

# X-ray and Electron imaging of Human Chromosomes

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# Outline

- X-ray Microscopy and Imaging
  - Scanning Transmission (STXM)
  - Coherent Diffractive Imaging (CDI)
  - X-ray Ptychography
- Serial Block Face SEM (“3-view”)
- Optical methods: STORM and FLIM

# Chromatin as a storage material (2016)

Blu-ray



2 Gbit/cm<sup>2</sup>

Magnetic  
Tape



20 Gbit/cm<sup>2</sup>

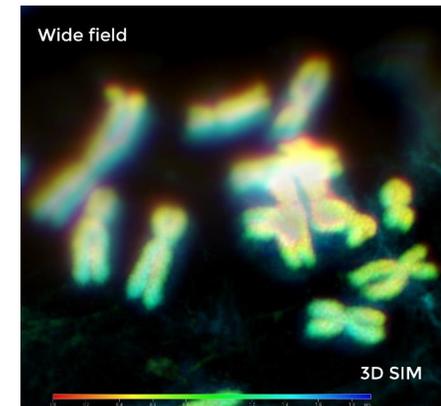
Magnetic  
Hard Drive



200 Gbit/cm<sup>2</sup>

Genome ~ 1.5  
Gbytes

Chromatin

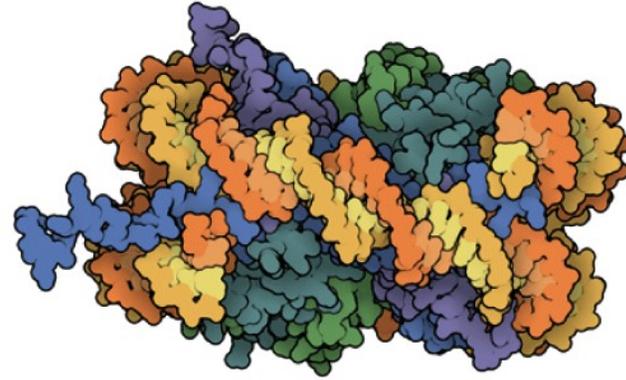
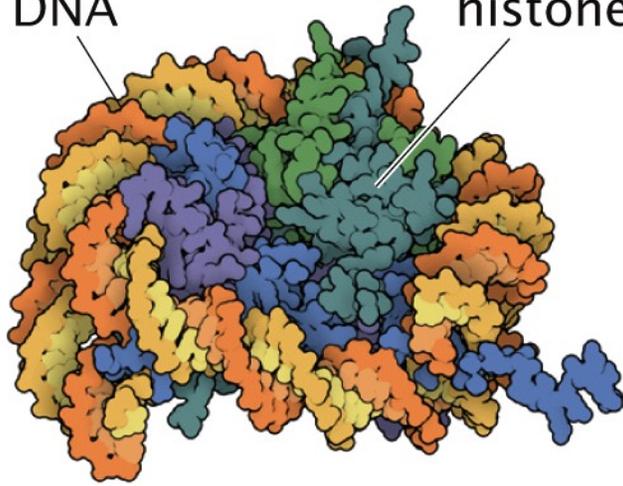


20,000,000  
Gbit/cm<sup>2</sup>

(A)

DNA

histone



(B)

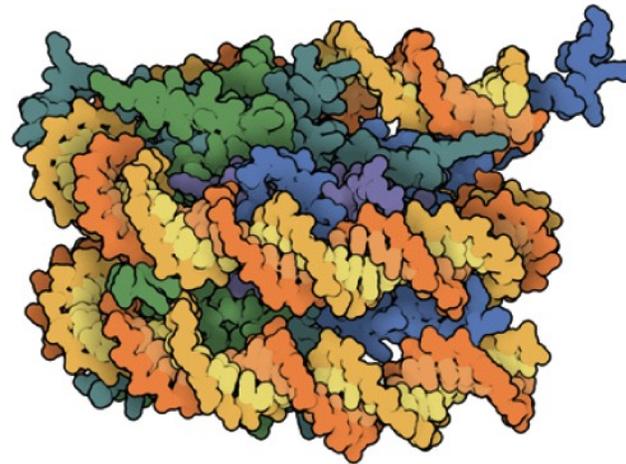
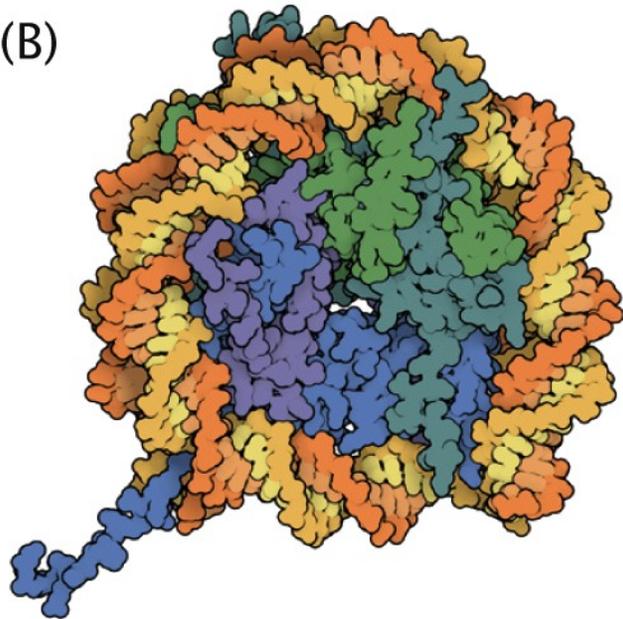
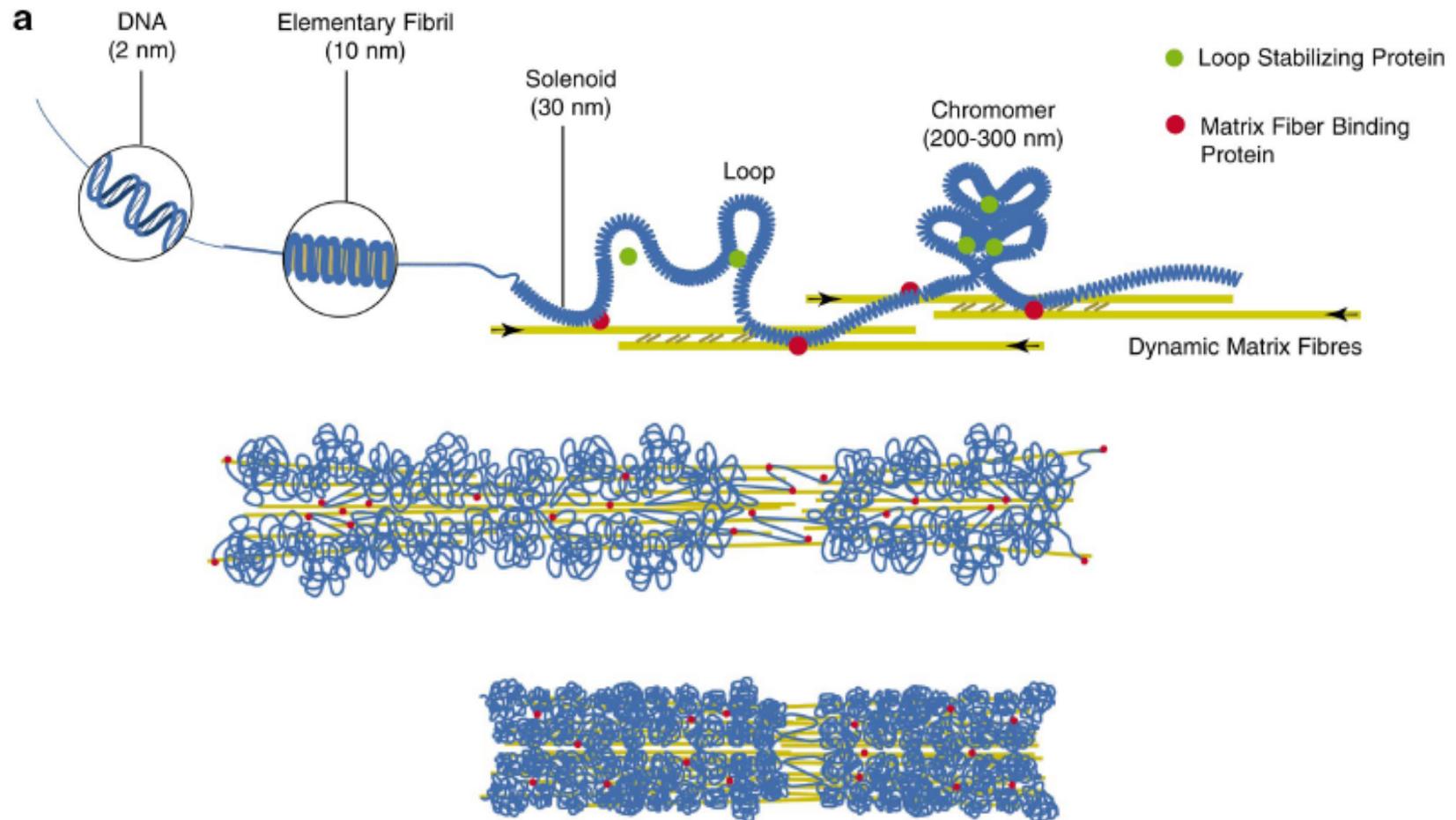


Figure 10.21ab Physical Biology of the Cell, 2nd Edition, © Garland Science 2019

# Chromosome architecture model

G. Wanner & H. Formanek, J. Struct Biol 132, 147–161 (2000)

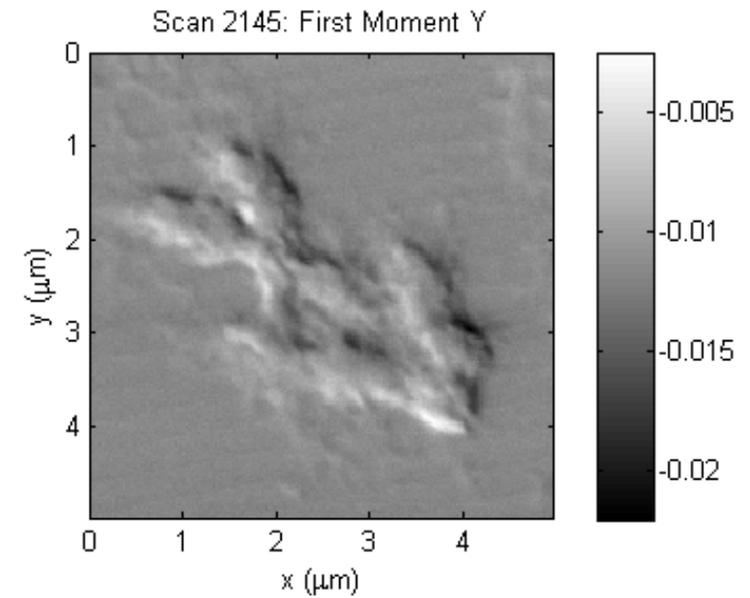
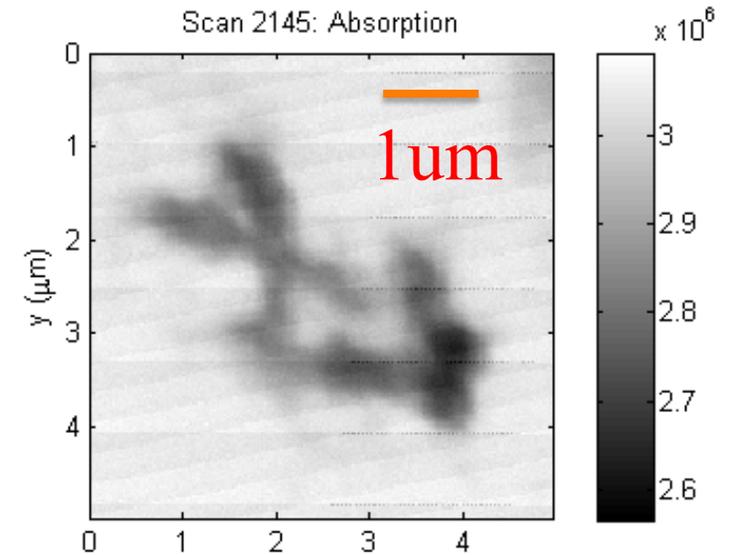
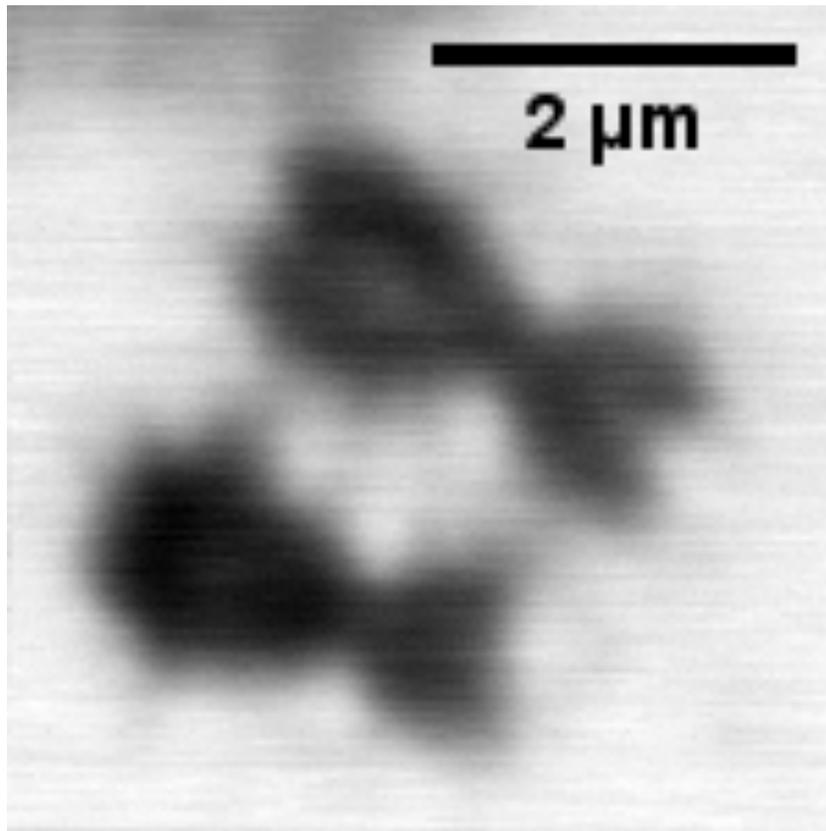


I. K. Robinson, Aga Khan, 2019

# Scanning Transmission X-ray Microscope

Graeme Morrison

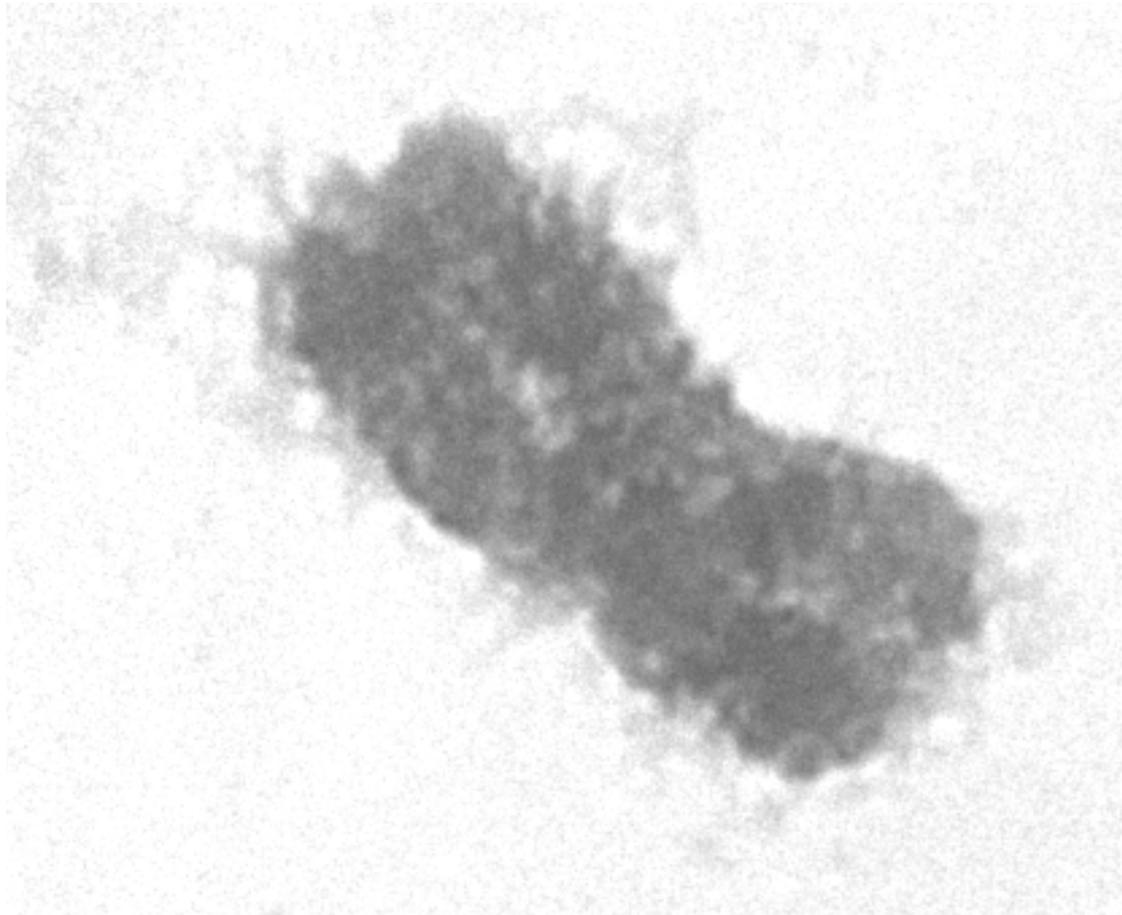
Elettra (500eV), Diamond I-08 (1100eV)



