Lecture 6

Cryo-electron microscopy

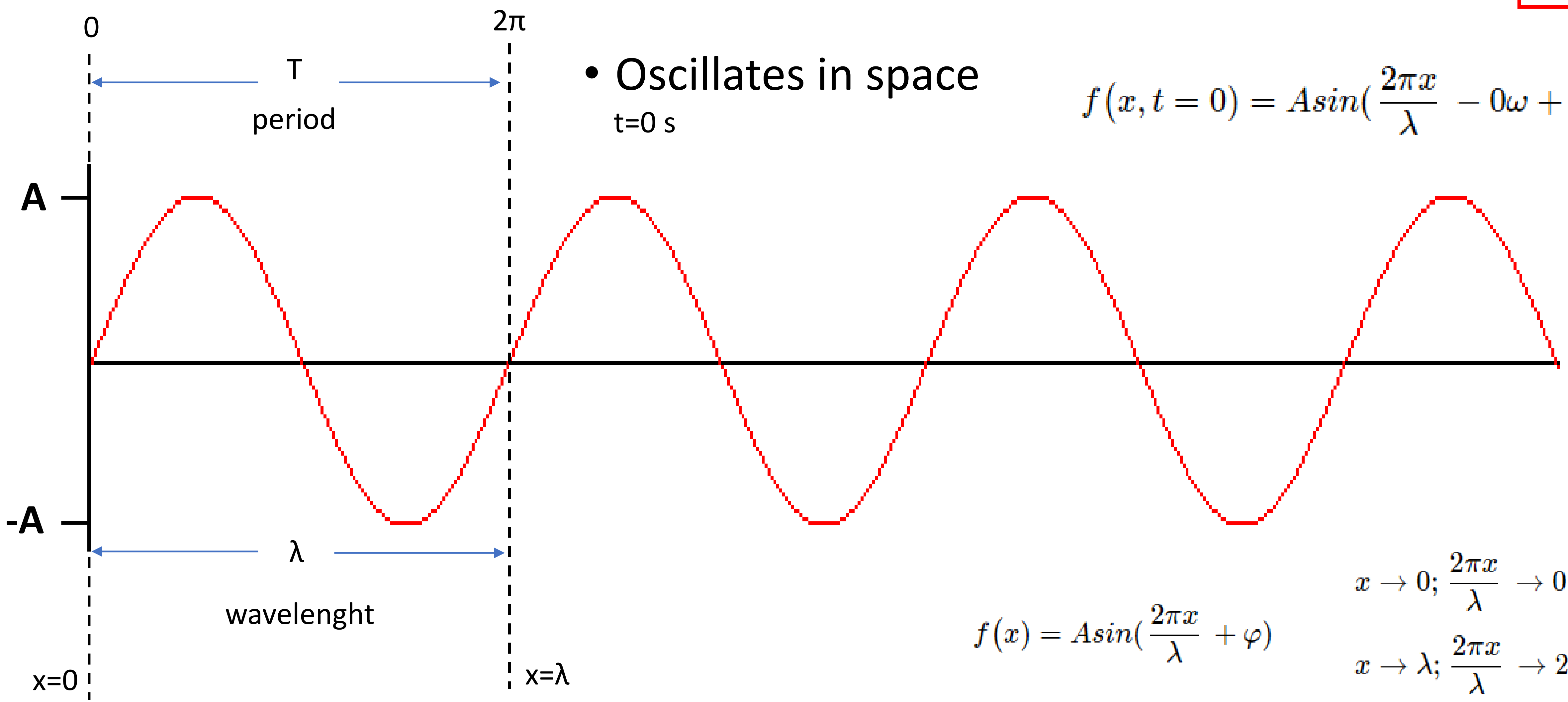
Spatial waves, Fourier transform, image formation
contrast transfer function

Tibor Füzik

Spatial wave

[redacted]

$$\boxed{k} = \frac{\omega}{v} = \frac{2\pi f}{v} = \boxed{\frac{2\pi}{\lambda}}$$



- Oscillates in space

t=0 s

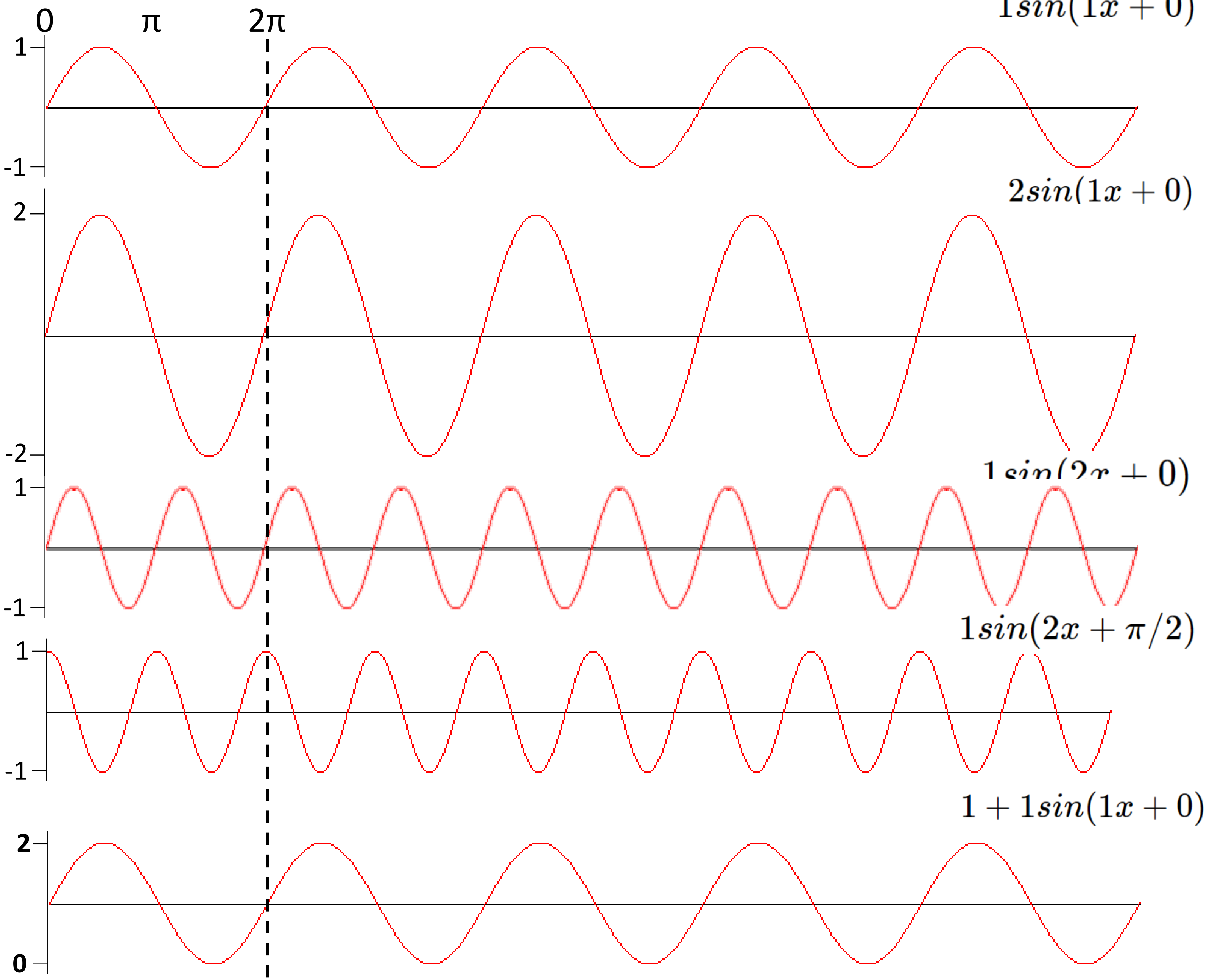
$$f\left(x, t=0\right) = Asin(\frac{2\pi x}{\lambda} - 0\omega + \varphi)$$

$$f\left(x\right) = Asin(\frac{2\pi x}{\lambda} + \varphi)$$

$$x \to 0; \frac{2\pi x}{\lambda} \to 0$$

$$x \to \lambda; \frac{2\pi x}{\lambda} \to 2\pi$$

Spatial wave



$$f(x) = Asin(kx + \varphi)$$

Amplitude

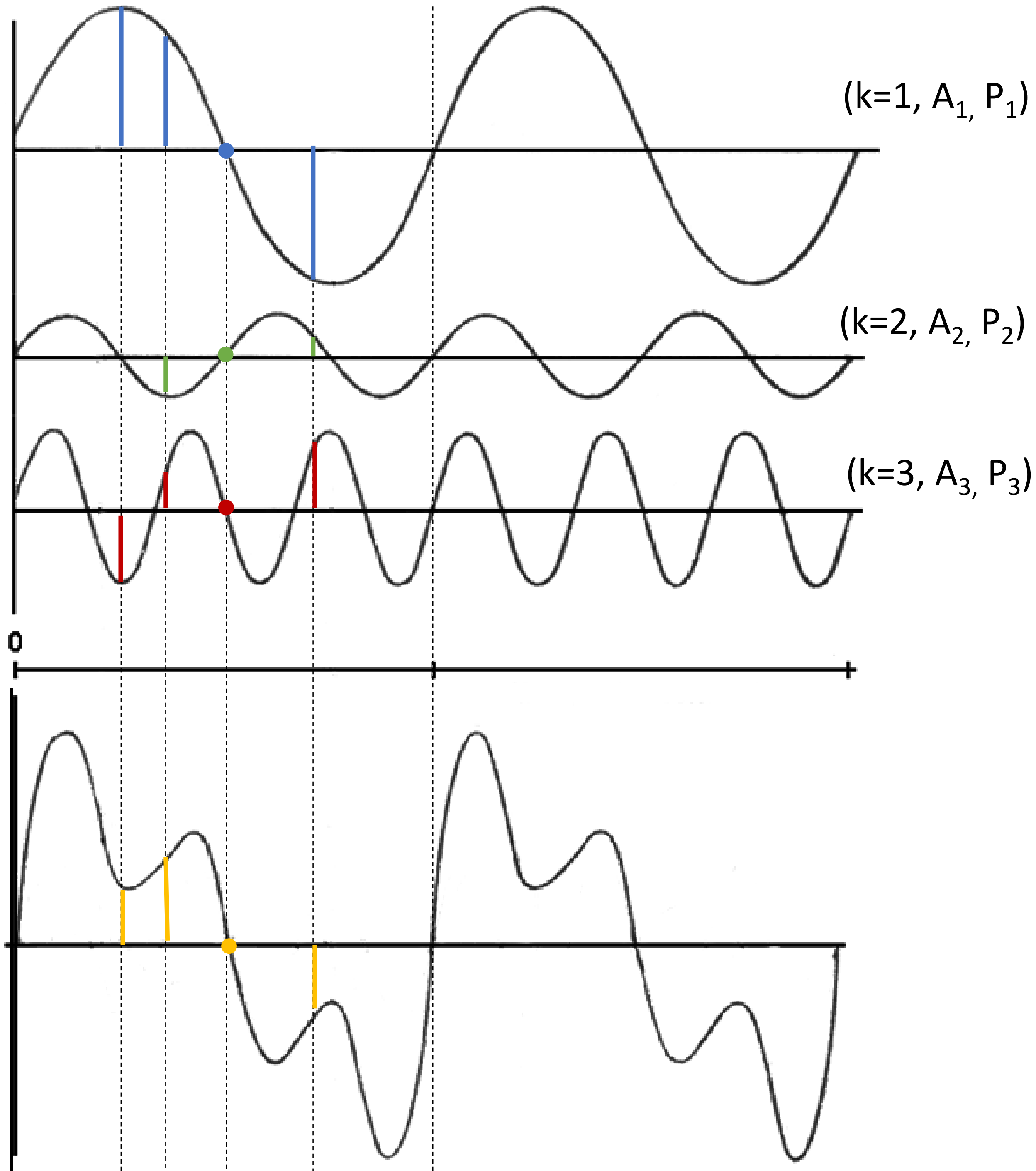
Spatial frequency

Phase shift

$$k = 2; k = \frac{2\pi}{\lambda}; \lambda = \frac{2\pi}{2} = \pi$$

$$f(x) = A_{\circ} + Asin(kx + \varphi)$$

http://www.maxmcarter.com/sinewave/generate_sinewave.php

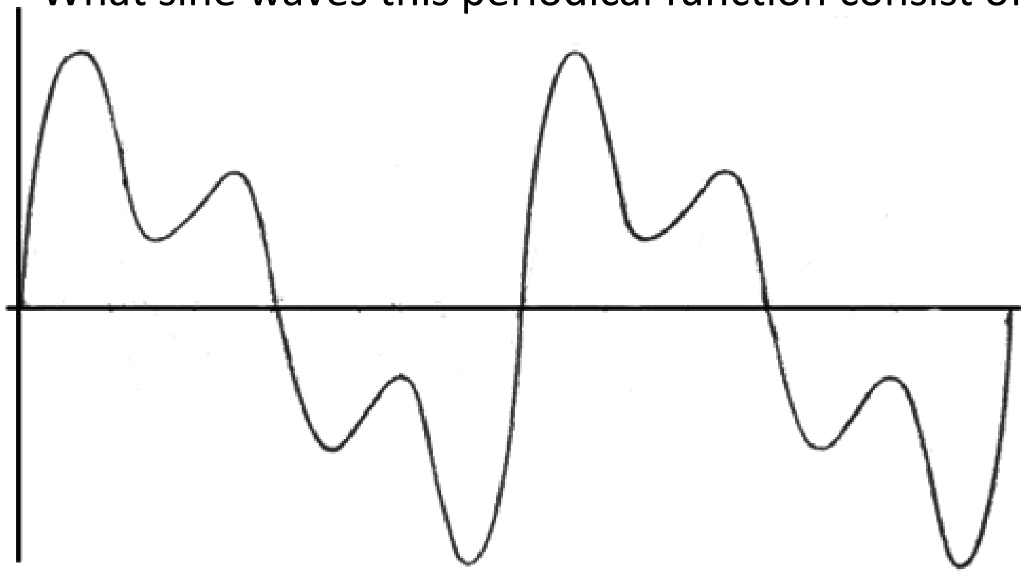


Adding sine waves

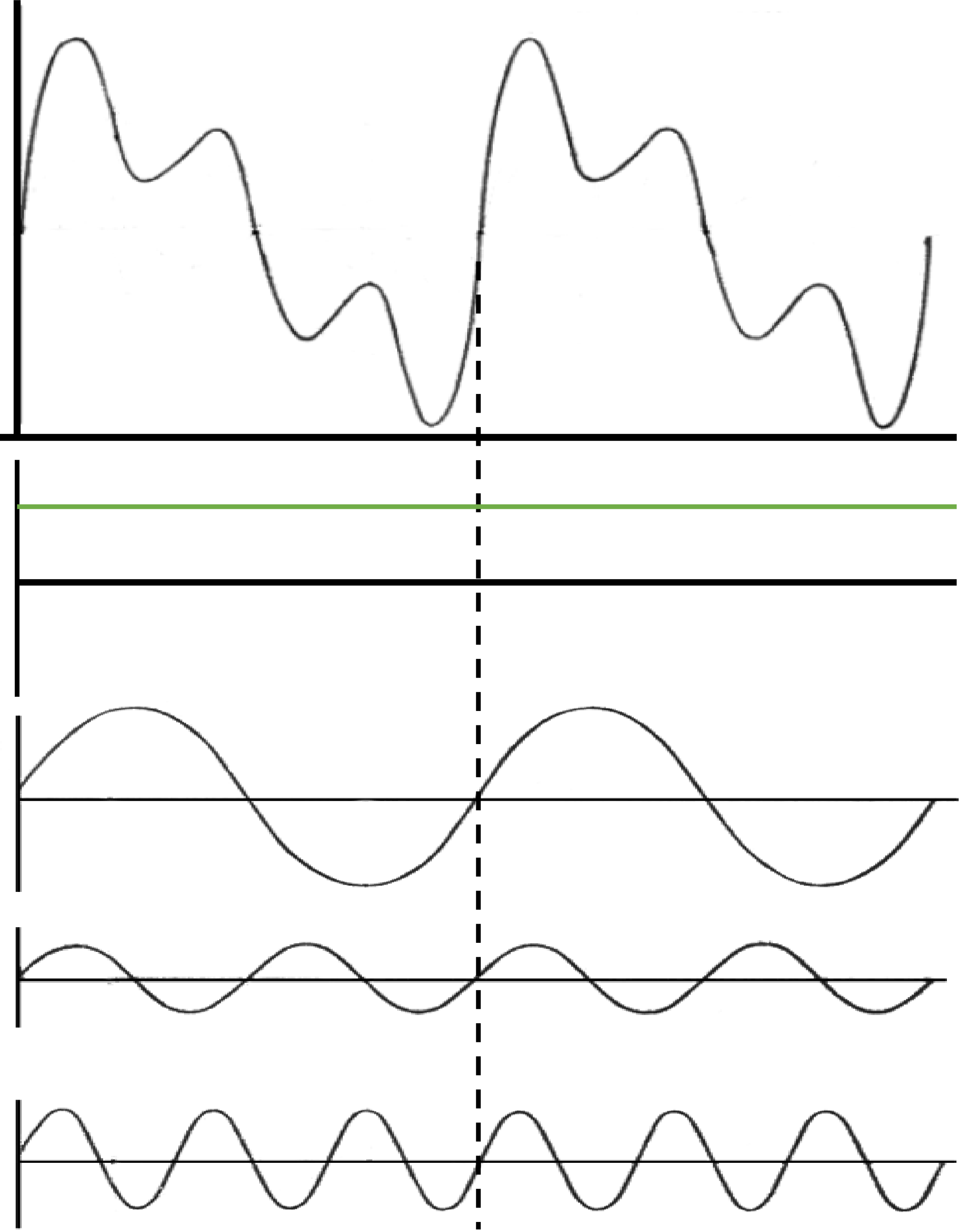
Every single complex wave we can construct by addition of series of single waves

Can we do the opposite way?

What sine waves this periodical function consist of?



Fourier decomposition



DC component (A_o)

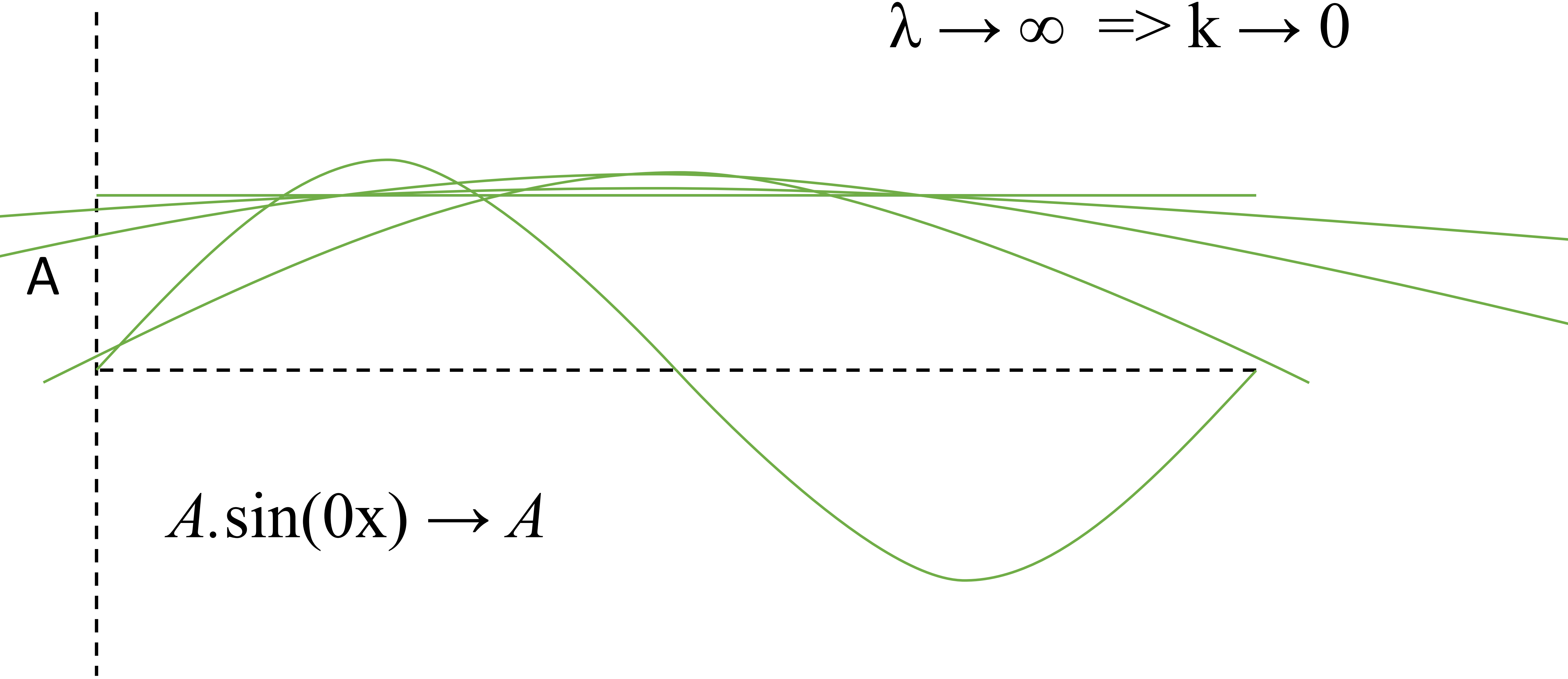
Fundamental frequency ($k=1$, A_1, P_1)

1st harmonics ($k=2$, A_2, P_2)

2nd harmonics ($k=3$, A_3, P_3)

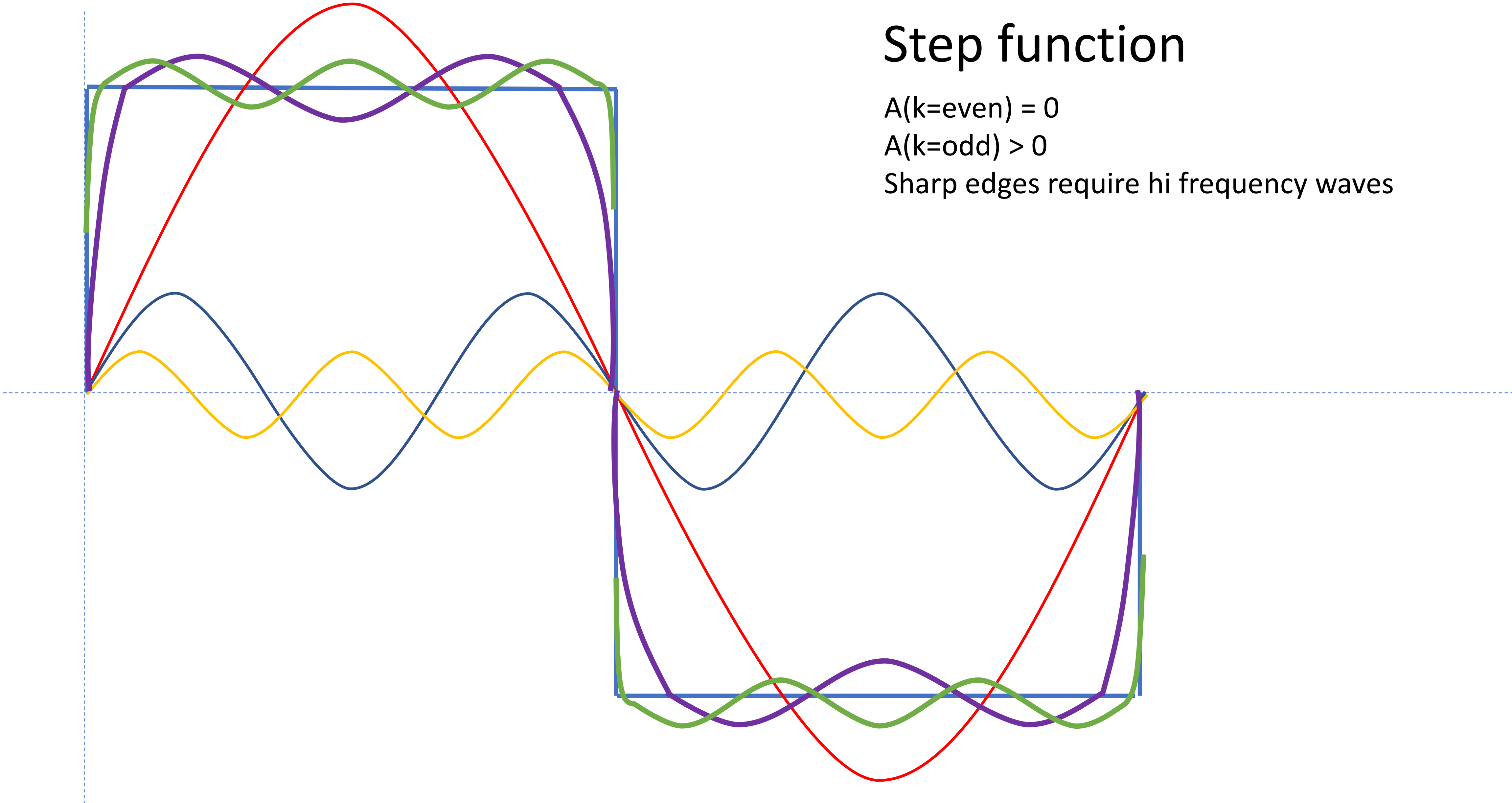
A constant function

$$\lambda \to \infty \;\; => k \to 0$$



$$A.\sin(0x) \to A$$

Square wave – Fourier decomposition



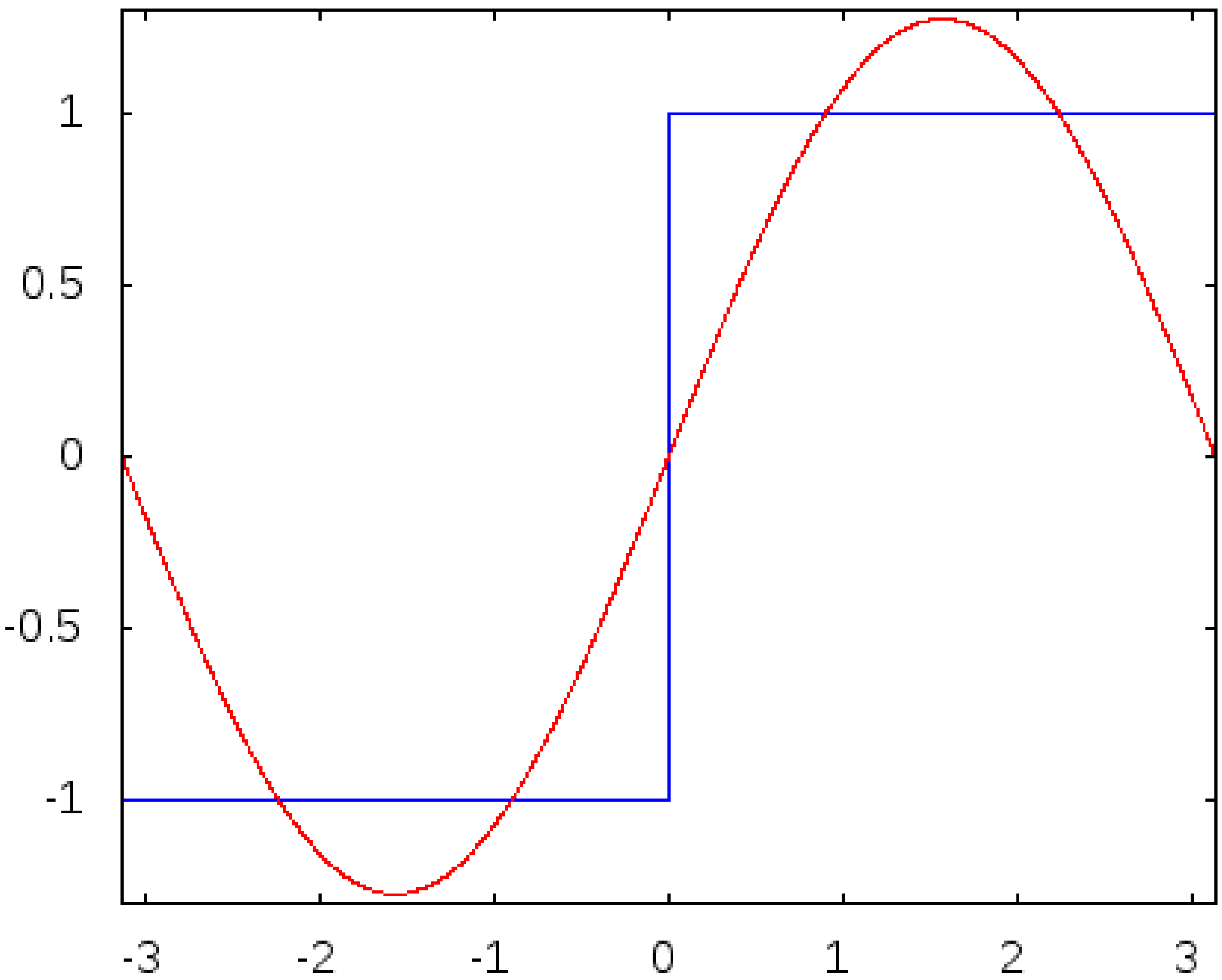
Step function

A(k=even) = 0

A(k=odd) > 0

Sharp edges require hi frequency waves

Step function



wikipedia.org

Fourier decomposition

Fourier transform

$$F(\omega) = \int_{-\infty}^{\infty} f(t) e^{-i\omega t} dt$$

Inverse Fourier transform

$$f(t) = \frac{1}{2\pi} \int_{-\infty}^{\infty} F(\omega) e^{i\omega t} d\omega$$

