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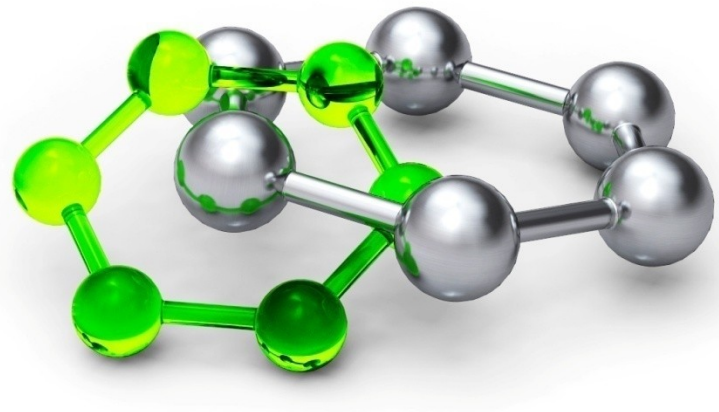
# C9940 3-Dimensional Transmission Electron Microscopy



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# Sylabus

- **Lecture 1: Anatomy of the electron microscope**
- **Lecture 2: Electron microscopy in biological applications, sample preparation for electron microscopy**
- **Lecture 3: Principles of image formation, contrast transfer function, image alignment in 2D**
- **Lecture 4: 3D reconstruction methods in TEM**
- **Lecture 5: Interpretation and optimization of cryo-EM maps**

# Resources

## Literature:

- J. Frank, Three-dimensional electron microscopy of macromolecular assembliesvisualization of biological molecules in their native state.
- J. Frank, Electron Tomography: Methods for Three-Dimensional Visualization of Structures in the Cell
- Williams et al., Transmission electron microscopy

## Video courses (youtube):

- Grant Jensen
- NRAMM SEMC
- Cryo-EM14 (LMB)
- Cryo-EM17 (LMB)

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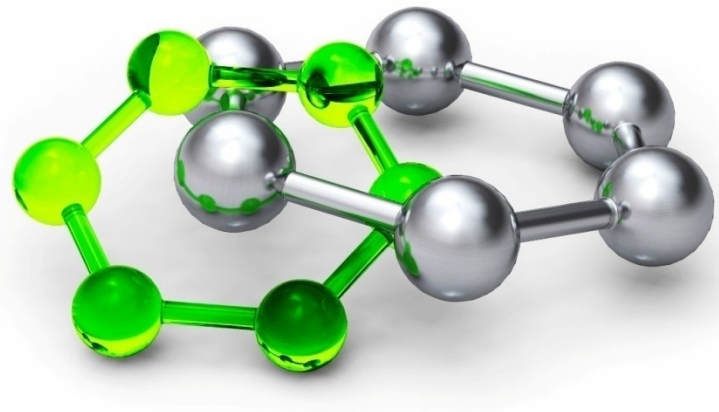
# C9940 3-Dimensional Transmission Electron Microscopy

## Lecture 1: Electron Microscope



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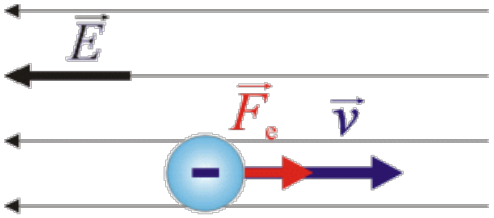
# Electron

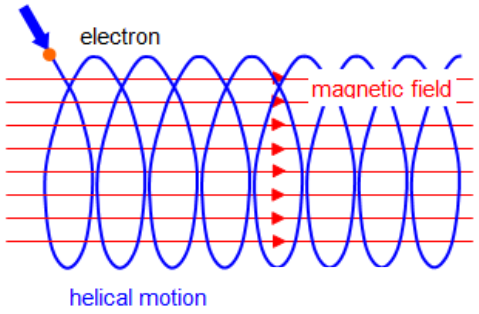
| | |
|---|---|
| **rest mass:** | **9.109 e-31 kg** |
| **charge:** | **-1.61 e-19 C** |
| **spin:** | **1/2** |

## Electron in electric and magnetic field

$$\mathbf{F} = q(\mathbf{E} + \mathbf{v} \times \mathbf{B})$$ (Lorentz force)







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# Electron

## Dual character of electron

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

$$\lambda_{\text{de Broglie}} = \frac{h}{p} = \frac{h \cdot c}{\sqrt{(e \cdot V_\text{a})^2 + 2 \cdot e \cdot V_\text{a} \cdot m_\text{e} \cdot c^2}}$$

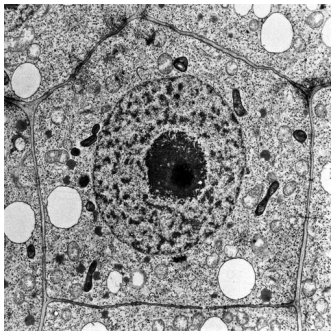
| Acceleration Voltage [kV] | Non-relativistic wavelength [pm] | Relativistic wavelength [pm] |
|---|---|---|
| 2 | 27.35 | 27.32 |
| 20 | 8.65 | 8.57 |
| 100 | 3.87 | 3.69 |
| 200 | 2.73 | 2.50 |
| 300 | 2.23 | 1.96 |

## Abbe diffraction limit

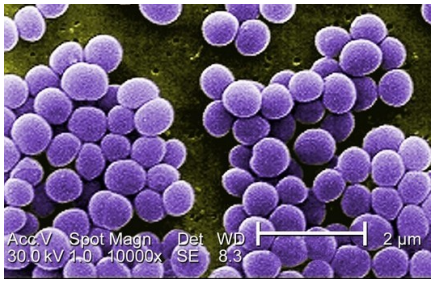
$$\Delta x \cong \frac{\lambda}{2n \sin \alpha}$$

# Scales in electron microscopy

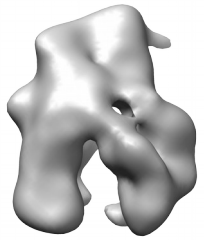

Tick (ESEM)



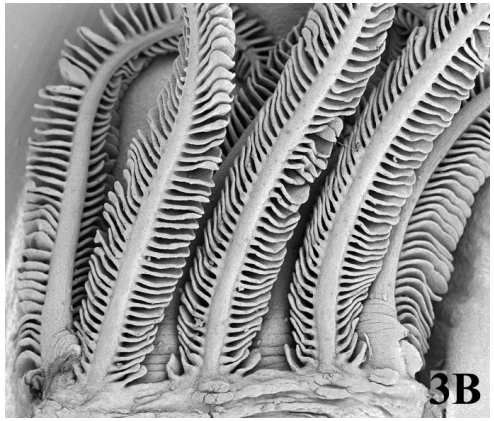
Plant cell (TEM)



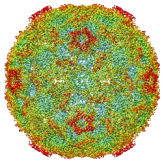

Bacteria (SEM)



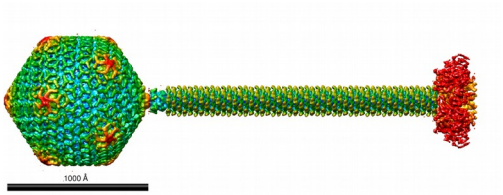
RNA polymerase (TEM)




Plant (SEM)



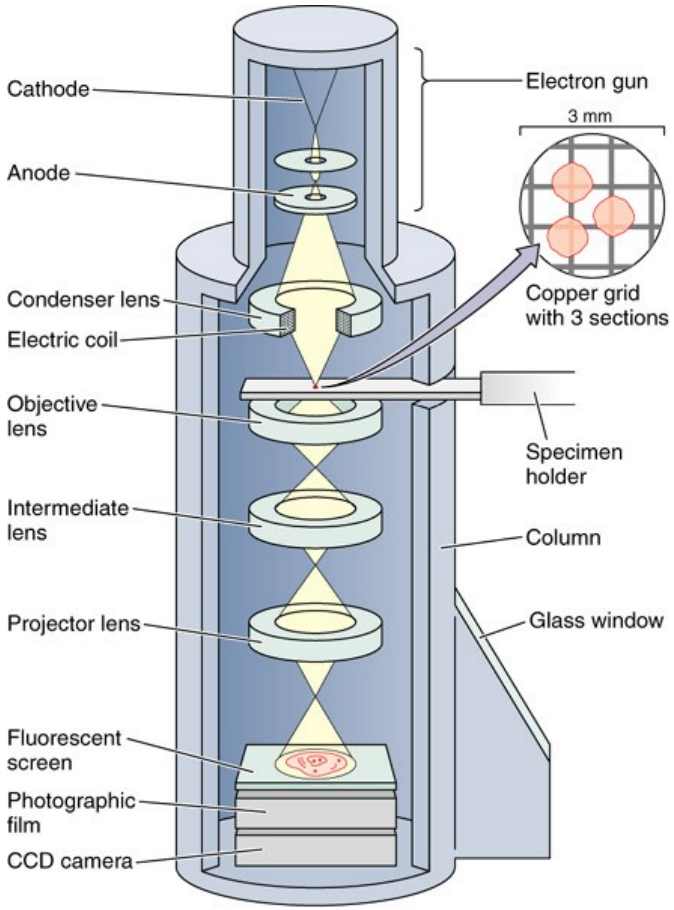
Virus (TEM)




Bacteriophage (TEM)

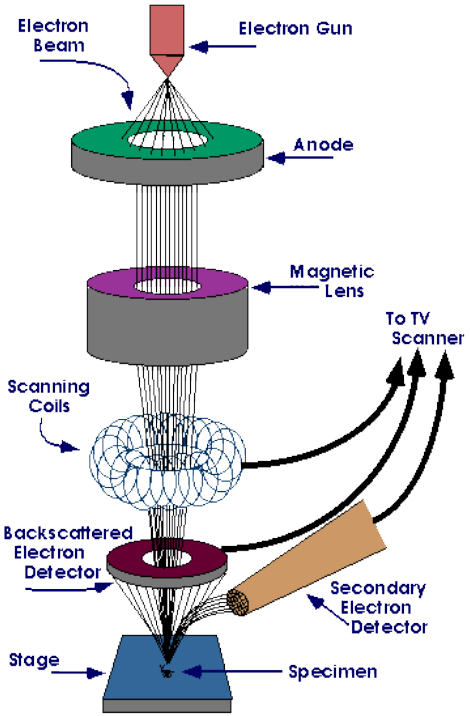
# Electron microscopes

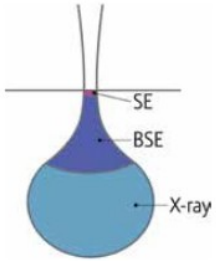
## TEM

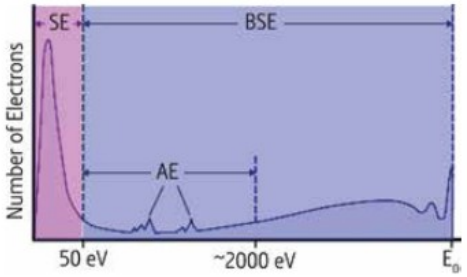



## STEM

## SEM



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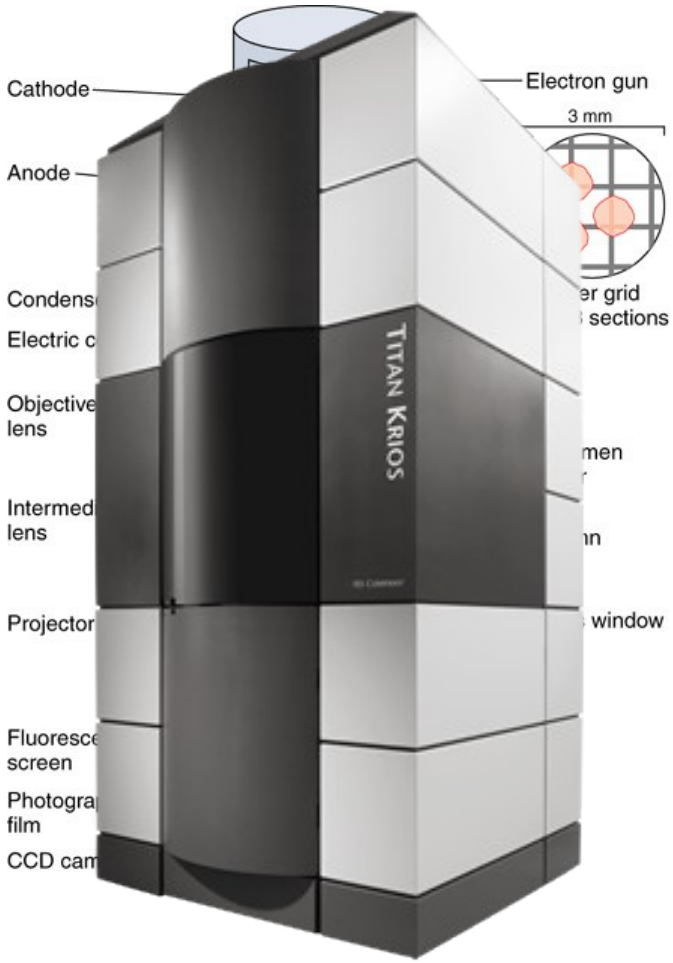
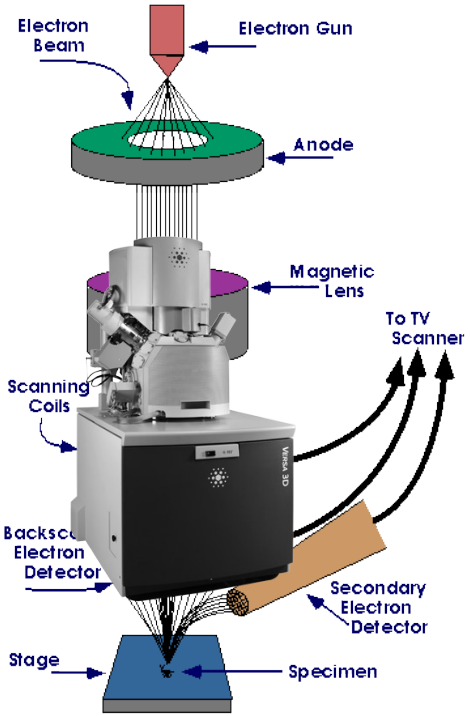
# Electron microscopes

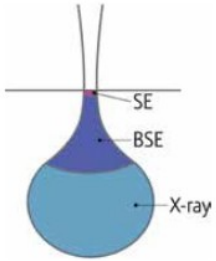
## TEM

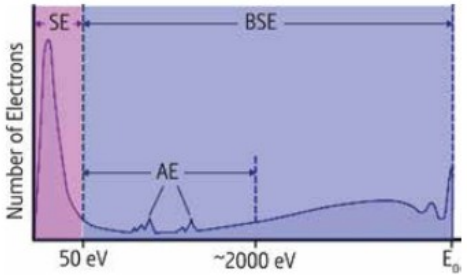



## STEM

## SEM



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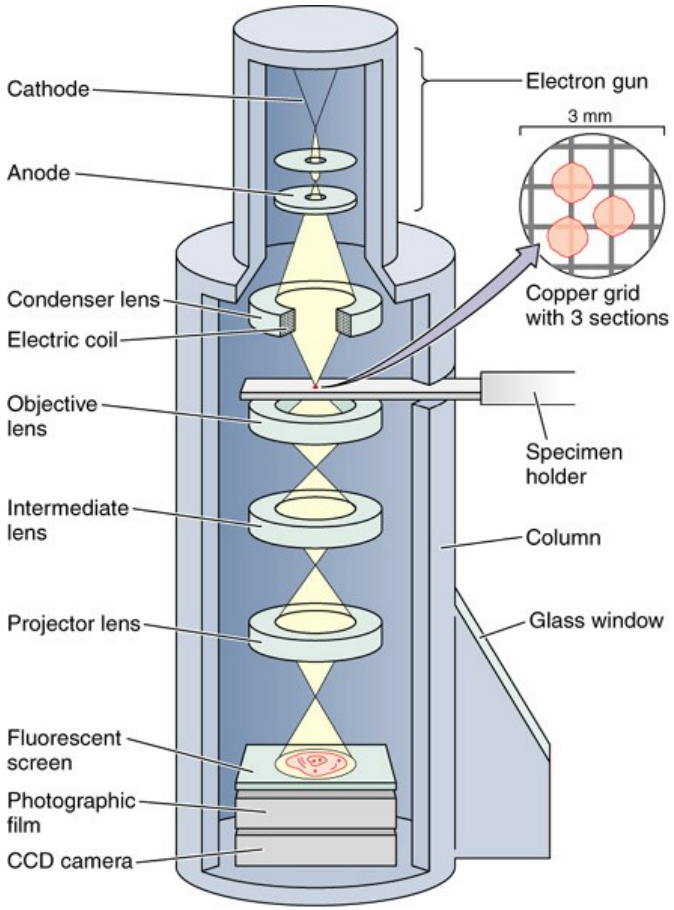
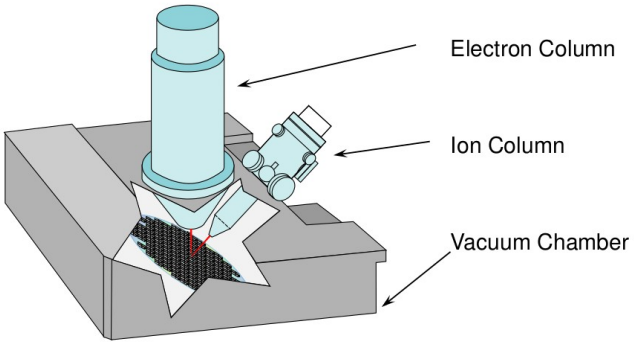
# Electron microscopes

