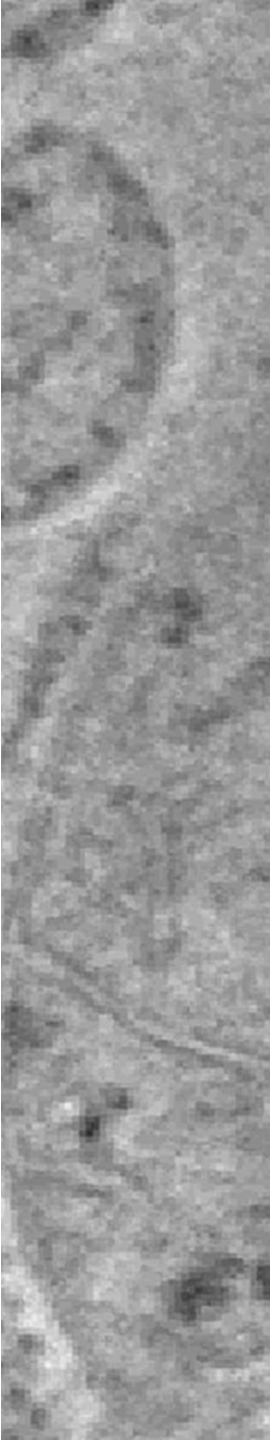
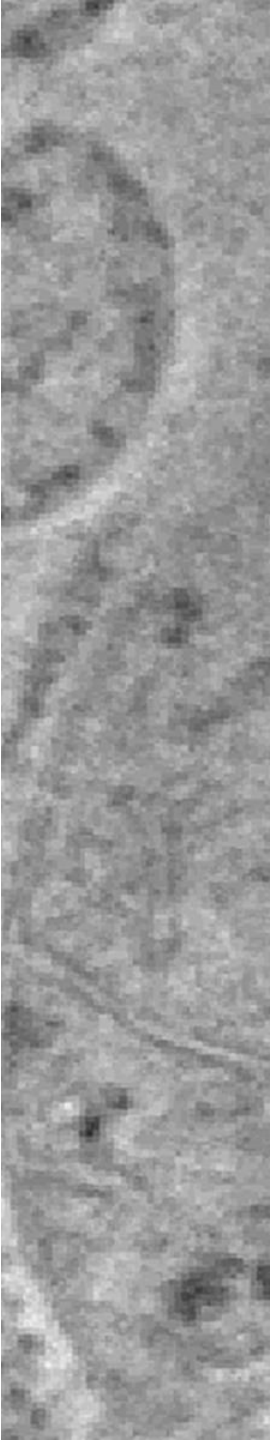


IMpaCT IMAGING LIFE FROM MOLECULES TO CELLS

An introduction to electron tomography & cryo-tomography

Online workshop: Introduction to Cryo-Tomography

Michael Elbaum
Dept of Chemical and Biological Physics
Weizmann Institute of Science



Definitions of Tomography

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Cambridge: the use of X-rays to make three-dimensional images of flat sections of the body.

Oxford: a way of producing an image of the inside of the human body or a solid object using X-rays or ultrasound.

Webster: a method of producing a three-dimensional image of the internal structures of a solid object (such as the human body or the earth) by the observation and recording of the differences in the effects on the passage of waves of energy impinging on those structures.

Wikipedia: Tomography is imaging by sections or sectioning through the use of any kind of penetrating wave. A device used in tomography is called a tomograph, while the image produced is a tomogram.

Joachim Frank: Tomography is a method for reconstructing the interior of an object from its projections.

A set of methods for reconstructing a 3D representation from multiple 2D recordings.

Projections vs Slices :: Cartesian vs Radial/Spherical

Radon/Radial Tomography

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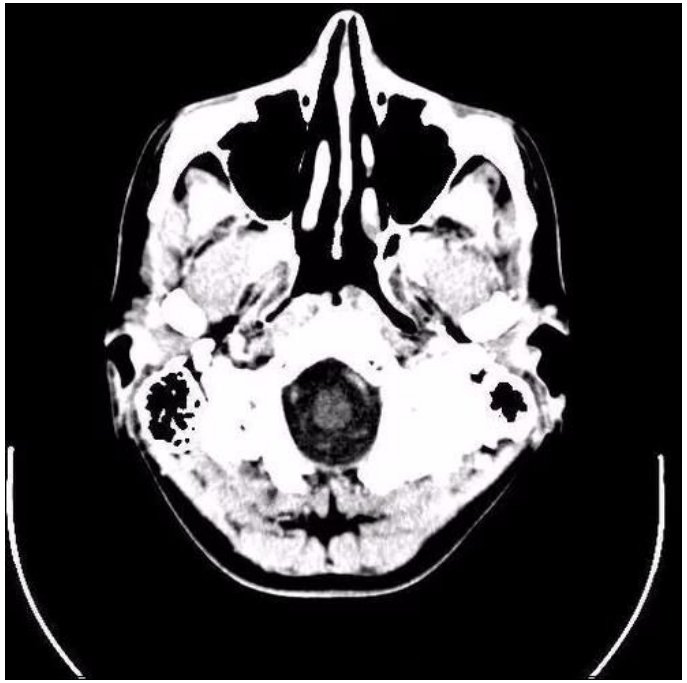
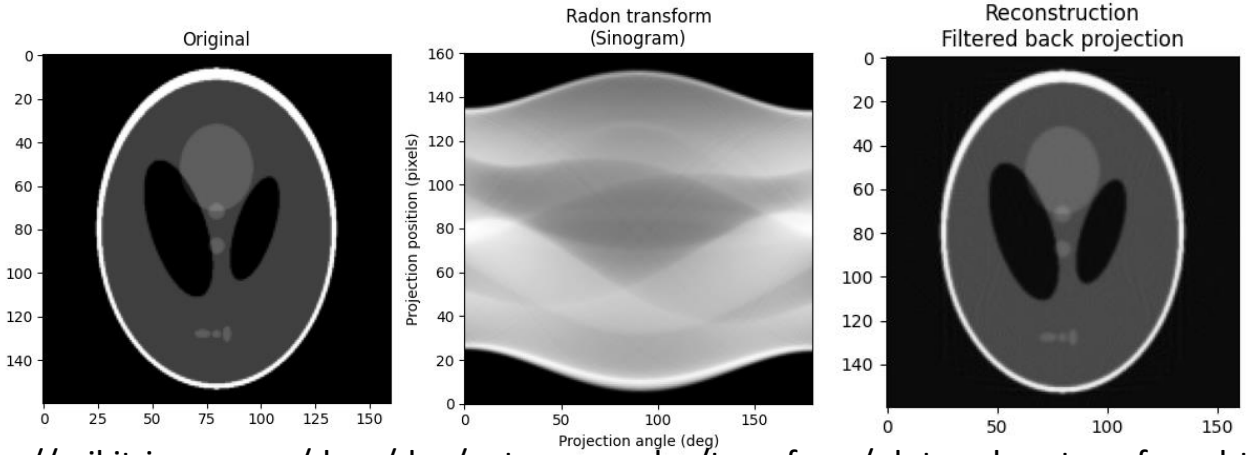
https://en.wikipedia.org/wiki/CT_scan



https://en.wikipedia.org/wiki/Computed_tomography_of_the_head



https://scikit-image.org/docs/dev/auto_examples/transform/plot_radon_transform.html

Cartesian Tomography

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- Serial sections by transmission EM
- Serial surfaces by scanning EM
- Array tomography

Cartesian Tomography

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Cartesian Tomography

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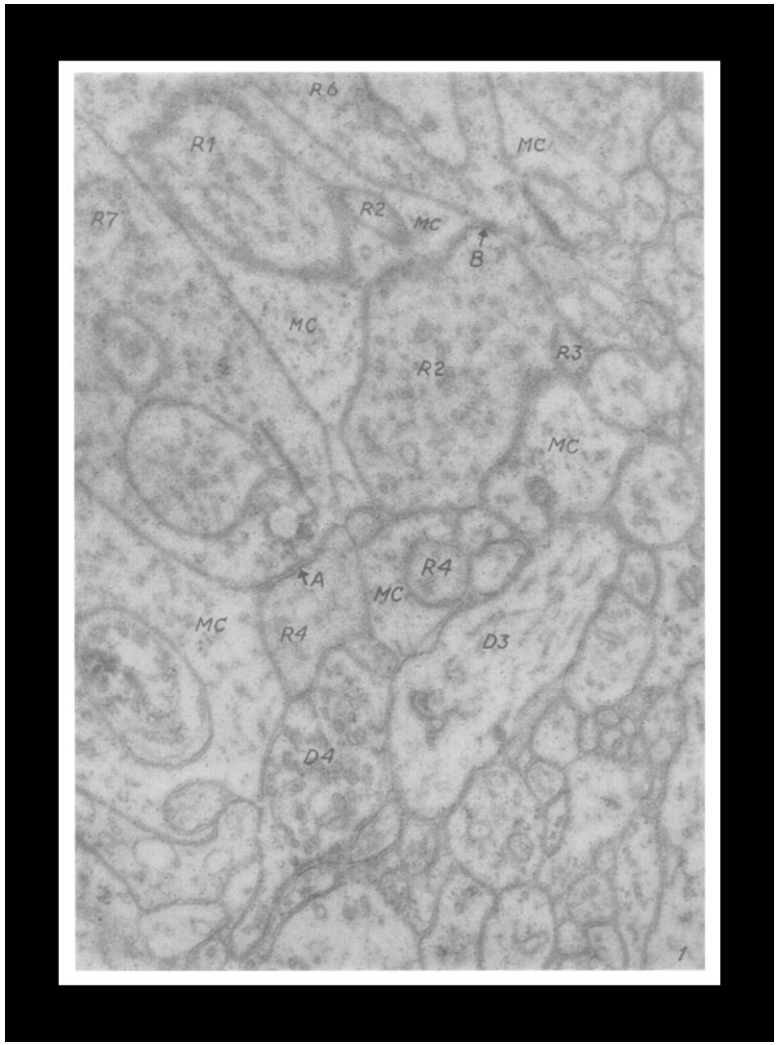
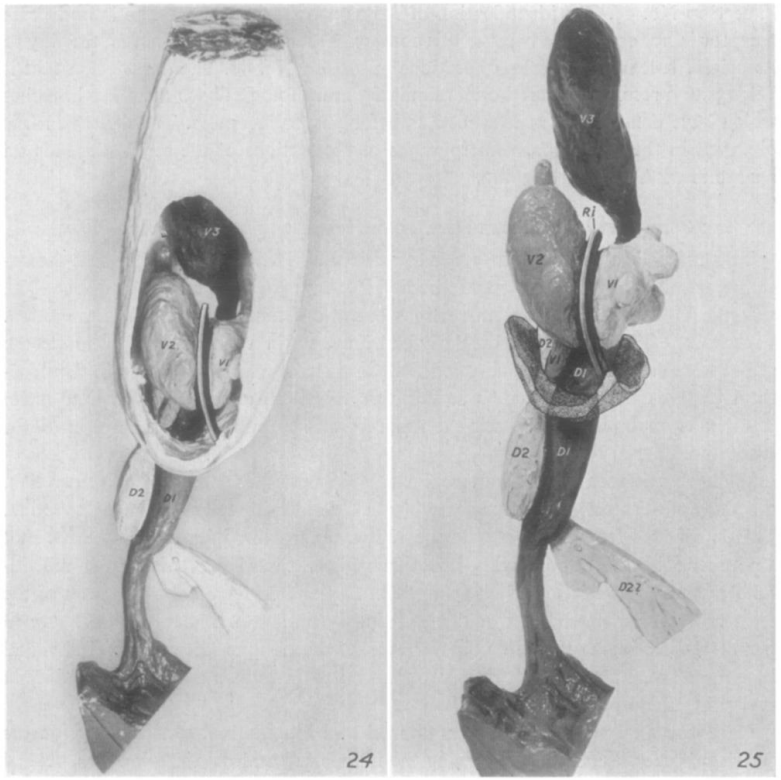
- On the importance of sample preparation…
- What did I do to the native state of the native state of the egg?
- Justification for cryo: fixation in fully hydrated state!

