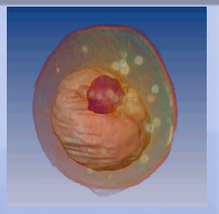

National Center for X-ray Tomography

University of California, San Francisco

&

Lawrence Berkeley National Laboratory

National Center for Research Resources, NIH

Office of Biological and Environmental Research, DOE

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UCSF University of California San Francisco

National Center for Research Resources

National Institute of General Medical Sciences

Office of Science U.S. Department of Energy

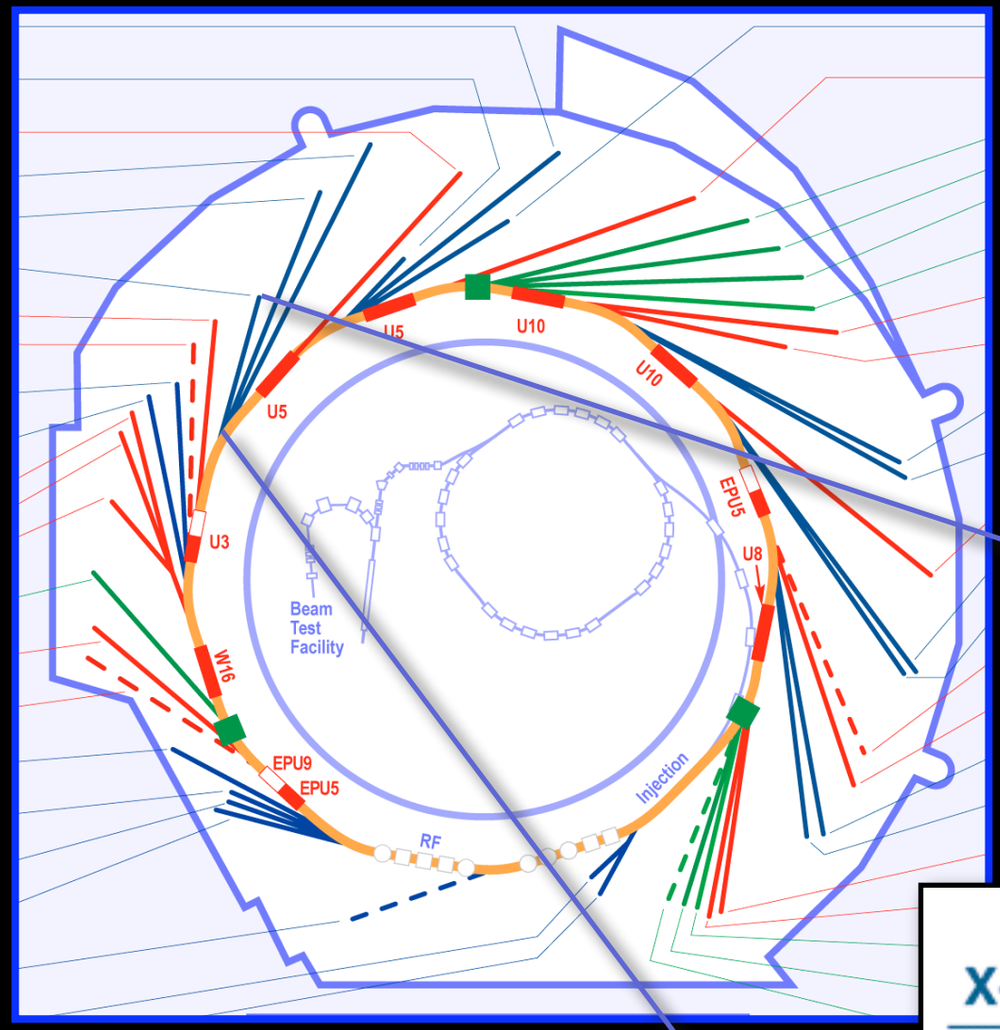
DOE Biological and Environmental Research Program

Berkeley Lab

Imaging Cells with Soft X-ray Microscopy

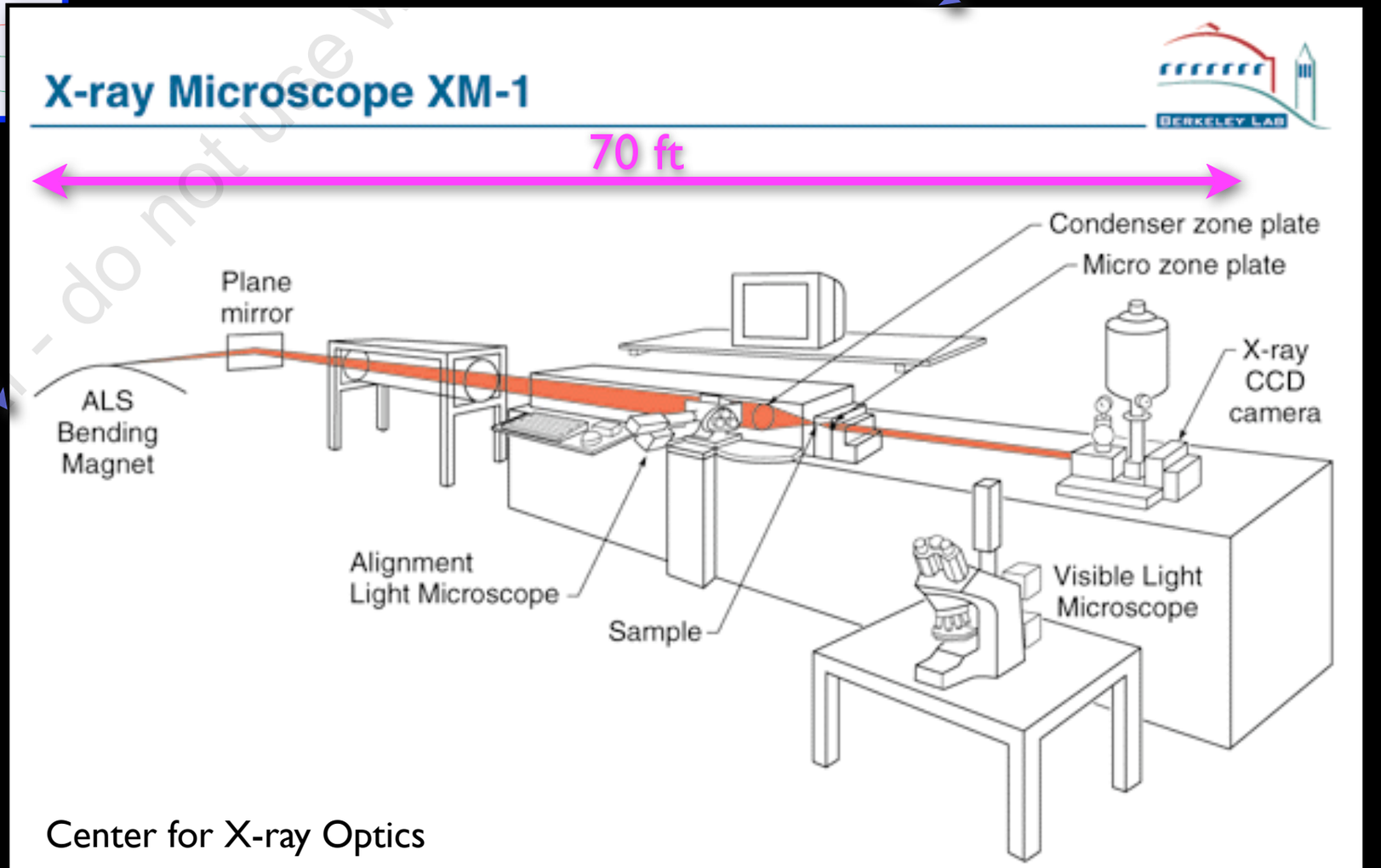
- Whole cells up to 10 µm thick
- Fully hydrated specimens
- Inherent contrast of organic material
- Quantitative - use linear absorption coefficient
- Localization of proteins and multi-protein complexes
- Better than 50 nm resolution - isotropic
- Fast - collect tomographic data set in < 5 min

Soft X-ray Microscope

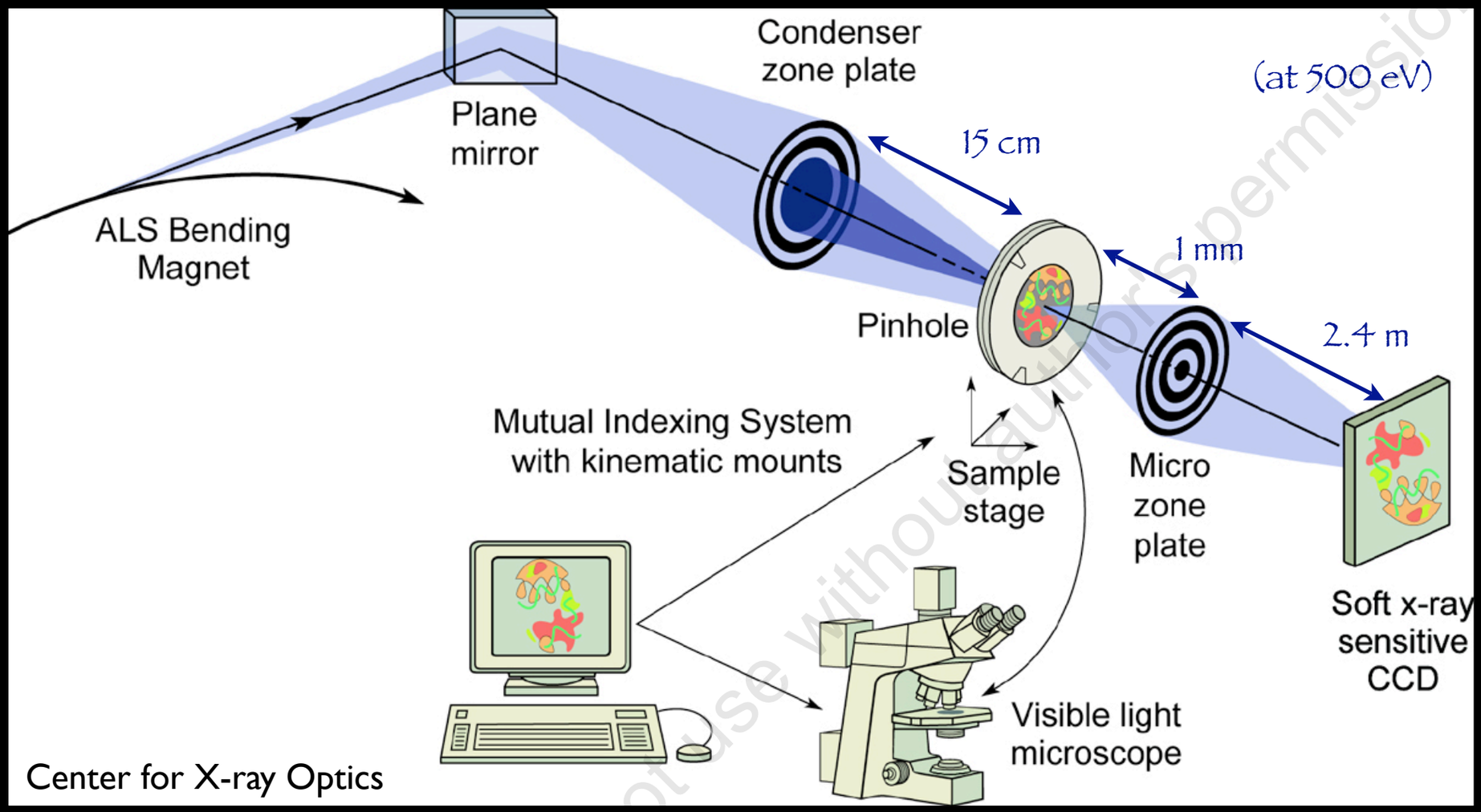


2.4 nm λ

517 eV



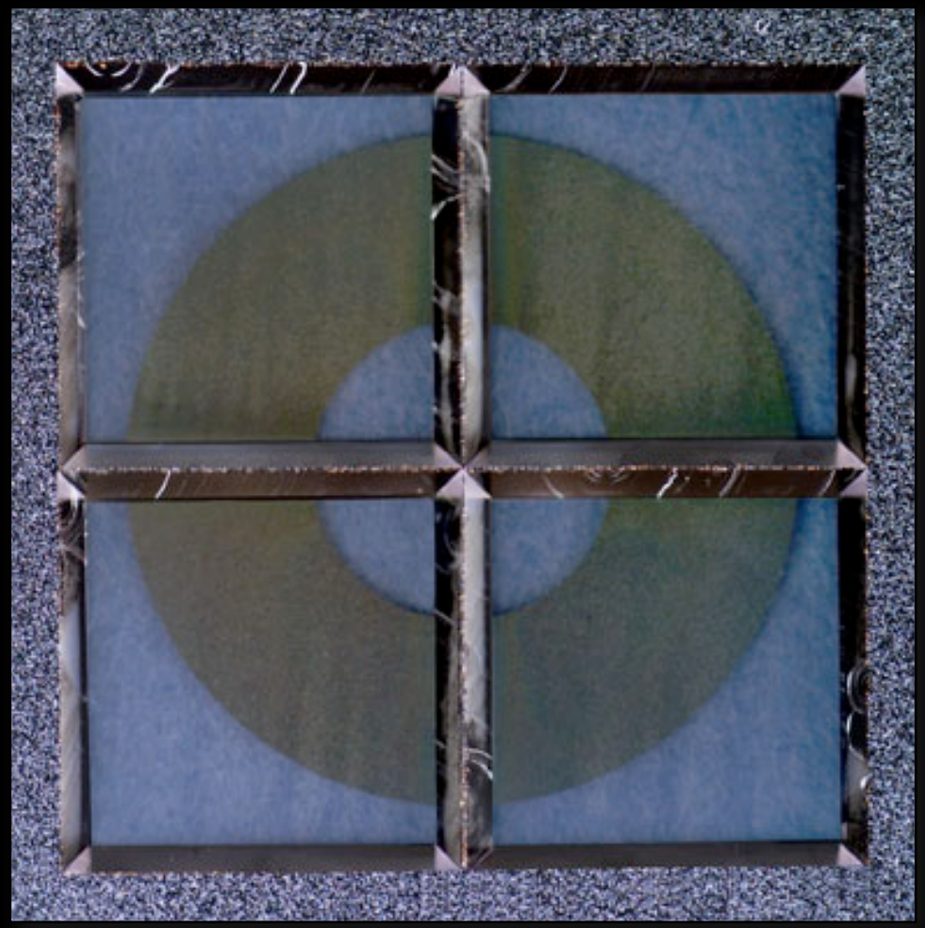
Center for X-ray Optics



- Condenser zone plate focuses source onto object
- Objective zone plate magnifies object onto CCD camera
- Focal length of the objective is ~ 1 mm
- CCD is ~ 2.4 meters away
- Magnification ~ 2400

