Electron microscopy in molecular cell biology I
Electron optics and image formation

Werner Kühlbrandt
Max Planck Institute of Biophysics
<table>
<thead>
<tr>
<th>Object</th>
<th>Measurement</th>
<th>Conversion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Galaxy</td>
<td>$10^6$ light years</td>
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## Objects of interest

<table>
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<tr>
<th>Biology</th>
<th>Example</th>
<th>Distance (m)</th>
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<tbody>
<tr>
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### Which objects can we see?

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Structural methods in molecular cell biology

- AFM
- NMR
- x-ray crystallography
- electron microscopy
- light microscopy

- 0.1nm=1Å
- 1nm
- 10nm
- 100nm
- 1µm
- 10µm
- 100µm
- 1mm
- 10mm

- atoms
- proteins
- bacteria
- organisms
- small molecules
- viruses
- cells

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Part 1: Electron optics
Microscopy with electrons
Microscopy with electrons
Microscopy with electrons

- Electrons are energy-rich elementary particles
Microscopy with electrons

- Electrons are energy-rich elementary particles
- Electrons have much shorter wavelength than x-rays (~ 3 pm = 3 x 10^{-12} m for 100kV electrons)
Microscopy with electrons

- Electrons are energy-rich \textit{elementary particles}.
- Electrons have much shorter \textit{wavelength} than x-rays
  \((\sim 3 \ \text{pm} = 3 \times 10^{-12} \ \text{m} \text{ for } 100\text{kV electrons})\)
- Resolution not limited by wavelength.
Microscopy with electrons

- Electrons are energy-rich elementary particles
- Electrons have much shorter wavelength than x-rays (~3 pm = 3 x 10^{-12} m for 100kV electrons)
- Resolution not limited by wavelength
- but by comparatively poor quality of magnetic lenses and radiation damage
Microscopy with electrons

- Electrons are energy-rich elementary particles
- Electrons have much shorter wavelength than x-rays (~3 pm = 3 x 10^{-12} m for 100kV electrons)
- Resolution not limited by wavelength
  - but by comparatively poor quality of magnetic lenses and radiation damage
- High vacuum is essential - no live specimens!
Electron microscopes

300 kV                      120 kV

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The transmission electron microscope
The transmission electron microscope

electron gun
The transmission electron microscope

electron gun

condenser
The transmission electron microscope

electron gun
condenser
objective lens
camera
The transmission electron microscope

electron gun
condenser
objective lens
projector lenses

Donnerstag, 3. Juli 14
The transmission electron microscope

electron gun
condenser
objective lens
projector lenses
projection chamber
The transmission electron microscope

electron gun
condenser
objective lens
projector lenses
projection chamber

Donnerstag, 3. Juli 14
The transmission electron microscope

electron gun
condenser
objective lens
projector lenses
projection chamber
viewing screen

Donnerstag, 3. Juli 14
The transmission electron microscope

- electron gun
- condenser
- objective lens
- projector lenses
- projection chamber
- viewing screen
- camera

Donnerstag, 3. Juli 14
The transmission electron microscope

electron gun
condenser
objective lens
specimen
projector lenses
projection chamber
viewing screen
camera

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The transmission electron microscope

electron source
specimen
objective lens
diffraction plane
image plane
focus plane
The transmission electron microscope
Electron sources

Thermionic emitters

Tungsten filament: emits electrons at ~ 3000 °C (melts at 3380 °C)
Large source size, poor coherence of electron beam

LaB$_6$ crystal: single crystal, emits electrons at ~ 2000 °C
Electrons are emitted from crystal tip -> smaller source size, better coherence
Energy spread $\Delta E \sim 1 - 2$ eV, depending on temperature

Field emitters

Oriented tungsten single crystal, tip radius ~ 100 nm
Small source size -> high coherence of electron beam, high brilliance
Requires very high vacuum ($10^{-11}$ Torr)
Electrons extracted from crystal by electric field applied to tip (extraction voltage)

Schottky emitter: Zr plated, heated to ~ 1200 °C to assist electron emission and prevent contamination, energy spread $\Delta E \sim 0.5$ eV

Cold field emitter: not heated, very low energy spread, best temporal coherence, but contaminates easily (frequent ‘flashing’ with oxygen gas)
Coherence

tungsten filament  field emission tip

incoherent electron beam  coherent electron beam

temporal coherence: all waves have the same wavelength (they are monochromatic)

spatial coherence: all waves are emitted from the same point source (as in a laser)
Electrons: particles or waves?

