



CEITEC

Central European Institute of Technology  
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# *Electron optics*

*C9940 3-Dimensional Transmission Electron Microscopy*  
*S1007 Doing structural biology with the electron microscope*

**February 23, 2015**



EUROPEAN UNION  
EUROPEAN REGIONAL DEVELOPMENT FUND  
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**OP Research and  
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*Correction/clarification from last week*

# Correction/clarification

First Siemens microscope, 1939



<http://ernst.ruska.de>

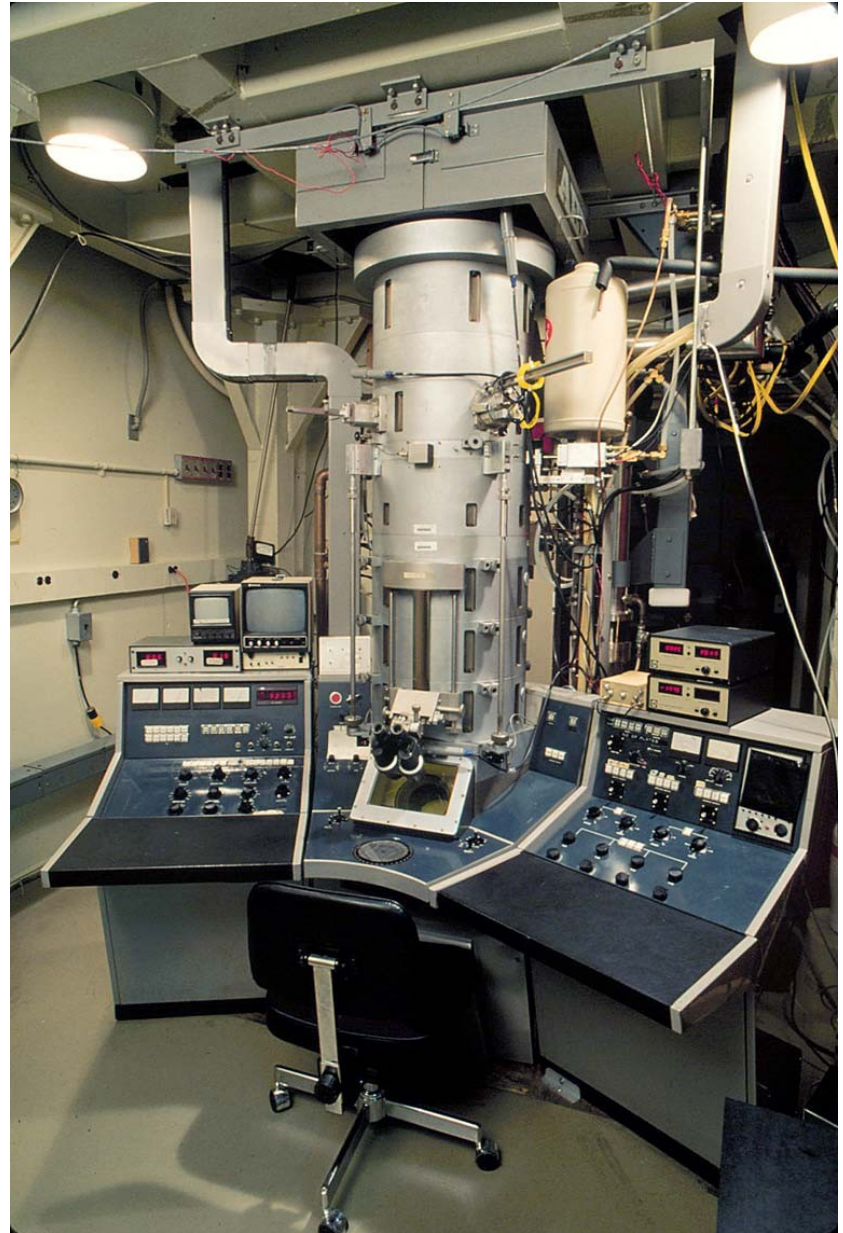
First **commercial** EM, 1937  
Metropolitan-Vickers EM1  
(EM2 shown)



<http://emu.msim.org.uk>

The first commercial electron microscope was actually by the British company Metropolitan-Vickers in 1937. However, the magnification was worse than for the light microscope, so the Siemens is considered “first.”

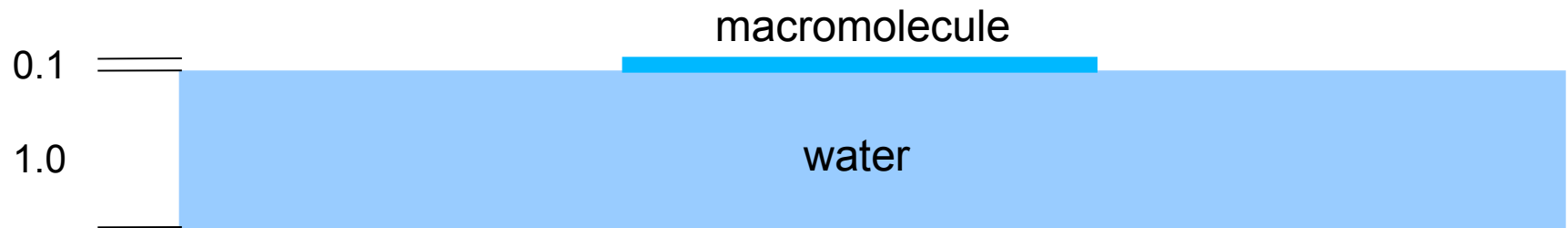
Metropolitan Vickers eventually became AEI, which built a 1.2 million volt EM-7.



