Image Formation in Transmission Electron Microscope

Principles of Transmission Electron Microscopy, Image Acquisition in TEM

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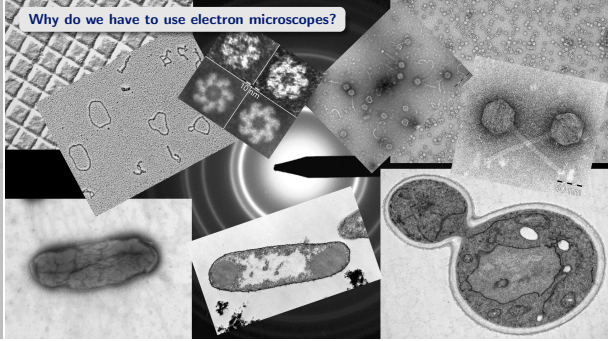
Czech Academy of Sciences

Why do we have to use electron microscopes?



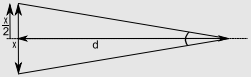
Human eye is an optical instrument

Human eye has spatial resolution of $\approx 1/60$ - $2/60$ degree

From simple geometry we can calculate the minimal size of an object in reading distance ($d \approx 25 - 30$ cm):

$\approx 80 - 160\ \mu$m

$\frac{x}{2} = \tan(\frac{\alpha}{2}) \times d$



It works in the visible spectrum range (420 nm - 780 nm)

Human eye
has a finite resolution

Optimal reading distance
d $\approx$ 25 - 30 cm

Human eye resolution
$\approx$ 1' - 2'

Minimal resolvable detail
$\approx$ 80 - 160 μm

Human eye responds
to visible spectrum

Why do we have to use electron microscopes?

If objects smaller than 100 μm have to be seen:

Their images, projected onto the retina, must be enlarged. For this purpose, microscopes are used. However, there is a physical barrier limiting the use of optical microscopes.

The wavelength of the light used in optical microscopy does not allow to resolve details smaller than a half of its wave length.

$\approx$ **280 nm** for ordinary light (*sunlight*);

$\approx$ **160 nm** for UV light

Abbe Limit: (d = lateral resolution) $d = \frac{0.5 \cdot \lambda}{n \cdot \sin\alpha} = 0.5 \cdot \frac{\lambda}{NA} = \frac{\lambda}{2NA}$

Rayleigh criterion

$$d = \frac{1.22 \cdot \lambda}{2 \cdot n \cdot \sin\alpha} = 0.61 \cdot \frac{\lambda}{n \cdot \sin\alpha}$$

- λ = wavelength of the light
- n = refractive index
- α = 1/2 angle of the cone of light

Electrons wave length range used in EM

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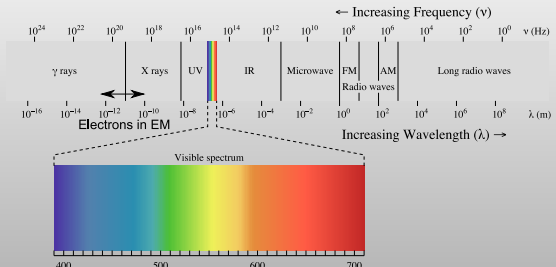

What's electron microscopy?

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It is a scientific method, which utilizes a beam of high energy electrons to study really small objects or details.

The answer is in the wavelength of the electrons.

$$d = \frac{1.22 \cdot \lambda}{2 \cdot n \cdot \sin\alpha} = 0.61 \cdot \frac{\lambda}{n \cdot \sin\alpha}$$

Why we have to use an electron microscope?
How small the objects are?
How small the objects might be?
What is the minimal and maximal size of the objects?

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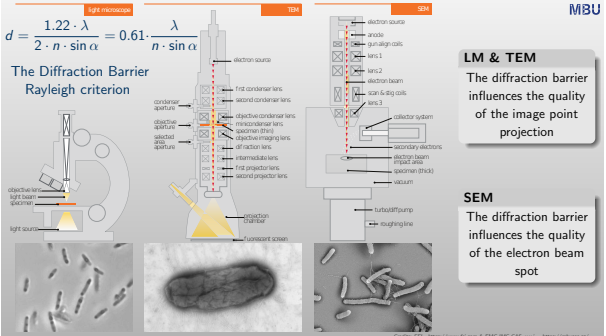
$$d = \frac{1.22 \cdot \lambda}{2 \cdot n \cdot \sin\alpha} = 0.61 \cdot \frac{\lambda}{n \cdot \sin\alpha}$$

