CIMST Summer School

Cryo-electron microscopy for structural biology

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1. Introduction
1-1. What can you see by electron microscopy? Which scale can electron microscopy deal with for biology? Example1: chromatin.
1-2. Single particle analysis and electron tomography: 3D Electron microscopy without crystallization. Example2: Dynamics of Clp protease.

2. Basics of transmission electron microscopy
2-1. 3D reconstruction is a backprojection from micrographs. You need to know the view angle of each projection.
2-2. Electron cryo-microscopy: ice embedded specimen. Why cryo?
2-3. Radiation damage

3. Single particle analysis
3-1. Example 3: Ribosome
3-2. Single particle analysis is a method for purified molecules. View angles of projections and 3D structure are determined simultaneously.
3-3. Strategy of single particle analysis: Projection matching
3-4. Example 4: Clp protease
3-5. Resolution: What does resolution mean? How to define resolution in single particle analysis?

4. Electron tomography
4-1. In electron tomography, images are acquired from the same view by tilting the specimen to fill the whole Fourier space for the 3D reconstruction.
4-2. Strategy of electron tomography. Example5: flagellum. Gold cluster for translational alignment.
4-3. Example6: Whole cell tomography
4-4. Cryo sample is sensitive for radiation damage. Since electron tomography requires multiple exposures, the radiation damage is serious and becomes the limitation of resolution.

References

Many examples of structural biology by EM can be seen in
Alberts et al. (2002) “Molecular biology of the cell, forth edition”, Garland Science. p. 560-570 (techniques), p. 949-969 (molecular motor research combining various methodologies including EM).

There are some (but, not many) descriptions in
Branden, C. and Tooze, J. (1999) “Introduction to structural biology, second edition” Garland.

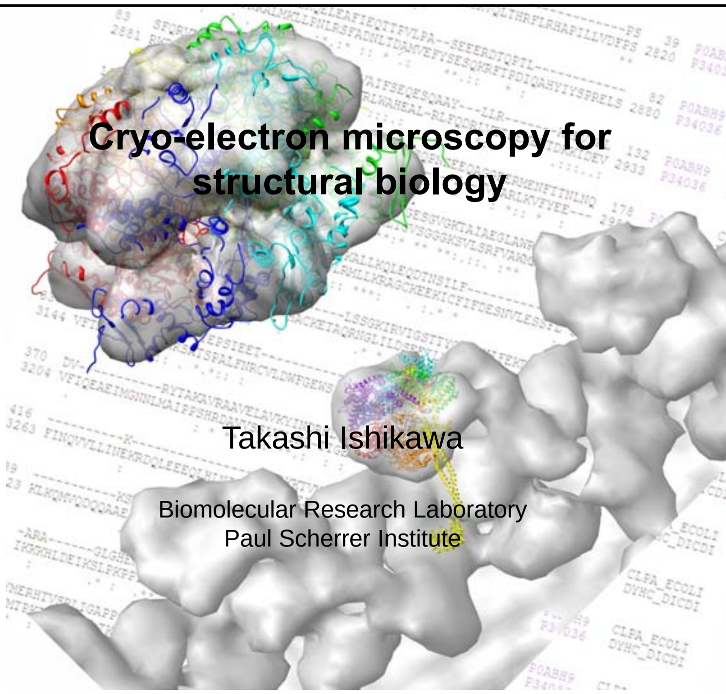
A good review on single particle analysis and electron tomography
Baumeister, W. and Steven, A.C. (2000) “Macromolecular electron microscopy in the era of structural genomics” Trends in Biochemical Sciences 25, 624-631.

Text books on single particle analysis
Frank, J. (1996) “Three-dimensional electron microscopy of macromolecular assemblies” Academic Press.

Frank, J. (edited) (1992) “Electron tomography” Plenum Press.

Technical aspects
Misell, D.L. (1978) “Practical methods in electron microscopy, vol.7: Image analysis, enhancement and interpretation” North-Holland.

Examples of high resolution electron microscopy with crystals
Kuhlbrandt W. and Williams, K.A. (1999) “ Analysis of macromolecular structure and dynamics by electron cryo-microscopy” Curr. Opin. Chem. Biol. 3, 537-543.

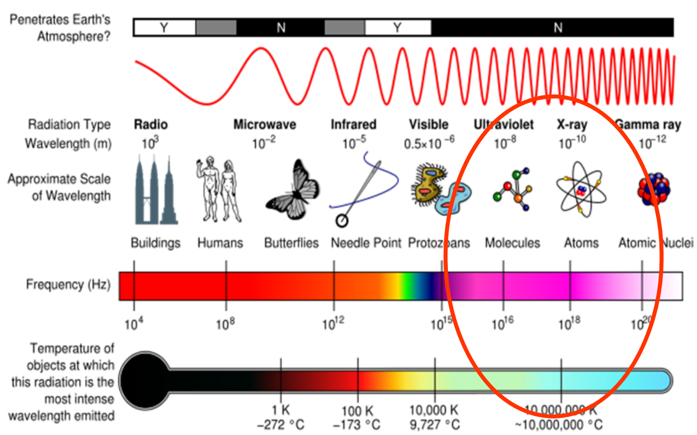


Cryo-electron microscopy for structural biology

Takashi Ishikawa

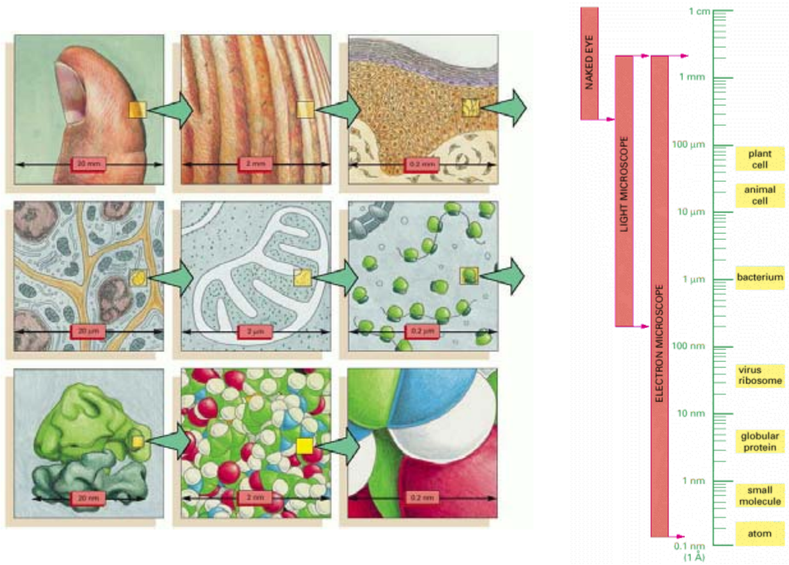
Biomolecular Research Laboratory
Paul Scherrer Institute

