Introduction of Molecular Electron Microscopy

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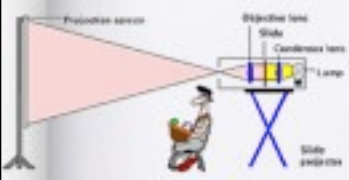
May 5, 2010

Tecnai F30 Polara from FEI company



Outlines

- **Basics of molecular electron microscopy**
  - **electron microscope**
  - **electron cryo-microscopy**
  - **3D reconstruction**
- Three different techniques of molecular EM
  - electron crystallography of membrane proteins
  - single particle analysis
  - electron tomography
- Applications of molecular EM
- Current status of molecular EM
- What can you get out of molecular EM?

Wave-particle duality of electron

De Broglie's hypothesis: $\lambda = \frac{h}{p}$

λ is wavelength, h is Planck's constant, and p is momentum.

$$eE_0 = \frac{h^2}{2m\lambda^2} \qquad \lambda = \frac{h}{\sqrt{2meE_0}}$$

E_0 = acceleration voltage
λ = wavelength

m = electron mass
e = electron charge
h = Planck's constant

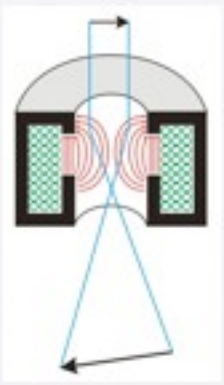
* Electron scattering - particle character of electron
* Image formation - wave character of electron

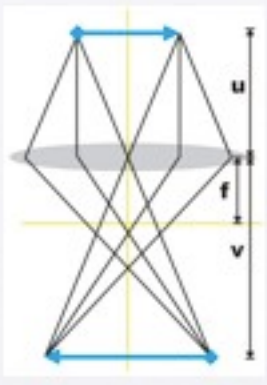
Electron v.s. X-ray

As particles:
• Electrons interact with an atom's shell electrons and nucleus, X-rays interact with only shell electrons.
• Electrons have much larger scattering cross-sections than X-rays. Thus, electron scattered much stronger than x-ray.

As wave:
• Electrons can be focused by electromagnetic lens, X-ray can not be focused by lens;

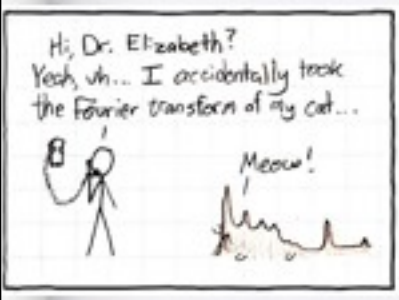
Electromagnetic lens

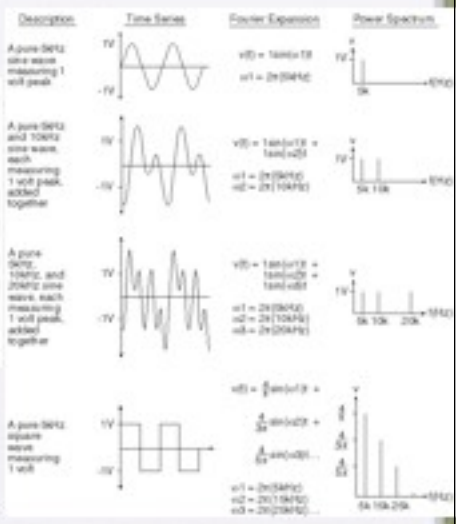




* The focal length of a electromagnetic lens can be easily adjusted by changing the lens current.

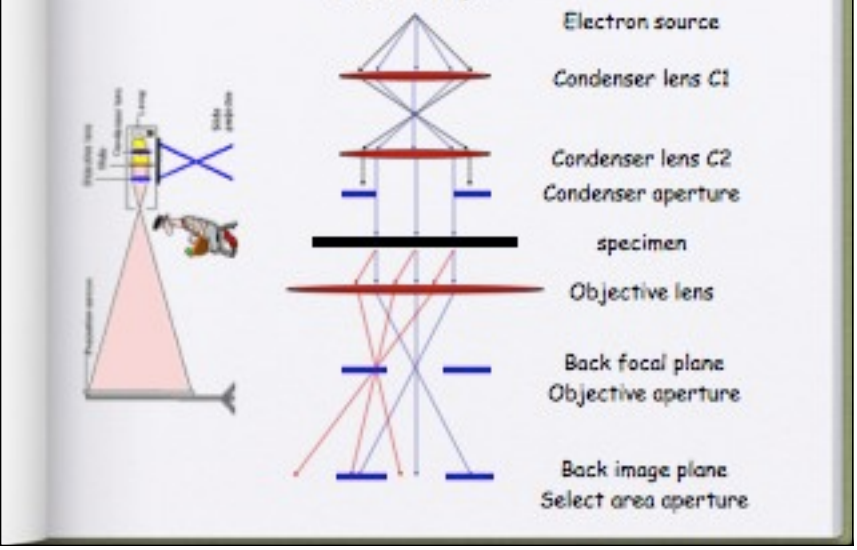
Fourier Transform





From web.http://mathworld.wolfram.com/FourierTransform.html

Optic system in an electron microscope



It is a microscope!

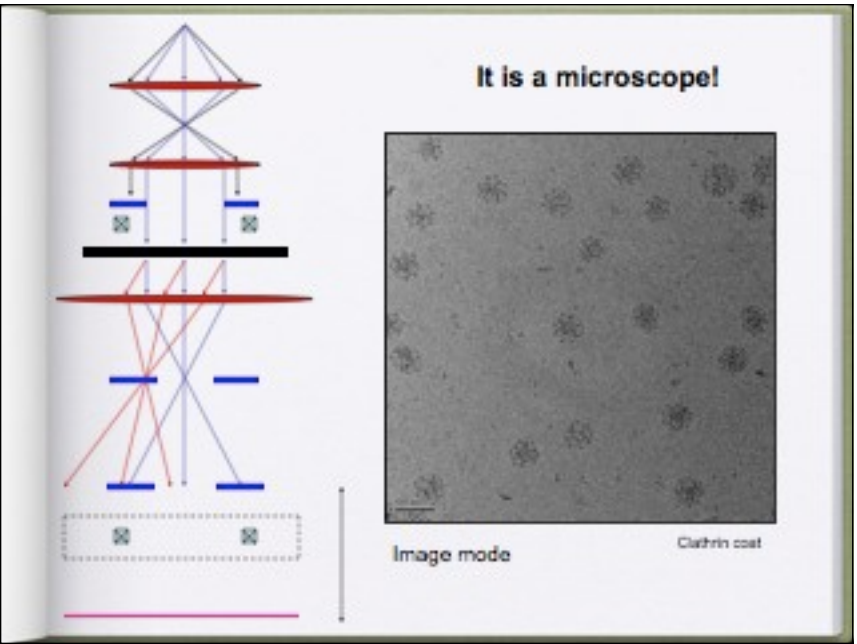
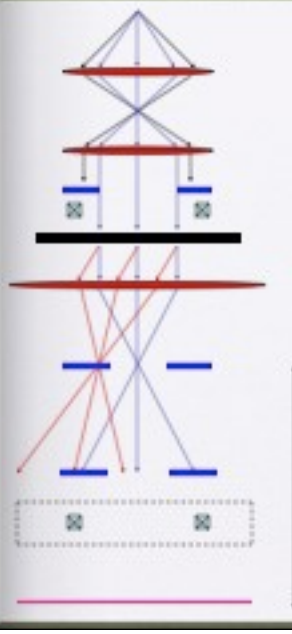
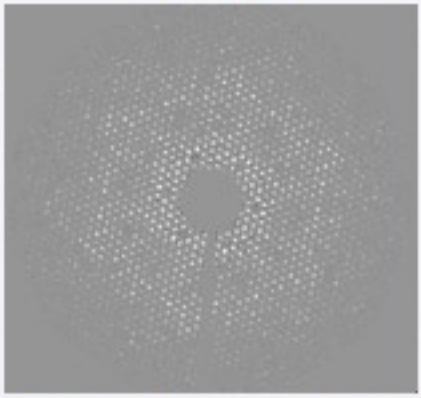


Image mode

Clathrin coat



It is also a diffractometer!



