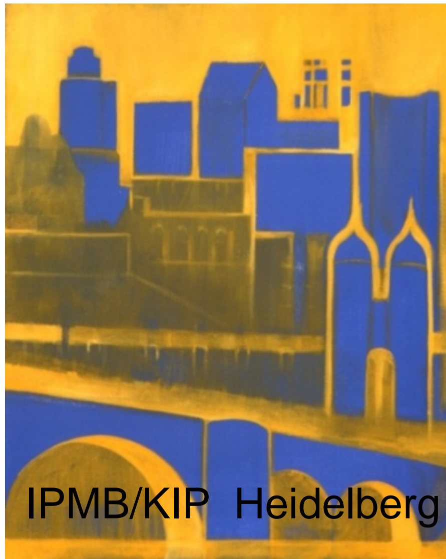
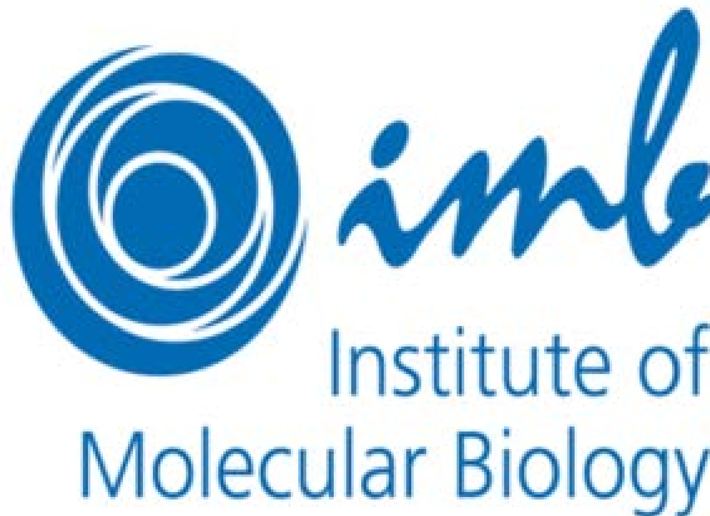
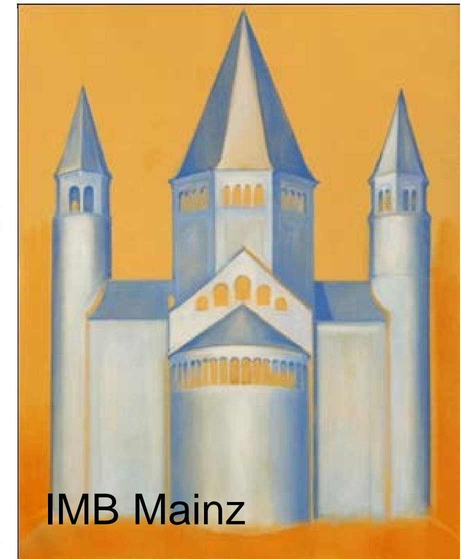
Lecture IMB January 15, 2019 Summary







Imaging the Cell Nucleus: Genome Architecture and Gene Regulation

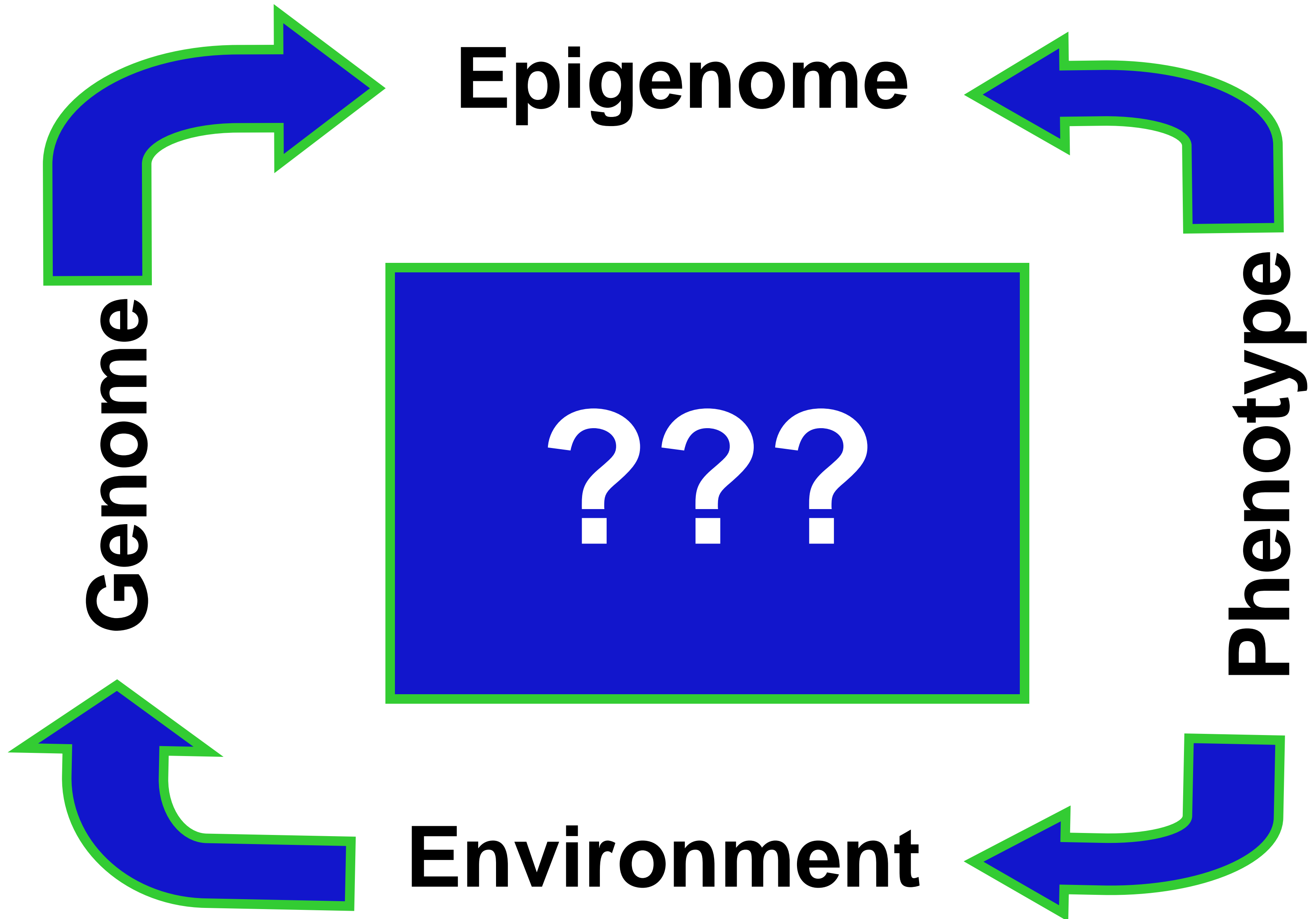
Christoph Cremer

Institute of Molecular Biology (IMB), Mainz

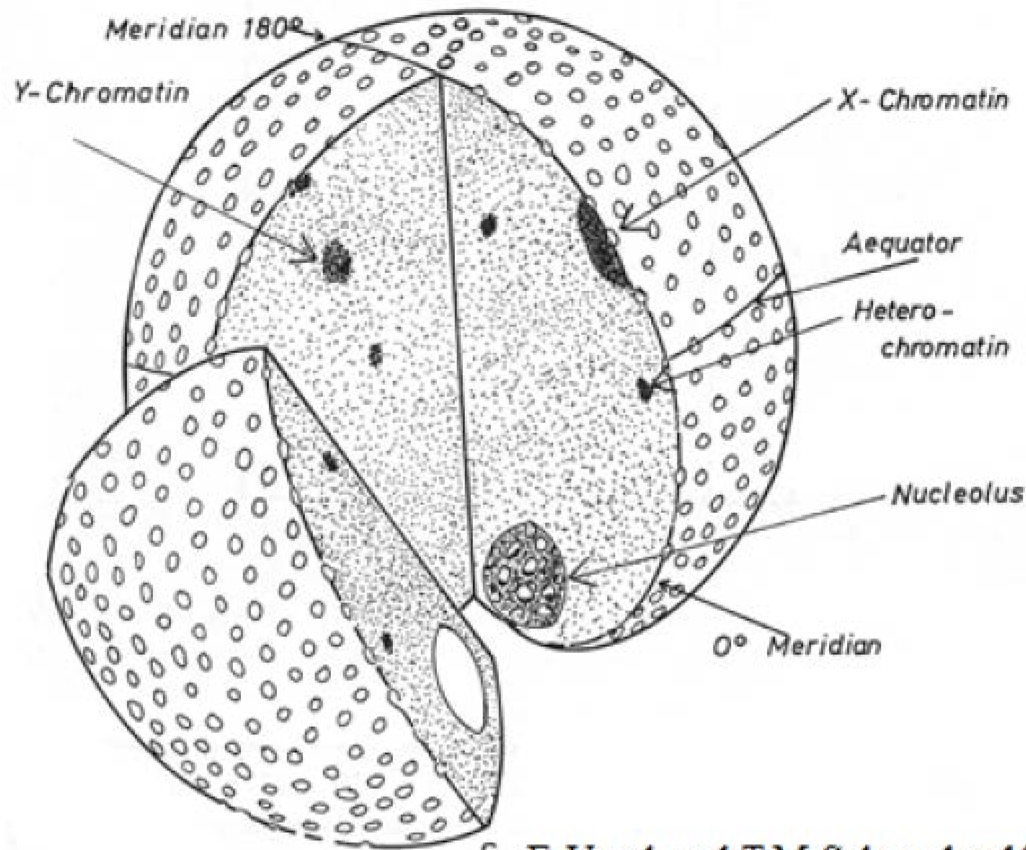
*Kirchhoff-Institute for Physics (KIP) and Institute for Pharmacy and Molecular Biotechnology (IPMB), University Heidelberg; Max-Planck Institute for Chemistry, Mainz

www.optics.imb-mainz.de

The phenotype is the result of exceedingly complex and interacting genetic, epigenetic and environmental networks



For many Decades, the Cell Nucleus was just a Bag with DNA and Proteins

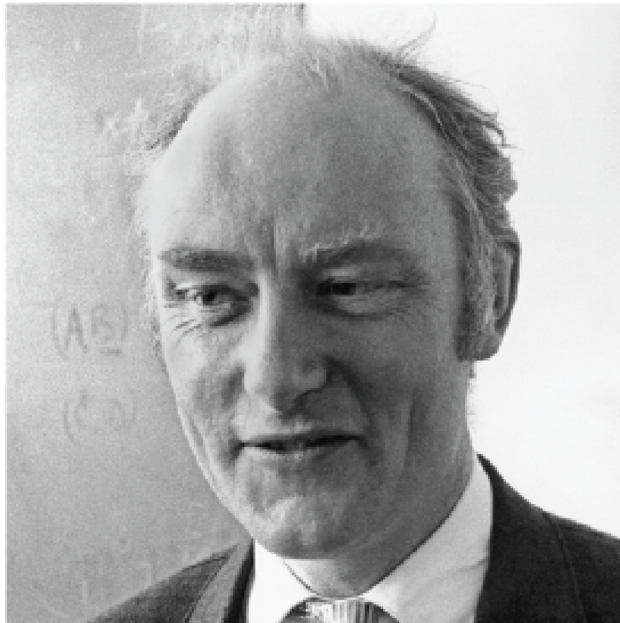


F. Vogel and T.M Schroeder 1974

Nuclear Genome Structure and Gene Regulation

1978: Francis H. C. Crick's view on the future of chromosome research

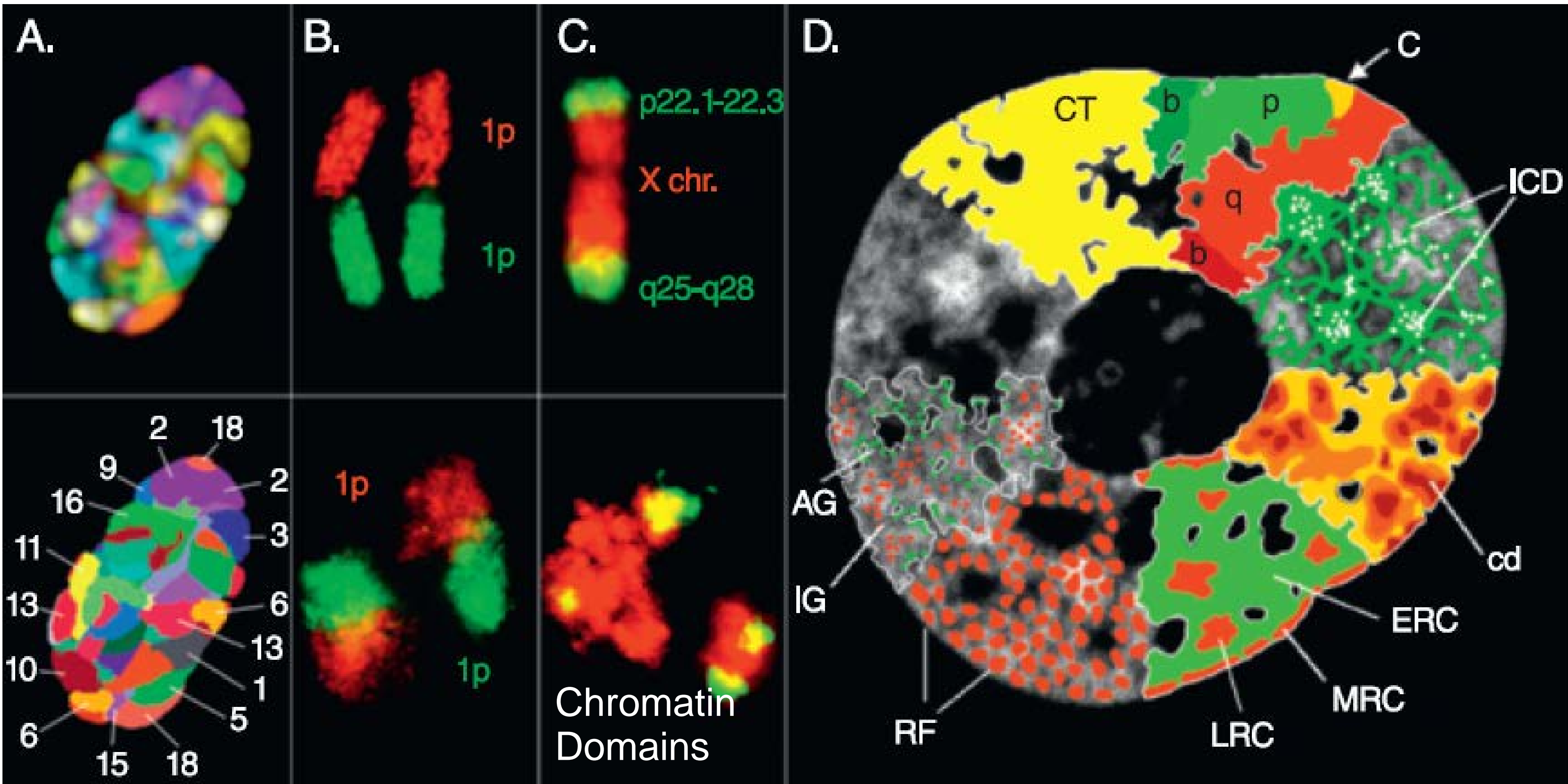
Chromosome Structure and Function. Future Prospects. Eur. J. Biochem. 83, 1 - 3



Francis Crick (1916 - 2004)

"The most general unanswered question appears to be: how much does the 3 D structure of the eukaryotic genome matter for expression, compared to the 1D structure? ... The methods of studying 3 D structures with precision are far more difficult than the methods available for sequencing DNA. Thus if it turns out ... that the 3D structure is not merely a packing device needed mainly for mitosis but is also of primary importance for gene expression, then ... we will need a more devious and ingenious plan of attack. Only time can show which alternative is preferred by nature and how difficult the problem will turn out to be."

State of the Art as elucidated by conventional Microscopy: The complex Organisation of Chromosomes results in a highly complex nuclear Genome Structure



Cremer&Cremer 2001; Bolzer et al. 2005

Christoph Cremer

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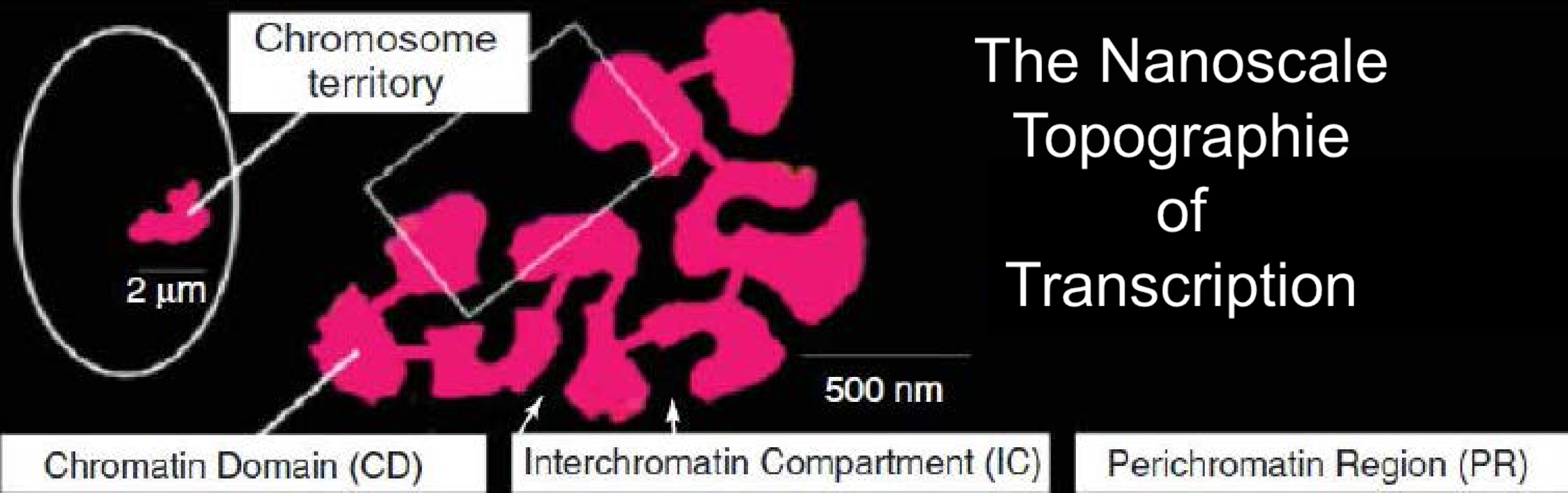


A recent (hypothetical) Model for the Nanoscale Structure of the Cell Nucleus:

The Active Nuclear Domain (ANC) – Inactive Nuclear Domain (INC) Model

Cremer et al. 2012, 2015

The Nanoscale Topographie of Transcription

Chromosome territory

2 µm

500 nm

Chromatin Domain (CD)

Interchromatin Compartment (IC)

Perichromatin Region (PR)

Perichromatin fibril (PF)

RNA polymerase II

Nascent RNA

Splicing factors

Splicing speckles

10 nm thick chromatin loop

Chromatin Domain (CD)

100 nm

IC

PR

PR

Genes

Genes

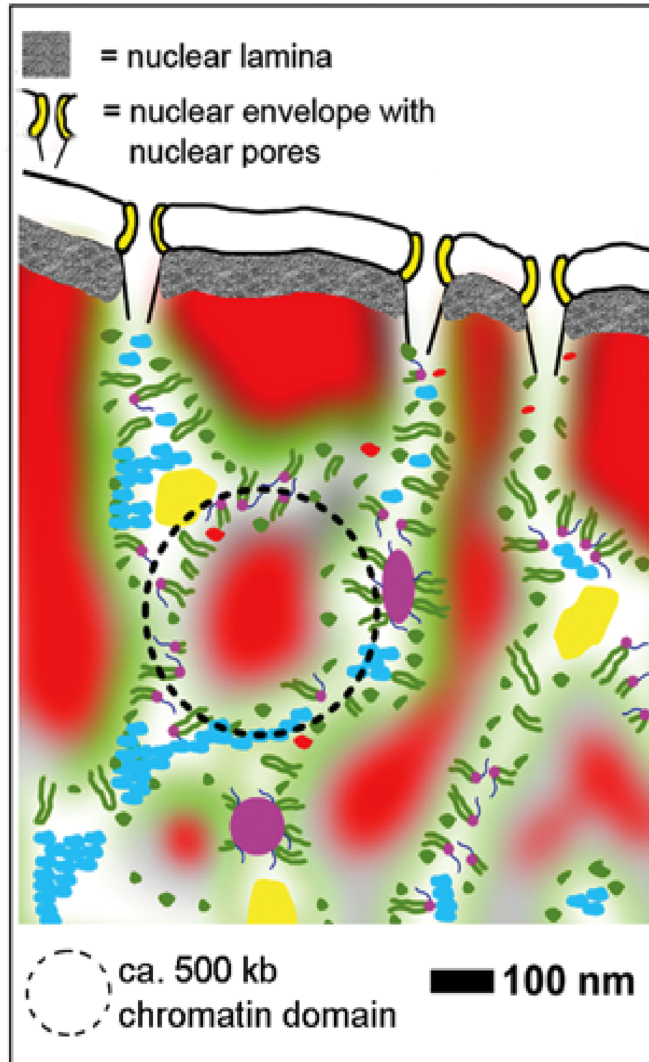
(a)

Cremer et al. 2012, 2015

_____________ **500 nm**

ANC – INC Network Model: Functional compartmentalization of chromatin

based on co-aligned active and inactive nuclear compartments



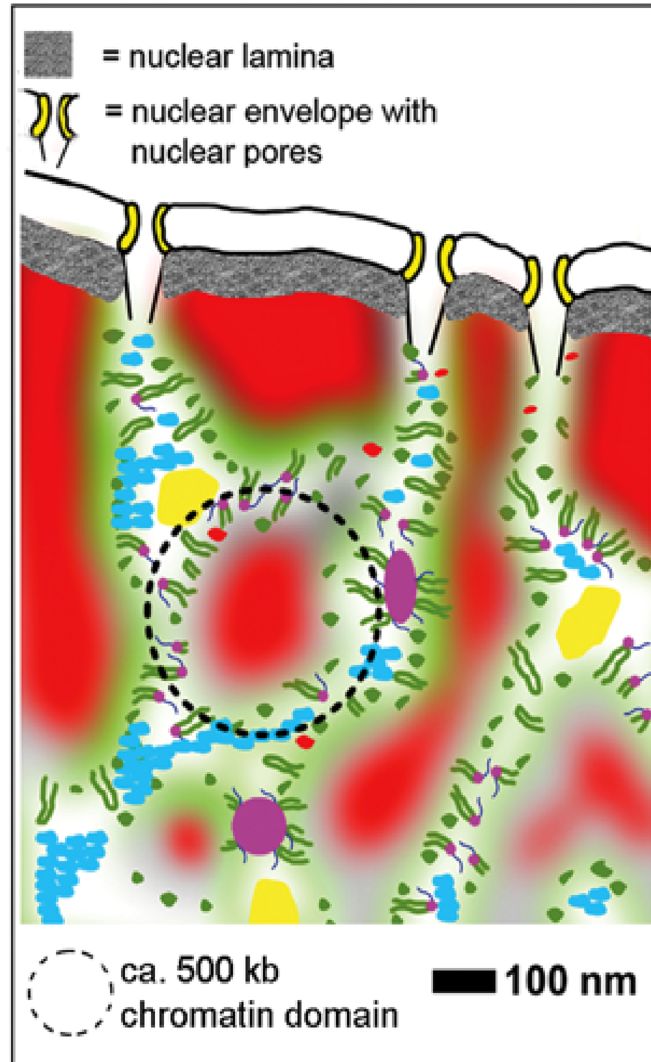
Two Structural Phases of nuclear Chromatin

Phase 1:
The Active Nuclear Domain (ANC): Low DNA density containing the transcriptionally active genes

Phase 2:
The Inactive Nuclear Domain (INC): High DNA Density containing the silenced genes

Adapted from Cremer et al. (2015) FEBS Letters 589: 2931-2943

ANC – INC Network Model: Functional compartmentalization of chromatin based on co-aligned active and inactive nuclear compartments



Some general Predictions:

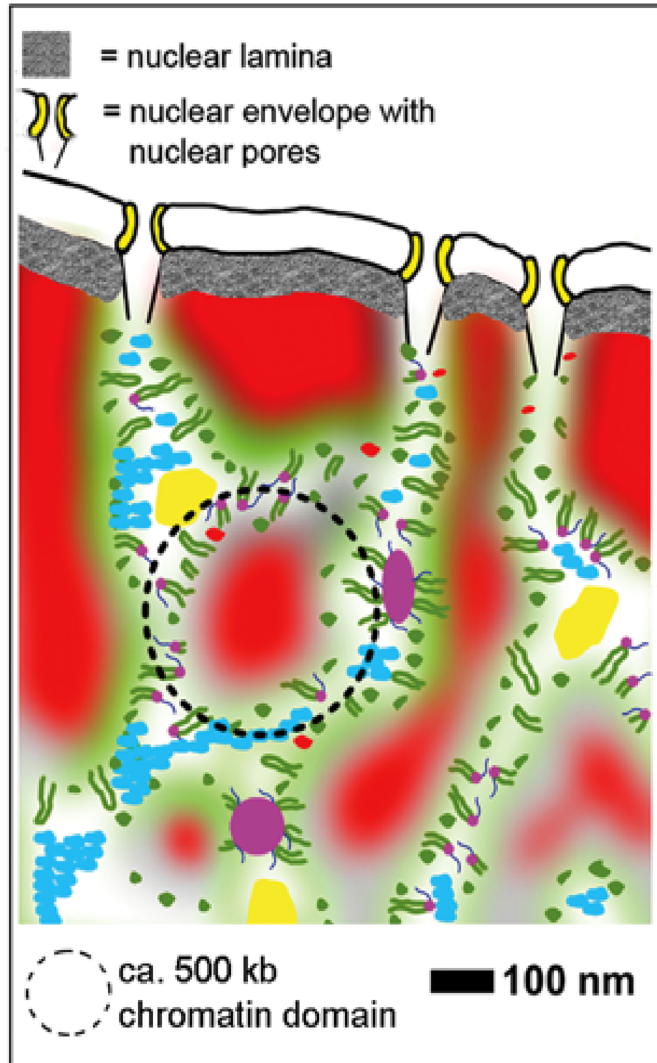
- **Epigenetic (biochemical) Modifications induce Nanostructure Changes**

