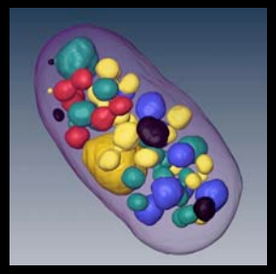
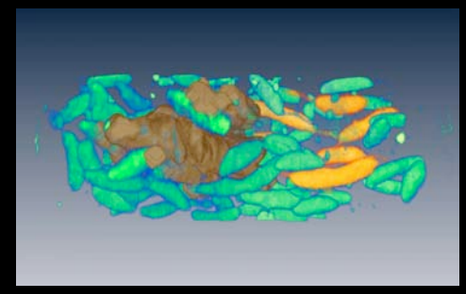




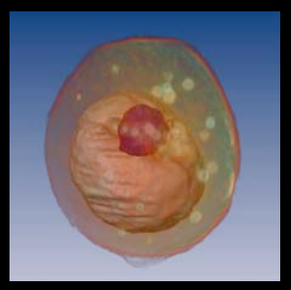
National Center for X-ray Tomography

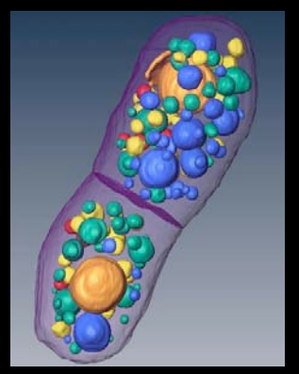
a Biomedical Technology Resource Center

University of California, San Francisco
Lawrence Berkeley National Laboratory

National Center for Research Resources, NIH
Office of Biological and Environmental Sciences, DOE

Carolyn A. Larabell
http://ncxt.lbl.gov





Cell Biology

Cell Structure/Function in normal vs. disease states

- Gene Expression ⇒ cell structure/function
- Protein Location ⇒ cell structure/function
- Subcellular structures - number, organization, and changes during cell cycle; normal vs. disease
  - Nucleus, mitochondria, endoplasmic reticulum, etc.
- Apoptosis - cell structure changes
- Stem cells
- Drug trafficking
- Cell response to chemotherapeutic agents

Cell Biology

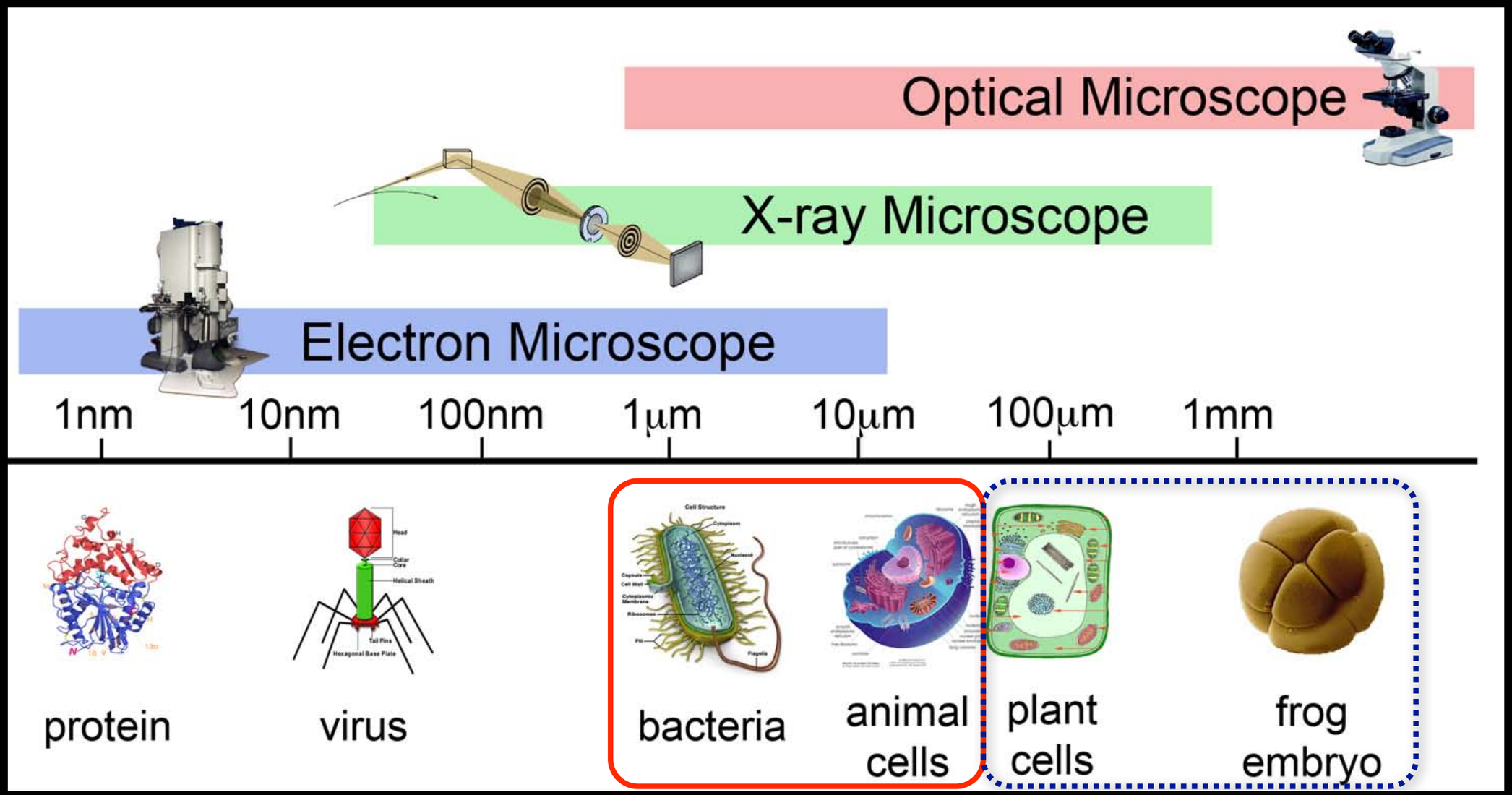
Imaging Technologies

- Light Microscopy (200-500 nm resolution)
  - dynamics
  - protein localizations (normal vs. disease)
  - examine whole cells
- Electron Microscopy (5-35 nm resolution)
  - structural changes (normal vs. disease)
  - protein localizations (normal vs. disease)
  - examine sections (50 nm thick)

X-ray Microscopy: Imaging at the Mesoscale

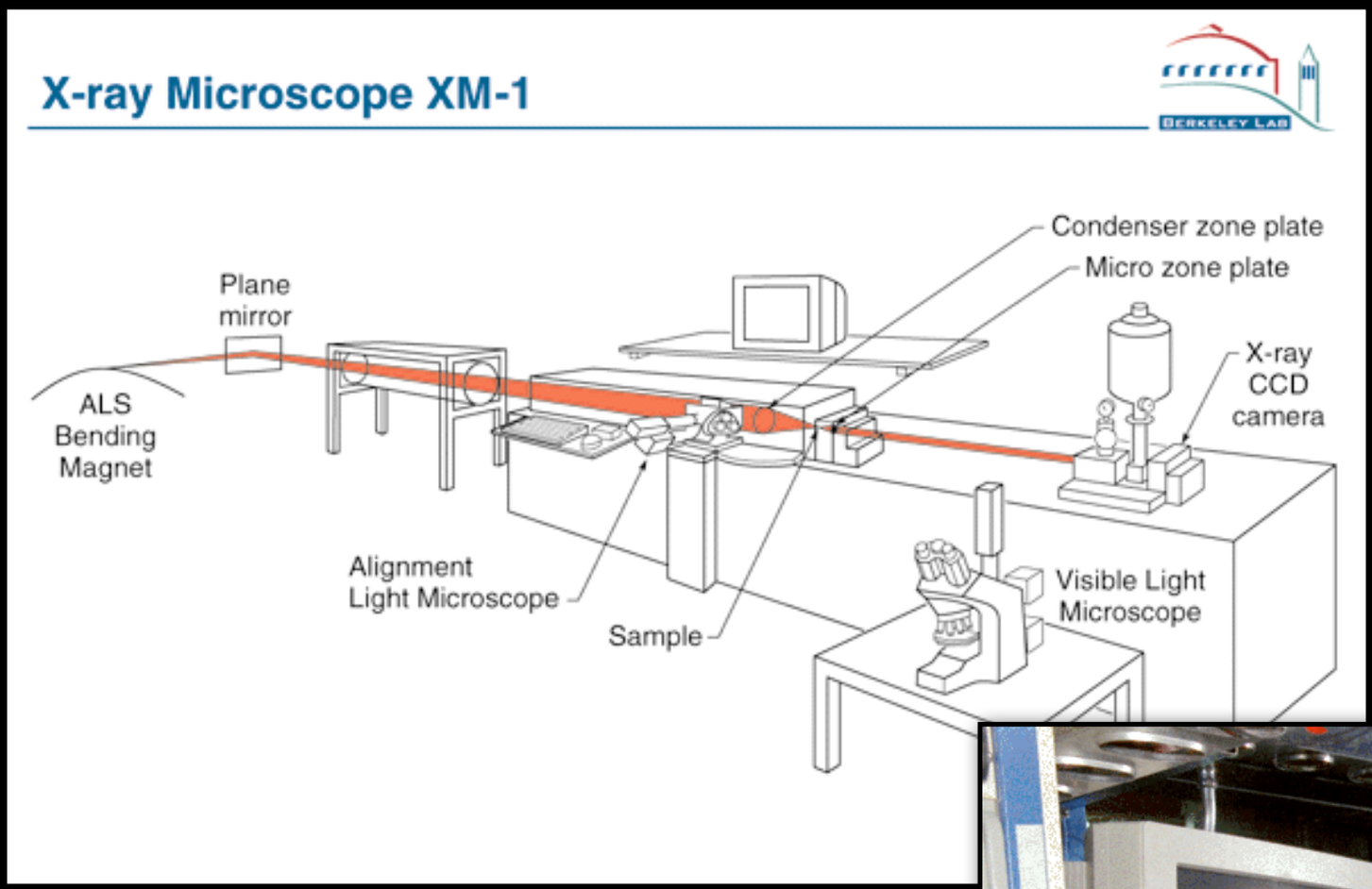
Structural Information from Whole Cells and Tissues

