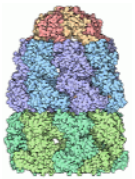
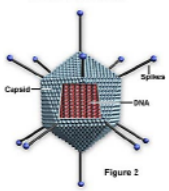
Imaging of Biological Systems

Size (m) 10^{-8} 10^{-7} 10^{-6} 10^{-5} 10^{-4} 10^{-3} 10^{-2} 10^{-1} 1

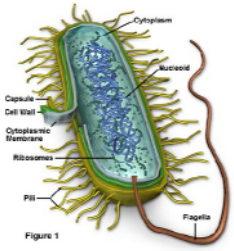
Molecules



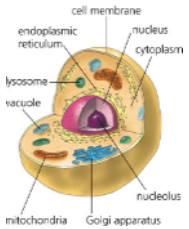
Complexes



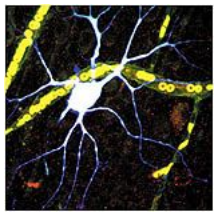
Viruses



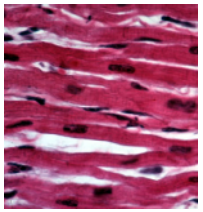
Bacteria



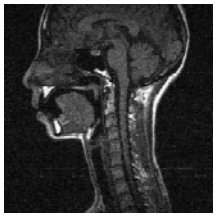
Cellular compartments



Cells



Tissues



Whole organisms

Cryo-electron microscopy

Light microscopy

X-ray crystallography
- atomic resolution
- needs protein crystals

MRI

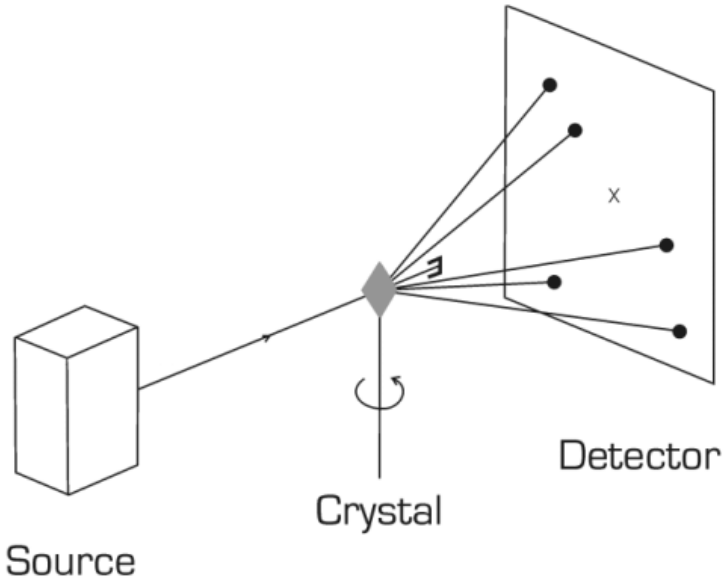
X-ray microscopy
- 20 nm resolution
- thick objects (compared to EM)

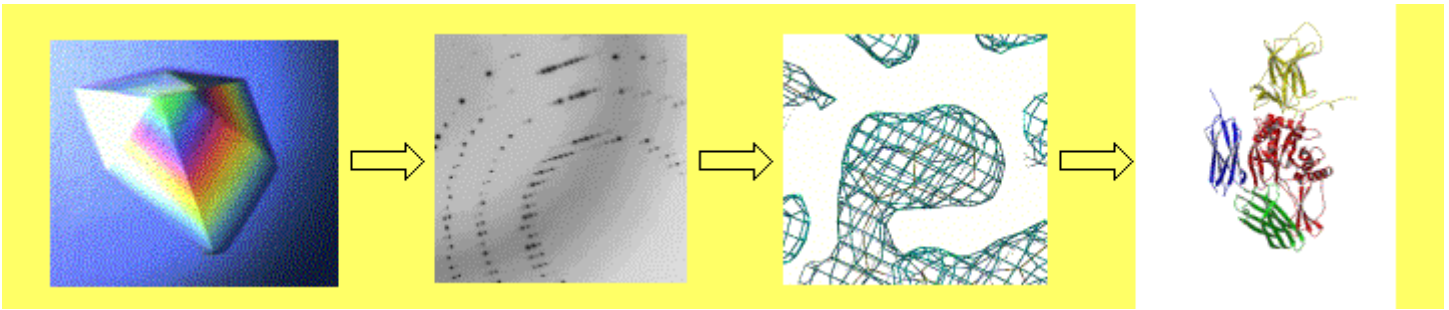
X-ray Crystallography

- **Fixed energy beam**

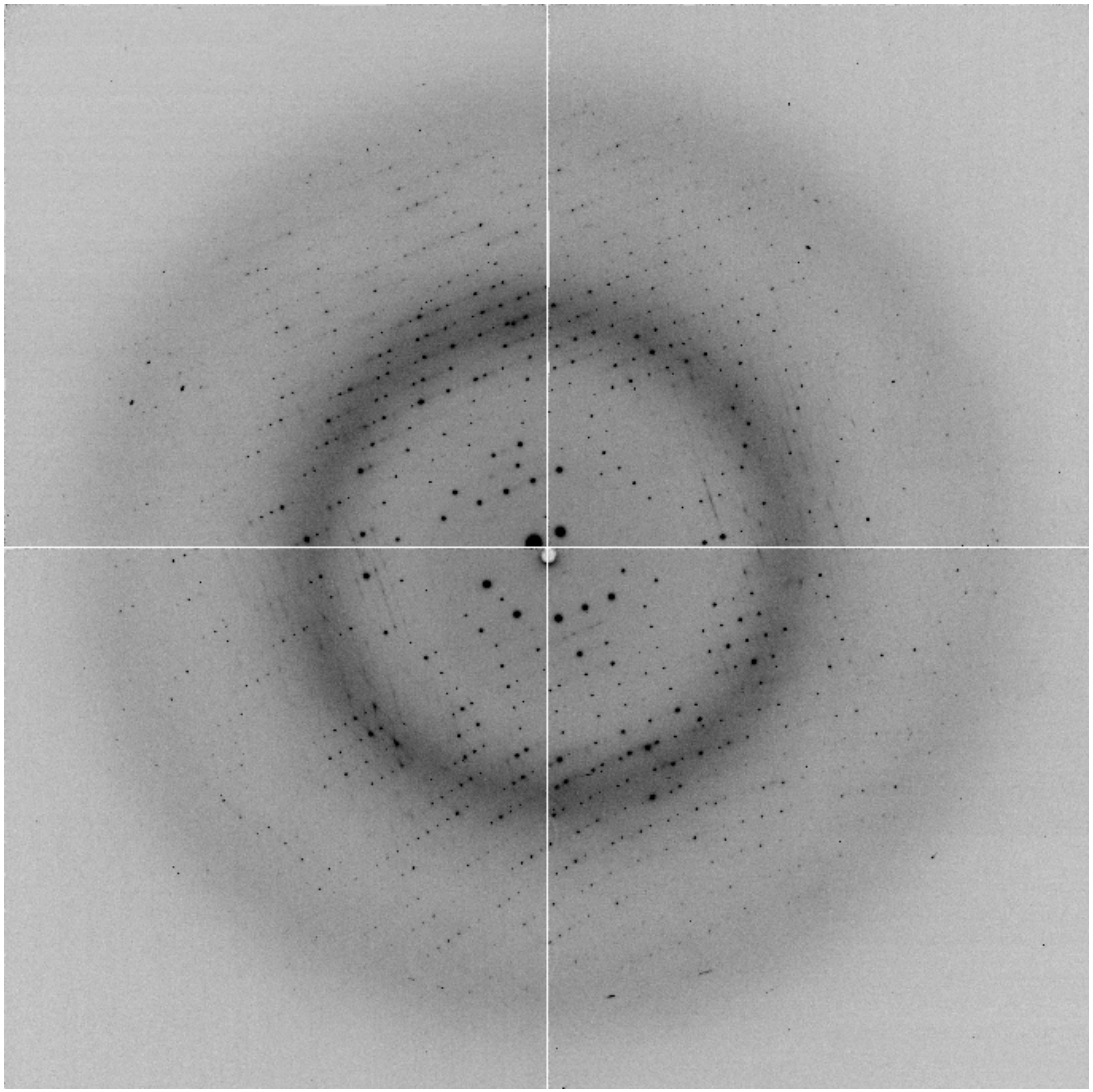
  - rotate crystal through small angle while recording data

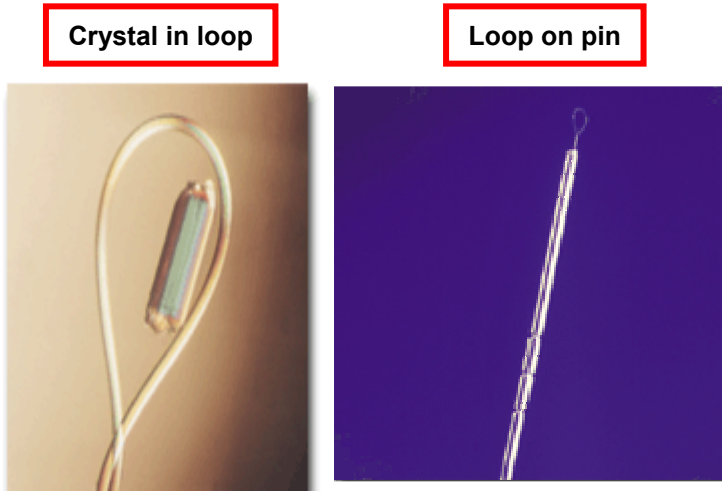
  - repeat N times through large angle given by crystal symmetry

