Introduction to Image Acquisition Methods
Joachim Weickert, Winter Term 2007 / 2008

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Lecture 13:
Electron Microscopy

Contents

- Electron Radiation
- Electron Optics
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Electron Radiation (1)

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Electron Radiation

- By electrical fields, electrons can be accelerated to high speeds (close to the speed of light). The energy of a moving electron is measured in **electron-Volts (eV)**.
- Focussed moving electrons form electron beams.
- Moving electrons behave as a type of radiation. They interact with matter, can be manipulated and detected and therefore be used to measure images.
- Radioactive β decay also emits electrons (or positrons, cf. PET, Lecture 10) radiation.
- High-energy electron radiation is ionising radiation and causes damage in biological tissue.

Electron Radiation (2)

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Wavelength

◆ According to quantum mechanics, electron radiation also has a wave character. The wavelength λ is given by

$$\lambda = \frac{h}{\sqrt{2m_0eU}\left(1 + \frac{eU}{2m_0c^2}\right)}$$

where

h	Planck's constant
m_0	electron's rest mass
e	electron charge
U	voltage by which the electrons are accelerated

Electron beams used in electron microscopy have wavelengths in the picometre range (10^{-12} m).

◆ Remember Abbe's formula (see Lecture 5) which links wavelength of radiation with achievable image resolution: Electron beams with small wavelengths can resolve finer structures than visible light.

Electron Radiation (3)

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Interaction with Matter

◆ **Elastic Scattering:** deflection (Ablenkung) of electrons by electrical fields of atomic nuclei.

- Incident electrons are scattered by small angles.
- Energy of scattered electrons does not change considerably.

◆ **Inelastic Scattering:** result of interactions of electrons with atomic shell electrons. Energy is transferred to shell electrons which are therefore excited to higher energy levels.

- Incident electrons can be deflected by large angles
- Incident electrons lose energy
- Energy transferred to shell electrons is reemitted as secondary electrons or X-ray photons

Electron Radiation (4)

◆ **Auger and Secondary Electrons:** Electrons which are set free from atomic shells as consequence of inelastic scattering processes.

- *Secondary electrons:* outer shell electrons hit by incident electrons.
- *Auger electrons:* outer shell electrons which receive energy indirectly, after an inner shell electron has been excited.

◆ **Mean free path** of electron radiation in matter ranges from a few nanometres to a few hundred nanometres. Even in air electron beams don't get far.

Electron Optics

Electron Optics

◆ **Deflection.** As charged particles, electrons change their propagation direction in electric fields. Electromagnetic coils can therefore be used as *electron lenses*.

- Deflection angles (thus, focal lengths etc.) strongly depend on the speed (thus, energy or wavelength) of electrons – high chromatic aberration.

◆ **Absorption.** Diaphragms (Blenden) can be used to constrain electron beams.

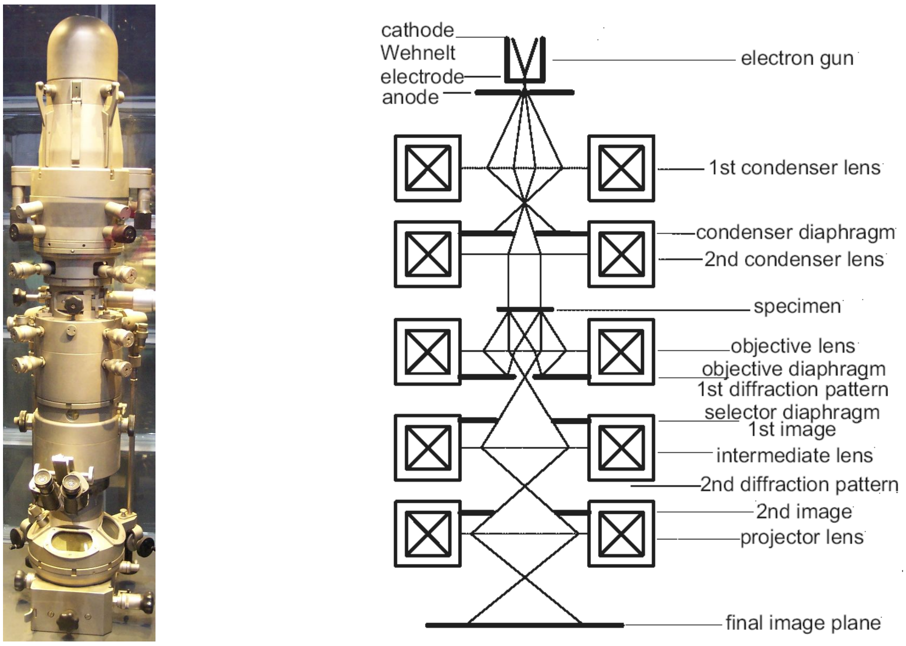
◆ **Diffraction.** Because of their wave nature, electron beams are also subject to diffraction.