<http://www.wadsworth.org>

# *Contrast transfer function*

# Why do we defocus?

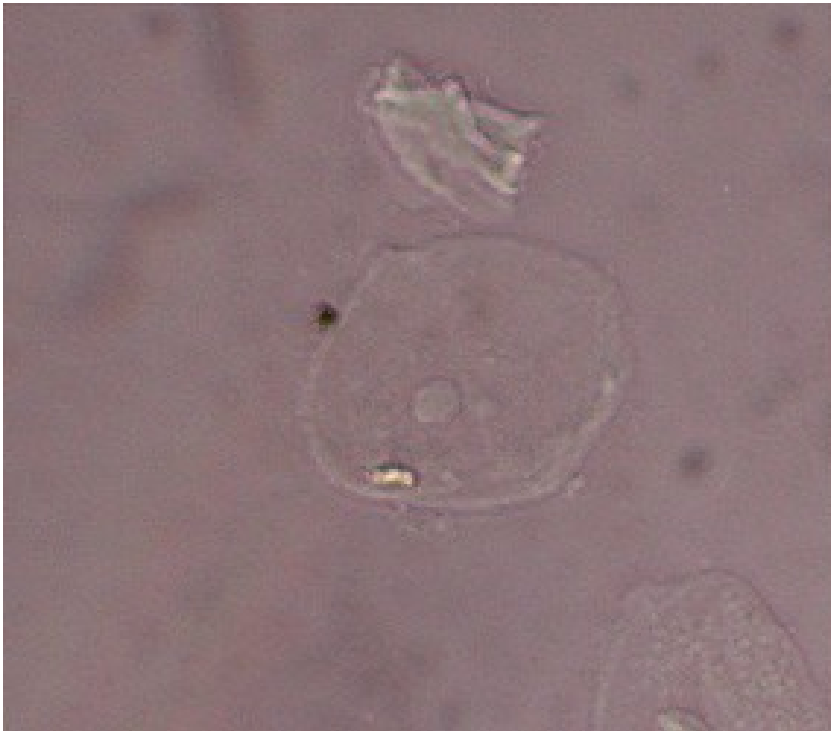


Typical amplitude contrast is estimated a 0.08-0.12  
(minus noise)

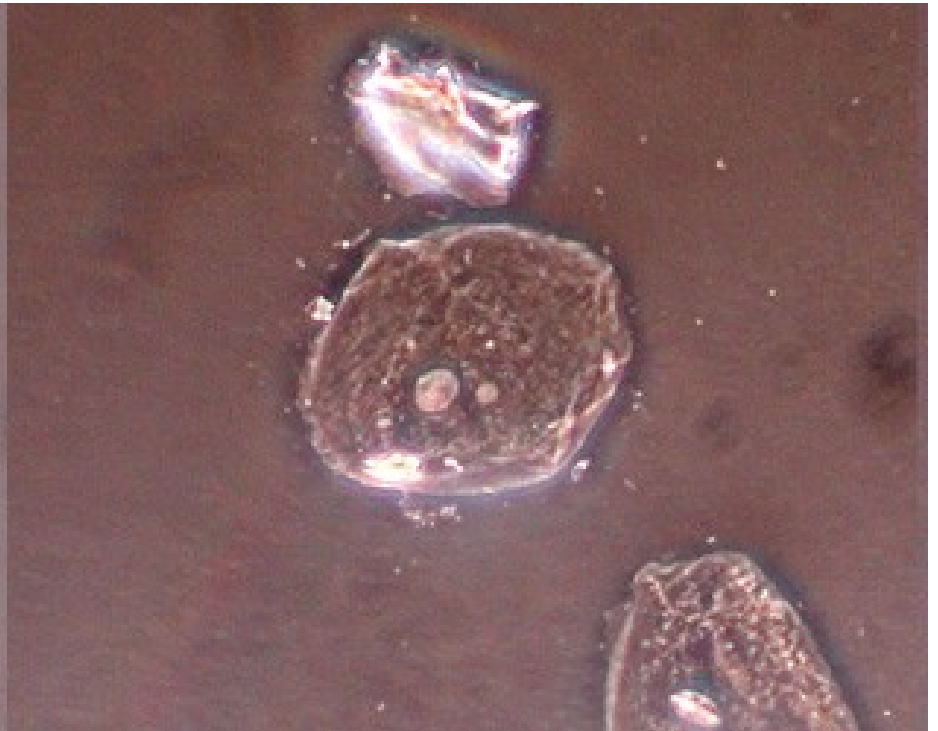
*Instead of amplitude contrast,  
we'll use phase contrast.*