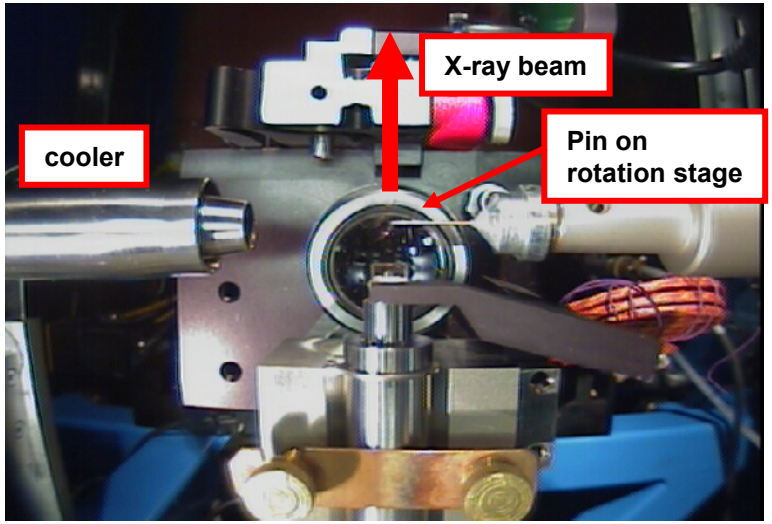




X-ray Crystallography



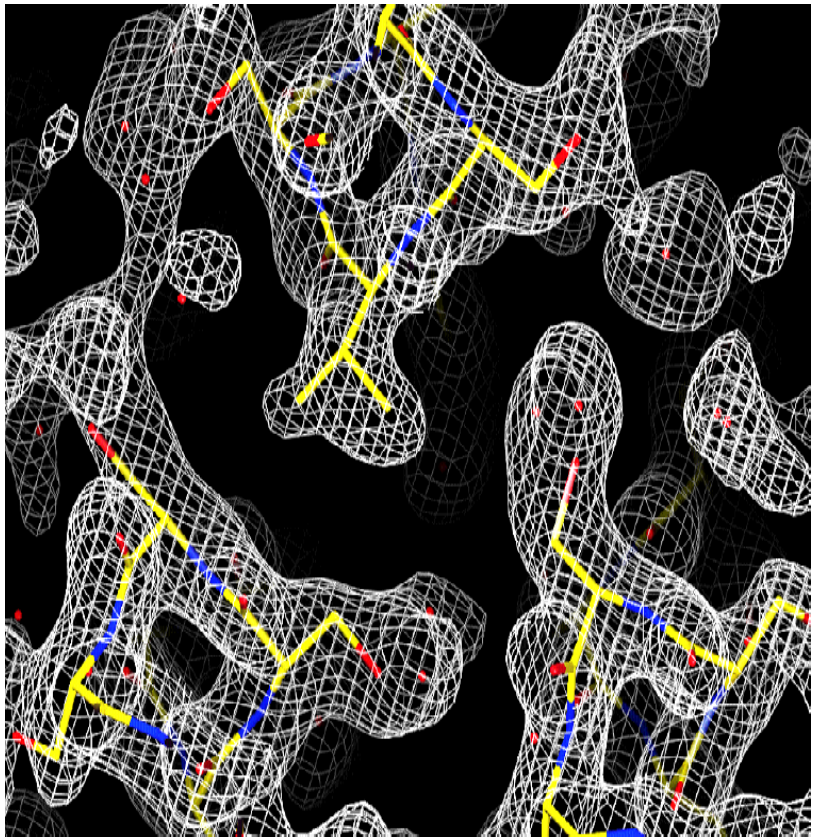


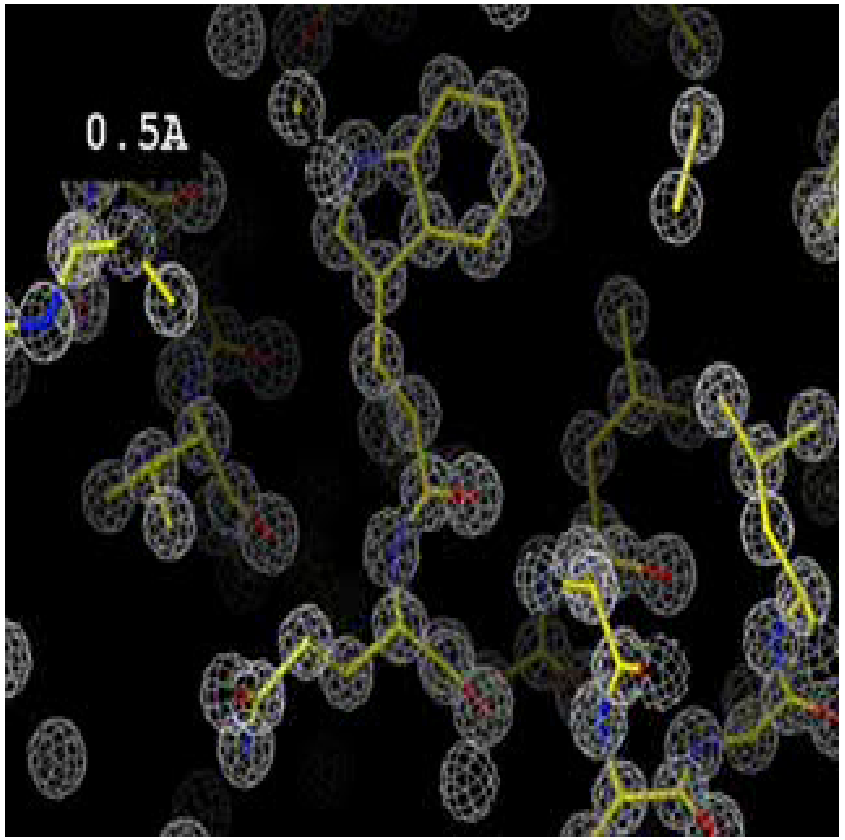


X-ray Crystallography

- determine electron density (ie. recover phase information)

- fit known sequence information into electron density





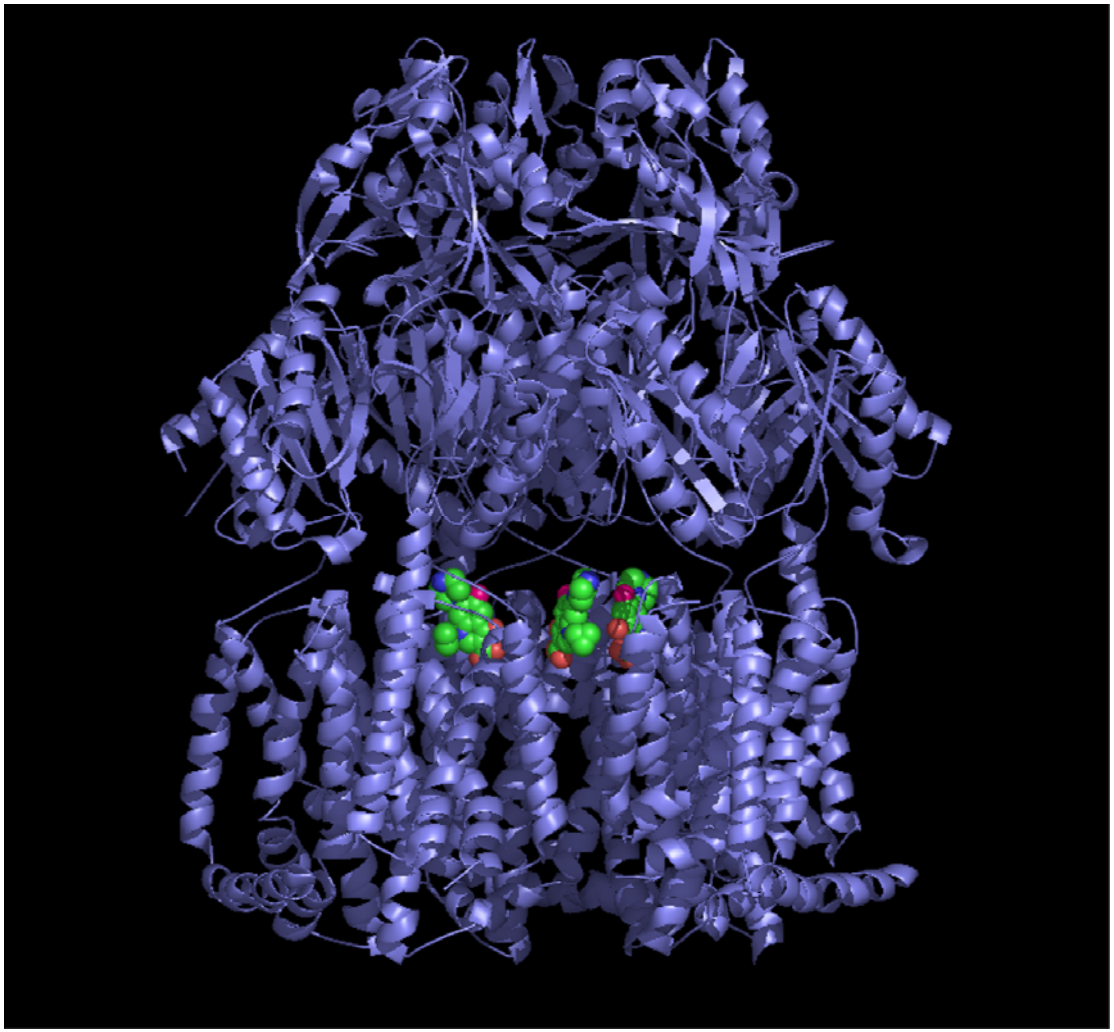
X-ray Crystallography

PS. Just skipped over 40 years of developments!

it used to be more difficult!