- Interference patterns can be generated.
- Phase differences between electron beams are relevant.

Transmission Electron Microscopy (1)

Transmission Electron Microscope (TEM)

In **transmission electron microscopes**, electron optics is used for image formation in a similar way like light optics in an optical microscope.



Left: Electron microscope *(Wikipedia)*. **Right:** Schematic ray path in a TEM in bright field imaging mode *(Stegmann et al. 1999)*.

Transmission Electron Microscopy (2)

◆ **Vacuum.** Due to the short mean free path of electrons in air, the entire raypath of an electron microscope is evacuated.

◆ **Beam Source.** Generation of electrons by cathode emission, and focused by a negatively charged Wehnelt cylinder.

◆ **Aperture.** Small apertures have to be used due to aberrations.

◆ **Image contrast.** There are two kinds of contrast phenomena:

- *Amplitude contrast* caused by absorption of scattered electrons
- *Phase contrast* due to interference phenomena.

Transmission Electron Microscopy (3)

Examples of Image Sensors in TEM

◆ **Photographic Emulsions.** These are directly sensitive to electron radiation.

◆ **Fluorescent Screens with TV Cameras.** Cameras coupled to fluorescent screens via fibre-optic plates. Sensitivity can be raised by image intensifiers.

- Allow real-time observation and digitisation
- Low dynamic range

◆ **Semiconductor Detectors.** CCD arrays etc. have to be coupled with scintillator screens which transform electrons into photons, and protect the semiconductor chip from the damaging electron beam. The scintillator deteriorates resolution.

Transmission Electron Microscopy (4)

Optical Limitations of TEM Imaging

◆ **Spherical Aberration.** Phase shift for electrons not parallel to the optical axis, caused by different pathlength. The strong spherical aberration in magnetic lenses requires compensation by a small objective diaphragm.

◆ **Abbe's Limit.** Because of the small diaphragm aperture, Abbe's formula (Lecture 5) limits the possible resolution.

◆ **Chromatic Aberration.** Voltage deviations in the electron source, current variations in the electron lenses, and inelastic scattering lead to variations in electron energy.

Quantification of Aberrations

Effects of the different aberrations are expressed by radii of *confusion disks (Zerstreuungskreise)*:
A sharp object point is mapped to a confusion disk of a certain radius because of each imperfection.

Transmission Electron Microscopy (5)

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Preparation and Dose

- Extremely thin slices of probes have to be prepared (thickness down to $50\,\mathrm{nm}$).
  - Biological specimens are often dehydrated and embedded in plastic substrates, or rapidly frozen before cutting in ultramicrotomes.
  - Anorganic specimens may be sawed first and then polished to final thickness.
- Probes are damaged by high-energy electron radiation.
  - As a consequence, imaging can be destructive.
  - To obtain correct images, and to take more than one image of the same probe, requires careful dose adjustment.
  - However, lower dose results in noisier images.

3-D Extensions

- Holographic techniques can be used.
- Stereoscopic imaging (series of two and more images) is possible.
- In principle, tomographic imaging would also be possible. Often, however, it is prevented by damages in the probe caused by the electron radiation.

Scanning Transmission Electron Microscopy

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Scanning Transmission Electron Microscopy (STEM)

- In a **scanning transmission electron microscope,** the object is scanned with a focussed electron beam. Transmitted electron radiation is detected with a detector of lower spatial resolution.
- Preparation of objects is analogous to that for TEM.
- With sharp focussing, higher resolutions than with TEM can be achieved. Resolutions down to atomic sizes are possible.
  (Diametre of a hydrogen atom: ca. $100\mathrm{pm}$).

