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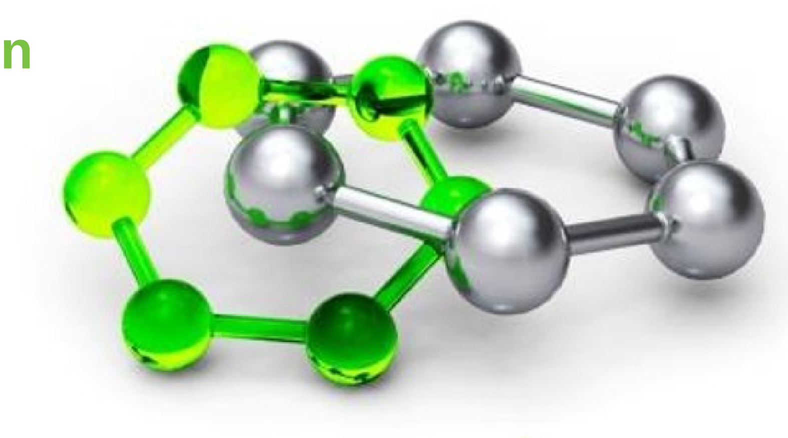
S1007 Doing structural biology with the electron microscope

C9940 3-Dimensional Transmission electron microscopy

Lecture 4: Principles of image formation



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OP Research and
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Task 1: What is the electron wavelength at acceleration voltage of 200kV?

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$U = 300\,kV$

$e = 1.61 \cdot 10^{-19}\,C$

$m_e = 9.31 \cdot 10^{-31}\,kg$

$E_p = U \cdot e \qquad E_K = \frac{1}{2} m v^2$

$$\lambda = \frac{h}{p} = \frac{h}{mv} = \frac{h}{\sqrt{2m \cdot Ue}}$$

$Ue = \frac{1}{2} m v^2$

$v = \sqrt{\frac{2Ue}{m}}$

$$m = \frac{m_0}{\sqrt{1 - \frac{v_0^2}{c^2}}} = \frac{m_e}{\sqrt{1 - \frac{2Ue}{mc^2}}}$$

$$\approx m_e\left(1 + \frac{Ue}{m_e c^2}\right)$$

$$\frac{h}{\sqrt{2 m_e Ue \frac{1+Ue}{mc^2}}} = 2{,}51\,pm$$

Task 2: How many electron are there in the microscope at one point? (U=300kV, I=1nA, column length: 2m)

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$U = 300\ kV$

$I = 1\ nA$

$l = 2\ m$

$e = 1.61 \cdot 10^{-19}\ C$

$c = 3 \cdot 10^{8}\ \frac{m}{s}$

$E_p = U \cdot e \qquad E_k = \frac{1}{2} m v^2$

$$v = \sqrt{\frac{2Ue}{m}}$$

$$t_0 = \frac{l}{v} = \sqrt{\frac{l^2 m}{2Ue}}$$

$$t = \frac{t_0}{1 - \frac{v^2}{c^2}} = \frac{t_0}{1 - \frac{2Ue}{mc^2}} \cong t_0 \left[1 + \frac{Ue}{mc^2}\right]$$

$$t = \sqrt{\frac{l^2 m}{2Ue}} \left[1 + \frac{Ue}{mc^2}\right]$$

$$I = \frac{n \cdot e}{t}$$

$$n = \frac{I \cdot t}{e} = \frac{I}{e} \sqrt{\frac{l^2 m}{2Ue}} \left[1 + \frac{Ue}{mc^2}\right] = 61{,}2$$

Task 3: What is the radiation damage (electron dose in e-/A2) of the specimen in SEM?
(U=2kV, I=5pA, dwell time: 1us, spot size: 5nm)

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(U=2kV, I=5pA, dwell time: 1us, spot size: 5nm)

$U = 2kV$

$I = 5pA$

$t = 1\mu s$

$d = 5\,nm$

$e = 1{,}61 \cdot 10^{-19}$

$$q = I \cdot t \Rightarrow q_e = \frac{It}{e}$$

$$S = \frac{\pi d^2}{4}$$

$$D\,(e/A^2) = \frac{q_e}{S} = \frac{4It}{\pi d^2 e} = 0{,}016$$

Outline

Image analysis I

- Fourier transforms
    - Why do we care?
    - Theory
    - Examples in 1D
    - Examples in 2D
- Digitization
- Fourier filtration
- Contrast transfer function
- Resolution

Fourier transforms

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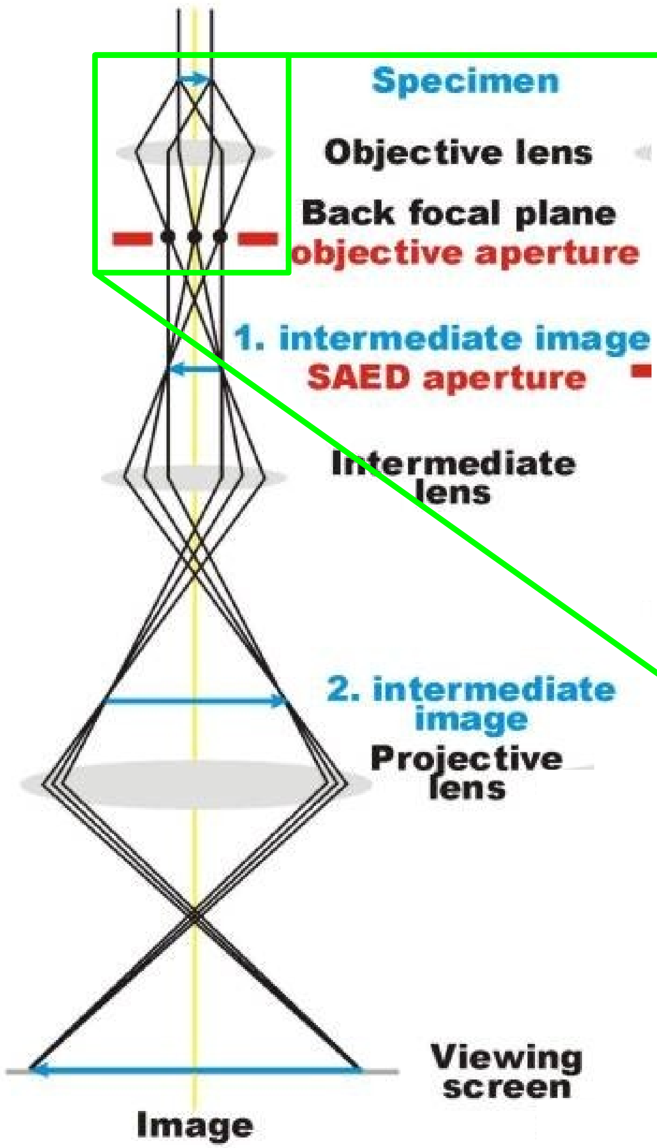
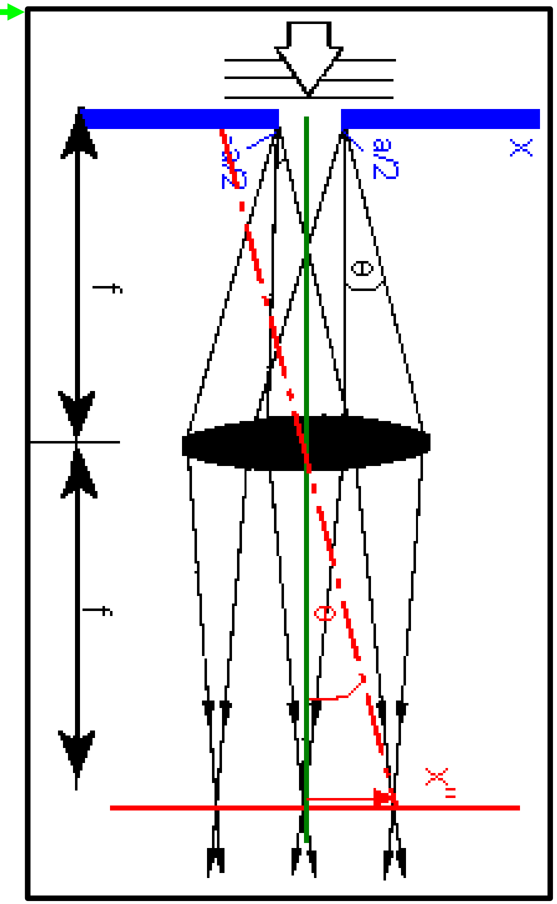
A quiz

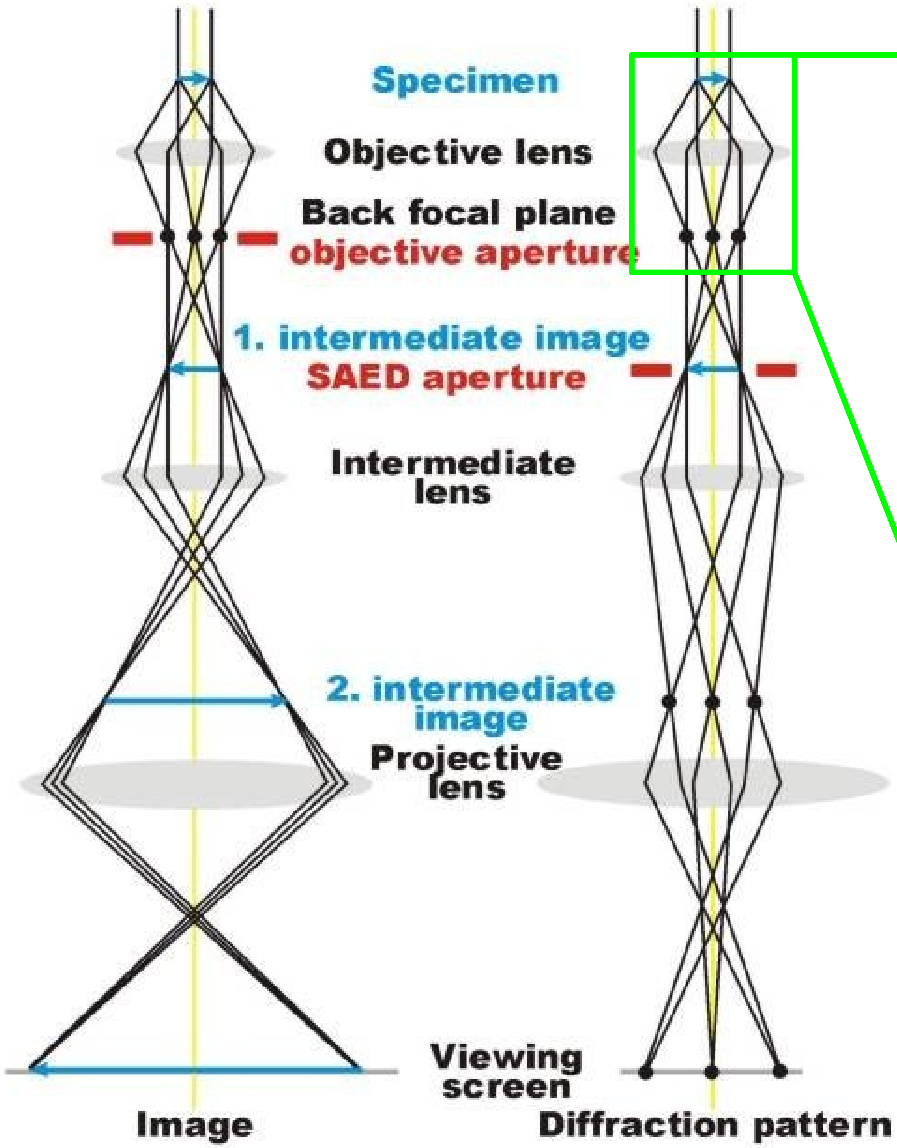


http://www.microscopy.ethz.ch

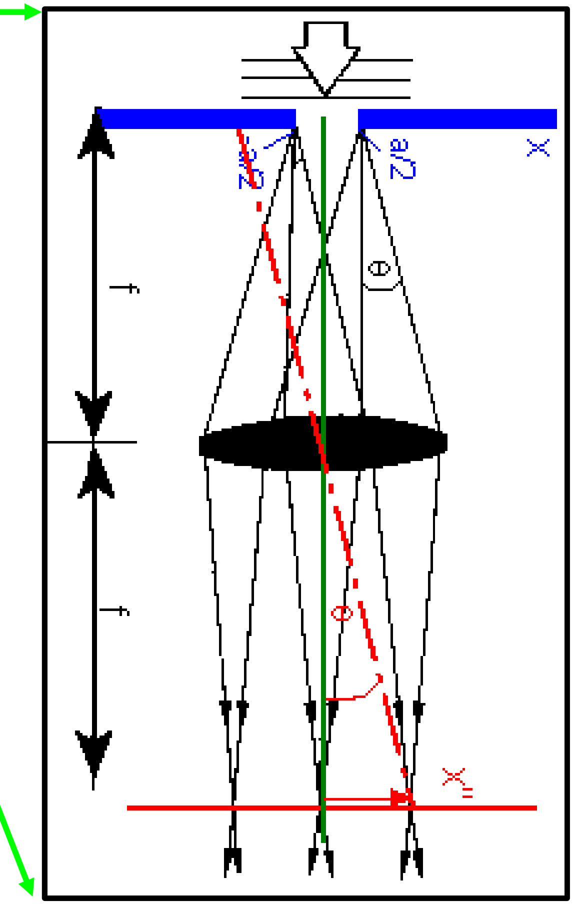


http://electron6.phys.utk.edu

Relationship between imaging and diffraction



http://www.microscopy.ethz.ch



http://electron6.phys.utk.edu

The only difference between microscopy and diffraction is that, in microscopy, you can (easily) focus the scattered radiation into an image.

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Relevance of Fourier transforms to EM

Fourier transform ~ diffraction pattern

see John Rodenburg's site, http://rodenburg.org

NOTE: $\nu = \alpha / \lambda$

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: Exponential form

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

f:	function which we are transforming (1D)
x:	axis coordinate
i:	√-1
k:	spatial frequency
F(k):	Fourier coefficient at frequency k

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Fourier transforms: Exponential form

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

Euler's Formula: $e^{i\phi}=\cos\phi+i\sin\phi$

$$F(k)=\underbrace{\int_{-\infty}^{\infty} f(x)\cos(-2\pi kx)\,dx}_{a}+i\underbrace{\int_{-\infty}^{\infty} f(x)\sin(-2\pi kx)\,dx}_{b}$$

$a \quad +i \quad b$

Fourier transforms: Sines + cosines

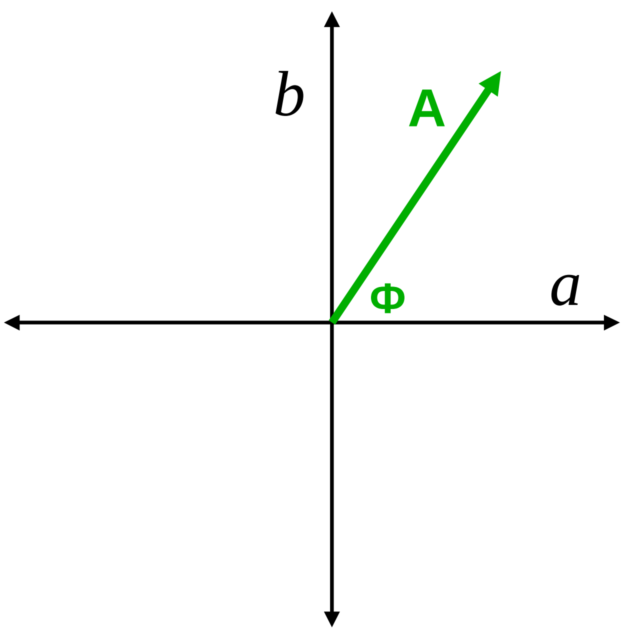
$$F(k) = \int_{-\infty}^{\infty} f(x) e^{-2\pi i k x} dx$$

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

(NOTE: This isn't the same a & b from the previous slide.)