- **Nanostructure Changes induce epigenetic Modifications (Mechanogenomics)**

- **Gene Regulation is based on highly dynamic spatial Genome Nanostructures**

Adapted from Cremer et al. (2015) FEBS Letters 589: 2931-2943

ANC – INC Network Model: Functional compartmentalization of chromatin based on co-aligned active and inactive nuclear compartments



Some general Predictions:

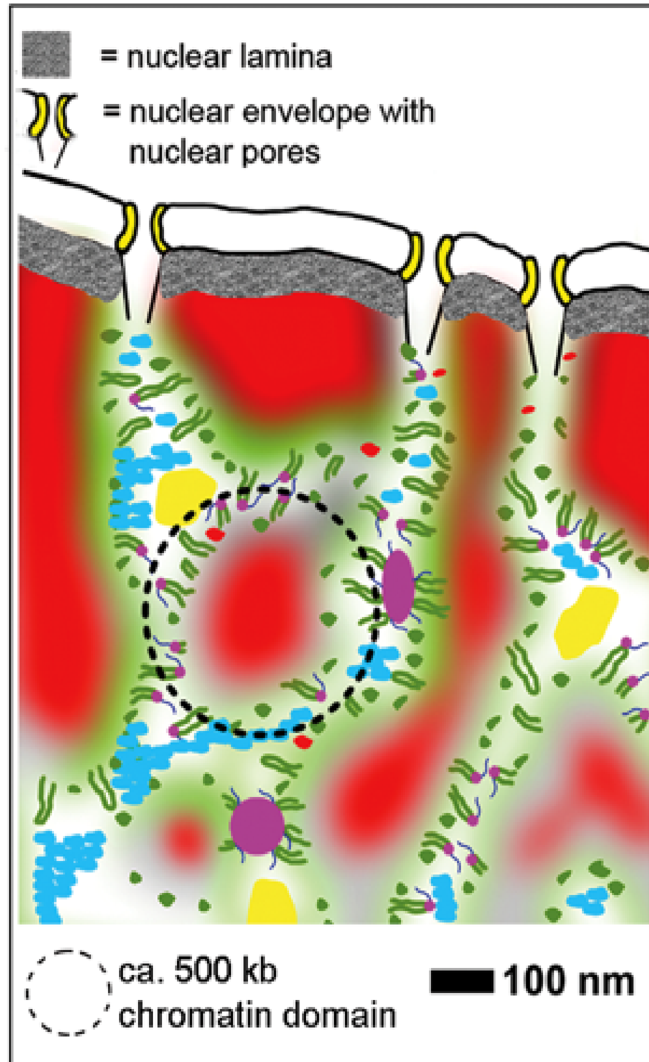
If gene regulation is based on highly dynamic spatial Genome Nanostructures,

then

Genome Nanostructure can be used to as a tool to identify epigenetically relevant features (cell type, stage, genome instability, environmental stress etc.)

Adapted from Cremer et al. (2015) FEBS Letters 589: 2931-2943

ANC – INC Network Model: Functional compartmentalization of chromatin based on co-aligned active and inactive nuclear compartments



A Long Term Perspective:

If Genome Nanostructure is known,

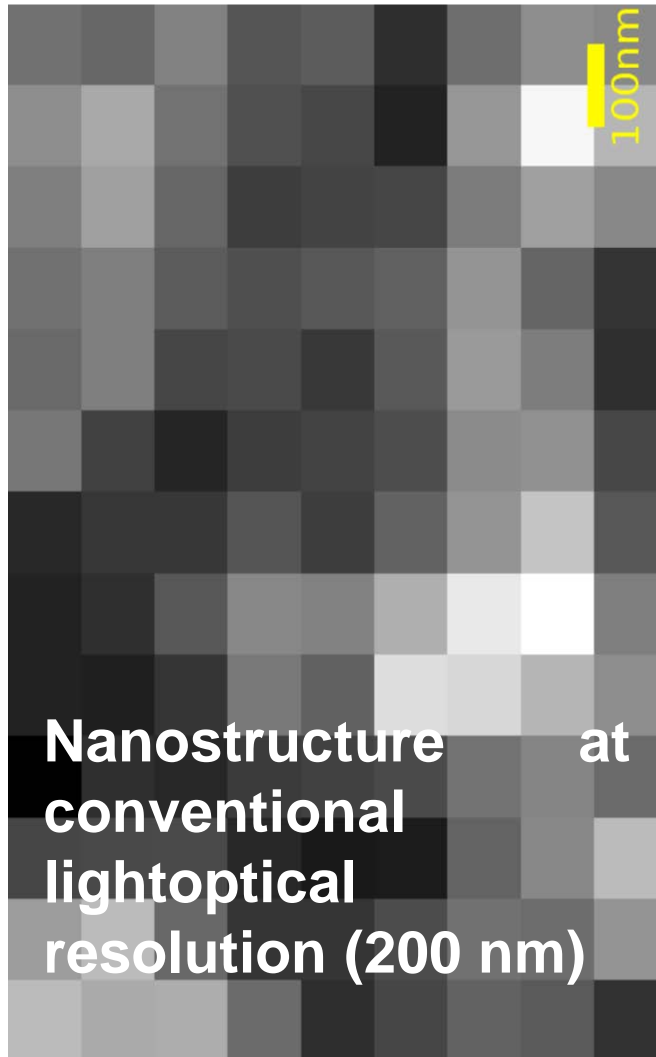
then

"Epigenetic" Drugs might be developed to modify Genome Nanostructure and hence control regulation of disease relevant genes

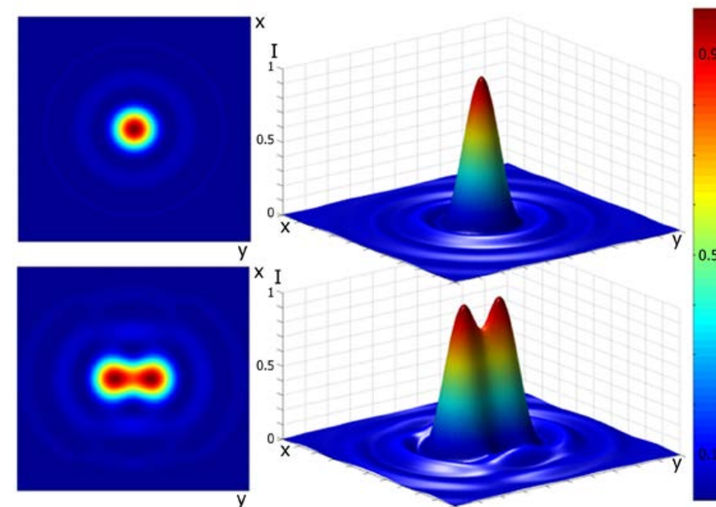
Adapted from Cremer et al. (2015) FEBS Letters 589: 2931-2943

"One of the greatest tragedies in life is the murder of a beautiful theory by a gang of brutal facts"*:

A fundamental Limitation of cellular Nanostructure Analysis



E. Abbe (1873)



Rayleigh (1896)

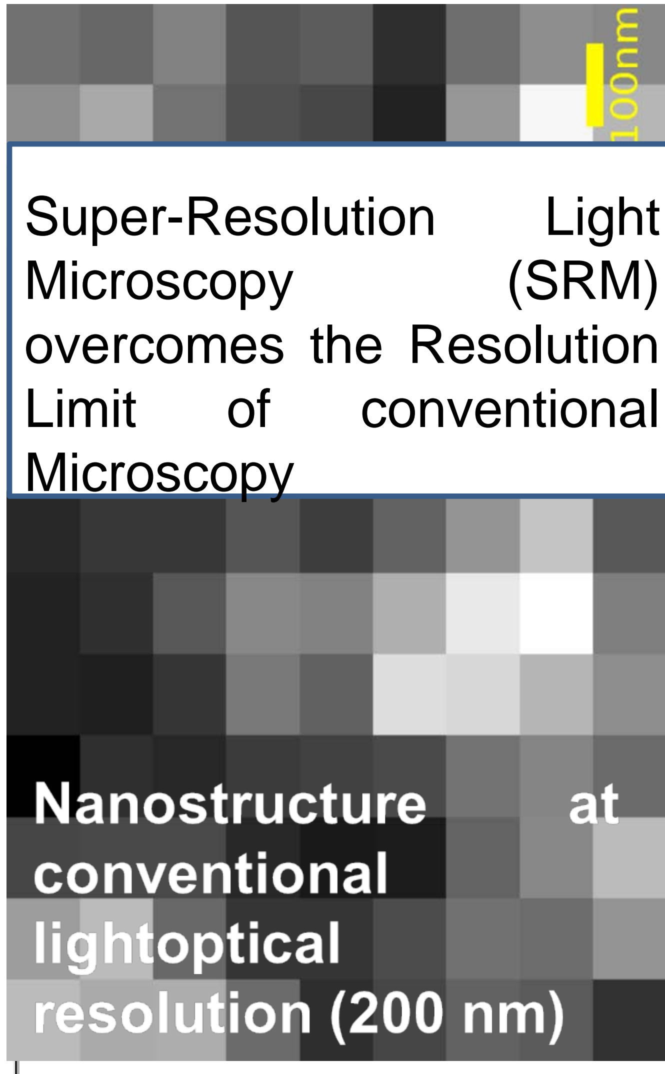
**Benjamin Franklin*

Challenge I:

Need for Superresolution Microscopy ("Nanoscopy") Methods

to study quantitatively the Nanoscale Architecture of the nuclear Genome and its functional Relation to active/inactive Gene Domains

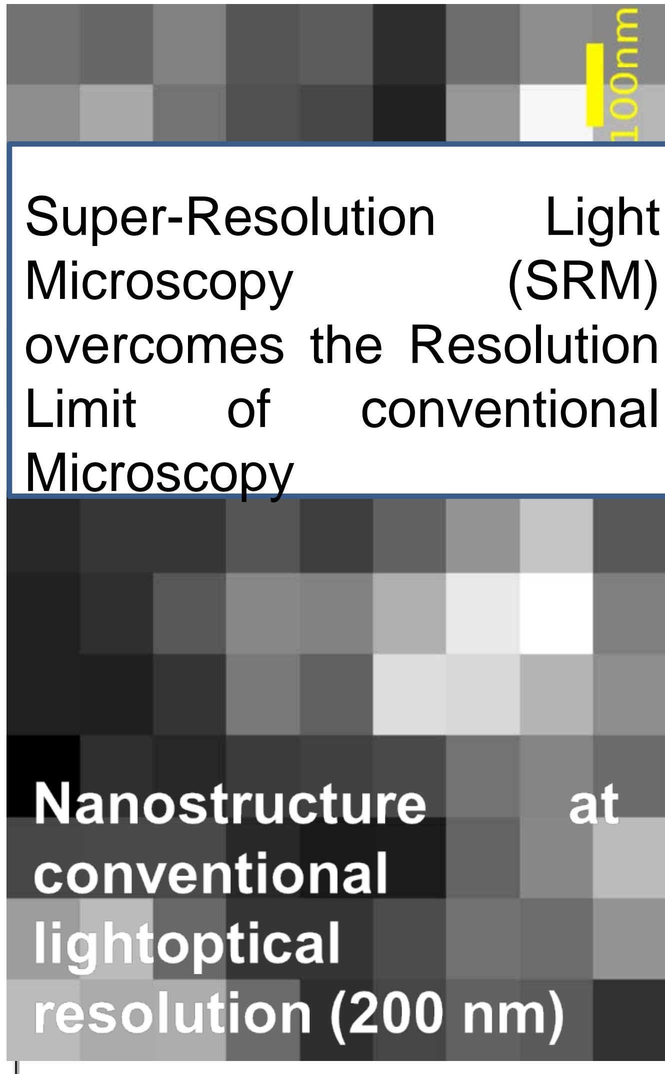
The Development of Super-Resolution Light Microscopy allows to analyse nuclear Genome Nanostructure



*Former Associate Cremer Lab
Numerous Joint Publications (1993ff) on confocal/4Pi/STED Microscopy

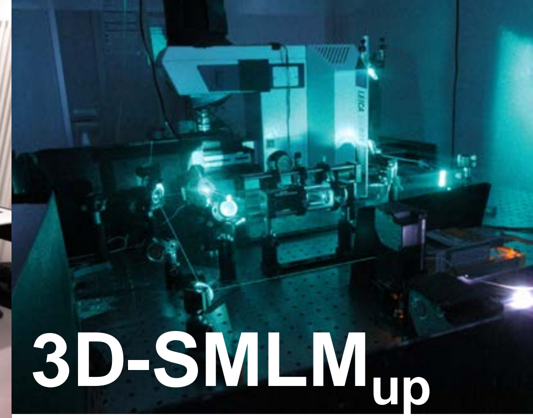
For History of SRM see:
Schermelleh et al. 2010; Cremer & Masters 2013; Fornasiero & Opazo 2015

The Development of Super-Resolution Light Microscopy allows to analyse nuclear Genome Nanostructure

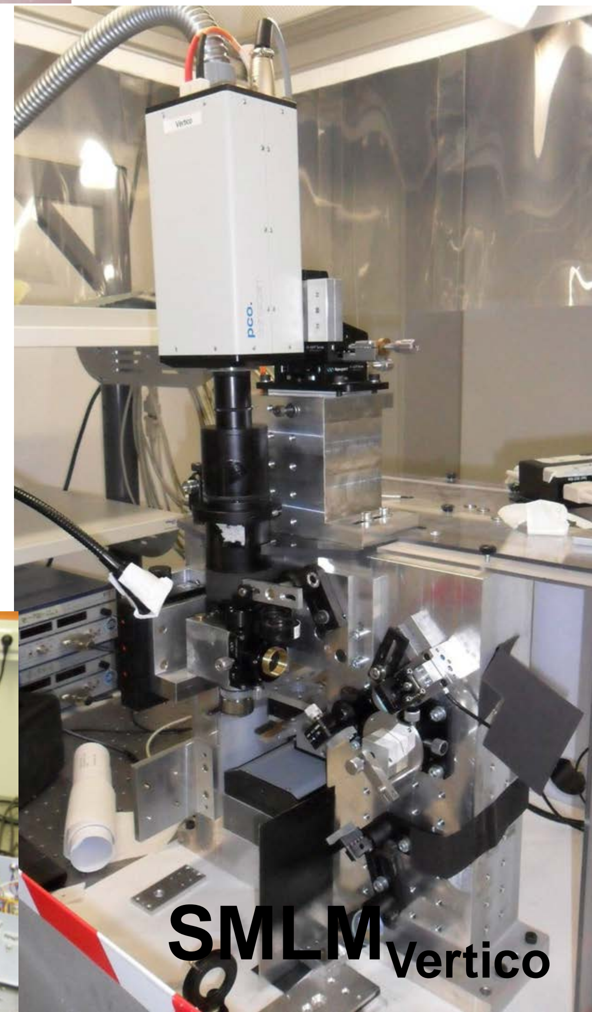


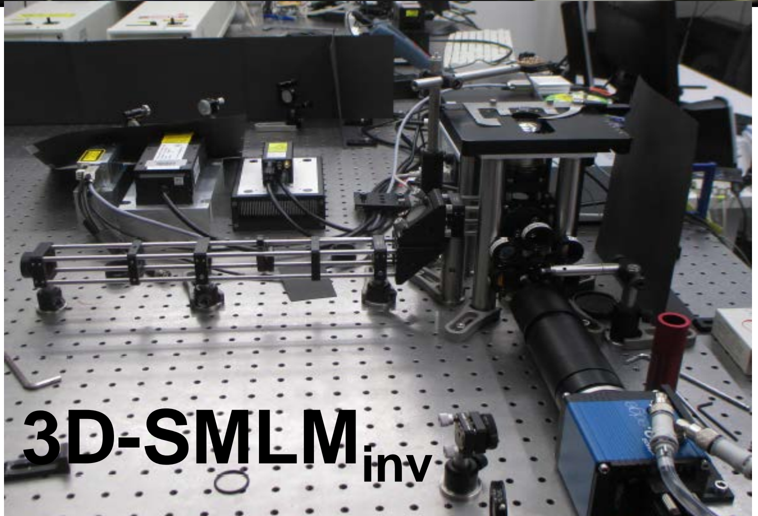
„This development [*super-resolving fluorescence microscopy*] is expected to revolutionize biology and medicine…“

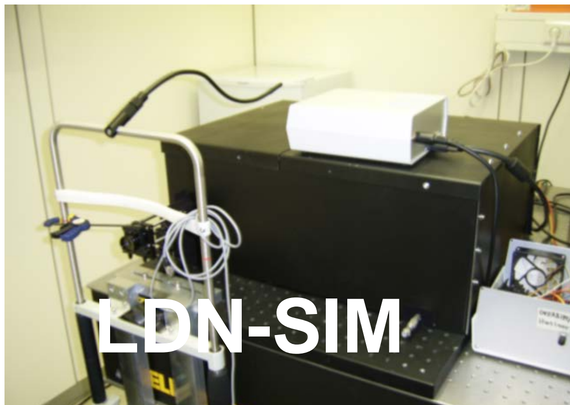
Justification of the Nobel Prize in Chemistry (2014)

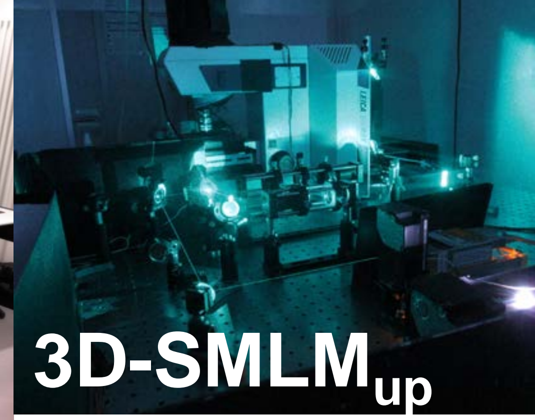


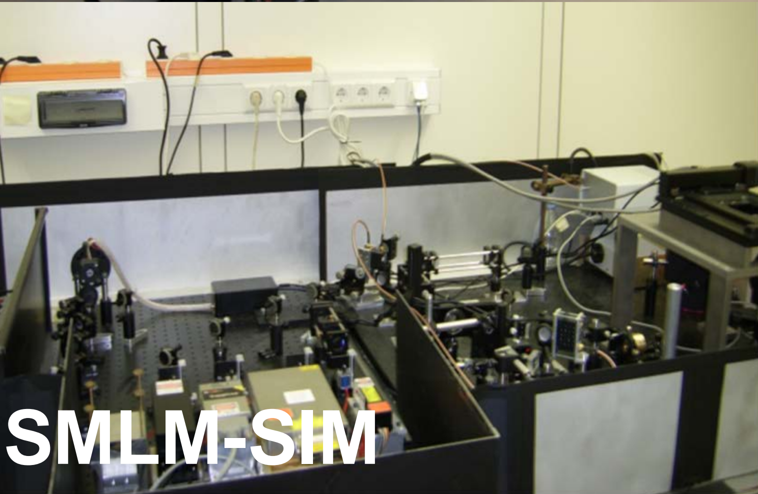
Super-Resolution Microscopes at IMB/Cremer-Lab





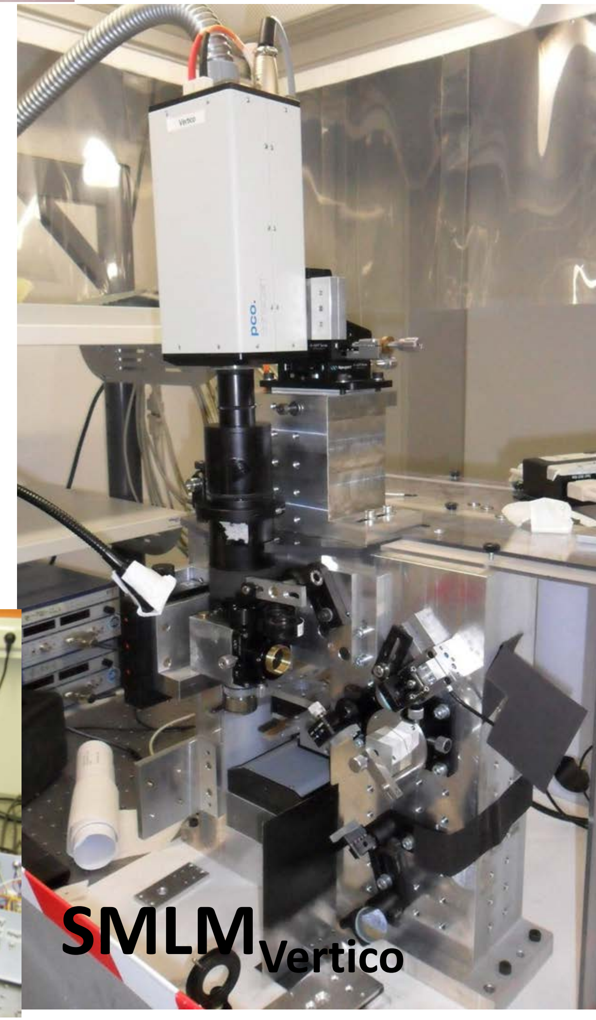


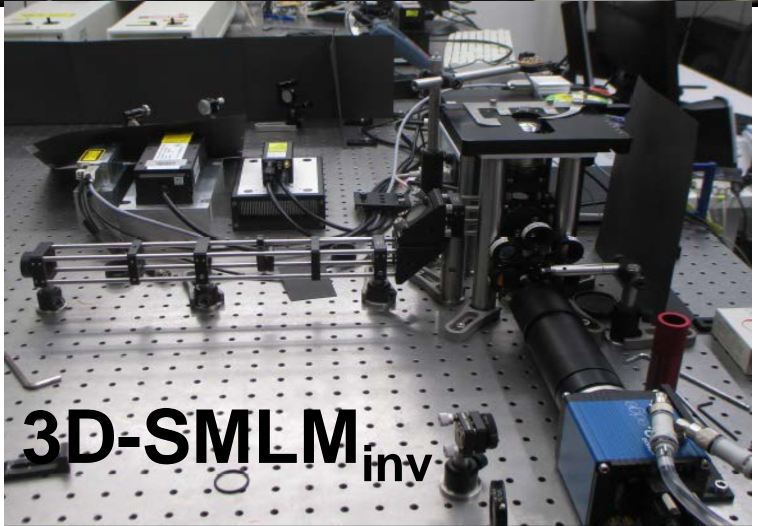


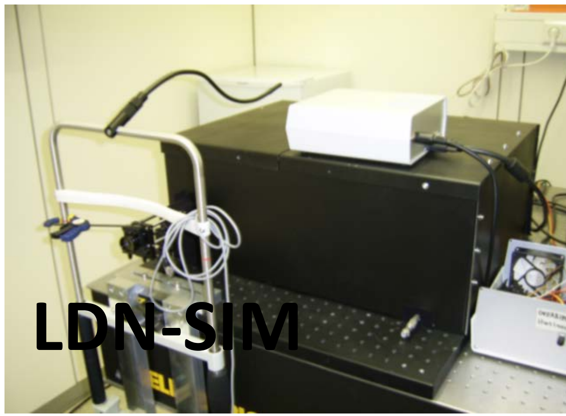


Best optical Resolution achieved at Cremer-Lab:

5 nm







Cremer-Lab: Development of alternative Super-resolution Microscopy Approaches especially useful in nuclear nanostructure analysis

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INTERNATIONAL PhD PROGRAMME MAINZ