# Phase contrast in light microscopy

Bright-field image



Phase-contrast image

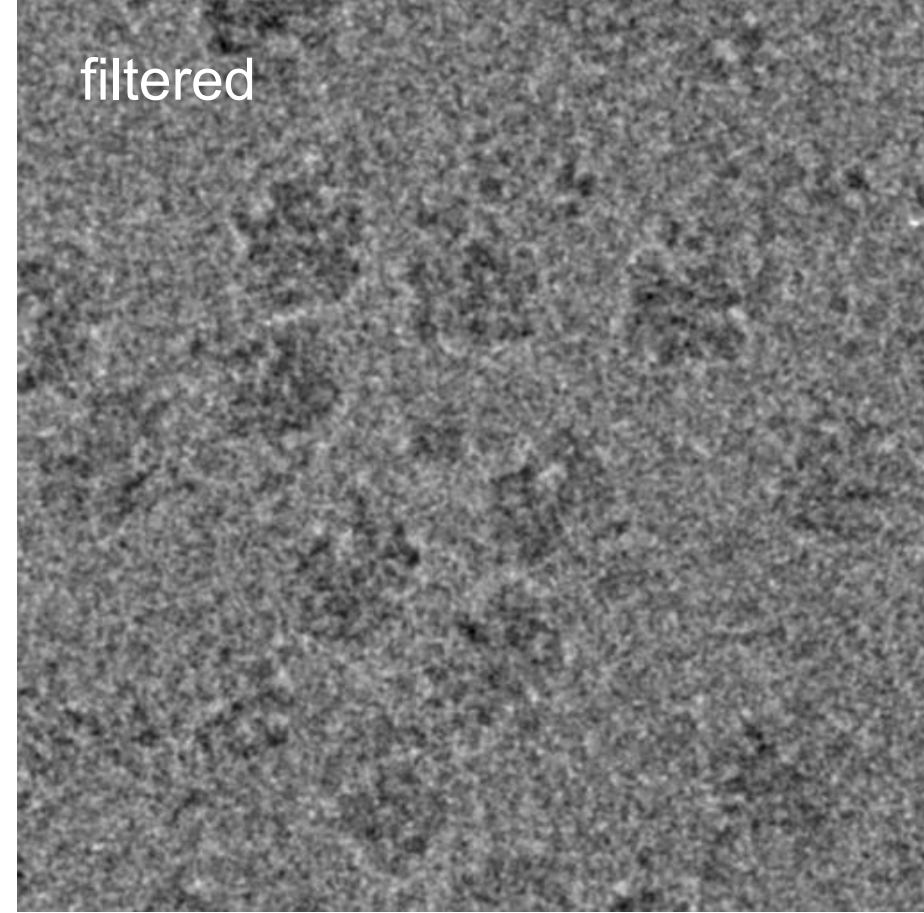
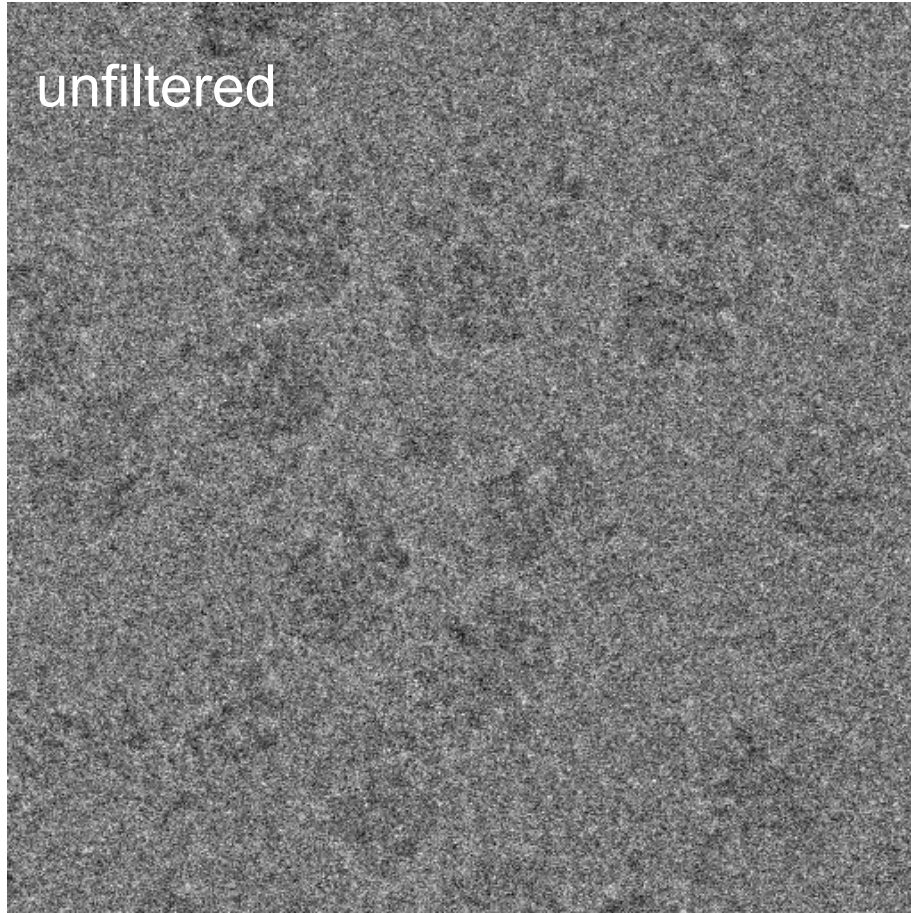


<http://www.microbehunter.com>



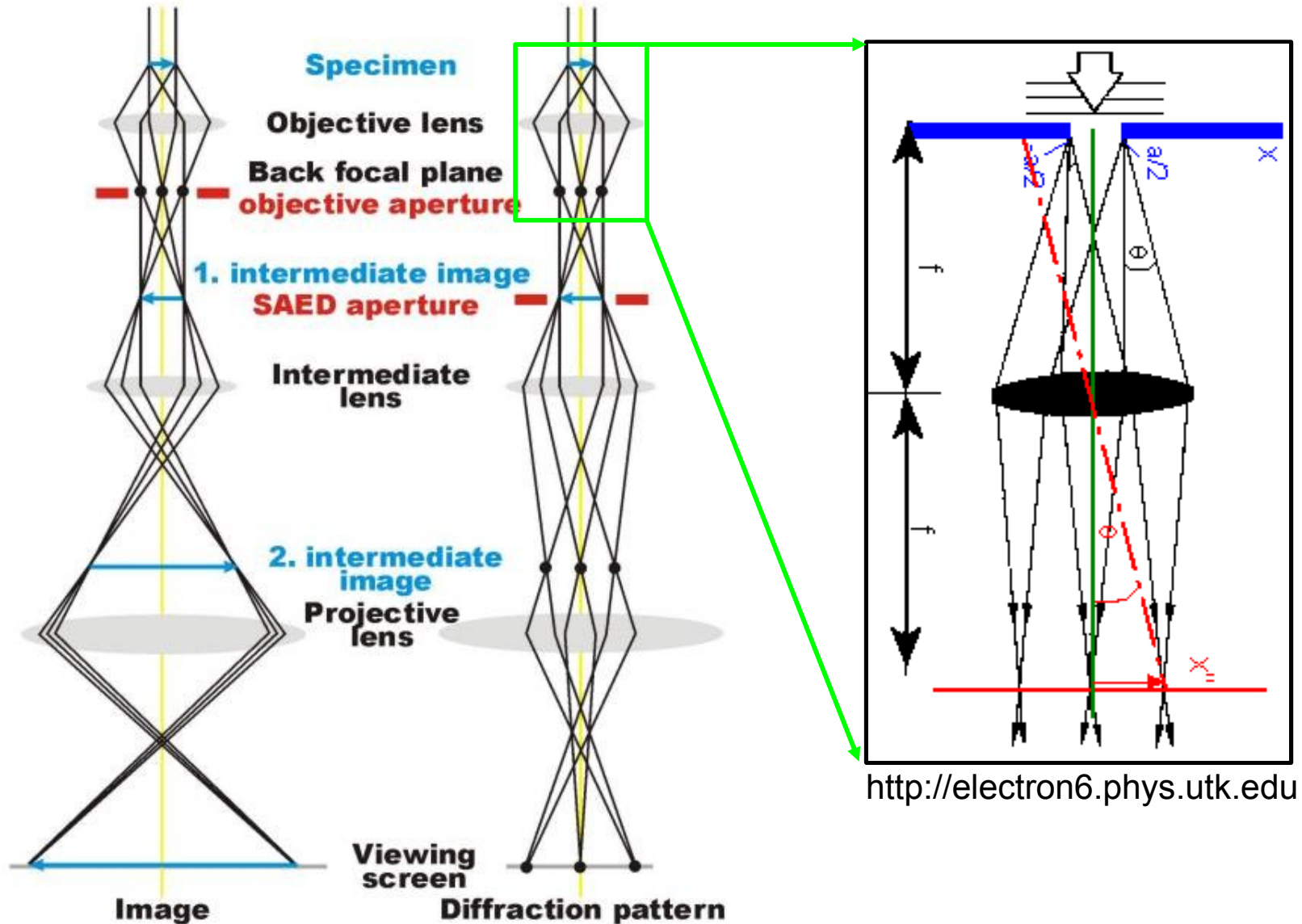
In EM, even with defocus, the contrast is poor.

