Novel methods for imaging chromosomes

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RCaH-MRC-Diamond
Bioimaging Workshop
March 2014

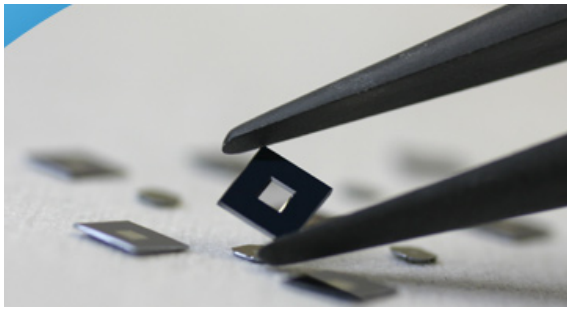
Outline

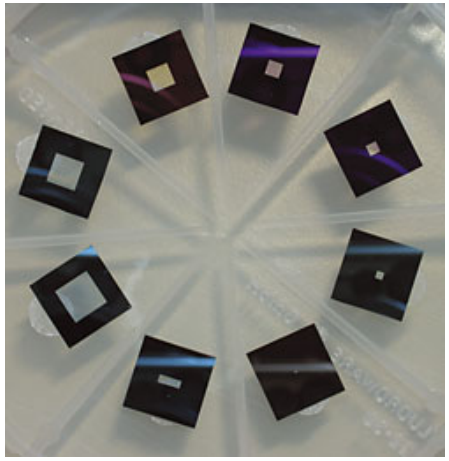
- X-ray Microscopy and Imaging
  - Scanning Transmission (STXM)
  - Coherent Diffractive Imaging (CDI)
  - X-ray Ptychography
- Serial Block Face SEM ("3-view")
- Optical methods using CLF

Chromosome sample preparation

Silicon Nitride membranes

Yusuf Mohammed and Neha Parmar

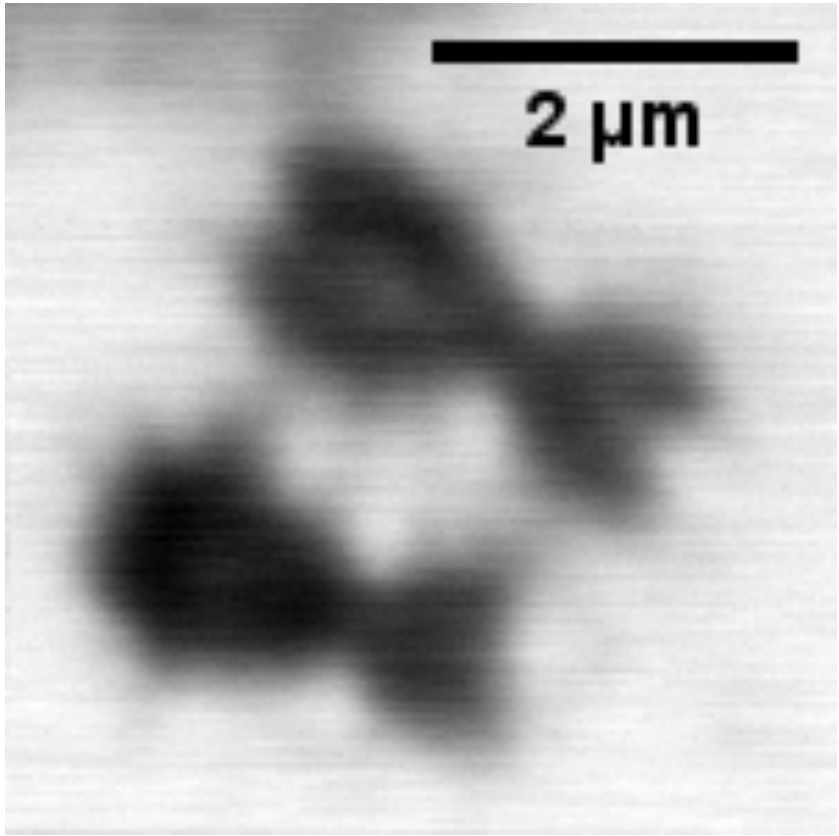




- Cell culture from Yoruba cell line (GM18507, passage 4)
- Synchronization in mitosis: thymidine+colcemid for 16 h
- Fixed in 3:1 methanol-acetic acid
- Crosslinked in 1% glutaraldehyde
- Air-dried onto 100nm silicon nitride membranes
- Nuclei stained with platinum blue 2.5 mM for 30 min

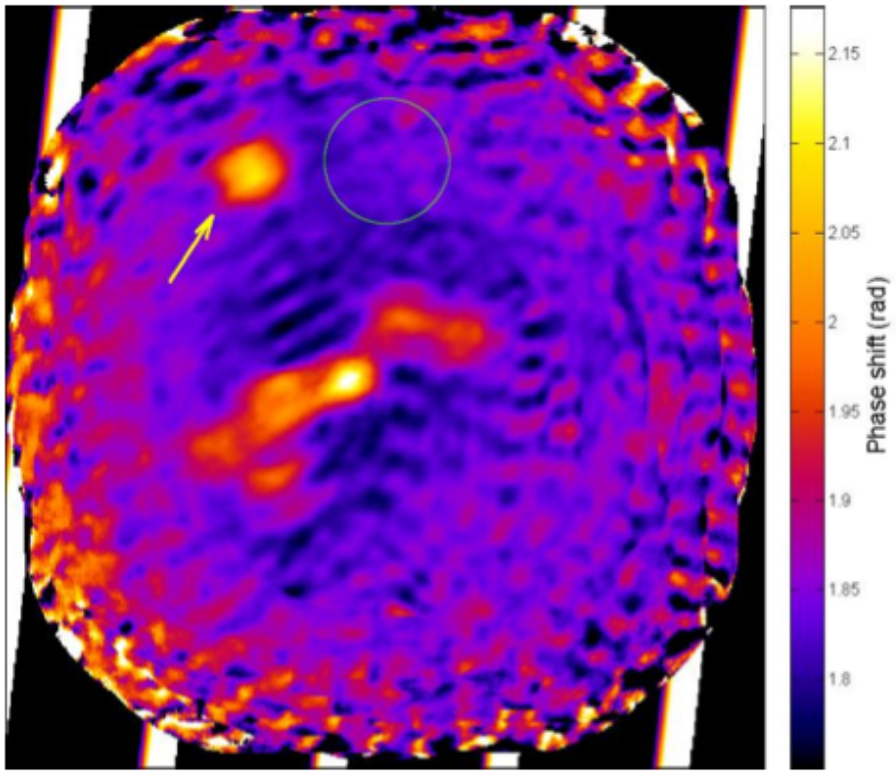
Scanning Transmission X-ray Microscope

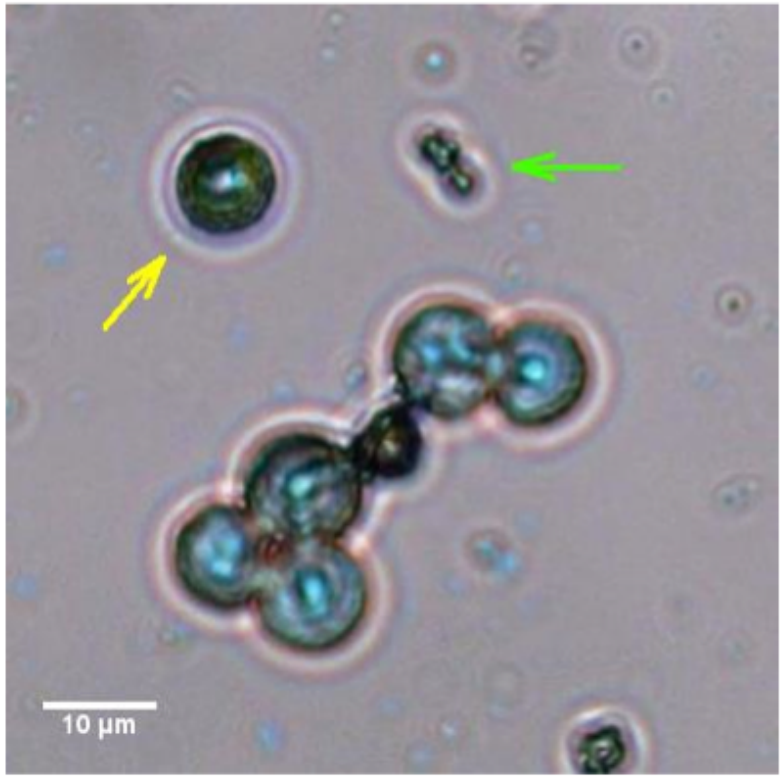
Absorption contrast, Elettra, Twin-Mic, Graeme Morrison
500eV, 30nm steps, 145nm probe, 40ms dwell (projection)



