EMBO Course on Image Processing for Cryo-EM

Lecture 2

Basic Concepts of Fourier Analysis

- and of convolution and correlation -

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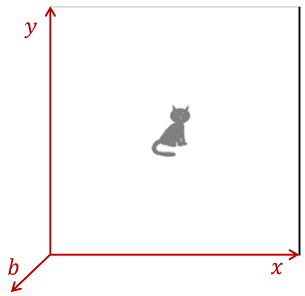
- MRC LMB -

London, 2nd September 2015

Fourier analysis

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



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

Fourier analysis

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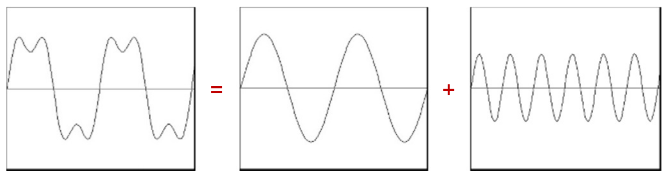
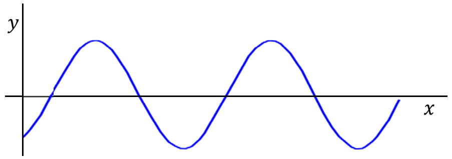
- simple 1D function -



Sine waves

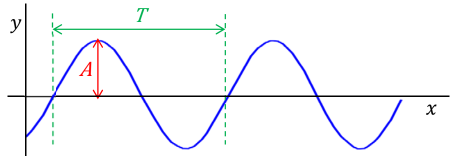
$$y = A \sin(2\pi x/T + \varphi)$$



simple periodic function

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) in x axis units, normally Ångstroms in the processing of EM images

$f = 1/T$ = frequency; complete repeats per x axis unit

Sine waves

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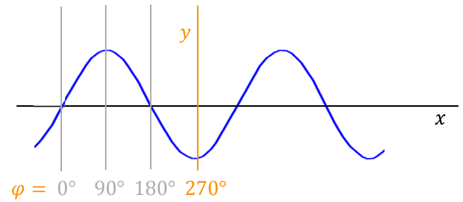

φ = phase, in degrees or radians, defines the oscillation stage at the wave origin

Sine waves

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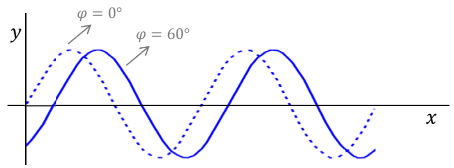

the phase defines the "relative position" of a wave

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

Sine waves

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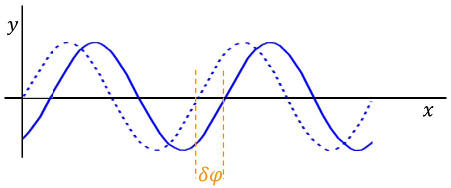
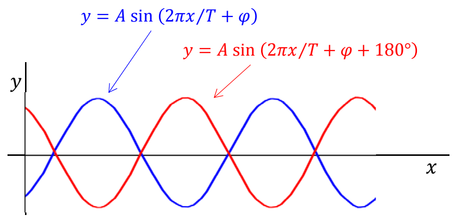


phase difference between two out-of-phase waves

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

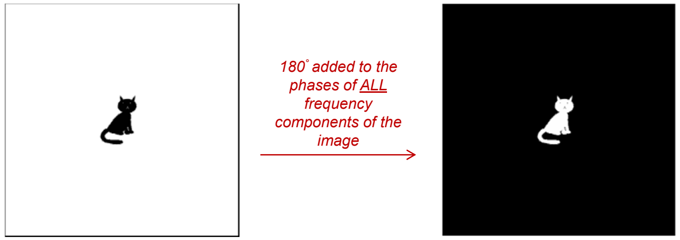


adding 180˚ to the phases of a sine wave results in its mirror function

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 (lecture 3) -

Sine waves



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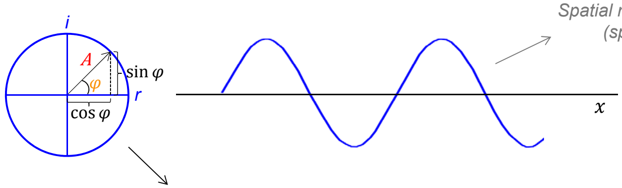