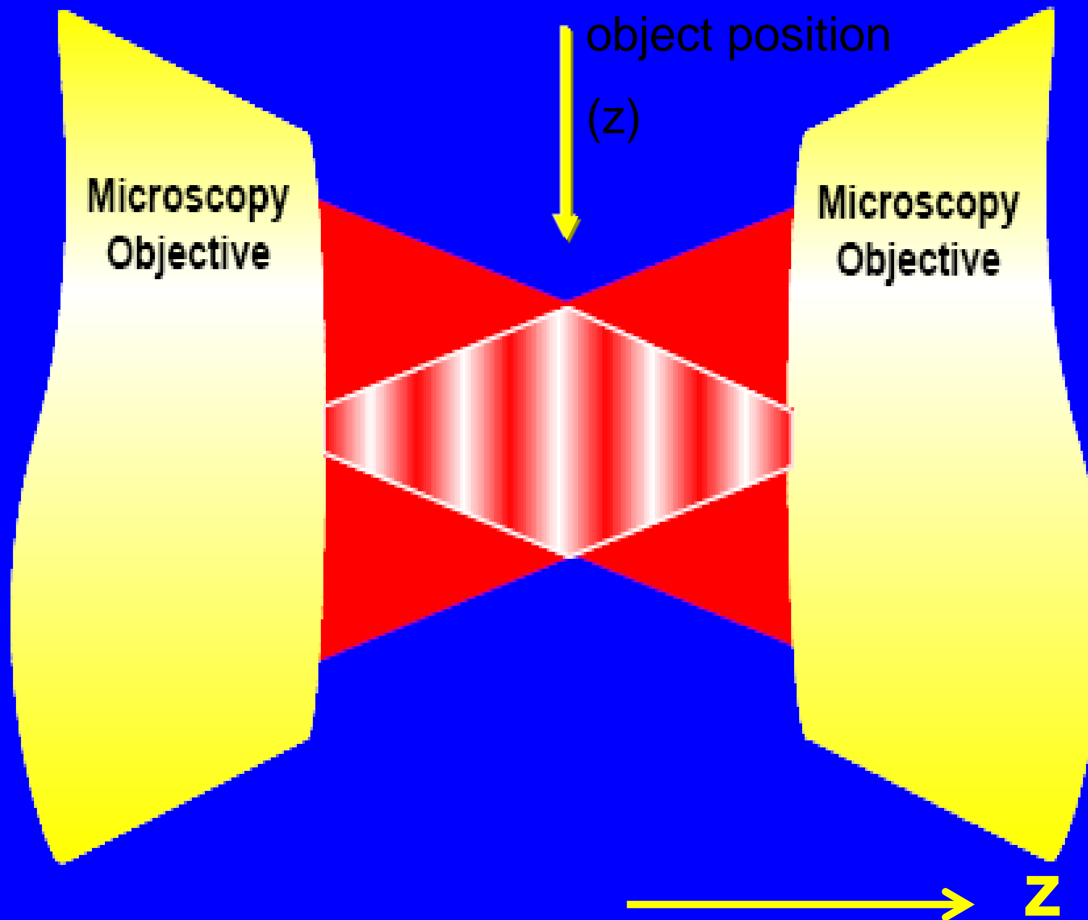
Structured

Illumination Excitation (SIE) Microscopy

Principle: Nanoscopy is based on Patterned Illumination Excitation (“Optical Lattice“)

Object is excited by a laser beam illumination pattern and fluorescence is registered multiple times using relative object-pattern positions

SIE Microscopy I



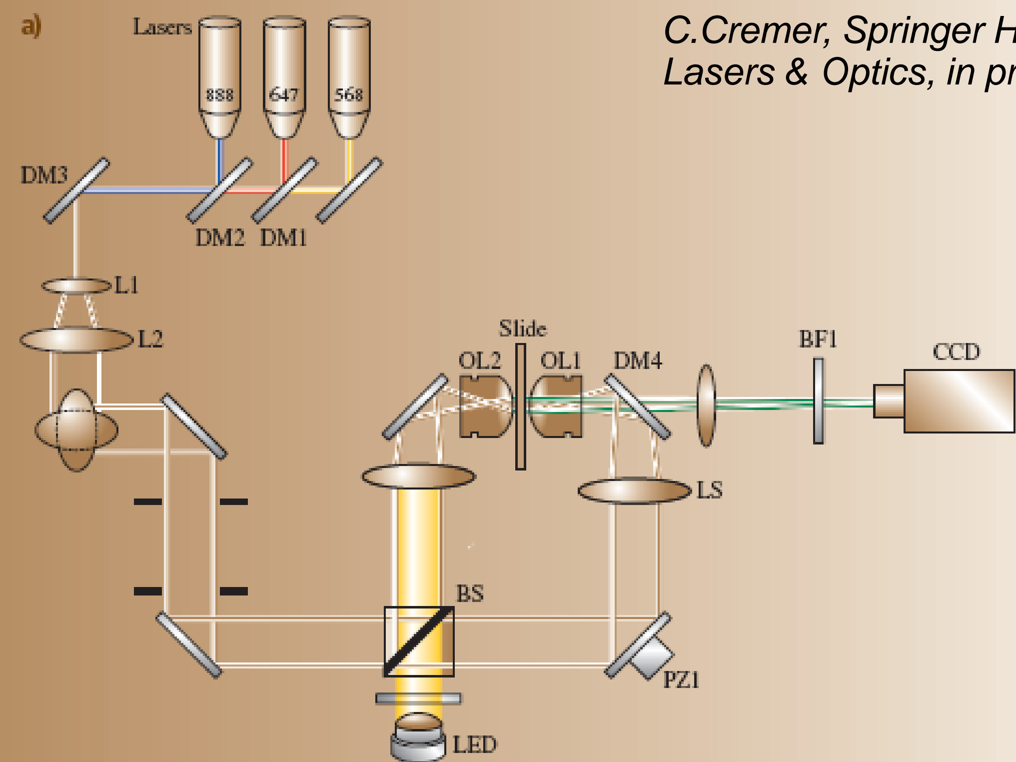
Baddeley et al. Nature Protocols (2007)

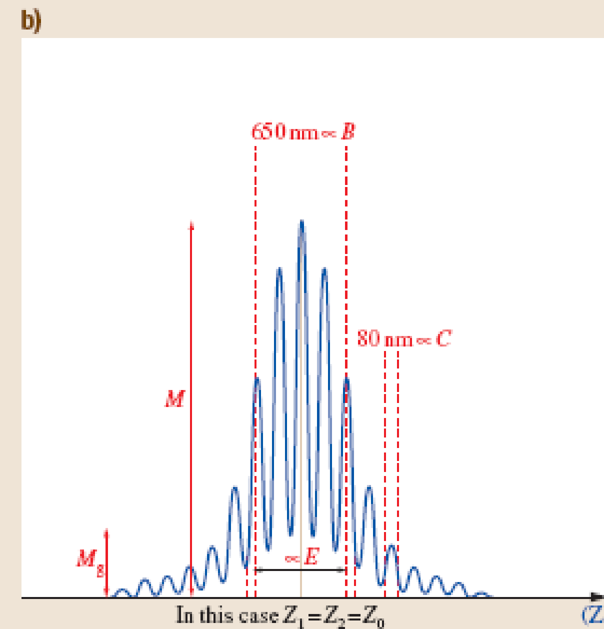
Principle: Standing Wave (SW)Field (Bailey et al. 1993)

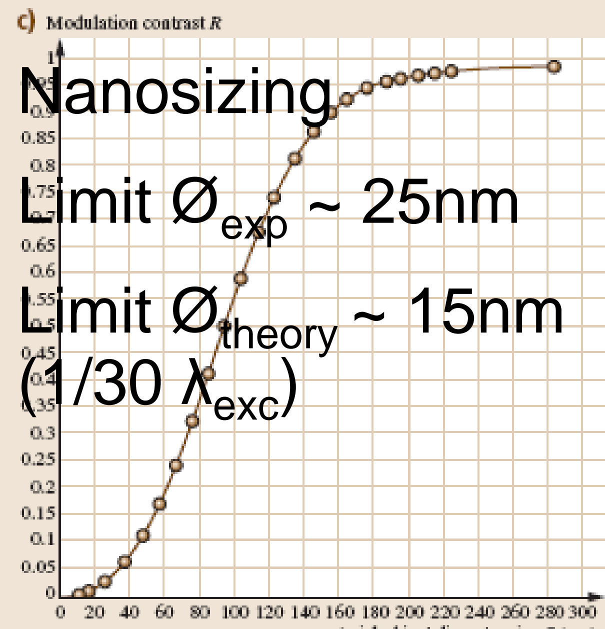
Novel Features: Movement of object in discrete steps Δz & imaging at each step

The CC-Lab Tripel Beam horizontal SMI Microscope

C.Cremer, Springer Handbook Lasers & Optics, in press







Nanosizing

Limit $Ø_{exp}$ ~ 25nm

Limit $Ø_{theory}$ ~ 15nm ($1/30\ \lambda_{exc}$)

Modulation Contrast

$R = M_g/M = f(Ø)$

Schneider et al. 1997;
Failla et al. 2001, 2002
Baddeley et al. 2007

Birk et al. 2008 (KIP)

SMI Measurements of geometrical Sizes of specifically labelled individual Gene Domains inside single human cell nuclei

gene region	probe length (kb)	hybridisation technique	number of measured regions	geometrical size (nm)
p53	**45**	**FISH**	**41**	**119±14**
p58	**80**	**FISH**	**28**	**123±10**
c-myc	**120**	**FISH**	**28**	**103±12**
abl	**0,4/186***	**triplehelical**	**42**	**77±22**

* 400bp in 31 oligonucleotides over 186kb

Hildenbrand et al. 2006 (CC-Lab)

Laterally Structured Illumination

KUNGL. VETENSKAPS AKADEMIEN
THE ROYAL SWEDISH ACADEMY OF SCIENCES

Scientific Background on the Nobel Prize in Chemistry 2014

SUPER-RESOLVED FLUORESCENCE MICROSCOPY

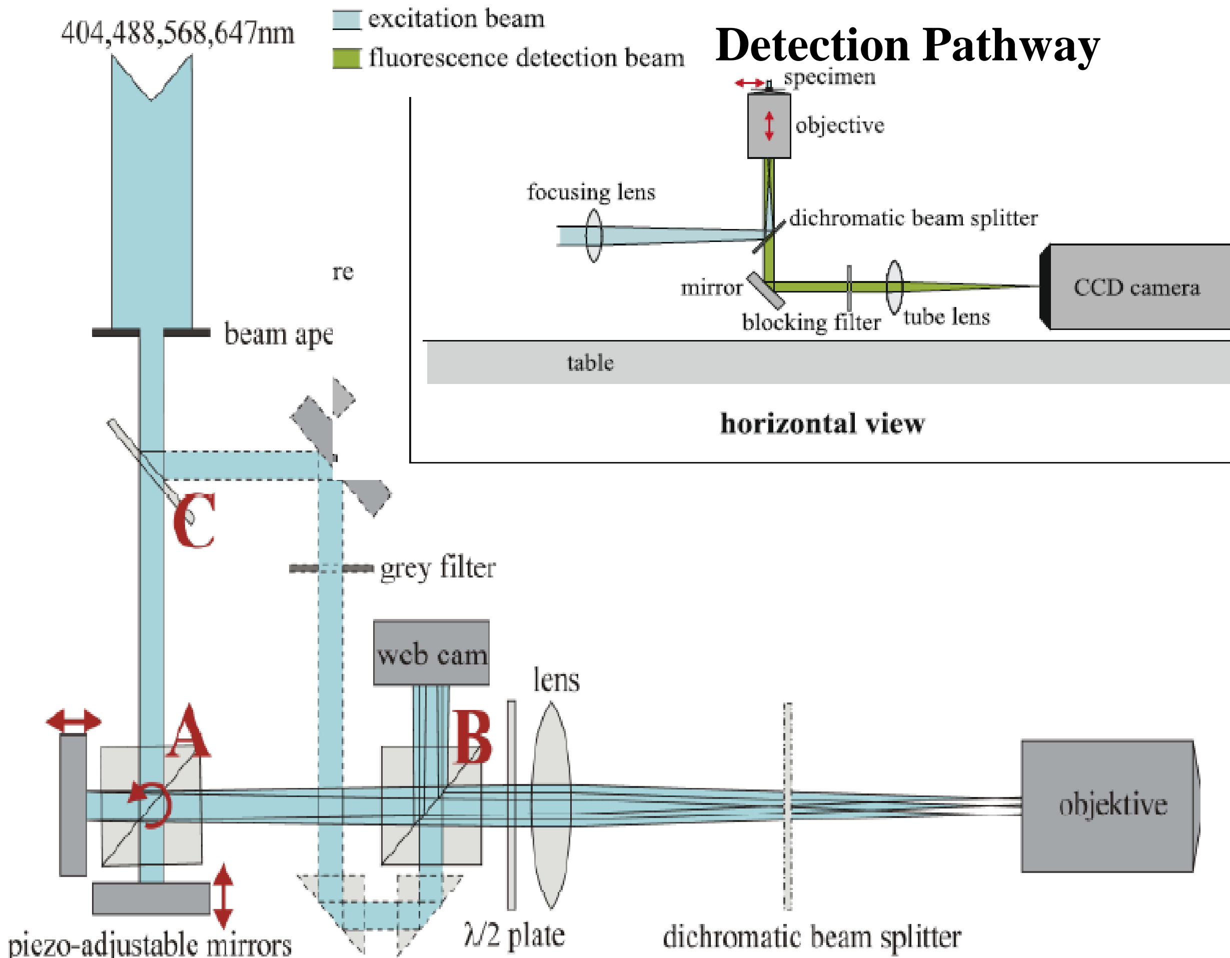
awarded to Erik Betzig, Stefan W. Hell and William E. Moerner

"Structured Illumination Microscopy (SIM) commonly uses the interference between two beams to create a sinusoidal pattern in the exciting light (Heintzmann and Cremer, 1999; Gustafsson, 2000)."

Rainer Heintzmann and Christoph Cremer (1999) Lateral modulated excitation microscopy: Improvement of resolution by using a diffraction grating. Proc. SPIE.3568:185-195.

Mats G. Gustafsson (2000) Surpassing the lateral resolution limit by a factor of two using structured illumination microscopy. J. Microsc.198:82-87.

A Multi-Frequency laterally Structured Interference Illumination Microscope (SIIM)



G. Best et al. 2011 (CC-Lab)

g = PSF * (ρ·I)

FT [g] =

OTF·(FT[ρ]*FT[I])

Image with 2x increase of cutoff frequency:

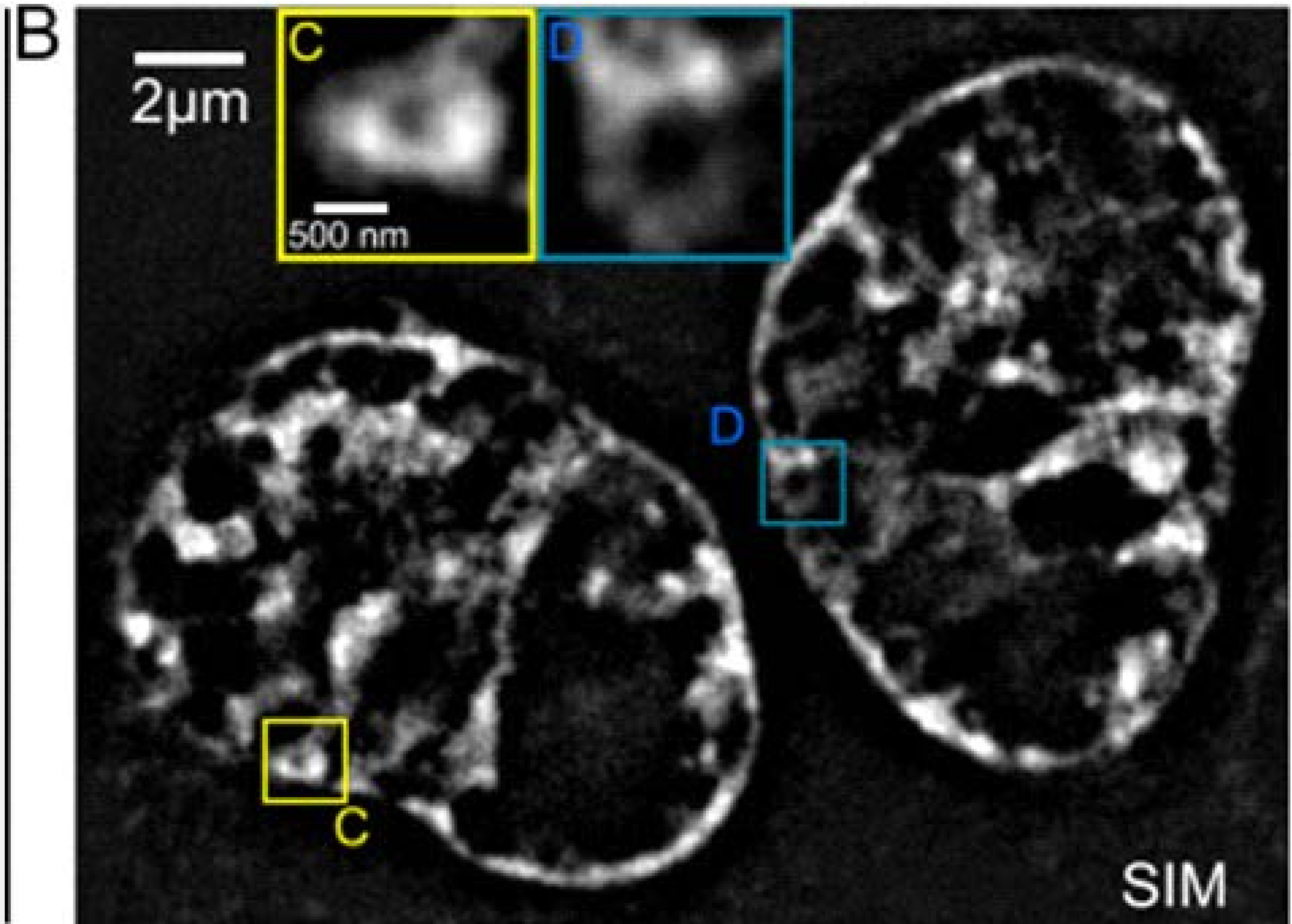
3 – 5 different phase positions

g image

ρ molecule distribution

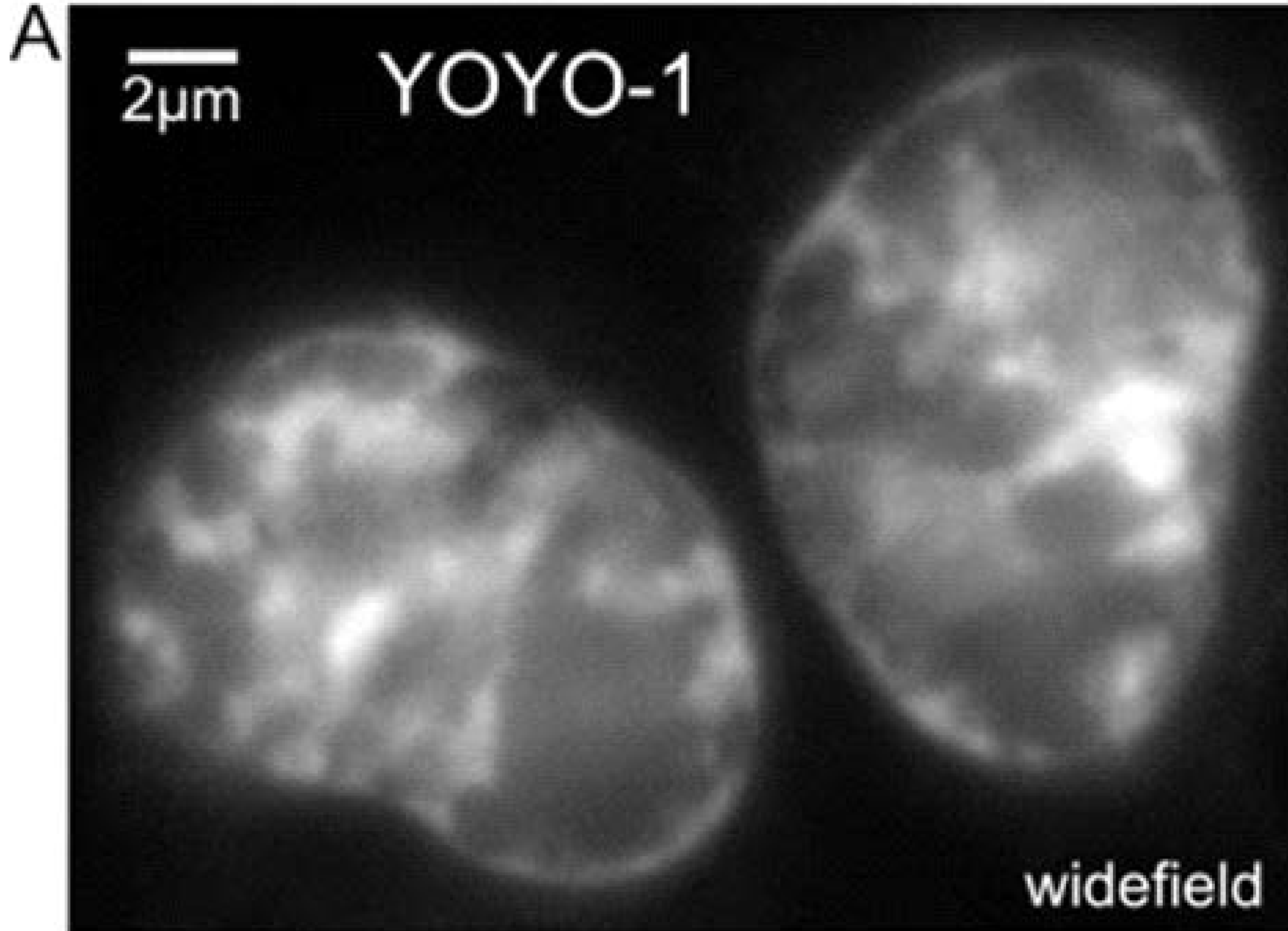
I Excitation Intensity

DNA Distribution in Cardiomyocyte Nucleus (HL-1), Structured Illumination with SIM System CC-Lab



Kirmes et al., Genome Biology (2015)

DNA Distribution in Cardiomyocyte Nucleus (HL-1), Conventional Microscopy



Cremer-Lab: Development of alternative Super-resolution Microscopy Approaches especially useful in nuclear nanostructure analysis

Christoph Cremer
c.cremer@imb-mainz.de

The basic Idea of super-resolving Localization Microscopy

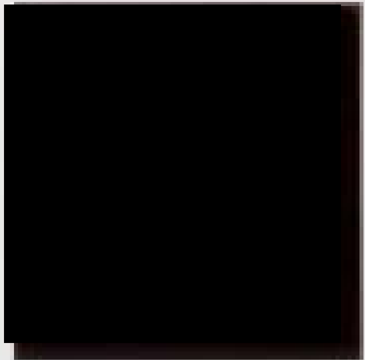
"Pointilistic" Image Formation

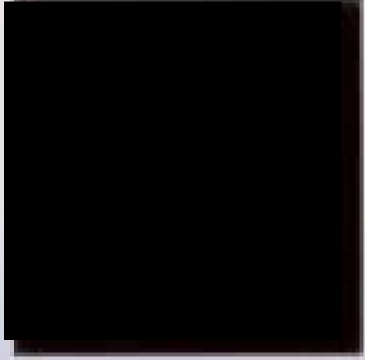
Microsoft Encarta

Spectrally Assigned Localisation Microscopy:

SALM/SPDM/SMLM/STORM/PALM/GSDIM etc.

