Lecture 13

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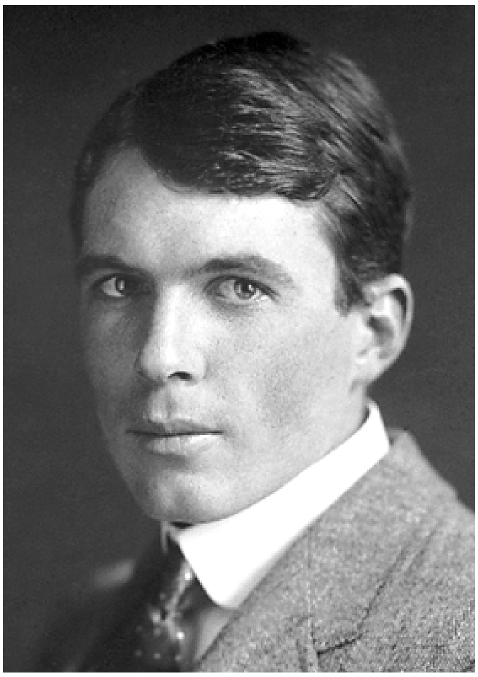
General principles of image processing in cryo-EM

Cryo EM & 3D Image Processing
8 July 2016
Thiruvananthapuram, India

Crystallography

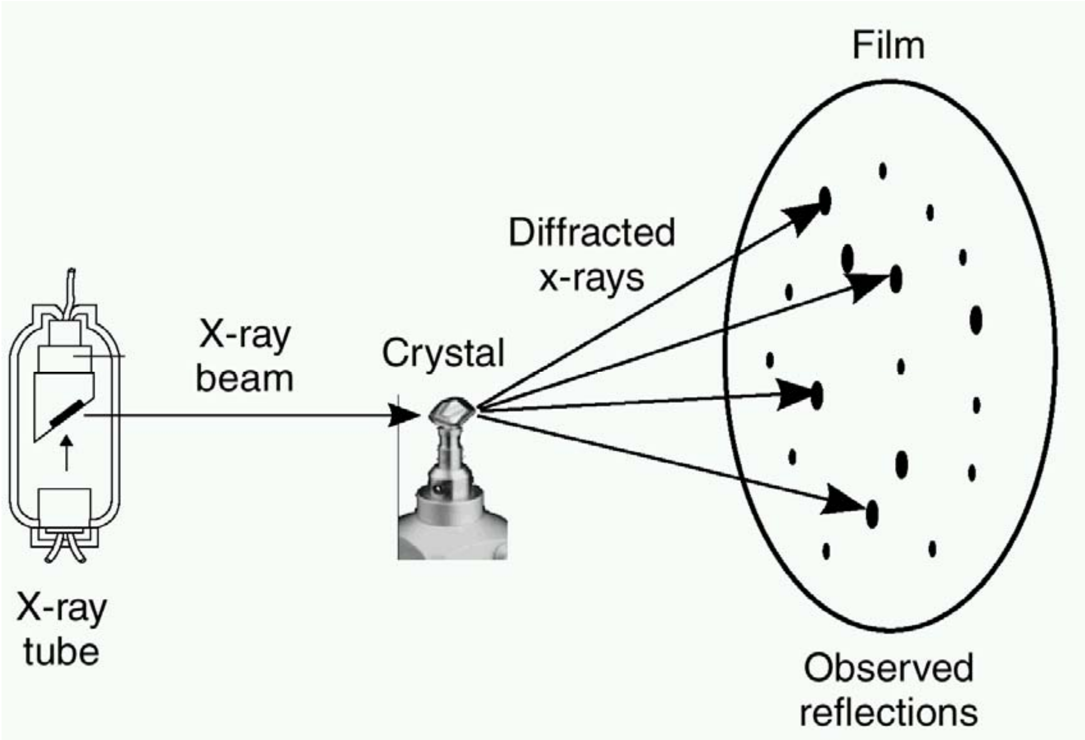
William Lawrence Bragg



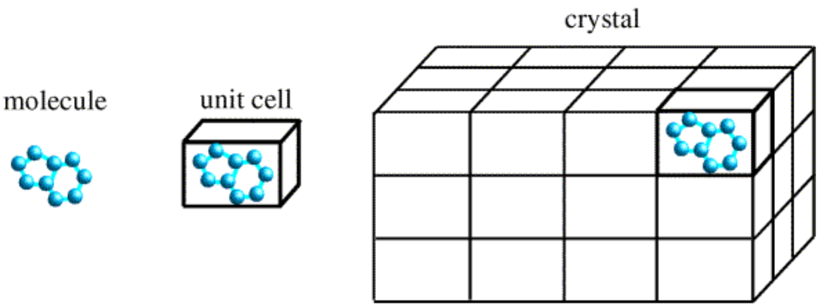
31 March 1890 – 1 July 1971

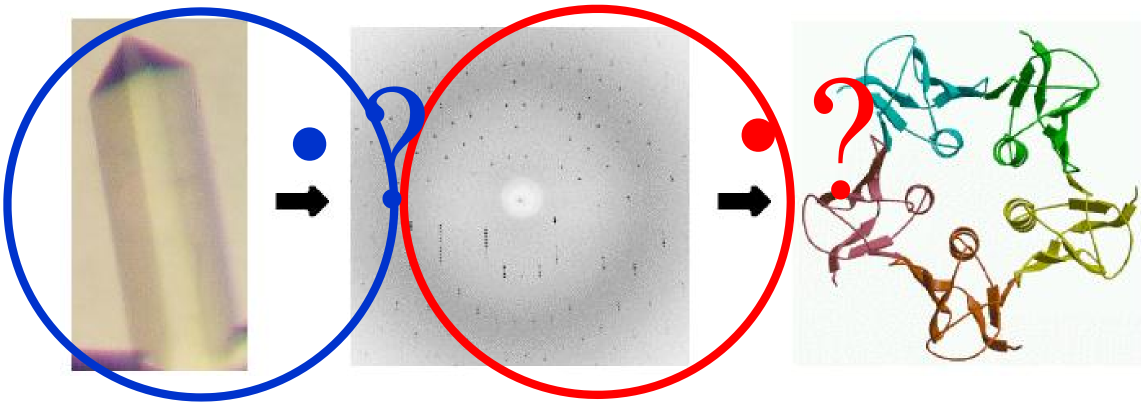
was an Australian-born British physicist and X-ray crystallographer, discoverer (1912) of the Bragg law of X-ray diffraction, which is basic for the determination of crystal structure. He was joint winner (with his father, William Henry Bragg) of the Nobel Prize in Physics in 1915: *"For their services in the analysis of crystal structure by means of X-ray"* an important step in the development of X-ray crystallography.

Crystallography



Crystallography



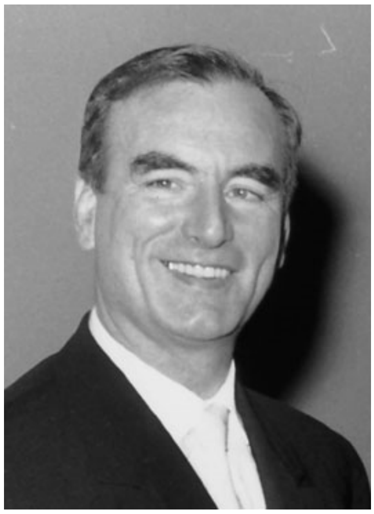


http://www-structmed.cimr.cam.ac.uk/Course/Overview/Overview.html

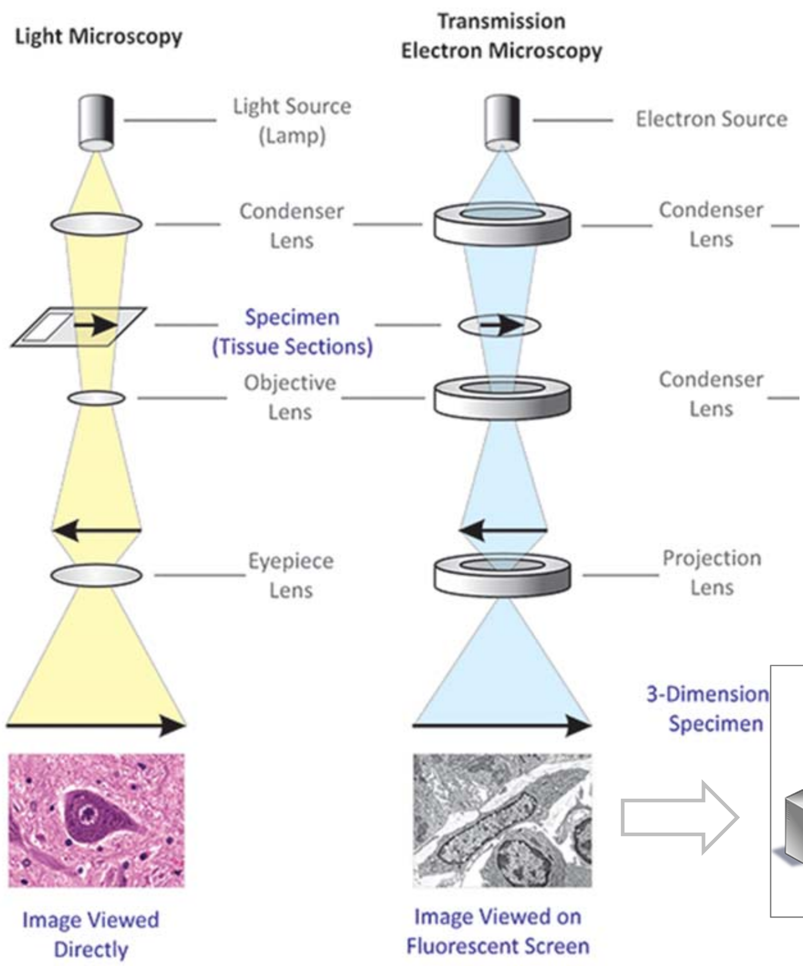
Electron microscopy

Ernst Ruska

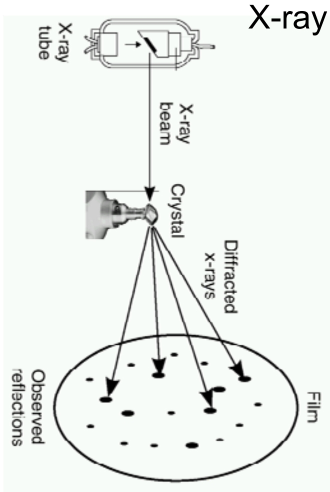
(25 Dec 1906 –27 May 1988)



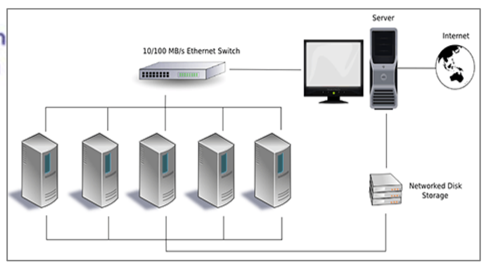
The **electron microscope** is a type of **microscope** that uses a beam of **electrons** to create an image of the specimen. It is capable of much higher magnifications and has a greater resolving power than a light **microscope**, allowing it to see much smaller objects in finer detail.



