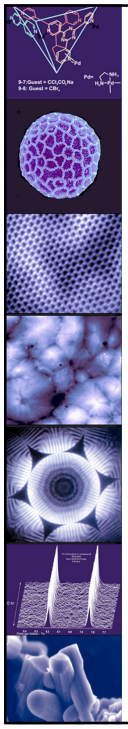
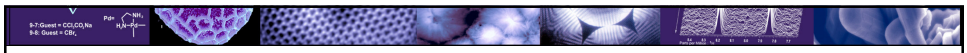


Imaging in Cryo-Electron Microscopy

Jaap Brink
JEOL USA, Inc.

JEOL

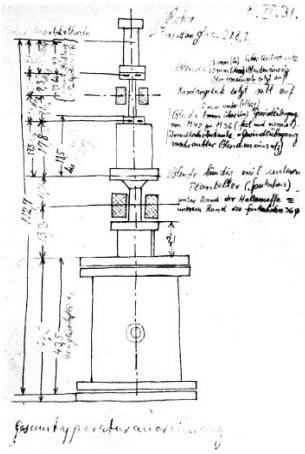


Outline

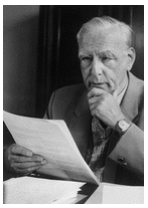
- Electron interactions
- Imaging theory
- Why are images not perfect?
- Analysis of cryo-EM data
- Future directions

JEOL

Early design by Ernst Ruska



1931

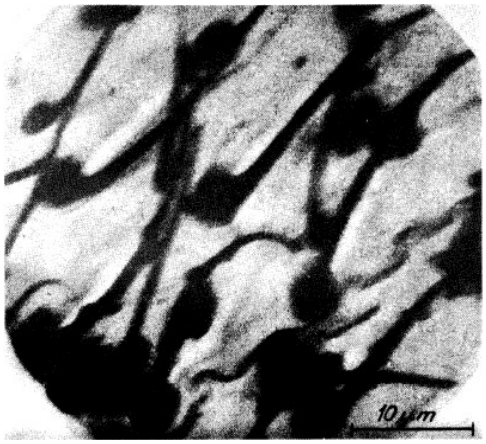


From Ernst Ruska's Nobel lecture (1986) and the nobelprize.org website.

Essentially, the electron microscope was developed out of work aimed at a efficient electron cathode-ray oscillograph under Ing. Max Knoll.

JEOL

First electron image

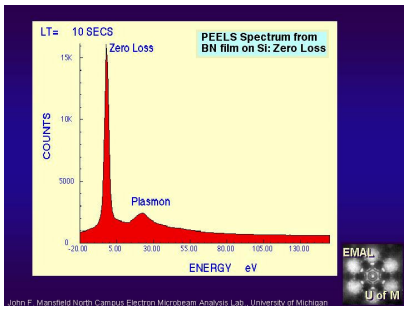


Wings of house fly

JEOL

Electron Interactions with specimen

- Elastic
- Inelastic
- None



J. Mansfield, U Michigan

Elastic interaction

- Mean free path – larger at higher kV
  - Single scattering event (kinematic)
- Angular dependency
  - Most of the electrons are scattered over large angles
  - Go either through the OL aperture (phase contrast) or are blocked (aperture contrast)
  - Without phase contrast, cryo-EM is not possible

Inelastic scattering

- All electrons that lose energy while interacting with the specimen:
  - Absorbance (EELS)
  - X-rays (EDS)
  - Other electrons (Auger)
  - Other processes (phonons, plasmons)
  - Radiation damage

Inelastic scattering I

- Beam damage:
  - Radiolysis
    - chemical bond breakage, free radical formation
    - loss of resolution
    - beam-induced motion
  - Knock-on damage
    - direct displacement of atoms by high energy electron

