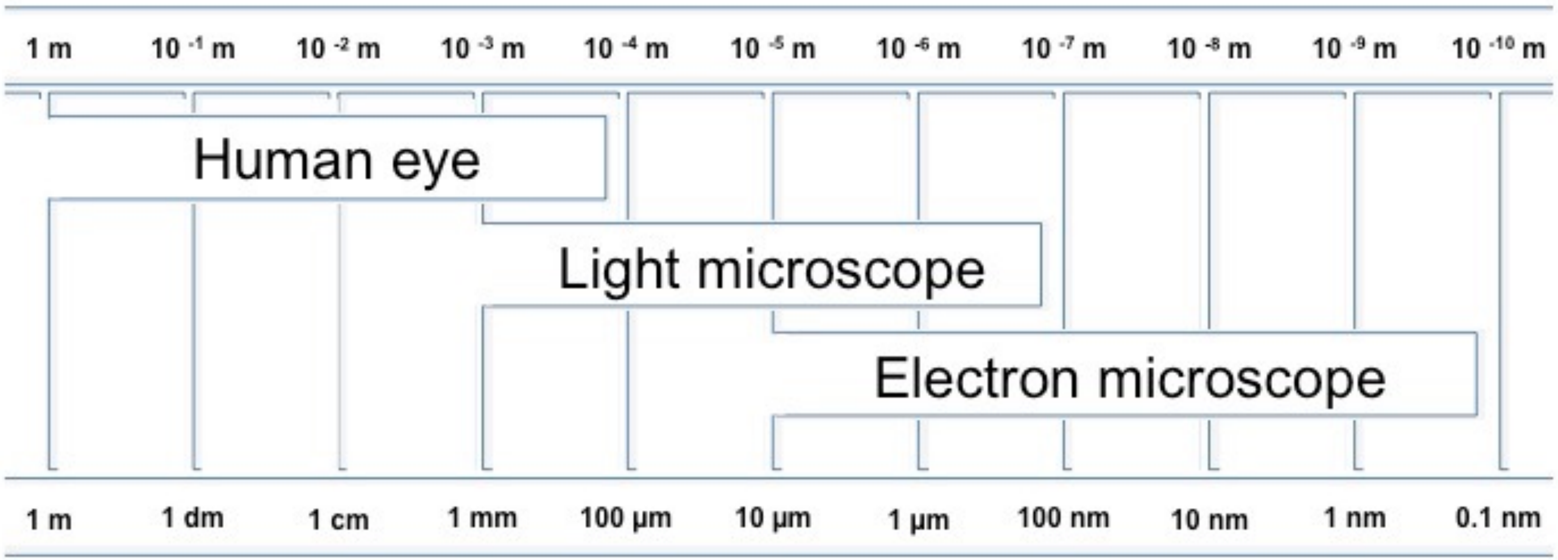
Electron microscopy – a focus on 3D techniques

Why electron microscopy?

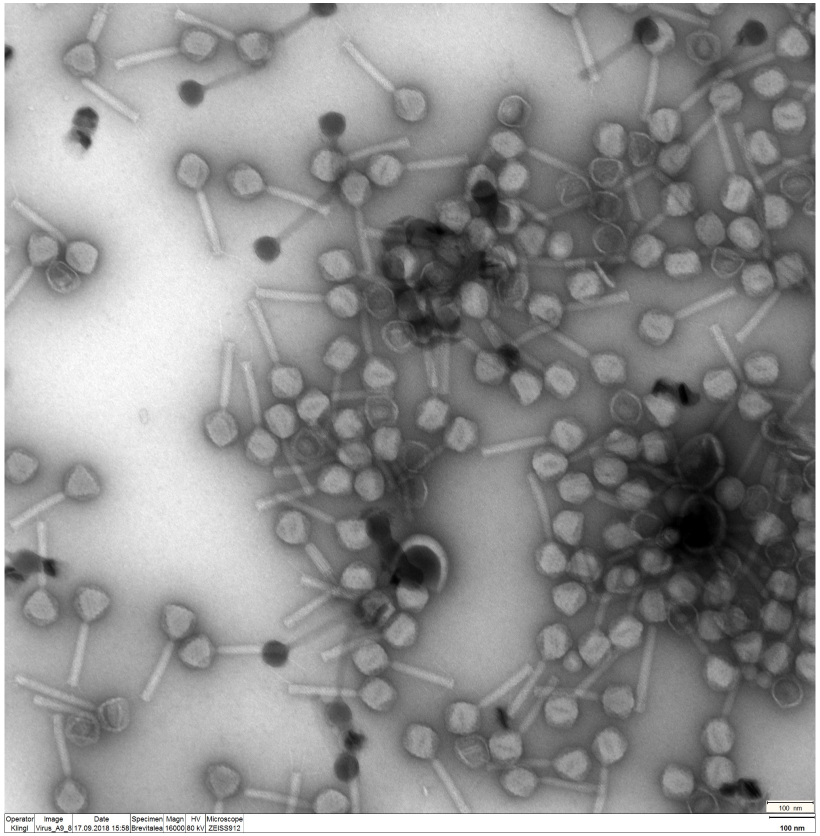
Visualization of cellular and subcellular structures



Virus

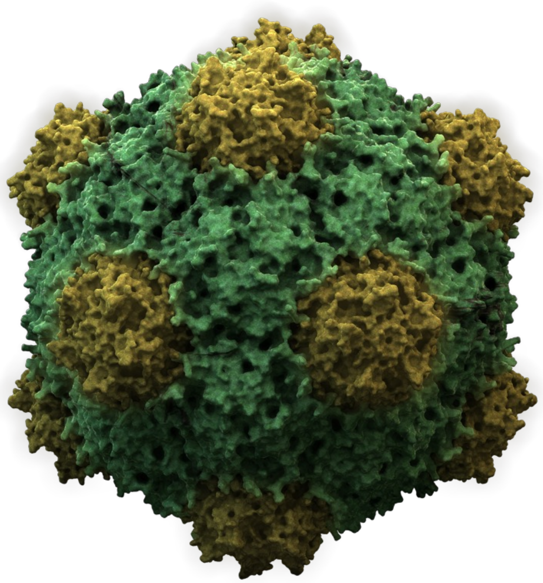
T4-Phage

Diameter: 90 nm
Length: 200 nm



Cowpea mosaic virus

Diameter: 28 nm



https://upload.wikimedia.org/wikipedia/commons/c/c0/CowpeaMosaicVirus3D.png
15.09.15

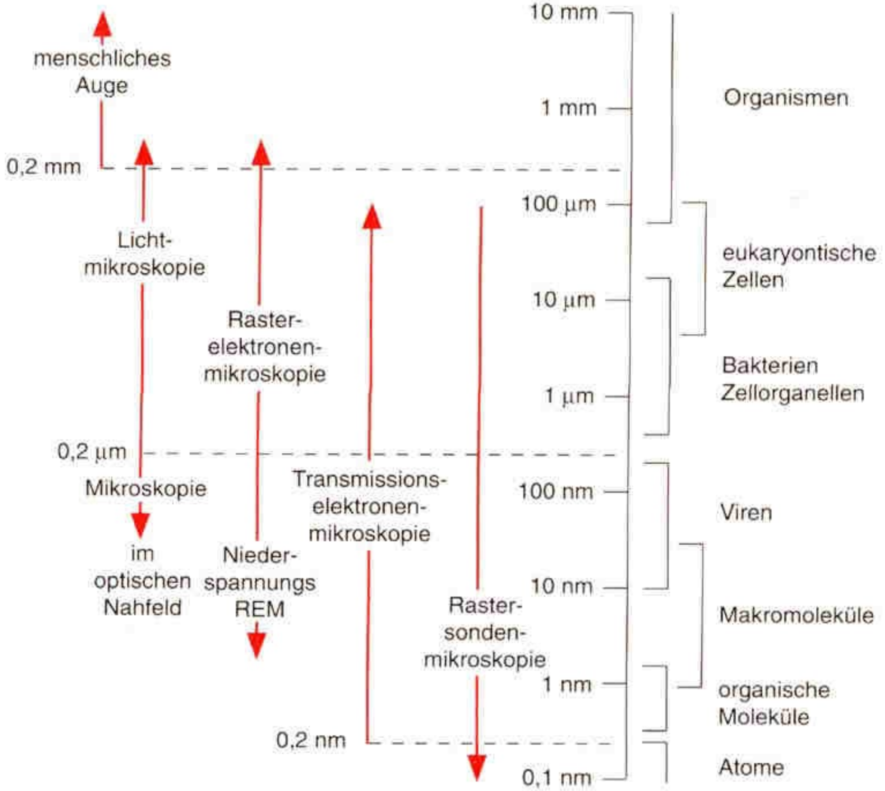
Why electron microscopy?

Das sub-mikroskopische Universum der Viren

Man stelle sich vor, man schrumpfe millionenfach. Könnte man das, so wäre man etwa

Maßstab–vom Atom zum Menschen

© Zoom Tool



© Paul Walther, Universität Ulm

1 mm = 1000 µm

10^{-3} m

1 µm = 1000 nm

10^{-6} m

1 nm = 1000 pm

10^{-9} m

1 pm = 1000 fm

10^{-12} m

1 fm = 1000 am

10^{-15} m

1 am (attometer) = 10^{-18} m

1 nm = 10 Å
1 Å = 10^{-10} m

The transmission electron microscope (TEM)

A transmission electron microscope (TEM) is a device using electron optics to produce magnified images of small, translucent objects.

The general arrangement of TEMs is similar to light microscopes.

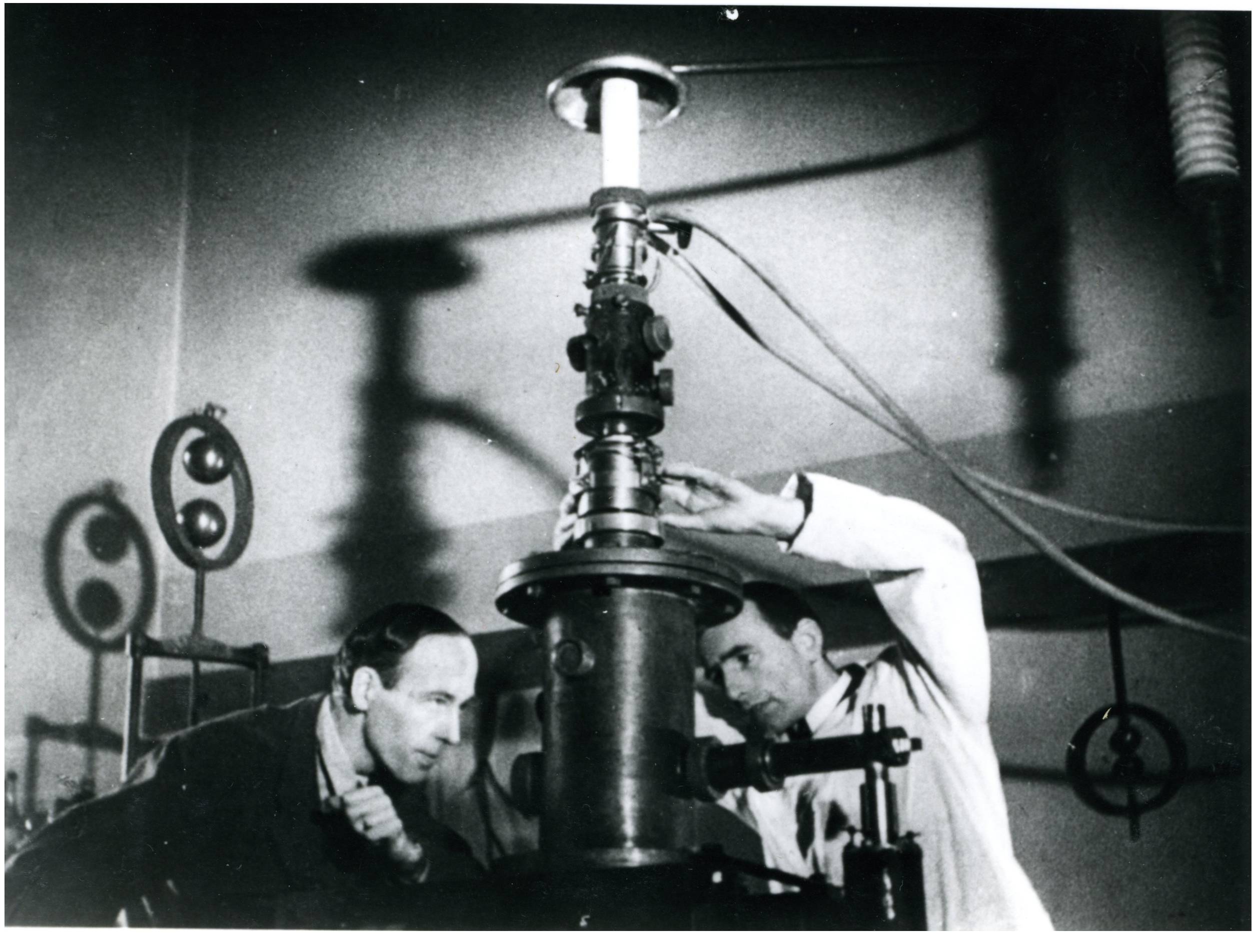
But: electron source instead of light bulbs and the electron beam is focused with electromagnetic lenses instead of glass lenses.

