LeRoy Eyring Center for Solid State Science
Arizona State University

Understanding the biological machinery by cryogenic TEM imaging and structure determination.

Presented by NCI Southwest
and
NACK Network

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NCI Southwest + NACK Network

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Today's Moderator and Host

Trevor Thornton
NNCI Director: Professor of Electrical Engineering
Arizona State University

Michael Lesiecki
Co-Principal investigator
NACK Support Center

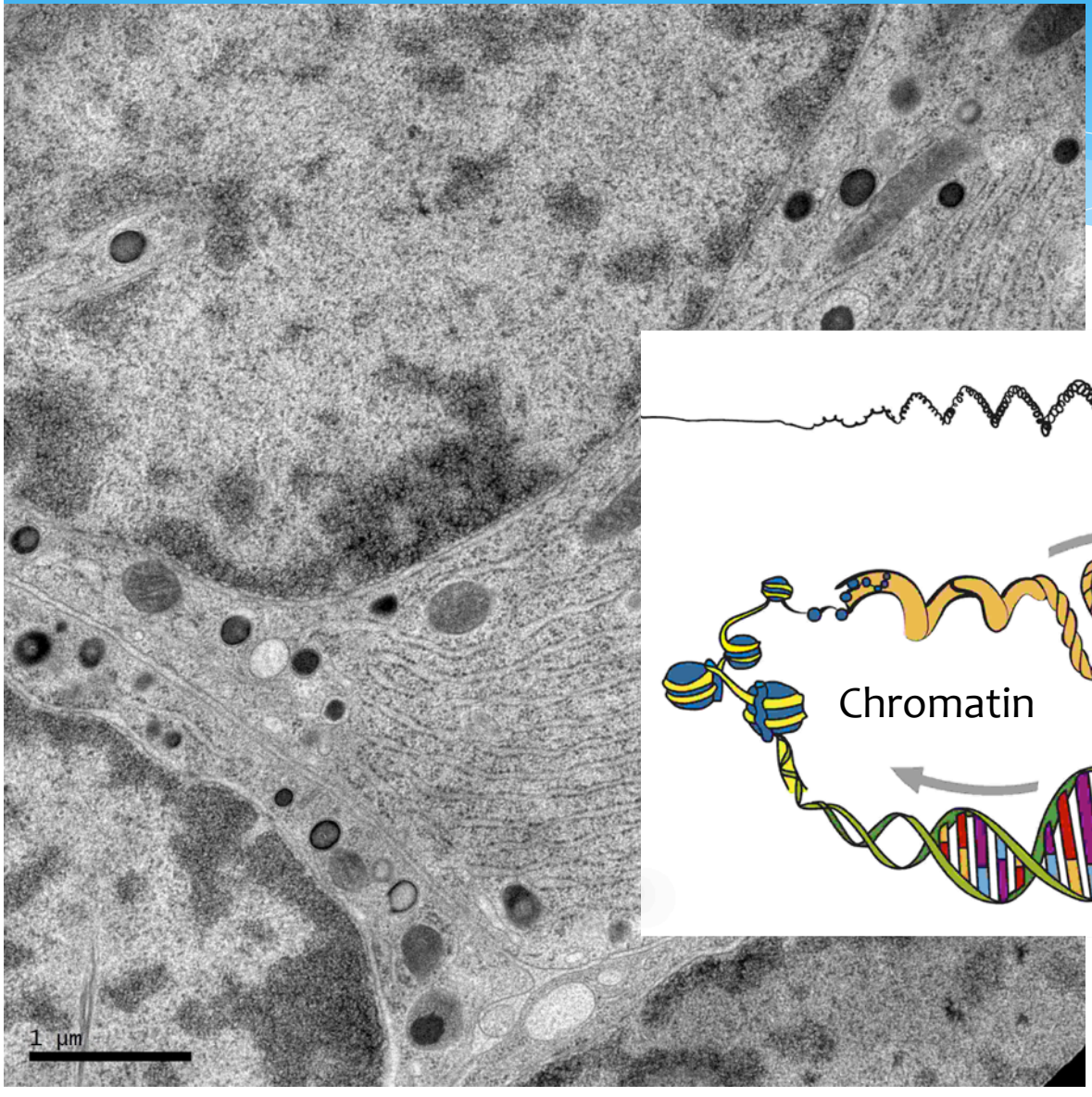
Today's Presenters

Dewight Williams
Associate Research Scientist
John M. Cowley Center for High Resolution Electron Microscopy

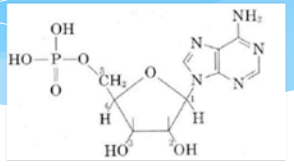
Katia March
Associate Research Scientist
John M. Cowley Center for High Resolution Electron Microscopy

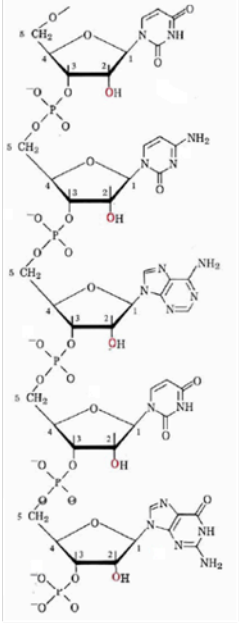
2017 Nobel Prize in Chemistry

Biological molecules:



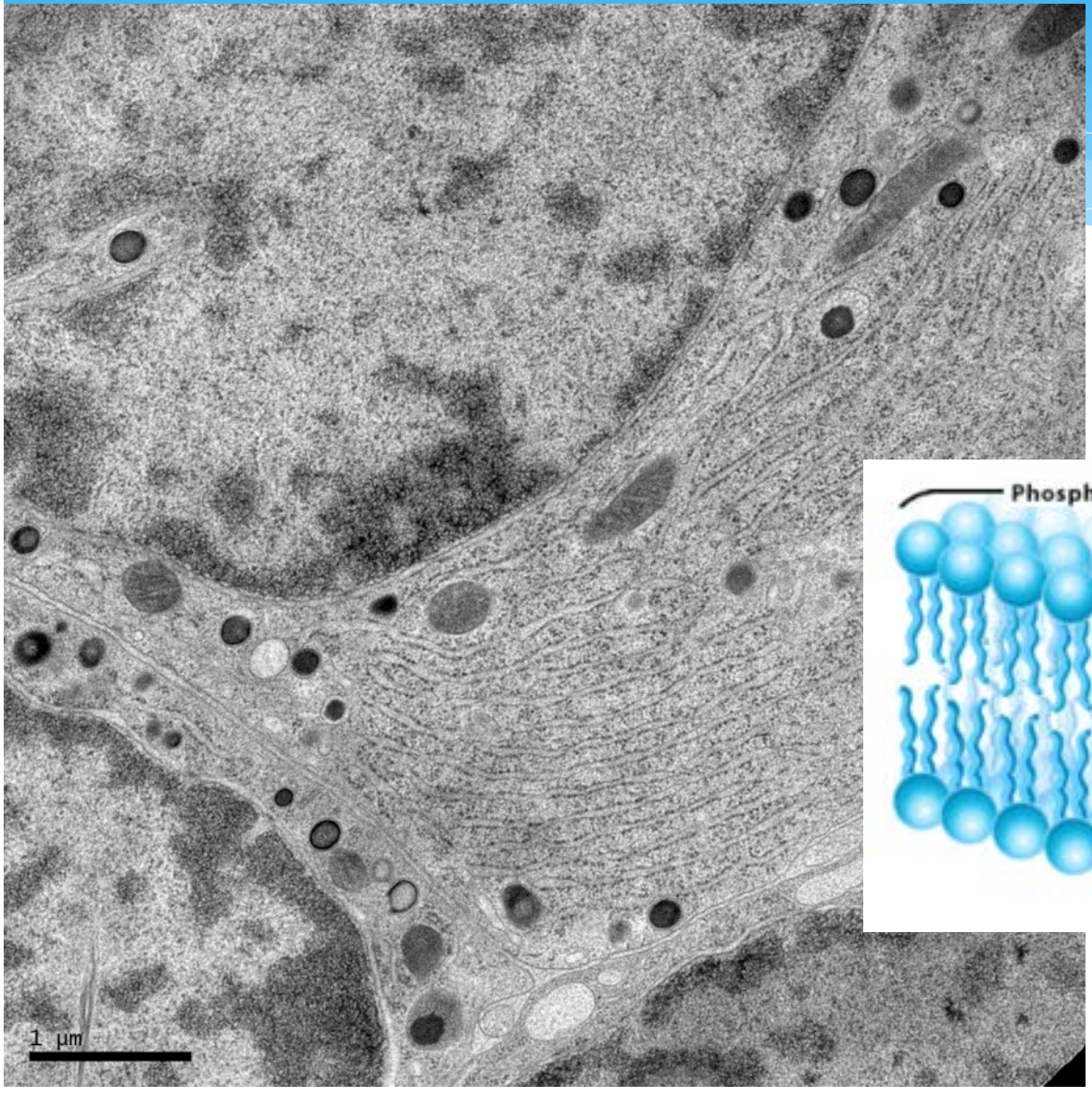
Nucleic acid

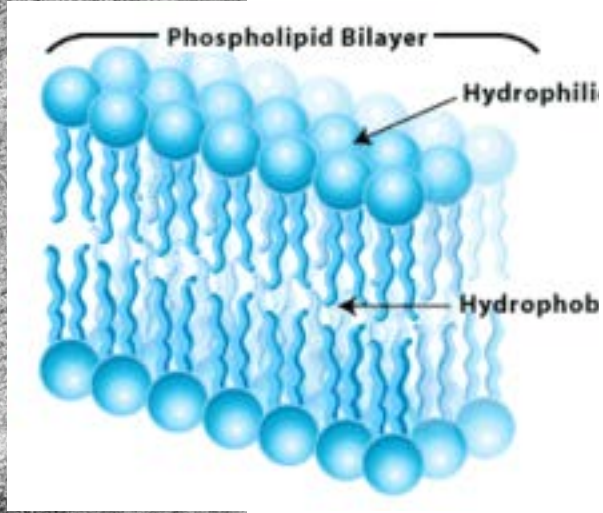


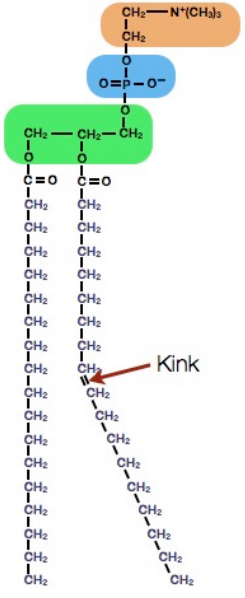


DNA

Biological molecules:







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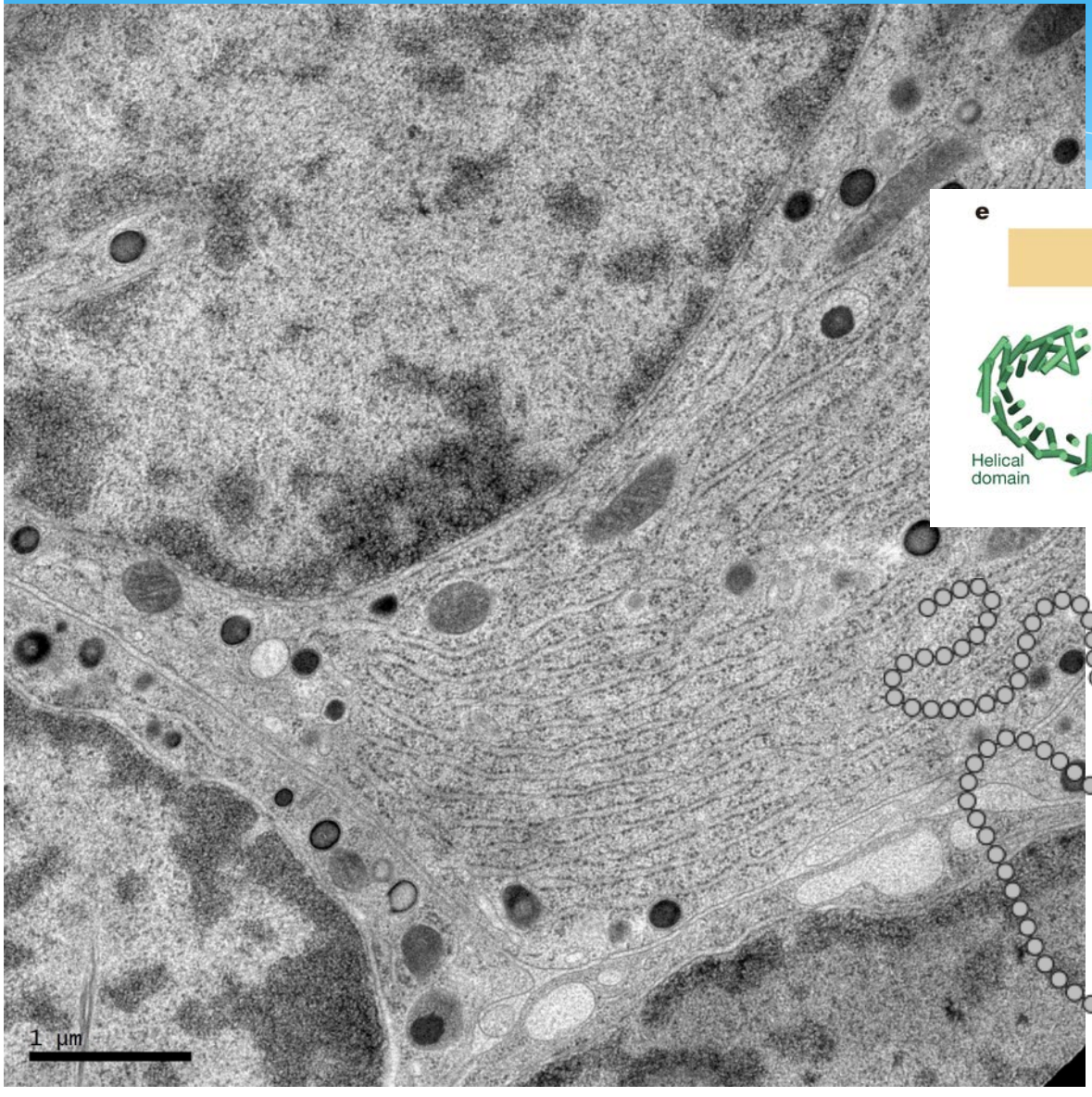
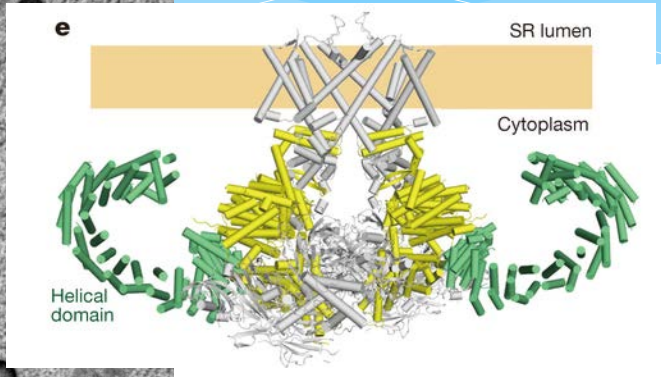
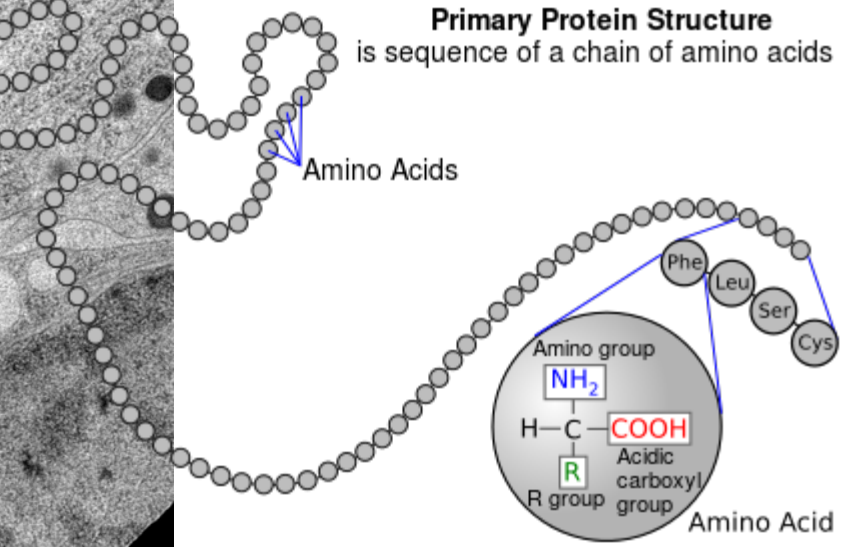




