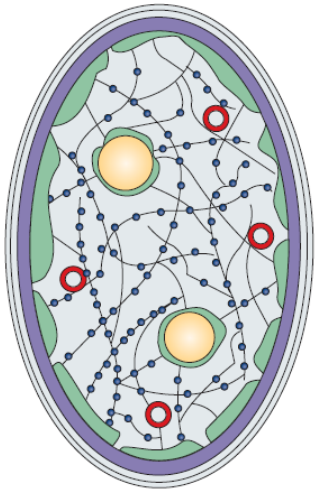
Alterations of chromatin organization induced by an oncogene investigated by super-resolution microscopy

Luca Lanzanò

Department of Physics and Astronomy 'Ettore Majorana', University of Catania, Italy

Nanoscopy, Istituto Italiano di Tecnologia, Genoa, Italy

Motivation



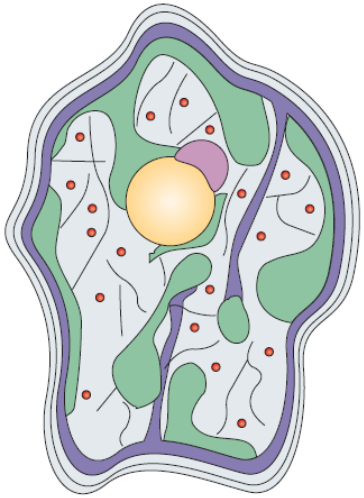
Normal cell nucleus

Oncogenes activation

???

Genomic instability

Alterations in:
DNA replication (“copy”)
DNA transcription (“read”)
DNA damage response (“repair”)



Cancer cell nucleus

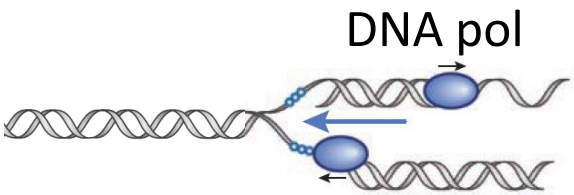
- Activation of oncogenes may promote alterations in the genome that lead to genomic instability, a hallmark of most cancers

It is important to study, at the molecular level, basic processes such as DNA replication and transcription and the role of their alterations in cancer

Motivation

- How can we observe the evolution of basic nuclear processes (e.g. replication, transcription) during the cell cycle?

- Next Generation Sequencing (NGS) methods provide a high-resolution map in space and time of replication and transcription across the genome



Example of Repli-Seq data

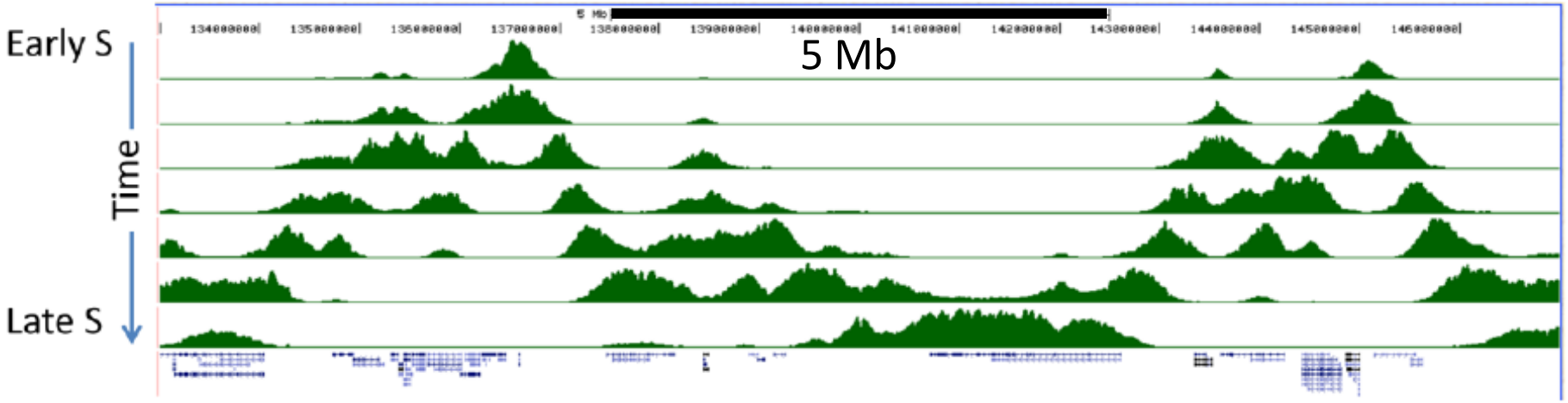
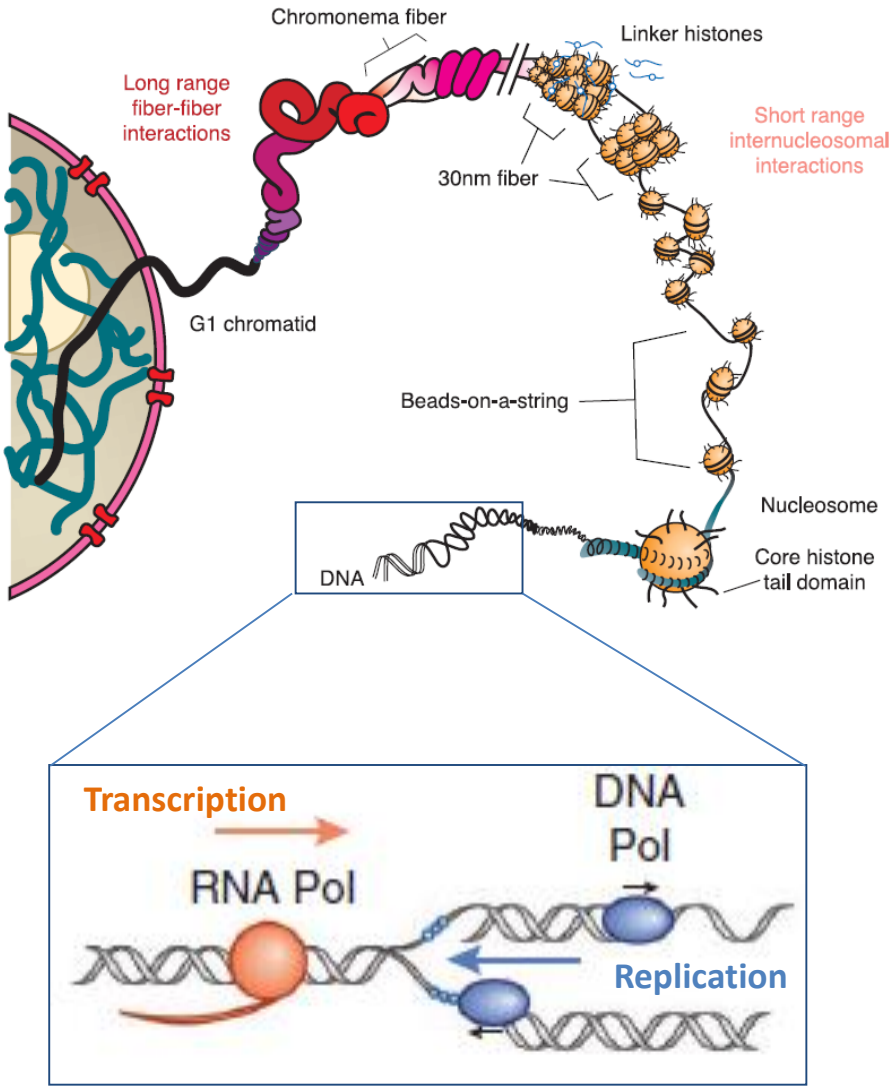


Image Credit: G.I Dellino

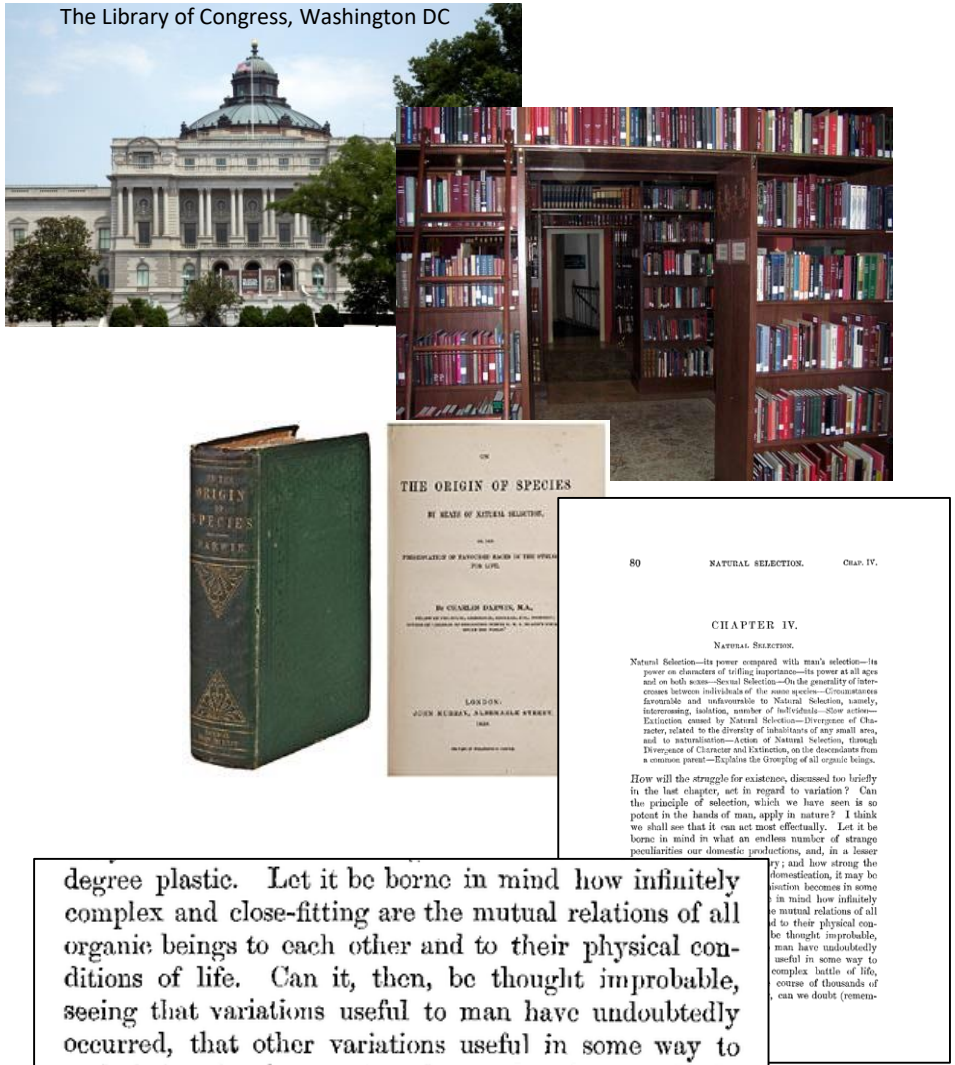
Motivation



However:

- Genomes are more than linear sequences: ~2m of DNA are packed within the small space of a nucleus ~10um
- NGS methods: NO intact nuclei / NO single cells

Motivation



However:

- Genomes are more than linear sequences: ~2m of DNA are packed within the small space of a nucleus ~10um
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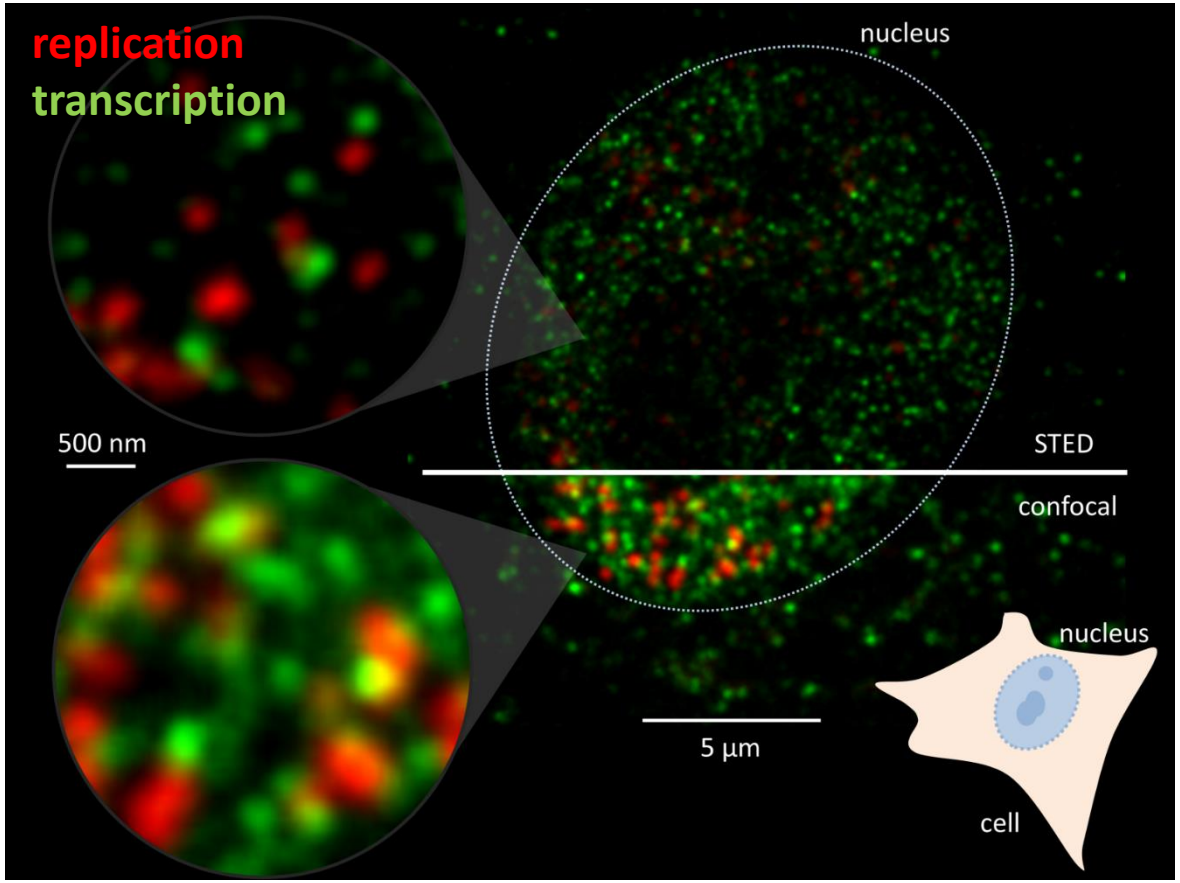
Optical microscopy in intact nuclei

- We can label specific molecules
- We can improve the spatial resolution

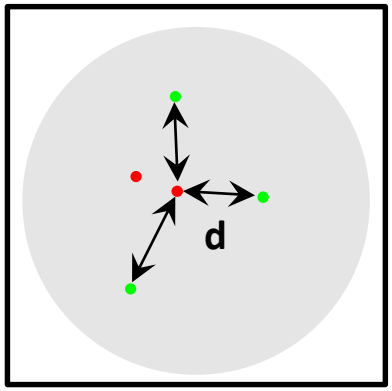


Resolution helps but…

How do we extract data from images?

Analysis of spatial organization at the nanoscale

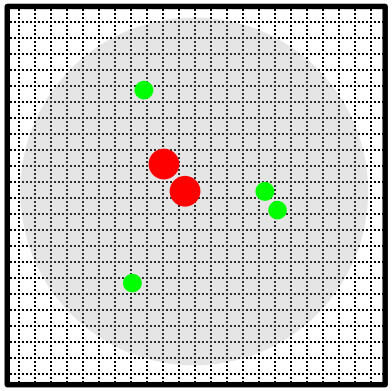
Object-based methods



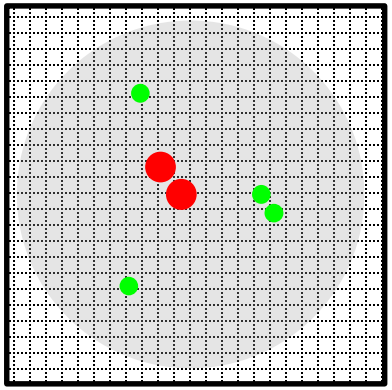
(x_i , y_i) (x_k , y_k)

- Full description of the spatial distribution:
  i) **Locations of higher proximity**
  ii) **Extraction of nanoscale distances**
- Require segmentation into objects
- Methods of choice for SMLM

SR Image



Pixel-based methods



$I_1(x,y)$ $I_2(x,y)$

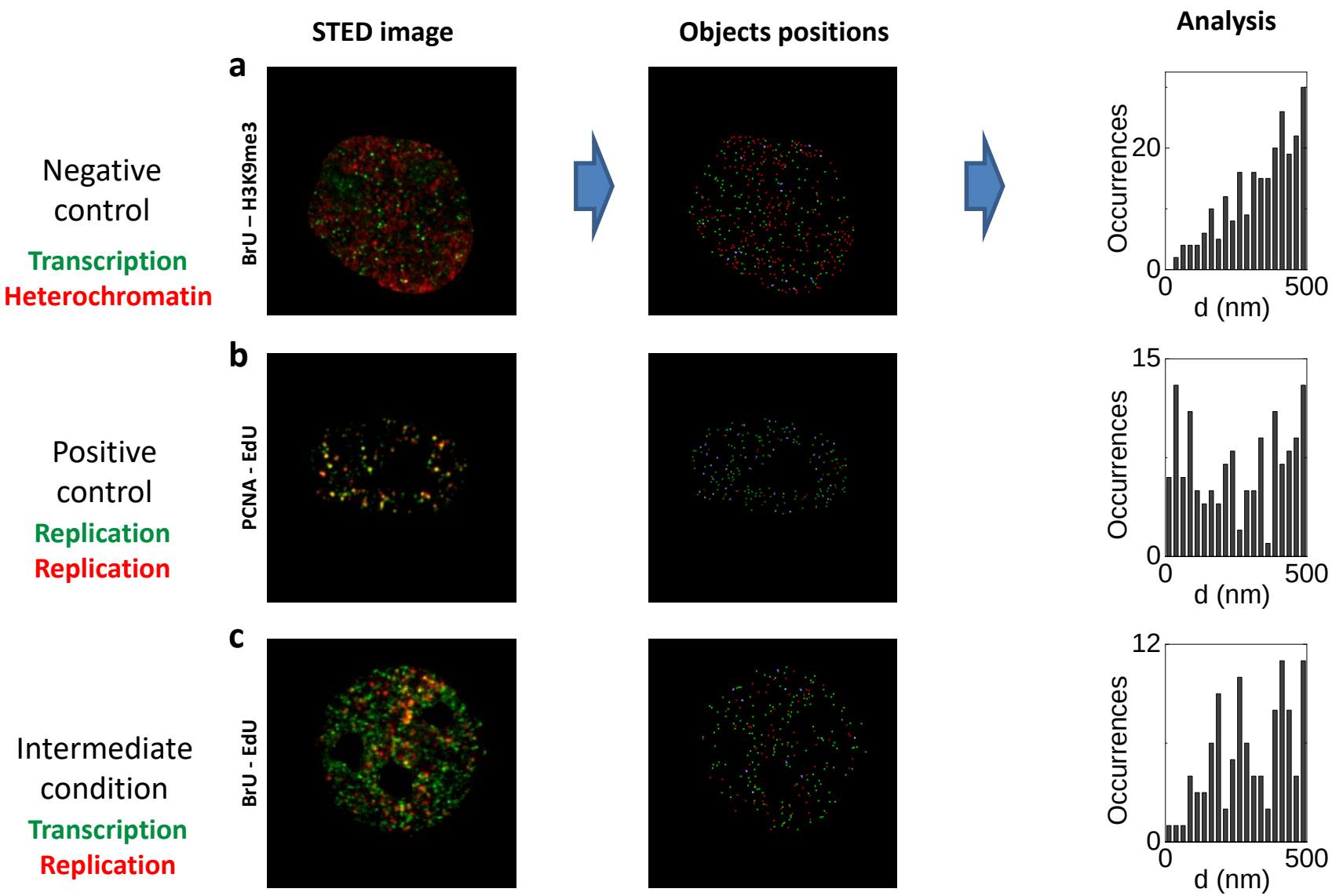
- Do not require segmentation
- Average information
- Pearson, Manders, etc
- **ICCS** (spatial correlations)