STRUCTURE OF THE AcrB PUMP

Bacterial Drug Transporter Reveals Secrets of Drug Resistance

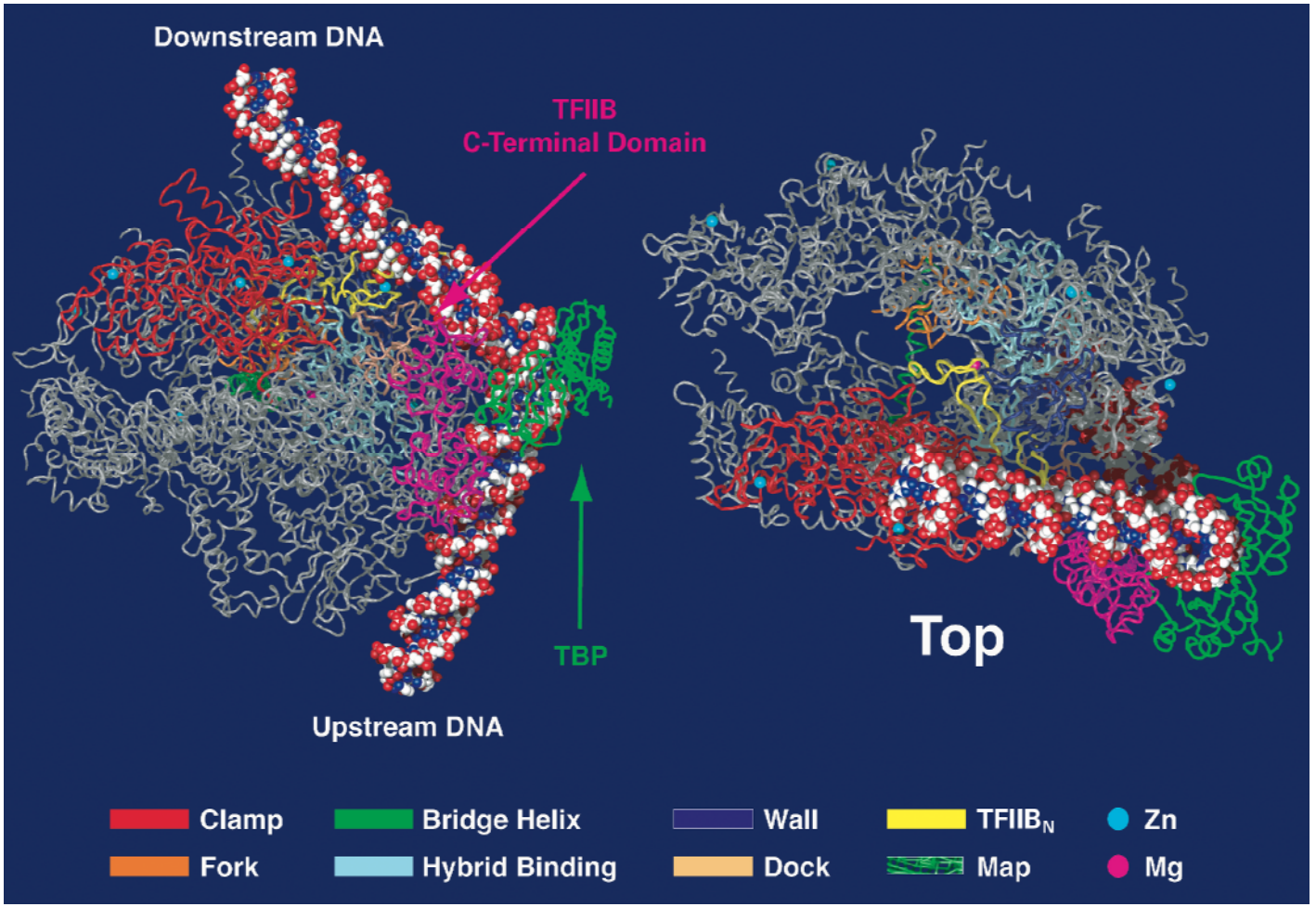


Even ciprofloxacin, an antibiotic used to treat a variety of bacterial infections including inhaled anthrax, is no match for AcrB. In this image, the green-colored drug is firmly ensnared in the protein's cavity.

E.W. Yu, H.I. Zgurskaya, H. Nikaido, D.E. Koshland, Jr. (University of California, Berkeley); G. McDermott (Berkeley Lab). [*Science* **300**, 976 (2003)]

STARTING THE RNA ASSEMBLY LINE

Transcription Initiation and Elongation by RNA Polymerase II



RNA polymerase II pre-initiation complex model.

K.D. Westover, D.A. Bushnell, R.E. Davis, and R.D. Kornberg (Stanford University). [*Science* **303**, 983 (2004); *Science* **303**, 1014 (2004)]

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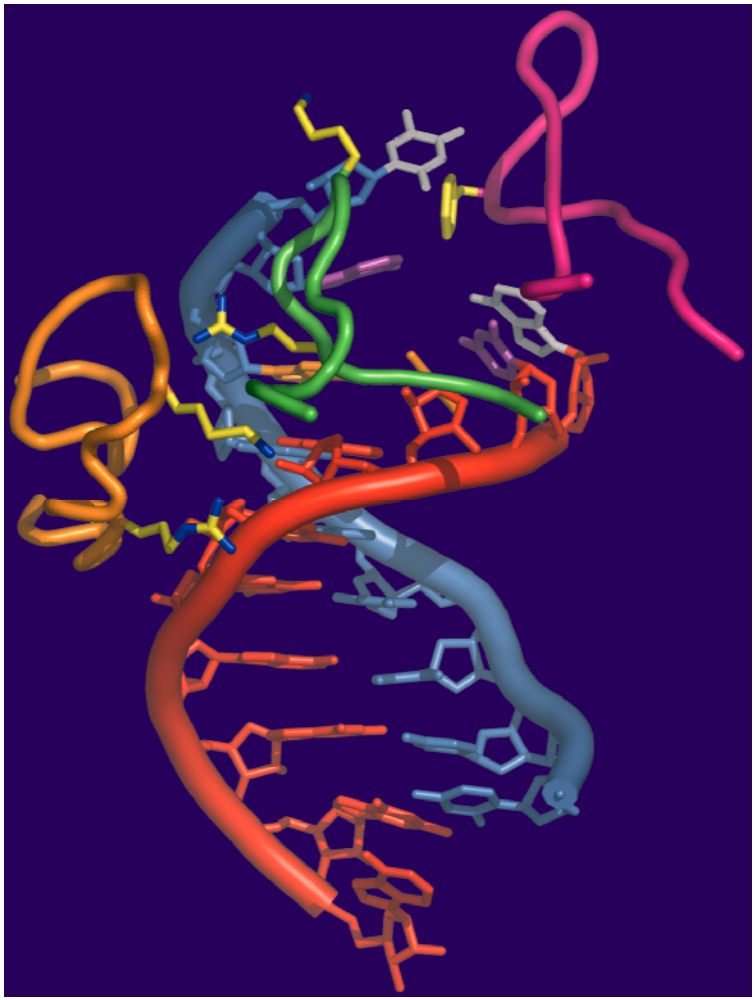

Close-up of strand separation. RNA (red) *and DNA* (blue) *strands are separated by a network of interactions with three protein loops. The loops limit the extent of RNA separation* (orange)*, stabilize the separated single strand, and form part of the RNA exit pore* (green and pink)*.*

K.D. Westover, D.A. Bushnell, R.E. Davis, and R.D. Kornberg (Stanford University). [*Science* **303**, 983 (2004); *Science* **303**, 1014 (2004)]

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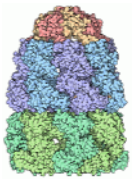

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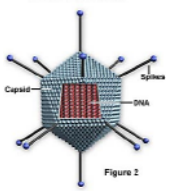

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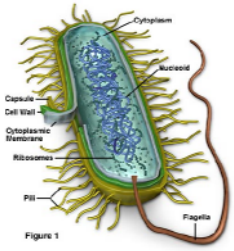

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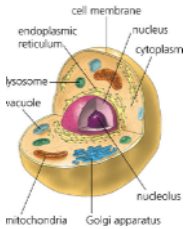
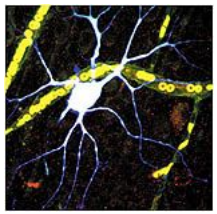
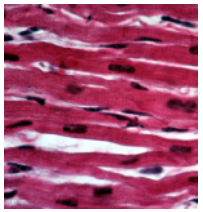


Cellular compartments



Cells



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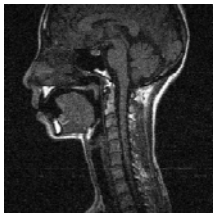