Imaging wavelength of transmission electron microscopy for biology



From wikipedia

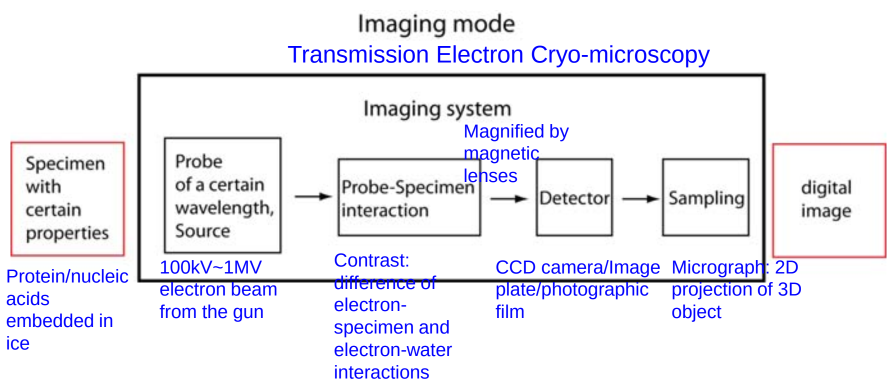
Our target: Biological macromolecules (Protein, Nucleic Acids)



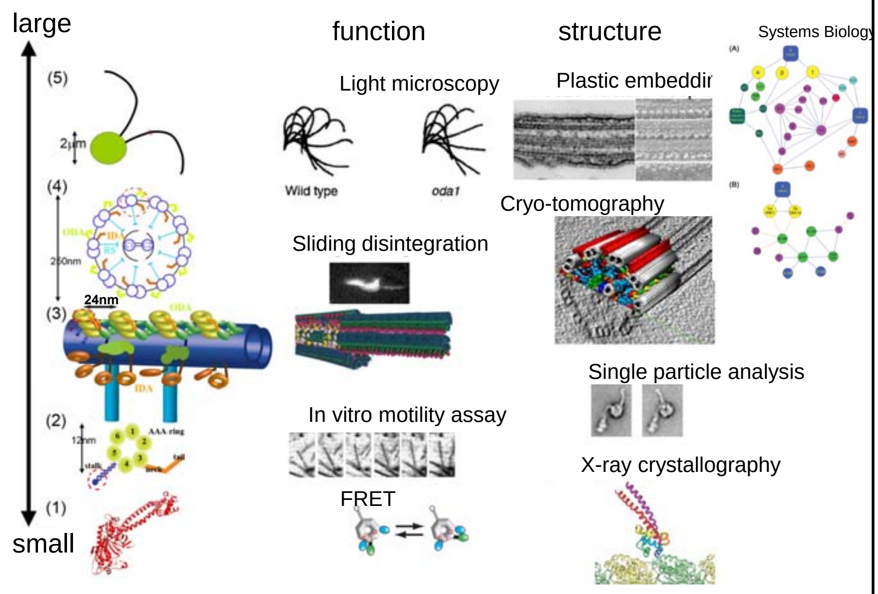
From "Molecular Biology of the Cell" Fourth Edition

Imaging:

"Information transfer chain"

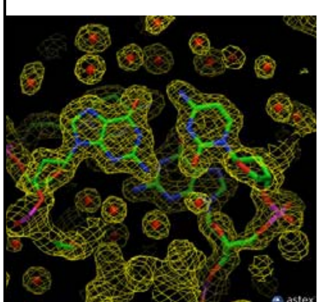


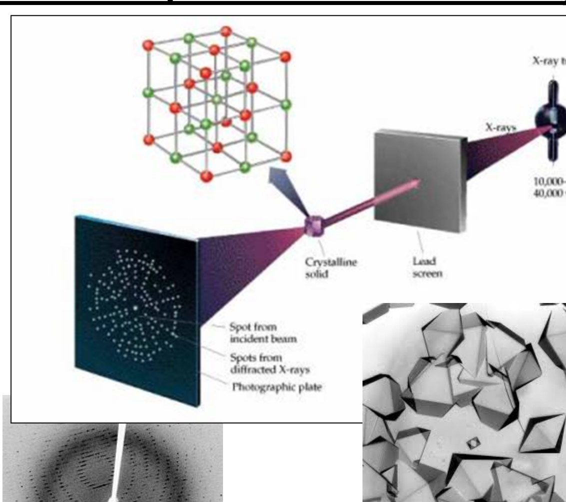
Interdisciplinary Approach

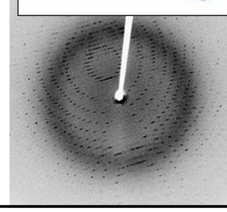


Ishikawa (2011) modified from “Dynein: Structure, Biology and Disease“ edited by S. King (Elsevier)

X-ray crystallography the most powerful tool for protein structural analysis





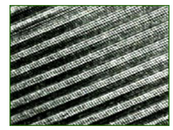


Atomic resolution
Difficult for large complexes
Difficult for flexible molecules

Transmission Electron Microscopy (TEM)

Material Science

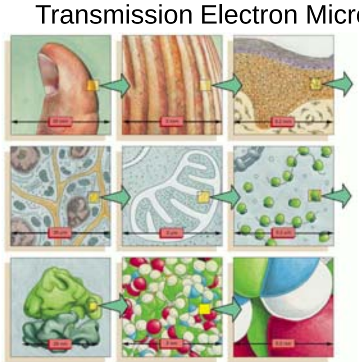
You can see atoms directly

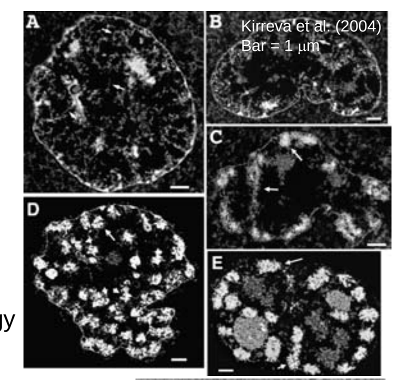


Biological Science

1. Low Contrast
2. High Sensitivity to the Electron Beam

Transmission Electron Microscopy (TEM)

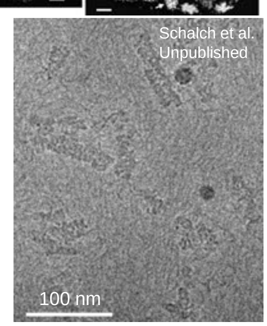




Biological Science

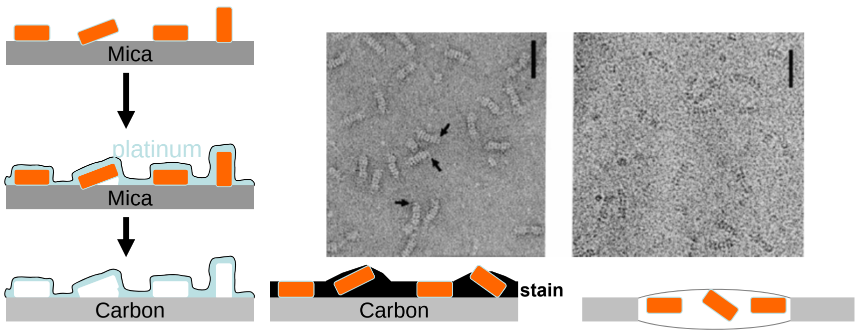
Cell Biology
(in vivo)

Molecular Biology
(in vitro)



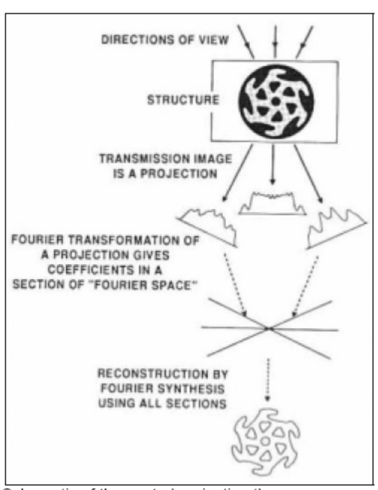
Various specimen preparations for biological macromolecules

We would like to observe molecules as close to the physiological conditions as possible.