X-ray

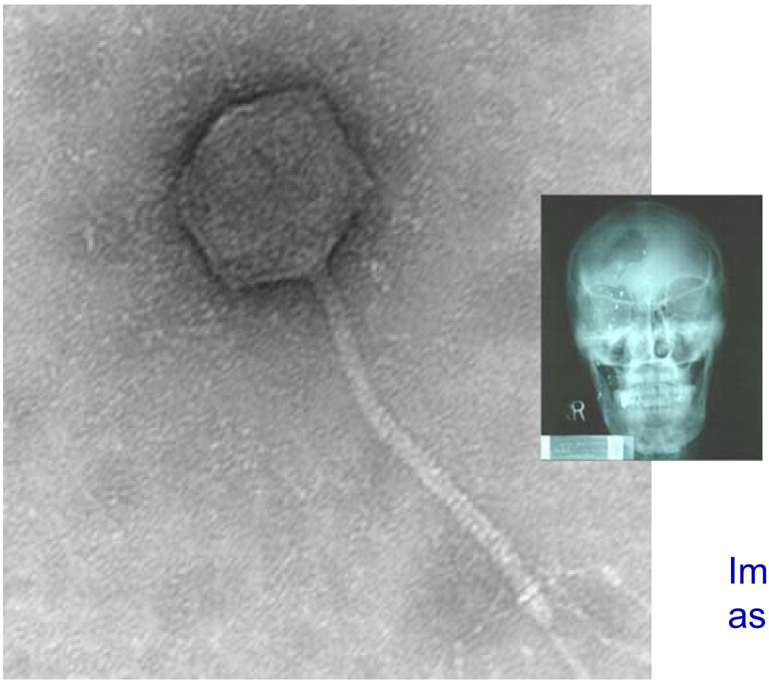


3-Dimension Specimen



Electron microscopy

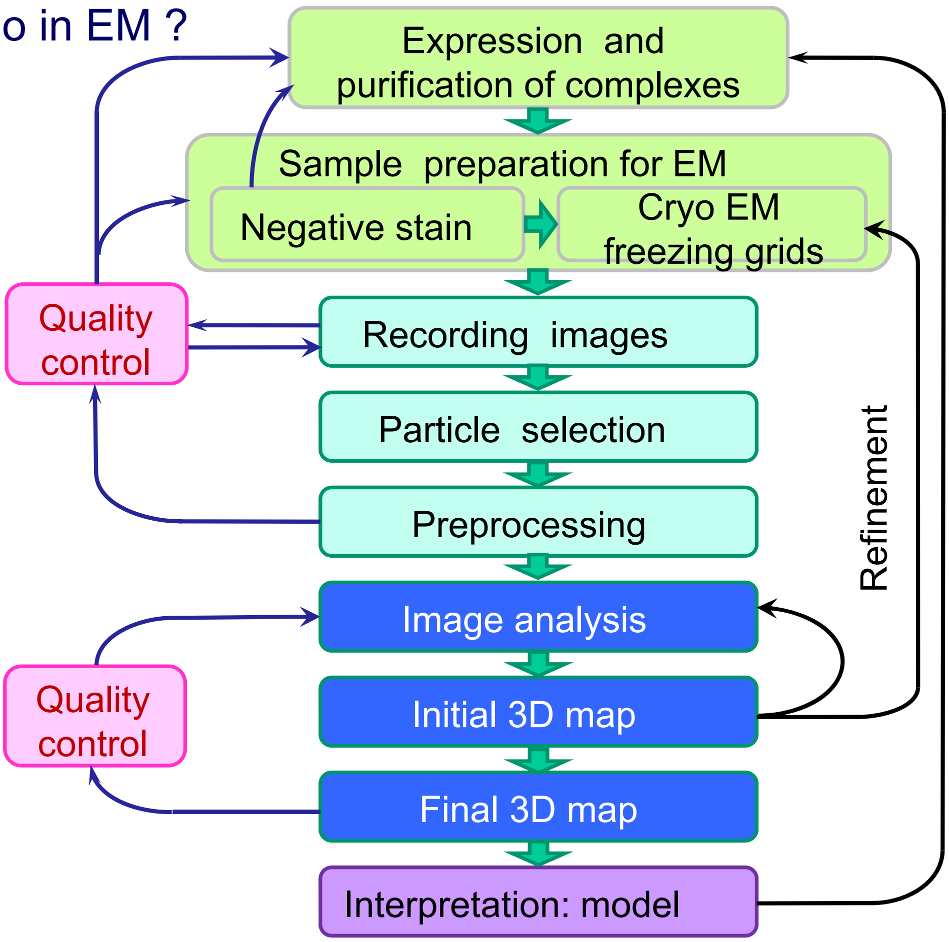
Images of phage in negative stain



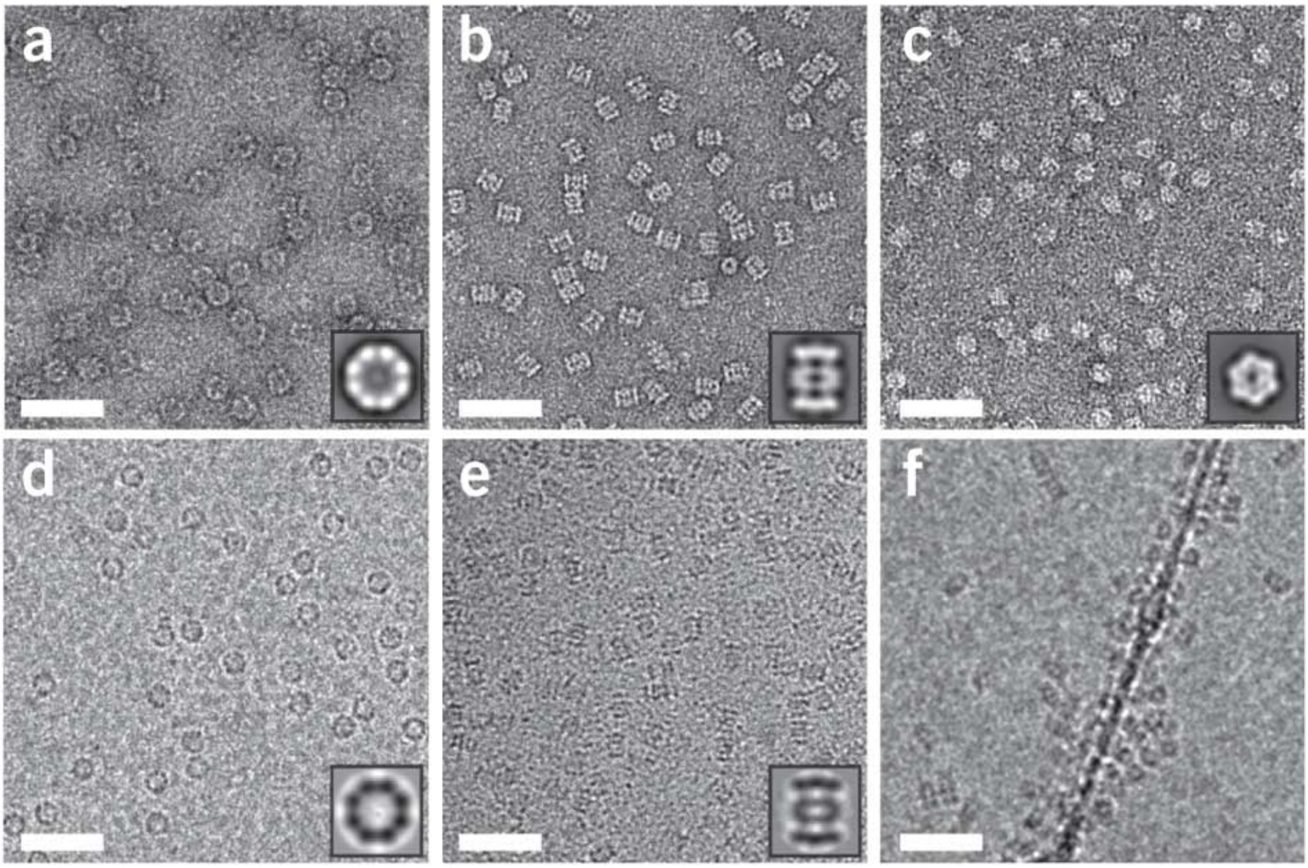
Good contrast !!!
But particles are distorted: collapsed flattened, since the samples became dry

Images can be considered as projections of an objects

What do we do in EM ?