I. K. Robinson, Aga Khan, I

# Transmission X-ray Microscope

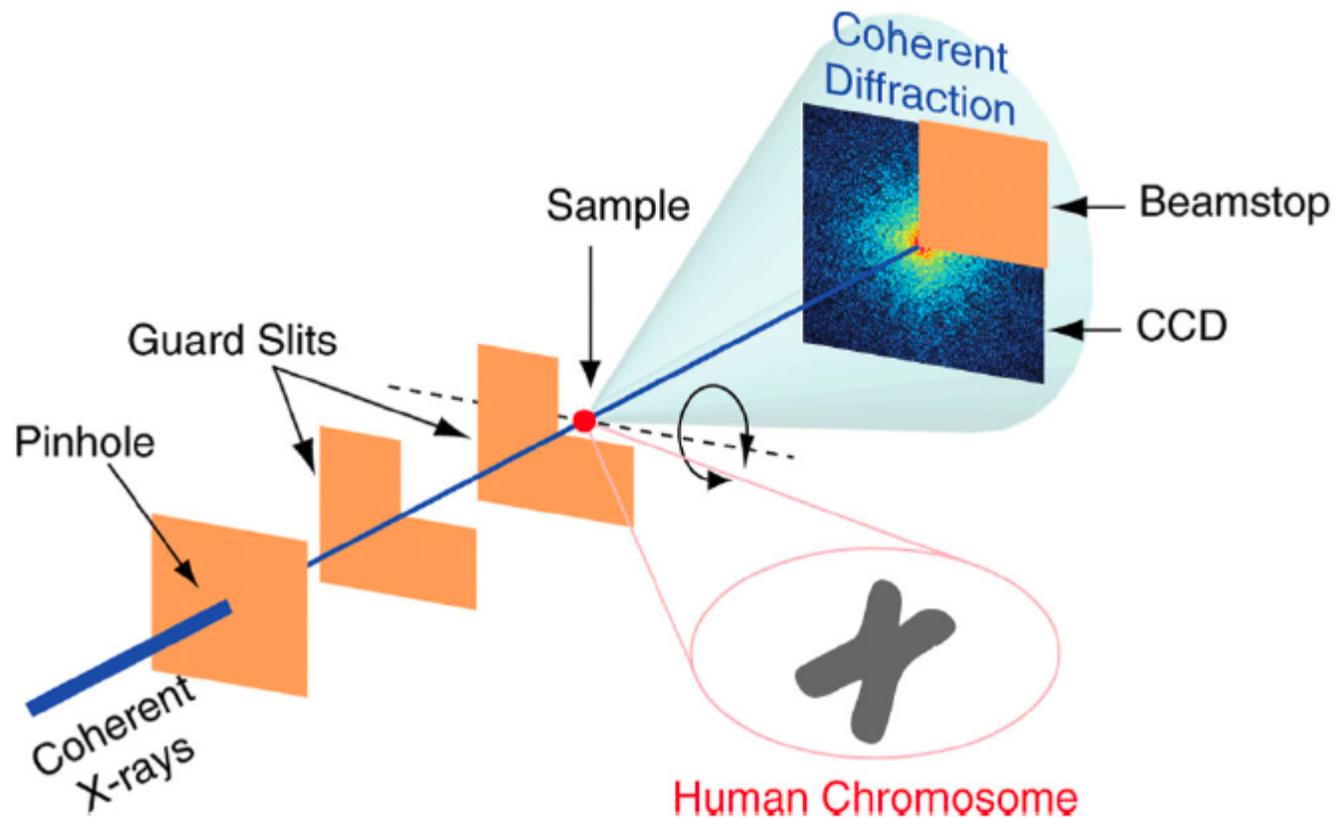
Bo Chen, Joerg Schwenke, Peter Guttman, Gerd Schneider, BESSY  
Glutataraldehyde fixation (1%), unstained



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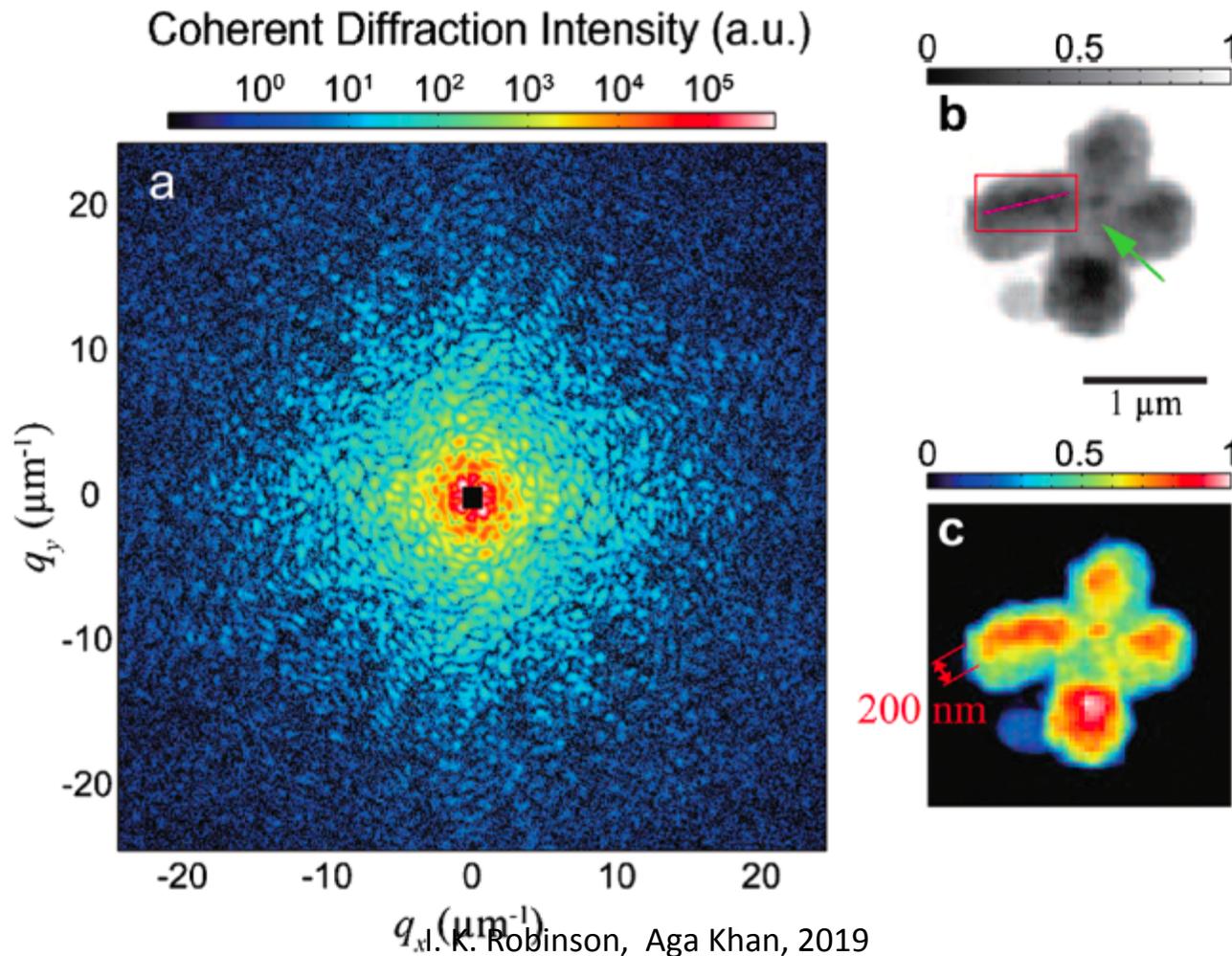
# Schematic in-line CDI setup

Y. Nishino et al PRL 102, 018101 (2009)



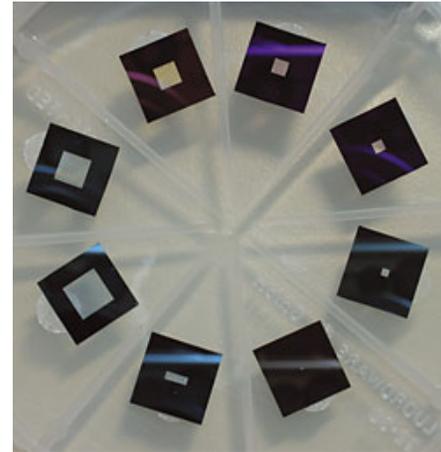
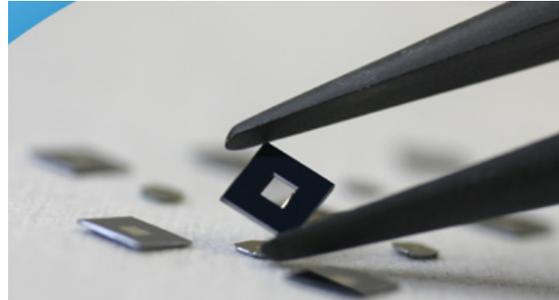
# Chromosome diffraction pattern

Yoshi Nishino et al PRL 102, 018101 (2009)



# Chromosome sample preparation

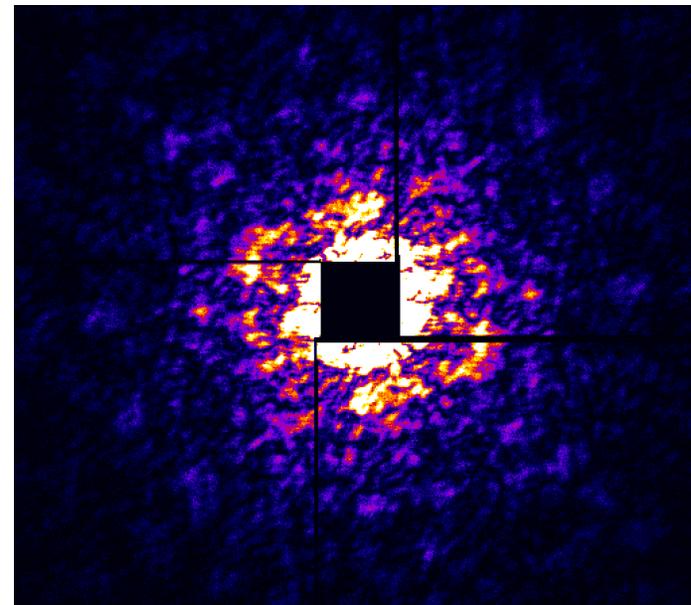
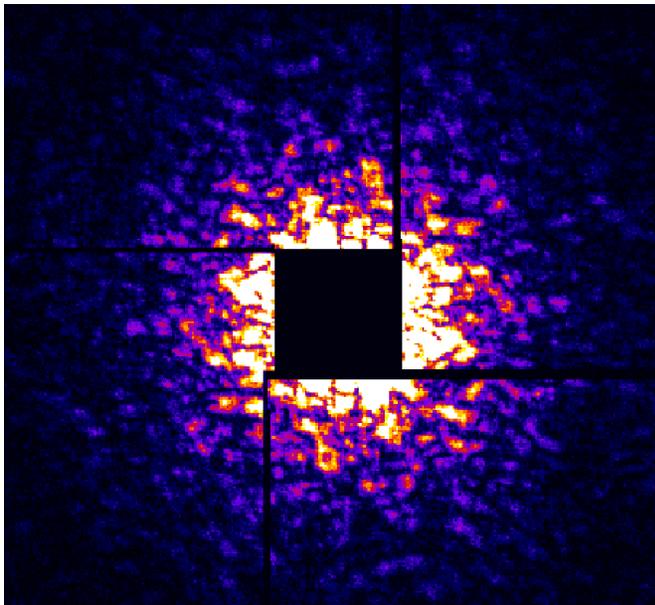
## Silicon Nitride membranes



- Cell culture from Yoruba cell line (GM18507, passage 4)
- Synchronization in mitosis: thymidine+colcemid for 16 h
- Fixed in 3:1 methanol-acetic acid
- Crosslinked in 1% glutaraldehyde
- Air-dried onto 100nm silicon nitride membranes
- Nuclei stained with platinum blue 2.5 mM for 30 min