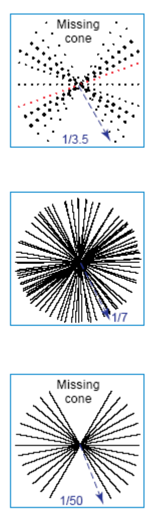


Shadowing | Negative Staining | **Cryo** (ice embedding)

Missing wedge, missing pyramid and missing cone

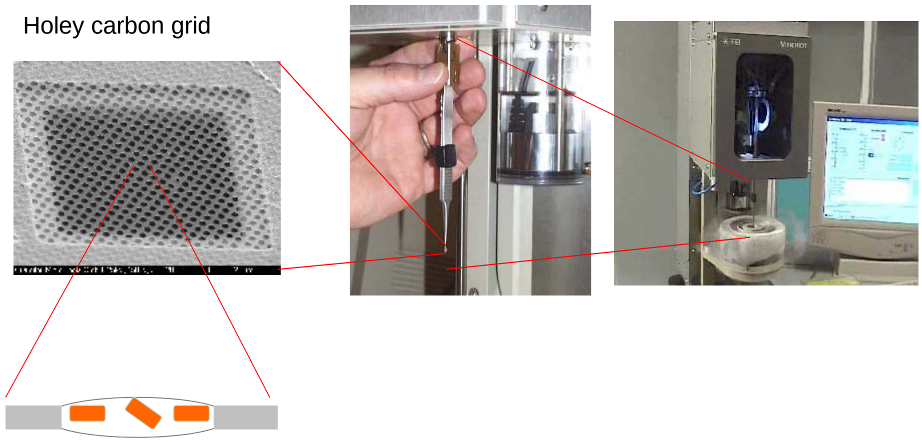


Schematic of the central projection theorem

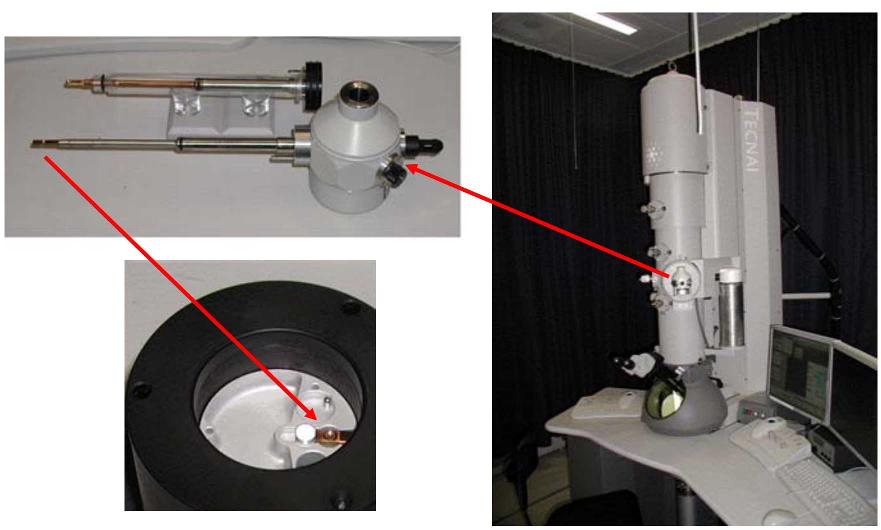


Baumeister, W. and Steven, A.C. (2000)

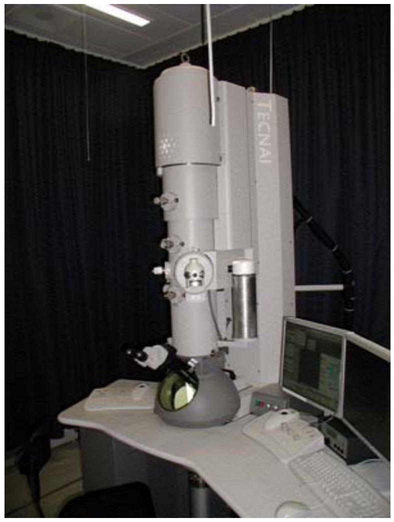
Cryo-EM: Ice-Embedding

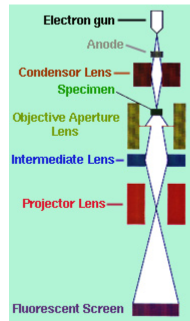


Cryo-holder



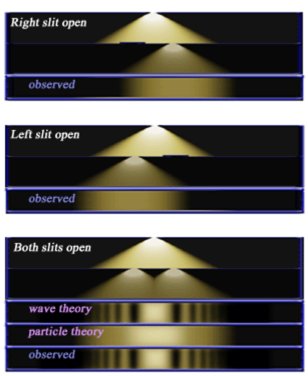
Transmission Electron Microscope

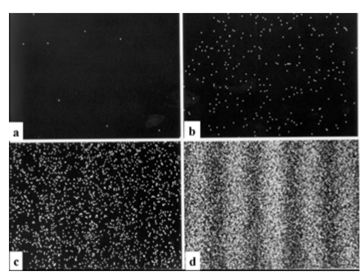




From the material of Virginia Tech.