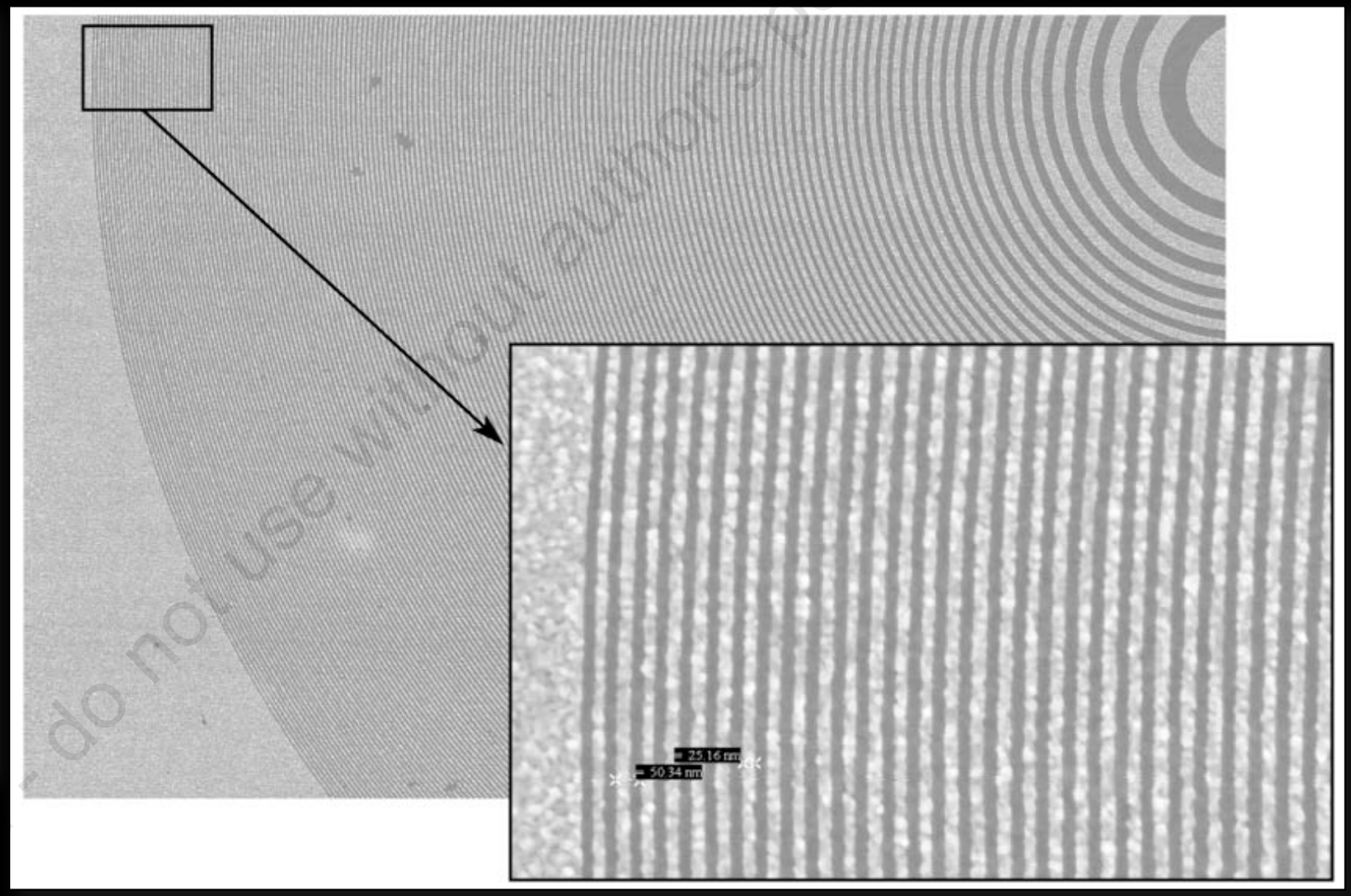
Zone Plate Lenses

Condenser Lens



diameter = 1 cm
No. of zones = 41,700
outer zone width = 60 nm
central stop diameter = 5 mm

Objective Lens

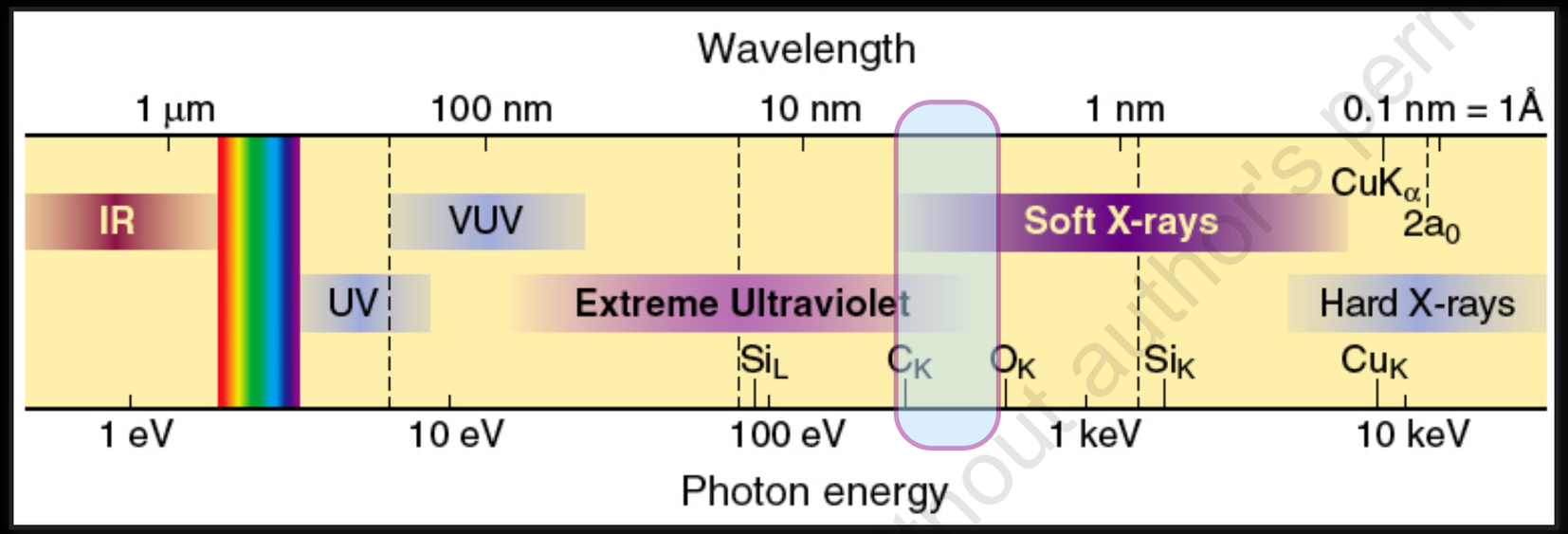


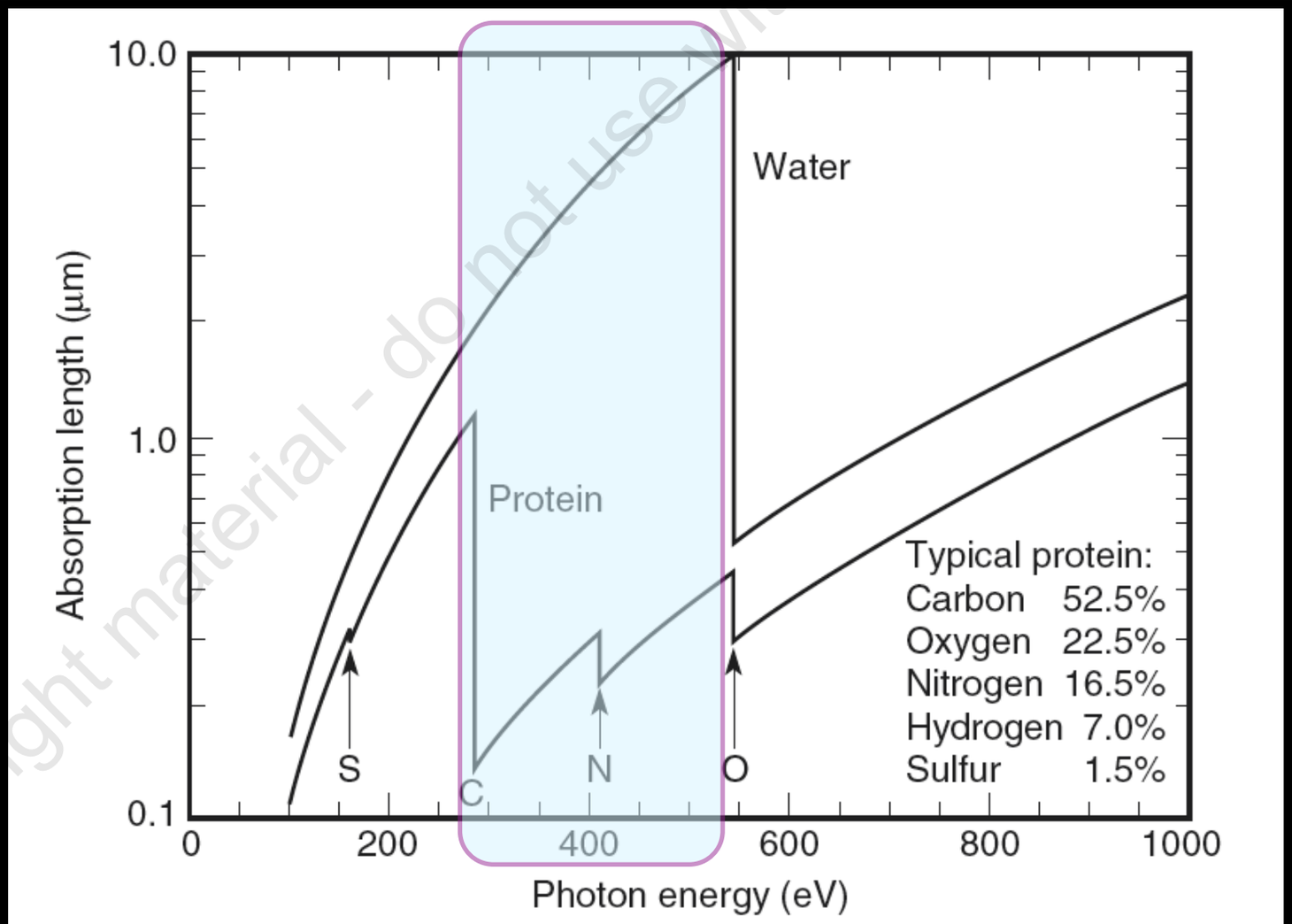
diameter = 63 μm
No. of zones = 628
outer zone width = 25 nm
nickel plating

E. Anderson, W. Chao, B. Harteneck, D. T. Attwood, Center for X-ray Optics

Image in Water Window - Natural Contrast

Between K shell absorption edges of oxygen (543 eV; 2.3 nm) and carbon (284 eV; 4.4 nm)