Jean-Baptiste Joseph Fourier

Every periodical function can be decomposed into sum of infinite number of sine waves

$$\omega = 2\pi f$$

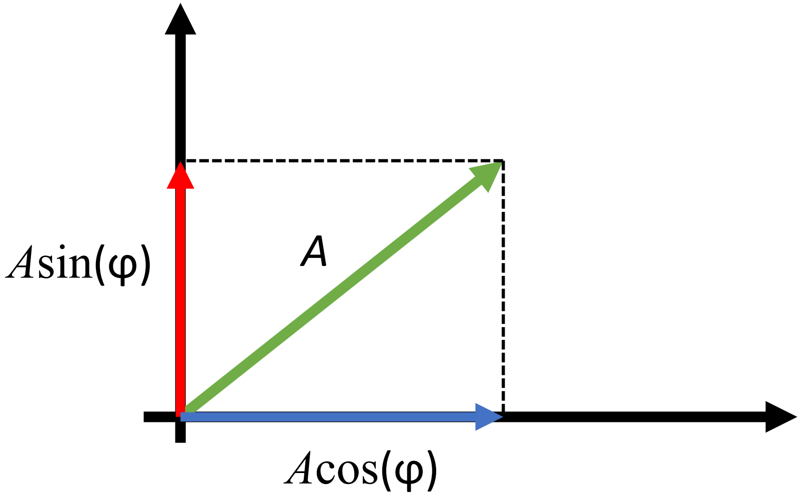
$$Ae^{i\alpha} = Acos(\alpha) + iAsin(\alpha)$$

Fourier decomposition of spatial waves

$$f(x) = \frac{A_0}{2} + \sum_{m=1}^{\infty} A_m cos(\frac{2\pi mx}{\lambda}) + \sum_{m=1}^{\infty} B_m sin(\frac{2\pi mx}{\lambda})$$

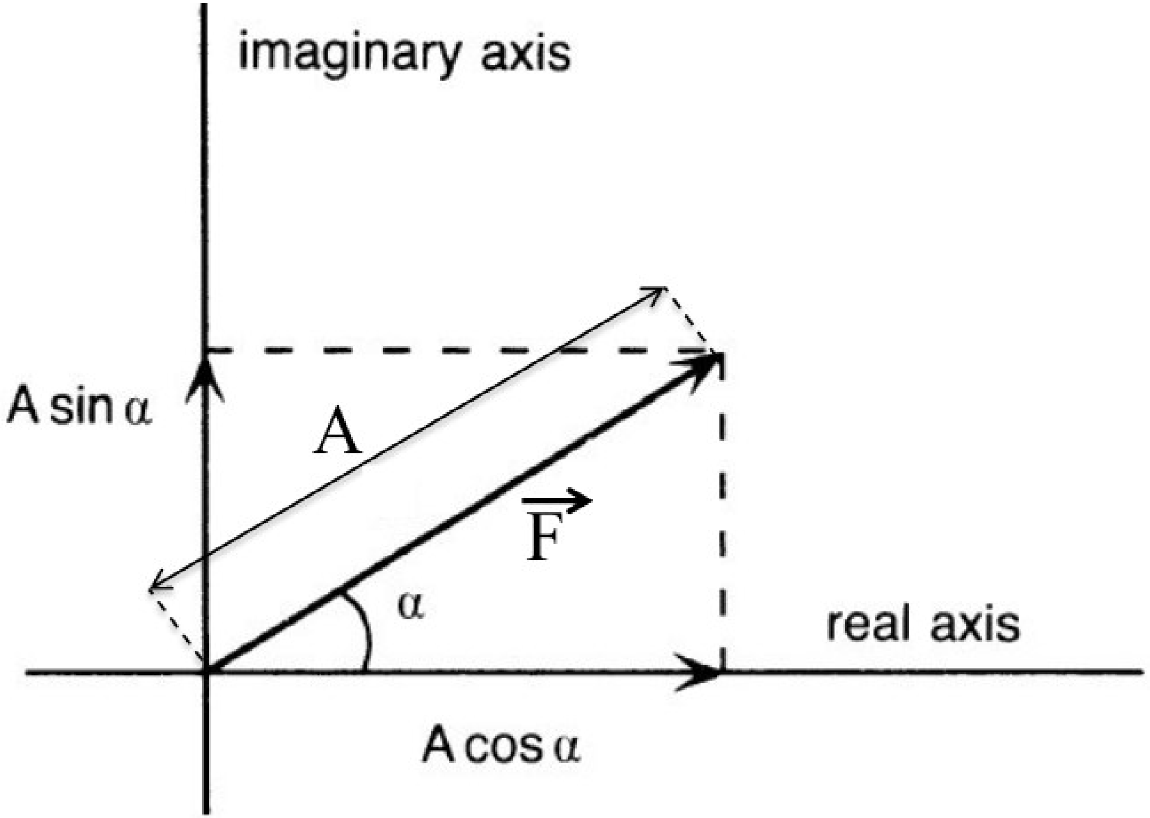
$$A_m = \frac{2}{\lambda}\int_0^{\lambda} f(x)cos(\frac{2\pi mx}{\lambda})dx$$

$$B_m = \frac{2}{\lambda}\int_0^{\lambda} f(x)sin(\frac{2\pi mx}{\lambda})dx$$



How can we store Fourier transform

Wave as a vector F



$$\vec{F} = Acos(\alpha) + iAsin(\alpha)$$

from Lecture 2

- Need to store waves (parameters of waves)
- Reciprocal space
  - series of wave functions
  - series of wave vectors

