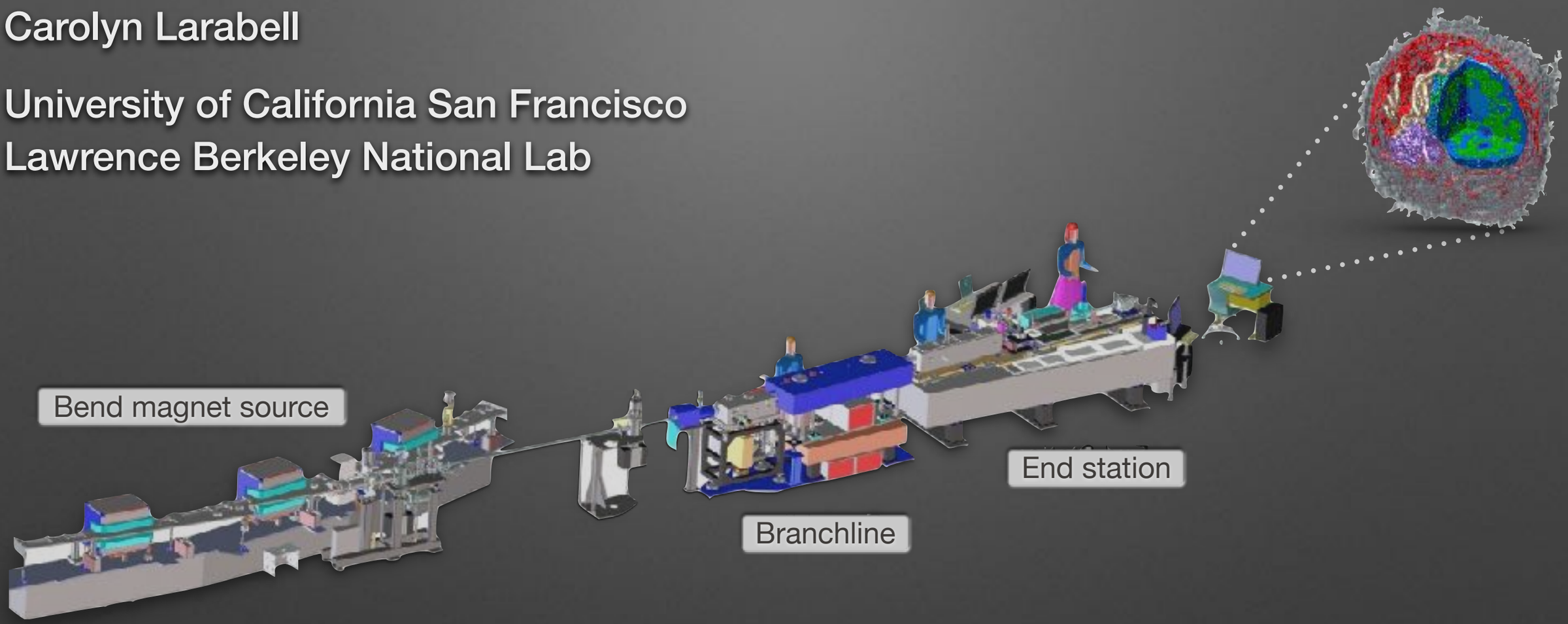
Soft X-ray Tomography (SXT)

Carolyn Larabell

University of California San Francisco
Lawrence Berkeley National Lab



National Center for X-ray Tomography

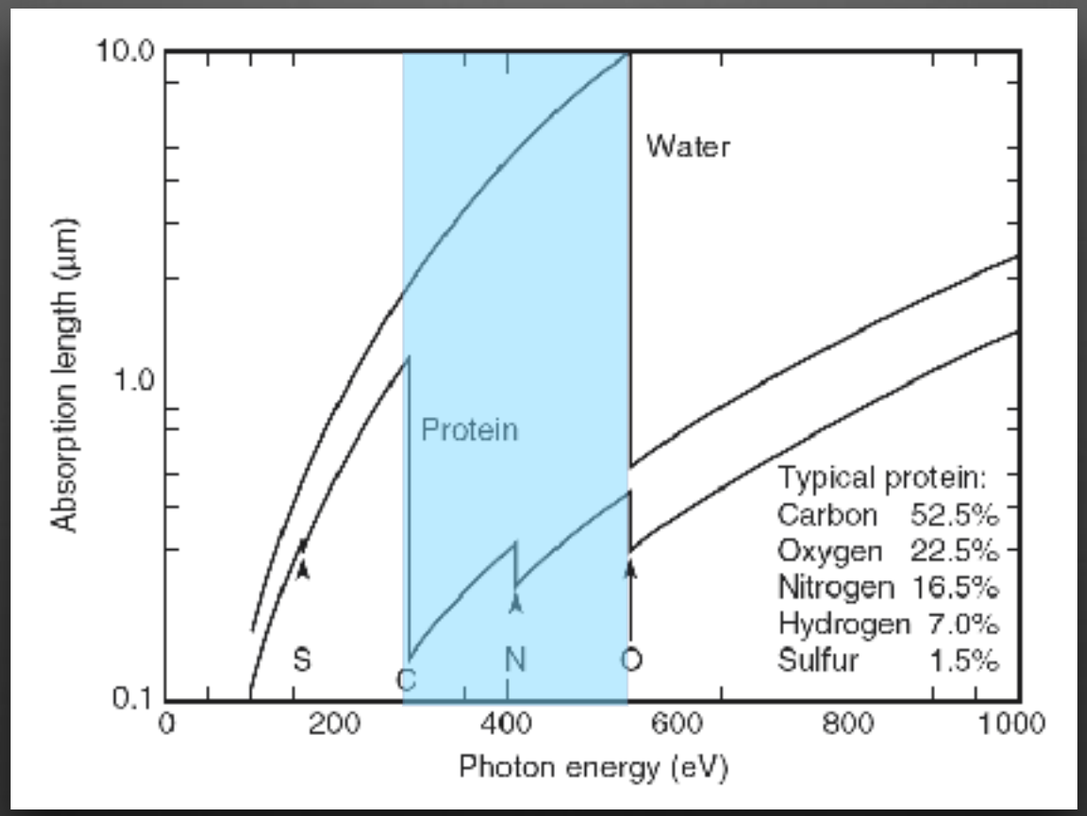
Supported by NIH-NIGMS and DOE-BER

Soft X-ray Tomography (SXT)

- Imaging whole, hydrated cells in the native state
- No fixatives, no stains
- Cryo-immobilized
- 50 nm isotropic resolution (not limit; source is 2.4 nm)
- See molecules using correlated fluorescence and x-ray tomography

Contrast: Imaging in the ‘water window’

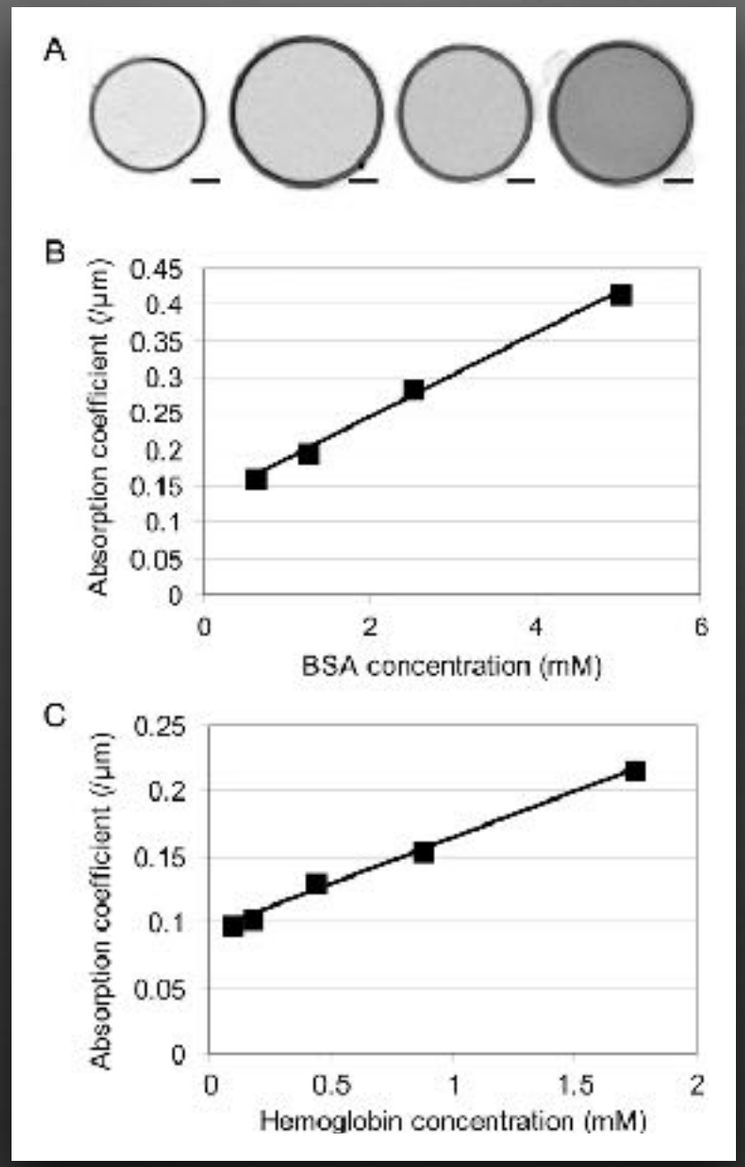
Between K shell absorption edges of C (284 eV) & O_2 (543 eV)



$\lambda = 2.4$ nm

Contrast: Imaging in the ‘water window’

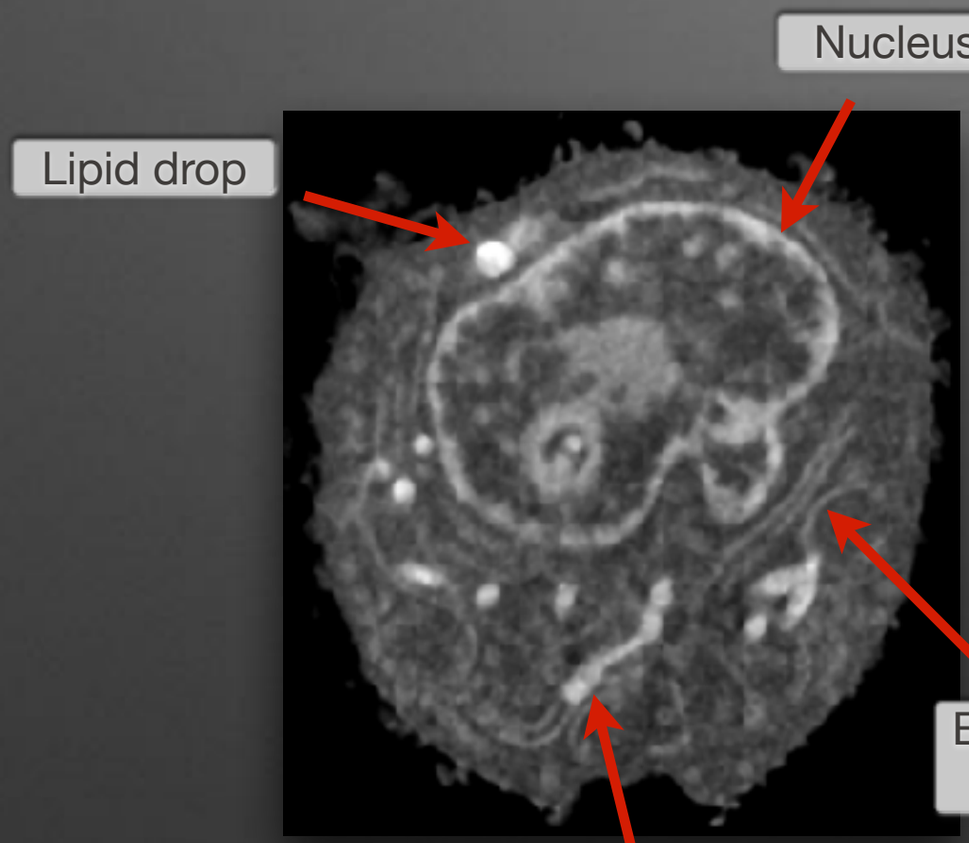
Absorption is linear with thickness & concentration



Hanssen et al (2012). *J. Struct. Biol.* 177, 224-232

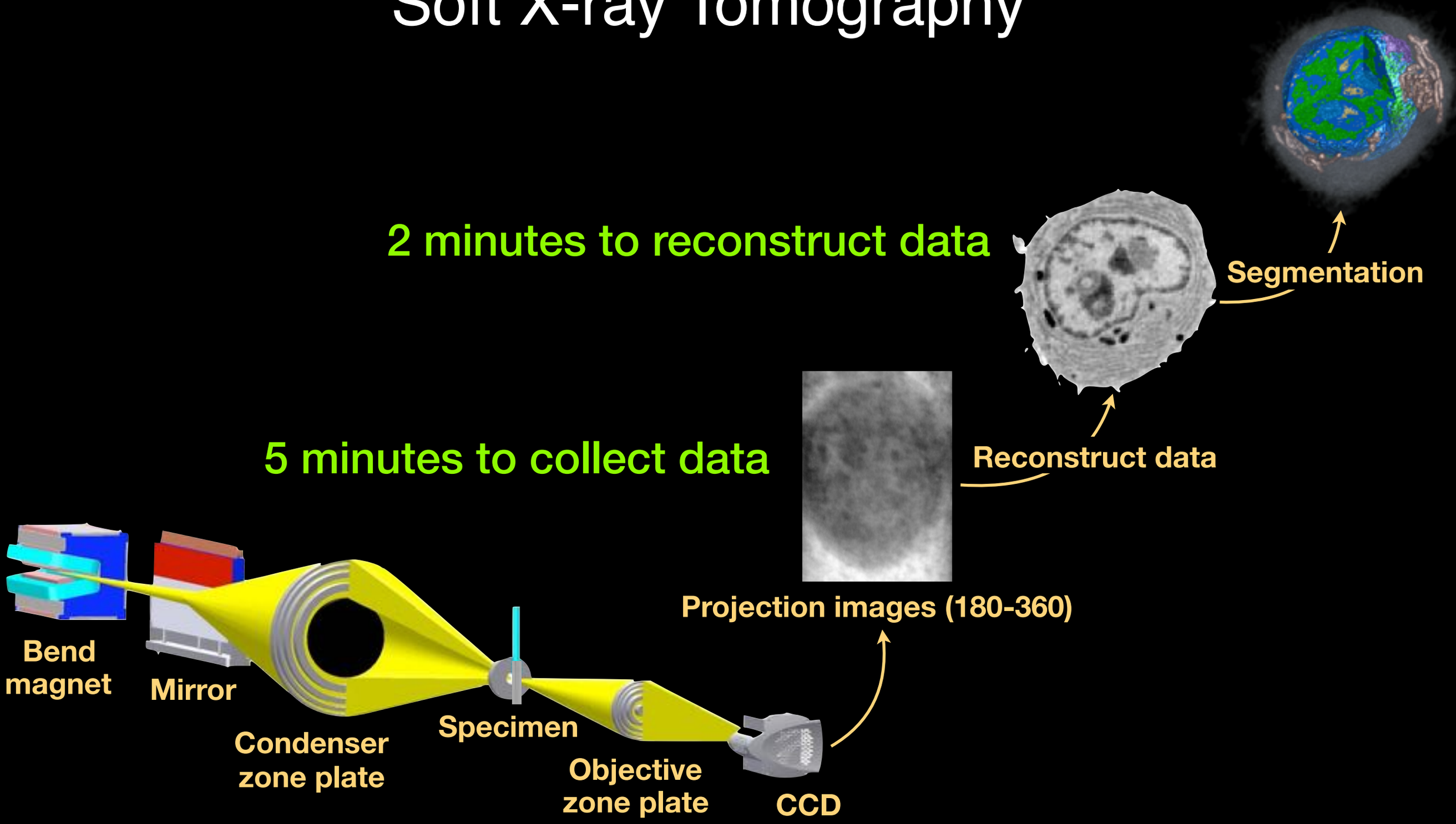
Contrast: Imaging in the ‘water window’

Absorption is linear with thickness & concentration



LAC Values (μm^{-1})

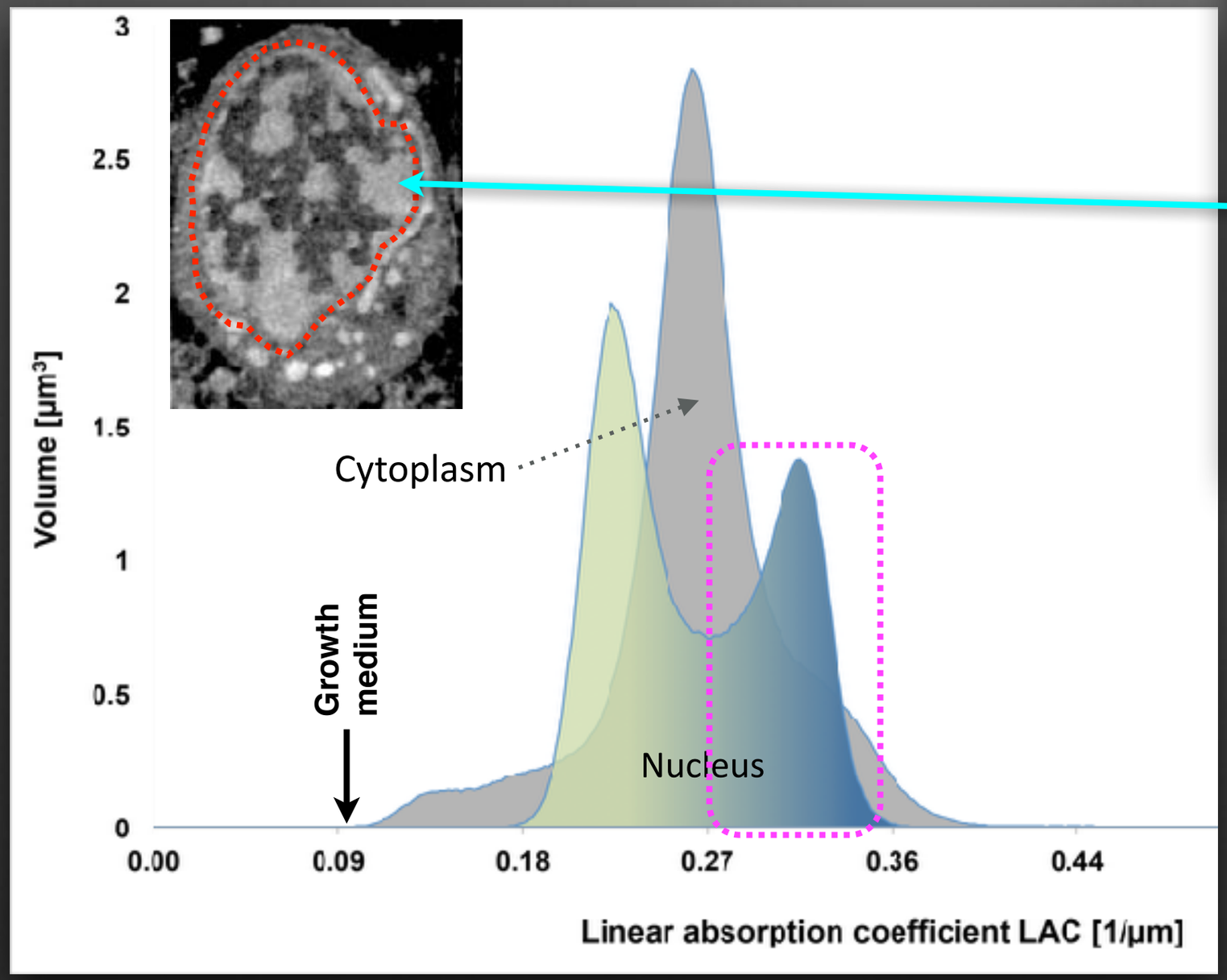
Soft X-ray Tomography

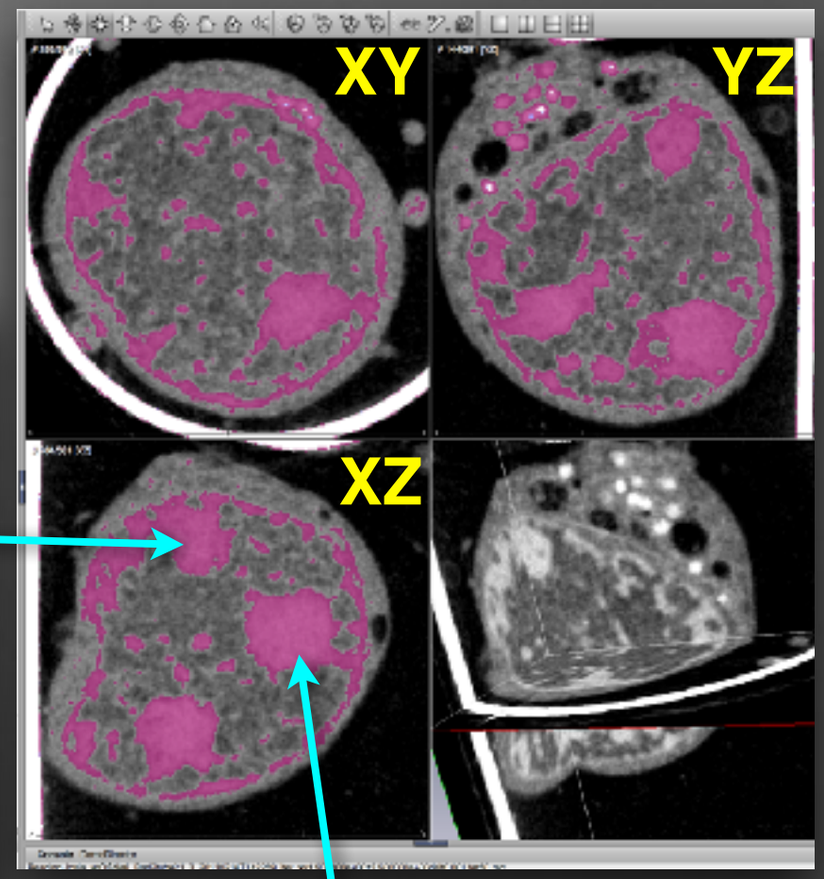


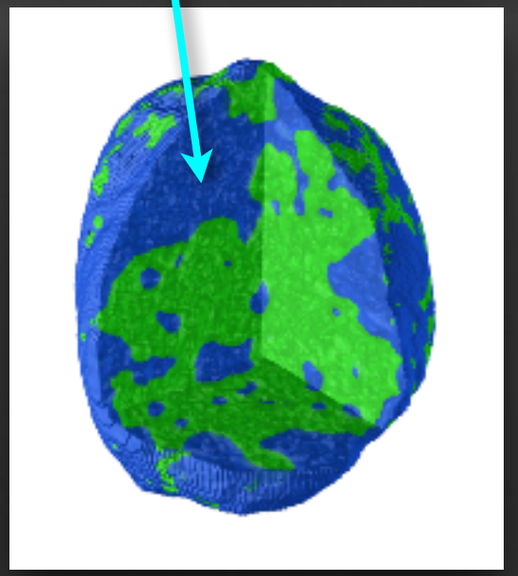
- Whole, hydrated cells in near-native state (cryo-immobilized)
- Natural, quantitative contrast; absorption of x-rays linear