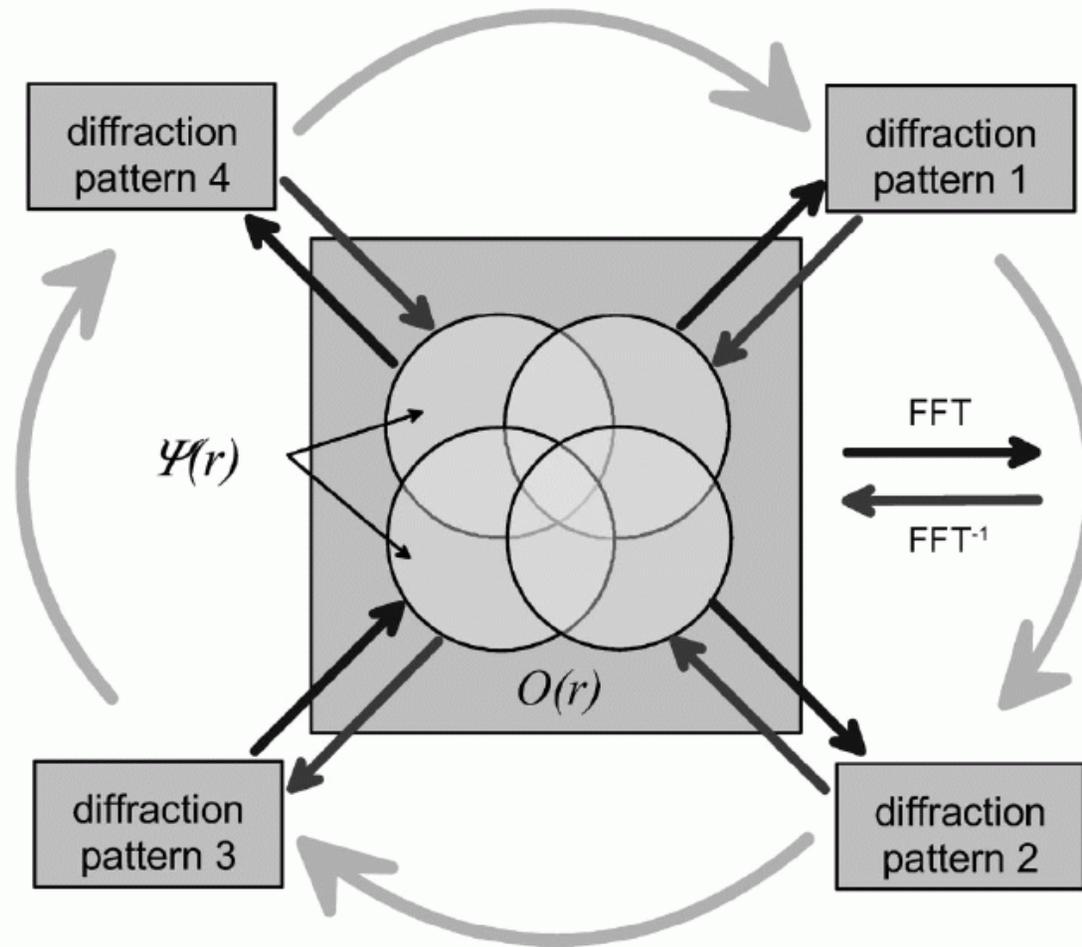
# Human Chromosome Sample M2 340600

J. Schwenke, F. Zhang, Y. Mohammed, C. Song, K. Fukui, SACLA



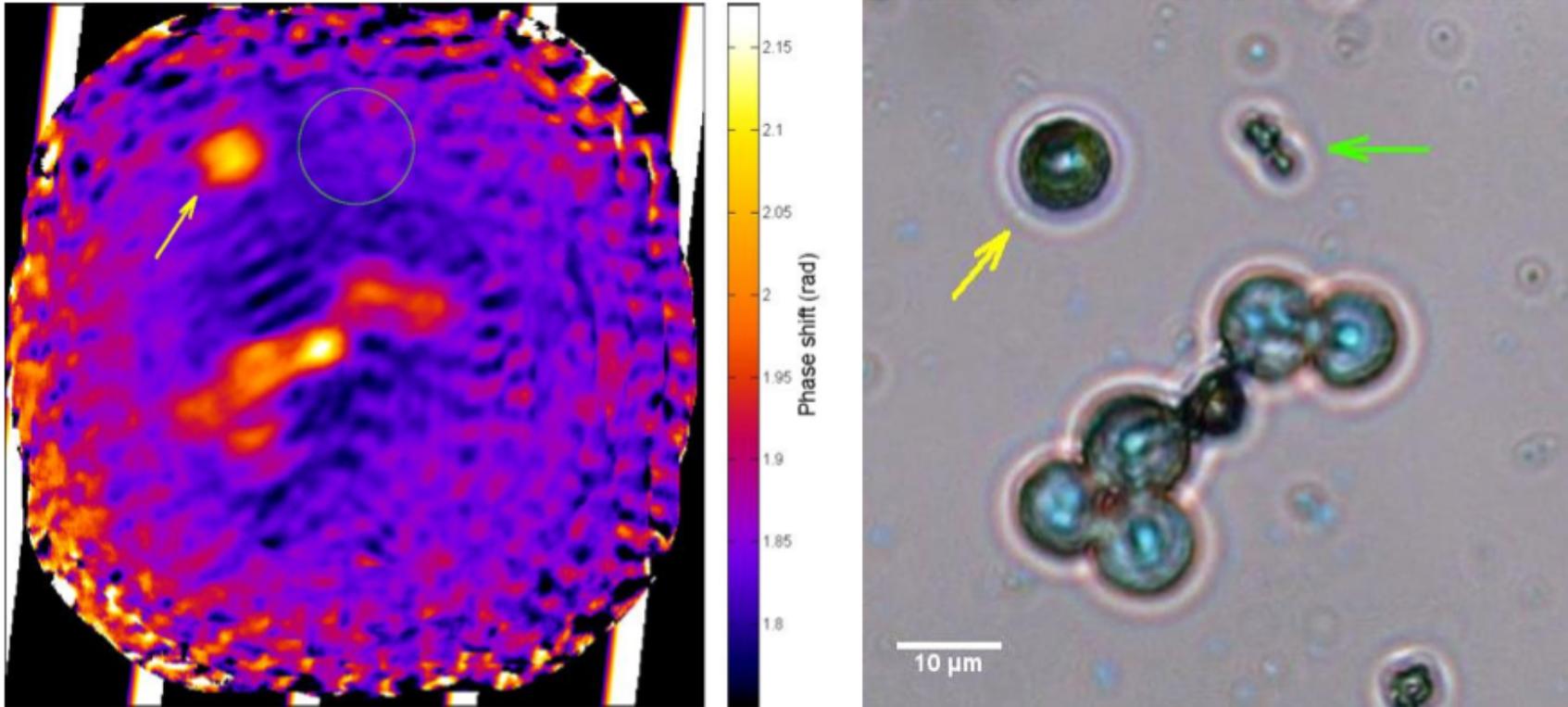
# X-ray Ptychography

J. M. Rodenburg et al, Phys. Rev. Lett. 98 034801 (2007)



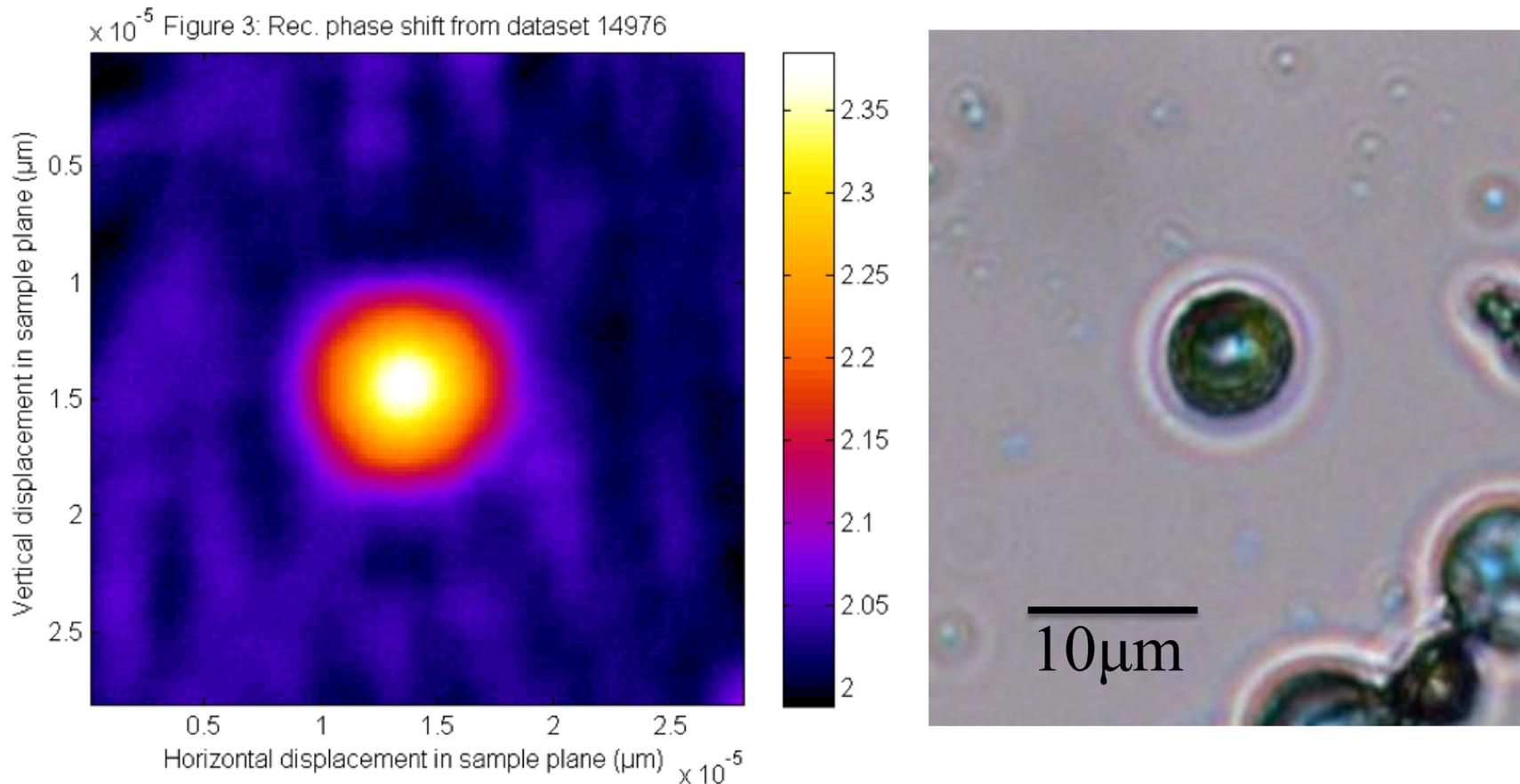
# Chromosomes and Nuclei

14939 X-ray ptychography, 25 $\mu$ m probe, Diamond I-13



# Mitotic Nucleus

14976 X-ray ptychography, Joerg Schwenke and Laura Shemilt, Diamond I-13

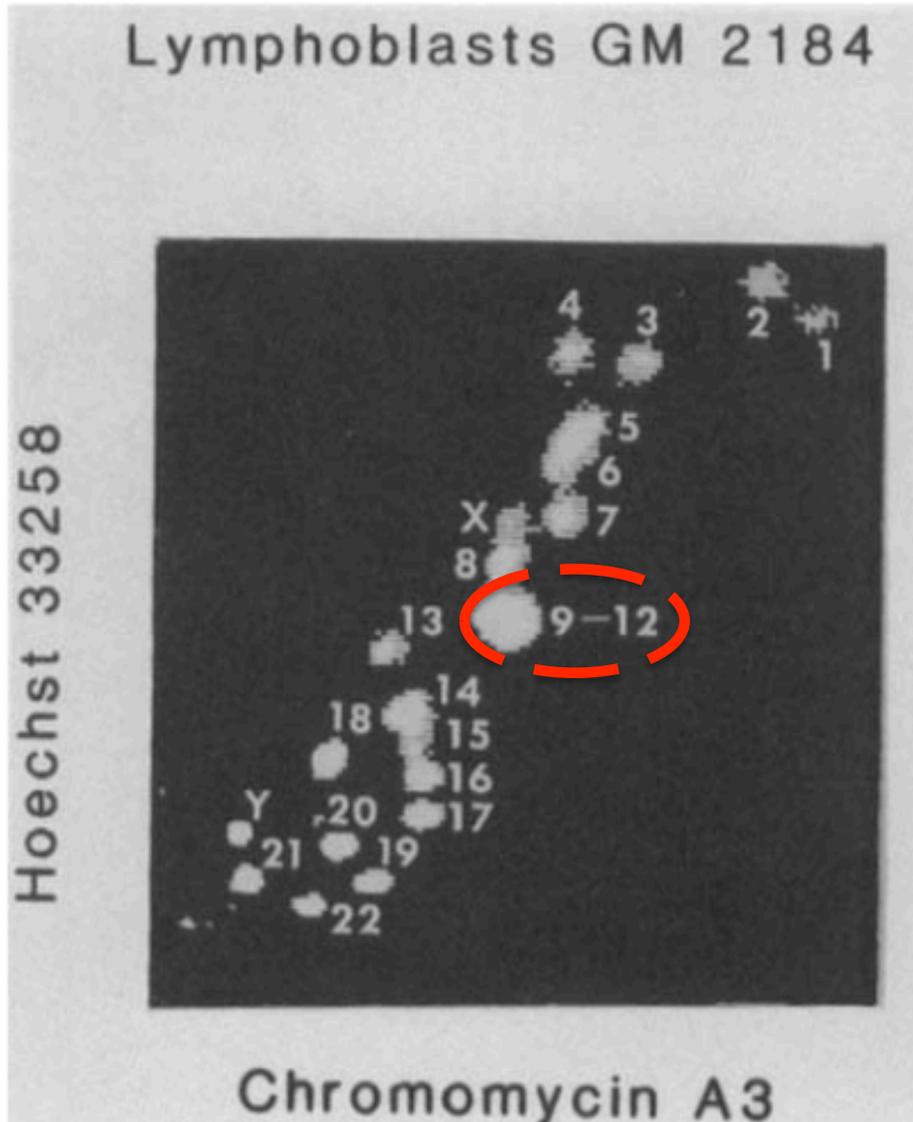


# Quantitative Phase Shift (14957)

Joerg Schwenke, Laura Shemilt, MY, GM, IKR to be published

- Max phase shift  $\varphi = \delta \times t \times 2\pi / \lambda = 0.22 \text{ rad}$
- Area of nucleus,  $A = 35 \mu\text{m}^2$
- Refractive index,  $\delta = 3.85 \times 10^{-6} \times \rho [\text{g/cc}]$  for DNA
- Mass of nucleus =  $A \times \rho \times t = 53 \times 10^{-12} \text{ g}$
- Genome =  $3.5 \text{ GBP} \times 4 \text{ copies} = 16 \times 10^{-12} \text{ g DNA}$
- 3.3-fold excess is made up of proteins, glutaraldehyde, stain, solvent etc

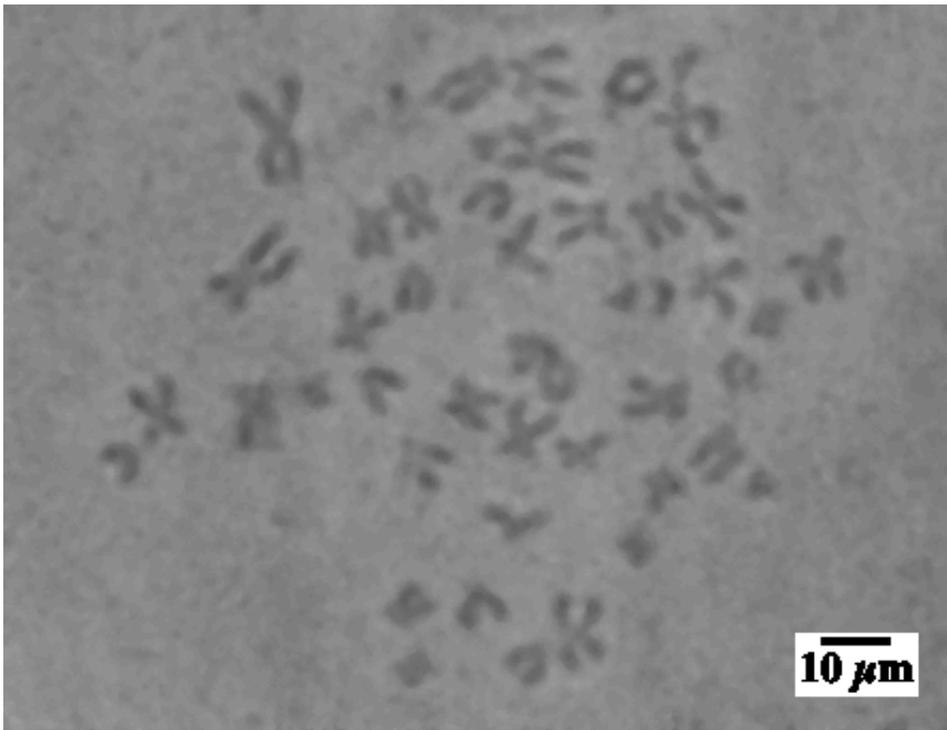
# Flow cytometry karyotype



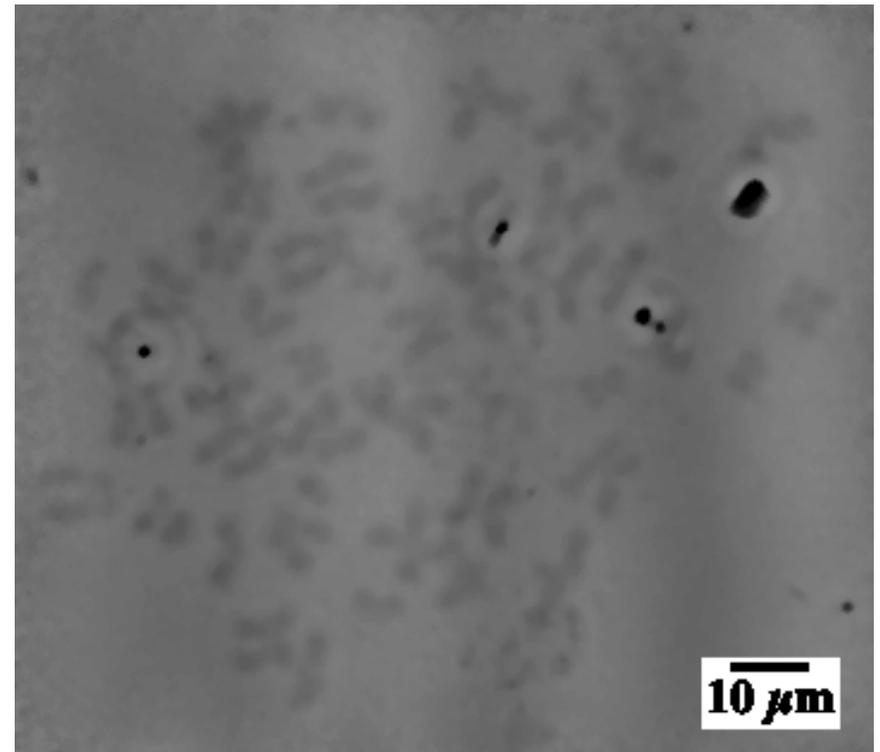
Human chromosome identification on the bases of DNA content using bivariate flow cytometry.

Cram, L.S et al., Cytometry Supplement, 1988, vol. 3, pp.94-100.