Bridging the gap between light and electron microscopy



Imaging Cells with Soft X-ray Microscopy

- Whole cells up to 15 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

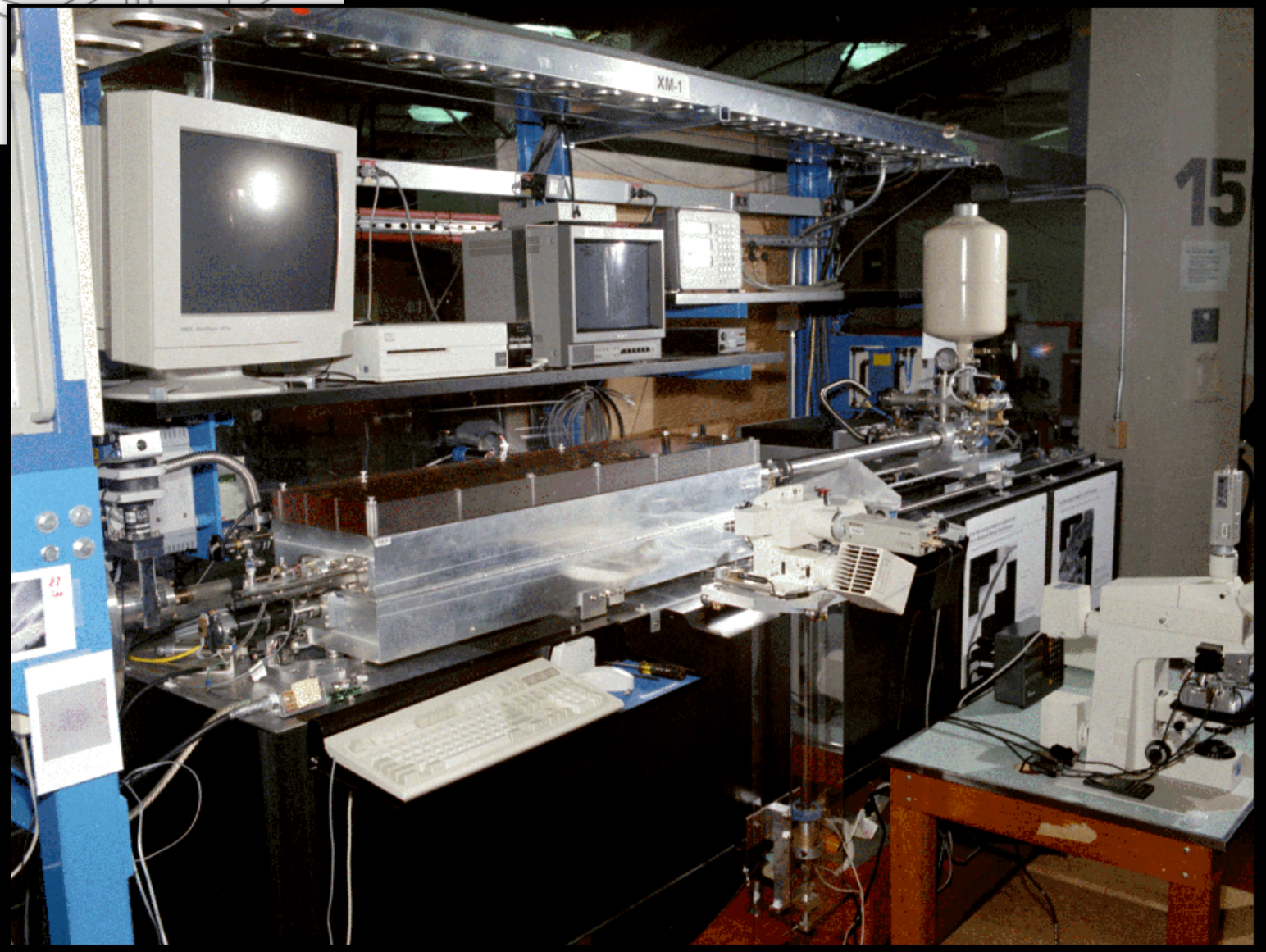


Soft X-ray Microscope (XM-1) at the ALS Lawrence Berkeley Lab

2.4 nm λ

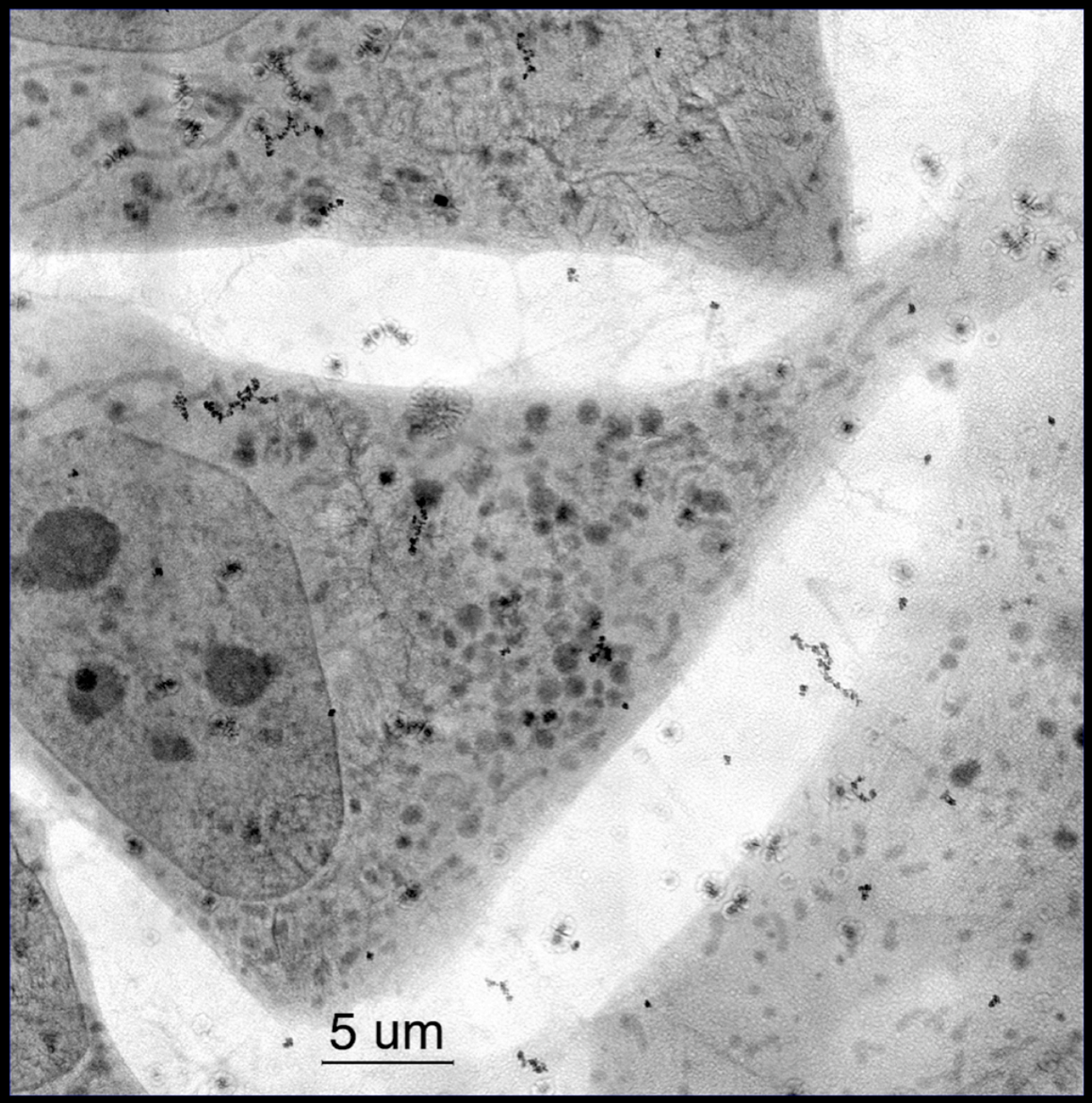
517 eV

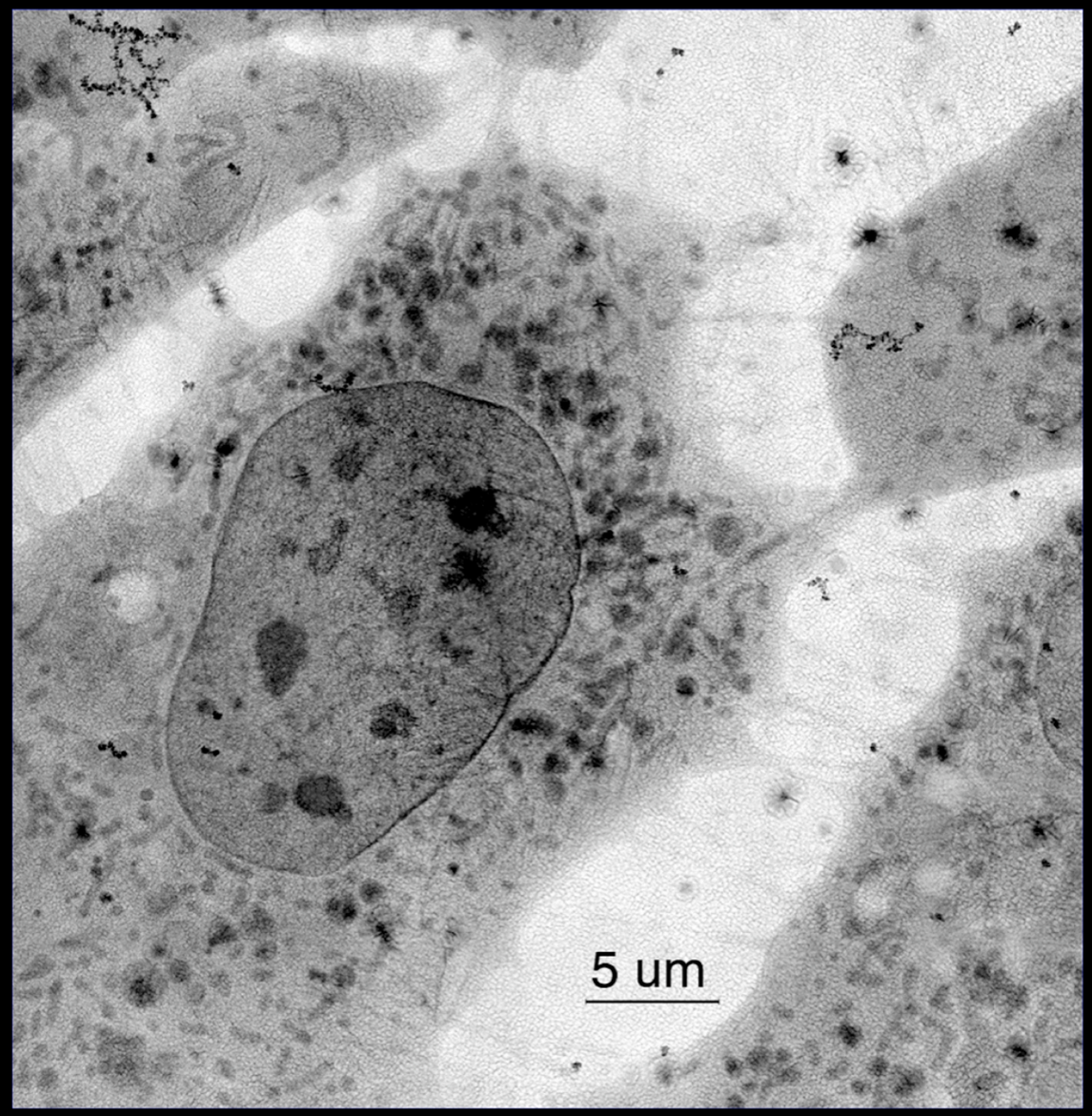
Center for X-ray Optics
David Attwood
Erik Anderson