The Diffraction Barrier
Rayleigh criterion



LM & TEM

The diffraction barrier influences the quality of the image point projection

SEM

The diffraction barrier influences the quality of the electron beam spot

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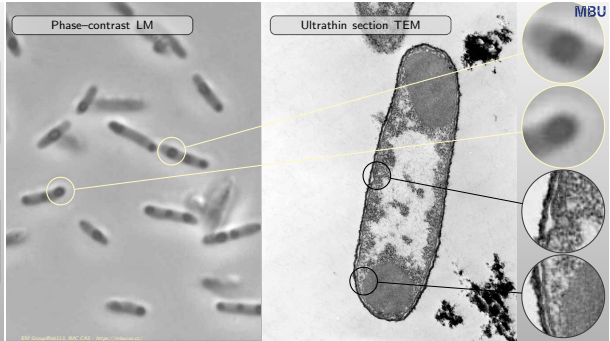
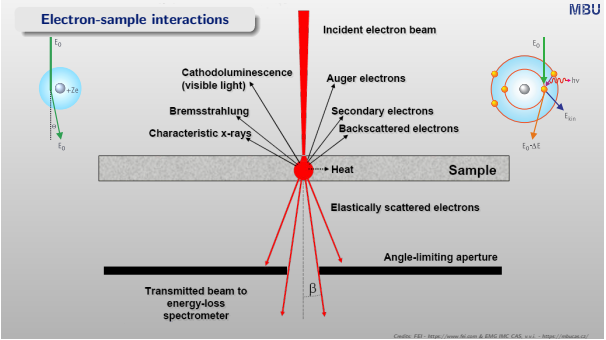


Electron-sample interactions

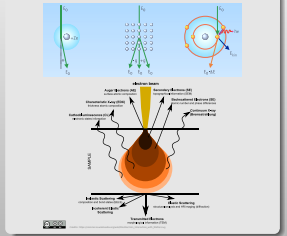


Physical Basics of Electron Microscopy

Electron Beam – Sample Interactions

- **Transmits electrons** - primary electrons, elastically and inelastically scattered electron TEM, STEM-BF, STEM-DF, EELS in STEM, ESI
- **Reflects electrons** - backscattered electrons - BSE
- **Emits electrons** - secondary electrons - SE
- **Absorbs electrons** - sample current
- **Emits electromagnetic radiation** - characteristic radiation - EDXS, EDXS imaging
- **Emits positively charged ions** - ESEM

Electron – Sample Interactions



Electron Microscopes = Electron Probe Instruments

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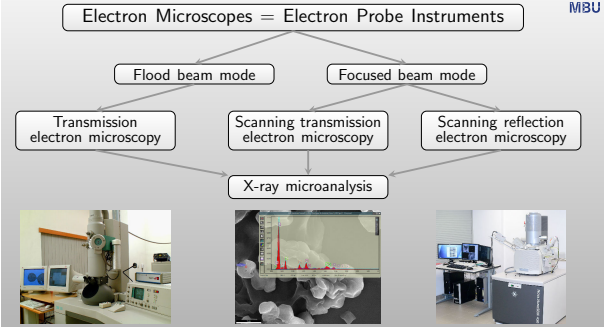

History of Electron Microscopy

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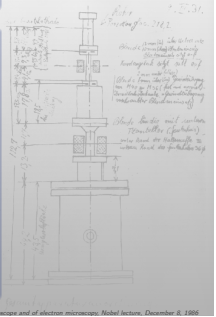
- 1926 **Hans Busch**
  - charged particles can be bent in a magnetic field as glass lenses bend visible light.
- 1931 **Ernst Ruska**
  - builds the first transmission electron microscope with resolution higher than a light microscope.
- 1935 **Max Knoll**
  - becomes the first researcher to scan a surface with an electron beam to obtain an image. Lacking lenses, his system has a resolution of ∼100 µm.
- 1938 **Manfred von Ardenne**
  - develops the first scanning transmission electron microscope, with an electron beam diameter on target of ∼10 nm.
- 1940 **Manfred von Ardenne**
  - differentiates secondary and backscattered electrons; demonstrates that reducing the beam energy improves contrast at the expense of resolution.
- 1942 **Vladimir Zworykin**
  - working SEM instrument with 50 nm resolving power at RCA.
- 1948 **Charles Oatley**
  - begins SEM development at Cambridge University, UK, spending some two decades on the project.
- 1960 **Fabian Pease**
  - in Oatley's group in Cambridge, UK, achieves SEM resolution of ∼10 nm.
- 1965 **Cambridge Scientific Instruments**
  - releases the first commercial SEM, the *Stereoscan Mark I*.
- 1972 **Albert Crewe at the University of Chicago, US, and researchers at Hitachi, Japan**
  - develop the first practical field emission (FE)-SEM.

