3-D views of nuclear organization and chromatin topology with x-ray tomography

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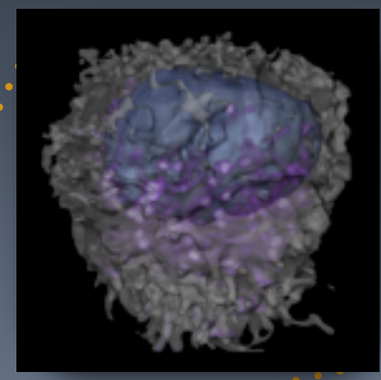
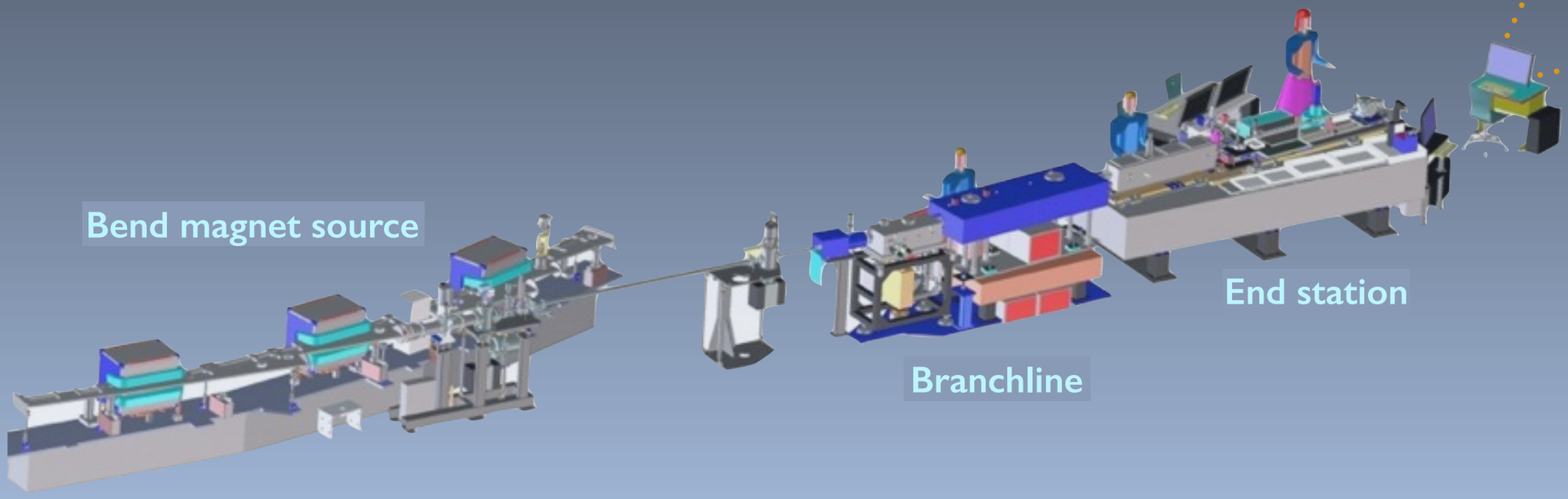
Lawrence Berkeley National Laboratory





National Center for X-ray Tomography

ncxt.lbl.gov

NIH-NIGMS
NIH Epigenomics Roadmap Grant
NIH-NIDA

DOE-Biological and Environmental Research
Gordon and Betty Moore Foundation

Why soft x-ray tomography?

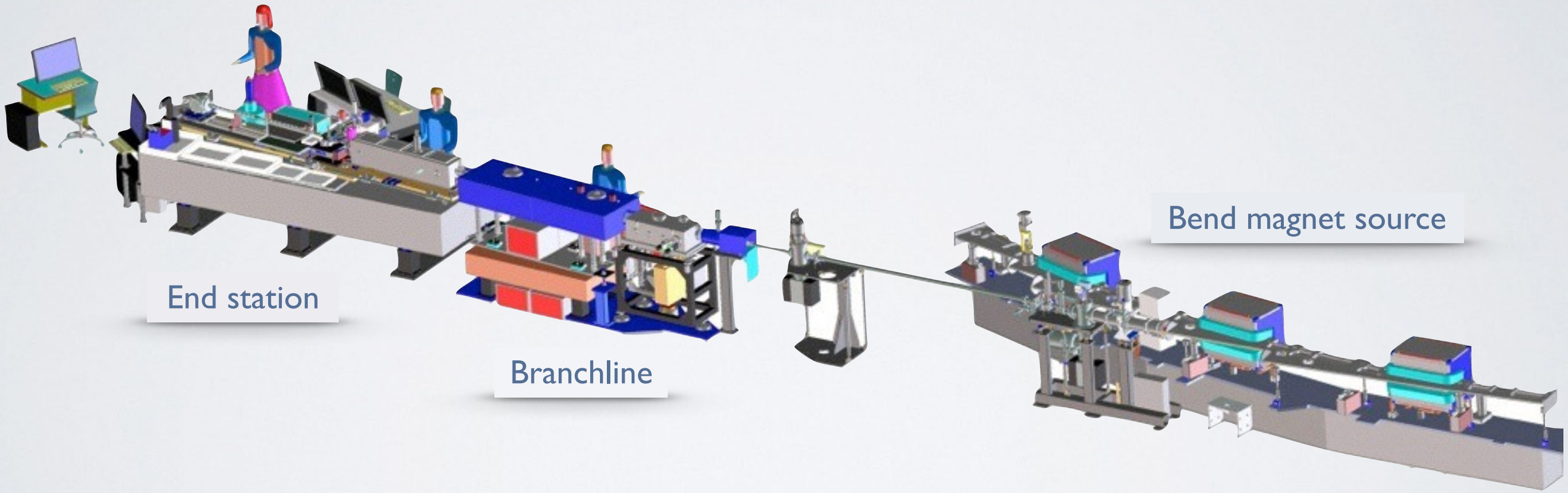
- Image biological specimens up to 15μm thick
- Specimens in near-native state
  - Hydrated
  - Cryo-immobilized
- High-contrast images without dyes or stains
- Quantitative
- Better than 50 nm resolution (isotropic)
- Localize molecules with respect to cell structures
  - Correlated fluorescence and x-ray tomography

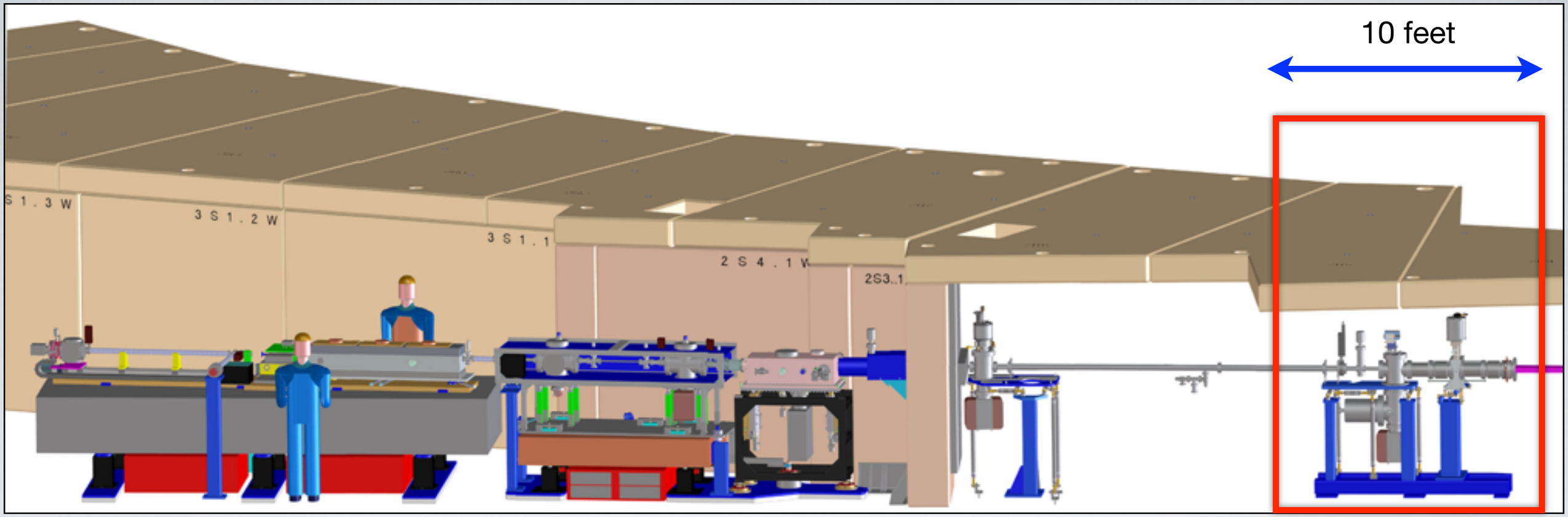
Lawrence Berkeley National Laboratory

Full-field transmission x-ray microscope

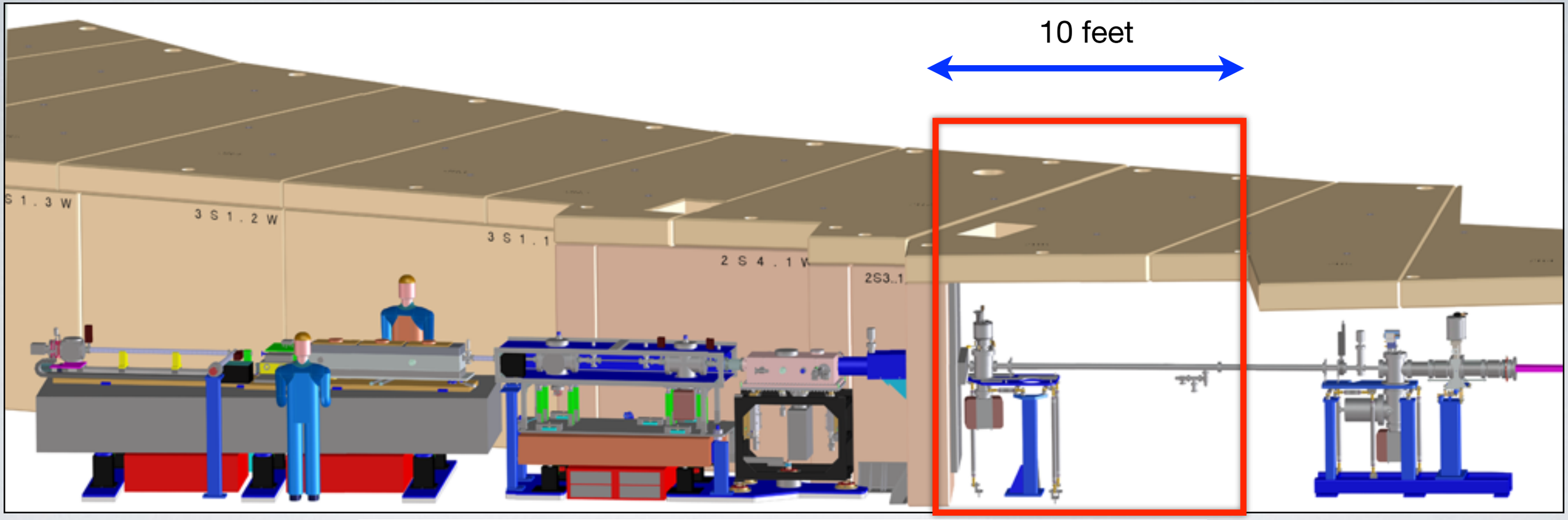
Whole, hydrated, unfixed, unstained cells

