MPI of Biochemistry: THE CAMPUS

Department of Molecular Structural Biology
In 1928, Dennis Gabor had rejected Leo Szilard’s suggestion to make a microscope based on electron waves with the response:

“What is the use of it? Everything under the electron beam would burn to a cinder!”

TEM HISTORY: An Anecdote...

“You know,” Szilard told Gabor over a café table in 1927, now that it is possible to make electron lenses, “why do you not make a microscope with electrons?” At smaller and smaller wavelengths, you would achieve much more detailed resolution than is possible with microscopes using light. Gabor and Szilard pondered his idea for a few minutes, then agreed it would serve no useful purpose. After all, you could not put living matter into the kind of vacuum tube needed to control electron beams. Besides, they concluded, so much power would be focused in the electron beam that it would incinerate any sample.

But as Gabor later realized, with that idle suggestion Szilard had grasped the possibility of an electron microscope at least a year before anyone else. And of the incinerated sample, Gabor later wrote, “Who would have dared to believe that the cinder would preserve not only the structure of microscopic bodies but even the shapes of organic molecules?” Gabor would be remembered years later as the inventor of holography, for which he received the 1971 Nobel Prize in physics.15
Laboratorium für Übermikroskopie
BAKTERIEN UND VIRUS IN ÜBERMIKROSKOPISCHER AUFWAHME
(mit einer Einführung in die Technik des Übermikroskops).
Von
B. von Borries, E. Ruska und H. Ruska.*

Aus dem Laboratorium für Elektronenoptik des Wernerwerks F der Siemens & Halske.
Aktiengesellschaft und der I. Medizinischen Universitätsklinik der Charité.

Ziel der Arbeit.
Durch das Übermikroskop können solche Krankheitserreger sichtbar gemacht werden, die bisher ohne Anwendung von Färbeverfahren wegen ihrer Kleinheit im Lichtmikroskop unsichtbar waren. Abb. 1 stellt das Virus der Mäuseektormelie in übermikroskopischer Aufnahme dar. Man kann
'Cryo-Electron Microscope': The concept

NEW APPROACHES IN CORRELATIVE STUDIES OF BIOLOGICAL ULTRASTRUCTURE BY HIGH-RESOLUTION ELECTRON MICROSCOPY

By
Professor H. Fernandez-Moran, M.D., Ph.D.
Committee on Biophysics
University of Chicago
Chicago 37, Illinois

Paper presented at ROYAL MICROSCOPICAL SOCIETY'S Celebration of the "Tercentenary of the Microscope in Living Biology"
April 9, 1963
Bethesda, Maryland

These "cryo-electron microscopes", operating at temperatures of 1 to 4 degrees Kelvin, would embody the following significant features: (a) highly stable superconducting electromagnetic lenses, with very ripple-free magnetic fields of a persistent current in the optimum case; (b) operation in ultra-high vacuum and low temperatures resulting in decisive advantages of minimized specimen contamination, specimen damage and thermal noise; (c) optimum conditions for both low voltage (i.e. 1 to 10 kV) and high voltage electron microscopy. In addition, the use of high-


Humberto Fernández-Morán
*1924 Maracaibo, Venezuela
†1999 Stockholm, Sweden
‘Cryo-Electron Microscope’: The concept

Humberto Fernández-Morán
*1924 Maracaibo, Venezuela
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Abstract. With use of an electronic picture-scanning device and a digital computer, electron micrographs taken of a specimen along several different directions can be superimposed to form a montage that is more informative than the component images. Preliminary results indicate that one may thus study unstained, unshadowed biological material at high resolution.

Because of their low inherent contrast in the electron microscope, isolated biological particles or tissue sections are usually prepared for observation by staining, negative staining, or shadowing with some electron-dense material. While such techniques have been of great value for observation of features coarser than 20 Å, finer details are thus destroyed, obscured, or simply not enhanced. Clearly, if recent improvements in electron microscopes are to be fully utilized in biology, contrast must be enhanced without drastic molecular alteration of the specimen or obscuration by extraneous material.