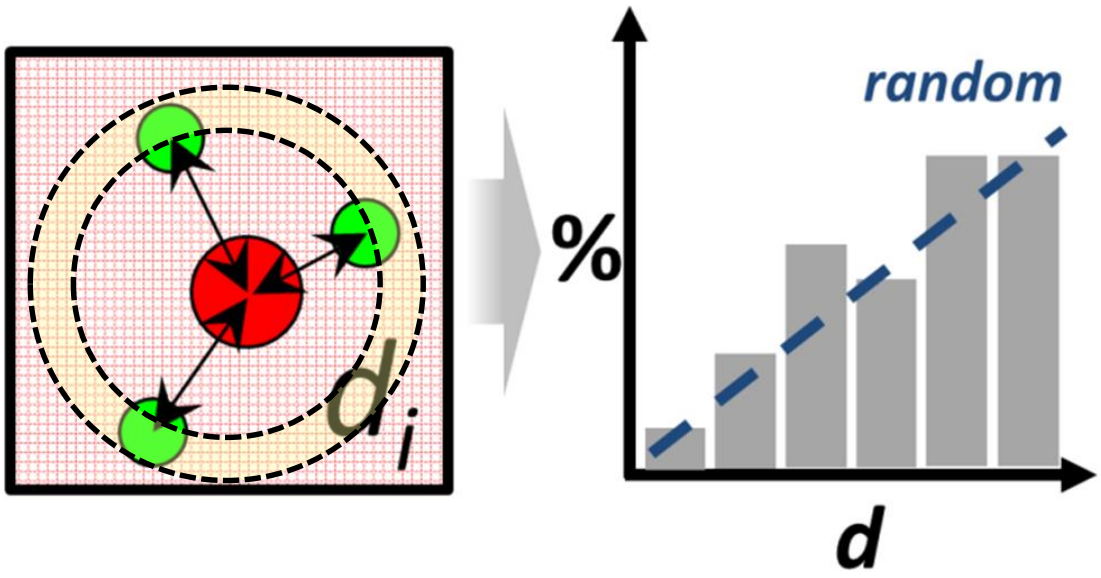
Dynamic methods

- FCCS (temporal correlations)
- **ICCS** (spatio-temporal correlations)

Lagache et al, *Cytom A* 2015 ; Arena et al, *WIREs Dev Biol* 2017

Object-based: Example

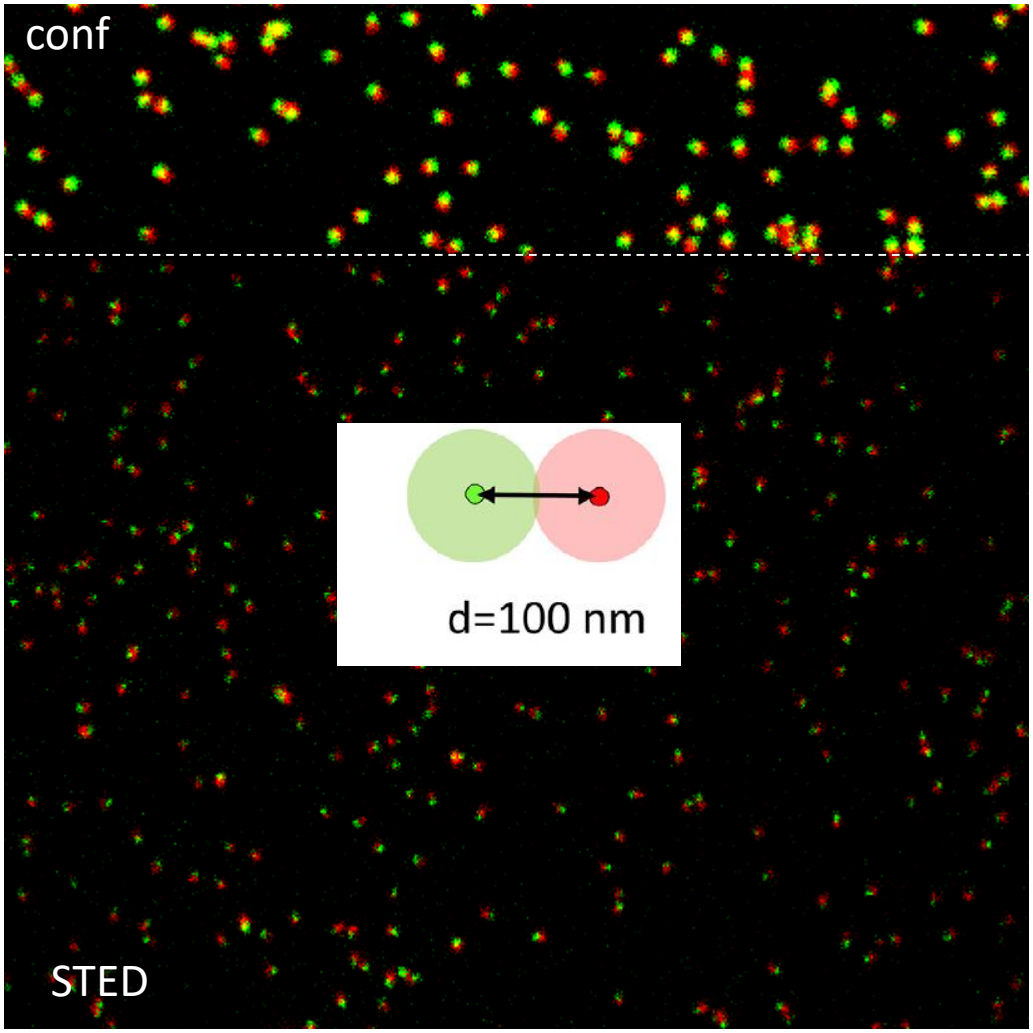


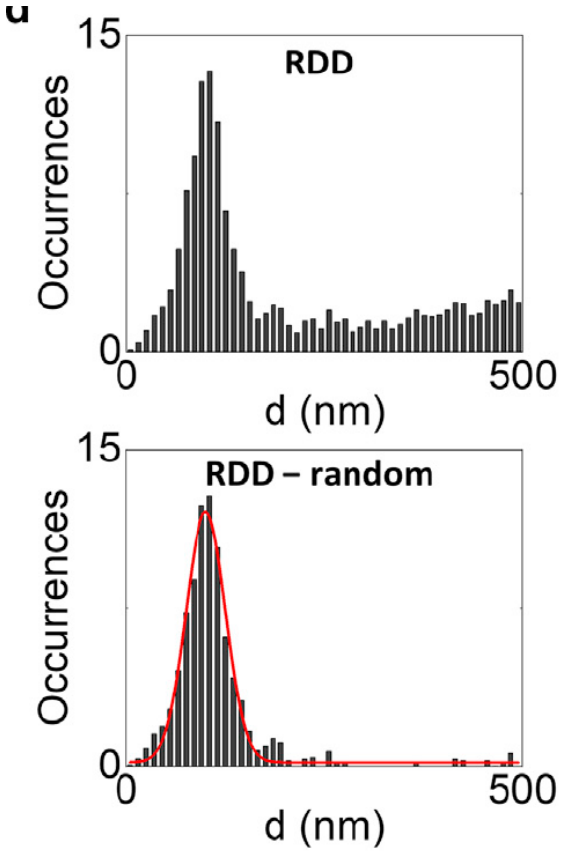


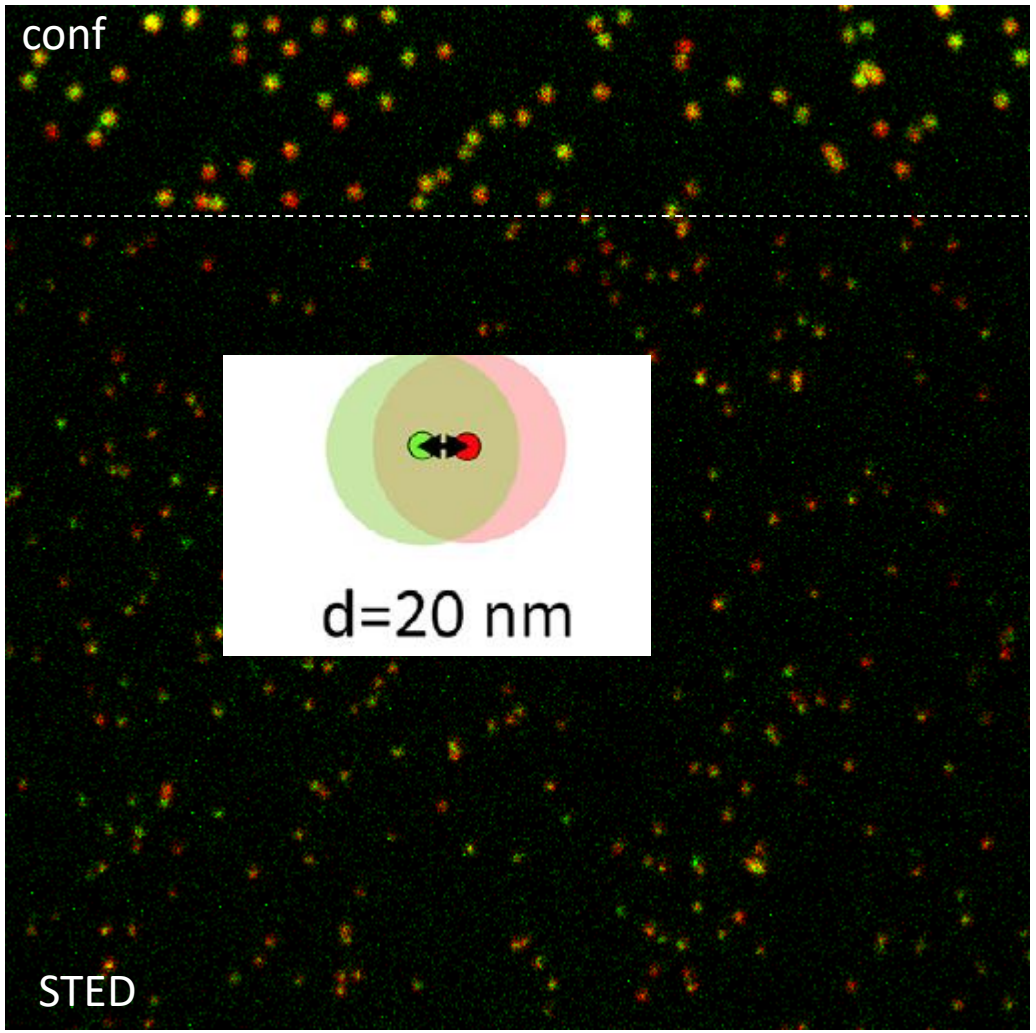
The area of the ring increases linearly with distance
N(d)~d

