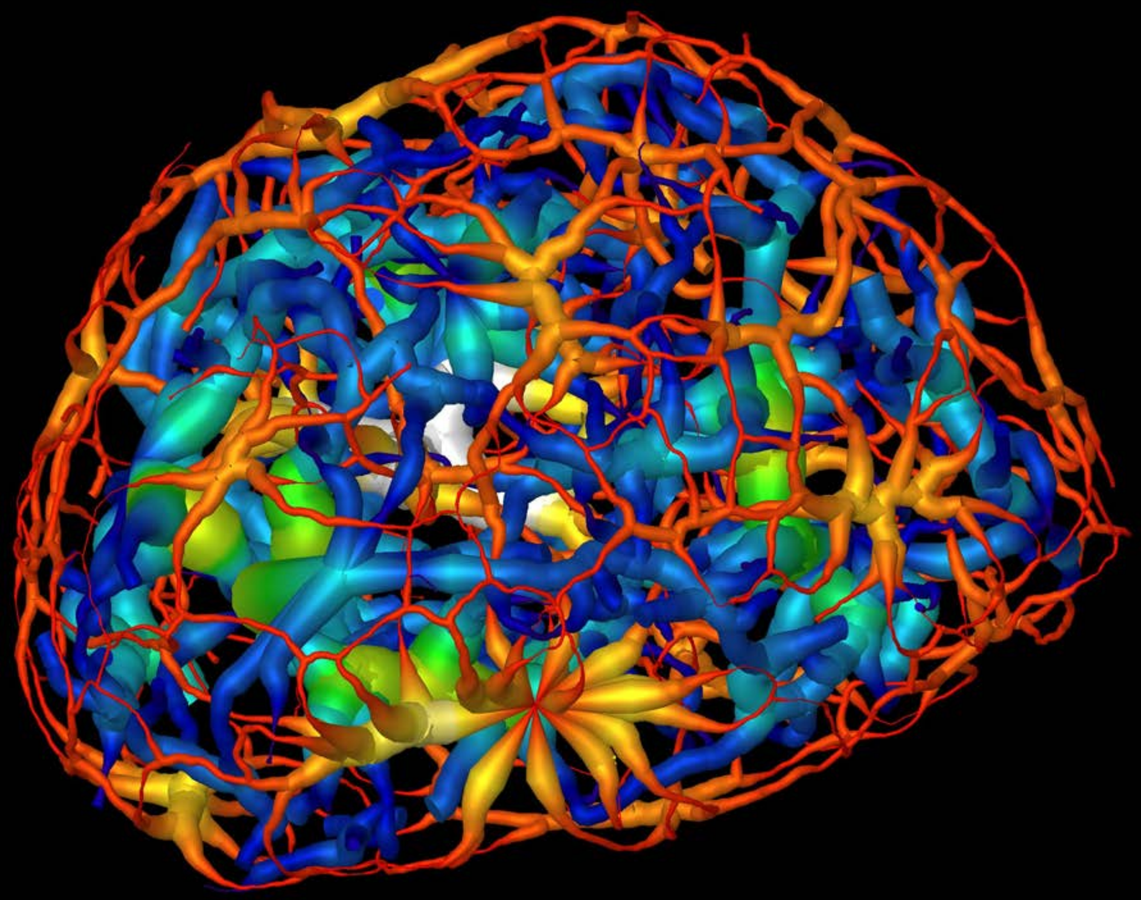
Soft X-ray Tomography (SXT)

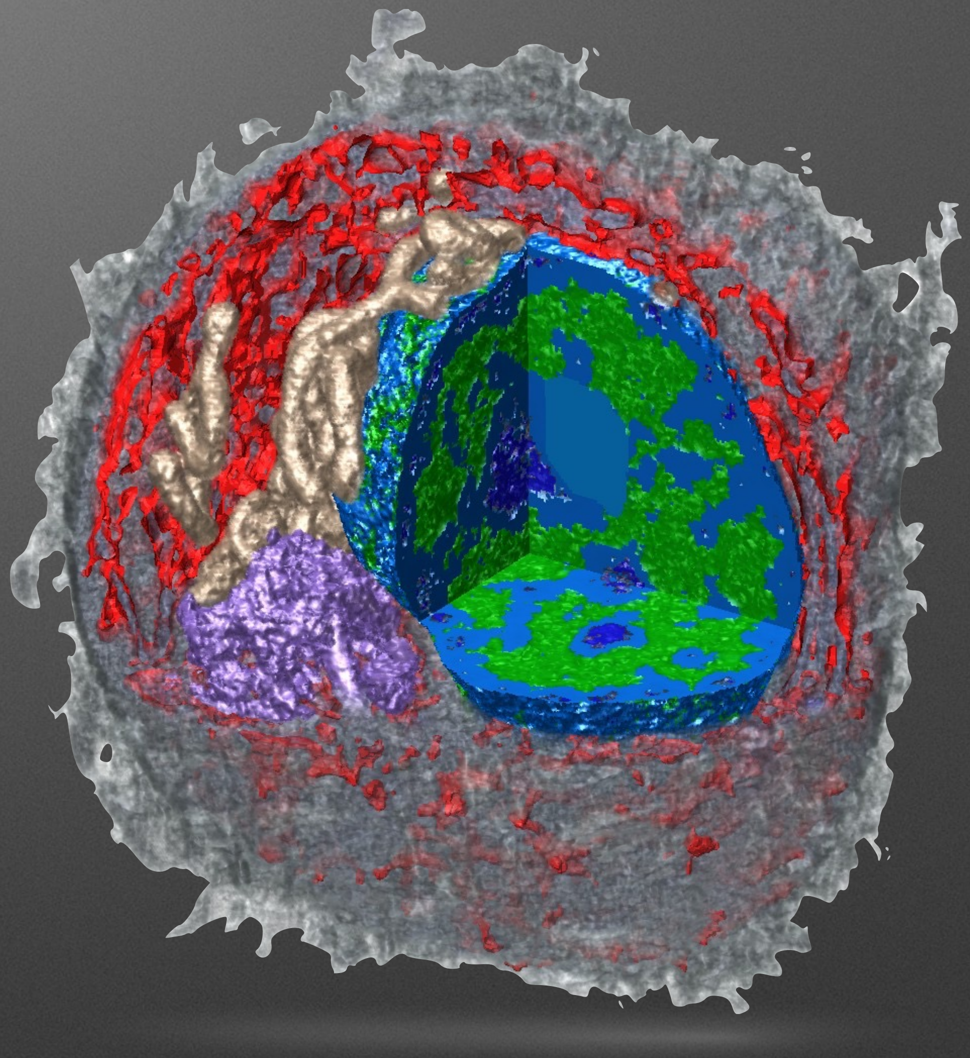


National Center for X-ray Tomography

Supported by NIH-NIGMS & DOE-BER

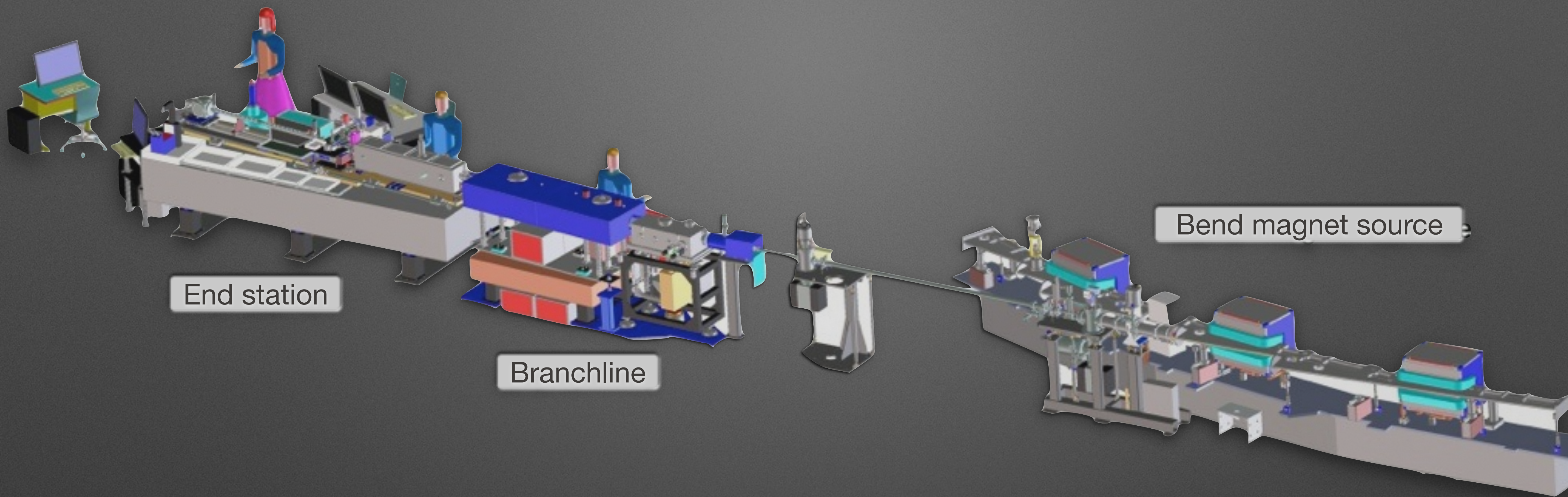
Soft X-ray Tomography (SXT)

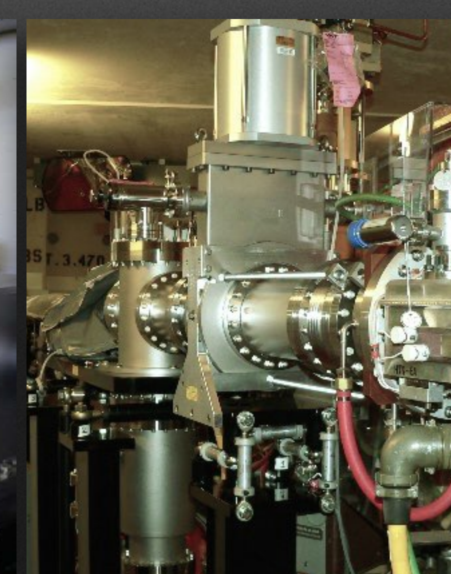
- Imaging whole, hydrated cells in the native state
- No fixatives, no stains
- Cryo-immobilized
- 50 nm isotropic resolution (not limit; source is 2.4 nm)
- See molecules using correlated fluorescence and x-ray tomography

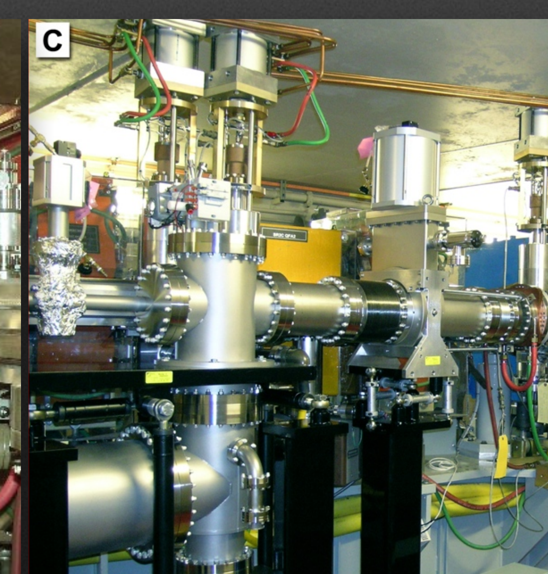


Lawrence Berkeley National Laboratory

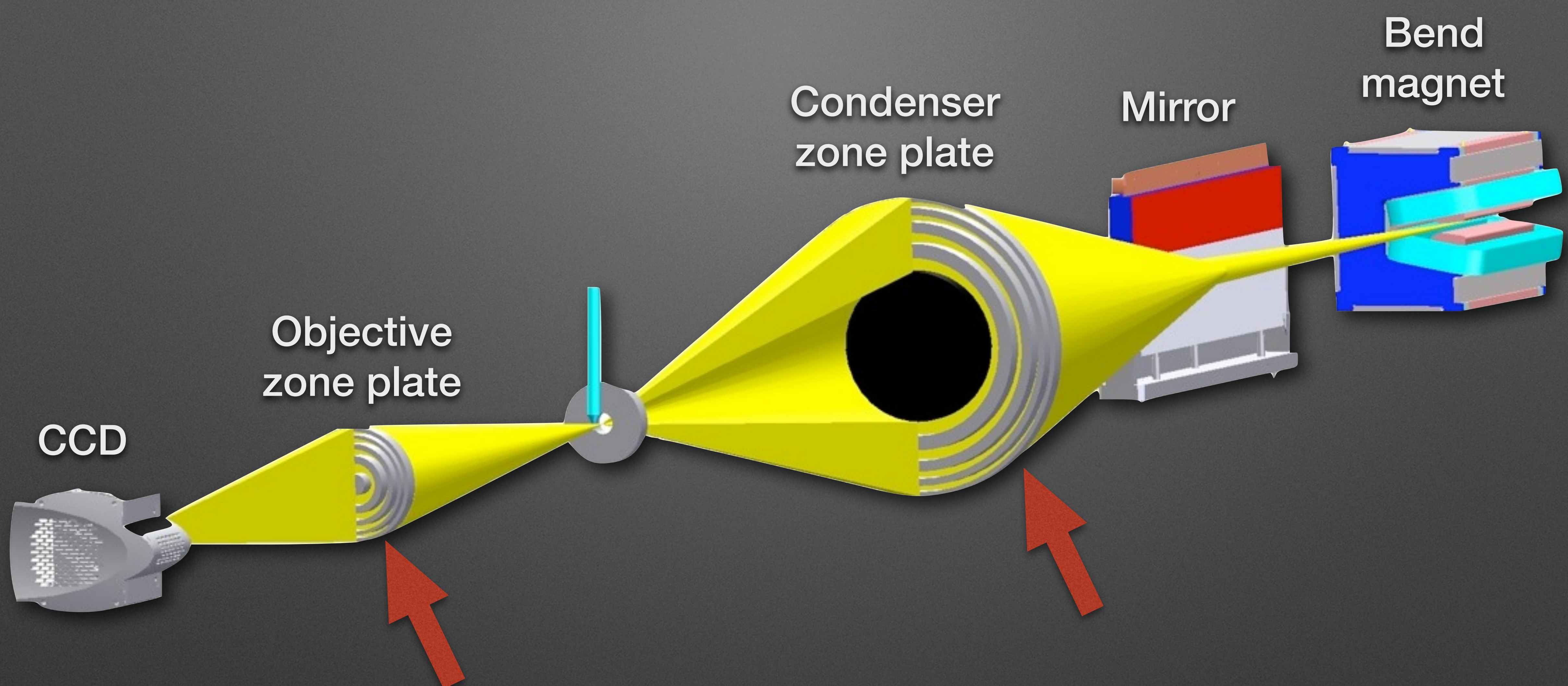
Soft x-ray microscope, Xm-2







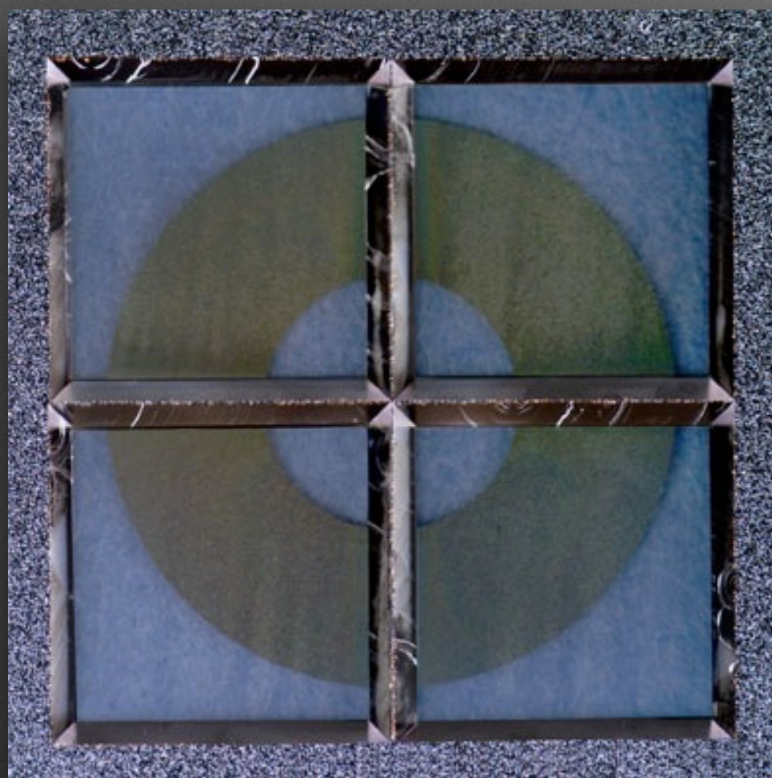
Soft x-ray microscope, Xm-2



- Condenser zone plate focuses source onto specimen
- Objective zone plate magnifies object onto CCD camera

Zone plate lenses - diffractive optics

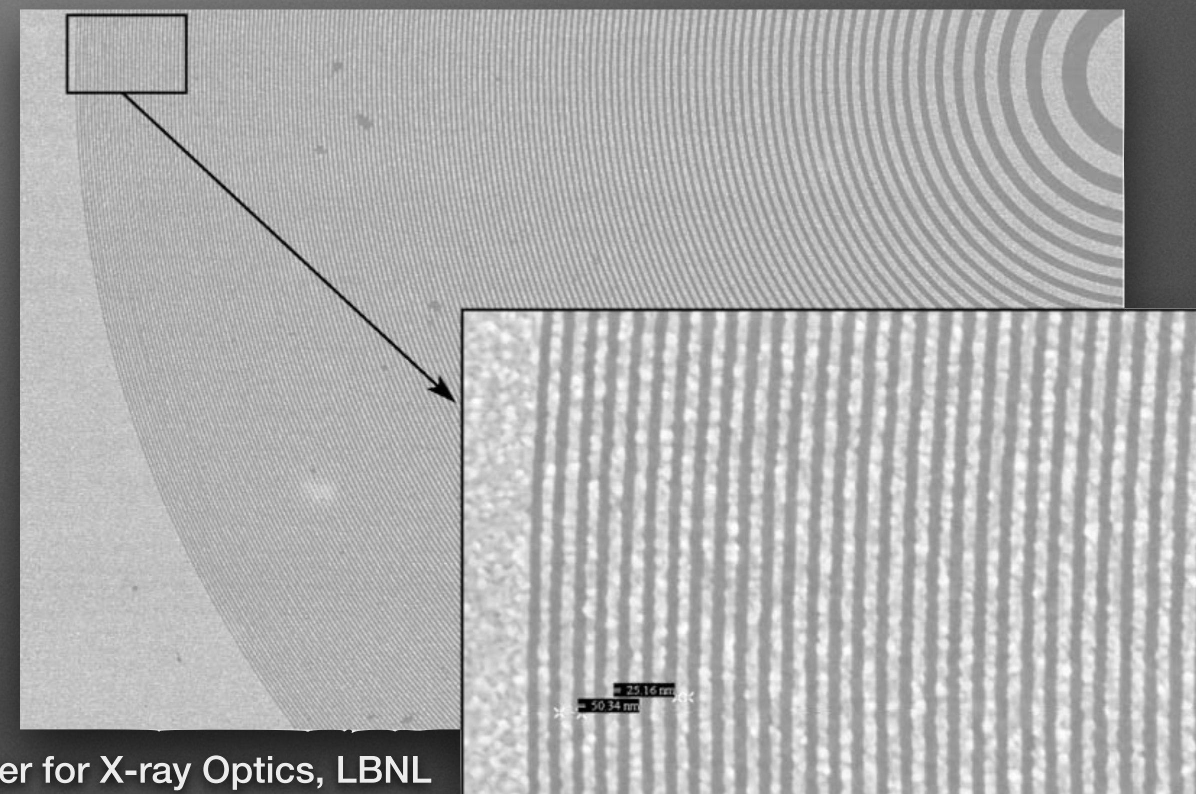
Condenser lens



Diameter = 1 cm

Outer zone width = 50 nm

Objective lens



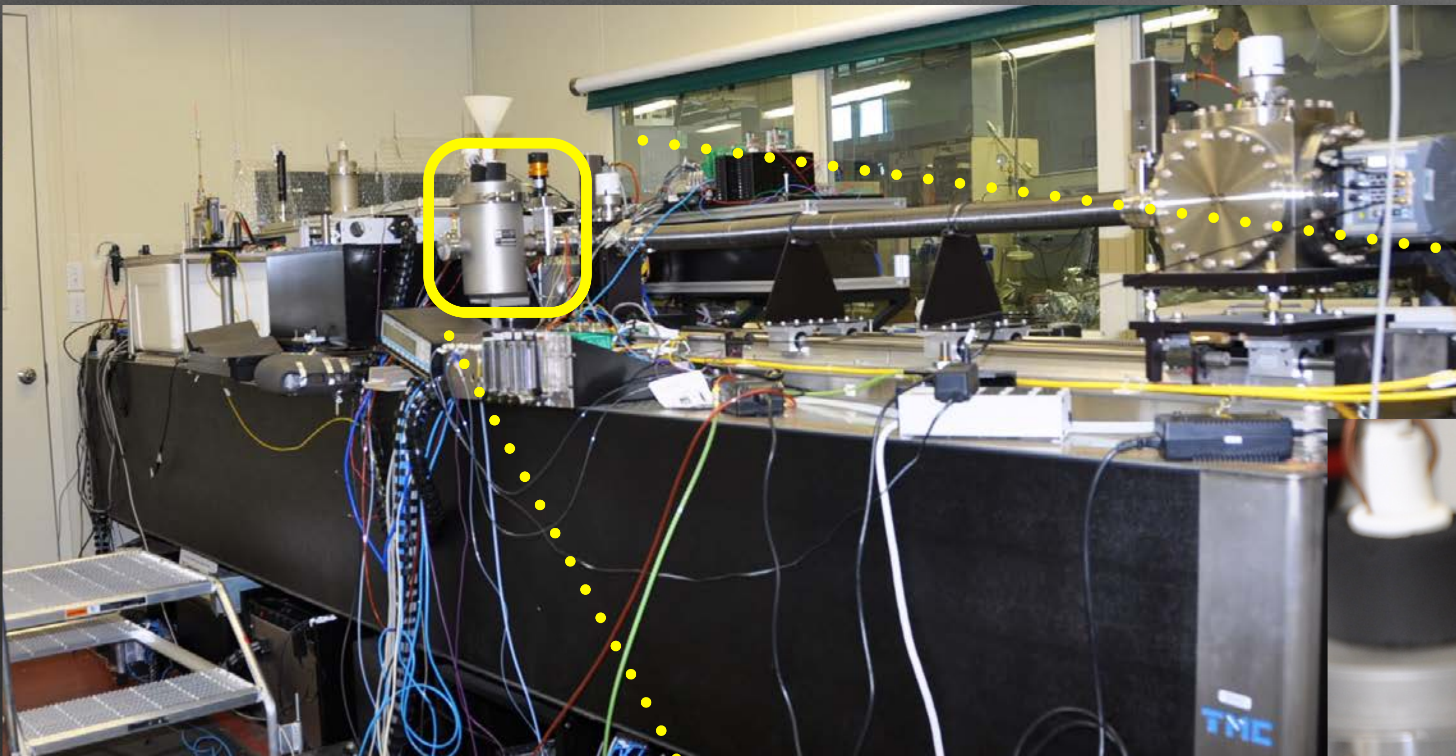
Center for X-ray Optics, LBNL

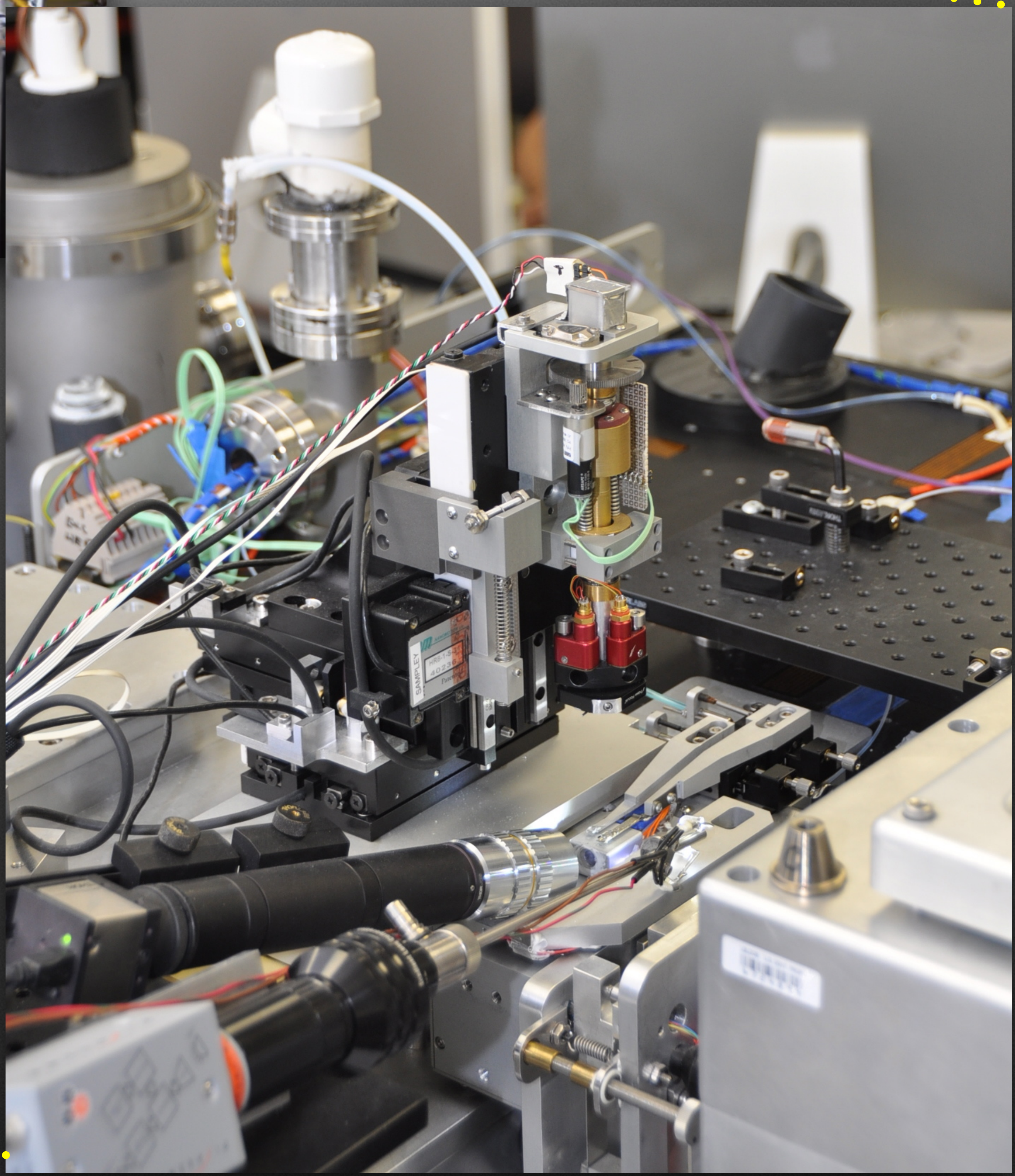
Diameter = 63 μm

Outer zone width = 50 nm

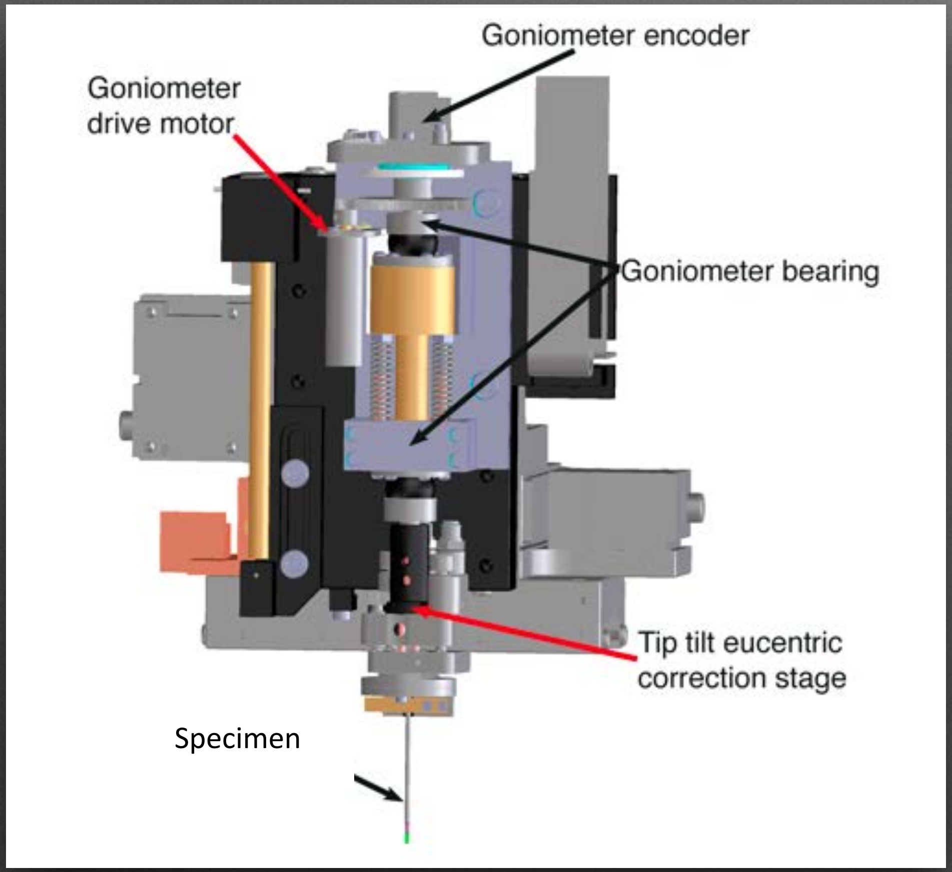
- Resolution determined by width of outermost zone of the lens
- As resolution of zone plate increases, depth of focus decreases