I have developed another kind of montage to reinforce weak image signals which requires no specimen symmetry. The several component images that are to form the montage are obtained by tilting the specimen in the electron microscope (3) and taking transmission micrographs of the same area along several different directions (hence the term polytropic). To produce such a montage, the component images appearing in the original micrographs must be transformed, by simple linear operations, into images that are superimposable. If the images are reduced to numerical data with a scan-
Tomography or the ‘polytropic montage’

Electron Microscopy of Unstained Biological Material: The Polytropic Montage

Abstract. With use of an electronic picture-scanning computer, electron micrographs taken of a specimen along a set of parallel planes can be superimposed to form a montage that is more informative than the component images. Preliminary results indicate that one can obtain unshadowed biological material at high resolution.

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Thus the polytropic montage seems to offer a means of determining the three-dimensional structures of low-contrast biological specimens at a resolution of 3 Å, or the best resolution attainable with existing electron microscopes. I have not yet reached this point, but preliminary efforts have produced images of tobacco mosaic virus comparable in fineness of detail to those obtained by shadowing a similar specimen with tungsten or rhenium (8). Still to be determined is the extent to which the fine details appearing in the montage represent real structures of the virus rather than residual noise that may have survived this attempt at its elimination.

Roger G. Hart
Biomedical Division, Lawrence Radiation Laboratory, University of California, Livermore 94550

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Electron microscopy of unstained biological material: The polytropic montage.
Science 1968, 159, 1464-1467
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Fig. 16. „Tomographischer Hähnchengrill“. Das Hähnchen H auf dem Drehspieß S wird durch das Feuer F gegrillt. Bei der Drehung werden in gleichen Winkelinkrementen $\Delta \alpha$ Aufnahmen mit paralleler Röntgenstrahlung R auf Platten P hergestellt. Im elektronenmikroskopischen Fall sind die Röntgenstrahlen durch Elektronenstrahlen zu ersetzen; die Parallelprojektion wird durch Abbildung bei hoher Tiefenschärfe erzeugt. Das „Grillen“ des Präparates besorgen (leider) die Elektronen selber.
Titan Krios

- Development start/a: 2004
- Development end/a: 2011
- Voltage/kV: 80-300
- Samples/#: 12
- FEG-type: Schottky
- Lenses: constant current
- Condenser: 3 lenses
- Energy filter: post-column

…starring Mike Strauss
CRYO-EM: The ‘Hurdles’

- Samples that have to be studied in their hydrated state to ensure structural preservation
- Suitable sample thickness to obtain molecular resolution
- Low-contrast due to weakly scattering building blocks
- Sensitivity to ionizing radiation

Vitrification
- Converts liquid water into amorphous ice

Sample Preparation
- Advance Micromachining
- Advance 3D Correlation

Poor Imaging Conditions
- Optimize Instrumentation
  e.g. Detectors & Phase Plates

Low Dose Methods
- Advance Automation
WHY?: Microscopy with electrons

Szilard was right: At smaller and smaller electron wavelengths, one can achieve much more

\[ \lambda_{\text{electrons}} = \frac{h}{mv} \approx \frac{h}{\sqrt{2m_0E(1 + \frac{E}{2m_0c^2})}} \]

<table>
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<tr>
<th>energy E [keV]</th>
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→ Transmission electron microscopes can resolve details down to 1 Ångström!
## TEM HISTORY: The past...

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<td>E. Ruska/ G. Binning and H. Rohrer</td>
<td>Received the Nobel Prize for TEM and STM</td>
</tr>
</tbody>
</table>

OUTLINE: The Instrument

Inside and outside the
<table>
<thead>
<tr>
<th></th>
<th>The Instrument</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>The “GUN”</td>
</tr>
<tr>
<td>2</td>
<td>Condenser System</td>
</tr>
<tr>
<td>3</td>
<td>Objective System</td>
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<tr>
<td>4</td>
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<td>5</td>
<td>Sample Holders</td>
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<td>6</td>
<td>Electron Detectors</td>
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<tr>
<td>7</td>
<td>Energy Filters</td>
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## OUTLINE: The Instrument

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**MECHANICS & VACUUM SYSTEM**
OUTLINE: The Instrument

1. The “GUN”
2. Condenser System
3. Objective System
4. The “STAGE”
5. Sample Holders
6. Electron Detectors
7. Energy Filters
Objective lens

Final image

Gun crossover

Double condenser

Specimen

Objective lens (OA)