Whole organisms

Cryo-electron microscopy

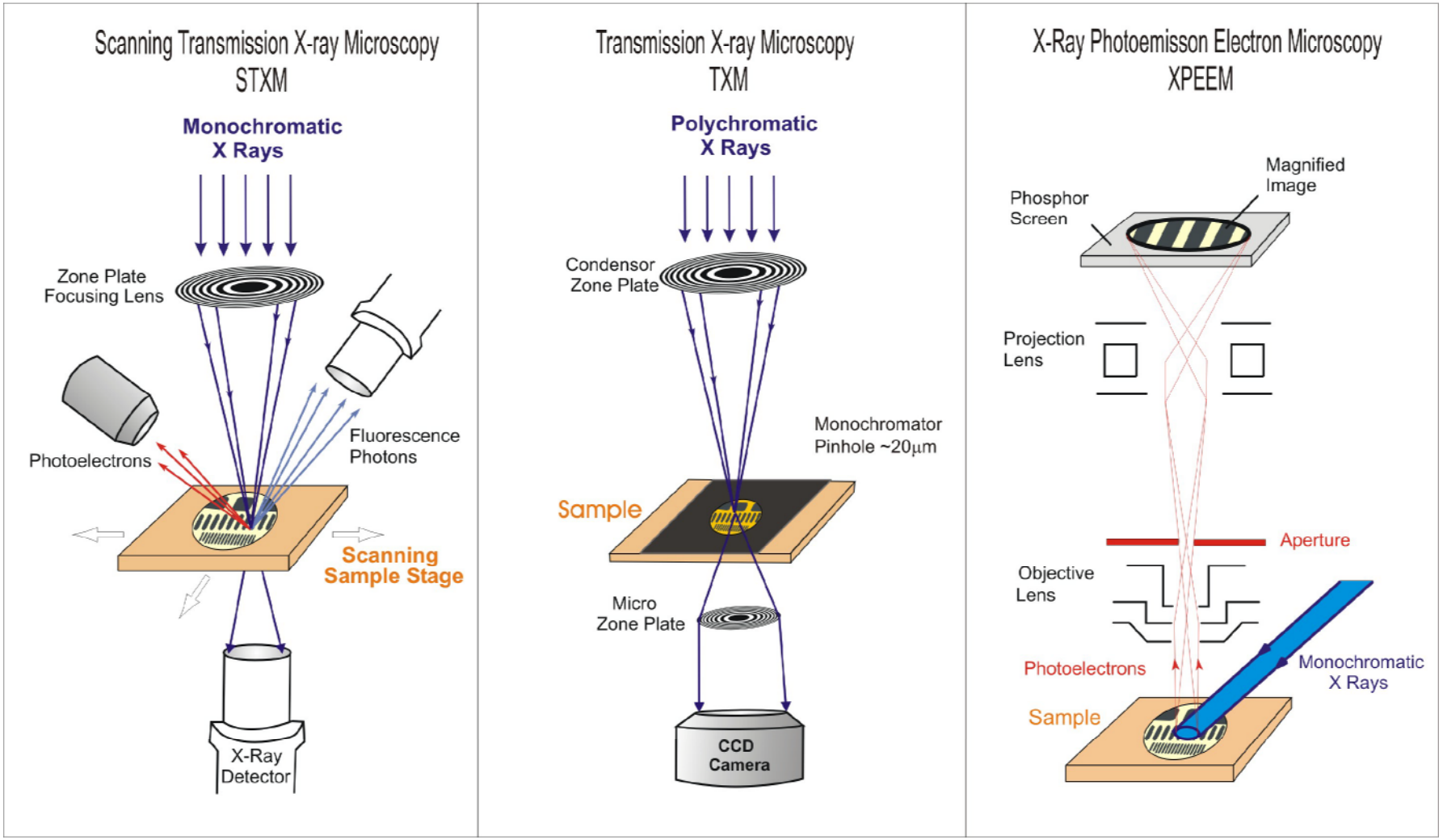
Light microscopy

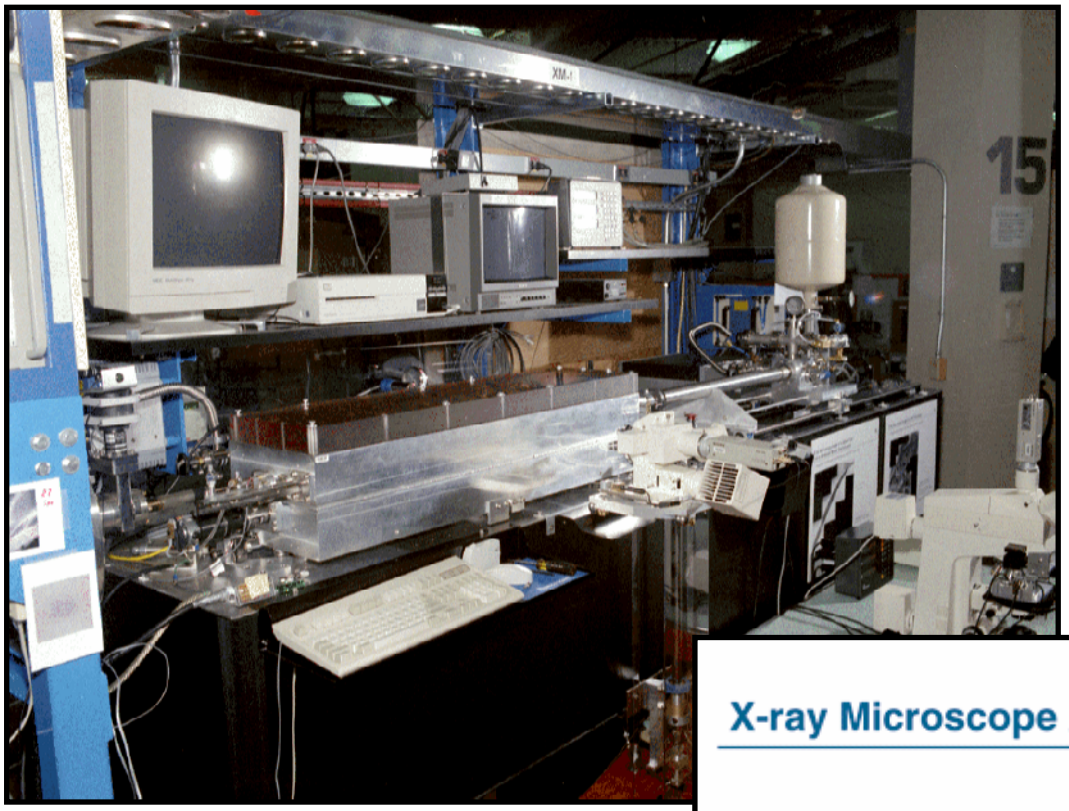
X-ray crystallography
- atomic resolution
- needs protein crystals

MRI

X-ray microscopy
- 20 nm resolution
- thick objects (compared to EM)

X-ray Microscopes

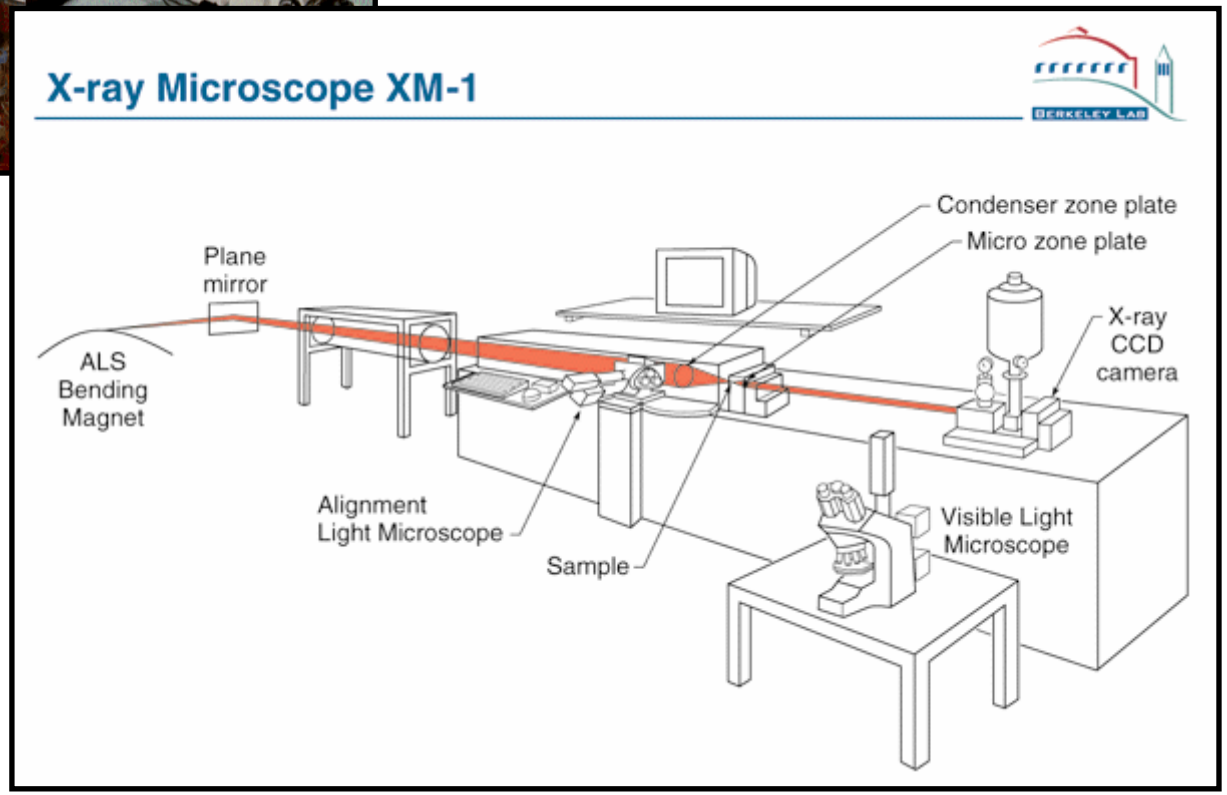




Beamline 6.1.2

Full-field Transmission X-ray Microscope (XM-1)