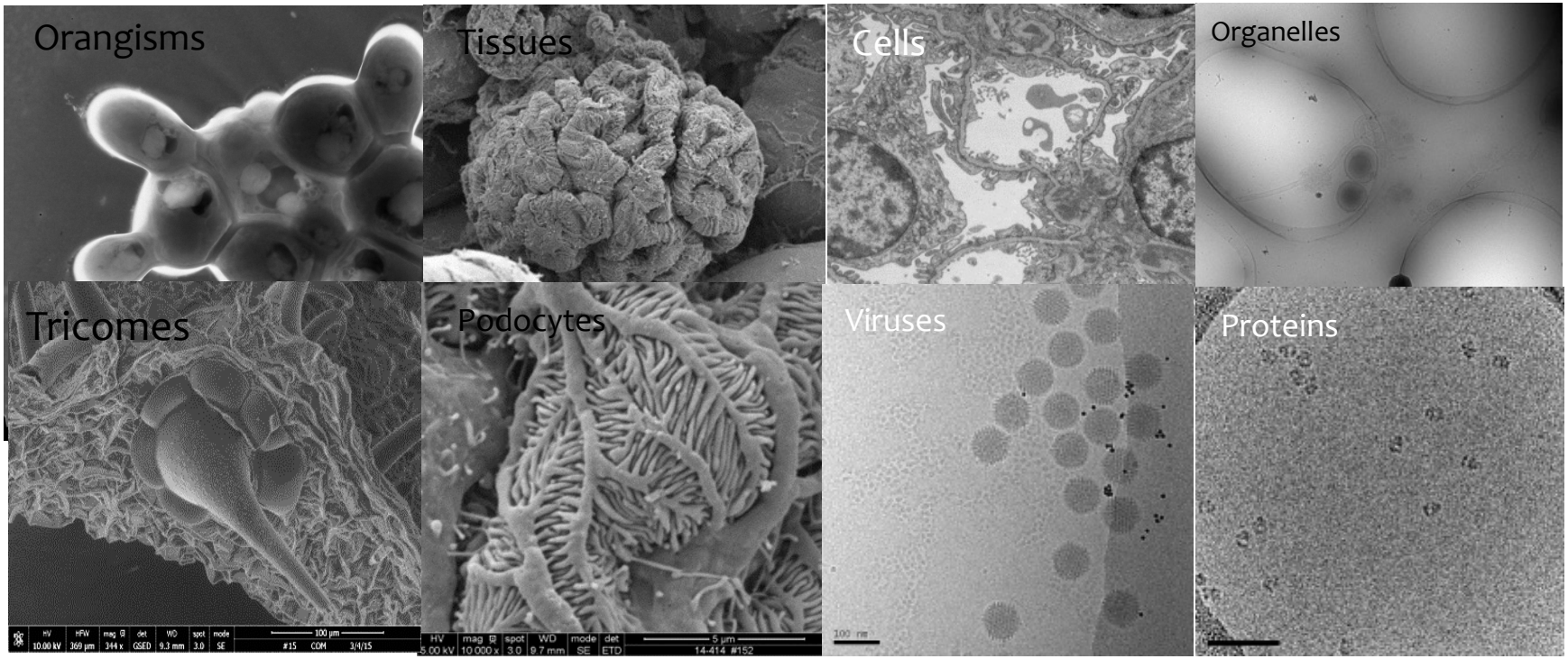
Primary Protein Structure
is sequence of a chain of amino acids



Biomolecular Fold Space:

* Traditionally protein structures were determined by
  * X-ray crystallography:
    * Captures only a single state because dependent on crystallization
  * NMR spectroscopy:
    * Captures dynamic states but limited size <60kDa
* A large number of private/public structure determination consortiums have solved ~150,000 protein structures add ~15,000 per year
  * Soon all protein fold patterns will be determined.
* Structure determination will soon look toward higher order assembly, dynamic and or conformational variation, as well as *in situ* assembly states.

EM imaging can investigate this higher order assembly



With image averaging methods, atomic resolution of complexes is possible

Why CryoEM?

* Biological chemistry occurs in water
* Biological molecules require water to properly organize
* Imaging in high vacuum is incompatible with hydration

* Best solution is freezing biology in vitreous (or water) ice. Jacques Dubochet

Cryogenic preservation

* As near native conditions as possible
* Water is frozen vitreously

* Plunge freezing in liquid nitrogen cooled ethane
  * up to 5 micrometer

* High pressure freezer
  * up to 500 micrometers

Plunge Freezing in detail

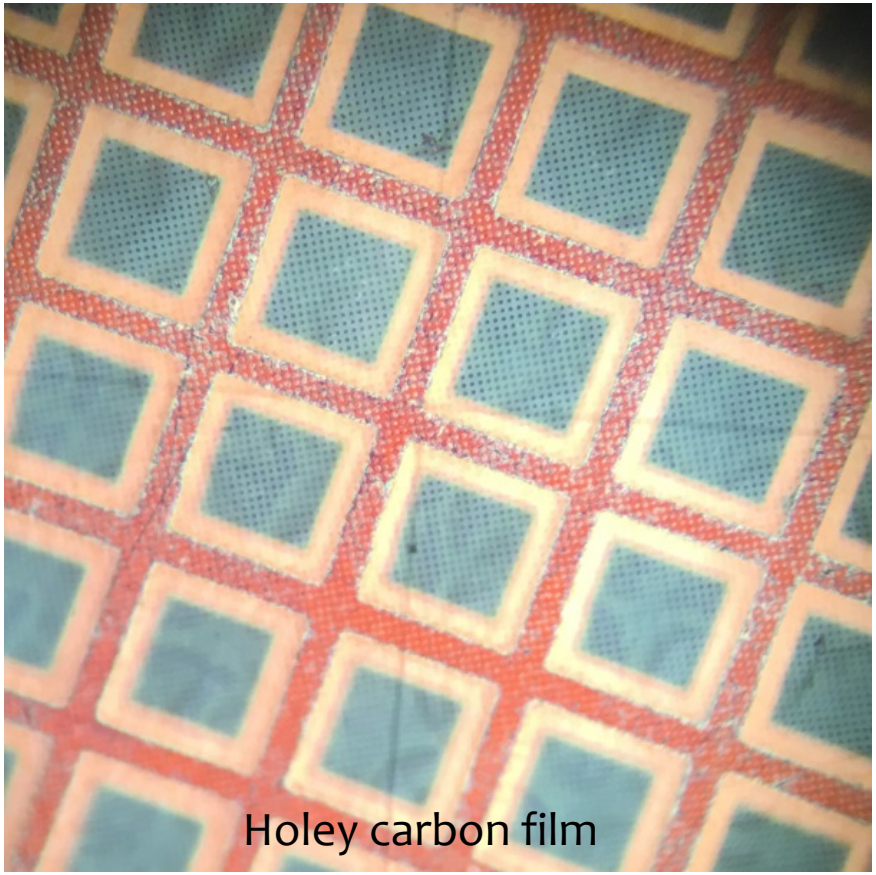
Glow discharge grids to make carbon hydrophilic

Apply protein solution to holey carbon grid (5 µL of 20-100 nM)

Blot away excess liquid

Rapidly plunge into liquid nitrogen cooled cryogen (liquid ethane)

Sample preserved in ultra thin vitreous ice



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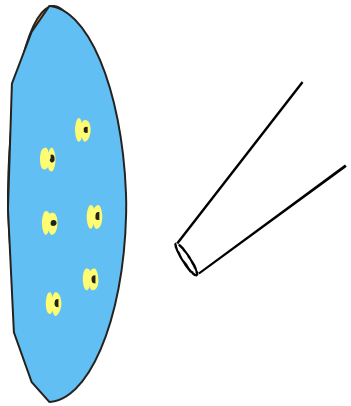

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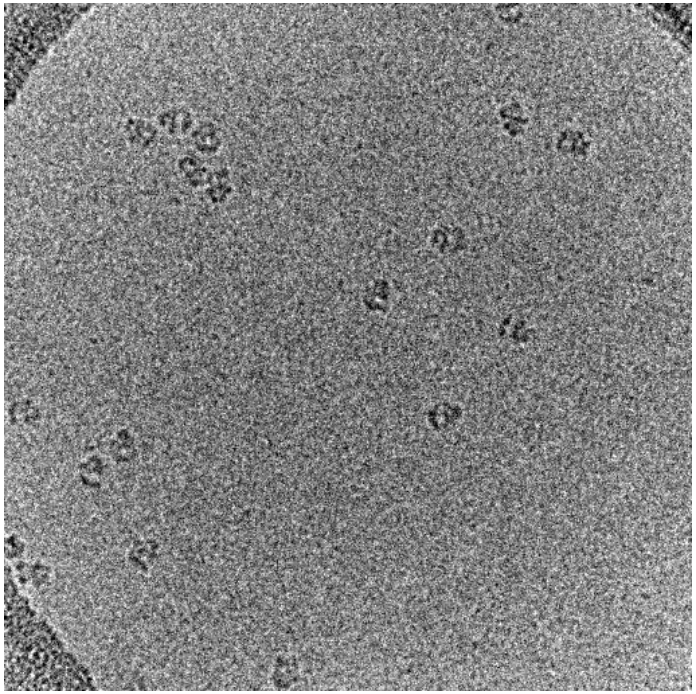
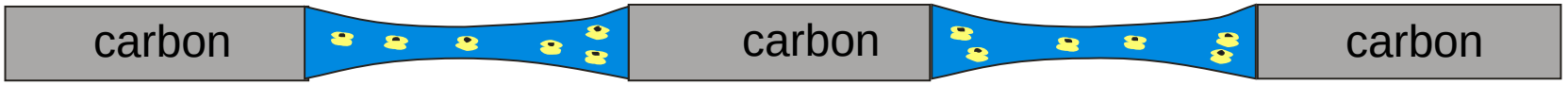
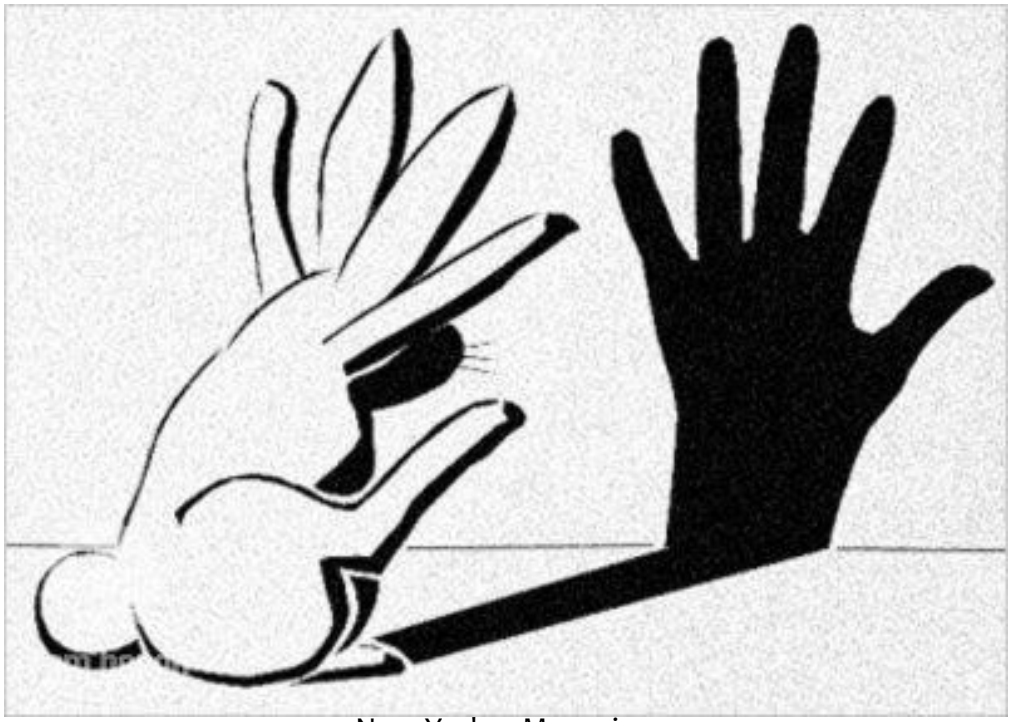