1st Image plane

Intermediate lens

Projectors

Final image
Cryo-EM and Cryo-ET: Instruments for high-resolution (HR) and high-throughput (HT)

Sample Preparation

- Molecules
- Cells
- Cryo-FIB milling
- Correlative LM/FM

Instruments for high-resolution (HR) and high-throughput (HT)

- Cryo-EM
  - Autoloader
  - $C_s$-corrector
  - Phase plate
  - Direct detection
  - Autotuning
  - HT-acquisition

- Cryo-ET
  - $C_c$-corrector
  - Energy Filter
  - Batch tomography

Automation and 3D data Analysis

- SPA
- Tomography
THE GUN: Types of Emitters

Thermionic Gun
- LaB$_6$ crystal or Tungsten

Field emission Gun (FEG)
- Tungsten tip coated with ZrO

100 nm

extract electrons
accelerate
image
Electrons are manipulated using electromagnetic lenses. The electron beam coming from the gun is focused and shaped with the help of condenser lenses and apertures. The objective lens and the projection system are used to obtain and magnify a diffraction pattern or the real image.
CONDENSER System: C2 ‘Intensity’

- When C2 is weak, Intensity knob turned fully anti-clockwise, the rays from the source spread all over the phosphor screen.
- Turn up C2, turning the Intensity knob clockwise, the rays get more and more concentrated on the screen, so what we see gets brighter and brighter.
- Then we reach the focal position: electrons coming from any particular point on the source all arrive at the same place, and so we can see a sharp image of the source.
- As we turn C2 yet higher, the beams spread out again into a circle shape, which eventually spills off the phosphor screen. As we turn it even higher, the screen gets darker and darker as the electrons get spread ever more thinly.
- When an electron lens is turned up (knob turned clockwise) above the focal position, it is said to be ‘over-focused’; when it is turned down it is said to be ‘under-focused’.
- When you are doing normal imaging, you should always run C2 (intensity) over-focused.

The best way to achieve parallel illumination is to point (focus) at something a very long distance away.
CONDENSER System: Ray Diagram

* IP = image plane
CONDENSER System: Ray Diagram

Increase strength of C2

Gun

'under-focus': weaker lens focuses after the image plane

C2

IP*

'* IP = image plane

'under-focused'
CONDENSER System: Ray Diagram

- Increase strength of C2

Gun

C2

IP*

* IP = image plane

- 'under-focus': weaker lens focuses after the image plane
- ‘over-focus’: strong lens focuses before the image plane

‘under-focused’

‘over-focused’
Condenser ... parallel illumination

increase strength of C2

convergent

divergent

parallel

C2

objective lens pre-field

objective lens
Parallel illumination on a Talos Arctica

Objective aperture is brought into focus in the front focal plane of the diffraction lens using defocus – crisp edges of the objective aperture (100 μm)
This is followed by spreading of the beam (Intensity) until the width of the gold powder diffraction rings are minimized.
The inserted beam stop occludes the unscattered electron beam.

Image collected in diffraction mode at a distance of D 850 mm
Deflection coils

• Each microscope has three sets of double deflection coils, gun, beam (above the objective lens) and the image deflection coils (below the objective).

• **Deflection coils** play an essential role in the alignment of the microscope and are used for aligning the gun, beam, objective lens, magnification system (image and diffraction shifts to the screen centre) and detector alignments (image or diffraction shifts to a detector that is situated off the optical axis).

• Most of the steps in the alignment procedures either align the deflection coils themselves or use the deflection coils to align another electron-optical element.

They play therefore an important role in many alignment steps.
Image shift/beam shift

on axis

- Beam deflection coils
- Minicondenser
- Objective
- Objective aperture
- Image deflection coils
Image shift/beam shift

on axis

off axis

Beam deflection coils

minicondenser

objective

objective aperture

Image deflection coils
Image shift/beam shift

- In two operational modes, the microscope provides an image shift coupled with a compensating beam shift.
- The compensation means that the beam stays centered on the area of the specimen observed, even if that area lies off the optical axis.
- These modes are the HR-TEM mode with its image shift and the Low-dose mode where an image shift is used for the Focus state (‘off axis’ state).
- With the image shift, the image can be displaced by up to about 10 µm in all directions (in Low-dose mode, approx. 300 nm in HR-TEM).
- The electron beam illuminates only a small area on the specimen. If that area is shifted off the screen, then the beam of course has moved with it. The Low-dose image shift corrects for this seeming beam shift by compensating the image shift with a beam shift.
- The coupling between image shift and beam shift must be calibrated in order to ensure that the beam stays on the field of view when the image is shifted.
- This calibration is done in the Image/beam alignment procedure.
Low Dose Acquisition scheme

