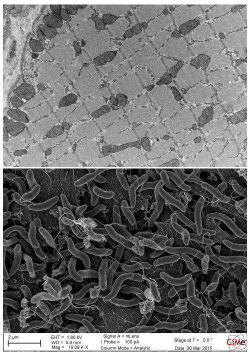
An Introduction to Transmission and Scanning Electron Microscopy

Graham Knott, BioEM Facility, Centre of Electron Microscopy, EPFL, Lausanne



BIOENG - 518

An Introduction to Transmission and Scanning Electron Microscopy

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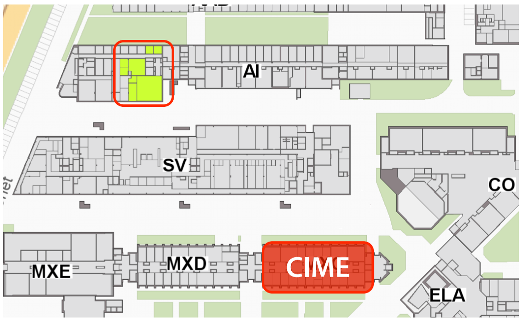
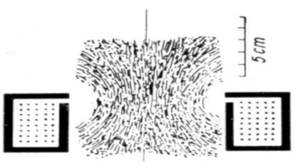
1. Electron lenses
2. Layout of the electron microscope
3. Generation of a TEM image
4. Generation of an SEM image



Magnetic fields could be used as lenses



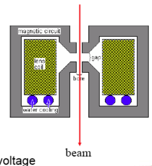
Electron optics was born in 1927, when Hans Busch showed that the elementary lens equation is applicable to short magnetic coils.

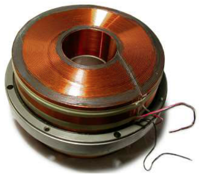
Magnetic fields could be used as lenses

- the focal length is given by:

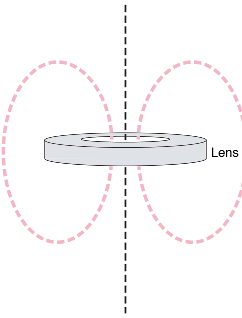
$$f = \frac{K \cdot U}{(N \cdot I)^2}$$

K : constant
U : accelerating voltage
N : windings
I : lens current

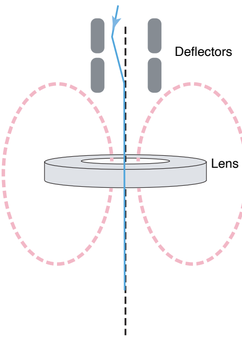




Components of an electron lens



Components of an electron lens



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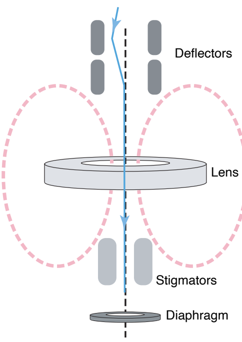

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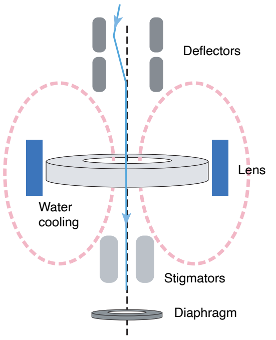
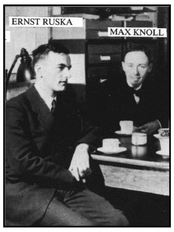
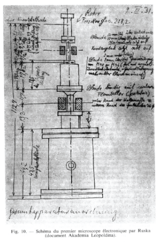


Ernst Ruska & Max Knoll 1932



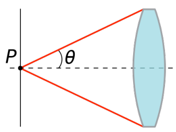


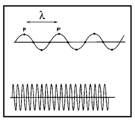
The resolving power of a microscope is proportional to λ/NA

λ = wavelength

NA = numerical aperture

NA = n sin θ (n = index of refraction)





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

$$v = \sqrt{\frac{2eU}{M_0}}$$

h = Planck's constant (6.624 X 10-27 erg/second)
m = mass of an electron (9.11 X 10-28 gram = 1/1837 of a proton)
v = velocity of the electron

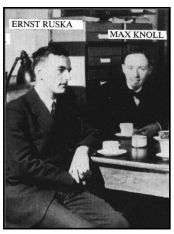
In electron microscopy the refractive index cannot exceed 1.0, the half angle is very small, and thus the only thing that can be adjusted is decreasing the wavelength of illumination

