Basics of Electron Microscopy

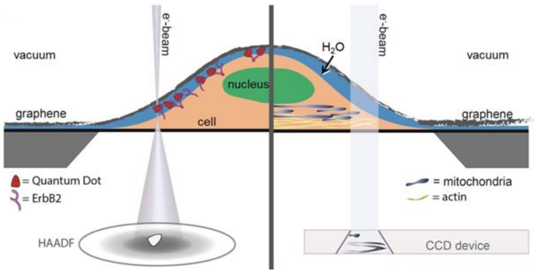
(of biological samples)

NanoBioMaterials2

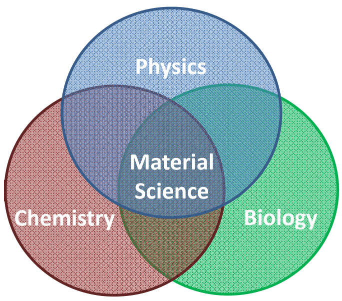
Dr. Indra Navina Dahmke

Innovative Electron Microscopy

INM - Leibniz Institute for New Materials



MATERIAL SCIENCE



Based on Marc A. Meyers

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OUTLINE

I. Why Electron Microscopy (EM)?

II. Properties of Electrons (e^-)

III. Basic Elements of Electron Microscopes

IV. Lens Aberrations

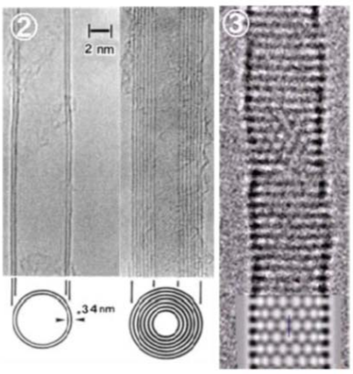
V. Interaction of e^--Beam with Sample > Signal Formation

VI. Limitations of EM

VII. EM of Biological Samples

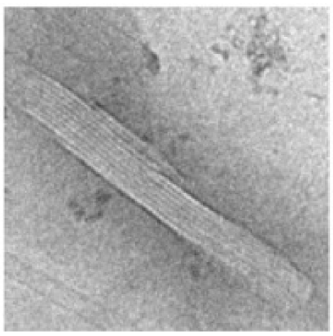
WHY ELECTRON MICROSCOPY (EM)?

CARBON NANOTUBE



Nano/Bio-Materialien:
Mikroskopie/Beugung, H. Schmidt, 2011

MICROTUBULE



Published in: Sercan Keskin; Niels de Jonge; *Nano Lett.* 18, 7435-7440.
DOI: 10.1021/acs.nanolett.8b02490

WHY ELECTRON MICROSCOPY (EM)?

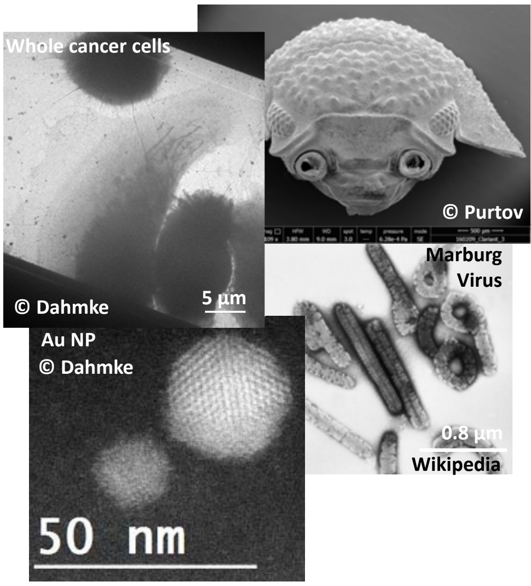
Table 1-1. Approximate sizes of some common objects and the smallest magnification M* required to distinguish them, according to Eq. (1.5).

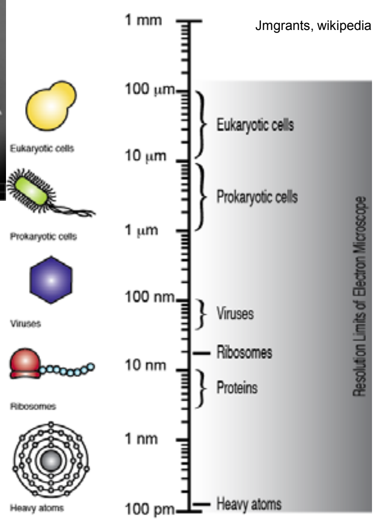
Object	Typical diameter *D*	M* = 75µm / *D*
Grain of sand	1 mm = 1000 µm	None
Human hair	150 µm	None
Red blood cell	10 µm	7.5
Bacterium	1 µm	75
Virus	20 nm	4000
DNA molecule	2 nm	40,000
Uranium atom	0.2 nm = 200 pm	400,000

Physical Principles of Electron Microscopy, R.F. Egerton, Springer, 2005

WHY ELECTRON MICROSCOPY (EM)?

WHY ELECTRON MICROSCOPY (EM)?





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WHY ELECTRON MICROSCOPY (EM)?

1. **Richness of detail**
2. **High spatial resolution**
3. **Analytical properties of e^--beam**

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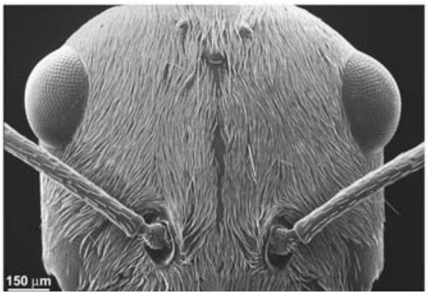
WHY ELECTRON MICROSCOPY (EM)?

1. Richness of detail



A scanning electron microsocope (SEM) view of the Saharan silver ant's head. Norman Nan Shi and Nanfang Yu, Columbia Engineering, 2018

2. High spatial resolution

3. Analytical properties of e^--beam

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WHY ELECTRON MICROSCOPY (EM)?

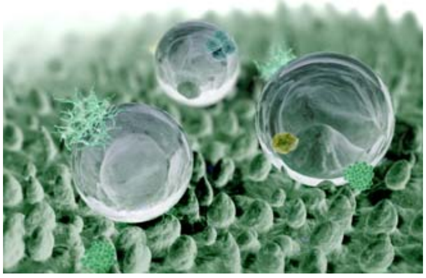
1. Richness of detail

2. High spatial resolution

Indian Lotus, Botanical Garden KIT, Karlsruhe. H. Zell, 2010, wikipedia

> LOTUS EFFECT ®

(1970 by botanist Barthlott, patented in 1998)



Animation of lotus effect, W. Thielicke, wikipedia

3. Analytical properties of e^--beam

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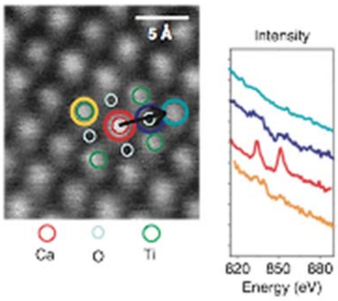
WHY ELECTRON MICROSCOPY (EM)?

1. Richness of detail

2. High spatial resolution

3. Analytical properties of e⁻-beam



- high-resolution imaging,
- electron diffraction
- X-ray spectroscopy (EDS/EDX)
- electron spectroscopy (EELS)

→ structural and chemical analysis with the high spatial resolution

Lupini et al.,Nanocharacterization, ed. Kirkland, E.J. & Hutchison, J.L., pp. 28-65 (Royal Society of Chemistry, Cambridge, 2007).

OUTLINE

RESOLUTION

(1) $d_{min} = \frac{\lambda}{n \sin\alpha}$ Abbe limit (central illumination)

- e.g. d_{min} compound light microscope with condensor:

~200 nm (n sinα = NA: 1.4 (oil immersion))

Ernst Abbe
(1840 – 1905)

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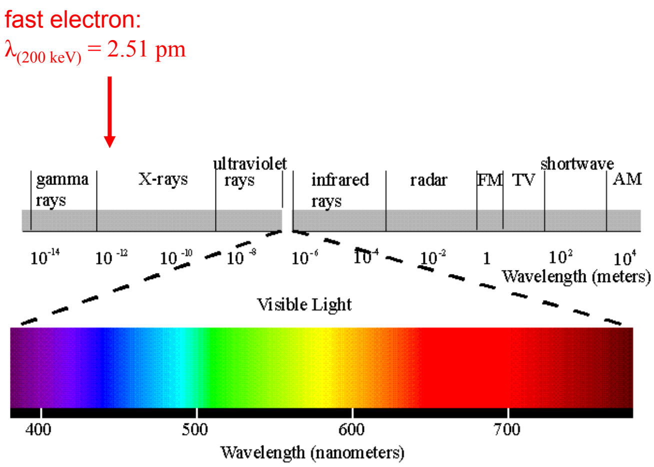
ELECTROMAGNATIC RADIATION



Nano/Bio-Materialien:
Mikroskopie/Beugung, H. Schmidt, 2011

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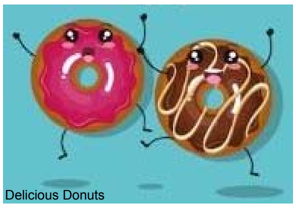
WAVENATURE OF ELECTRONS

INM

(2) $\lambda = \frac{h}{p} = \frac{h}{m_0 v}$

De Broglie wave equation



„Wir sind auf der selben Wellenlänge!!!"