# X-ray phase image of stained chromosome spreads

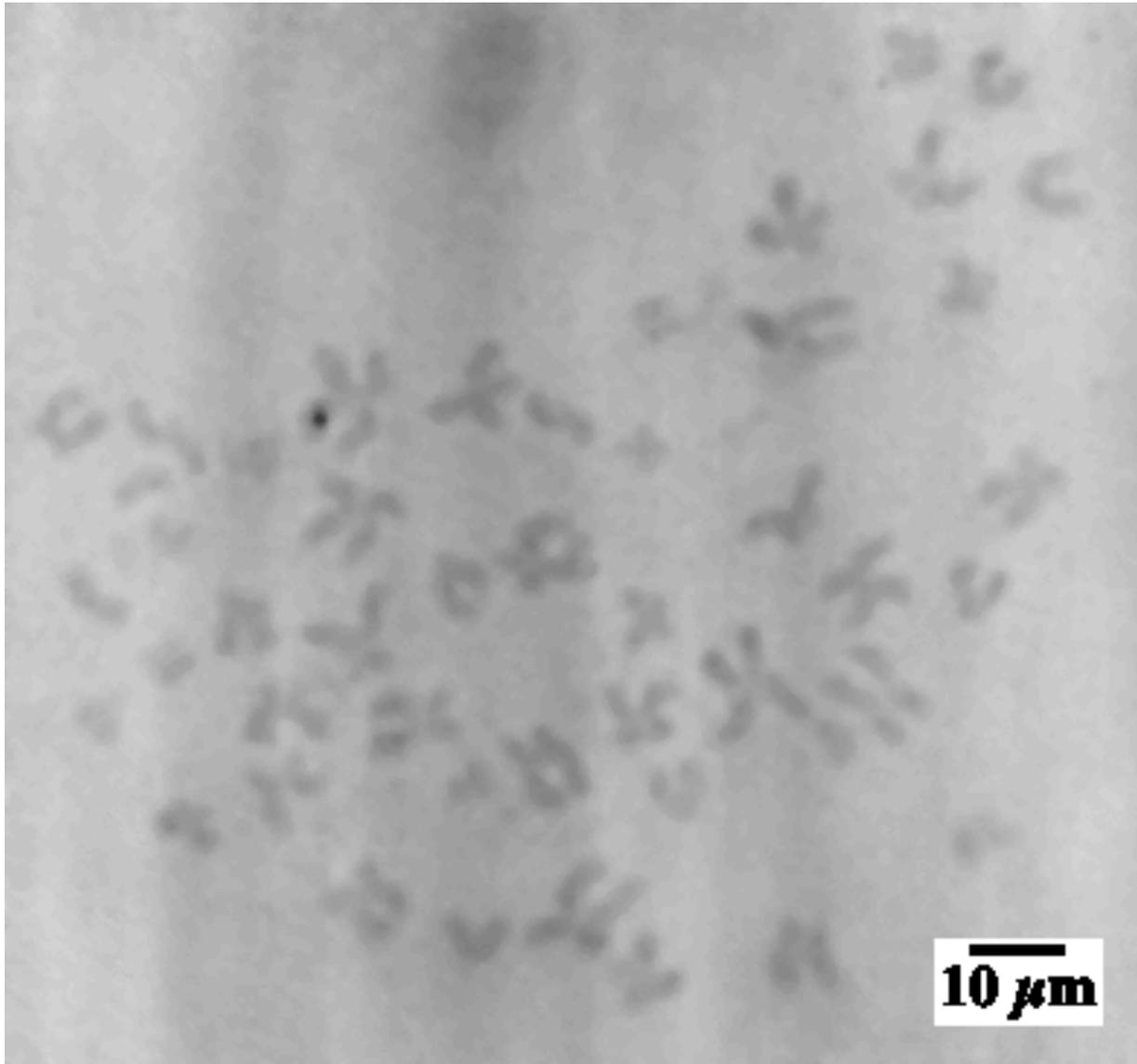


1% Uranyl acetate



5mM Platinum stain

# Unstained chromosome spread



Archana Bhartiya

Image size:  
64μm x 64μm

Step size:  
1μm x 1μm

Environment:  
room temperature

# Mass ratio calculation

**Equation used to calculate mass of the chromosomes**

$$M = \sum_j \frac{\phi_j}{\lambda r_0} P_x P_y$$

M= Number of electrons.

$\sum_j \phi_j$  = Phase shift (took sum of the values of the pixels measured by ImageJ software).

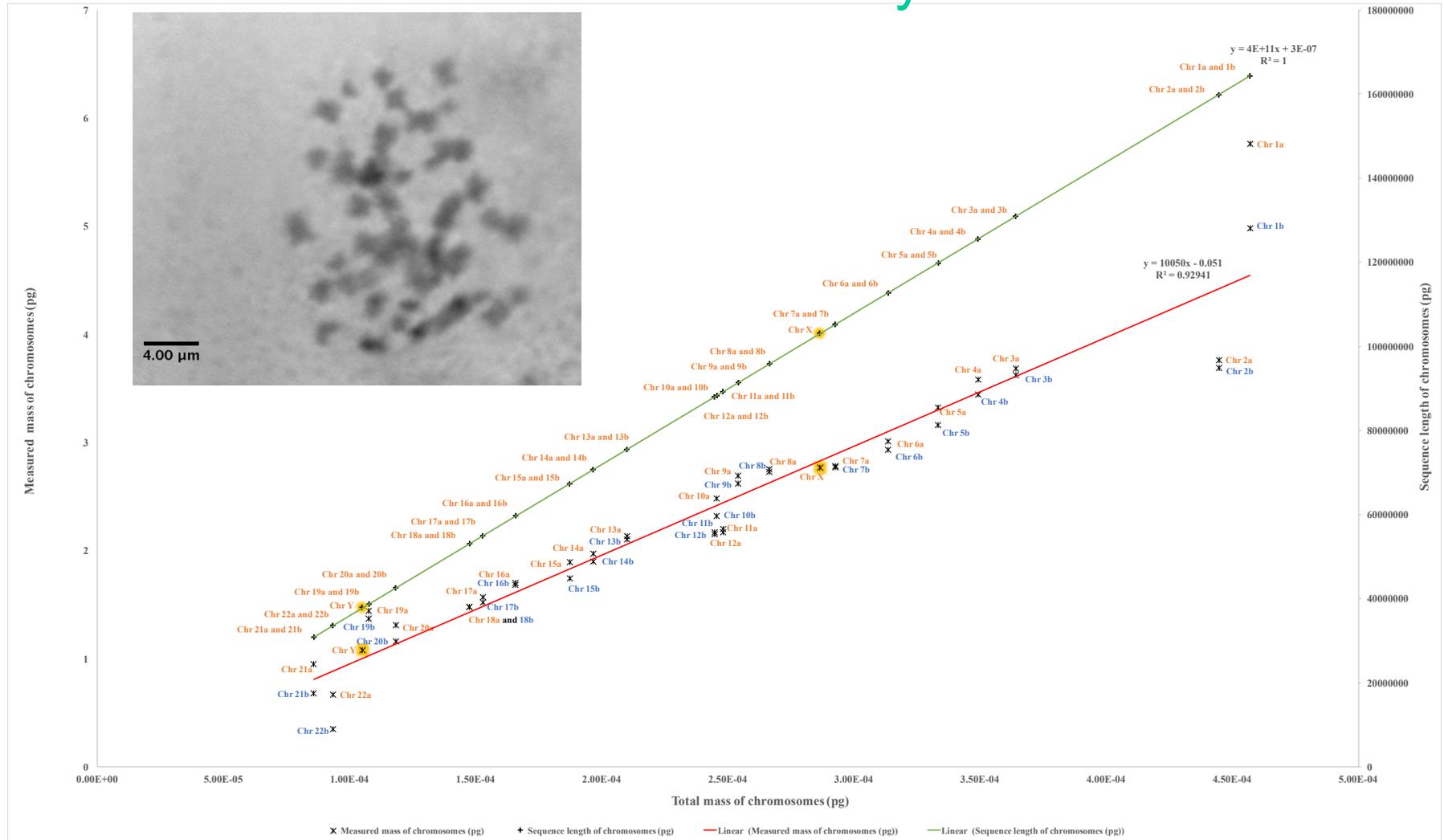
$\lambda$ = Wavelength of X-rays beam.

$r_0$ = Classical radius of the electron.

$P_x P_y$ = Real space pixel size.

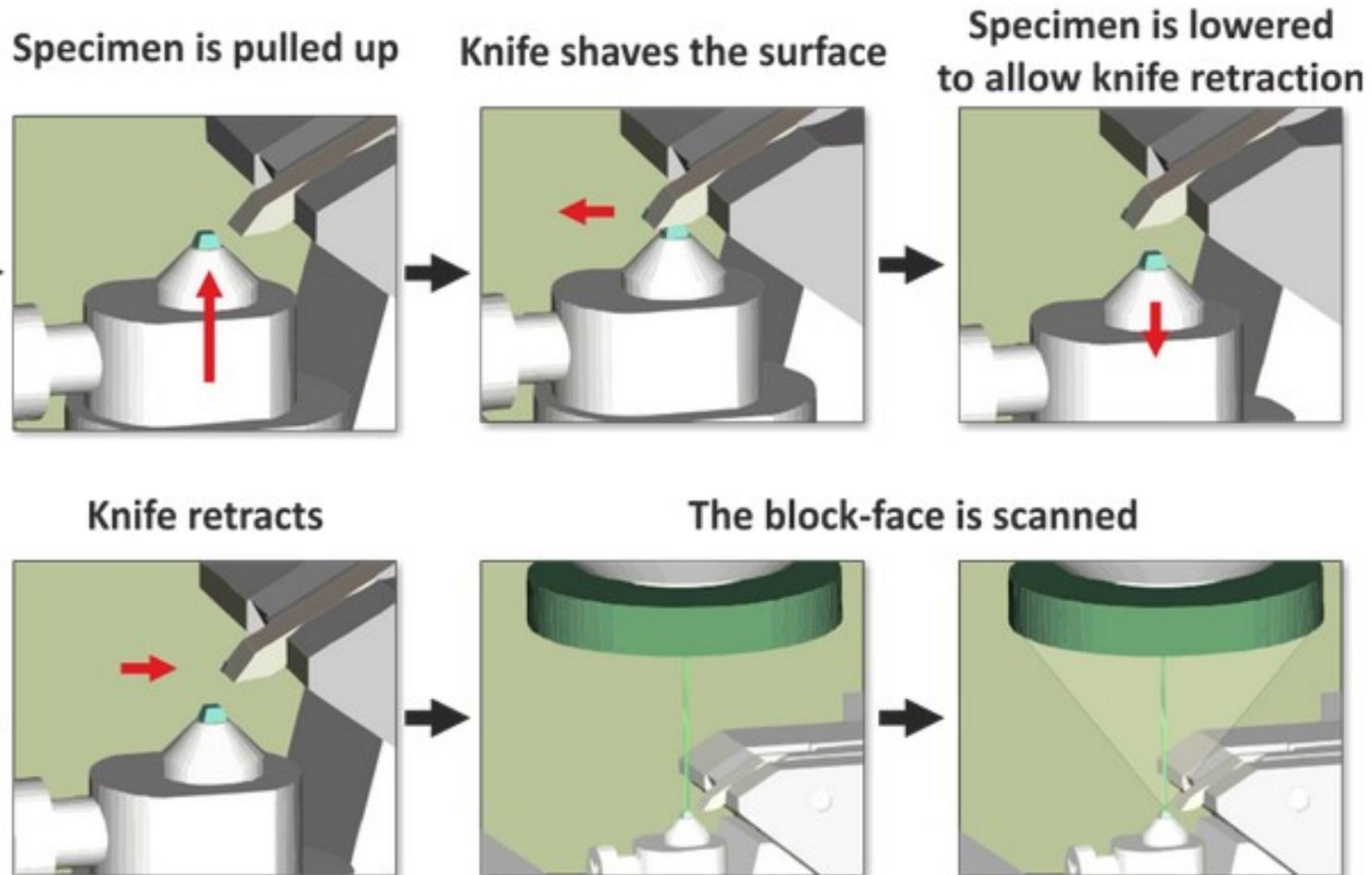
# Karyotype of human chromosomes

## Archana Bhartiya



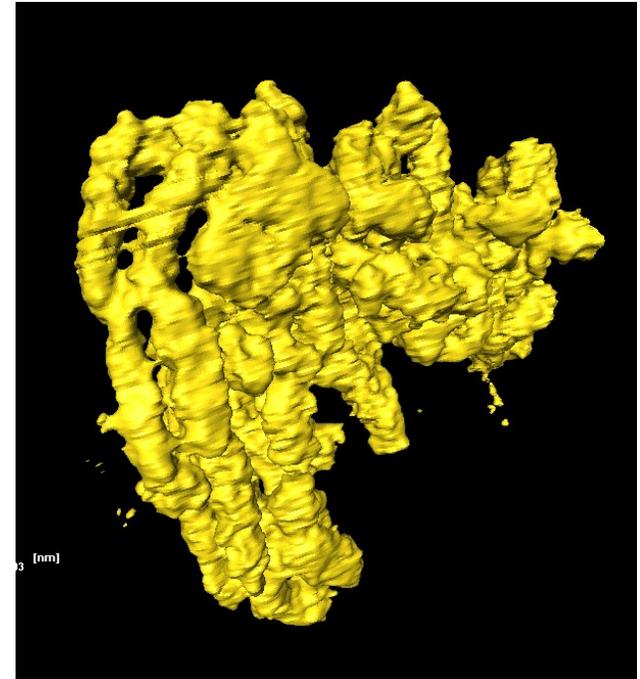
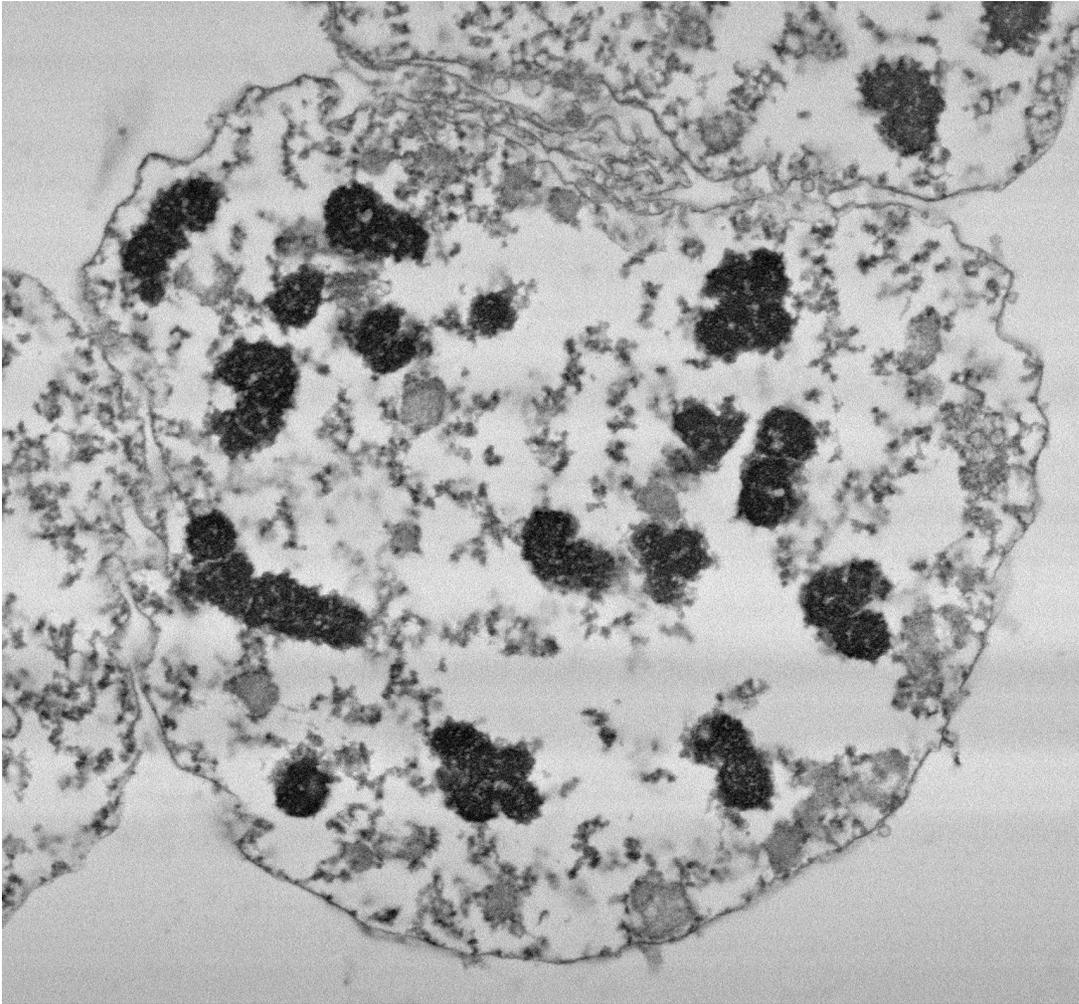
# Serial Block Face SEM (3-view)