Chromosomes and Nuclei

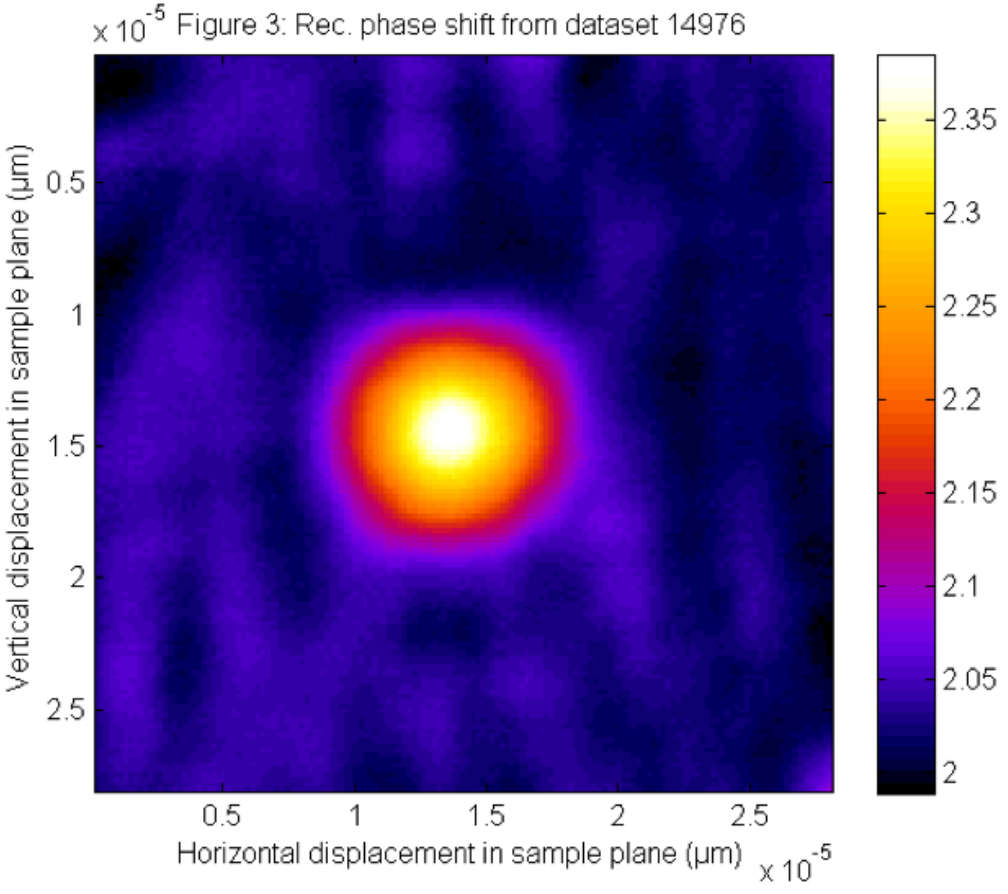
14939 X-ray ptychography, 25µm probe, Diamond I-13

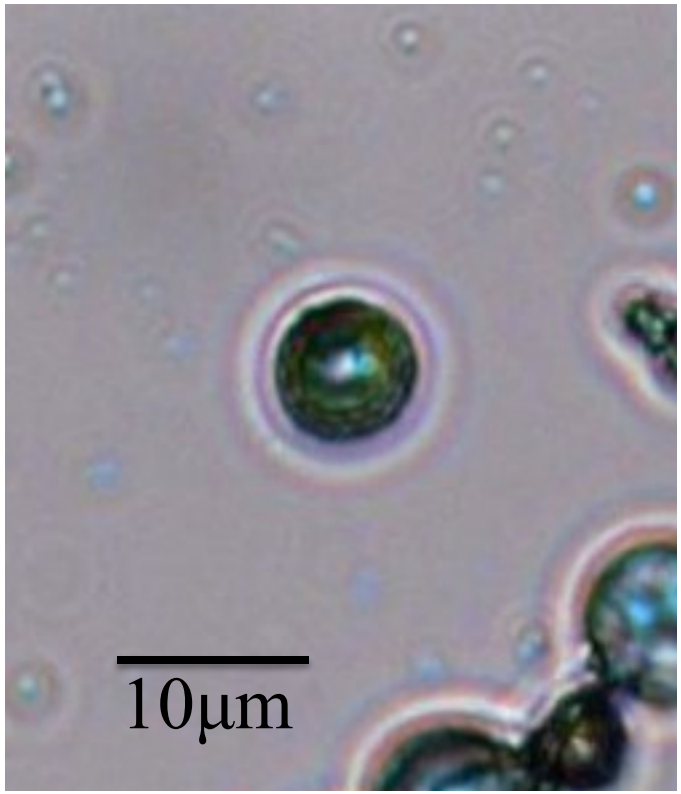




Mitotic Nucleus

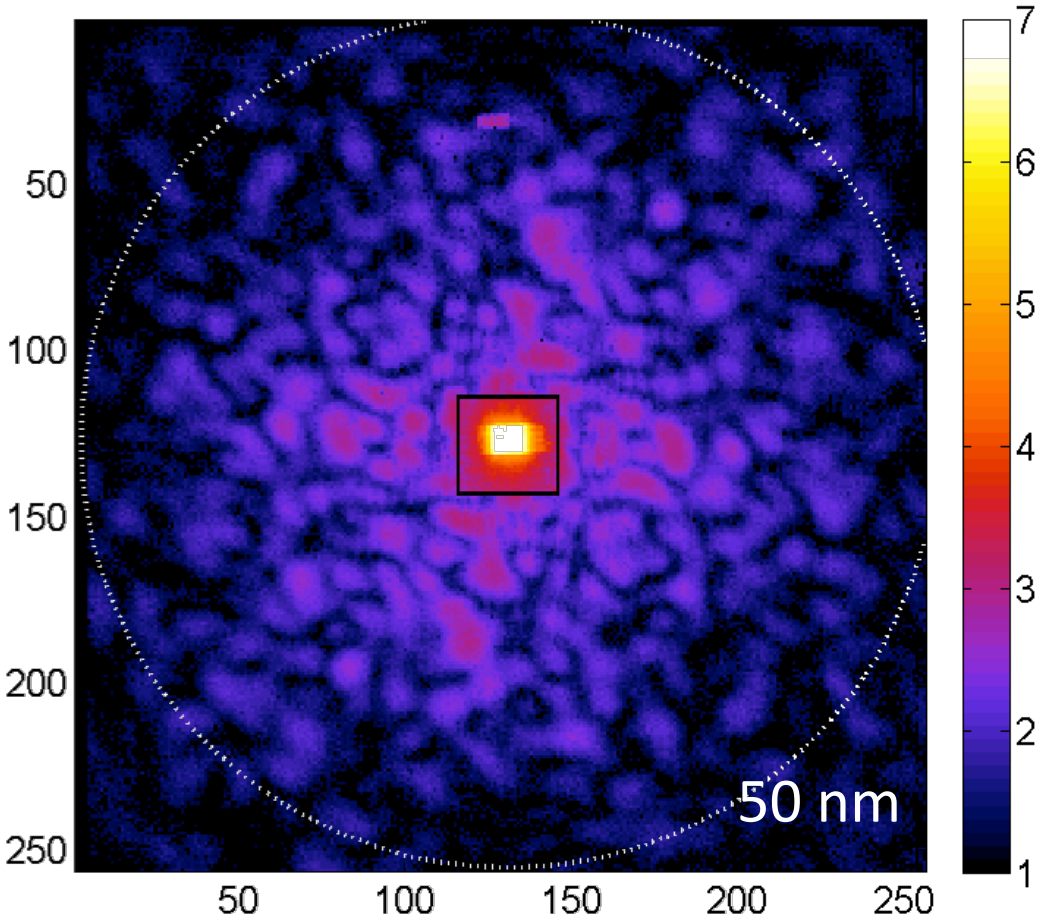
14976 X-ray ptychography, Joerg Schwenke and Laura Shemilt, Diamond I-13



