

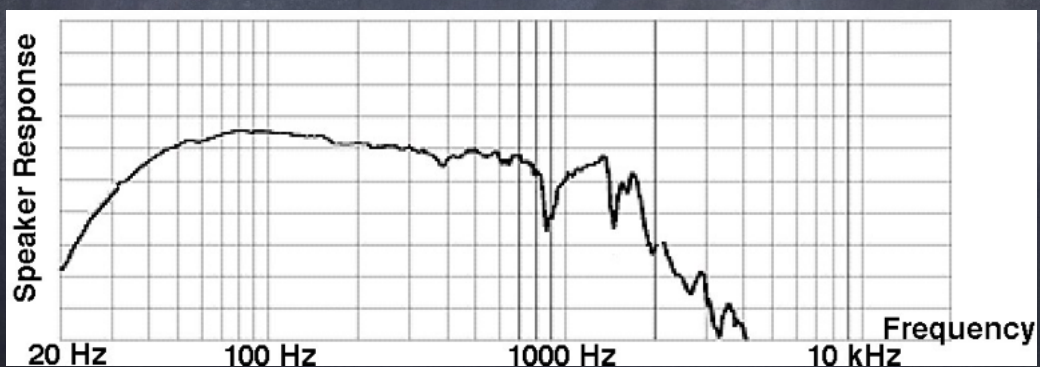
CTF

Henning Stahlberg,
Biozentrum, Uni Basel, Switzerland
c-cina.org

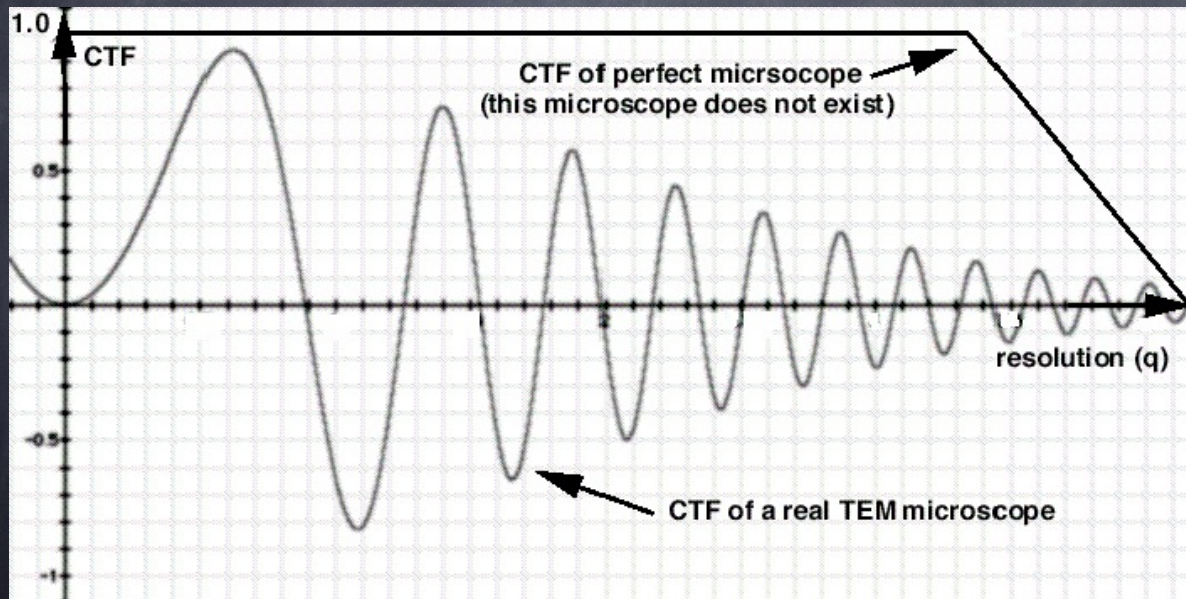
2dx Workshop
Basel, August 23, 2016



Transfer Function



Transfer Function of a TEM



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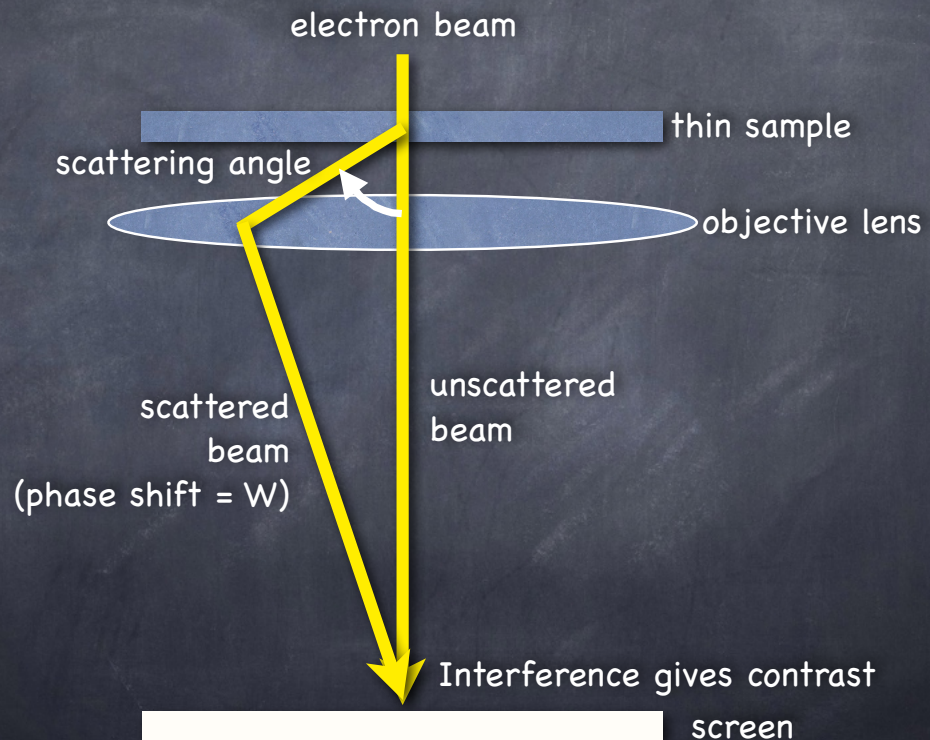
Otto Scherzer

(Mar. 9, 1909 – Nov. 15, 1982)

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Contrast



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Scherzer Formula

$$\gamma(\mathbf{u}) = \frac{2\pi W}{\lambda} = \frac{\pi}{2} [C_s \lambda^3 \mathbf{u}^4 - 2\Delta z \lambda \mathbf{u}^2]$$

$\gamma(\mathbf{u})$: Wave aberation function

(phase shift of scattered vs. non-scattered wave)

$\sin(\gamma(\mathbf{u}))$: phase contrast transfer function

$\cos(\gamma(\mathbf{u}))$: amplitude contrast transfer function

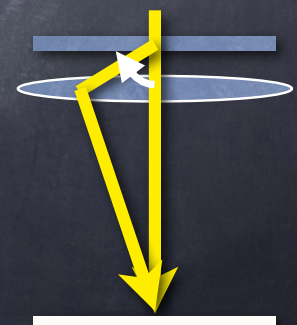
u : scattering vector (\approx scattering angle)

W : wave aberation

λ : electron wavelength

Δz : defocus

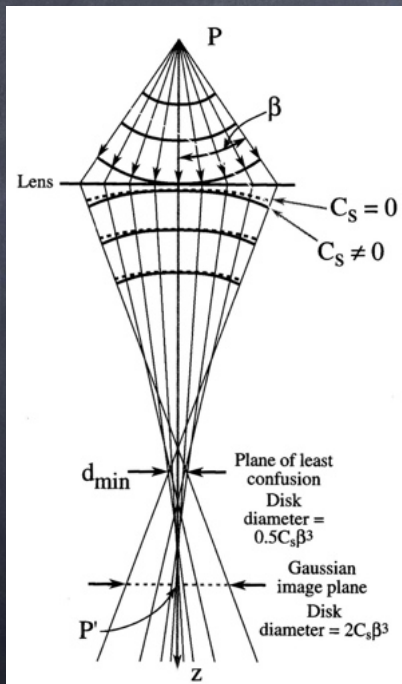
C_s : spherical abberation constant



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Spherical Aberration



C_s

Limits resolution beyond $\sim 2 \text{ \AA}$

Electrons further from the axis are more strongly bent back towards the axis.

