Basic concepts of Fourier Analysis

EMBO Course on Image Processing for Cryo-Electron Microscopy 2019

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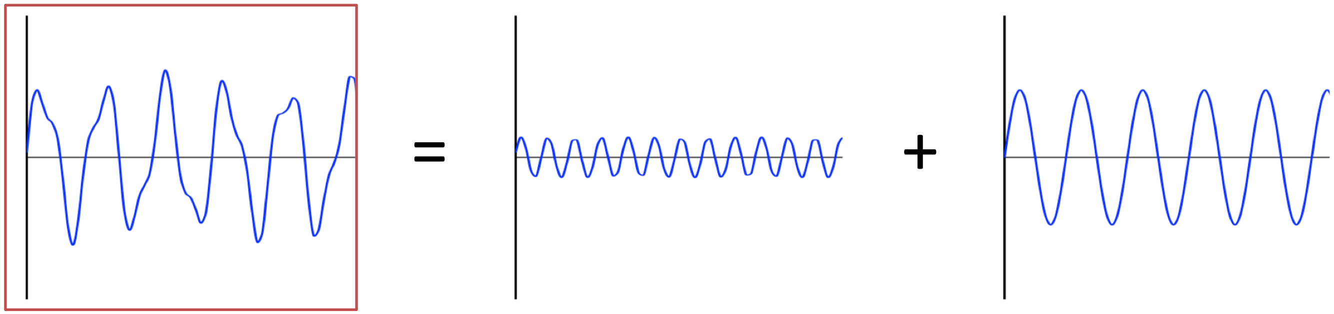
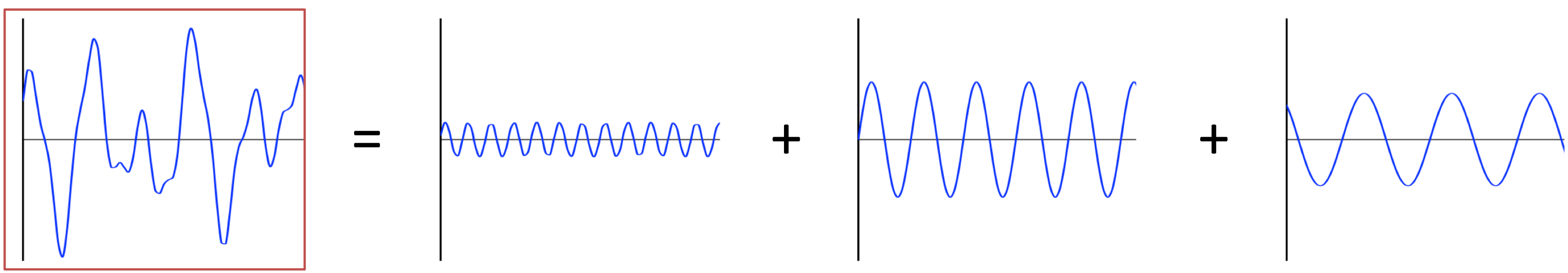
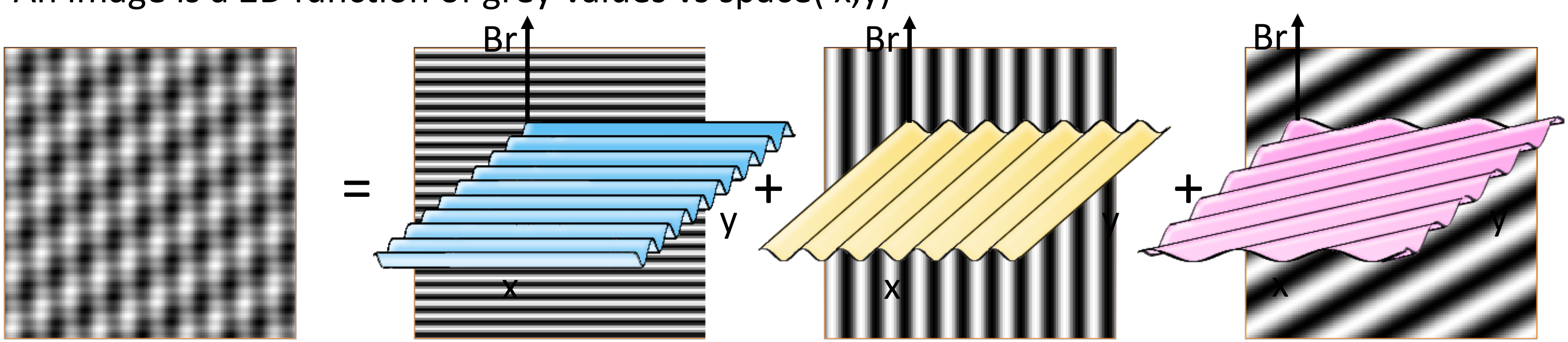
Every function can be expressed as the sum of sine waves





An image is a 2D function of grey-values vs space(x,y)



image

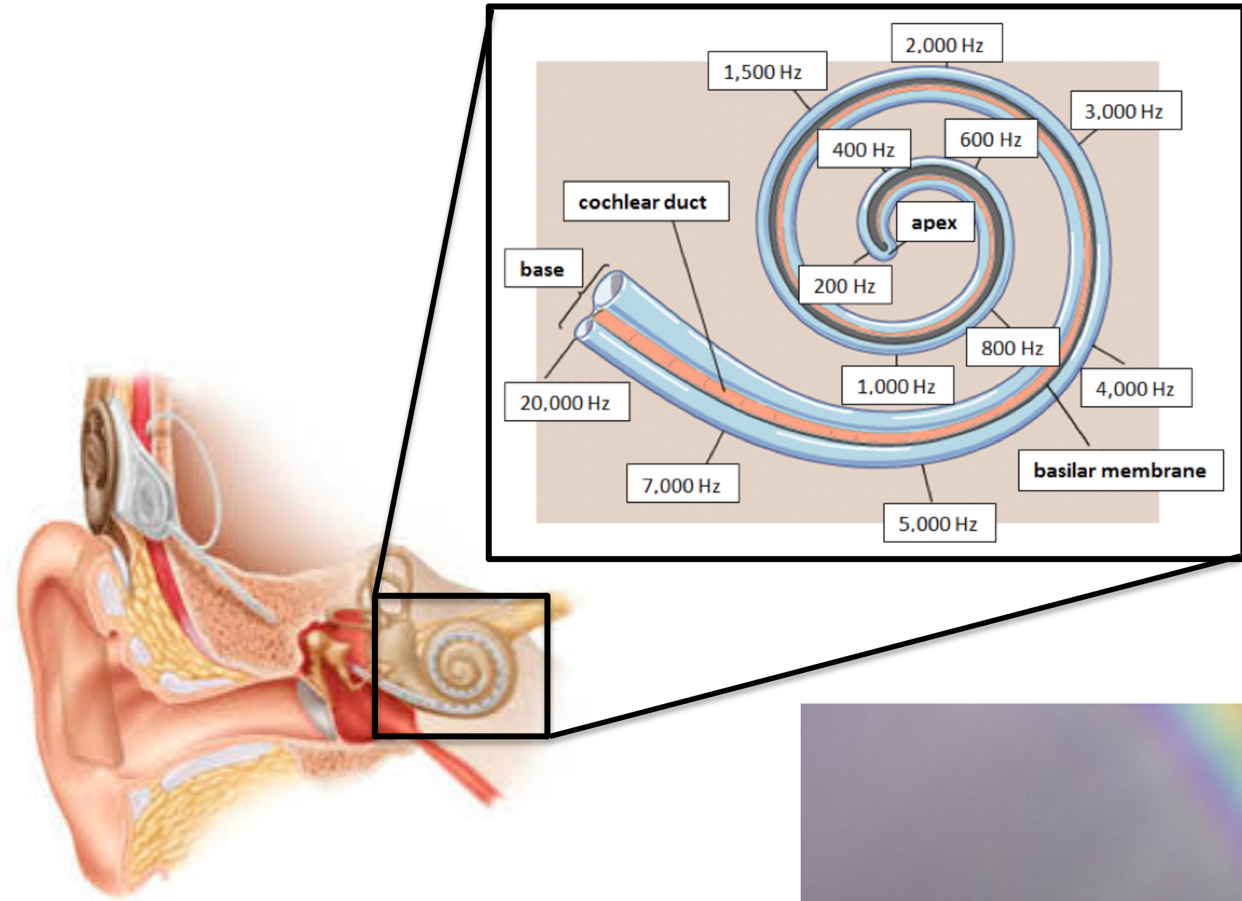
Every ~~function~~ can be expressed as the sum of sine waves

Sine component n

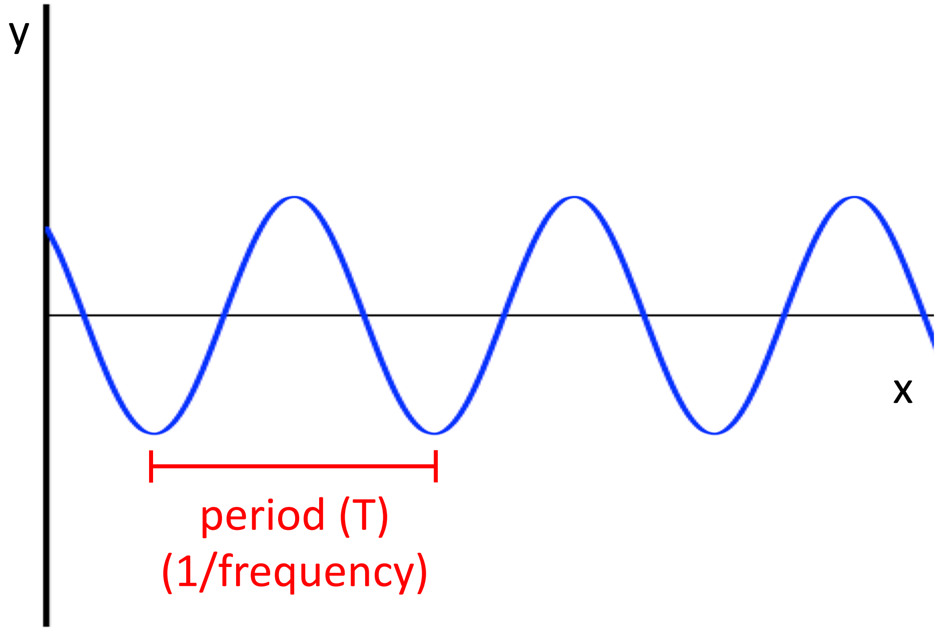
Cumulative sum

Quote: "The profound study of nature is the most fertile source of mathematical discoveries"

Natural Fourier analysers



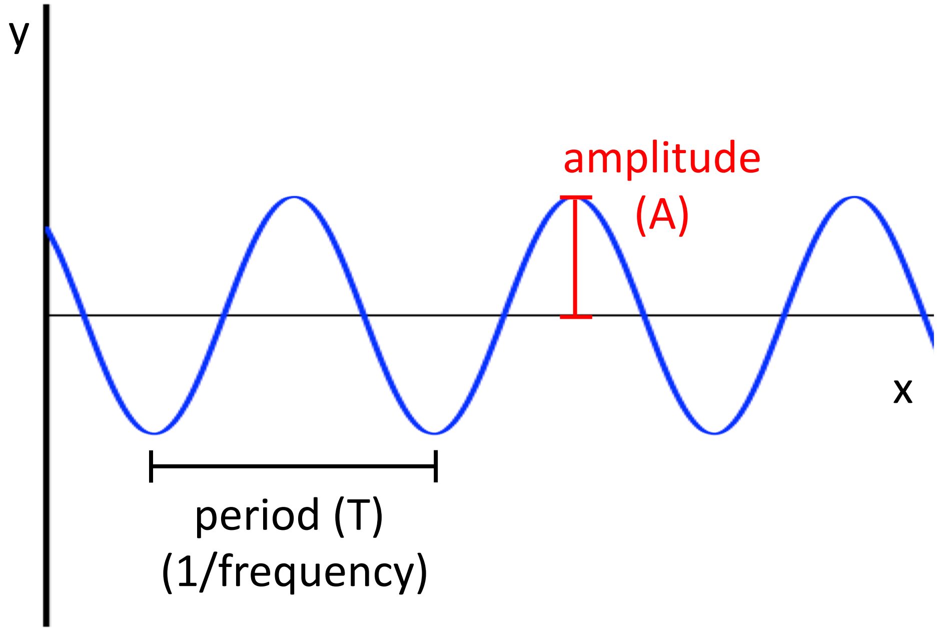
Description of sine waves



$$y = A \sin (2\pi x/T + \phi)$$

period or wavelength: units necessary to describe one cycle
(in case of images these are space units, e.g. nm, or pixels)

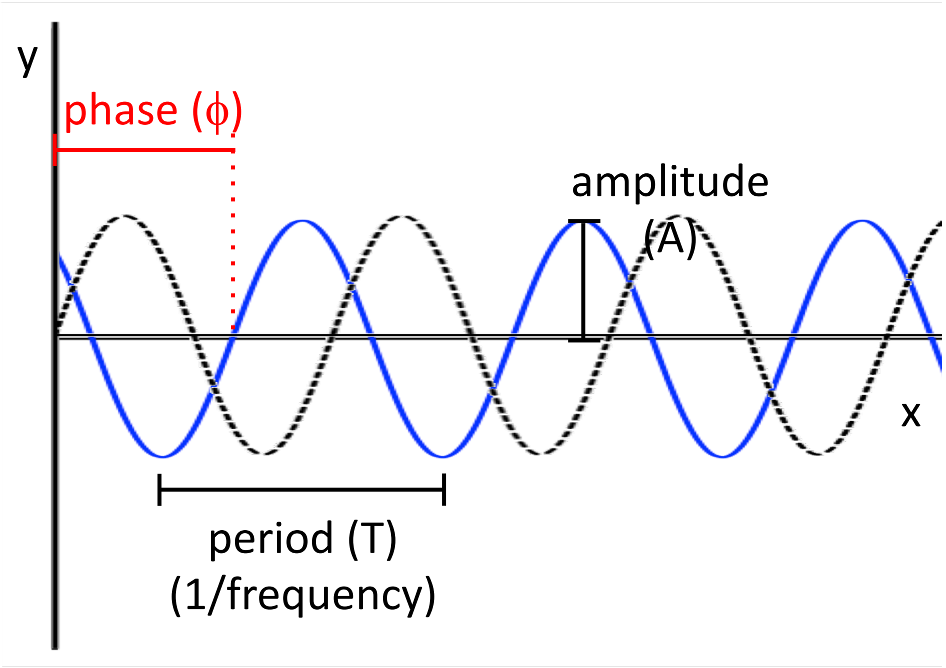
Description of sine waves



$$y = A \sin(2\pi x/T + \phi)$$

amplitude: maximum value of the sine function
(in case of images these are greyvalues, or brightness)

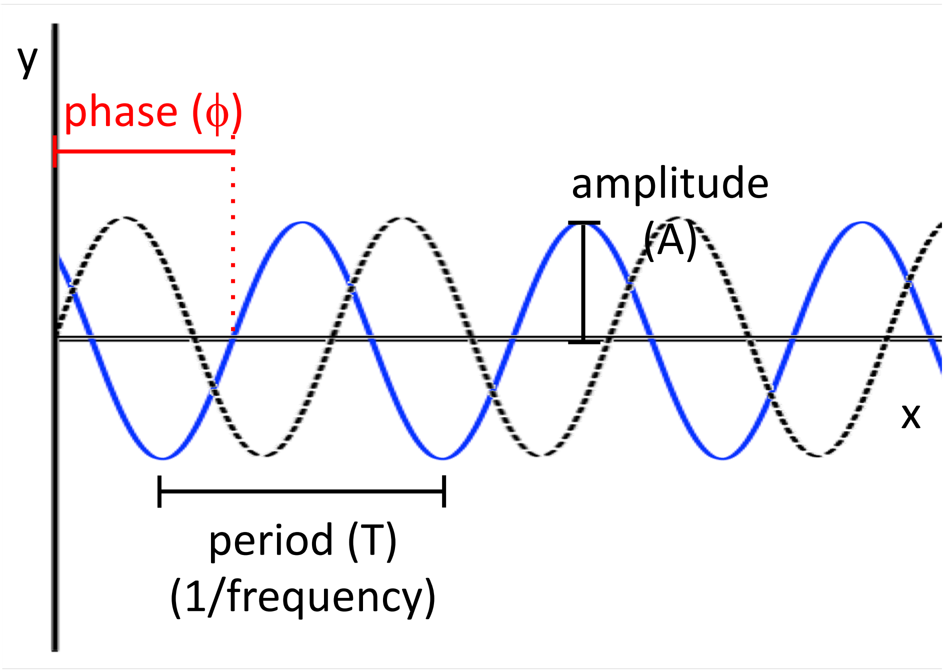
Description of sine waves



$$y = A \sin(2\pi x/T + \phi)$$

phase: defines the x position of the "zero", i.e. the relative position of a wave with respect to one where y(0)=0. Defined as an angle (degrees or radians)