Step 1: Only fluorescence of 'red' molecule is registered



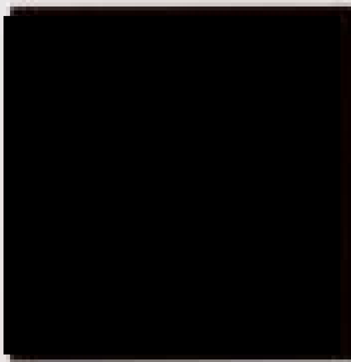


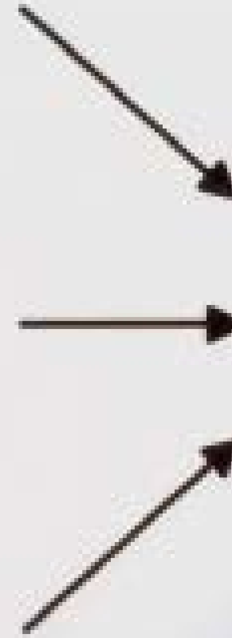
'Red' Fluorescence: 'Bright State'
This "ON"signal is registered by detector

'Green'/'Blue' Fluorescence: 'Dark State' with respect to detector
These "OFF"Signals NOT registered

Cremer et al. 1996, 1999, 2002
After Graph Lecture C.Cremer 2002, The Jackson Lab/Univ. Maine

Step 2: Only fluorescence of ‘green‘ molecule is registered



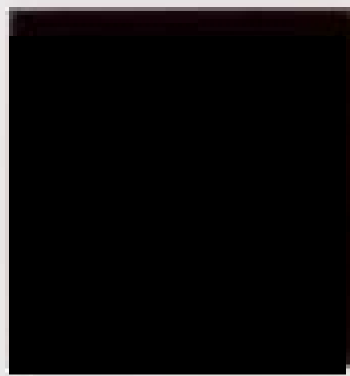


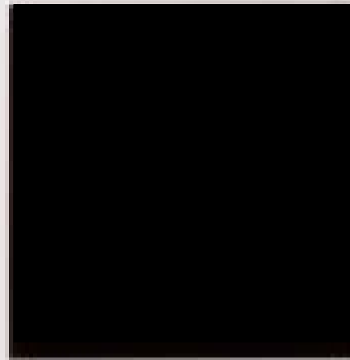
‘Green‘ Fluorescence: ‘Bright State‘
This “ON“ Signal is registered by detector

‚Red‘/‘Blue‘Fluorescence ‘Dark State‘ with respect to detector
These “OFF“Signals NOT registered

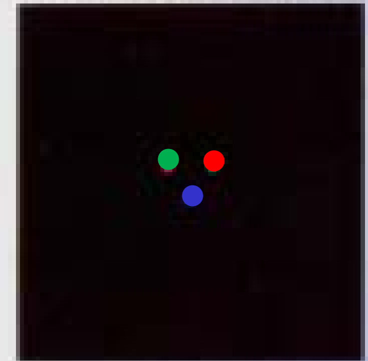
Cremer et al. 1996, 1999, 2002
After Graph Lecture C.Cremer 2002, The Jackson Lab/Univ. Maine

Step 3: Only fluorescence of 'blue' molecule is registered





Localization Map

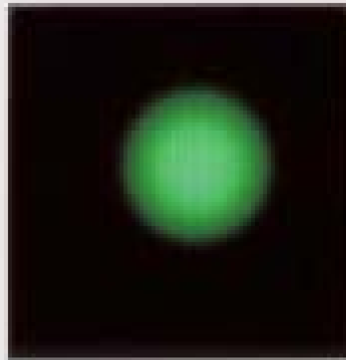


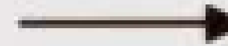
'Blue' Fluorescence: 'Bright State'
This "ON" Signal is registered by detector

Nuclear Nanostructure: Different 'spectral signatures' allow optical resolution of specific adjacent DNA Sequences, or DNA + other molecues

Cremer et al. 1996, 1999, 2002
After Graph Lecture C.Cremer 2002, The Jackson Lab/Univ. Maine

Assignment of all barycenter positions to localization map





bary centre reconstruction

Localization map

Step 3:
Localization Assignment

*Different 'signatures' allow also to identify molecule type!**
** e.g. short DNA sequence*

First ideas on fluorescence based Localization Microscopy: If all molecules (assumed distances 50 nm) simultaneously emit fluorescence light of <u>different</u> frequencies, and diffraction patterns are registered <u>independently,</u> optical resolution <u>is possible, due to OPTICAL ISOLATION</u> *Cremer et al. 1996, 1999, 2002*

General Approach:

Optical isolation* of individual diffraction discs of individual point sources/molecules

+

Insertion of positions into joint localization map

“Make DARK the molecules you do not want to see“

Betzig 1995; Cremer et al. 1996, 1999

First Application to nuclear Genome Architecture

Journal of Microscopy, Vol. 199, Pt 2, August 2000, pp. 96–105.
Received 14 June 1999; accepted 21 February 2000

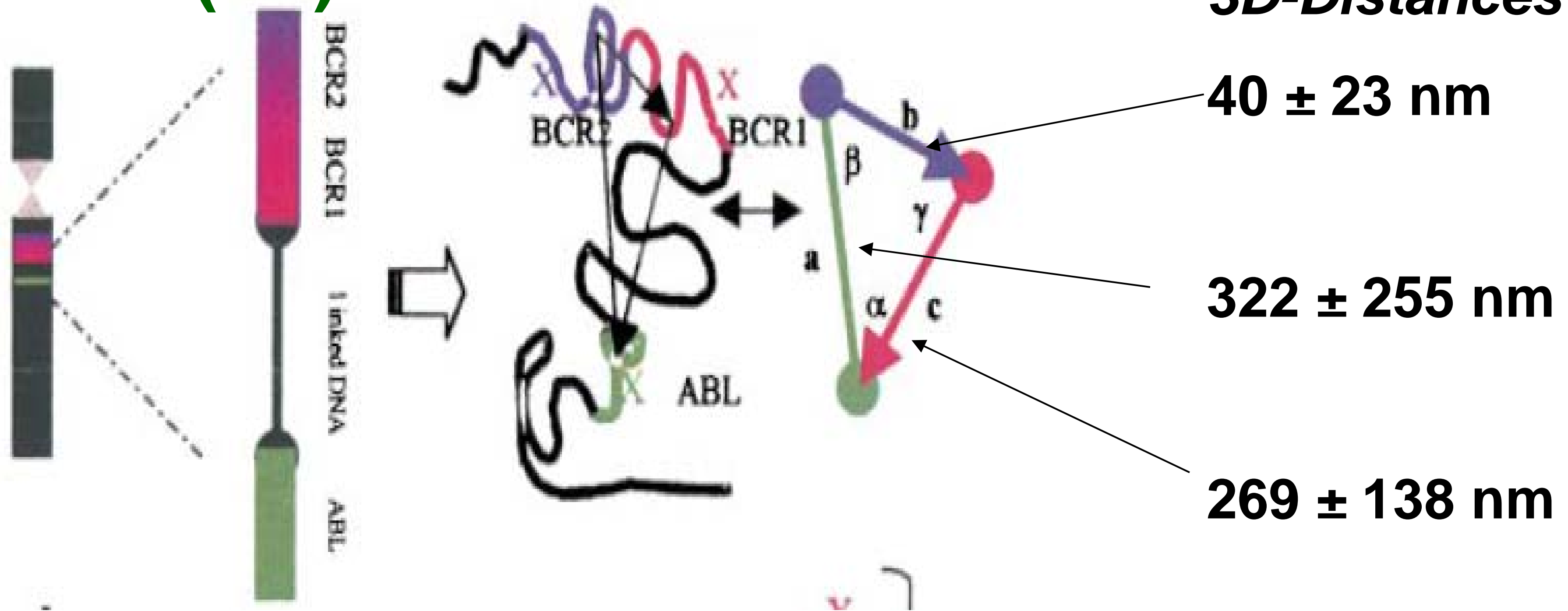
Three-dimensional spectral precision distance microscopy of chromatin nanostructures after triple-colour DNA labelling: a study of the *BCR* region on chromosome 22 and the Philadelphia chromosome

A. ESA*†[1], P. EDELMANN*†, G. KRETH*†, L. TRAKHTENBROT‡, N. AMARIGLIO‡, G. RECHAVI‡, M. HAUSMANN*[2] AND C. CREMER*†
*Applied Optics and Information Processing, Kirchhoff Institute for Physics (KIP), University of Heidelberg, Albert-Ueberle-Str. 3–5, 69120 Heidelberg, Germany
†Interdisciplinary Center of Scientific Computing (IWR), University of Heidelberg, INF 368, Germany
‡Institute of Hematology, The Chaim Sheba Medical Centre, 52621 Tel Hashomer, Israel

Method: Specific labelling of three adjacent sites in the Leucemia translocation breakpoint region
Fluors: Cy5, FITC, TRITC

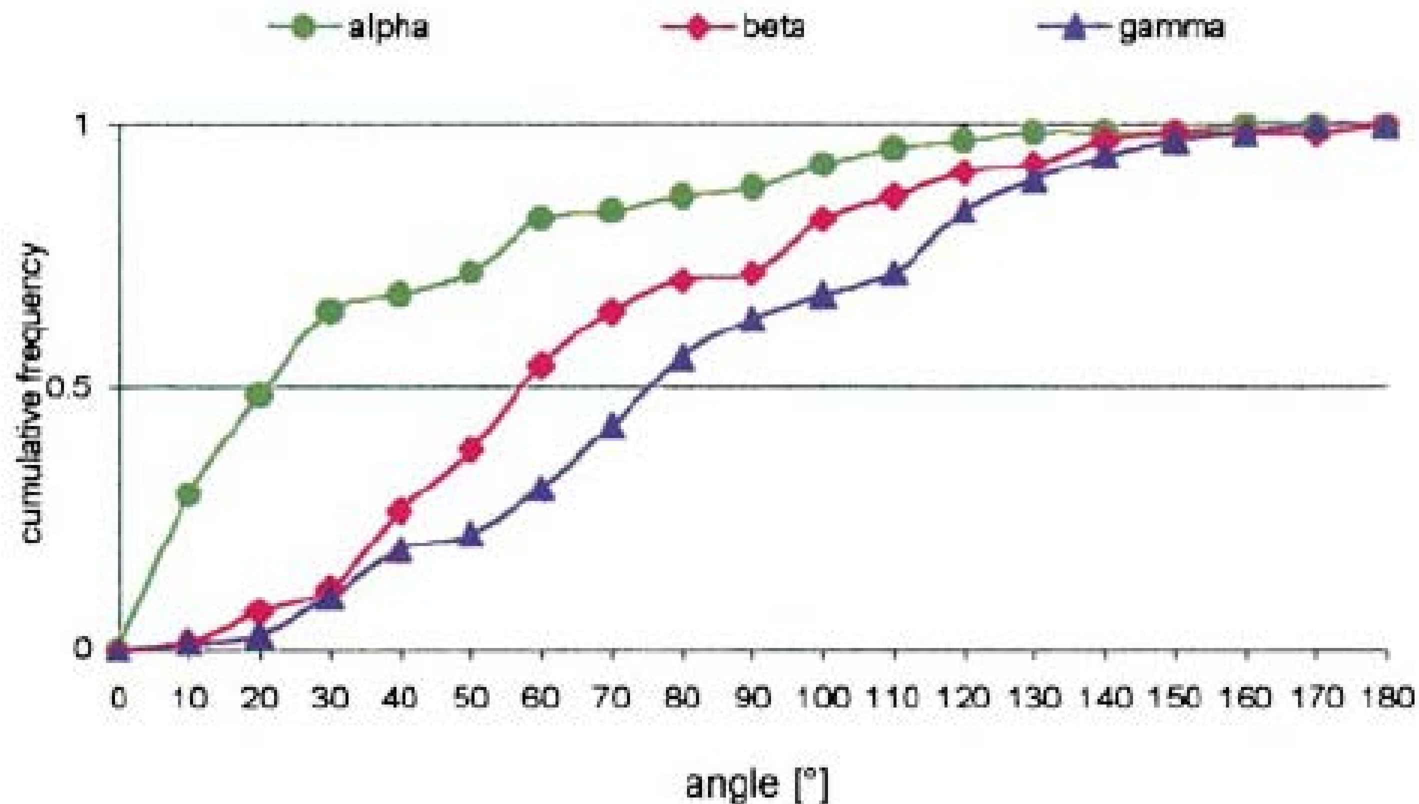
“Proof-of-Principle“ Application : 3- color 3D-Localization Microscopy (SPDM) of t(9;22) Translocation Breakpoint: Different sites on the same Molecule (DNA) in bone marow cell nuclei of CML Patients

σ_{loc} (3D) 17 ± 10 nm (CLSM)
Optical (3D) Resolution ~ 40 nm



A. Esa, P. Edelmann, L. Trakthenbrot, N. Amariglio, G. Rechavi, M. Hausmann, C. Cremer (2000), J. Microscopy 199: 96 – 105 (2000).

First Localization Microscopy Measurements of Angles in 3D inside intact Cell Nuclei indicate Non-Random Genome Nanostructure



Esa et al. 2000

„Dr. Dark“

Optical Isolation in Far Field Fluorescence Microscopy: Labeling of Biomolecules with

Appropriate spectral signatures

<u>Spectral signature</u>:

Generally: Any photophysical property useful for photonic discrimination, e.g.

- **Abs/Emission spectrum**
- **Fluorescence Lifetimes**
- **Luminescence**

Cremer et al. 1996, 1999

Desirable for Nanoscale Analyis of Nuclear Chromatin Distribution:

Multiple Differences in spectral signature to allow enhanced <u>STRUCTURAL resolution</u>

Realization: Stochastic emissions in space and time of the <u>same</u> type of fluorophores by

Bright-Dark Transitions,‘Blinking‘:
BALM, FPALM/PALM, GSDIM, PAINT, SPDM$_{Phymod}$, STORM/dSTORM,…

Lidke 2005, Betzig 2006, Hess 2006, Rust 2006; Bock 2007, Egner 2007, Huang 2008, Reymann 2008, Lemmer 2008, 2009; Biteen 2008, Fölling 2008, Heilemann 2008, Kaufmann 2009; Baddeley 2009, 2010, 2011, Matsuda 2010, Pertsinidis 2010, Löschberger 2012,…..

Study of nuclear Genome Architecture at the Nanoscale at high structural resolution:

Single Molecule Localization Microscopy (SMLM) with

single Standard Fluorophors*:

Switch Standard DNA Dyes between ‘bright‘ states and ‘dark‘ states

Bright-Dark transitions e.g.

- differences in Absorption/Emission spectrum („color“change) by „photoswitching“;
- differences in Intensity („spatial switching“)

**Szczurek et al. 2014; Kirmes et al. Genome Biology 2015; Żurek-Biesiada Ex. Cell Res. 2016; Szczurek et al. 2016, 2017, 2018;*

Desirable for Localization Microscopy of Imaging of NanoSTRUCTURES

Multiple Differences in spectral signature to allow STRUCTURAL resolution

Generally: Any photophysical property useful for photonic discrimination:

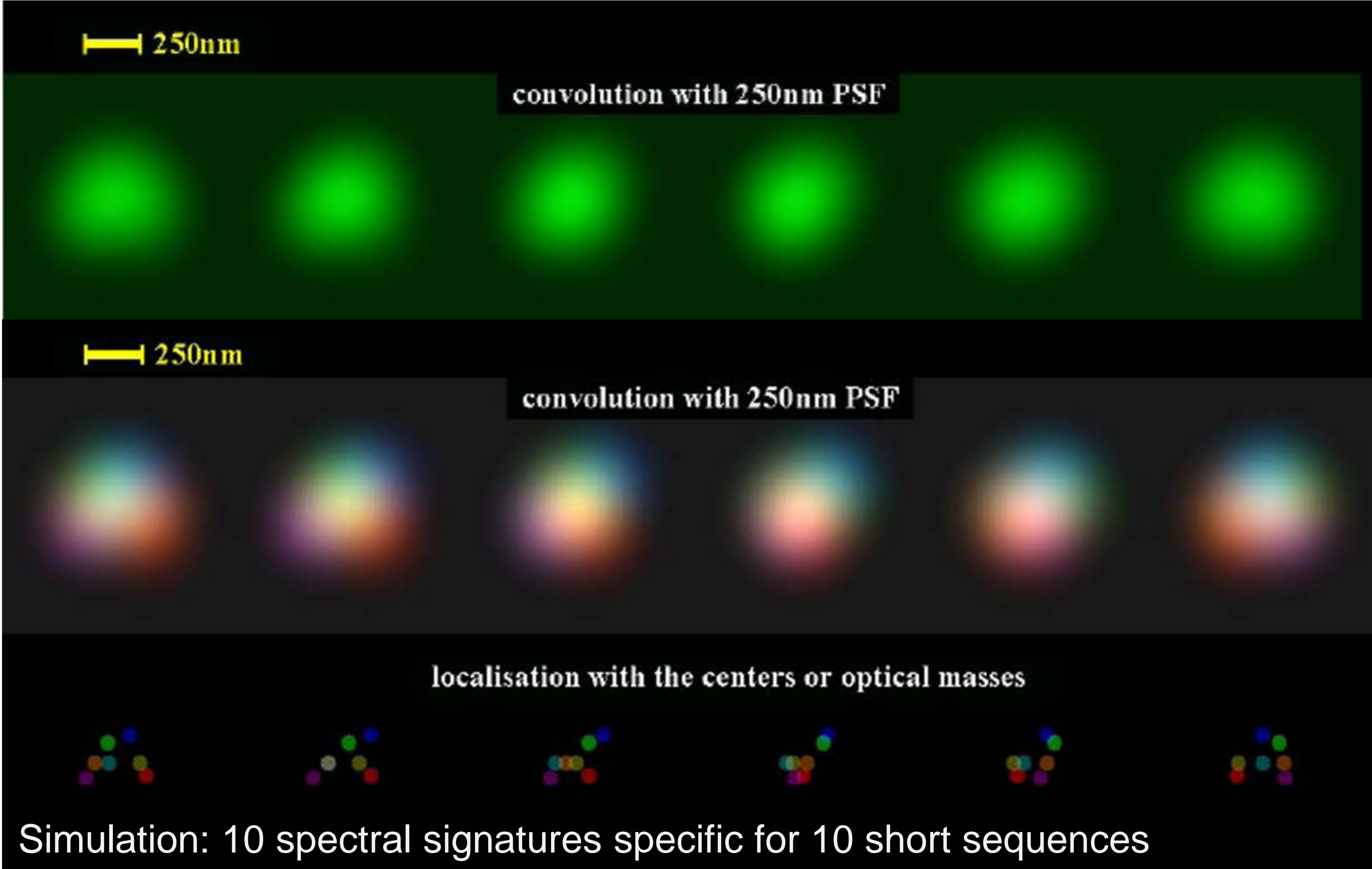
„Make DARK the molecules you do not want to see“

Solution I:

Labeling of target molecules with multiple photostable fluorophors (e.g. differences in absorption, emission, fluorescence life times, Raman spectra, Antibunching)

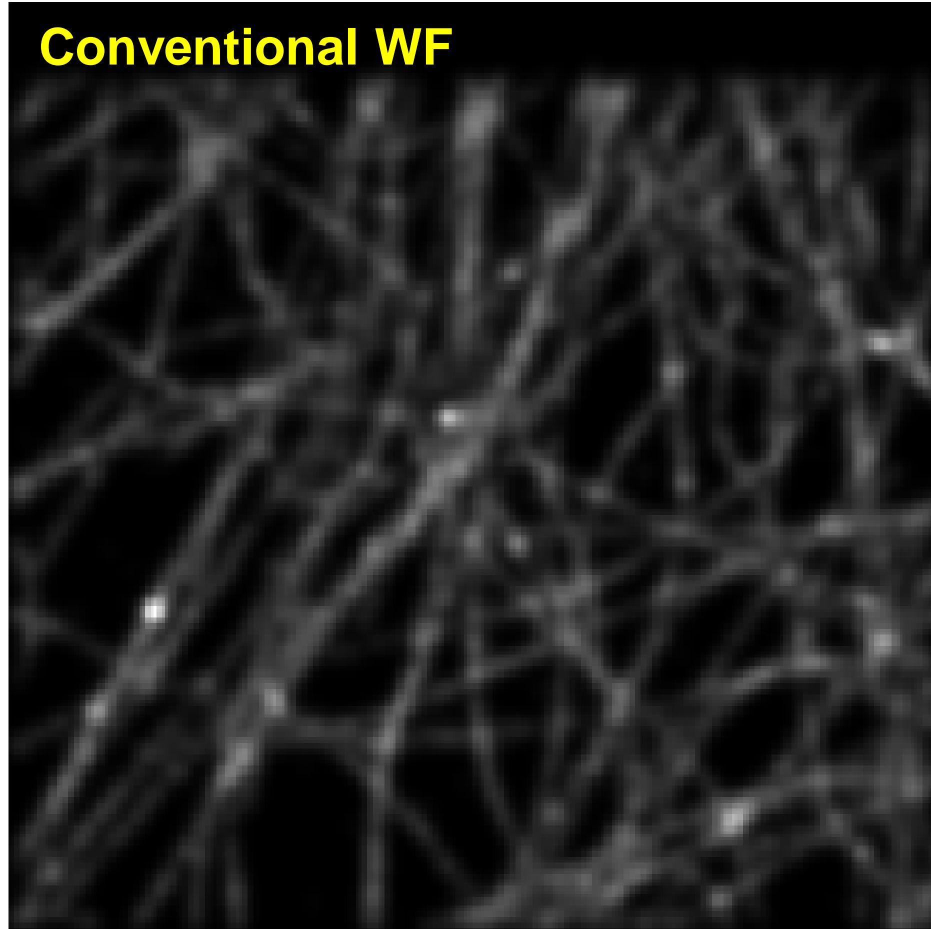
Cremer et al. 1999, 2002

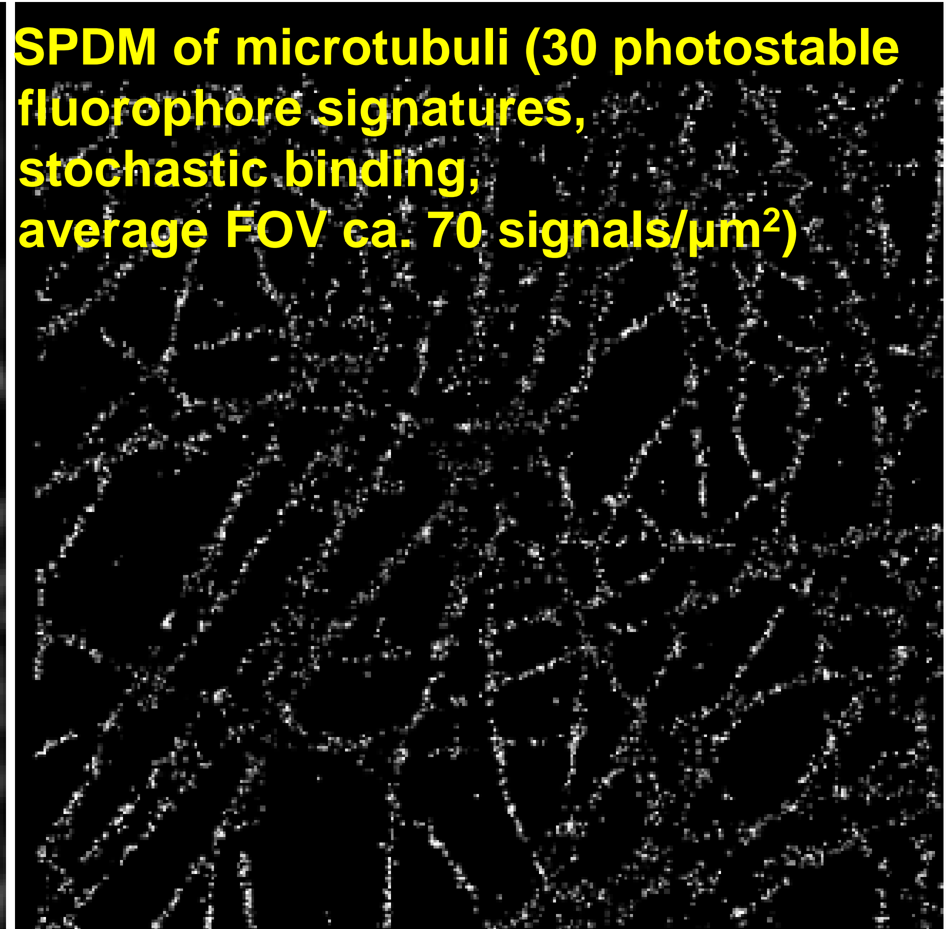
Localization Microscopy with photostable fluorophores (Betzig 1995; Cremer et al. 1996) allows structural super-resolution also **without** photoswitching
Simulation: Specifically labelled short DNA Sequences



Cremer et al. 1999

SPDM using photo-stable Fluorophores: A Virtual Microscopy Study





C.Cremer et al. 2002; U.Birk & C. Cremer (2017)

Desirable for Localization Microscopy of Imaging of NanoSTRUCTURES

Multiple Differences in <u>spectral signature to allow STRUCTURAL resolution</u>

Generally: Any photophysical property useful for photonic discrimination:

„Make DARK the molecules you do not want to see“

Solution II: Photoswitching

(Bright-Dark Transitions,‘Blinking‘):

BALM, FPALM/PALM, GSDIM, SPDM, STORM/dSTORM,…

Lidke 2005, Betzig 2006, Hess 2006, Rust 2006; Bock 2007, Egner 2007, Huang 2008, Reymann 2008, Lemmer 2008, 2009; Biteen 2008, Fölling 2008, Heilemann 2008, Kaufmann 2009; Baddeley 2009, 2010, 2011, Matsuda 2010, Pertsinidis 2010, Löschberger 2012,…..