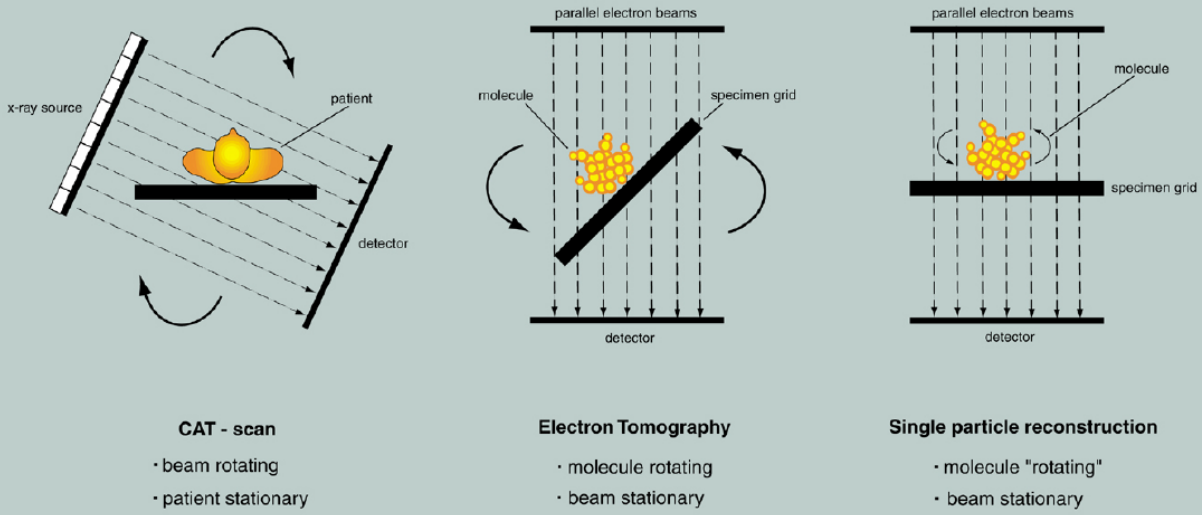


TEM images are projection images



New Yorker Magazine comics

2D projections to 3D structures



CAT - scan

- beam rotating
- patient stationary

Electron Tomography

- molecule rotating
- beam stationary

Single particle reconstruction

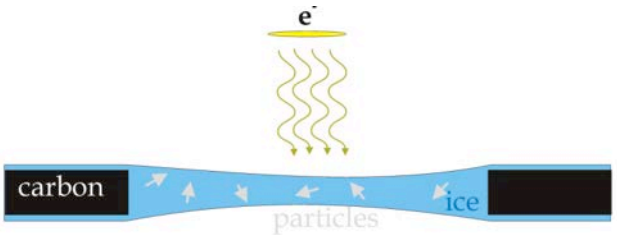
- molecule "rotating"
- beam stationary

From Frank, 3D EM of macromolecular Assemblies

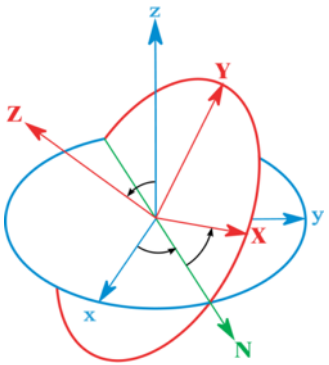
Single particle reconstruction

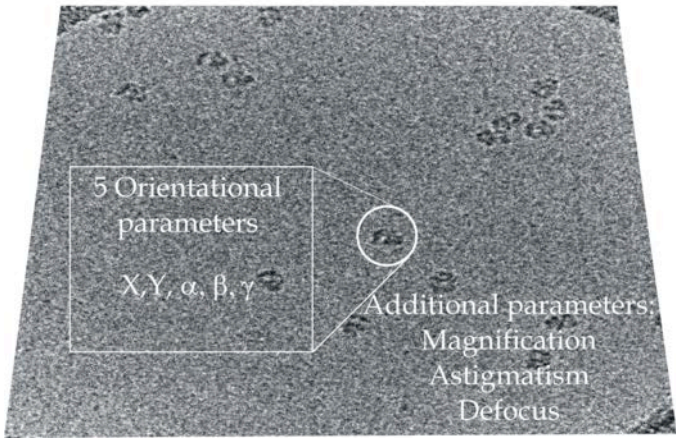
10-30 electrons per Angstrom^2

Orientations unknown so computationally intensive

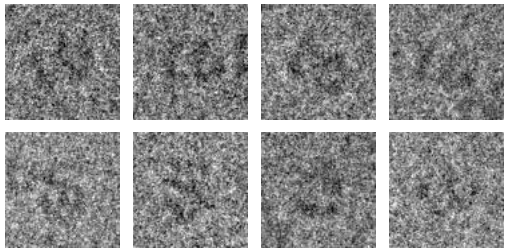


2D-projection images



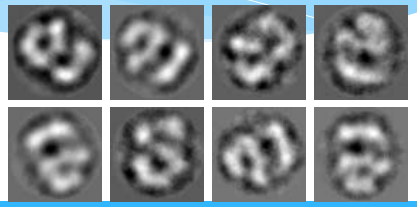


SPR: overview



Particle stack

Translational and rotational Alignment of particles



Multivariate statistical analysis and classification

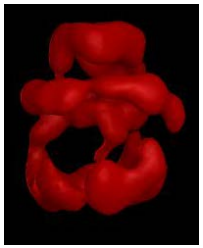
Now with references

Common lines

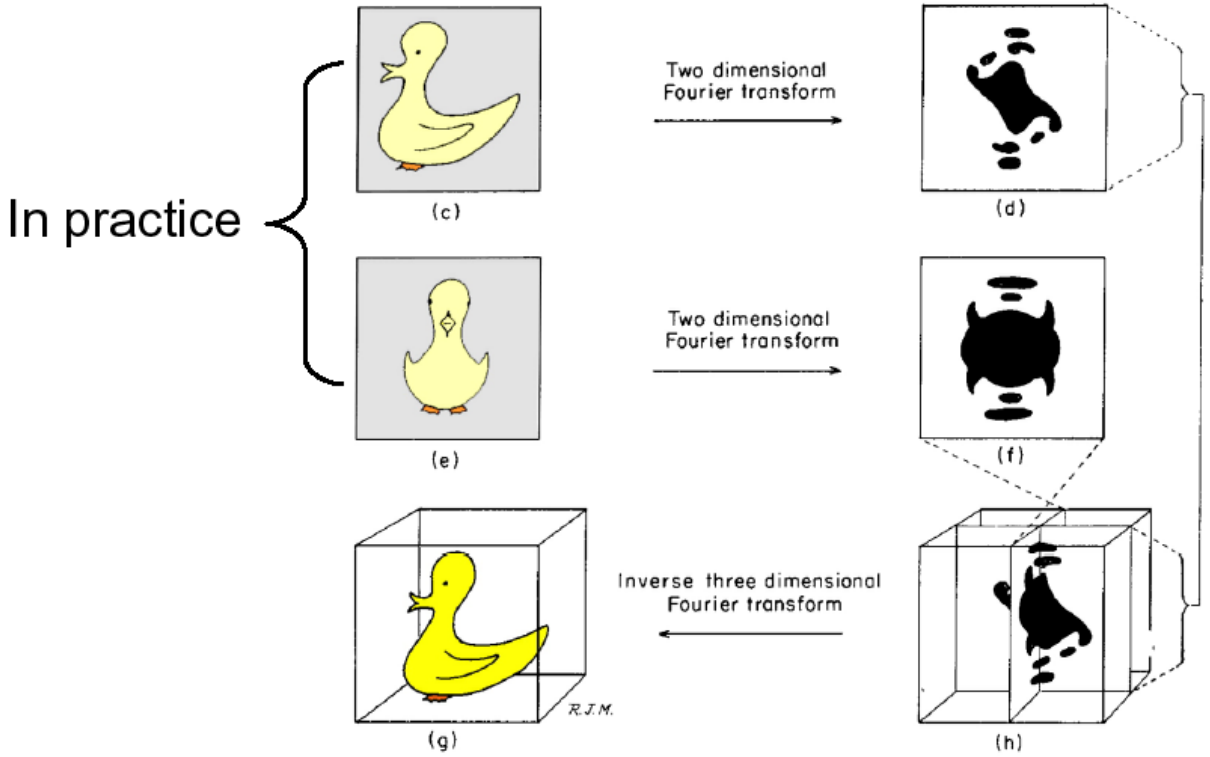
Angular and translational assignment to each class sum image