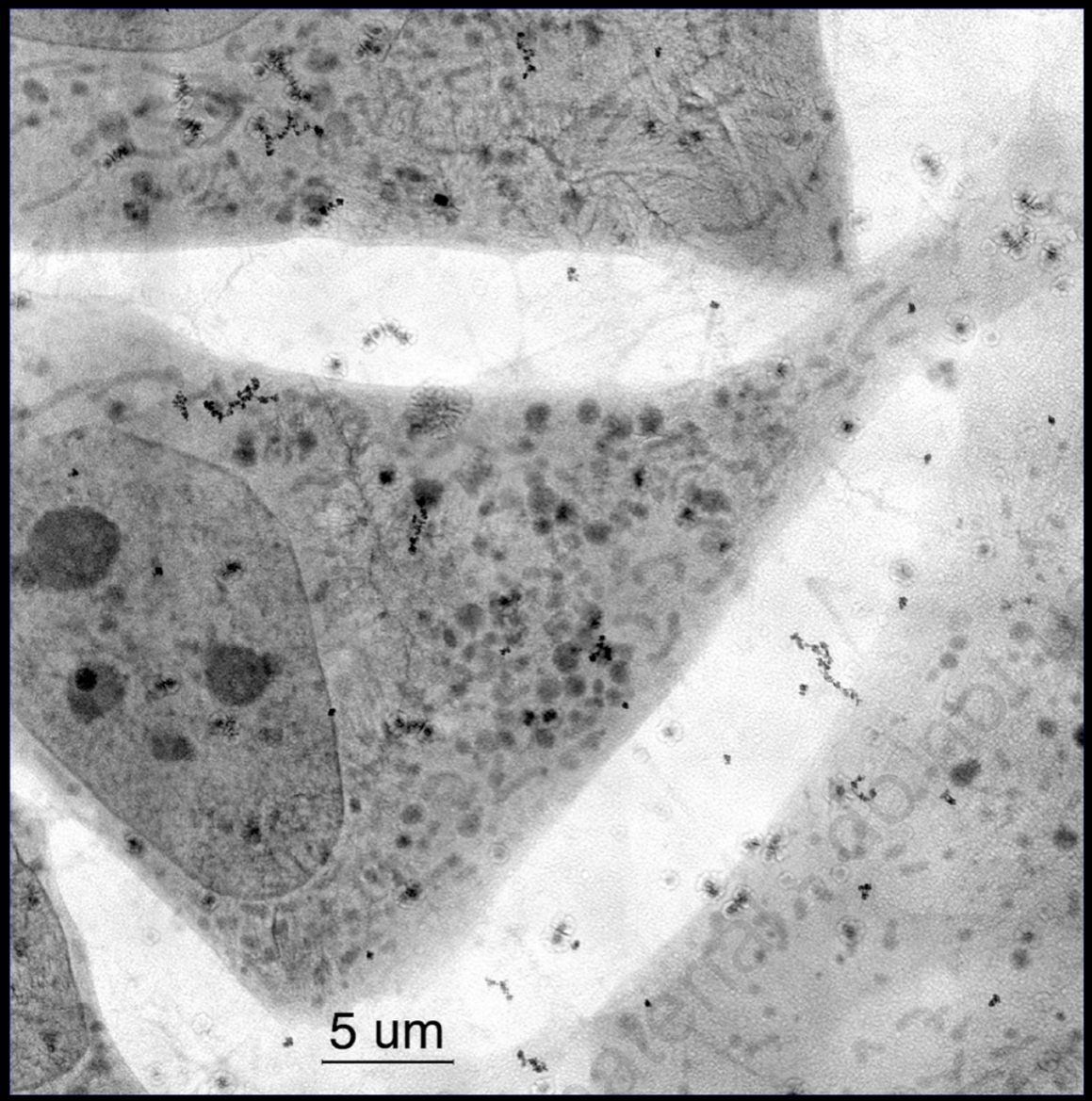


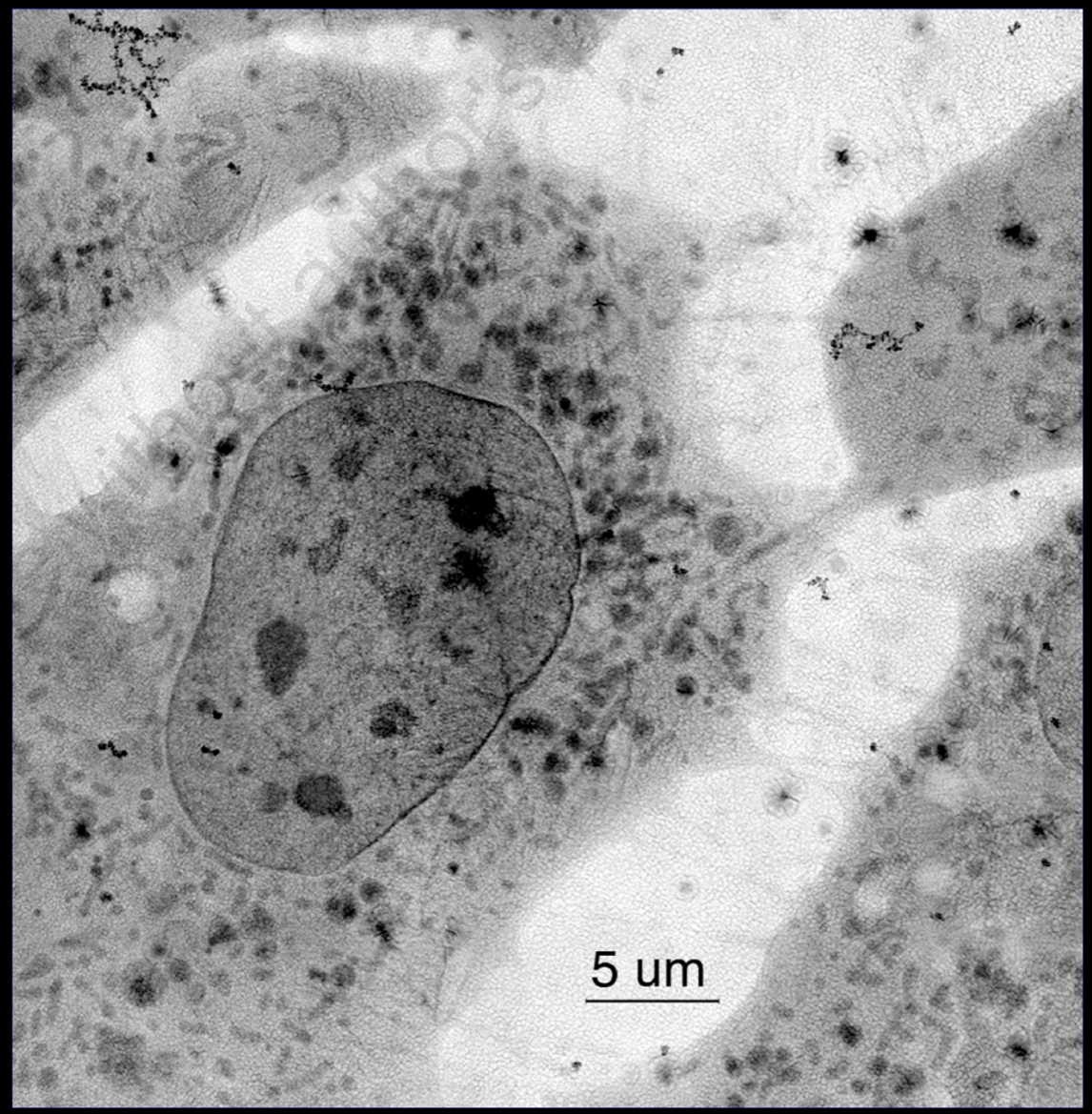


2.4 nm λ

517 eV

Cryo X-ray Microscopy of NIH 3T3 Fibroblasts

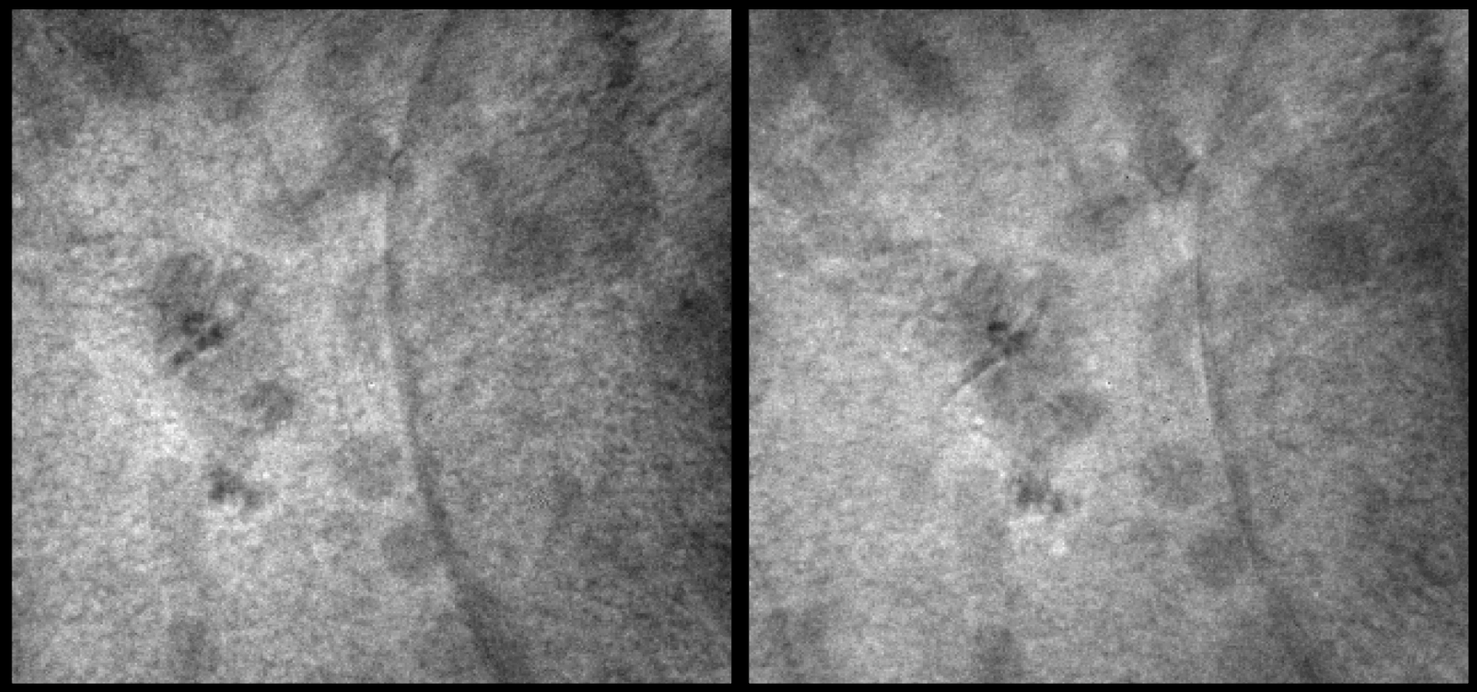




No chemical fixatives or contrast enhancement reagents

Meyer-Ilse, W., Hamamoto, D., Nair, A., Lelievre, S.A., Denbeaux, G., Johnson, L., Pearson, A.L., Yager, D., LeGros, M.A., and Larabell, C.A. (2001). J. Microscopy. 201, 395-403.

Nuclear envelope of NIH 3T3 fibroblast



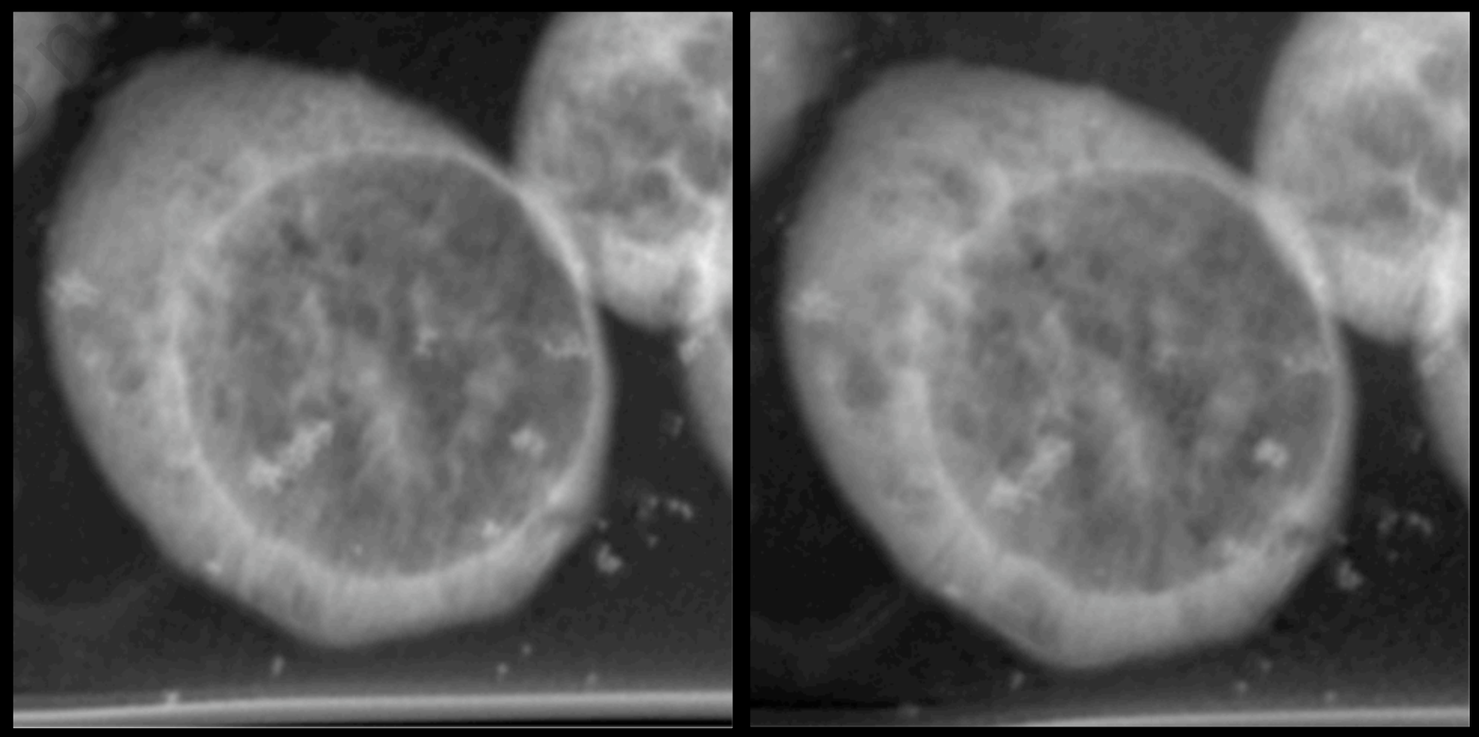
1 sec

130 sec

- Specimen must be cryofixed to prevent radiation damage
- Must *maintain* low temperature (77 K) during data collection

Cryo-preserved specimens tolerate hundreds of images (1sec/ image) without apparent radiation damage