- „We share the same wave length!!!"

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WAVELENGTH OF ELECTRONS IN EM

INM

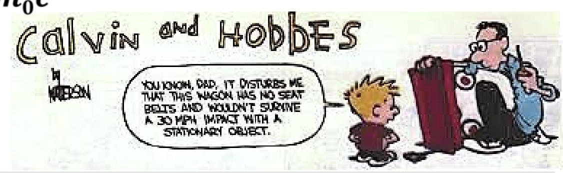
(3) $E = \frac{1}{2} m v^2$

(4) $p = m_0 v = 2 m_0 E^{1/2}$

(5) $\lambda = \frac{h}{m_0 v} = \frac{h}{\sqrt{2 m_0 E}}$

(6) $\lambda = \frac{h}{\sqrt{2 m_0 E (1 + \frac{E}{2 m_0 c^2})}}$

Relativistic effects (E≥100 keV)



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WAVELENGTH OF ELECTRONS IN EM

INM

V (keV)	λ (nm)	v/c
10	0.012	0.195
50	0.0055	0.414
100	0.0039	0.548
1000	0.0012	0.941

Excursus velocity (v) vs. velocity of light (c):
The rapidity (θ) is an alternative measure for relativistic velocity and defined as $\theta = artanh\ (v/c)$. For non-relativistic velocities the rapidity approximates v/c.

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INM

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ELECTRON MICROSCOPES - HISTORY

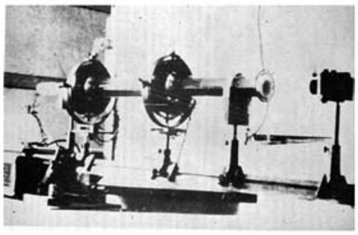
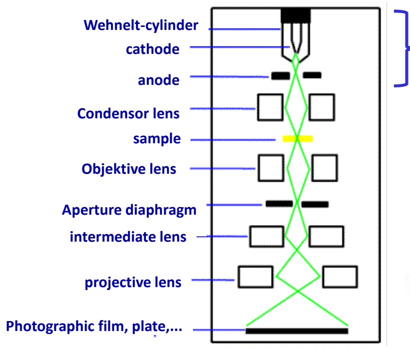


Figure 1-8. Early photograph of a horizontal two-stage electron microscope (Knoll and Ruska, 1932). This material is used by permission of Wiley-VCH, Berlin.

Physical Principles of Electron Microscopy, R.F. Egerton, Springer, 2005

ELECTRON MICROSCOPE – WEHNELT CAP



Based on http://daten.didaktikchemie.uni-bayreuth.de/

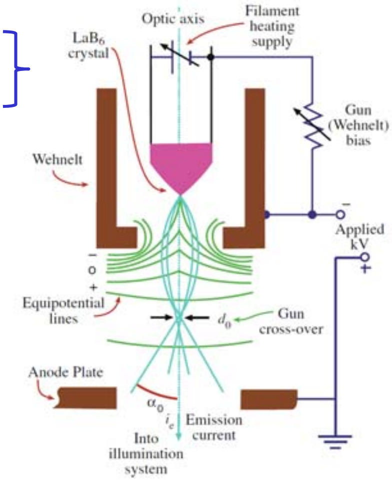
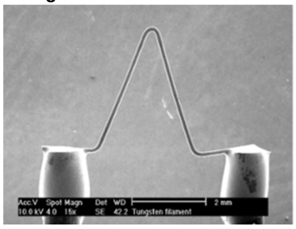


FIGURE 5.1. Schematic diagram of a thermionic electron gun. A high voltage is placed between the cathode and the anode, modified by a potential on the Wehnelt which acts as the grid in a triode system. The electric field from the Wehnelt focuses the electrons into a crossover, diameter d_0 and convergence/divergence angle α_0 which is the true source (object) for the lenses in the TEM illumination system.

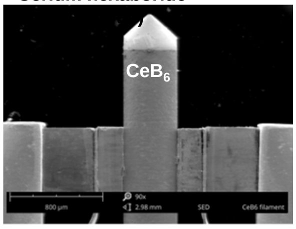
Transmission Electron Microscopy, Williams & Carter, Springer 2nd Ed., 2009

ELECTRON MICROSCOPE - GUN

Tungsten filament

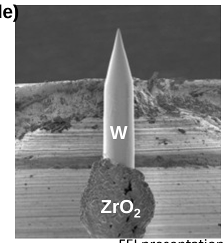


Cerium hexaboride



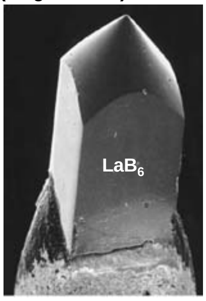
https://blog.phenom-world.com/

Schottky FEG (Tungsten with Zirkoniumoxide)

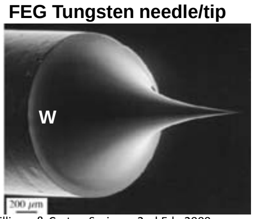


FEI presentation

Lanthanum hexaboride (single cristal)

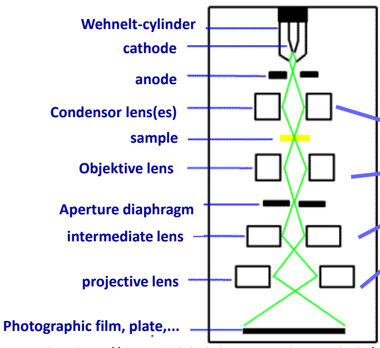


FEG Tungsten needle/tip



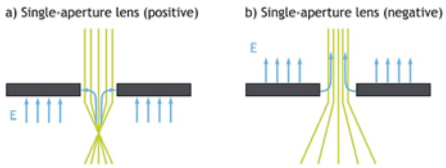
Transmission Electron Microscopy, Williams & Carter, Springer 2nd Ed., 2009

ELECTRON MICROSCOPE - LENSES

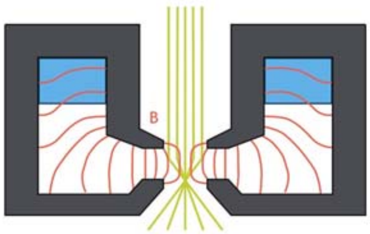


Based on http://daten.didaktikchemie.uni-bayreuth.de/

Electrostatic lens

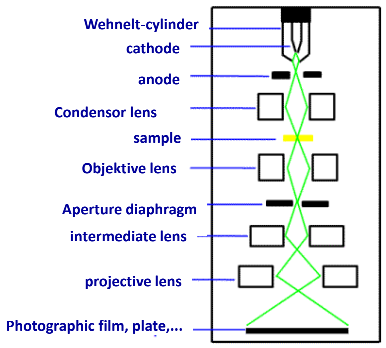


Magnetic lens



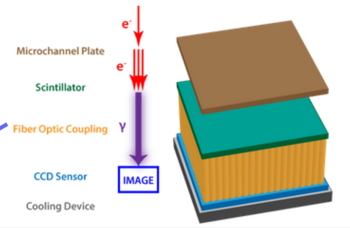
https://blog.phenom-world.com/

ELECTRON MICROSCOPE – DETECTION



Based on http://daten.didaktikchemie.uni-bayreuth.de/

charge-coupled device (CCD)



http://www.directelectron.com/products/lv-series

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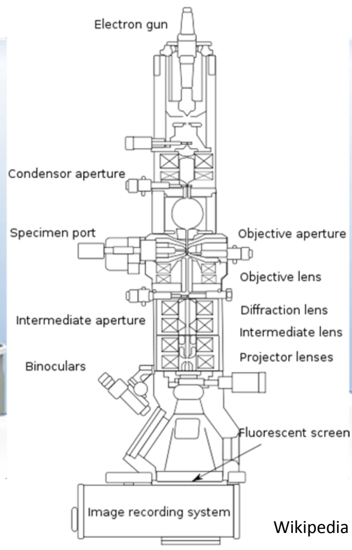
ELECTRON MICROSCOPE - SETUP

http://www.jeol.de/electronoptics/produktuebersicht/



Wikipedia

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OUTLINE

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Lens aberrations

- Chromatic aberration
- Spherical aberration
- Astigmatism
- Diffraction error

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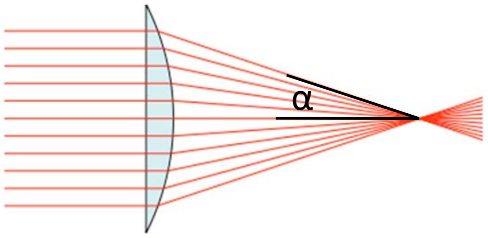
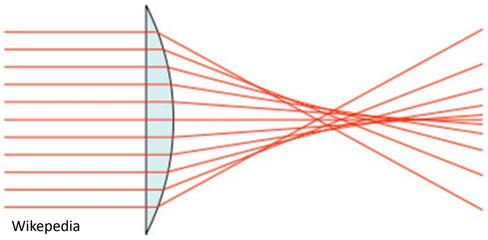
Lens aberrations

> Spherical aberration

$$d_s = C_s \alpha^3$$



Ideal lens



Real lens

Wikepedia

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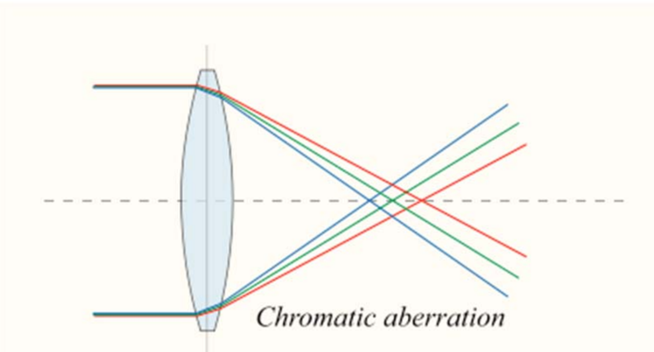
Lens aberrations