Inside the TEM: vacuum is necessary.

The first TEM was built in 1931 by Ernst Ruska and Max Knoll.

In 1986, Ernst Ruska was awarded the Nobel Prize.

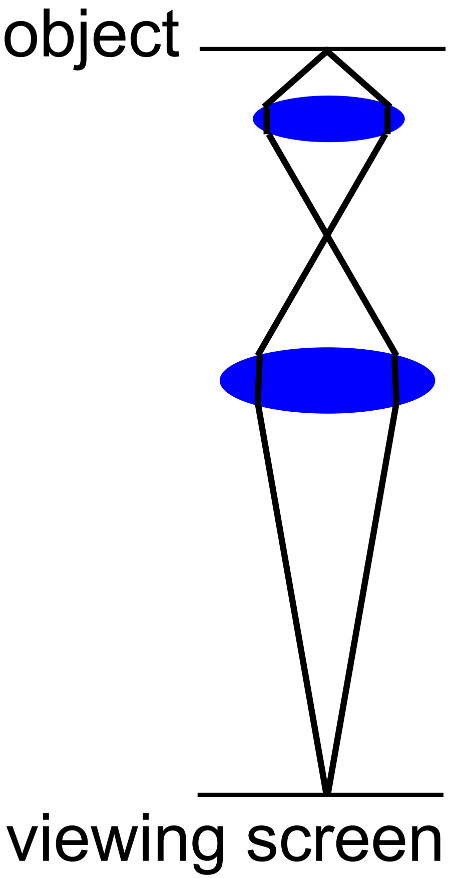
first transmission electron microscope

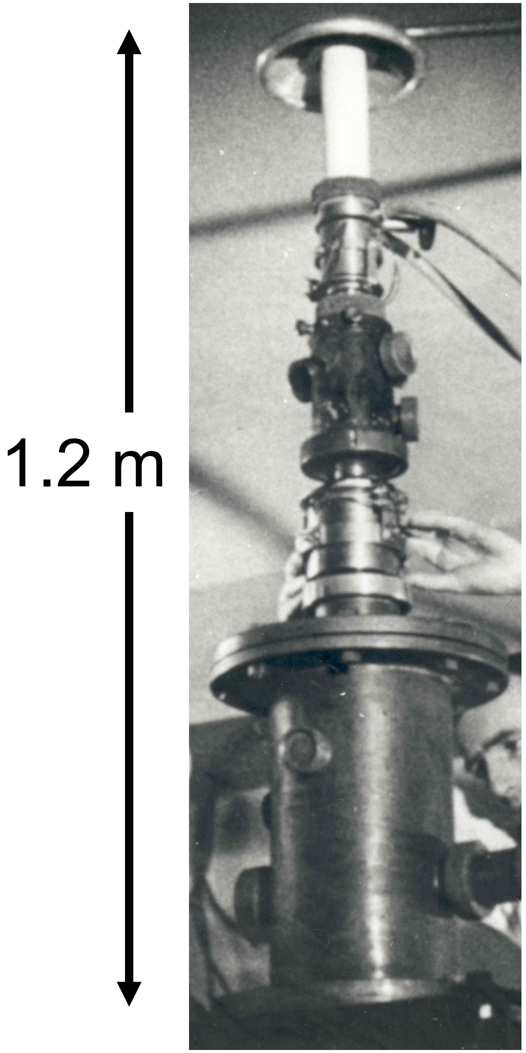


M. Knoll und E. Ruska am ersten Elektronenmikroskop

M. Knoll, E. Ruska: Beitrag zur geometrischen Elektronenoptik I u. II,
Ann. Physik 12 (1932) 607-640, 641-661 (eingegangen 10.9.1931)

first transmission electron microscope





1931

magnification: 16 ×

resolution:

< light microscope

1933

magnification: 12,000 ×

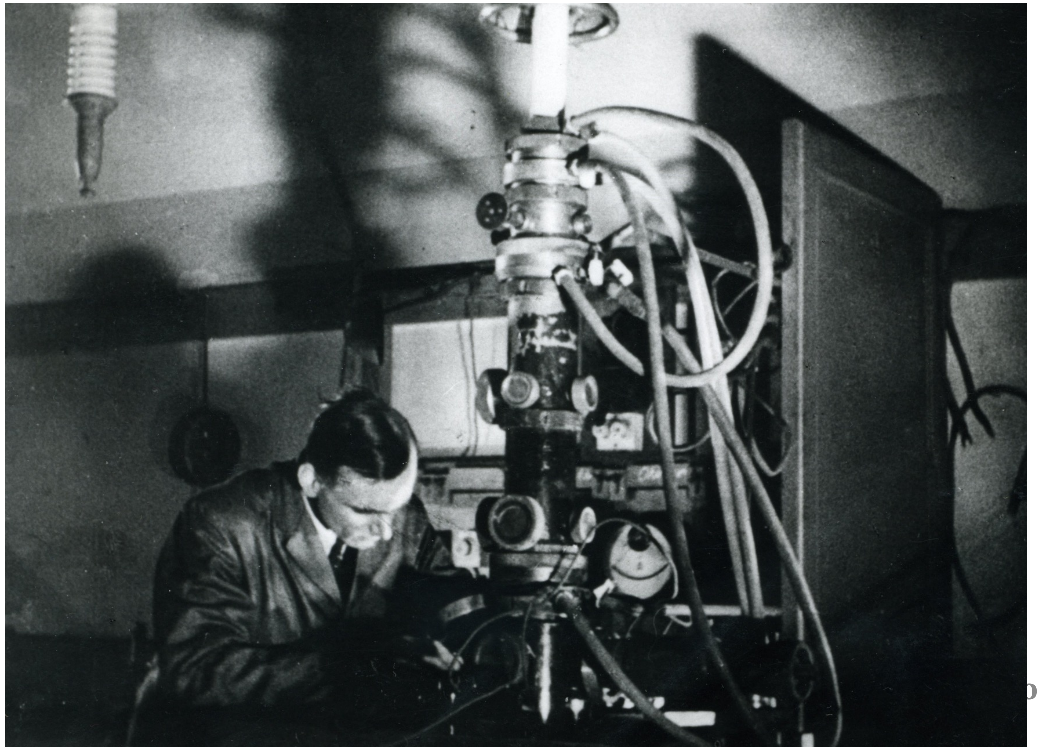
resolution:

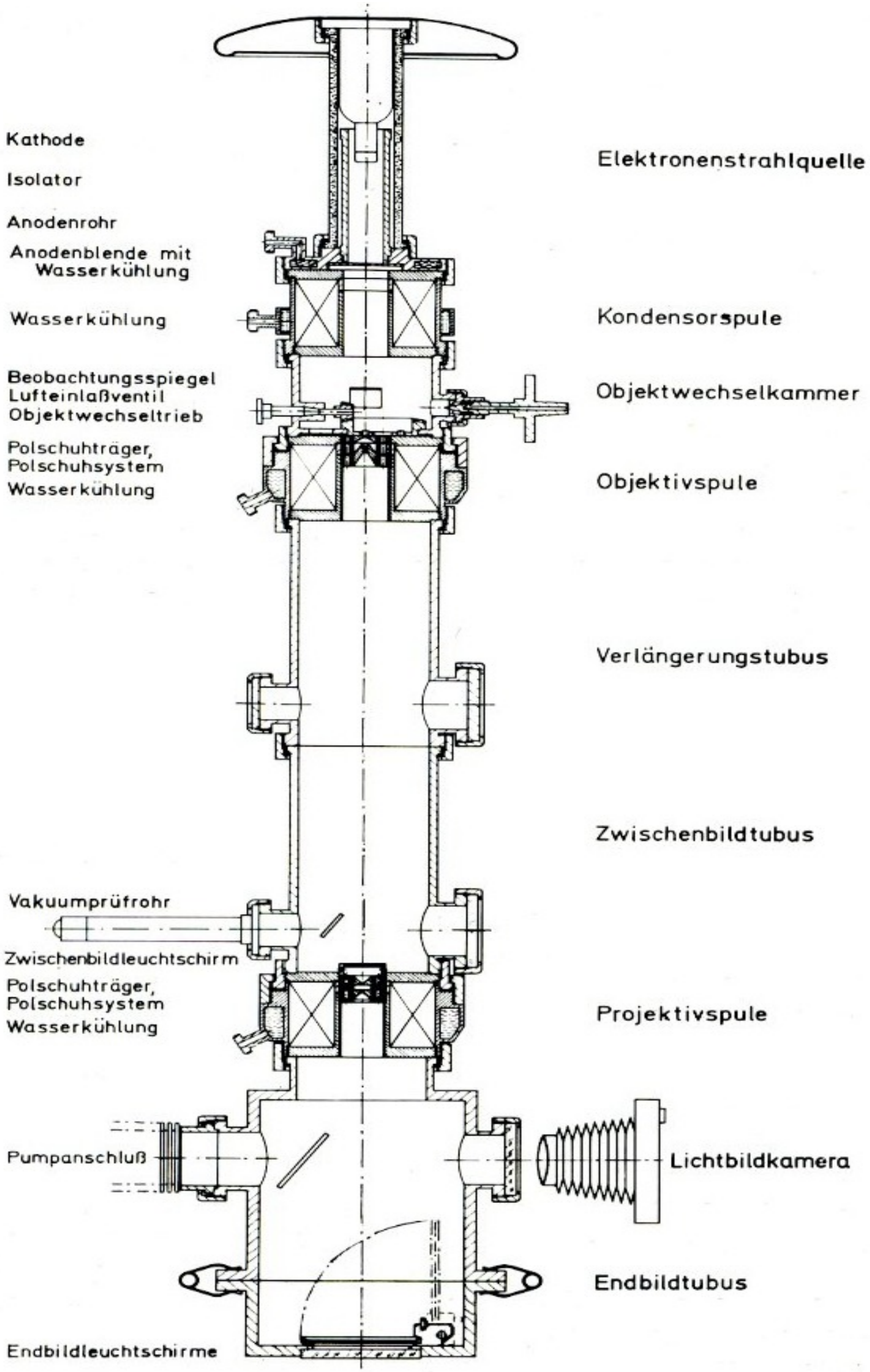
>> light microscope

invention of electron microscope

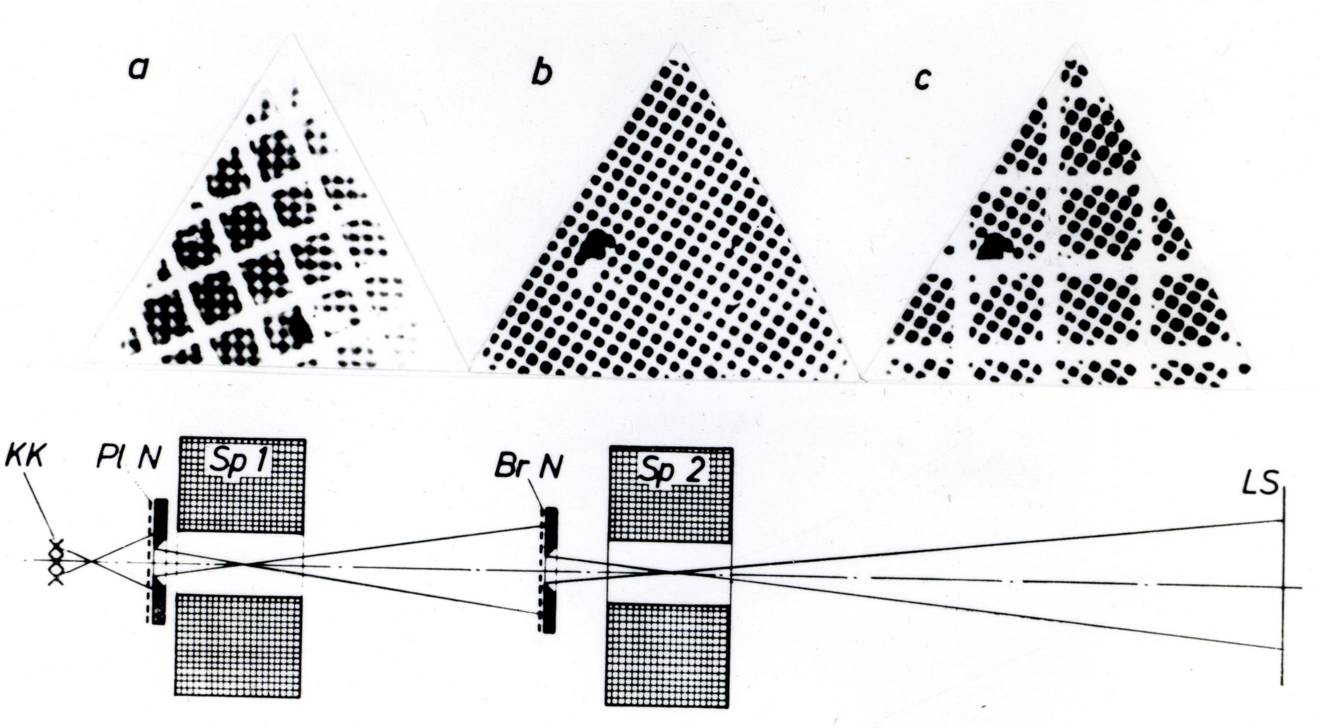
First electron microscope
having higher magnification than light
microcope