- change tilt angle
- correct 'image shift'
- correct focus
- acquisition
- **PVP** - Pre Vac Pump (rotary pump)
- **ODP** - Oil Diffusion Pump
- **Turbo (TMP)** - Turbo Molecular Pump
- **IGP** - Ion Getter Pump
• PVP - Pre Vac Pump (rotary pump)
• ODP - Oil Diffusion Pump
• Turbo (TMP) - Turbo Molecular Pump
• IGP - Ion Getter Pump
- PVP - Pre Vac Pump (rotary pump)
- ODP - Oil Diffusion Pump
- Turbo (TMP) - Turbo Molecular Pump
- IGP - Ion Getter Pump
## System Status

### Lens

<table>
<thead>
<tr>
<th>Component</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Condenser 1</td>
<td>42.83%</td>
</tr>
<tr>
<td>Condenser 2</td>
<td>38.41%</td>
</tr>
<tr>
<td>Condenser 3</td>
<td>52.82%</td>
</tr>
<tr>
<td>Minicondenser</td>
<td>-97.39%</td>
</tr>
<tr>
<td>Objective</td>
<td>78.22%</td>
</tr>
<tr>
<td>Diffraction</td>
<td>60.23%</td>
</tr>
<tr>
<td>Intermediate</td>
<td>52.11%</td>
</tr>
<tr>
<td>Projector 1</td>
<td>49.39%</td>
</tr>
<tr>
<td>Projector 2</td>
<td>91.03%</td>
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</tbody>
</table>

### Gun Deflector

<table>
<thead>
<tr>
<th>Component</th>
<th>X</th>
<th>Y</th>
<th>Perp X</th>
<th>Perp Y</th>
<th>All</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gun tilt</td>
<td>-0.0367</td>
<td>-0.0743</td>
<td></td>
<td></td>
<td>0.0005</td>
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<tr>
<td>Gun shift</td>
<td>0.0513</td>
<td>-0.0639</td>
<td></td>
<td></td>
<td>0.1086</td>
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<td>Spot-dep. shift</td>
<td>0.0310</td>
<td>-0.0143</td>
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<td>0.0613</td>
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<tr>
<td>Gun tilt pp</td>
<td>4.5000</td>
<td>4.5000</td>
<td>0.0472</td>
<td>0.0239</td>
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<tr>
<td>Gun shift pp</td>
<td>3.4400</td>
<td>3.4400</td>
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</tr>
</tbody>
</table>

### Condenser Def. X

<table>
<thead>
<tr>
<th>Component</th>
<th>X</th>
<th>Y</th>
<th>Perp X</th>
<th>Perp Y</th>
<th>All</th>
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<tbody>
<tr>
<td>Condenser tilt</td>
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<td>Condenser shift</td>
<td>-0.2478</td>
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<td>0.0441</td>
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<tr>
<td>Condenser tilt pp</td>
<td>2.0000</td>
<td>2.0000</td>
<td>0.0240</td>
<td>0.1360</td>
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</tbody>
</table>

### Beam Deflector X

<table>
<thead>
<tr>
<th>Component</th>
<th>X</th>
<th>Y</th>
<th>Perp X</th>
<th>Perp Y</th>
<th>All</th>
</tr>
</thead>
<tbody>
<tr>
<td>DF tilt</td>
<td>0.0000</td>
<td>0.0000</td>
<td></td>
<td></td>
<td>0.0616</td>
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<tr>
<td>User shift</td>
<td>0.0011</td>
<td>0.0003</td>
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<td>0.0716</td>
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<tr>
<td>Rot Center</td>
<td>-0.1948</td>
<td>0.0929</td>
<td></td>
<td></td>
<td>0.0157</td>
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<tr>
<td>Align shift</td>
<td>0.1117</td>
<td>-0.1194</td>
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<td></td>
<td>0.0836</td>
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<tr>
<td>Beam tilt pp</td>
<td>4.6718</td>
<td>4.6609</td>
<td>-0.1087</td>
<td>0.1562</td>
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<tr>
<td>Beam shift pp</td>
<td>4.2876</td>
<td>4.3086</td>
<td>0.0317</td>
<td>-0.0457</td>
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</tbody>
</table>