Spatial representation of a wave (space vs amplitude)

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

real component is $A\cos\varphi$

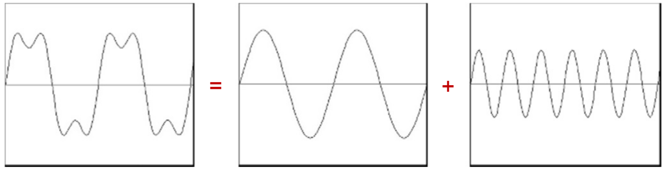
imaginary component is $A\sin\varphi$

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

Fourier analysis

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

- simple 1D function -

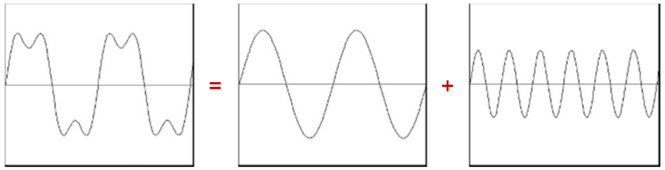


$f(x)$ $\quad$ $\sin x$ $\quad$ $1/3 \sin 3x$

$$\underbrace{f(x) = \sin x + 1/3 \sin 3x}_{}$$

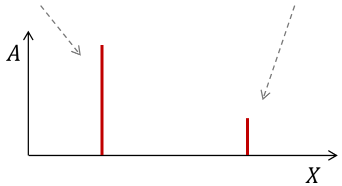
Fourier series for $f(x)$

Fourier analysis



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

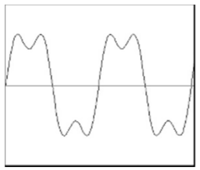
the components of a Fourier series can be represented as a function of amplitude (A) vs frequency (X)

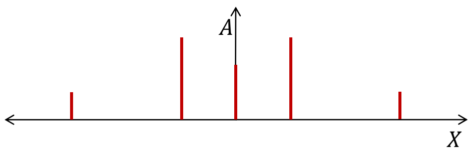


$$\underbrace{f(x) = \sin x + 1/3 \sin 3x}_{}$$

Fourier series for $f(x)$

Fourier transform





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

$\leftrightarrow$ *Fourier transform*

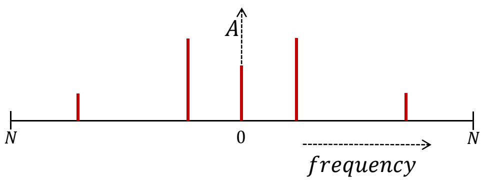
$F(X)$
Fourier space
(frequency domain)

Fourier transform of a function:

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

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

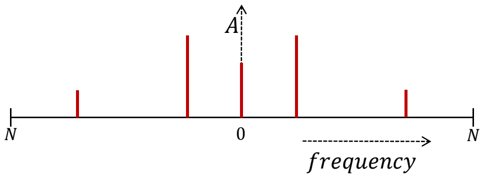
Fourier transform



Fourier transform of a function:

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

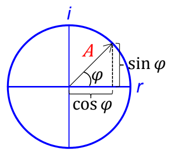
Fourier transform



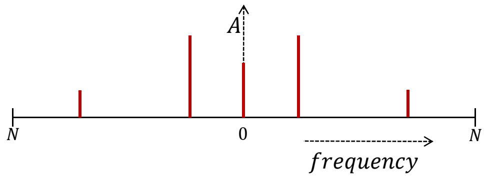
Fourier transform of a function:

- strictly, a plot of the Fourier transform (as a function of frequency *vs* amplitude) corresponds to the amplitude spectrum of the image, 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



