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Image analysis I

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

March 2, 2015

EUROPEAN UNION
EUROPEAN REGIONAL DEVELOPMENT FUND
INVESTING IN YOUR FUTURE

2007–13
OP Research and
Development for Innovation

Syllabus, original

Week	Date	Instructor	Topic
1	02/16	D. Nemecek & T. Shaikh	Introduction/Tour/History
2	02/23	D. Nemecek & T. Shaikh	Electron Optics
3	03/02	D. Nemecek	Specimen preparation
4	03/09	T. Shaikh	Image analysis I
5	03/16	T. Shaikh	Image analysis II
6	03/23	T. Shaikh	3D reconstruction
7	03/30	T. Shaikh	Single-particle reconstruction
			(Easter)
8	04/13	D. Nemecek	Tomography I
9	04/20	D. Nemecek	Tomography II
10	04/27	D. Nemecek	Visualization/Segmentation
11	05/04	D. Nemecek	Hybrid methods
12	05/11	T. Shaikh	Computer practicals
13	05/18	D. Nemecek & T. Shaikh	Journal club

Syllabus, updated

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Correction/clarification from Feb. 16

Correction/clarification

First Siemens microscope, 1939

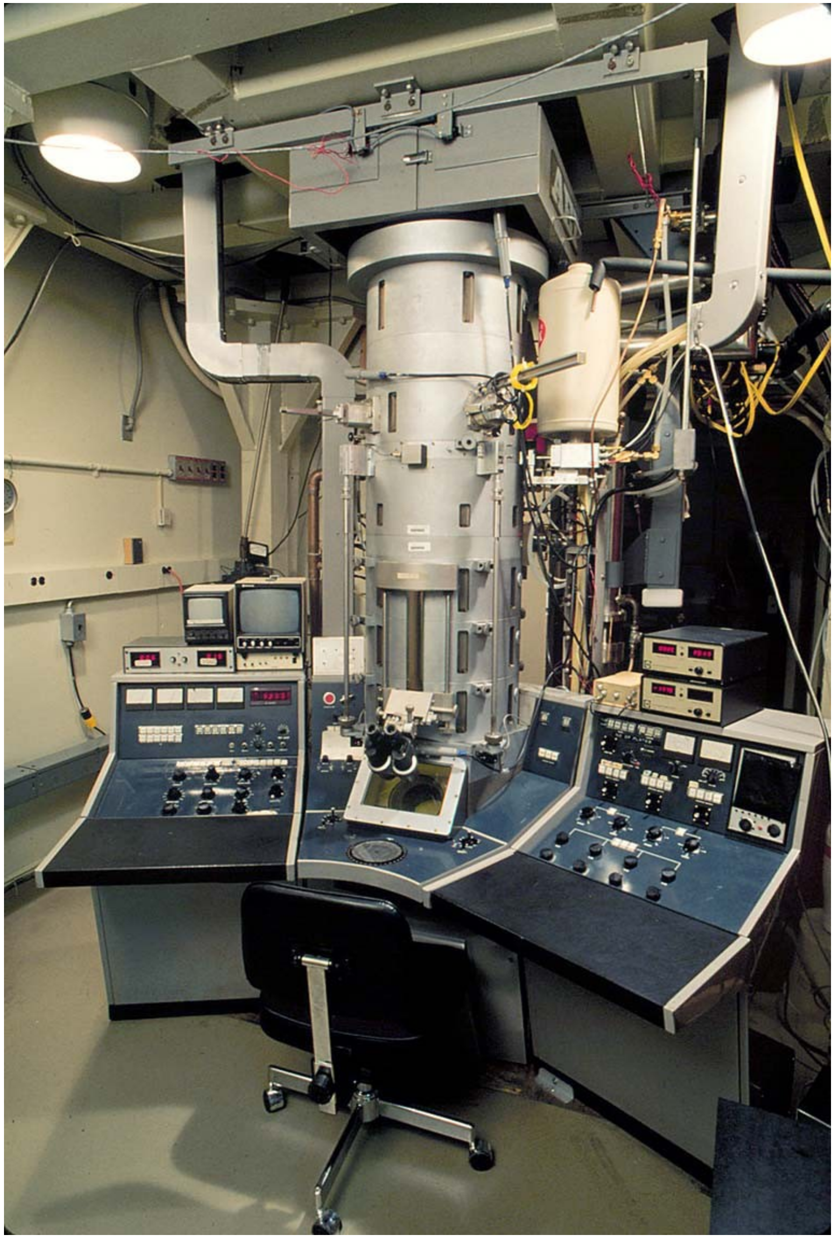
http://ernst.ruska.de

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

Outline

Correction/clarification form Feb. 16

Intro to image analysis

- Relationship between imaging and diffraction
- Fourier transforms
    - Theory
    - Examples in 1D
    - Examples in 2D
- Fourier filtration
- Contrast transfer function
- Resolution

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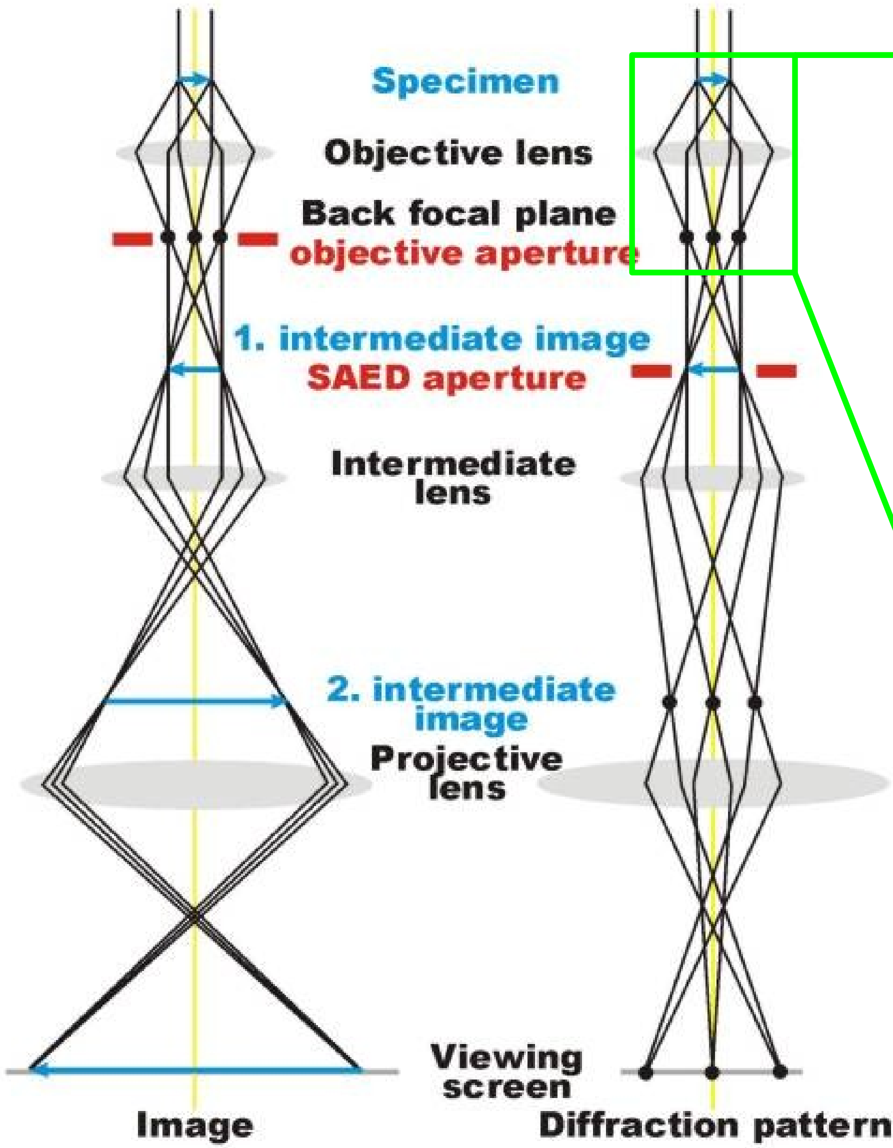
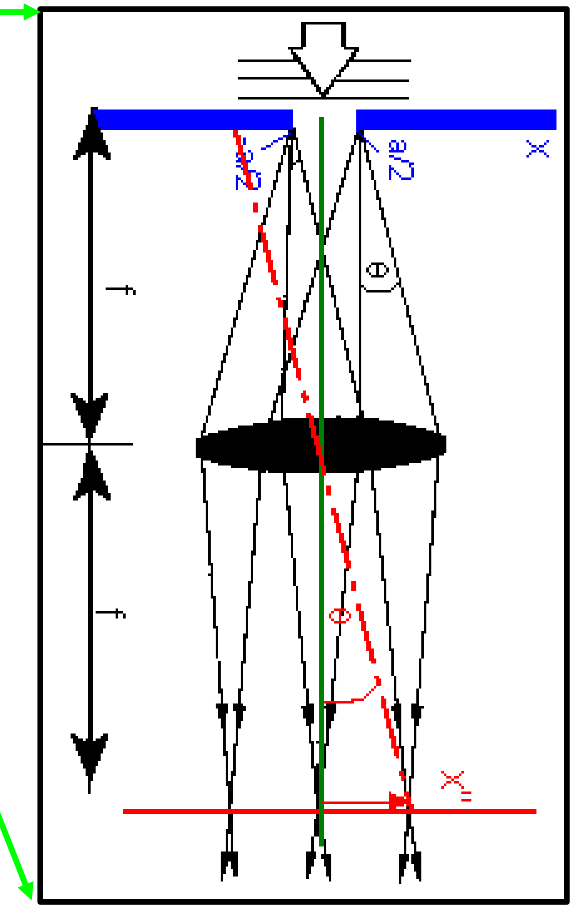
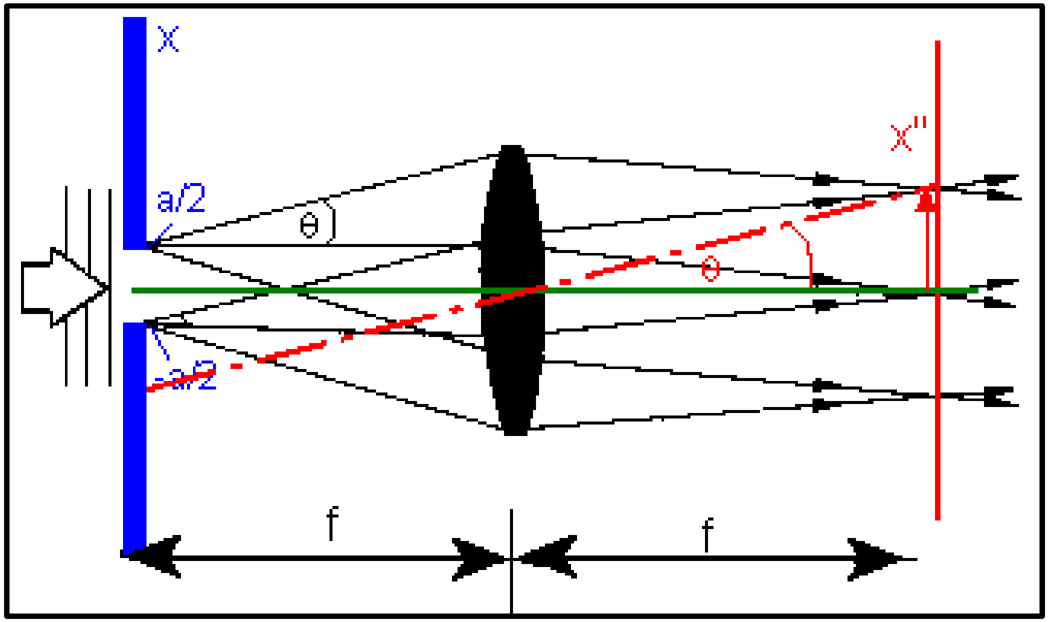
Relationship between imaging and diffraction



http://www.microscopy.ethz.ch



http://electron6.phys.utk.edu



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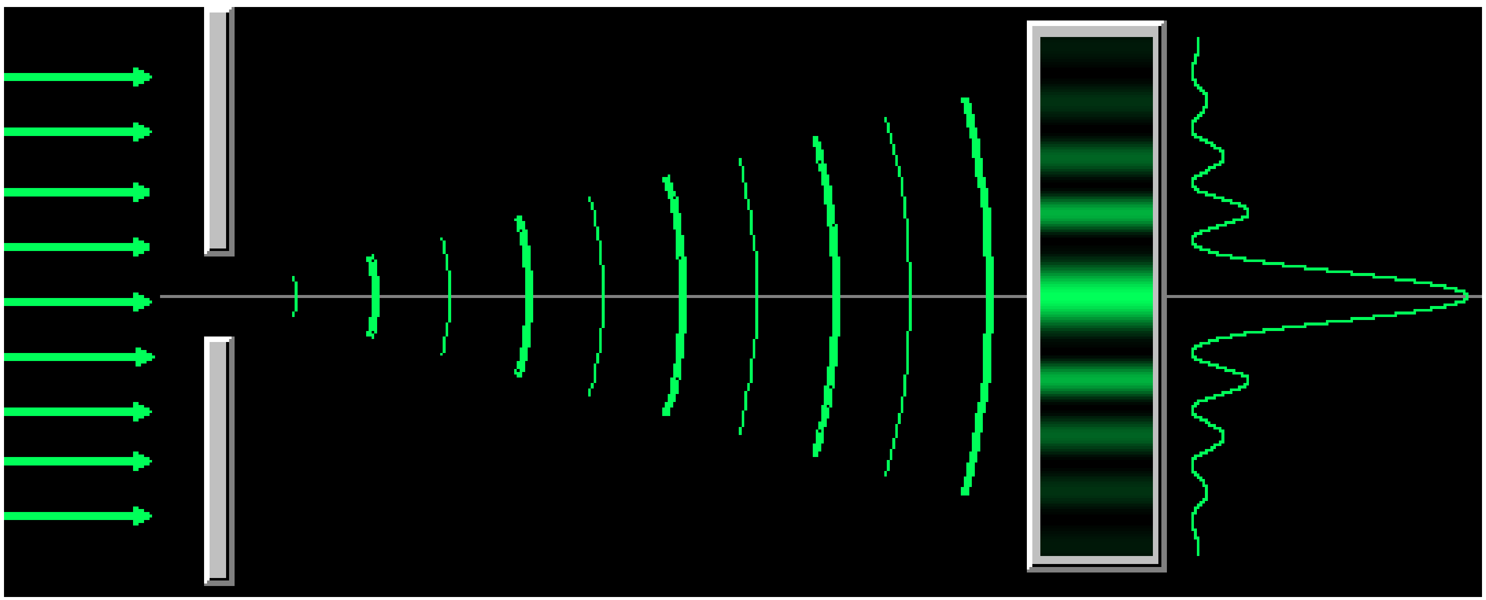
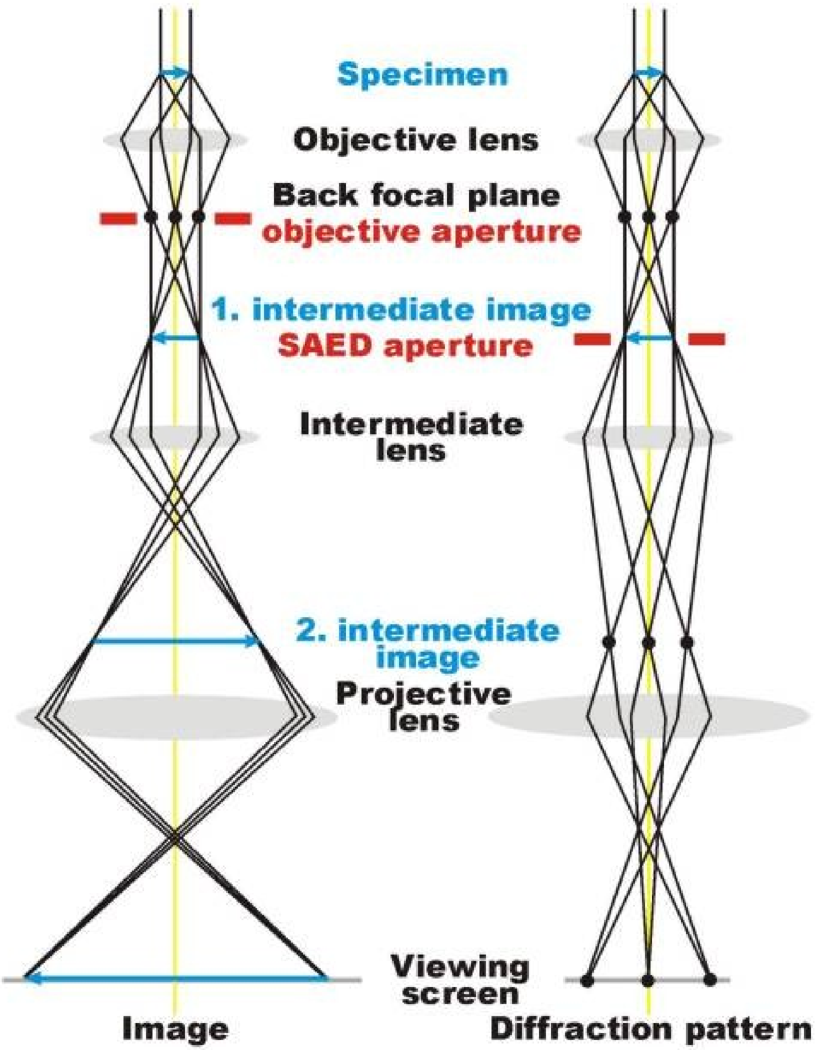
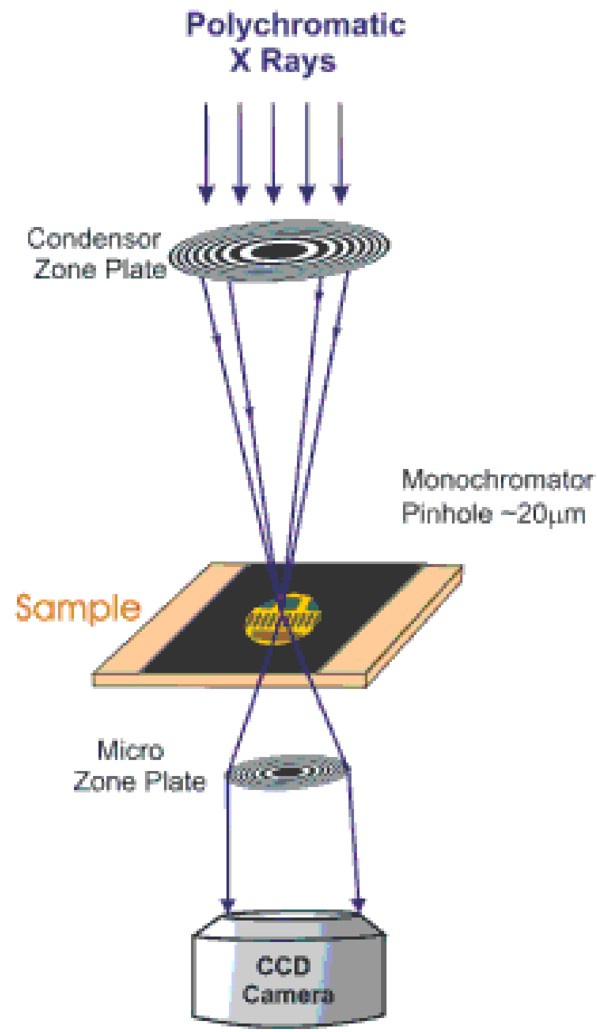