> Chromatic aberration

$$d_c = C_c \frac{\Delta E}{E} \alpha$$



Wikepedia

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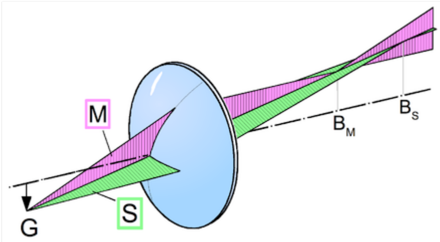
Lens aberrations

> Astigmatism

> paraxial rays are focused to a line instead of a point



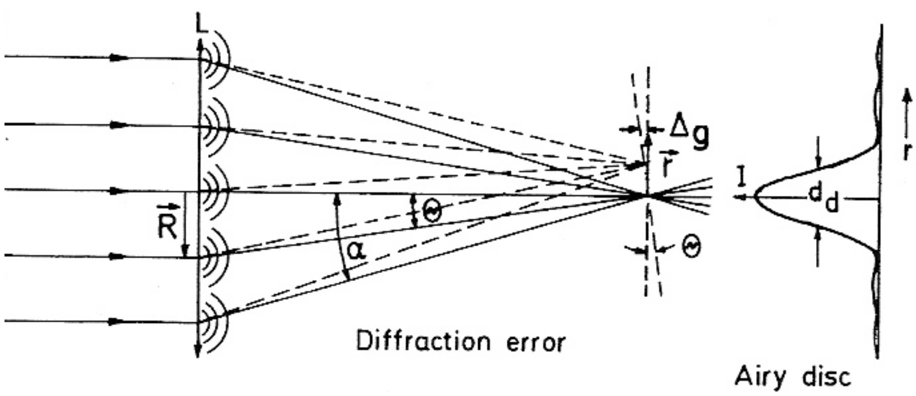
M: meridional plane
S: sagittal plane

Wikipedia

Lens aberrations

> Diffraction error

$$d_d = 0.6\lambda/\alpha$$



OUTLINE

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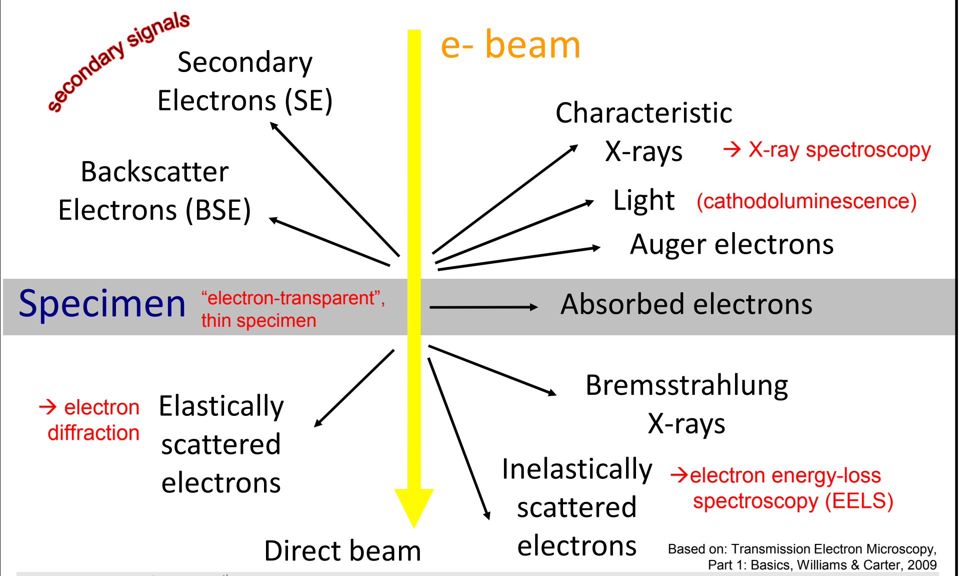
TYPES OF SIGNAL



Based on: Transmission Electron Microscopy, Part 1: Basics, Williams & Carter, 2009

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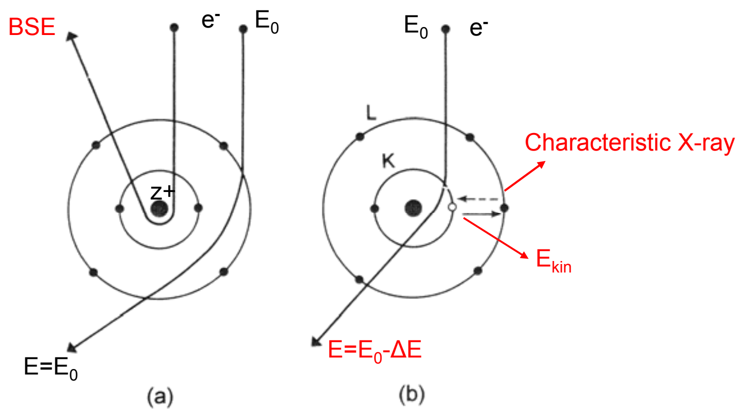
ELECTRON SCATTERING



elastic scattering:
(Coulomb attraction by nucleus)

→ change of momentum
→ no energy transfer

inelastic scattering:
(Coulomb repulsion by inner-shell electrons)

→energy-loss ΔE
→ excitation of electrons

Based on: Nano/Bio-Materialien: Mikroskopie/Beugung, H. Schmidt, 2011

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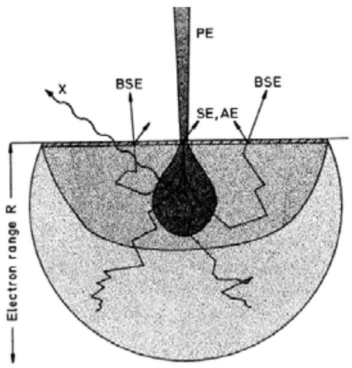
ENERGY TRANSFER



Scanning Electron Microscopy, L. Reimers, Springer 2nd Ed., 1998

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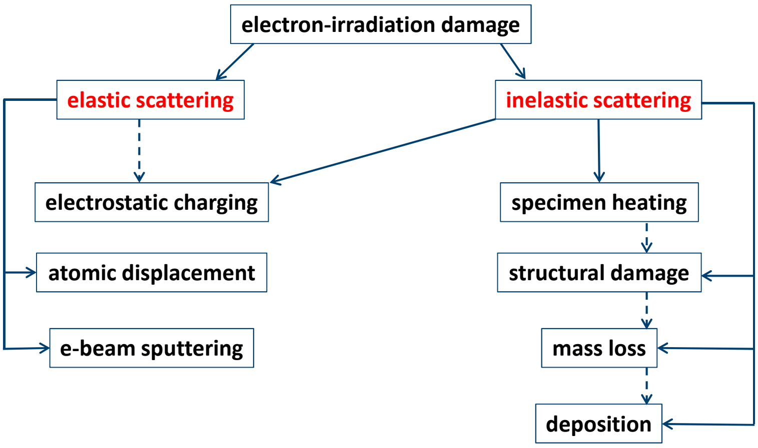
BEAM DAMAGE



Based on Fig. 1, Eggerton et al., 2004, Micron

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SCANNING EM (SEM)

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SETUP SCANNING EM (SEM)

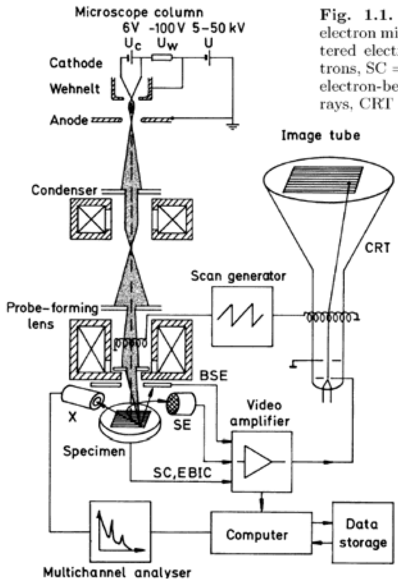

Fig. 1.1. Principle of the scanning electron microscopoe (BSE = backscattered electrons, SE = secondary electrons, SC = specimen current, EBIC = electron-beam-induced current, X = x-rays, CRT = cathode-ray tube)

