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

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 ~10μm

• NGS methods: NO intact nuclei / NO single cells
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
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

- 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

Pixel-based methods

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

Dynamic methods

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

Object-based: Example

- **Negative control**
  - Transcription
  - Heterochromatin

- **Positive control**
  - Replication
  - Replication

- **Intermediate condition**
  - Transcription
  - Replication

**STED image**

- a. BrU - H3K9me3
- b. PCNA - EdU
- c. BrU - EdU

**Objects positions**

**Analysis**

- Occurrences vs. d (nm) for each condition:
  - Negative control
  - Positive control
  - Intermediate condition
The area of the ring increases linearly with distance \( N(d) \sim d \)

Uncorrelated particles (random) = linear
Object-based example: nanorulers

STED

conf

$\text{d}=100\text{ nm}$

$\text{Occurrences}$

$\text{d (nm)}$

$\text{Occurrences}$

$\text{d (nm)}$

RDD

RDD - random
d = 20 nm

Confocal

STED
Object-based: Example

**STED image**

**a**

Negative control

Transcription

Heterochromatin

BrU – H3K9me3

**b**

Positive control

Replication

PCNA – EdU

**c**

Intermediate condition

Transcription

Replication

BrU – EdU

**Cumulative results**

BrU – H3K9me3

Occurrences vs d (nm)

PCNA – EdU

Occurrences vs d (nm)

BrU – EdU

Occurrences vs d (nm)
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)

\[ G_{ij}(\delta_x, \delta_y) = \frac{\langle I_i(x,y)I_j(x+\delta_x,y+\delta_y) \rangle}{\langle I_i(x,y) \rangle \langle I_j(x,y) \rangle} - 1 \]

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

- \( i\neq j \): 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)

\[ G_{ij}(\delta_x, \delta_y) = \frac{\langle I_i(x,y)I_j(x+\delta_x,y+\delta_y) \rangle}{\langle I_i(x,y) \rangle \langle I_j(x,y) \rangle} - 1 \]

Colocalized

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

Negative control
Transcription
Heterochromatin

Positive control
Replication
Replication

Intermediate condition
Transcription
Replication

Analysis
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.
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

0.99

Cells=176

0.41

Cells=210

0.02

Cells=91

PML 8h + Alexa 488
PML 8h + Atto 594

H3k9me2
H3k9me3

H3K9me2
Pol2

3 μm

10 μm
Application: the model of oncogene activation

Acute promyelocytic leukemia (APL)

Chromosome 15
PML

Chromosome 17
RARα

PML Nuclear Bodies
PML

PML Cell cycle
Apoptosis
Gene regulation
DNA repair
Senescence

Cell cycle
Apoptosis
Gene regulation
DNA repair
Senescence

Transcription blocked

Transcription activated

U937-PR9 cells:
In vitro model of APL
Non-adherent 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-RARα speckles in the activated sample

confocal

QuICS

PML

3 µm

QuICS

PML

3 µm

R=260 nm
B=2.4e+03
N=0.21

R=150 nm
B=60
N=0.07
Object analysis on PML spots

- Size of spots decreases, number of spots increases
- Heterogeneous response
Imaging of Pol2 (transcription foci) – (Leica Stellaris 8)
ICCS of PML vs transcription

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

Elena Cerutti
Morgana D’Amico
Elisabetta Di Franco
Anna Privitera

TRIDEO ID. 17215: Super-resolution imaging of the organization of transcription and replication during oncogene-induced replicative stress
My First AIRC Grant ID. 21931: Optical nanoscopy to investigate the origin and evolution of oncogene-induced genomic damage