CT Scans of Single Cells

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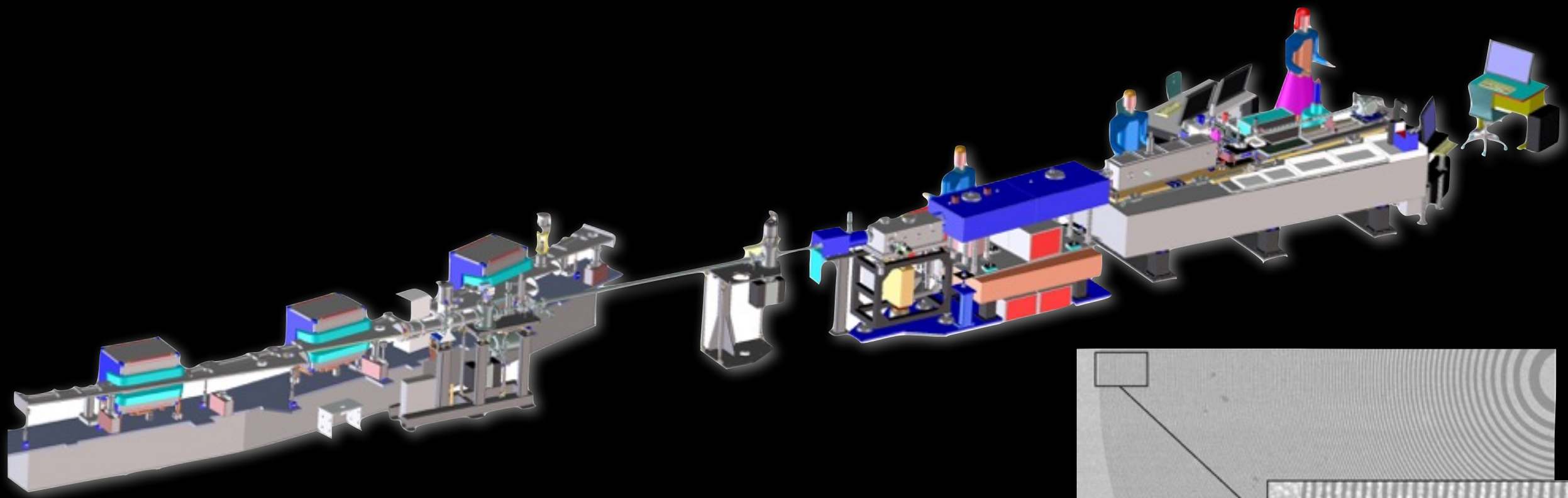
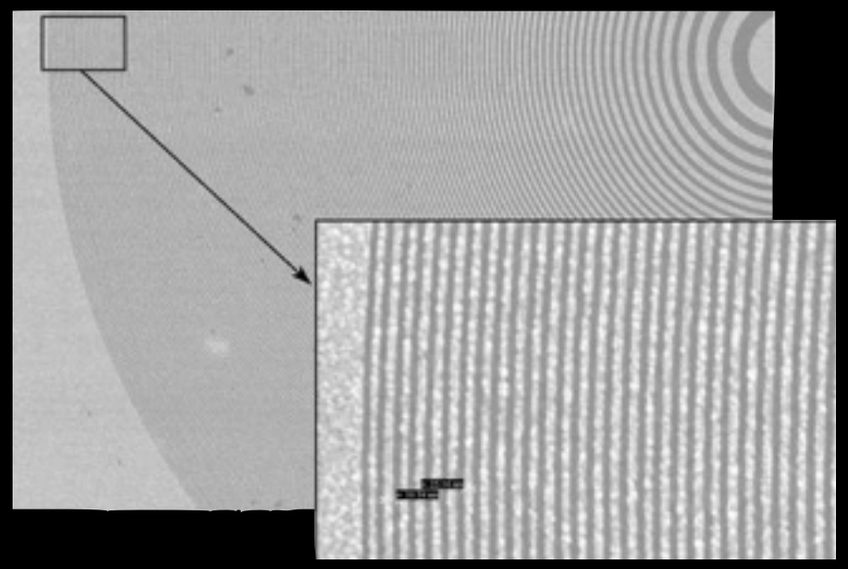
Soft X-ray Tomography

- Whole, hydrated cells
- Cell structures in near-native state (cryo-immobilized)
- Natural contrast - absorption imaging
- Label-free imaging
- Better than 50nm **isotropic** resolution
- See molecules using correlated fluorescence and x-ray

Soft X-ray Tomography





- **Light source:** synchrotron radiation ⇢ 517 eV, 2.4 nm λ
- **Optics:** zone plates (nano-fabricated Nickel Fresnel lenses)
- **Specimen preparation:** cryo-immobilization
- **Contrast mechanism:** x-ray absorption by cellular components

Specimen preparation

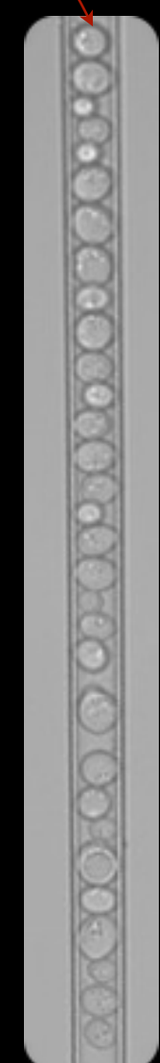
Specimen holder

1 mm

300 µm

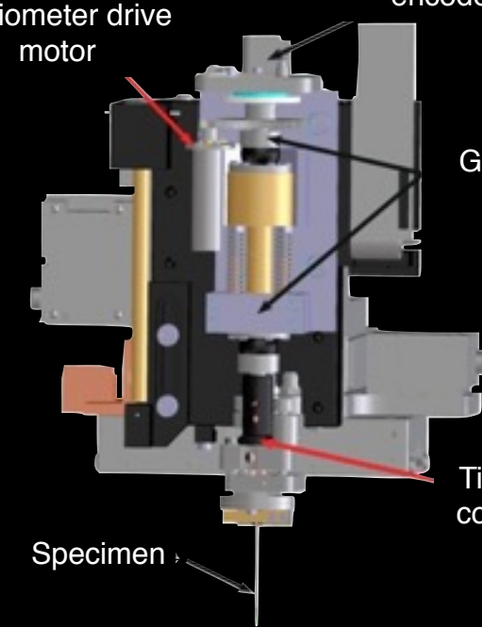
2-20 µm

Specimen



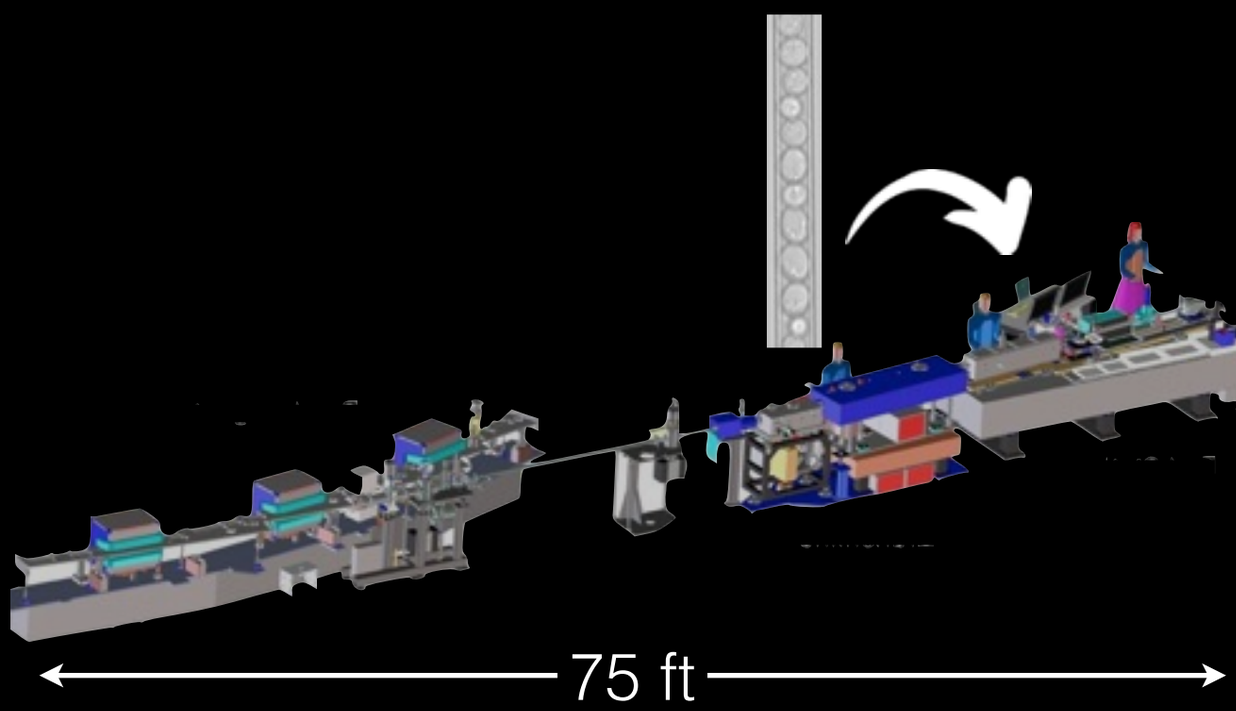
Rapidly freeze

Fast - 5 min / cell

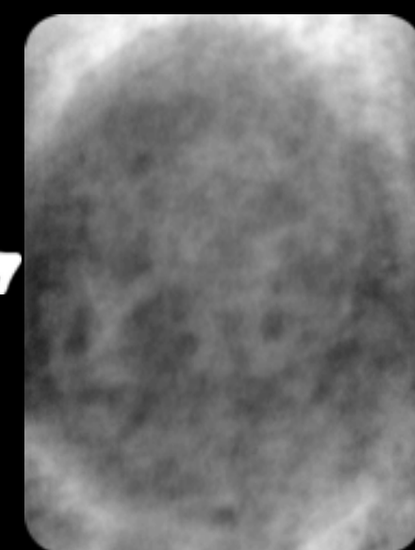


Le Gros MA, McDermott G, Cinquin BP, Smith EA, Do M, Chao WL, Naulleau PP, and Larabell CA (2014). J Synchrotron Radiation. 21, 1370-1377.