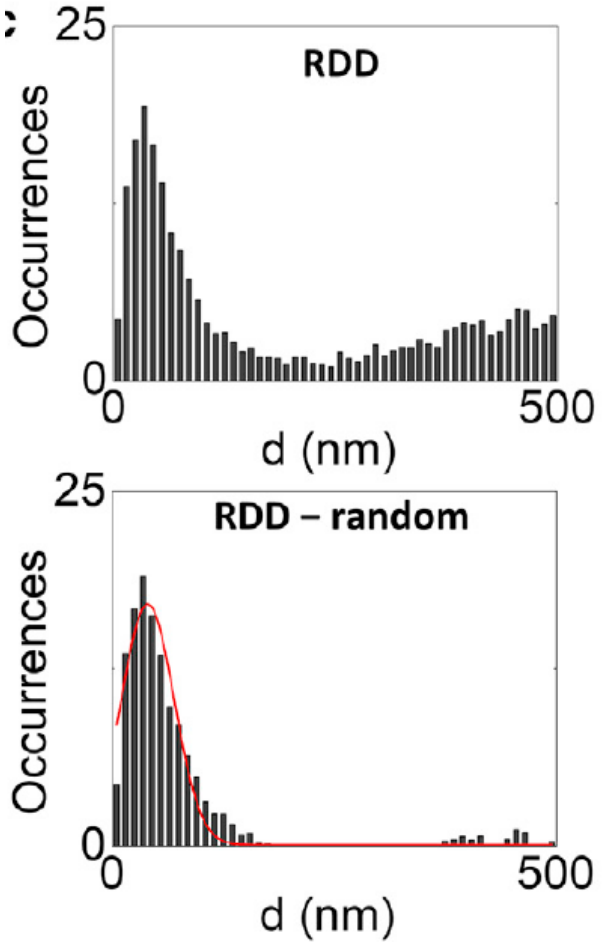
Uncorrelated particles (random) = linear

Object-based example: nanorulers

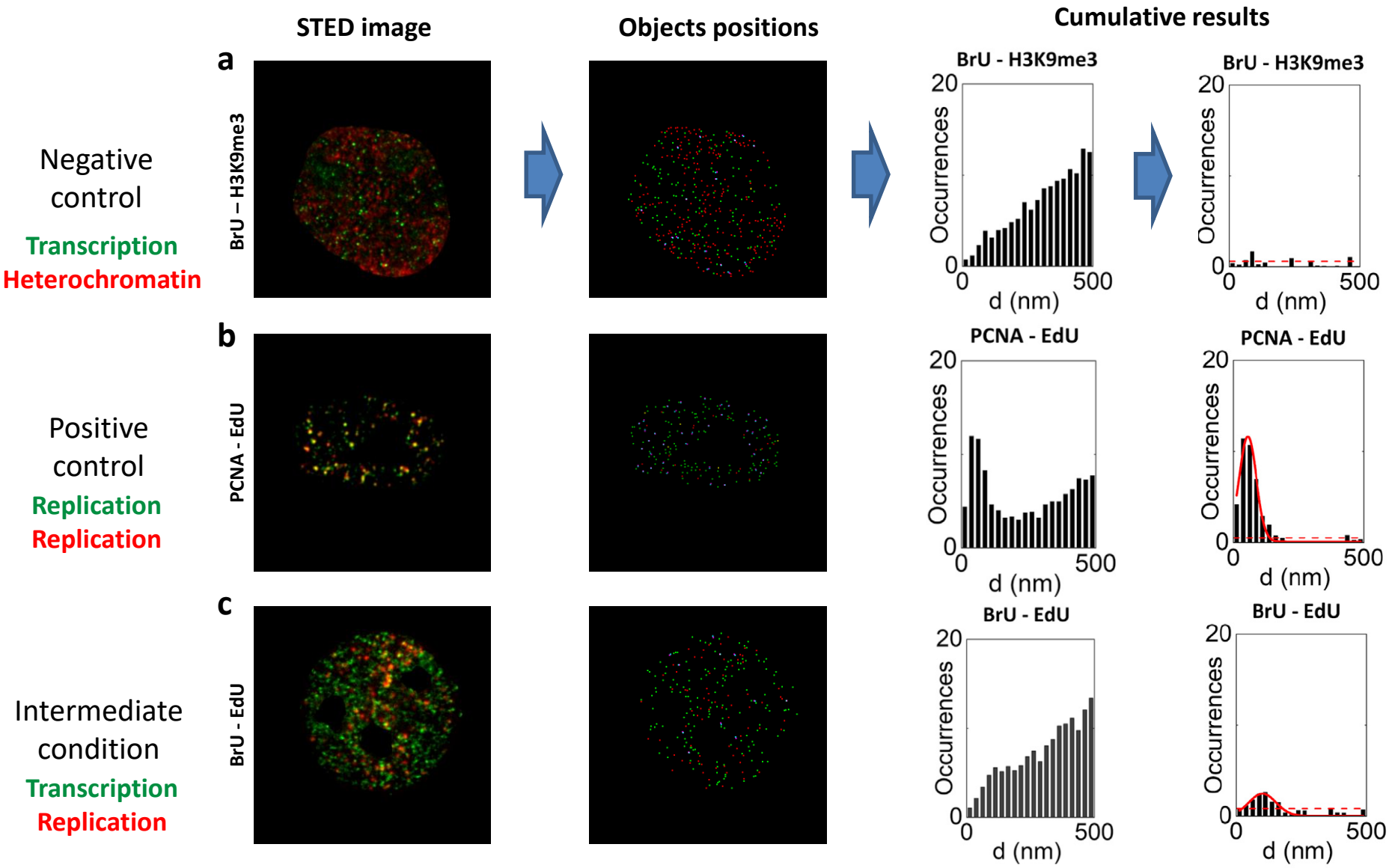


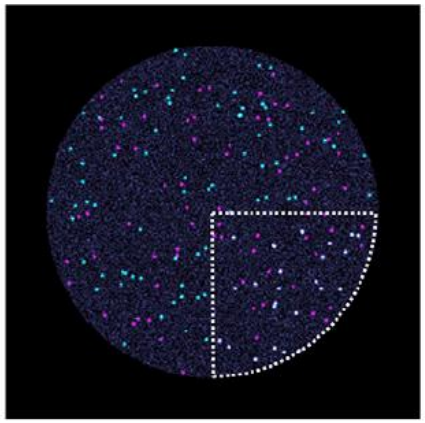


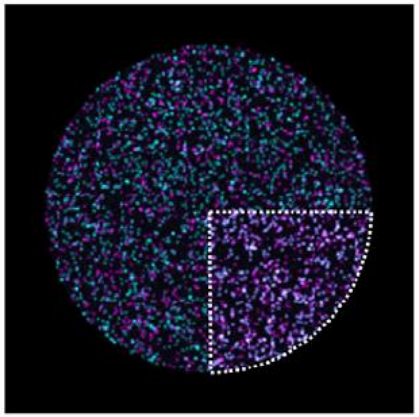

conf
d=20 nm
STED


RDD
Occurrences
25
0
0
500
d (nm)
RDD – random
Occurrences
25
0
0
500
d (nm)

Object-based: Example

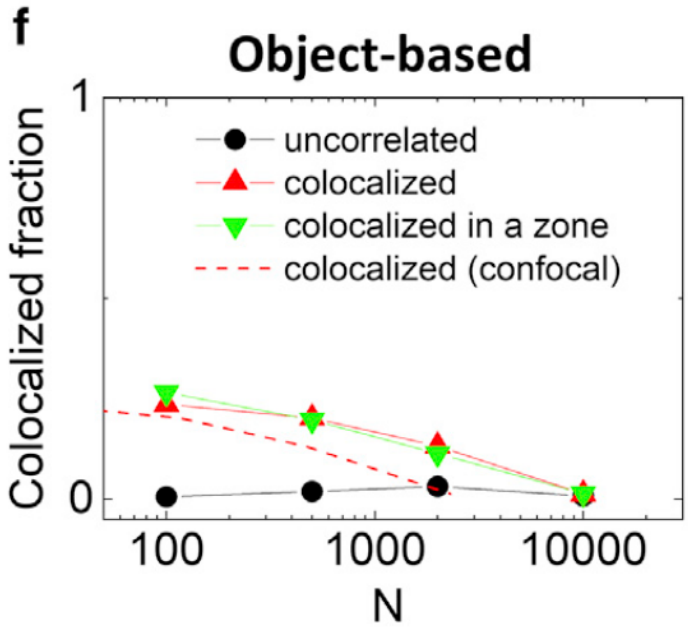






Simulations with 25% of colocalized particles

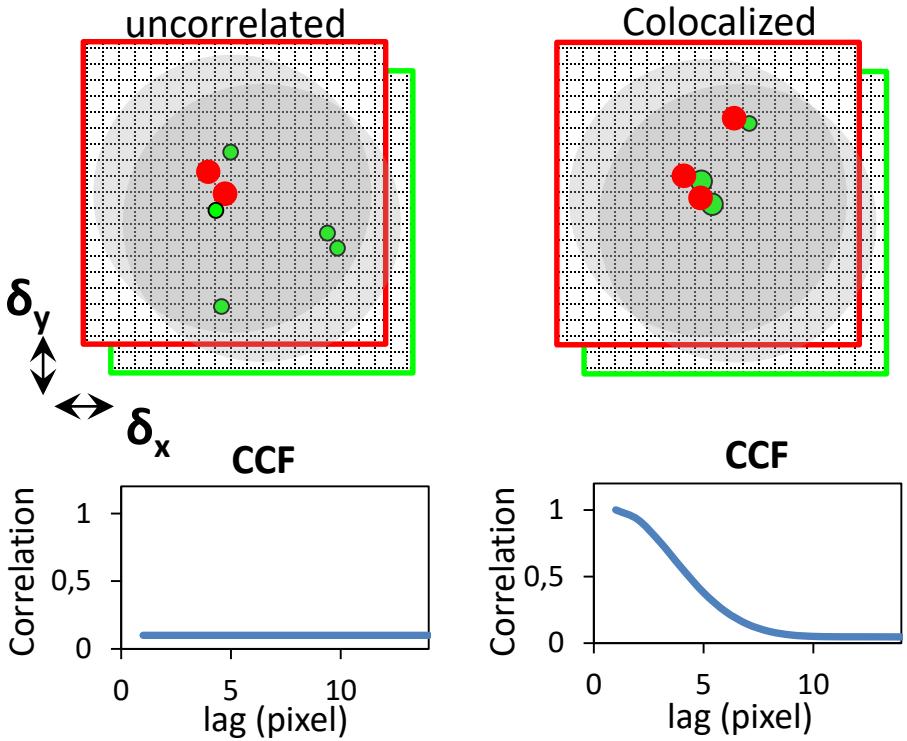
Different density



- Full description of the spatial distribution
- Segmentation less accurate at high concentration of particles