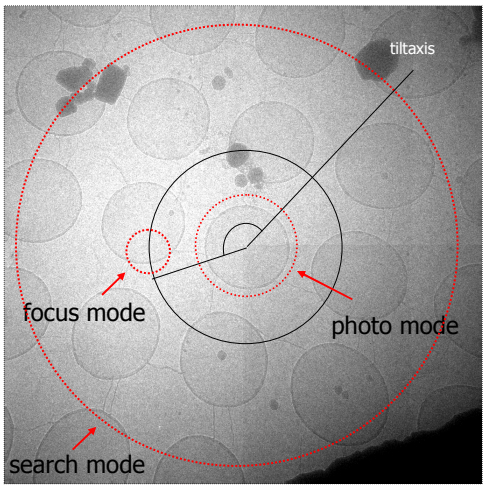
Inelastic scattering II

- Typical exposure requires 20 electrons/Å^2 on the specimen
- Equivalent to 10^7 – 10^8 rad in terms of radiation dose
- No biological specimen can survive this exposure
- Limited beneficial effect of lower specimen temperatures

JEOL

Imaging Techniques in cryo-TEM

- Low intensity search mode, < 0.01 e/Å^2/s
- Off-axis focus mode w. high intensity 50-100 e/Å^2/s
- Record mode w. beam blanking on film/CCD



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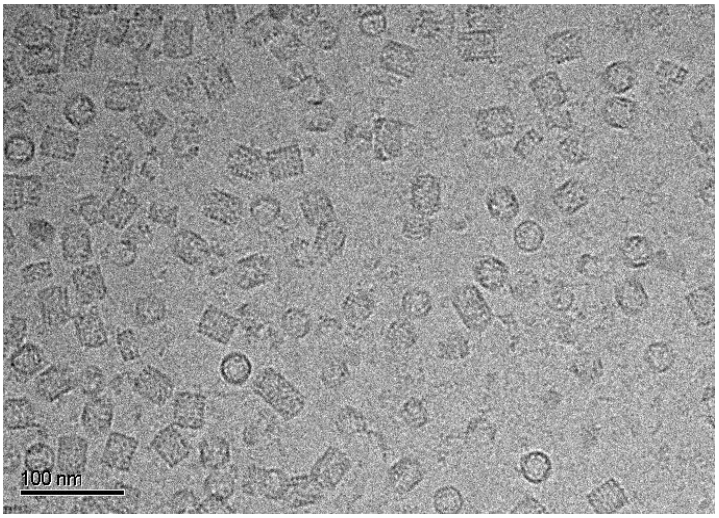
KLH in amorphous ice



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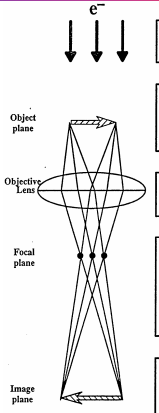
Wave Theory in EM



Object Coulomb potential function $V(x_o, y_o, z_o)$

Object transmitted wave function $\Psi_o(x_o, y_o)$

$\Psi_o(x_o, y_o) = 1 + i\sigma v(x_o, y_o)$

$v(x_o, y_o) = \int V(x_o, y_o, z_o)\, dz_o$

← Weak phase approx.

Phase shift $\gamma(S)$ *introduced by objective lens*

$\gamma(S) = 2\pi(\frac{1}{4} C_s \lambda^3 S^4 - \frac{1}{2} \Delta Z \lambda S^2)$

← Aberration function

Diffraction wave function $\Psi_d(S_x, S_y)$

$\Psi_d(S_x, S_y) = F(S_x, S_y)\ exp\ (i\gamma(S))$

$F(S_x, S_y) = \mathcal{F}\ [\Psi_o(x_o, y_o)]$

Diffraction intensity $I_d(S_x, S_y) = \Psi_d(S_x, S_y)\ \Psi_d^*(S_x, S_y)$

← Diffraction description

Image wave function $\Psi_i(x_i, y_i)$

$\Psi_i(x_i, y_i) = \mathcal{F}^{-1}\ [\Psi_d(S_x, S_y)]$

Image intensity $I_i(x_i, y_i)$

$I_i(x_i, y_i) = \delta(0,0) - 2\sigma v(x_i, y_i) * \mathcal{F}^{-1}\ [sin\ \gamma(S)]$

← Image description

Computed diffraction wave function $T(S_x, S_y)$

$T(S_x, S_y) = \mathcal{F}\ [I_i(x_i, y_i)]$

$= \delta(0,0) - 2\ F(S_x, S_y)\ sin\ \gamma(S)$

JEOL

Image Contrast

The Fourier Transform of an Electron Micrograph: Effects of Defocussing and Aberrations, and Implications for the Use of Underfocus Contrast Enhancement