Electron microscopy

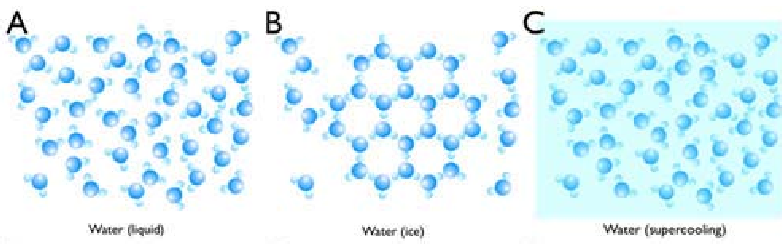


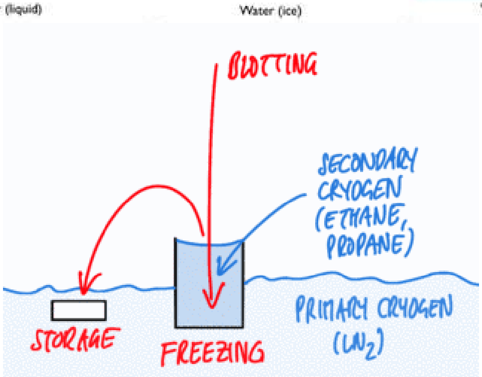
Electron microscopy

Jacques Dubochet

Robert Glaeser

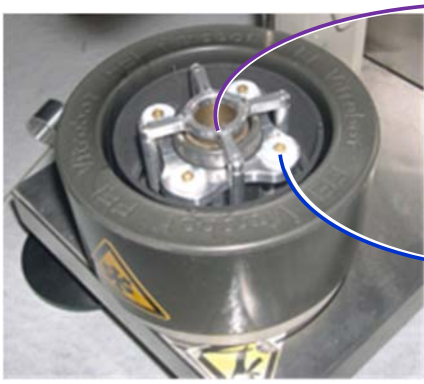
CryoEM → 1988
Sample preparation





Electron microscopy

Sample preparation for EM

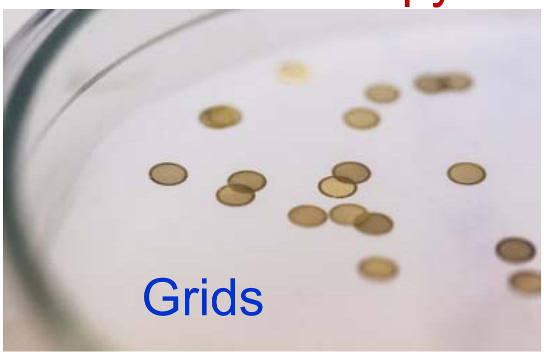


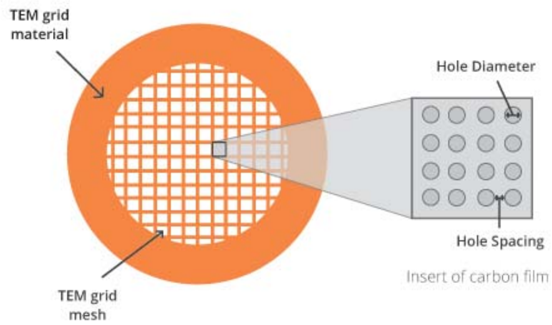
Liquid ethan

Liquid nitrogen

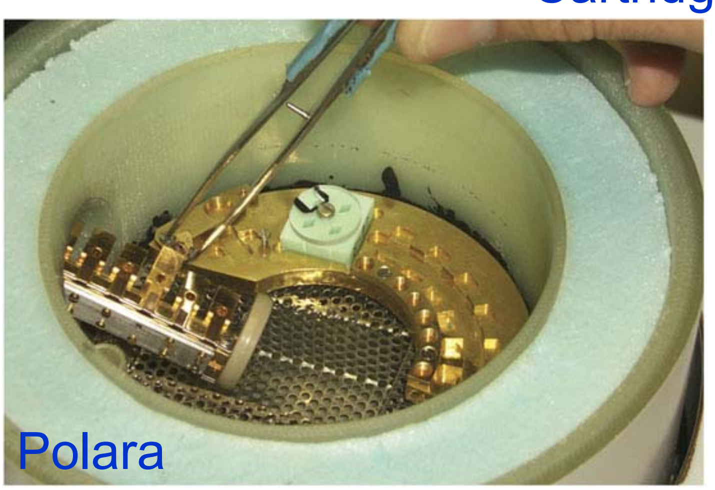
Electron microscopy

Sample preparation



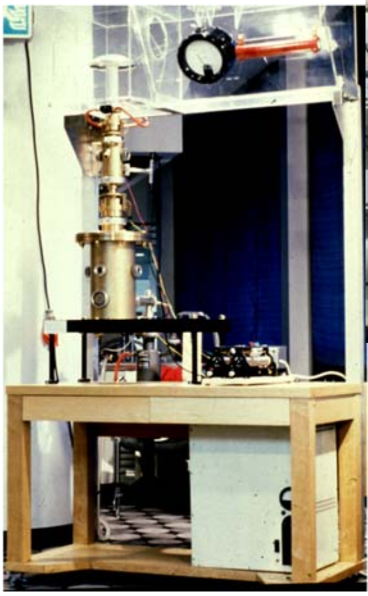


Cartridges



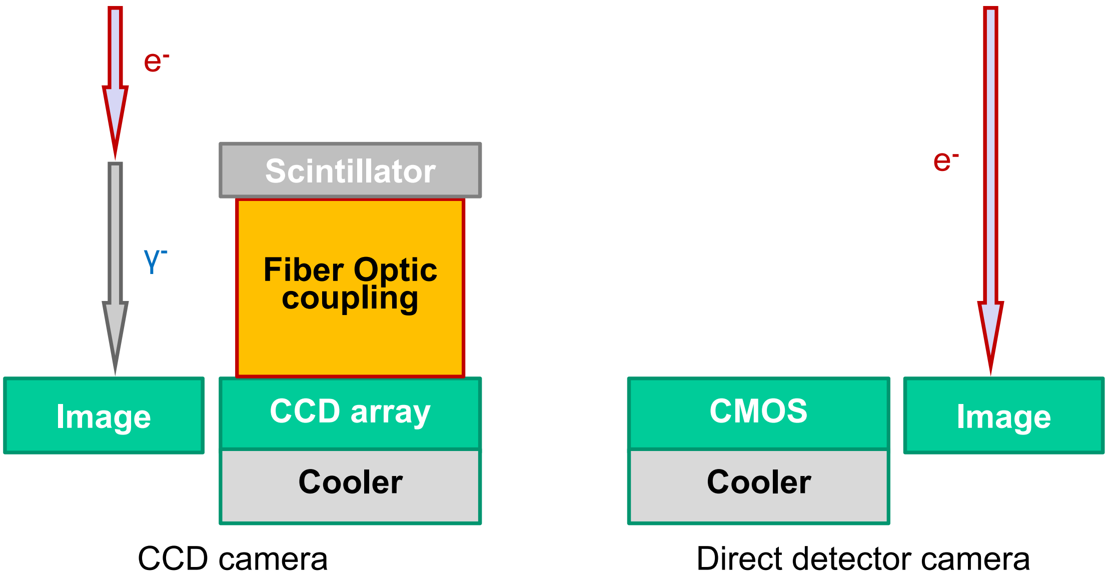
Electron microscopy

Ruska
Microscope
~1935



FEI
Polara, FEG
~1995

Recording images

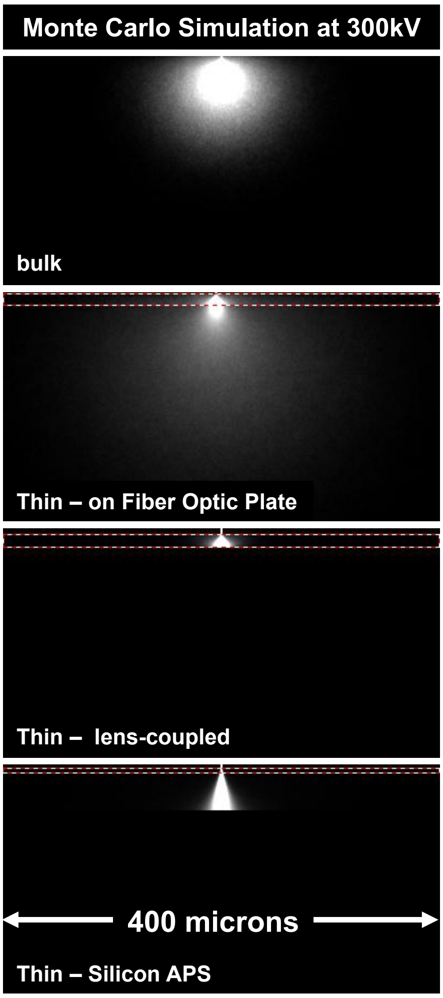


CCD (charge-coupled device) and **CMOS** (complementary metal-oxide semiconductor) image sensors have the the same starting point -- they have to **convert light into electrons**.

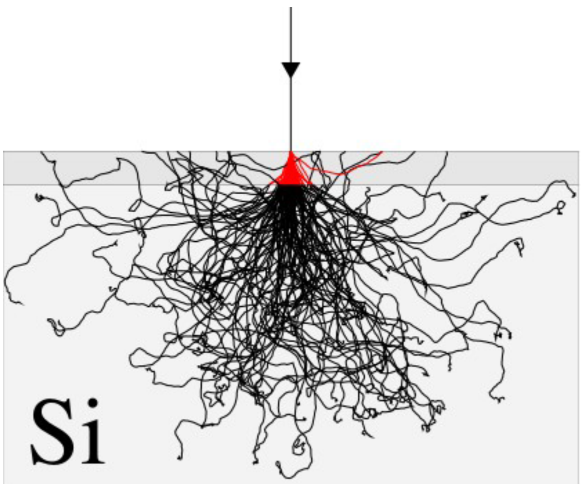
In electron microscopy it was an additional step: Electrons were converted into photons.

The sensors used in a digital camera (and microscopes) represent a 2-D array of thousands tiny solar cells, each of which transforms the light from one small portion of the image into electrons. Both CCD and CMOS devices perform this task using a variety of technologies.

The next step **is to read the value** (accumulated charge) of each cell in the image.