Diffraction mode

bacteriorhodopsin

Observing biological sample by electron microscopy

Strong scattering power means two things:

1 high vacuum of microscope column;

2 strong scattering with protein sample;

Problems:

1 dehydration of biological sample;

2 radiation damage by high energy beam;

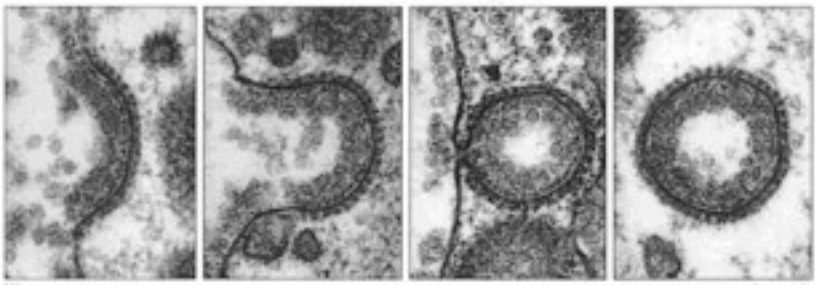
Cellular Electron Microscopy

To look at cellular organization by using electron microscope:

Sample are prepared by fixation, dehydration, plastic embedding, thin section and staining;

A typical electron micrograph of a biological sample (plastic embedding/thin section/negative staining)

Formation of Clathrin-Coated Vesicles



- 2500 every minute
- CCV uncoat within seconds

Cellular Electron Microscopy

To look at cellular organization by using electron microscope:
Sample are prepared by fixation, dehydration, plastic embedding, thin section and staining;

Molecular Electron Microscopy

To look at structures of individual molecules:
look at the purified sample directly by electron microscope
* negative staining;
* embedding sample in vitreous ice (frozen hydrated sample);

Electron cryomicroscopy - cryoEM

Taylor K and Glaeser RM (1974) "Electron diffraction of frozen, hydrated protein crystals" *Science* **186**, 1036-1037

Fig. 1. Electron diffraction pattern of a catalase crystal which was frozen in liquid nitrogen and observed on a specimen stage cooled with liquid nitrogen. The resolution of the photographic reproduction is 4.5 Å, although that of the diffraction pattern on the original plate was 3.4 Å.

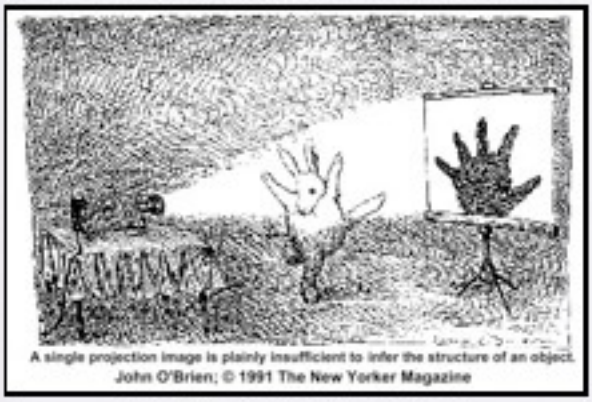
Taylor and Glaeser (1974)

Cryo-electron microscopy

- Against dehydration:
  - glucose/trehalose embedding: using glucose to substitute water, thus maintain hydration in the high vacuum.
  - frozen hydration: using plunge freezing to avoid crystal ice. Mostly for single particle;

- Against radiation damage:
  - Low-temperature: LN2 (~80K) or LHe (~10K); Challenges to the instrumentation;
  - Low-electron dose: Low-dose imaging; Results in extremely noisy images, challenges for the data processing;

Images obtained from electron microscope are projections of three dimensional object

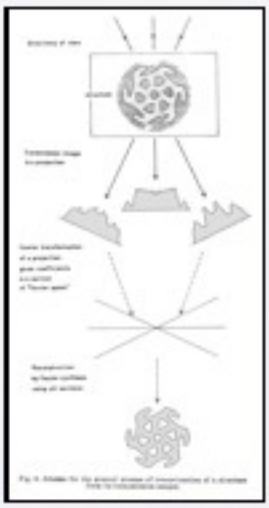


A single projection image is plainly insufficient to infer the structure of an object.
John O'Brien; © 1991 The New Yorker Magazine

Principle of 3D reconstruction of biological object from projection images

Central Section Theorem :
Fourier transform of a 2D projection equals the central section through its 3D Fourier transform perpendicular to the direction of projection.

DeRosier, D. and Klug, A. (1968)
"Reconstruction of three dimensional structures from electron micrographs" *Nature* ***217*** 130-134



DeRosier and Klug (1968)

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3D electron microscopy

There are three distinct 3DEM techniques:

* Electron crystallography: to study membrane proteins that forms 2D crystal;

* Single particle averaging: to study protein that have defined (homogeneous) shapes;

* Electron tomography: to study large assemblies or organelles with unique shapes that can not be averaged;

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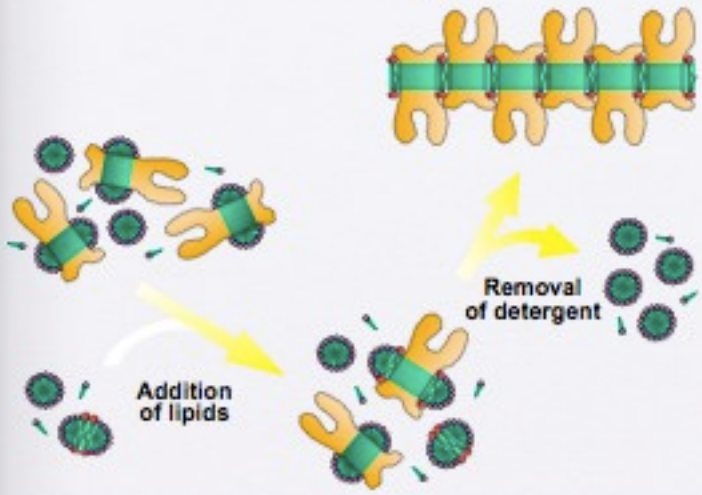
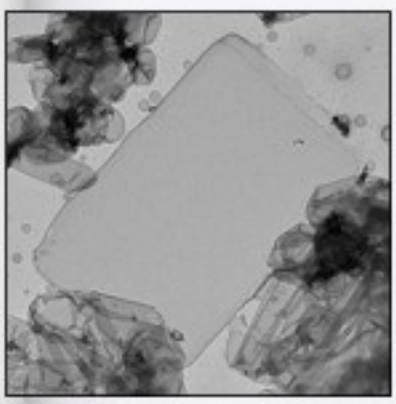
Crystallization of a membrane protein into 2-dimensional crystal



2D crystal



A good 2D crystal!

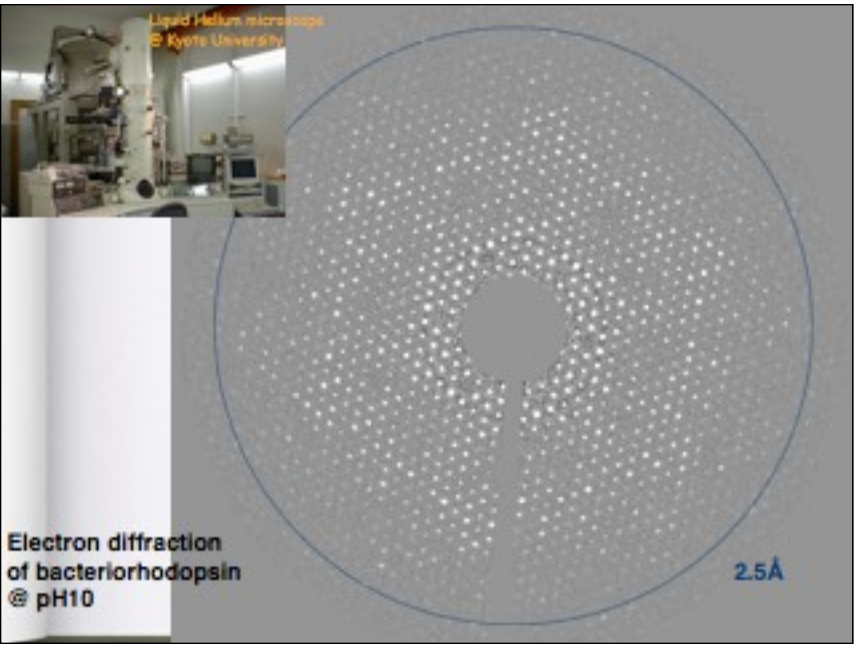
Typically, only one image or one diffraction pattern can be taken from one single 2D crystal.