Segmenting structures

Plot histogram of all voxels



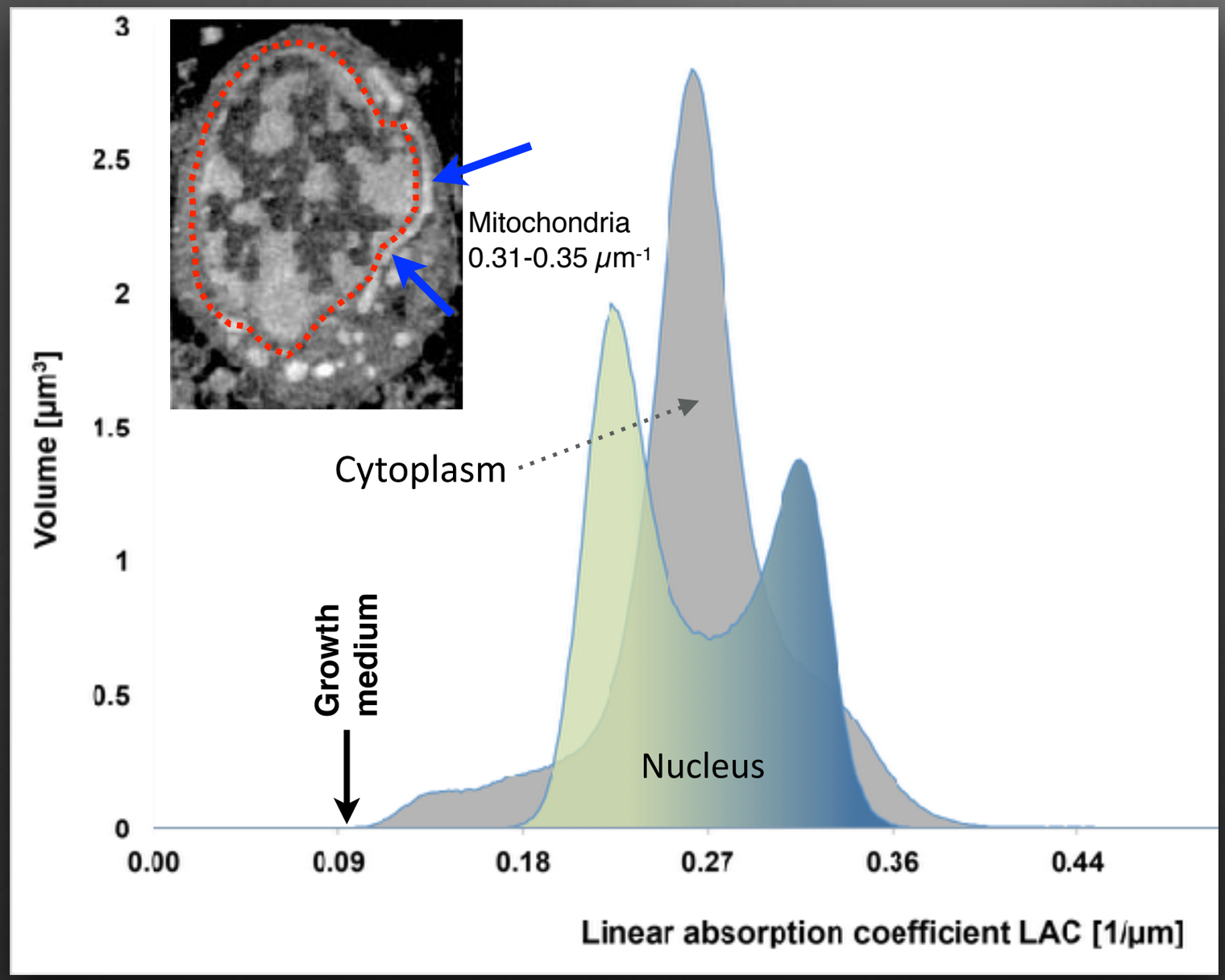


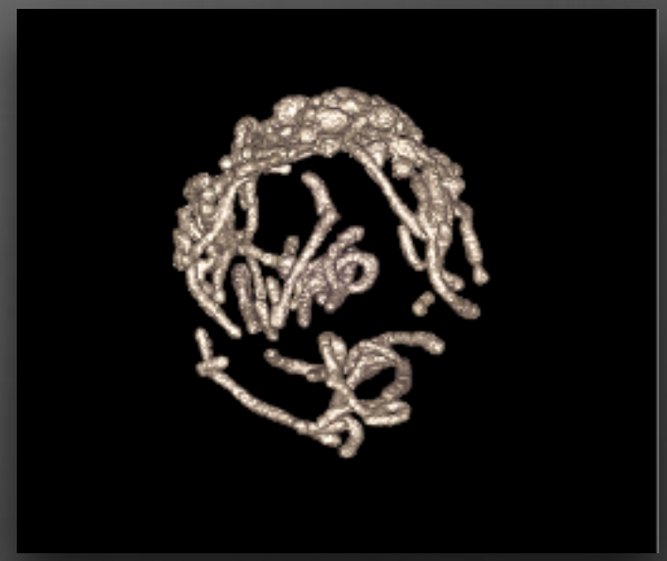


Le Gros et al. (2016) Cell Reports. 17(8), 2125-2136

Segmenting structures

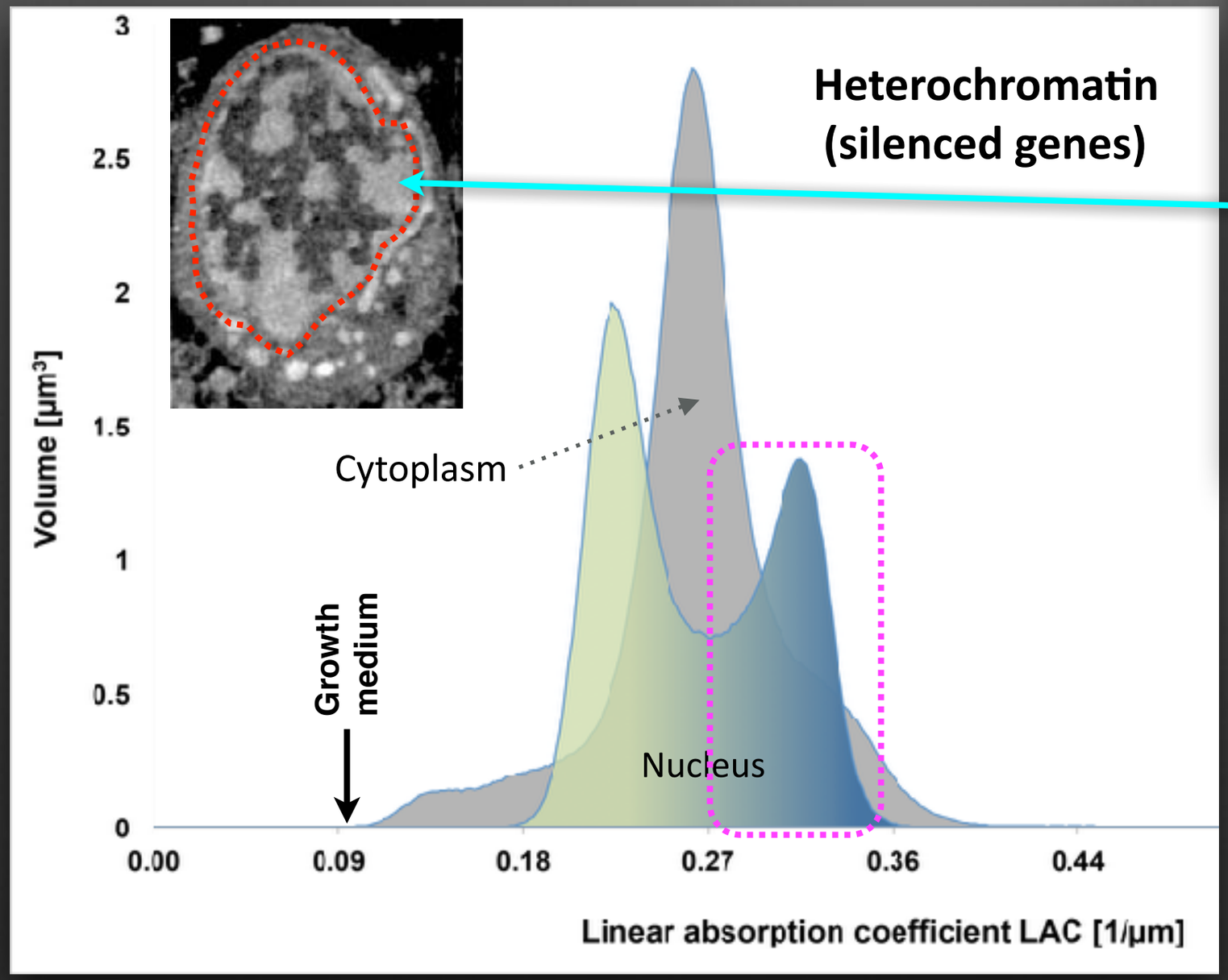
Plot histogram of all voxels

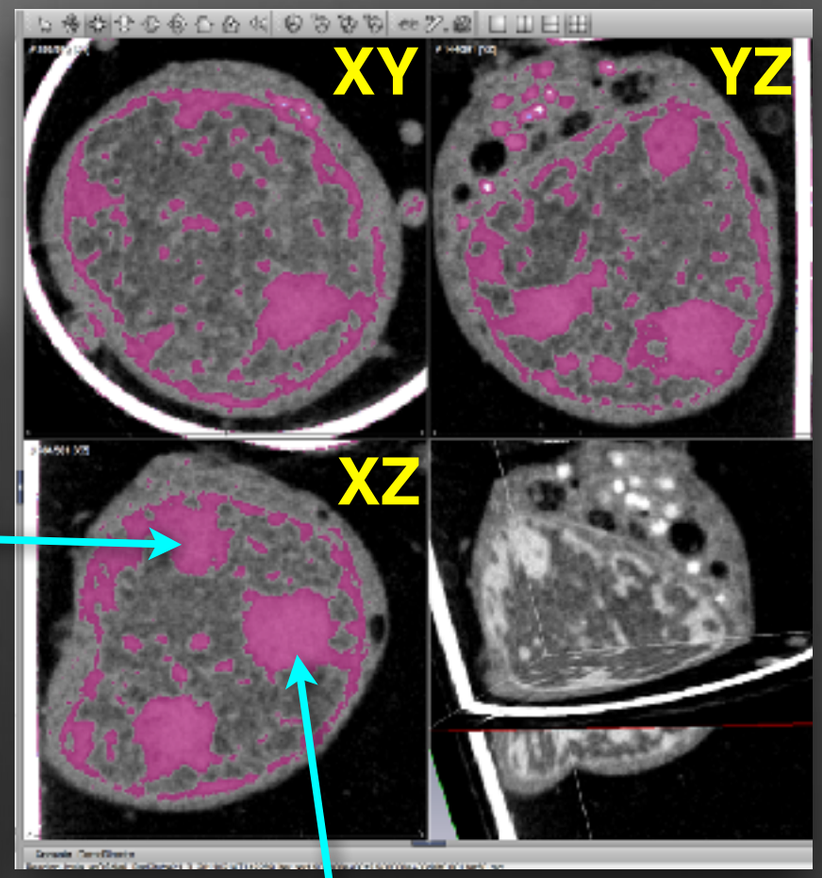


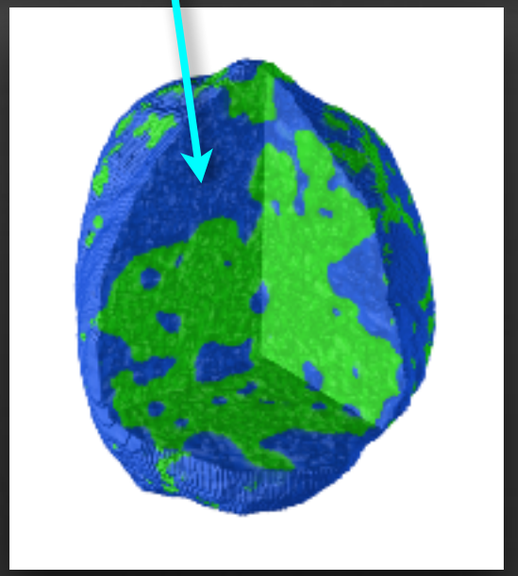


Segmenting structures

Plot histogram of all voxels





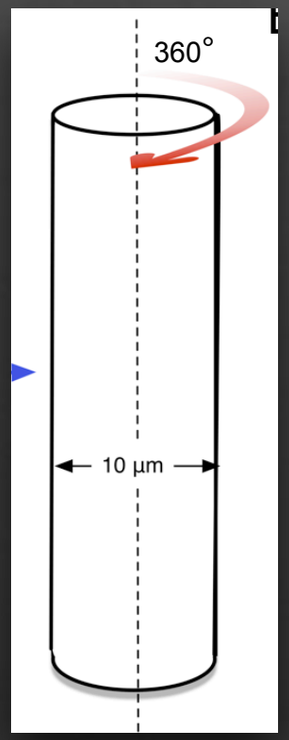


Le Gros et al. (2016) Cell Reports. 17(8), 2125-2136

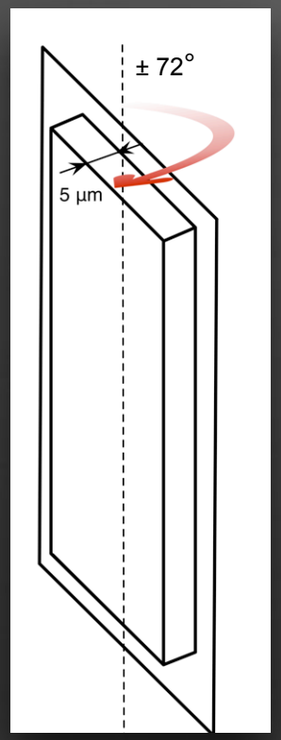
Isotropic resolution

Full rotation vs. limited tilt

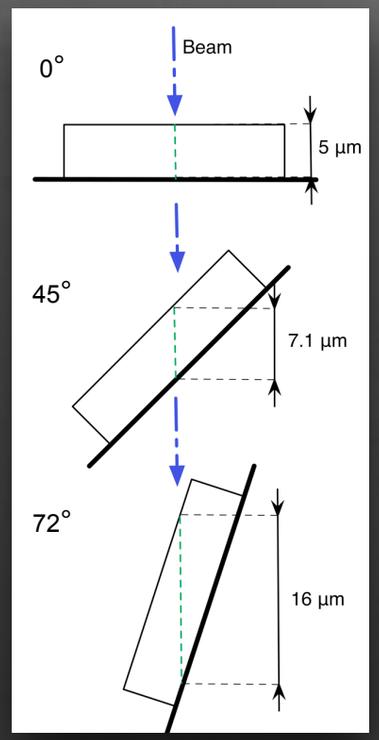
± 90°



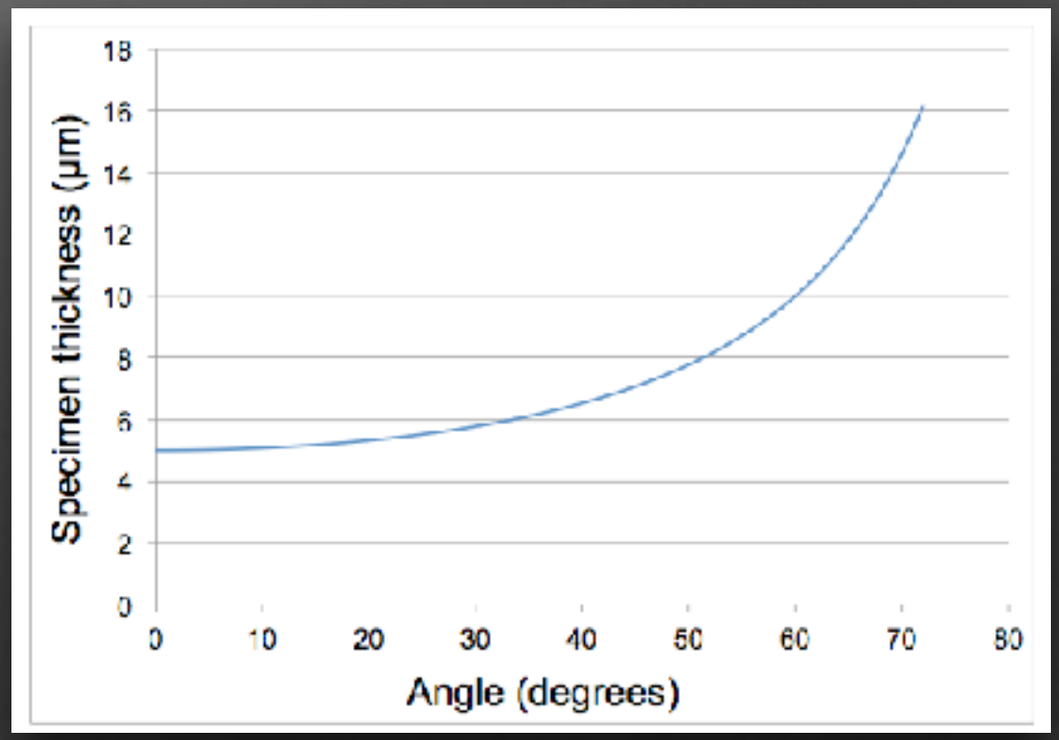
± 75°



16 µm thick
at ± 75°

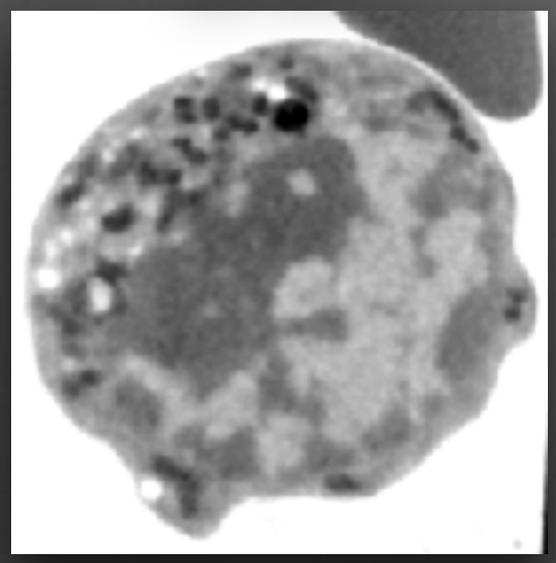
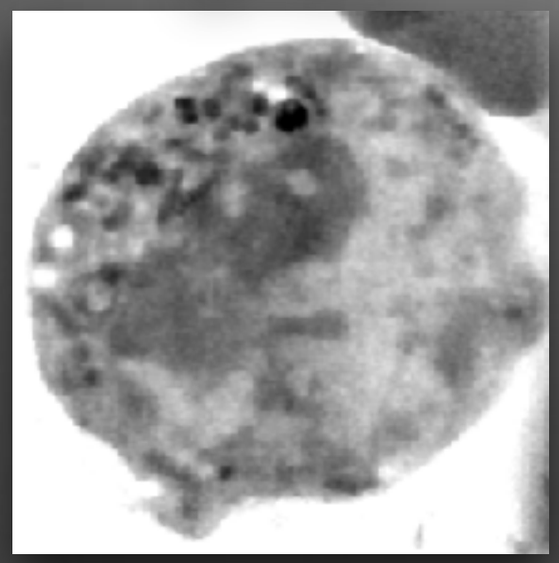
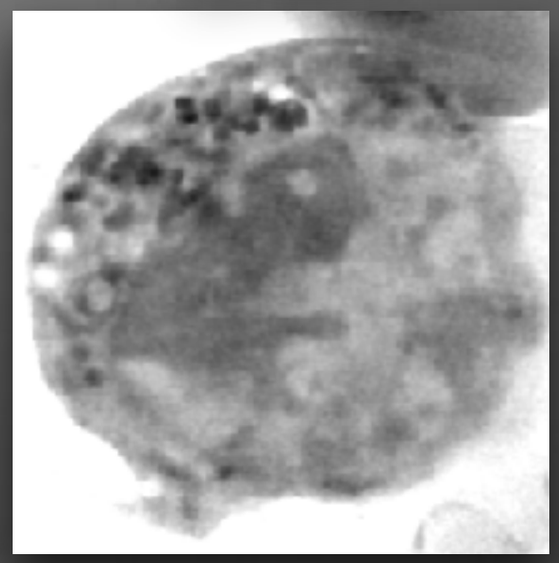
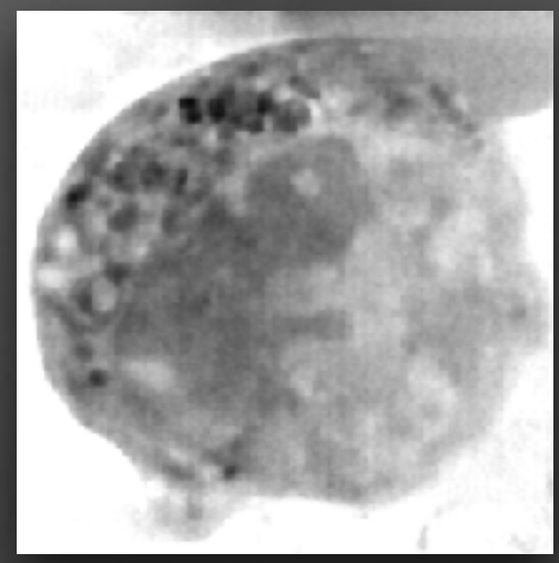
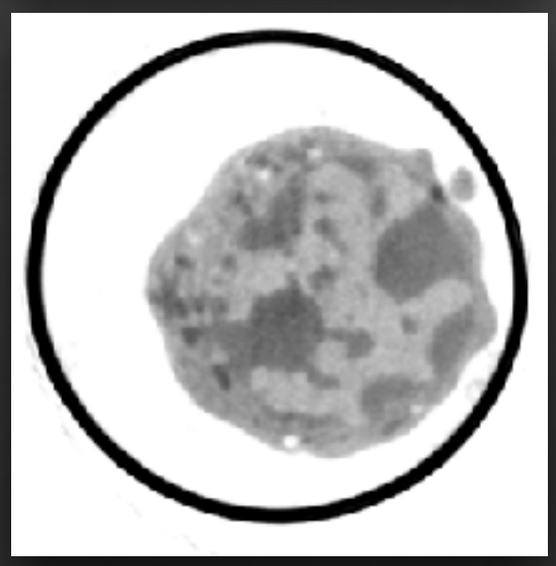
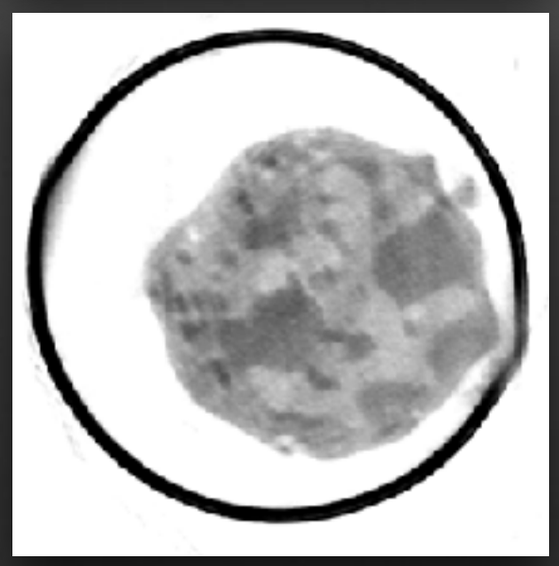
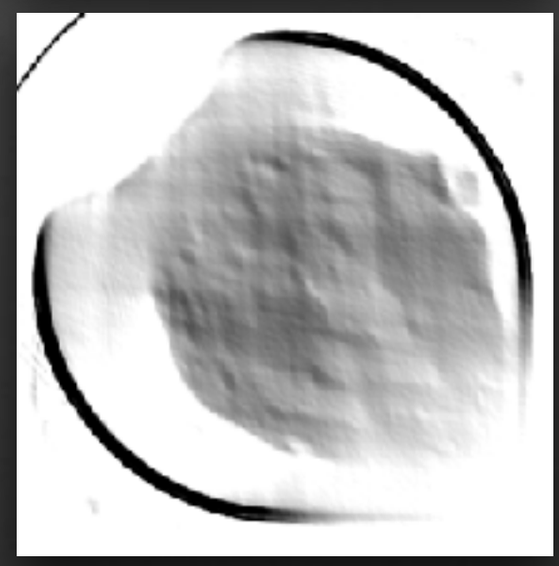


Specimen thickness
increases with tilt



Cinquin et al. (2014). J Cellular Biochemistry. 115:2009-216

Full rotation vs. limited tilt

	180° rotation (± 90°)	150° rotation (± 75°)	120° rotation (± 60°)	90° rotation (± 40°)
X-Y				
X-Z				

Cinquin et al. (2014). J Cellular Biochemistry. 115:2009-216

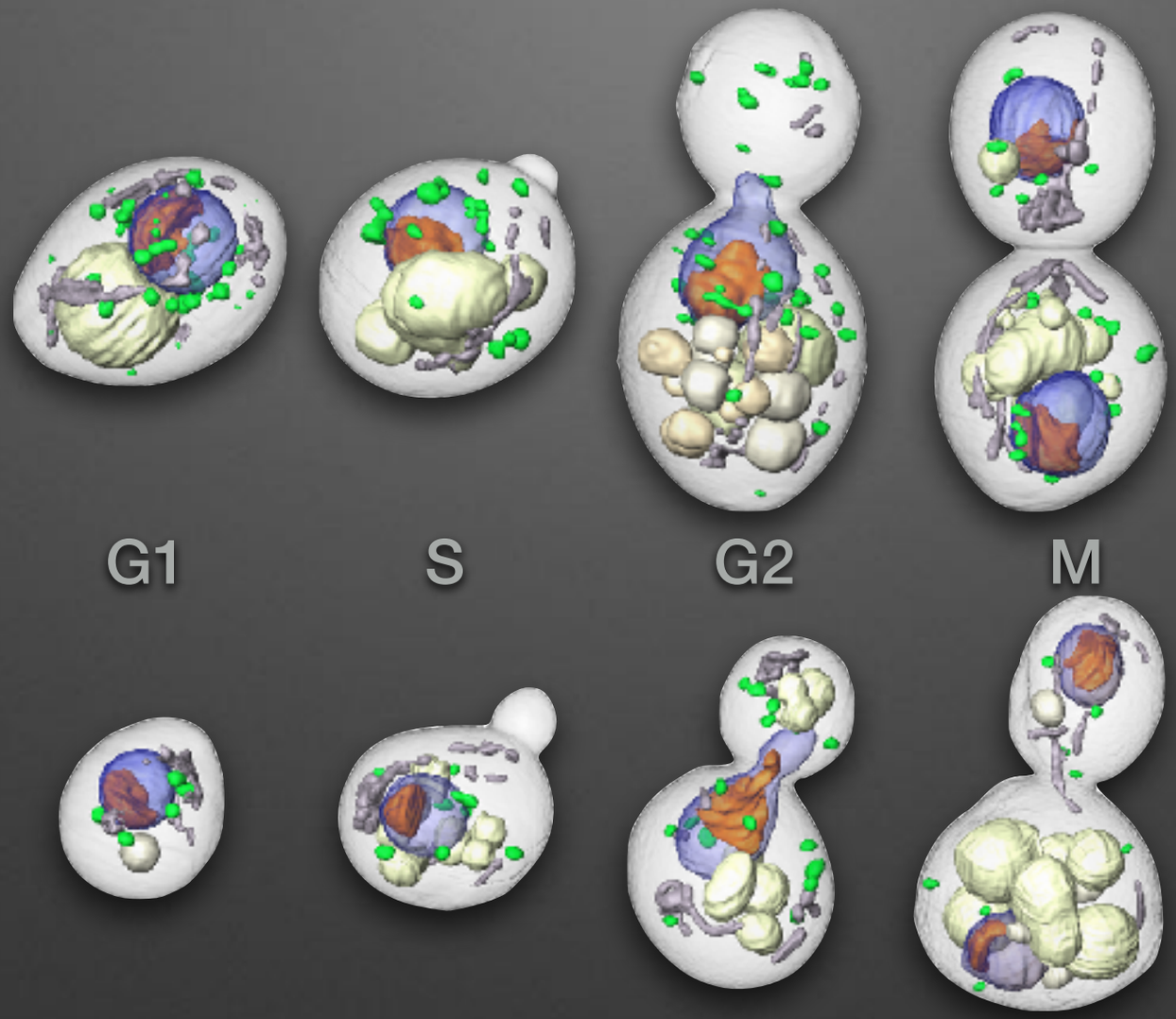
Phenotypic consequences of knocking out a gene