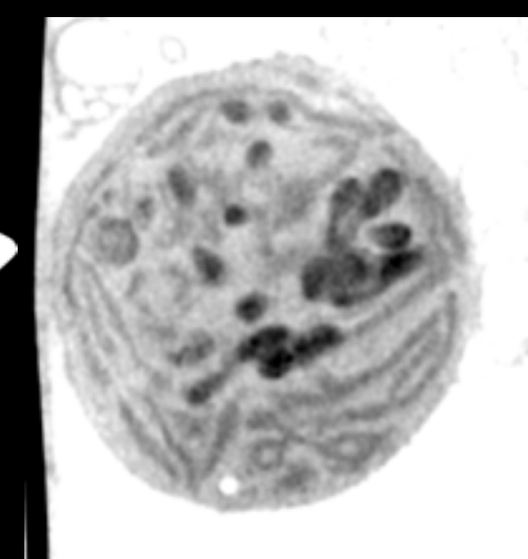
Soft X-ray Tomography



Projection image

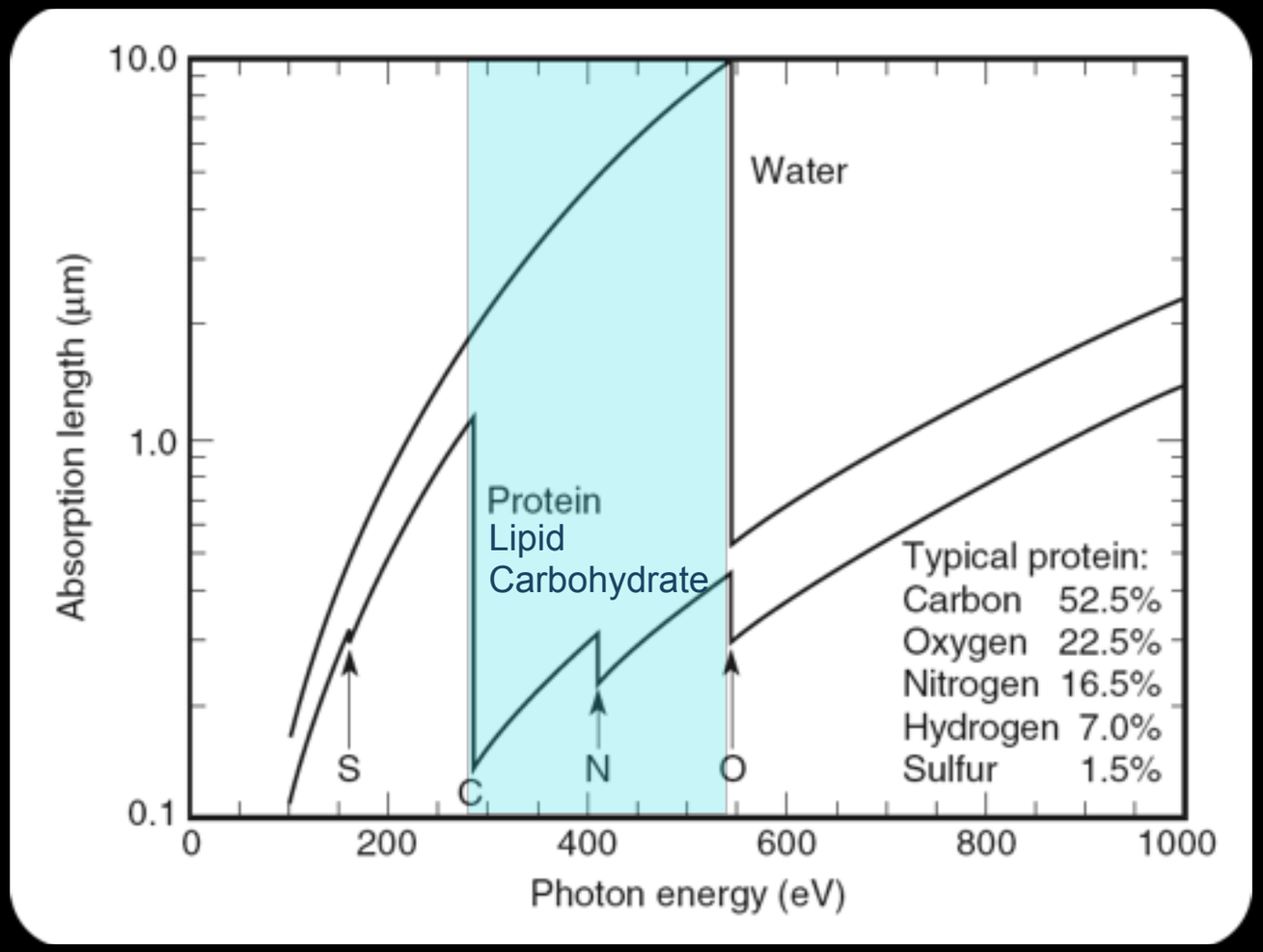


Virtual section



The contrast mechanism - ‘water window’ imaging

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

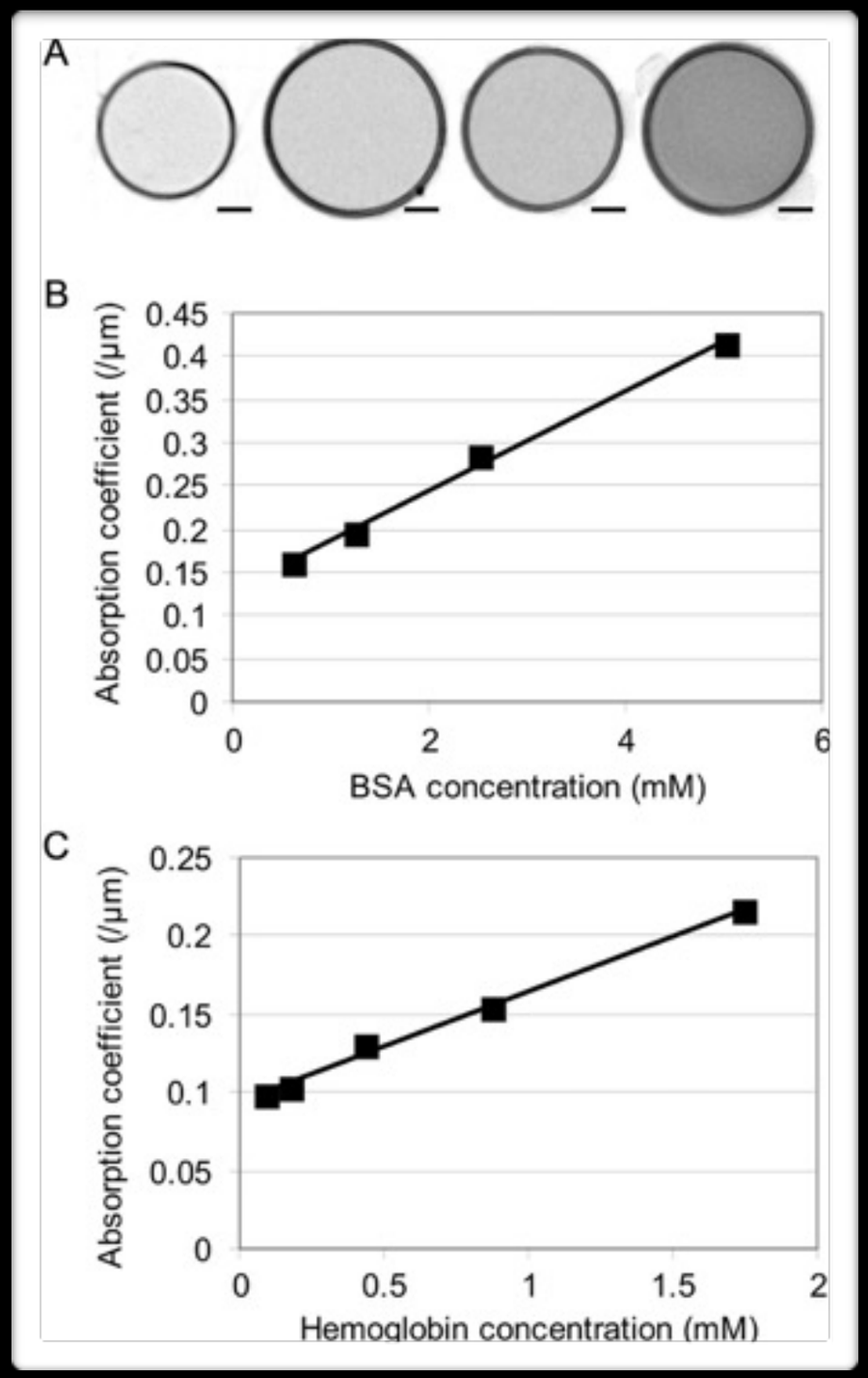


2.4 nm

517 eV

The contrast mechanism: Linear absorption coefficient

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



Alcohol oxidase
crystal in yeast cell

Calculated LAC ➟ 0.625 μm^{-1}

Measured LAC ➟ 0.626 μm^{-1}

LAC Values (μm^{-1})