By Harold P. Erickson and A. Klug

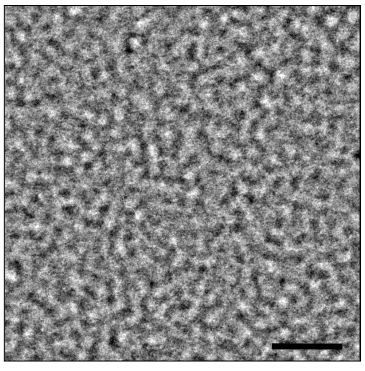
Medical Research Council Laboratory of Molecular Biology, Cambridge, England

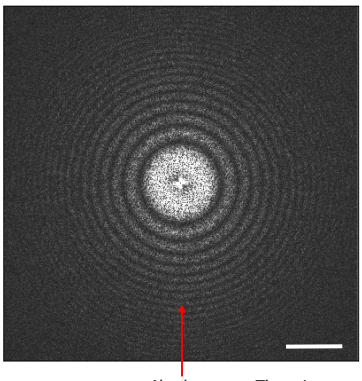
(Vorgetragen von H. P. Erickson anläßlich der Diskussionstagung der Deutschen Bunsen-Gesellschaft für Physikalische Chemie e. V. am 16. März 1970 in Hirschegg)

The effects of defocussing and spherical aberration in the electron microscope image are most simply and directly displayed in the Fourier transform of the image. We have investigated the process of image formation by determining the changes in the transform of the image of thin crystals of catalase, which has discrete diffraction maxima in the resolution range of 100 Å to 20 Å, as a function of defocussing. The experimentally measured changes in amplitude and phase of these diffraction maxima have been measured and compared with the predictions of a first order theory of image formation. The theory is generally confirmed, and the transfer function of the microscope is completely determined by finding the relative contributions from phase and amplitude contrast. A "true" maximum contrast image of the catalase molecule, compensated for the effects of defocussing, is reconstructed from the set of micrographs in the focal series. The relation of this compensated image to individual underfocussed micrographs, and the use of underfocus contrast enhancement in conventional electron microscopy, is discussed.

Ber. Bunsen-Gesell. (1970) 74(11), 1129-1137

300 kV Image Power Spectrum





Also known as Thon rings

What does a diff. pattern reveal?

$$T(S_x, S_y) \bullet T^*(S_x, S_y) =$$
$$F^2(S_x, S_y) \bullet \sin^2 \gamma(S_x, S_y) \bullet E^2(S_x, S_y) + N^2(S_x, S_y)$$

JEOL

What does a diff. pattern reveal?

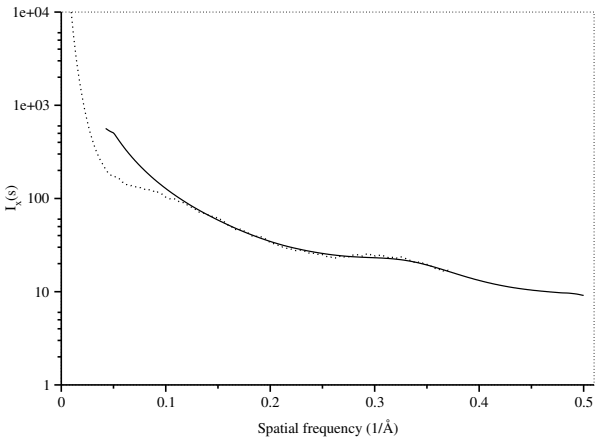
$$T(S_x, S_y) \bullet T^*(S_x, S_y) =$$
$$F^2(S_x, S_y) \bullet \sin^2 \gamma(S_x, S_y) \bullet E^2(S_x, S_y) + N^2(S_x, S_y)$$

Specimen's structure factor

Conveniently obtained from X-ray solution scattering data, e.g. 1D scattering plots of HSV-1, p22, or (with some pain) from electron diffraction patterns

NCMI Cryo-EM Workshop 2006

JEOL



Structure factor from carbon film from 300 kV electron diffraction

What does a diff. pattern reveal?