Structural organization of *S. cerevisiae*

Haploid

Diploid

G1 S G2 M

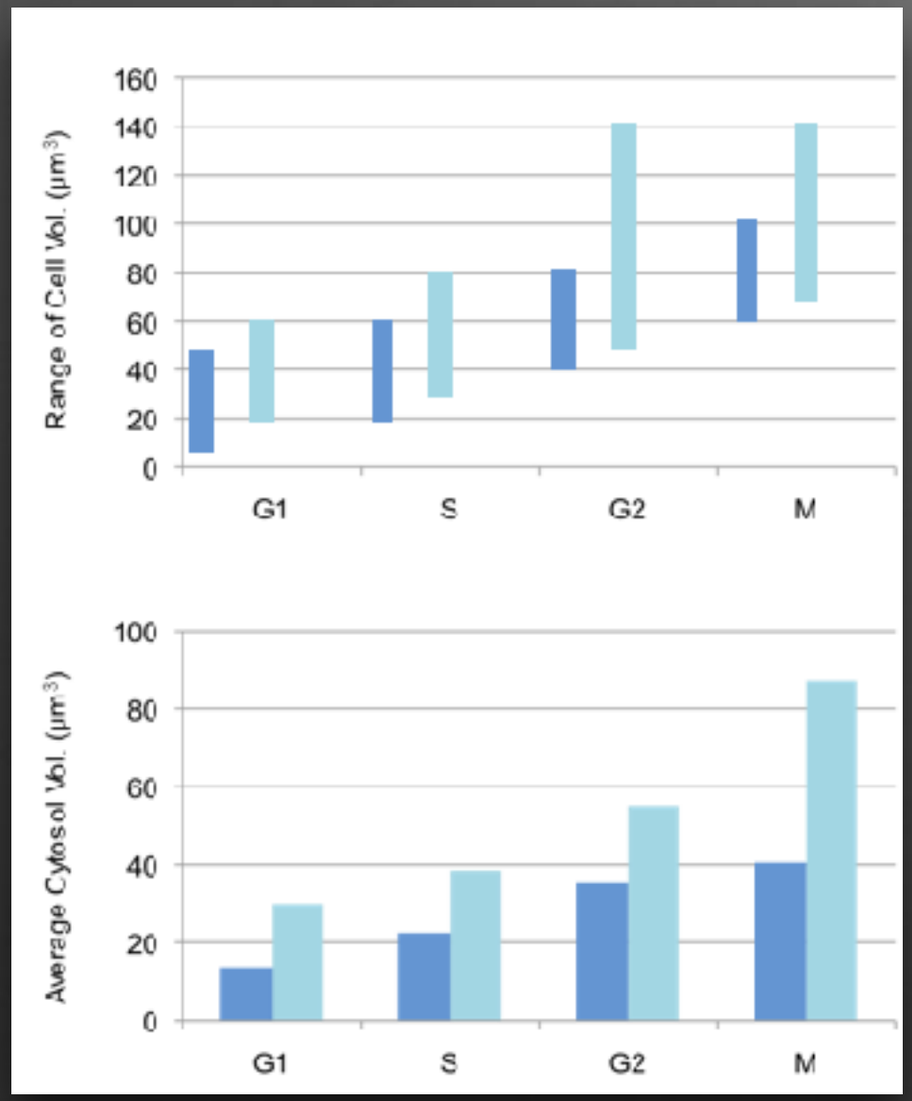


Nucleus
Nucleolus
Mitochondria
Vacuole
Lipid

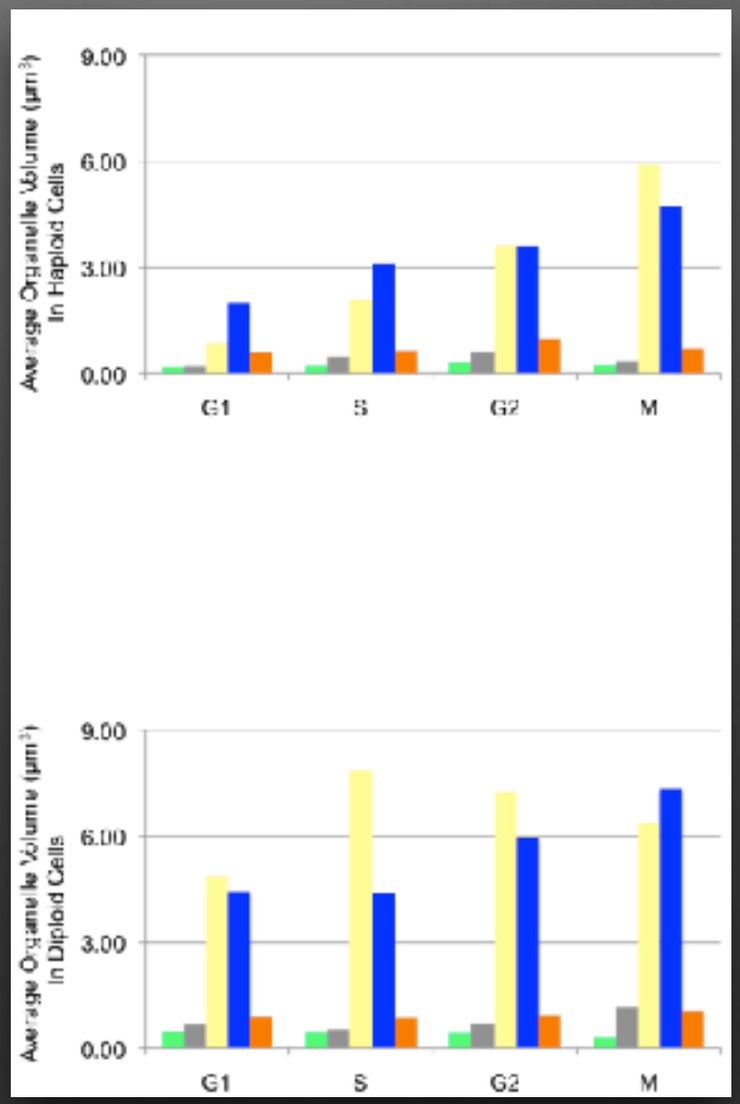
Uchida et al. (2011) Yeast. 28, 227-236

Quantitative analysis of *S. cerevisiae*

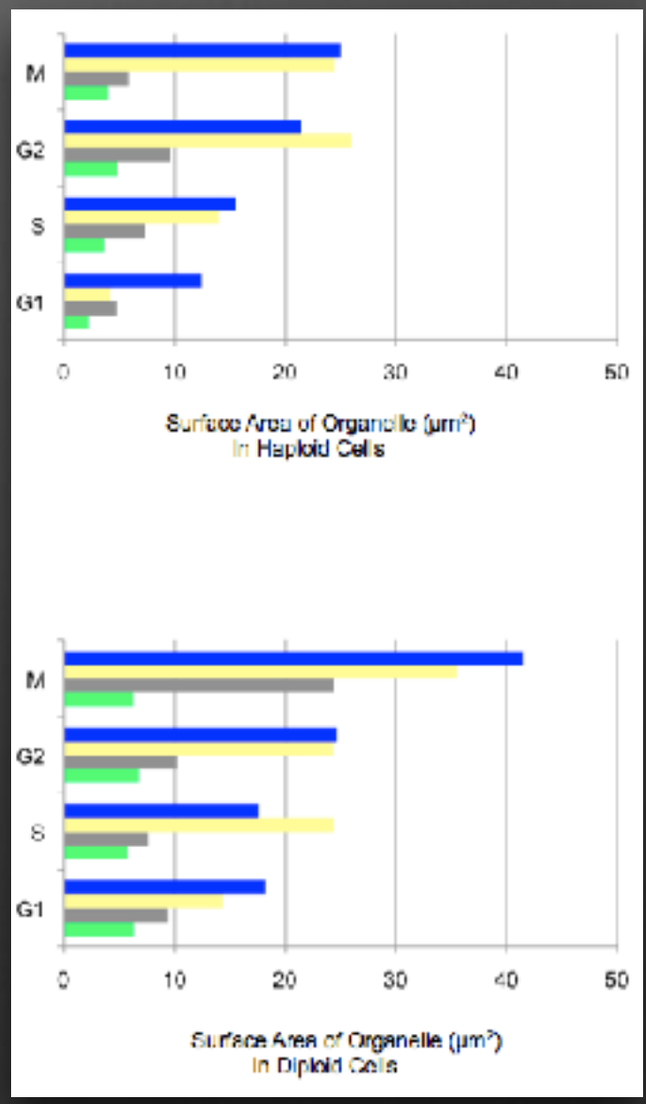
Cell volume



Organelle volume



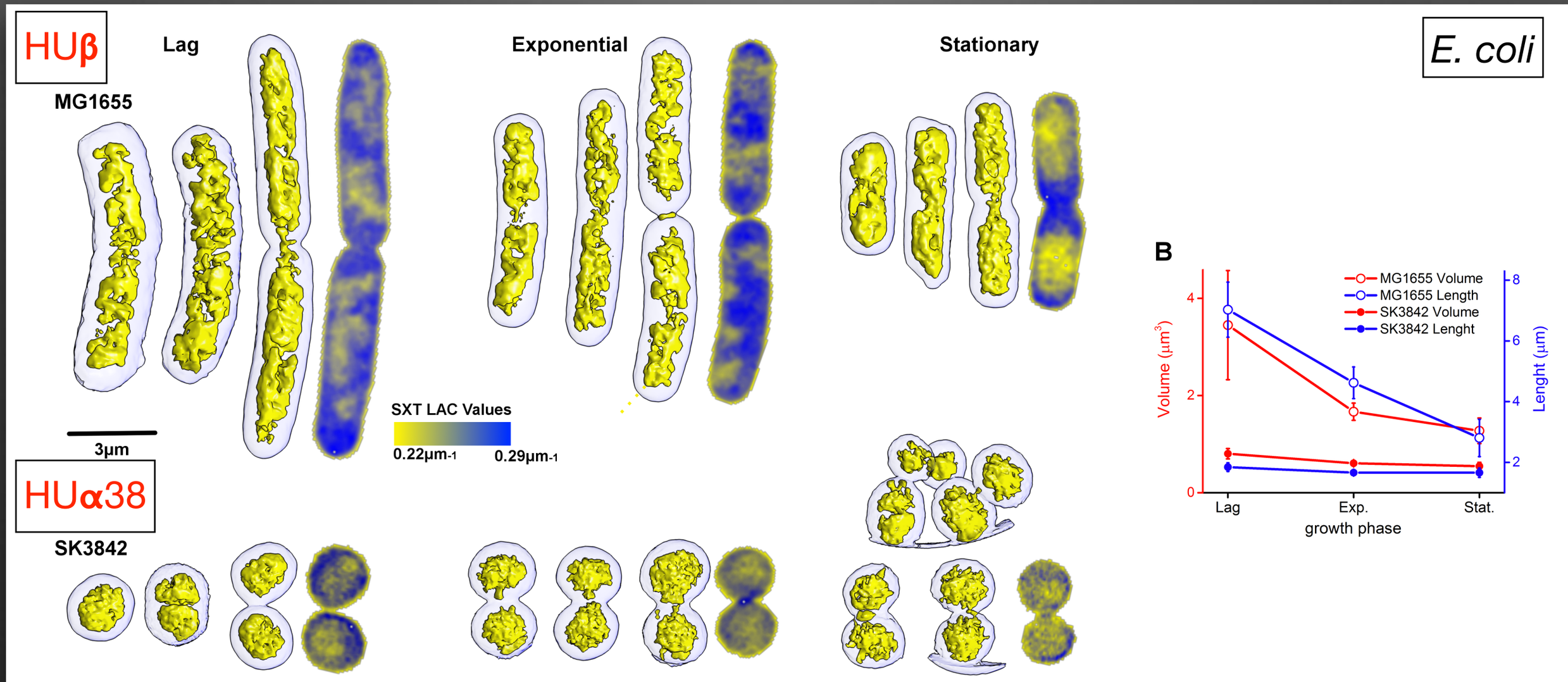
Organelle surface area



Uchida et al. (2011) Yeast. 28, 227-236

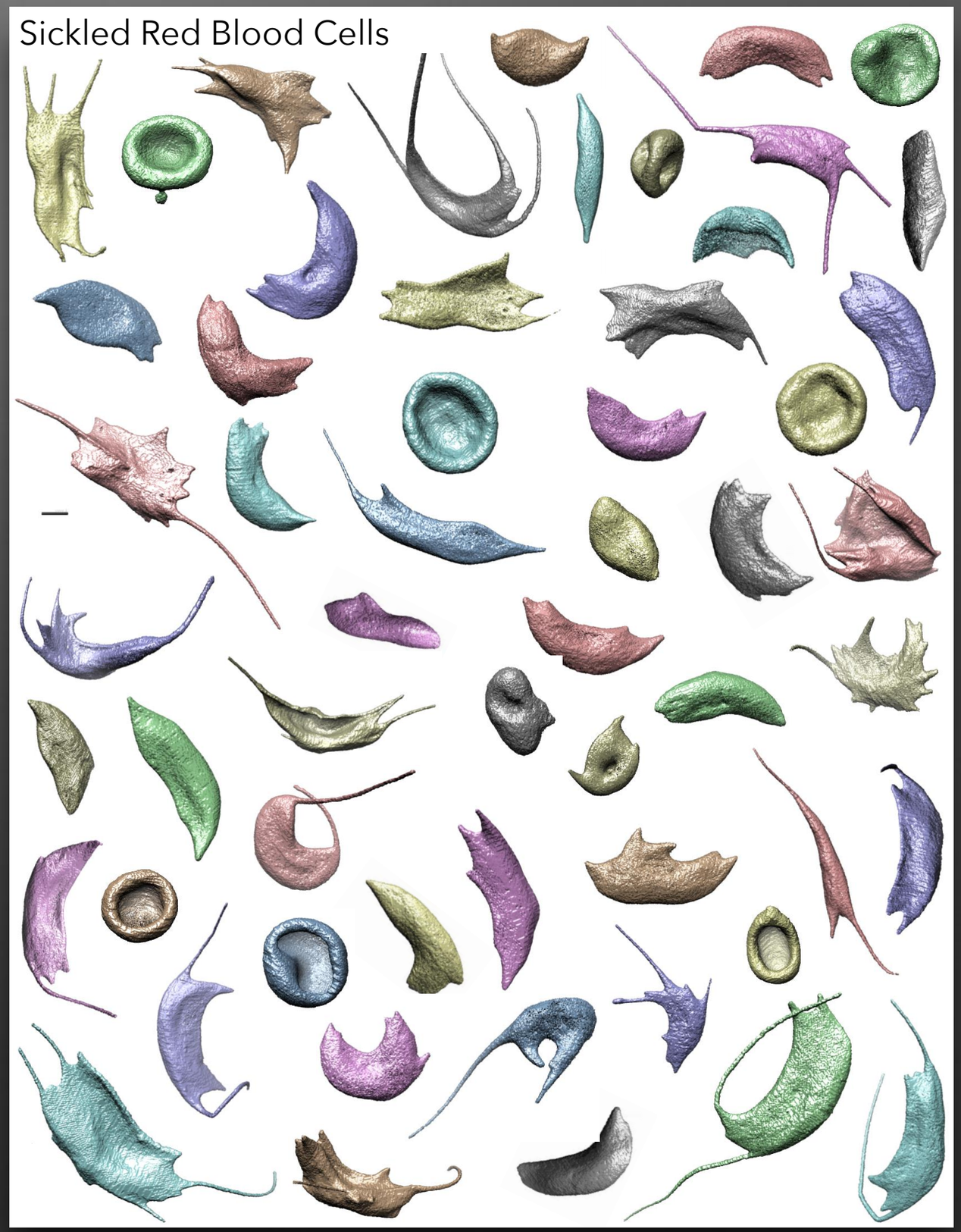
HU multimerization shift controls nucleoid compaction

HU - histone like protein



Hammel et al., (2016) Science Advances. doi: 10.1126/sciadv.1600650.