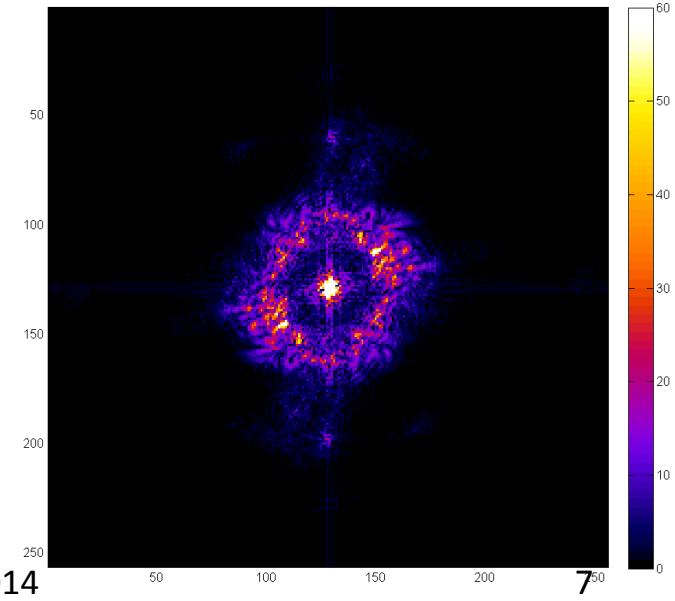


Chromosome Ptychography

Laura Shemilt, Joerg Schwenke, Yusuf Mohammed, 34-ID-C



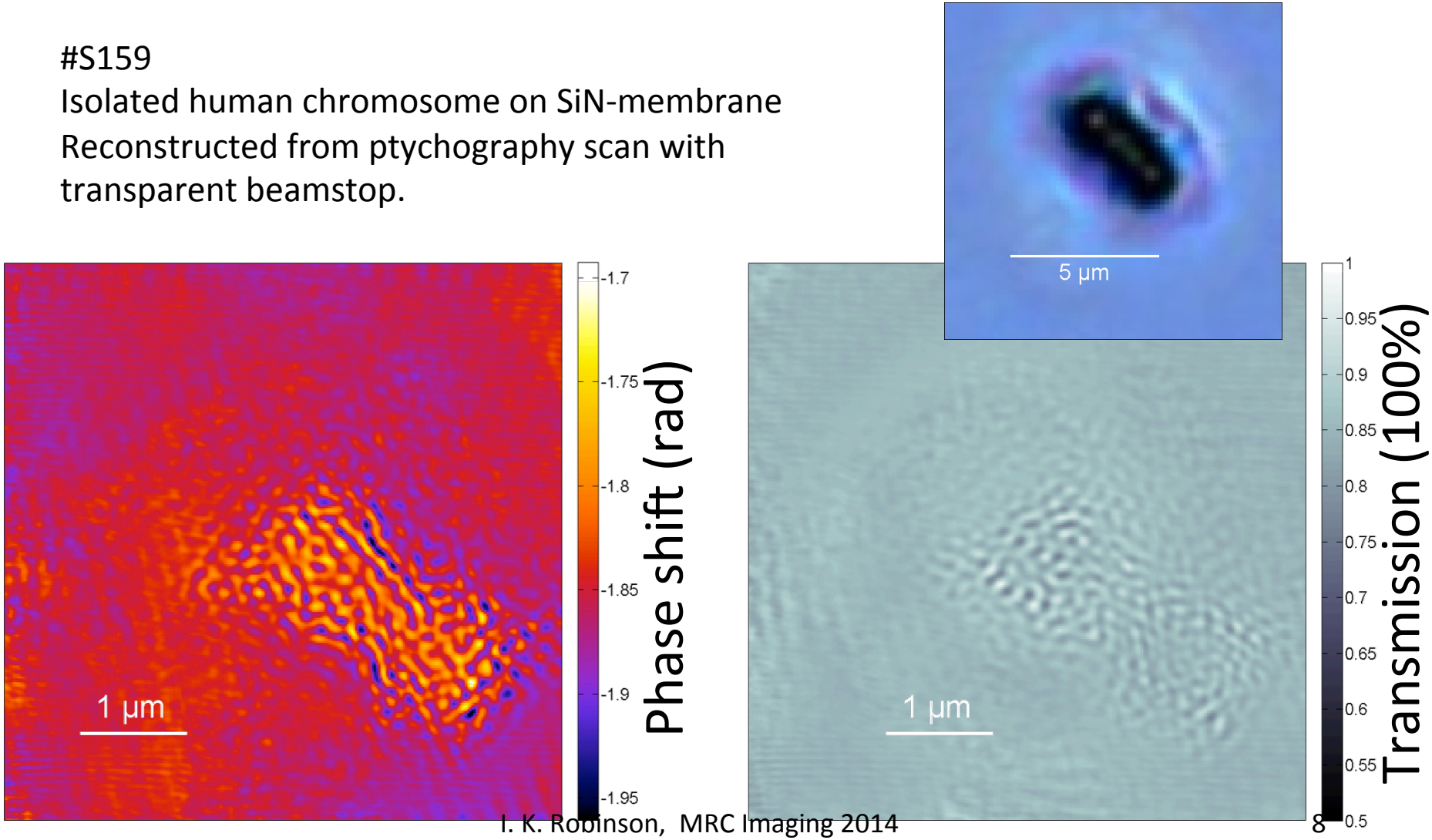
#S152, frame 1
10s exposure
6.5 keV
Medipix2, 55 µm
1.851 m distance



Chromosome Ptychography

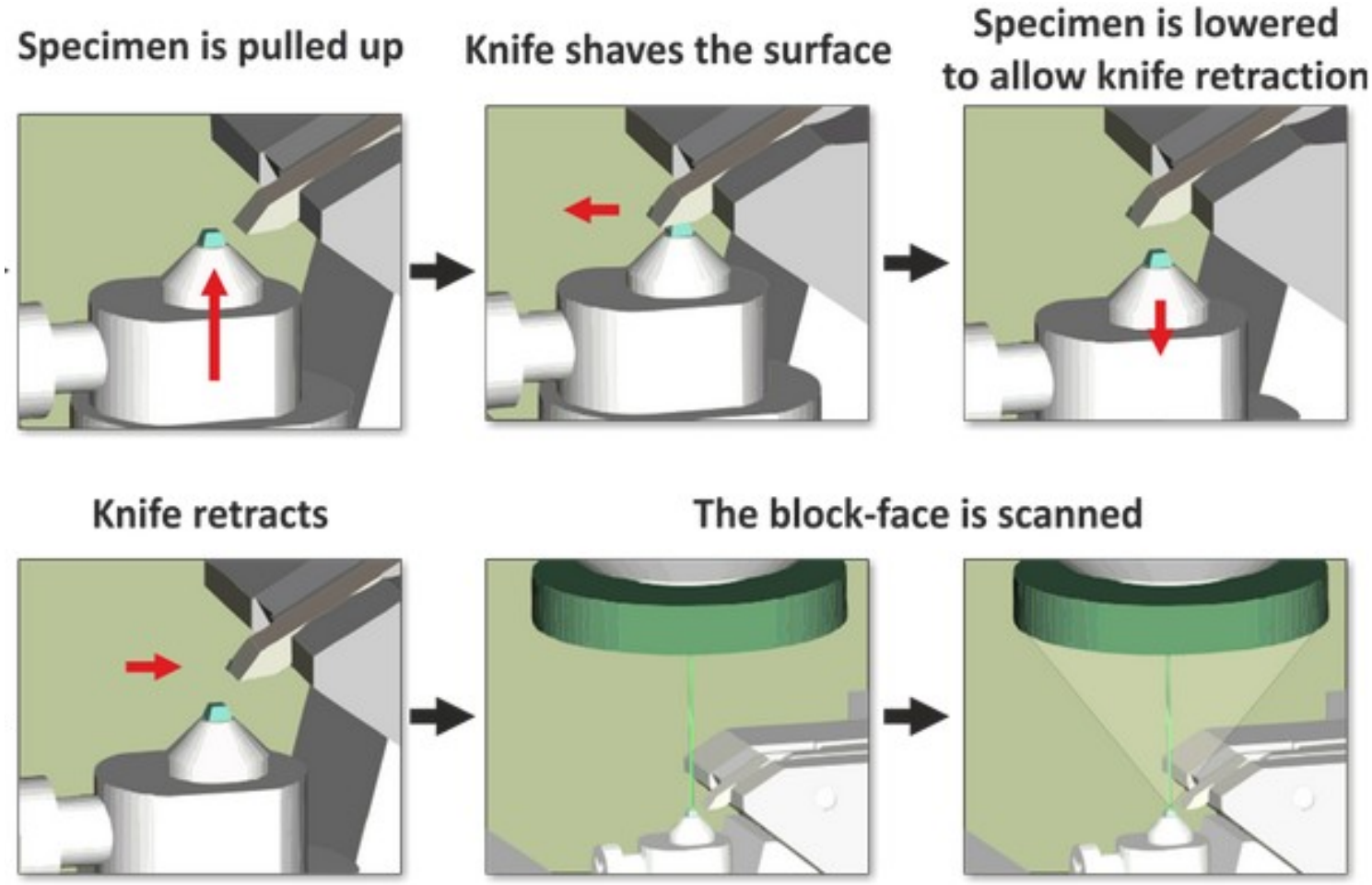
Laura Shemilt, Joerg Schwenke, Yusuf Mohammed, Argonne

#S159
Isolated human chromosome on SiN-membrane
Reconstructed from ptychography scan with transparent beamstop.