Yeast

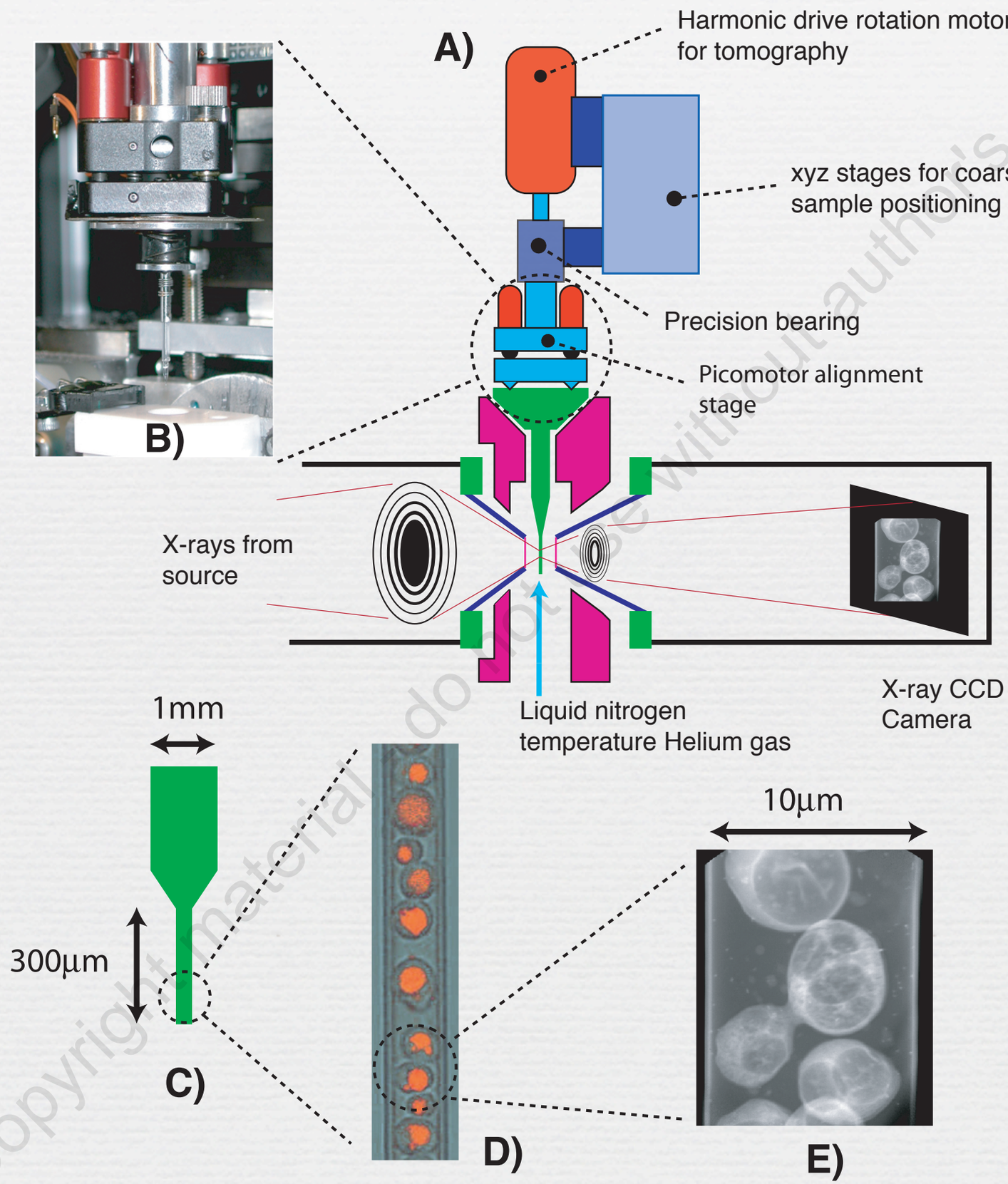


20 sec

1,020 sec

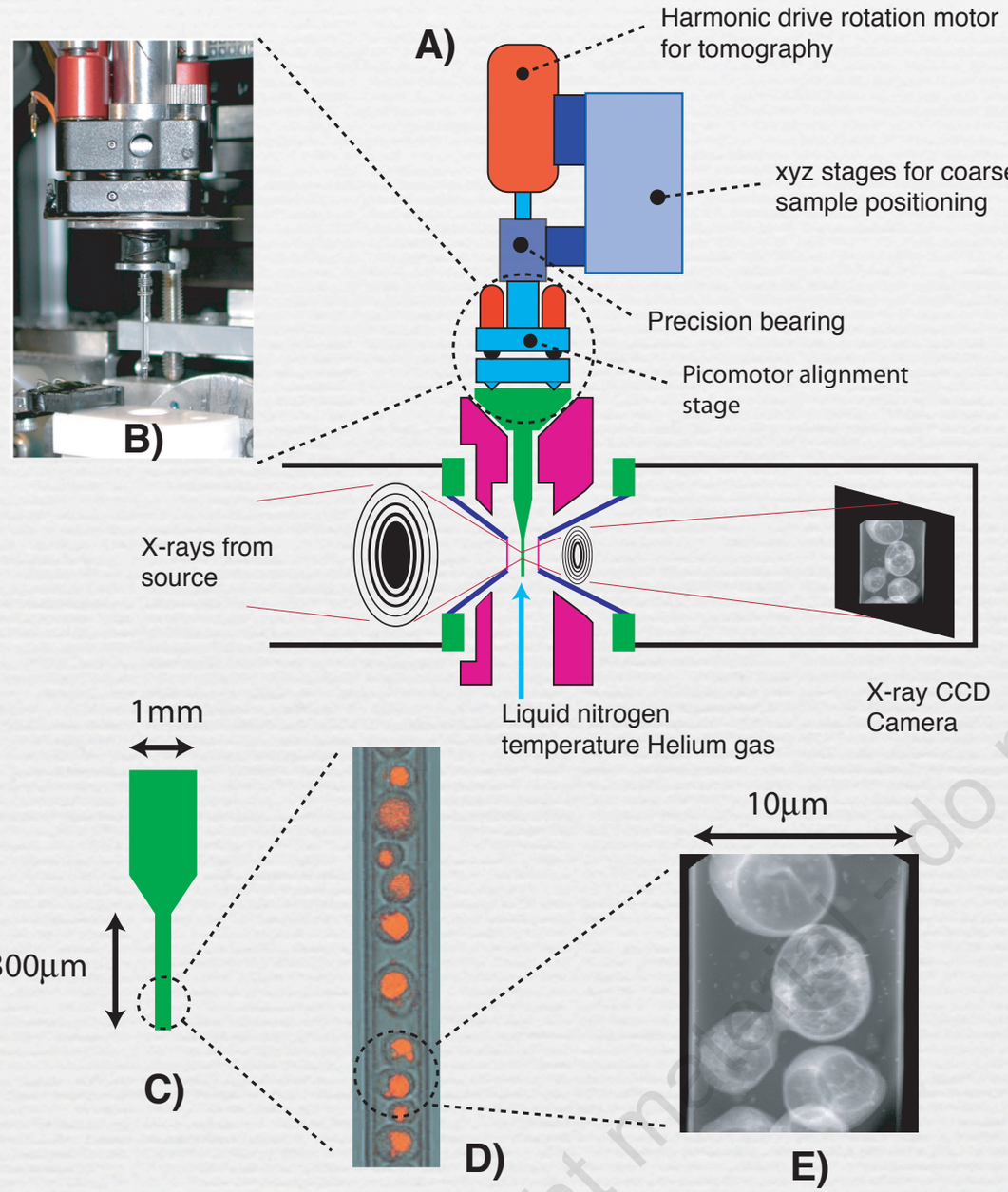
Cryo X-ray Tomography

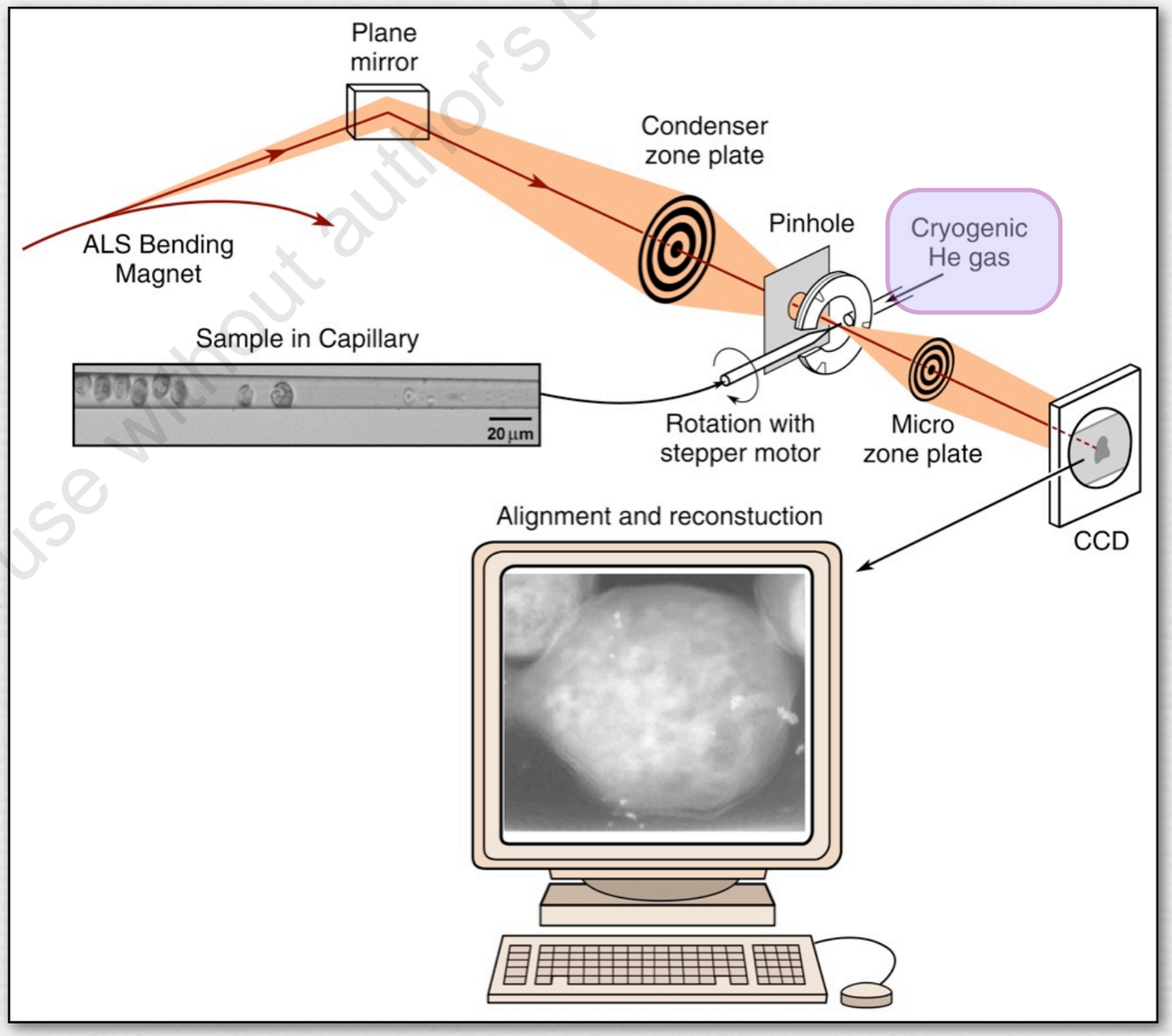
Specimen at atmospheric pressure



Mark Le Gros, Gerry McDermott, Carolyn Larabell; Current Opinion in Structural Biology. 2005, 15:1-8.

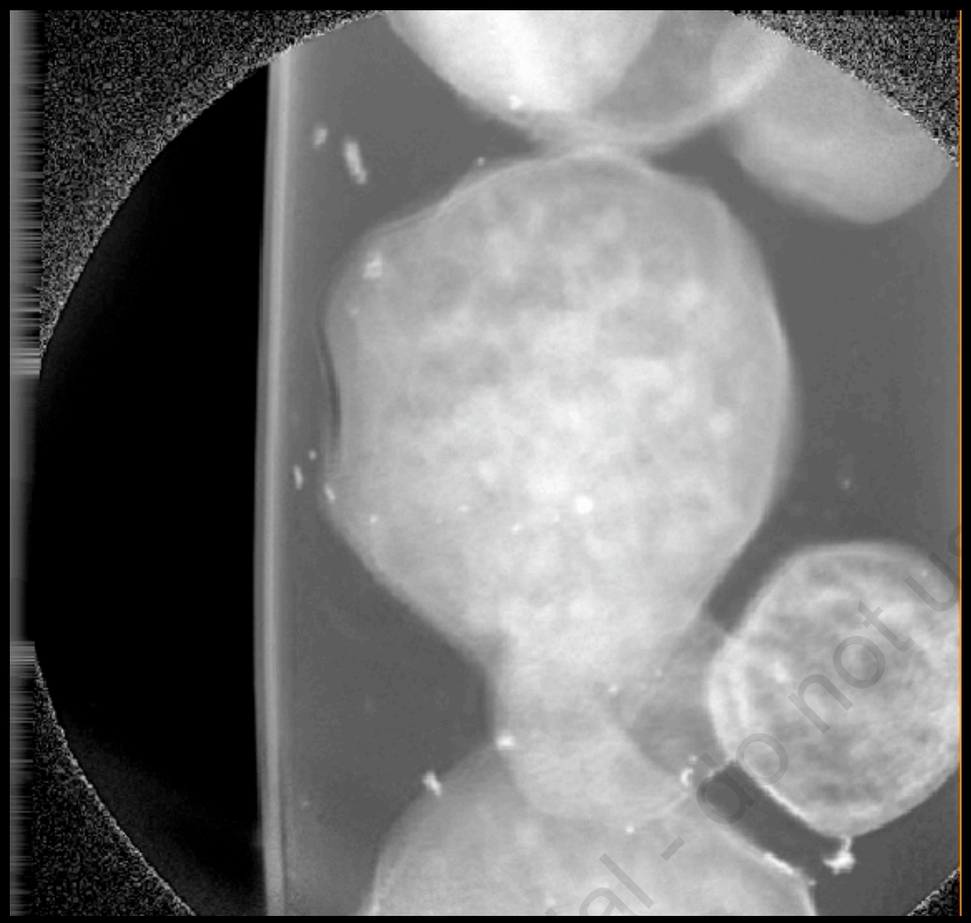
Cryo X-ray Tomography





Saccharomyces cerevisiae

45 images collected at 4-degree intervals



Projection images

(60 nm gold balls as fiducial markers)

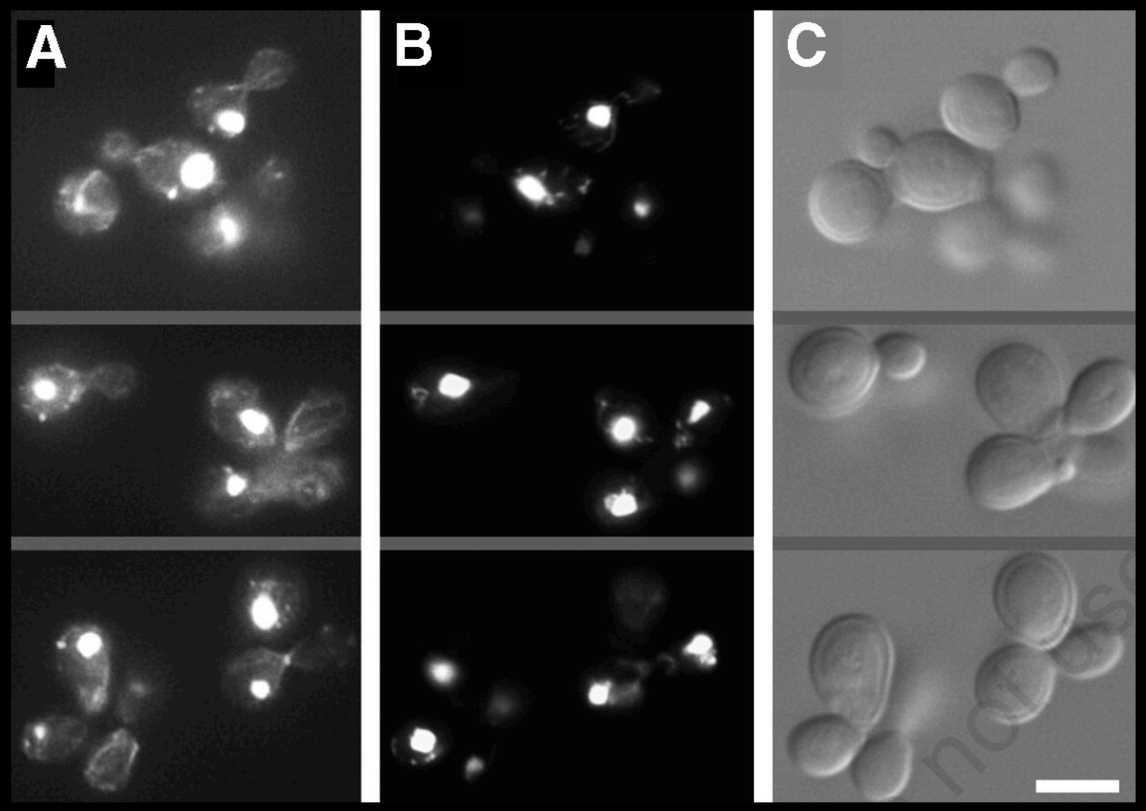
Sections through the reconstructed data

Yeast bud = 3 microns diameter

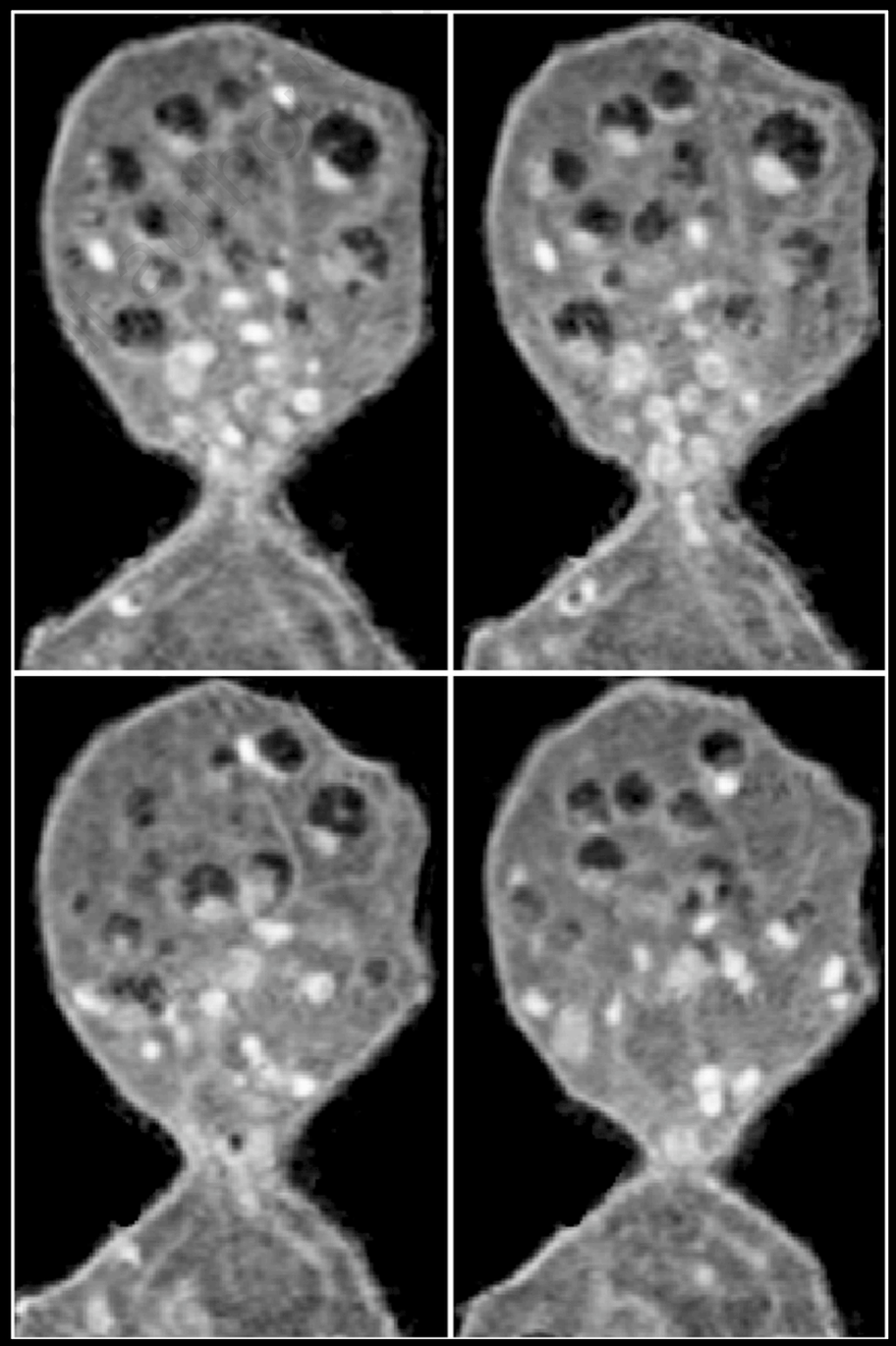
C.A. Larabell & M.A. Le Gros (2004). Molecular Biology of the Cell, 15(3), 956-962