Electron Microscopy Unit, University of Helsinki



# Serial Block Face SEM

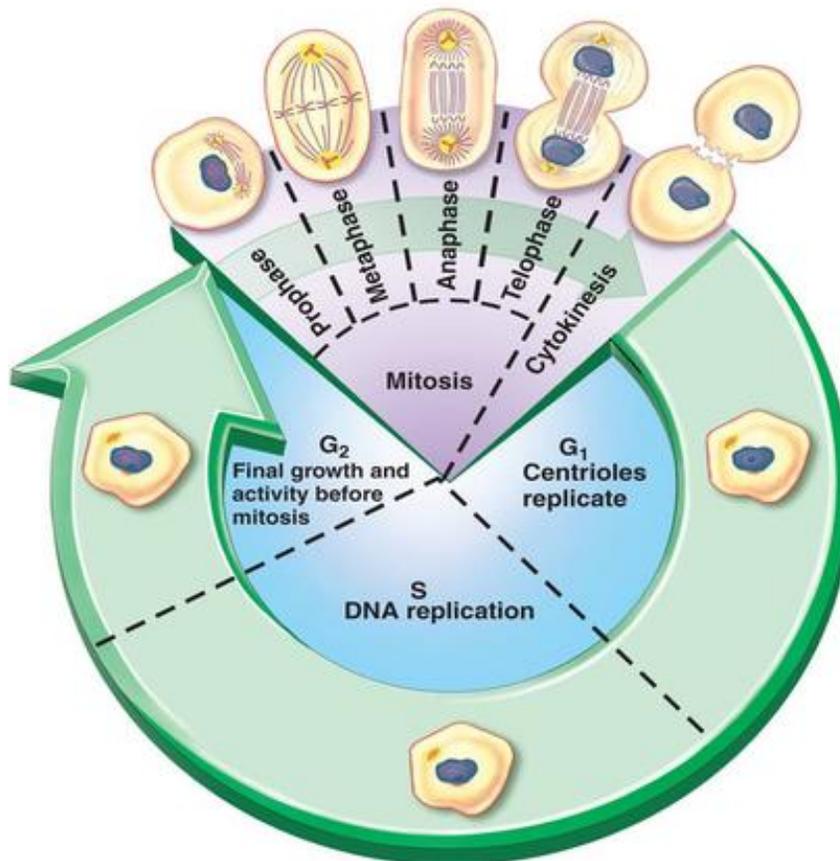
Bo Chen and Yusuf Mohammed



I. K. Robinson, Aga Khan, 2019

# Preparation favours Prophase

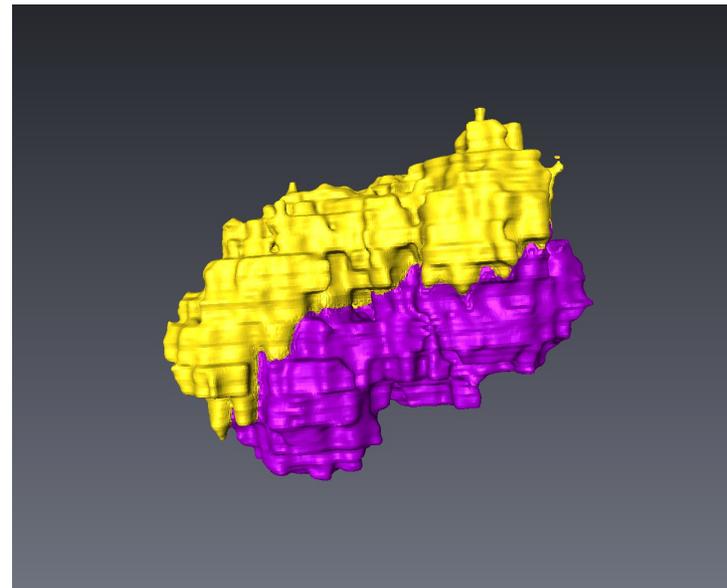
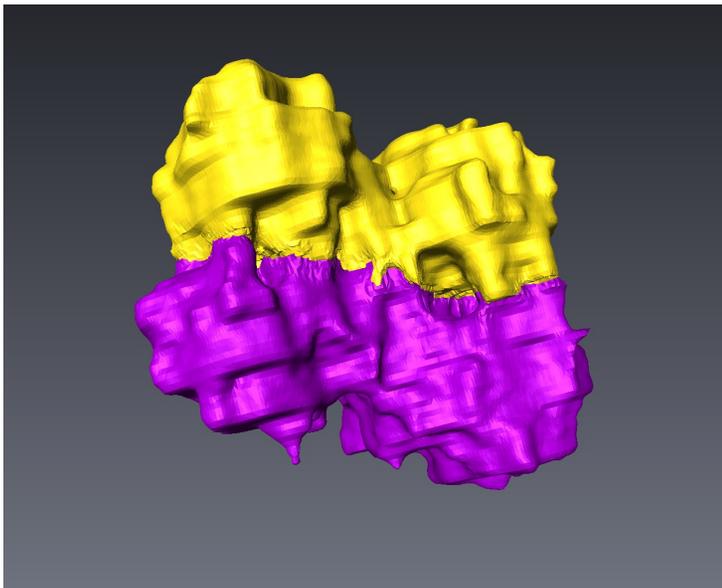
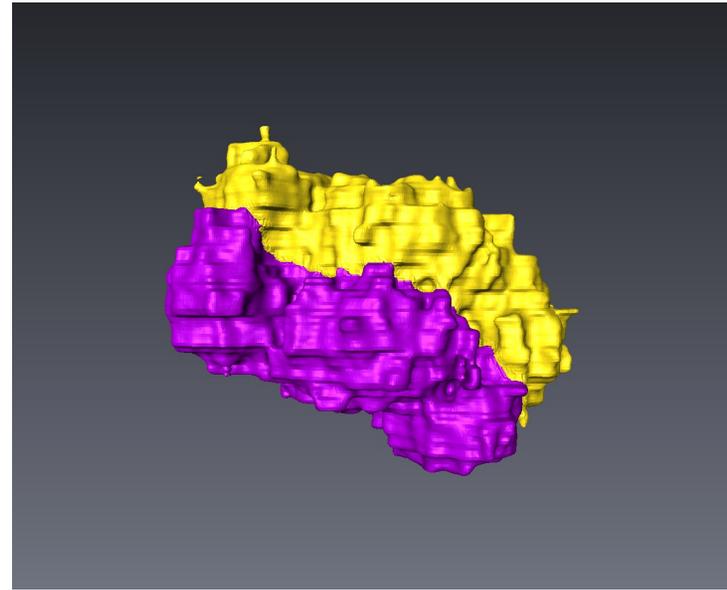
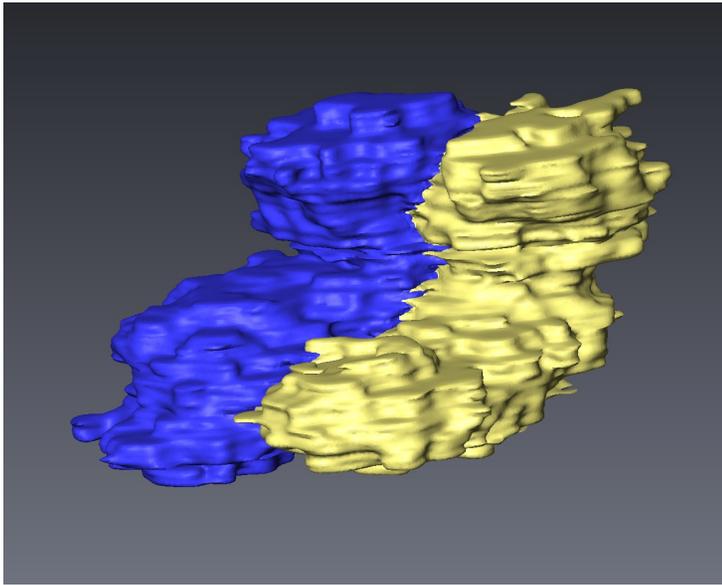
“Human Physiology” Wiki, by A. Rausch and C. Kortleever



- Prophase
  - condensed chromosomes
  - nuclei still present
- Prometaphase
  - nuclear membrane dispersed
- Metaphase
  - spindle assembly

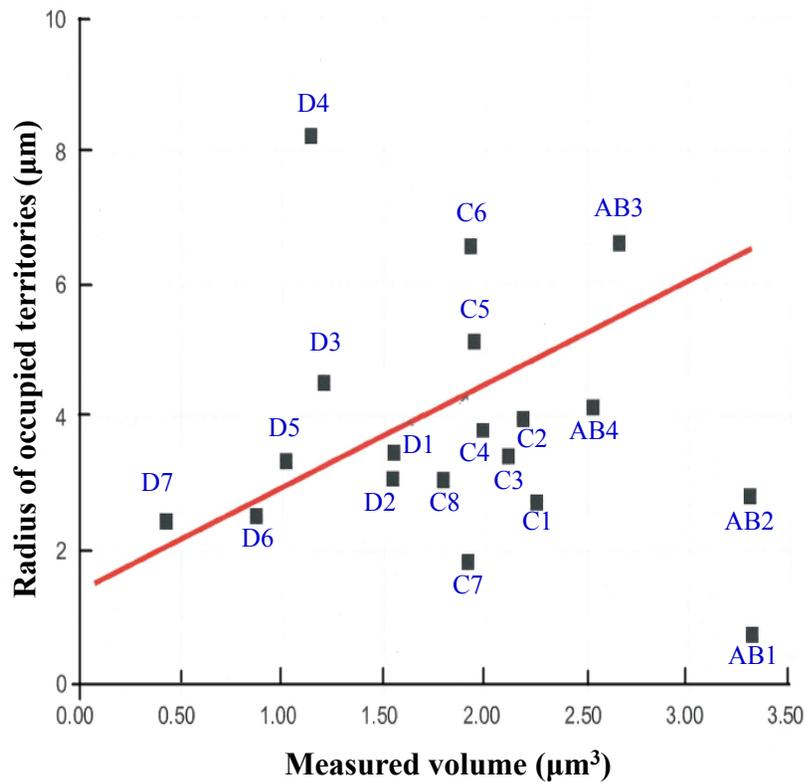
# Segmented Prophase Chromatids

Bo Chen and Yusuf Mohammed, Sci. Adv. 3 e1602231 (2017)

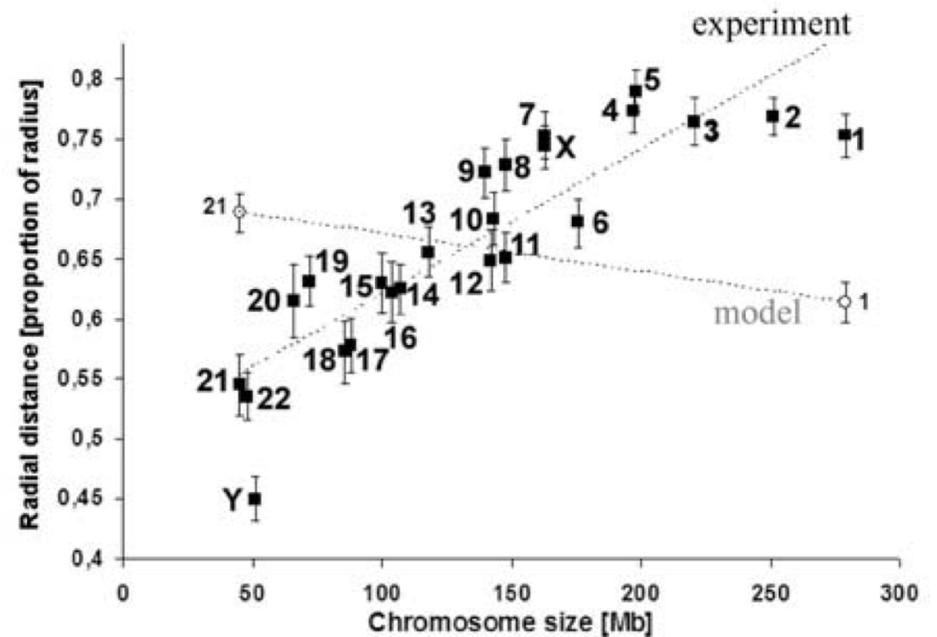


# Smaller Chromosomes at Nuclear Interior, following Territories

Th. Cremer et al, PloS Biology 3 157 (2005)



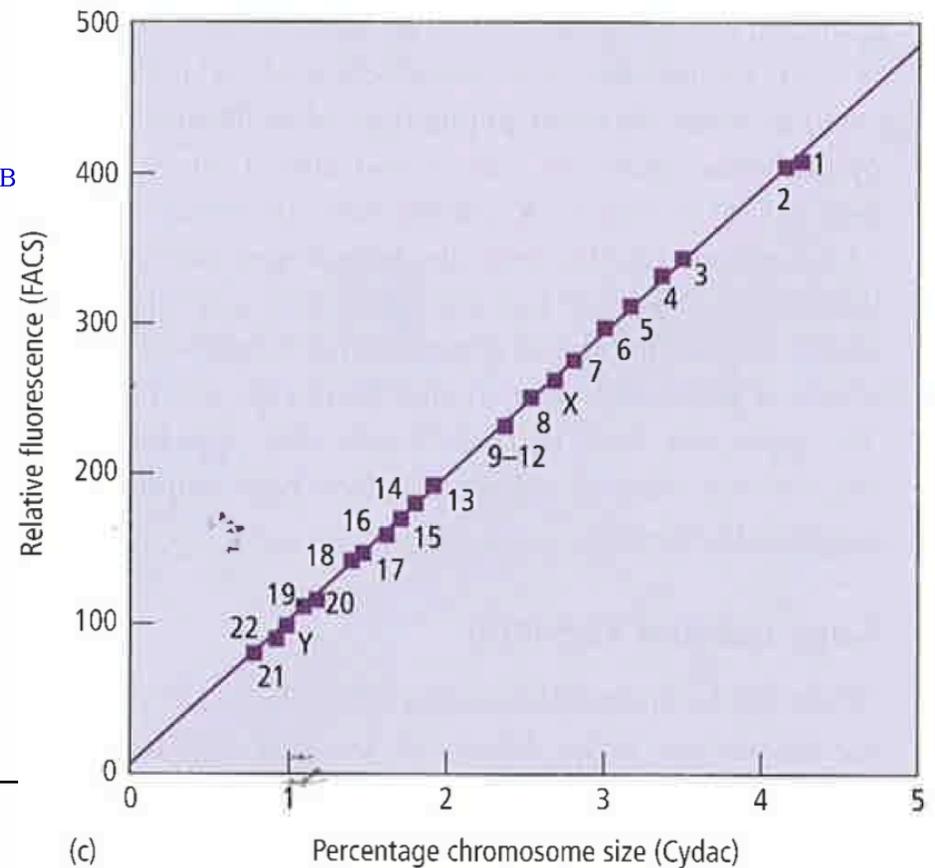
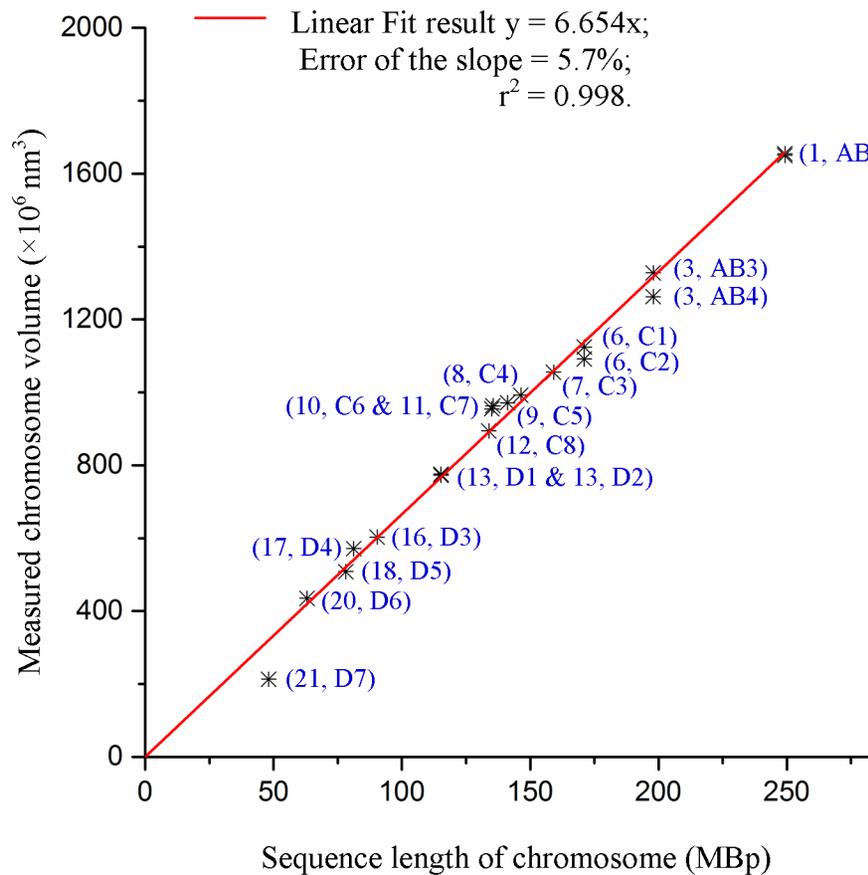
**A** Normalized radial distances of CTs in experimental and SCD model nuclei



# Volume Karyotype

Questionable identification of chromosome  
"Essential Medical Genetics"

E. Tobias, M. Connor & M. Fergusson-Smith



# 3D Chromatid Volume Analysis

Accounting for measured, volumes **per base pair**

Methanol-Acetic Acid embedding in Epoxy Resin

Bo Chen, Mohammed Yusuf, to be published

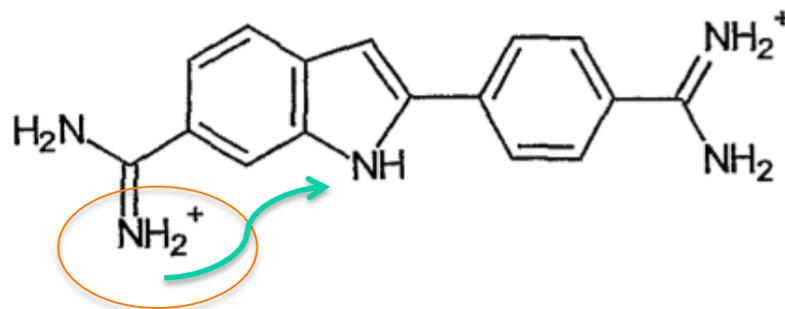
Measured volume	slope of line	6.65nm <sup>3</sup>
DNA alone	2nm diam x 0.34nm	1.06nm <sup>3</sup>
Nucleosome (140BP)	11nm diam x 6nm	4.07nm <sup>3</sup>
Histones H2-H4	subtract	3.01nm <sup>3</sup>
Non-histone protein	65% histone	1.62nm <sup>3</sup>
Total filled	<b>86%</b> of measured	5.69nm <sup>3</sup>