Wavelength, 2.4 nm
Photon energy, 517 eV

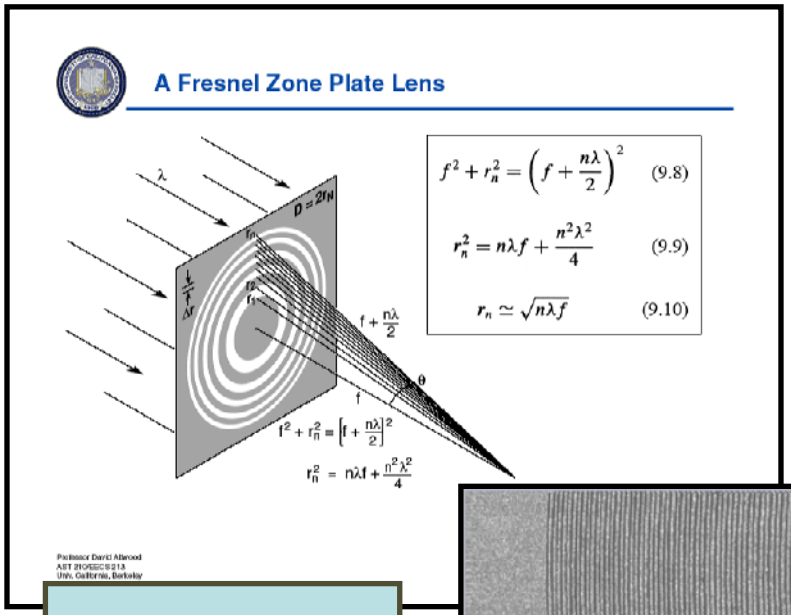


Center for X-ray Optics

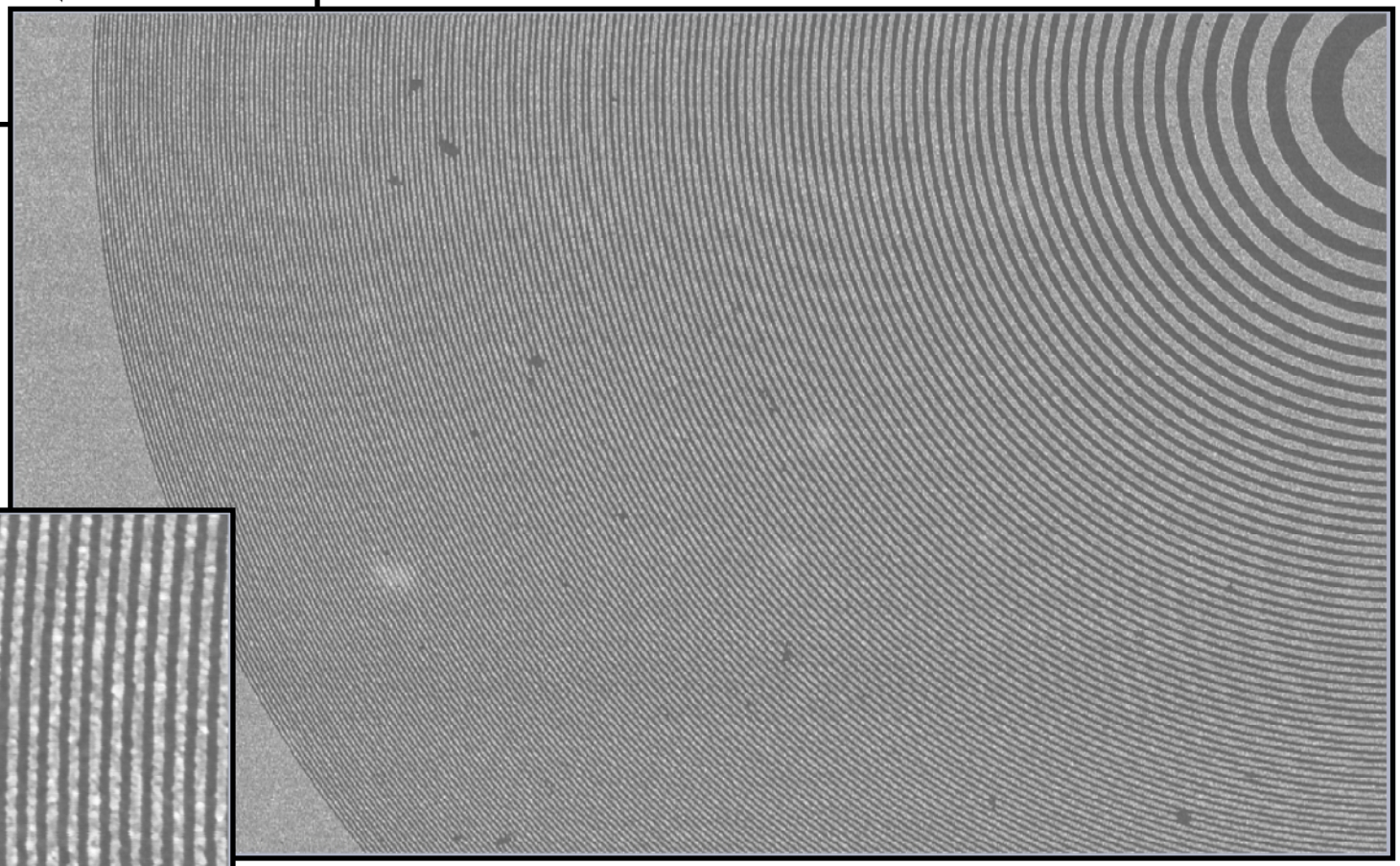
Erik Anderson
David Attwood

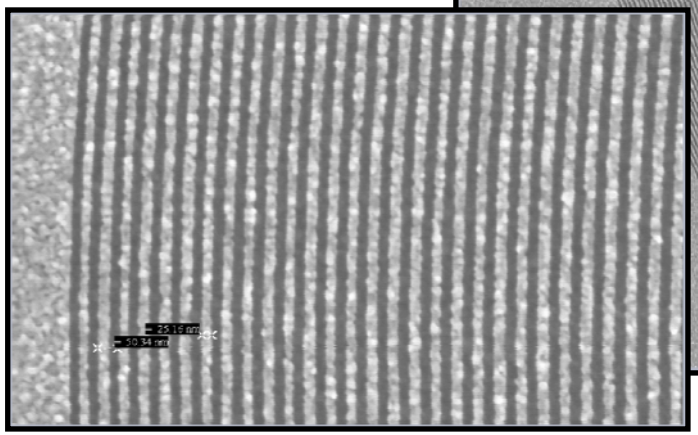
Zone Plate Lenses
20 nm Resolution

E. Anderson, D. Attwood, A. Liddle, D. Olynick, B. Harteneck, W. Chao; Center for X-ray Optics



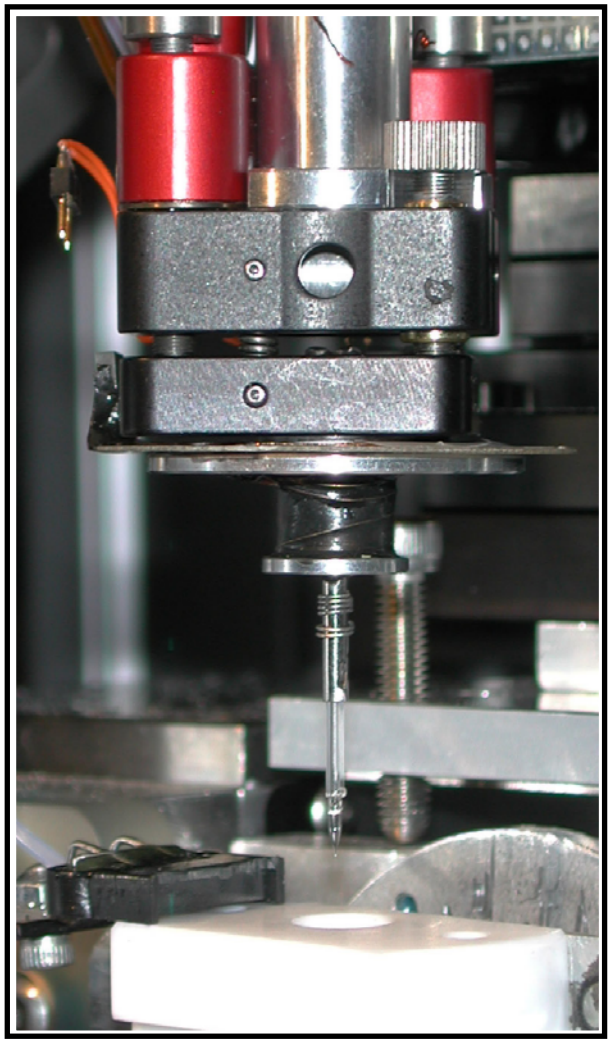
Dr = 25 nm
D = 63 um
N = 618 zones
f = 650 um
NA = 0.05
@ 2.4 nmλ

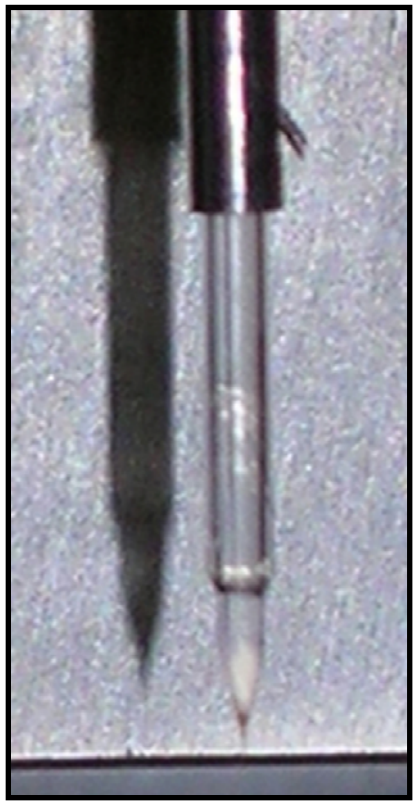


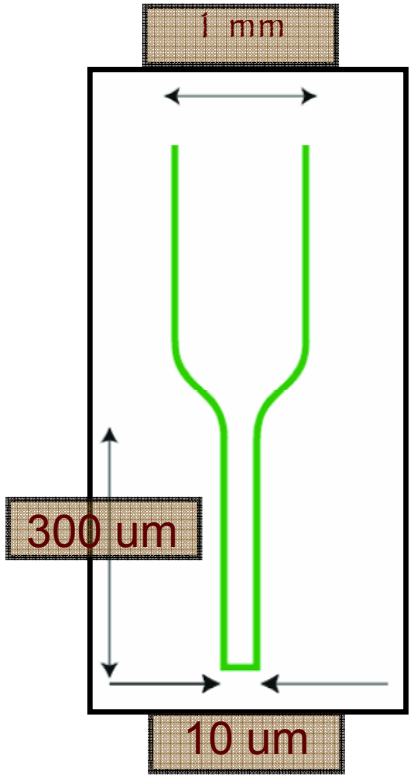


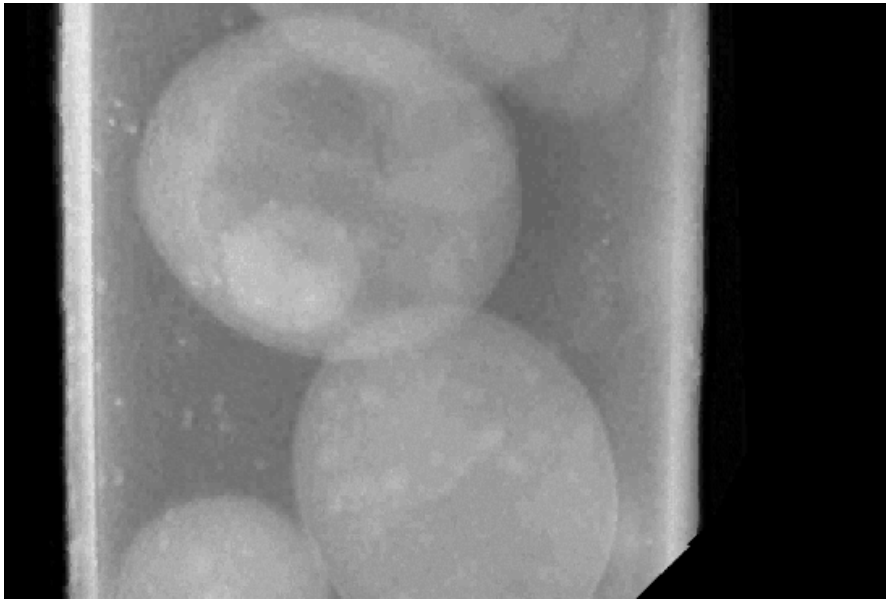
Cryo X-ray Tomography

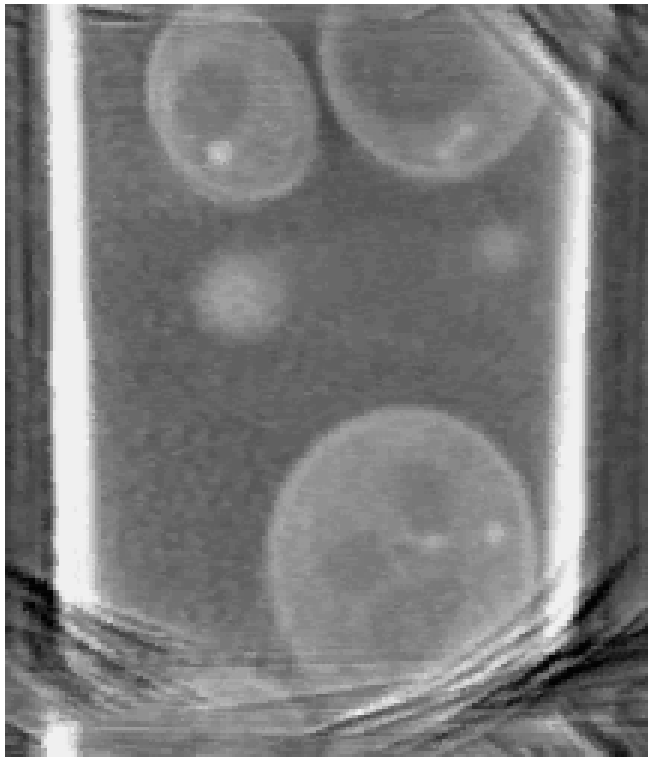
C.A. Larabell & M. A. Le Gros (2004).
Molecular Biology of the Cell, 15(3), 956-962