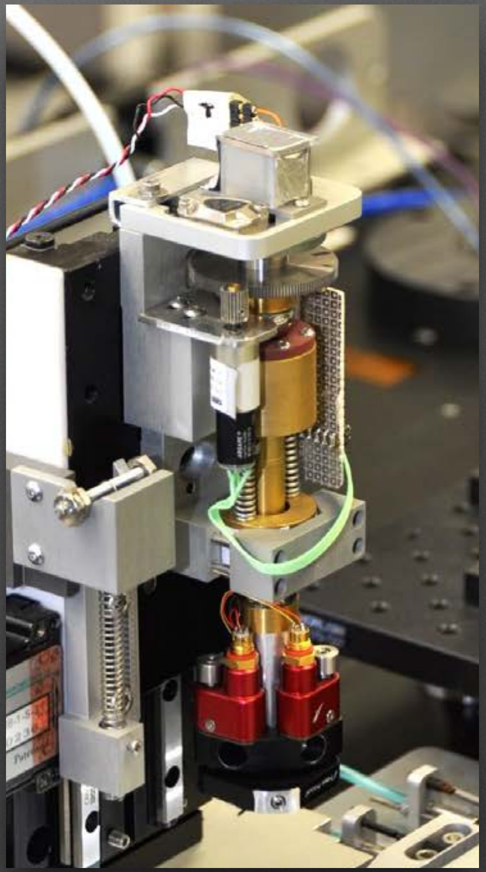
Specimen stage

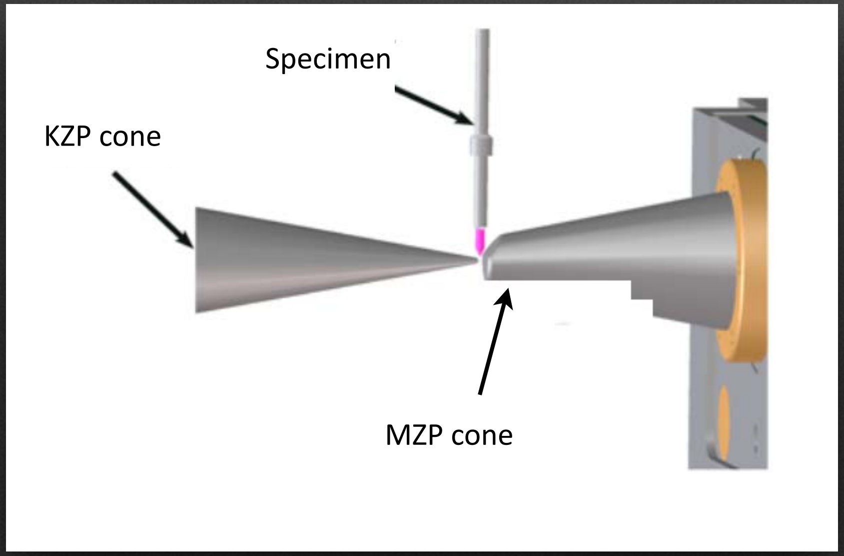


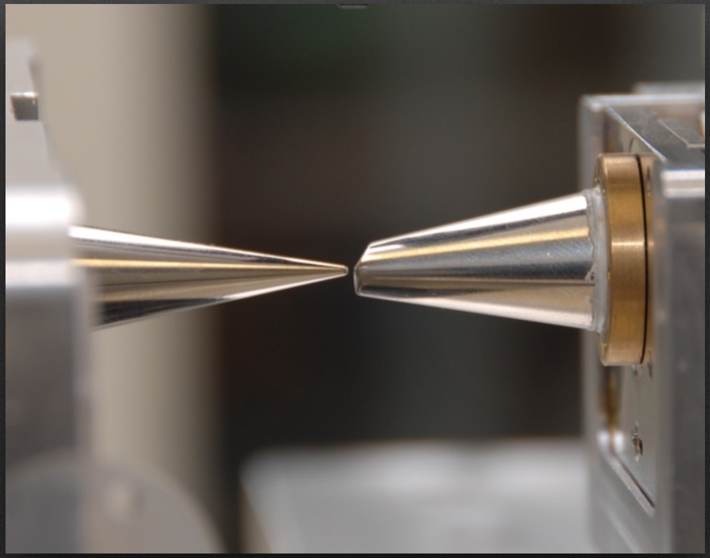


Specimen stage



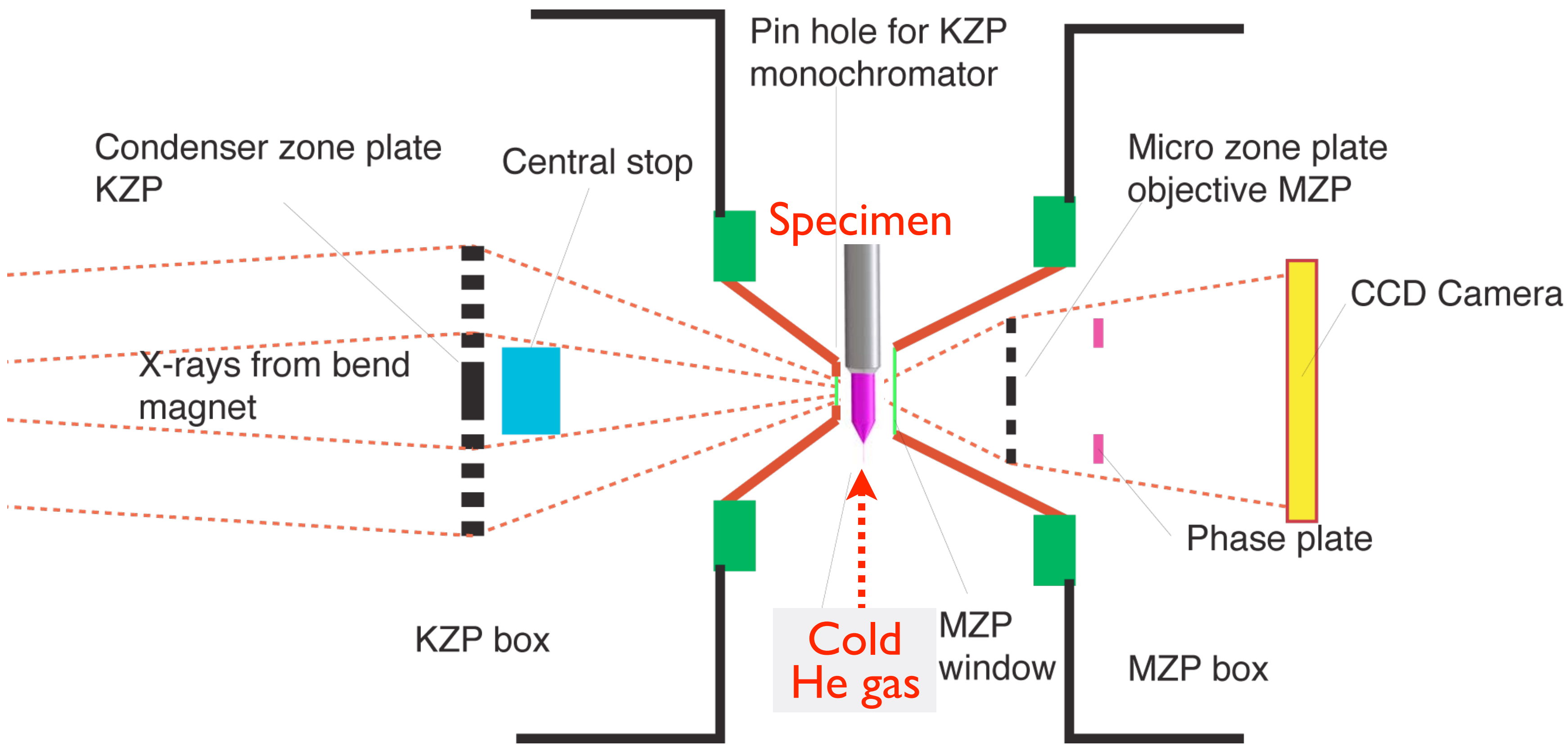


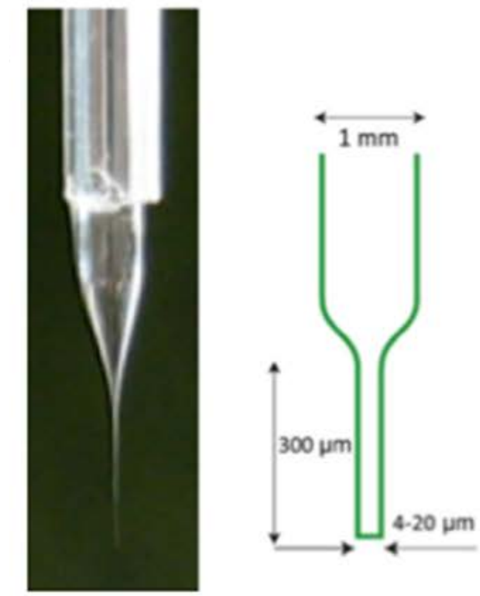


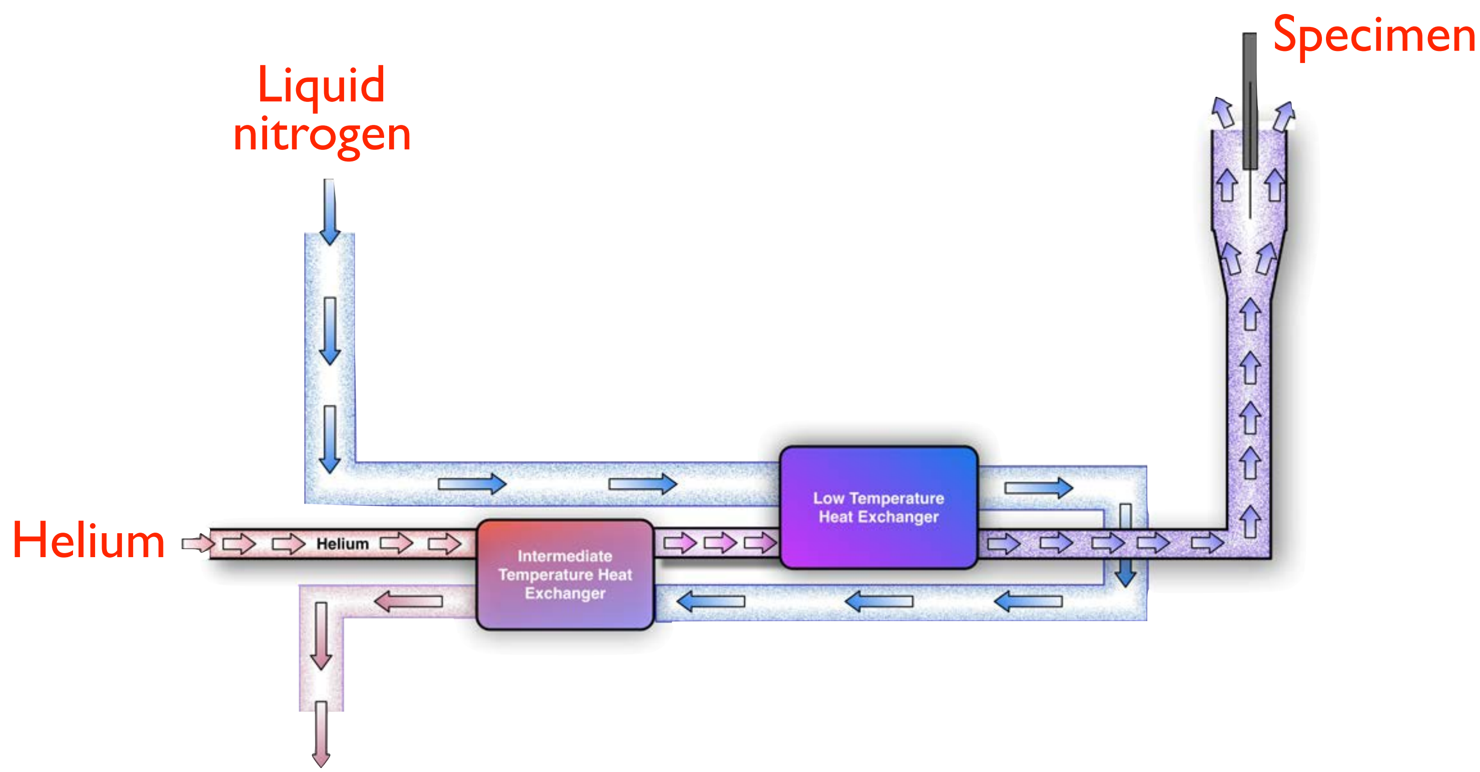


Le Gros MA, McDermott G, Cinquin BP, Smith EA, Do M, Chao WL, Naulleau PP, and Larabell CA (2014). J Synchrotron Radiation. 21, 1370-1377.

Specimen stage



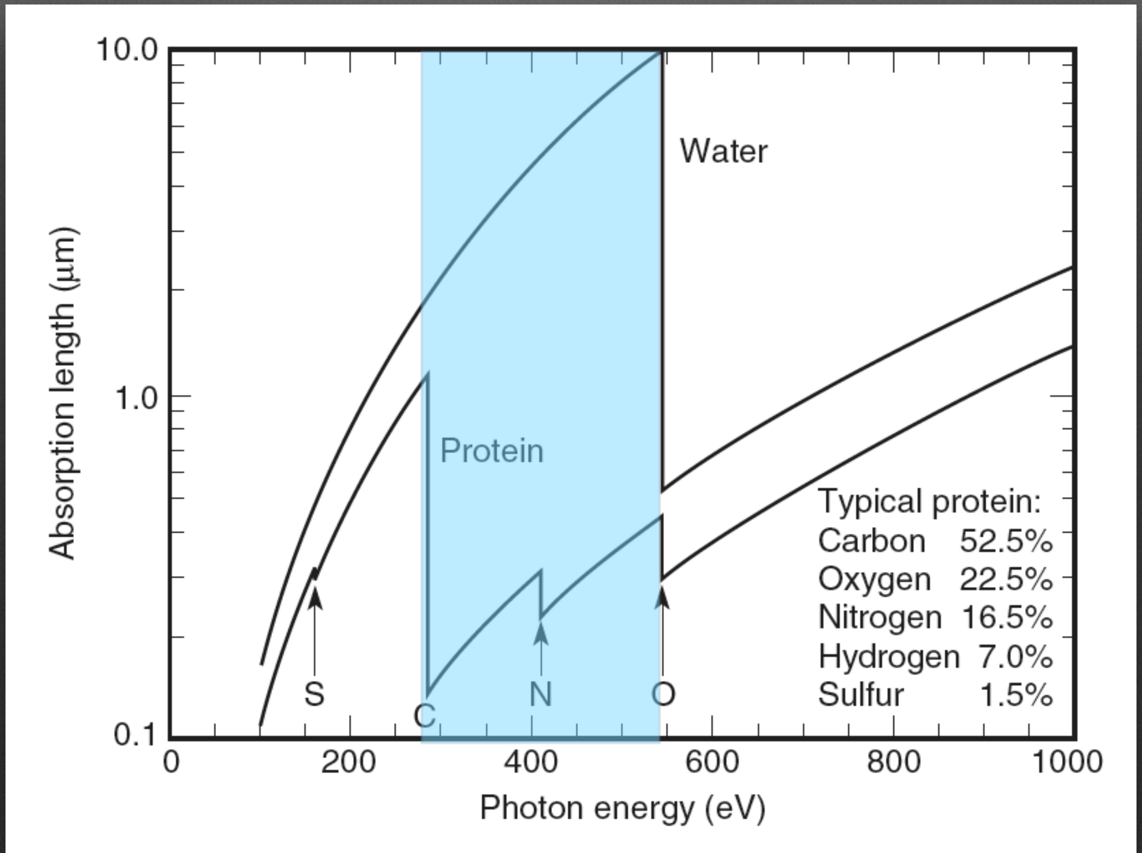




Le Gros et al (2014). J Synchrotron Radiation. 21, 1370-1377.

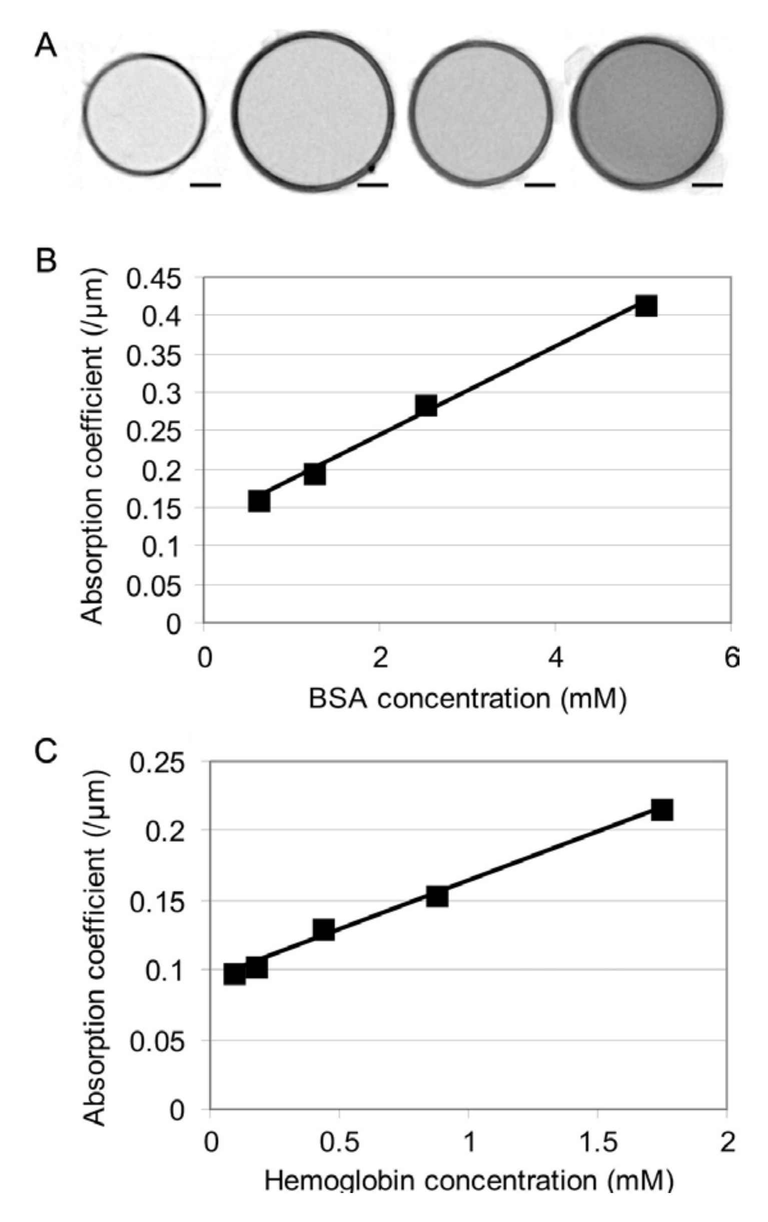
Contrast: Imaging in the ‘water window’

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



Contrast: Imaging in the 'water window'

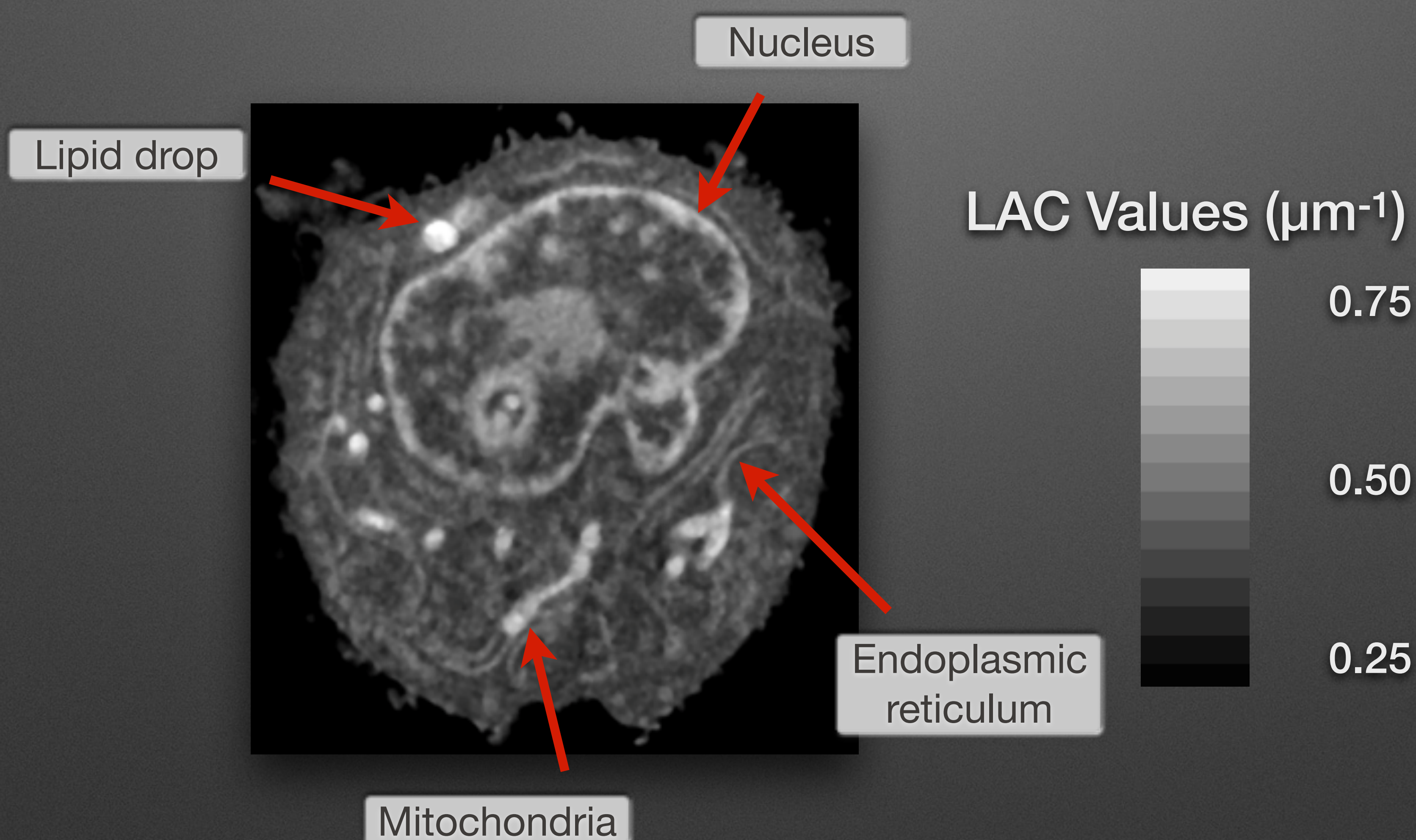
Absorption is linear with thickness & concentration



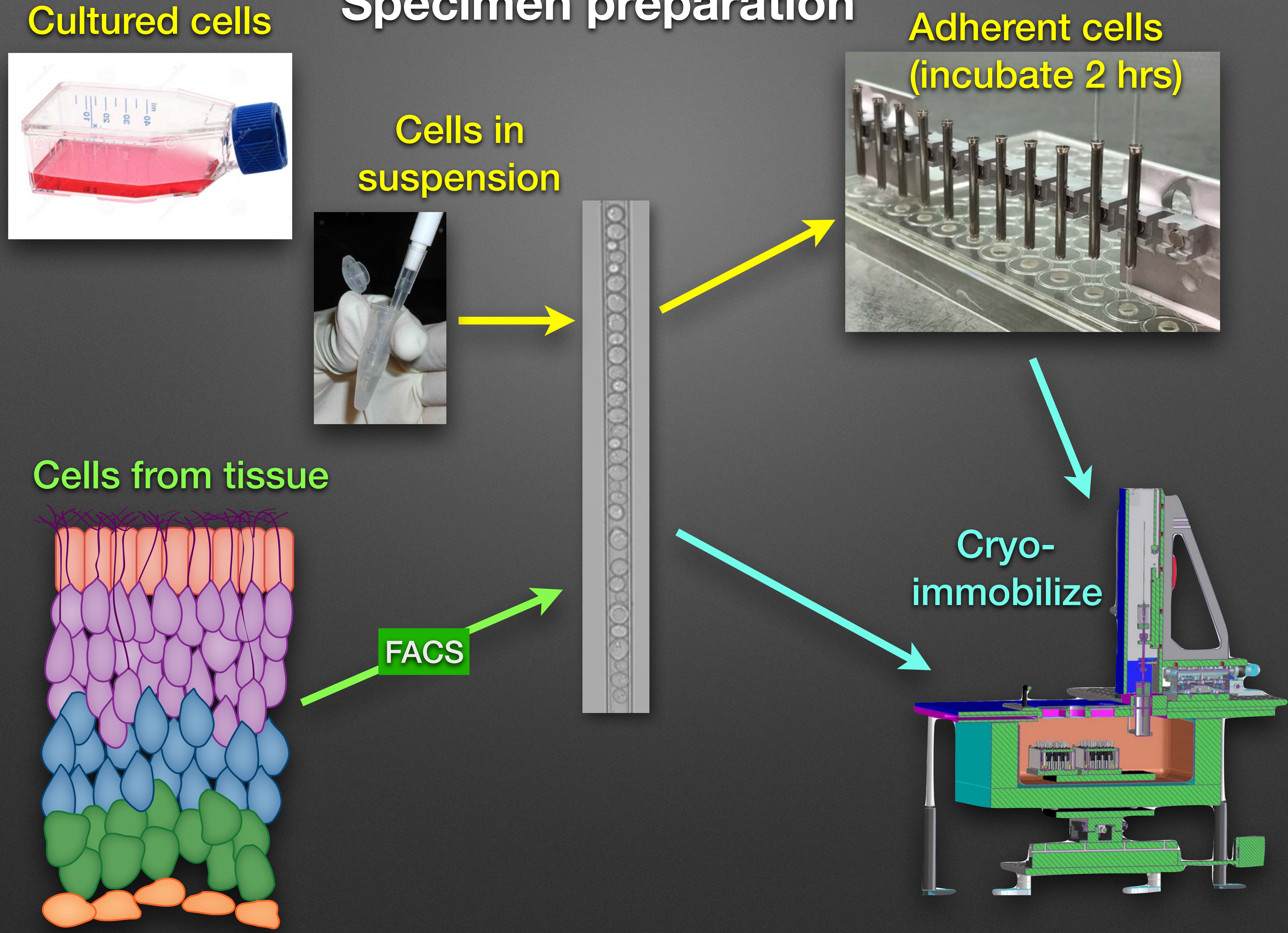
Hanssen et al (2012). *J. Struct. Biol.* 177, 224-232

Contrast: Imaging in the 'water window'

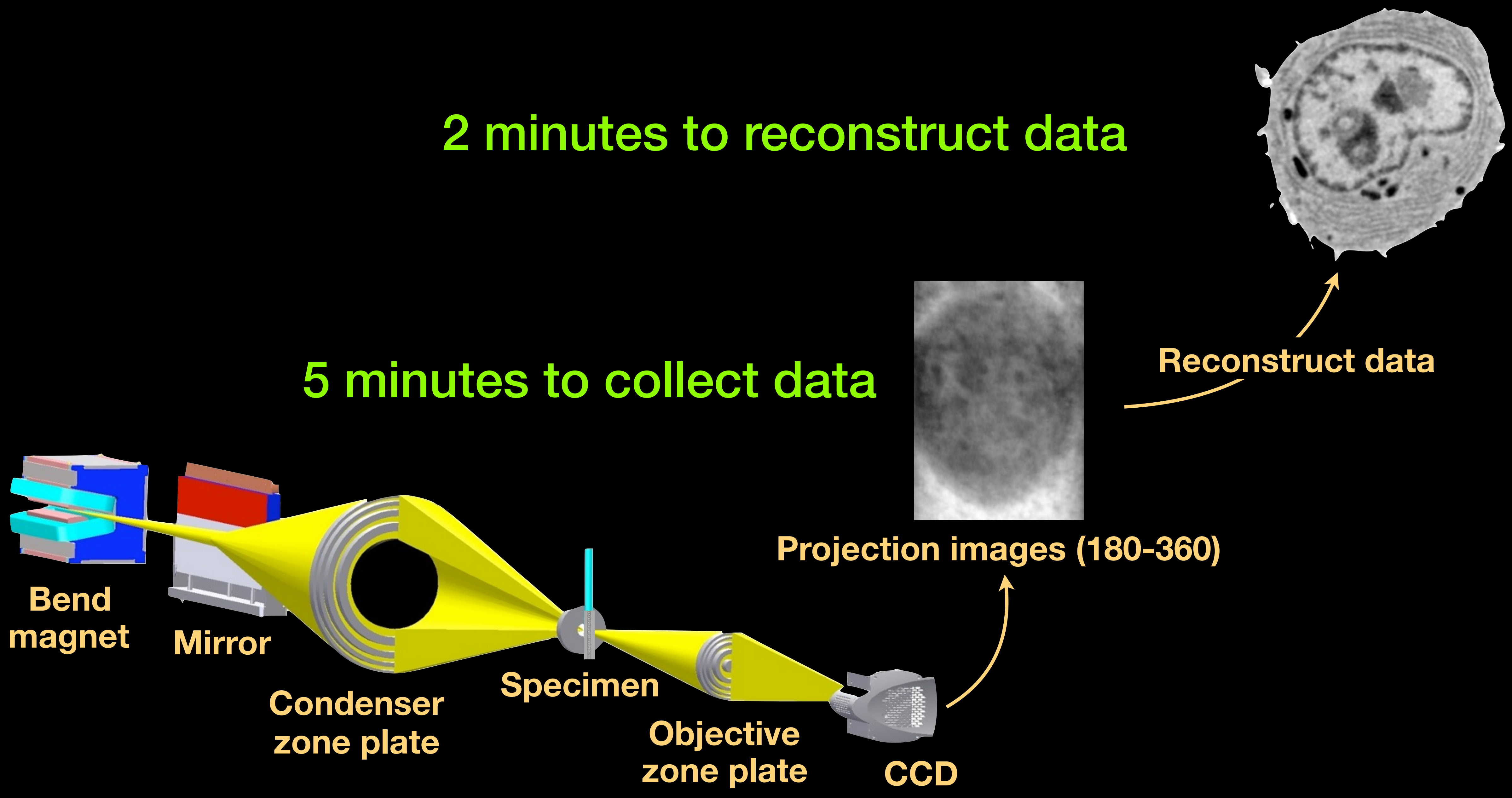
Absorption is linear with thickness & concentration



Specimen preparation



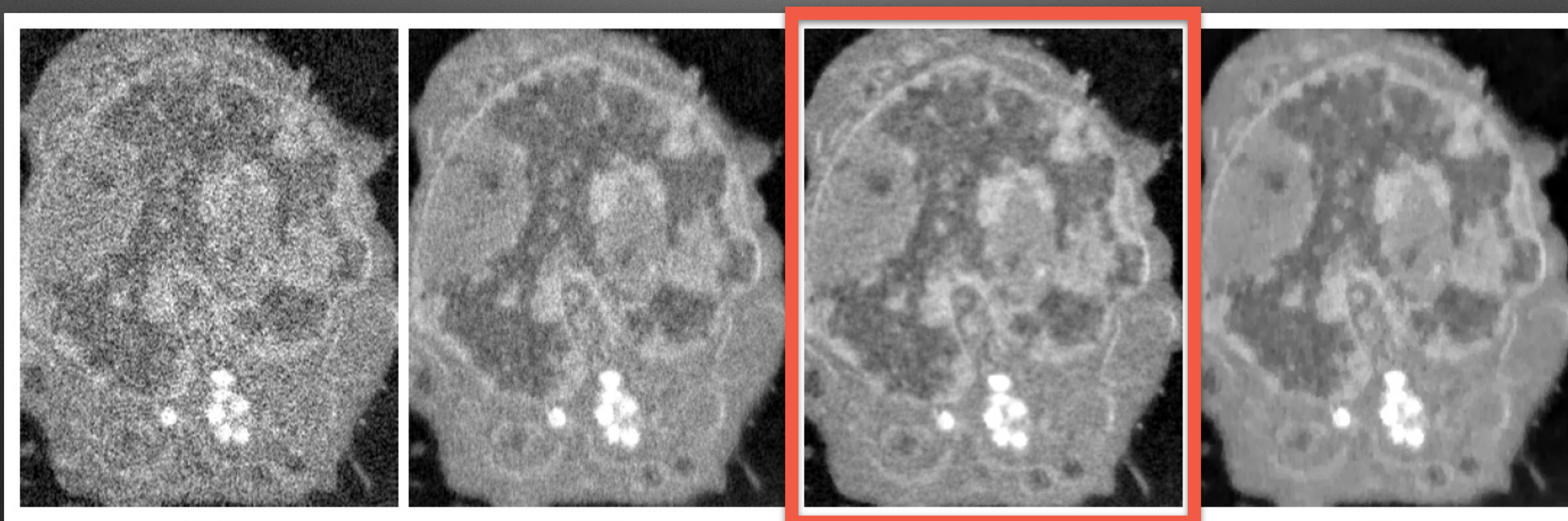
Soft X-ray Tomography



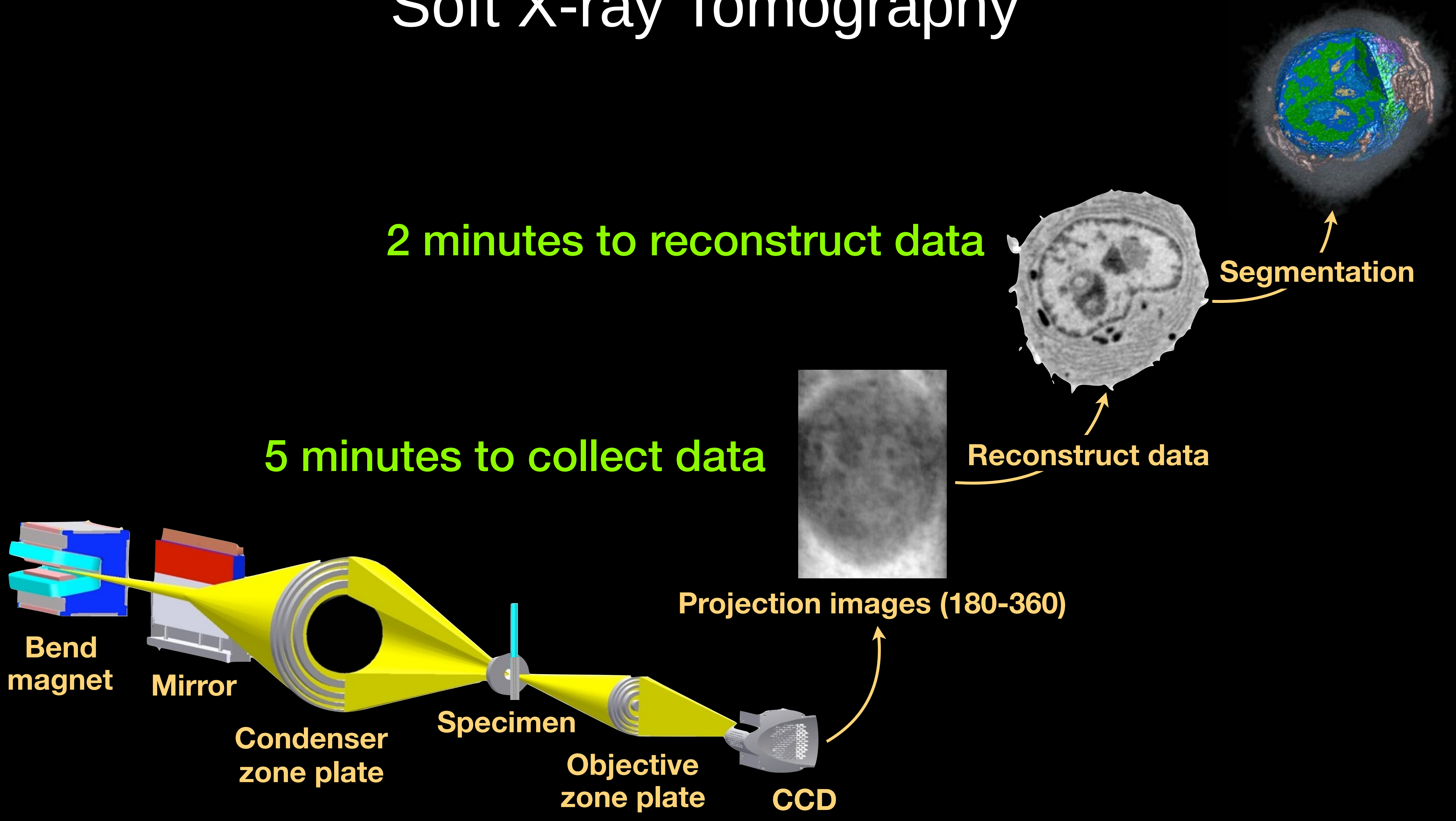
- Whole, hydrated cells in near-native state (cryo-immobilized)
- Natural, quantitative contrast; absorption of x-rays linear

Comparing reconstruction methods

Filtered back projection (FBP)	Conjugate Gradient Least Squares	Penalized-Likelihood	L1 regularized Conjugate Gradient Least Squares
Kremer et al. 1996	*Parkinson et al. 1996*	*Stayman & Fessler 2004*	*Vandeghinste et al. 2011)*



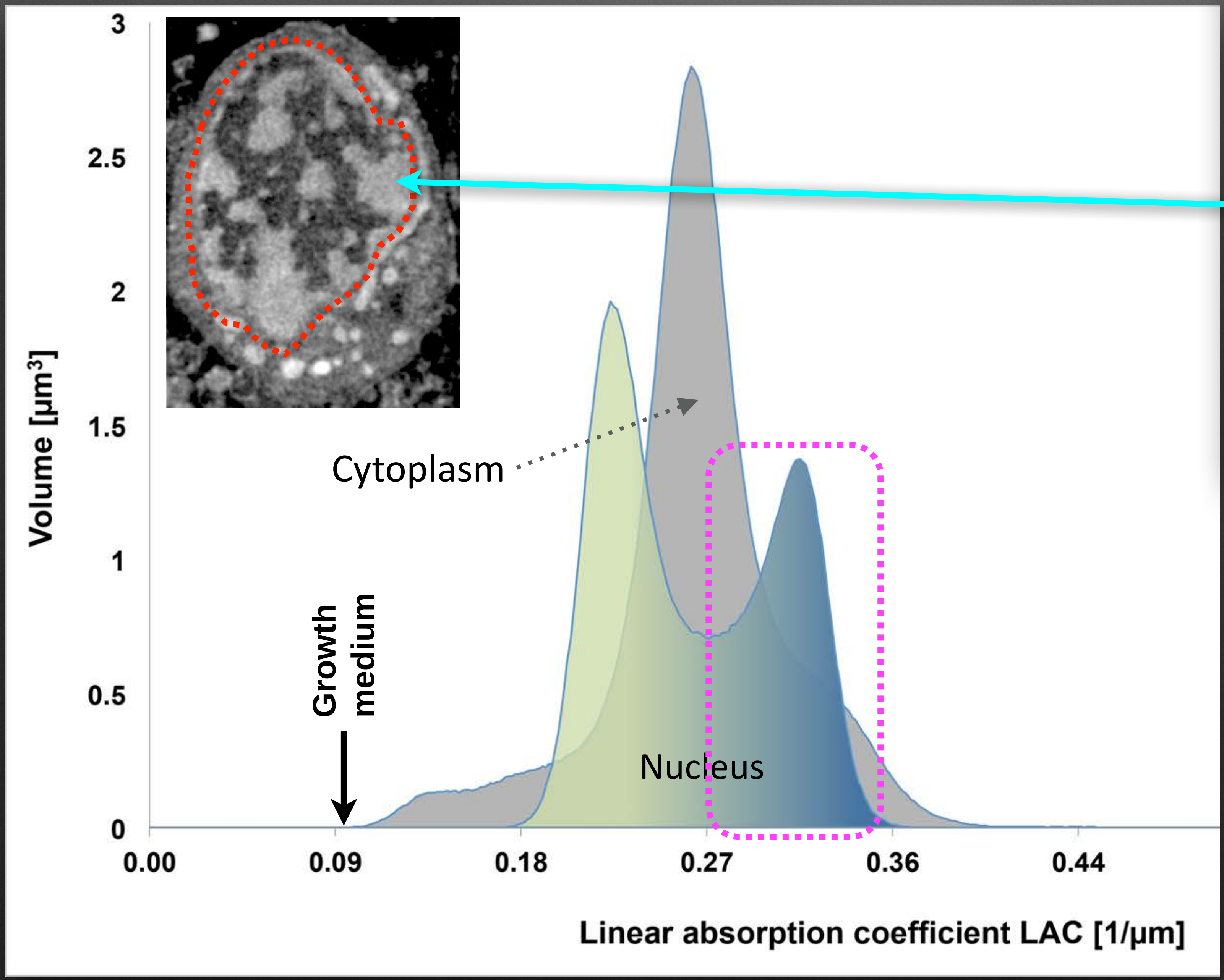
Soft X-ray Tomography

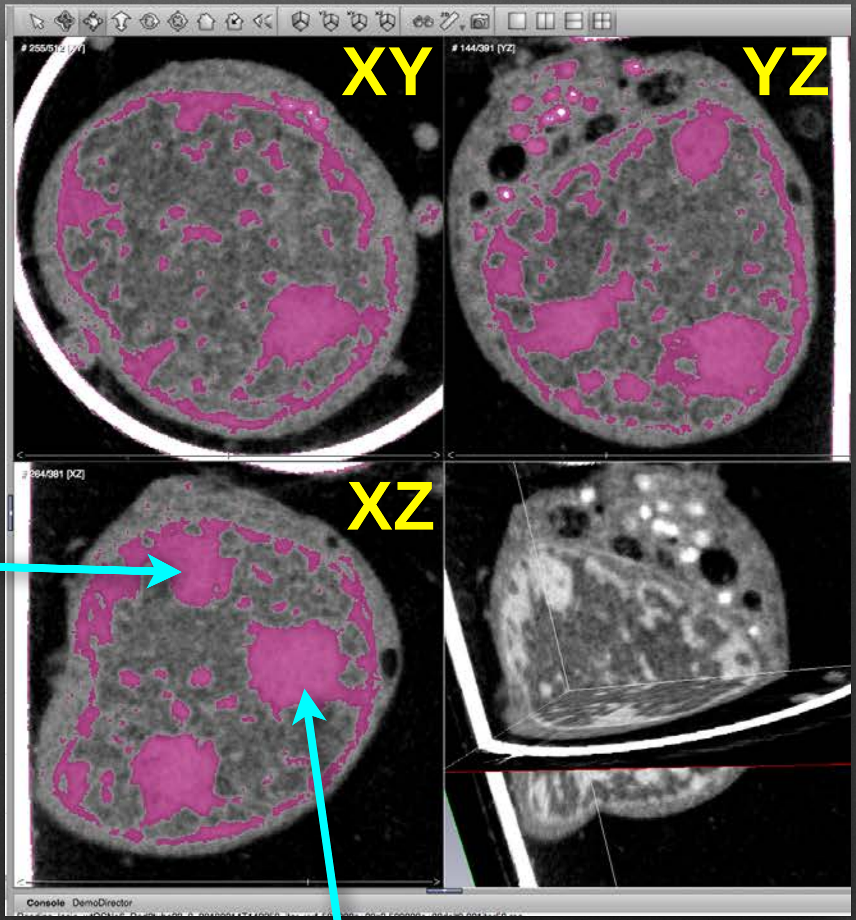


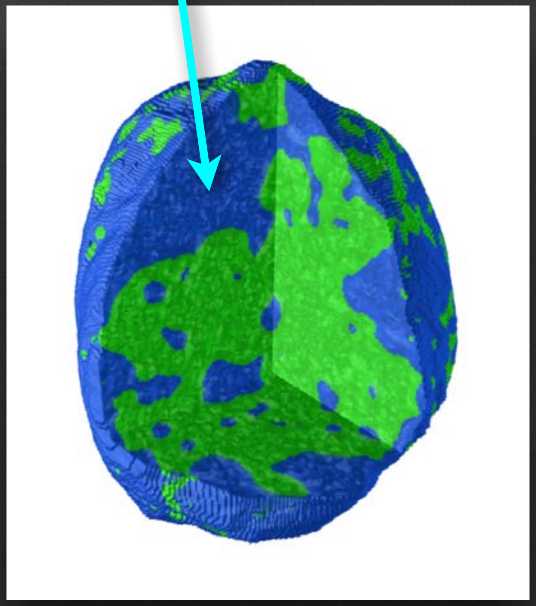
- Whole, hydrated cells in near-native state (cryo-immobilized)
- Natural, quantitative contrast; absorption of x-rays linear