Challenge II :

Study Genome Architecture at the Nanoscale at high structural resolution:

Single Molecule Localization Microscopy (SMLM) with

Standard Fluorophors:

Switch Standard Dyes between ‘bright‘ states and ‘dark‘ states

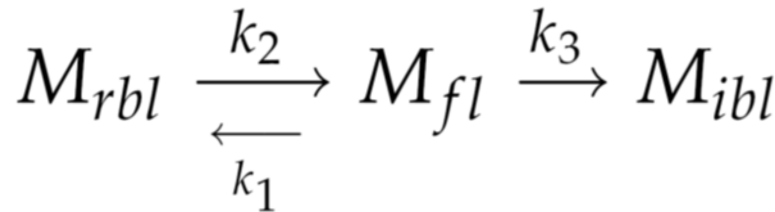
SPDM with Physically Modified Fluorochromes*

($SPDM_{PHYMOD}$)

* Related approaches: BALM, STORM, dSTORM, GSDIM,…

Standard Fluorophores can be induced to ‘blink’

A mechanism for optical isolation of single standard fluorophores in the Photoswitching Mode



$$M_{rbl} \underset{k_1}{\overset{k_2}{\rightleftarrows}} M_{fl} \xrightarrow{k_3} M_{ibl}$$

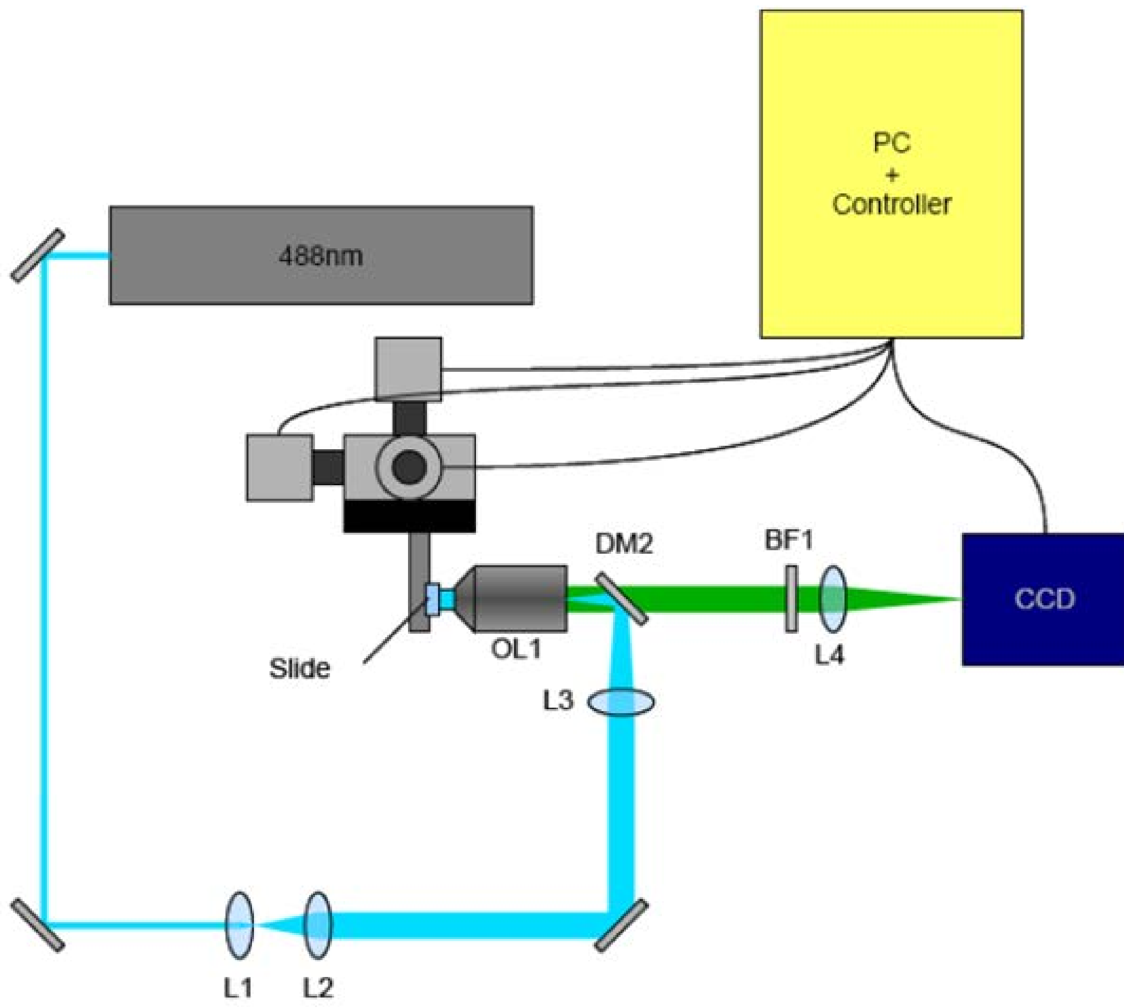
Creemers et al., 2000;Sinnecker et al. 2005

Spectral Precision Distance/Spatial Position Determination Microscopy *with physically modified Fluorophores* **($SPDM_{Phymod}$): Combination of suitable chemical environment + ‘high intensity‘ illumination** (typically using only one laser wavelength/dye)

Reymann et al. 2008; Lemmer et al. 2008, 2009; Kaufmann et al. 2009

Christoph Cremer
c.cremer@imb-mainz.de

A minimum Laseroptical Setup for SPDM-SMLM with photophysically modified fluorophores (SPDM$_{Phymod}$)



Reymann et al.,2008

(submitted Feb 2008,

published May 2008)

Lemmer et al, 2008,2009

Kaufmann et al., 2009

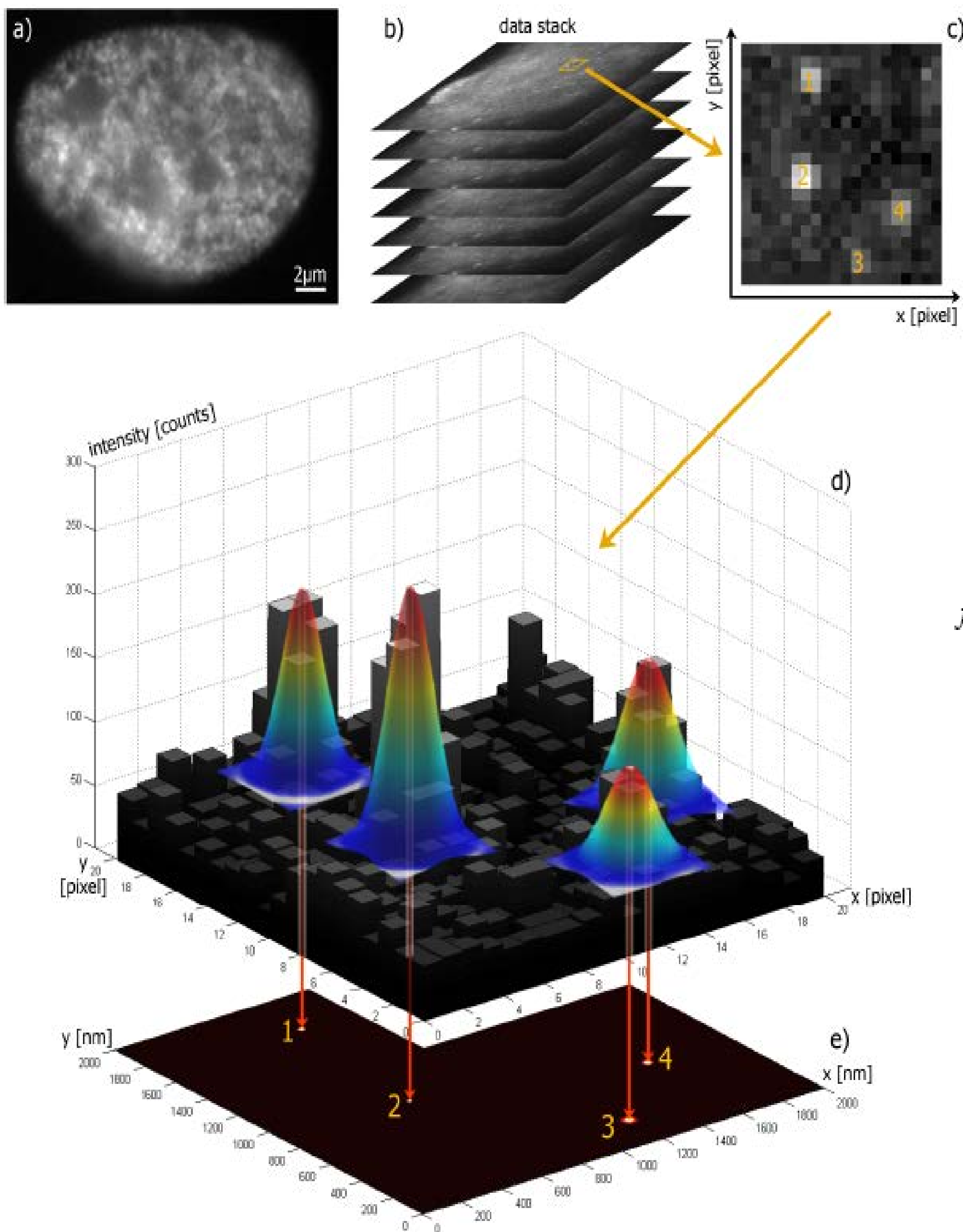


Figure 4. Example for the experimental localization of single histone proteins by SPDM. See text for details.

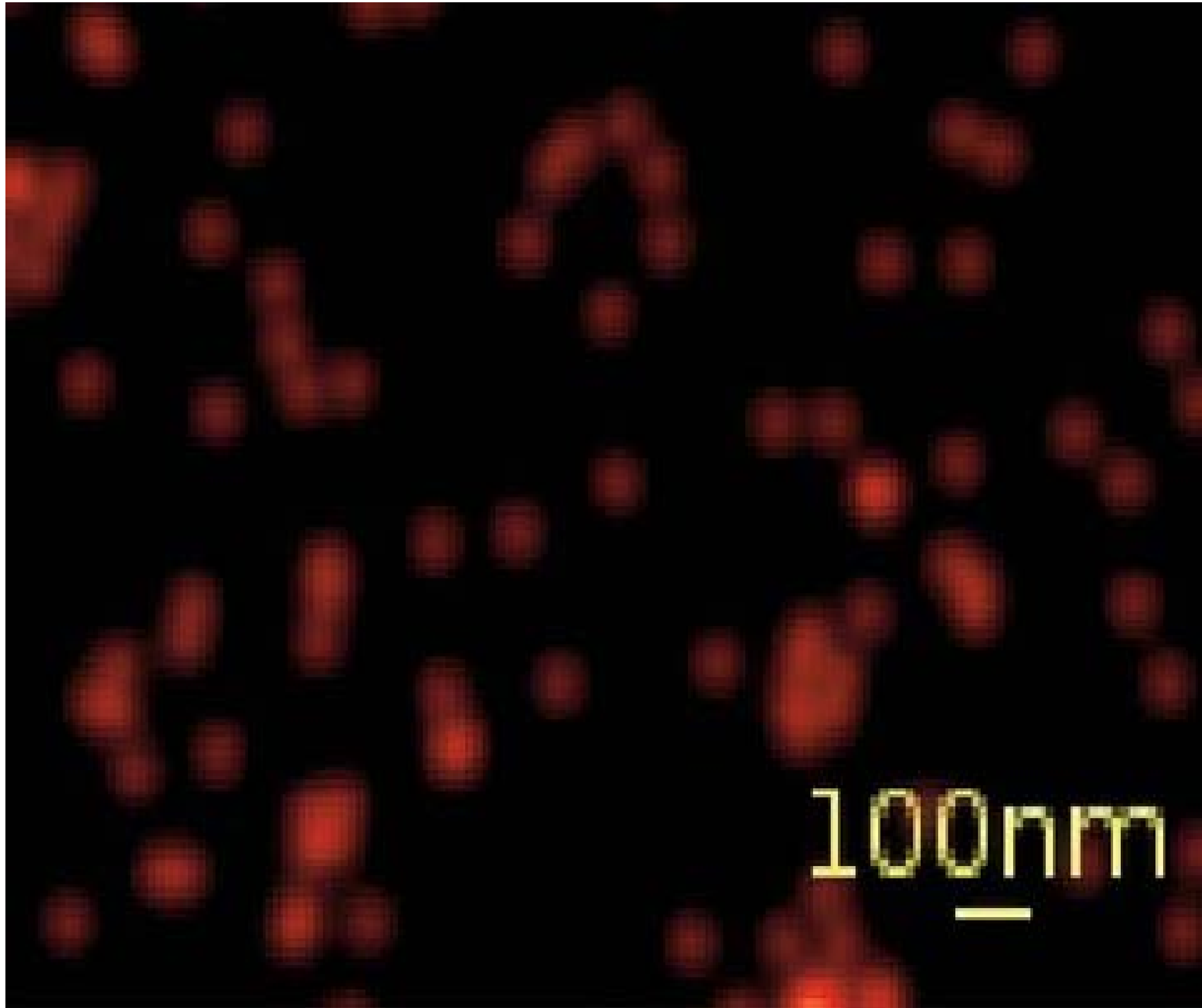
Determination of optically isolated Molecule Positions from Image Data: Adaptation of 2D Gaussians

$$f(x,y) = p_1 \exp\left(-\frac{(x-p_2)^2+(y-p_3)^2}{2p_4^2}\right) + p_5 + p_6(x-p_2) + p_7(y-p_3)$$

Best Single Molecule Localization Precision obtained in Biostructures at CC-Lab ca. 2 nm

Kaufmann et al. (2009)

2D-SPDM of p62 Labelled Nucleopore Complexes in Human Osteosarcoma (U2OS) Cells*



Localization Accuracy ~ 15 nm

Optical Resolution (OR):

OR ~ 35 nm

Total No. of

p62s detected per cell

(upper half):

~ 4800

*ALEXA488

Reymann et al., *Chromos. Res.* **16** (May 2008)

Challenge III:

Development of dedicated Localization Microscopy Systems*

to study

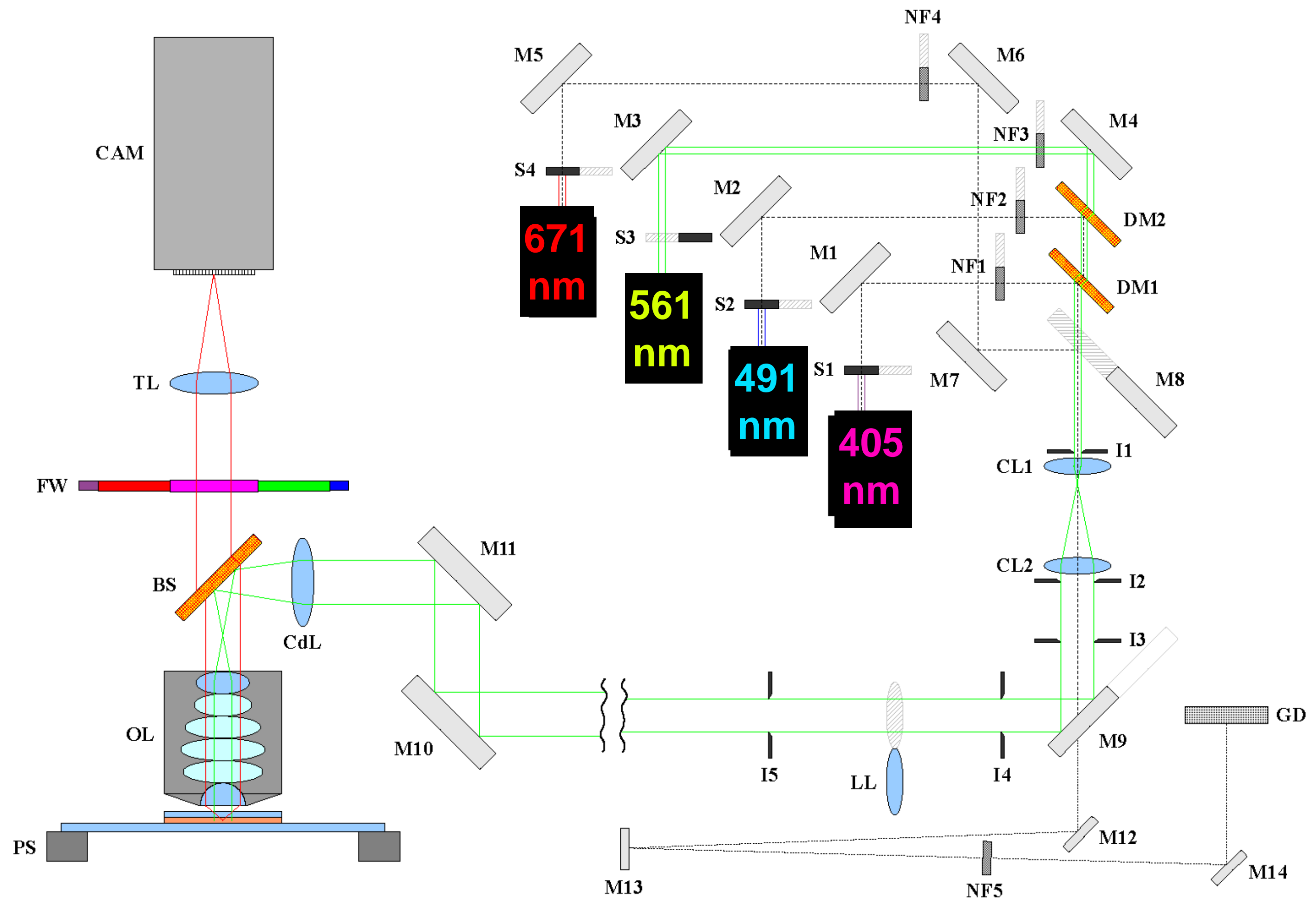
Genome Architecture at the Nanoscale

at high structural resolution

**including appropriate data evaluation algorithms*

The Vertico SPDM microscope features 4 excitation wavelengths: Potential for localization microscopy with 6 different molecule types simultaneously

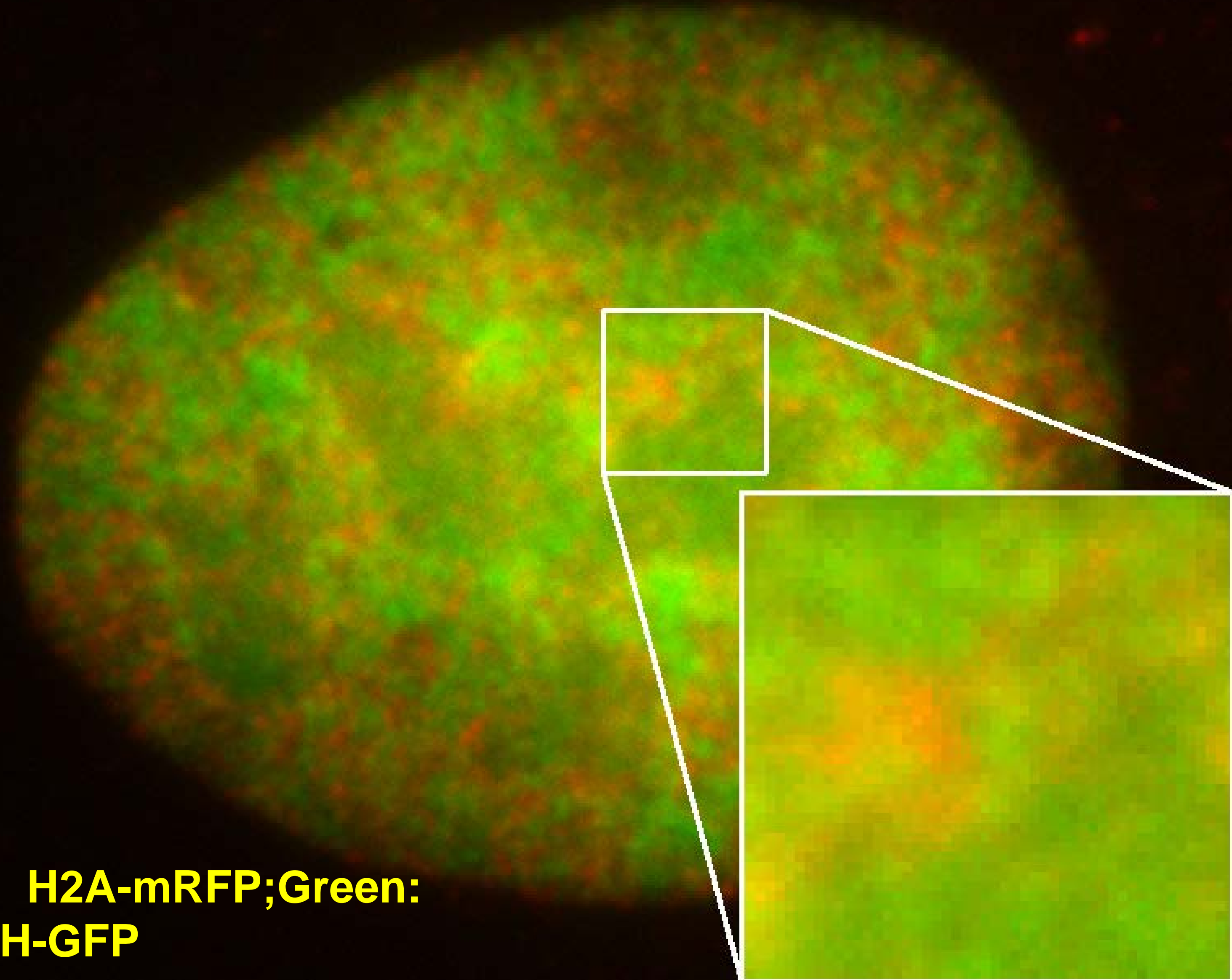
Optical resolution (best value) 5 nm ($\sim 1/100\lambda_{exc}$)



U. Birk, H.-K. Lee, C.Cremer/IMB

Eyample for Dual Color Localization Microscopy of intracellular single Molecules:

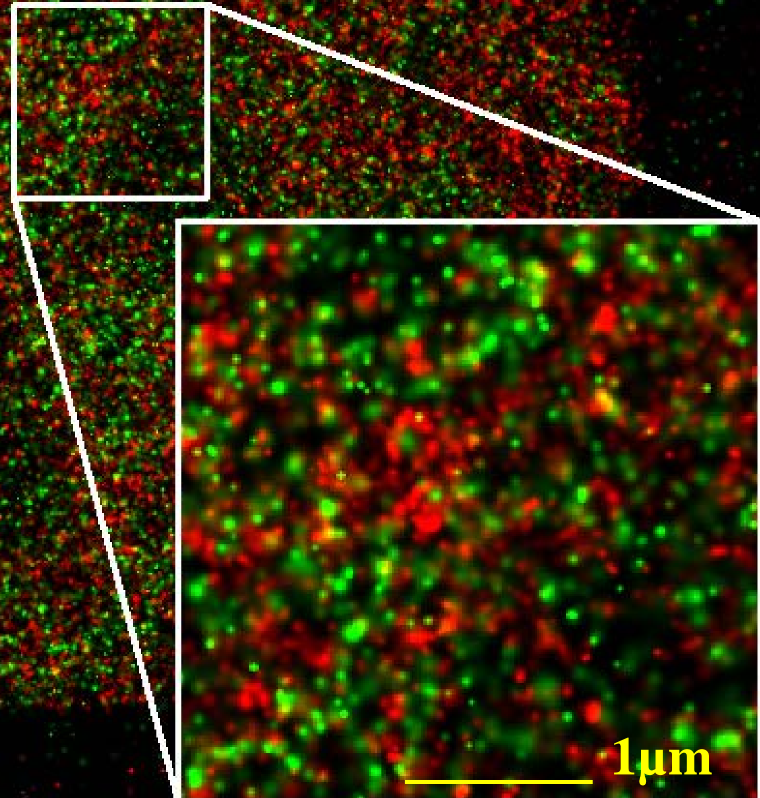
Distribution of Histones and Chromatin Remodelling Proteins in Somatic Cell Nuclei



Gunkel et al. 2009

SPDM Analysis of Histone-Chromatin Remodelling Factor Distribution

2µm



Red: H2A-mRFP, σ_{mean} = 25nm, N = 70,000; Green: Snf2H-GFP; σ mean = 30nm; N = 37,000

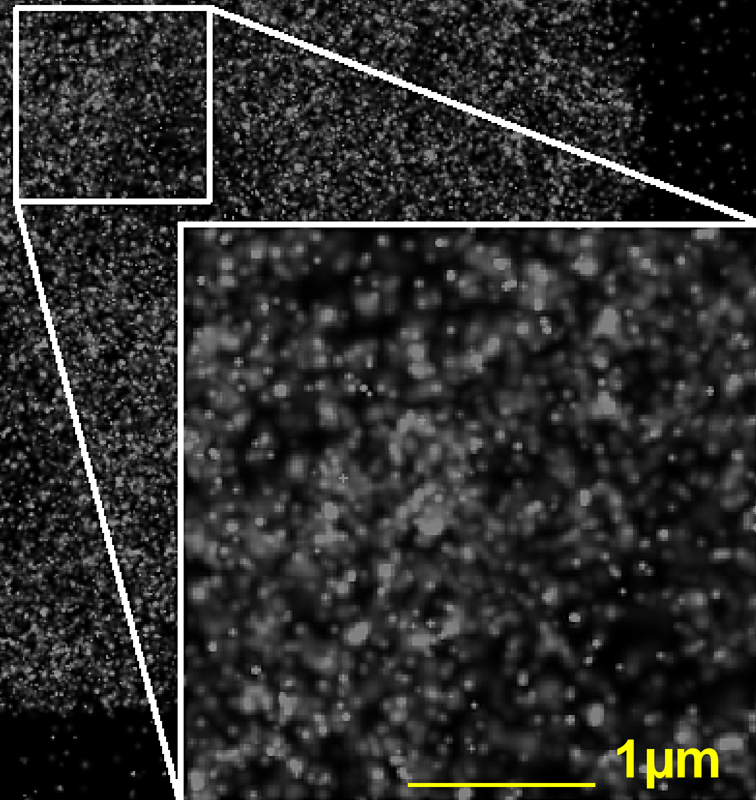
Gunkel et al. 2009

Gunkel et al. (2009)

2µm

Monocolor 'BLINKING' based Localization Microscopy often is not sufficient

Gunkel et al. KIP



Molecule Types Labelled:

1)H2A-mRFP, σ_{mean} = 25nm,

N = 70,000;