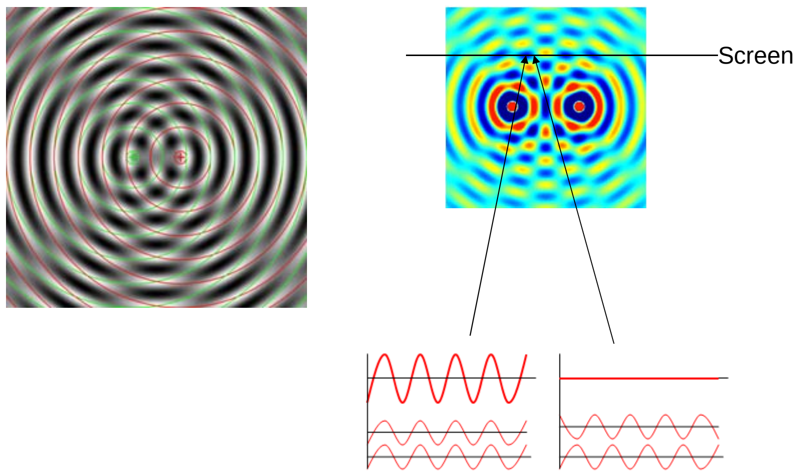
Wave–particle duality of electrons

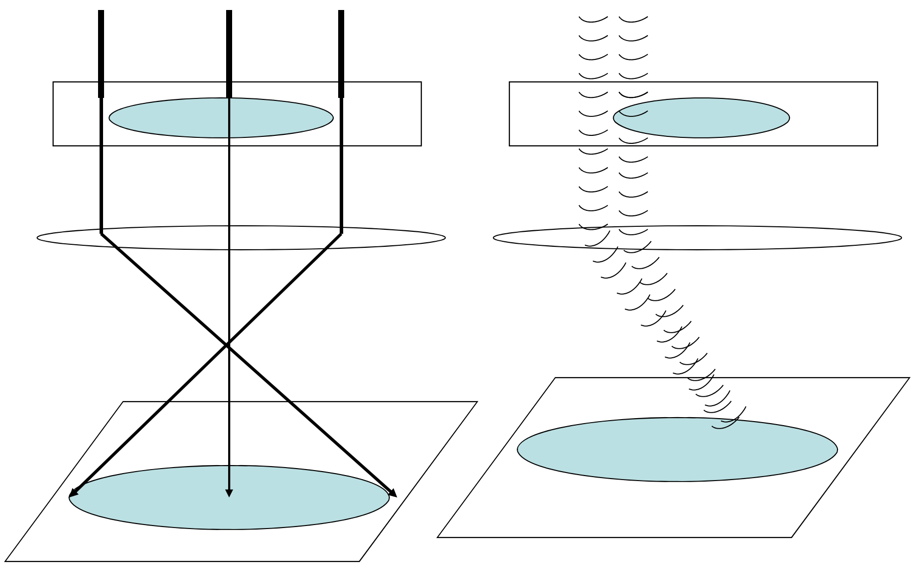




L de Brogli proposed and G. P. Thomson proved that electron has wave-particle duality.

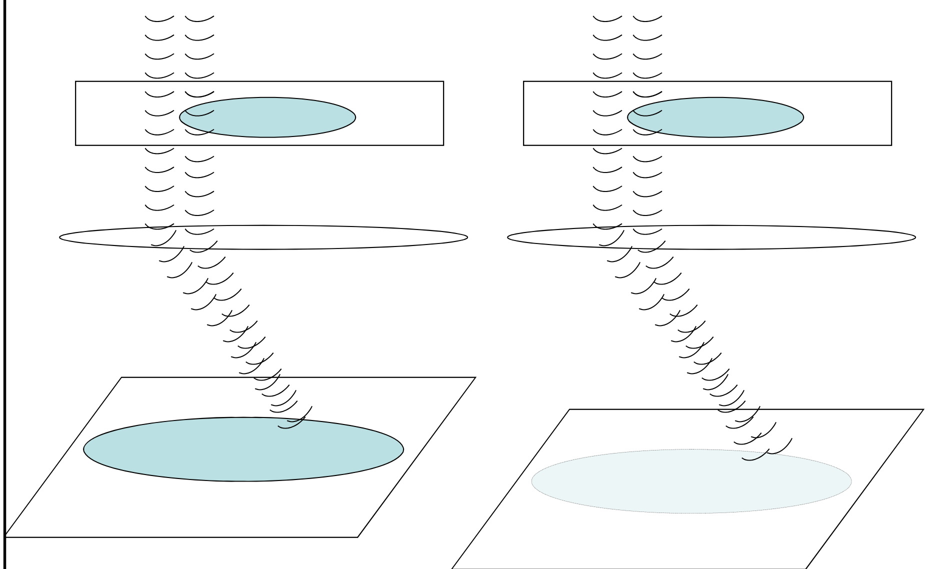
Interference of waves





Amplitude contrast (very weak with cryo-EM)

Phase contrast



Defocus: interference generates contrast

In focus: no interference, no phase constrast

Image formation for cryo-EM

$\mathcal{F}$(Image(ν)) = $\mathcal{F}$(Proj(r)) CTF(ν) Env(ν)
ν: spatial frequency

Proj(r) = $\int \rho(x,y,z)\,dz$
ρ: density of the object

$CTF(\nu) = -\{(1-F_{amp}{}^2)^{1/2}\sin(\chi(\nu)) + F_{amp}\cos(\chi(\nu))\}$
phase contrast   amplitude contrast

$\chi(\nu) = -2\pi\,[C_s\lambda^3\nu^4 / 4 - \Delta f\lambda\nu^2 / 2]$
Δf : defocus
λ: wavelength of electron
ν: spatial frequency
F_{amp} << 1 for cryo

Env: Envelop decay (gaussian), dependent on the coherence of the beam
$Env(\nu)=\exp[-(\pi\alpha/\lambda)^2(Cs\lambda^3\nu^3+\Delta f\lambda\nu)^2]$

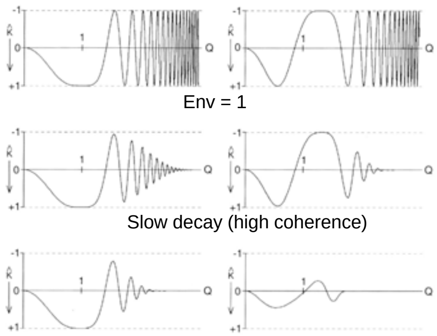
Image(ν) = $\mathcal{F}$(Proj(r)) CTF(ν) Env(ν)

Env: Envelop decay, dependent on the coherence of the beam

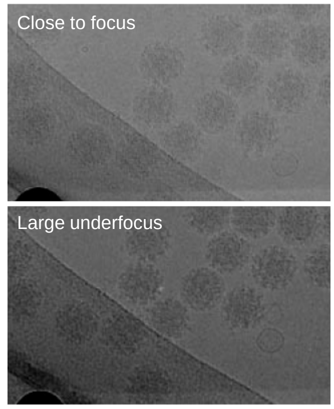
PCTF(ν) = -sin(χ(ν))

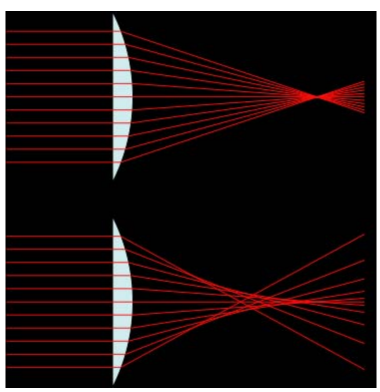
$\chi(\nu) = -2\pi\ [C_s\lambda^3\nu^4 / 4 - \Delta f\lambda\nu^2 / 2]$

Close to focus   Large underfocus



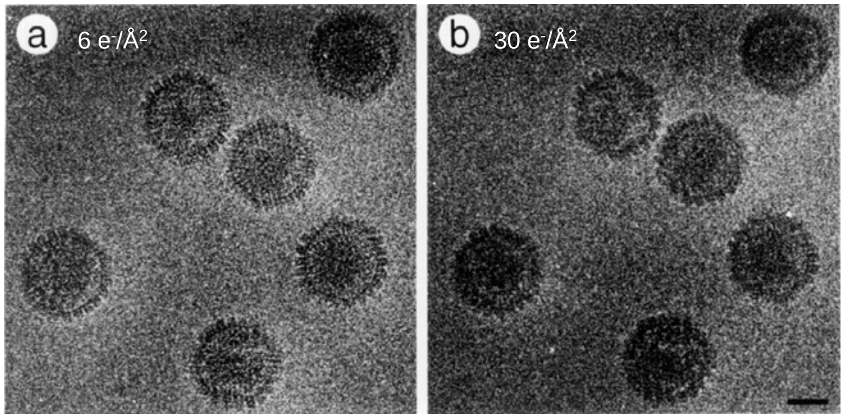
Fast decay (low coherence)