Image of glucose embedded 2D crystal with low dose imaging

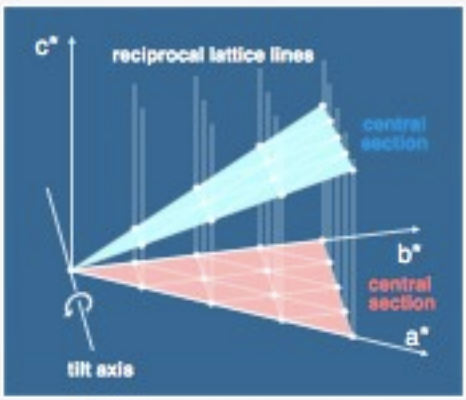
an image of bacteriorhodopsin 2D crystal embedded in glucose

Fourier transform gives both phase and amplitude

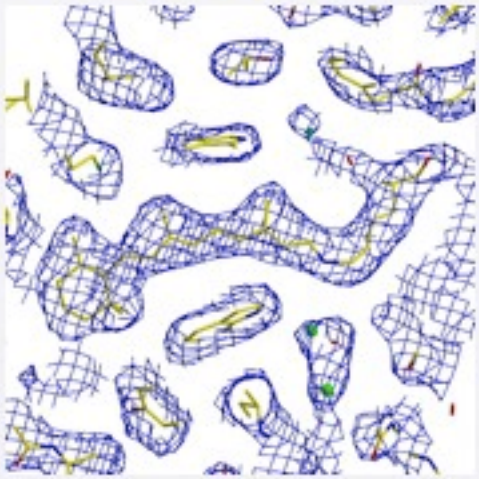
Fourier transform from image in previous slide.



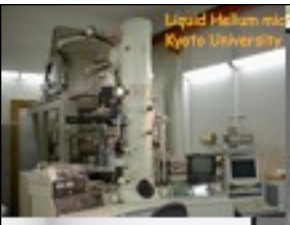
3D reconstruction of 2D crystals

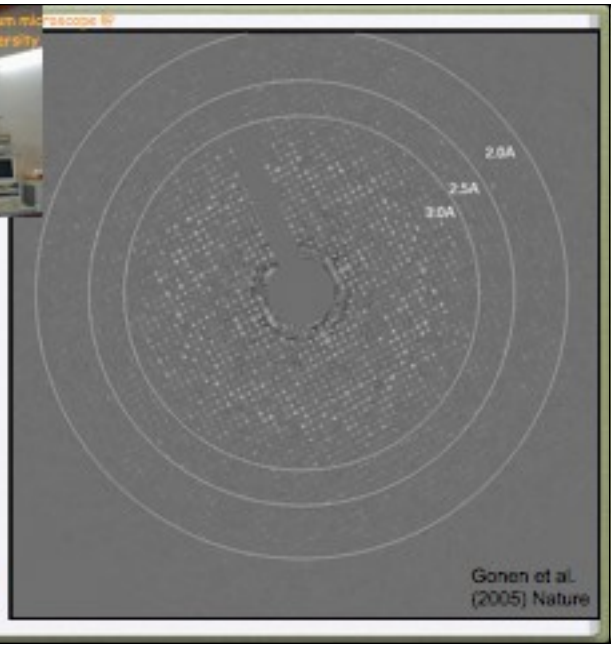


A part of the density map of bacteriorhodopsin at a resolution of 2.5Å



$(2F_o - F_c)$ map

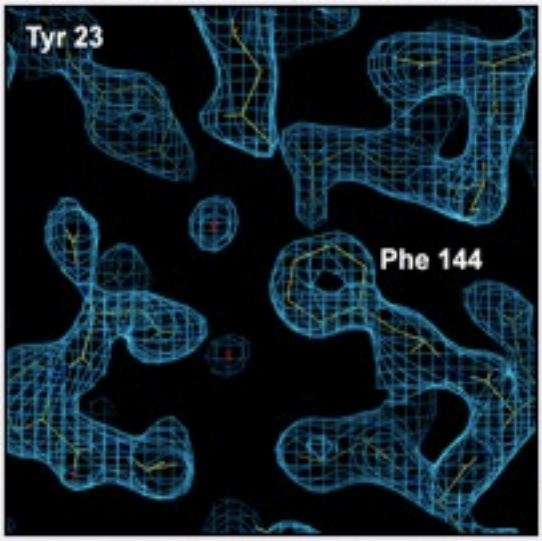




Electron diffraction of aquoporin 0

Gonen et al. (2005) Nature

Density map of AQP0 at a resolution of 1.9Å



Gonen et al. (2005) Nature

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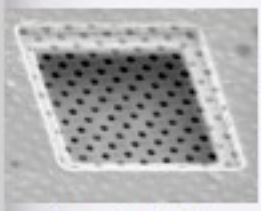
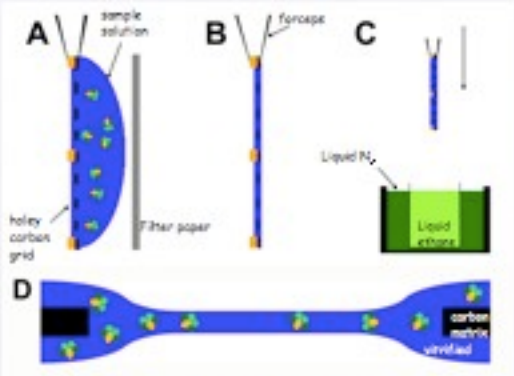
Frozen hydrated sample preparation for single particle cryoEM

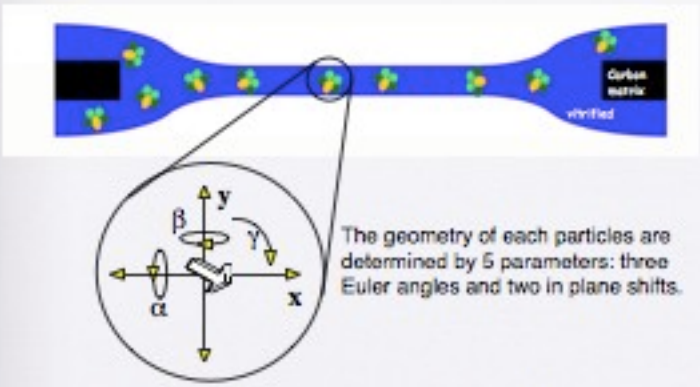
Adrian M, Dubochet J, Lepault J & McDowall AW (1984) Cryo-electron microscopy of viruses. *Nature* **308**, 32-36.





Quantifoil grid **Plunge freezing**

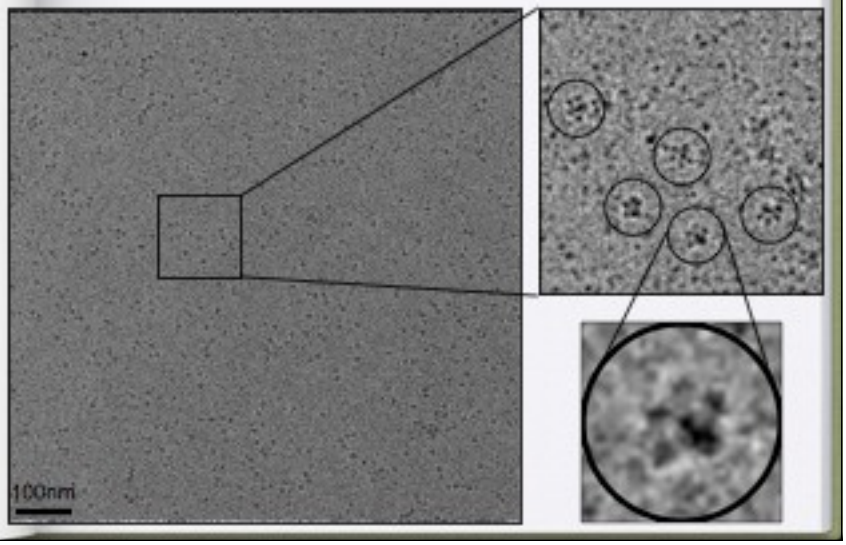
Single particle cryoEM of macromolecular assemblies



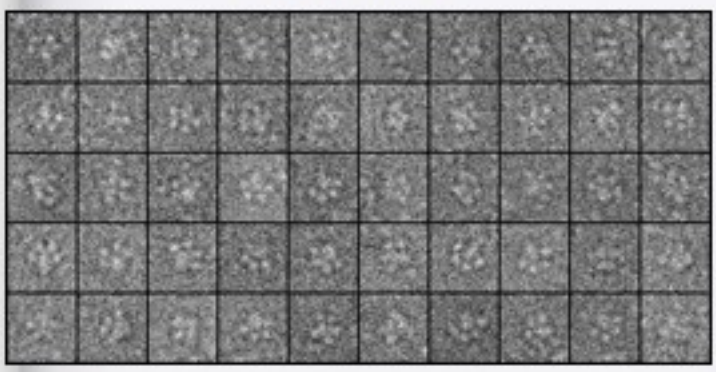
The geometry of each particles are determined by 5 parameters: three Euler angles and two in plane shifts.