E. Ruska: Über Fortschritte im Bau und in der
Leistung des magnetischen Elektronenmikroskops,
Z. Physik 87, (1934) 580-602 (eingeg.: 12.12.1933)





Experimental proof of imaging using magnetic lenses (7.4.1931)



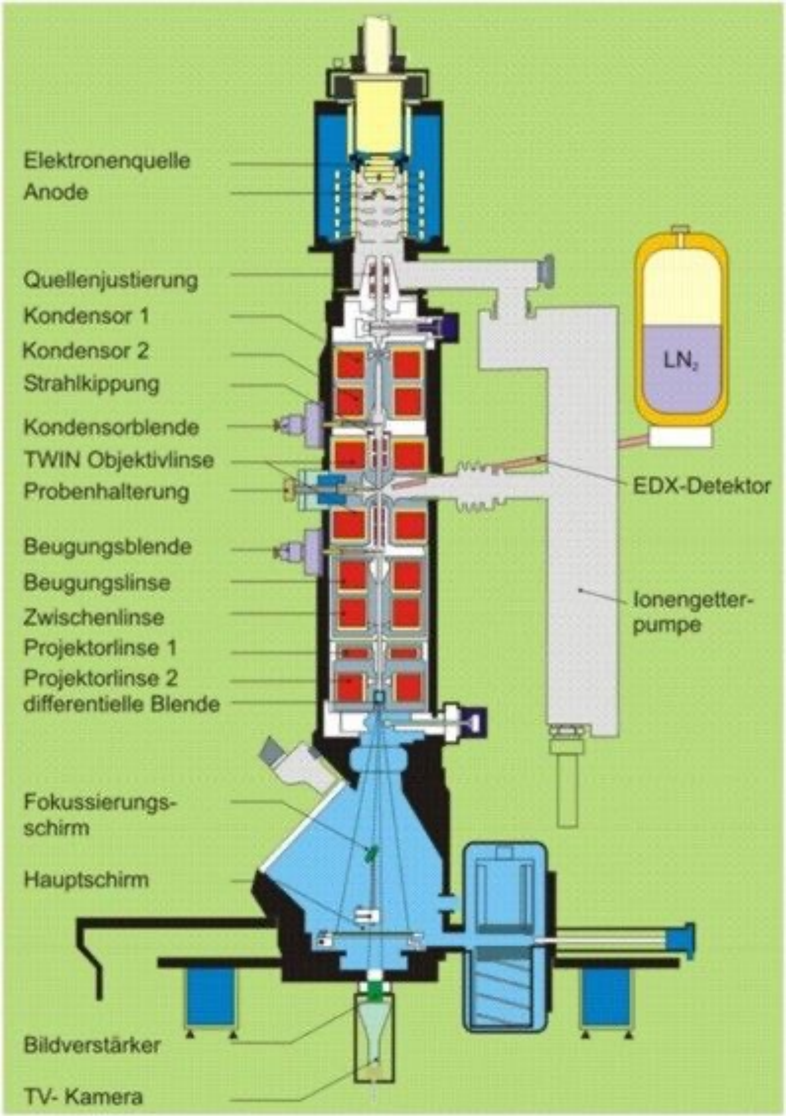
a) One-step image of platinum-grid in front of coil 1 formed by coil 1; M_{el} 13,0 :1

b) One-step image of bronze-grid in front of coil 2 formed by coil 2; M_{el} 4,8 :1

c) Two-step image of platinum-grid in front of coil 1 formed by coil 1 and coil 2; M_{el} 17,4 :1 together with the one-step image of the bronze-grid image from coil 2; M_{el} 4,8 :1

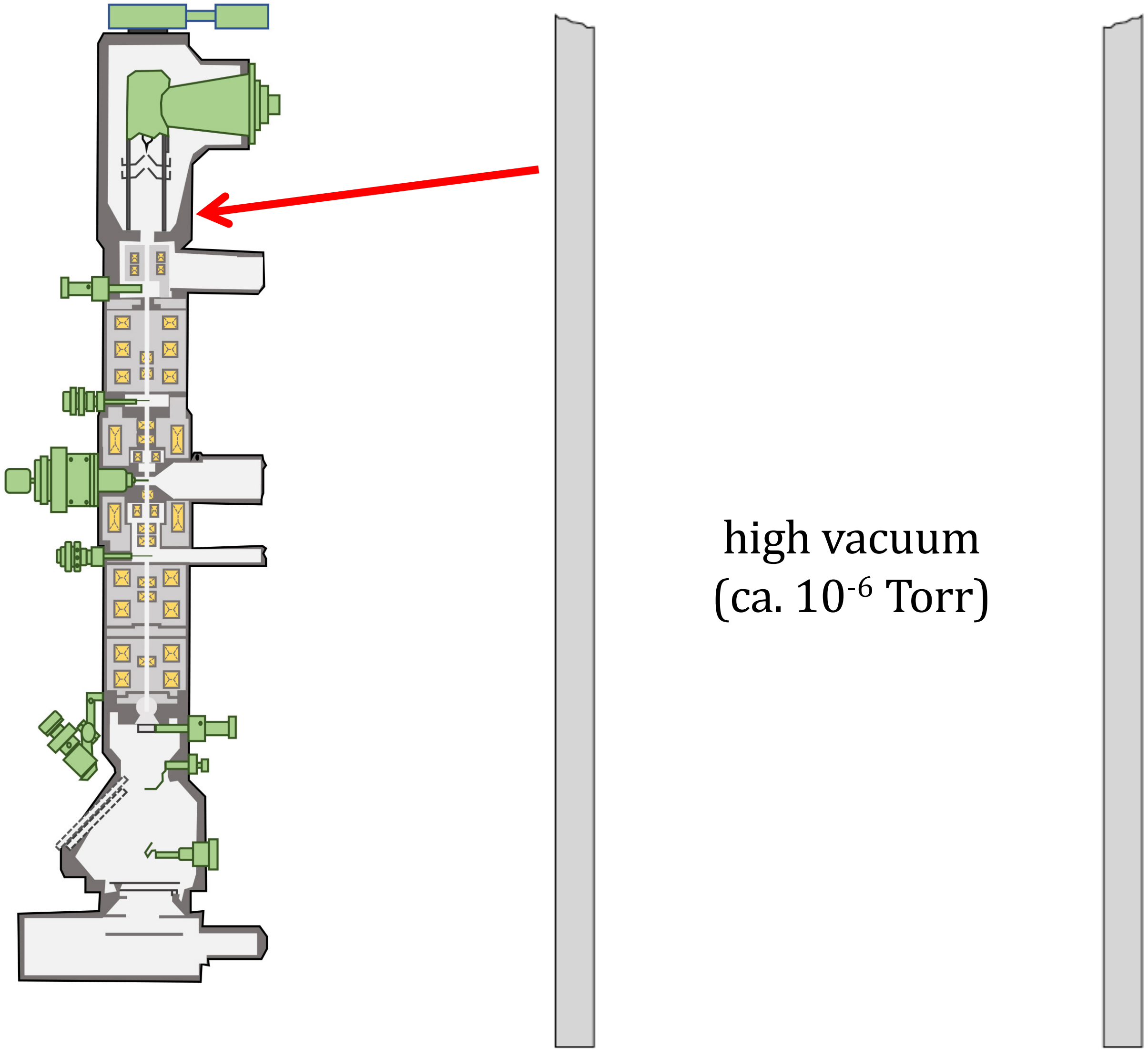
The electron microscope: vacuum, cathodes, lenses

General composition of a TEM



Schematischer Aufbau eines TEM CM200 (nach Philips-Firmenschrift)
http://www.msz.ovgu.de/Labore/Transmission_Elektronen_Mikroskopie+TEM/TEM+Philips.print

The column



high vacuum
(ca. 10^{-6} Torr)

1 Torr = 1,3 mbar

Why do we need high vacuum in EM?

Cathode

Utilization of high tension without vacuum results in short-circuits caused by ionization.

The cathode interacts with gas molecules at high temperatures („burn through“).

Column

Any deflection of beam electrons (= scattering) in the vacuum reduces the resolution.

Conclusion: The better the vacuum, the higher the resolution.

The „mean free path“ of electrons should be as large as possible.

The „mean free path“ is the average distance, travelled by a gas molecule, before colliding with another gas molecule. The lower the pressure, the lower the amount of gas molecules→ the „mean free path“ becomes larger
Unit: [m], symbol: I; p x I = const., but different for distinct gasses

Vaccum: a two-component system

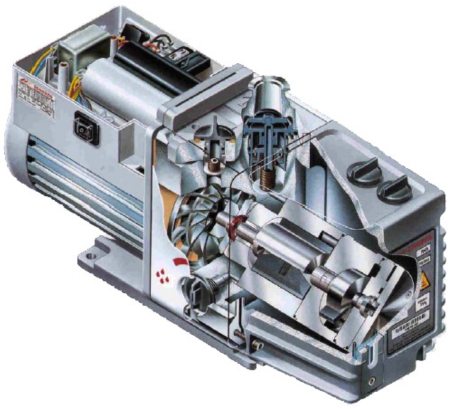
a simple example, up to 10 - 30 mbar

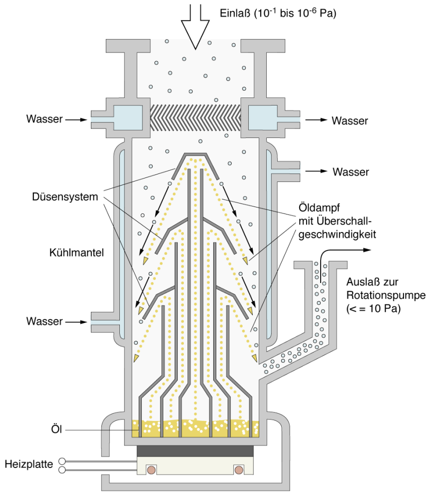
A rotary pump

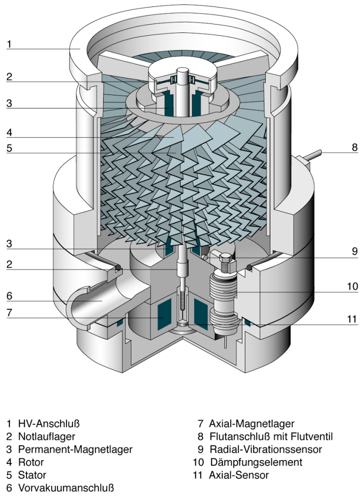
combined with

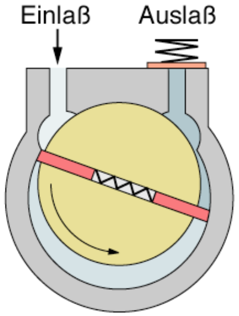
high-vaccum pump: turbomolecular pump, oil-diffuson pump, ion-getter-pump

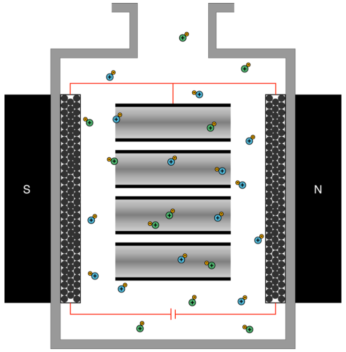
Drehschieberpumpe











Vaccum: a two-component system

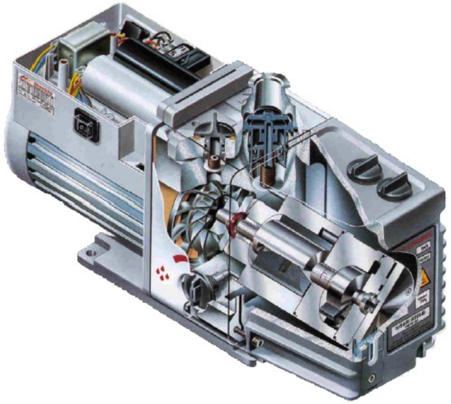
a simple example, up to 10 - 30 mbar

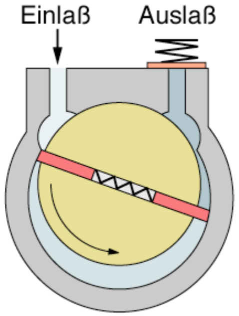
A rotary pump

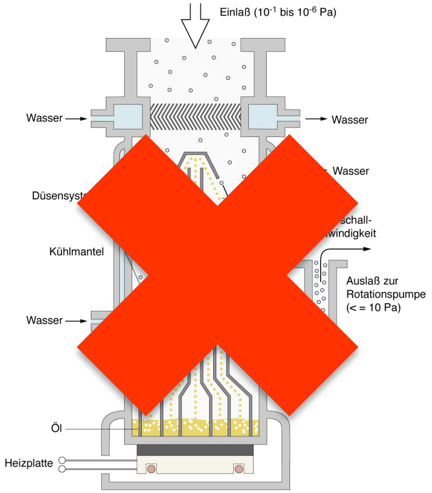
combined with

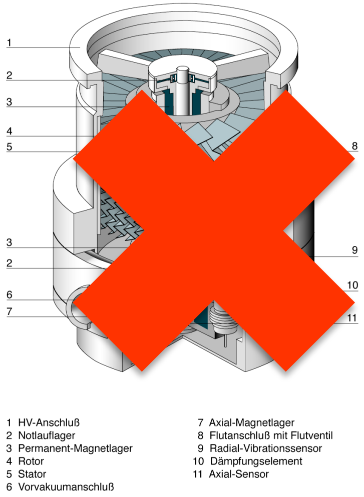
high-vaccum pump: ~~turbomolecular pump~~, ~~oil-diffuson pump~~, ion-getter-pump