Testing drugs for sickle cell disease



Wah Chiu

Michele Darrow

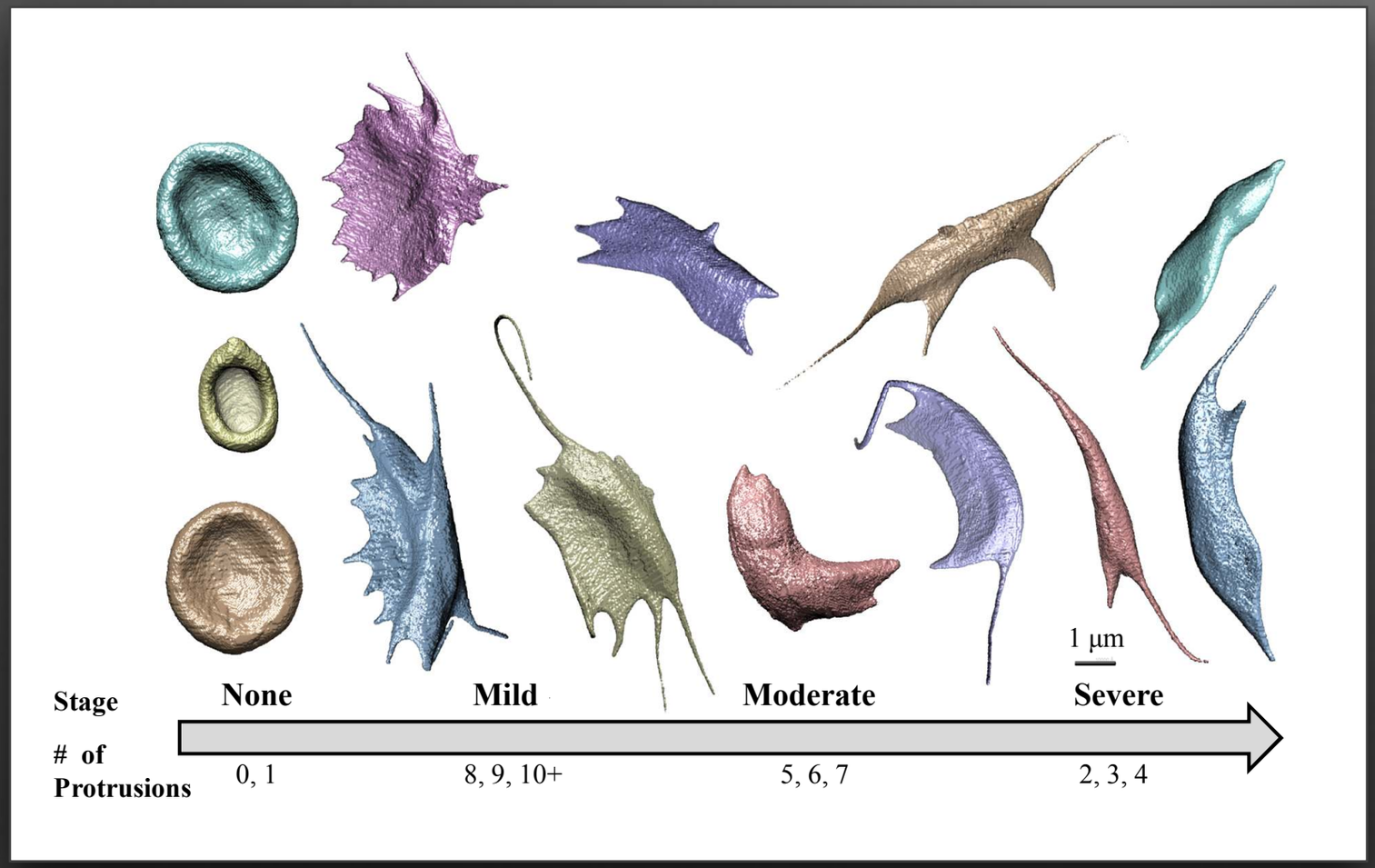
Baylor College of Medicine

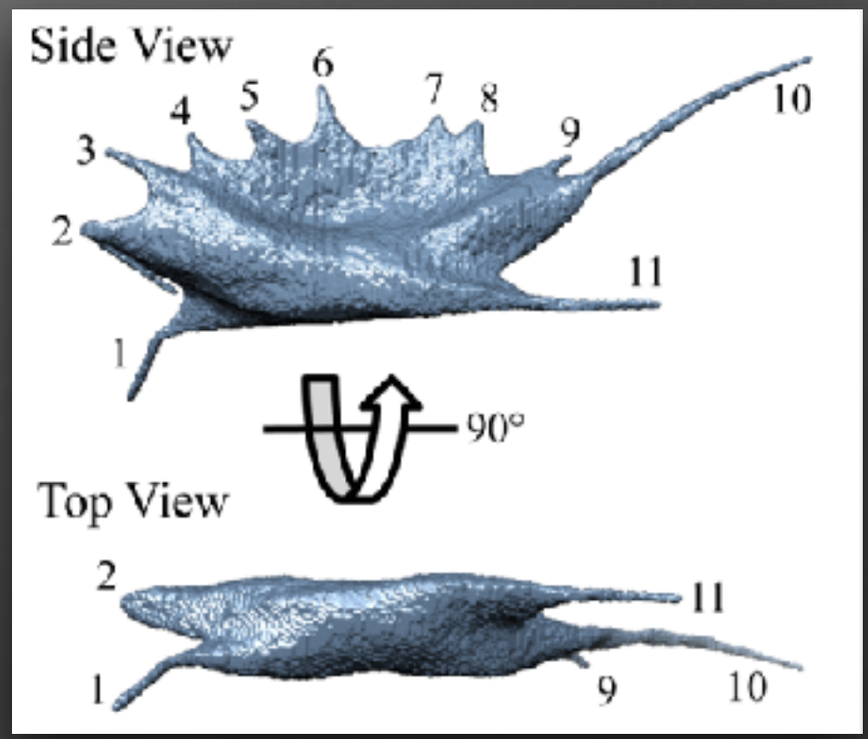
Yang Xia

University of Texas Health Science Center

Darrow et al. (2016) J. Cell Science. 129, 3511-3517

Testing efficacy of drugs to reverse sickling



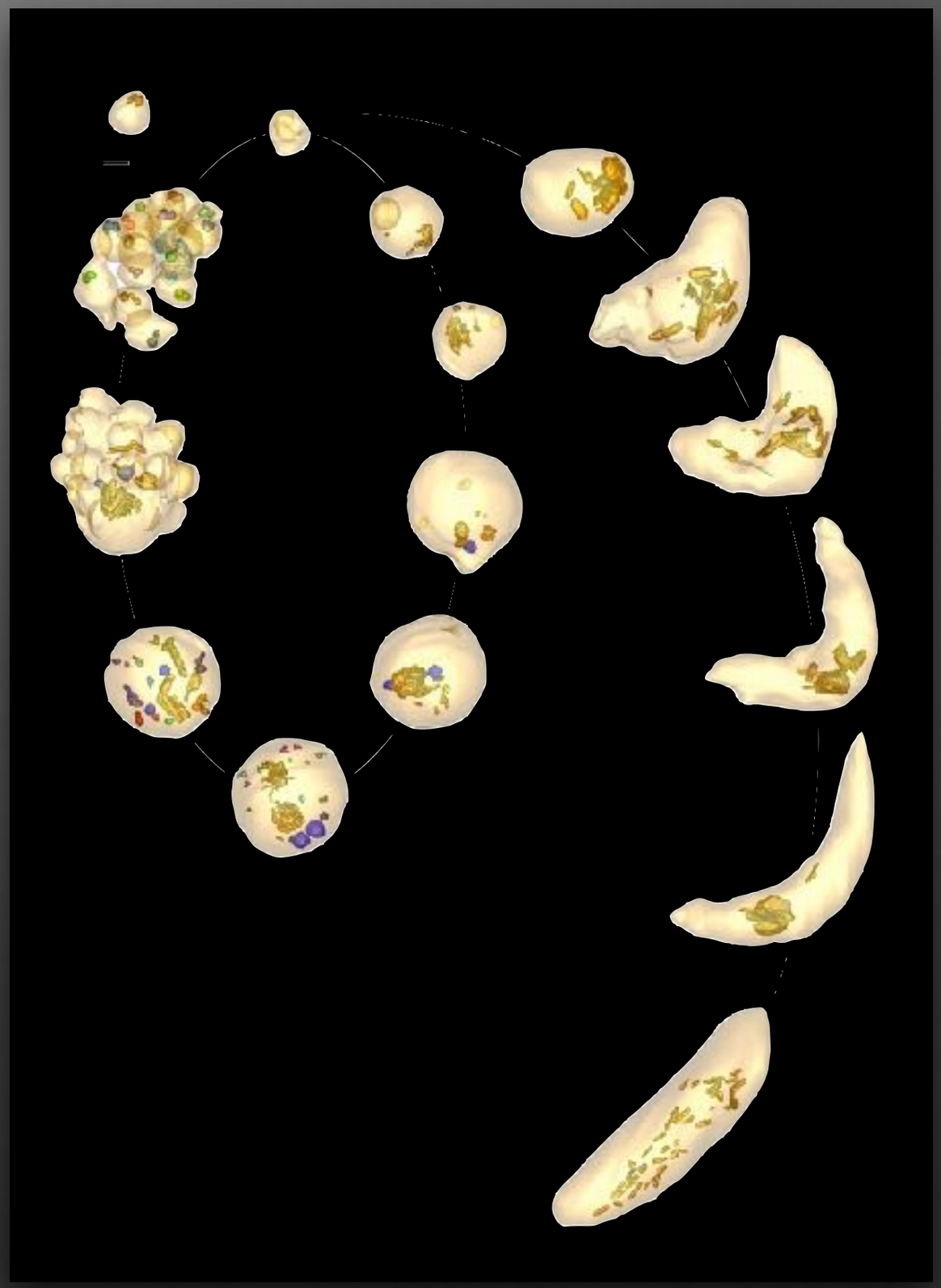


Darrow et al. (2016) J. Cell Science. 129, 3511-3517

Malaria-infected red blood cells

Leann Tilley
Eric Hanssen

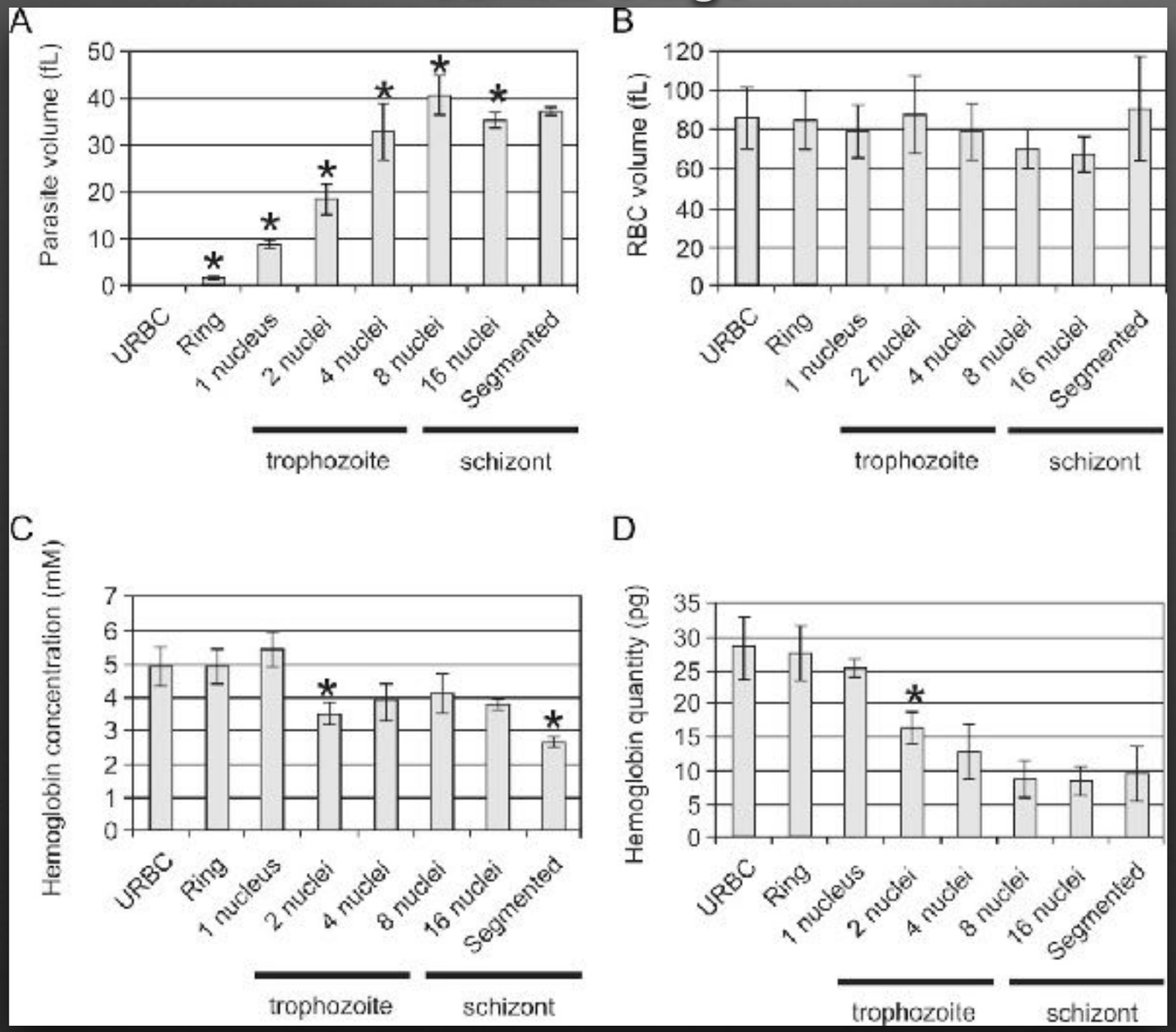
University of Melbourne
Australia

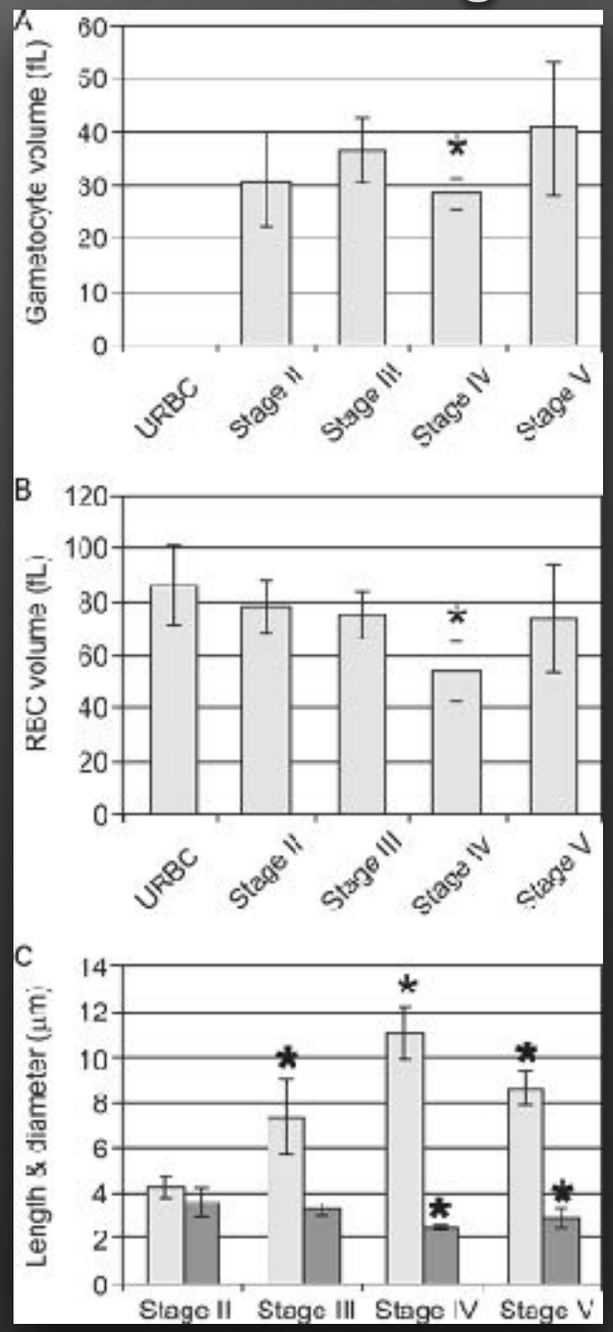


Malaria-infected RBC

Sexual stage

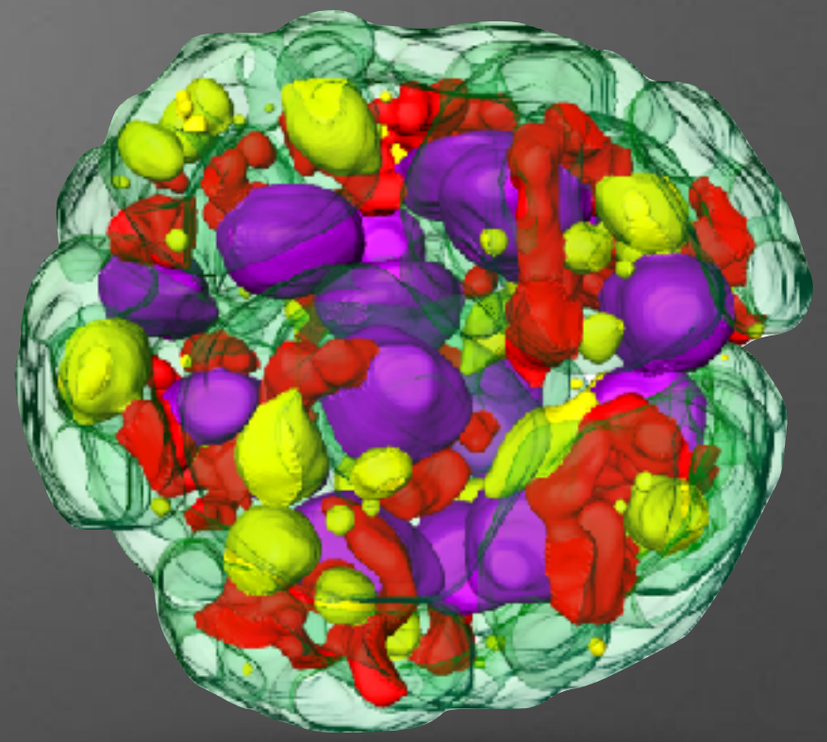
Asexual stage





Hanssen et al. (2012) J. Structural Biol. 177, 224-232

Photosynethisis, Bioenergy

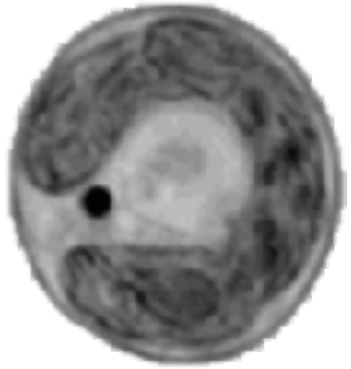


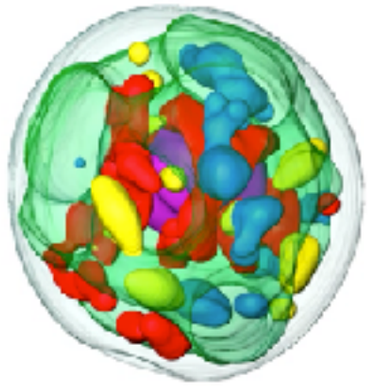
Krishna Niyogi

University of California