http://web.utk.edu

How do X-ray microscopes work?

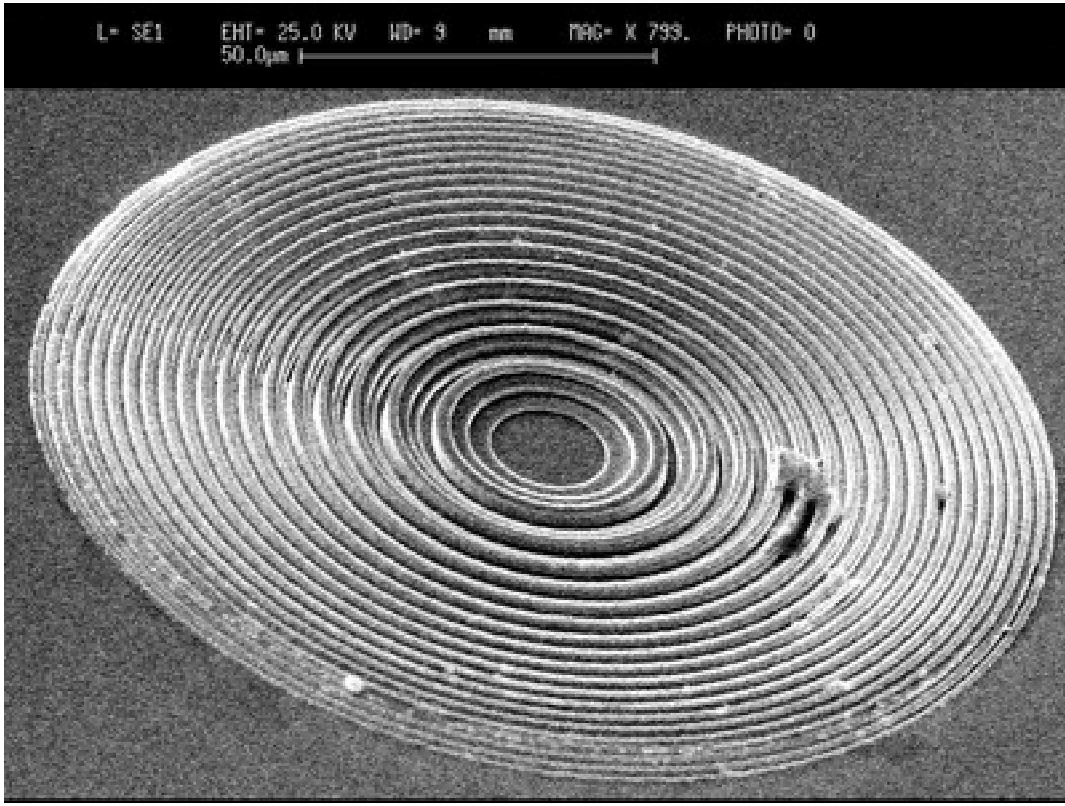


http://www.microscopy.ethz.ch

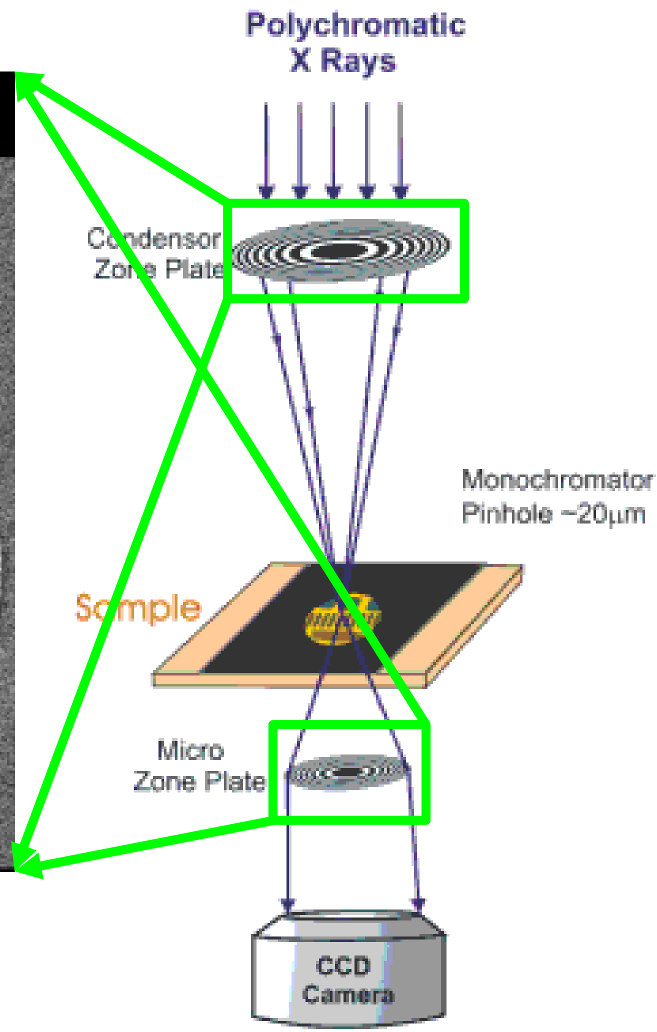


http://ssrl.slac.stanford.edu

How do X-ray microscopes work?



Fabrizio...Barrett, 1999, Nature



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Fourier series

A Fourier series is an expansion of a periodic function f(x) in terms of an infinite sum of sines and cosines

$$f(x) = \frac{1}{2}a_0 + \sum_{n=1}^{\infty} a_n \cos(nx) + \sum_{n=1}^{\infty} b_n \sin(nx)$$

Fourier transforms: Definition

$$F(k) = \int_{-\infty}^{\infty} f(x) e^{-2\pi ikx} dx$$

f: function which we are transforming (1D)

x: axis coordinate

i: √-1

k: spatial frequency

F(k): Fourier coefficient at

Fourier transforms: Definition

$$F(k) = \int_{-\infty}^{\infty} f(x) e^{-2\pi i k x} dx$$

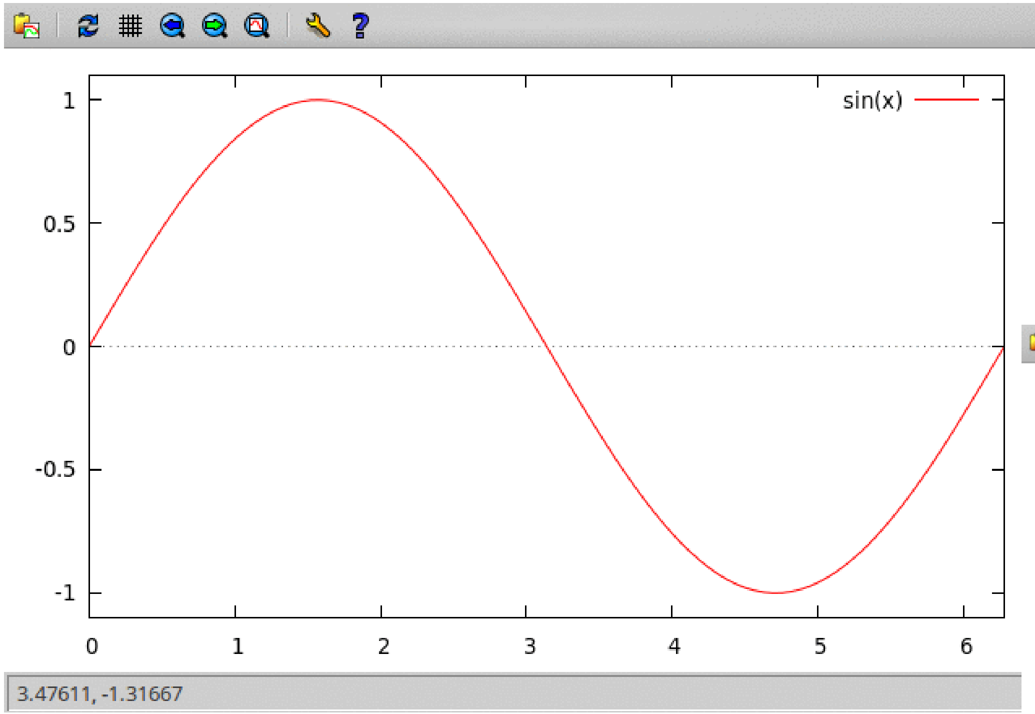
$$F(k) = a\cos(-2\pi kx) + ib\sin(-2\pi kx)$$

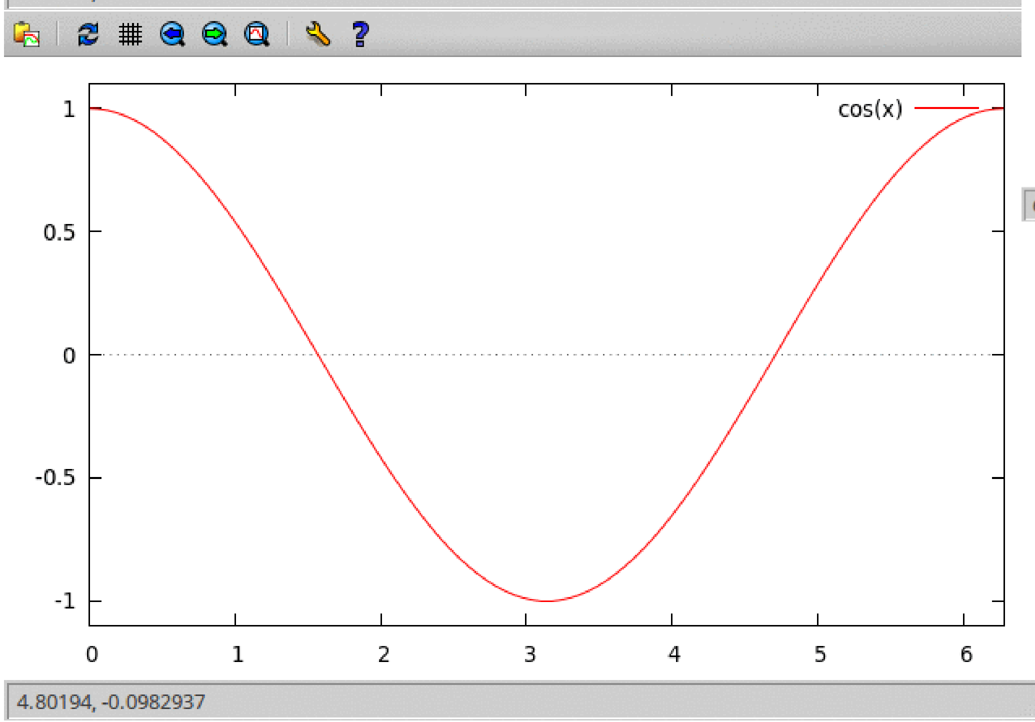
Fourier coefficients, discrete functions