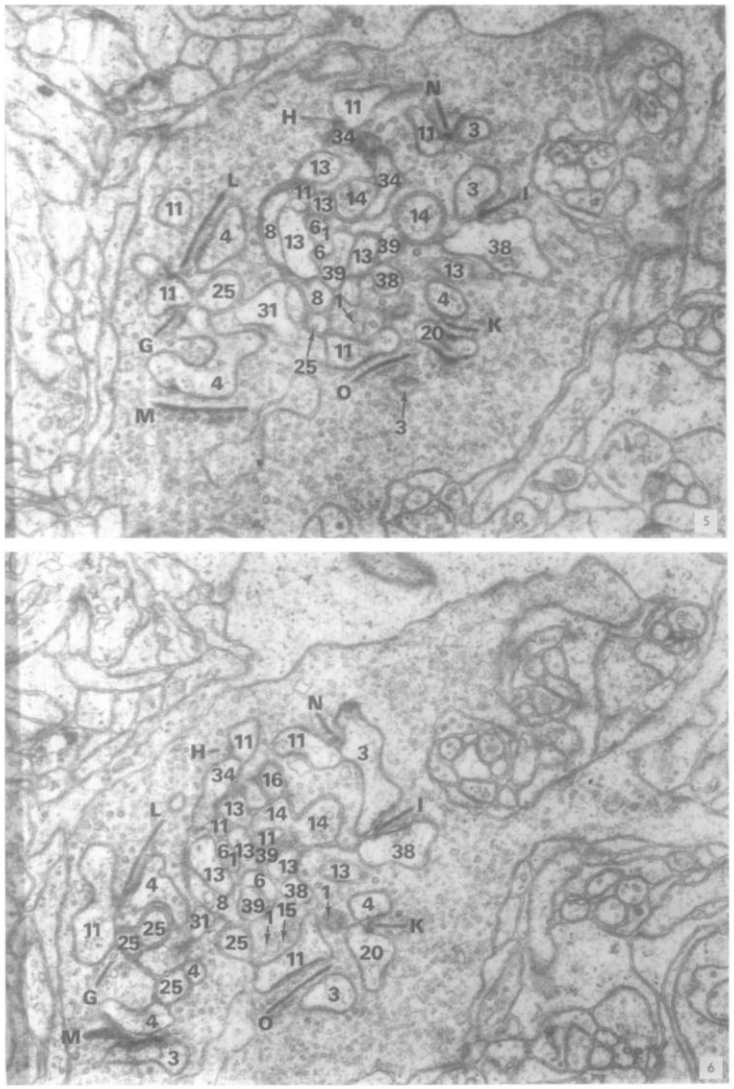
Serial Sections (1958)

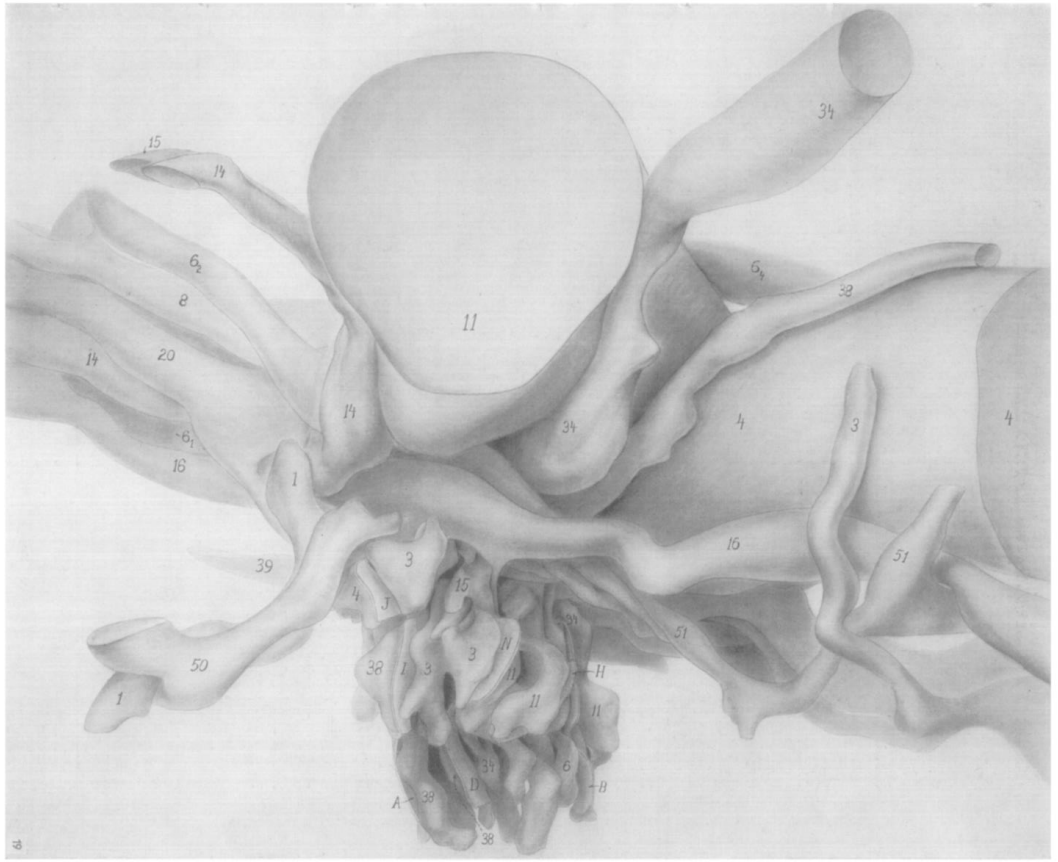




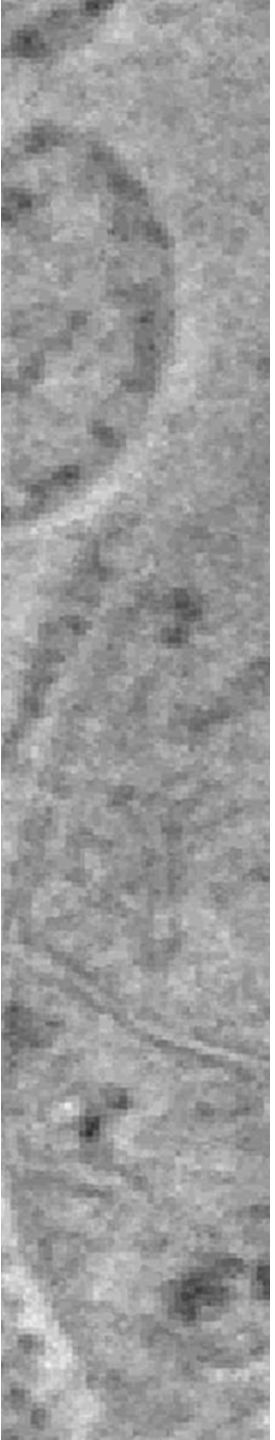
Sjostrand, F. Ultrastructure of Retinal Rod Synopses of the Guinea Pig Eye as Revealed by Three Dimensional Reconstructions from Serial Sections. J Ultrastructure Research 2, 122-170 (1958)

Serial Sections (1974)





Sjostrand, F. A Search for the Circuitry of Directional Selectivity and Neural Adaptation through Three-Dimensional Analysis of the Outer Plexiform Layer of the Rabbit Retina.
J Ultrastructure Research 49,60-156 (1974)



Three R's of Tomography

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Recording:	raw data, slices/sections, projections
Reconstruction:	2D $\rightarrow$ 3D, alignment, transforms
Rendering:	representations, re-slicing, solid models, transparency who's in front? segmentation, interpretation

Wherefore electrons?

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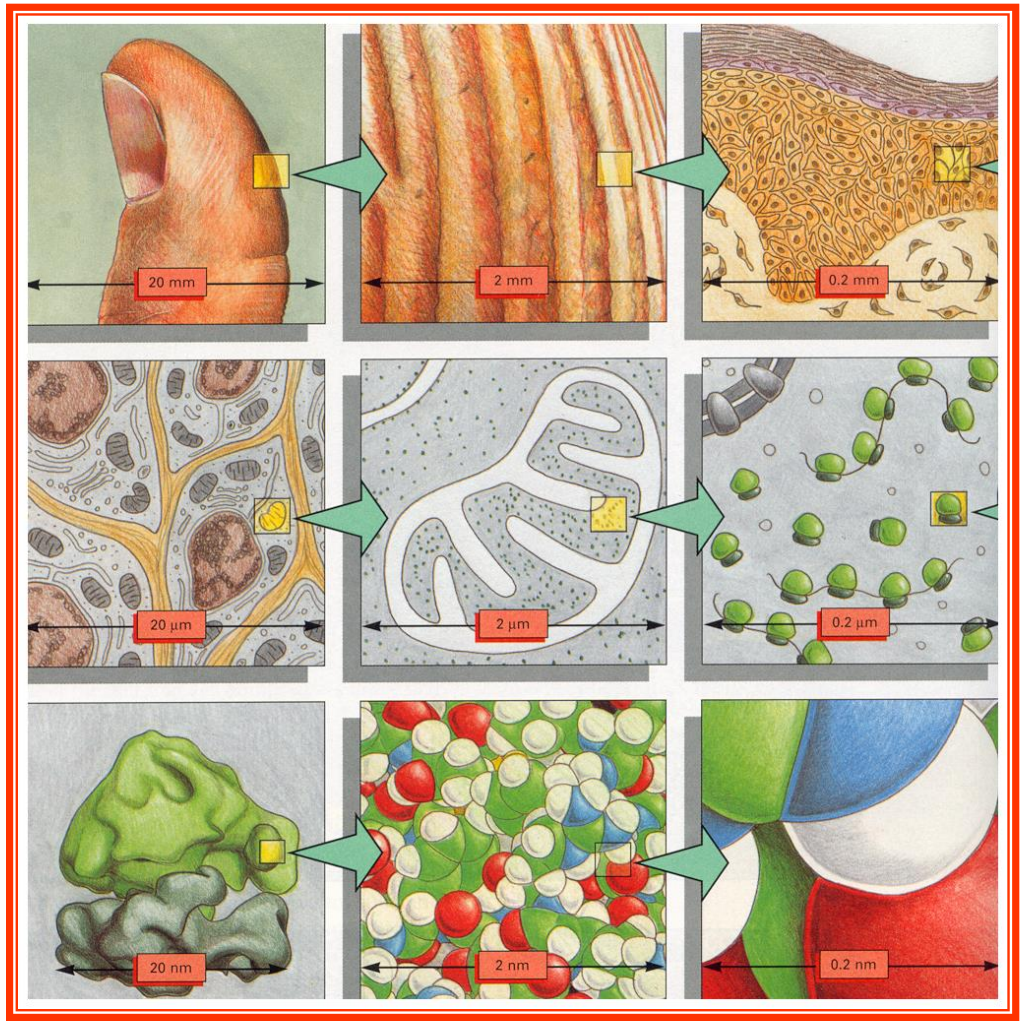

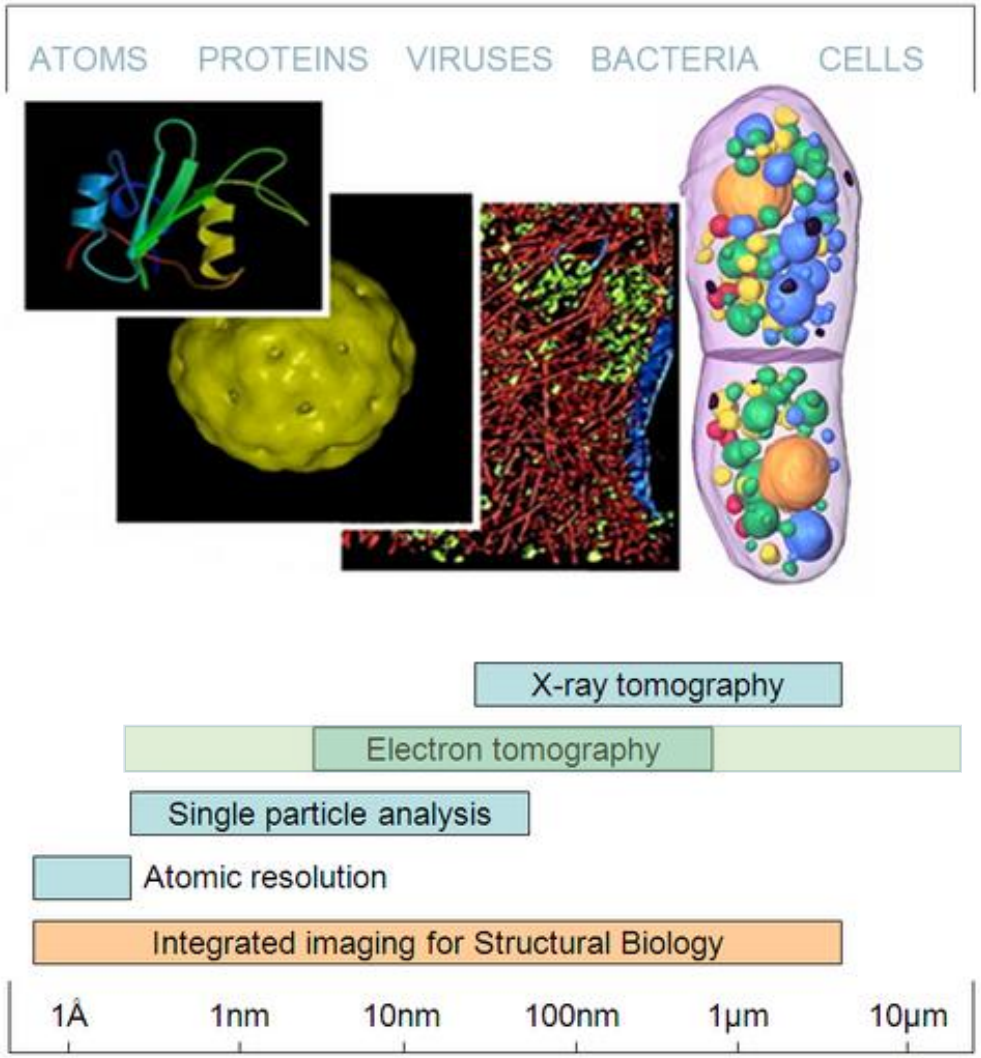

Wherefore electrons?