Single particles are randomly oriented in vitreous ice

Human TfR-Tf complex in vitrified ice



Individual TfR-Tf Complexes in Vitrified Ice



50 out of 36,266 particles

Single particle EM

* Classifications and multi-reference alignment to produce class averages that have higher signal-to-noise ratio;

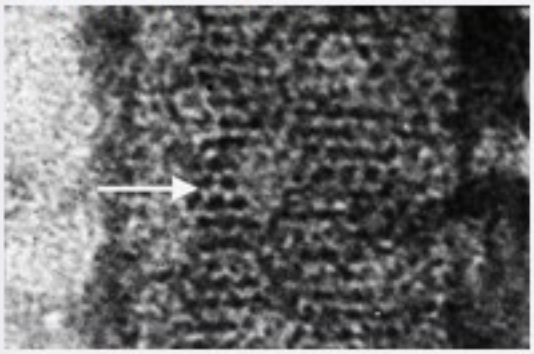
* 3D reconstruction using 2D class averages;

Image averaging is powerful and interesting!

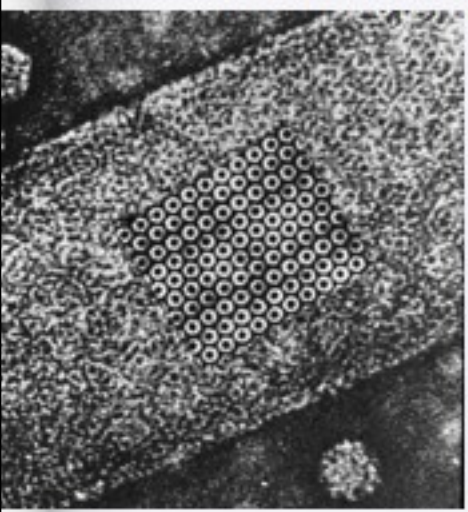
From Joachim Frank

Image averaging

Cryo-EM images are very noisy; have extremely low signal-to-noise ratio. Averaging of a large number of images are necessary to improve the SNR.



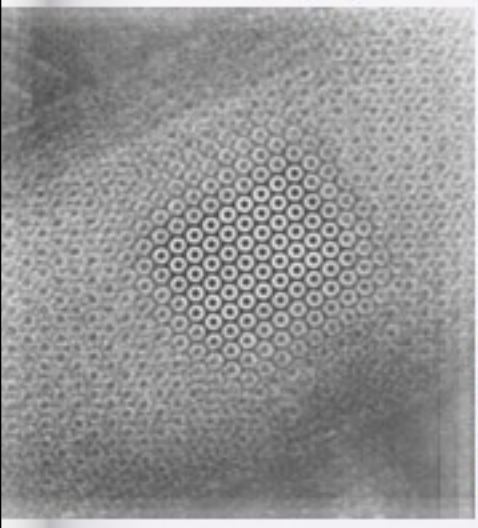
Averaging in a darkroom



Photographic image superposition (averaging) by Roy Markham, who shifted image and added to the original in darkroom.

The trick is to know how much and along which direction to shift the image for superposition.

Averaging in a computer!



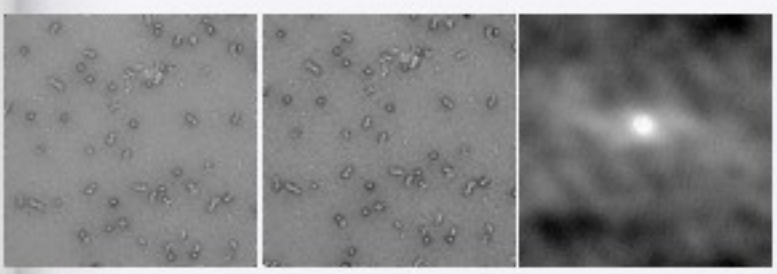
David DeRosier used Markham's lattice to determine how much to shift by, and performed averaging by using Adobe Photoshop.

What about single molecules

A single particle image data set is a collection of images, each contains projection images of one molecules. The orientations and position of particles in all images are different. Before averaging, one needs to:

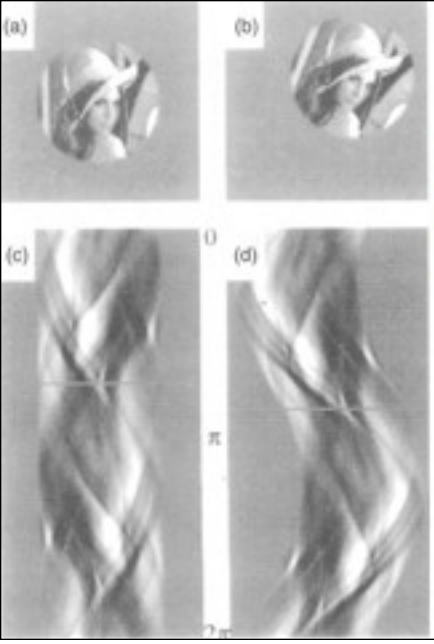
- judge how similar is the two particles: *cross-correlation coefficient*;
- shifts/rotates one particle to match another by maximizing ccc: *alignment*;
- separate different particles: *classification*;

How cross-correlation looks like



CCF

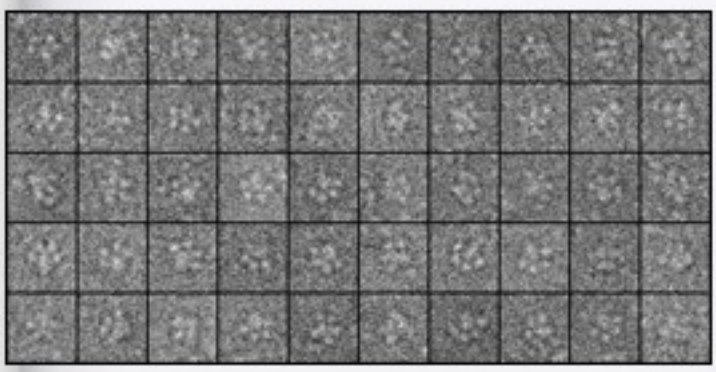
The image size is 1024X1024. The peak in the CCF is at (445,500). How much is the shift?



Radon transform

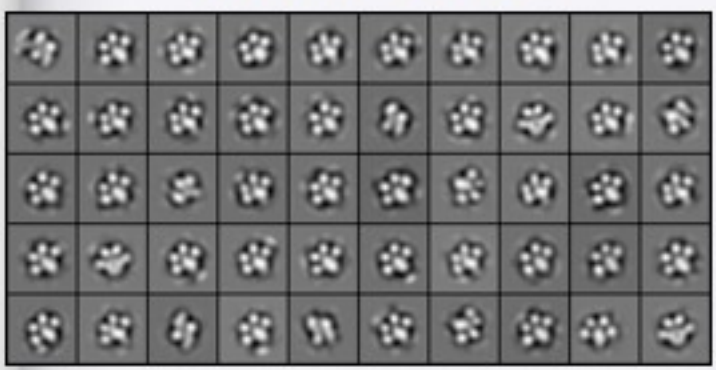
Radon transform is an efficient way for determining angular relationship between two images, but it only works well in images with high SNR.

Individual TfR-Tf Complexes in Vitrified Ice



50 out of 36,266 particles

Class Averages of Vitrified TfR-Tf Complexes



50 out of 200 classes

Demonstration of reference induced bias

Note: The averaged image after reference based alignment is strongly biased towards the reference.

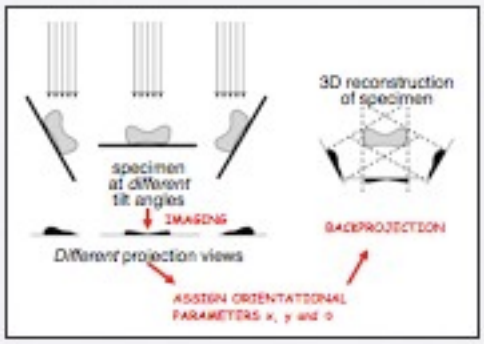
100 images 1000 images reference

From Niko Gorigorieff

3D reconstruction

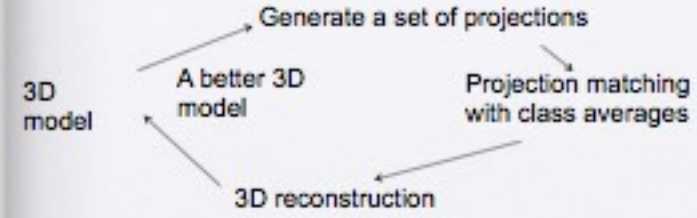
Assume we have already a number of class averages, they represent the projections of a 3D object in different orientations. And we know (can determine) these relative orientations of each class averages. We can reconstruct the 3D object - 3D reconstruction.