$$T(S_x, S_y) \bullet T^*(S_x, S_y) =$$
$$F^2(S_x, S_y) \bullet \sin^2 \gamma(S_x, S_y) \bullet E^2(S_x, S_y) + N^2(S_x, S_y)$$

Contrast Transfer Function

Oscillatory function describing the level of contrast of a particular spatial frequency

Contrast Transfer Function

$$CTF = \sqrt{1-Q^2}\sin(\chi(s) - Q\cos(\chi(s))$$

$$\chi(s) = 2\pi(-\frac{C_s\lambda^3 s^4}{4} + \frac{\Delta Z\lambda s^2}{2})$$

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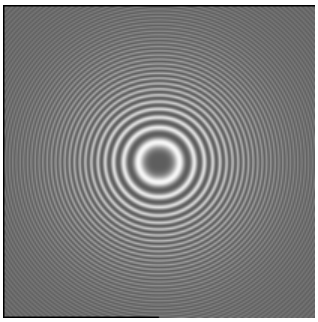
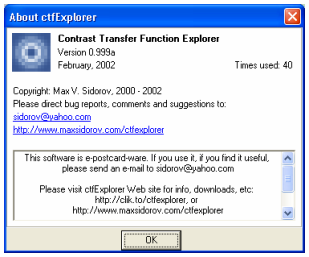
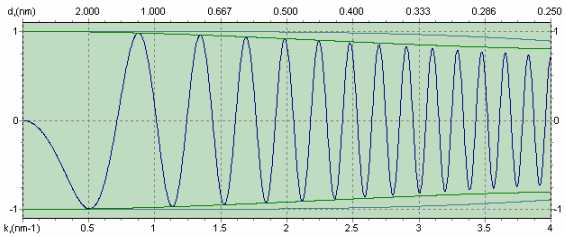
Contrast Transfer Function

- CTF-explorer (Max Sidorov)
- example: JEOL 3200FSC

ΔZ = 1.0 μm, Cs 4.1mm, Cc 3.4 mm
ΔE = 0.7eV
α = 0.06 mrad







JEOL

What does a diff. pattern reveal?

$$T(S_x, S_y) \bullet T^*(S_x, S_y) =$$

$$F^2(S_x, S_y) \bullet \sin^2 \gamma(S_x, S_y) \bullet E^2(S_x, S_y) + N^2(S_x, S_y)$$

Total Envelope Function

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Envelope Functions

- Decay in the power spectrum due to coherence limitations:
  - Spatial – ∝ illumination angle
  - Temporal – ∝ energy spread
- Can also be caused by "environmental factors":
  - Drift from the holder
  - Acoustic coupling of the cryo-holder

JEOL

Envelope Functions II

- Guassian type source:

$$G_{sc}(s) = \exp(-\pi^2\alpha^2(C_s\lambda^2 s^3 - \Delta Z s)^2)$$

  - α dominates total envelope at ΔZ ≥ 0.5 µm
- Guassian type fluctuations:

$$G_{tc}(s) = \exp(-(\frac{\pi^2}{16\ln 2}C_c^2\lambda^2\left(\frac{\Delta E}{E}\right)^2 s^4))$$

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Envelope Functions III

- Guassian type fluctuations :

$$G_{ol}(s) = \exp(-(\frac{\pi^2}{4\ln 2}C_c^2\lambda^2\left(\frac{\Delta I}{I}\right)^2 s^4))$$

- Sinosoidal type fluctuations:

$$G_{lm}(s) = J_0(\pi\Delta f\lambda s^2)$$

- Drift:

$$G_{tm}(s) = \sin(\pi s\Delta r)\Big/\pi s\Delta r$$

JEOL

What does a diff. pattern reveal?

$$T(S_x, S_y) \bullet T^*(S_x, S_y) =$$

$$F^2(S_x, S_y) \bullet \sin^2 \gamma(S_x, S_y) \bullet E^2(S_x, S_y) + N^2(S_x, S_y)$$

Total noise component

From a variety of sources (Gaussian, exponential, other). Some difference has been observed between energy-filtered and unfiltered data.

