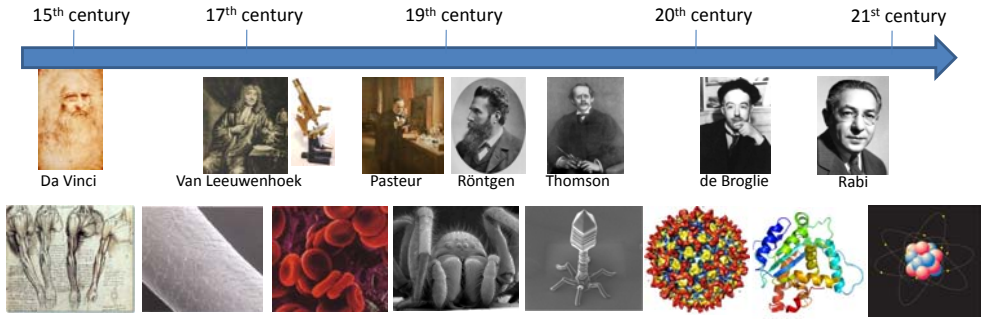
Que peut-on voir avec un microscope électronique à transmission ?

What can we see with a TEM ?

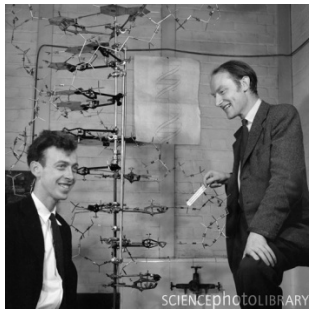
Pierre-Damien COUREUX
Ecole polytechnique
Rénafobis

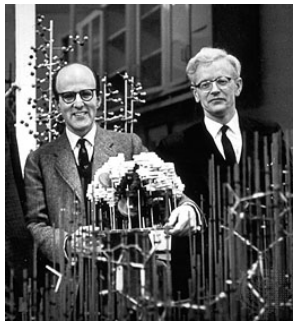
Oléron – 22 mai 2016

Origin of research in biology

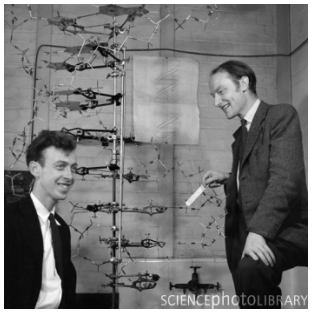


Structure/function studies

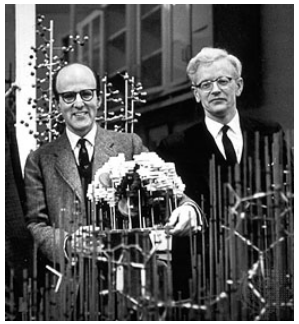




Structure/function studies



Watson/Crick (1953)

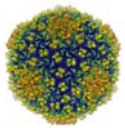


Perutz/Kendrew (1959)

Structural biology today

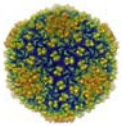
X-ray crystallography 

Nuclear magnetic resonance (NMR) 

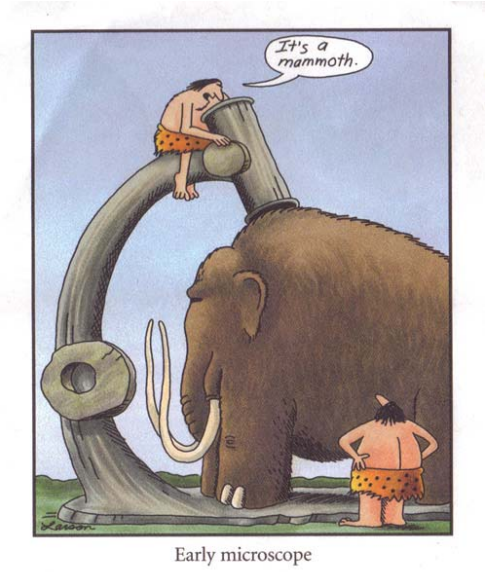
Electron microscopy (EM) 

SAXS, …

Electron microscopy



History

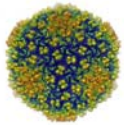


Early microscope

1600-1700

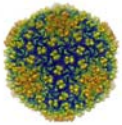

Van Leeuwenhoek

Electron microscopy History

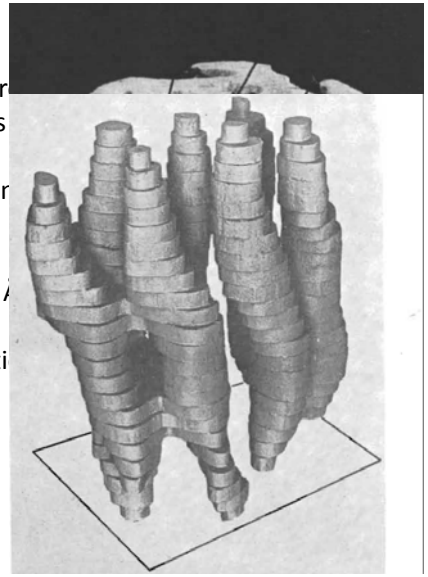


DATES	NAMES	HIGHLIGHTS
1897	J.J. Thomson	Electron discovery
1924	L. de Broglie	Identification of electron wavelength in movement
1926	H. Busch	Characterization of lens effects on magnetic and electric fields with electrons
1929	E. Ruska	Ph.D on magnetic lenses
1931	Knoll & Ruska	Building the first electron 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

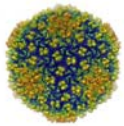


DATES	NAMES	Highlights
1960-1970	France/Japon	High voltage micr material sciences
1968	de Rosier et Klug	3D reconstructior from EM images
1975	Unwin and Henderson	3D structure at 7
1981	Dubochet et al	Sample preparati



EM in the 1990-2000s

For what for?

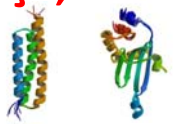


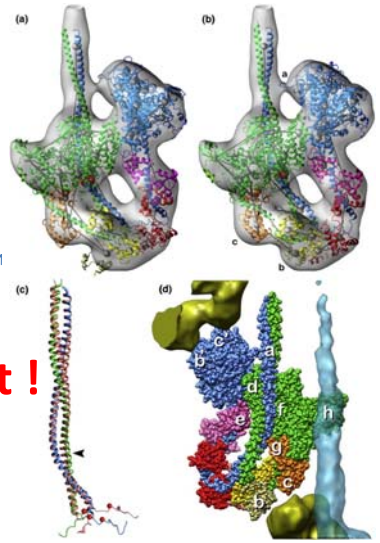
To combine structural informations from other methods (X-ray crystallography, NMR, SAXS, MS…) to link the structure and function of a protein (or a macromolecular complex)

X-ray crystallography

Mais ça, c'était avant !

