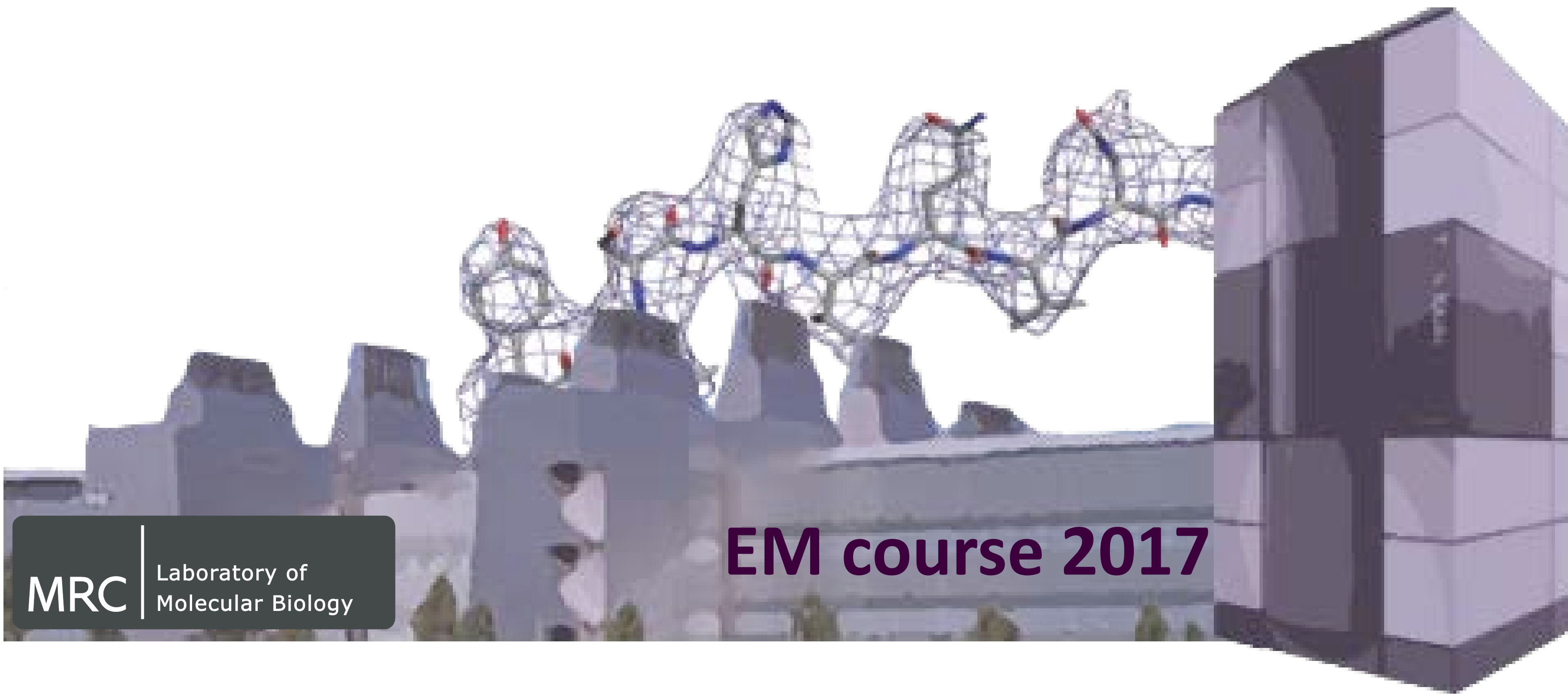
3. Image formation, Fourier analysis and CTF theory

Paula da Fonseca



- Agenda -

Overview of:

- Introduction to Fourier analysis
  - Sine waves
  - Fourier transform (simple examples of 1D functions)
  - Fourier transform of images
  - Why is it useful for image processing?

- Image formation
  - Weak phase approximation

- The contrast transfer function

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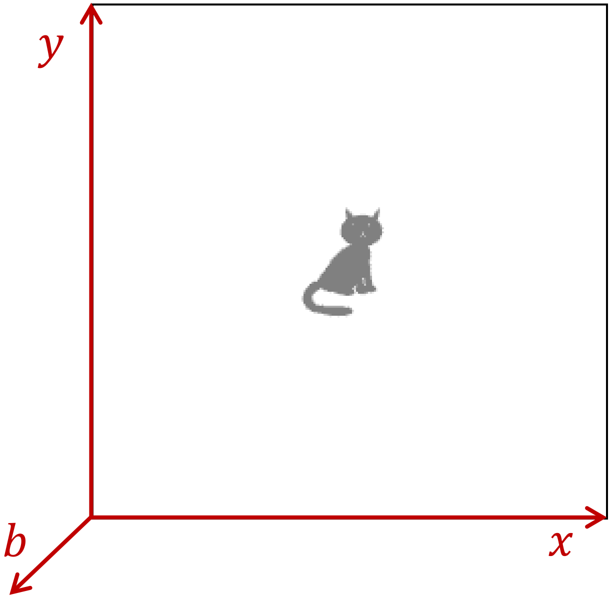
Introduction to Fourier analysis

- Every (monochromic) image is a 2D function of space (x,y) vs brightness (b)



Introduction to Fourier analysis

Fourier theory: any function (like our images) can be expressed as a sum of a series of sine waves.

Jean-Baptiste Joseph Fourier
1768 – 1830

Introduction to Fourier analysis

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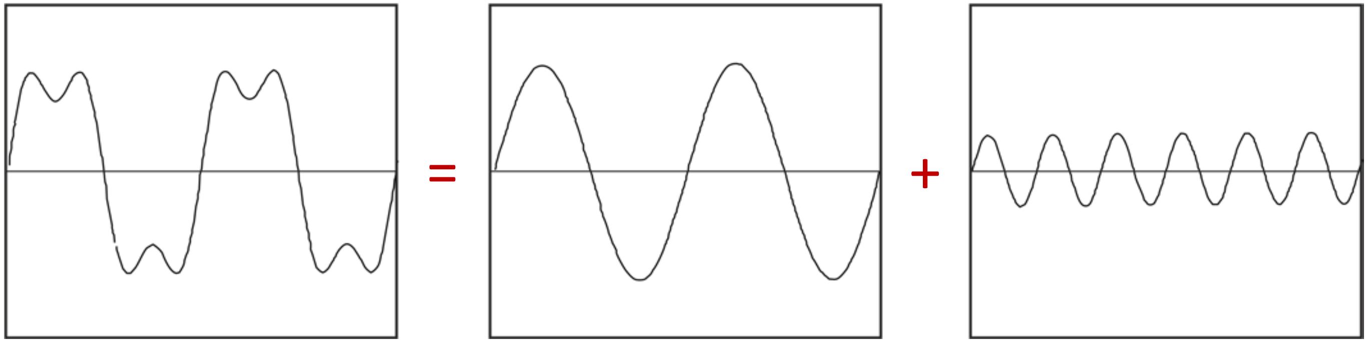

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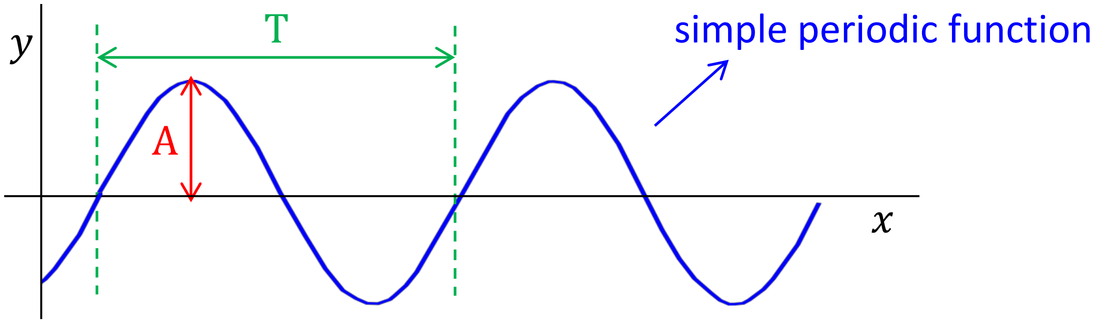
Sine waves

some basic concepts

Sine waves

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



A = amplitude (maximum extent of the wave, the units correspond to brightness in the case of an image)

T = period (repeat, cycle, wavelength; length of one repeat in x axis units, normally Ångstroms in the processing of EM images)

$f = 1/\mathrm{T}$ = frequency (repeats per x axis unit; normally per Ångstrom in the processing of EM images)

Sine waves

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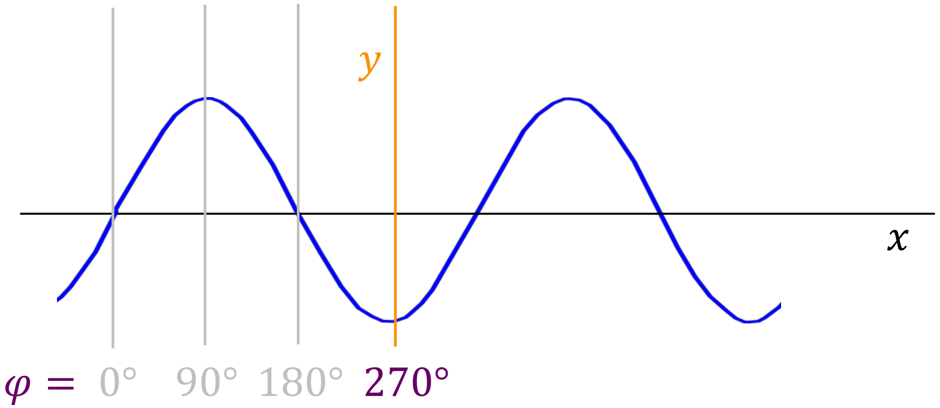
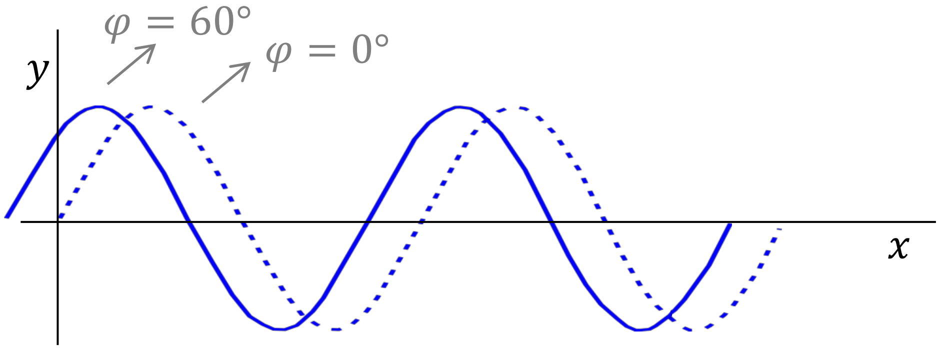


φ = phase, in degrees or radians, defines the oscillation stage at the wave origin

Sine waves

$$y = A \sin\left(2\pi x/T + \varphi\right)$$



the phase defines the "relative position" of a wave

φ = phase, in degrees or radians, defines the oscillation stage at the wave origin

Sine waves

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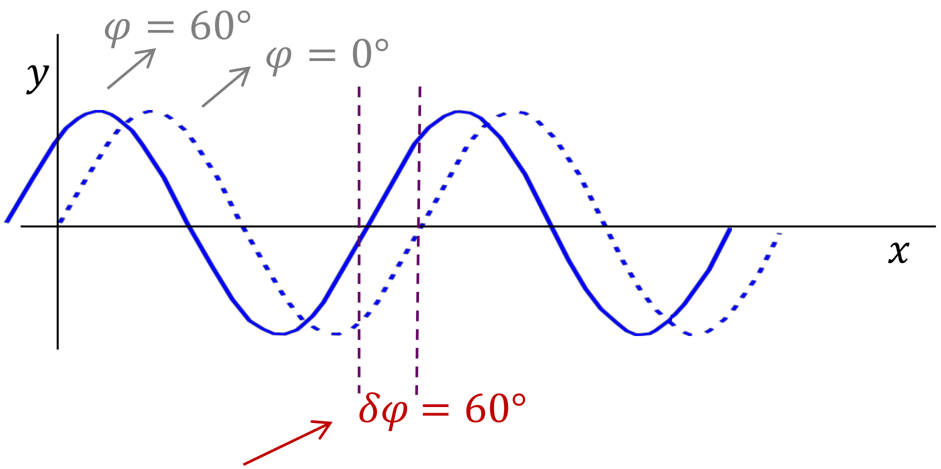
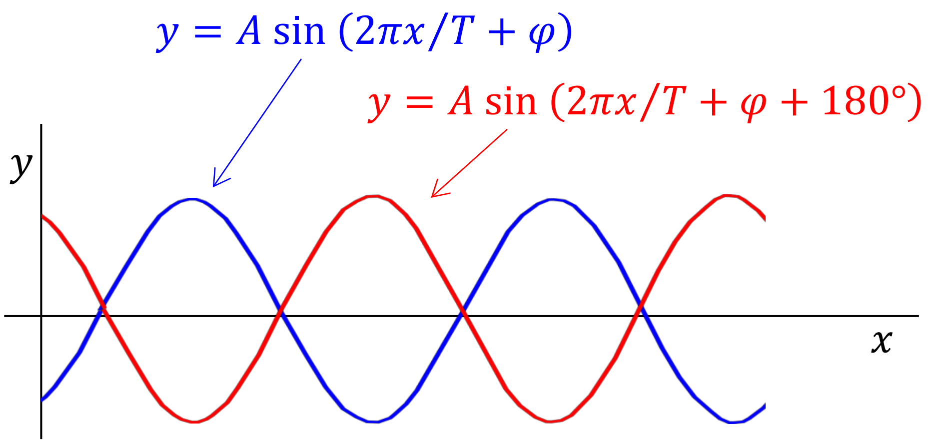


phase difference between two out-of-phase waves

phase shift = addition of a constant (in degrees or radians) to wave phase

φ = phase, in degrees or radians, defines the oscillation stage at the wave origin

Sine waves

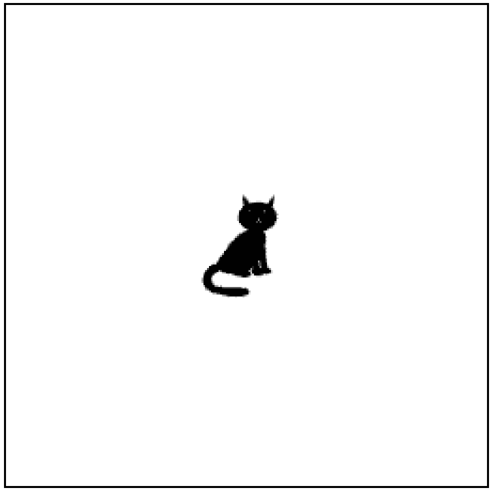


adding 180° to the phases of a sine wave results in its mirror function

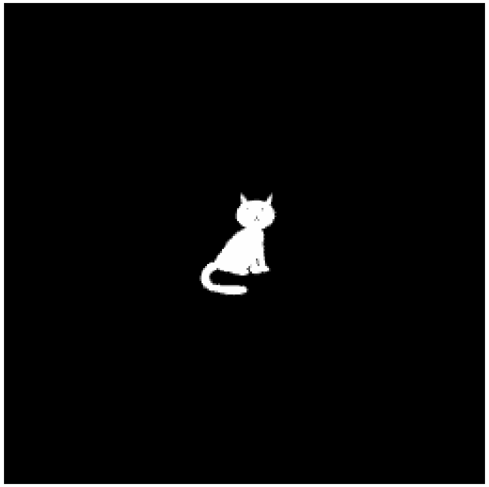
in image processing, adding 180° to the phase of a wave component of an image results in the contrast reversal (white becomes black and black becomes white) of the contribution of that wave for the image

\- relevant for CTF correction -

Sine waves



180° added to the phases of ALL frequency components of the image →



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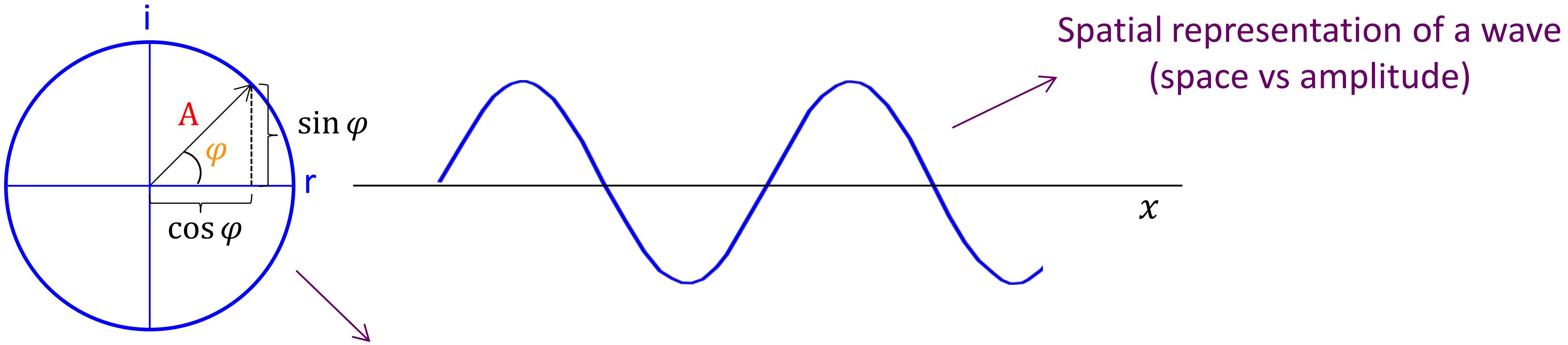
Sine waves

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

Argand Diagram



the amplitude and phase of a sine wave can be represented as a complex number where:

real component is $A\cos\varphi$

imaginary component is $A\sin\varphi$

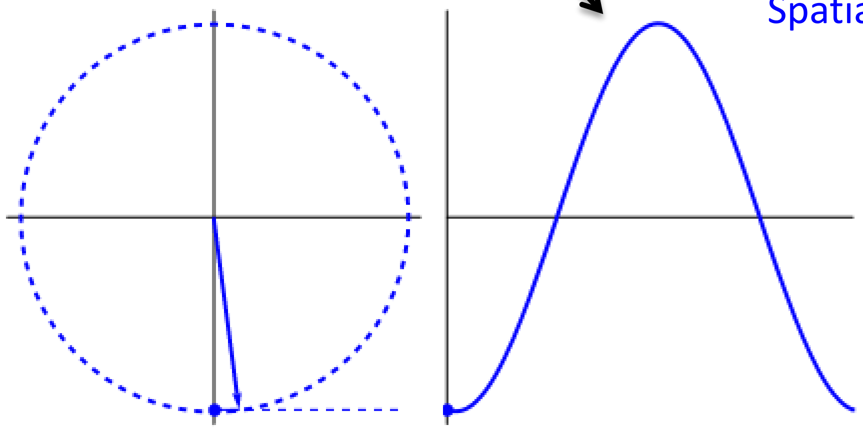
$$e^{i\varphi} = A(cos\varphi + isin\varphi)$$

Sine waves

$$y = A\sin\left(2\pi x/T + \varphi\right)$$

Spatial representation of a wave (space vs amplitude)

Argand Diagram



$$e^{i\varphi} = A(cos\varphi + isin\varphi)$$

(animation taken from: https://en.wikipedia.org/wiki/Phasor)

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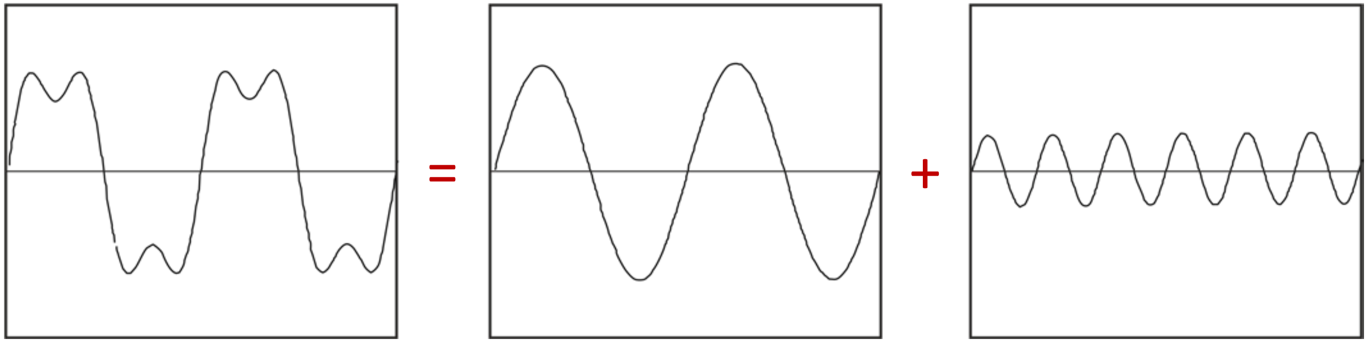
Fourier transform

(simple 1D functions)

Fourier theory: any function (like our images) can be expressed as a sum of a series of sine waves.