Berkeley & HHMI

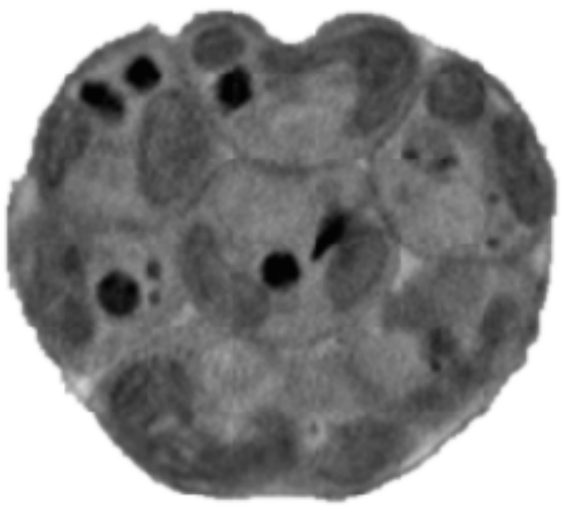
Chromochloris zofingiensis

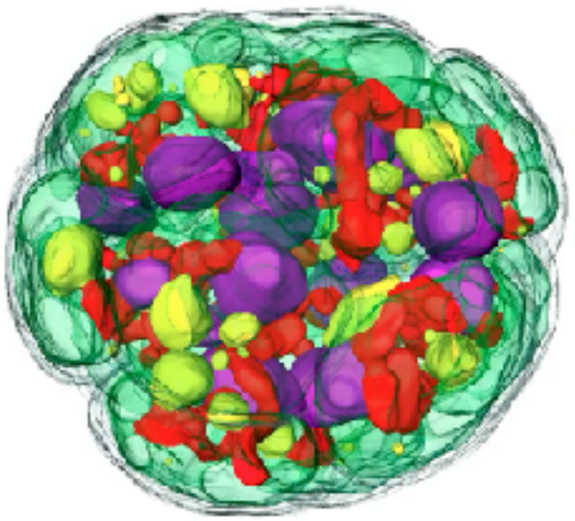
Single cell





16 cells



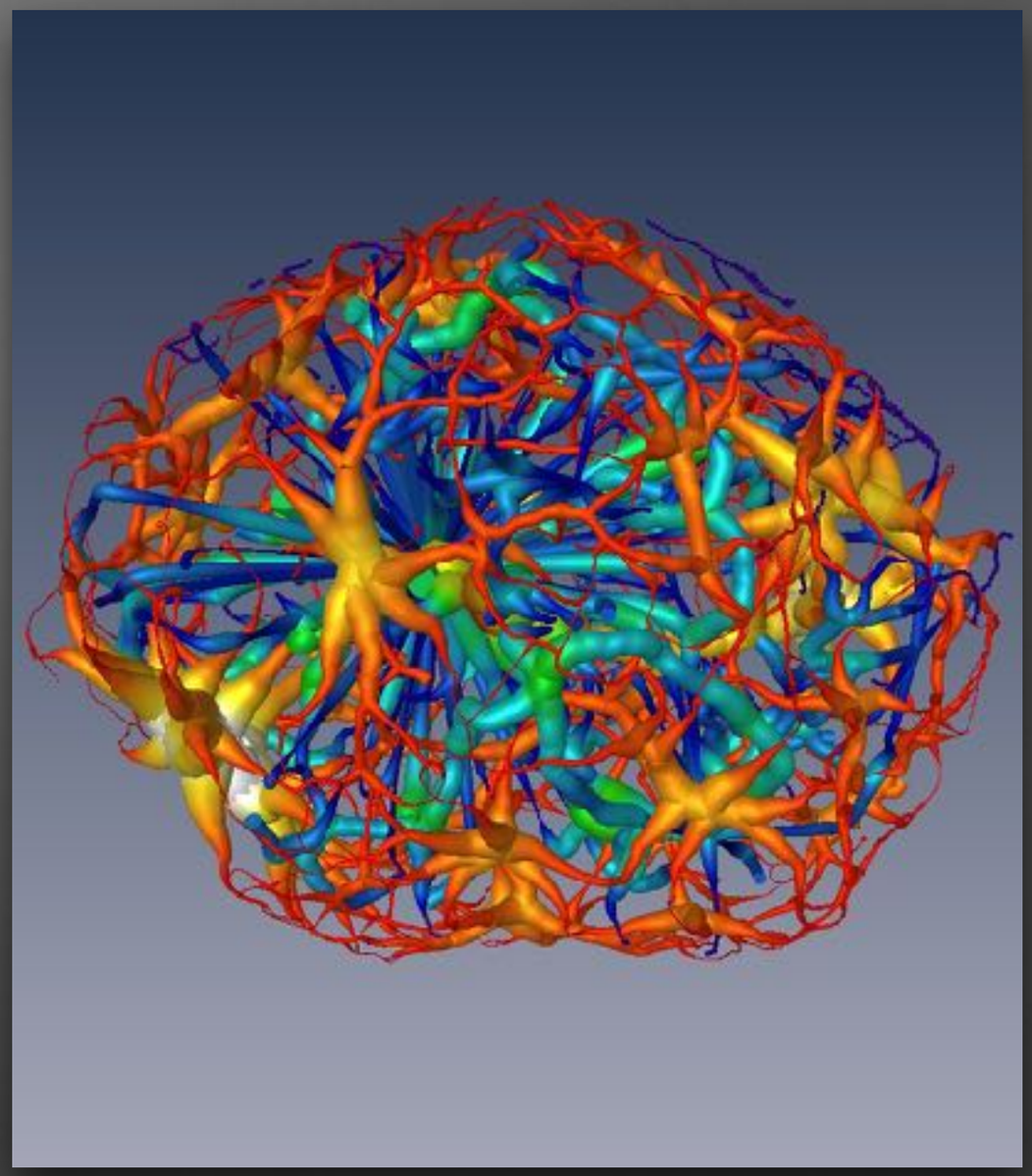


- Chloroplast
- Starch
- Mitochondria
- Nucleus
- Lipid bodies

Roth et al., PNAS, in revision.

Role of nuclear organization in gene expression

Chromatin condensation during neurogenesis



Stavros Lomvardas

Columbia University

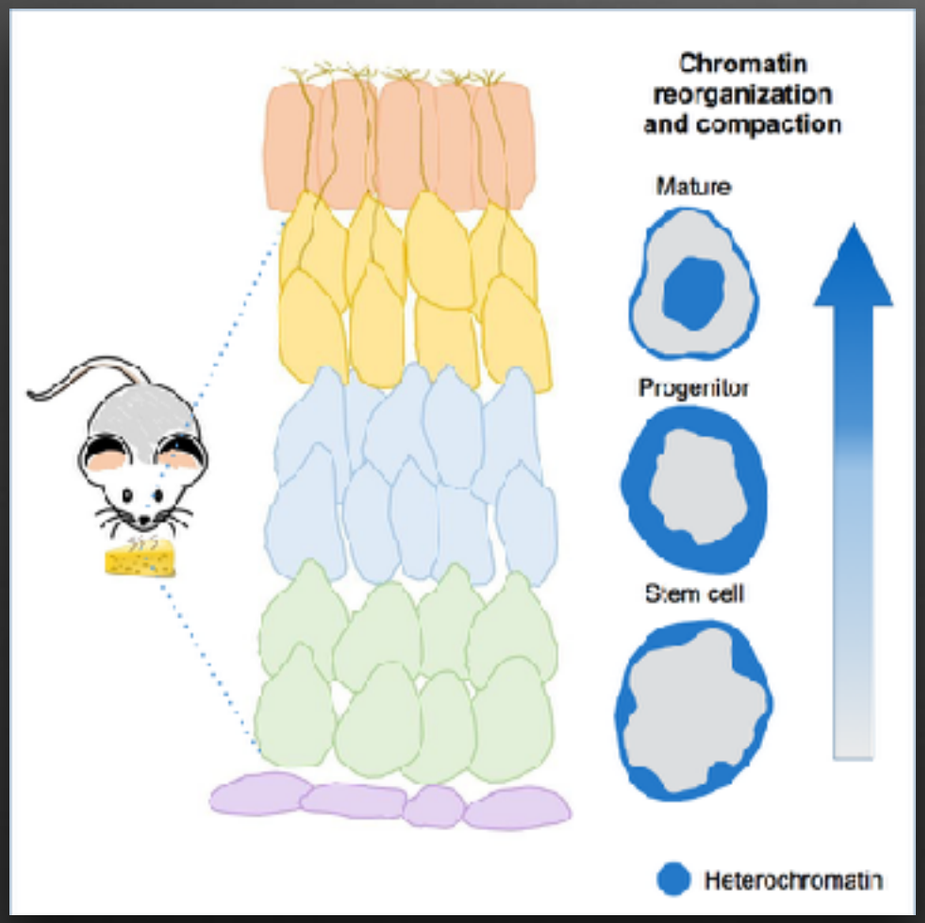
Mark Le Gros & Carolyn Larabell

University of California

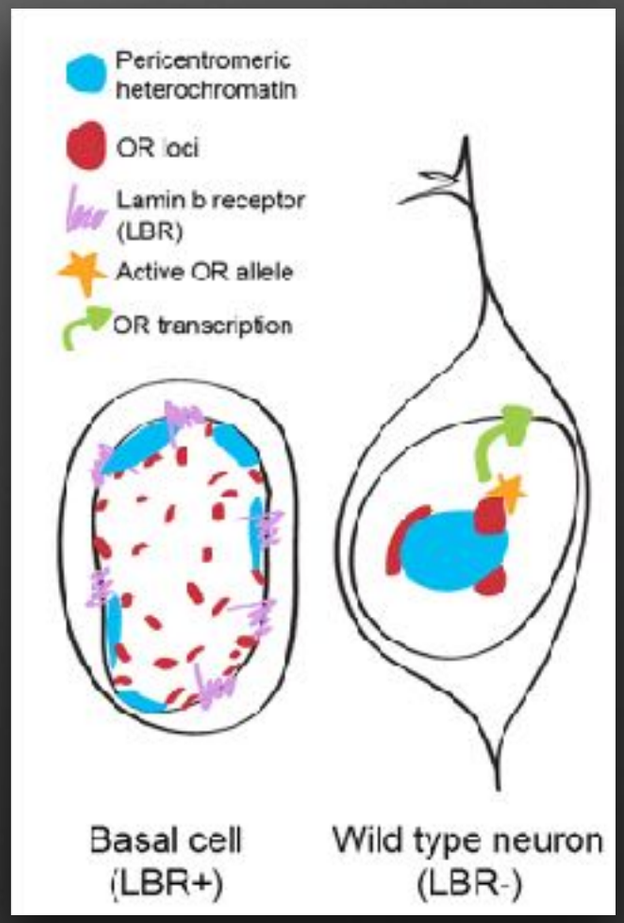
San Francisco

Neurogenesis: from stem cell to neuron

- About 1200 Olfactory Receptor (OR) genes found in 18 mouse chromosomes
- Each neuron transcribes *one* out of ~2400 OR alleles
- Allele selection occurs during neurogenesis



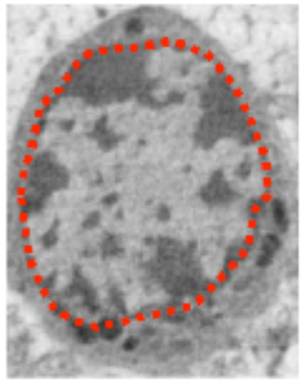
Le Gros et al. (2016) Cell Reports. 17(8), 2125-2136