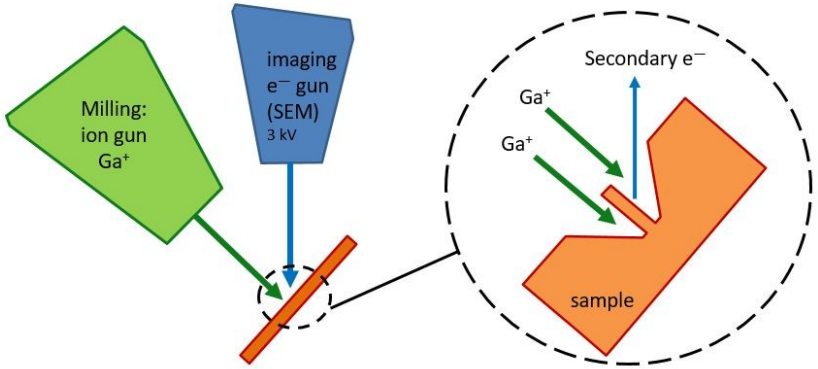
## TEM



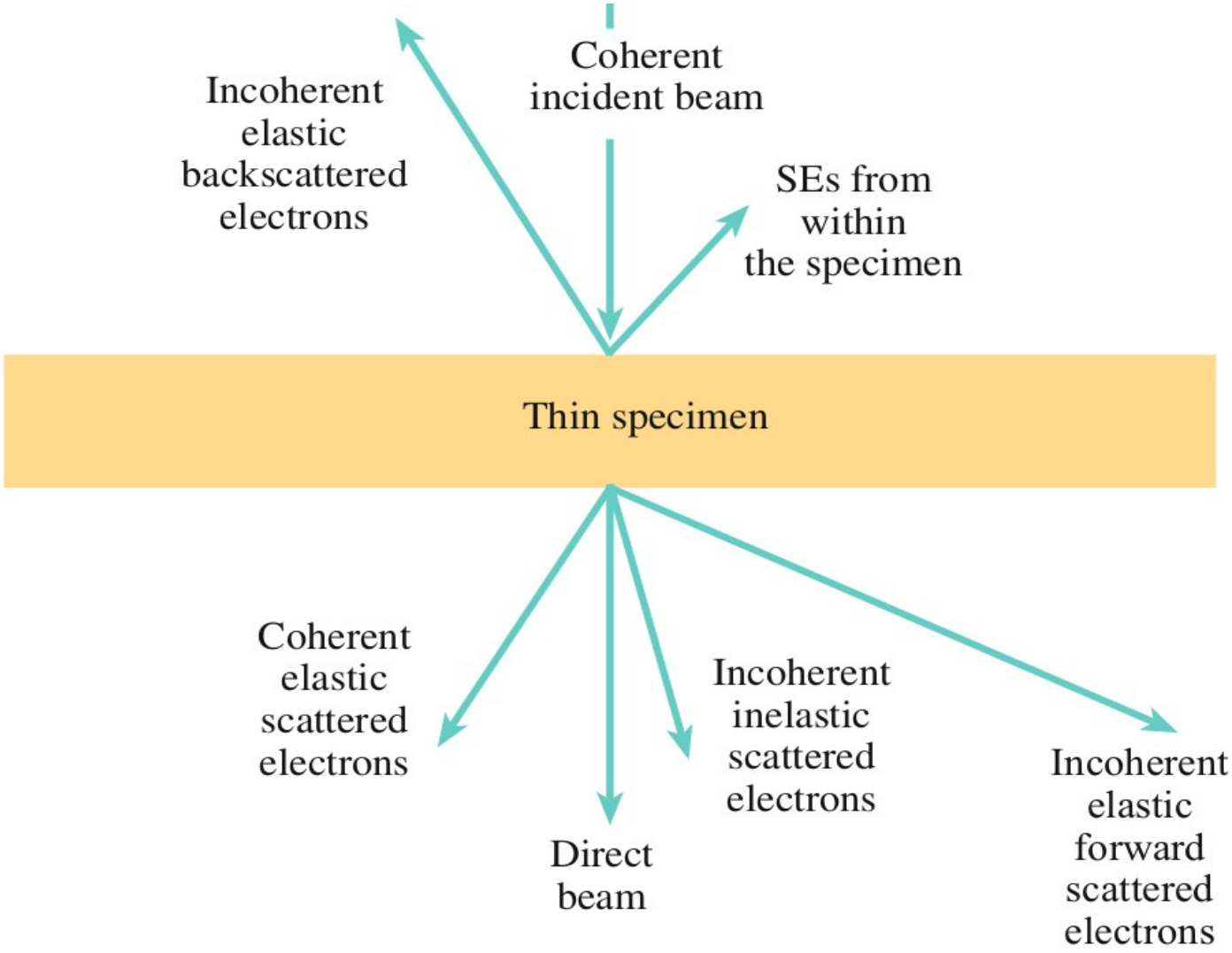

## FIB/SEM

## STEM



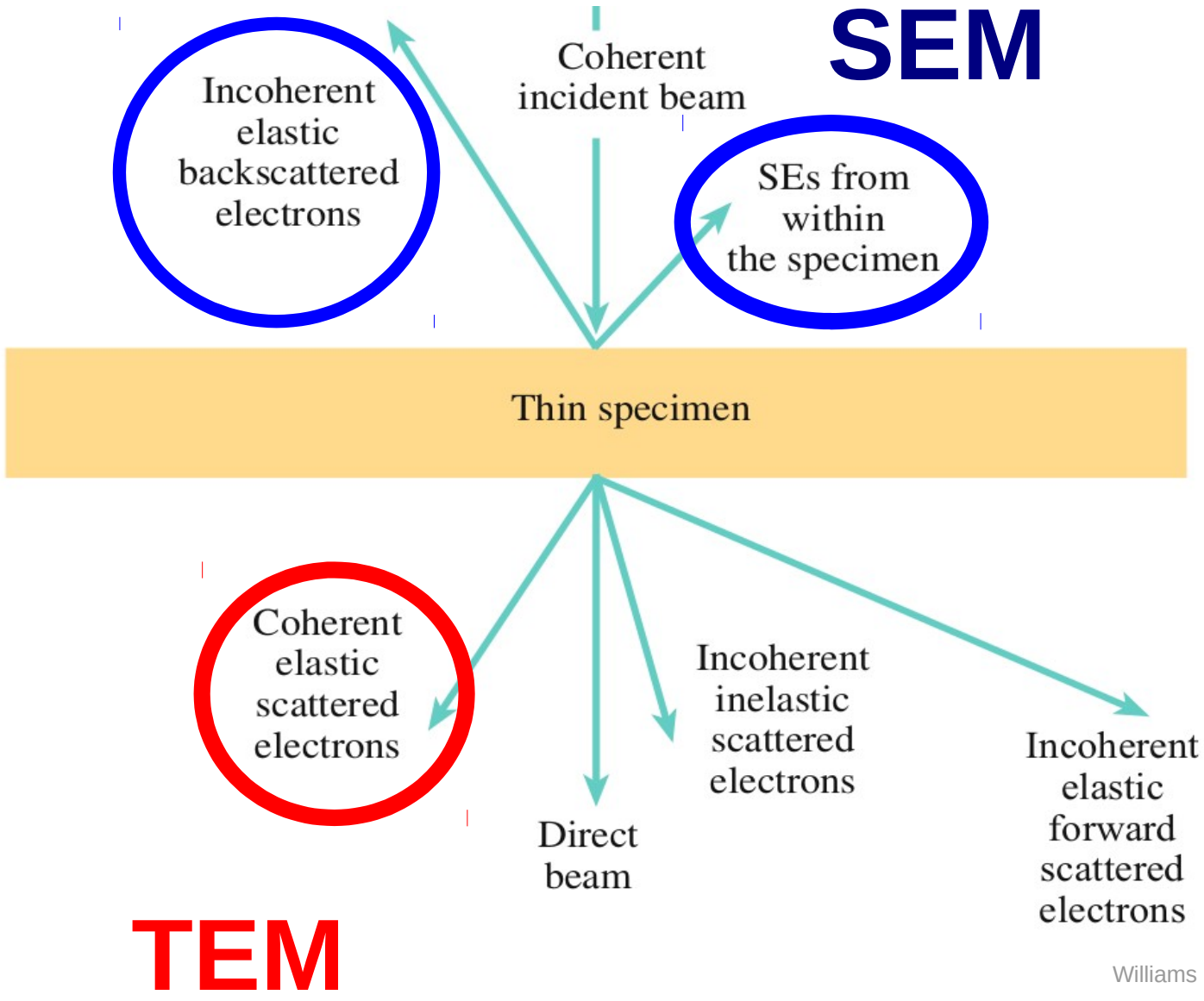



# Interaction of electrons with matter



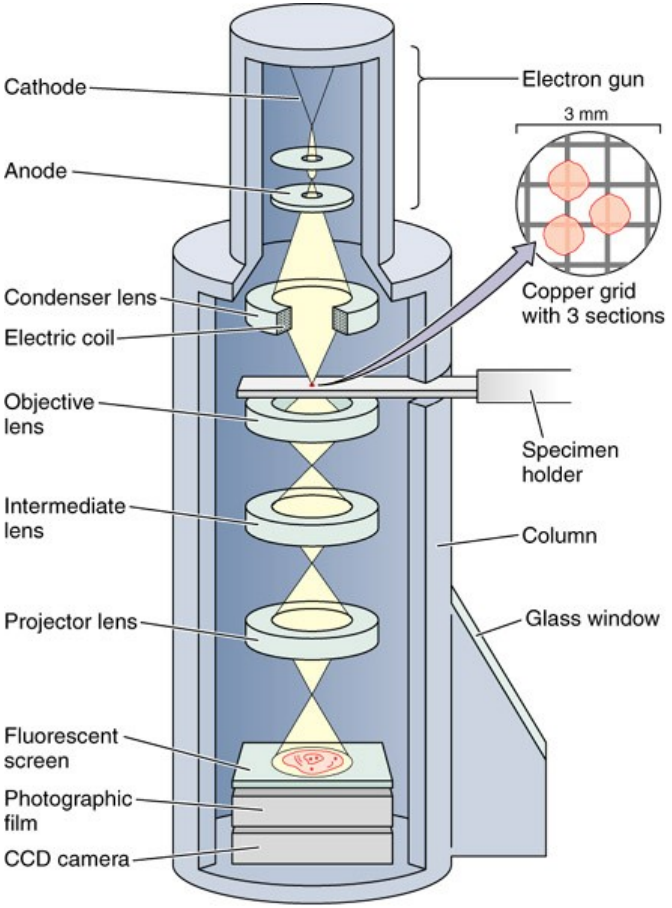

Williams et al. 2009

# Interaction of electrons with matter

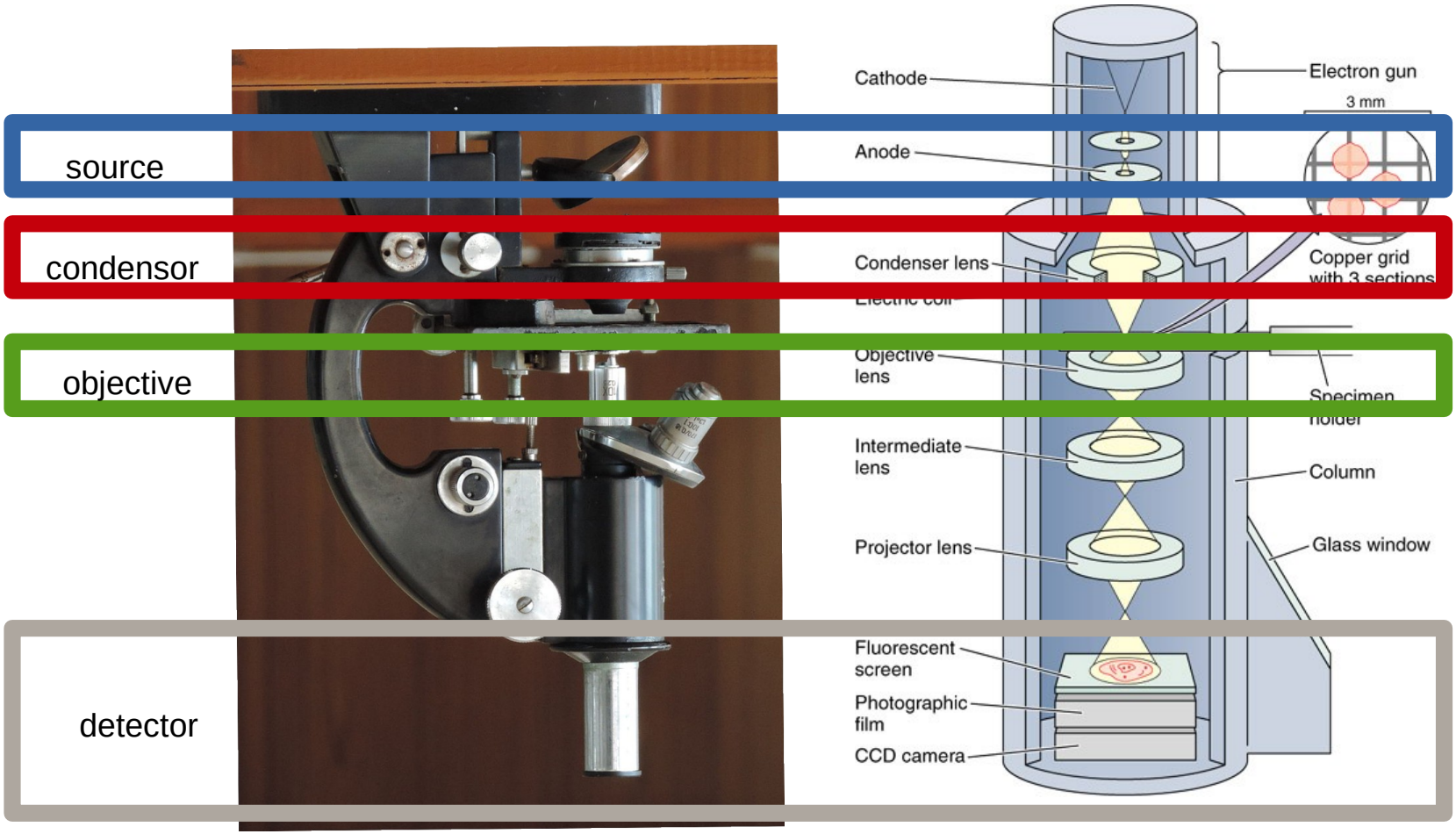



Williams et al. 2009

# Optical vs. TEM microscope



# Optical vs. TEM microscope

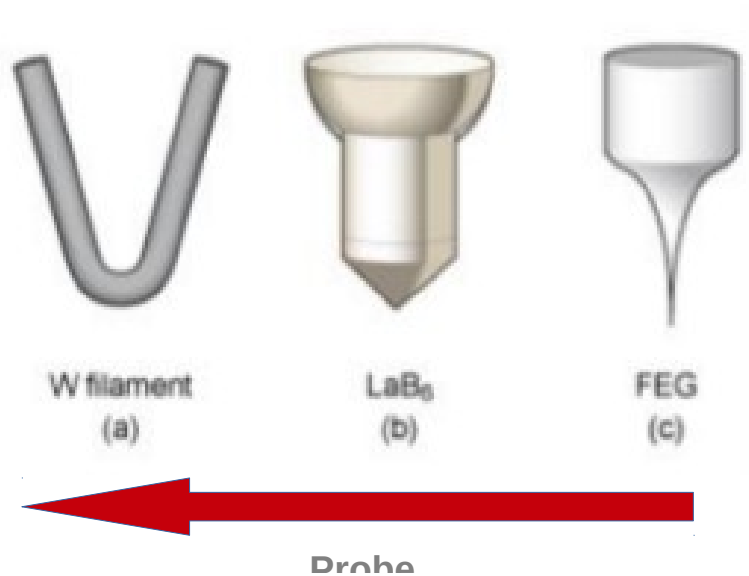


# Electron source

- thungsten filament

- LaB6 crystal

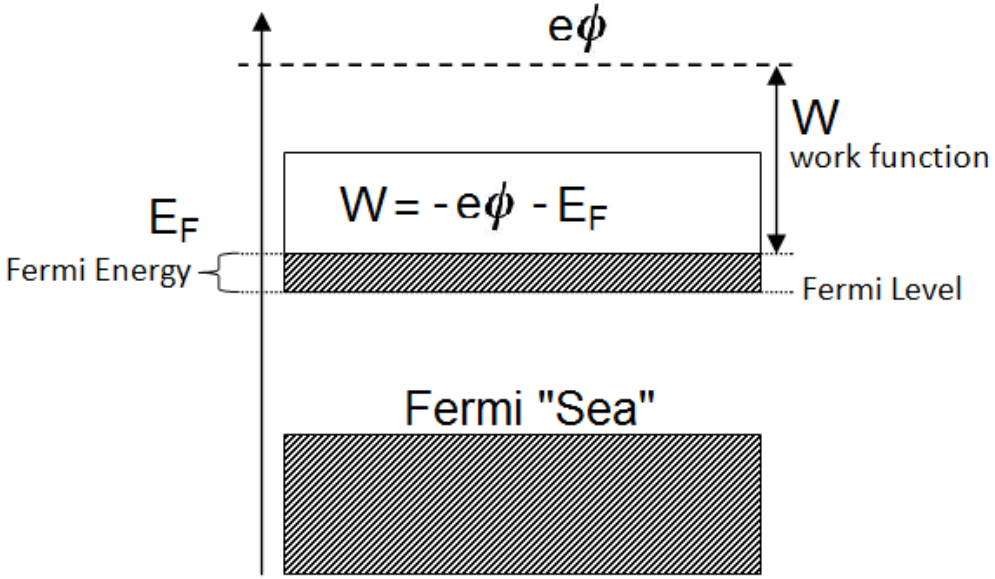
- Field Emission Gun




Work function

$$W = -e\phi - E_F$$

$$\phi = V - \frac{W}{e}$$



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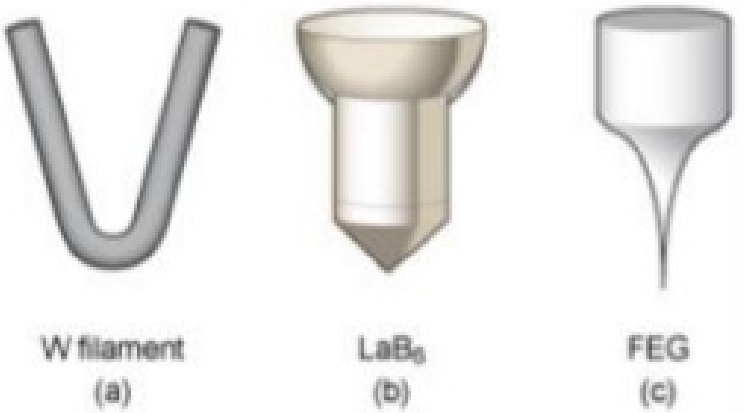
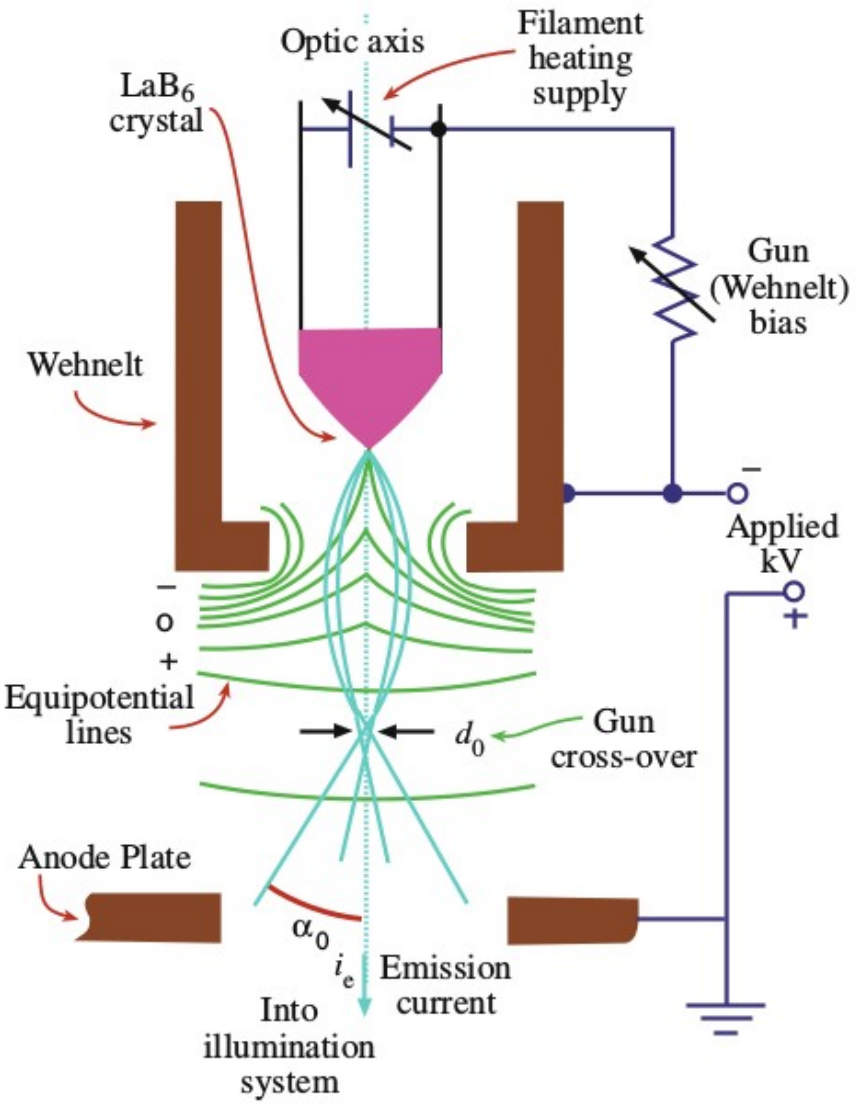
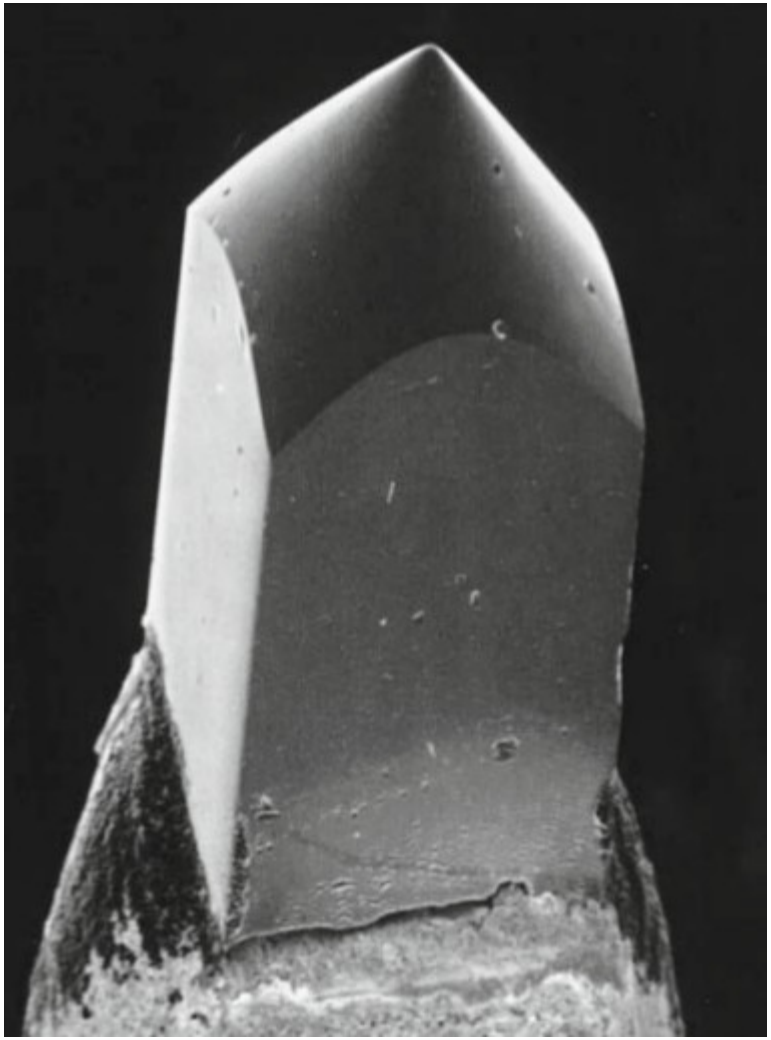


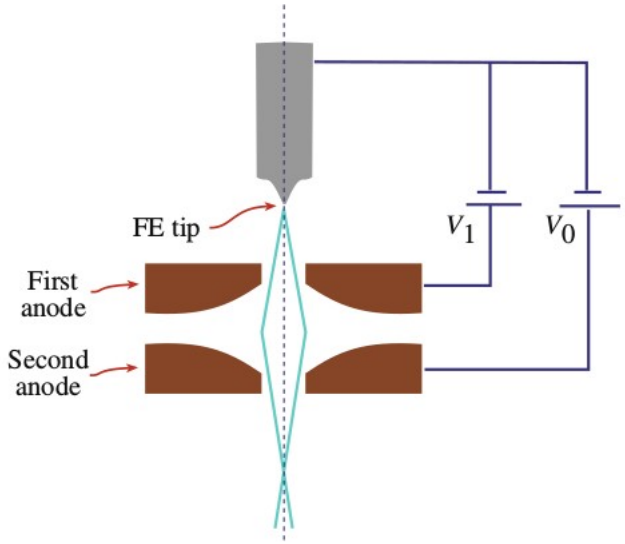

| | Units | Tungsten | $LaB_6$ | Schottky FEG | Cold FEG |
|---|---|---|---|---|---|