Scanning Electron Microscopy, L. Reimers, Springer 2nd Ed., 1998

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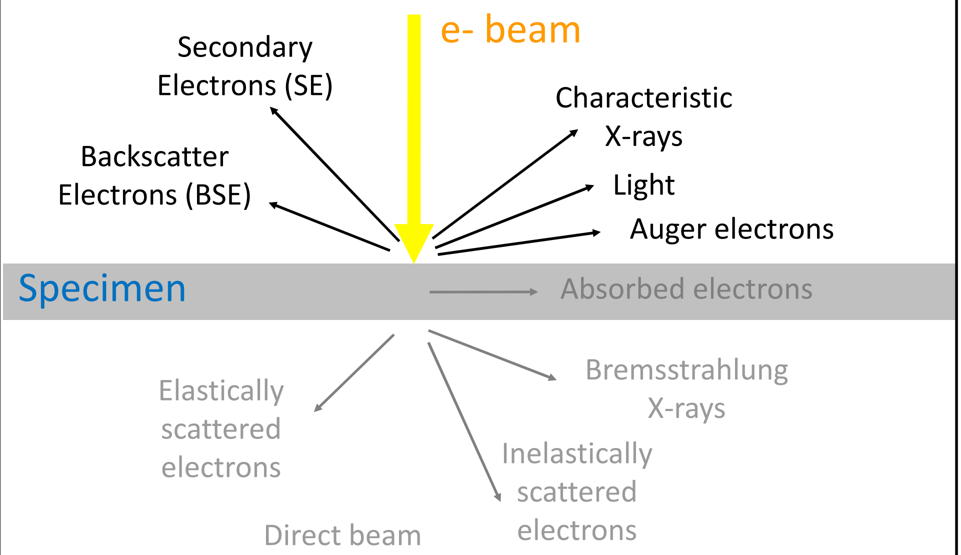
SIGNAL DETECTION WITH SEM



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SIGNAL DETECTION WITH SEM

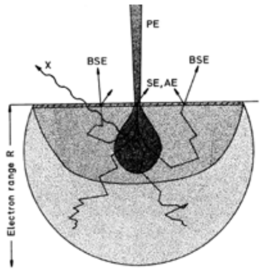

Fig. 1.4. Origin and information depth of secondary electrons (SE), backscattered electrons (BSE), Auger electrons (AE) and x-ray quanta (X) in the diffusion cloud of electron range R for normal incidence of the primary electrons (PE)

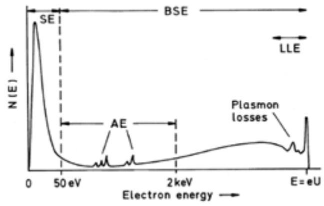

Fig. 1.5. Schematic energy spectrum of electrons emitted consisting of secondary electrons (SE) with $E_{SE} \lesssim 50$ eV, low-loss electrons (LLE) with energy losses of a few hundreds of eV, backscattered electrons (BSE) with $E_{BSE} > 50$ eV and peaks of Auger electrons (AE)

Scanning Electron Microscopy, L. Reimers, Springer 2nd Ed., 1998

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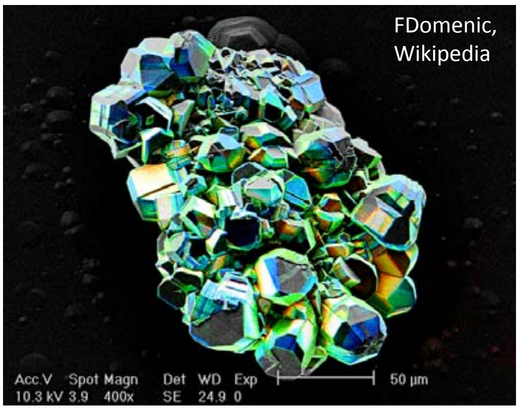
CATHODOLUMINESCENCE IN SEM



Color cathodoluminescence overlay on SEM image of an InGaN polycrystal. The blue and green channels represent real colors, the red channel corresponds to UV emission.

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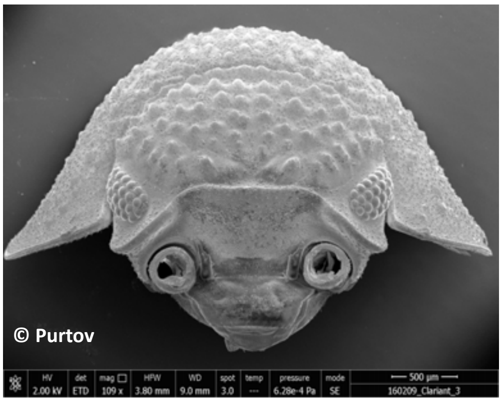
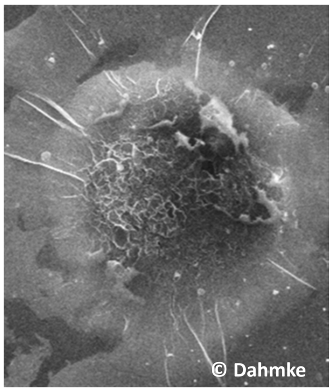
SEM IMAGES





Secondary electrons
Backscatter electrons
X-rays, Cathodoluminesce

} IMAGING OF SURFACE (THICK SAMPLES)
> ca. 1 nm RESOLUTION

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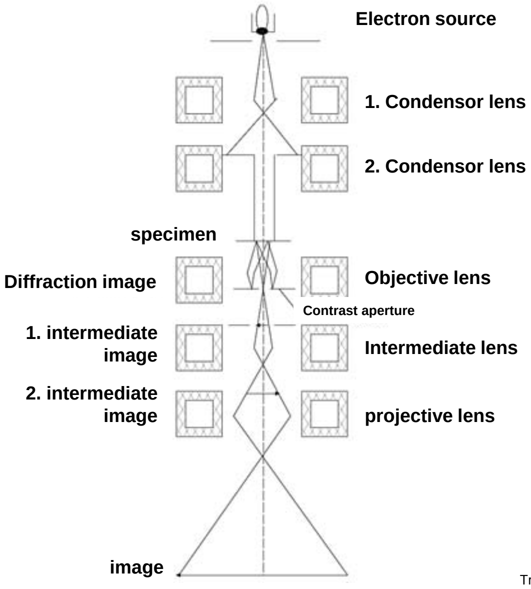
PATH OF RAYS IN TRANSMISSION EM (TEM)



Based on: fig. 2, Praktikum für Fortgeschrittene Transmissionselektronenmikroskopie, J. Schmauch, 2017

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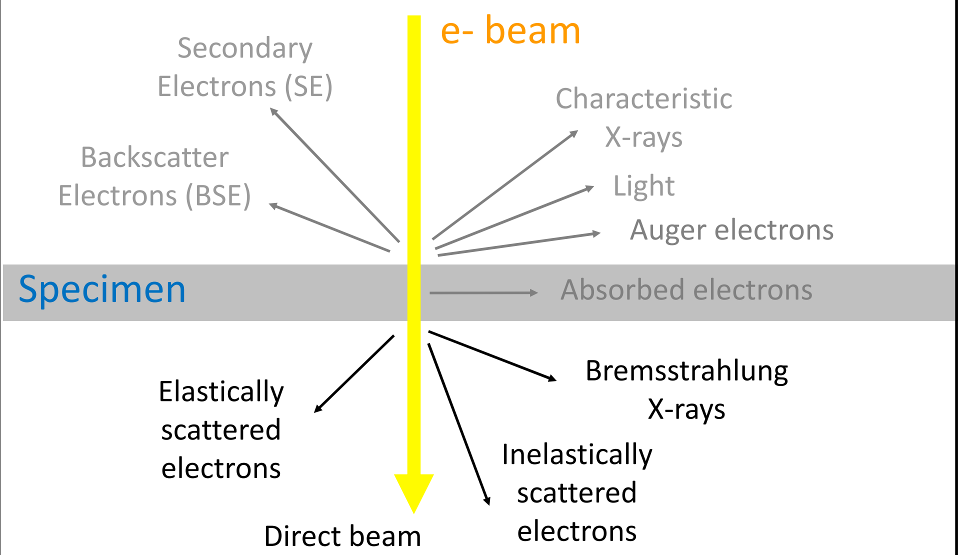
SIGNALDETECTION WITH TEM



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IMAGING MODES IN TEM

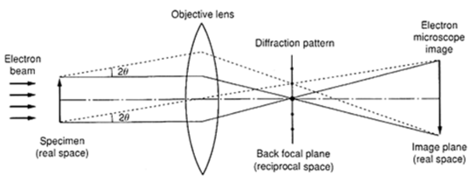
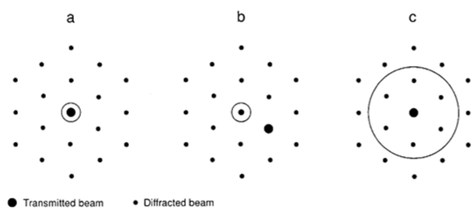


Fig. 1.1. Optical ray diagram with an optical objective lens showing the principle of the imaging process in a transmission electron microscope

Modes of Operation in TEM:
- Image Mode (BF/DF)
- Diffraction Mode (SAD, CBED)
- **Energy filtered TEM (EFTEM)**



Electron diffraction pattern in back focal plane

bright-field BF | dark-field DF | Lattice Imaging

(essentially diffraction contrasts)

(interference contrasts showing lattice periodicity of crystal structure)

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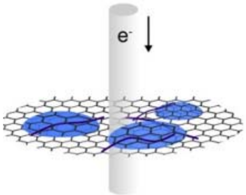
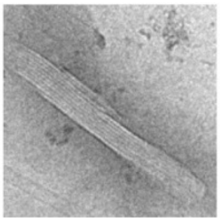
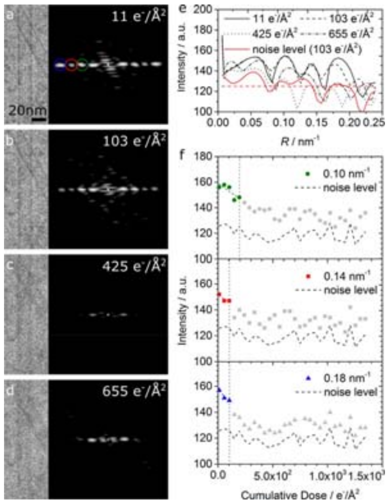
DIFFRACTION FOR ANALYSIS OF MICROTUBULES