Back projection:



How to determine the relative orientations?

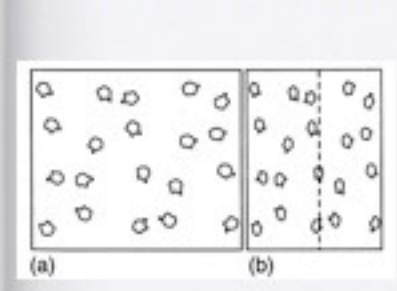
Model based projection matching: by matching of class averages with the calculated projections of the 3D object in known directions - a refinement procedure.

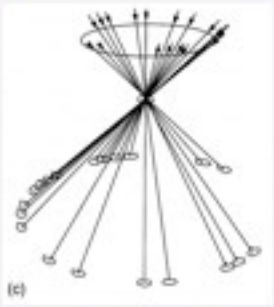


Where do you get the first model?

Random conical tilt

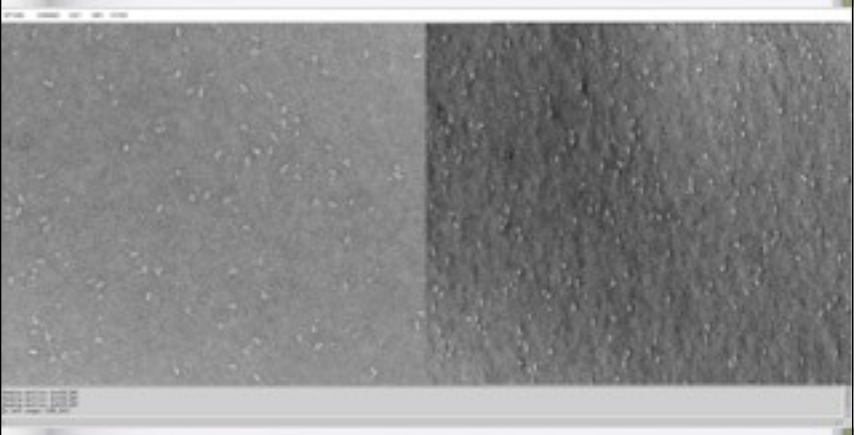
A pair of images are taken from the same specimen area for the random conical tilt 3D reconstruction.





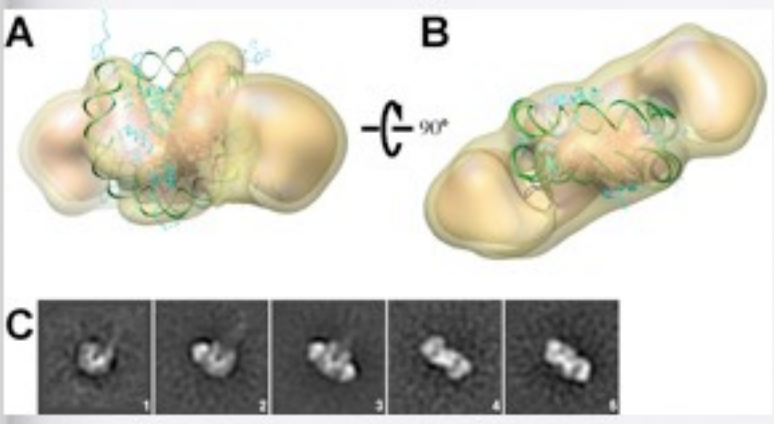
From Joachim Frank

Random conical tilt



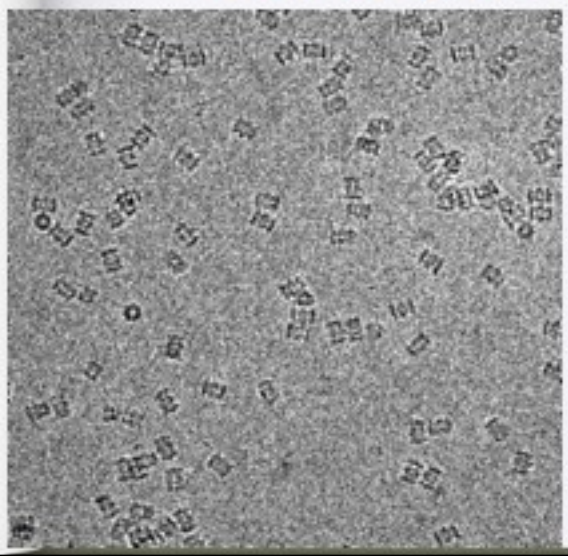
A tilt-pair image of SNF2h-nucleosome complex

Random conical tilt



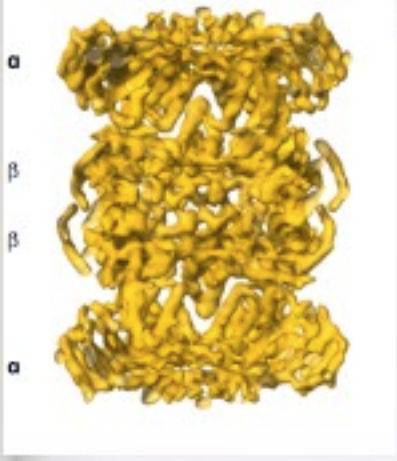
A tilt-pair image of SNF2h-nucleosome complex

T. acidophilum 20S proteasome

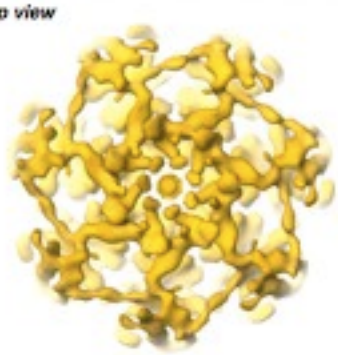


Structure of archaeal 20S CP

Side view



Top view



** Density map filtered to 6.8 Å;*

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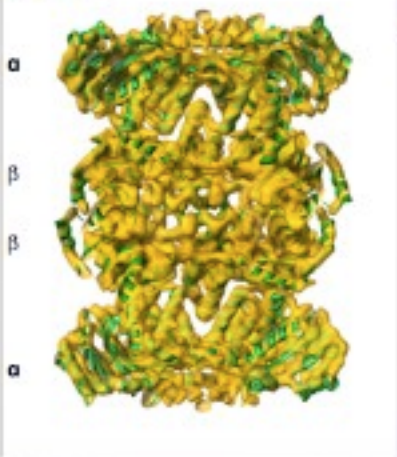

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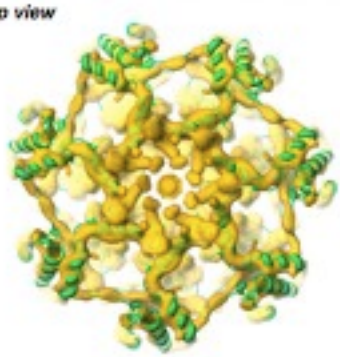
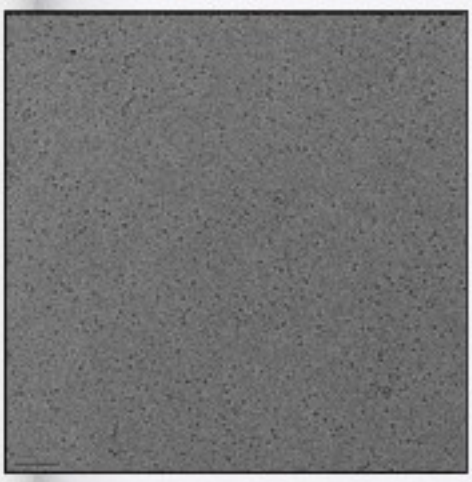
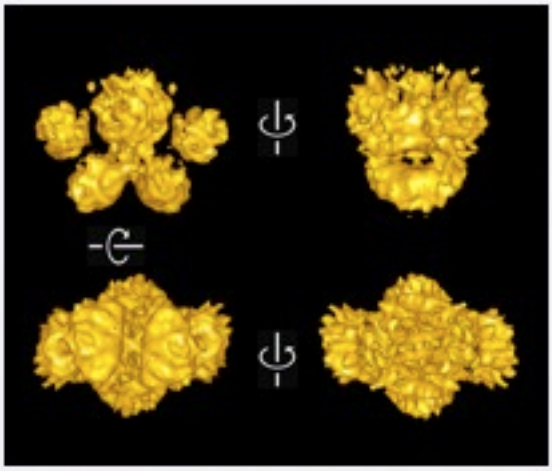