Colocalization by image cross-correlation spectroscopy (ICCS)

$$G_{i,j}(\delta_x, \delta_y) = \frac{\langle I_i(x,y) I_j(x+\delta_x, y+\delta_y)\rangle}{\langle I_i(x,y)\rangle \langle I_j(x,y)\rangle} - 1$$



i=j autocorrelation function (ACF)
-size and number of particles in one channel

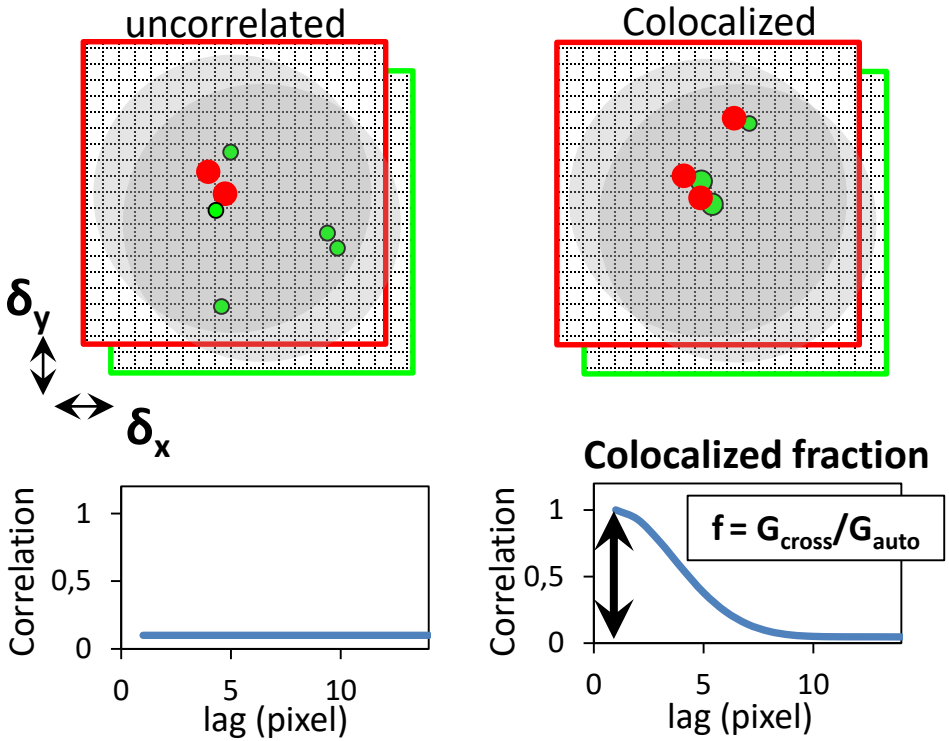
i≠j cross-correlation function (CCF)
-correlated particles

2D function (under conditions of symmetry can be reduced to 1D)

Comeau et al, Biophys J 2006
Oneto et al, Biophys J 2019 github.com/llanzano/ICCS

Colocalization by image cross-correlation spectroscopy (ICCS)

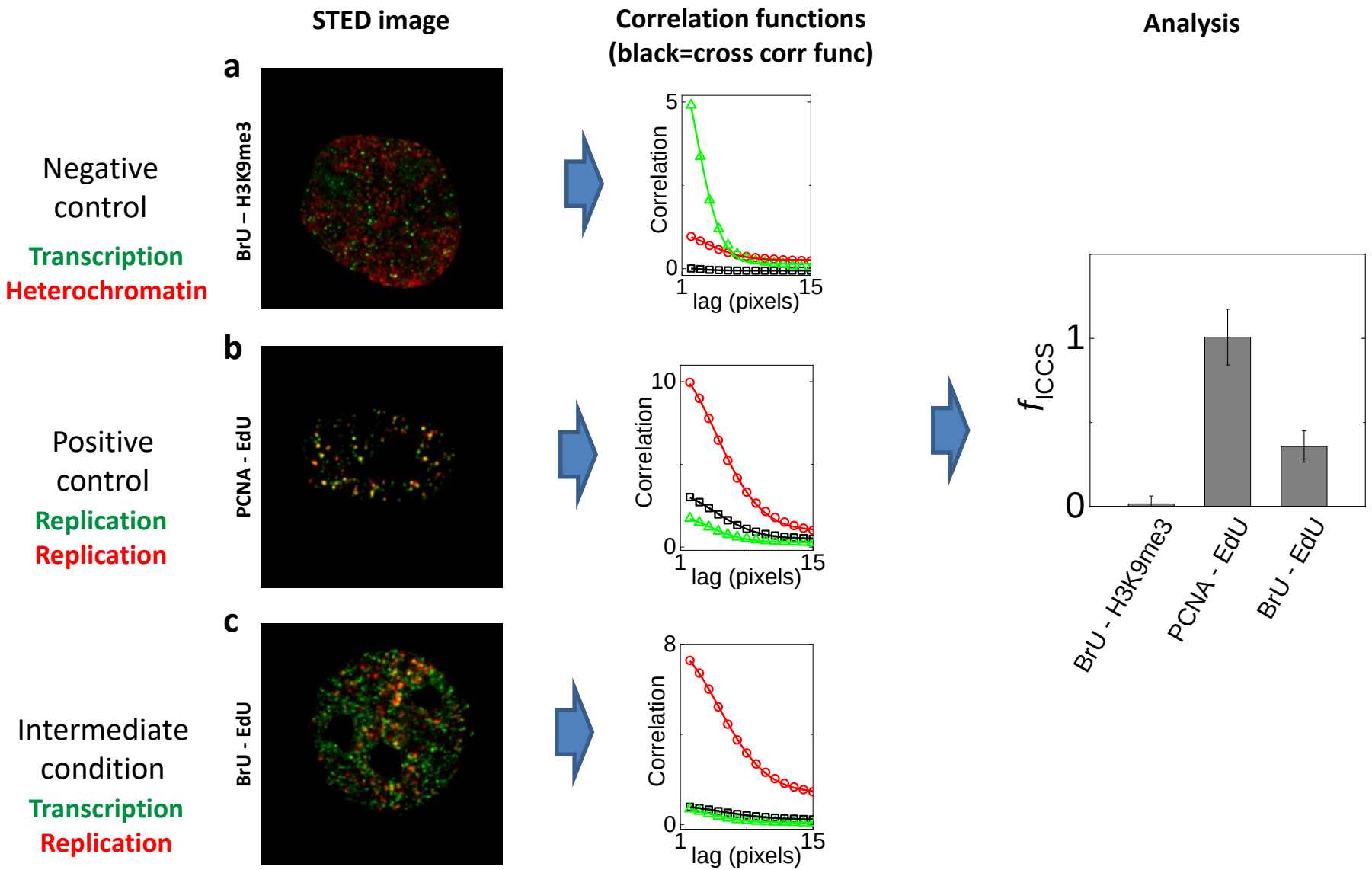
$$G_{i,j}(\delta_x, \delta_y) = \frac{\langle I_i(x,y) I_j(x+\delta_x, y+\delta_y)\rangle}{\langle I_i(x,y)\rangle \langle I_j(x,y)\rangle} - 1$$

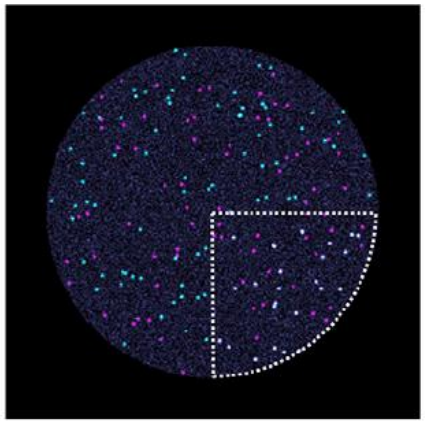


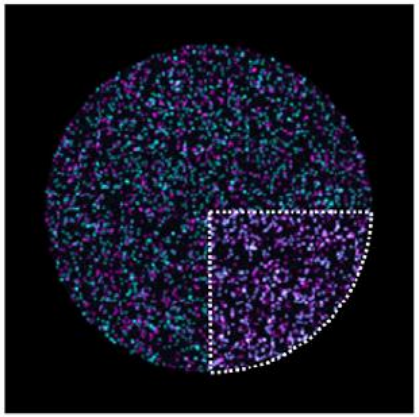
Perfectly colocalized	f=1
Uncorrelated	f=0
Anti-correlated	f=-1

Comeau et al, Biophys J 2006
Oneto et al, Biophys J 2019 github.com/llanzano/ICCS