Registration of 300KeV electrons -> prons and cons

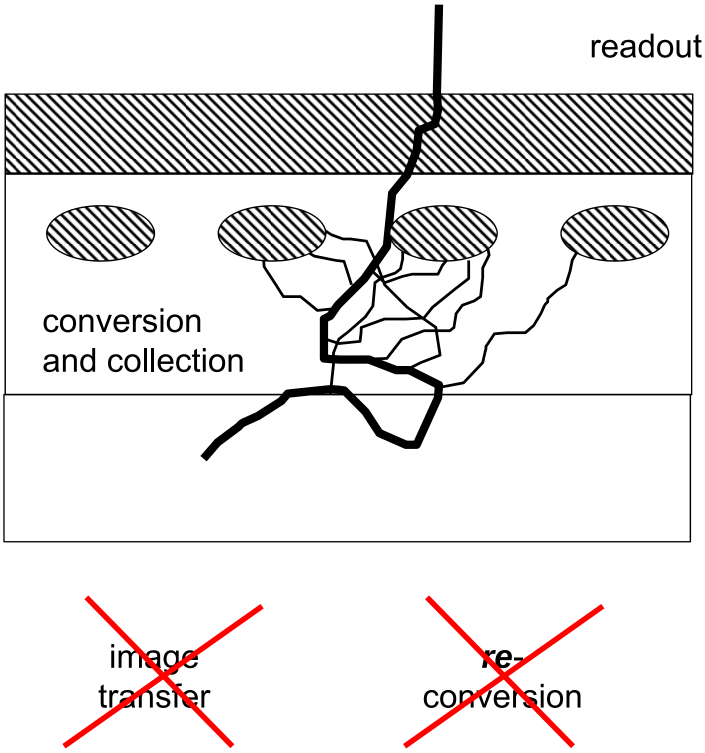


Gatan, P. Mooney

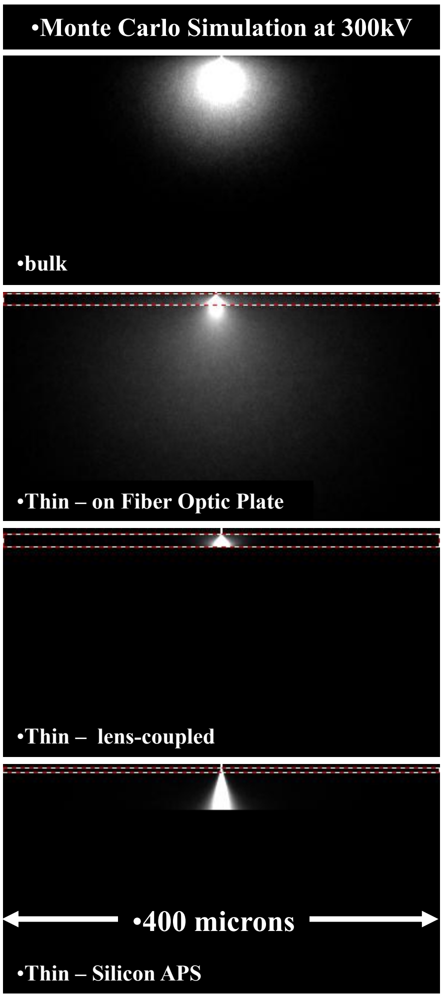
Direct-detection of electrons

Gatan K2

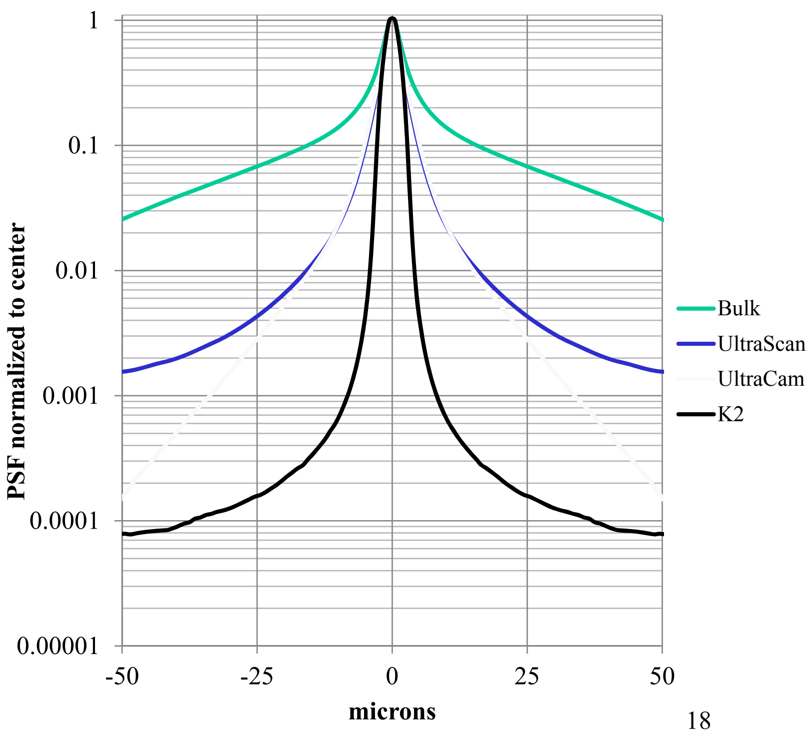
DE



Gatan, P. Mooney



The 300kV TEM Electron:



Gatan, P. Mooney

In a **CCD** device,

the charge is actually transported across the chip and read at one corner of the array. An analogue-to-digital converter turns each pixel's value into a digital value.

http://www.microscopyu.com/articles/digitalimaging/ccdintro.html

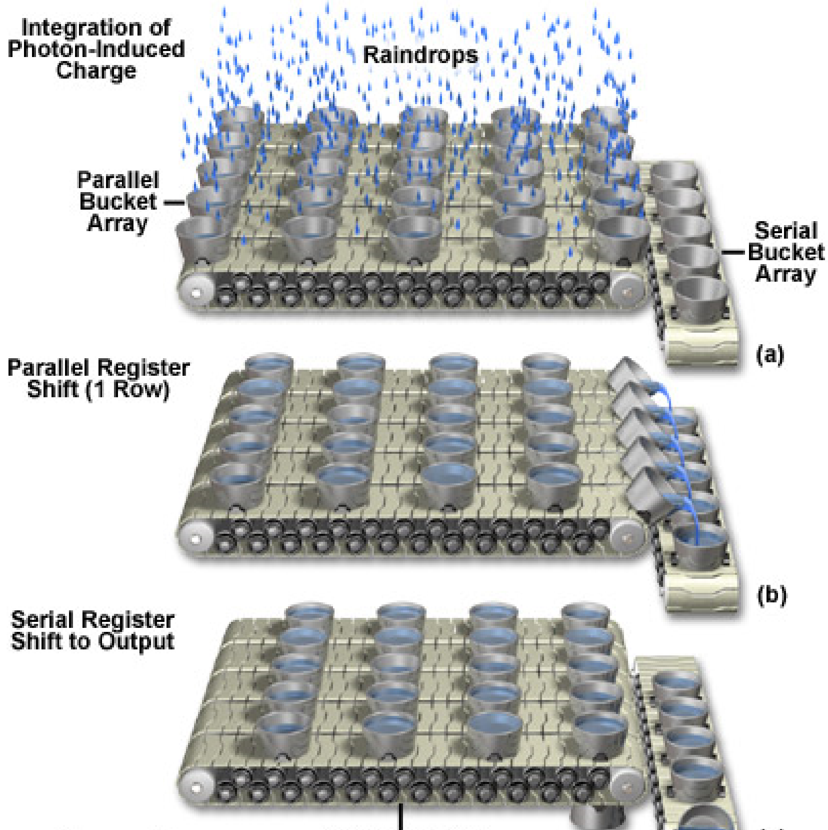
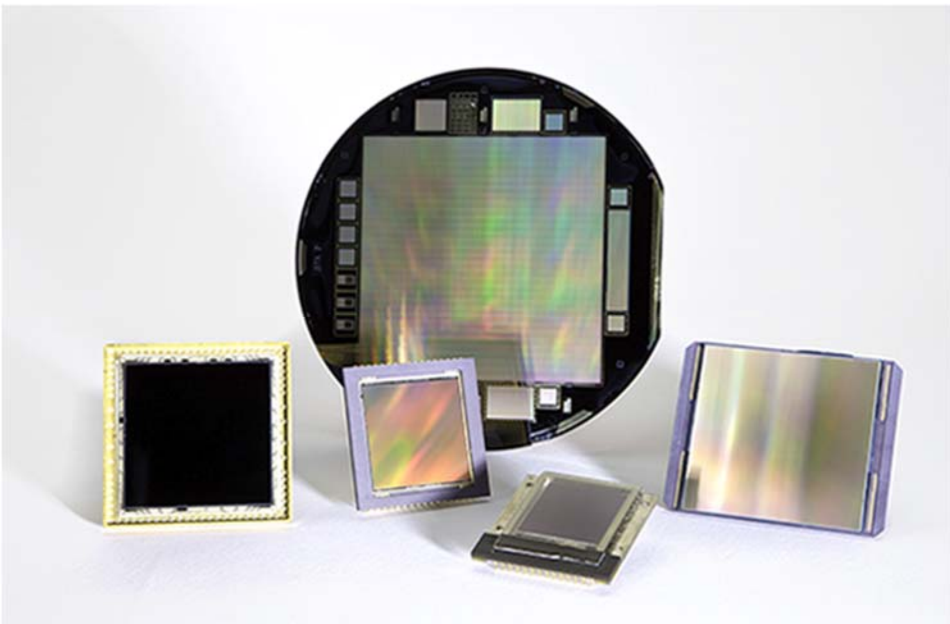


Figure 5

Bucket Brigade CCD Analogy

In most **CMOS** devices, there are several transistors at each pixel that amplify and move the charge using more traditional wires. The CMOS approach is more flexible because each pixel can be read individually