Beam of 100 KeV has a wavelength of 0.0389 - theoretical resolution of 0.0195 Angstroms

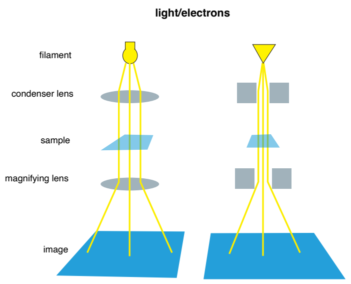
In reality most TEMs will only have an actual resolution of around 2.4 Angstroms at 100KeV

The First Electron Microscope

Ernst Ruska & Max Knoll 1932



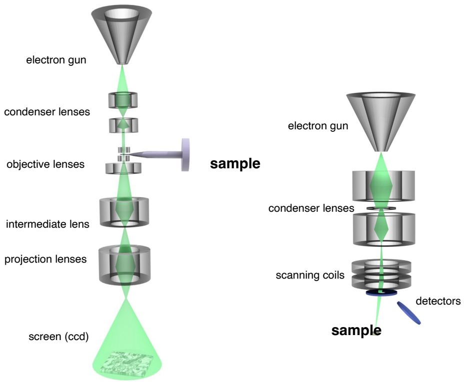
Light microscopy and TEM Analogy



EPFL

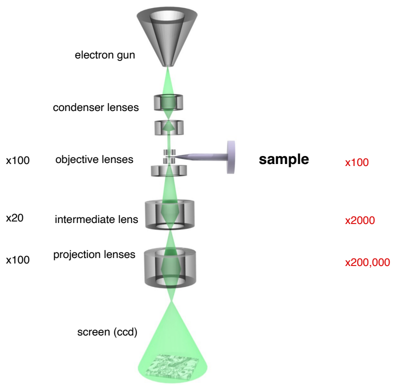
Transmission electron microscope

Scanning electron microscope

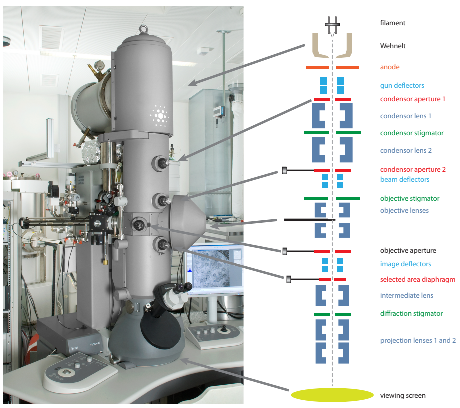


EPFL

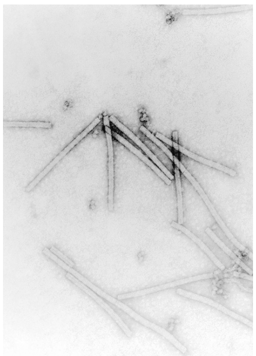
Transmission electron microscope



EPFL



EPFL



Helmut Ruska, 1939, imaged the tobacco virus

Technique - negative staining

-virus dried to a thin film

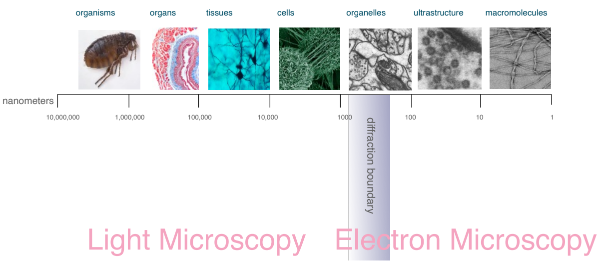
-solution of electron dense stain applied

-dried

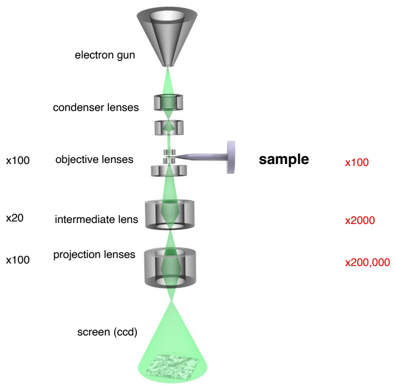
-imaged

Scale of biological structures

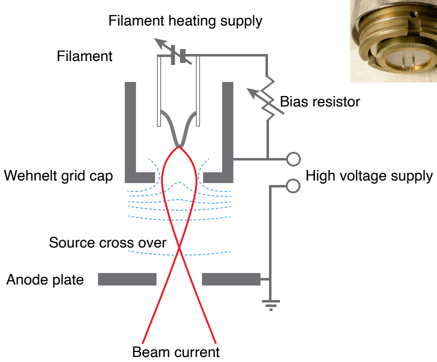
Why we need electron microscopes to see biological structures