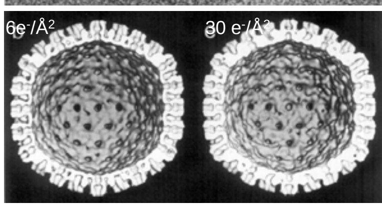


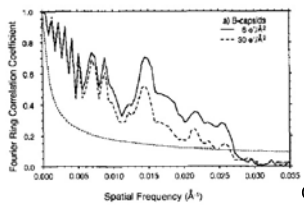


Spherical Aberration

Biological specimen (especially ice-embedded) is very sensitive to the radiation damage

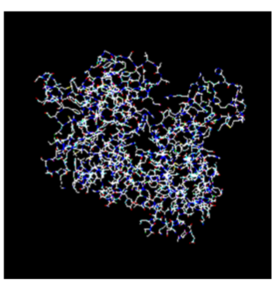


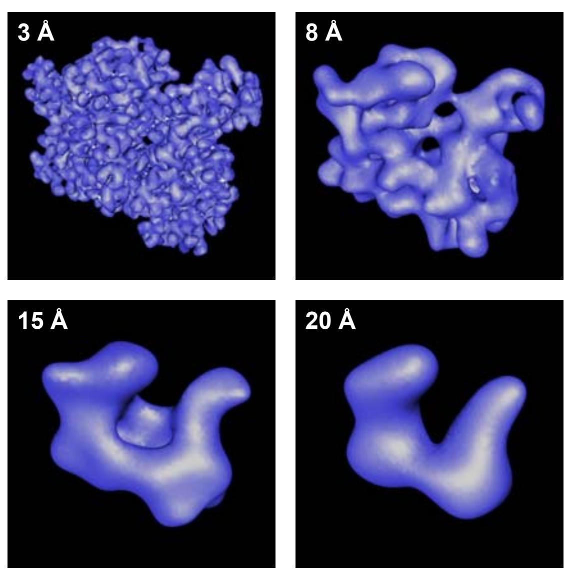




Conway et al. (1993)

Resolution





Various methodologies of 3D electron microscopy

1. 2D crystal
2. Helical reconstruction
3. Single particle analysis
4. Electron tomography

<u>2D crystal: electron microscopy and electron diffraction</u>

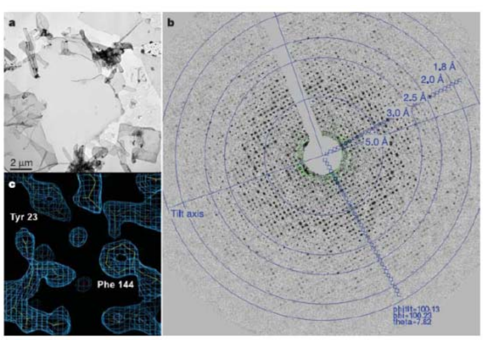
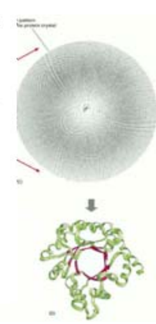
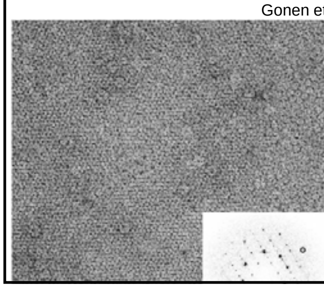


Figure 1 | Electron crystallography of AQP0 junctions. **a**, Double-layered AQP0 2D crystals were often several micrometres in size. **b**, A typical electron diffraction pattern recorded from an untilted AQP0 2D crystal prepared by the carbon sandwich technique[13], showing diffraction spots to a resolution beyond 2 Å. **c**, Region of the final $2F_o - F_c$ map of AQP0 refined to 1.9 Å resolution. Two aromatic residues, Tyr 23 and Phe 144, that line the water pore in AQP0 are represented by doughnut-shaped densities.

Gonen et al. (2005) Nature 438, 633.



“Molecular Biology of the Cell”



In successful cases, you can build atomic models directly from EM data.

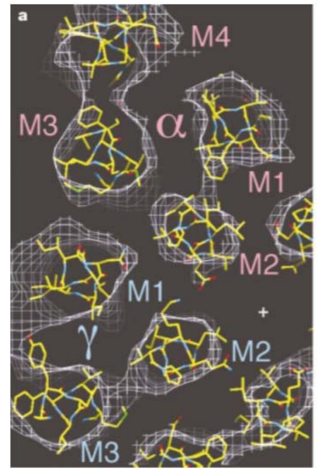
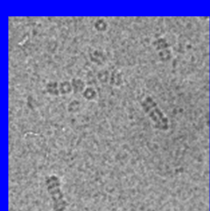


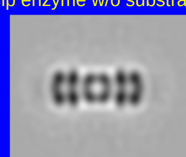
Figure 1 Cross-section of a tubular crystal, at low resolution. The receptor protein projects from either side of the membrane, visible as two concentric rings of density, 30 Å apart. A single receptor, cut centrally, is shown at the top. The membrane-spanning pore and the N-terminal ligand-binding domain, shaping a large central vestibule, are outlined by red and green rectangles, respectively. The surfaces encompassing the hydrophobic core of the membrane are assumed to lie along the centres of the rings of density[48].

Miyazawa et al. (2003) Nature 423, 949.

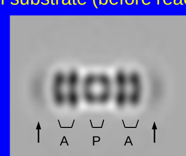
Single particle analysis: 2D averaging
Advantage to reconstruct without crystallization: Free solution condition



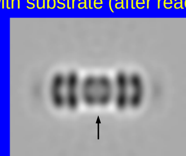
Clp enzyme w/o substrate



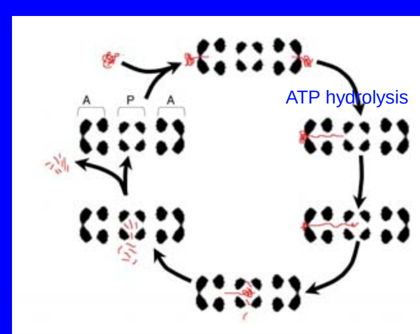
with substrate (before reaction)



