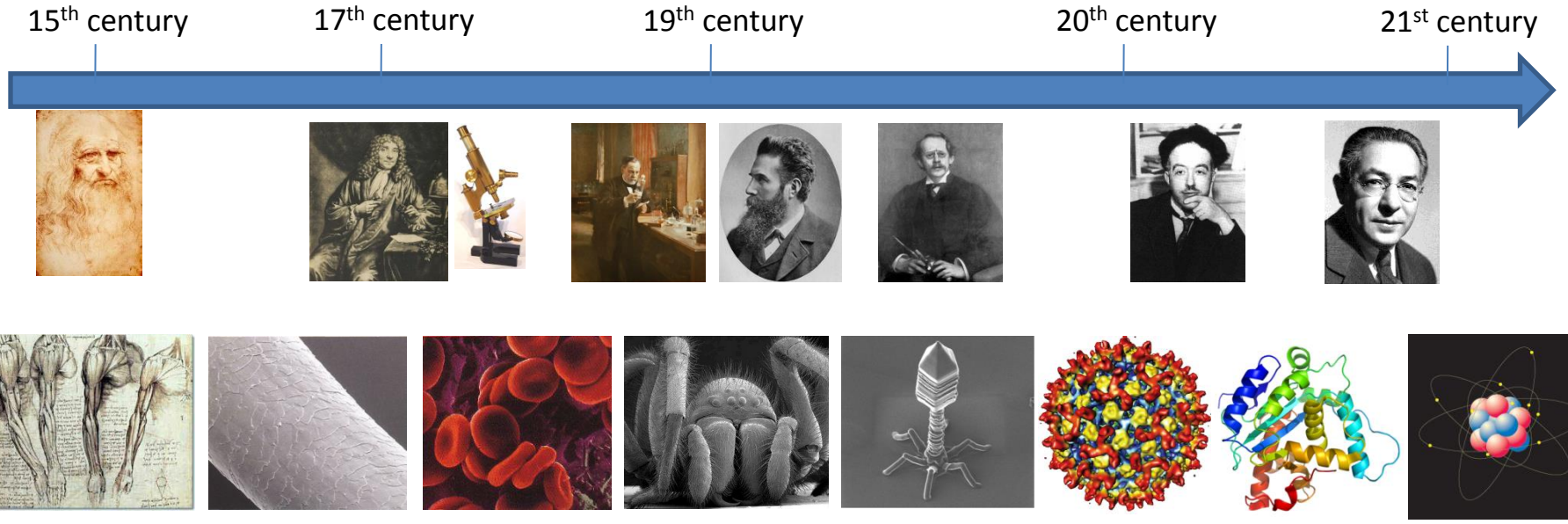
Que peut-on voir avec un microscope électronique à transmission ?

What can we see with a TEM ?

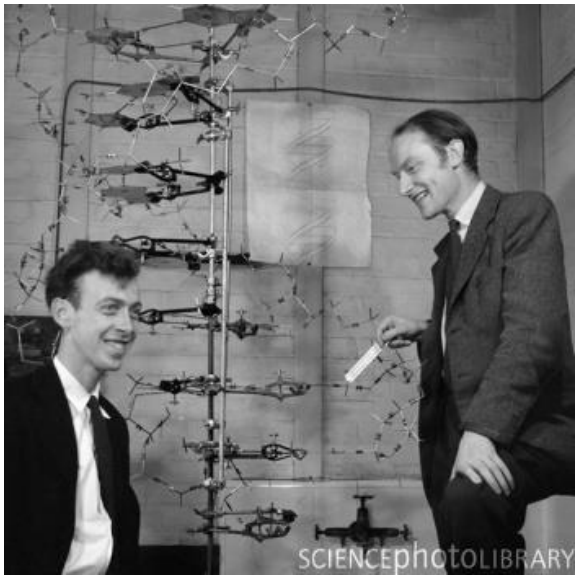
Pierre-Damien COUREUX
Ecole polytechnique
Rénafobis

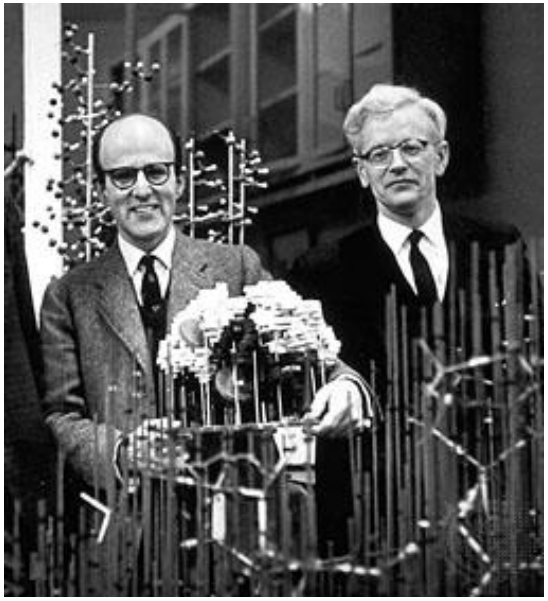
Oléron – 7 juin 2015

Origin of research in biology

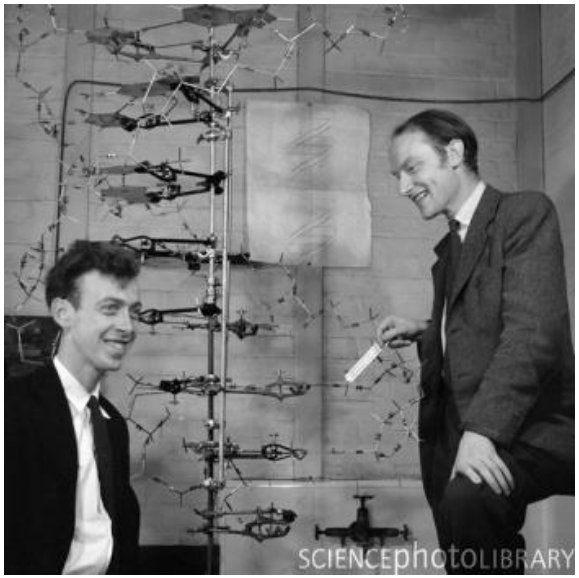


Structure/function studies

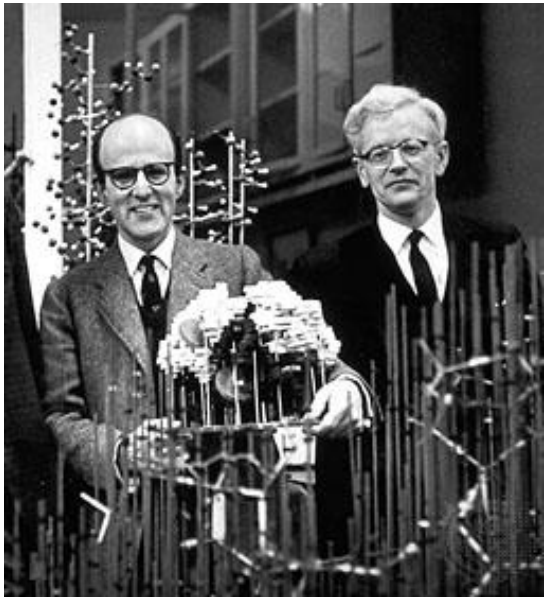




Structure/function studies

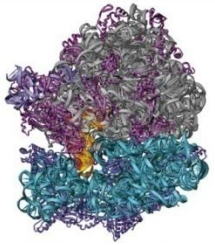


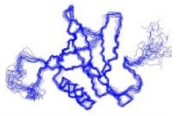
Watson/Crick (1953)

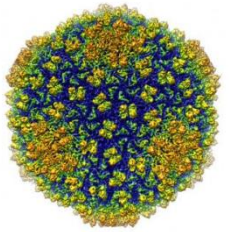


Perutz/Kendrew (1959)

Structural biology today

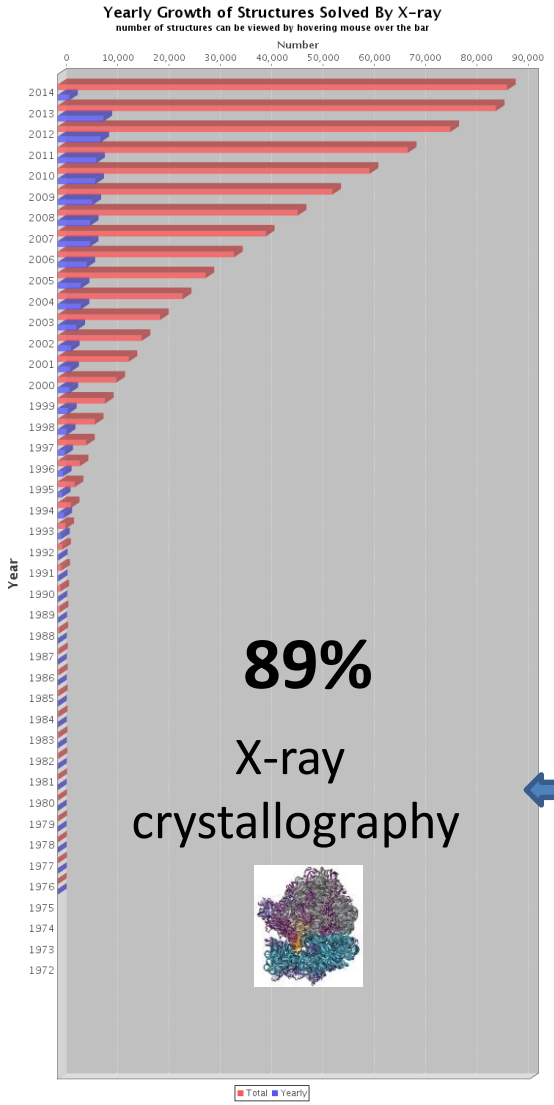
X-ray crystallography 

Nuclear magnetic resonance (NMR) 

Electron microscopy (EM) 

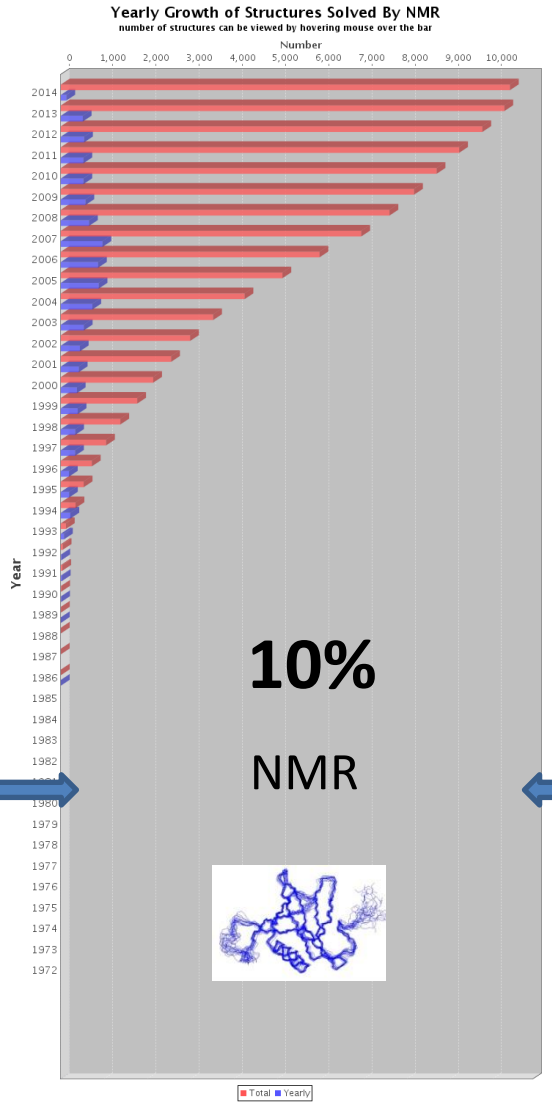
SAXS, …

Structural biology statistics



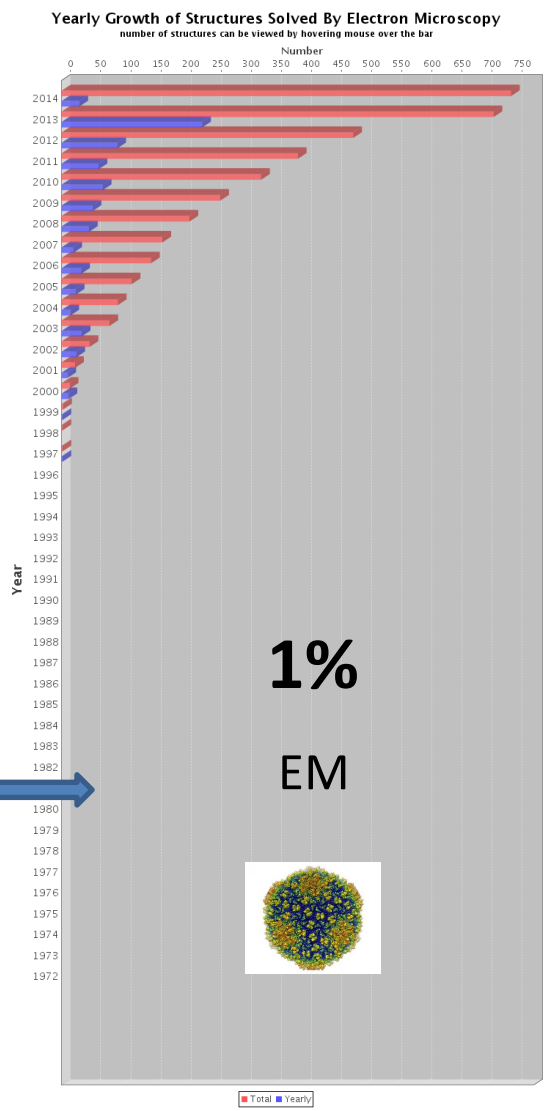
89%

X-ray crystallography



10%

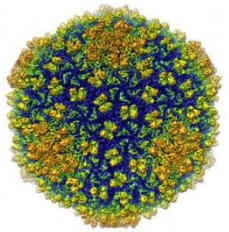
NMR



1%

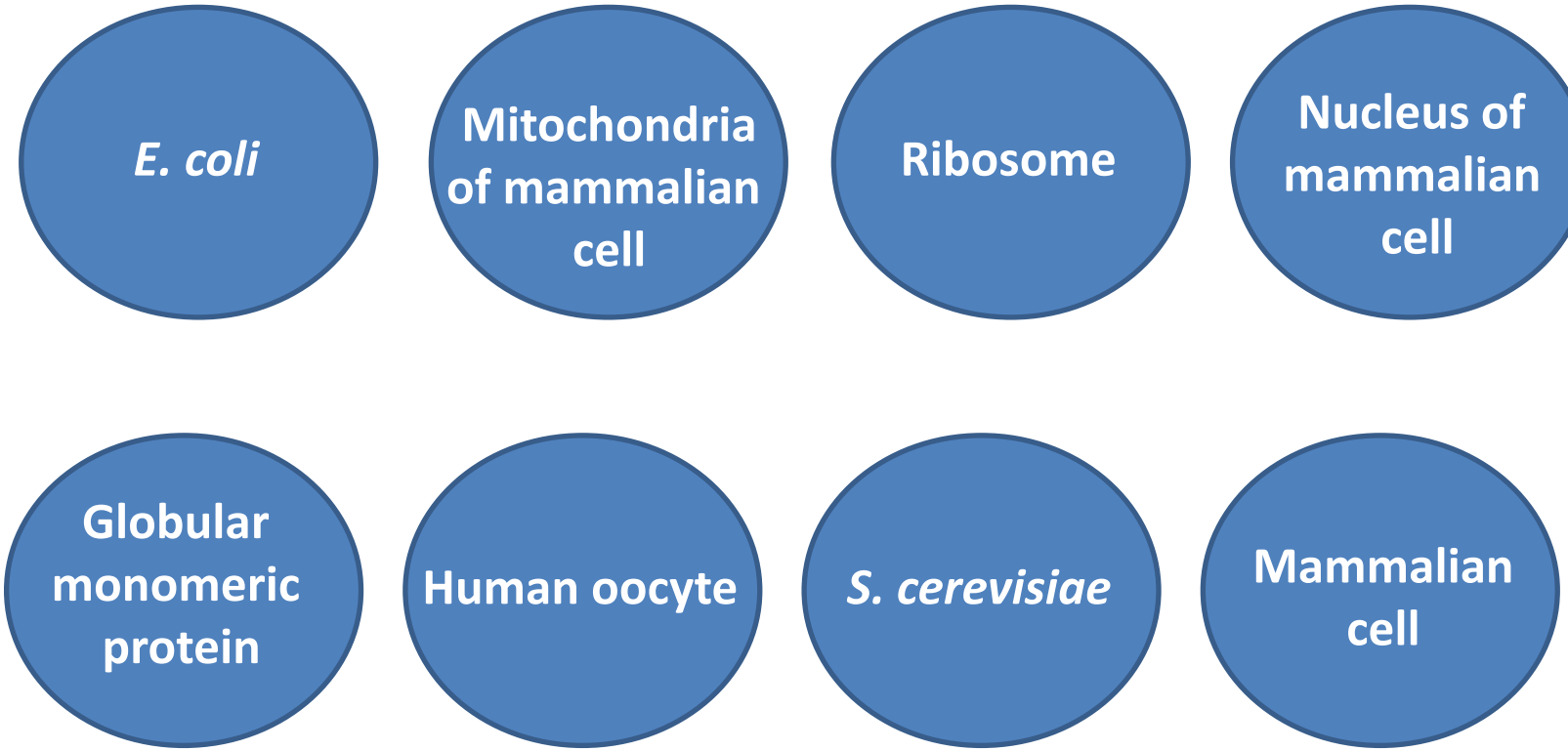
EM

Electron microscopy



- What type of biological material can we study (cell, protein, atom) ?
- Difference optical microscopy vs electronic microscopy ?
- Which microscopy ? For what for?
- Composition of an electron microscope
- What structural informations can you get?

