Image data collection of two-dimensional crystals

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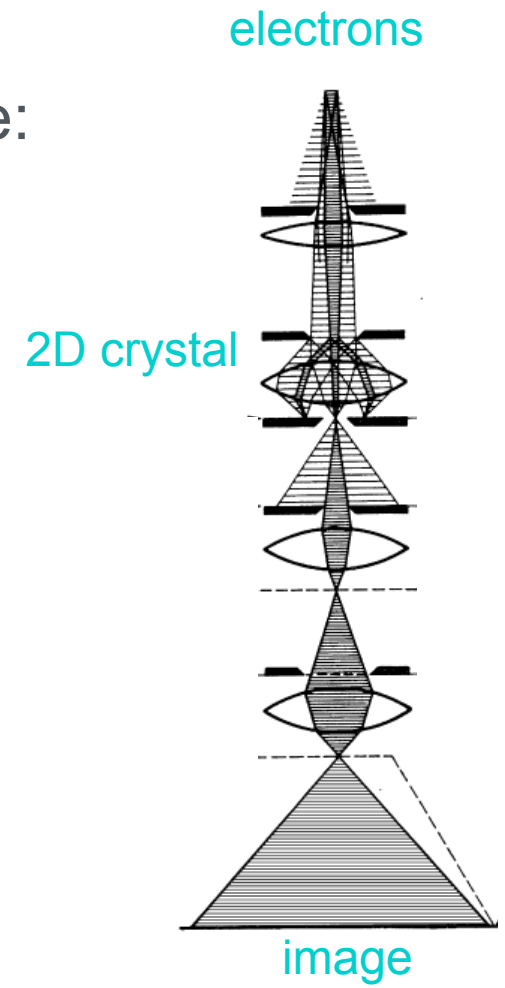
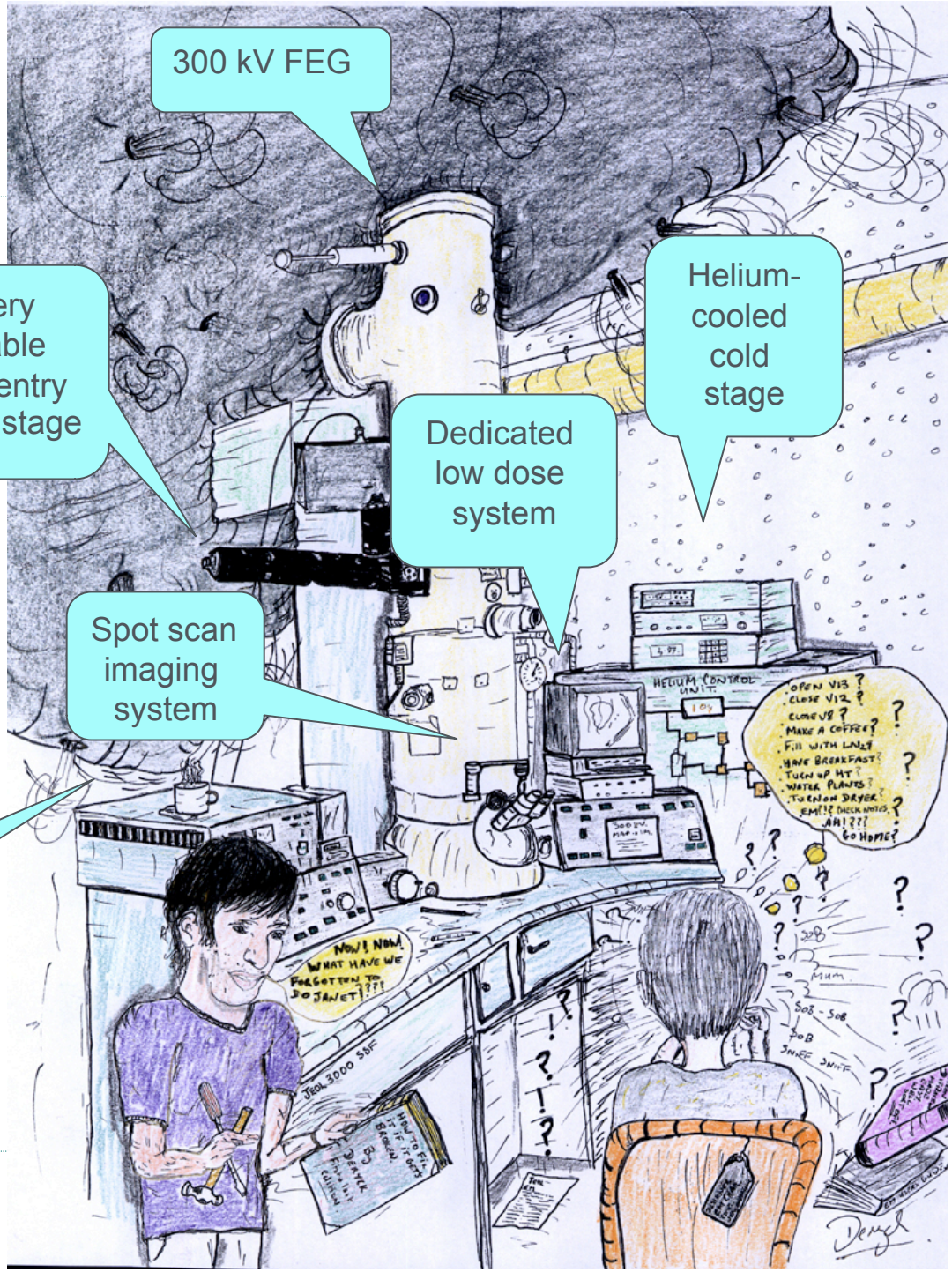
Image data collection of 2D crystals

Factors that limit the resolution of an image:

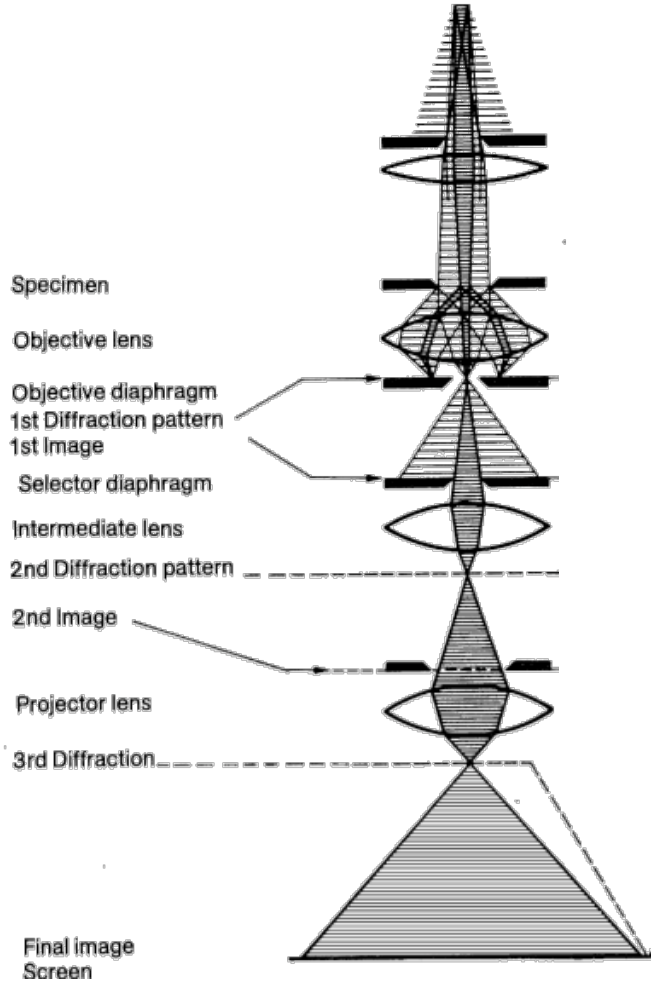
- ✧ Radiation damage
- ✧ Drift
- ✧ Contamination
- ✧ Specimen charging
- ✧ Variation in voltage
- ✧ Variation of lens currents
- ✧ Lack of coherence of the electron beam



JEOL 3000 SFF



Imaging in the electron microscope



The basic design of an EM is much like a light microscope, but the resolution is limited not by the wavelength but by chromatic and spherical aberration of the objective lens.