*E. coli* 70S ribosomes, field width  $\sim 1440\text{\AA}$ .

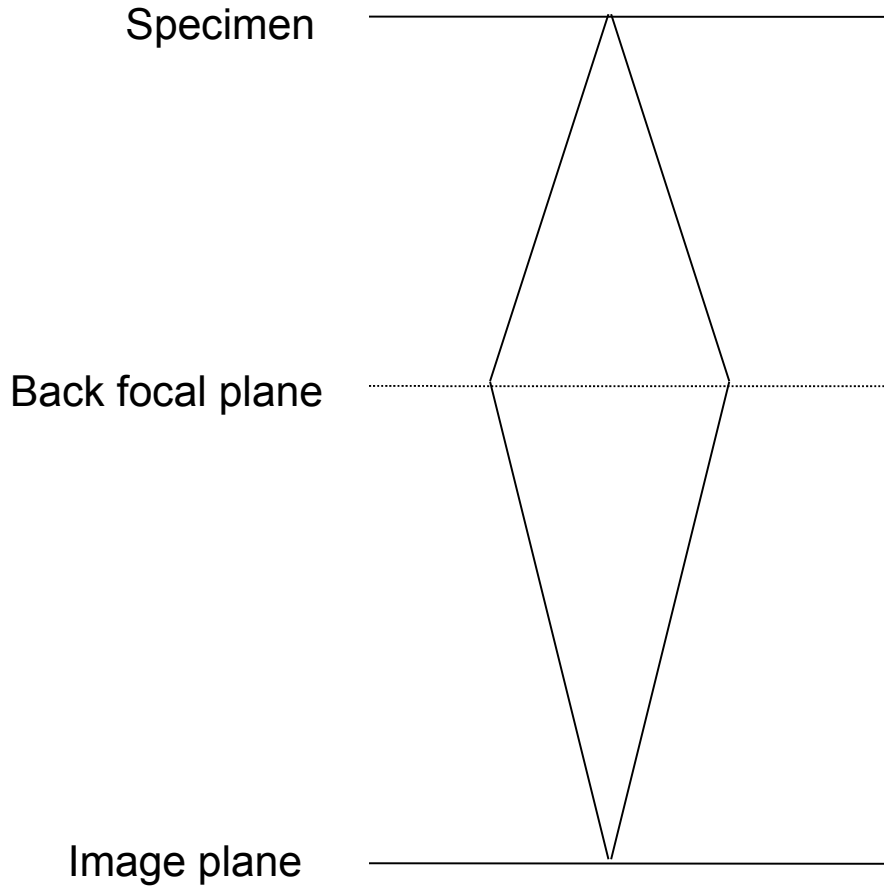


Signal-to-noise ratio for cryoEM typically given to be between 0.07 and 0.10.