0.75

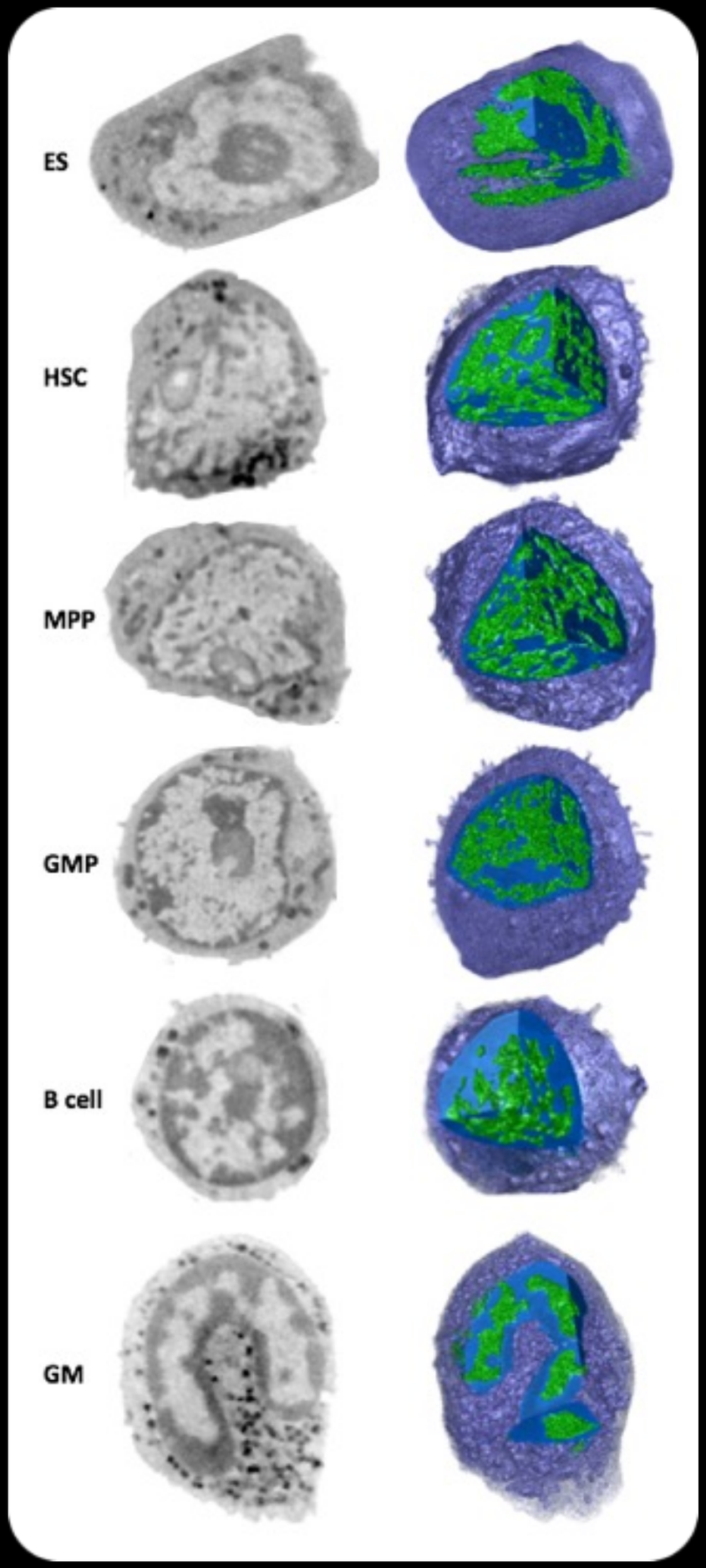
0.50

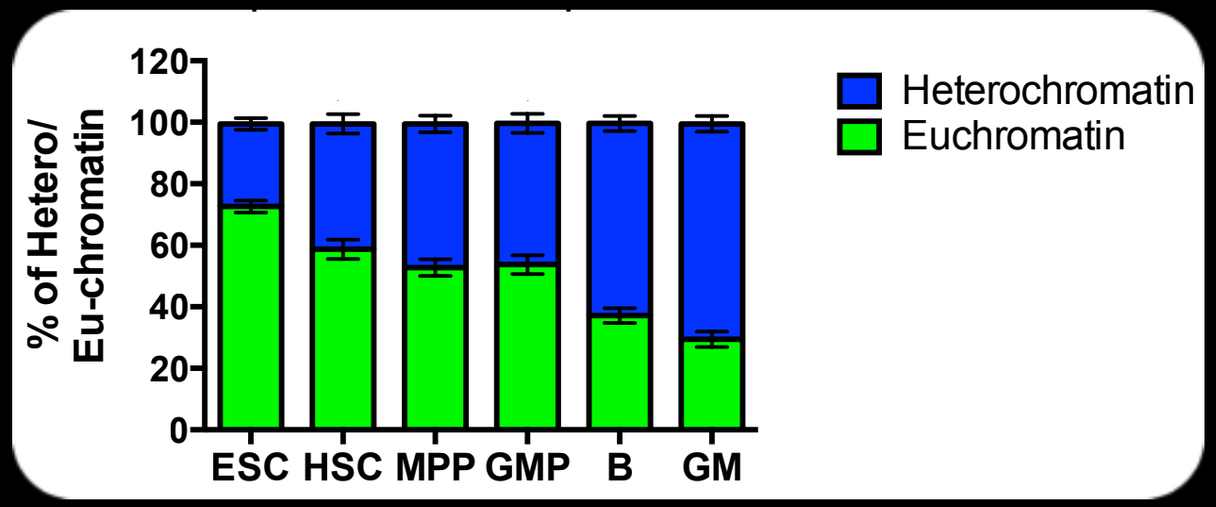
0.25

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

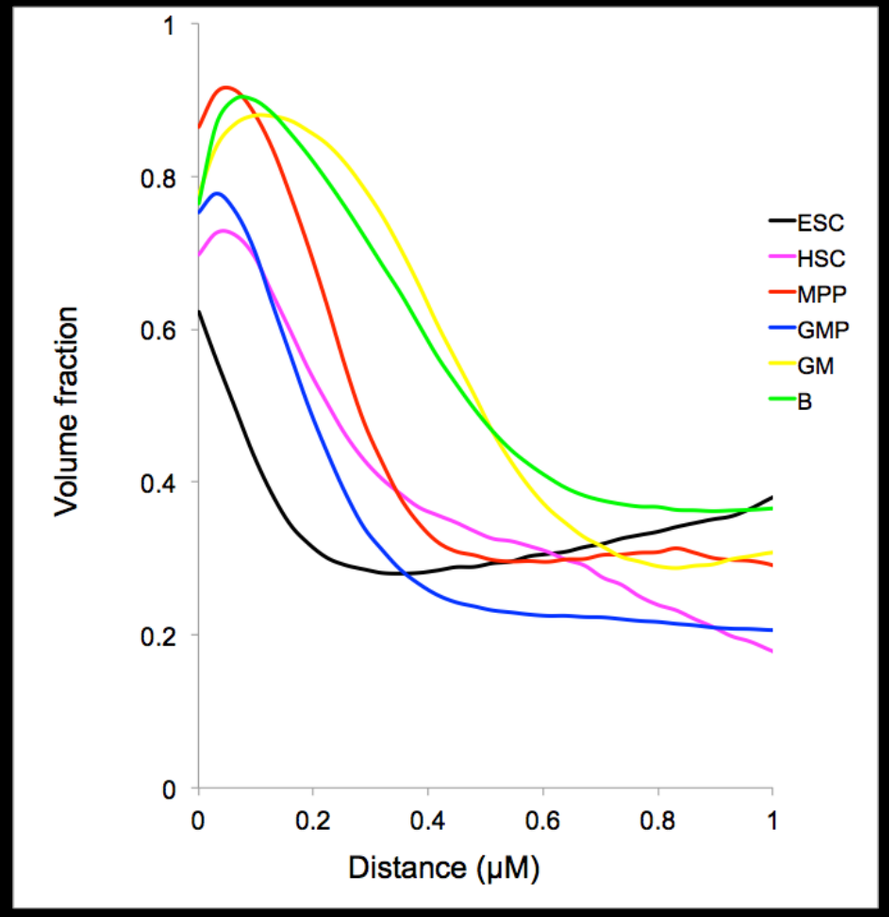
Chromatin reorganization during hematopoiesis

Chromatin reorganization during hematopoiesis



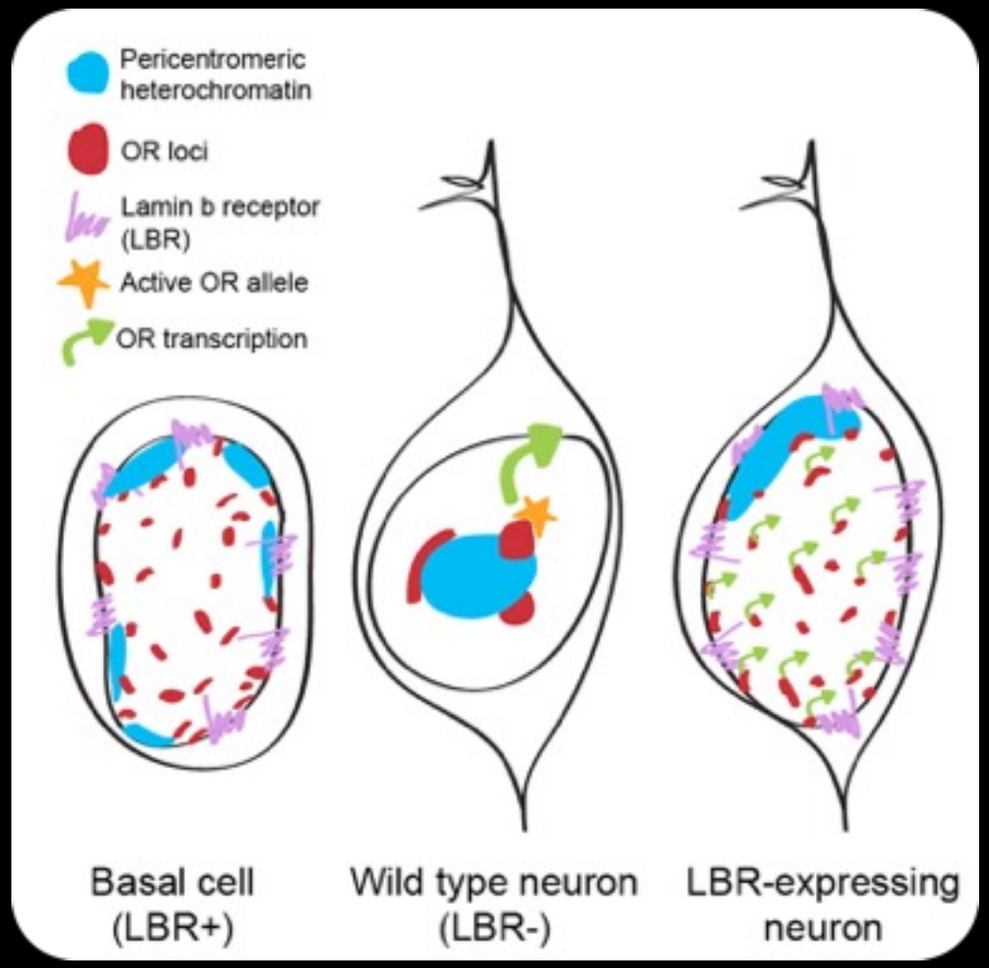


Spatial distribution of heterochromatin (outer 1 μm)