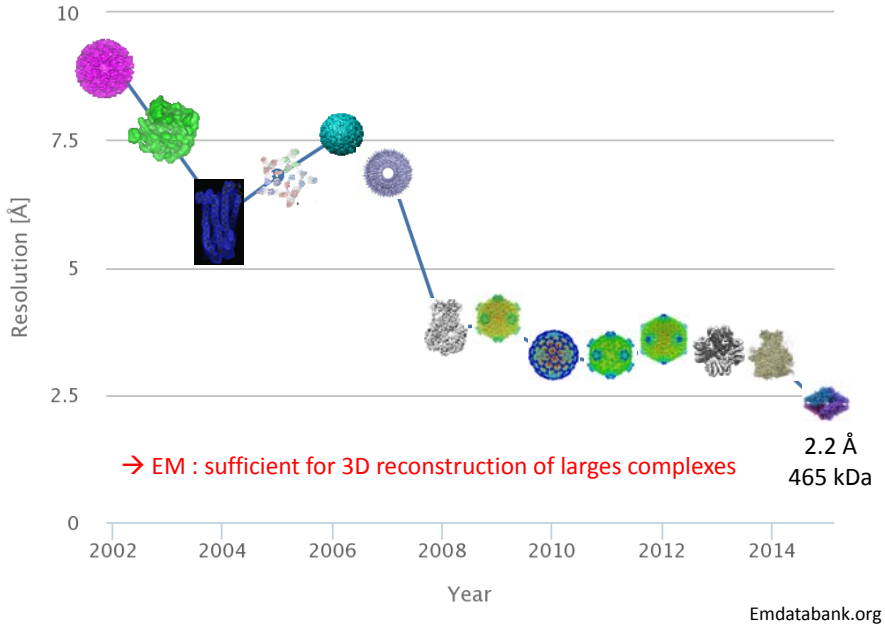
NMR





Alamo et al, 2008

Resolution trends

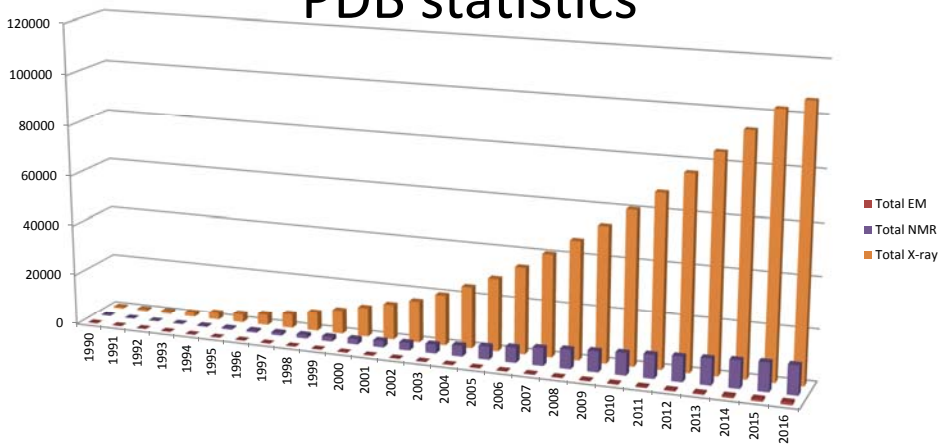


→ EM : sufficient for 3D reconstruction of larges complexes

2.2 Å
465 kDa

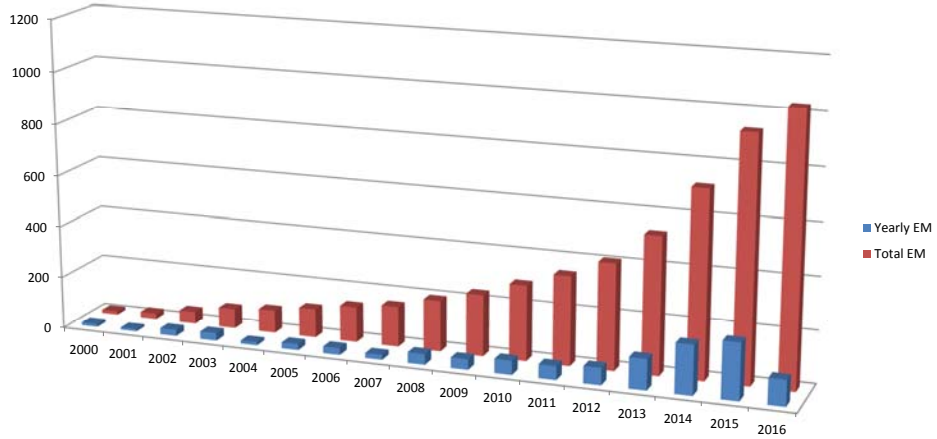
Emdatabank.org

PDB statistics



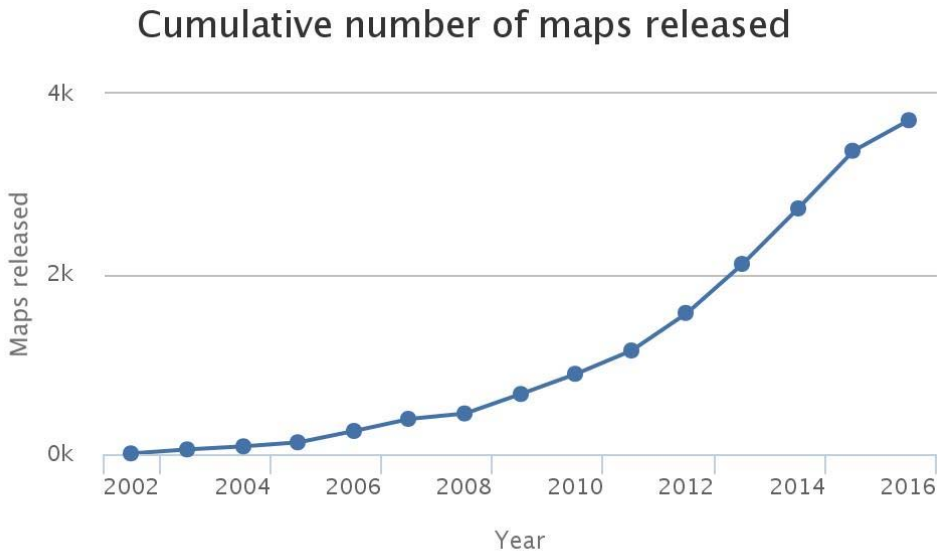
89% X-ray crystallography ⟷ **10%** NMR ⟷ **1%** EM

PDB statistics



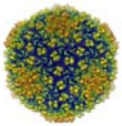
89% X-ray crystallography ⟷ **10%** NMR ⟷ **1%** EM

EMDB statistics



www.emdatabank.org

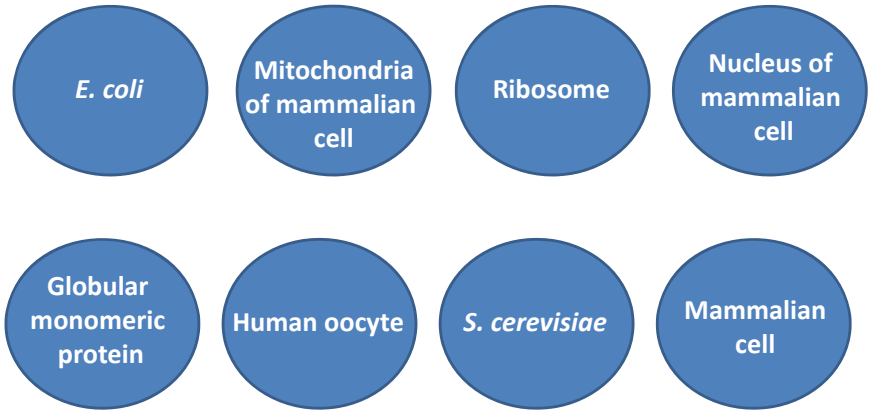
Electron microscopy



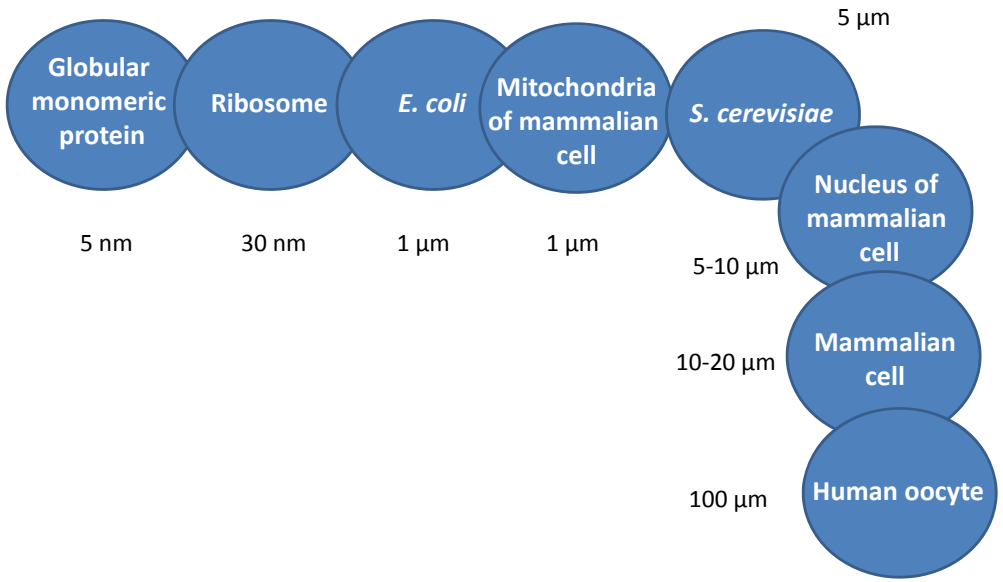
- What type of biological material can we study (cell, protein, atom) ?
- Difference optical microscopy vs electronic microscopy ?
- Which microscopy ? For what for?
- Composition of an electron microscope
- What structural informations can you get?