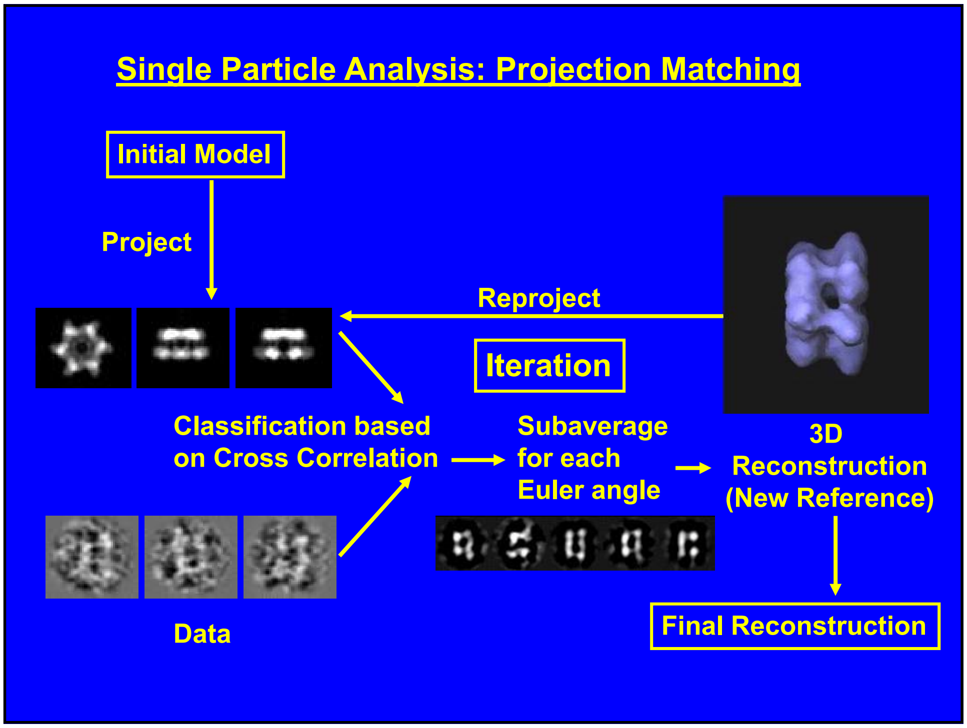
with substrate (after reaction)

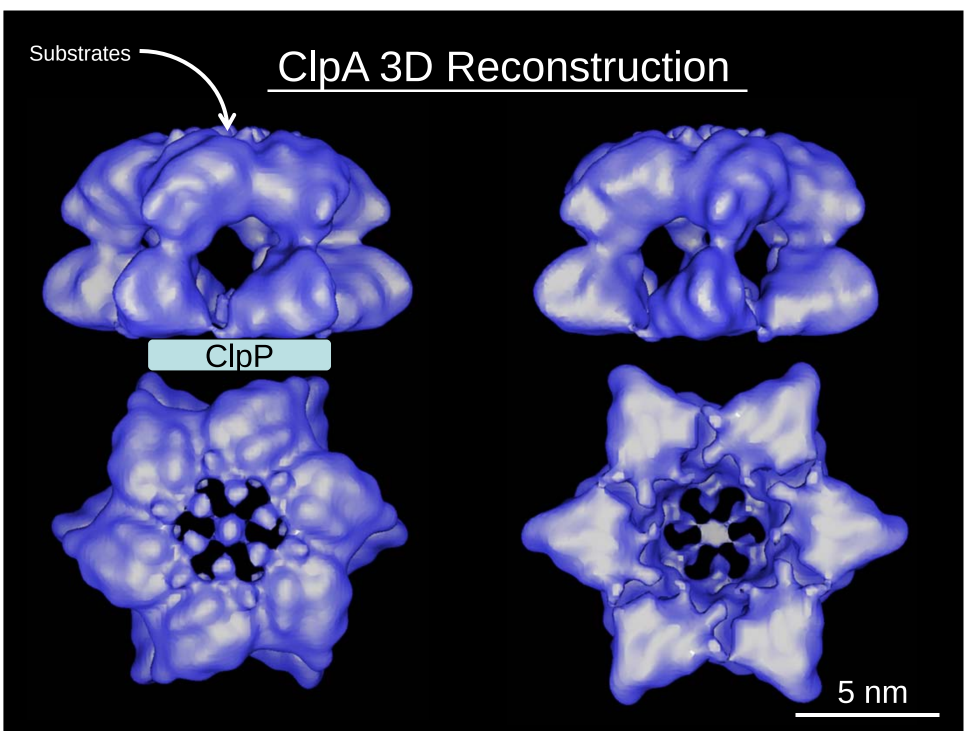


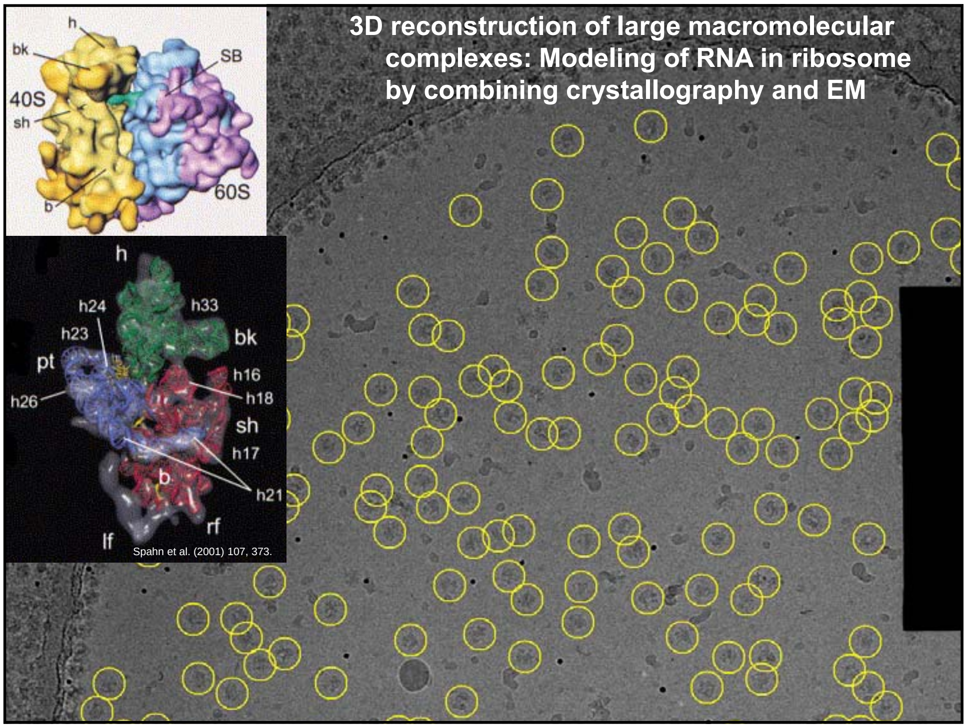
20 nm



Ishikawa et al. (2001)

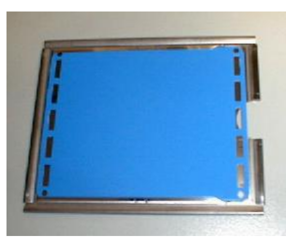

Single Particle Analysis: Projection Matching
Initial Model
Project
Reproject
Iteration
Classification based
on Cross Correlation
Subaverage
for each
Euler angle
3D
Reconstruction
(New Reference)
Data
Final Reconstruction


Substrates
ClpA 3D Reconstruction
ClpP
5 nm

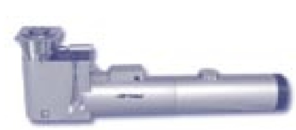


7. Detectors (Photographic Films, Imaging Plates, Digital Camera)

Photographic Films



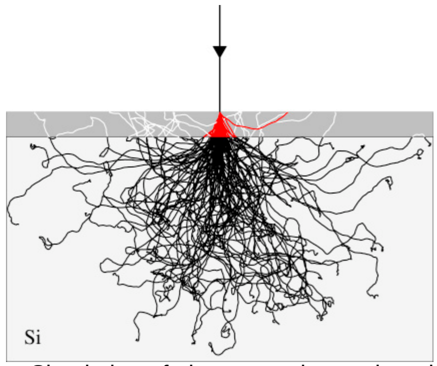
Imaging Plates



Digital Camera

Two important parameters to evaluate detectors:
(1) Resolution (how many microns one pixel is; how sharp one pixel is)
(2) Size (how many pixels in one image)

Point Spread Function determines Resolution of the Detector



Simulation of electron pathways in a detector

McMullen et al. (2009) Ultramicroscopy 109, 1144.