Fourier transform

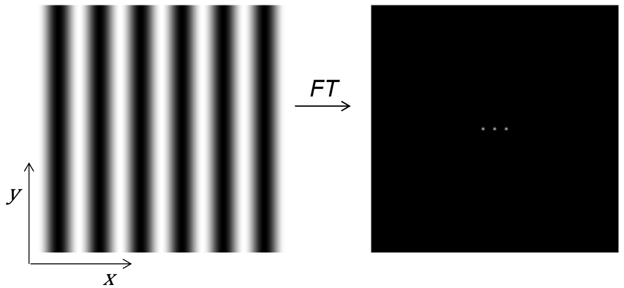


Fourier transform of a function:

- strictly, a plot of the Fourier transform (as a function of frequency *vs* amplitude) corresponds to the amplitude spectrum of the image, as the phase components of the Fourier transform are omitted
- 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
- the Fourier transform of a function can be fully inverted :

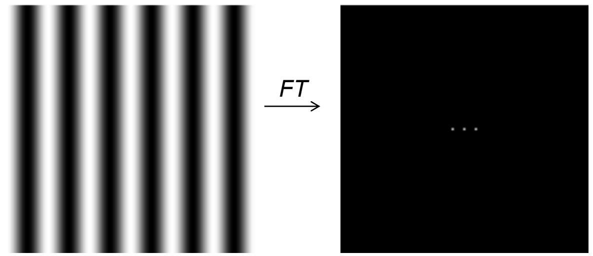
$$f(x) \xrightarrow[\text{F. t.}]{\text{forward}} F(X) \xrightarrow[\text{F. t.}]{\text{inverse}} f(x)$$

Fourier transform of images



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

Fourier transform of images

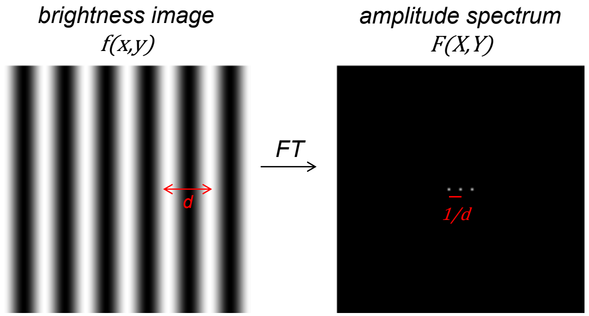


Fourier transform of a digital image:

- encodes all the spatial frequencies present in an image, from zero (i.e. no modulation) to N (Nyquist frequency)
- Nyquist frequency is the highest spatial frequency that can be encoded in a digital image

$$N = 1/(2 \times (image\ pixel\ size))$$

Fourier transform of images

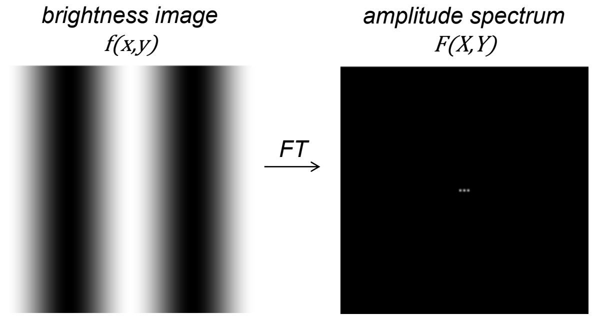


Fourier transform of a digital image:

- in image processing of cryo-EM images, frequency corresponds to spacing and it is normally described in $(1/\text{Å})$ units
- a brightness image with closely spaced features will give a Fourier transform with wide spacings

- Fourier space is also known as reciprocal space -

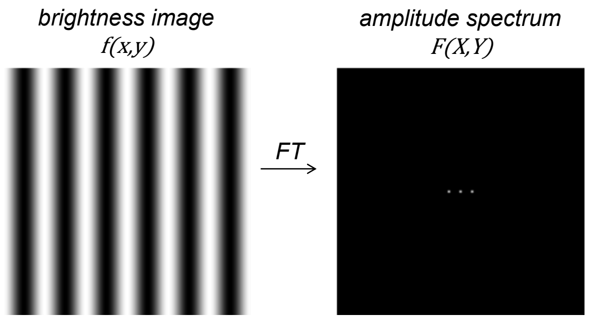
Fourier transform of images



decreasing the period of a wave component of the real image will increase the distance between its Fourier terms