Segmenting structures

Plot histogram of all voxels



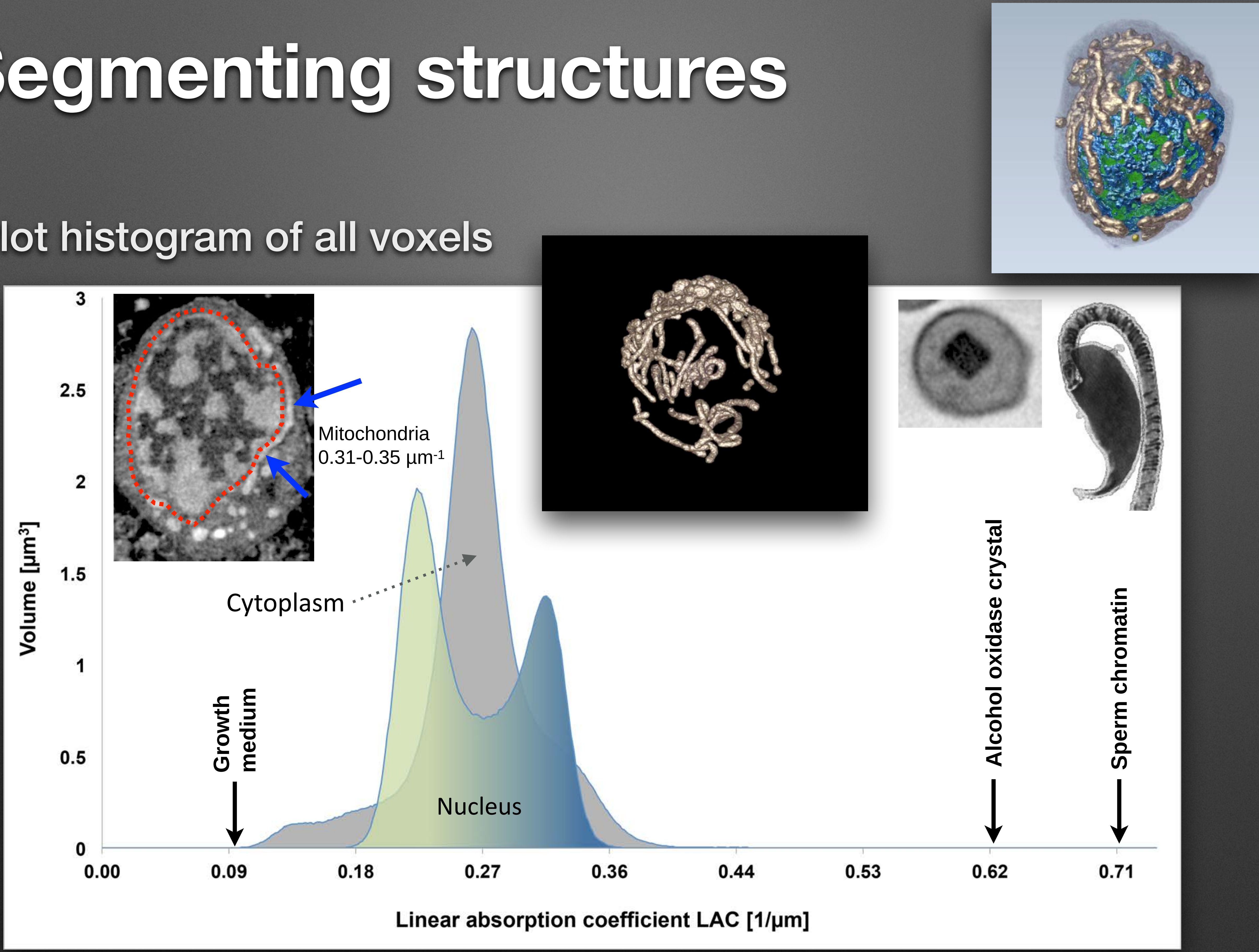




Le Gros et al. (2016) Cell Reports. 17(8), 2125-2136

Segmenting structures

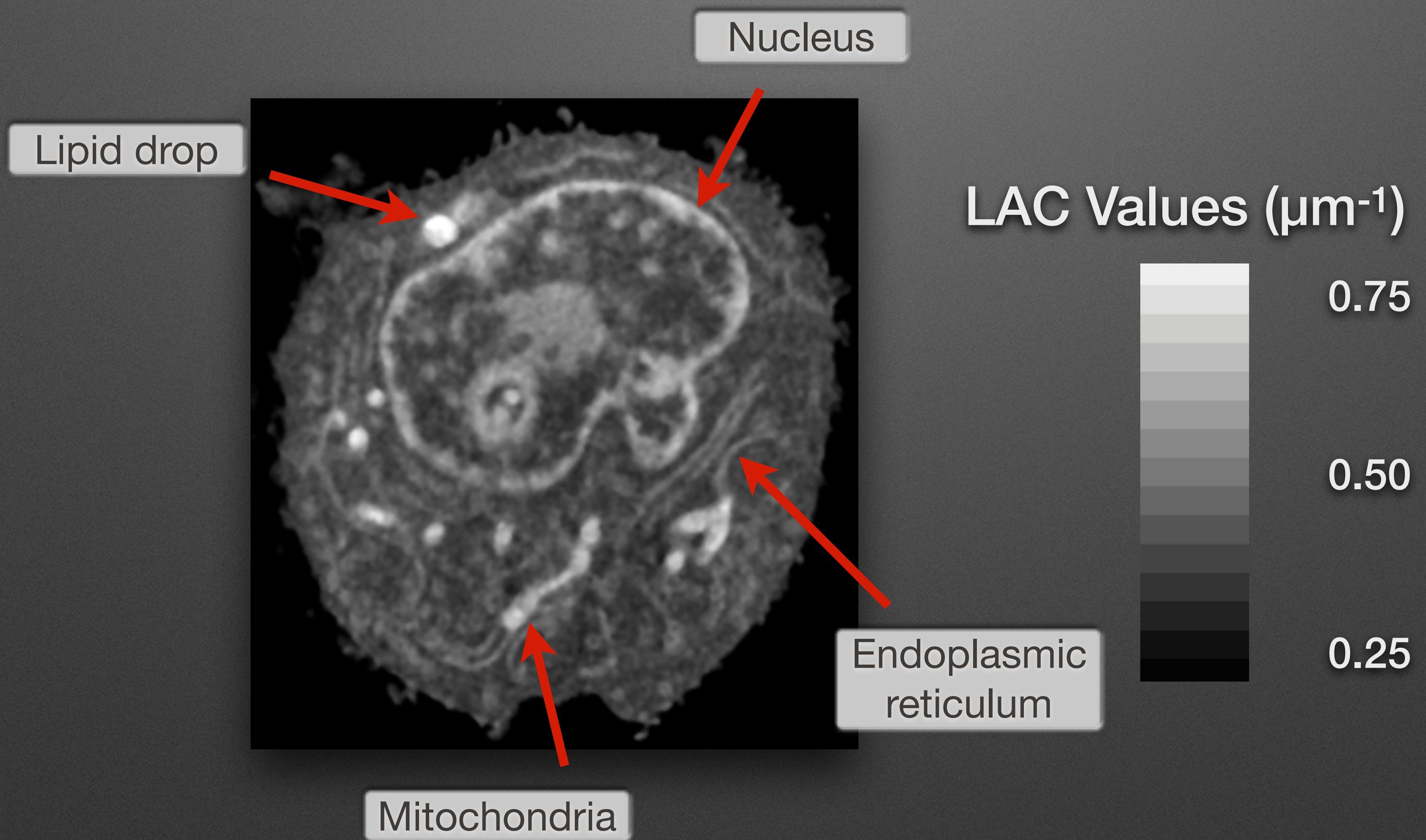
Plot histogram of all voxels



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

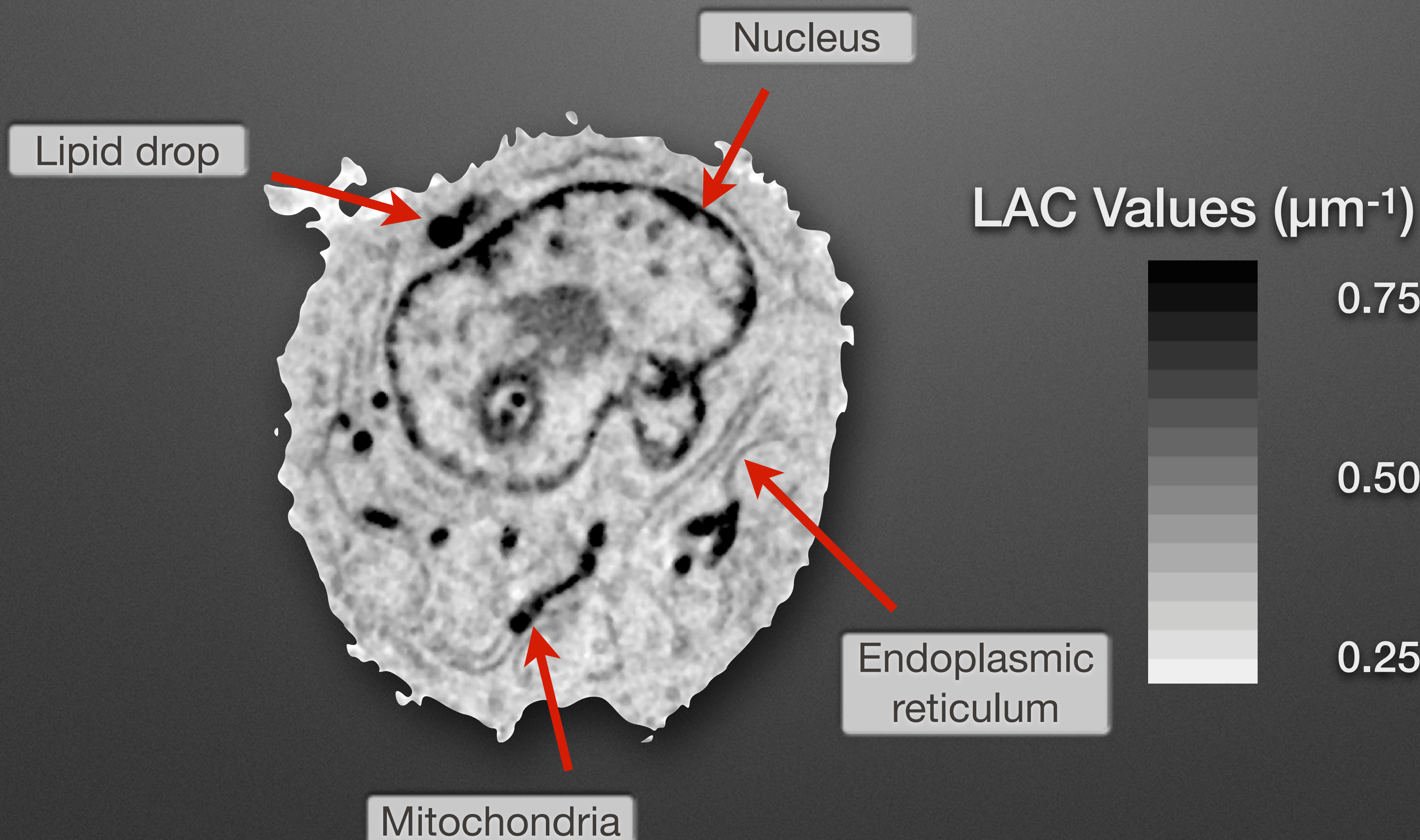
Contrast: Imaging in the ‘water window’

Absorption is linear with thickness & concentration

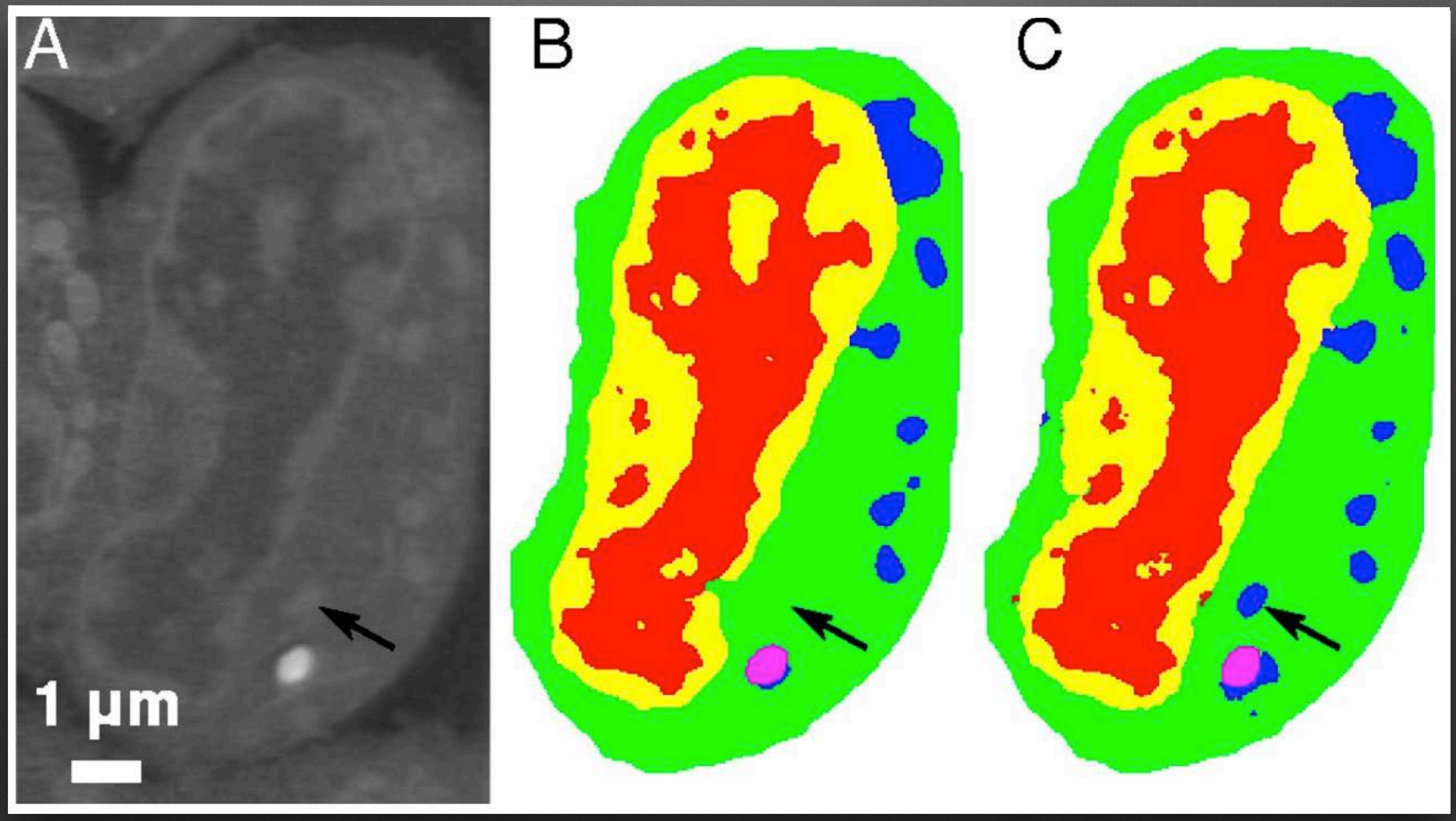


Contrast: Imaging in the 'water window'

Absorption is linear with thickness & concentration



Segmentation - machine learning

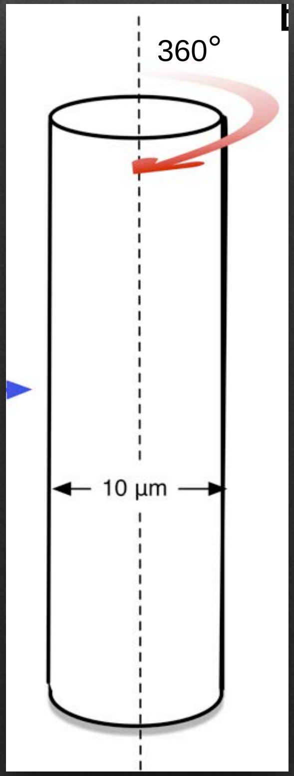


Pelt & Sethian. (2018). PNAS doi/10.1073/PNAS.1715832114.

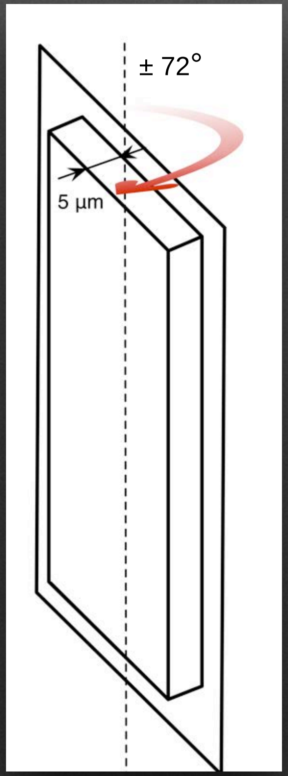
Isotropic resolution

Full rotation vs. limited tilt

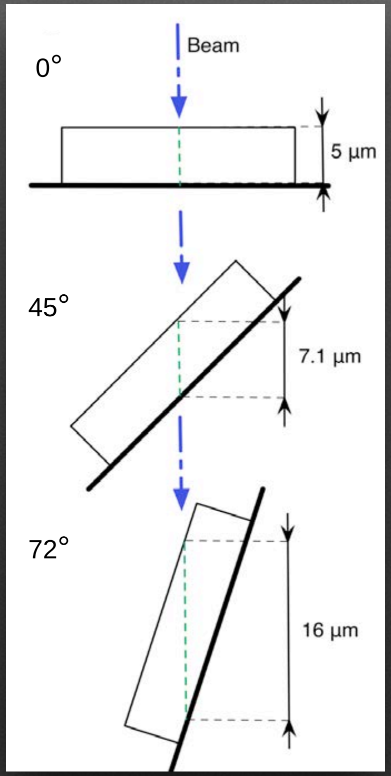
± 90°



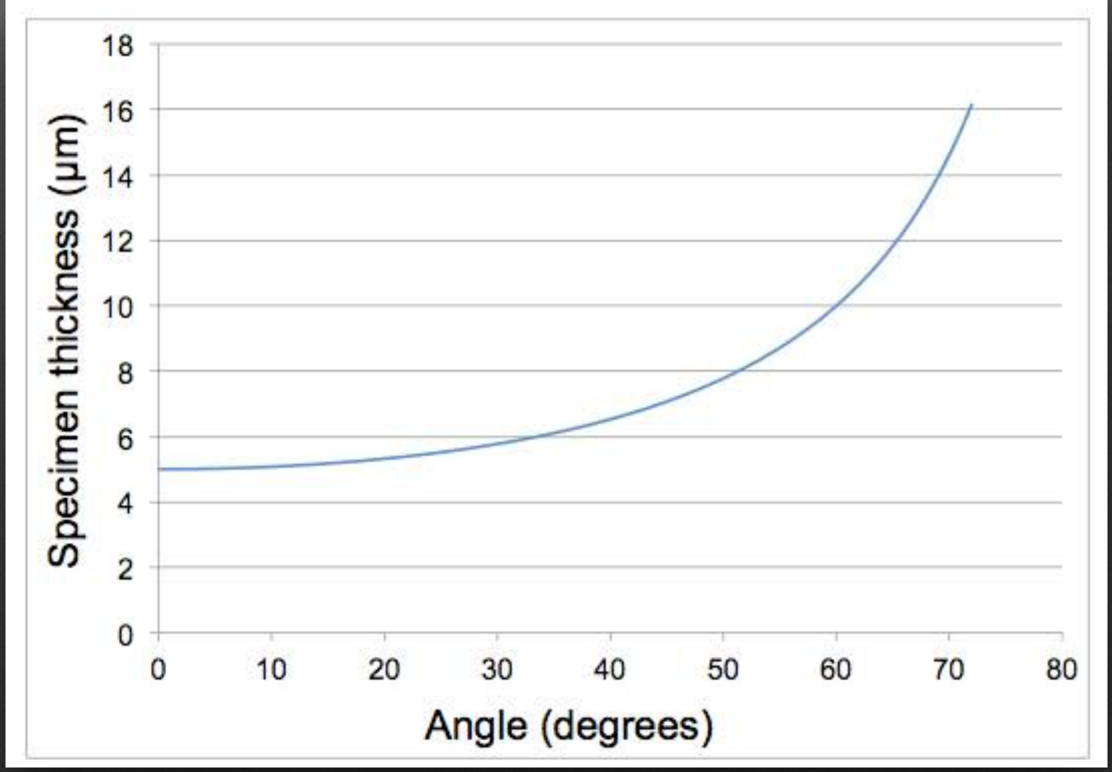
± 75°



16 µm thick
at ± 75°



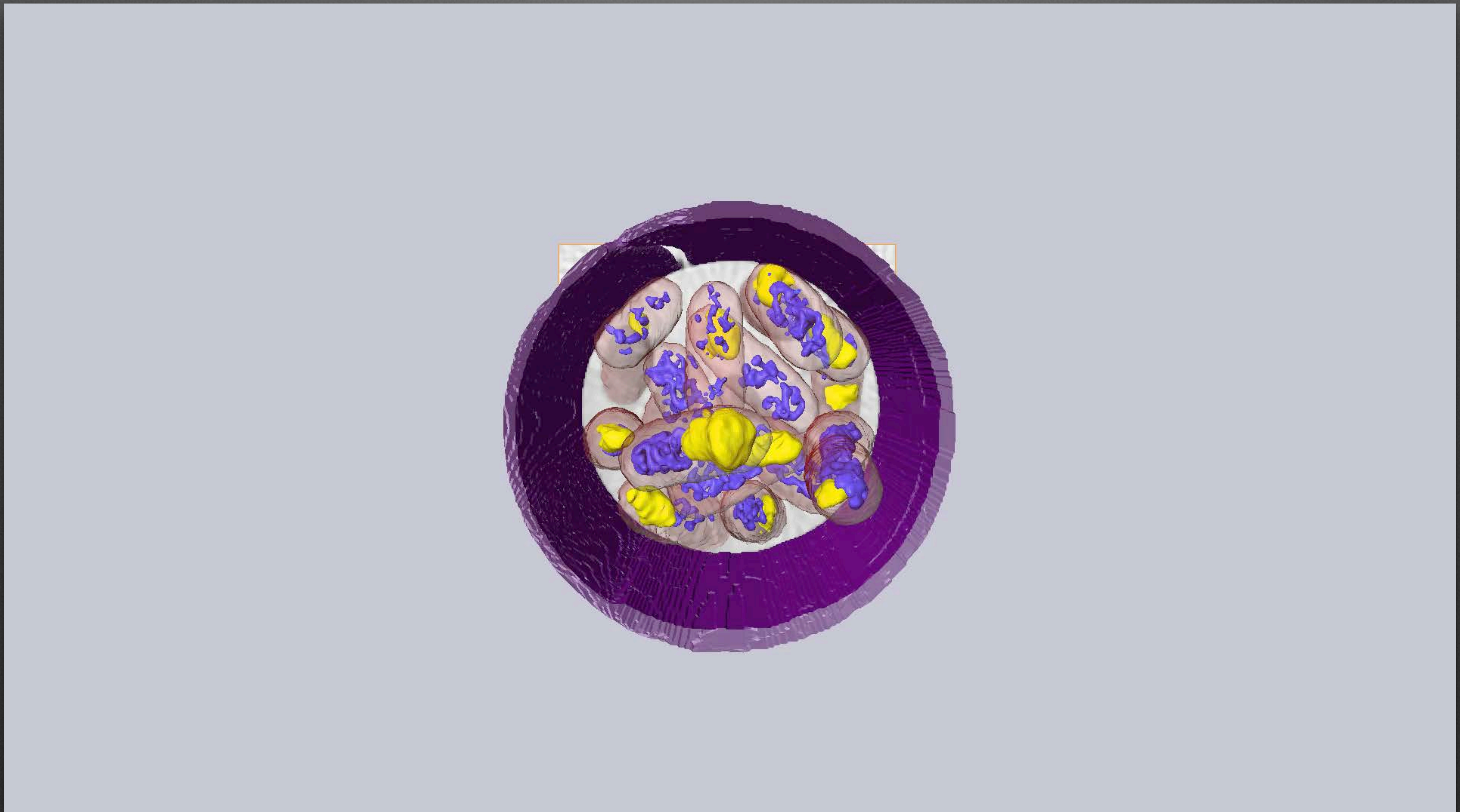
Specimen thickness
increases with tilt



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

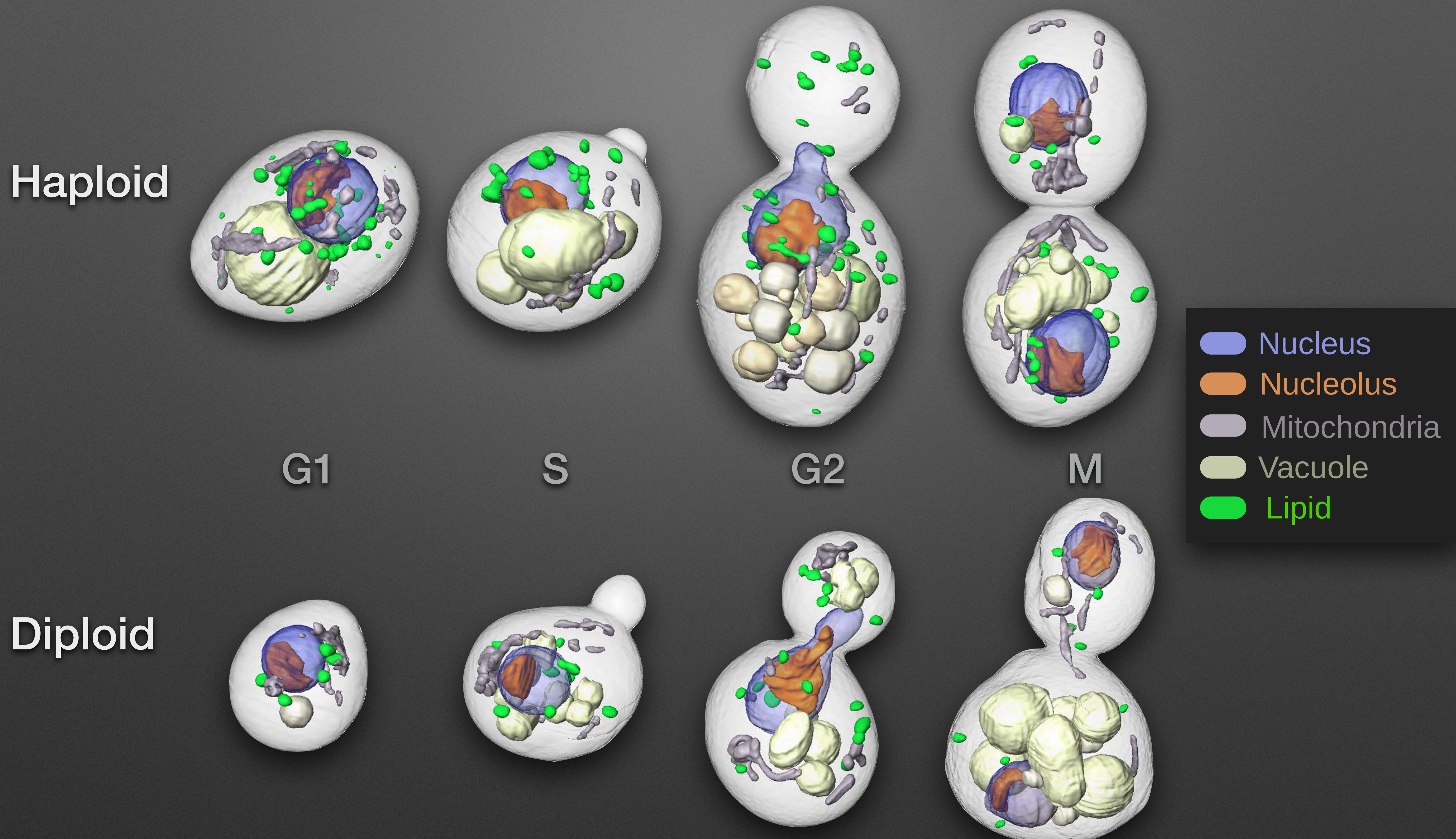
Biological applications

Segmentation of *E. coli*



YFP, inclusion bodies

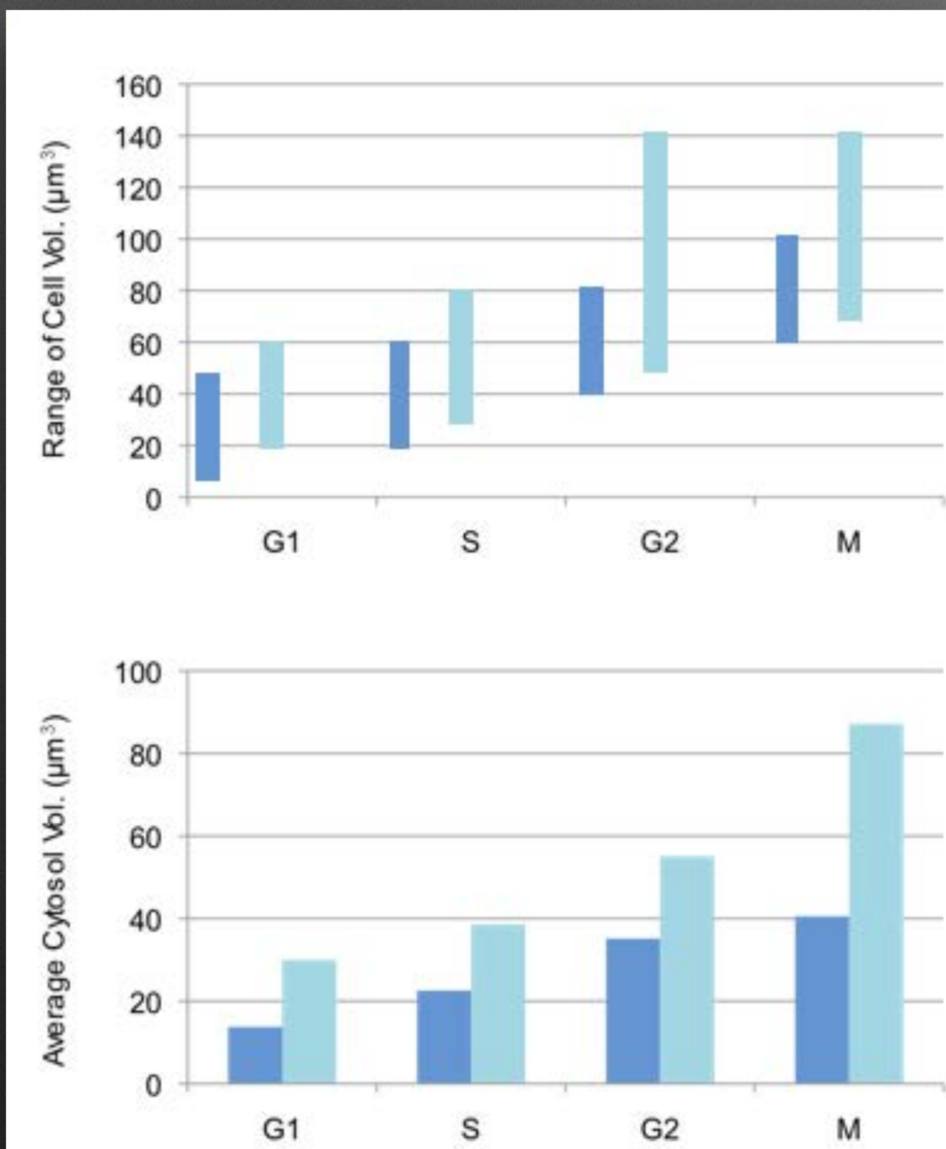
Structural organization of *S. cerevisiae*