Description of sine waves



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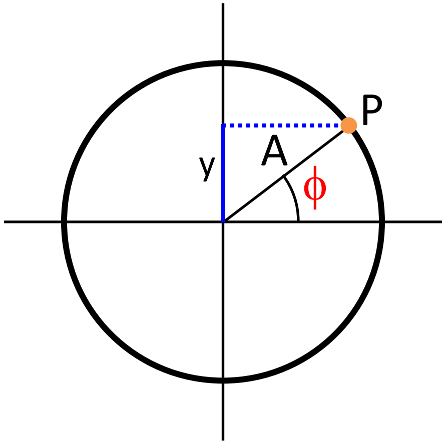
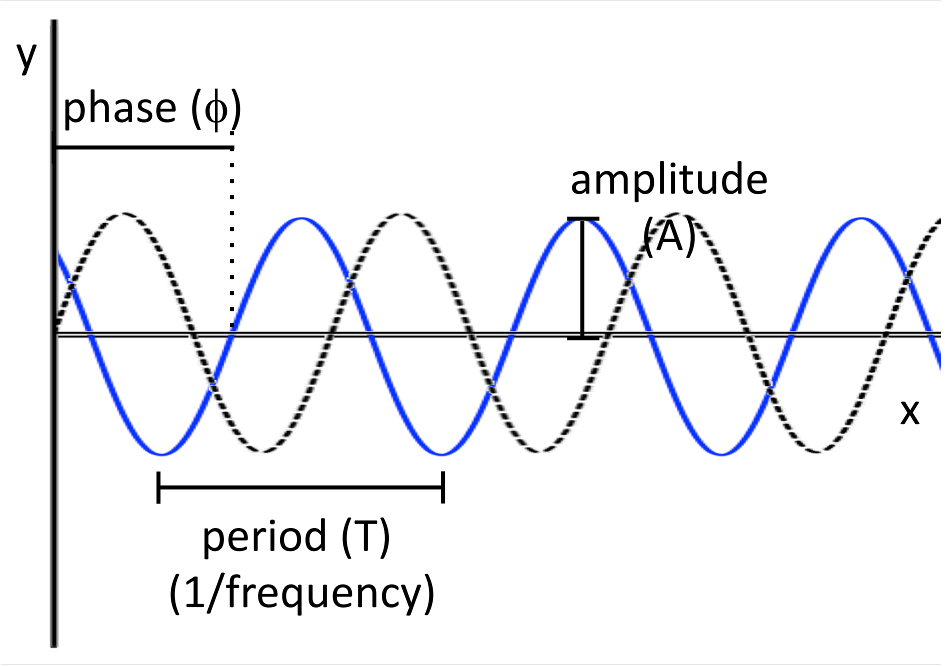


Alternative representation of sine functions: the projection on y of a point undergoing circular motion describes sinusoidal oscillations.

Description of sine waves

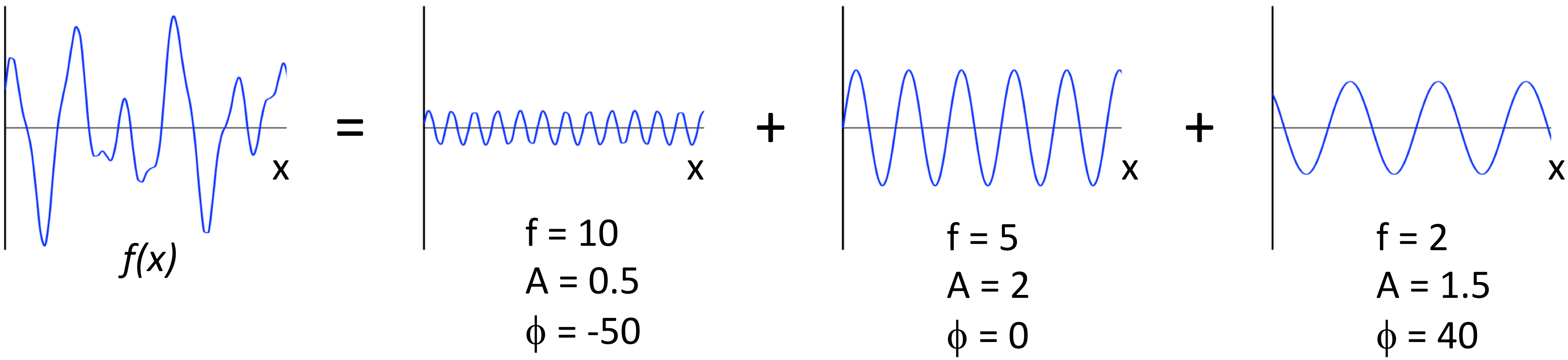


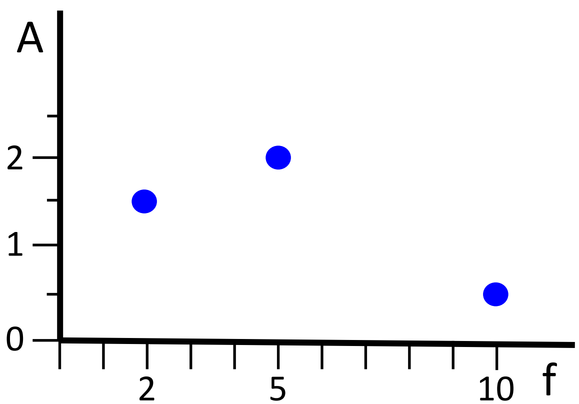
Each component sine wave has a certain frequency, amplitude, and phase.

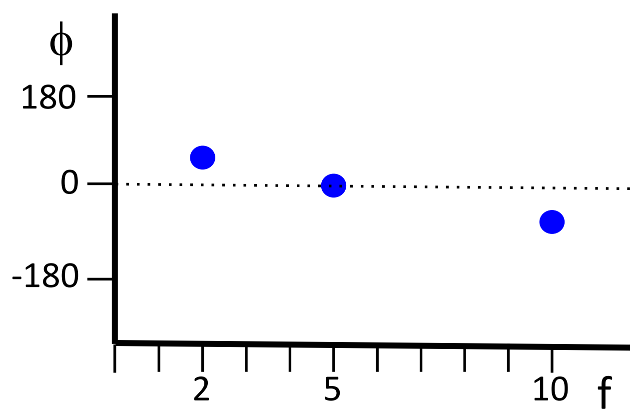
For a given frequency there is one component sine wave (as if you combine sine waves with the same frequency you obtain another sine wave)

We can describe a function by defining amplitudes and phases of all its components as function of frequency.

Representing sine components in the frequency domain



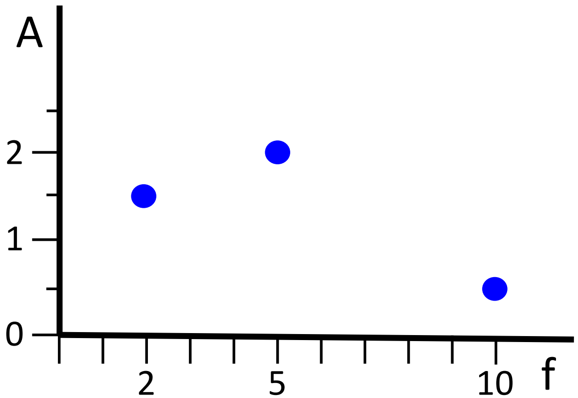


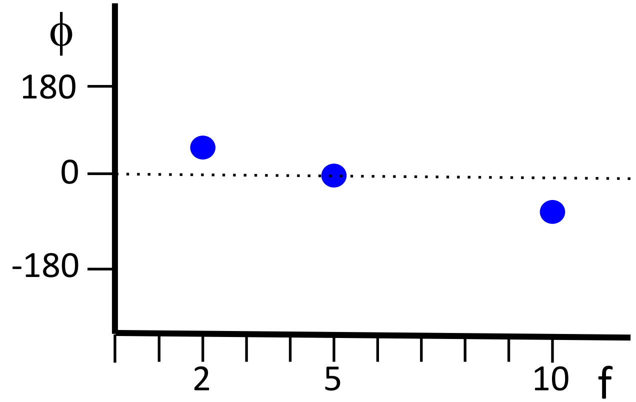


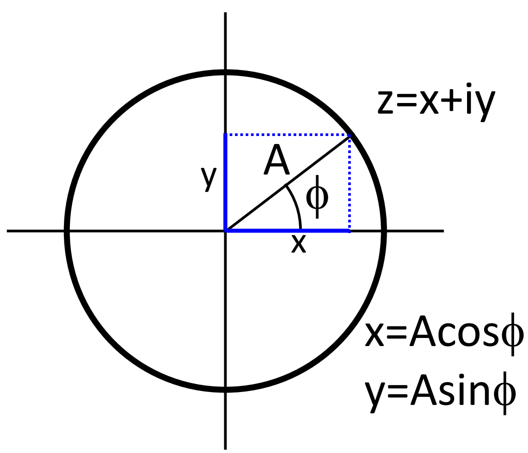
Phases and amplitudes can be represented together as complex number (**$Ae^{i\phi}$**)

We can represent *f(x) as* $Ae^{i\phi}$ of all its components as function of frequency = **FOURIER TRANSFORM**

Representing sine components in the frequency domain



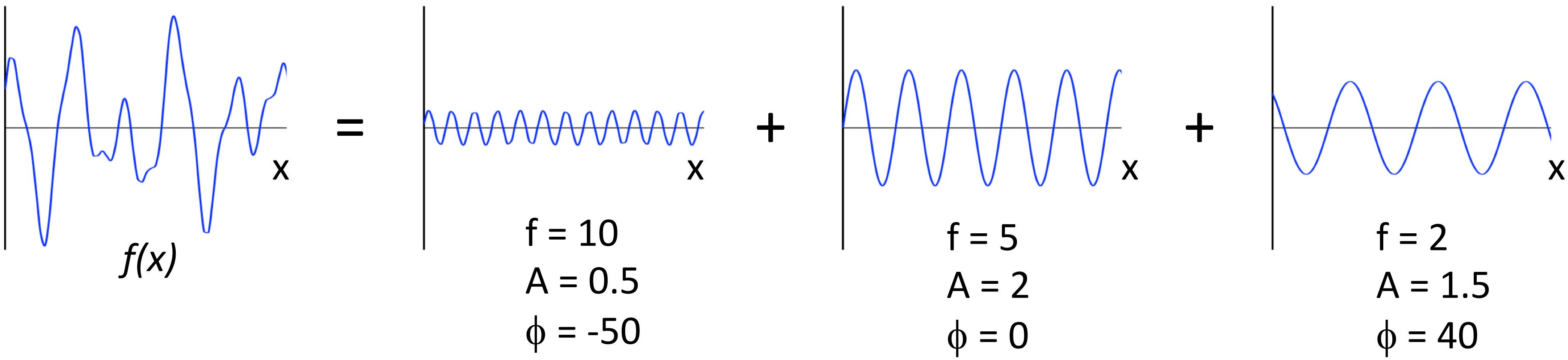


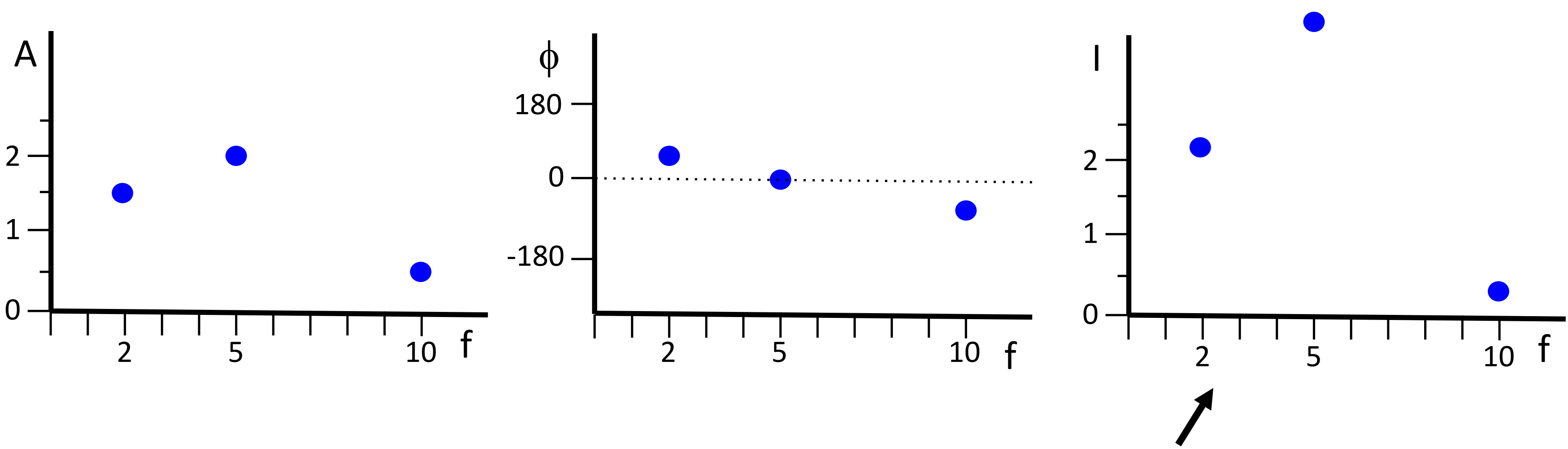


$z = A(\cos\phi + i\sin\phi) = Ae^{i\phi}$

Euler formula

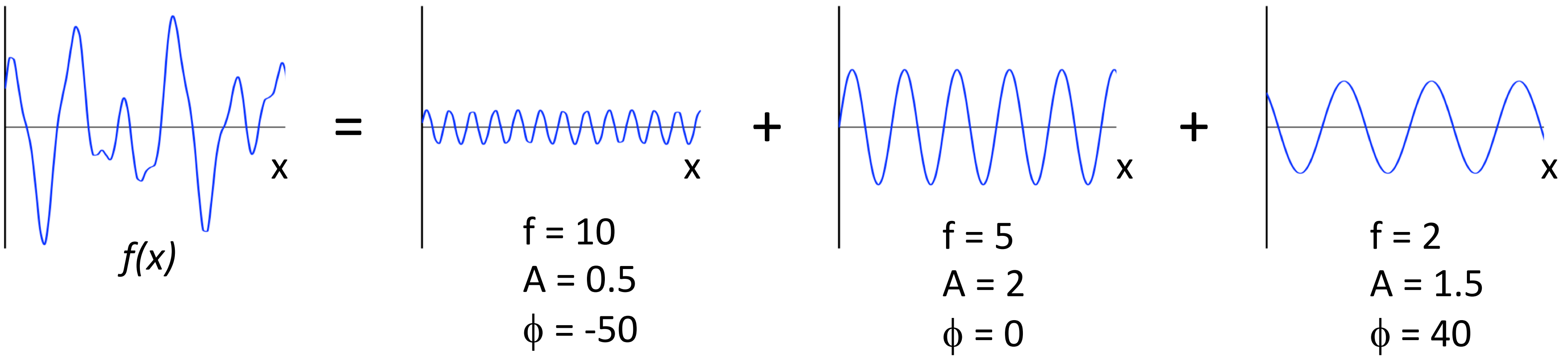
Representing sine components in the frequency domain