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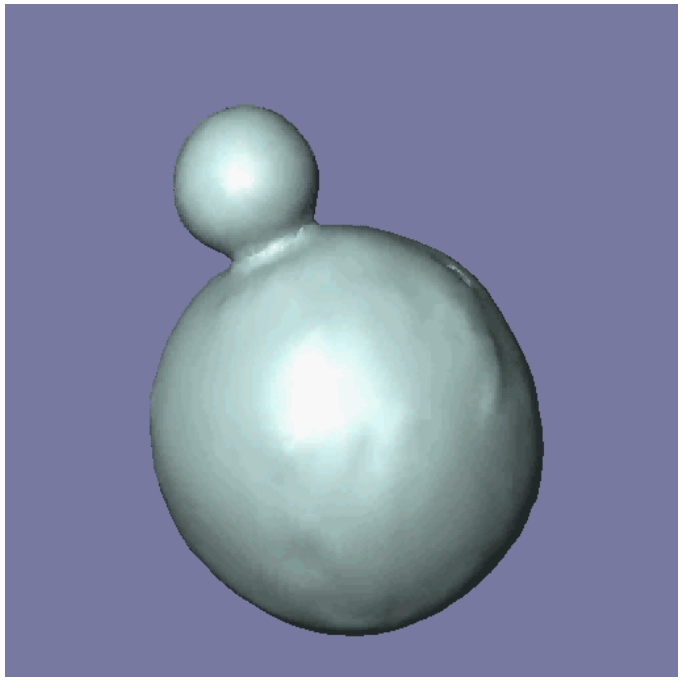

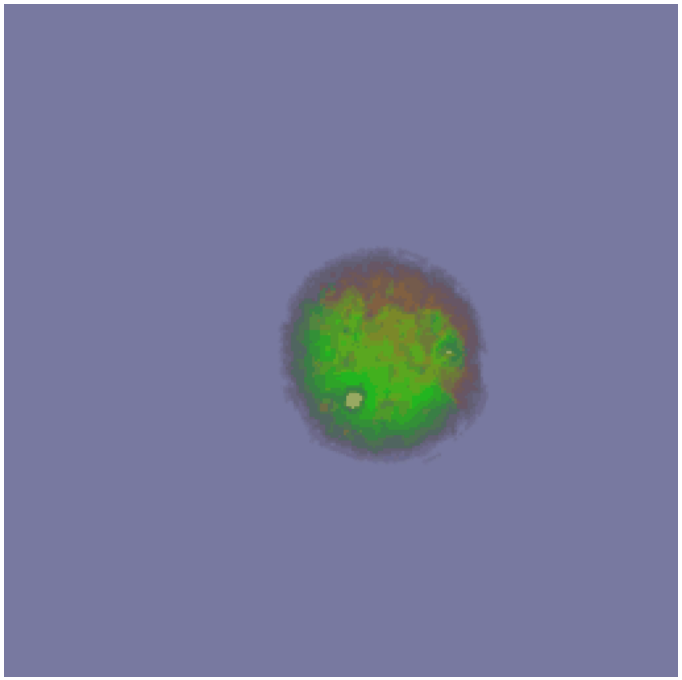

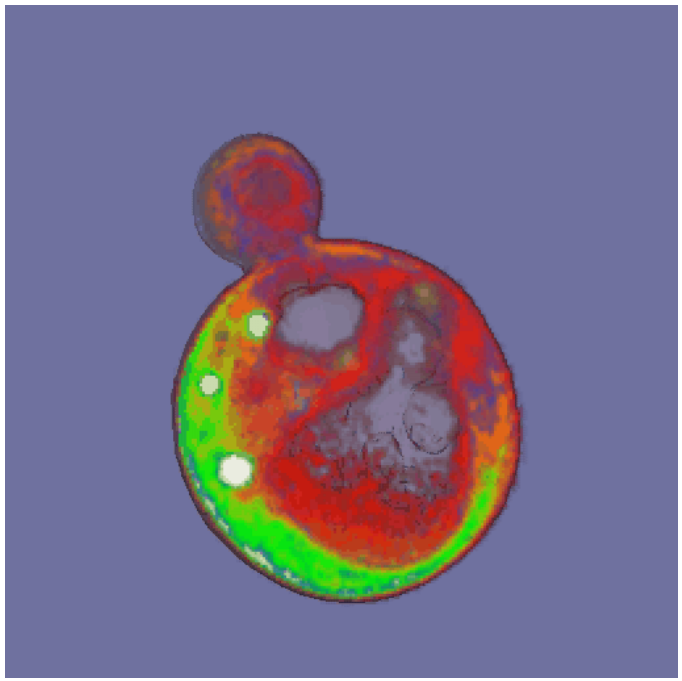

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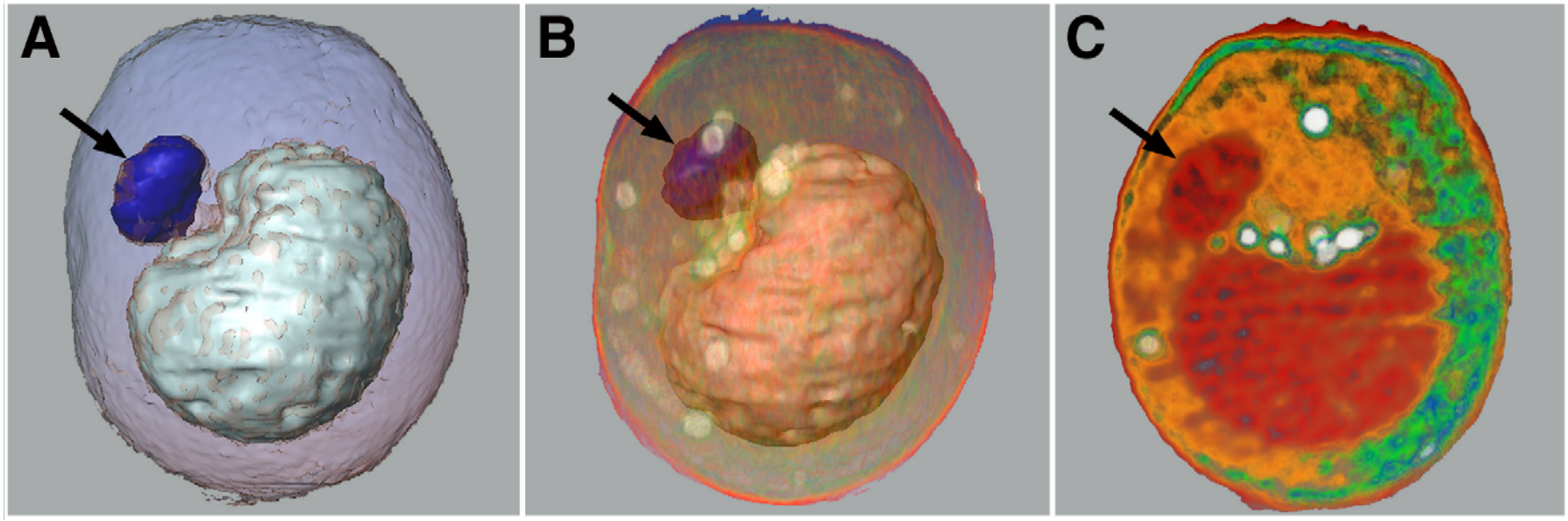
Saccharomyces cerevisiae



Reconstructed data using different volume analysis algorithms.

A) Combination of translucent outer surface & opaque surfaces highlight internal organelles; arrow points to nucleus that has been color-coded blue

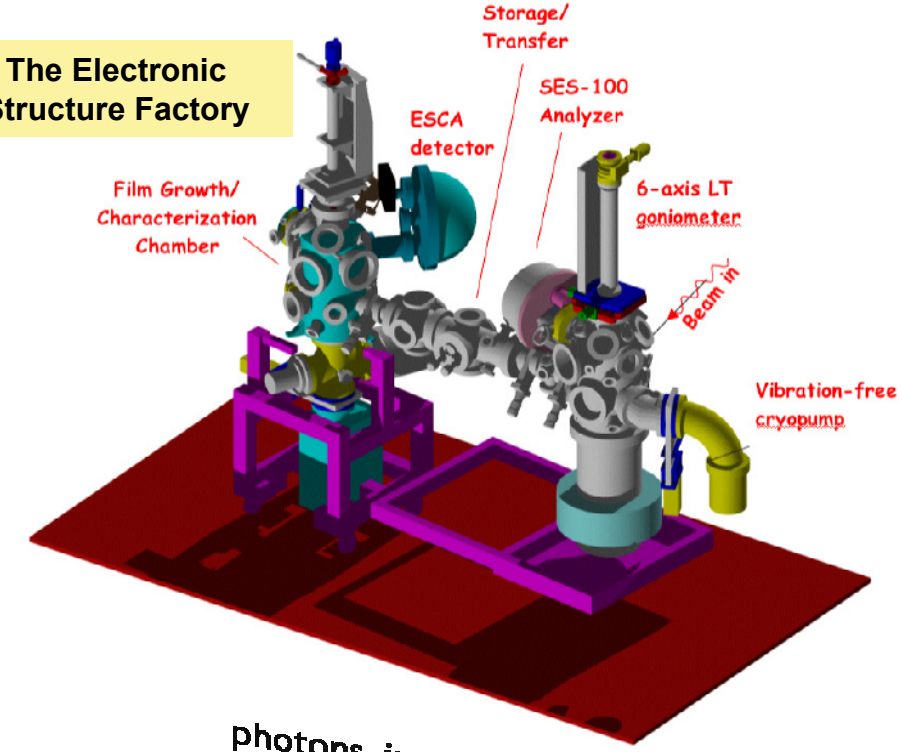
B) Surface views combined with volume rendering
- lipid droplets white
- surface of large vacuole pink

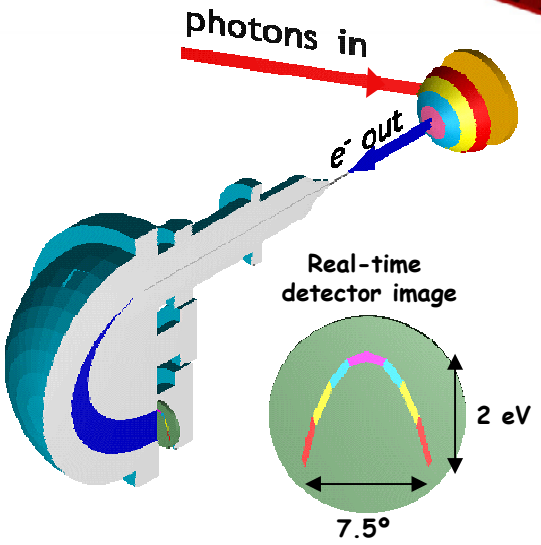
C) Computer section that was volume-rendered according to x-ray absorption
- lipid droplets white
- internal structures of vacuole and nucleus red
- other cytoplasmic structures appear green and orange.