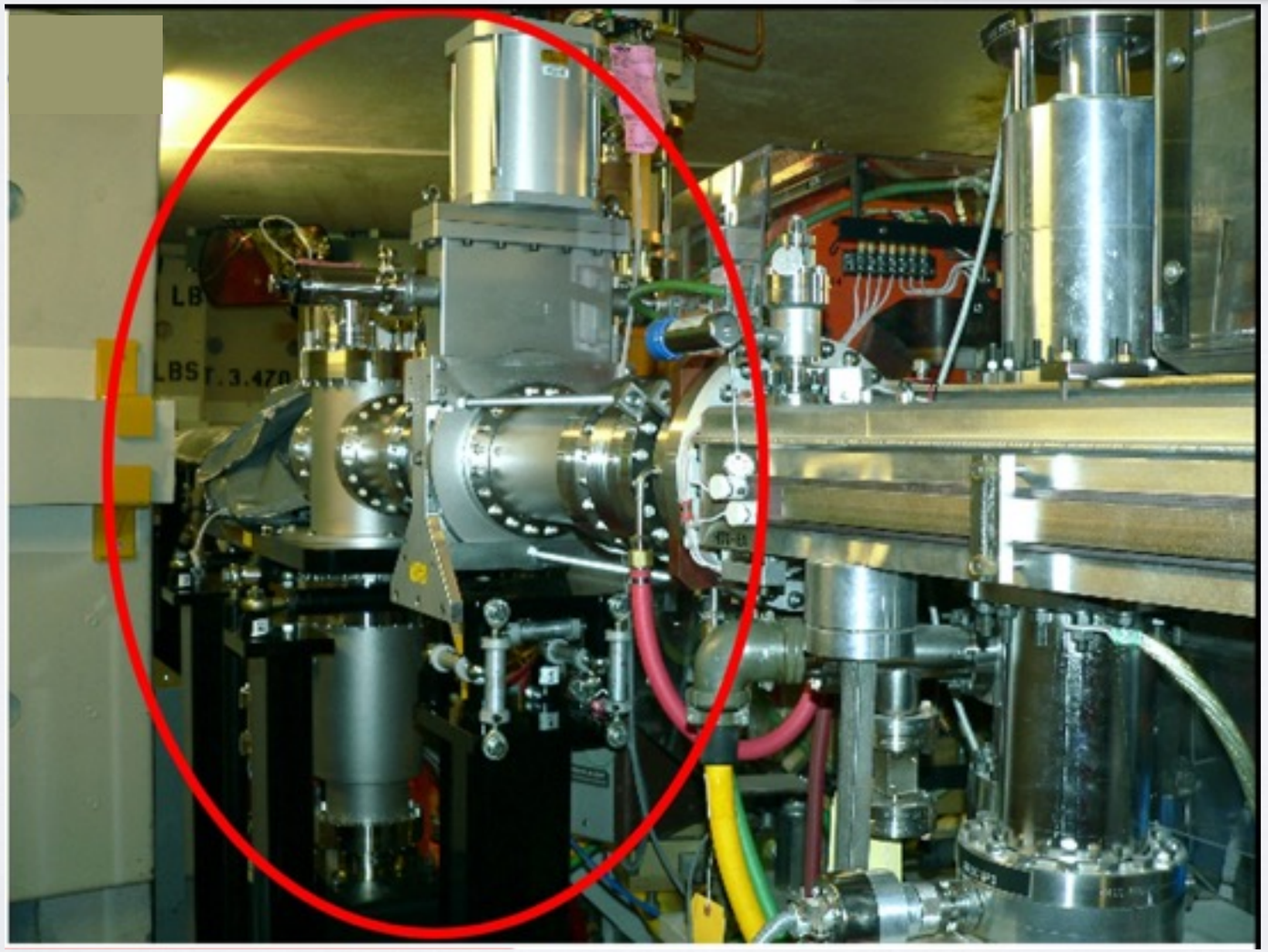
50 nm isotropic resolution



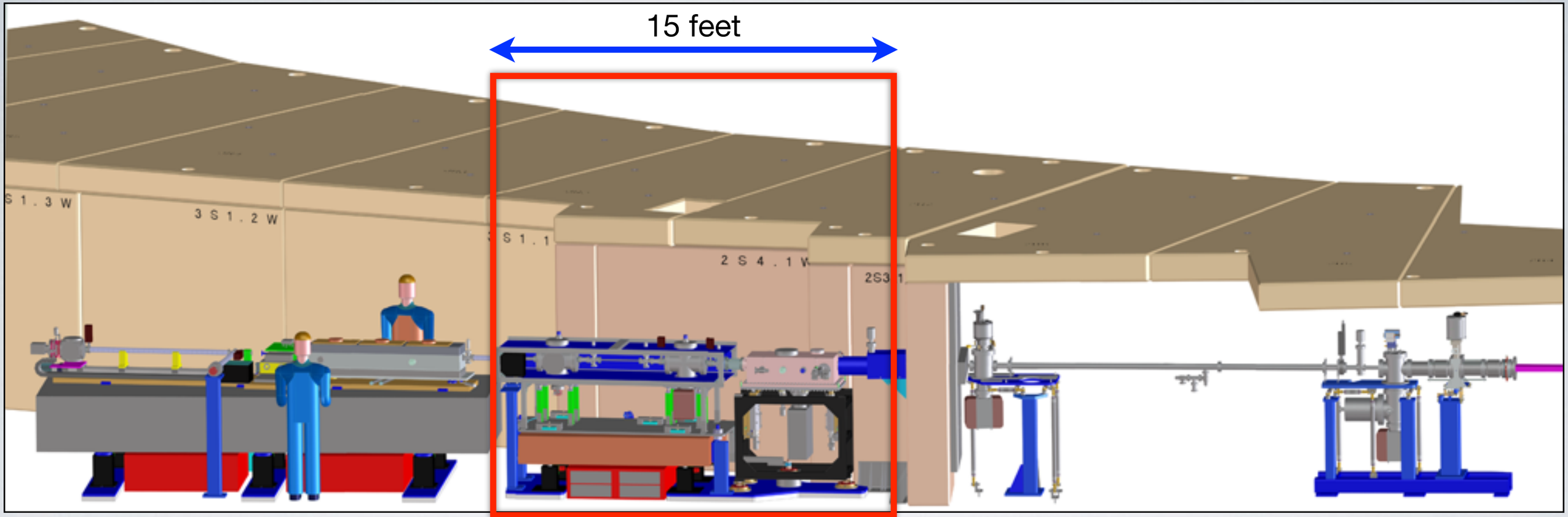


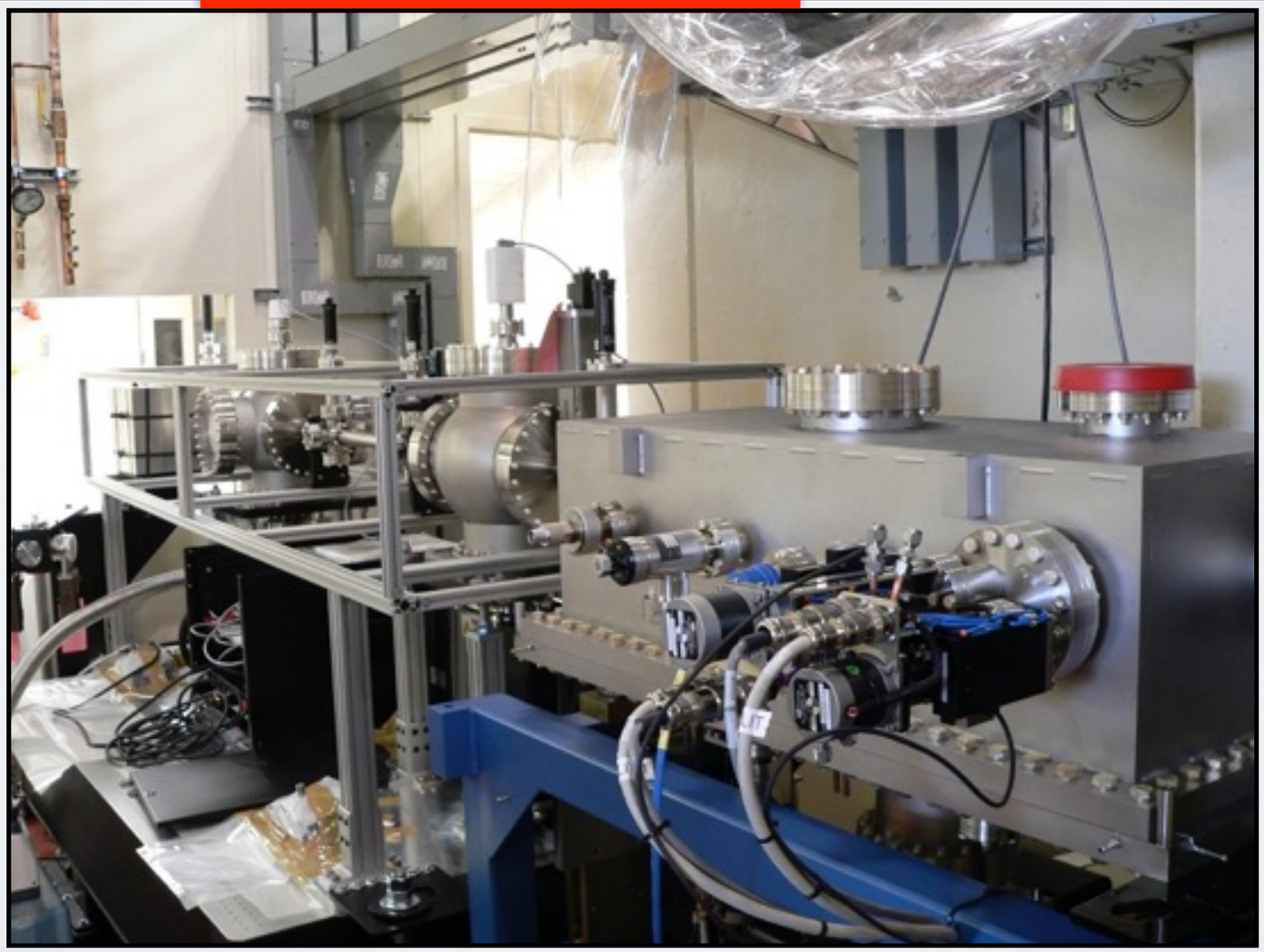
photon shutter



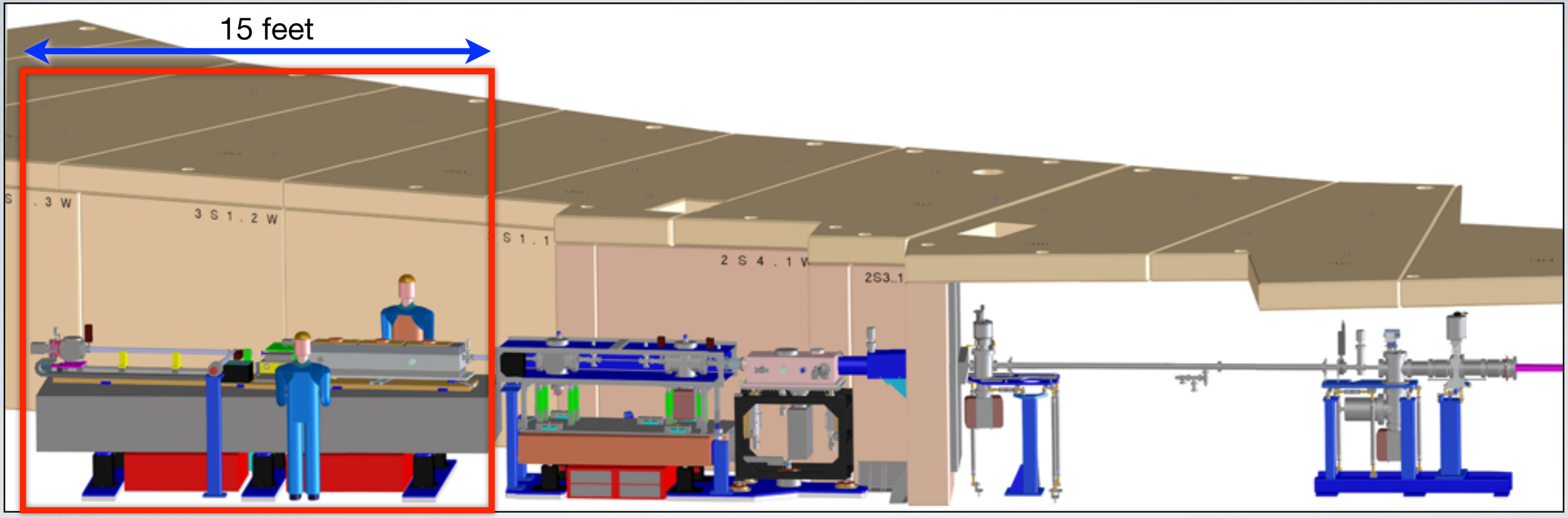


personnel
safety shutter



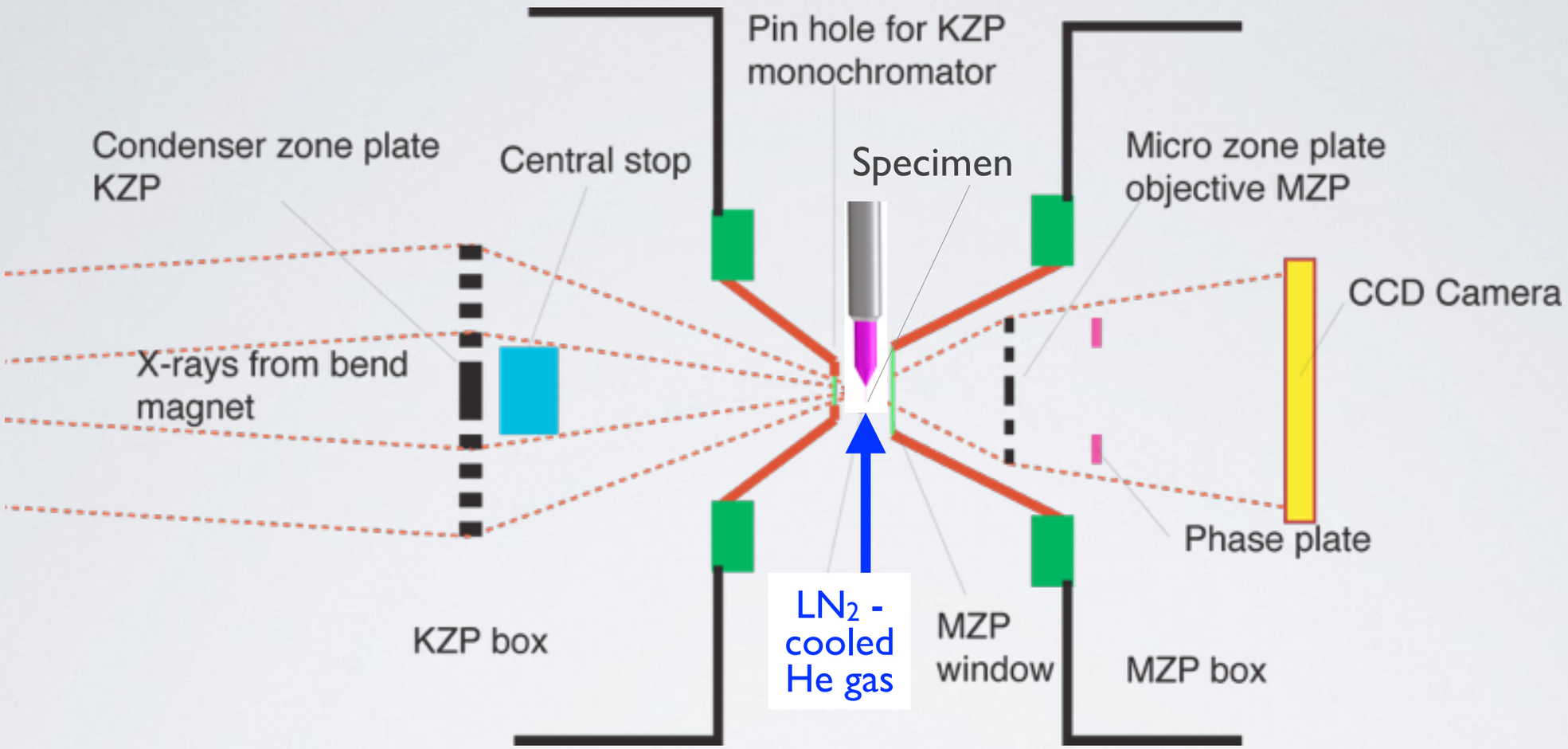


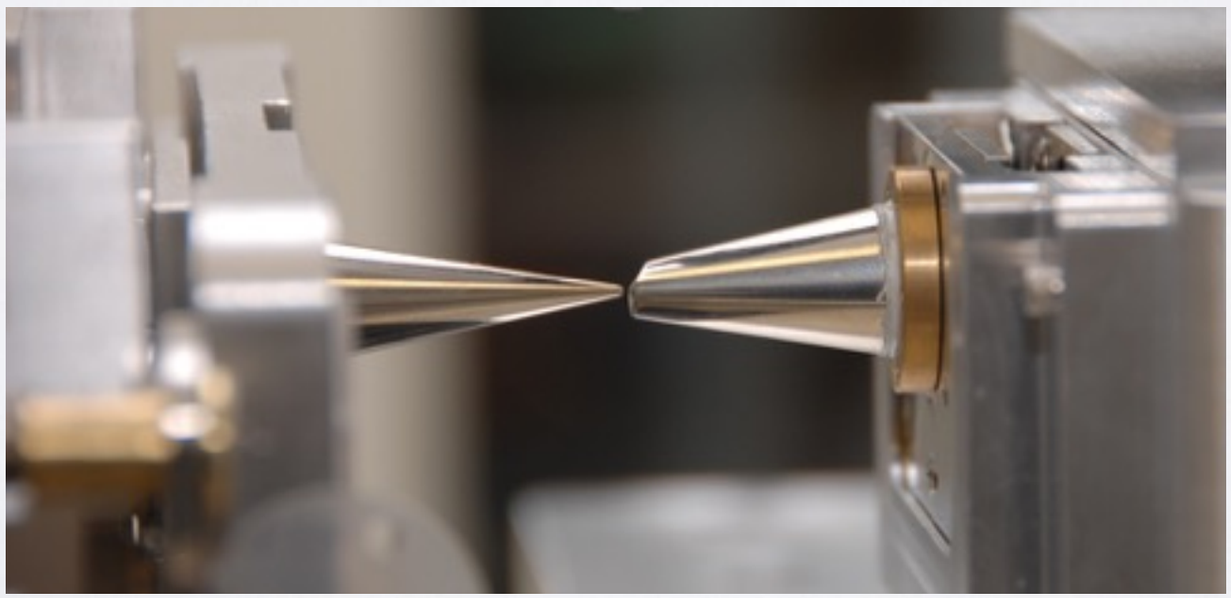
mirror and branchline



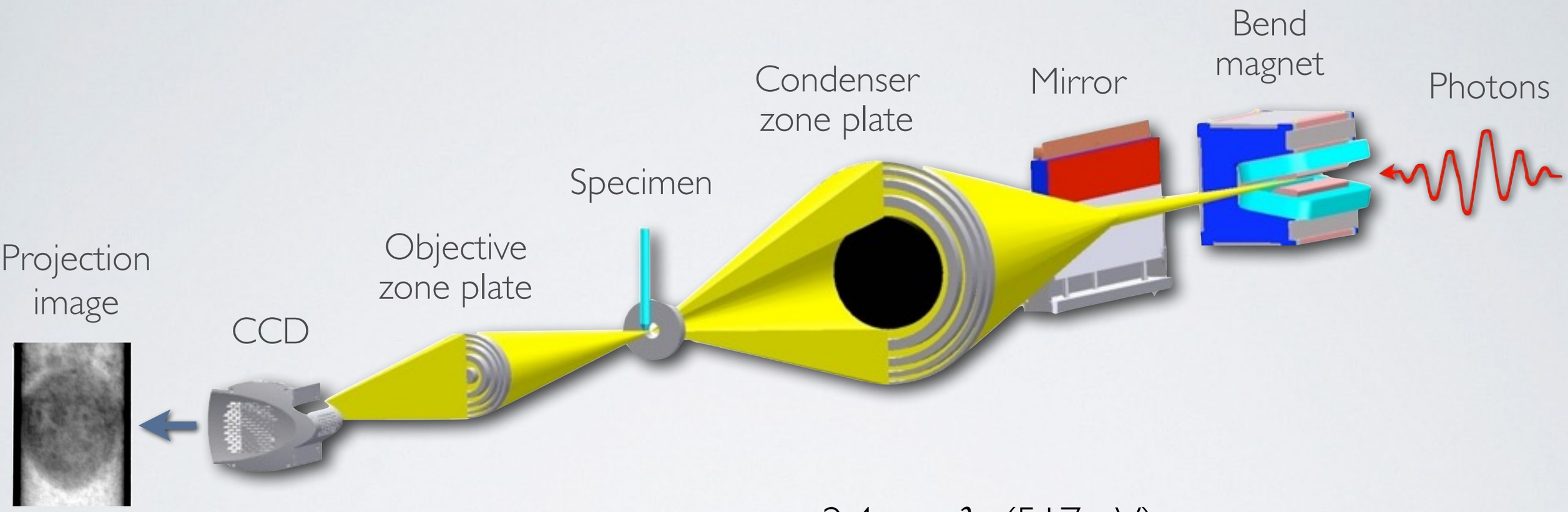
X-ray microscope end station

Specimen stage





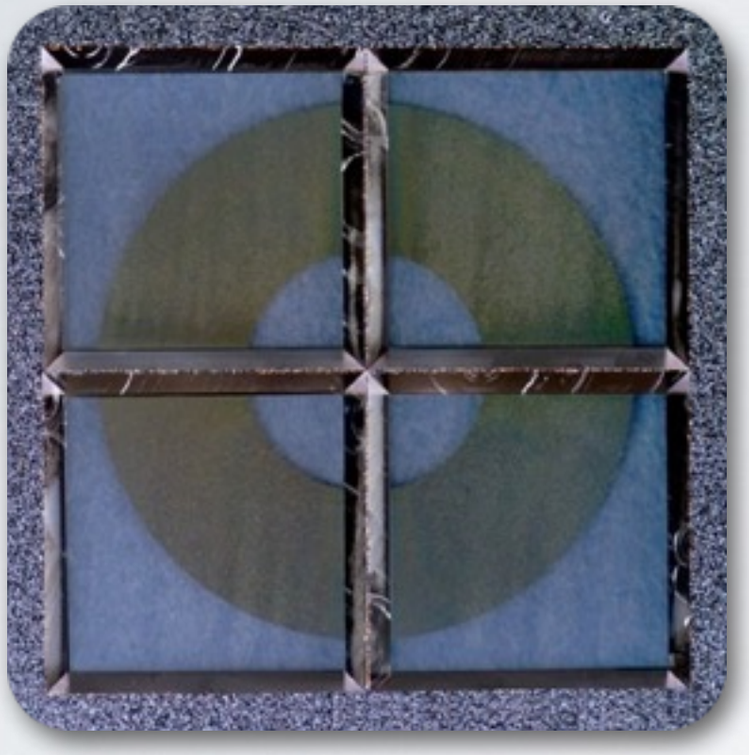
Soft x-ray tomography



- 2.4 nm λ (517 eV)
- Condenser zone plate focuses source onto specimen
- Objective zone plate magnifies object onto CCD camera

Zone plate lenses - diffractive optics

Condenser lens



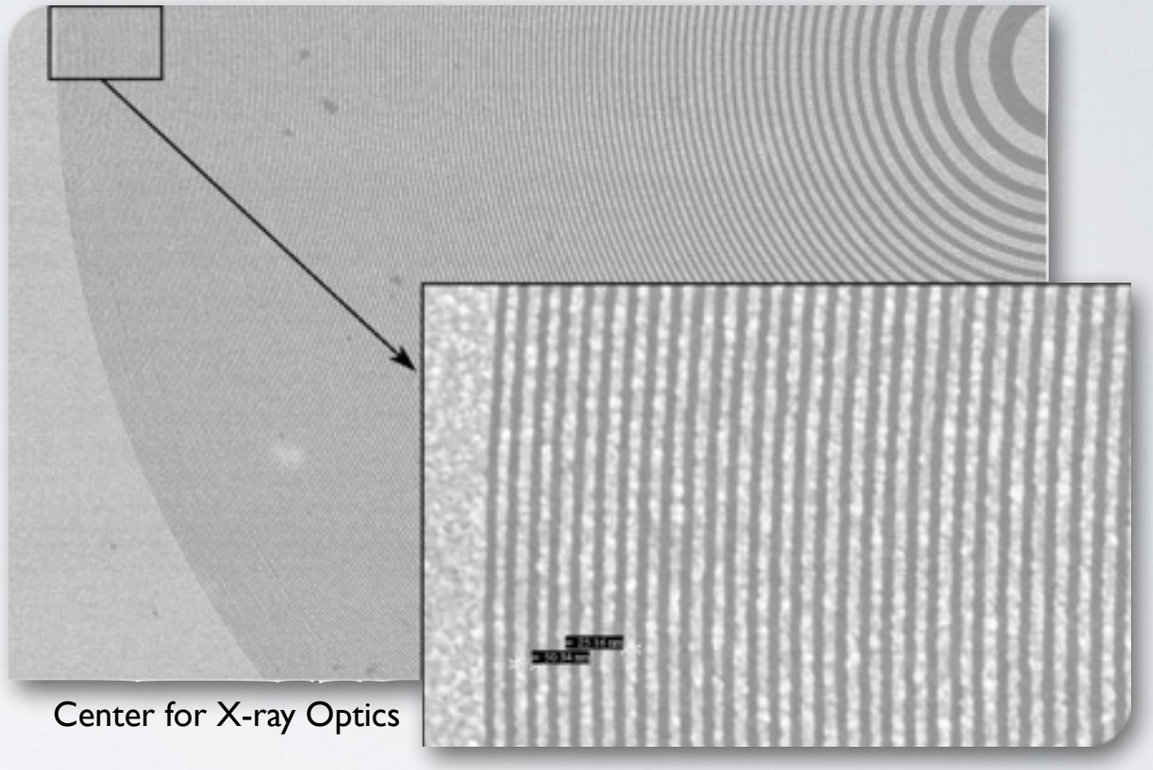
Diameter = 1 cm

No. of zones = 41,700

Outer zone width = 50 nm

Central stop diameter = 5 mm

Objective lens



Diameter = 63 μm

No. of zones = 628

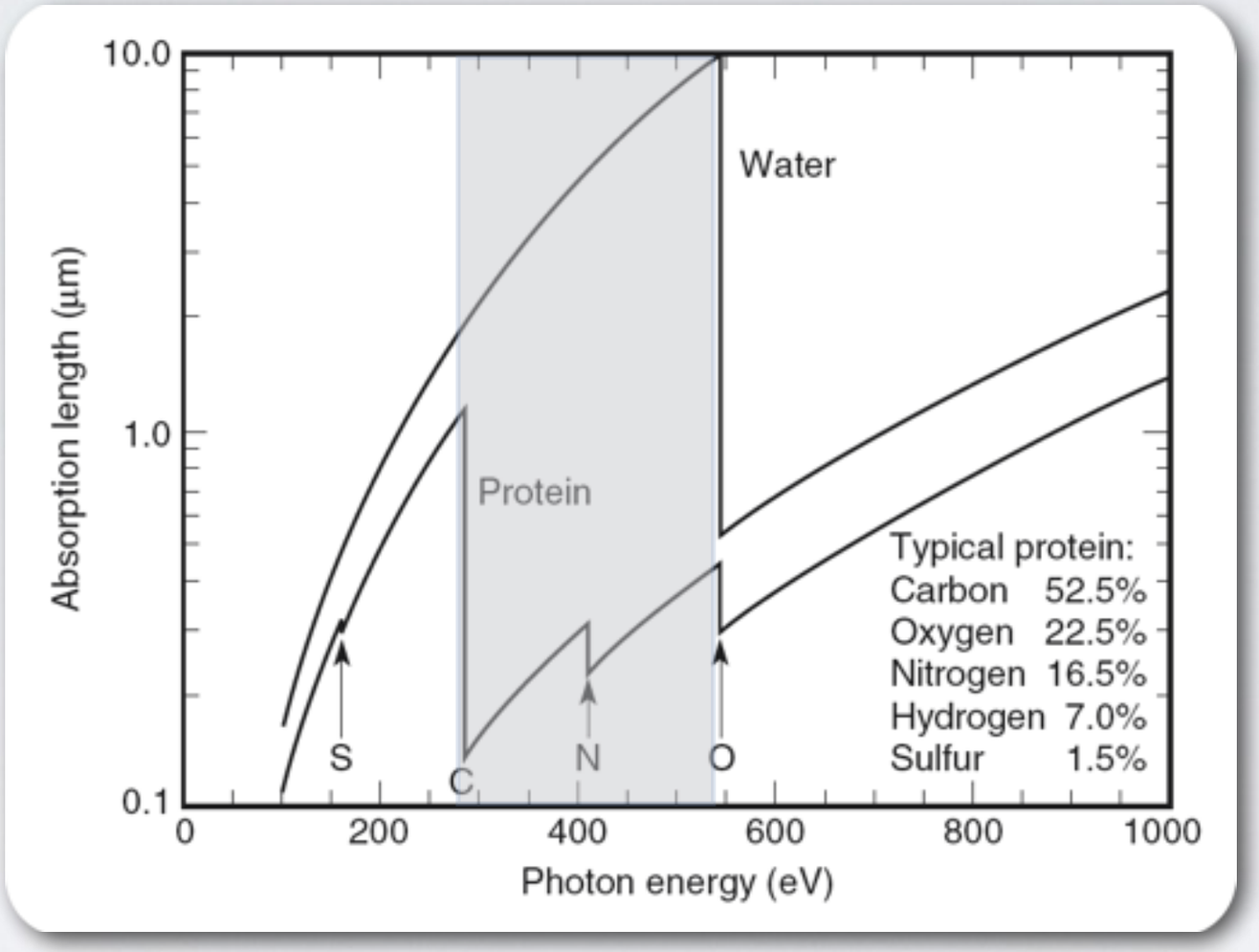
Outer zone width = 25 nm

Nickel plating

Imaging in the water window

Image between K shell absorption edges of C (284 eV, 4.4nm) & O_2 (543 eV, 2.3nm)

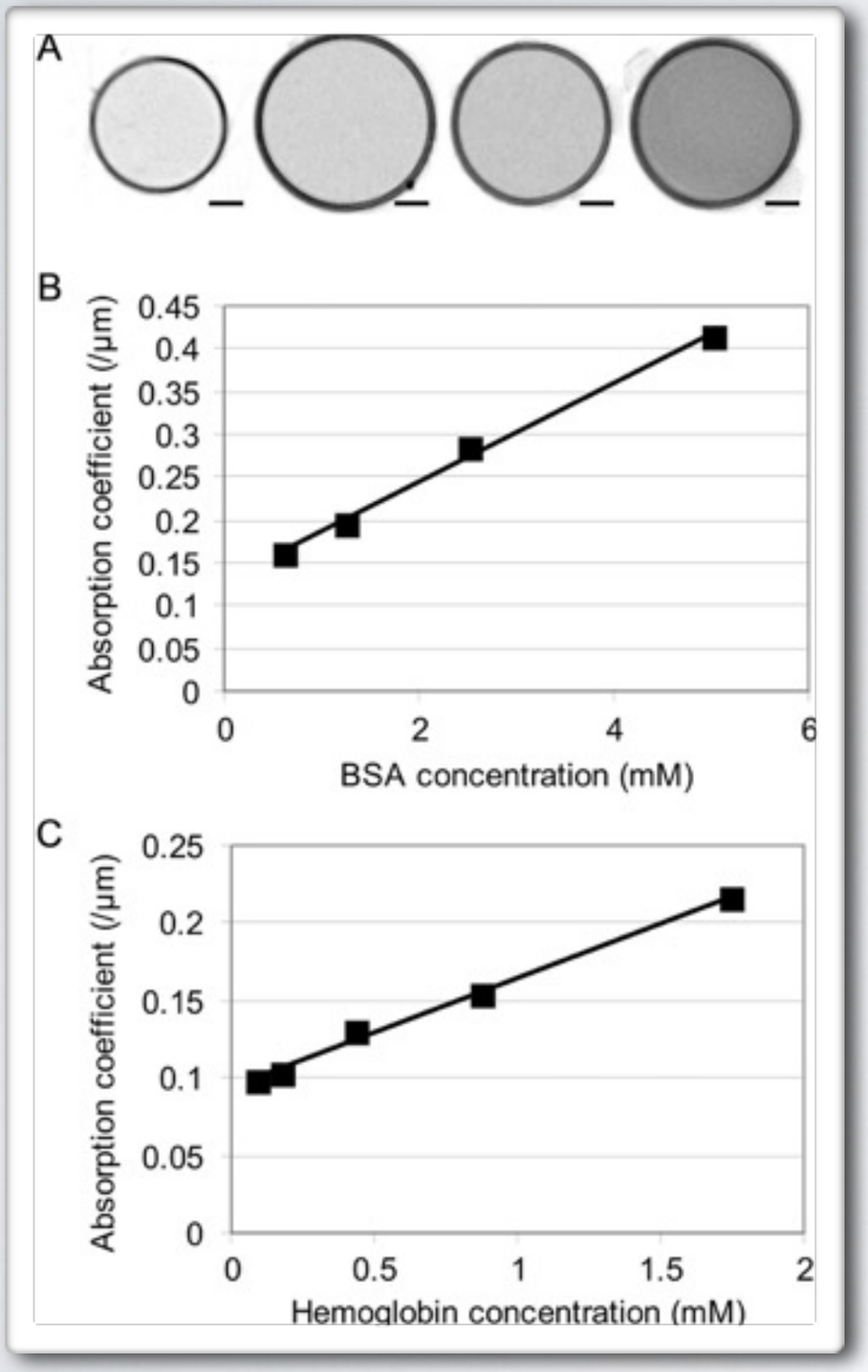
Absorption adheres to Beer-Lambert's law and is linear with thickness & concentration