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.
# Electron parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Expression</th>
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<tbody>
<tr>
<td>Rest mass</td>
<td>( m_0 = 9.1091 \times 10^{-31} \text{ kg} )</td>
</tr>
<tr>
<td>Charge</td>
<td>( Q = -e = -1.602 \times 10^{-19} \text{ C} )</td>
</tr>
<tr>
<td>Kinetic energy</td>
<td>( E = eU, 1 \text{ eV} = 1.602 \times 10^{-19} \text{ Nm} )</td>
</tr>
<tr>
<td>Rest energy</td>
<td>( E_0 = m_0 c^2 = 511 \text{ keV} = 0.511 \text{ MeV} )</td>
</tr>
<tr>
<td>Velocity of light</td>
<td>( c = 2.9979 \times 10^8 \text{ m s}^{-1} )</td>
</tr>
<tr>
<td>Planck’s constant</td>
<td>( h = 6.6256 \times 10^{-34} \text{ N m s} )</td>
</tr>
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</table>

<table>
<thead>
<tr>
<th>Non-relativistic ( E \ll E_0 )</th>
<th>Relativistic ( E \sim E_0 )</th>
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</thead>
<tbody>
<tr>
<td>Mass</td>
<td>( m = m_0 )</td>
</tr>
<tr>
<td>Energy</td>
<td>( m = m_0 (1 - \frac{v^2}{c^2})^{-1/2} )</td>
</tr>
<tr>
<td>Energy</td>
<td>( E = eU = \frac{1}{2} m_0 v^2 )</td>
</tr>
<tr>
<td>Energy</td>
<td>( mc^2 = m_0 c^2 + eU = E_0 + E )</td>
</tr>
<tr>
<td>Velocity</td>
<td>( v = c \sqrt{1 - \frac{1}{(1 + \frac{E}{E_0})^2}} )</td>
</tr>
<tr>
<td>Velocity</td>
<td>( v = c \left[1 - \frac{1}{(1 + \frac{E}{E_0})^2}\right]^{1/2} )</td>
</tr>
<tr>
<td>Momentum</td>
<td>( p = m_0 v = (2m_0 E)^{1/2} )</td>
</tr>
<tr>
<td>Momentum</td>
<td>( p = mv = [2m_0 E (1 + E / 2E_0)]^{1/2} )</td>
</tr>
<tr>
<td>de Broglie wavelength</td>
<td>( \lambda = \frac{h}{p} = h (2m_0 E)^{-1/2} )</td>
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<td>( \lambda = \frac{h}{c} (2EE_0 + E^2)^{1/2} )</td>
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Electrons in an electrostatic (Coulomb) field

The force produced by an electrostatic field $E$ on a particle of (negative) charge $e^-$ is

$$F = -eE$$

This means that the electron is accelerated towards the anode. The kinetic energy $E$ [eV] of the electron after passing through a voltage $U$ [V] is

$$E = eU$$

The wavelength of an electron moving at velocity $v$ (de Broglie wavelength) is

$$\lambda = \frac{h}{mv}$$

where $h$ is Planck’s constant, $m$ the rest mass of the electron. Both the mass and the velocity of the electron are energy dependent. Relativistic treatment is necessary for acceleration voltages $> 80$ kV.

Electron beams are monochromatic except for a small thermal energy spread arising from the temperature of the electron emitter.

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de Broglie wavelength

<table>
<thead>
<tr>
<th>voltage</th>
<th>velocity</th>
<th>wavelength</th>
</tr>
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<tbody>
<tr>
<td>100 kV</td>
<td>1.64 x 10^8 m/s</td>
<td>3.7 pm = 0.037 Å</td>
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<tr>
<td>200 kV</td>
<td></td>
<td>2.5 pm = 0.025 Å</td>
</tr>
<tr>
<td>300 kV</td>
<td></td>
<td>2.0 pm = 0.02 Å</td>
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Resolution limit in crystallography

Reciprocal space!