Cryo X-ray Microscopy of NIH 3T3 Fibroblasts

No chemical fixatives or contrast enhancement reagents



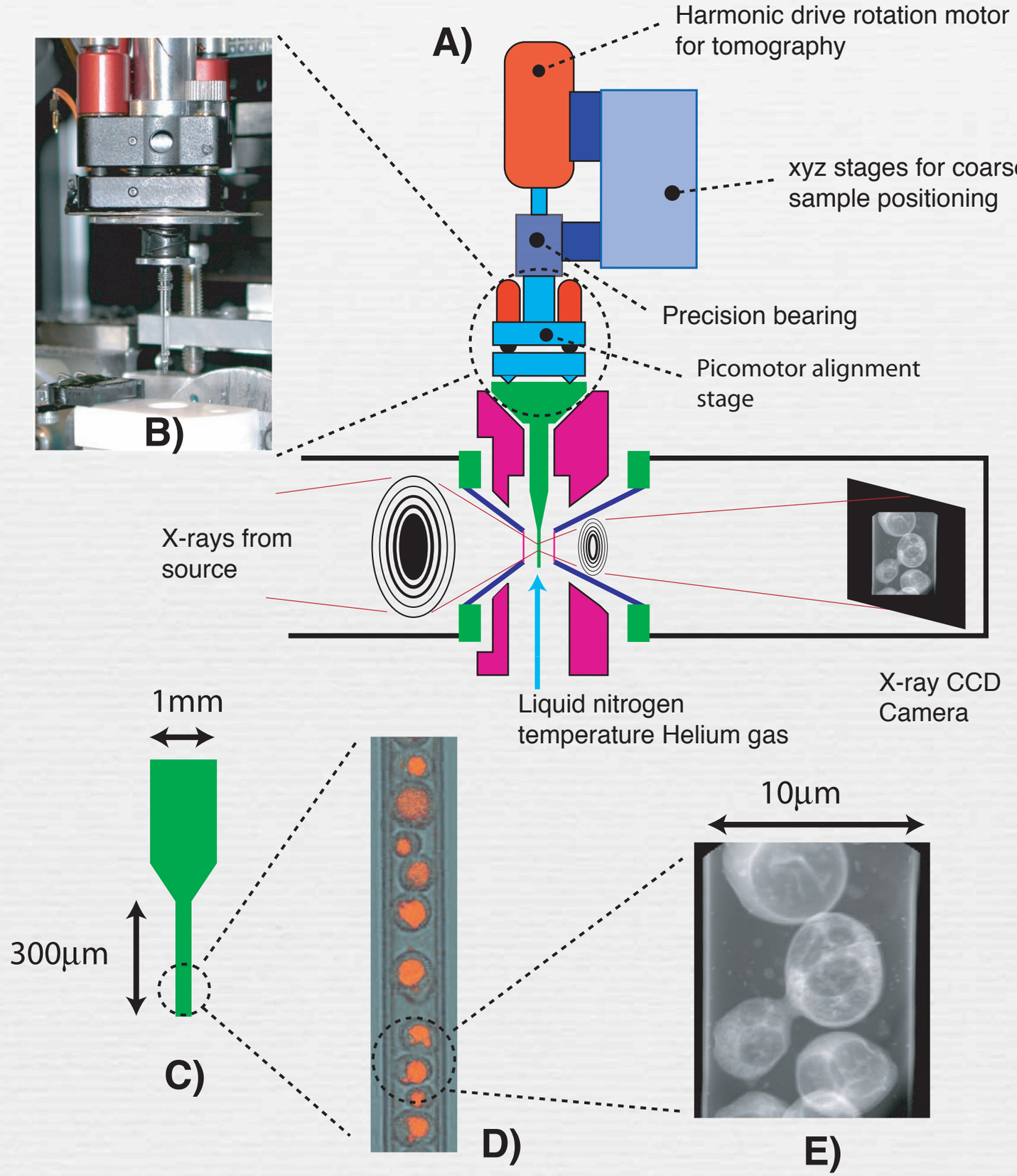


Projection images: tomography required to retrieve 3D information

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.

Cryo X-ray Tomography

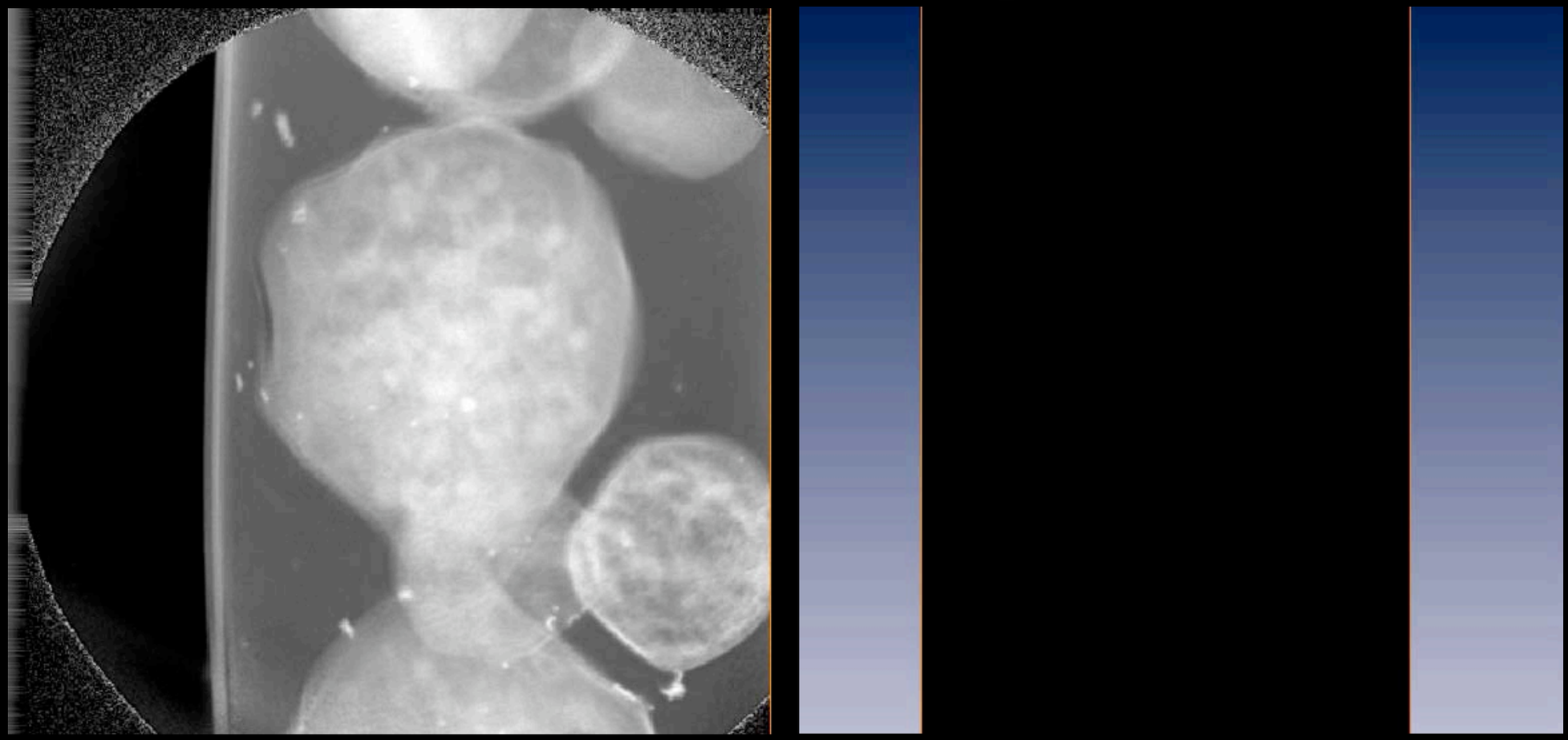
Specimen at atmospheric pressure



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

Saccharomyces cerevisiae

45 images collected at 4-degree intervals



Projection images

(60 nm gold balls as fiducial markers)