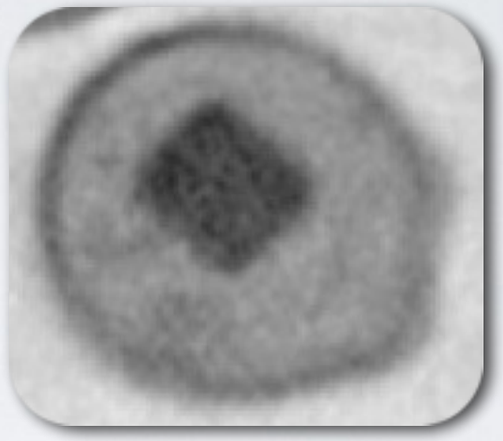


2.4 nm

517 eV

Linear absorption coefficient





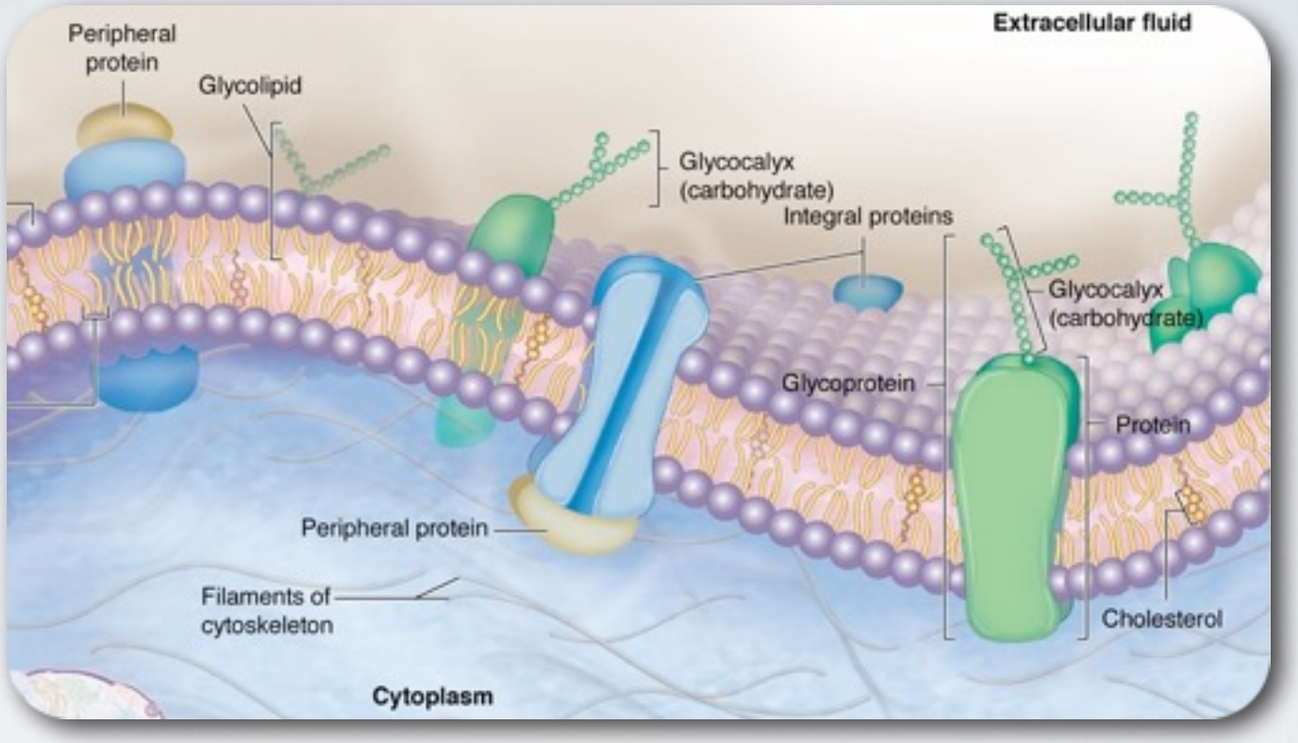
Alcohol oxidase crystal in yeast cell

Calculated LAC ➟ 0.625 μm^{-1}

Measured LAC ➟ 0.626 μm^{-1}

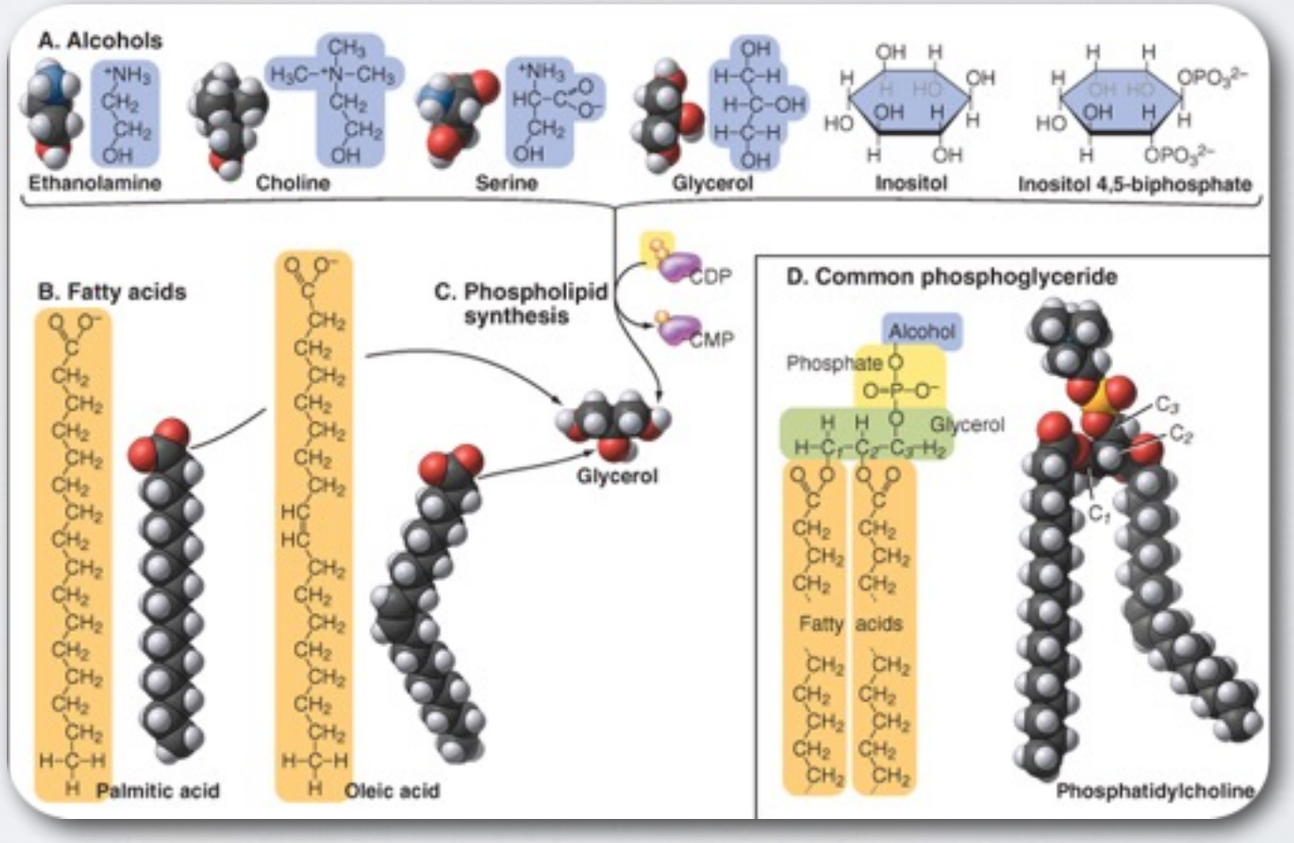
Imaging in the water window

Membrane
(lipid bilayer)



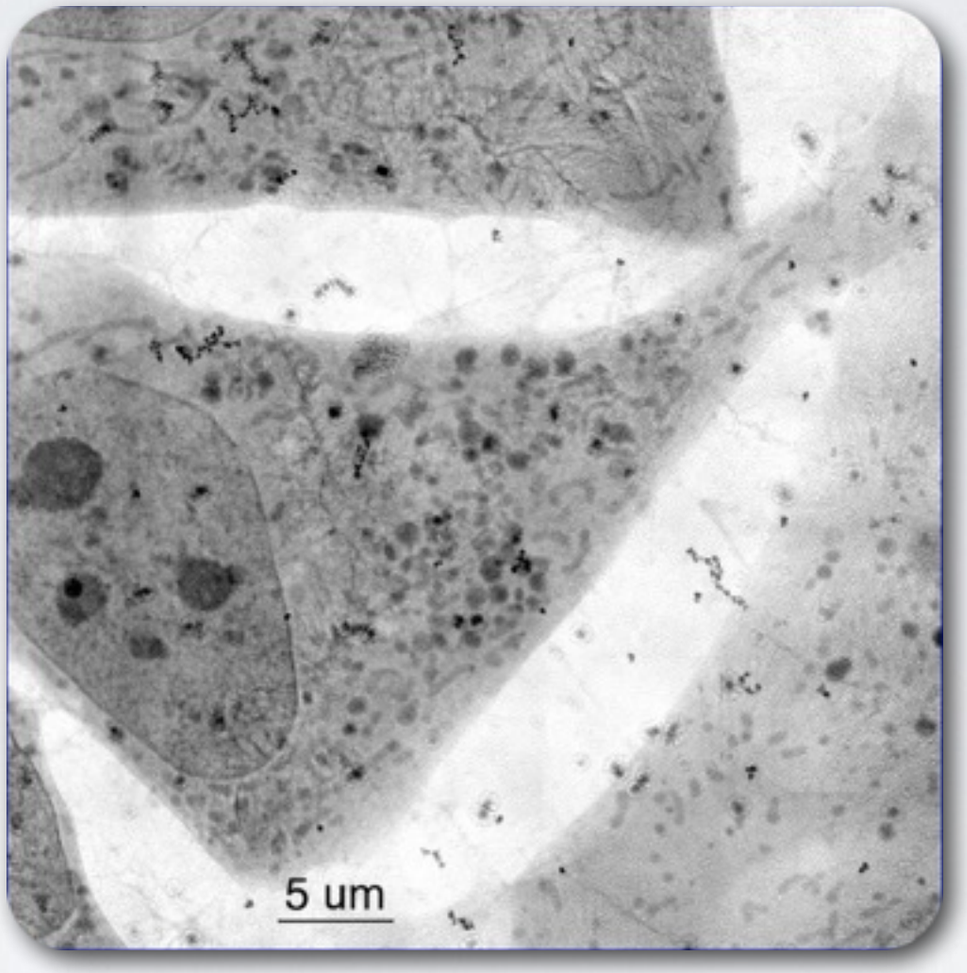
Membrane lipids

tightly packed
carbon molecules



Imaging in the water window

High carbon content structures have high contrast



Specimen preparation

Specimen holder

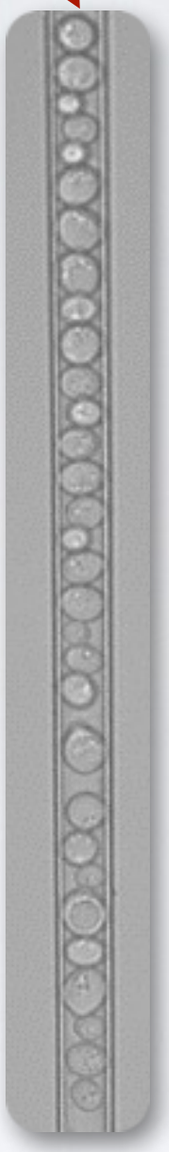
1 mm

300 µm

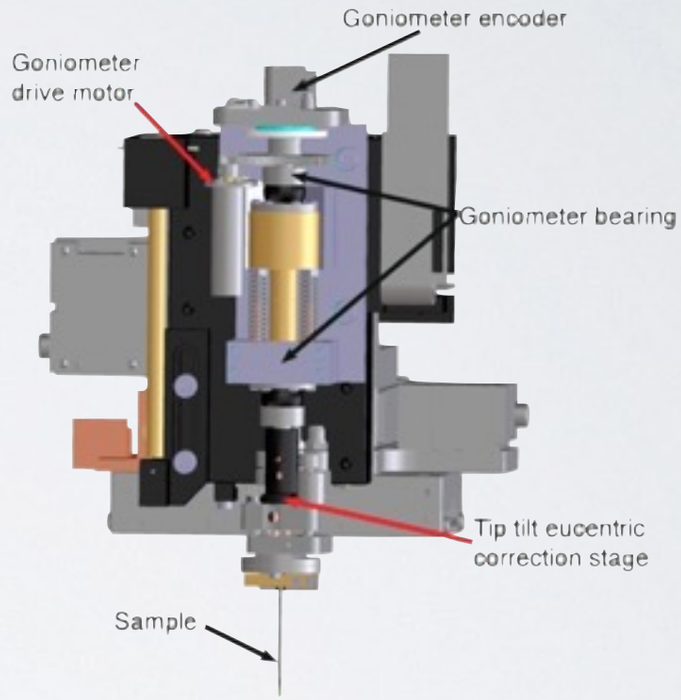
2-20 µm

Specimen

Rapidly freeze



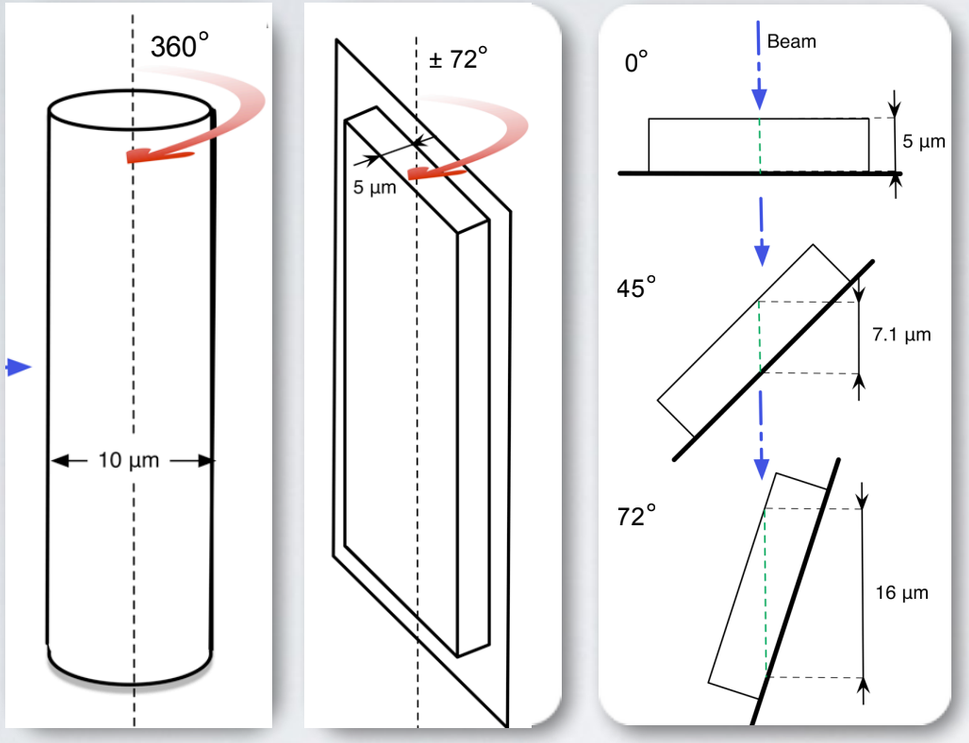
Fast - 5 min / cell



Full rotation vs. limited tilt

Full rotation
(± 90°)

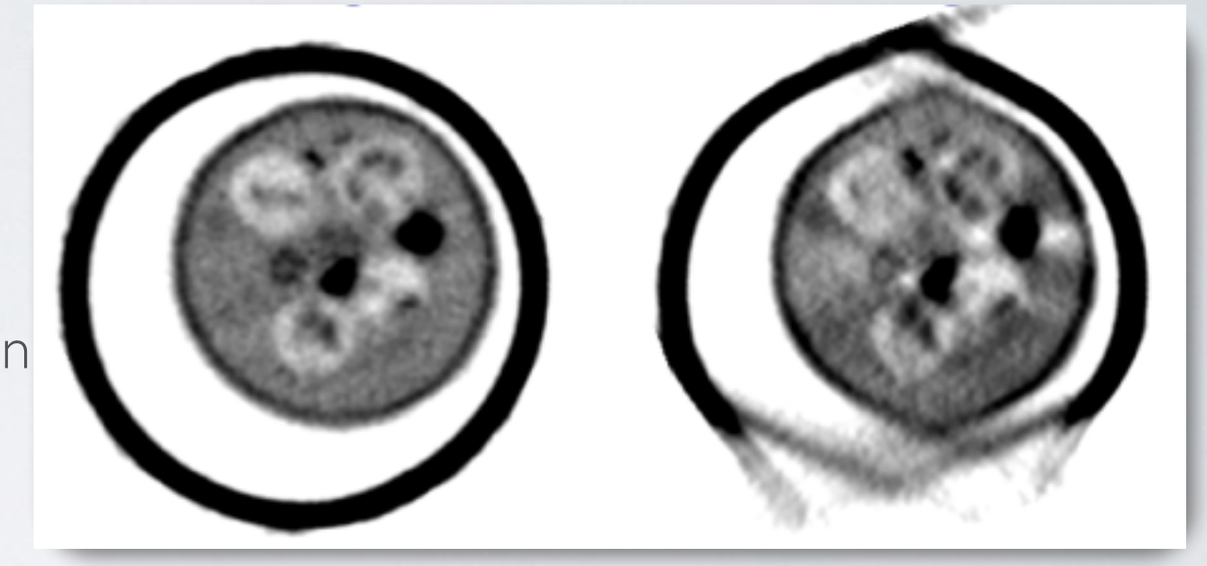
Flat specimen
limited tilt



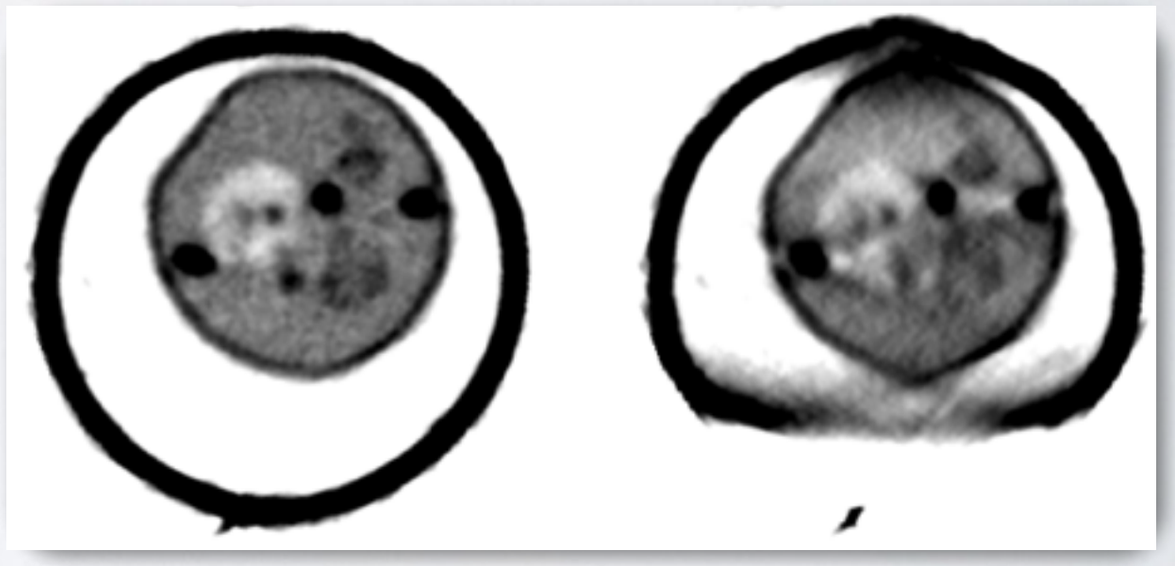
Filtered back
projection
reconstruction

Full rotation
(± 90°)

Limited tilt
(± 70°)