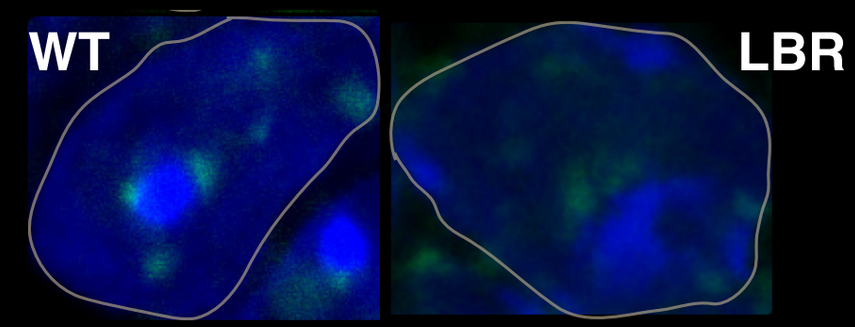


Ugarte et al. (2015) *Stem Cell Reports*. 5(5), 728-740

Gene selection - structural organization of nucleus critical

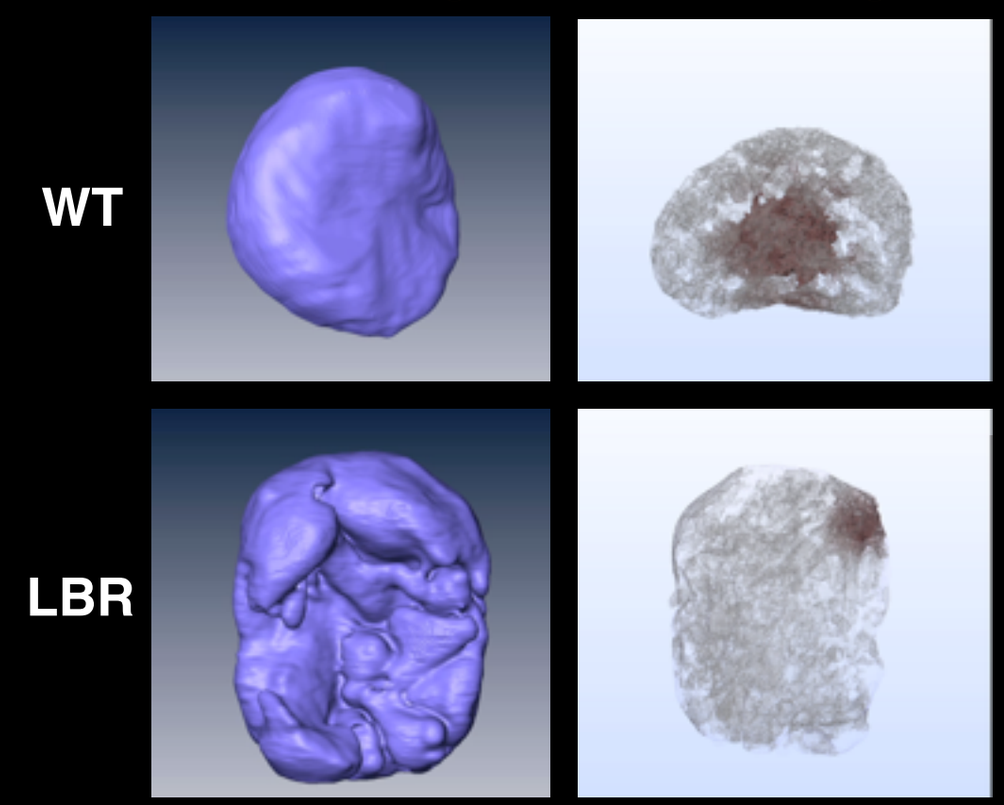


Lamin B Expressing



SXT - surface rendering

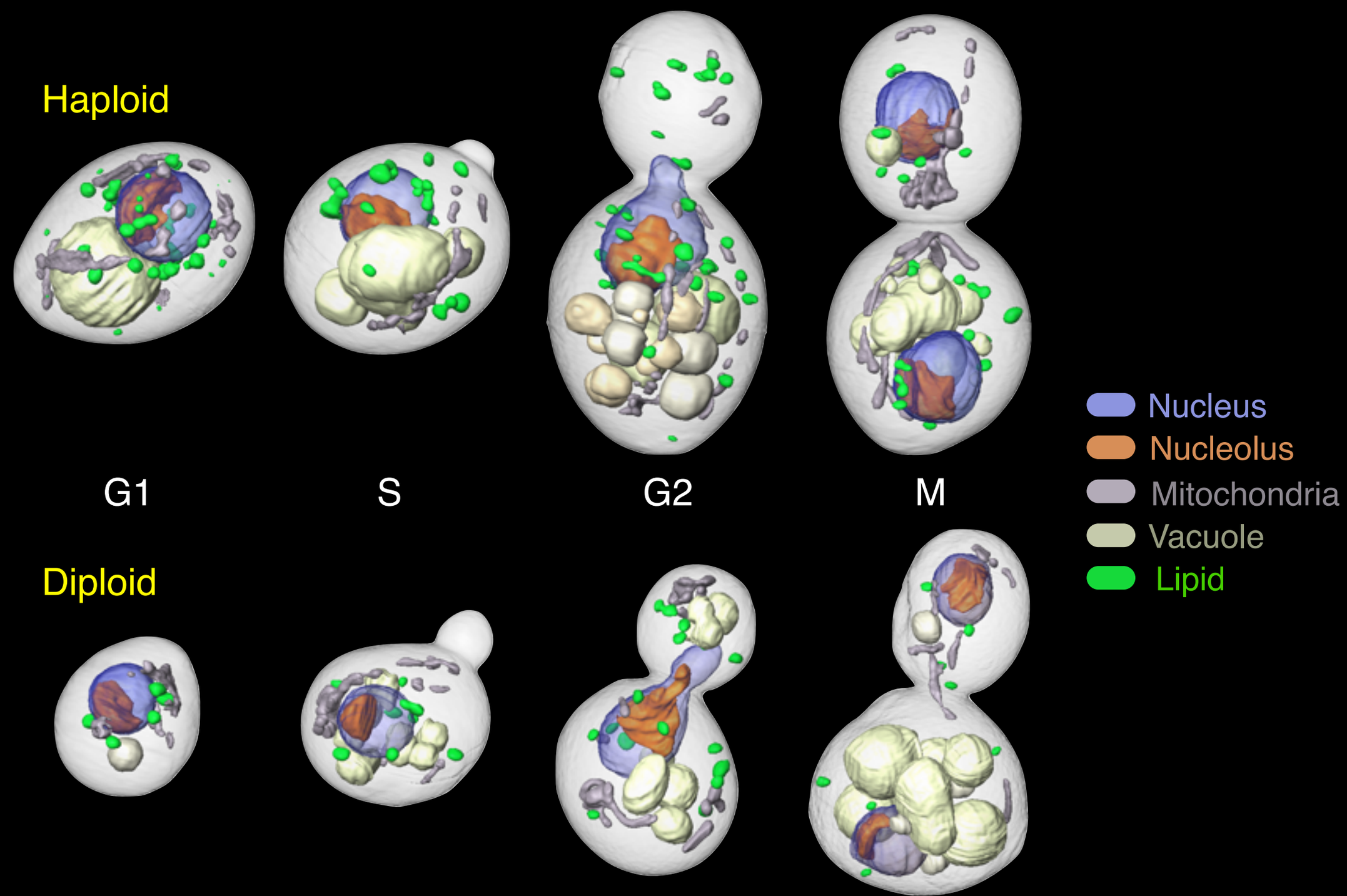
SXT - volume rendering



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.

Phenotypic consequences of genetic mutations

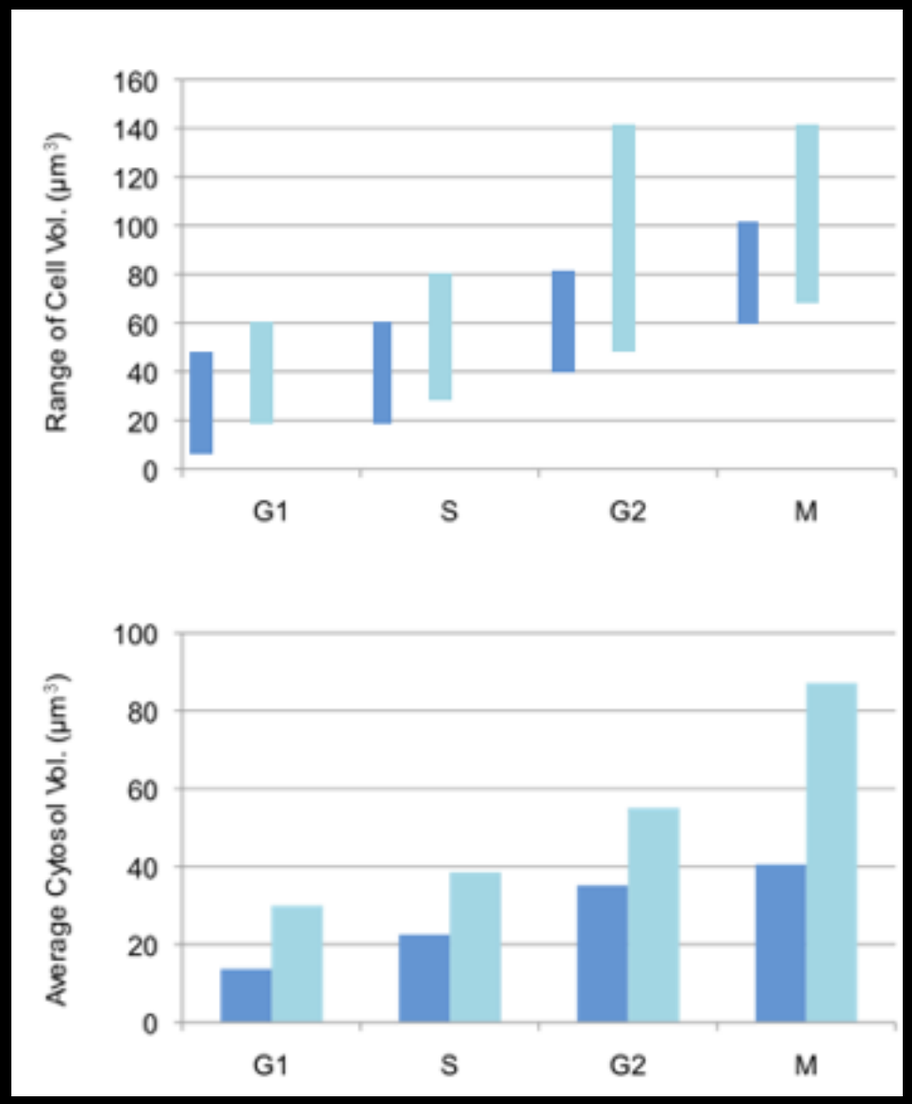
Structural organization of *S. cerevisiae*



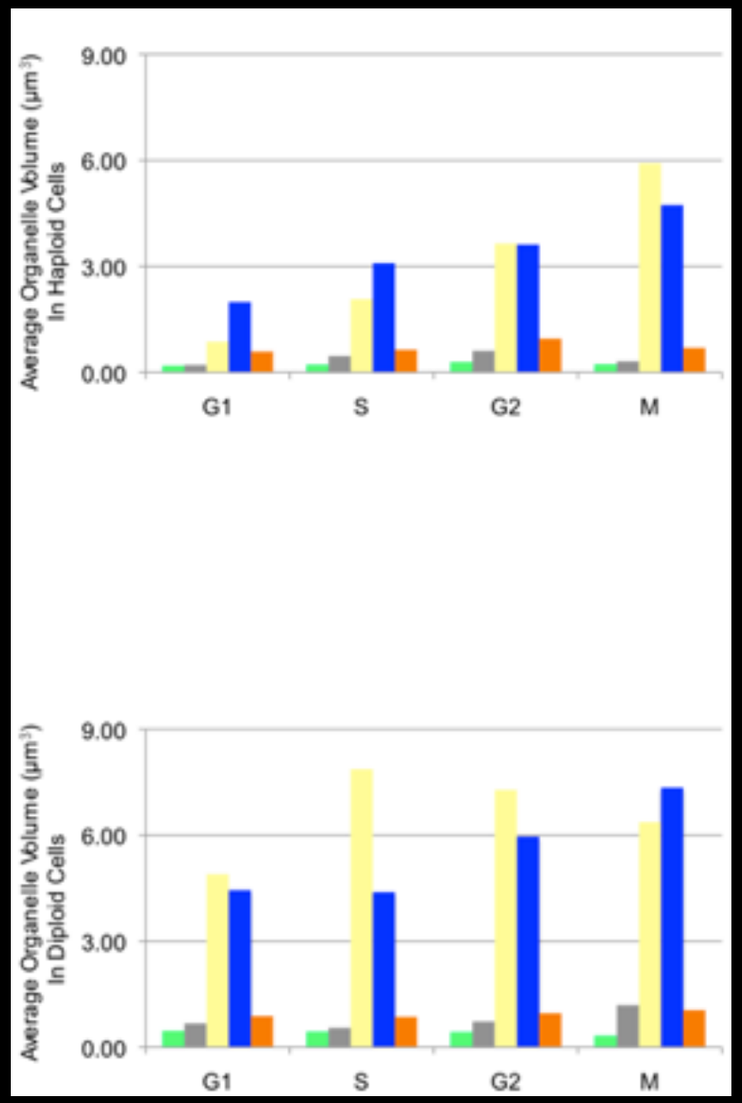
Uchida M, Sun Y, McDermott G, Knoechel C, Le Gros MA, Parkinson D, Drubin DG, and Larabell CA (2011). Yeast. 28:227-236