Size of objects



What is the resolution that you can achieve ?

- To summarize, it's the smallest detail that you can observe. ⟺ X-ray crystallography, NMR ?

Wavelength of light

Rayleigh criterion:

$$d = 0{,}61\ \lambda / n \sin(\alpha)$$

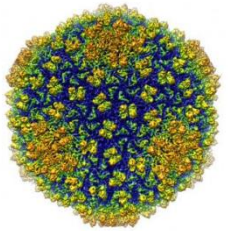
Numerical aperture of the lens

distance

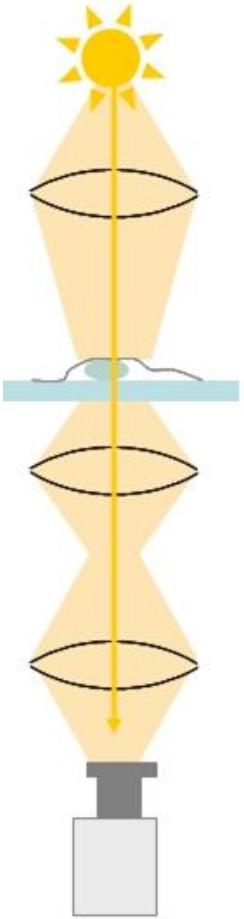
Refraction index of studied matter

To simplify: $d \approx 0.5\ \lambda$

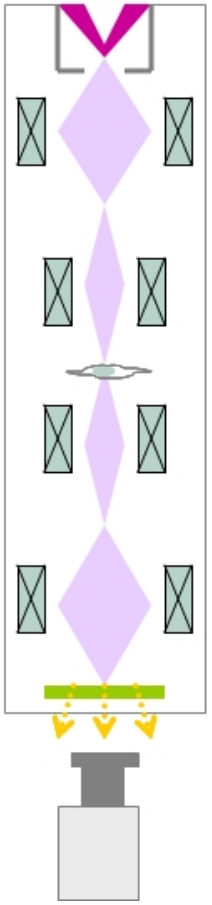
Optical vs electron microscope



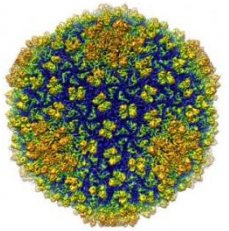
OM



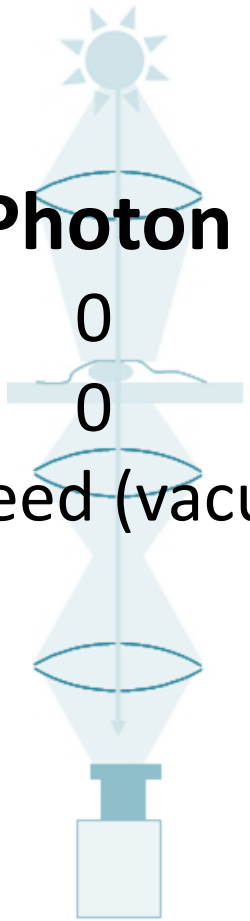
TEM

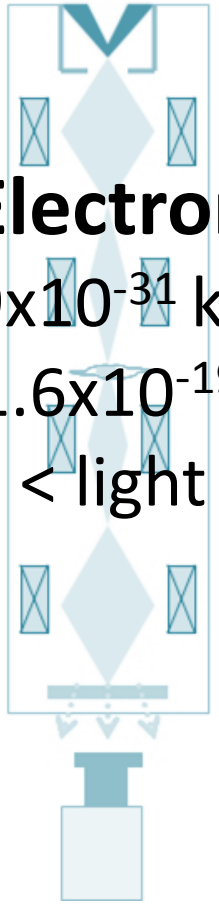


Particules sources

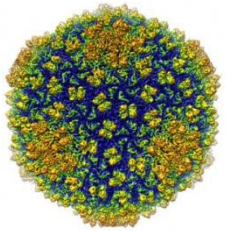


	Photon	**Electron**
Mass	0	9×10^{-31} kg
Charge	0	-1.6×10^{-19} C
Speed	light speed (vacuum)	$0 \leq s <$ light speed





Wave-particle duality

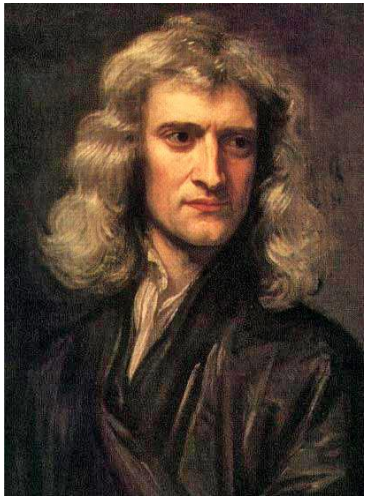


Relation Acceleration tension - wavelength

$$E_c = \frac{1}{2} m v^2$$

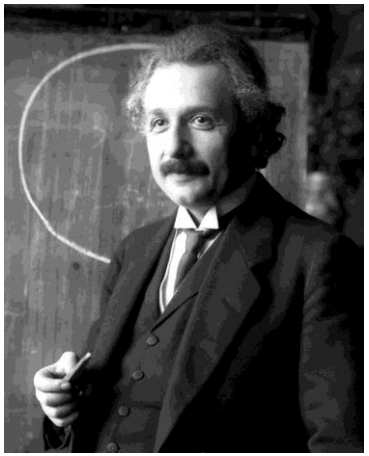
$$\lambda = \frac{h}{mv}$$

$$m = \frac{m_0}{\sqrt{1 - \frac{v^2}{c^2}}}$$



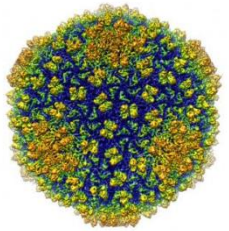
Isaac Newton

Louis de Broglie



Albert Einstein

Wave-particle duality

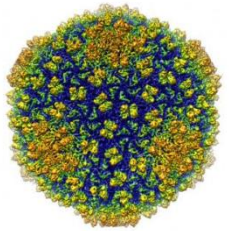


Relation Acceleration voltage - wavelength

$$E_c = \frac{1}{2} m v^2 \qquad \lambda = \frac{h}{mv} \qquad m = \frac{m_0}{\sqrt{1 - \frac{v^2}{c^2}}}$$

$$\lambda = \frac{1.23}{\sqrt{V + 10^{-6} V^2}} \, nm$$

Wave-particle duality



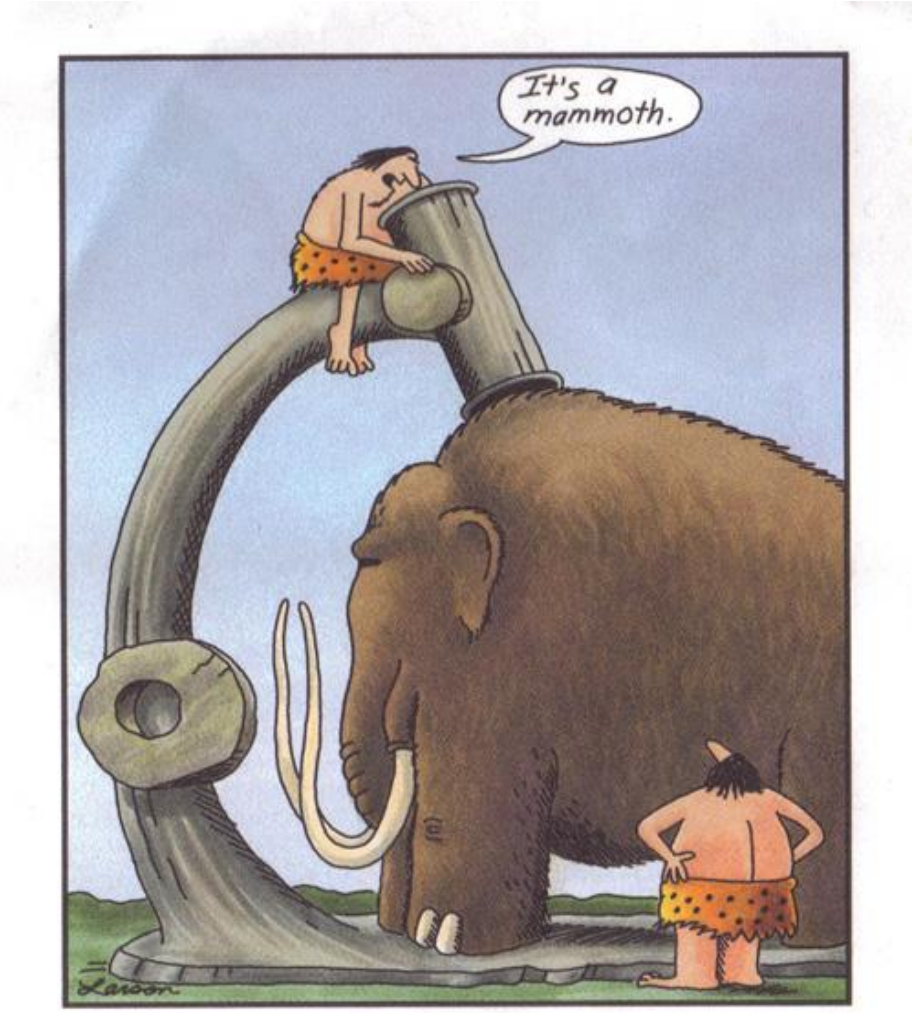
Relation Acceleration voltage - wavelength

V	λ (nm)	Theoretical resolution (nm)
10,000	0.01223	0.00611
50,000	0.00536	0.00268
100,000	0.00370	0.00185
1,000,000	0.00086	0.00043

80-300 kV

Electron microscopy

History

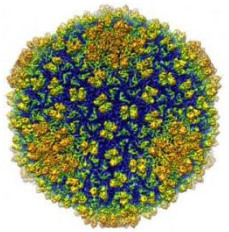


Early microscope

1600-1700

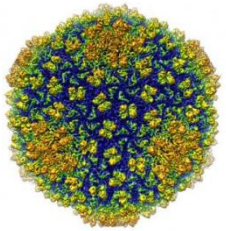
Van Leeuwenhoek

Electron microscopy History

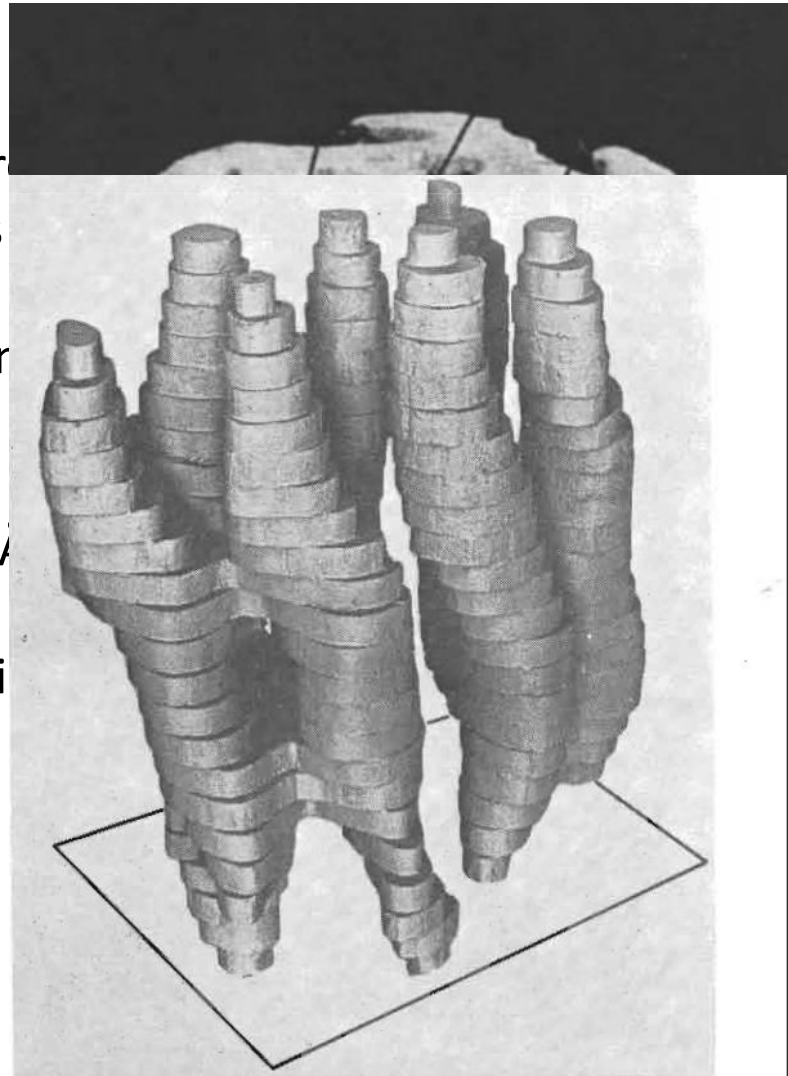


DATES	NAMES	HIGHLIGHTS
1897	J.J. Thomson	Electron discovery
1924	L. de Broglie	Identification of electron wavelength in movement
1926	H. Busch	Characterization of lens effects on magnetic and electric fields with electrons
1929	E. Ruska	Ph.D on magnetic lenses
1931	Knoll & Ruska	Building the first electron microscope
1934	Driest & Muller	Resolution better than optical microscope
1938	von Borries & Ruska	First microscope (Siemens) - 10 nm resolution
1940	RCA	Commercial microscope - 2.4 nm resolution
1945		resolution better than 1.0 nm

Electron microscopy History

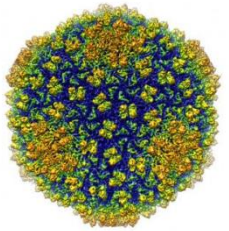


DATES	NAMES	Highlights
1960-1970	France/Japon	High voltage micr material sciences
1968	de Rosier et Klug	3D reconstructior from EM images
1975	Unwin and Henderson	3D structure at 7 A
1981	Dubochet et al	Sample preparati



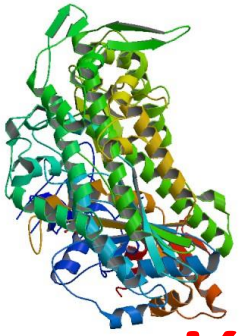
EM in the 1990-2000s

For what for?



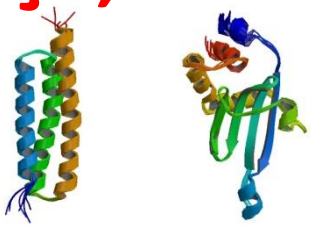
To combine structural informations from other methods (X-ray crystallography, NMR, SAXS, MS…) to link the structure and function of a protein (or a macromolecular complex)

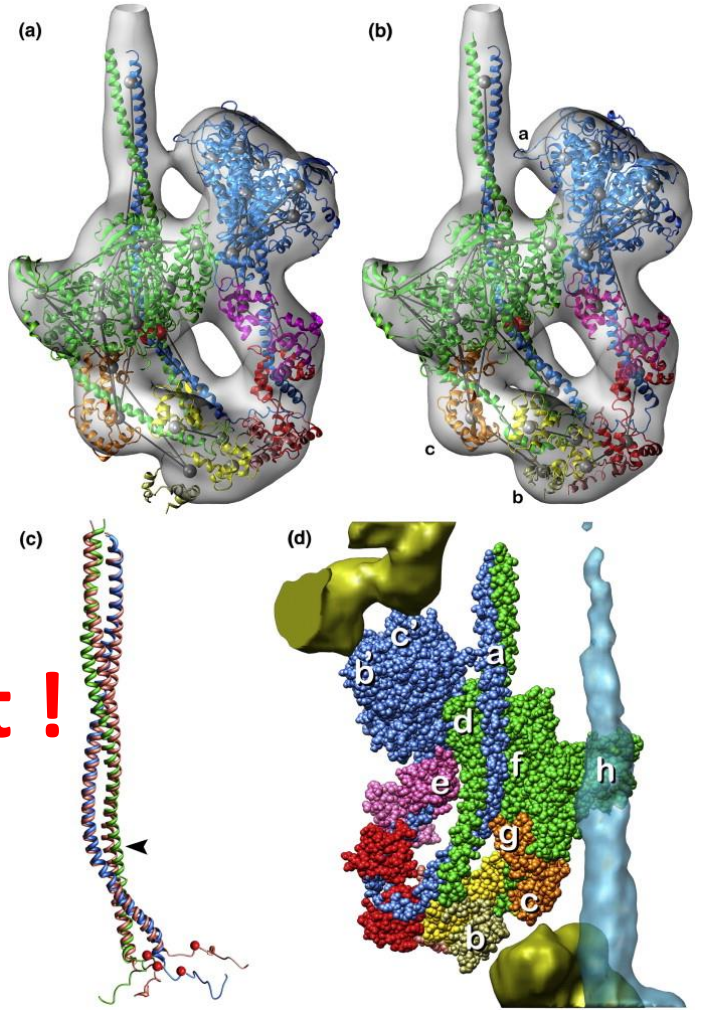
X-ray crystallography



Mais ça, c'était avant !