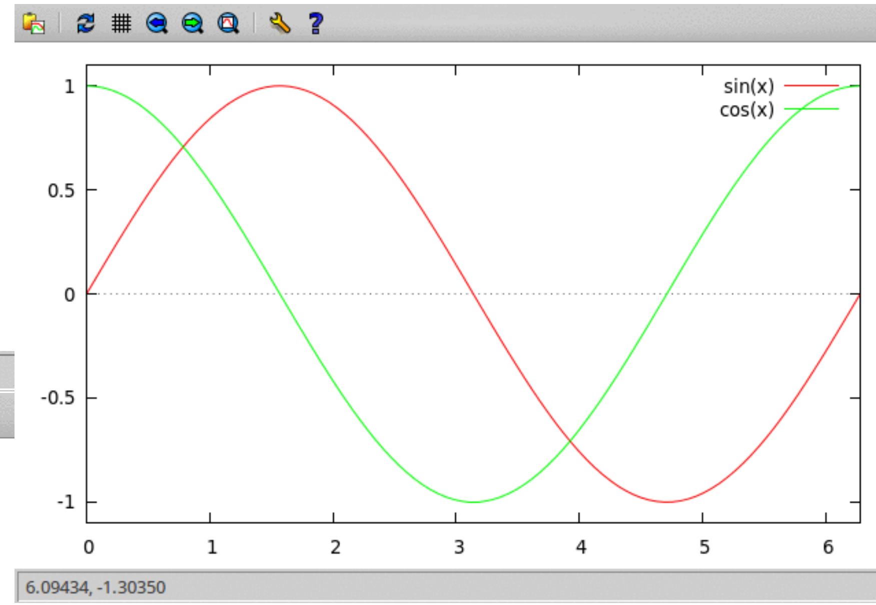
$$f(x) = \frac{1}{2}a_0 + \sum_{n=1}^{\infty} a_n \cos(nx) + \sum_{n=1}^{\infty} b_n \sin(nx)$$

$$a_n = \frac{1}{\pi}\int_{-\pi}^{\pi} f(x)\cos(nx)\,dx$$

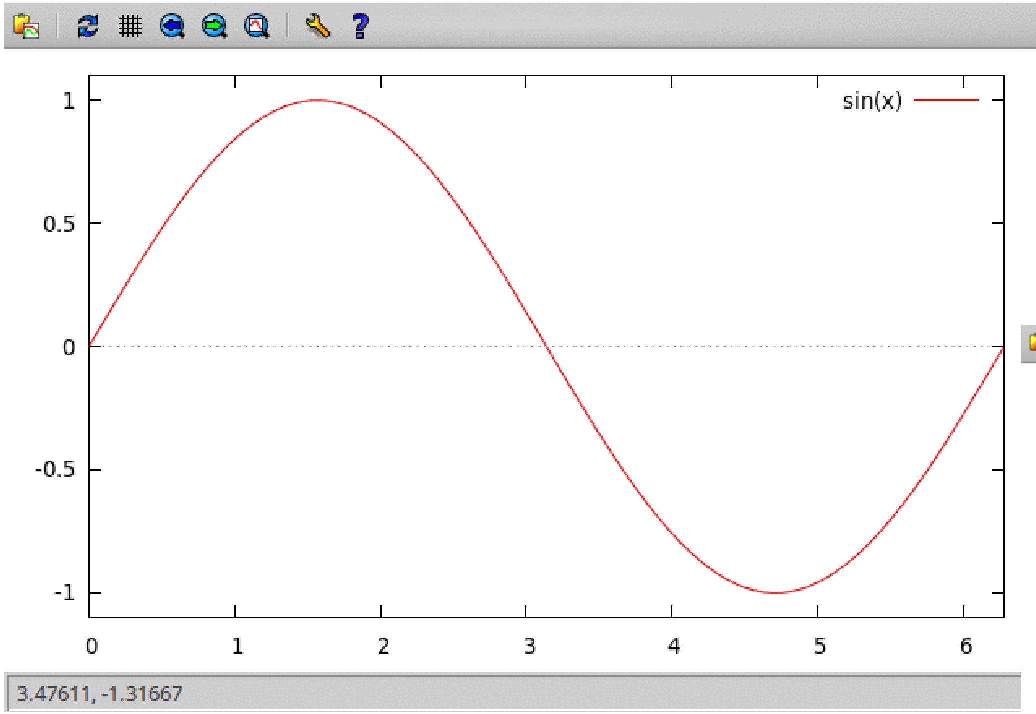
$$b_n = \frac{1}{\pi}\int_{-\pi}^{\pi} f(x)\sin(nx)\,dx$$

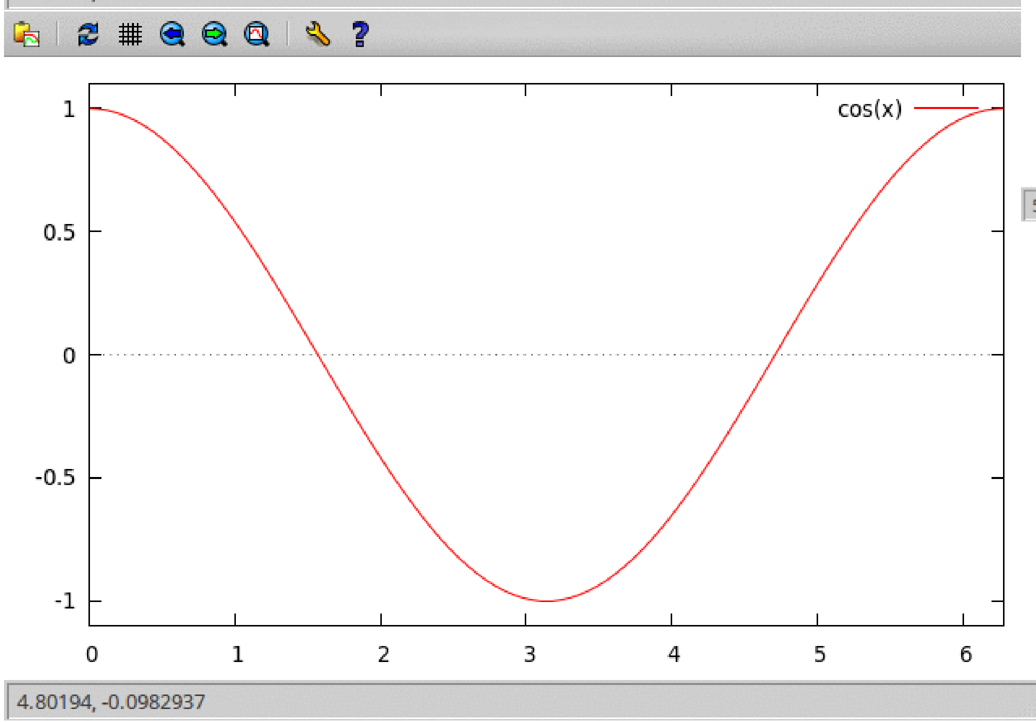


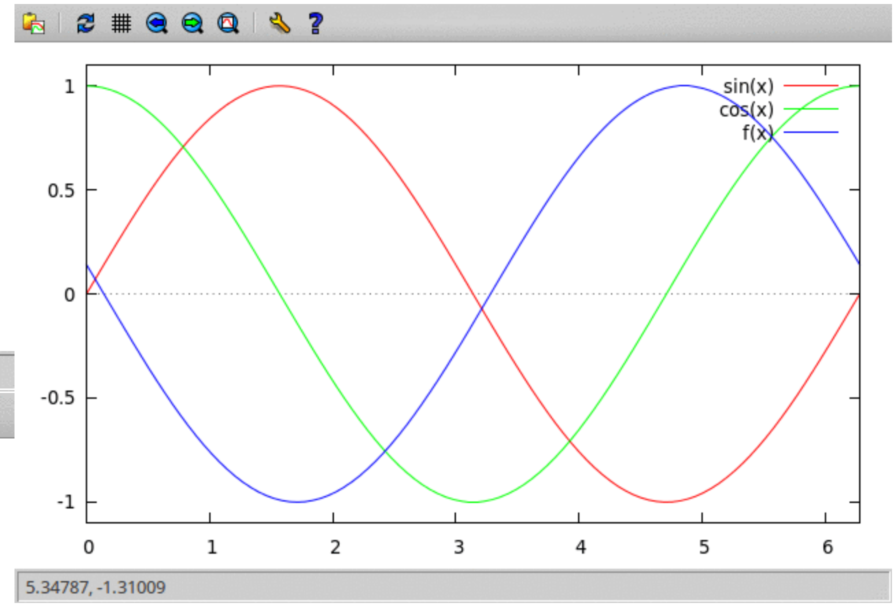




sine and cosine are orthogonal.
The inner product of the two is zero.

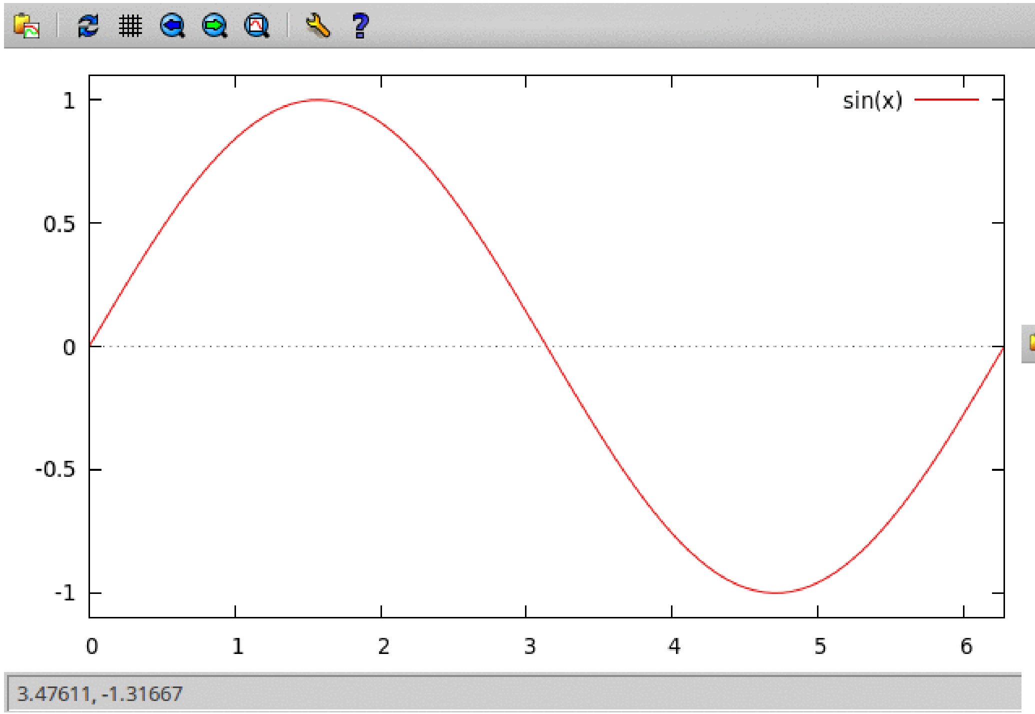


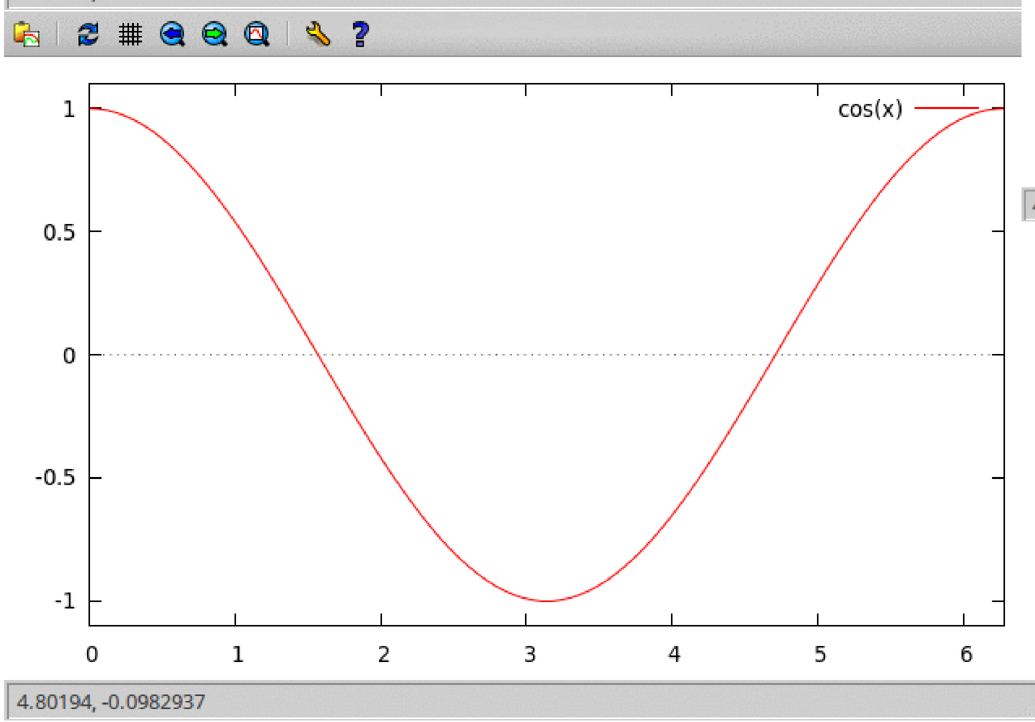


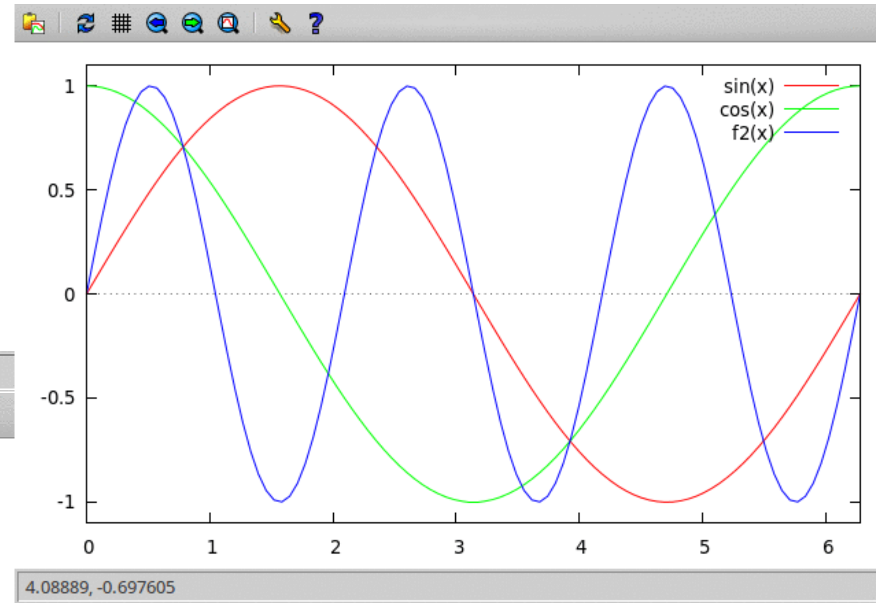


A function with the same frequency but with an offset will have some components of both sine and cosine.

That is, *a* and *b* will be non-zero.

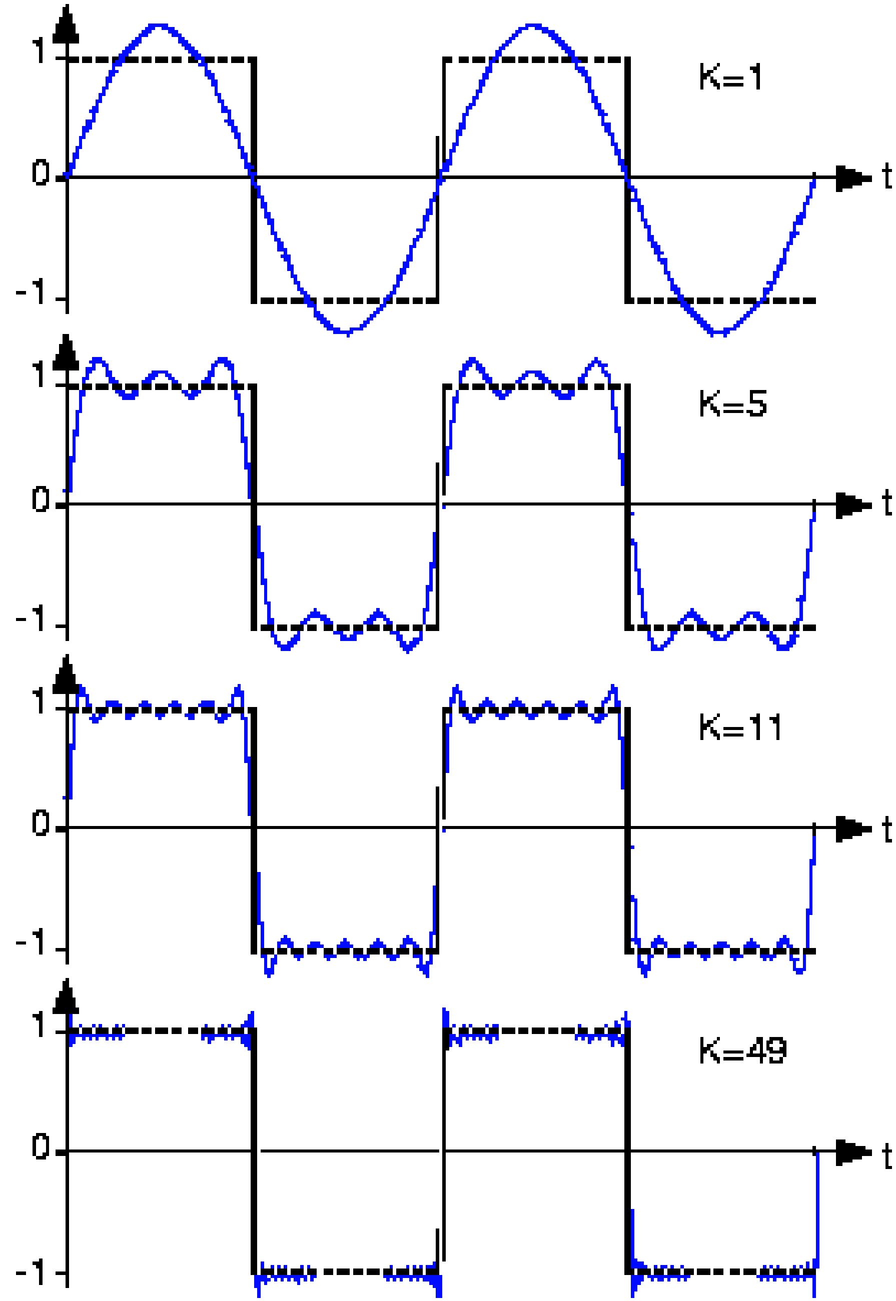






A function with a different frequency will have coefficients of zero.