Quantitative information

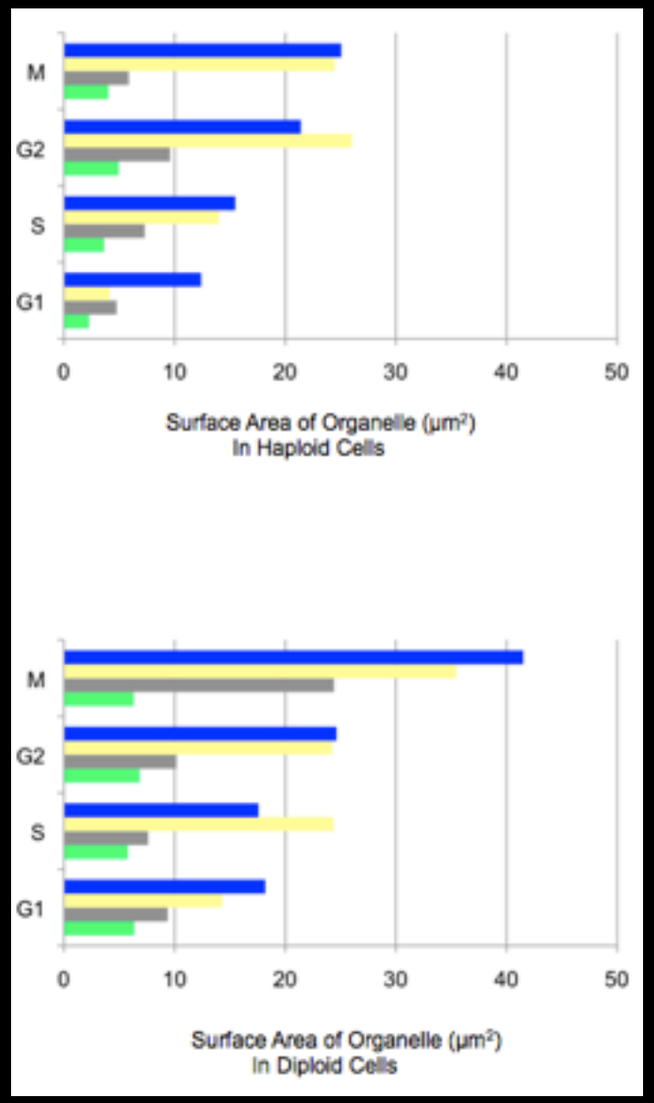
Cell volume



Organelle volume



Organelle surface area



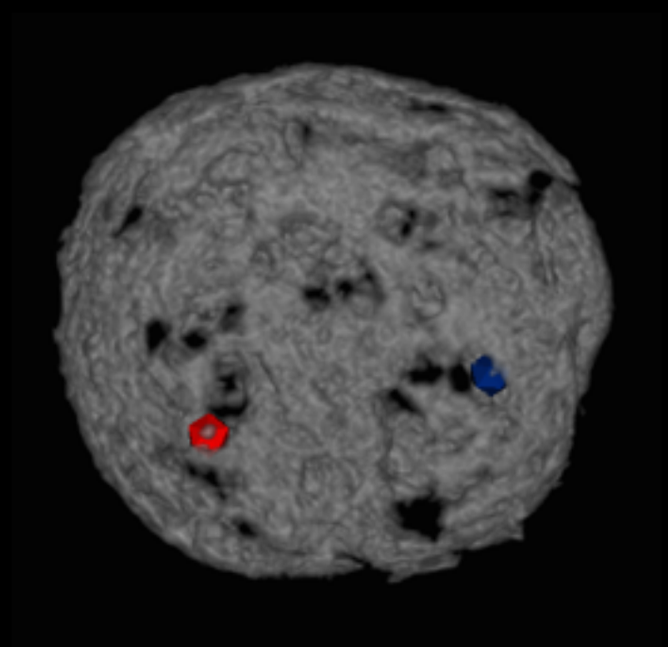
Uchida M, Sun Y, McDermott G, Knoechel C, Le Gros MA, Parkinson D, Drubin DG, and Larabell CA (2011). Yeast. 28:227-236

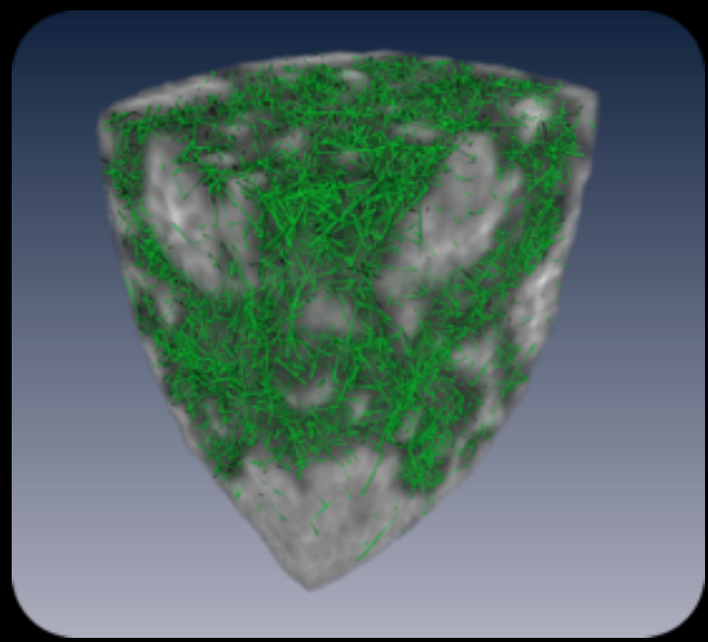
Modeling

Modeling movement of molecules through chromatin

How does volume exclusion due to chromatin influence the time required for regulatory proteins to find DNA binding sites?

Path of molecule through euchromatin

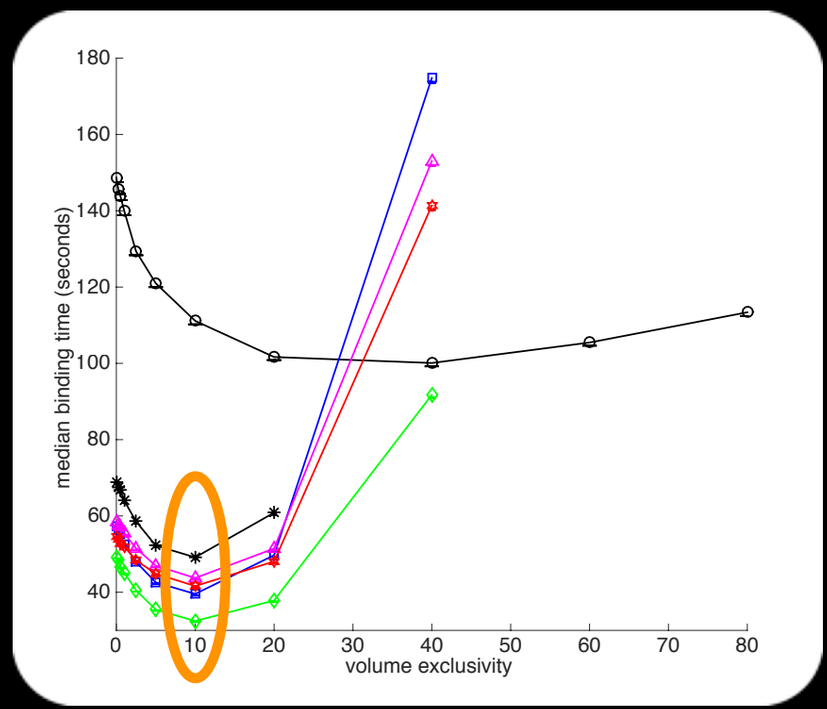




protein

binding site

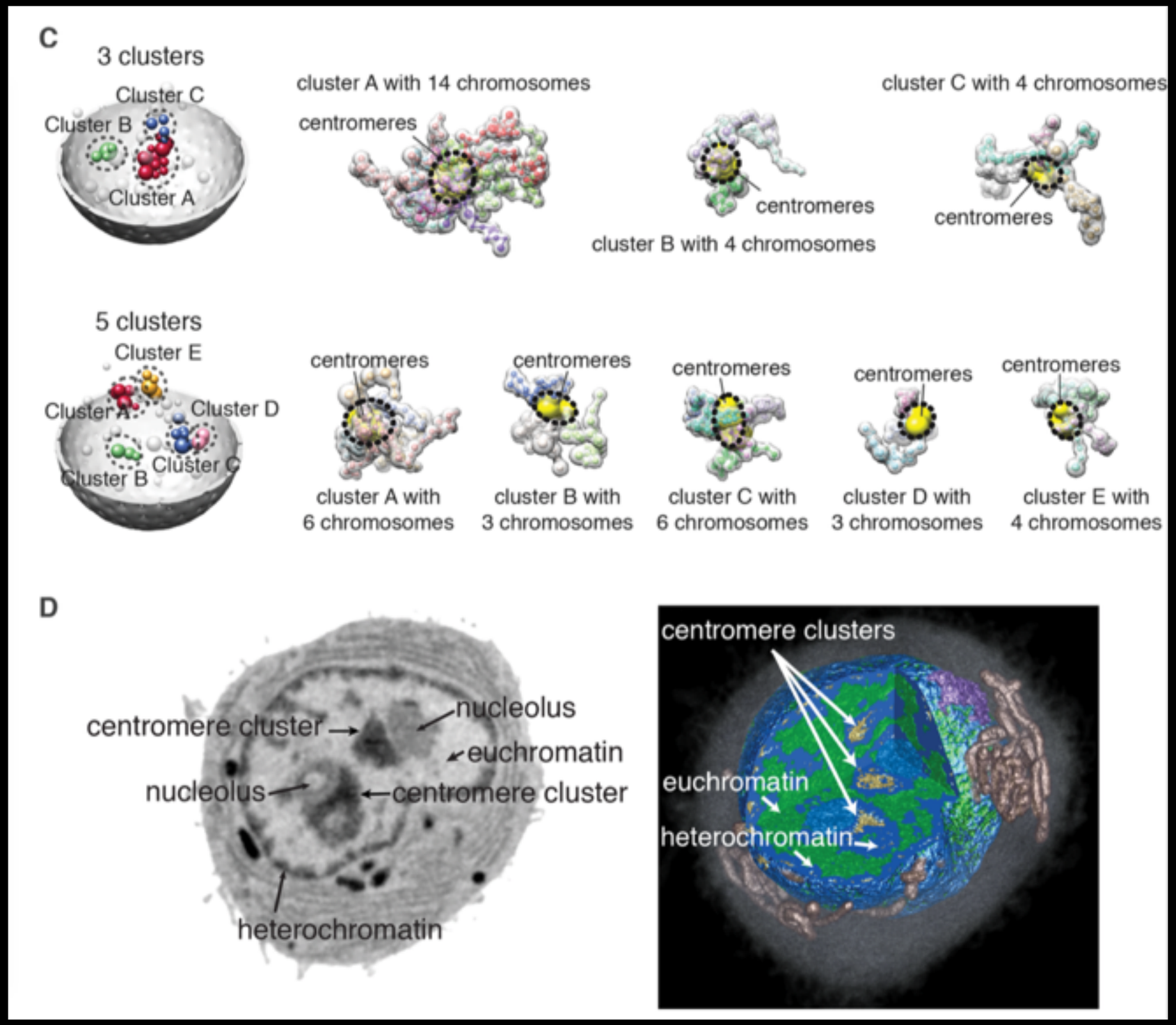
Median search times



SA Isaacson, CA Larabell, MA Le Gros, DM McQueen, and CS Peskin. (2013) Bull. Math. Biol.751, 2093-2117

Do M, Isaacson SA, McDermott G, Le Gros MA, Larabell CA. (2015) Arch. Biochem. Biophys. 2015 Aug; 581:111-121