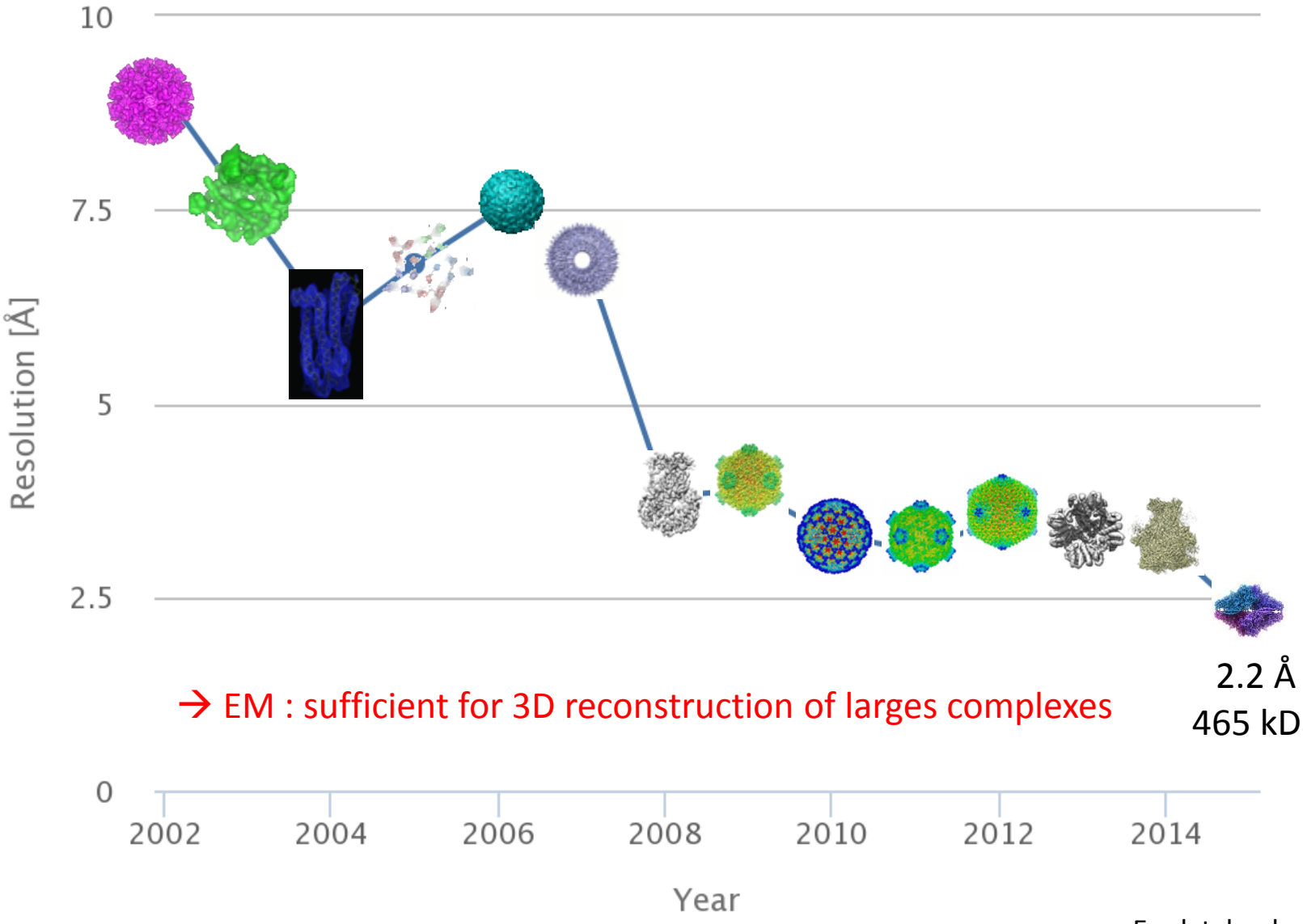
NMR





Alamo et al, 2008

Resolution trends



Emdatabank.org

EM in april 2015

Structure of the human 80S ribosome.
Khatter H, Myasnikov AG, Natchiar SK, Klaholz BP.
Nature. 2015 Apr 30;520(7549):640-5.

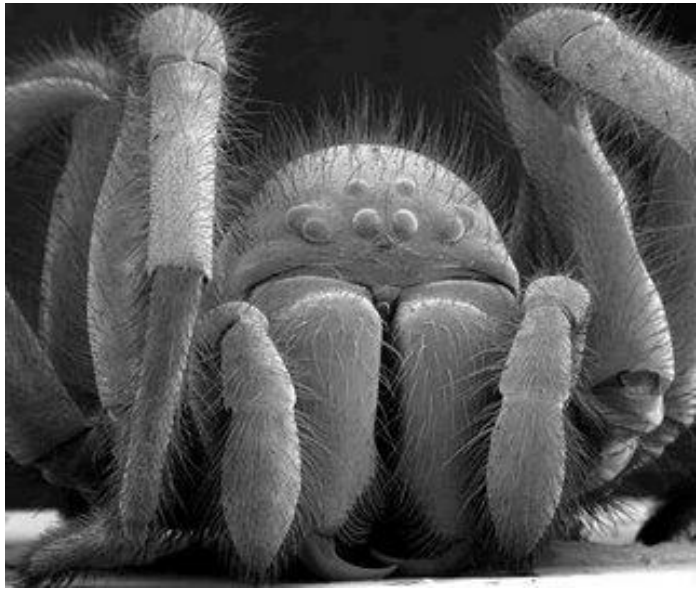
The structure of the human mitochondrial ribosome.
Amunts A, Brown A, Toots J, Scheres SH, Ramakrishnan V.
Science. 2015 Apr 3;348(6230):95-8.

The complete structure of the 55S mammalian mitochondrial ribosome.
Greber BJ, Bieri P, Leibundgut M, Leitner A, Aebersold R, Boehringer D, Ban N.
Science. 2015 Apr 17;348(6232):303-8.

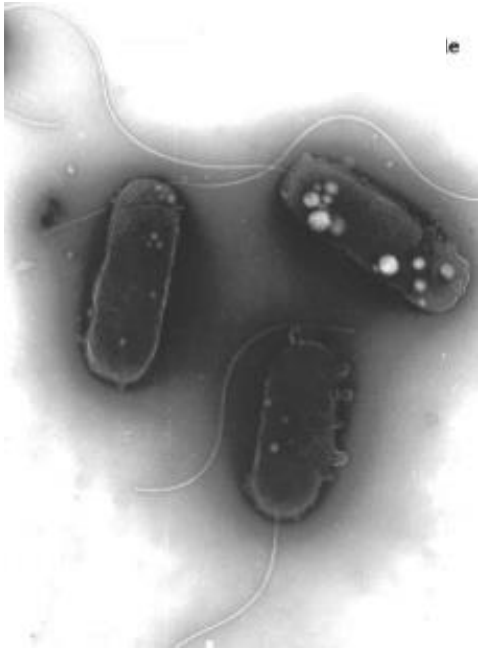
Structure of the E. coli ribosome-EF-Tu complex at<3 Å resolution by Cs-corrected cryo-EM.
Fischer N, Neumann P, Konevega AL, Bock LV, Ficner R, Rodnina MV, Stark H.
Nature. 2015 Apr 23;520(7548):567-70.

Electron microscopy types

Scanning electron microscopy (SEM)

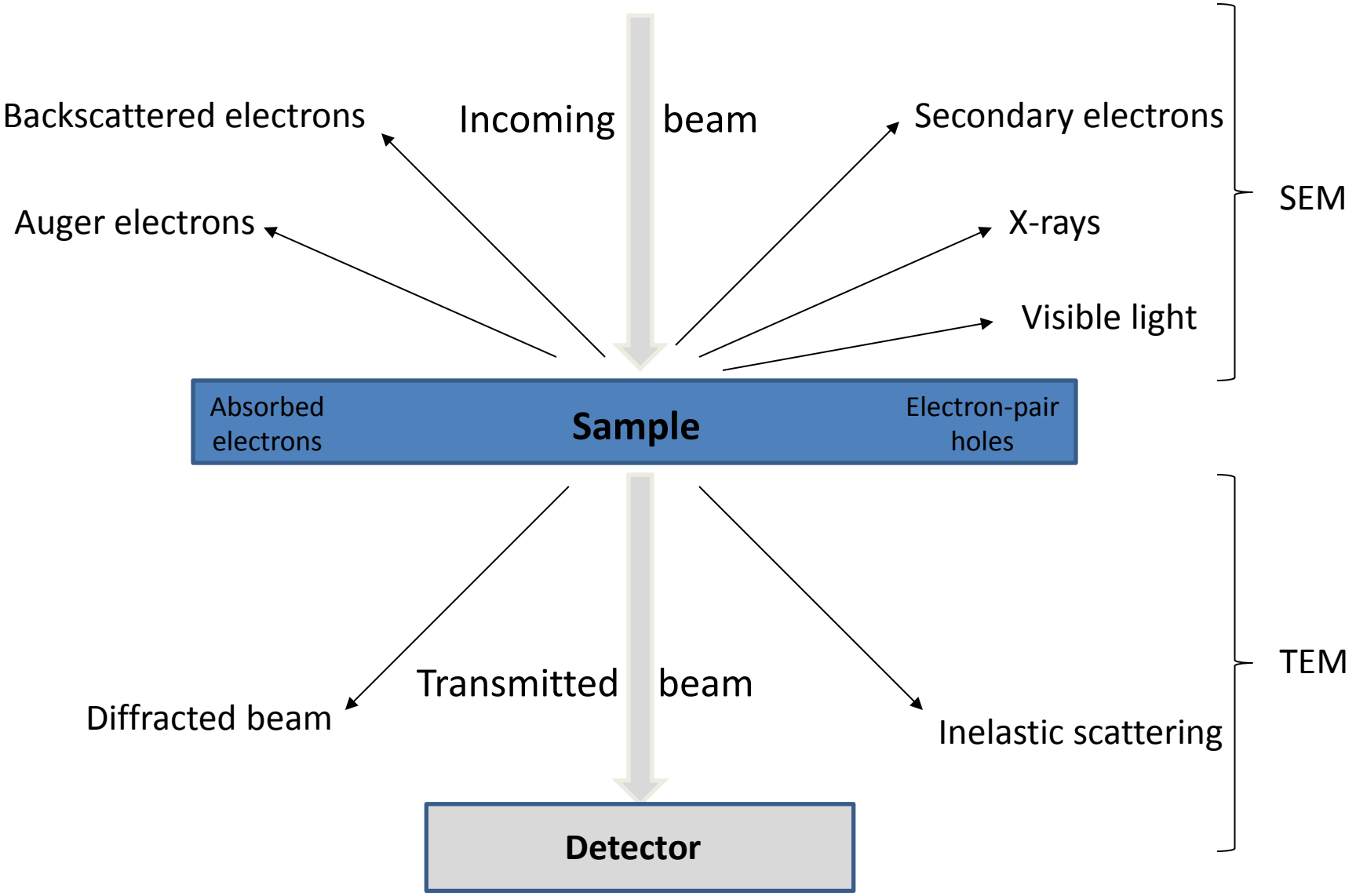


Transmission electron Microscopy (TEM)

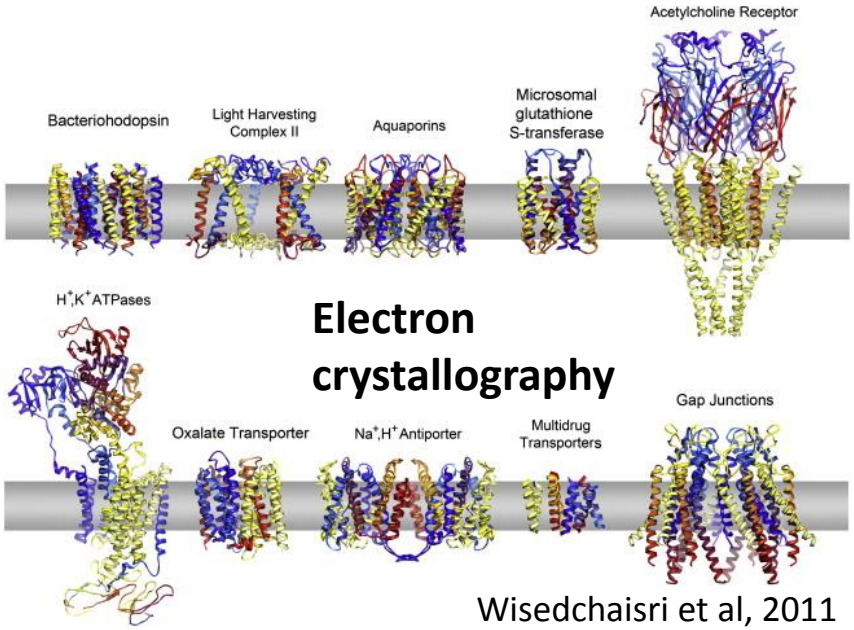


≠ Atomic force microscopy (AFM)

How is an image formed?

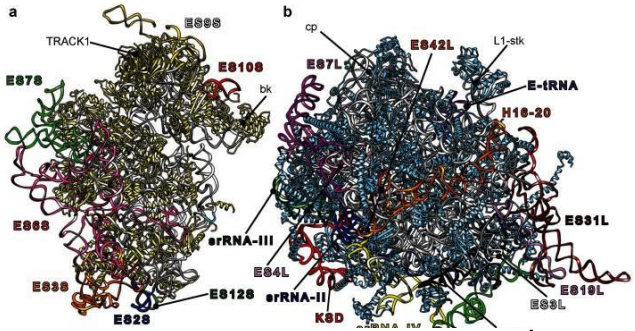


Fields in transmission electron microscopy



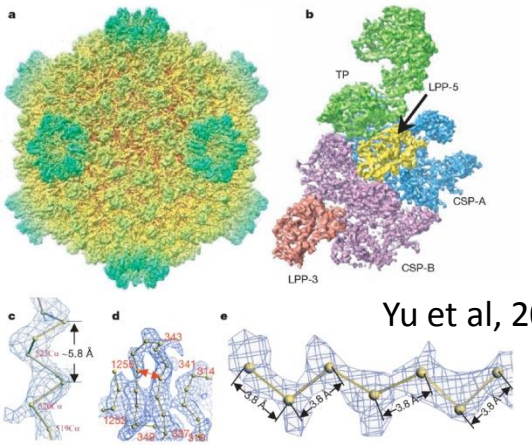
Electron crystallography

Wisedchaisri et al, 2011



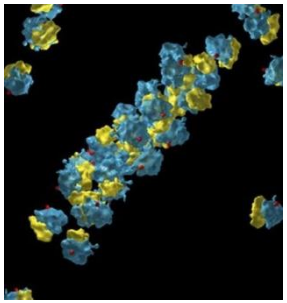
Hashem et al, 2013

Single particles with low degree of symmetry



Yu et al, 2008

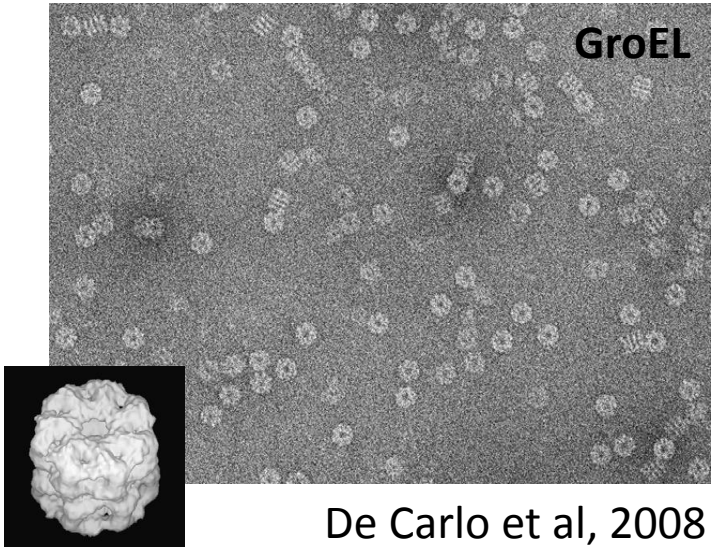
Single particles with high degree of symmetry



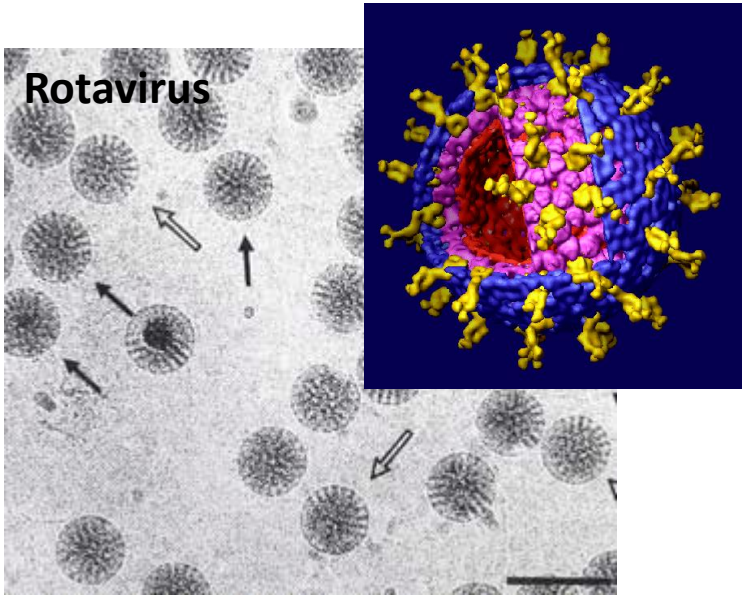
Brandt et al, 2010

Tomography

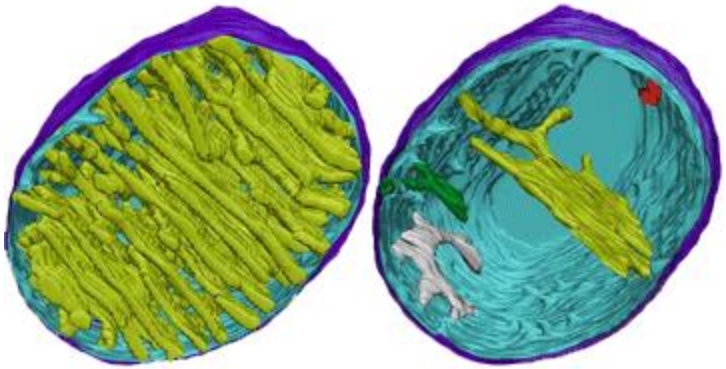
What can we see with a TEM?