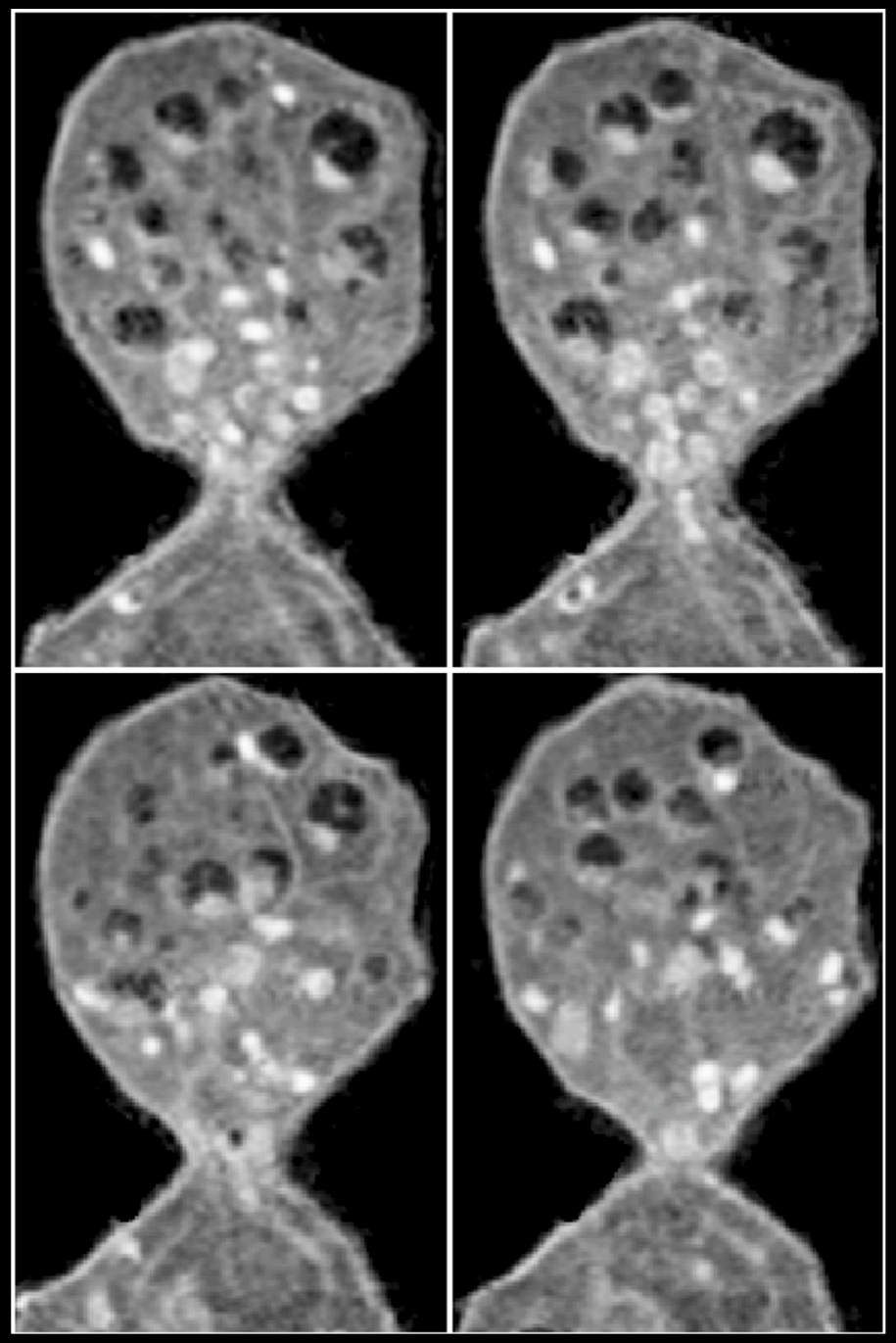
Sections through the reconstructed data

Yeast bud = 3 microns diameter

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

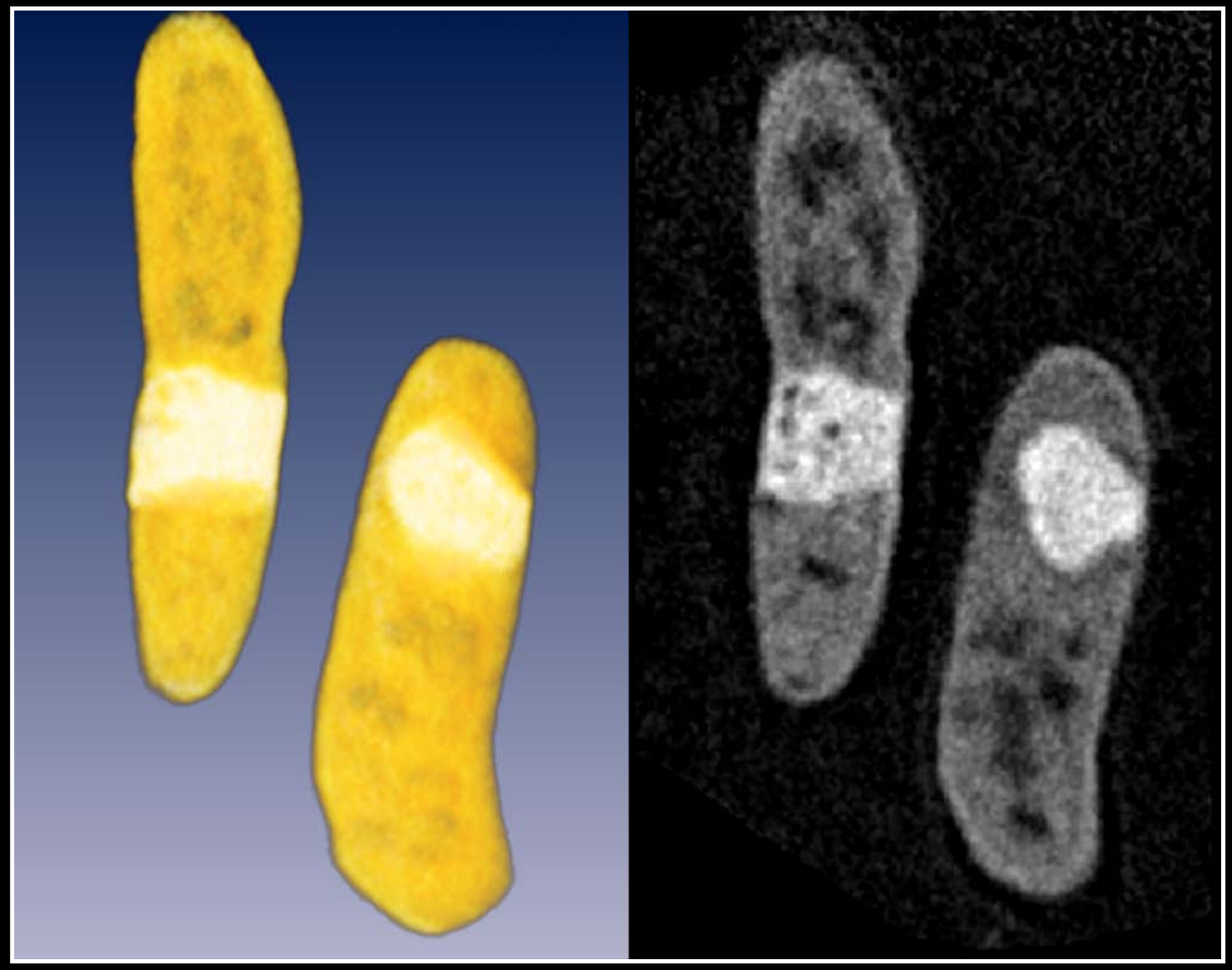
Saccharomyces cerevisiae

X-ray Tomography: Sections through reconstructed data



Yeast bud = 3 microns diameter

E. coli

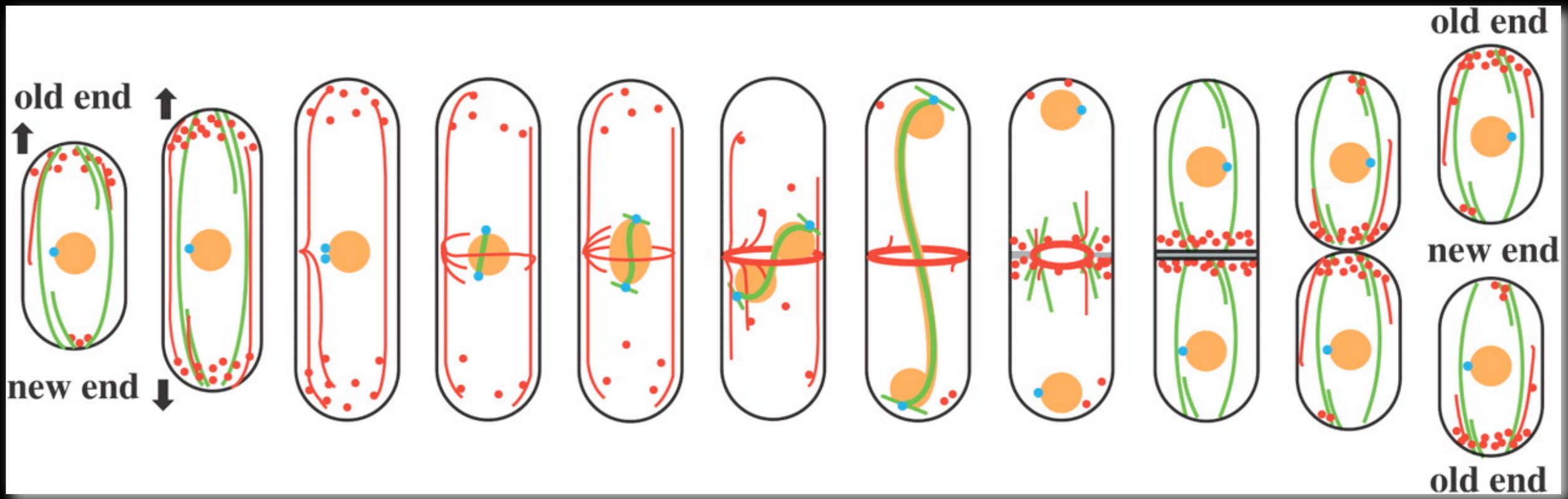


Volume rendered data set

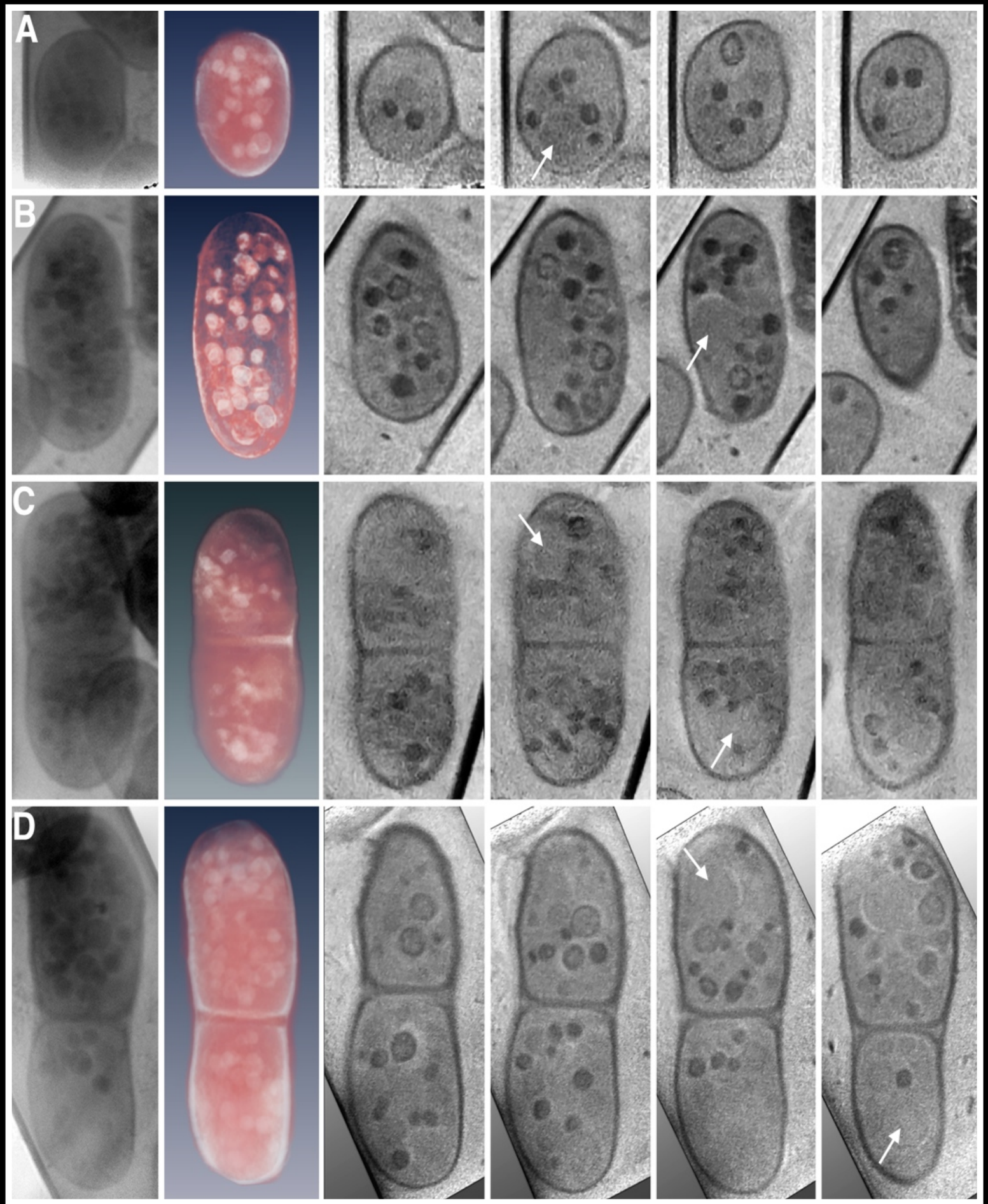
Computer sectioning through the reconstructed data