History of Electron Microscopy

ERNST RUSKA: Nobel Lecture 1986

... Knoll and I simply hoped for extremely low dimensions of the electrons. As engineers we did not know yet the thesis of the "material wave" of the French physicist de Broglie that had been put forward several years earlier (1925). Even physicists only reluctantly accepted this new thesis.
When I first heard of it in summer 1931, I was very much disappointed that now even at the electron microscope the resolution should be limited again by a wavelength (of the "Materiestrahlung"). I was immediately heartened, though, when with the aid of the de Broglie equation I became satisfied that these waves must be around five orders of magnitude shorter in length than light waves. Thus, there was no reason to abandon the aim of electron microscopy surpassing the resolution of light microscopy. ...



Credit: Ernst Ruska – The development of the electron microscope and of electron microscopy, Nobel lecture, December 8, 1986

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Essential Background

Image Formation in Transmission Electron Microscope

Image Acquisition in Transmission Electron Microscope

Transmision Electron Microscope - The Main Parts

The electronics

High voltage sources; Sources for electromagnetic lenses; Regulation circuits; PC based control

Vacuum part

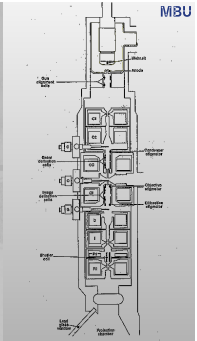
Two or three stage system; modern construction usually with IGP or turbomolecular pumps

Imaging part

Electron beam generation and manipulation; Image generation and magnification

Image recording part

Classic photographic or digital recording (TV CCD cameras; slow-scan CCD cameras; Pixel Image Plate)



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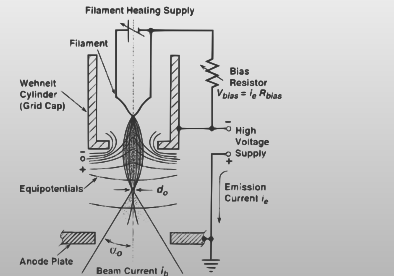
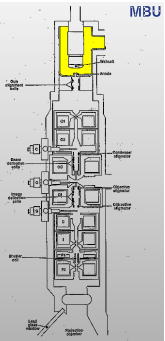
Classical Tungsten Electron Source (Cathode)





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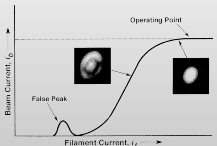
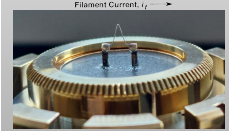
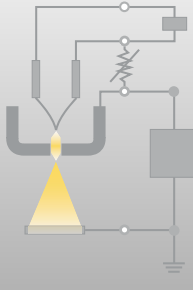
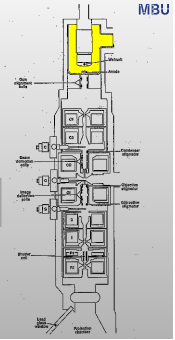
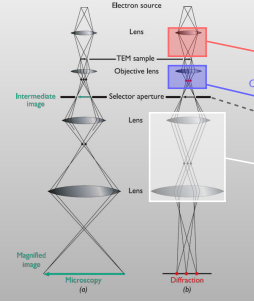


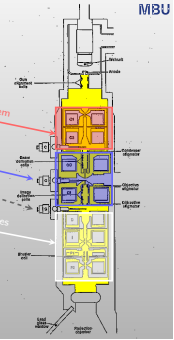




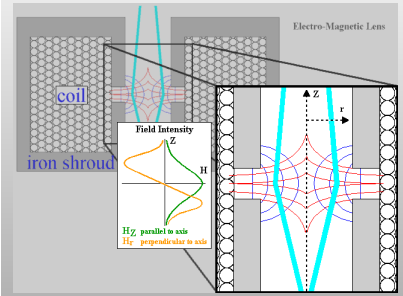


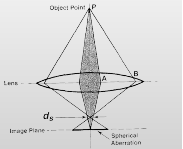
TEM Column and System of Electromagnetic Lenses