The higher the spatial frequencies (i.e., higher resolution) that are included, the more faithful the representation of the original function will be.



http://cnx.org

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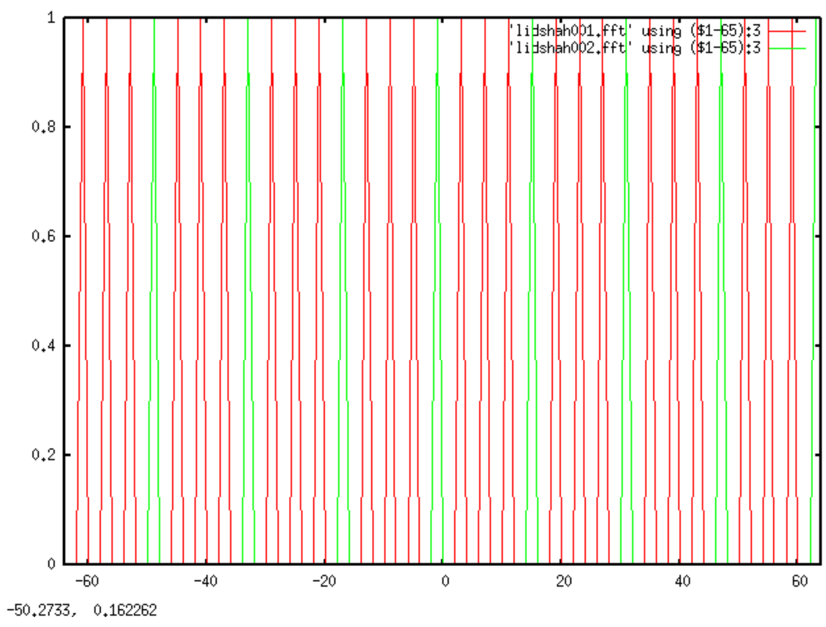
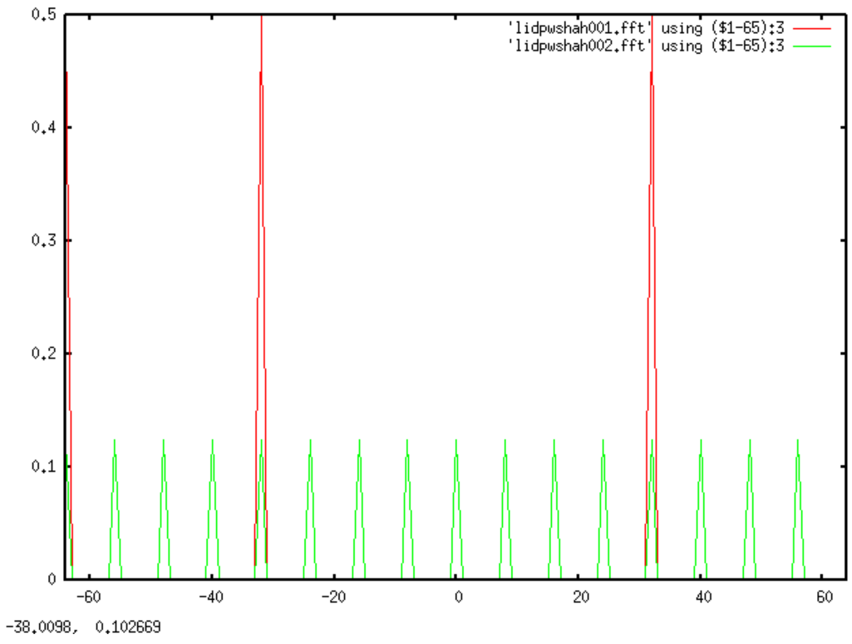
Some properties

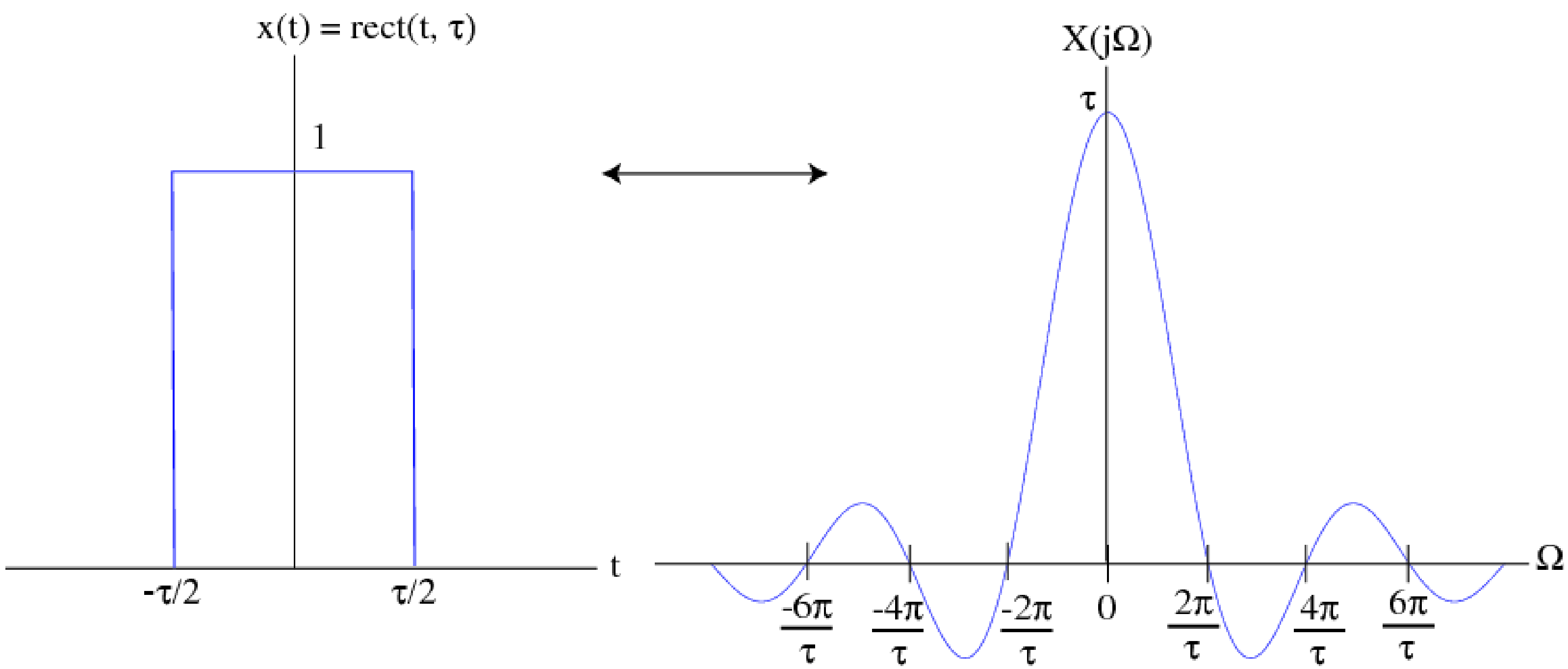
- As *n* increases, so does the spatial frequency, *i.e.*, the "resolution."
  - For example, $\sin(2x)$ oscillates faster than $\sin(x)$
- Computation of a Fourier transform is a completely reversible operation.
  - There is no loss of information.
- Fourier terms (or coefficients) have amplitude and phase.
- The diffraction pattern is the physical manifestation of the Fourier transform
  - Phase information is lost in a diffraction pattern.
  - An image contains both phase and amplitude information.

Some simple 1D transforms: a 1D lattice



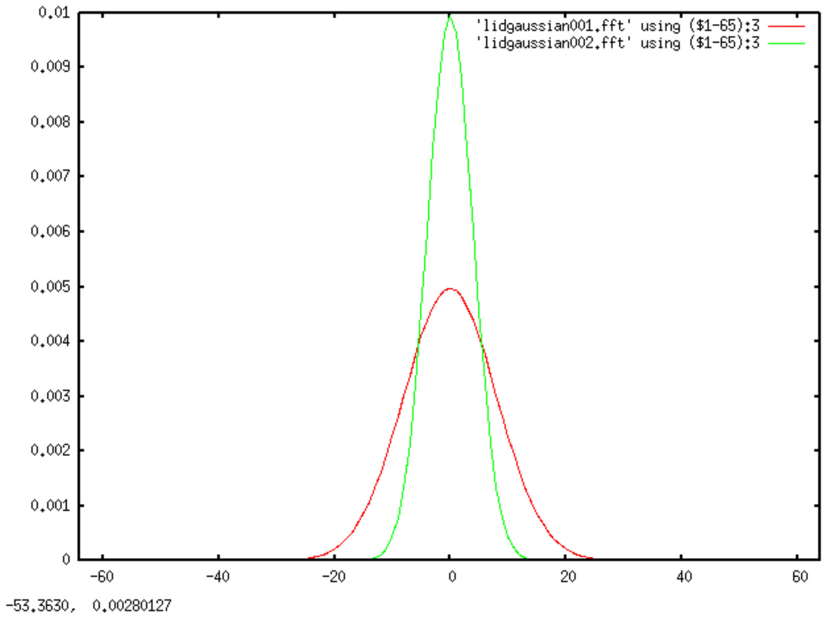


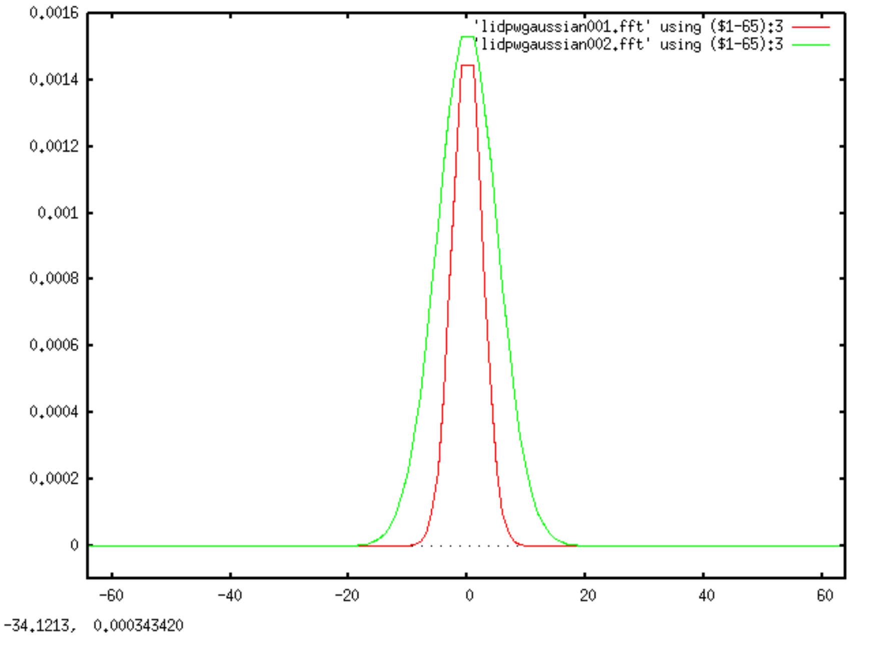
Some simple 1D transforms: a box



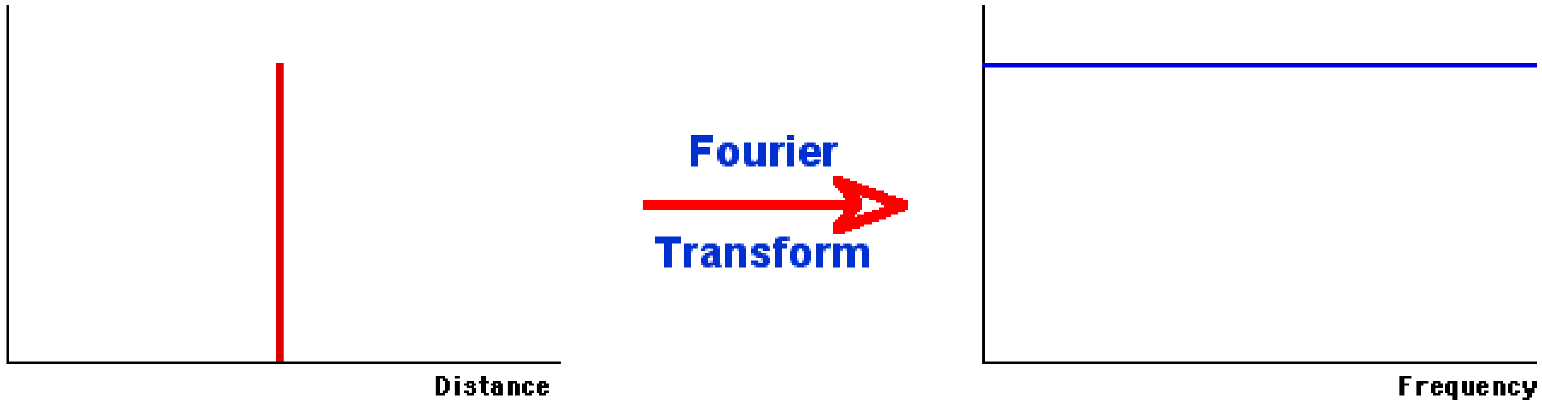
http://cnx.org

Some simple 1D transforms: a Gaussian



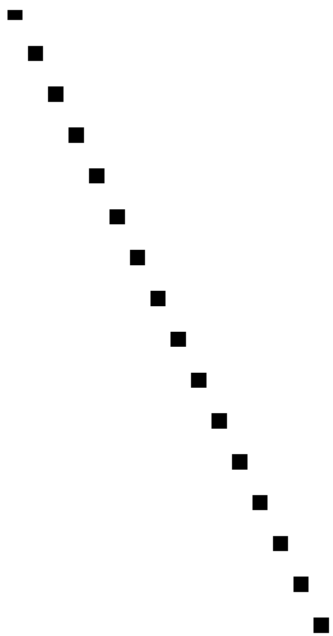


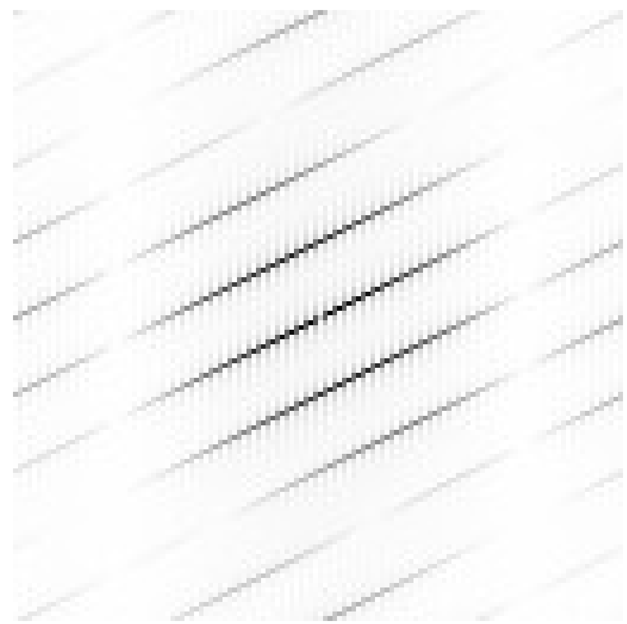
Some simple 1D transforms:
a sharp point (Dirac delta function)