# DAPI as probe of DNA conformation

Ana Estandarte, Chris Lynch and Stan Botchway

**DAPI** as the fluorescent probe

- High quantum yield ( $\phi = 0.92$ ) when bound to chromosomes
- More stable than other fluorochromes

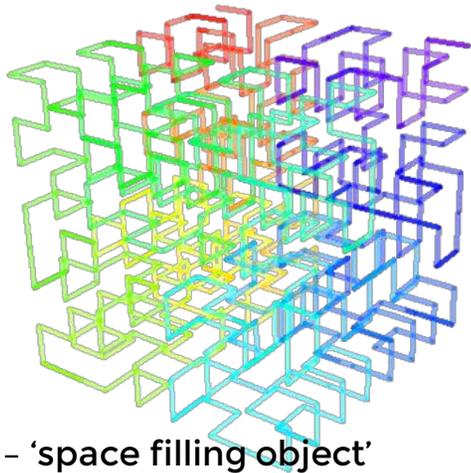


In water, proton transfer causes quenching  
4',6-diamidino-2-phenylindole

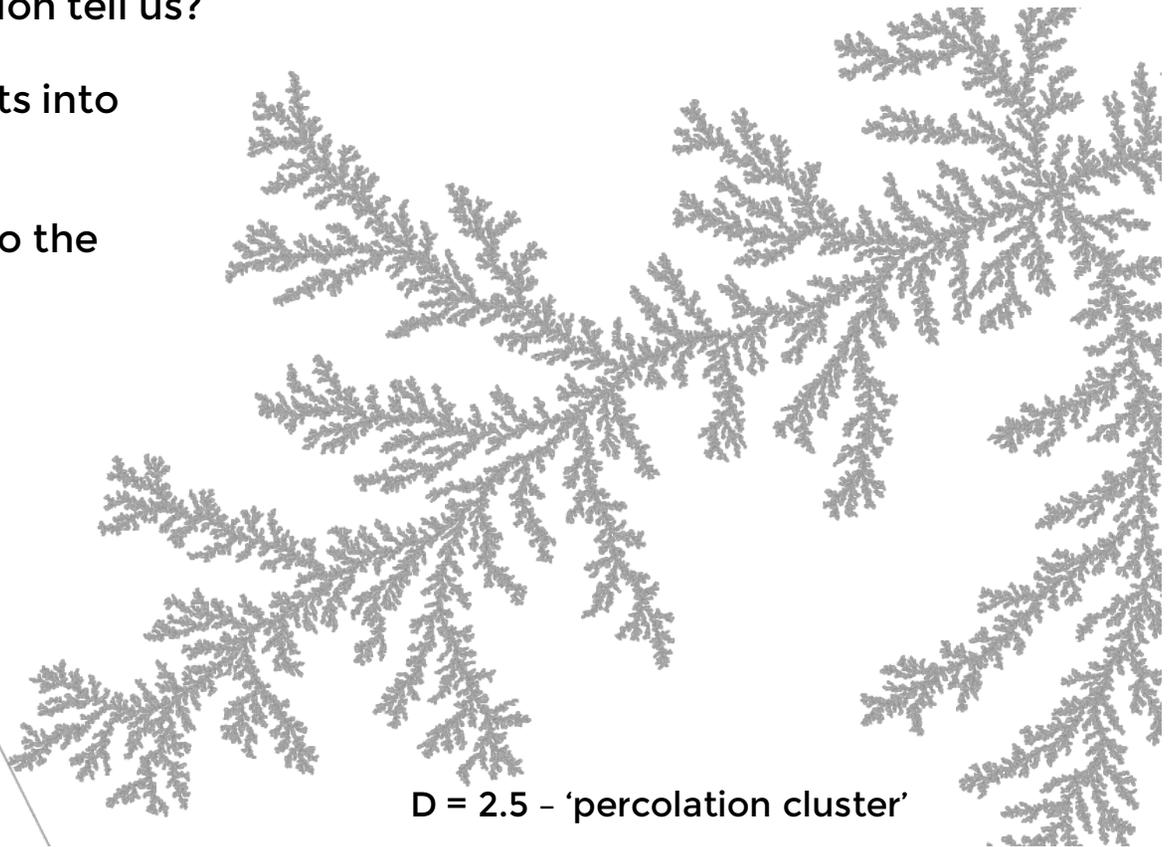
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# What is a fractal dimension?

- What does the fractal dimension tell us?
- Used to describe how a line fits into space
- Ratio of the change in detail to the change in scale



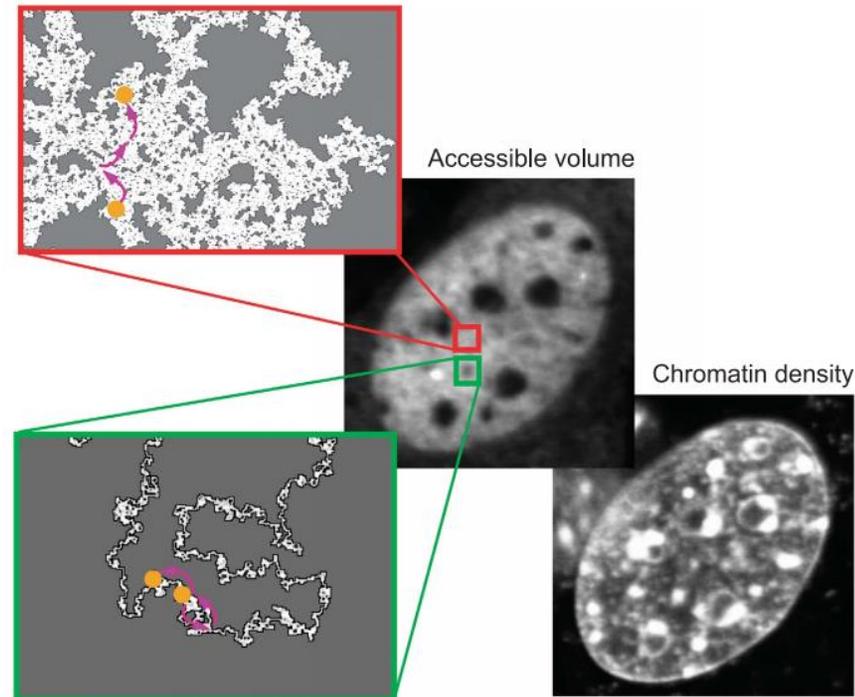
D = 3 - 'space filling object'



D = 2.5 - 'percolation cluster'

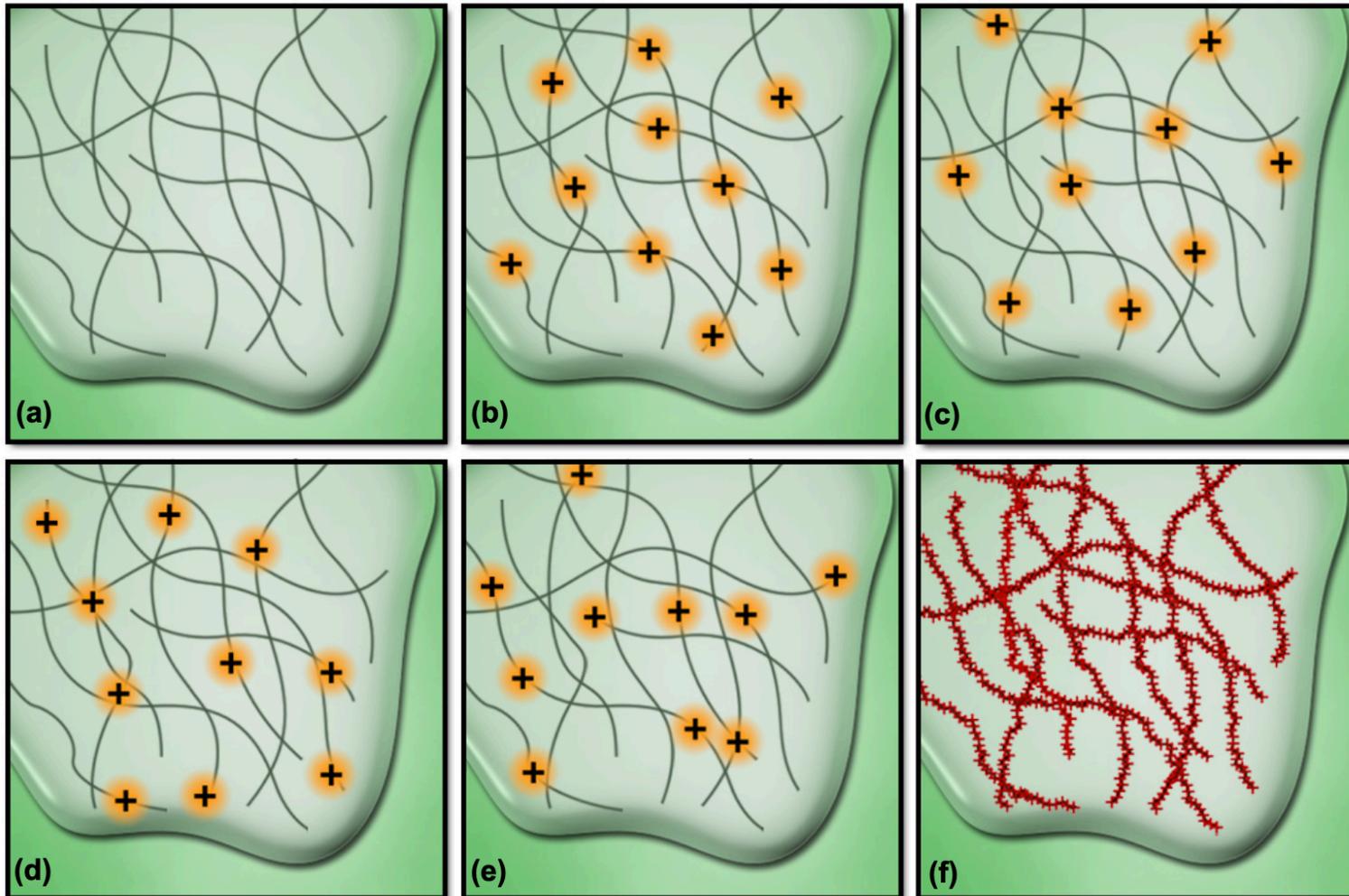
# Heterochromatin and Euchromatin

- *Bancaud, EMBO Journal, 2009:* Different diffusion coefficients for particles in heterochromatin (2.2) and euchromatin (2.6)
- Chromatin acts as an obstacle to diffusion of particles in the nucleus (transcription machinery, maintenance proteins etc.)
- *Hinde (2011)* suggests that organisation plays a role in gene regulation. 'Fractal' nature of chromatin provides automatic control of diffusion rate in the nucleus

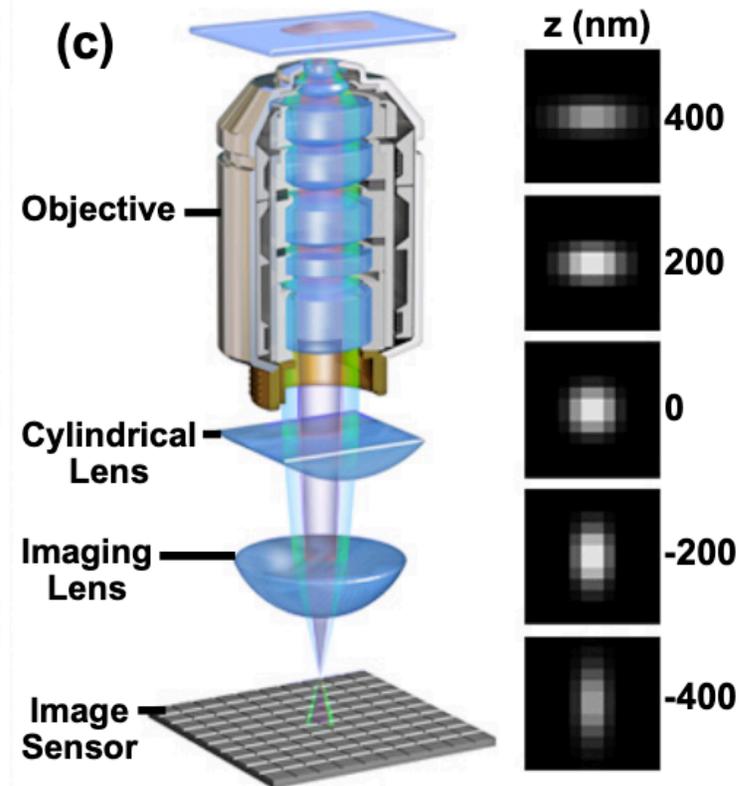
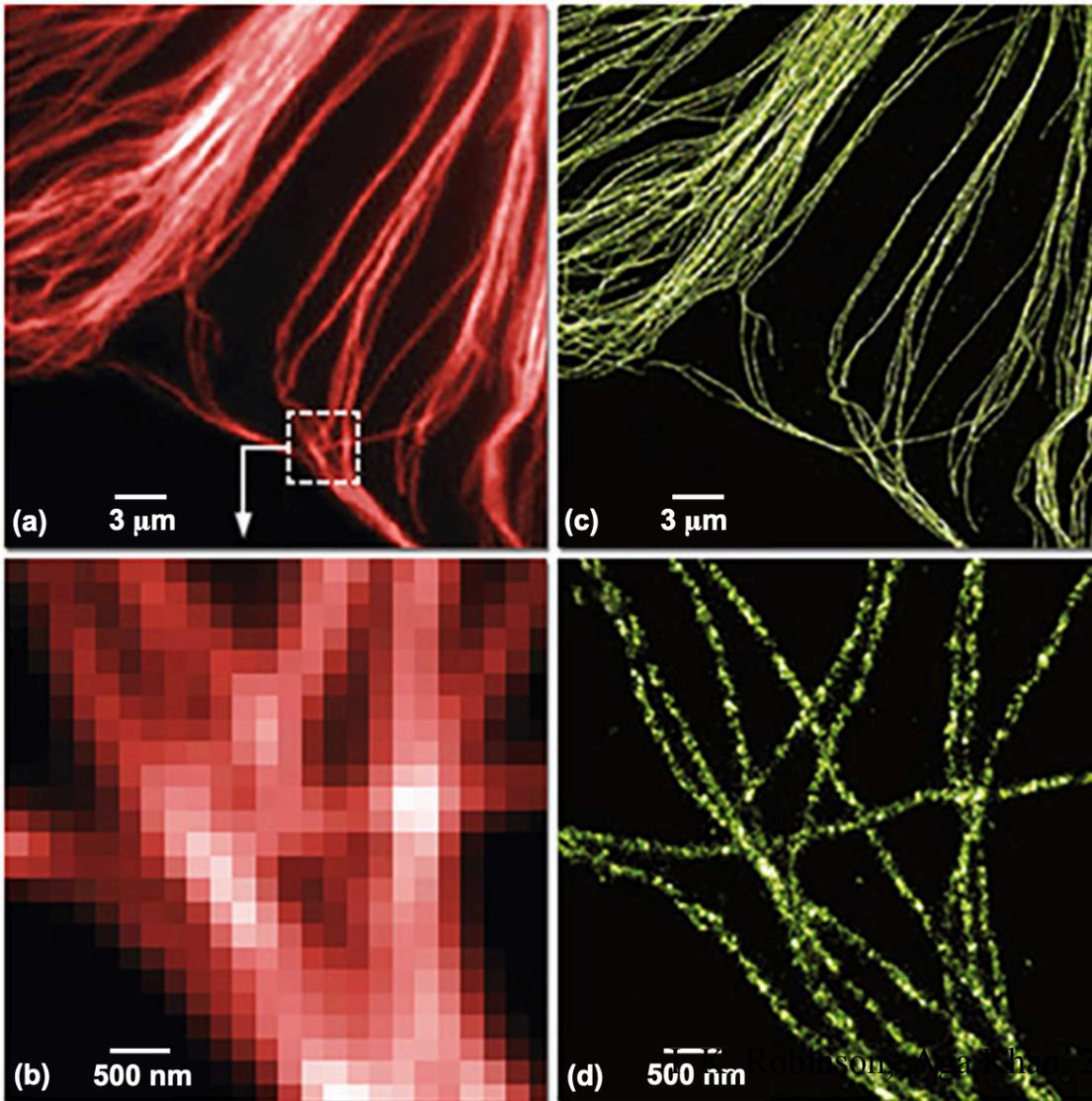