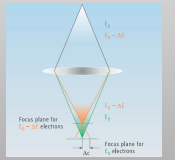




Electromagnetic Lens and Its Errors

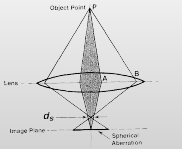


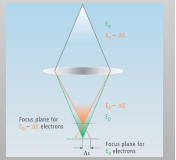




Three main types of aberration

- *Geometrical aberrations*, which occur when electrons passing through a lens travel too far from, or at too large an angle to, the axis of the lens. The important example of this is *Spherical Aberration*.
- *Chromatic aberrations*, which describe the errors associated with the manner in which a lens focuses electrons of different energy or how the focus behaves when the excitation of the lens is varied.
- *Mechanical or parasitic aberrations*, which are associated with small mechanical or electrical imperfections in lenses. An example of this type of aberration would be stigmatic error (i.e. when the focal length of a lens is different in the X and Y planes).





Credit: Joy, 2005; Hawkes and Kasper, 1988, 2017

Spherical Aberration and Diffraction on Aperture

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Spherical aberration: $d_s = \frac{1}{2} c_s \alpha^3$; $\frac{1}{2} c_s = constant$

Spherical aberration causes rays from one object point to not cross in a corresponding image point in the image space.

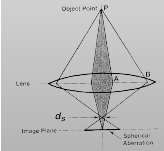
Diffraction on Aperture

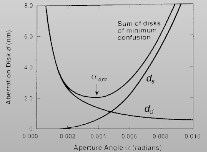
Rayleigh criterion

$$d = \frac{1.22 \cdot \lambda}{2 \cdot n \cdot \sin\alpha} = 0.61 \cdot \frac{\lambda}{n \cdot \sin\alpha}$$

Spherical aberration vs. diffraction

Spherical aberration and diffraction act in opposite fashion !!!





Credit: Goldstein et al., 2003

Chromatic Aberration

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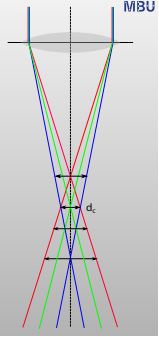
Depends on current and voltage: $d_c = c_c \alpha \Delta E / E_0$

Electrons of different wavelength (velocity, energy) leaving a point in object space are not brought to a focus at the same point in image space.

- fluctuations in high tension supply (usually less than 1×10^{-5} in stabilized circuits)
- variation in velocity of electrons emitted by the cathode (about $\pm$ 3.5 ppm)
- energy losses due to inelastic collisions in the specimen (minimized using thin specimens)



- For **thin specimens**, chromatic aberration is not a major limit to resolution in the images.
- For **thick specimens**, chromatic aberration is a major limit to resolution in the images.
- In **electron tomography** special devices are used to correct the chromatic aberration caused by energy losses due to inelastic collisions of the electrons in a thick sample.

Credit: Goldstein et al., 2003

Monochromator Technology in Libra200MC (Zeiss)

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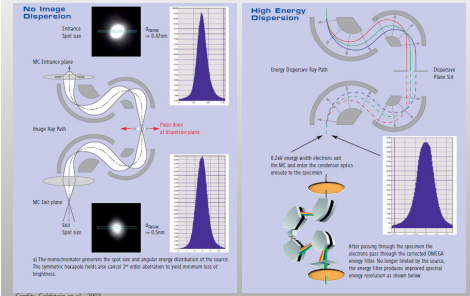

Credit: Goldstein et al., 2003

Monochromator Technology in Libra200MC (Zeiss)

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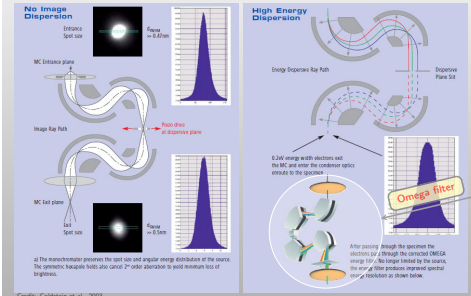

Credit: Goldstein et al., 2003

Electromagnetic Lens and Astigmatisms

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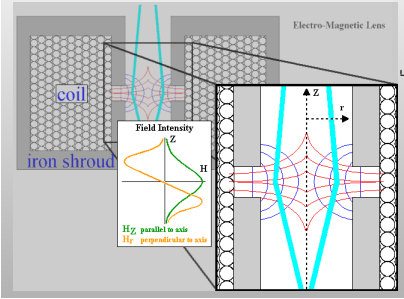