Fourier transforms: Definition

$$F(k)=\underbrace{\int_{-\infty}^{\infty} f(x)\cos(-2\pi kx)\,dx}_{a}\;\underbrace{+i}_{+i}\;\underbrace{\int_{-\infty}^{\infty} f(x)\sin(-2\pi kx)\,dx}_{b}$$



Amplitude, A: $\sqrt{a^2+b^2}$

Phase, Φ: $\arctan\dfrac{b}{a}$

Fourier coefficients, discrete functions

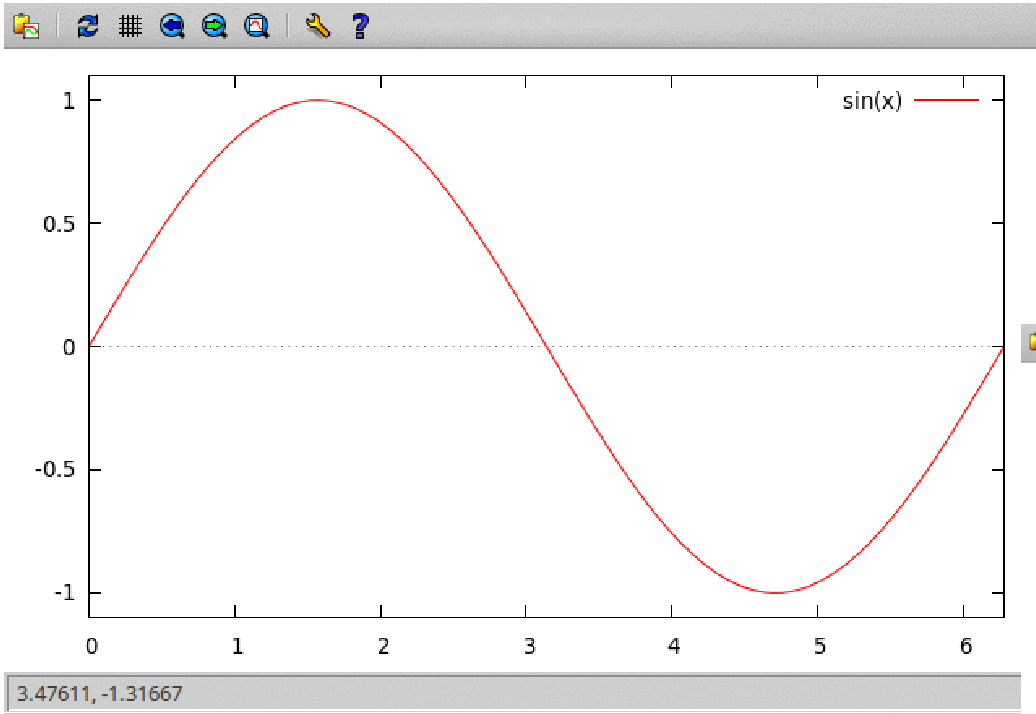
$$f(x) = \frac{1}{2}a_0 + \sum_{n=1}^{\infty} a_n \cos(nx) + \sum_{n=1}^{\infty} b_n \sin(nx)$$

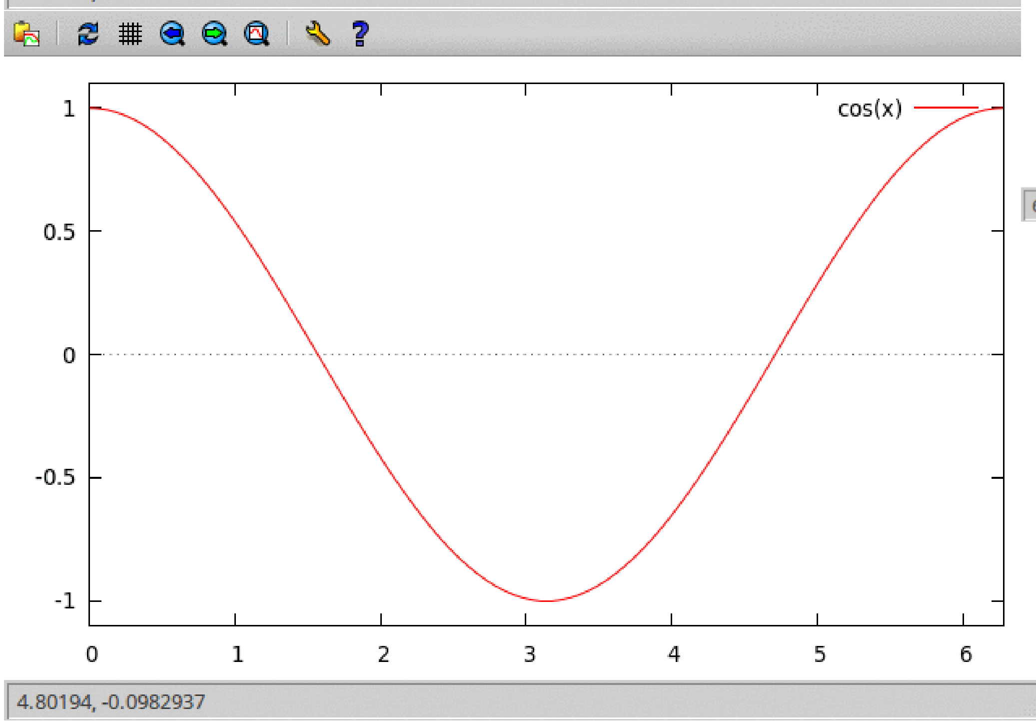
$$a_n = \frac{1}{\pi}\int_{-\pi}^{\pi} f(x)\cos(nx)dx$$

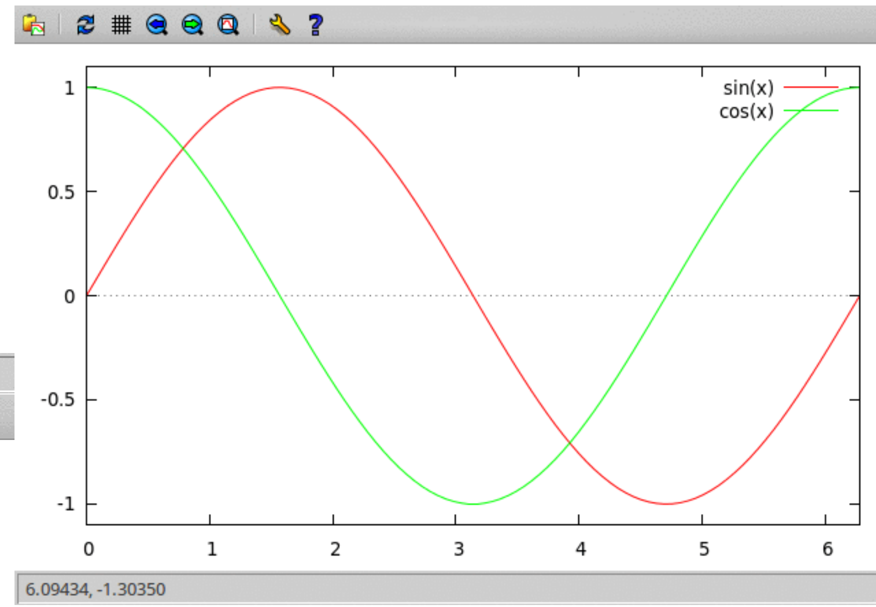
$$b_n = \frac{1}{\pi}\int_{-\pi}^{\pi} f(x)\sin(nx)dx$$

Task 1: Calculate Fourier transform coefficients of following signal?

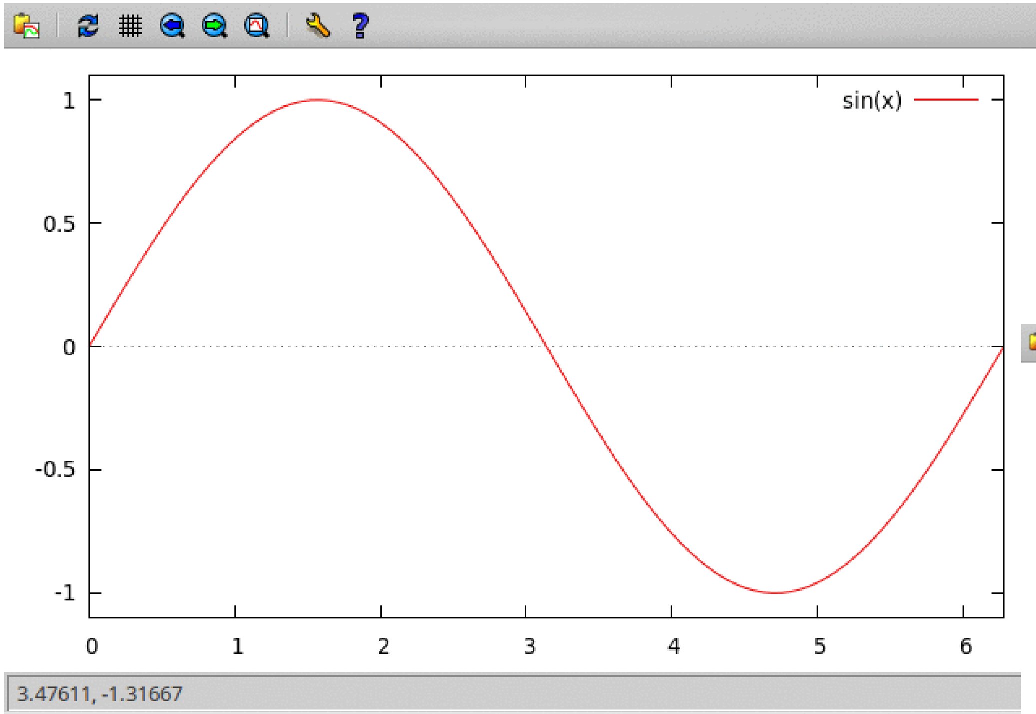
8 5 3 7 0 -1 9 1

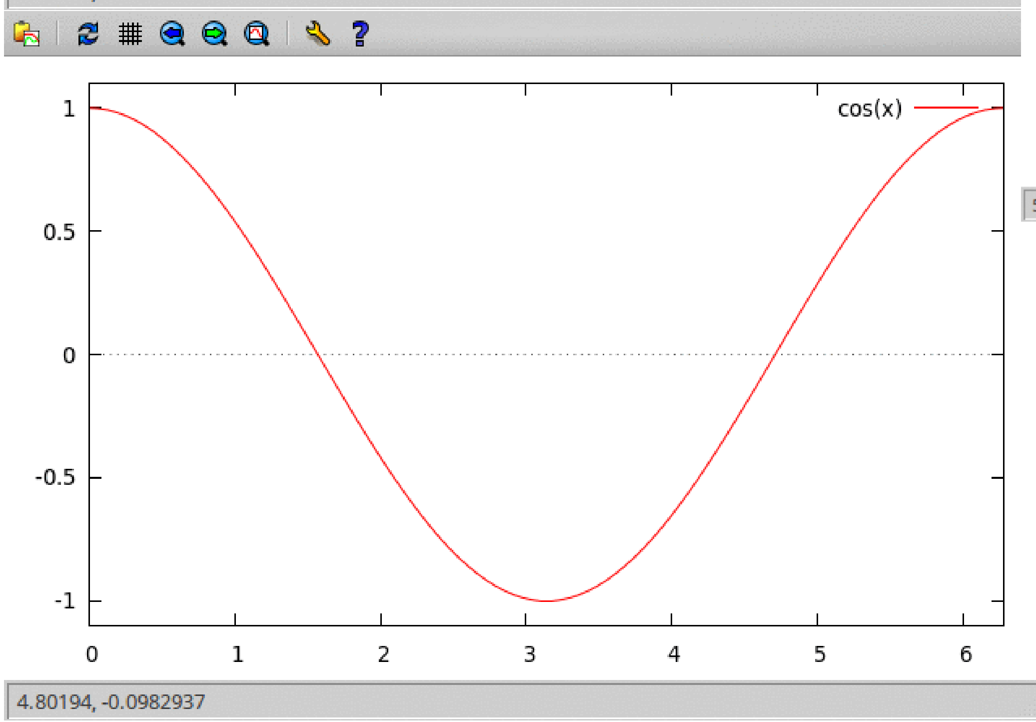


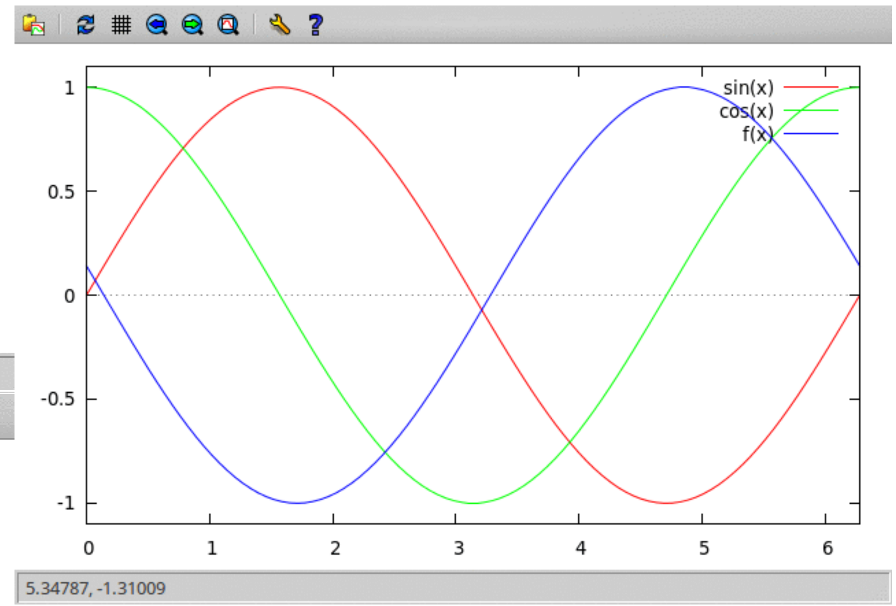




sine and cosine are orthogonal.
The inner product of the two is zero.

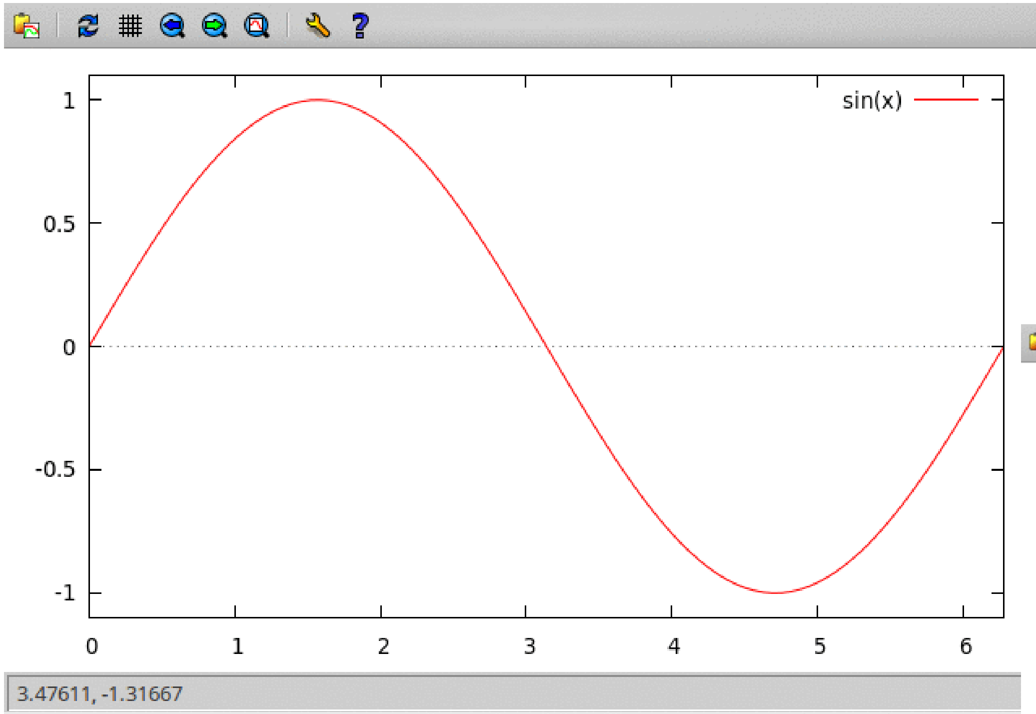


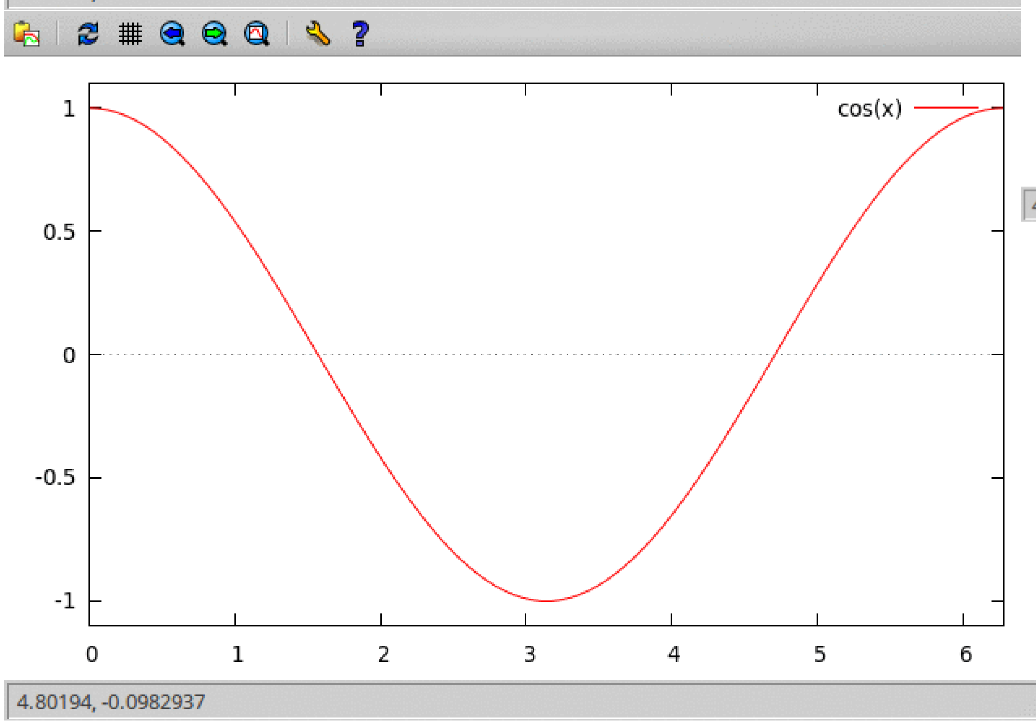


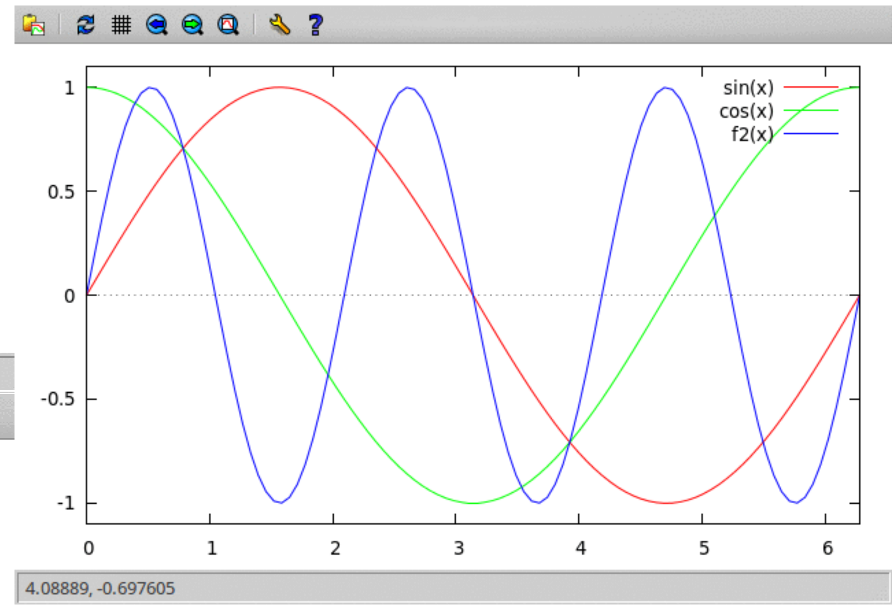


A function with the same frequency but with an offset will have some components of both sine and cosine.