| Work function, $\Phi$ | eV | 4.5 | 2.4 | 3.0 | 4.5 |
| Richardson's constant | $A/m^2K^2$ | $6 \times 10^9$ | $4 \times 10^9$ | | |
| Operating temperature | K | 2700 | 1700 | 1700 | 300 |
| Current density (at 100 kV) | $A/m^2$ | 5 | $10^2$ | $10^5$ | $10^6$ |
| Crossover size | nm | $> 10^5$ | $10^4$ | 15 | 3 |
| Brightness (at 100 kV) | $A/m^2sr$ | $10^{10}$ | $5 \times 10^{11}$ | $5 \times 10^{12}$ | $10^{13}$ |
| Energy spread (at 100 kV) | eV | 3 | 1.5 | 0.7 | 0.3 |
| Emission current stability | %/hr | <1 | <1 | <1 | 5 |
| Vacuum | Pa | $10^{-2}$ | $10^{-4}$ | $10^{-6}$ | $10^{-9}$ |
| Lifetime | hr | 100 | 1000 | >5000 | >5000 |

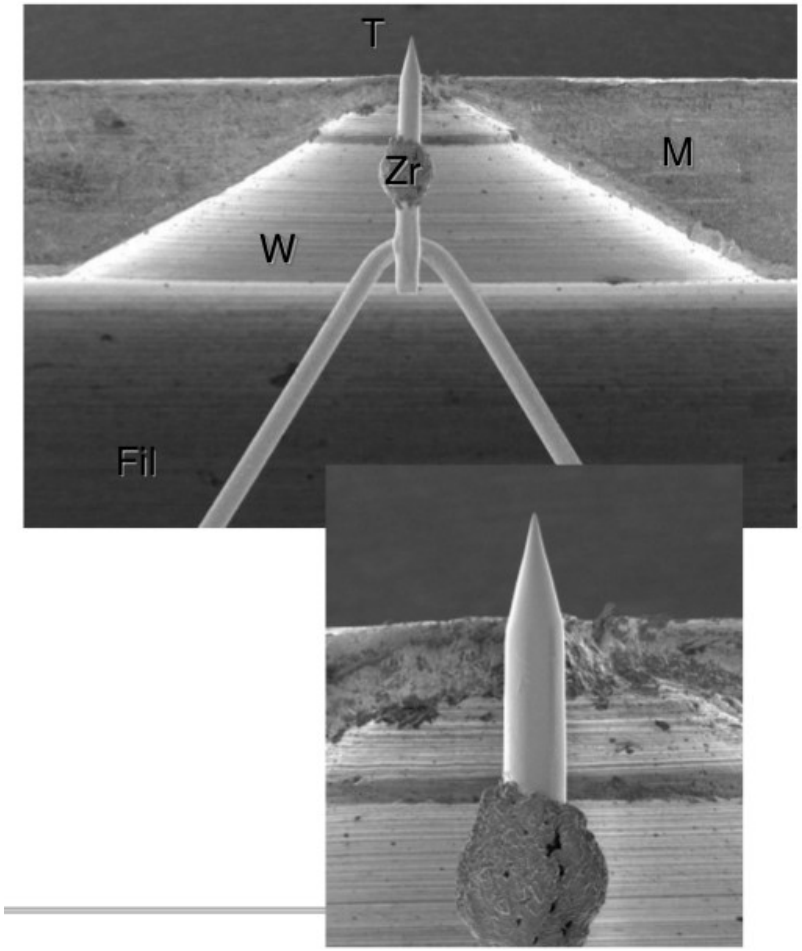
# Electron source - LaB6

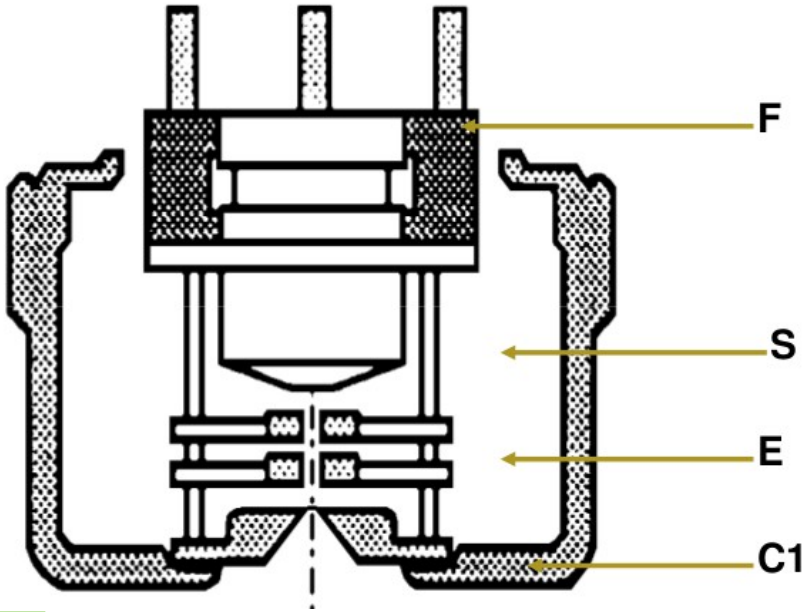


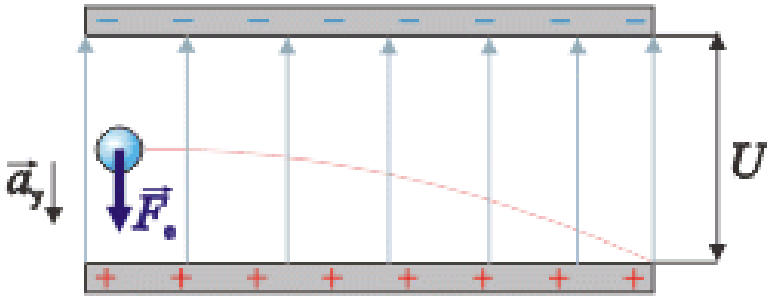
# Electron source - FEG



# Electron source - accelerator

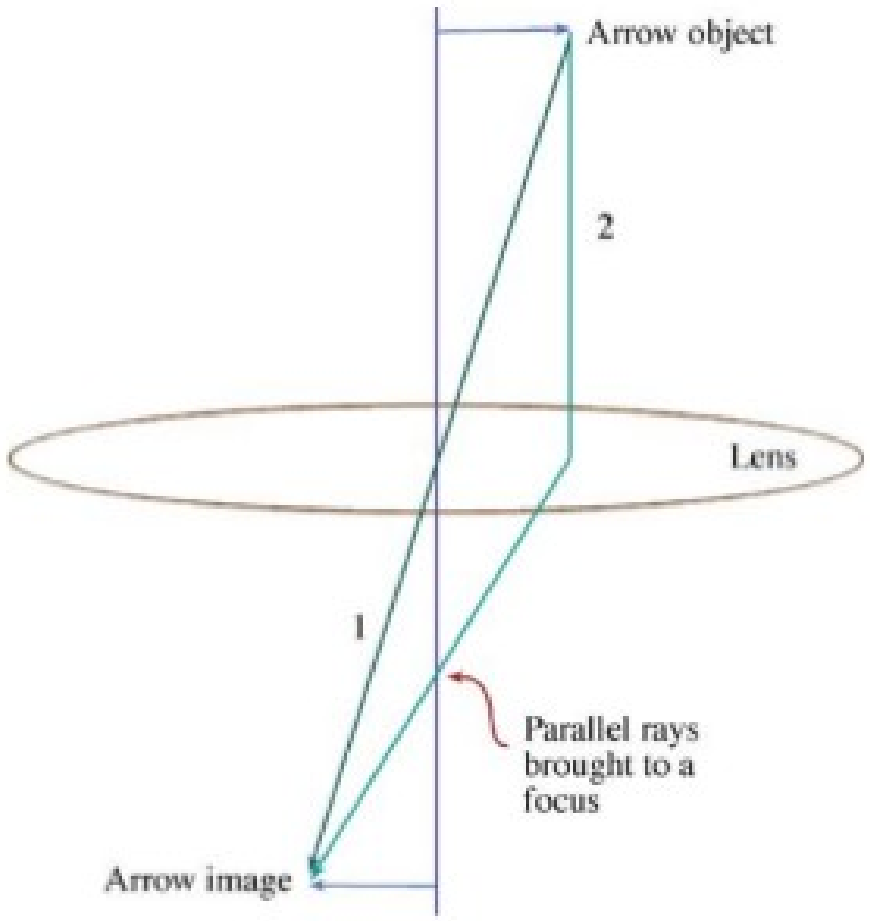

$E_k = \frac{1}{2}mv^2$

E=U.
e

$E_k = \frac{p^2}{2m}$

U=300kV =>
λ=1.97pm
U=200kV ??