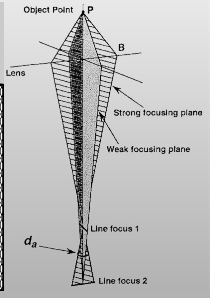

Astigmatism and Stigmator of Electromagnetic Lens

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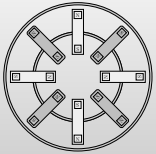
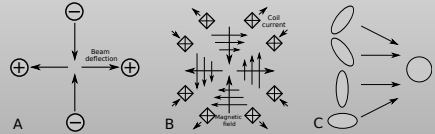
Astigmatism = Lens asymmetry

Neither the homogeneity of available magnetic materials nor the accuracy of machining these metals into lens pole pieces is adequate for the direct production of lenses capable of displaying the theoretical resolving power estabilished by the *Spherical aberration-Diffraction limit*. Asymmetry, resulting from lack of *axial symmetry*, has the effect of producing images in which the focal level varies with direction.





Strong Astigmatism

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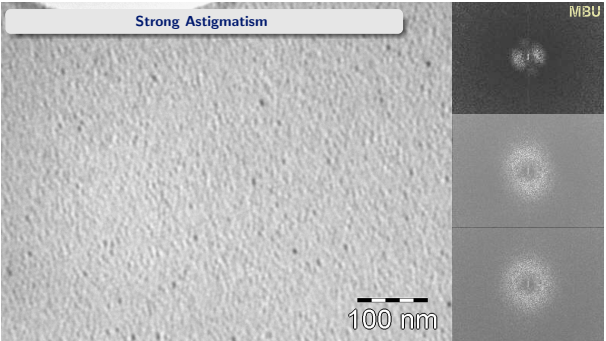

Unadjusted Astigmatism

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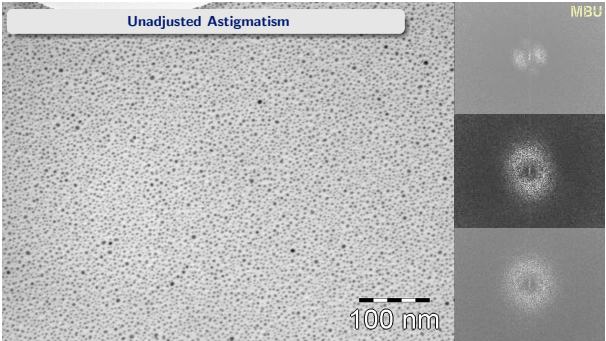
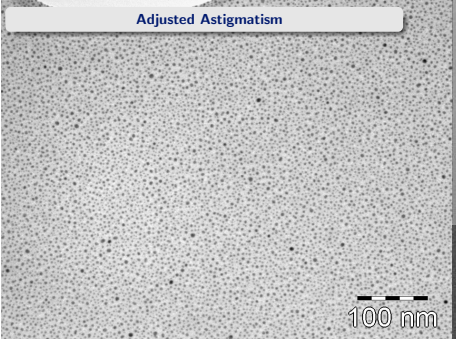
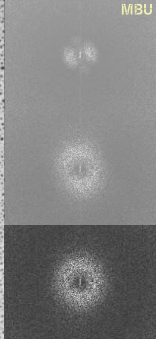
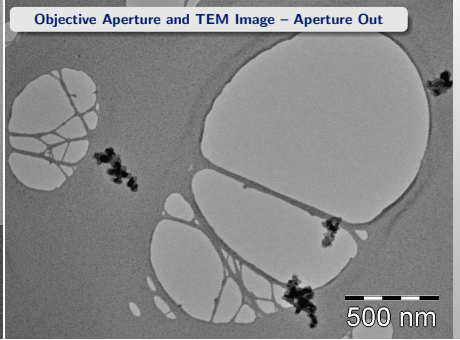