*Bancaud, EMBO Journal, 2009*

# STORM: how does it work?

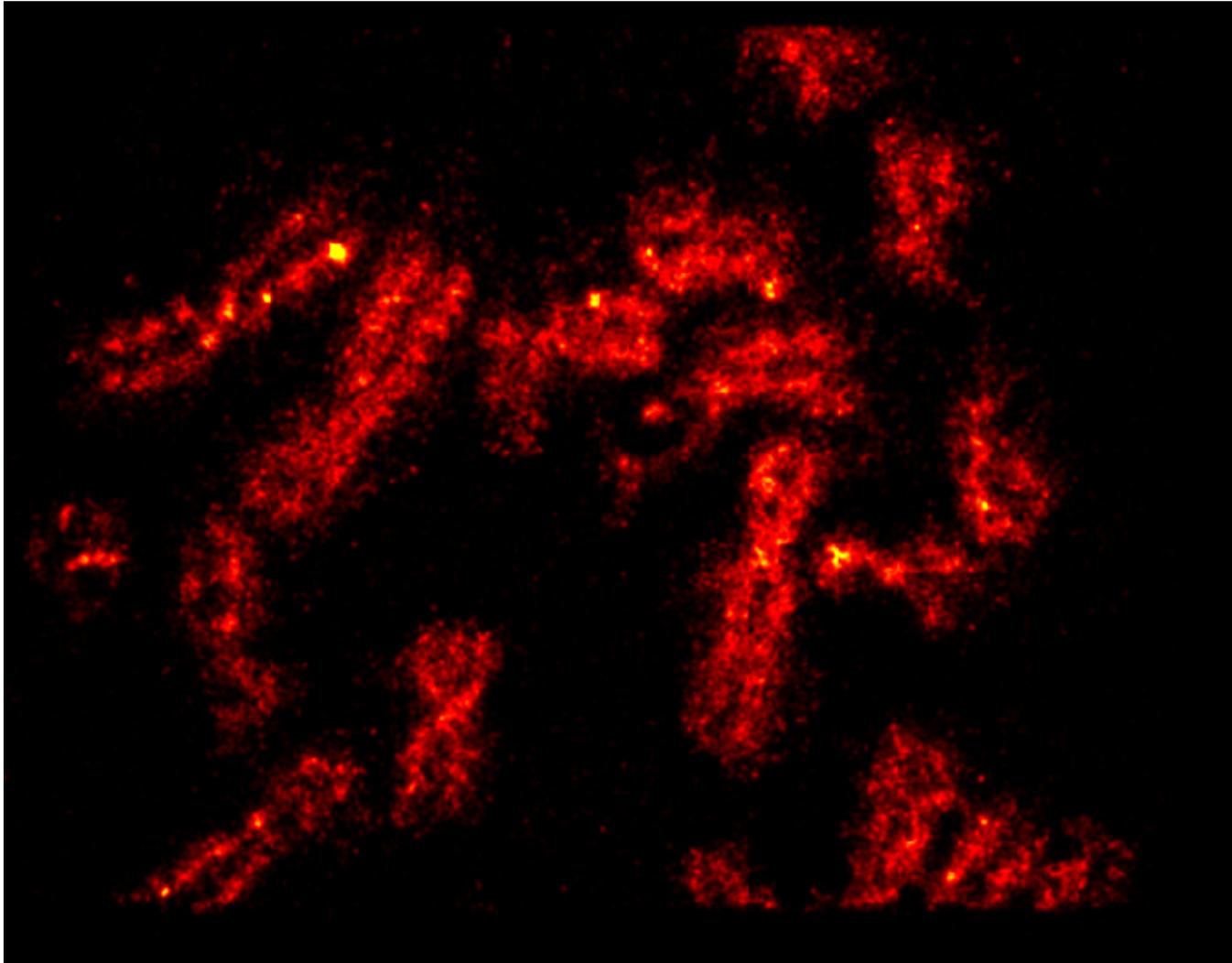


# N-STORM for 3D imaging (Nikon)



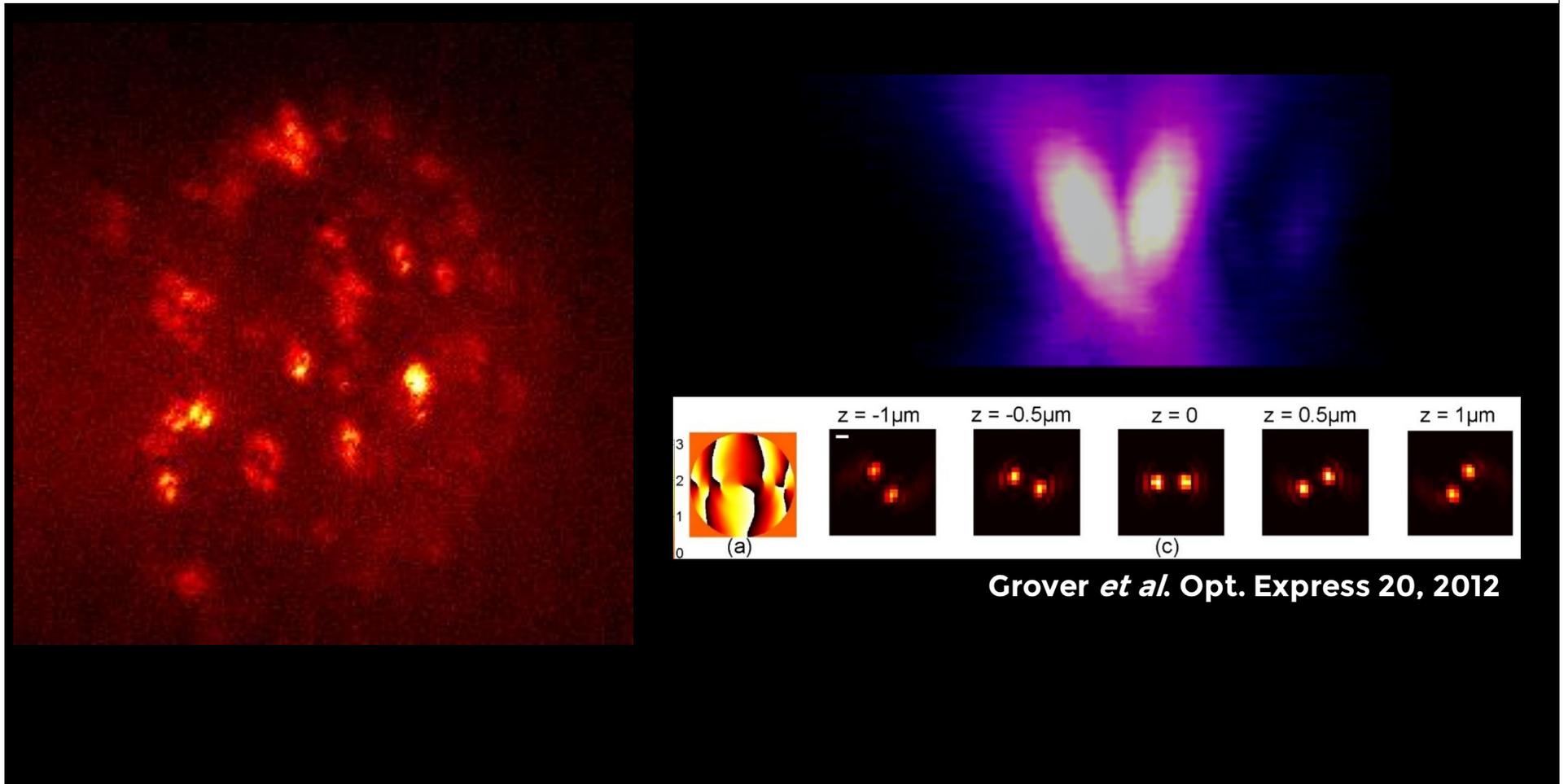
# Super-resolution Chromosome Images

Christophe Lynch and Steven Webb



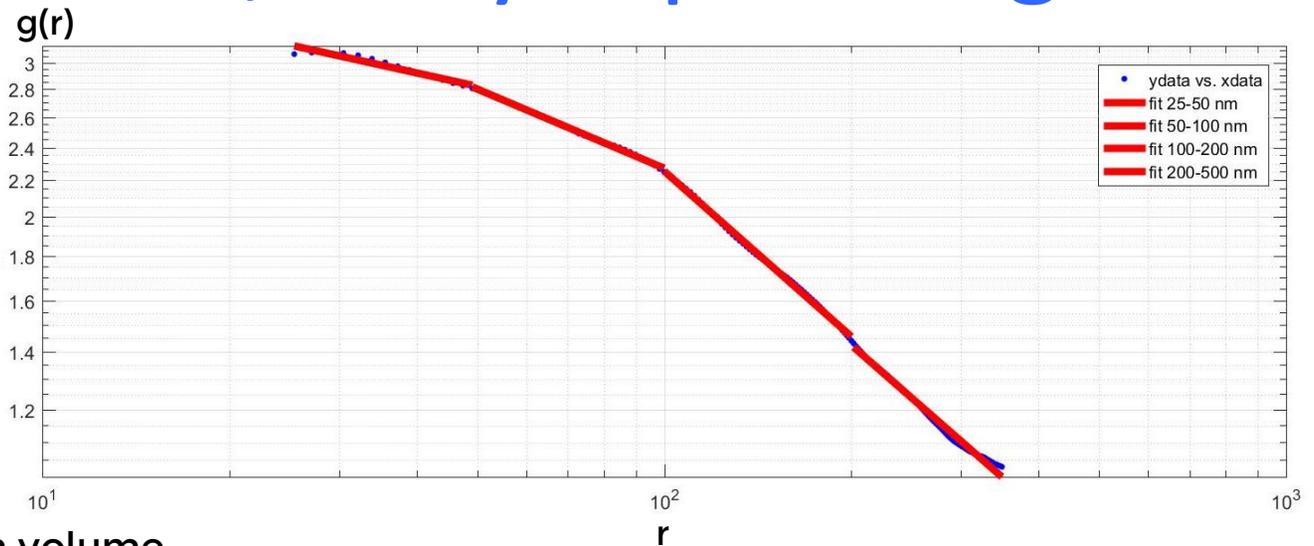
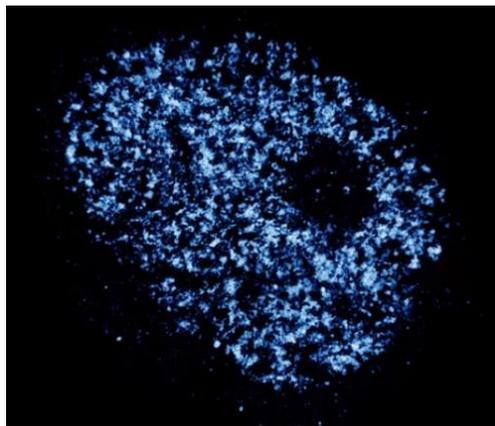
I. K. Robinson, Aga Khan, 2019

# 3D imaging with Double-Helix PSF



# Christophe Lynch

## Interphase, early replicating



PCF in  $1\ \mu\text{m} \times 1\ \mu\text{m} \times 1\ \mu\text{m}$  volume  
 Implemented in custom MATLAB scripts

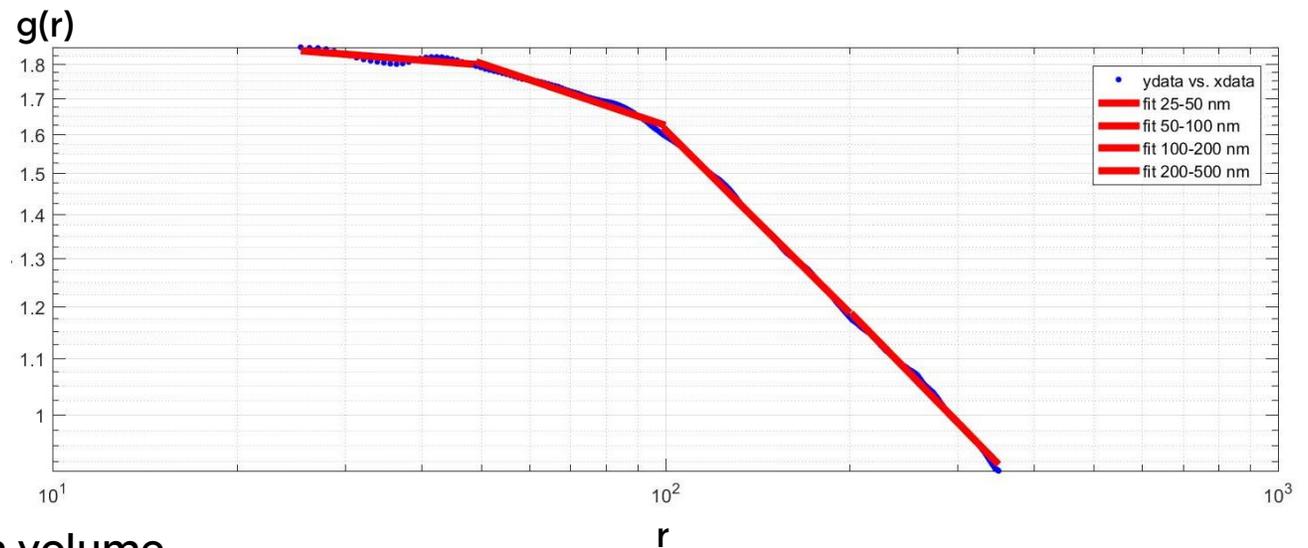
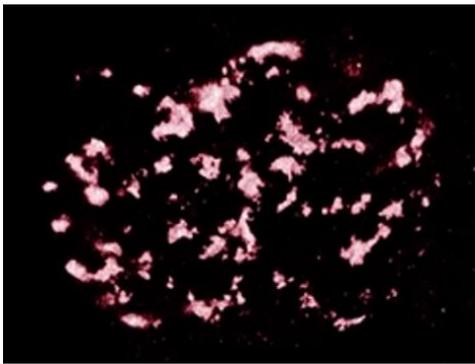
Fit to power-law at four different length scales

$$g(r) \sim A \cdot r^B, D = B + 3$$

Average values across 7 nuclei, 5 VOIs each

25-50 nm	50-100 nm	100-200 nm	200-500 nm
D = 2.81	2.67	2.54	2.60

# Interphase, late replicating



PCF in  $1\ \mu\text{m} \times 1\ \mu\text{m} \times 1\ \mu\text{m}$  volume  
Implemented in custom MATLAB scripts

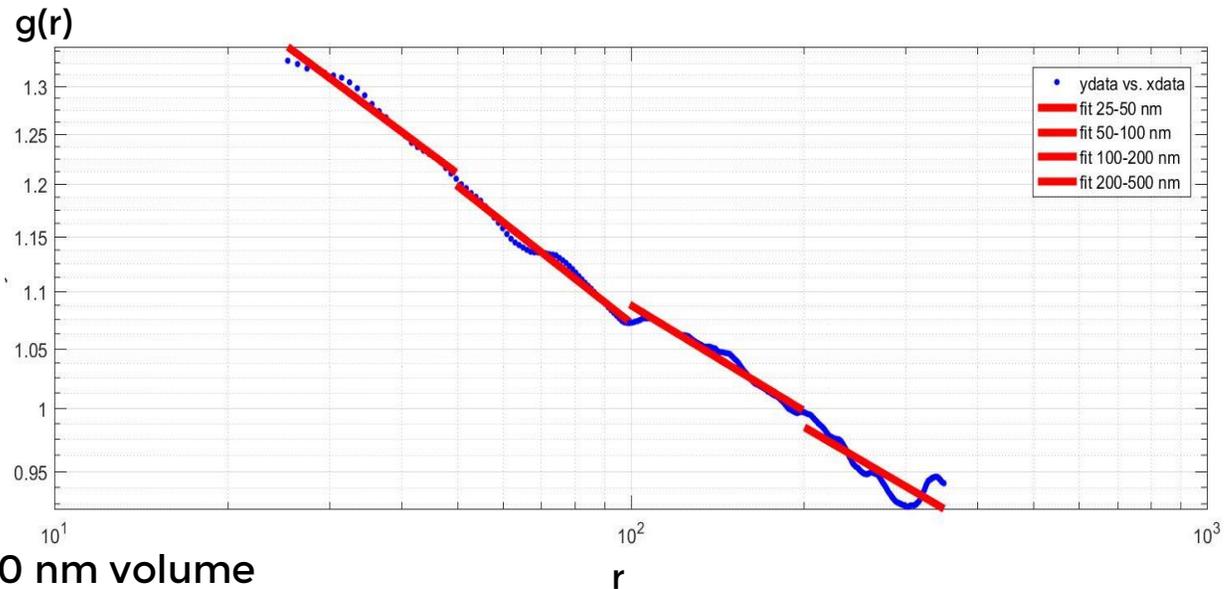
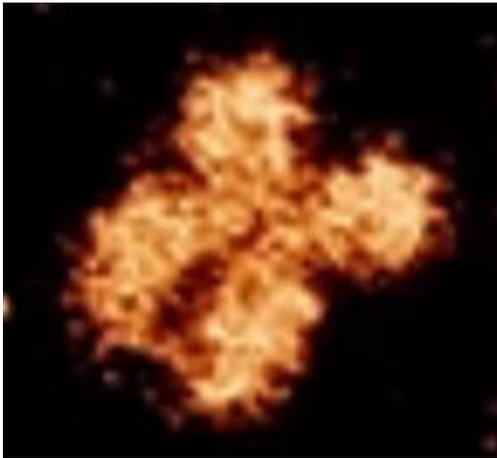
Fit to power-law at four different length scales