# Relationship between imaging and diffraction

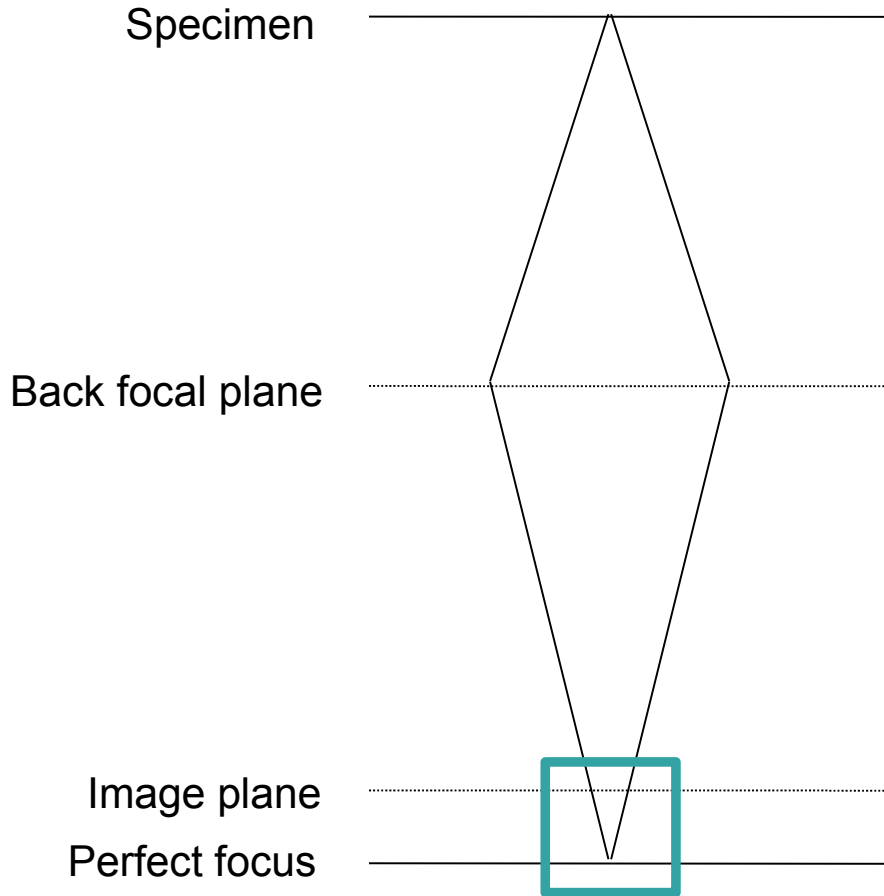


# Optical path

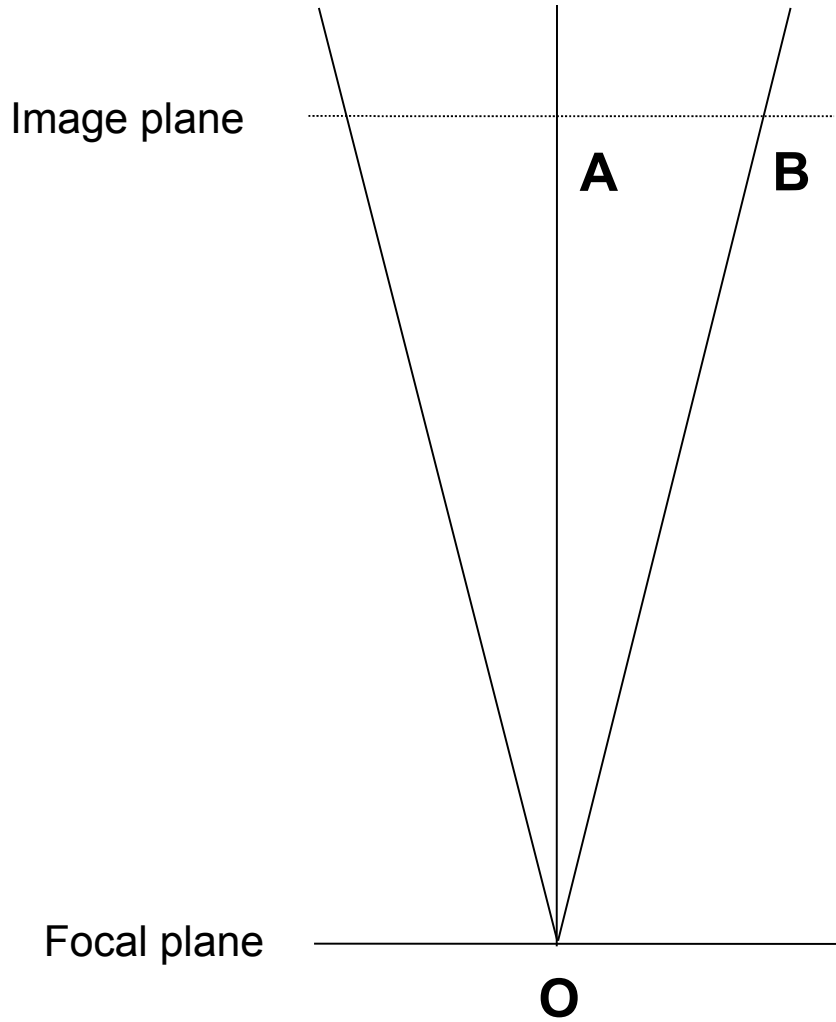


At focus, all we would see is amplitude contrast.

# Optical path with defocus



# Optical path with defocus



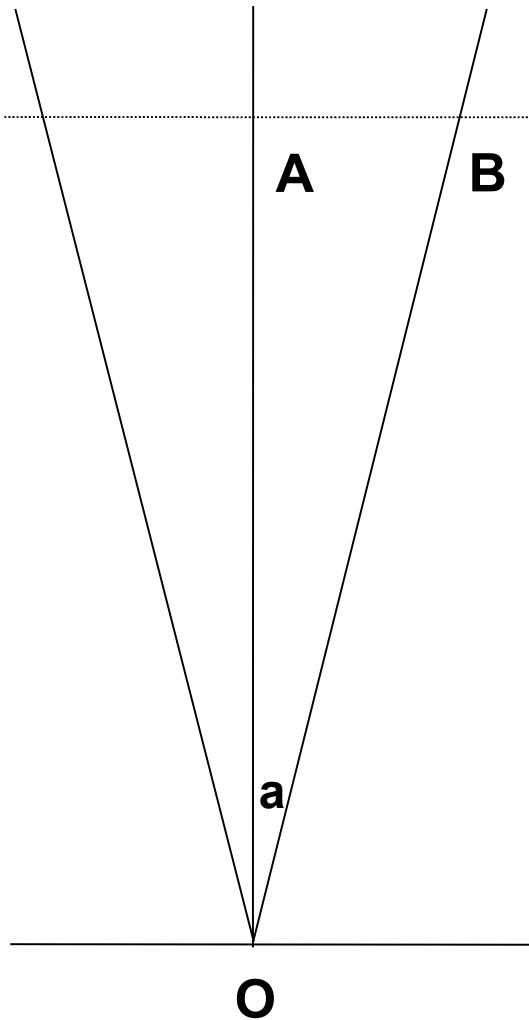
**OA** path of unscattered beam

**OB** path of scattered beam

The length **OA** is also the amount of defocus  $\Delta f$

What is the path difference between the scattered and unscattered beams?