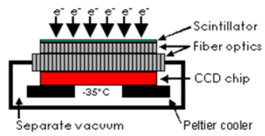
More secondary electron -> wider spread
Less secondary electron -> less sensitivity

Photographic Films

Advantages:
Wide area (15kx18k), high point spread function

Disadvantages:
Low sensitivity, Fog, Narrow dynamic range, Inconvenience

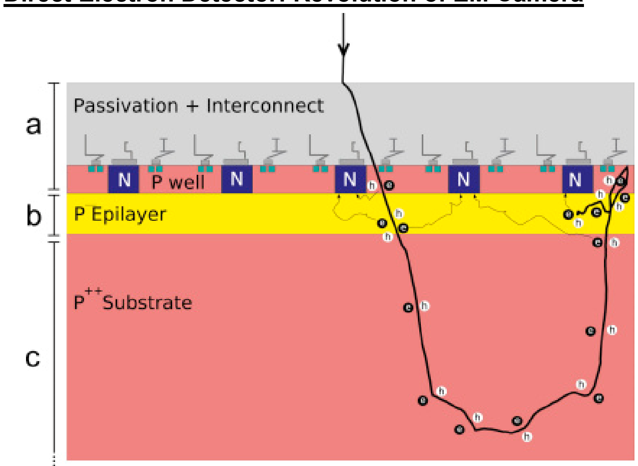
Digital (CCD) Camera



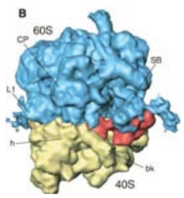
Advantages:
High sensitivity, High linearity, Convenience

Disadvantages:
Narrow area (4kx4k)
Poor point spread function (because of the scintillator)

Direct Electron Detector: Revolution of EM Camera



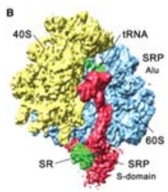
McMullen et al. (2009) Ultramicroscopy 109, 1126.



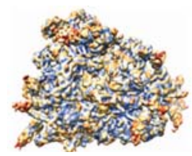
11.5A (Spahn et al. 2004)

Progress of single particle analysis

Getting close to near atomic resolution



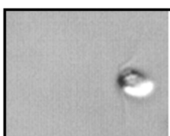
Halic et al. 2006



Gogala et al. 2014

8A with a CMOS camera/negative

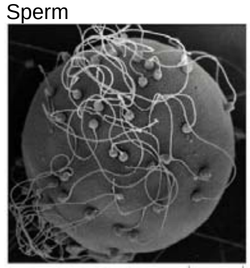
Beckmann lab (4.8A with a direct camera)



Green algae
Chlamydomonas

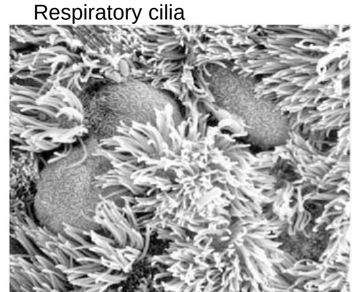
Flagella and motile cilia in vertebrates

Sperm



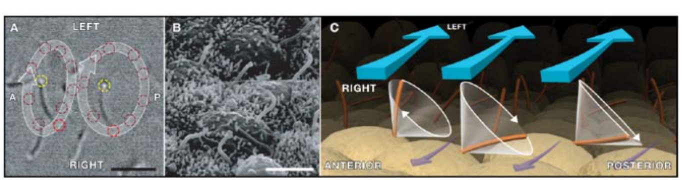
Molecular Biology of the Cell

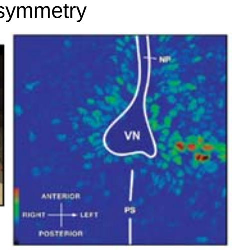
Respiratory cilia



Eley et al. (2005) Curr. Op. Genetics Development 15, 308

Nodal cilia generate unidirectional flow and determine left-right asymmetry

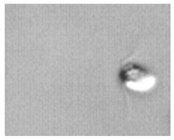




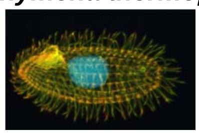
Hirokawa et al. (2006) Cell 125, 33.

Eukaryotic flagella/cilia have motility by themselves

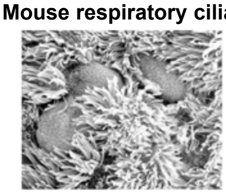
Chlamydomonas reinhardtii



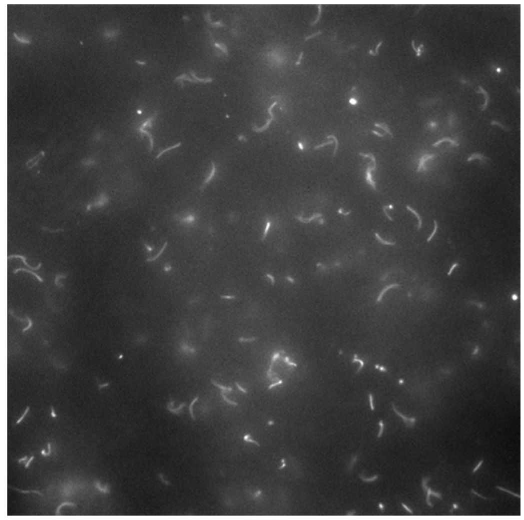
Tetrahymena thermophila



Mouse respiratory cilia

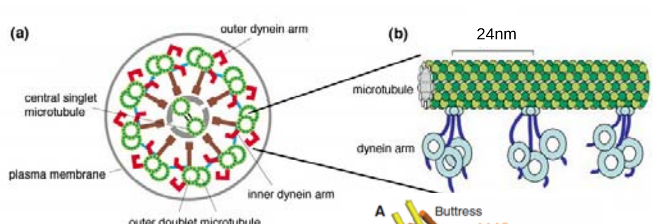


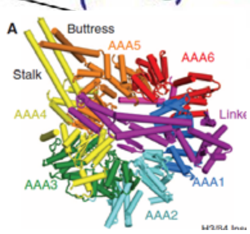
Isolated flagella+ATP

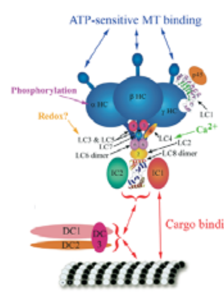


Dr. S. Toba (KARC, NICT, Japan)

Web page of S. King Lab.







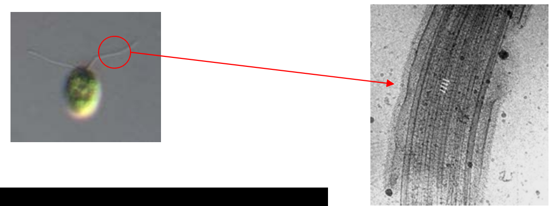
Sakato & King (2004) J. Struct. Biol. 146, 58.