** Density map filtered to 6.8 Å;*

Human Transferrin receptor - transferrin complex

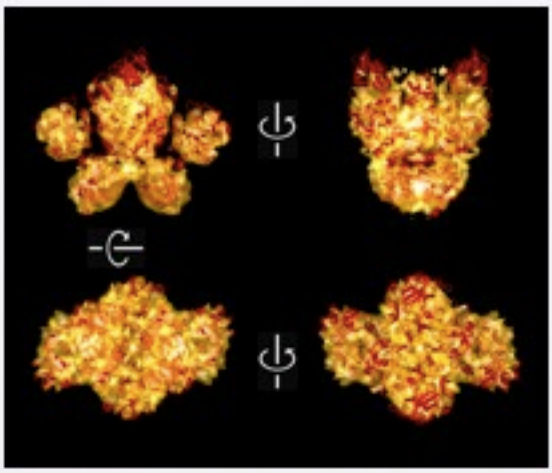


Structure of TfR-Tf complex at a 7.5Å resolution



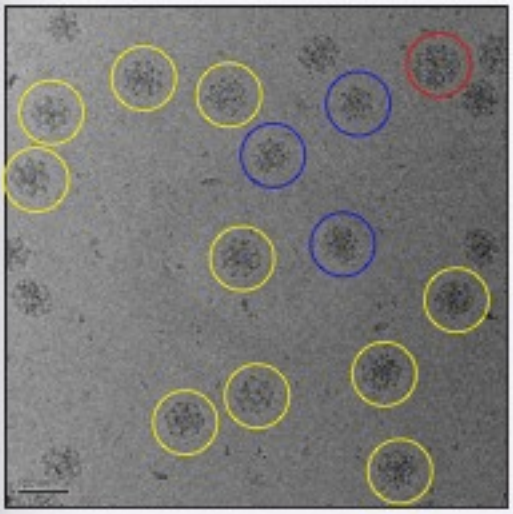
Cheng et al. (2004) Cell

Docking the atomic structures



Cheng et al. (2004) Cell

In vitro assembled clathrin coats in vitrified ice

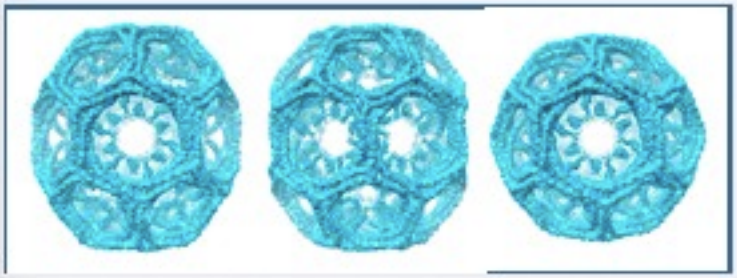


Tetrahedral coats

D6 barrel coats

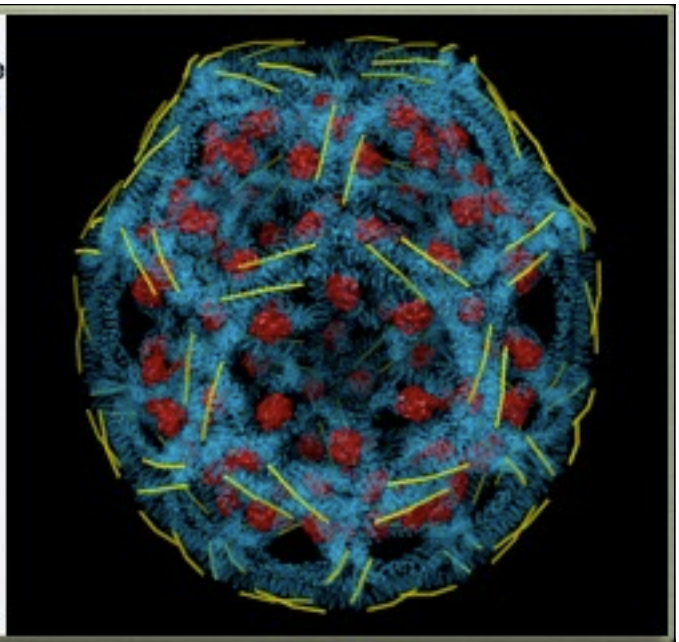
Icosahedral coats

Structure of a clathrin coat at 7.9 Å resolution



Fotin et al. (2004) Nature

The complete clathrin coat with light chain and auxilin



Fotin et al. (2004) Nature

3D electron microscopy

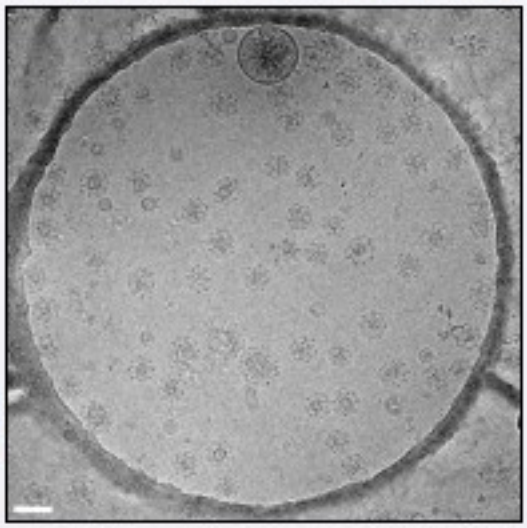
There are three distinct 3DEM techniques:

* Electron crystallography: to study membrane proteins that forms 2D crystal;

* Single particle averaging: to study protein that have defined (homogeneous) shapes;

* **Electron tomography: to study large assemblies or organelles with unique shapes that can not be averaged;**

Clathrin coated vesicles in vitrified ice



Clathrin coated vesicle purified from calf brain

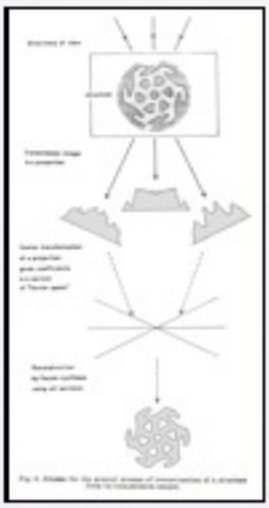
100nm

Principle of 3D reconstruction of biological object from projection images

Central Section Theorem :
Fourier transform of a 2D projection equals the central section through its 3D Fourier transform perpendicular to the direction of projection.

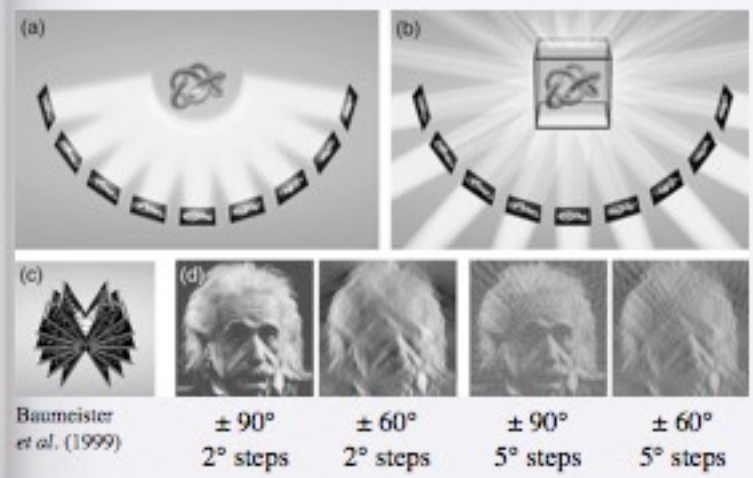
DeRosier, D. and Klug, A. (1968) "Reconstruction of three dimensional structures from electron micrographs" *Nature* ***217*** 130-134

Hart, R.G. (1968) "Electron microscopy of unstained biological material: the polytropic montage" *Science* ***159*** 1464-1467



DeRosier and Klug (1968)