Saccharomyces cerevisiae

Light Microscopy



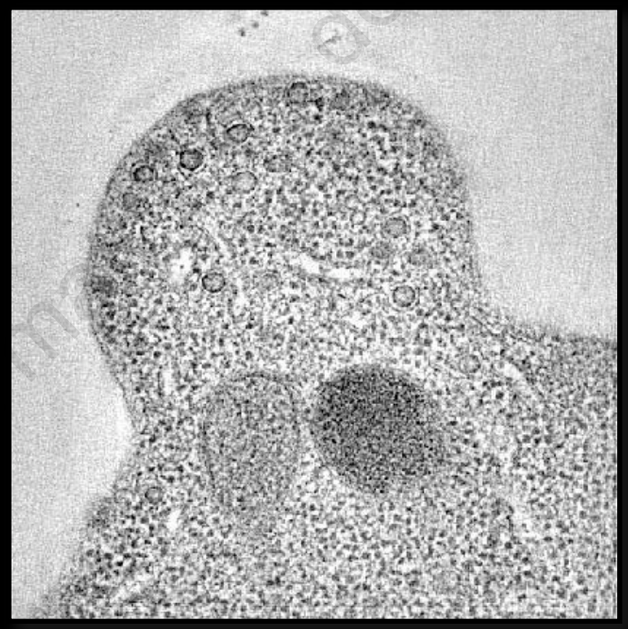
X-ray Tomography



Yeast bud = 3 microns diameter

Electron Tomography

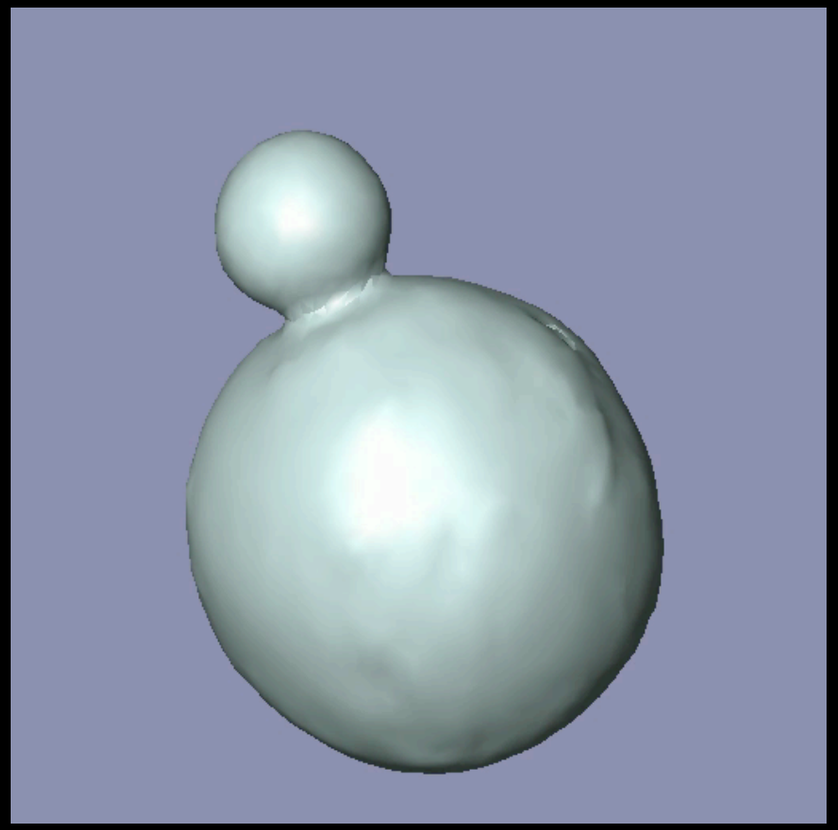
250 nm thick section



O'Toole et al., Methods in Enzymology 2002

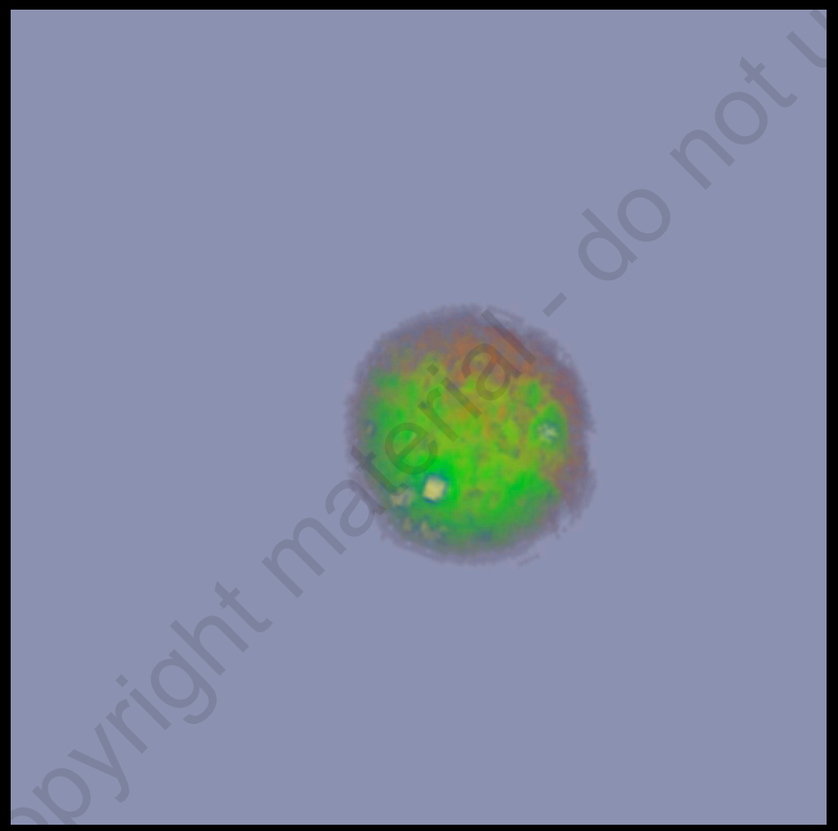
Saccharomyces cerevisiae

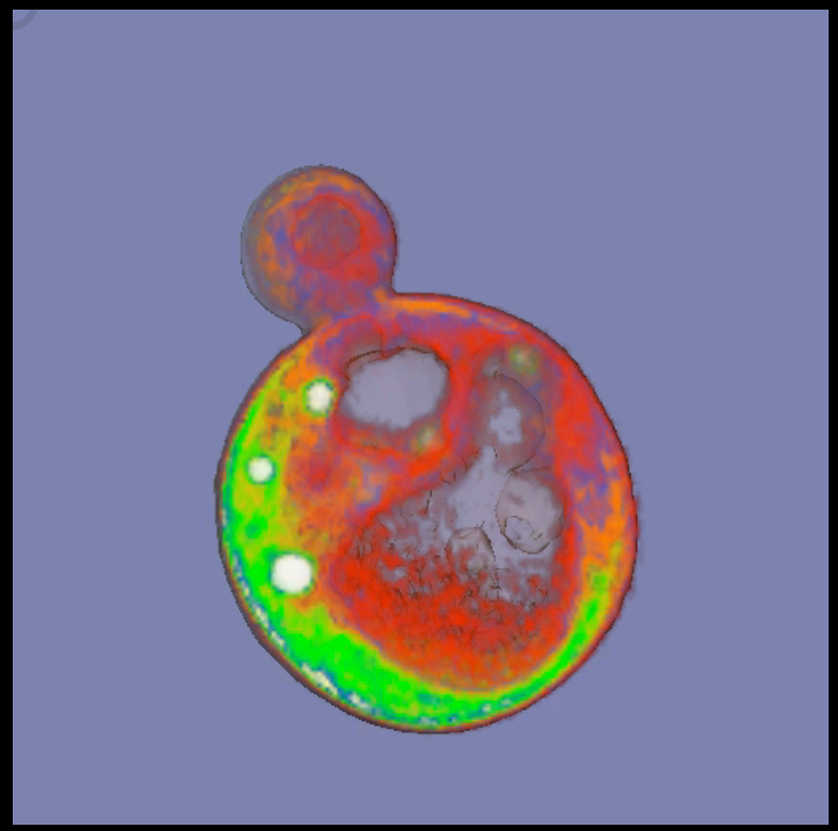
Opaque surface



Transparent surface

Volume rendered, color coded using absorption coefficient



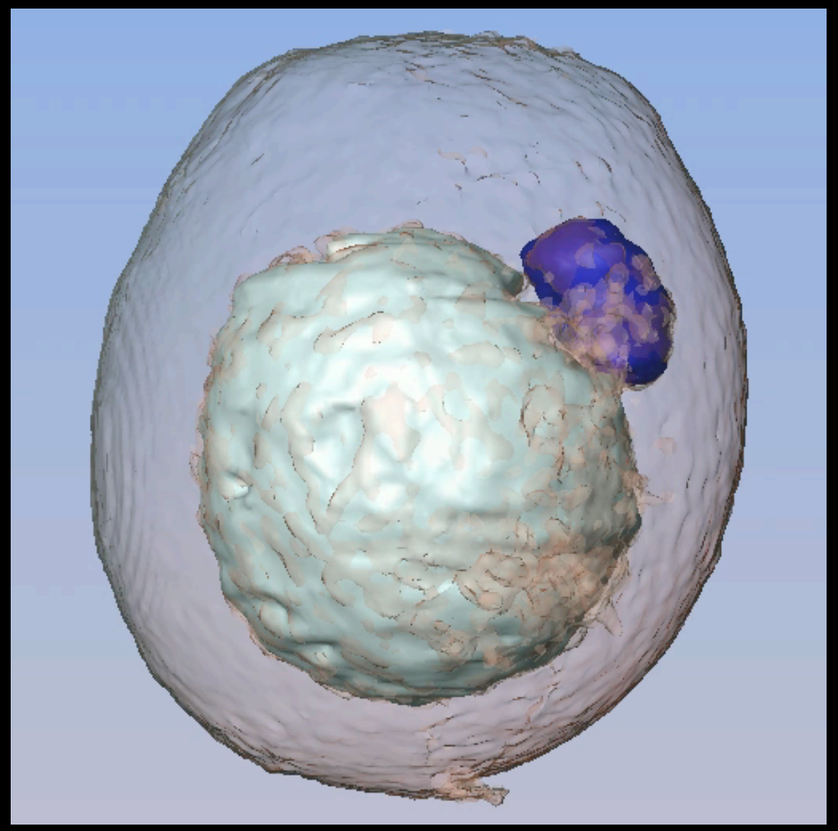


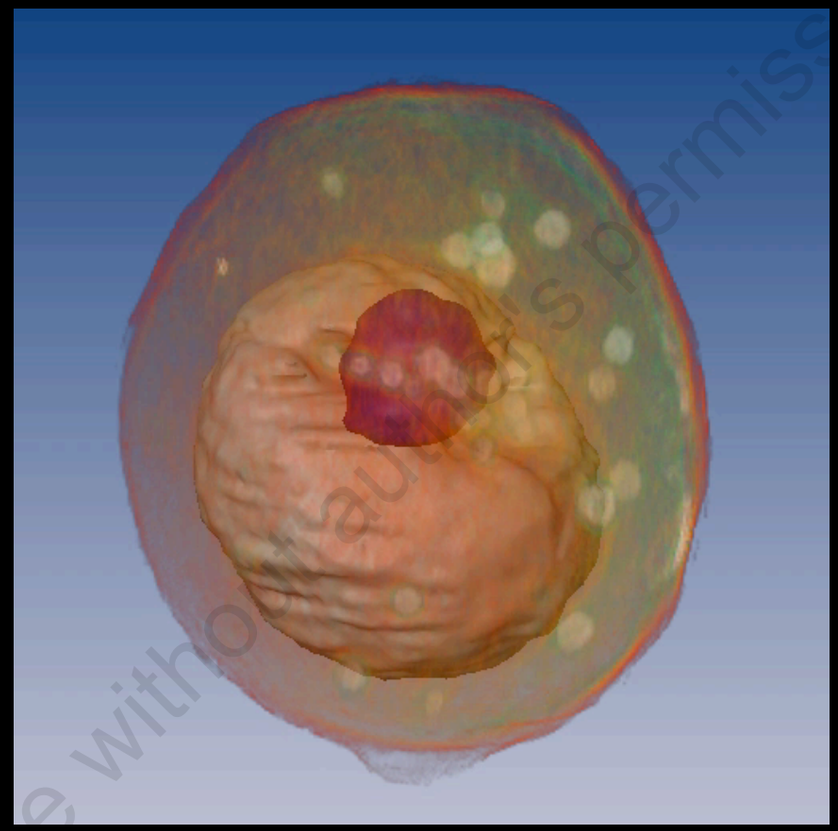
Absorption coefficient superimposed on transparent surface view

C.A. Larabell & M.A. Le Gros (2004). Molecular Biology of the Cell, 15(3), 956-962

Saccharomyces cerevisiae

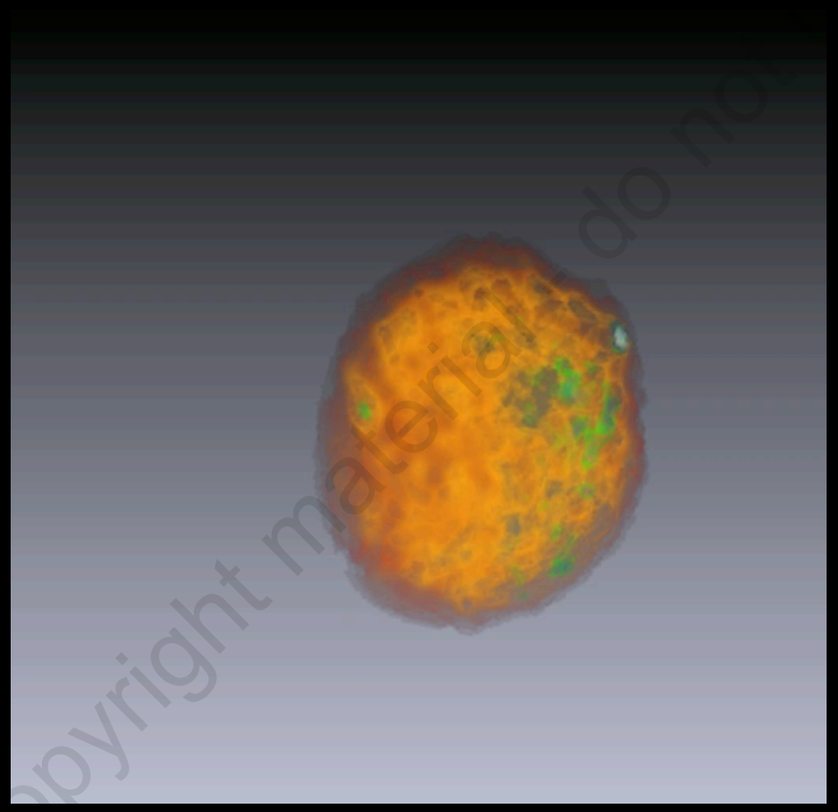
Translucent outer surface, opaque surfaces show internal organelles; blue = nucleus