Ewald sphere

Crystal

Detector

Reciprocal lattice planes

Highest possible resolution:

\[ \frac{1}{d} = \frac{2}{\lambda} \]

\[ d = \frac{\lambda}{2} \]

\[ \theta = 90^\circ \]

\[ \sin 2\theta = \frac{1}{d} / (1/n\lambda) \]

\[ \Rightarrow 2d \times \sin \theta = n\lambda \quad \text{Bragg's law} \]
Resolution limit

Diffraction (Rayleigh) limit: \( \sim 0.5 \lambda \)

- \( \sim 150 \text{ nm} = 1,500 \text{ Å} \) for visible light
- 0.5 Å for x-rays
- 0.01 Å = 1 pm for 300 kV electrons
The transmission electron microscope

electron source
specimen
objective lens
diffraction plane
image plane
focus plane
Electron-specimen interactions

Incident beam of energy $E$

- Elastic
- Inelastic

$h \nu$

$E - \Delta E$

Electron-specimen interactions

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Elastic and inelastic scattering cross sections for electrons, x-rays and neutrons

Electron scattering factors

Large elastic cross section
- $\sim 10^6$ higher than for x-rays
  high signal per scattering event

... but even larger inelastic cross section
- $\frac{\sigma_{\text{inel}}}{\sigma_{\text{el}}} \approx \frac{18}{Z}$
  high radiation damage, especially for light atoms, e.g. C:
  3 electrons scattered inelastically per elastic event

Good ratio of elastic/inelastic scattering
- 1000 times better than for 1.5 Å x-rays
Considerations for biological specimens

- Biological specimens consist mainly of light atoms (H, N, C, O), which suffer most from radiation damage
Considerations for biological specimens

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• This makes it essential to minimize the electron dose to about ~25 e/Å² for single-particle cryoEM
Considerations for biological specimens

- Biological specimens consist mainly of light atoms (H, N, C, O), which suffer most from radiation damage.
- This makes it essential to minimize the electron dose to about \( \sim 25 \text{ e/Å}^2 \) for single-particle cryoEM.
- Low electron dose means low image contrast.
The transmission electron microscope

electron source
specimen
objective lens
diffraction plane
image plane
focus plane
Electrons in a magnetic field

Lorentz force on a charge $e^-$ moving with velocity $\mathbf{v}$ in a magnetic field $\mathbf{B}$ (vectors are bold):

$$\mathbf{F} = -e (\mathbf{v} \times \mathbf{B})$$

The direction of $\mathbf{F}$ is perpendicular on $\mathbf{v}$ and $\mathbf{B}$. $\mathbf{v}$, $\mathbf{B}$, and $\mathbf{F}$ form a right-handed system (right-hand rule, where $v$ = index finger, $B$ = middle finger and $c$ = thumb).

In a homogenous magnetic field, if

$$\mathbf{v} = 0 \text{ then } \mathbf{F} = 0$$

$\mathbf{v}$ is perpendicular to $\mathbf{B}$: the electron is forced on a circle with a radius depending on the field strength $|\mathbf{B}|$.

$\mathbf{v}$ is parallel to $\mathbf{B}$: no change of direction.

Note that unlike the electric field, the magnetic field does not change the energy of the electron, i.e. the wavelength does not change!
Passing a current through a coil of wire produces a strong magnetic field in the centre of the coil. The field strength depends on the number of windings and the current passing through the coil.
Electromagnetic Lens

Pole pieces of soft iron concentrate the magnetic field
Electromagnetic Lens

Pole pieces of soft iron concentrate the magnetic field
Electromagnetic Lens
Electromagnetic Lenses

Electromagnetic lenses are comprised of windings of wire through which electric current is applied. This creates a strong magnetic field through which negatively-charged electrons must pass.

Due to the magnetic field, the electrons follow a helical trajectory which converges at a fine focal point after it emerges from the lens. (DC-powered magnets behave similar to converging glass lenses)

Field Strength determines the focal length which varies with:

\[(\text{focal length}) \ f = K \left(\frac{V}{i^2}\right)\]

- \(K\) = constant based on the number of turns of lens coil wire and the geometry of the lens.
- \(V\) = accelerating voltage
- \(i\) = milliamps of current put through the coil

Potentiometer controls which vary the current to the various lenses are the means by which focus and magnification of the electron beam are achieved.
Magnification steps

EM grid

2mm x 2mm

60x
Magnification steps

EM grid

grid square with holey carbon film

2mm x 2mm

20 µm x 20 µm

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Magnification steps

- EM grid: 2mm x 2mm
- Grid square with holey carbon film: 20 µm x 20 µm
- Hole: 2µm x 2µm