- simple 1D function -



$f(x)$ $\sin x$ $1/3 \sin 3x$

$$f(x) = \sin x + 1/3 \sin 3x$$

Fourier series for $f(x)$

Fourier transform

(simple 1D functions)

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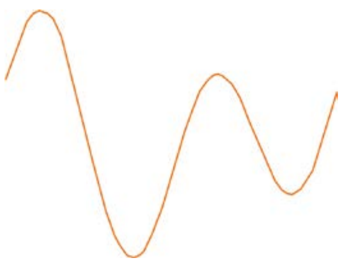
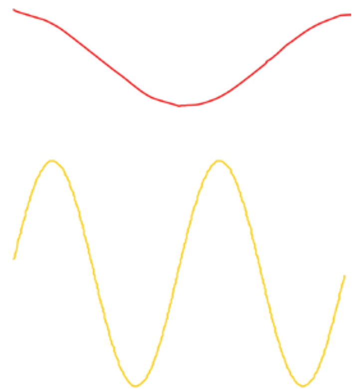


= A + B

A

B



different functions are decomposed into different Fourier series

Fourier transform

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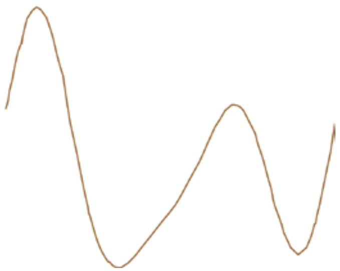
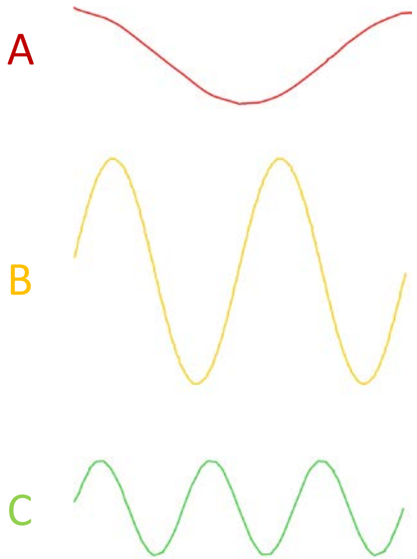


= A + B + C



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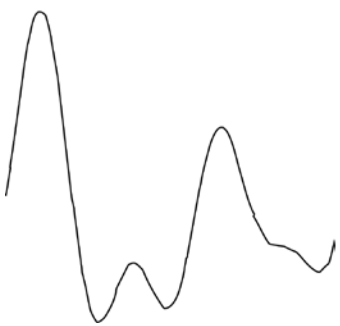
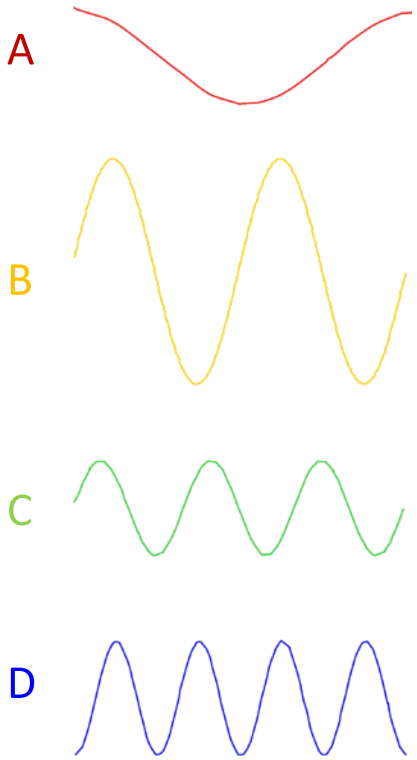


= A + B + C + D



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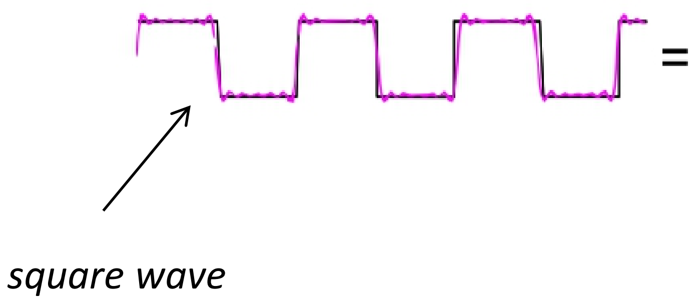
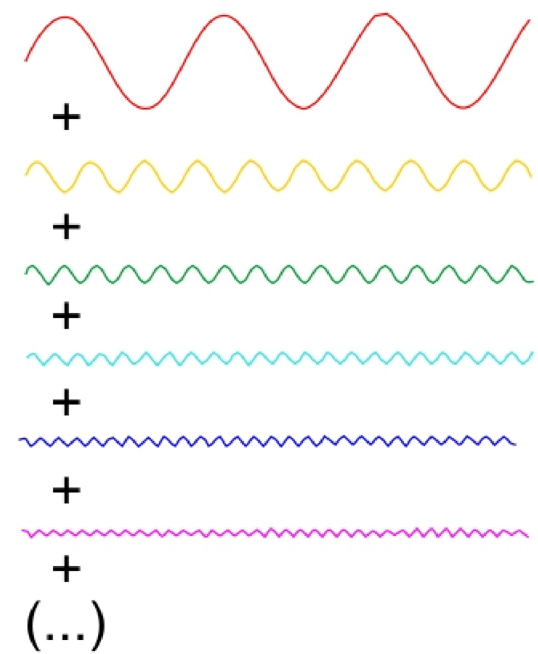


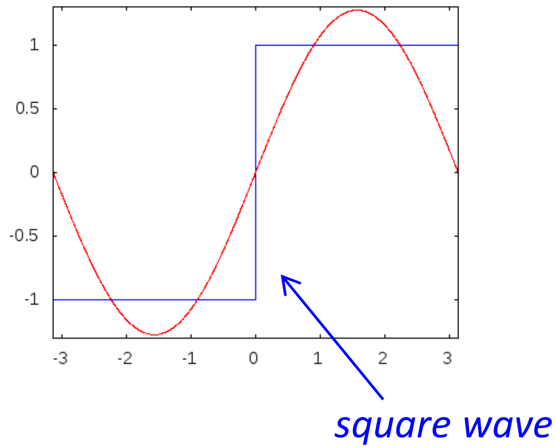


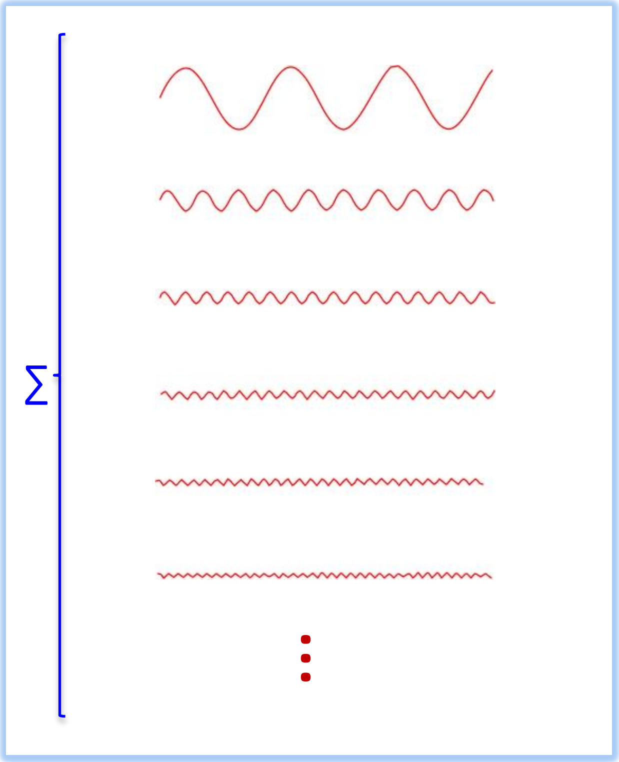
discontinuous functions are decomposed into series of infinite number of sine waves

Fourier transform

(simple 1D functions)

Fourier theory: any function (like our images) can be expressed as a sum of a series of sine waves.





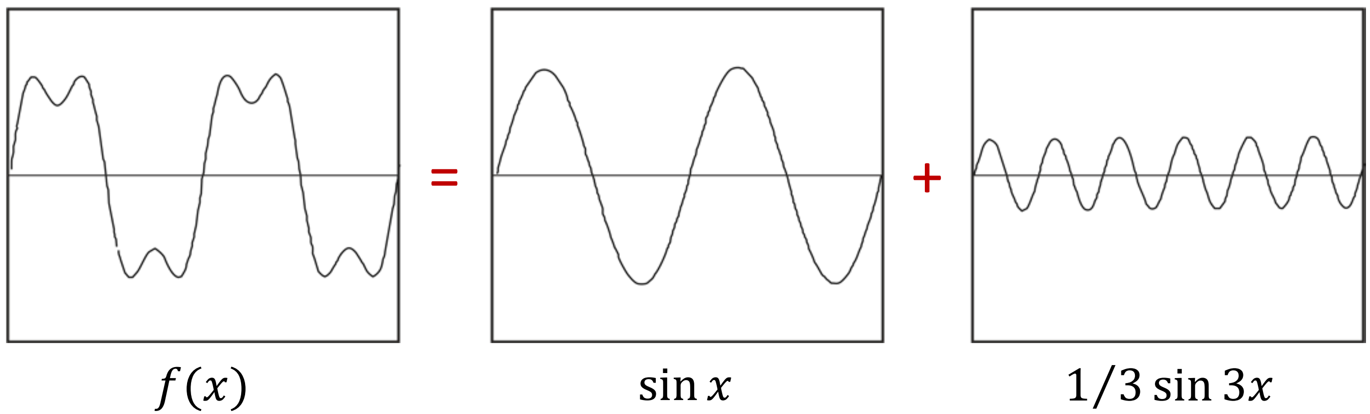
square wave

discontinuous functions are decomposed into series of infinite number of sine waves

(animation taken from: https://en.wikipedia.org/wiki/Square_wave)

Fourier transform

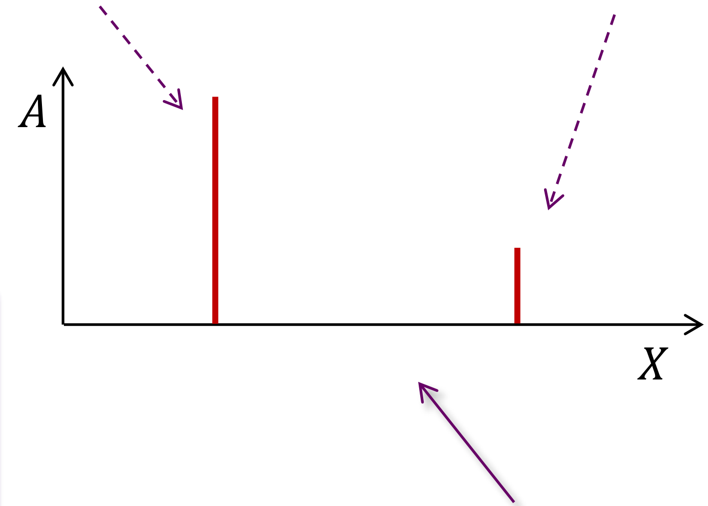
(simple 1D functions)



$$f(x) = \sin x + 1/3 \sin 3x$$

Fourier series for $f(x)$

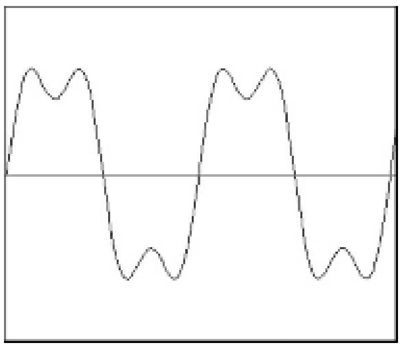
$f(x)$ is decomposed into a Fourier series that can be represented in a plot of amplitude (A) *vs* frequency (X)

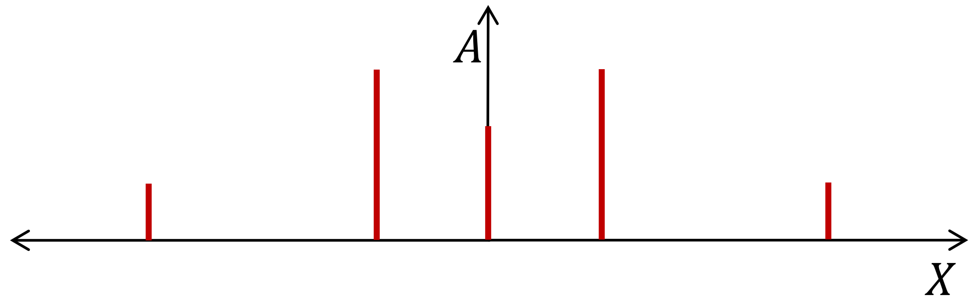


spectrum of $f(x)$
(frequency domain)

Fourier transform

(simple 1D functions)





$f(x)$
real space
(spatial domain)

⟷
Fourier transform

$F(X)$
Fourier space
(frequency domain)

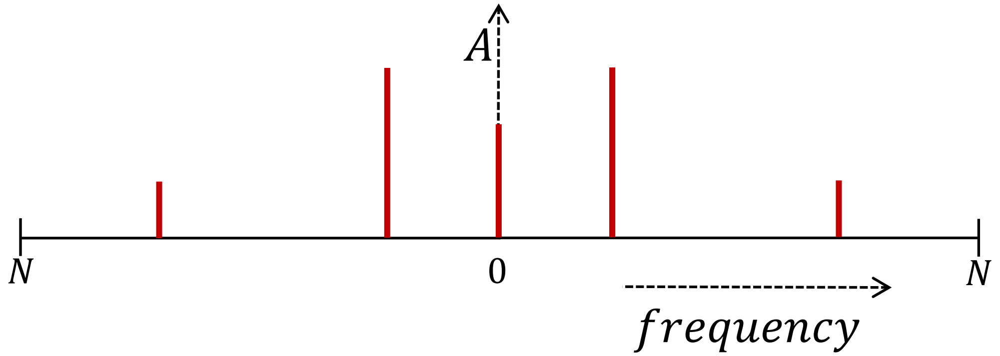
Fourier transform:

- continuous function (in frequency domain) that encodes all the spatial frequencies that define the transformed real space function:

$$F(X) = \int_{-\infty}^{\infty} F(x) e^{-i2\pi xX} dx$$

Fourier transform

(simple 1D functions)

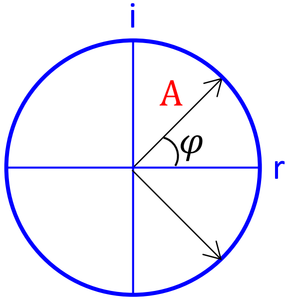


Fourier transform:

- the term at zero frequency represents the average amplitudes across the whole function
- for mathematical reasons, the Fourier transform of a real (non-complex) function is reflected across the origin, with the frequency increasing in both directions
- each Fourier component has a mirror (equivalent) component (Friedel symmetry)

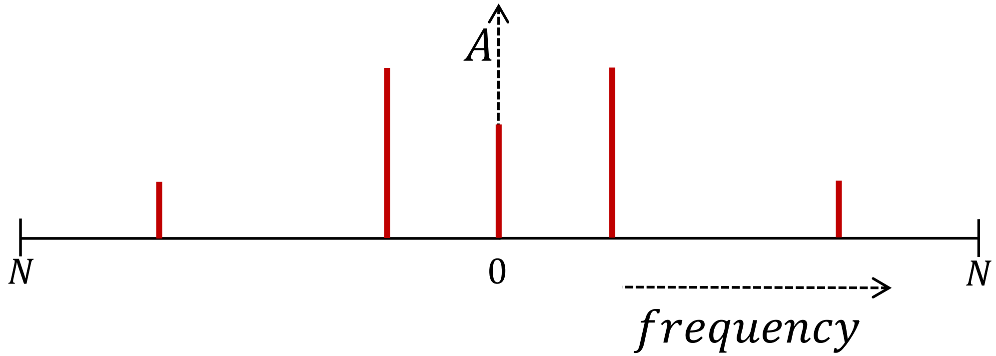
Friedel pairs have the same amplitude, but differ in phase:

on an Argand diagram they are reflected across the real axis



Fourier transform

(simple 1D functions)

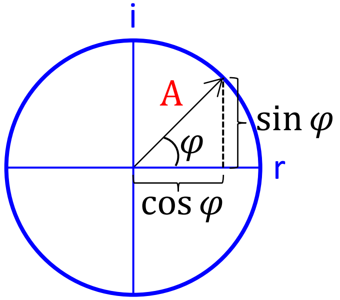


Fourier transform:

- strictly, a plot of the Fourier transform representing frequency vs amplitude corresponds to the amplitude spectrum of the real space function, as the phase components of the Fourier transform are omitted

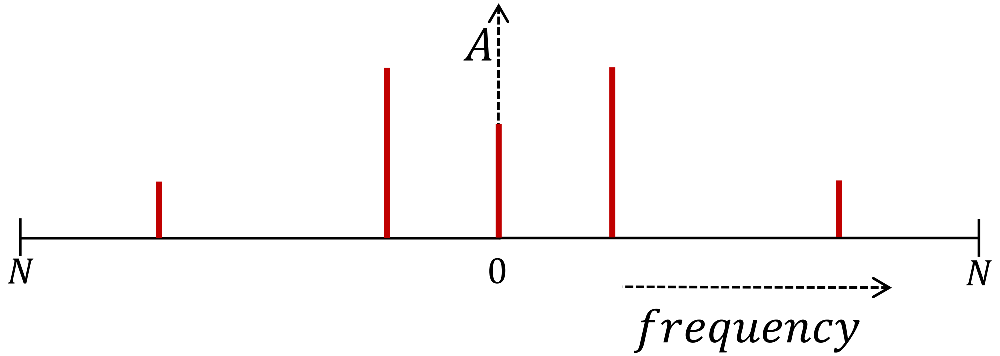
each Fourier term corresponds to a sine wave, that can be represented as a complex number defined by an amplitude and phase

$$e^{i\varphi} = A(cos\varphi + isin\varphi)$$



Fourier transform

(simple 1D functions)



Fourier transform:

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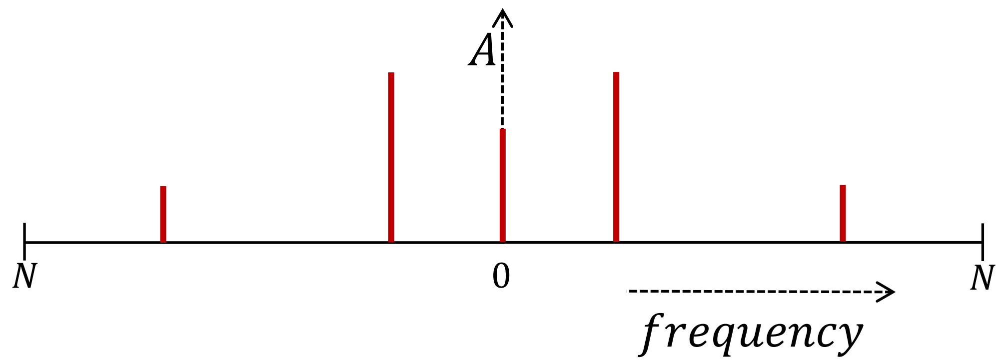
- it is common to a plot a Fourier transform as a function of intensity vs frequency ($\text{intensity} = \text{amplitude}^2$); such plot is known as a power spectrum

Fourier transform

(simple 1D functions)



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the Fourier transform of a function can be fully inverted :

$f(x)$ spatial domain → forward Fourier transform → $F(X)$ frequency domain → inverse Fourier transform → $f(x)$ spatial domain

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Fourier transform of images

Every (monochromic) image is a 2D function of space (x,y) vs brightness (b)