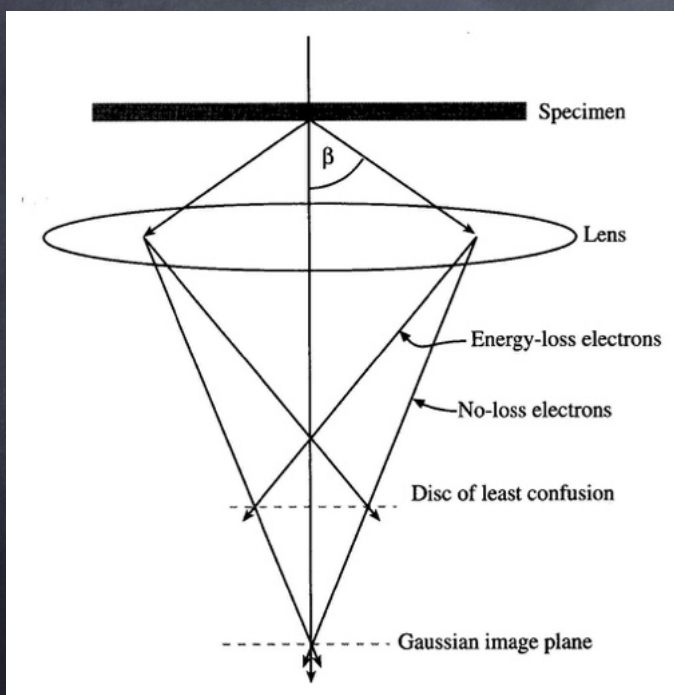
Or:

Electron waves further from the axis receive more phase shift.

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Chromatic Aberration



C_c

Limits resolution beyond $\sim 0.5 \text{ \AA}$

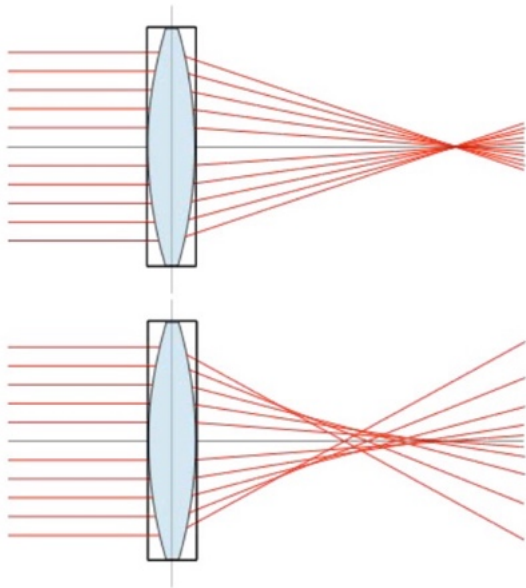
Electrons of lower energy are bent more strongly than those of zero-loss energy.

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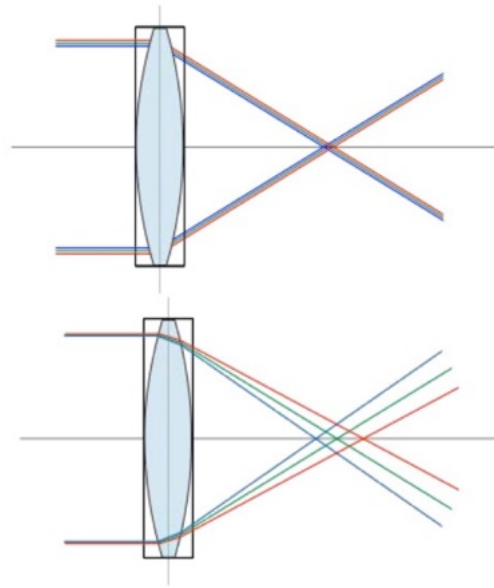
Lens aberrations

C_s -Spherical aberration



Rays from the outer rim of the lens are focussed more tightly than the inner ones.

C_c -Chromatic aberration



Different wavelengths are focussed more tightly than others.

Carsten Sachse, EMBL Talk, 2008

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CTF

$$\text{CTF}(\mathbf{u}) = \{ A * \cos(\gamma(\mathbf{u})) - \sqrt{1-A^2} * \sin(\gamma(\mathbf{u})) \} * E(\mathbf{u})$$

$$\gamma(\mathbf{u}) = \frac{2\pi W}{\lambda} = \frac{\pi}{2} [C_s \lambda^3 \mathbf{u}^4 - 2\Delta z \lambda \mathbf{u}^2]$$

$\sin(\gamma(\mathbf{u}))$: phase contrast transfer function

$\cos(\gamma(\mathbf{u}))$: amplitude contrast transfer function

\mathbf{u} : scattering vector (\approx scattering angle)

A : Amplitude contrast fraction. (neg. stain: use 0.07)

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Envelope functions

$$E(\mathbf{u}) = E_s(\mathbf{u}) \cdot E_c(\mathbf{u}) \cdot E_d(\mathbf{u}) \cdot E_v(\mathbf{u}) \cdot E_D(\mathbf{u})$$

with

$E_s(\mathbf{u})$: angular spread of the source

α = opening angle

$$E_s(u) = \exp\left[-\left(\frac{\pi\alpha}{\lambda}\right)^2 \left(\frac{\delta X(u)}{\delta u}\right)^2\right] = \exp\left[-\left(\frac{\pi\alpha}{\lambda}\right)^2 (C_s \lambda^3 u^3 + \Delta f \lambda u)^2\right]$$

$E_c(\mathbf{u})$: chromatic aberration

$$E_c(u) = \exp\left[\frac{1}{2}(\pi\lambda\delta)^2 u^4\right] \quad \delta = C_c \sqrt{4\left(\frac{\Delta I_{obj}}{I_{obj}}\right)^2 + \left(\frac{\Delta E}{V_{acc}}\right)^2 + \left(\frac{\Delta V_{acc}}{V_{acc}}\right)^2}$$

$E_d(\mathbf{u})$: specimen drift

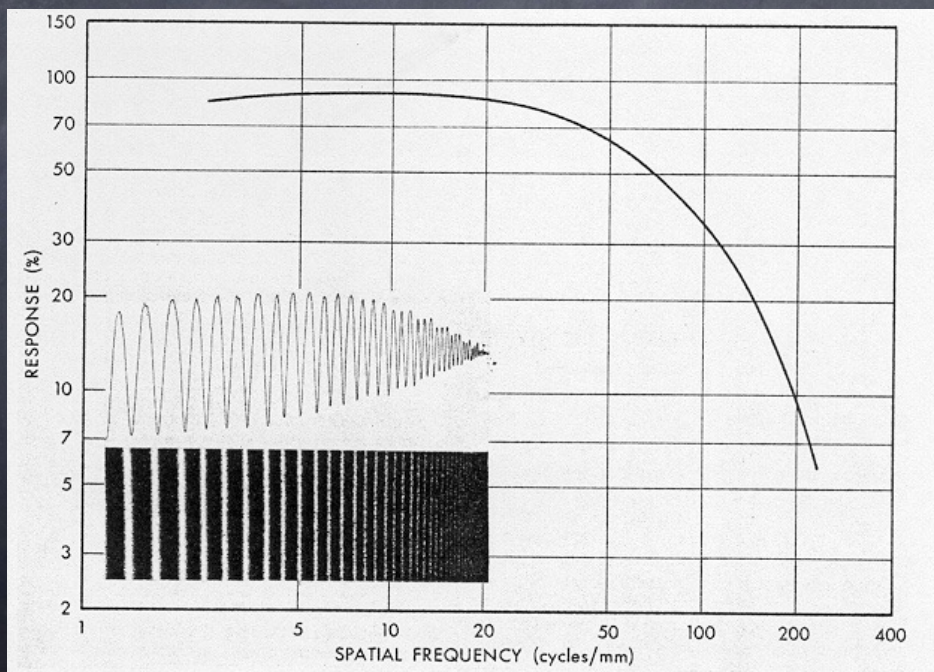
$E_v(\mathbf{u})$: specimen vibration

$E_D(\mathbf{u}) = MTF_D(u)$: detector

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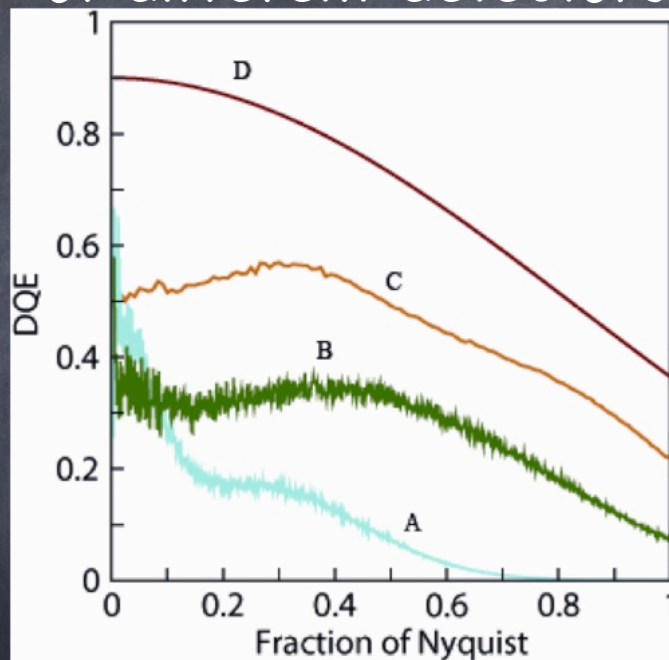
Modulation Transfer Function (MTF) of photographic film