ICCS Example

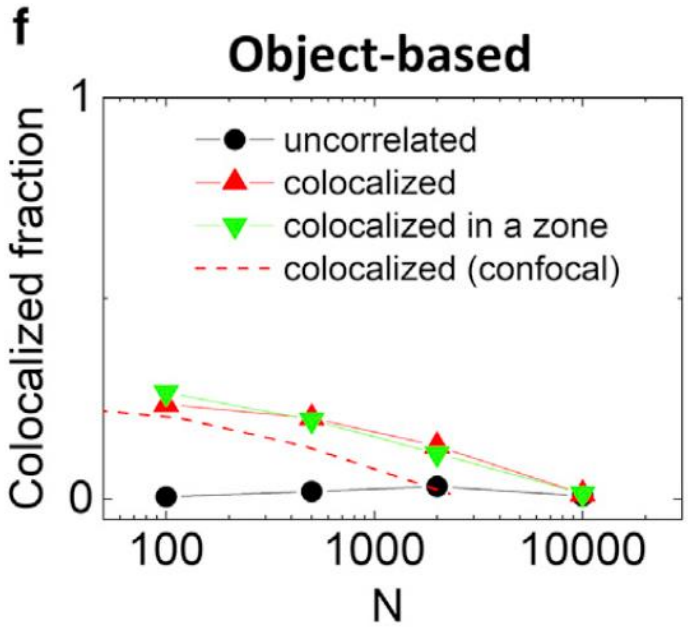


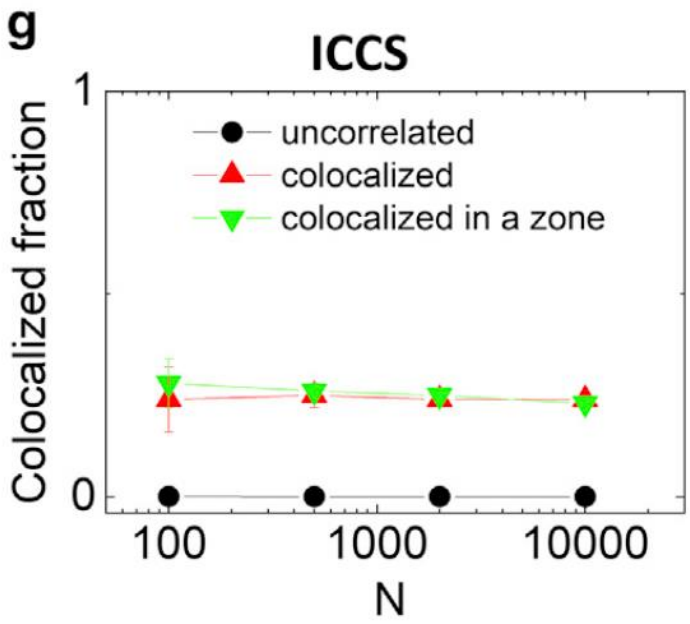


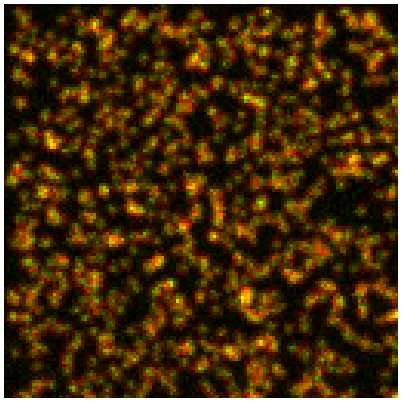


Simulations with 25%
of colocalized particles

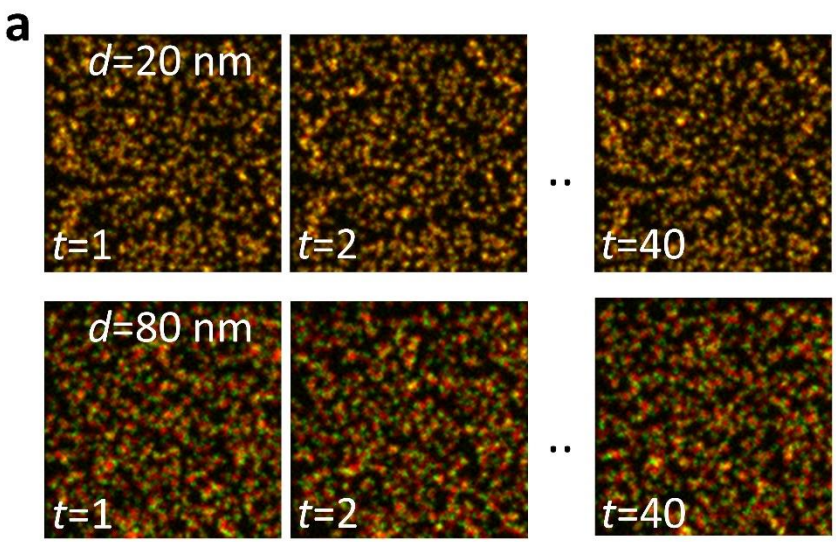
Different density

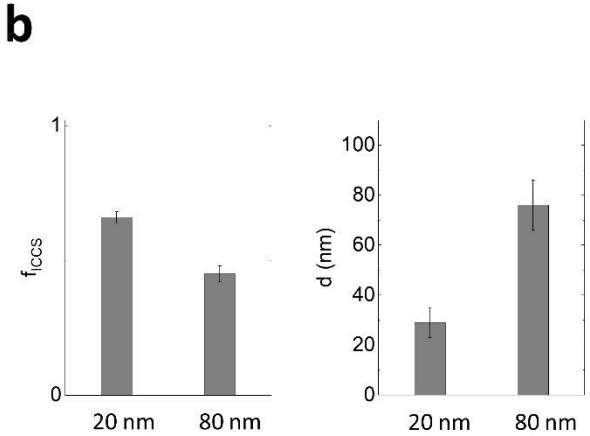




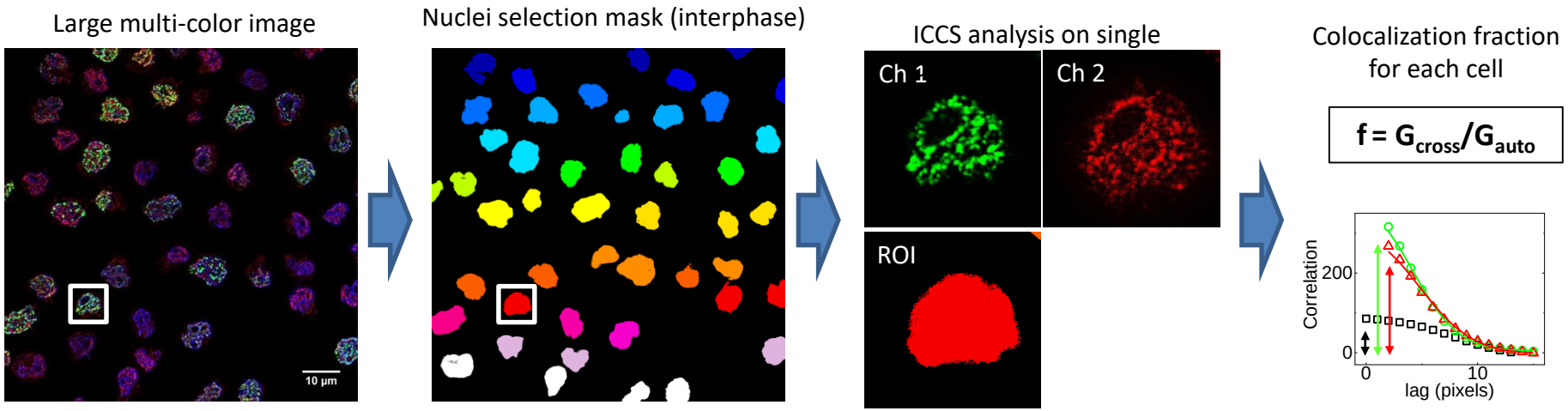


- ICCS is a dynamic method
- Can also be applied to images of particles (molecules) in motion
- Same formalism of techniques like RICS, STICS, etc





Analysis on large number of cells

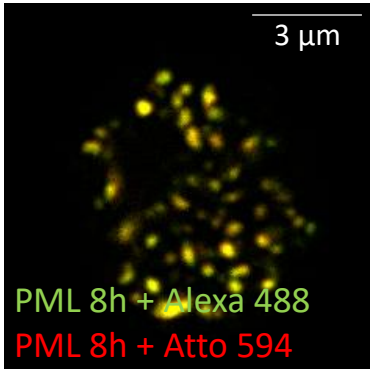


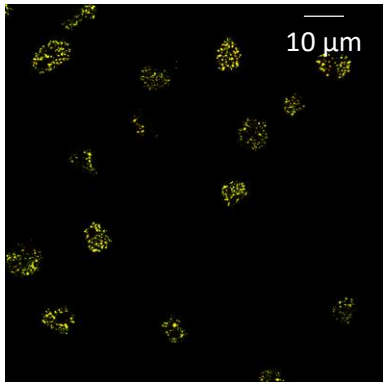
We adapted the ICCS algorithm to the analysis of a large number of cells:

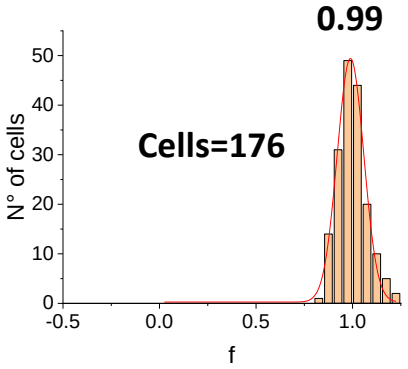
- Single cells from a large field of view are automatically selected and analyzed sequentially
- The spatial auto- (ACF) and cross-correlation function (CCF) are calculated and fitted
- A value of colocalization fraction f is extracted for each cell, normalizing the amplitude of the CCF to that of the ACFs