2 microns long
0.5 um diameter

Schizosaccharomyces pombe



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



New cell

Adult cell

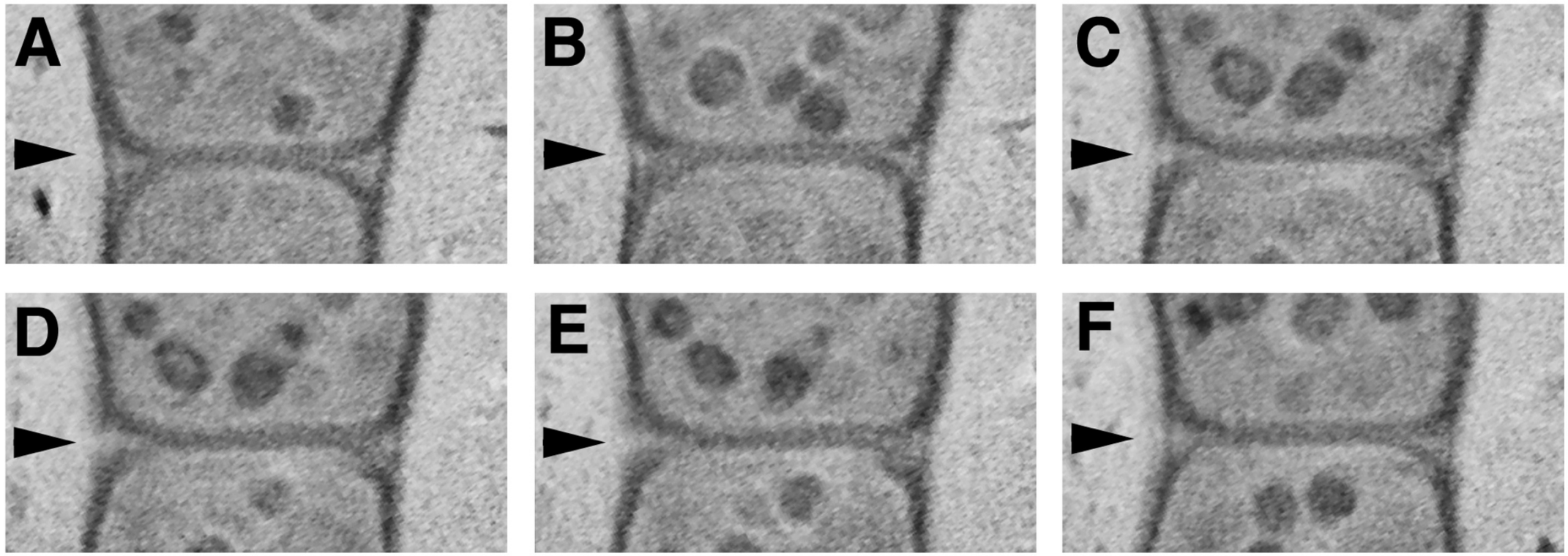
Dividing cell
(early)

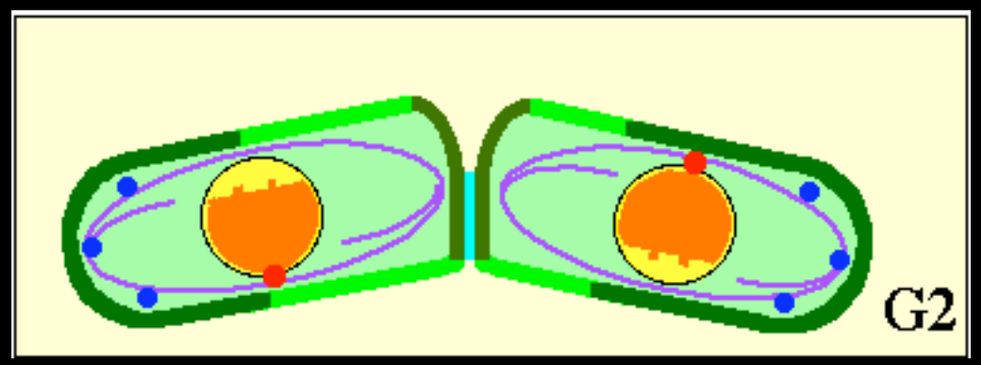
Dividing cell
(late)

W. Gu, L. D. Etkin, M.A. Le Gros, and C.A. Larabell. (2007) Differentiation. 75:529-535

Schizosaccharomyces pombe

Cell wall dissolution

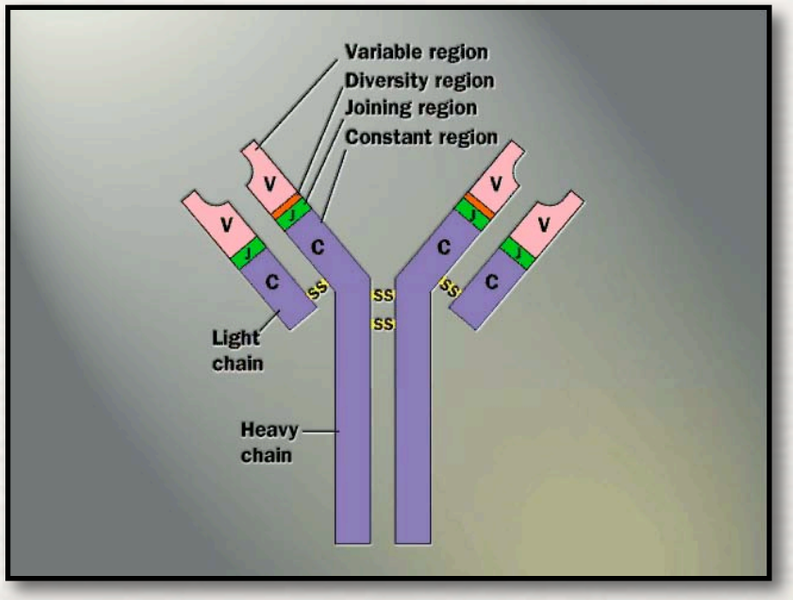




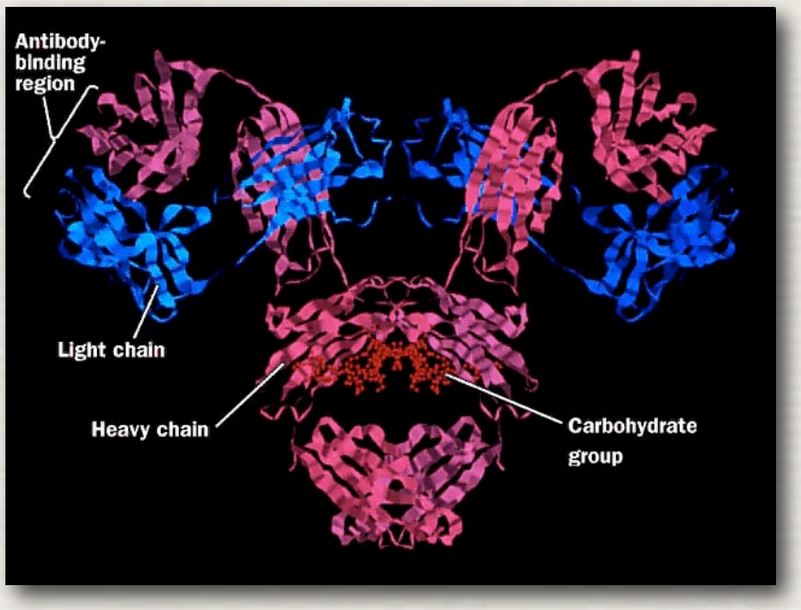
W. Gu, L. D. Etkin, M.A. Le Gros, and C.A. Larabell. (2007) Differentiation. 75:529-535

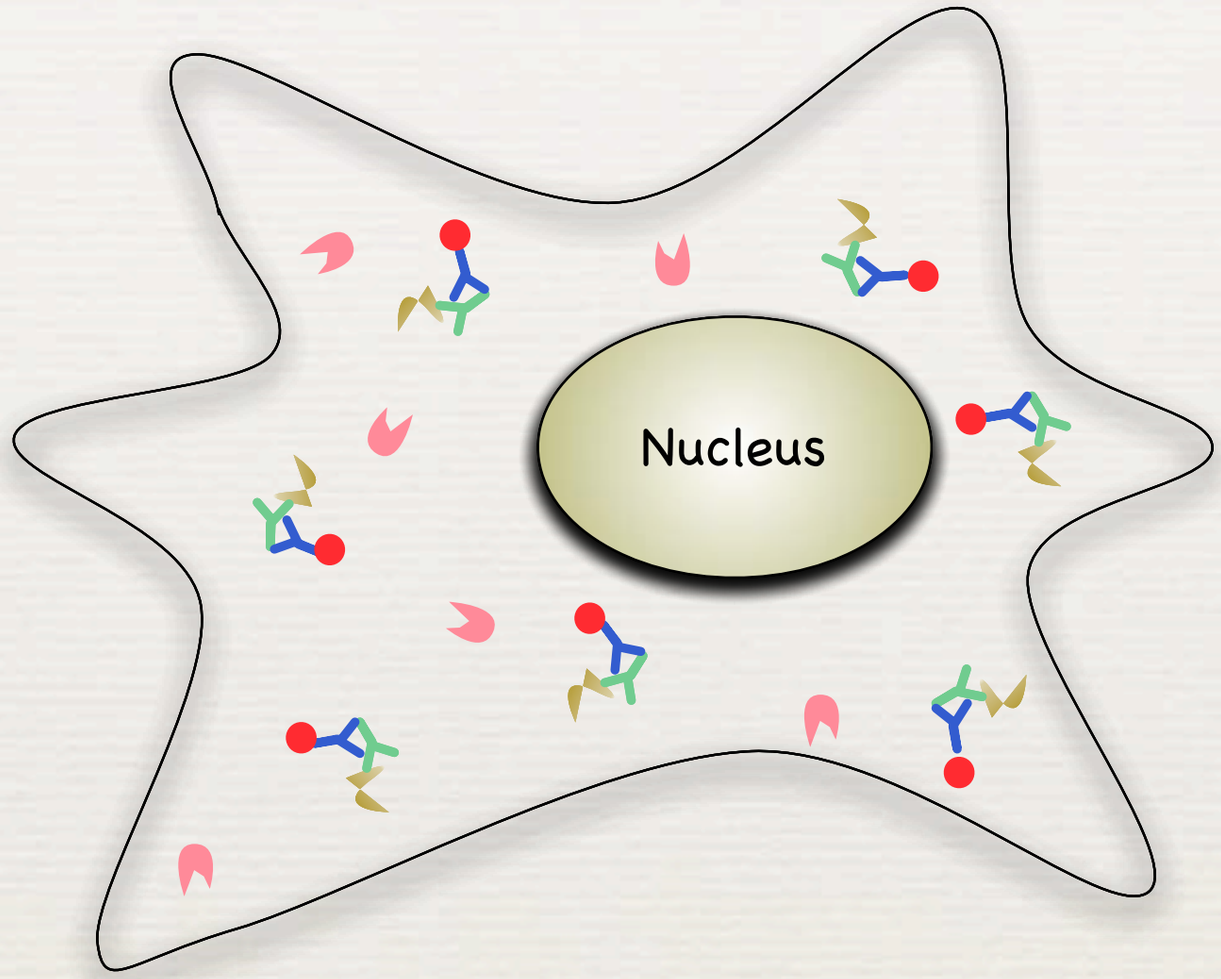
Localization of Proteins - X-ray Microscopy