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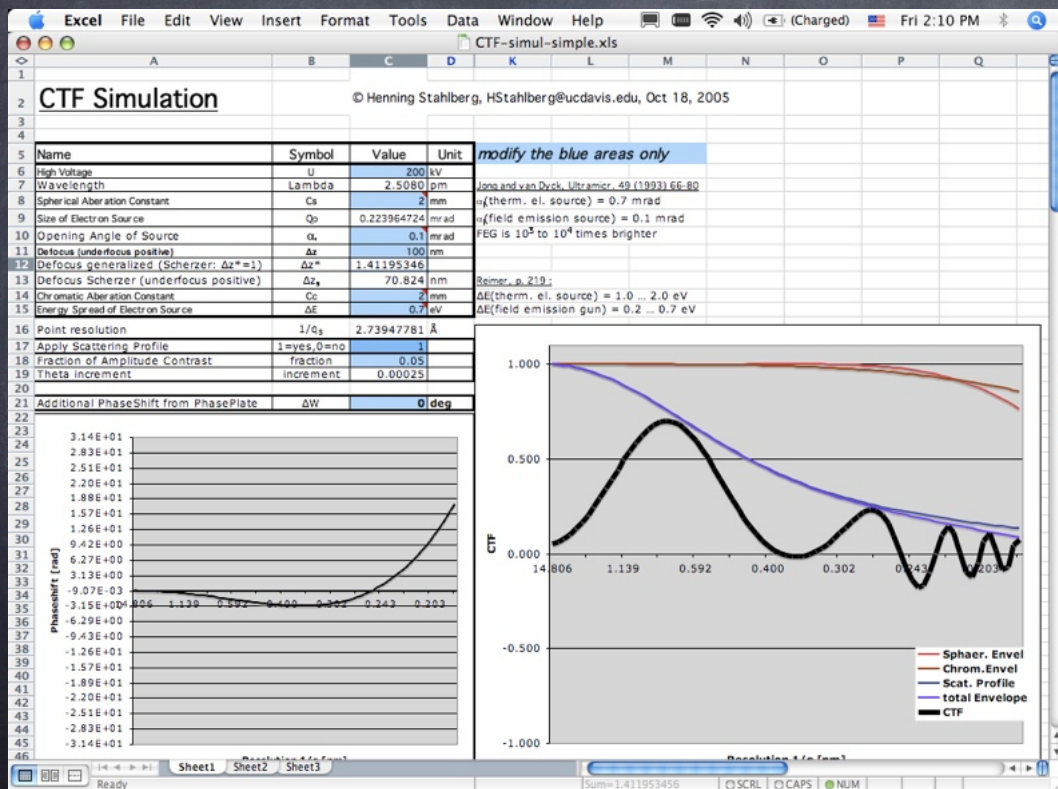
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Detector Quantum Efficiency (DQE) of different detectors

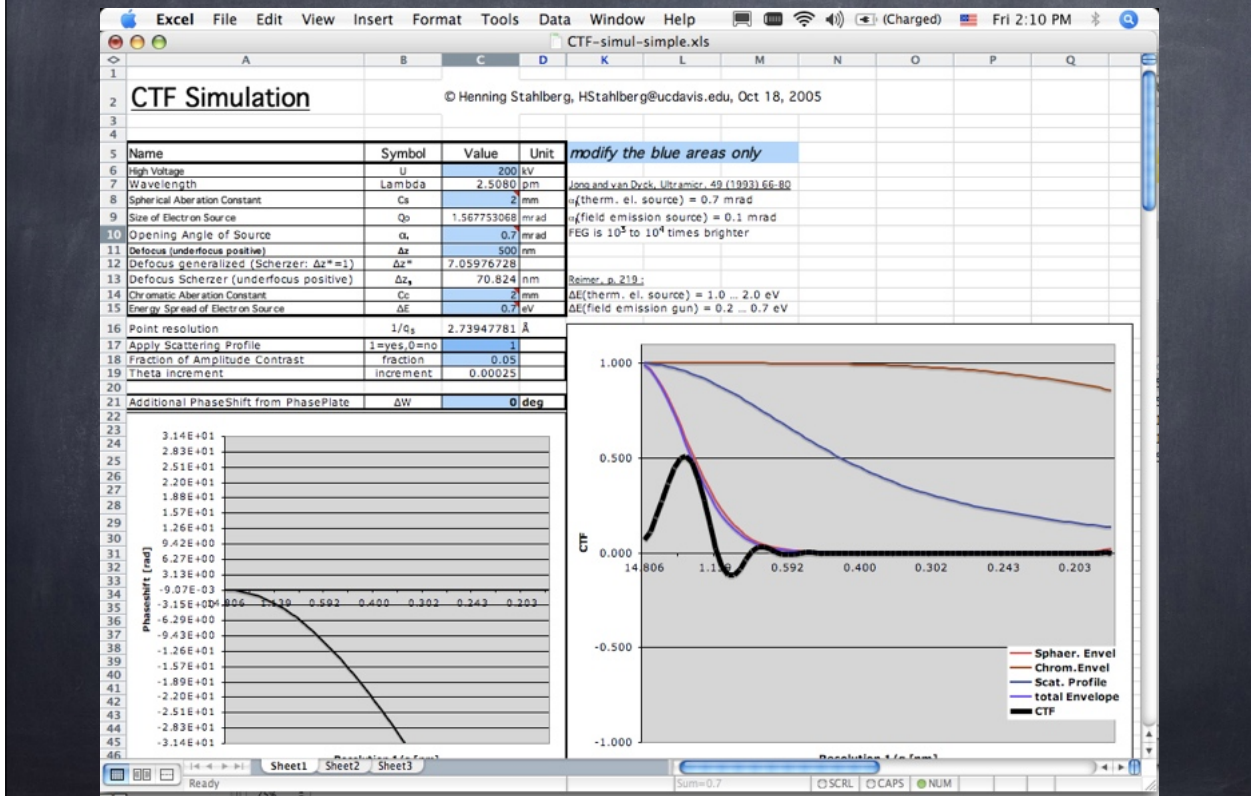


Spatial frequency dependence of the DQE for four different electron detectors in a 300 keV electron microscope: (A) current generation CCD detector, (B) Kodak SO-163 film, (C) backthinned CMOS detector, (D) a proposed detector. (R. Henderson, 2012)