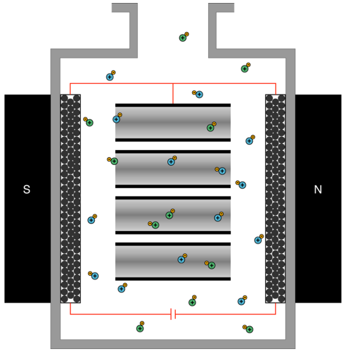
Drehschieberpumpe



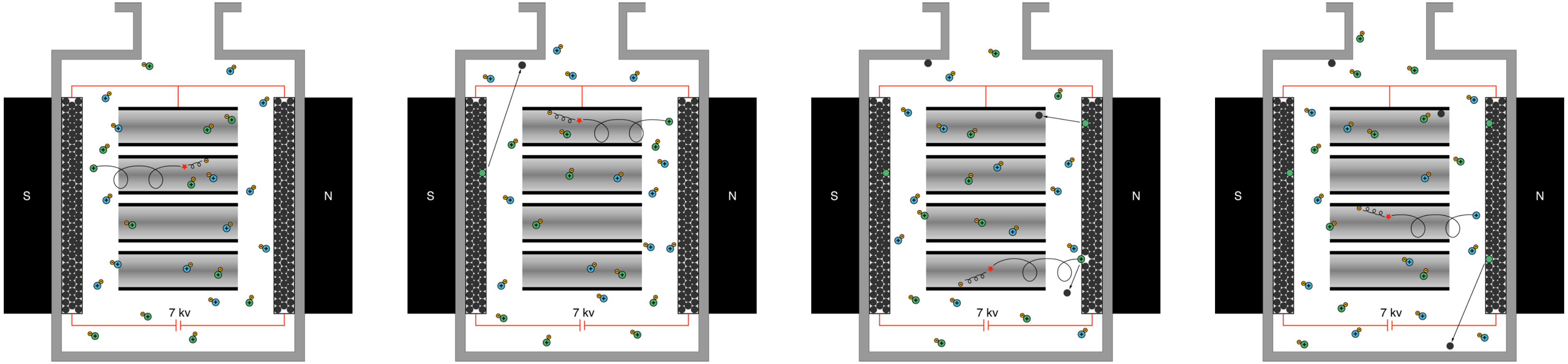


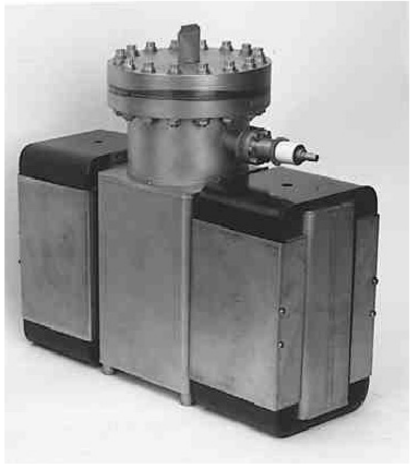






Ion getter pump

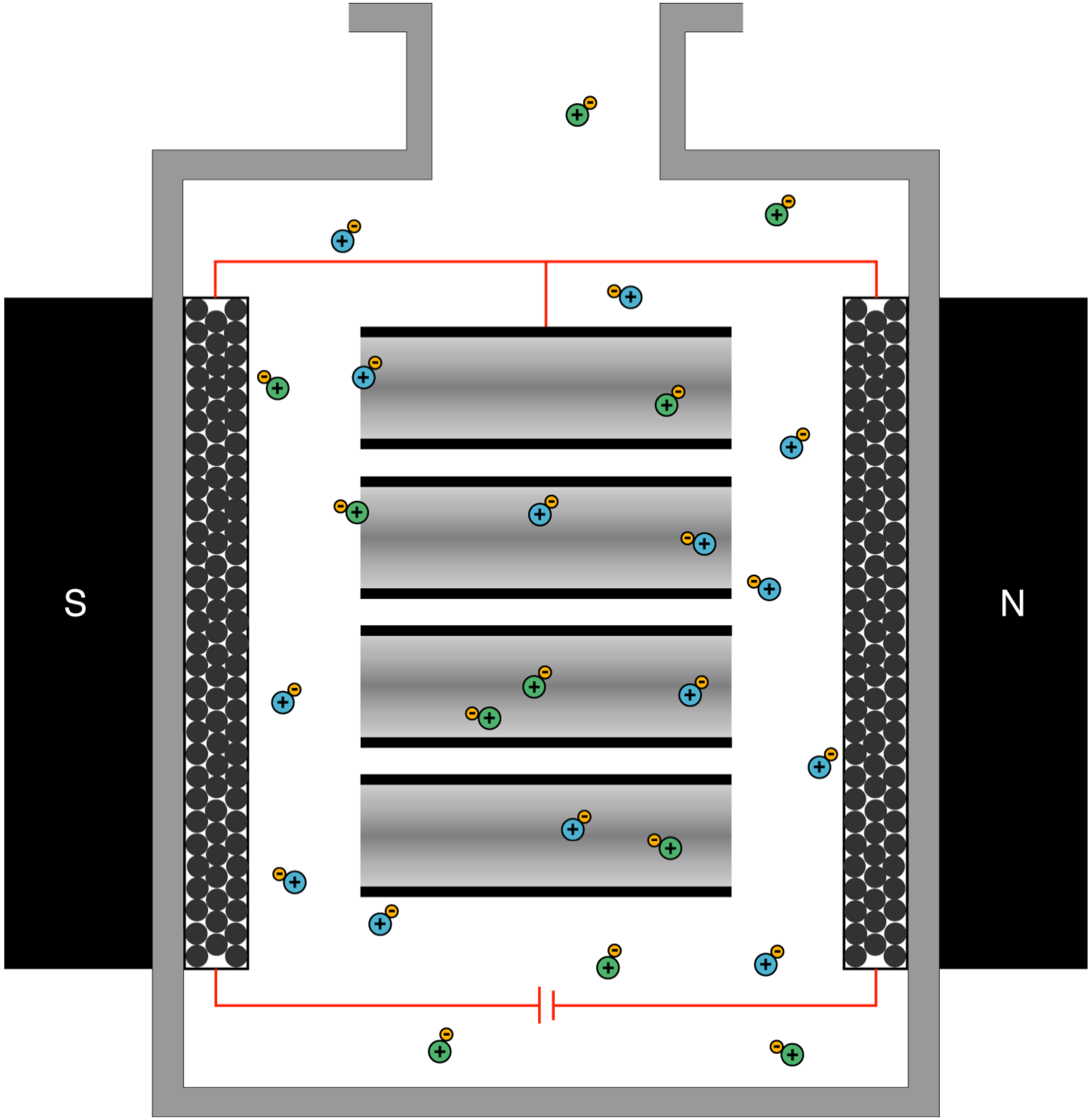




Gas ions are generated inside using magnets and a pipe system. (Up to 10^{-11} mbar)

Ion getter pump

(up to 10^{-11} mbar)



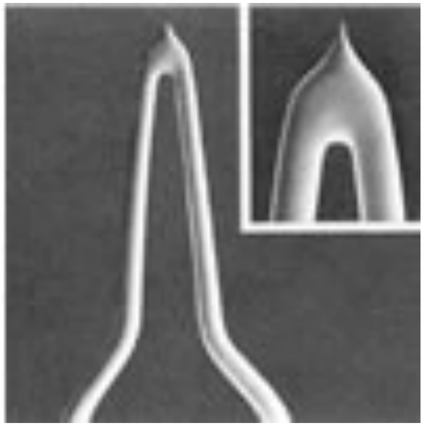
Thermoionic Emitters: Hairpin cathode

https://www.microtonano.com/de/EBS-Wolfram-EM-Kathoden.php

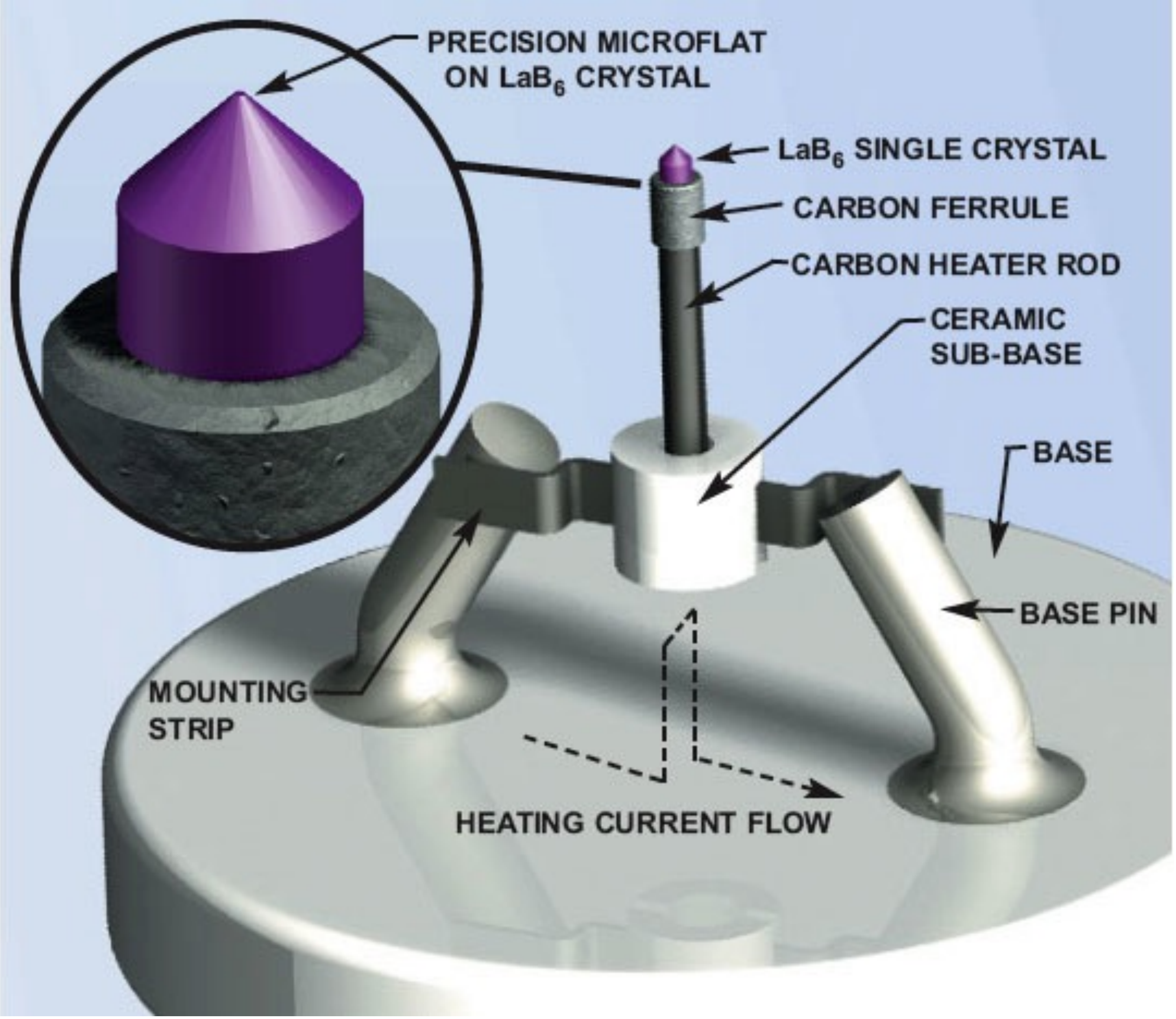
http://scienceservices.de/de/verbrauchsmaterial-werkzeuge/kathoden