Size of objects

Size of objects



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:

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

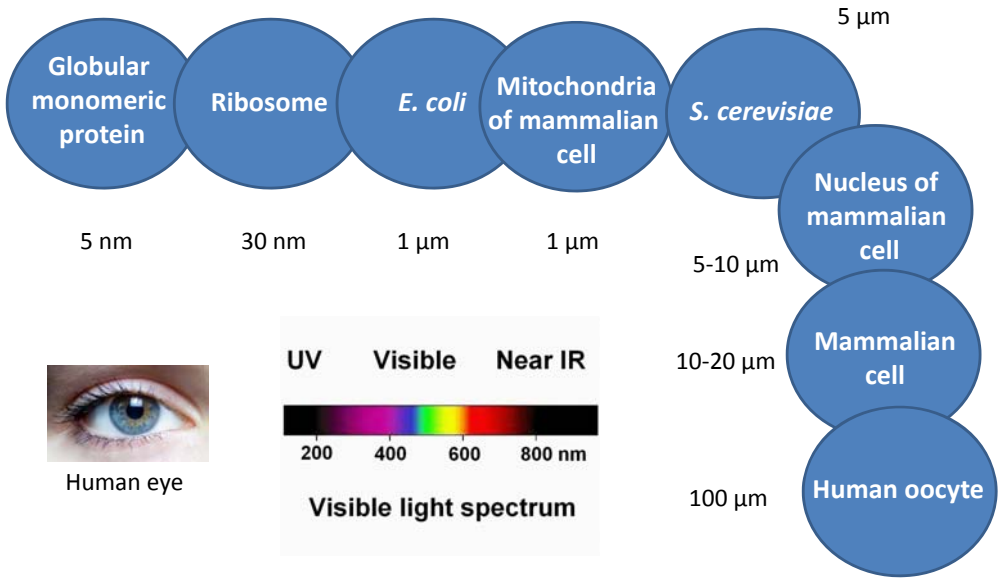
Numerical aperture of the lens

distance

Refraction index of studied matter

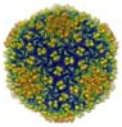
To simplify: $d \approx 0.5\ \lambda$

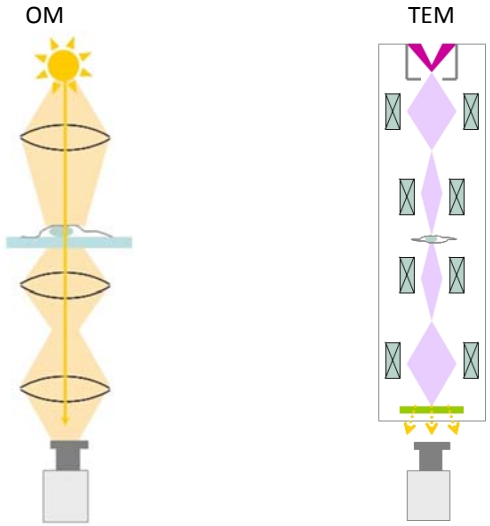
Size of objects



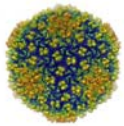
Human eye

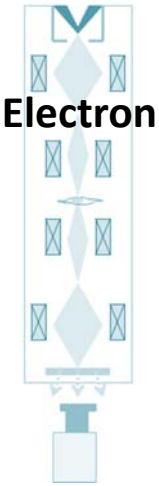
Optical vs electron microscope





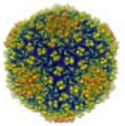
Particules sources

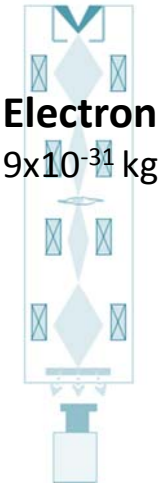




	Photon	**Electron**
Mass		
Charge		
Speed		

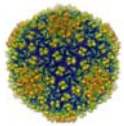
Particules sources

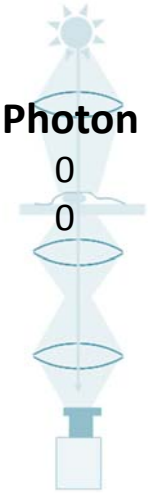


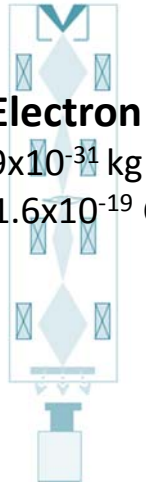


	Photon	**Electron**
Mass	0	$9x10^{-31}$ kg
Charge		
Speed		

Particules sources

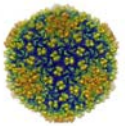


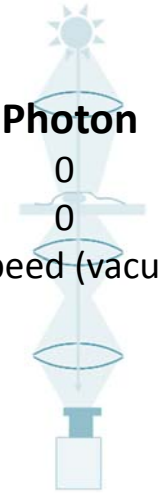


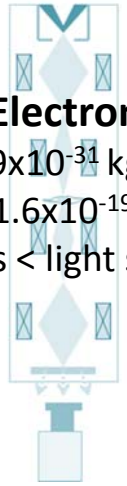


	Photon	**Electron**
Mass	0	$9x10^{-31}$ kg
Charge	0	$-1.6x10^{-19}$ C
Speed		

Particules sources





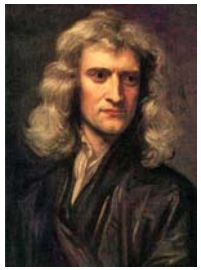


	Photon	**Electron**
Mass	0	$9x10^{-31}$ kg
Charge	0	$-1.6x10^{-19}$ C
Speed	light speed (vacuum)	$0 \leq s <$ light speed

Wave-particle duality

Relation Acceleration voltage - wavelength

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



Isaac Newton

Louis de Broglie

Albert Einstein

Wave-particle duality

Relation Acceleration voltage - wavelength

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

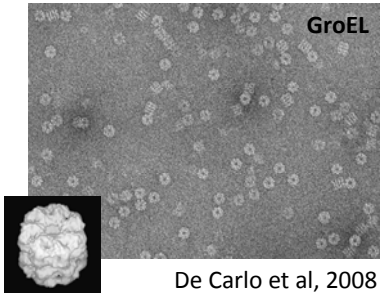
$$\lambda = \frac{1.23}{\sqrt{V + 10^{-6}V^2}}\,nm$$

Wave-particle duality

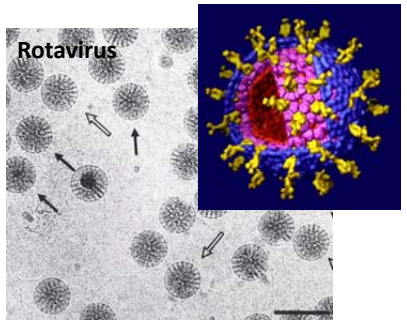
Relation Acceleration voltage - wavelength

	V	λ (nm)	Theoretical resolution (nm)
	10,000	0.01223	0.00611
	50,000	0.00536	0.00268
80-300 kV	100,000	0.00370	0.00185
	1,000,000	0.00086	0.00043

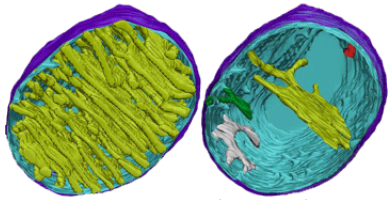
What can we see with a TEM?