Published in: Sercan Keskin; Niels de Jonge; *Nano Lett.* 18, 7435-7440.
DOI: 10.1021/acs.nanolett.8b02490

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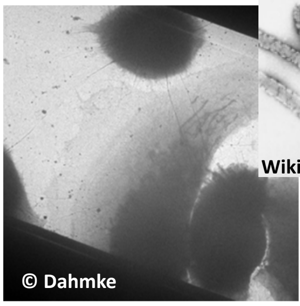
TEM IMAGES



Direct e-beam
Elastically scattered e-
Inelastically scattered e-
X-rays

IMAGING OF INTERIOR (THIN SAMPLES)
> ca. 0.1 nm RESOLUTION,

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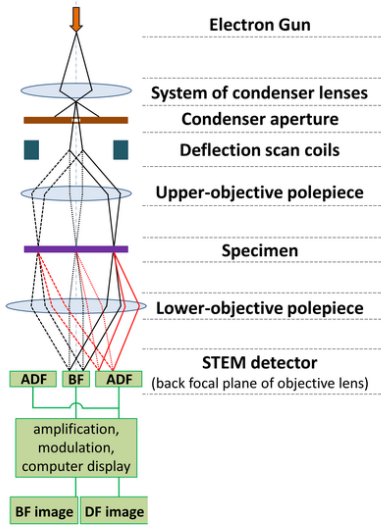
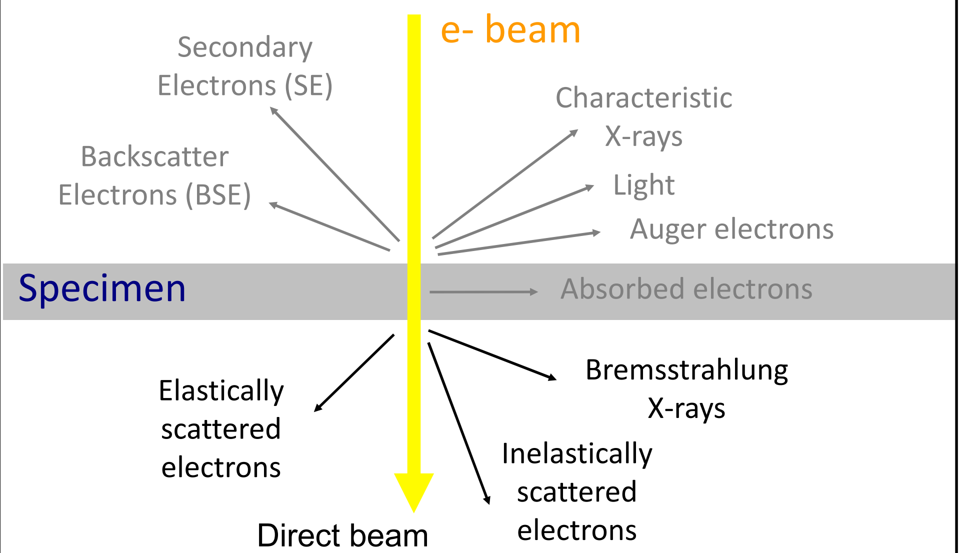
PATH OF RAYS IN SCANNING TRANSMISSION EM (STEM)

STEM mode

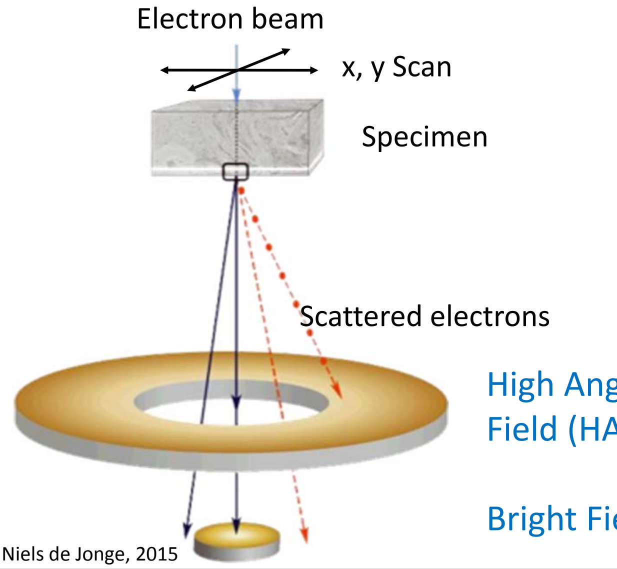


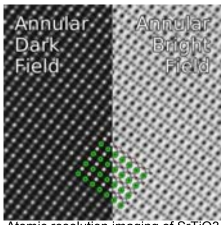
SIGNAL DETECTION WITH STEM



SCANNING TRANSMISSION EM (STEM) DETECTOR

INM

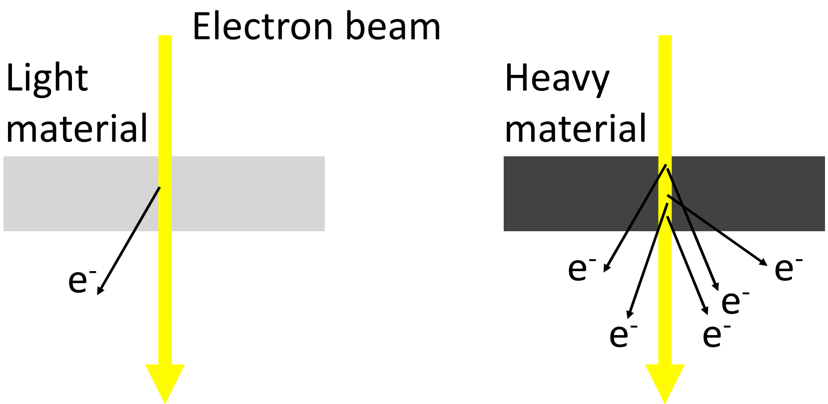




Atomic resolution imaging of SrTiO3, using annular dark field (ADF) and annular bright field (ABF) detectors. Overlay: strontium (green), titanium (grey) and oxygen (red). Wikipedia, Magnunor

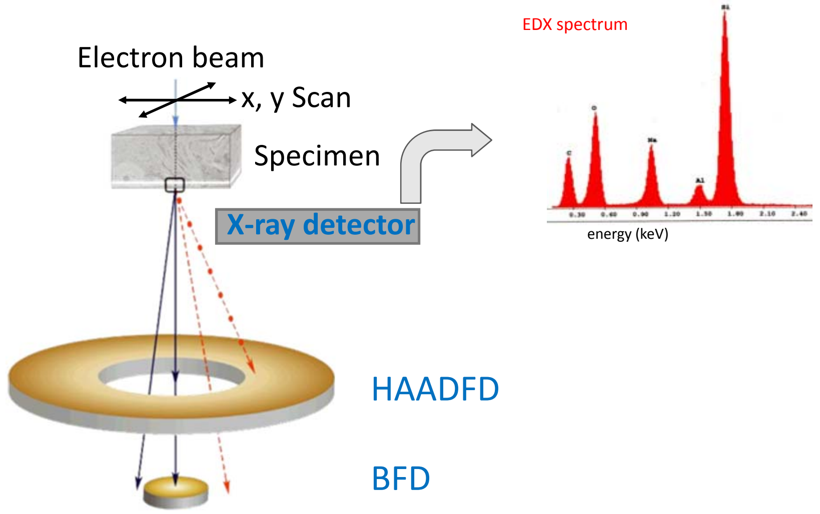
STEM IMAGES

INM



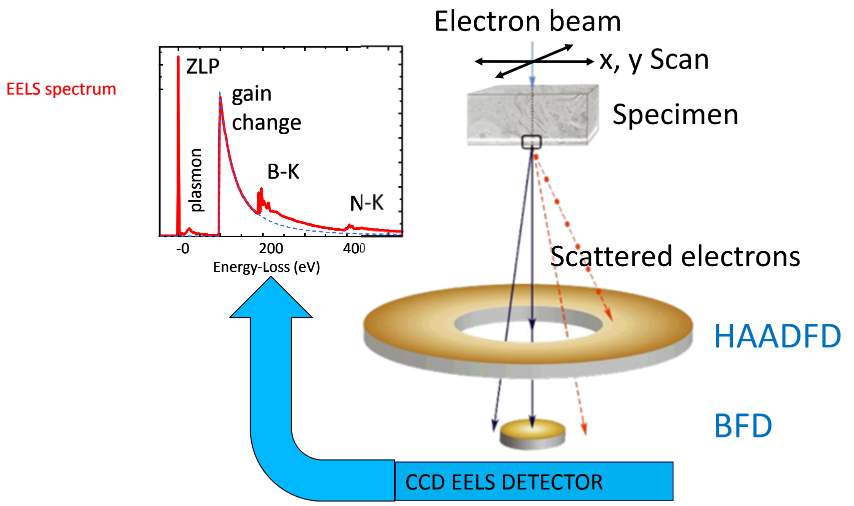
Contrast depends on atomic number (Z-contrast)

Energy-dispersive X-ray spectroscopy (EDX)