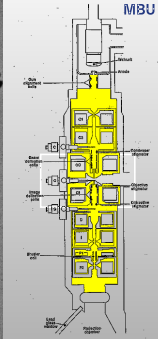
Adjusted Astigmatism



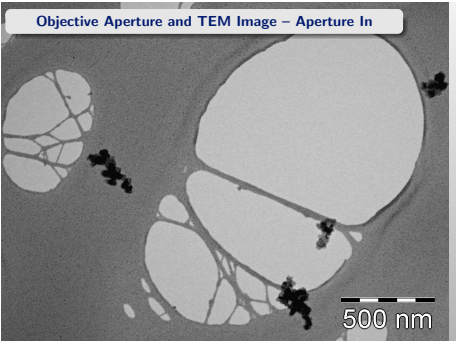


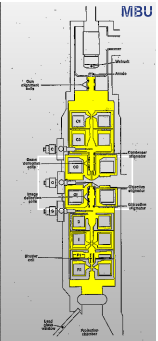
Objective Aperture and TEM Image – Aperture Out



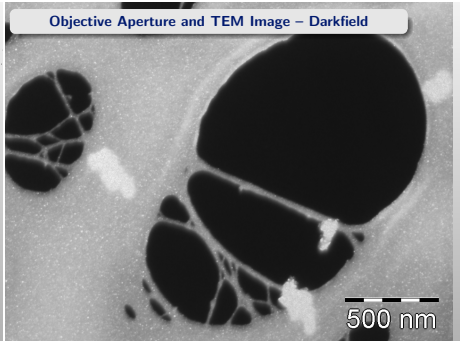


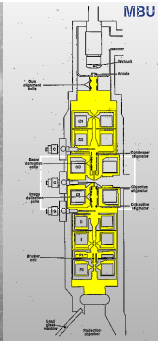
Objective Aperture and TEM Image – Aperture In



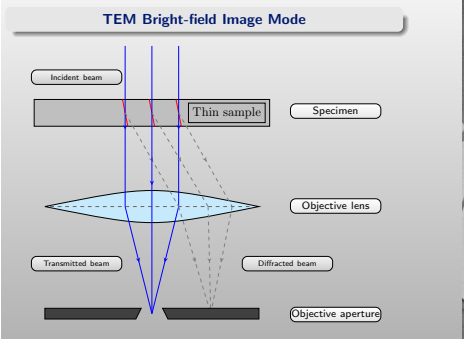


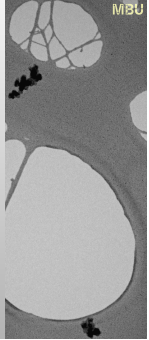
Objective Aperture and TEM Image – Darkfield



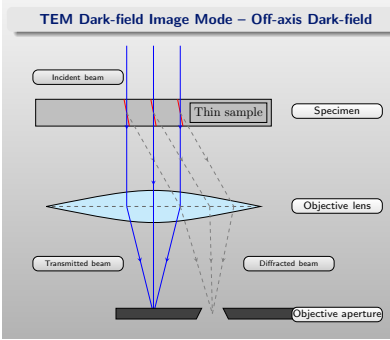


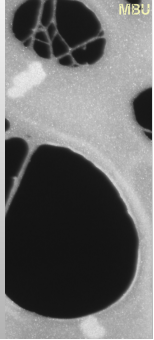
TEM Bright-field Image Mode



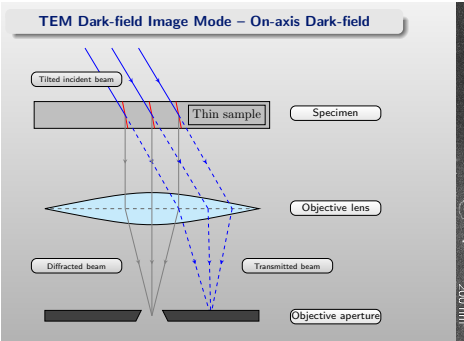


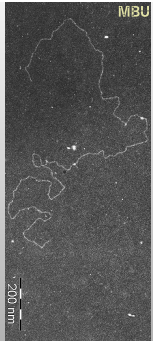
TEM Dark-field Image Mode – Off-axis Dark-field



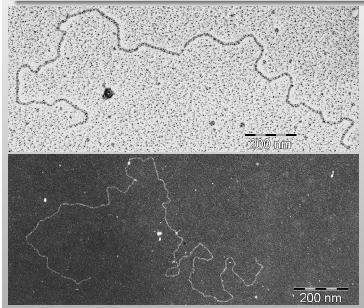


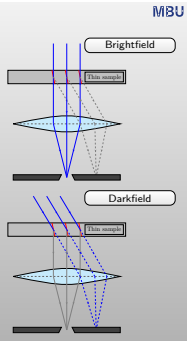
TEM Dark-field Image Mode – On-axis Dark-field