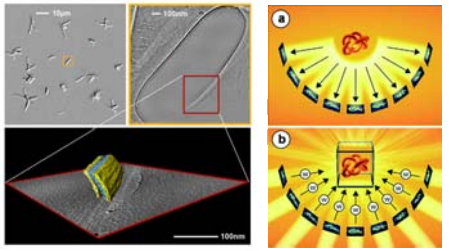
De Carlo et al, 2008



Tihova et al, 2001



Perkins et al, 1997



Cryo-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.

2016 – Method of the year

SPECIAL FEATURE | COMMENTARY METHOD OF THE YEAR

How good can cryo-EM become?

Robert M Glaeser

The suddenness with which single-particle cryo-electron microscopy (cryo-EM) has emerged as a method for determining high-resolution structures of biological macromolecules invites the questions, how much better can this technology get, and how fast is that likely to happen? Though we can rightly celebrate the maturation of cryo-EM as a high-resolution structure-determination tool, I believe there still are many developments to look forward to.

While cryo-EM now produces very exciting biological results by enabling the determination of atomic-resolution macromolecular structures[1], the outcome is still quite far from what physics would allow. Ultimately, the amount of image

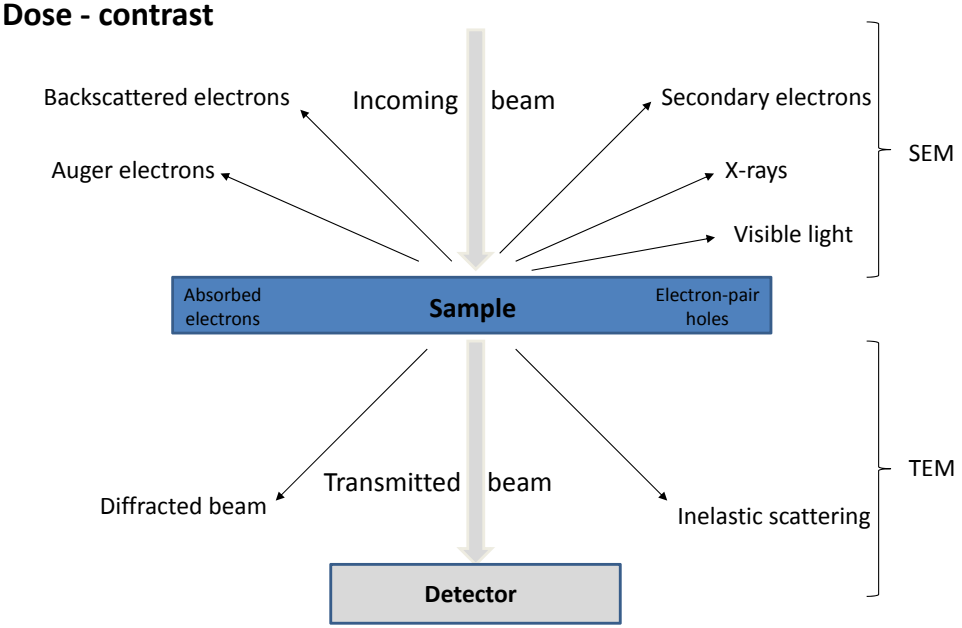
avoid having the transmitted electrons be scattered multiple times) but not less than the size of the particle itself, typically 10–30 nm. Preparation of such thin samples is currently achieved by placing a few microliters of the specimen onto a hydrophilic EM

may be so severe that the project cannot go forward. When this happens, one is forced to ask what the root cause might be.

One possibility is that the specimen may have been less homogeneous than was initially thought. In this case further biochemi-

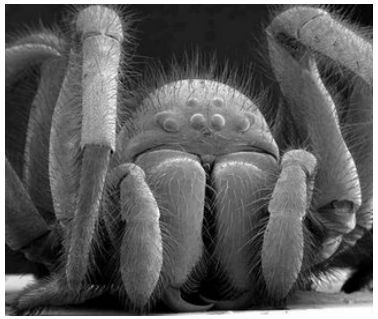
How is an image formed?

Dose - contrast



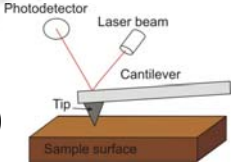
Electron microscopy types

Scanning electron microscopy (SEM)

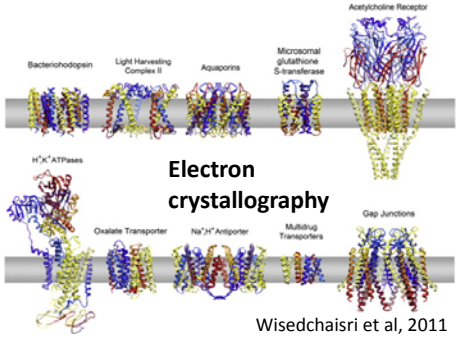


Transmission electron Microscopy (TEM)

≠ Atomic force microscopy (AFM)

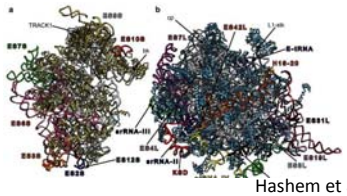


Fields in transmission electron microscopy



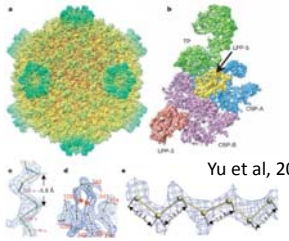
Electron crystallography

Wisedchaisri et al, 2011



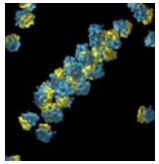
Hashem et al, 2013

Single particles with low degree of symmetry



Yu et al, 2008

Single particles with high degree of symmetry

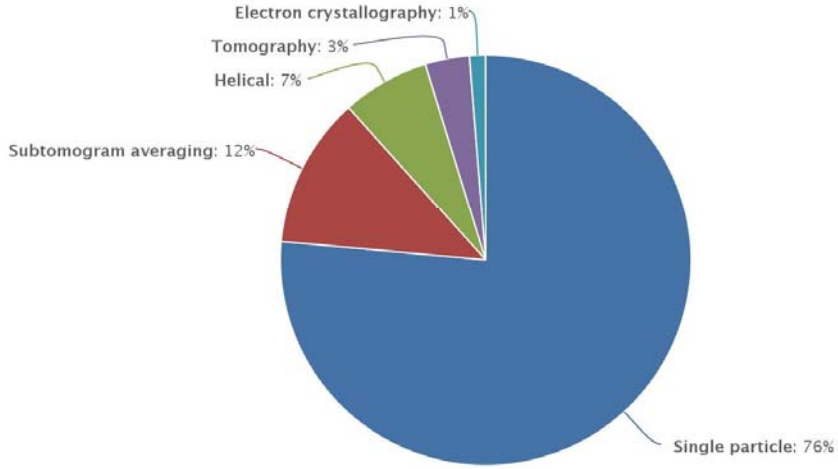


Brandt et al, 2010

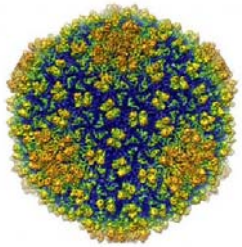
Tomography

Fields in transmission electron microscopy

Distribution of released maps (3706 in total) as a function of technique used



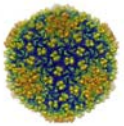
Structure of a protein complex obtained by electron microscopy



<u>Advantages :</u>

- 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

How does a modern TEM look like ?