# Path difference as a function of $\Delta f$



$$OB - OA$$

$$OB = OA / \cos(a)$$

$$\frac{OA}{\cos(a)} - OA$$

$$OA \times \left( \frac{1}{\cos(a)} - 1 \right)$$

Expressed in the number of wavelengths  $\lambda$

$$OA \times \left( \frac{\frac{1}{\cos(a)} - 1}{\lambda} \right)$$

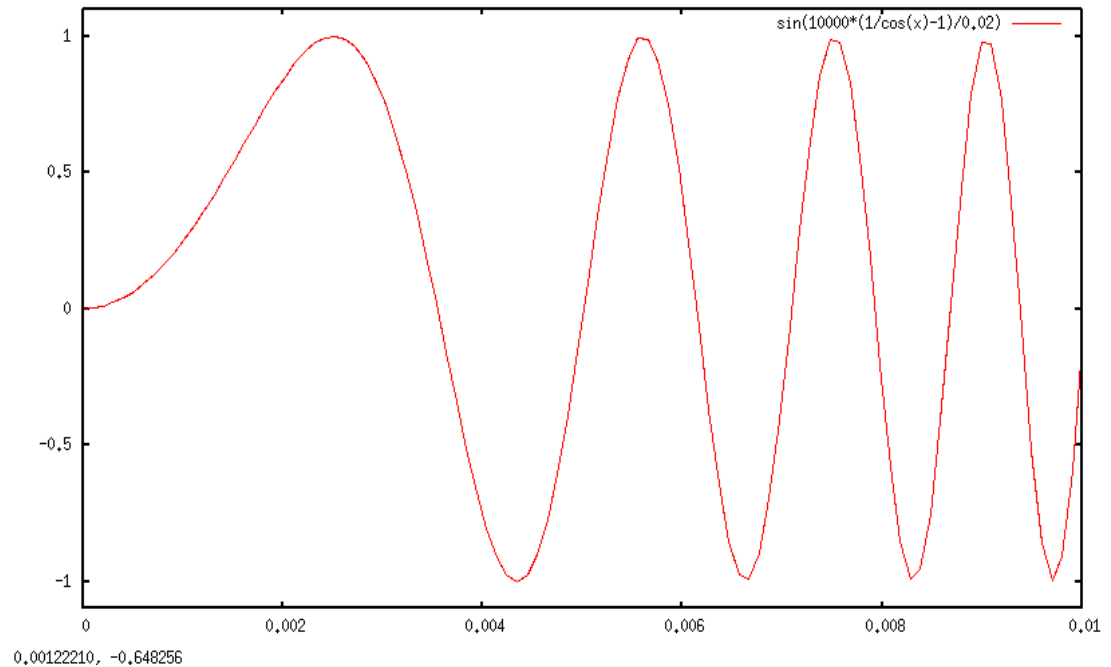
Phase difference is the sine

$$\sin \left( \frac{OA \times \left( \frac{1}{\cos(a)} - 1 \right)}{\lambda} \right)$$

# Some typical values

$$\sin\left(\frac{OA \times \left(\frac{1}{\cos(a)} - 1\right)}{\lambda}\right)$$

$$\begin{aligned} OA &= \Delta f = 10,000 \text{ \AA} \\ \lambda &= 0.02 \text{ \AA} \\ a &< 0.01 \end{aligned}$$



A more precise formulation of the CTF can be found in  
Erickson & Klug A (1970). Philosophical Transactions of the Royal Society B. 261:105.

# Proper form the CTF

$$-\sin\left(\frac{\pi}{2}C_s k^4 + \pi\Delta f\lambda k^2\right)$$

where:

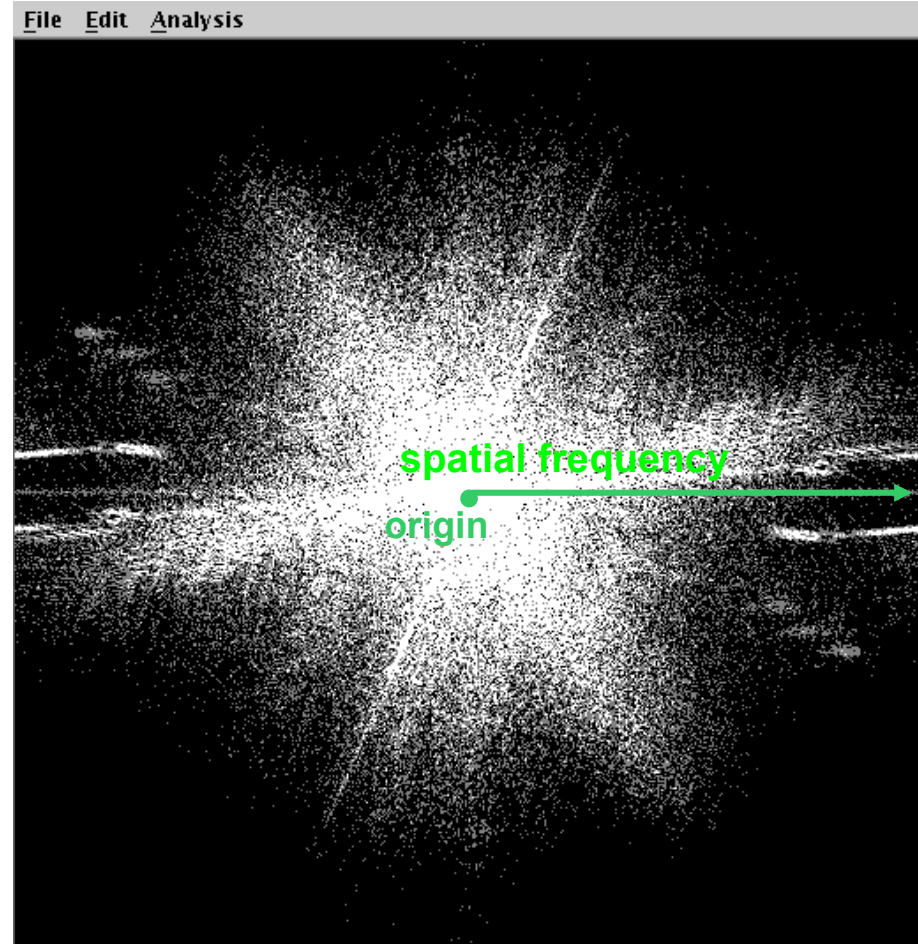
- ◆  $C_s$ : spherical aberration
- ◆  $k$ : spatial frequency (resolution)