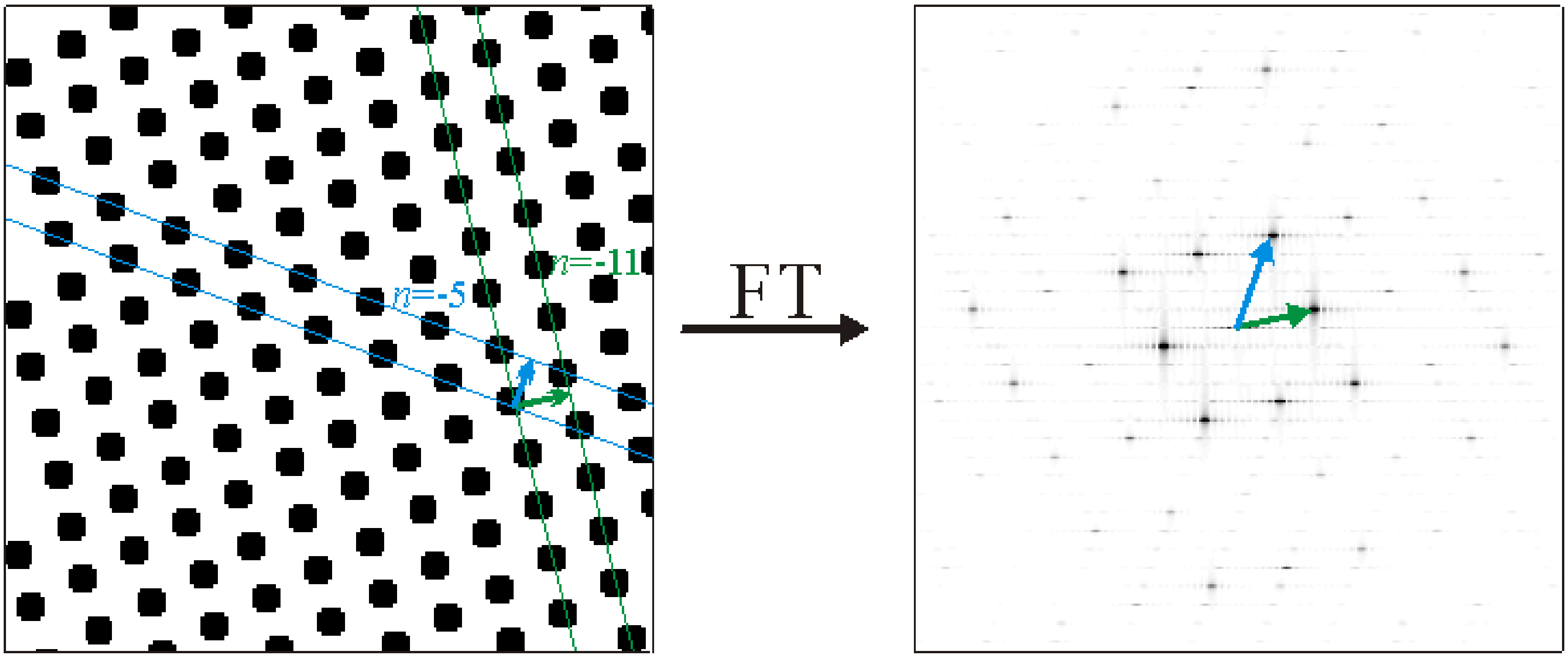
http://en.labs.wikimedia.org/wiki/Basic_Physics_of_Nuclear_Medicine/Fourier_Methods

Some simple 2D Fourier transforms: a row of points

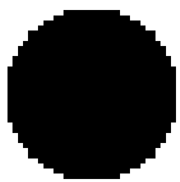


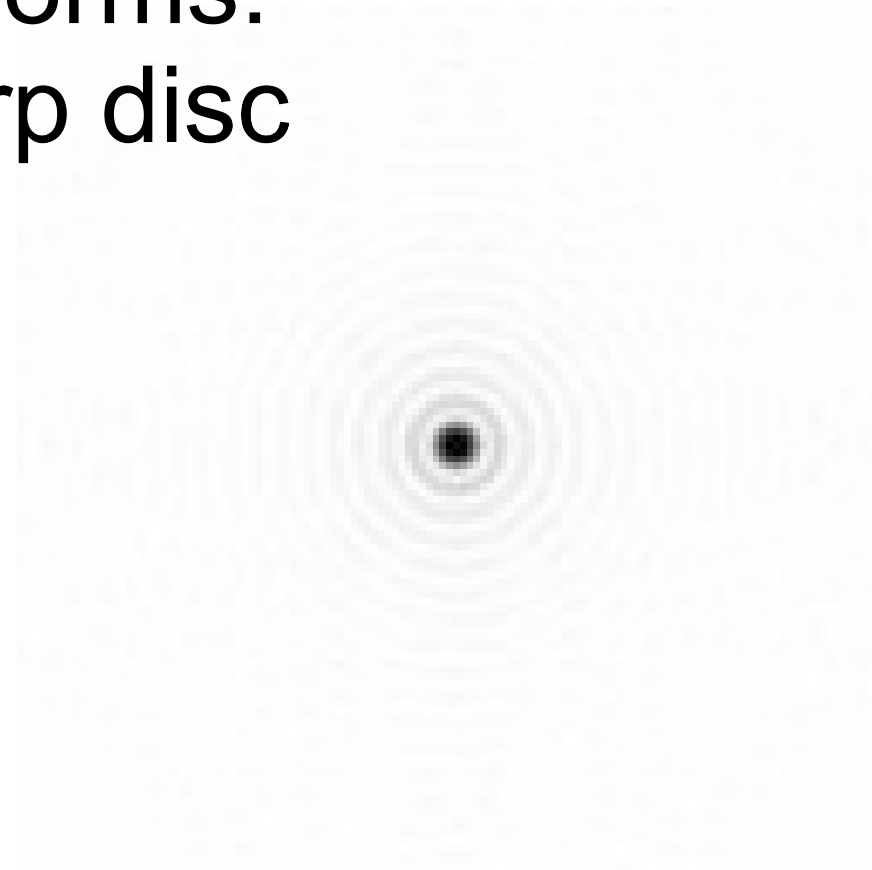


Some simple 2D Fourier transforms: a 2D lattice

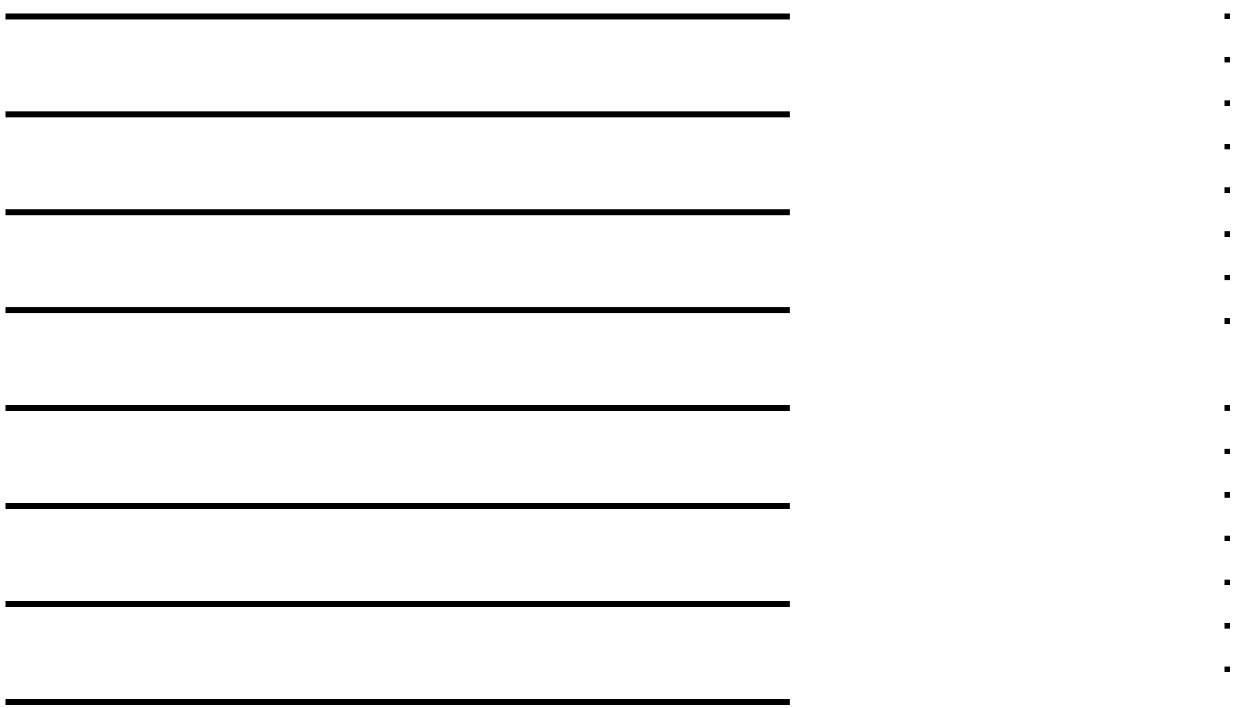


Some simple 2D Fourier transforms: a sharp disc



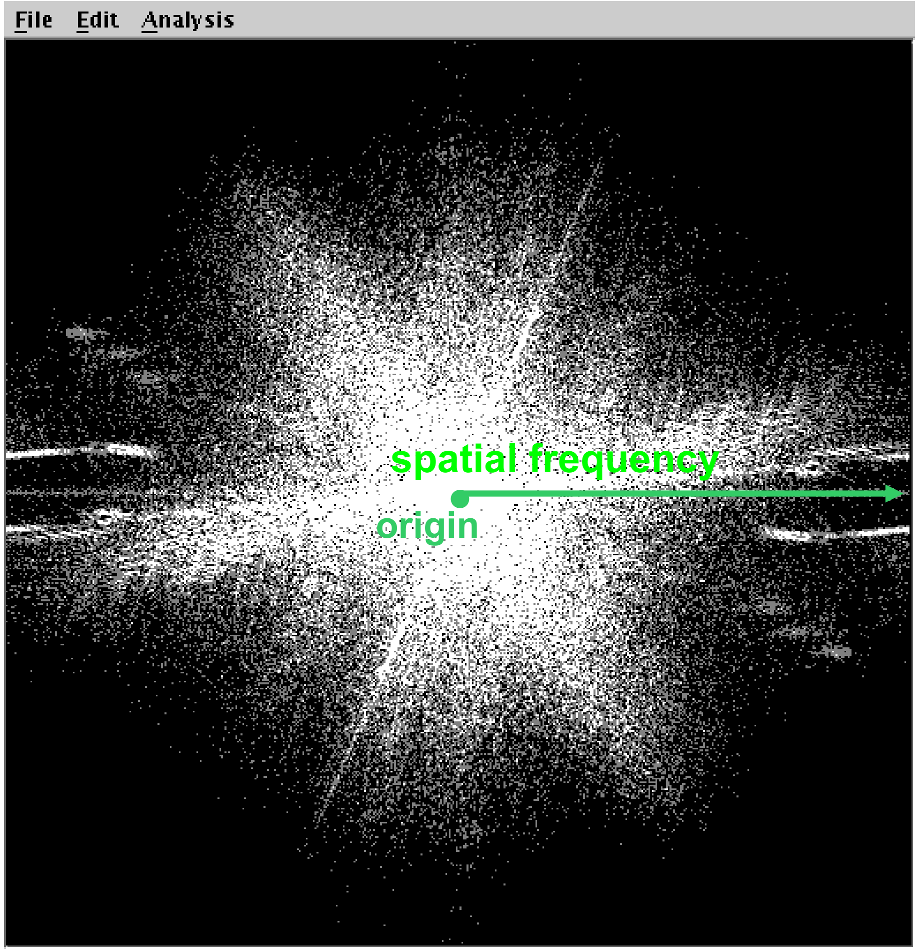


Some simple 2D Fourier transforms: a series of lines

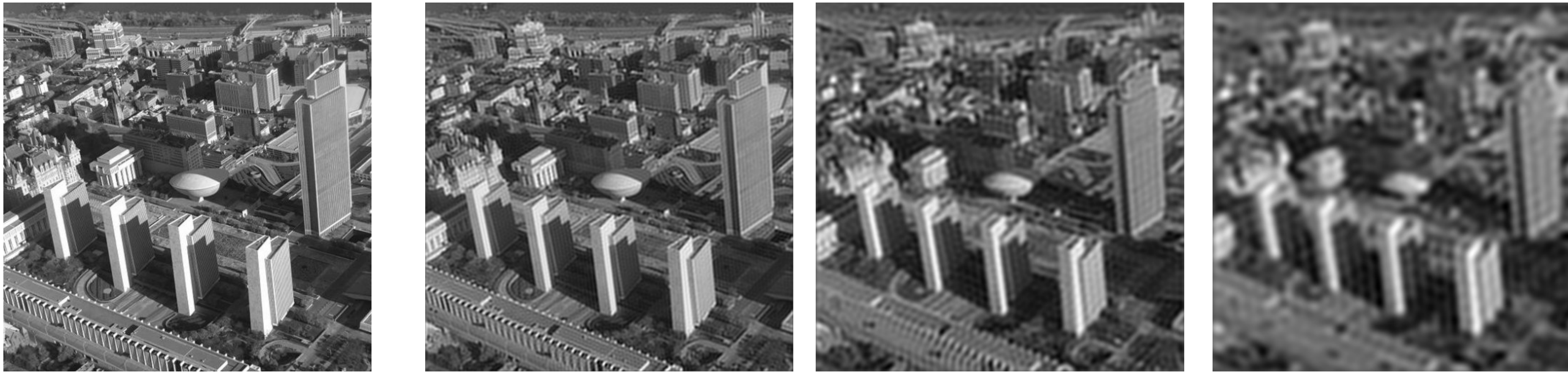


What do we mean by spatial frequency?