Images of unstained biological samples are formed by phase contrast. Wave theory assumes that the electron wave is coherent: a monochromatic plane wave. In practice the performance of the EM is affected by partial coherence of the electron beam.

Partial coherence

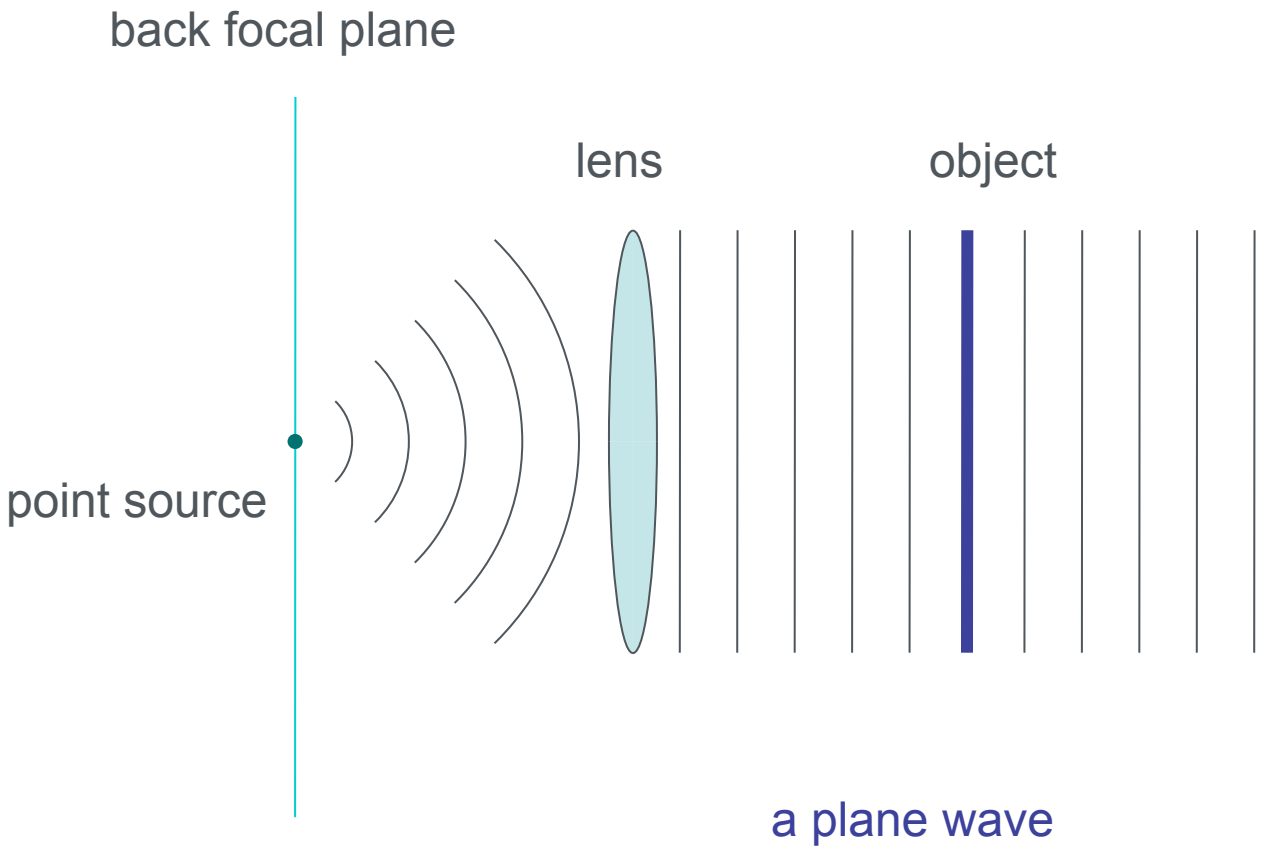
Perfect temporal coherence:

- All electrons have the same wavelength (monochromatic illumination).
- In practice, the gun has an energy spread (~2.5 eV for tungsten filament, 1 eV for FEG).
- Temporal coherence is determined by energy spread divided by accelerating voltage, so higher voltage gives better coherence.
- Instability of the high voltage or the objective lens current also causes loss of coherence.

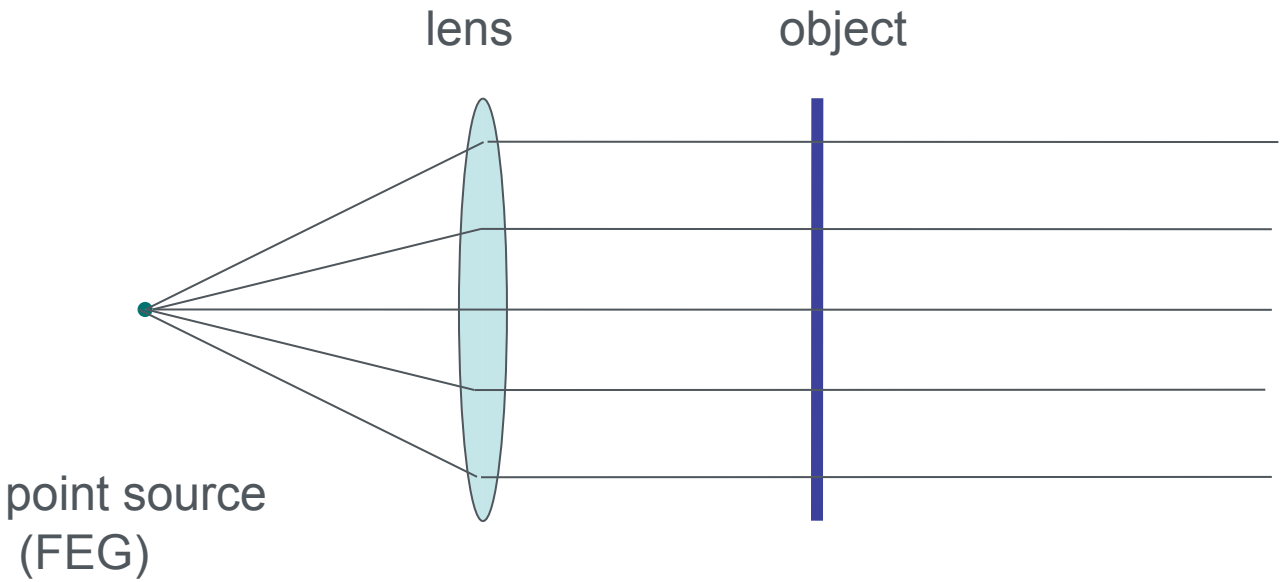
Perfect spatial coherence:

- All electrons have the same direction (plane wave).
- This would only be true if the source is a point, which is never the case.