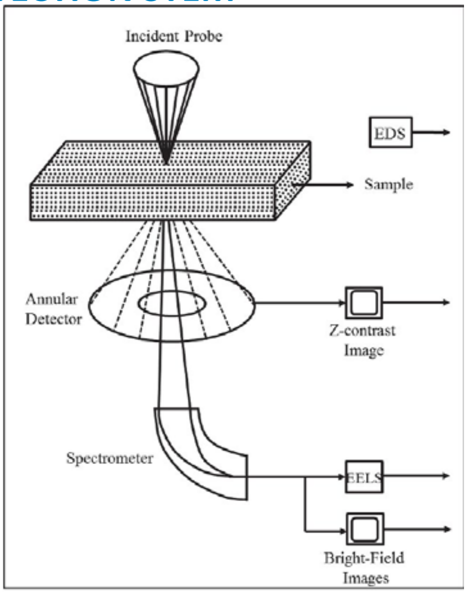
based on Niels de Jonge, 2015

Electron energy loss spectroscopy (EELS)



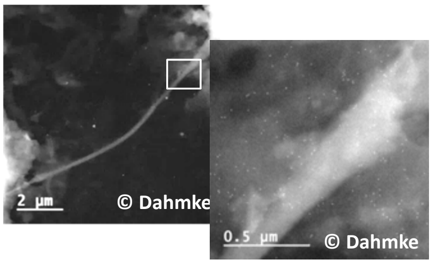
based on Niels de Jonge, 2015

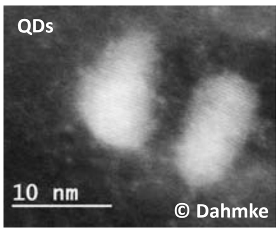
SIGNAL DETECTION STEM



Schematic of Scanning Transmission Electron Microscopy (STEM) by Subarna Khanal; 2006

STEM IMAGES





Direct e- beam
Elastically scattered e-
Inelastically scattered e-
X-rays

IMAGING OF INTERIOR (THIN SAMPLES)
ca. 0.1 nm RESOLUTION

Summary

(Analytical) TEM/STEM:

- materials characterization (nanostructure, crystal structure, chemical composition)
- Atomic resolution in (S)TEM imaging (0.08 nm in Cs-corrected system)
- EDX and EELS analysis with high spatial resolution
- Element mapping by energy filtered imaging (EFTEM) in TEM,
  and/or EDX/EELS spectroscopic imaging in STEM
- drawback: elaborate sample preparation

(Analytical) SEM:

- High-resolution surface imaging of bulk samples (1 nm in SE imaging at 15 kV)
- Topographical and compositional information by SE and BSE imaging
- Quantitative element and phase analysis by EDX (drawback: limited spatial resolution in bulk samples)

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OUTLINE

I. Why Electron Microscopy (EM)?

II. Properties of Electrons (e^-)

III. Basic Elements of Electron Microscopes

IV. Lens Aberrations

V. Interaction of e^--Beam with Sample > Signal Formation

VI. Limitations of EM

VII. EM of Biological Samples

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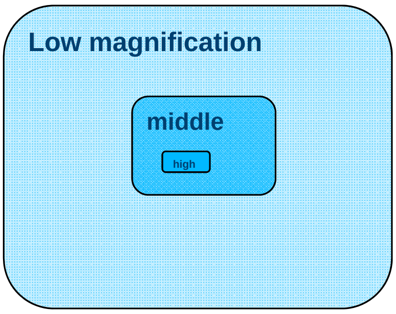
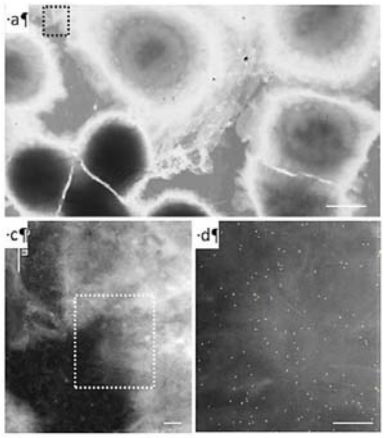
LIMITATIONS OF EM

INM

IN GENERAL:

- SMALL FIELD OF VIEW (bad sampling)





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LIMITATIONS OF EM

INM

IN GENERAL:

- SMALL FIELD OF VIEW (bad sampling)
- PROJETION LIMITATIONS (2D image from 3D sample)

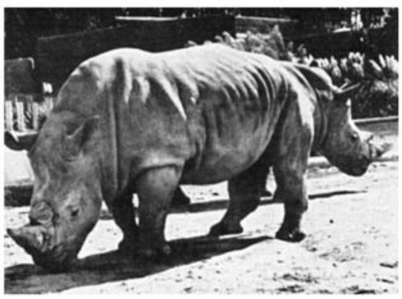

FIGURE 1.7. Photograph of two rhinos taken so that, in projection, they appear as one two-headed beast. Such projection artifacts in reflected-light images are easily discernible to the human eye but similar artifacts in TEM images are easily mistaken for 'real' features.

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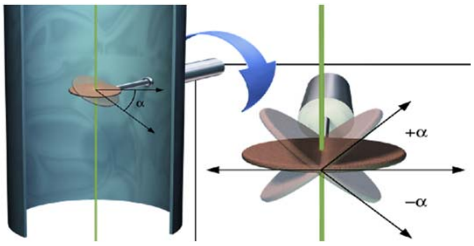
LIMITATIONS OF EM

IN GENERAL:

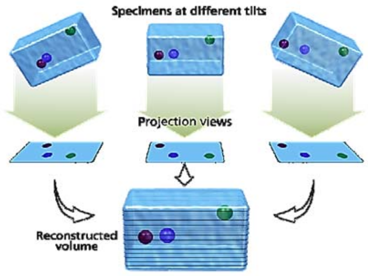
- SMALL FIELD OF VIEW (bad sampling)
- PROJETION LIMITATIONS (2D image from 3D sample)

Tomography of sample



DRAWBACK: EVEN SMALLER SAMPLING AREA

3D reconstruction



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LIMITATIONS OF EM

IN GENERAL:

- SMALL FIELD OF VIEW (bad sampling)
- PROJETION LIMITATIONS (2D image from 3D sample)
- SPECIMEN PREPARATION (THE THINNER THE BETTER (<100 nm)
- E- BEAM HAS HIGH ENERGY > BEAM DAMAGE OF SAMPLE

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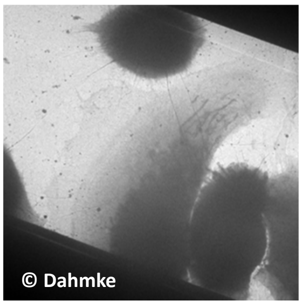
LIMITATIONS OF EM

REGARDING BIOLOGICAL SAMPLES (SOFTMATTER):

- HYDRATED SAMPLES ARE NOT COMPATIBLE WITH VACUUM
- ORGANIC SAMPLES SHOW LOW CONTRAST (low Z-number)
- NO CONDUCTIVITY
- HIGHER SENSITIVITY TO BEAM DAMAGE
- BULKY SAMPLE AND COMPLEX



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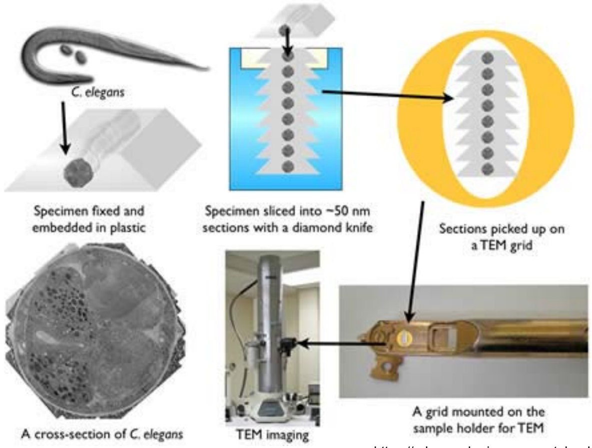
PREPARATION OF BIOLOGICAL SPECIMENS

CLASSICAL WORKFLOW TEM:

KILL > FIX > DEHYDRATE > IMBED > THIN CUTTING (SLICING/SECTIONING) > CONTRAST / SPECIFIC LABELING > IMAGING



https://advanced-microscopy.utah.edu/education/electron-micro/

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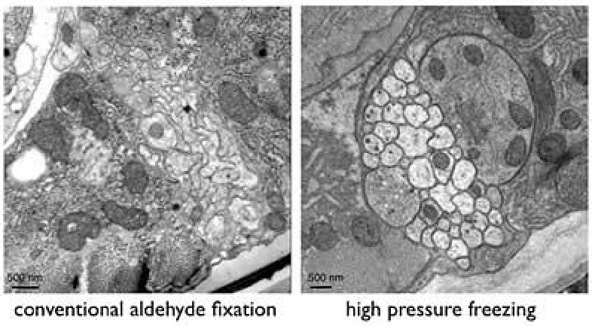
CONVENTIONAL FIXATION VS. HIGH PRESSURE FREEZING



https://advanced-microscopy.utah.edu/education/electron-micro/

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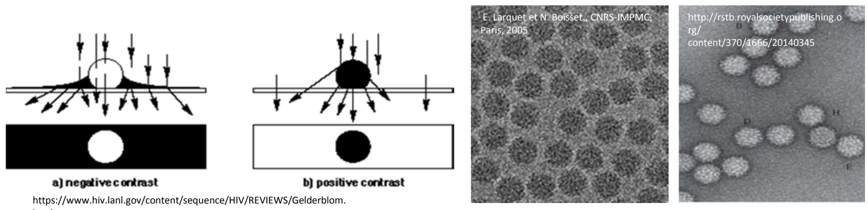
PREPARATION OF BIOLOGICAL SPECIMENS