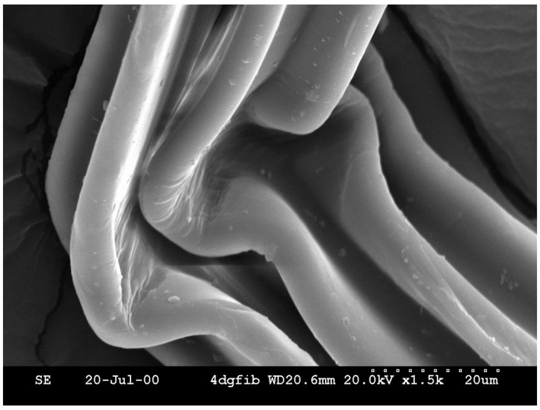
Scanning Electron Microscopy (1)

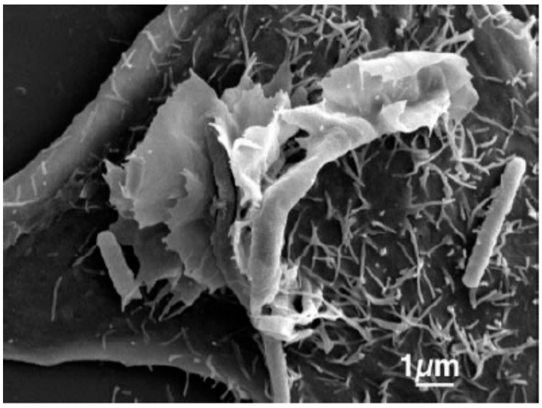
Scanning Electron Microscope (SEM)

- Images surfaces of massive objects.
- Surface is scanned with a focussed electron beam.
- Electrons emitted from the surface are detected; no high spatial resolution is required from the detector
- Detected electrons can be
  - Secondary electrons
  - Auger electrons
  - Back-scattered electrons – i.e. elastically scattered primary electrons with high energy which can come from rather deep in the probe.
- Resolution is worse than with STEM (due to a larger volume involved in the absorption/emission process) but SEM have excellent in-depth sharpness and give valuable structural information from object surfaces.

Scanning Electron Microscopy (2)

SEM Image Examples

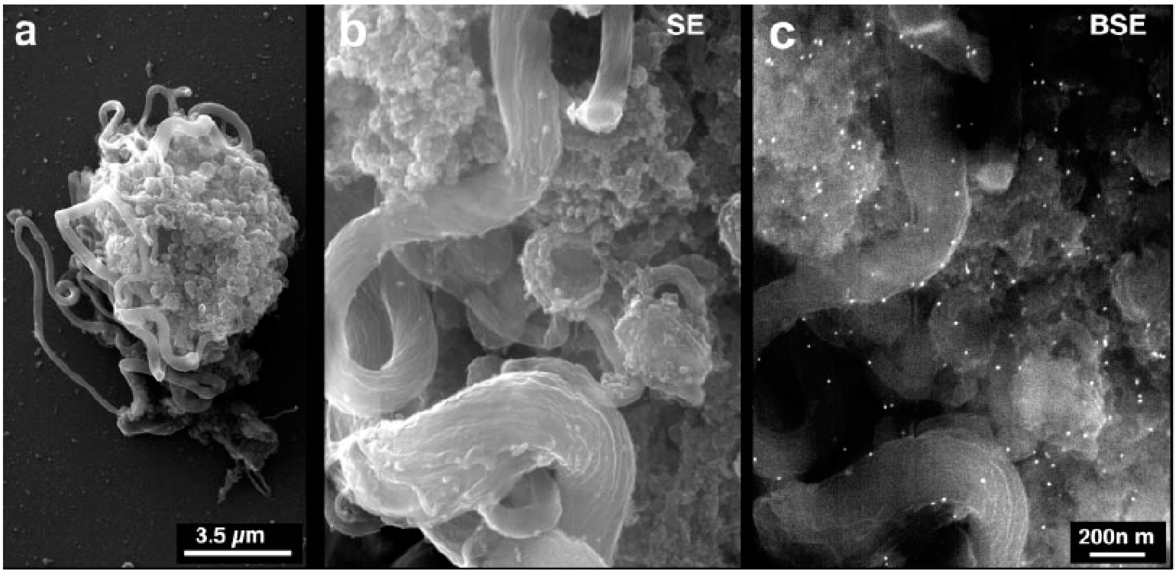




SEM images. **Top left:** Ant head *(United States Geological Survey – copied from Wikipedia)*. **Top right:** Polyester fibre *(Wikipedia)*. **Bottom left:** Bacteria infecting a cell. *(Stegmann et al. 1999)*