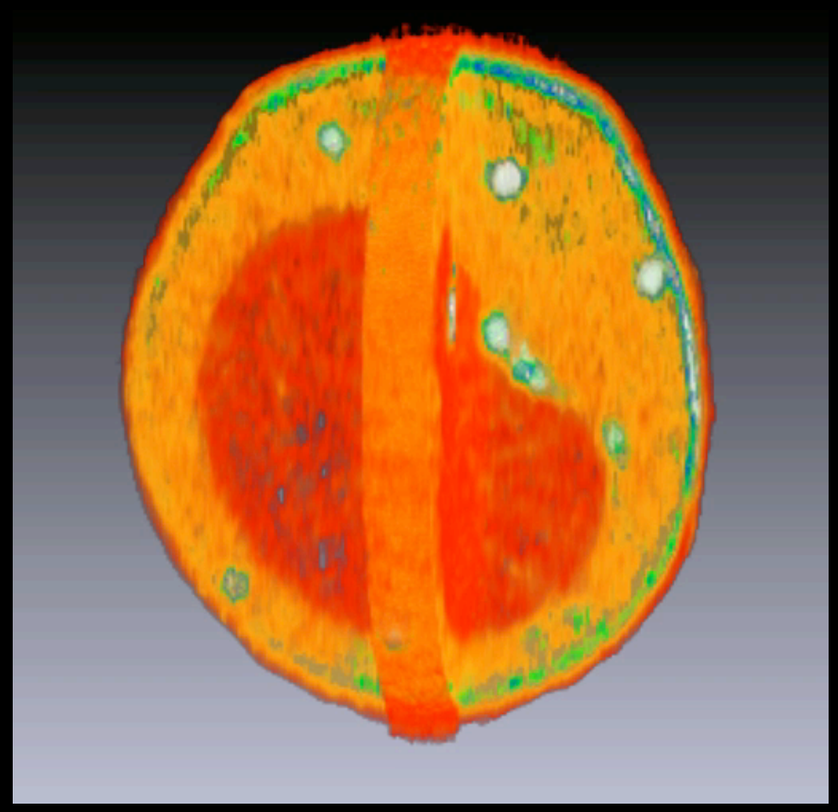




Volume rendered; Translucent outer surface; Opaque surfaces show internal organelles

Volume rendered; Color-coded using x-ray absorption coefficient

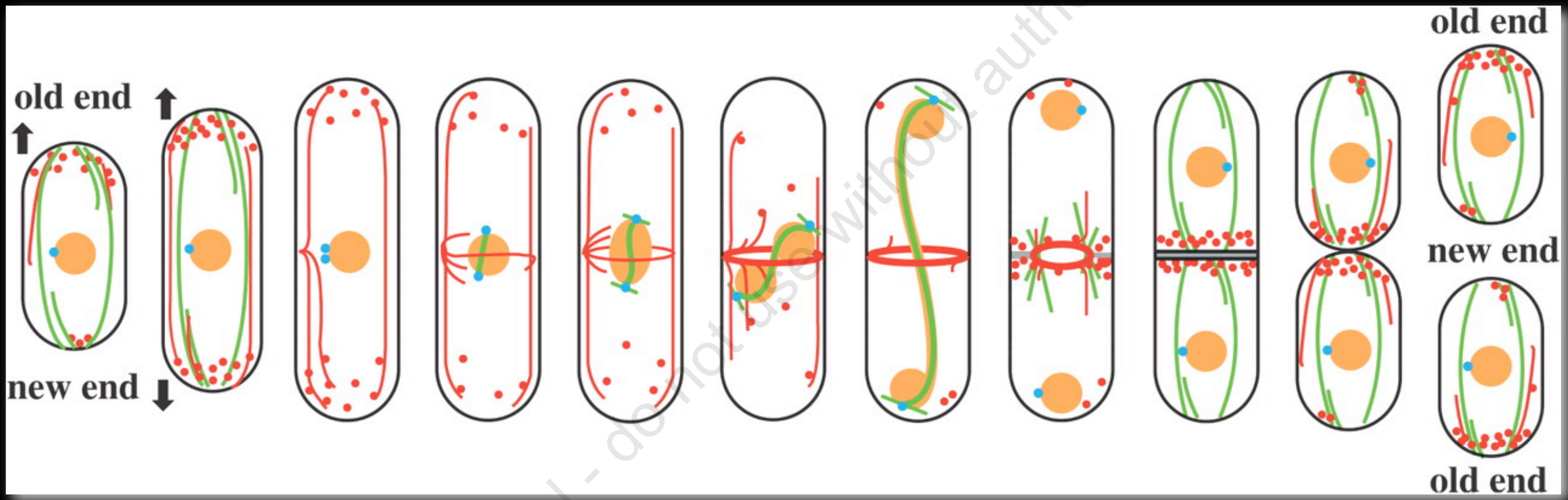




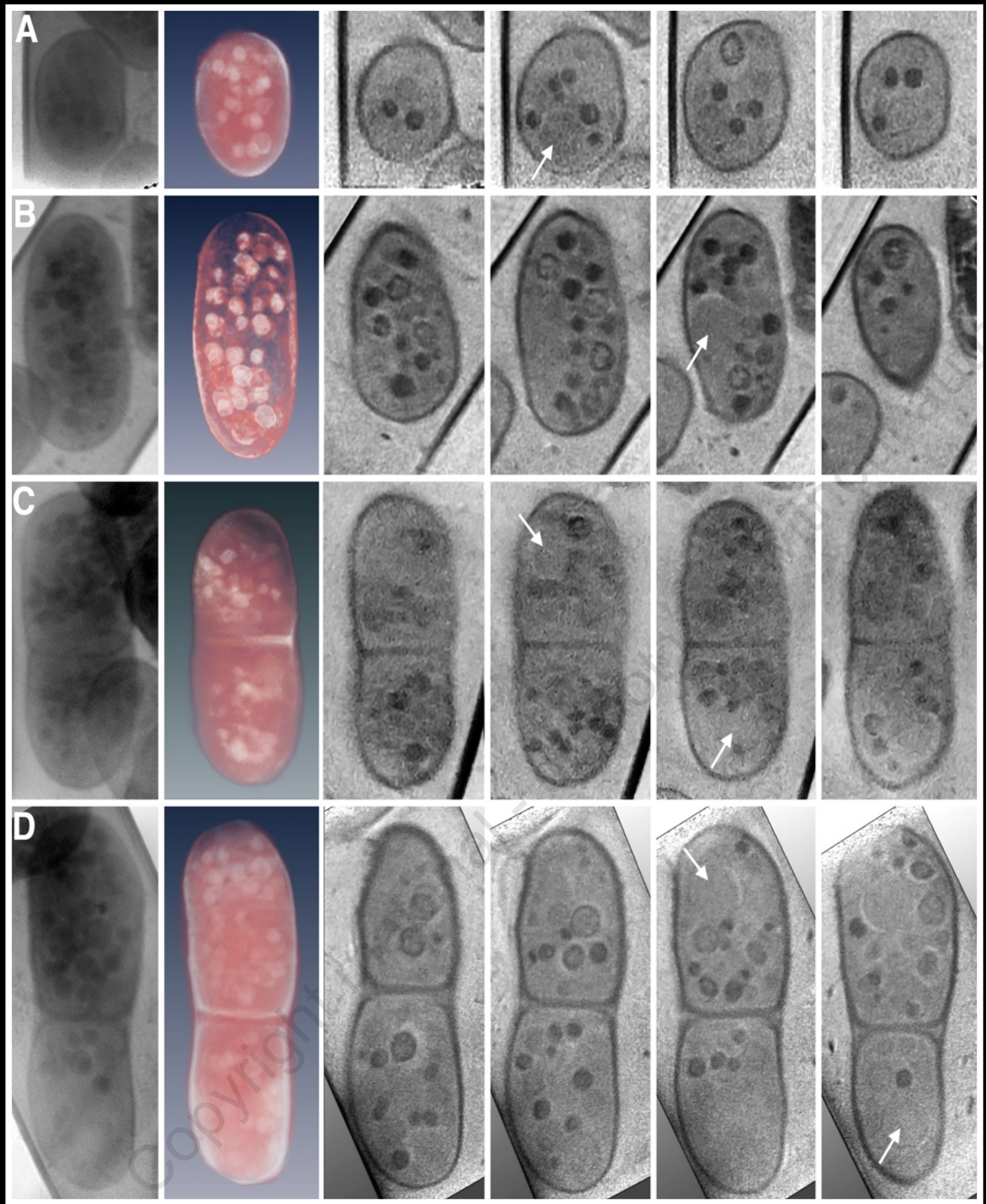
Volume rendered; Color-coded using x-ray absorption coefficient

C.A. Larabell & M.A. Le Gros (2004). Molecular Biology of the Cell, 15(3), 956-962

Schizosaccharomyces pombe



Osumi et al. (2006) Journal of Electron Microscopy 55(2), 75-88



New cell

Adult cell

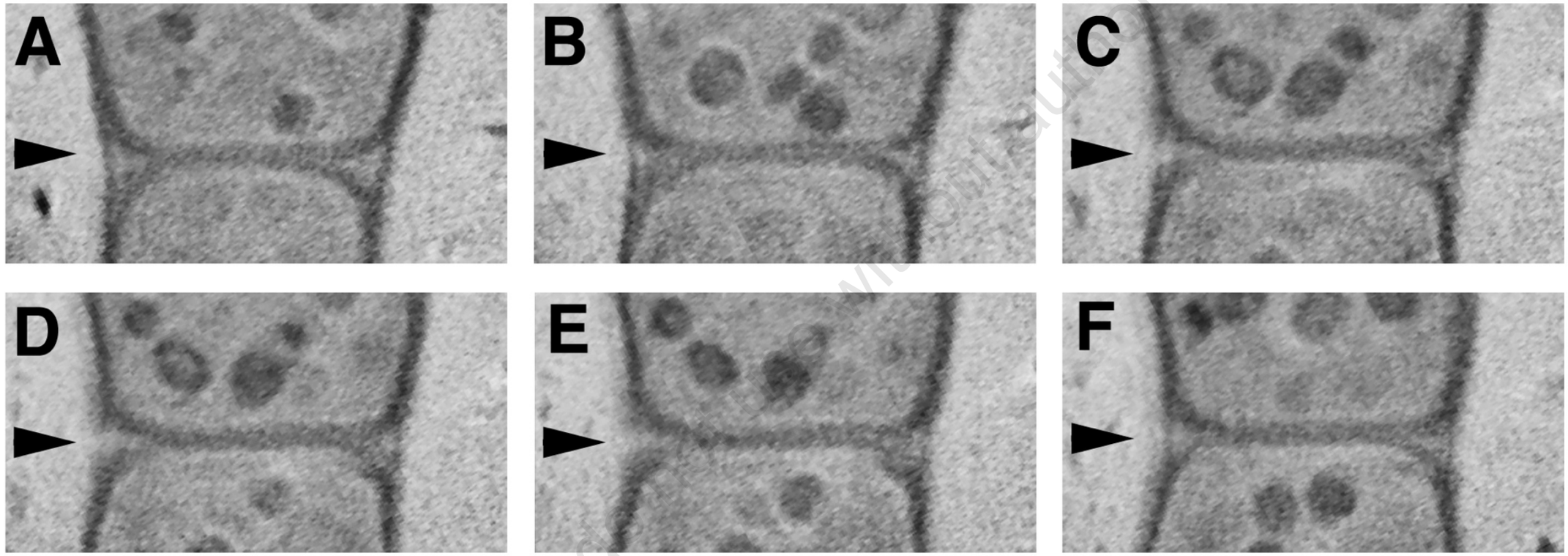
Dividing cell
(early)

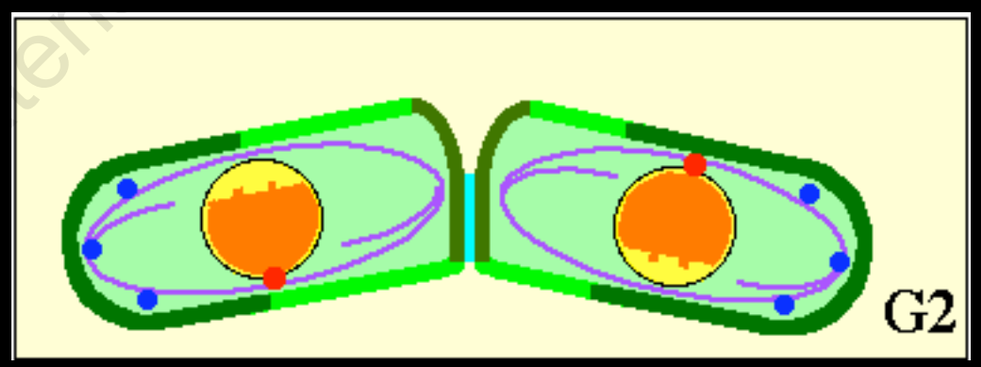
Dividing cell
(late)

W. Gu, L. D. Etkin, M. A. Le Gros, and C. A. Larabell. (2007) Differentiation. In press

Schizosaccharomyces pombe

Cell wall dissolution





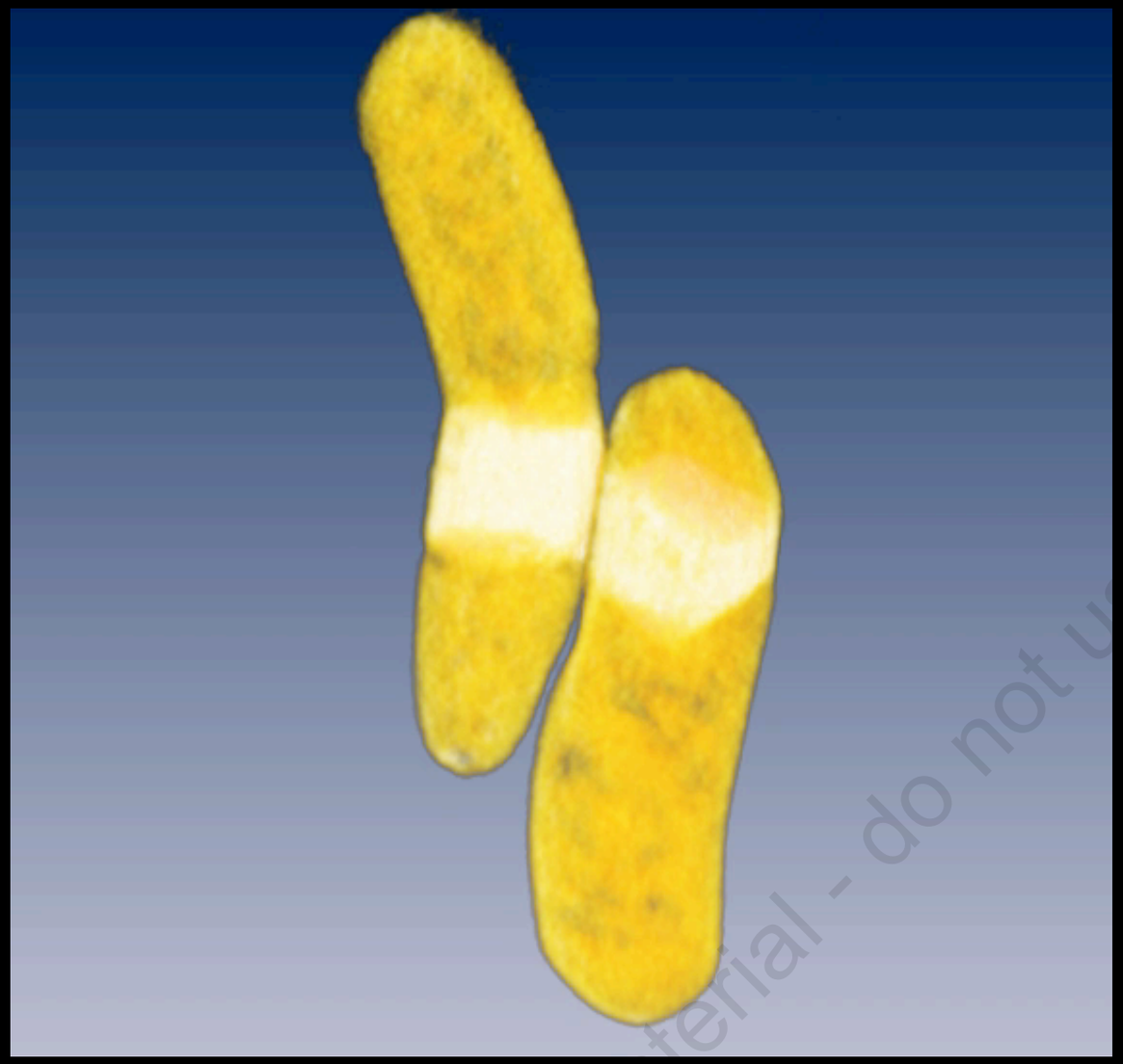
W. Gu, L. D. Etkin, M.A. Le Gros, and C.A. Larabell. (2007) Differentiation. In press