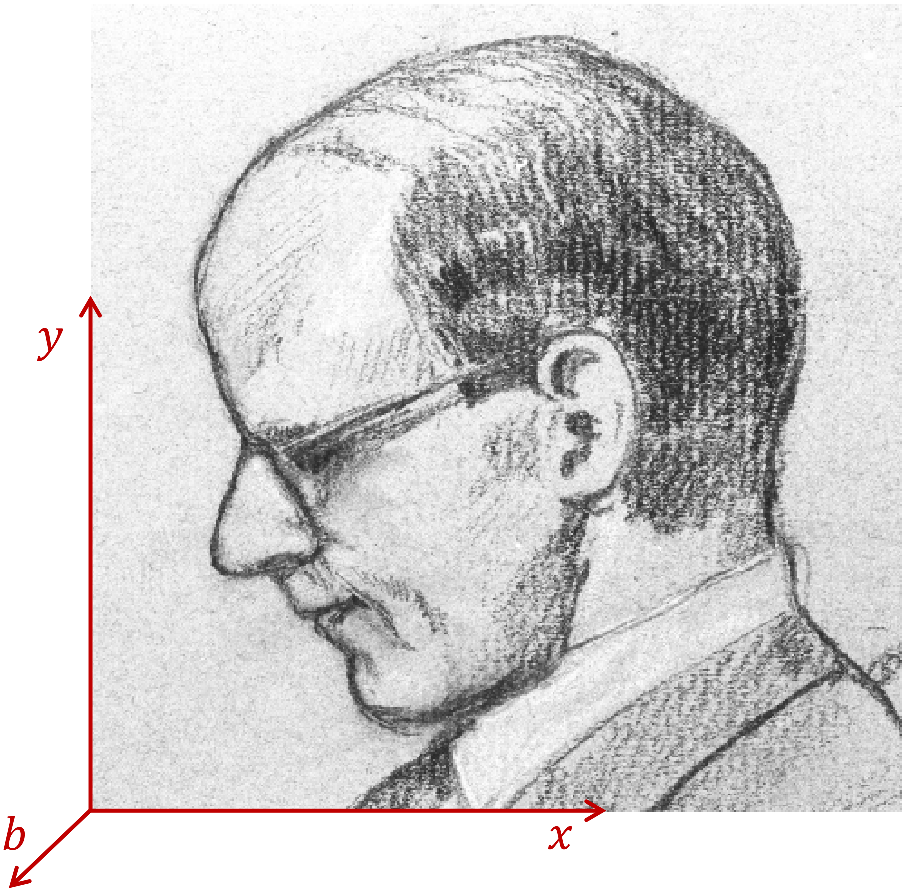
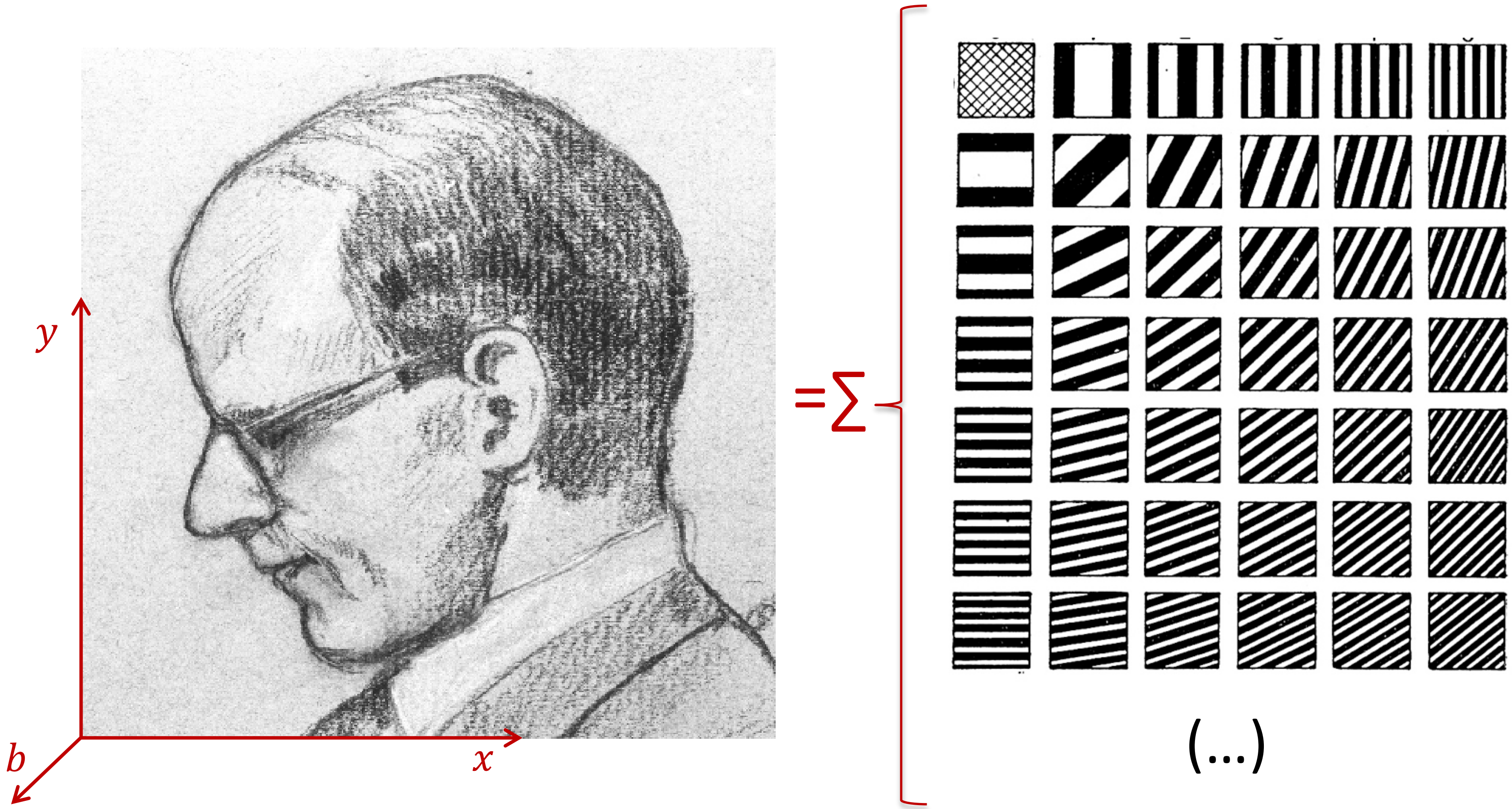


Image of Max Perutz provided by Tony Crowther (adapted from Sjors' slides)

Fourier transform of images

the Fourier transform decomposes a 2D image into a series of 2D sine waves



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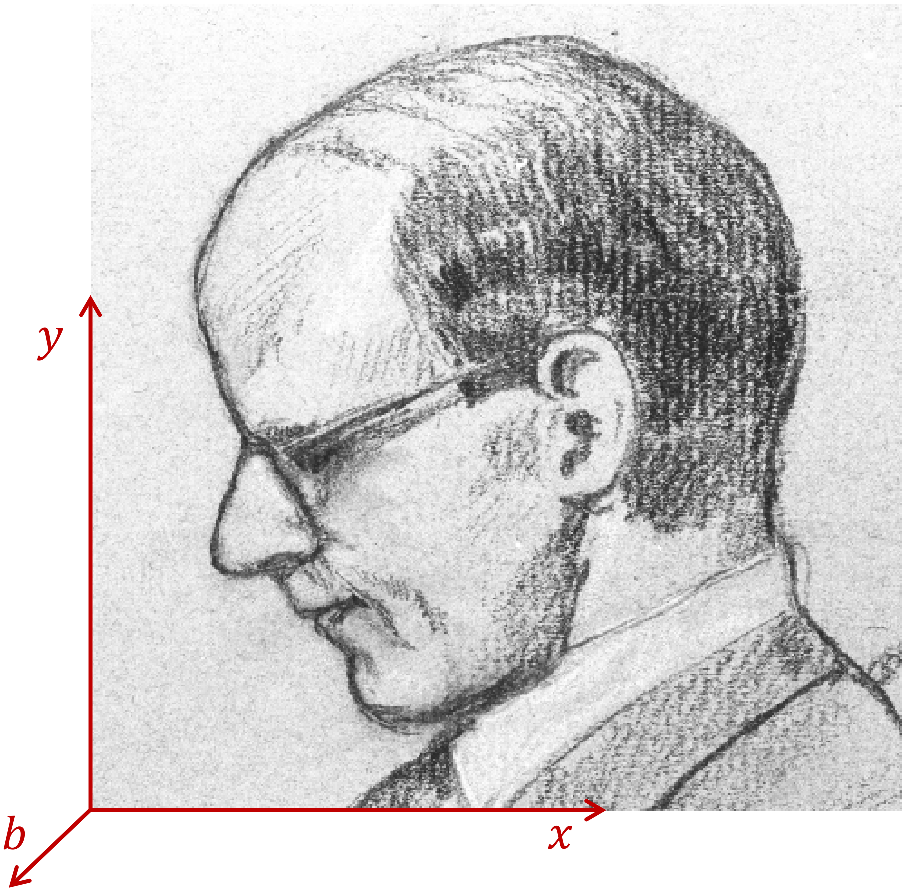

the same applies for 3D volumes
(or any n-D waveform)

2D image $\longrightarrow$ $\sum$ (2D sinusoids)
3D volume $\longrightarrow$ $\sum$ (3D sinusoids)
n-D function $\xrightarrow[\text{Fourier transform}]{}$ $\sum$ (n-D sinusoids)

$$F(X,Y) = \int_{-\infty}^{\infty} F(x,y) e^{-i2\pi(xX+yY)} dxdy$$

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Fourier transform of images

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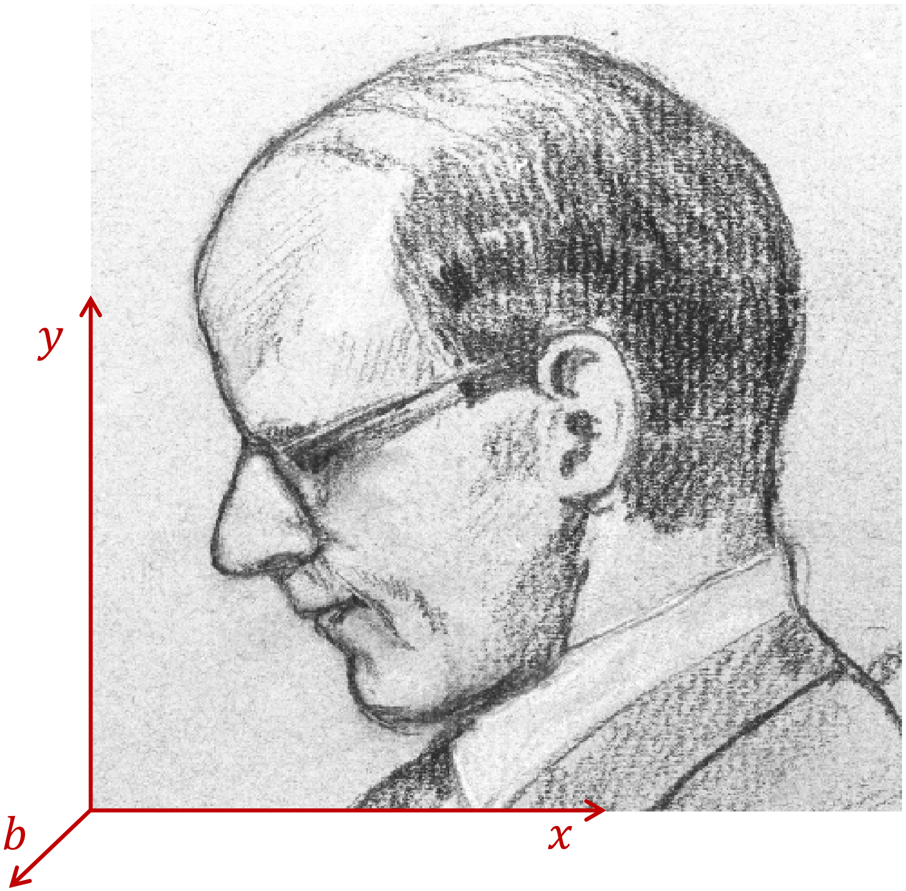


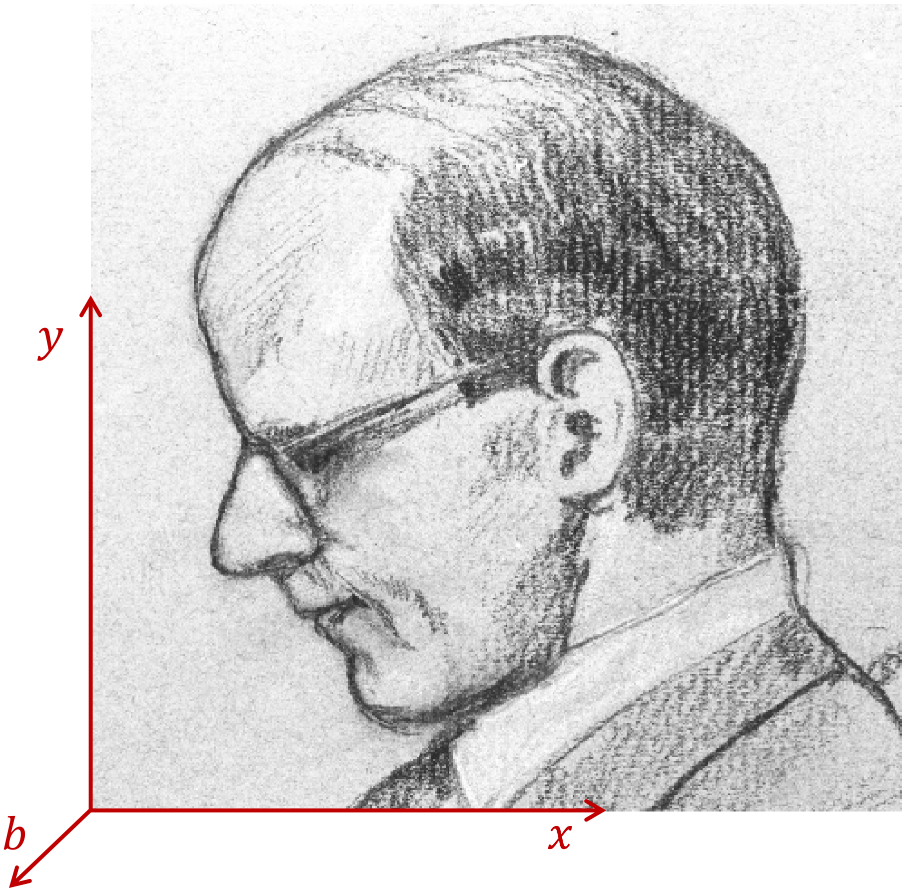
Fourier transform of a digital image:

encodes all the spatial frequencies present in an image, from zero (i.e. no modulation) to N (Nyquist frequency)

Image of Max Perutz provided by Tony Crowther (adapted from Sjors' slides)

Fourier transform of images

the Fourier transform decomposes a 2D image into a series of 2D sine waves



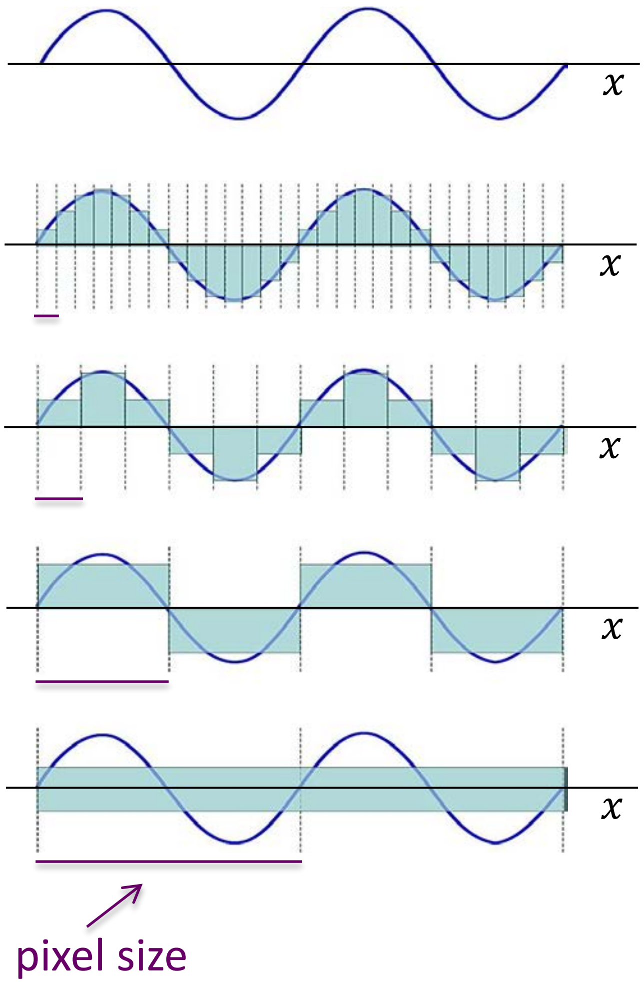
digital image - not continuous

- its intensity function is only known at discrete points:
  the image pixels

Image of Max Perutz provided by Tony Crowther (adapted from Sjors' slides)

Fourier transform of images

the Fourier transform decomposes a 2D image into a series of 2D sine waves

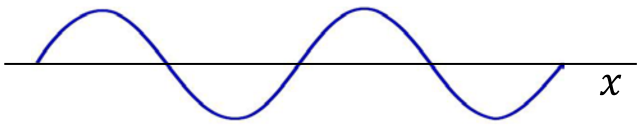


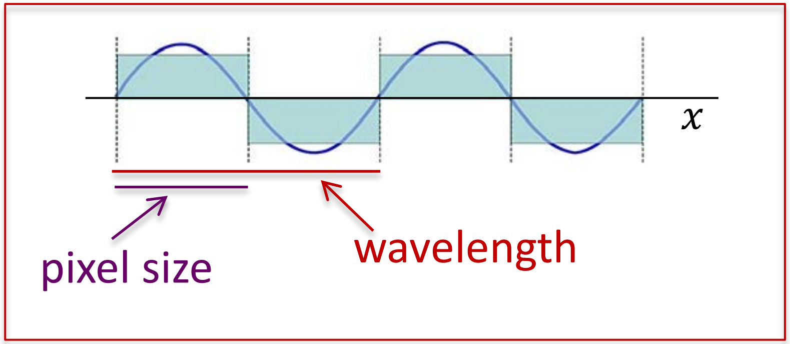
fine sampling (small pixel size compared with the wavelength) results in good representation of the wave

sampling too coarse to represent the wave

Fourier transform of images

the Fourier transform decomposes a 2D image into a series of 2D sine waves

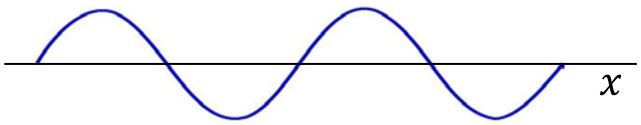


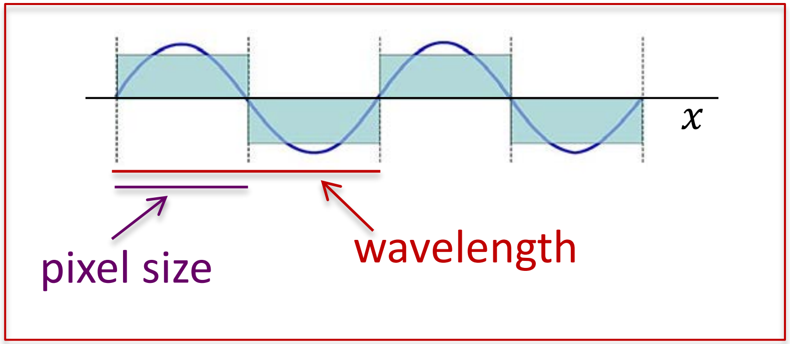


a waveform can only be decomposed into Fourier components with wavelengths that are at least 2x the sampling rate (pixel size)

Fourier transform of images

the Fourier transform decomposes a 2D image into a series of 2D sine waves



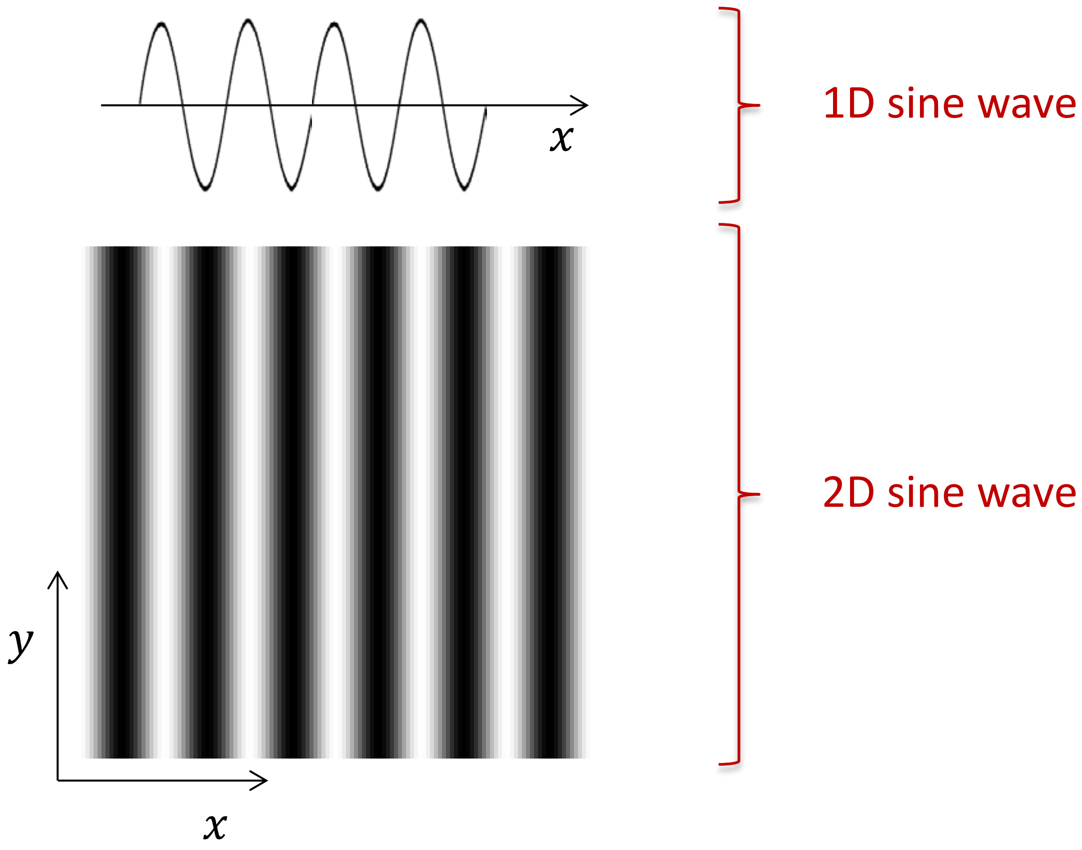


A waveform represented with a pixel size $=\upsilon$ can only be decomposed into Fourier components with wavelengths $\geq 2\upsilon$

½ υ is the Nyquist frequency

Nyquist frequency is the highest spatial frequency that can be encoded in a digital image