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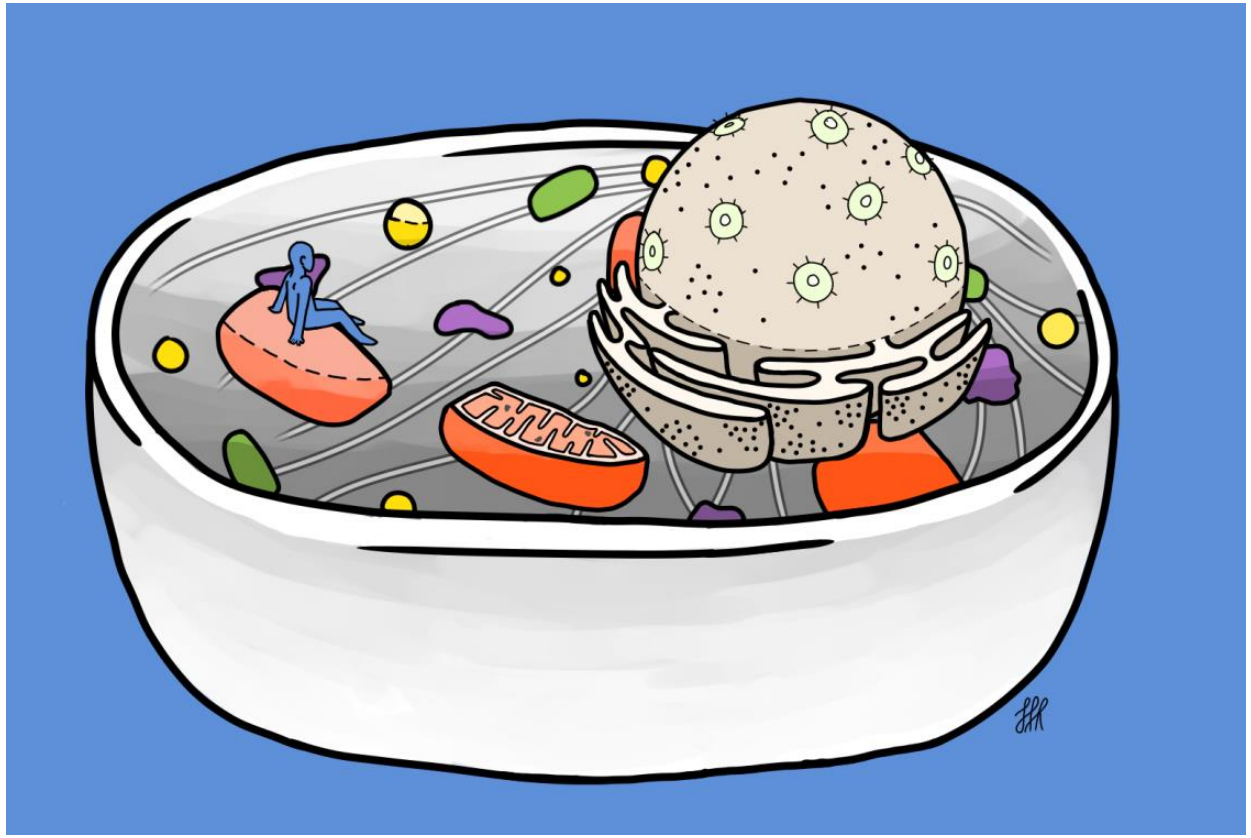
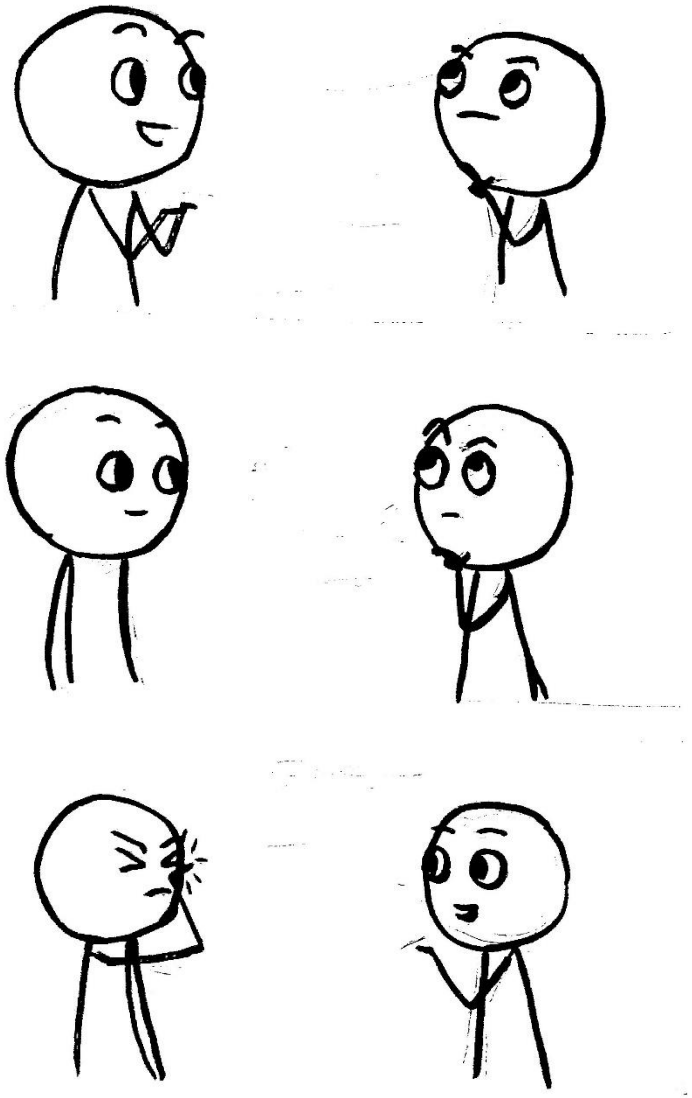
Pro: From tissues to cells to molecules
From microns to Angstroms
High or low resolution

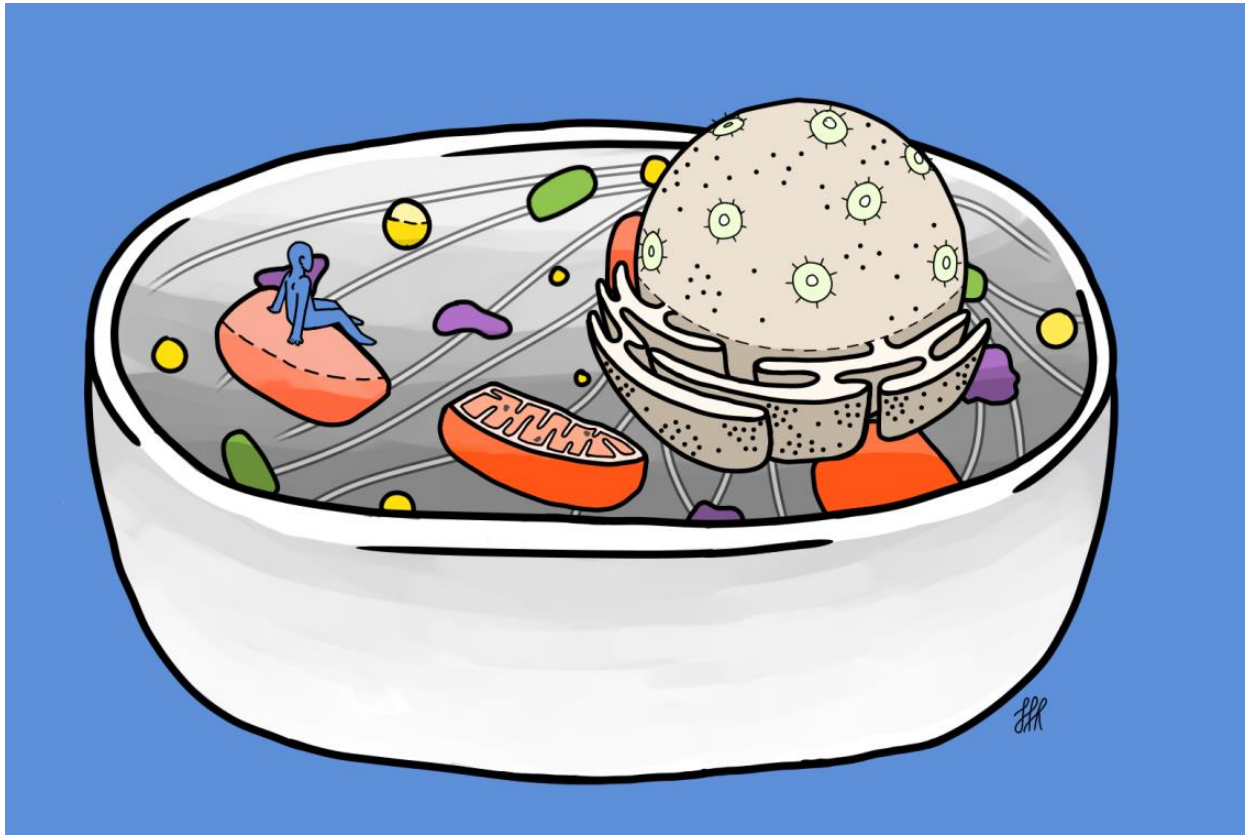
Con: Vacuum
Contrast
Identification



Wherefore electrons?

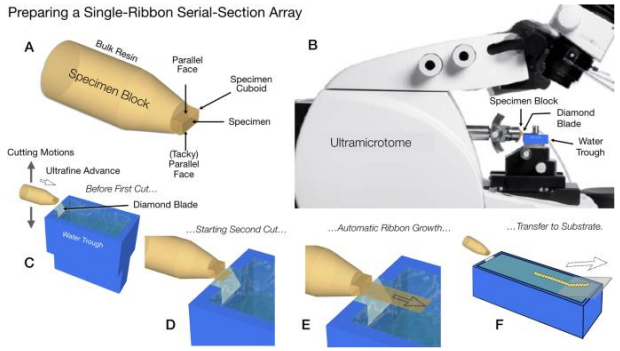
IMpaCT IMAGING LIFE FROM MOLECULES TO CELLS