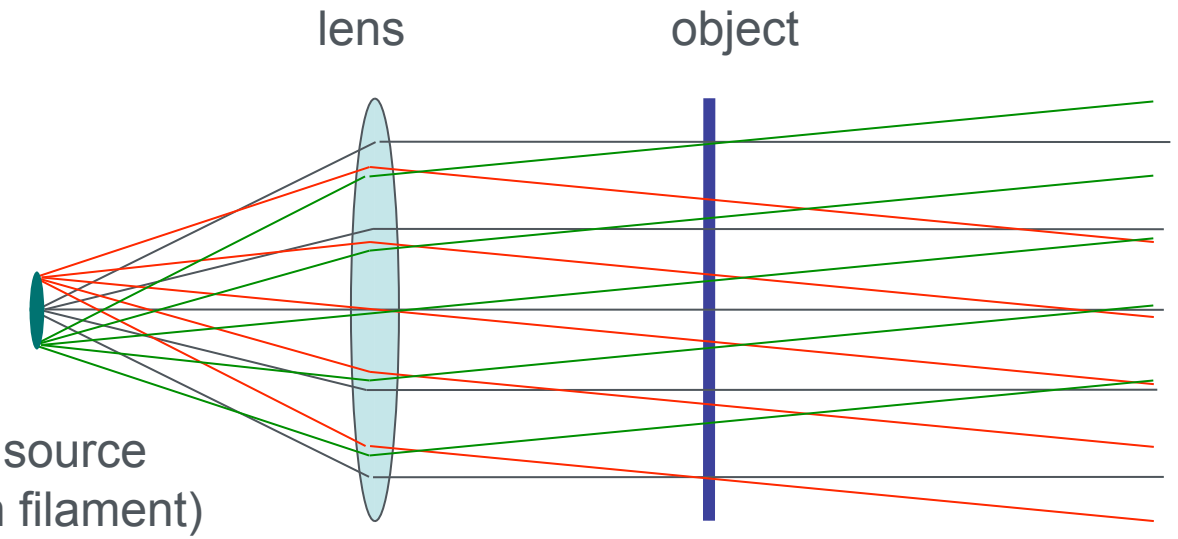
Spatial coherence



Spatial coherence



Spatial coherence



Not a plane wave – loss of spatial coherence

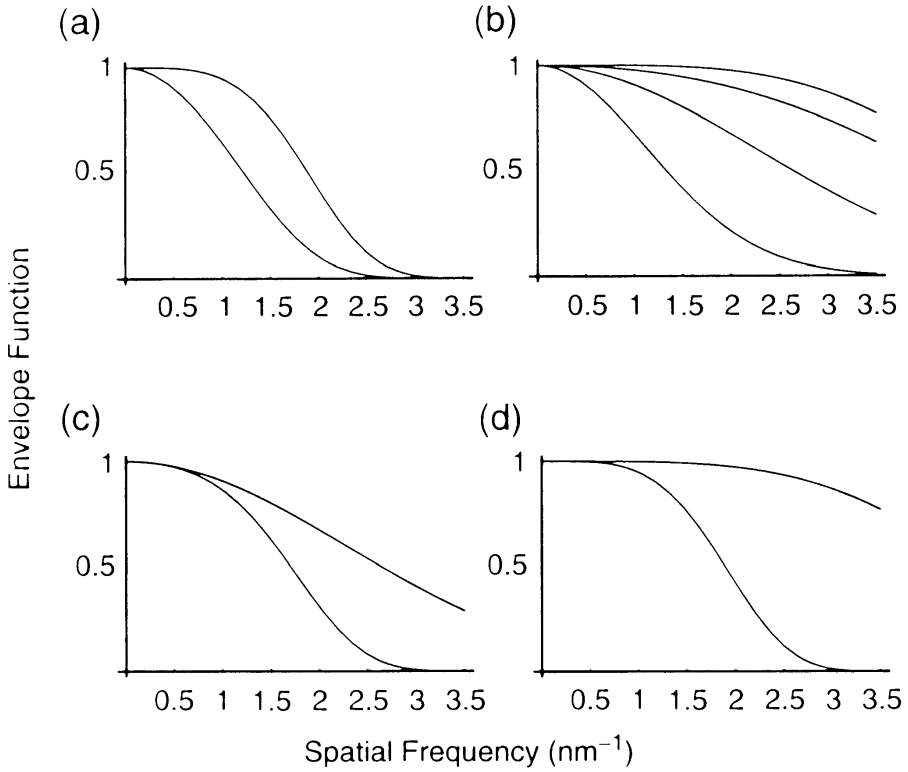
Effect of partial coherence

Partial coherence imposes an envelope function on the CTF: the signal gets lower at higher resolution.

(a) 100 kV; 100 and 2000 nm defocus
(b) 300 kV; 100, 500,1000 and 2000 nm defocus
(c) 1000 nm defocus; 100 and 300 kV
(d) 100 nm defocus; 100 and 300 kV

For best results use

- ✧ FEG
- ✧ high voltage
- ✧ low defocus



Glaeser et al., 2007

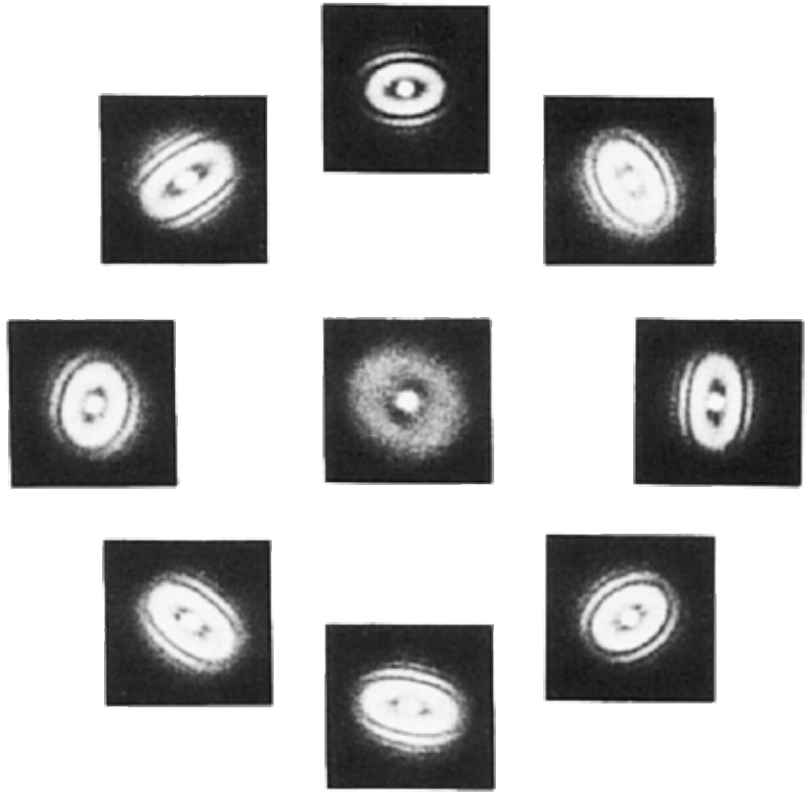
Microscope alignment

Make sure the beam is aligned with the optical axis of the objective lens

- Introduce a grid and set it to the eucentric height of the specimen stage.
- Focus the objective lens.
- Align the condenser aperture.
- Gun tilt alignment
- Gun shift alignment.
- Pivot point alignment.
- Rotation center alignment (the beam passes through the center of the objective lens, there is no movement during focusing).

Beam tilt

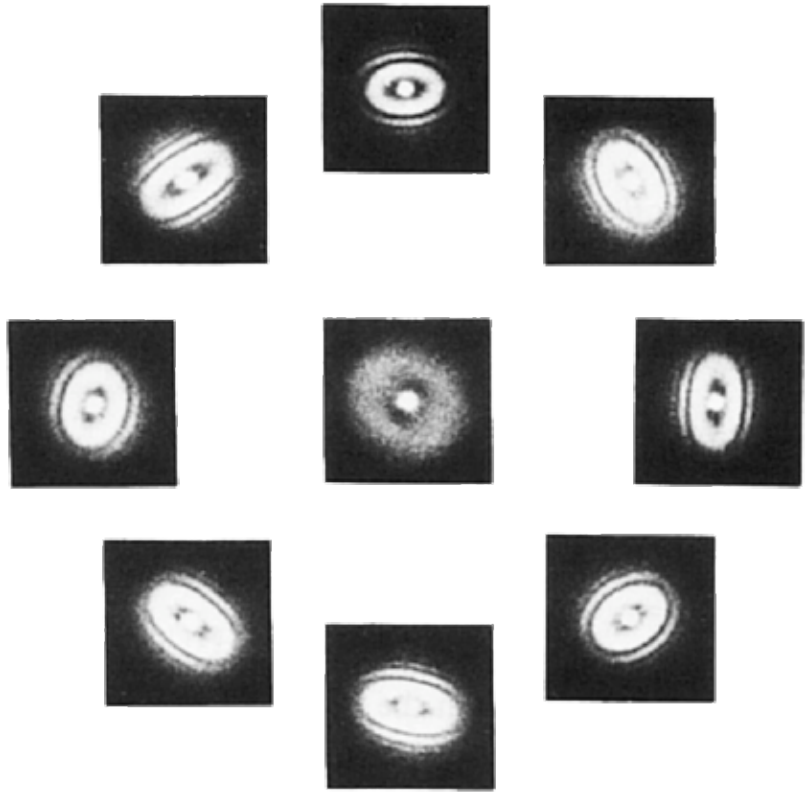
- If the beam is not exactly parallel to the optical axis of the EM, a shift in the phases is introduced which increases with the cube of the spatial frequency and the square of the wavelength.
- This effect is thus stronger at lower voltage.
- Beam tilt becomes important at <5Å resolution at 300 kV.
- Beam tilt can be adjusted with the aid of a “Zemlin tableau”: tilting the beam in opposite directions results in different distortions when the beam was misaligned to start with.