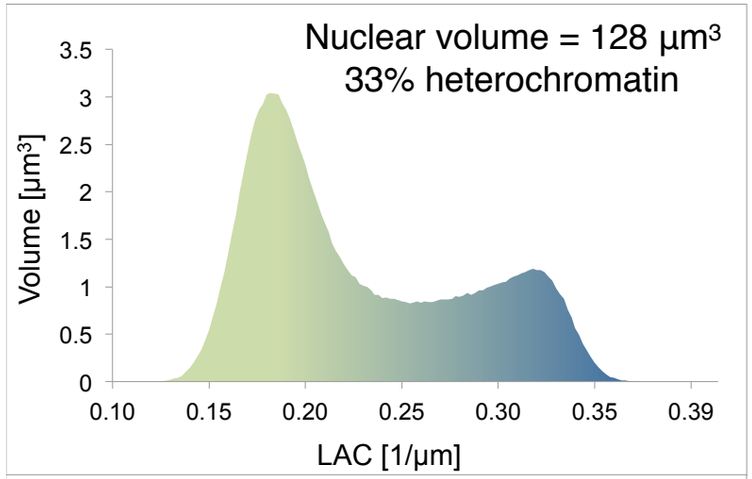


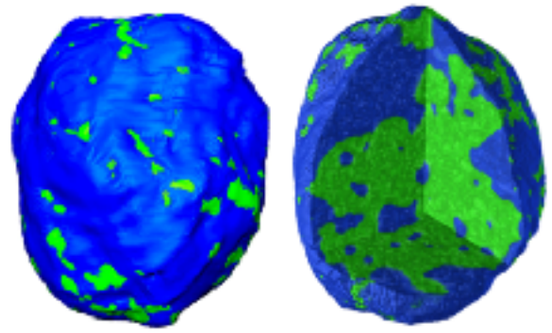
Clowney et al. Cell. 151, 724-737

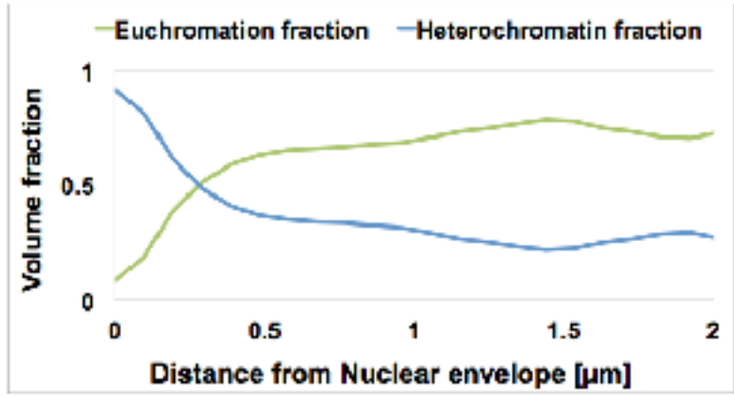
From stem cell to neuron

Stem cell

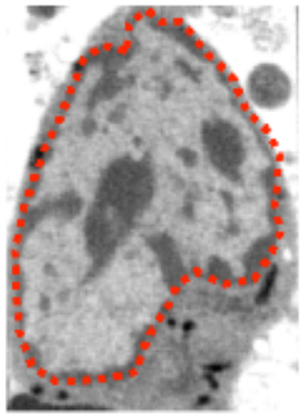


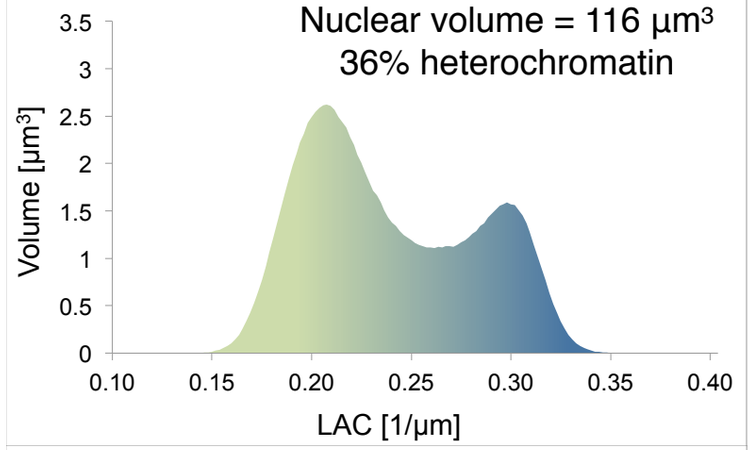


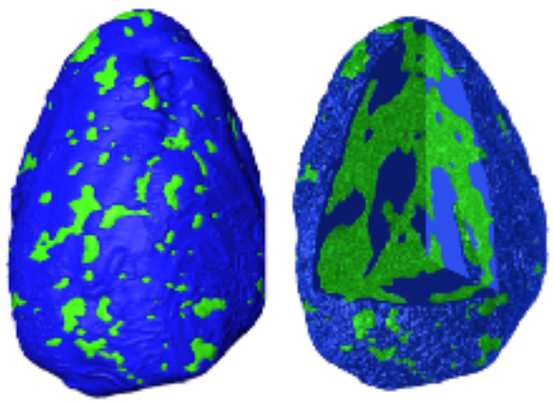


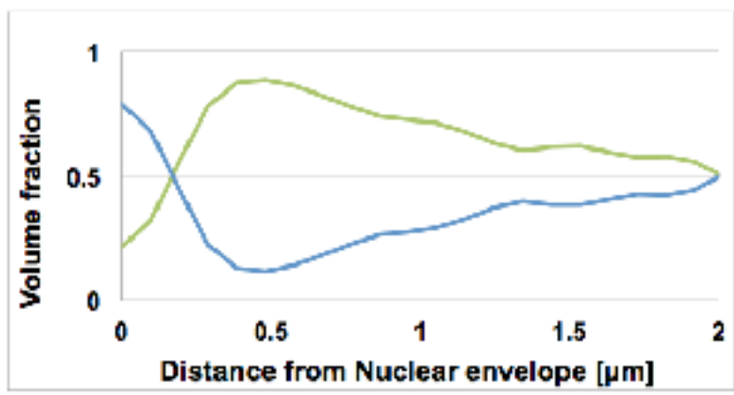


Progenitor

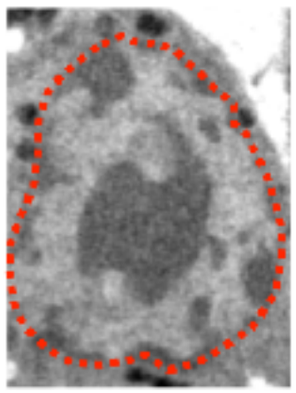


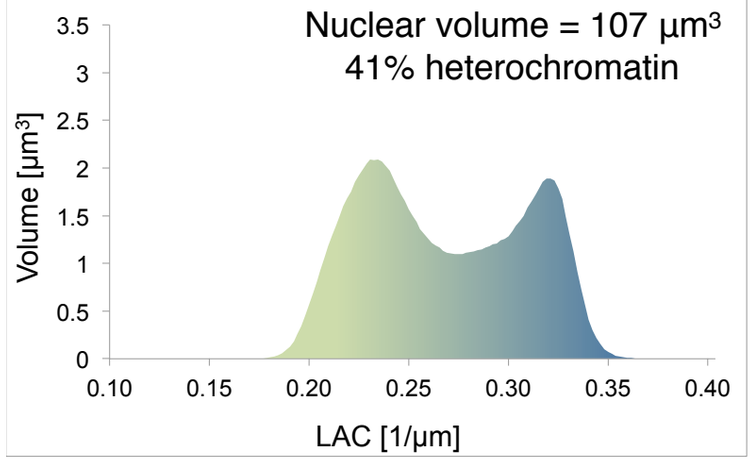


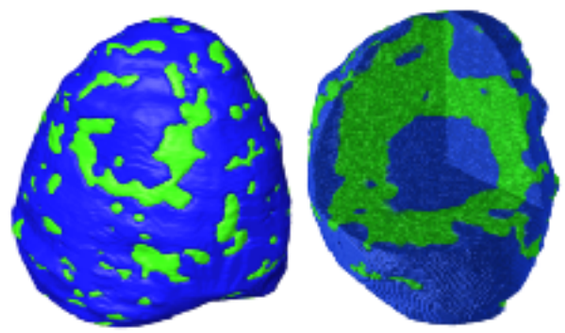


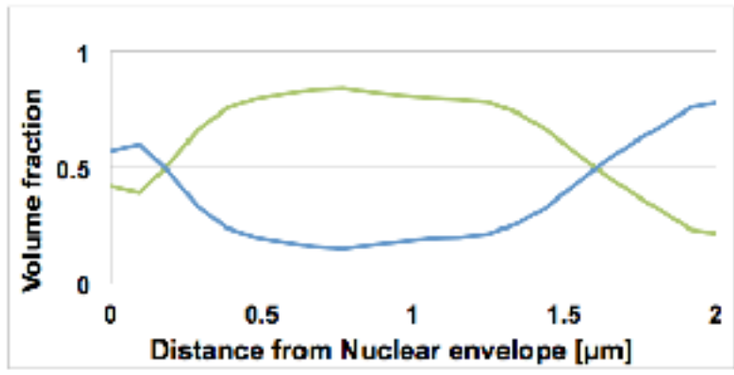


Mature







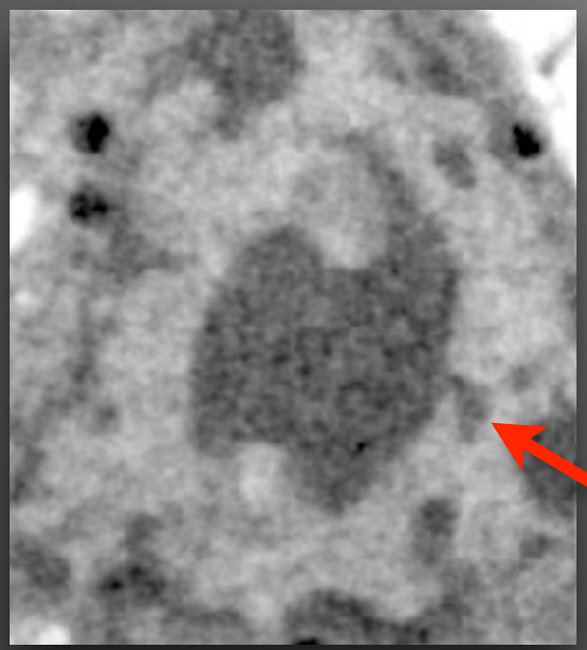


Le Gros et al. (2016) Cell Reports. 17(8), 2125-2136

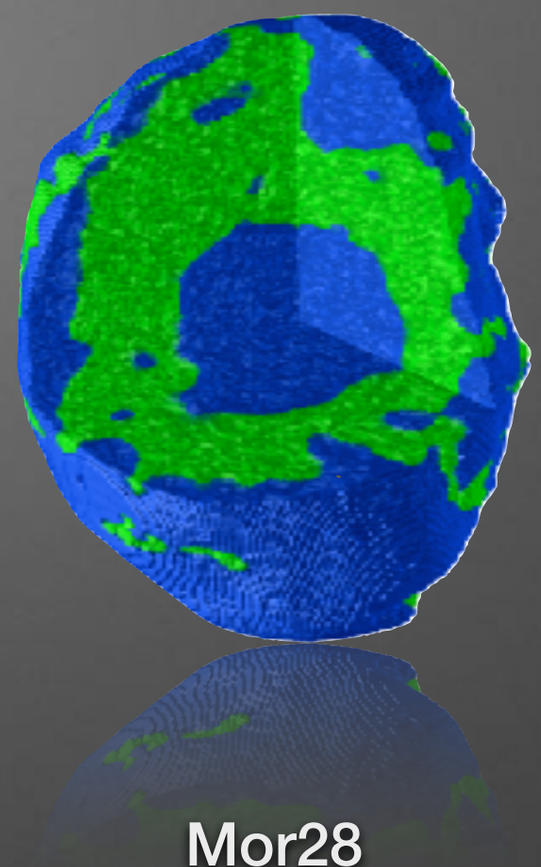
Heterochromatin
Euchromatin

Nuclear structure and gene selection

SXT

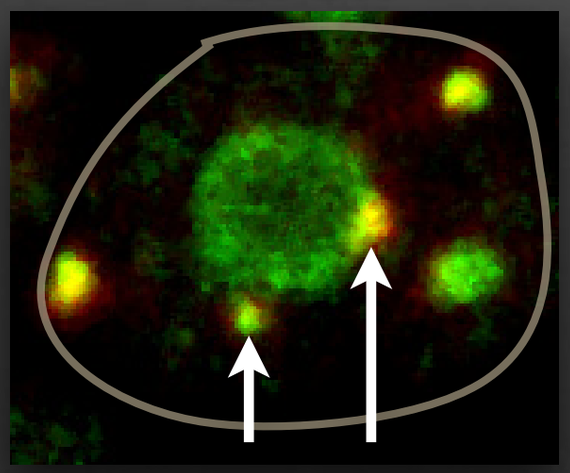


Silenced genes



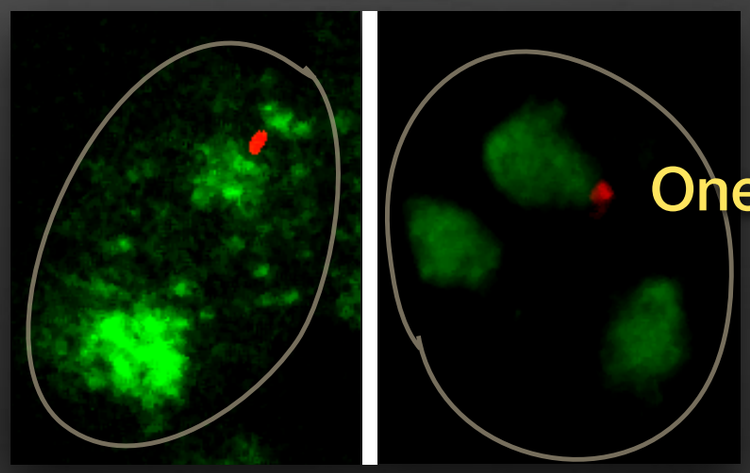
FISH

Pan OR



Silenced genes

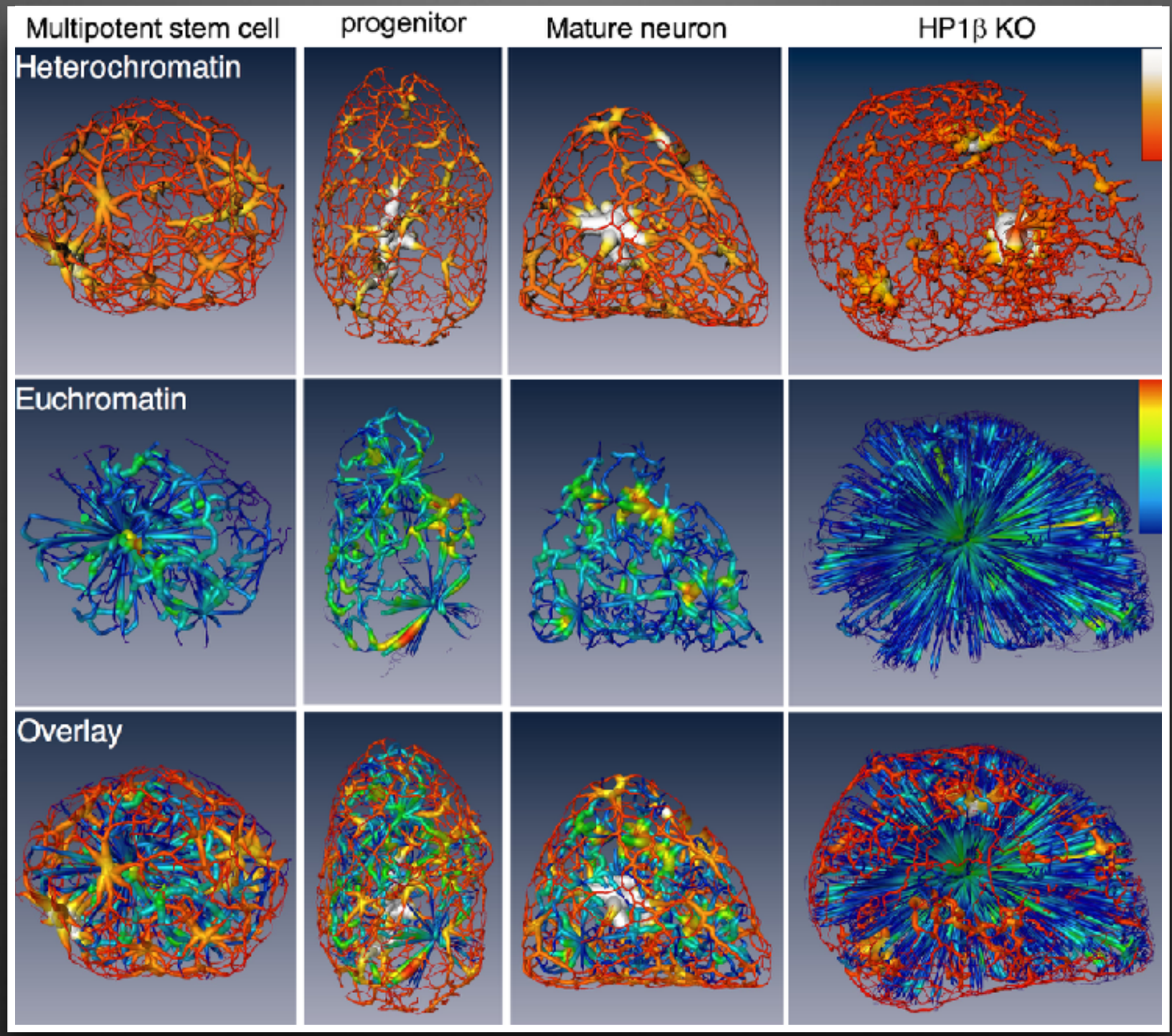
Mor28



One active allele

Clowney et al. Cell. 151, 724-737

Chromatin networks



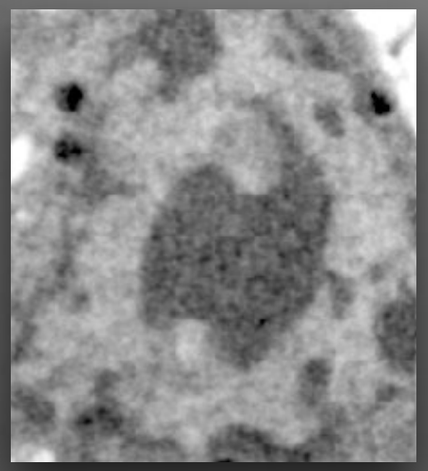
Le Gros et al. (2016) Cell Reports. 17(8), 2125-2136.

3D organization of the nucleus critical for normal differentiation

Nuclear structure and gene selection

Wild type cell

SXT



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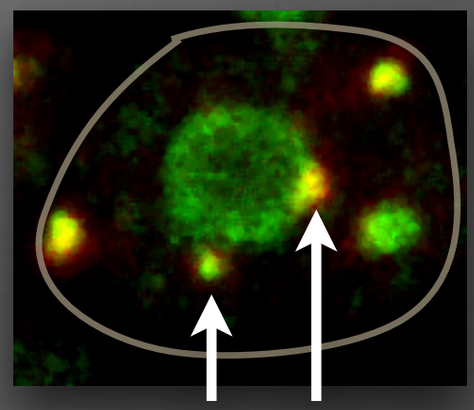
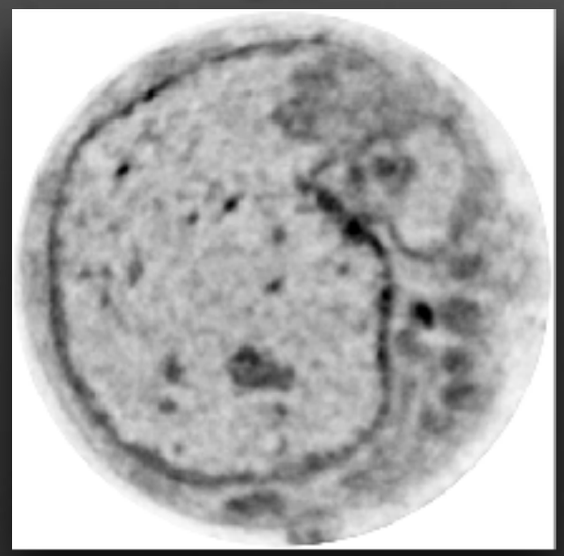