ICCS on large number of cells

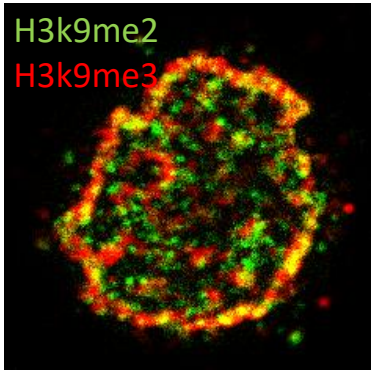
Positive control

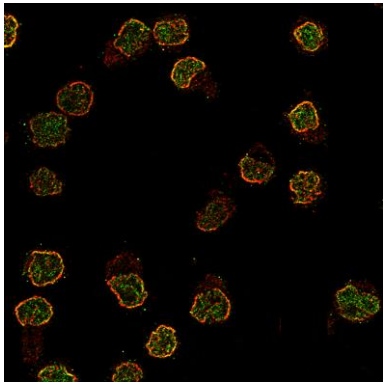


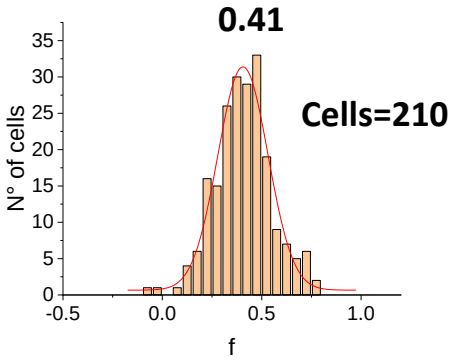




Intermediate condition

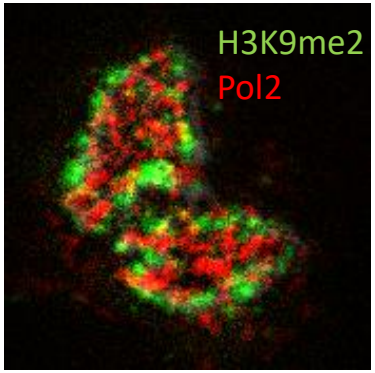


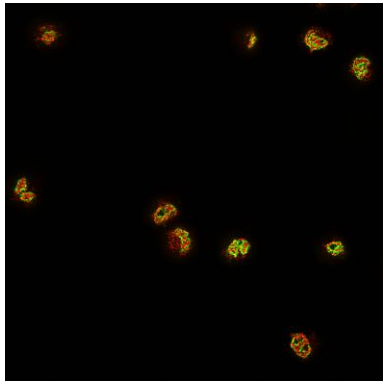


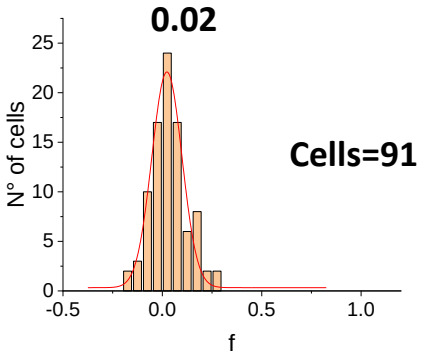


Negative control

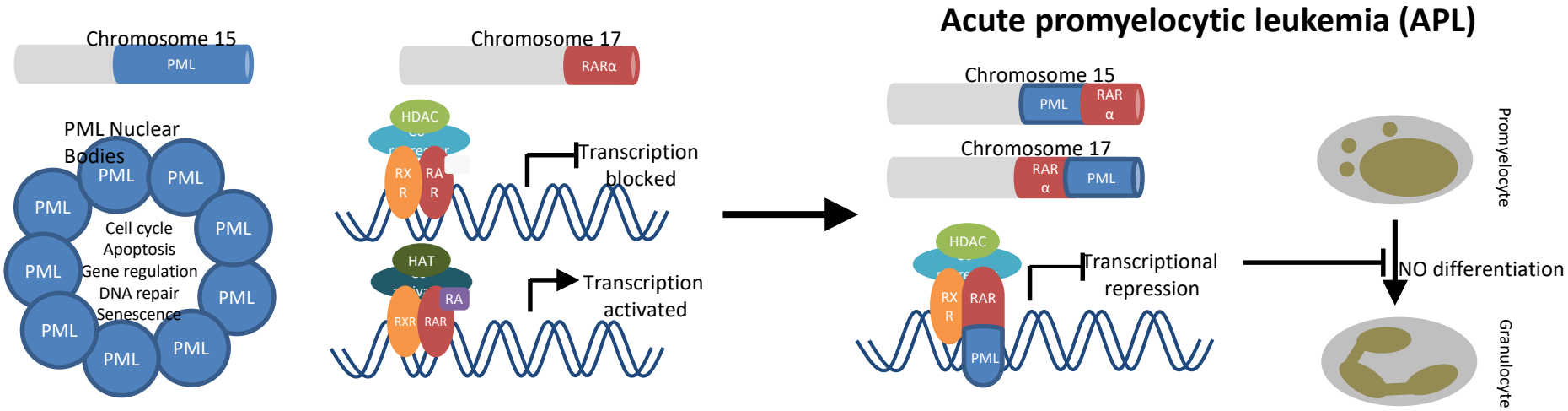
Transcription
Heterochromatin



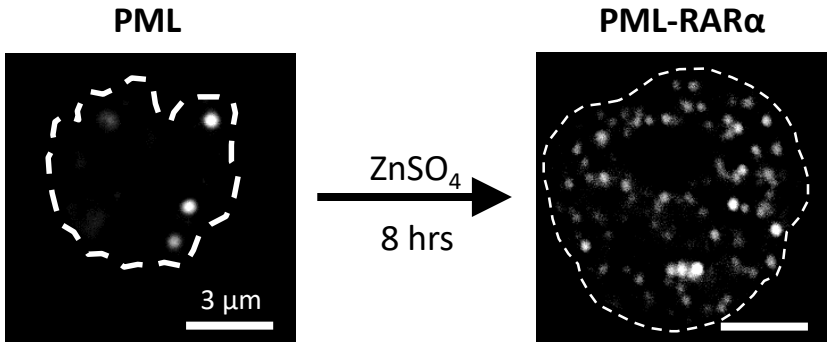




Application: the model of oncogene activation



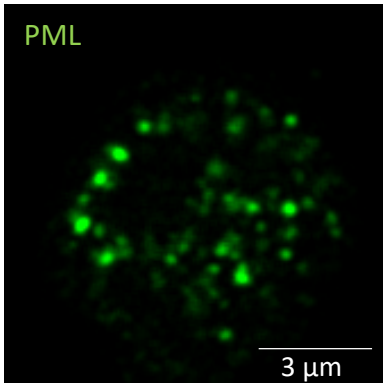
U937-PR9 cells:
In vitro model of APL
Non-adherent cells



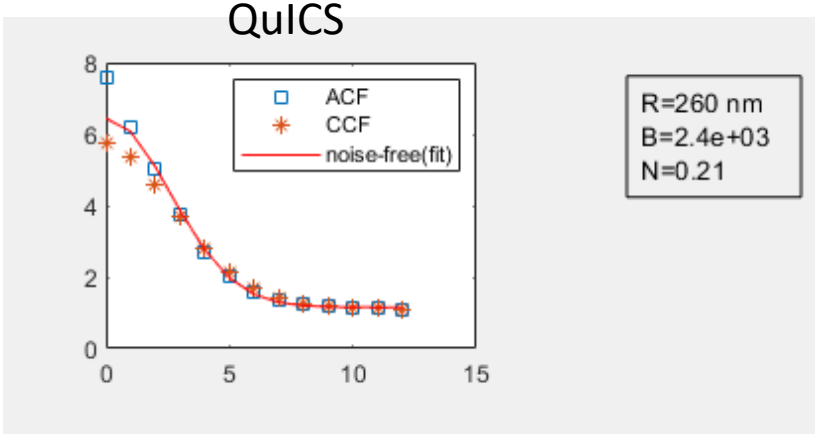
Question:
Does the oncogene (PML-RARα) affect the spatio-temporal organization of nuclear processes?