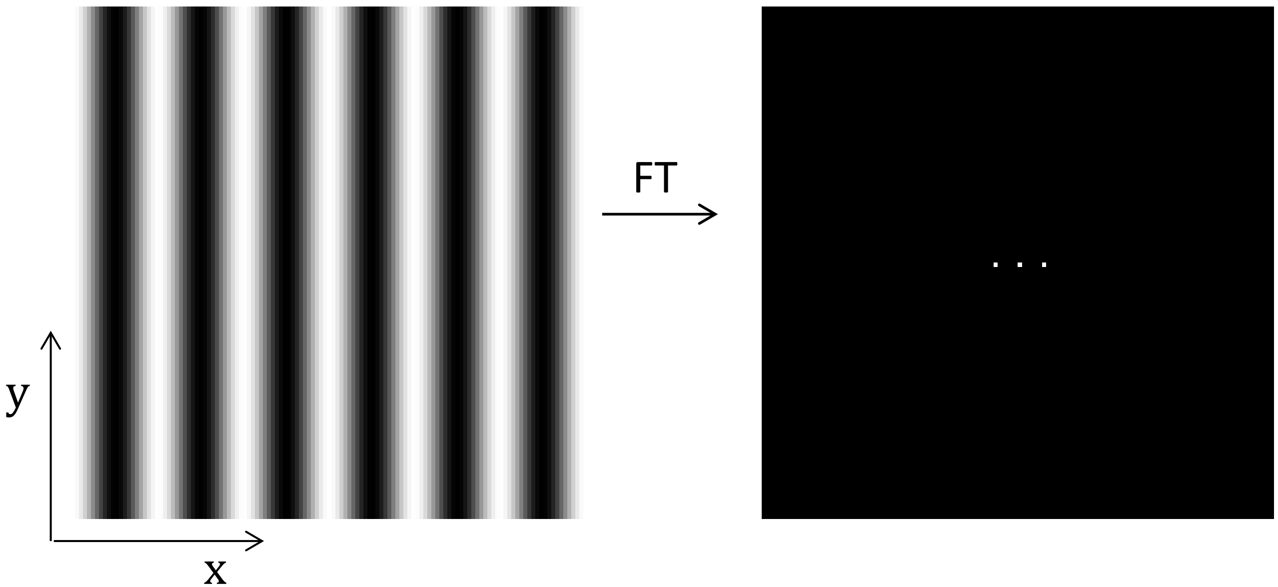
Fourier transform of images



Fourier transform of images

(some properties)

brightness image $f(x,y)$ | amplitude spectrum $F(X,Y)$



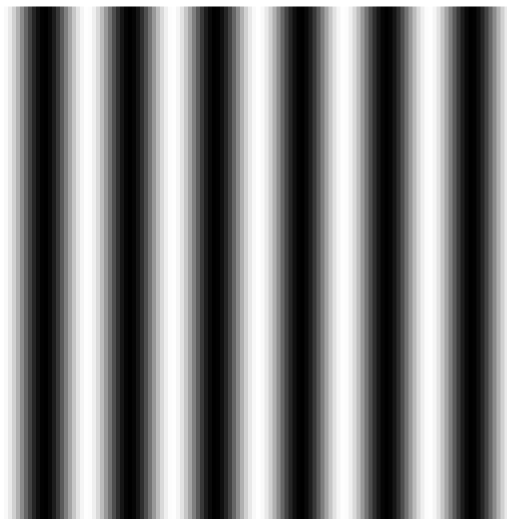
$$F(X,Y) = \int_{-\infty}^{\infty} F(x,y) e^{-i2\pi(xX+yY)} dxdy$$

Fourier transform of images

(some properties)

brightness image

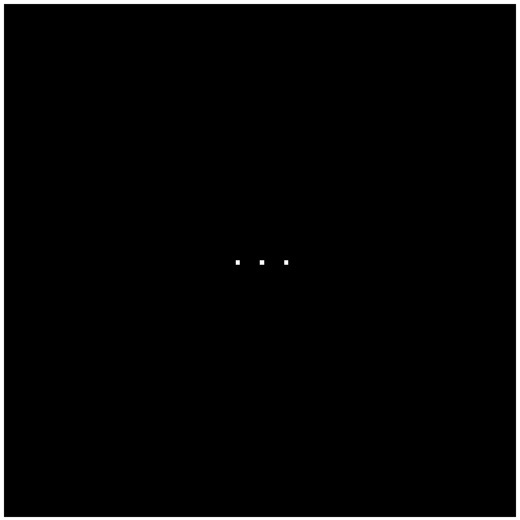
$f(x,y)$



FT →

amplitude spectrum

$F(X,Y)$



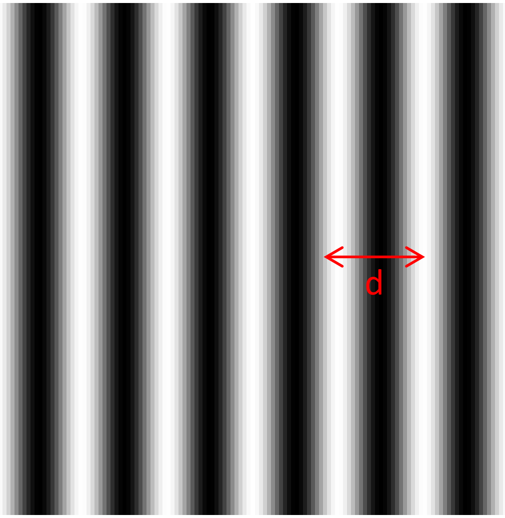
in image processing of cryo-EM images, frequency corresponds to spacing and it is normally described in $(1/\text{Å})$ units

Fourier transform of images

(some properties)

brightness image

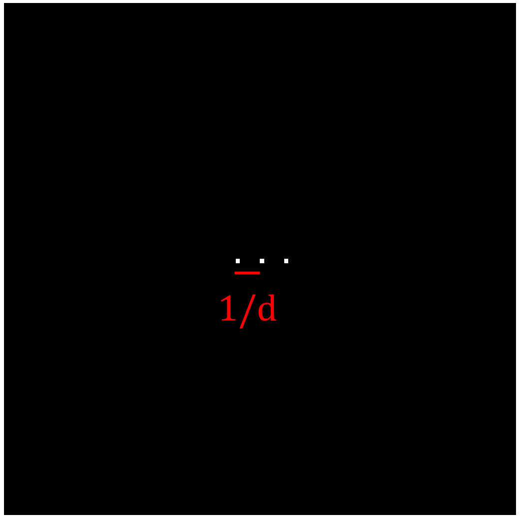
f(x,y)



FT →

amplitude spectrum

F(X,Y)



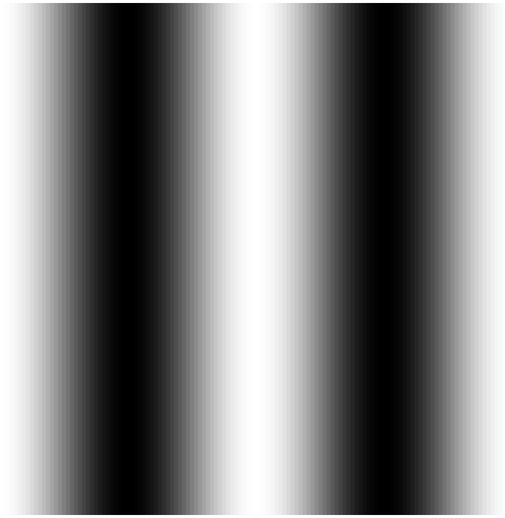
a brightness image with closely spaced features (high frequency information) will result in a amplitude spectrum with wide spacings

- Fourier space is also known as reciprocal space -

Fourier transform of images

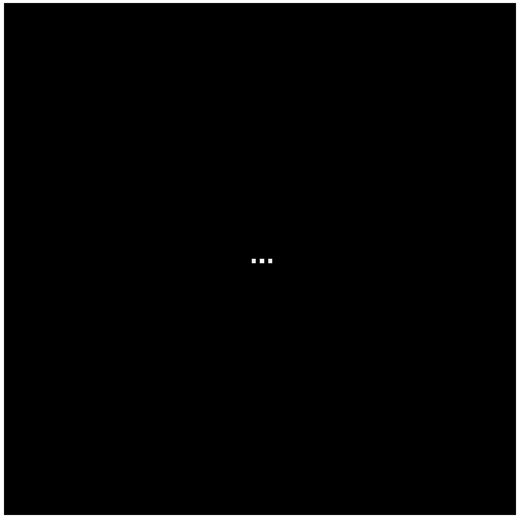
(some properties)

brightness image
f(x,y)



FT →

amplitude spectrum
F(X,Y)



a brightness image with closely spaced features (high frequency information) will result in a amplitude spectrum with wide spacings

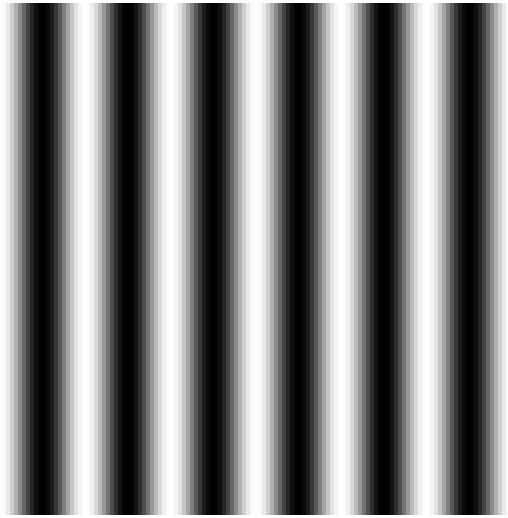
- Fourier space is also known as reciprocal space -

Fourier transform of images

(some properties)

brightness image

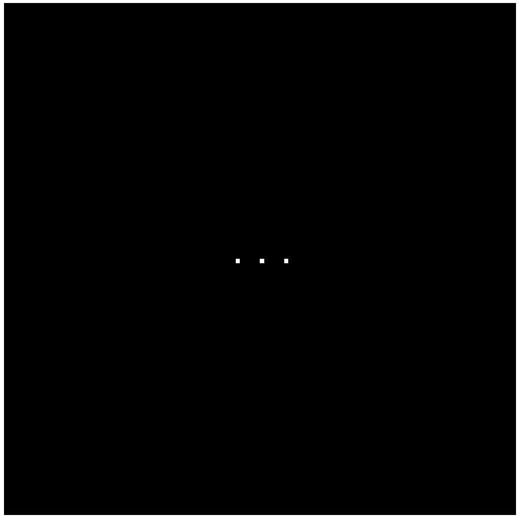
f(x,y)



FT →

amplitude spectrum

F(X,Y)



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Fourier transform of images

(some properties)

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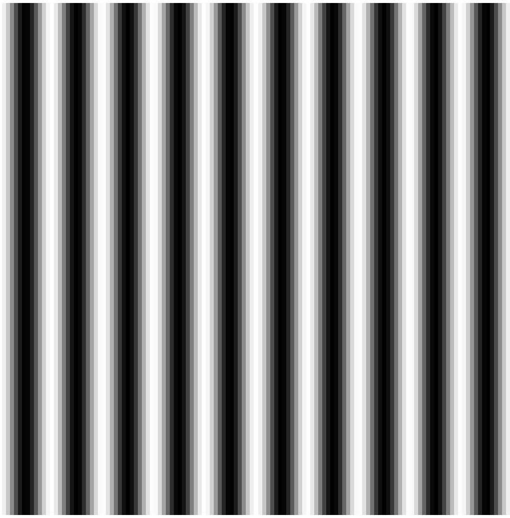

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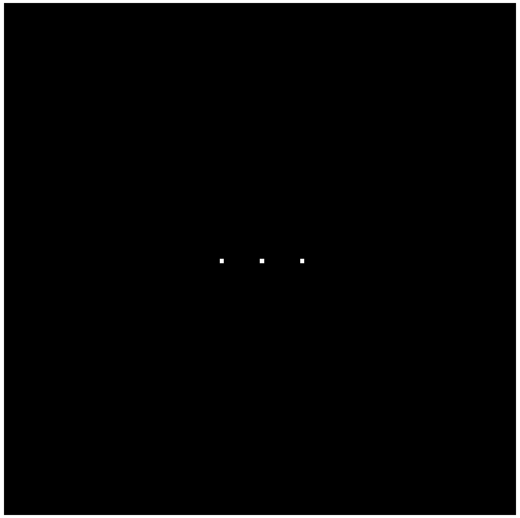
amplitude spectrum
F(X,Y)



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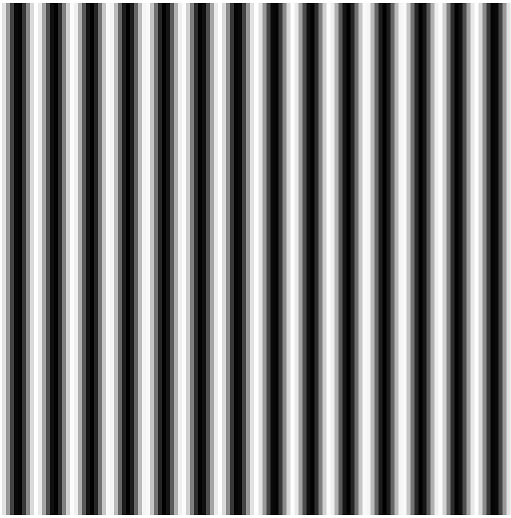
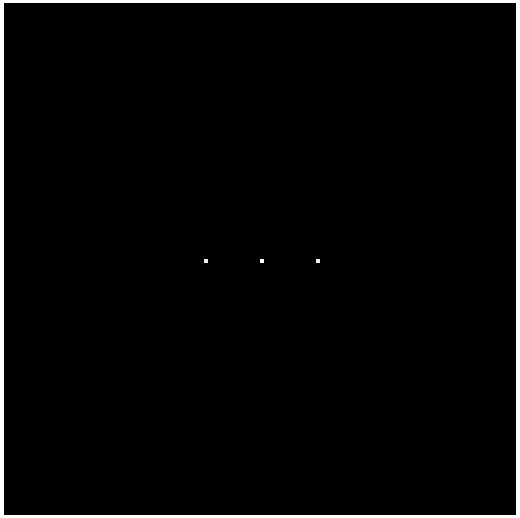


FT →

amplitude spectrum
F(X,Y)



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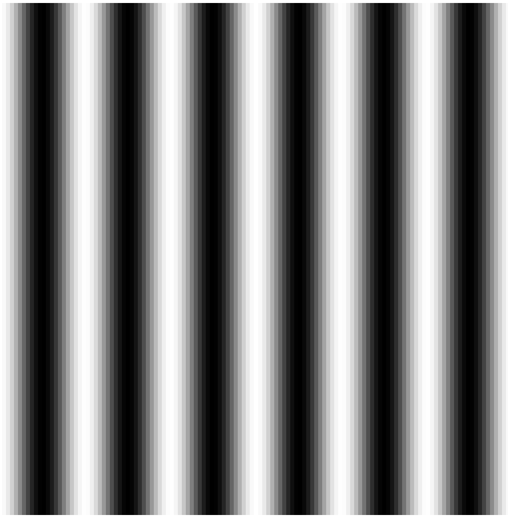
Fourier transform of images

(some properties)

brightness image

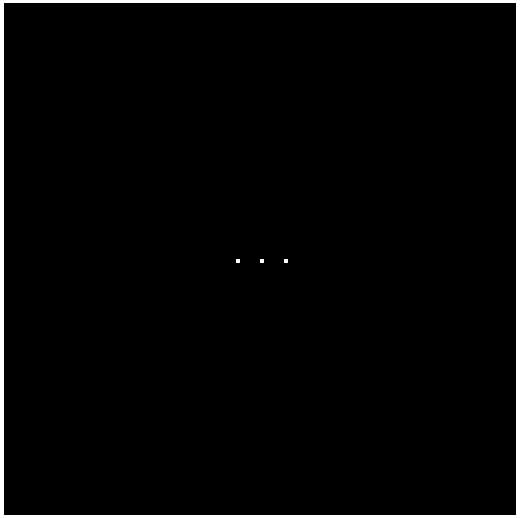
$f(x,y)$



FT $\longrightarrow$

amplitude spectrum

$F(X,Y)$



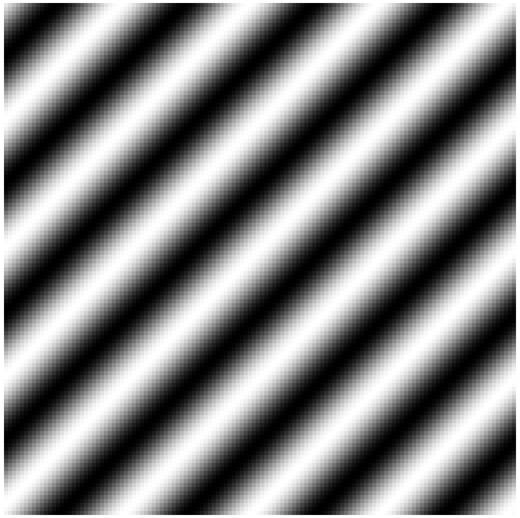
a rotation of the real space image results in a rotation of its transform

Fourier transform of images

(some properties)

brightness image

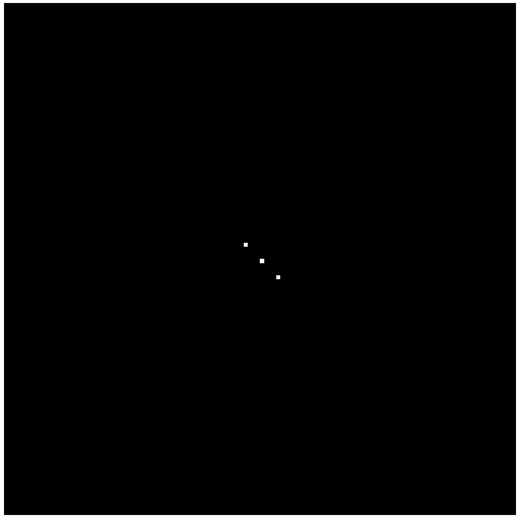
f(x,y)



FT →

amplitude spectrum

F(X,Y)



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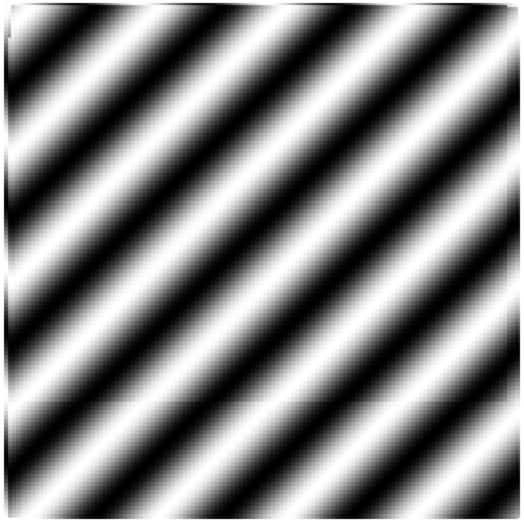
Fourier transform of images

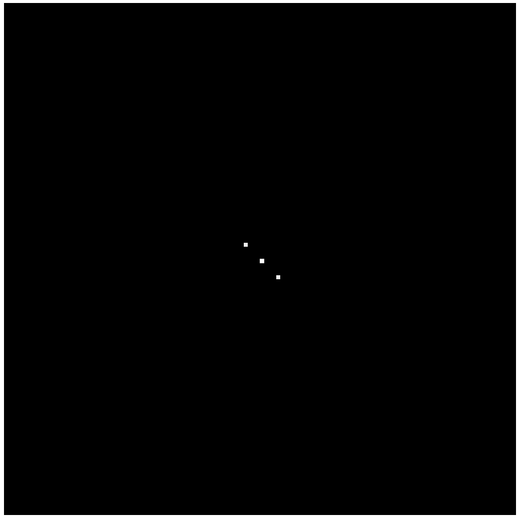
(some properties)

brightness image
f(x,y)



FT →

amplitude spectrum
F(X,Y)



a rotation of the real space image results in a rotation of its transform

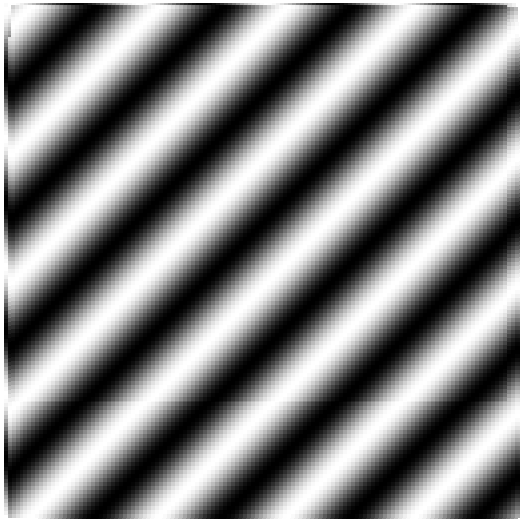
a translation of the real space image will produce a phase shift of its Fourier terms, with no observed changes in the corresponding amplitude and power spectra
(where the phase components of the Fourier transform are not represented)

Fourier transform of images

(some properties)

brightness image

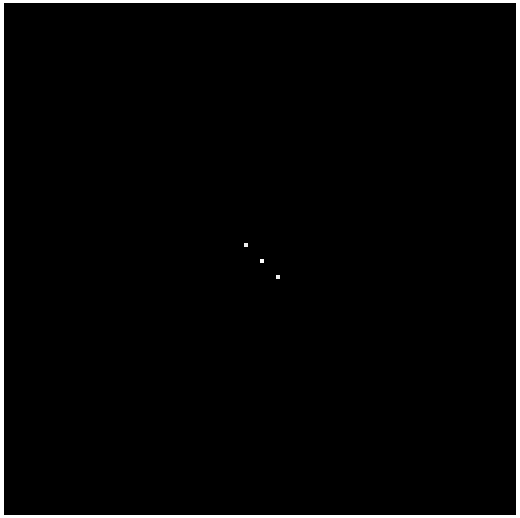
f(x,y)



FT →

amplitude spectrum

F(X,Y)



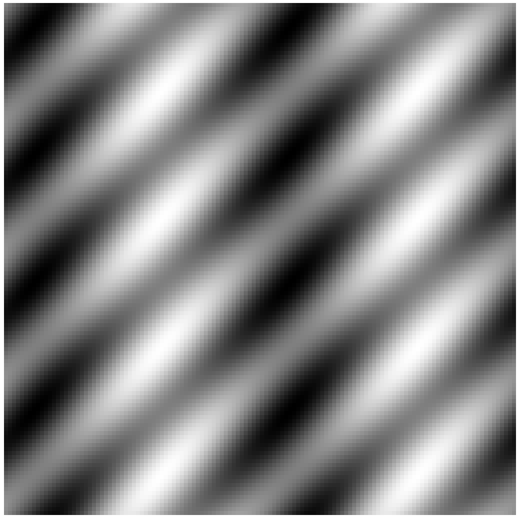
simple image formed by a single sinusoidal component