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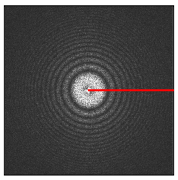
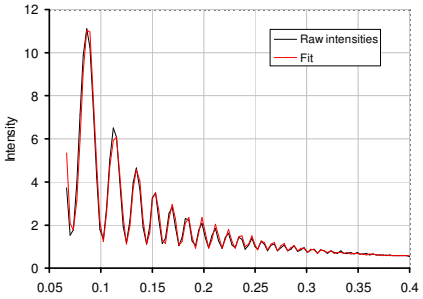
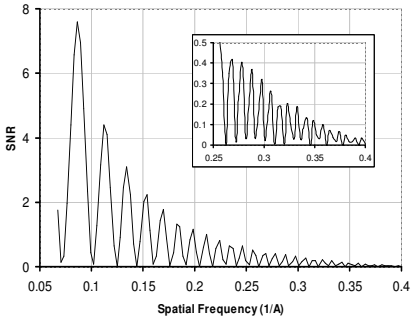
Fitting the intensities



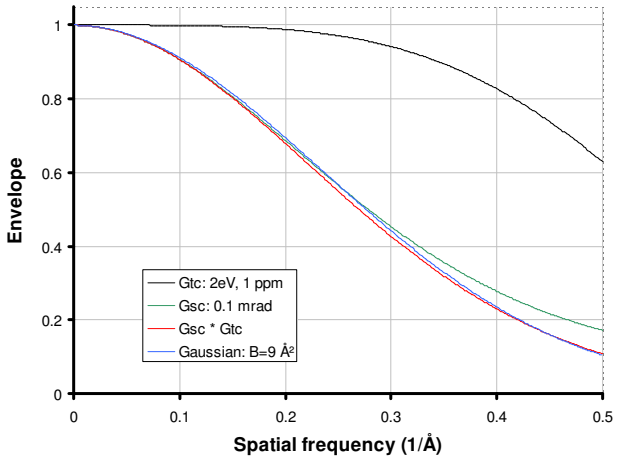
JEOL3000SFF, ΔZ = 0.97 µm





JEOL

Simple Guassian as total envelope



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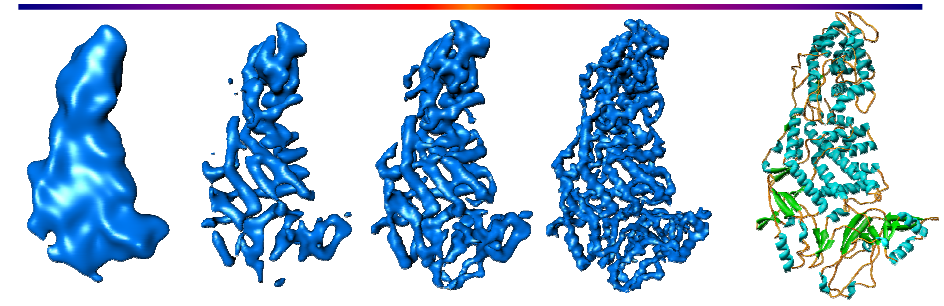
Structure at various resolutions



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Resolution

The Theoretical Resolution Limit of the Electron Microscope

O. SCHERZER
Signal Corps Engineering Laboratories, Fort Monmouth, New Jersey
(Received June 14, 1948)

The resolving power of the electron microscope and the contrast in the image are calculated for different conditions of focusing, illumination and aperture. These conditions can change the limit of resolution by a factor of about 3. The contrast in the image of an atom is appreciably increased by defocusing and spherical aberration. Nevertheless, the contrast improves when the numerical value of the aberration constant is diminished. The effect of different methods of spherical correction is discussed briefly.

Appl. Physics (1949) 20, 20-29

Theorem: Chromatic and spherical aberrations are unavoidable for rotationally symmetric lenses. In addition, all aberrations add in quadrature, meaning one cannot combine lenses with suitable aberrations to cancel them in an attempt to pursue higher resolution.

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Aberrations I

- Spherical aberration

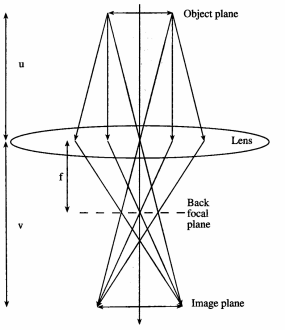

Figure 6.3. A complete ray diagram for a finite object, symmetrically positioned around the optic axis. All rays emerging from a point in the object (distance u from the lens) that are gathered by the lens converge to a point in the image (distance v from the lens) and all parallel rays are focused in the focal plane (distance f from the lens).