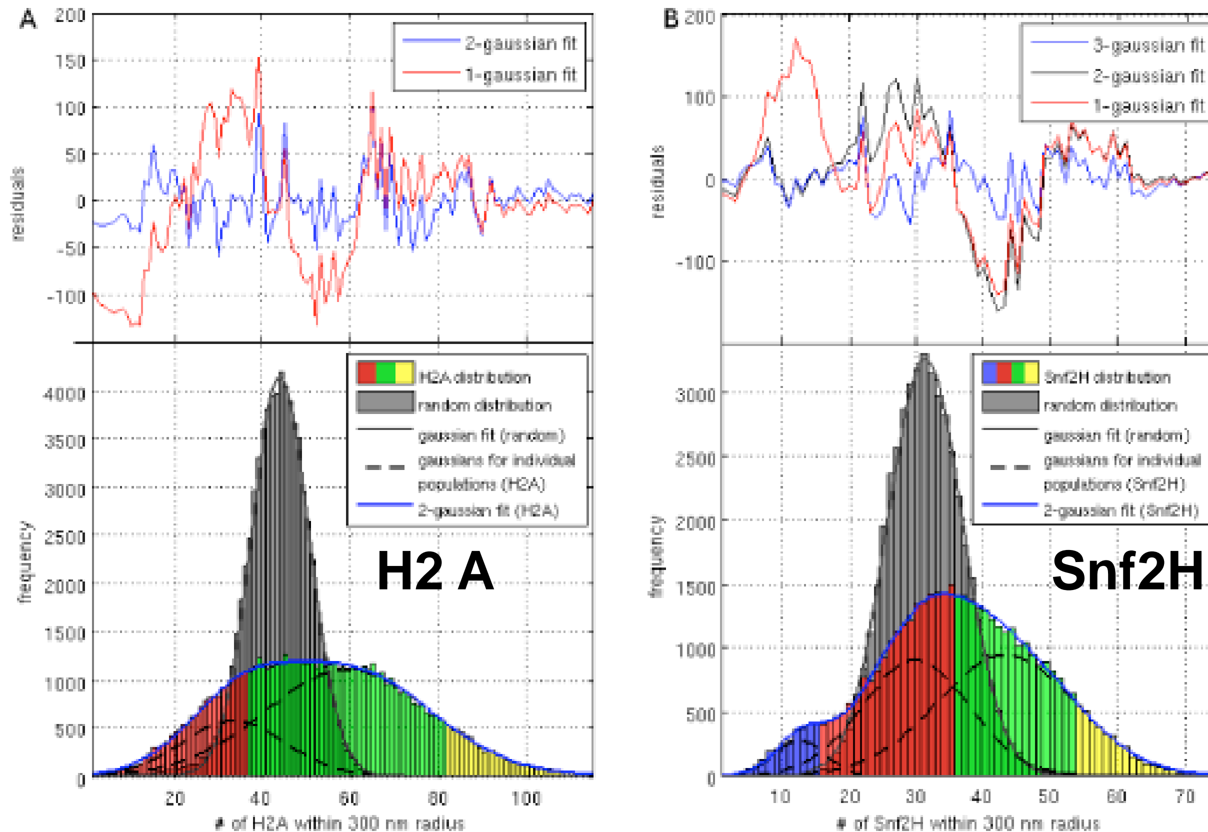
2) Snf2H-GFP;

σ mean = 30nm; N = 37,000

Gunkel et al. 2009

Dual Color Localization Microscopy of Nuclear Protein Distribution

A quantitative Evaluation of single Molecule Positions inside a Cell Nucleus



Analysis of Protein Densities. A Histogram of the number of neighbors (of the same color) within a circle of 300 nm radius was plotted for H2A (A) and Snf2H (B). As a reference, the same histograms were plotted for random distributions with equal particle densities (gray curve)

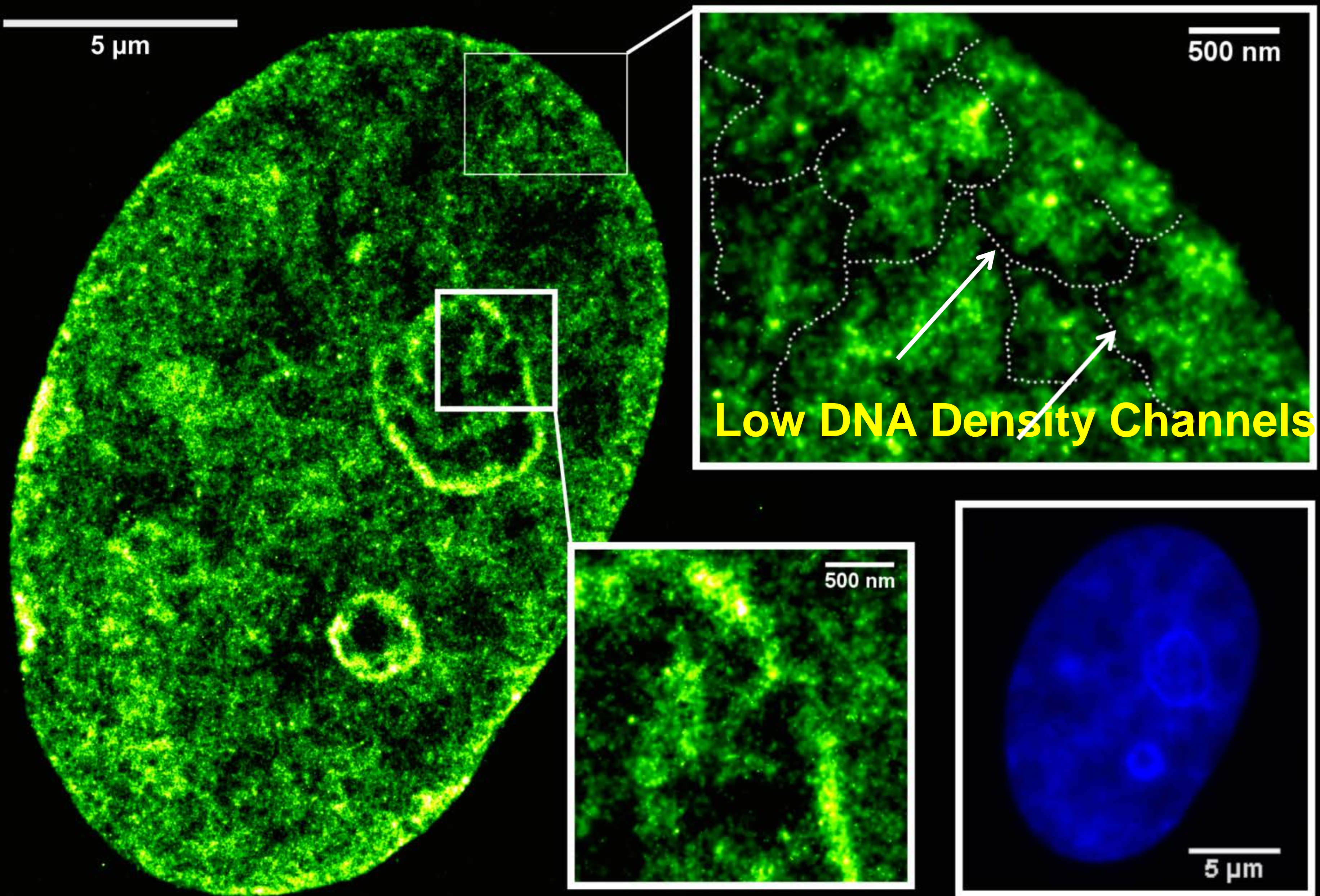
Gunkel et al.2009

Localization Microscopy of
Functional nuclear Genome Architecture:

Quantitative Nanoscale Analysis of

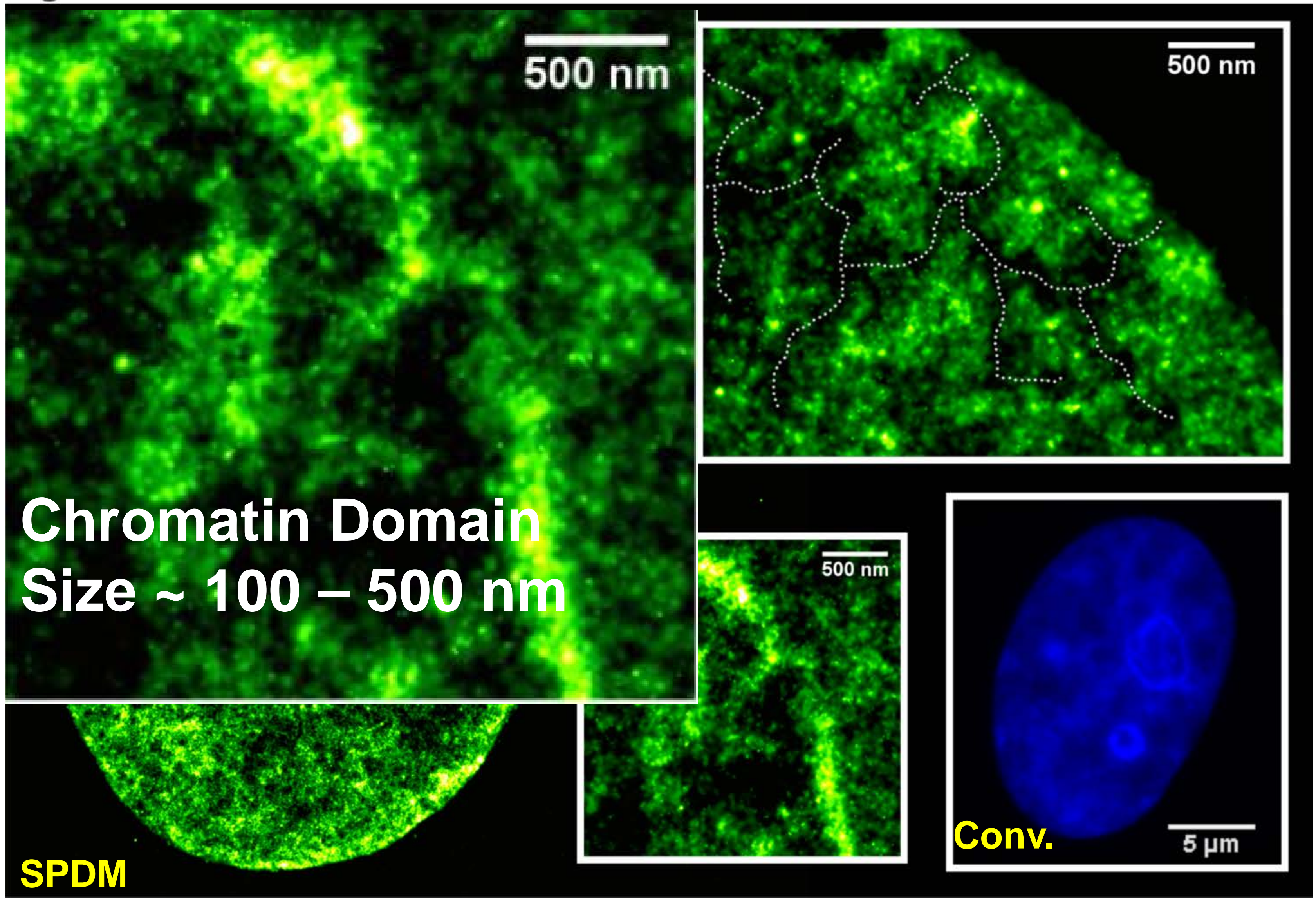
the DNA Distribution

Strategy I:
Permanently bound DNA Dyes



D. Żurek-Biesiada et al. 2015

SPDM allows to image individual small Chromatin Domains

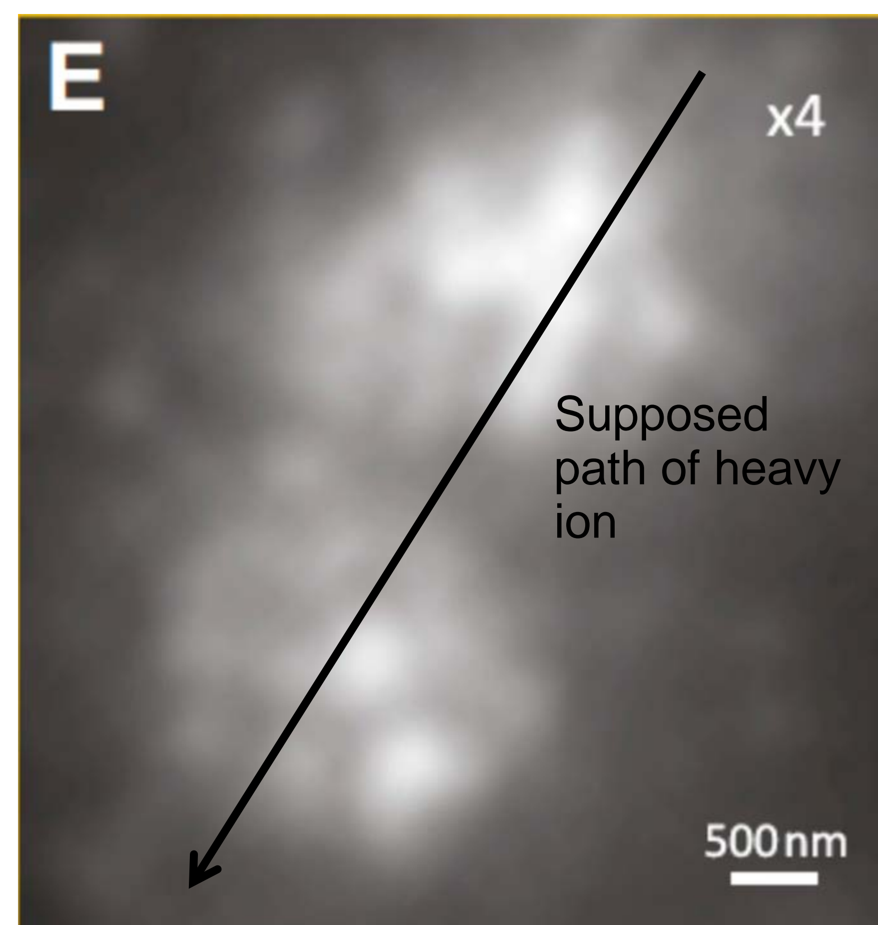


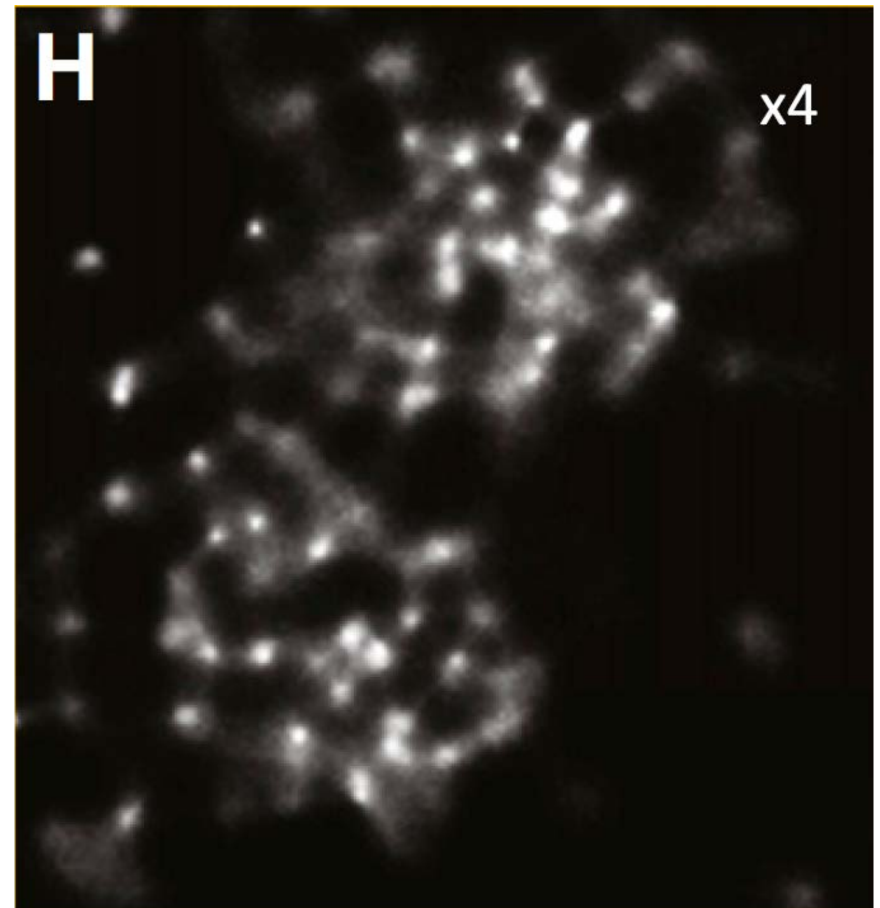
D. Żurek-Biesiada et al. 2015

Challenge IV:

Super-Resolution Microscopy of nuclear Genome Nanostructure under the influence of environmental modifications

Super Resolution Microscopy of ionizing Radiation induced Repair





SMLM of high energy heavy ions (part of cell nucleus): Visualization of Nanoscale Repair Cluster Distribution von hochenergetischen Teilchen

R. Perez et al. (2015)

Super-Resolving Localisation Microscopy of Nanostructures of the nuclear Genome:

An Application in Ischemia Research*

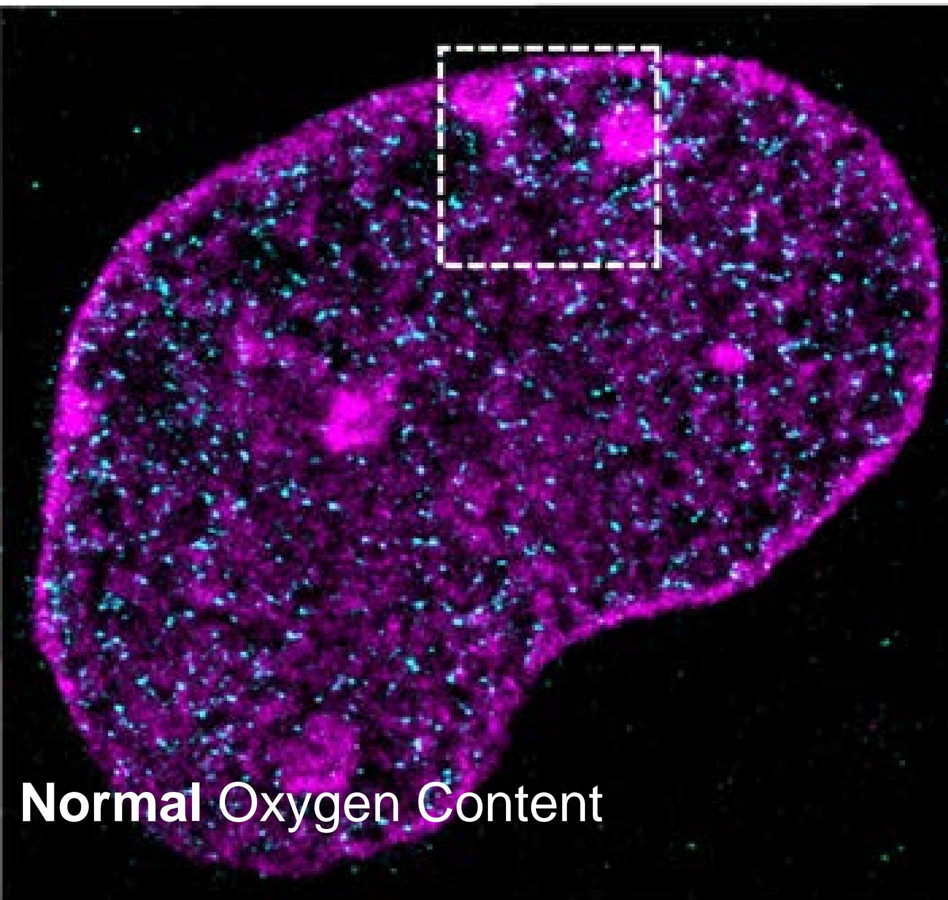
(Infarct, Stroke)

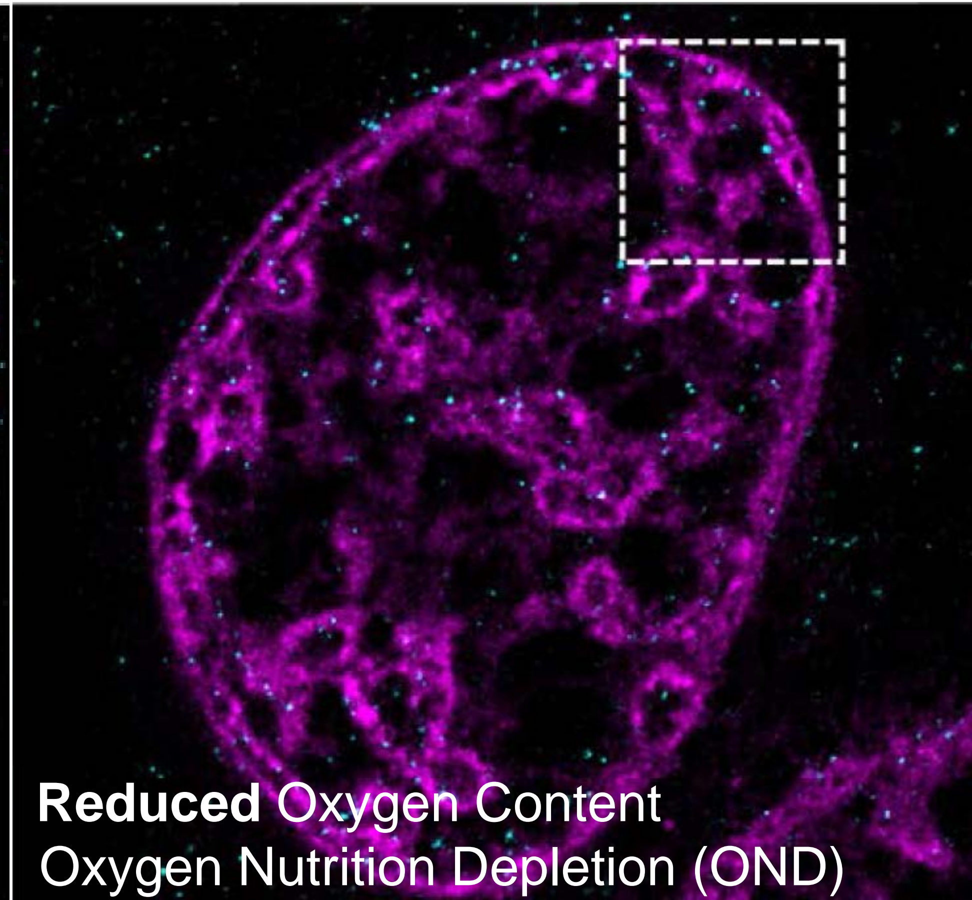
Kirmes et al., Genome Biology (2015)

Localization Microscopy of Myocard Cells at hypoxic (ischemic) Conditions

- DNA (purple)
- H3K14ac (blue)

OND: ATP level & Transcription reduced ca. 10 fold

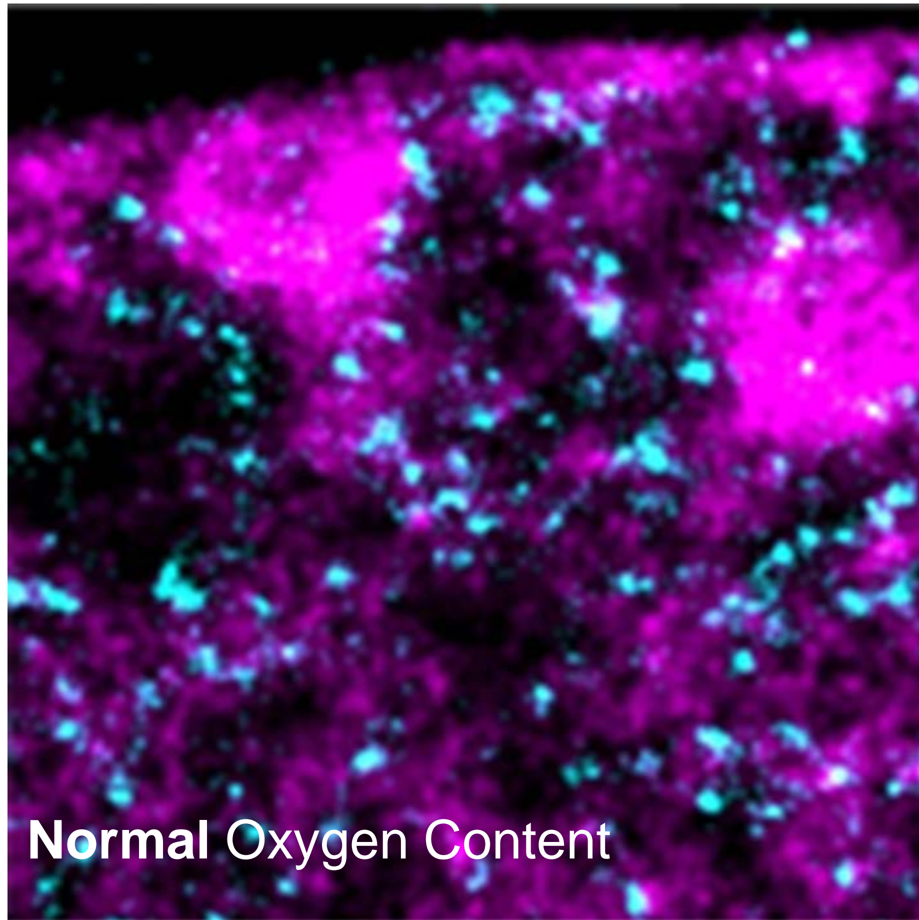


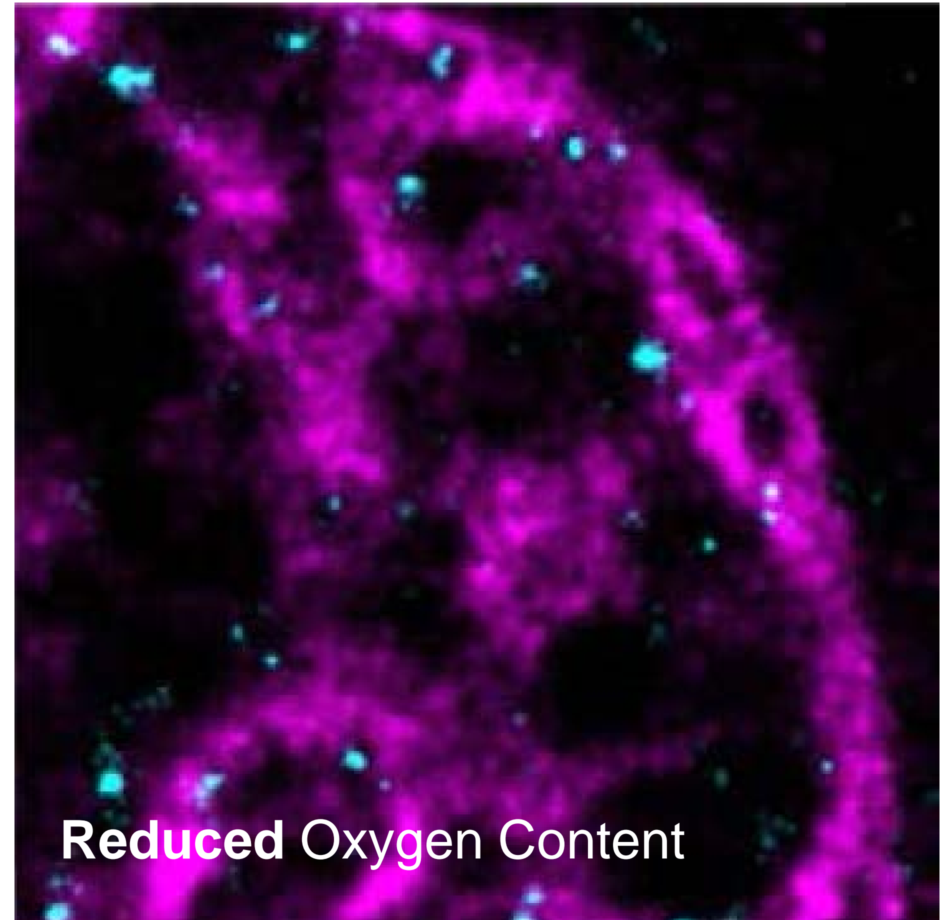


Kirmes et al., Genome Biology 2015

Localization Microscopy (SPDM) of nuclear Genome Nanostructure at hypoxic (ischemic) Conditions