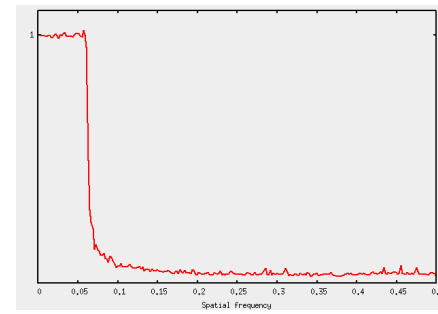
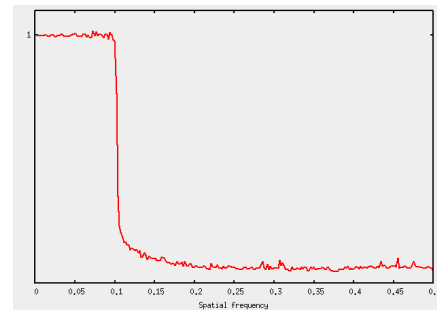
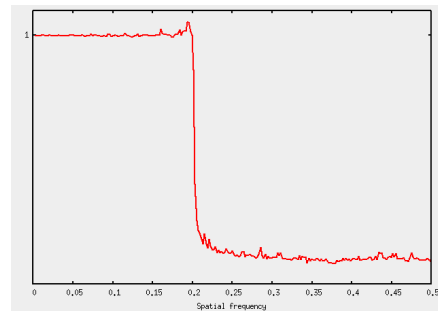
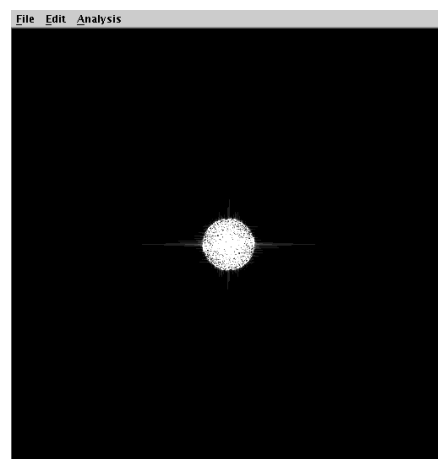
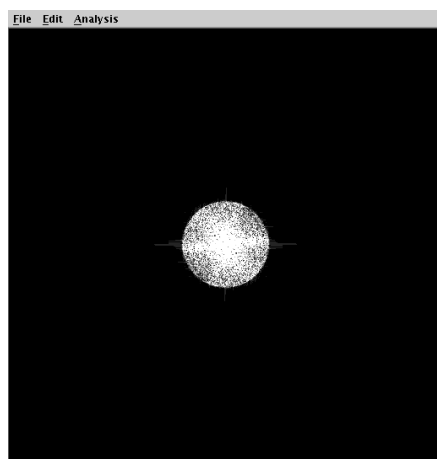
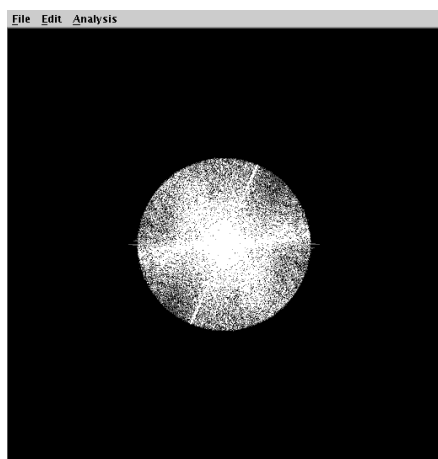
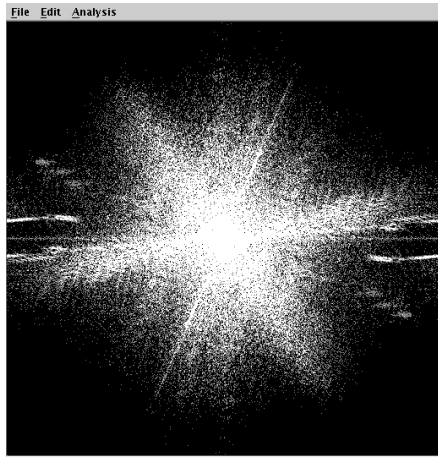