Immuno-Labeling



Immunoglobulin (IgG)



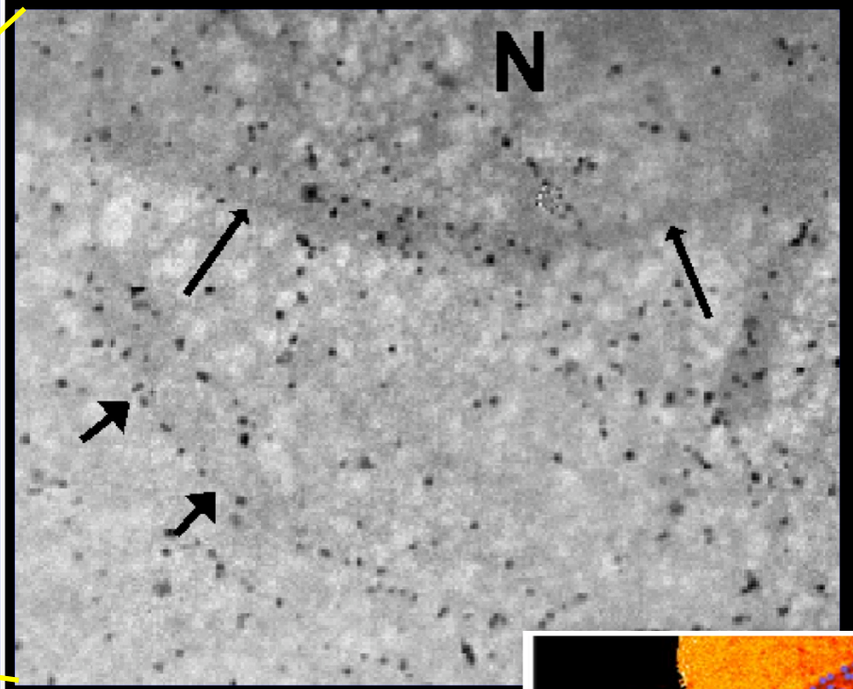


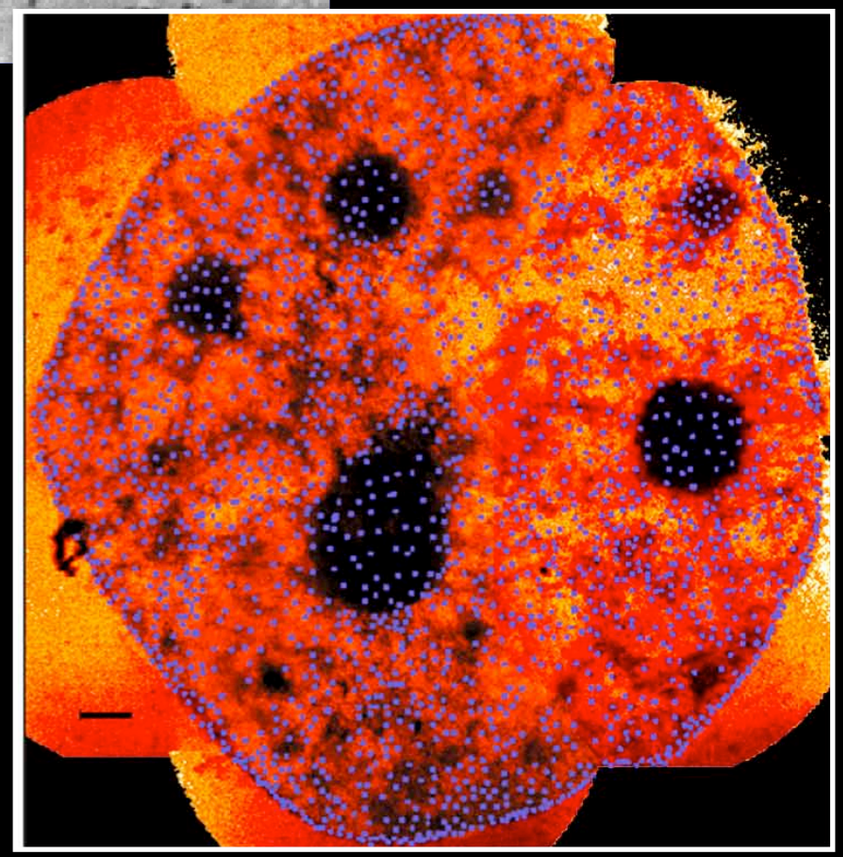
Primary antibodies recognize the protein

Secondary antibodies recognize the primary antibodies; tagged with X-ray Probe

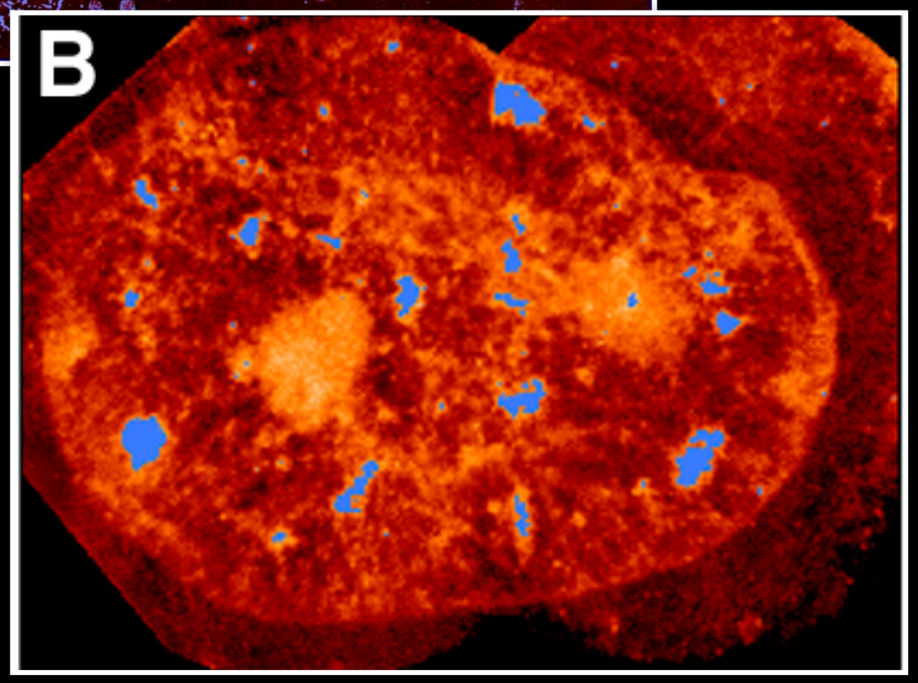
Microtubules

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

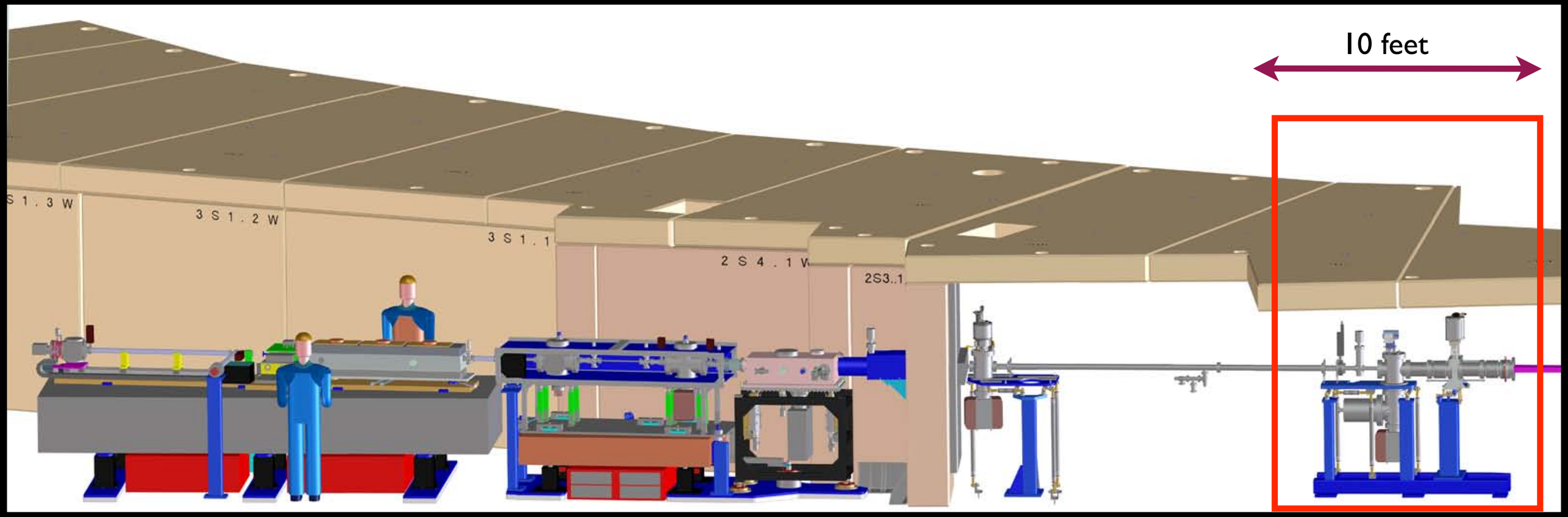
LETTERS

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

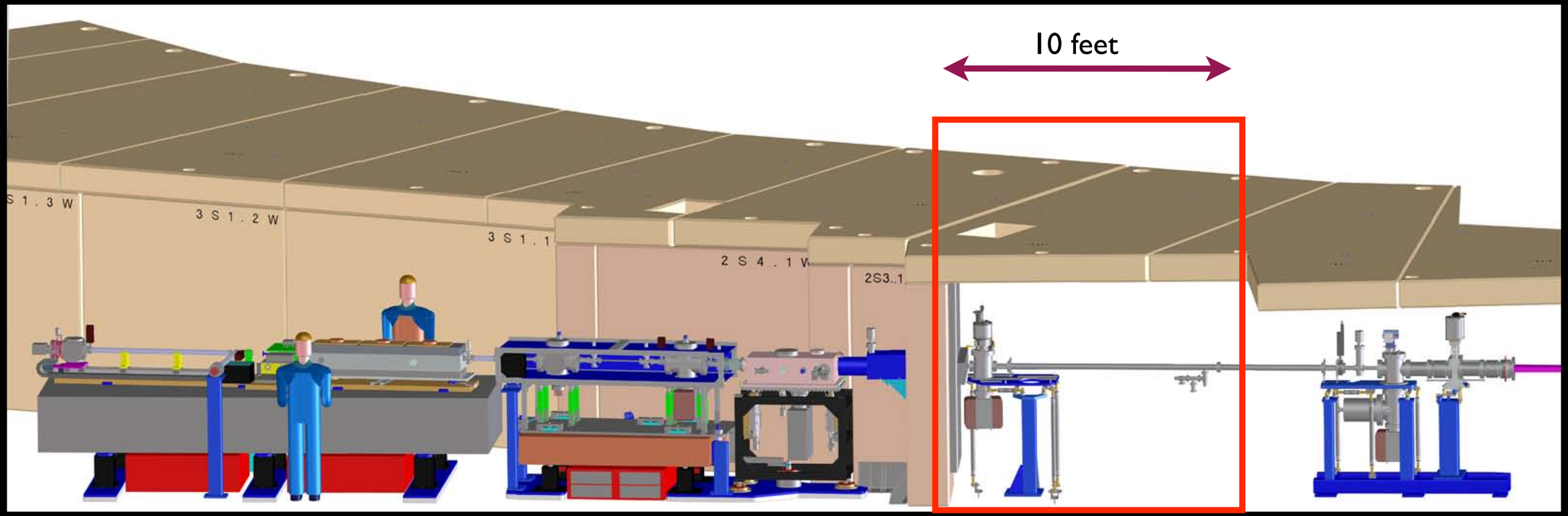
Weilun Chao[1,2], Bruce D. Harteneck[1], J. Alexander Liddle[1], Erik H. Anderson[1] & David T. Attwood[1,2]