2 ways of wave vector representation

  - as amplitudes and corresponding phases
  - as complex numbers

Complex Numbers

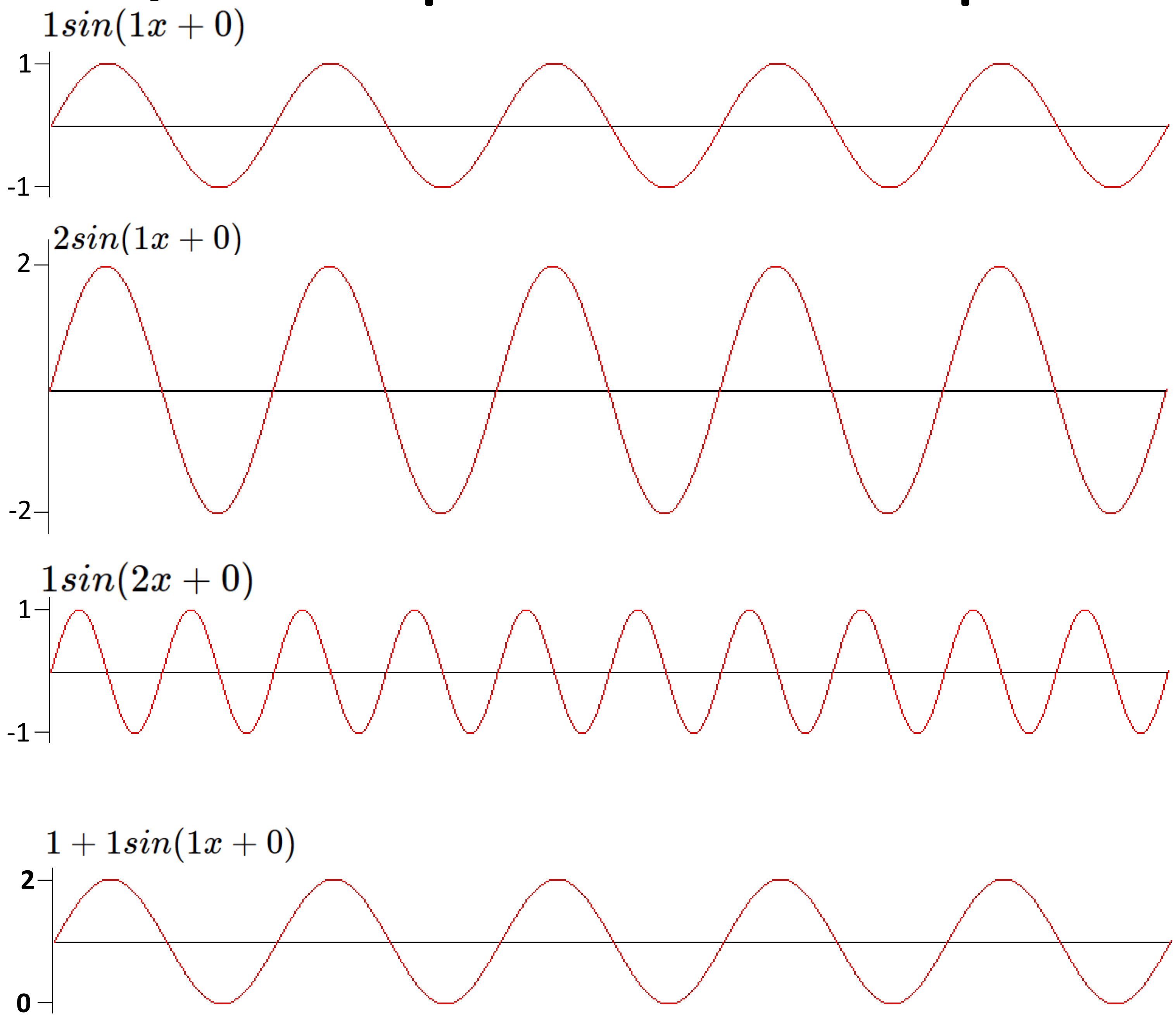
Addition

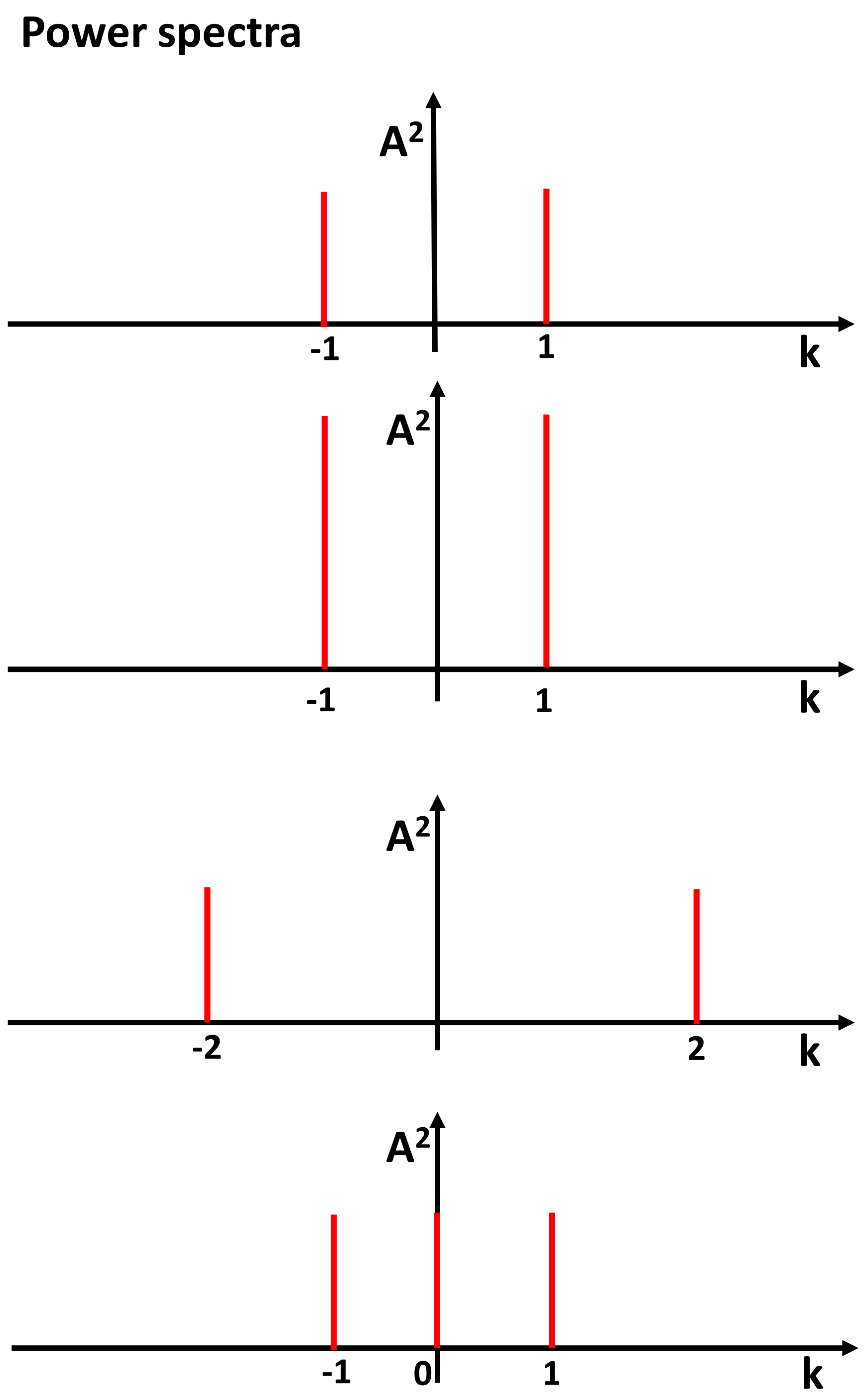
$$(a+bi)+(c+di)=(a+c)(b+d)i$$

Multiplication

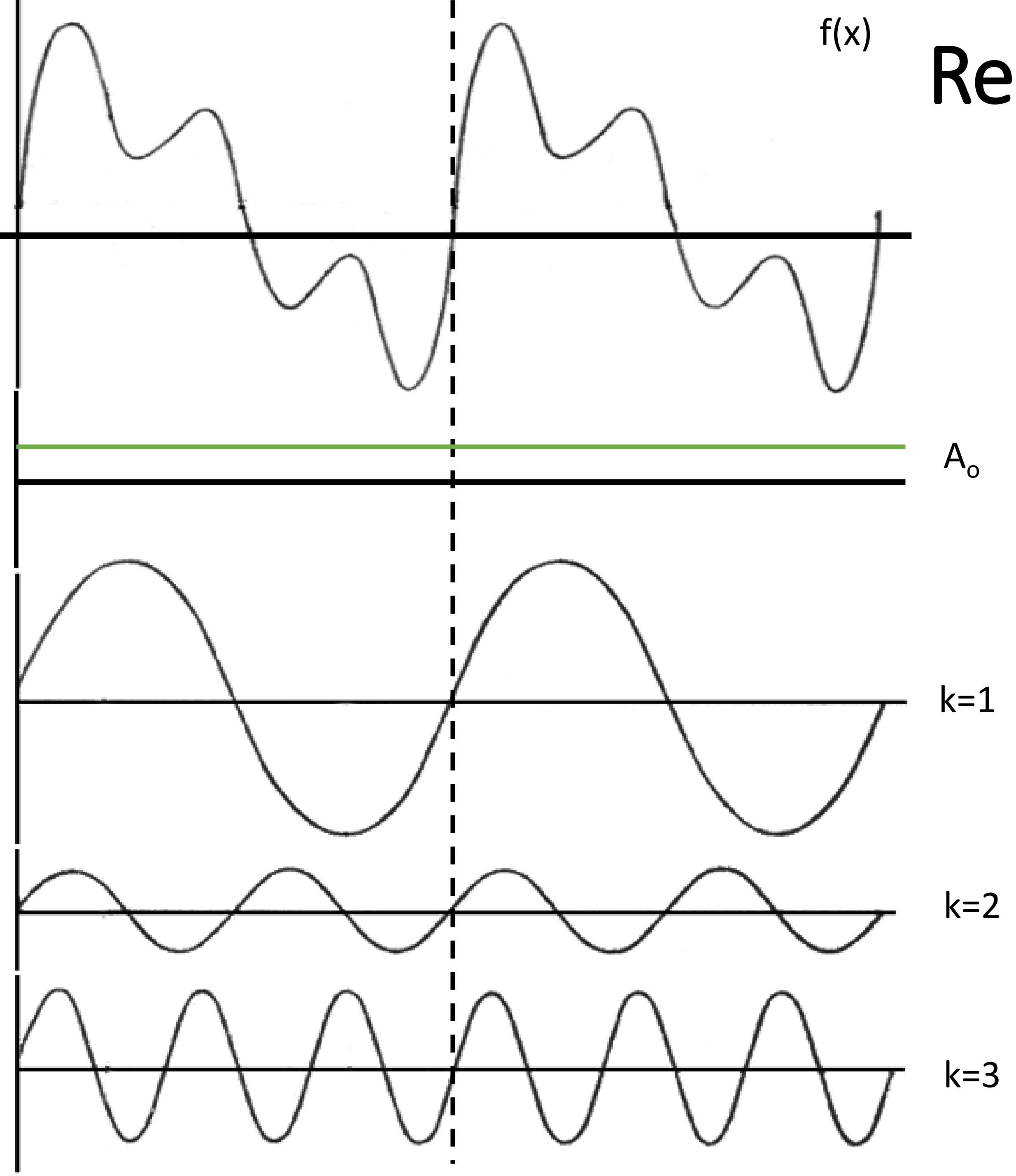
$$(a+bi)(c+di)=(ac-bd)+(ad+cd)i$$

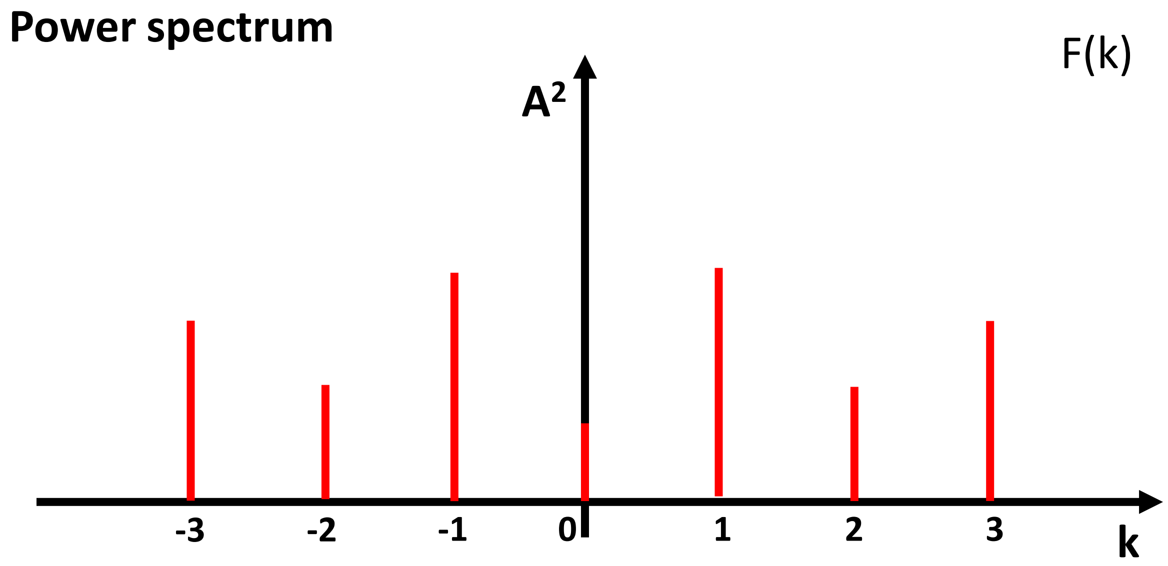
Reciprocal space – Power spectra



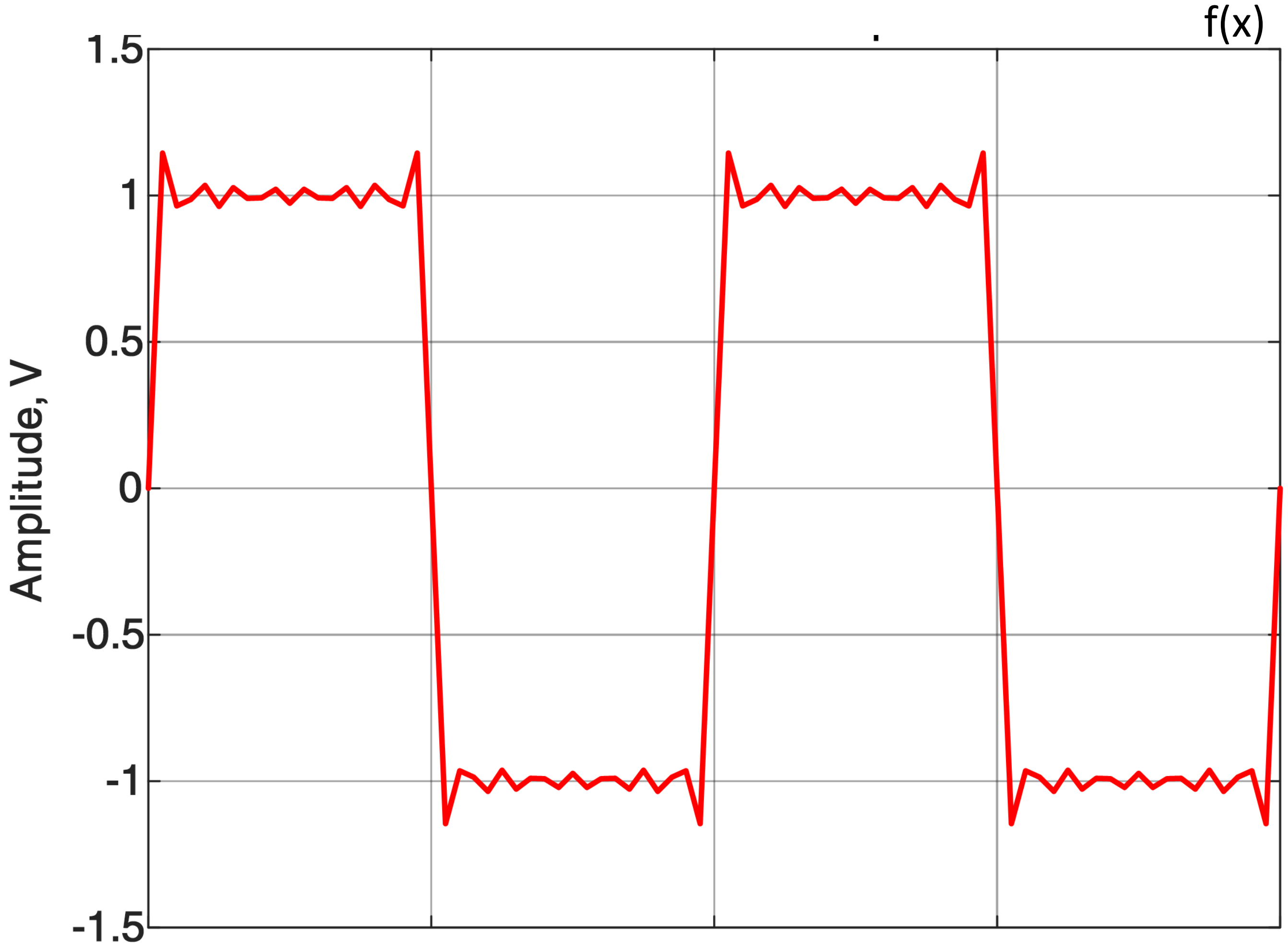


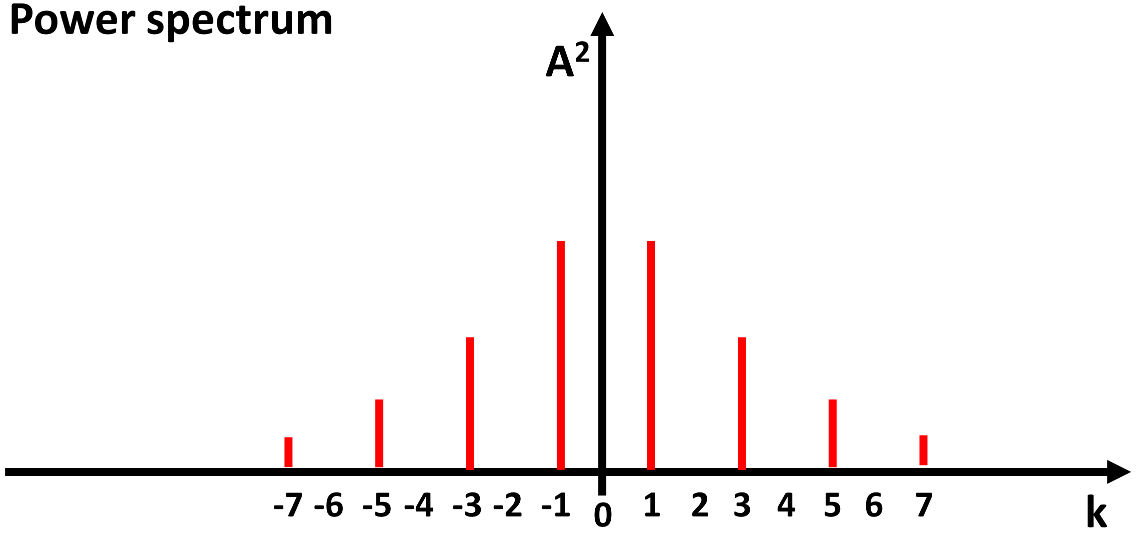
Reciprocal space – clpx function

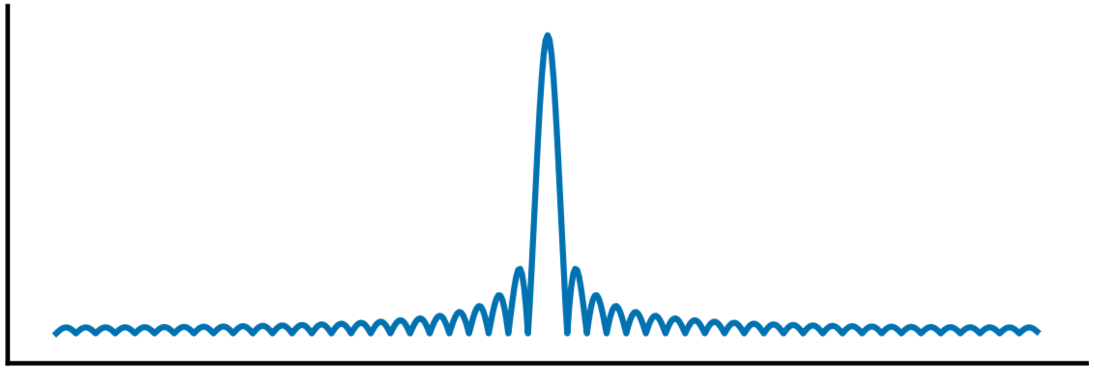




Reciprocal space – step function

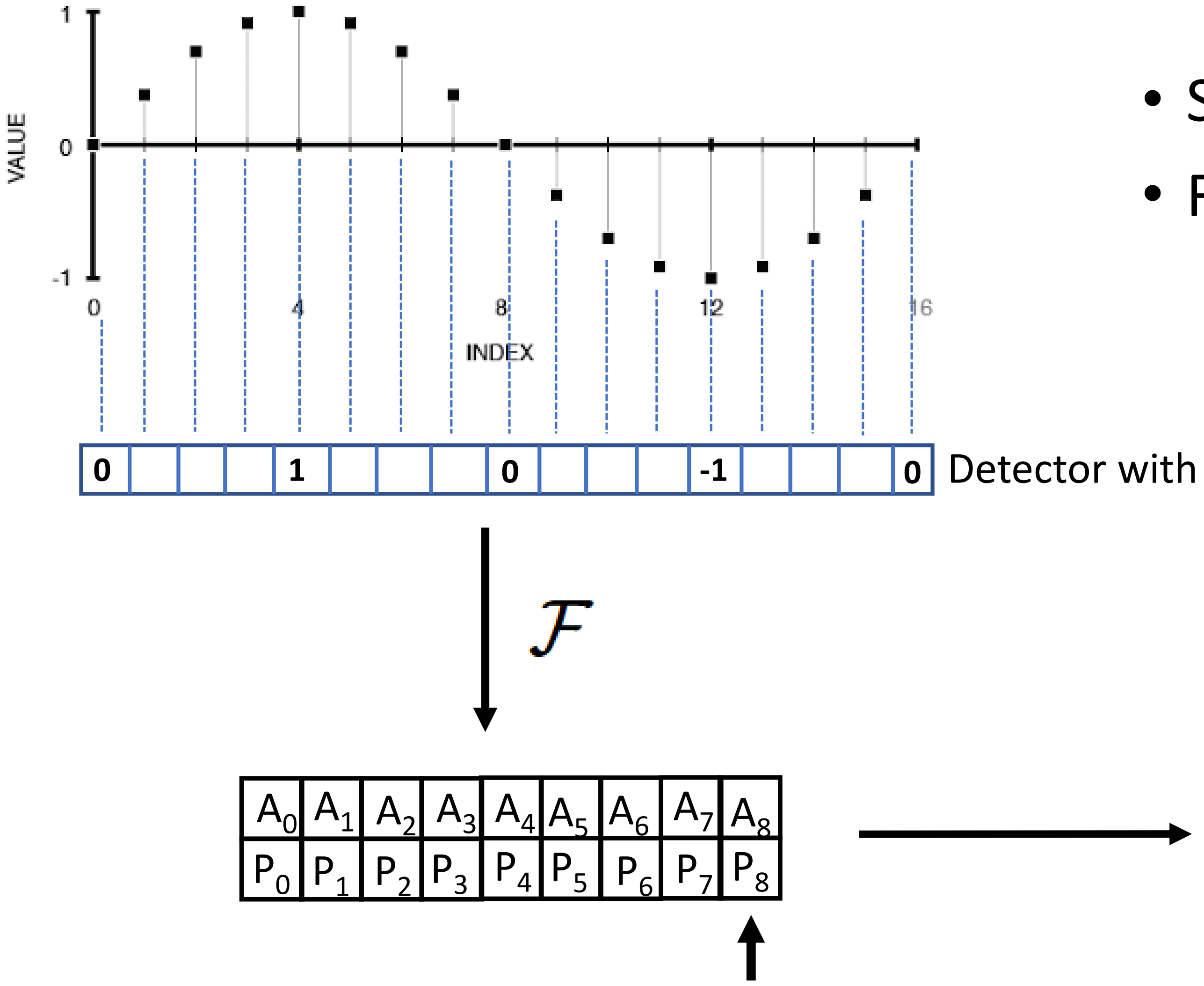






Fourier transform of 1D discrete waves

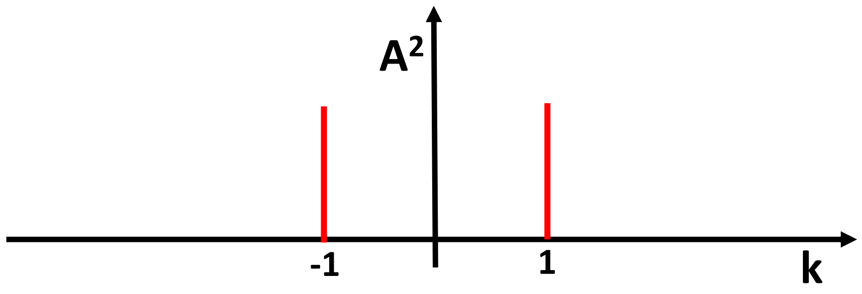
- Sampling cause discretization of the wave
- Finite number of Fourier components