*What do we mean by spatial frequency?*



From Wikipedia

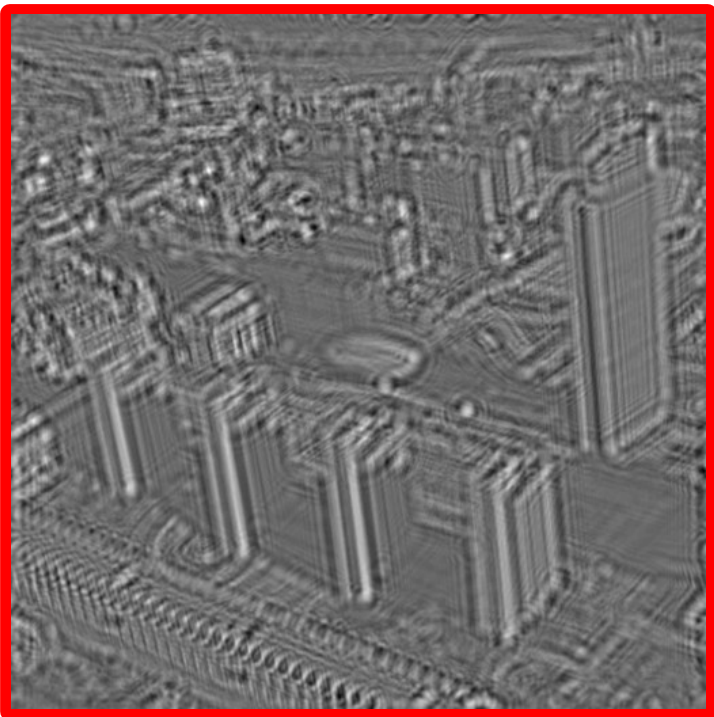
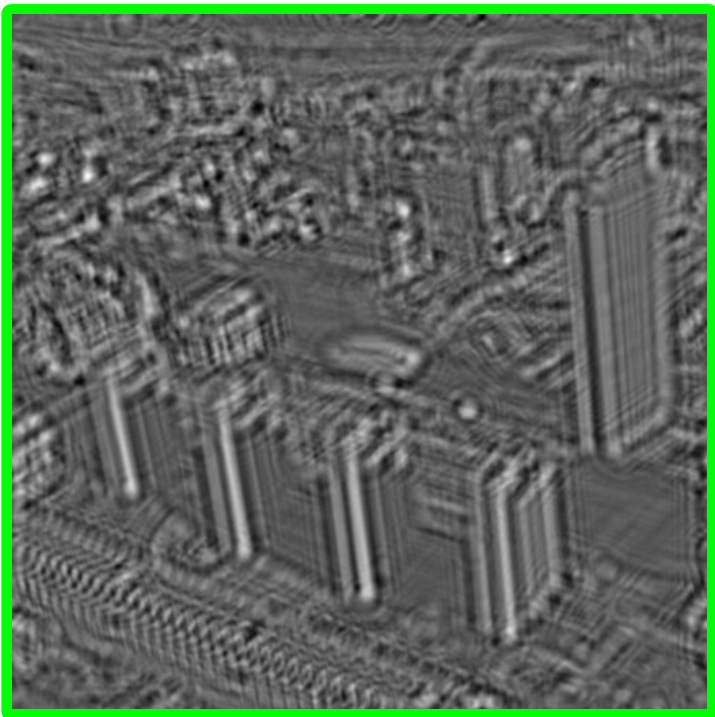
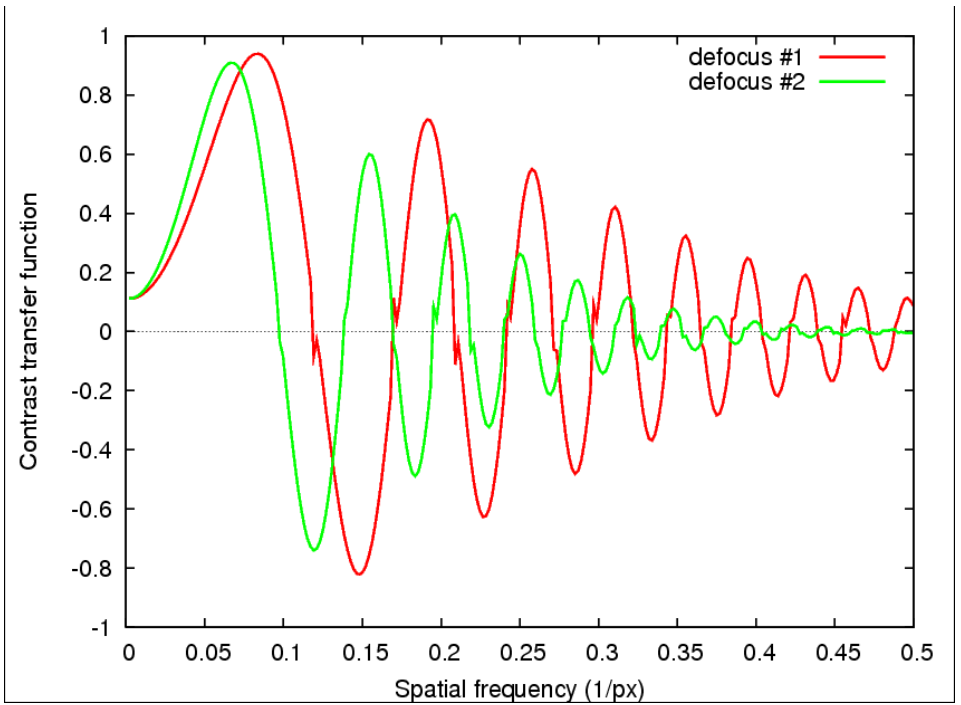


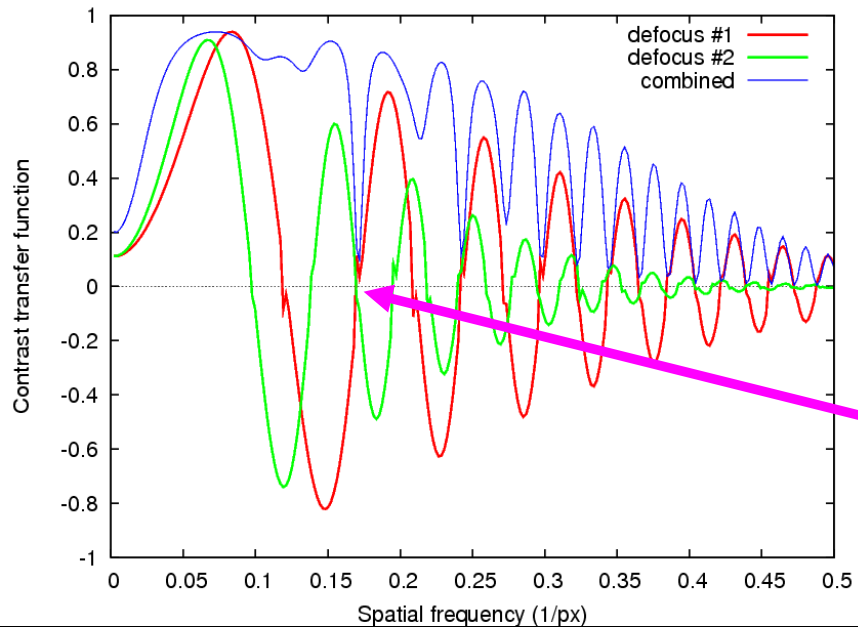


diff α Pmrt ceprs re wo P

*How does the CTF affect an image?*

original





Still a zero present

combined



original



# Thank you for your attention



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