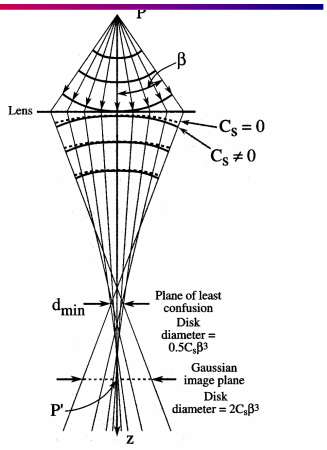

Figure 6.11. Spherical aberration in the lens causes wavefronts from a point object P to be spherically distorted. The point is thus imaged as a disk with a minimum radius in the plane of least confusion and a larger disc at P′ in the Gaussian image plane.

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Aberrations II

- Chromatic aberration

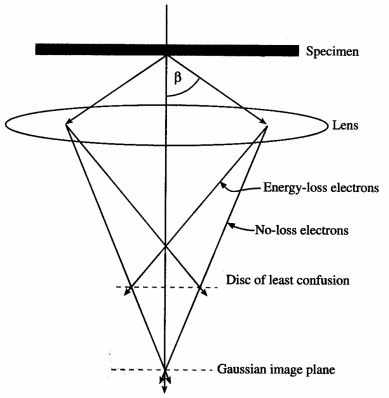

Figure 6.12. Chromatic aberration results in electrons with a range of energies being focused in different planes. Electrons emerging from the specimen with no loss of energy are less strongly focused than those that suffered energy loss in the specimen, so a point is imaged as a disk.

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New directions

- Resolution limitations:
  - Lenses
  - Specimen

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New directions II

- Resolution limitations & goals:
  - Lenses – make them better
  - Specimen – make it less susceptible

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New directions III

- Resolution limitations & fixes:
  - Lenses – Cs correctors, phase plates
  - Specimen – spot-scan imaging, conductivity

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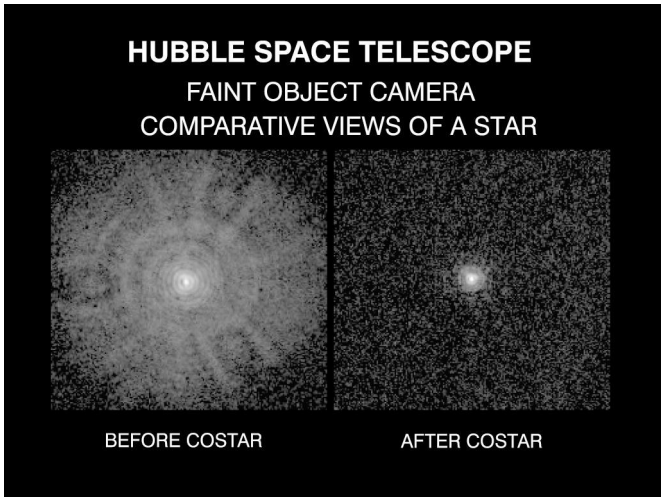
Are correctors useful for cryo-EM?



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C_S Correctors

C_s correctors are used with TEM and STEM. There is a separate corrector for each imaging mode. The object of the corrector is to eliminate or greatly reduce spherical aberration.

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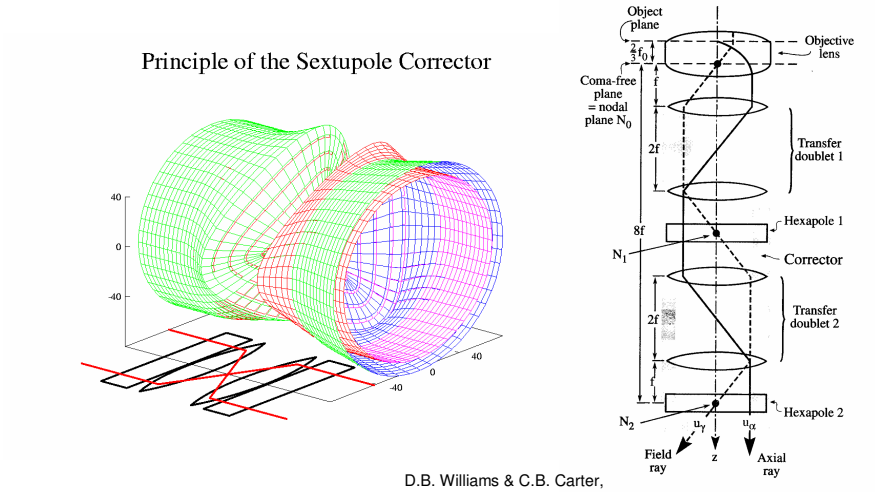
Principle of the Cs corrector