Zemlin et al., 1978

Beam tilt

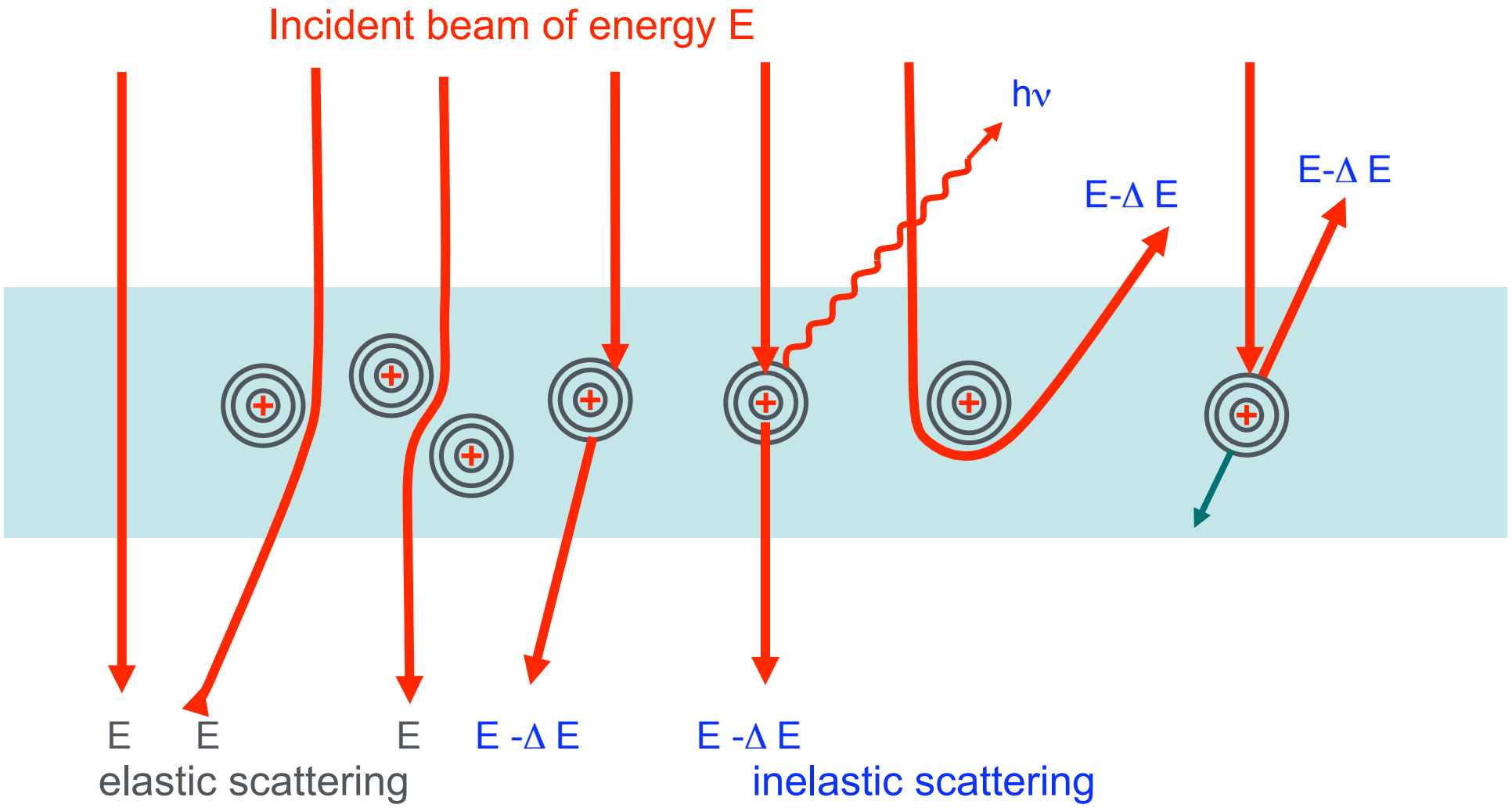
- If the beam is not exactly parallel to the optical axis of the EM, a shift in the phases is introduced which increases with the cube of the spatial frequency and the square of the wavelength.
- This effect is thus stronger at lower voltage.
- Beam tilt becomes important at <5Å resolution at 300 kV.
- Beam tilt can be adjusted with the aid of a “Zemlin tableau”.
- For two-D crystals, beam tilt can be corrected computationally. This should be done for resolutions better than 5 Å.



Zemlin et al., 1978

Radiation damage

Electron-specimen interactions



Inelastic scattering and radiation damage

Elastic scattering does not deposit energy in the specimen and thus does not cause radiation damage.
By inelastic scattering part of the electron's kinetic energy is transferred to the specimen. This is the main cause of radiation damage.

Energy is lost to the specimen through excitation of

•molecular vibrations	(0.02 - 1 eV)
•lattice vibrations (phonons)	(~20 eV)
•electrons in covalent bonds	(1 - 50 eV): bond breakage, formation of radicals
•inner shell electrons	(up to 1000 eV): emission of x-rays, secondary electrons, ionisation

➢Inelastically scattered electrons have lost some of their energy to the specimen and therefore have longer wavelengths. They are not focused by the objective lens in the same plane as elastically scattered electrons.
➢Breaking of bonds causes formation of fragments and radicals and eventually mass loss, resulting in a loss of resolution with accumulating electron dose.
➢The proportion of inelastically scattered electrons is higher for lighter atoms (C,N,O).