Scanning Electron Microscopy (4)

SEM Image Examples



SEM images of an insect cell labelled with gold-marked antibodies against a membrane receptor. **(a) Left:** Total view. **(b) Middle:** Detail imaged with secondary electrons. **(c) Right:** Same detail imaged with back-scattered electrons. Single metal clusters can be seen such that the receptors can be localised on the membrane. *(M. Cyrclaff, copied from Stegmann 1999)*

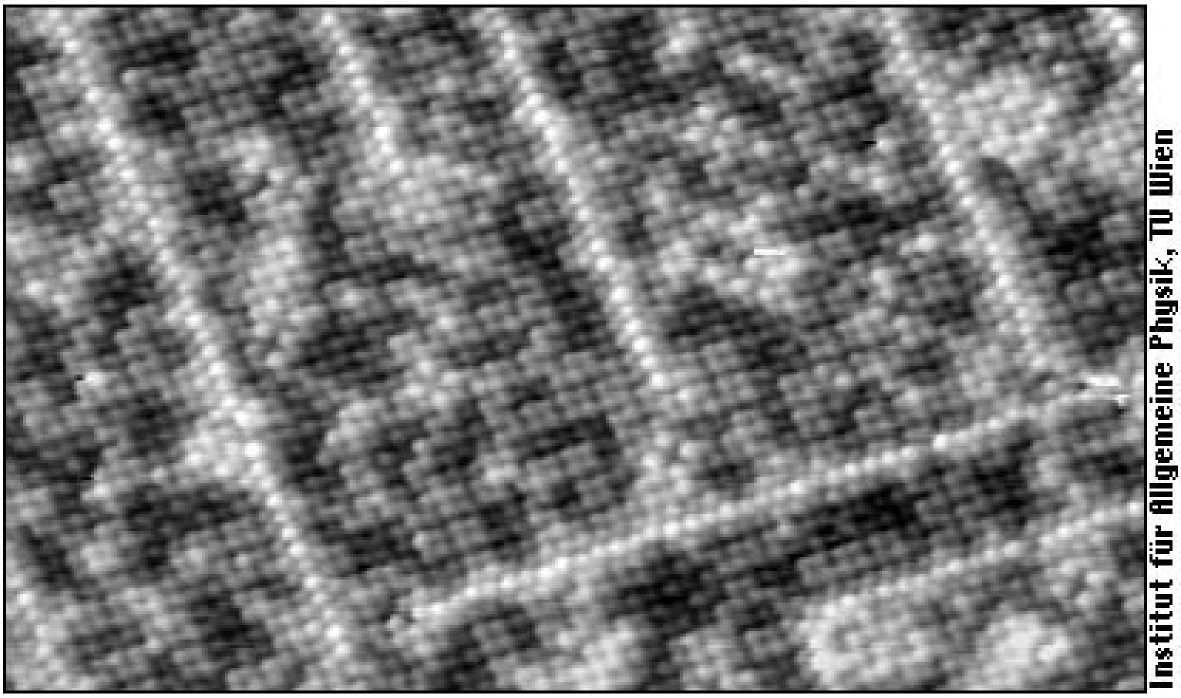
Scanning Tunneling Microscope (1)

Scanning Tunneling Microscope (STM)