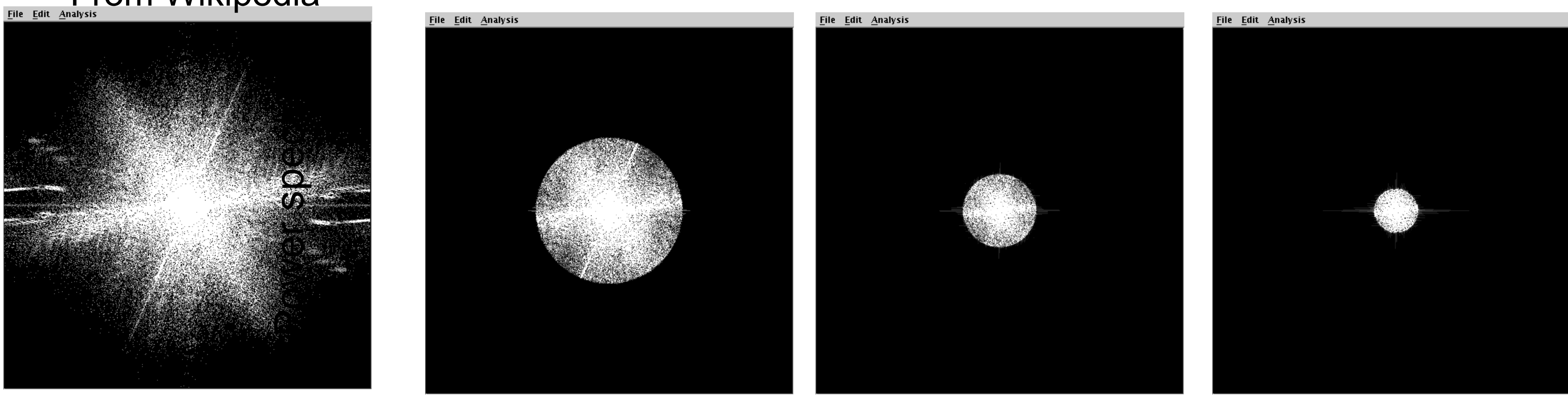
From Wikipedia



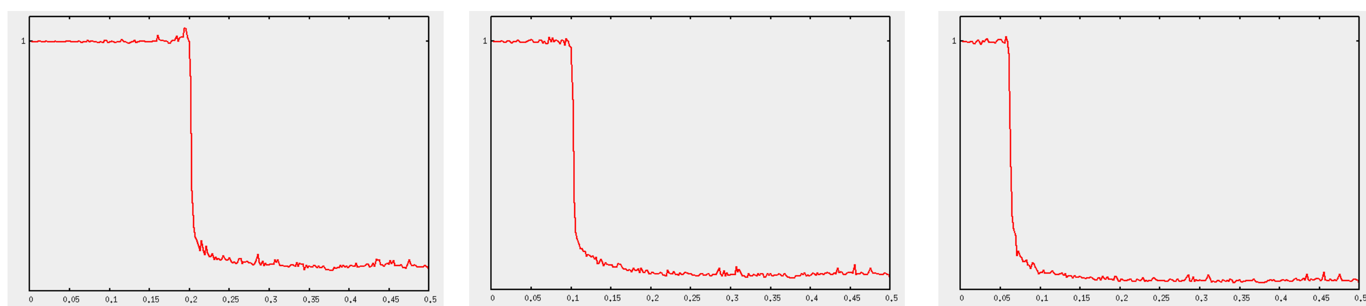
Fourier filtration

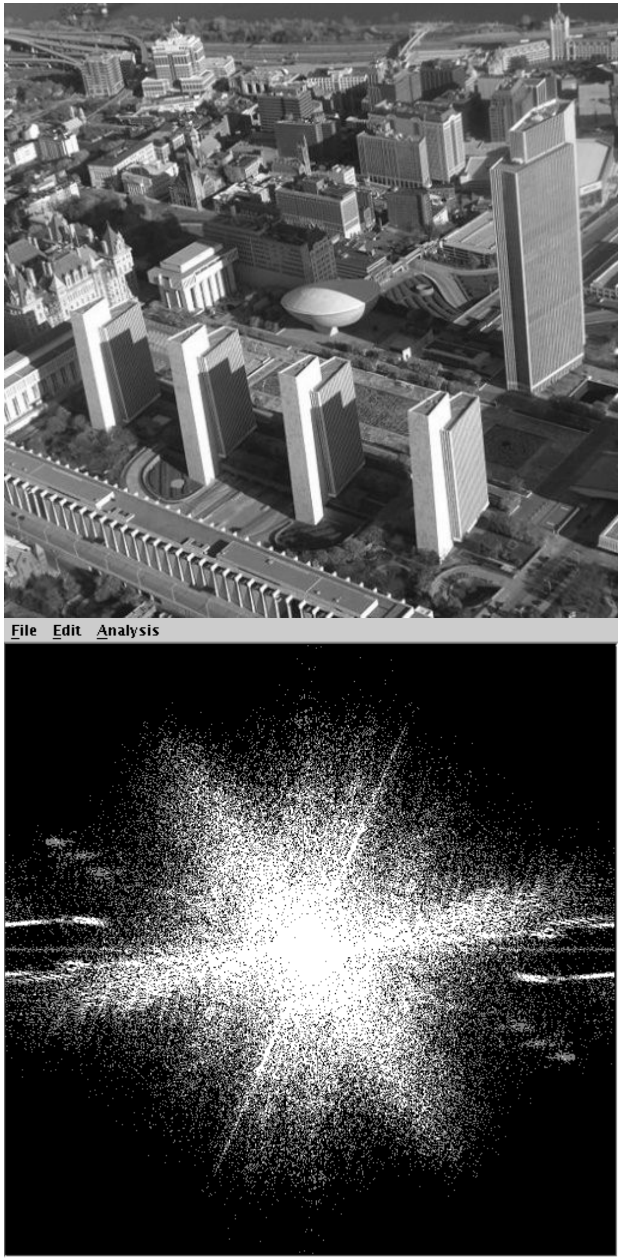


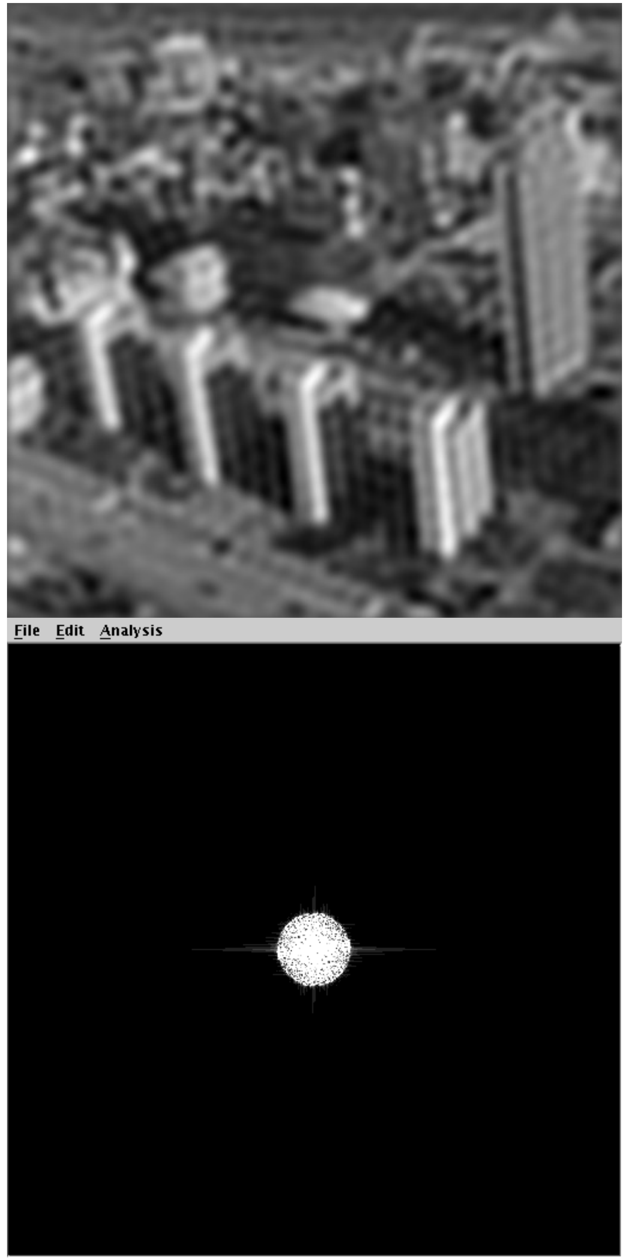
From Wikipedia



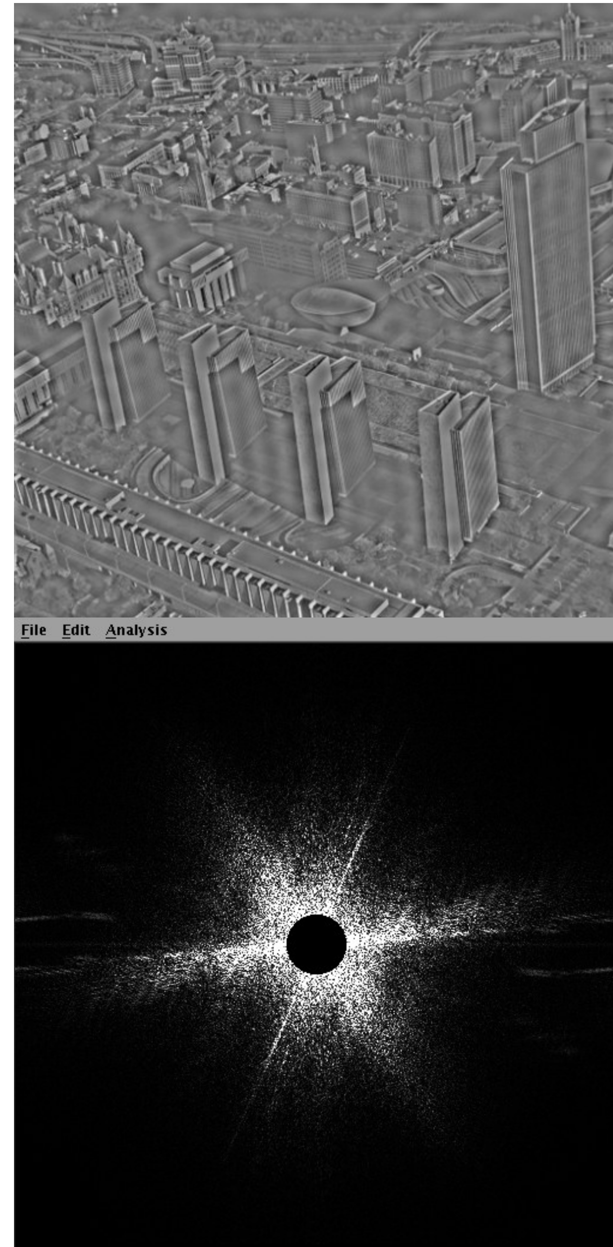
Profile







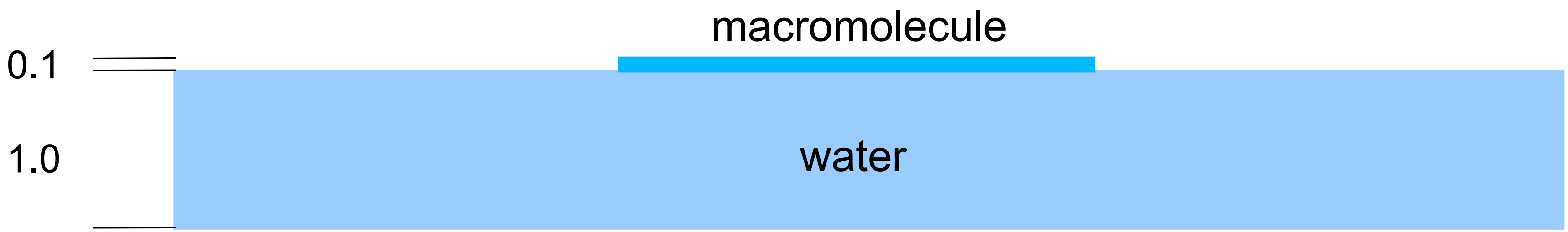
A “low-pass” filter



A “high-pass” filter

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.

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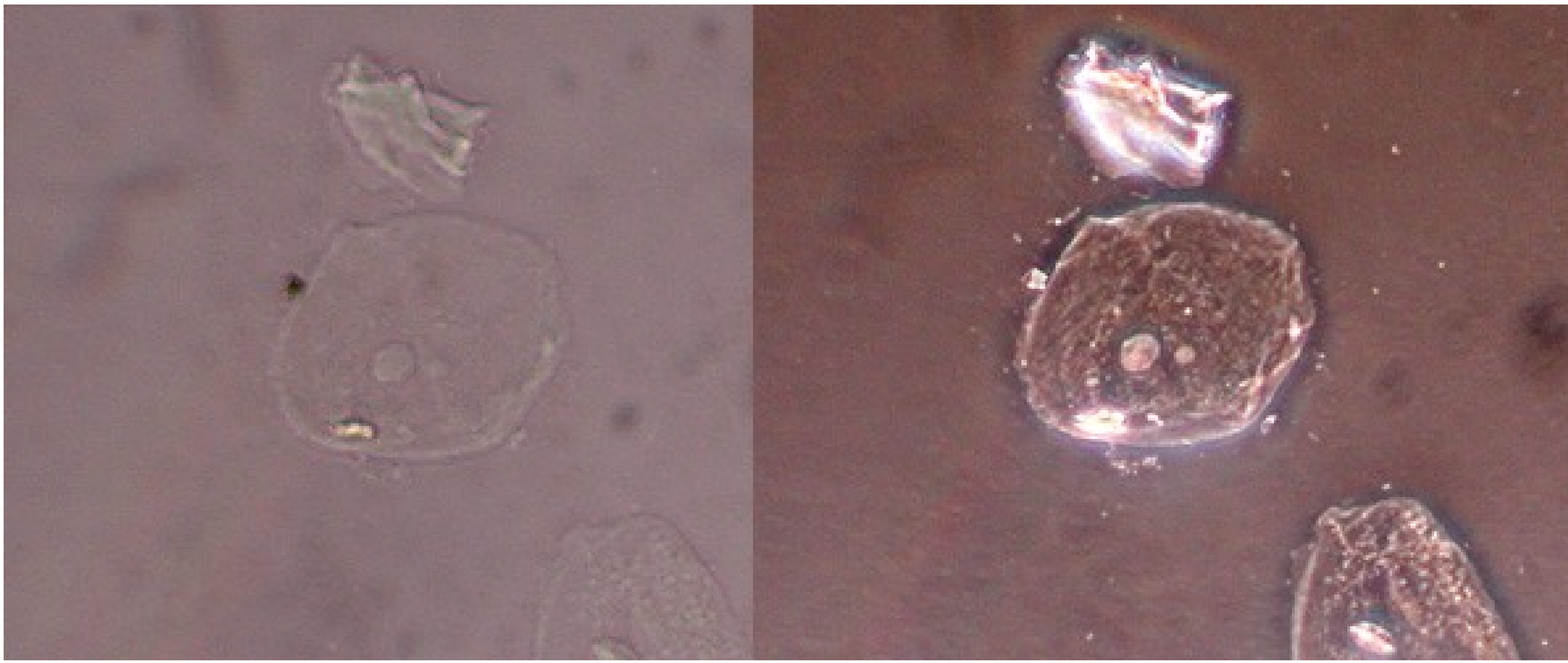
Phase contrast in light microscopy

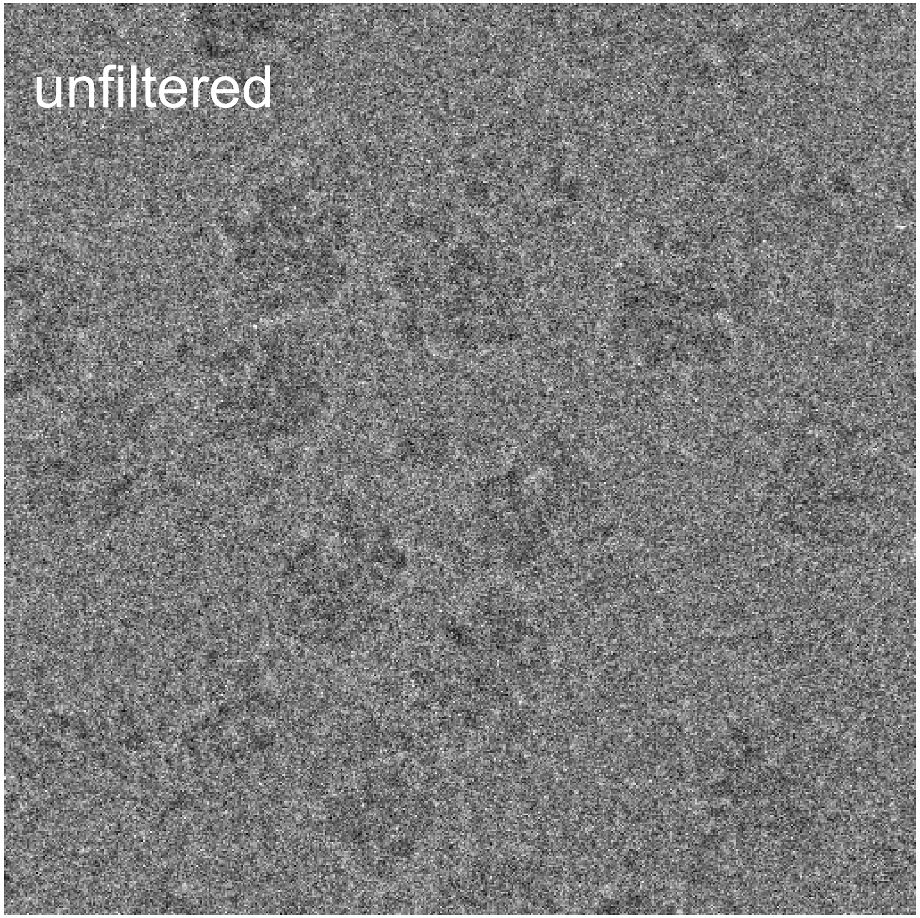
Bright-field image　　Phase-contrast image