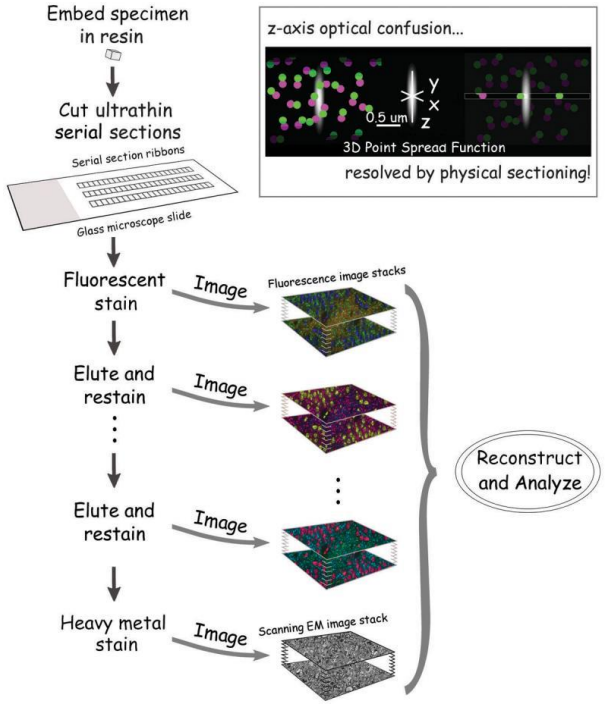




Array Tomography

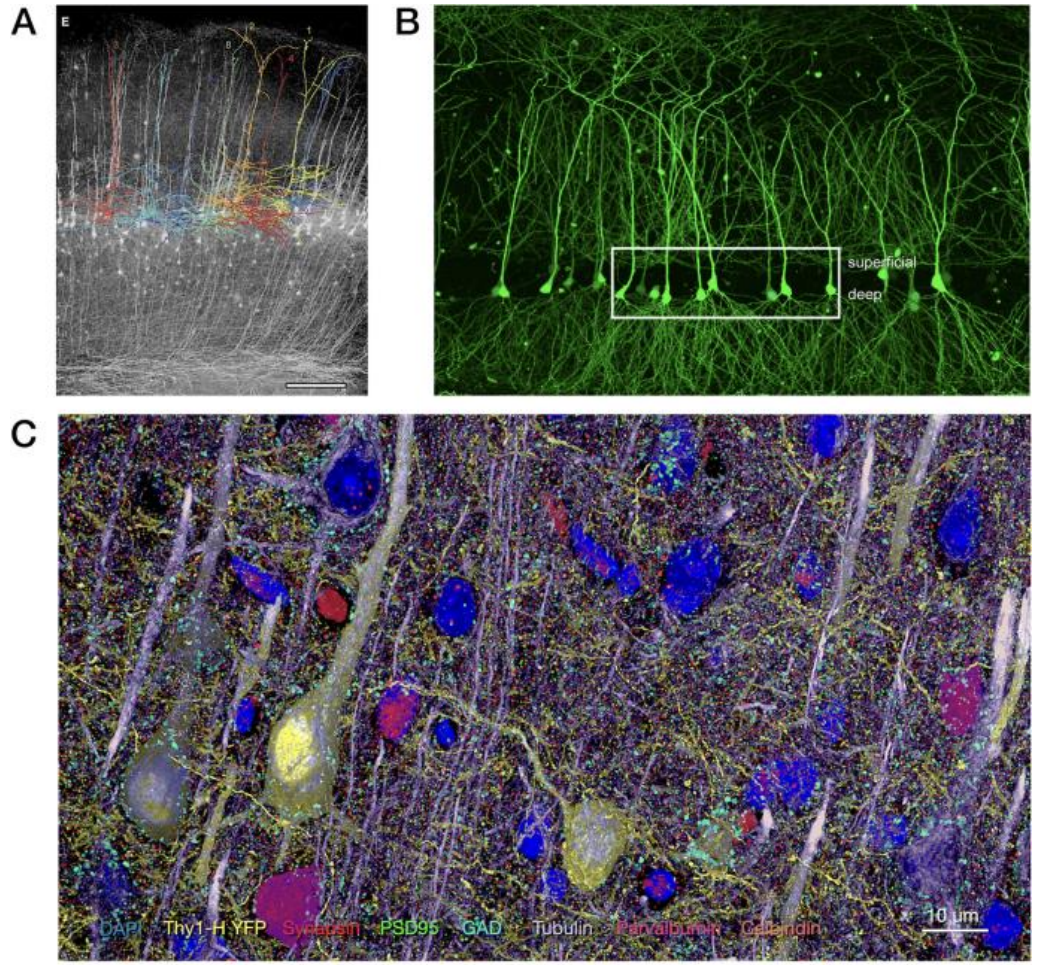
IMpaCT IMAGING LIFE FROM MOLECULES TO CELLS





Micheva K.D. and Smith, S.J. Array tomography: a new tool for imaging the molecular architecture and ultrastructure of neural circuits. Neuron 55, 25-36 (2007).

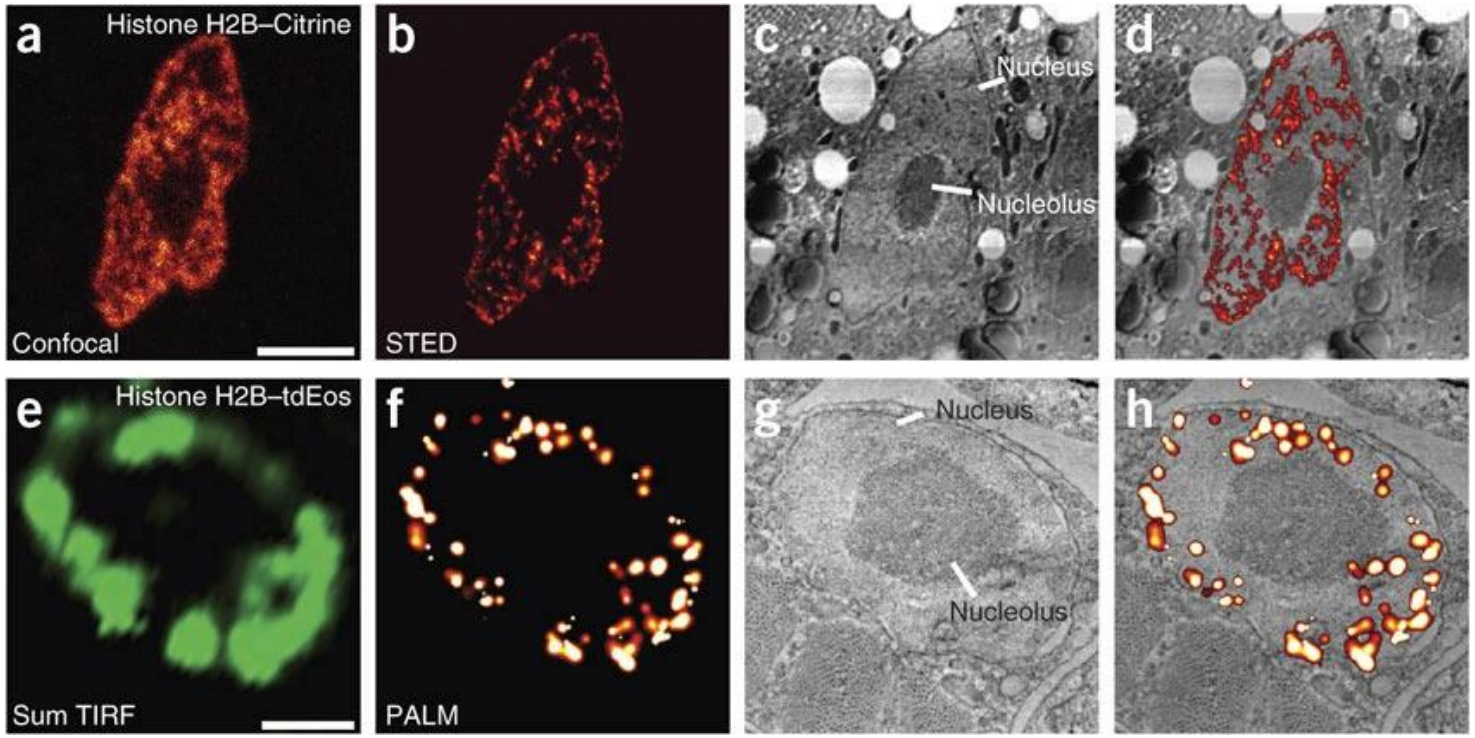
Smith, S.J. Q&A: Array tomography. *BMC Biol* **16,** 98 (2018)



See Movie S2 in https://bmcbiol.biomedcentral.com/articles/10.1186/s12915-018-0560-1

SEM & Super-Resolution

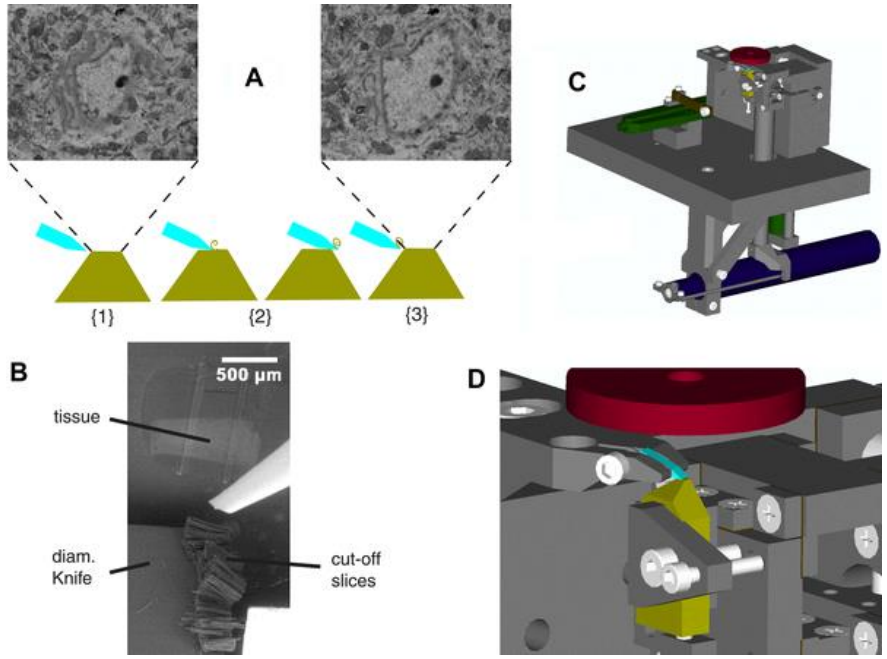
IMPaCT IMAGING LIFE FROM MOLECULES TO CELLS

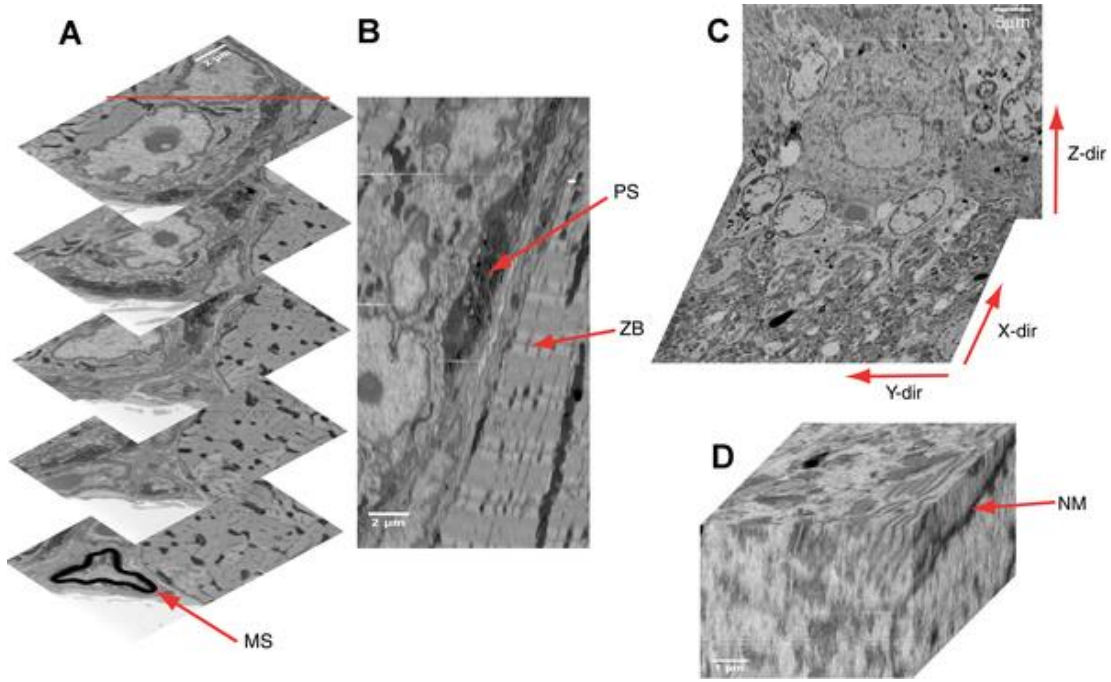


Watanabe, S., Punge, A., Hollopeter, G. *et al.* Protein localization in electron micrographs using fluorescence nanoscopy. *Nat Methods* **8,** 80–84 (2011).