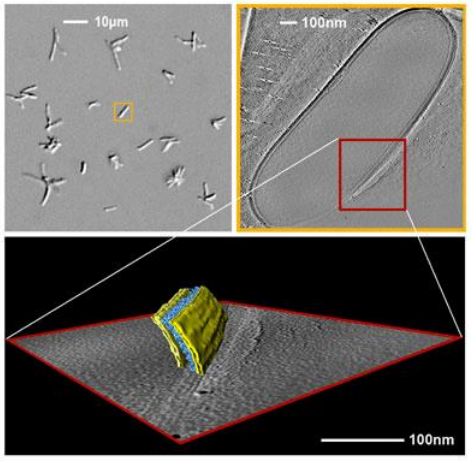
De Carlo et al, 2008

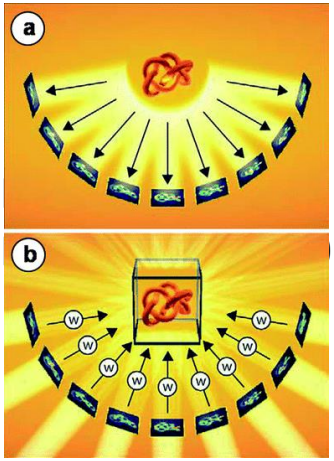


Tihova et al, 2001

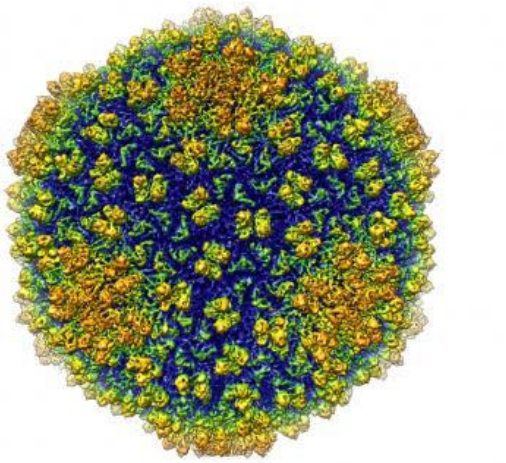


Perkins et al, 1997





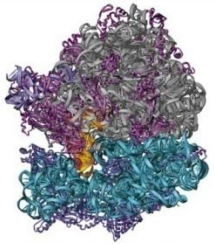
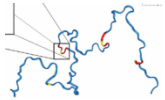
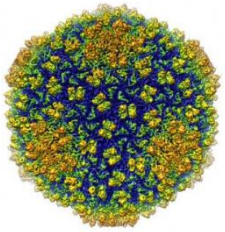
Structure of a protein complex obtained by electron microscopy



Advantages :

- 3D structure of the protein complex
- (very) large complexes (protein → cell)
- No crystal needed !
- Small amount of material

Quantity needed for an experiment

X-ray crystallography		~200 µM	~100 µL
NMR		~200 µM	~200 µL
Electron microscopy		~0.2 µM	~50 µL

Composition of a TEM

Electron gun

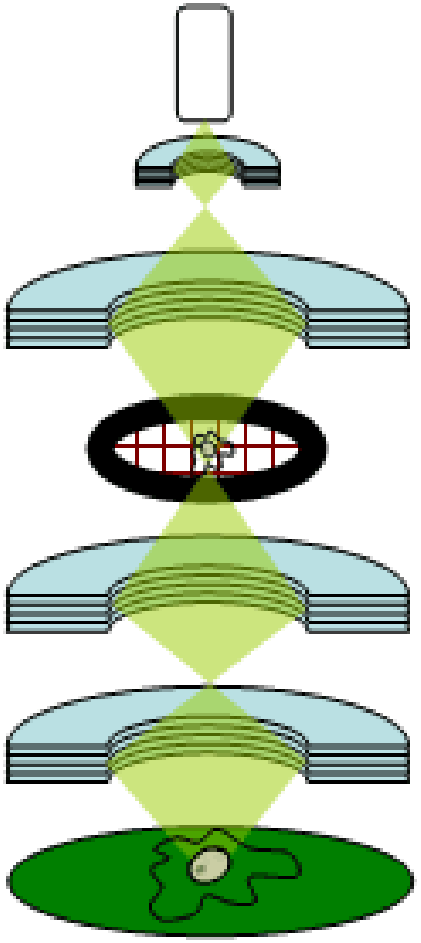
Condenser lens

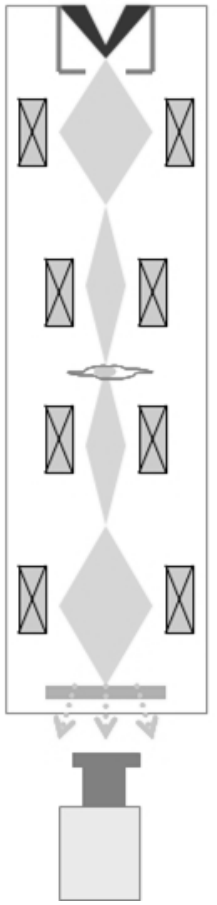
Sample grid

Objective lens

Projection lens

Phosphor screen





Microscope drawbacks

And solutions…

Stability:

- Mechanical vibrations
- Acoustic vibrations
- Magnetic fields
- Electron source
- Lens current

How does a modern TEM look like ?

JEOL -3200 FSC (Japon)

FEI-Titan Krios (USA)

Composition of a TEM

Electron gun

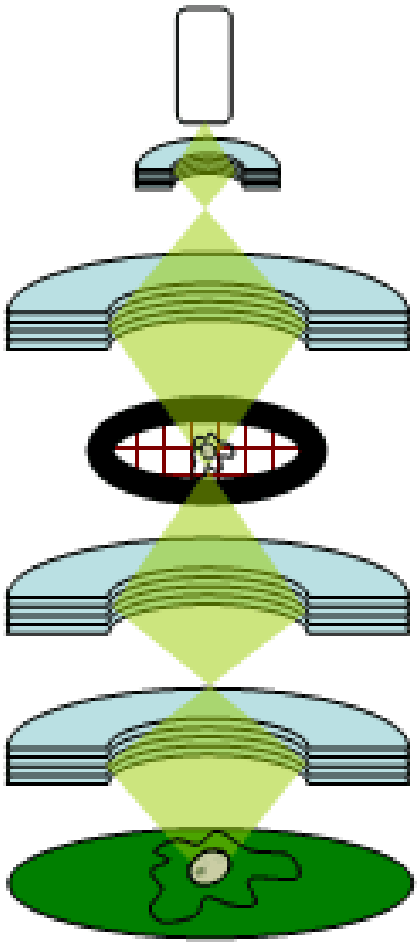
Condenser lens

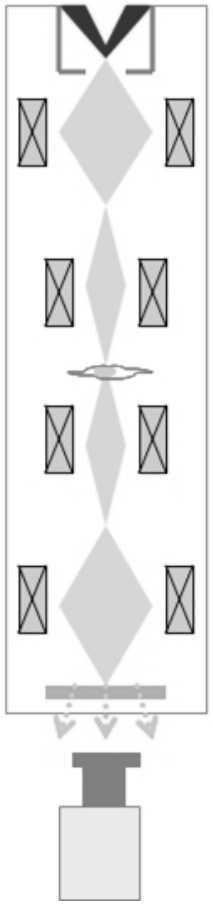
Sample grid

Objective lens

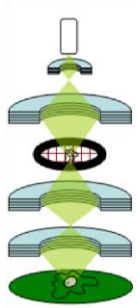
Projection lens

Phosphor screen



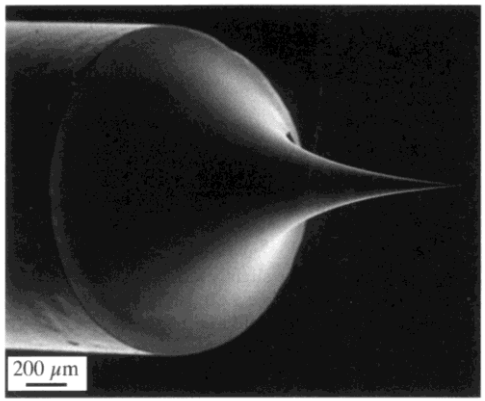


Source of electrons



- Thermoionic gun

- Field emission gun (FEG)



Field emission gun (FEG)

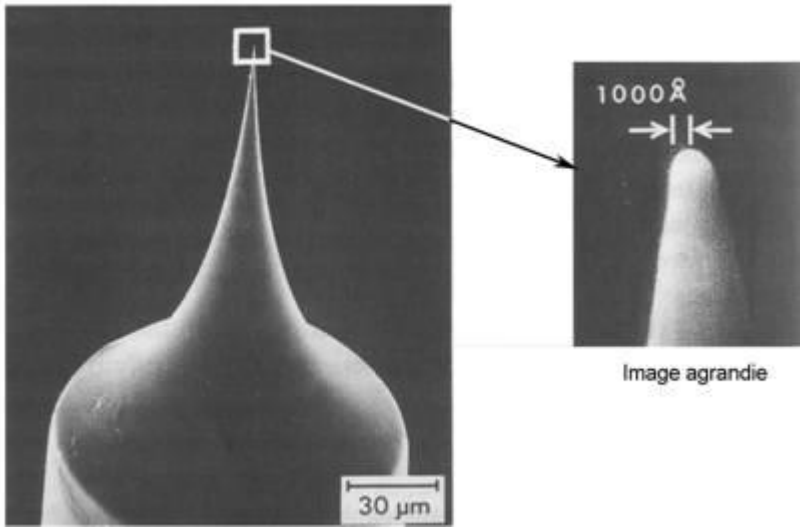
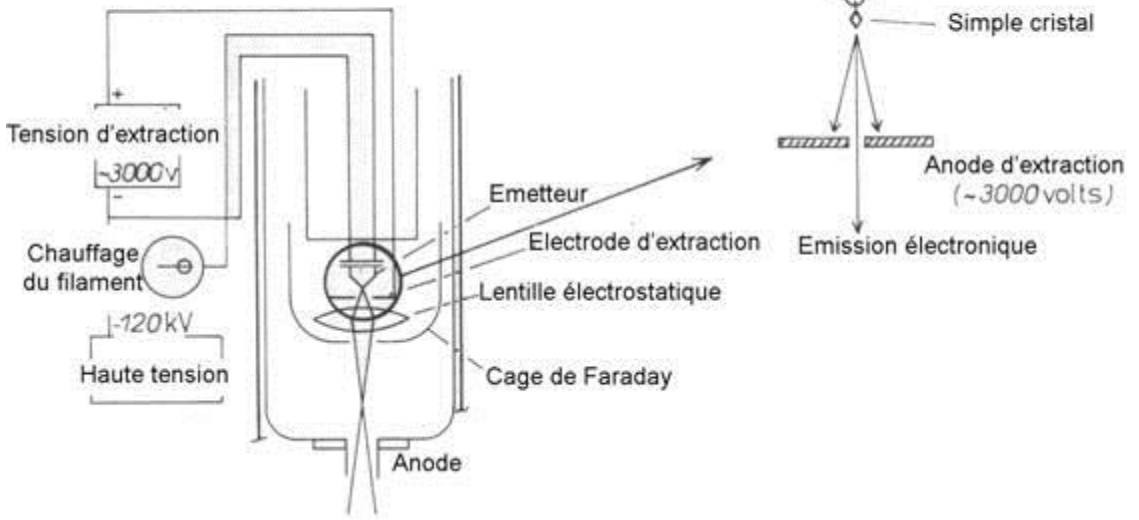


Image agrandie



Composition of a TEM

Electron gun

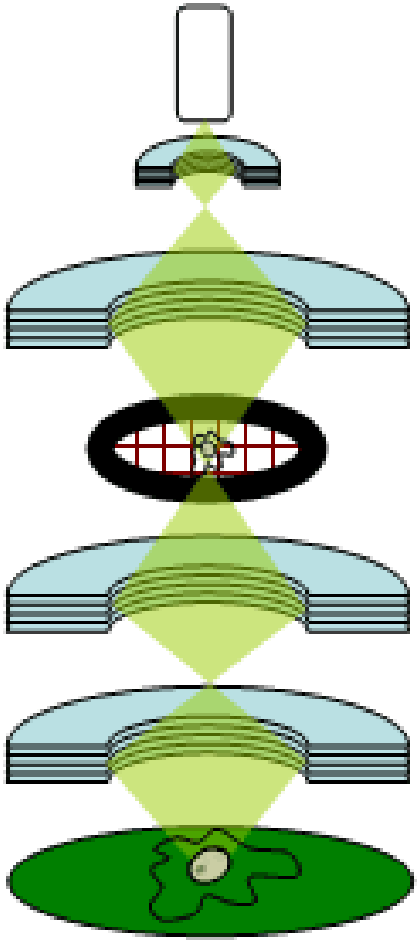
Condenser lens

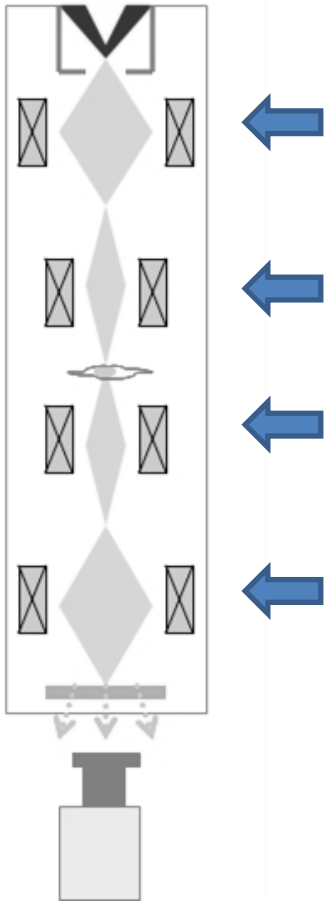
Sample grid

Objective lens

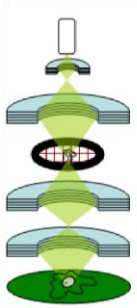
Projection lens

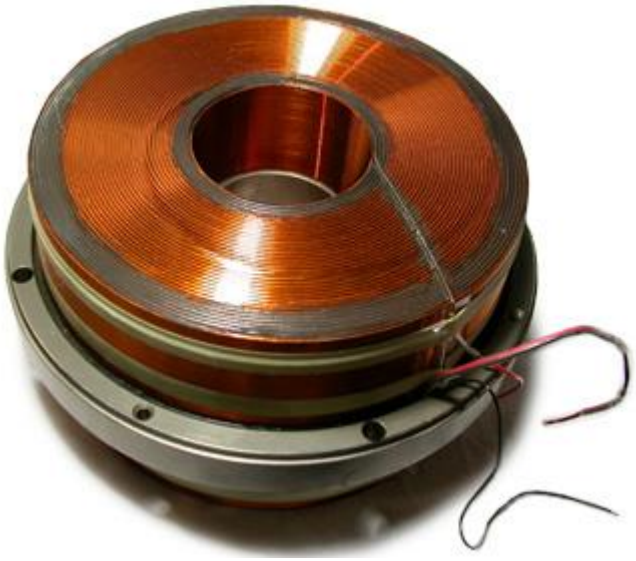
Phosphor screen

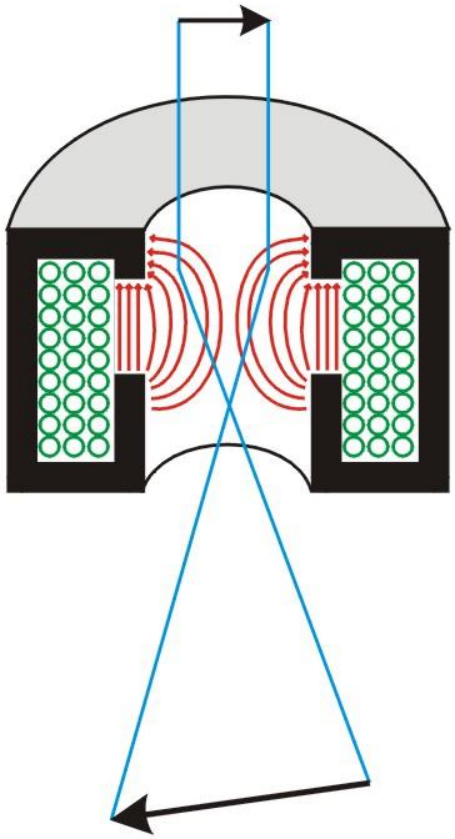




Electromagnetic lens







Microscope drawbacks

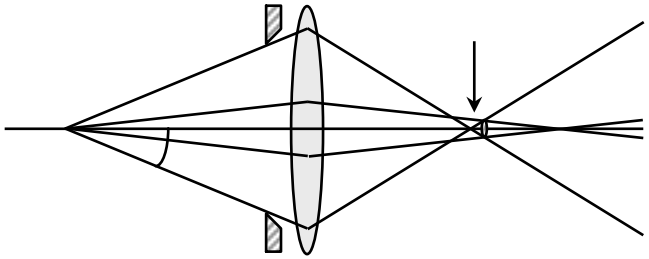
And solutions…

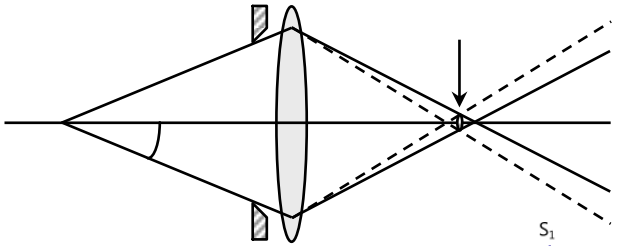
Stability:

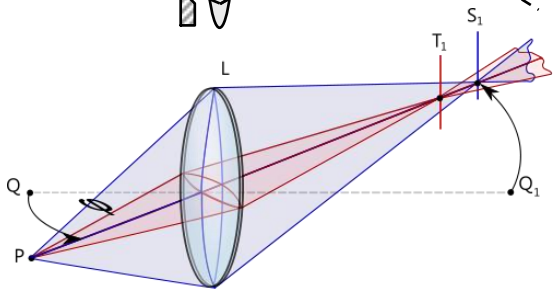
- Mechanical vibrations
- Acoustic vibrations
- Magnetic fields
- Electron source
- Lens current

Lens:

- Spherical aberration
- Chromatic aberration
- Astigmatism

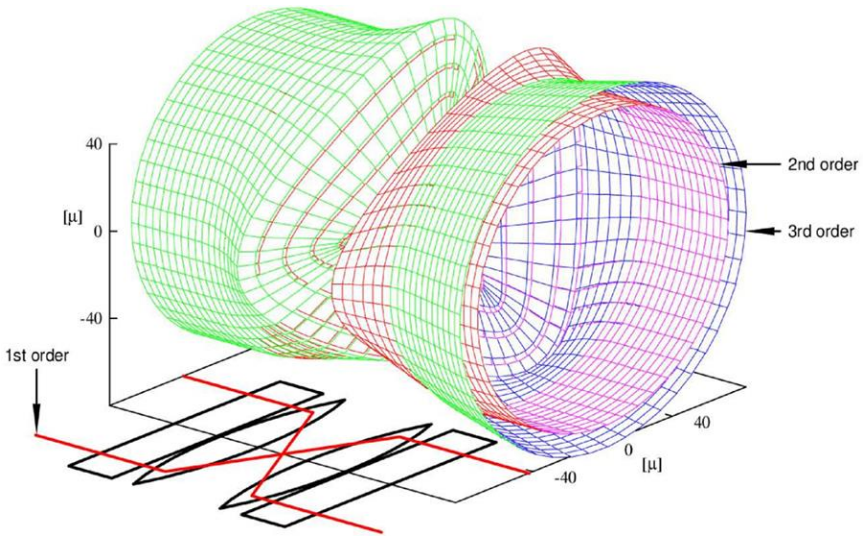




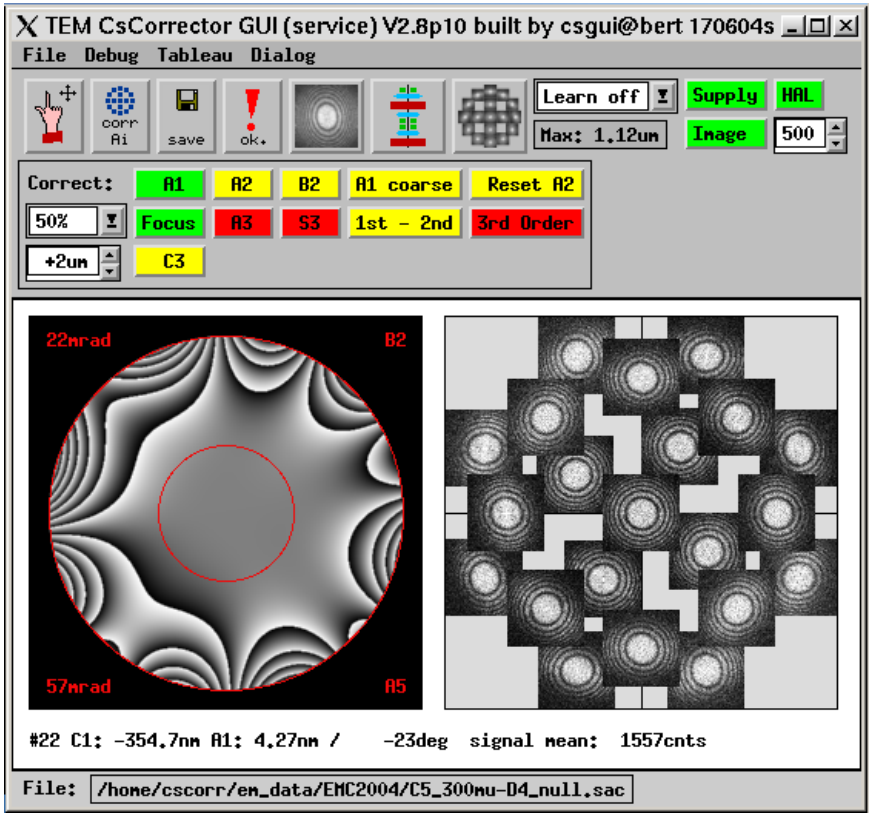


Lens spherical aberration

Correction



http://salve-project.de



http://www.ceos-gmbh.de/English/products/residualsCEXCOR.html

Composition of a TEM

Electron gun

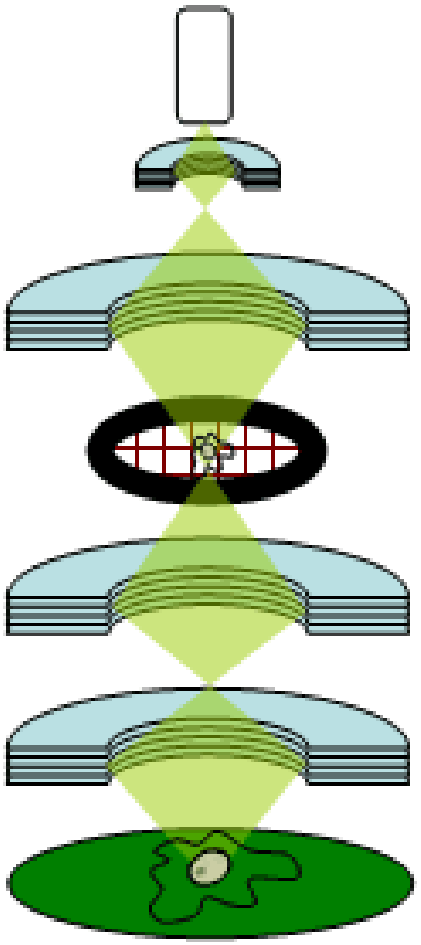
Condenser lens

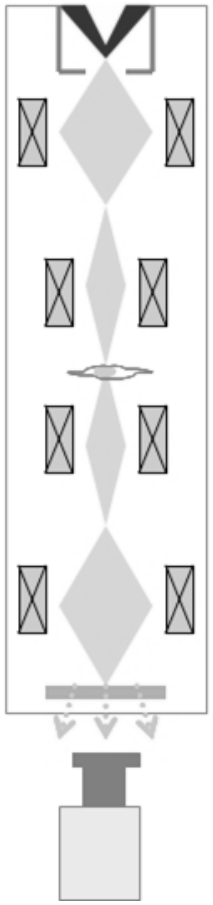
Sample grid

Objective lens

Projection lens

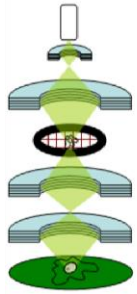
Phosphor screen

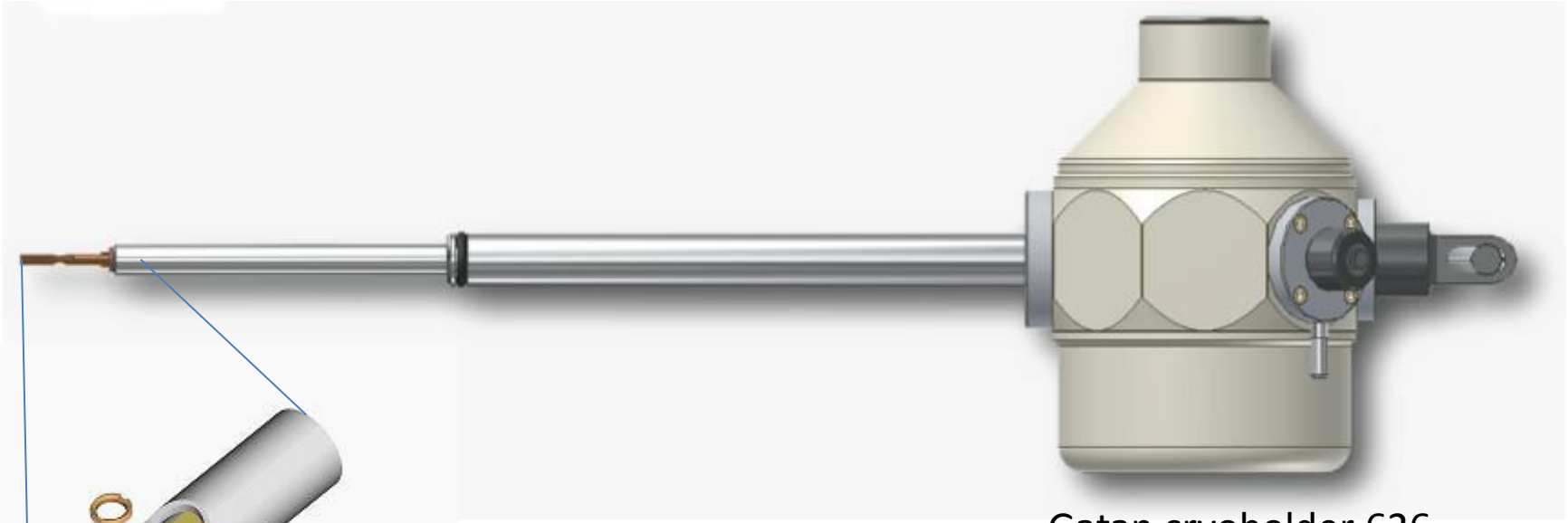




Sample holder

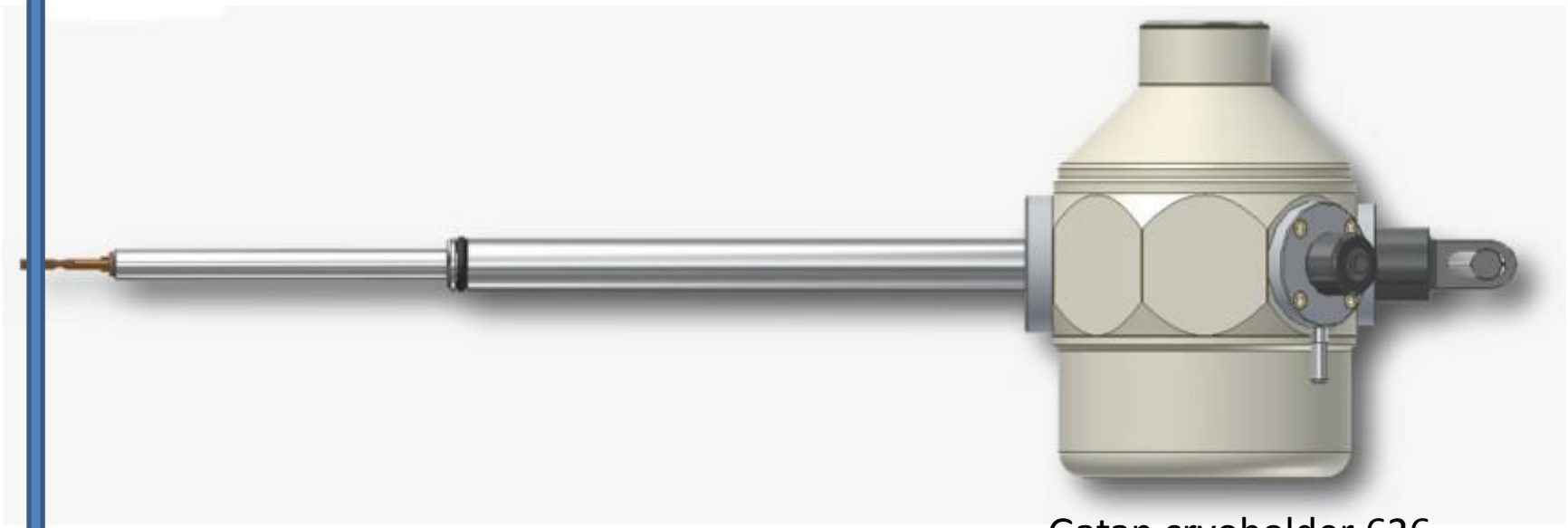
3 mm





Gatan cryoholder 626

Sample holder



Gatan cryoholder 626

Inside the microscope

Outside the microsocpe

Autoloader (FEI)