Reconstruction of 3D volume

reprojection

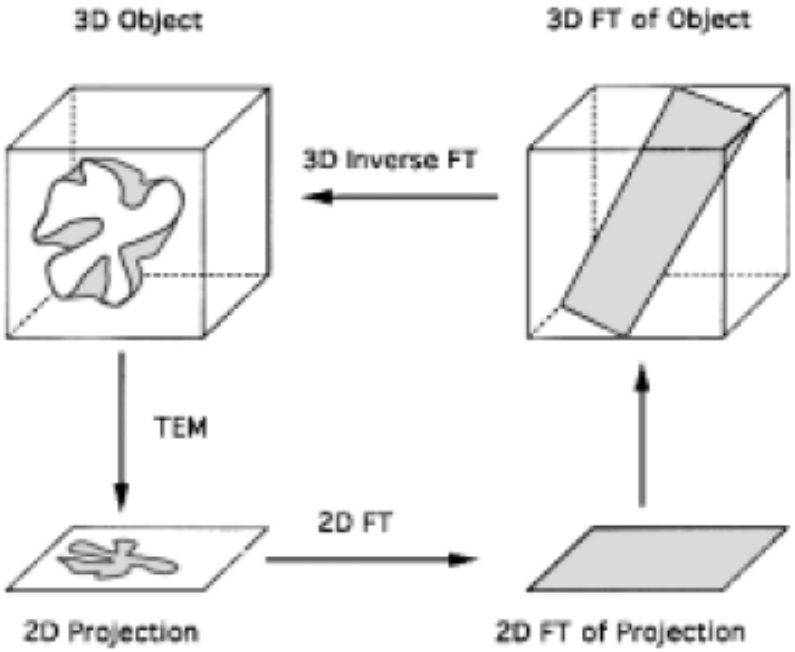


Central Slice Theorem



From Lake (1972), p.174

Computing a 3D Reconstruction

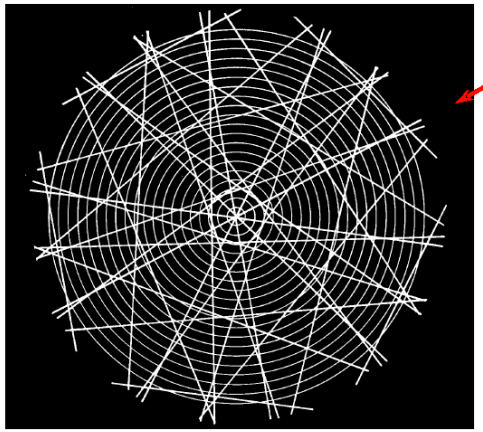


Low Dose cryoTEM images have weak phase information per particle image, so 100,000's to millions of views are required

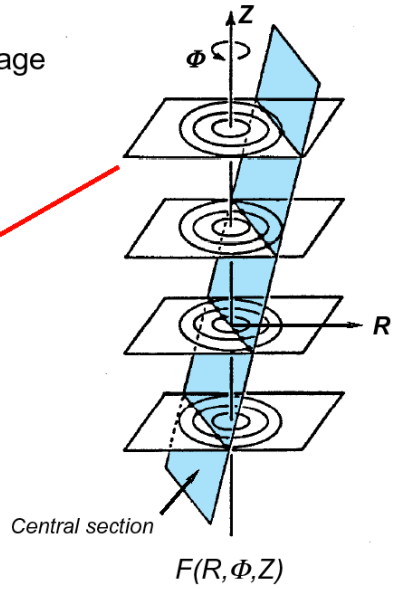
Compute 3DR

Steps:

1. Compute 2D FFT of each particle image
2. Combine all 2D FFTs to build up 3D Fourier-Bessel transform



Crowther, DeRosier and Klug, 1970, p.329



Adapted from Crowther (1971) Fig. 4, p.223

Reconstructions are computationally intensive

Reconstructions use to require 100's of CPUs only available on a high performance computing cluster

Recent improvement in code and GPU utilization has allowed reconstructions on high end workstations

cisTEM, Relion, cryoSparc

Ice behaves different than other materials in the electron beam

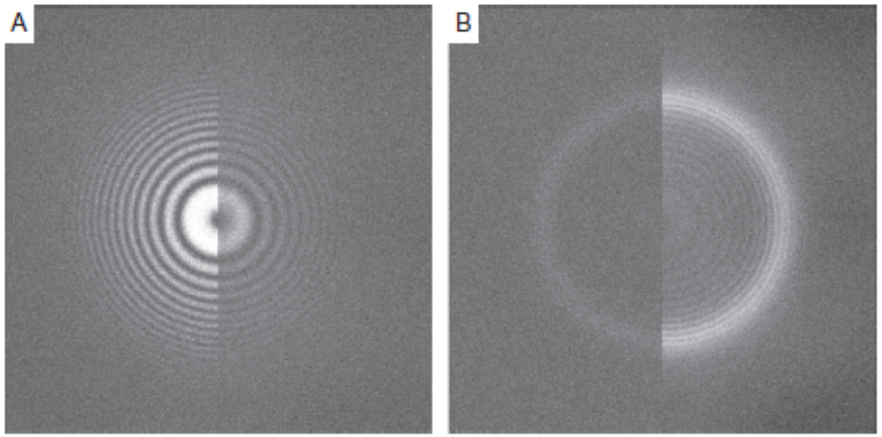
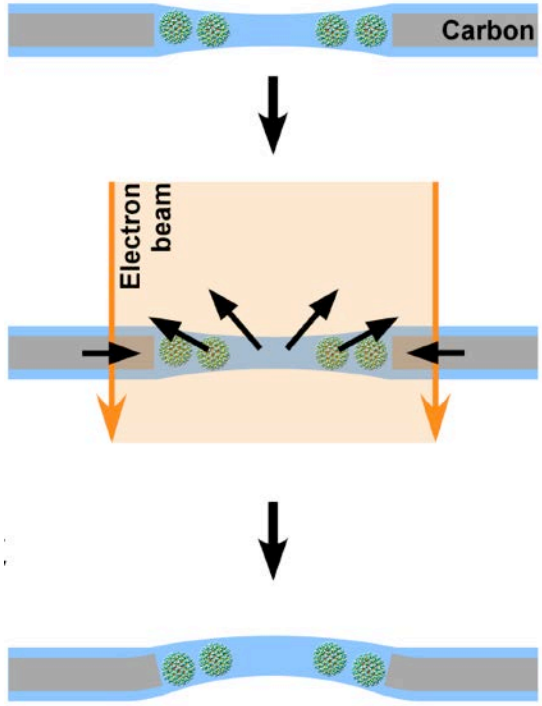


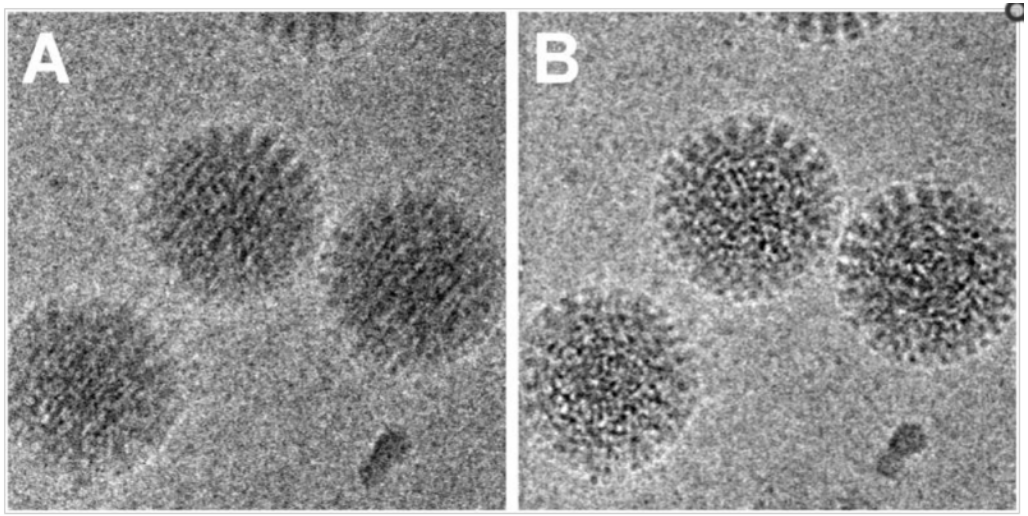
Fig. 4 Example illustrating that the power spectra of (A) amorphous carbon and (B) amorphous ice are dramatically different. In both cases images were obtained as dose-fractionated movies, using far greater electron exposures than could be tolerated by biological specimens, in order to improve the statistical definition of the power spectra. Each panel is, furthermore, split into two half-plane images in which the power spectrum of the coherent sum of frames is shown on the left half, and the "incoherent sum" of power spectra of individual frames is shown on the right half. *This figure was kindly prepared by Dr. Greg McMullan, using the same data published in McMullan, G.,*

Imaging protein in Ice



Beam induced motion

Direct electron detectors make possible



Uncorrected

Motion corrected

Brilot et al. J Struct Biol. 2012 March ; 177(3): 630–637.