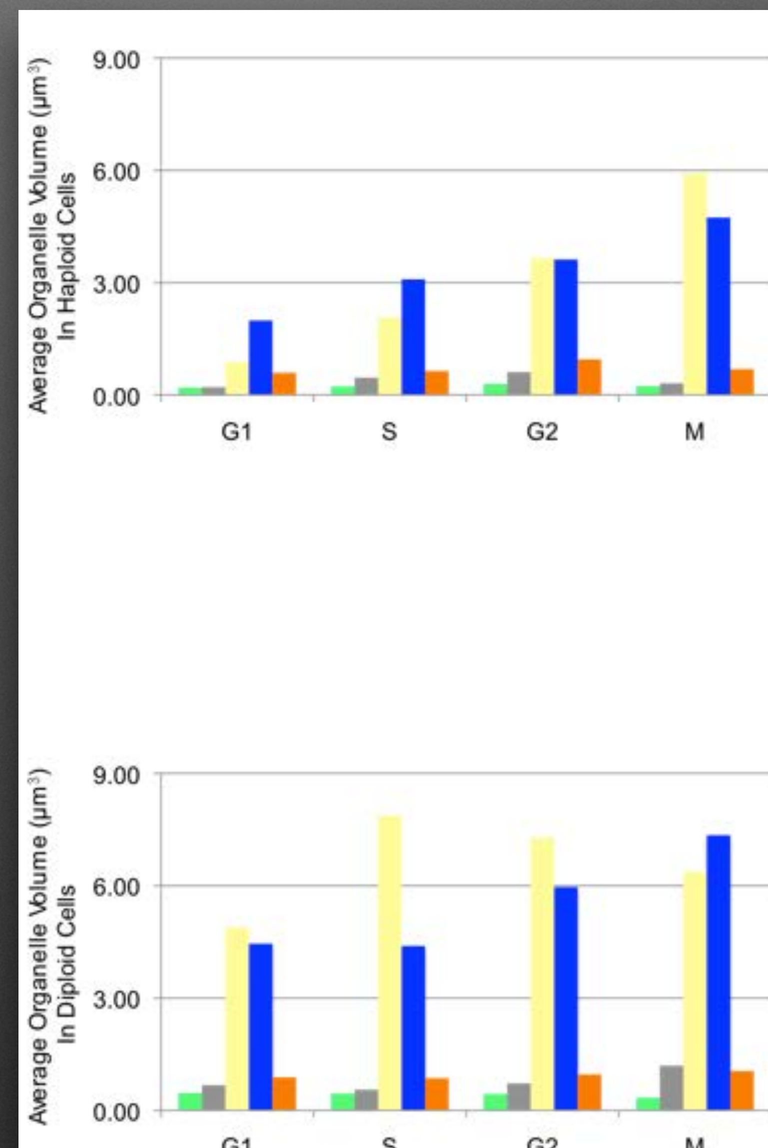
Uchida et al. (2011) Yeast. 28, 227-236

Quantitative analysis of *S. cerevisiae*

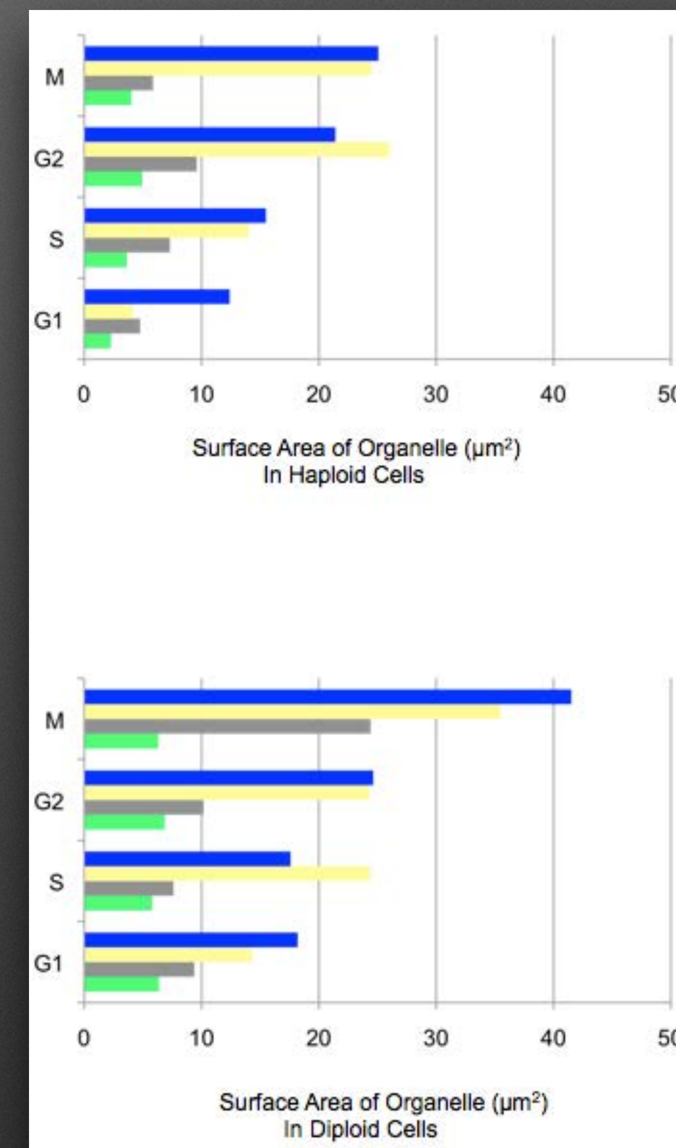
Cell volume



Organelle volume



Organelle surface area

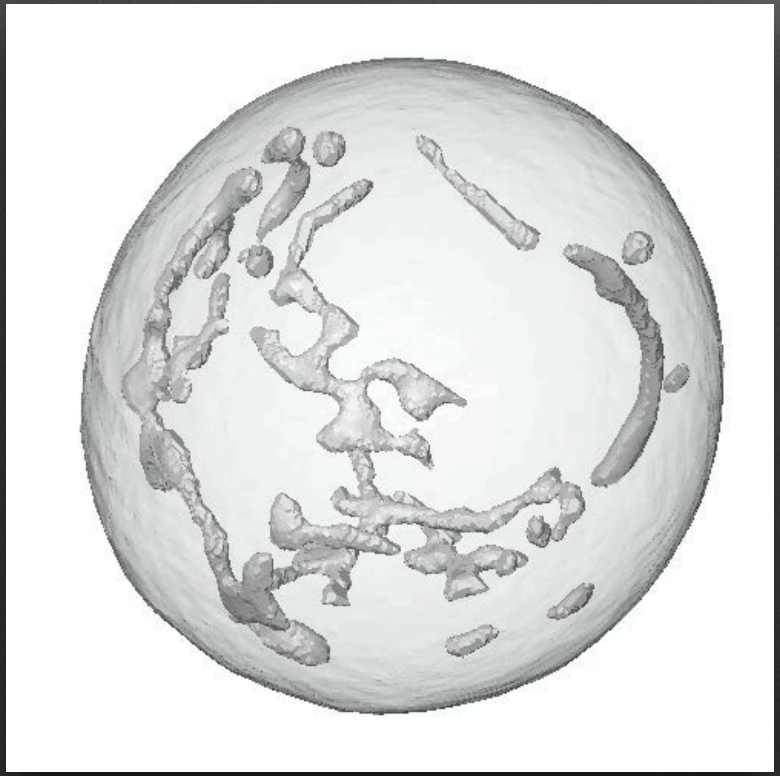


Uchida et al. (2011) Yeast. 28, 227-236

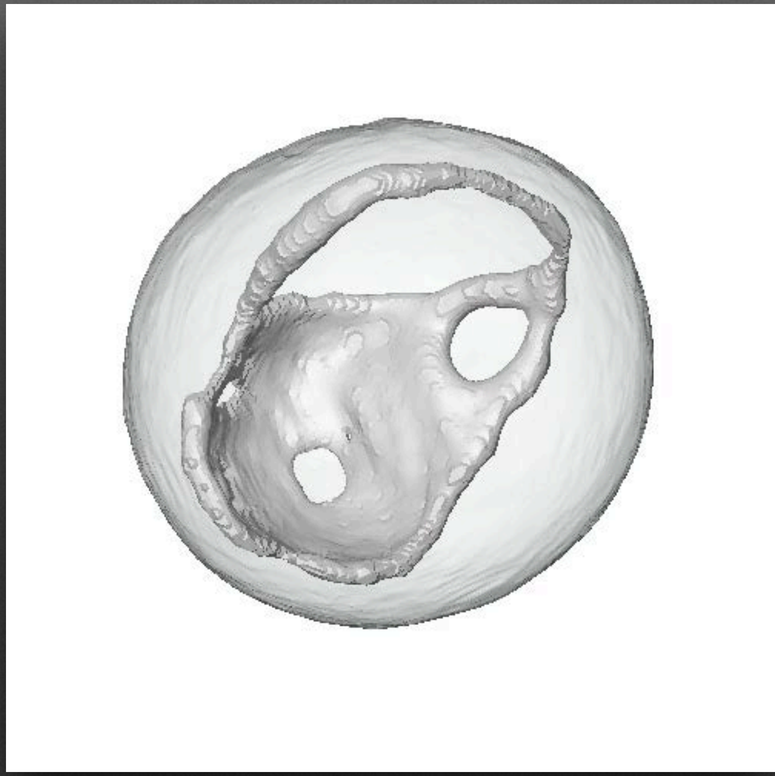
Phenotypic consequences of genetic knockouts

Mitochondria

Wildtype

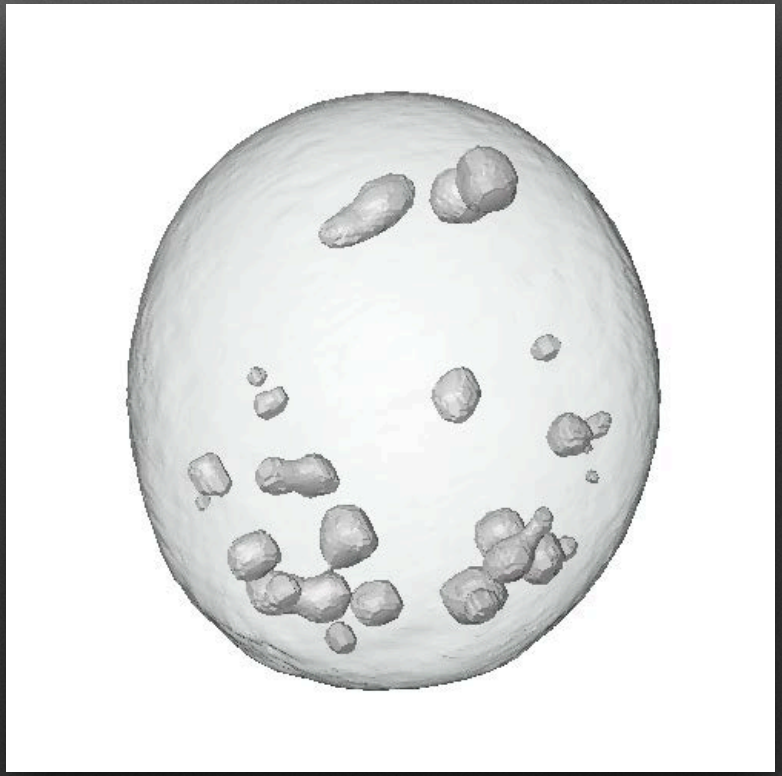


dnm1 KO



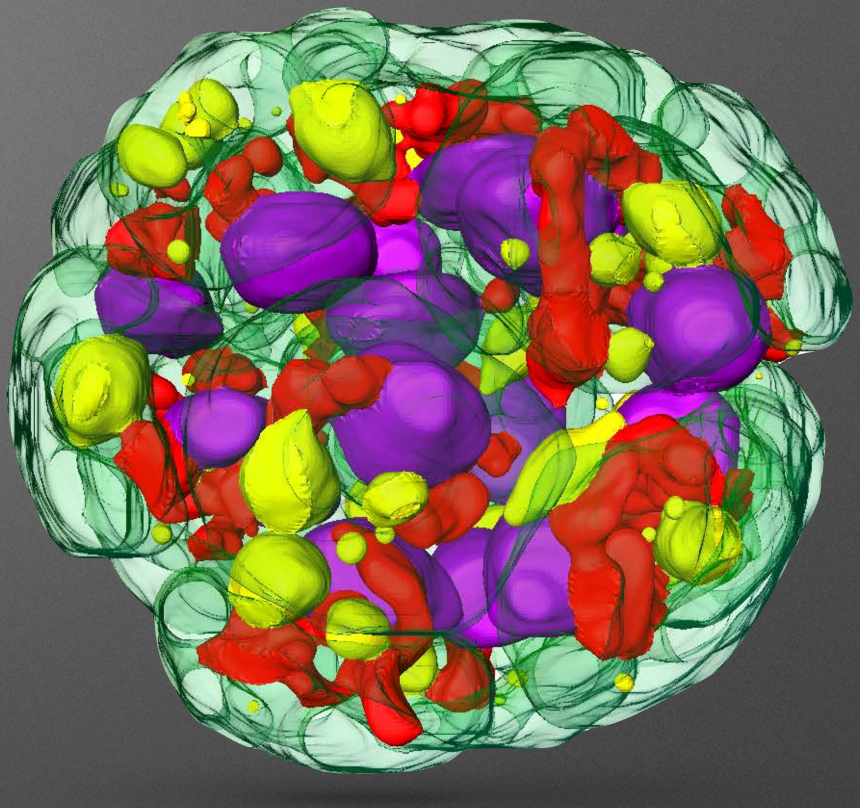
Blocks fission

fzo1 KO



Blocks fusion

Photosynethisis, Bioenergy



Krishna Niyogi

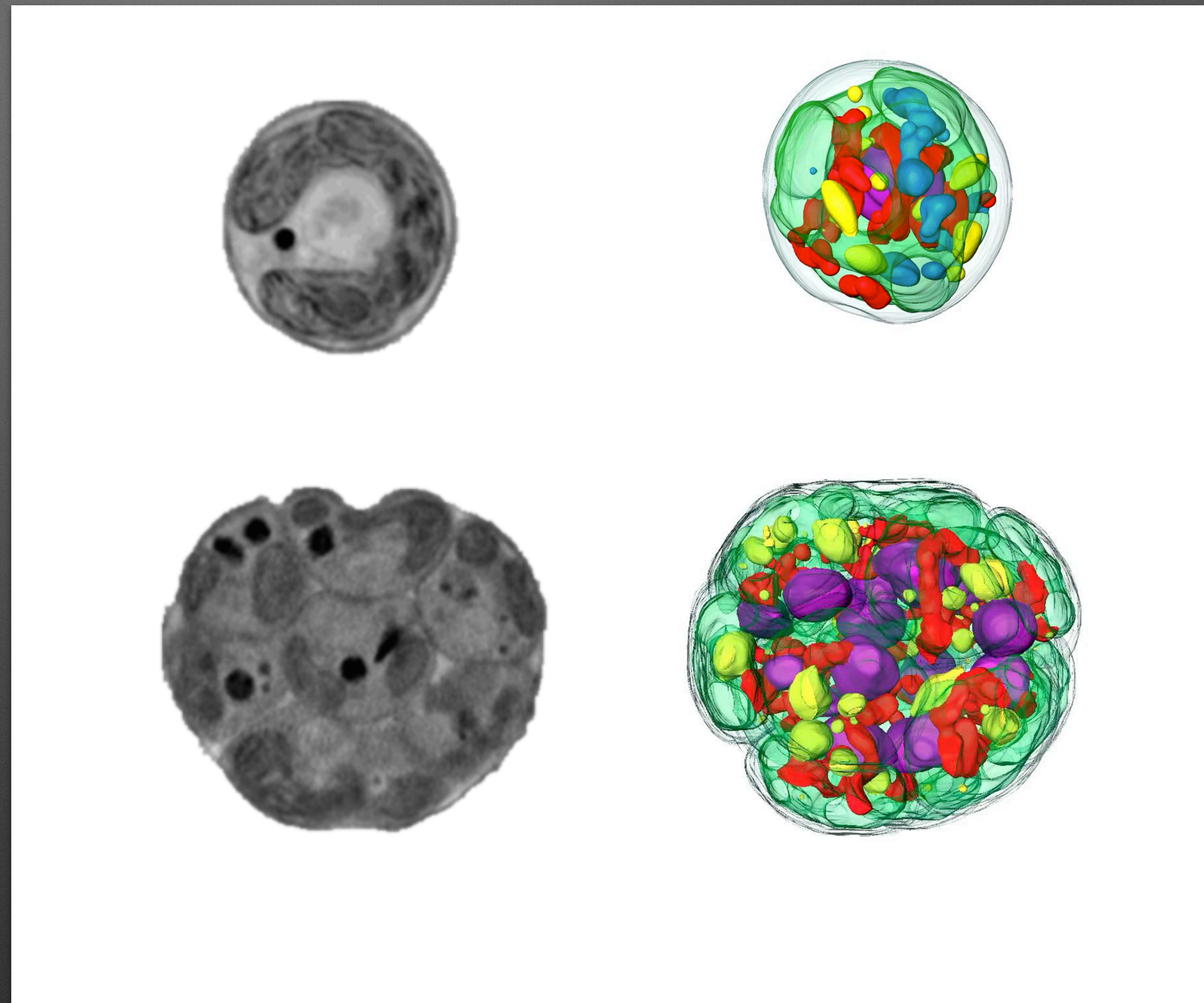
University of California

Berkeley & HHMI

Chromochloris zofingiensis

Single cell

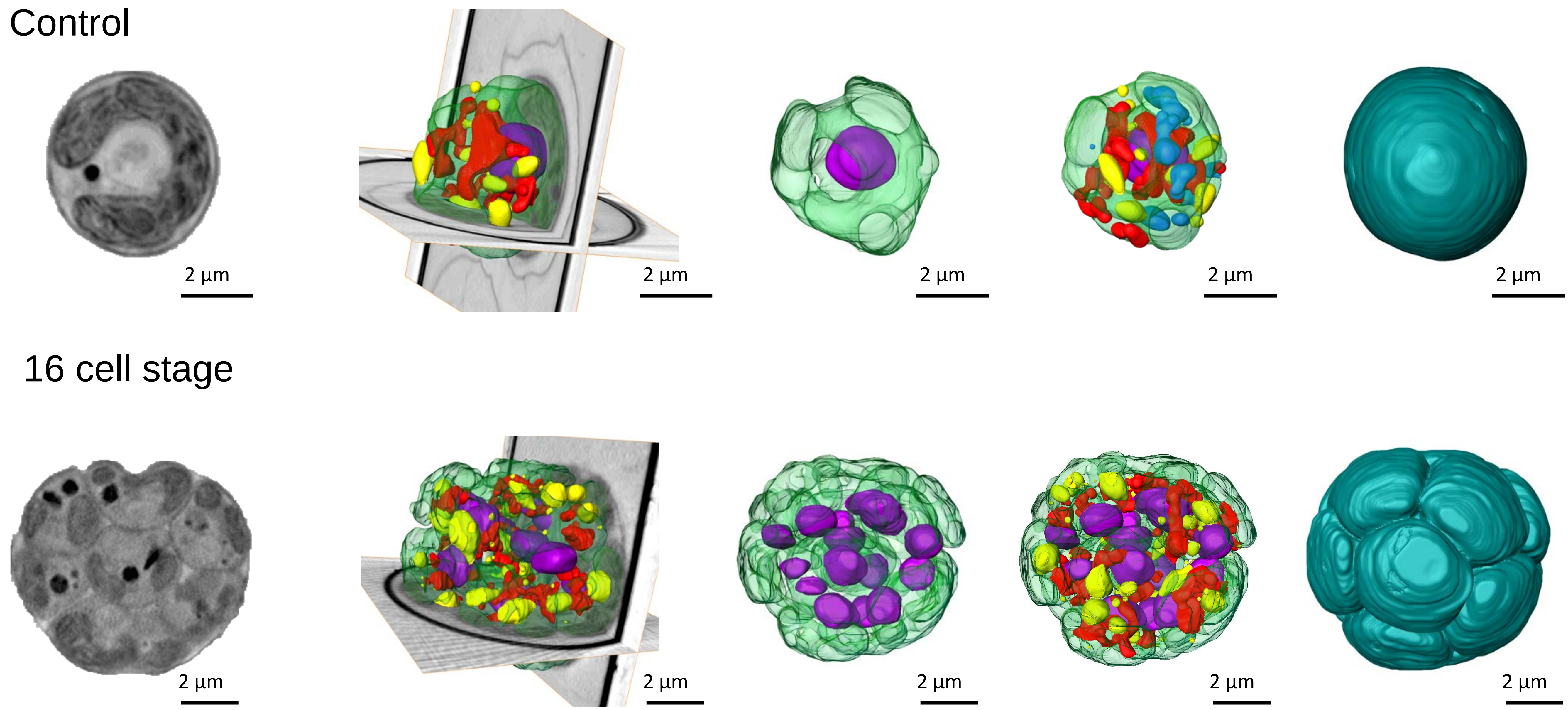
16 cells



- Chloroplast
- Starch
- Mitochondria
- Nucleus
- Lipid bodies

Roth et al. (2017) PNAS. E4296-E4305, doi/10.1073/pnas.1619928114

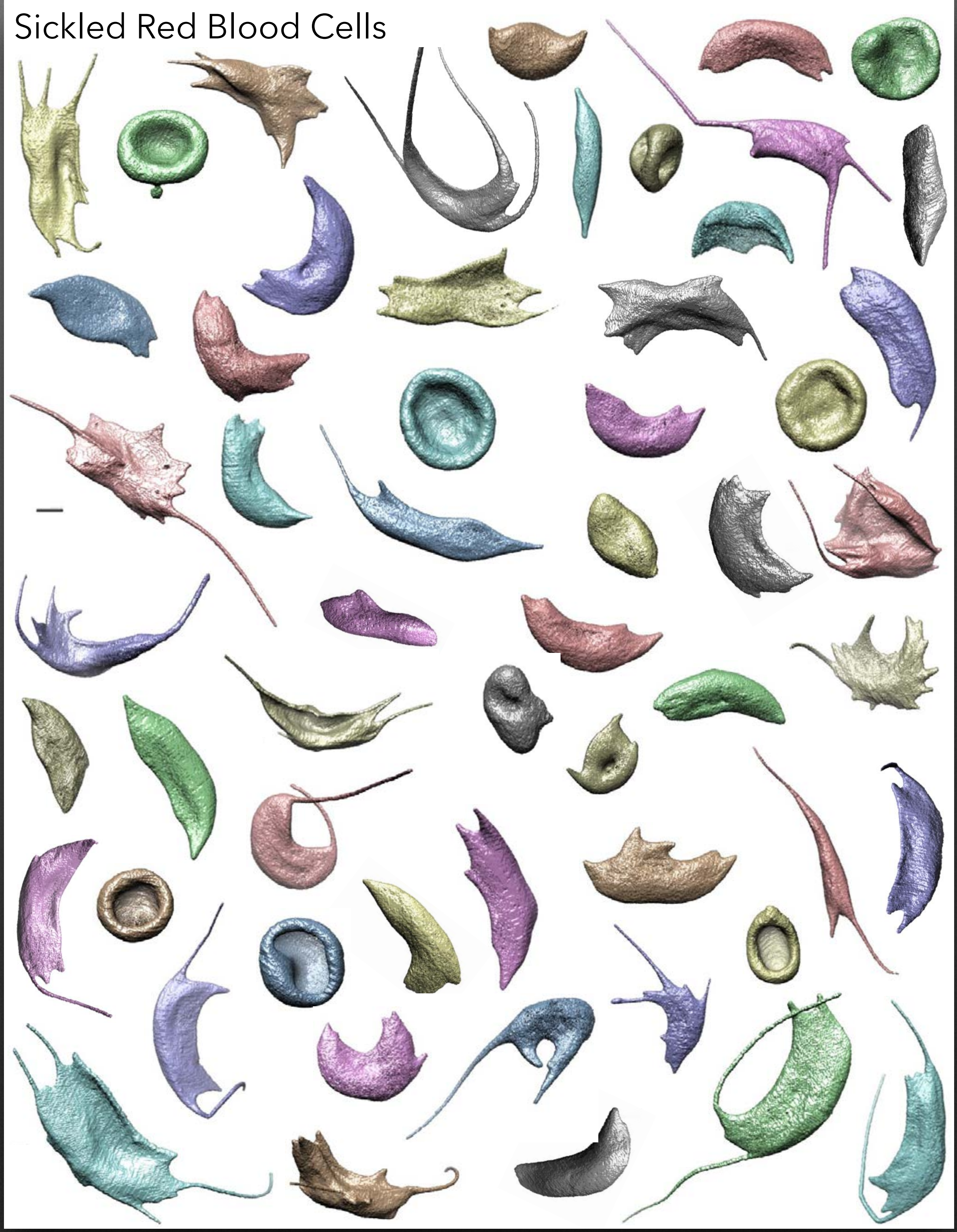
Chromochloris zofingiensis



- Chloroplast
- Starch
- Mitochondria
- Nucleus
- Lipid bodies

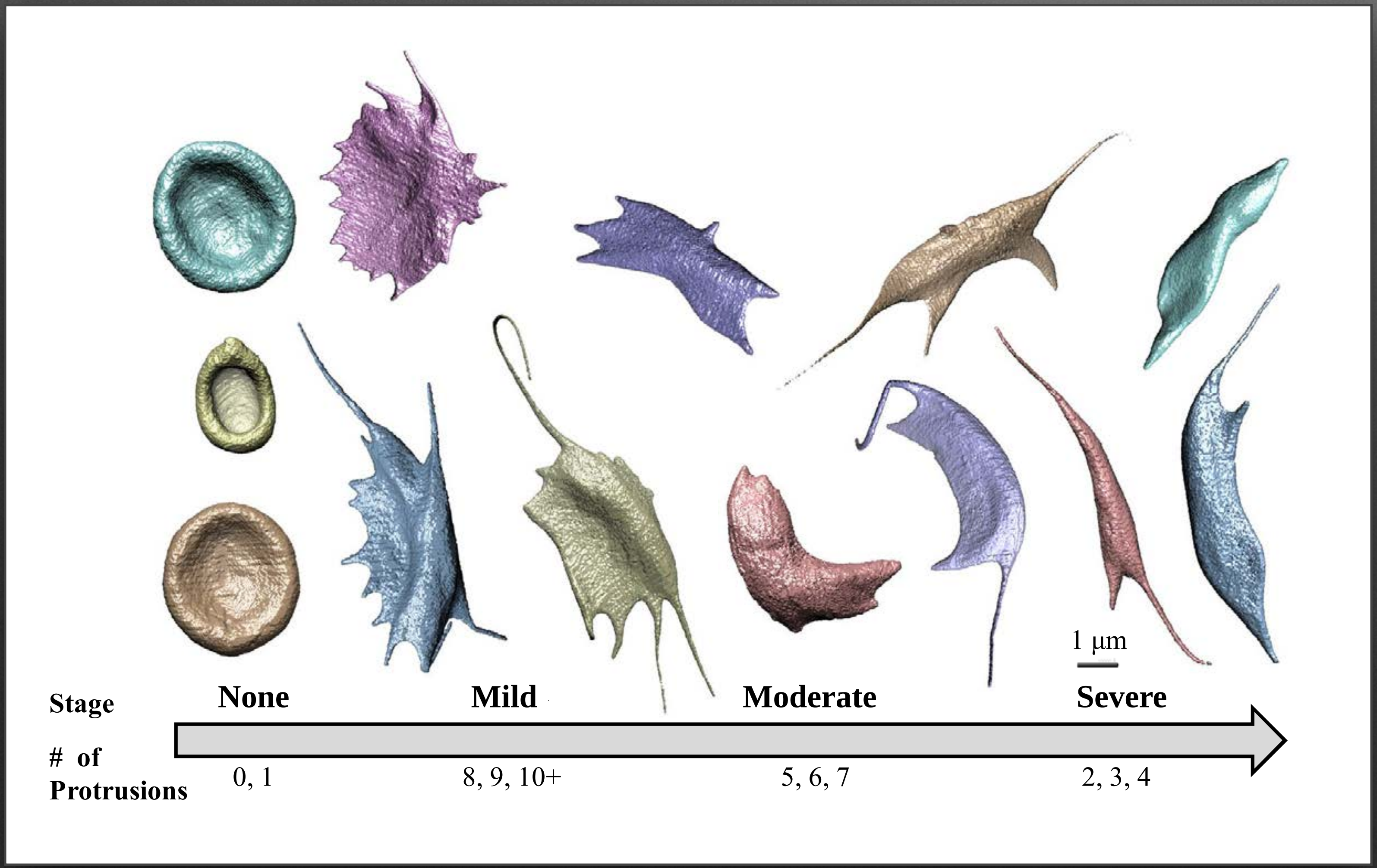
Roth et al. (2017) PNAS. E4296-E4305, doi/10.1073/pnas.1619928114

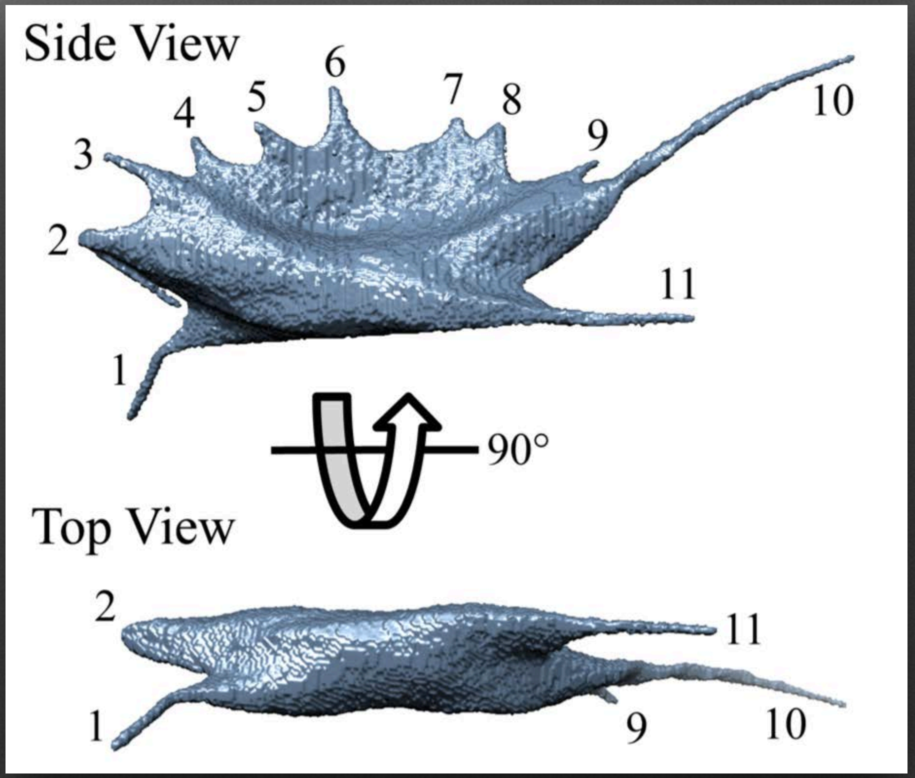
Testing drugs to treat sickle cell disease