CTF simulation

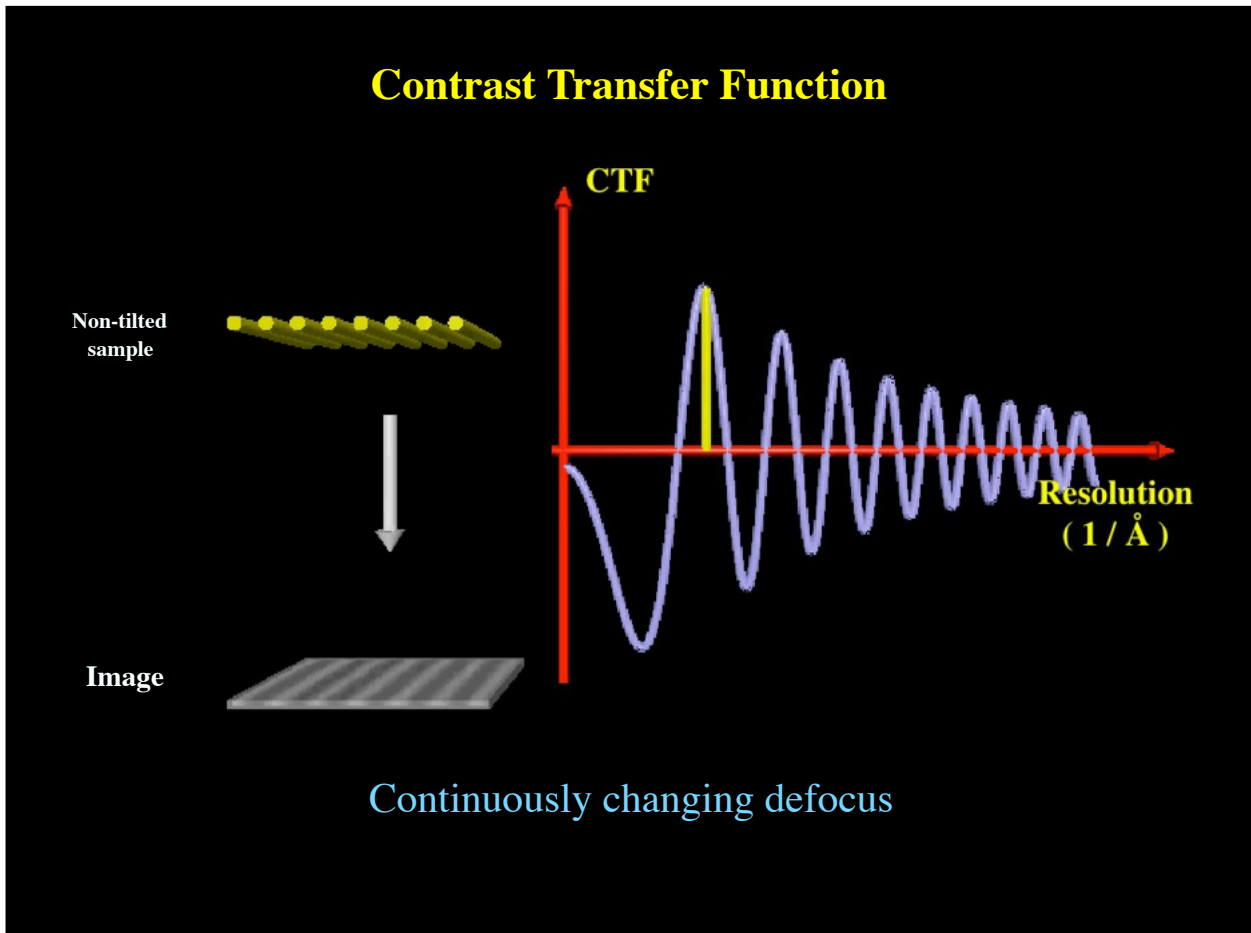


Excel sheet available on
<http://c-cina.org>



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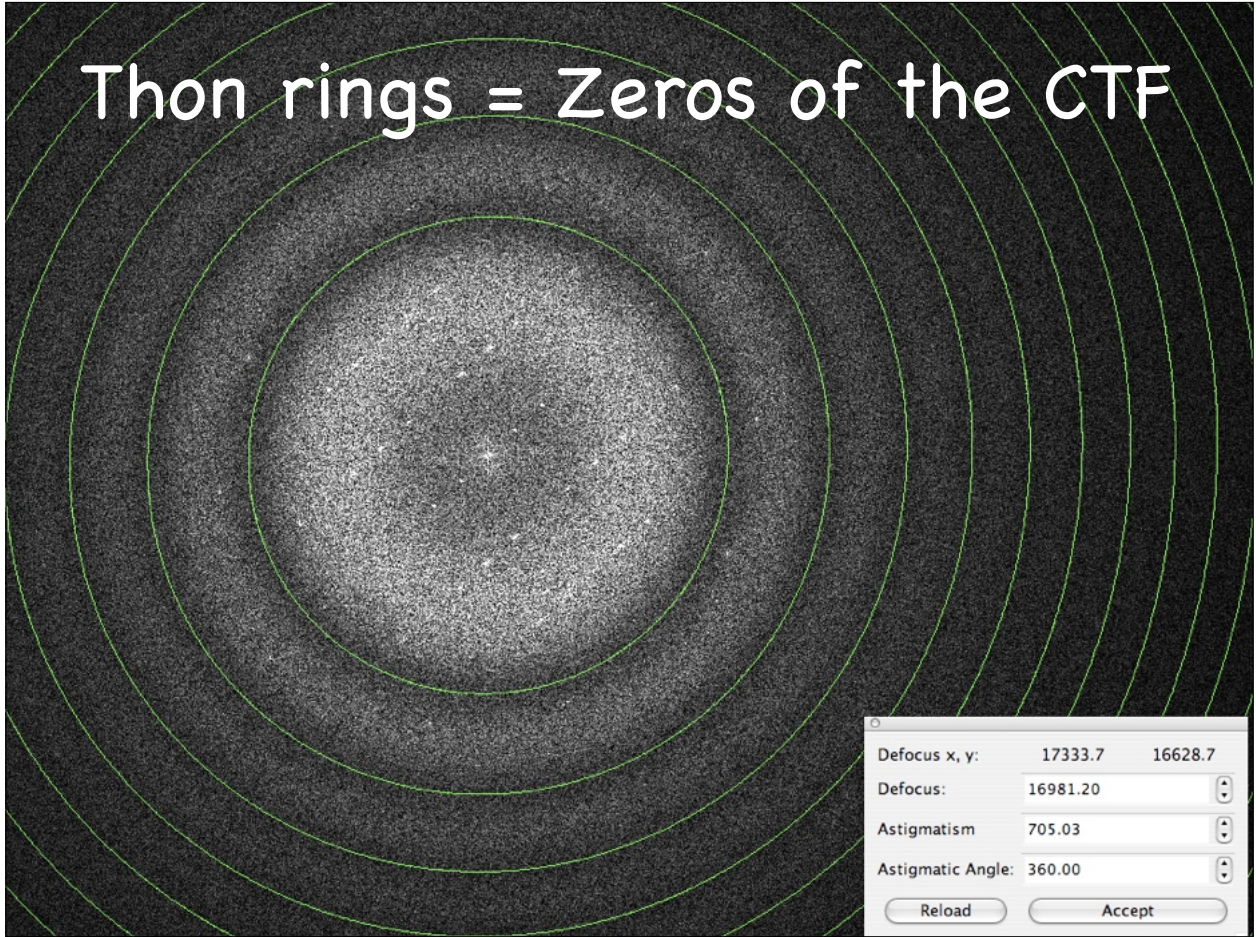
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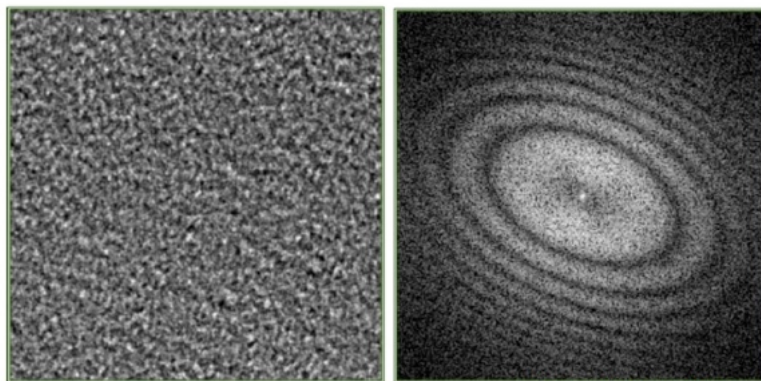
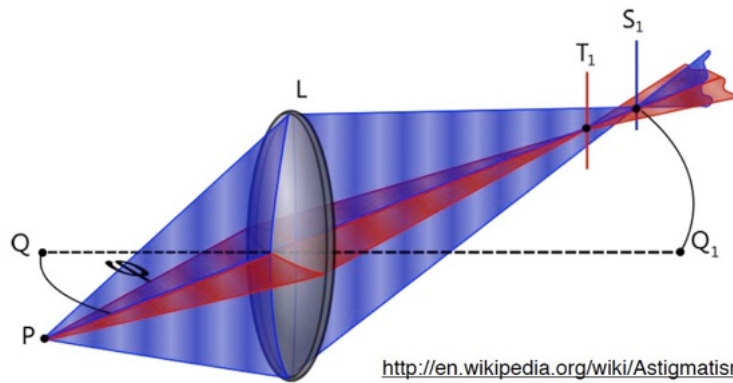
Thon rings = Zeros of the CTF