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

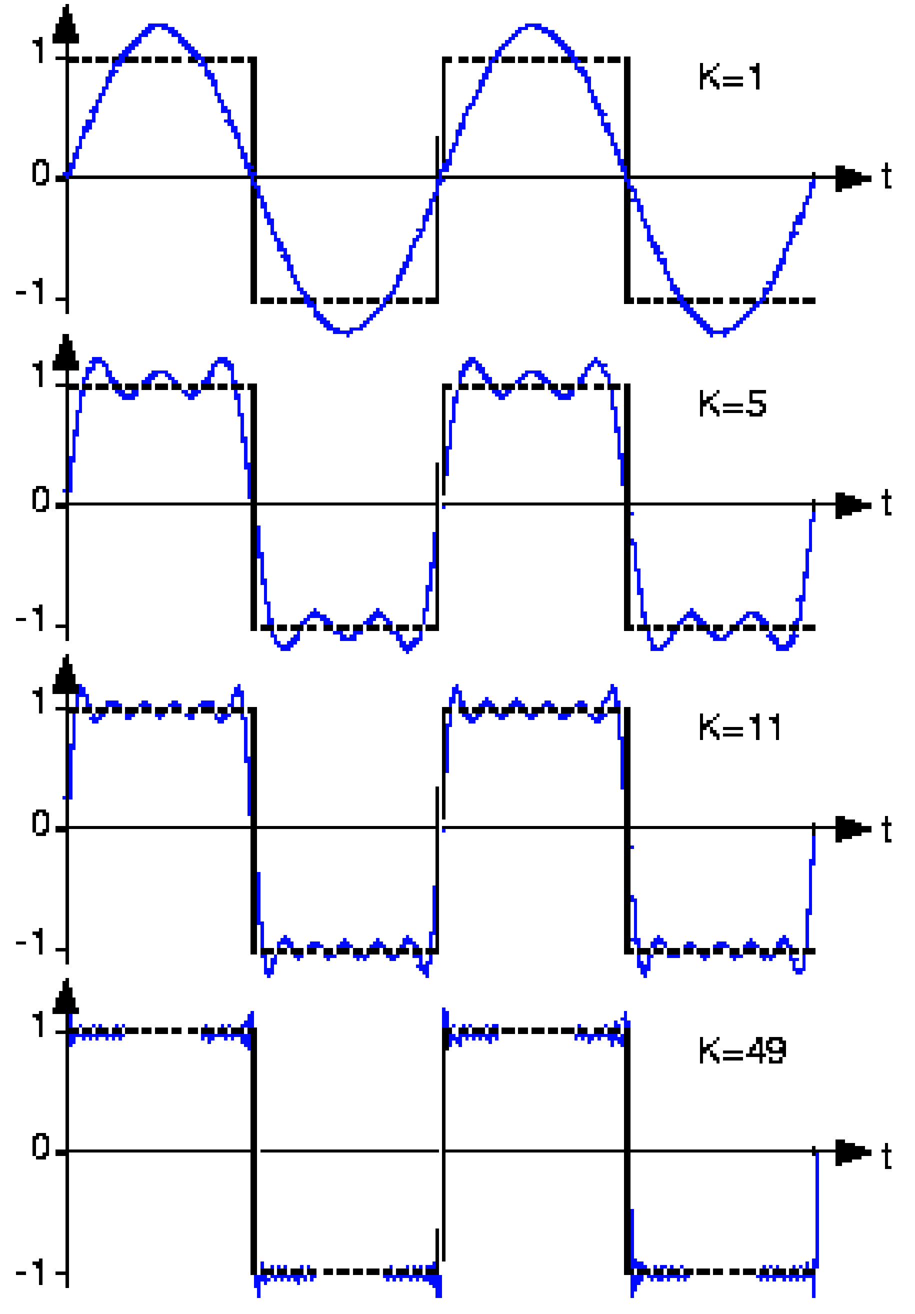






A function with a different frequency will have coefficients of zero.

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

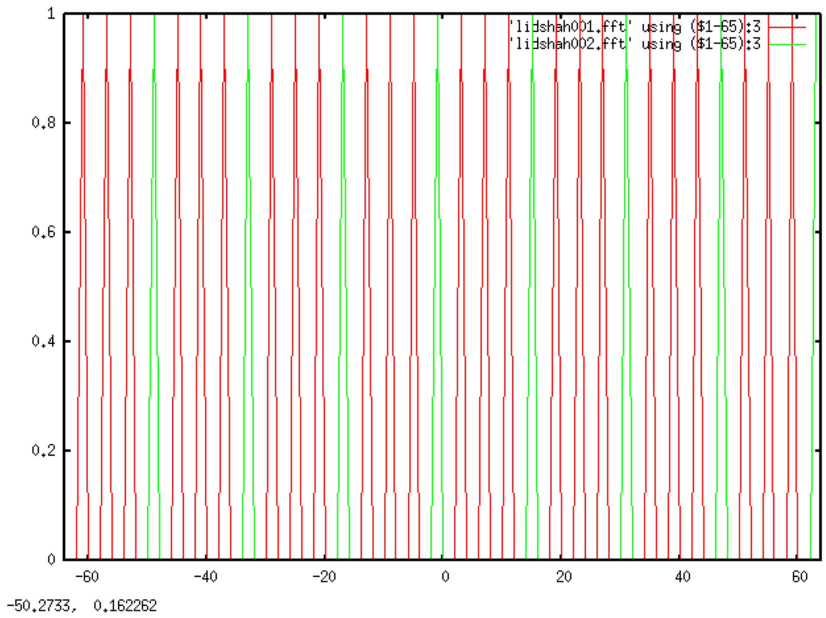


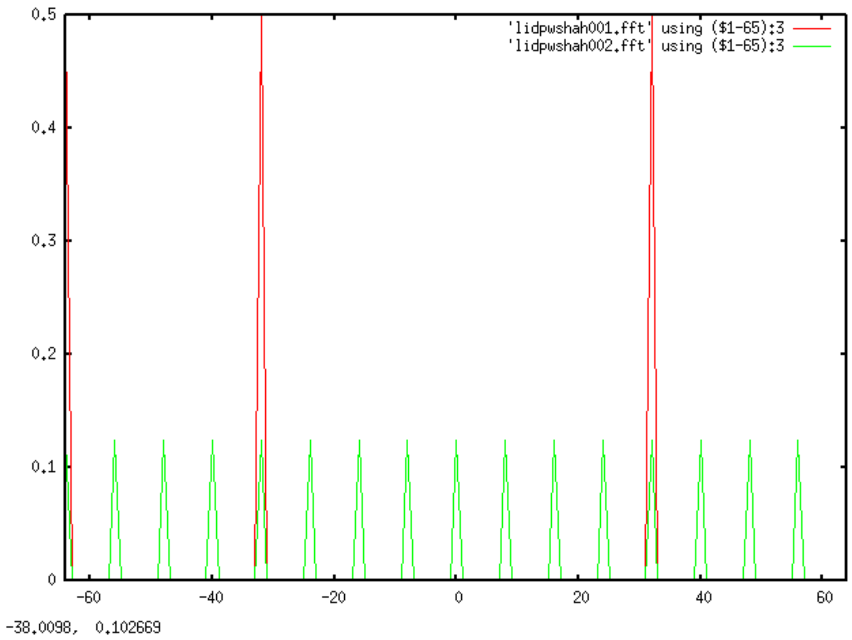
http://cnx.org

Some properties

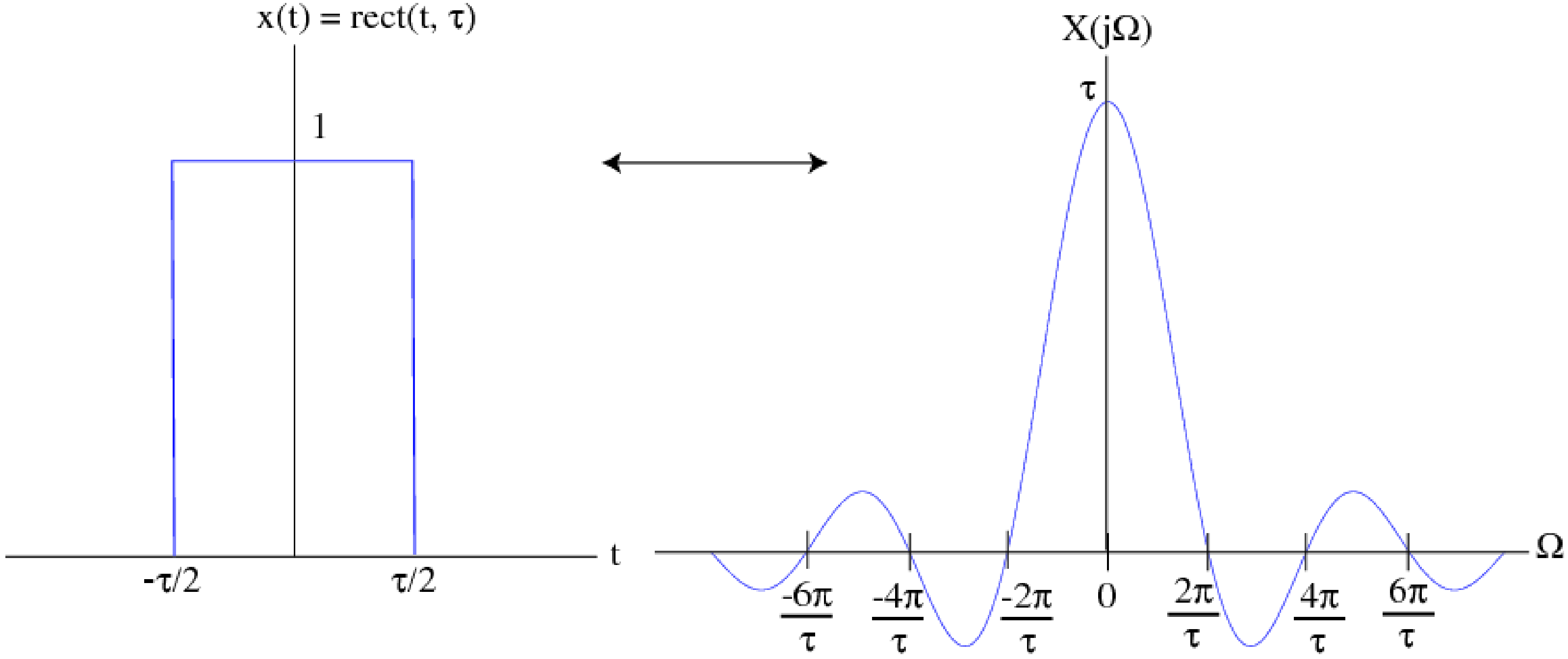
- As *n* increases, so does the spatial frequency, *i.e.*, the “resolution.”
  - For example, sin(2*x*) oscillates faster than sin(*x*)
- Computation of a Fourier transform is a completely reversible operation.
  - There is no loss of information.
- Fourier terms (or coefficients) have amplitude and 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





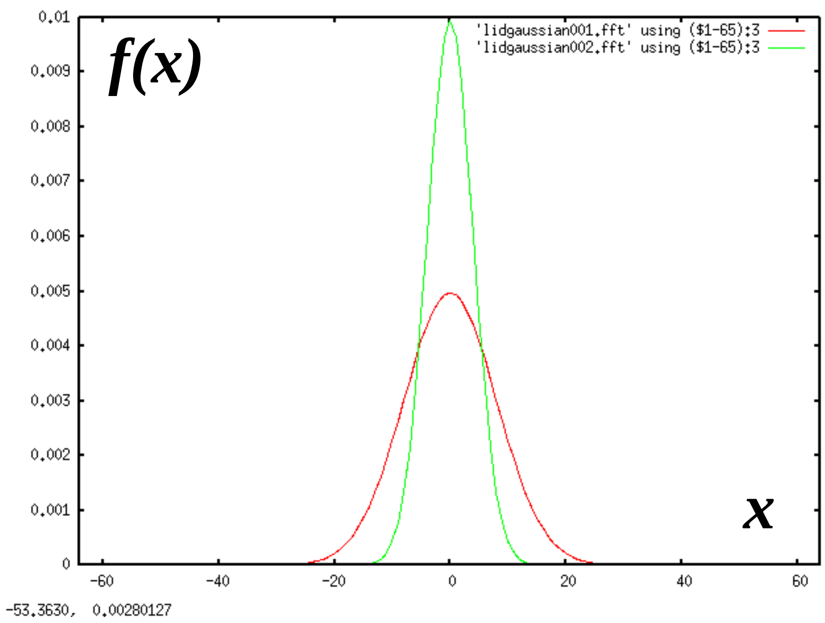
Some simple 1D transforms:
a box

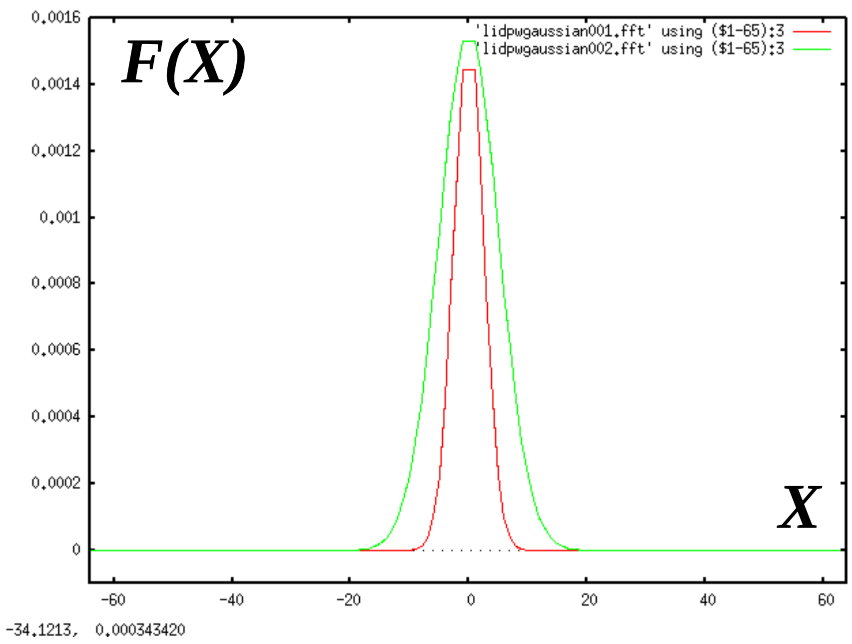


http://cnx.org

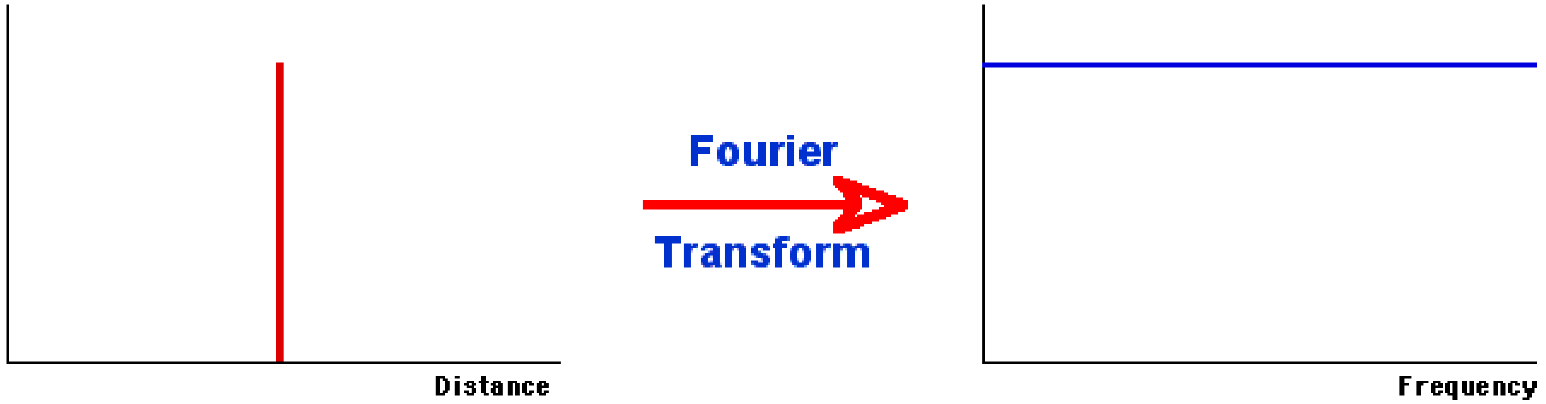
Later, you will learn that multiplying a step function is bad, because of these ripples in Fourier space.

Fourier transforms: plot of a Gaussian



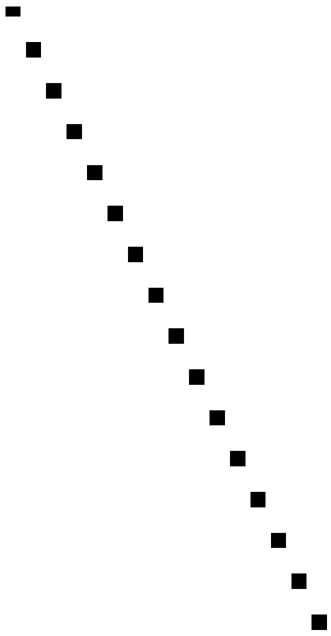


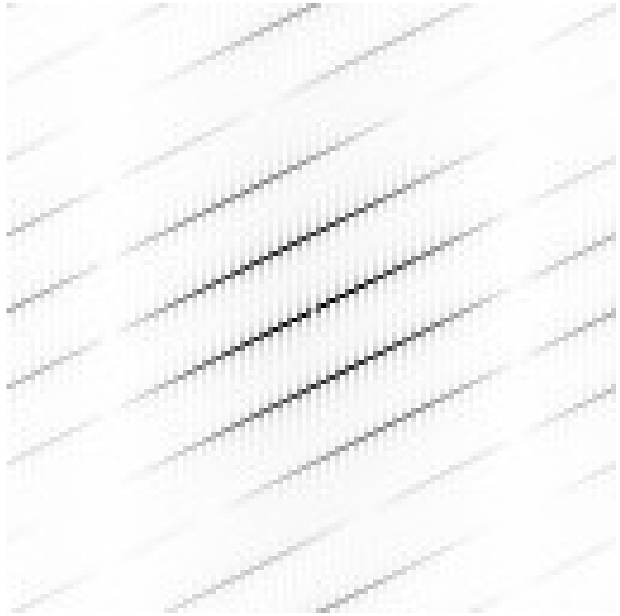
Some simple 1D transforms:
a sharp point (Dirac delta function)



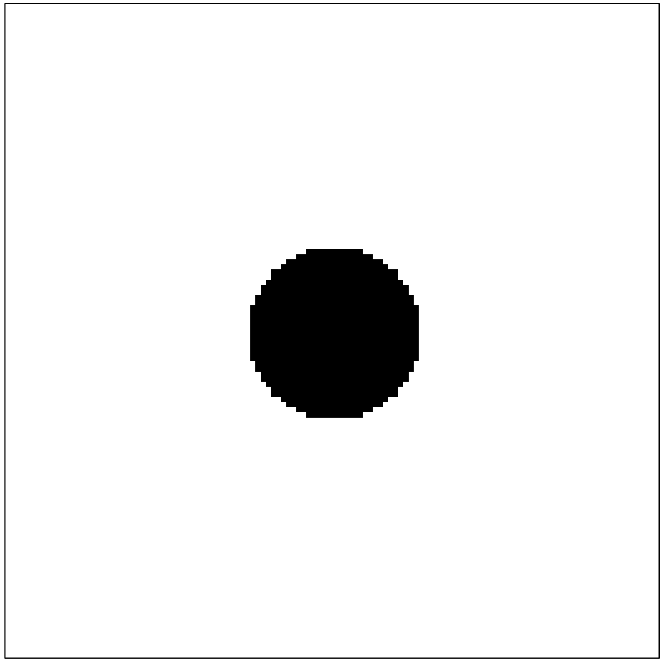
http://en.labs.wikimedia.org/wiki/Basic_Physics_of_Nuclear_Medicine/Fourier_Methods