Detector with discrete pixels

Nyquist component

Power spectrum



Reciprocal space

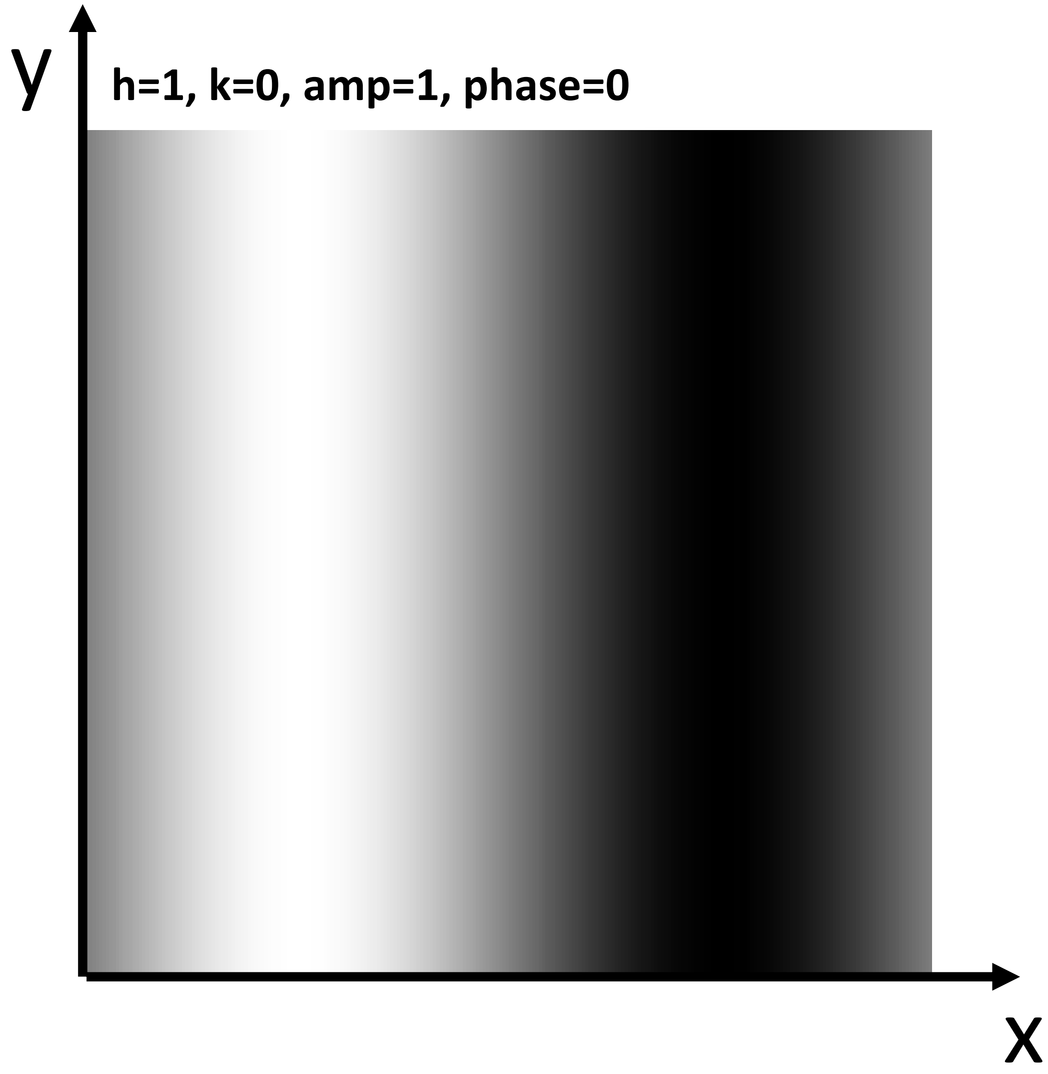
2D waves

1D wave

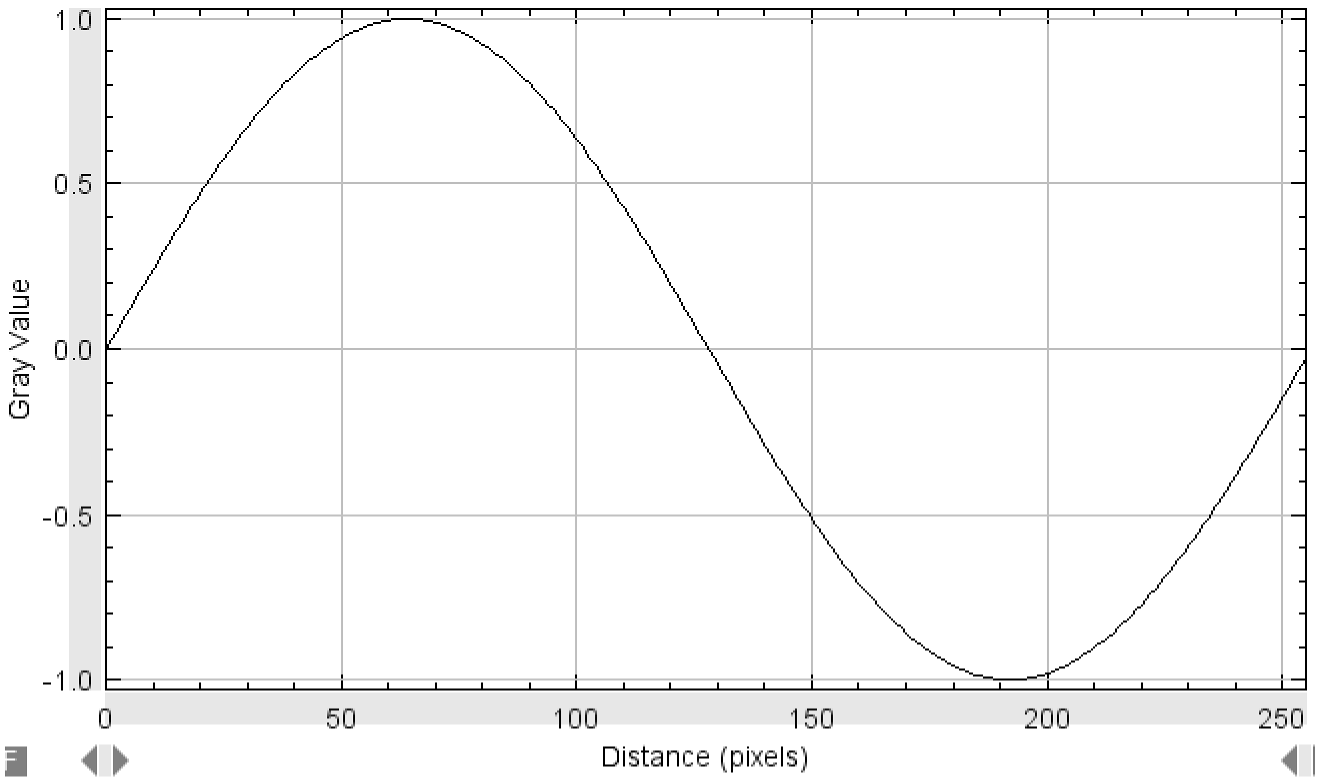
k -> number of wave periods

2D wave

h, k -> number of wave periods per x, y



Profile plot



2D waves

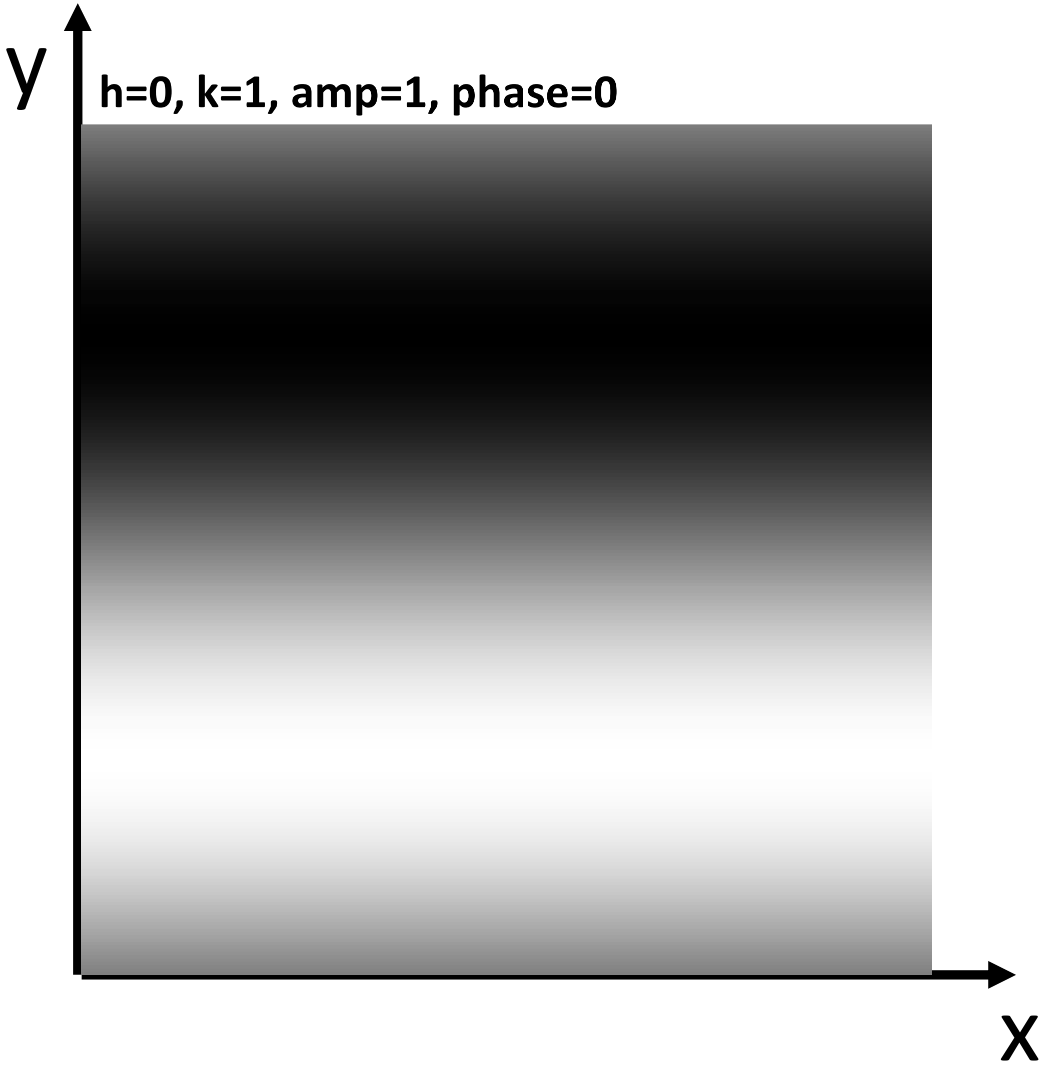
1D wave

k -> number of wave periods

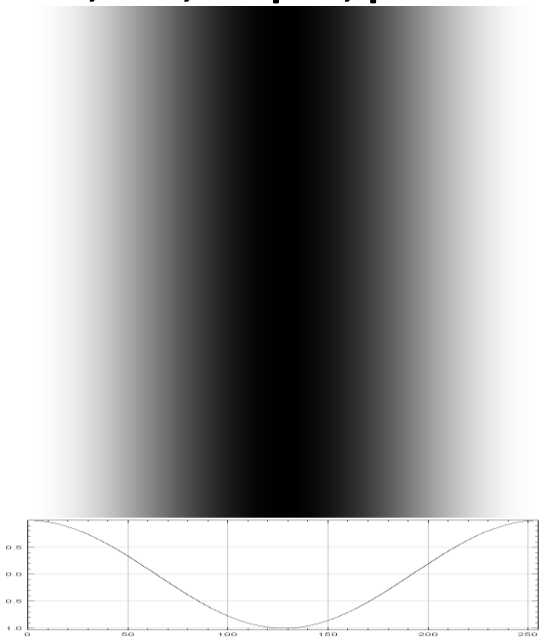
2D wave

h, k -> number of wave periods per x, y

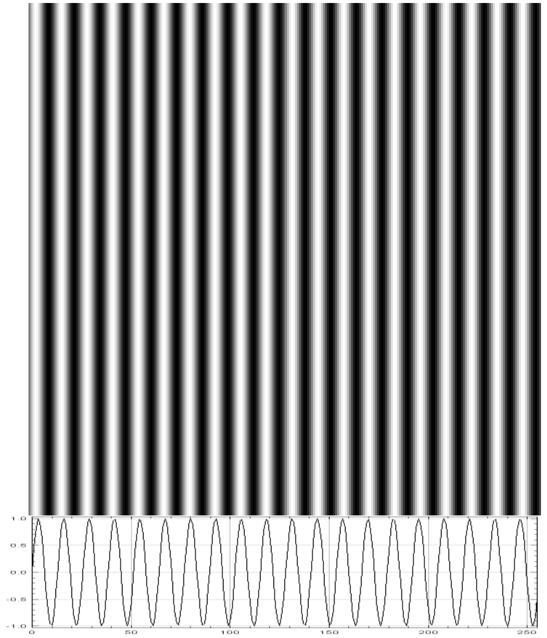
h=0, k=1, amp=1, phase=0



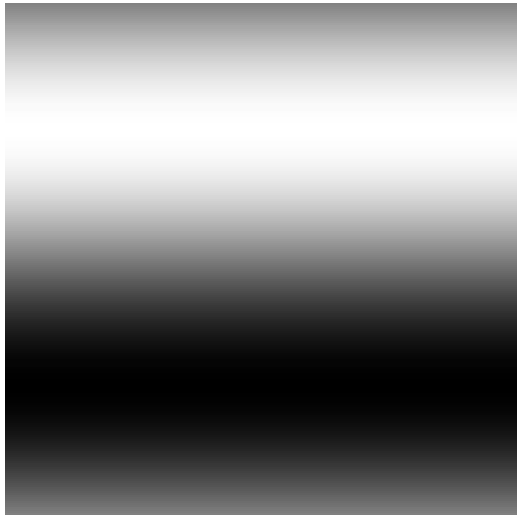
h=1, k=0, amp=1, phase=90



h=20, k=0, amp=1, phase=0



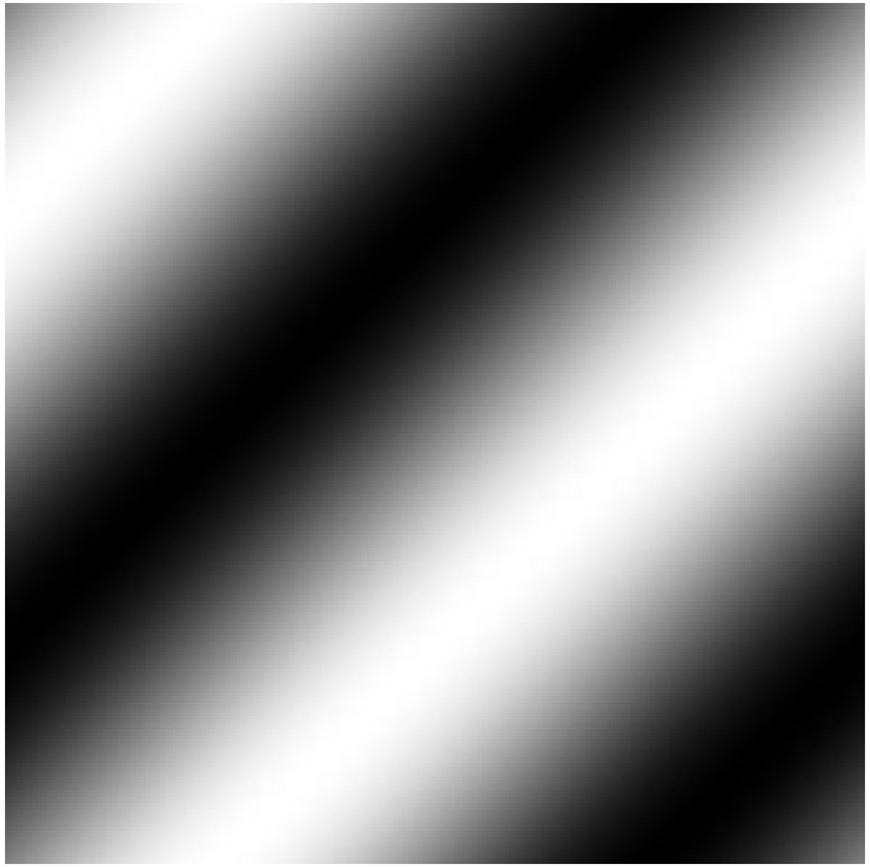
h=0, k=-1, amp=1, phase=90



2D waves

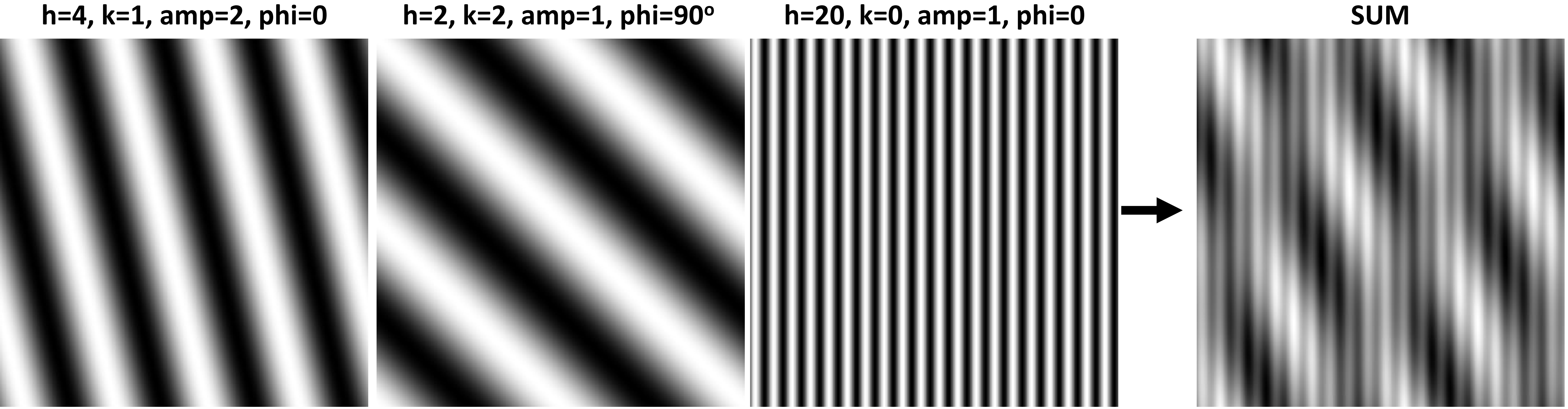
h=1, k=1, amp=1, phase=0

h=1, k=-1, amp=1, phase=0

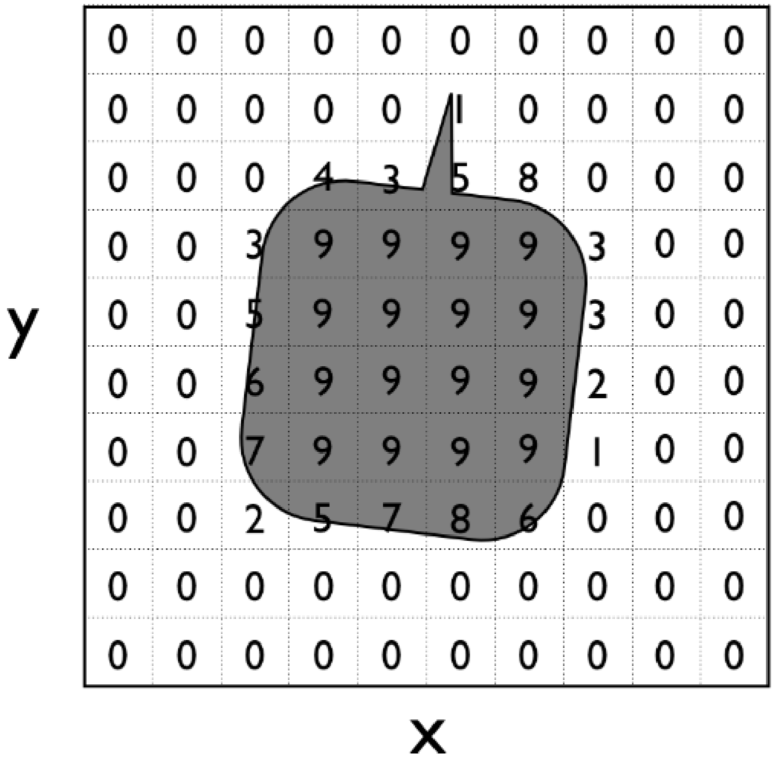


h=2, k=7, amp=1, phase=0

Combining 2D waves

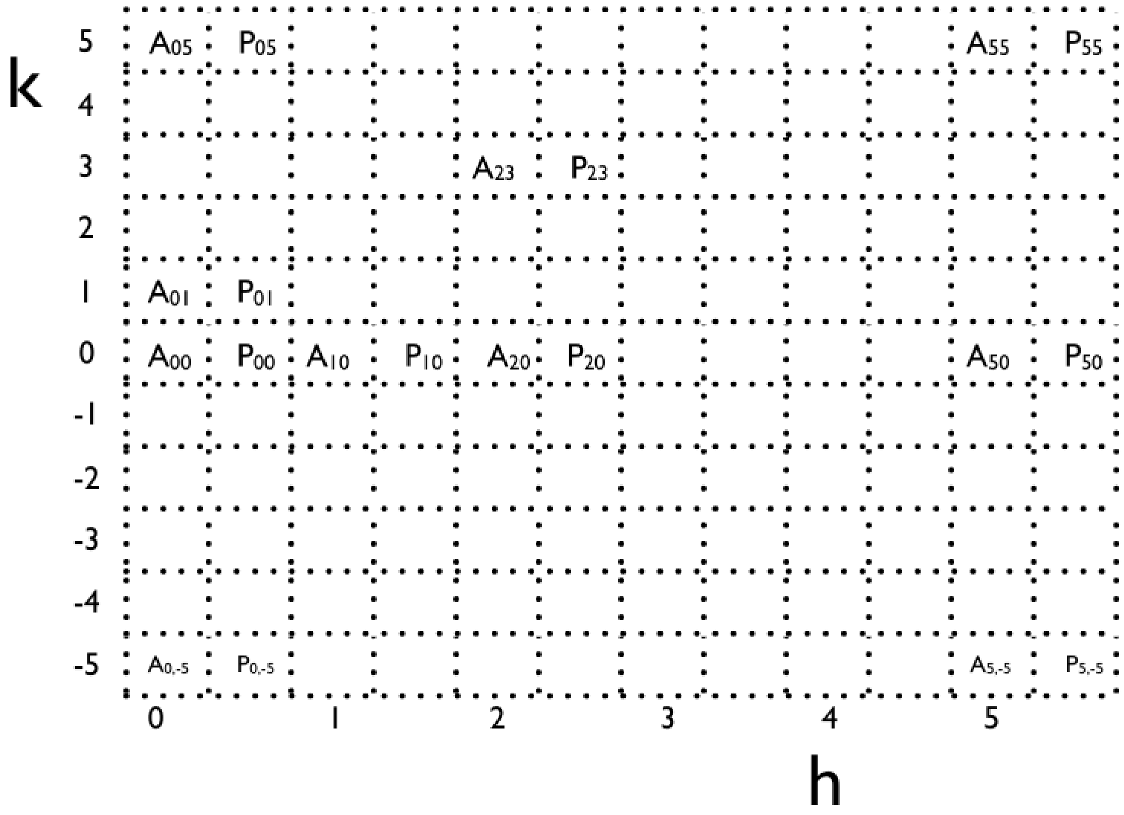


Fourier transform of 2D waves



Fourier transform →

← Inverse FT



N^2 numbers

10x10 (x,y,z) samples

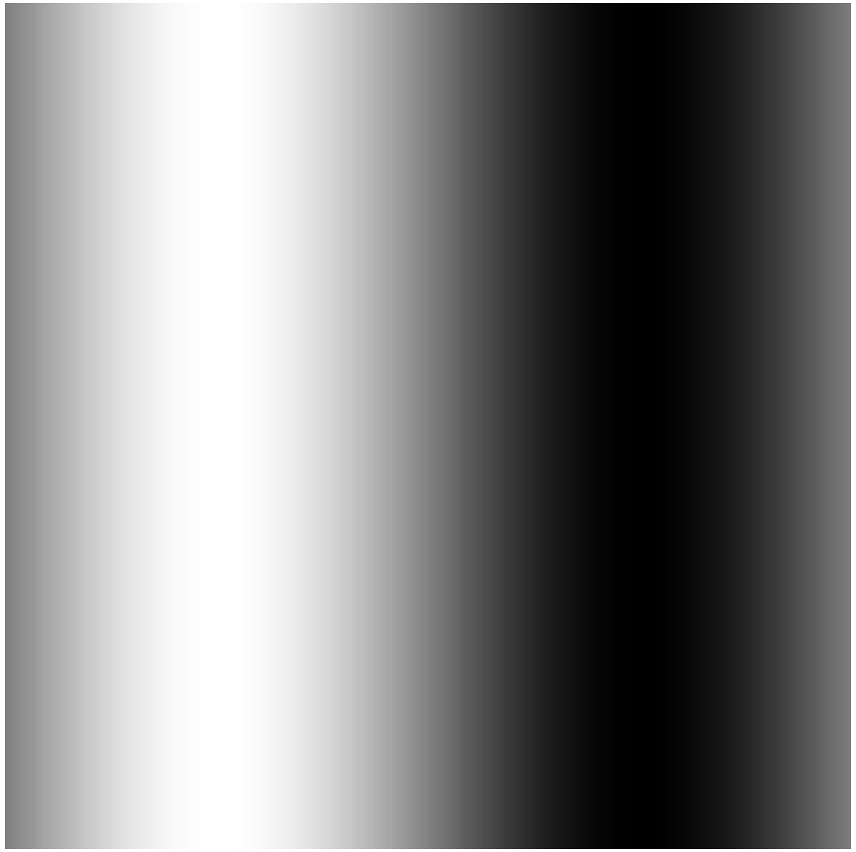
$\sim N^2$ numbers

Grant Jensen

2D Fourier transform of simple 2D waves

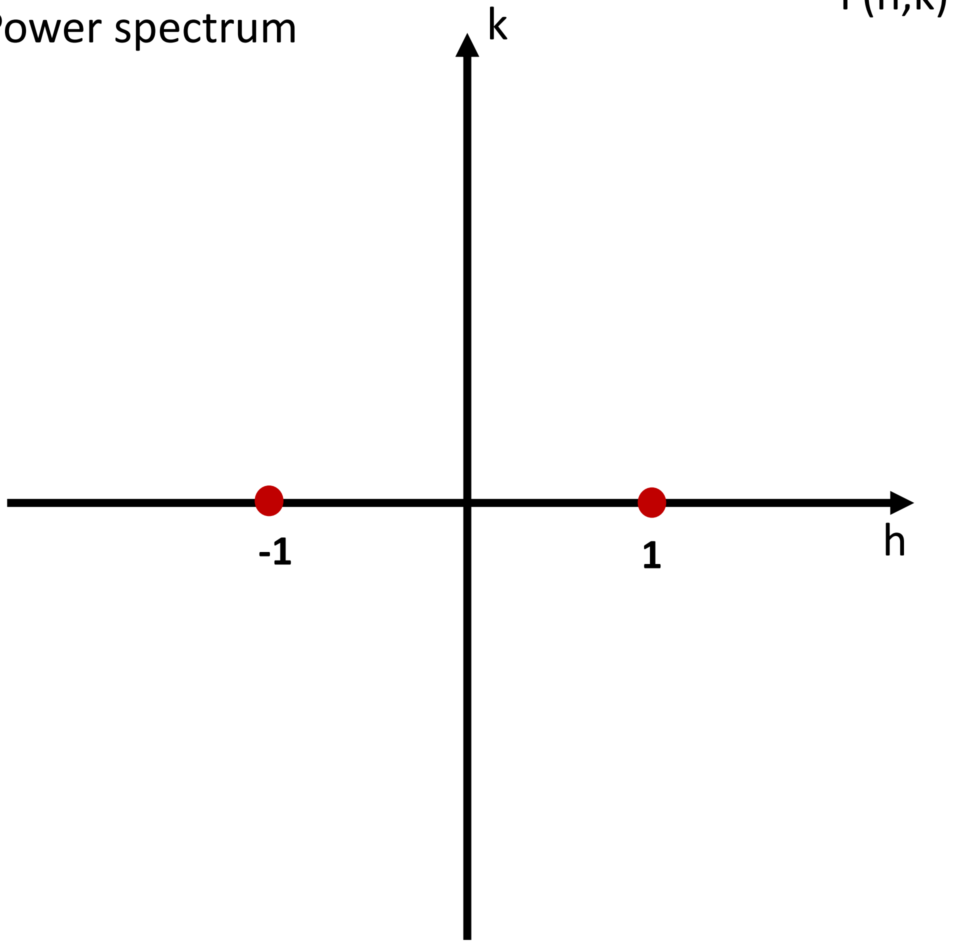
f(x,y)

h=1, k=0, amp=1, phase=0



Power spectrum

F(h,k)



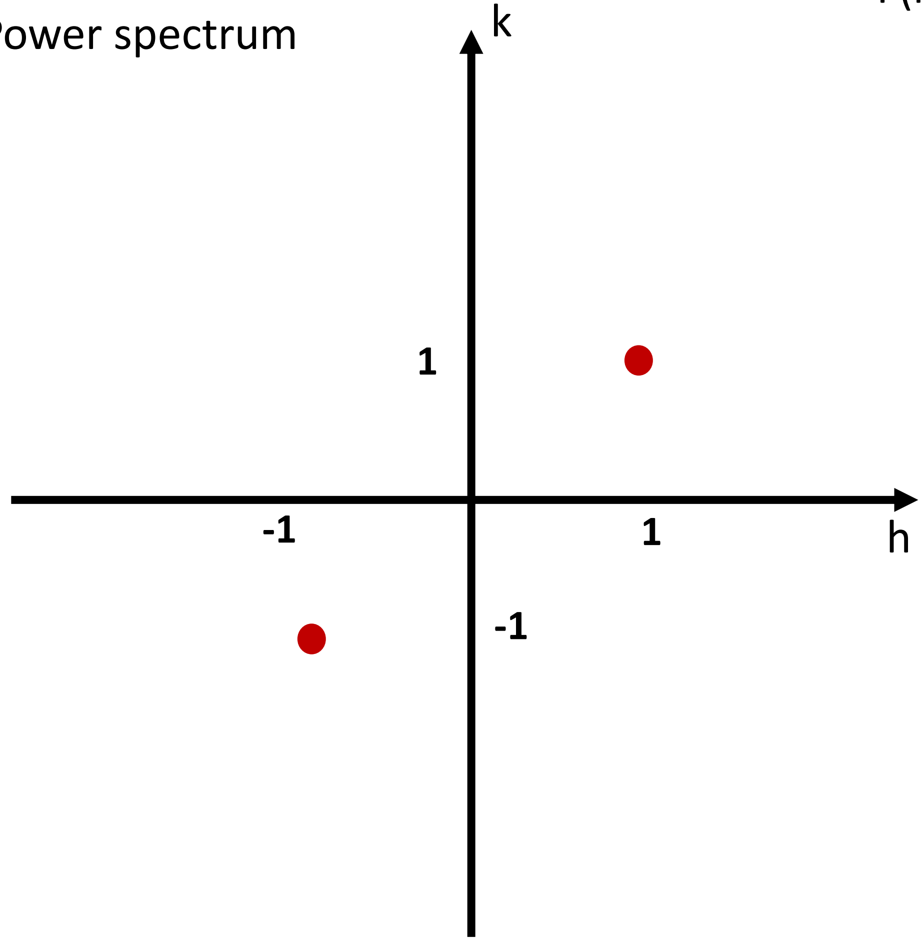
2D Fourier transform of simple 2D waves

f(x,y)

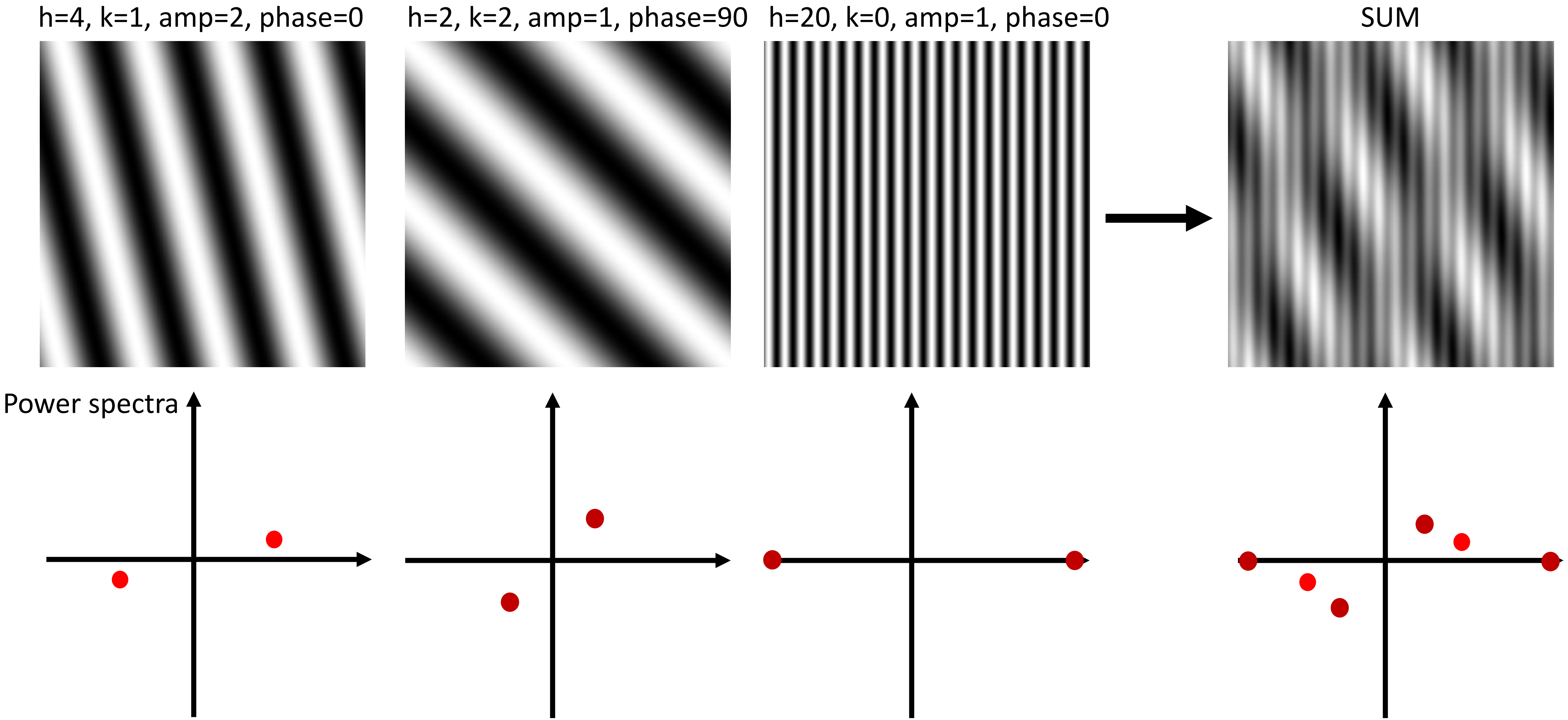
h=1, k=1, amp=1, phase=0

Power spectrum

F(h,k)