Microbial Communities

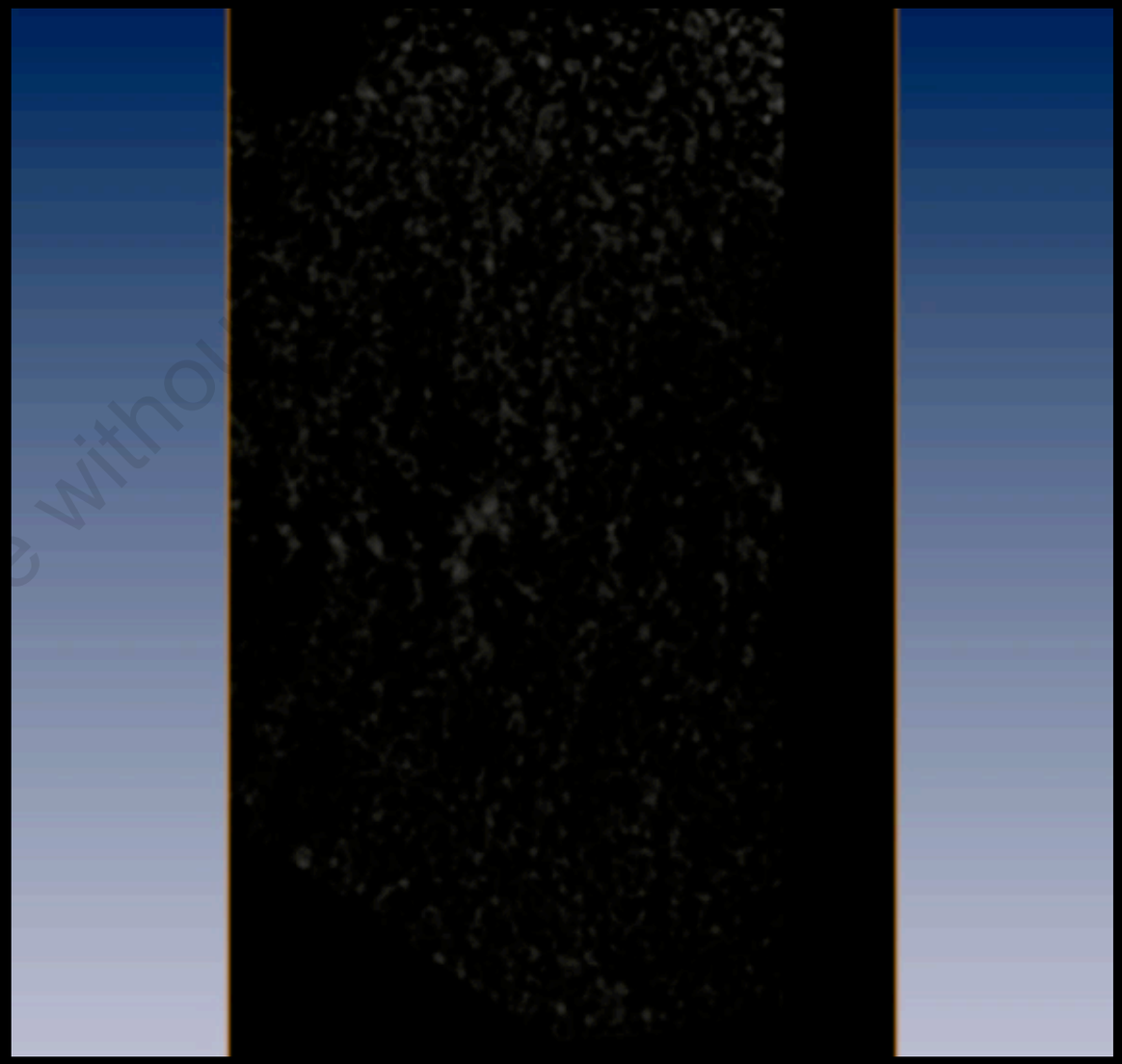
Caulobacter crescentus

D.Y. Parkinson, M.A. Le Gros, and C.A. Larabell

E. coli



Volume rendered data set

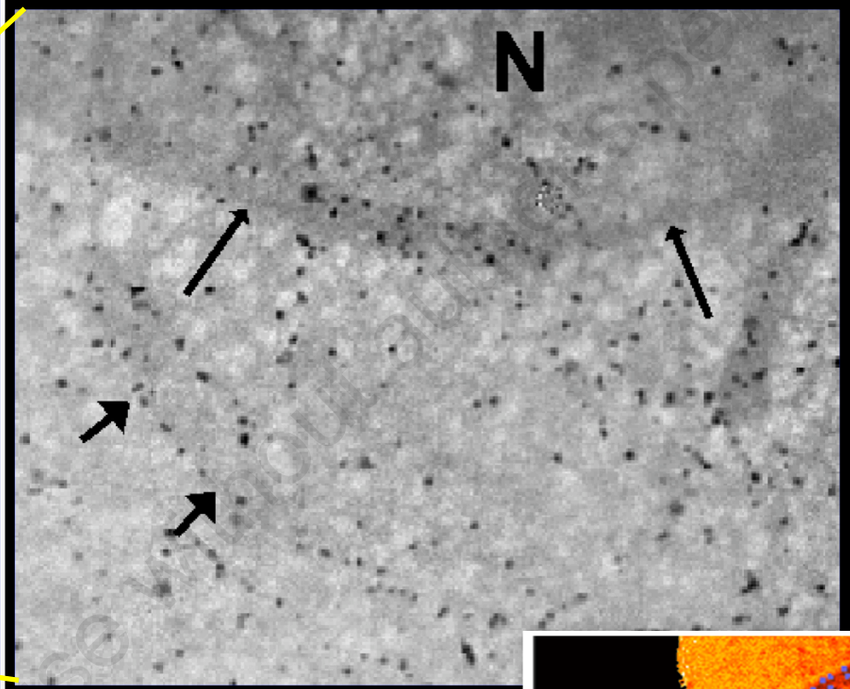


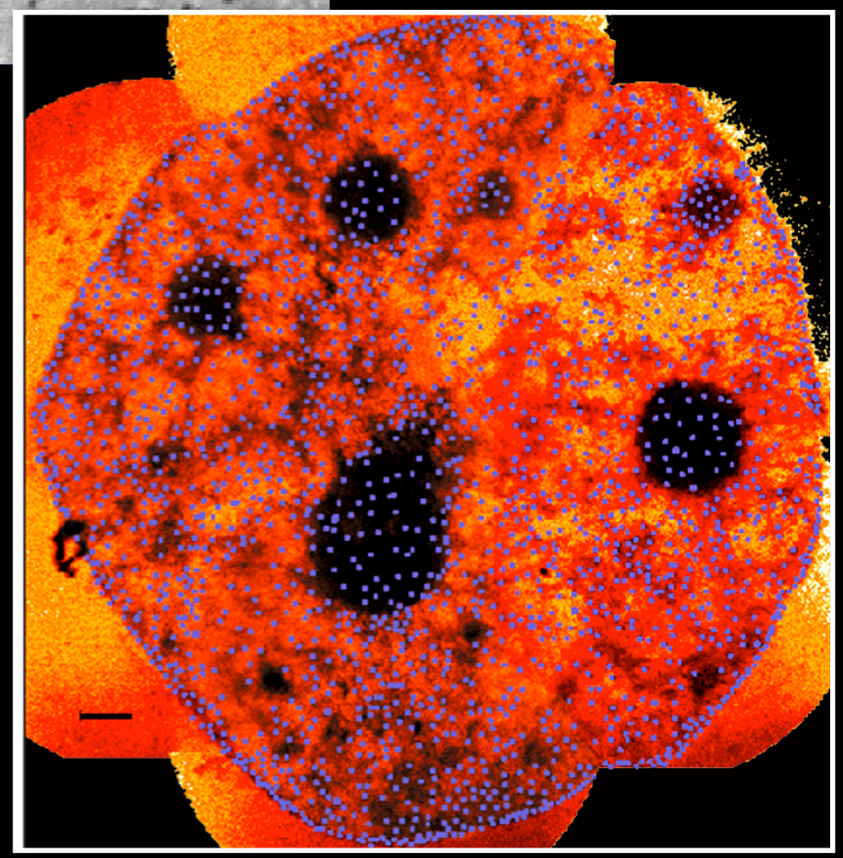
Computer sectioning through the reconstructed data

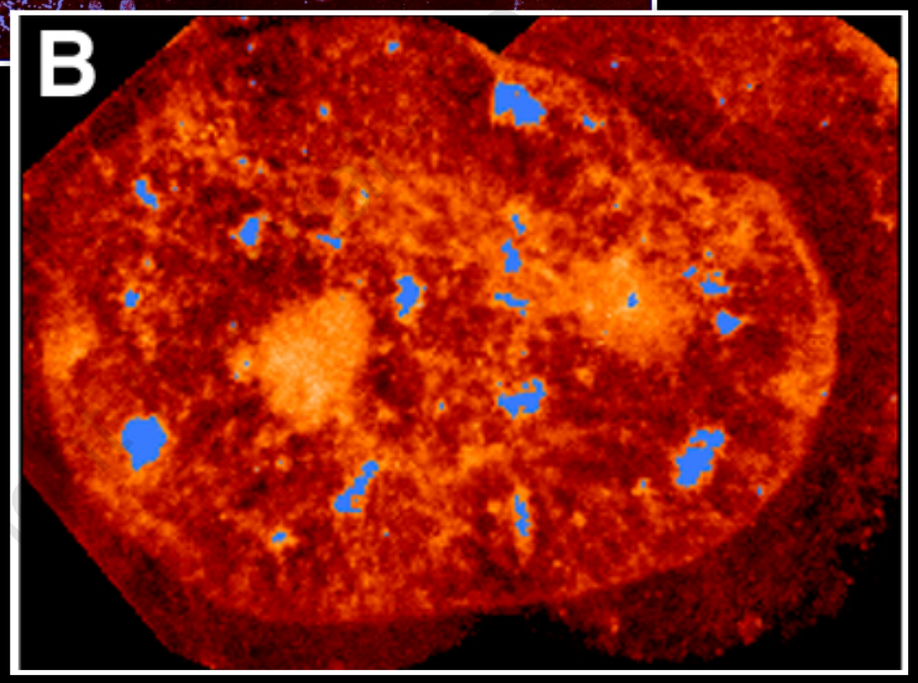
2 microns long
0.5 um diameter

Microtubules

Immunolocalization with 1.4 nm gold particles, enhanced with silver or gold







Splicing Factors

Nuclear Pore Complex

Meyer-Ilse, et al. (2001). J. Microscopy. 201, 395-403.

Achieving Better Resoloution

- Automated cryo-rotation stage enables collection of hundreds of images - will yield better resolution
- Better resolution zone plates

nature Vol 435|30 June 2005|doi:10.1038/nature03719

LETTERS

Soft X-ray microscopy at a spatial resolution better than 15 nm

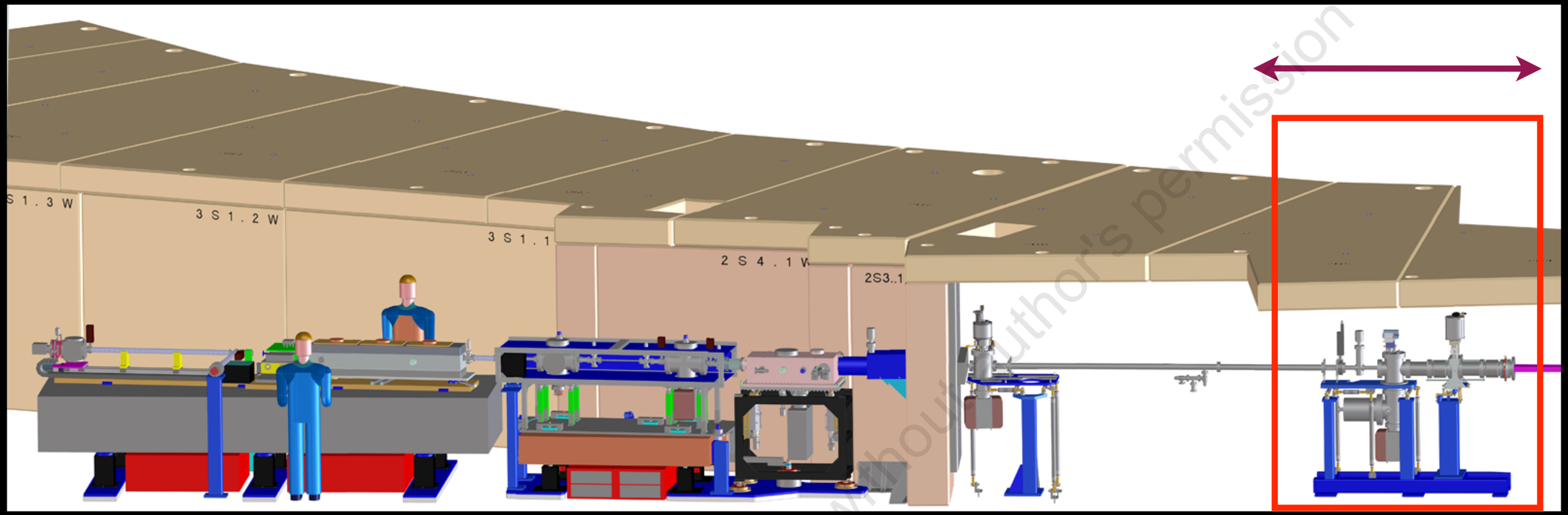
Weilun Chao[1,2], Bruce D. Harteneck[1], J. Alexander Liddle[1], Erik H. Anderson[1] & David T. Attwood[1,2]