D.B. Williams & C.B. Carter,
Transmission Electron Microscopy (1996) pg. 469

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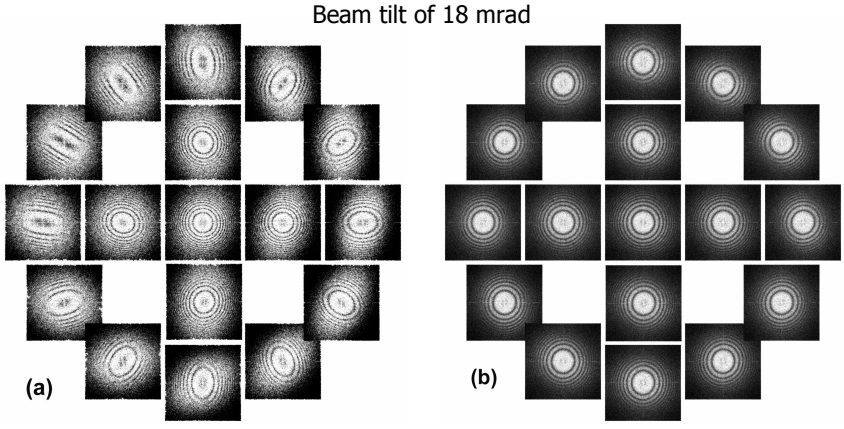
Effect of Cs correction

Beam tilt of 18 mrad



Uncorrected Corrected

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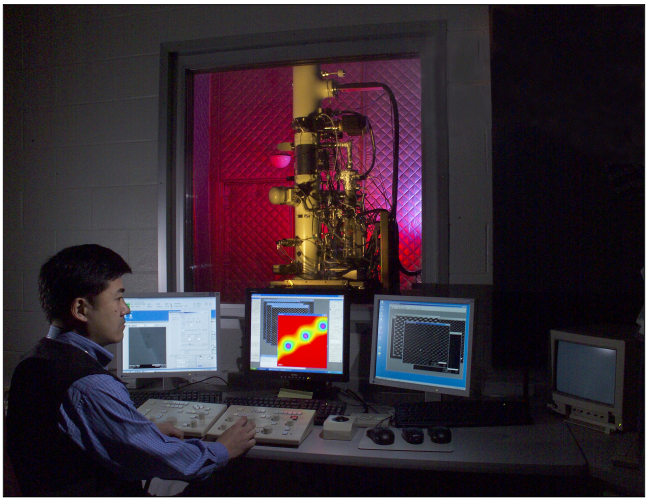
ACEM at ORNL



View from control room into instrument room

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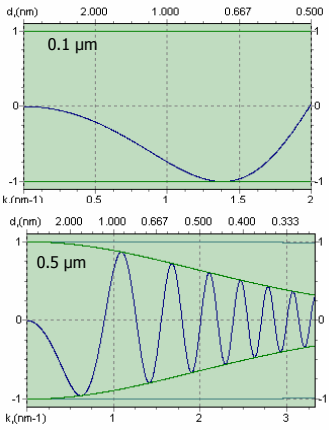
Cs Correctors in Biology I

- Current interest focused on sub-10 Å.
- Specimen are low Z elements requiring large defocus for imaging.
- Extensive ringing in phase CTF