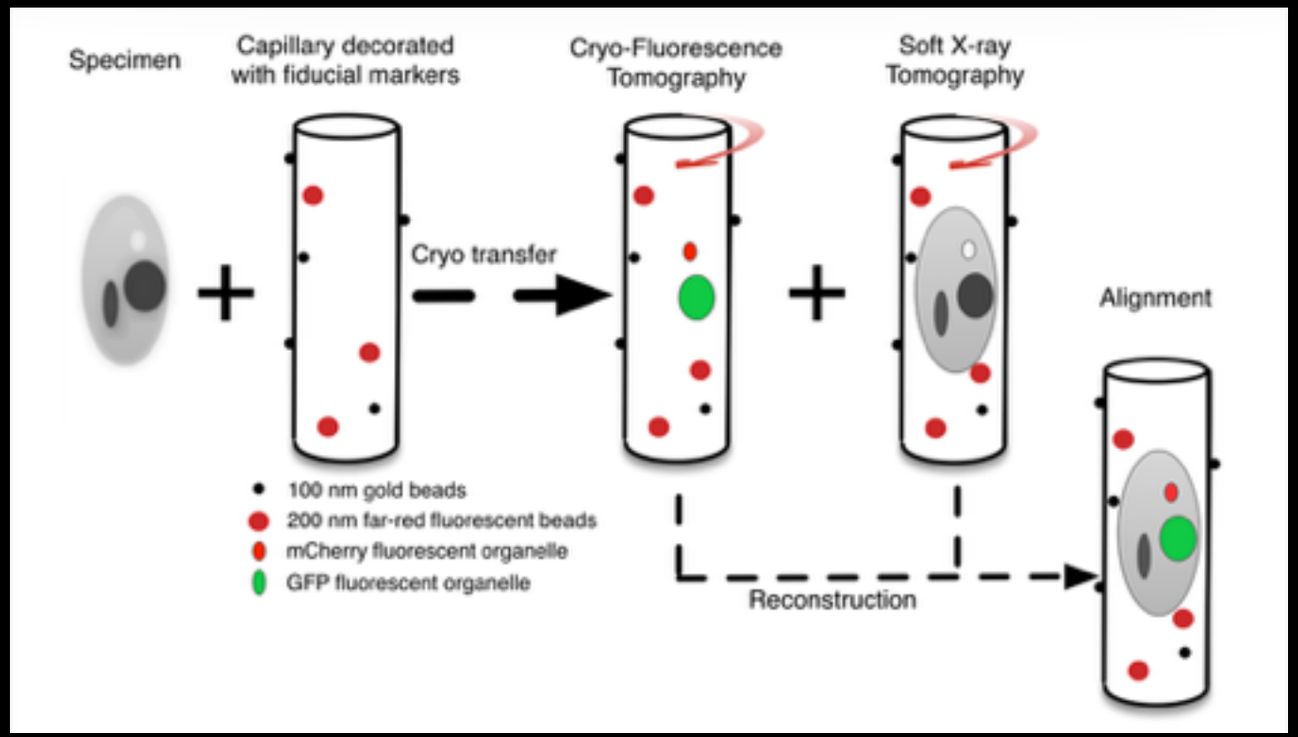
Population–based 3D genome structure analysis reveals driving forces in spatial genome organization

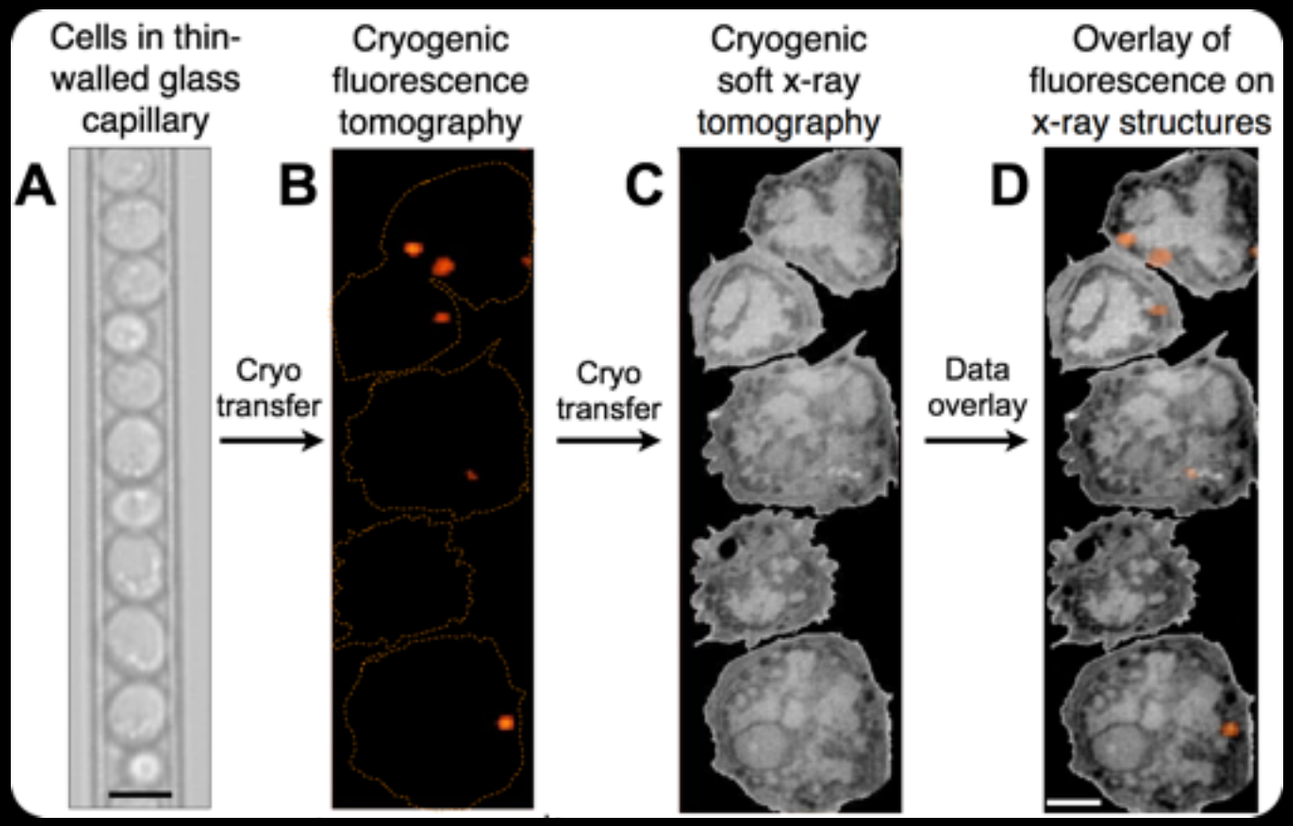


Tjong H, Li W, Kalhor R, Dai C, Hoa S, Gong K, Zhou Y, Li H, Zhou XJ, Le Gros MA, Larabell CA, Chen L, and Alber F. PNAS. 2016 Mar 22; 113 E1663-1672.

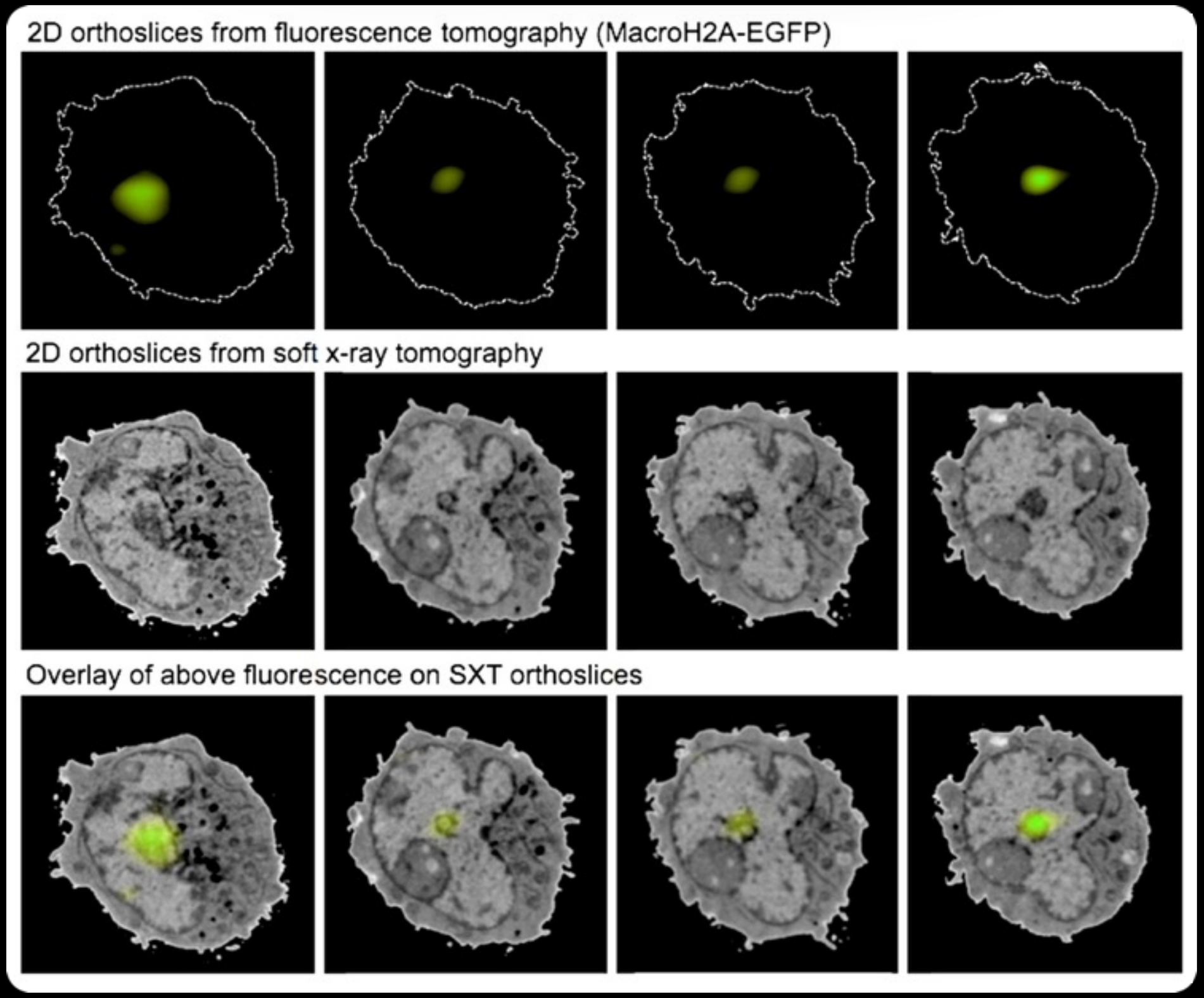
Correlated fluorescence and x-ray tomography

Cryo Confocal Tomography





Inactive X chromosome



Smith EA, McDermott G, Do M, Leung K, Panning B, Le Gros MA and Larabell CA (2014). Biophysical Journal. 107(8) 1988-96.