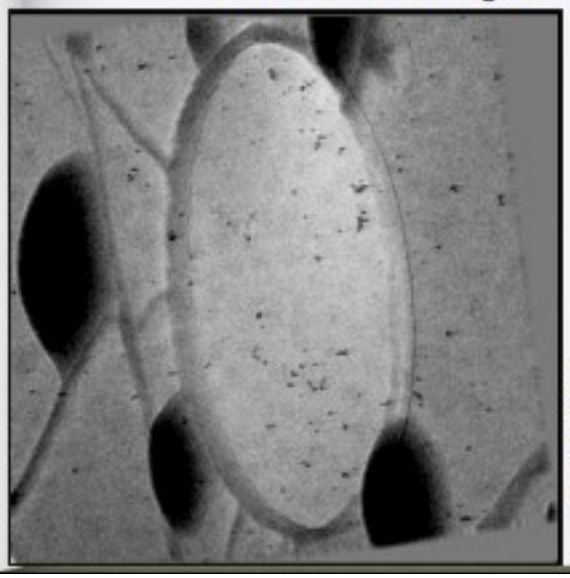
Electron Tomography



Baumeister *et al.* (1999)

± 90°	± 60°	± 90°	± 60°
2° steps	2° steps	5° steps	5° steps

A tilt series after alignment

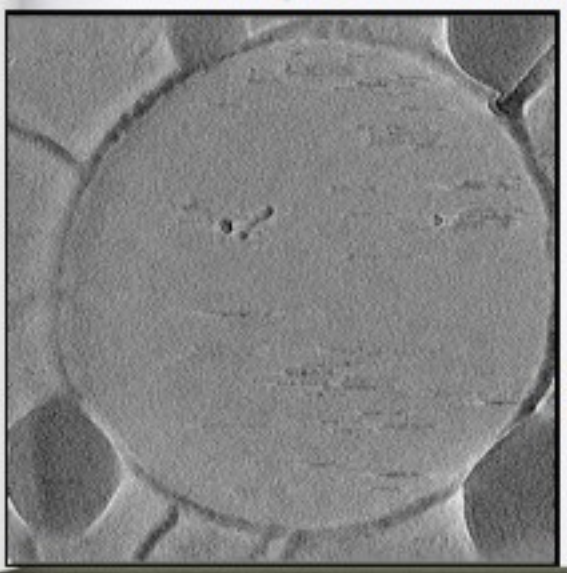


Aligned tilt series of clathrin-coated vesicles

* 10nm colloid gold;
* tilt range: -55° ~ +67°
* defocus: -7μm;
* magnification: 29kX;
* 7.75Å/pixel in CCD;
* binning 2×2;

Cheng et al. (2007) JMB

Tomogram of coated vesicles

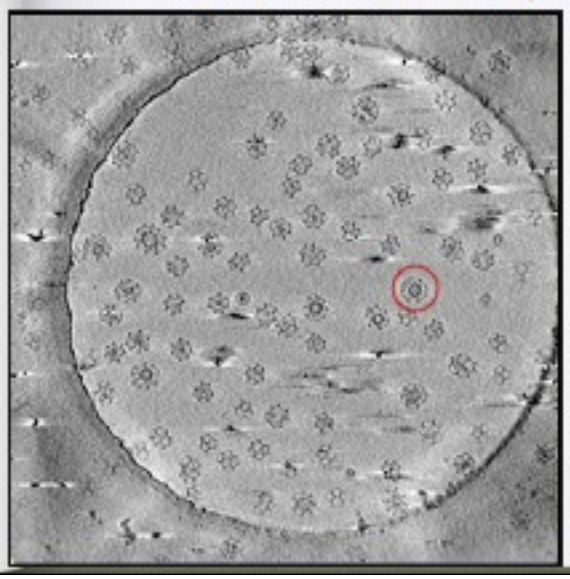


Slice view of tomogram

* IMOD;
* pixel size: 15.5Å;
* thickness: 1,550Å;

Cheng et al. (2007) JMB

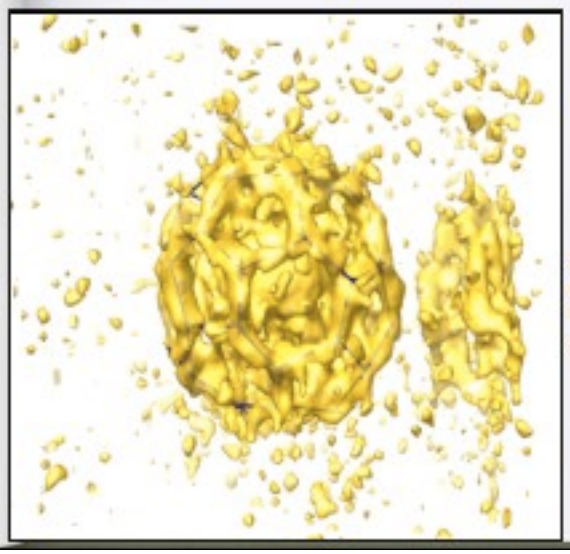
Central section of tomogram



Central slice of 15.5 nm thick from tomogram

Cheng et al. (2007) JMB

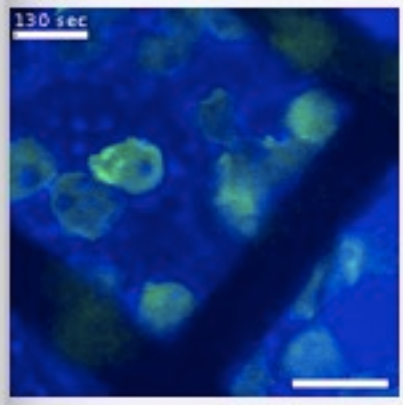
Reconstruction of coated vesicles

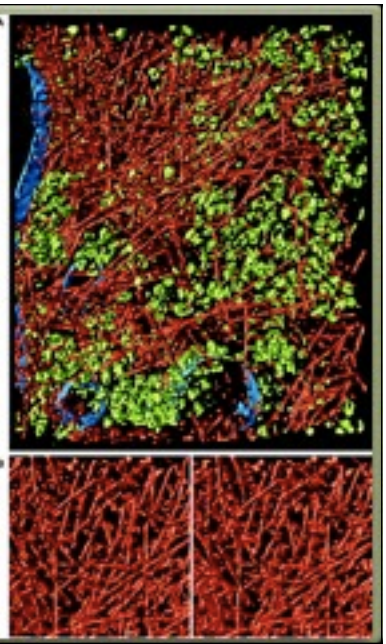


3D density map of clathrin-coated vesicle filter by 3×3×3 median filter

Cheng et al. (2007) JMB

Electron cryo-Tomography of whole cell





Medalia et al. (2002) Science

Outlines

- Basics of molecular electron microscopy
  - electron microscope
  - electron cryo-microscopy
  - 3D reconstruction
- Three different techniques of molecular EM
  - electron crystallography of membrane proteins
  - single particle analysis
  - electron tomography
- **Applications of molecular EM**
- Current status of molecular EM
- What can you get out of molecular EM?

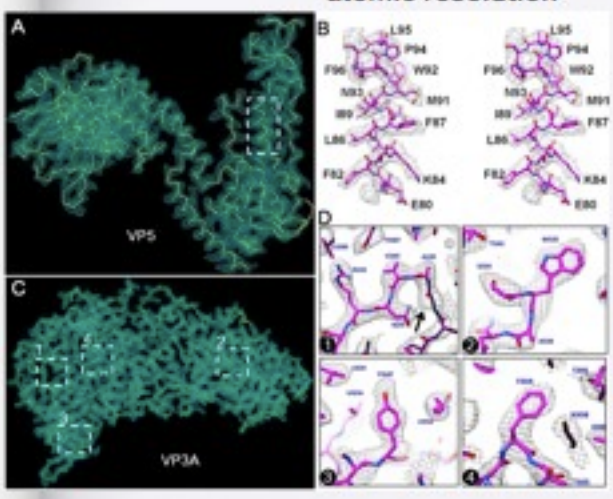
Molecular electron microscopy

* Electron crystallography: atomic resolution;

* Single particle EM: sub-nanometer level for most large and stable complex, reveal secondary structural features; For icosahedral virus, ATOMIC resolution, ~3.3A;

* Electron tomography: can look at individual assembly or cell, but at lower resolution (~ 2nm);

Single particle cryoEM reached near atomic resolution



3.3 A CryoEM structure of a nonenvelope virus ... Zhang, et al. (2010) Cell

What do we want to achieve by molecular electron microscopy?

To achieve near atomic resolution of macromolecular complexes/machineries by the molecular single particle cryoEM and routinely;

* To determine structures of macromolecules that are too large to be crystallized for x-ray crystallography and NMR spectroscopy.
* To understand complex biological processes in molecular/ atomic details.