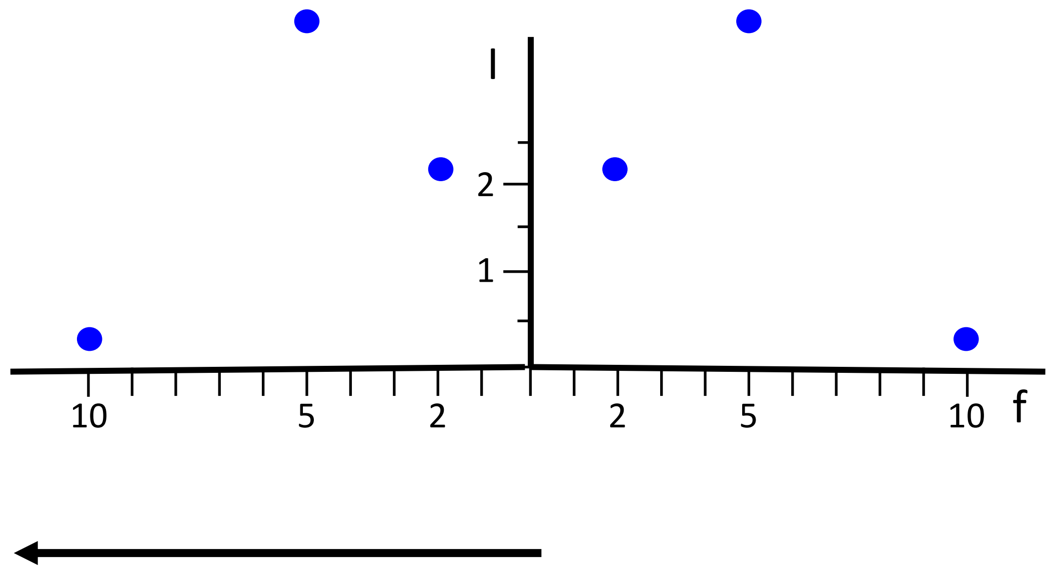




For representation we use the intensity
(average power per unit: $|Ae^{i\phi}|^2$)
A^2

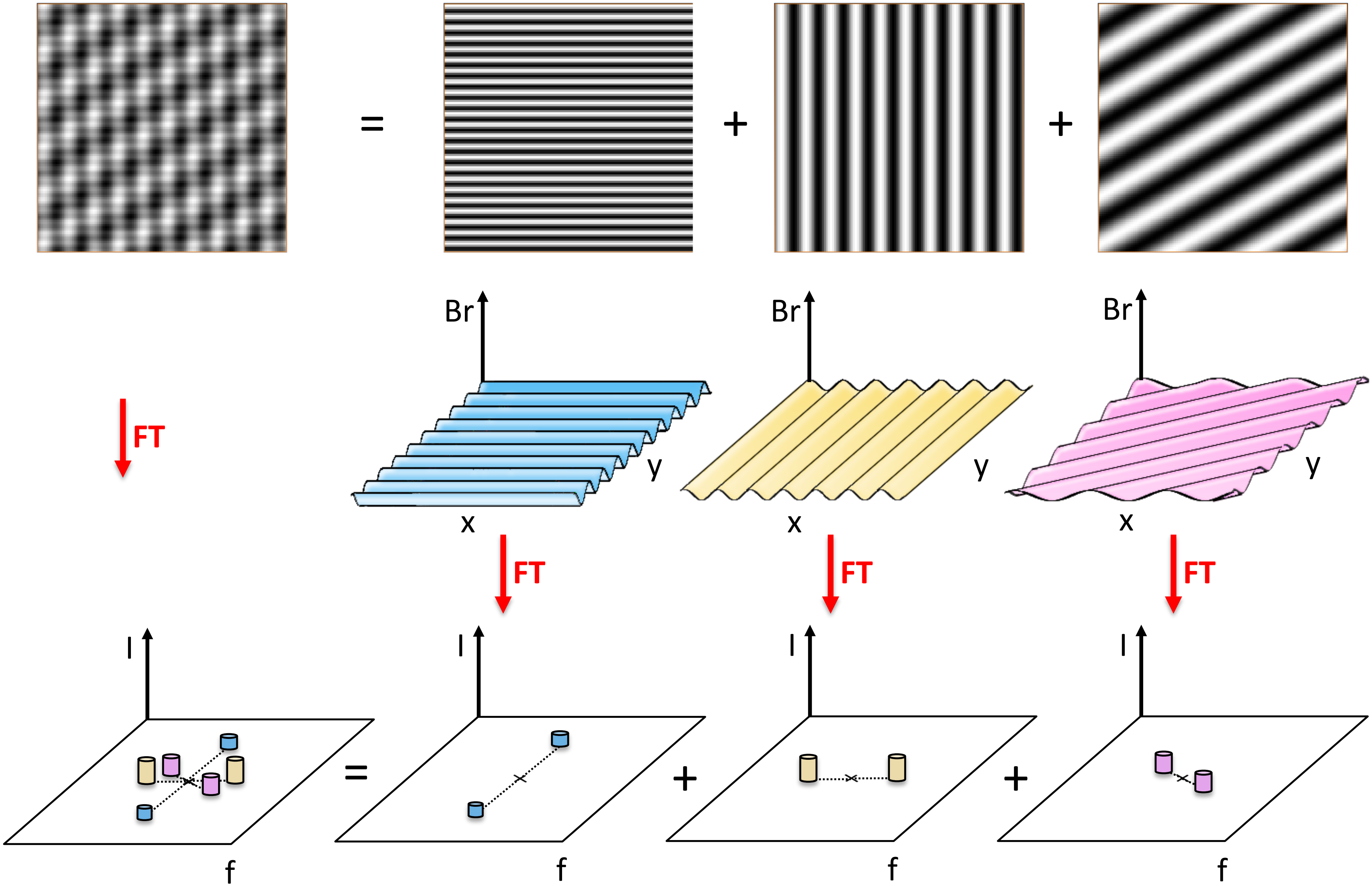
Representing sine components in the frequency domain

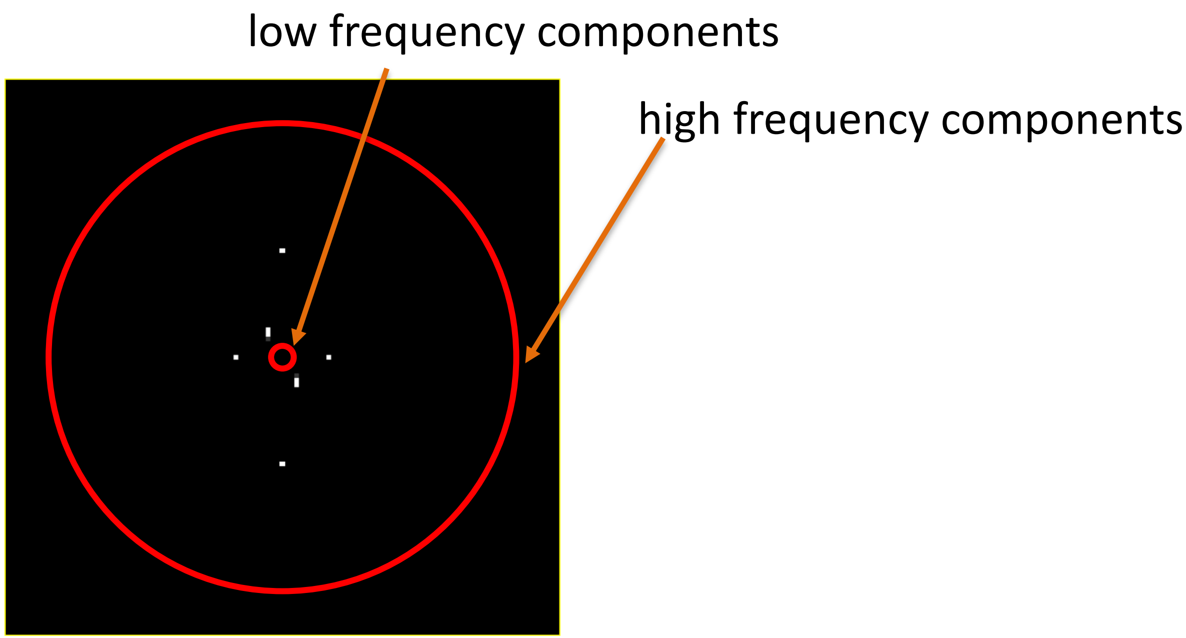




Intensities of FT are always centro-symmetric

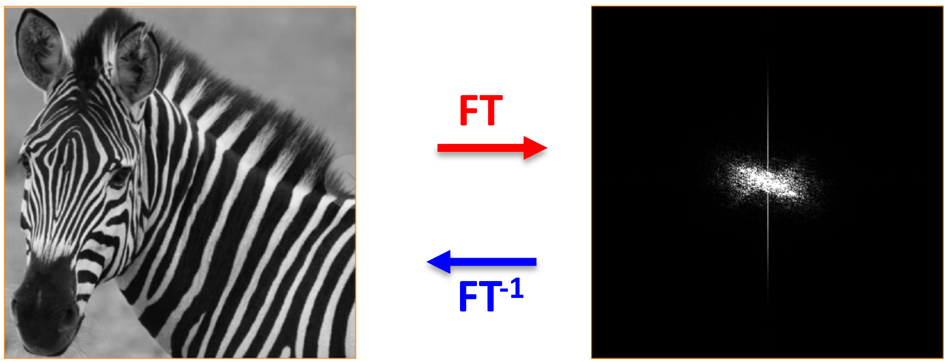
Images = 2D functions (grey-values/brightness VS space)




low frequency components
high frequency components

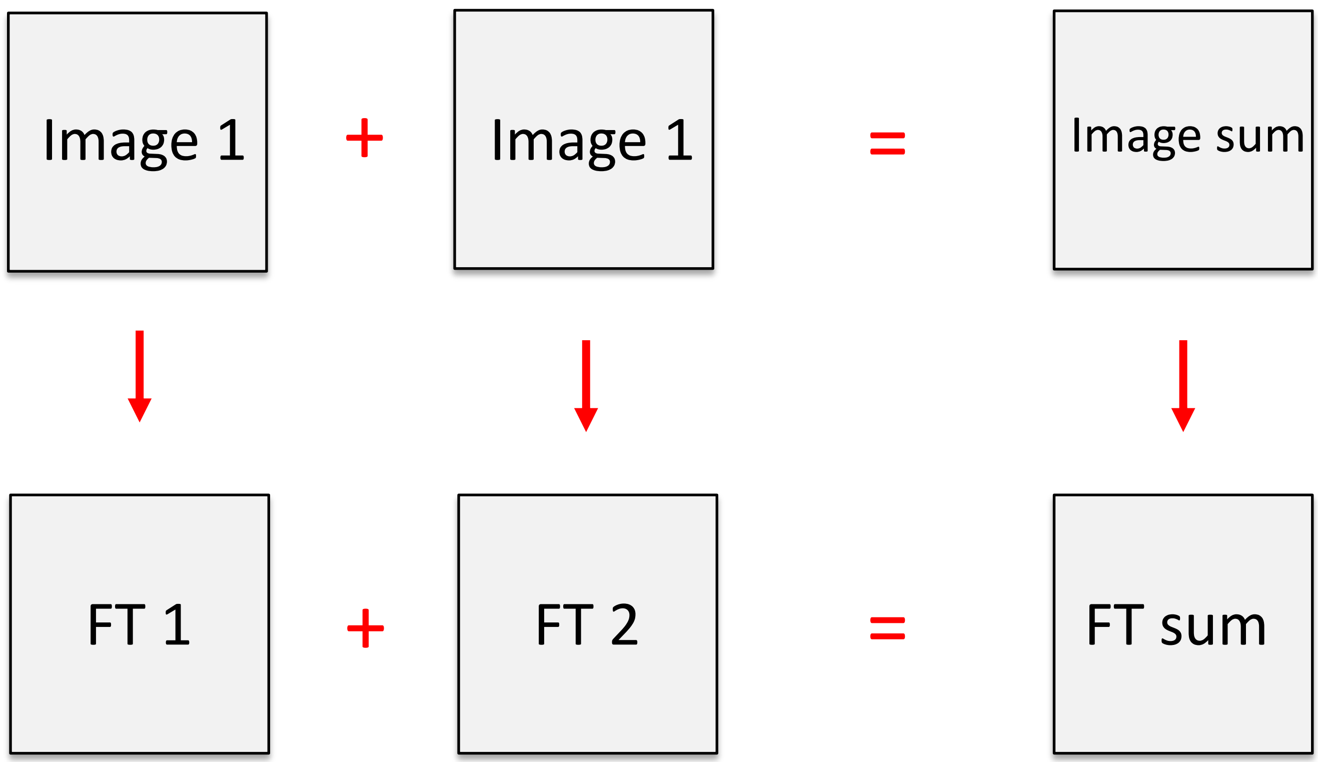
Fourier Inversion Theorem

The FT of the FT of a function recovers the original function

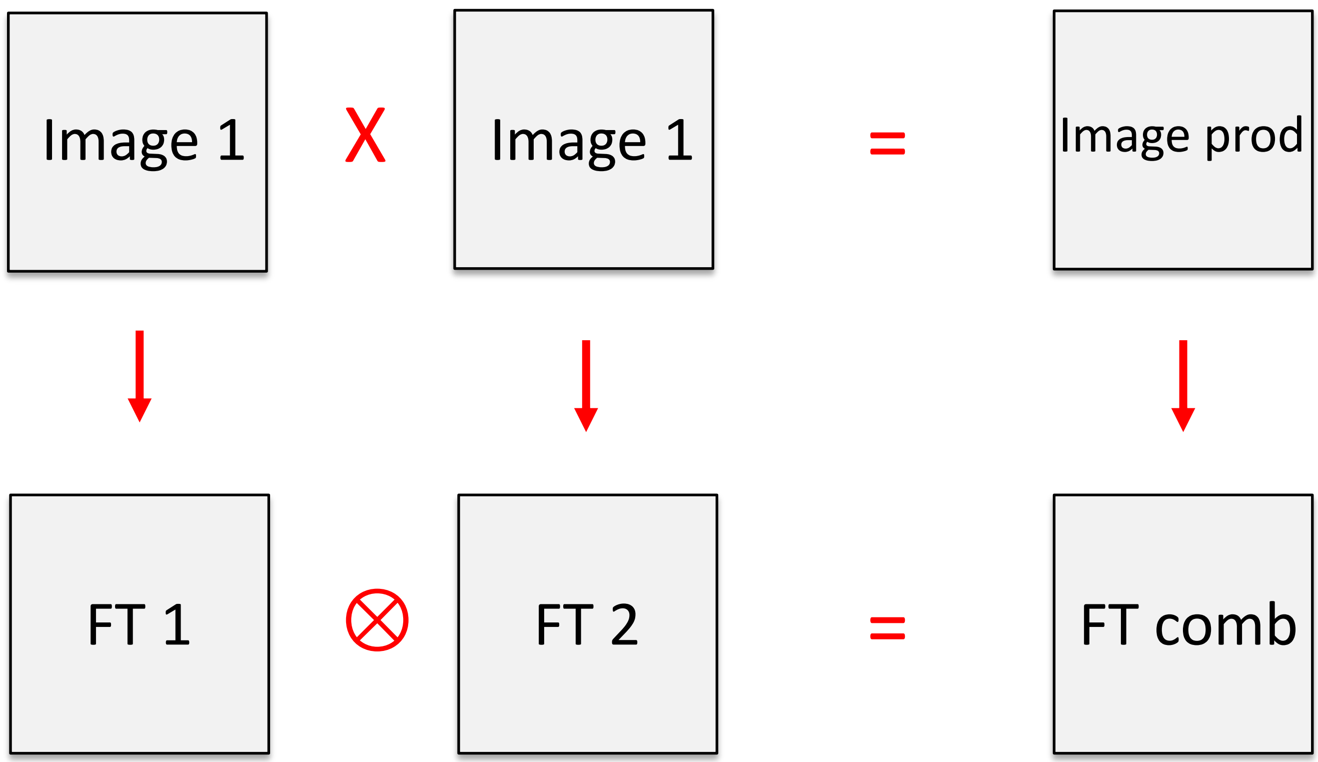


A fundamental property in image processing as we can perform operations on the image OR on its transform according to convenience.