Fourier transform of images

(some properties)

brightness image

$f(x,y)$



FT →

amplitude spectrum

$F(X,Y)$

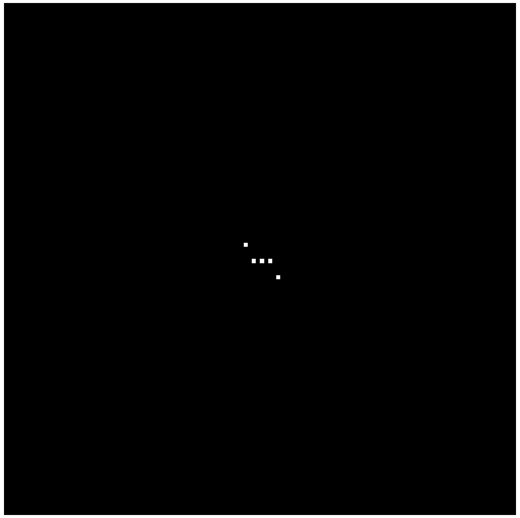


image formed by two sinusoidal components

Fourier transform of images

(some properties)

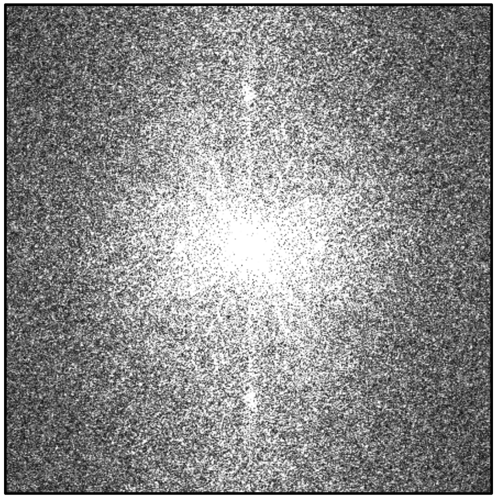
brightness image

$f(x,y)$

FT →

amplitude spectrum

$F(X,Y)$

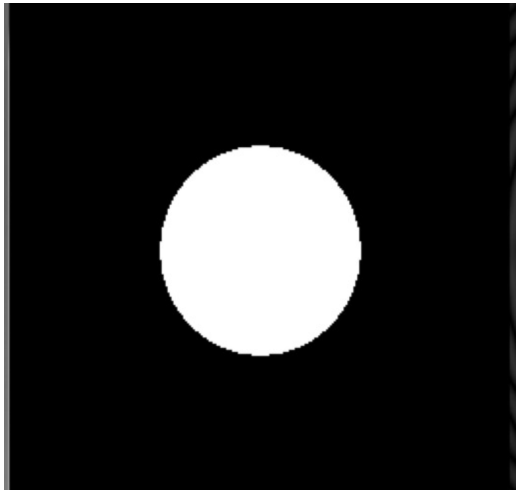


images formed by multiple sinusoidal components

Fourier transform of images

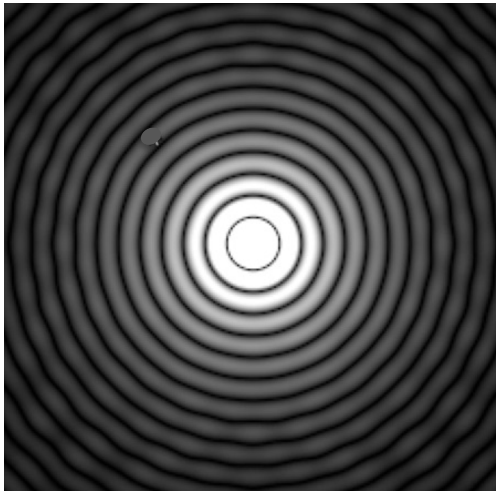
(some properties)

brightness image
f(x,y)



FT →

amplitude spectrum
F(X,Y)

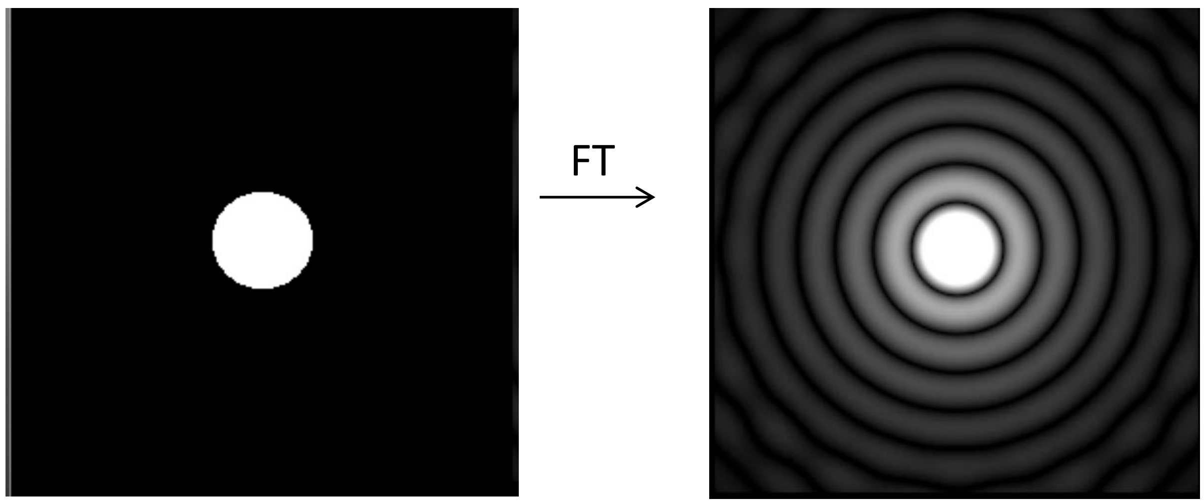


the Fourier transform of a sharp aperture results in an amplitude spectrum formed of “ripples”

Fourier transform of images

(some properties)

brightness image $f(x,y)$ — amplitude spectrum $F(X,Y)$



the Fourier transform of a sharp aperture results in an amplitude spectrum formed of "ripples"

Fourier transform of images

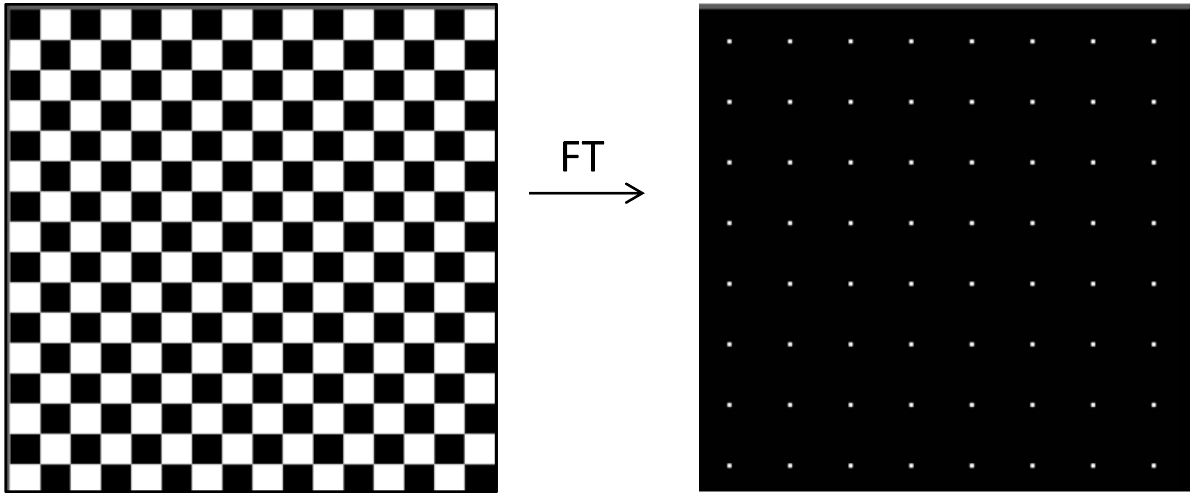
(some properties)

brightness image

f(x,y)

amplitude spectrum

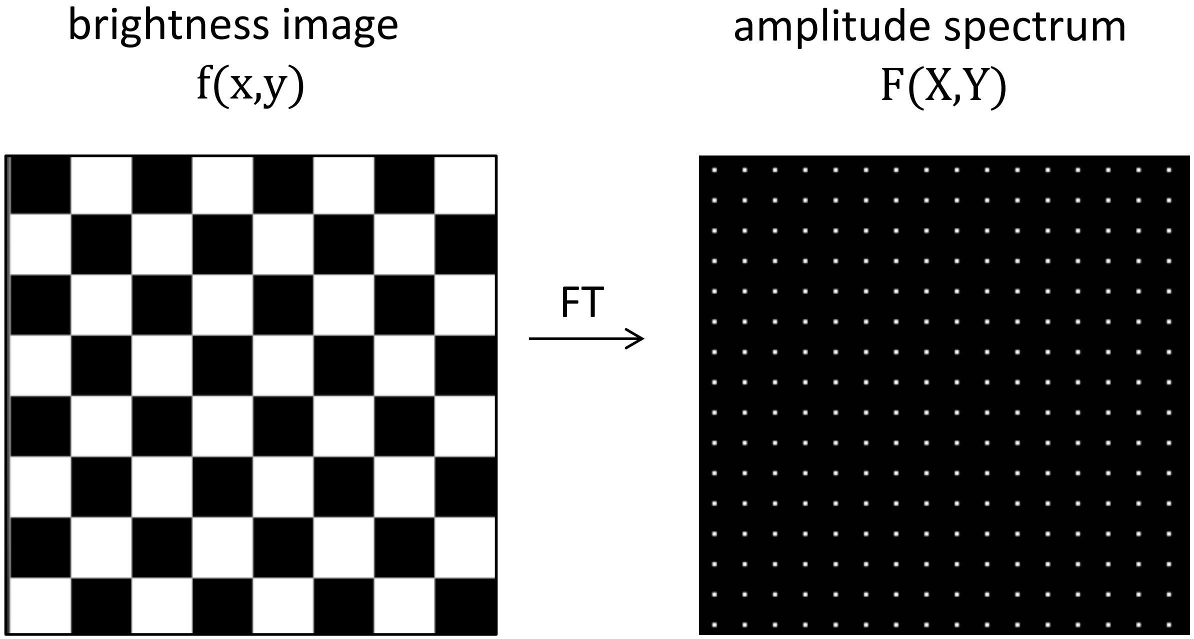
F(X,Y)



the Fourier transform of a lattice pattern is another lattice

Fourier transform of images

(some properties)



the Fourier transform of a lattice pattern is another lattice

- Agenda -

<u>Overview of:</u>

- Introduction to Fourier analysis
    - Sine waves
    - Fourier transform (simple examples of 1D functions)
    - Fourier transform of images
    - Why is it useful for image processing?
- Image formation
    - Weak phase approximation
- The contrast transfer function

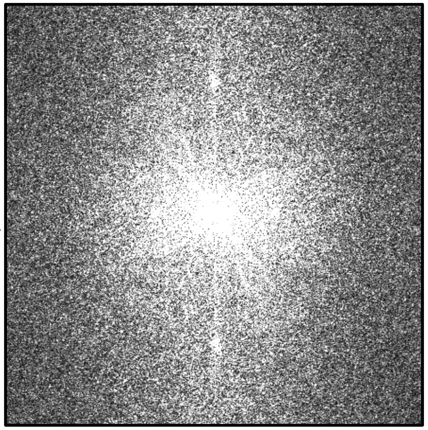
Fourier analysis

(why is it useful for image processing)

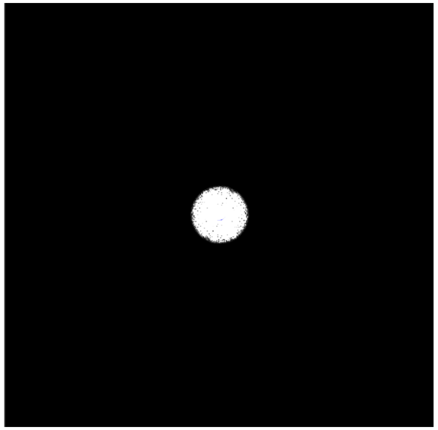
brightness image
$f(x,y)$

FT $\rightarrow$

Fourier transform
$F(X,Y)$



mask $\rightarrow$



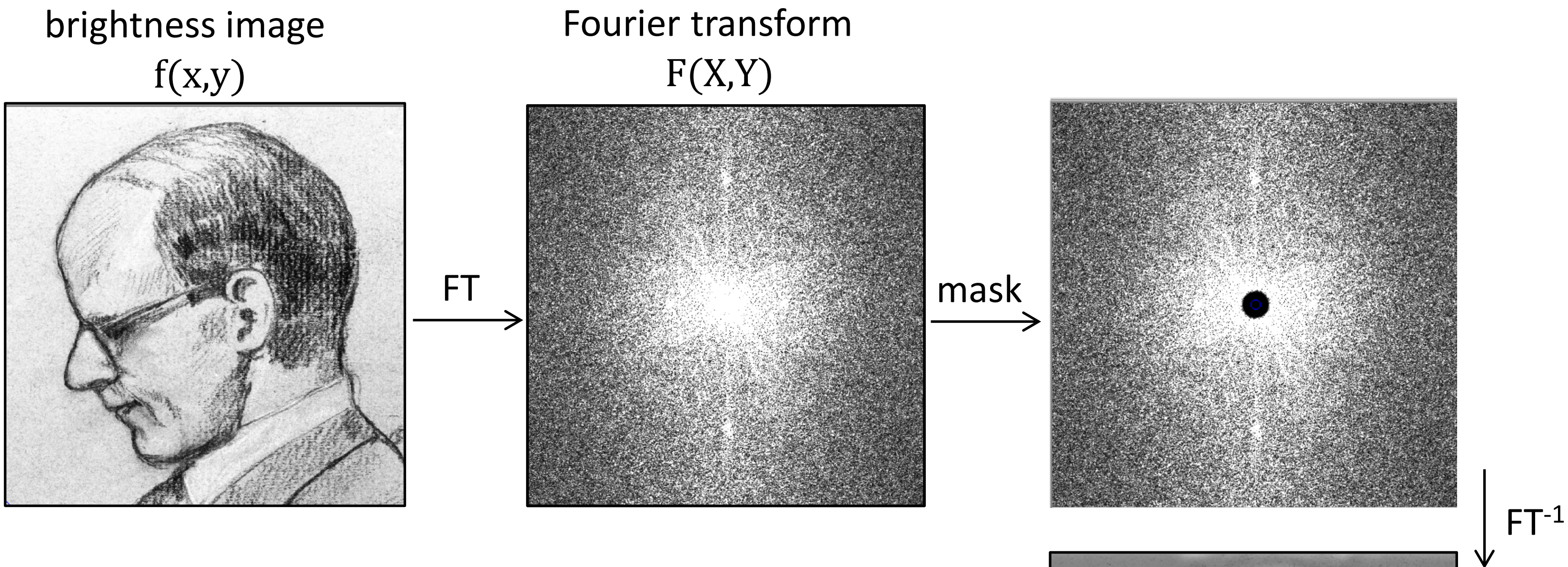
$\downarrow$ FT^{-1}

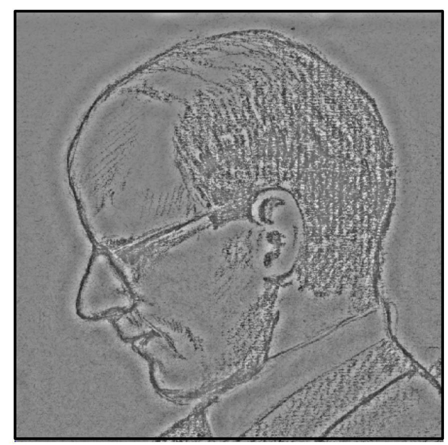
low-pass filtering

masking the high frequencies of the Fourier transform (only low frequencies pass the filter) results in the removal of fine detail in the inverse Fourier transform

Fourier analysis

(why is it useful for image processing)





high-pass filtering

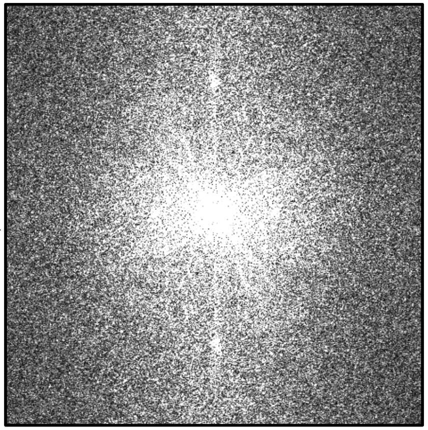
masking the low frequencies of the Fourier transform (only high frequencies pass the filter)
results in the removal of the coarse features in the inverse Fourier transform

Fourier analysis
(why is it useful for image processing)

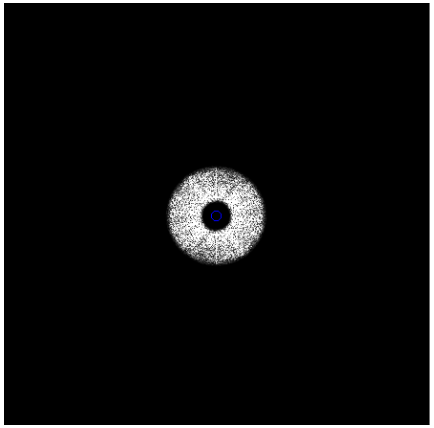
brightness image
f(x,y)

FT →

Fourier transform
F(X,Y)



mask →



↓ FT^{-1}

band-pass filtering

masking both the higher and lower frequencies of the Fourier transform (only a band of frequencies pass the filter)

Fourier analysis

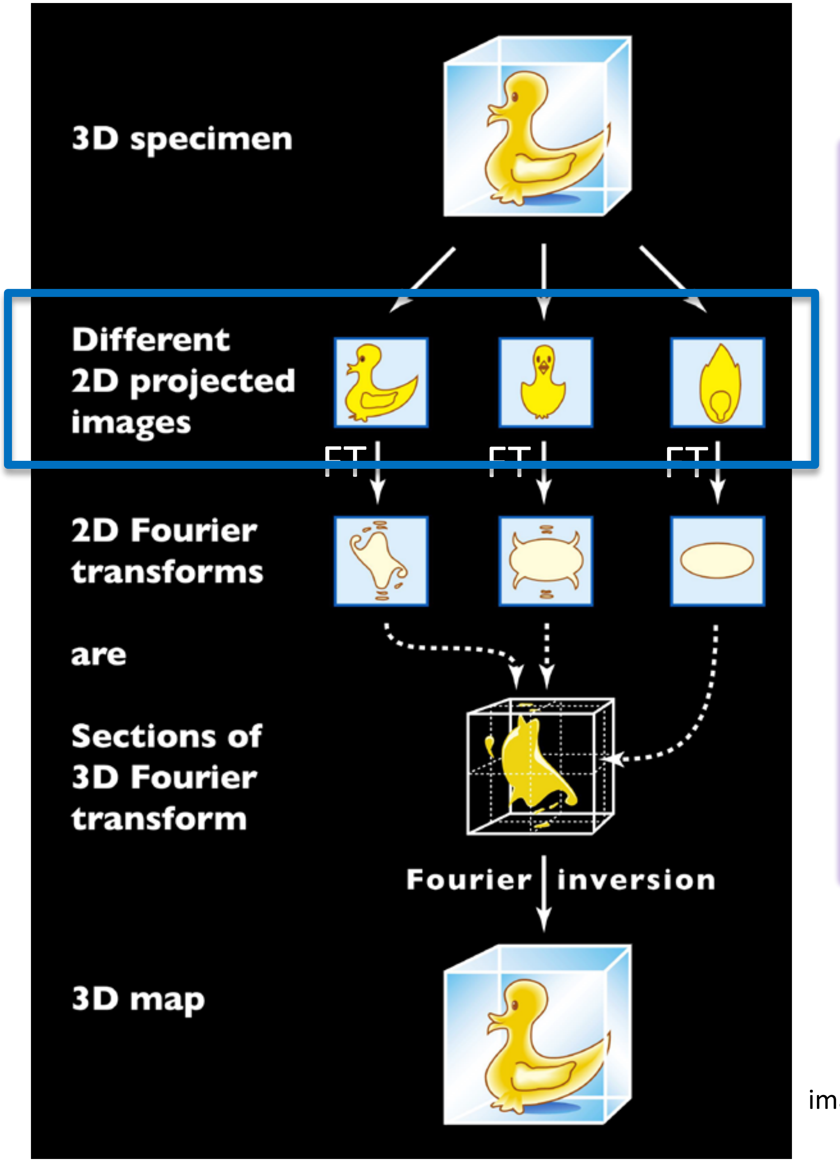
(why is it useful for image processing)

projection-slice theorem,
central slice theorem or Fourier slice theorem

- The F.T. of a 2D projection of a 3D object is a central slice through the 3D F.T. of that object
- Vector normal to slice = projection direction

Fourier analysis

(why is it useful for image processing)



projection-slice theorem,
central slice theorem or Fourier slice theorem

- the Fourier transform of a 2D projection of a 3D object is a central slice through the 3D Fourier transform of that object
- vector normal to slice = projection direction
- 3D reconstruction from 2D projection images ("our recorded images")

image from: Baker and Henderson; *International Tables for Crystallography* (2012) vol. F, Chapter 19.6 - 593

Fourier analysis

(why is it useful for image processing)

- faster calculations -

Fourier analysis

(why is it useful for image processing)

- basic concepts of cross-correlation -

$$ccf = \int f(x)g(x-t)dx$$

cross correlation is a measure of similarity between two functions (like our images) over a range of relative shifts

identifies the similarity between two functions as one function (“template”) is shifted over the other function

much faster operation in Fourier space

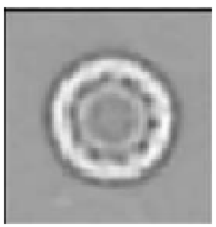
$$ccf = \underbrace{iFT}_{\text{inverse Fourier transform}}\left(F(x) \times \overline{G}(x)\right)$$

inverse Fourier transform — *complex conjugate of* $G(x)$

Fourier analysis

(why is it useful for image processing)