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

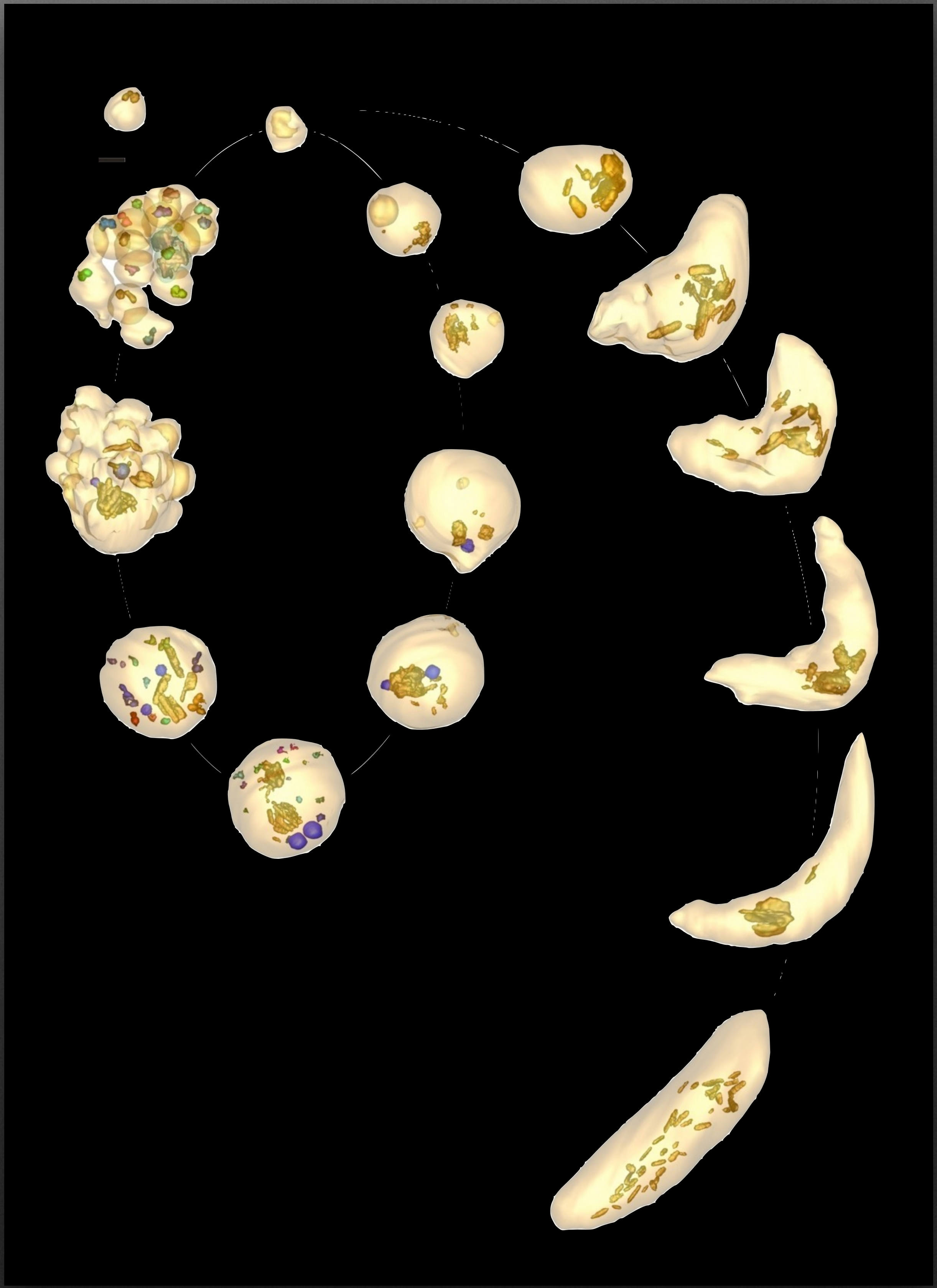
Malaria-infected red blood cells

Leann Tilley

Eric Hanssen

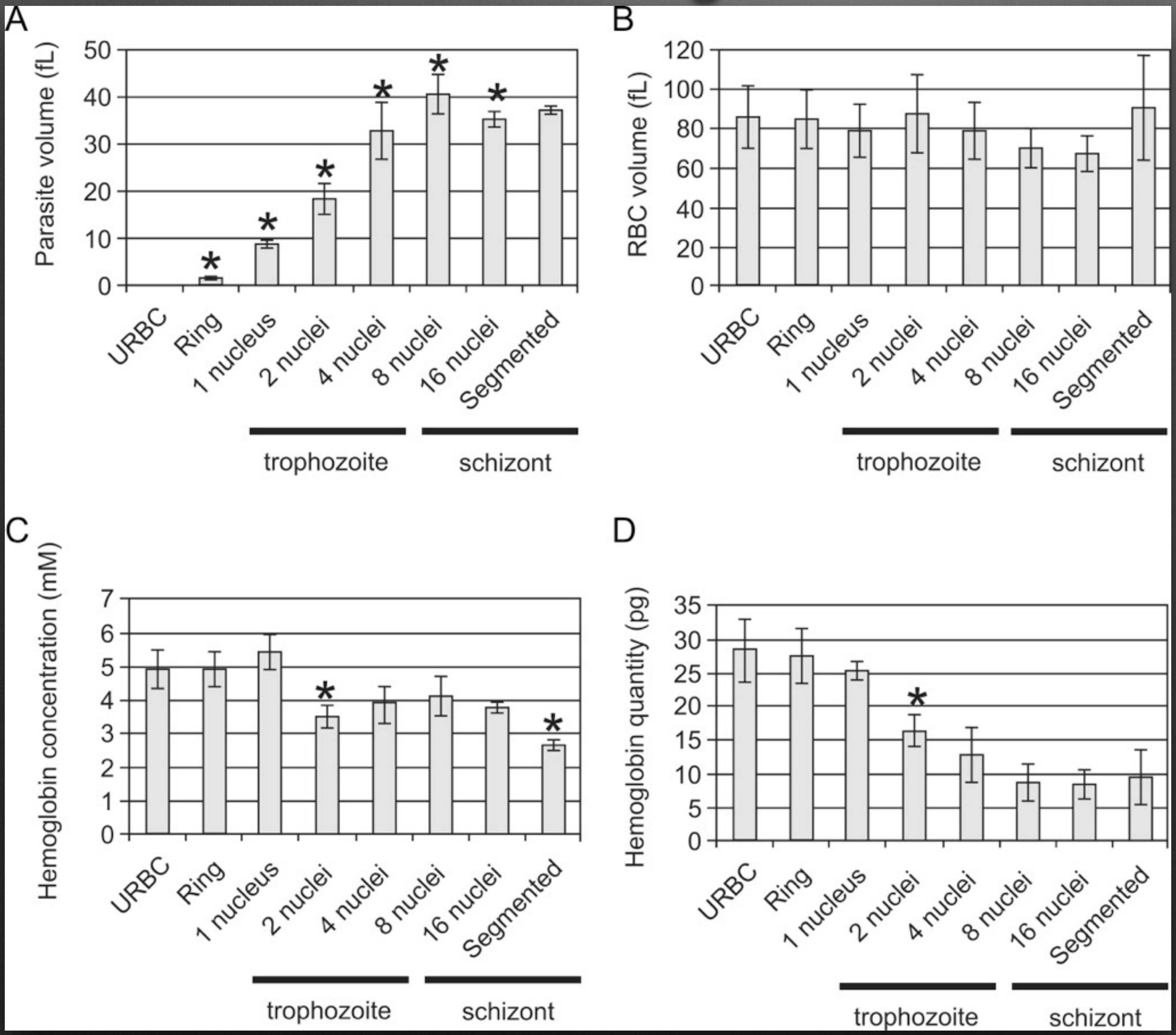
University of Melbourne

Australia

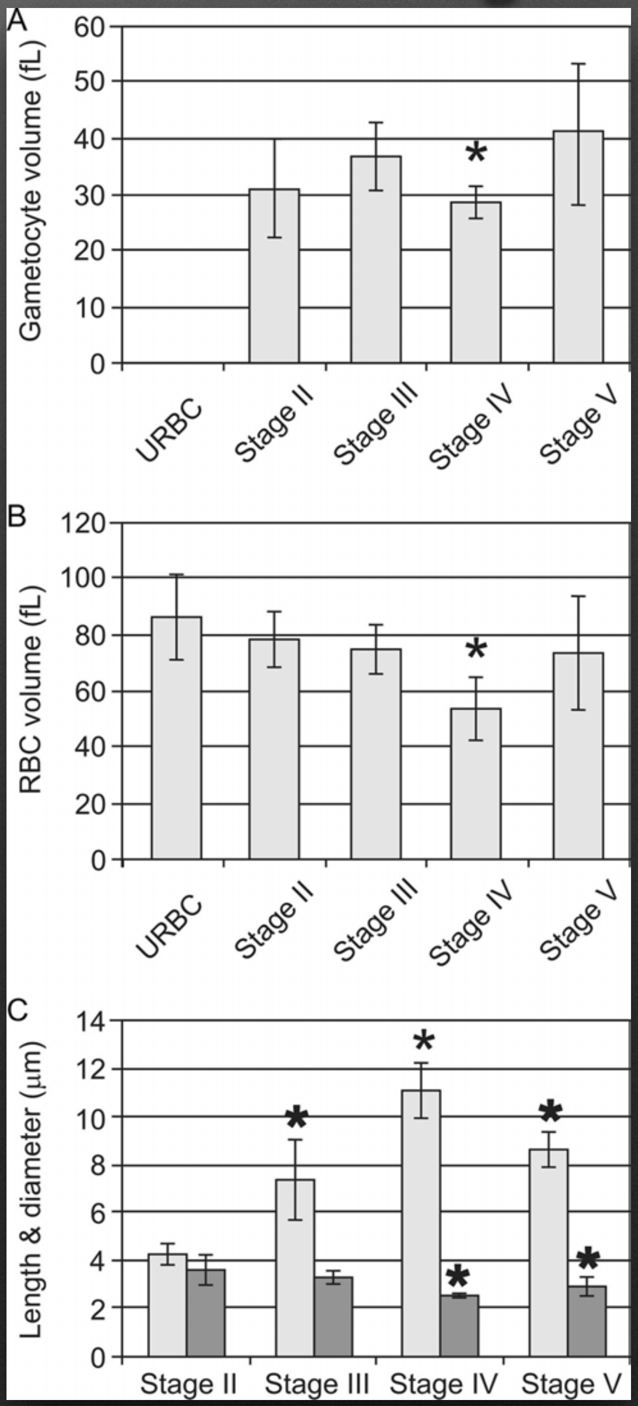


Malaria-infected RBC

Sexual stage



Asexual stage

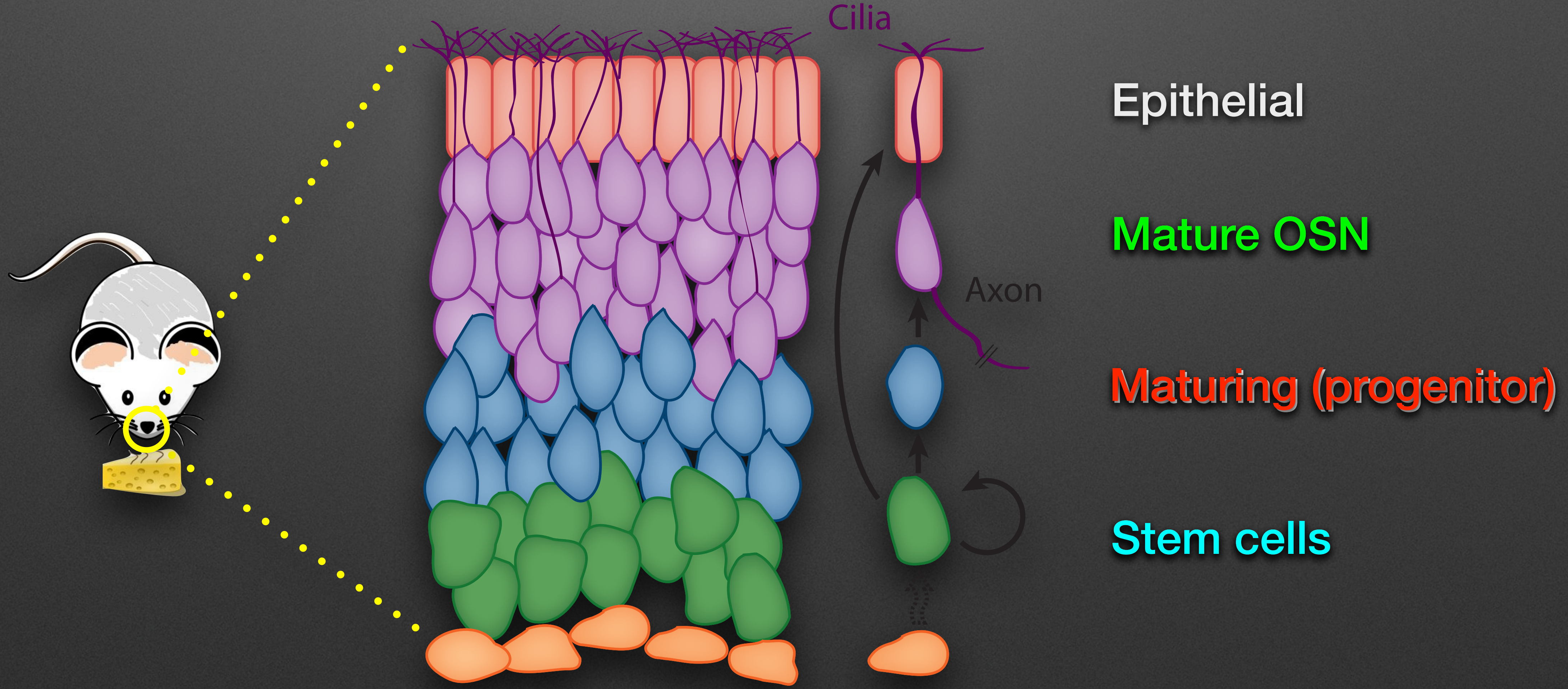


Hanssen et al. (2012) J. Structural Biol. 177, 224-232

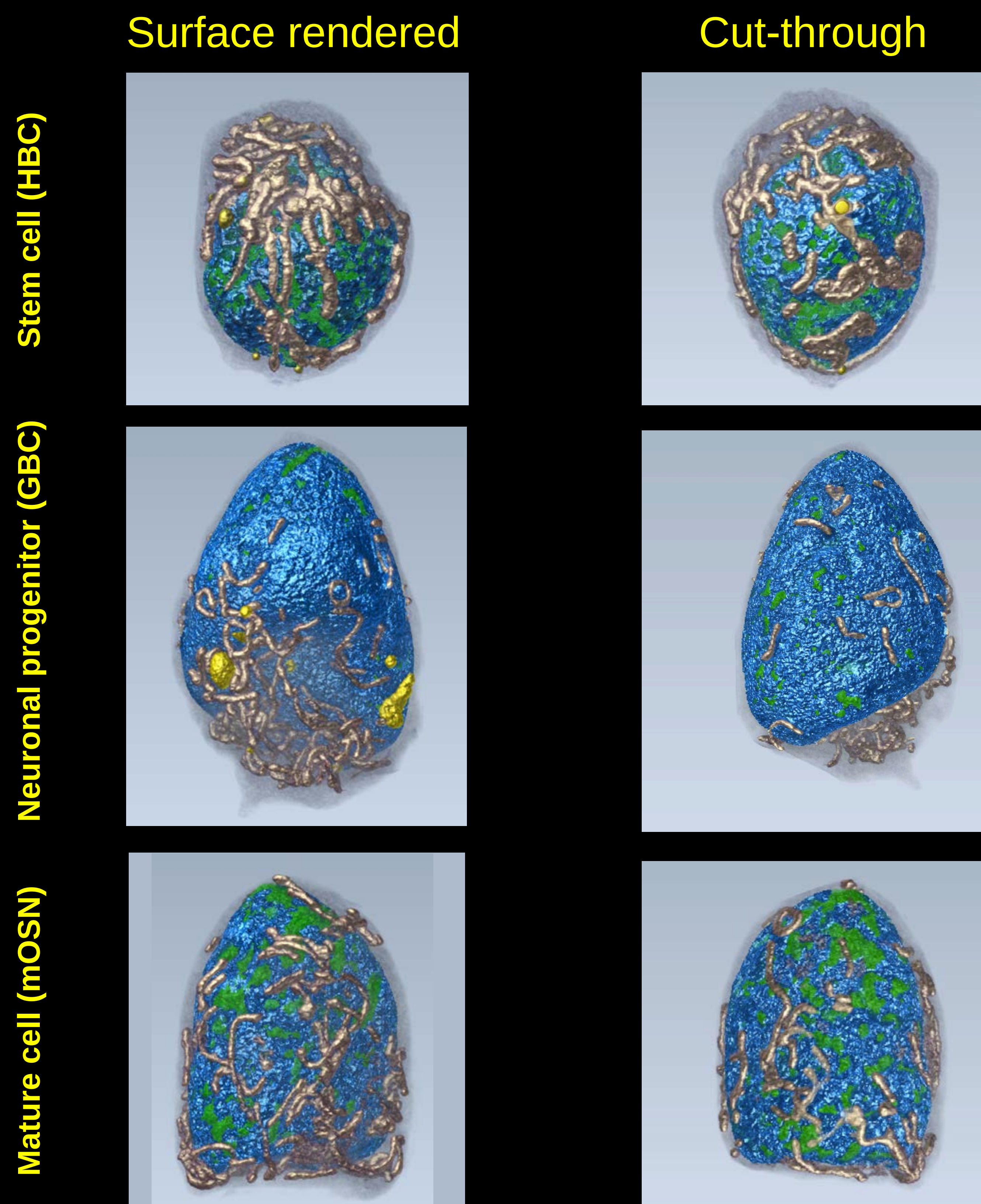
Role of nuclear organization in gene expression

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

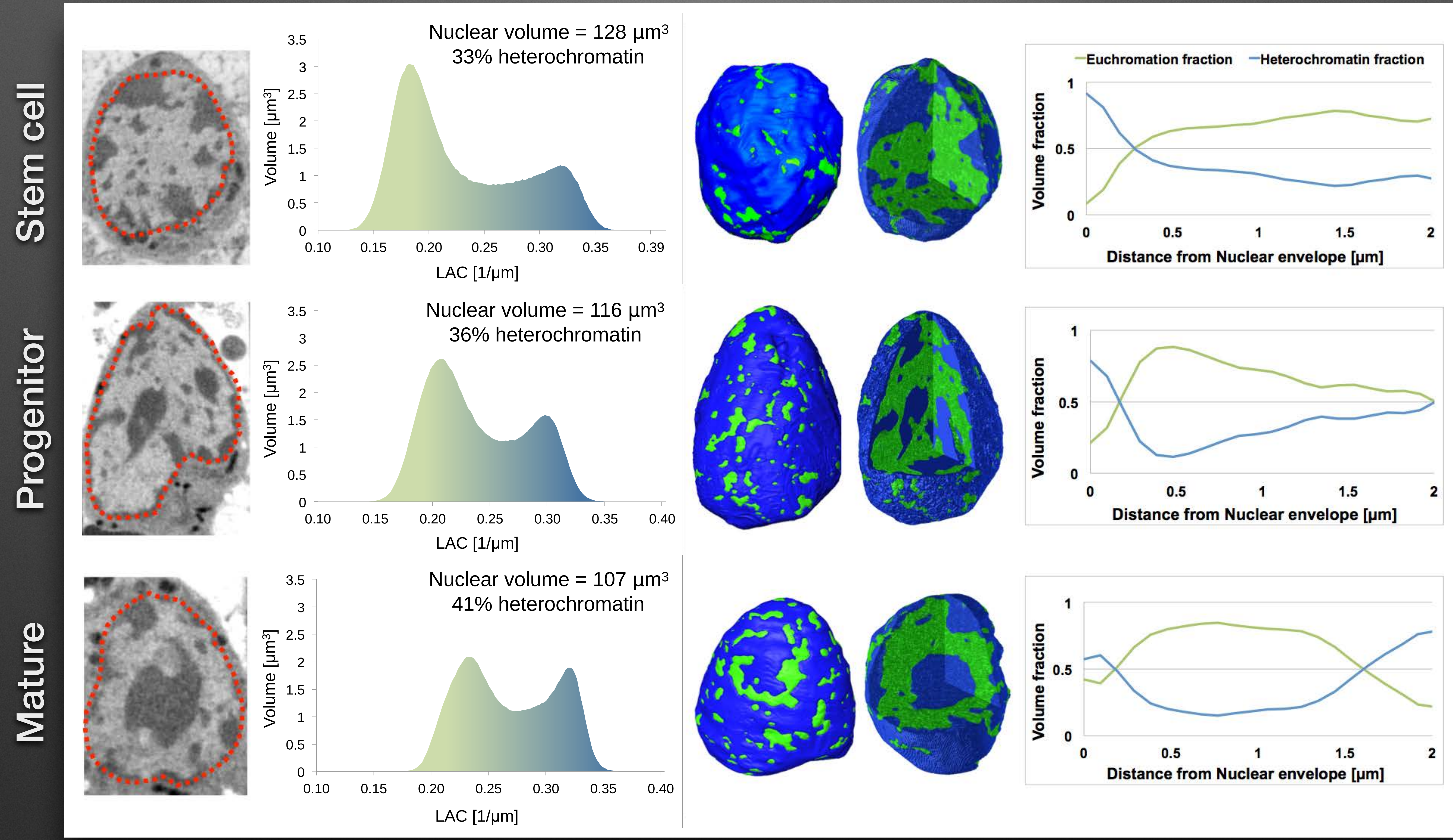


Heterochromatin organization during neurogenesis



Le Gros MA, Clowney EJ, Magklara A, Yen A, Markenscoff-Papadimitriou E, Colquitt B, Myllys M, Kellis M, Lomvardas S, and Larabell CA. In review.

From stem cell to neuron

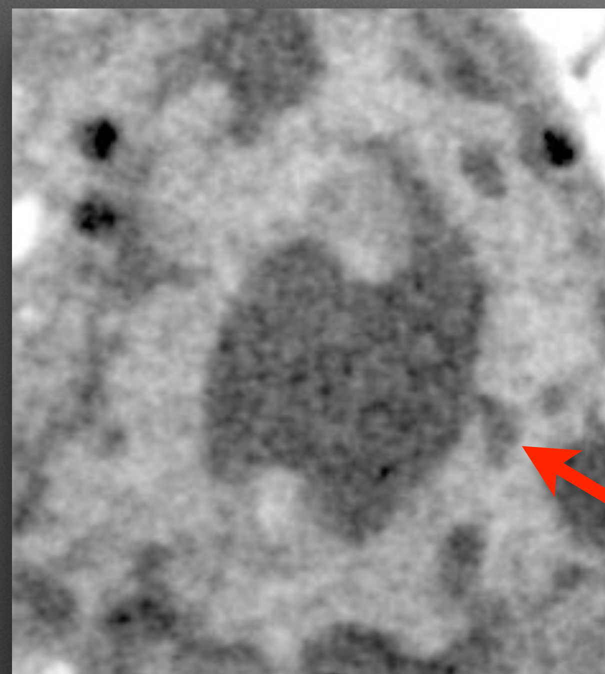


Le Gros et al. (2016) Cell Reports. 17(8), 2125-2136

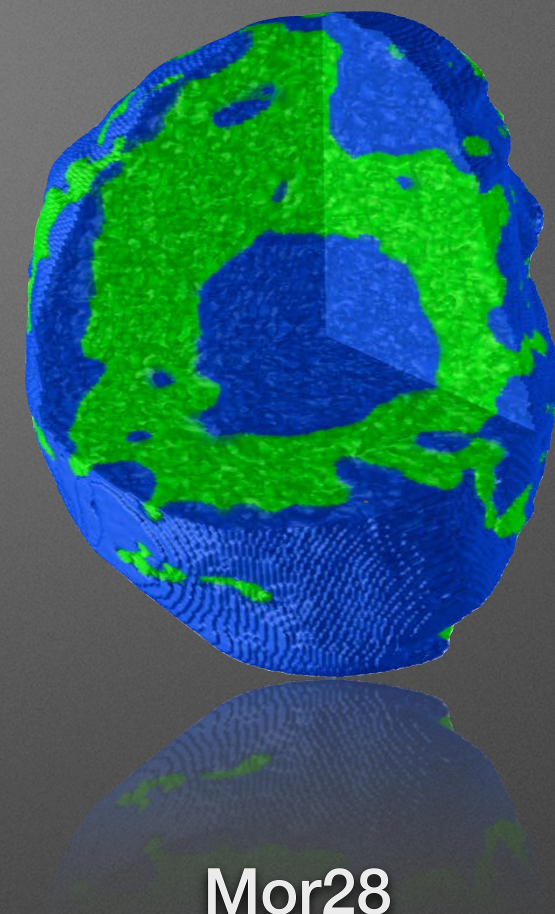
Heterochromatin
Euchromatin

Nuclear structure and gene selection

SXT

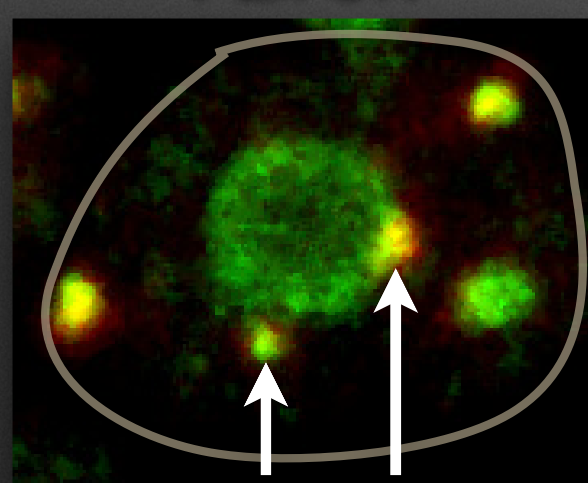


Silenced genes



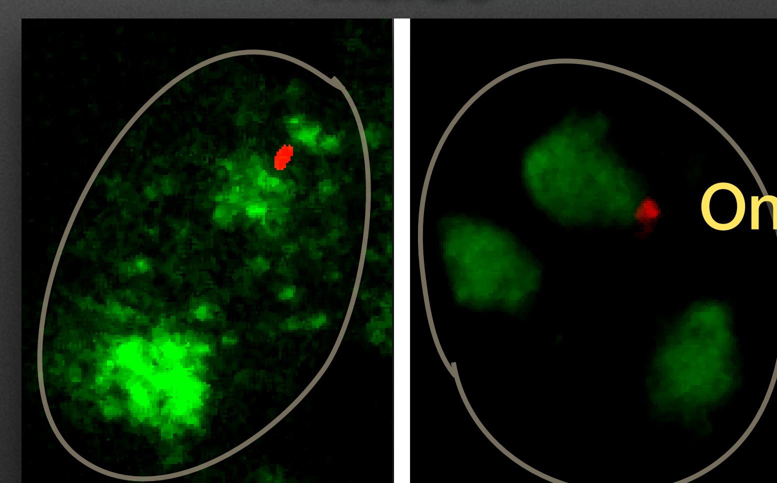
FISH

Pan OR



Silenced genes

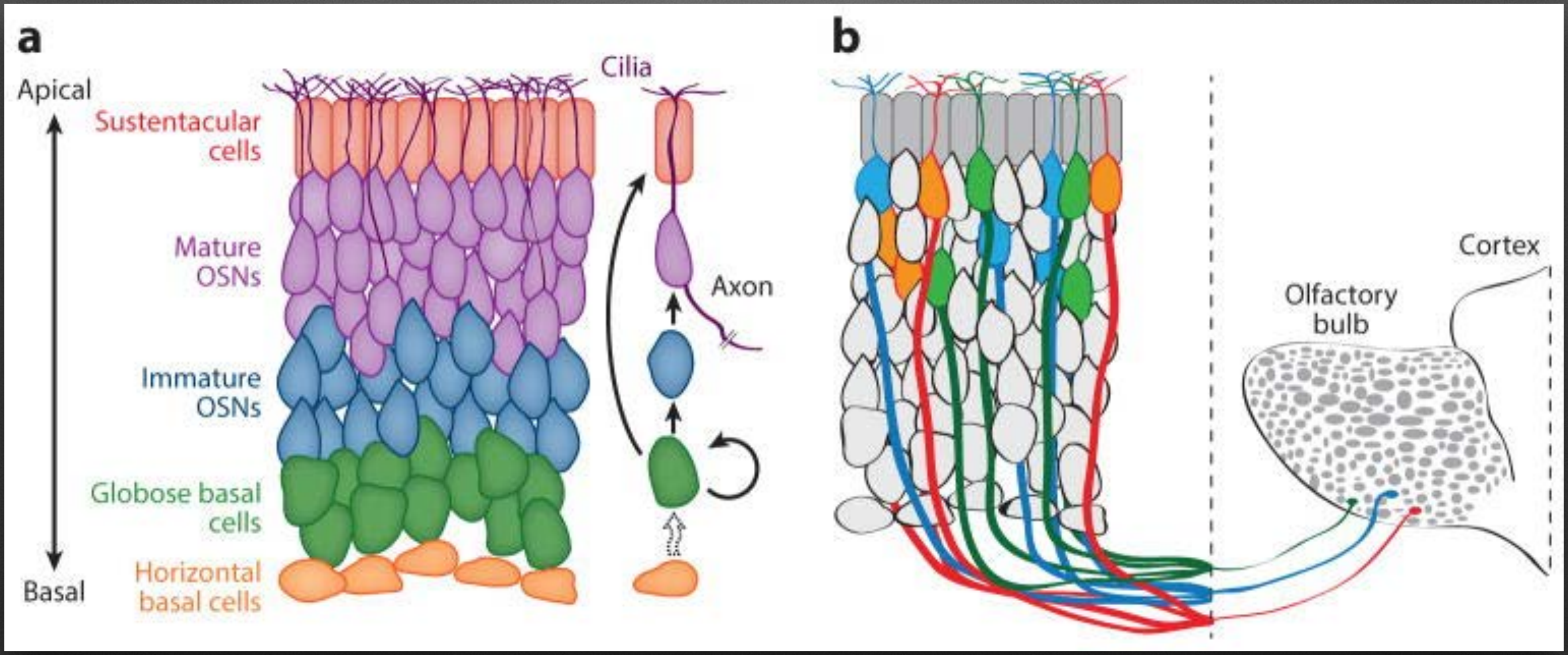
Mor28



One active allele

Clowney et al. Cell. 151, 724-737

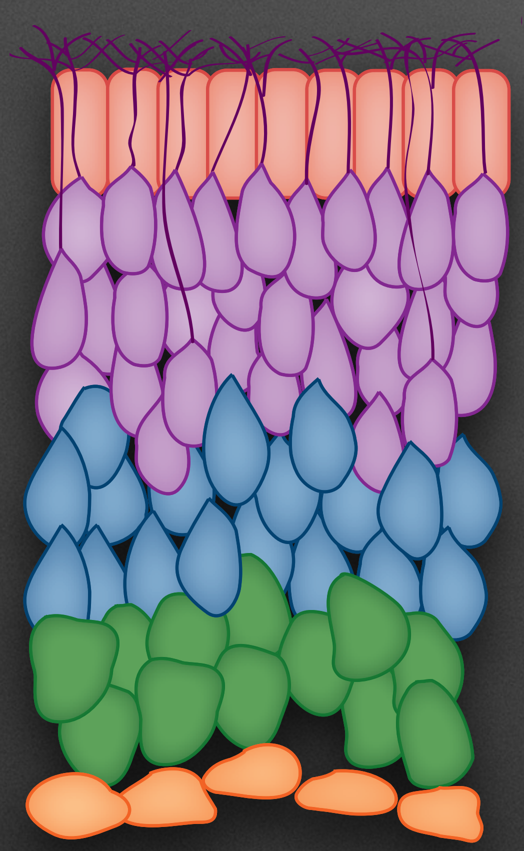
Glomeruli specificity

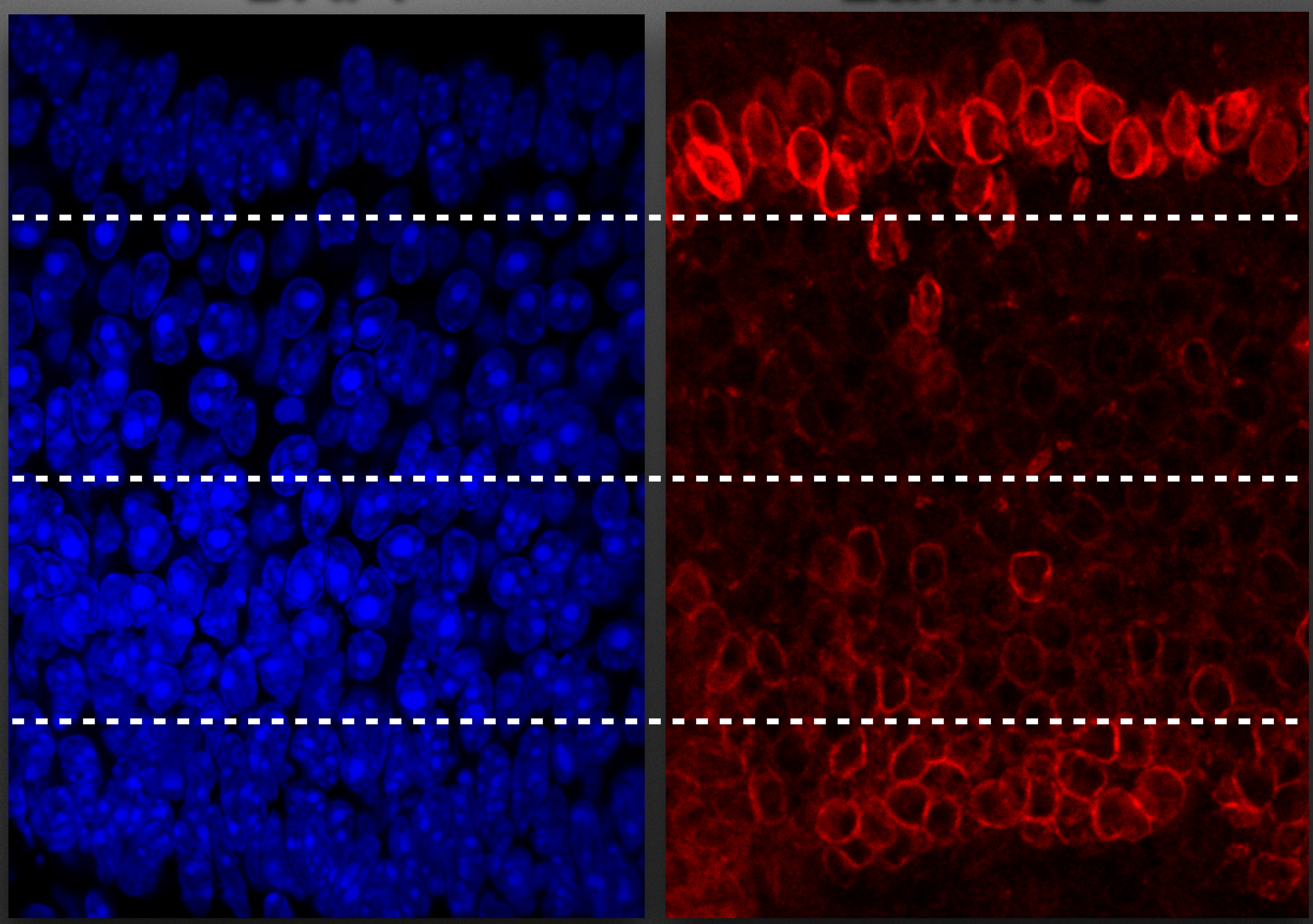


K Monahan and S. Lomvardas (2015) Ann Rev Cell Dev Biol. 31, 721-40

Does 3D organization of the nucleus matter?