2D Fourier transform of simple 2D waves



3D waves

1D wave

k -> number of wave periods

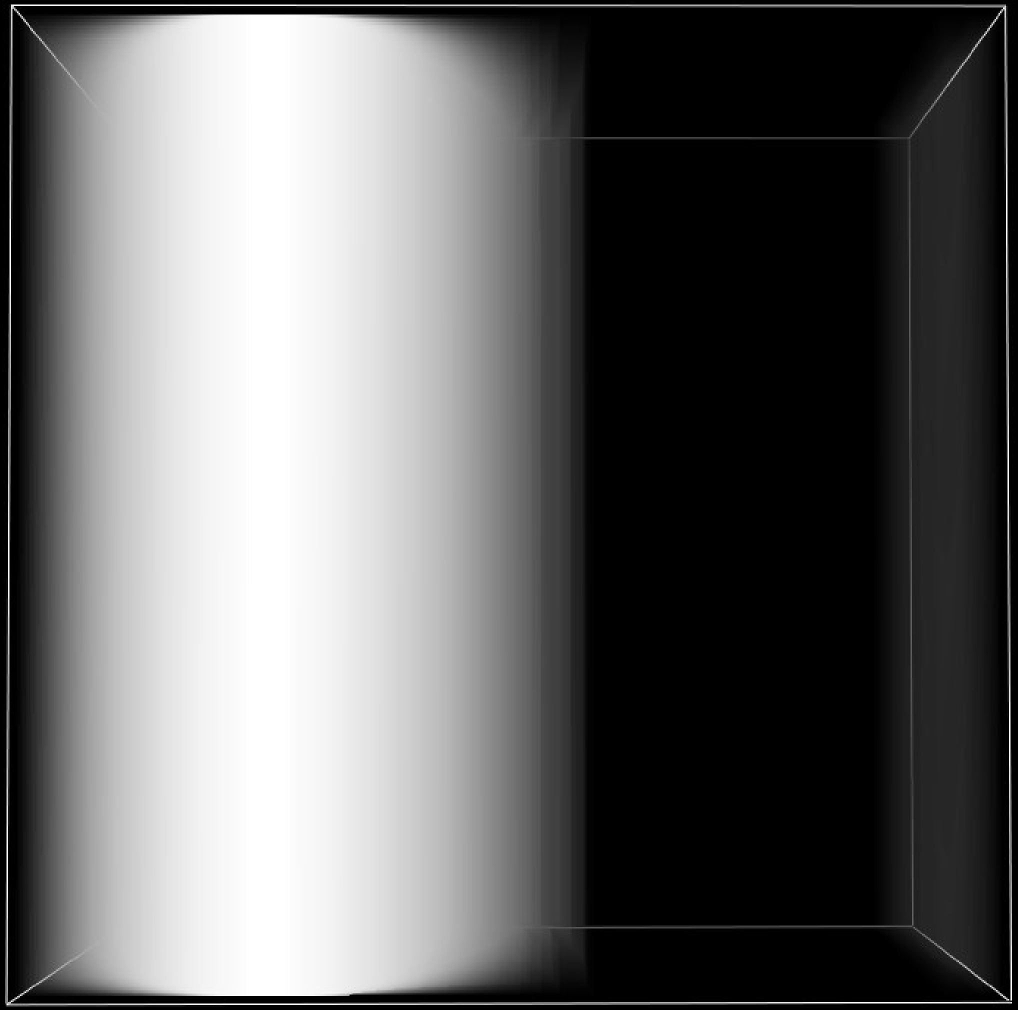
2D wave

h, k -> number of wave periods per x, y

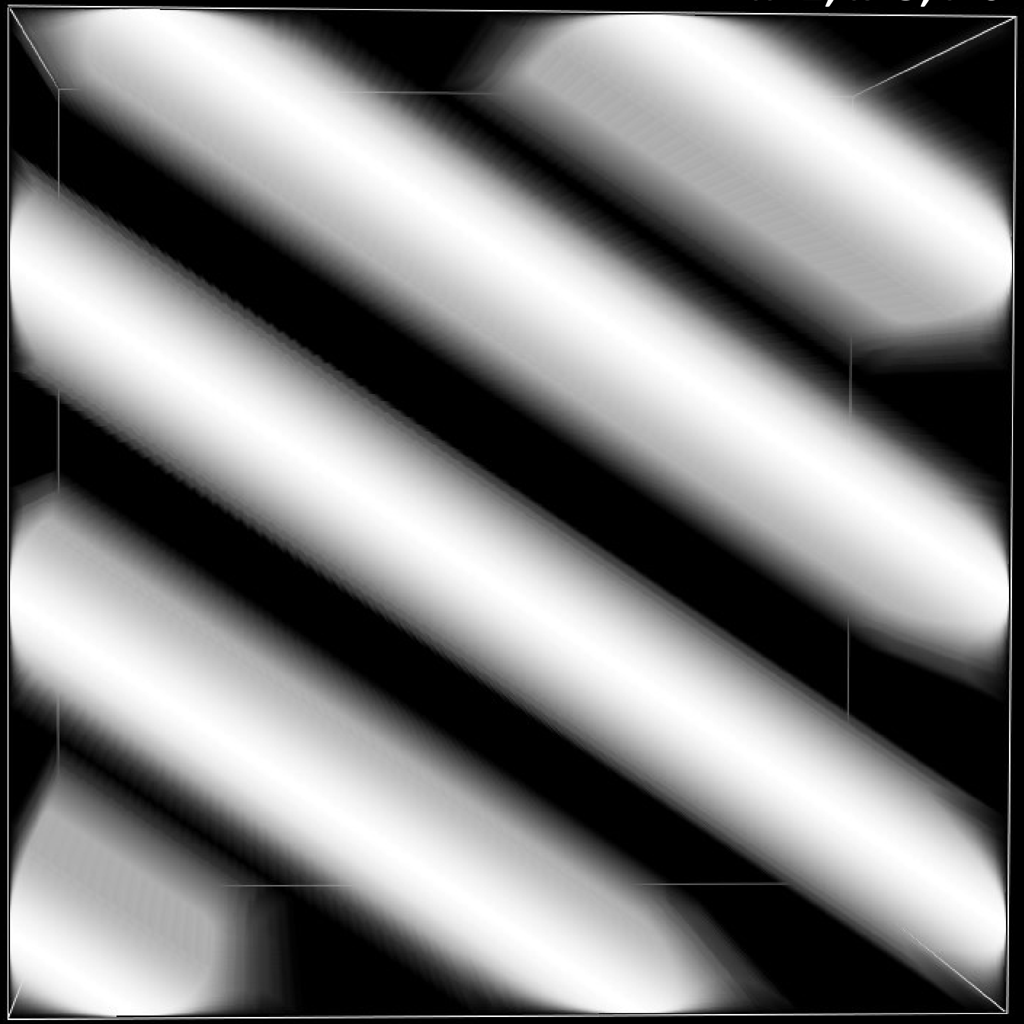
3D wave

h, k, l -> number of wave periods per x, y, z

h=1; k=0; l=0

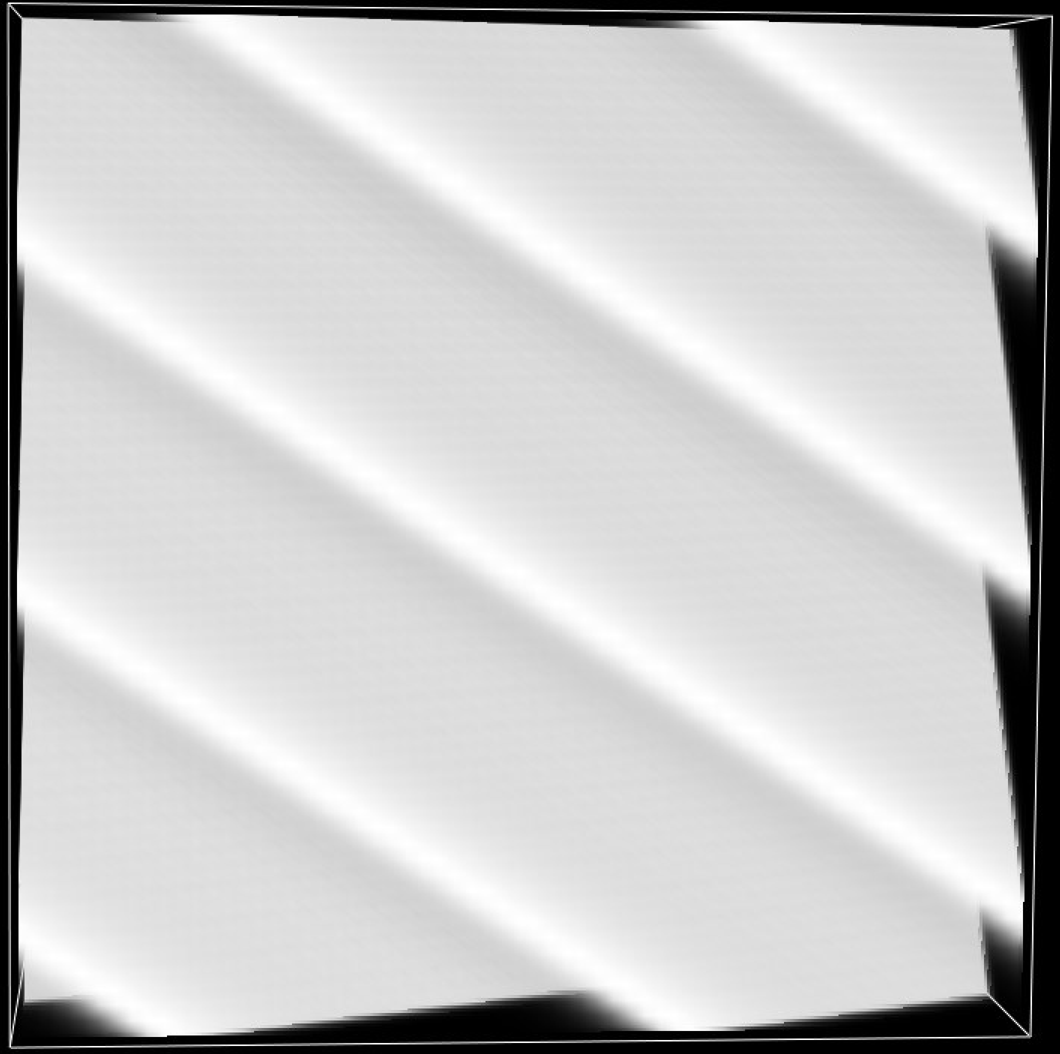


h=2; k=3; l=0

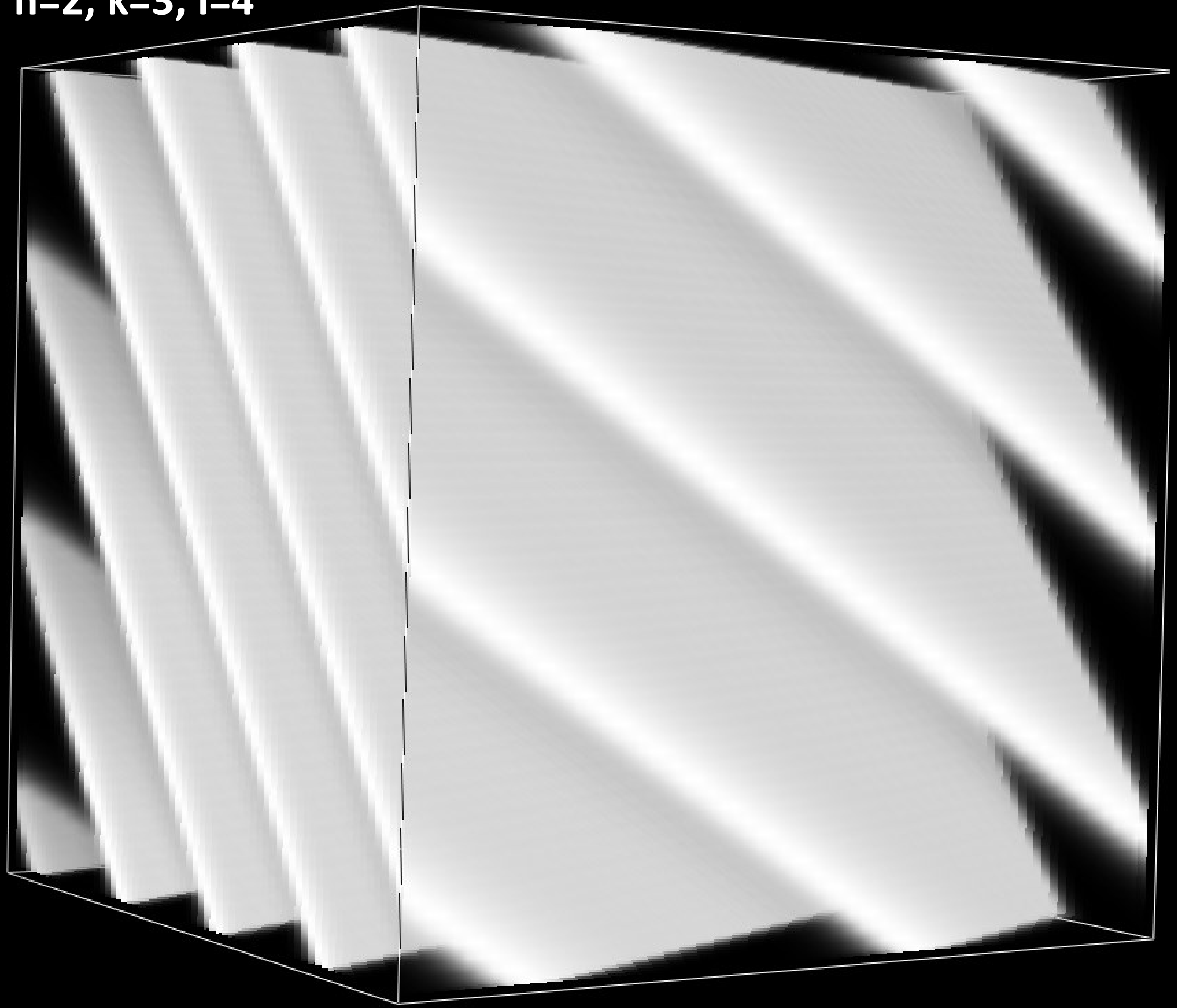


3D waves

h=2; k=3; l=4

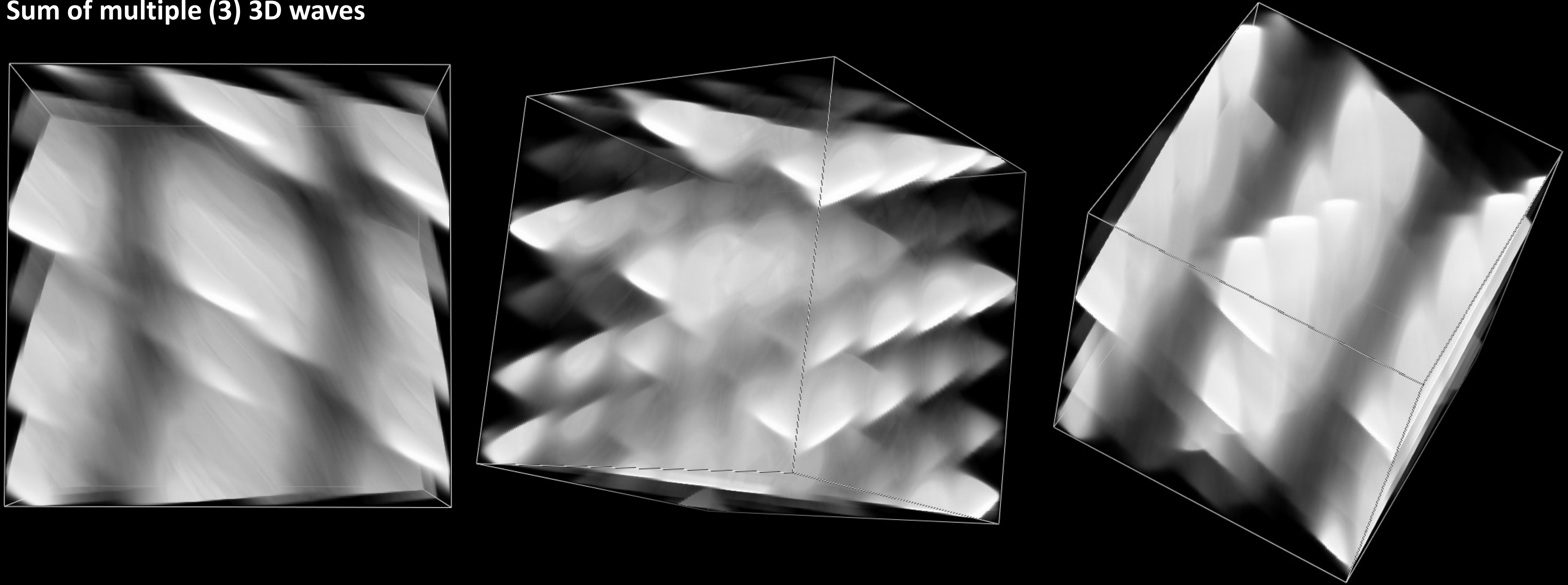


h=2; k=3; l=4



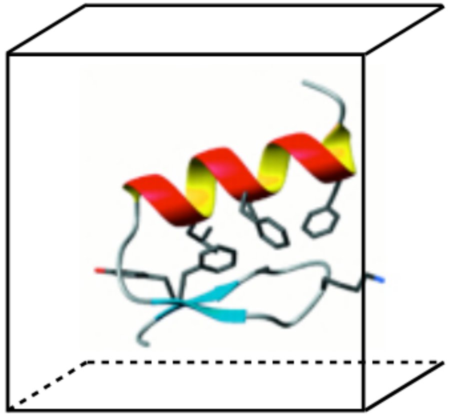
Sum of 3D waves

Sum of multiple (3) 3D waves



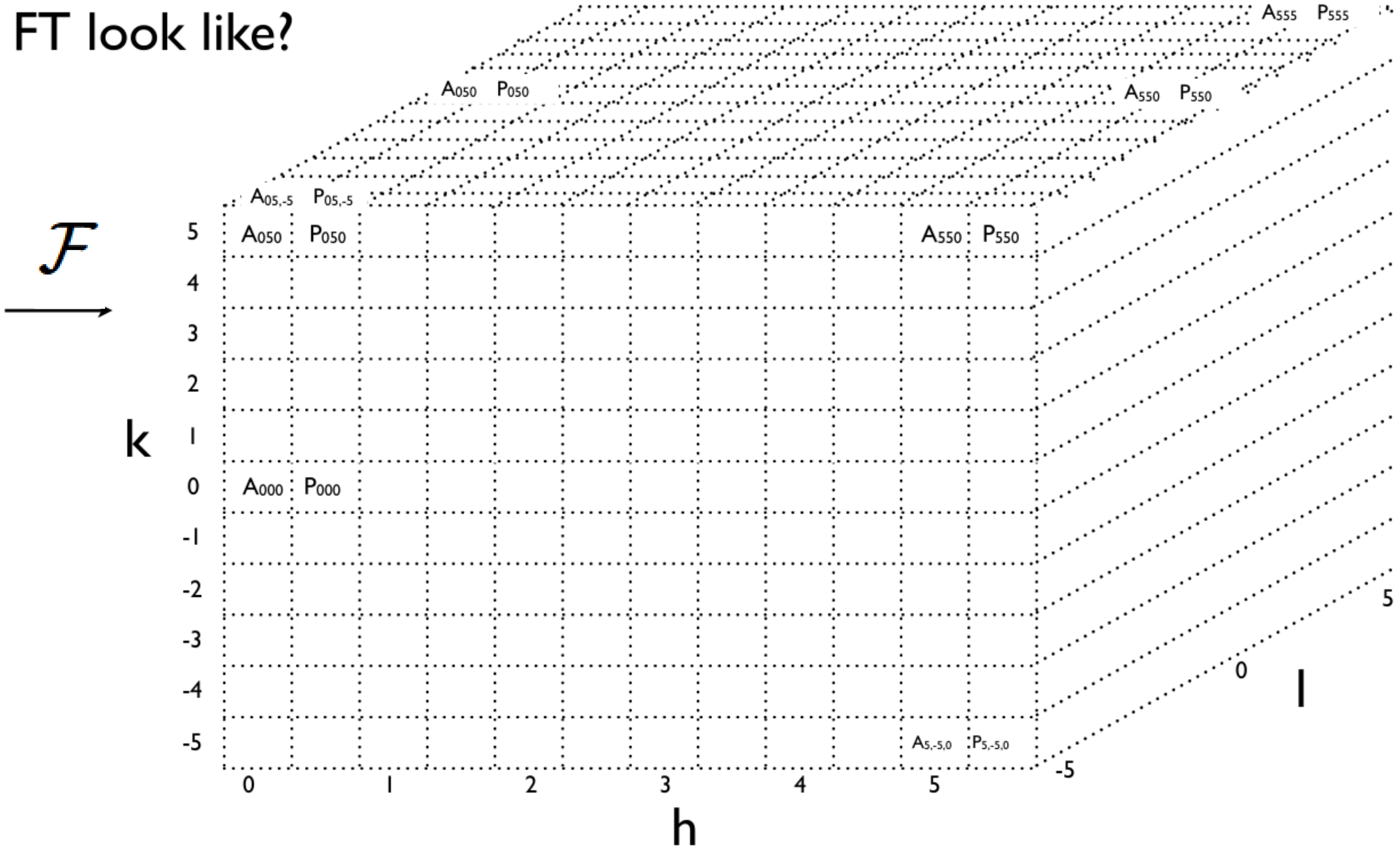
3D Fourier transform

What does a 3-D FT look like?



N^3 numbers

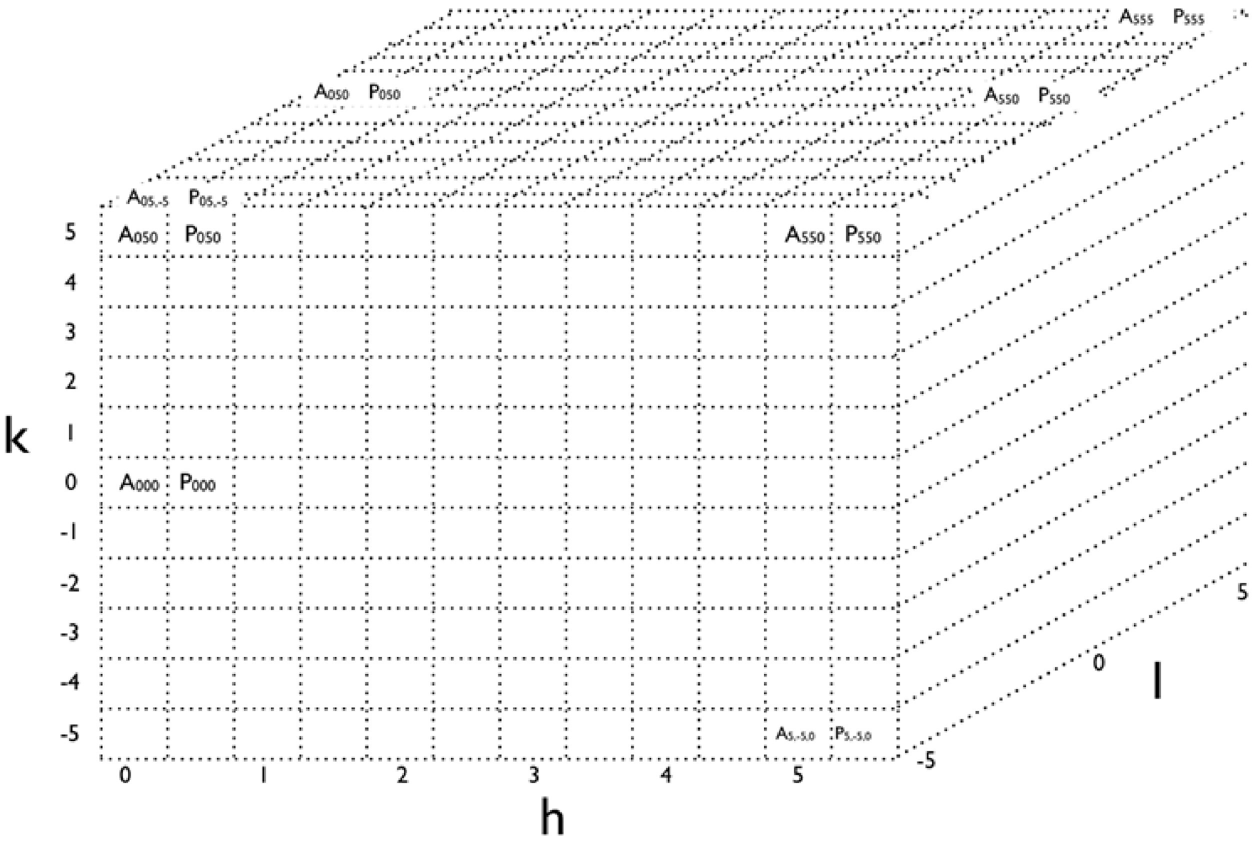
10x10x10 (x,y,z) samples



~N^3 numbers

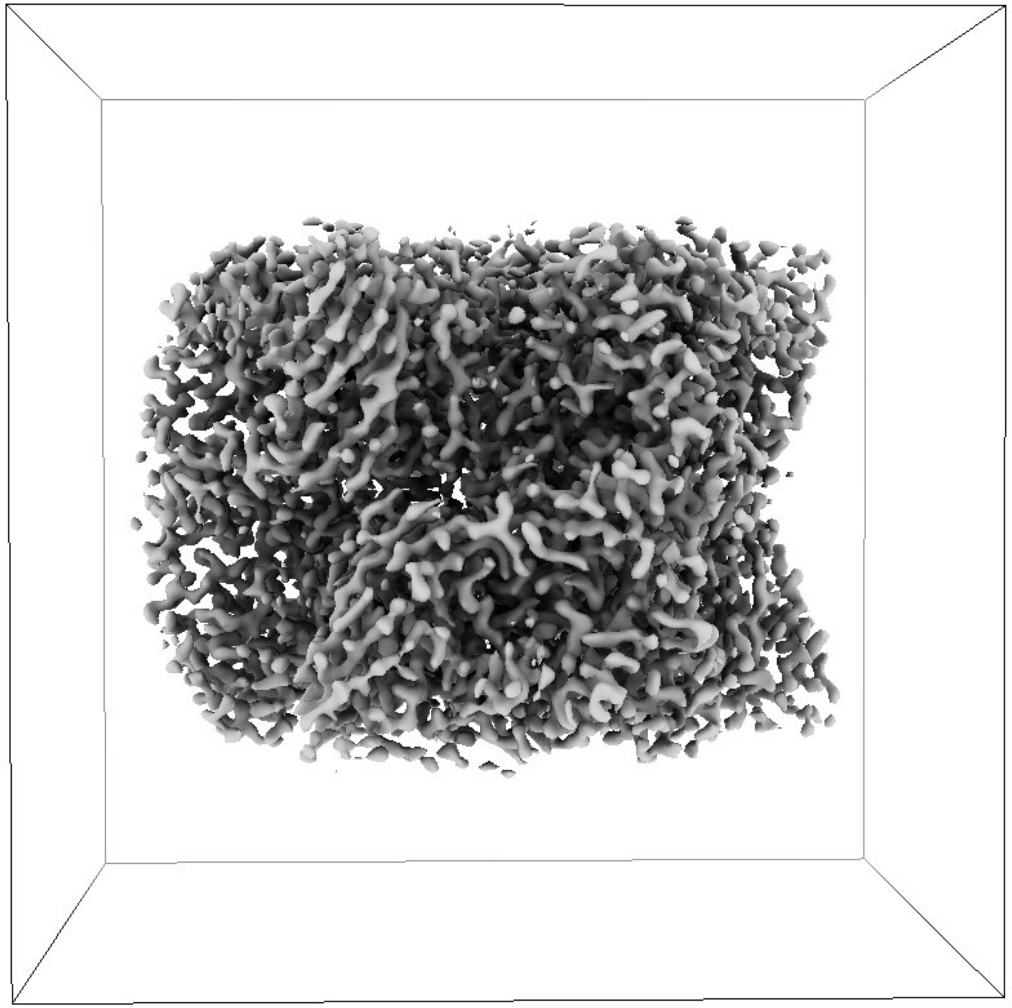
Grant Jensen

3D reconstruction



Reciprocal space

$\xrightarrow{\mathcal{F}^{-1}}$



Real-space

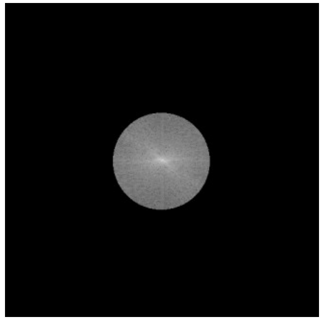
$$\rho(x\,y\,z) = \frac{1}{V}\sum_h \sum_k \sum_l \left|F(h\,k\,l)\right| \exp\left[-2\pi i(hx + ky + lz) + i\alpha(h\,k\,l)\right]$$

from Lecture 3

Good to know about reciprocal space

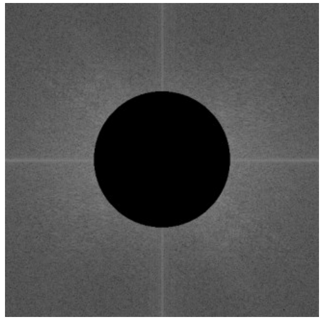
- Every single point in reciprocal space affects all the points in real-space
- Every single point in real-space affects all the points in reciprocal space
- More far from the center of the power spectrum – higher the spatial frequency
- While only amplitudes are represented in the power spectrum, the underlying phases are equally important

Letting the low freq. pass



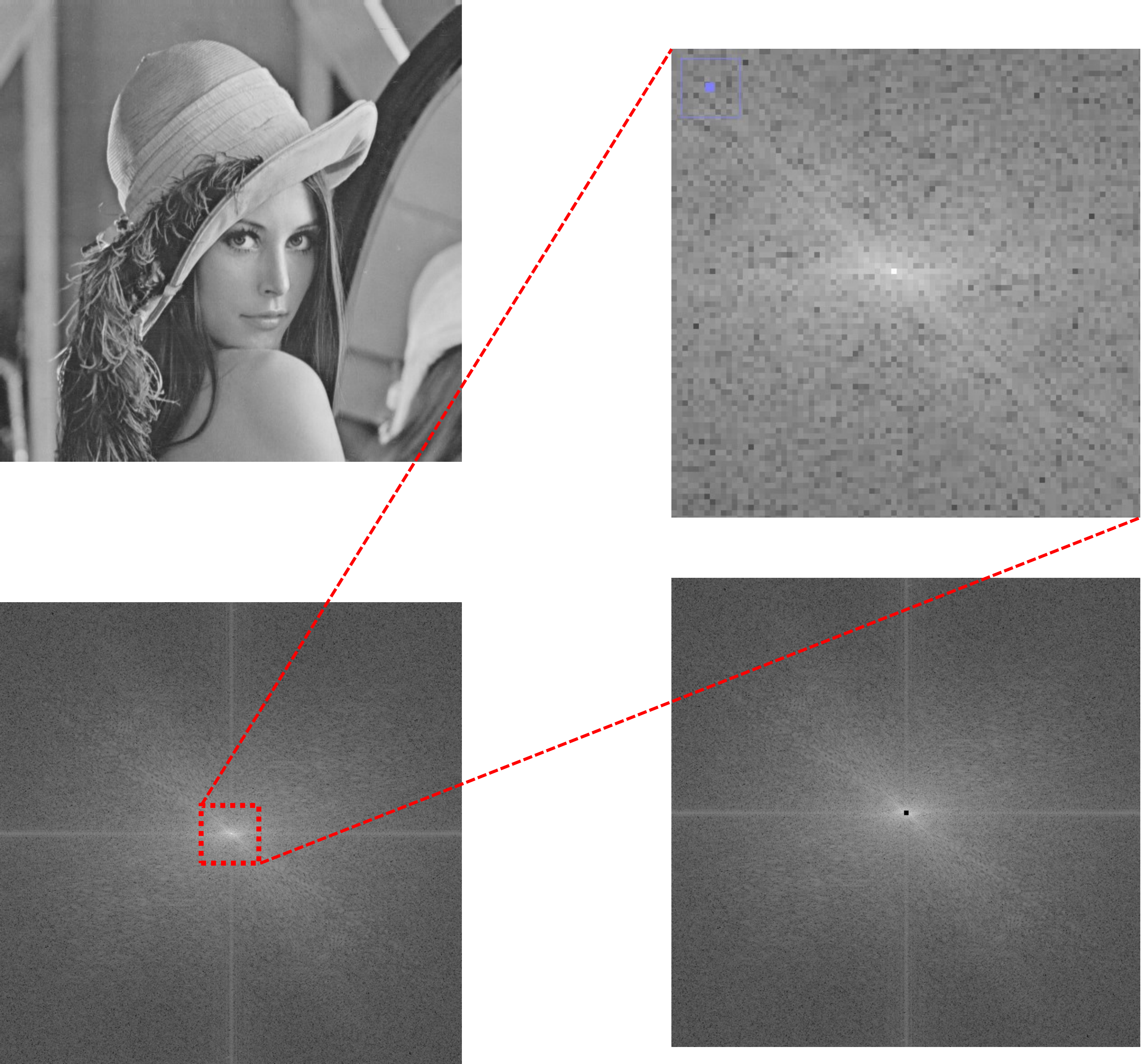
Low-pass filter

Letting the hi freq. pass



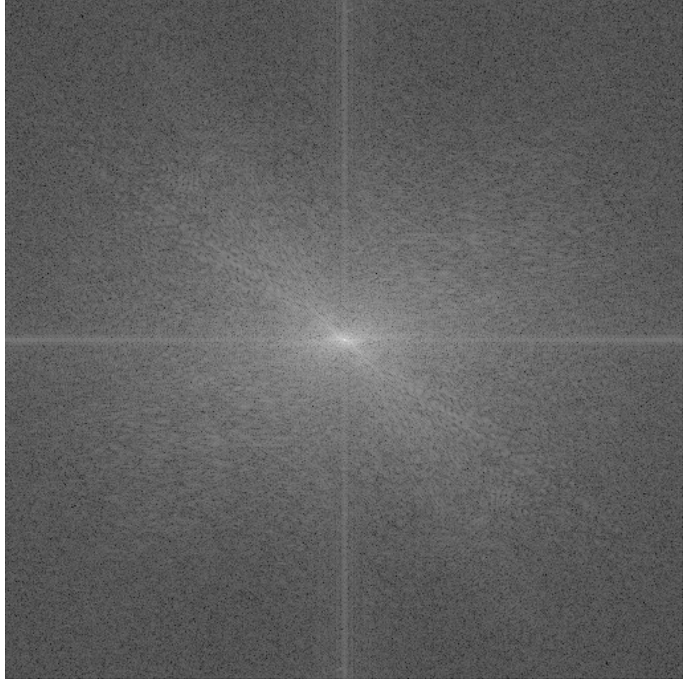
Hi-pass filter

DC component



DC component removed

Image rotation



Real-space **Reciprocal-space**

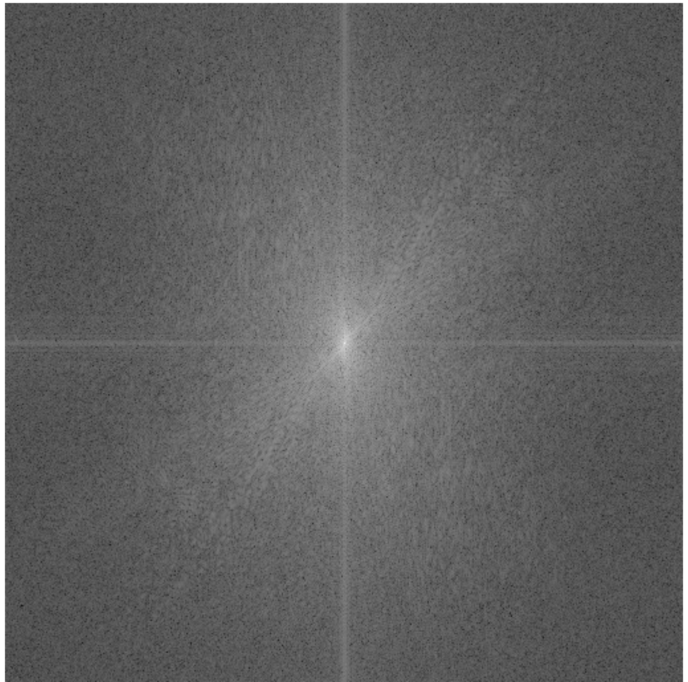
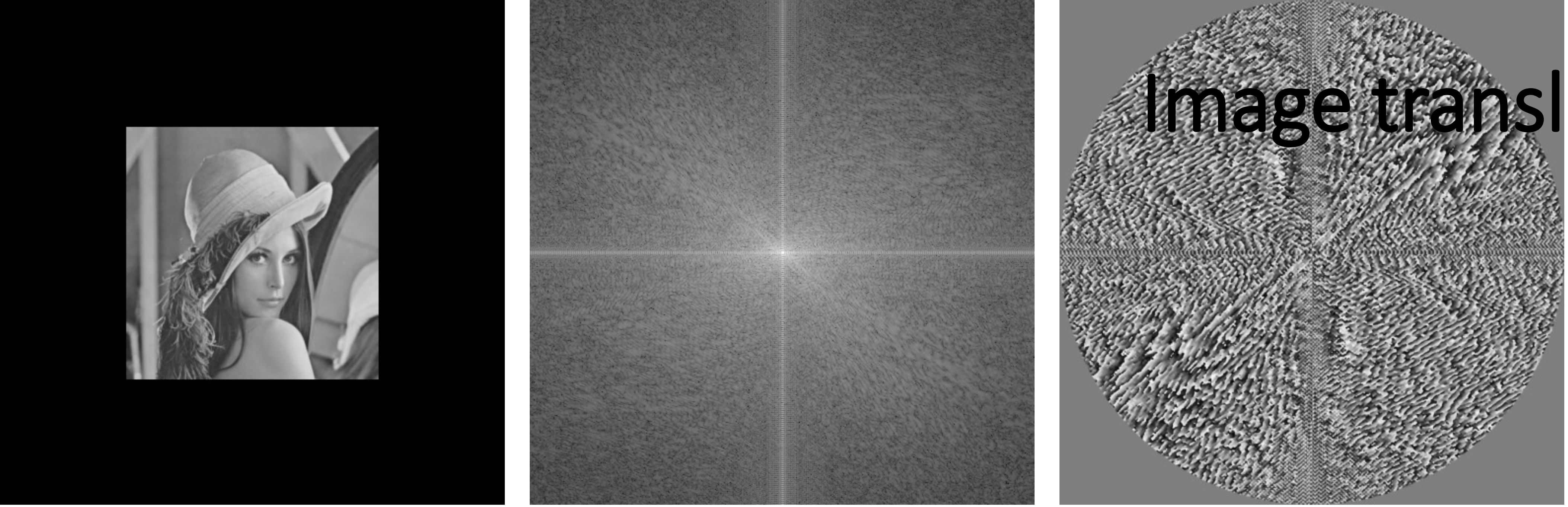
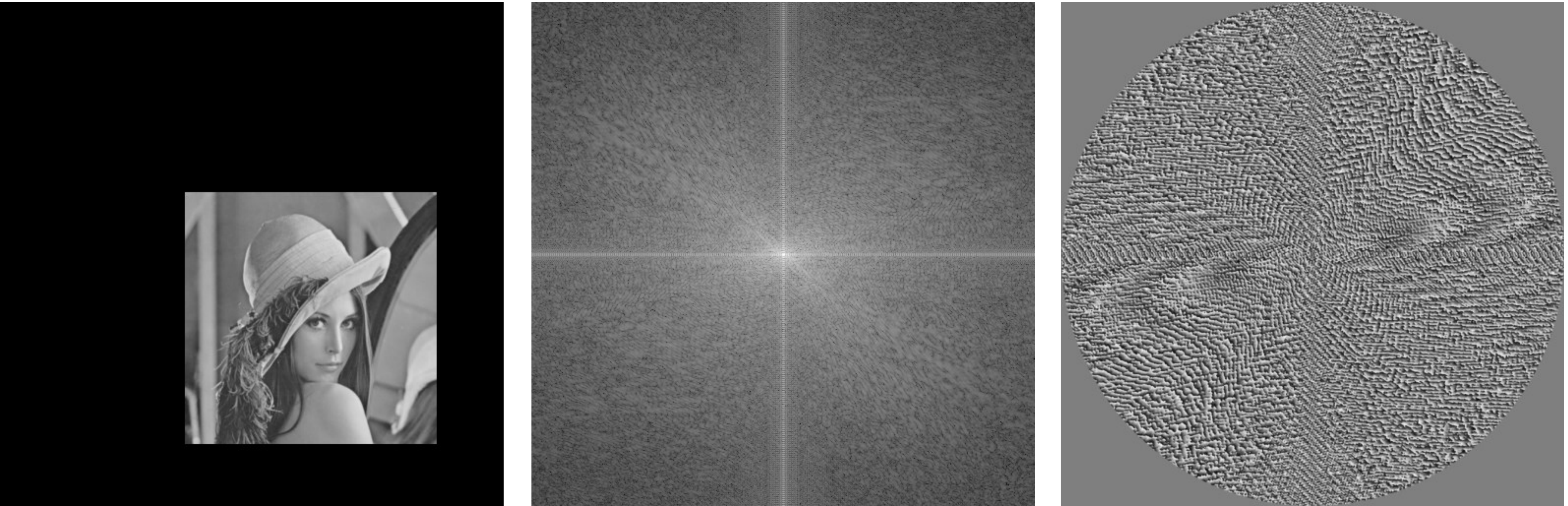