lancet cathode

tip cathode



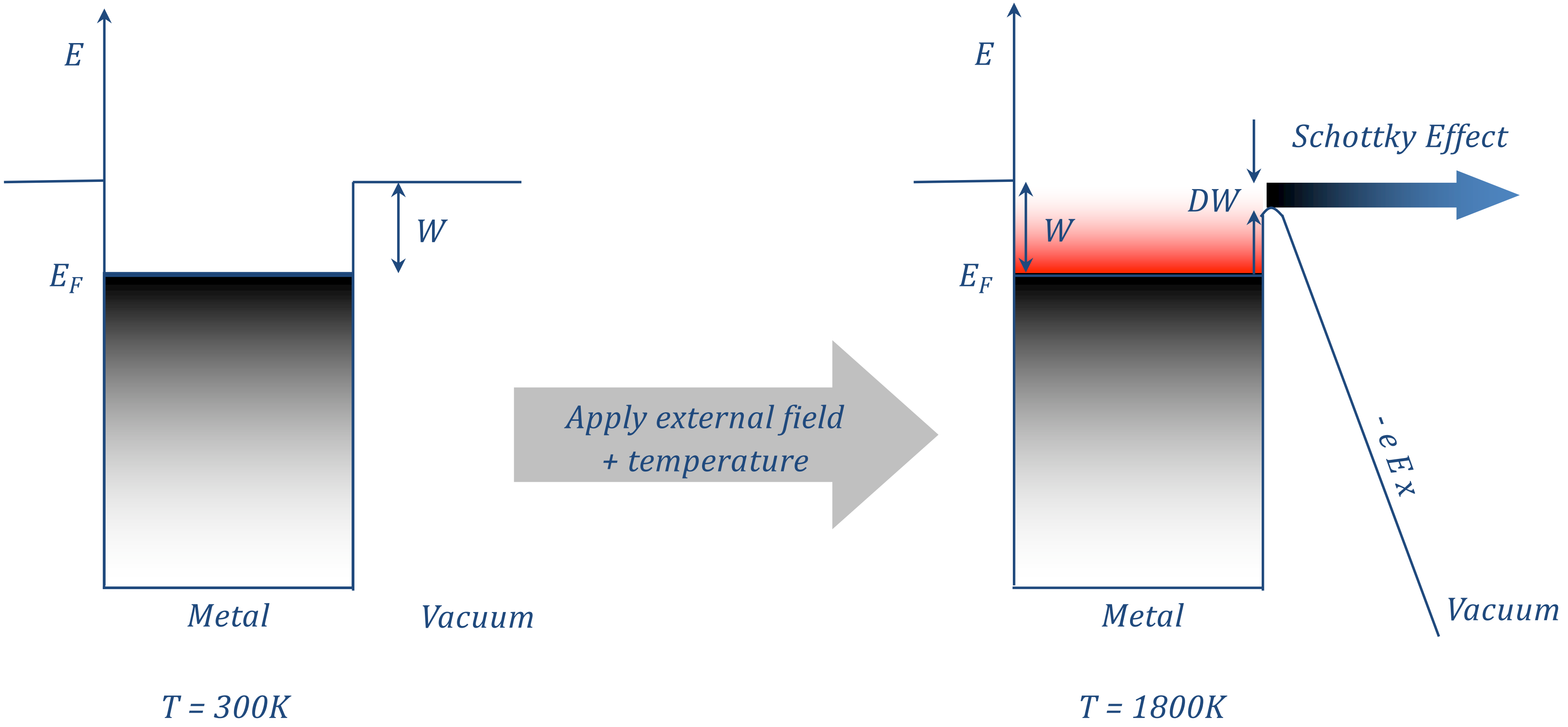
Thermoionic Emitters: LaB_6 cathode



© Plant Development

Emission System: Schottky - (Field) Emitter

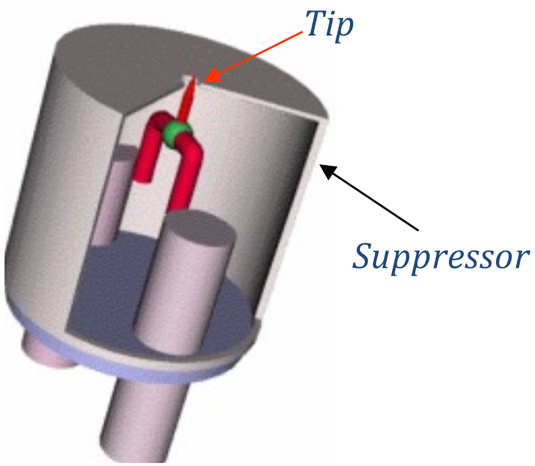
Material: ZrO / W (100)



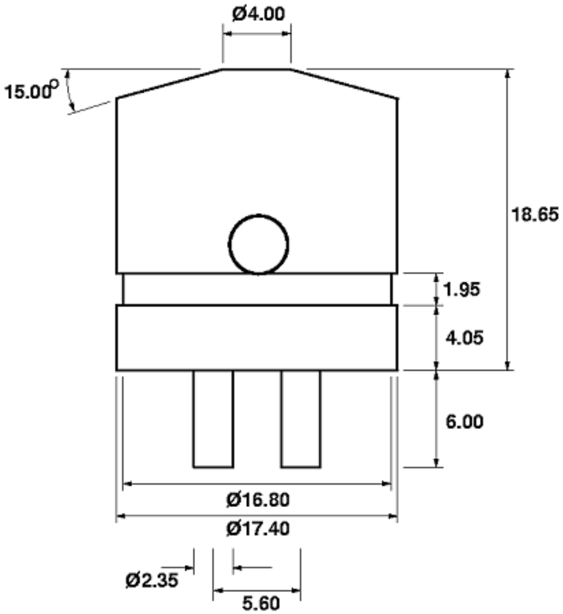
Emission mechanism of a Schottky field emitter:

By covering the tip with ZrO and applying a strong electrical field the work function W is decreased (Schottky Effect). Electrons from the Fermi level E_F are lifted to a higher energy level by heating the emitter and overcome the work function W below the vacuum level

Emissionssysteme: Schottky - (Field) Emitter



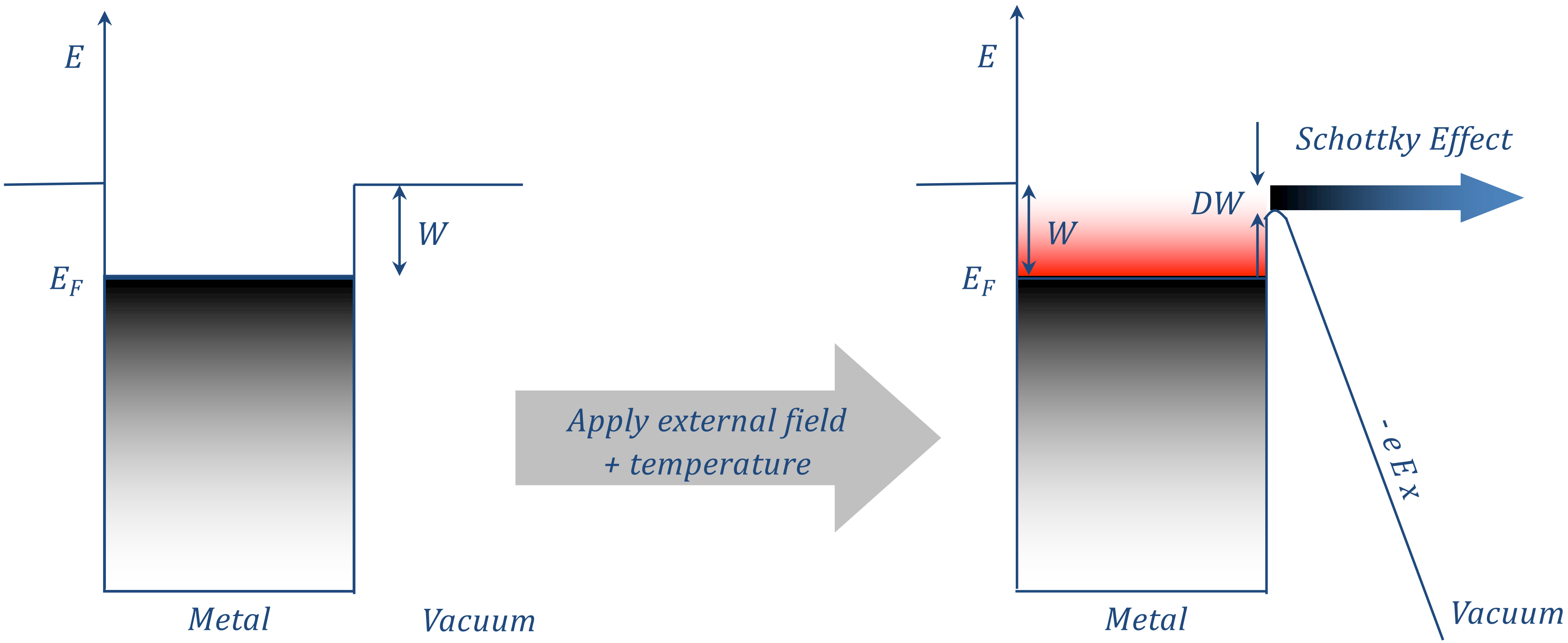
Schottky Emitter – Suppressor Cartridge



A single crystal tungsten wire with a sharp end etched to a small radius (red in the sketch) is mounted on a tungsten hairpin (also red).
A current through the filament is used to maintain the **tip** at a temperature of 1750 - 1850 K.
The tip just penetrates a hole in a cylindrical **suppressor** electrode mounted around the assembly.
Electrons are emitted from the tip due to both thermal excitation and the electric field at the tip due to the potential difference between it and an **extractor** electrode (not shown).
Electrons from the filament are repelled by the potential on the suppressor.

Emission Systeme: Schottky - (Field) Emitter

Material: ZrO / W (100)



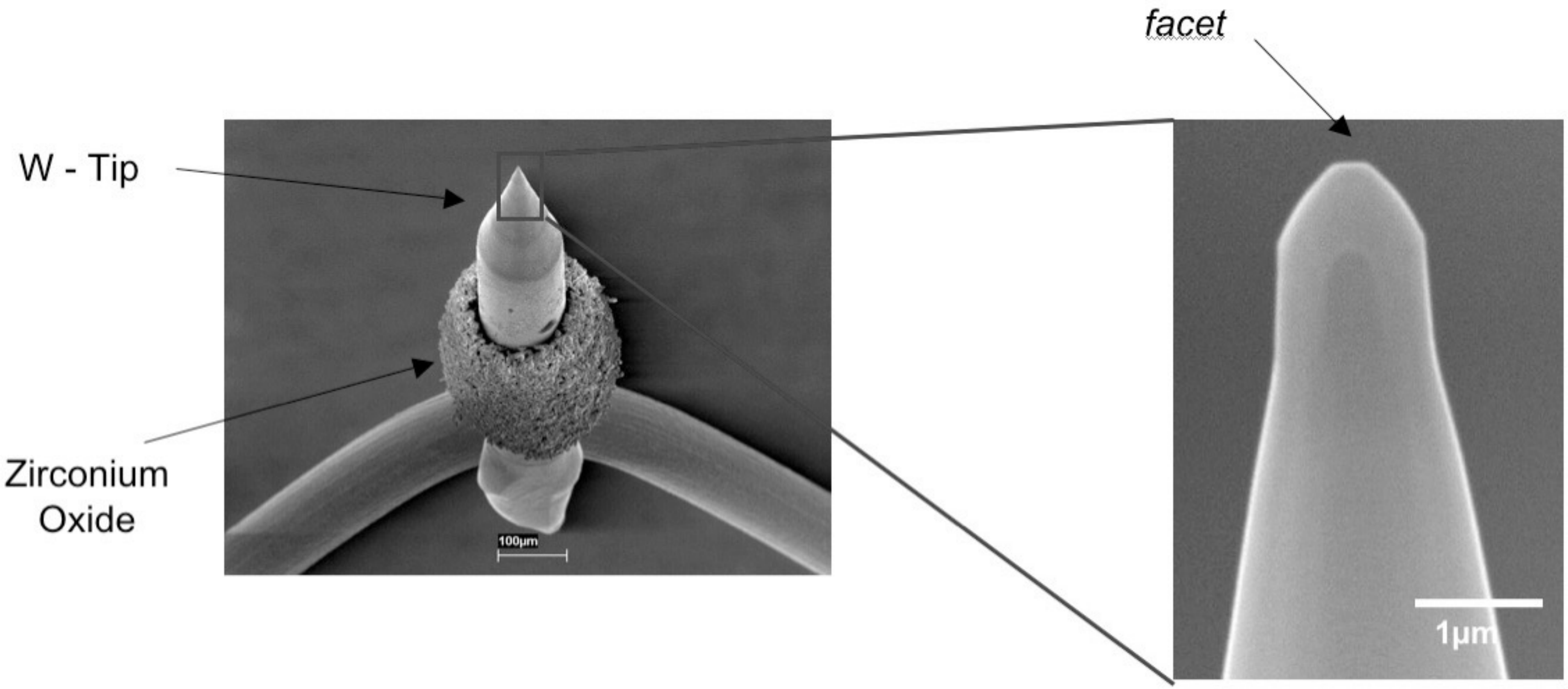
T = 300K

T = 1800K

Emission mechanism of a Schottky field emitter:

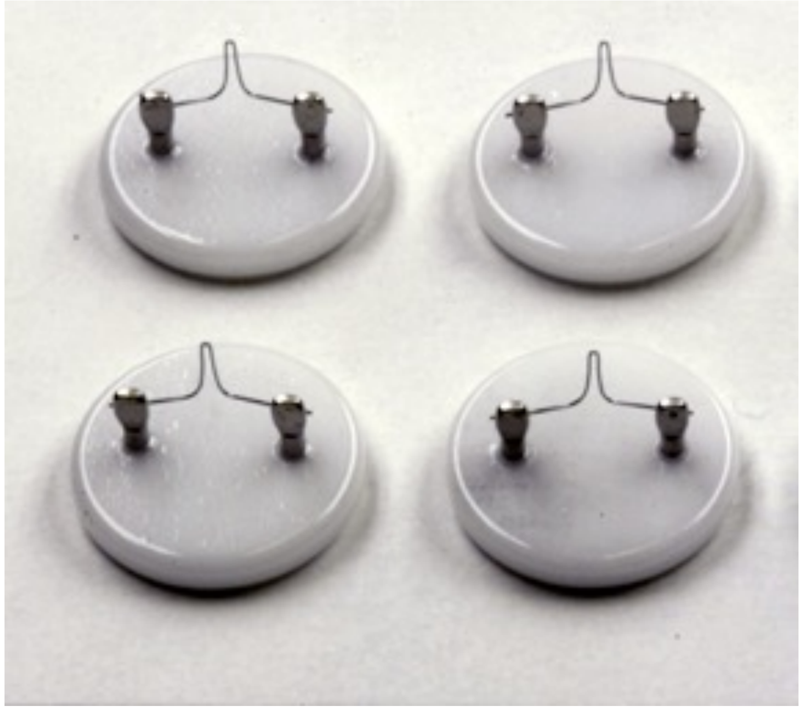
By covering the tip with ZrO and applying a strong electrical field the work function W is decreased (Schottky Effect). Electrons from the Fermi level E_F are lifted to a higher energy level by heating the emitter and overcome the work function W below the vacuum level

Schottky Emitter



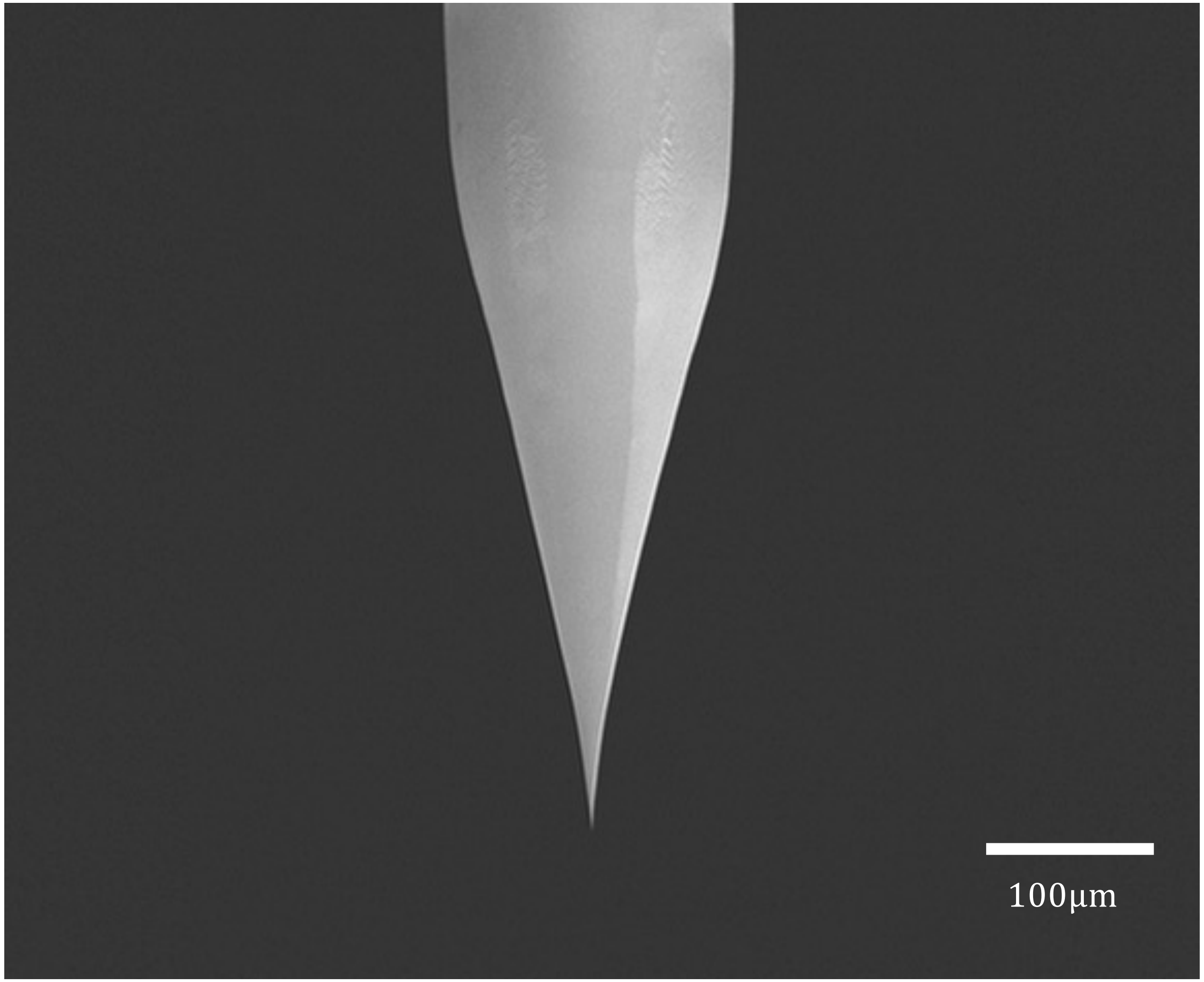
SEM image of a Schottky tip.
Emission occurs from the crystalline facet (horizontal at the top) that is about 0.3 µm across.

hairpin cathode



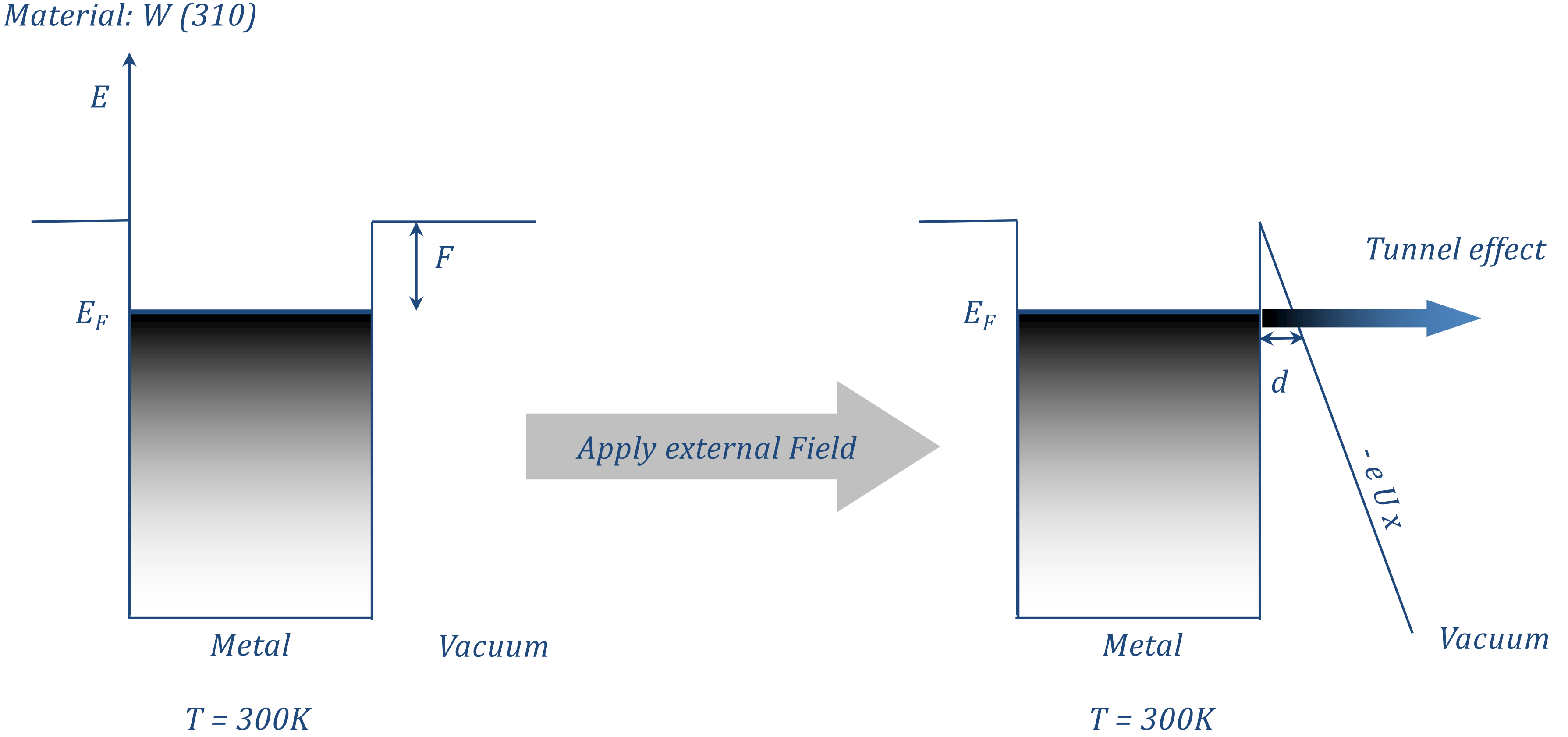
field emission cathode

Emission systems: cold field emission



Cold field emission(SEM)

Emission systems: Cold Field Emitters



Emission mechanism of a cold field emitter:

A strong electrostatic field is applied to a sharp tip (r < 100nm), the field strength U/r increases to values larger than 10^7 V/cm. This electrostatic field decrease the width of the potential barrier ***d*** *in front of the emitter material to a few tens of a nm. In this case electrons from the Fermi level* $\mathbf{E_F}$ *can penetrate through the barrier by the quantum mechanical "tunnel effect" and are emitted into the vacuum.*

Potential energy metal vacuum boundary

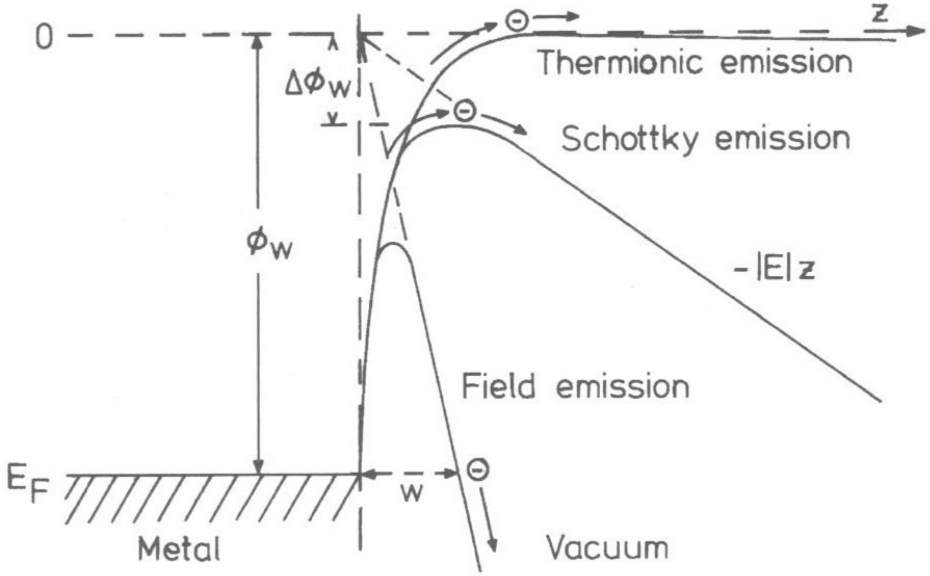


Fig. 4.1. Potential energy $V(z)$ of electrons at the metal–vacuum boundary. Electrons with energies beyond the Fermi energy E_{F} have to overcome the barriers ϕ_{w} and $\phi_{\mathrm{w}} - \Delta\phi_{\mathrm{w}}$ for thermionic or Schottky emission or can tunnel through the barrier of width w for field emission.