- DNA (purple)
- H3K14ac (blue)

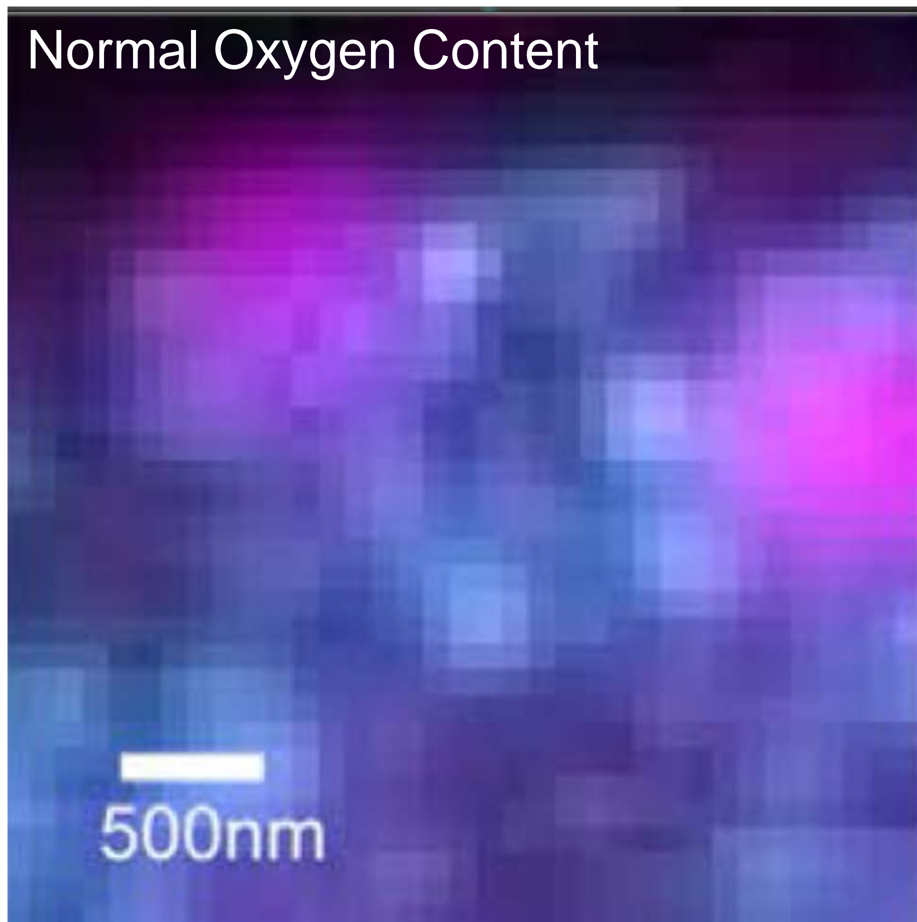


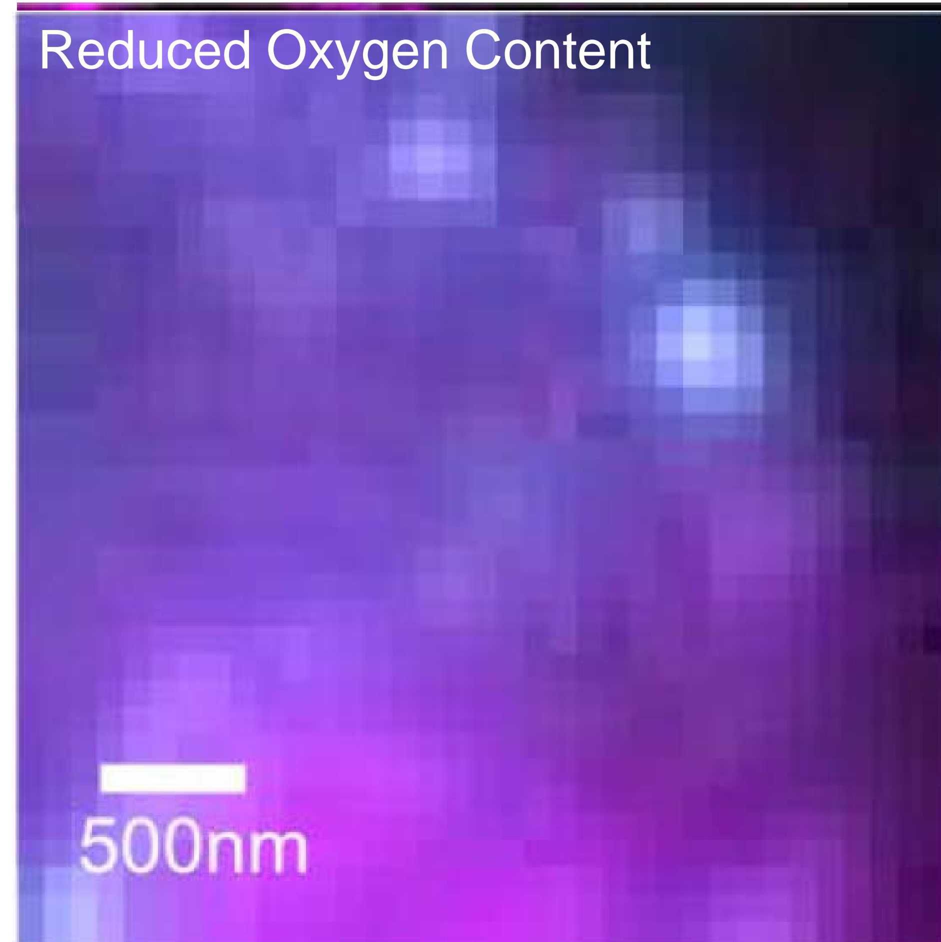


Kirmes et al., Genome Biology 2015

Conventional Microscopy of nuclear Genome Nanostructure at hypoxic (ischemic) Conditions

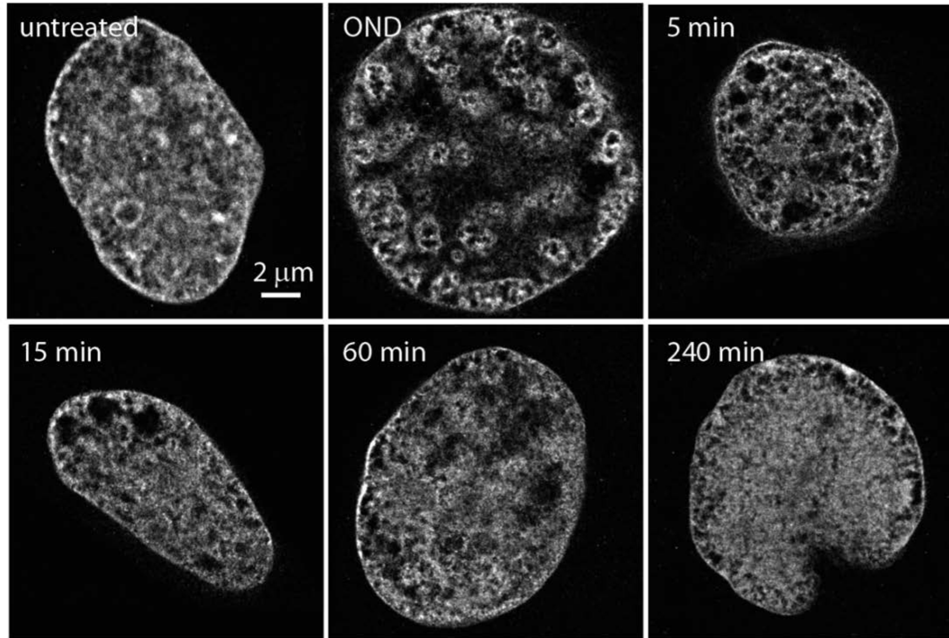
- DNA (purple)
- H3K14ac (blue)

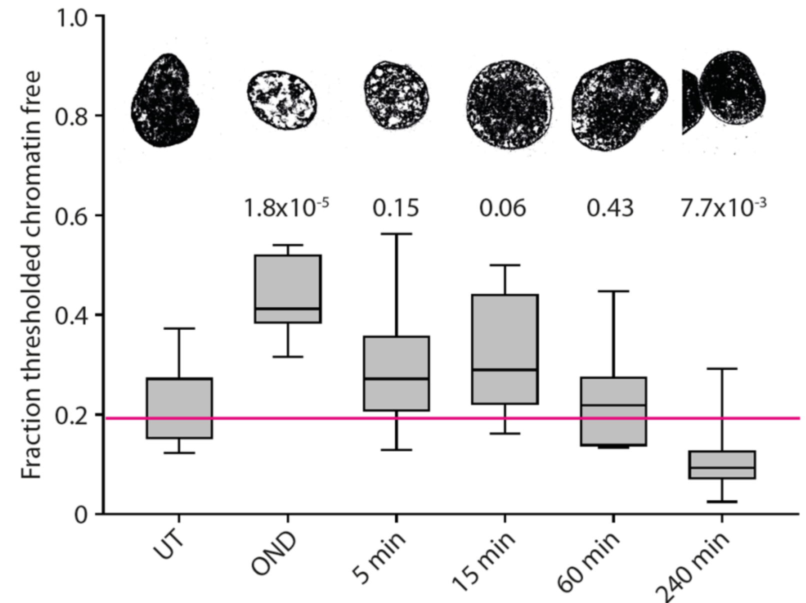




Kirmes et al., Genome Biology 2015

Time dependent changes in chromatin distribution can be quantified on the nanoscale



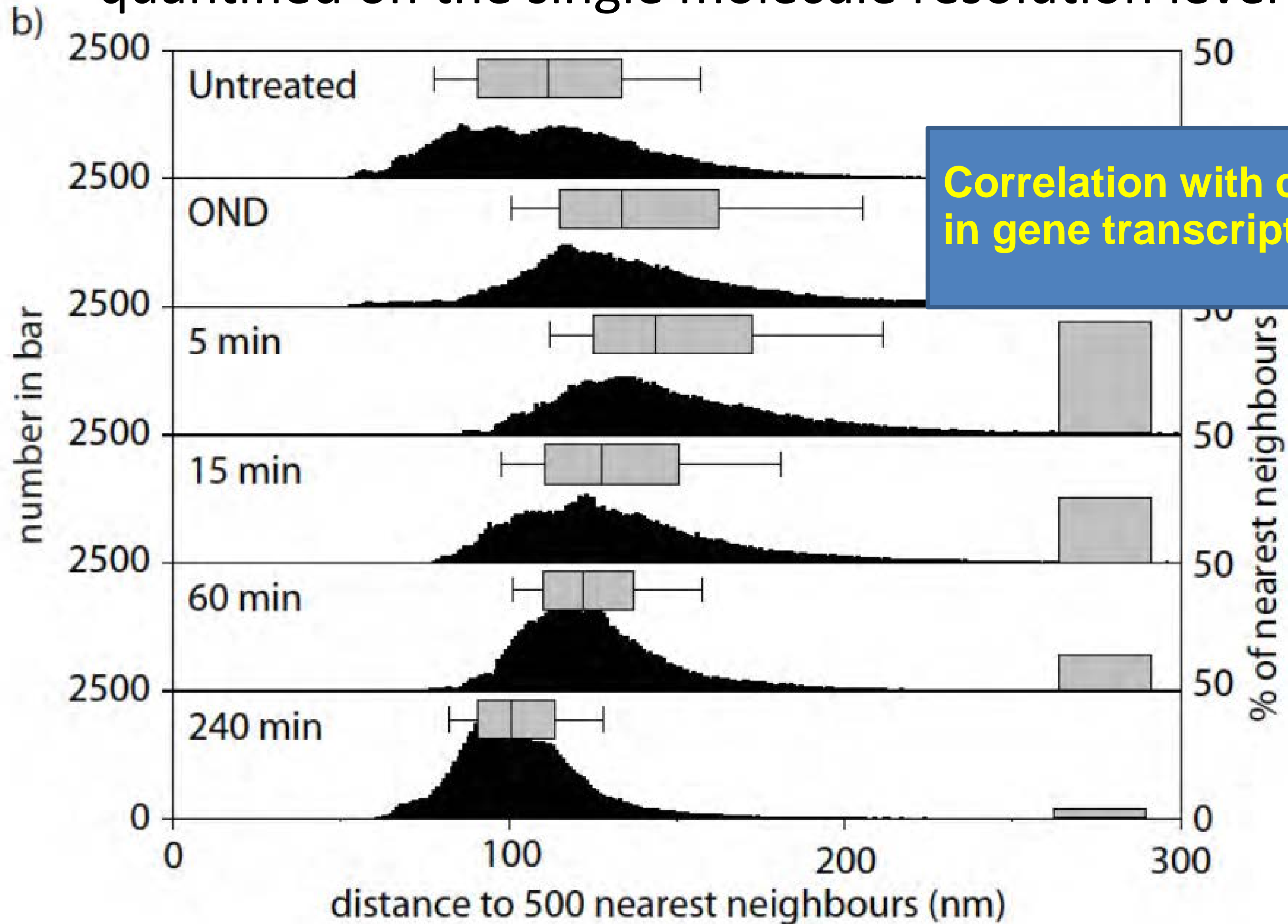


After OND, the IC-Compartment (LOW DNA Density) is enlarged

n=10 cells per bar

Kirmes et al., Genome Biology 2015

Time dependent changes in chromatin nanostructure can be quantified on the single molecule resolution level



Correlation with change in gene transcription

Kirmes et al., Genome Biology 2015

Challenge V:

Super-Resolution Microscopy of nuclear Genome Nanostructure: Towards resolution on the single nucleosome level

Improvement of Binding Activated Localization Microscopy (BALM) to image nuclear DNA at very high Structural Resolution: **Phase Dependent BALM (pBALM)**

A.Szczurek

Desirable for Localization Microscopy of Imaging of NanoSTRUCTURES

Multiple Differences in spectral signature to allow STRUCTURAL resolution

Generally: Any photophysical property useful for photonic discrimination:

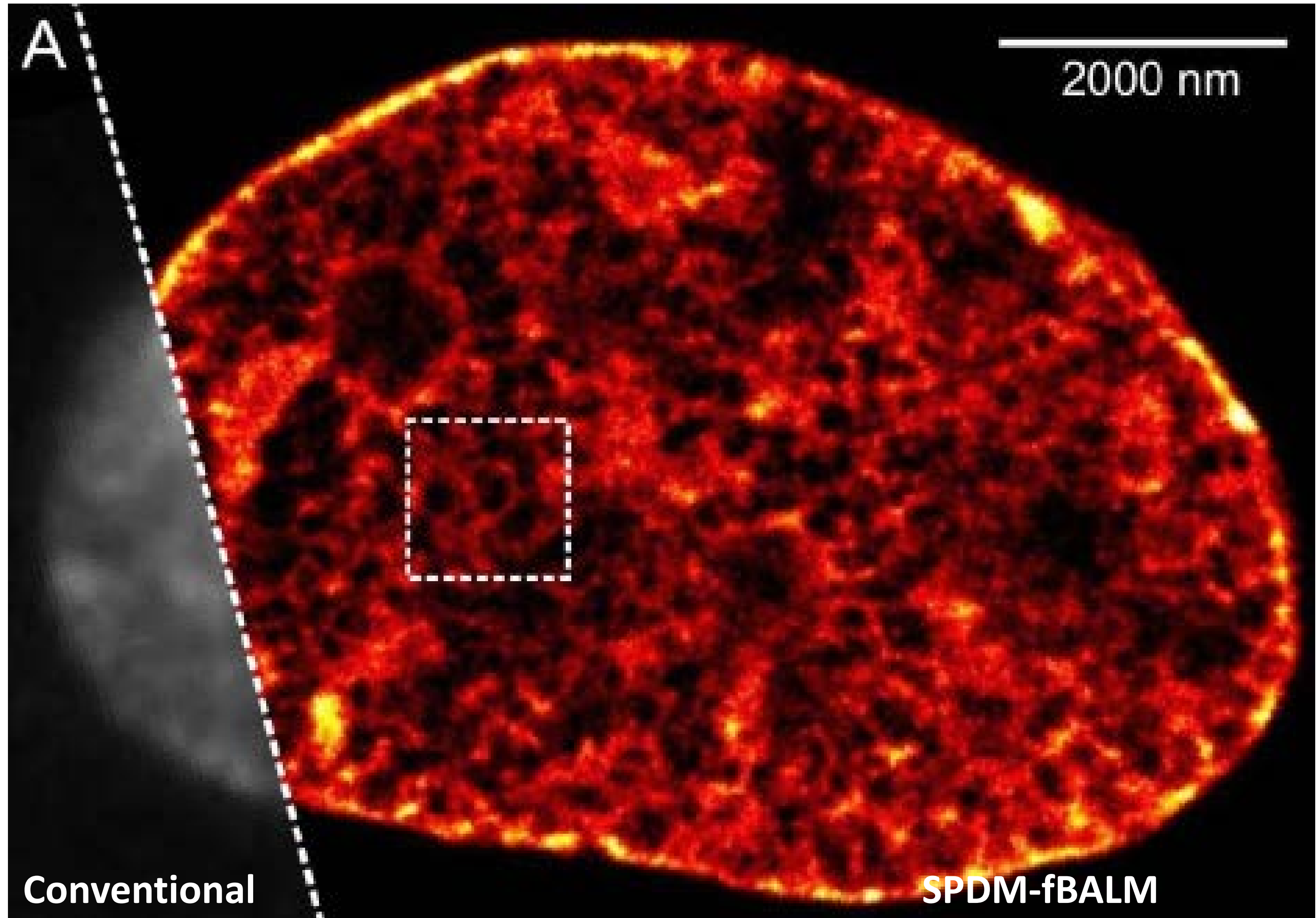
„Make DARK the molecules you do not want to see“

Solution III: Spatial switching

Bright-Dark Transitions,‘Blinking induced by vicinity of fluorophore to target‘: BALM/FBALM

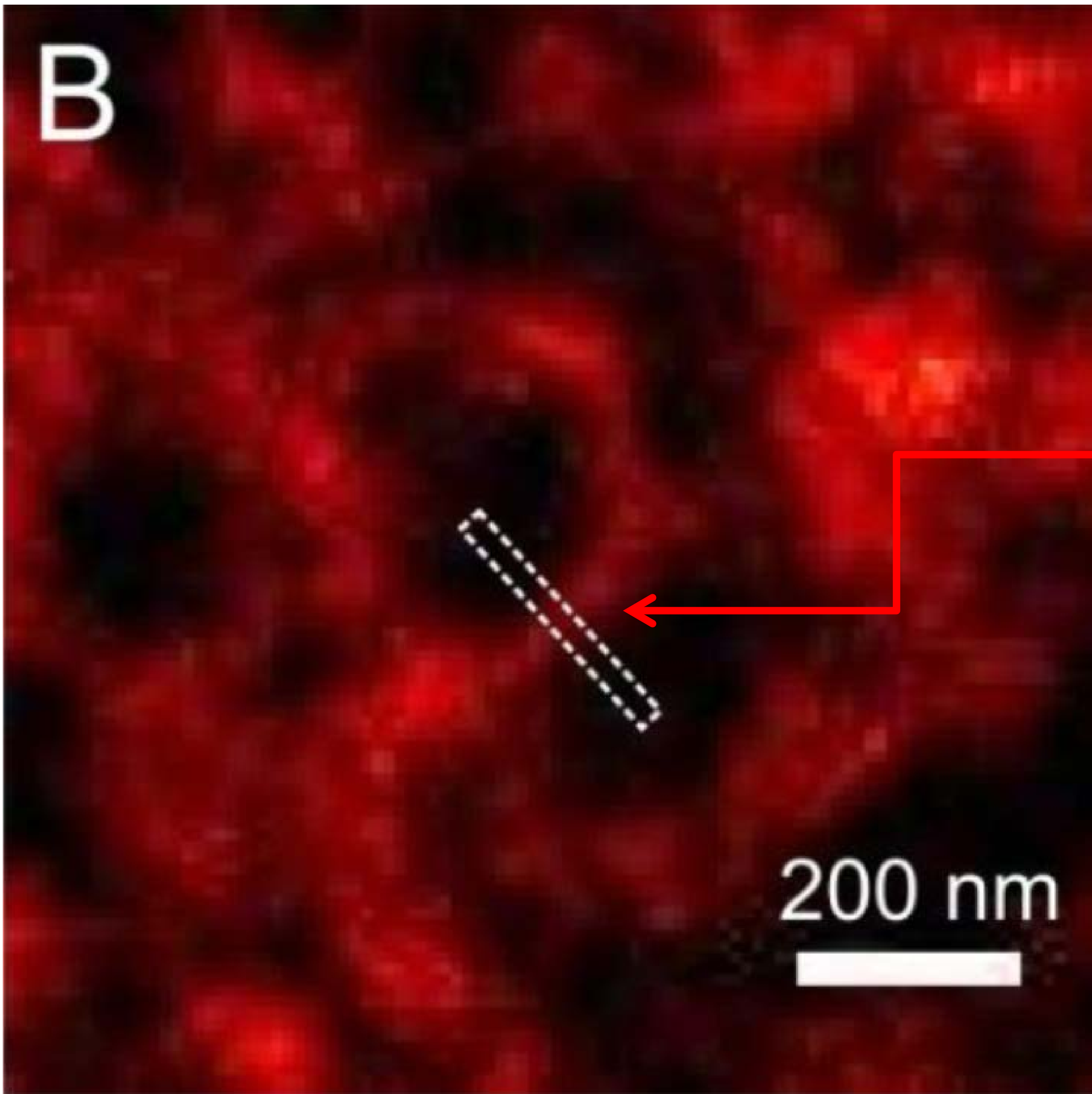
Single Molecule Localization Microscopy of nuclear Genome Nanostructure: Toward resolution of single small nucleosome cluster distribution

SPDM-fBALM: Nuclear DNA Distribution in a HL-1 murine myocardiac cell line



A. Szczurek et al., NAR (2017)

Localization Microscopy (SPDM-fBALM) of intranuclear DNA Distribution indicates complex Nanostructures in the sub 100 nm Range