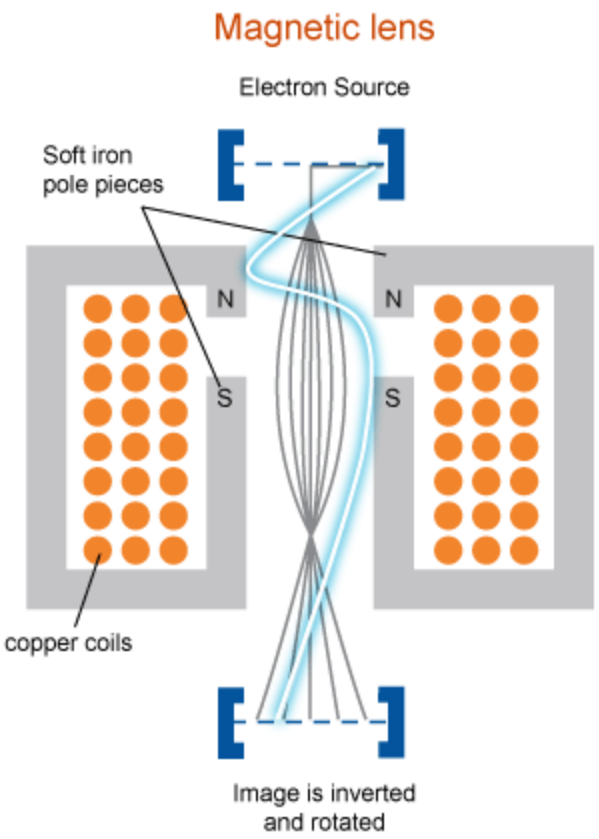
# Lenses – ray diagram

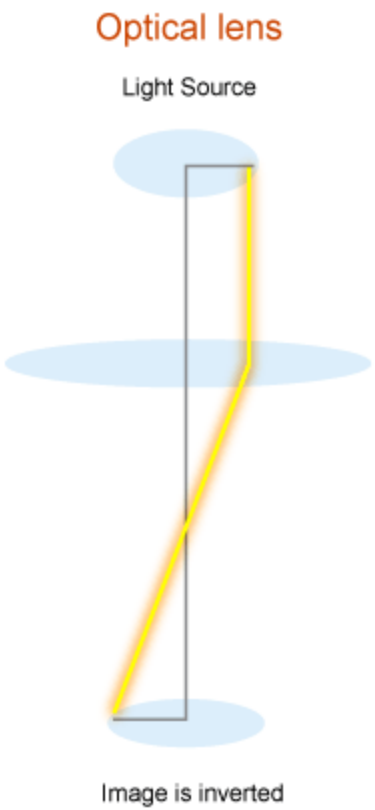


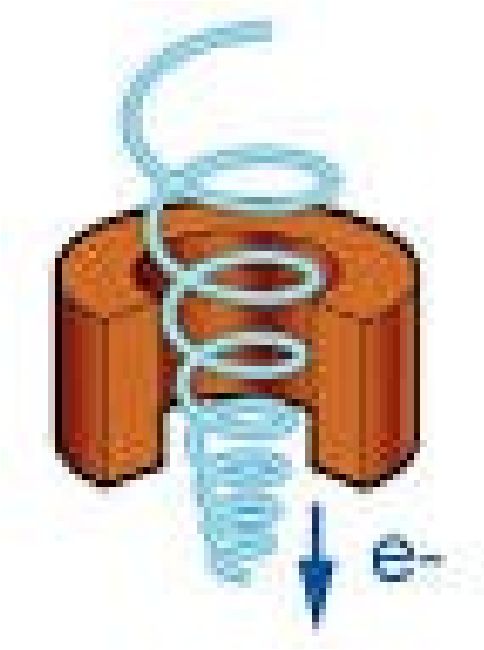
# Electromagnetic lenses

Lorentz force:

$$\mathbf{F} = q(\mathbf{E} + \mathbf{v} \times \mathbf{B})$$

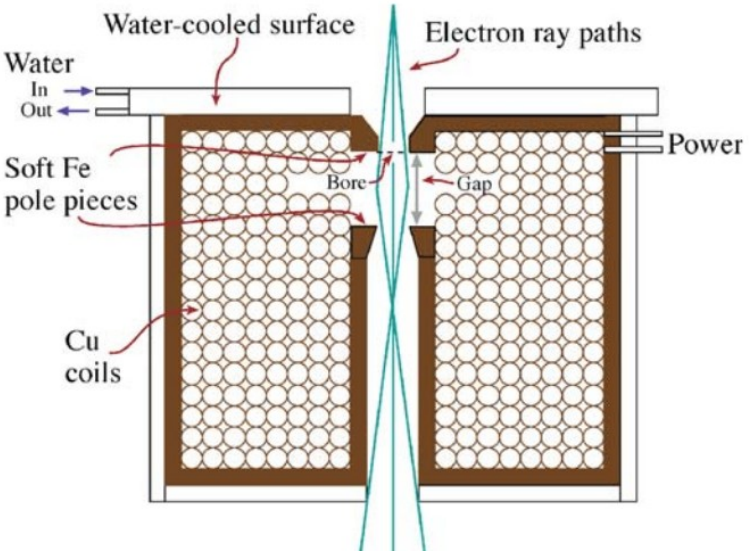


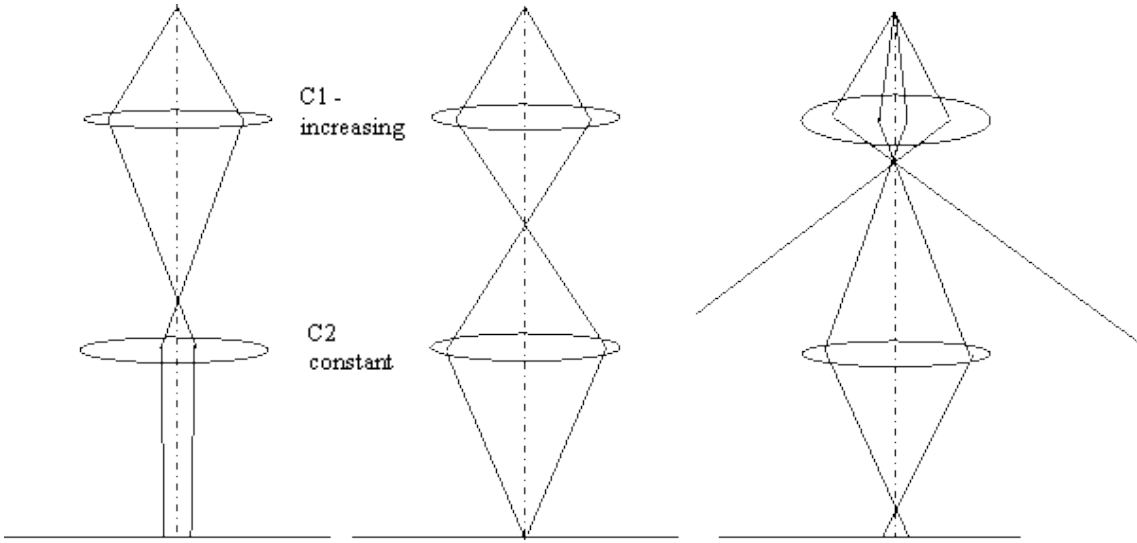




Magnetic lenses rotate image

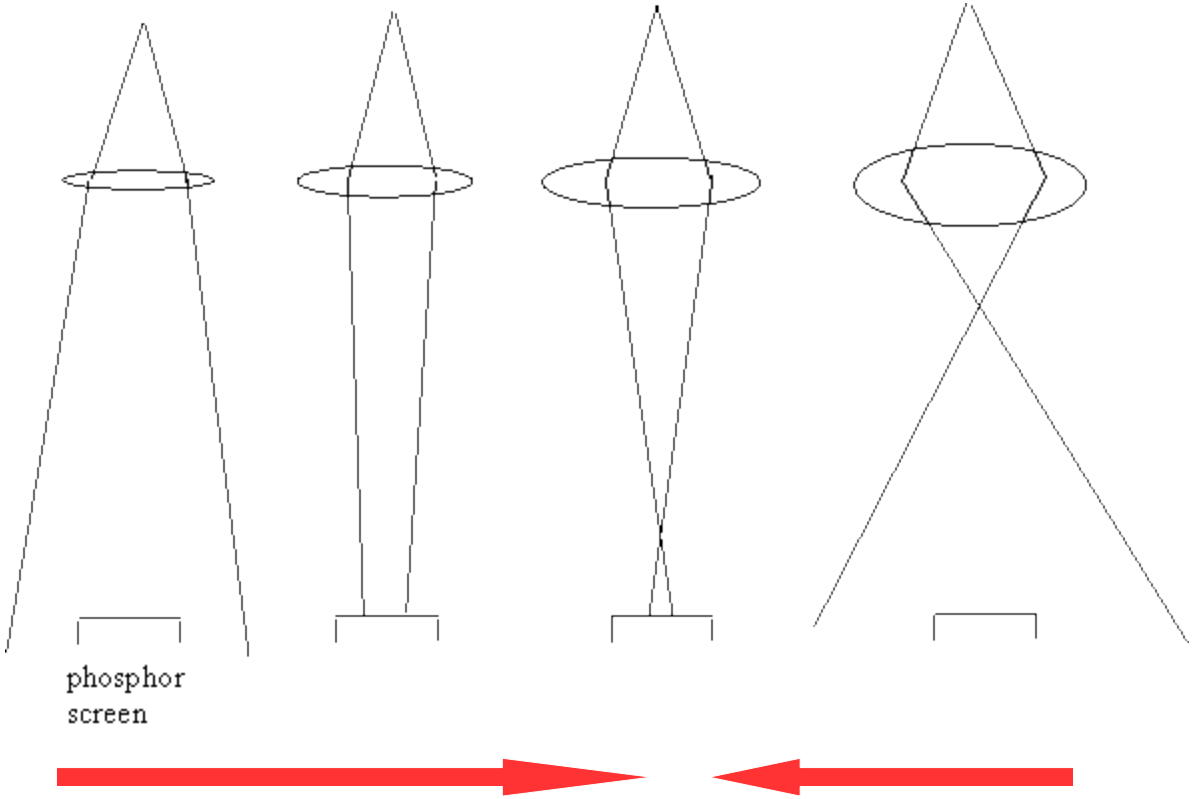
# Electromagnetic lenses



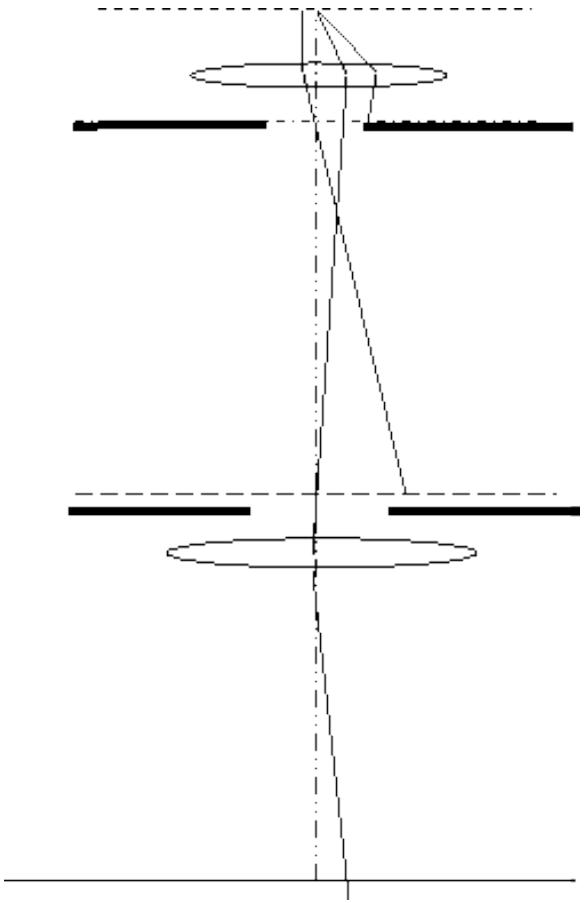

**Power of the magnetic lens can be changed**

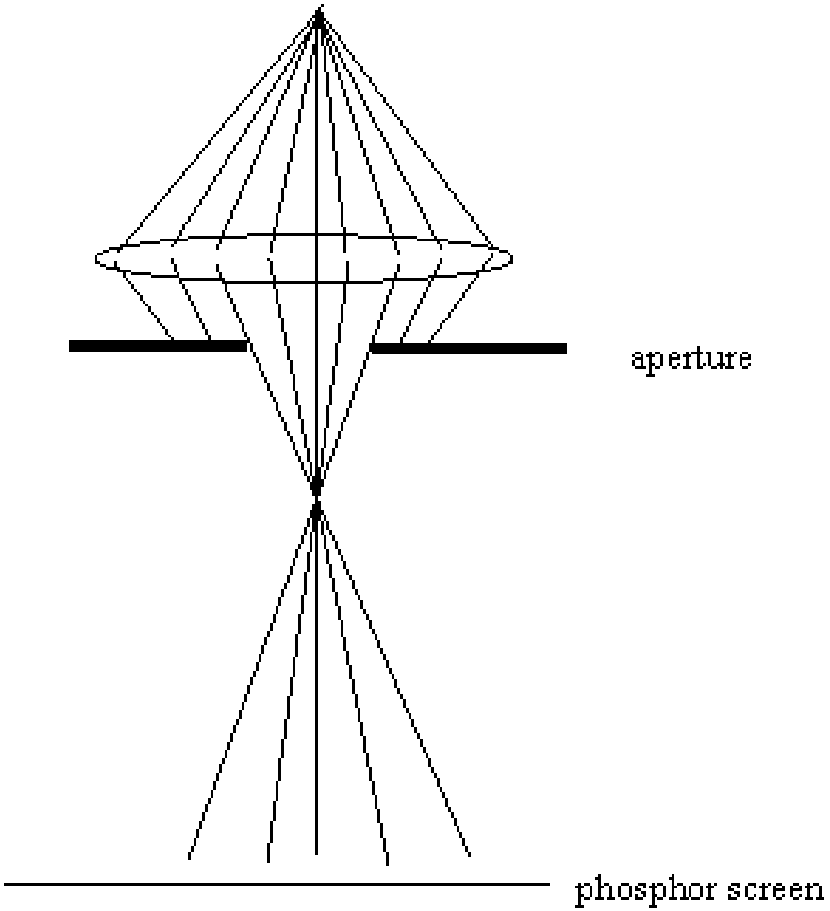
# Electromagnetic lenses




**Illumination on the detector changes with change of lens power**

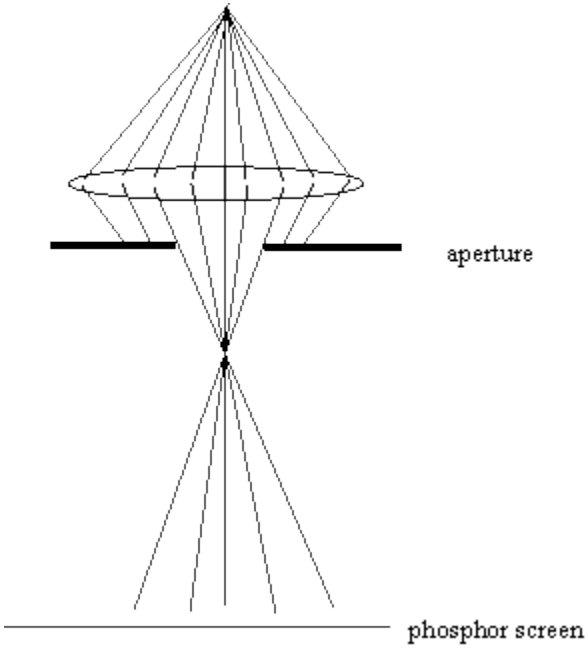
# Lens assembly - appertures

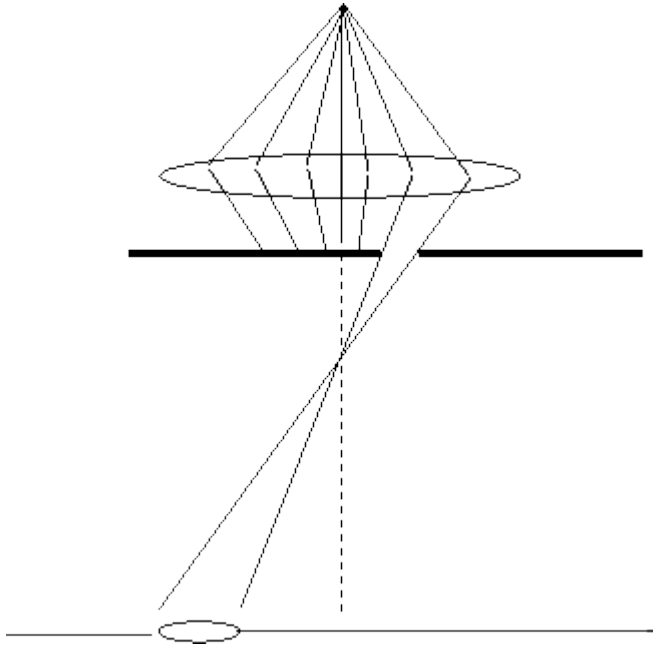




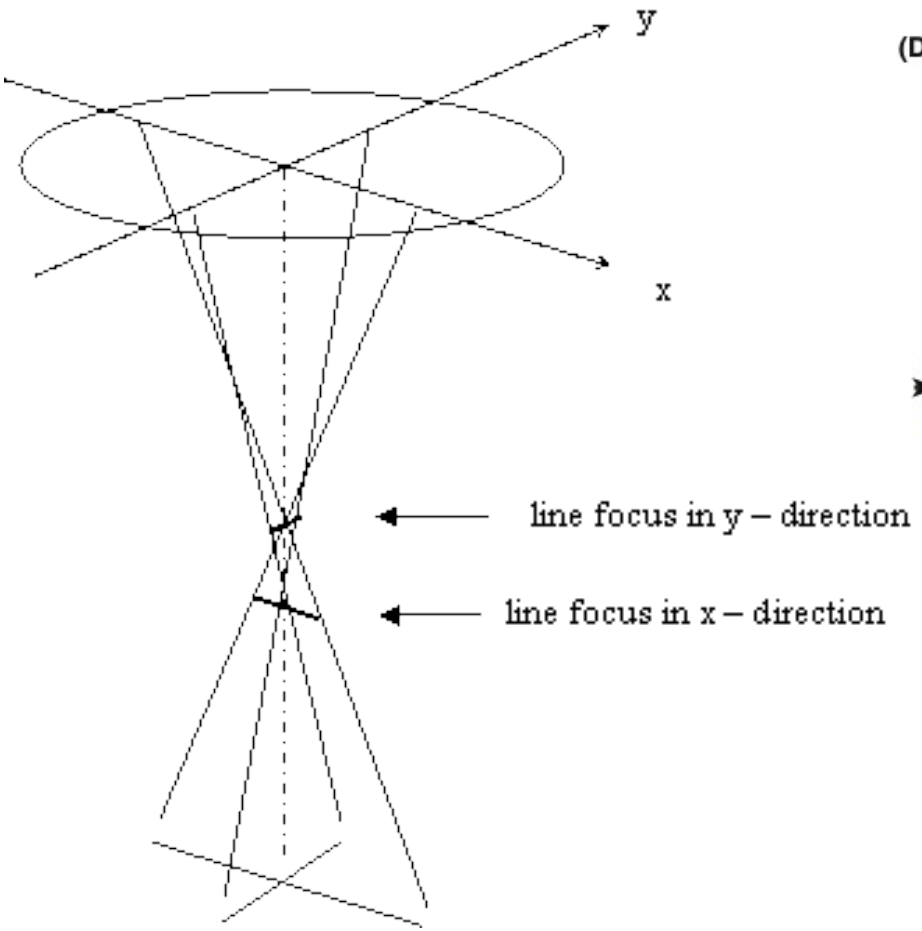

**Apperture size: ~100um**

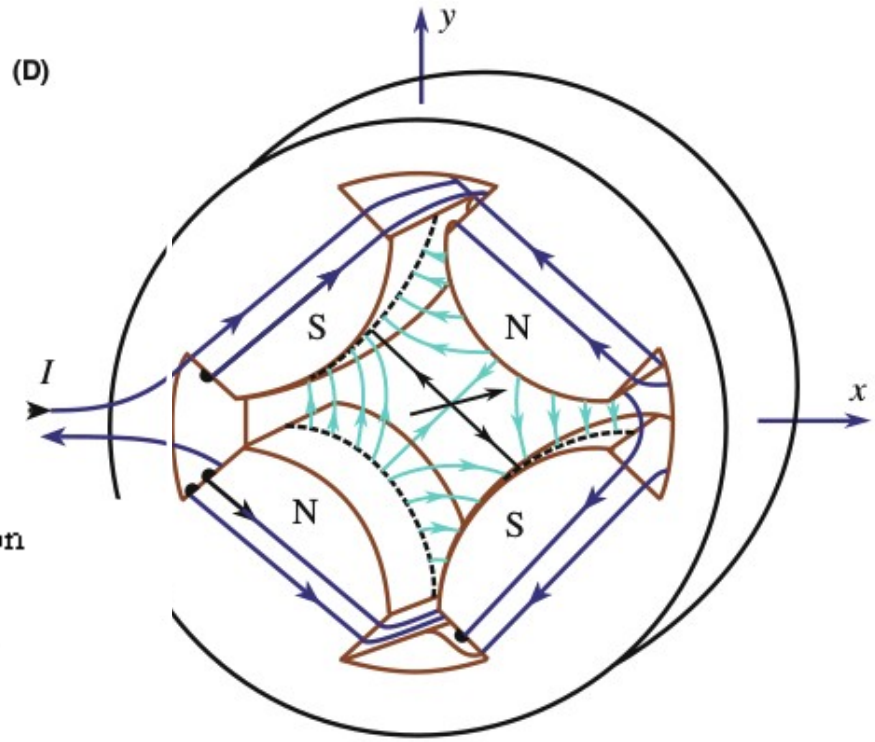
# Lens assembly - appertures



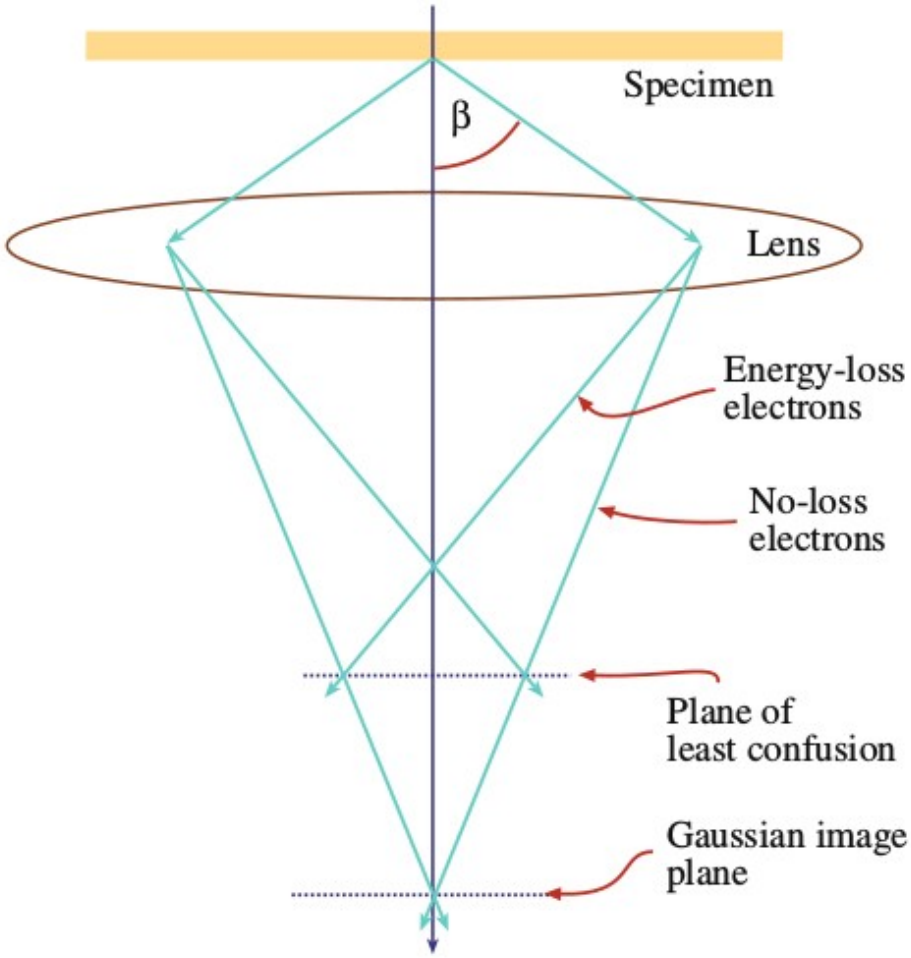



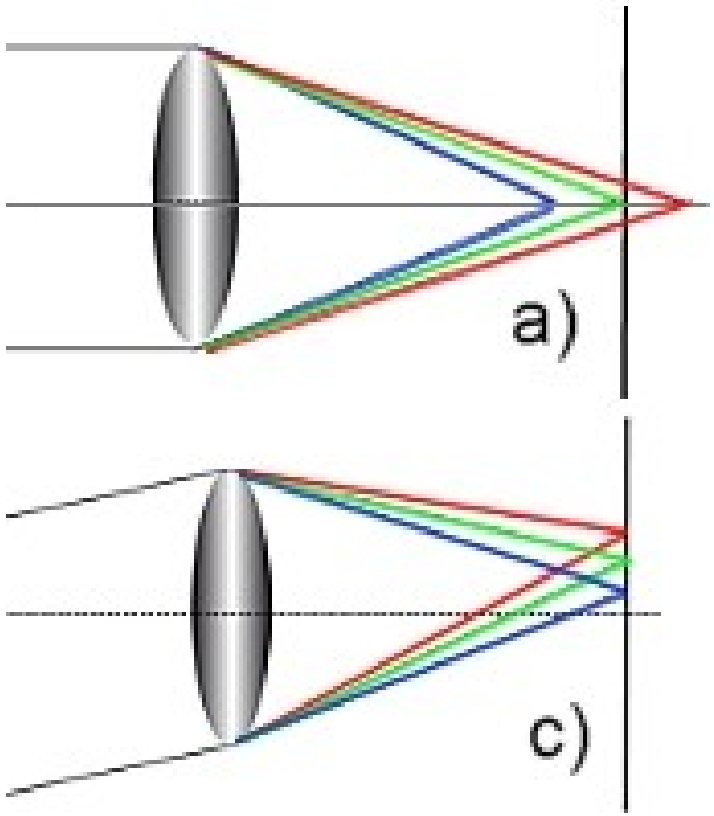
# Lens assembly - stigmators



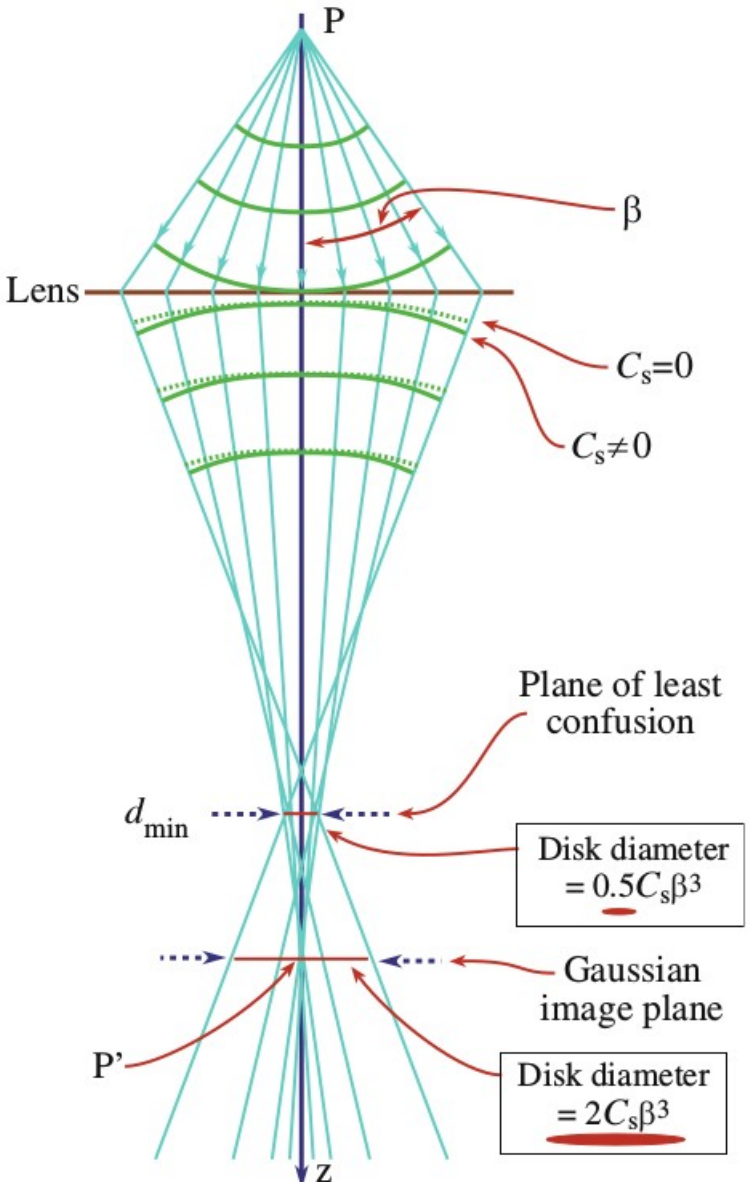



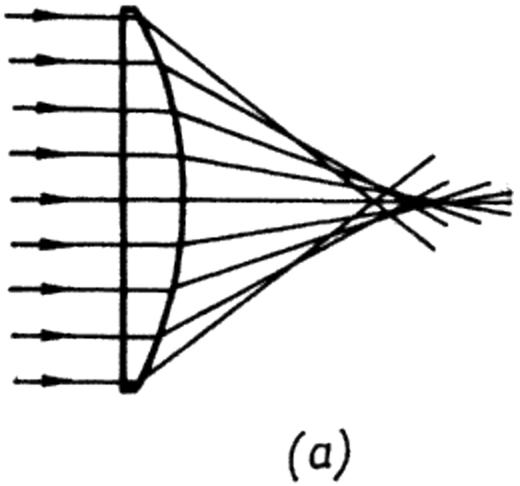
# Lens aberrations - chromatic



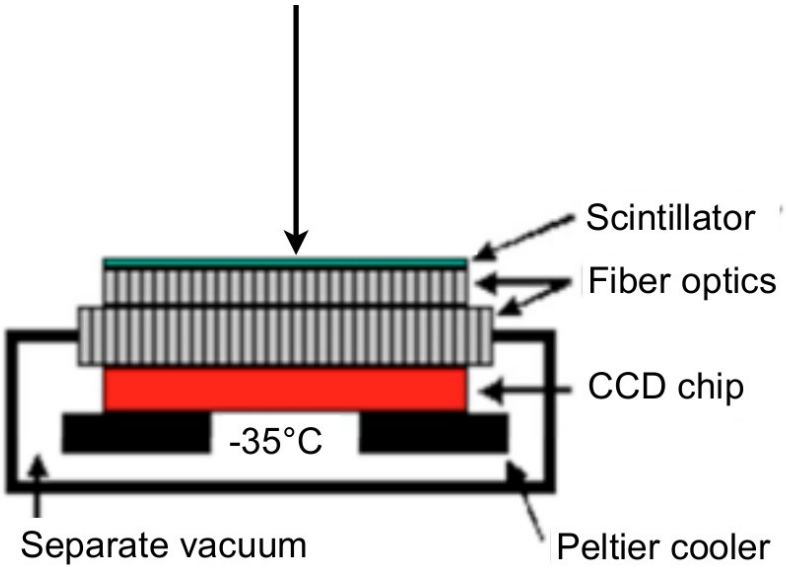
# Lens aberrations - spherical



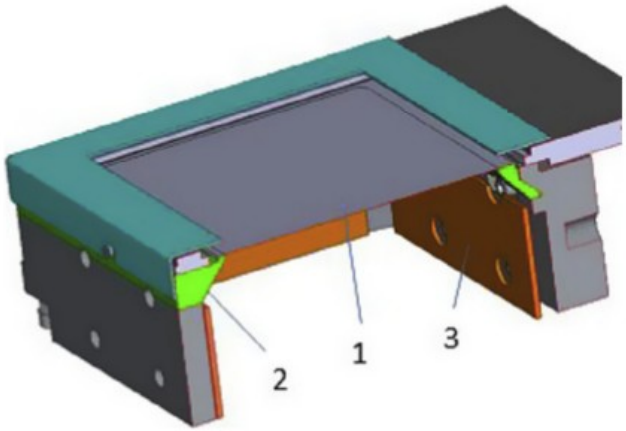



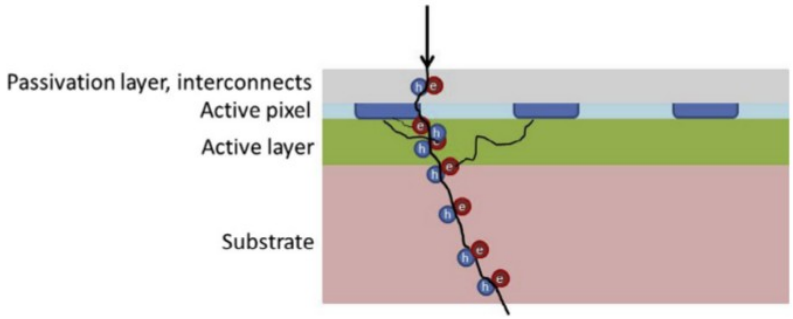
(a)