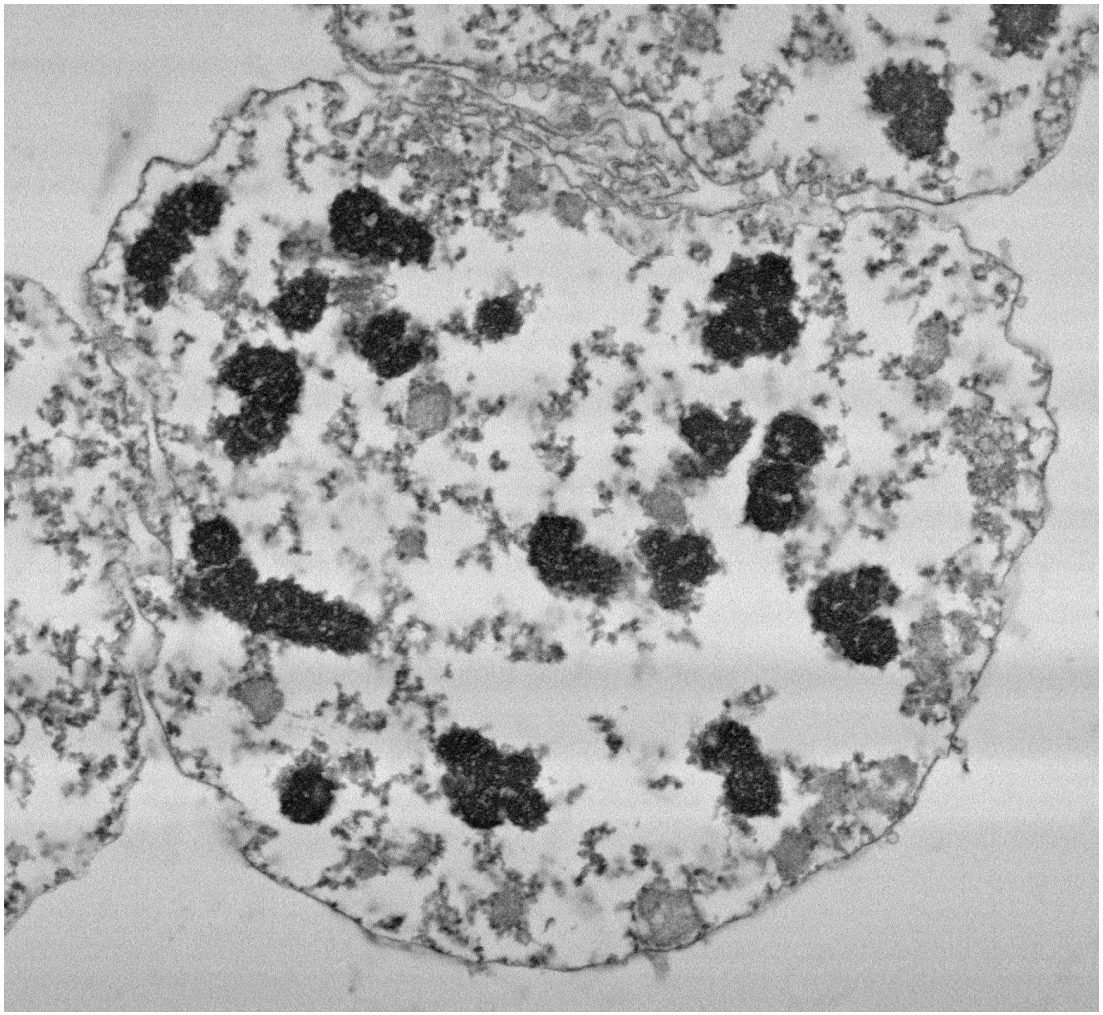
Serial Block Face SEM (3-view)

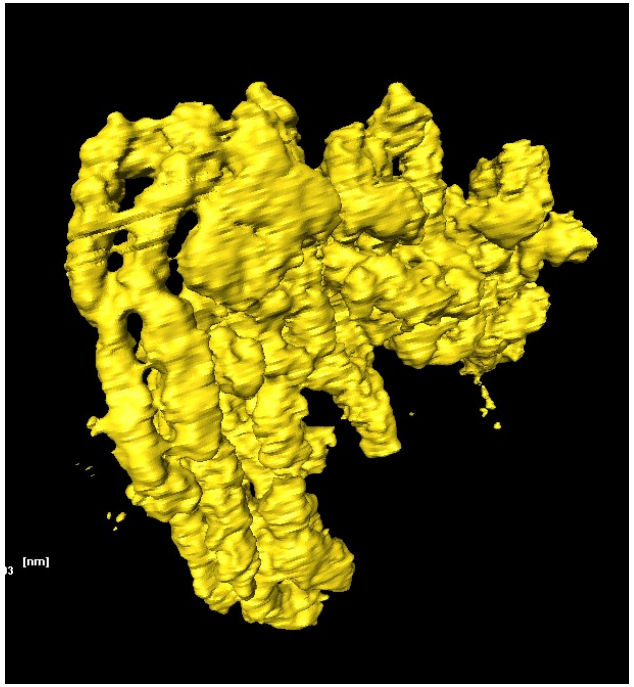
Electron Microscopy Unit, University of Helsinki