- Fourier space is also known as reciprocal space -

Fourier transform of images



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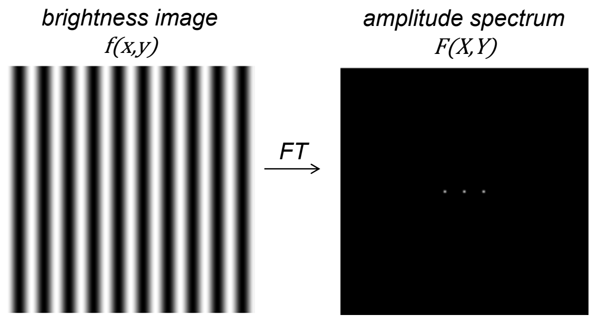

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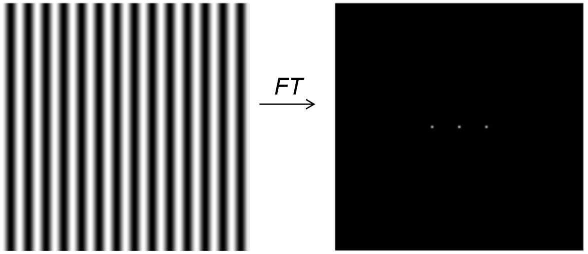
Fourier transform of images

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



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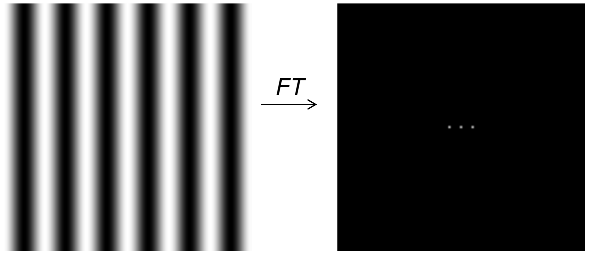

a rotation of the real space image results in a rotation of its transform

Fourier transform of images

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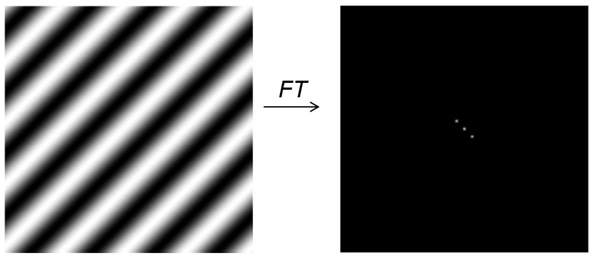

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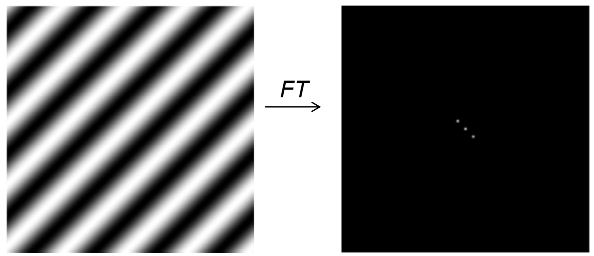
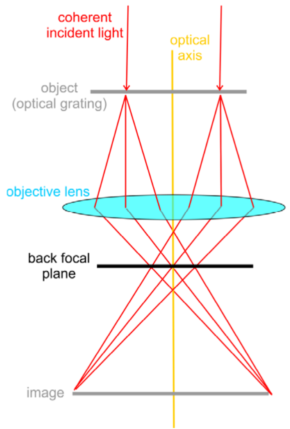