$$g(r) \sim A \cdot r^B, D = B + 3$$

Average values across 6 nuclei, 5 VOIs each

25-50 nm	50-100 nm	100-200 nm	200-500 nm
D = 2.94	2.85	2.65	2.38

# Interphase, early replicating Single chromosome



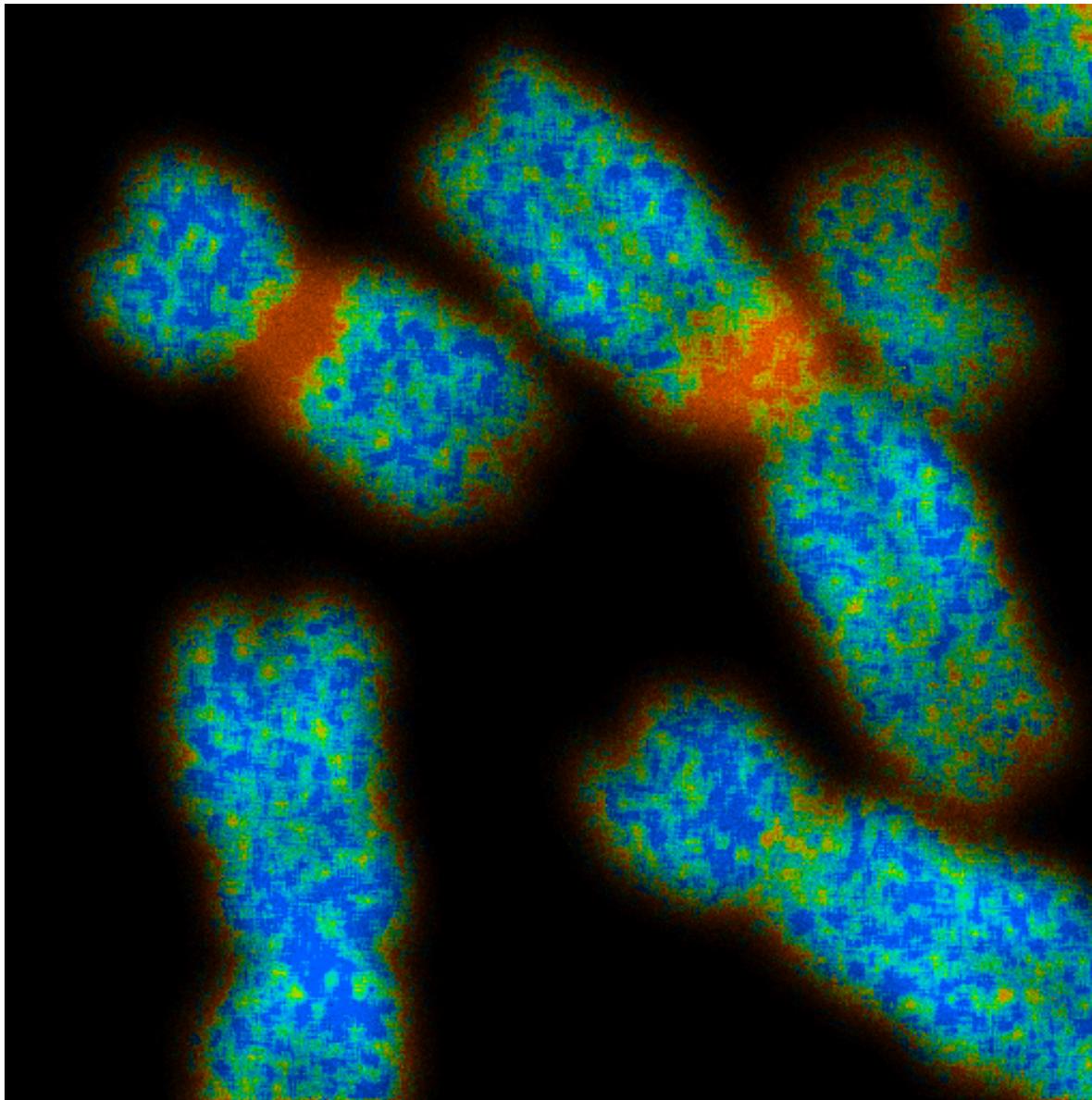
PCF in 500 nm x 500 nm x 500 nm volume  
Implemented in custom MATLAB scripts

Fit to power-law at three different length  
scales

$$g(r) \sim A \cdot r^B, D = B+3$$

Average values across 6 nuclei, 5 VOIs each

25-50 nm	50-100 nm	100-200 nm
D = 2.87	2.86	2.86



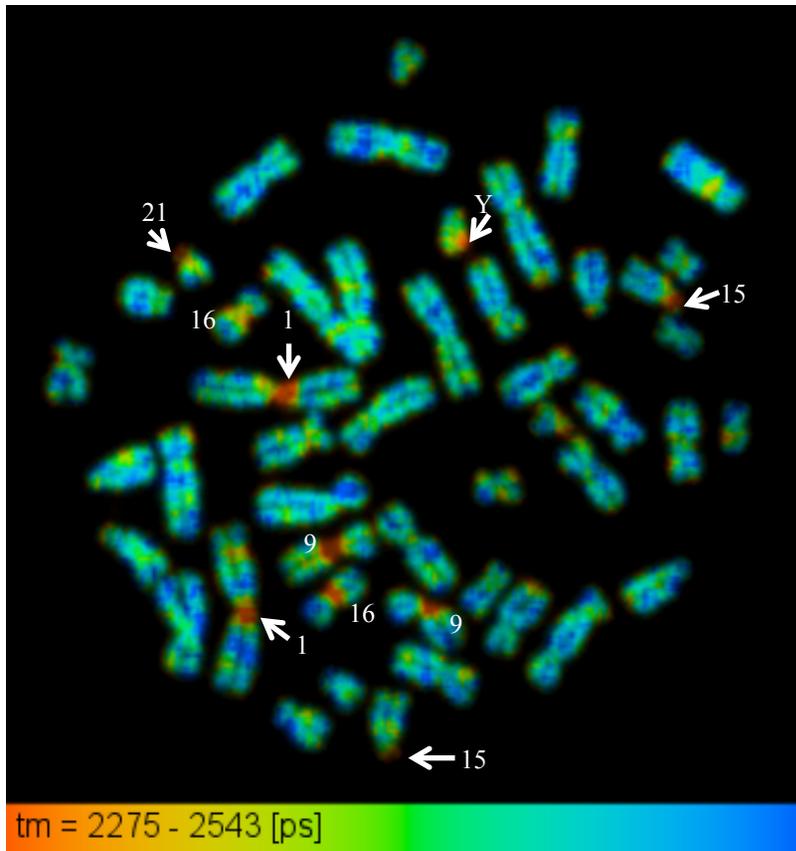
tm = 2605 - 3063 [ps]

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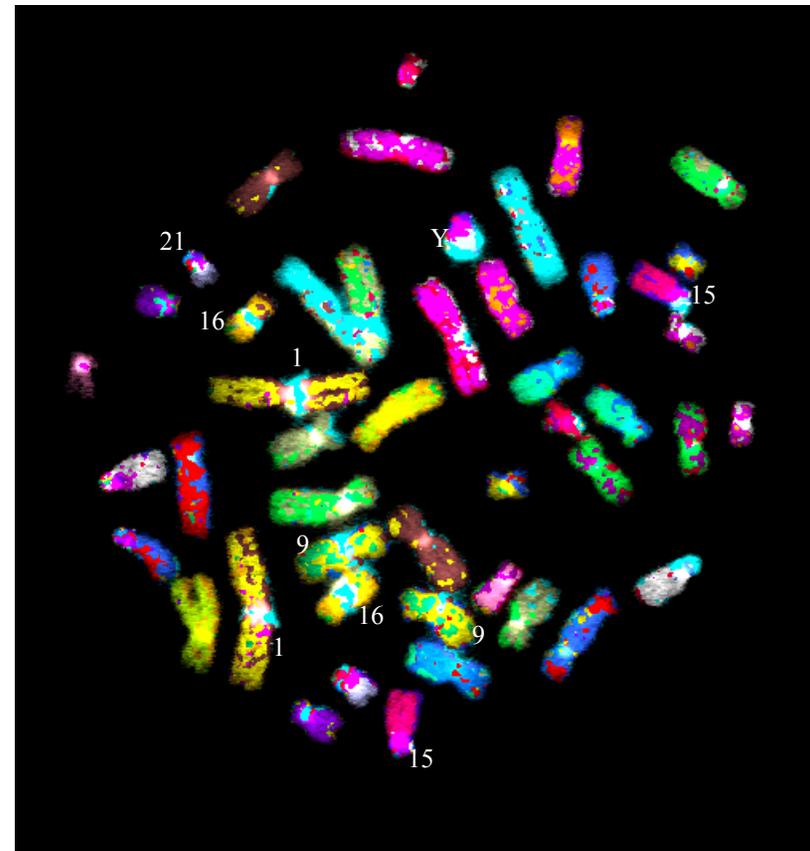
FLIM  
Ana Estandarte  
512x512 pixels  
FOV: 10  $\mu\text{m}$

# Heterochromatic Blocks

Ana Estandarte, Yusuf M and Stan Botchway



**Lifetime Image**



**MFish**

- Short lifetimes observed at chromosomes 1, 9, 15, 16, and Y
- In some spreads: other acrocentric chr (21, 22, 13, 14) and chr 20

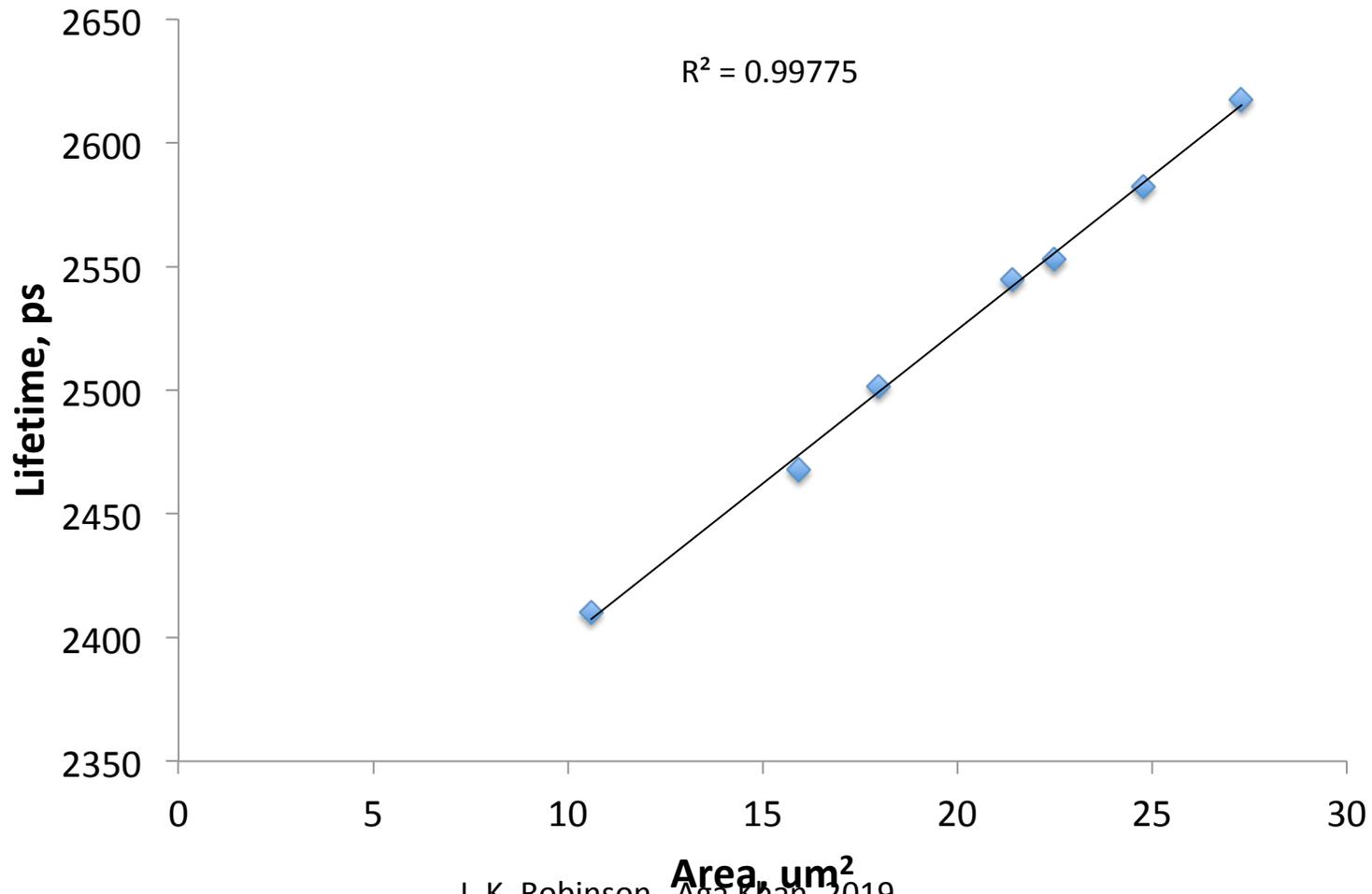
I. K. Robinson, Aga Khan, 2019

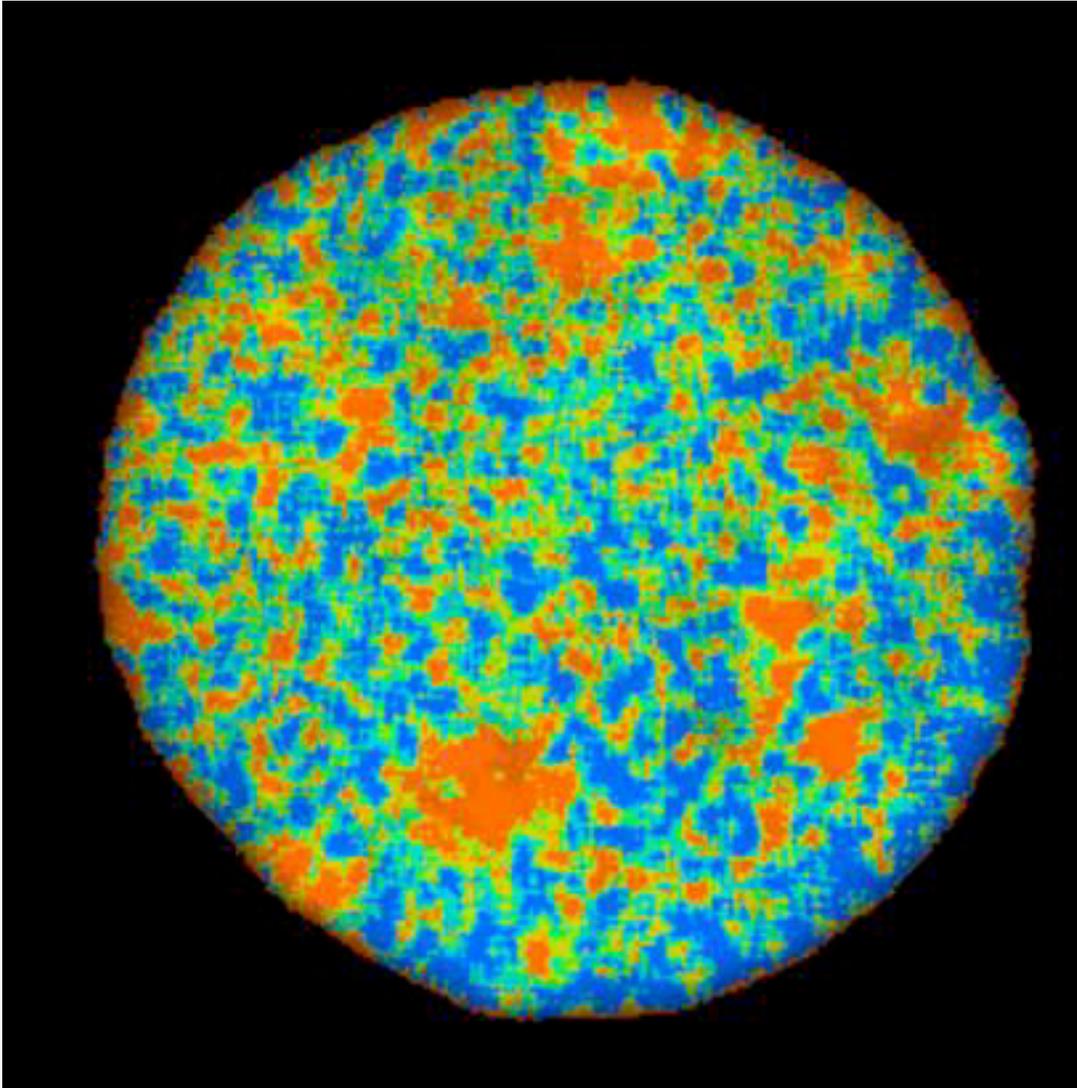
39

# Lifetime scales with Condensation

Heterochromatic regions – whole chromosome area

Ana Estandarte and Stan Botchway





## Interphase Nucleus

500 nm z-step  
size, 5 $\mu$ m FOV

# Chromosome Imaging at RCaH

- X-ray CDI and Ptychography
- Mass and Volume Karyotype plots
- Serial Block Face SEM – very promising
  - “Territories” preserved in prophase
  - Tight packing of material
- STORM super-resolution
- Fluorescence lifetime imaging (FLIM)