Reimer & Kohl (2008). Transmission Electron Microscopy. Springer Verlag

Tip radius of pointed cathode

Cathode	W-filament	LaB_6	Schottky-FE	Cold-FEG
Diameter	100 µm	100 µm	0.3 µm	< 0.1 µm

Cathode parameters: overview

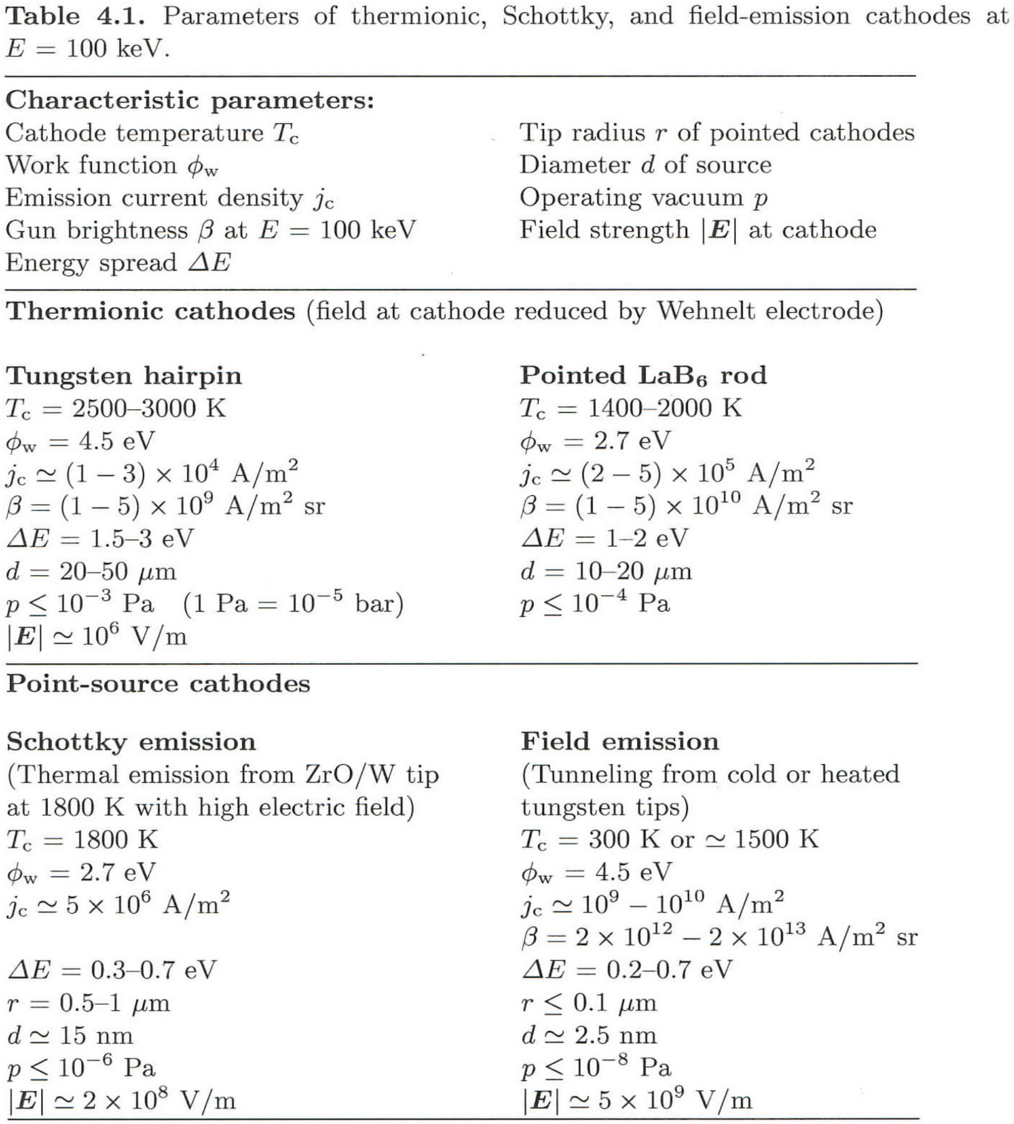
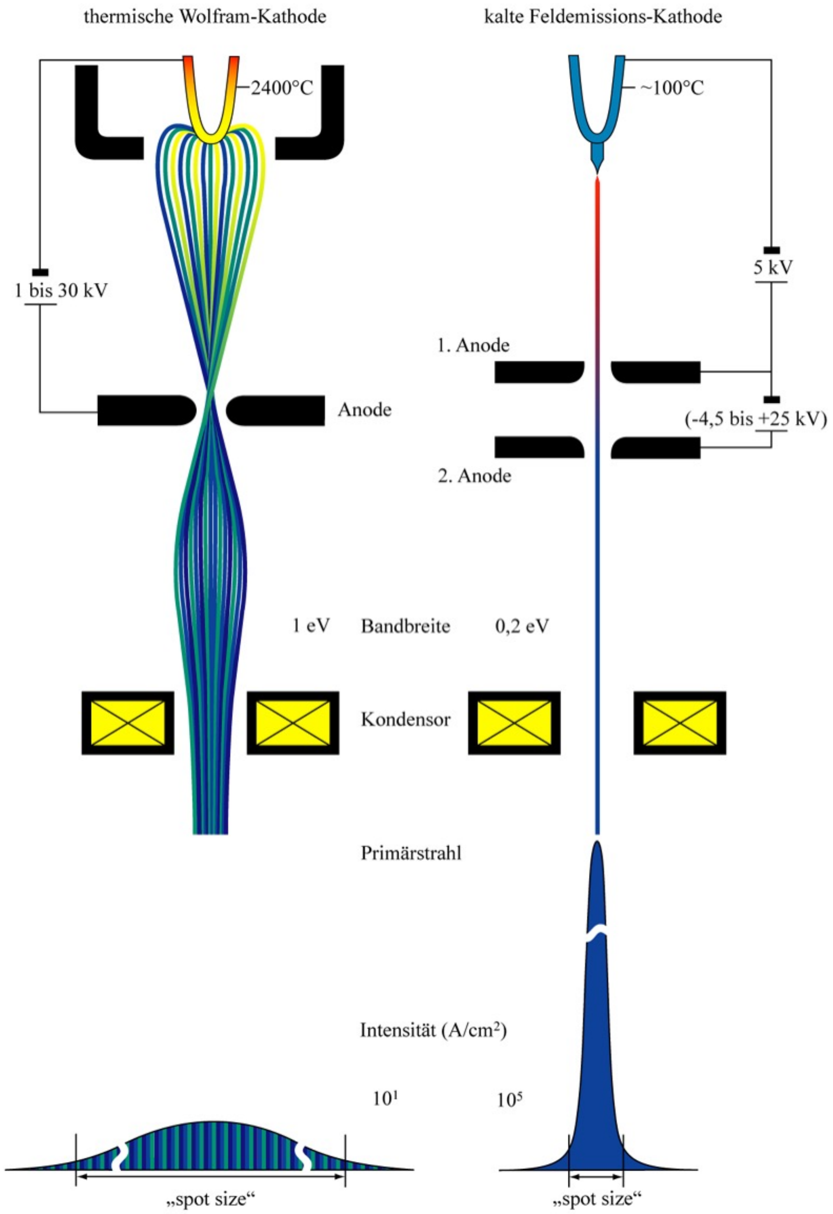


Table 4.1. Parameters of thermionic, Schottky, and field-emission cathodes at $E = 100$ keV.

Characteristic parameters:	
Cathode temperature T_c	Tip radius r of pointed cathodes
Work function ϕ_w	Diameter d of source
Emission current density j_c	Operating vacuum p
Gun brightness β at $E = 100$ keV	Field strength $\|\boldsymbol{E}\|$ at cathode
Energy spread ΔE	
Thermionic cathodes (field at cathode reduced by Wehnelt electrode)	
Tungsten hairpin	**Pointed LaB$_6$ rod**
$T_c = 2500$–3000 K	$T_c = 1400$–2000 K
$\phi_w = 4.5$ eV	$\phi_w = 2.7$ eV
$j_c \simeq (1-3) \times 10^4$ A/m^2	$j_c \simeq (2-5) \times 10^5$ A/m^2
$\beta = (1-5) \times 10^9$ A/m^2 sr	$\beta = (1-5) \times 10^{10}$ A/m^2 sr
$\Delta E = 1.5$–3 eV	$\Delta E = 1$–2 eV
$d = 20$–50 μm	$d = 10$–20 μm
$p \leq 10^{-3}$ Pa (1 Pa = 10^{-5} bar)	$p \leq 10^{-4}$ Pa
$\|\boldsymbol{E}\| \simeq 10^6$ V/m	
Point-source cathodes	
Schottky emission	**Field emission**
(Thermal emission from ZrO/W tip at 1800 K with high electric field)	(Tunneling from cold or heated tungsten tips)
$T_c = 1800$ K	$T_c = 300$ K or $\simeq 1500$ K
$\phi_w = 2.7$ eV	$\phi_w = 4.5$ eV
$j_c \simeq 5 \times 10^6$ A/m^2	$j_c \simeq 10^9 - 10^{10}$ A/m^2
	$\beta = 2 \times 10^{12} - 2 \times 10^{13}$ A/m^2 sr
$\Delta E = 0.3$–0.7 eV	$\Delta E = 0.2$–0.7 eV
$r = 0.5$–1 μm	$r \leq 0.1$ μm
$d \simeq 15$ nm	$d \simeq 2.5$ nm
$p \leq 10^{-6}$ Pa	$p \leq 10^{-8}$ Pa
$\|\boldsymbol{E}\| \simeq 2 \times 10^8$ V/m	$\|\boldsymbol{E}\| \simeq 5 \times 10^9$ V/m

Reimer & Kohl (2008). Transmission Electron Microscopy. Springer Verlag


thermische Wolfram-Kathode
kalte Feldemissions-Kathode
2400°C
~100°C
1 bis 30 kV
5 kV
1. Anode
Anode
(-4,5 bis +25 kV)
2. Anode
1 eV
Bandbreite
0,2 eV
Kondensor
Primärstrahl
Intensität (A/cm²)
10¹
10⁵
„spot size“
„spot size“

Resolution: light microscope (LM)

Formula 1:

$$d = \frac{\lambda}{n \cdot \sin \frac{\alpha}{2}}$$

Formula 2:

$$n.A. = \frac{n \cdot \sin \frac{\alpha}{2}}{2}$$

When using the numerical apertur:

Formula 3:

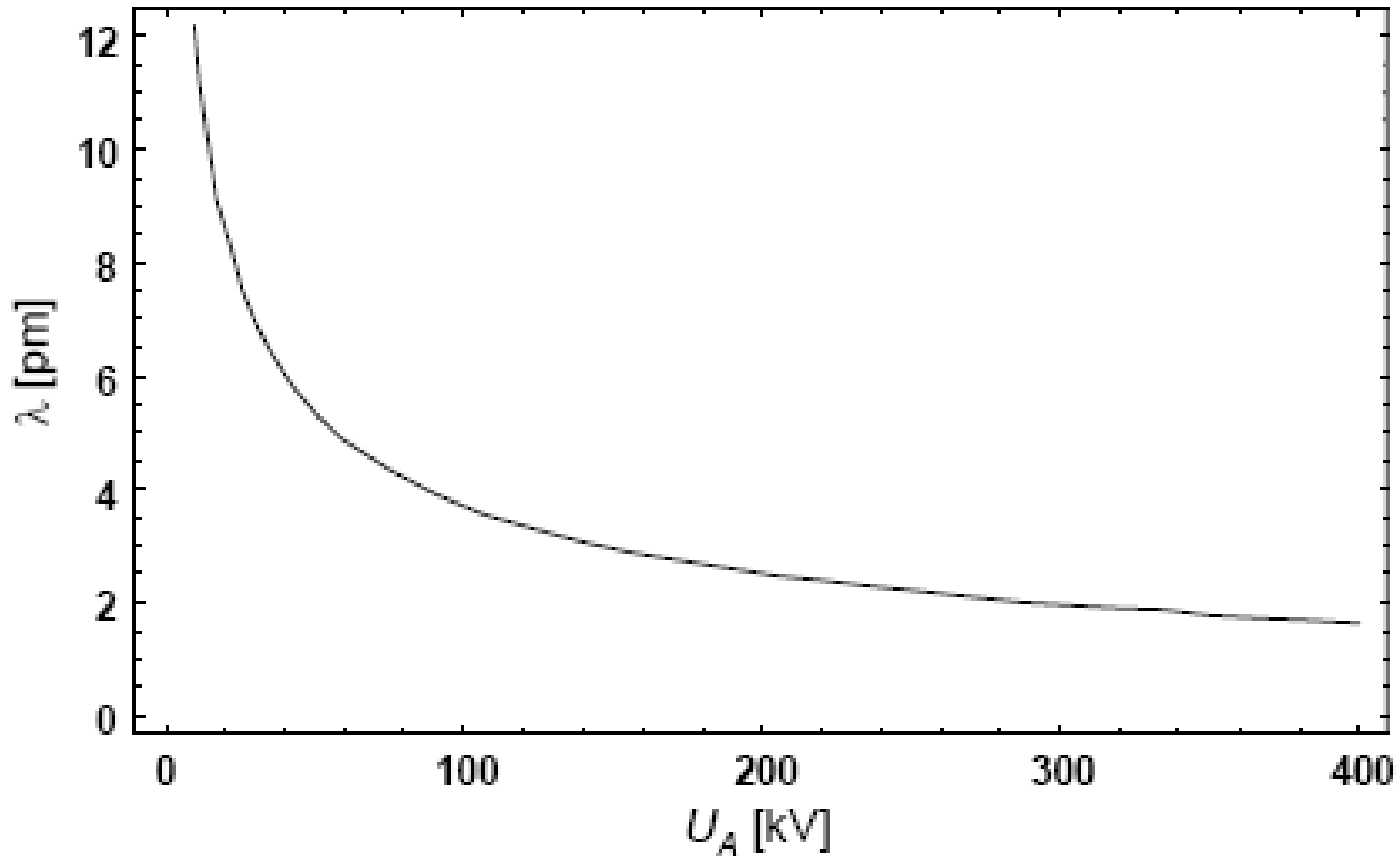
$$d = \frac{\lambda}{2 \cdot n.A.}$$

α = half aperture angle of the objectivelens
n = refractive index of the immersion medium
(air: n = 1; Immersion oil: n = 1,518)
d = distance of two image points
λ = wavelength

The useful magnification is the product of 500x to 1000x the numerical aperture .