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Magnification steps

EM grid

2mm x 2mm

grid square with holey carbon film

20 µm x 20 µm

hole

2µm x 2µm

FAS in vitreous buffer

0.2 µm x 0.2 µm
The transmission electron microscope

electron source
specimen
objective lens
diffraction plane
image plane
focus plane
Electron diffraction

Bragg’s law: $n\lambda = 2d \sin\theta$
Electron diffraction

Bragg’s law: \( n\lambda = 2d \sin\theta \)
Interference

- **Constructive Interference**
- **Destructive Interference**

**Superposition**

1\(^{st}\) wave

2\(^{nd}\) wave
Elastic scattering by atoms (Rayleigh scattering)

n is an integer:
scattered waves in phase, constructive interference

n is not an integer:
scattered waves out of phase, destructive interference

Bragg’s law: $n\lambda = 2d \sin \theta$
2D crystals of LHC-II

Electron diffraction pattern: amplitudes, no phases

3.2 Å
The image is generated by interference of the direct electron beam with the diffracted beams.

- Specimen
- Objective lens
- Objective diaphragm
- 1st Diffraction pattern
- 1st Image
- Selector diaphragm
- Intermediate lens
- 2nd Diffraction pattern
- 2nd Image
- Projector lens
- 3rd Diffraction

Final image Screen

a) Bright field imaging
b) Selected area diffraction

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Part 2:

Image formation
The transmission electron microscope

electron source
specimen
objective lens
diffraction plane
image plane
focus plane
Electron-specimen interactions

Incident beam of energy $E$

- Elastic
- Inelastic

$h\nu$

$E - \Delta E$

$Z = 0$

$Z = t$

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Contrast mechanisms

Image

- Amplitude contrast
- Phase contrast

No interaction

Amplitude object

Phase object

Electron wave

- Modified amplitude, unchanged phase
- Modified phase, unchanged amplitude

Shorter wavelength within object

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Origin of the phase shift

The potential $V(x,y,z)$ experienced by the scattered electron upon passing through a sample of thickness $t$ causes a phase shift

$$V_i(x,y) = \int_0^t V(x,y,z)dz$$

The vacuum wavelength $\lambda$ of the electron changes to $\lambda'$ within the sample:

$$\lambda = \frac{h}{\sqrt{2meE}} \quad \rightarrow \quad \lambda' = \frac{h}{\sqrt{2me(E + V(x,y,z))}}$$

When passing through a sample of thickness $dz$, the electron experiences a phase shift of:

$$d\varphi = 2\pi \left( \frac{dz}{\lambda'} - \frac{dz}{\lambda} \right)$$

(how many times does $\lambda$ fit into the thickness $dz$?)

(by how many degrees out of $2\pi = 360^\circ$ does this change the phase $\phi$?)

$$d\varphi = \frac{\pi}{\lambda E} V(x,y,z)dz = \sigma V(x,y,z)dz$$
The weak phase approximation

As we have seen, the phase shift is proportional to the projected potential of the specimen:

\[ \varphi = \sigma \int V(x, y, z) dz = \sigma V_t(x, y) \]

The potential \( V \) of the specimen shift modifies the phase of the incident plane wave \( \Psi_{\text{incident}} \) to:

\[ \Psi_{\text{exit}} = \Psi_{\text{incident}} e^{-i\varphi} = \Psi_{\text{incident}} e^{-i\sigma V_t} \]

For \( V_t << 1 \) and thus \( \varphi << 2\pi \) as for thin biological specimens:

\[ i = e^{i\pi/2} = \cos(\pi/2) + i\sin(\pi/2) \]

\[ \Psi_{\text{exit}} \approx \Psi_{\text{incident}} (1 - i\varphi) = \Psi_{\text{incident}} - i\Psi_{\text{incident}} \varphi = \Psi_{\text{incident}} - i\Psi_{\text{scattered}} \]

→ For thin biological specimens there is a linear relation between the transmitted exit wave function and the projected potential of the sample:

\[ \Psi_{\text{exit}} \approx \underbrace{\Psi_{\text{incident}}}_{=1(\text{background})} (1 - i\varphi) = 1 - i\varphi = 1 - i\sigma V_t \]
Taylor series