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Thon rings can reveal astigmatism



Orlova, CTF talk 2004

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CTFFIND3 determines Defocus and Astigmatism

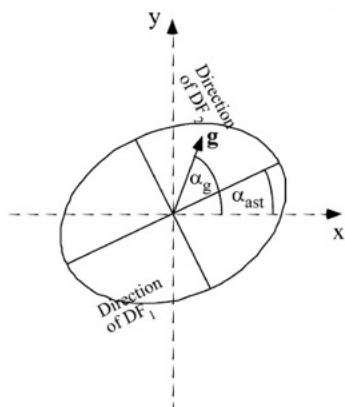
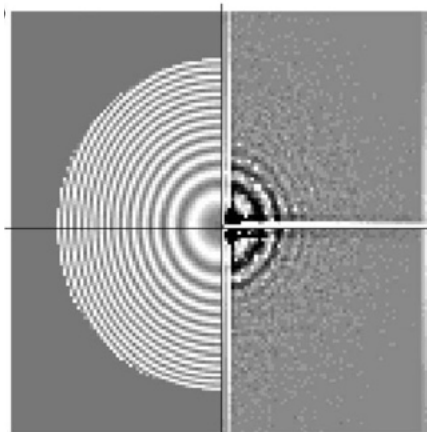


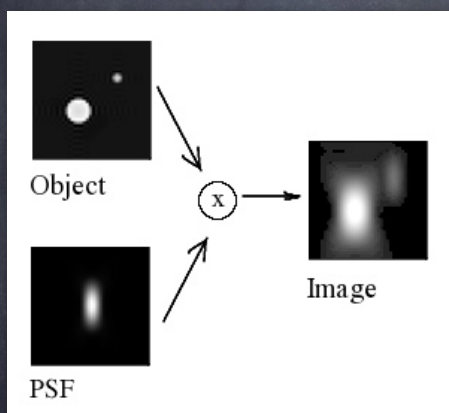
Fig. 3. Definitions for the CTF parameters DF_1 , DF_2 , and α_{ast} . The angle α_g of the scattering vector $g = k' - k$ (k , wave vector of the incident wave; k' , wave vector of the scattered wave) is used in Eq. (6), indicating the point where the CTF is evaluated.



Mindell & Grigorieff, JSB 2003

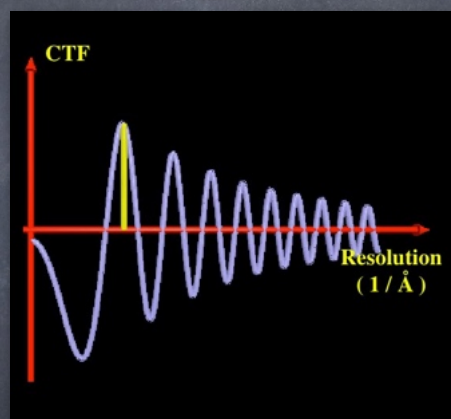
Real Space:
Point Spread Function

$$\text{Object} \otimes \text{PSF} = \text{Image}$$



Fourier Space:
Contrast Transfer Function

$$\text{FT}(\text{Object}) \cdot \text{CTF} = \text{FT}(\text{Image})$$

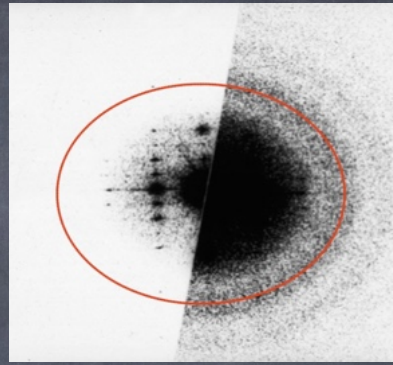


$$\text{FT}(\text{PSF}) \approx \text{CTF}$$

$$\text{PSF} \approx \text{FT}(\text{CTF})$$

What the FFT can tell us

(After David deRosier, 2006)



Spot positions	Unit cell size and shape
Spot size	Size of coherent domains
Intensity relative to background	Signal to noise ratio
Distance to farthest spot	Resolution
Amplitude and Phase of spots	Structure of molecules
Radius of Thon rings	Amount of defocus
Ellipticity of Thon rings	Amount of astigmatism
Assymmetric intensity of Thon rings	Amount of instability
Direction of assymetry	Direction of instability

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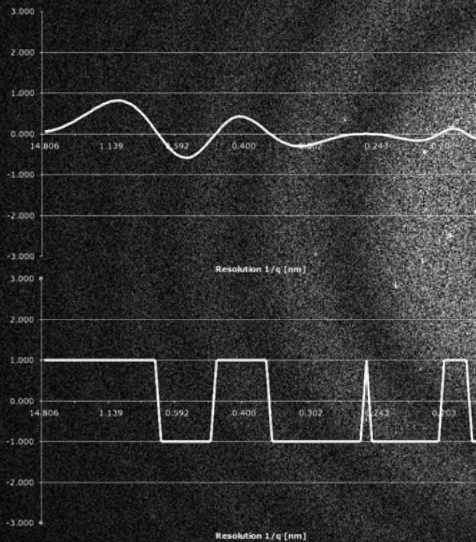
Can we correct the CTF ?

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CTF correction: Phase flipping

$$\text{NewImage} = \text{FT}^{-1} \{ \text{FT}(\text{Image}) \bullet \text{sign}(\text{CTF}) \}$$



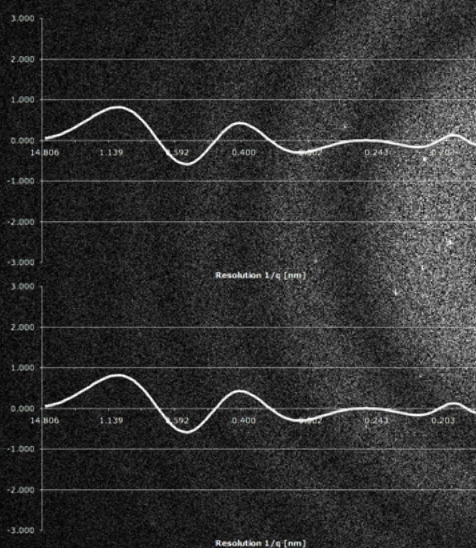
“Invert the phase behind every second Thon ring”

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CTF correction: multiply by CTF

$$\text{NewImage} = \text{FT}^{-1} \{ \text{FT}(\text{Image}) \bullet \text{CTF} \}$$

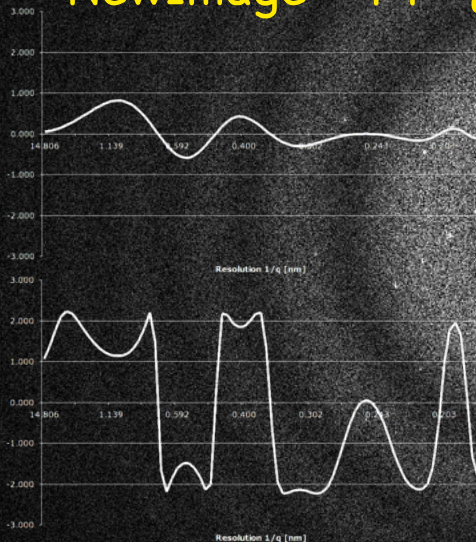


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CTF correction: Wiener Filter

$$\text{NewImage} = \text{FT}^{-1} \left\{ \text{FT}(\text{Image}) \bullet \frac{\text{CTF}}{\text{CTF}^2 + N^2} \right\}$$



“Divide by the CTF (sort of...)”