DNA Imaging in the Brightfiels and Darkfield





Calibration of TEM Image Magnification: Replica Grating

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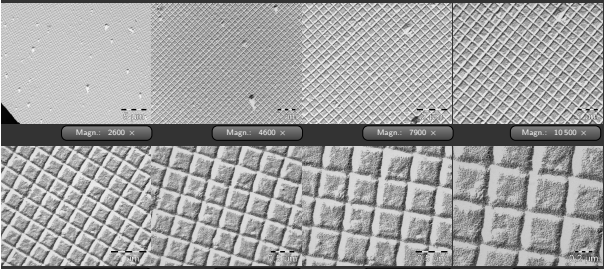

Focus serie: from +504 nm to −672 nm, step 168 nm

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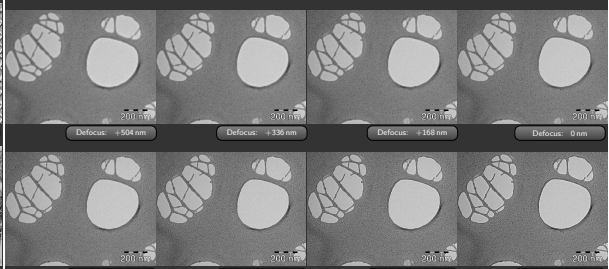

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Essential Background

Image Formation in Transmission Electron Microscope

Image Acquisition in Transmission Electron Microscope

Image Acquisition in TEM

Classical Photography - plates, planfilms, roll films; 35mm films

Resolution of photographic emulsion:
Standard: 70-90 lines/mm; High resolution: 140-180 lines/mm;
For electron microscopy: up to 300 lines/mm (line spacing approx. 3.33 µm);

Digital recording - CCD Cameras & CMOS Cameras

TV CCD - Image recorded in 8 bits (256 grayscale, 2^8)

- Output according current TV Norms: CCIR with 625 lines (Europe) and NTSC norm with 480 lines (USA, Japan)
- Frame grabbers convert analog TV signal to digital: 756x574px (PAL) or 640x480 (NTSC)

Slow scan CCD - Image recorded in 12bits, 14bits or 16bits

- Image size ranged from 1024x1024px up to 4096x4096px
- Generally it is not possible to view image in real time at full resolution

Direct Detection Cameras

- Image size up to 8k x 8k px
- Generally it is not possible to view image in real time at full resolution

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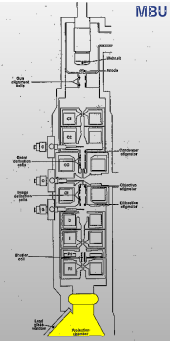

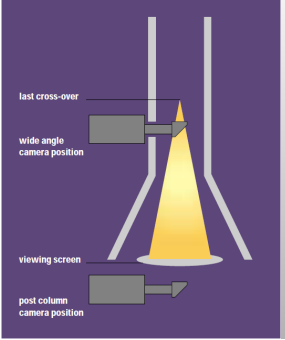

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CCD Cameras and TEM Column

Credit: Digital Cameras for Electron Microscopy, Soft Imaging System GmbH

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On-line Shading-Correction (SC)

$$SC = \frac{(Original\ image) - (Offset\ image)}{Gain\ Image} \times (mean\ grey\ value\ of\ Gain\ image)$$

An Important Step in Image Acquisition

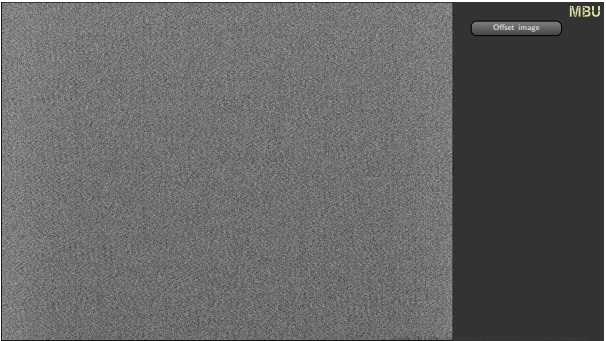
The Online shading correction corrects fixed image structures resulting from the imaging system during acquisition process.