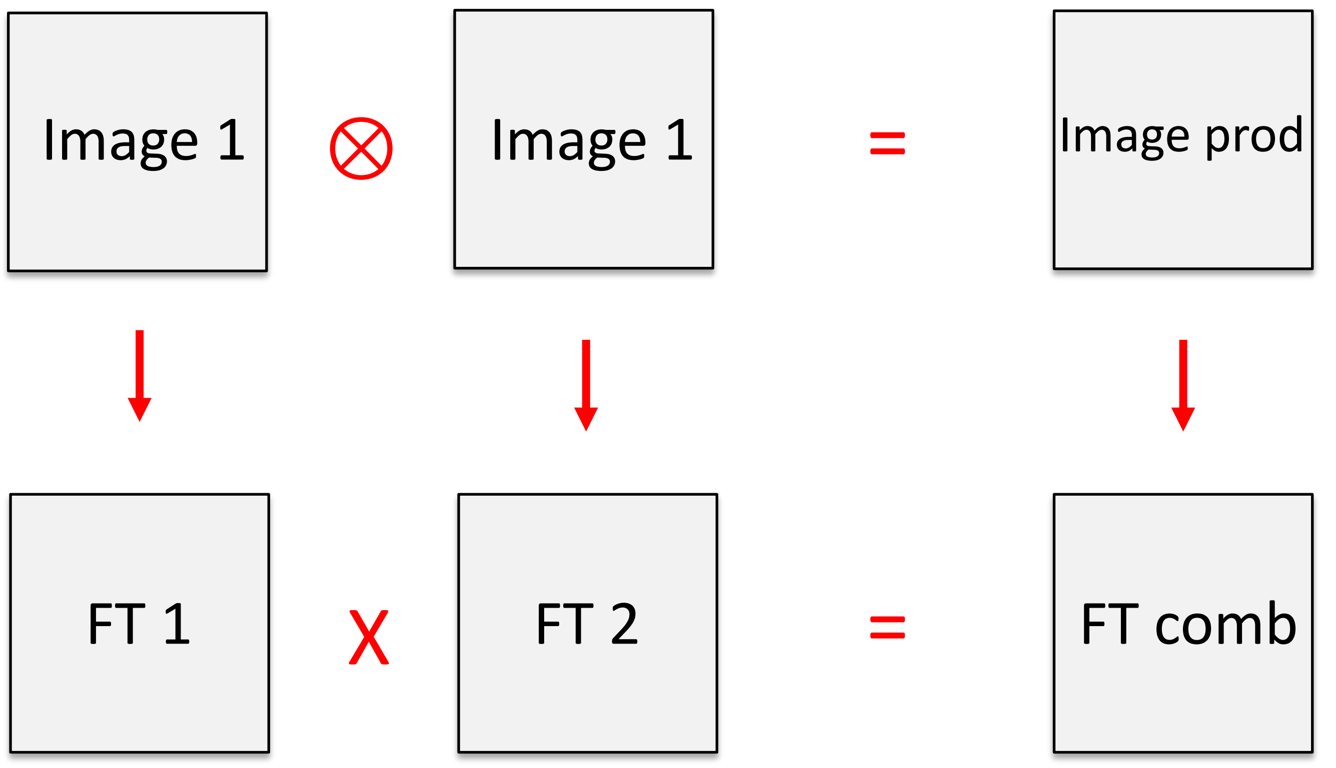
Summing images



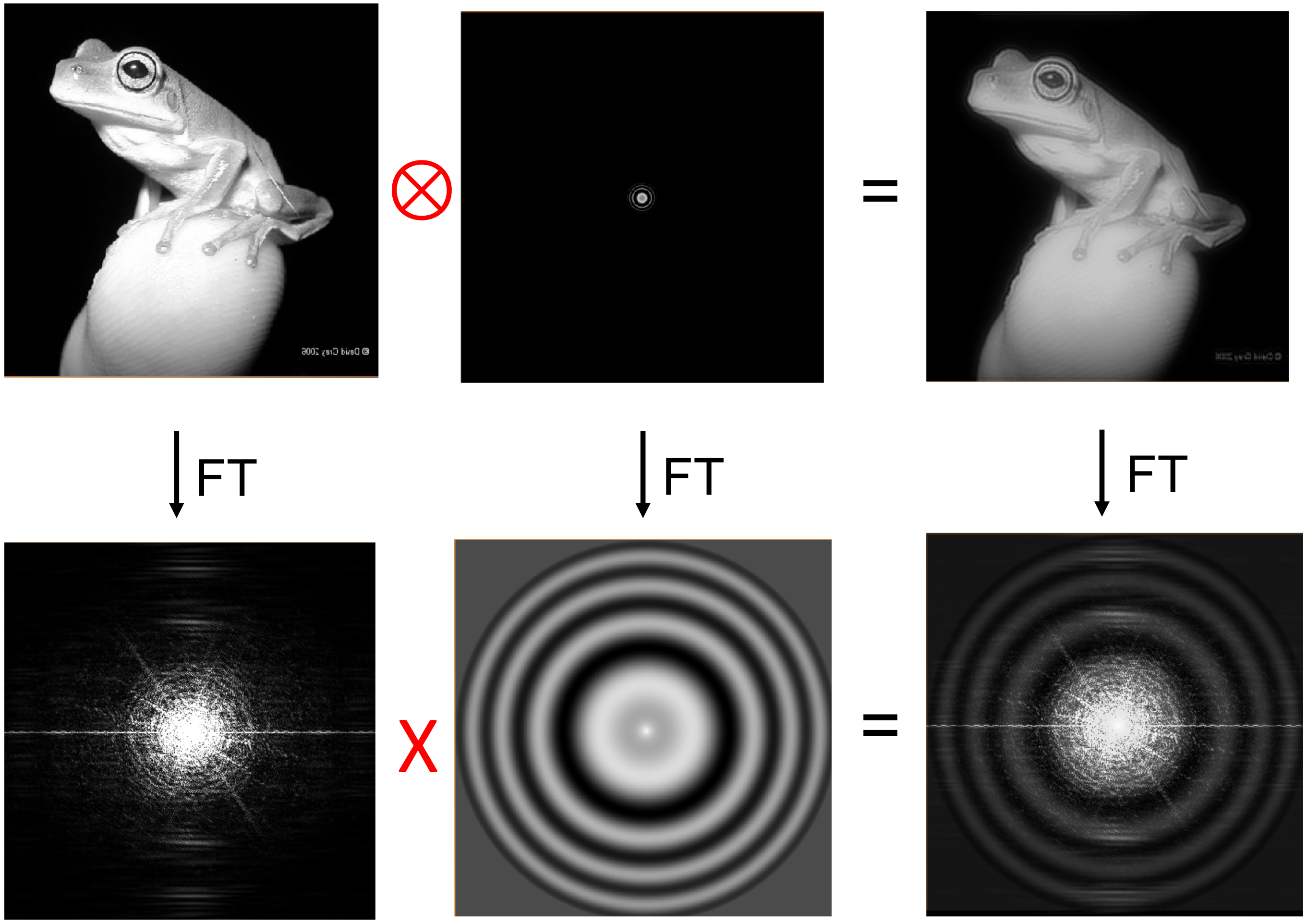
Multiplying images



Multiplying images

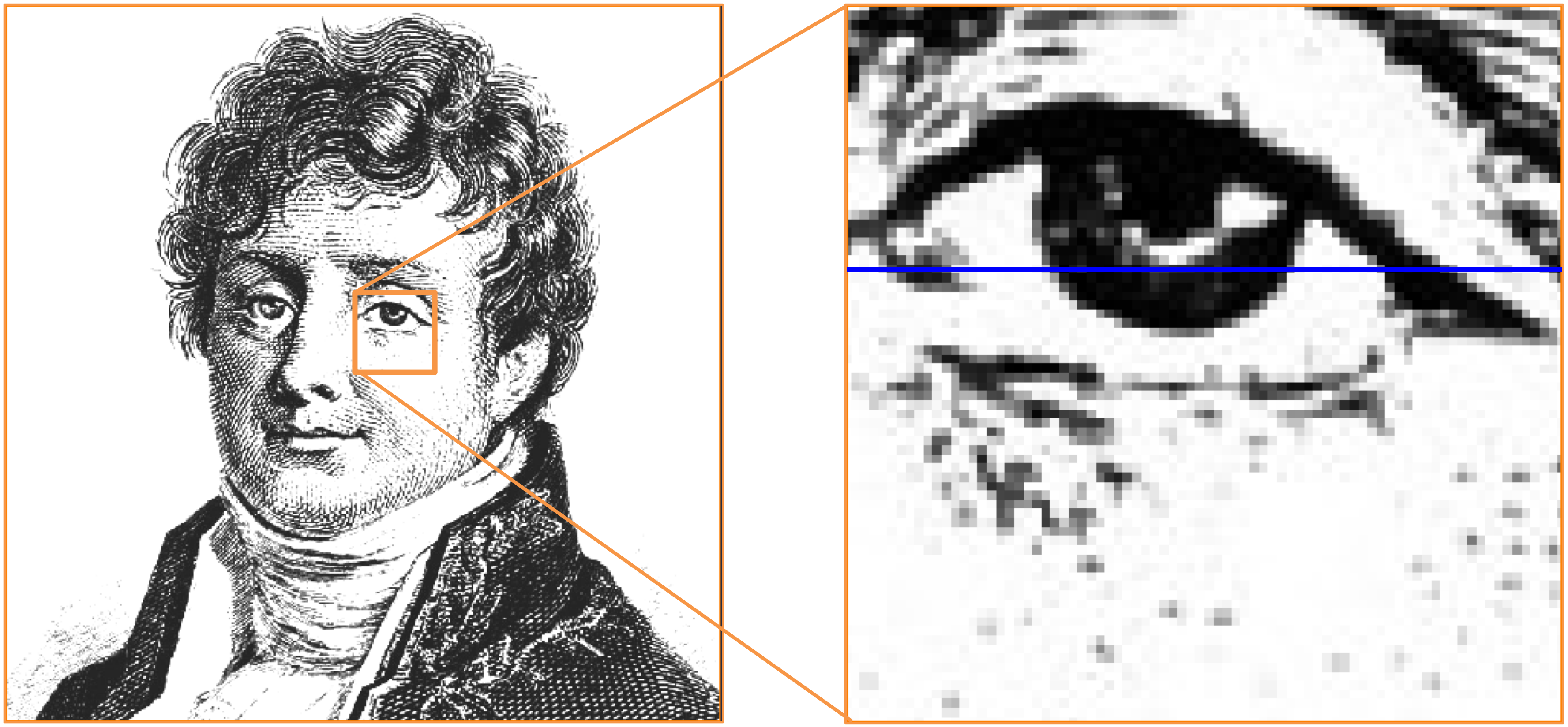


Convolution is multiplication in Fourier space

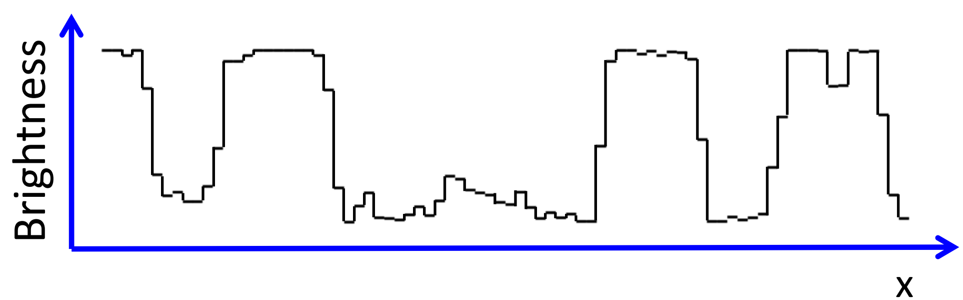


Elena Orlova’s lecture

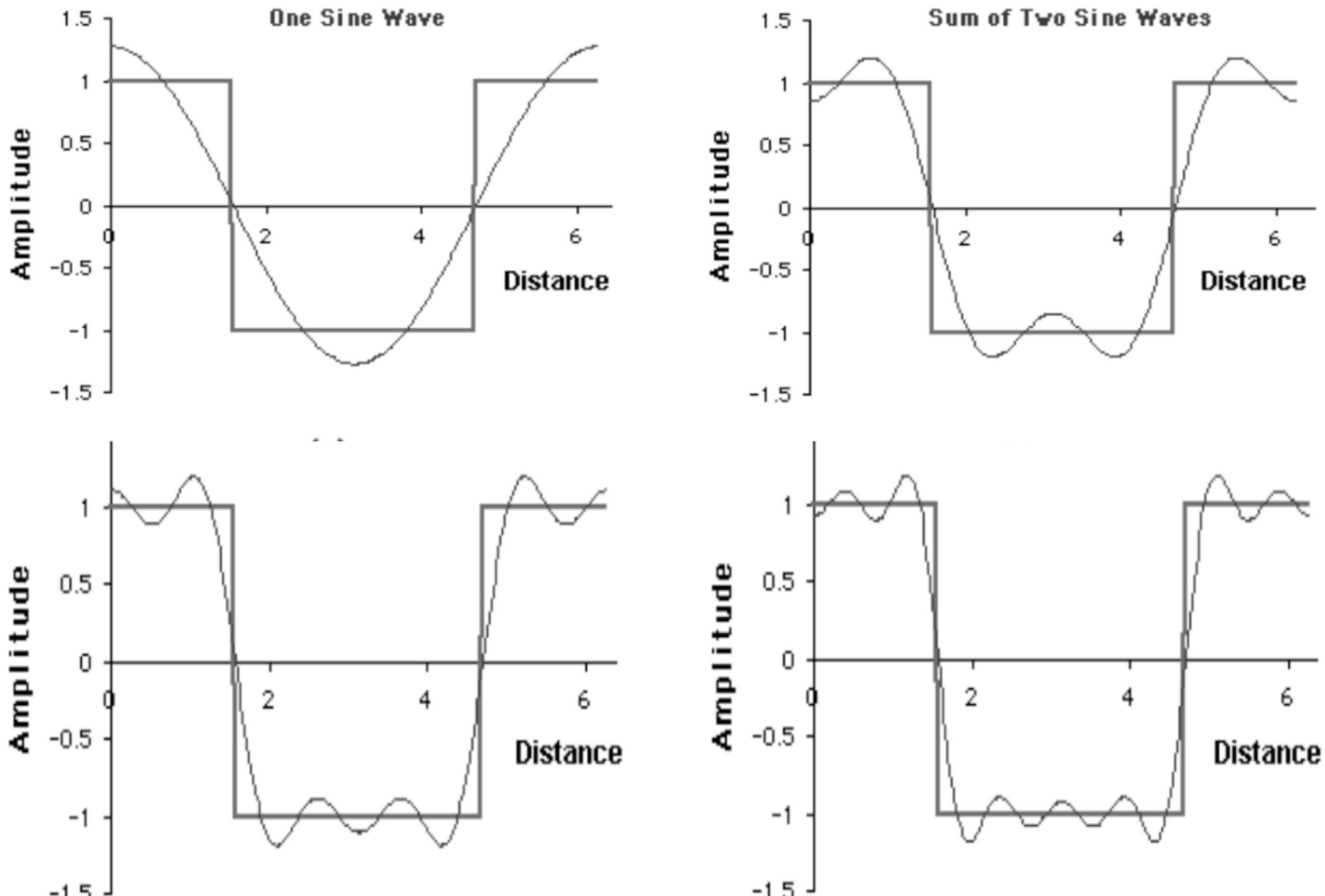
Digital Images and Fourier Transforms



We need an infinite amount of Fourier components to represent a discrete function