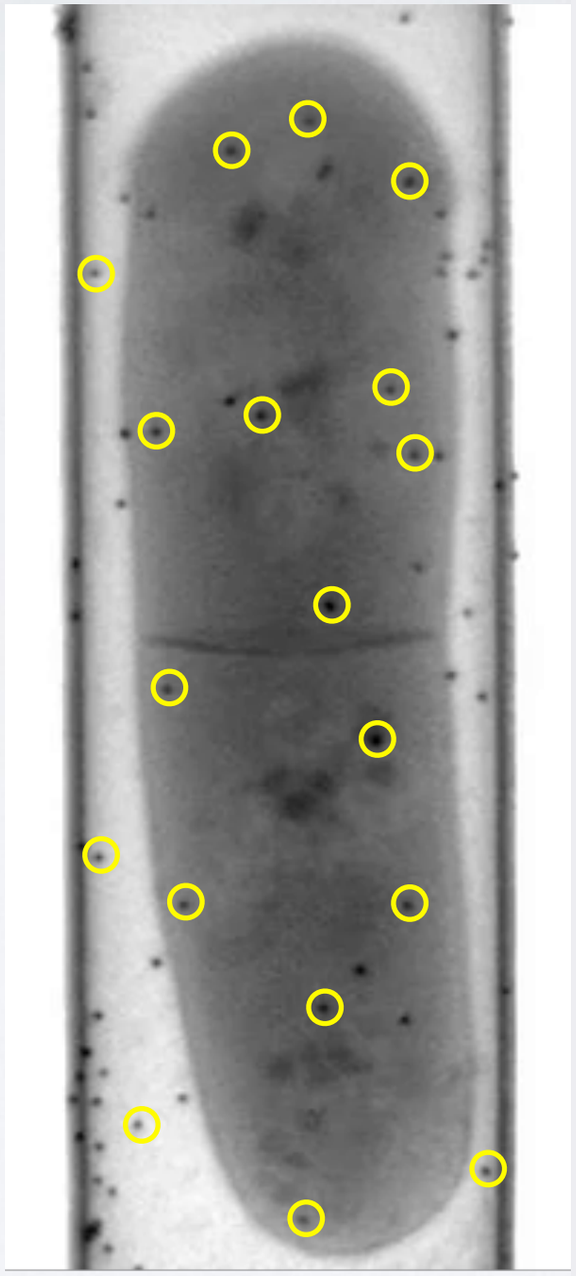
Iterative
algorithm
reconstruction



Smith EA et al. (2013) J. Structural Biology. 184:12-20

Aligning projection images

Gold particles to align images

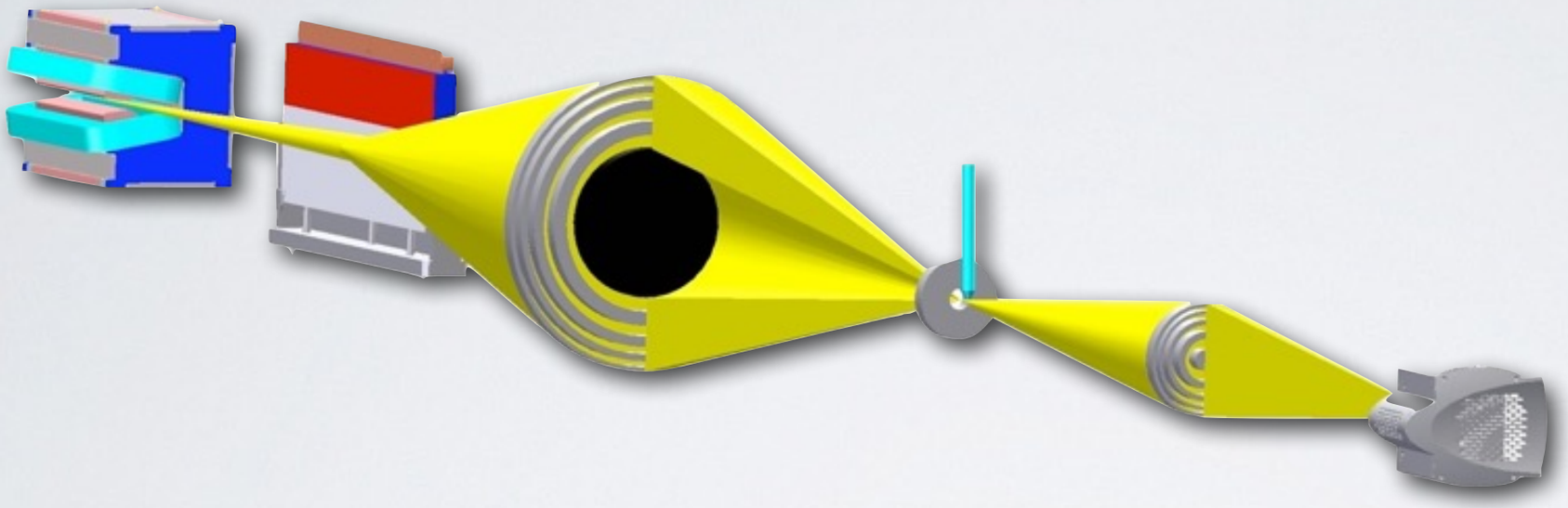


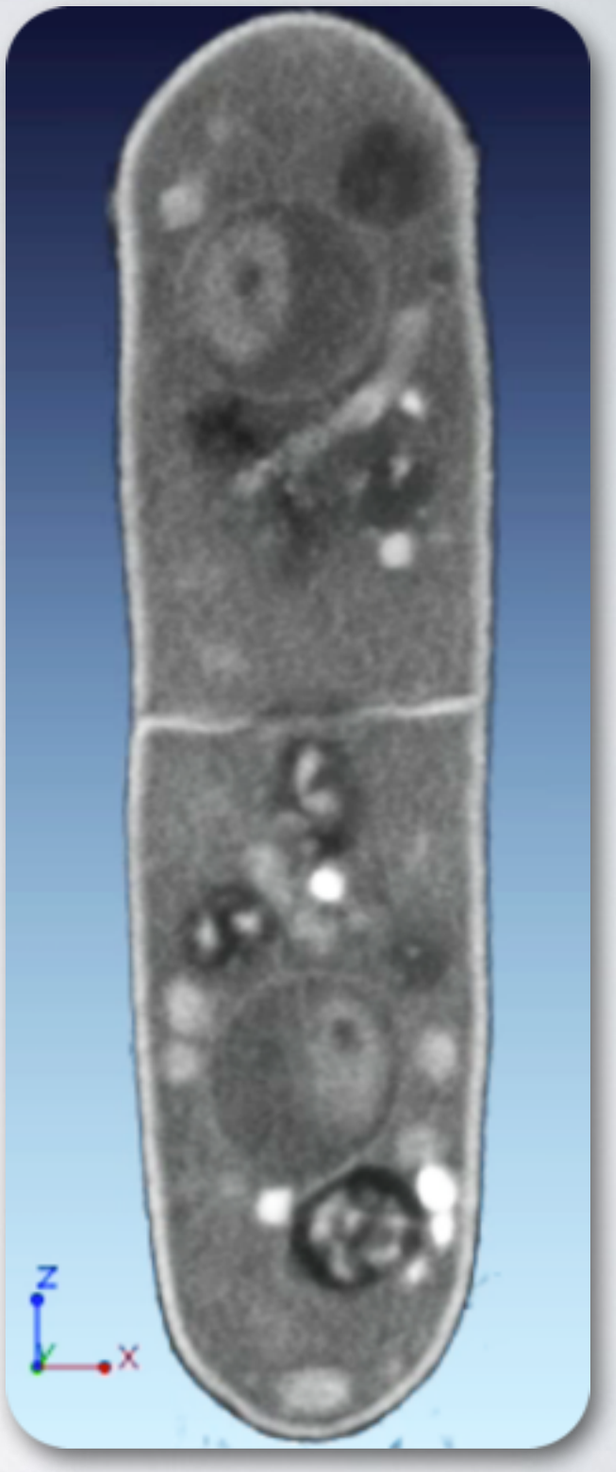
Tomographic reconstruction

- Filtered back projection
- Iterative algorithms

Orthoslices from tomographic reconstruction

Negative images





Segmentation

S. pombe organelles segmented, color-coded

Nucleolus

Nucleus

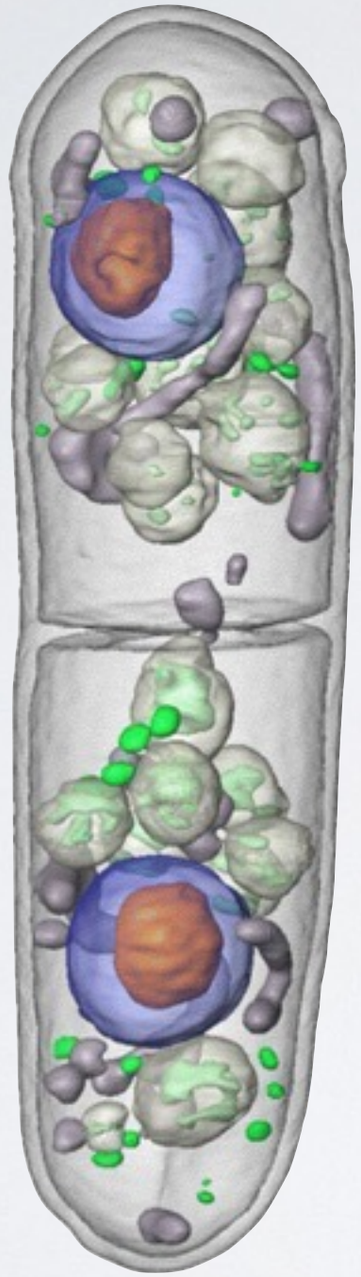
Lipids

Mitochondria

Vacuoles

Contents of vacuoles

S. pombe organelles - quantitative data

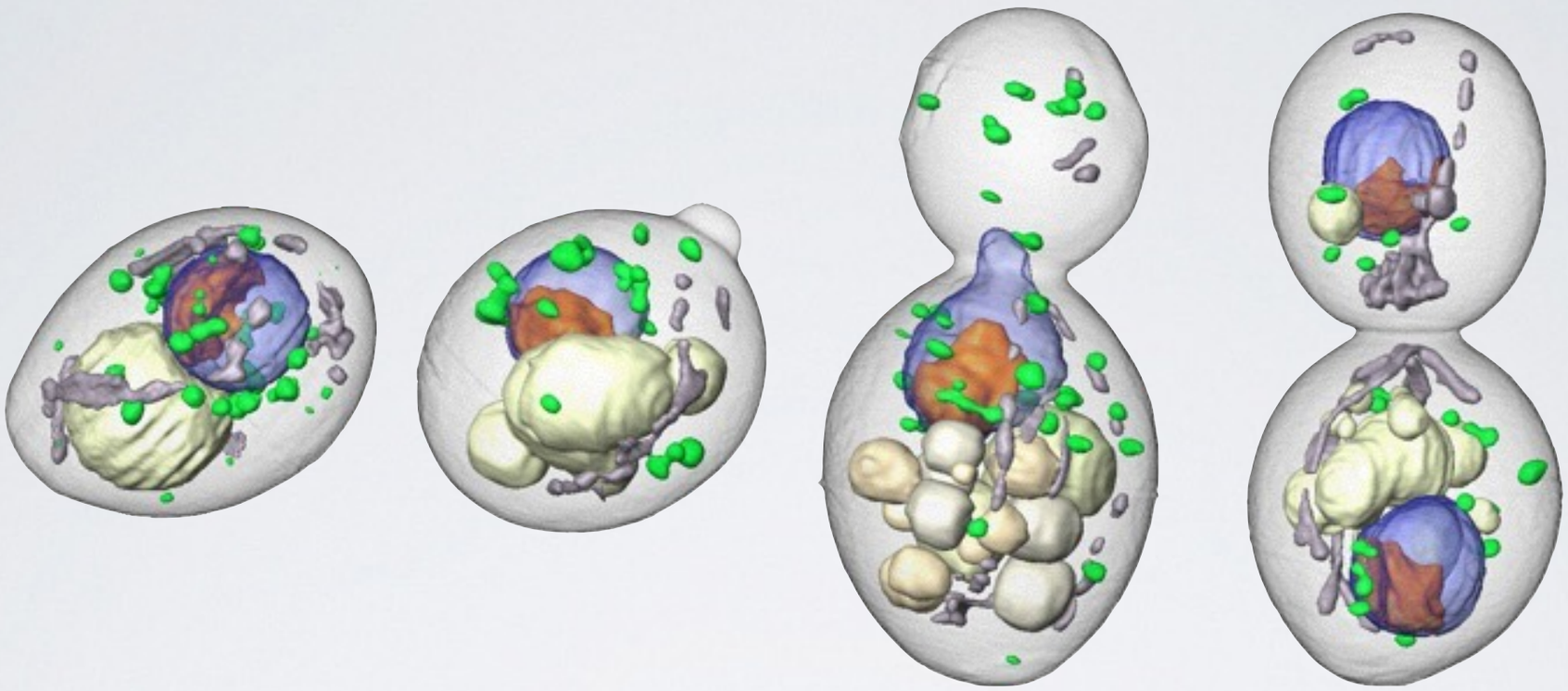


	Volume (μm^3)	Average LAC (μm^{-1})
Lipids	0.45	0.72
Mitochondria	2.97	0.42
Nuclei	4.59, 4.90	0.31
Nucleoli	0.74, 0.71	0.37
Endosomes	13.9	0.23
Endosome inclusions	1.15	0.42

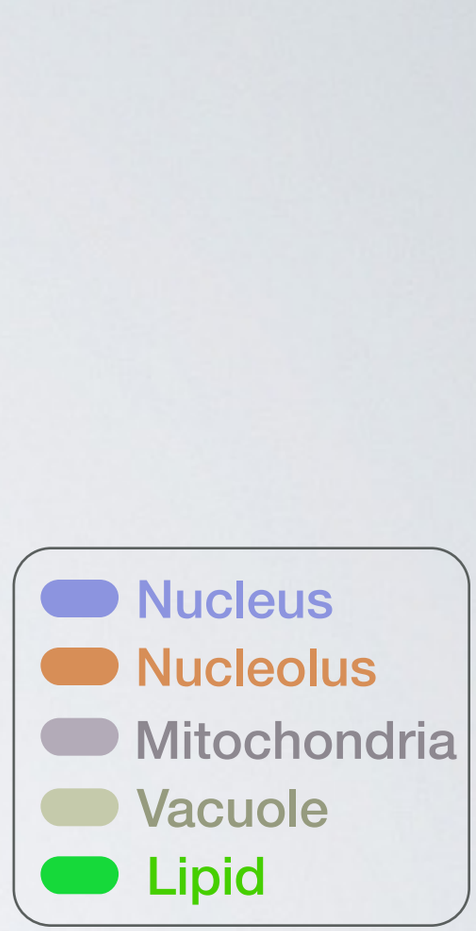
Phenotypic consequences of genetic manipulations

Normal structure of *S. cerevisiae*

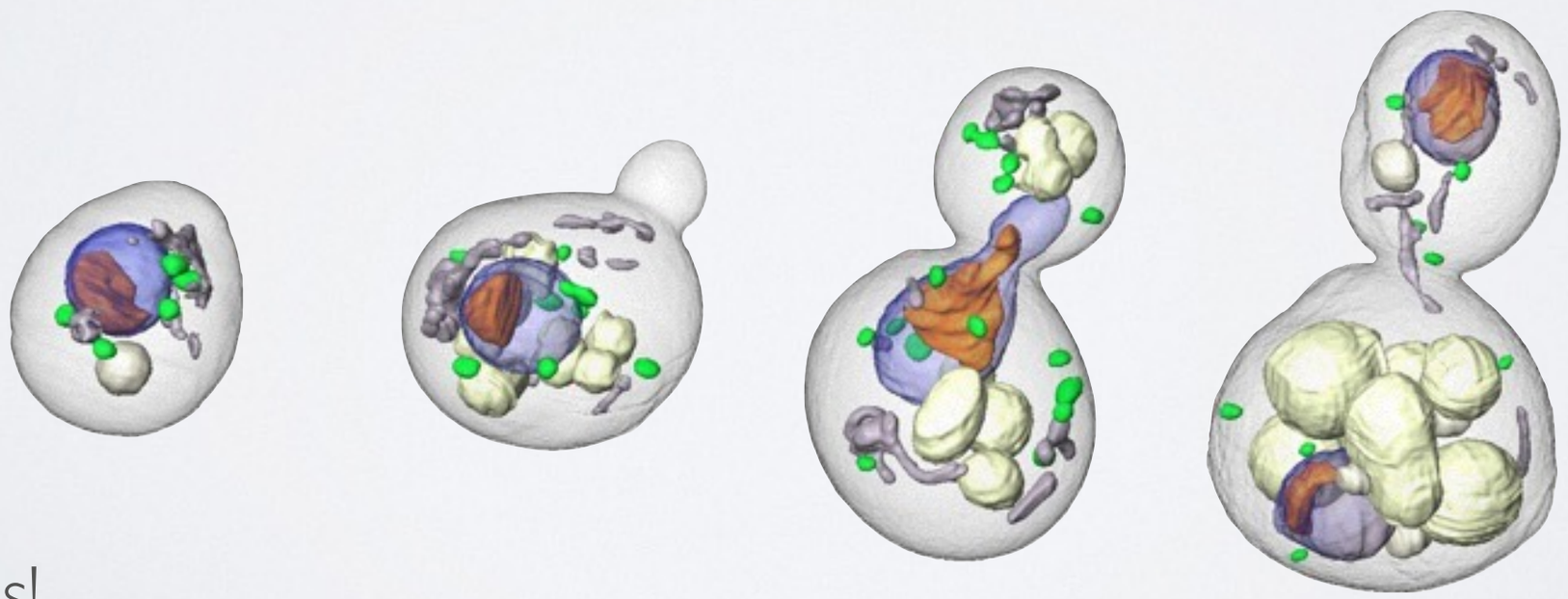
Diploid



G1 S G2 M



Haploid

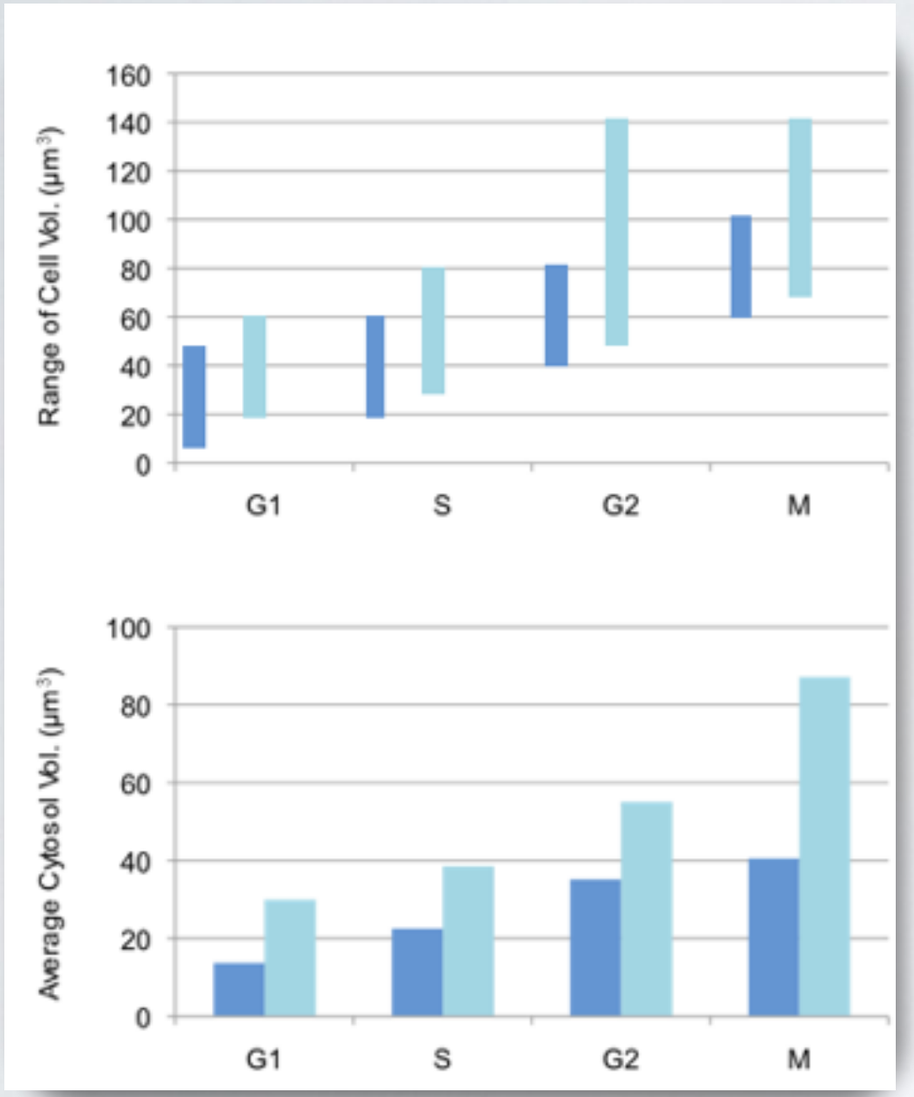


250 cells!