Serial Block Face SEM

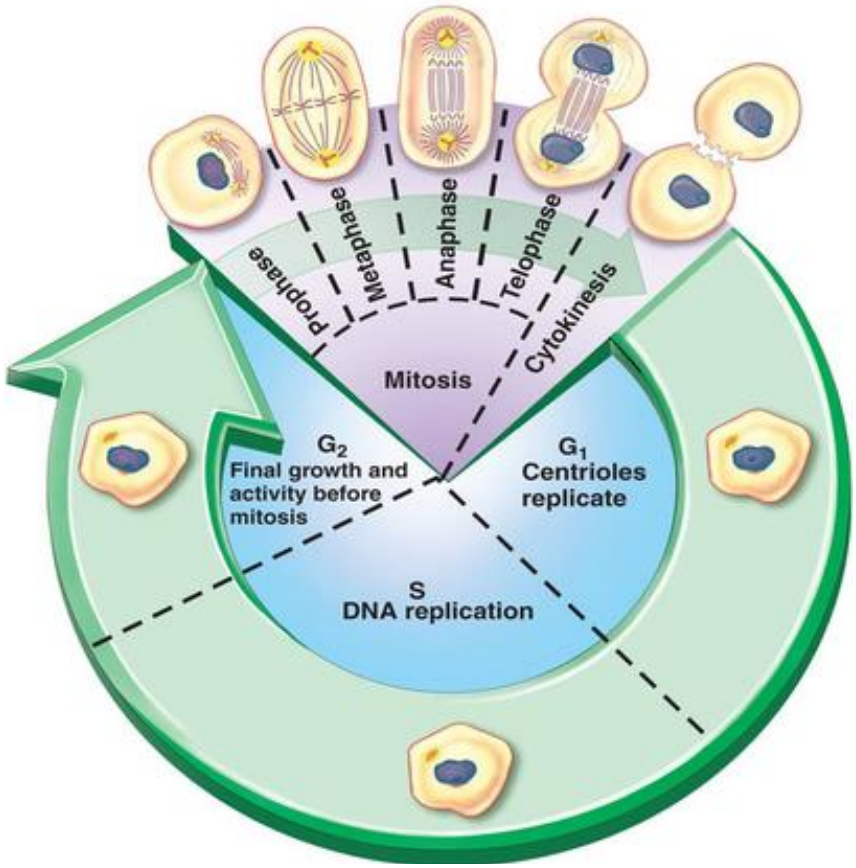
Bo Chen, Yusuf Mohammed, Sherman Ip





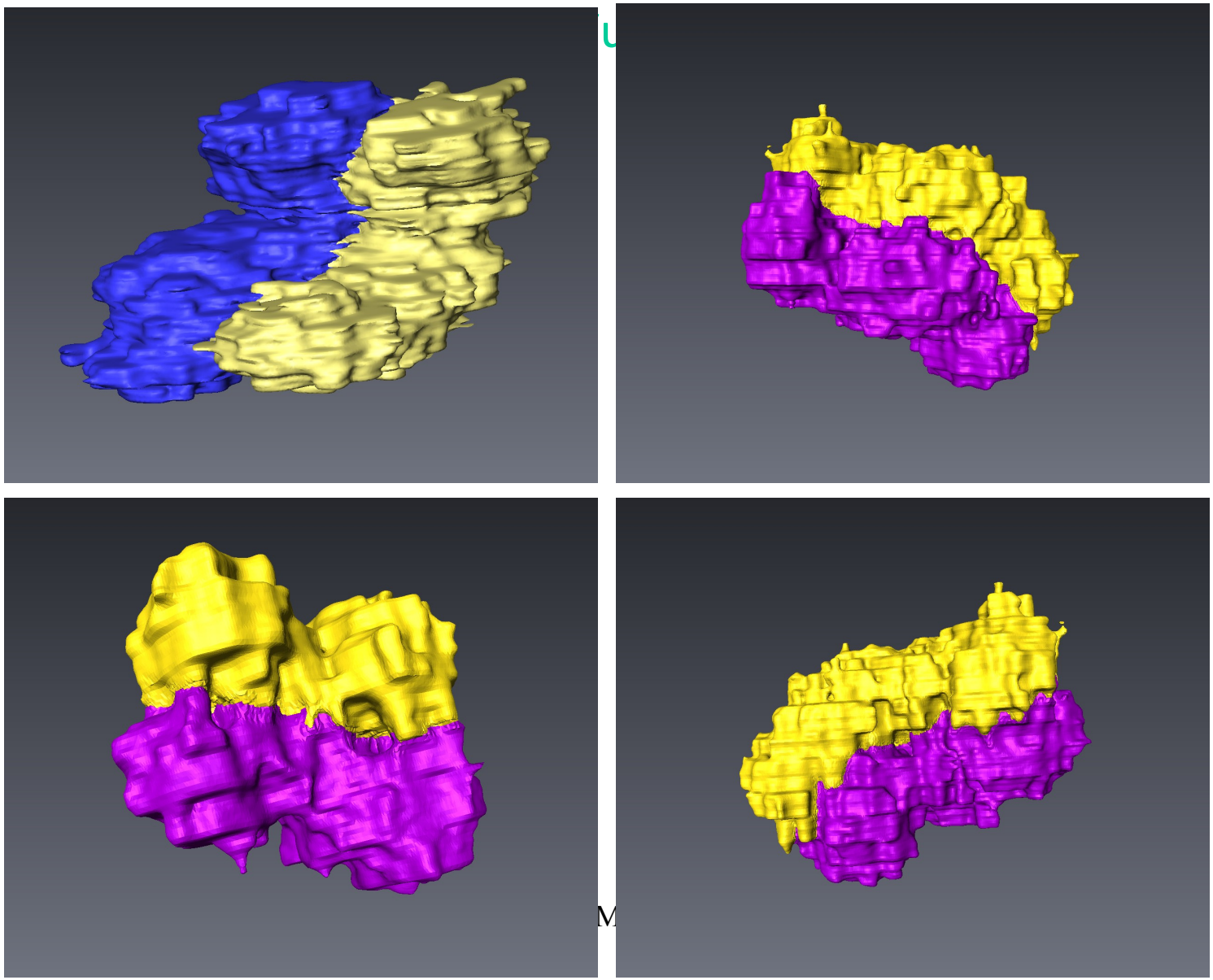
Preparation favours Prophase

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



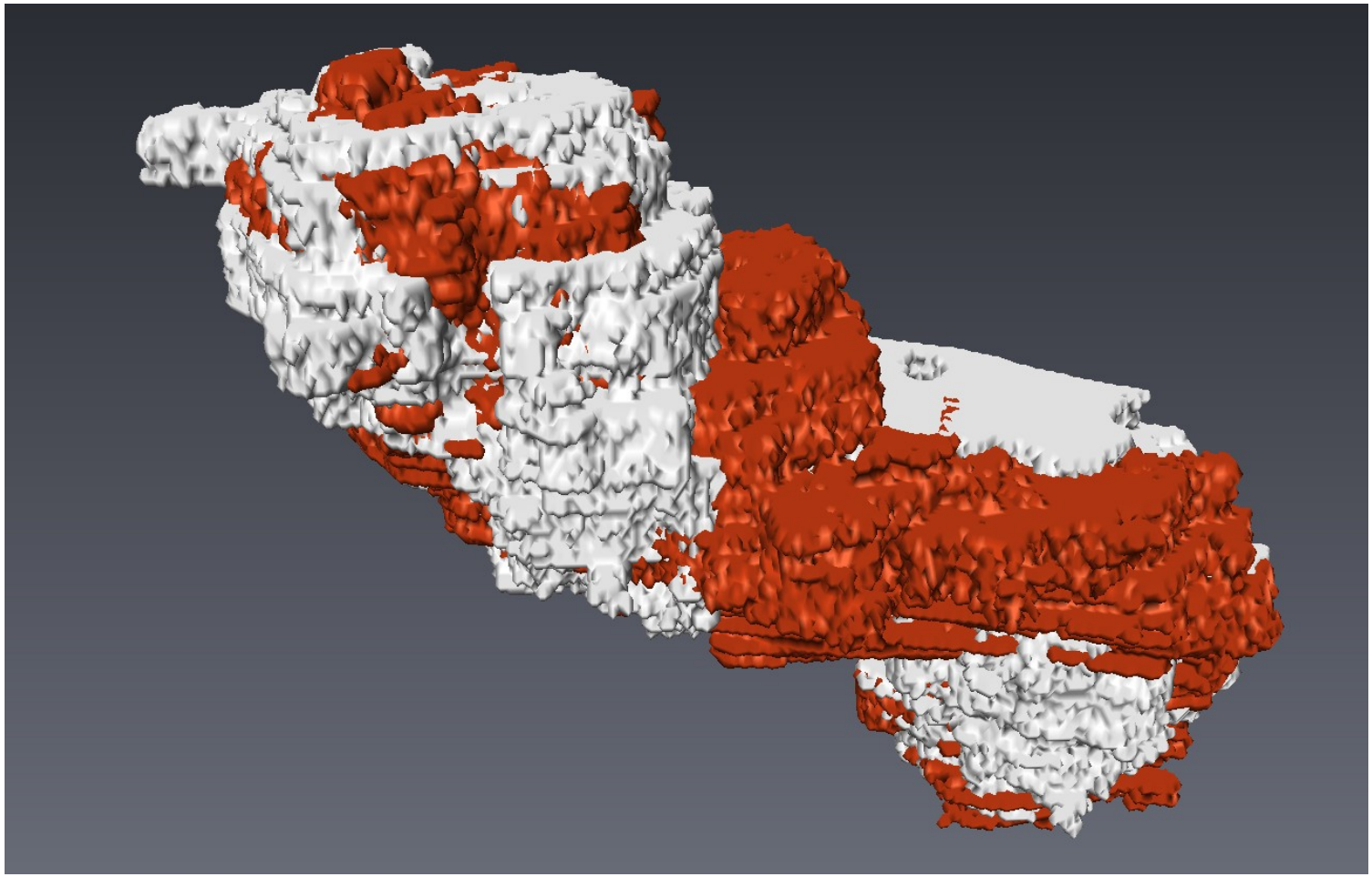
- Prophase
  - condensed chromosomes
  - nuclei still present
- Prometaphase
  - nuclear membrane dispersed
- Metaphase
  - spindle assembly