### Image Deflector X

<table>
<thead>
<tr>
<th>Component</th>
<th>X</th>
<th>Y</th>
<th>Perp X</th>
<th>Perp Y</th>
<th>All</th>
</tr>
</thead>
<tbody>
<tr>
<td>Image-Beam shift</td>
<td>0.0858</td>
<td>0.0638</td>
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<td></td>
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<tr>
<td>User diff. shift</td>
<td>0.0000</td>
<td>0.0000</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>User image shift</td>
<td>0.0000</td>
<td>0.0000</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Align diff. shift</td>
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<td>-0.0026</td>
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<tr>
<td>Align image shift</td>
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<tr>
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<td>Mag. corr.</td>
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<tr>
<td>X-over corr.</td>
<td>0.0000</td>
<td>0.0000</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>
AutoLoader - components

Microscope Column ("Tecnai & Titan" technology)
RT – AutoLoader (EU, FP6 ‘HT-3DEM’)
Cryo - AutoLoader (EU, FP6 ‘Interaction Proteome’)

AND…

Manual Loading Station

Capsule
Cassette
TITAN KRIOS: AUTOLOADER

CHARACTERISTICS

- robotic sample/grid handling (12 grids)
- sample transfer to the EM in liquid nitrogen (capsule)
- sample exchange fully automated (within 160 seconds)
TITAN KRIOS: AUTOLOADER
TITAN KRIOS: AUTOGRIDS

CHARACTERISTICS

• Suitable for robotic sample/grid handling
• ‘Smallest’ possible TEM specimen holder
• Pre-requisite for in-column rotation 90° (e.g. for dual-axis tomography)
Electron-sample interactions

**SEM**
- Backscattered e-
- Cathodoluminescence
- Secondary e-
- X-rays
- Auger e-

**TEM**
- **elastically scattered e-** (1-10°)
- **inelastic scarttered e-** (<1°)
- unscattered e-

*Only the direction of the electron is changed*
*The energy and the direction of the electron is changed*
Signal transfer

3 transfer functions
...but we only consider two

i. Gun – electron spread, $U_0$

ii. Objective lens – $C_S$, $C_C$, $\Delta f$

}\[ \text{Contrast Transfer Function} \]

iii. Detector – point spread, noise

}\[ \text{Modulation Transfer Function} \]
Electron optical aberrations

spherical aberration
Electron optical aberrations

spherical aberration
Electron optical aberrations

spherical aberration

Lens plane
Electron optical aberrations

spherical aberration

Lens plane
Electron optical aberrations

spherical aberration
Electron optical aberrations

spherical aberration

\[ r_{sph} = C_s \cdot \beta^3 \]

- \( \beta \) is the maximum semi-angle of collection of the objective lens aperture
- \( C_s \) is the spherical aberration coefficient of the lens

Plane of least confusion
disk diameter = 0.5 \( C_s \beta^3 \)

Gaussian image plane
Electron optical aberrations

chromatic aberration

Lens plane
Electron optical aberrations

chromatic aberration
Electron optical aberrations

chromatic aberration
Electron optical aberrations

chromatic aberration

Focus for $E_0$
Electron optical aberrations

chromatic aberration
Electron optical aberrations

chromatic aberration

- Focus for $E - \Delta E$
- Focus for $E_0$
- Lens plane
- Fast electrons
- Slow electrons
Electron optical aberrations

chromatic aberration

\[ r_{chr} = C_c \cdot \frac{\Delta E}{E_0} \cdot \beta \]

- \( \beta \) is the maximum semi-angle of collection of the objective lens aperture
- \( C_c \) is the chromatic aberration coefficient of the lens
- \( \Delta E \) is the energy loss and \( E_0 \) is the initial beam energy
Electron optical aberrations

chromatic aberration

\[ r_{chr} = C_c \cdot \frac{\Delta E}{E_0} \cdot \beta \]