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

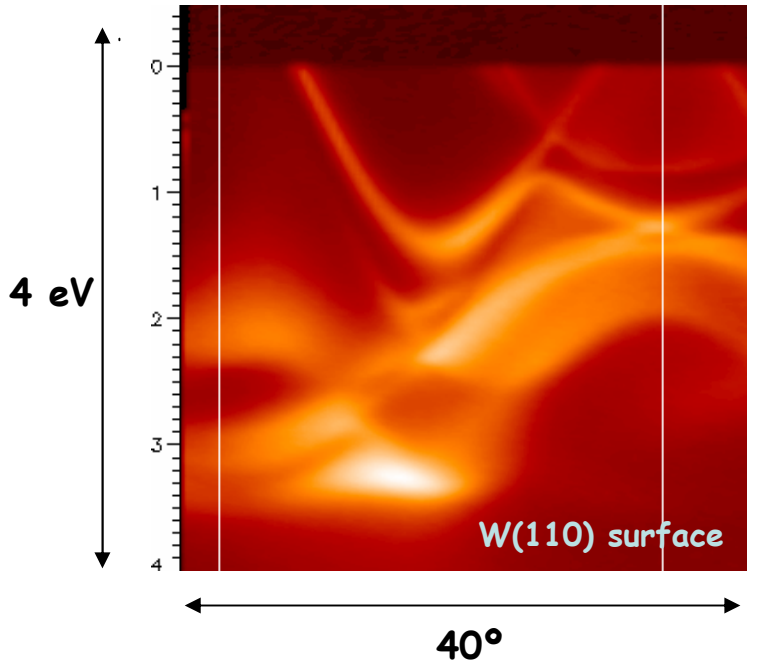
Angle Resolved Photo-Electron Spectroscopy (ARPES)

The Electronic Structure Factory





"2-Dimensional" (E vs k_x)
Bandstructure Measurement
Built up by tiling detector images



Eli Rotenberg et al, BL 7.0

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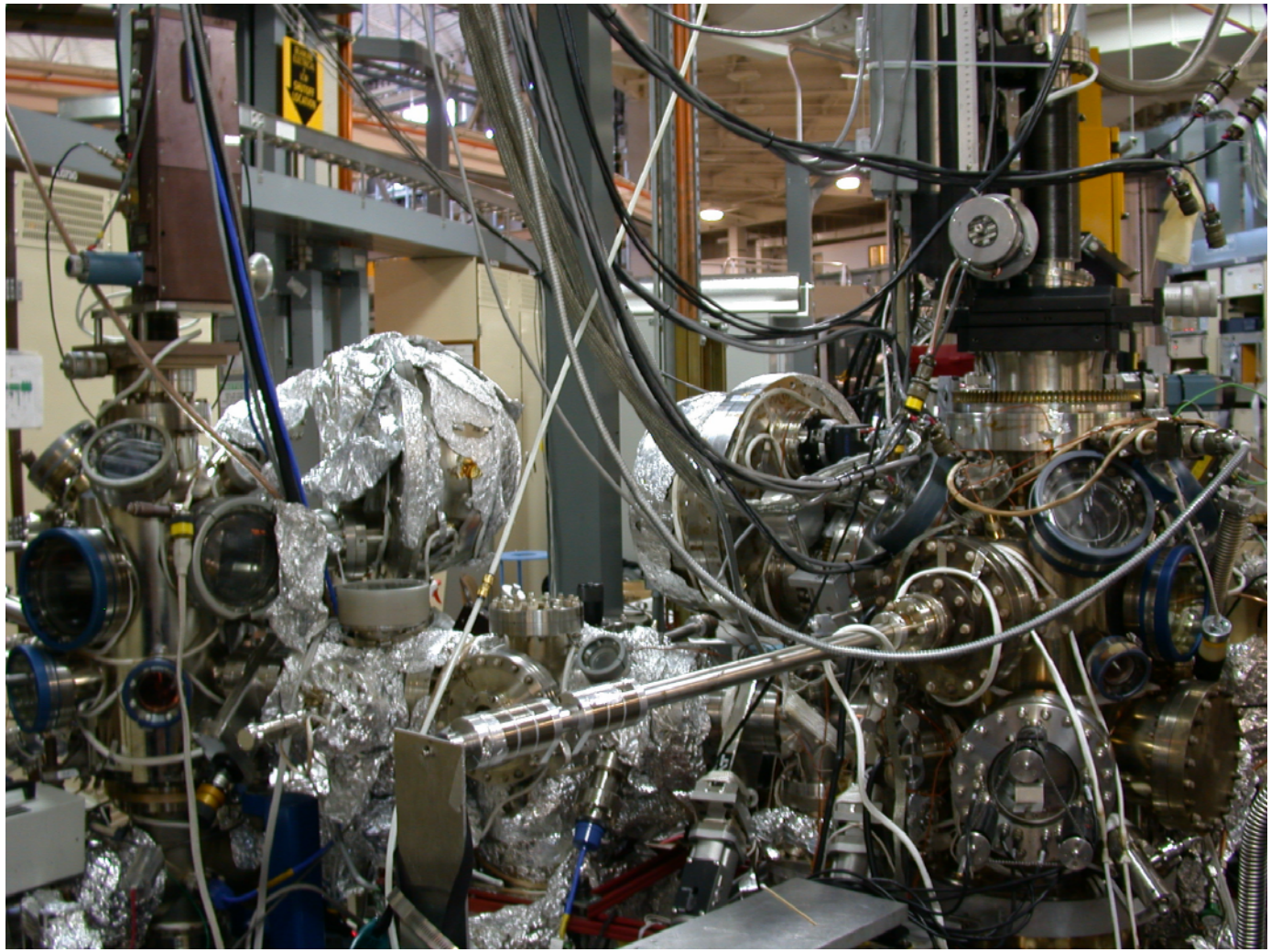
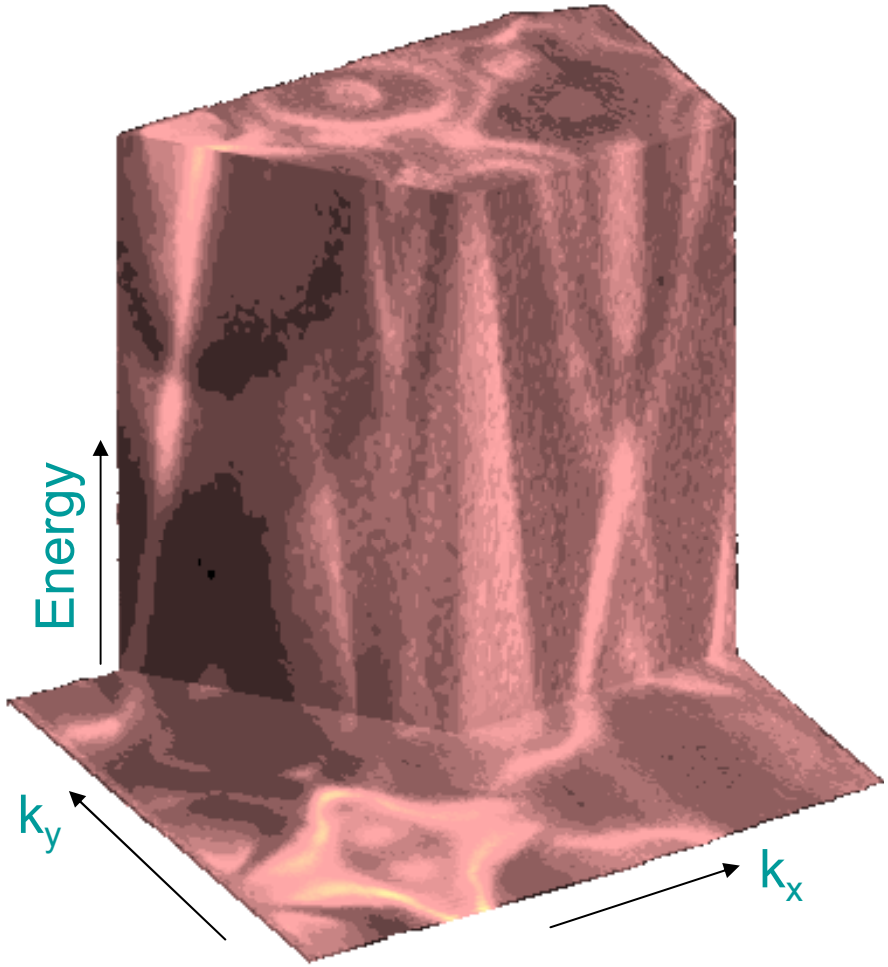
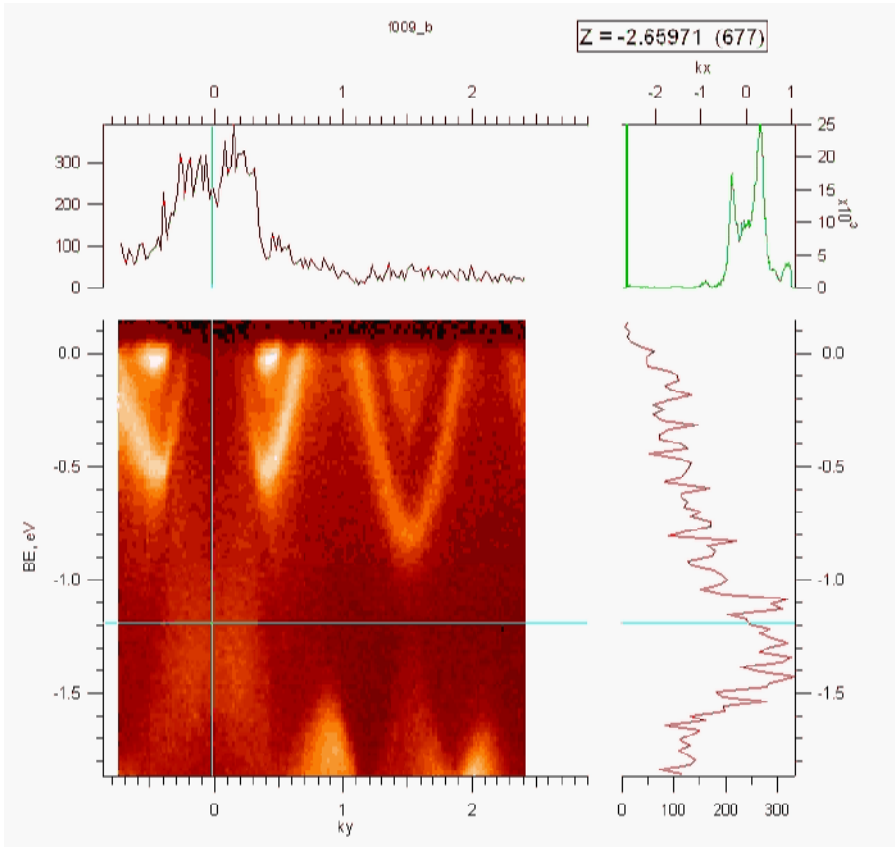