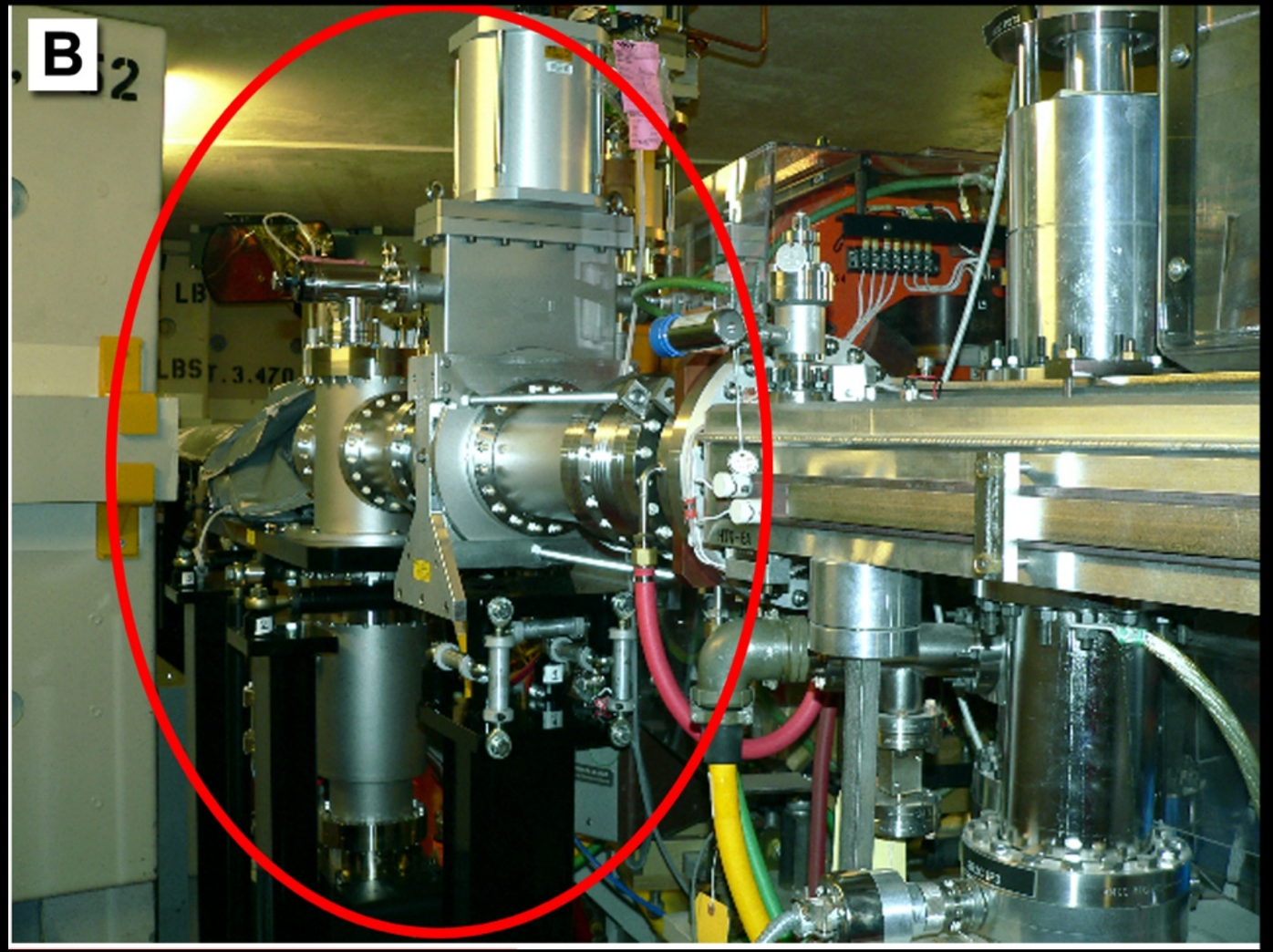
National Center for X-ray Tomography

- 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


10 feet
3 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


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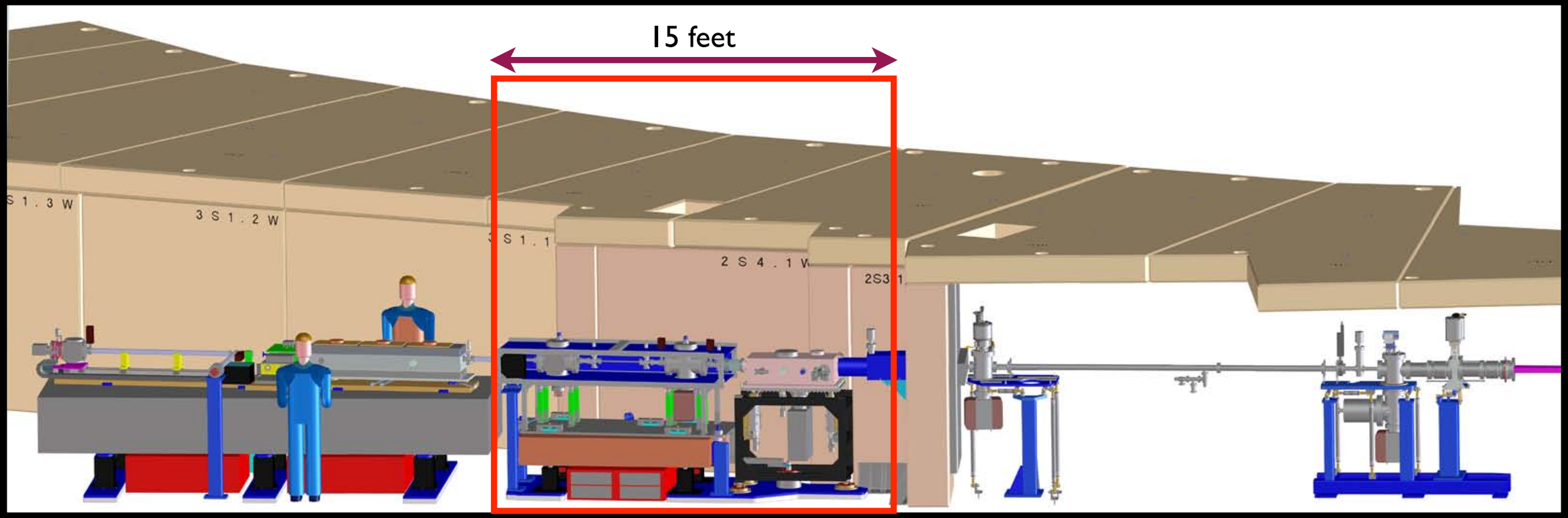
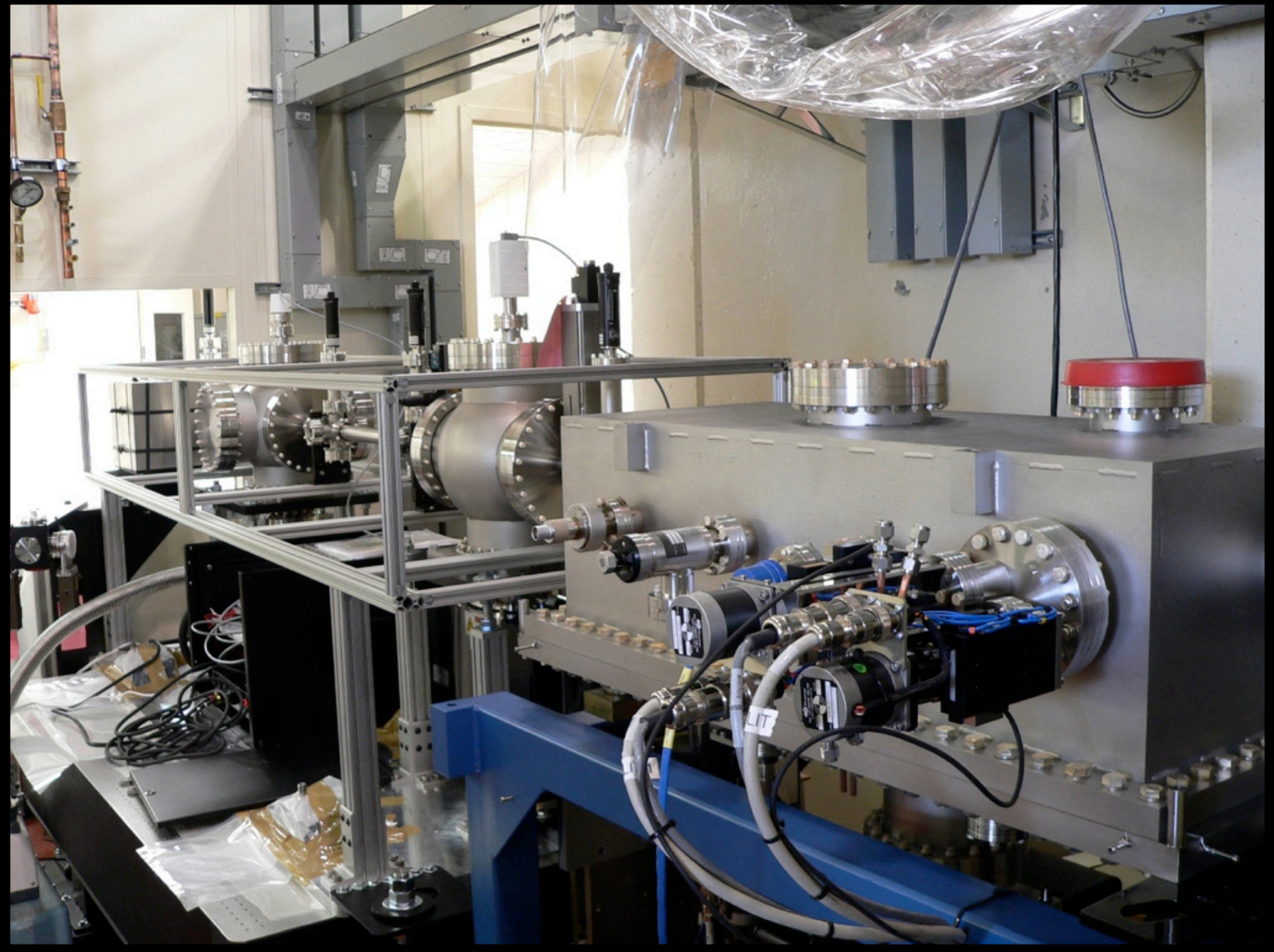
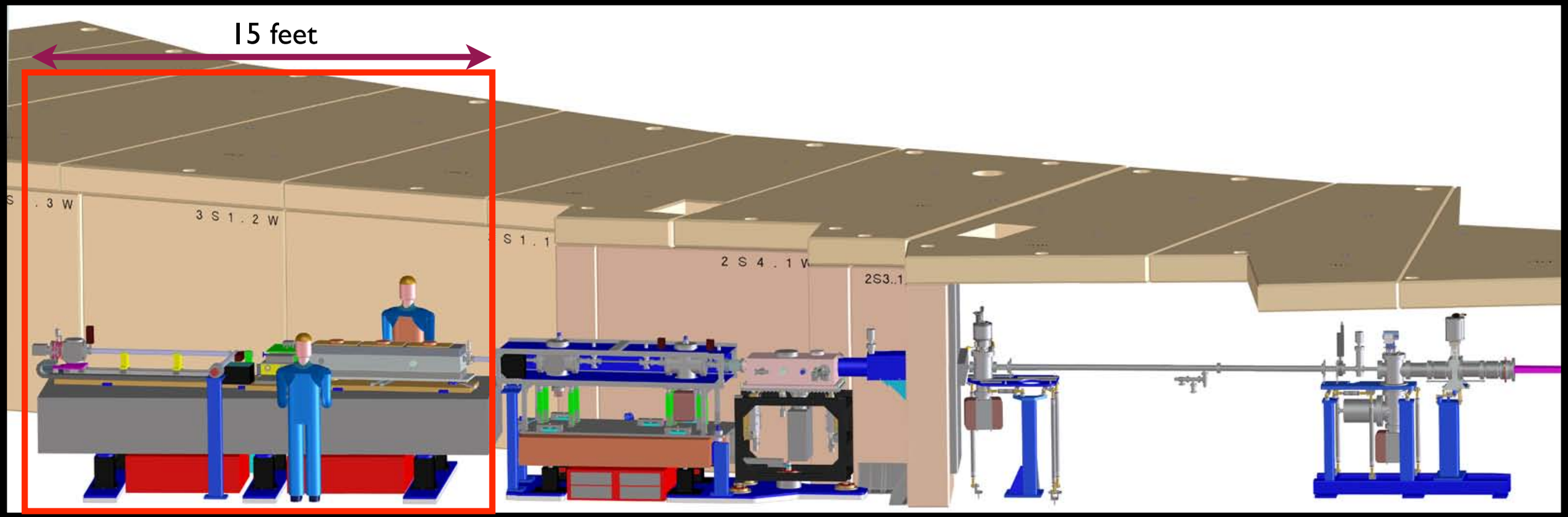
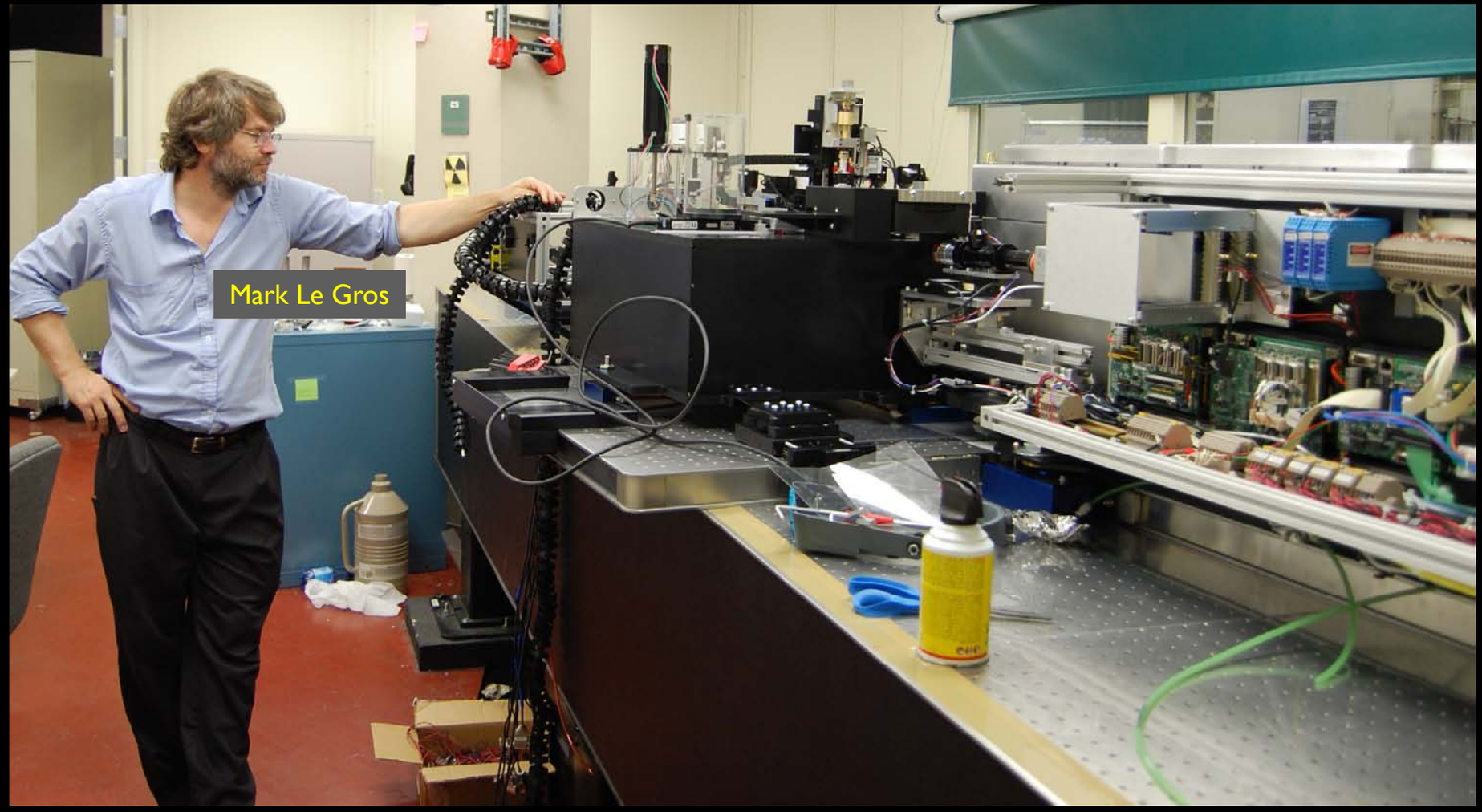
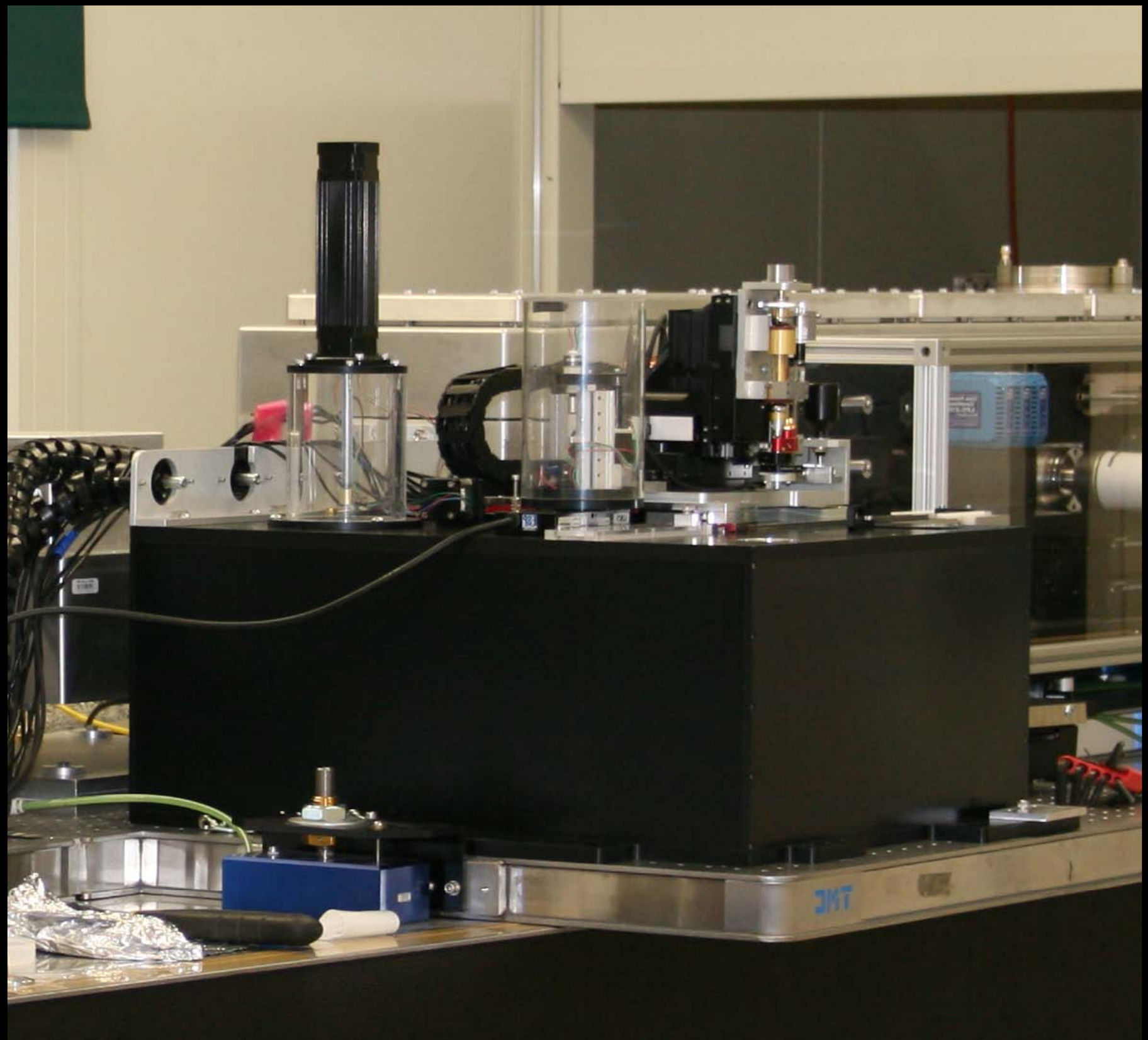

15 feet
3 S 1 . 2 W
2 S 4 . 1 W




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


Mark Le Gros

Robotic cryo-rotation stage



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Weilun Chao
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Bruce Harteneck
Anne Sakdinawat
Werner Meyer-Ilse (1954-1999)

Visiting Scientists

Hong-Tae Kim
Eva Pereiro Lopez
Larry Etkin (1946-2006)

Office of Biological & Environmental Research, Department of Energy
National Center for Research Resources, National Institutes of Health
National Institute of General Medical Sciences, National Institutes of Health