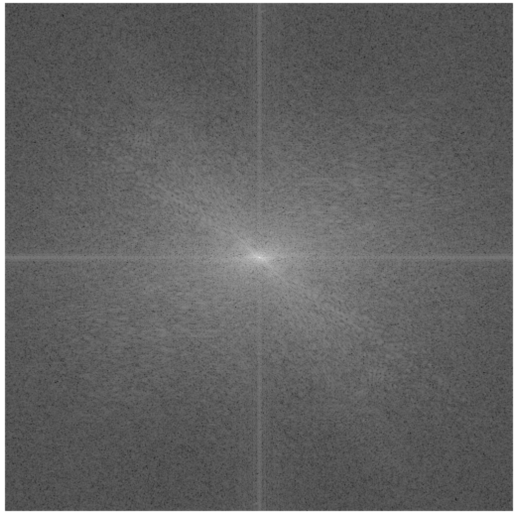
Image translation



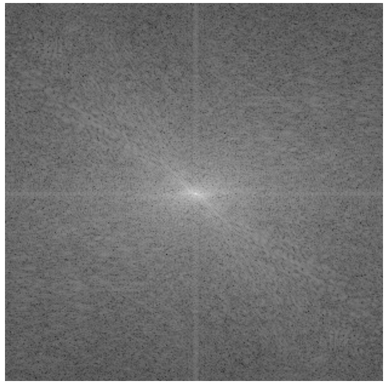
Real-space **Reciprocal-space** **Reciprocal-space phases**



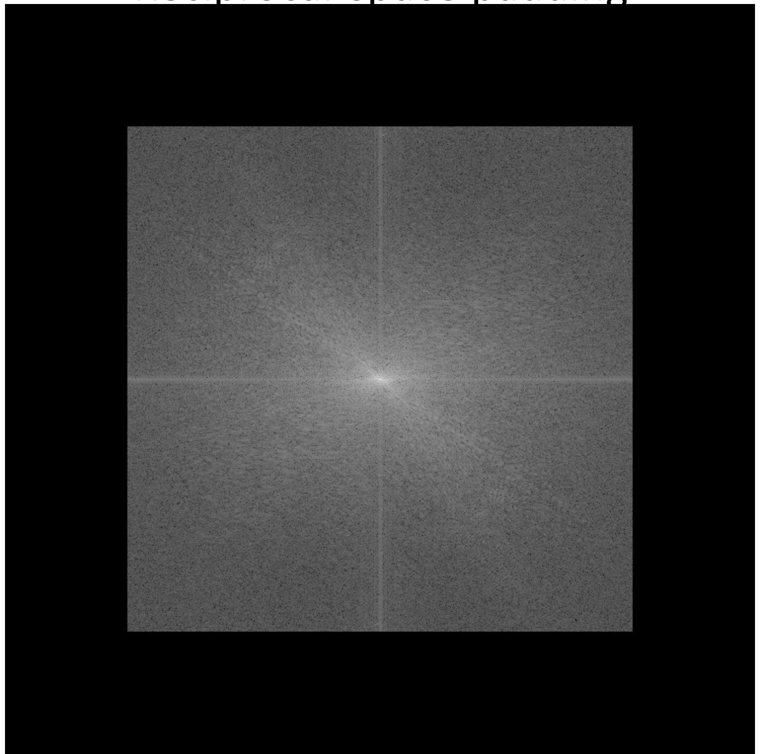
Fourier space cropping, padding



Reciprocal-space cropping



Reciprocal-space padding



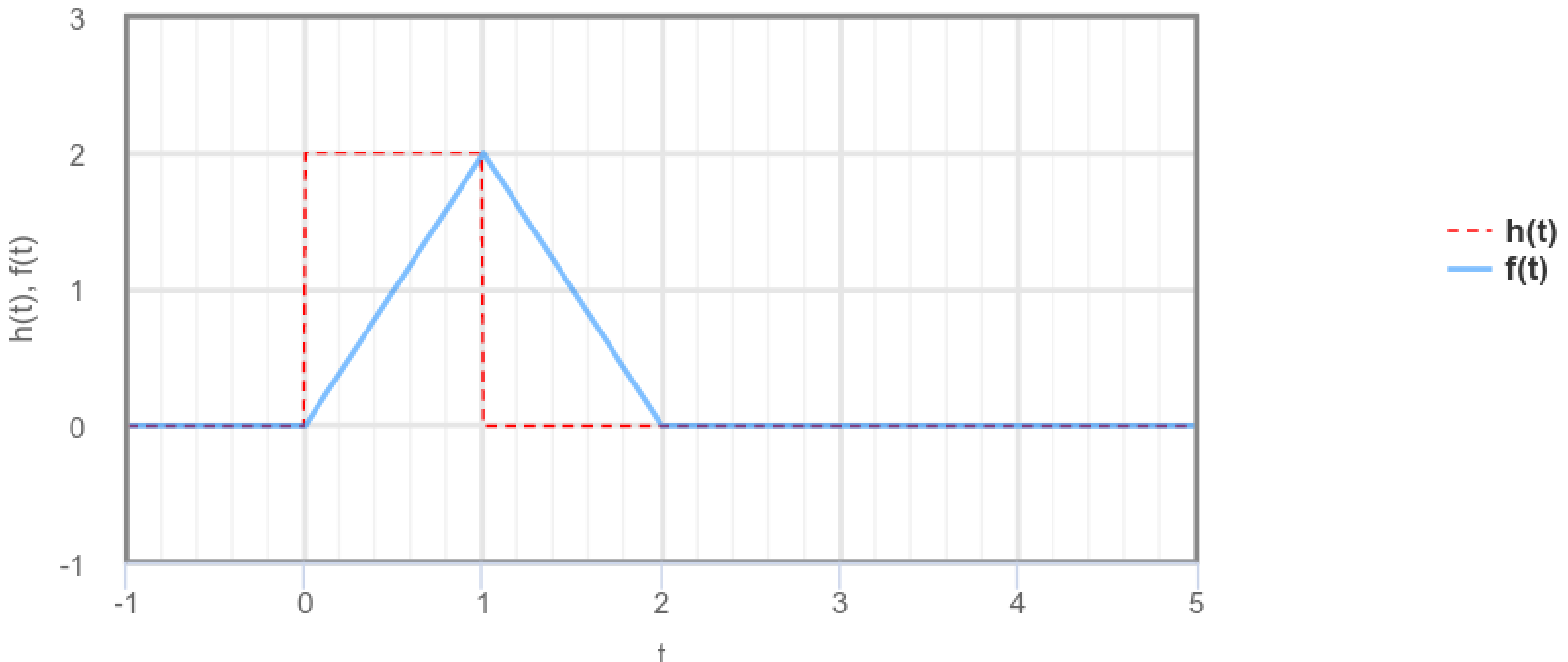
Downscaling (~lowpass)

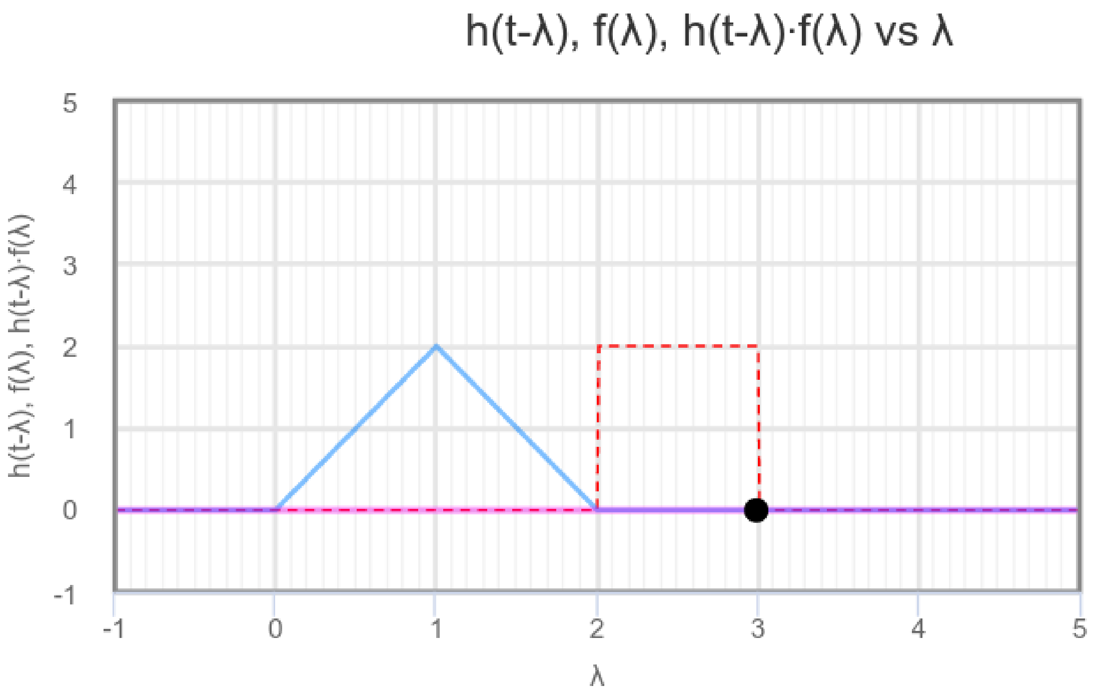
Upscaling (without adding information)

Convolution

- **Convolution is a mathematical operation on two functions (f and g) that produces a third function (f*h) that expresses how the shape of one is modified by the other.**
- **f*h ~ “pass the function f over the function g take the area under”**
- **Convolution is commutative operation**

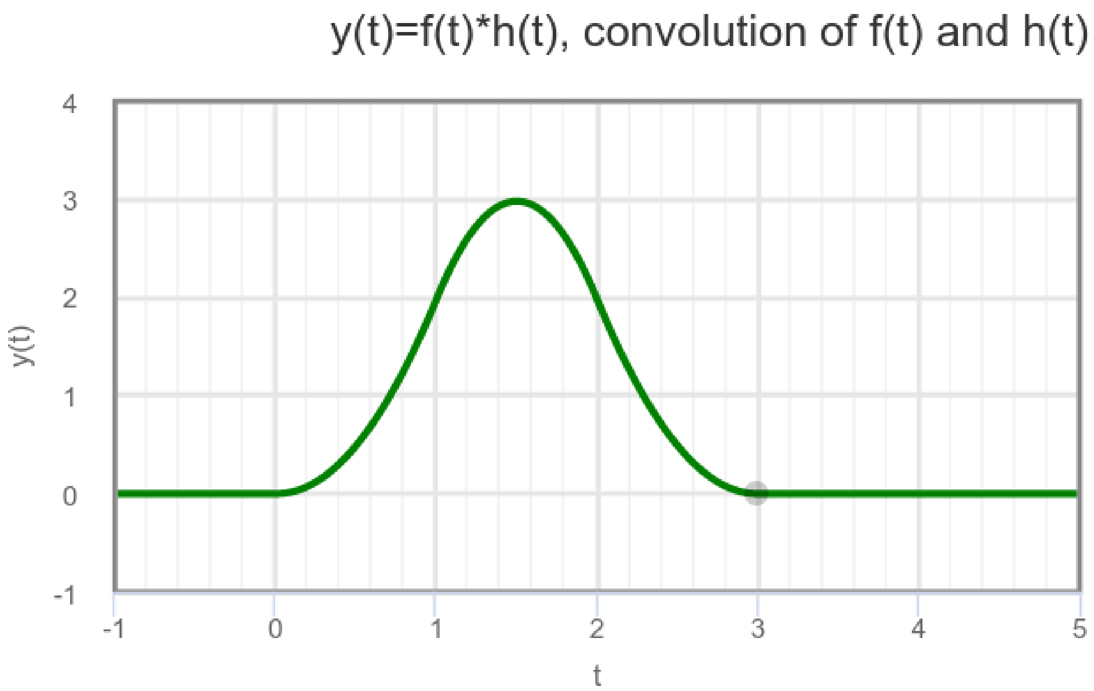
$$g(i) = f \otimes h = \int_{-\infty}^{\infty} f(x)h(i-x)dx$$





Convolution

$$g(i) = f \otimes h = \int_{-\infty}^{\infty} f(x)h(i-x)dx$$



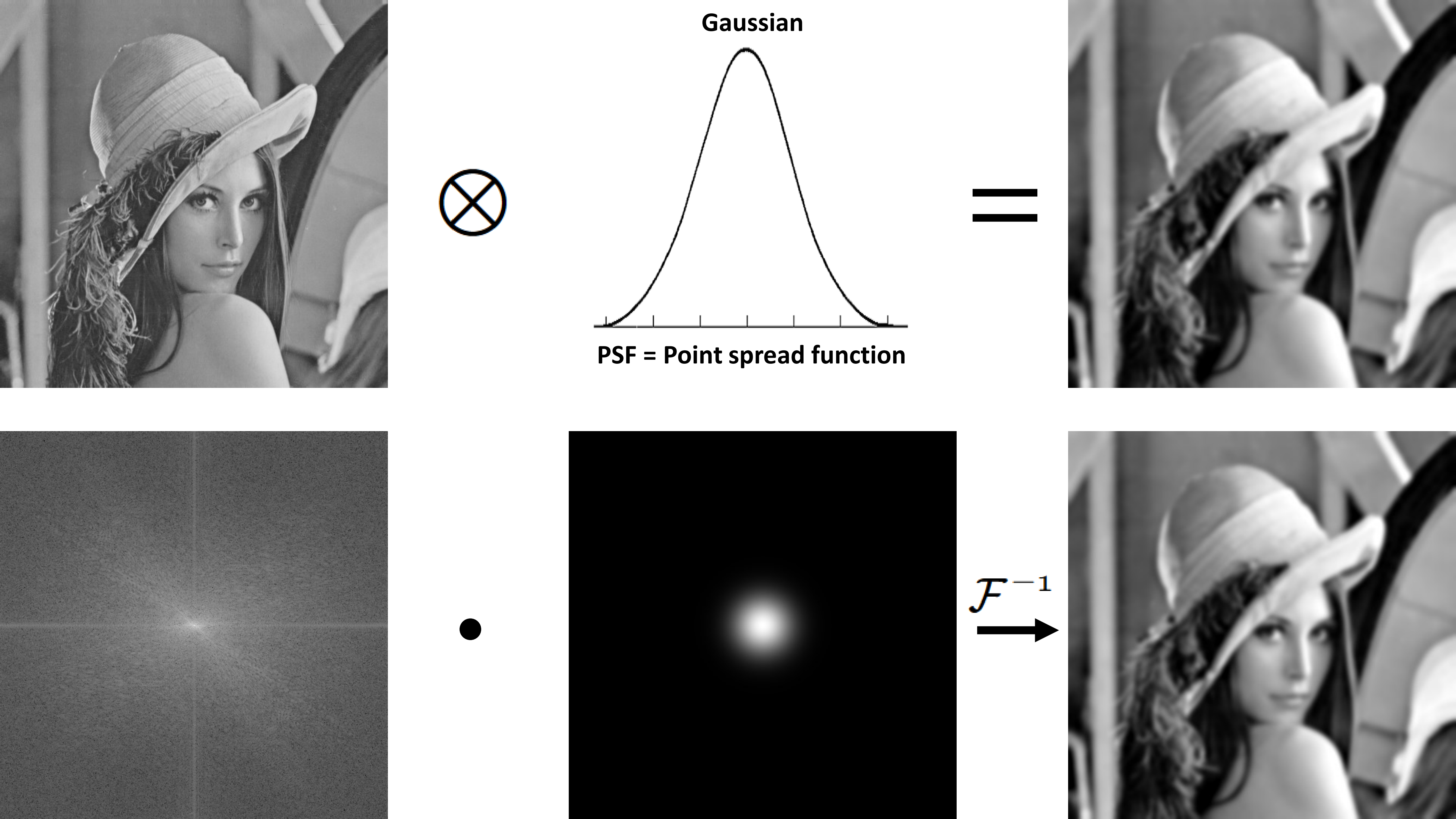
Convolution theorem

$$g = f \otimes h$$

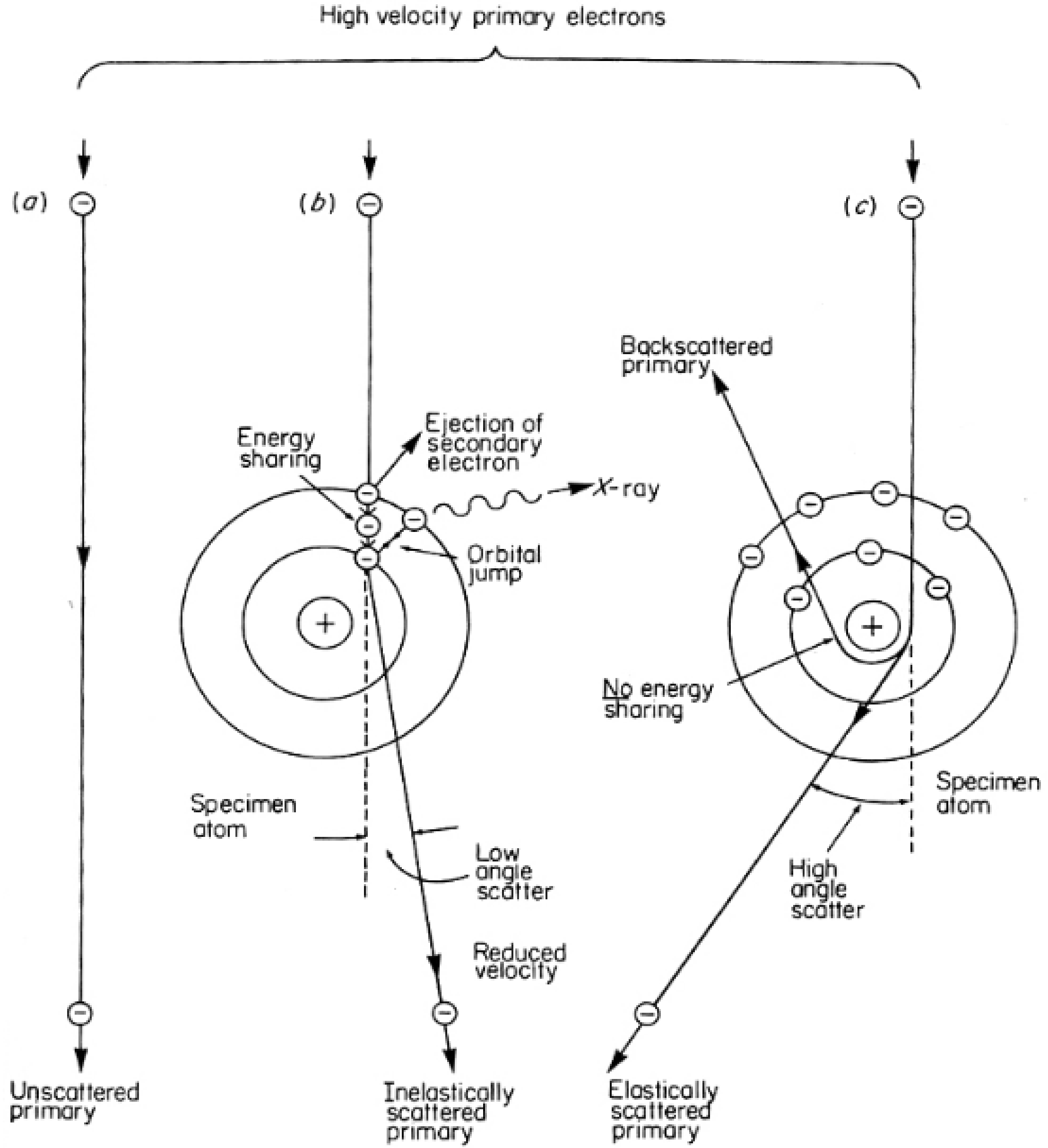
$$\mathcal{F}\{g\} = \mathcal{F}\{f\} \bullet \mathcal{F}\{h\}$$

$$g = f \otimes h = \mathcal{F}^{-1}\{\mathcal{F}\{f\} \bullet \mathcal{F}\{h\}\}$$

https://lpsa.swarthmore.edu/Convolution/CI.html


Gaussian
PSF = Point spread function
$\mathcal{F}^{-1}$

Electron scattering



Electron scattering – TEM image formation

Braggs law

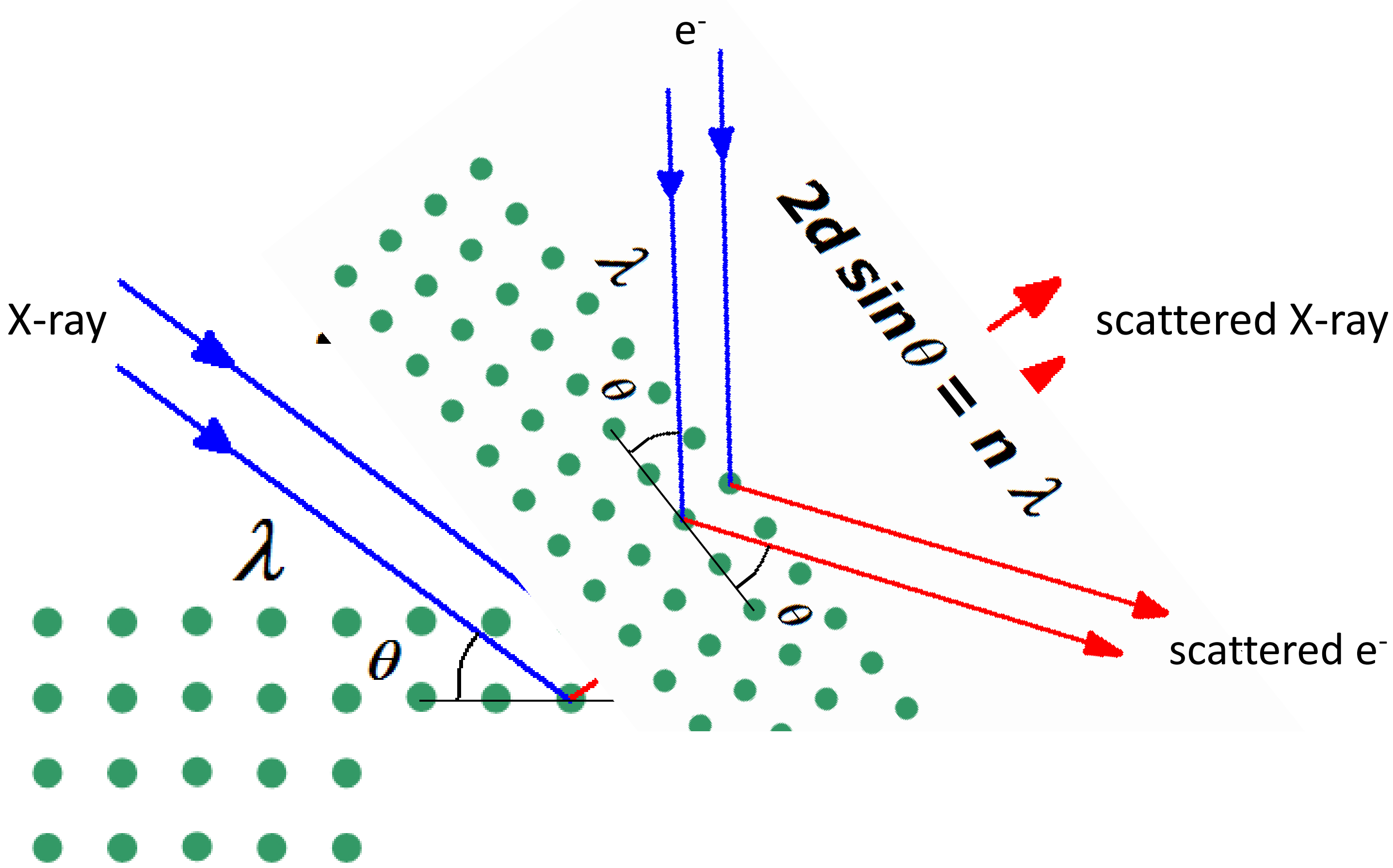
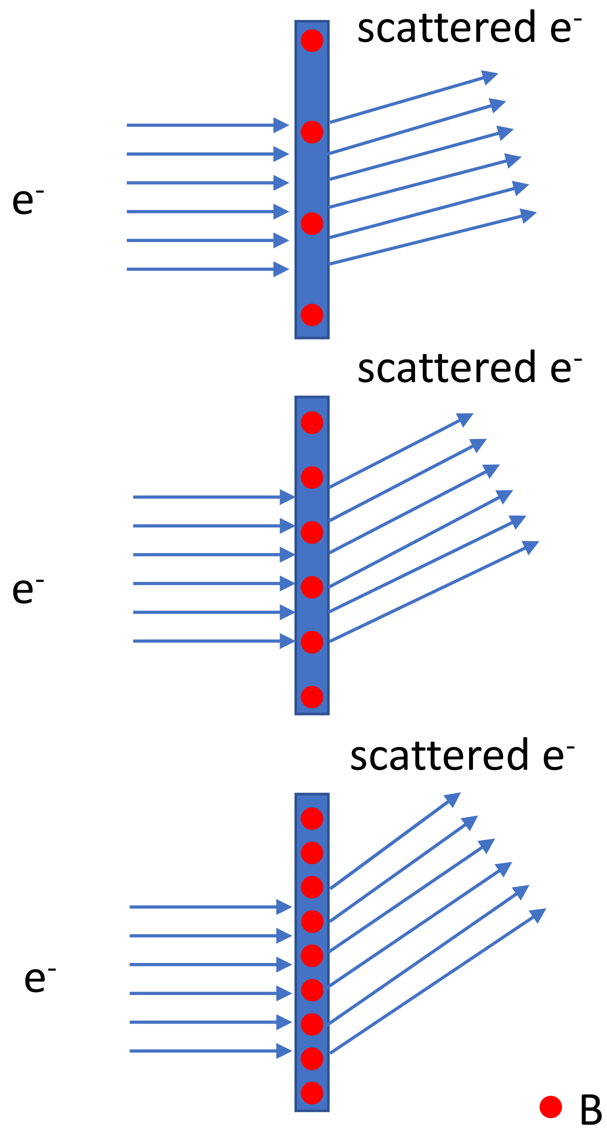
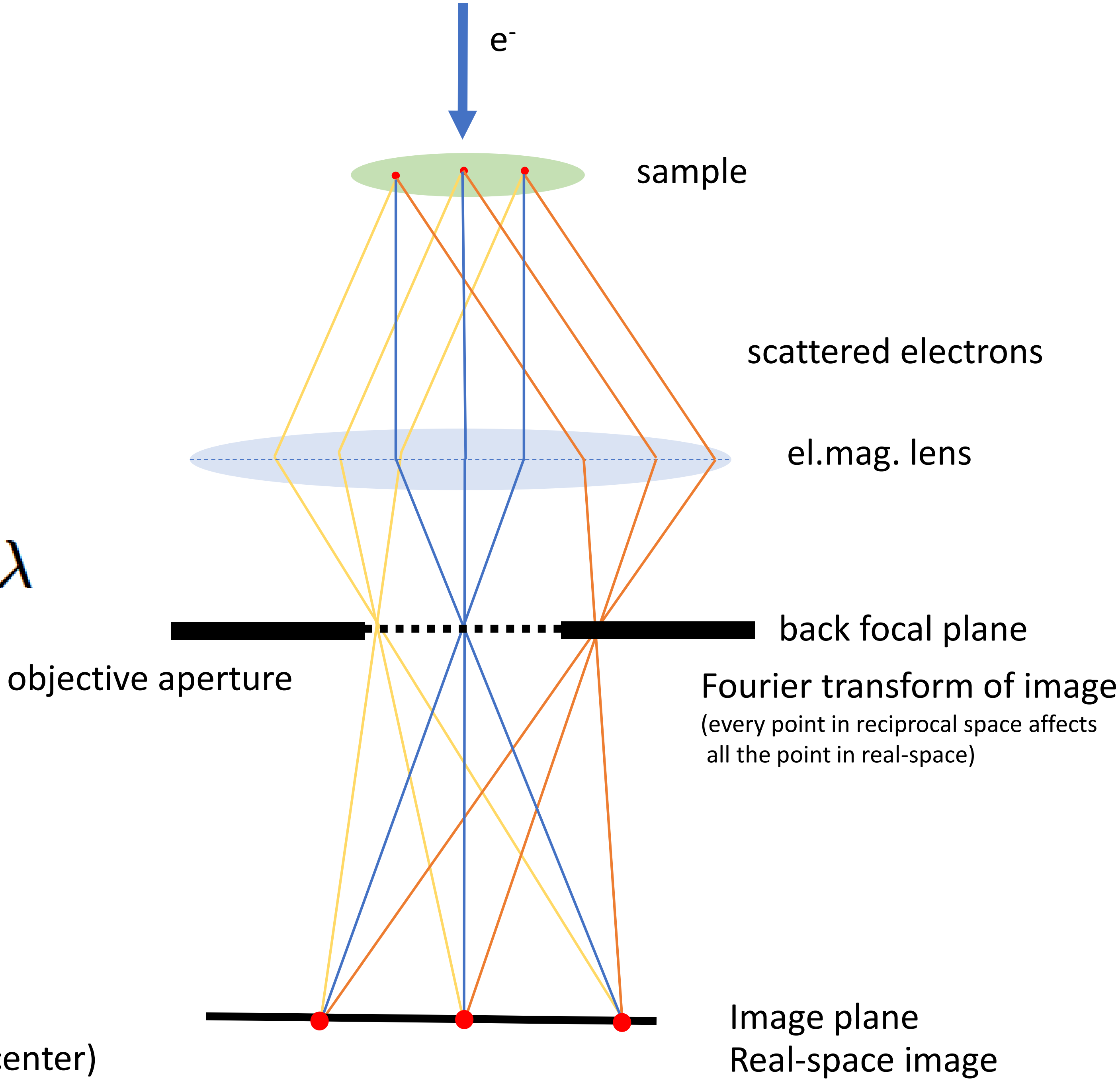