Eli Rotenberg et al, BL 7.0

Angle Resolved Photo-Electron Spectroscopy (ARPES)

- 3d and 4d datasets built up from angle scanning the sample manuipulator

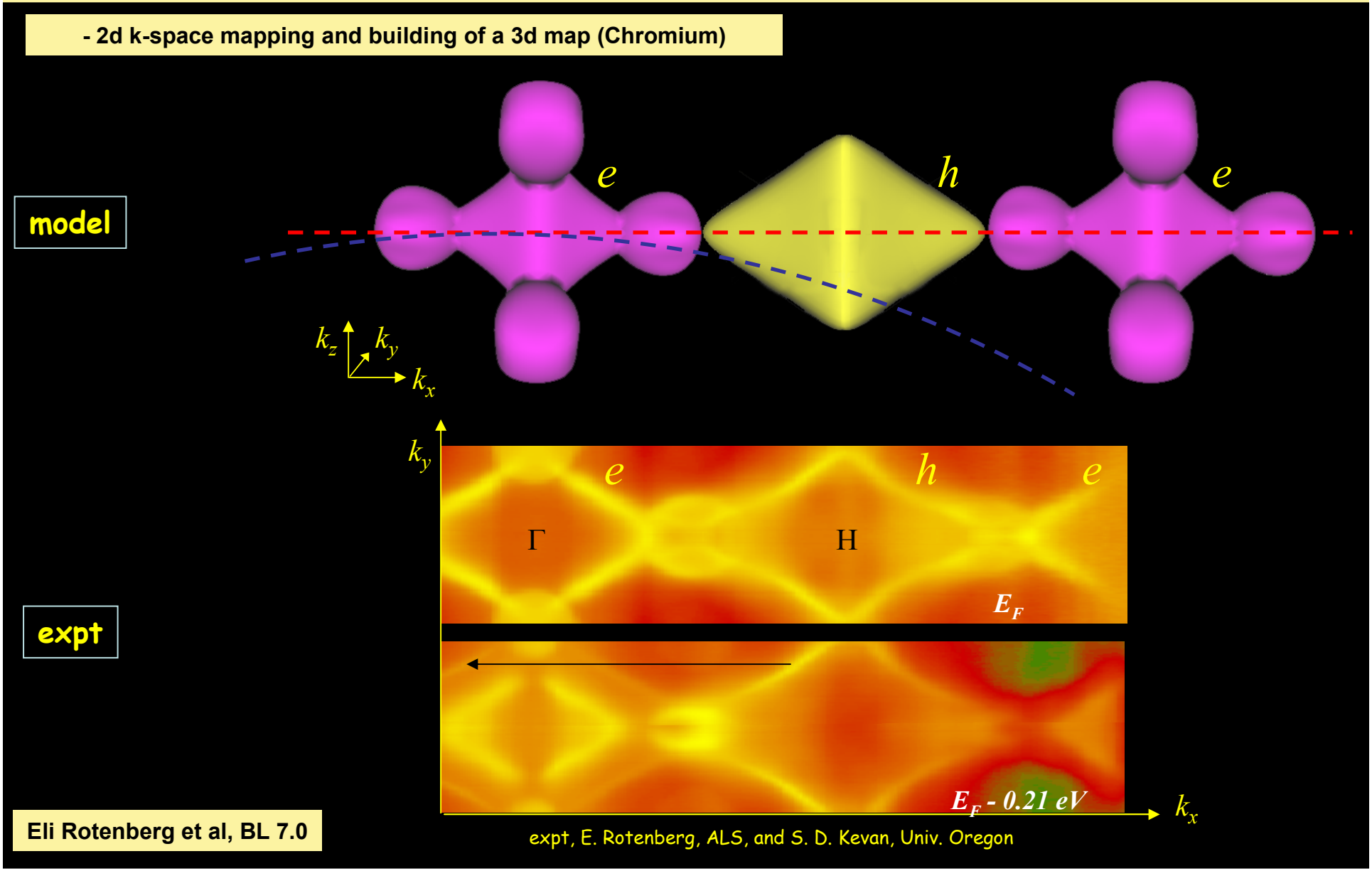




Eli Rotenberg et al, BL 7.0

Angle Resolved Photo-Electron Spectroscopy (ARPES)

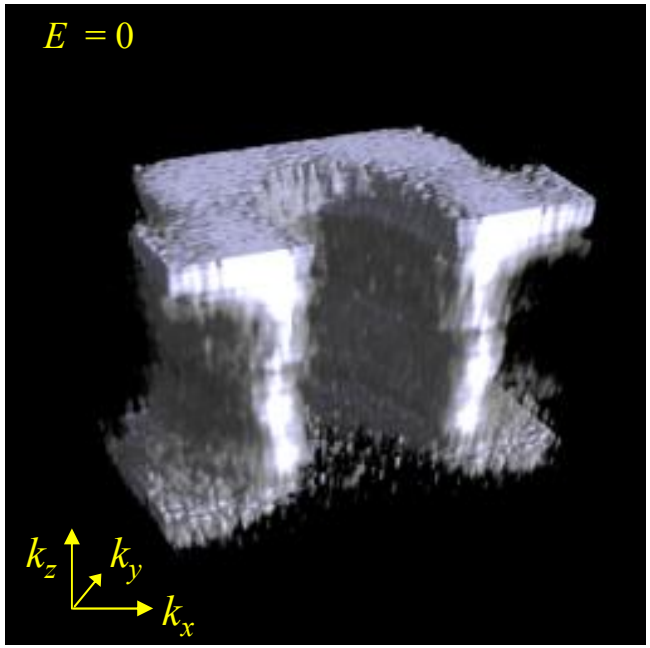
- 2d k-space mapping and building of a 3d map (Chromium)



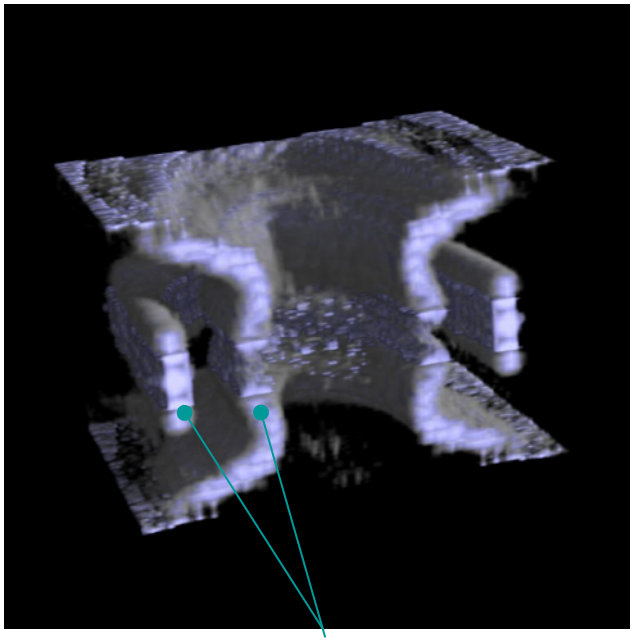
Angle Resolved Photo-Electron Spectroscopy (ARPES)

- 3-D Fermi Surface (extracted from 4-D data)
  - For example, rare earth ferromagnet Terbium

Paramagnetic
T=240°K



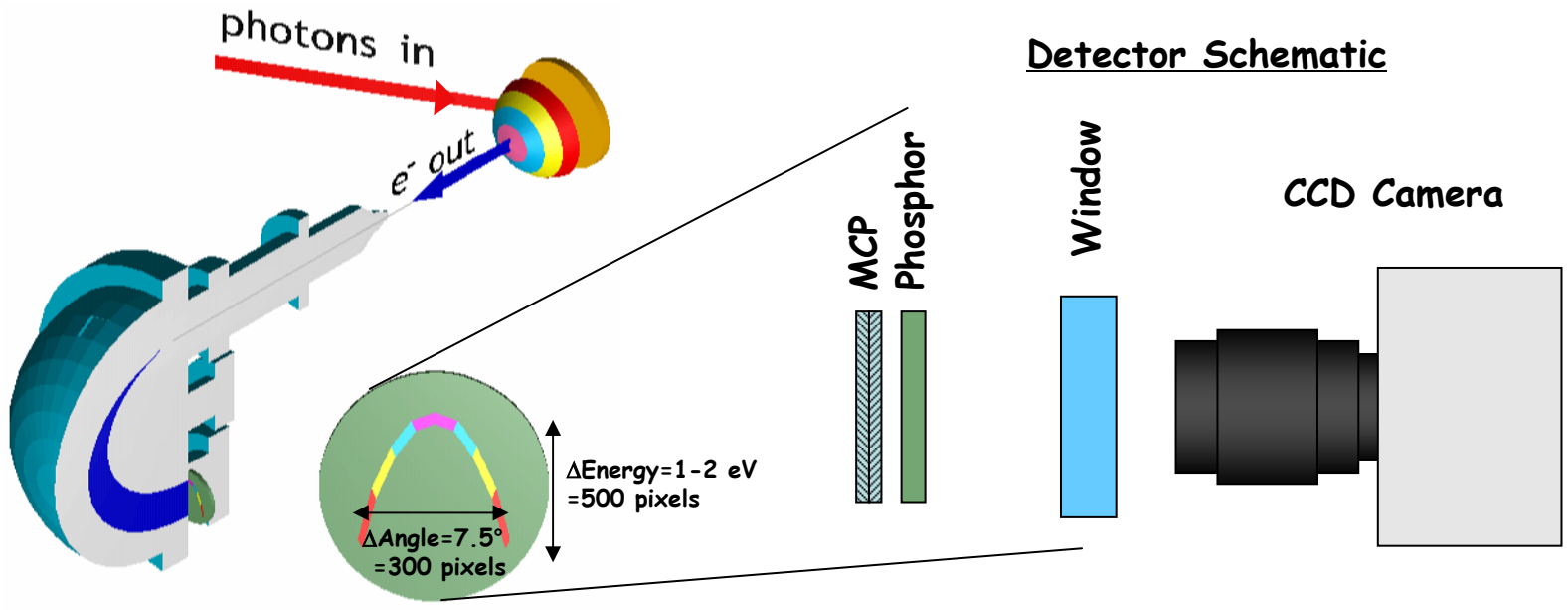
Ferromagnetic
T=30°K



exchange-split Fermi Surface

Fermi surface data courtesy of K. Starke, FU Berlin

Angle Resolved Photo-Electron Spectroscopy (ARPES)



- Count Rate is the most significant detector limitation
  - Could be improved with faster phosphors, lower readout noise of CCD, faster data collection and processing

- Angle Resolution limited approx. equally by
  - sizes of electron spot on phosphor, # pixels on CCD camera, photon beam on sample