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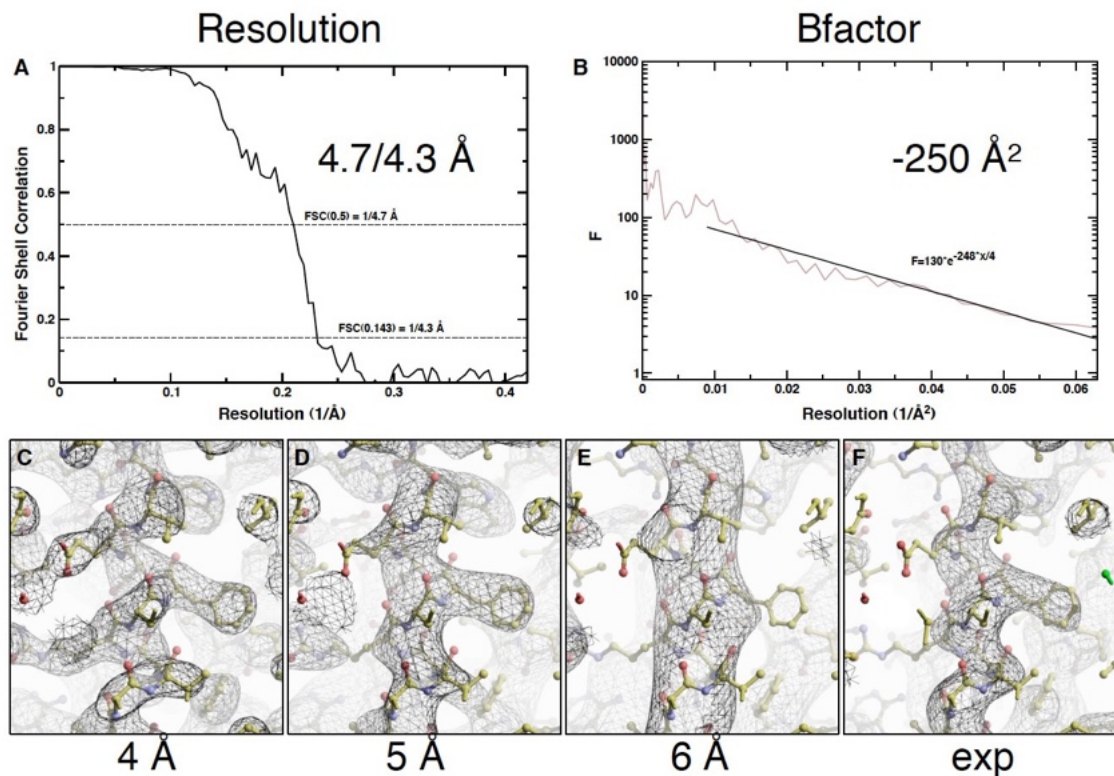
Methods of CTF Correction

- Phase flipping
- CTF multiplication
- Wiener filtering
(Böttcher et al. 1997, Penczek et al., 1997)
- CTF multiplication of 2D image, and Wiener filtration of 3D volume
(Grigorieff 1998, Sachse et al. 2007)
- In 2dx currently most successful:
Phase flipping before unbending.
Amplitude correction is taken over by PCO.

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B-factor correction at high resolution



Carsten Sachse, EMBL Talk, 2008

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Defocus varies on an image of a tilted sample

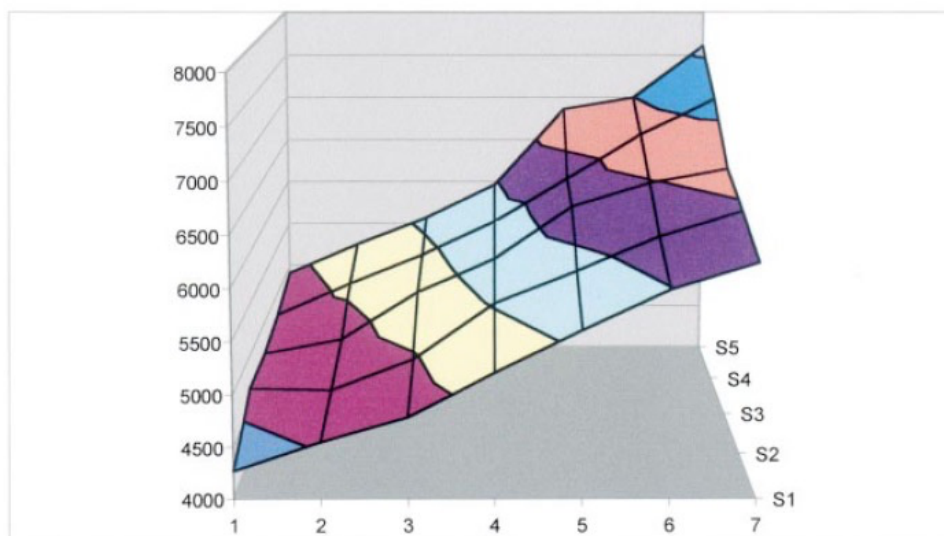


Fig. 6. Position dependent PhCTF determination. This graph illustrates a typical nominal '0°' tilt on our CM200 cryo-EM/Gatan cryo-holder system. The system exhibits a systematic 6° tilt with respect to the nominal tilt angles that, if not corrected for, causes a defocus spread of almost 3000 Å. After our diagnostic analysis, the holder is now systematically used at a nominal -6° tilt to compensate for this effect. However, the defocus difference between front and back of the plot of ~ 600 Å – perpendicular to the tilt axis of the goniometer – is not correctable with the current set up. Moreover, due to a recent repair of this particular holder, the nominal '0°' tilt position requires recalibration.

van Heel et al., Quart. Rev. Biophys. 2000

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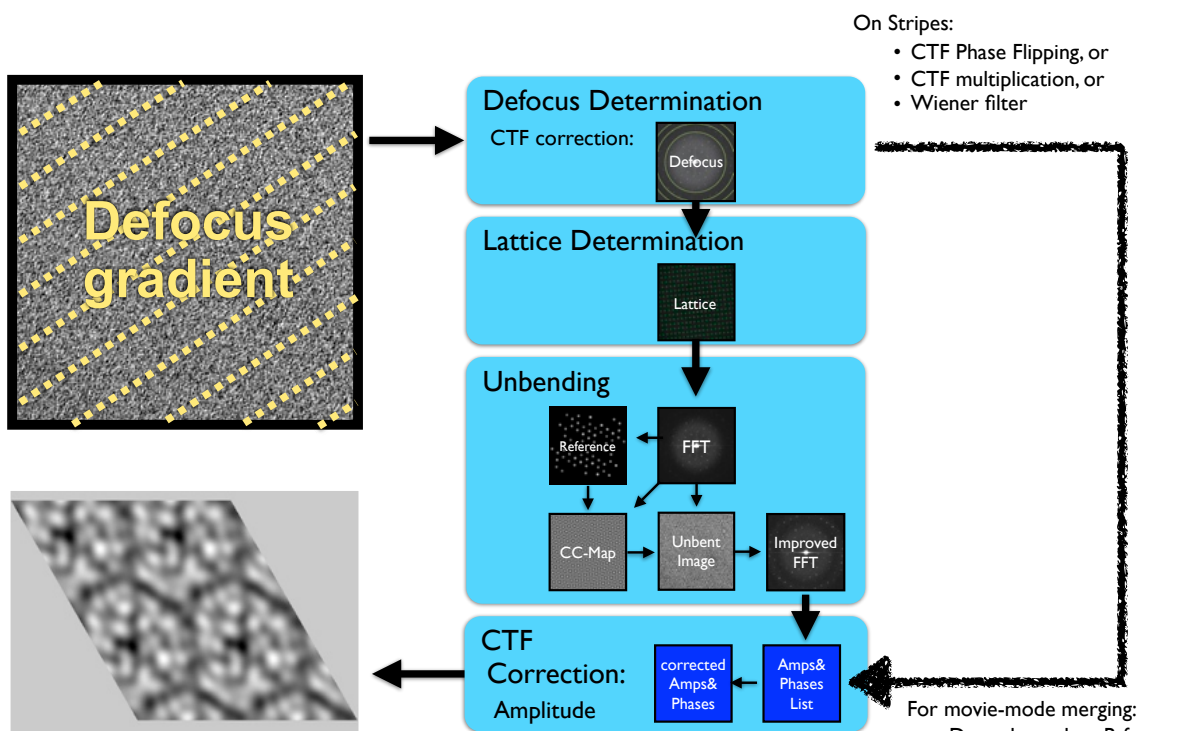
In 2dx, we apply CTFFIND3 in 7x7 locations to measure the tilt geometry



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In 2dx, we correct the CTF before any further image processing by Phase flipping in stripes