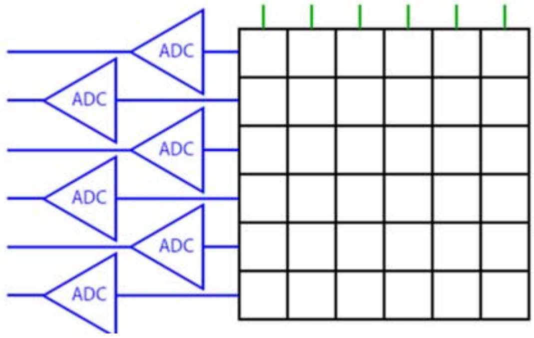
Collect Movies Instead of Static Images

Excellent sensitivity and SNR

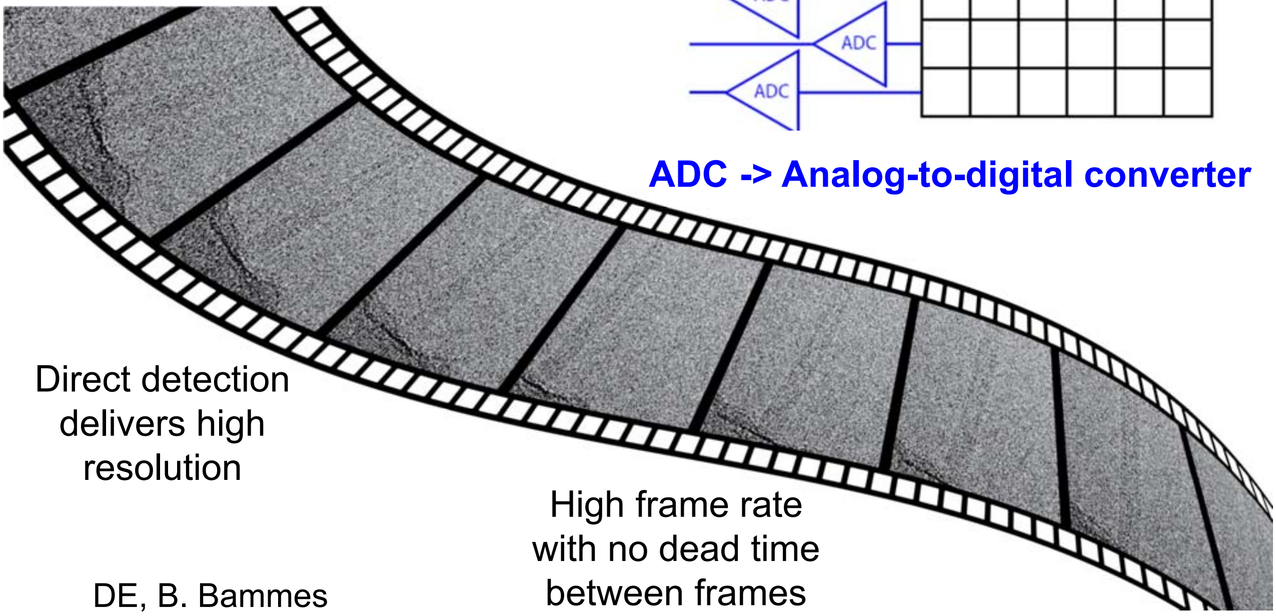
so that each raw frame contains usable information

The second secret is fast readout of frames

25-40 frames/second



ADC -> Analog-to-digital converter



Direct detection delivers high resolution

High frame rate with no dead time between frames

DE, B. Bammes

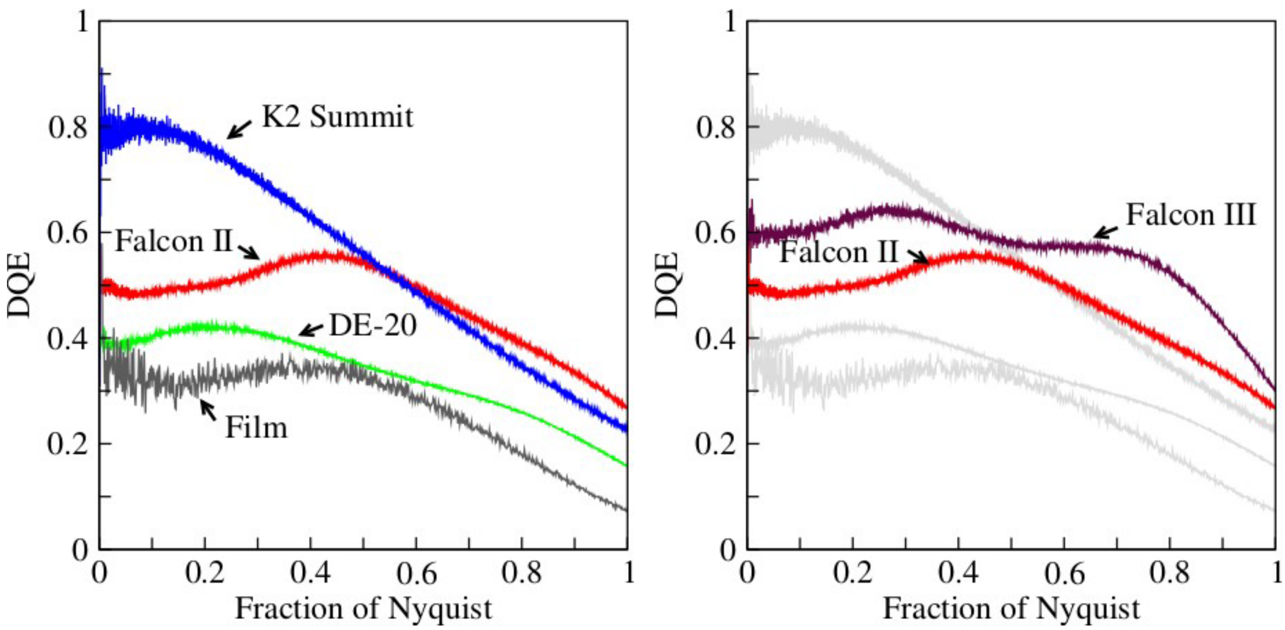
Camera Comparison

The definition of DQE:

$$DQE(N, \mathbf{r}) = \left(\frac{SNR_{out}(N, \mathbf{r})}{SNR_{in}(N, \mathbf{r})}\right)^2$$

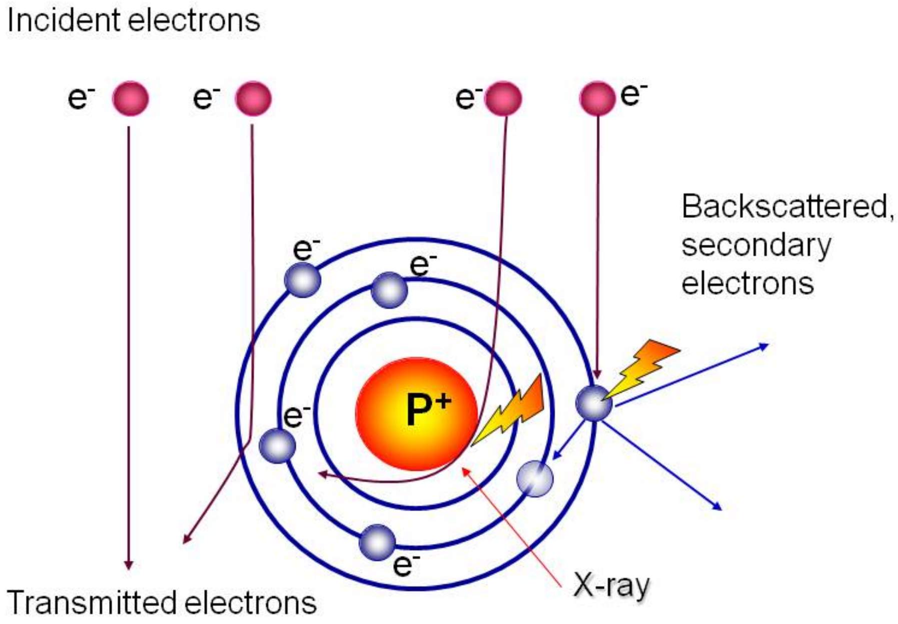
N - # electrons/pxl

r – spatial frequency



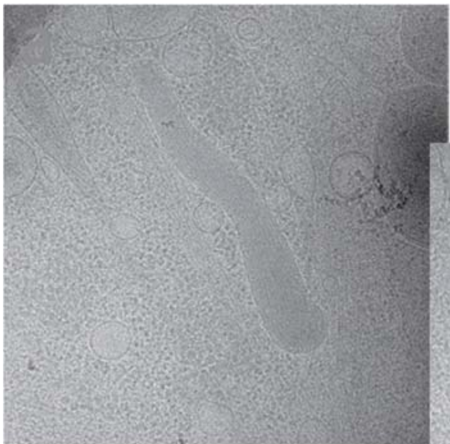
McMullan et al., 2014, Ultramicroscopy, 147, 156-163

Interaction of the electron beam with the sample

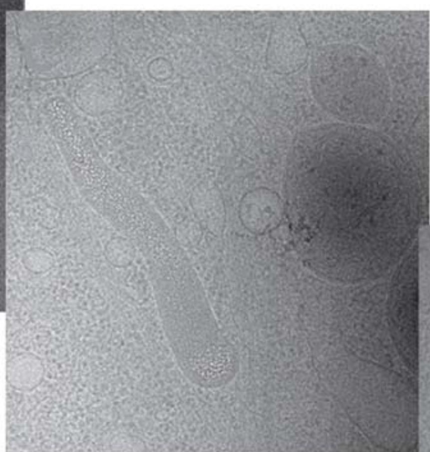


- In an <u>elastic</u> collision of the electron with the atom the electron will be scattered through an angle Q. The kinetic energy of the incident electron is not changed significantly.
- In an <u>inelastic</u> collision a part of the kinetic energy is transferred to the atom and transformed into another kind of energy.