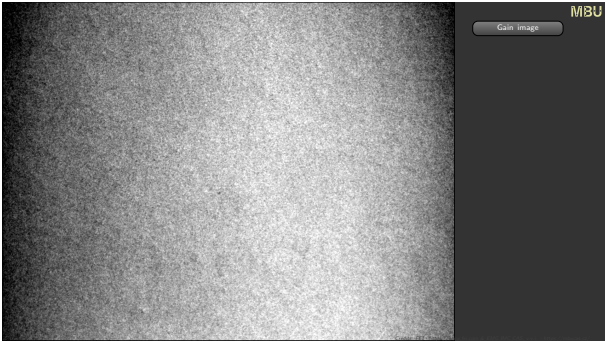
Offset image

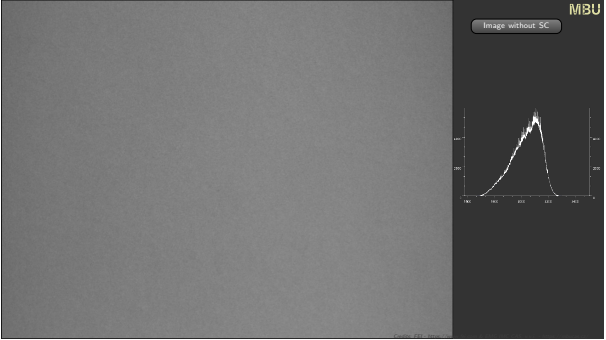
A camera offset image is acquired without any illumination. An offset correction is necessary when using a CCD camera in order to compensate for a non-homogenous dark-current signal. A shading correction involves subtracting the offset image from the original image.

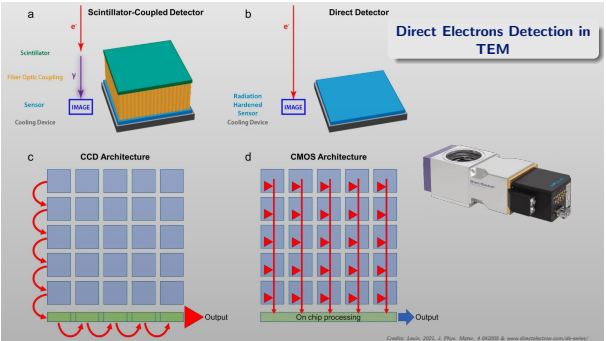
Gain image

The camera gain image is acquired at homogeneous illumination of scintilator (without any specimen). The original image is divided by the gain image during shading correction.









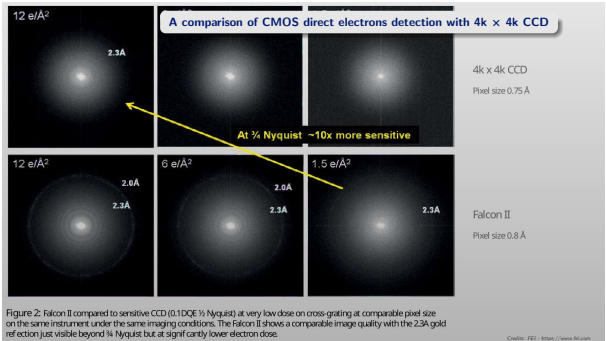


Figure 2: Falcon II compared to sensitive CCD (0.1DQE ½ Nyquist) at very low dose on cross-grating at comparable pixel size on the same instrument under the same imaging conditions. The Falcon II shows a comparable image quality with the 2.3A gold ref ection just visible beyond ¼ Nyquist but at signif cantly lower electron dose.

Laboratory of Molecular Structure Characterization, Institute of Microbiology, Czech Academy of Sciences

Electron Microscopy Group – past lineup

Oldřich Benada benada@biomed.cas.cz

Macromolecular complexes, Ultrastructure of prokaryotes - TEM,
High resolution SEM of biological surfaces, X-ray microanalysis

Olga Kofroňová *already retired* kofra@biomed.cas.cz

Scanning electron microscopy of biological surfaces

Zdeněk Žižka *part-time senior employee* zizka@biomed.cas.cz

Advanced optical microscopy techniques

Zuzana Večerková *technician* zuzka@biomed.cas.cz

Sample preparation for TEM, Ultrathin sectioning, Essential laboratory management

Tereza Juříková, *PhD student, Faculty of Science, Charles University, Prague* tereza.jurikova@biomed.cas.cz

HR SEM - Host-pathogen interactions in fungal and bacterial infections; Dual culture systems: bacteria-bacteria and bacteria-fungi; Basic and advanced optical microscopy techniques

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