Image formation in TEM



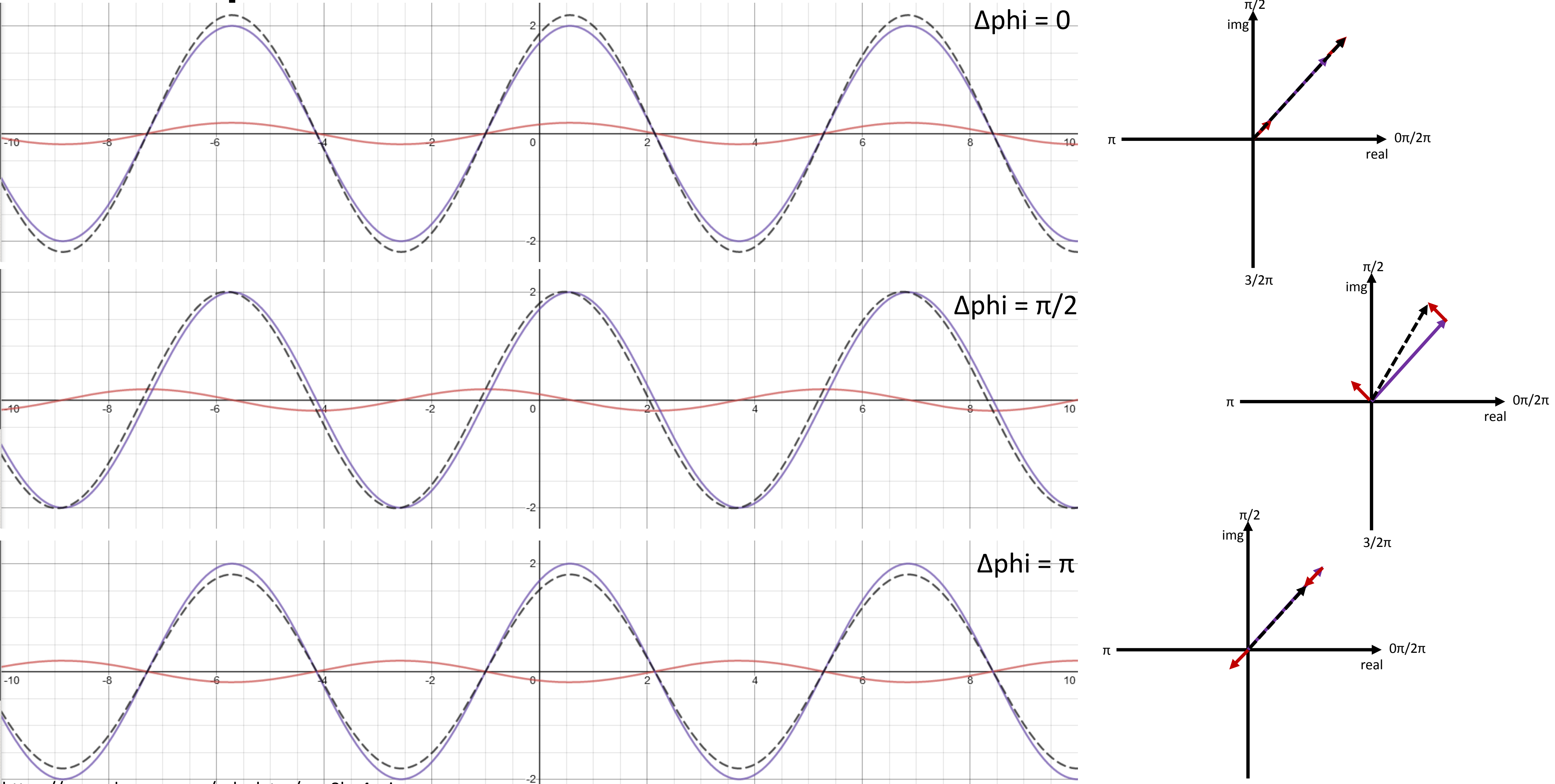
$$2d sin\theta = n\lambda$$

● Bragg plane (scattering center)



Sum of phase shifted waves

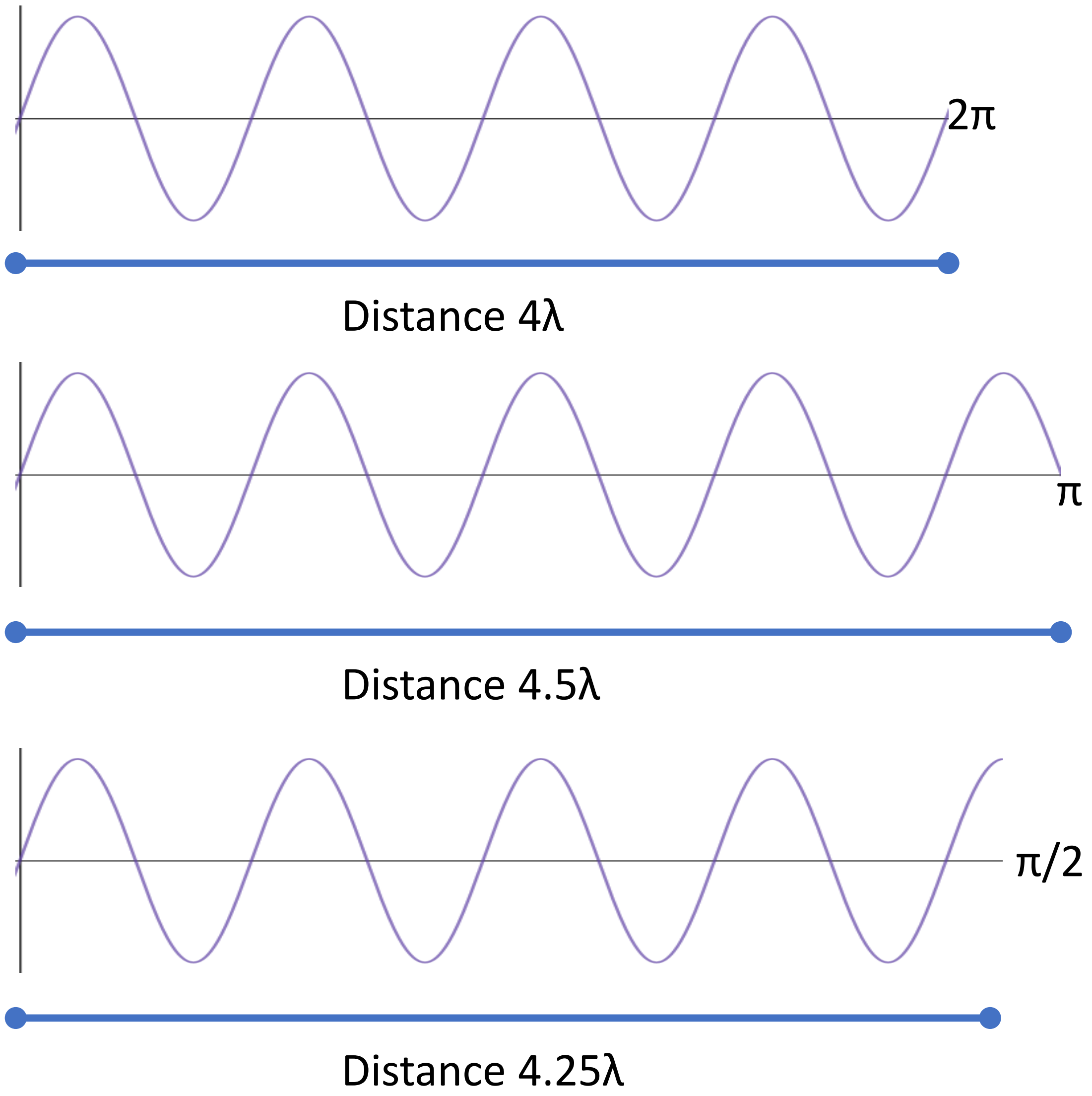
Argand diagram

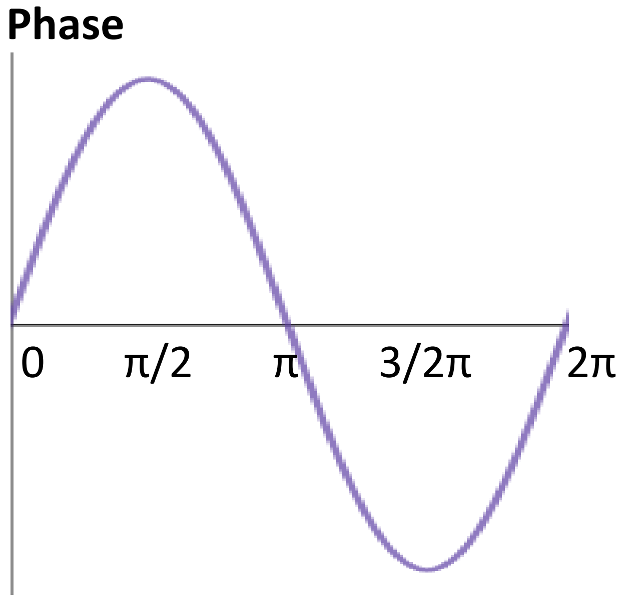


https://www.desmos.com/calculator/cwc8bn4snj

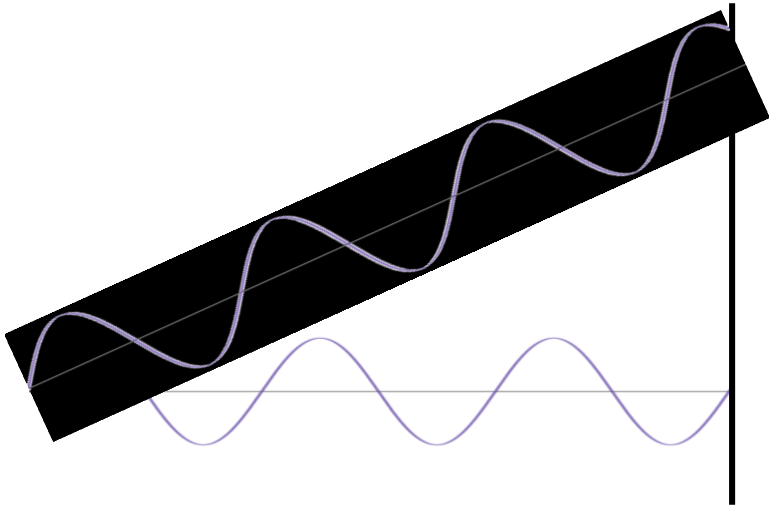
Phase change during wave propagation

- When a wave propagates in space, it continuously changes its phase





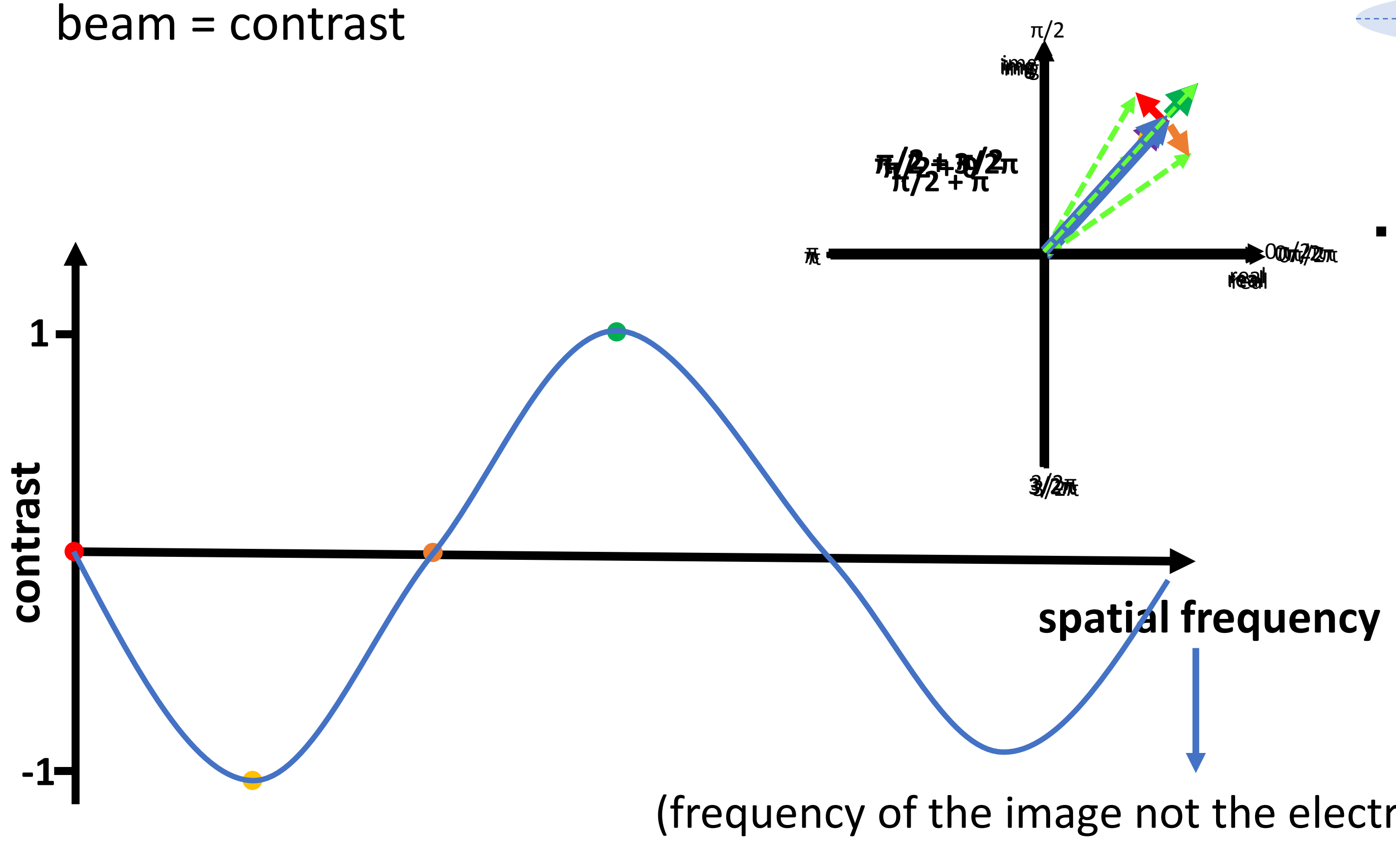
Distance	Phase shift
n*λ	0, 2π
n*λ + λ/4	π/2
n*λ + λ/2	π
n*λ + ¾λ	3/2π

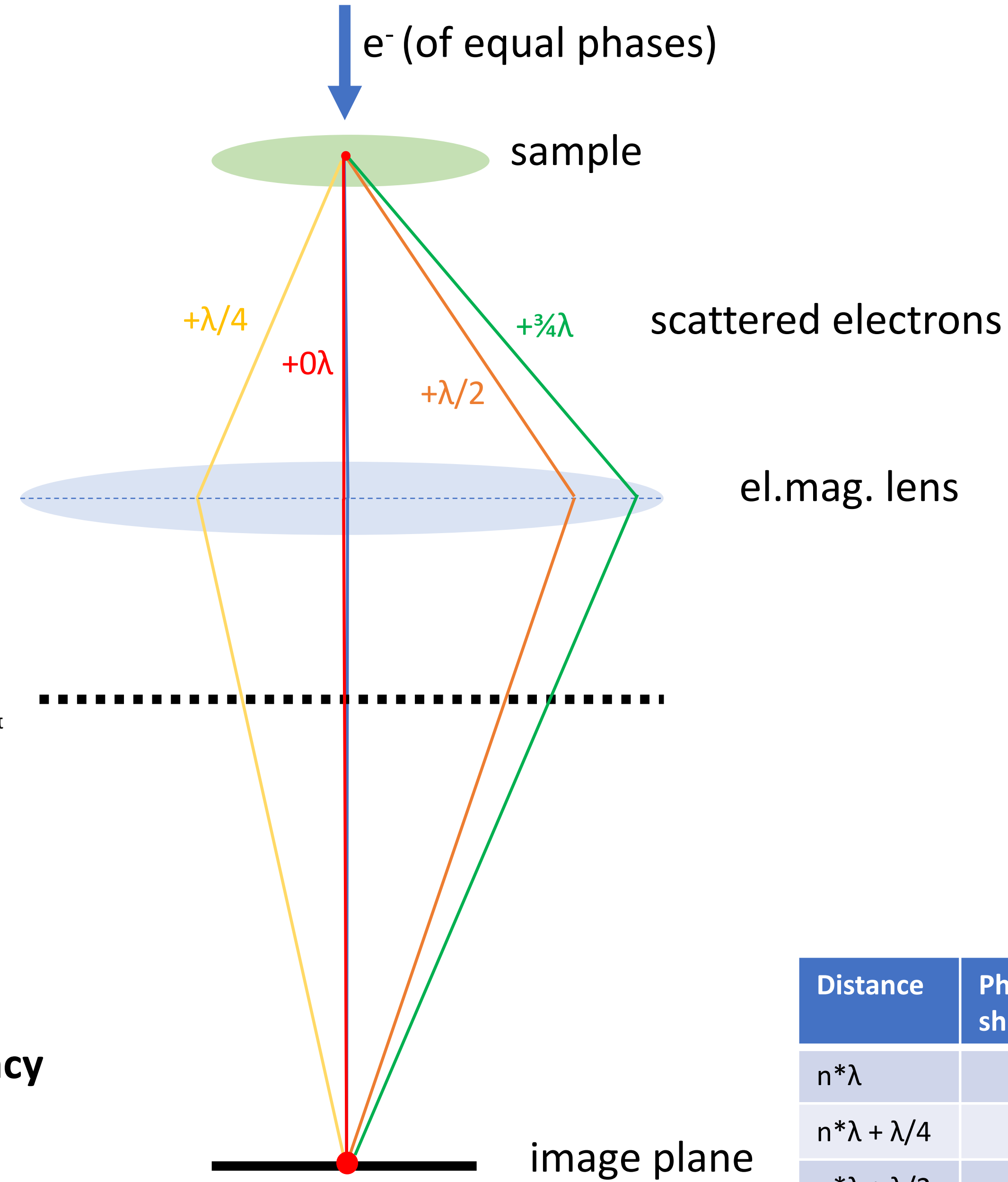


Scattered electron travels an extra distance

Contrast transfer function

- detectors detect intensity (Amp^2) not phases
- when e$^-$ scatters π/2 phase-shift is introduced
- Un-diffracted beam = non-scattered e$^-$
- intensity of un-diffracted beam >> diffracted
- Increase/decrease of intensity relative to un-diffracted beam = contrast





Distance	Phase shift
n*λ	0, 2π
n*λ + λ/4	π/2
n*λ + λ/2	π
n*λ + ¾λ	3/2π

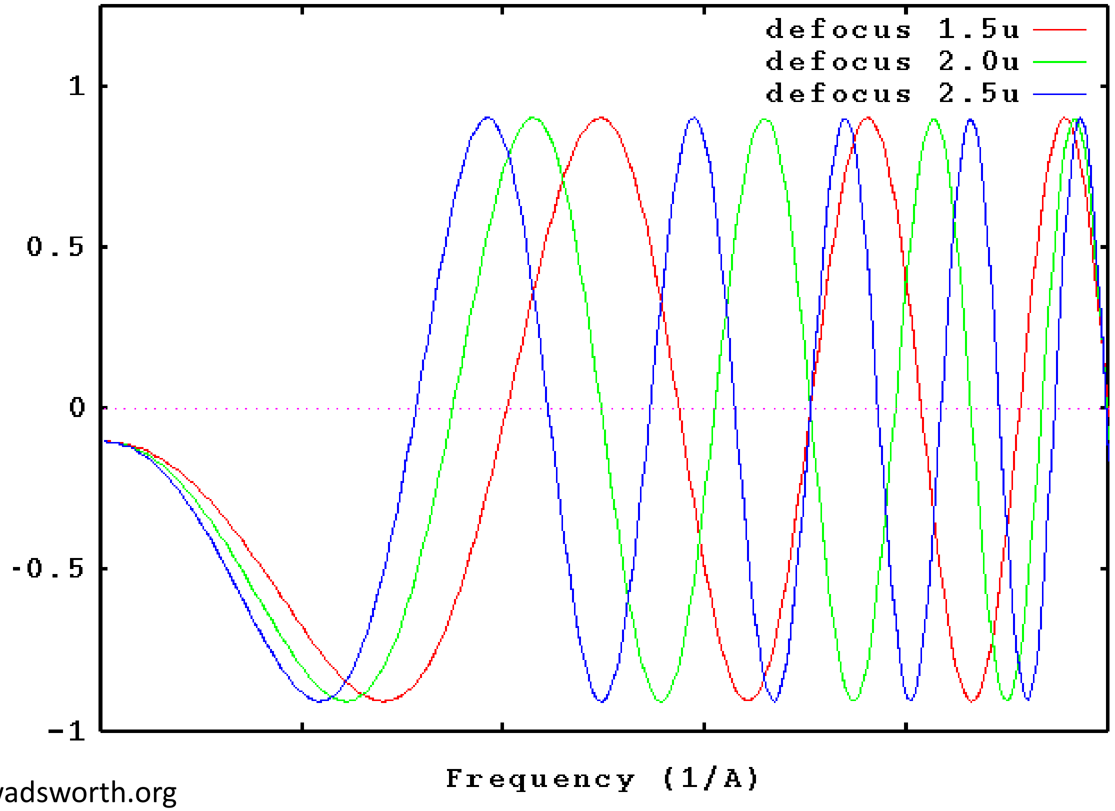
Contrast transfer function (CTF)

$$CTF = sin(-\pi \Delta z \lambda k^2 + \frac{\pi C_s \lambda^3 k^4}{2})$$

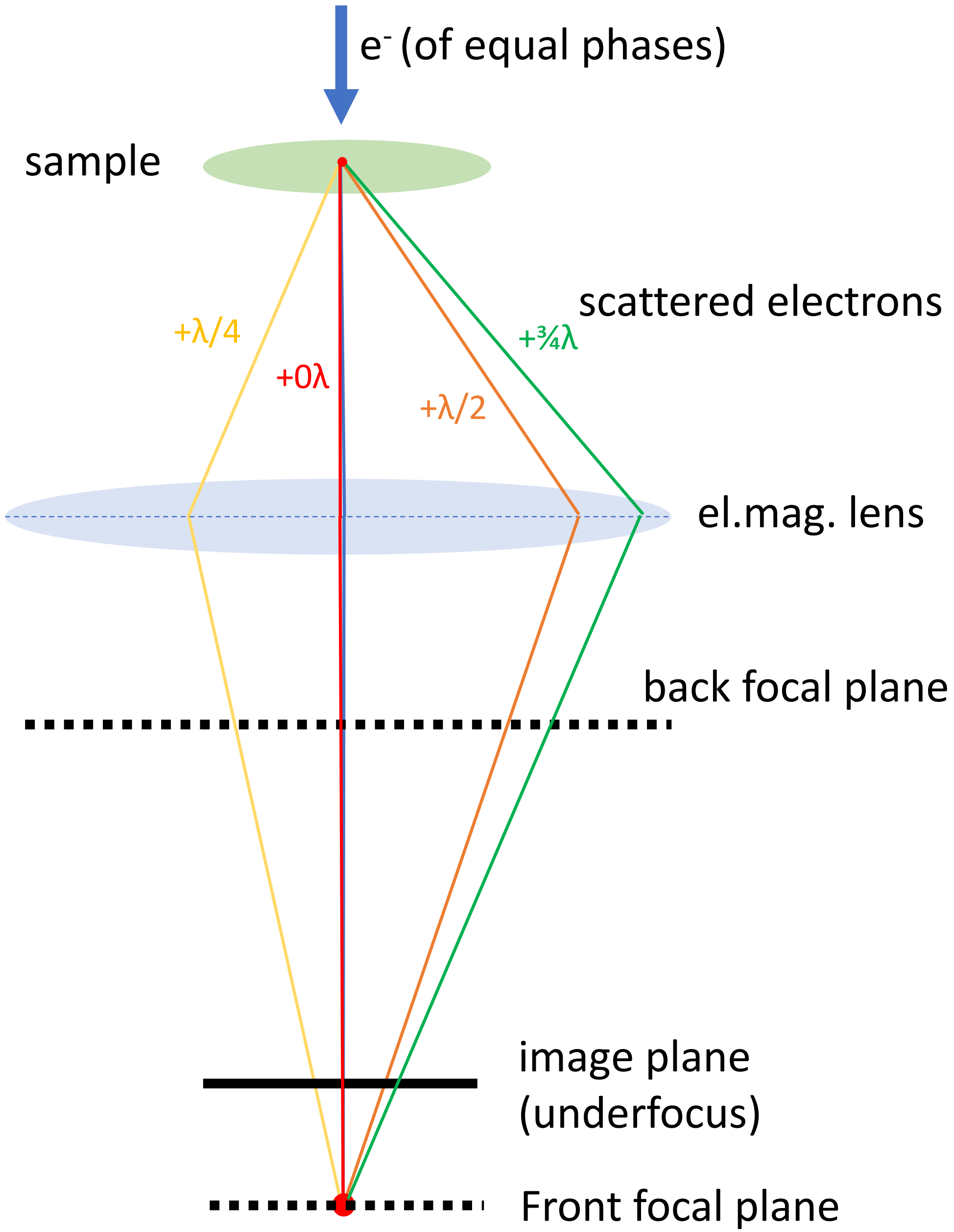
defocus

wavelength (e^-)