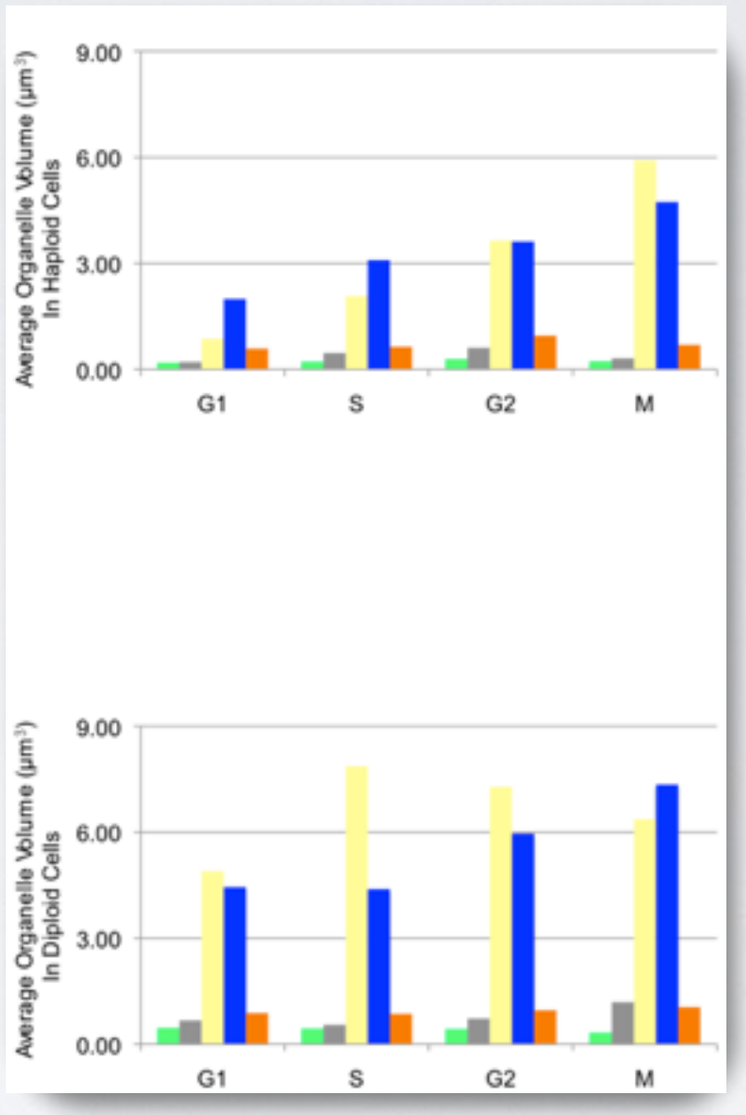
Uchida et al. (2011) Yeast 28:227-236

Normal structure of *S. cerevisiae*

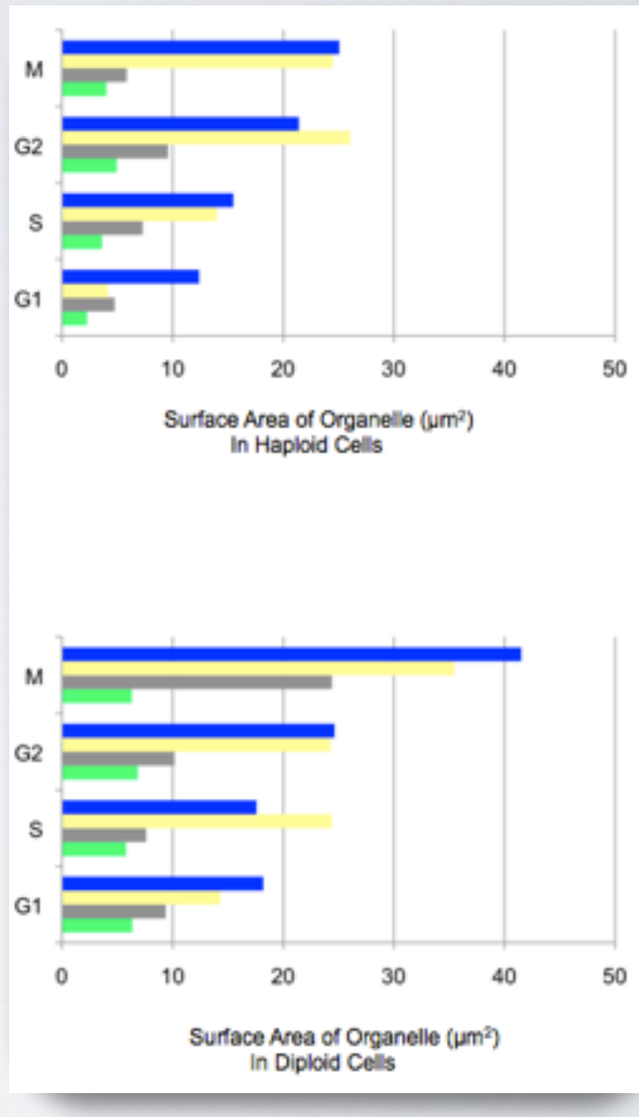
Cell volume



Organelle volume



Organelle surface area



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

Gene knockouts affecting mitochondria

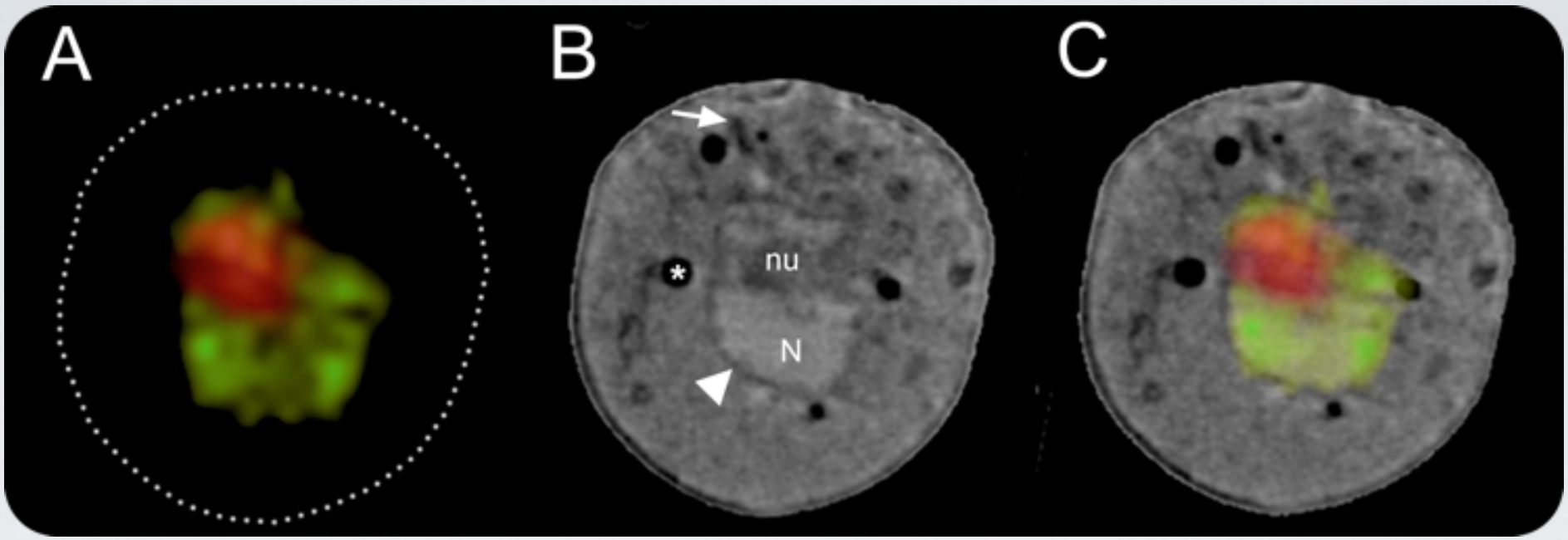
Wildtype

dnm1 KO

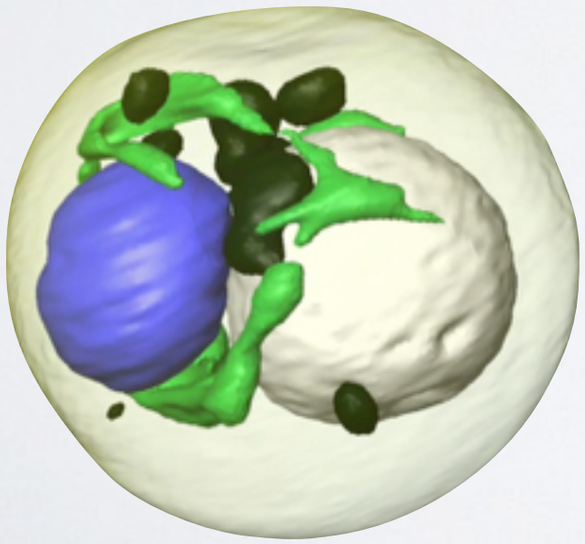
fzo1 KO

Jennifer Lippincott-Schwartz, NIH
Arnold Seo, NIH

Regulation of nucleus size and shape

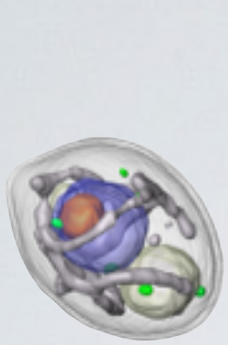


Cinquin BP, Do M, McDermott G, Walters AD, Myllys M, Smith EA, Cohen-Fix O, Le Gros MA and Larabell CA. (2014). J. Cellular Biochemistry. 115(2):209-216.



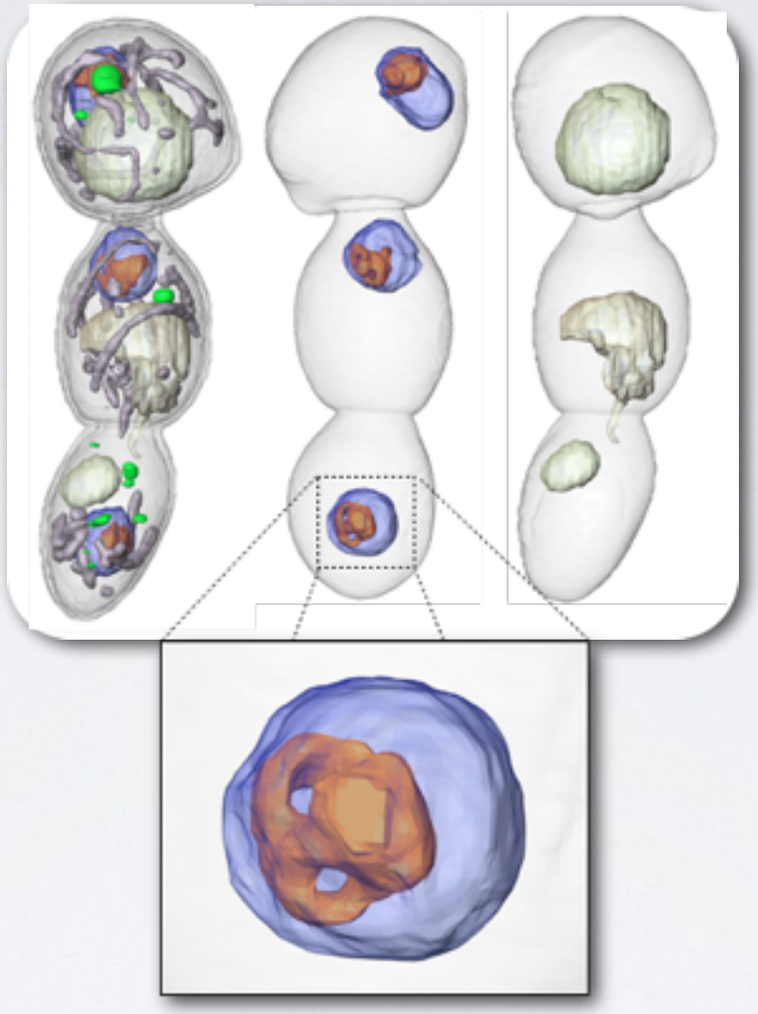
Walters, A.D., May, C.K., Dauster, E., Cinquin, B.P., Smith, E.A., Robellet, X., D'Amours, D., Larabell, C.A., Cohen-Fix, O. (2014). The yeast polo kinase, Cdc5, regulates the shape of the mitotic nucleus. *Current Biology.* In press.

Effect of peptoids on pathogenic yeast

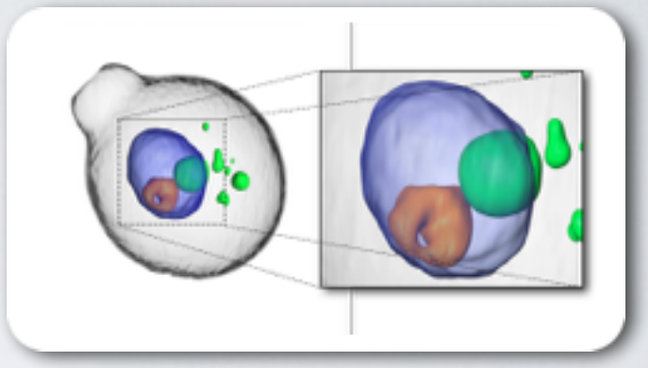


Pathogenic

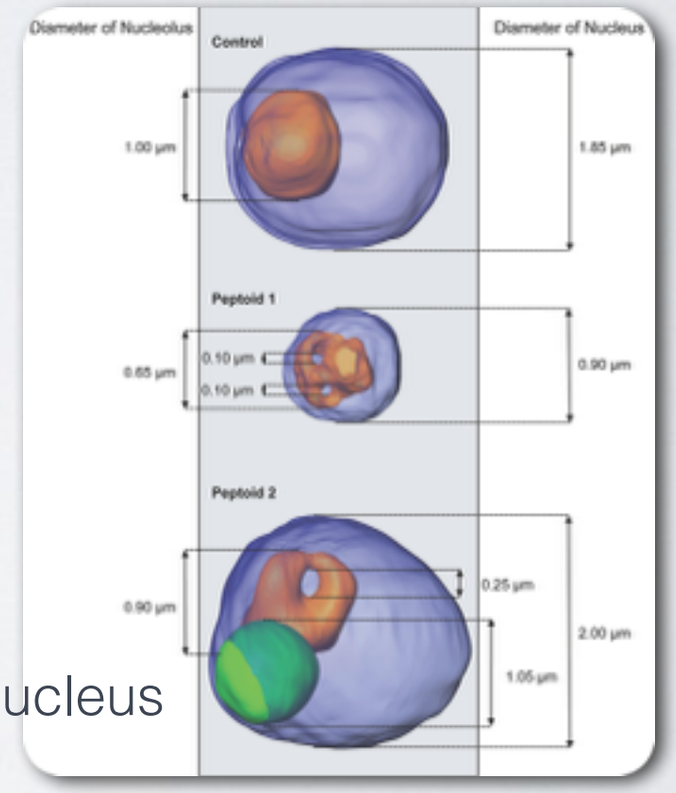
Peptoid 1 treated



Peptoid 2 treated



Effects on nucleus

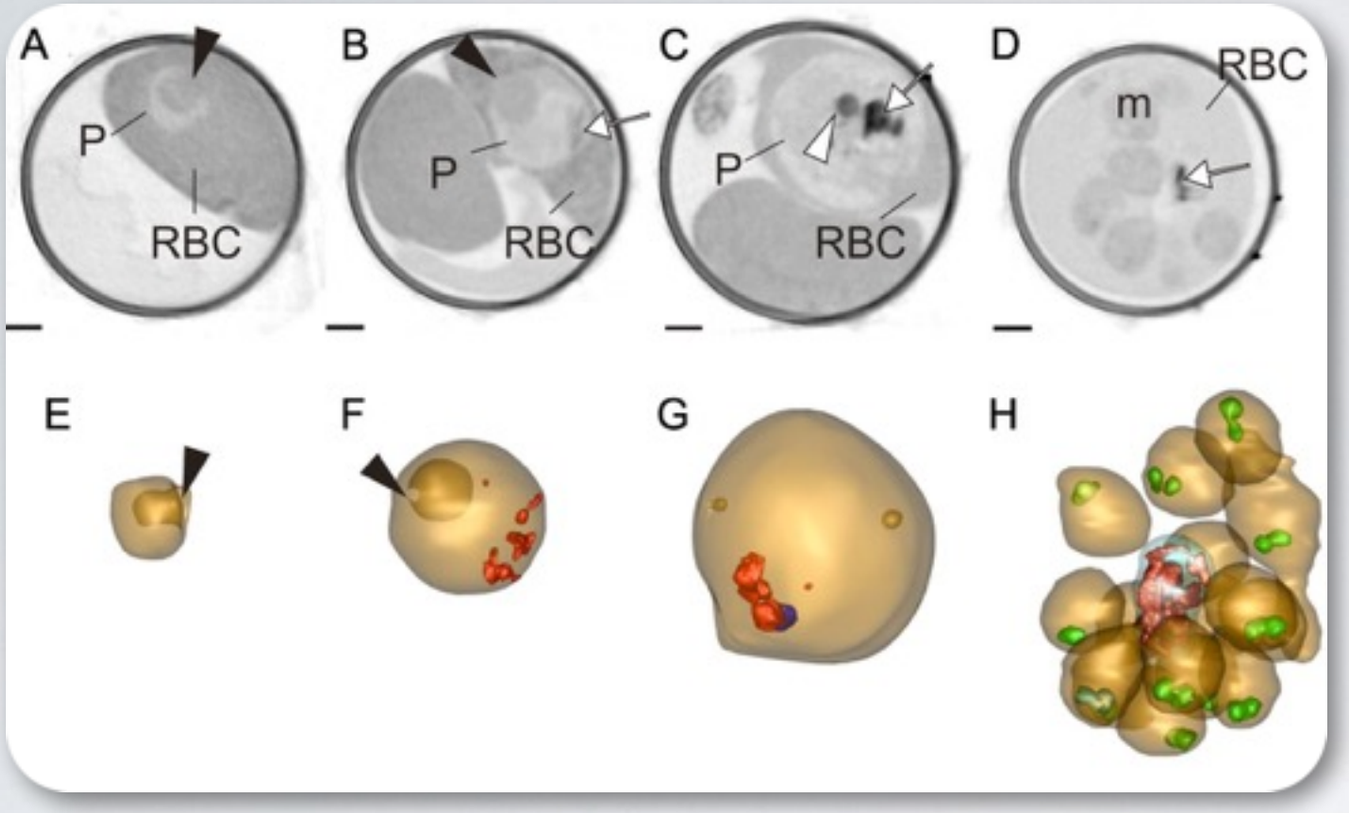


Lipid in nucleus

M Uchida, G McDermott, M Wetzler, MA Le Gros, M Myllys, C Knoechel, A Barron, CA Larabell. (2009). PNAS. 206(46):19375-19380

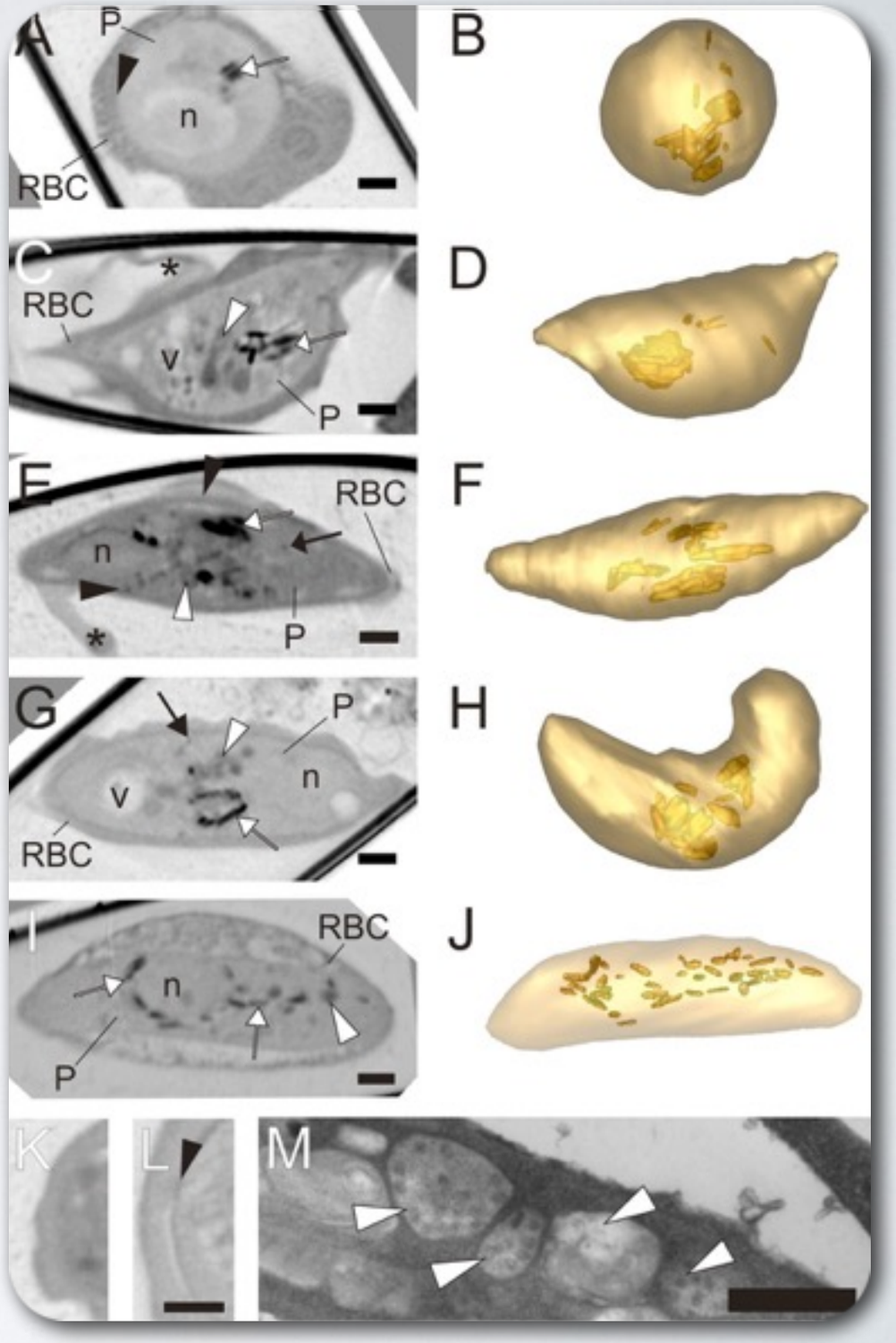
Host - parasite interactions

Sexual stage



- Total volume of infected RBC remains constant
- Parasite occupies ~ 50% of RBC volume.
- 70% of RBC hemoglobin digested during parasite development