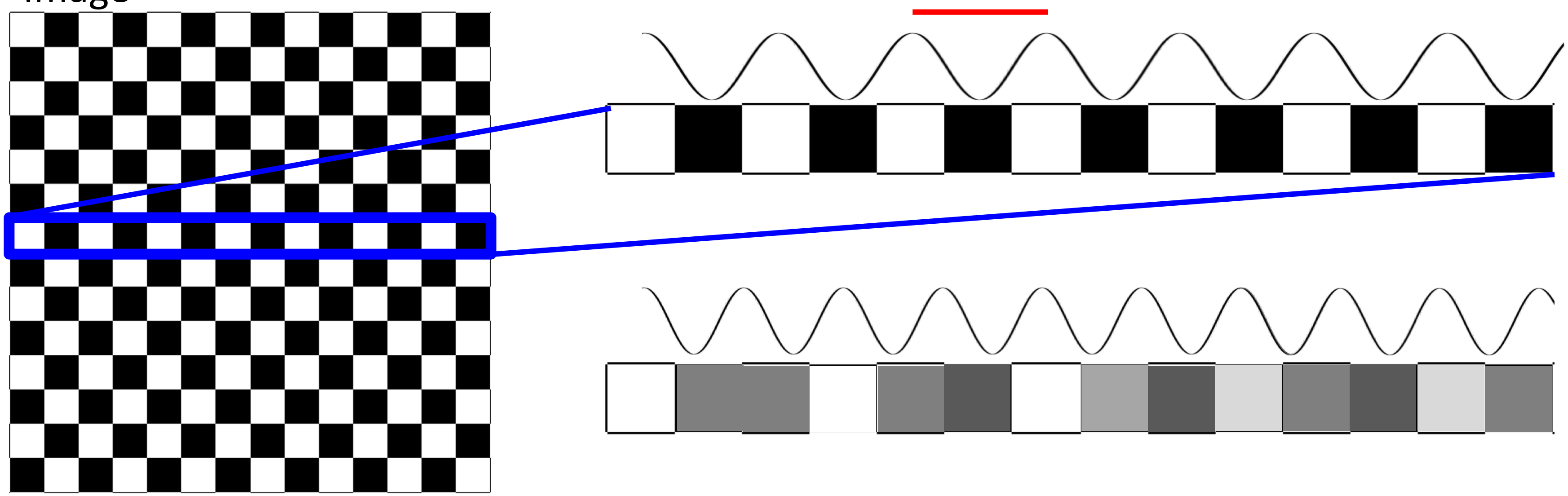
Digital Images and Fourier Transforms



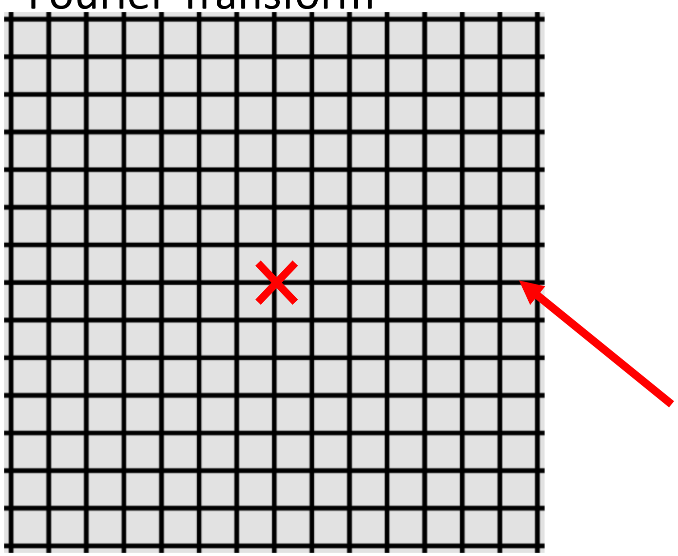
from Peter Rosenthal

The Nyquist limit

Image



Fourier Transform

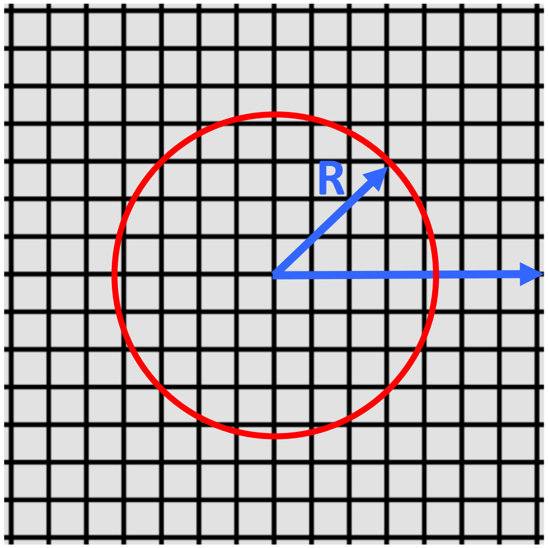


The highest possible frequency that can be represented in the digital world is **1/(2 pixels)**

Nyquist frequency

$$\frac{1}{2*\text{pixels}}$$

A formula to relate real and reciprocal space



FT

box = size of your image (=size of FT)

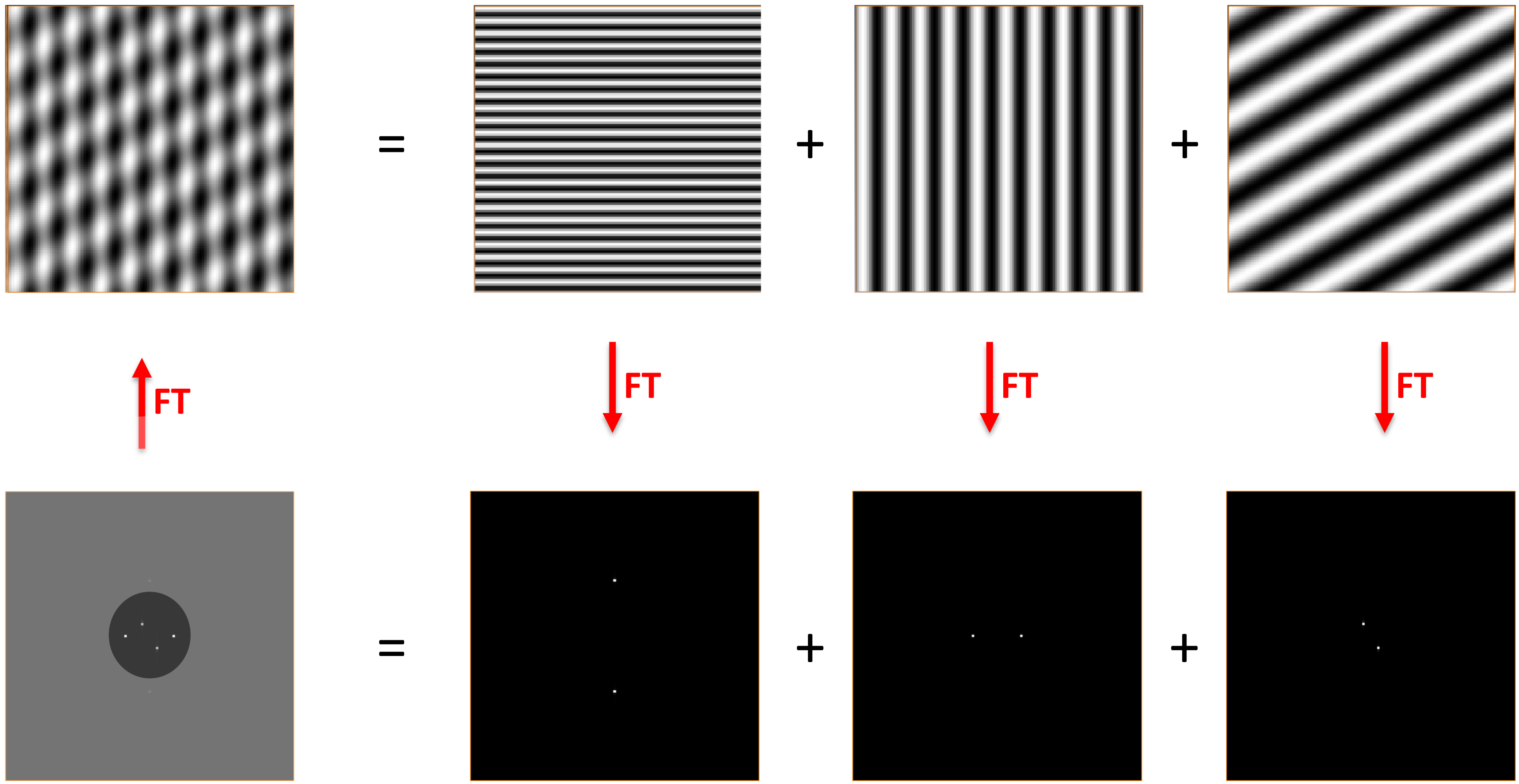
R = distance from center of FT

pixelsize = size of one pixel in the real image

resolution = spacing (wavelength, period) corresponding to the spatial frequency being represented at radius R in the FT

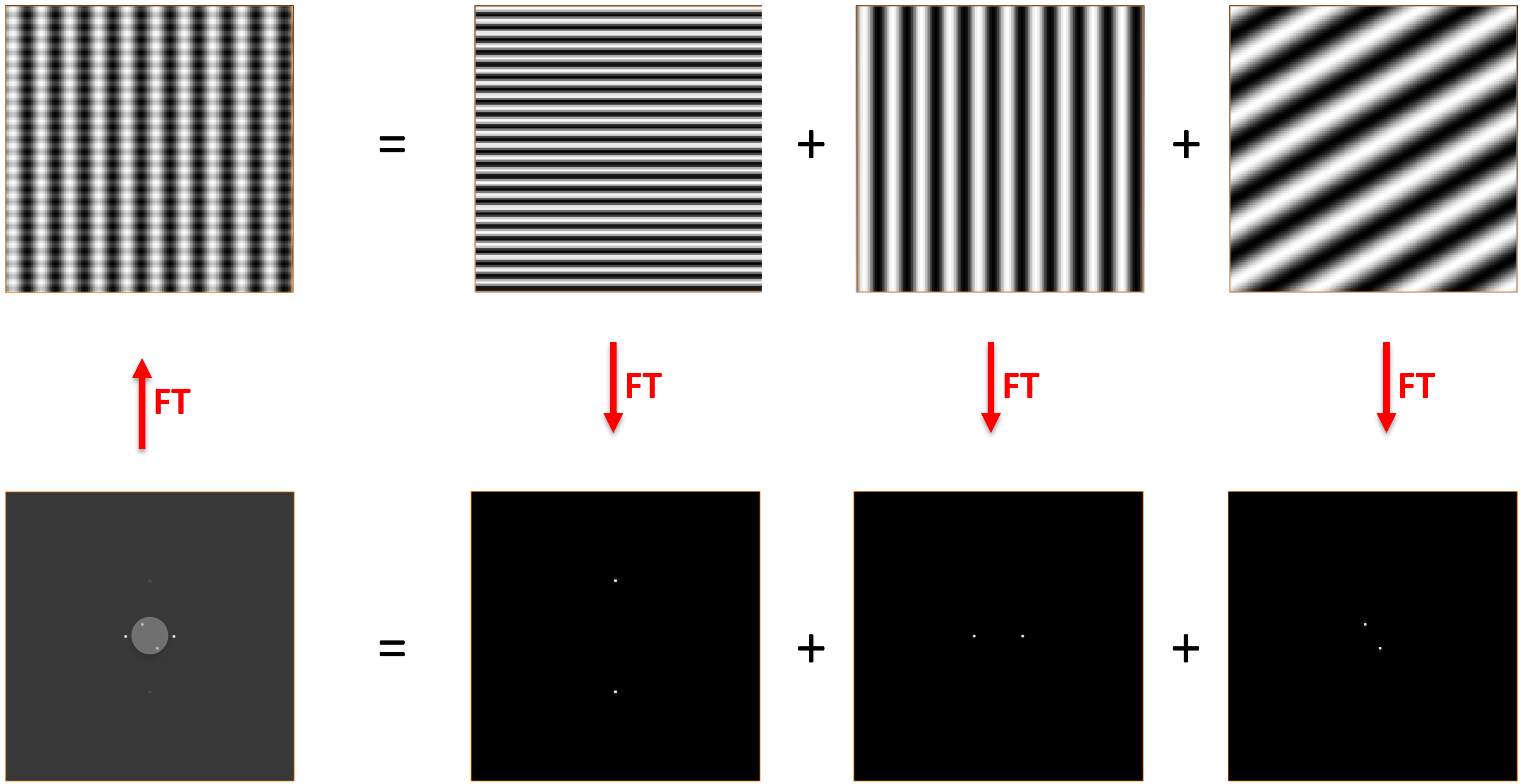
$$\frac{\text{box}}{2} : R = \frac{1}{2*\text{pixelsize}} : \frac{1}{\text{resolution}}$$

Applying filters in Fourier space



Low-pass filter

Applying filters in Fourier space



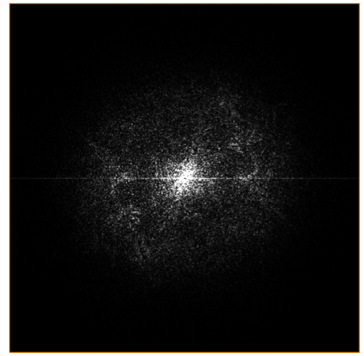
High-pass filter

Low-pass filtering

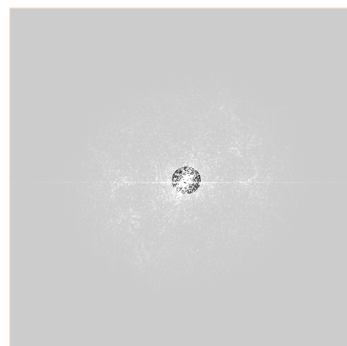
50 cm

750 pix

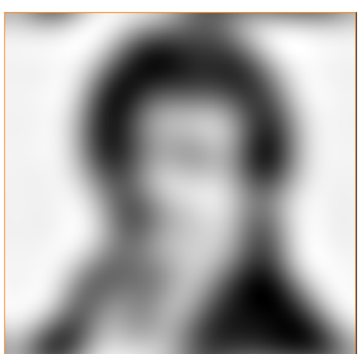
FT →



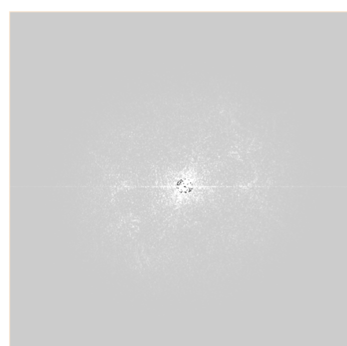
← FT^{-1}



filter_resolution = 2 cm
box = 750 pixels
pixelsize = 0.067 cm/pixel
R = 25 pixels



← FT^{-1}



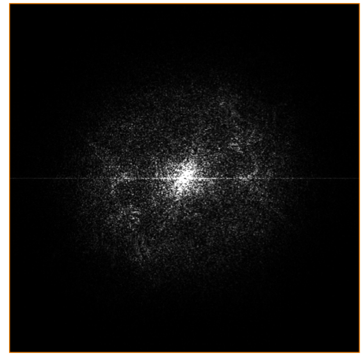
filter_resolution = 10 cm
box = 750 pixels
pixelsize = 0.067 cm/pixel
R = 5 pixels

High-pass filtering

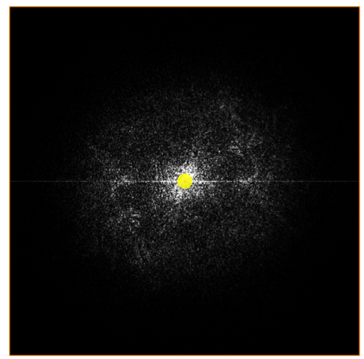
50 cm

750 pix

FT



FT^{-1}



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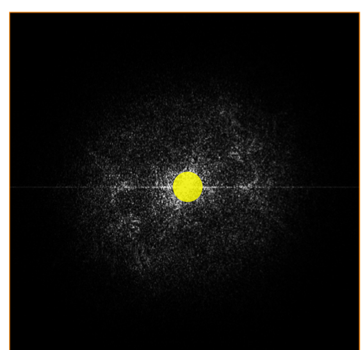
FT^{-1}