JEOL -3200 FSC (Japon)

FEI-Titan Krios (USA)

Composition of a TEM

Electron gun

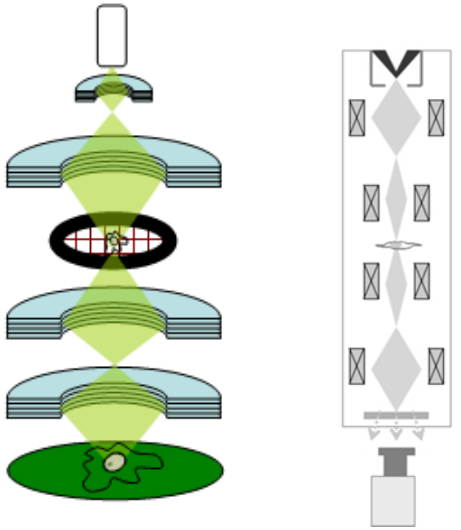
Condenser lens

Sample grid

Objective lens

Projection lens

Phosphor screen



Composition of a TEM

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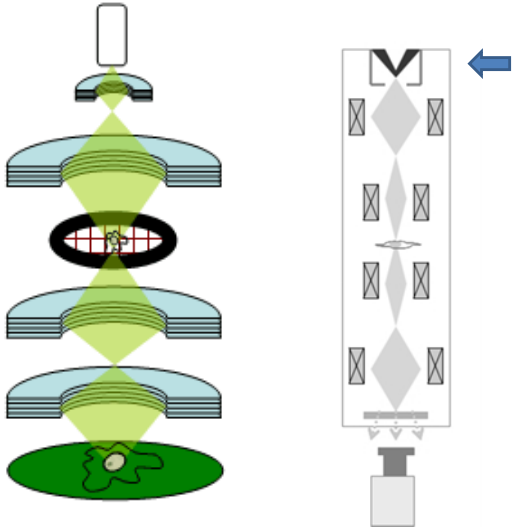
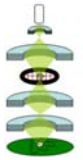
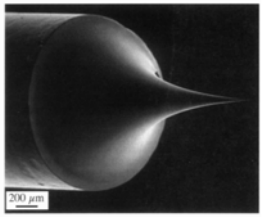


Source of electrons

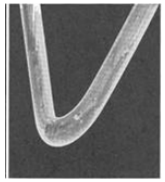


- Thermoionic gun

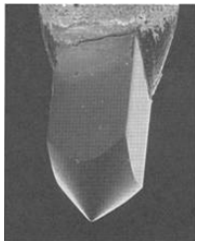
- Field emission gun (FEG)



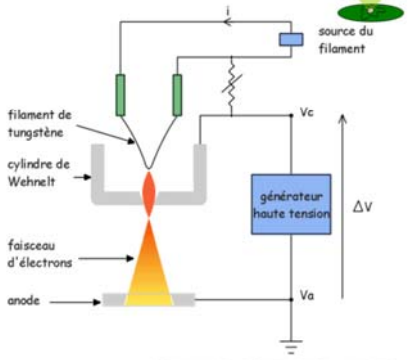
Thermoionic gun

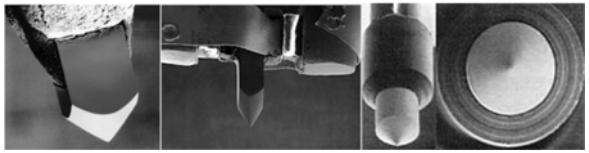


(W)

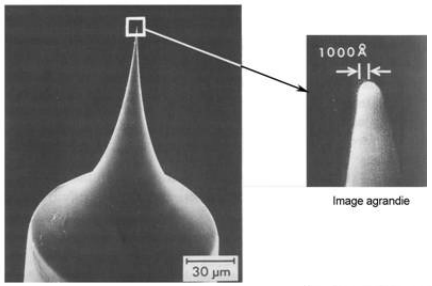


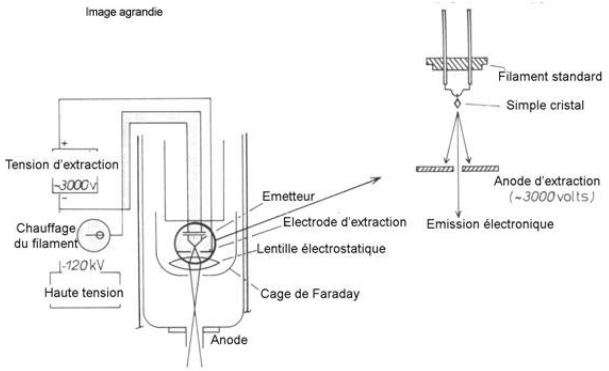
(LaB_6 ou CeB_6)





Field emission gun (FEG)





Composition of a TEM

Electron gun

Condenser lens

Sample grid

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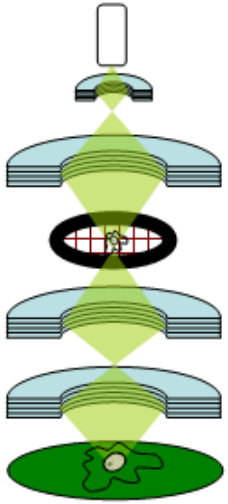
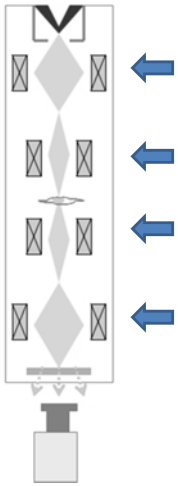
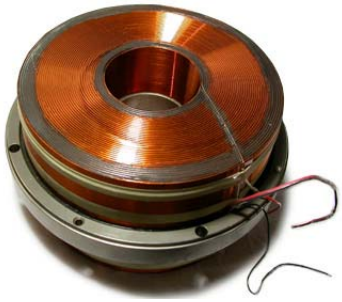
Phosphor screen

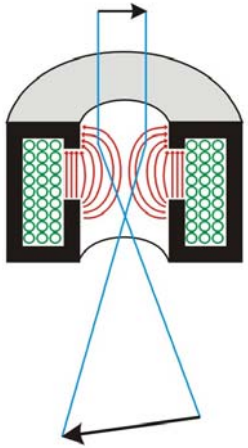




CTF

Electromagnetic lens



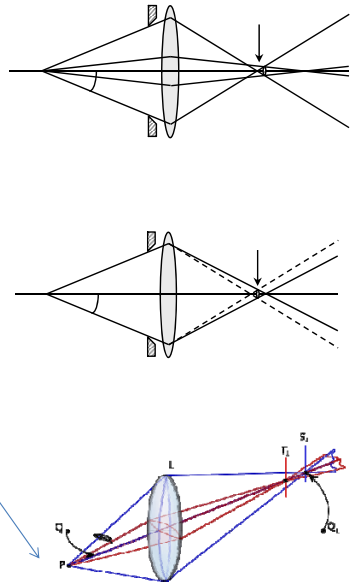


Microscope drawbacks

And solutions…

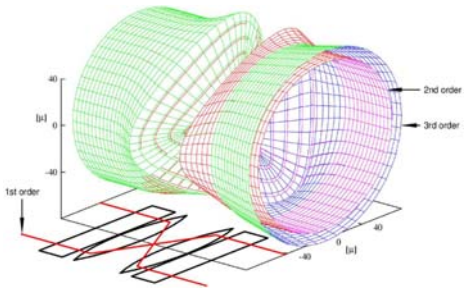
Lens:

- Spherical aberration
- Chromatic aberration
- Astigmatism

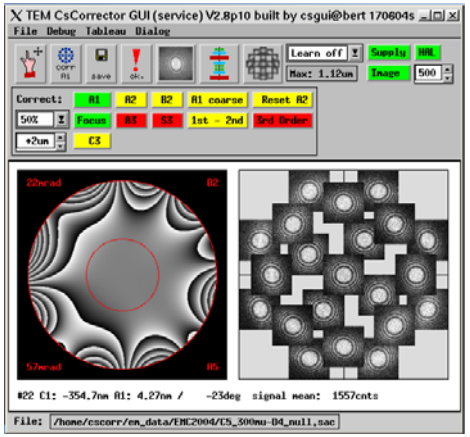


Lens spherical aberration

Correction



http://salve-project.de



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

Composition of a TEM

Electron gun

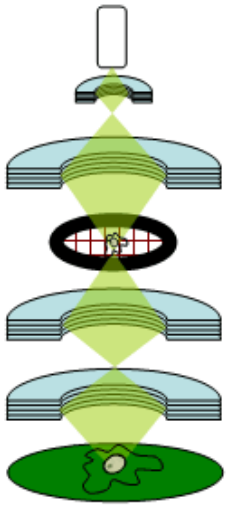
Condenser lens

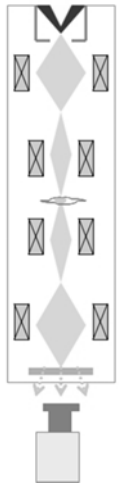
Sample grid

Objective lens

Projection lens

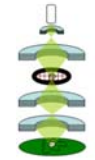
Phosphor screen





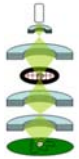
Sample holder

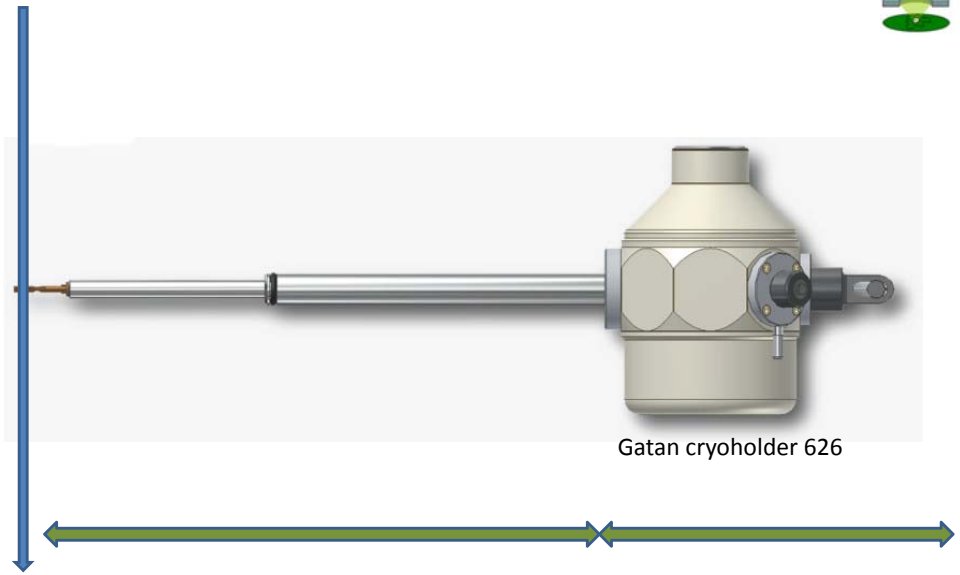
3 mm



Gatan cryoholder 626

Sample holder





Gatan cryoholder 626

Inside the microscope

Outside the microsocpe

Microscope drawbacks

And solutions…

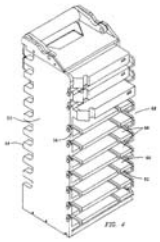
Lens:

- Spherical aberration
- Chromatic aberration
- Astigmatism

Stability:

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

Autoloader (FEI)



Composition of a TEM

Electron gun

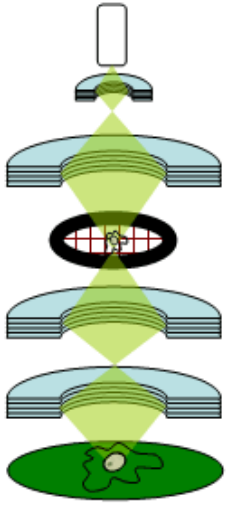
Condenser lens

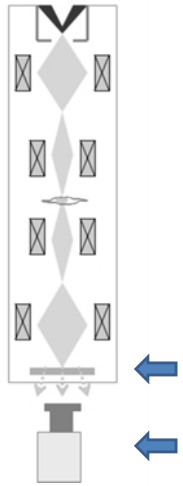
Sample grid

Objective lens

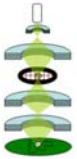
Projection lens

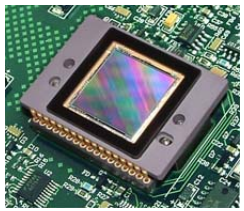
Phosphor screen





Visualization





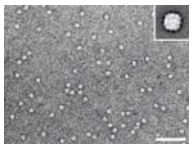
Screen

Negatives

Digital detector

Digitalization of images

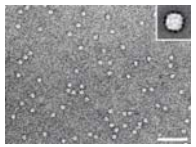
Film sensitive to electrons



Pixel size = 8 µm
1 image / s

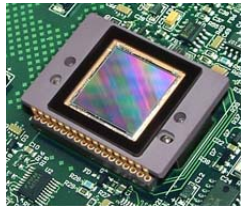
Digitalization of images

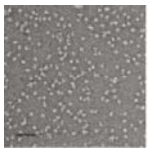
Film sensitive to electrons



Pixel size = 8 µm
1 image / s

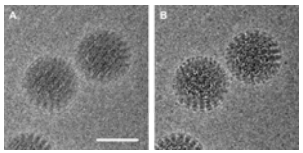
CCD





14 µm
1 image / s

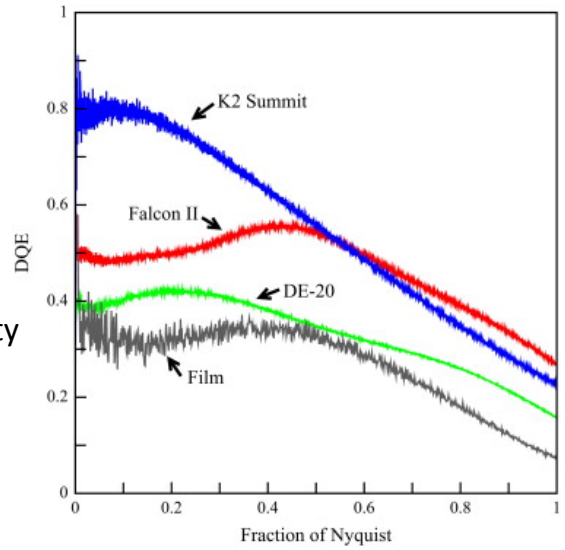
DED



5 µm
20 images / s

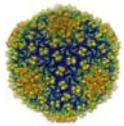
Sensitivity Film vs Digital detector

Detective quantum efficiency ≈ Sensitivity



McMullan et al, 2014

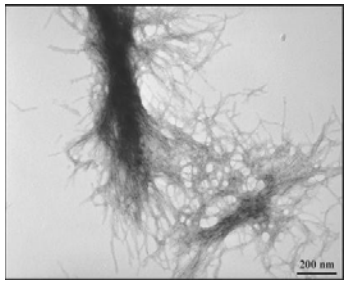
Electron microscopy



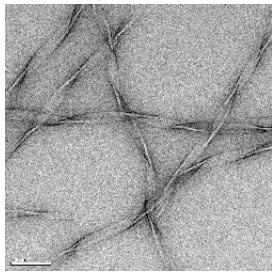
What structural informations
can you get ?

Qualitative analysis

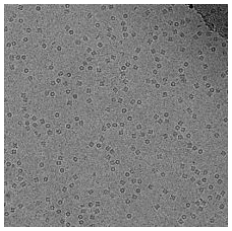
TEM



Agreggation



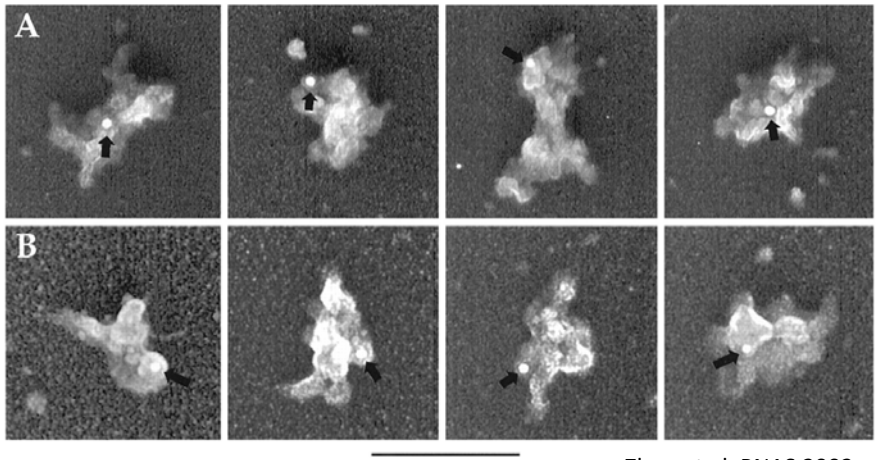
Filaments



Soluble protein

Localize a protein in a complex

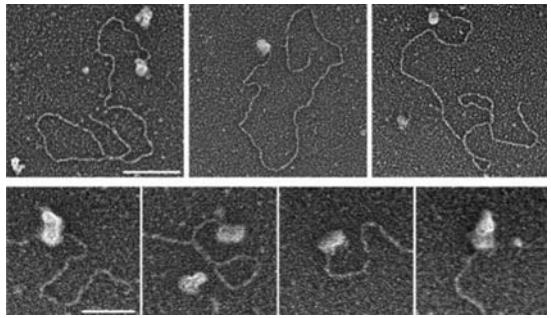
Human spliceosome (SEM)