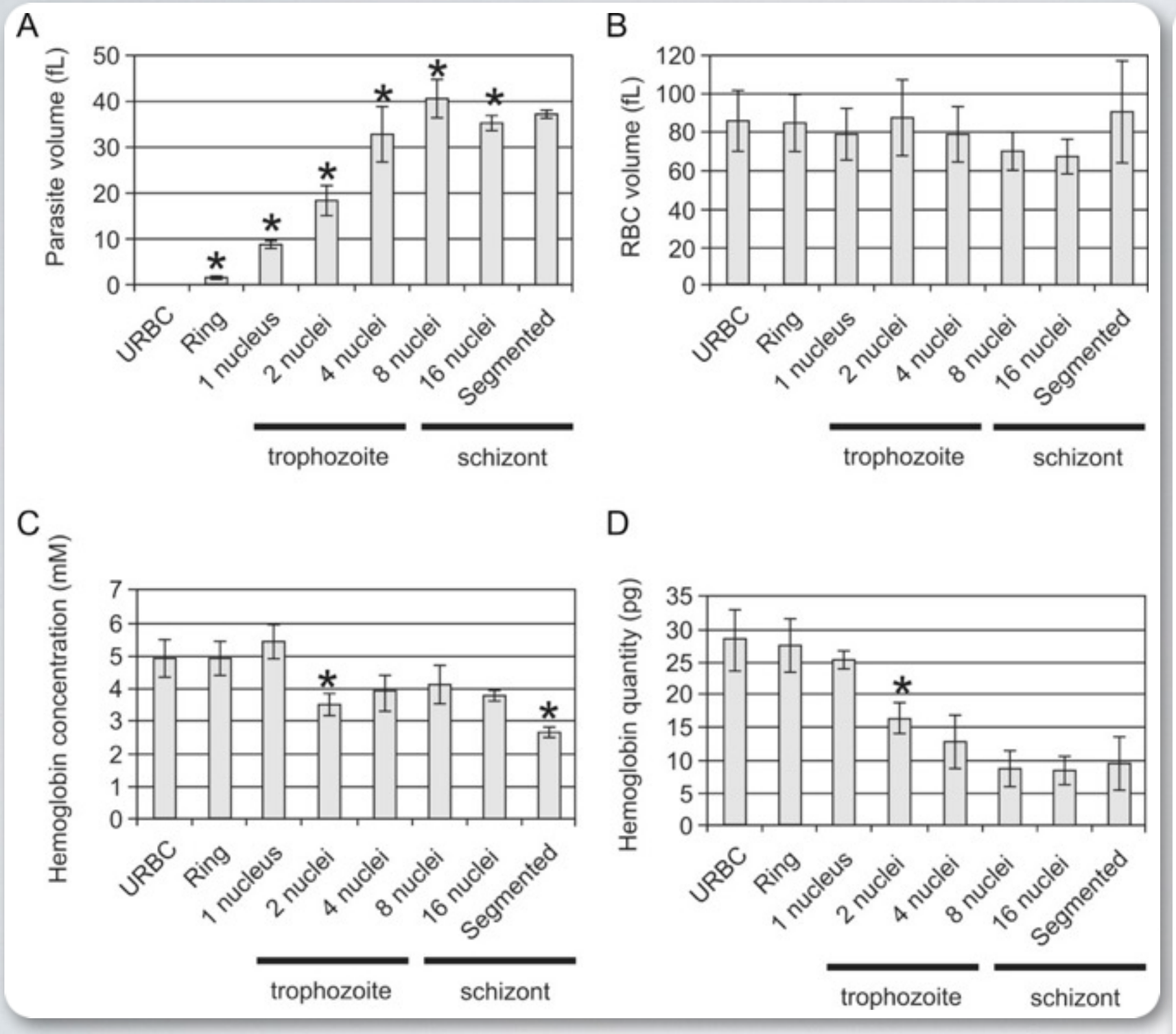
Asexual stage



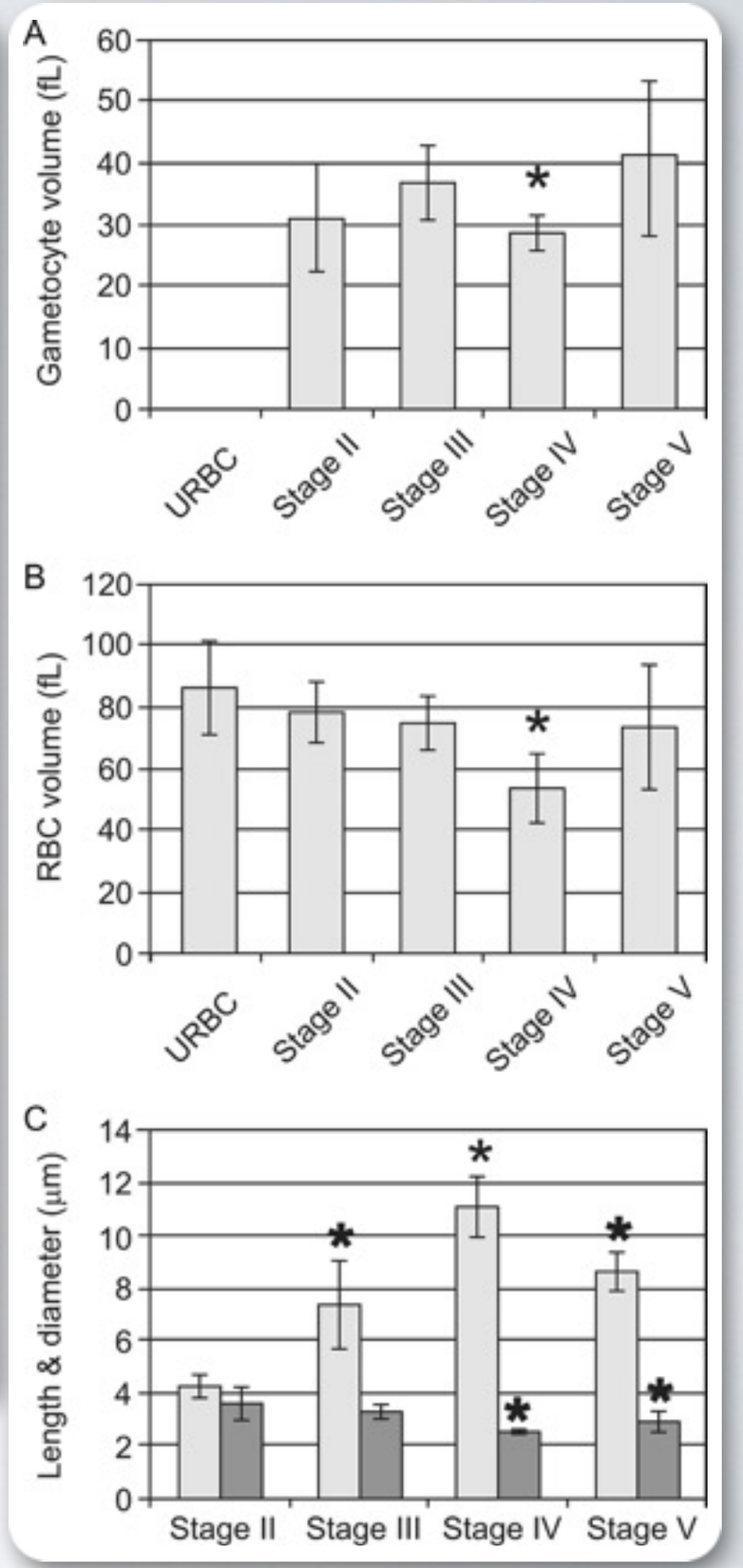
E Hanssen et al. J. Structural Biology (2012) 177:224-234

Malaria, *p. falciparum*

Sexual stage



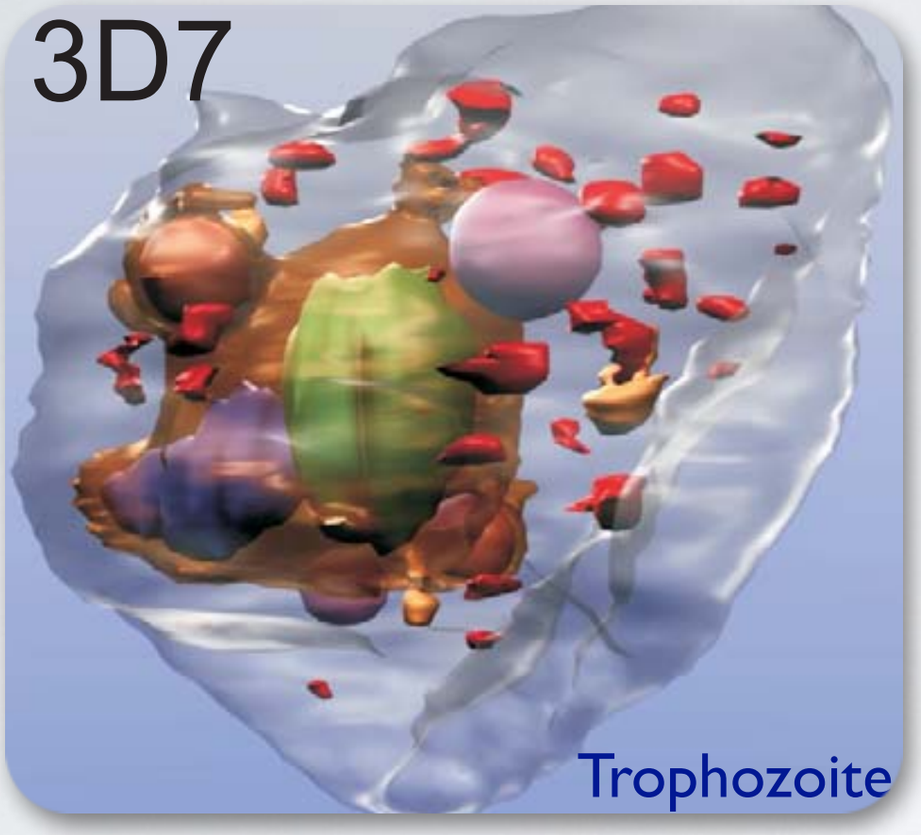
Asexual stage

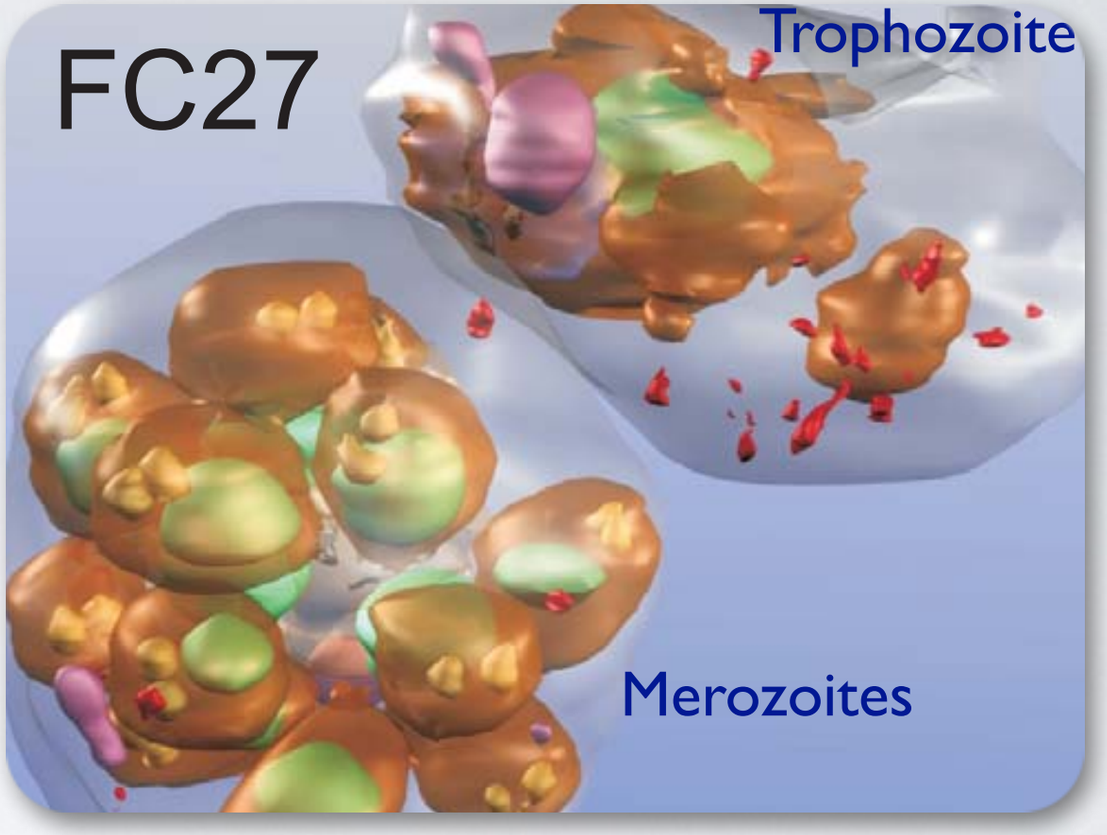


E Hanssen et al. J. Structural Biology (2012) 177:224-232.

Malaria, *p. falciparum*

How do parasite adhesion proteins get to blood cell surface?





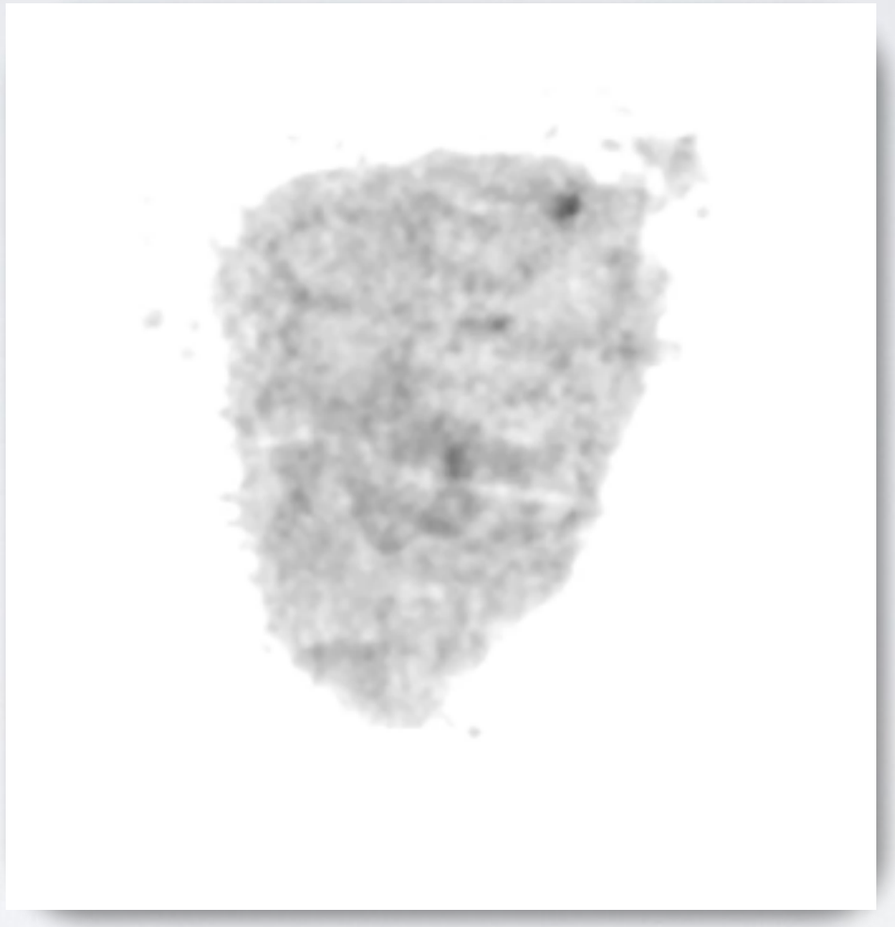
- Parasite
- Nucleus
- Hemoglobin
- Maurer's clefts
- Rhoptry
- Digestive vacuole

E Hanssen, C Knoechel, N Abu Bakar, N Klonas, S Deed, MA Le Gros, CA Larabell & L Tilley, J. Structural Biology (2011) 173:161-168.

Lymphocytes

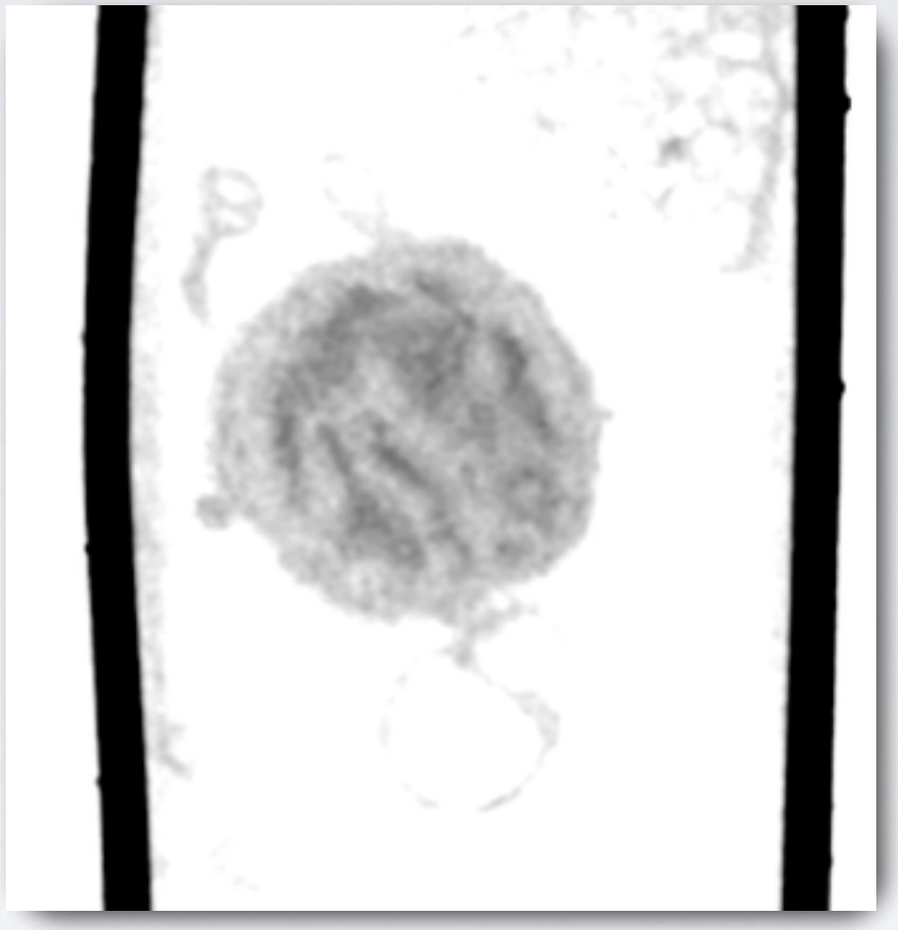
SXT of human lymphoblastoid cell (Encode GM12878)

Flipping through orthoslices



SXT of human lymphoblastoid cell (Encode GM12878)

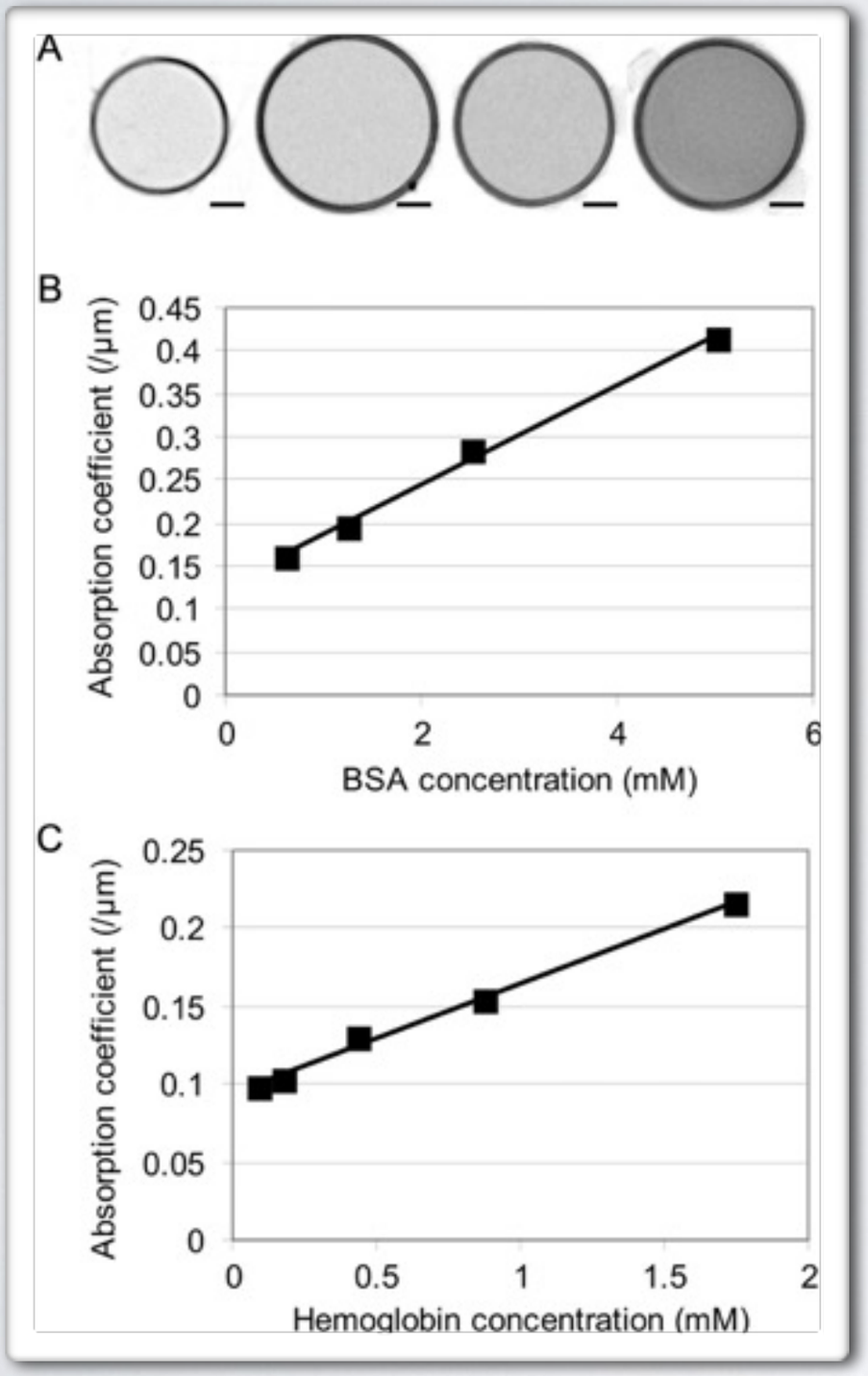
Flipping through orthoslices

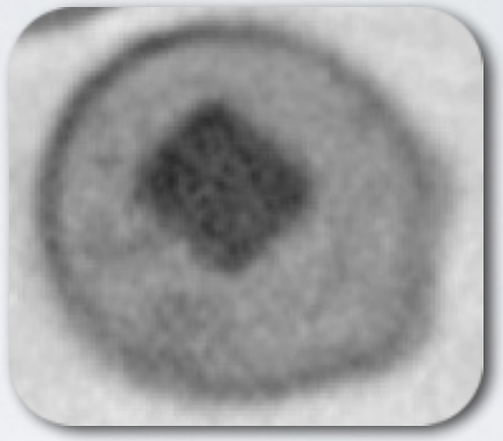


SXT of human lymphoblastoid cell (Encode GM12878)