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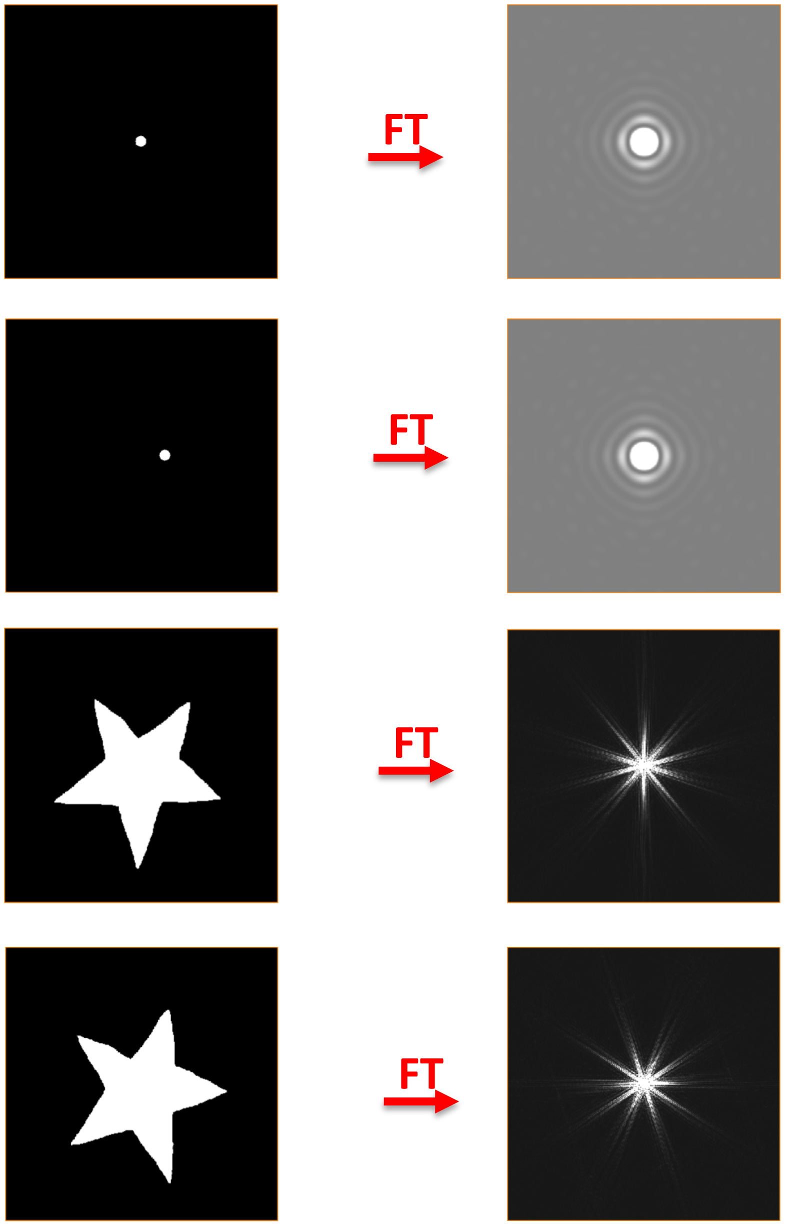
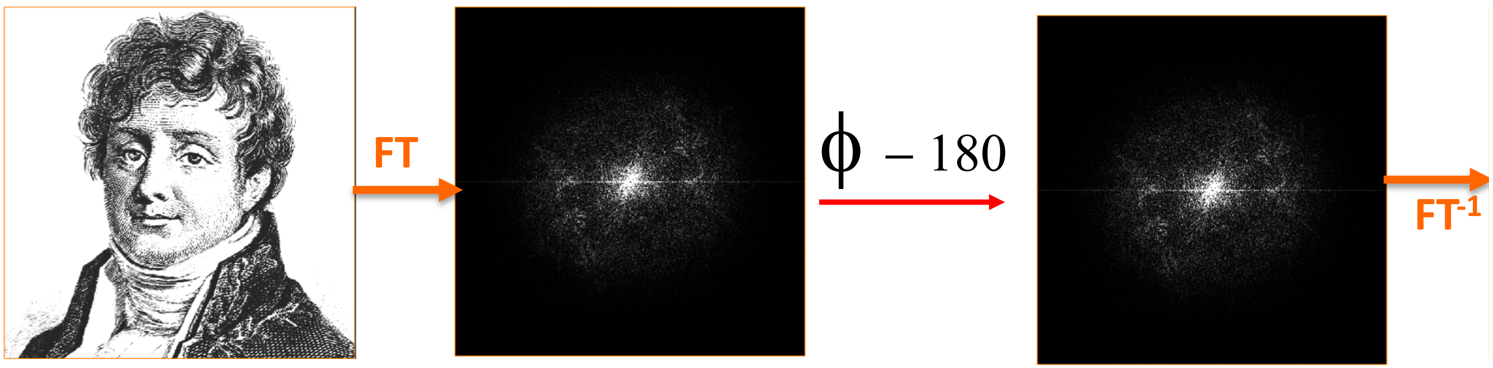
pixelsize = 0.067 cm/pixel
R = 25 pixels

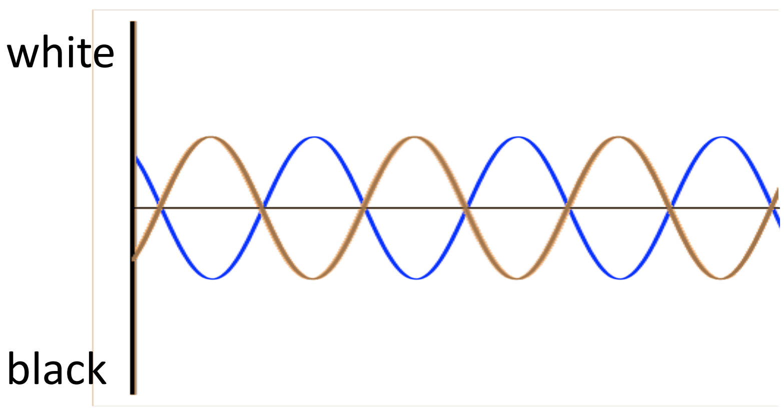
Shifts and Rotations



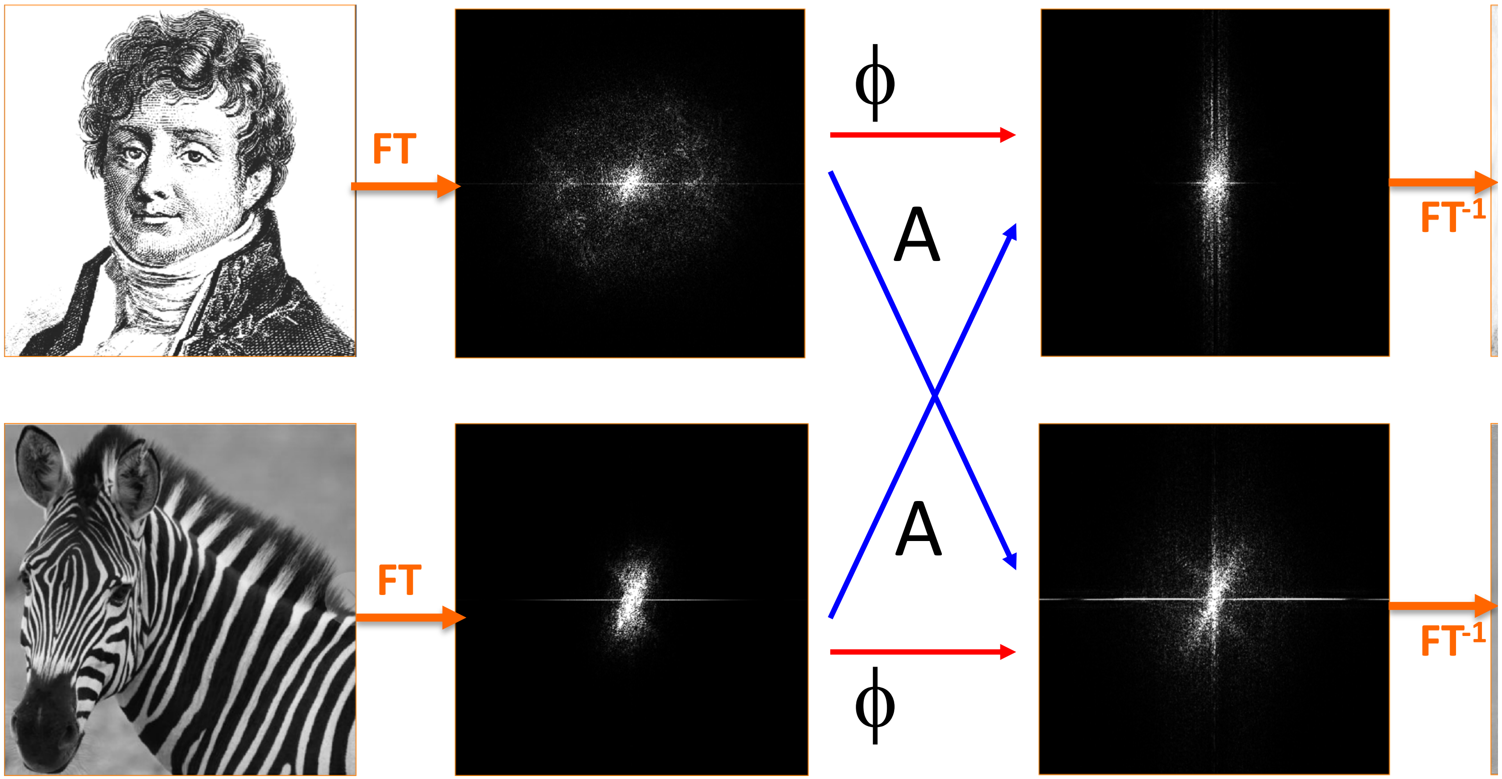
The amplitude spectra are the same.
The phases are different though!

What happens if the phase is inverted?

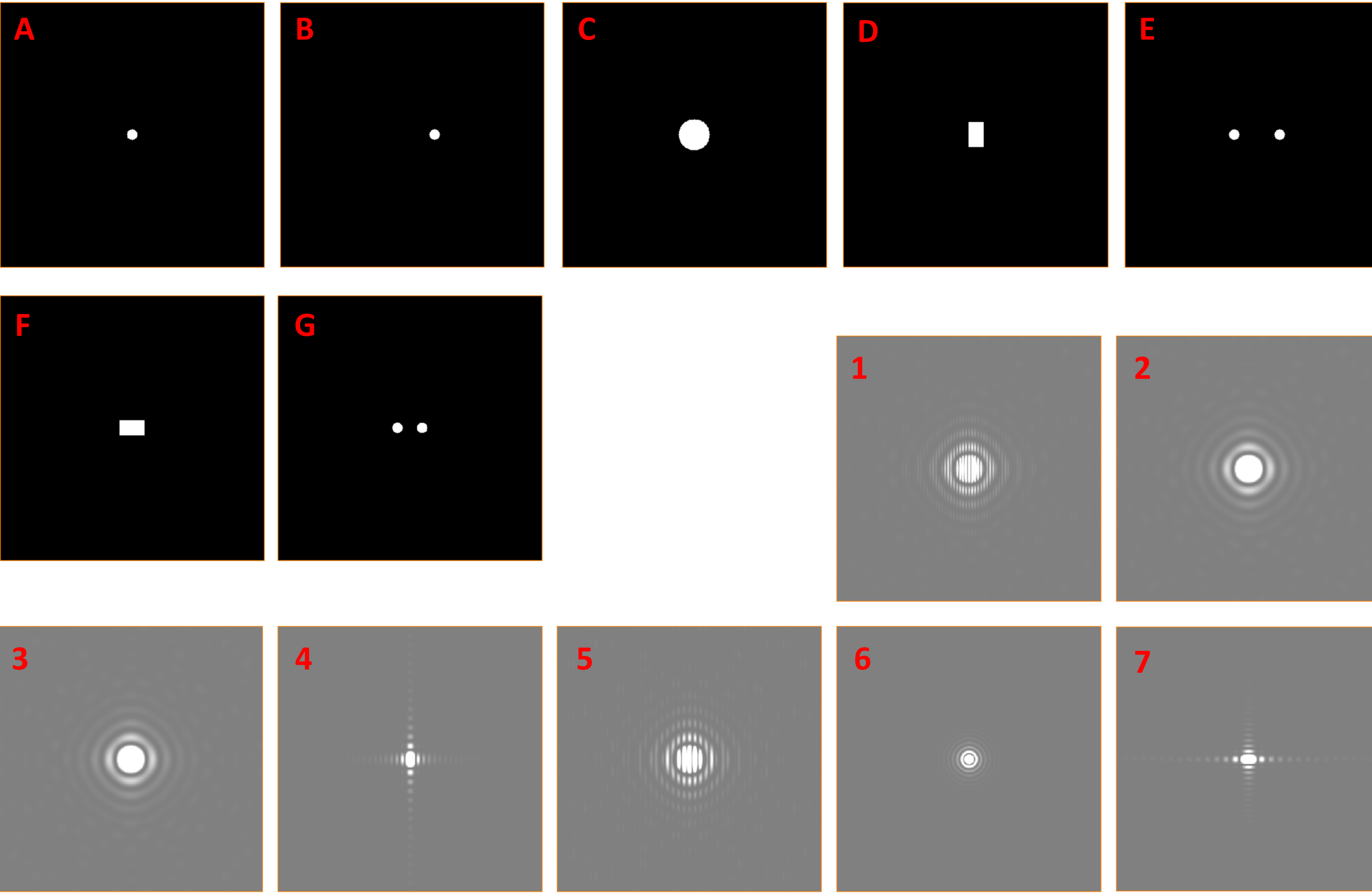




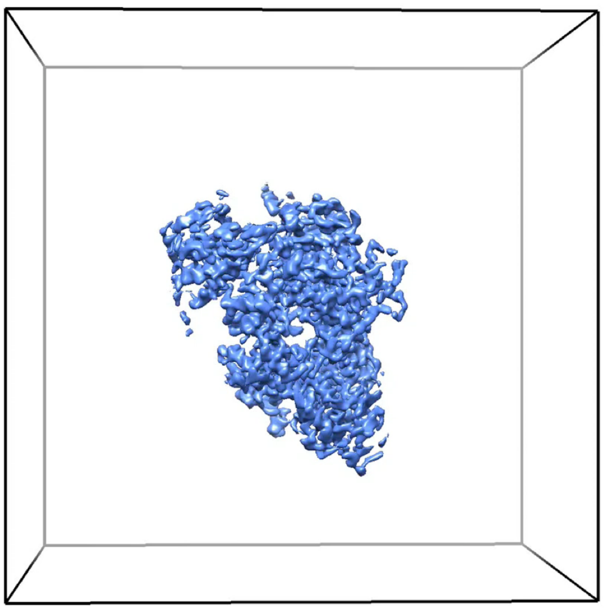
Are phases or amplitudes more important?