# Detectors




**CCD – charge coupled device**



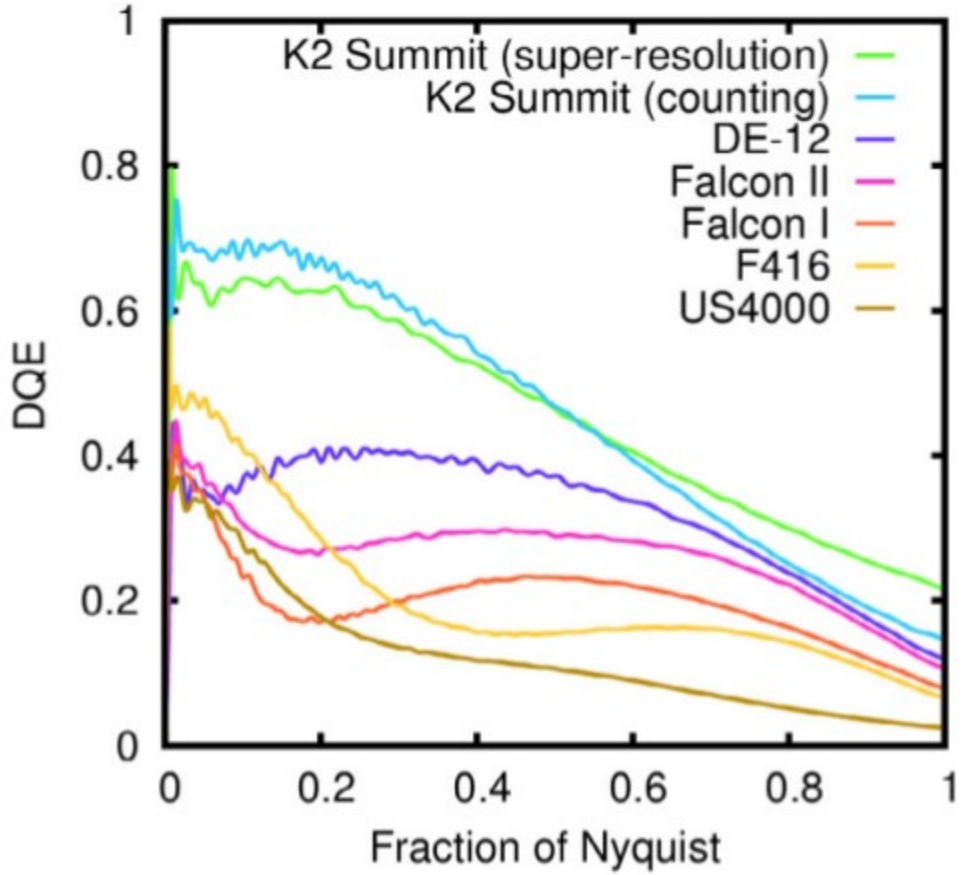

**CMOS – complementary metal oxide semiconductor**



CEITEC

# Detectors

**DQE – detective quantum efficiency**




**- probability to detect an electron**

**- DQE ~ sin(x)/x**



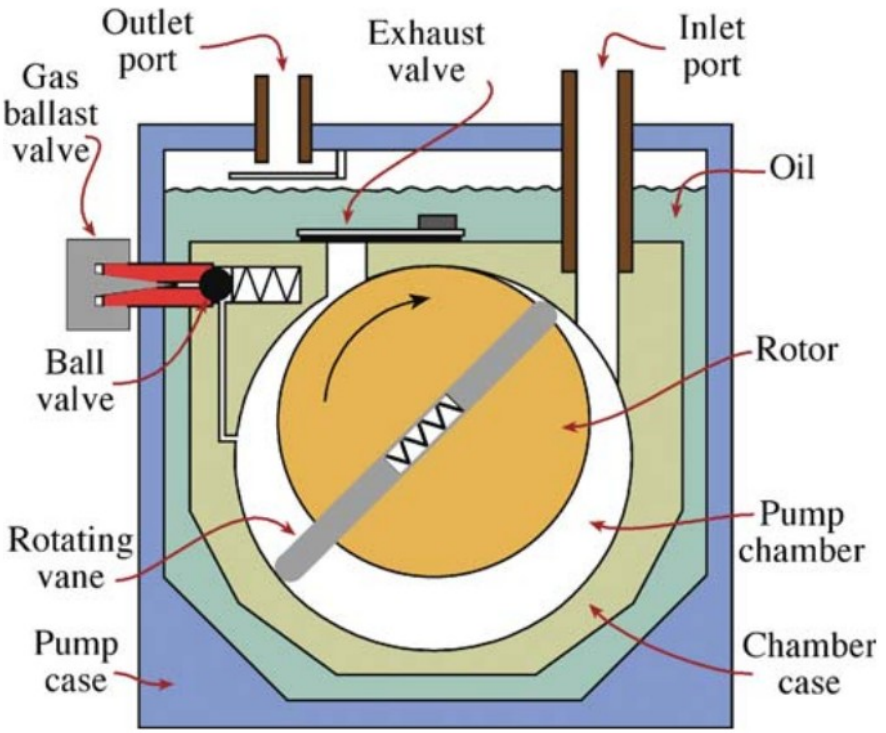
CEITEC

# Vacuum system

- roughing pump ($10^{5}$ – $10^{-4}$ Pa)

- turbo molecular pump ($10^{-2}$ – $10^{-8}$Pa)

- ion getter pump ( up to $10^{-9}$Pa)



# Vacuum system

- roughing pump ($10^{5}$ – $10^{-4}$ Pa)

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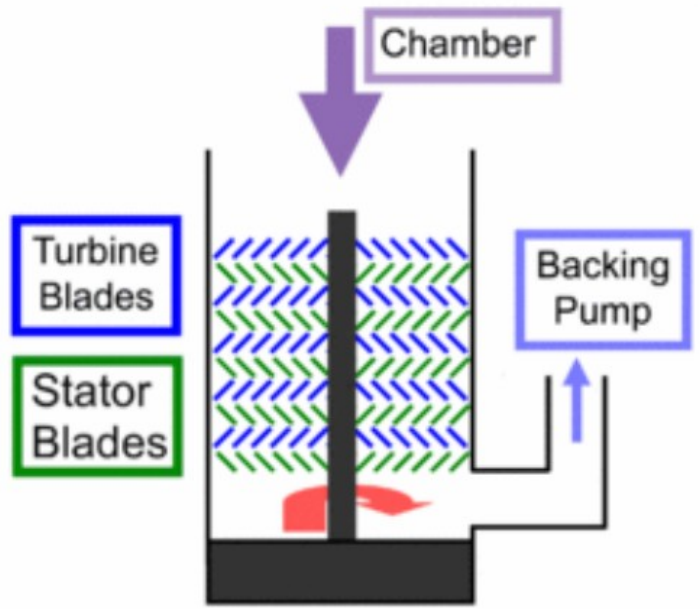
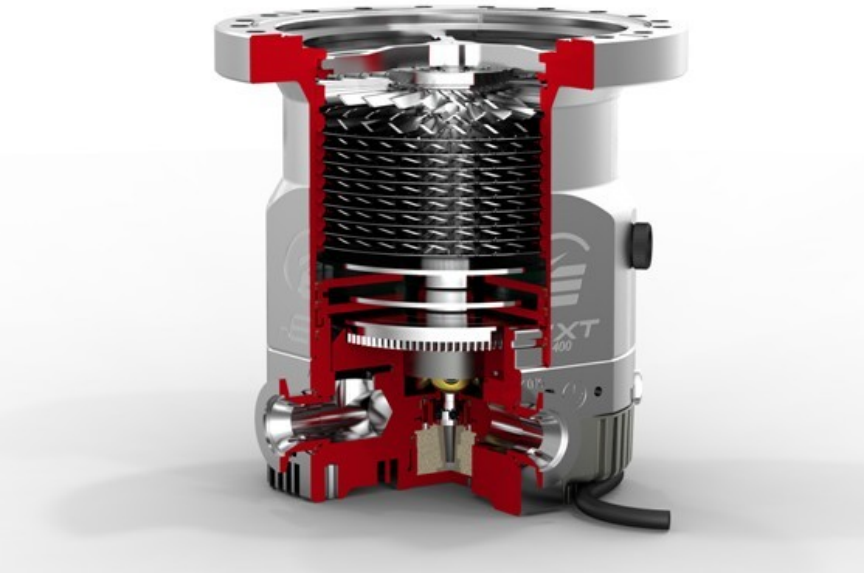


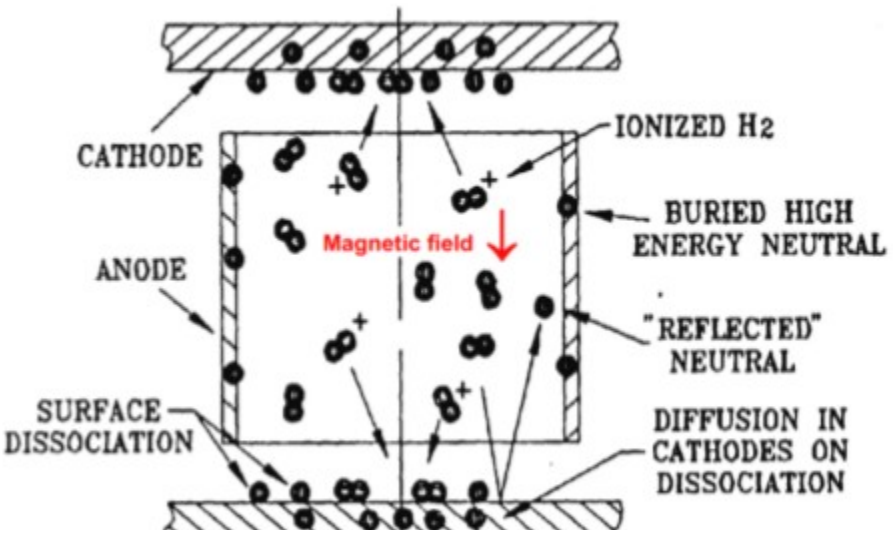
90.000 rpm

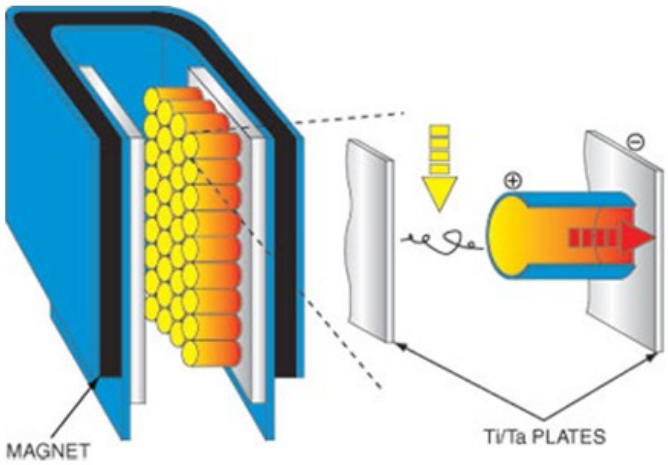
# Vacuum system

- roughing pump ($10^{5}$ – $10^{-4}$ Pa)

- turbo molecular pump ($10^{-2}$ – $10^{-8}$Pa)

- ion getter pump ( up to $10^{-9}$Pa)