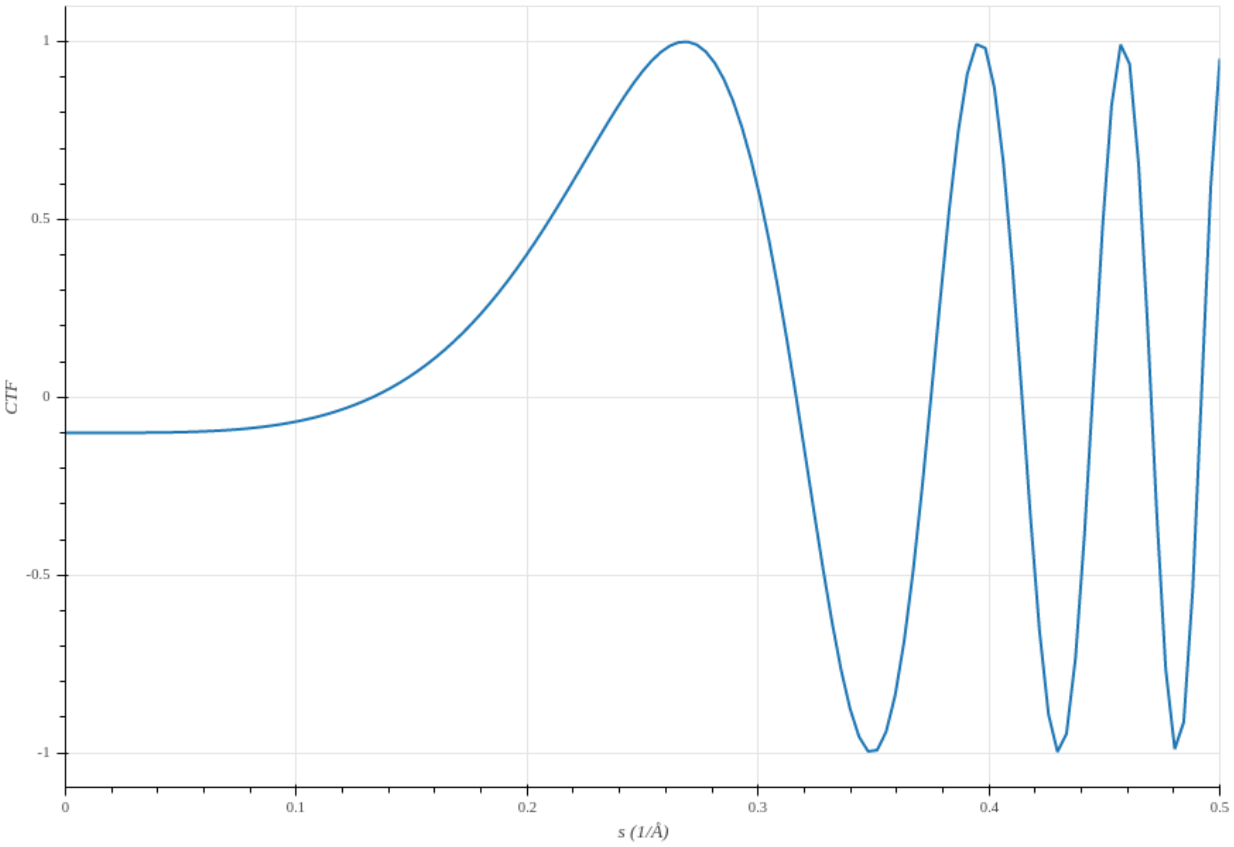
spherical aberration



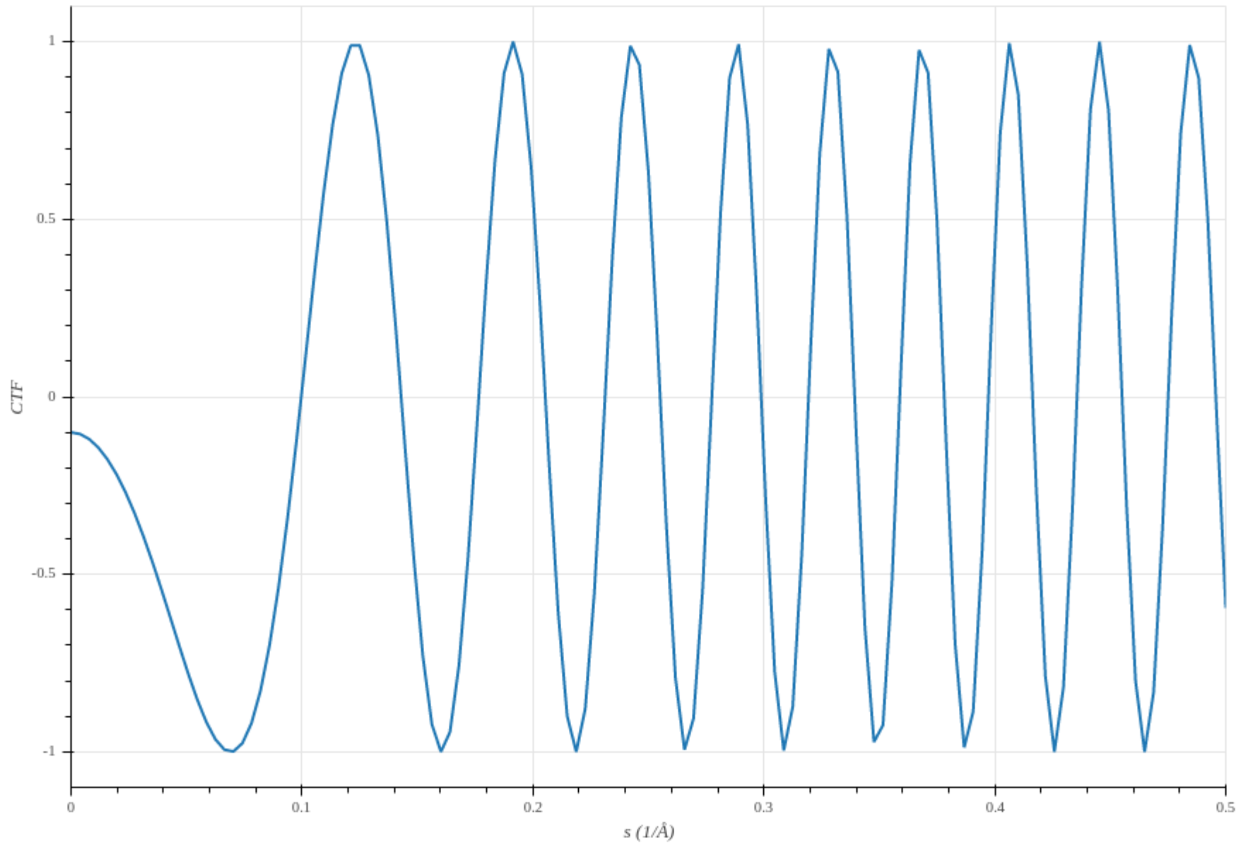
spider.wadsworth.org



In focus images suffer from low contrast



0 µm = in focus

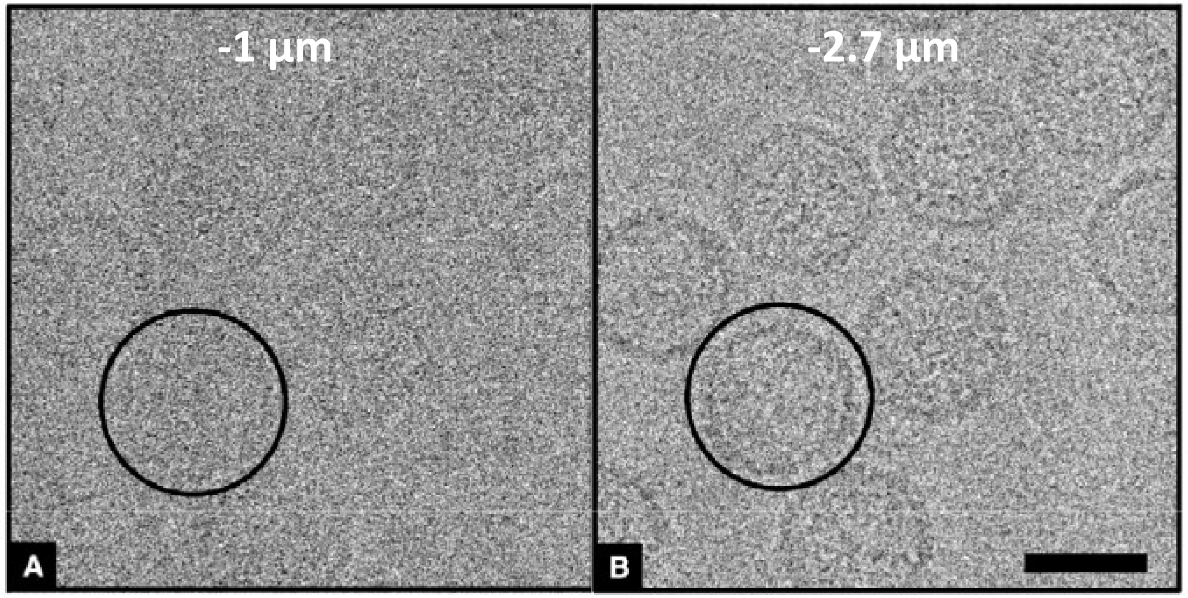


-0.5 µm

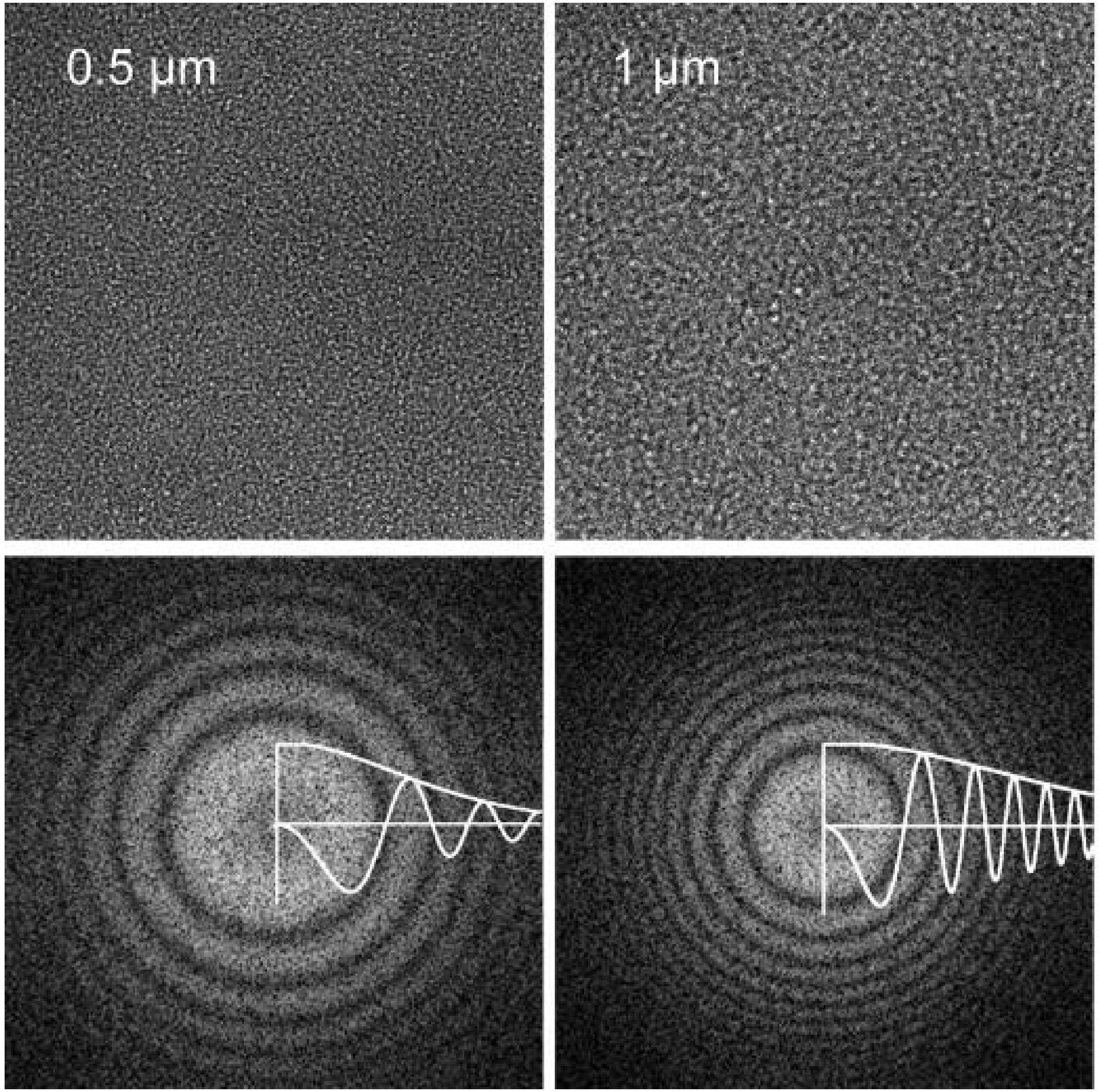
https://ctf-simulation.herokuapp.com/

Contrast transfer function (CTF)

- Electron microscope images are convoluted by a point spread function
- Point spread function in EM is represented by CTF in Fourier space
- CTF has zero values (information loss)

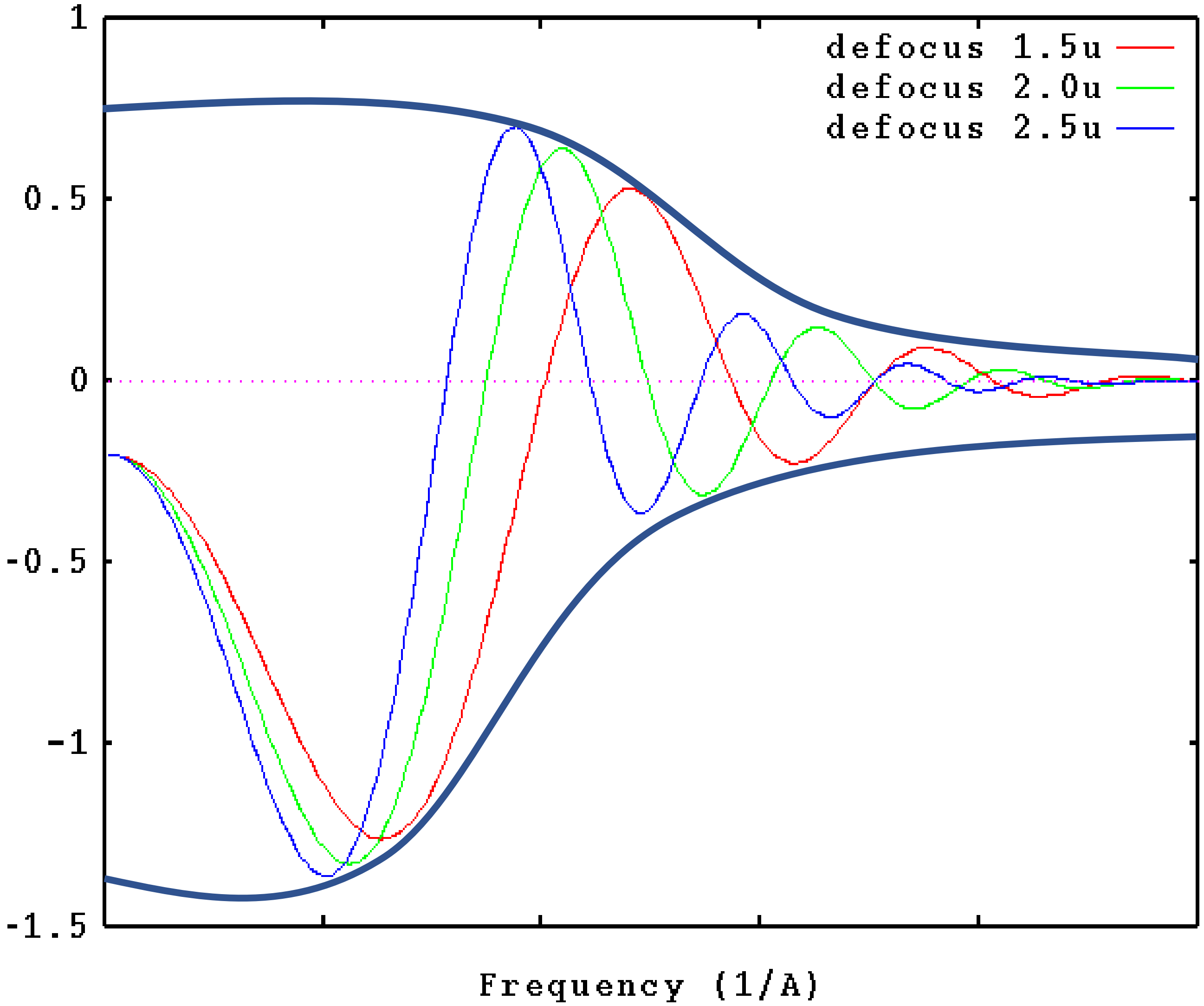


Thuman-Commike and Chiu, Micron



Orlova, Saibil 2011

Envelope function



- Hi frequencies in CTF are damped
- Envelope function
  - Chromatic aberrations
  - Focus spread
  - Energy spread
  - Variance in hi-tension
  - Defocus
  - Coherence of the electron beam

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Point spread function of TEM

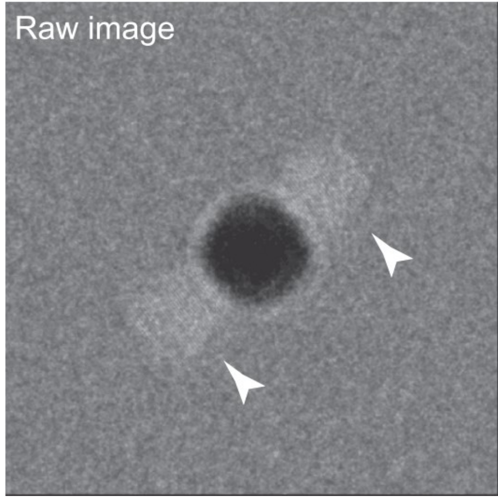
Every single point in image is the convolution of PSF and the object

$$I = O \otimes PSF$$

Image **Object** **Point spread function**

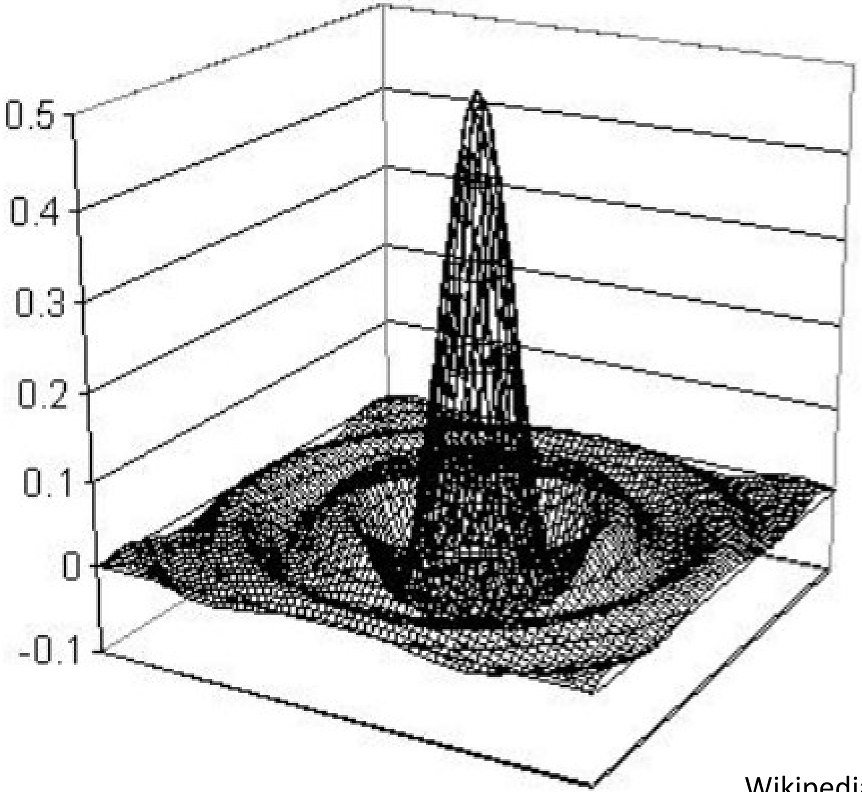
$$PSF = \mathcal{F}(CTF)$$

$$CTF = \mathcal{F}(PSF)$$



Russo&Henderson, 2018

2D point spread function



Wikipedia

CTF correction

$$I = O \otimes PSF$$

Convolution theorem

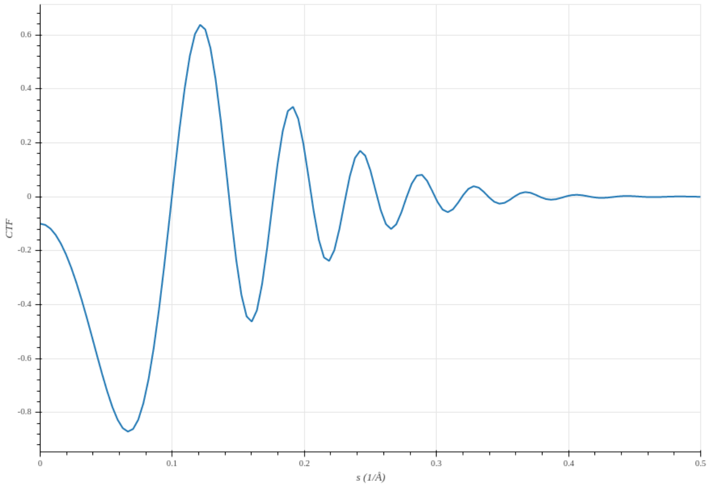
$$\mathcal{F}(I) = \mathcal{F}(O).\mathcal{F}(PSF)$$

$$\mathcal{F}(I) = \mathcal{F}(O).CTF$$

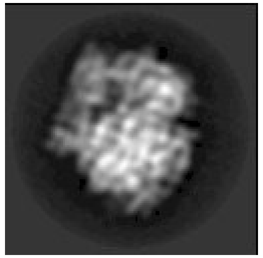
What was the shape of the original object represented by the image ?

$$\mathcal{F}(O) = \mathcal{F}(I)/CTF$$

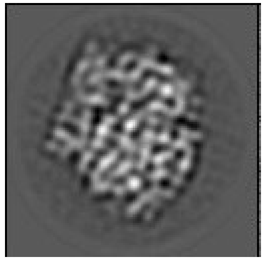
$$O = \mathcal{F}^{-1}(\mathcal{F}(I)/CTF)$$



Real-space | Reciprocal-space | Real-space

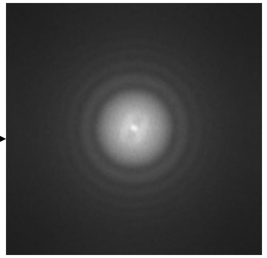


object



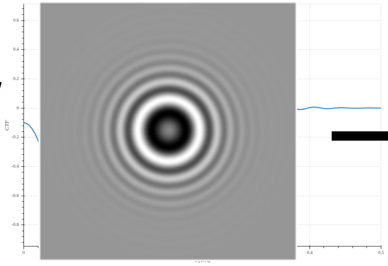
PSF convoluted image

$\mathcal{F}$

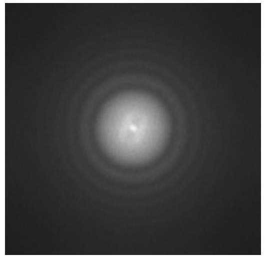


$\mathcal{F}(I)$

/

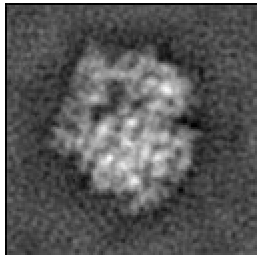


CTF



$\mathcal{F}(I)/CTF$

$\mathcal{F}^{-1}$



CTF corrected image

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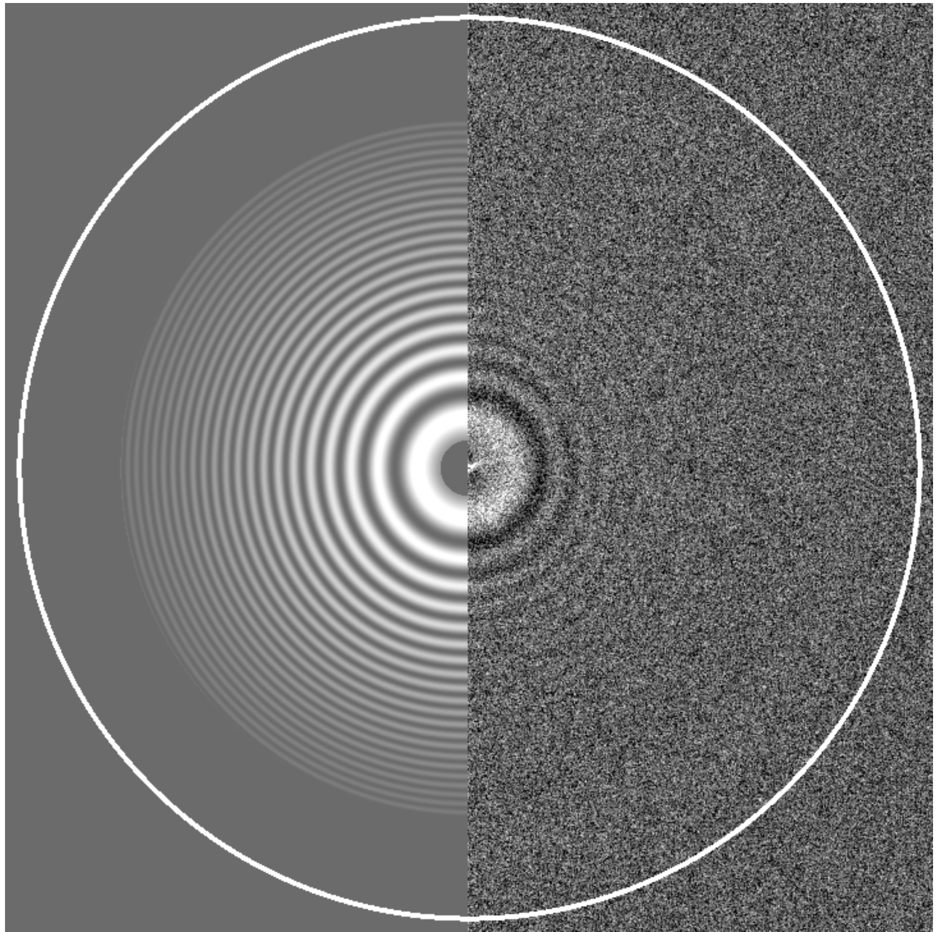
Estimation of CTF

- CTF function of the image is unknown
- Simulate/fit CTF that represents the Amp oscillation of the F(I)
- Find the parameters of the CTF curve (mainly defocus)



What we have learned…..

- Spatial waves: 1D, 2D, 3D
- Fourier transform of spatial waves: 1D, 2D, 3D
- Inverse Fourier transform
- Reciprocal space and its properties
- TEM image formation: phase contrast
- CTF and its properties
- Point spread function and CTF correction

The end

Age 21

Age 69

1972 → **2019**

"age filter"

"time convolution"

Lena Forsén (*31 March 1951)