Semi-automatic segmentation

Linear absorption coefficient





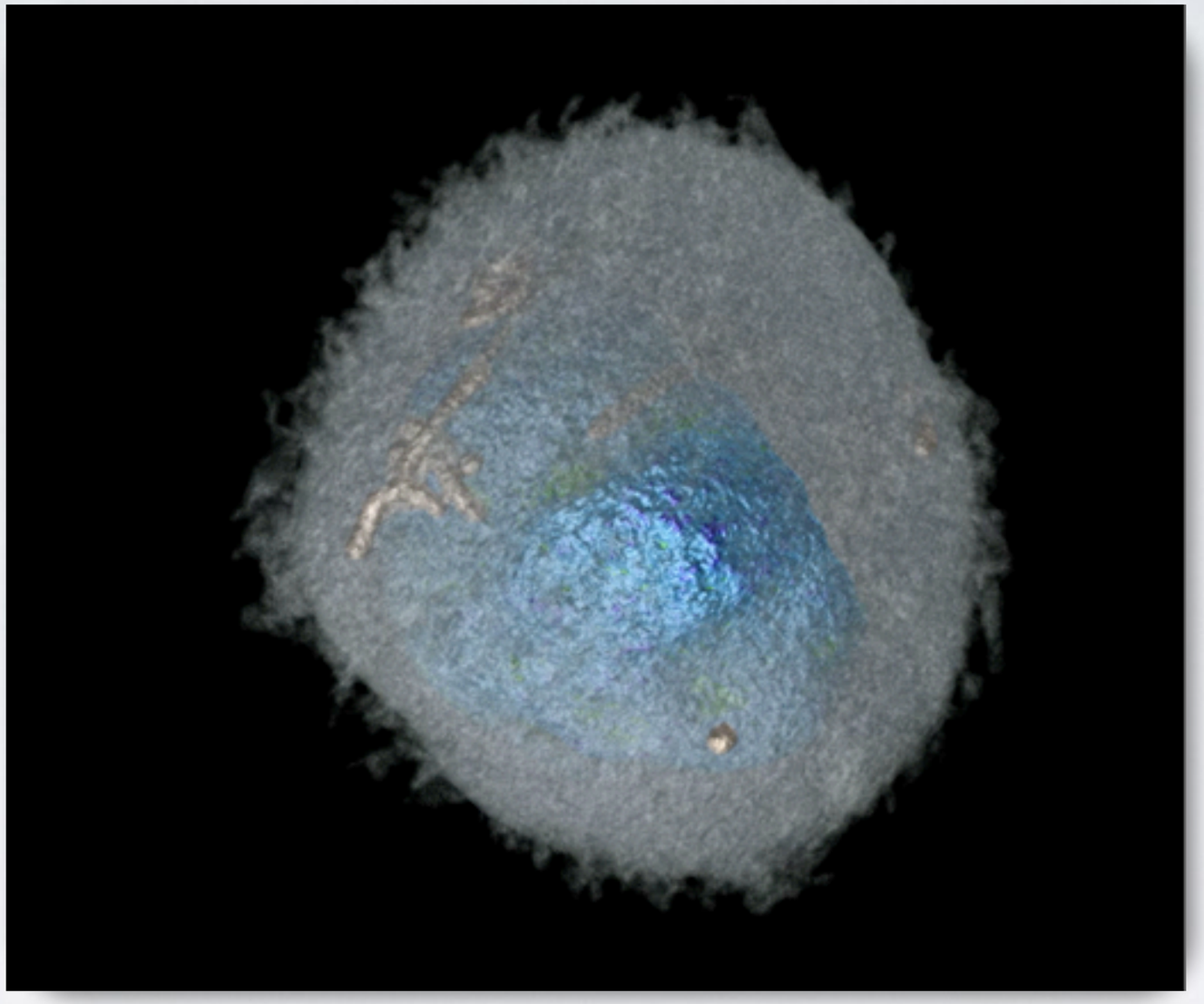
Alcohol oxidase crystal in yeast cell

Calculated LAC ➟ 0.625 μm^{-1}

Measured LAC ➟ 0.626 μm^{-1}

SXT of human lymphoblastoid cell (Encode GM12878)

Volume rendered, segmented



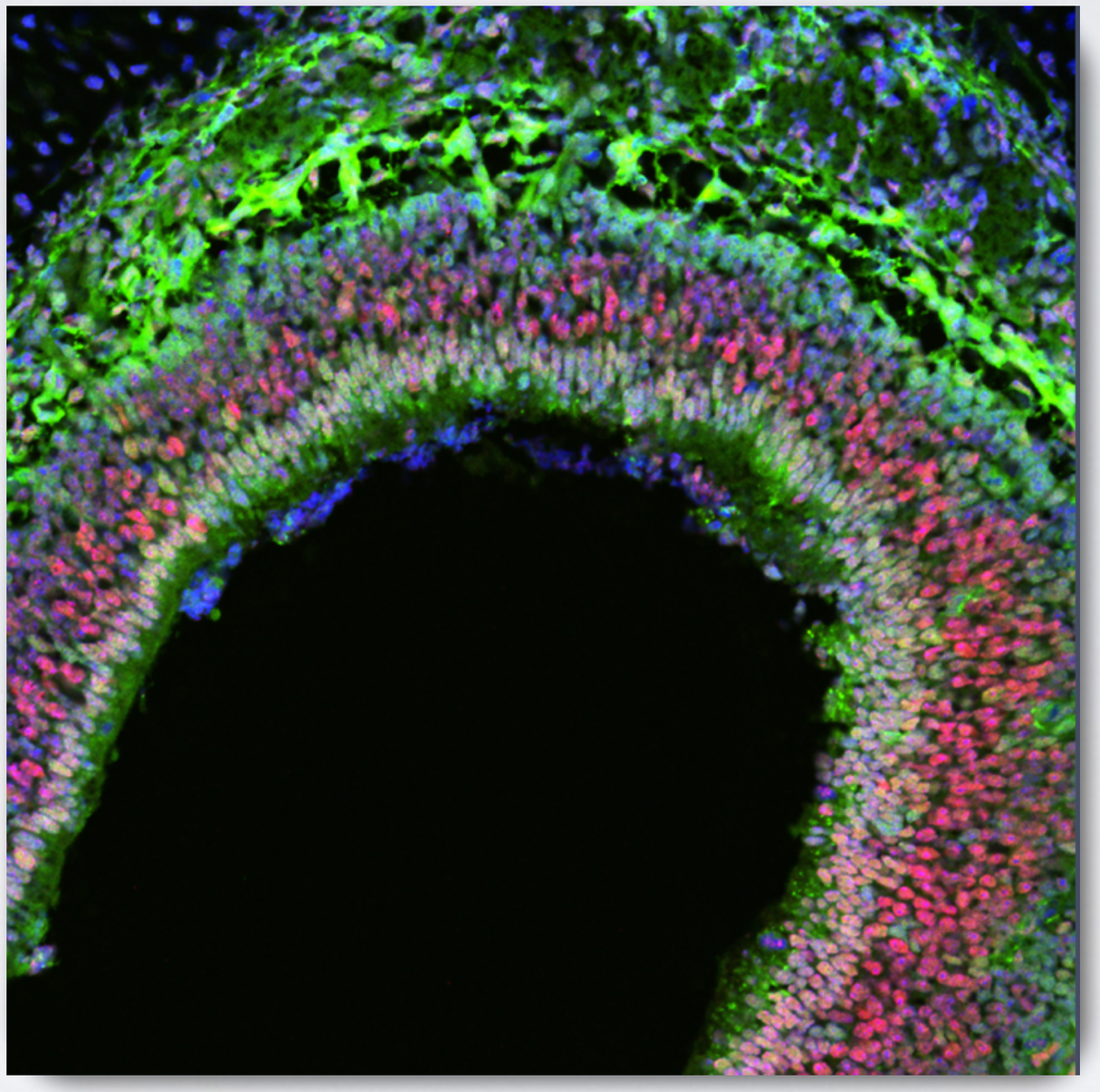
Cytoplasm - grey
Heterochromatin - blue
Euchromatin - green
Pericentromeric heterochromatin - gold
Mitochondria - bronze
Endoplasmic reticulum - red

Nuclear organization

Olfactory sensory neuron (OSN) differentiation

Mouse olfactory epithelium

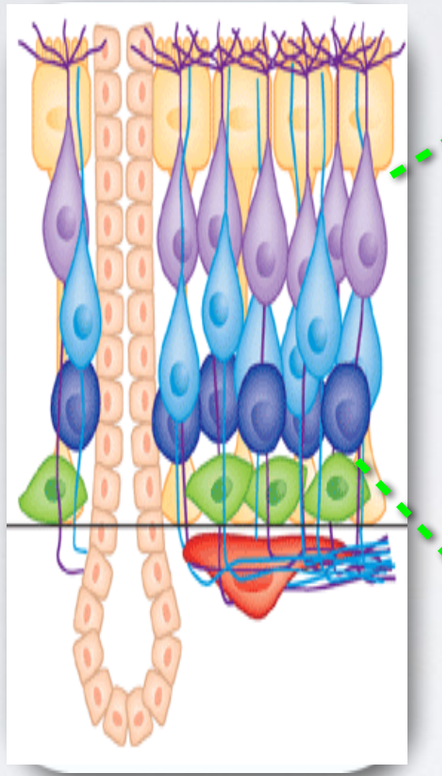
~ 1400 genes for olfaction in mice; each cell expresses just one

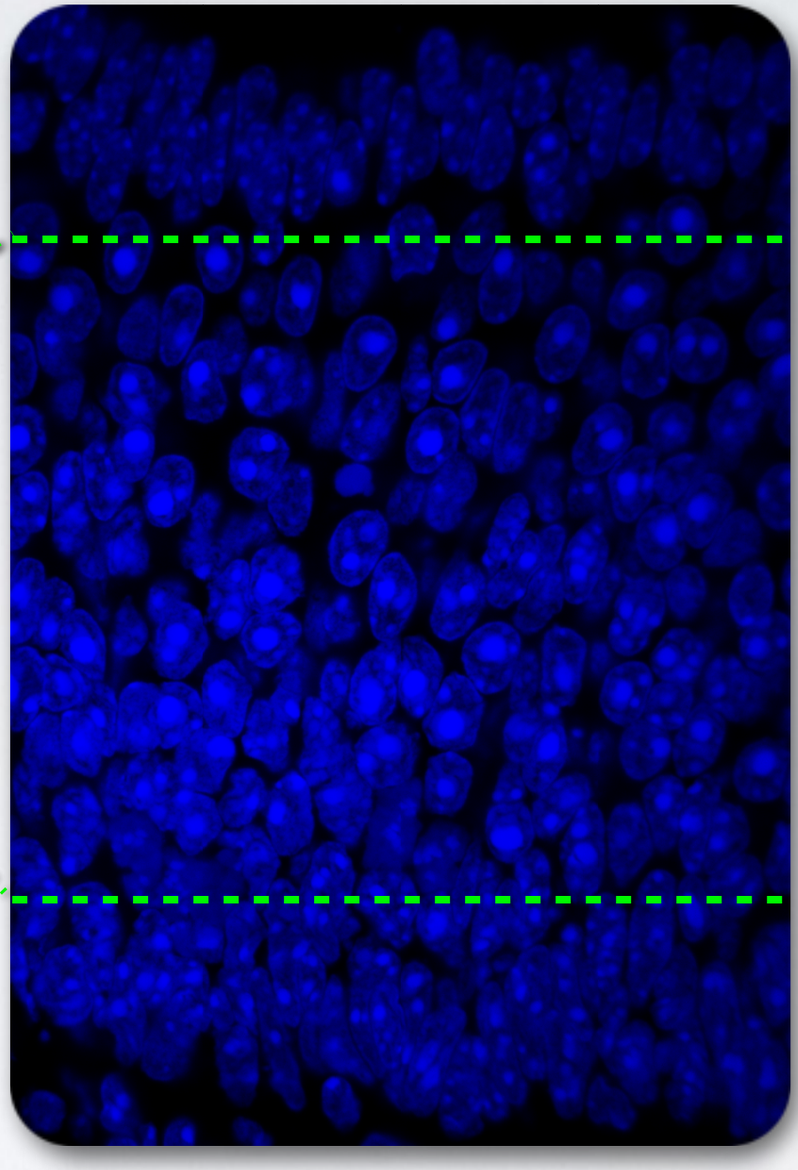


Stavros Lomvardas, UCSF

OR foci are silenced

Heterochromatin in nucleus center





Epithelial cells

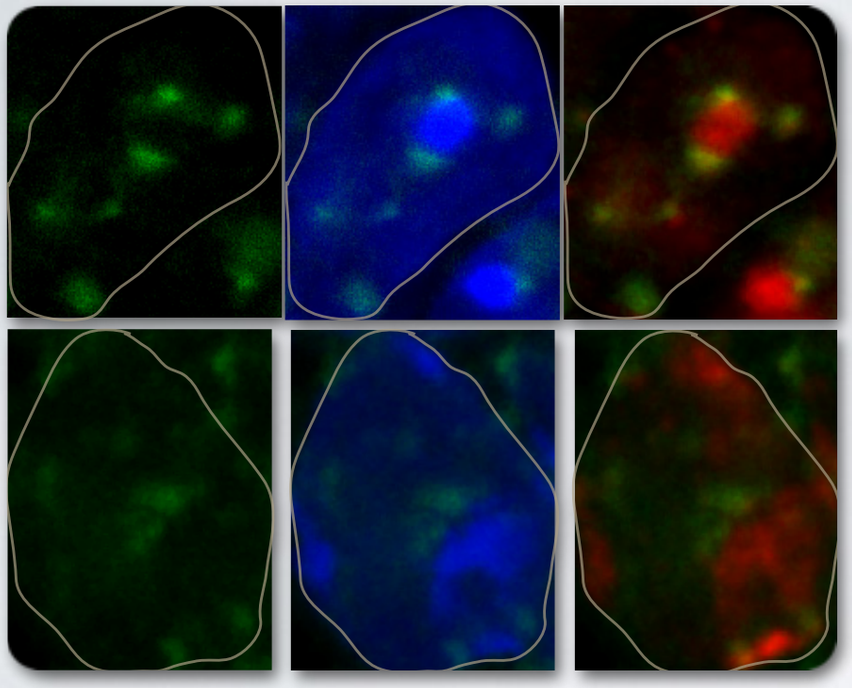
Olfactory sensory neurons

Stem cells

EJ Clowney, MA Le Gros, CP Mosley, FG Clowney, EC Markenskoff-Papadimitriou, M Myllys, G Barnea, CA Larabell and S Lomvardas. (2012) Cell 151, 724-737.

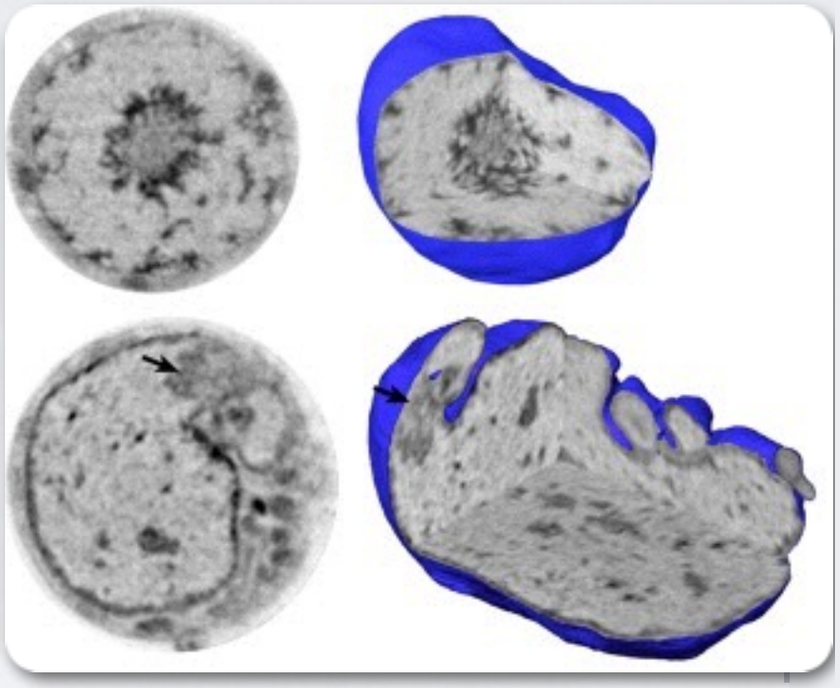
Structural organization critical for OR silencing

FISH

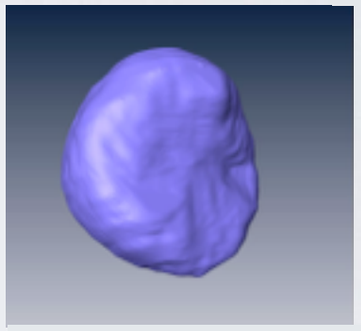


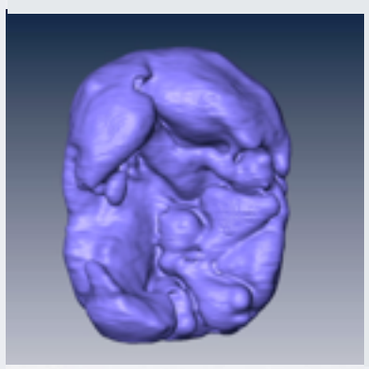
Pan OR DAPI HP1β

SXT orthoslices

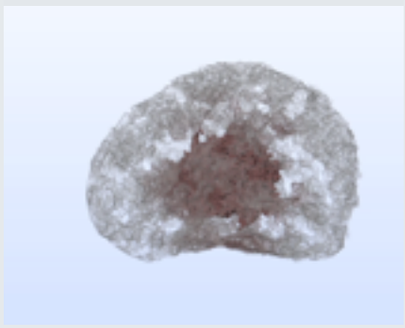


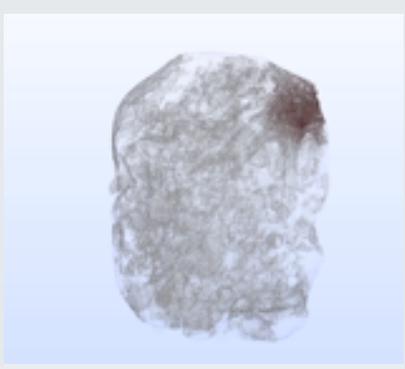
SXT surface rendering





SXT volume rendering



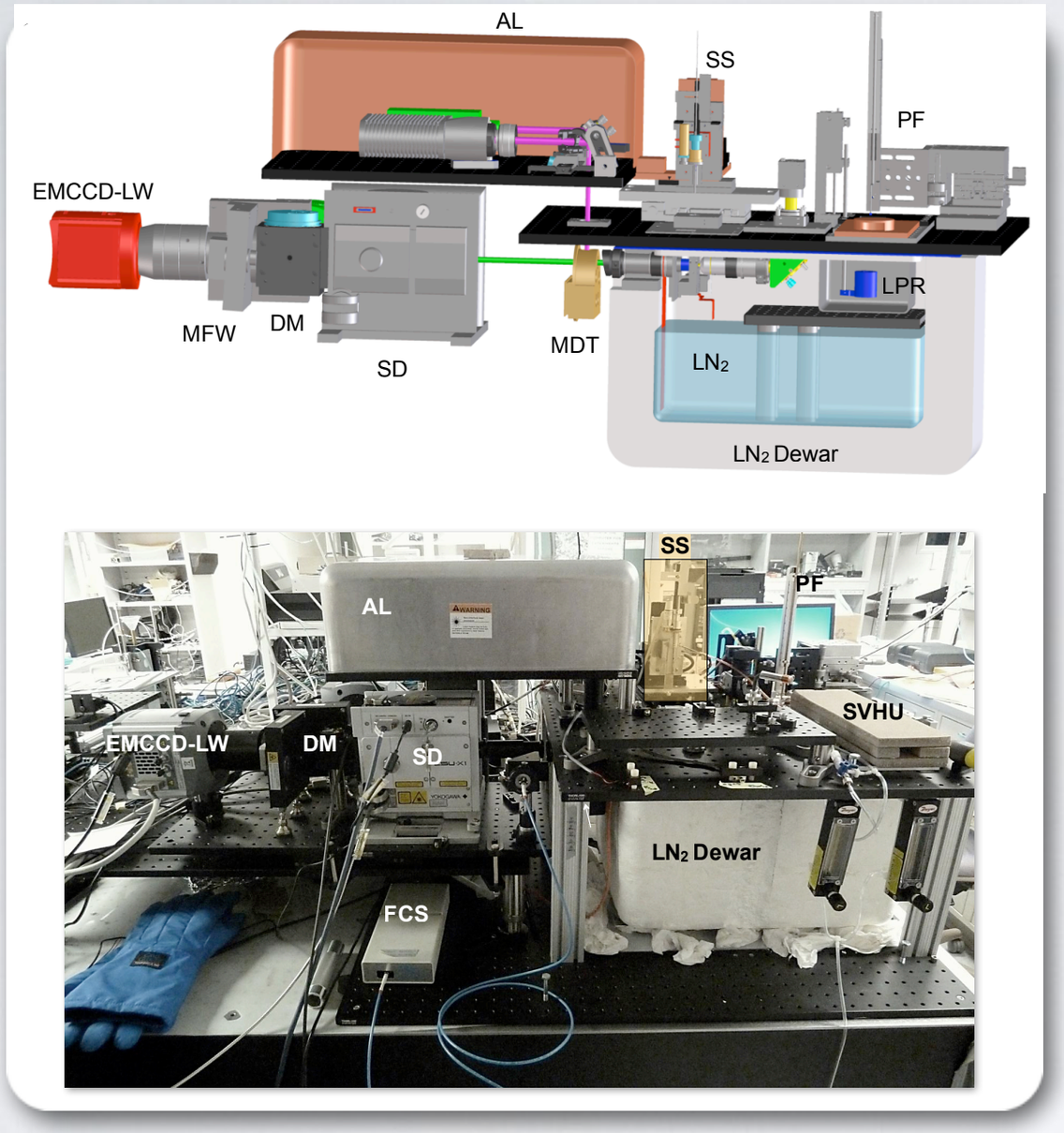


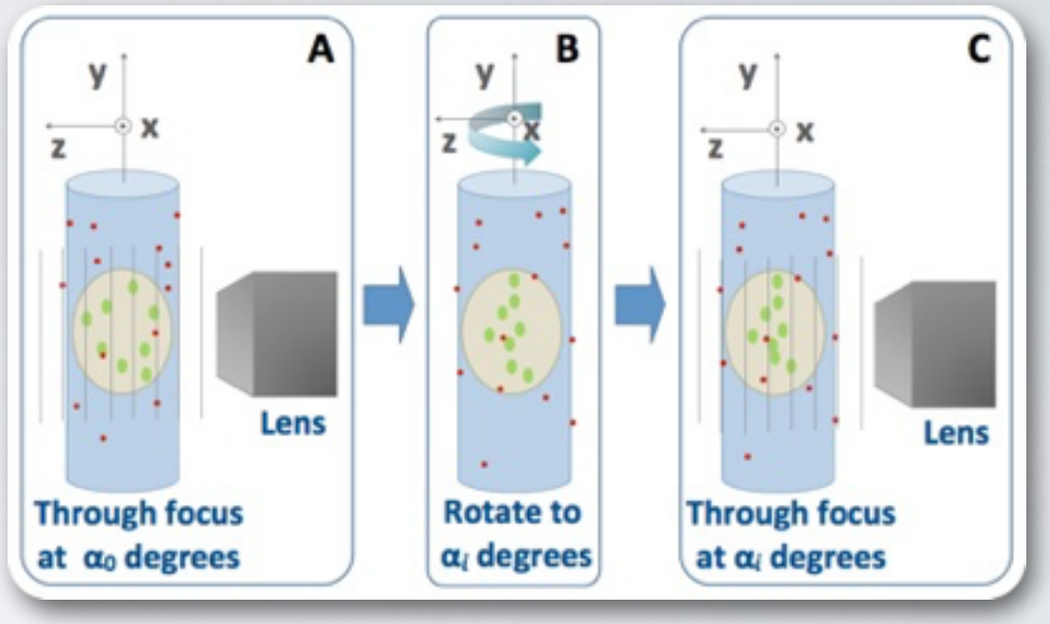
EJ Clowney, MA Le Gros, CP Mosley, FG Clowney, EC Markenskoff-Papadimitriou, M Myllys, G Barnea, CA Larabell and S Lomvardas. (2012) Cell 151, 724-737.