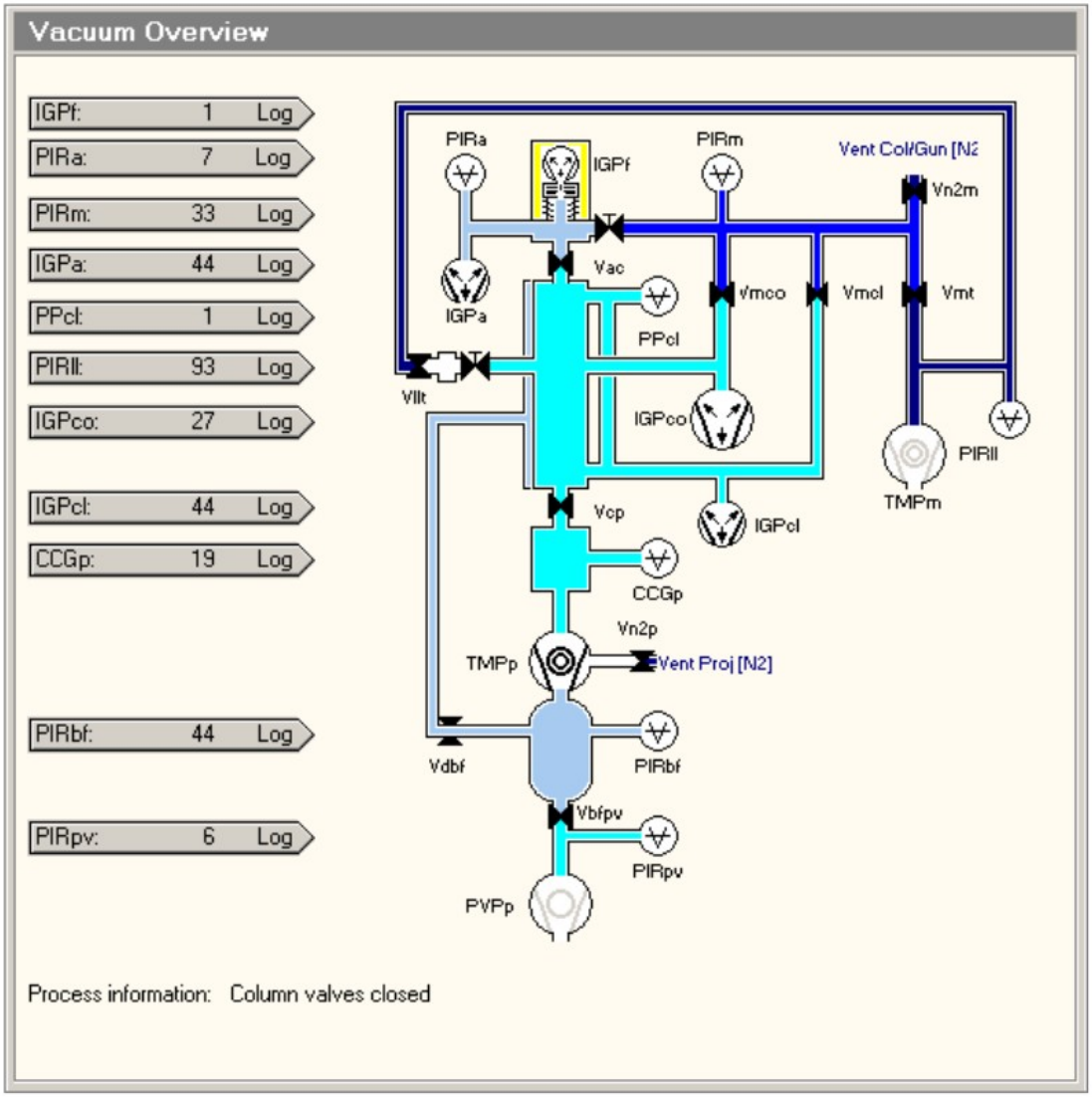


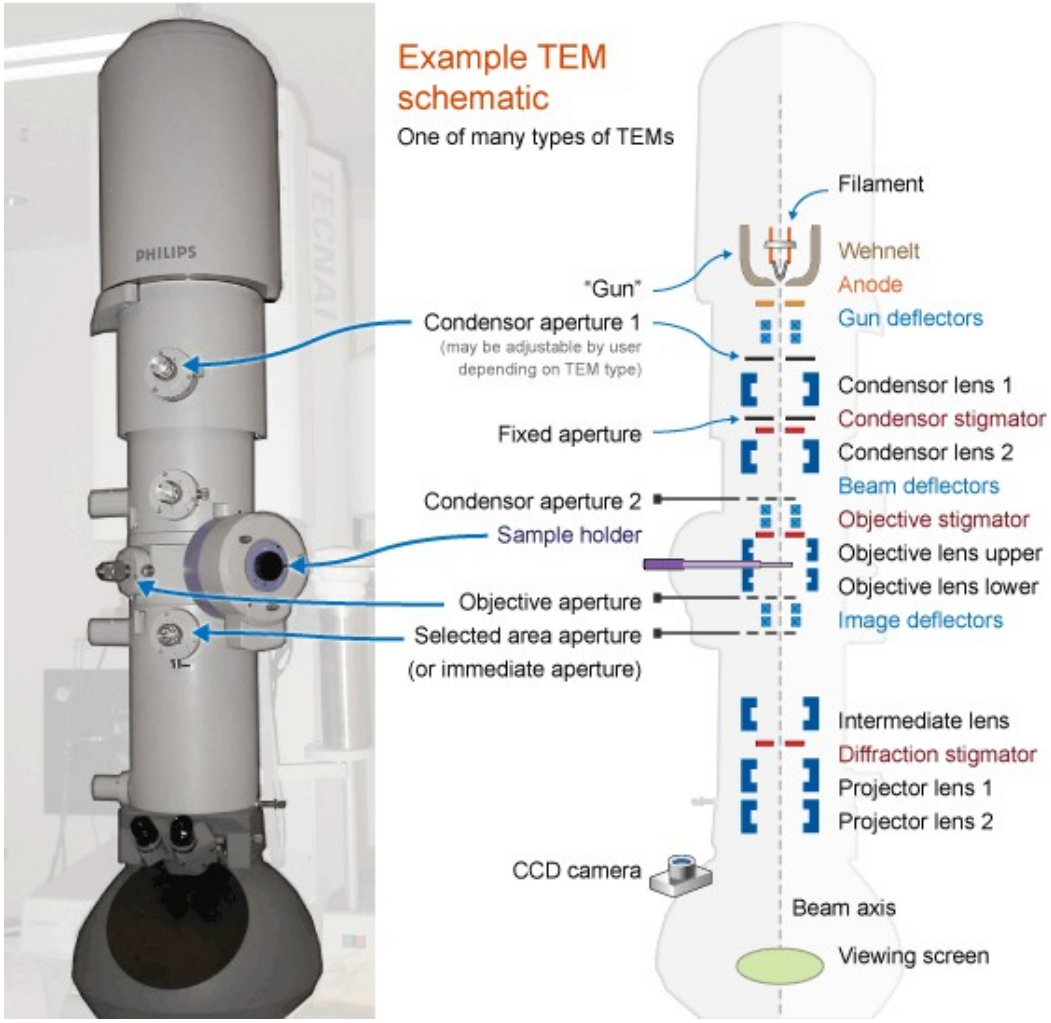

Principle of Operation

External View

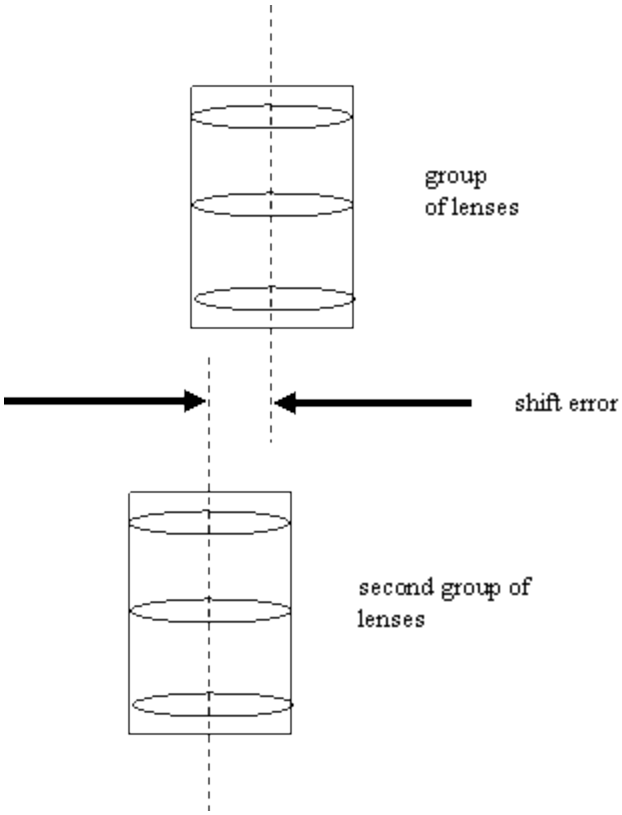
# Vacuum system

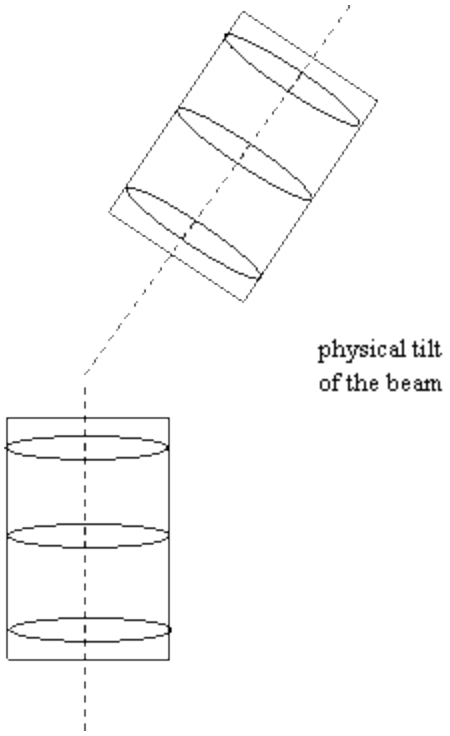


# TEM

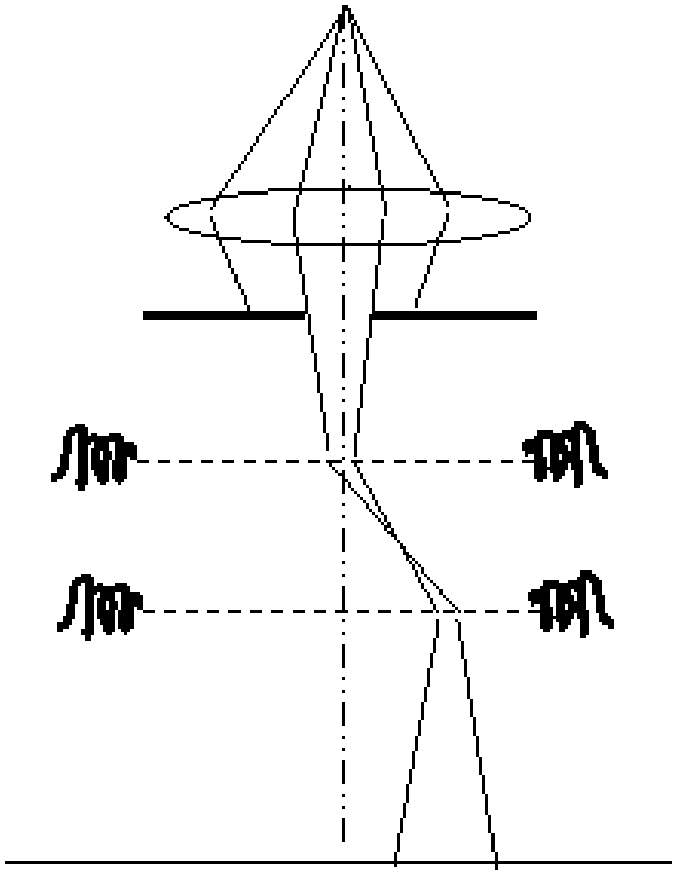

PHILIPS
Example TEM schematic
One of many types of TEMs
Filament
Wehnelt
Anode
"Gun"
Gun deflectors
Condensor aperture 1
(may be adjustable by user depending on TEM type)
Condensor lens 1
Condensor stigmator
Fixed aperture
Condensor lens 2
Beam deflectors
Condensor aperture 2
Objective stigmator
Sample holder
Objective lens upper
Objective lens lower
Objective aperture
Image deflectors
Selected area aperture
(or immediate aperture)
Intermediate lens
Diffraction stigmator
Projector lens 1
Projector lens 2
CCD camera
Beam axis
Viewing screen

# TEM


group
of lenses
shift error
second group of
lenses

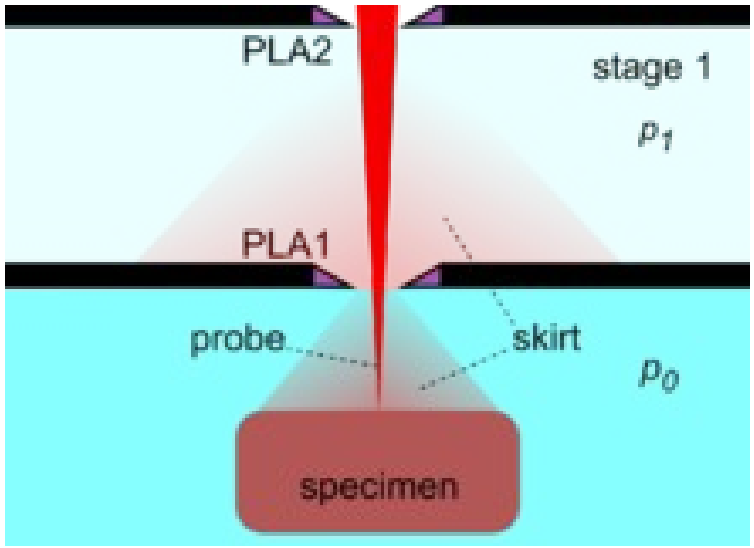

physical tilt
of the beam

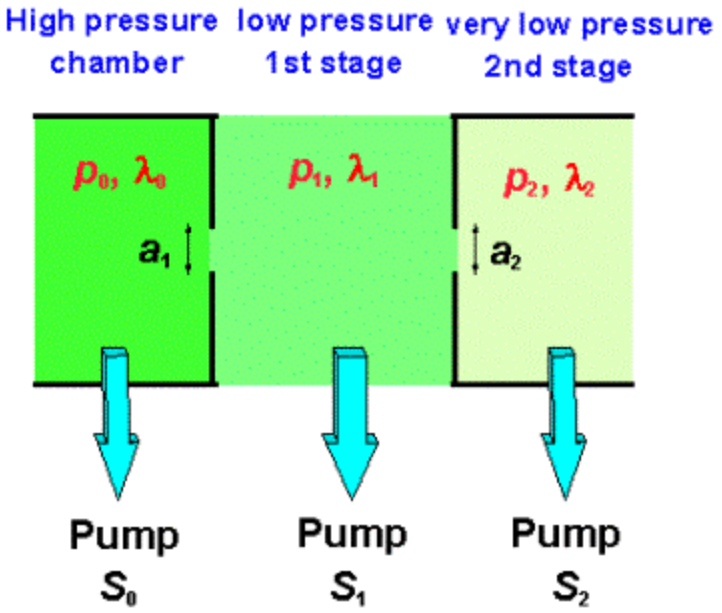
# TEM



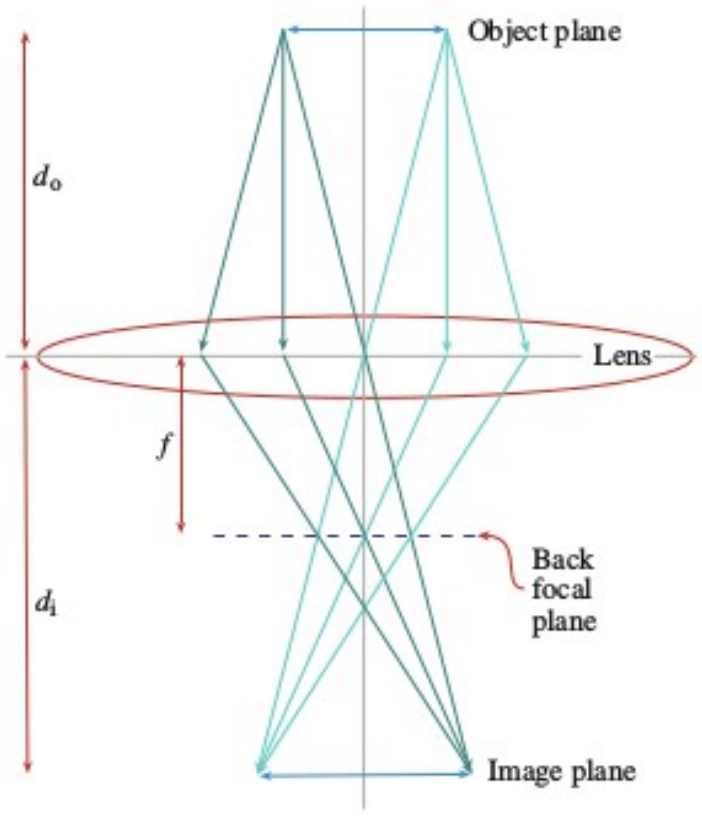
# TEM

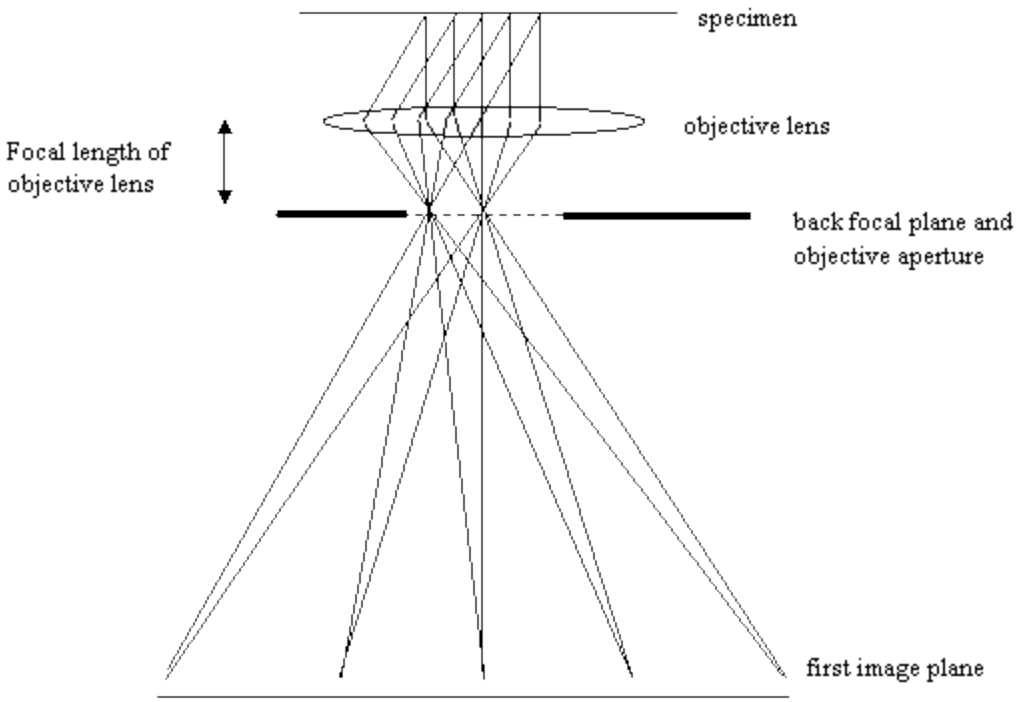
**Differential pumping apperture**



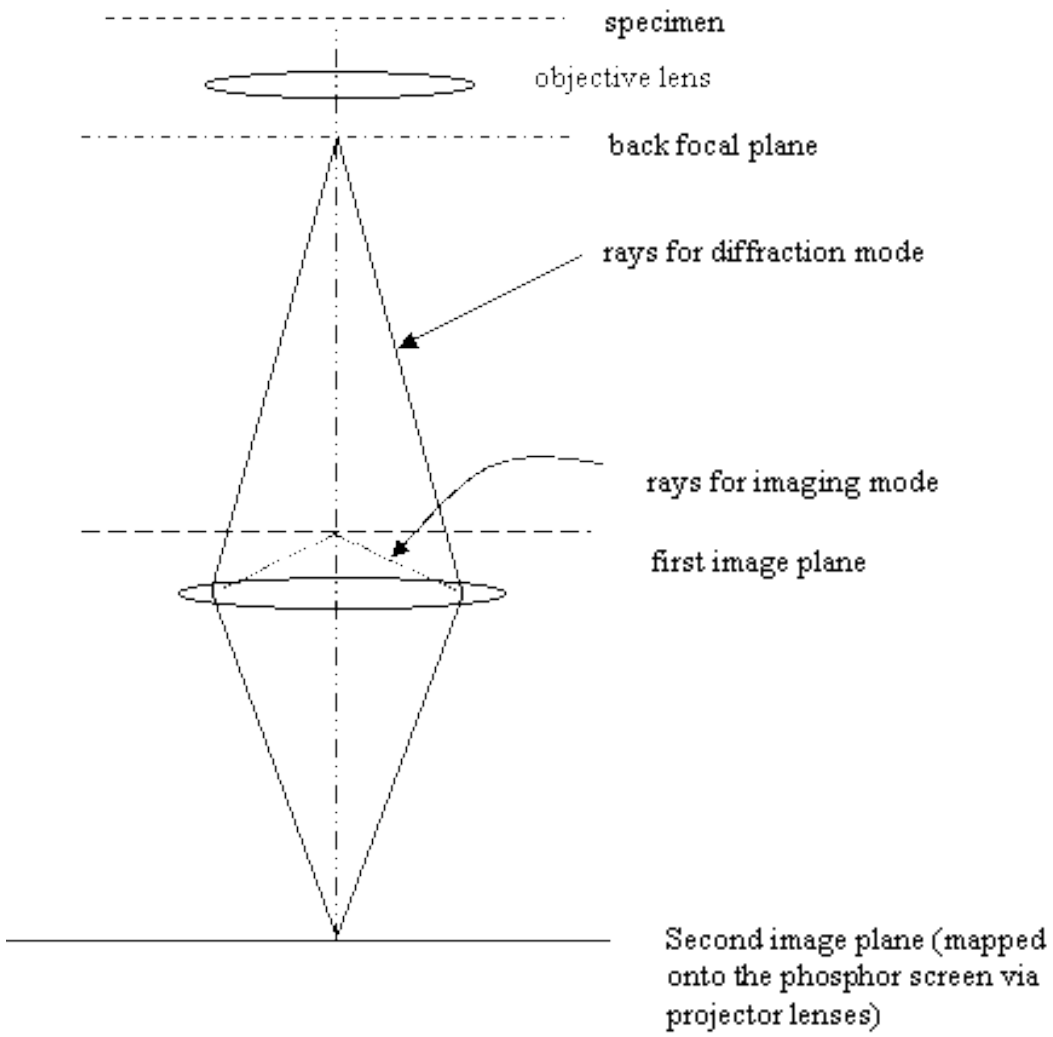



# TEM

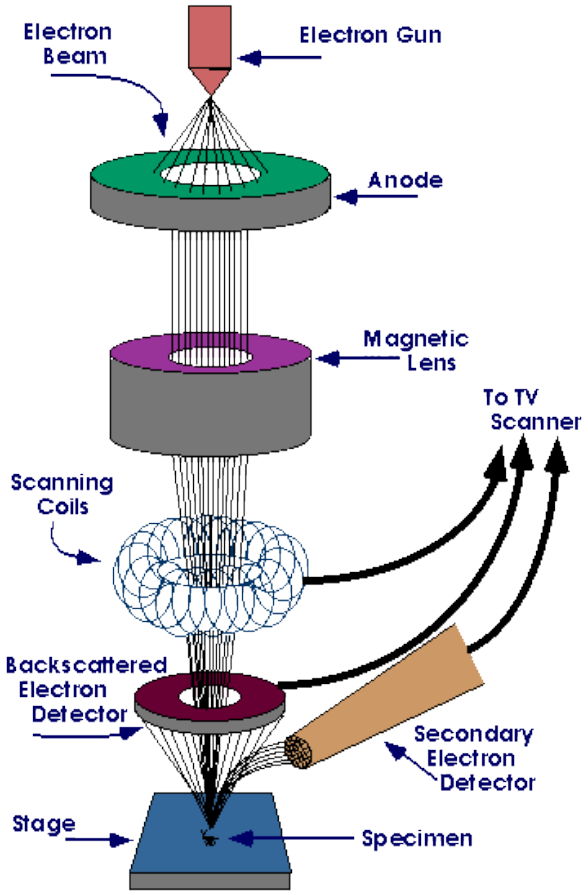

Object plane
$d_o$
Lens
$f$
$d_i$
Back
focal
plane
Image plane

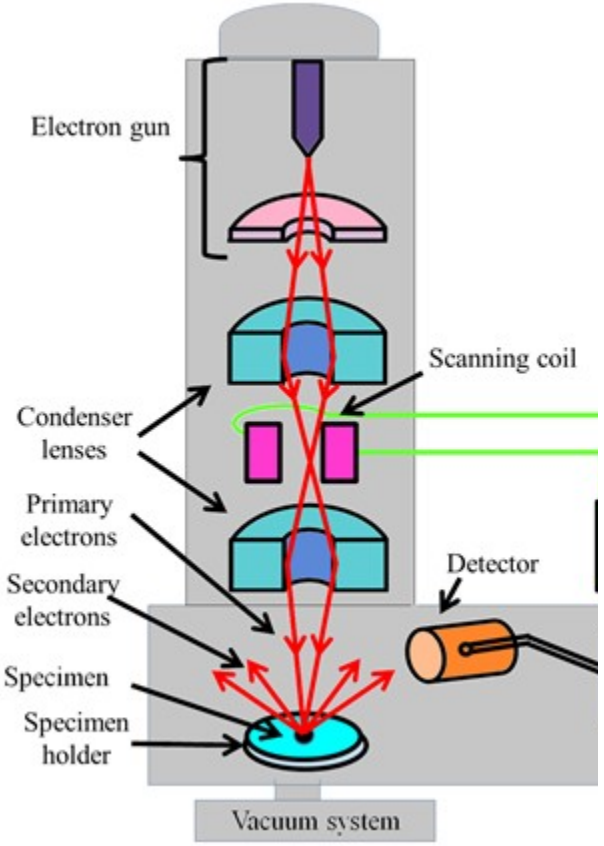

specimen
objective lens
Focal length of
objective lens
back focal plane and
objective aperture
first image plane