Imaging Cells with Soft X-ray Microscopy

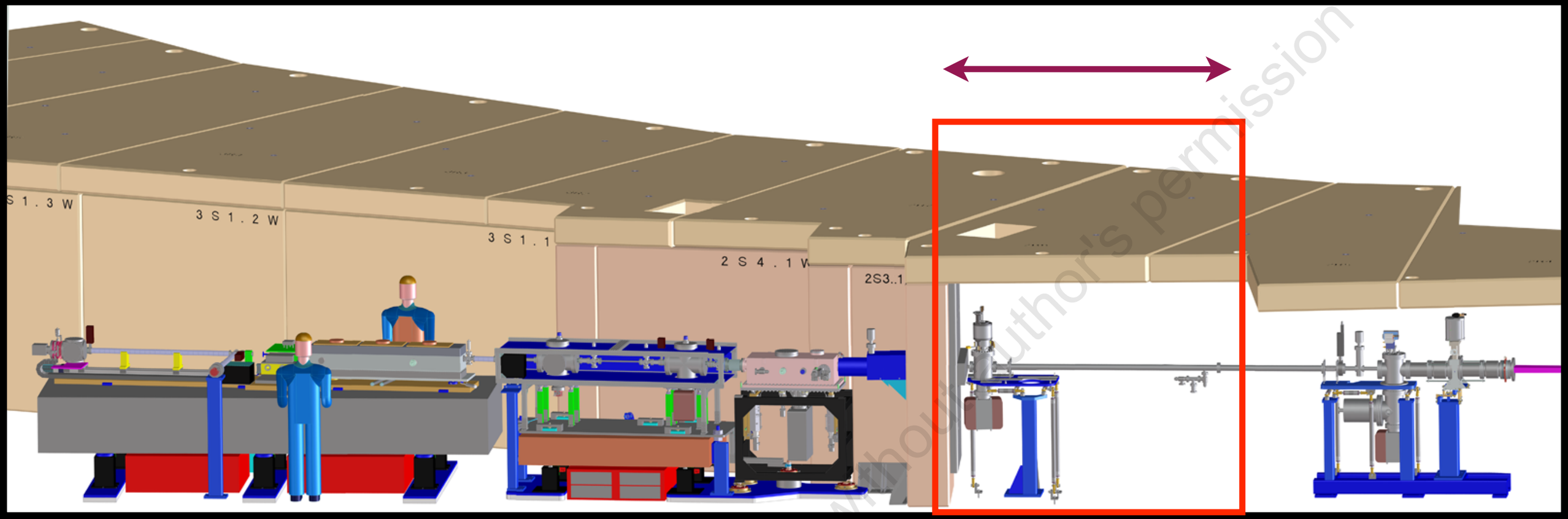
- Whole cells up to 10 µm thick
- Fully hydrated specimens
- Inherent contrast of organic material
- Quantitative - use linear absorption coefficient
- Localization of proteins and multi-protein complexes
- Better than 50 nm resolution - isotropic
- Fast - collect tomographic data set in < 5 min

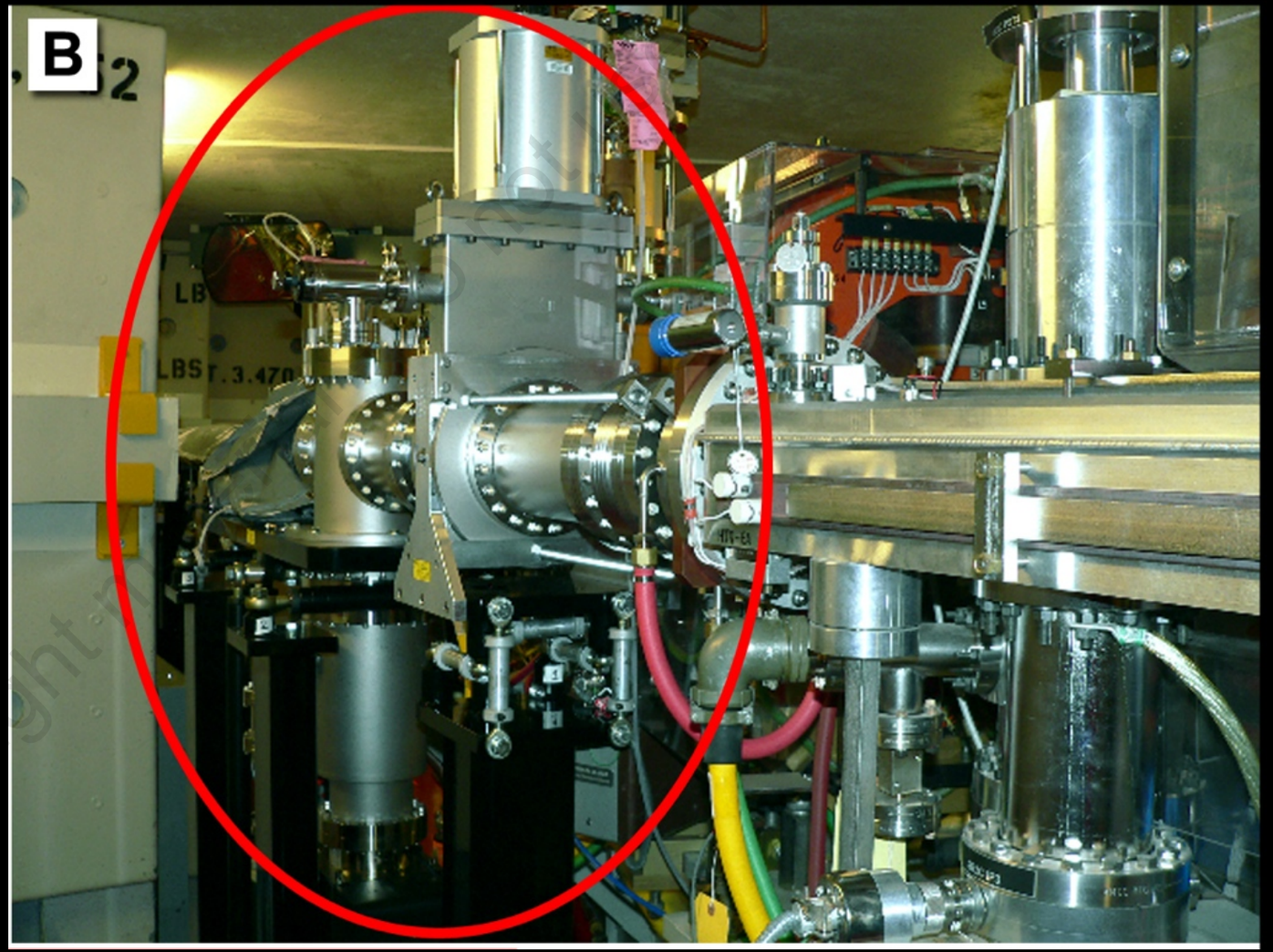
National Center for X-ray Tomography

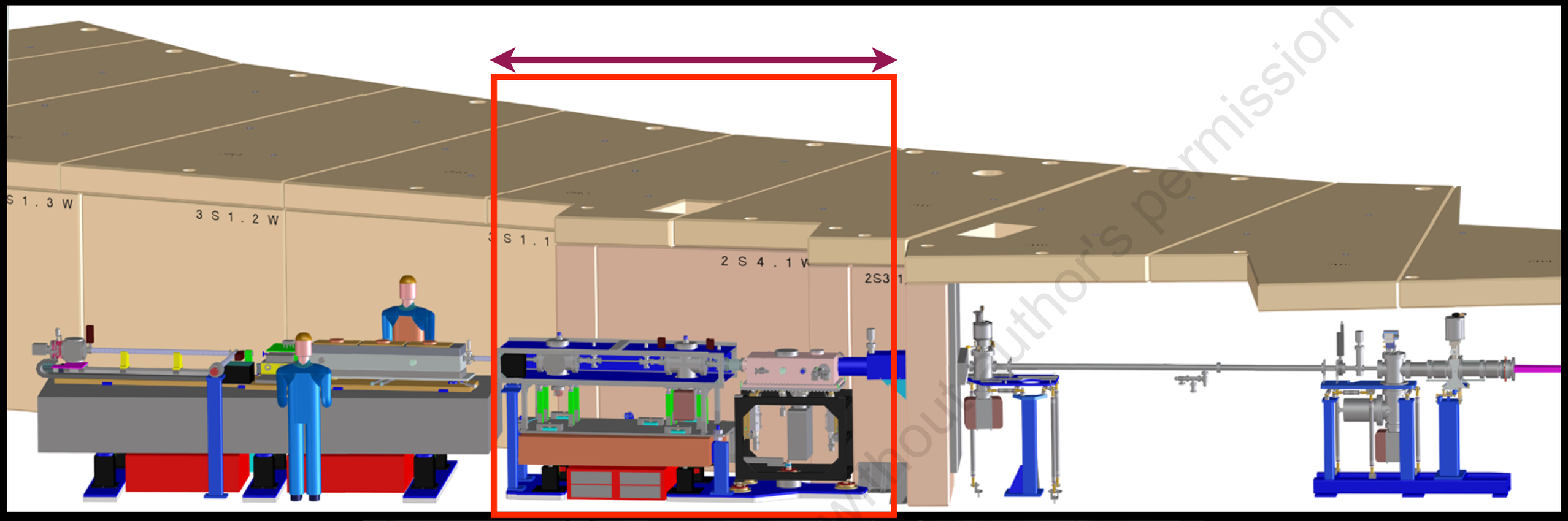
- Funded by NCRR/NIH and OBER/DOE
  - National Center for Research Resources, National Institutes of Health
  - Office of Biological and Environmental Research, Department of Energy
- Construct new beamline and microscope: XM-2
- Development of x-ray tomography of biological cells
- Make available to scientific community

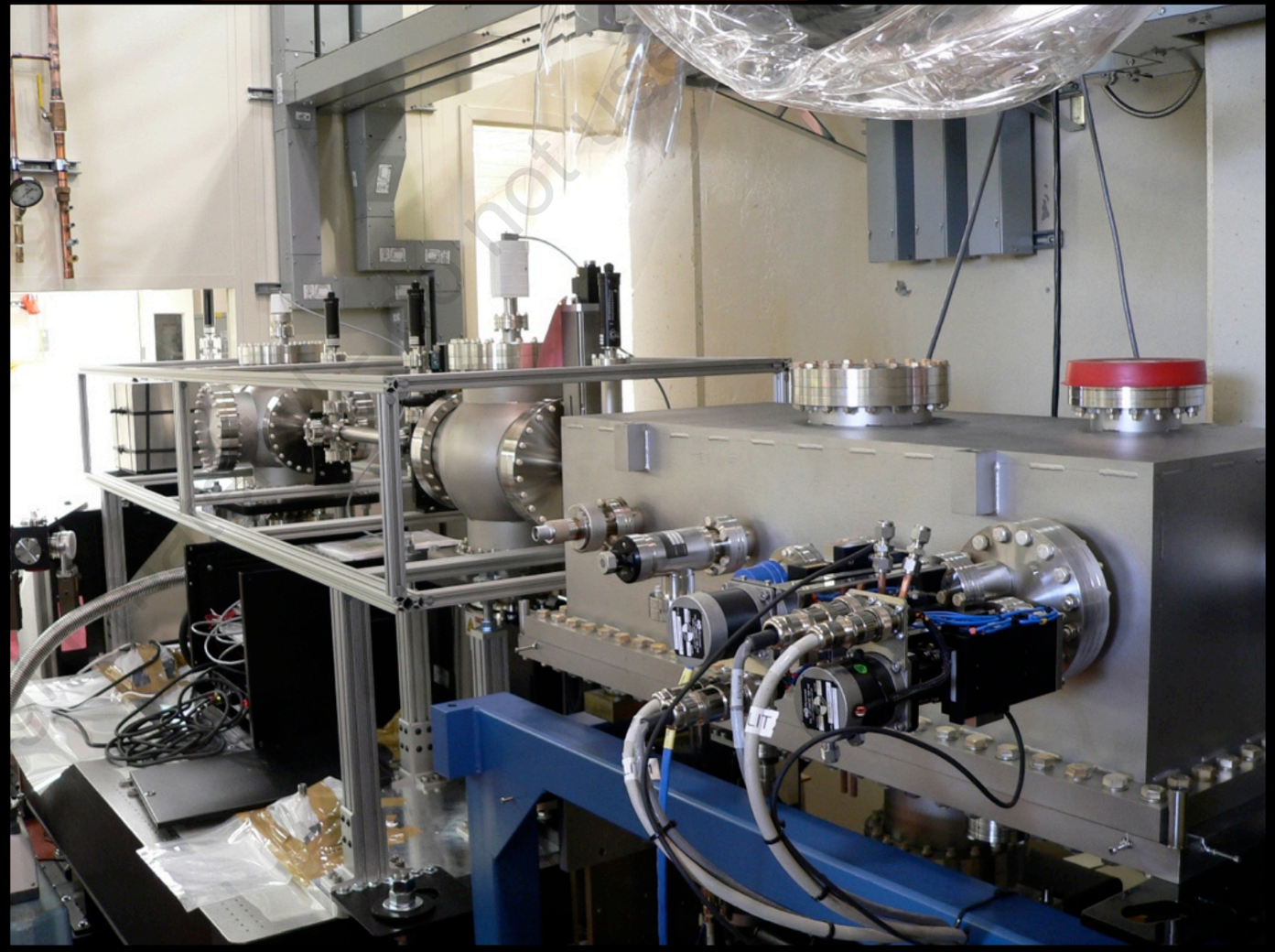

S 1 . 3 W
3 S 1 . 2 W
3 S 1 . 1
2 S 4 . 1 W
2S3..1

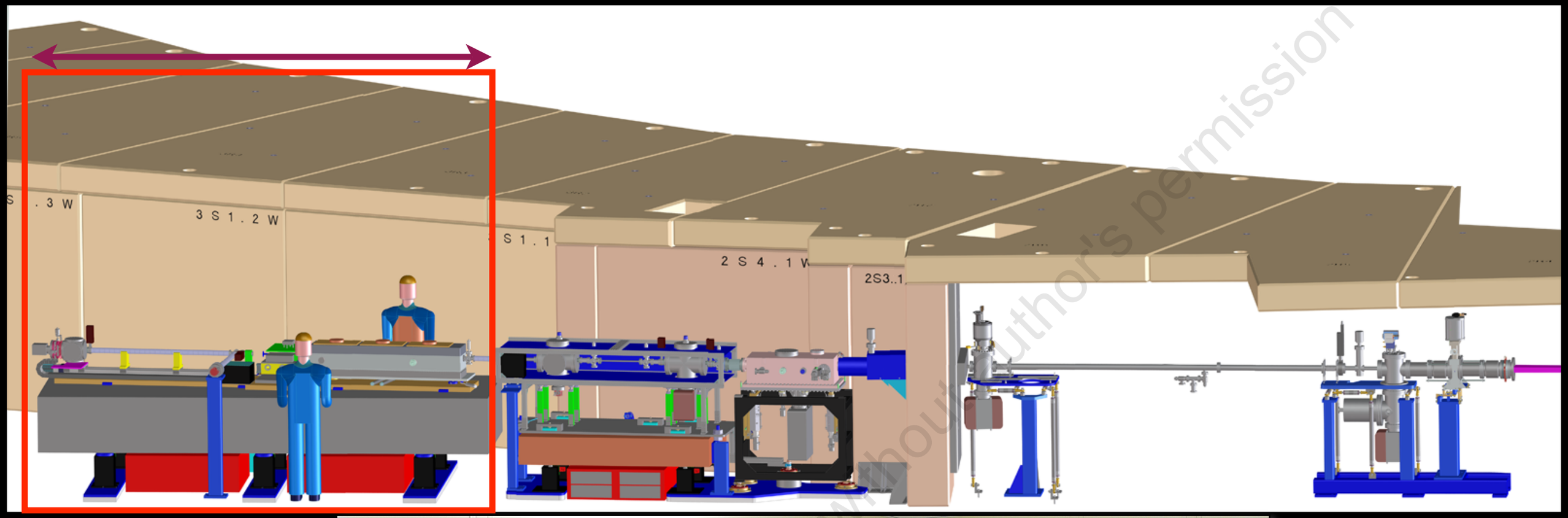

C
11.01.2005 15:27


3 S 1 . 3 W
3 S 1 . 2 W
3 S 1 . 1
2 S 4 . 1 W
2S3..1


B


S 1 . 3 W
3 S 1 . 2 W
3 S 1 . 1
2 S 4 . 1 W
2S3 1





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National Center for Research Resources, National Institutes of Health
National Institute of General Medical Sciences, National Institutes of Health