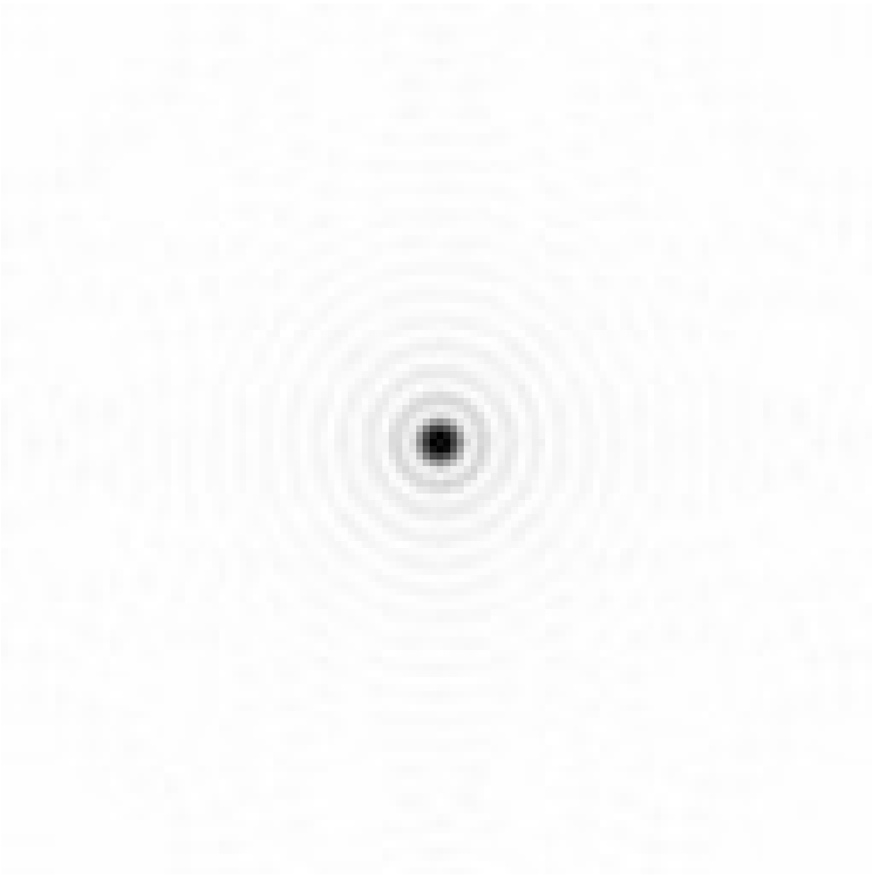
Some simple 2D Fourier transforms: a row of points



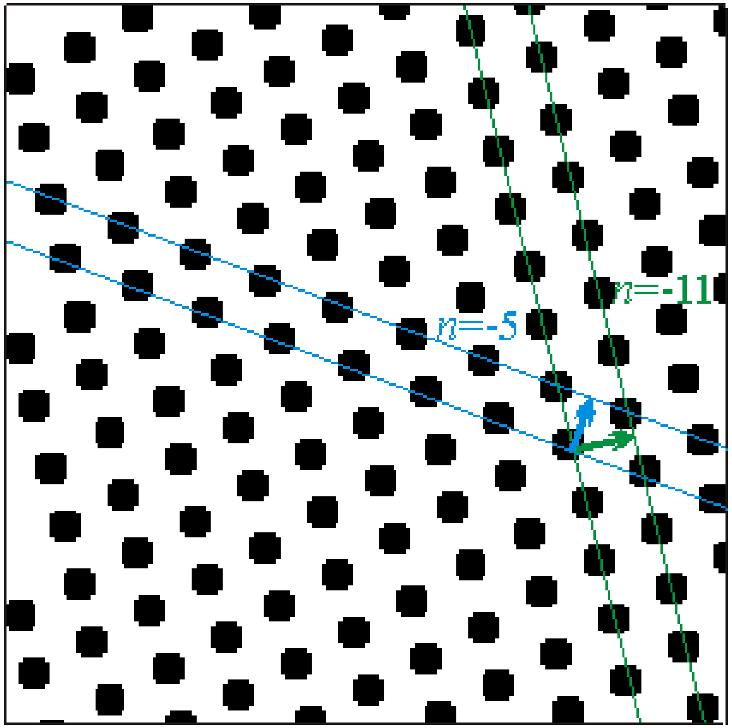


Some simple 2D Fourier transforms: a sharp disc

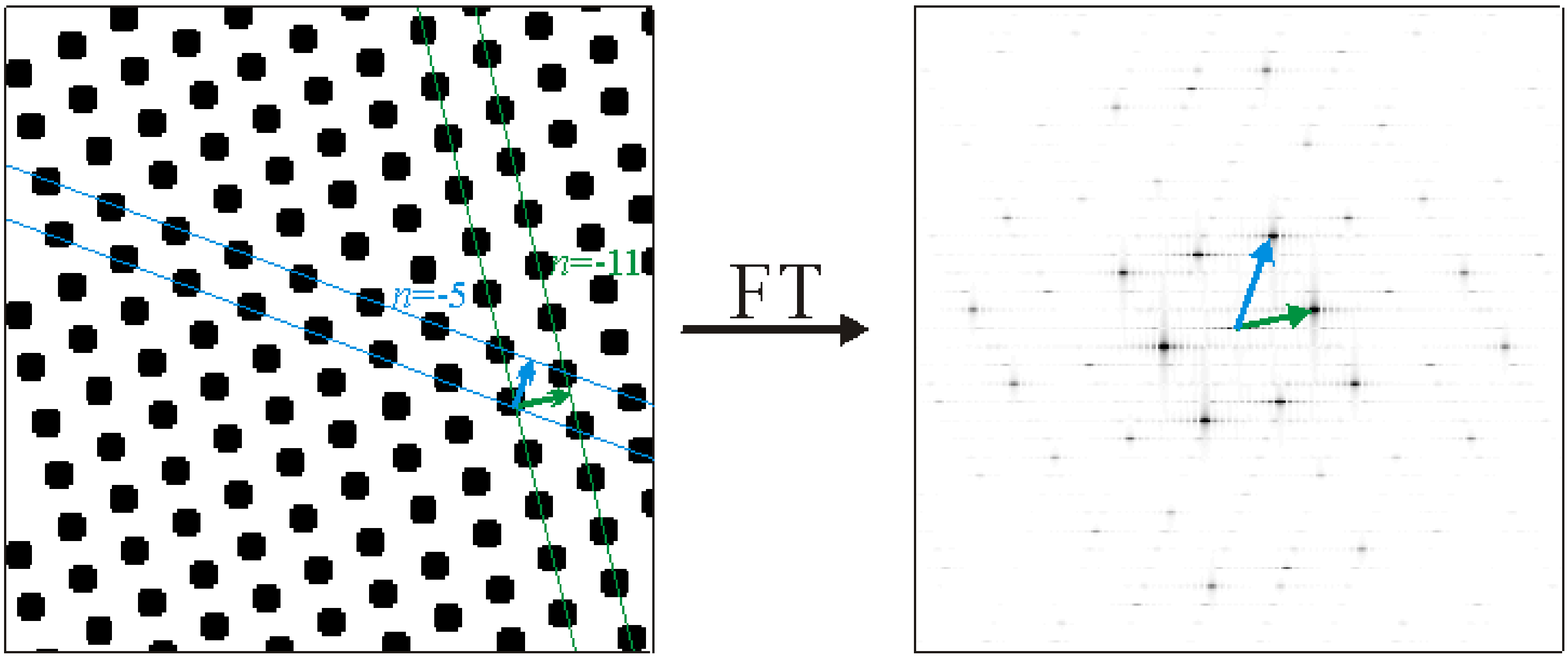




Some simple 2D Fourier transforms: a 2D lattice



Some simple 2D Fourier transforms: a 2D lattice



Some simple 2D Fourier transforms: a helix

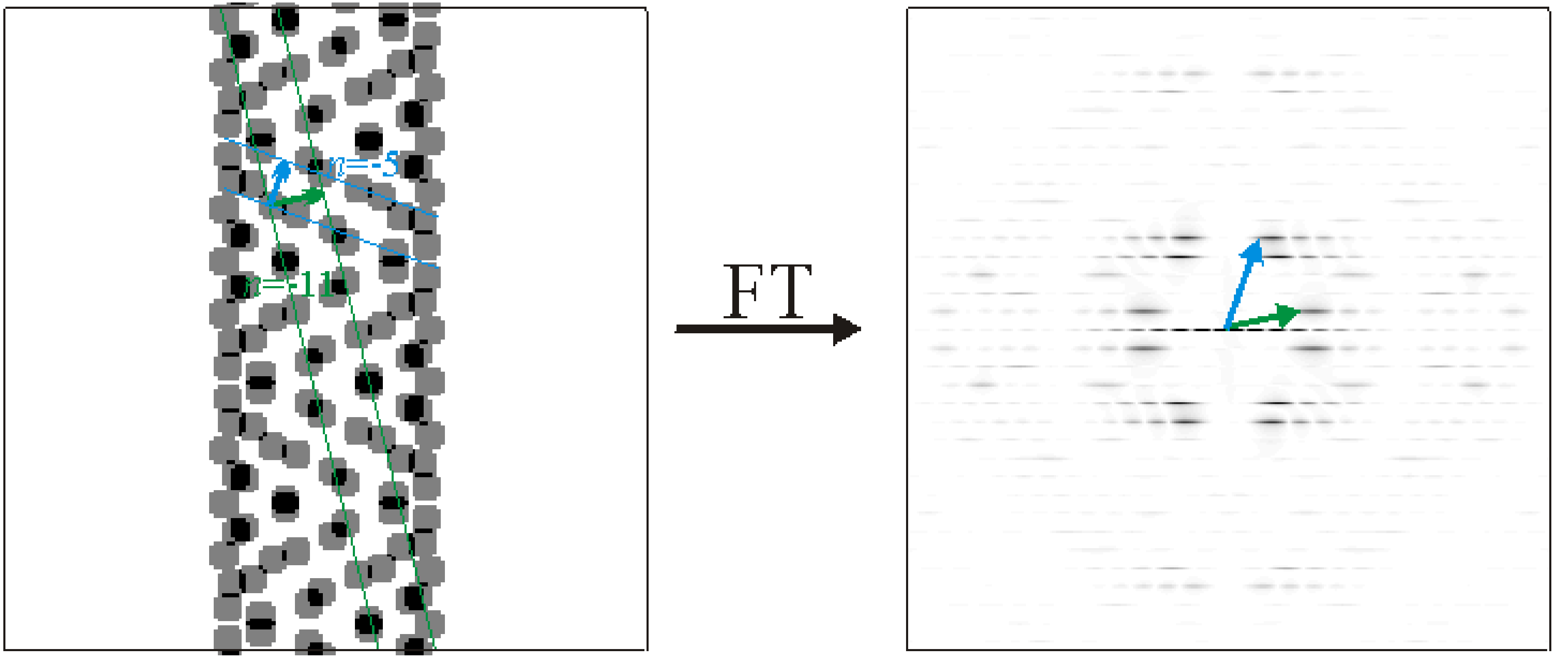


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Image analysis I

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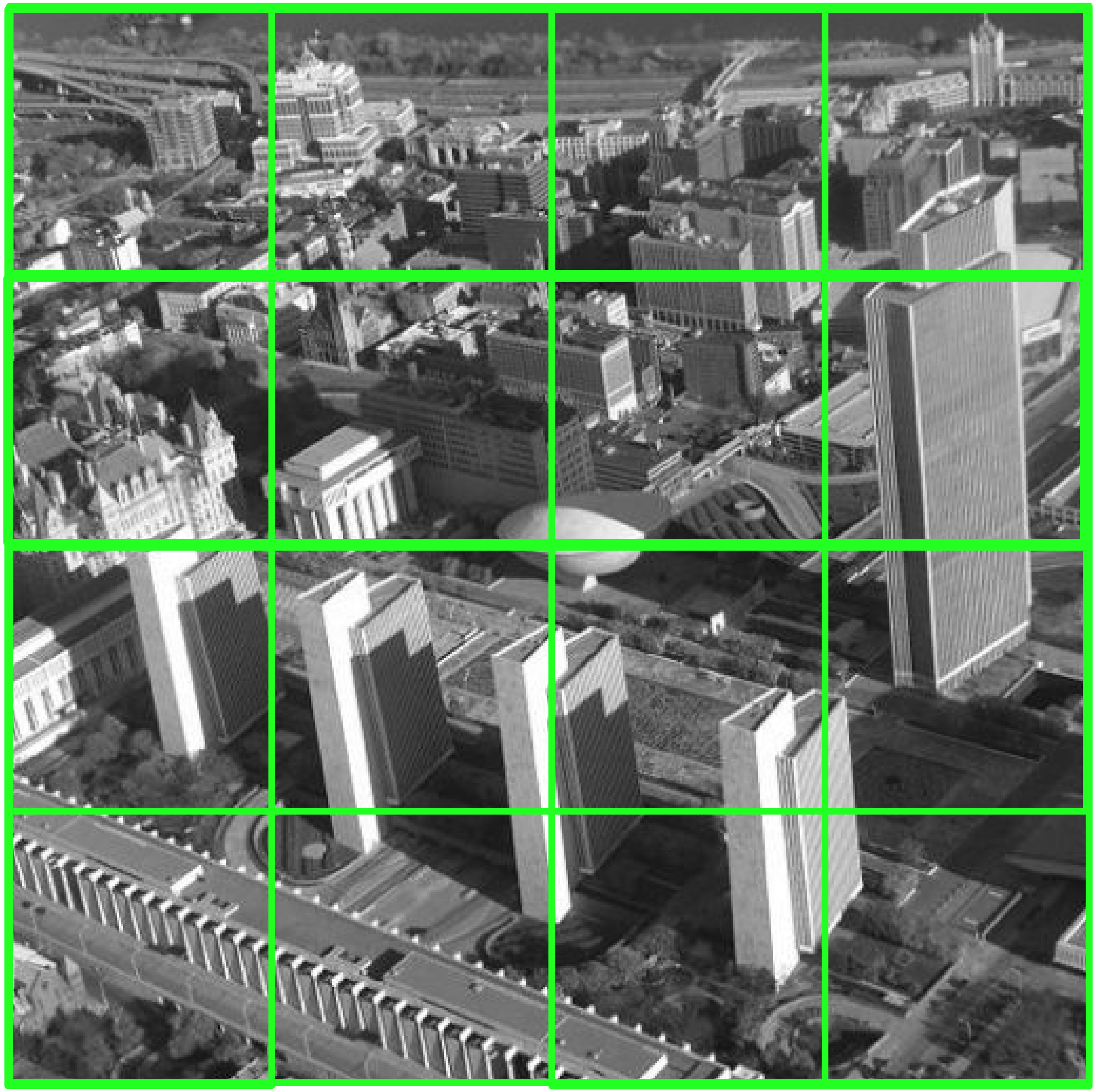
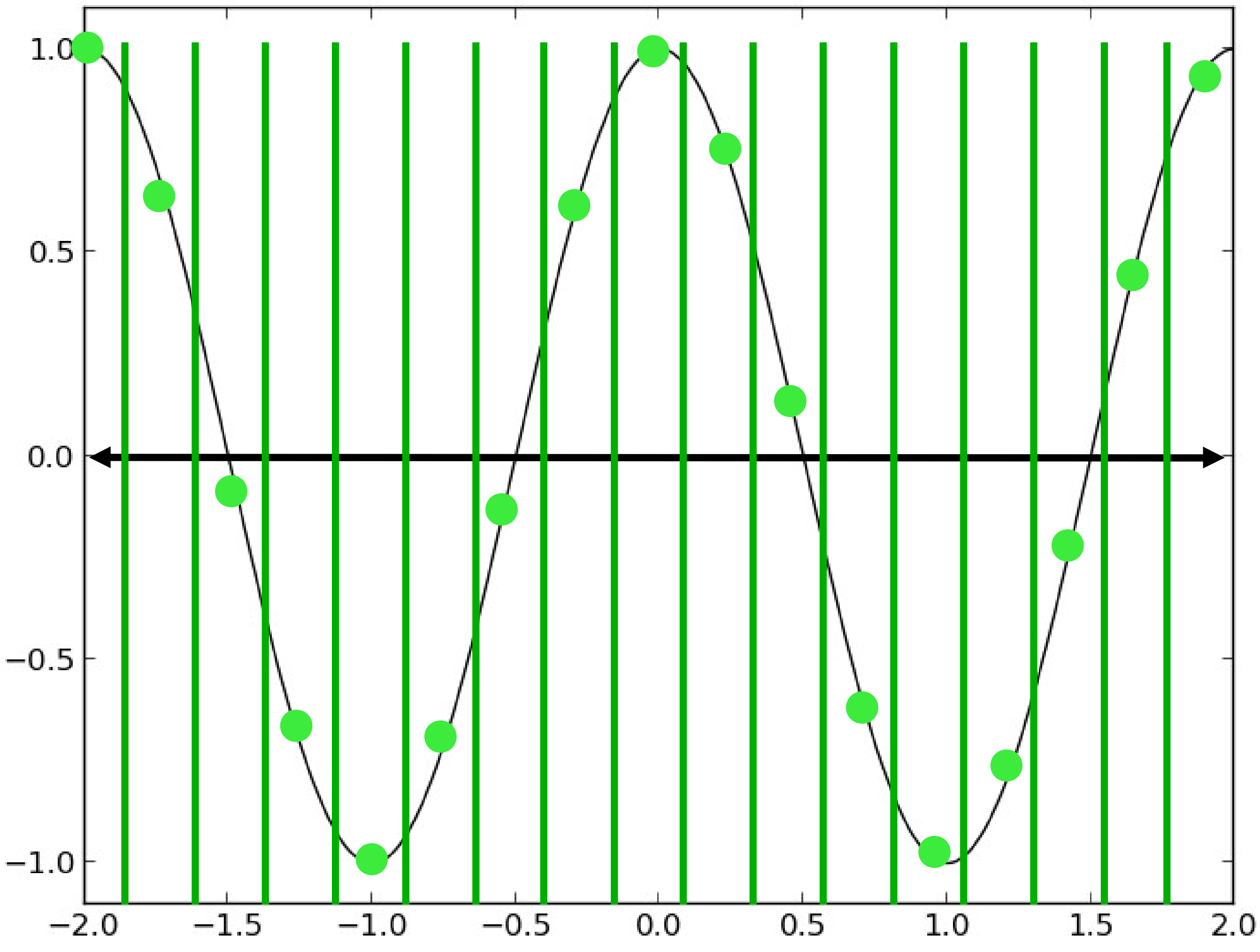
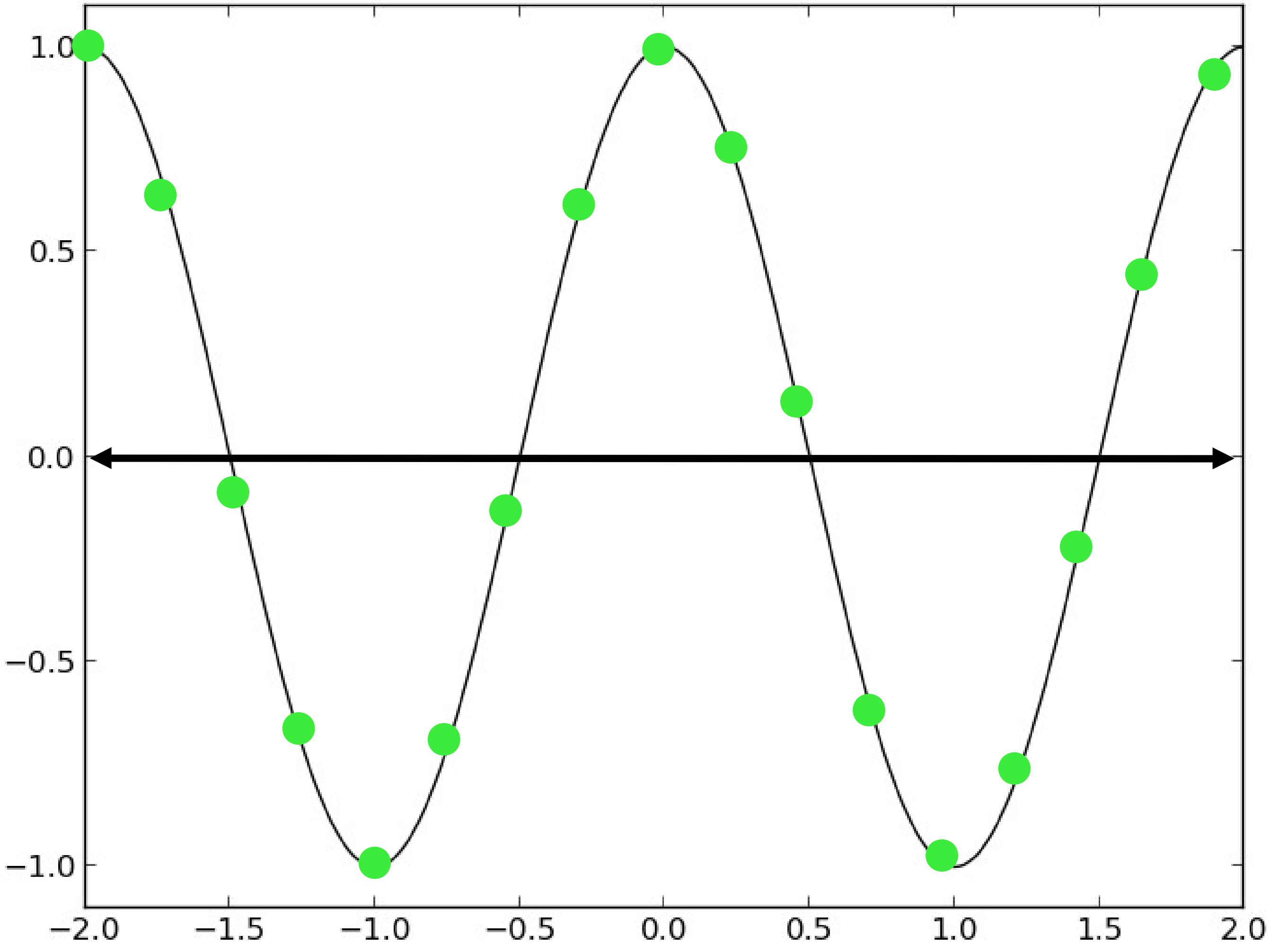
Digitization in 2D