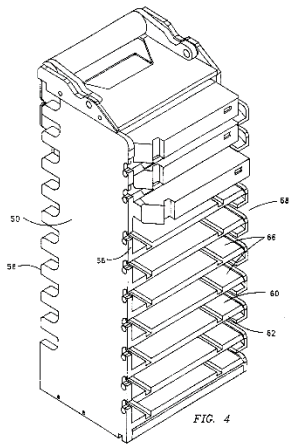


FIG. 4

Composition of a TEM

Electron gun

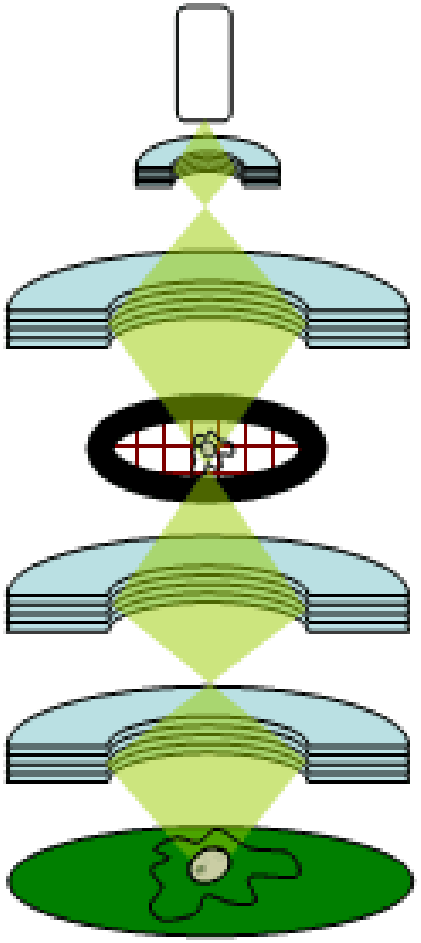
Condenser lens

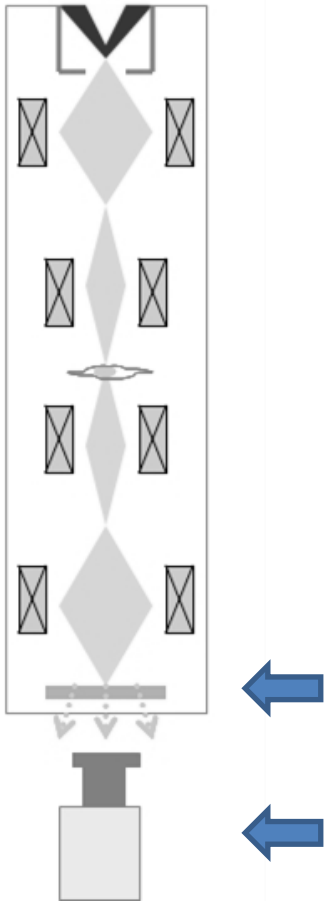
Sample grid

Objective lens

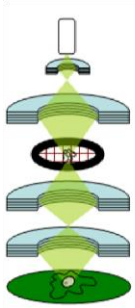
Projection lens

Phosphor screen



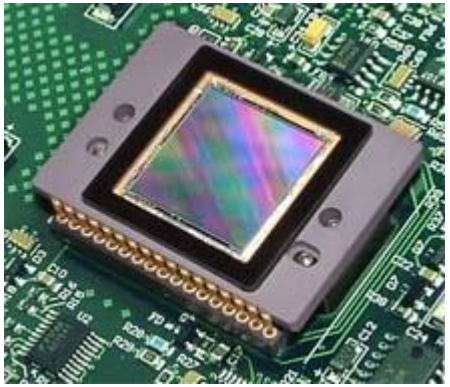


Visualization



Screen

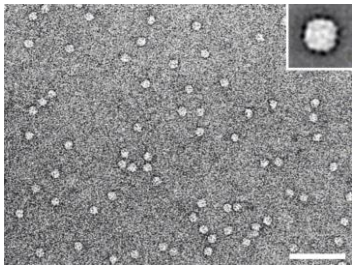
Negatives



Digital detector

Digitalization of images

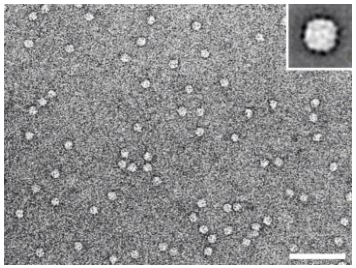
Film sensitive
to electrons



Pixel size = 8 µm
1 image / s

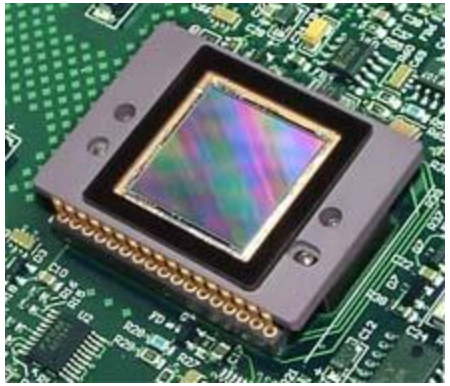
Digitalization of images

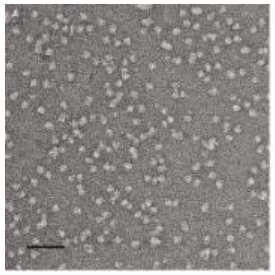
Film sensitive to electrons



Pixel size = 8 µm
1 image / s

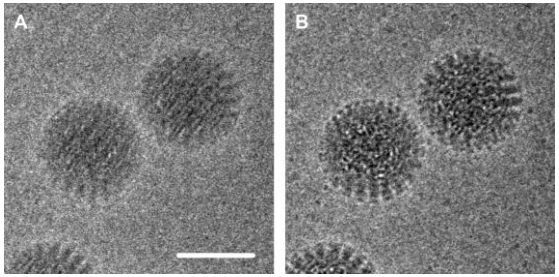
CCD





14 µm
1 image / s

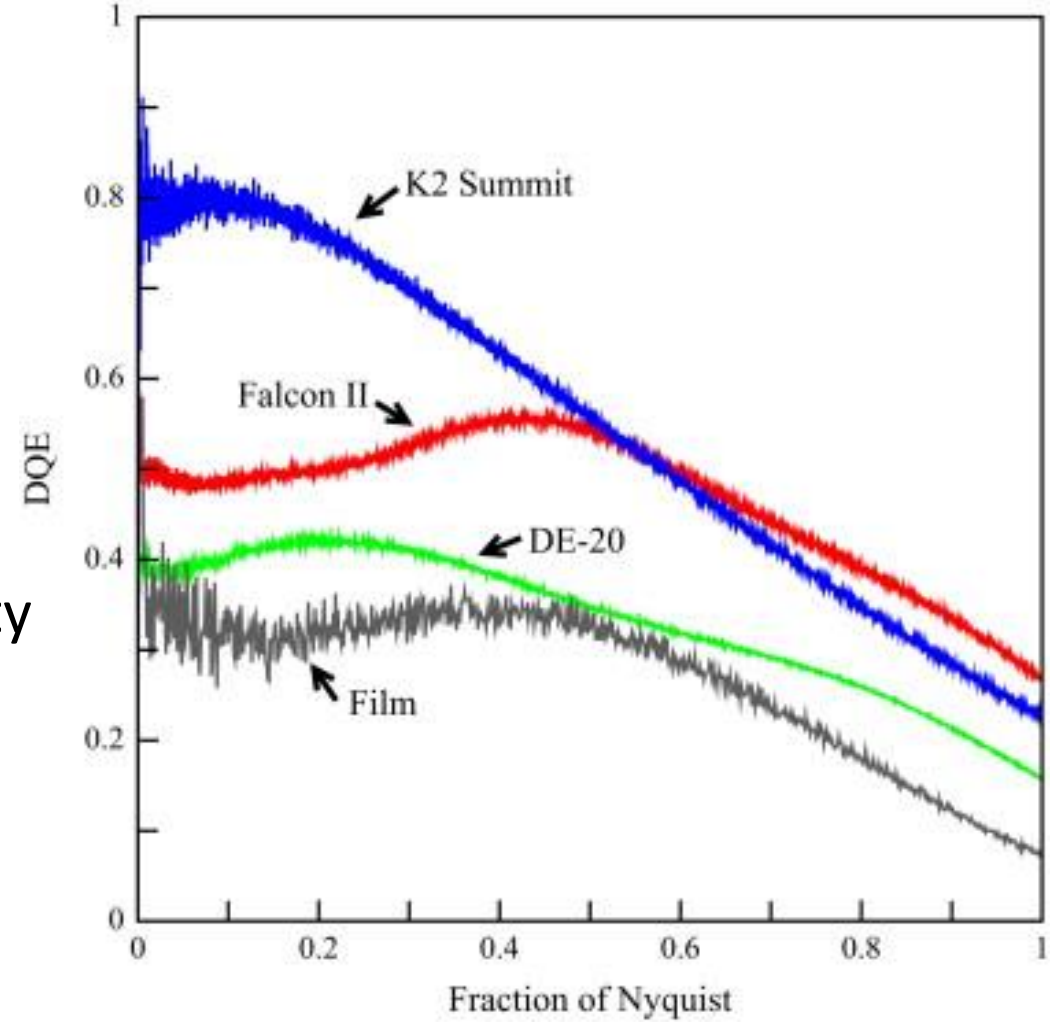
DED



5 µm
20 images / s

Sensitivity Film vs Digital detector

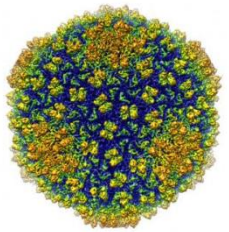
Detective quantum efficiency ≈ Sensitivity



(1/Resolution)

McMullan et al, 2014

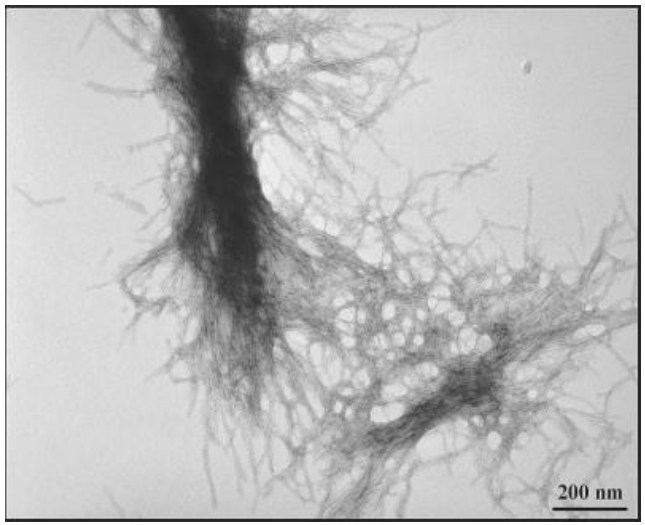
Electron microscopy



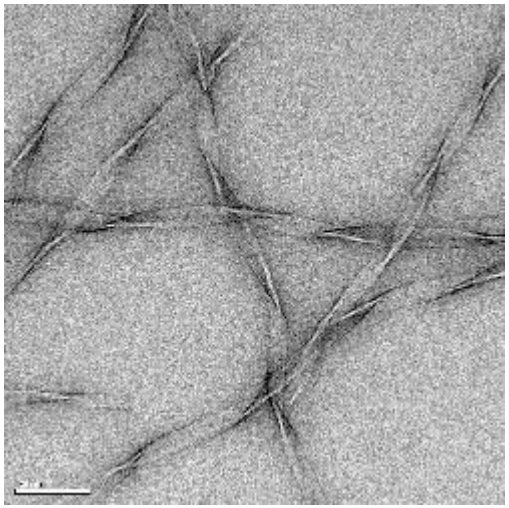
What structural informations can you get ?

Qualitative analysis

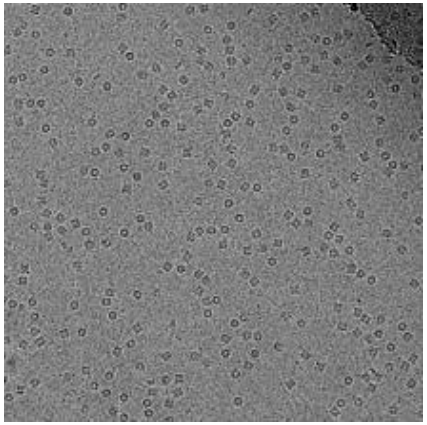
TEM



Agreggation



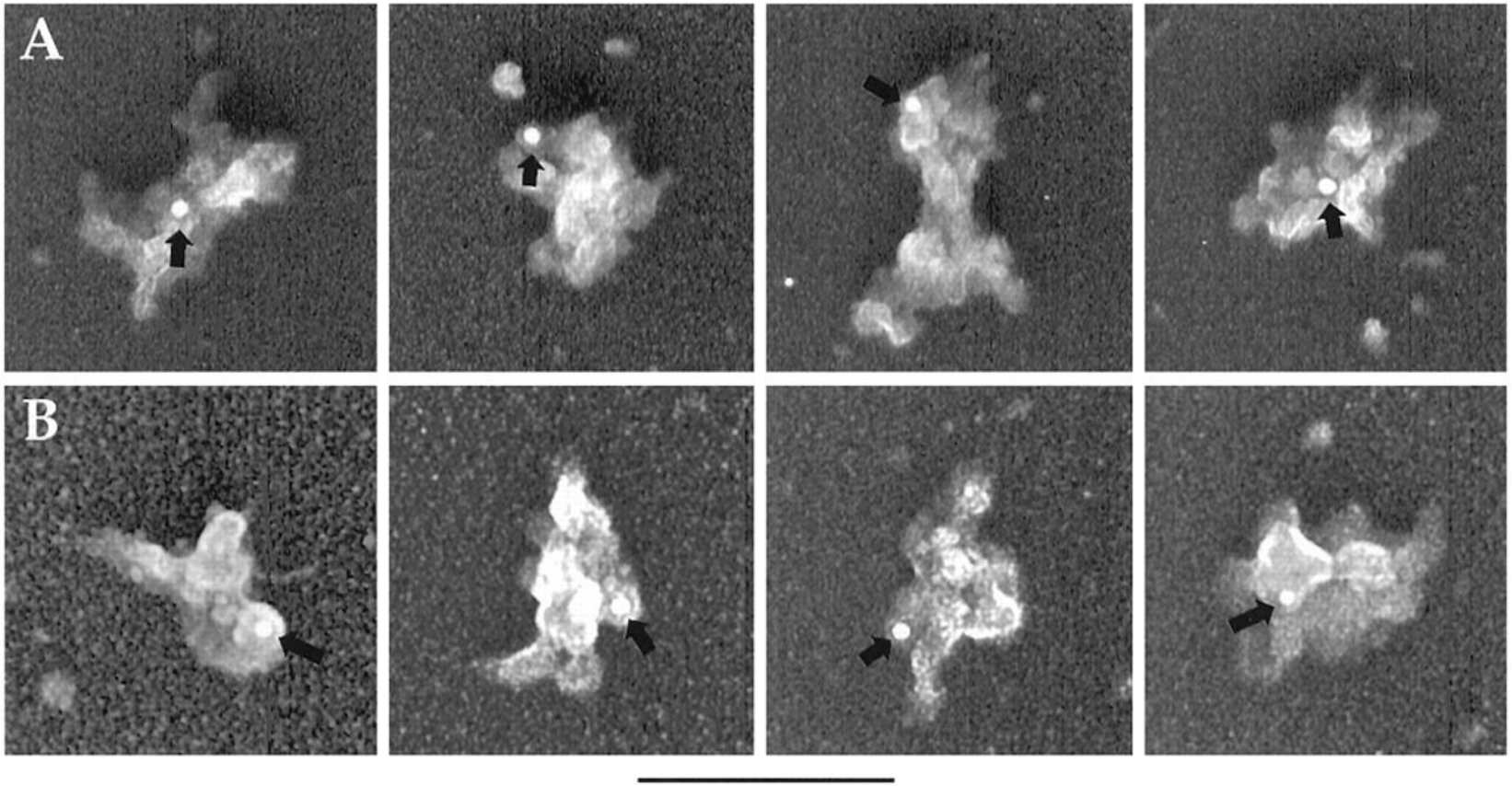
Filaments



Soluble protein

Localize a protein in a complex

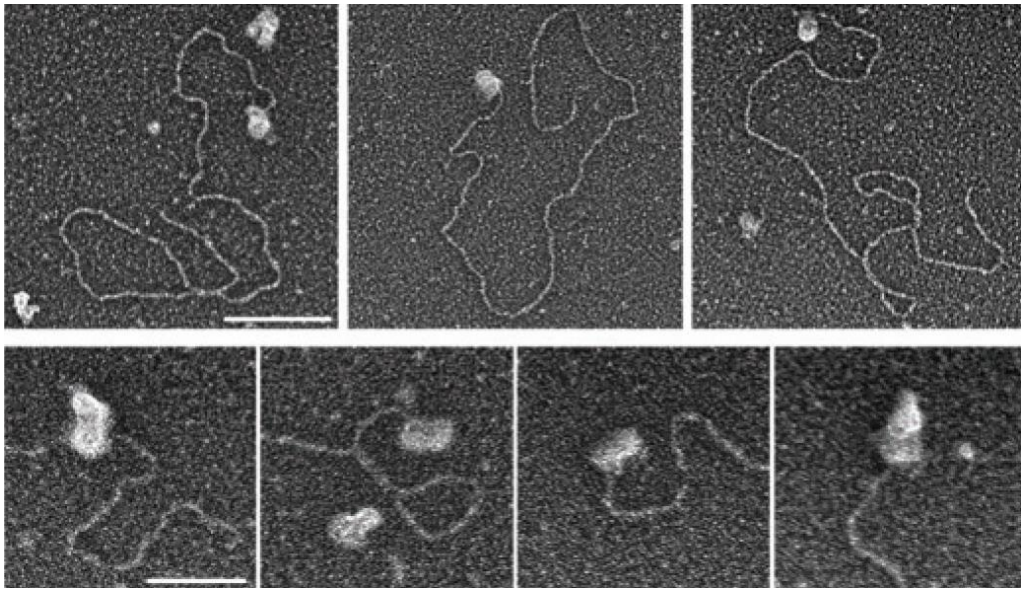
Human spliceosome (SEM)