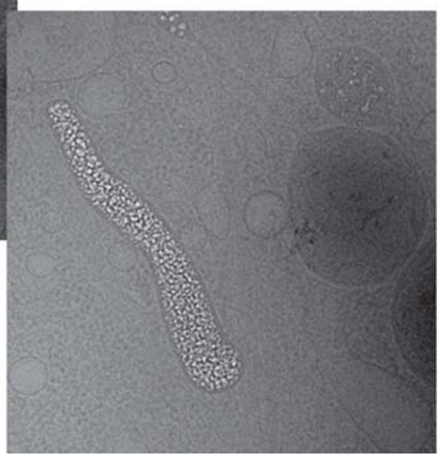
Data collection : low dose



•70 $e^-/Å^2$



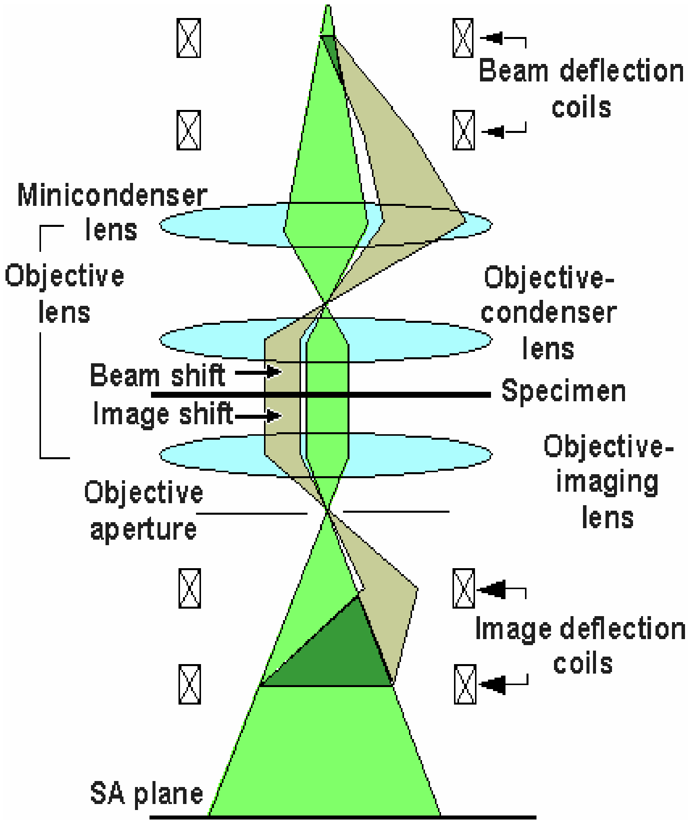
•100 $e^-/Å^2$



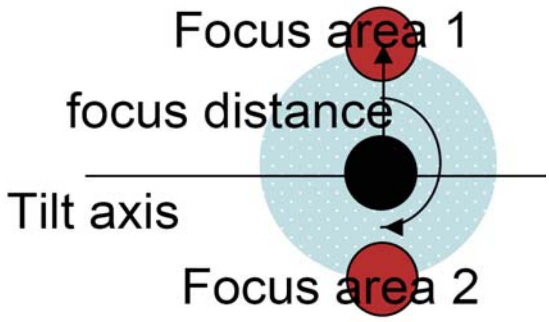
•140 $e^-/Å^2$

Low dose system -> 1971

Data collection : low dose

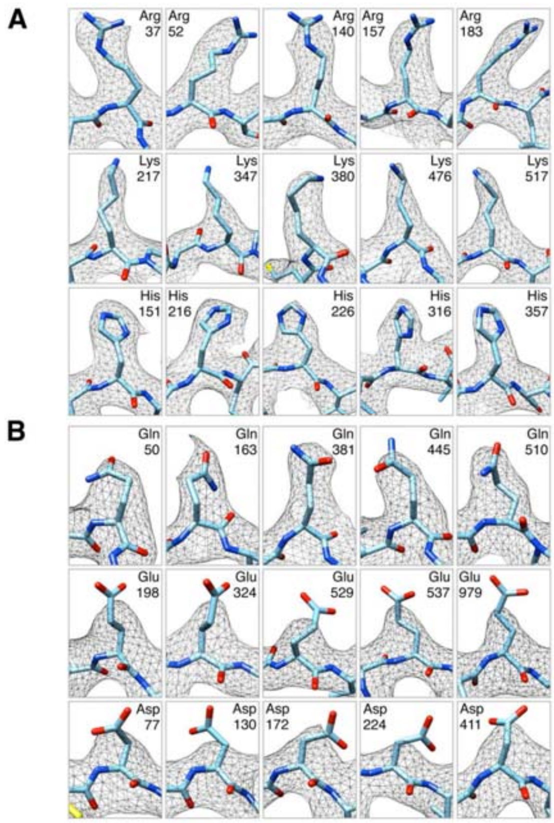


•Nigel Unwin



Variation in observed side-chain densities between positively, neutral and negatively charged residues.

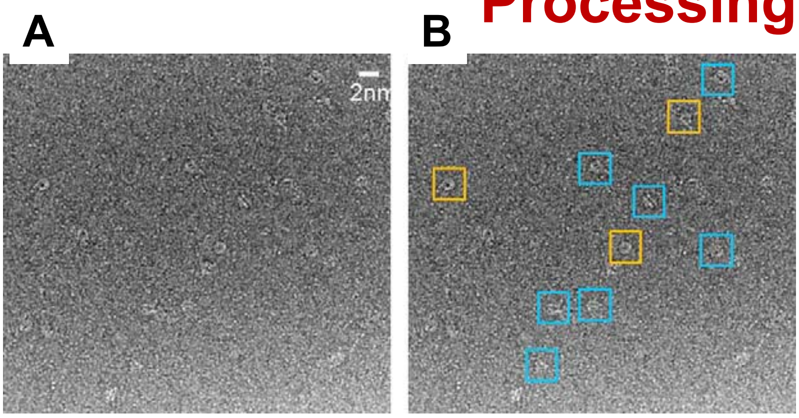
(A) Densities observed for a set of Arg, Lys and His residues (shown in stick representation).
(B) Comparison of densities observed for a set of Gln and Glu residues, as well as Asp residues to indicate preferential loss of density for the negatively charged side-chain in comparison to the similarly sized, but neutral side-chains.

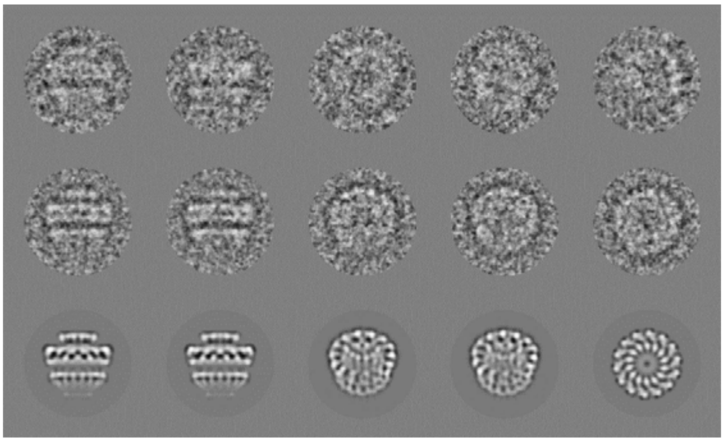


β-galactosidase

Bartesaghi et al., Proc Natl Acad Sci U S A. 2014 Aug 12;111(32)