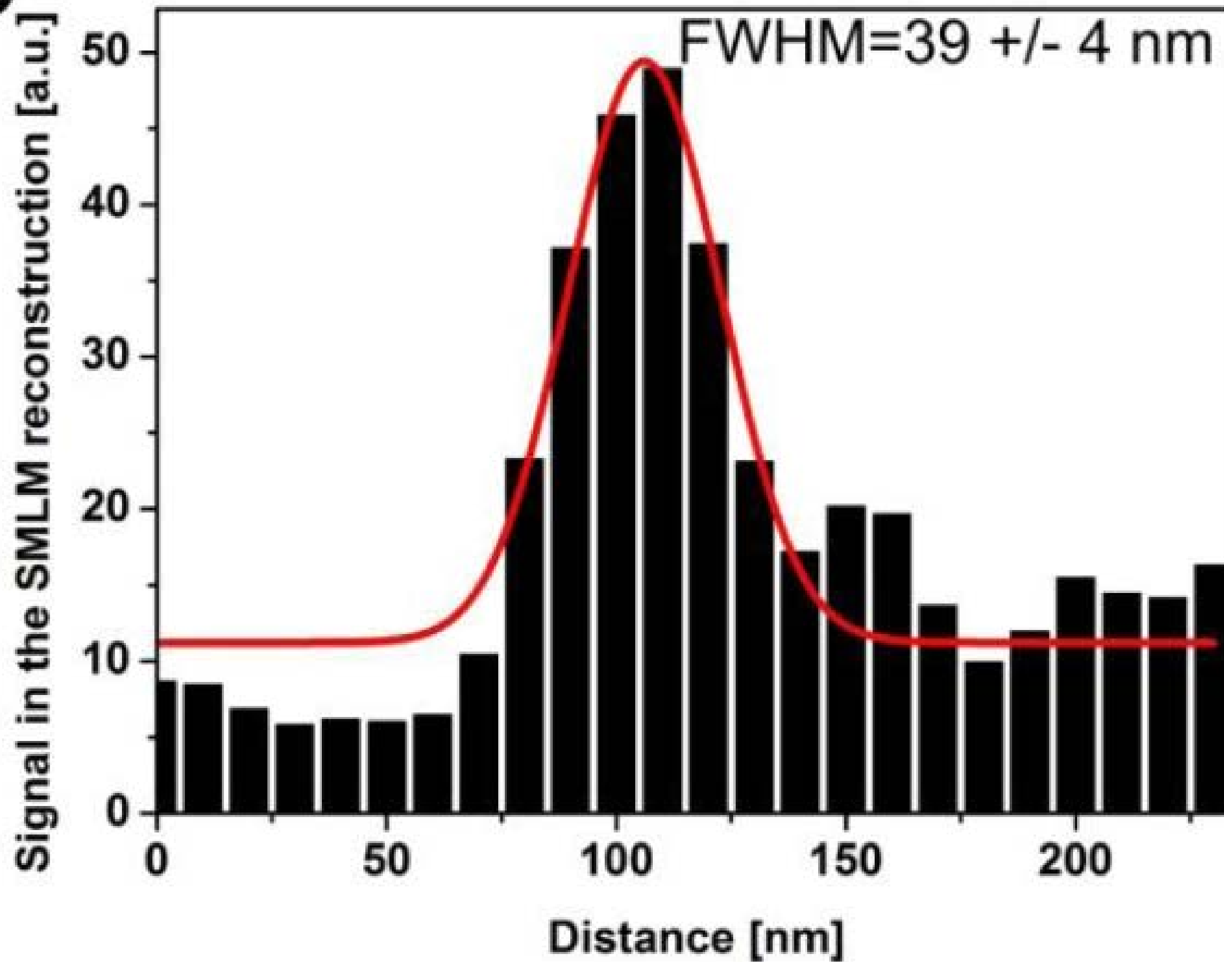
HL-1 murine myocardiac cell line (ischemic conditions)

Small condensed chromatin nanostructure

A. Szczurek et al., NAR (2017)

SPDM-fBALM indicates intranuclear super-resolution DNA imaging with structural resolution down to 40nm

HL-1 murine myocardiac cell line

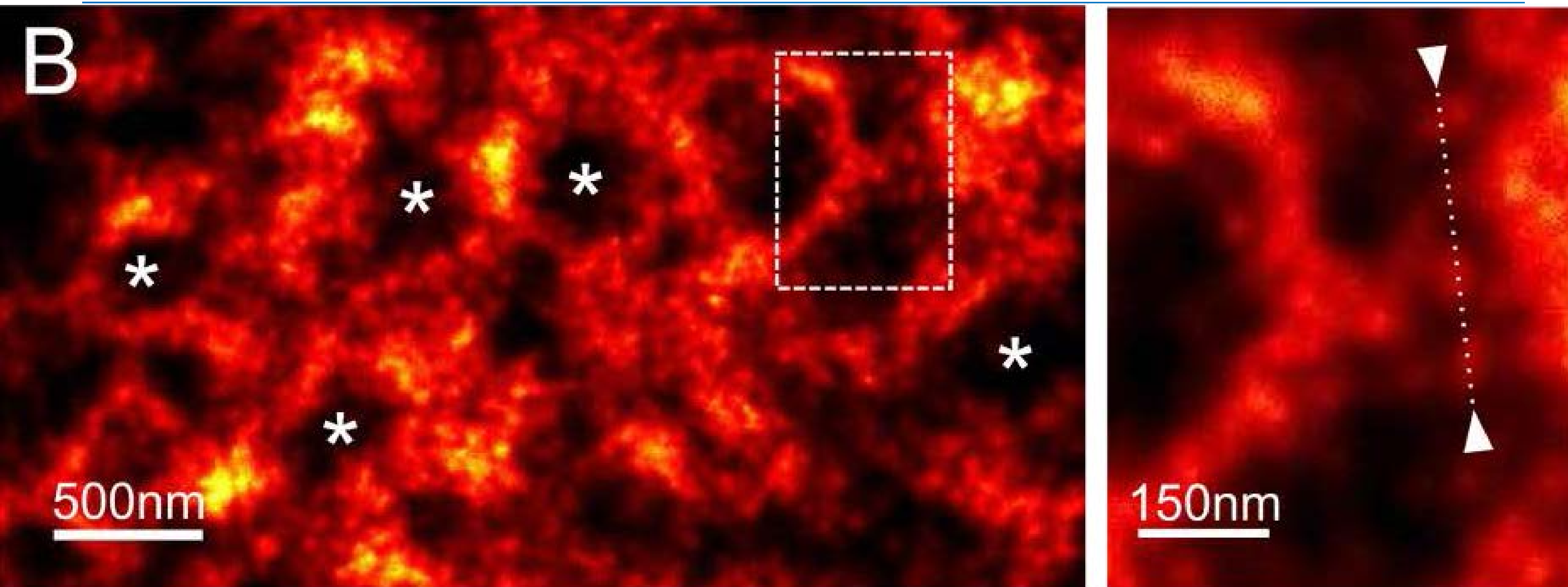


Intensity Distribution across a small condensed DNA region

A. Szczurek et al., NAR (2017)

SPDM-fBALM of Nuclear Genome Nanostructure

Institute of Molecular Biology

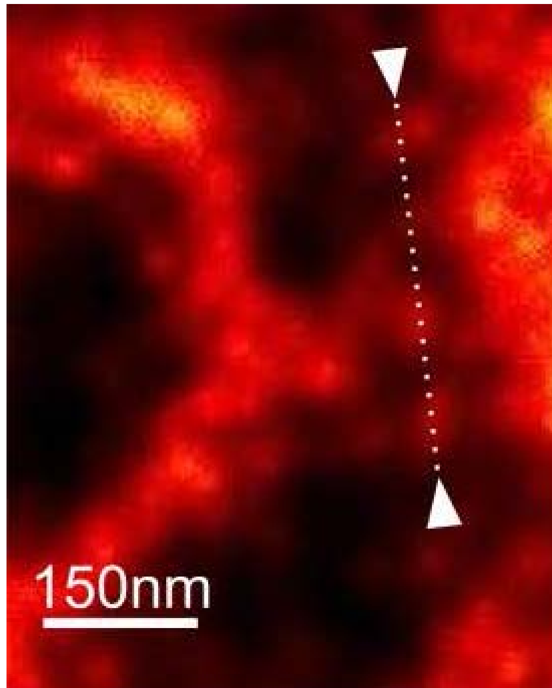


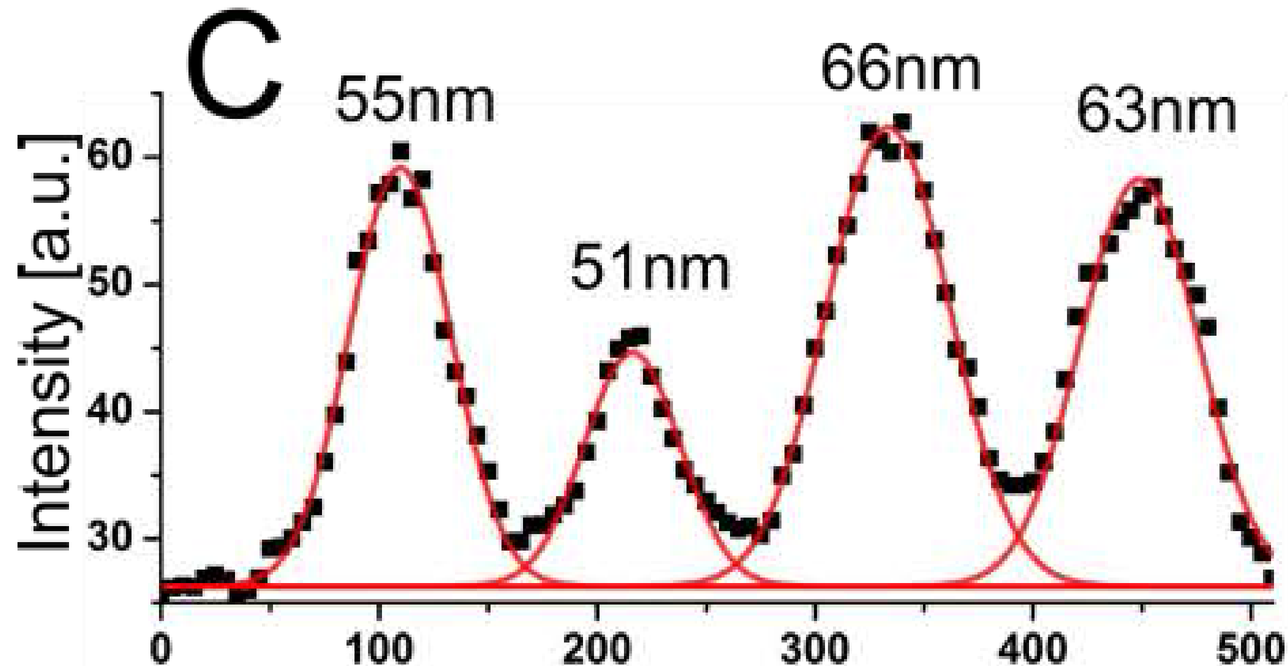
Region of interest indicated in an ischemic HL-1 myocardiac cell nucleus. Asterisks show void intranuclear regions with very low DNA signal density, likely an interchromatin/ANC compartment.

A.Szczurek et al., NAR (2017)

SPDM-fBALM of Nuclear Genome Nanostructure

imb Institute of Molecular Biology



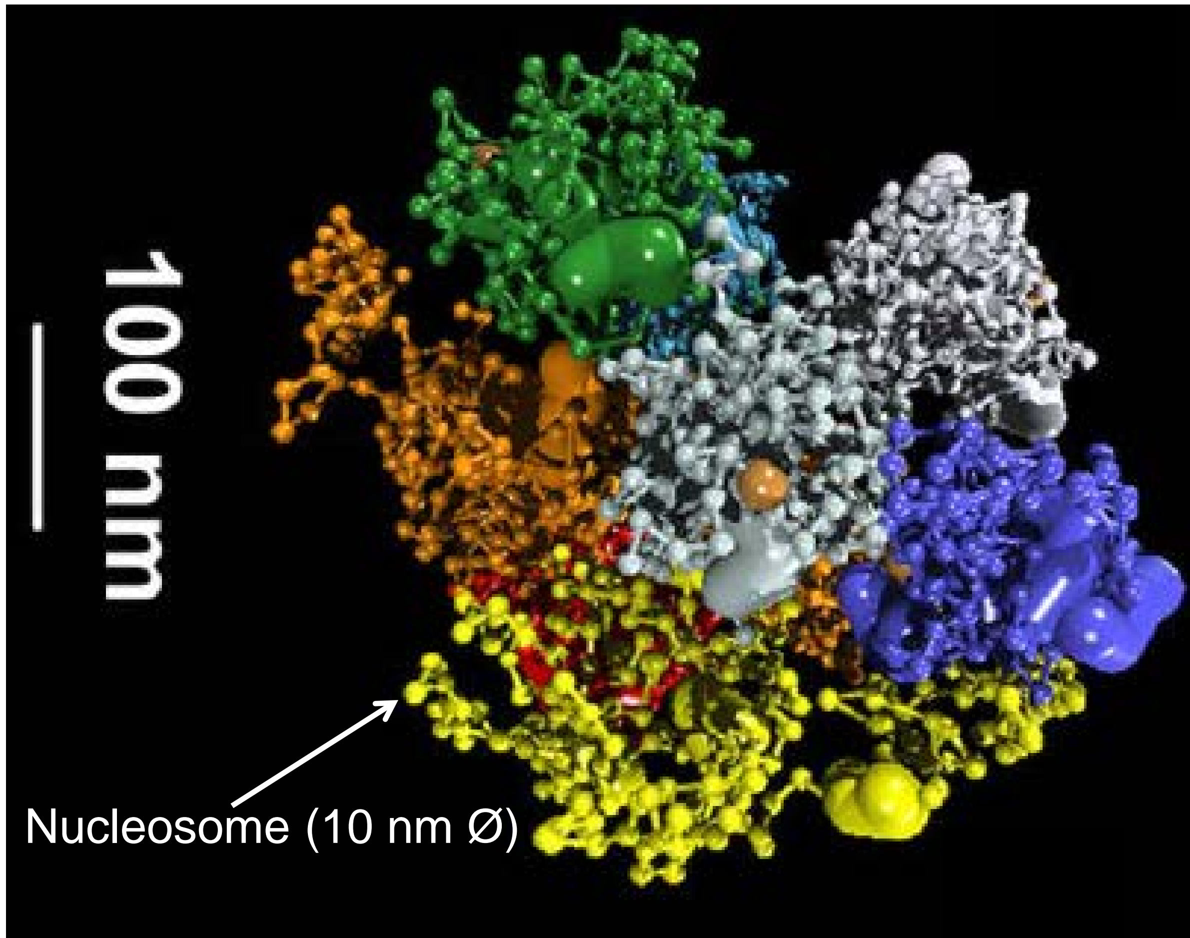


Signal intensity integrated between arrow-heads. Red line: Multiple gaussian fit. Black squares: actual signal intensities in the DNA density image. The numbers correspond to the Full-Width-at- Half-Maximum (FWHM) for each of the peaks fitted with a gaussian curve.

A. Szczurek et al., NAR 2017

SMLM Perspectives: Further Enhancement of optical & structural Resolution of Nucleosome Clusters

Institute of Molecular Biology



Example for Goal:
Analysis of individual Nucleosome **Arrangements**
(< 10 nm 3D Resolution Level required)

Strategy to achive this goal:

Combination of various approaches, e.g.
Localization Microscopy
+
Structured Illumination

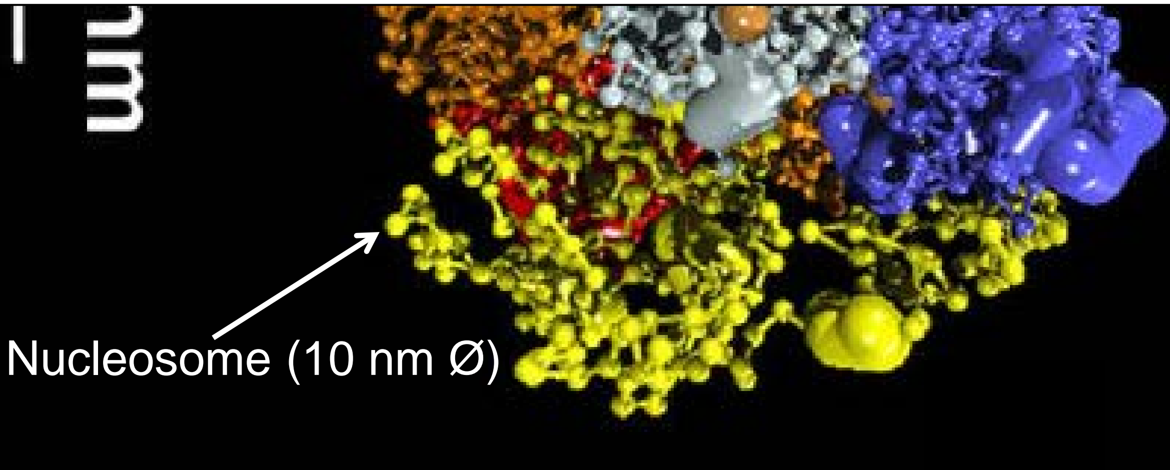
SMLM Perspectives: Further Enhancement of optical & structural Resolution of Nucleosome Clusters

Institute of Molecular Biology

Numerical Simulations (CC-Lab)

Optical 3D Resolution SIM/SMI + SMLM ~ 1 nm ($1/600\ \lambda_{exc}$)

Albrecht et al. 2001; Rossberger et al., 2014; Best et al. 2015



Example for Goal:
Analysis of individual Nucleosome **Arrangements**
(< 10 nm 3D Resolution Level required)

Strategy to achive this goal:

Combination of various approaches, e.g.
Localization Microscopy
+
Structured Illumination

Conclusions I

- Single Molecule Localization Microscopy (SMLM) allows to image nuclear structures with a resolution down to the lower nanometer range

- SMLM currently allows to determine the 3D positions up to 6 million of individual molecules in a single nucleus (laterally ~ 10 nm, axially ~ 50 nm; this precision may be considerably enhanced (present best value 2 nm for intranuclear site)

- SMLM opens an avenue to test models of genome organization down to molecular resolution and thus contributes to the development of a better quantitative understanding of nuclear structure and function.

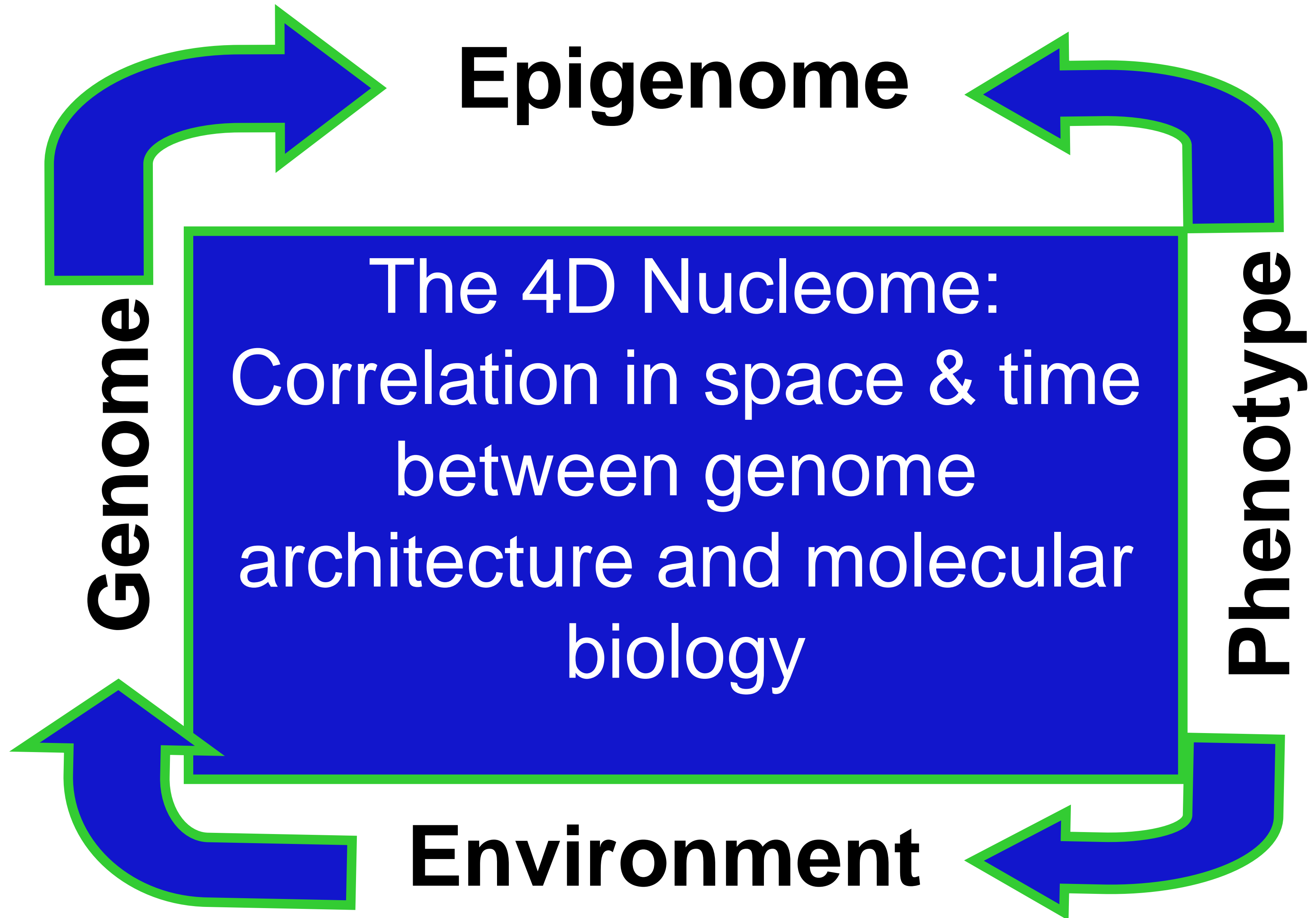
- SMLM complements Molecular Biology Approaches to unravel functional nuclear nanostructure

Conclusions II

Single Molecule Imaging of nuclear Genome nanostructure may be extended to biomedical applications, e.g.

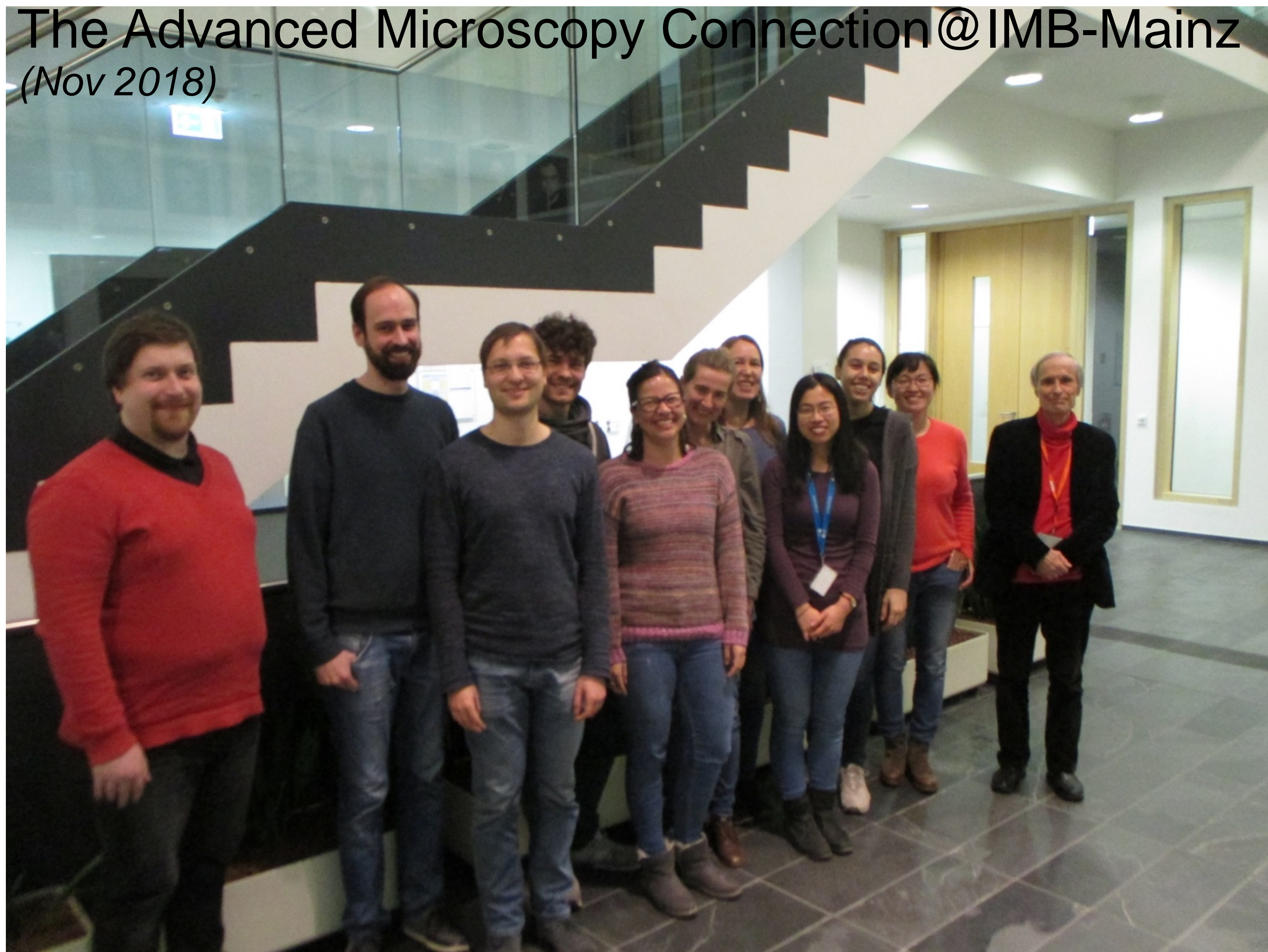
- Diagnostics of metastatic cancer clones in tissues;
- Biological dosimetry of tissue cells (interphase)
- Nuclear nanoscale monitoring of drug distribution
- Monitoring of epigenetically induced modifications of brain cells
- Development of epigenetic drugs

The phenotype is the result of exceedingly complex and interacting genetic, epigenetic and environmental networks



The Advanced Microscopy Connection@IMB-Mainz

(Nov 2018)



Florian Schock, Marton Gelleri, Jan Neumann, Felix Schreiber, Maria Contreras Gerenas; Sandra Ritz*, Maria Hanulova*, Shih-Ya Chen, Renata Pandolfo, Xiaomin Li, Christoph Cremer (from left)

**Core Facility IMB*

For former members see www.optics.imb-mainz.de

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