Nuclear structure and gene selection



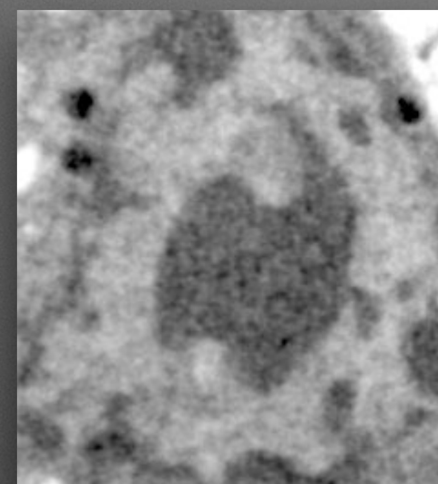


Clowney et al. Cell. 151, 724-737

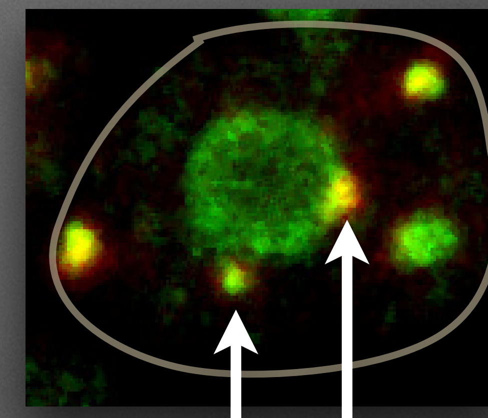
Nuclear structure and gene selection

Wild type cell

SXT



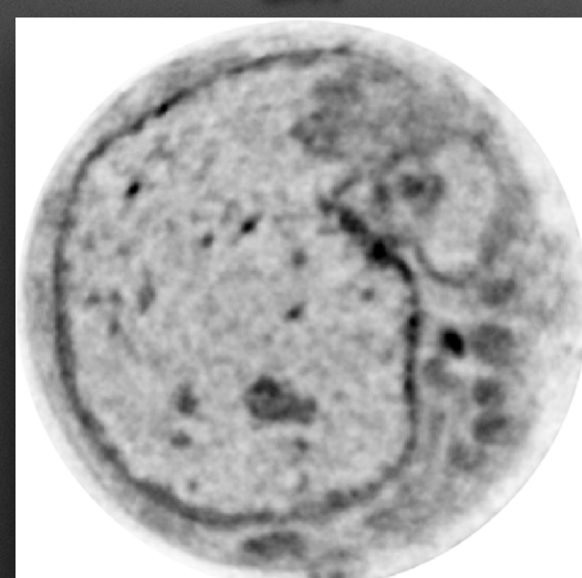
FISH



Silenced genes

LBR expressing cell

SXT



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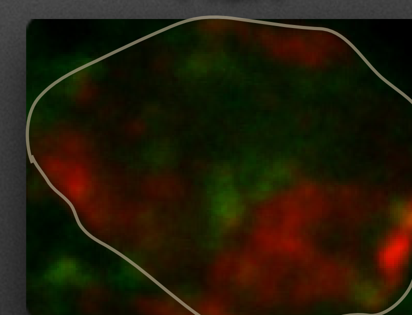
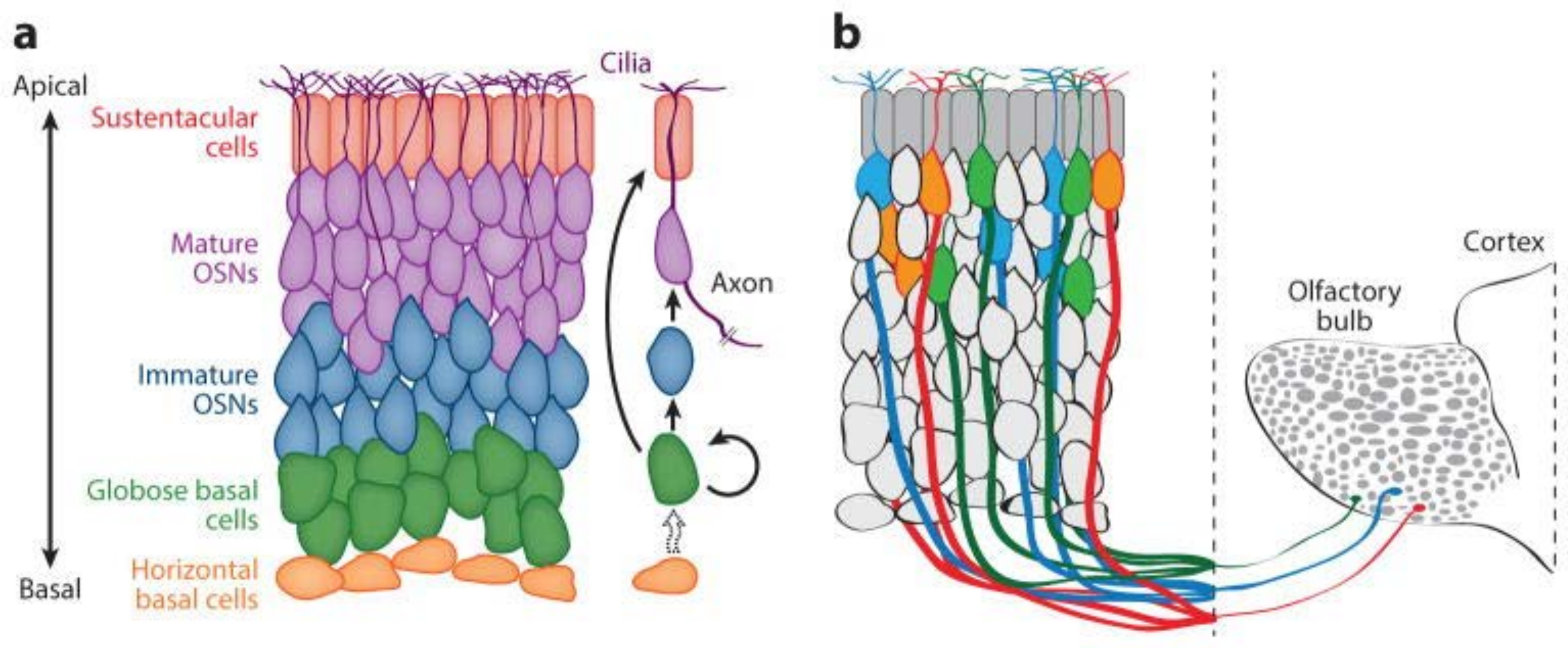
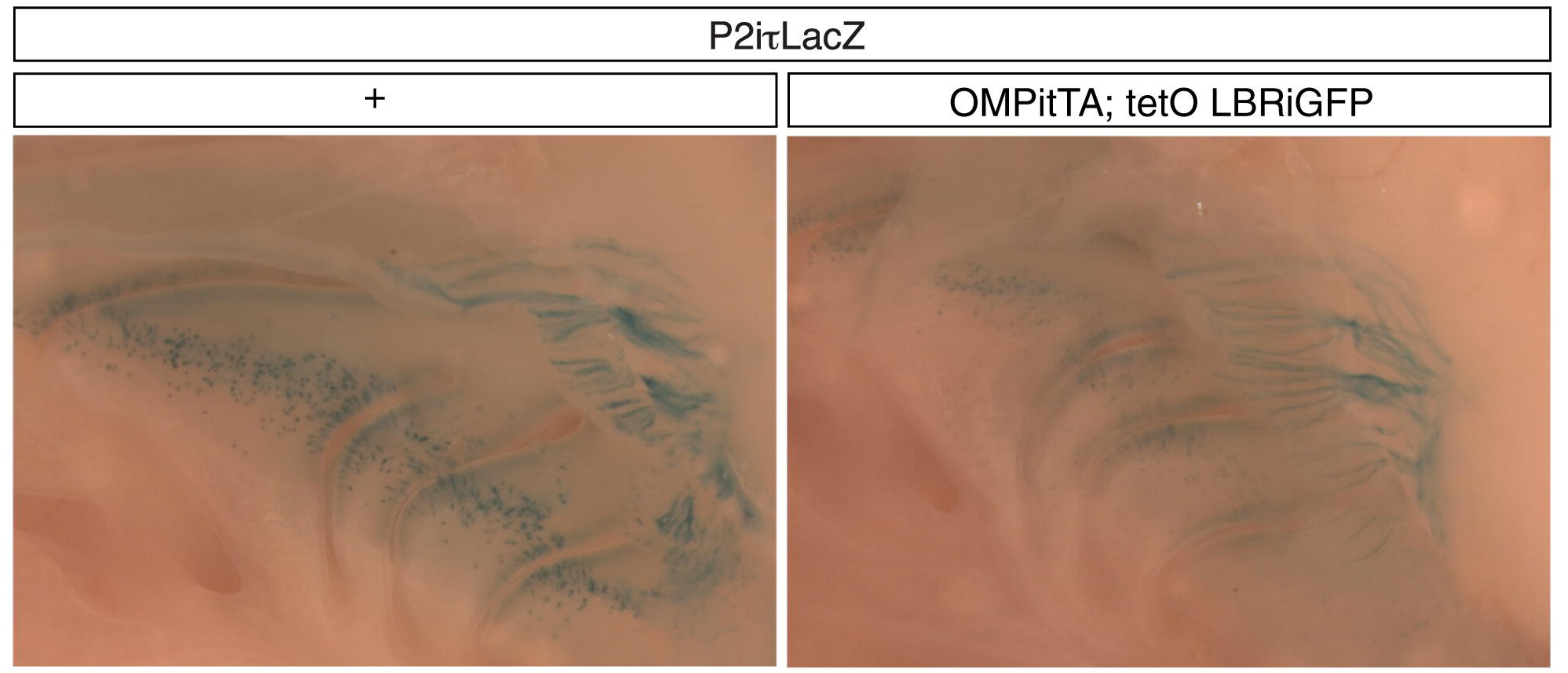


OR expression disrupted

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

Glomeruli specificity

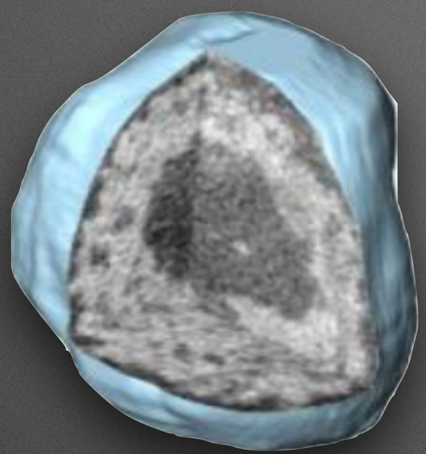


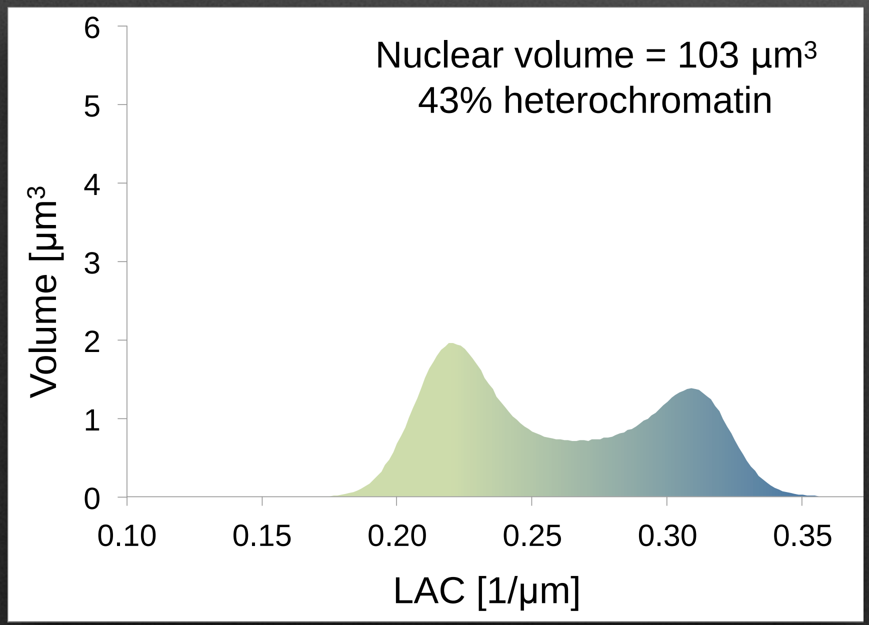


Clowney et al. Cell. 151, 724-737

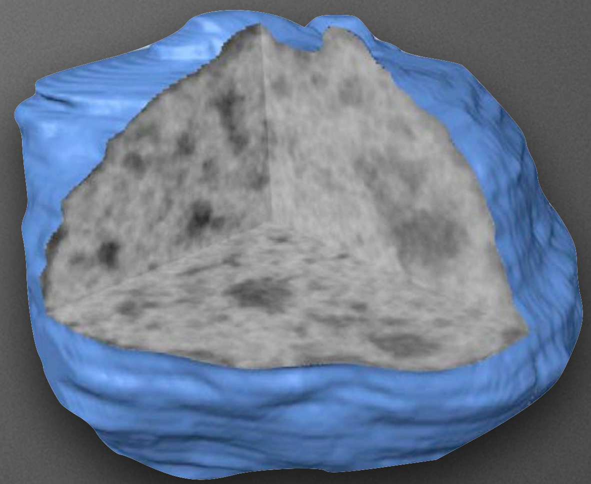
Nuclear structure and gene selection

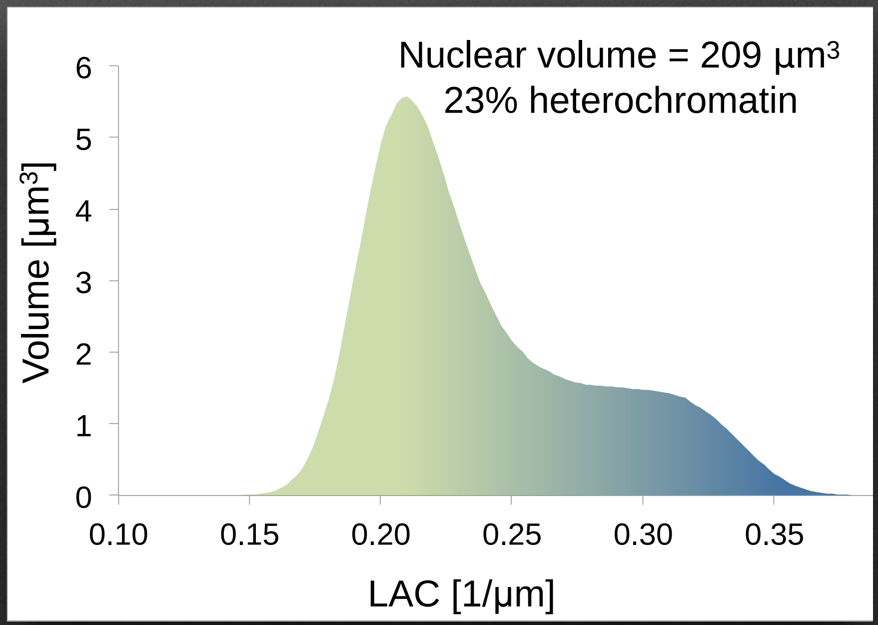
Wild type





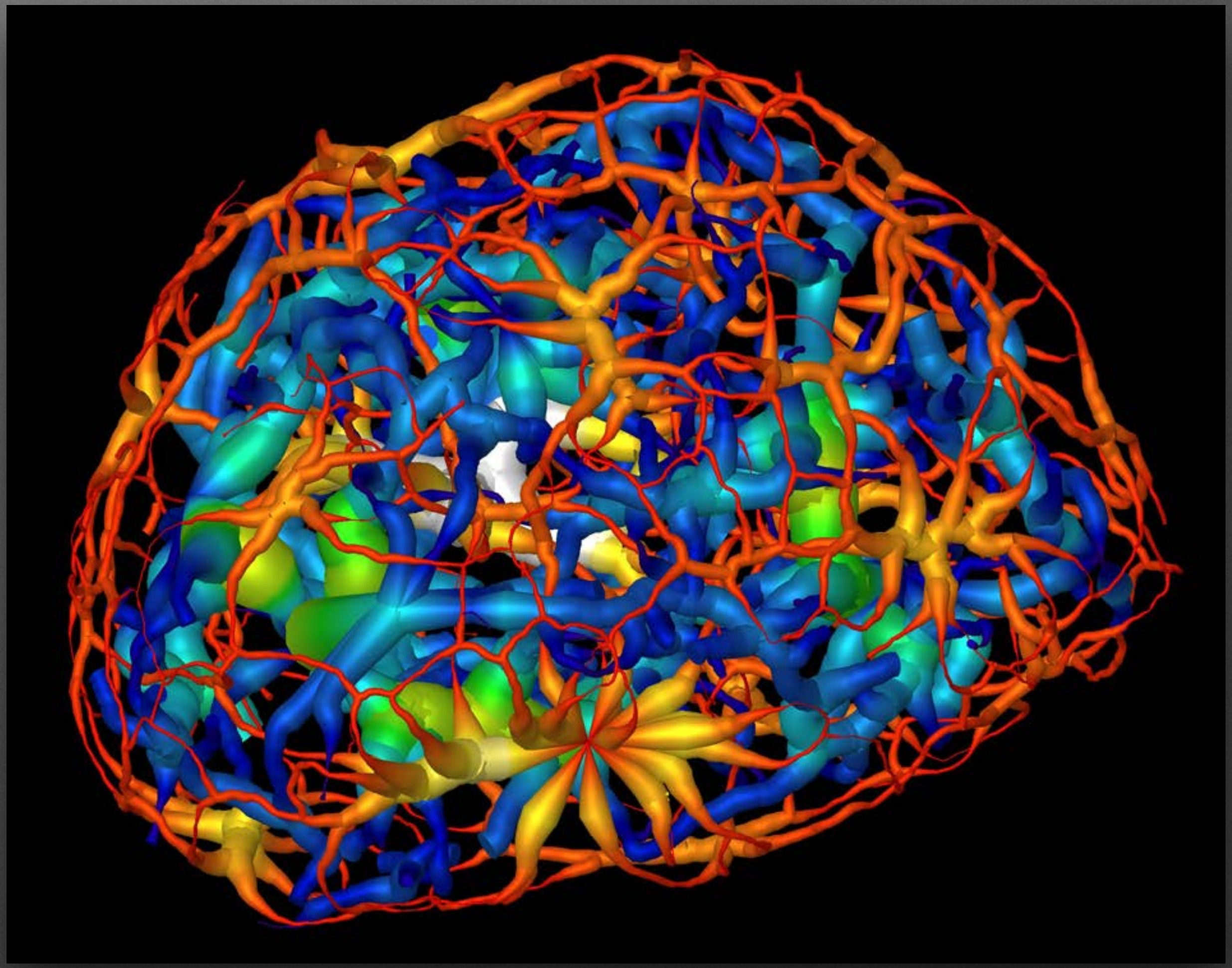
Hp1ß KO





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

Chromatin condensation during neurogenesis

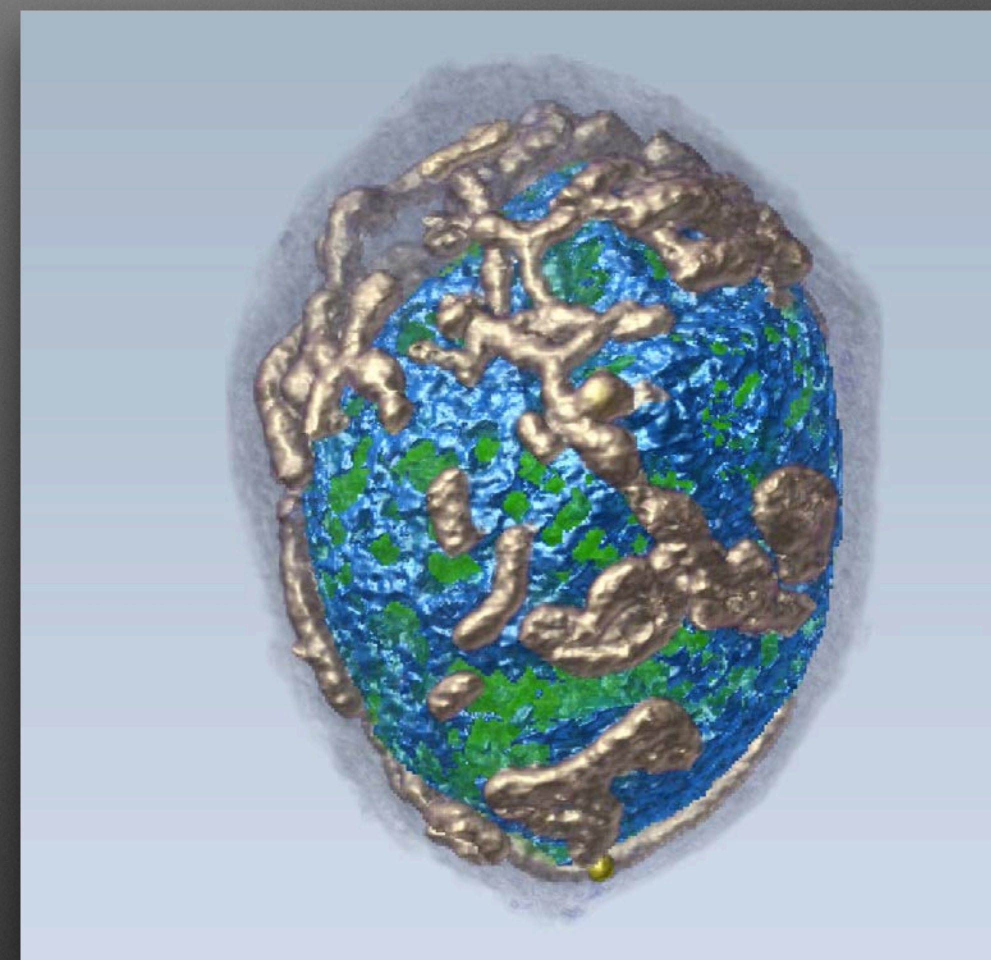


Stavros Lomvardas
Columbia University

Chromatin networks

TEM

No islands of heterochromatin

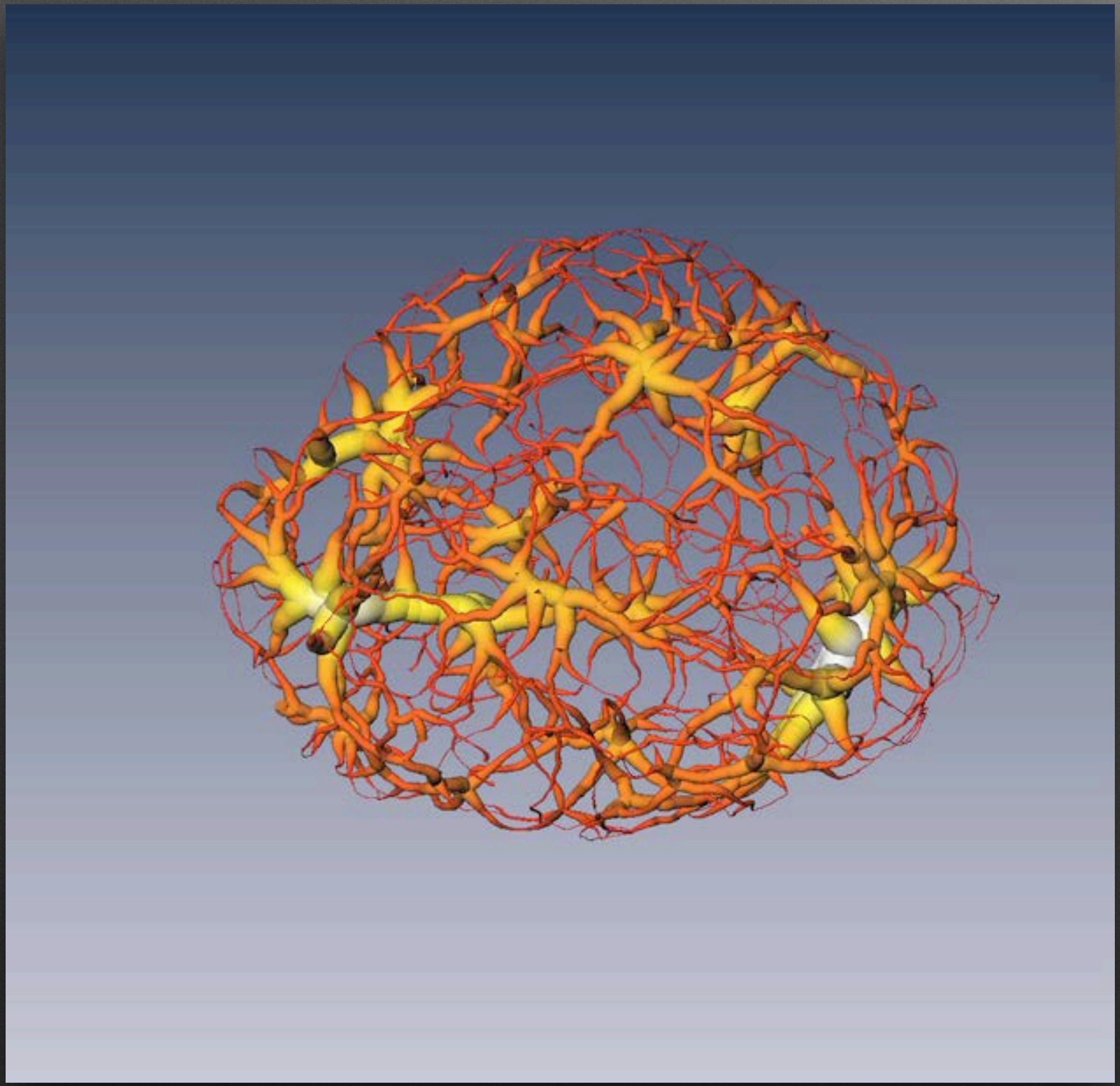


Heterochromatin
Euchromatin

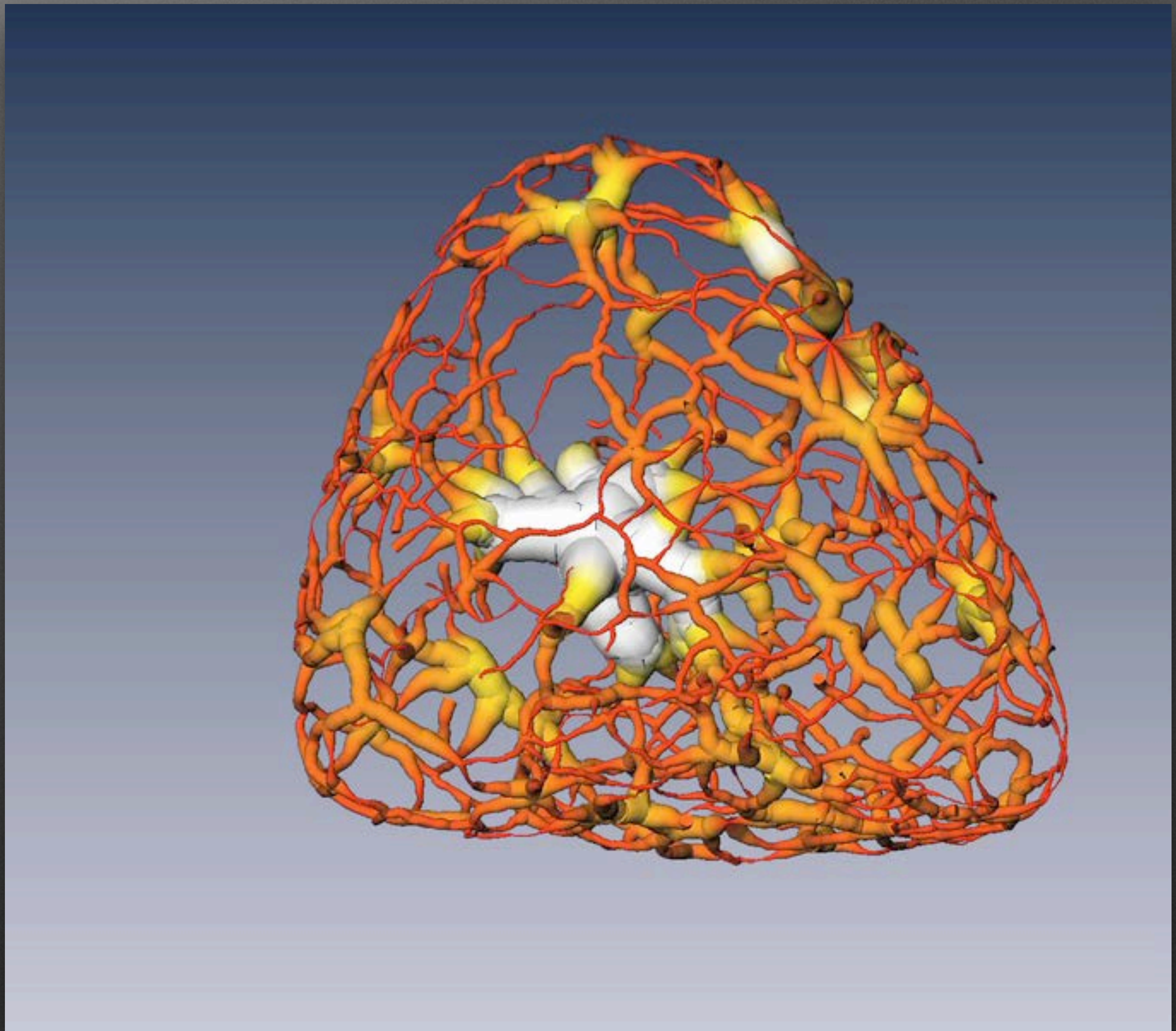
Le Gros et al. (2016) Cell Reports. 17(8), 2125-2136

Chromatin networks

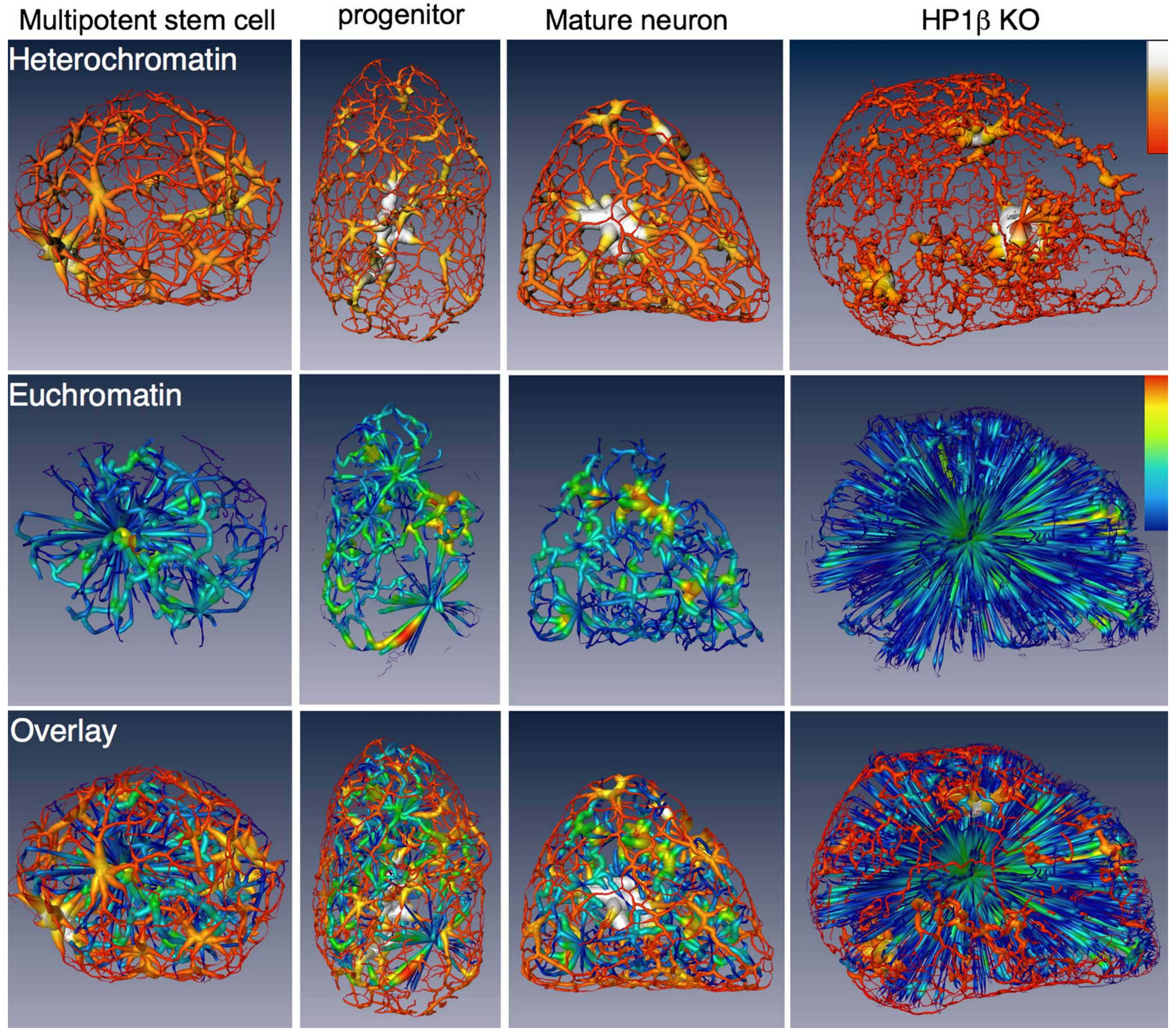
Stem cell



Differentiated cell



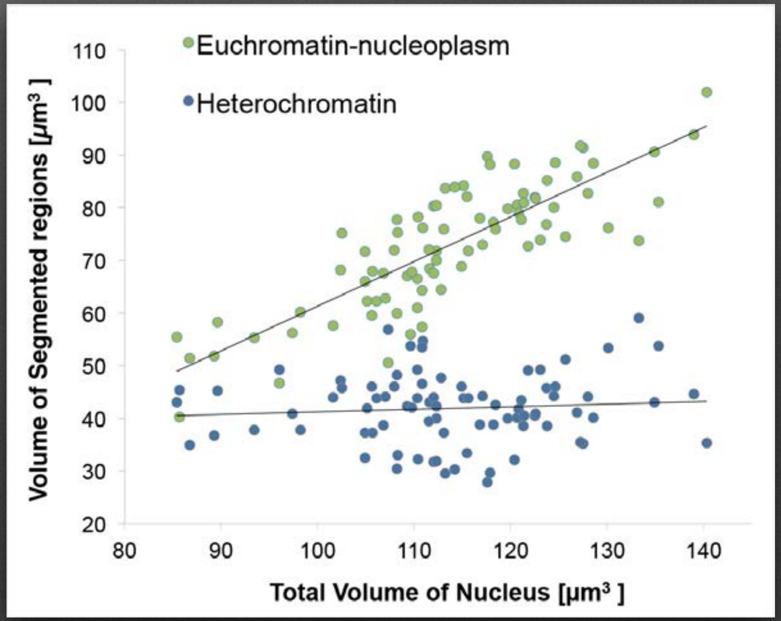
Le Gros et al. (2016) Cell Reports. 17(8), 2125-2136



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

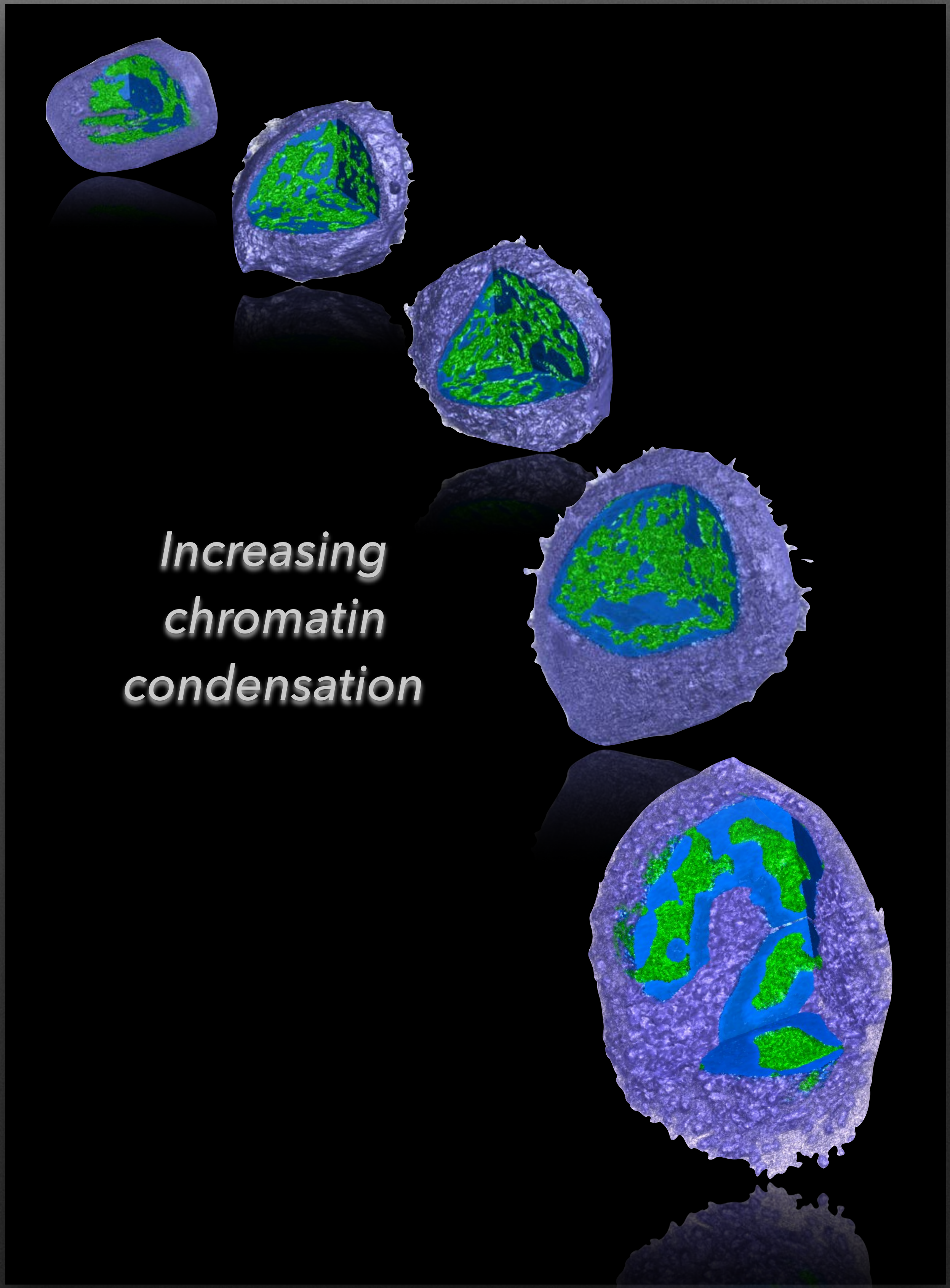
New information about the nucleus

- Heterochromatin ~ 30% more compacted (crowded) than euchromatin
- Increased compaction of heterochromatin during differentiation
- Chromatin networks; no islands of chromatin
- Nuclear volume directly proportional to euchromatin volume (active gene region)