- \( \beta \) is the maximum semi-angle of collection of the objective lens aperture
- \( C_c \) is the chromatic aberration coefficient of the lens
- \( \Delta E \) is the energy loss and \( E_0 \) is the initial beam energy

...opposite to light optics, where 'blue/fast rays' are bent more strongly!
Thermionic emission

- Incoherent electron beam
Coherence

Thermionic emission

- Incoherent electron beam

Lens plane
Coherence

**Thermionic emission**

- Incoherent electron beam

Lens plane
Coherence

Field emission

- Coherent electron beam
Coherence

Field emission

• Coherent electron beam

**temporal coherence:**
all waves have the same wavelength
→ they are monochromatic

**spatial coherence:**
all waves are emitted from the same point → as in a laser

Electron beams are monochromatic except for a small thermal energy spread arising from the temperature of the electron emitter.
According to the dualism of elementary particles in quantum mechanics, electrons can be considered as particles or waves.

Both models are valid and used in electron optics.

Electrons can be regarded as particles to describe scattering.

The wave model is more useful to describe diffraction, interference, phase contrast and image formation.
Objective lens

Image

FFT
Phase contrast can be described by the **Phase Contrast Transfer Function** (short CTF), which highly depends on the instrument and the imaging conditions.

\[
\chi(u) = \pi \cdot \Delta f \cdot \lambda u^2 + \frac{\pi}{2} \cdot C_s \cdot \lambda^3 u^4
\]

**Defocus**  
**Spherical Aberration**
Friedrich Thon with the Siemens ELMISKOP ST100F

Krisch, B., Mueller, K.-H., Schliepe, R., and Willasch, D.
The ELMISKOP ST100F, a high-performance transmission scanning electron microscope.
The Contrast Transfer Function

\[ T(k) = -\sin \left[ \frac{\pi}{2} C_s \lambda^3 k^4 + \pi D f \lambda k^2 \right] \]

Dependent on: \( \lambda_e, k, C_s, Df \)
The Contrast Transfer Function

\[ T(k) = -\sin\left[\frac{\pi}{2} C_s \lambda^3 k^4 + \pi Df \lambda k^2\right] \]

Dependent on: \( \lambda_e, k, C_s, Df \)
The Contrast Transfer Function

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The Contrast Transfer Function

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Dependent on: \(\lambda_e, k, C_s, Df\)
The Contrast Transfer Function

\[ T(k) = -\sin\left[ \frac{\pi}{2} C_s \lambda^3 k^4 + \pi D_f \lambda k^2 \right] \]

Dependent on: \( \lambda_e, k, C_s, D_f \)
The Contrast Transfer Function

\[ T(k) = -\sin\left[\frac{\pi}{2} C_s \lambda^3 k^4 + \pi D f \lambda k^2 \right] \]

Dependent on: \( \lambda_e, k, C_s, Df \)
The Contrast Transfer Function

- Information transfer is limited by the spatial damping envelope $E_s(u)$
- Spatial damping depends on $Df$
- Images at large underfocus: rapid phase changes of the CTF-scrambles amplitudes and phases
The Contrast Transfer Function

Object
The Contrast Transfer Function
The Contrast Transfer Function

Object $\ast$ CTF $\ast$ Image
The Contrast Transfer Function

Object $\ast$ CTF $=$ Image

Low defocus

[Graph showing the contrast transfer function with an example of low defocus]

Object

Object
The Contrast Transfer Function

Object $\ast$ CTF $\ast$ Image

Low defocus

High defocus
The Contrast Transfer Function

Low defocus

High defocus

Object $\ast$ CTF $\ast$ Image
The Contrast Transfer Function

Object \( \ast \) CTF \( \ast \) Image

Low defocus

High defocus

\[ \frac{1}{0.2 \text{ nm}^{-1}} = 5 \text{ nm} \]
The Contrast Transfer Function

Object $\ast$ CTF $\ast$ Image

$\text{Low defocus}$

$\text{High defocus}$

$\frac{1}{0.2 \text{ nm}^{-1}} = 5 \text{ nm}$
Electron-sample interactions

- **elastic scattering**: interaction with the nucleus; $E$, $\lambda$ constant; scattering angle depending on $Z$ and $U$; cross section $\approx Z^{3/2}$