Electron dose

How to measure radiation damage: fading of electron diffraction pattern

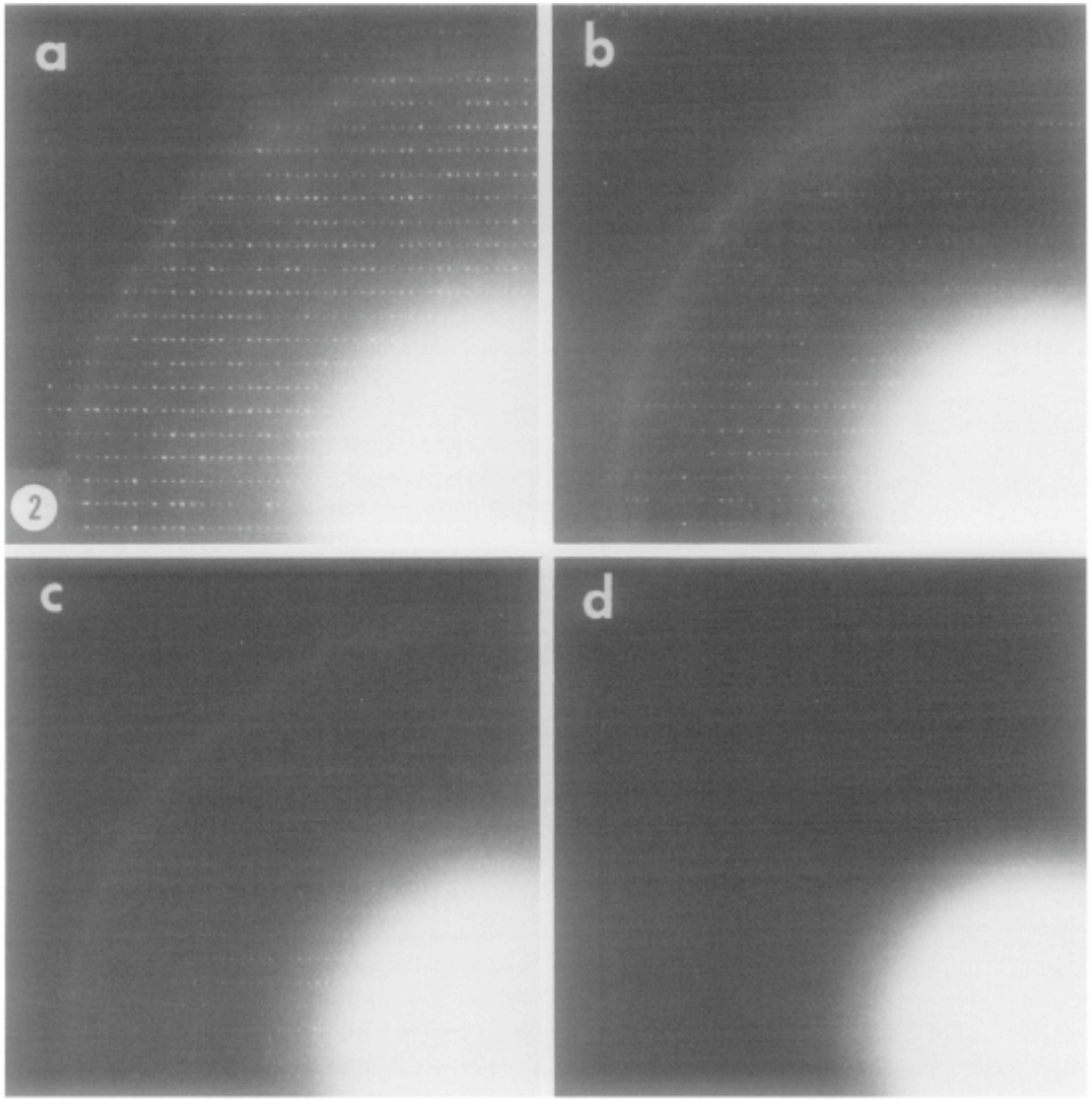
a: initial: 2.8 Å

b: 2.5 $e^-/Å^2$

c: 5 $e^-/Å^2$

d: 11 $e^-/Å^2$: 8.5 Å

Taylor & Glaeser, 1976



Electron dose

Complete loss of crystalline diffraction:

Frozen hydrated catalase at 100 kV: 27 +/- 9 $e^-/Å^2$

Catalase at room temperature: 2 $e^-/Å^2$

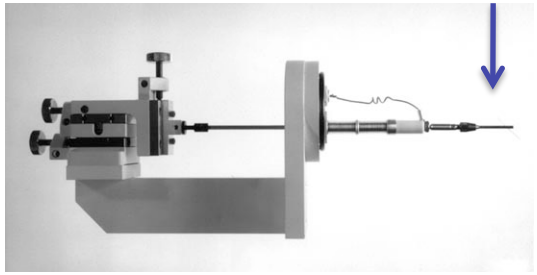
(higher dose at higher voltages)

- Liquid nitrogen temperature allows a 5-10x higher electron dose (cage effect/bubbling)
- Liquid helium may allow another 2x higher dose.

Taylor & Glaeser, 1976; Chiu et al., 1986

Measuring the electron dose

Faraday cup



Measure the current to calculate the number of electrons hitting the cup.

$$N/t = I/e$$

N: number of electrons observed in a time t (in seconds),

I: measured current (in amperes)

e: elementary charge (1.60×10^{-19} C)

Measuring the electron dose

Exposure meter:

- ✧Measures electrons hitting the screen ($e^-/\mu m^2$).
- ✧Calibrated to give a suitable film density (exposure in seconds to give OD=1) or showing a direct measurement in $e^-/\mu m^2$.
- ✧The dose *on the specimen* depends on the magnification!
- ✧Higher magnification, same exposure: exposure on a smaller area, so higher electron dose!
- ✧The dose is measured per *area,* so it increases as a *square* of the magnification.

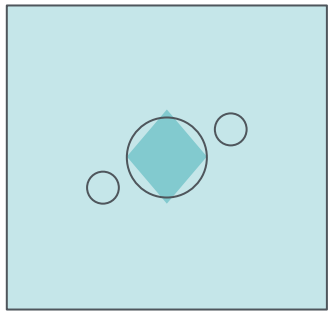
Magnification

- Optimal magnification depends on the expected resolution and pixel size:
  - 6 Å resolution, 7 µm pixel: 2 Å/pixel, 35,000x
  - 3 Å resolution, 7 µm pixel: 1 Å/pixel, 70,000x
- Lower magnification gives a better intensity of the film.
- Remember to adjust the exposure on the detector if you change magnification, to keep the electron dose on the specimen constant.

The low dose system

- Search mode: low magnification, minimal dose
- Focus mode: image shift, high magnification
- Image mode

Do all alignments in imaging mode!



Setting up the low dose system

Search mode:

Low magnification or defocused diffraction mode

Advantages of defocused diffraction:

- High contrast
- No objective lens adjustments
- Very low magnification

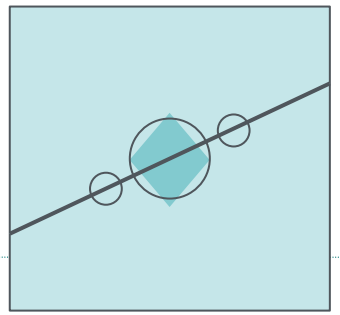
Disadvantage:

- Absolute magnification not known.

Setting up the low dose system

Focus mode:

- High magnification for easy focusing (200,000-300,000x).
- Minimum image shift (depends on the magnification of the imaging mode and detector size), typically 2-3 µm.
- Shift along the tilt axis for tilted specimens.



Setting up the low dose system

Image mode:

- Magnification
- Spot size
- Centering and spreading of the beam
- Set the correct exposure

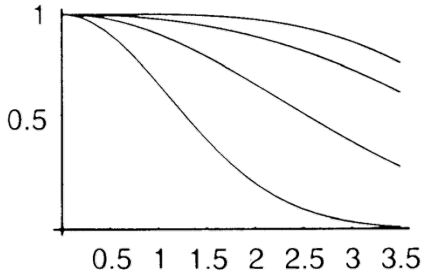
✓ Toggle through the modes a few times until all settings are stable.

✓ Make sure the focus stays constant when changing magnification (parafocal alignment).

Focusing and defocus

- Focus by minimizing the phase contrast on a clean area of carbon film, then set the desired defocus and take the image.
- For 2D crystals, low-resolution contrast to localize the molecules is not needed, so high defocus is not necessary.
- Low defocus gives a higher signal at high resolution (envelope function).
- For high-resolution work, a low defocus (100-400 nm) should be used.