Zhou et al. PNAS 2002

DNA-proteins interactions

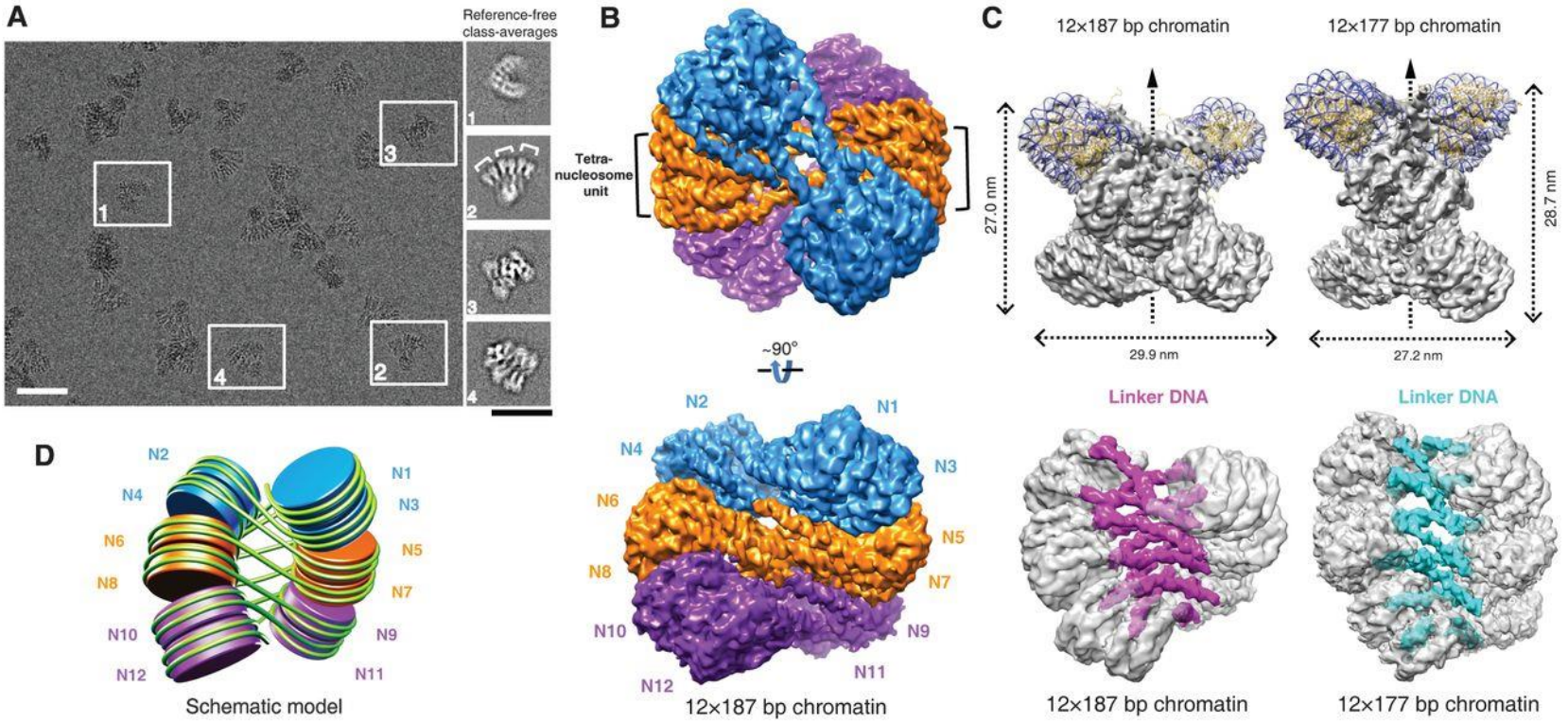
SEM



Thorslund et al, 2010

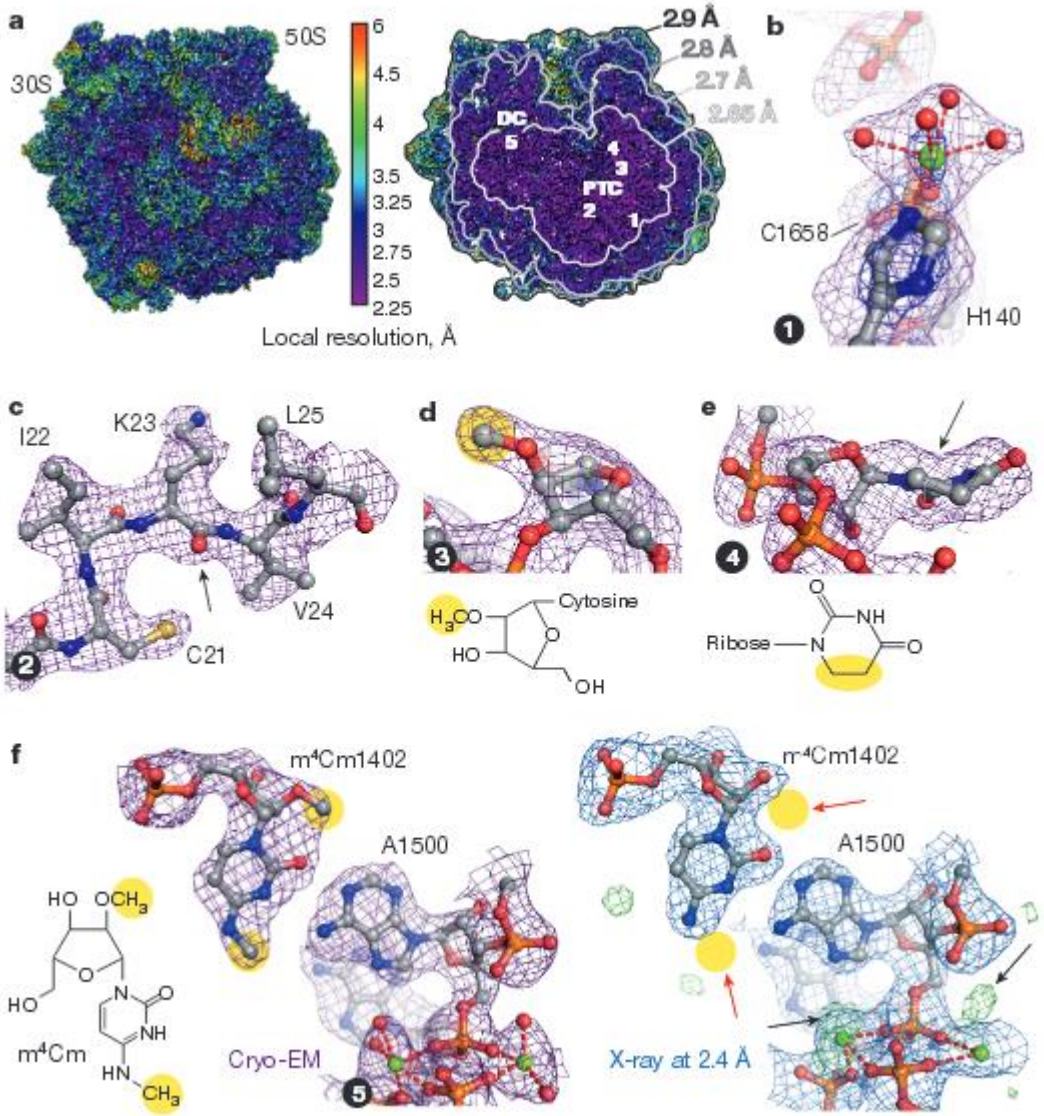
DNA-proteins interactions

TEM



Song et al, 2014

RNA-proteins interactions



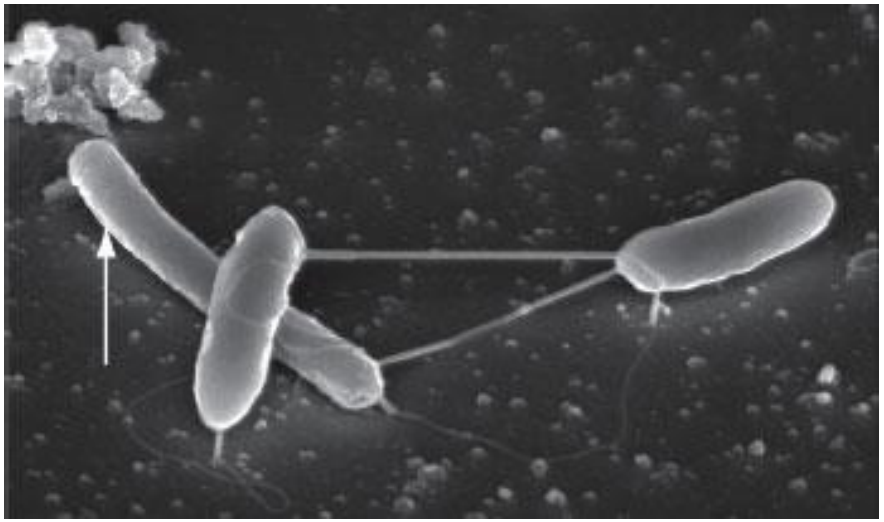
TEM

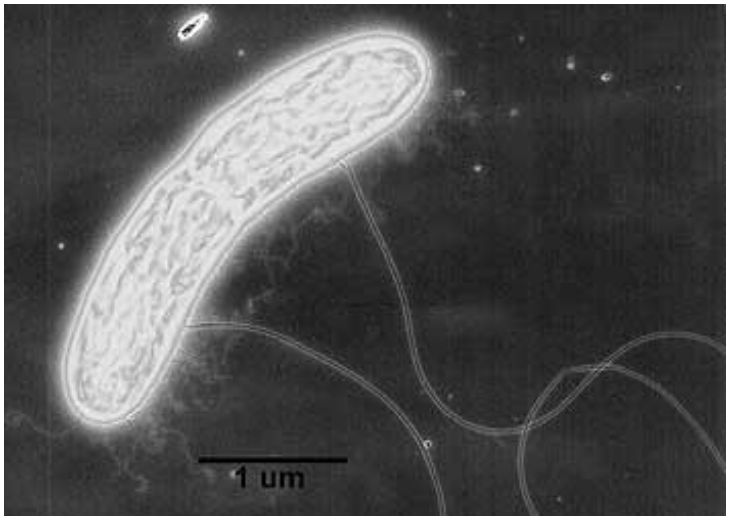
< 3 Å résolution

Fischer et al, Nature, 2015

Nanowires

SEM & TEM

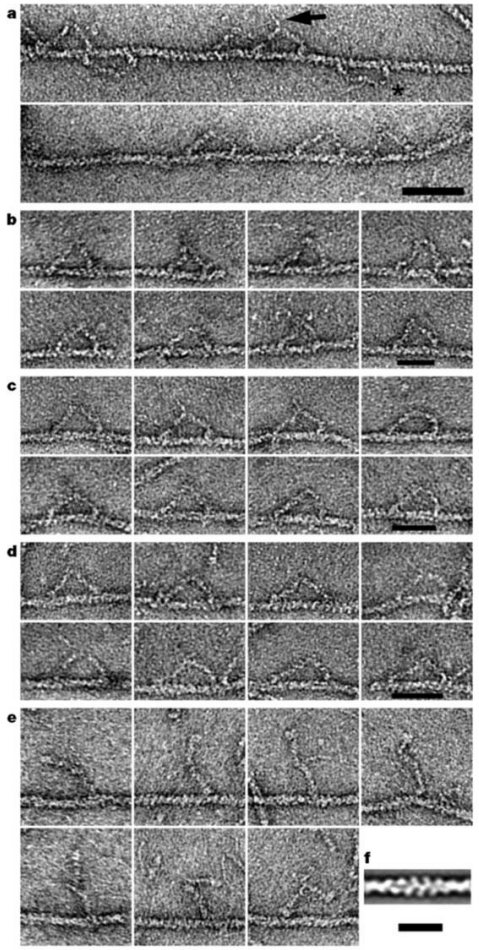




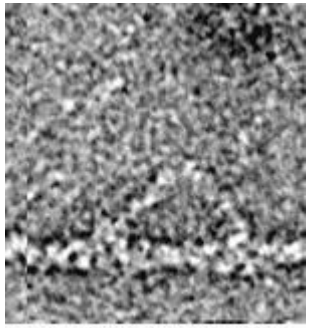
Geobacter metallireducens

Conformational changes

TEM



Walker et al, Nature, 2000

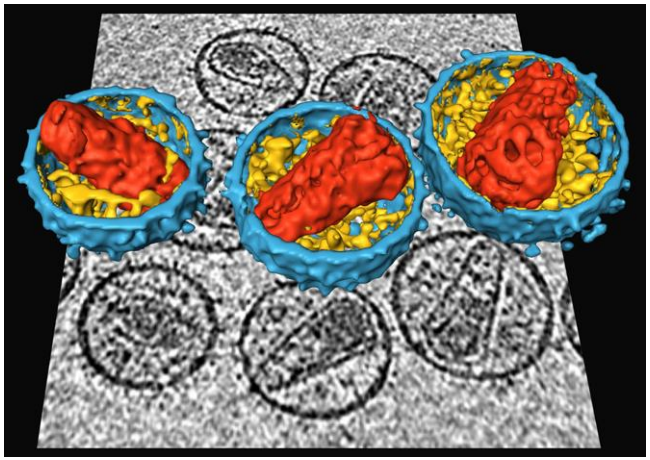


The Muscle Group, Leeds 2000

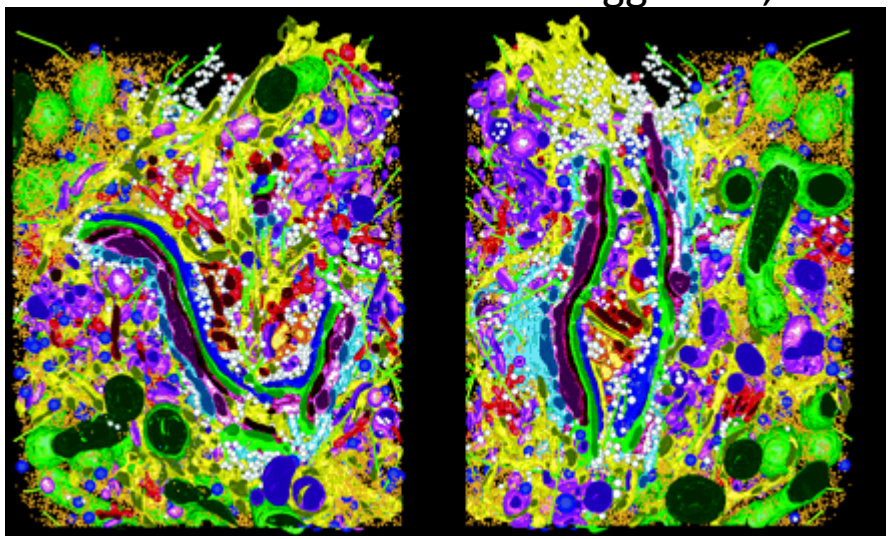
Tomography

Baumeister, 2004

TEM

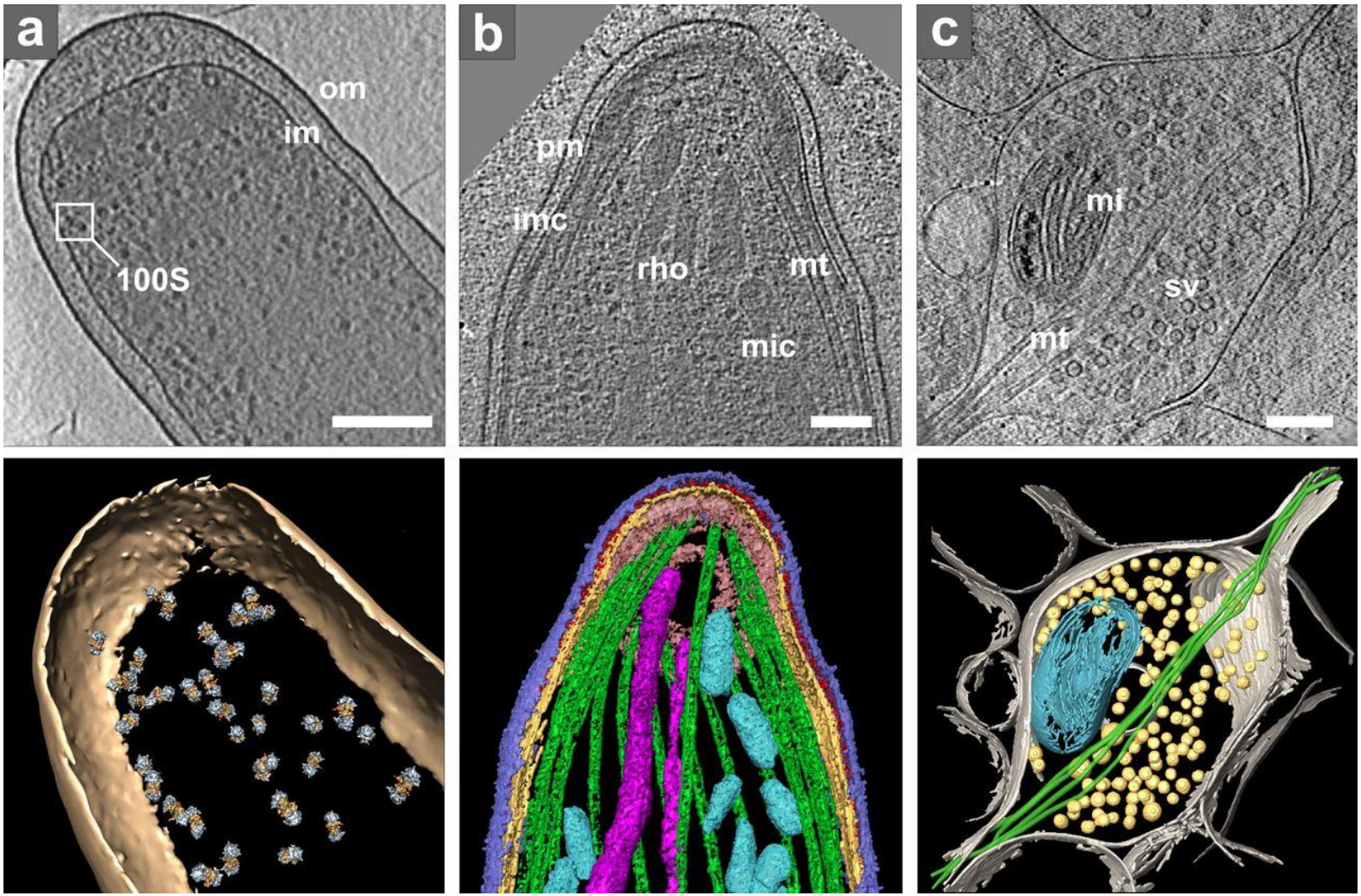


Briggs et al, 2006



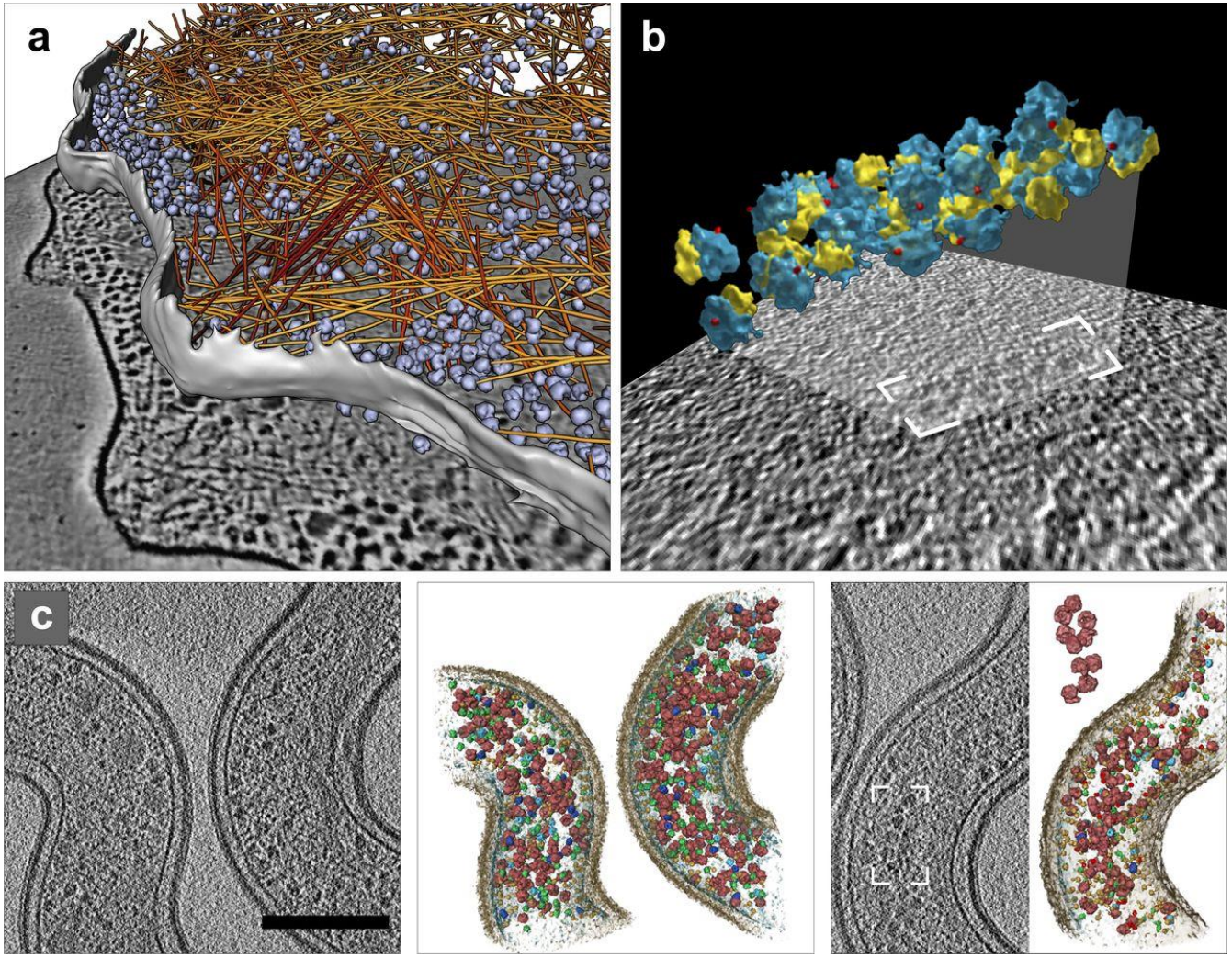
Marsh *et al, 2001*

Cryo-ET on whole cell or sections