Serial Block Face SEM

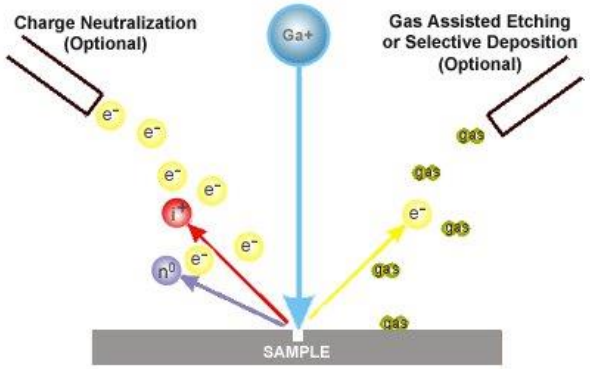
IMAGING LIFE FROM MOLECULES TO CELLS

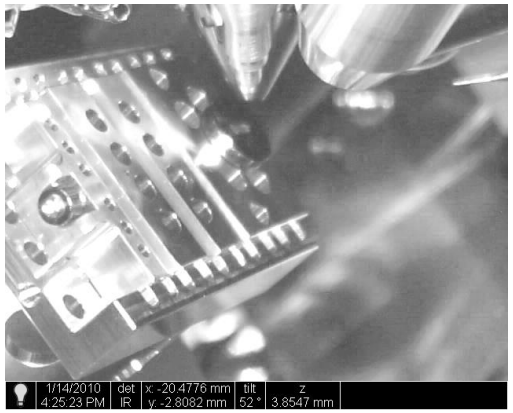


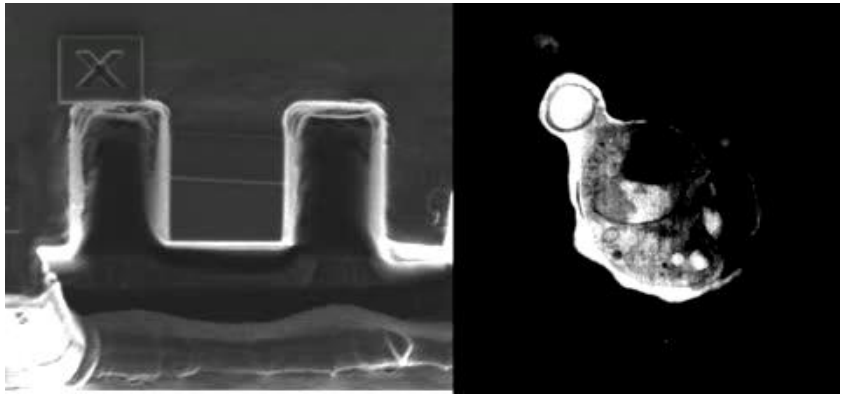


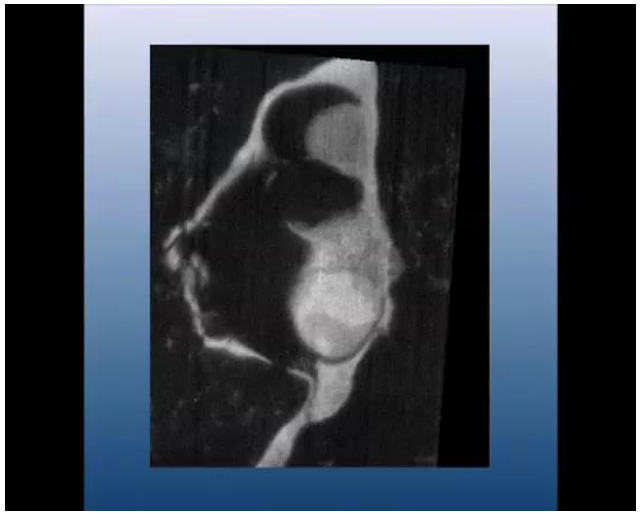
Denk W, Horstmann H (2004) Serial Block-Face Scanning Electron Microscopy to Reconstruct Three-Dimensional Tissue Nanostructure. PLOS Biology 2(11): e329.

Focused Ion Beam - SEM









Heymann et al, Site-specific 3D imaging of cells and tissues with a dual beam microscope. J Struct Biol. (2006)

Weiner, A, Dahan-Pasternak, N. 3D nuclear architecture reveals coupled cell cycle dynamics of chromatin and nuclear pores in the malaria parasite Plasmodium falciparum. Cell Microbiol. 13, 967-977 (2011).

Cryo 3D FIB - SEM

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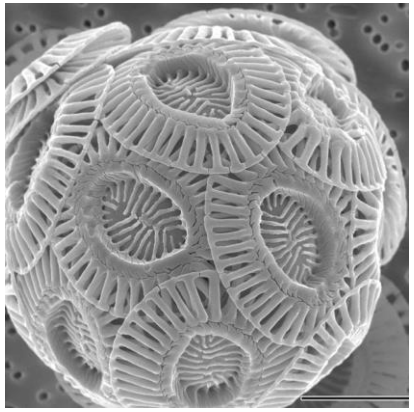
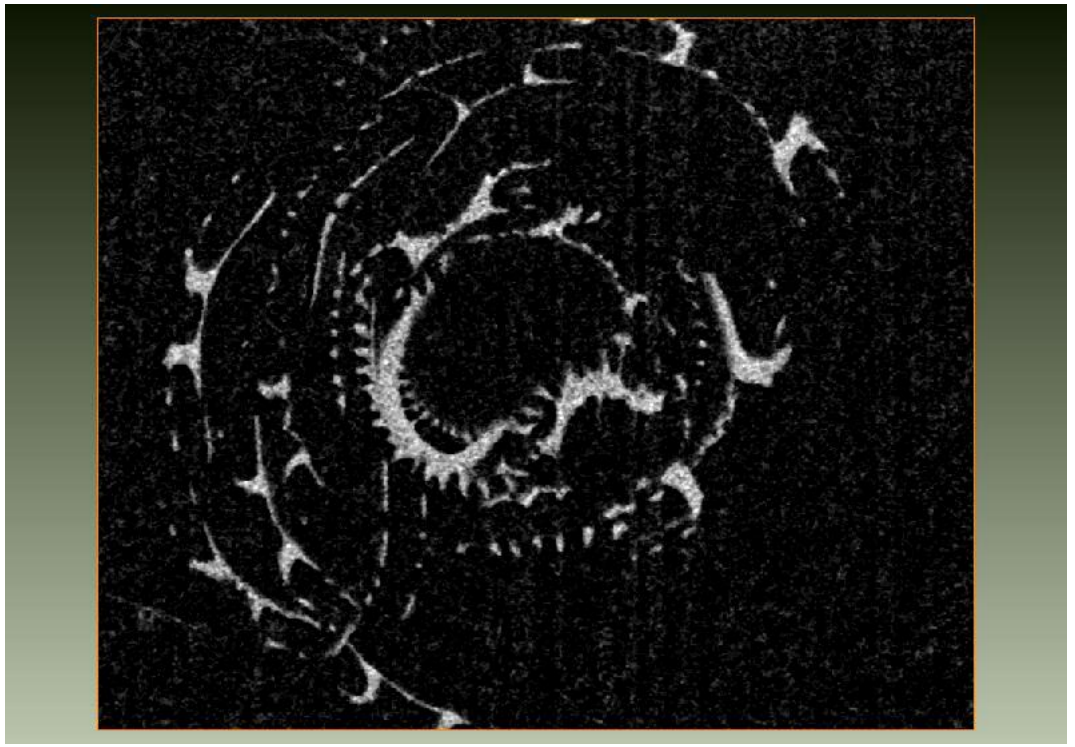
Emiliania huxleyi coccolithophore



Taylor, A. PLoS Biology, June 2011

NASA Landsat, wikipedia



Sviben, S., Gal, A., Hood, M. et al. A vacuole-like compartment concentrates a disordered calcium phase in a key coccolithophorid alga. Nat Commun 7, 11228 (2016).

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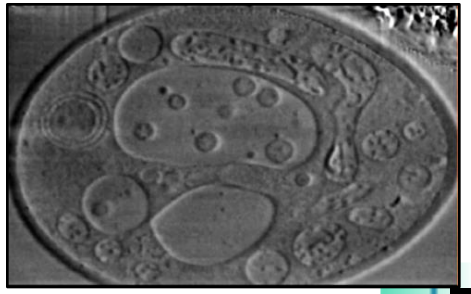
IMAGING LIFE FROM MOLECULES TO CELLS

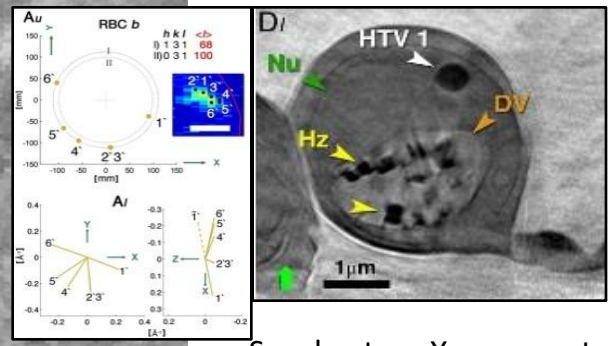
Cryo-microscopy across the scales



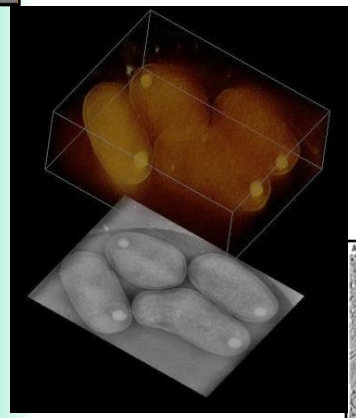
SERIAL SURFACE

Pereman & Rechav, unpubl.

SOFT X-RAY TOMO



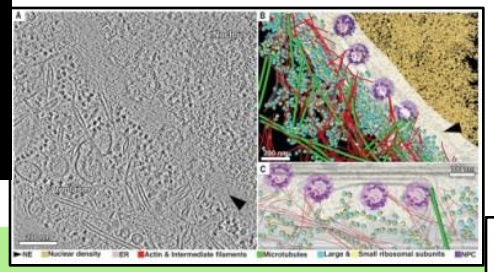
Synchrotron X-ray cryo-tomo
Spectroscopy & Diffraction
Kapishnikov et al, 2012



CSTET

Wolf et al, 2014

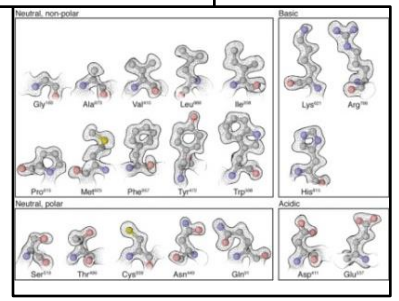
PHASE PLATE TOMO



Mahamid et al, 2016

SUB-TOMO AVERAGING

Turoňová et al, 2017



Barteghesi et al, 2018

SINGLE PARTICLE ANALYSIS

Volume (μm^3)

Resolution (1/nm)

Composition (1/ρ)