Segmented Prophase Chromatids



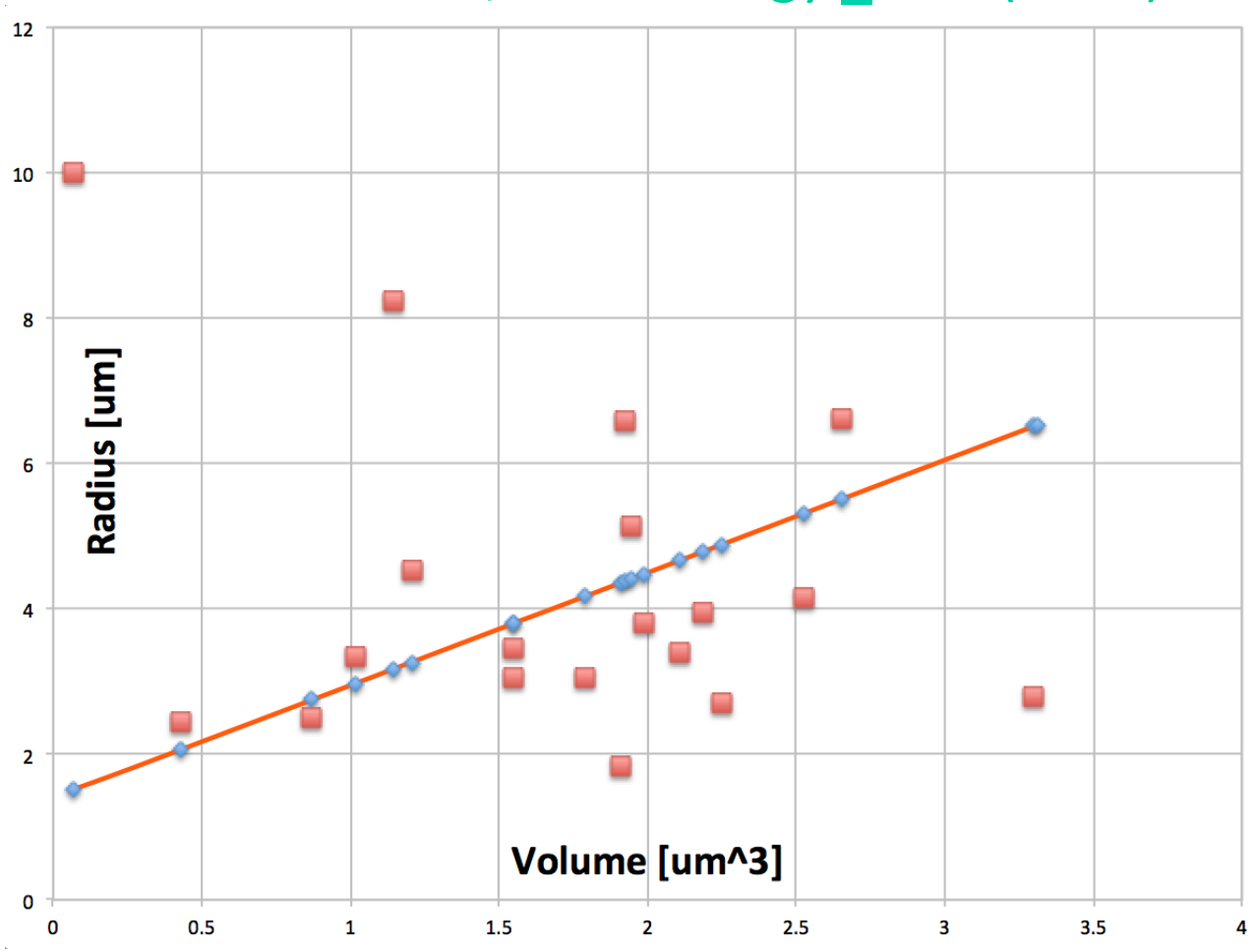
Segmented Prophase Chromatids

Bo Chen and Yusuf Mohammed



Smaller Chromosomes at Nuclear Interior, following Territories

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



3D Chromatid Volume Analysis

Accounting for measured, volumes per base pair

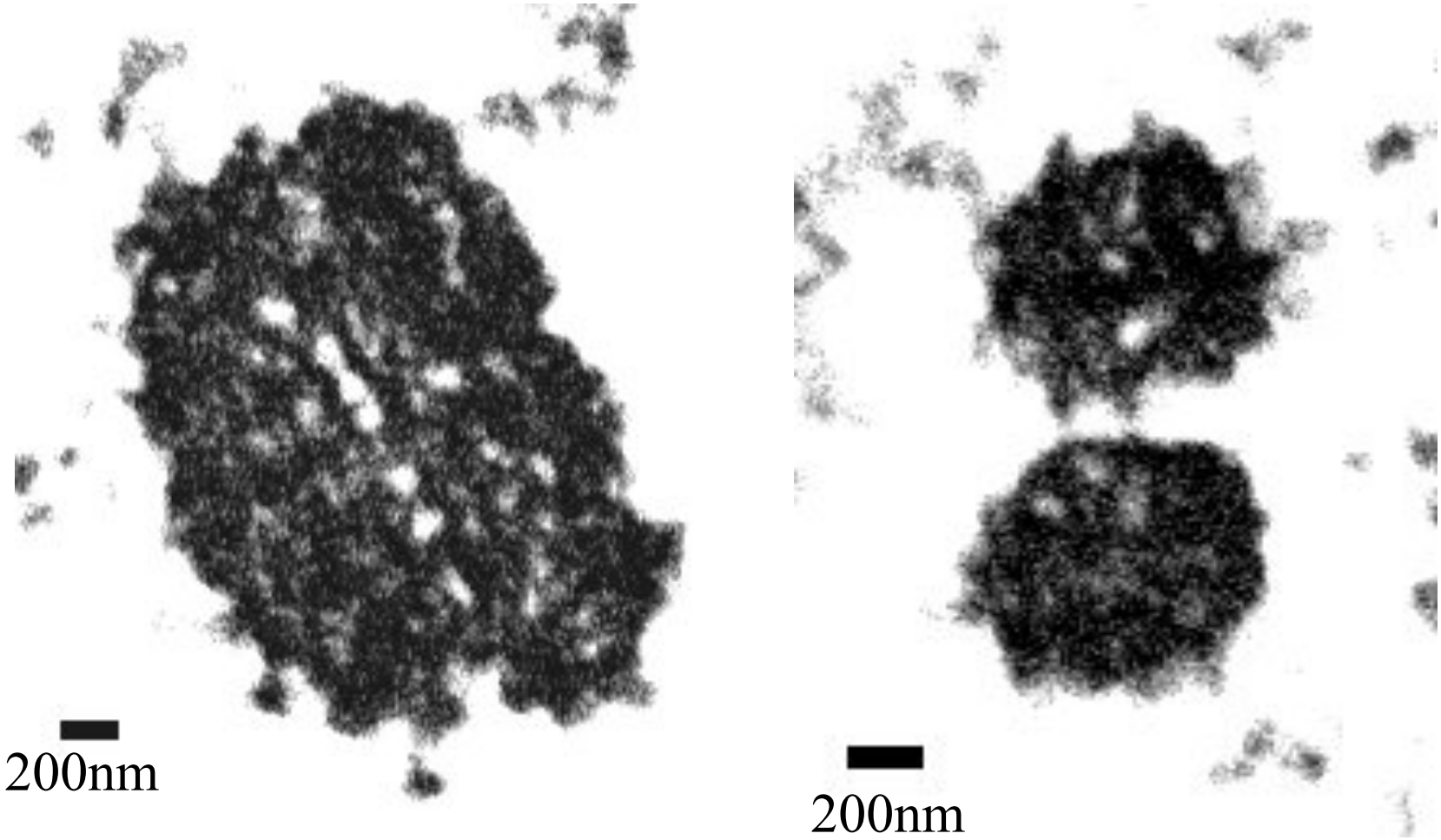
Methanol-Acetic Acid embedding in Epoxy Resin

Preliminary data (Bo Chen, Yusuf Mohammed, in progress)

Chromosome 1	250MBP per chromatid	6.4nm^3
DNA alone	2nm diam x 0.34nm	1.06nm^3
Nucleosome (140BP)	11nm diam x 6nm	4.07nm^3
Histones H2-H4	subtract	3.01nm^3
Non-histone protein	65% histone	1.62nm^3
Total filled	89% of measured	5.69nm^3