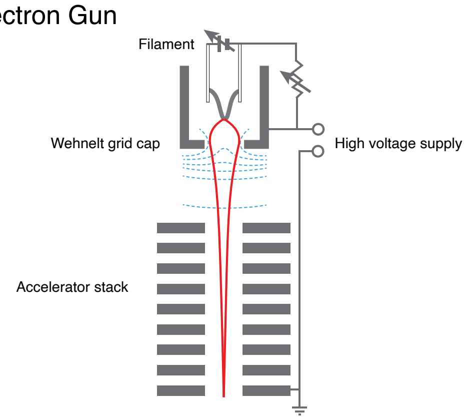
Transmission electron microscope



Electron Gun



Electron Gun

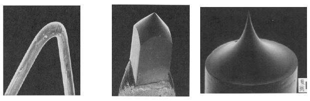


EPFL

Electrons need to be emitted from the same place and with the same speed (coherence)

- Spatial coherence - all electrons need to come from the same place
- Temporal coherence - all electrons need to have the same speed (same wave length)

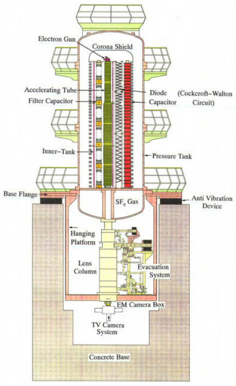
EPFL



Emission	Thermionic		Field emission
	tungsten	LaB6	FE
Current density (A/cm²)	2	100	10'000
Energy spread (eV)	1-5	0.5-3.0	0.2-0.3
Operating lifetime	>50	>500	>1000
Vacuum (torr)	10^{-4}-10^{-5}	10^{-6}-10^{-7}	10^{-9}-10^{-10}
Operating temperature (K)	2700	2000	300
Effective crossover size (nm)	50	10	0.01