a rotation of the real space image results in a rotation of its transform

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

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

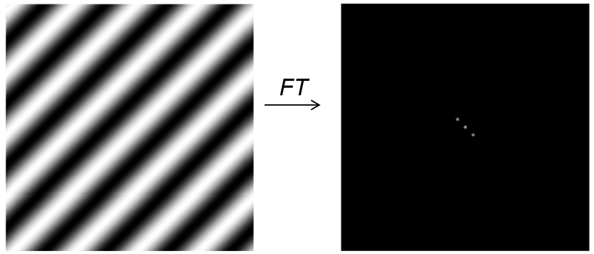
↓ FT^{-1}

image

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

Fourier transform of images

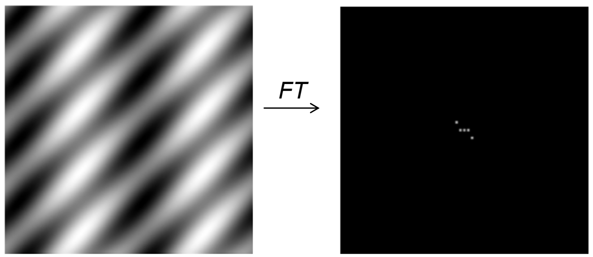
brightness image $f(x,y)$ *amplitude spectrum* $F(X,Y)$



simple image formed by a single sinusoidal component

Fourier transform of images

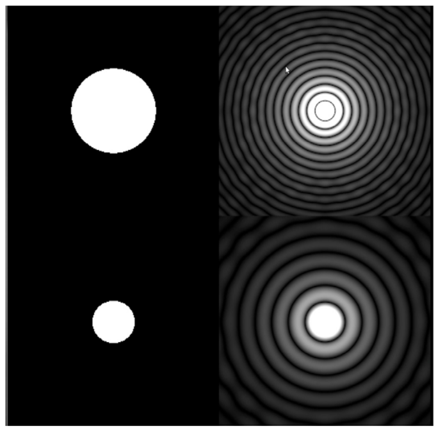
brightness image $f(x,y)$ *amplitude spectrum* $F(X,Y)$



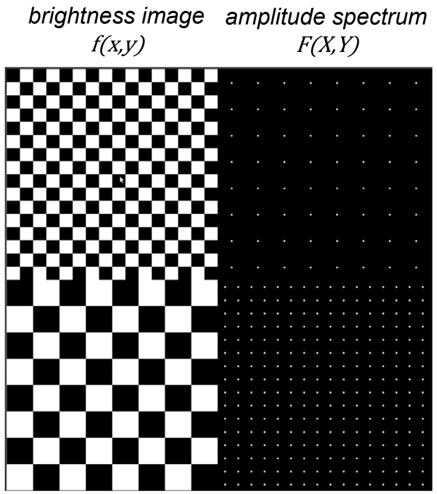
images formed by multiple sinusoidal components

Fourier transform of images

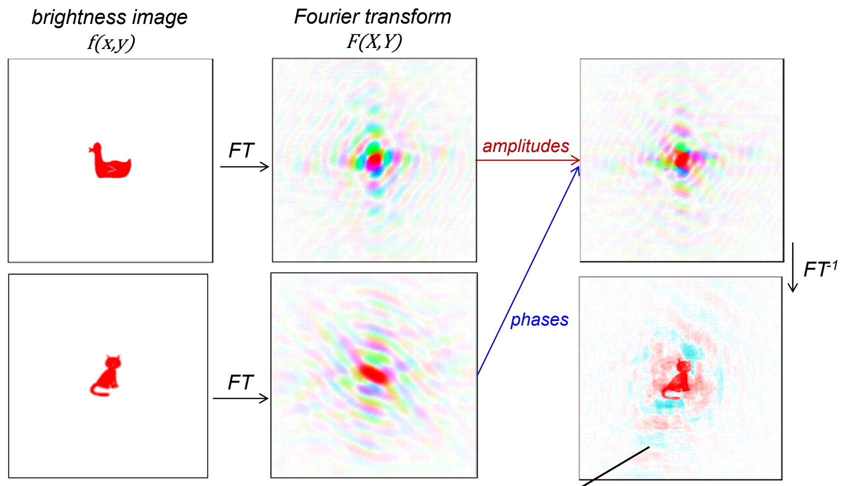
brightness image $f(x,y)$ *amplitude spectrum* $F(X,Y)$