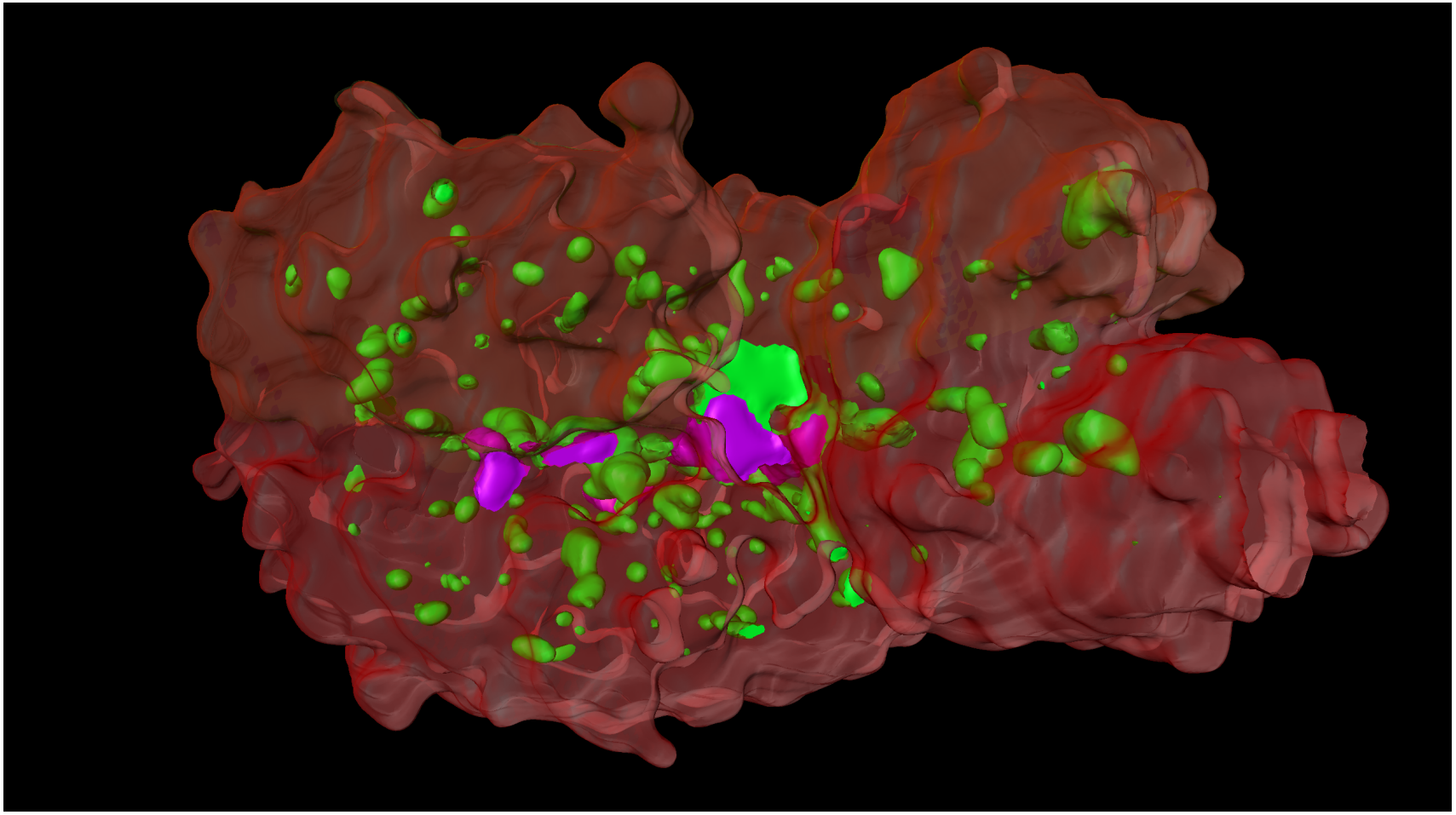
Prospect of Higher Resolution

Chromosomes AB1 and C1



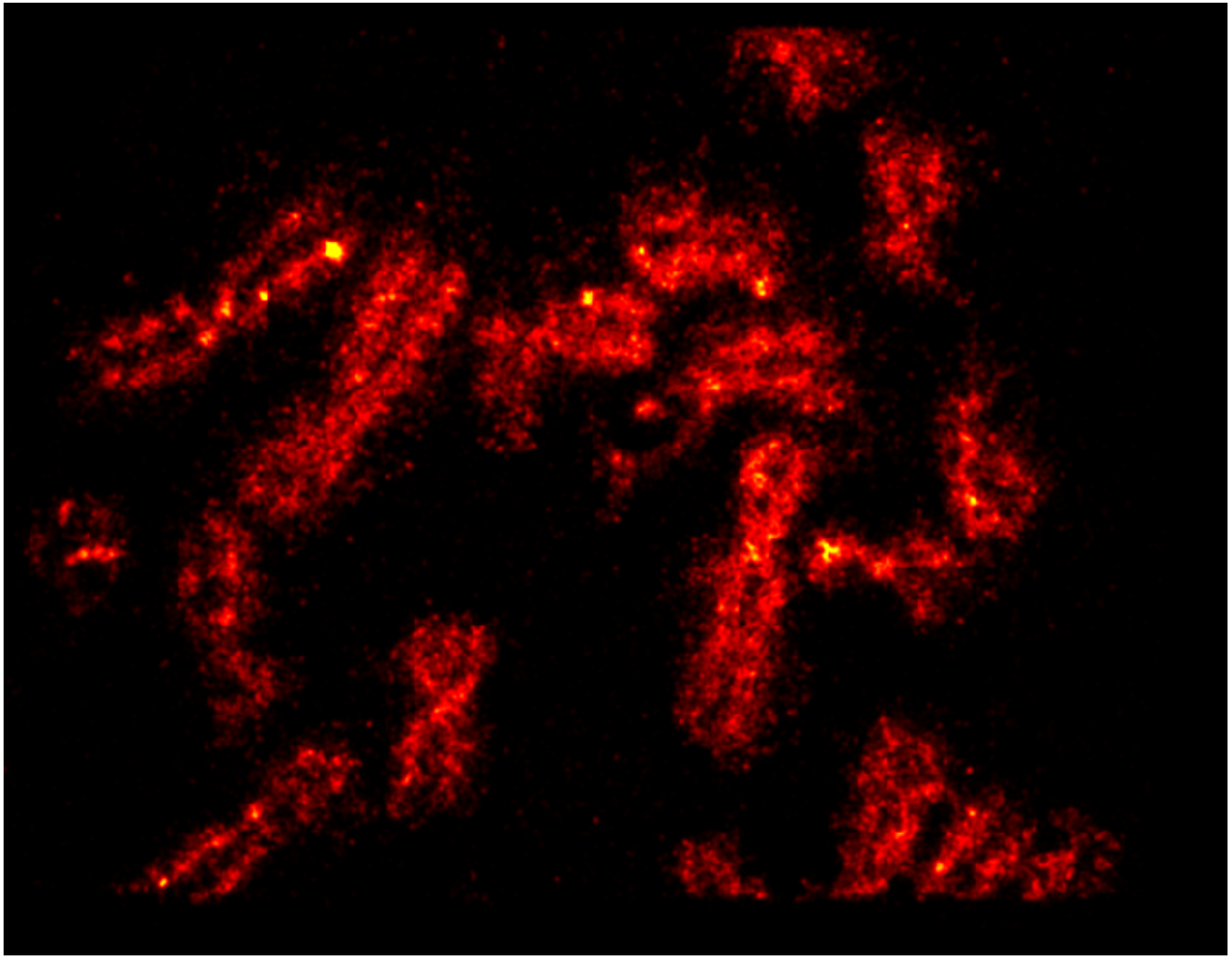
Prospect of Higher Resolution

Chromosome AB1, Bo Chen, internal voids



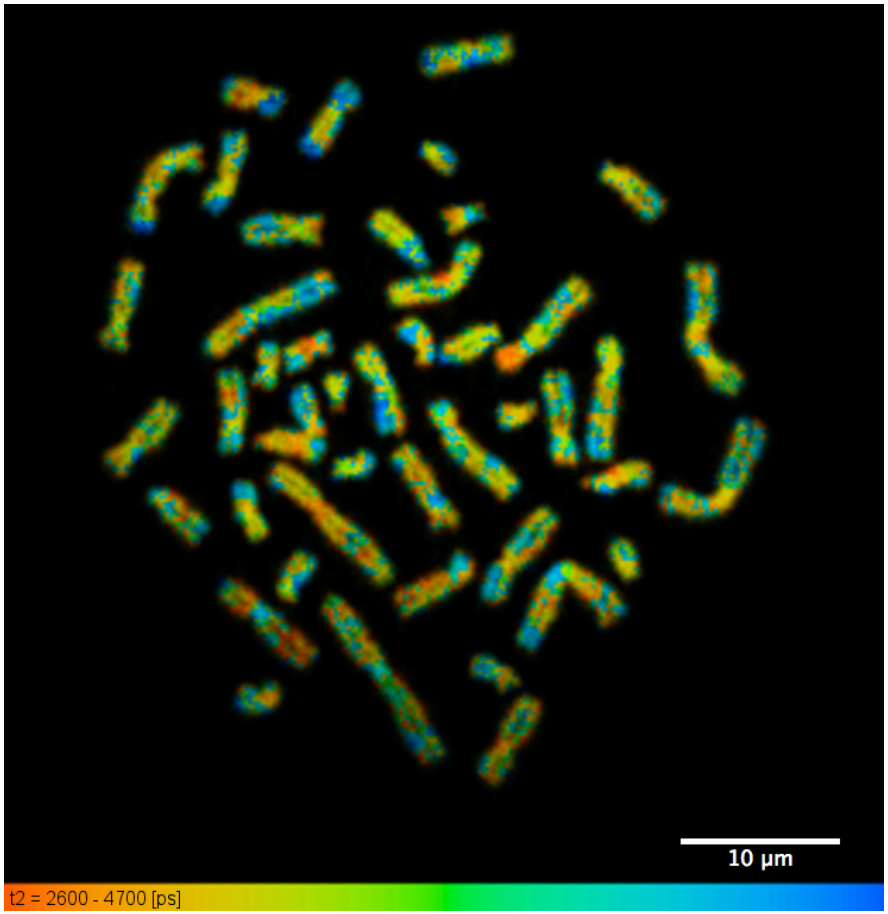
Super-resolution Chromosome Images

Christophe Lynch and Steven Webb

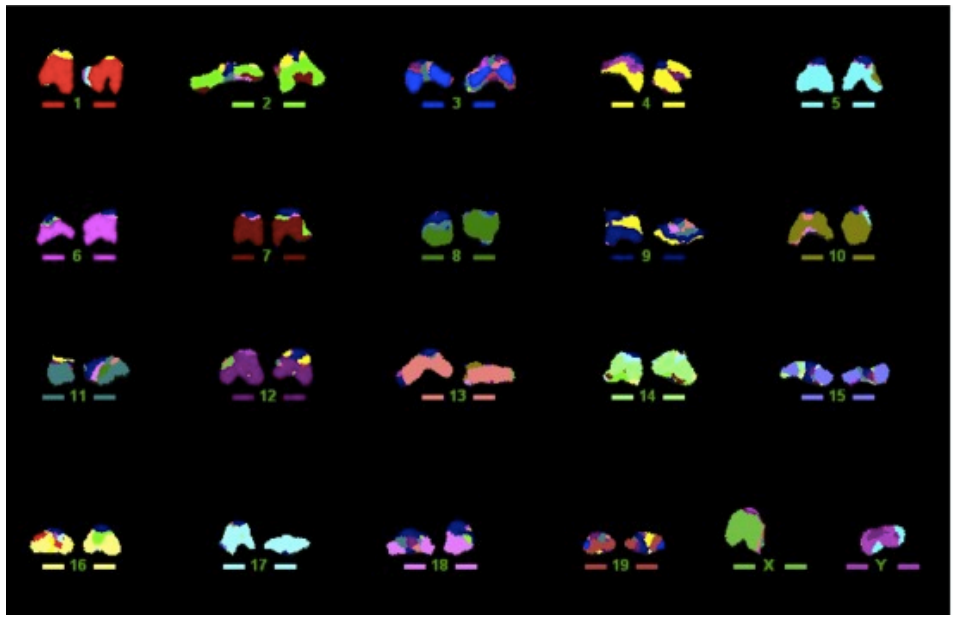


Fluoresence Lifetime Imaging

Ana Estandarte and Stan Botchway

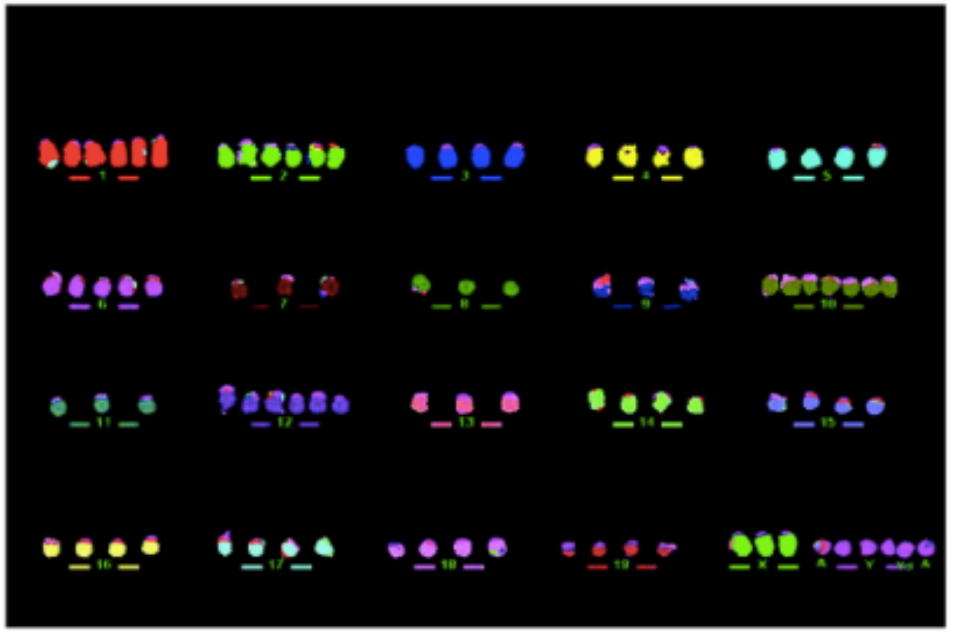


2.6ns 4.7ns



Yusuf M et al, BMC Biotechnol. 2011 11:121

Mouse normal karyotype
40 chromosomes



Ashrafian H et al, Cancer Res. 2010 70(22):9153-65

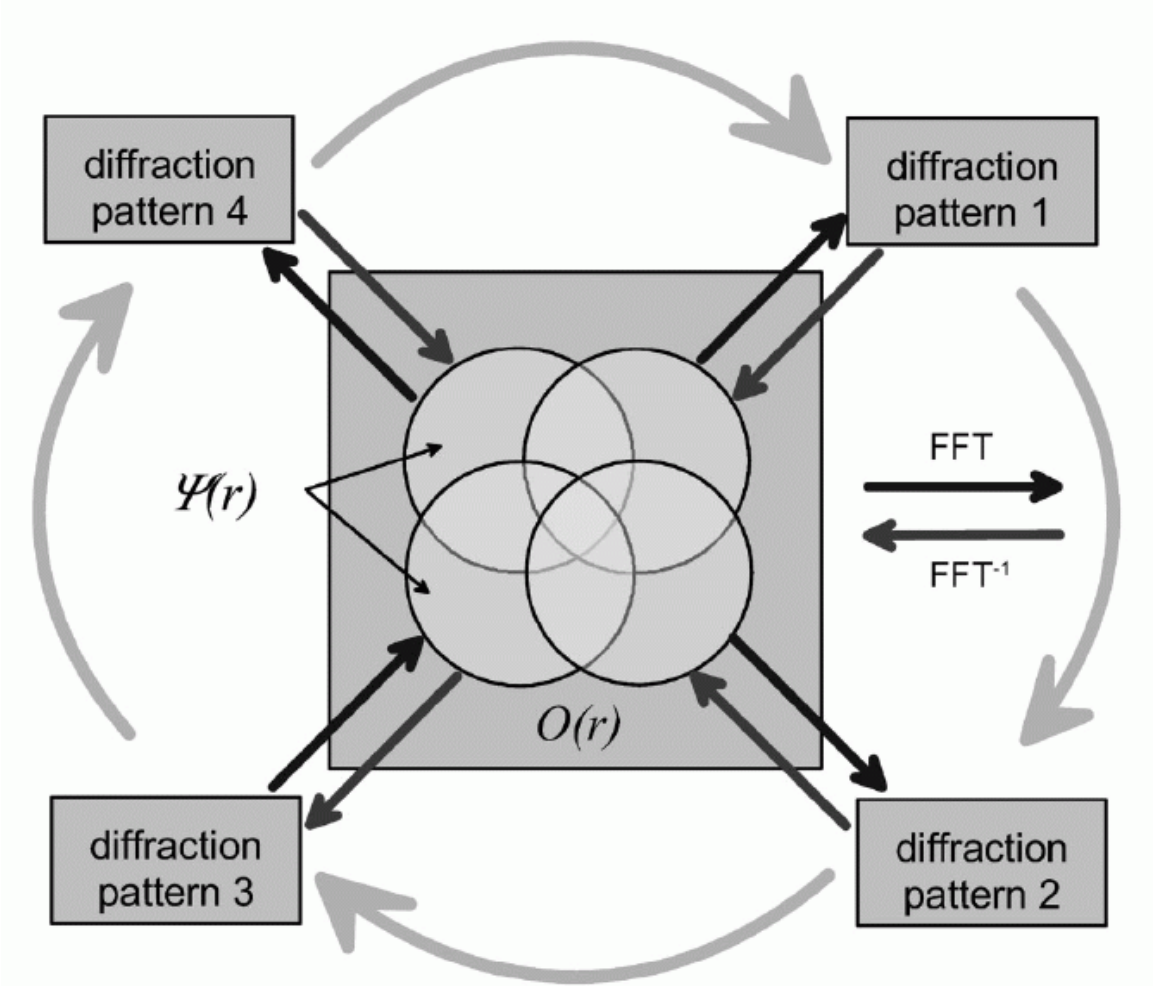
Mouse abnormal karyotype
90 chromosomes

Chromosome Imaging at RCaH

- X-ray CDI and Ptychography
- Serial Block Face SEM – very promising
  - "Territories" preserved in prophase
  - Tight packing of material
- STORM super-resolution
- Fluorescence lifetime imaging (poster)
- Mouse karyotyping by MFISH

X-ray Ptychography

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



Quantitative Phase Shift (14957)

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

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

Barley Chromosome FIB-SEM

Elizabeth Shroeder-Reiter, Martinsreid

Journal of Structural Biology 165 97–106 (2009)