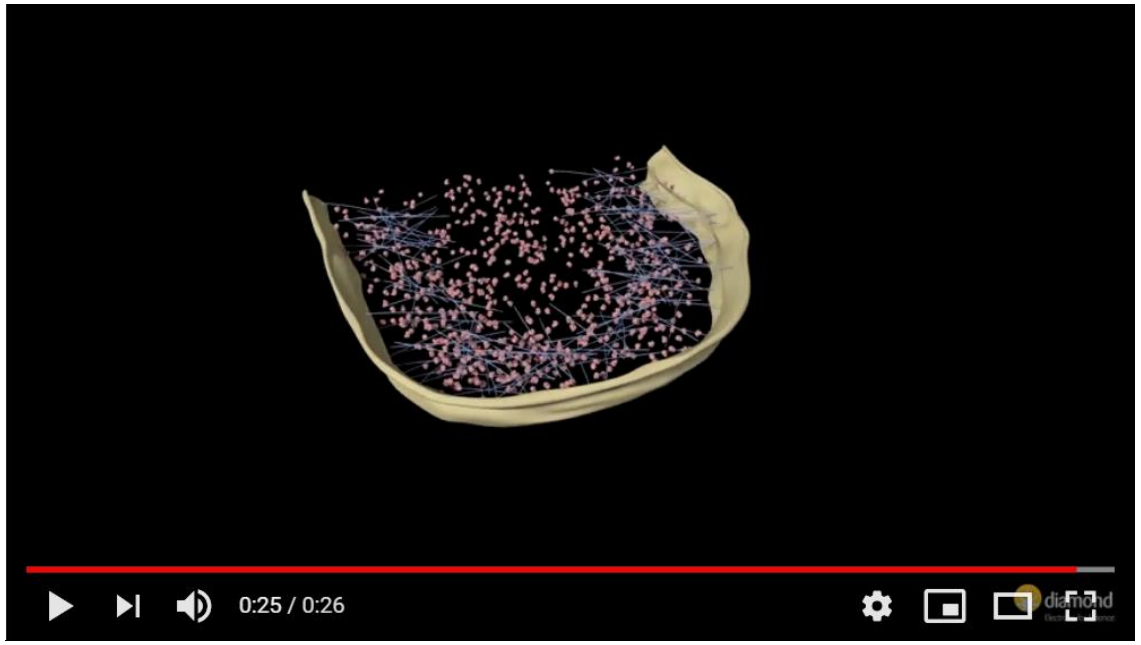
cryo preserves morphology

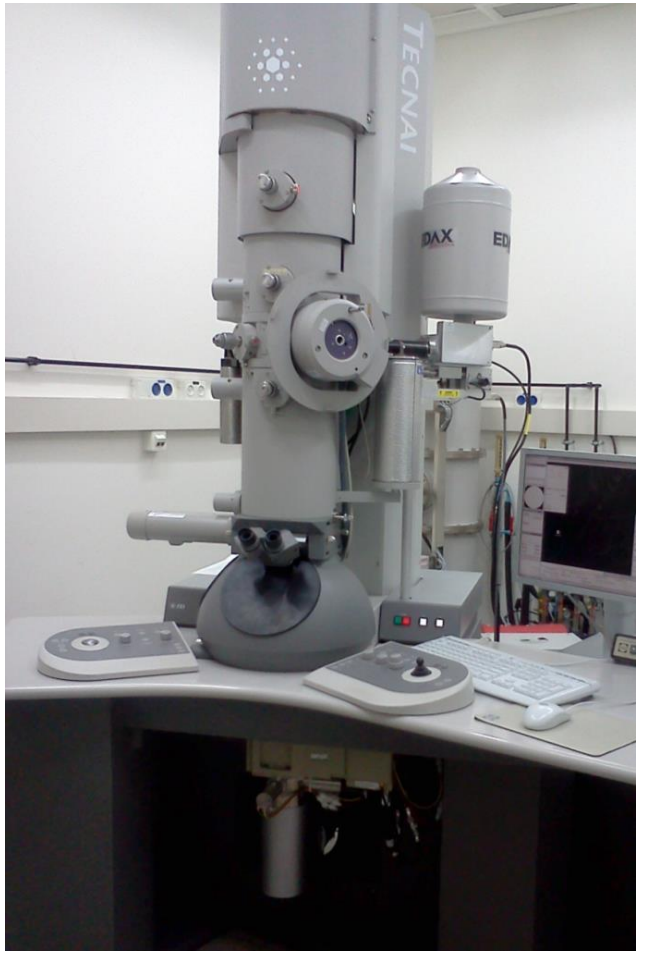
cryo preserves composition

Tilt Tomography in the TEM

IMPACT IMAGING LIFE FROM MOLECULES TO CELLS

https://youtu.be/frgjc-ZNhOY

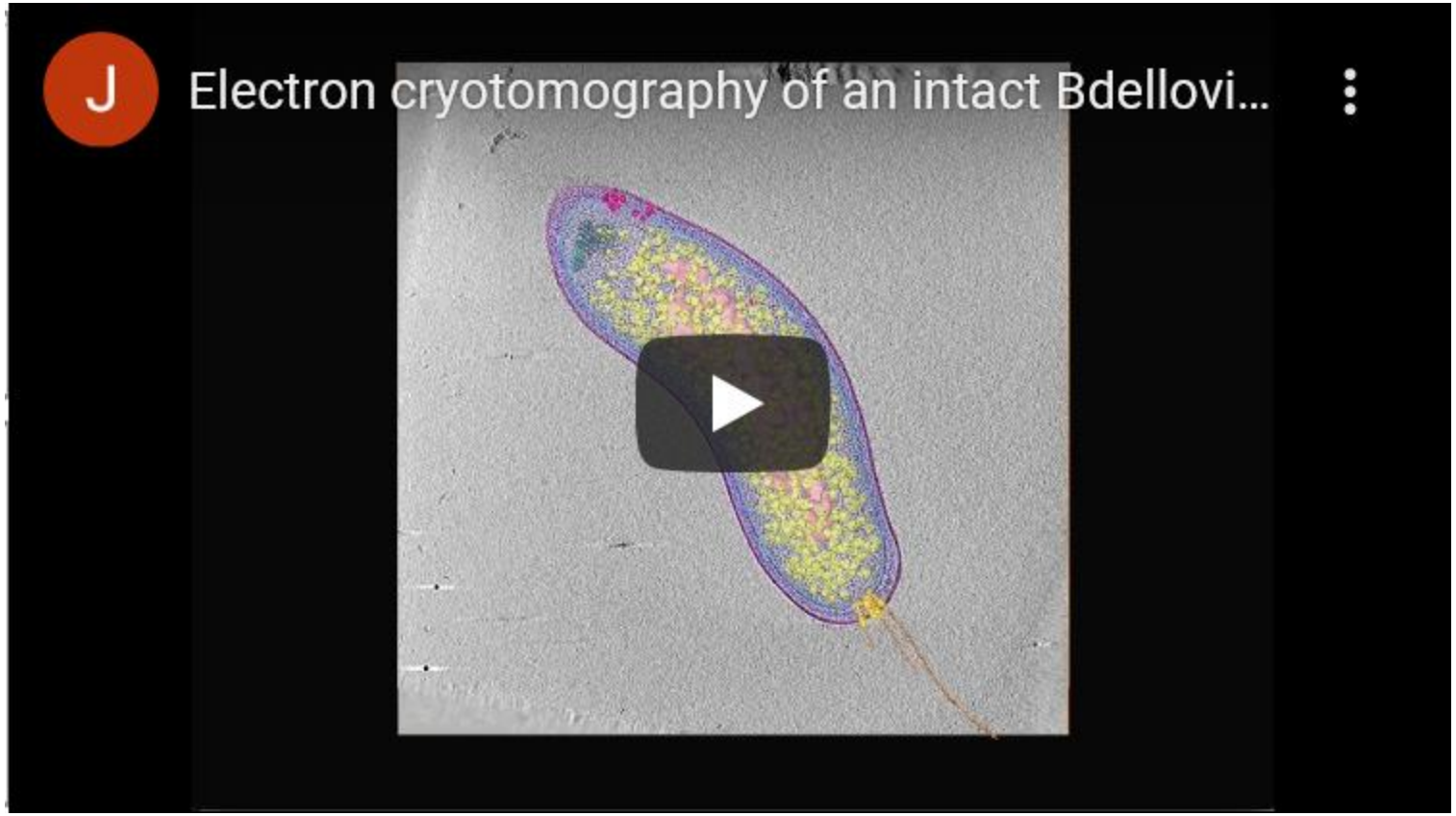




3 R's: Record, Reconstruct, Render

Tilt Tomography in the TEM

IMPACT IMAGING LIFE FROM MOLECULES TO CELLS



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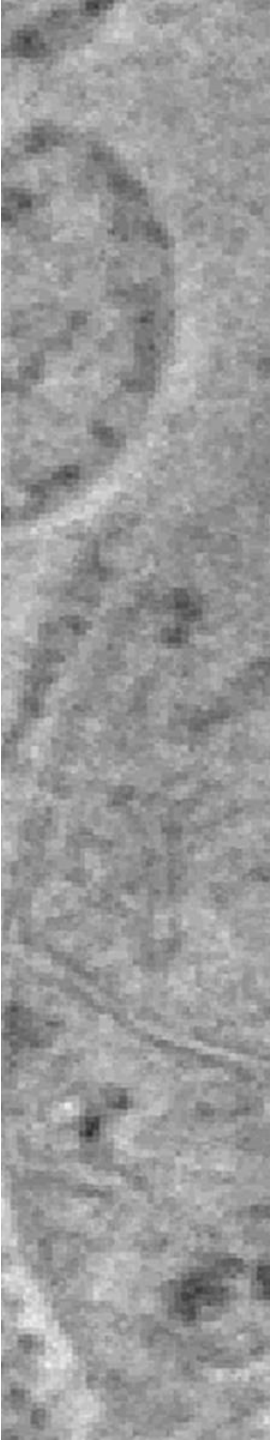


Tilt Tomography in the TEM

IMPACT IMAGING LIFE FROM MOLECULES TO CELLS

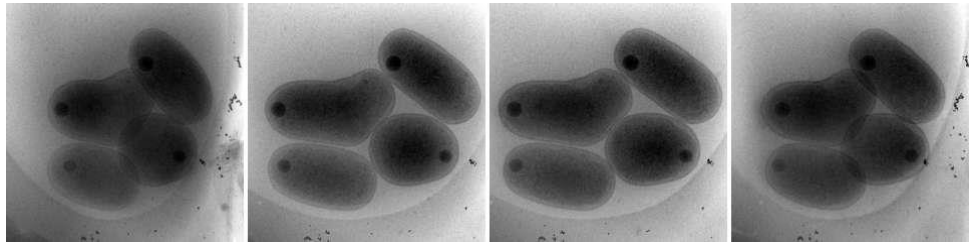
Record: Wide Field Transmission Electron Microscopy
Phase Contrast by Defocus or Phase Plate Optics
Zero-loss Energy Filter
others: Scanning Transmission EM, Soft X-ray Microscopy

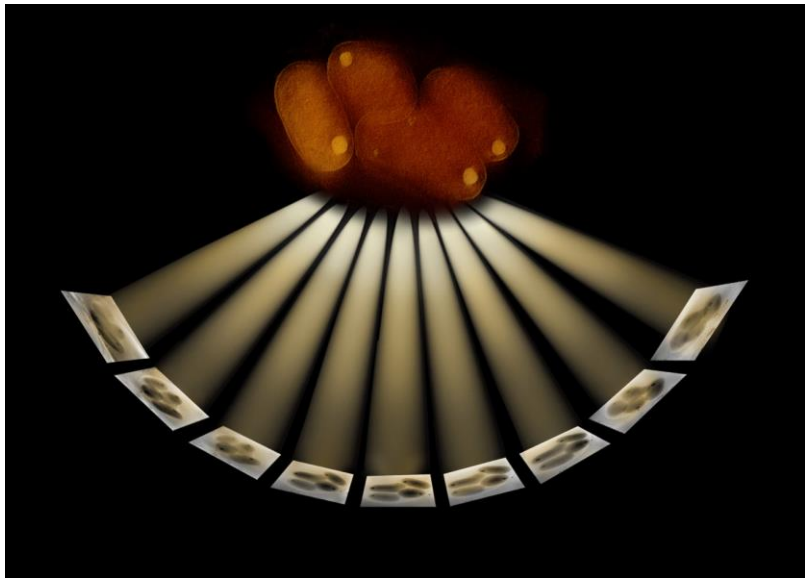
what does the image tell us about the sample?
what generates the contrast?
that is what we'll reconstruct!