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

Nuclear reorganization during hematopoiesis



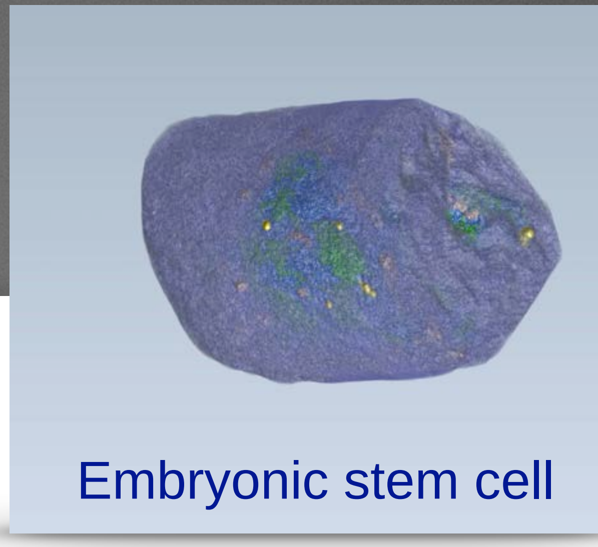
Camilla Forsberg

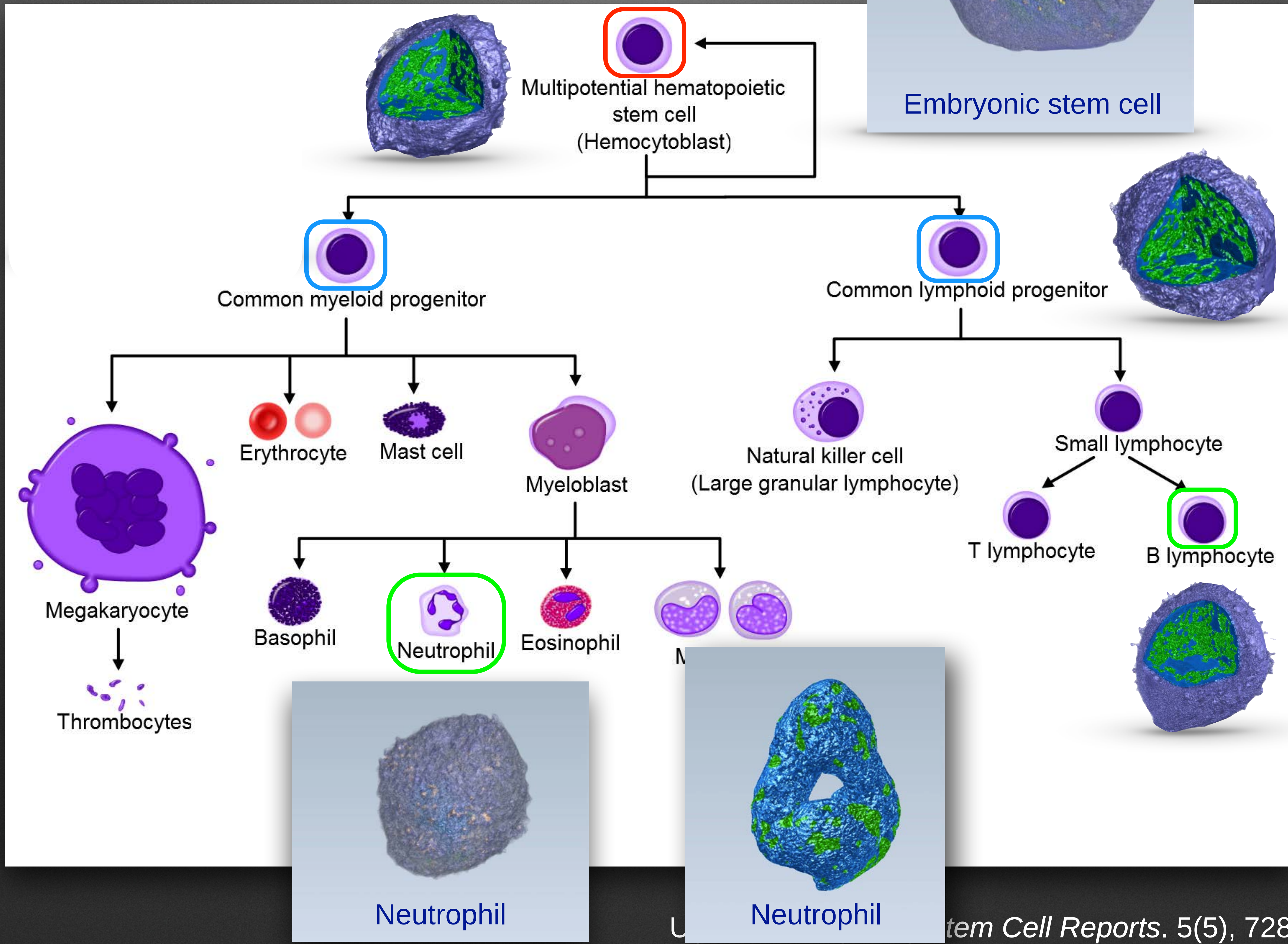
University of California

Santa Cruz

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

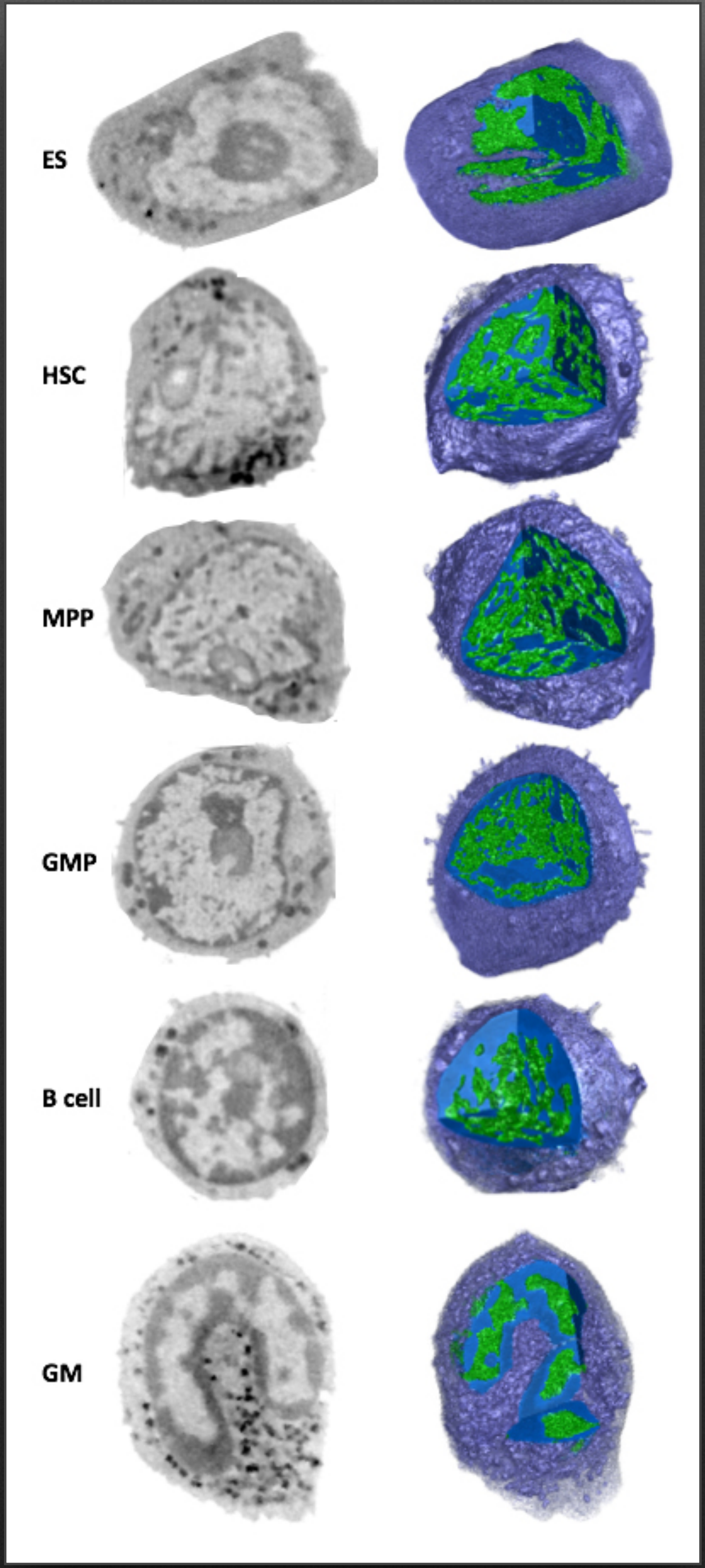
Hematopoiesis:
from stem cell to blood cell





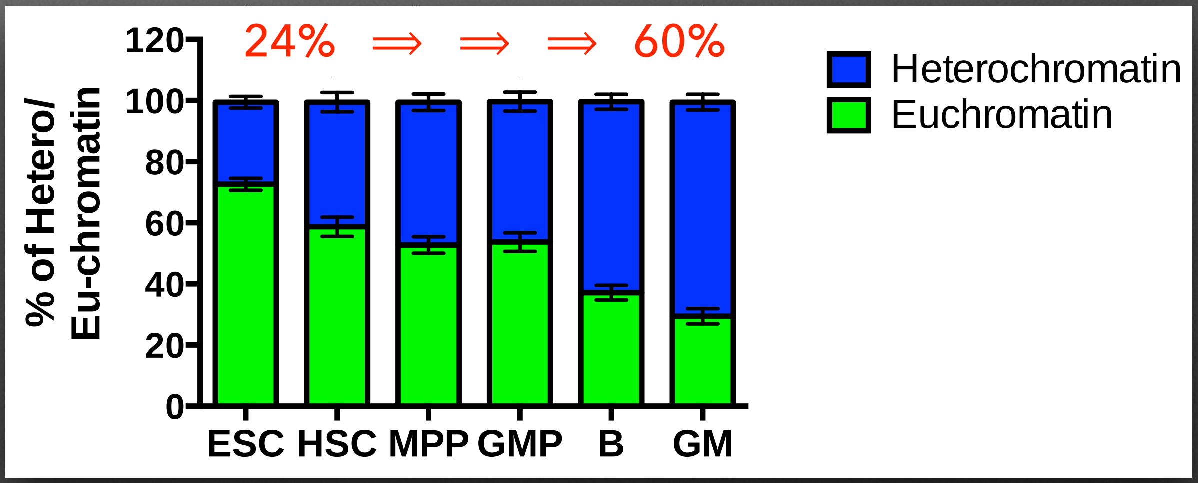
U… …tem Cell Reports. 5(5), 728-740

From stem cell to blood cell



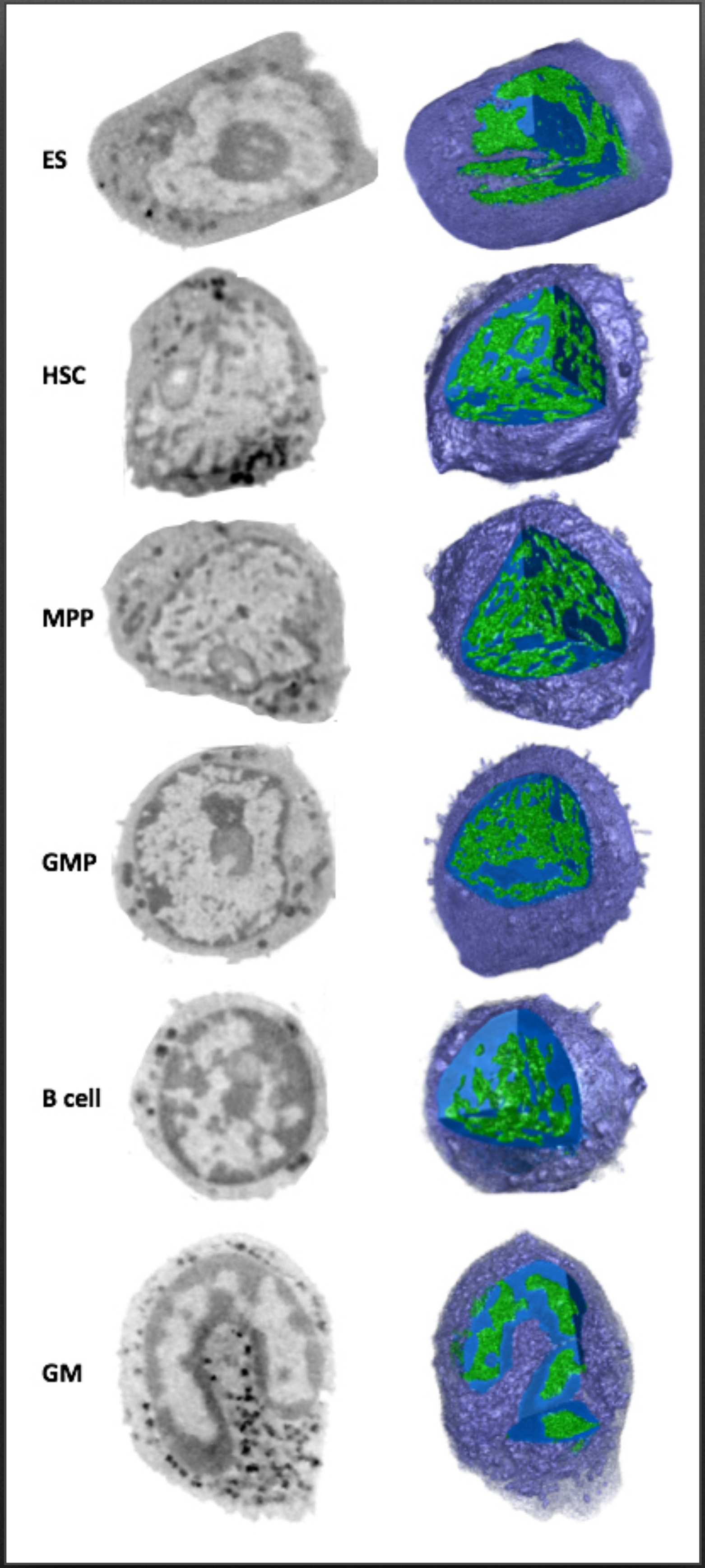
During differentiation:

Percent heterochromatin increases



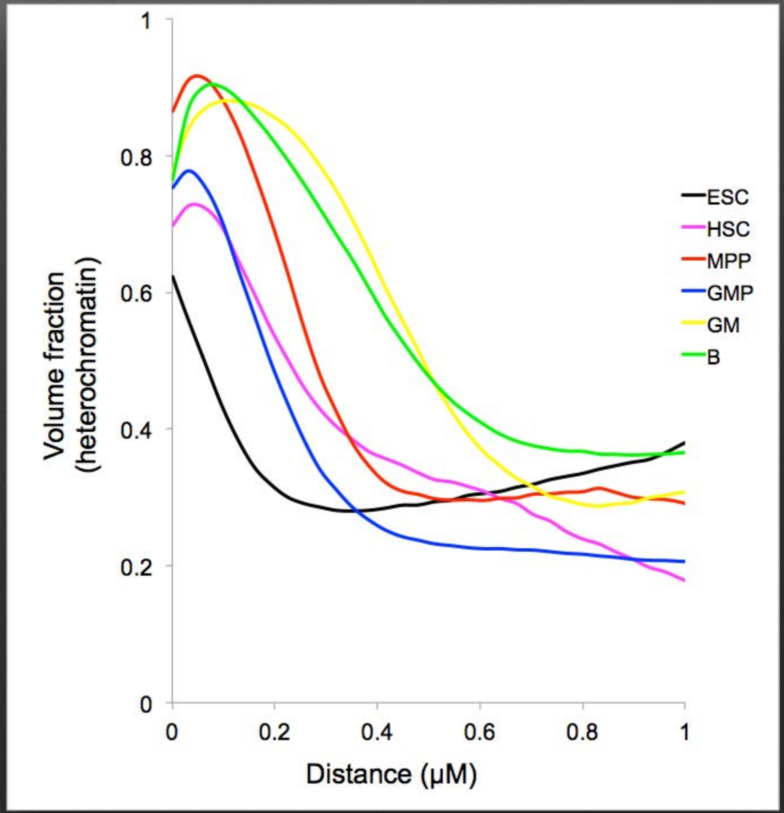
Ugarte et al. (2015) Stem Cell Reports. 5(5), 728-470

From stem cell to blood cell



During differentiation:

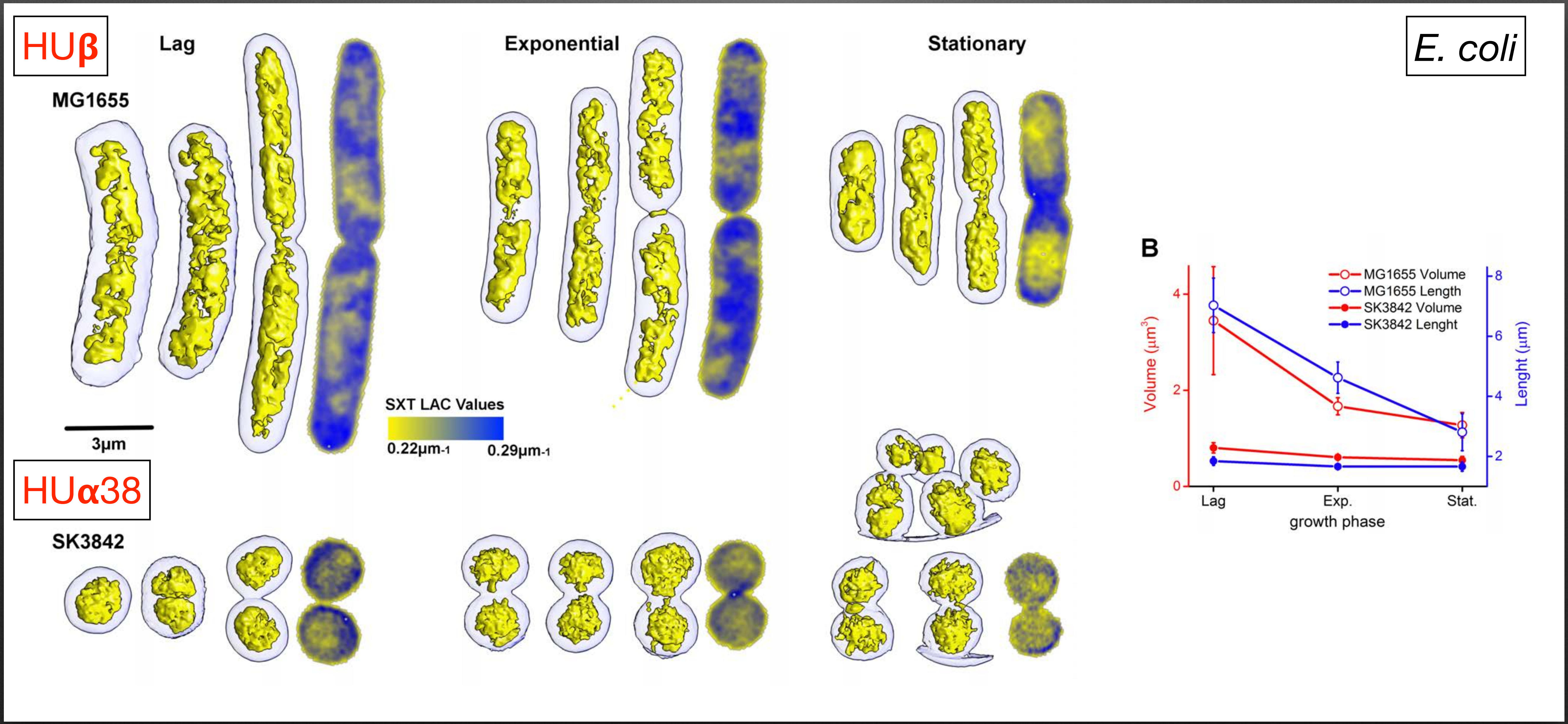
Nuclear volume decreases and peripheral heterochromatin thickens



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

HU multimerization shift controls nucleoid compaction

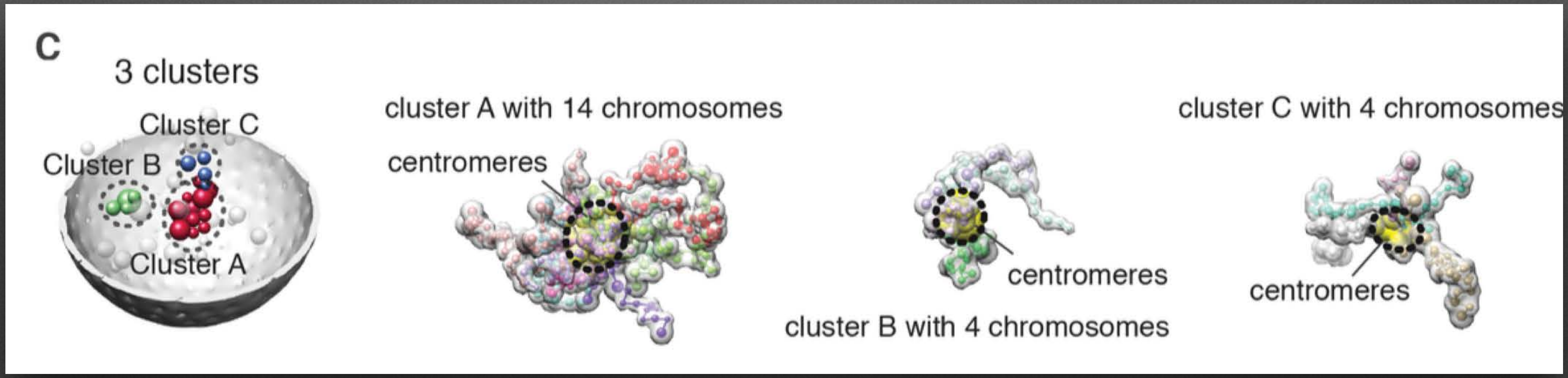
HU - histone like protein

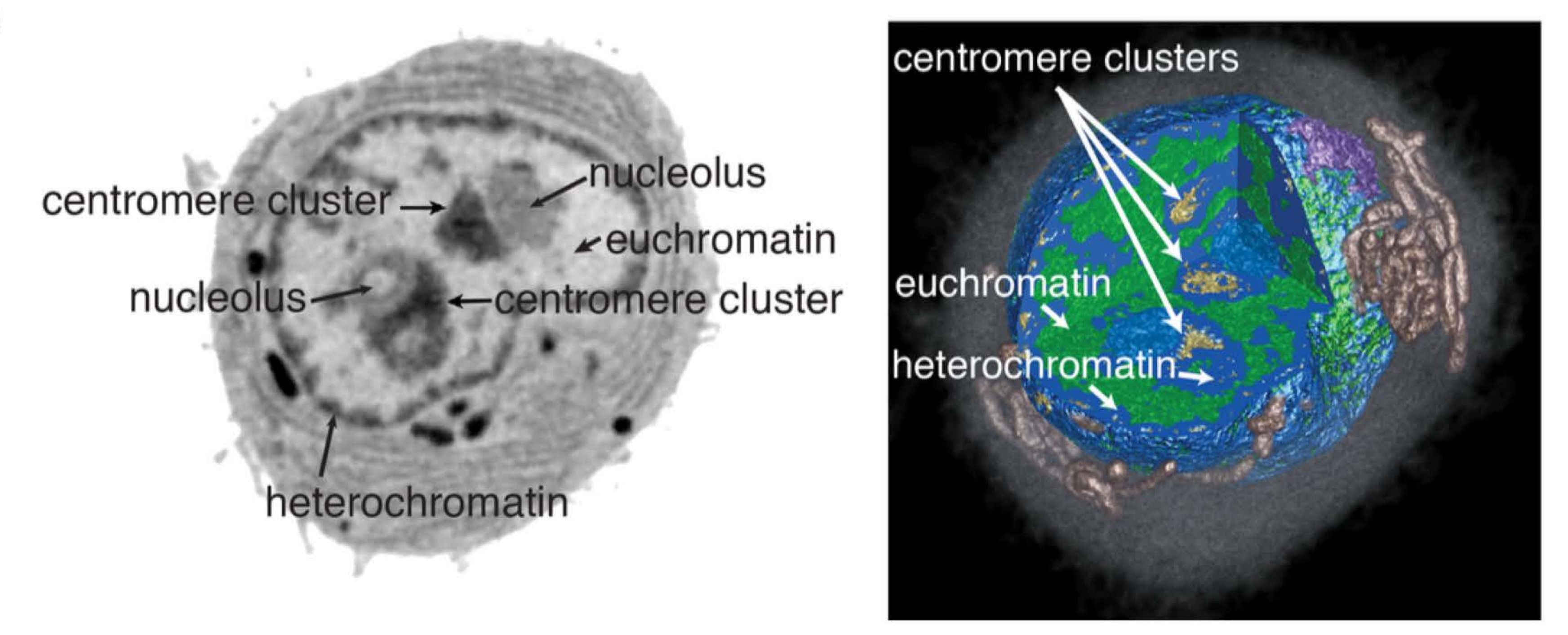


Hammel et al., (2016) Science Advances. doi: 10.1126/sciadv.1600650.

Topology of the human genome

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



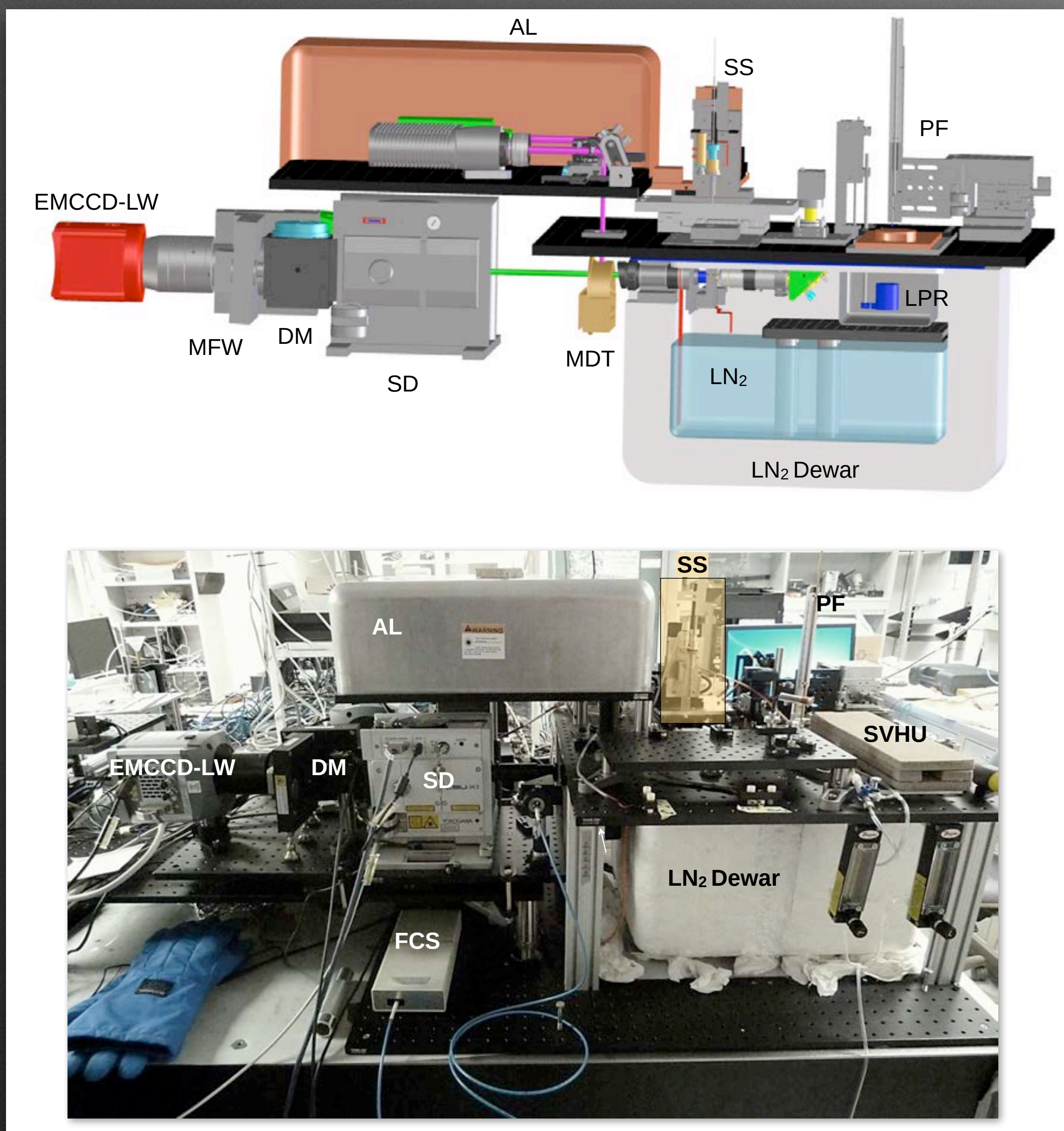


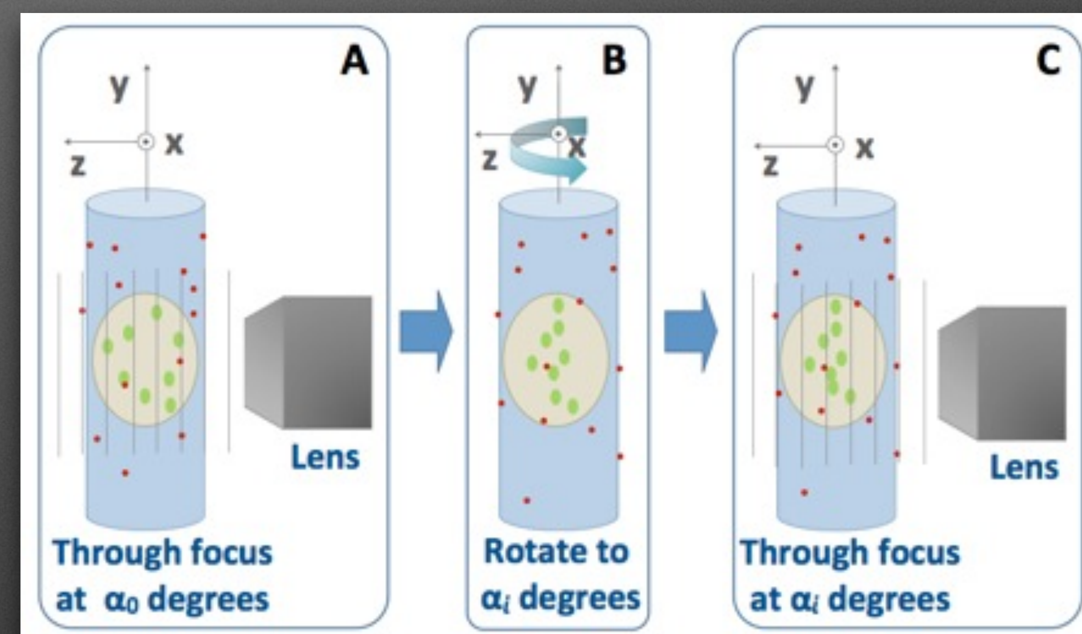
Tjong et al. (2016) PNAS. Mar 22; 113 E1663-1672

Imaging molecules in context

Correlated fluorescence and x-ray tomography

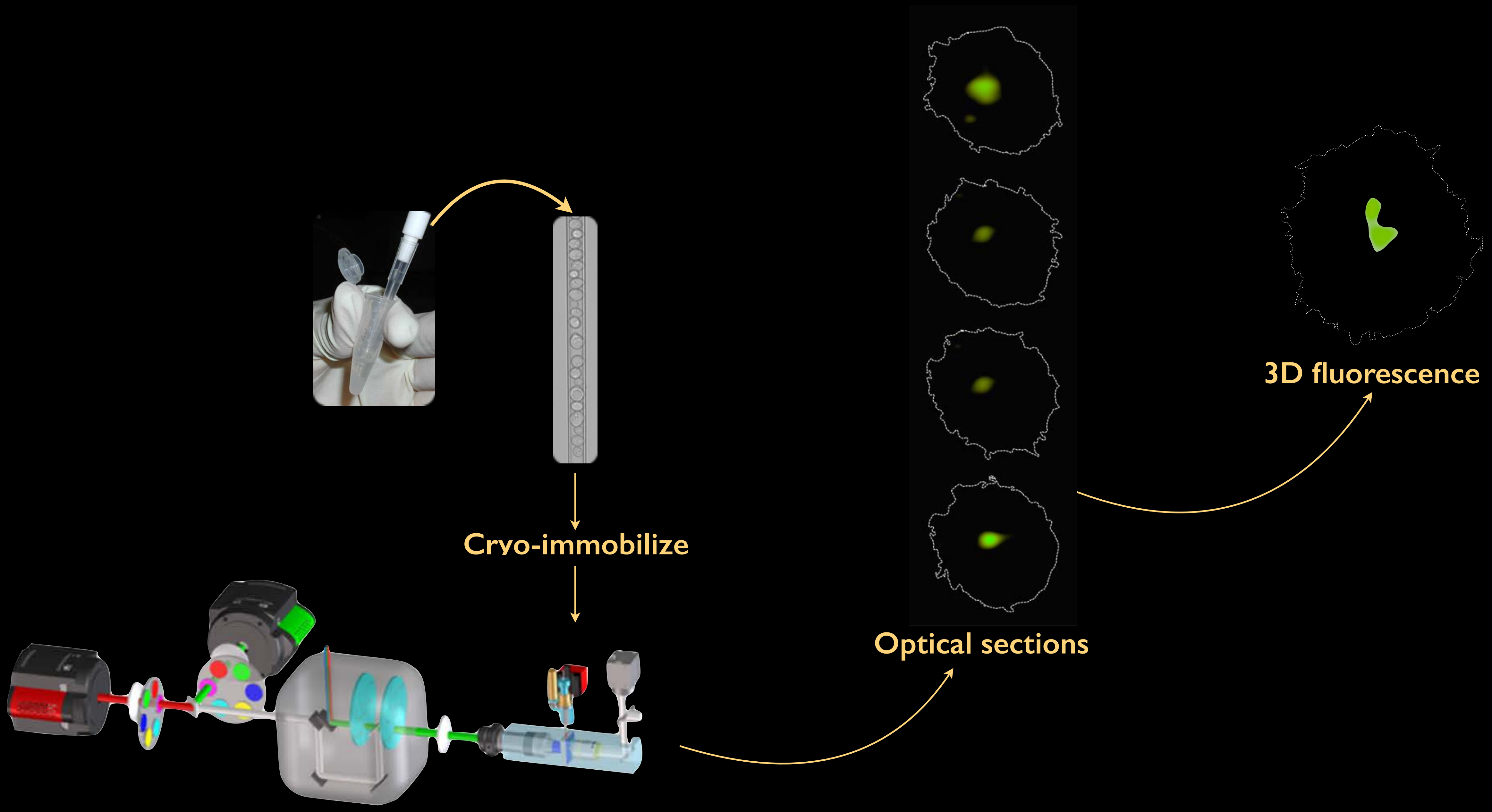
Cryo confocal tomography





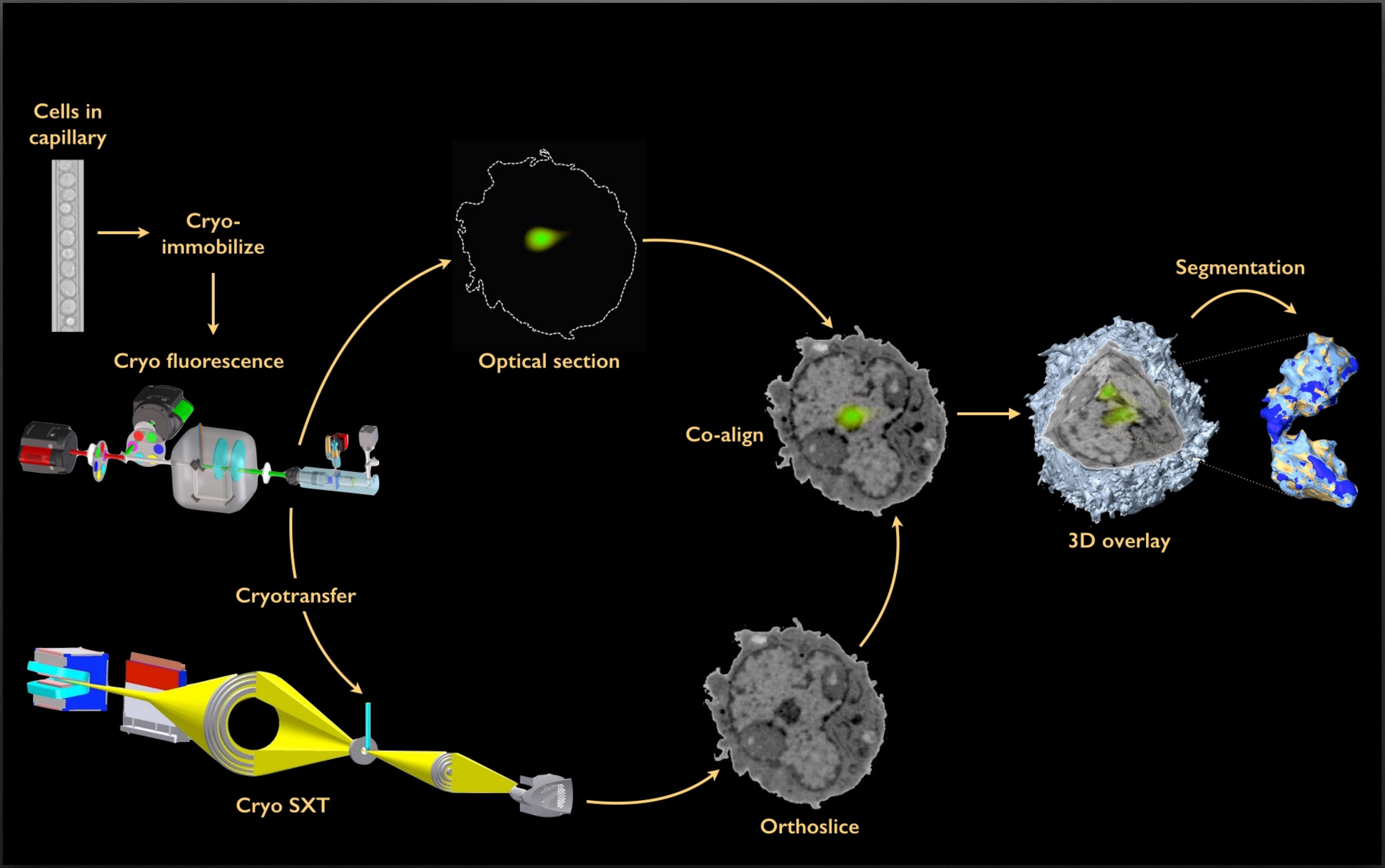
Le Gros et al. (2009) J. Microscopy. 235(1), 1-8