For example, the objective lens 40x of our light microscopes from the practical course has a numerical aperture of 0.65.
When working with a wavelength of 550 nm (green), the resulting value for d = 423 nm or 0.423 µm. The useful magnification is 325x - 650x . With the 10x eyepiece used in these microscopes, we have a total magnification of 400x, which is perfectly within the useful range of magnification.

Resolution in the electron microscope: electron wavelength

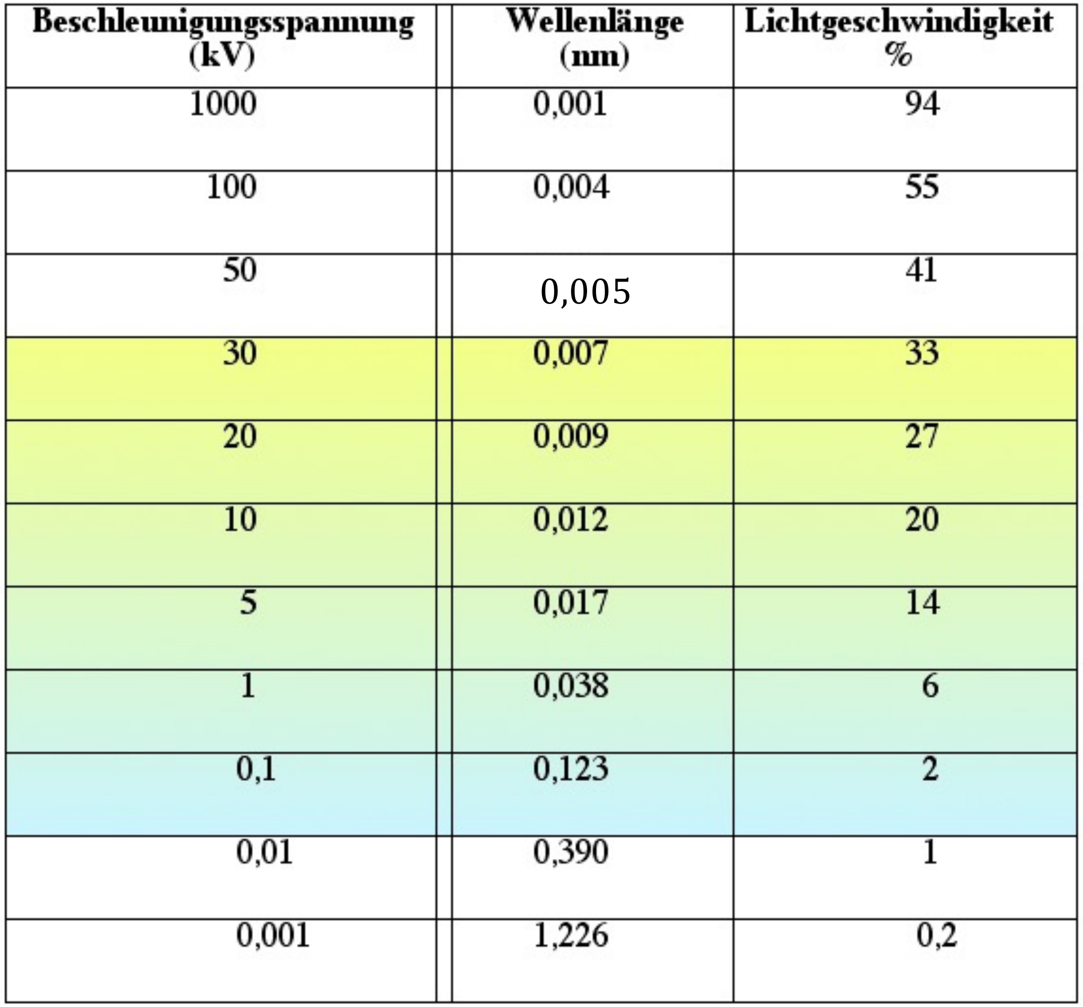


V = 300kV => λ = 0.00197 nm => resolution only >0.1 nm??

„magnetic lenses of TEMs have similar quality as bottom of bottle of champagne would have for light microscope“

Acceleration voltage – wave length and speed of electrons

Acceleration voltage **wave length λ** **speed of light**

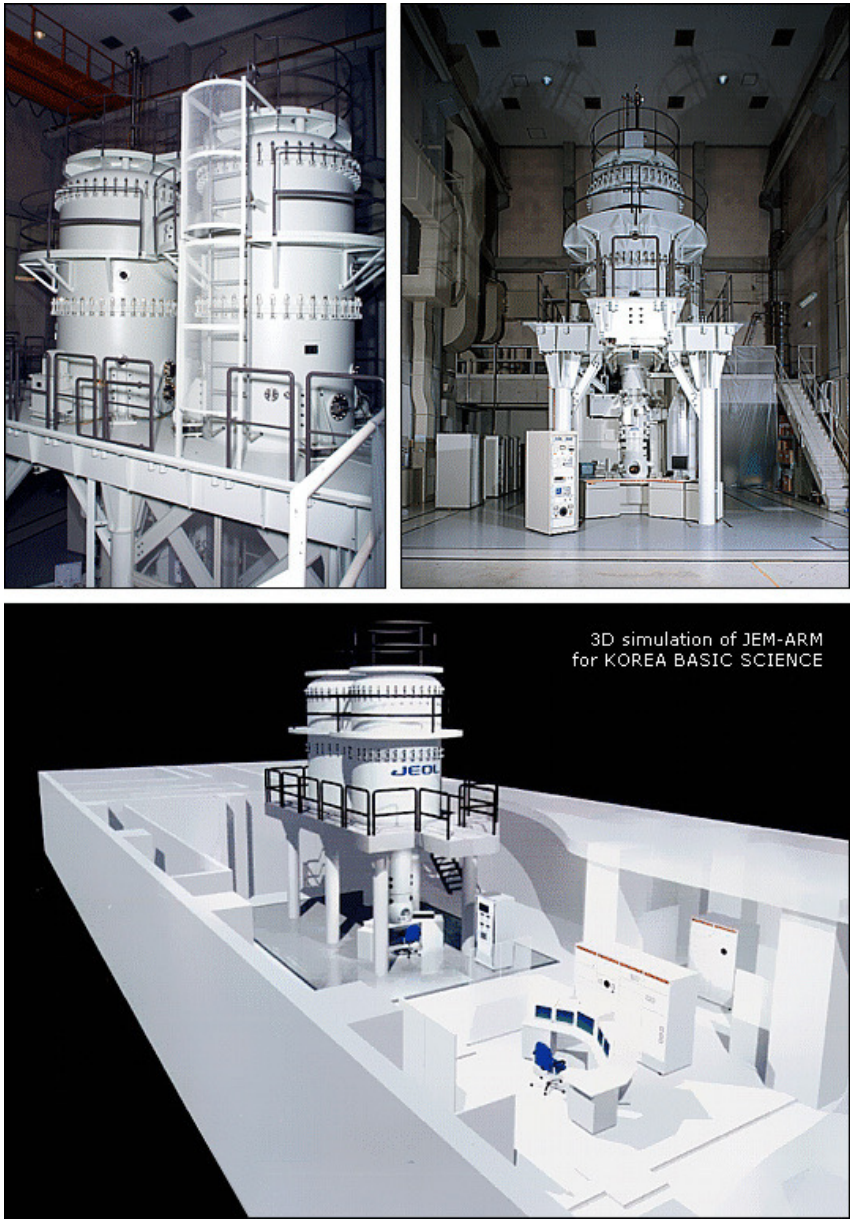


Beschleunigungsspannung (kV)	Wellenlänge (nm)	Lichtgeschwindigkeit %
1000	0,001	94
100	0,004	55
50	0,005	41
30	0,007	33
20	0,009	27
10	0,012	20
5	0,017	14
1	0,038	6
0,1	0,123	2
0,01	0,390	1
0,001	1,226	0,2

$$\boxed{d = k \sqrt[4]{\lambda^3 C_S}}$$

d resolution
k factor
λ wave length (electrons)
C_s spherical aberration

Resolution in the electron microscope: electron wavelength



https://www.jeol.de/electronoptics-en/products/electron-and-ion-optics/transmission-electron-microscopes/mev-tem/jem-arm1300s.php#tab_f793241eda2a96853a23470cf7c66e46_2

Specifications:

- Accelerating voltage: 400 to 1.300 kV
- Point resolution: 0.12 nm
- Line resolution: 0.10 nm
- Magnification range: x200 to max. 2.000.000

Example**: JEOL JEM-ARM1300S** Transmission Electron Microscope

Resolution in the electron microscope: electron wavelength

https://www.jeol.de/electronoptics-en/products/electron-and-ion-optics/transmission-electron-microscopes/200-kv-tem-feg-tem/jem-f200.php#tab_f793241eda2a96853a23470cf7c66e46_3

Specifications:

- Accelerating voltage: 20 to 200 kV
- Point to point resolution: 0.19 nm
- Line resolution: 0.10 nm
- STEM-HAADF image: 0.14 nm
- Magnification range:

  TEM: x20 to x2.000.000

  STEM: x200 to x150.000.000

Example**: JEOL JEM-F200** Multi-purpose Transmission Electron Microscope

Resolution in the electron microscope: electron wavelength

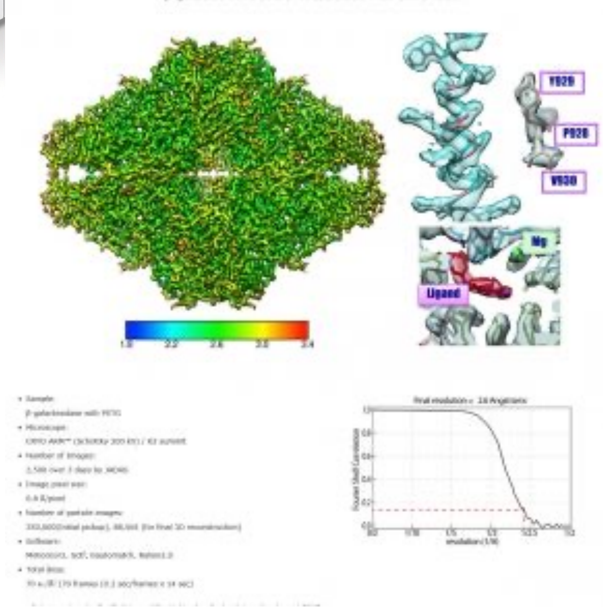
https://www.jeol.de/electronoptics-en/products/electron-and-ion-optics/transmission-electron-microscopes/300-kv-tem-feg-tem/jem-arm300f.php#tab_f793241eda2a96853a23470cf7c66e46_1

Specifications:

- Accelerating voltage: Maximum 300 kV
- TEM lattice resolution: 0.05 nm (with spherical aberration corrector for image forming system)
- STEM resolution: 0.063 nm (spherical aberration corrector for illumination system)

Example**: JEOL JEM-ARM300F** Atomic Resolution Transmission Electron Microscope

Resolution in the electron microscope: electron wavelength



https://www.jeol.de/electronoptics-en/products/electron-and-ion-optics/transmission-electron-microscopes/200-kv-tem-feg-tem/jem-z200fsc-cryo-arm-200.php#tab_f793241eda2a96853a23470cf7c66e46_4

Specifications:

- Accelerating voltage: 200 kV
- In-column Omega energy filter
- LN_2 cooling
- Automated specimen exchange
- Magnification range: x200 to max. 2.000.000

Example**: JEOL JEM-Z200FSC (CRYO ARM^{TM} 200)** Field Emission Cryo-Electron Microscope

Whats next?

SEM in general:

- Sample preparation
- Signal detection and imaging

Specific SEM techniques:

- ESEM
- Array-tomography
- Multi beam SEM
- SBF-SEM
- FIB/SEM

High-resolution TEM techniques:

- Cryo-EM
- Analytical EM
- TEM-Tomography
- Single particle analysis
- STEM