# TEM

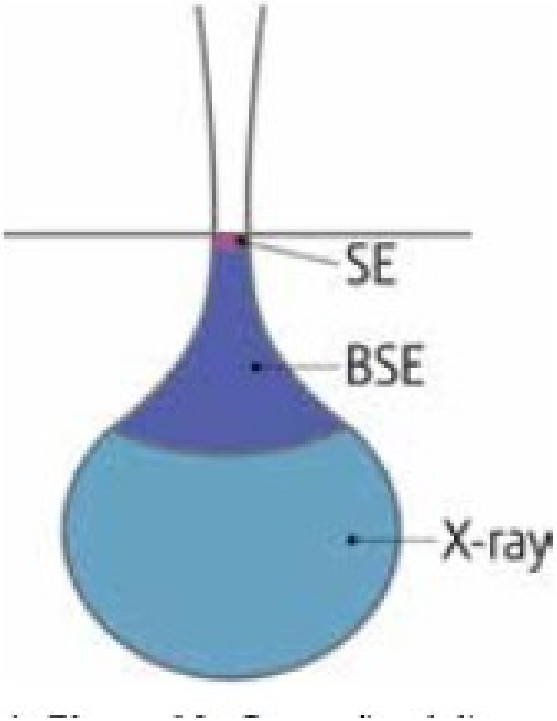

specimen
objective lens
back focal plane
rays for diffraction mode
rays for imaging mode
first image plane
Second image plane (mapped onto the phosphor screen via projector lenses)

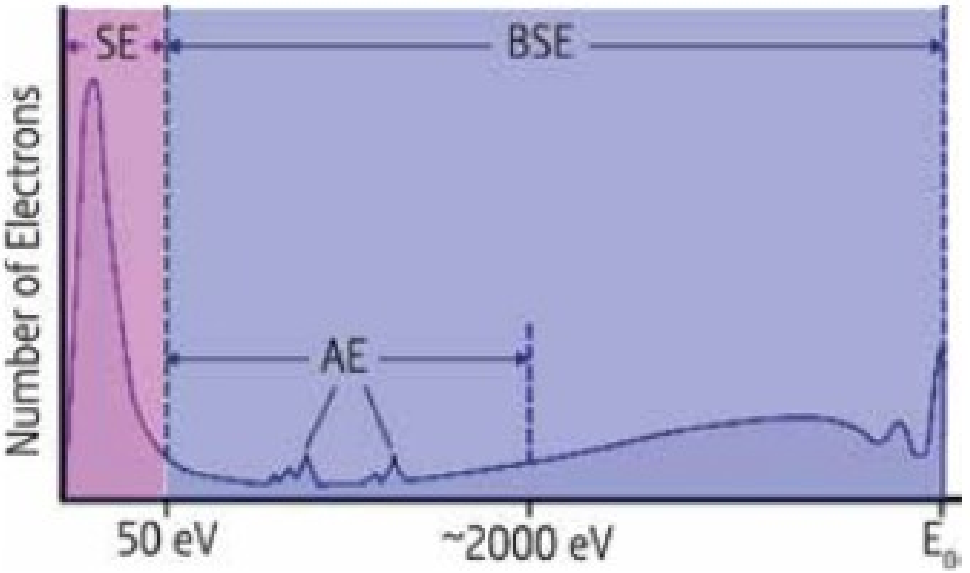
# SEM


Electron Beam
Electron Gun
Anode
Magnetic Lens
To TV Scanner
Scanning Coils
Backscattered Electron Detector
Secondary Electron Detector
Stage
Specimen

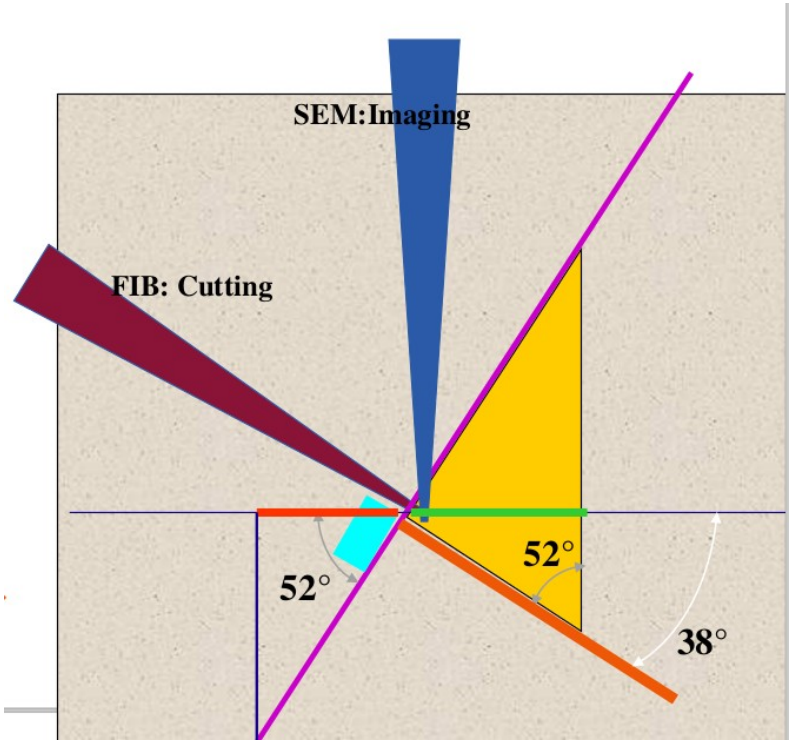

Electron gun
Scanning coil
Condenser lenses
Primary electrons
Detector
Secondary electrons
Specimen
Specimen holder
Vacuum system

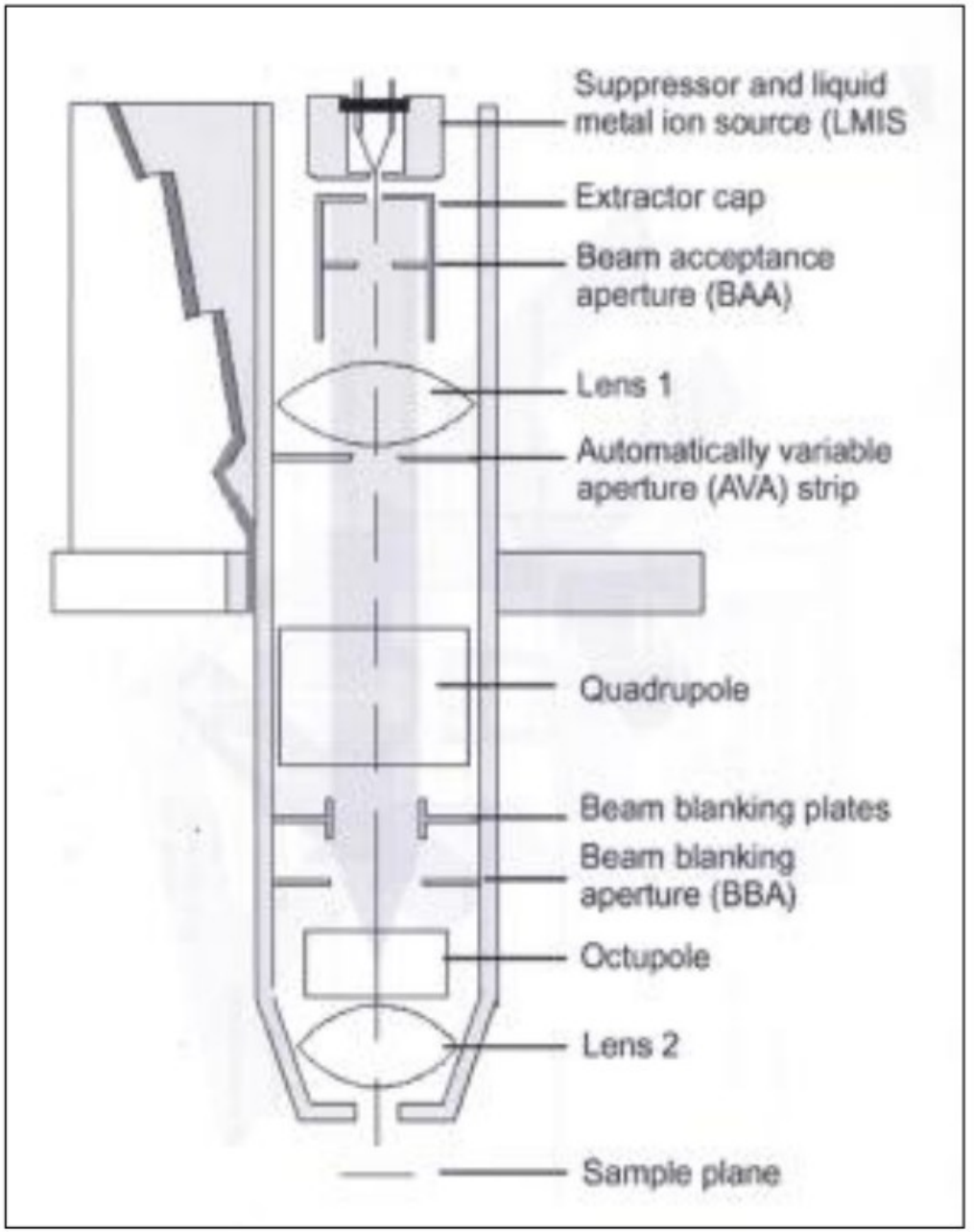
# SEM



# Dual beam FIB/SEM

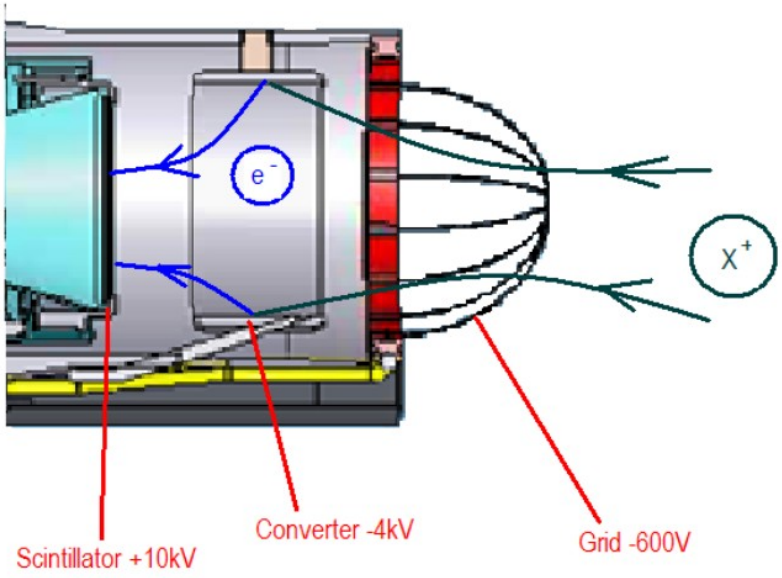


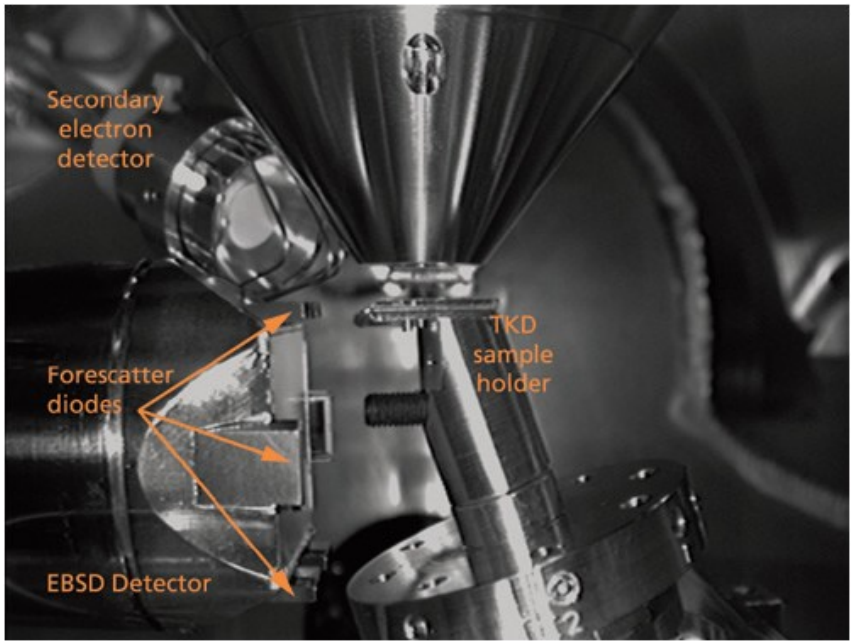



# SEM - detection

- Everhart-Thornley Detector (ETD)

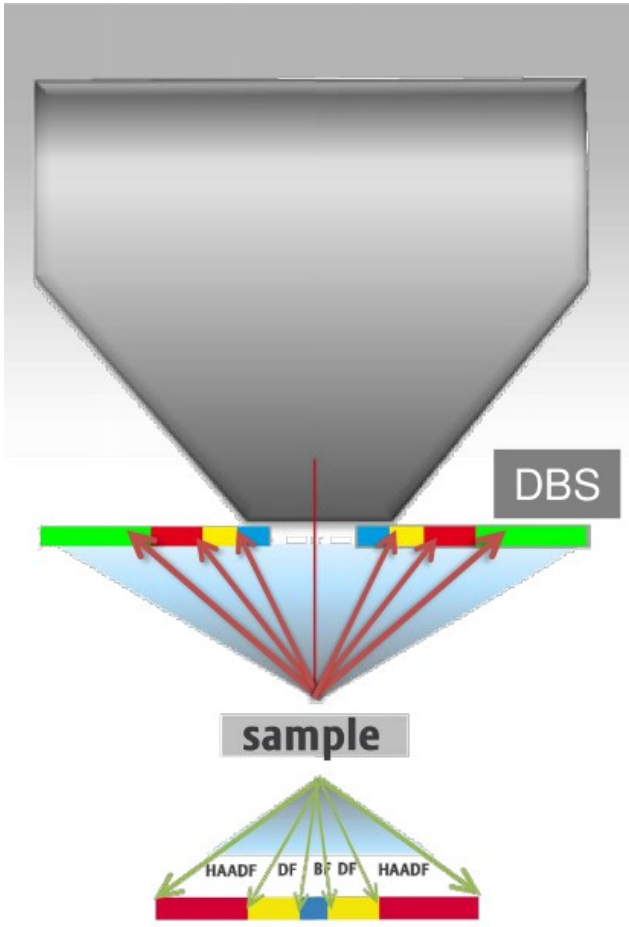
- Ion Convertion to Electron Detector (ICE)



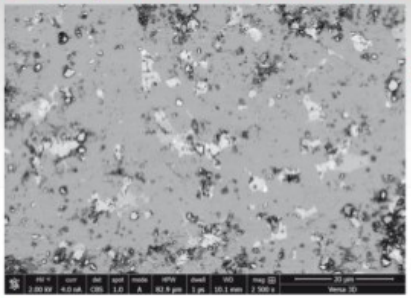
# SEM - detection

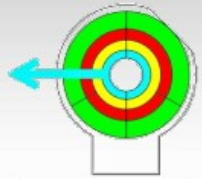
- Concentric Backscatter detector




The Directional Backscatter Electron detector* (DBS) allows collection of surface or compositional information through a Concentric Backscatter mode (CBS) to filter signal from various angles (which can be selected by segment, working distance and/or Beam Deceleration*). A range of angles can be precisely selected based on imaging conditions to reveal unique information.

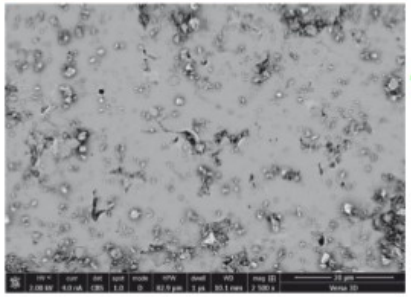
**Composition and material contrast**

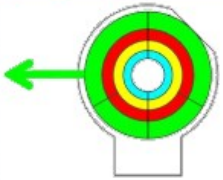




Inner rings collect signal on-axis with the primary beam which contains most channeling or atomic contrast information.

**Surface information and topographic contrast**





Outer rings collect large angle BSE signal, containing mostly topographic information