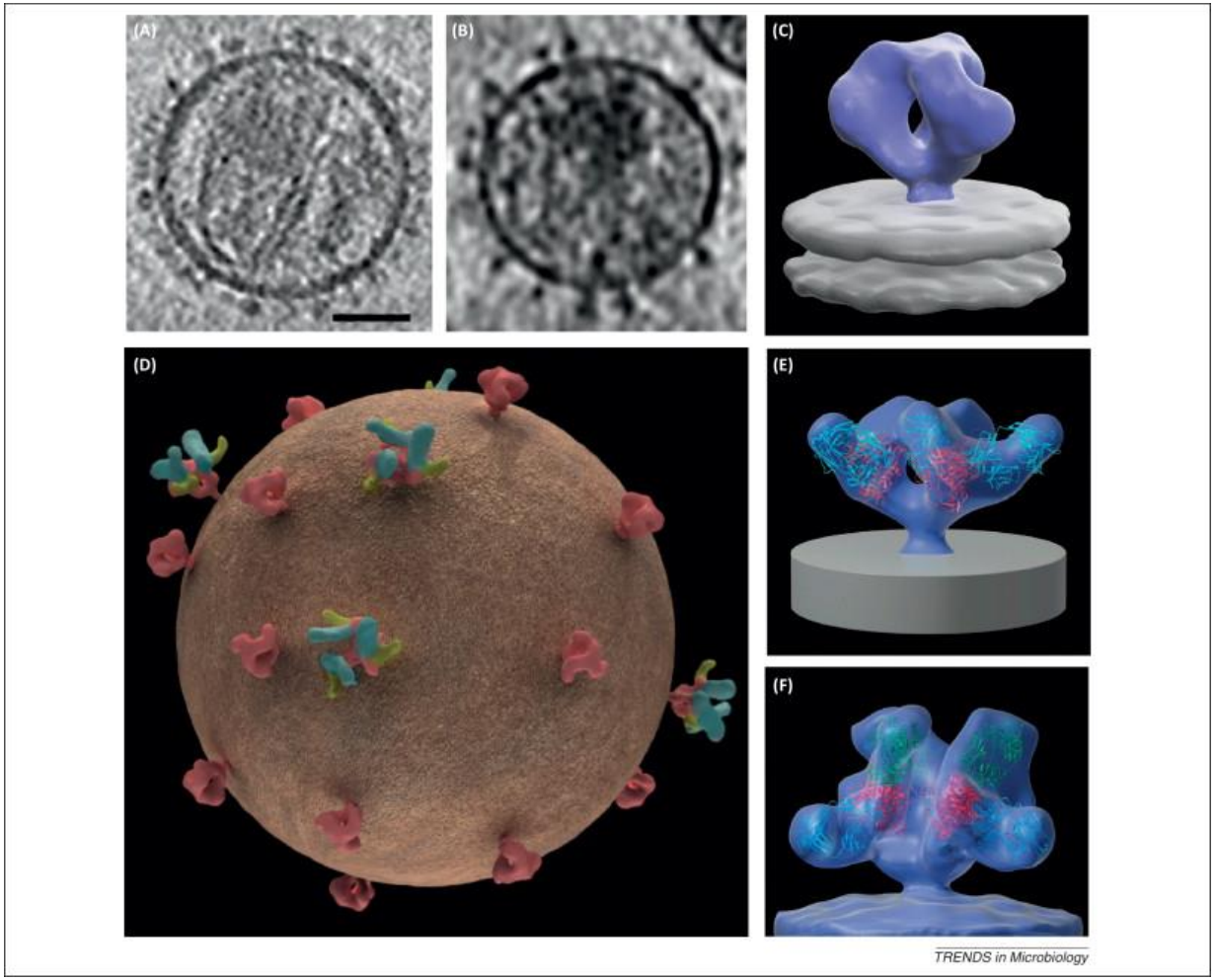
Lučić et al, 2013

Segmentation – Template matching



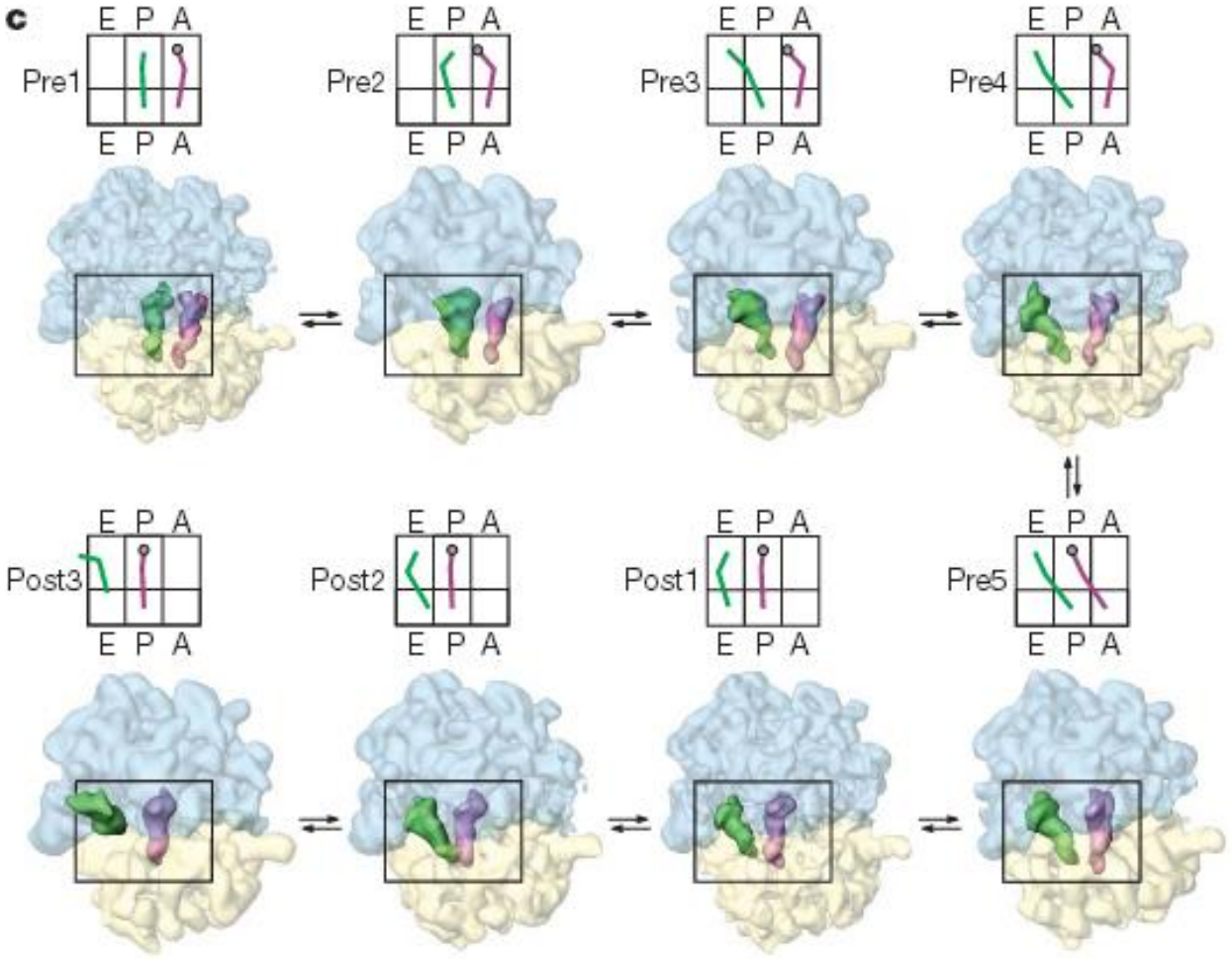
Lučić et al, 2013

Sub-tomogram averaging



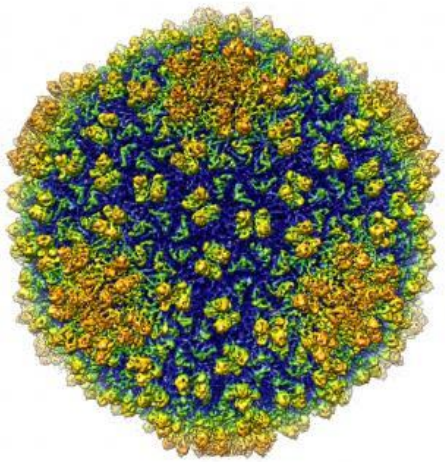
Earl et al, 2013

Dynamic of a mechanism



Fischer et al, 2010

Structure of a protein complex obtained by electron microscopy



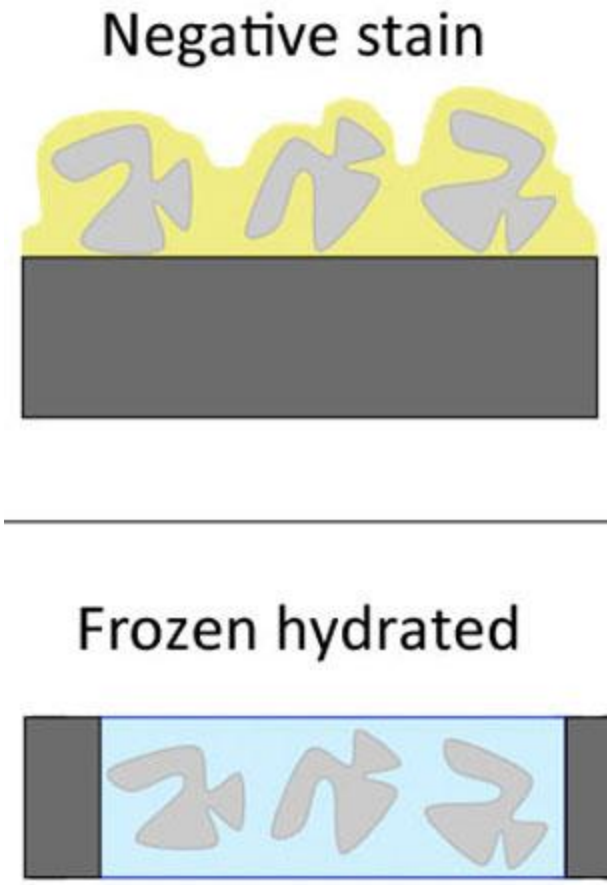
Advantages :

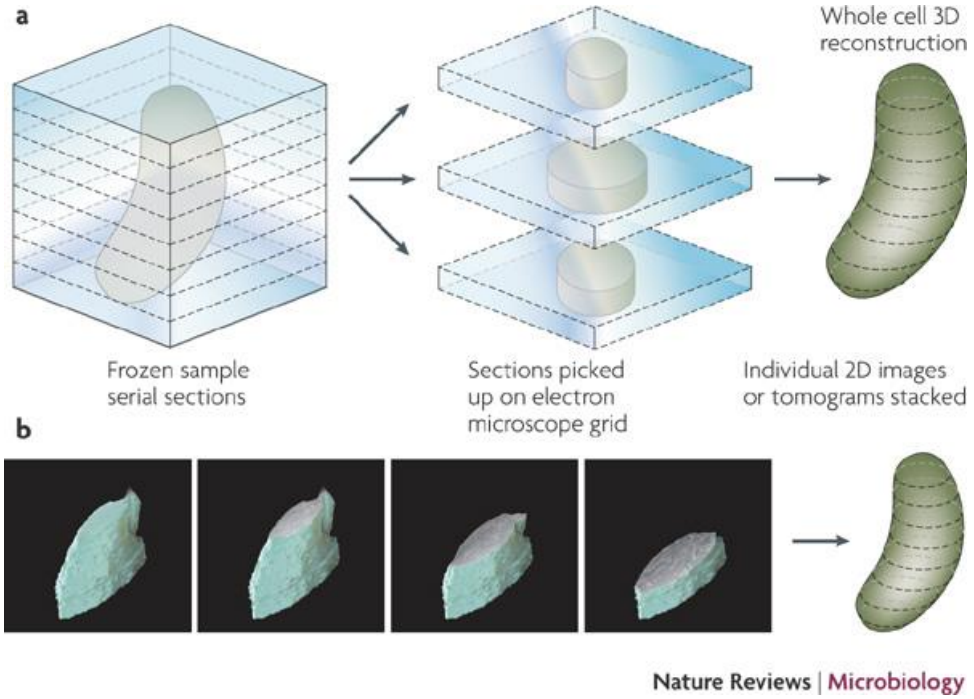
- 3D structure of the protein complex
- (very) large complexes
- No crystal needed !
- Small amount of material

Drawbacks :

- Size limit (>100-300 kDa)
- High computing power
- Signal/noise ratio very low
- Dose /contrast compromise

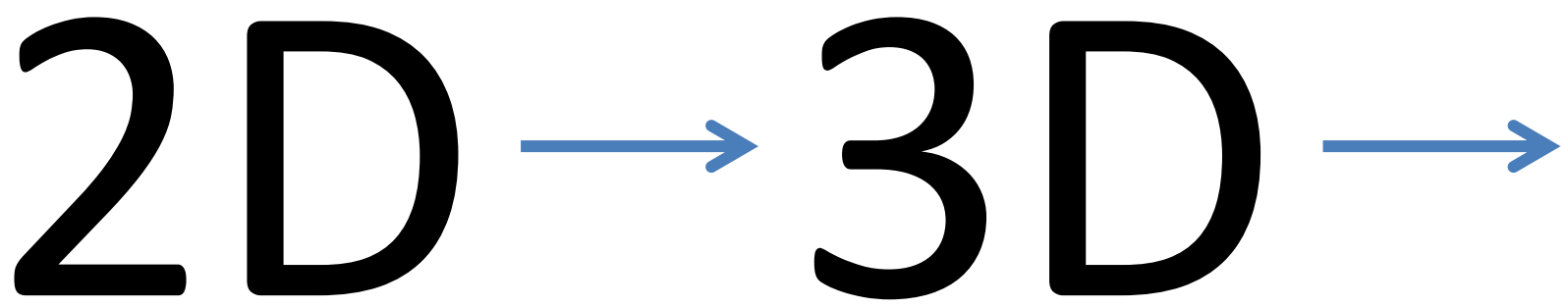
Sample preparation





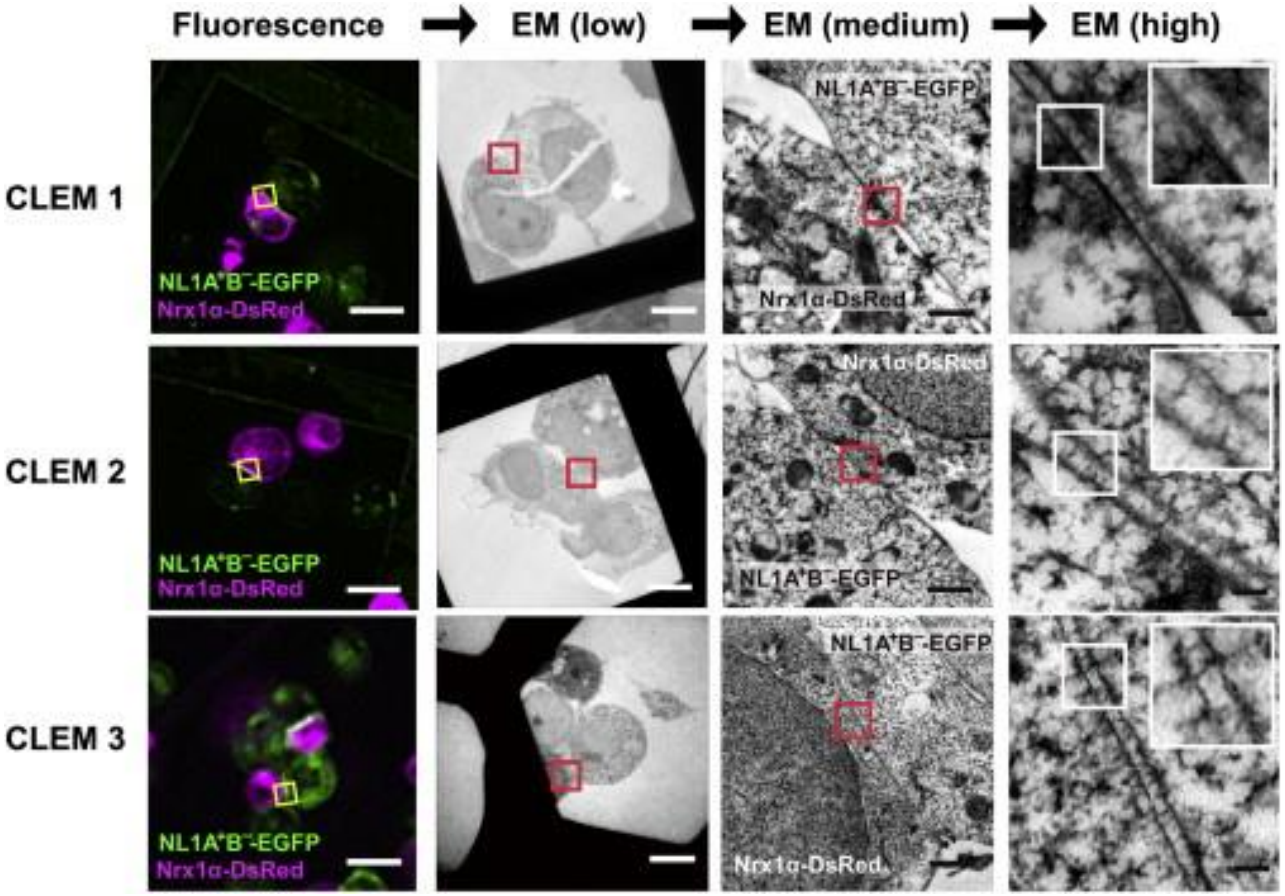
Durand et al, 2013

Data processing



CLEM

Correlative light-electron microscopy



Tanaka et al, Cell Reports, 2012

Questions ?