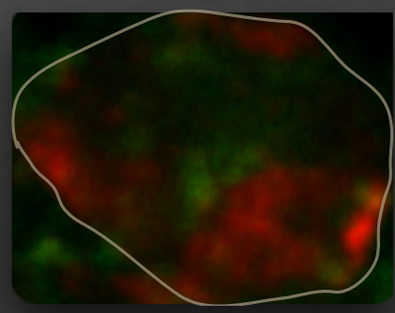
Silenced genes

LBR expressing cell

SXT



FISH

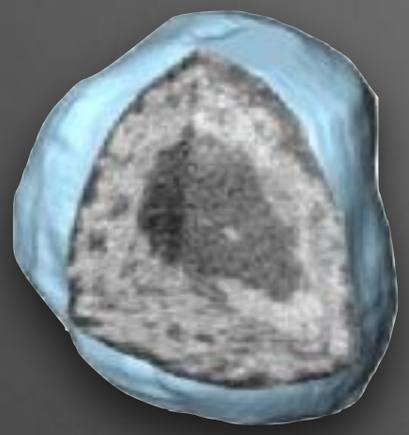


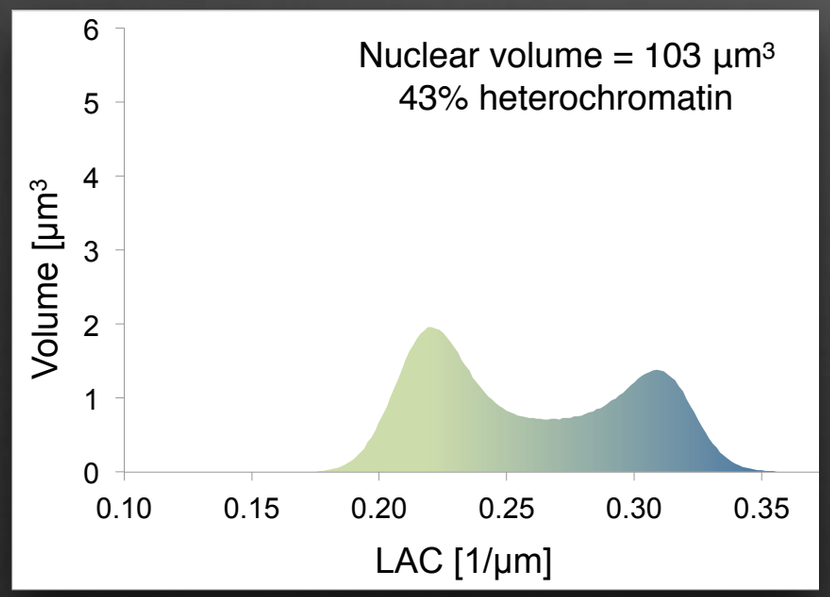
OR expression disrupted

Clowney et al. (2012) Cell. 151, 724-737

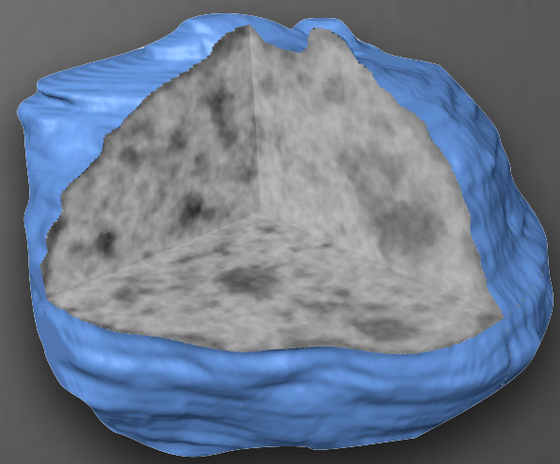
Nuclear structure and gene selection

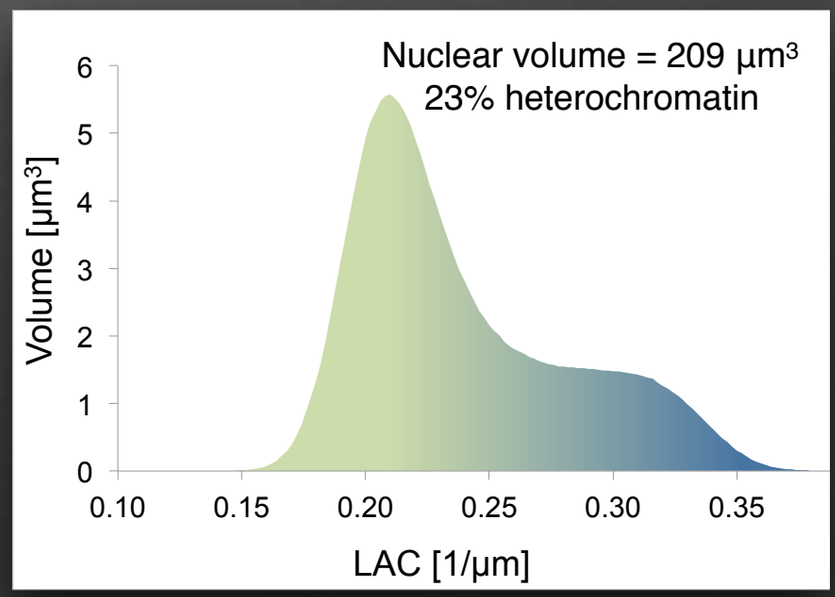
Wild type





Hp1ß KO

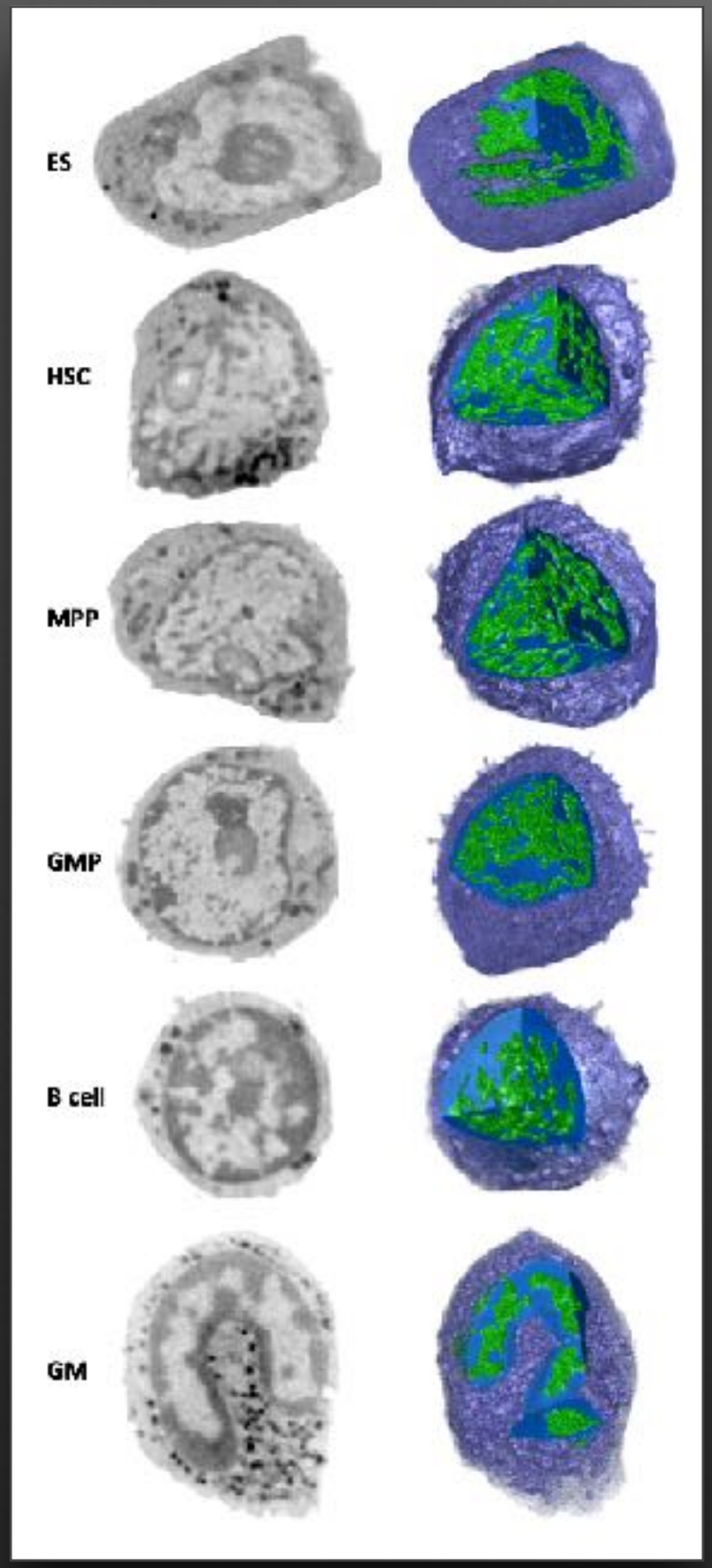




Le Gros et al. (2016) Cell Reports. 17(8), 2125-2136

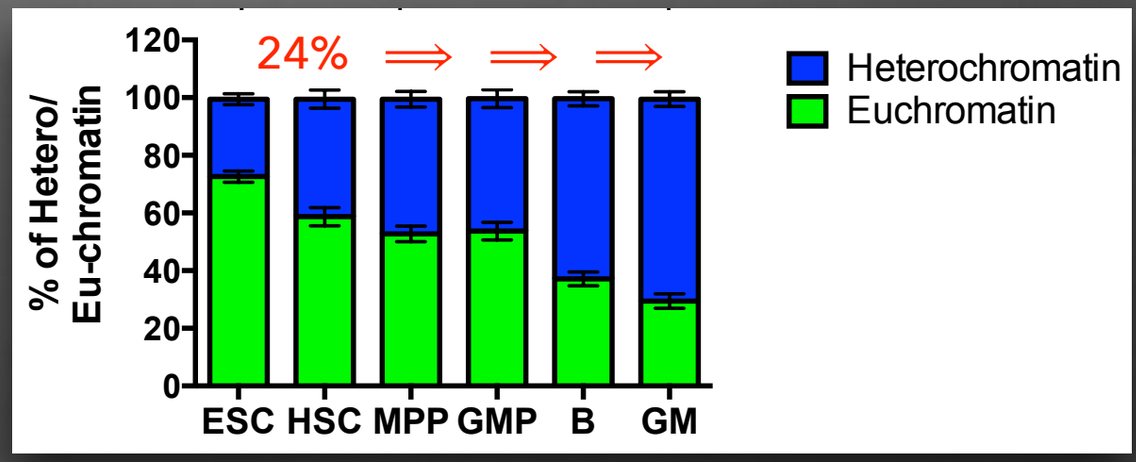
Nuclear organization

From stem cell to blood cell



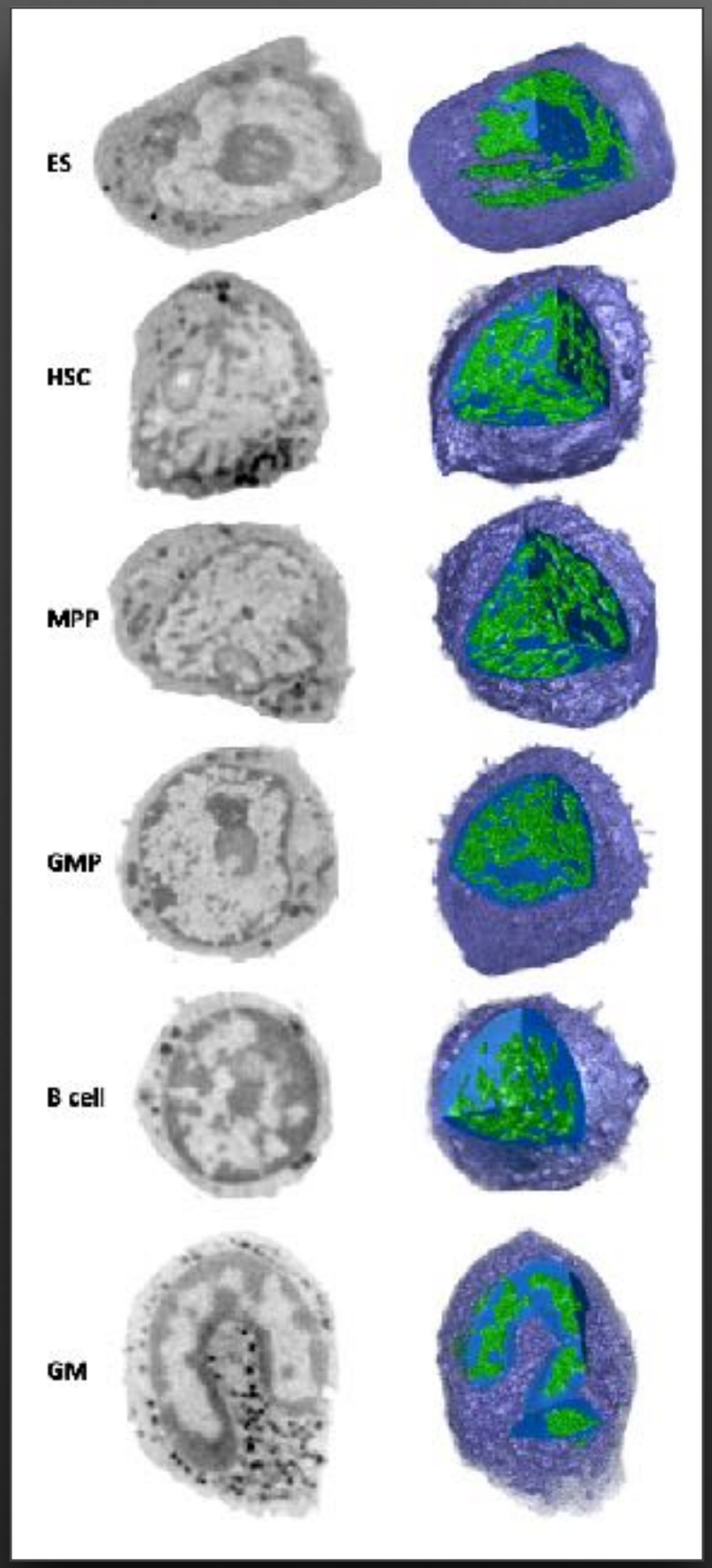
During differentiation:

Percent heterochromatin increases



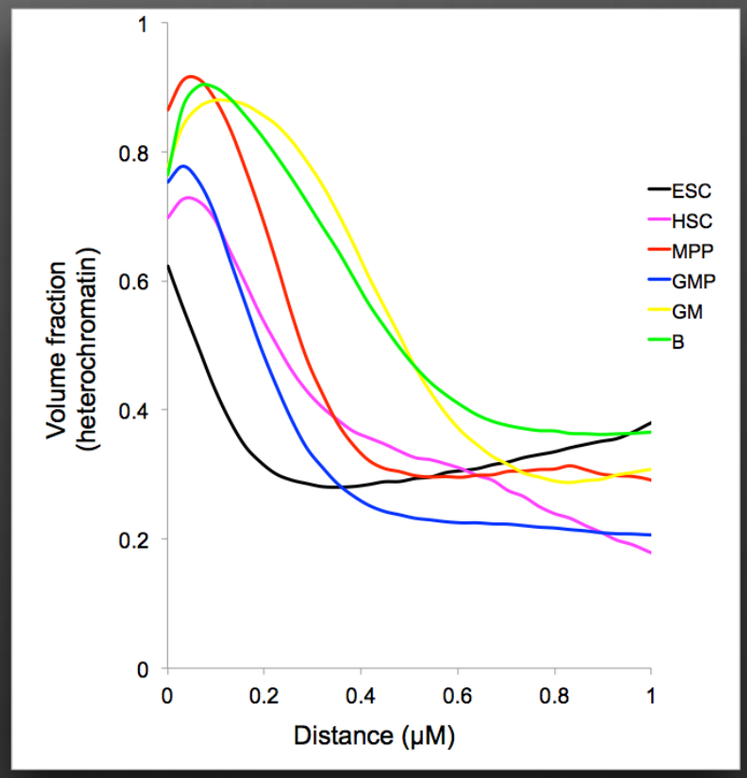
Ugarte et al. (2015) Stem Cell Reports. 5(5), 728-470

From stem cell to blood cell



During differentiation:

Nuclear volume decreases and peripheral heterochromatin thickens

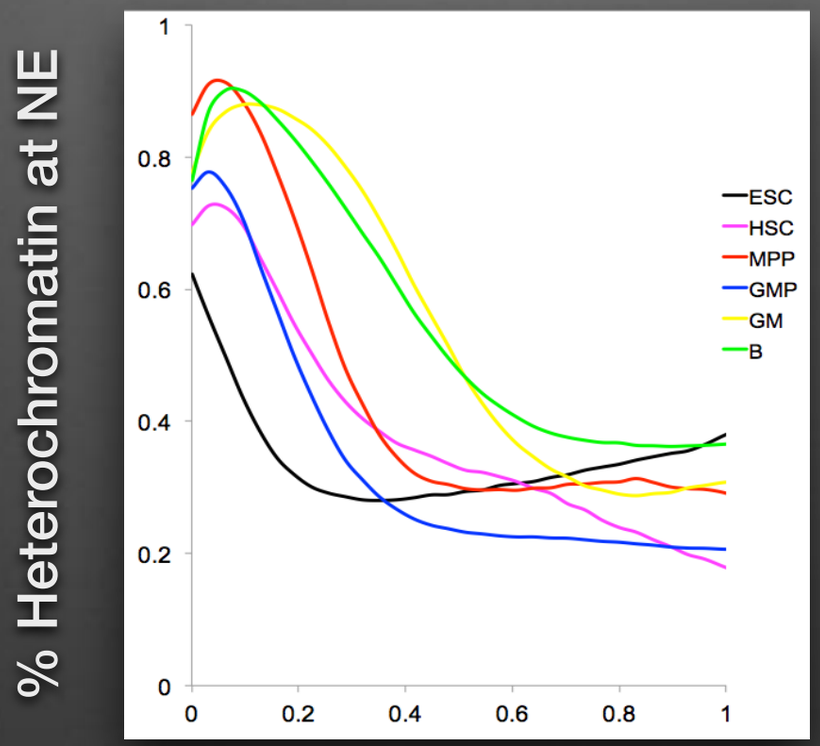


Ugarte et al. (2015) Stem Cell Reports. 5(5), 728-470

Peripheral heterochromatin

- Increases during differentiation
- Decreased in transformed and tumor cells

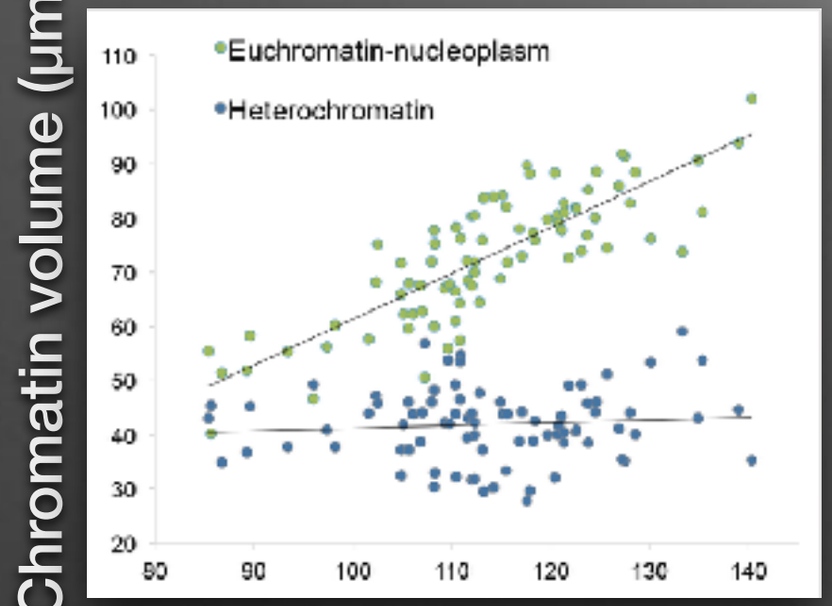
Hematopoiesis



Nuclear volume

Proportional to volume of euchromatin region

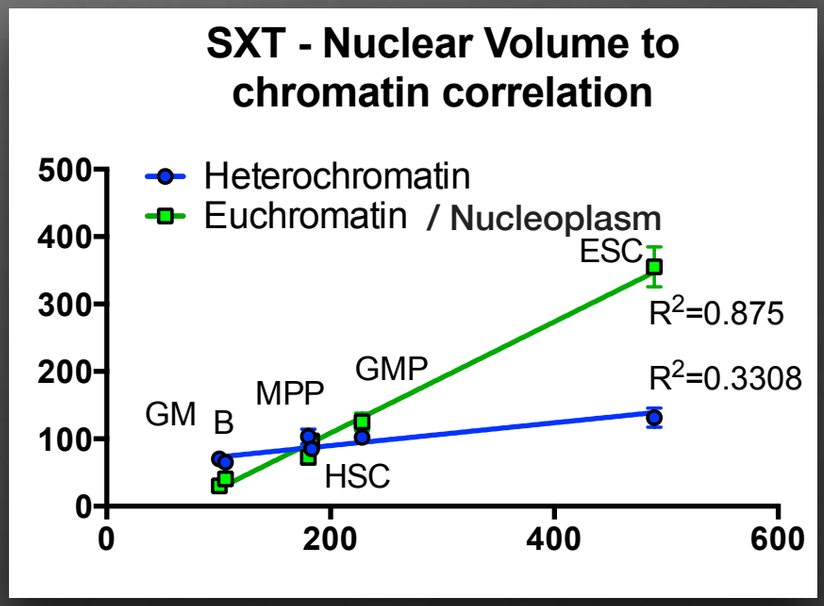
Neurogenesis



Nuclear volume (µm³)

Le Gros et al. (2016) Cell Reports. 17(8), 2125-2136

Hematopoiesis

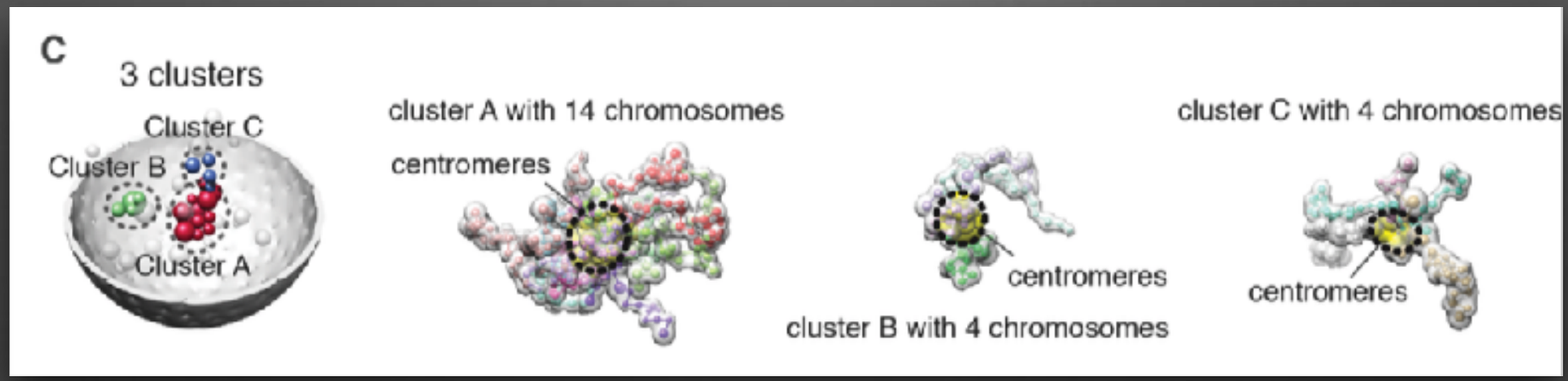


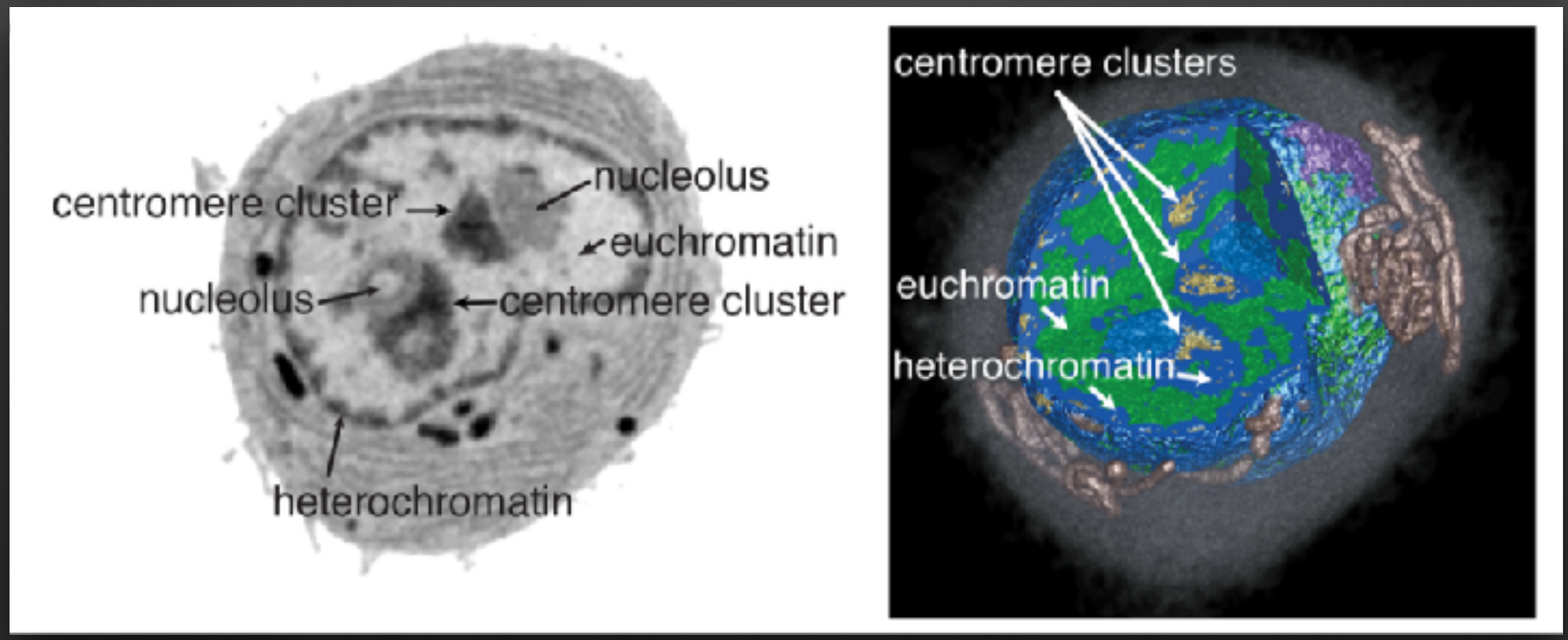
Nuclear volume (µm³)