Digitization in 1D: Sampling



Digitization: Is our sampling good enough?



Here, our sampling is good enough.

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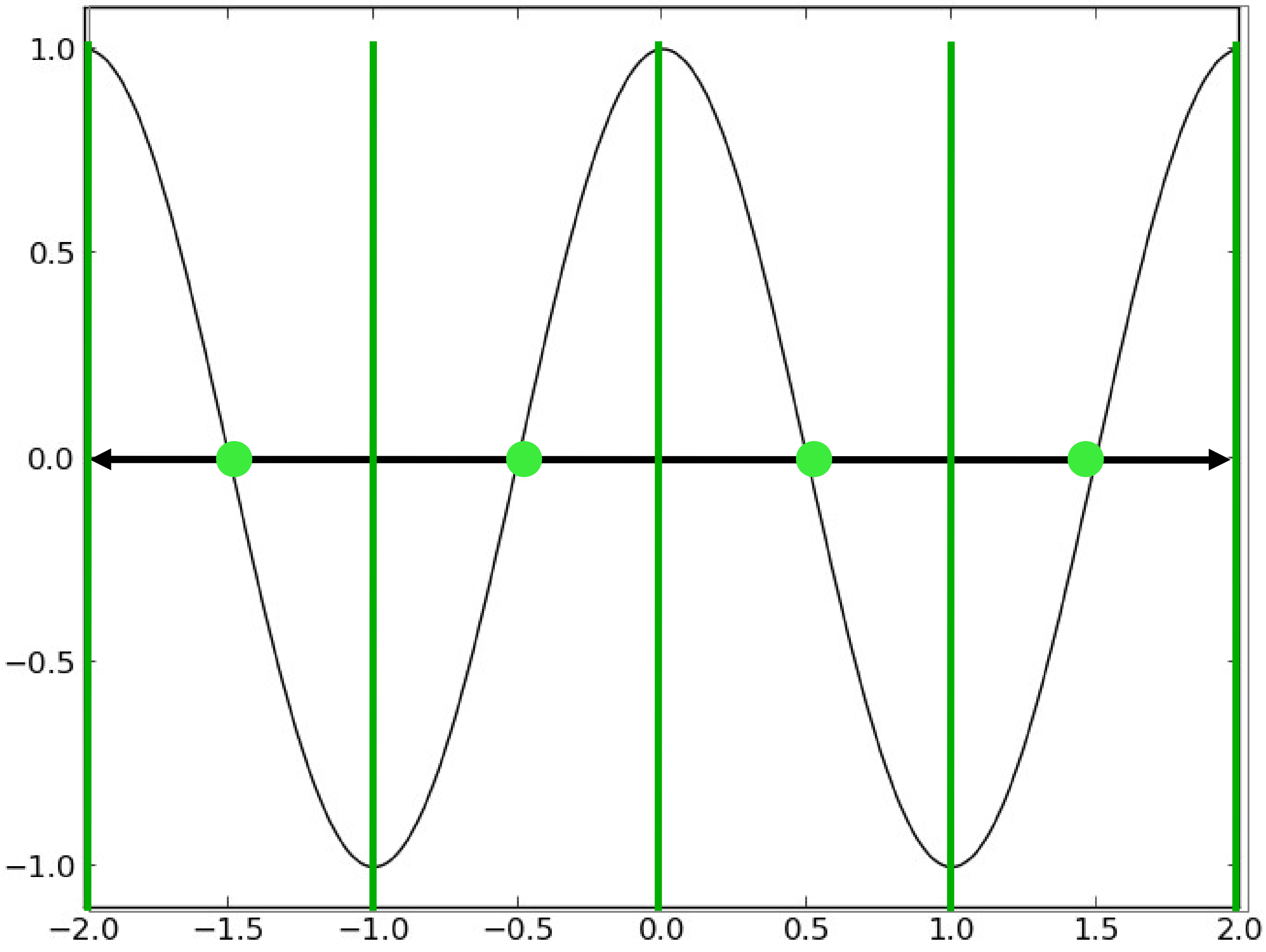
Digitization in 1D: Bad sampling



Discrete Fourier Transform

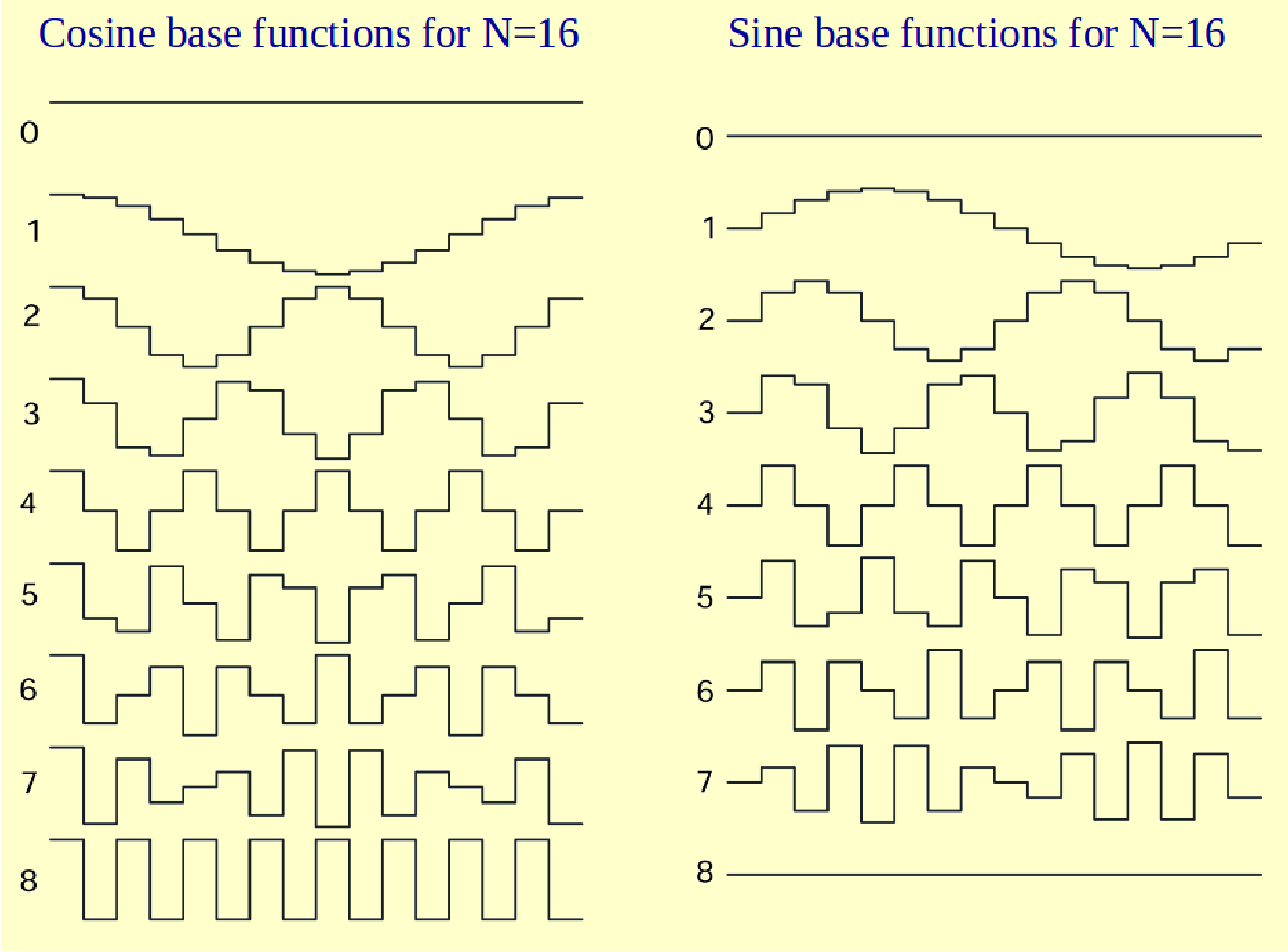
- 1D discrete Fourier transform of function f(x)

$$\Phi(\omega_x) = \sum_{x=0}^{N-1} f(x)\, e^{-i(\frac{2\pi}{N}\omega_x x)}$$

- 1D inverse discrete Fourier transform of function $\Phi(\omega_x)$

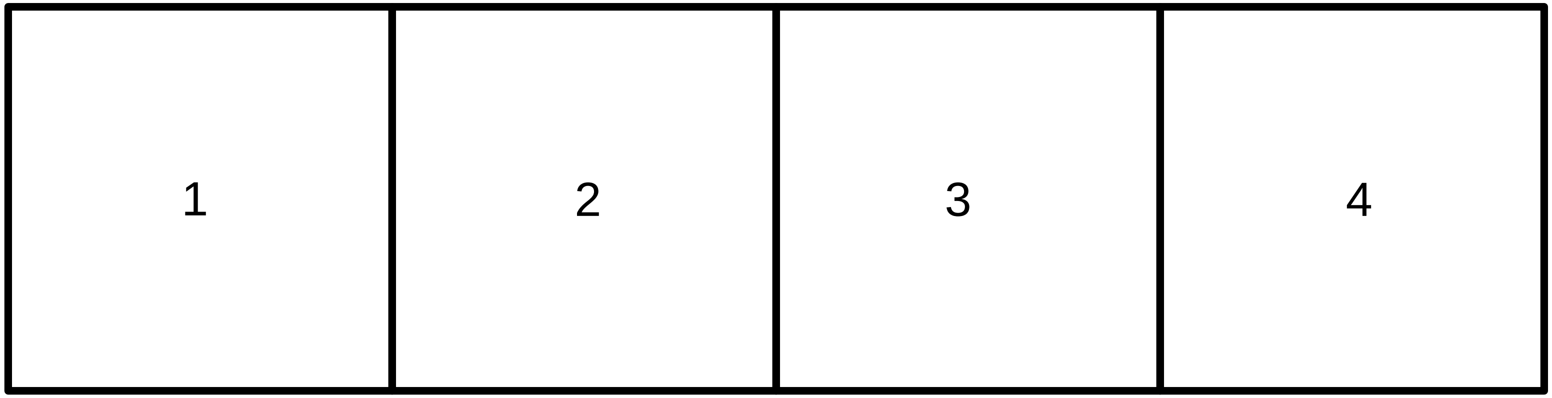
$$f(x) = \frac{1}{N} \sum_{\omega_x=0}^{N-1} \Phi(\omega_x)\, e^{i(\frac{2\pi}{N}\omega_x x)}$$

Discrete Fourier Transform



Task 2: Show that Discrete Fourier Transform is periodic?

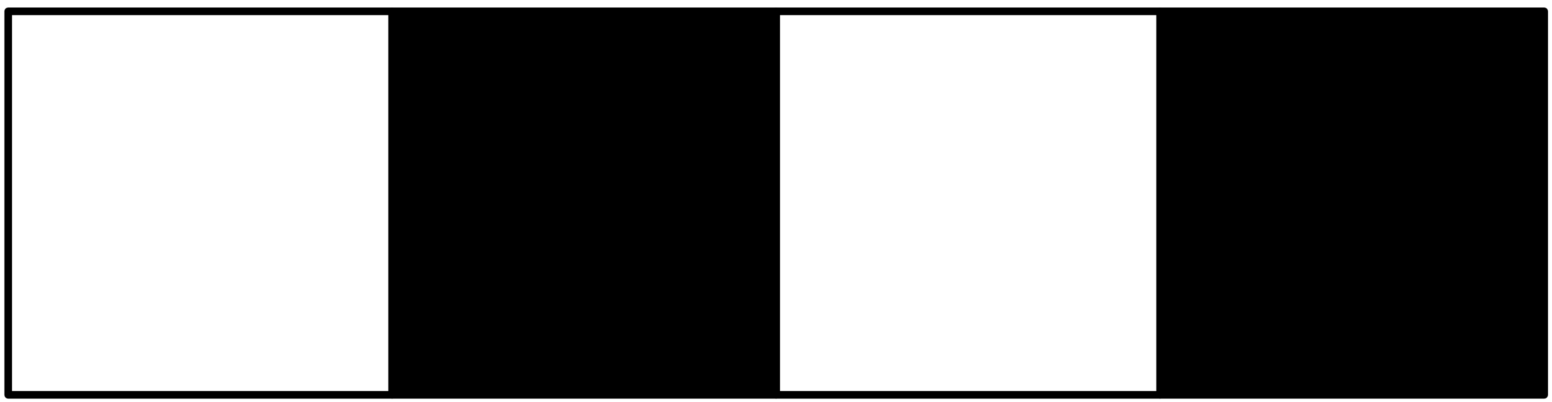
What's the best resolution we can get from a given sampling rate?



A 4-pixel "image"

In other words, what is the most rapid oscillation we can detect?

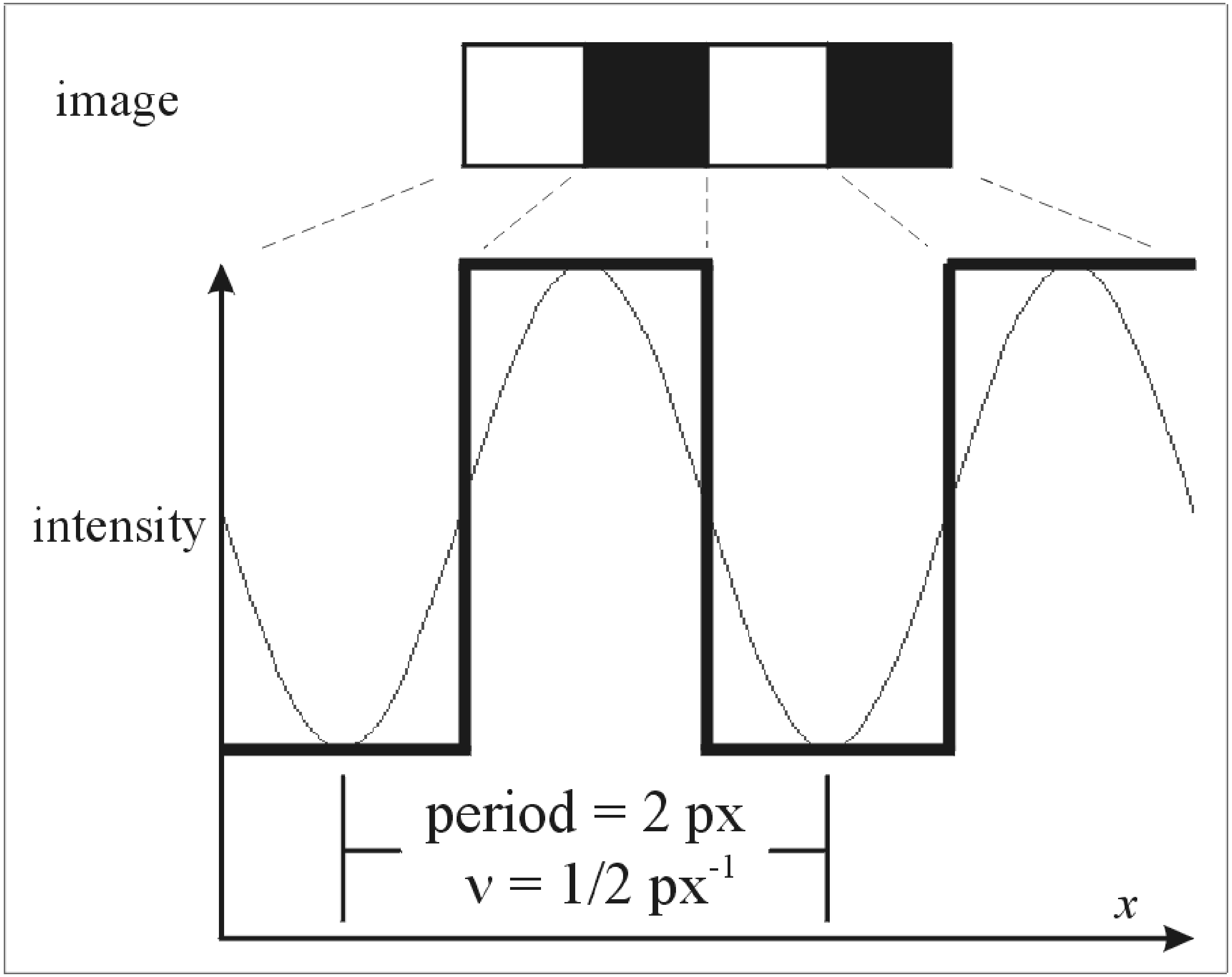
What's the best resolution we can get from a given sampling rate?