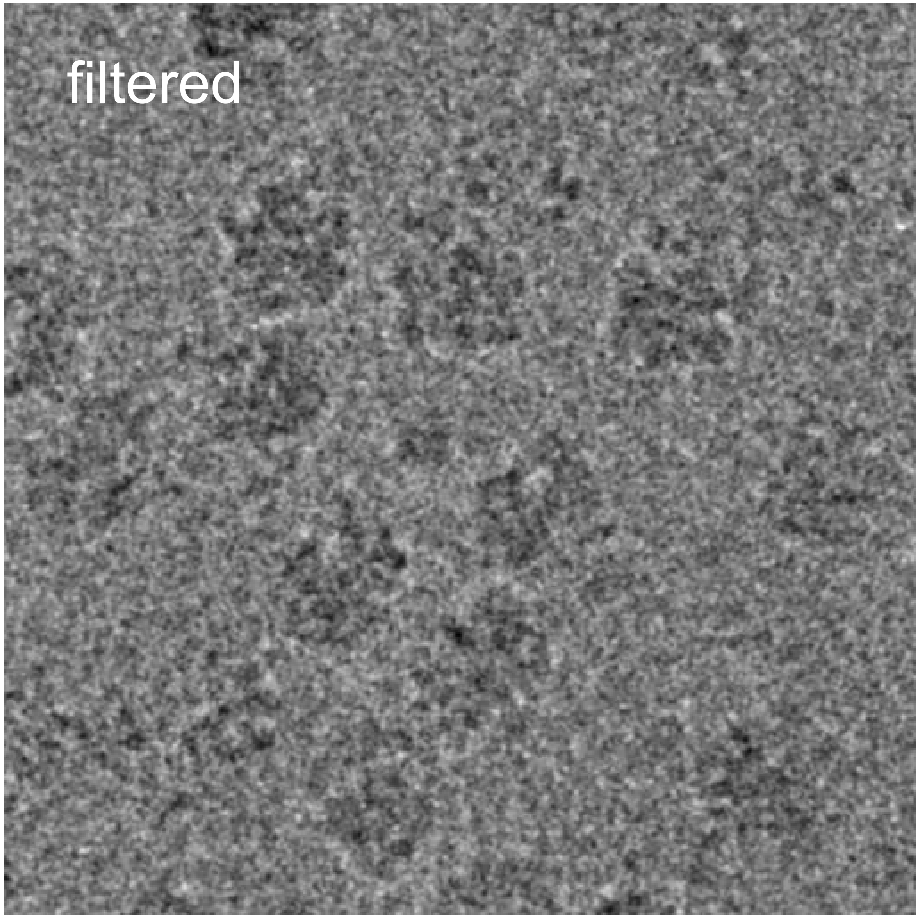


http://www.microbehunter.com

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

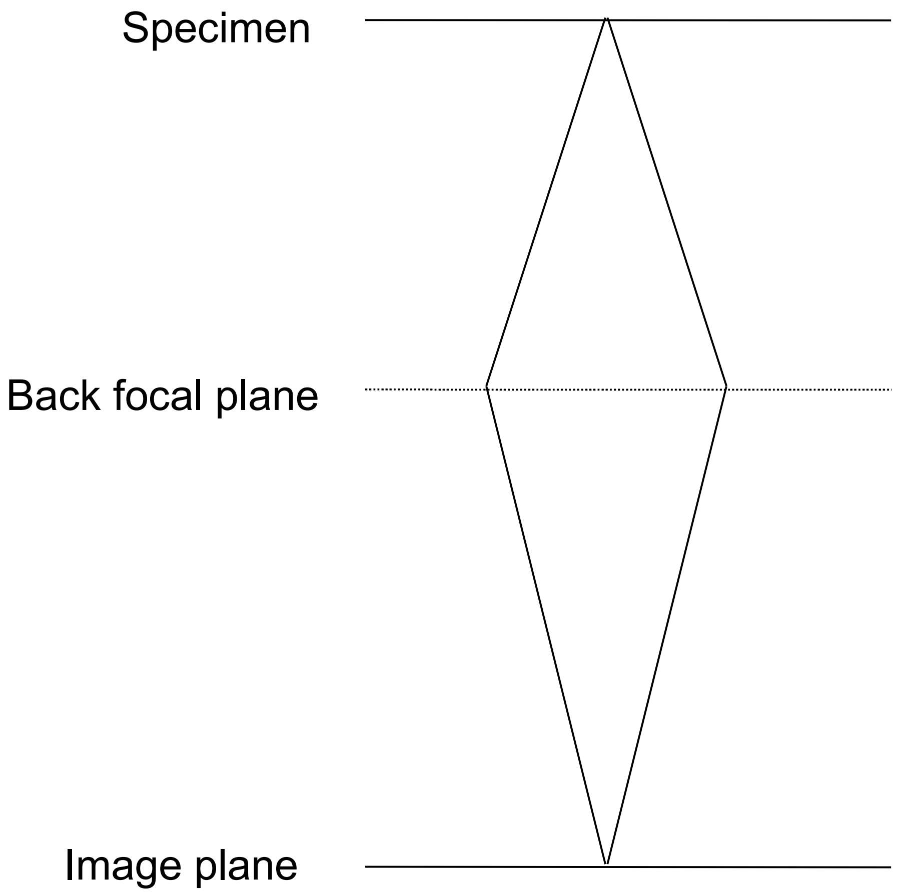
E. coli 70S ribosomes, field width ~1440Å.





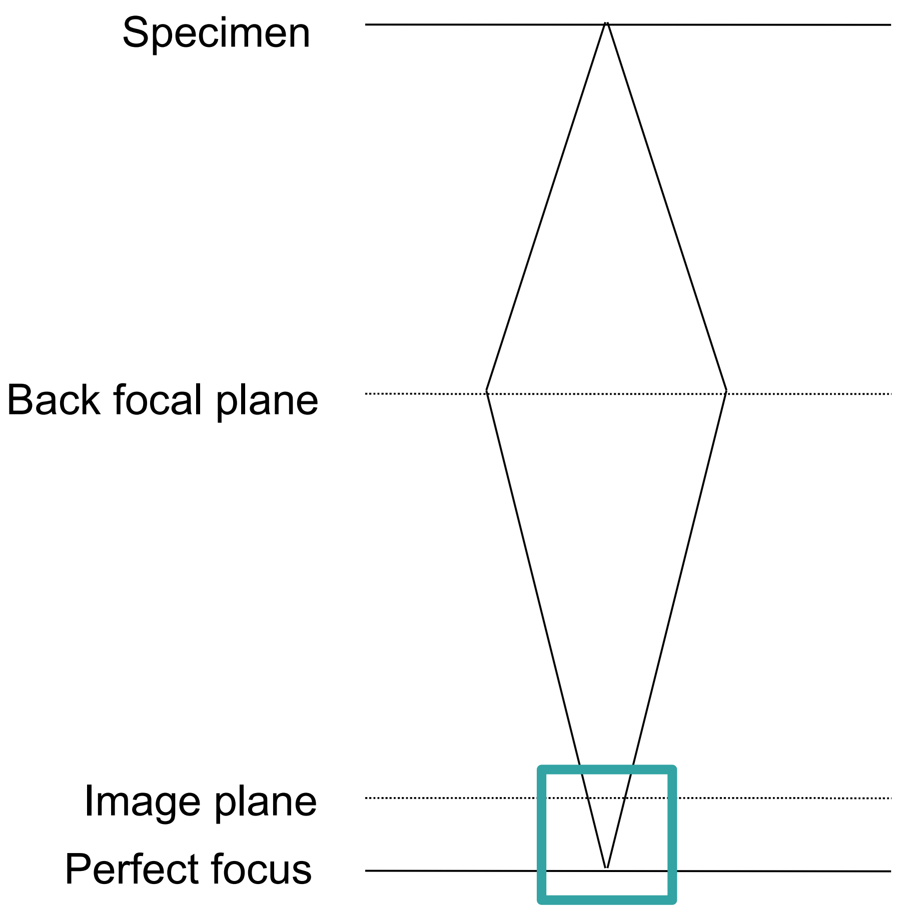
Signal-to-noise ratio for cryoEM typically given to be between 0.07 and 0.10.

Optical path

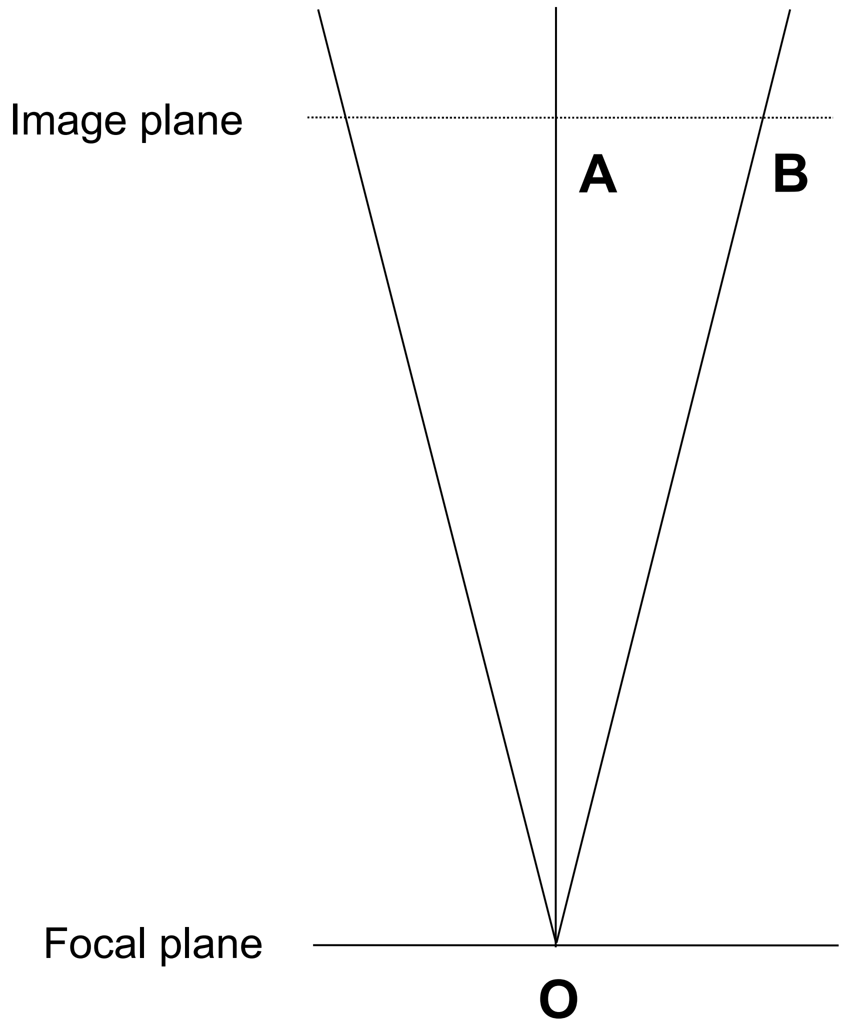


At focus, all we would see is amplitude contrast.

Optical path with defocus



Optical path with defocus



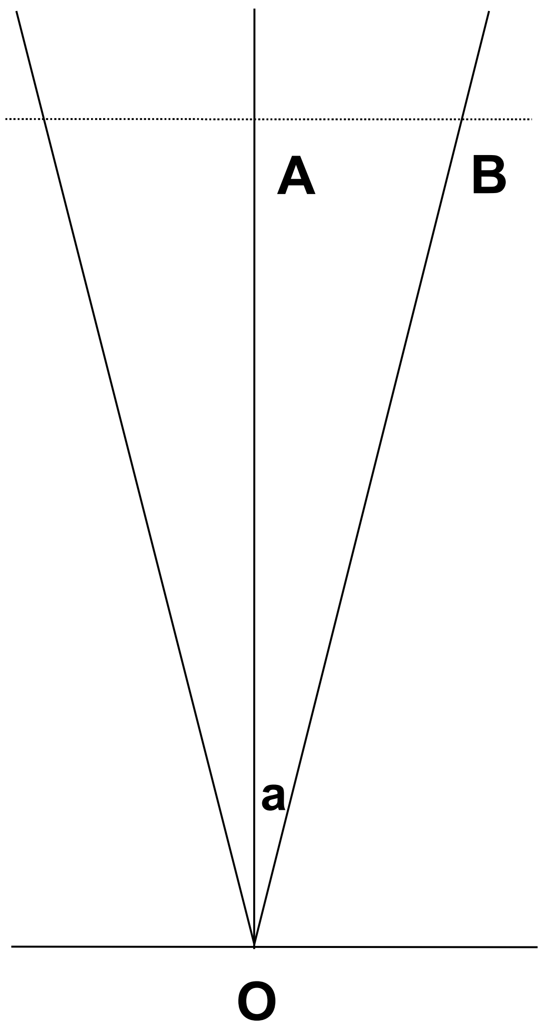
OA path of unscattered beam

OB path of scattered beam

The length **OA** is
also the amount of defocus Δf

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

Path difference as a function of Δf



$$OB - OA$$

OB = OA/cos(a)

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

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

Expressed in the number of wavelengths λ

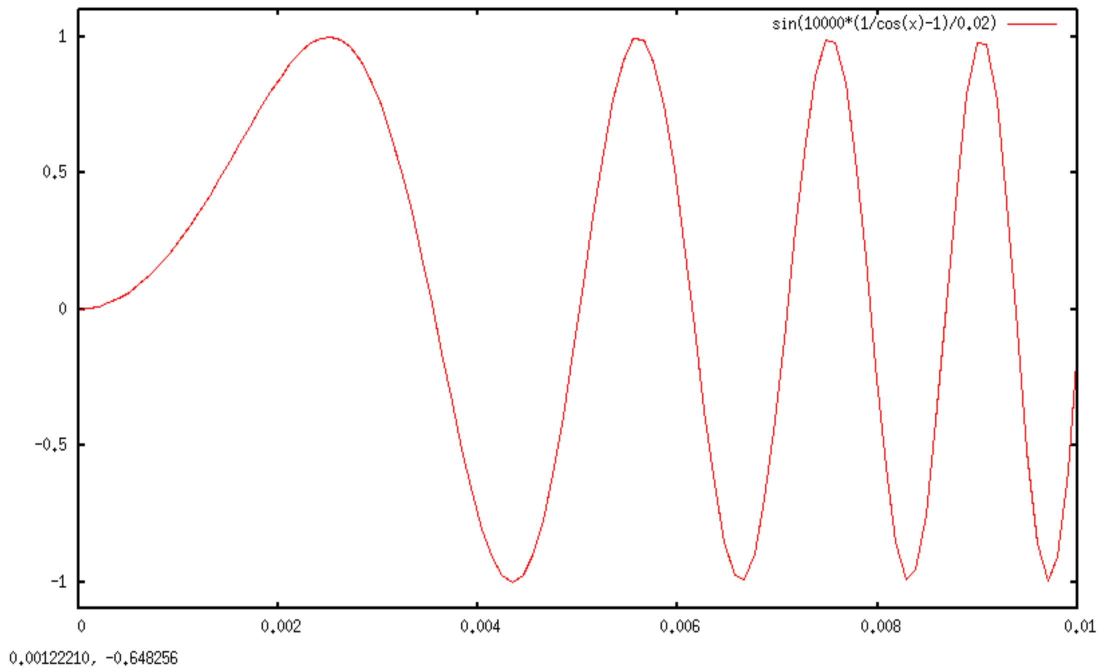
$$OA \times \left(\frac{\frac{1}{\cos(a)} - 1}{\lambda}\right)$$

Phase difference is the sine

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

Some typical values

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



OA = Δf = 10,000 Å
λ = 0.02 Å
a < 0.01

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

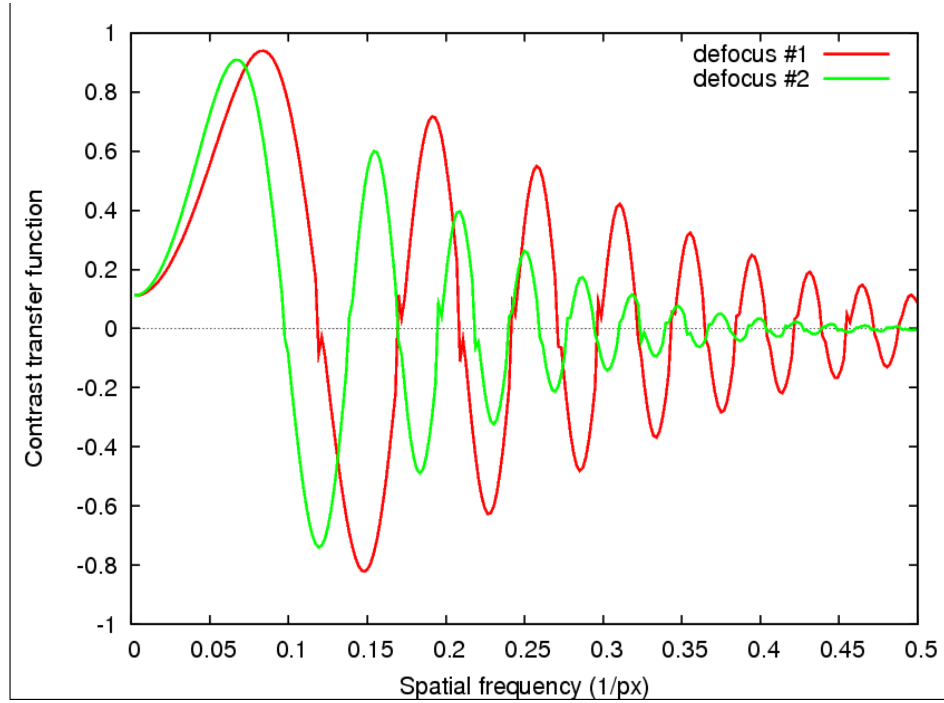
$$-\sin\left(\frac{\pi}{2}C_s k^4 + \pi \Delta f \lambda k^2\right)$$