Processing of images



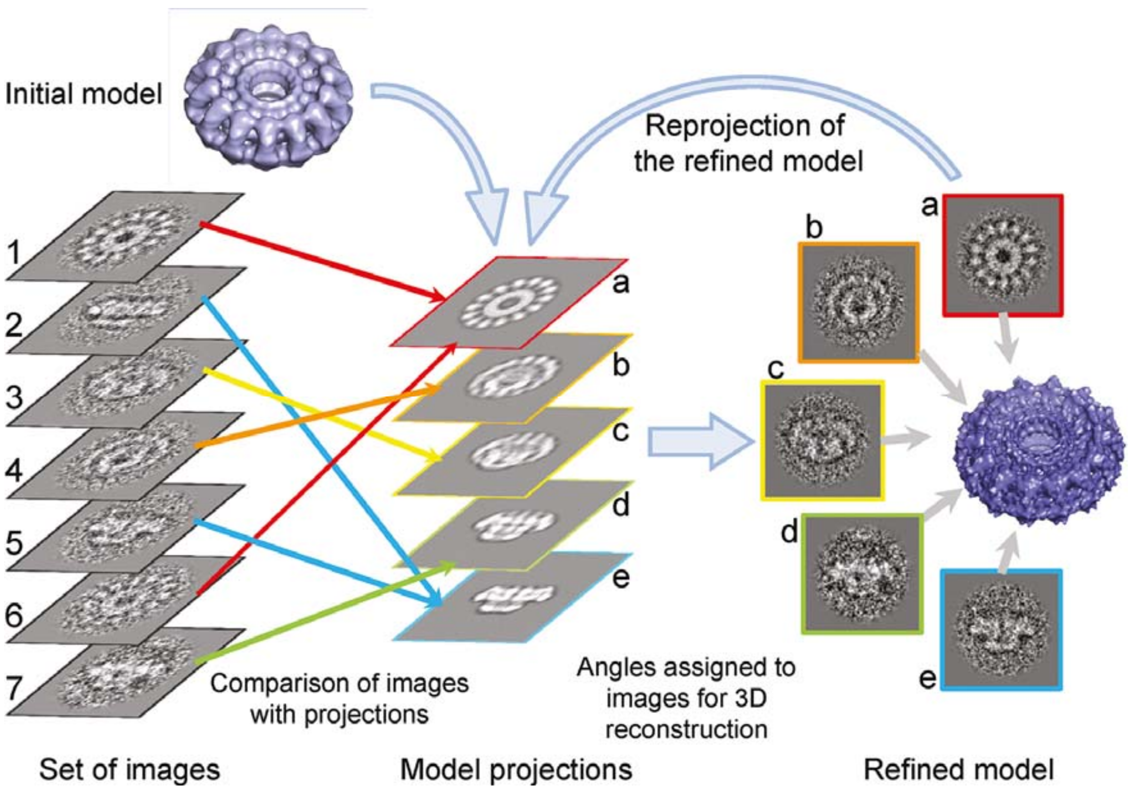


Single images

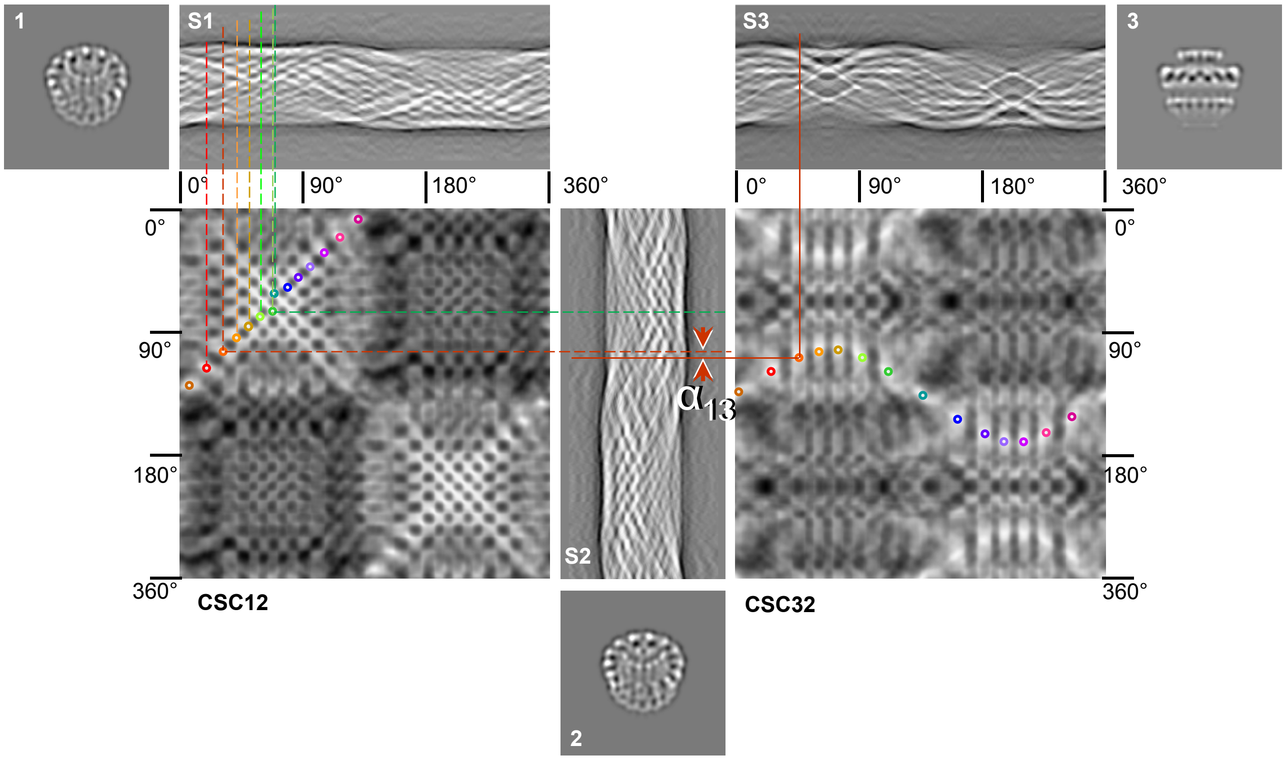
Classes

Re-projections

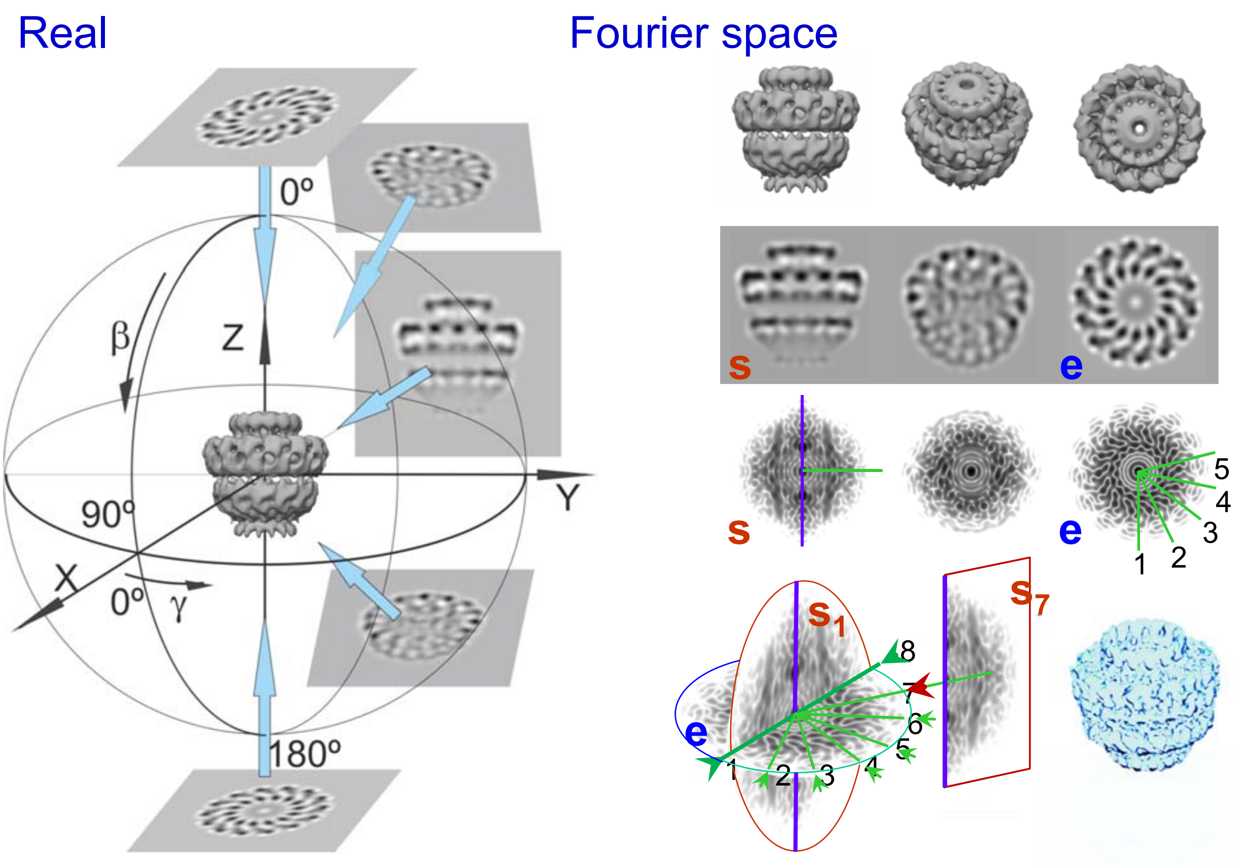
Projection matching



Angular reconstitution



3B reconstruction

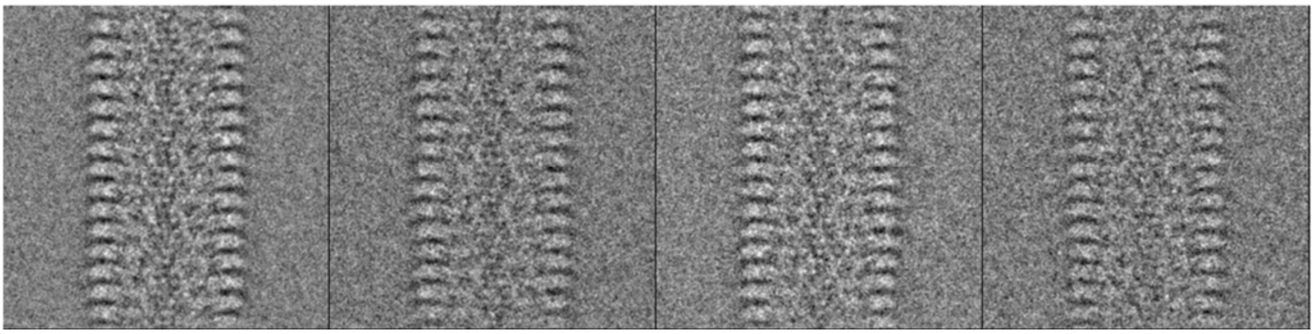


Direct Comparison Between Direct Detectors

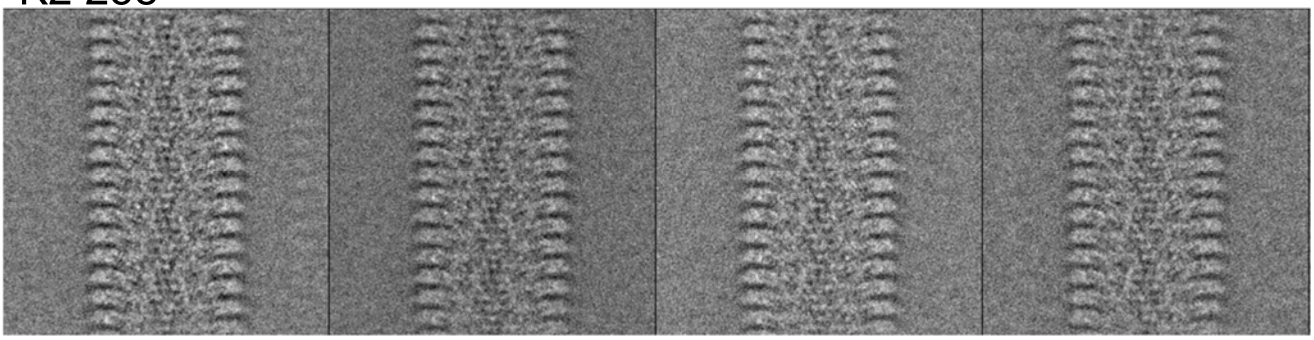
- ✓Comparison between Direct Electron DE--20 and Gatan K2-Summit.
- ✓Identi cal cryo--EM Experiment on two different cameras.
- ✓Same microscope (FEI Polara 300 kV).
- ✓Same specimen preparation (TMV).
- ✓Similar imaging conditions and number of particles.
- ✓Same image processing