2100F; UHR p/p

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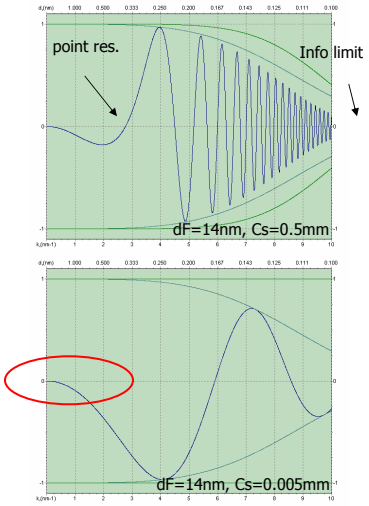
Cs Correctors in Biology II

- With Cs corrector the point resolution goes towards information limit.
- Also, larger p/p gap is possible thus allowing for environmental cells.
- No penalty for tilted illumination since coma is absent, thus allowing for “optical sectioning”
- Loss of contrast at low resolution can be compensated with phase plate.



Different Phase Plates

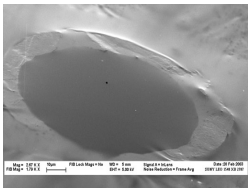
- Zernike phase plate – carbon film on BFP, small hole for the unscattered, direct beam
- Boersch phase plate or Einsellens – device in BFP w. floating inner ring at elevated potential

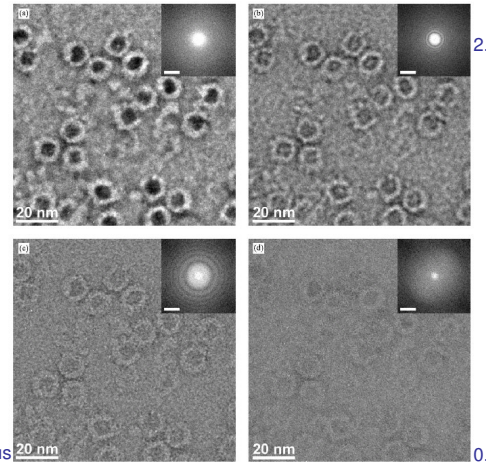
- Provides in-focus phase contrast, with increased transfer of lower spatial frequencies.
- Very suitable for frozen-hydrated specimens.

Zernike Phase Plate

Negatively-stained ferritin

Phase plate





Conventional, 2.5 µm underfocus

Conventional, 0.5 µm underfocus

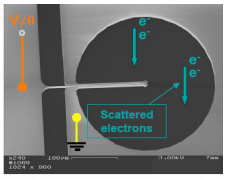
Conventional, 0.1 µm underfocus

Danov & Nagayama, Ultramicroscopy (2001) 90:85-89

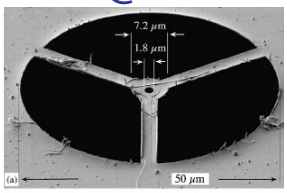
JEOL

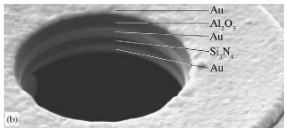
Boersch Phase Plate

- Proposed in 1947
- Actively researched by Schröder @ MPG & Glaeser @ LBL

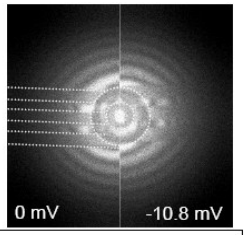


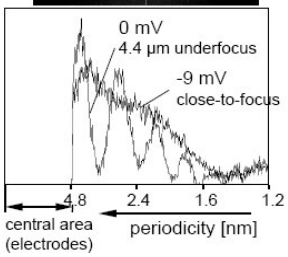
Glaeser





Schröder





JEOL

Specimen-related Limitations

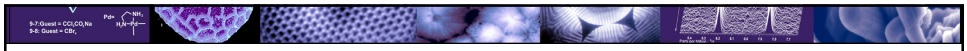
- Charging:
  - SE emission
  - Very noticeable with tilted specimens
- Beam-induced specimen movement
  - Image amplitudes are only 1-10% of the theoretical values (Henderson & Glaeser)

JEOL

Specimen-related Limitations II

- Charging:
  - Adjust beam size carefully (Unwin)
  - Carbon-coat specimen (Glaeser, Brink)
  - BFP OLA
  - New support films, e.g. TiSi alloys
- Beam-induced specimen movement
  - Spot-scan imaging technique
  - Higher kV (Brink & Chiu)
  - Sturdier support films ?

JEOL



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