- basic concepts of cross-correlation -



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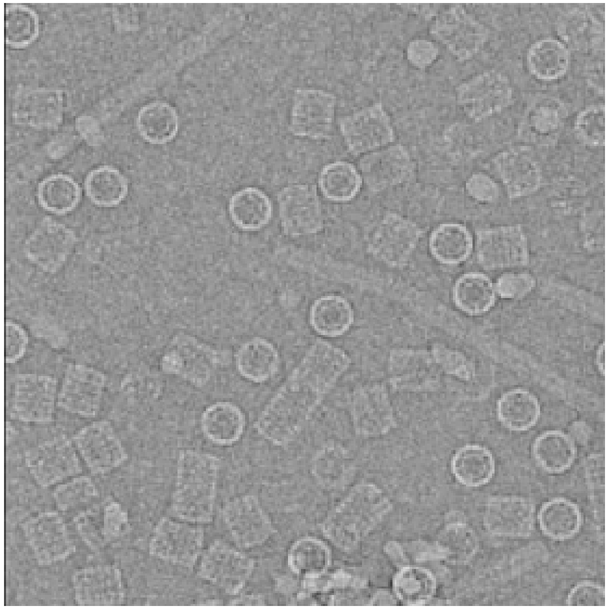

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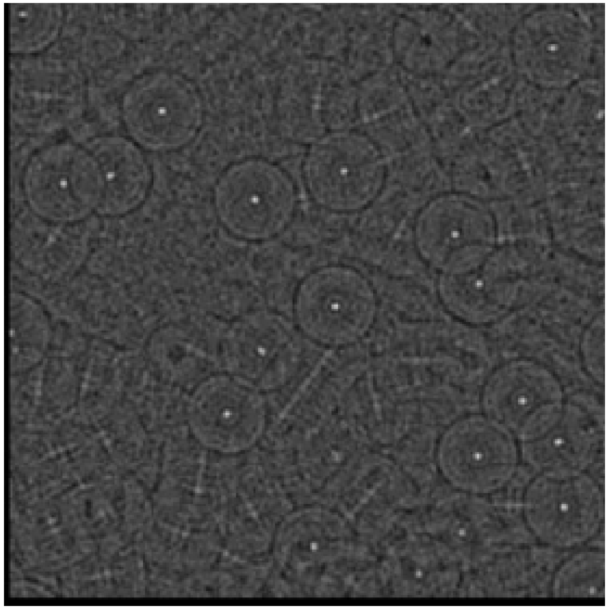


Fourier transform

Fourier transform

inverse Fourier transform

$F(x)$

$G(x)$

$\longrightarrow$

$F(x) \times \overline{G}(x)$

(adapted from Sjors' slides)

Fourier analysis

(why is it useful for image processing)

- basic concepts of cross-correlation -

Examples of usage of correlation in the processing of cryo-EM images include:

- particle picking
- alignment of images
- projection matching
- resolution estimates
- …

Fourier analysis

(why is it useful for image processing)

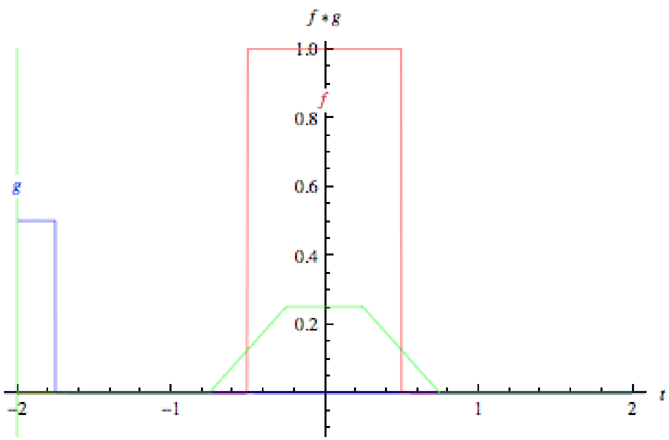
- basic concepts of convolution -

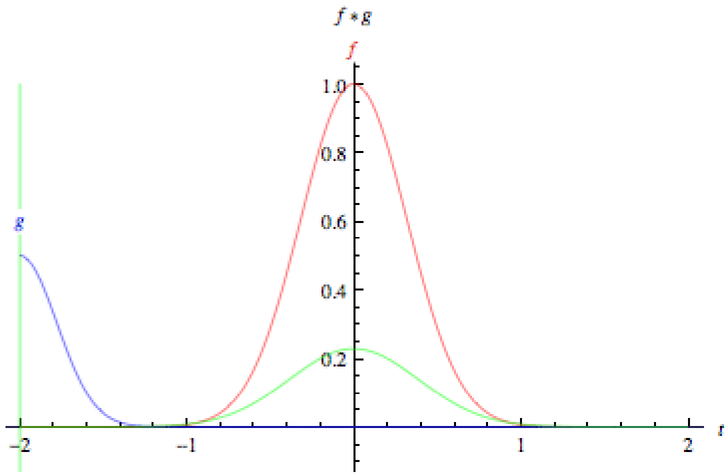
$$f(x) \otimes g(x) = \int f(x) g(t-x) dx$$

convolution of a function f(x) with a second function g(x) is the integral of their products after one function is reversed and shifted (by t)

expresses the amount of overlap of one function as it is shifted over another function

- it therefore "blends" one function with another -





(animations taken from: http://mathworld.wolfram.com/Convolution.html)

Fourier analysis

(why is it useful for image processing)

- basic concepts of convolution -

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expresses the amount of overlap of one function as it is shifted over another function

- it therefore "blends" one function with another -

much simpler operation in Fourier space

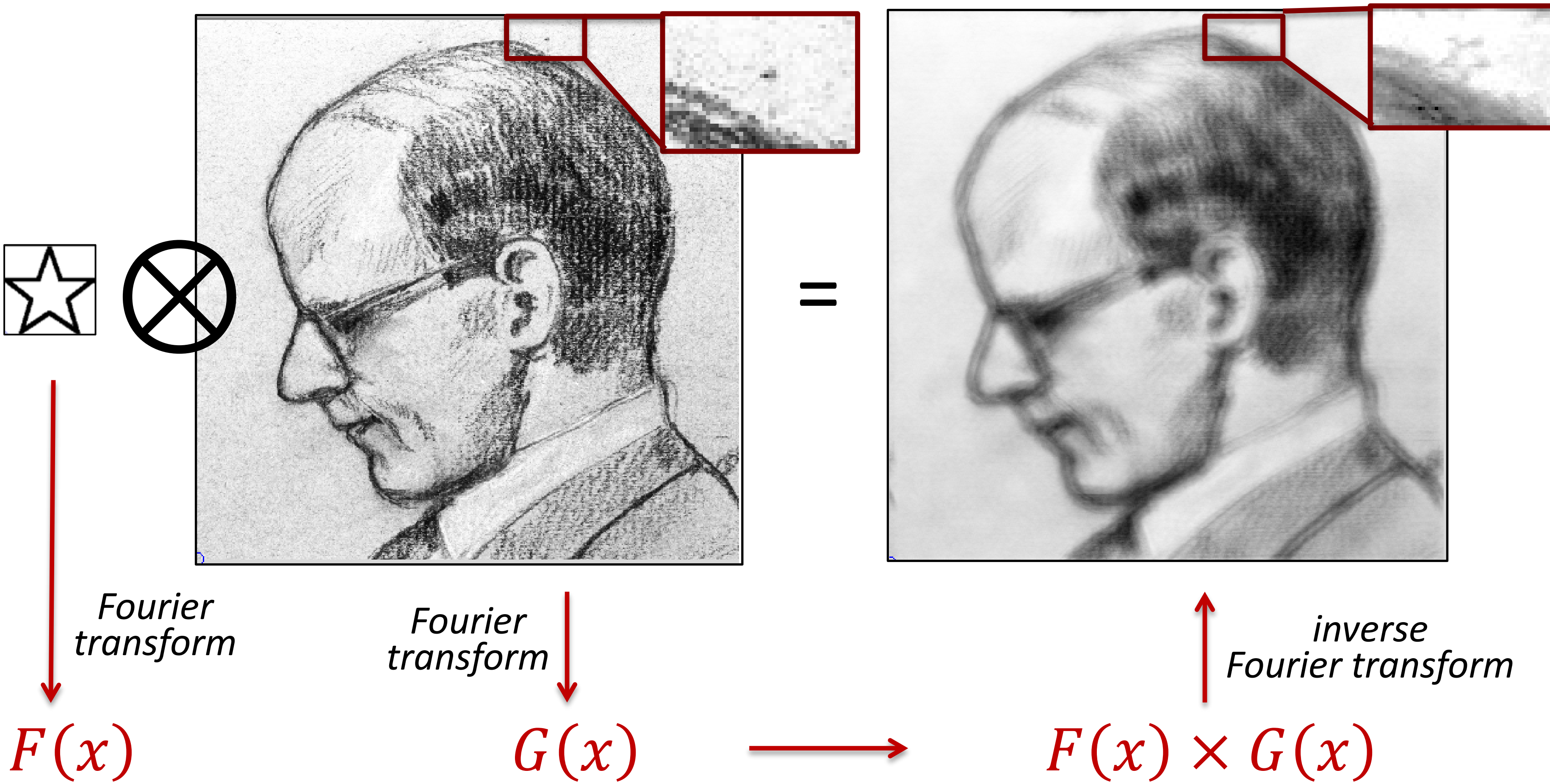
$$f(x) \otimes g(x) = \underbrace{iFT}_{\text{inverse Fourier transform}} (F(x) \times G(x))$$

inverse Fourier transform

Fourier analysis

(why is it useful for image processing)

- basic concepts of convolution -

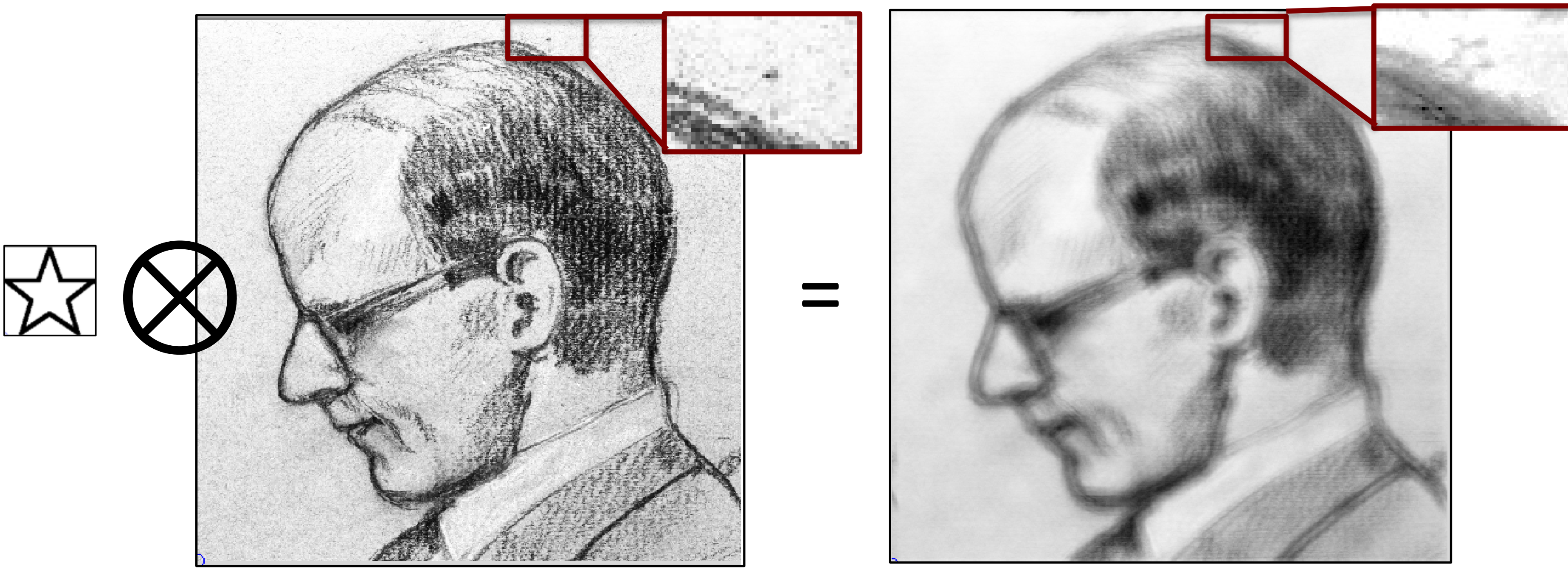


(adapted from Sjors' slides)

Fourier analysis

(why is it useful for image processing)

- basic concepts of convolution -



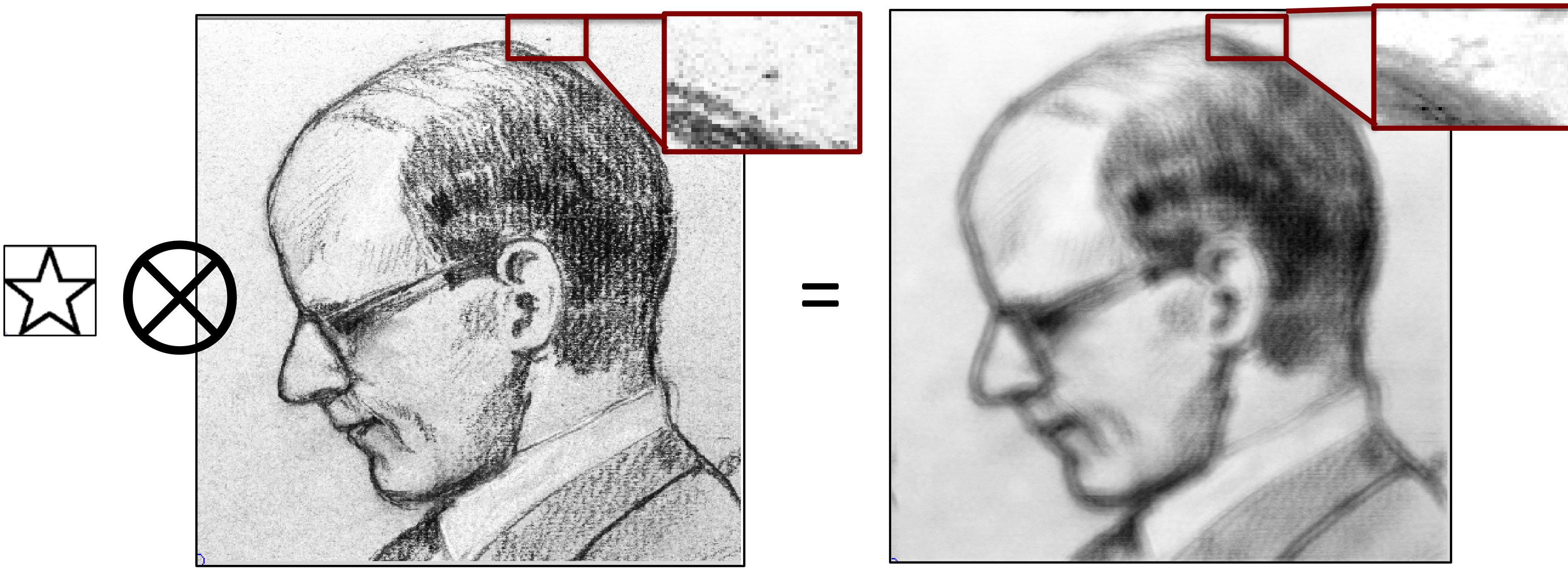
convolution combines two functions such that a copy of one function is placed at each point in space, weighted by the value of the second function at the same point

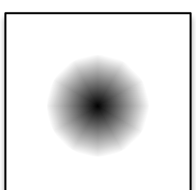
(adapted from Sjors' slides)

Fourier analysis

(why is it useful for image processing)

- basic concepts of convolution -





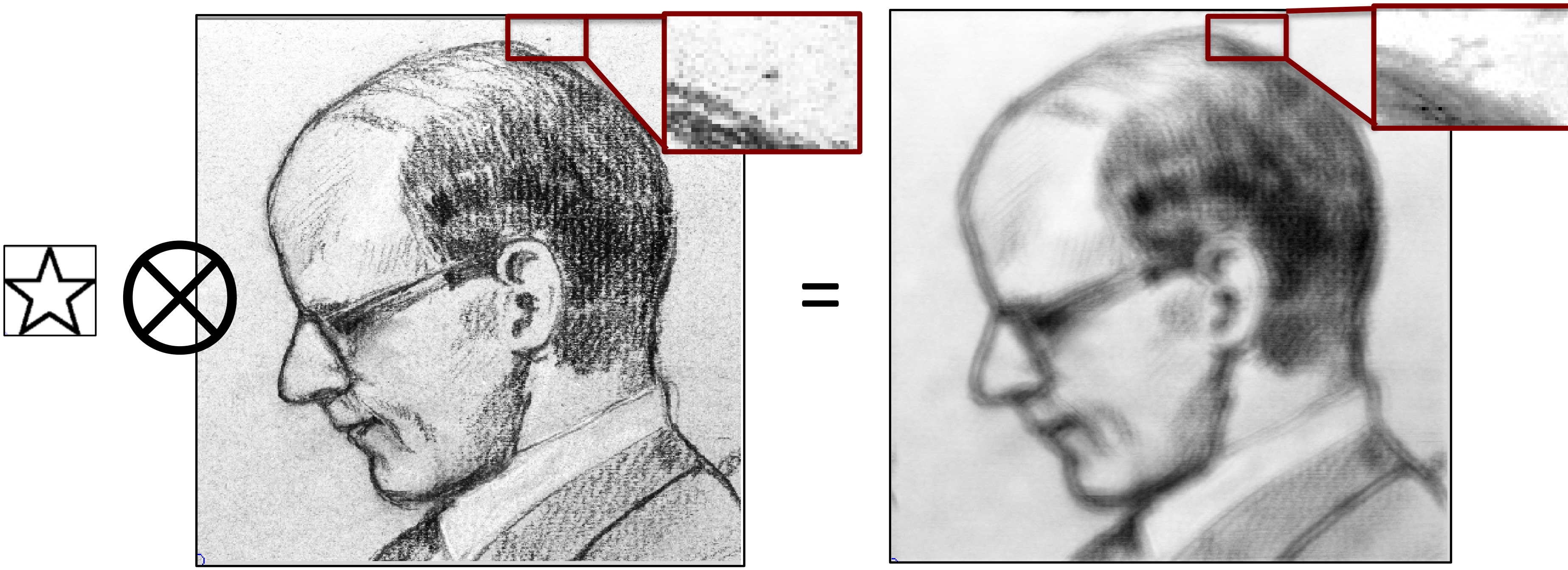
Point spread function - describes the response of an imaging system to a point source or point object (how an optical system sees a point)

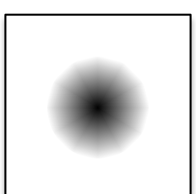
(adapted from Sjors' slides)

Fourier analysis

(why is it useful for image processing)

- basic concepts of convolution -





in cryo-EM, images are blurred by convolution with a point spread function arising from imperfections in the imaging system

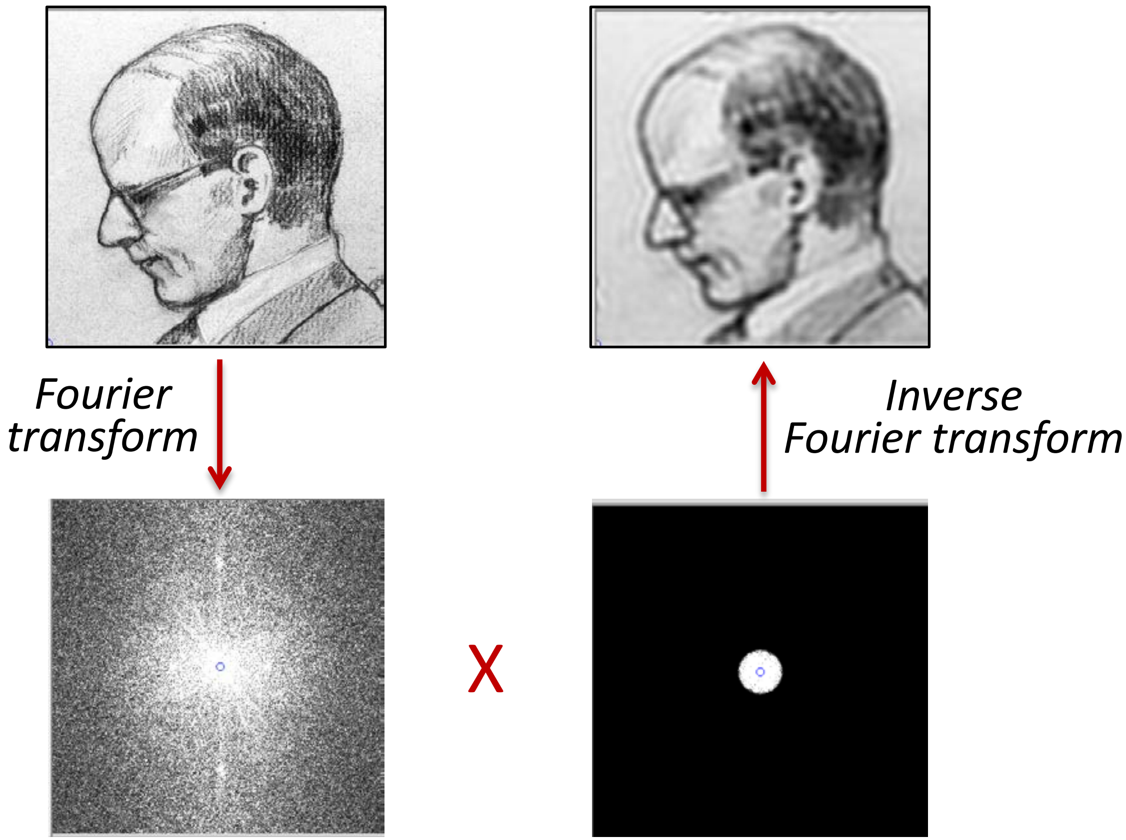
(adapted from Sjors' slides)

Fourier analysis

(why is it useful for image processing)

- basic concepts of convolution -

Fourier filtering is a convolution



(adapted from Sjors' slides)