Any function can be approximated by a finite number of polynomial terms

$$\sum_{n=0}^{\infty} \frac{f^{(n)}(a)}{n!} (x-a)^n$$

$$\sin(x) \approx x - \frac{x^3}{3!} + \frac{x^5}{5!} - \frac{x^7}{7!} + O(x^n)$$

$$e^x \approx 1 + x + \frac{x^2}{2!} + \frac{x^3}{3!} + O(x^n)$$

$$e^{-i\varphi} \approx 1 - i\varphi + \frac{\varphi^2}{2!} - i\frac{\varphi^3}{3!} + O(\varphi^n)$$

$$\approx 1 - i\varphi \text{ if } \varphi << 1$$

...taken from Wikipedia...
Taylor series

Any function can be approximated by a finite number of polynomial terms

\[ \sum_{n=0}^{\infty} \frac{f^{(n)}(a)}{n!} (x-a)^n \]

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\[ e^{-i\phi} \approx 1 - i\phi + \frac{\phi^2}{2!} - i\frac{\phi^3}{3!} + O(\phi^n) \approx 1 - i\phi \text{ if } \phi << 1 \]
Phase contrast

\[
\text{exit wave} = \text{scattered wave} + \text{unscattered wave}
\]

Scattered wave is 90° out of phase with unscattered wave.

Vector notation. Vector length shows amplitude (not drawn to scale)

Cosine wave notation (amplitudes not drawn to scale)
Phase contrast

exit wave = scattered wave + unscattered wave

A perfect lens in focus gives minimal phase contrast
Phase contrast

exit wave  =  scattered wave  +  unscattered wave

A perfect lens in focus gives minimal phase contrast

Since $\psi_{\text{scattered}} = \varphi \psi_{\text{unscattered}}$ with $\varphi \ll 1$:

$\psi_{\text{scattered}} \ll \psi_{\text{unscattered}}$

$I_{\text{image}} = |\psi_{\text{exit}}|^2 = |\psi_{\text{unscattered}} + i\psi_{\text{scattered}}|^2 \approx |\psi_{\text{unscattered}}|^2 = I_{\text{incident}}$
Phase contrast

\[
\psi_{\text{exit}} = \psi_{\text{unscattered}} + i\psi_{\text{scattered}}
\]

Lens aberrations cause an additional phase shift that generates phase contrast

Donnerstag, 3. Juli 14
Lens aberrations

Spherical aberration

Chromatic aberration

Donnerstag, 3. Juli 14
Defocus generates an additional phase shift

In a perfect lens, the number of wavelengths from object to focal point is the same for each ray, independent of scattering angle (Fermat’s principle)
Defocus generates an additional phase shift

In a perfect lens, the number of wavelengths from object to focal point is the same for each ray, independent of scattering angle (Fermat’s principle)

Lens with spherical aberration
Defocus generates an additional phase shift

In a perfect lens, the number of wavelengths from object to focal point is the same for each ray, independent of scattering angle (Fermat’s principle)

Lens with spherical aberration

Donnerstag, 3. Juli 14
Defocus generates an additional phase shift

In a perfect lens, the number of wavelengths from object to focal point is the same for each ray, independent of scattering angle (Fermat’s principle)

Perfect lens

Lens with spherical aberration

Donnerstag, 3. Juli 14
Defocus generates an additional phase shift

In a perfect lens, the number of wavelengths from object to focal point is the same for each ray, independent of scattering angle (Fermat’s principle)

Spherical aberration causes a path length difference $\chi$ which depends on the scattering angle

$\chi = \frac{3\lambda}{2}$
Depending on its phase shift, the scattered wave reinforces or weakens the exit wave.

\[ \Psi_{\text{exit}} = \psi_{\text{unscattered}} + i\psi_{\text{scattered}} \]

Scattered and unscattered wave are in phase: exit wave gets stronger.

Scattered and unscattered wave are out of phase by \( \pi = 90^\circ \): exit wave gets weaker.

Scattered wave is shifted by \( \pi/2 = 90^\circ \) relative to unscattered wave: exit wave is nearly same as unscattered wave, therefore phase contrast is minimal.
Optimal (Scherzer) focus

The phase shift $\chi(\theta)$ introduced by the spherical aberration $C_s$ of the objective lens depends on the scattering angle $\theta$ and on the amount of defocus $\Delta f$.

$$\chi(\theta) = \left(\frac{2\pi}{\lambda}\right) \left[ \Delta f \frac{\theta^2}{2} - C_s \frac{\theta^4}{4} \right]$$