A 4-pixel “image”

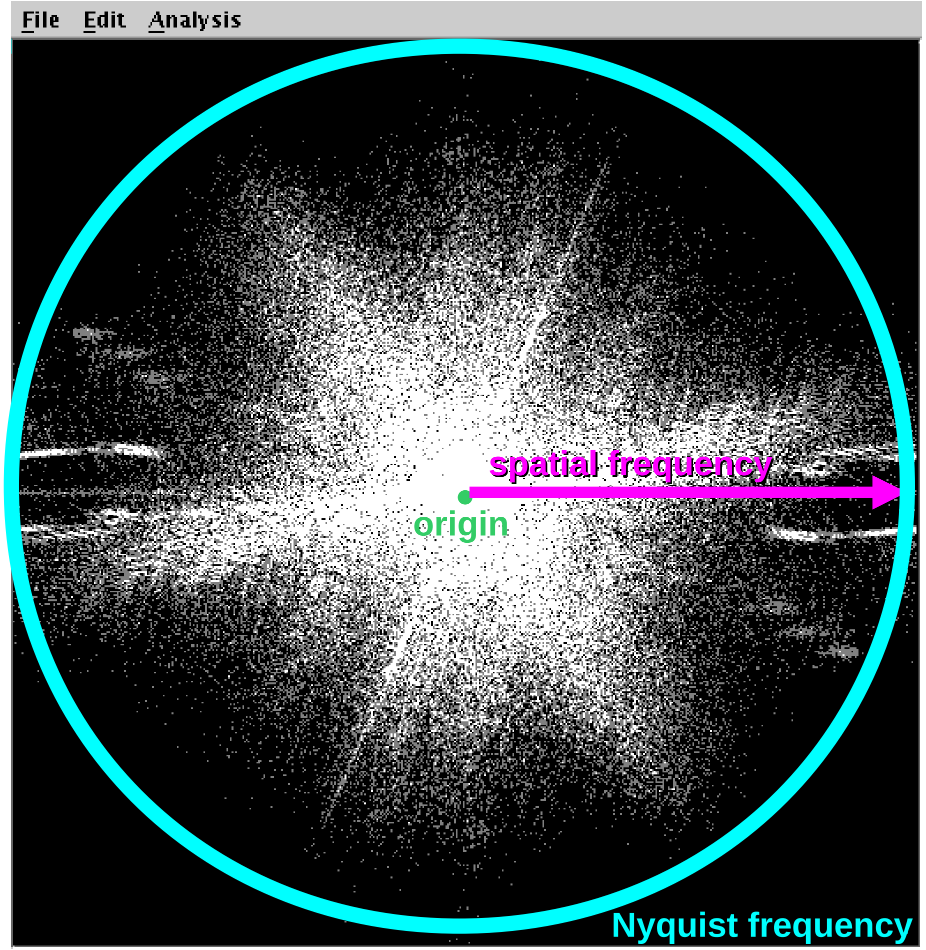
In other words, what is the most rapid oscillation we can detect?

ANSWER: Alternating light and dark pixels.



The period of this finest oscillation is 2 pixels.
The spatial frequency of this oscillation is 0.5 px^{-1}.
The finest detectable oscillation is what is known as “Nyquist frequency.”
The edge of the Fourier transform corresponds to Nyquist frequency.

Nyquist frequency



The period of this finest oscillation is 2 pixels.
The spatial frequency of this oscillation is 0.5 px^{-1}.
The finest detectable oscillation is what is known as “Nyquist frequency.”
The edge of the Fourier transform corresponds to Nyquist frequency.

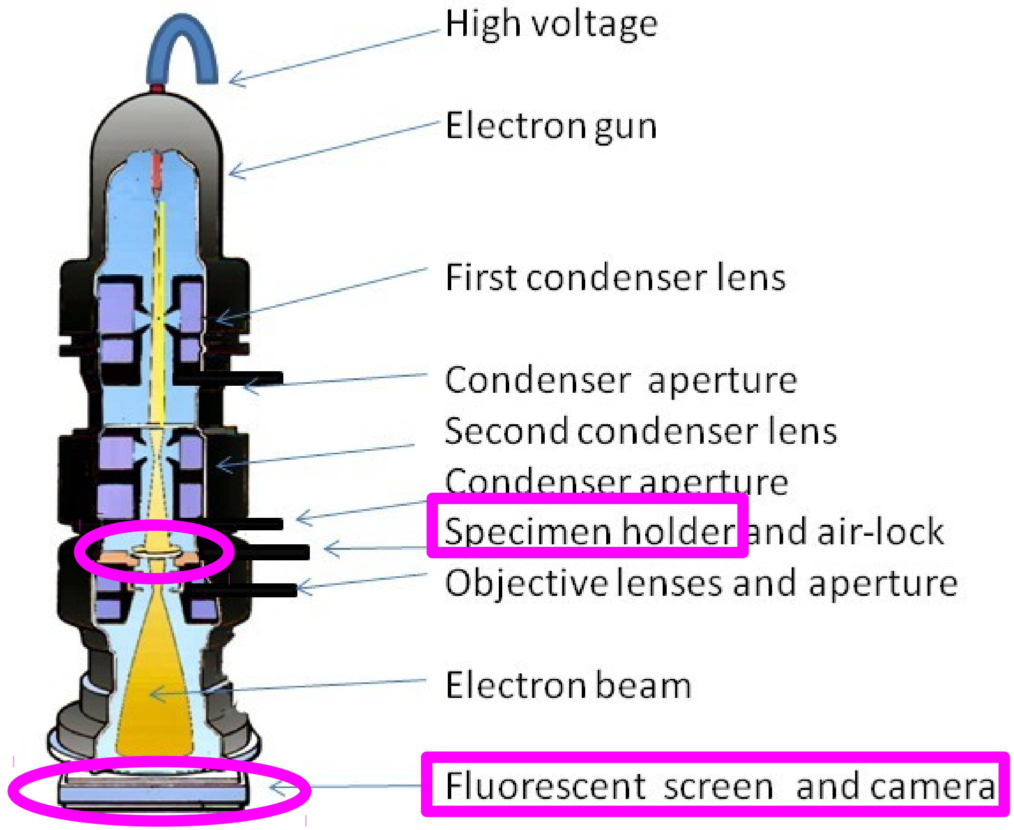
What do we mean by pixel size?

Typical magnification: 50,000X
Typical detector element: 15µm
(pixel size on the camera scale)

Pixel size on the specimen scale:
15×10^{-6} m/px / 50000 =
3.0×10^{-10} m/px = **3.0 Å/px**

In other words,
the best resolution we
can achieve (or, the
finest oscillation we
can detect) at 3.0 Å/px
is **6.0 Å**.

It will be worse due to interpolation,
so to be safe, a pixel should be 3X
smaller than your target resolution.



Transmission Electron Microscope

http://www.en.wikipedia.org

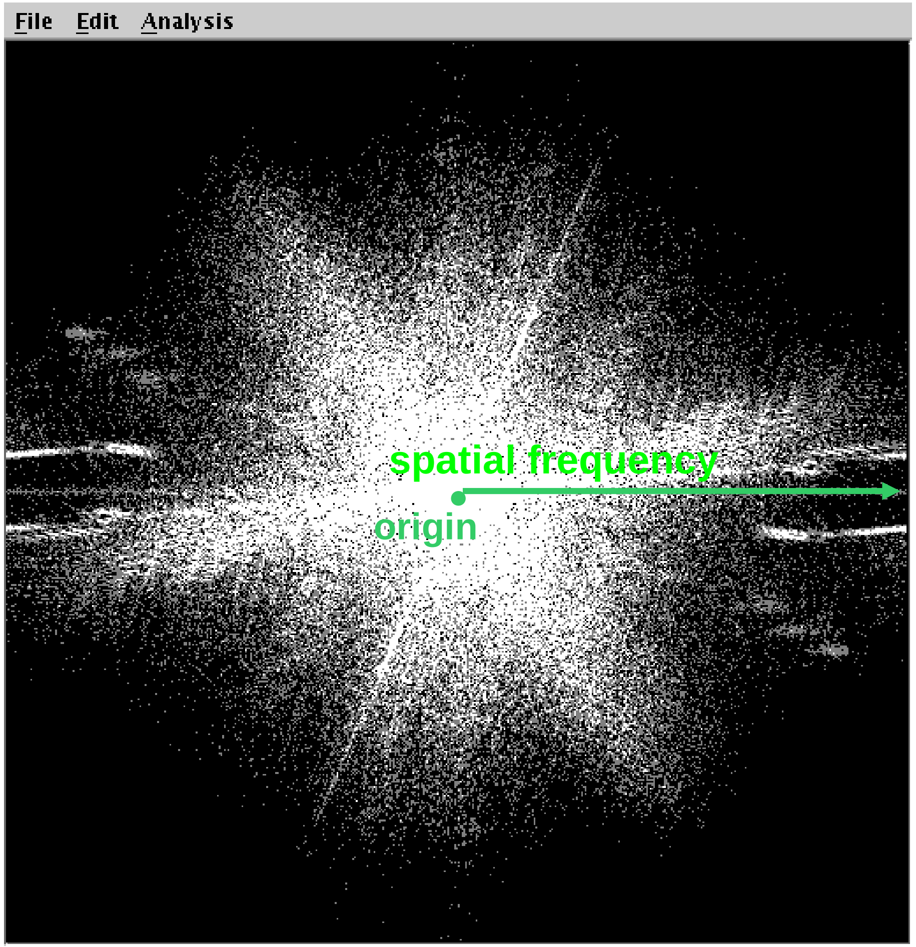
What happens if you're not oversampled enough?

Aliasing

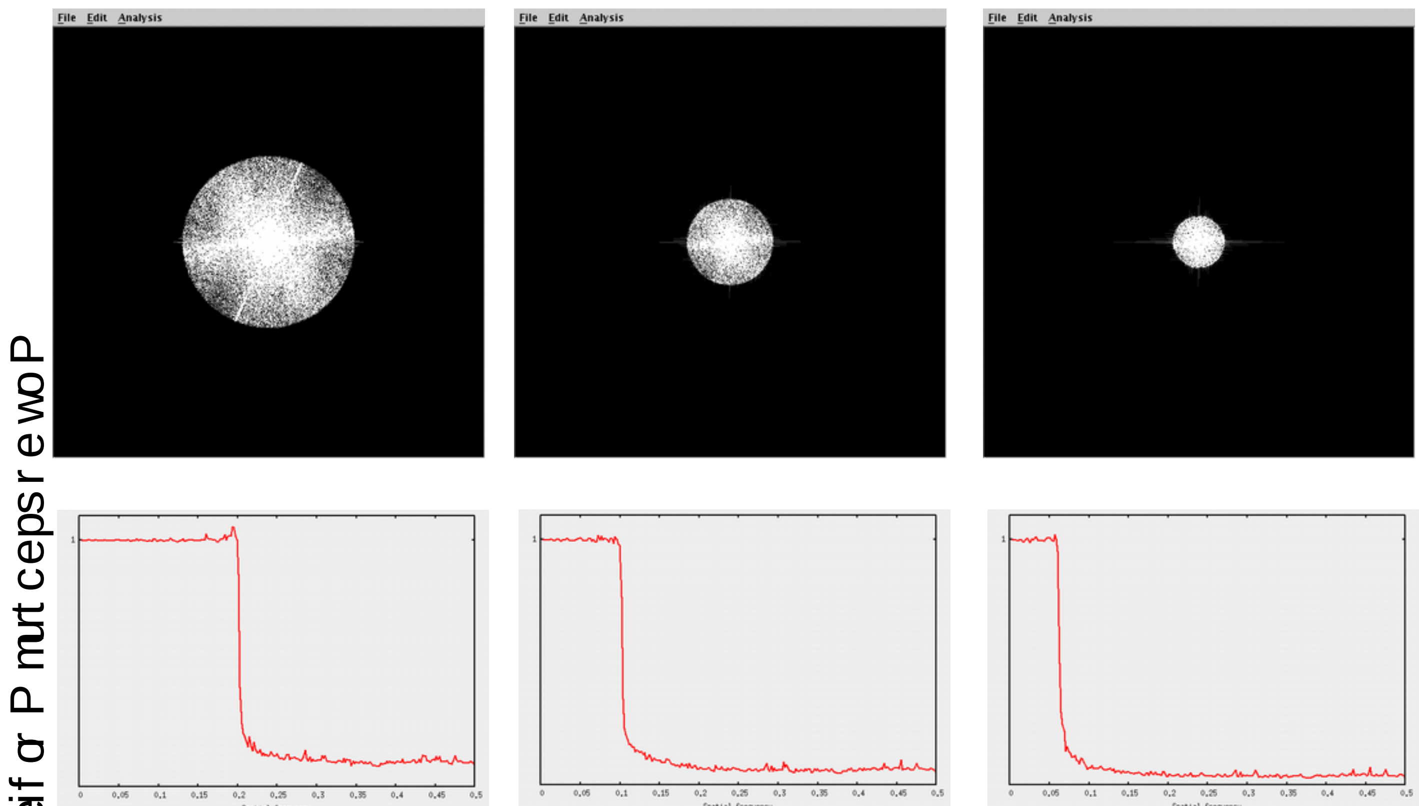
https://www.youtube.com/watch?v=6LzaPARy3uA

What do we mean by spatial frequency?