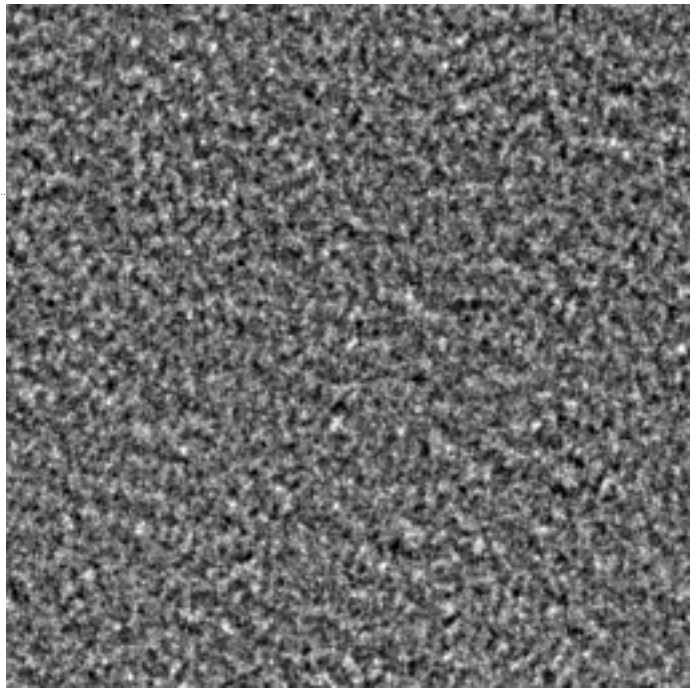
Envelope at 300 kV and 100,500,1000 and 2000 nm defocus

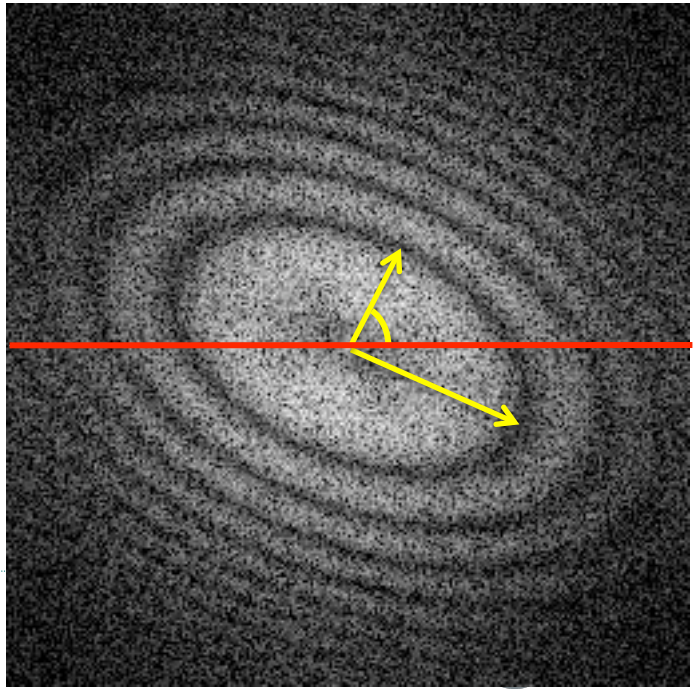


Glaeser et al., 2007

Astigmatism

- Focus is different in two directions.
- Thonrings are elliptical.
- Astigmatism correction:
  - On the carbon grain
  - Using live FFT on CCD camera

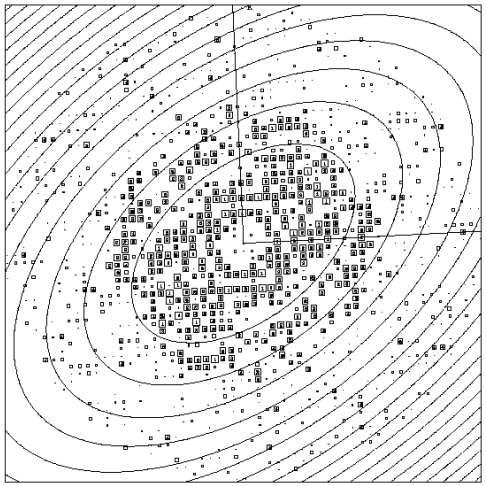




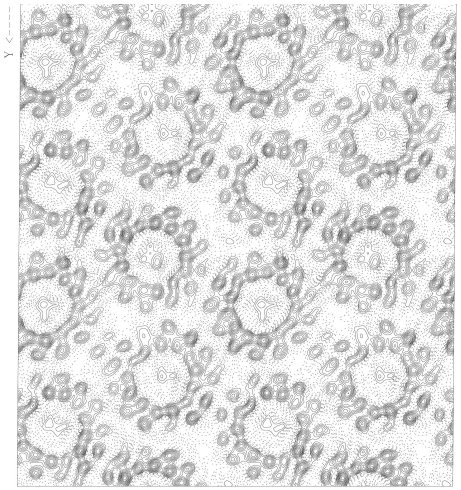
Astigmatism

Focusing can only be done accurately after astigmatism correction.

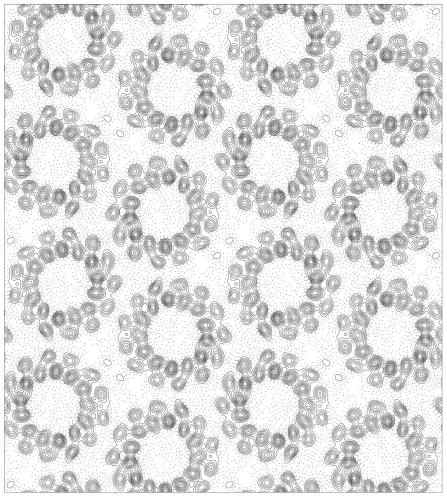
Astigmatism is not a problem for 2D crystal images and is taken into account by the CTF correction.



2800Å,8000Å,38°



Unsymmetrised map



$p22_12_1$ applied

Matthies et al., JMB 2009

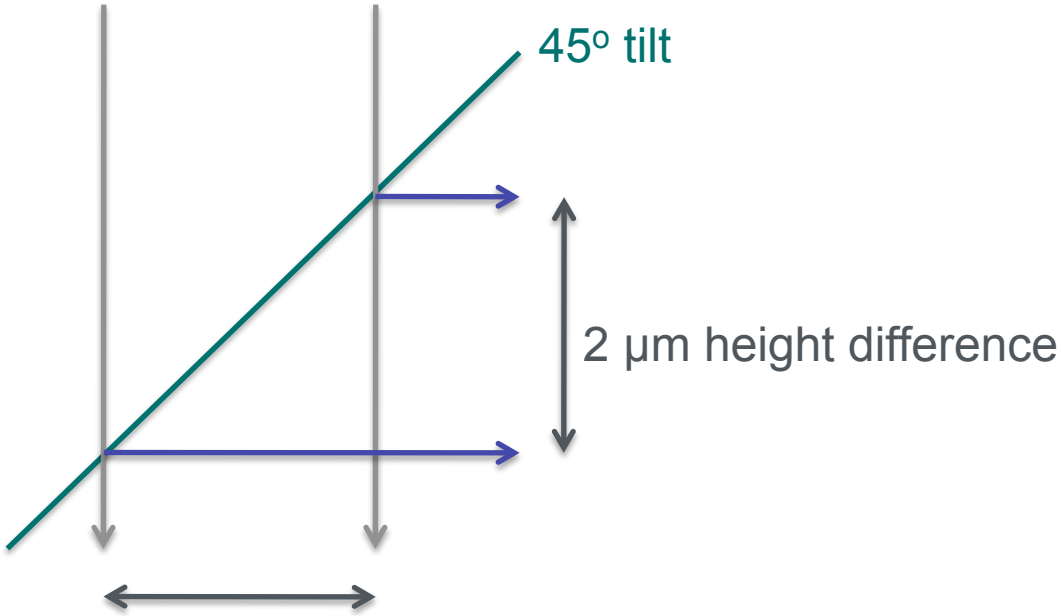
Tilted crystals

Special considerations:

- Focus gradient
- Electron dose
- Crystal flatness
- Specimen charging

Tilted crystals

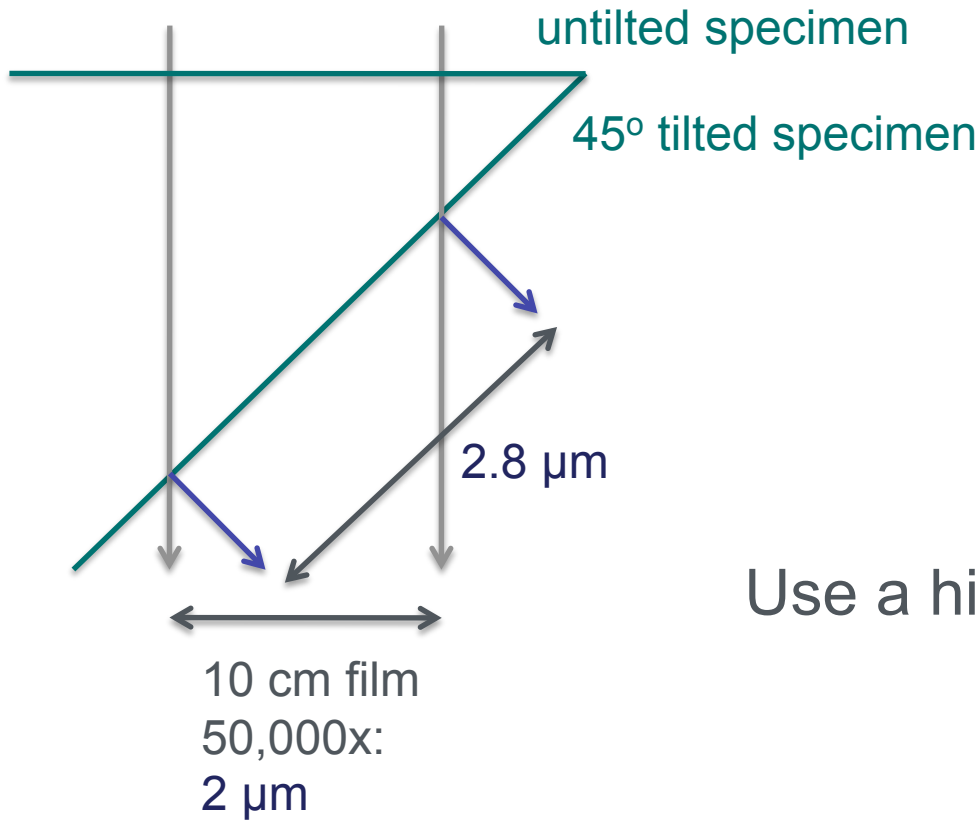
Focus gradient:



Use at least 1 µm defocus

Tilted crystals

Electron dose:



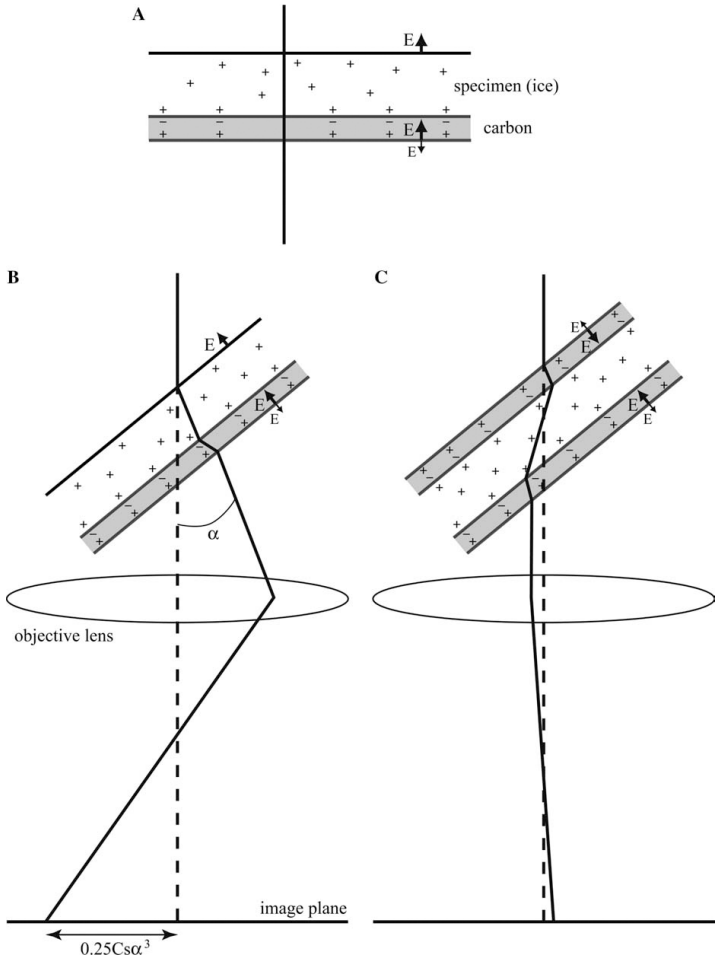
Use a higher dose at higher tilt

Beam-induced specimen movement

Electron irradiation of beam-sensitive specimens leads to breaking of covalent bonds, mass loss, specimen charging, specimen heating.

The effects on the image are most severe in tilted crystals.

Beam-induced specimen charging of tilted samples causes an image shift



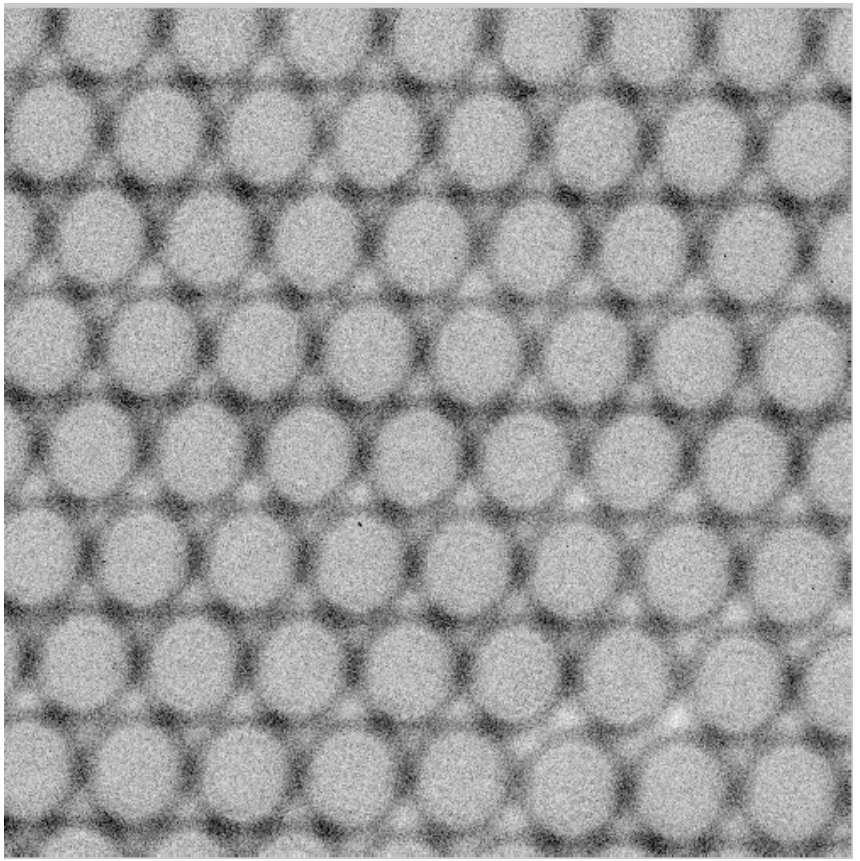
Gyobu et al., JSB 2004

- Beam-induced image shift can be compensated by using a symmetric carbon sandwich.
- Charging and beam-induced movements can be reduced by illuminating a very small area at a time: spot-scan imaging.