The spatial frequency $R$ (measured in Å $^{-1}$) is the reciprocal of the distance between any two points of the specimen. For small scattering angles, $R \approx \theta / \lambda$. With this approximation, the phase shift expressed as a function of $R$ is

$$\chi(R) = \left(\frac{\pi}{2}\right) \left[ 2\Delta f R^2 \lambda - C_s R^4 \lambda^3 \right] \quad \text{(Scherzer formula)}$$

At Scherzer focus, the terms containing $\Delta f$ and $C_s$ nearly add up to 1, and a positive phase shift of $\chi \approx +\pi/2$ applies to a wide resolution range.
Defocus and spherical aberration have similar but opposite effects.
Contrast transfer in exact focus

Scherzer focus

high underfocus

(Erikson and Klug, 1971)
The phase Contrast Transfer Function $B(\theta)$ of the electron microscope is defined as

$$B(\theta) = -2 \sin \chi(\theta)$$

For any particular defocus $\Delta f$, the CTF indicates the range of scattering angles $\theta$ in which the object is imaged with positive (or negative) phase contrast.

For scattering angles where CTF = 0, there is no phase contrast.

As spherical aberration $C_s$ is constant, $\Delta f$ can be adjusted to produce maximum phase contrast of $\pm \pi/2$ for a particular range of scattering angles.
Fourier transforms of amorphous carbon film images

0.5 $\mu m$ underfocus

1 $\mu m$ underfocus

FT

FT
Fourier transforms of amorphous carbon film images

Contrast Transfer Function, CTF
CTF as a function of defocus

Movie by Henning Stahlberg, UC Davis

Donnerstag, 3. Juli 14
CTF as a function of defocus

Movie by Henning Stahlberg, UC Davis
The transmission electron microscope
DQE of direct electron detectors

$$DQE = \frac{SNR^2_o}{SNR^2_i}$$

Donnerstag, 3. Juli 14

CCD vs. direct detector

300 kV electrons

conventional CCD detector

Falcon-II direct electron detector

Richard Henderson, Greg McMullan (MRC-LMB Cambridge, UK)
CCD vs. direct detector

300 kV electrons

Conventional CCD detector
- Scintillator
- Fiber optics
- CCD chip
- Separate vacuum
- Peltier cooler

Falcon-II direct electron detector
- 4000 x 4000 14 µm pixels
- read out 17 times per second

Richard Henderson, Greg McMullan (MRC-LMB Cambridge, UK)
Fast readout overcomes drift

Frame displacement in Å
The Resolution Revolution

Werner Kühlbrandt

Precise knowledge of the structure of macromolecules in the cell is essential for understanding how they function. Structures of large macromolecules can now be obtained at near-atomic resolution by averaging thousands of electron microscope images recorded before radiation damage accumulates. This is what Amunts et al. have done in their research article on page 1485 of this issue (1), reporting the structure of the large subunit of the mitochondrial ribosome at 3.2 Å resolution by electron cryo-microscopy (cryo-EM). Together with other recent high-resolution cryo-EM structures (2–4) (see the figure), this achievement heralds the beginning of a new era in molecular biology, where structures at near-atomic resolution are no longer the prerogative of x-ray crystallography or nuclear magnetic resonance (NMR) spectroscopy.

Ribosomes are ancient, massive protein-RNA complexes that translate the linear genetic code into three-dimensional proteins. Mitochondria—semi-autonomous organelles that supply the cell with energy—have their own ribosomes, which closely resemble those of their bacterial ancestors. Many antibiotics, such as erythromycin, inhibit growth of bacteria by blocking the translation machinery of bacterial ribosomes. When designing new antibiotics, it is essential that they do not also

Near-atomic resolution with cryo-EM. (A) The large subunit of the yeast mitochondrial ribosome at 3.2 Å reported by Amunts et al. In the detailed view below, the base pairs of an RNA double helix and a magnesium ion (blue) are clearly resolved. (B) TRPV1 ion channel at 3.4 Å (2), with a detailed view of residues lining the ion pore on the four-fold axis of the tetrameric channel. (C) F$_{430}$-reducing [NiFe] hydrogenase at 3.36 Å (3). The detail shows an α helix in the FhxA subunit with resolved side chains. The maps are not drawn to scale.

Photographic film works in principle much better for high-resolution imaging, but is incompatible with rapid electronic readout and high data throughput, which are increasingly essential.

Some 10 years ago, Henderson and Faruqi realized that it should be possible to design a
The Resolution Revolution

Werner Kühlbrandt

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