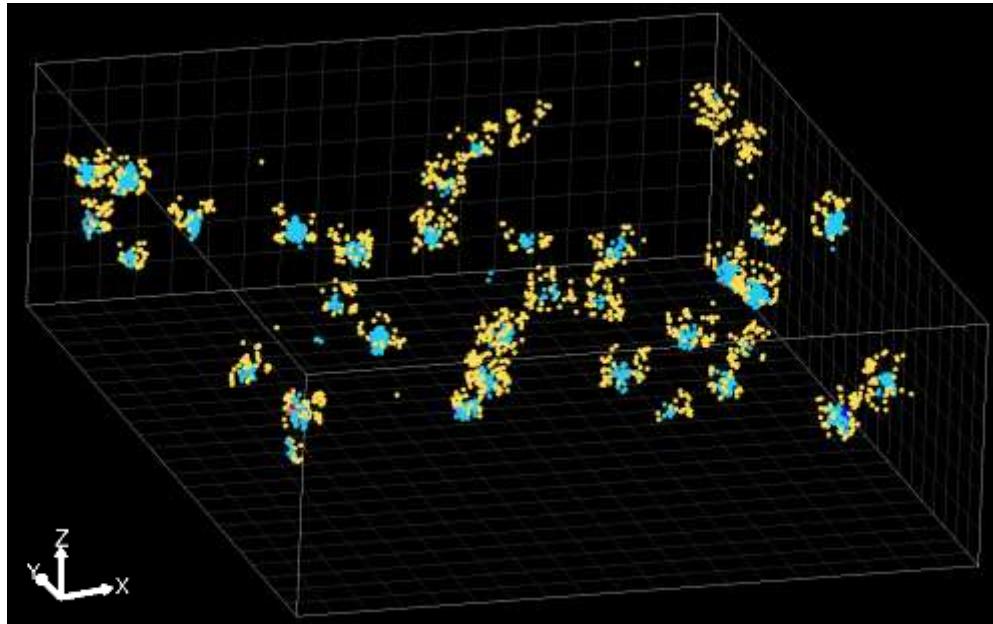


R. Galland



C. Butler

# Experimental controls

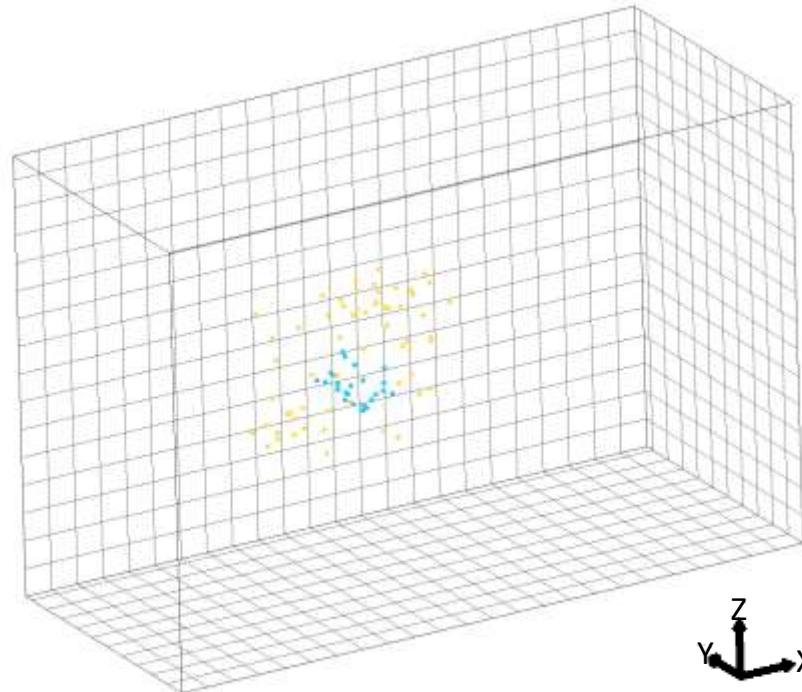


P. Hoess

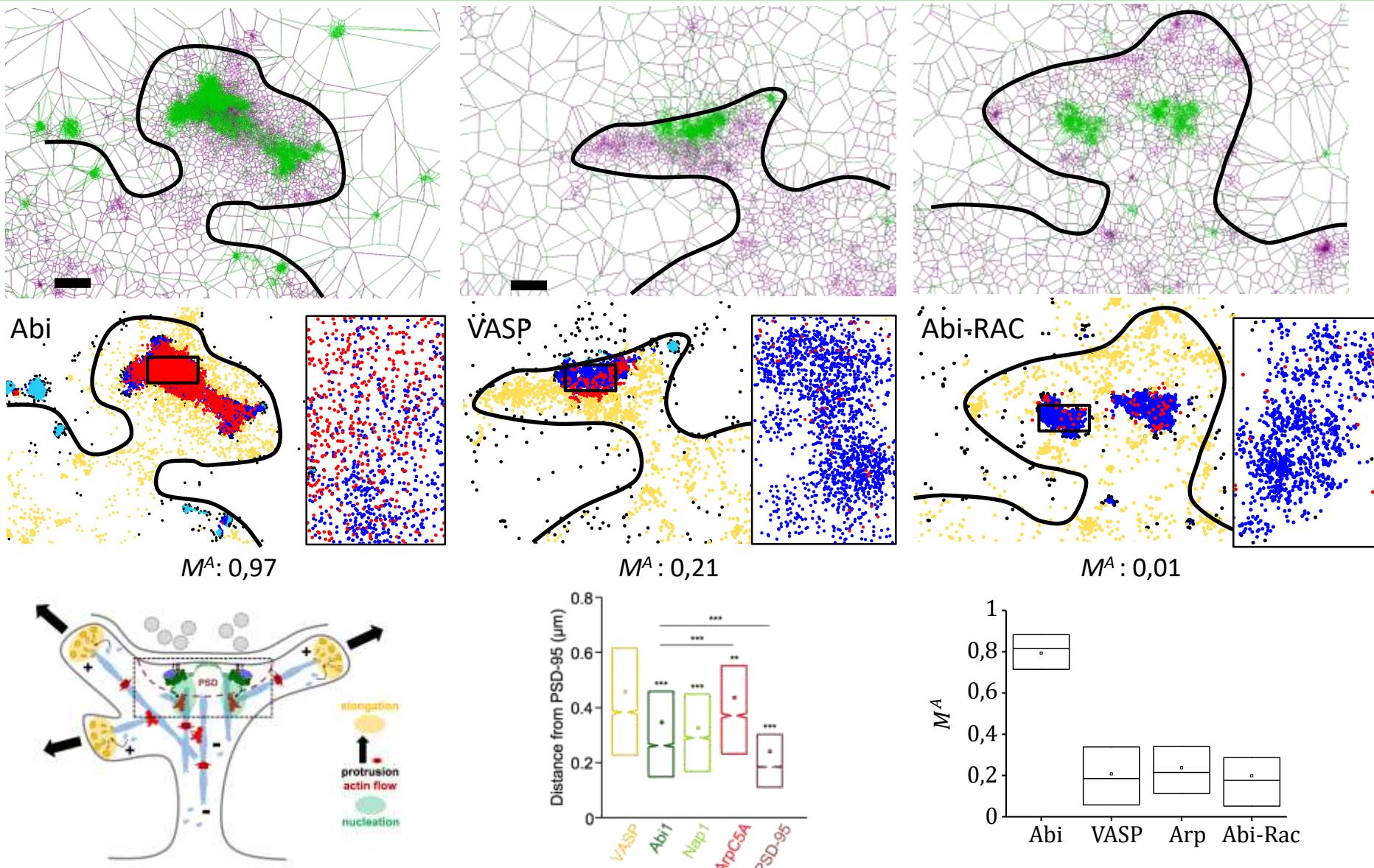


J. Ries

EMBL



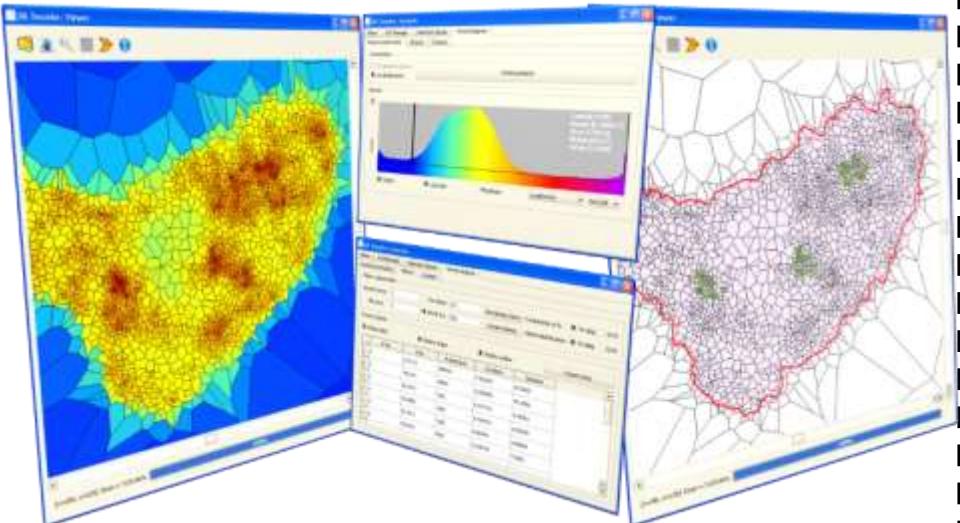
# Colocalisation analysis of λSMLM



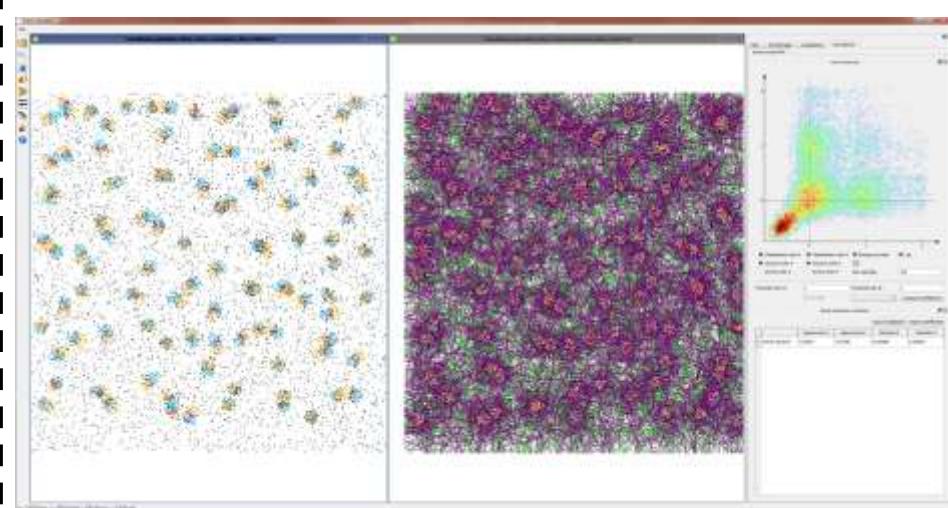
# Software availability



SR-Tesseler



Coloc-Tesseler



<https://github.com/flevet/SR-Tesseler/releases>

- 1-click Windows installer
- Code-source available



<https://github.com/flevet/Coloc-Tesseler/releases>

- 1-click Windows installer
- Code-source still pending

# Misc.

## Quantitative Imaging of the Cell, IINS, Bordeaux



## IINS, Bordeaux

- **O. Rossier**
- **G. Giannone**
- **E. Hosy**
- **D. Choquet**

## EMBL, Heidelberg

- **J. Ries**
- **P. Hoess**



### SR-Tesseler

Source-code : <https://github.com/flevet/SR-Tesseler>

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image.sc

Forum: <https://forum.image.sc/> (tag: « tesseler »)