Ugarte et al. (2015) Stem Cell Reports. 5(5), 728-470

Topology of the human genome

3D structural models of the human genome at 4Mb resolution





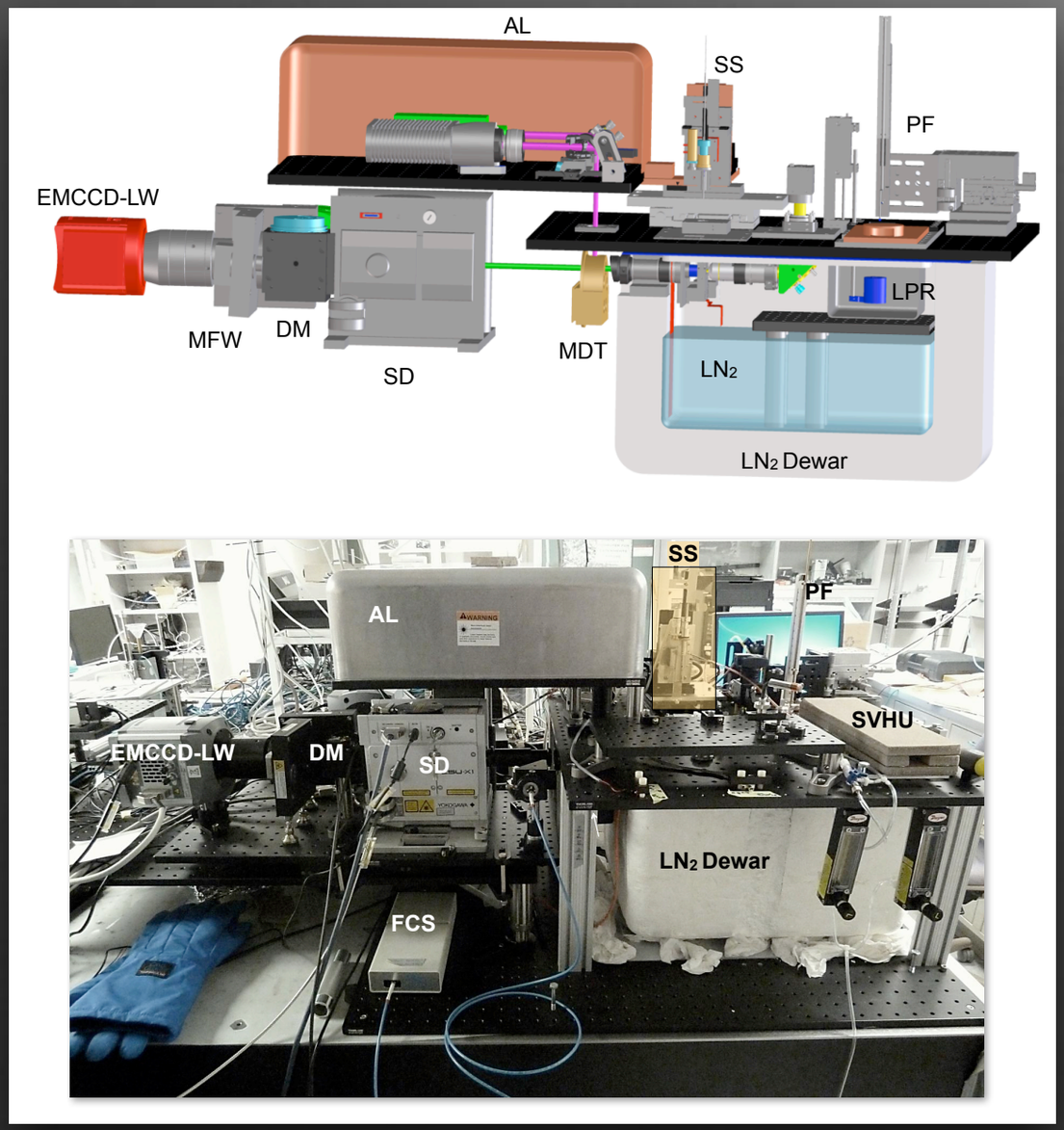
Future

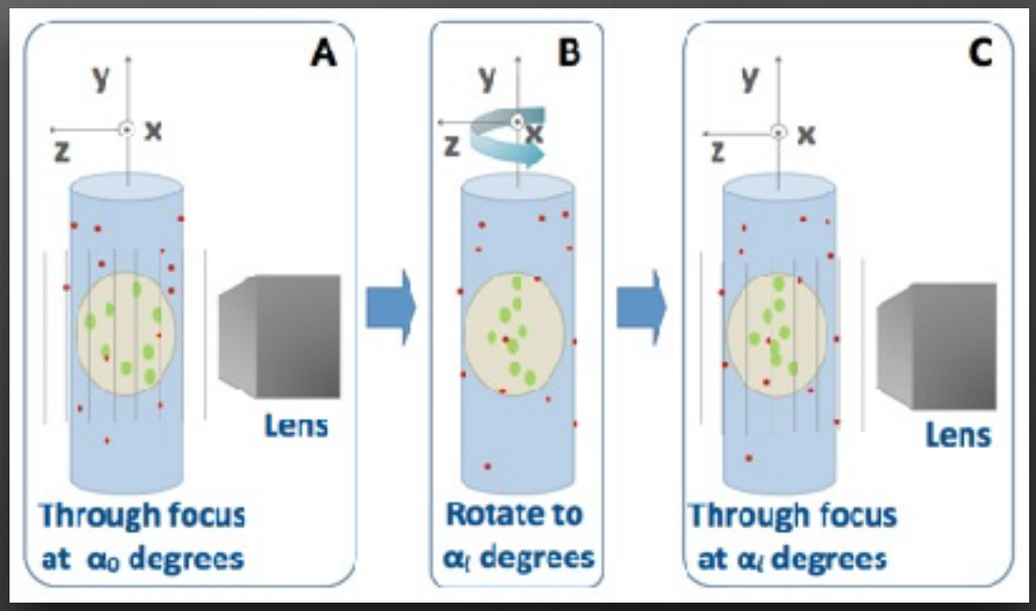
Tjong et al. (2016) PNAS. Mar 22; 113 E1663-1672

Correlated fluorescence and x-ray tomography

Fluorescence, super-resolution, etc.

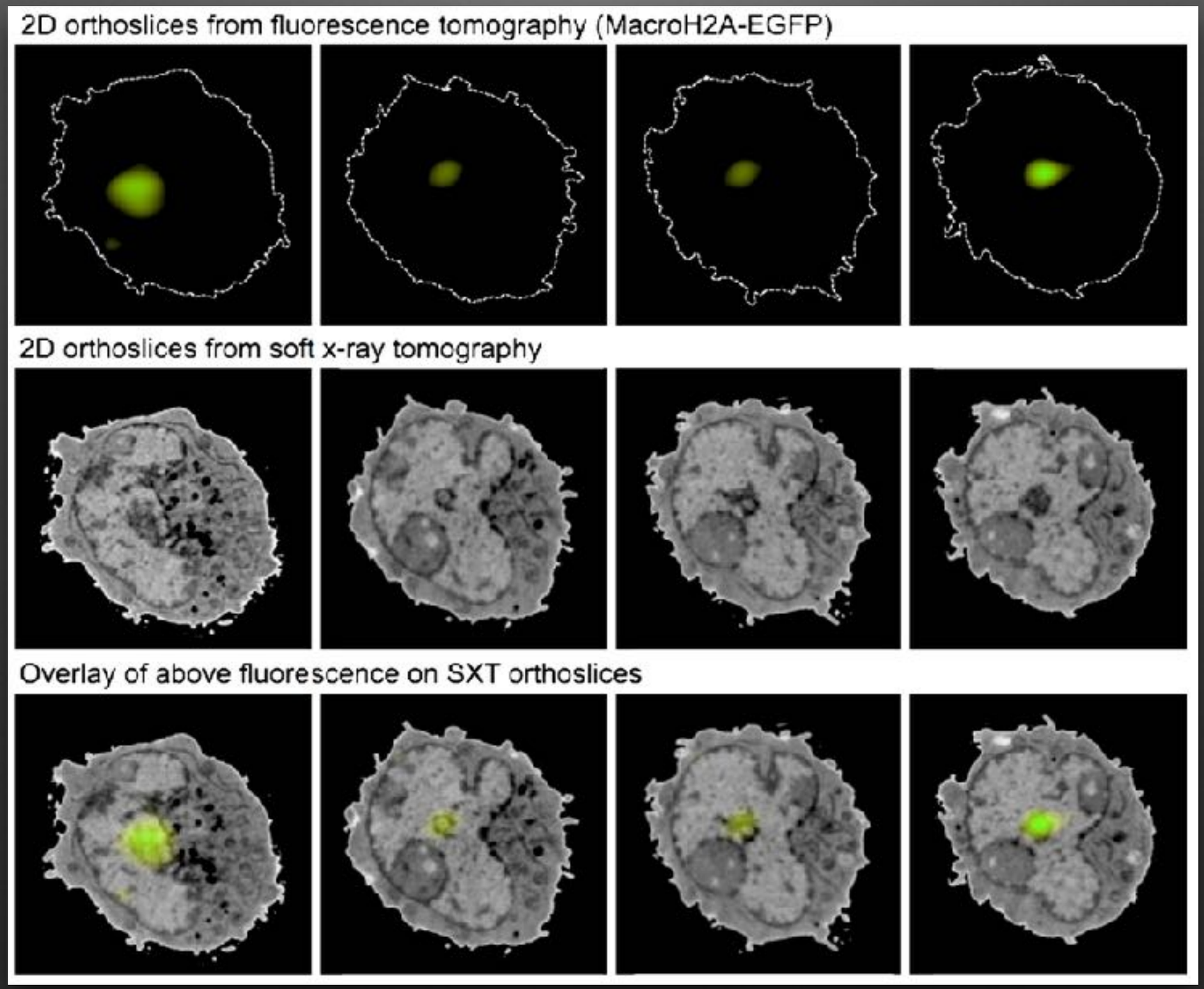
Cryo confocal tomography





Le Gros et al. (2009) J. Microscopy. 235(1), 1-8

Inactive X chromosome

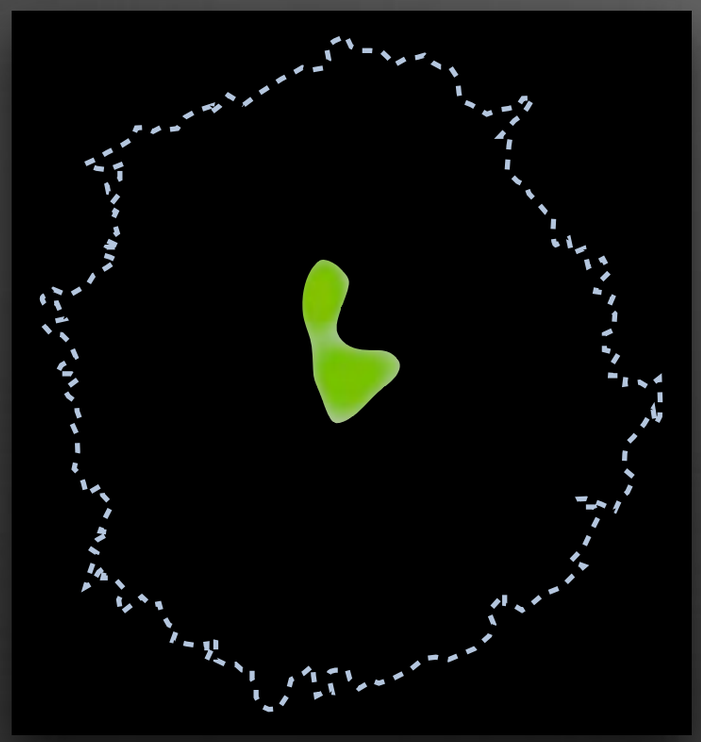


Smith et al. (2014) Biophysical Journal. 107(8), 1988-96

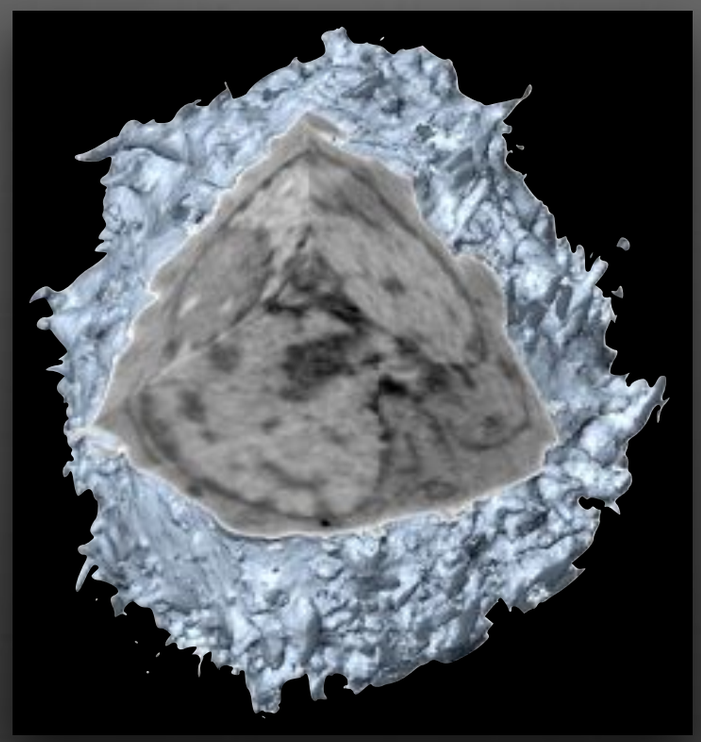
Correlated fluorescence and x-ray tomography

Inactive X chromosome

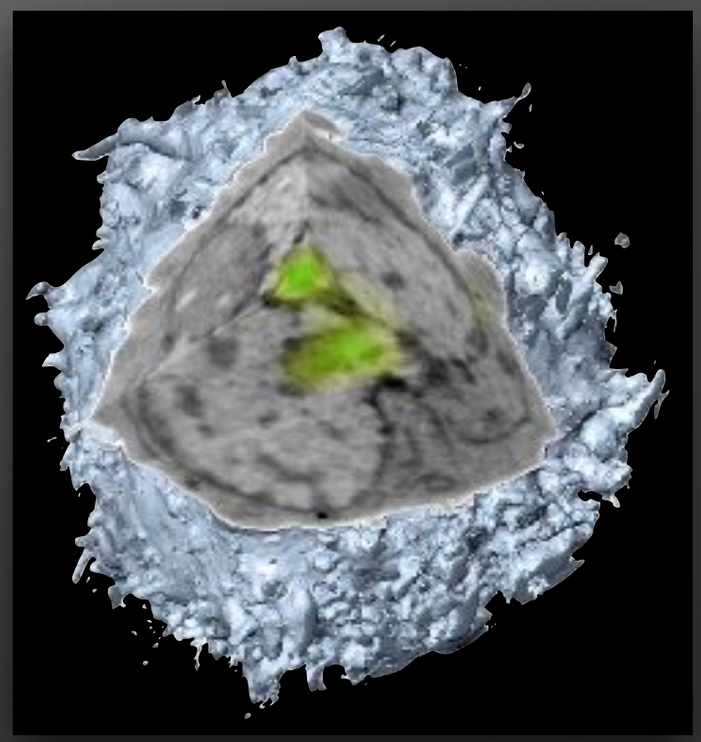
Fluorescence



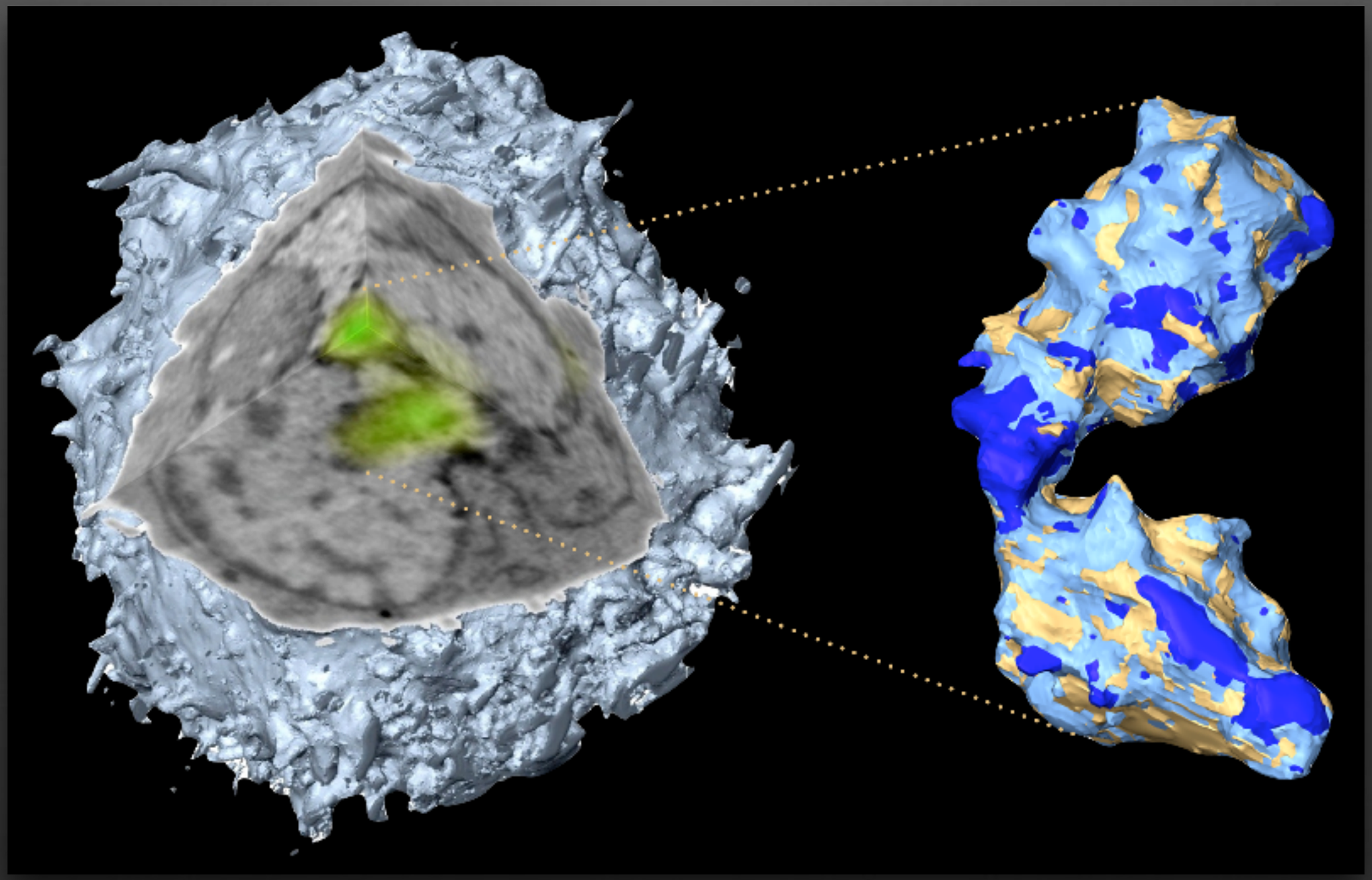
X-ray



Overlay



Correlated cryo fluorescence & x-ray tomography

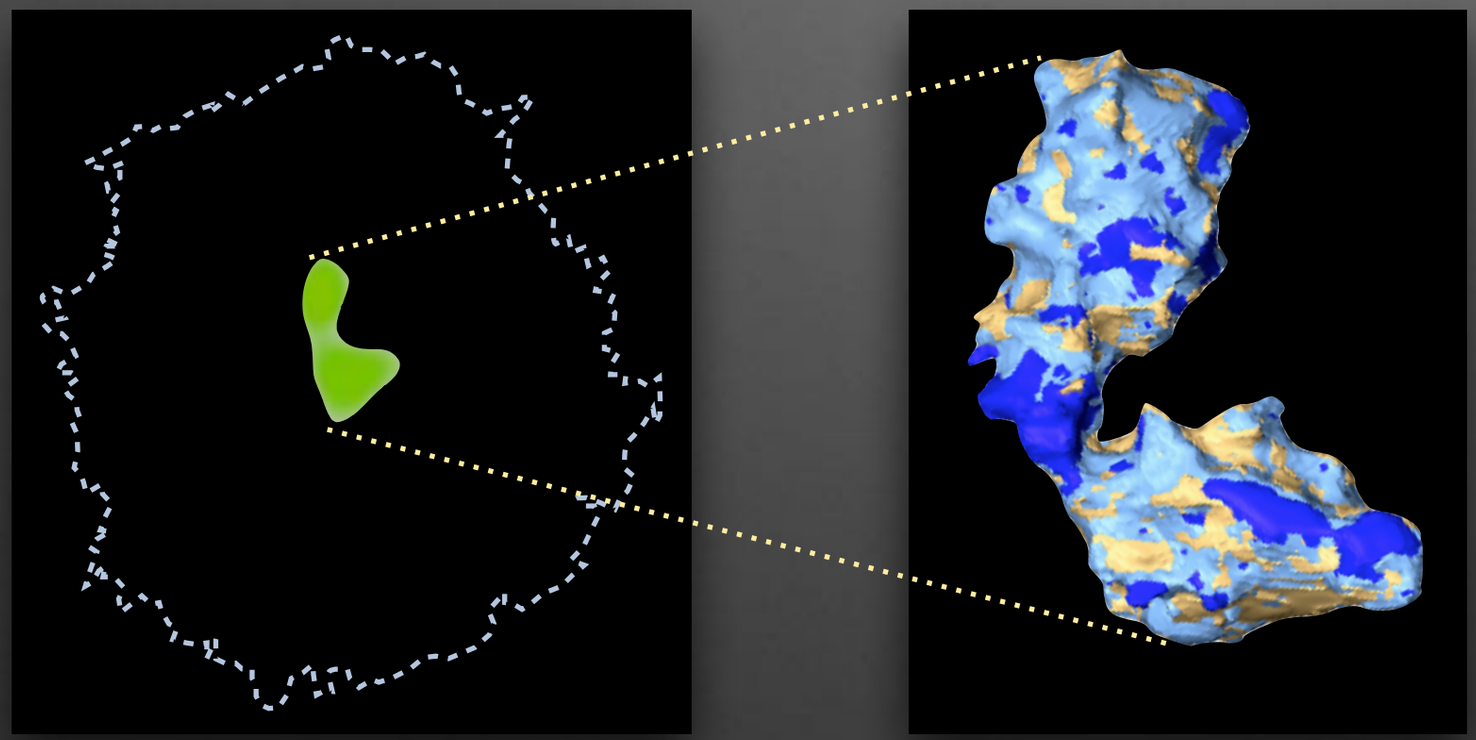


Smith et al. (2014) Biophysical J. 107, 1988-96

Inactive X chromosome

Fluorescence

X-ray



High LAC
0.34-0.36 μm^{-1}

Medium LAC
0.32-0.34 μm^{-1}

Low LAC
0.30-0.32 μm^{-1}

National Center for X-ray Tomography

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