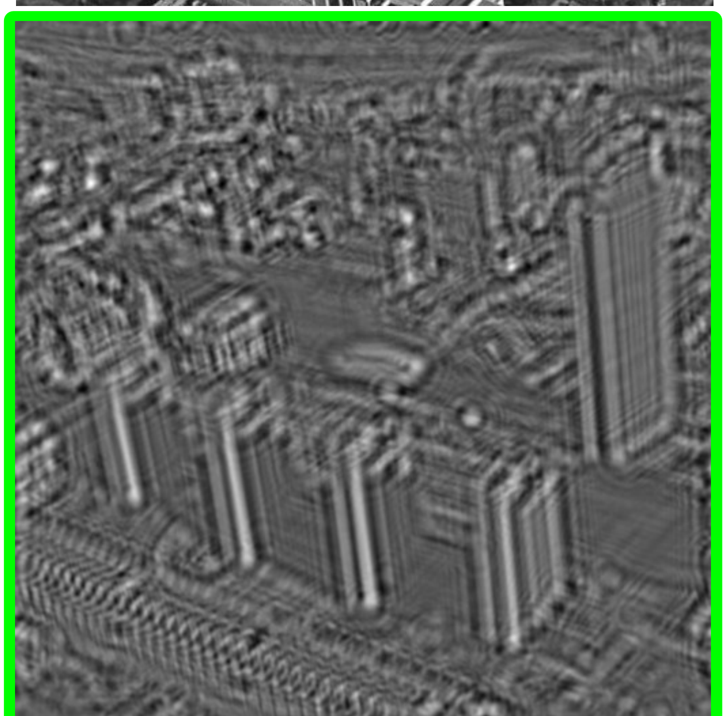
where:

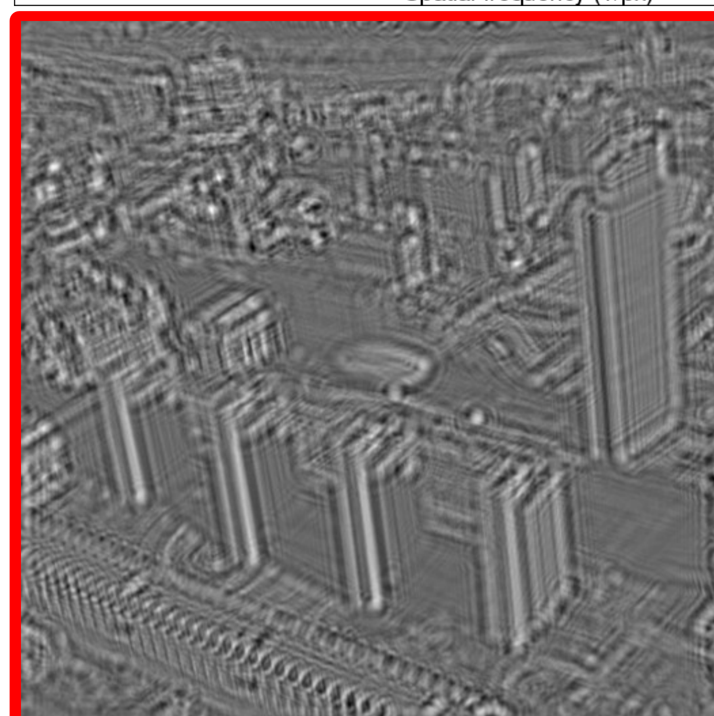
- C_s: spherical aberration
- k: spatial frequency (resolution)

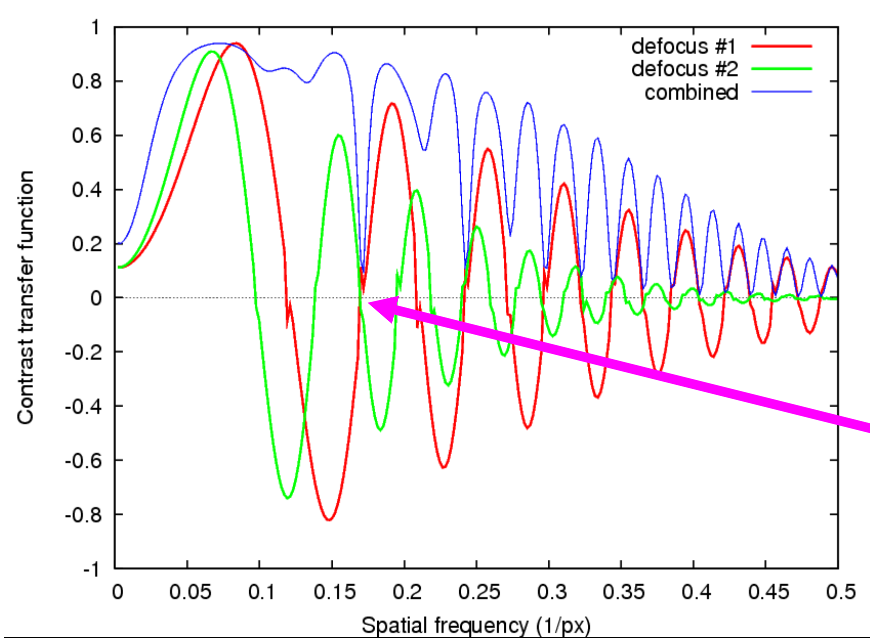
How does the CTF affect an image?

original


defocus #1
defocus #2
Contrast transfer function
Spatial frequency (1/px)
1
0.8
0.6
0.4
0.2
0
-0.2
-0.4
-0.6
-0.8
-1
0
0.05
0.1
0.15
0.2
0.25
0.3
0.35
0.4
0.45
0.5







Still a zero present

combined

original

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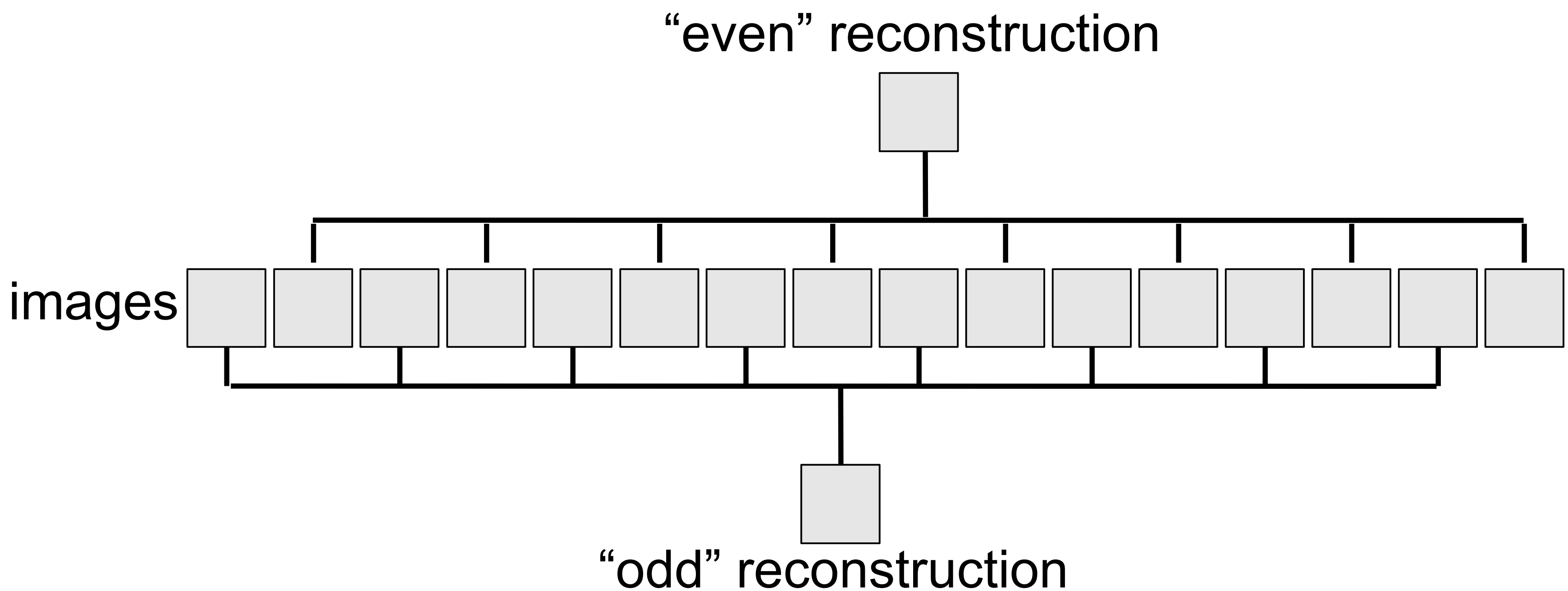
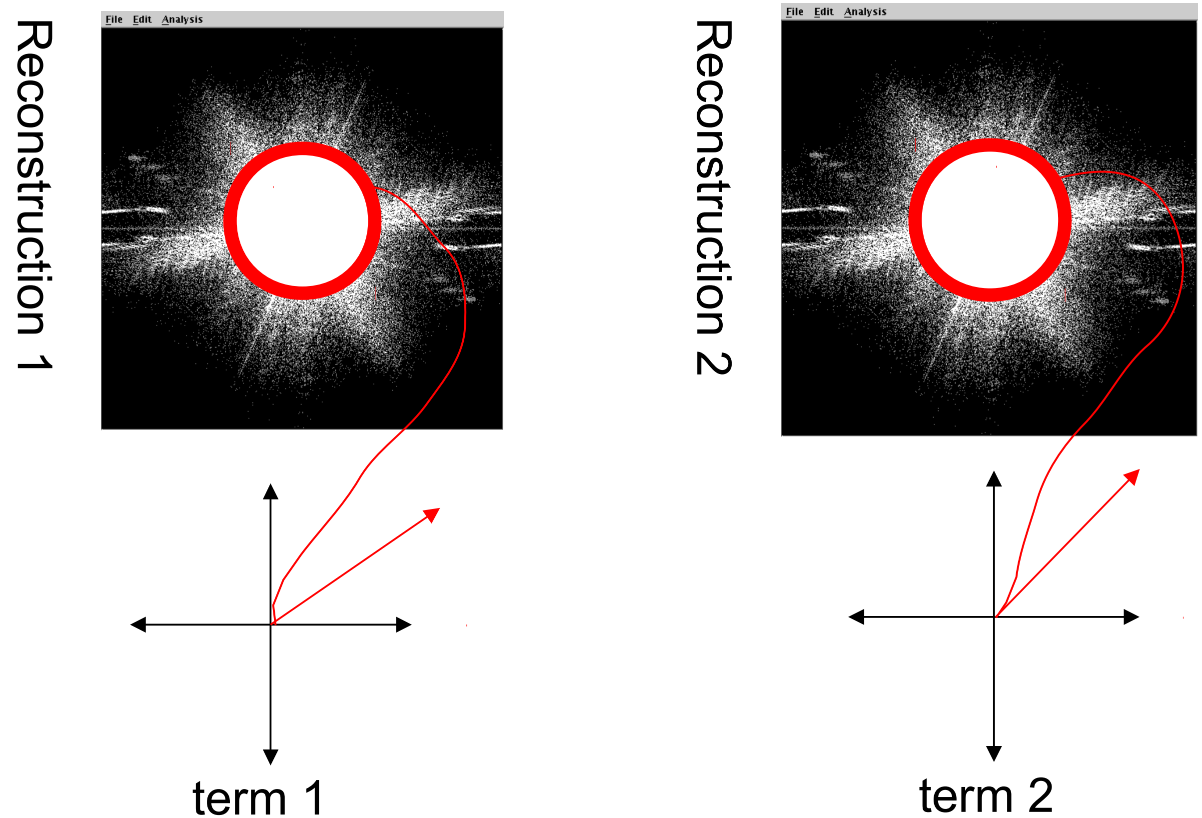
How do we evaluate the quality of a reconstruction?

We split the data set into halves and compare them.



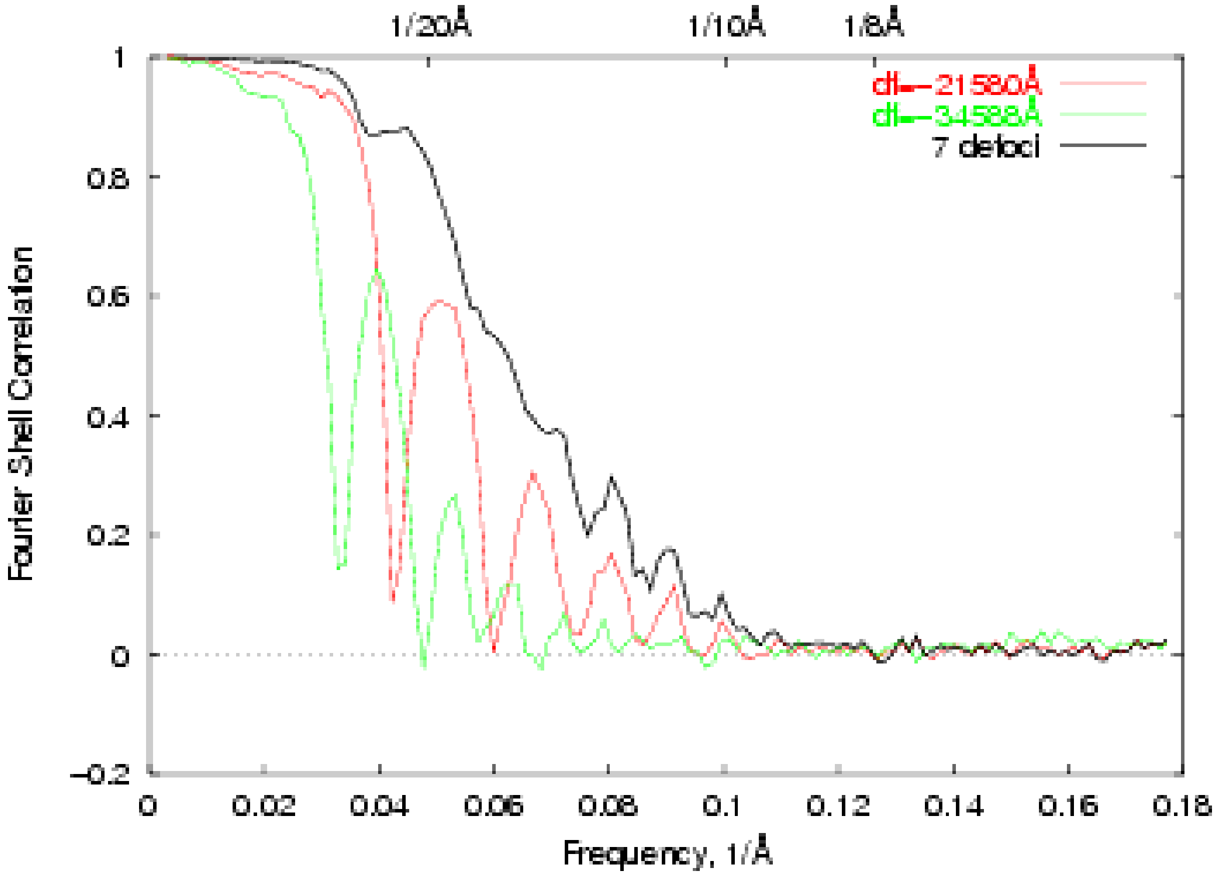
Now, <u>how</u> do we compare the two half-set reconstructions?

Fourier Shell Correlation (FSC)



Properties:
- Fourier terms have amplitude + phase.
- Correlation values range from -1 to +1.
- Noise <u>should</u> give an average of 0.
- The comparison is done as a function of spatial frequency (or "resolution")

Fourier Shell Correlation;
A better example



It is controversial what single number to use to describe this curve, but a common practice is to report the value where the FSC=0.5 as the nominal resolution.

Thank you for your attention

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