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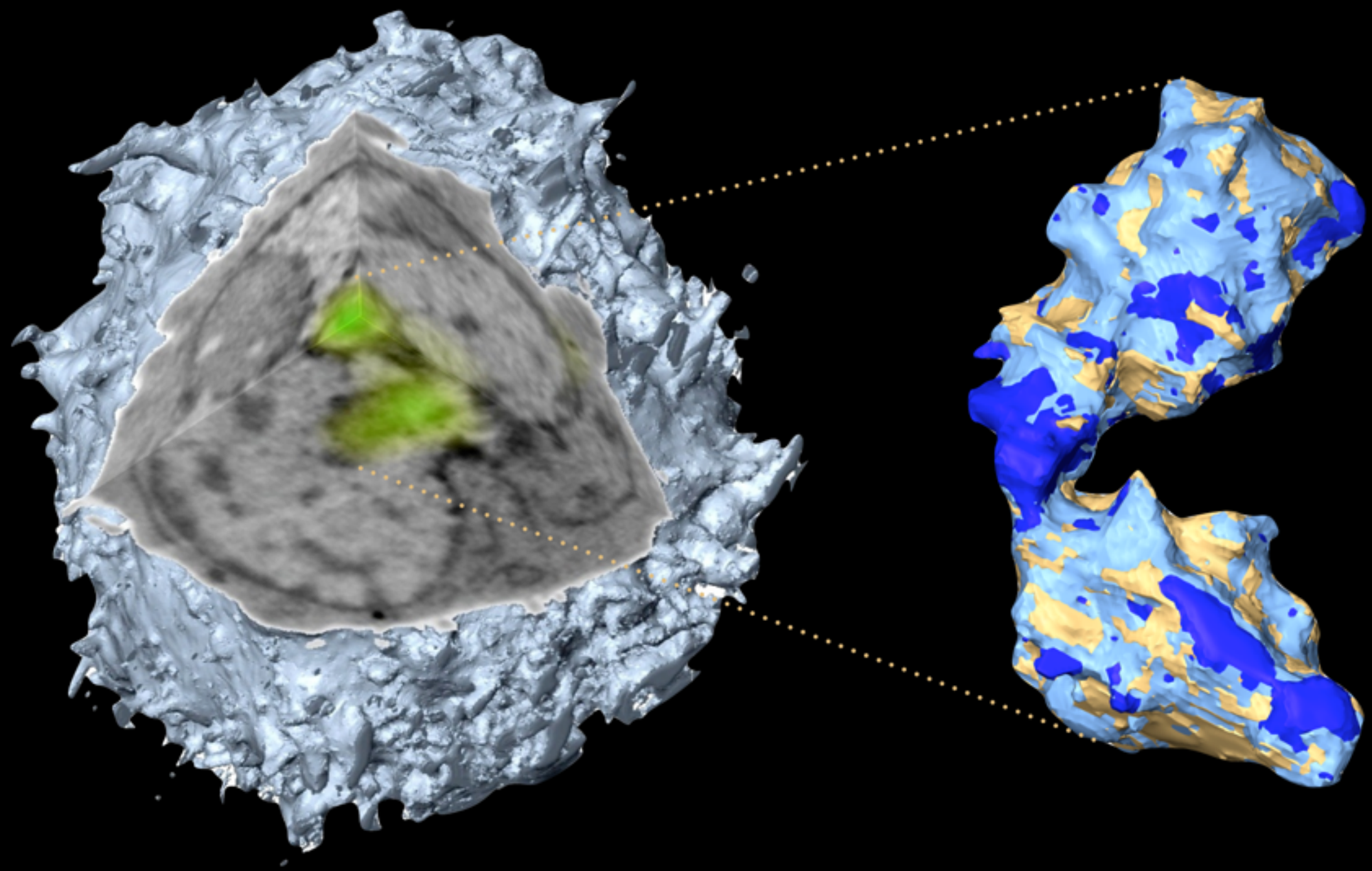

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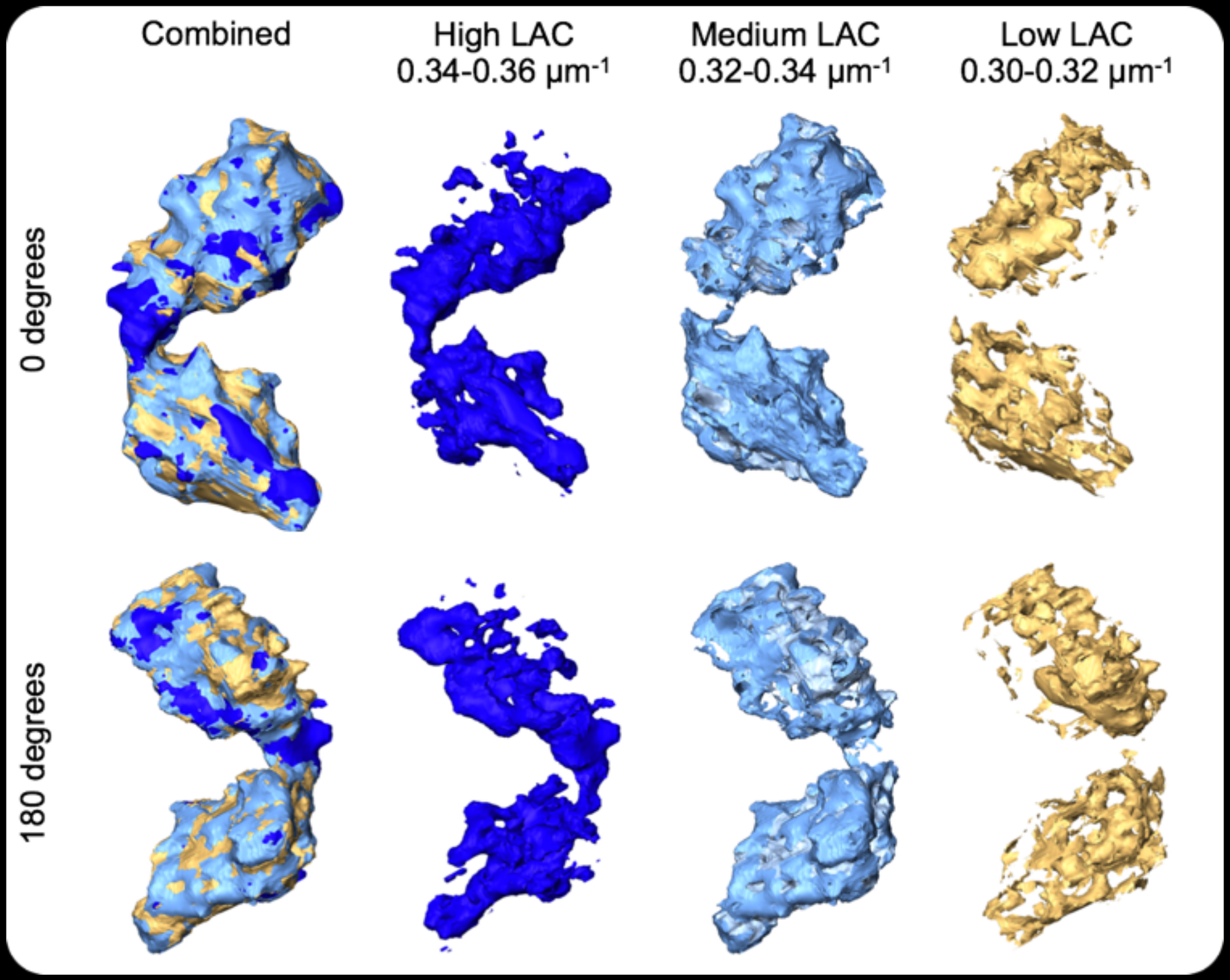

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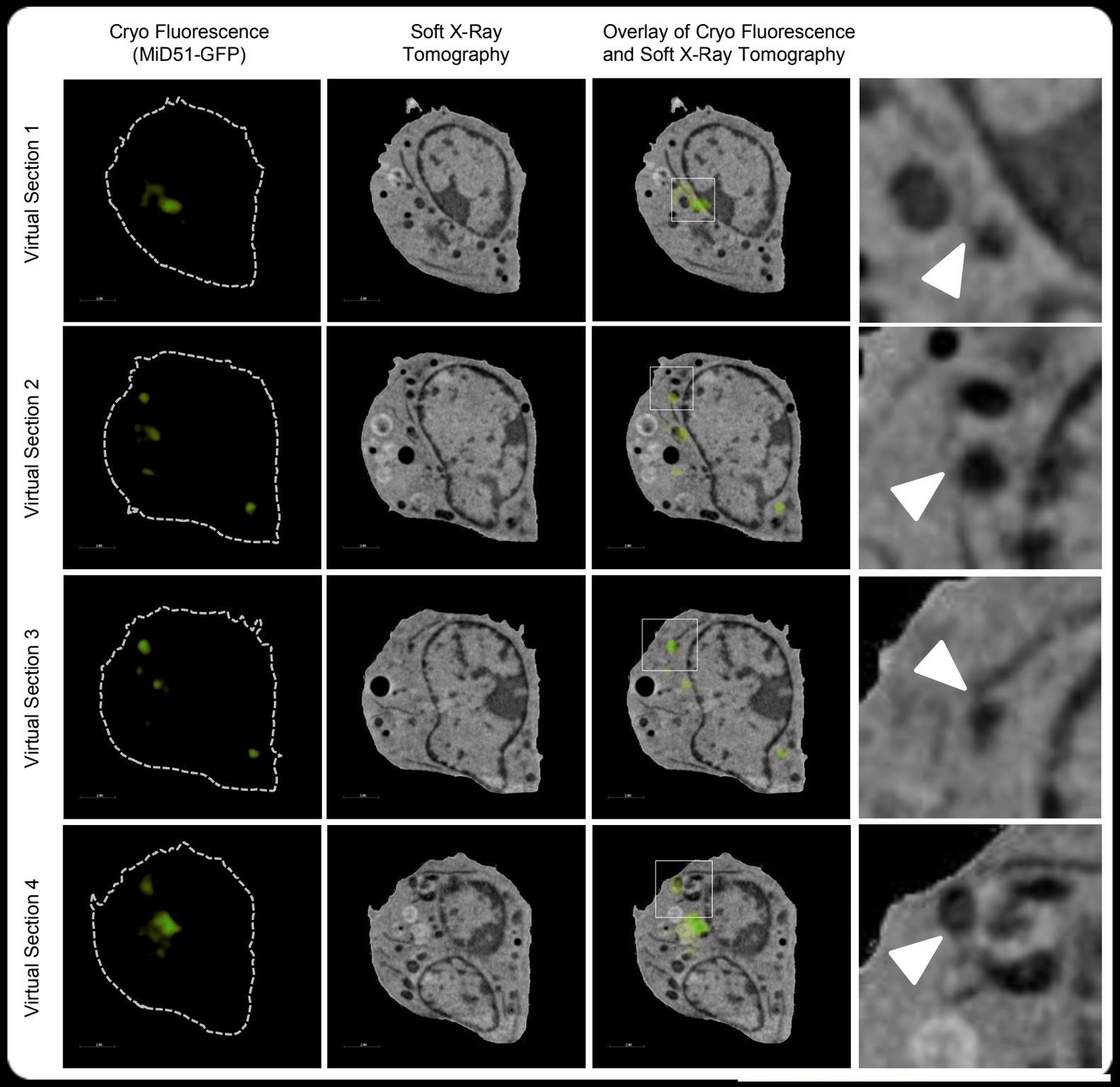
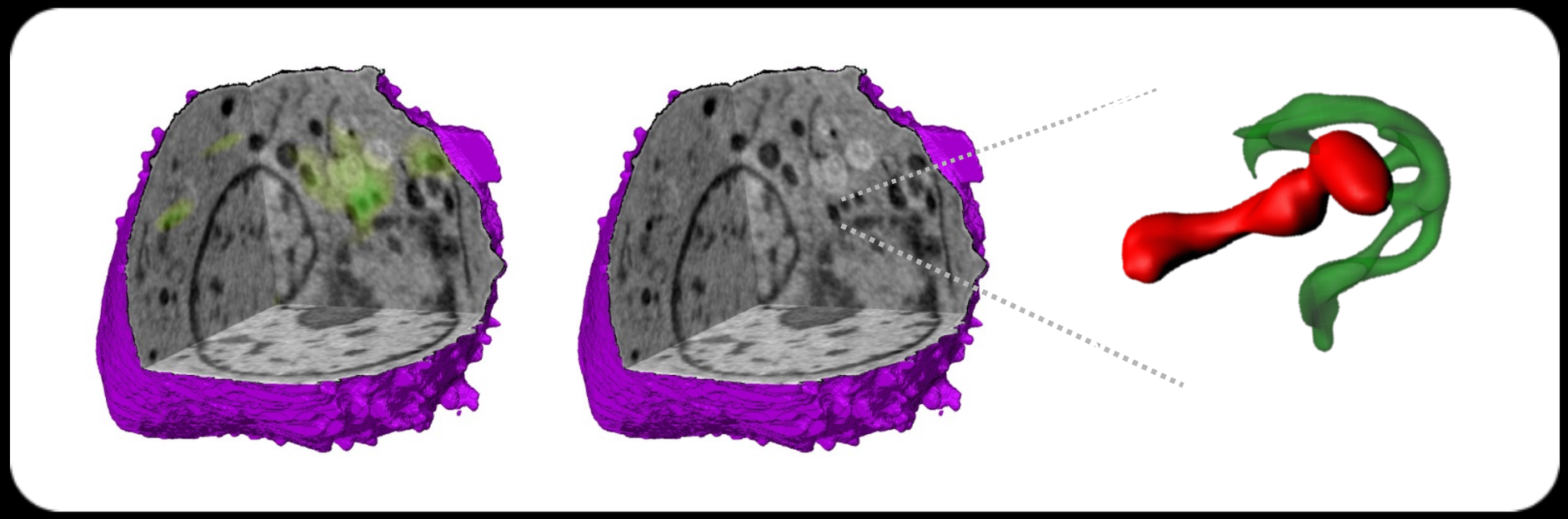
MiD51-GFP fluorescence foci at ER - mitochondria contact sites