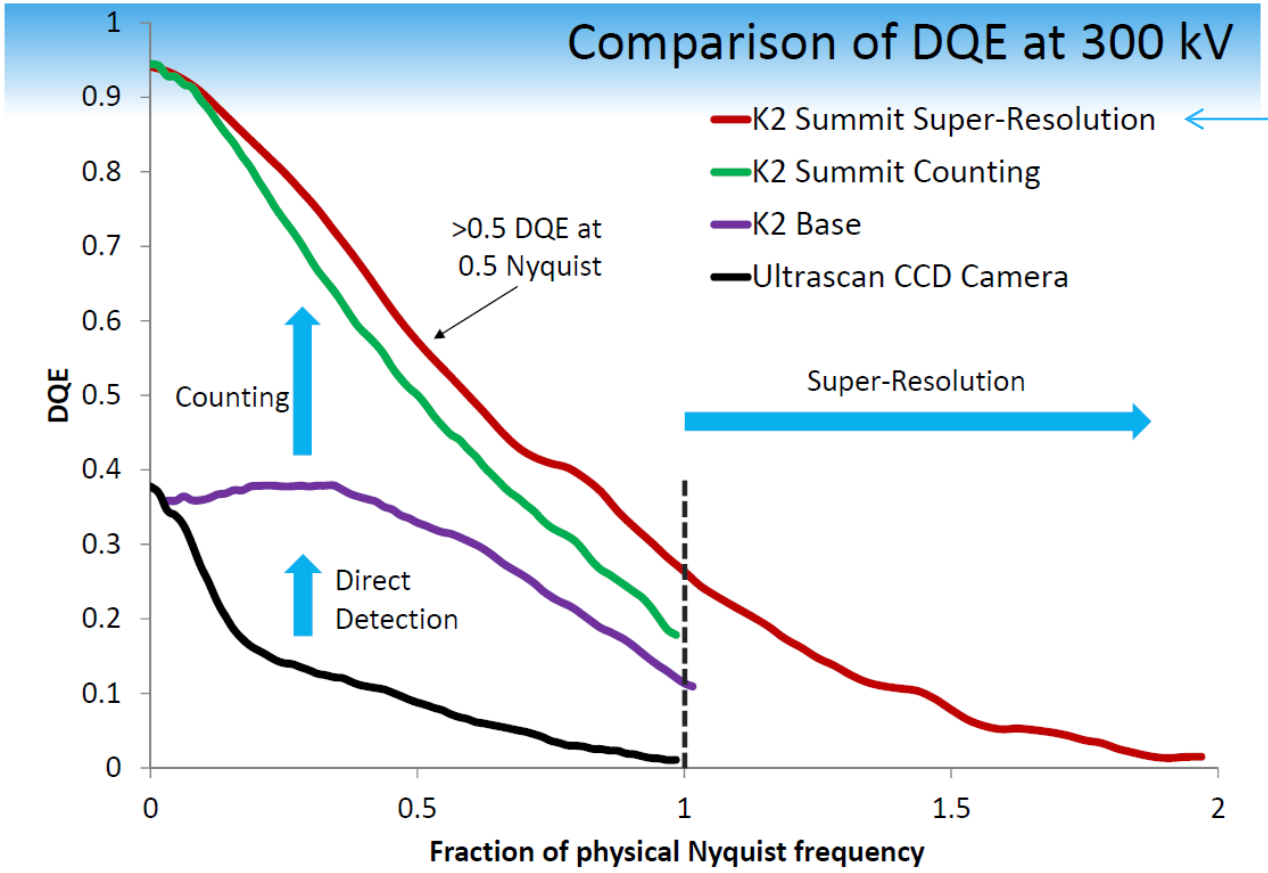
Direct electron detectors: CMOS APS

Thinned CMOS sensor

electron - electron conversion

* Improved DQE especially at low frequencies
  * Direct electron counting modes improve low frequency contrast
* High speed read out as movies (50 frames a second)
  * correction of beam induced motion
  * Specific dose selection
  * Spatial frequency filtering based on beam damage

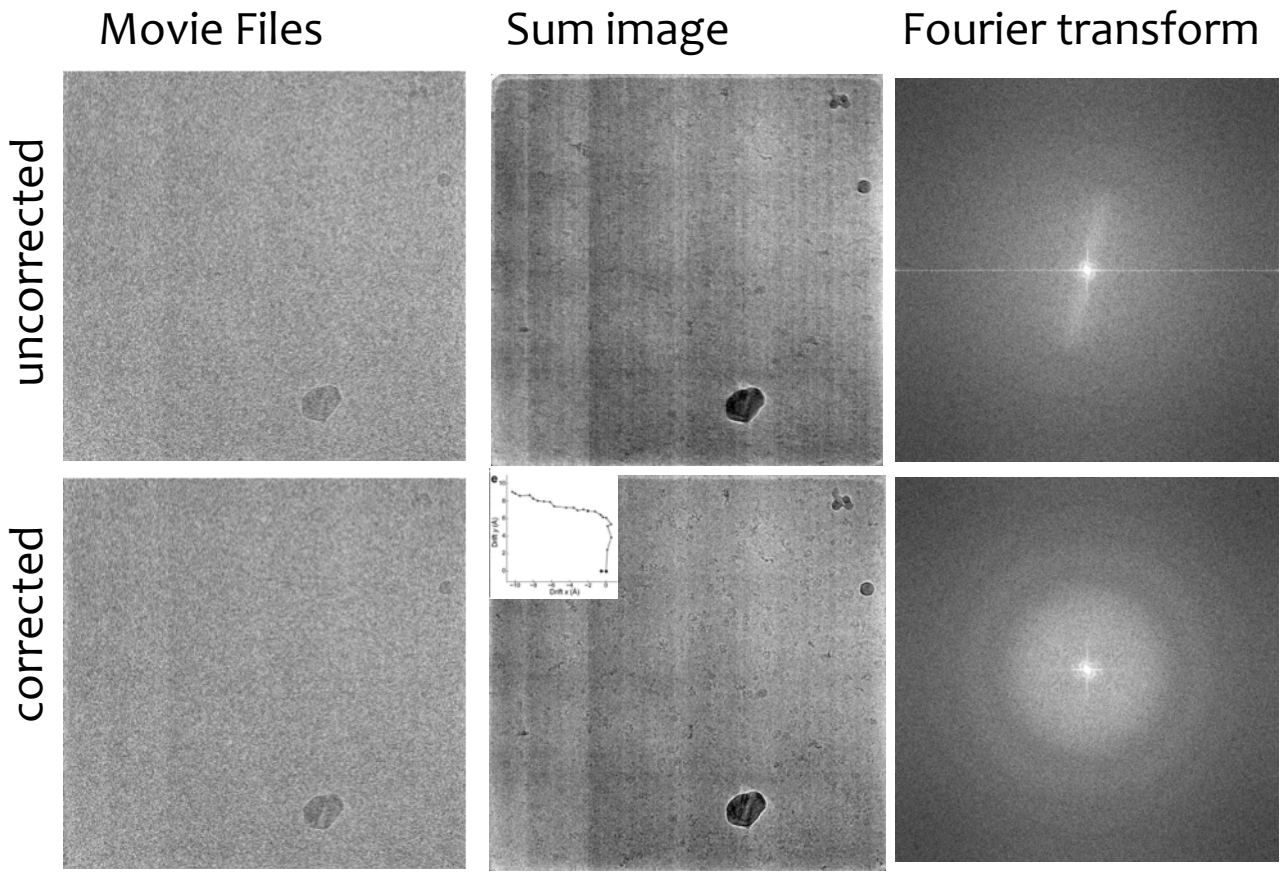
Counting modes have high low frequency DQE