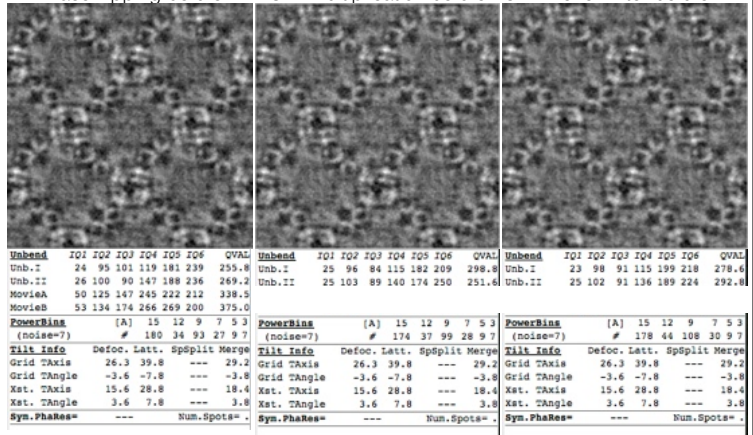
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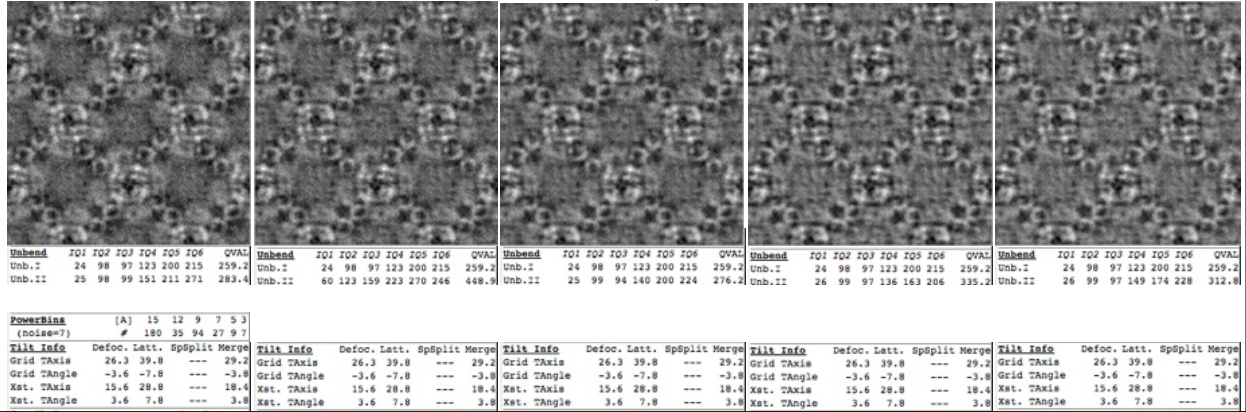
(Almost) Non-Tilted 2D Crystal

Image: 2014-05-26_14-33-45_0020025100
 Tilt axis: 29.2°
 Tilt angle: -3.8°
 Defocus: 9462.4, 10.179.7, 81.92°

1 Phase flipping before 2 CTF multiplication before 3 Wiener Filter before



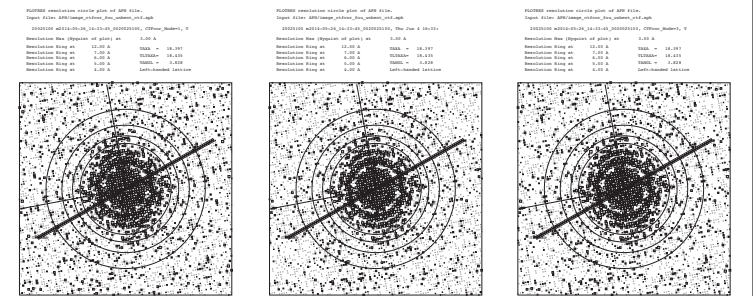
0 CTF correction after 4 TTF correction after 5 Phase flipping after 6 CTF multiplication after 7 Wiener Filter after