INM

> CONTRAST > IMAGING



https://www.hiv.lanl.gov/content/sequence/HIV/REVIEWS/Gelderblom.html

PREPARATION OF BIOLOGICAL SPECIMENS

INM

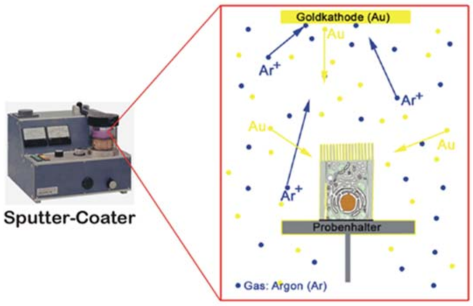
EXAMPLES:

- Sputter Coating
- Block Face EM
- Freeze Fractioning
- Liquid Phase EM

PREPARATION OF BIOLOGICAL SPECIMENS

EXAMPLES:

SPUTTER COATING



http://www.unikassel.de/fb19/cellbio/wwwSeiten/REM_Pr%E4p.htm

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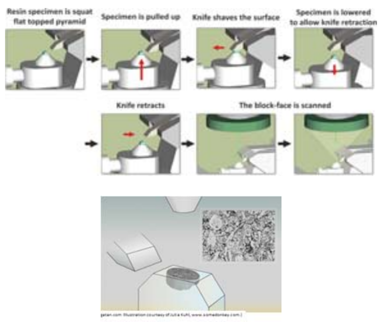
PREPARATION OF BIOLOGICAL SPECIMENS

EXAMPLES:

Block Face Scanning EM



http://www.biocenter.helsinki.fi/bi/em/emu_techniques_sbem.html

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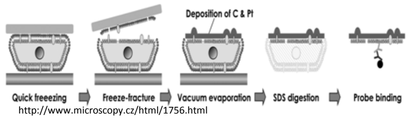
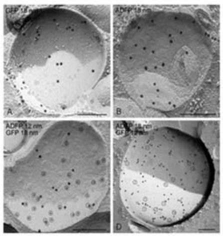
PREPARATION OF BIOLOGICAL SPECIMENS

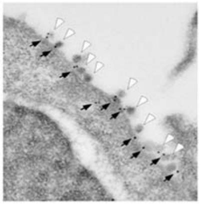
EXAMPLES:

FREEZE FRACTIONING



http://www.microscopy.cz/html/1756.html





(https://www.researchgate.net/publication/5227147_GFP-tagged_proteins_visualized_by_freeze-fracture_immuno-electron_microscopy_A_new_tool_in_cellular_and_molecular_medicine)

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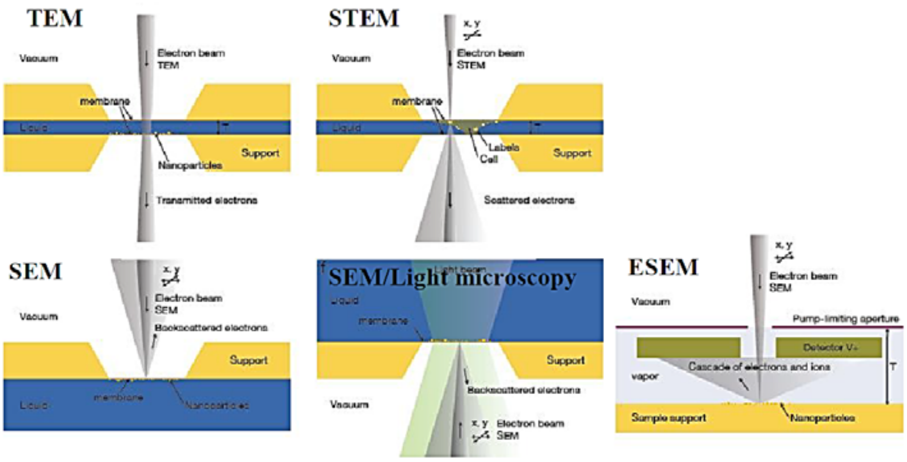
LIQUID PHASE EM – THIN FILM WINDOWS



Liquid cell transmission electron microscopy for in situ studies of crystal growth, F. Ross, lecture

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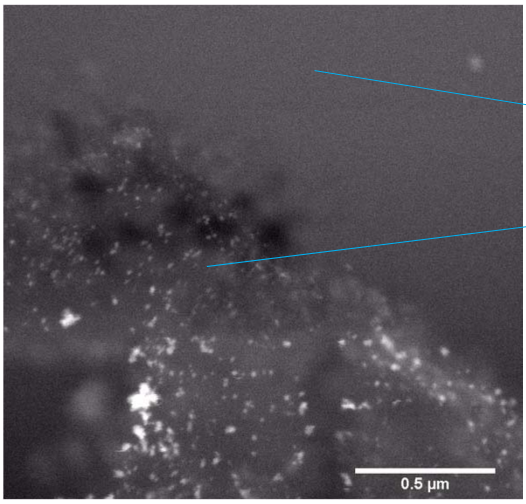
LIQUID PHASE EM

Pll-Latex window in liquid, dark field



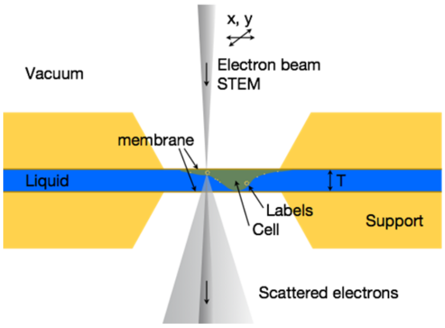
Charging effects?

Latex particles sintering?

Estimated electron dose 2800 e/nm^2/frame

170329_PL fallingdown_000-019 (7sfps)

(S)TEM OF HYDRATED CELLS IN LIQUID CELL

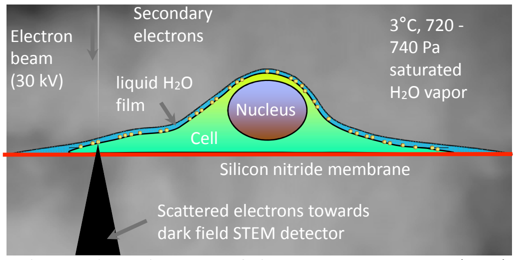


de Jonge & Ross, Nature Nanotechnology 6, 695 (2011)

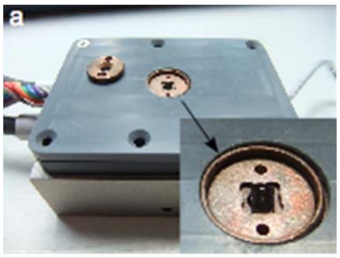
Protochips Inc.

DENS solutions

ENVIRONMENTAL SEM (ESEM) OF HYDRATED CELLS

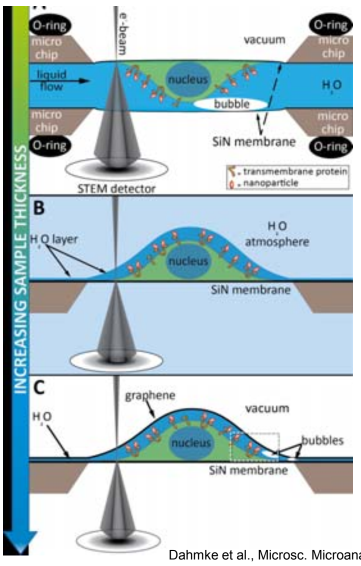


Peckys, Baudoin, Eder, Werner & de Jonge, Sci. Rep. 3, 2626 (2013)



Bogner et al., Ultramicroscopy (2005)

LIQUID PHASE EM



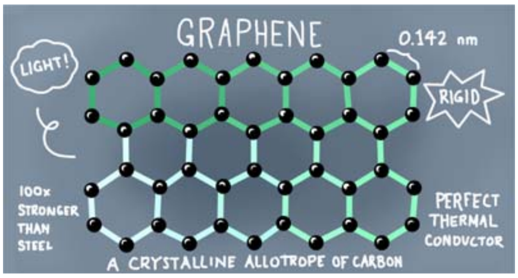
Dahmke et al., Microsc. Microanal. (2016)

Provides environment for hydrated biological samples and processes in material science (*in situ*), e.g. electrochemical reactions, Nano-particle assembly…

GRAPHENE

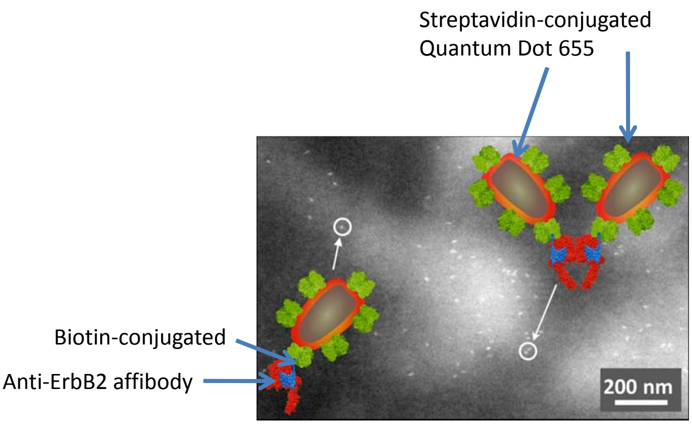
Single atom-thick layer of Carbon atoms:

- **Impermeable to liquids and gases**
- **Thermically and electrically conductive**
- **Electron transparent**