("Billiard ball against wall")

no energy transfer

generates image contrast

$0 \sim 0,1$ rad
• **Inelastic scattering**: interaction with the electron ‘shells’; Energy loss (20-100 eV); $E$ small, $\lambda$ large; scattering angle depending on $Z$ and $U$; cross-section $\approx Z^{1/2} - Z^{3/2}$
Energy Filter
Energy filter

1. Update principle
2. Show what is in an in-column filter and in an post-column filter
3. Show an image of a multipole (as also used for Cs/Cc correction)  
   - what is the advantage of multipoles vs. Hexa- and Quadrupoles?
4. Show an image of a Jeol in-column and a new Gatan Quantum system
Inelastic scattering

E = \Delta E - E_K

\Delta E \geq E_K

Ejected electron

Electron with energy loss

Magnetic Prism

Electrons with different energy loss

\Delta E_1

\Delta E_2

\Delta E_3

Energy dispersive plane

Magnetic field

Beam electron
Inelastic scattering

- The amount of inelastic scattering increases with specimen thickness (dependent on the incident electron energy and on the material the beam is interacting with).
- In general the heavier the material, the stronger the interaction.
- Inelastic **Mean Free Path (MFP, $\lambda_{\text{in}}$)**, which is a dimension that indicates the path-length inside the specimen material wherein all electrons (statistically speaking) will have undergone one inelastic scattering event.
  - Typical mean free path values are in the order of:
    - 0.050-100 nm for 120kV electrons
    - 100-200 nm for 200kV and
    - 150-300 nm for 300kV.
- Above one mean free path it is still possible to zero-loss ‘filter’ images.
Energy filtering... setup

- Entrance aperture
- Differential pumping
- Aperture (Ø 200 nm)
- Viewing chamber
- Entrance aperture
- 90° prism
- Image coupled
- Diffraction pattern
- Energy dispersive Aperture (‘slit’)

- 3mm
- 2 mm
- mask

GIF Entrance aperture
Energy filtering... setup

Image coupled / Diffraction coupled

Diffraction pattern / Image

Differential pumping
Aperture (Ø 200 nm)

Viewing chamber

Entrance aperture

90° prism

GIF Entrance aperture

3mm  2 mm  mask

Image / Diffraction pattern

Image / Spectrum

Energy dispersive Aperture (‘slit’)
Energy filtering... setup

- Entrance aperture
- Differential pumping
  - Aperture (Ø 200 nm)
- Viewing chamber
- Image coupled / Diffraction coupled
  - Diffraction pattern / Image
  - Image / Diffraction pattern
- 90° prism
- Cross-over alignment
- Entrance aperture
- GIF Entrance aperture
- Image / Spectrum
- Energy dispersive Aperture (‘slit’)

3mm 2 mm mask
Energy filtering... setup

Image coupled / Diffraction coupled

Diffraction pattern / Image

Differential pumping
Aperture (Ø 200 nm)

90° prism

Viewing chamber

Entrance aperture

9, 25 or 49 holes

Energy dispersive
Aperture (‘slit’)

Image / Spectrum

Image / Diffraction pattern

cross-over alignment

3mm  2 mm  mask

GIF Entrance aperture

9, 25 or 49 holes
Electron Energy-Loss Spectrum (EELS)
Electron Energy-Loss Spectrum (EELS)

Energy loss [eV]

Intensity [a.u.]

Optical properties and electronic structure

Zero loss  Valence loss

x25
Electron Energy-Loss Spectrum (EELS)

![Electron Energy-Loss Spectrum (EELS)](image)

- Zero loss
- Valence loss
- Core loss

- Optical properties and electronic structure

Energy loss [eV]

Intensity [a.u.]

Si-L$_{2,3}$
Electron Energy-Loss Spectrum (EELS)

- Zero loss
- Valence loss
- Core loss

Intensity [a.u.]