Imaging of PML-RARa speckles in the activated sample

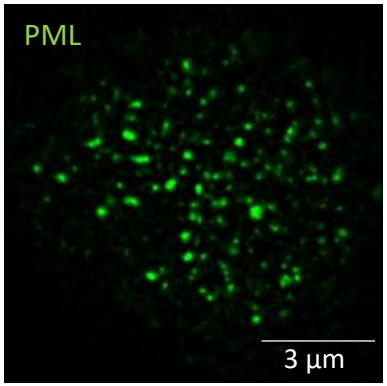
confocal



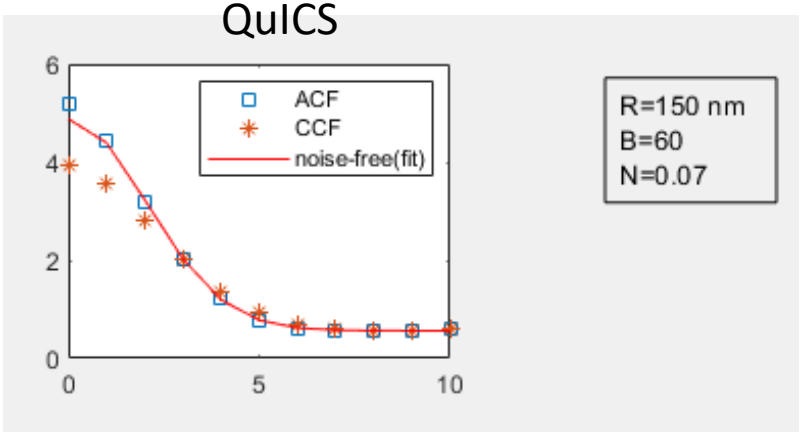
QuICS



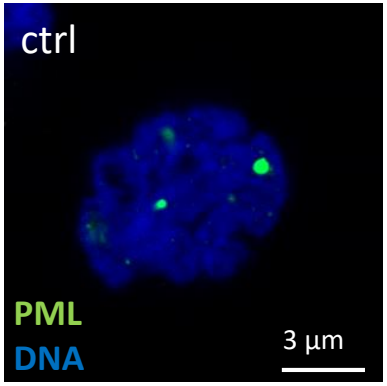
Tau-STED

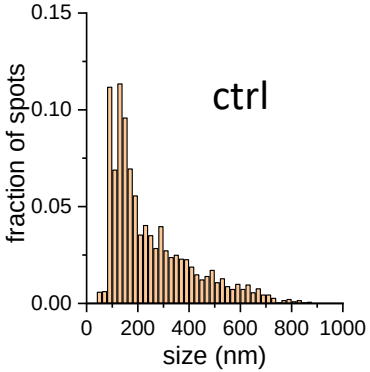


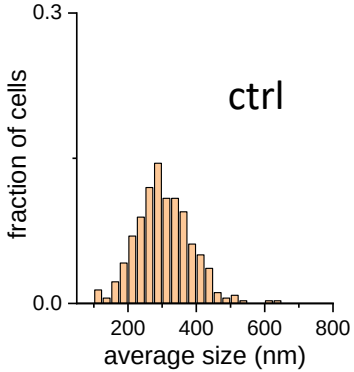
QuICS

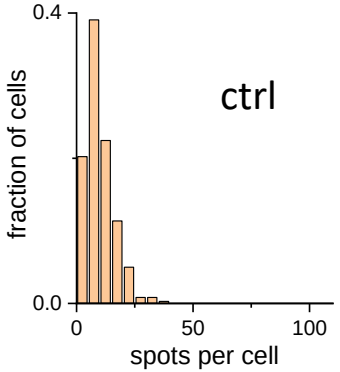


Object analysis on PML spots

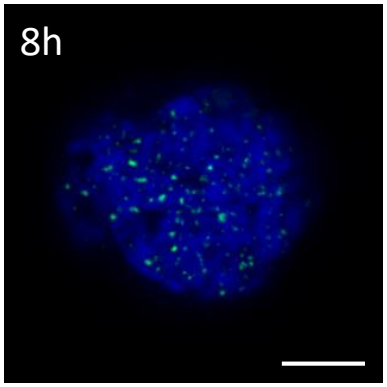


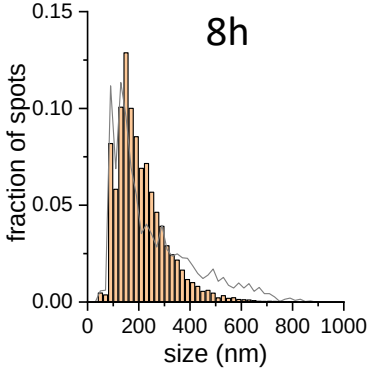


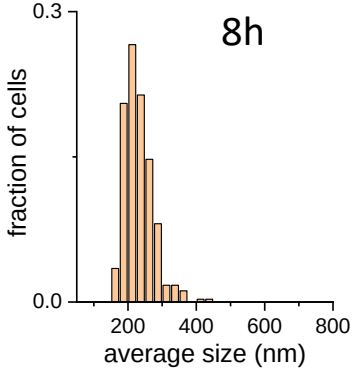


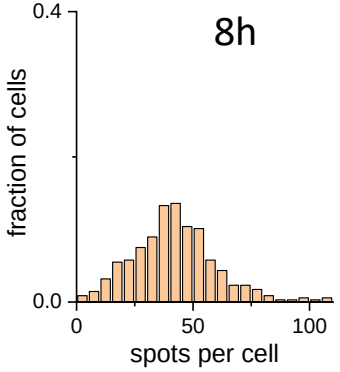


Induction of PML-RARα





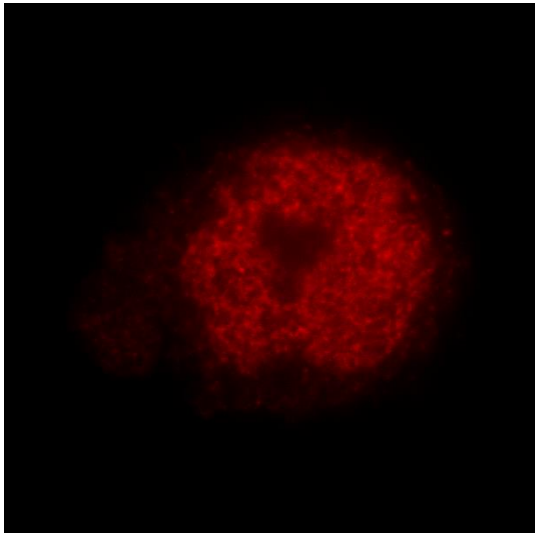




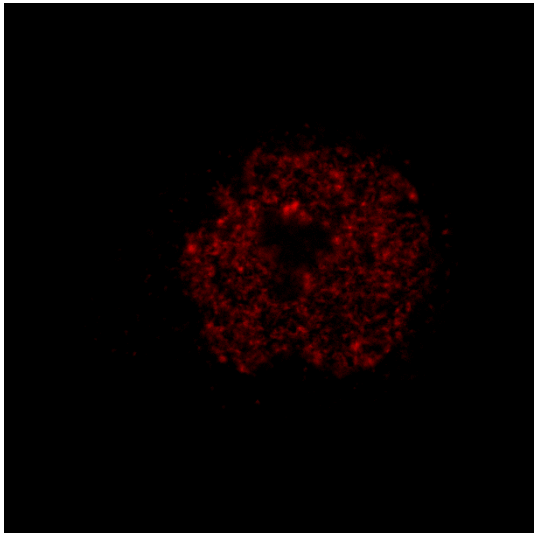
- Size of spots decreases, number of spots increases
- Heterogeneous response

Imaging of Pol2 (transcription foci) – (Leica Stellaris 8)

STED

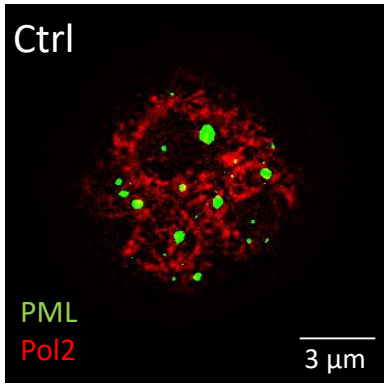


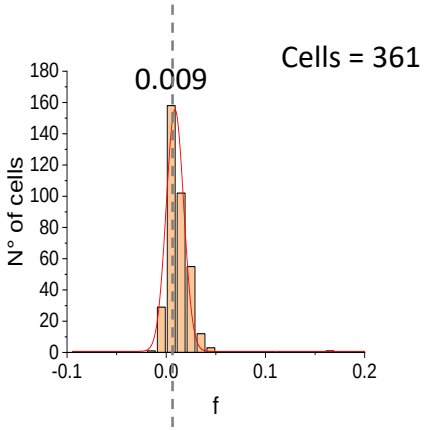
Tau-STED

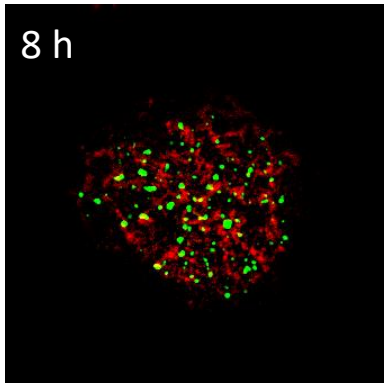


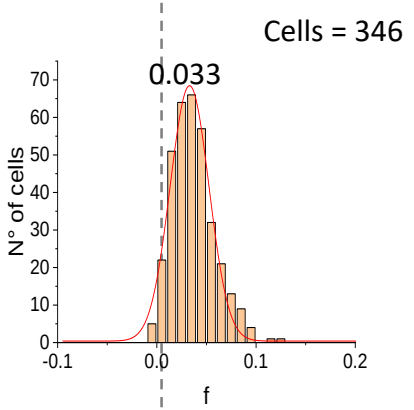
ICCS of PML vs transcription

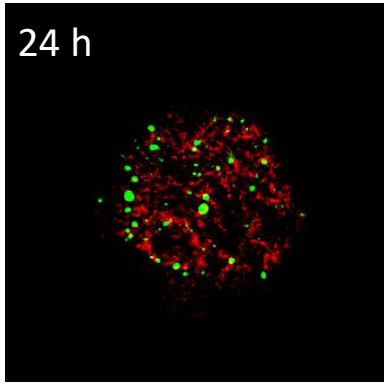
Time after Zinc treatment

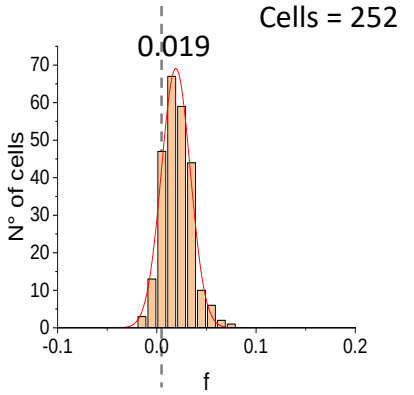






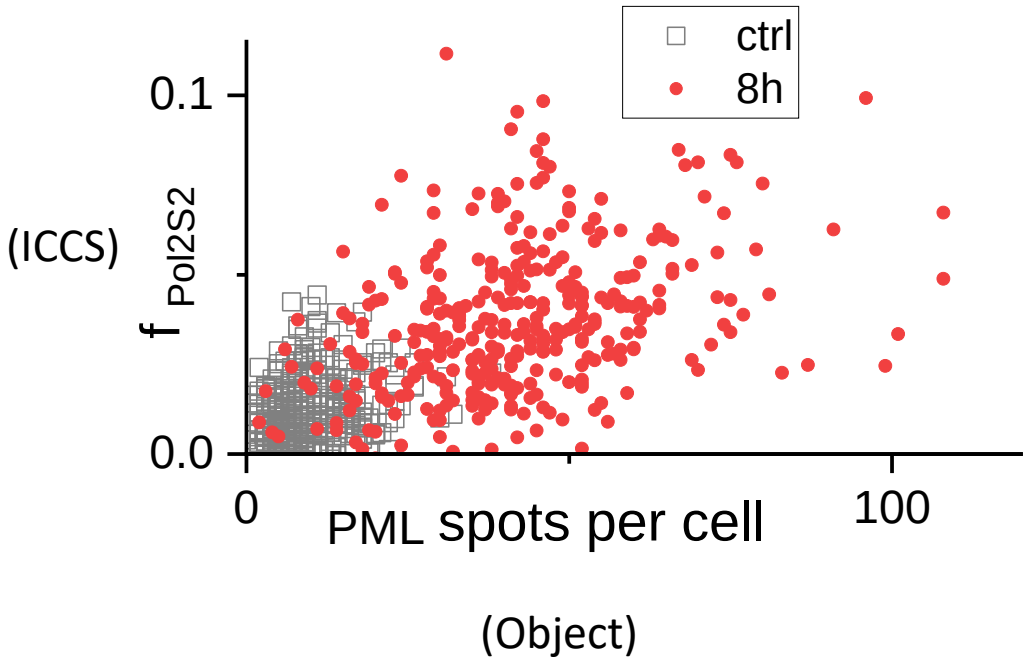




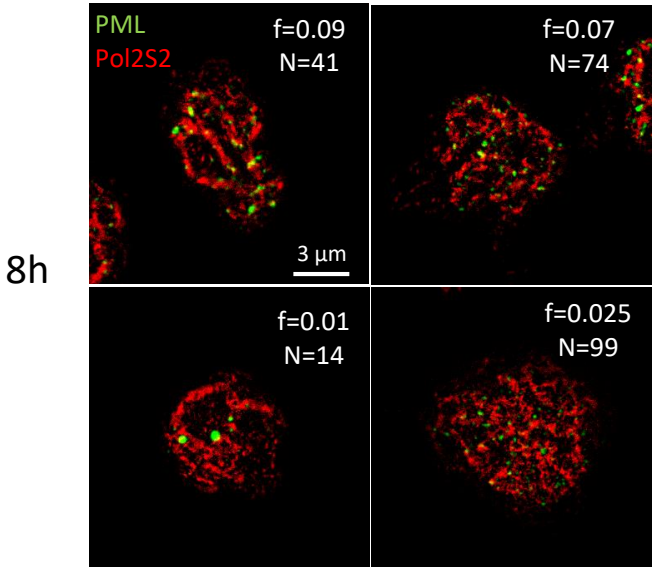


- Before activation, PML does not colocalize with pol2 foci
- After activation, a fraction of PML-RARα colocalizes with pol2 foci

ICCS + object analysis



Activated sample



- In the activated sample, the cells do not respond homogeneously!

Summary

- Correlation spectroscopy can be used to extract average parameters from nanoscopy images (e.g. colocalization) together with object-based analysis
- These tools can be applied to the study of nanoscale organization of the genome
- The oncogene PML-RARα induces an altered distribution of PML in the nuclear space, potentially leading to increased DNA damage

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iit NANOSCOPY

G Vicidomini

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