Outlines

- Basics of molecular electron microscopy
  - electron microscope
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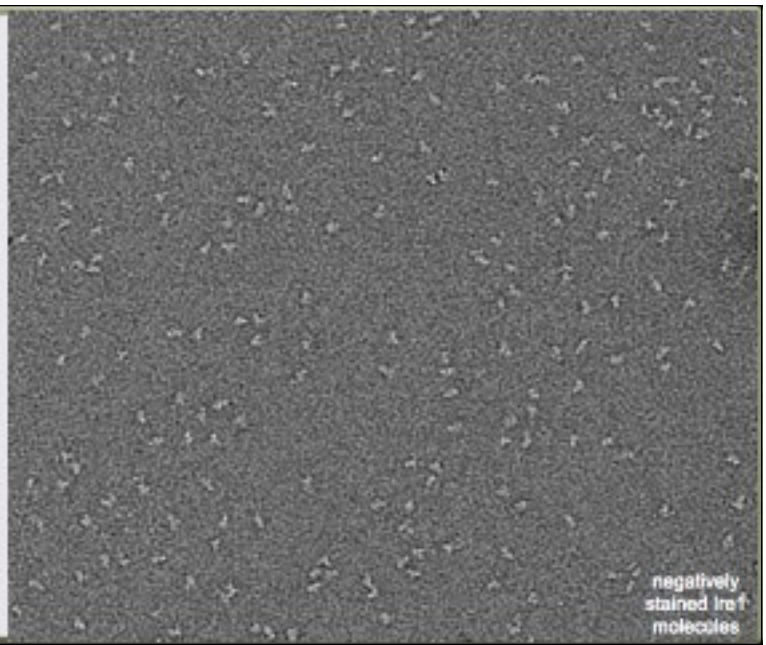
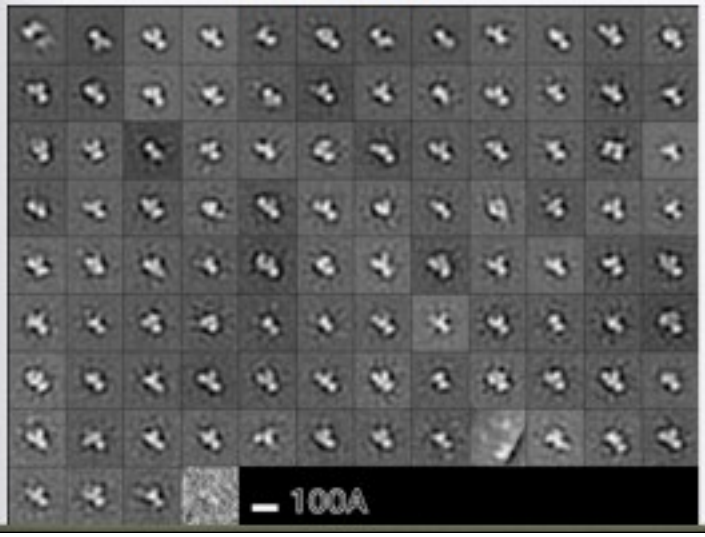
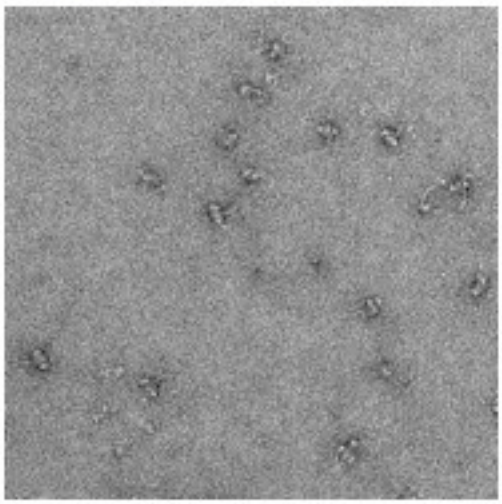
  - single particle analysis
  - electron tomography
- Applications of molecular EM
- Current status of molecular EM
- **What can YOU get out of molecular EM?**



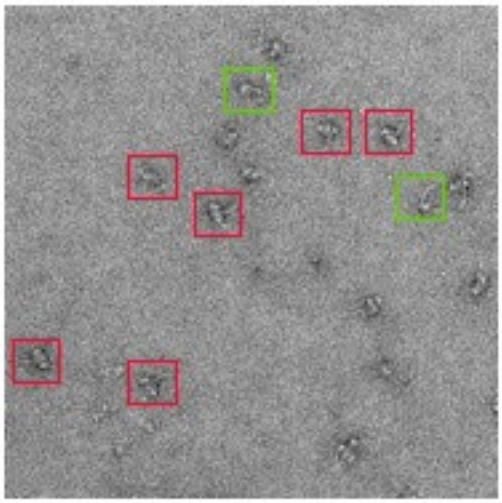
class averages after multi-reference alignment and classification



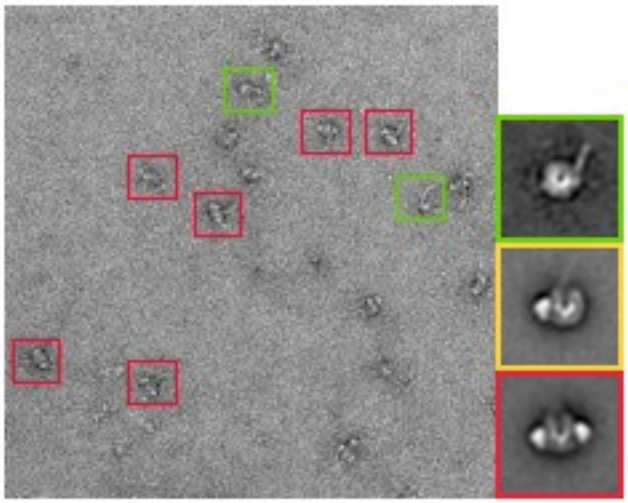
SNF2h-nucleosome complex



SNF2h-nucleosome complex

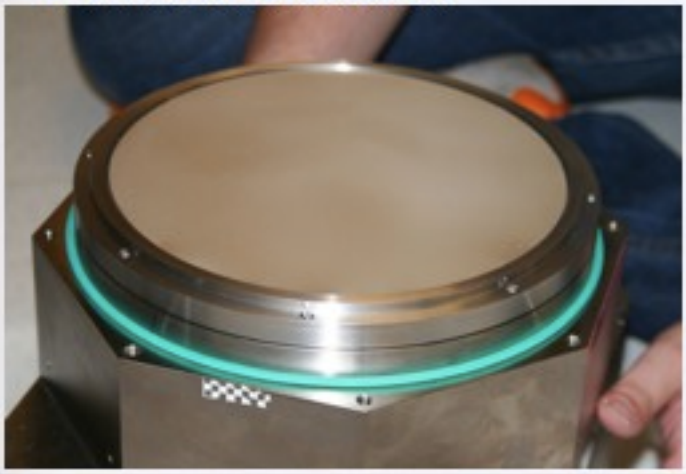


SNF2h-nucleosome complex



Recent cryoEM technology development at UCSF

- Instrumentation: 8K x 8K camera

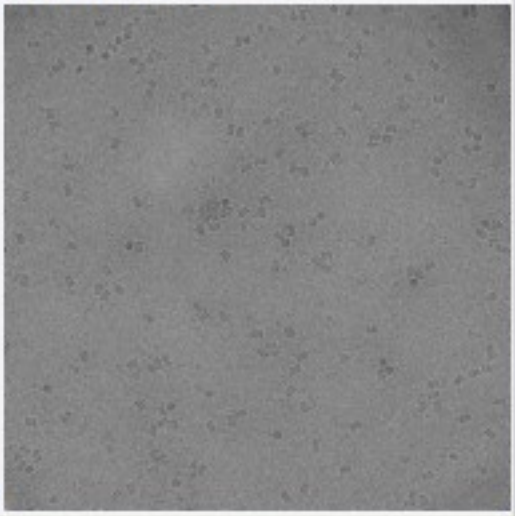


Recent cryoEM technology development at UCSF

- Instrumentation: 8K x 8K camera on a TF20

Recent cryoEM technology development at UCSF

• Instrumentation:
A first image from
the 8K x 8K camera



yeast 20S proteasome

Use molecular EM in your research

• exam the quality of your purification
• verify your hypothesis
• obtain addition information about your proteins: such as oligomeric status of your protein, formation of complex, etc
• Or if you are really really serious, get high resolution structure by cryoEM!

Facility at UCSF:
Keck Advanced Microscopy Laboratory
Yifan Cheng - S312B (ycheng@ucsf.edu)
David Agard - S412D (agard@msg.ucsf.edu)