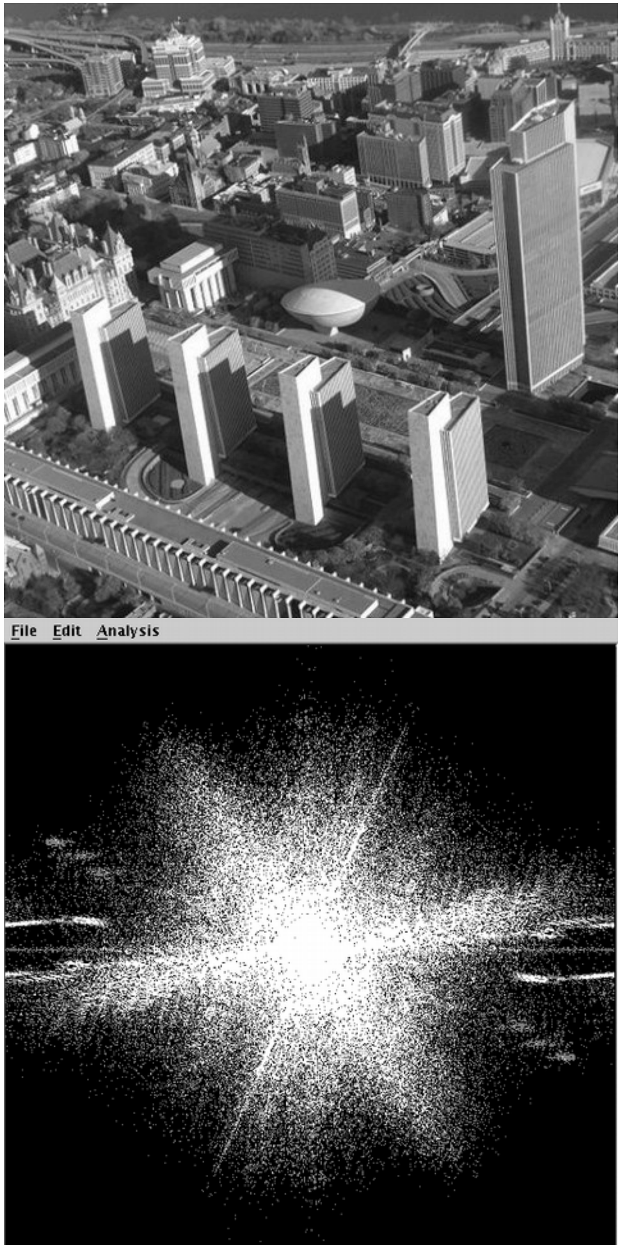
From Wikipedia

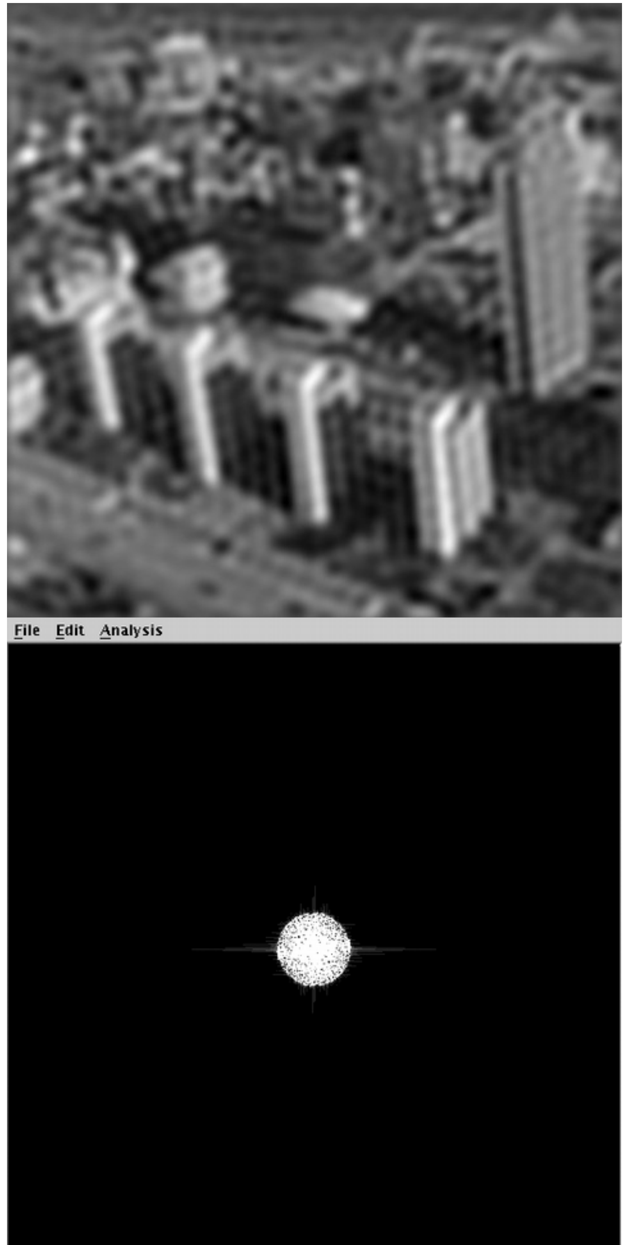


From Wikipedia

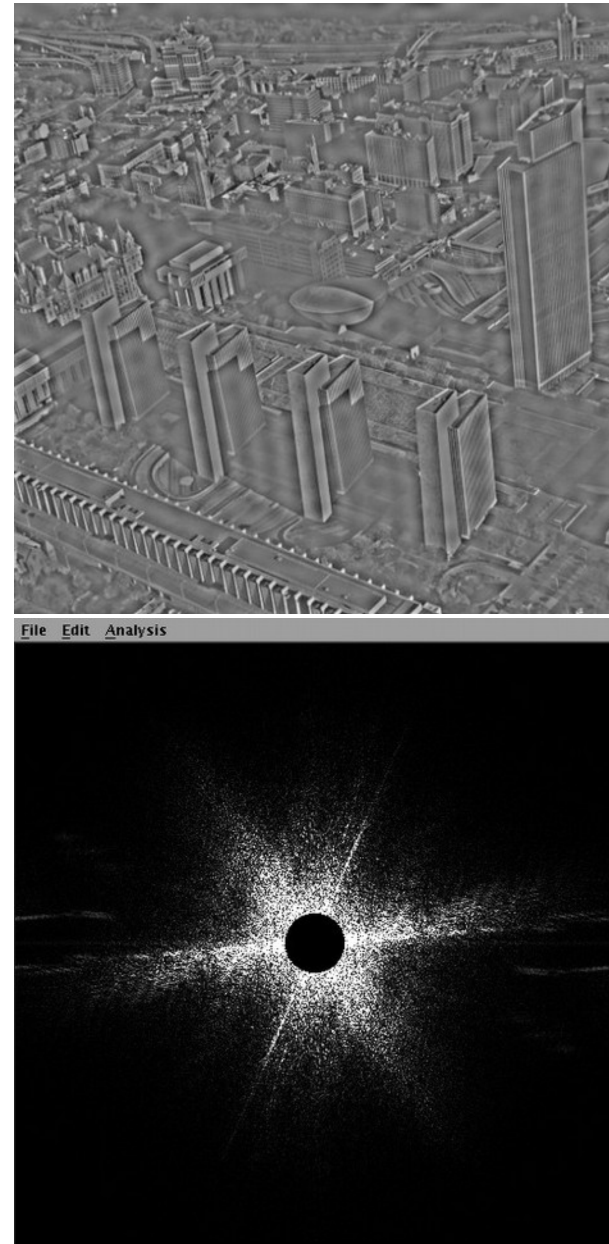


Fourier filtration





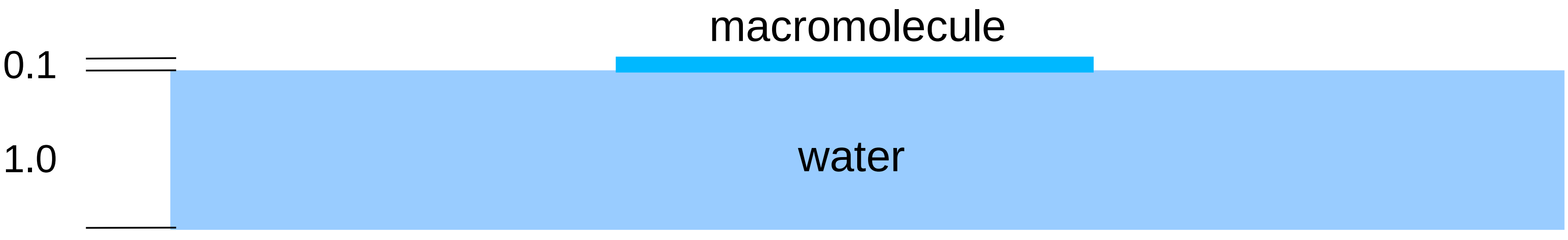
A “low-pass” filter



A “high-pass” filter

Contrast transfer function

Why do we defocus?

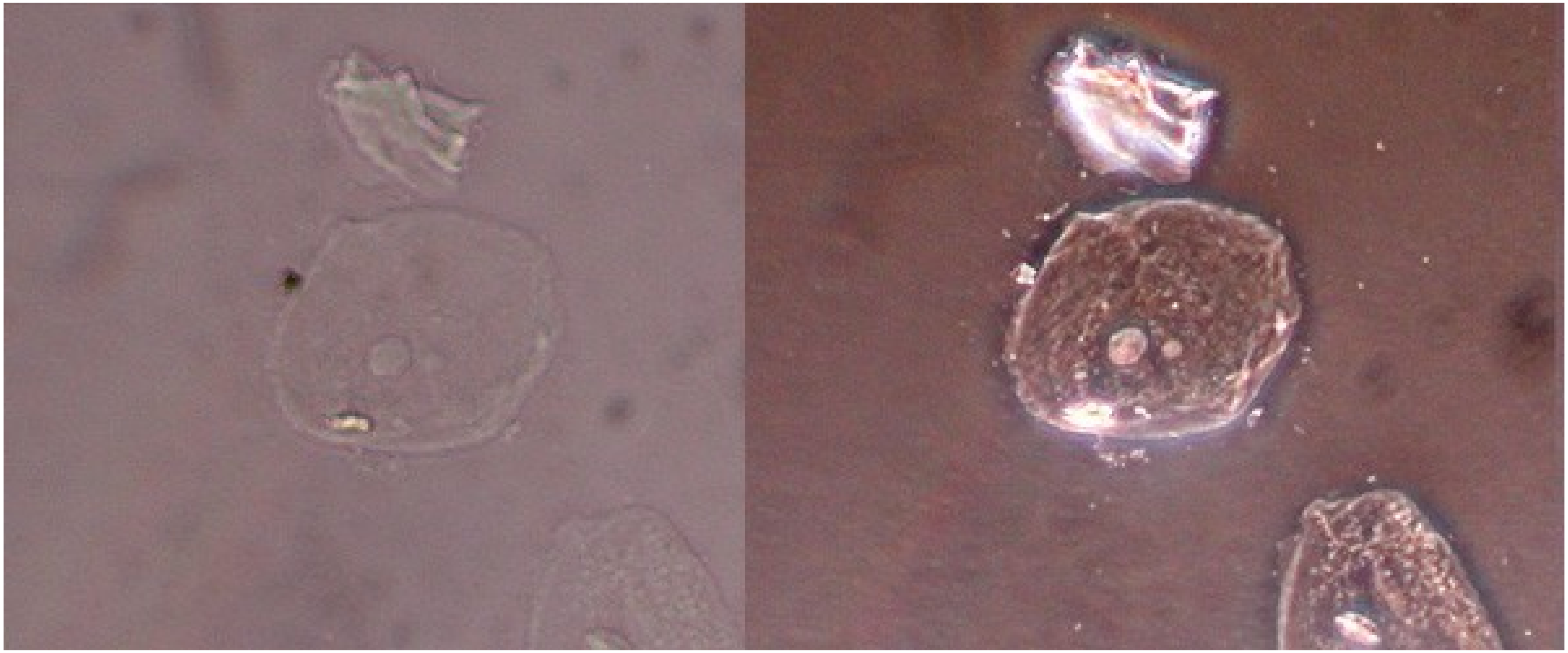


Typical amplitude contrast is estimated a 0.08-0.12
(minus noise)

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

Phase contrast in light microscopy

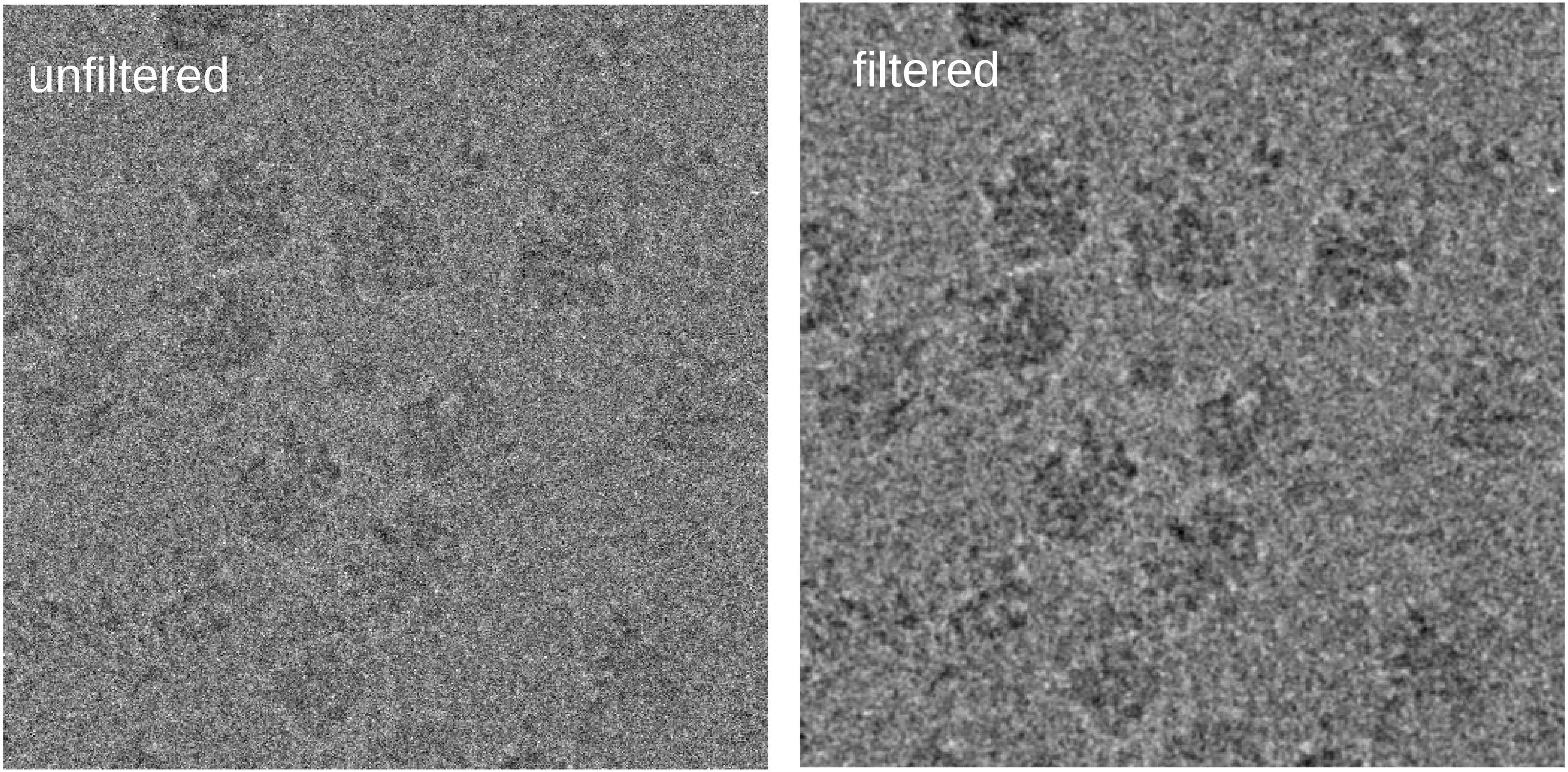
Bright-field image　　　　Phase-contrast image



http://www.microbehunter.com

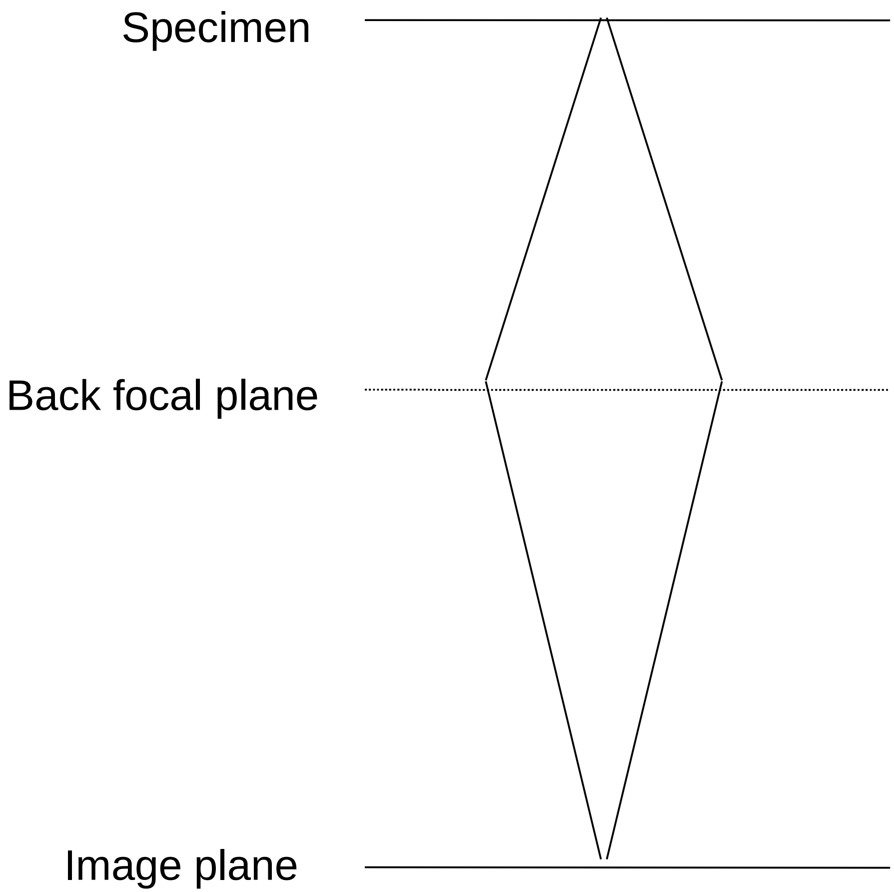
In EM, even with defocus, the contrast is poor.

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



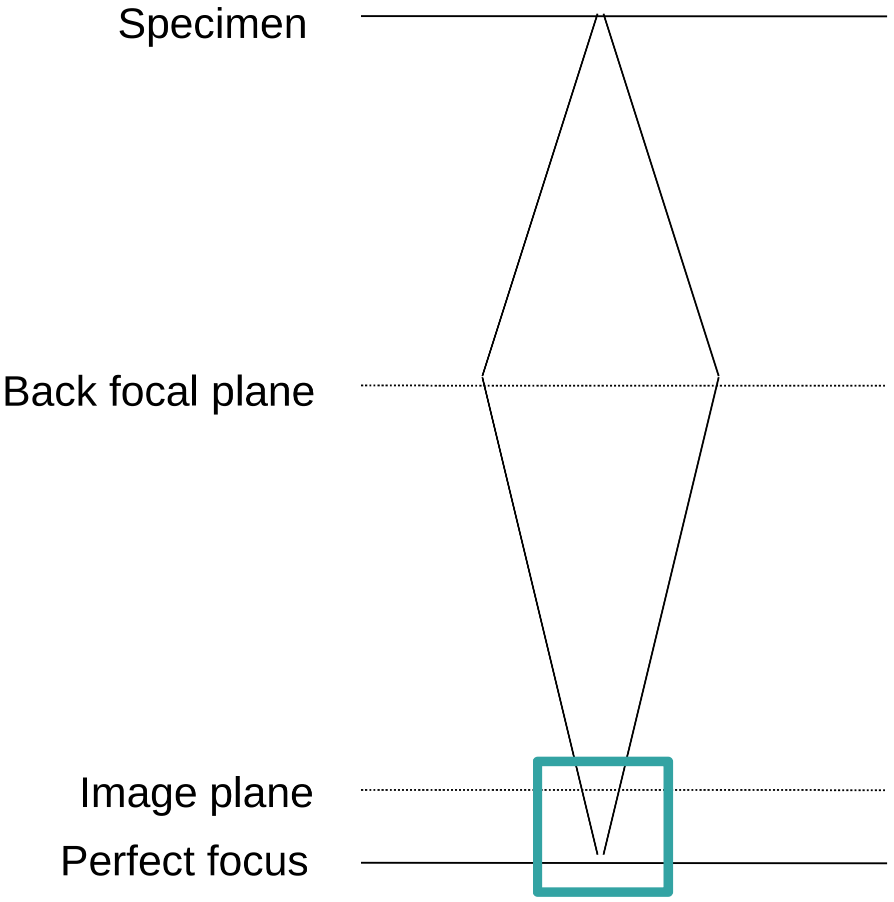
Signal-to-noise ratio for cryoEM typically given to be between 0.07 and 0.10.