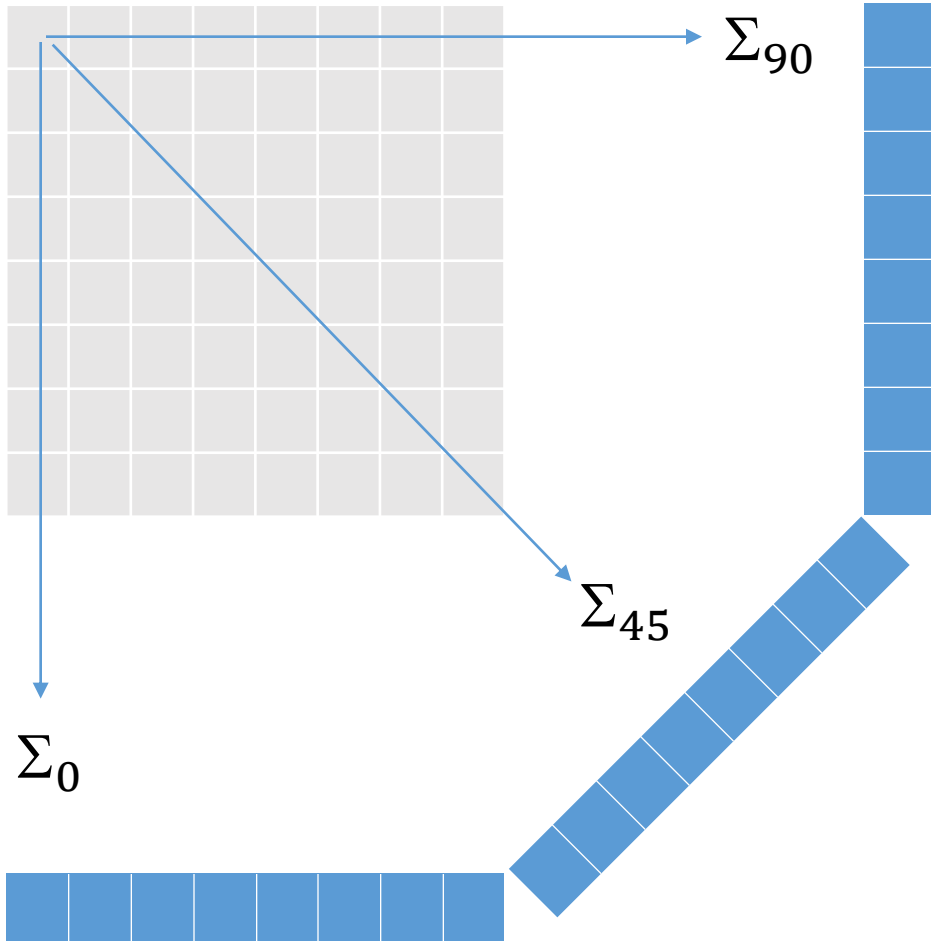
Tilt Tomography in the TEM

IMPACT IMAGING LIFE FROM MOLECULES TO CELLS

Reconstruct: Invert Projections







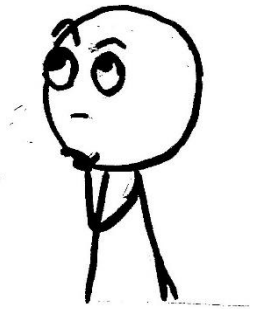
Tilt Tomography in the TEM

IMpaCT IMAGING LIFE FROM MOLECULES TO CELLS

Reconstruct: Invert Projections

$N \times N$ pixels $\rightarrow N^2$ unknowns

N measurements per projection



never enough data …

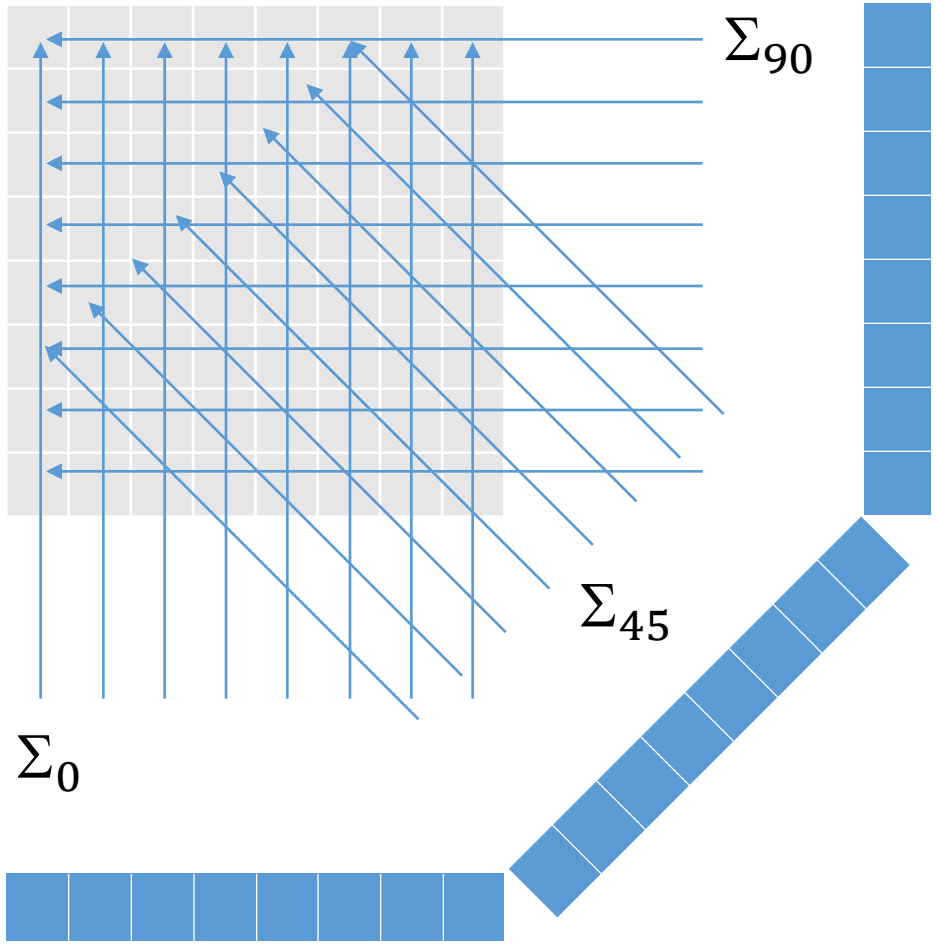
… all methods approximate!

Back-projection

reverse the hands of time & sum

filters compensate missing info

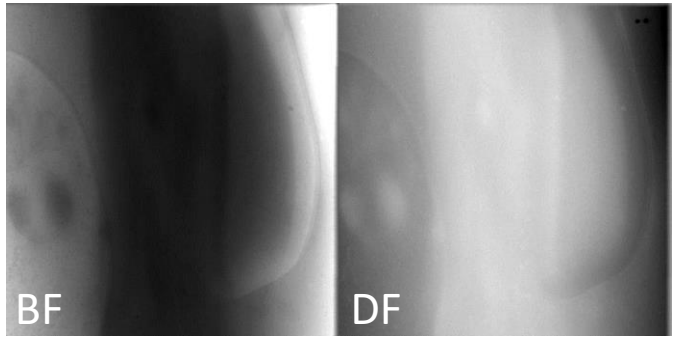
Cryo-EM limited by sample damage

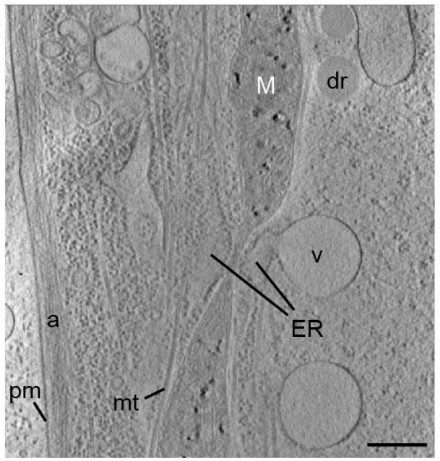


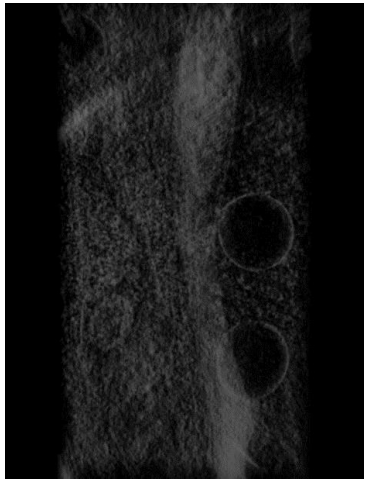
Tilt Tomography in the (S)TEM

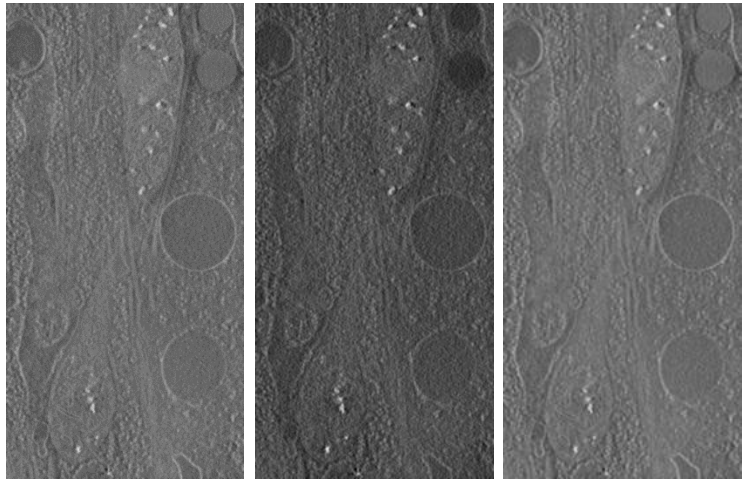
IMpaCT IMAGING LIFE FROM MOLECULES TO CELLS

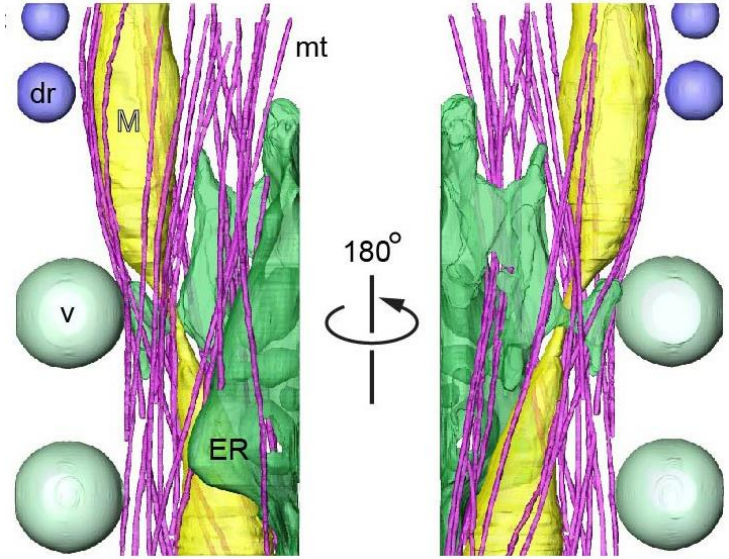
Render: segmentation, manual or automated
point out features of interest (hide others)









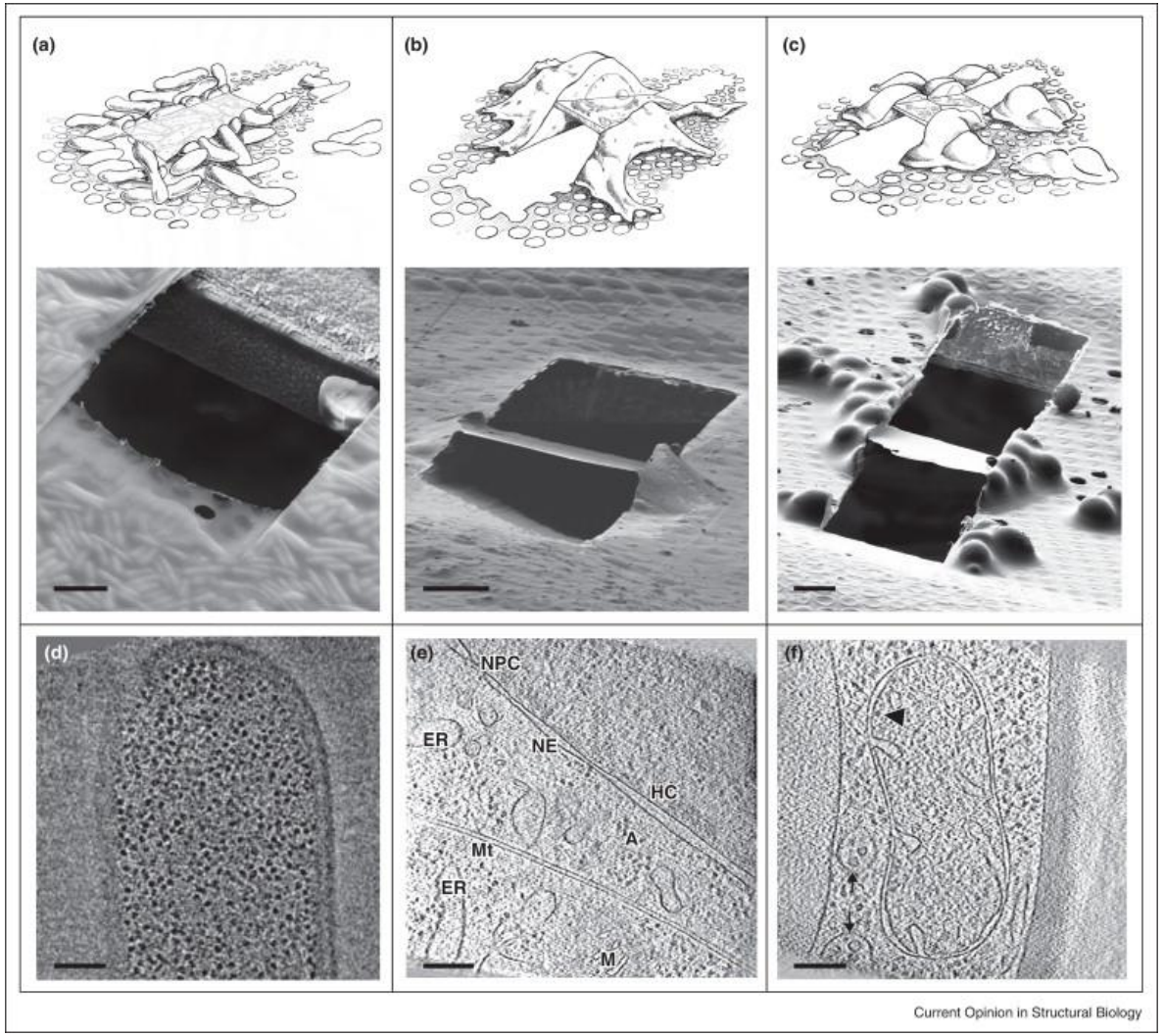


Wolf, SG et al, 3D visualization of mitochondrial solid-phase calcium stores in whole cells. eLife 2017;6:e29929