Angle averages after 6 rounds of alignment

DE 25e

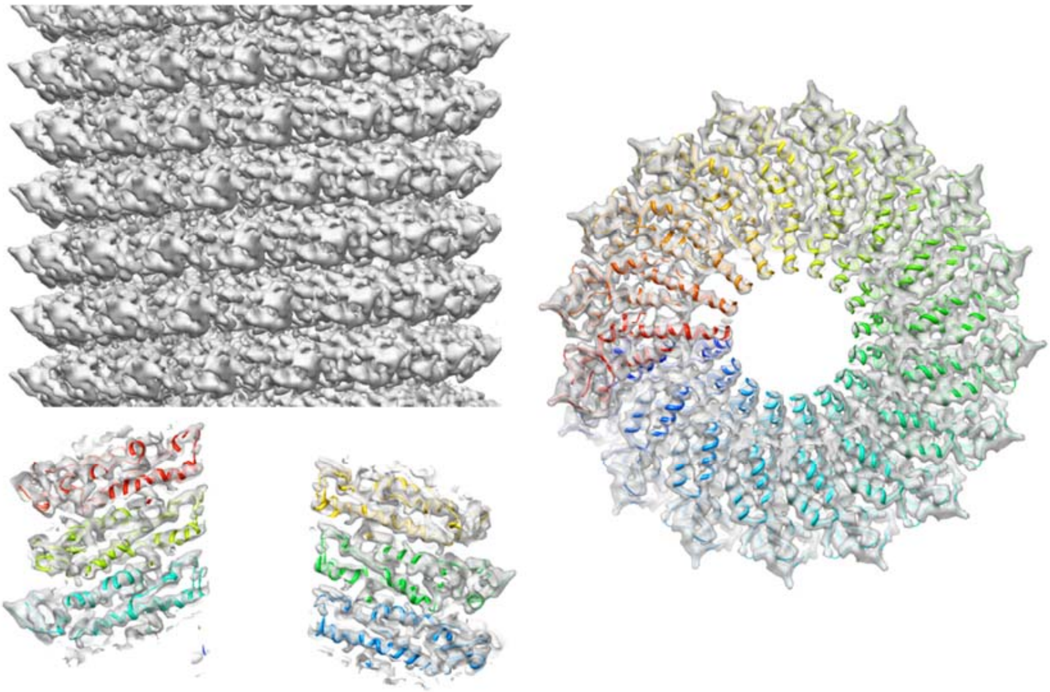


K2 25e



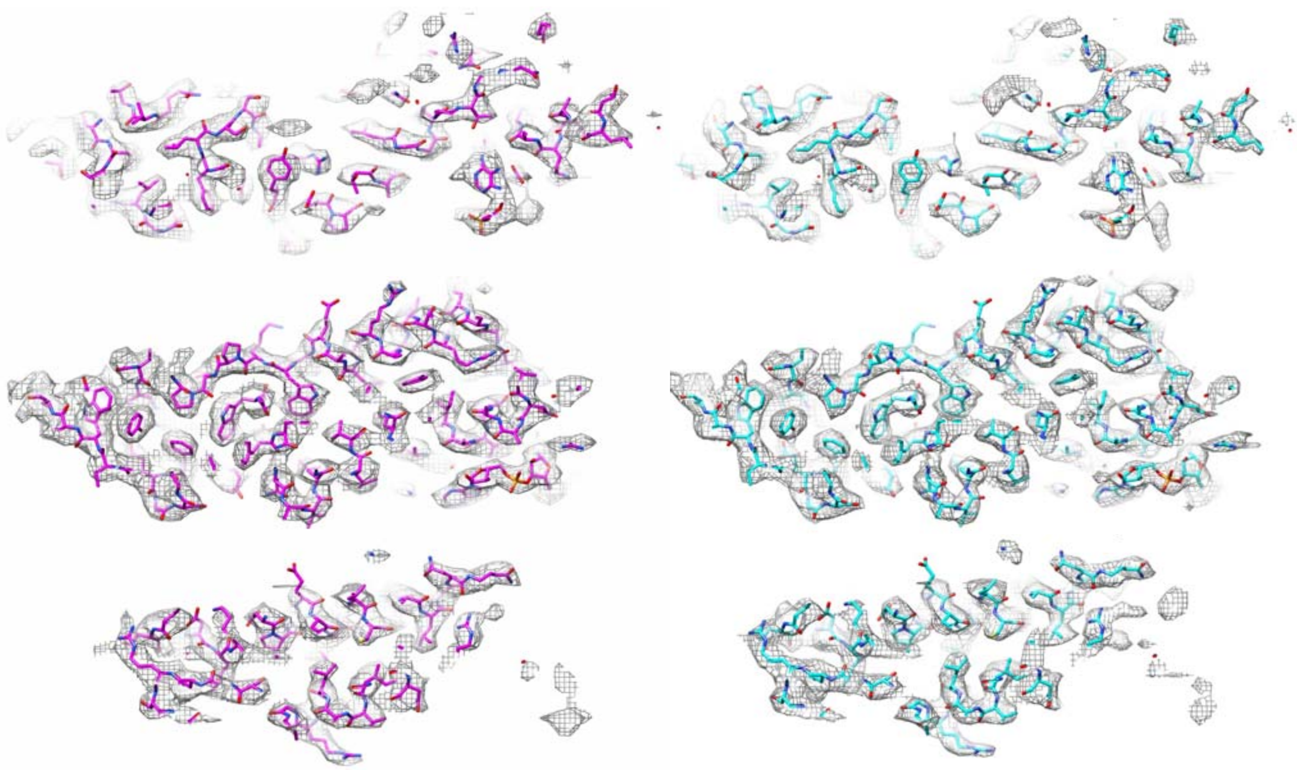
Angles alpha 90, beta 90 and Gamma 0-4 (1° spacing)

Tobacco mosaic virus



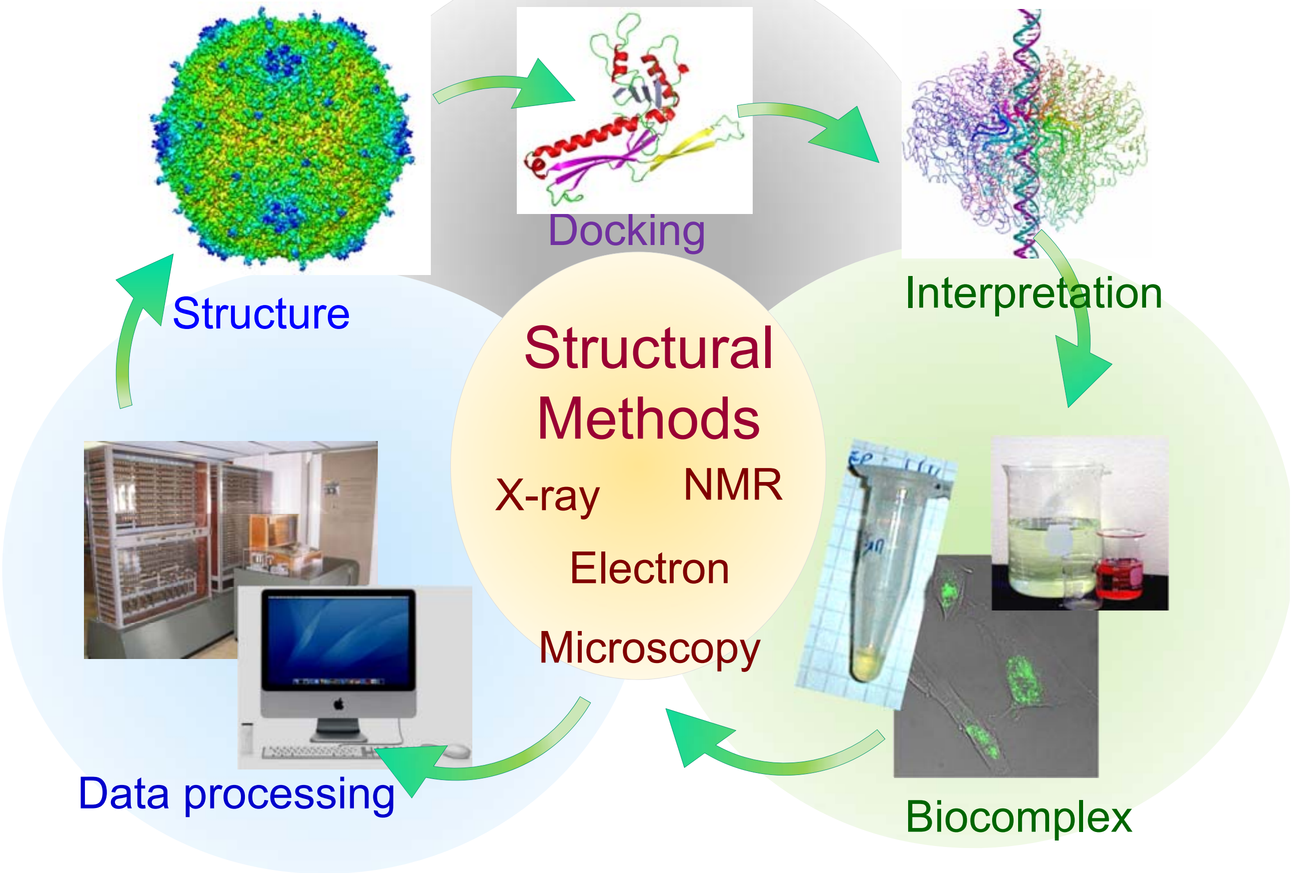
D. Clare and E.Orlova, J Struct Biol. 2010 Sep;171(3):303-8

DE 25 electrons   K2 25 electrons



Both maps look very similar!

How do we learn about structural features of the biological complexes and their components ?



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