- An STM is not an electron microscope in strict sense but a relative to it.
- Surface structures are directly scanned by an extremely fine needle. The point of this needle consists only of a single atom.
- The needle is moved across the (electrically conductive) surface at a distance of ca. $1\,\mathrm{nm}$ (note that collision would destroy the needle).
- This distance is kept constant, with a precision of about $2\,\mathrm{pm}$, by a piezoelectric servo element according to a measured tunneling current.
- The tunneling current is an effect from quantum physics: Electrons that actually should not be able to move through the gap between needle and surface can bridge this gap by the *tunnel effect*. When a low voltage is applied, these electrons form the tunneling current which is highly sensitive to the width of the gap.
- The servo control information makes up precise depth profile. Horizontal resolutions of ca. $100\,\mathrm{pm}$ and vertical resolutions of less than $100\,\mathrm{pm}$ can be achieved. Single atoms can be imaged!
- An STM typically operates at very low temperatures

Scanning Tunneling Microscope (2)

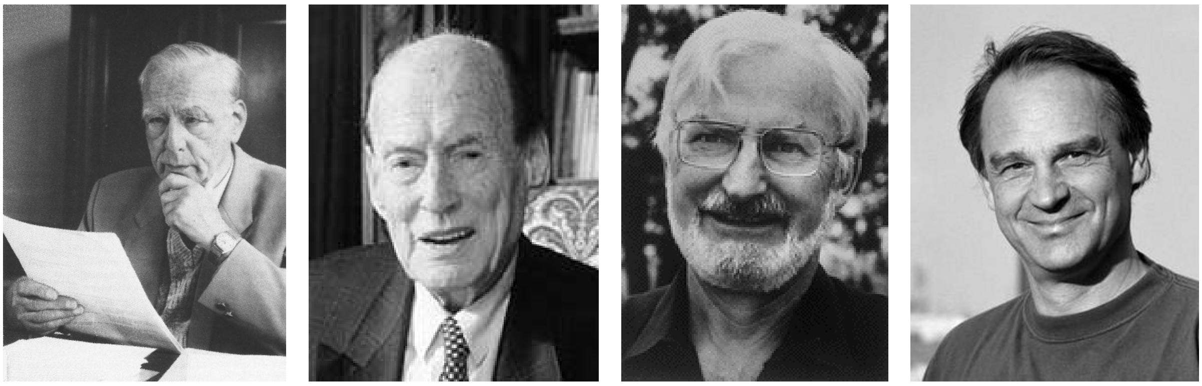
STM Image Examples



Crystal surface of platinum and nickel atoms, and carbon atoms which can not be seen directly. *(Institut für Allgemeine Physik, Vienna University of Technology)*

History of Electron and Tunneling Microscopy

Inventors



(a) Left: Ernst Ruska (1906–1988). He has built the first TEM in 1931, and commercial ones in 1938. **(b) Left middle:** Manfred von Ardenne (1907–1997) developped the STEM in the 1930s. **(c) Left right:** Heinrich Rohrer (b. 1933) **(d) Right:** Gerd Binnig (b. 1947). Binnig and Rohrer developed the scanning tunneling microscope in 1982. Ruska, Binnig and Rohrer received the 1986 Nobel Prize for Physics. *(Images: www.th.physik.uni-frankfurt.de/˜jr/gif/phys/ruska.jpg – barrierefrei.dresden.de – nobelprize.org – Wikipedia)*

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Summary

- Electron beams also have a wave-like character that depends on their energy.
- This is used in electron miscroscopy where wavelengths in the picometer range are possible, allowing higher resolution than light microscopy.
- One can design electron optics.
- In the transmission electron microscope (TEM), electron optics is used in a similar way as conventional optics in a light microscope.
- The scanning transmission electron microscope (STEM) the object is scanned with a focussed electron beam, yielding resolutions in the atom range.
- The scanning electron microscope (SEM) also uses focussed electron beams, but measures emitted (instead of transmitted) electrons.
- The scanning tunneling microscope (STM) scans objects with a very fine needle with a single atom on the top. It is steered by a piezoelectric element exploiting the tunnel effect.

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References

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- Scanning tunneling microscopy web pages at Institute for General Physics (Institut für Allgemeine Physik), Vienna University of Technology, Scanning Tunnel Microscopy page, http://www.iap.tuwien.ac.at/www/surface/STM_Gallery/index.htmlx
- G. Binnig, H. Rohrer, *Scanning Tunneling Microscope,* U.S. Patent 4343993, 1982, http://www.nanoworld.org/museum/ Scanning%20tunneling%20microscope%20(US4343993).htm