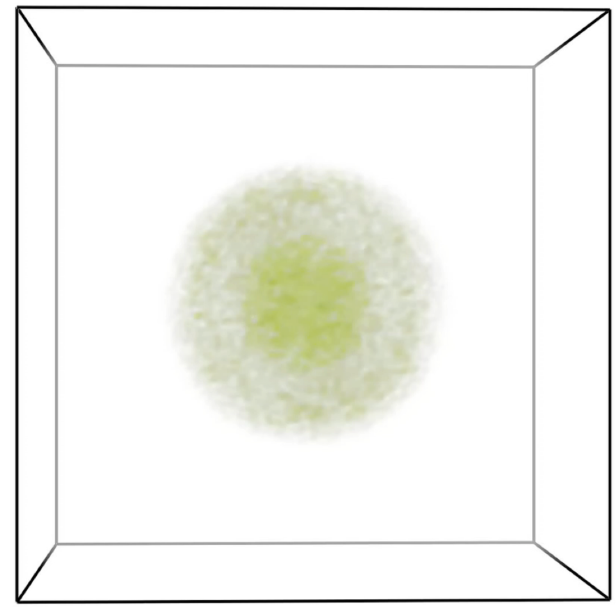
Match the image with its FT



Fourier transform of 3D functions

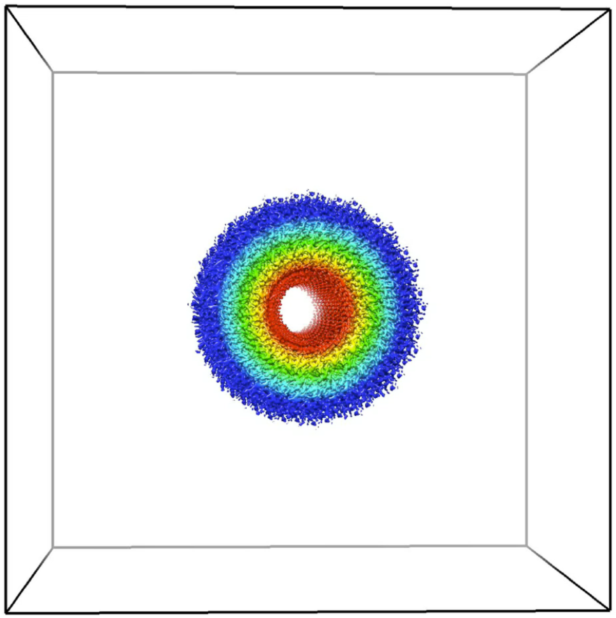


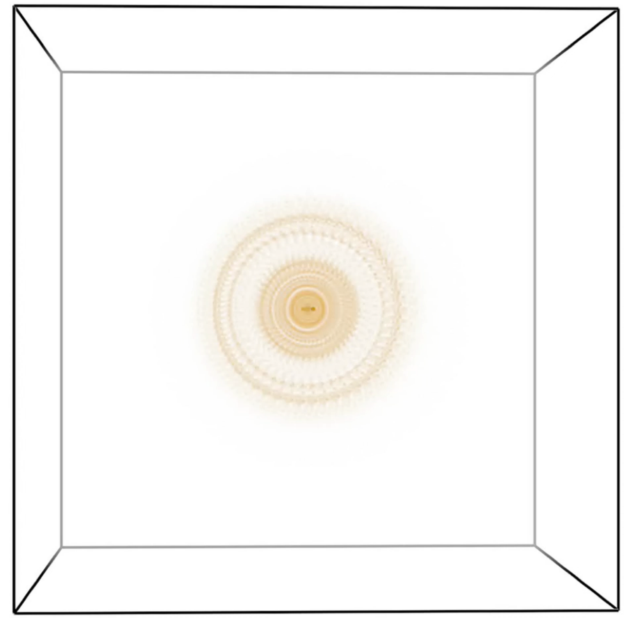
The 3D structure of a macromolecule is described as electron potential as a function of x,y and z (the MAP)



We can calculate its Fourier Transform

Fourier transform of 3D functions

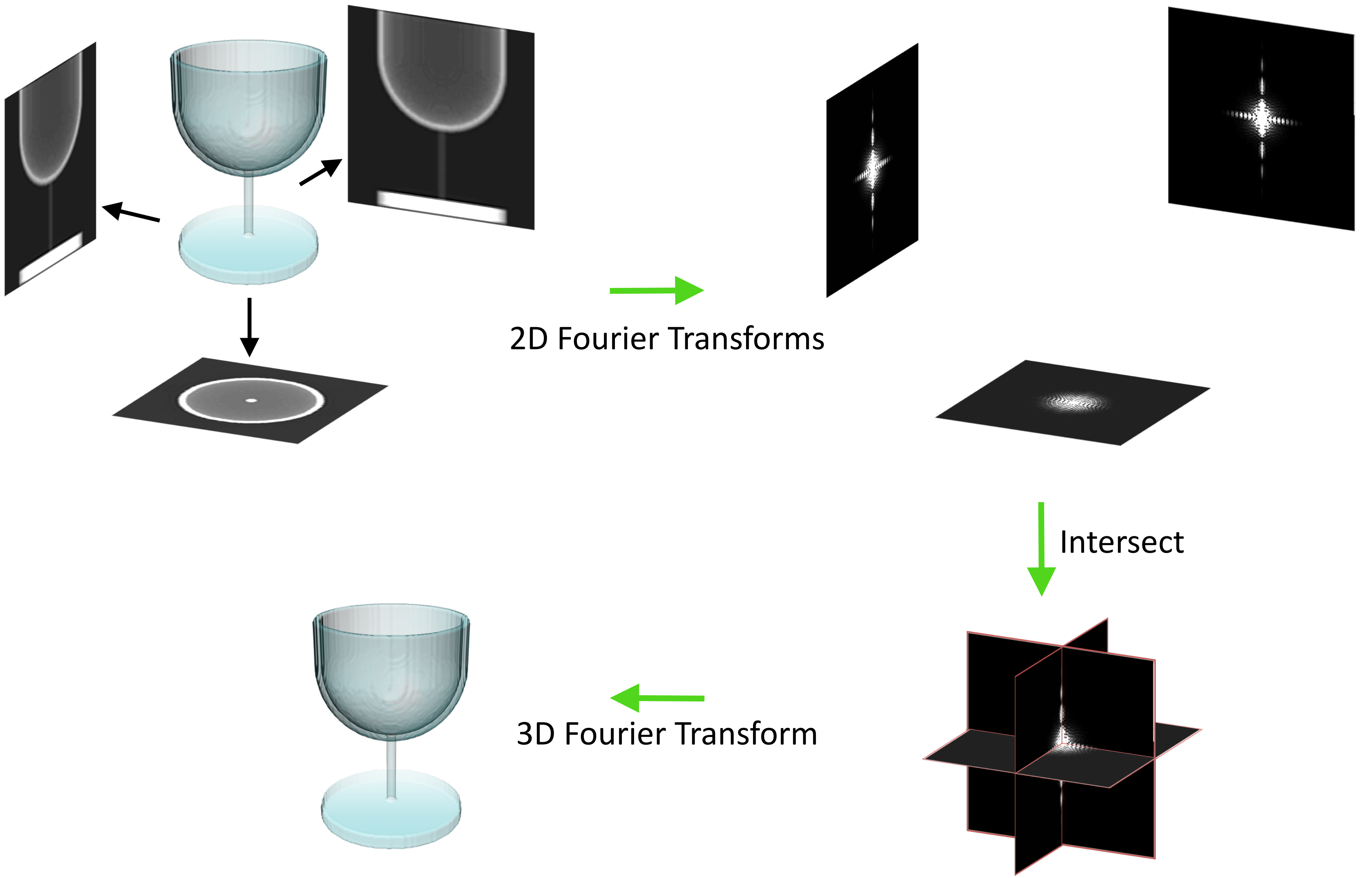




The 3D structure of a macromolecule is described as electron potential as a function of x,y and z (the MAP)

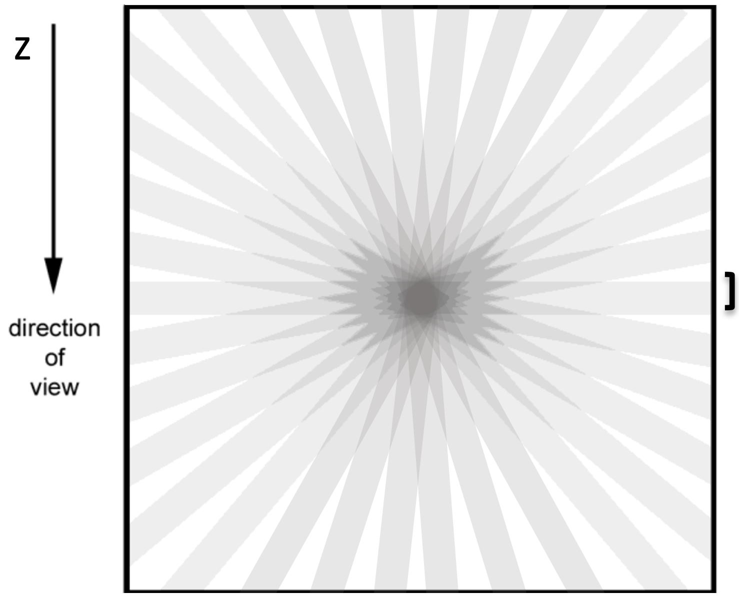
We can calculate its Fourier Transform

Projection theorem



Projection theorem

To calculate a 3D
One could ‘back-project’ in real space or intersect the FTs of all projections



planes in fourier transform are 1 pixel thick

Weighted back-projections: need of weighting down low resolution

original image

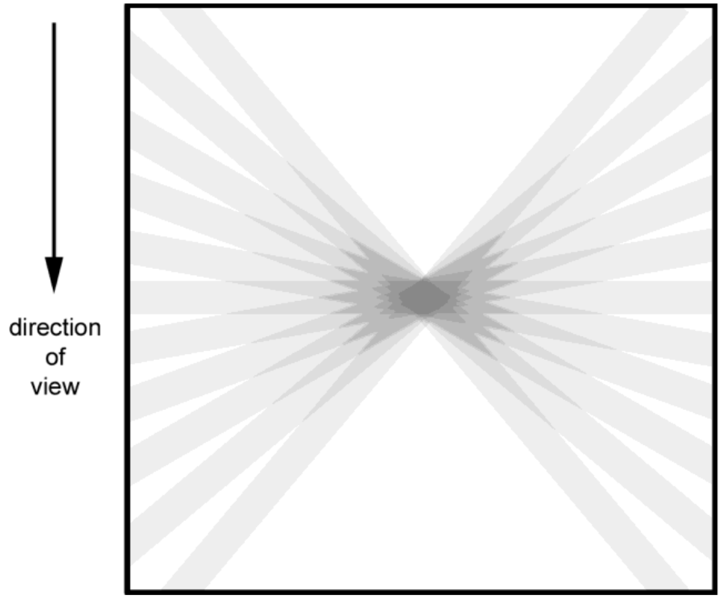
back-projection

weighted back-projection

Reconstruction – <u>weighted</u> backprojections

Anisotropy

What if we do not have projections from all angles?
Incomplete Fourier sampling (e.g. tomography)



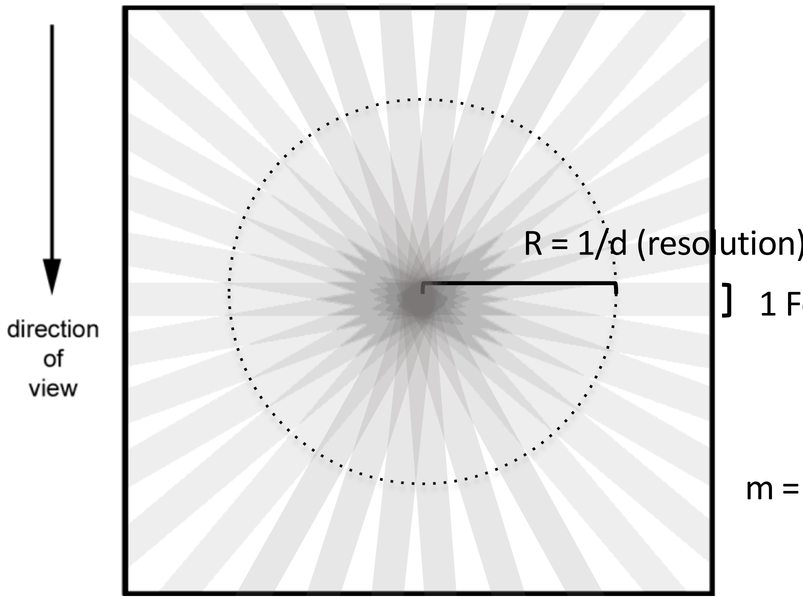
Distribution of information in Fourier space of a tomographic reconstruction:

- missing wedge (anisotropy)

original image

anisotropic

The Crowther criterion



] 1 Fourier pixel = 1/D (thickness of box)

m = number of views

$$m = \pi D/d$$

```
 Auto-refine: Angular step= 0.46875 degrees; local searches= true
 Auto-refine: Offset search range= 1.1295 pixels; offset step= 0.3765 pixels
 CurrentResolution= 7.15487 Angstroms, which requires orientationSampling of at least 4.09091 degrees
for a particle of diameter 200 Angstroms
 Oversampling= 0 NrHiddenVariableSamplingPoints= 1395
```

D=20 nm

d = 0.715 nm

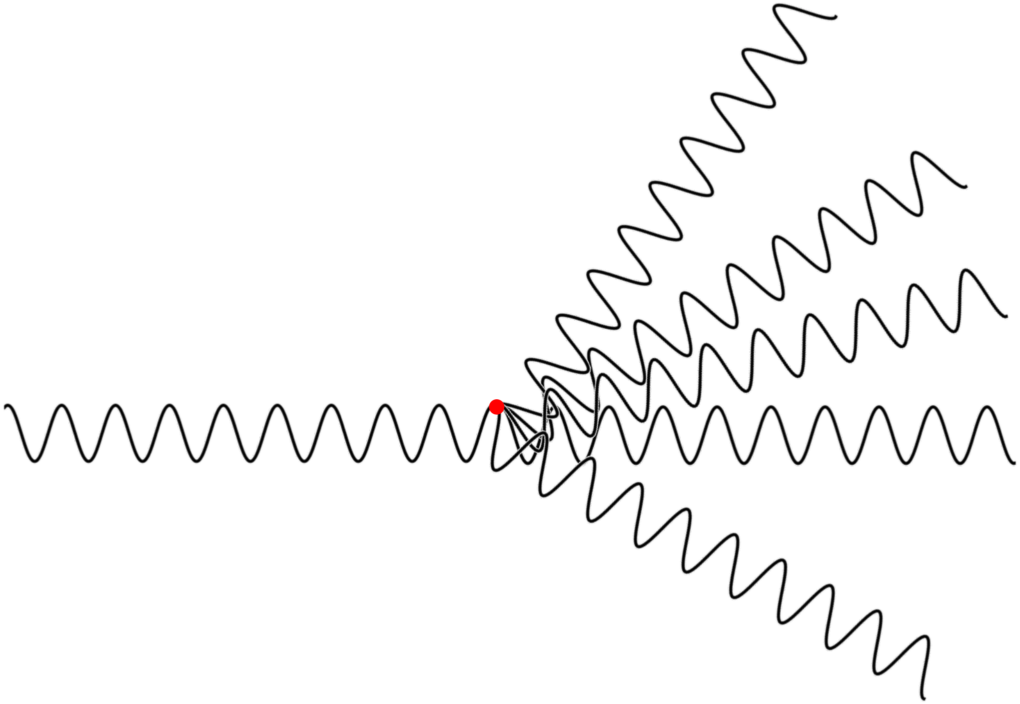
m = 87.83 => increments of 360/87.83 = 4.09°

How to resample maps

You have a map downloaded from EMDB, which has a pixel size of 1.7 A/pixel and a box size of 128^3 pixels.
You want to use it as a starting model for your new dataset, which was collected at 1.3 A/pixel and was extracted in 160^2 pixel boxes.