Carter group
Kon group (2013)

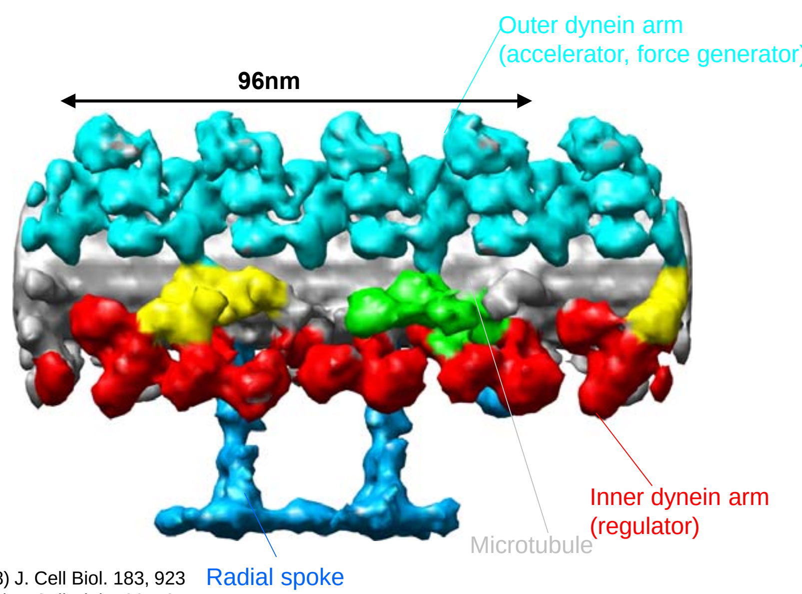
Axonemal

Dynein arms in flagella are highly complexed force-generating apparatus composed of various components and produce bending motion

Cryo electron tomography

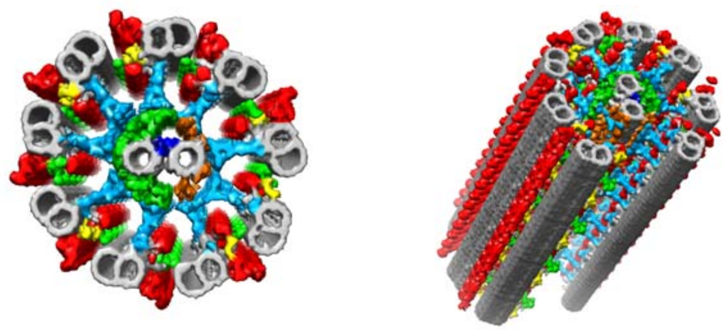


96nm periodic unit on the microtubule doublet from the axoneme



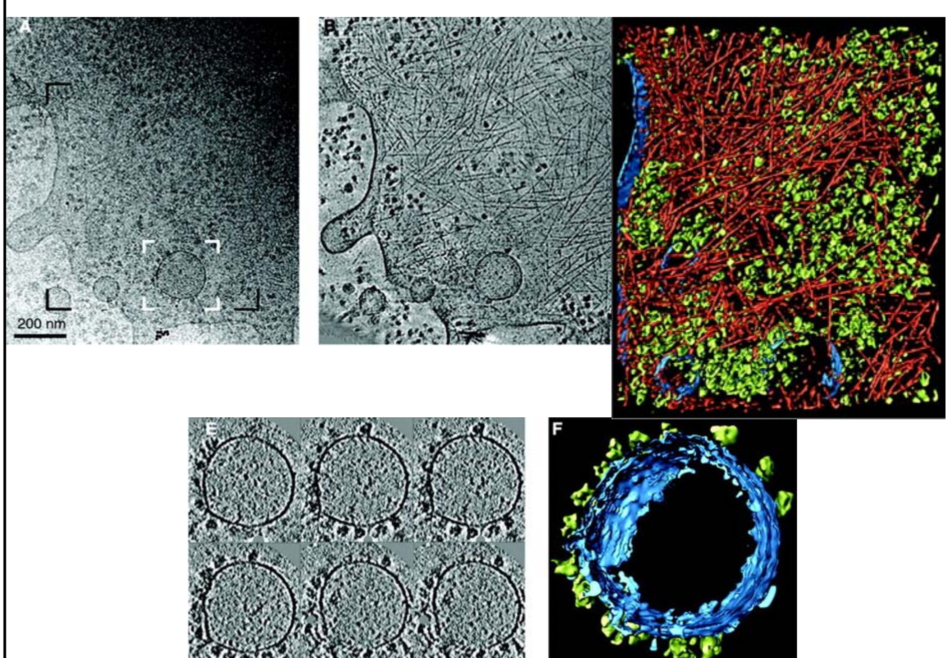
Bui et al. (2008) J. Cell Biol. 183, 923
Bui et al. (2009) J. Cell Biol. 186, 437
Pigino et al. (2011) J. Cell Biol. 195, 673
Bui et al. (2012) J. Cell Biol. 198, 913

Flagella Structure reconstructed by Electron Tomography

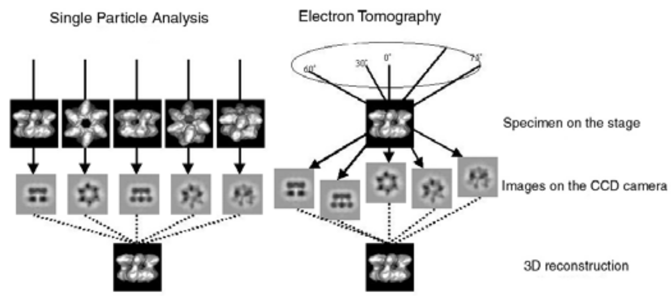


Modified from Bui et al. (2009) J. Cell Biol. 186, 437

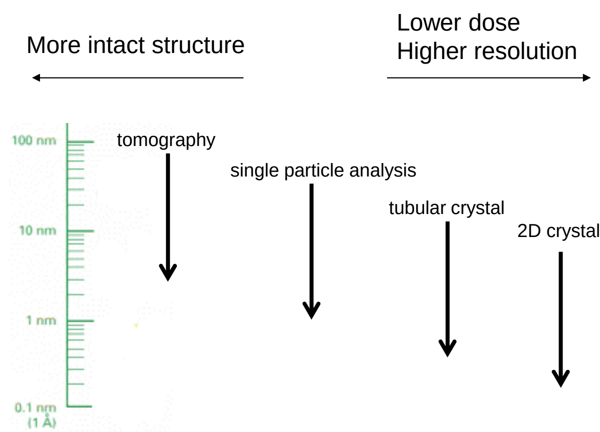
Example: Whole cell tomography



Electron Tomography



	Single particle analysis	Electron tomography
Principle to obtain multiple views	Merged many particles with various views in solution	Take micrographs of one particle tilted at various angle in the microscope
Crystallization	Not needed	Not needed
Structural heterogeneity	Averaged out	Visualized individually
Current resolution	High (up to 8 Å)	Low (currently 25 Å)
Missing information	None / Missing cone	Missing wedge



Resolution of various methodology of 3D cryo-TEM