Elgass, K., Smith, E.A., Le Gros, M.A., Larabell, C.A., Ryan, M.T. (2015). J. Cell Science. 128(15), 2795-804.

MiD51-GFP fluorescence foci at ER - mitochondria contact sites

Correlated cryo confocal and x-ray tomography



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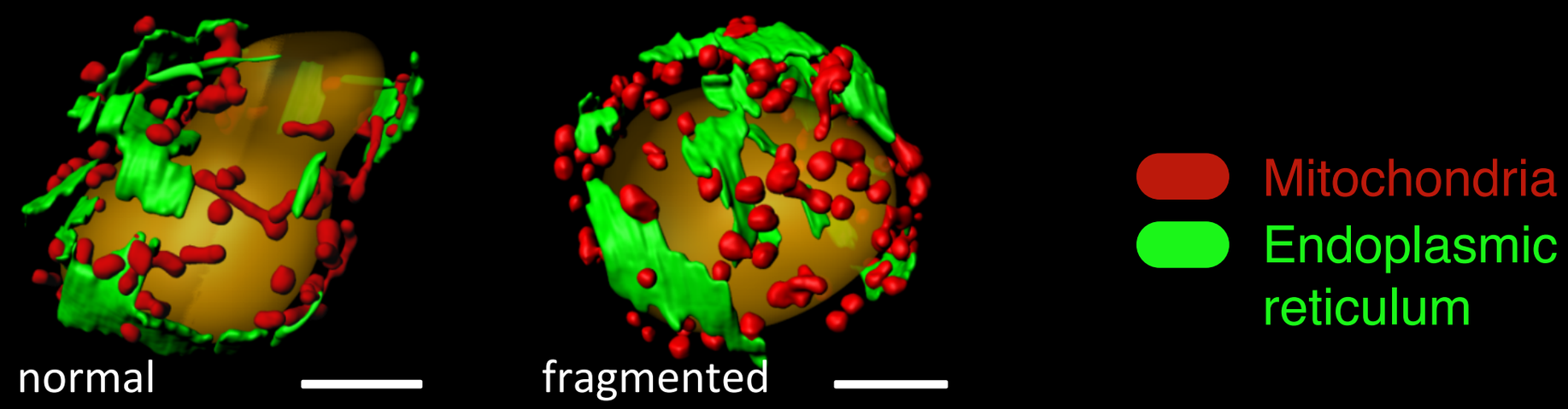

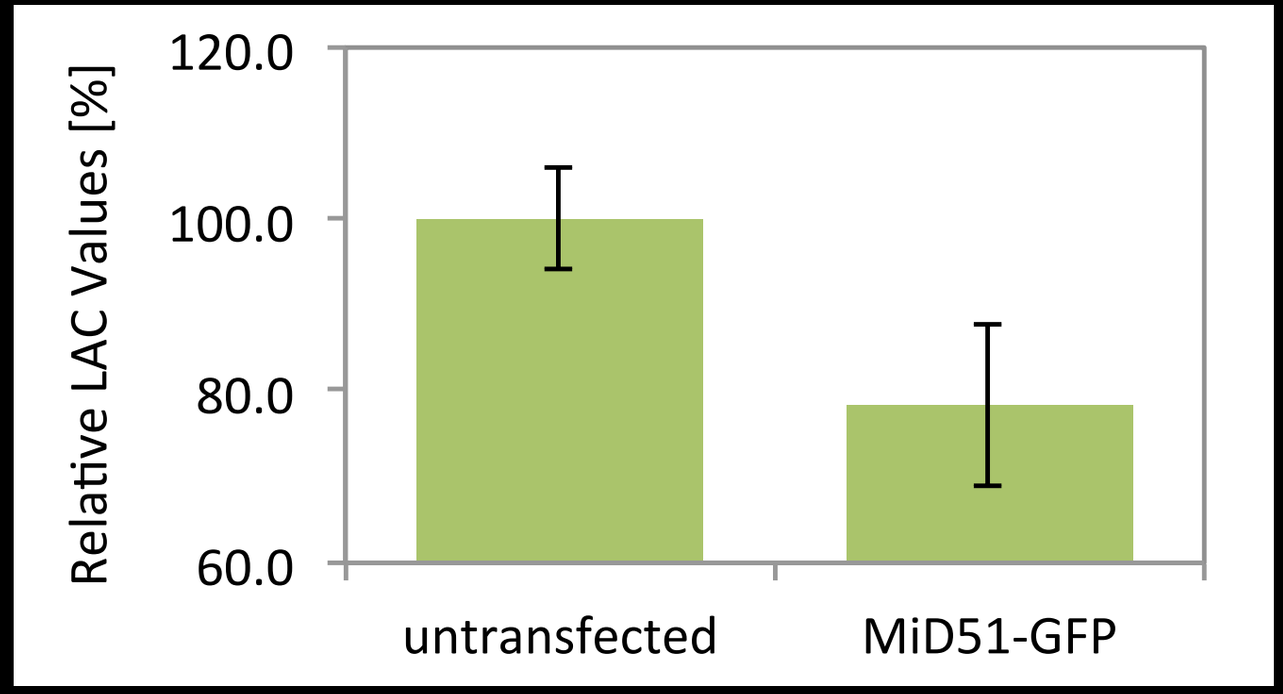

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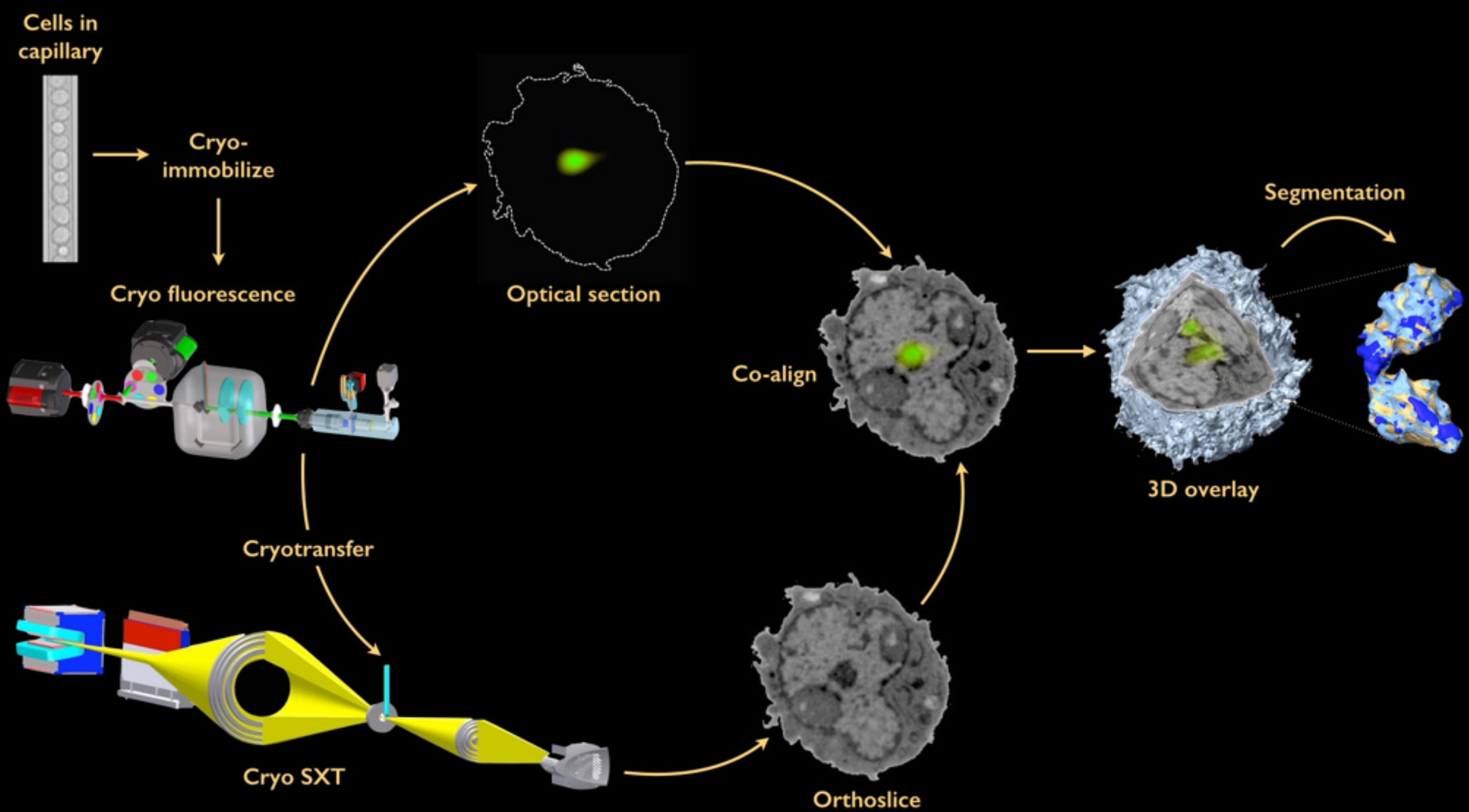
Correlated fluorescence and x-ray tomography

Localizing molecules with respect to cell structures



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National Center for X-ray Tomography

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