FIB lamellae

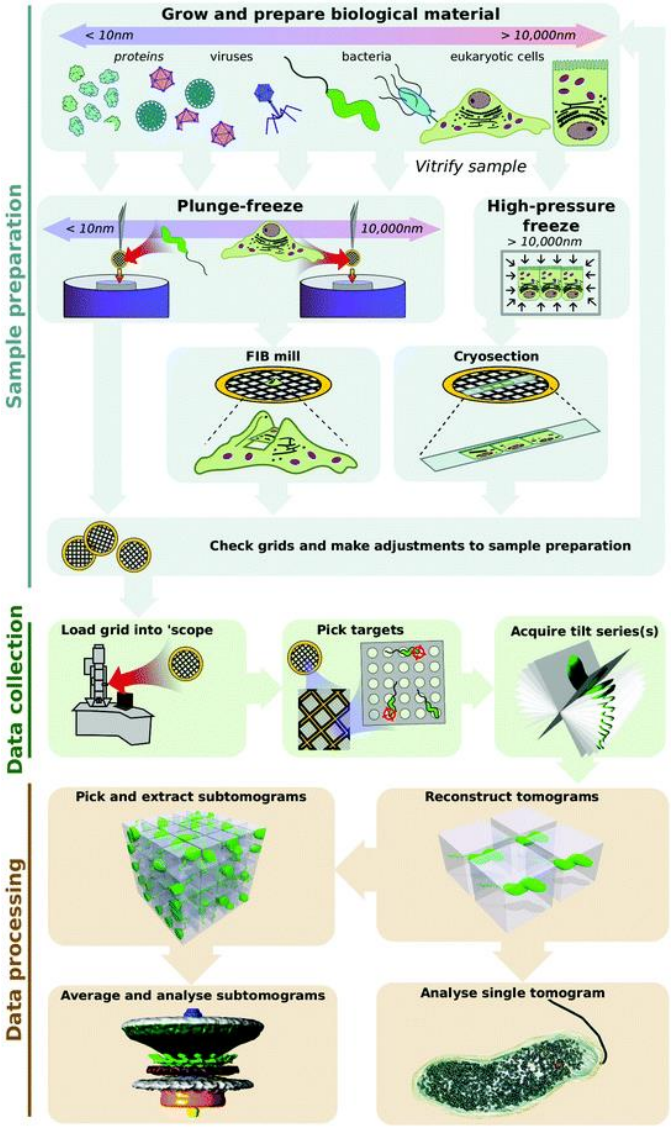
Windows in the Cell

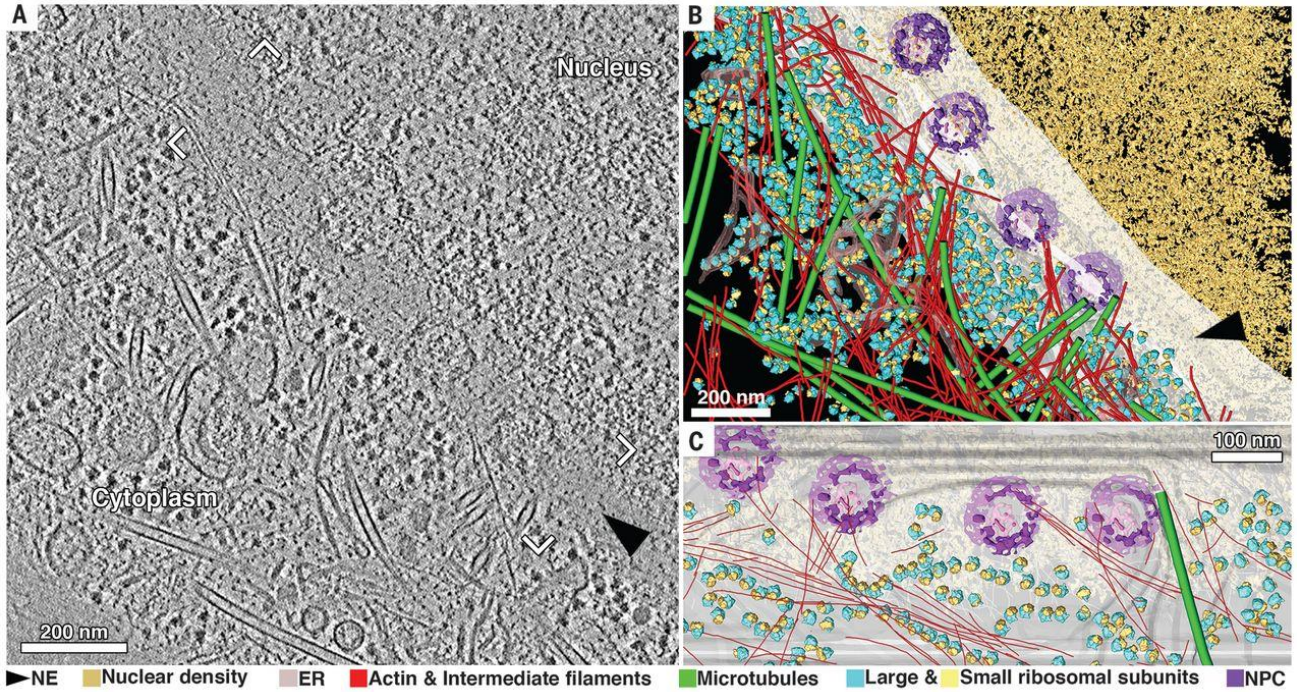
Gain resolution by thinning
Lose 3D
Limits in vitrification



Villa et al, Opening windows into the cell: focused-ion-beam milling for cryo-electron tomography. Curr Opin Struct Biol 23, 771-777 (2013).

FIB lamellae





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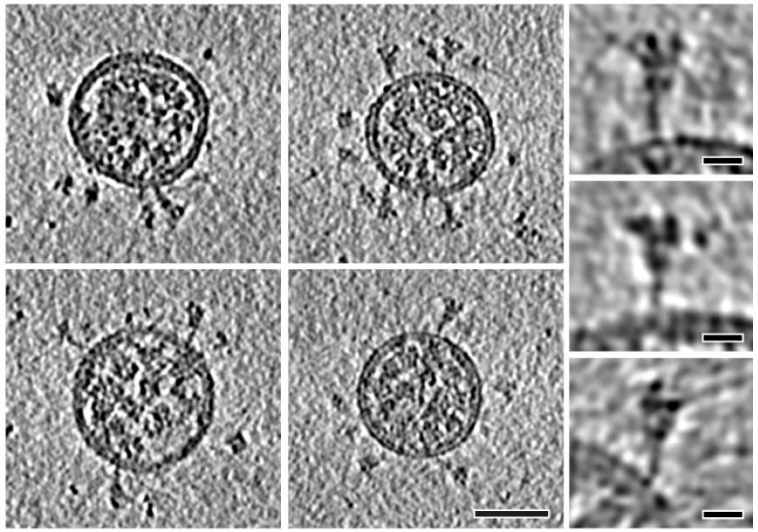
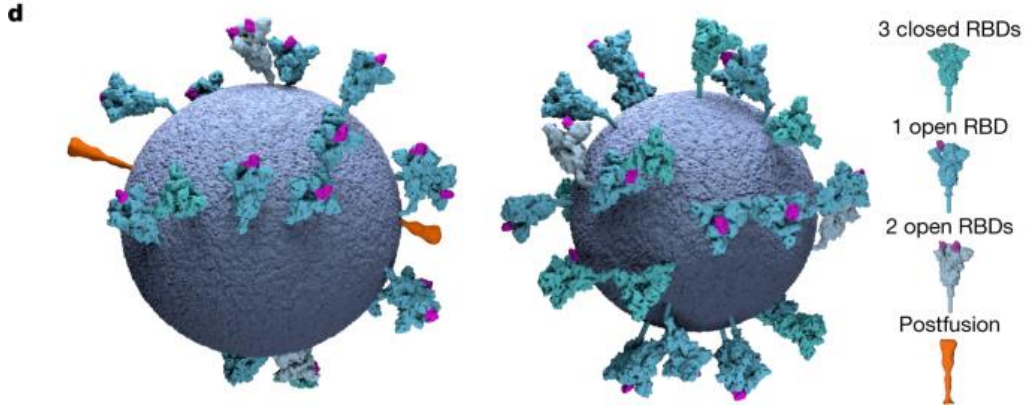
Ferreira J.L., Matthews-Palmer T.R.S., Beeby M. (2018) Electron Cryo-Tomography. In: Hanssen E. (eds) Cellular Imaging. Biological and Medical Physics, Biomedical Engineering. Springer, Cham.

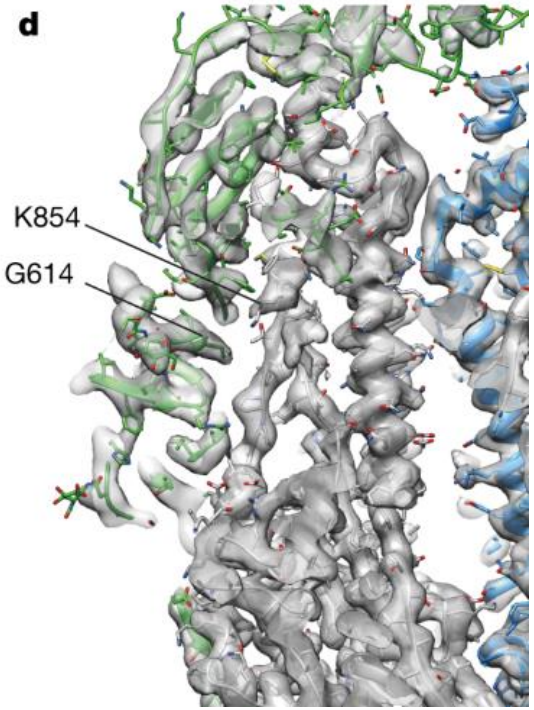
Sub-tomogram averaging

IMPACT IMAGING LIFE FROM MOLECULES TO CELLS

3D Single Particle Analysis







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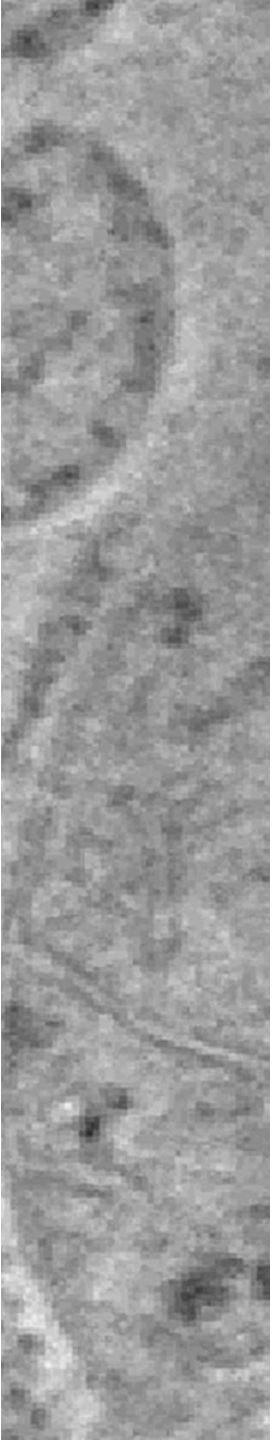

Summary

IMpaCT IMAGING LIFE FROM MOLECULES TO CELLS

Cartesian Tomography:

- Serial Sections in Transmission EM
- Serial Surface in Scanning EM
  - Microtome in situ (Serial Block Face)
  - Focused Ion Beam milling (3D FIB-SEM)

Radon Tomography:

- Thinnest Samples by Transmission EM
  - Cell sections (windows) by FIB-milling
  - Macromolecules by subtomogram (subvolume) averaging
- Extended Thickness by Scanning Transmission EM (CSTET), Soft X-ray Tomography (SXT)

Recording, Reconstruction, Rendering & Representation
Contrast generation governs interpretation.
The book is still open on new methods!