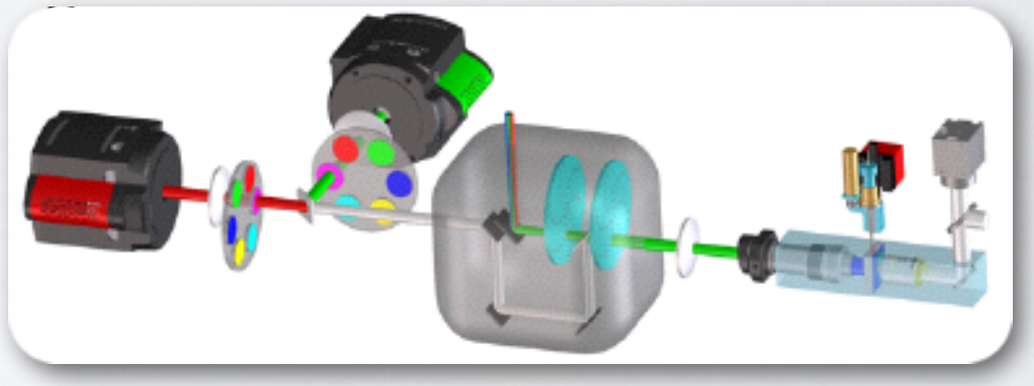
Unpublished data will be presented

Putting molecules in context

Cryo confocal tomography

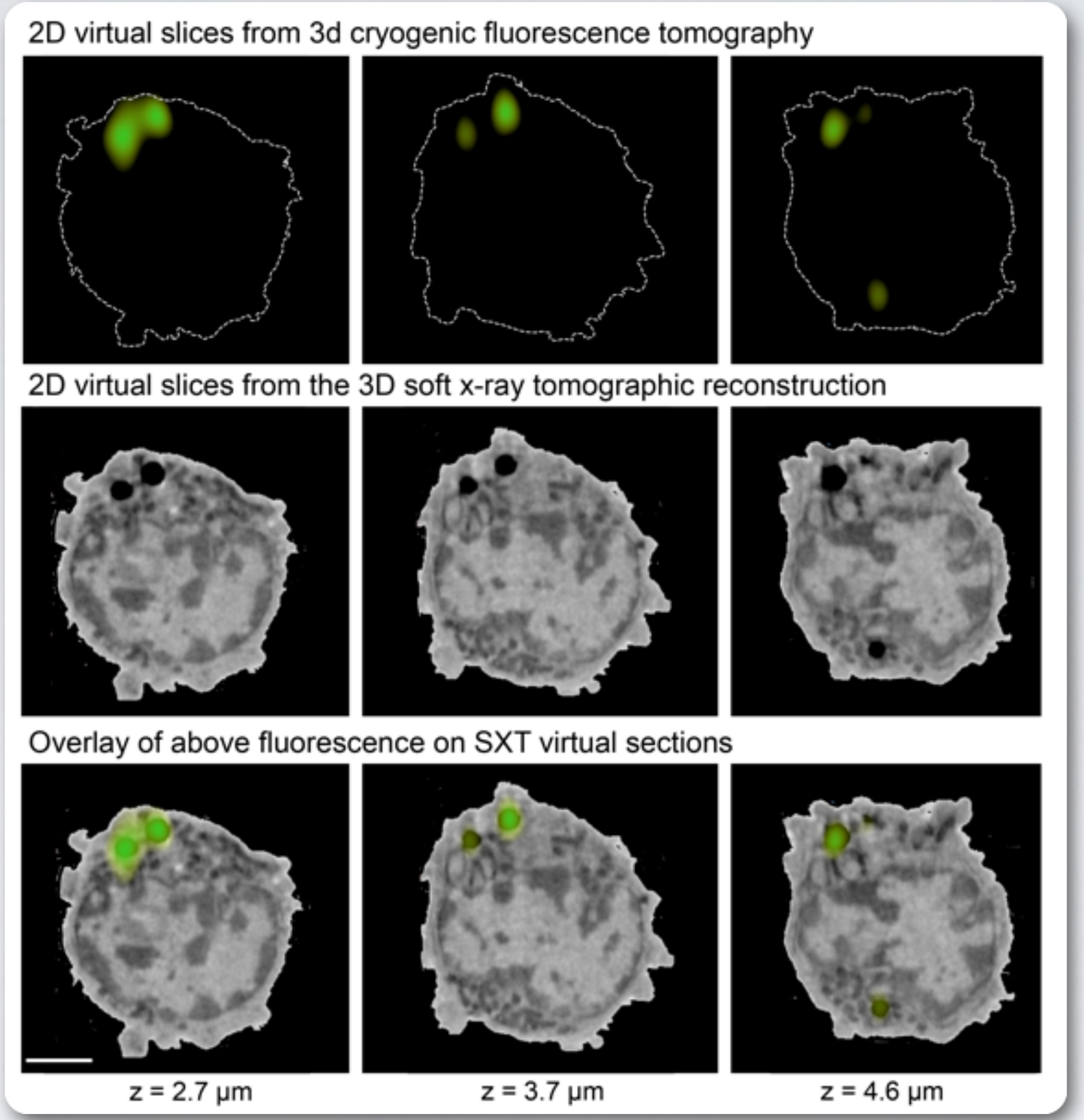






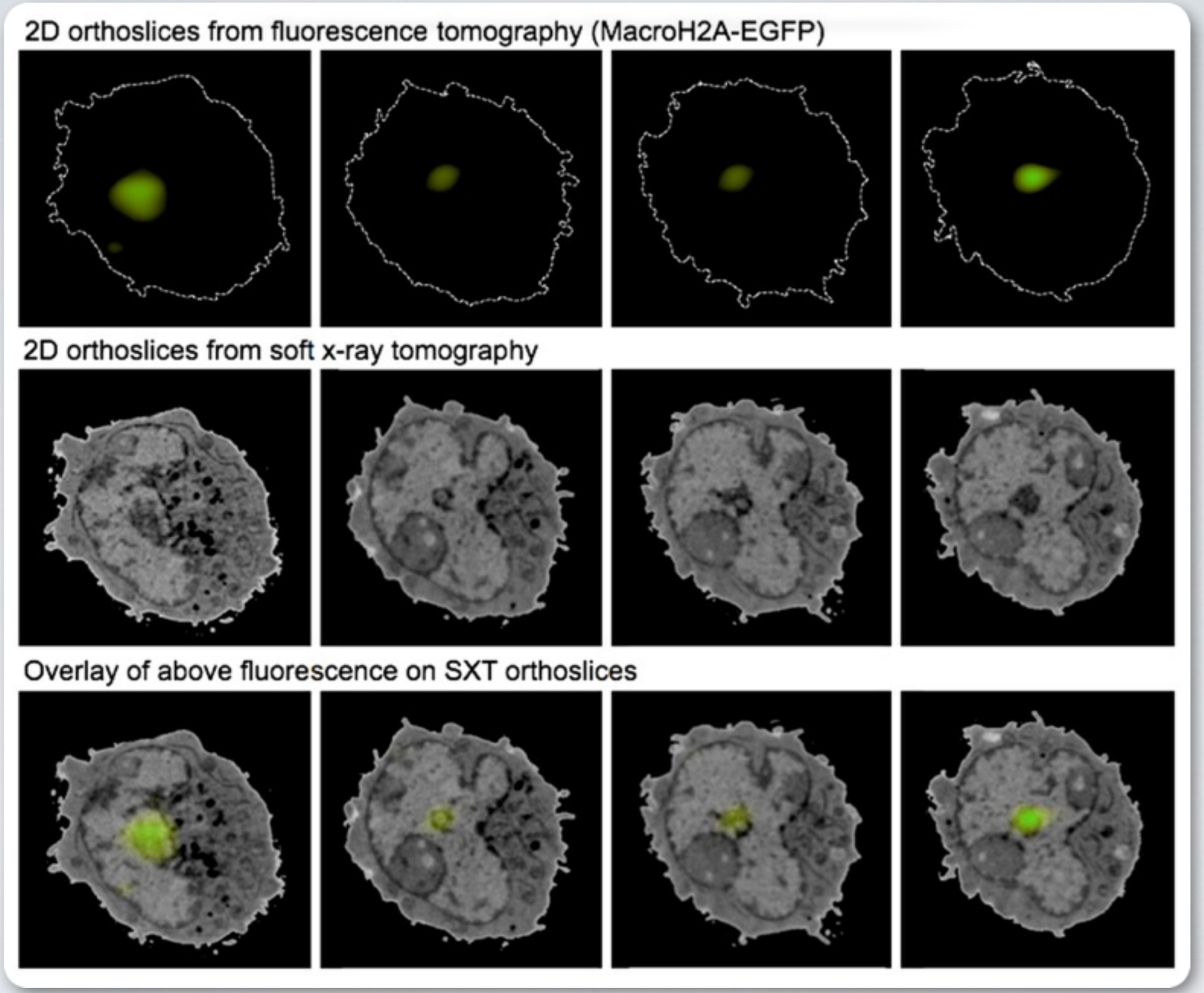
MA Le Gros et al. (2009). J. Microscopy 235(1):1-8

BODIPY labeled lipids in lymphoma cell



Smith EA, McDermott G, Do M, Leung K, Panning B, Le Gros MA and Larabell CA (2014). Quantitatively imaging chromosomes using correlated cryo-fluorescence and soft x-ray tomographies. *Biophysical Journal.* 107(8) 1988-96.

Inactive X chromosome



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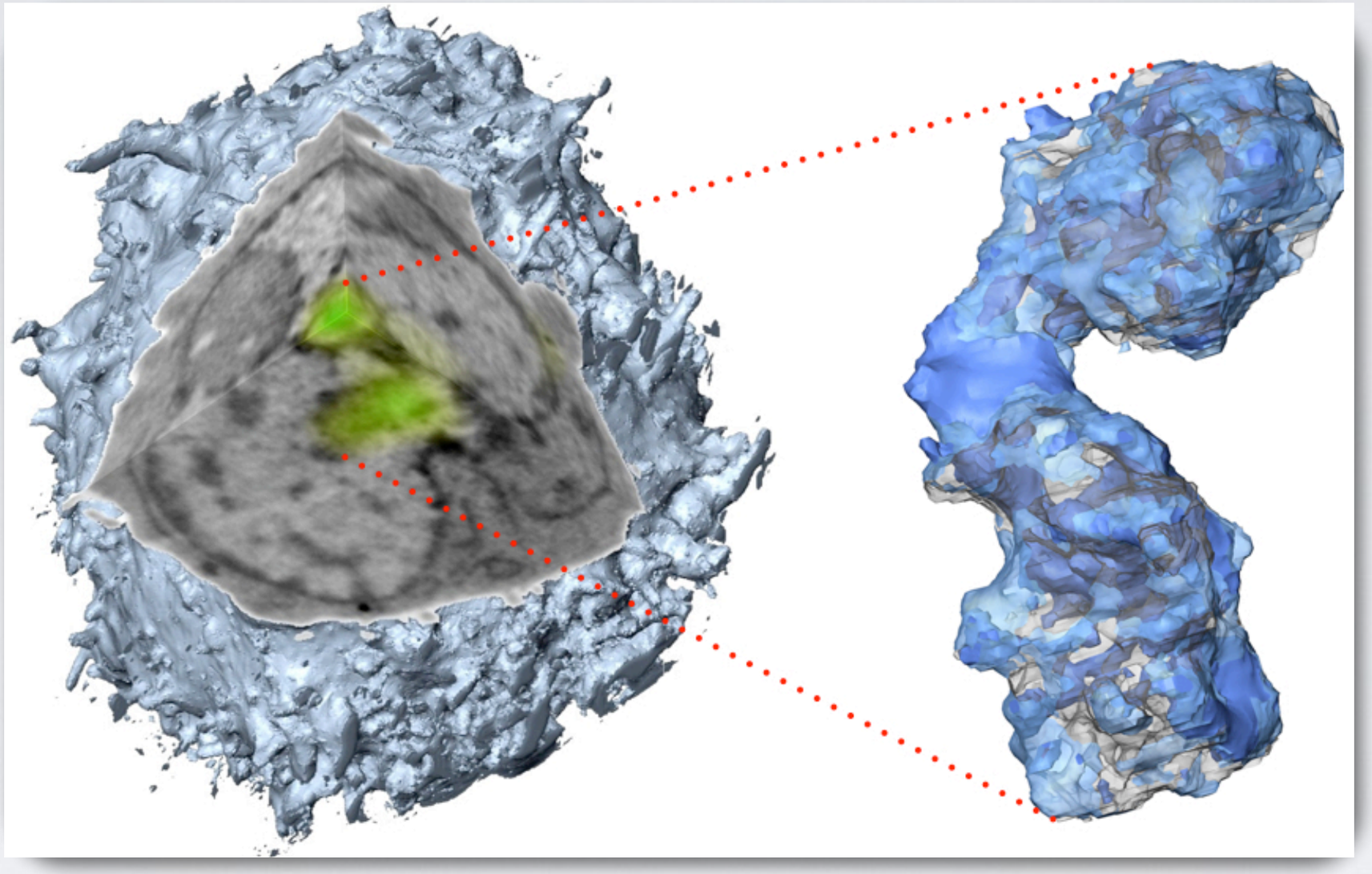

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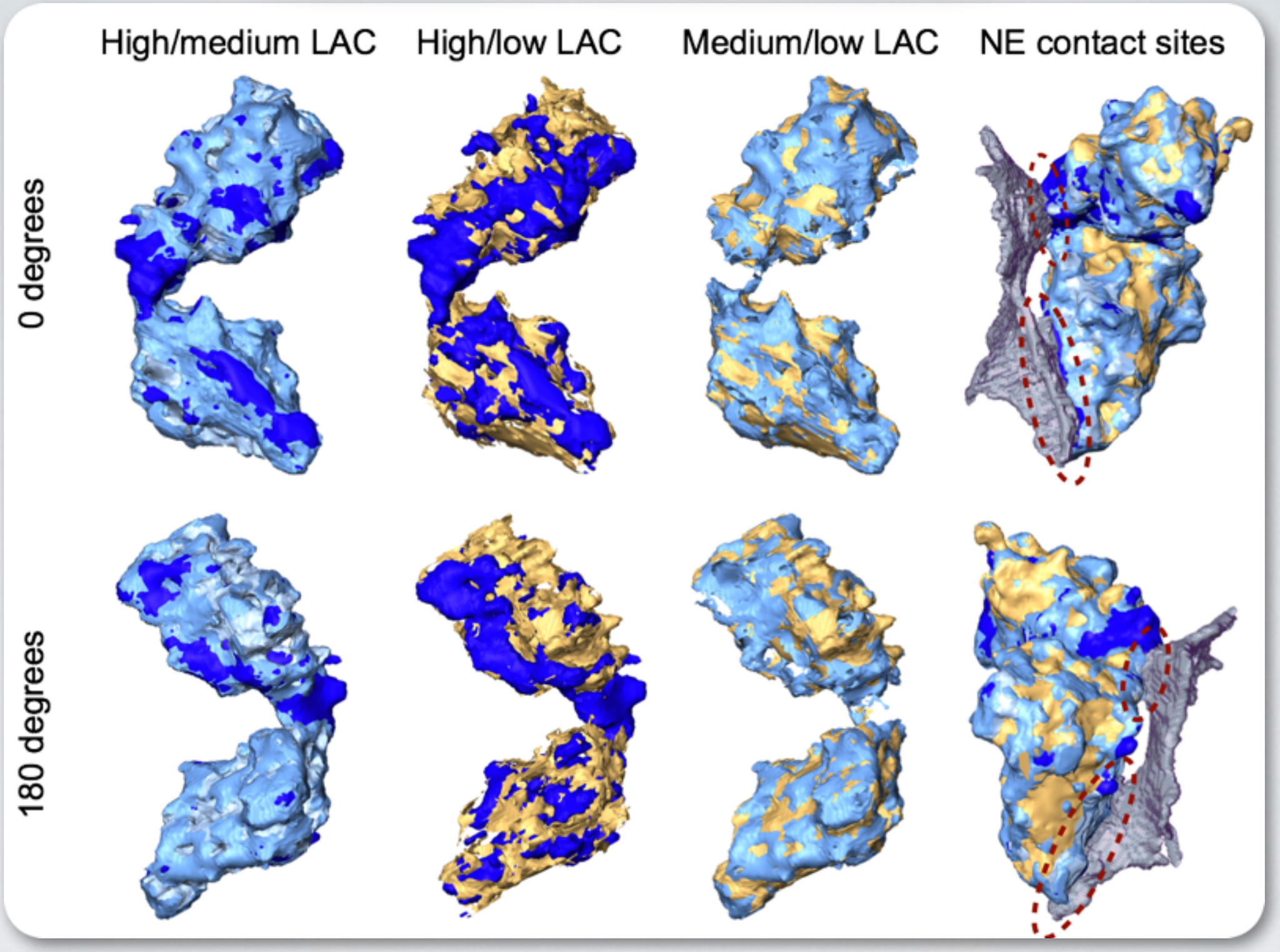

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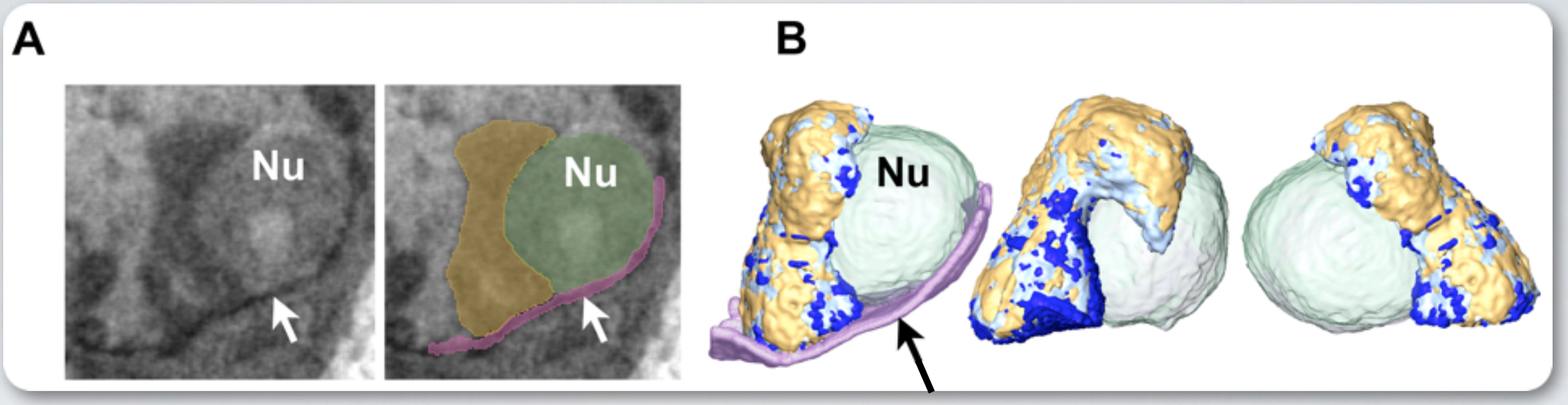

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