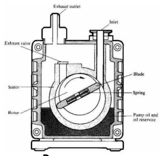
EPFL

3 million volt microscope, Osaka, Japan



EPFL

Vacuum Pumping Systems

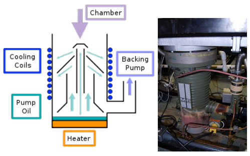


Rotary pump

10^{-1} - 10^{-2} mbar

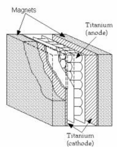
Turbomolecular pump

10^{-5} - 10^{-7} mbar



Oil diffusion pump

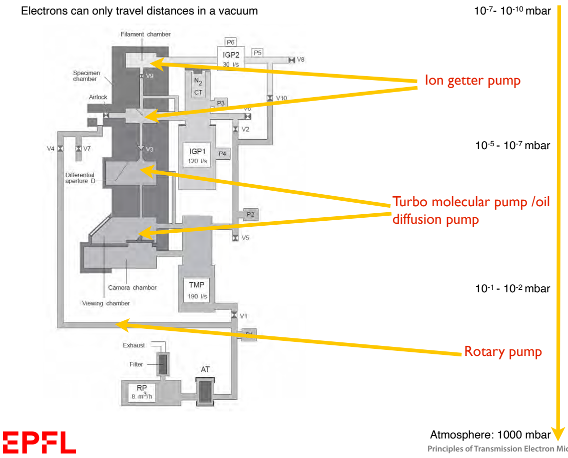
10^{-5} - 10^{-7} mbar

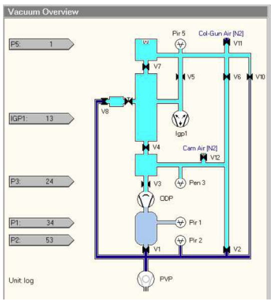


ion getter pump

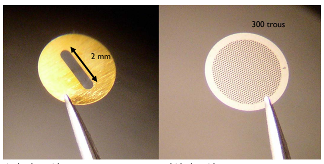
10^{-7}- 10^{-10} mbar

Electrons can only travel distances in a vacuum



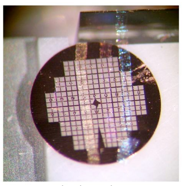


Typical EM grids for holding sections

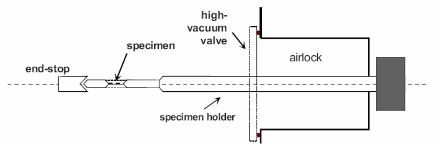


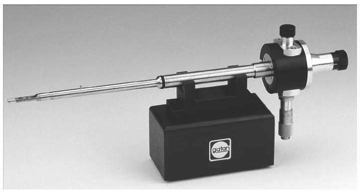
single slot grid

multi hole grid

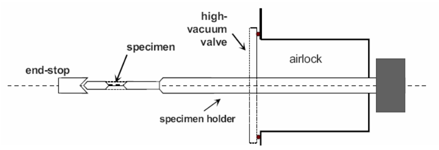


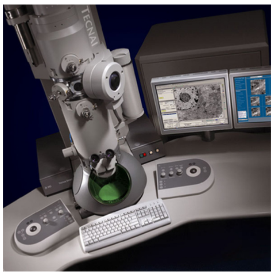
cryo sections placed on grid





Specimen size:
•3 mm in diameter!
•Ca. 100 nm in thickness
•(electron transparent





Specimen size:
•3 mm in diameter!
•Ca. 100 nm in thickness
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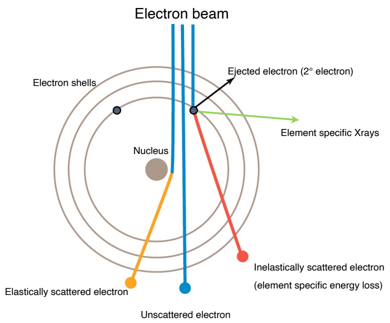
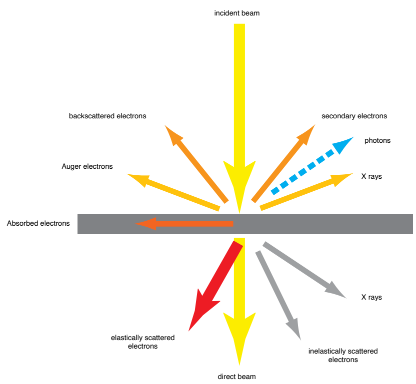
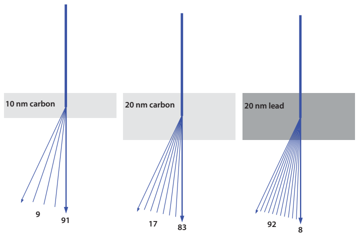
High energy electrons transmitting through a sample

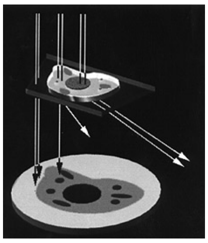


TEM / SEM interaction of electrons with the sample

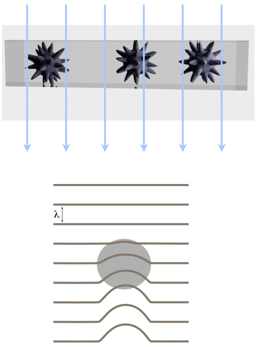


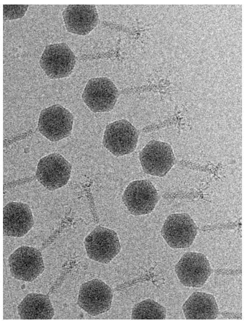
Electrons passing through the sample are deviated depending on the composition of the sample - **amplitude contrast imaging**





Phase contrast imaging - frozen hydrated sample



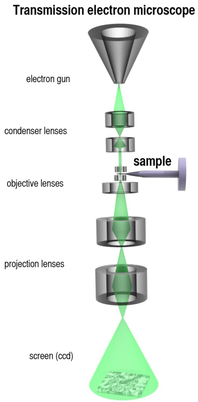


- Good preservation
- High resolution
- Time resolution

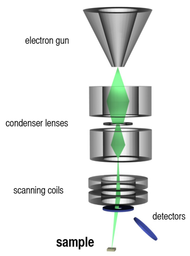
Image capture devices

- Fluorescent screen
- TV camera
- Photographic film
- CCD camera
- Direct electron detector

Transmission electron microscope



Scanning electron microscope



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