Fourier analysis
(why is it useful for image processing)

- basic concepts of convolution -

Examples of usage of convolution on the image processing of cryo-EM images includes:

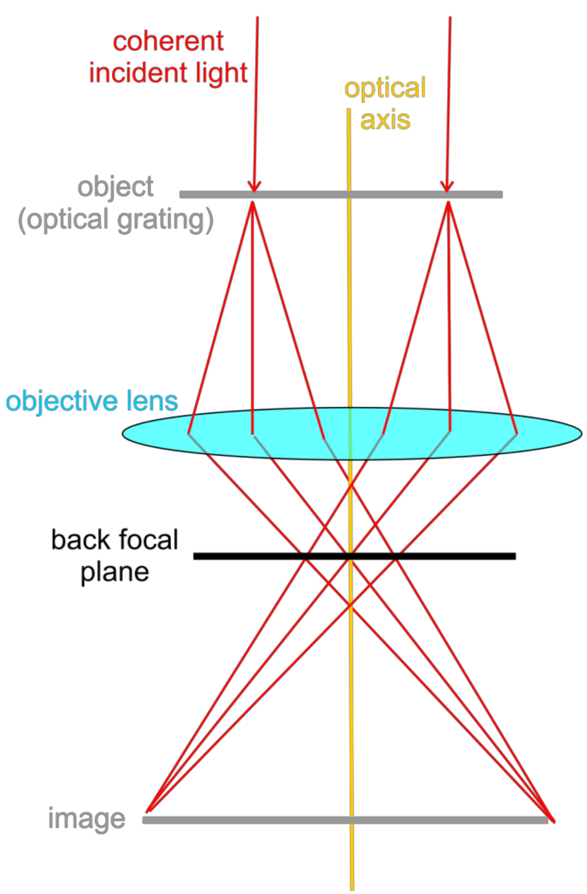
- Fourier filtering (as described earlier)
- to describe the point spread function of the optical system
- to describe the effects of the contrast transfer function
- …

Fourier analysis
(why is it useful for image processing)

in optics, the diffraction pattern obtained at the back focal plane corresponds to the power spectrum of the original object

Fourier analysis
(why is it useful for image processing)

in optics, the diffraction pattern obtained at the back focal plane corresponds to the power spectrum of the original object



object exit wave

↓ FT

diffraction pattern = image power spectrum

↓ FT^{-1}

image

the image in the projection chamber cannot be directly obtained mathematically from the diffraction pattern, as this contains no phase information

- Agenda -

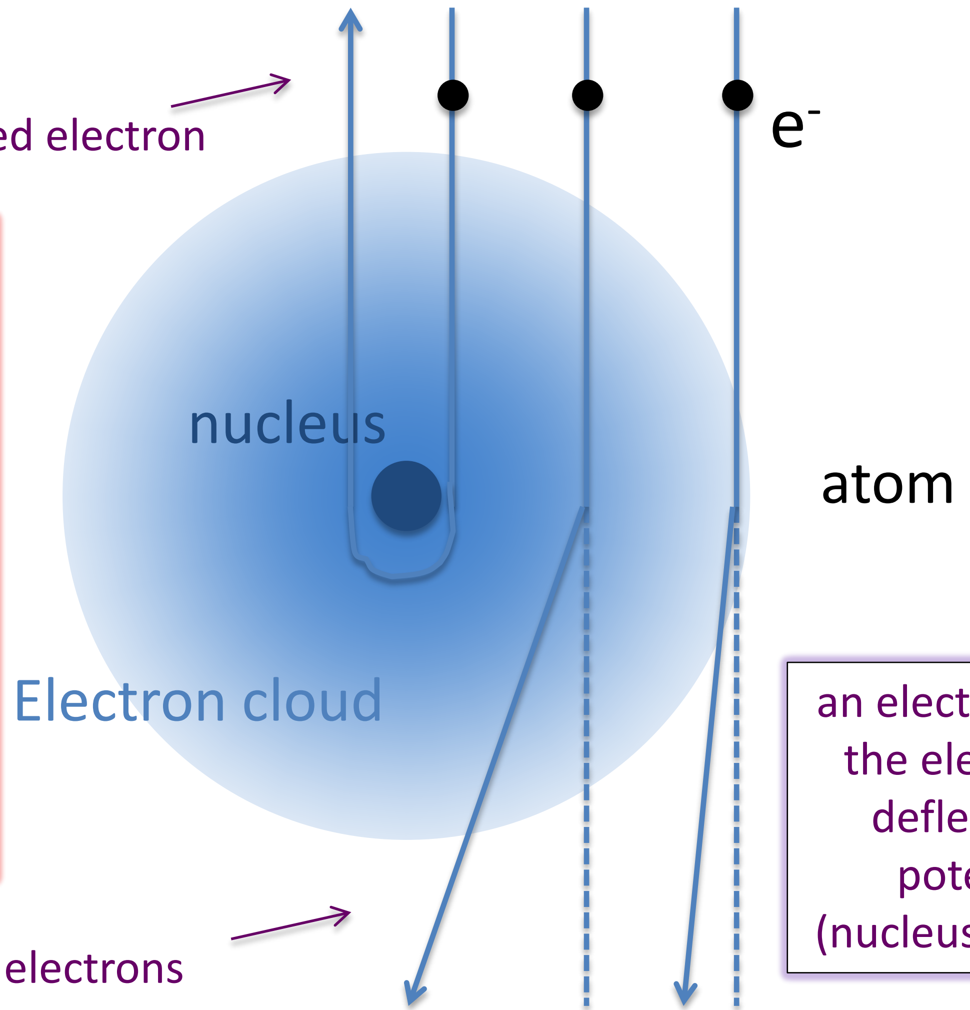
<u>Overview of:</u>

- Introduction to Fourier analysis
  - Sine waves
  - Fourier transform (simple examples of 1D functions)
  - Fourier transform of images
  - Why is it useful for image processing?

- Image formation
  - Weak phase approximation

- The contrast transfer function

Image formation



elastic scattering:
- electrons maintain their energy and contribute to image formation

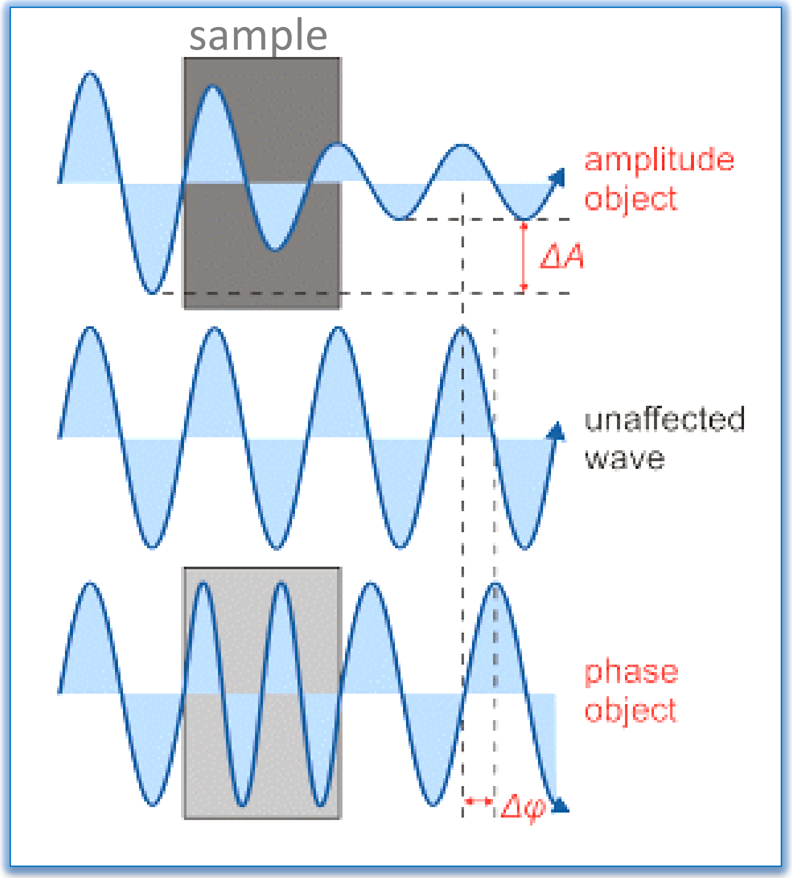
inelastic scattering:
- electrons loose energy; the deposited energy causes radiation damage

an electron that passes the electron cloud is deflected by local potential fields (nucleus and electrons)

(adapted from Sjors' slides)

Image formation

- electron: the wave-particle duality -

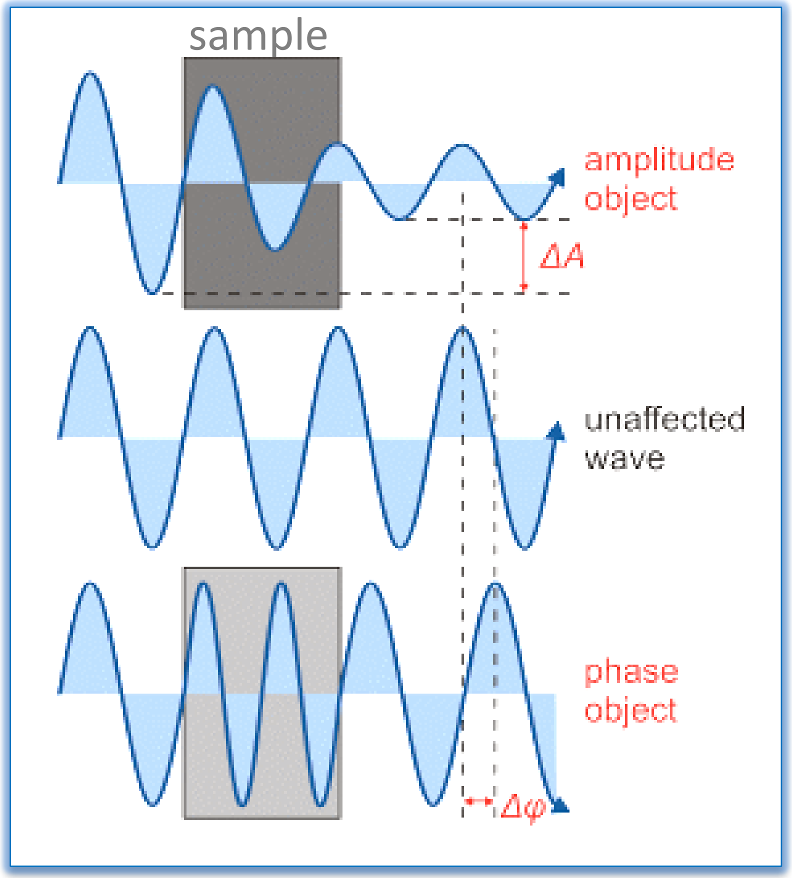


scattering results in changes of both amplitudes and the phases of an incident wavefront

(image taken from: http://home.uni-leipzig.de/pwm/web/?section=introduction&page=phasecontrast)

Image formation

- electron: the wave-particle duality -



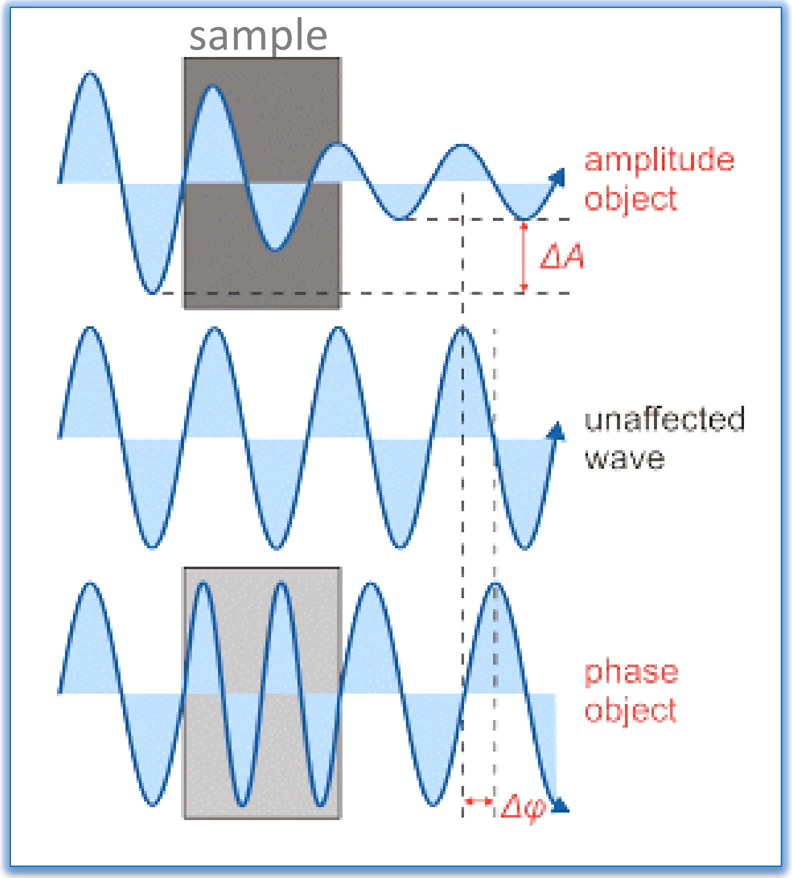
amplitude object:

- "electron as a particle"
- changes in the wavefront amplitudes
- deflected electrons lead to regions of reduced amplitude
- analogous to visible light detection; "detection of object envelope"
- applicable to strongly scattering and thick samples
- proteins scatter too weakly for significant amplitude contrast
- it can be enhanced by embedding in heavy atoms (negative stain), but resolution is compromised

(image taken from: http://home.uni-leipzig.de/pwm/web/?section=introduction&page=phasecontrast)

Image formation

- electron: the wave-particle duality -



<u>phase object</u>:

- "electron as a wave"
- changes in the wavefront phases
- protein samples in cryo-EM

"the weak phase object approximation"

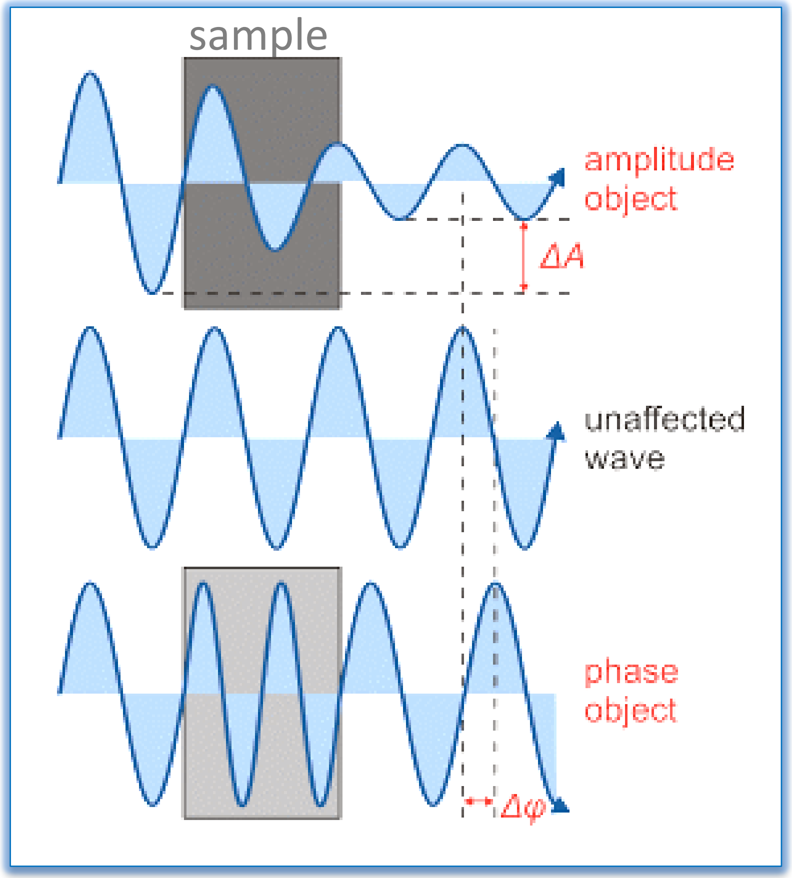
thin and weakly scattering samples (like proteins in vitreous ice) do not change the amplitudes of an incident electron wavefront, but slightly change its phases

contrast in the image is linearly related to the projected object potential

(image taken from: http://home.uni-leipzig.de/pwm/web/?section=introduction&page=phasecontrast)

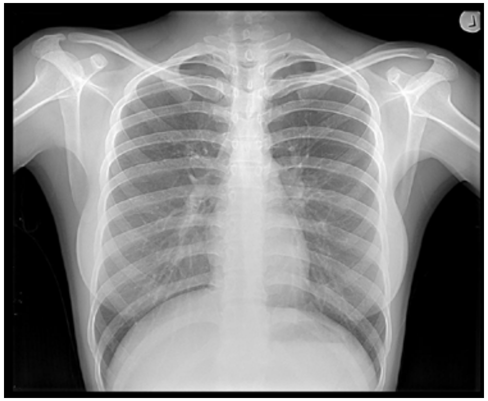
Image formation

- electron: the wave-particle duality -



phase object:

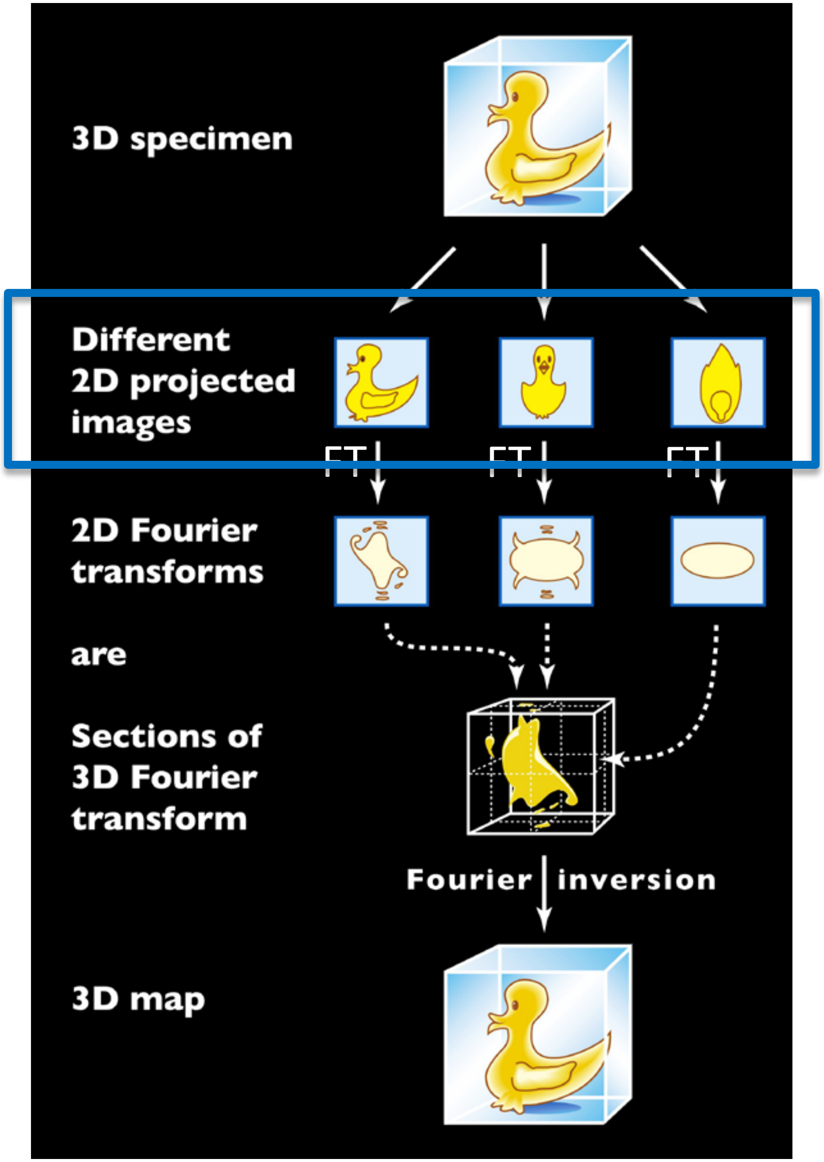
- "electron as a wave"
- changes in the wavefront phases
- protein samples in cryo-EM



contrast in the image is linearly related to the projected object potential

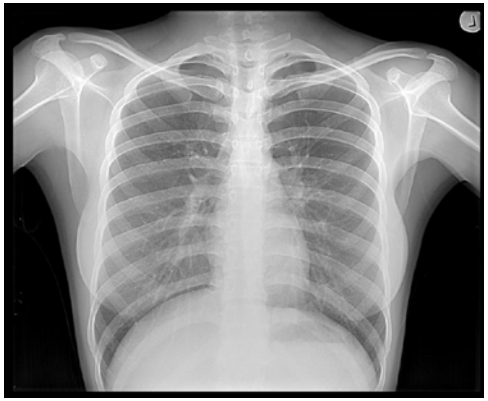
(image taken from: http://home.uni-leipzig.de/pwm/web/?section=introduction&page=phasecontrast)

Image formation



phase object:

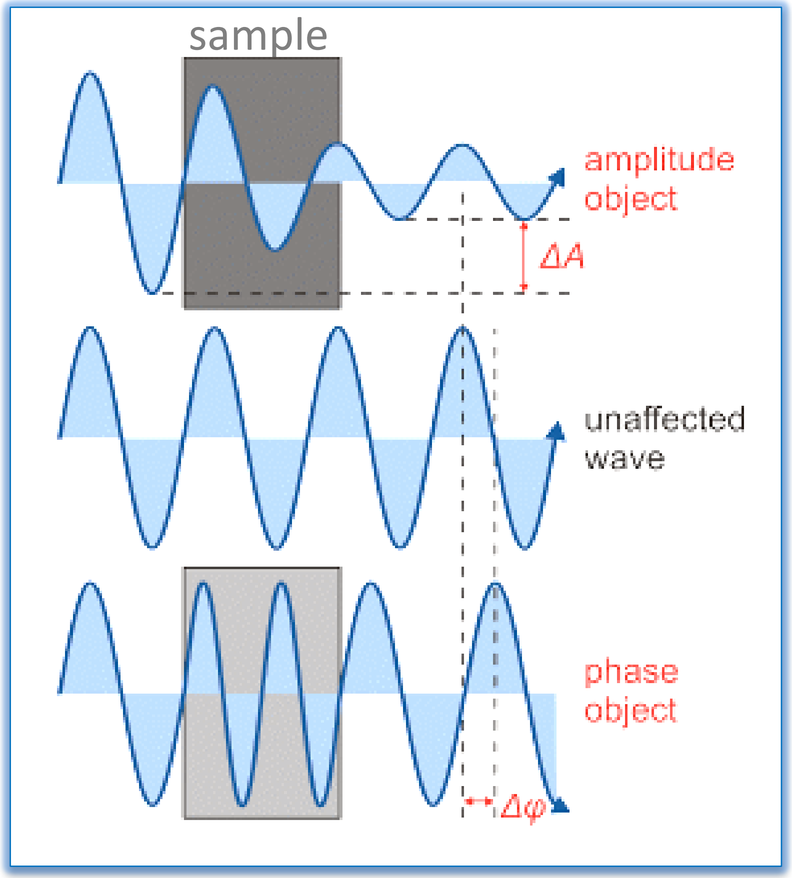
- "electron as a wave"
- changes in the wavefront phases
- protein samples in cryo-EM



validates the application of the projection-slice theorem in cryo-EM image processing

Image formation

- electron: the wave-particle duality -



phase object:

- "electron as a wave"
- changes in the wavefront phases
- protein samples in cryo-EM

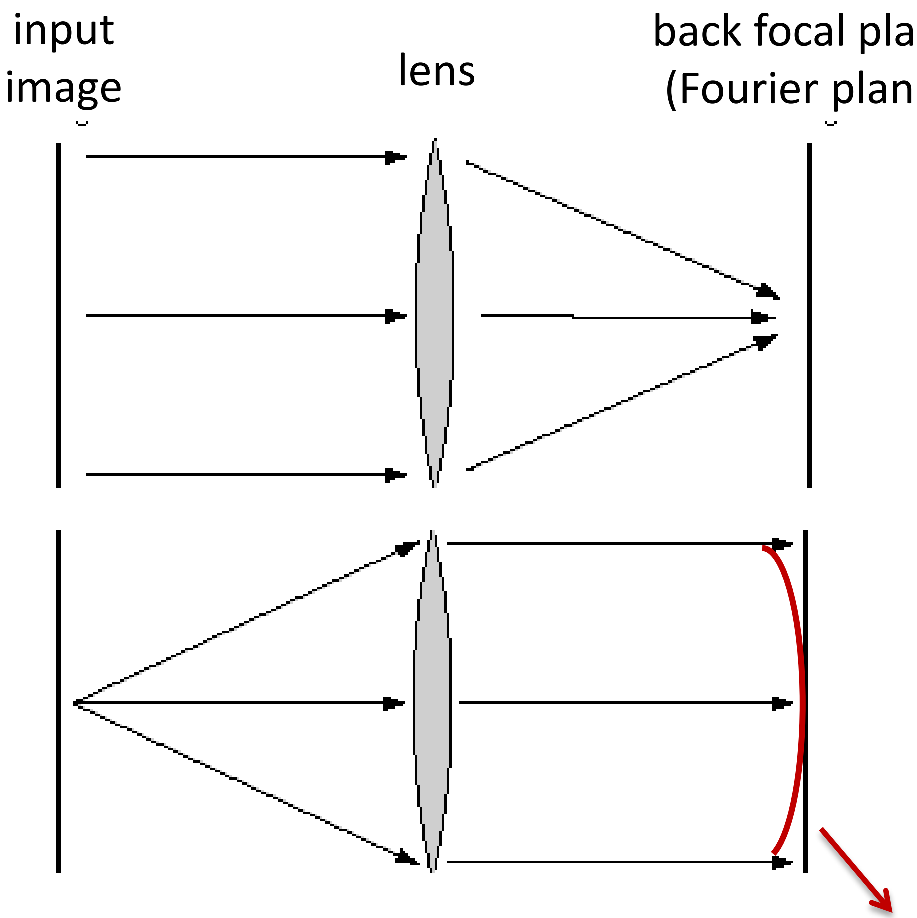
detectors can only record amplitudes

- in a in focus and perfect (aberration free) optical system phase objects are invisible to detectors -

amplitude contrast can be achieved by introducing interferences in the wavefront

(image taken from: http://home.uni-leipzig.de/pwm/web/?section=introduction&page=phasecontrast)

Image formation



Parallel rays from the entire input image are focused onto the single central point of the Fourier image, where it defines the average brightness of the input image.

Every point of the input image is spread uniformly over the Fourier image, where constructive and destructive interference will produce the Fourier representation.

underfocus causes a “delay” in the electrons scattered to higher angles, leading to interferences in the back focal plane that result in enhanced amplitude contrast

(adapted from Sjors’ slides)

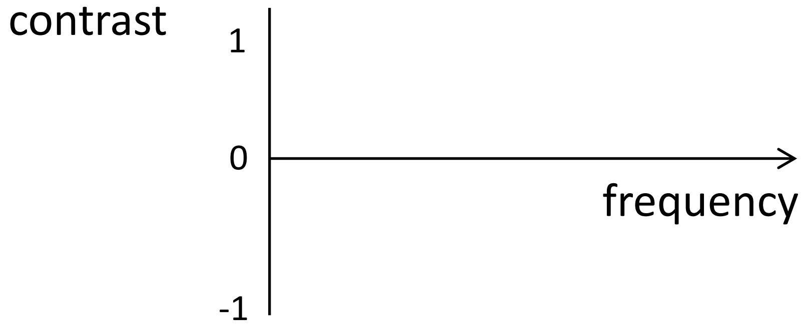
- Agenda -

Overview of:

- Introduction to Fourier analysis
  - Sine waves
  - Fourier transform (simple examples of 1D functions)
  - Fourier transform of images
  - Why is it useful for image processing?
- Image formation
  - Weak phase approximation
- The contrast transfer function

The contrast transfer function

representation of frequency dependent contrast transfer



contrast = 1   maximum contrast
contrast = 0   no contrast
contrast = -1   reversed contrast

The contrast transfer function

Contrast transfer function (CTF):

mathematically describes how aberrations in a transmission electron microscope (TEM) modify the image of a sample - gives the phase changes of diffracted beams with respect to the direct beam

"it is the Fourier transform of the optical system point spread function"

$$\mathrm{T}(k) = -\sin\left[\frac{\pi}{2} C_S \lambda^3 k^4 + \pi \Delta f \lambda k^2\right]$$

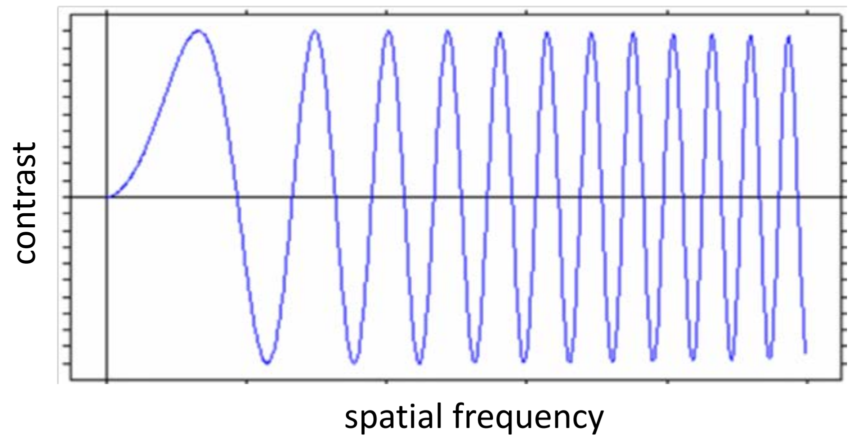
it depends on:

k – spatial frequency

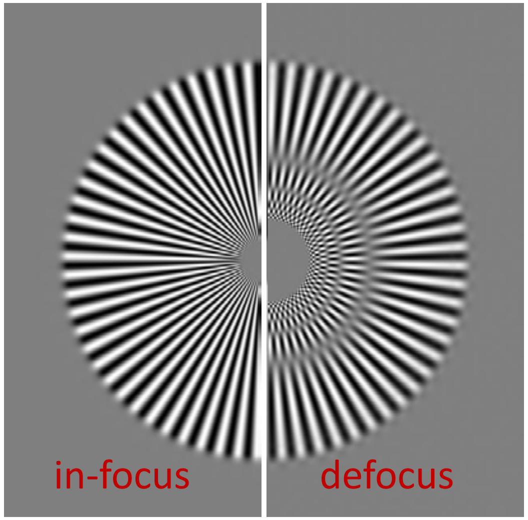
C_S - the quality of objective lens defined by spherical aberration coefficient
λ - wave-length defined by accelerating voltage
Δf - the defocus value

The contrast transfer function

- Contrast created by defocusing (underfocus)
- Introduces frequency dependent phase reversals described by the contrast transfer function (CTF):

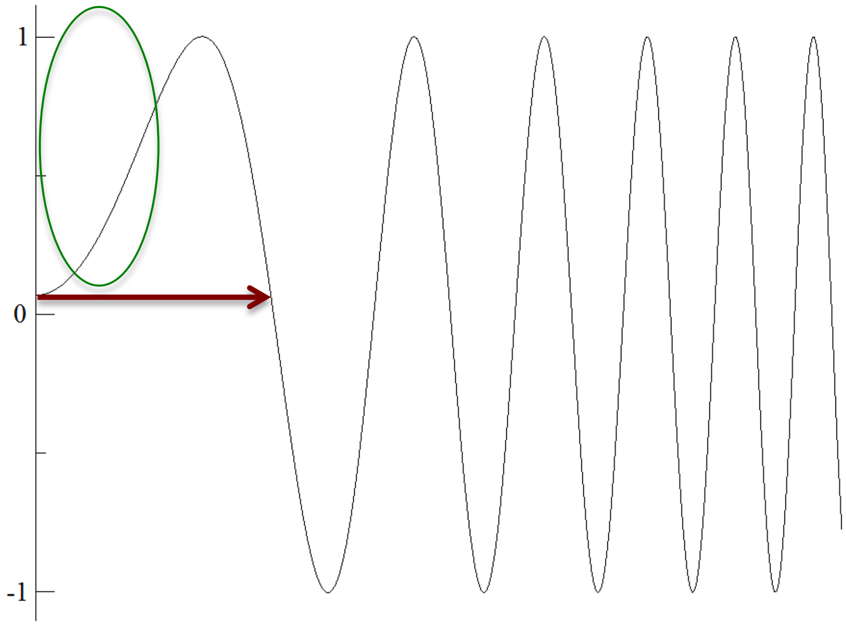


Siemens star



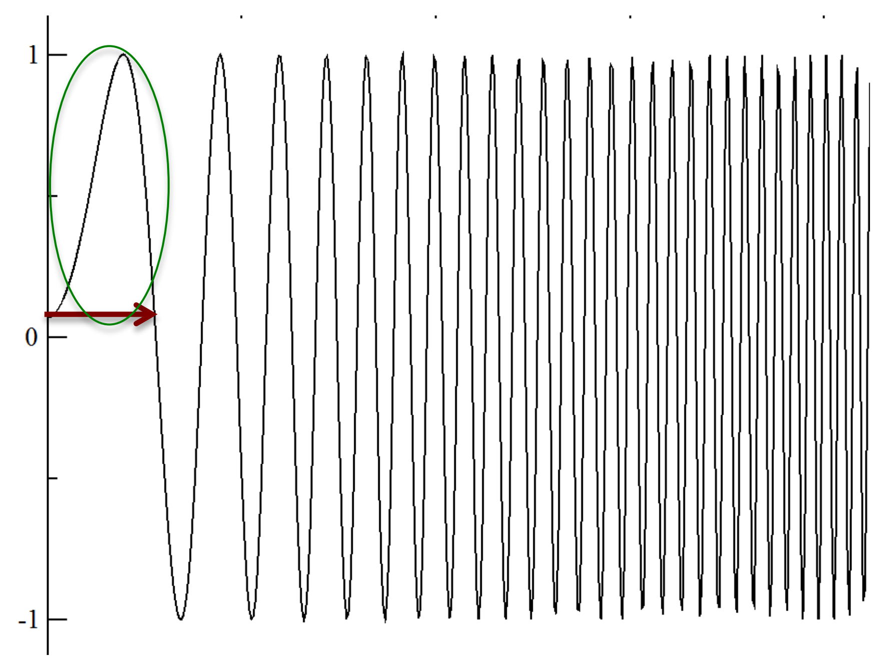
frequency dependent contrast reversals

The contrast transfer function



← Low defocus

High defocus →

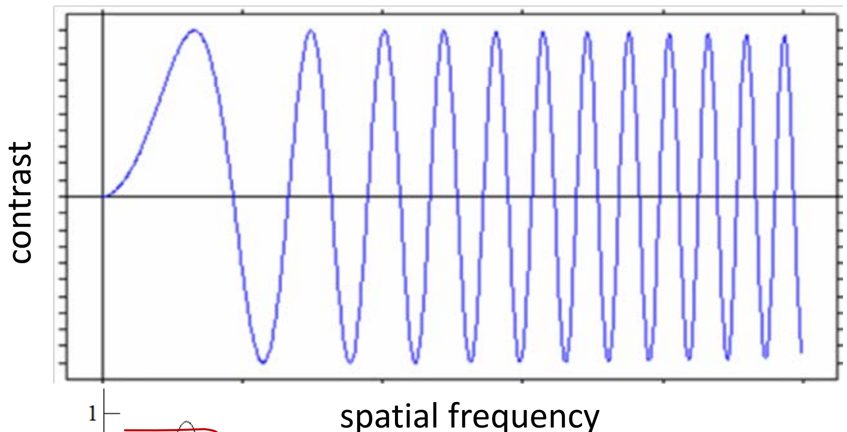


–
- First zero-crossing at lower frequency
- Much faster modulations
- Often stronger envelope

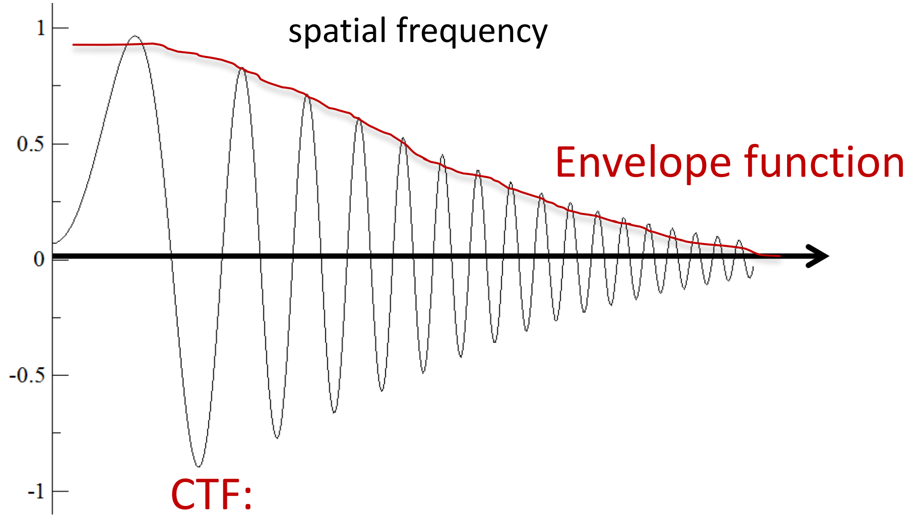
+
- Stronger low-frequency signal!
- Different zero-crossings

(Sjors' slide)

The contrast transfer function



contrast created by defocusing in a perfect (aberration free) optical system



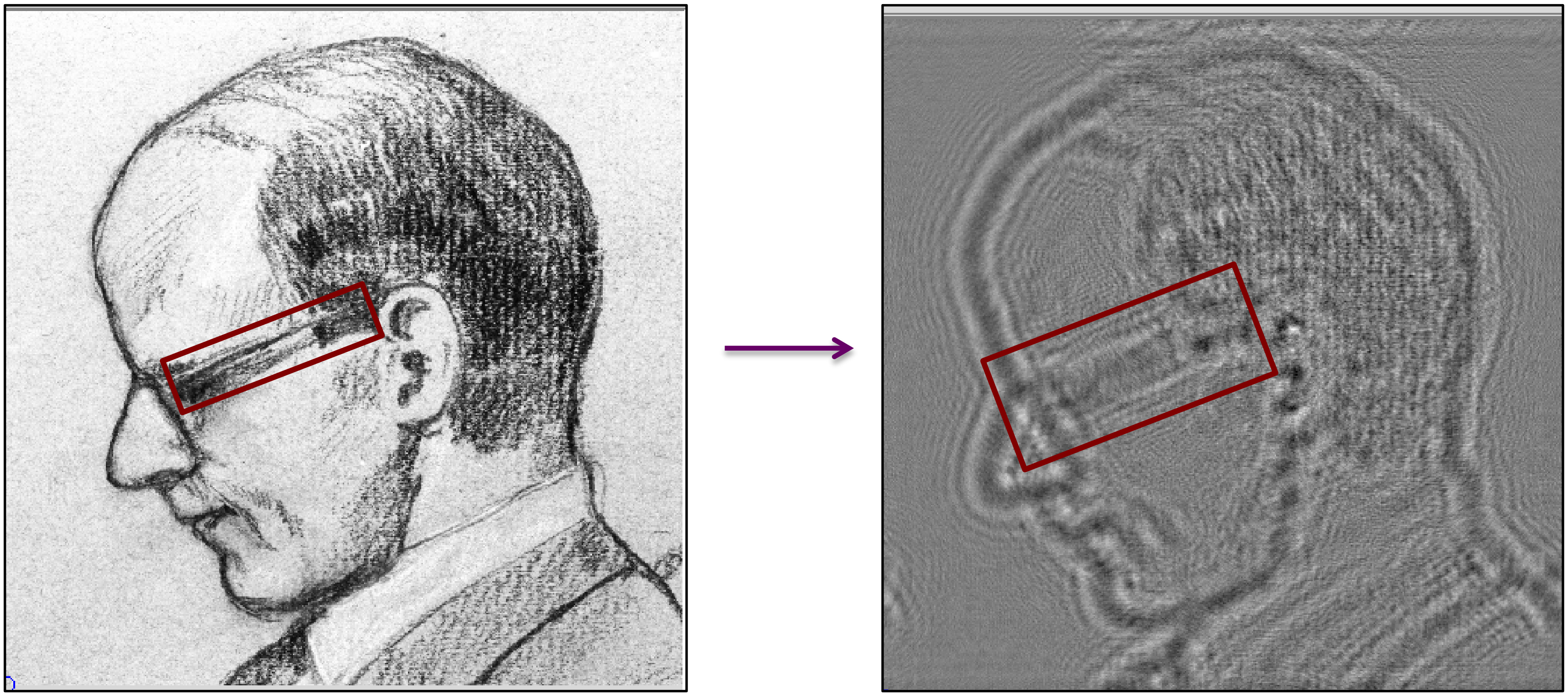
- Caused by imperfect imaging
- Acts as a low-pass filter!

CTF:

"it is the Fourier transform of the optical system point spread function"

(adapted from Sjors' slides)

The contrast transfer function



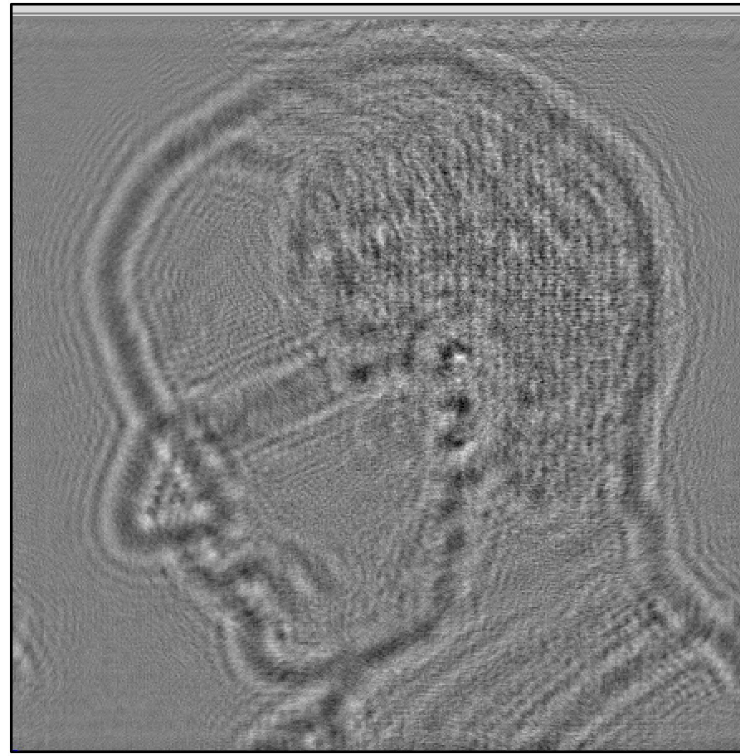
Black-white   grey

Convolution of an image with the "system point spread function"
- defocus dependent delocalisation -

(adapted from Sjors' slides)

The contrast transfer function

Black-white



grey

but we can correct for some of the contributors for the CTF (e.g. Cs and defocus)

- we can do CTF correction! -

(adapted from Sjors' slides)

The contrast transfer function

- Williams D.B. & Carter, C.B. Transmission electron microscopy (Plenum Press, New York, 1996)
- Spence, J. C. H. High-resolution electron microscopy (Oxford University Press, 2003).
- Glaeser, R. M. Electron crystallography of biological macromolecules (Oxford University Press, 2007).
- Frank, J. Three-dimensional electron microscopy of macromolecular assemblies (Oxford University Press, 2006).