Spot scanning

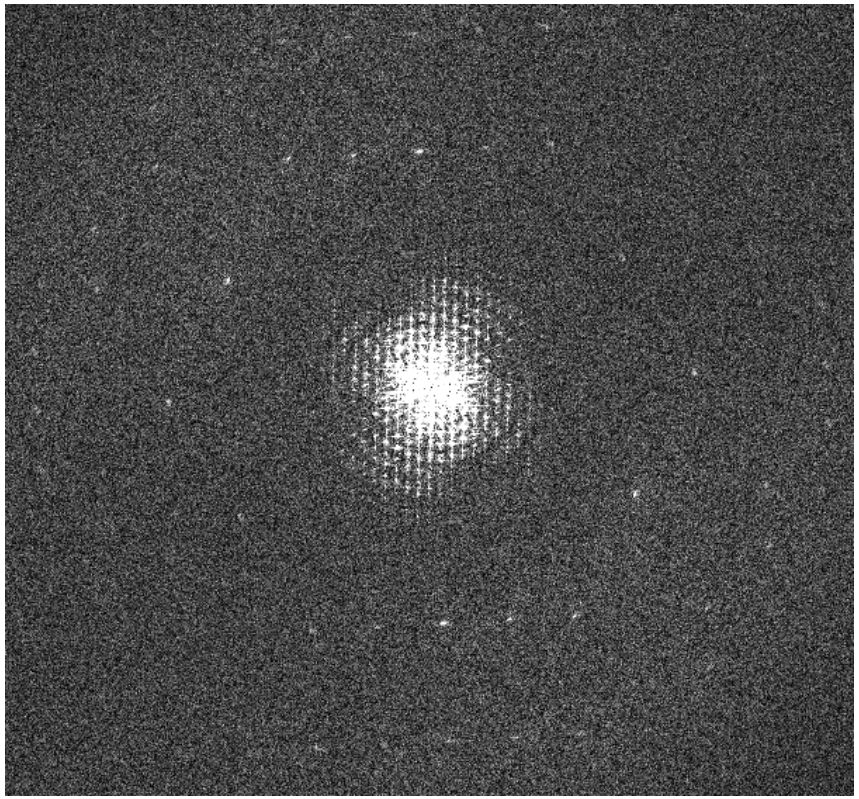
Spot size: 40-100 nm

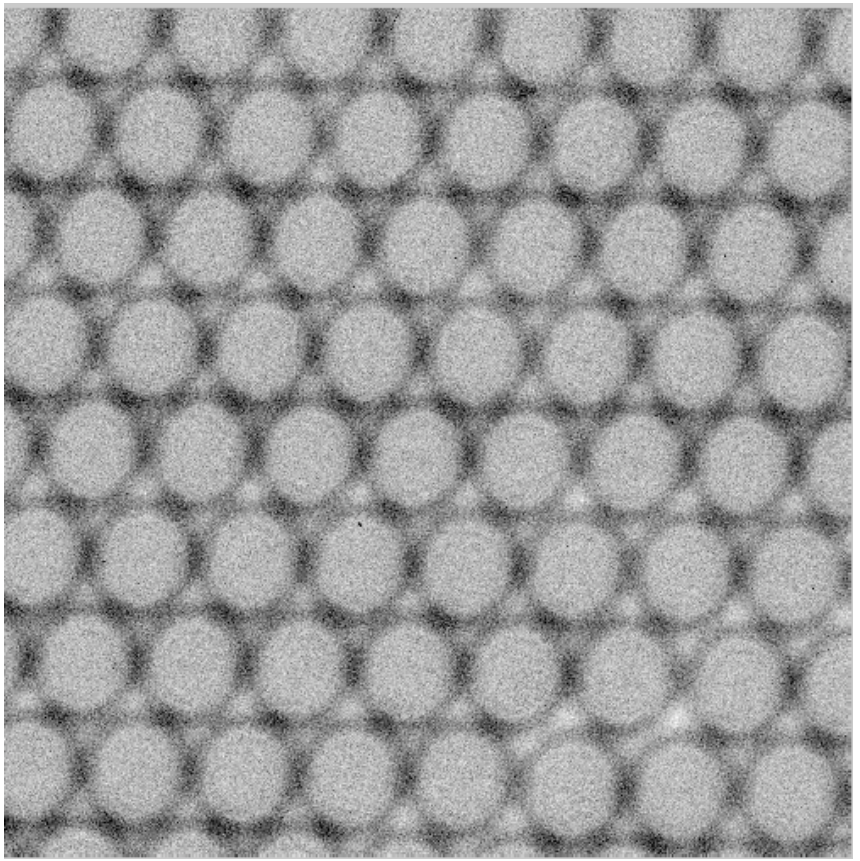
Exposure time per spot:
30-100 ms



Henderson & Glaeser, Ultramicroscopy 1985
K.H. Downing, Science 1991

Spot scanning

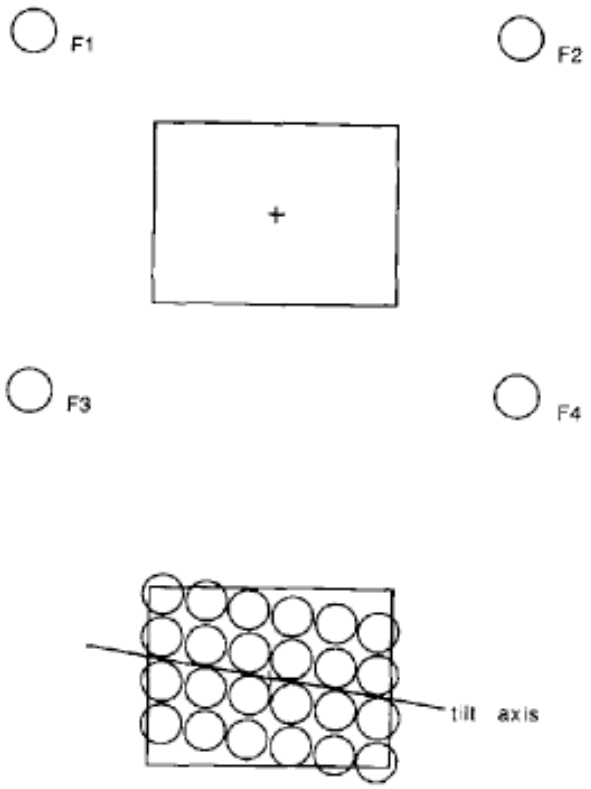




Henderson & Glaeser, Ultramicroscopy 1985
K.H. Downing, Science 1991

Spot scan imaging gives a much higher yield of good images, especially for tilted crystals.

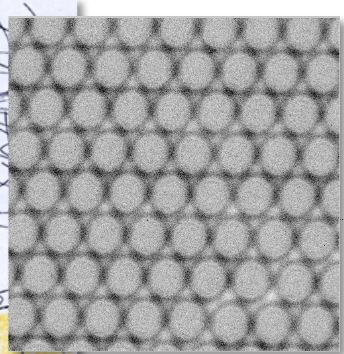
Spot scanning with dynamic focus

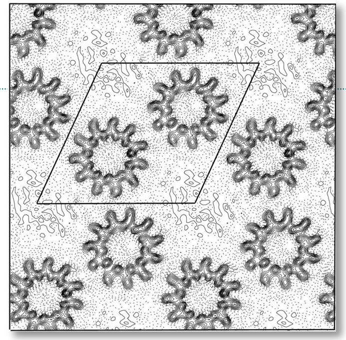


K.H. Downing, Ultramicroscopy 1992

- Focus at four corners of the area of interest.
- Calculate direction of tilt axis and defocus gradient.
- Set up spot scan patterns along the tilt axis and change defocus for each row.


HELIUM CONTROL UNIT
OPEN V13 ? CLOSE V12 ? CLOSE V8 ? MAKE A COFFEE? FILL WITH LN2? HAVE BREAKFAST? TURN UP HT? WATER PLANTS? TURN ON DRYER? EM!!? CHECK NOTES. AH!??? GO HOME?
NOW! NOW! WHAT HAVE WE FORGOTTON TO DO JANET!???
JEOL 3000 SSF
SOB - SOB
MUM
SNIFF SNIFF



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Some references

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A very basic primer on electron optics:

http://www.rodenburg.org/guide/index.html

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