Energy loss [eV]

Optical properties and electronic structure

Bonding and oxidation state

Si-L_{2,3}
Electron Energy-Loss Spectrum (EELS)

- **Intensity [a.u.]**
- **Energy loss [eV]**

Key features:
- **Zero loss**
- **Valence loss**
- **Core loss**

- **Optical properties and electronic structure**
- **Bonding and oxidation state**

**Lines and Peaks:**
- **Si-L\(_{2,3}\)**
- **C-K**
- **O-K**

EELS spectrum with annotations for different types of losses.
Electron Energy-Loss Spectrum (EELS)

Optical properties and electronic structure
Bonding and oxidation state
Concentration

Zero loss  Valence loss  Core loss

Energy loss [eV]
Danev R, Buijsse B, Khoshouei M, Plitzko JM and Baumeister W.

Volta potential phase plate for in-focus phase contrast transmission electron microscopy.
Proceedings of the National Academy of Sciences of the United States of America 2014, 111 (44): 15635-15640
Imaging modes: **Phase Contrast TEM**

**Conventional TEM**

**Zernike Phase Plate**

**Volta Phase Plate**

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Danev R, Buijsse B, Khoshouei M, Plitzko JM and Baumeister W.  
**Volta potential phase plate for in-focus phase contrast transmission electron microscopy.**  
Proceedings of the National Academy of Sciences of the United States of America 2014 (doi/10.1073/pnas.1418377111)
Volta Phase Plate: **Beam spots**

Day 1

Day 1?

ZPP hole

Day 3,
Day 1 spot has disappeared!

ZPP hole

Day 3

Day 1?
Beam-induced phase shift series

- 12 nm thick carbon film; beam current: 1.0 nA; beam diameter: 1 \( \mu \text{m} \); 30 sec conditioning
Beam-induced phase shift series

- 12 nm thick carbon film; beam current: 1.0 nA; beam diameter: 1 μm; 30 sec conditioning
Volta Phase Plate

• The carbon surface is in a chemical equilibrium with the residual gases in the vacuum.
• Electron irradiation breaks the bonds and causes local de-termination of the surface.
• Unsaturated dangling bonds cause excess of electrons on the surface.

surface chemistry hypothesis
Volta Phase Plate

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• Electron irradiation breaks the bonds and causes local de-termination of the surface.
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surface chemistry hypothesis
Volta Phase Plate

- The carbon surface is in a chemical equilibrium with the residual gases in the vacuum.
- Electron irradiation breaks the bonds and causes local de-termination of the surface.
- Unsaturated dangling bonds cause excess of electrons on the surface.

\[ V_{\text{Volta}} = V_{\text{Inner}} - V_{\text{Surface}} \]

∞

\[ V_{\text{Volta}} \]

\[ V_{\text{Surface}} \]

\[ V_{\text{Inner}} \]

-1 nm
VPP: Holder and phase plate

250 °C
Phase plates improve the contrast

Conventional cryo-EM
1.5 um defocus

VPP cryo-EM
in-focus
Images look different at different phase shifts.
Phase shift effect on resolution

- ca. 21,000 particles from each phase shift range were analyzed and reconstructed.
- all micrographs were > 50 Å high-pass filtered.

<table>
<thead>
<tr>
<th>phase shift</th>
<th>&lt; 0.32 π</th>
<th>~ 0.5 π</th>
<th>&gt; 0.7 π</th>
</tr>
</thead>
<tbody>
<tr>
<td>resolution</td>
<td>2.91 Å</td>
<td>2.78 Å</td>
<td>2.83 Å</td>
</tr>
<tr>
<td>B-factor</td>
<td>-104</td>
<td>-89</td>
<td>-98</td>
</tr>
</tbody>
</table>
How to align the phase plate

- find large empty area
- leave SerialEM in LowDose
- set C2 aperture to 50 $\mu$m - center
- normalize all lenses
- Beam settings $\rightarrow$ switch off ‘AutoZoom’
- select ‘adjust MF x-y back focal plane’

Remarks:
- use ‘dirty' phase plate area $\rightarrow$ drift is normal
- wait until drift settles

Find on plane conditions
How to align the phase plate

- Alignments → Align PPoints → PhasePlate \( \mu P \)
- stay slightly ‘out of focus’ → Clockwise (CW) → to be able to see
- ignore message to do so
- minimise movement in x and y

Remarks:
- do not adjust ‘Diff shift pivot points’ stay in real space
- skip ‘Diff’ nonsense → do basically only step 6 and 7
ACHTUNG
ELEKTRONEN-MIKROSKOP