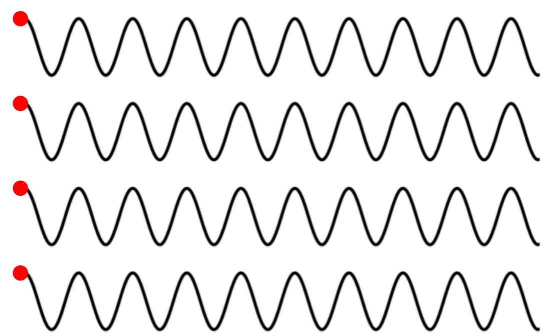
How do you resample your map? (not with relion_image_handler…)

Diffraction and Fourier Transform



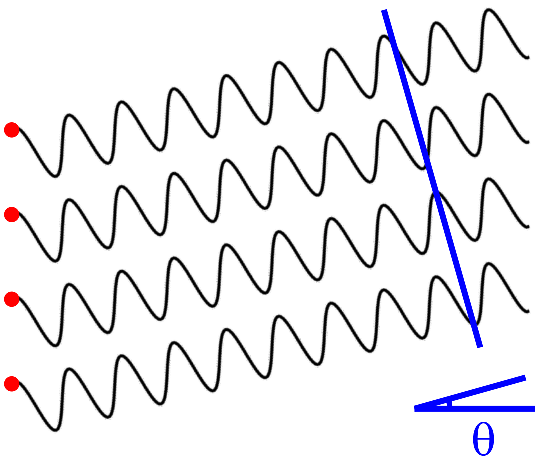
towards
objective lens
and detector

Diffraction and Fourier Transform



Unscattered wave

Diffraction and Fourier Transform



towards
objective lens
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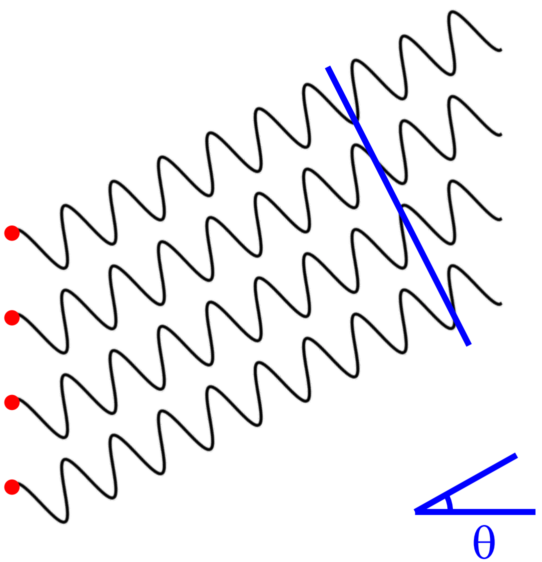
Scattered wave at angle θ

destructive interference

Diffraction and Fourier Transform



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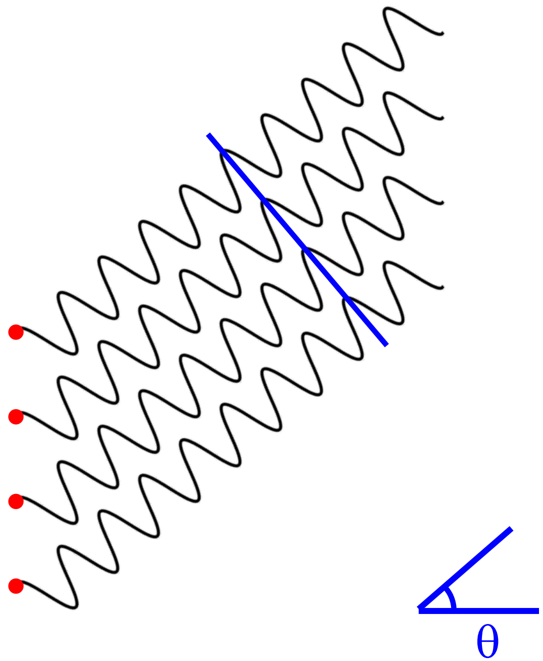
destructive interference

Diffraction and Fourier Transform



towards
objective lens
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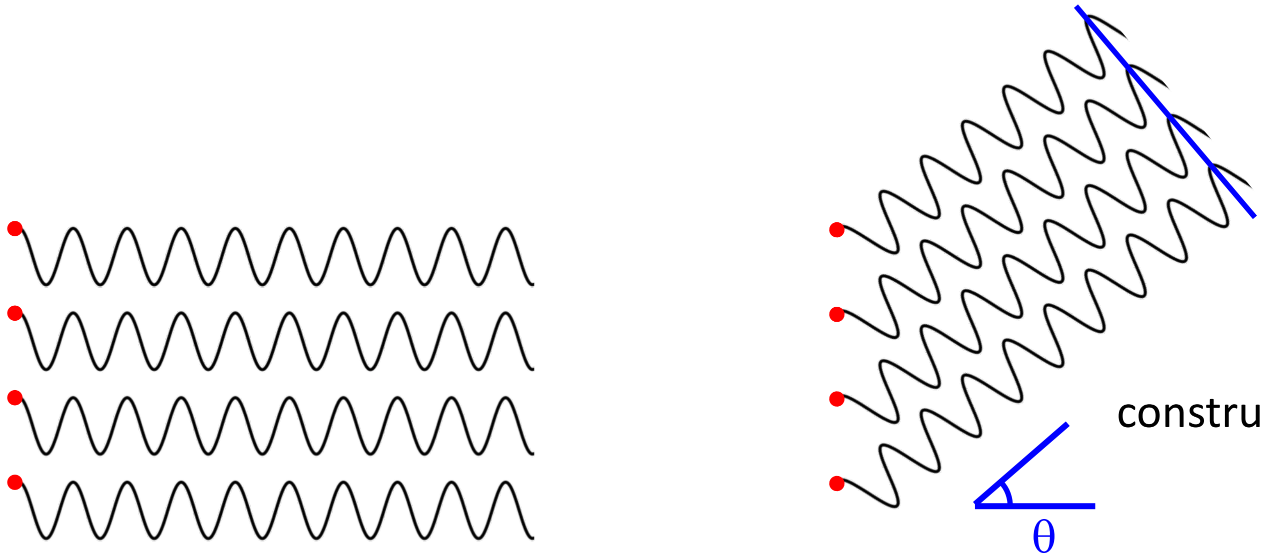
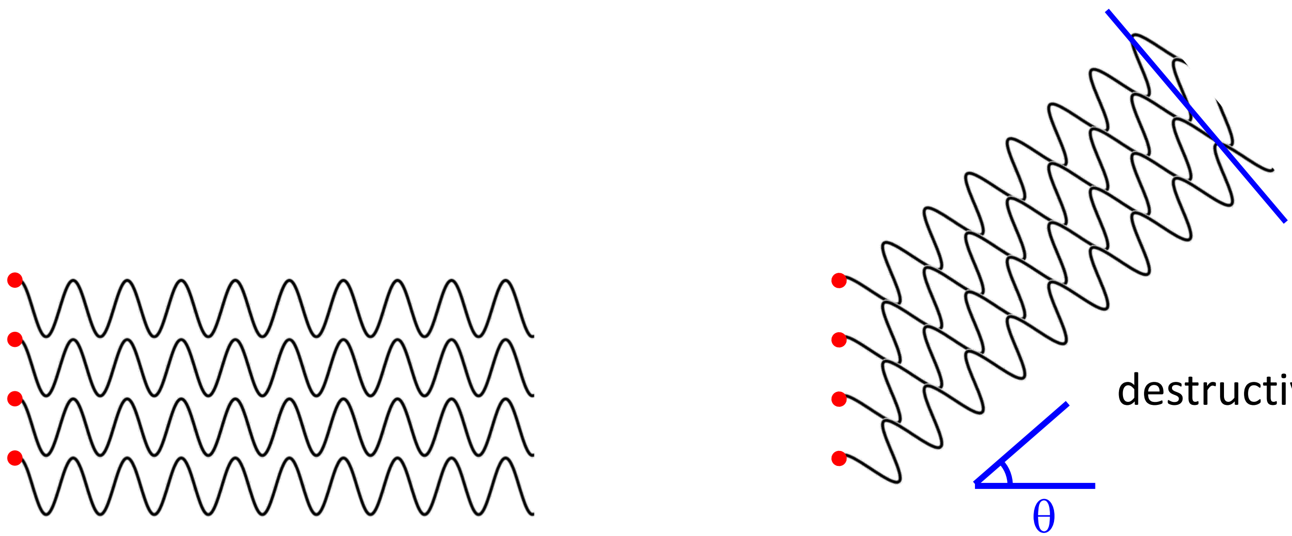
Scattered wave at angle θ

constructive interference

Diffraction and Fourier Transform

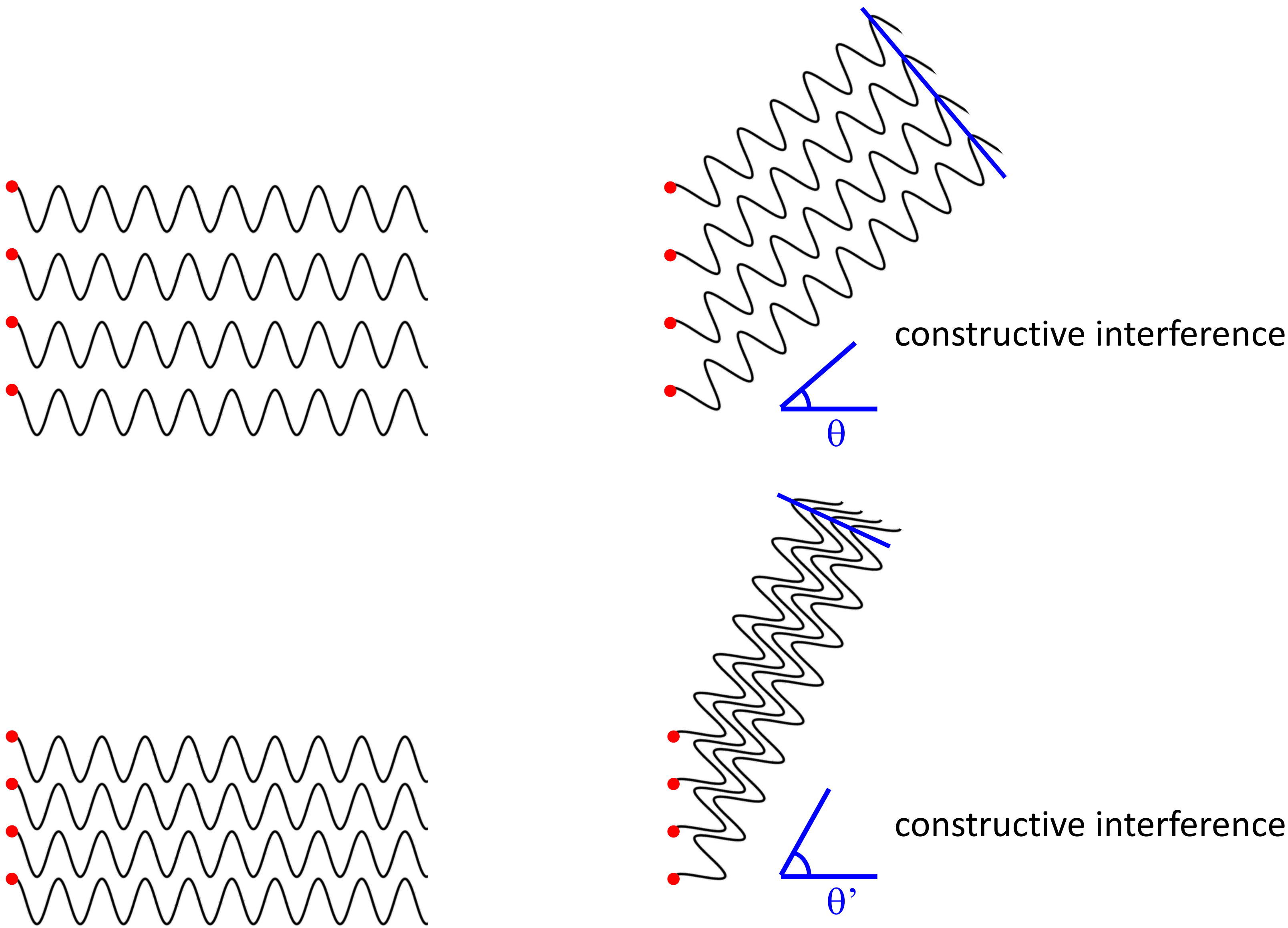


constructive interference



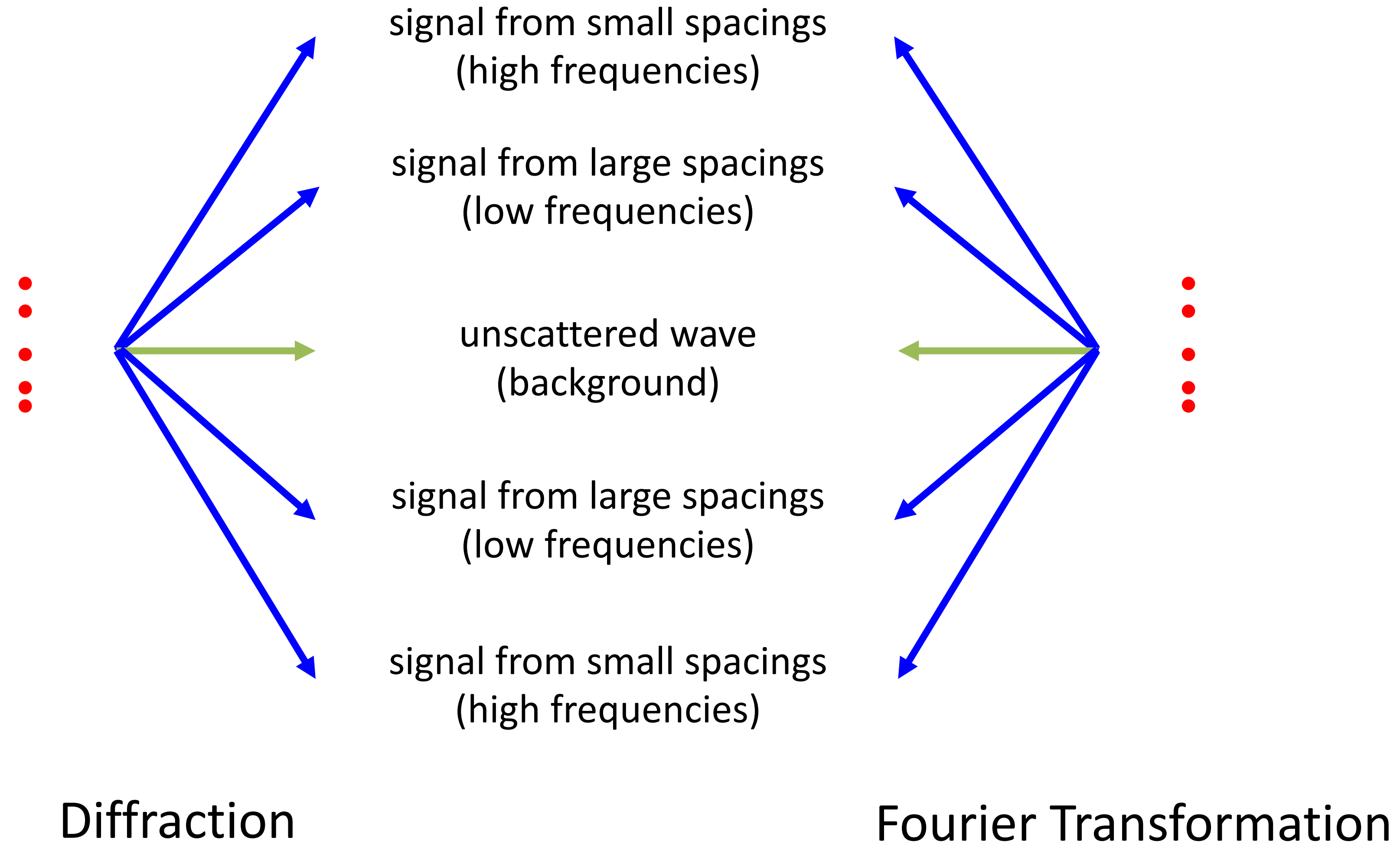
destructive interference

Diffraction and Fourier Transform



Diffraction and Fourier Transform

For a given spacing between scattering objects, there is one angle at which scattered waves are in phase with each other and produce a signal. The smaller the spacing the higher the angle.



When do we need to think in fourier space

High and Low-pass Filtering, denoising

CTF detection and correction

Spectral SNR considerations (relion T value, Wiener Filter)

Resolution assessment (Fourier Shell Correlation)

Sharpening

…and at almost every step in image processing!