Zhou et al. PNAS 2002

DNA-proteins interactions

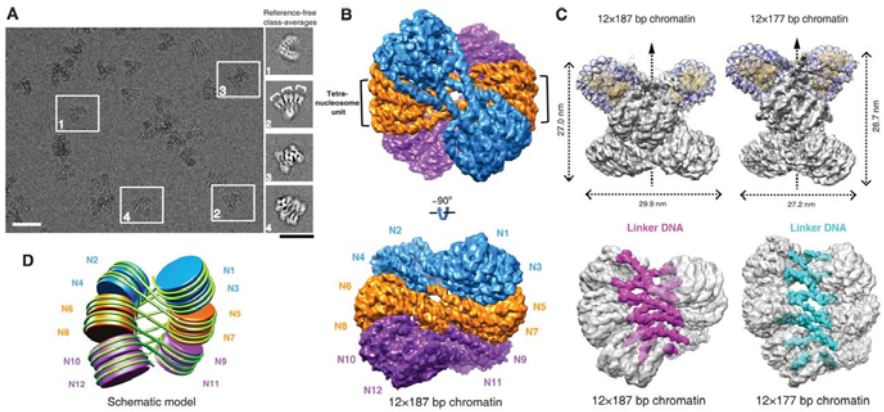
SEM



Thorslund et al, 2010

DNA-proteins interactions

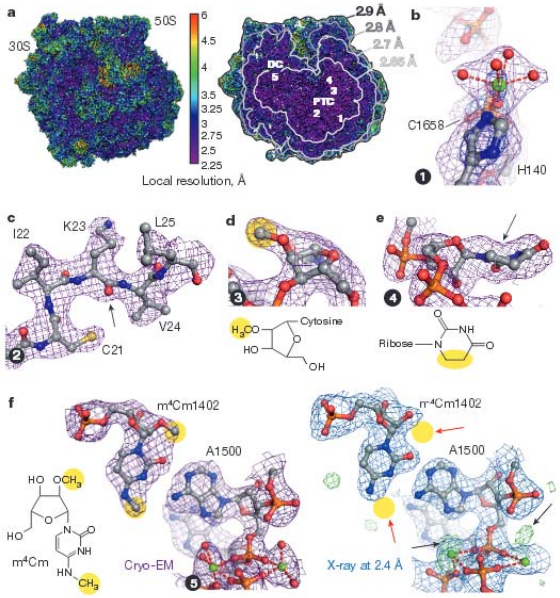
TEM



Song et al, 2014

RNA-proteins interactions

TEM

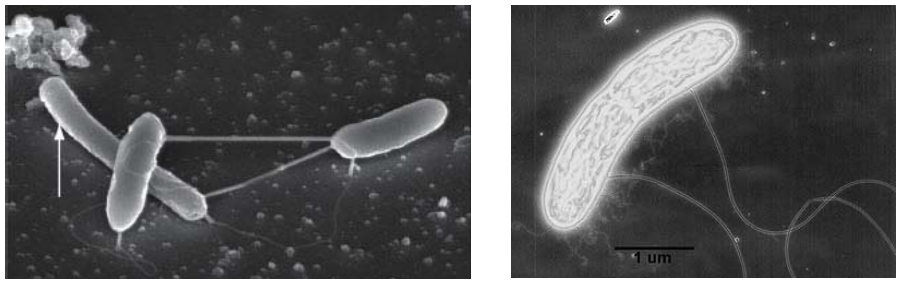


< 3 Å résolution

Fischer et al, Nature, 2015

Nanowires

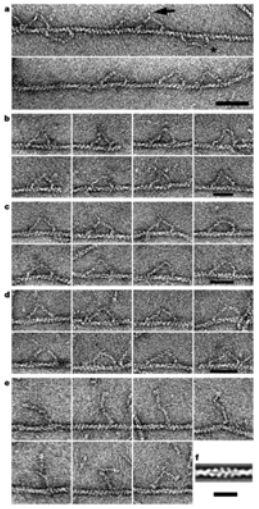
SEM & TEM



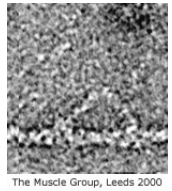
Geobacter metallireducens

Conformational changes

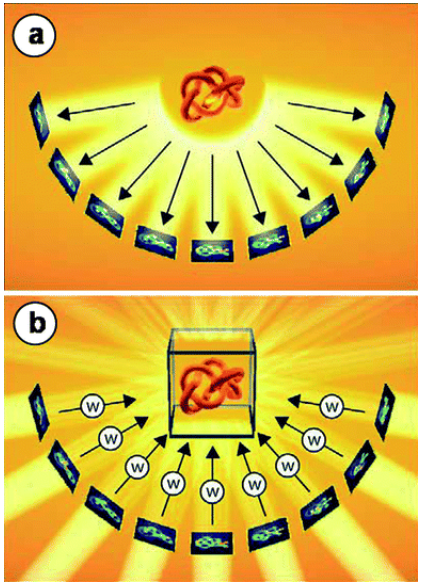
TEM



Walker et al, Nature, 2000

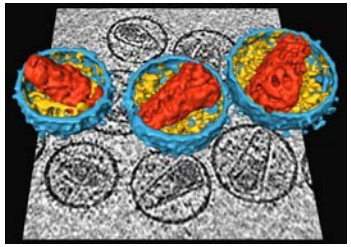


Tomography

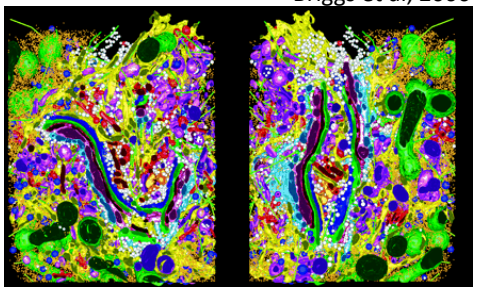


Baumeister, 2004

TEM

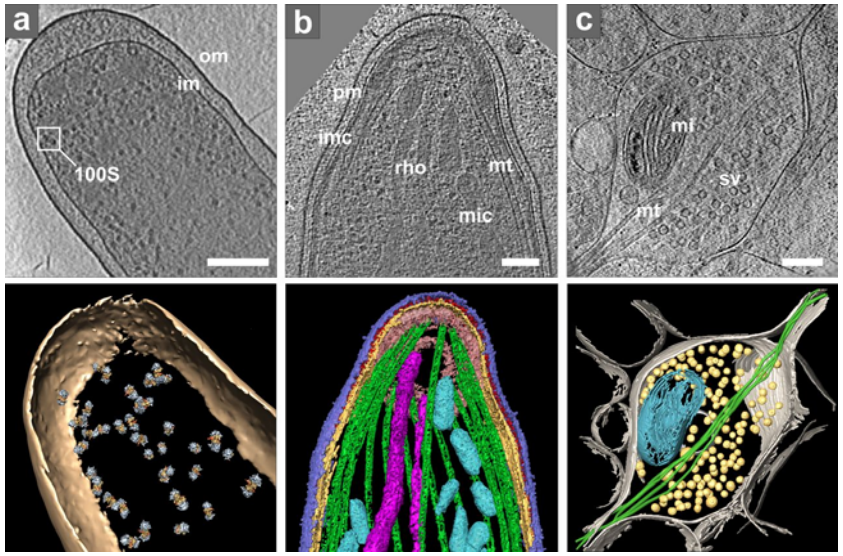


Briggs et al, 2006



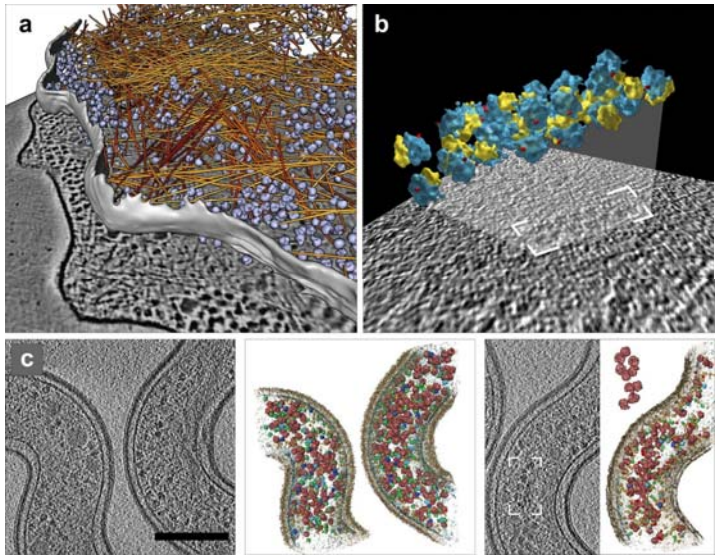
Marsh *et al, 2001*

Cryo-ET on whole cell or sections



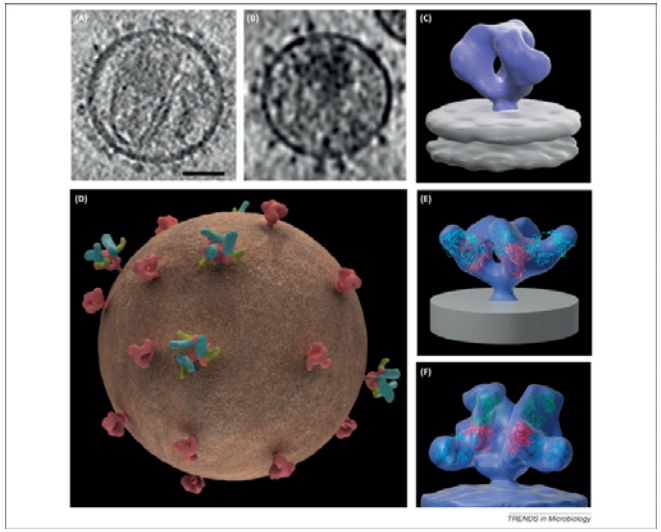
Lučić et al, 2013

Segmentation – Template matching