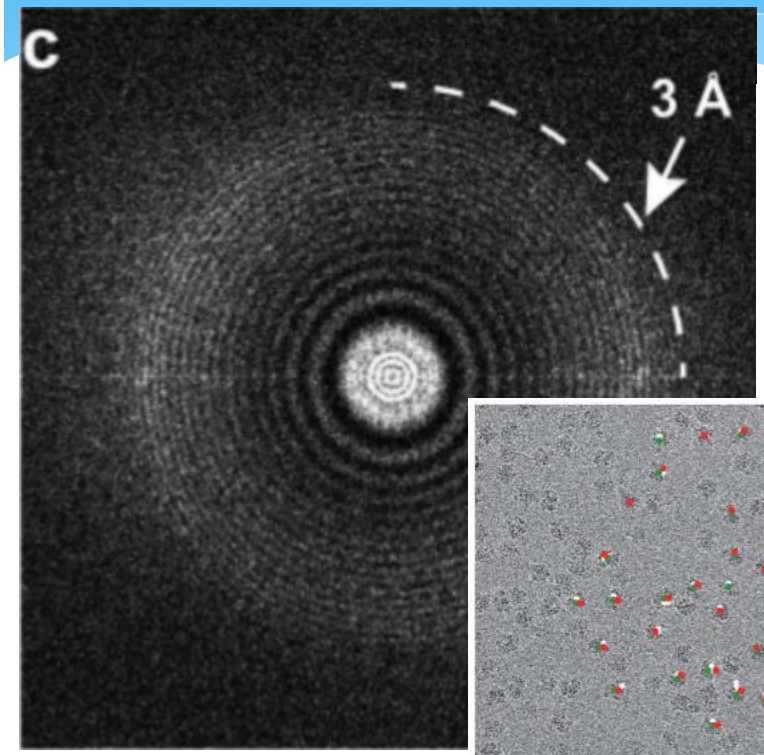
Centroid localization

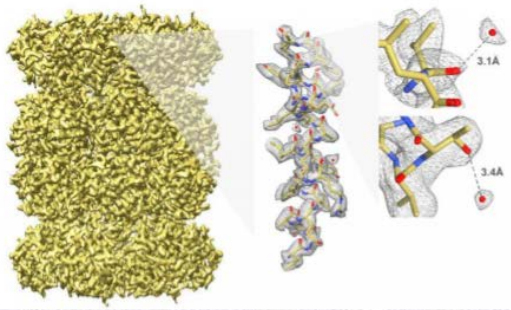
Source Gatan

Movies: motion correction and dose weighting

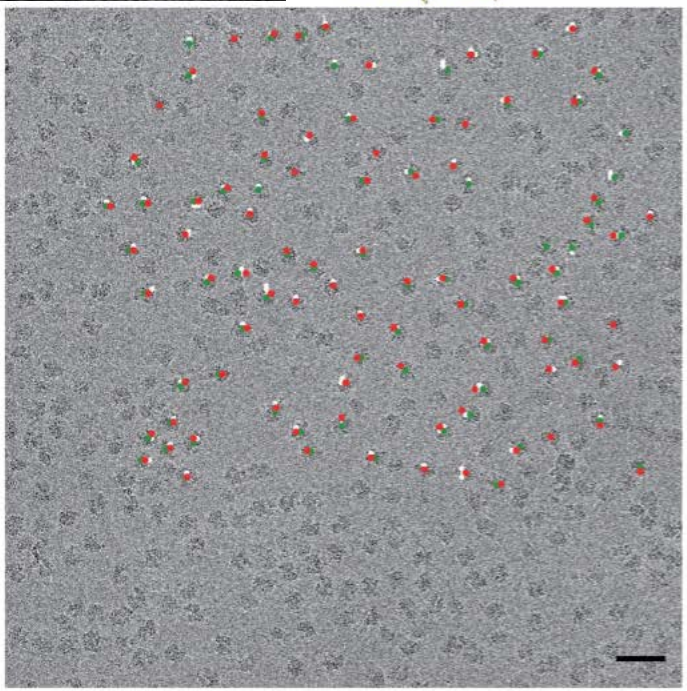


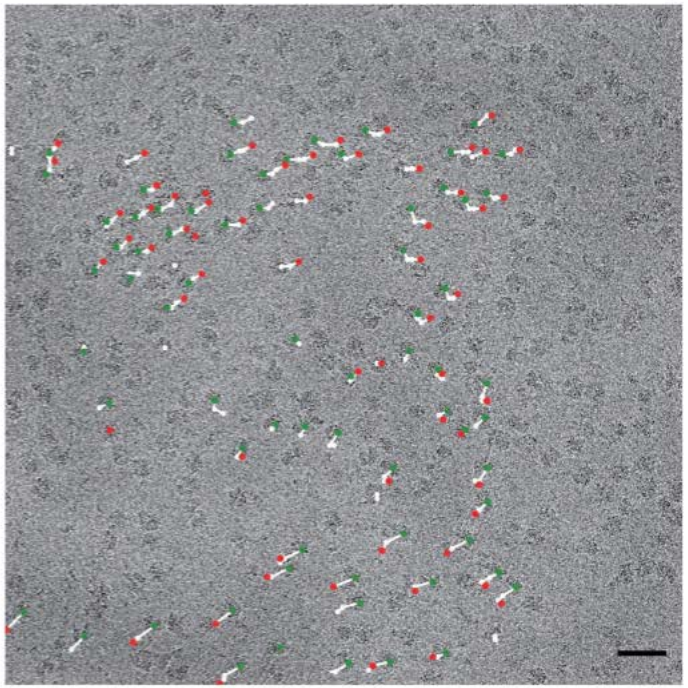
Motion Correction during reconstruction: Particle polishing





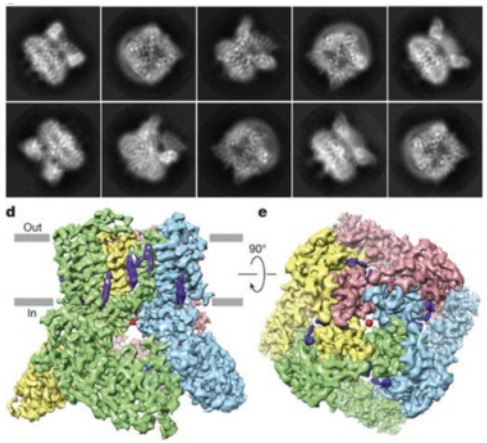
Gatan K2 Summit
20S Proteasome
2.8 Å
49,954 particles
Campbell *et al.* 2015





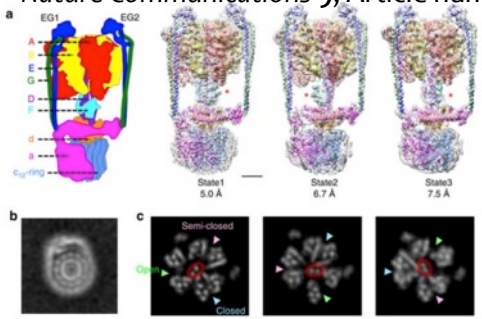
Solving protein assemblies is now routine.

Nature **553,** 233–237 (11 January 2018)



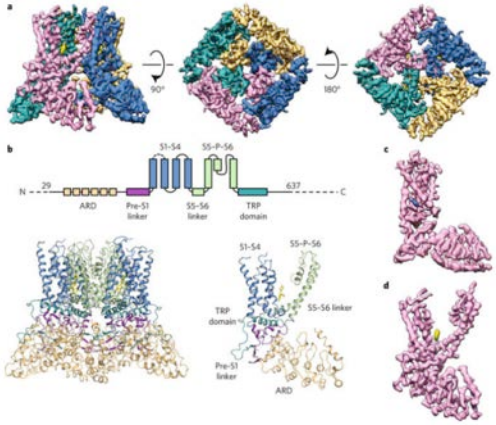
TRPV6

Nature Communications **9,** Article number: 89 (2018)



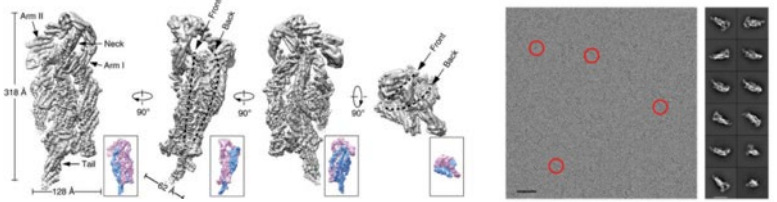
ATP synthase

Nature Structural & Molecular Biology **25,** 53–60 (2018)



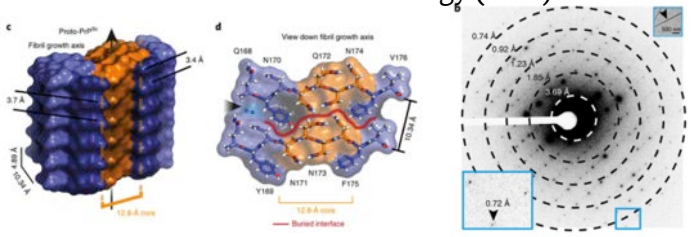
TRPV5

Nature Structural & Molecular Biology (2018)