Optical path

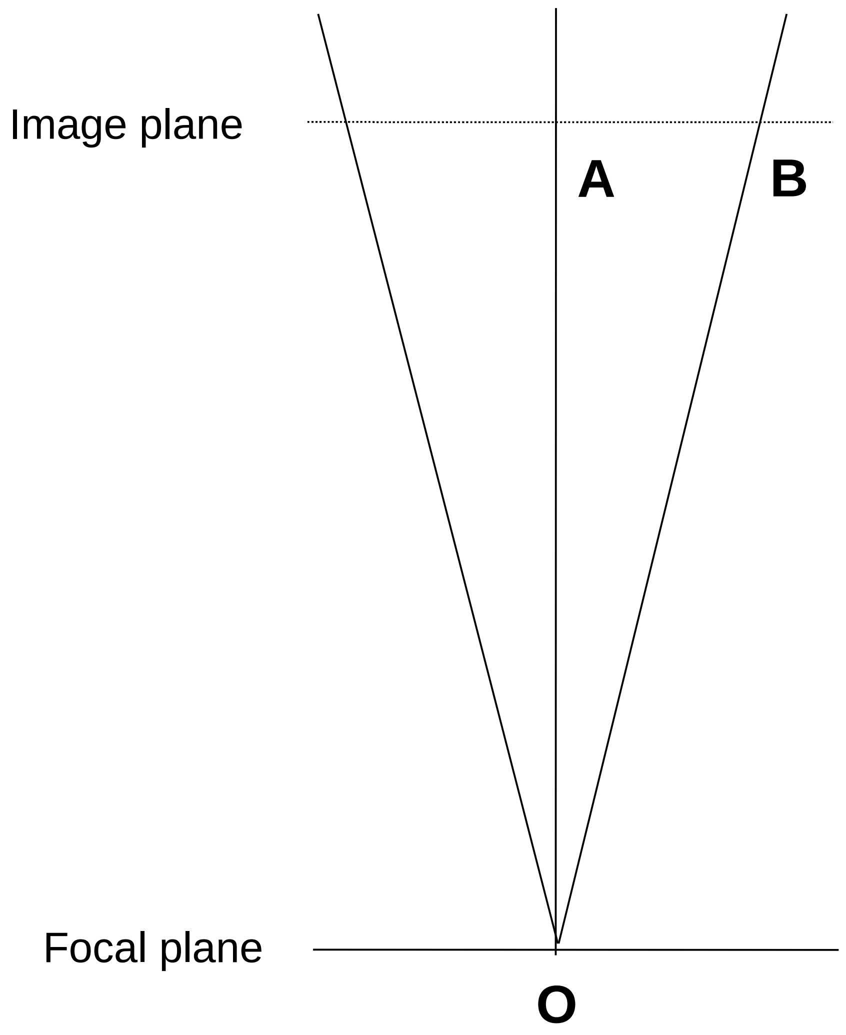


At focus, all we would see is amplitude contrast.

Optical path with defocus



Optical path with defocus



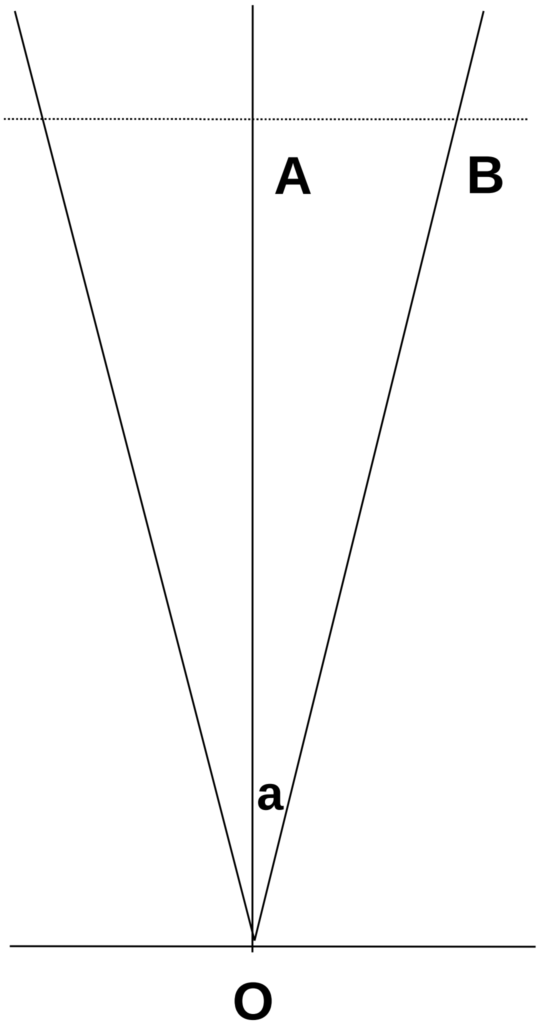
OA path of unscattered beam

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

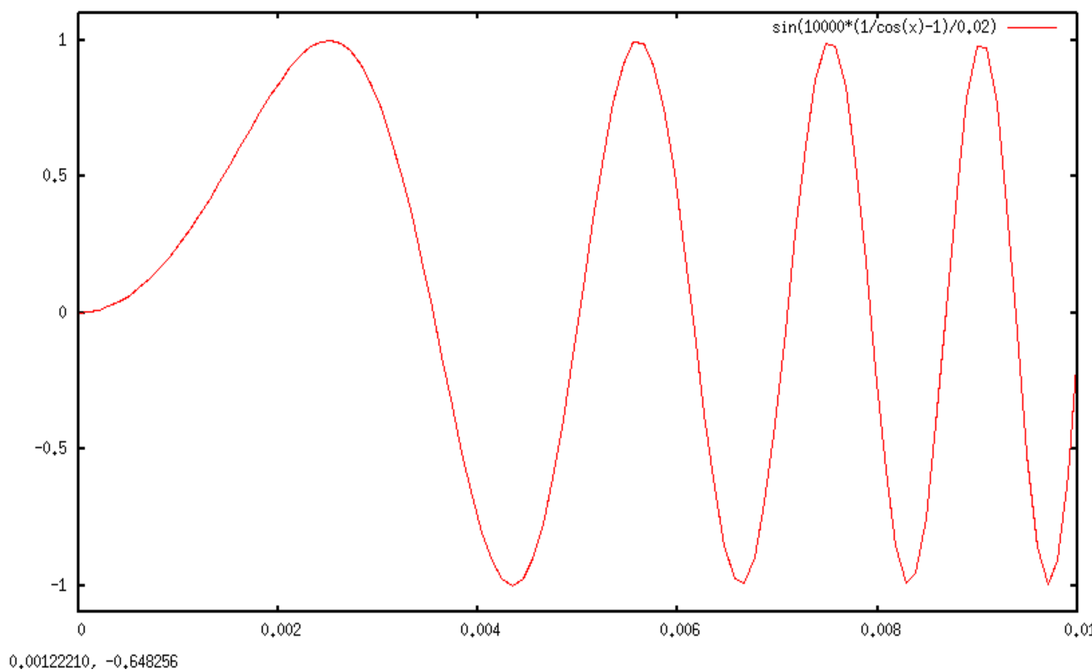
$$OA \times \left(\frac{\frac{1}{\cos(a)} - 1}{\lambda}\right)$$

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

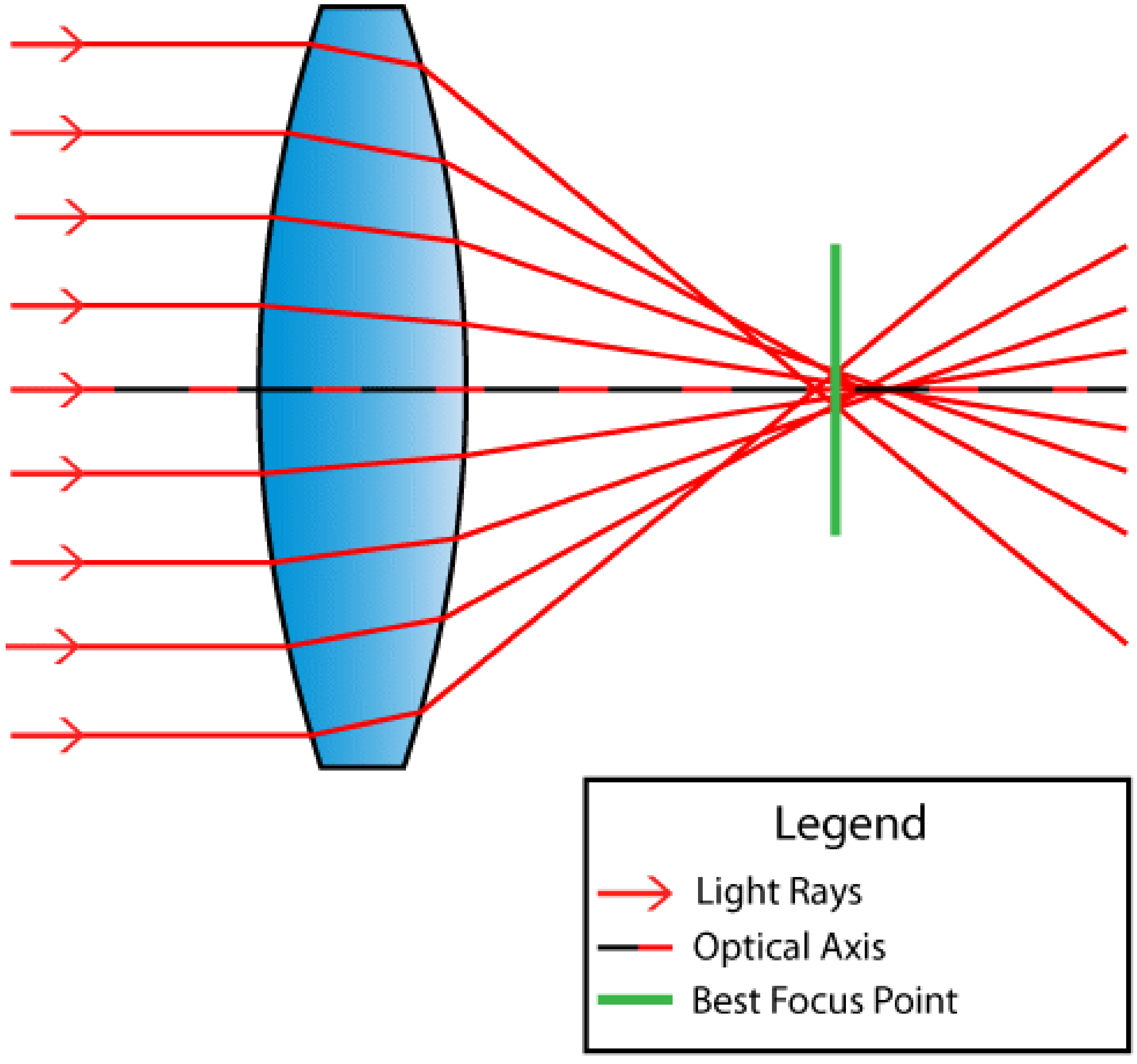
A more precise formulation of the CTF can be found in
Erickson & Klug A (1970). Philosophical Transactions of the Royal Society B. 261:105.

QUICK QUIZ:

What other example did we discuss where rays scattered at different angles experienced different path lengths?

Lens with Spherical Aberration

photographylife.com



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Proper form the CTF

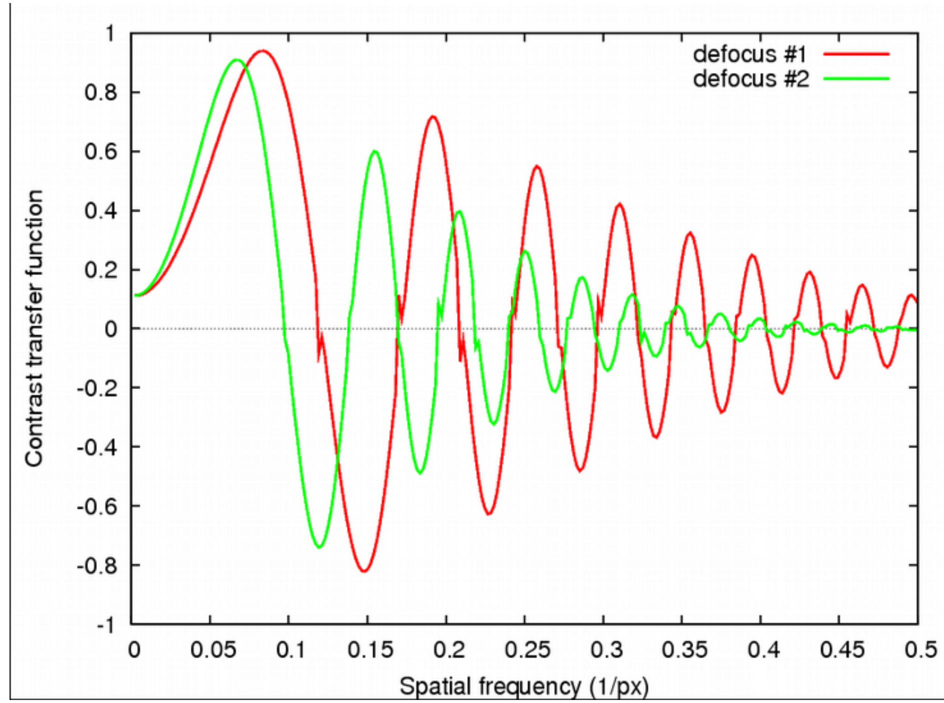
$$-\sin\left(\frac{\pi}{2}C_s k^4 + \pi \Delta f \lambda k^2\right)$$

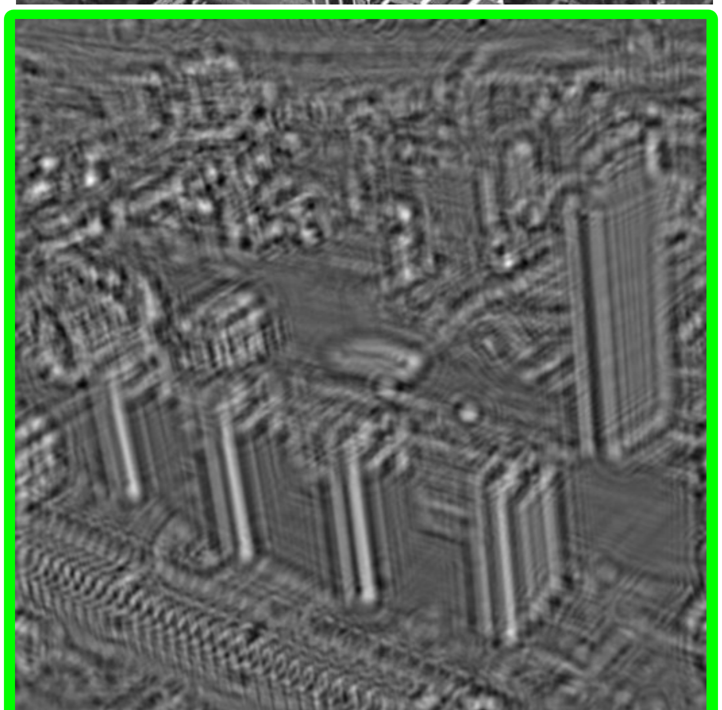
where:

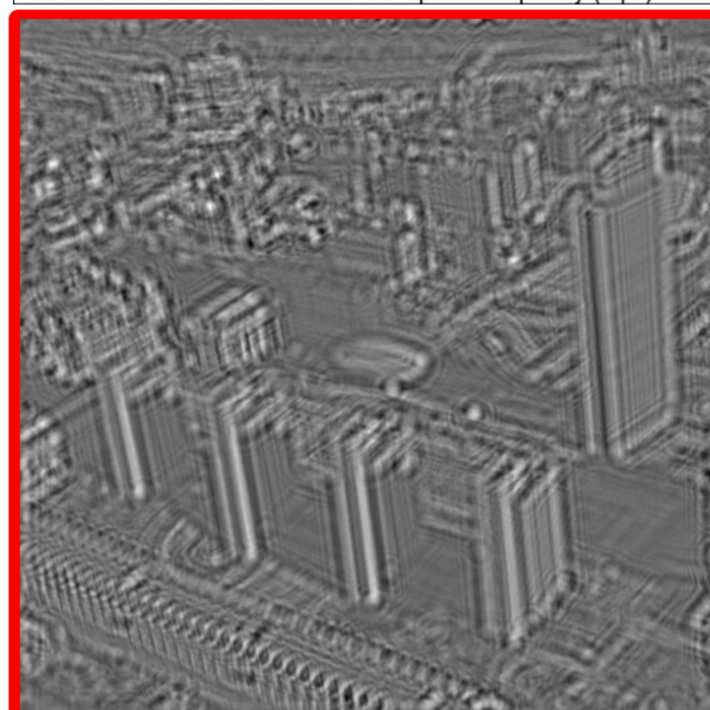
- C_s: spherical aberration
- k: spatial frequency (resolution)

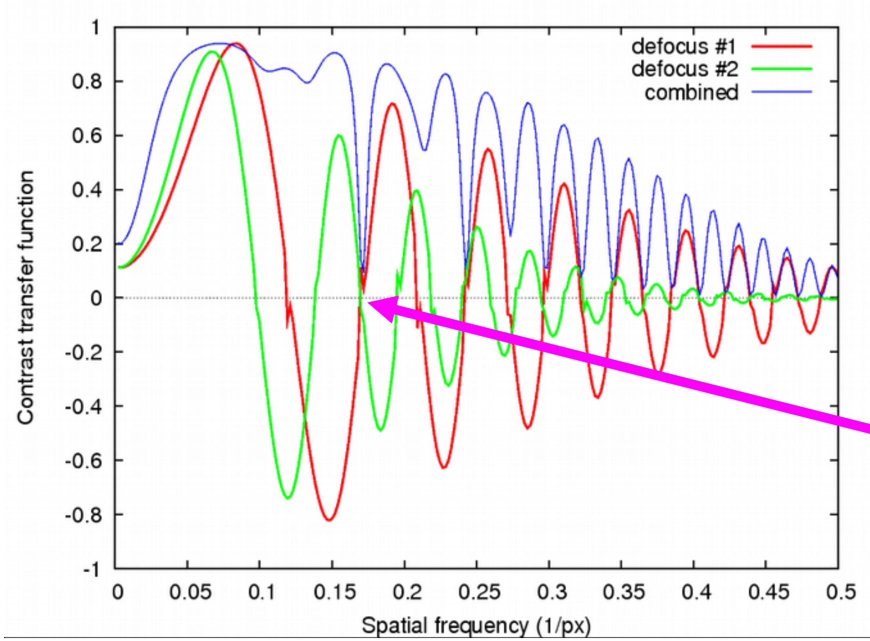
How does the CTF affect an image?

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

QUICK QUIZ:

What would happen if you collected all of your images at the same defocus?

Thank you for your attention

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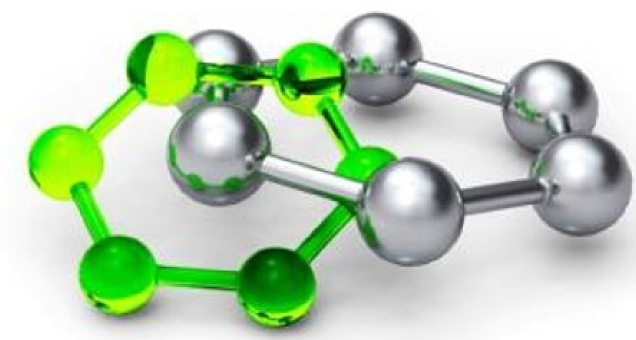

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