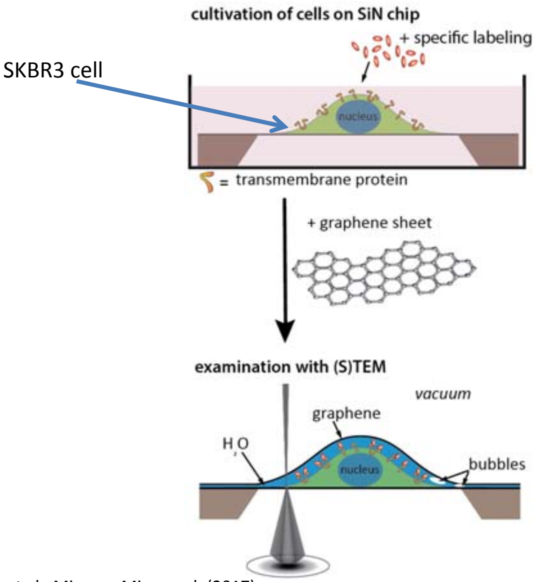
http://images.google.de/imgres?imgurl=http://graphenewholesale.com/wp-content/uploads/2015/05/

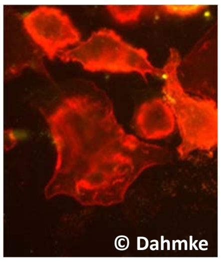
LABELING OF MEMBRANE PROTEINS

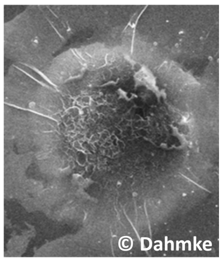


Peckys, D.B., Korf, U. & de Jonge, Science Advances 1:e1500165, 2015.

EXPERIMENTAL SET-UP

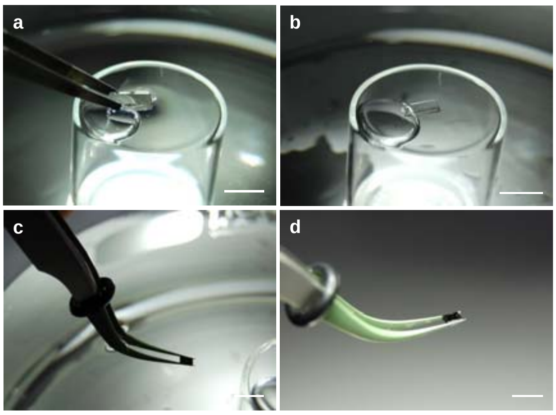






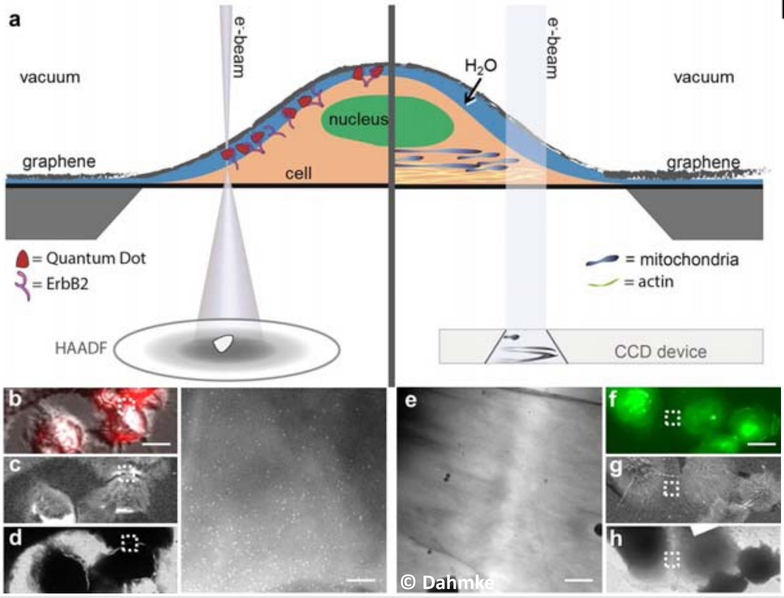
Dahmke et al., Microsc. Microanal. (2017)

GRAPHENE DEPOSITION ON HYDRATED SKBR3



Dahmke et al., ACS Nano (2017)

STEM versus TEM



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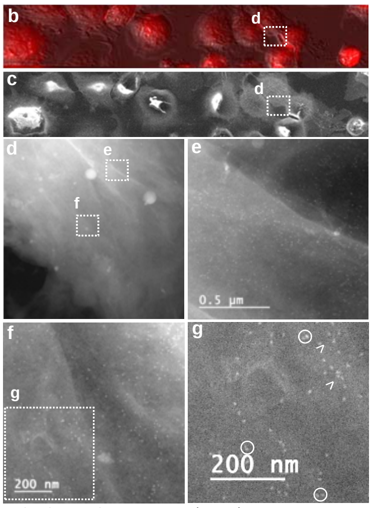
CORRELATIVE LIGHT AND ELECTRON MICROSCOPY



Dahmke et al., ACS Nano (2017)

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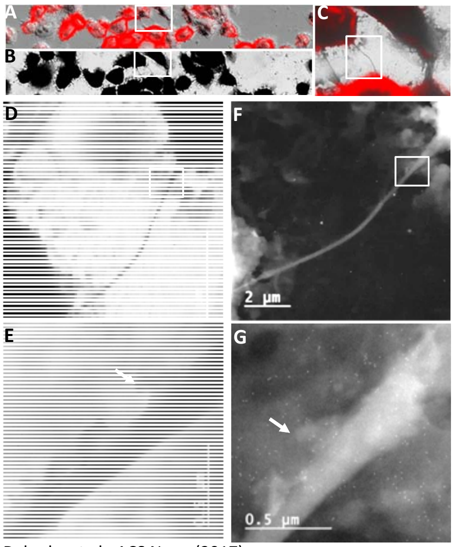
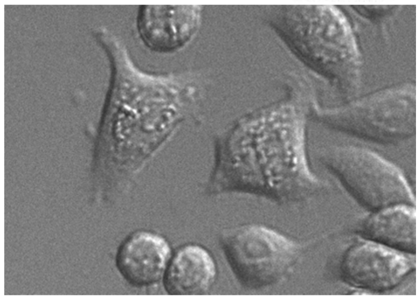
TUNNELING NANOTUBES (TNTS)



Dahmke et al., ACS Nano (2017)



Dahmke et al., ACS Nano (2017)

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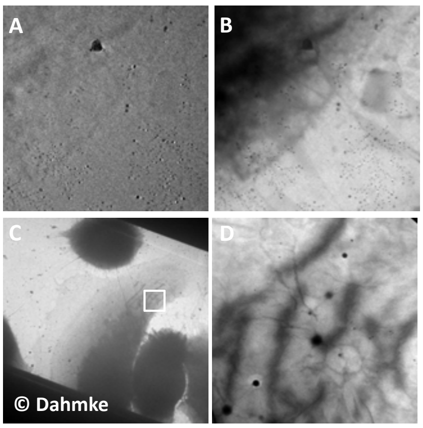
VIZUALIZATION OF CELLULAR STRUCTURES WITH LOW CONTRAST IN TEM



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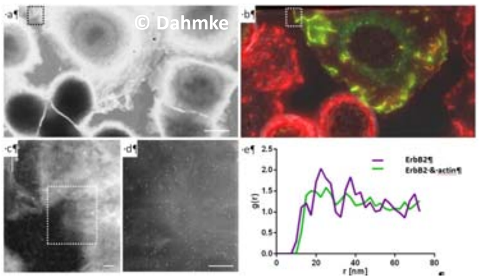
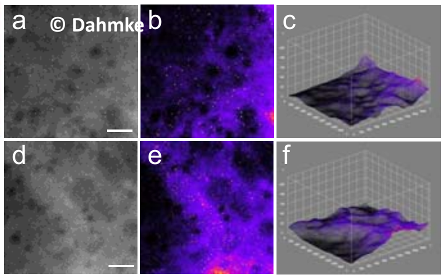
QUANTITATIVE ANALYSIS





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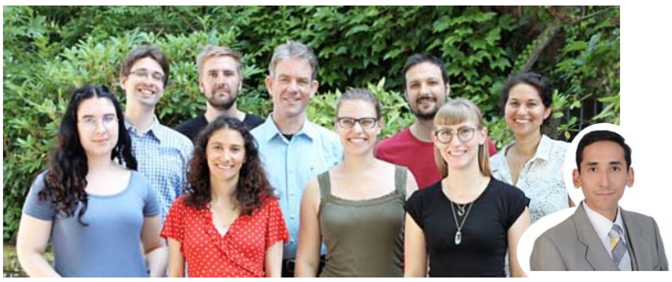

THANK YOU FOR YOUR ATTENTION!

Acronyms used in TEM/STEM:

- **TEM:** Transmission Electron Microscopy
- **BF/DF:** Bright-Field/Dark/Field TEM imaging
- **FEG:** Field Emission Electron Gun
- **EDS:** Energy-Dispersive X-ray Spectroscopy
- **STEM:** Scanning Transmission Electron Microscopy
- **EELS:** Electron Energy-Loss Spectroscopy
- **ESI:** Electron Spectroscopic Imaging (by EELS)
- **SIX:** Spectroscopic Imaging by X-ray
- **EFTEM:** Energy-Filtered TEM/STEM
- **HAADF:** High-Angle Annular Dark Field (STEM imaging)
- **Z-Contrast:** Atomic-Number Contrast in HAADF-STEM

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