Yeast Exocyst

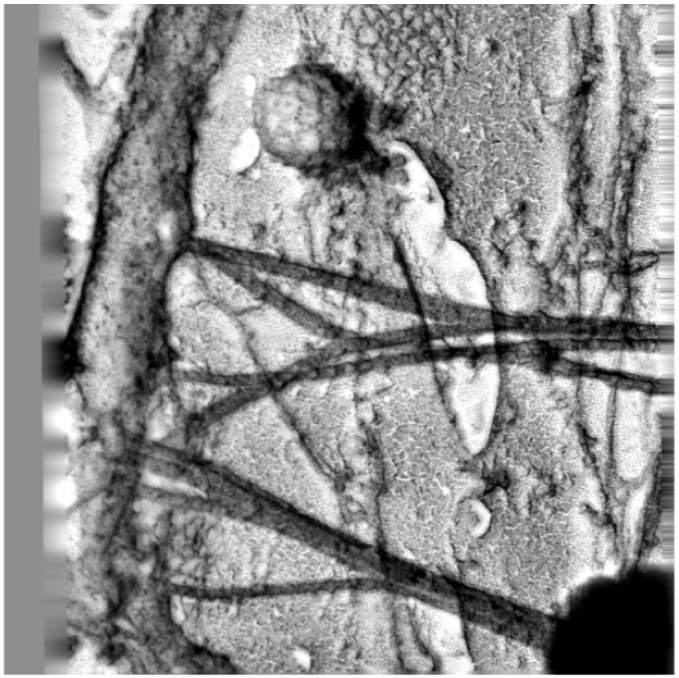
Nature Structural & Molecular Biology (2018)



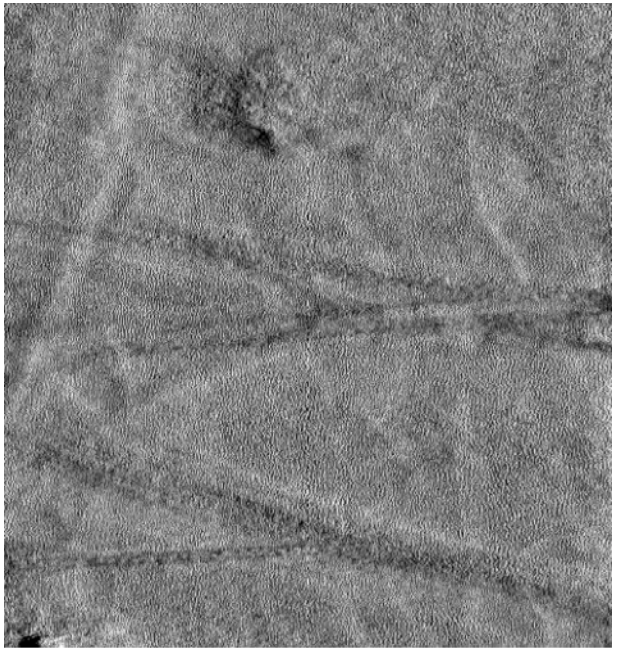
Prion filament

Building up biological assembly

Tatyana Svitkina

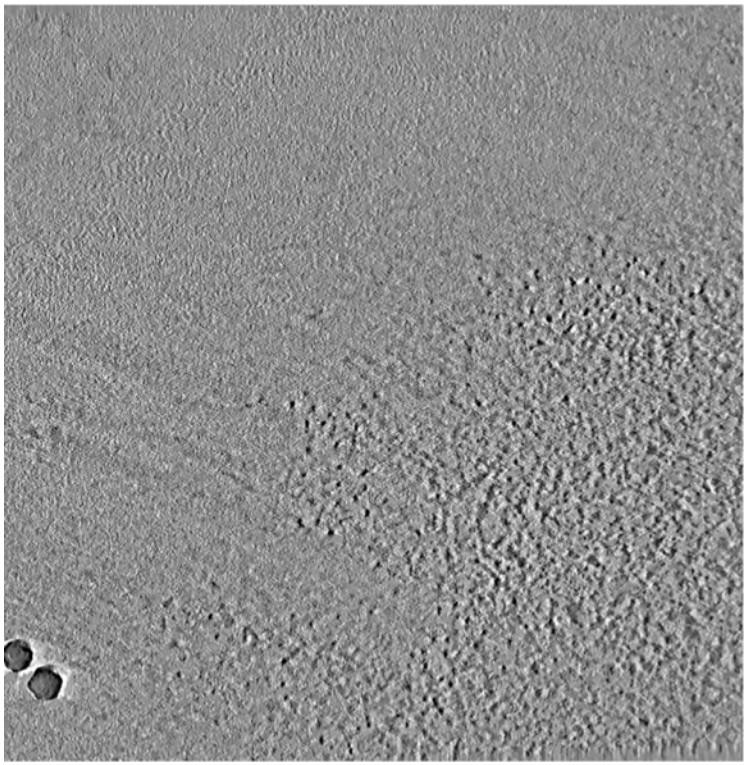


Movie 3: Tilt series



Movie 4: Tomogram

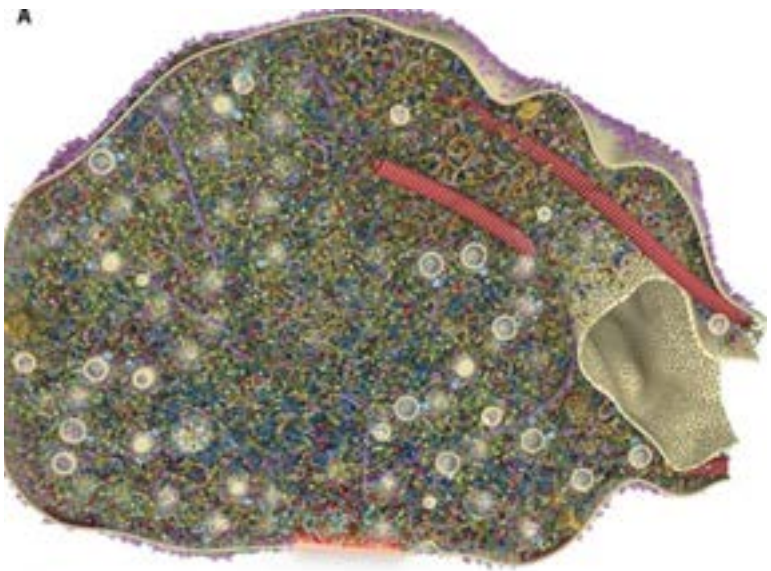
Unstained cryogenic material

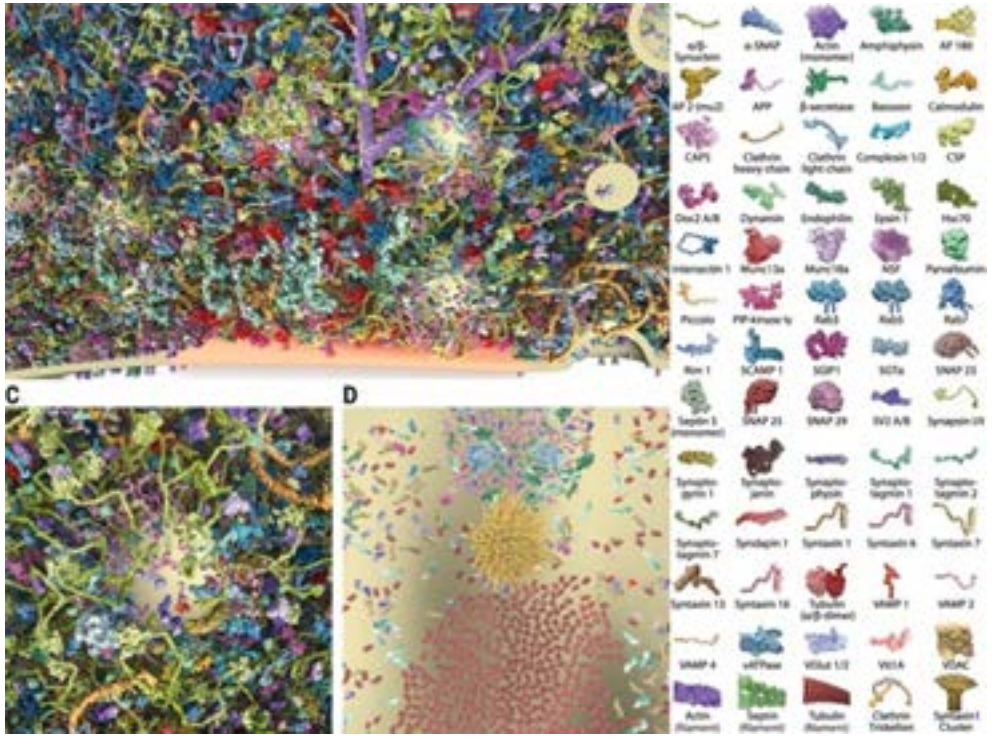


Play Movie

Julia Mahamid Max Planc Martinreid from Science 351, 969-972, 2016

Segmentation and template matching in volumes.





Wilhelm et al. 2014

Future:

* How do we preserve and image thick cellular or tissue volumes?
* 4D TEM and conformational dynamics?
* Can we discern molecules when connected or layered?

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Understanding the biological machinery by cryogenic TEM imaging and structure determination.

January 26, 2018 at 1 PM EST

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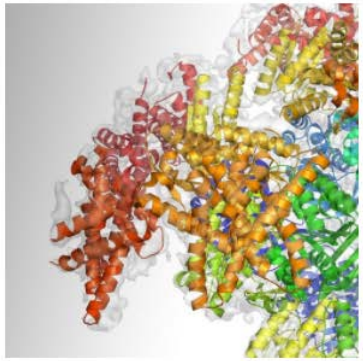

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