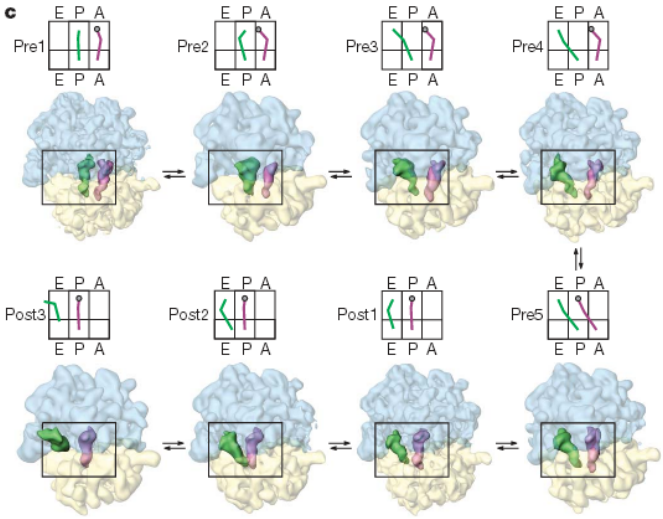
Lučić et al, 2013

Sub-tomogram averaging



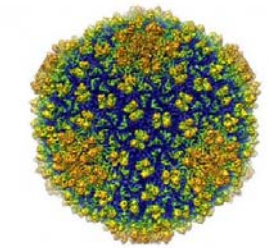
Earl et al, 2013

Dynamic of a mechanism



Fischer et al, 2010

Structure of a protein complex obtained by electron microscopy



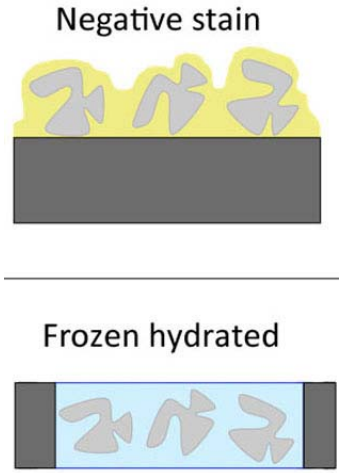
Advantages :

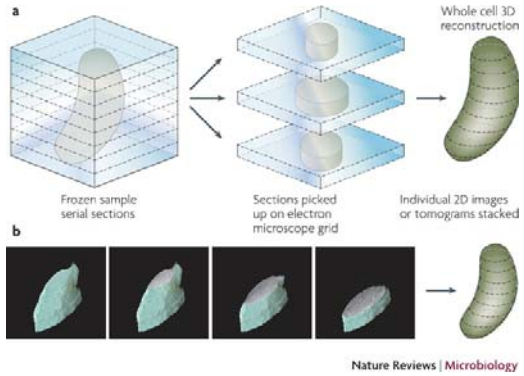
- 3D structure of the protein complex
- (very) large complexes
- No crystal needed !
- Small amount of material

Drawbacks :

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

Sample preparation

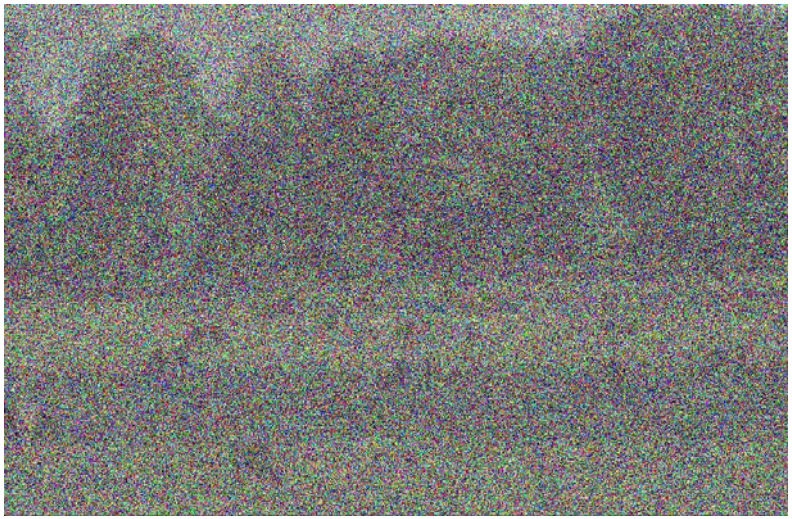




Durand et al, 2013

Signal/noise ratio (>>1)

Signal/noise ratio (≈1)



Drawbacks

Sample

- Dose – contrast
- Noise
- BIM

Microscope

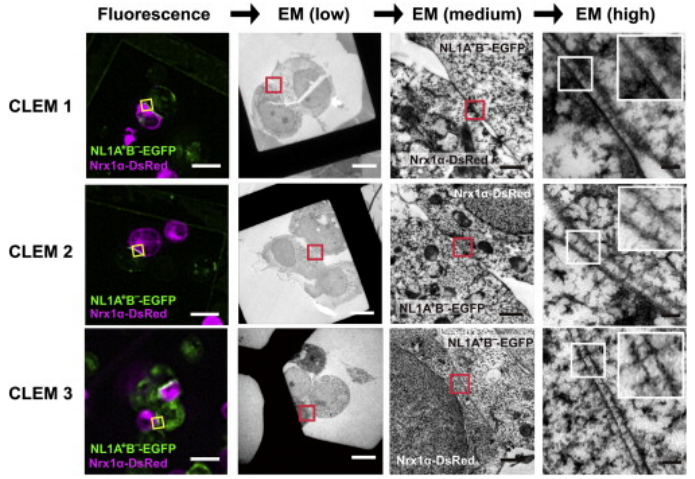
- Spherical aberration
- Chromatic aberration
- Astigmatism
- Signal modulation (CTF)

Data processing

2D → 3D →

CLEM

Correlative light-electron microscopy



Tanaka et al, Cell Reports, 2012

Questions ?