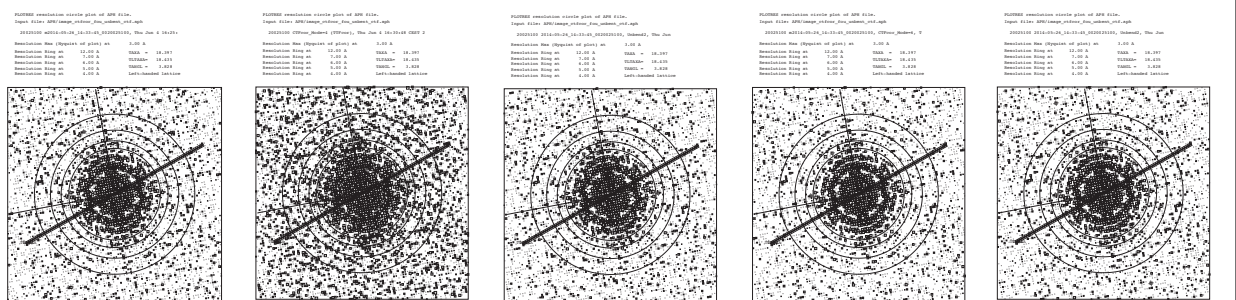
(Almost) Non-Tilted 2D Crystal

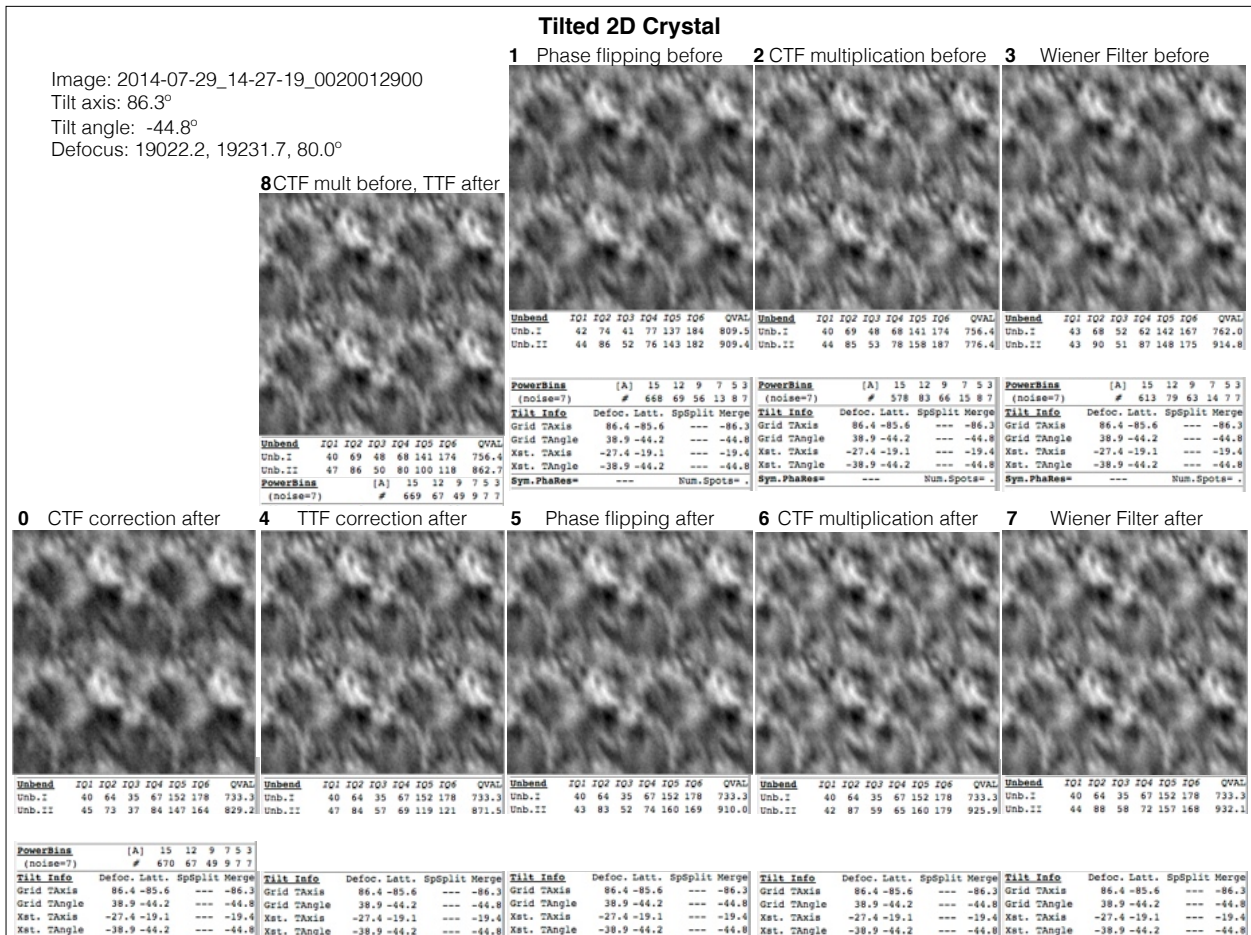
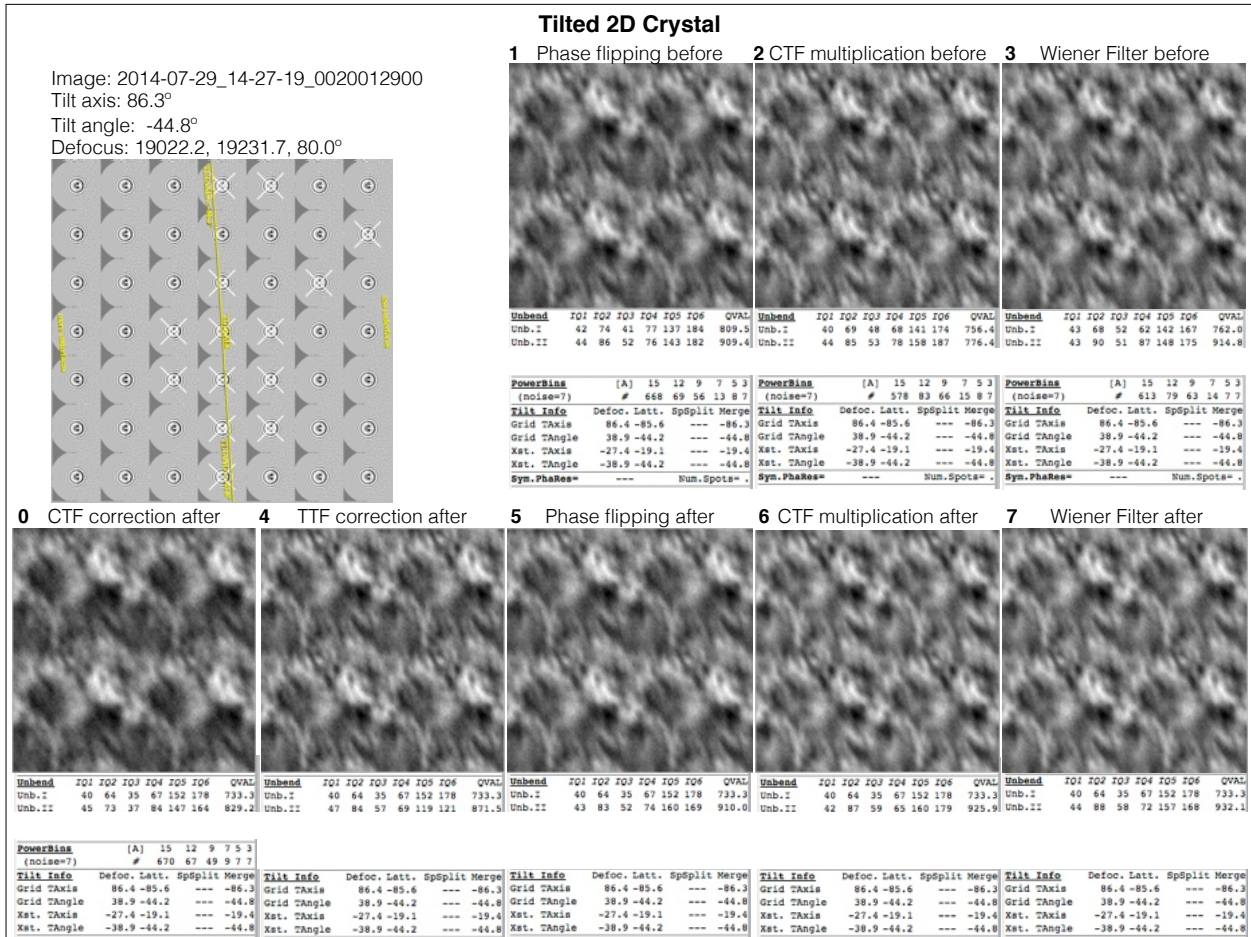
Image: 2014-05-26_14-33-45_0020025100
 Tilt axis: 29.2°
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 Defocus: 9462.4, 10.179.7, 81.92°

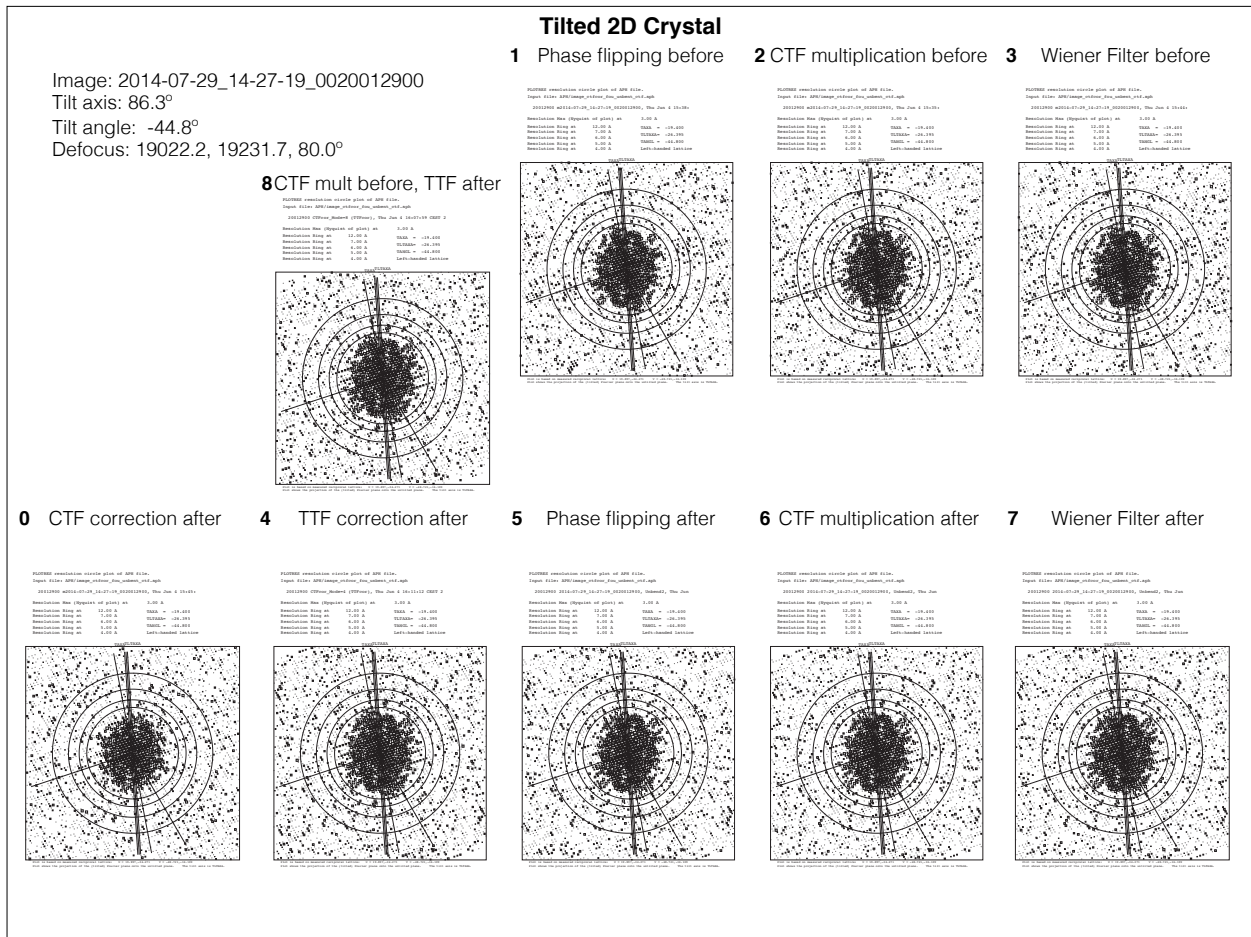
1 Phase flipping before 2 CTF multiplication before 3 Wiener Filter before



0 CTF correction after 4 TTF correction after 5 Phase flipping after 6 CTF multiplication after 7 Wiener Filter after







Conclusions CTF

- The CTF defines the transfer of contrast from the sample onto the image.
- The PSF defines the impact on the image from a point in the sample. $PSF = FFT(CTF)$.
- CTF needs to be fitted and corrected.
- CTF for tilted samples can be done in various ways. In 2dx, recommendation is Phase Flipping before unbending.