Cryo-light tomography



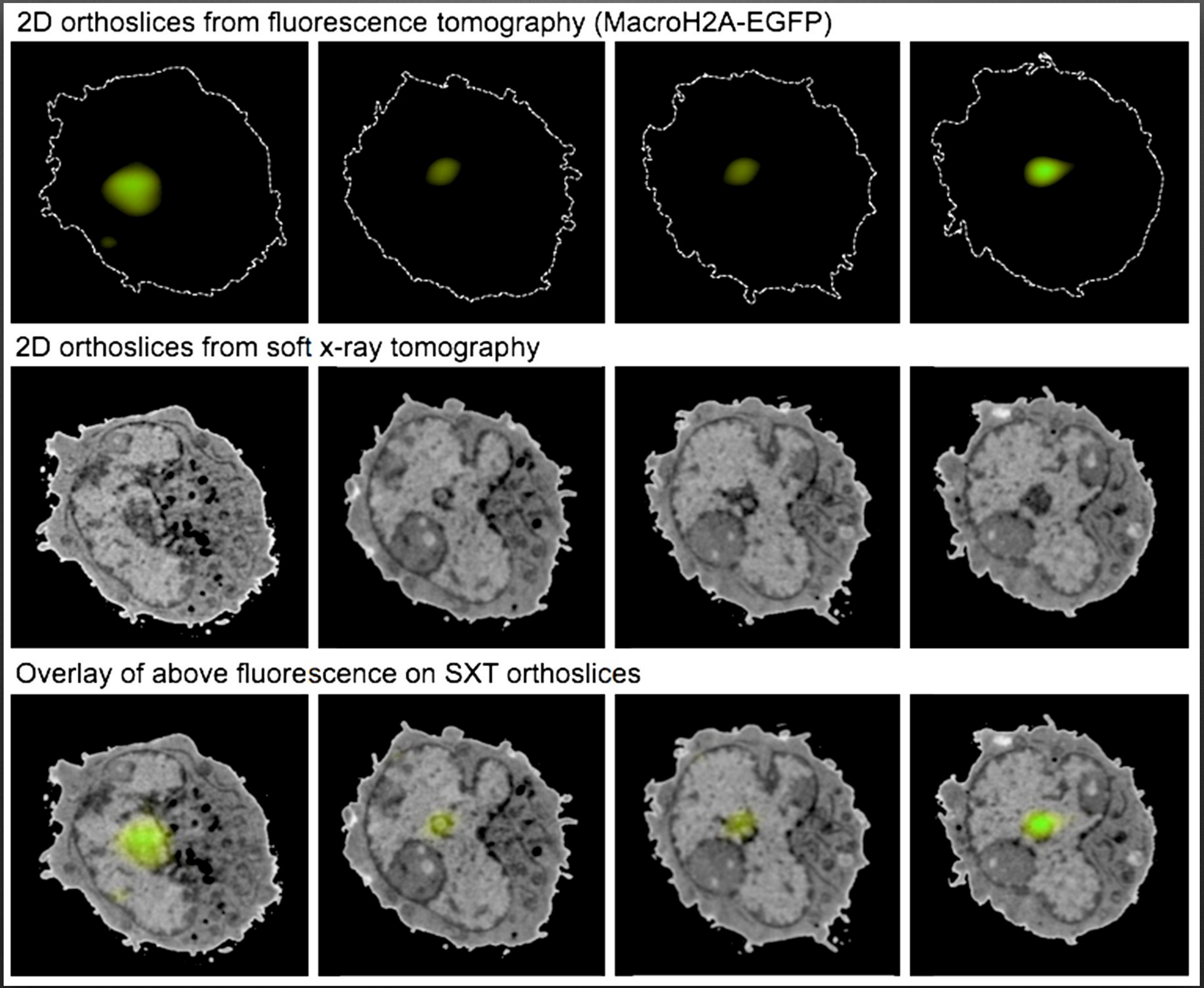
Do et al. (2015) Arch Biochem & Biophys 581:111-121.

Correlated fluorescence and x-ray tomography



Do et al. (2015) Arch Biochem & Biophys 581:111-121.

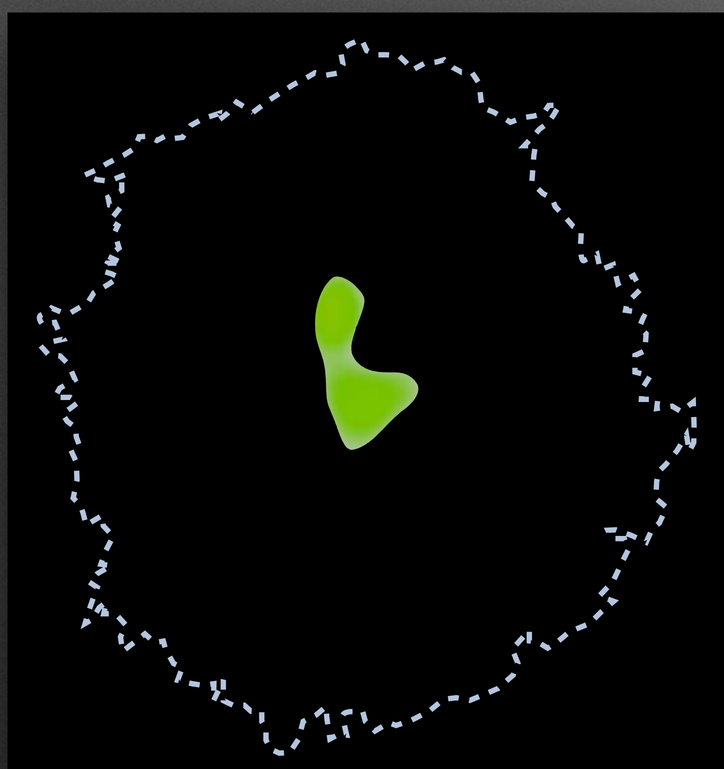
Inactive X chromosome



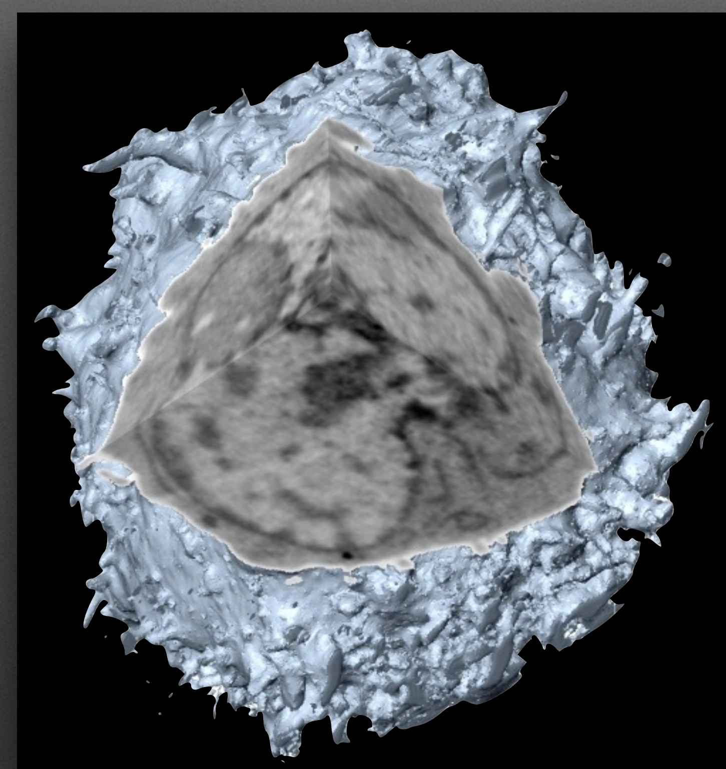
Smith et al. (2014) Biophysical Journal. 107(8), 1988-96

Inactive X chromosome

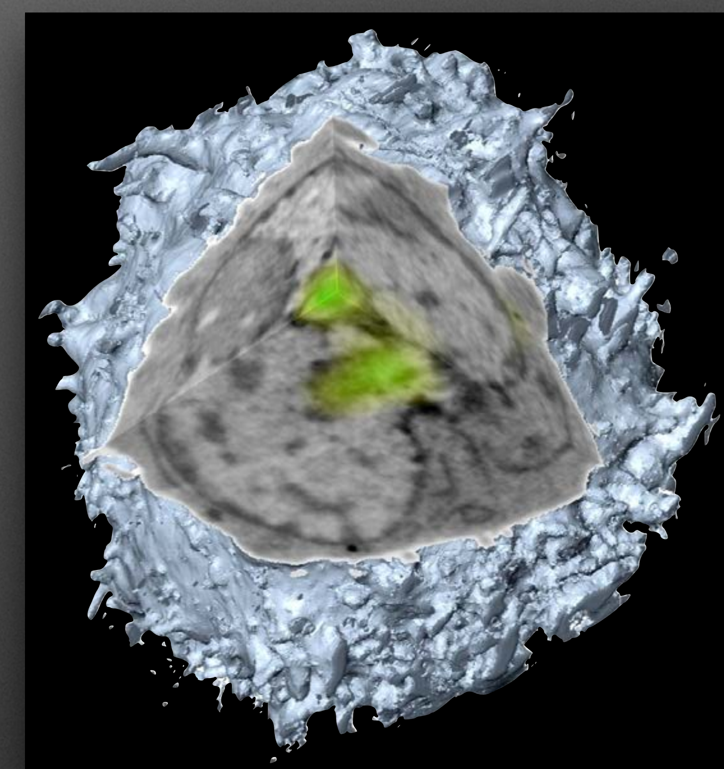
Fluorescence



X-ray

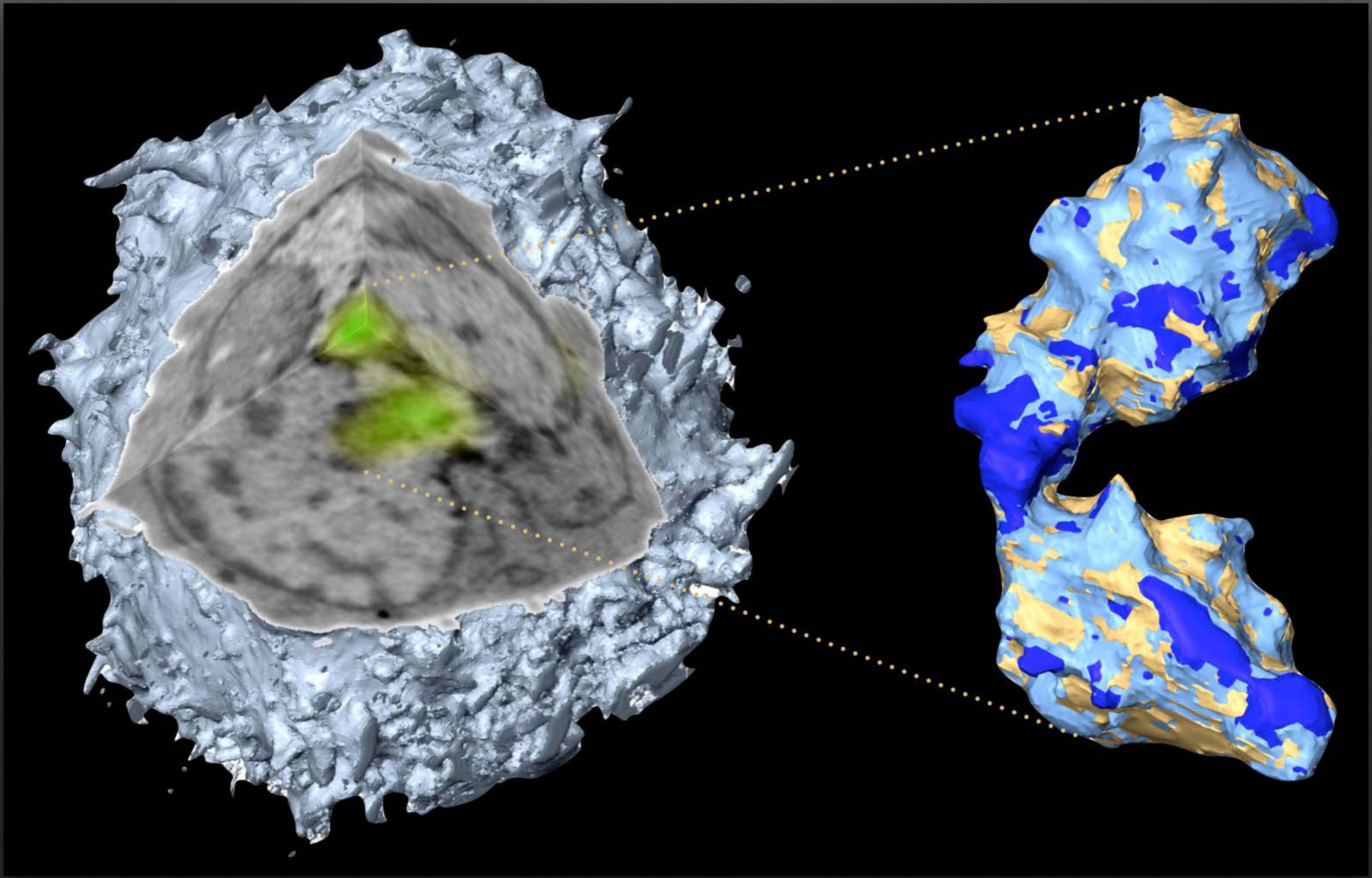


Overlay



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

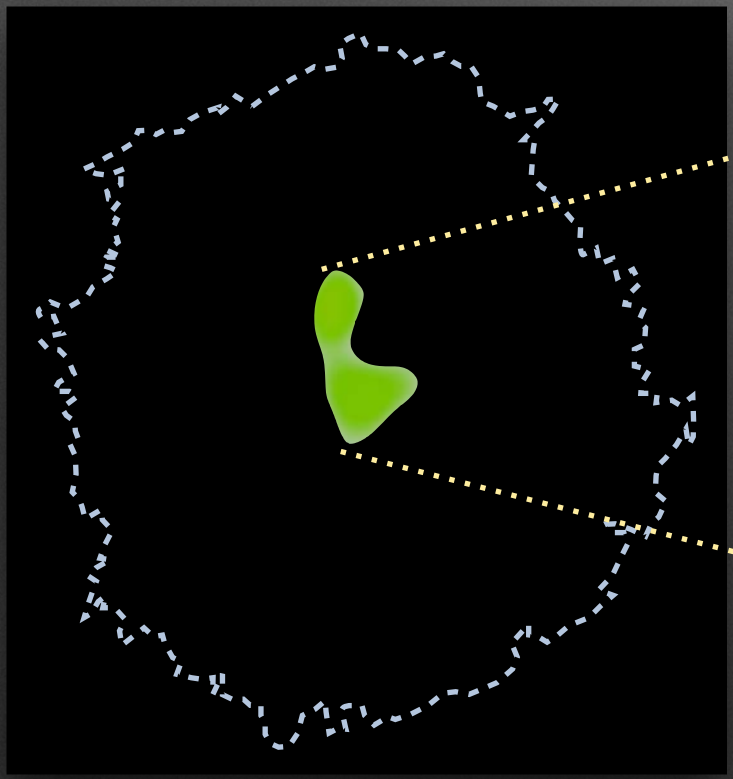
Inactive X chromosome



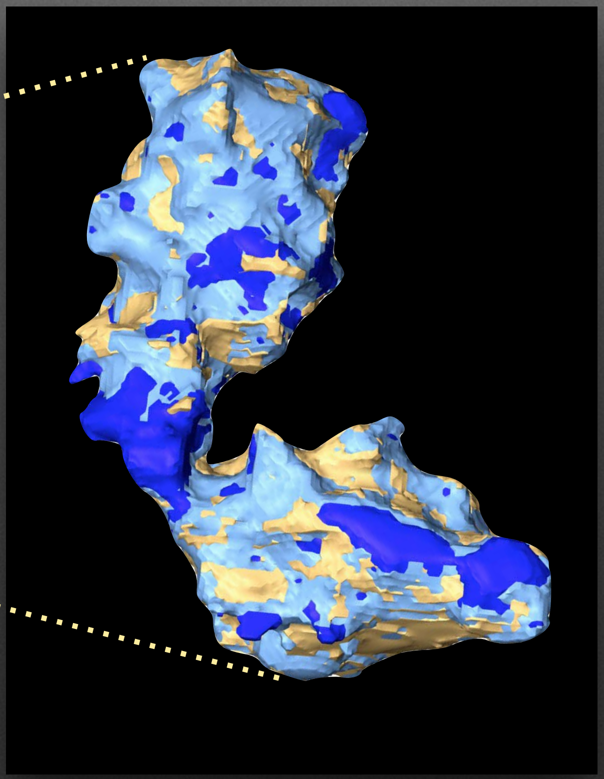
Smith et al. (2014) Biophysical Journal. 107(8), 1988-96

Inactive X chromosome

Fluorescence



X-ray



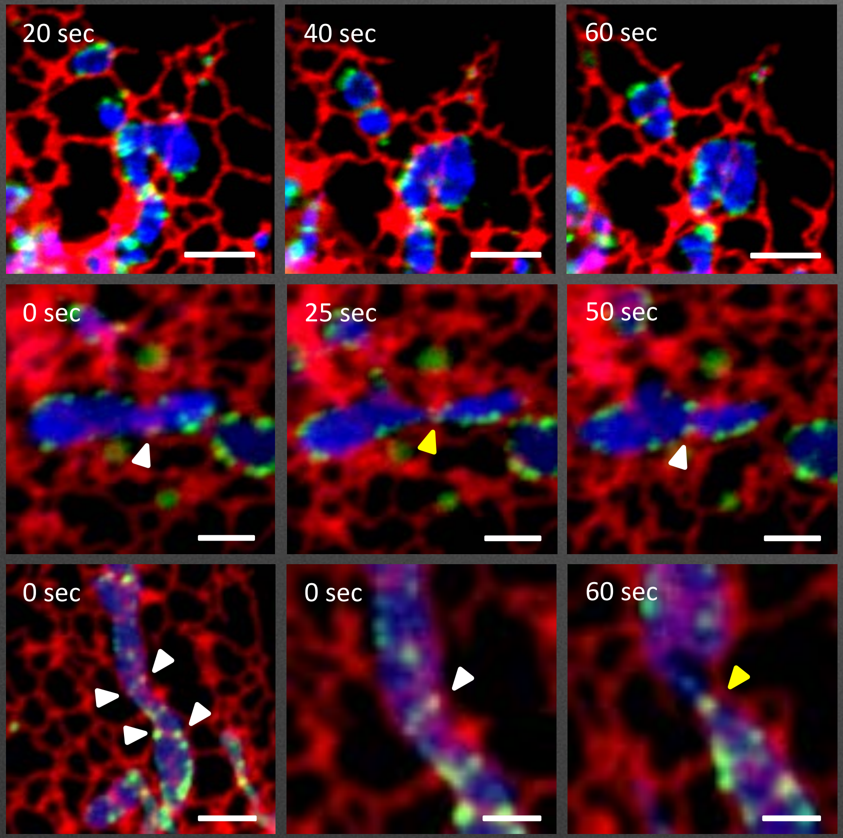
High LAC
0.34-0.36 μm^{-1}

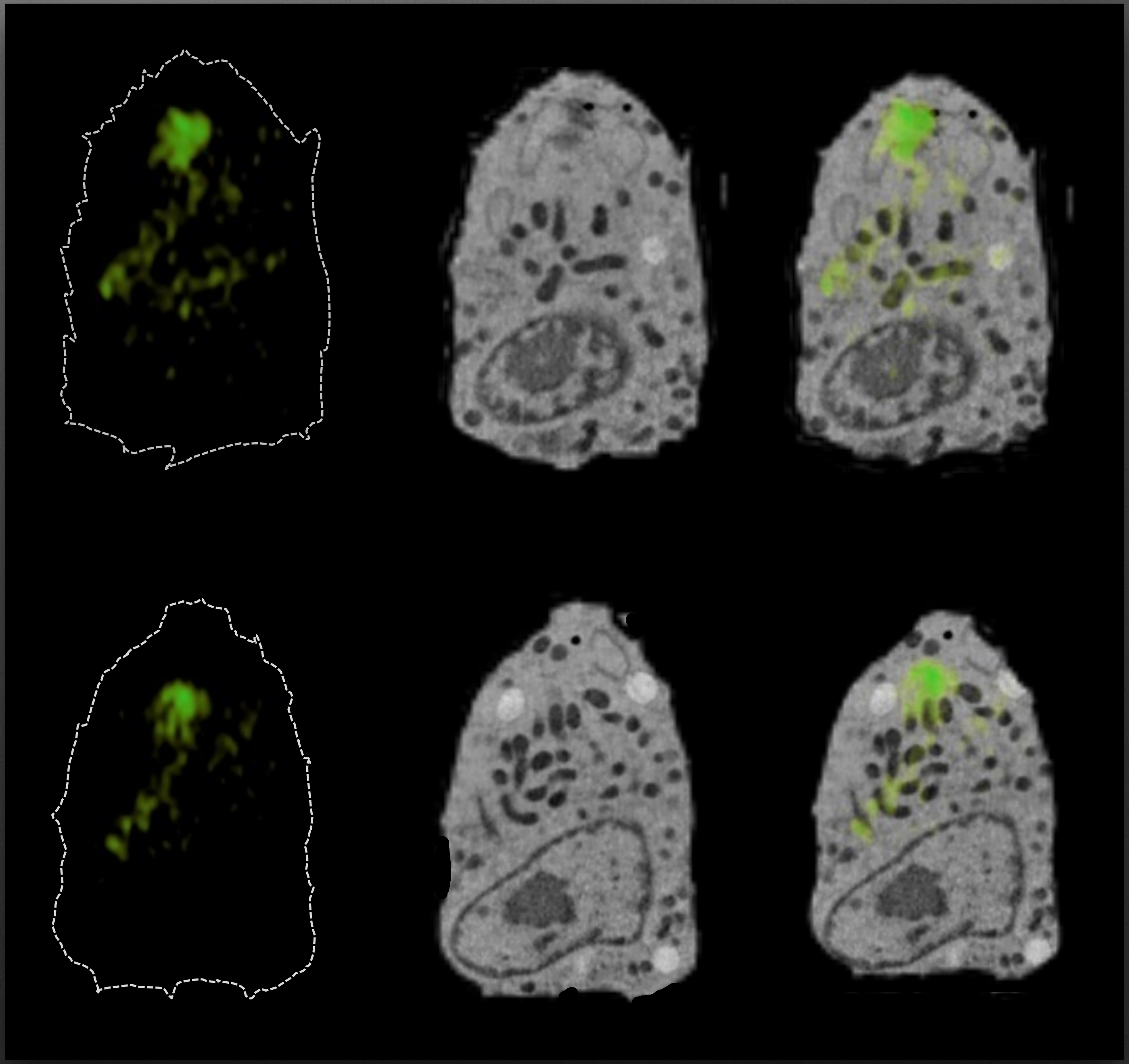
Medium LAC
0.32-0.34 μm^{-1}

Low LAC
0.30-0.32 μm^{-1}

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

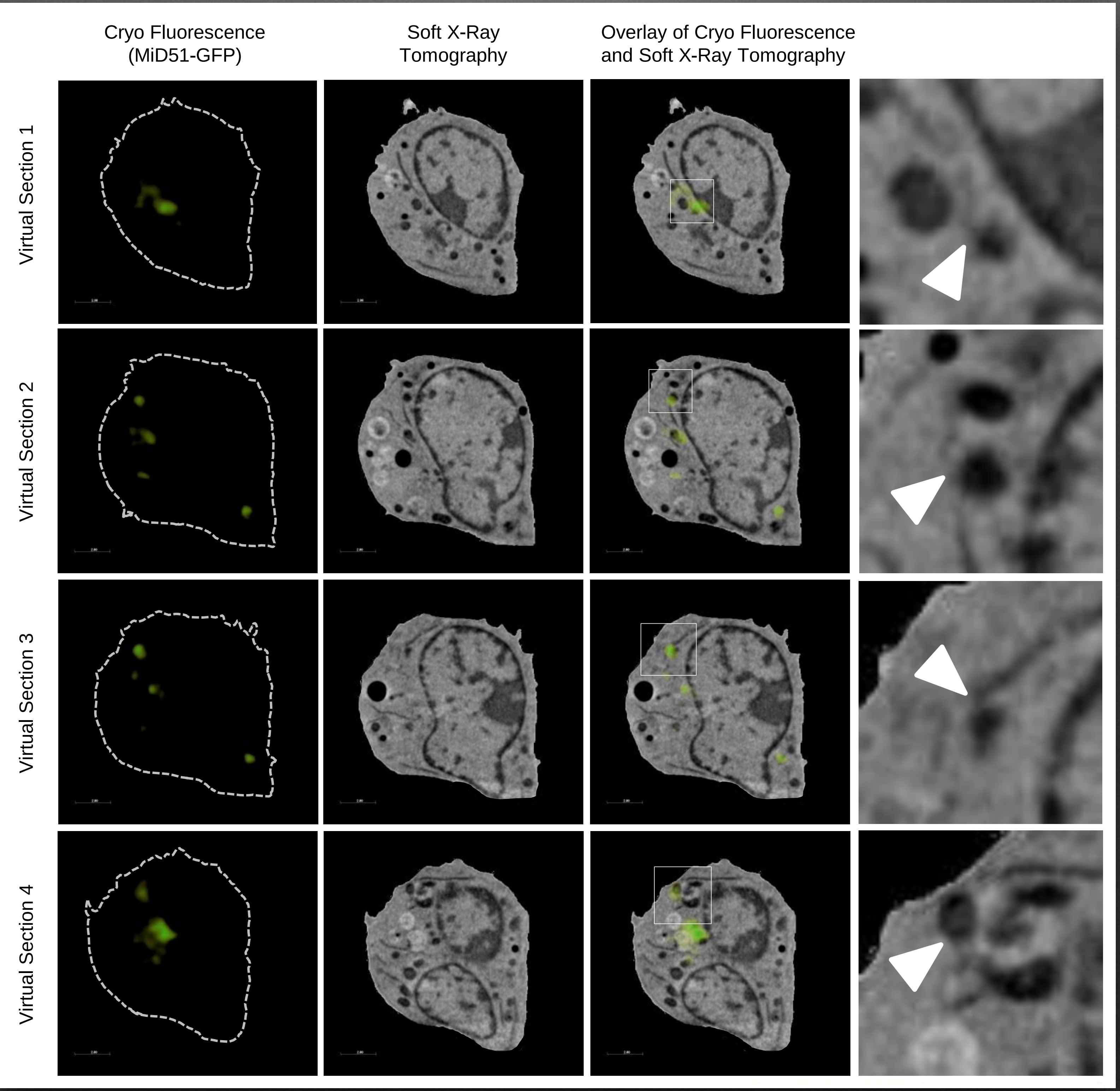
Mid51-GFP foci at ER - mitochondria contact sites





Elgass et al. (2015) J. Cell Science. 128(15), 2795-2804

Mid51-GFP foci at ER - mitochondria contact sites



Elgass et al. (2015) J. Cell Science. 128(15), 2795-2804

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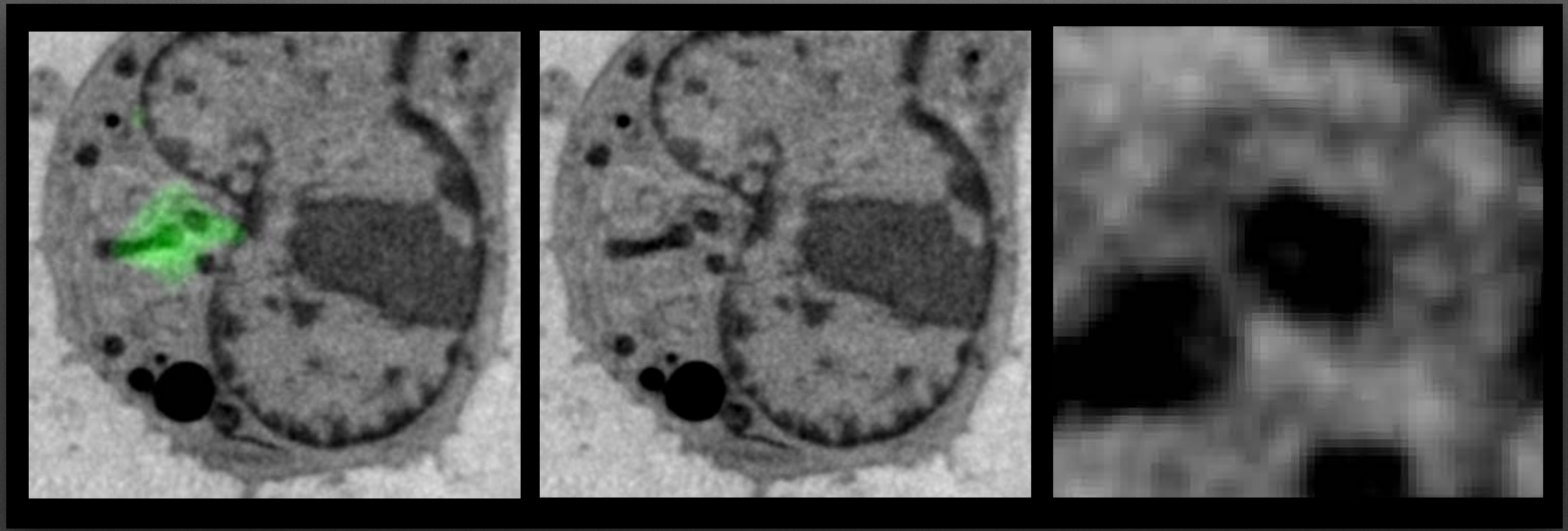

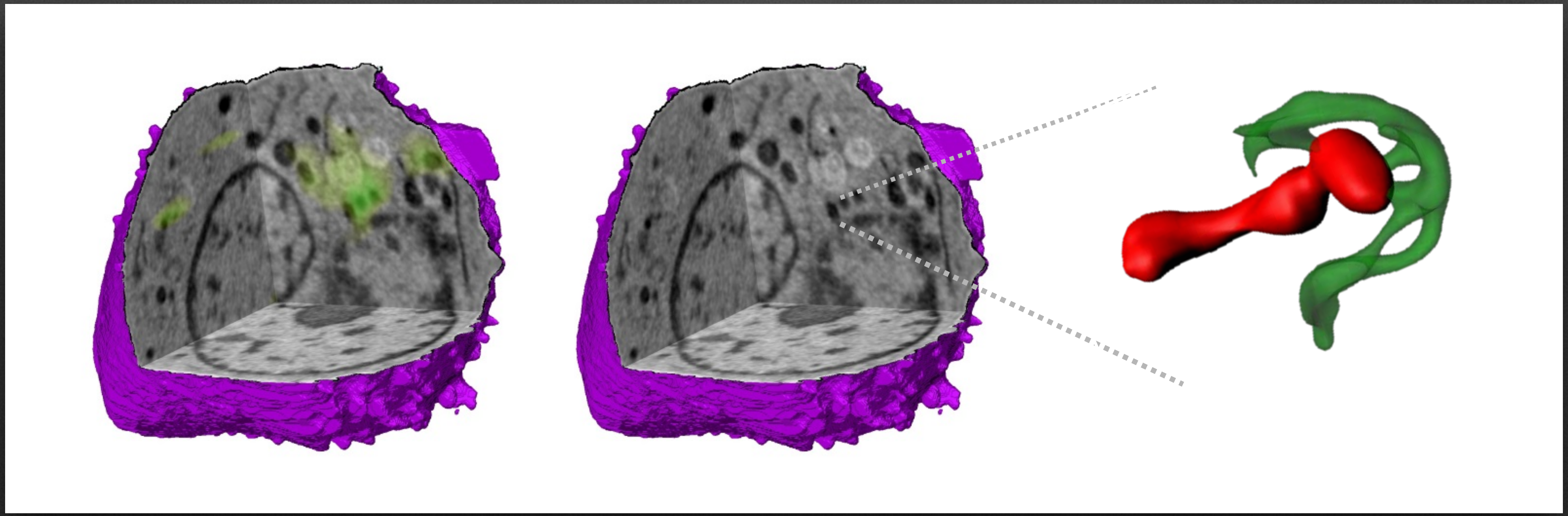

Elgass et al. (2015) J. Cell Science. 128(15), 2795-2804

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http://ncxt.lbl.gov

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