Fourier transform of images

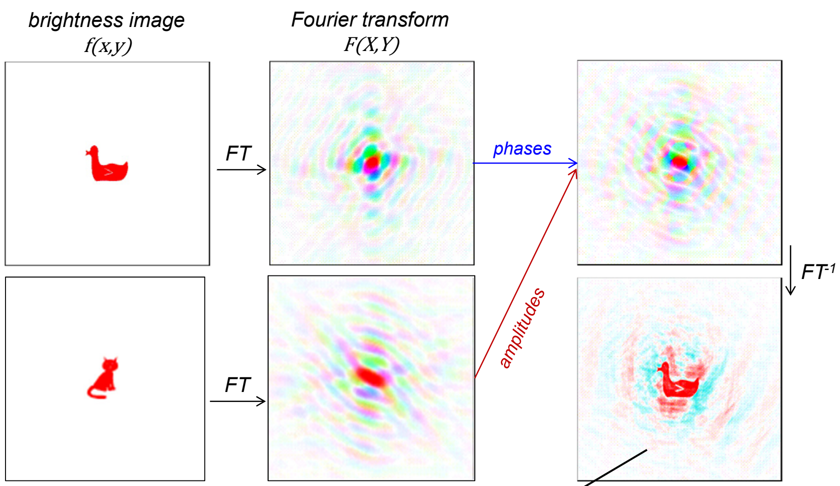


Fourier transform of images



The image contributing the phases dominates the inverse Fourier transform

Fourier transform of images



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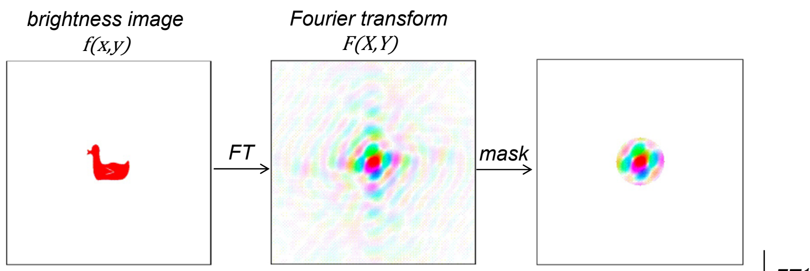
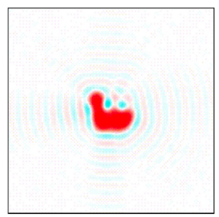
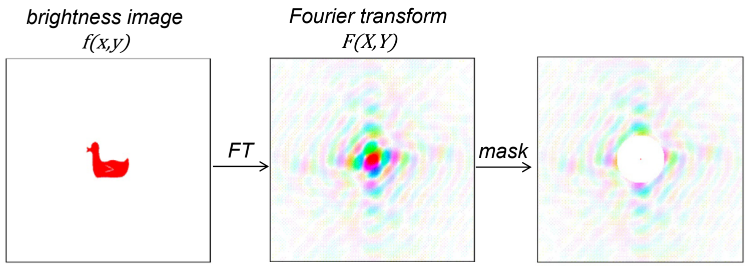


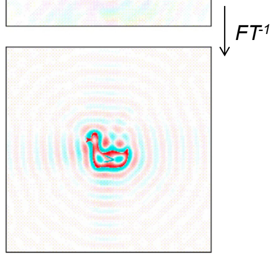
low-pass filtering

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 transform of images

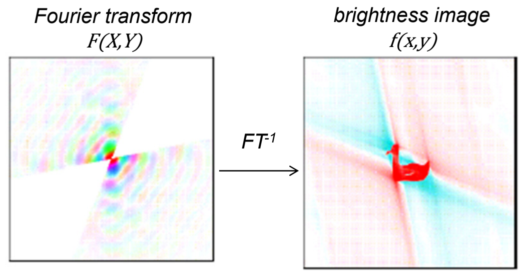




high-pass filtering

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 transform of images

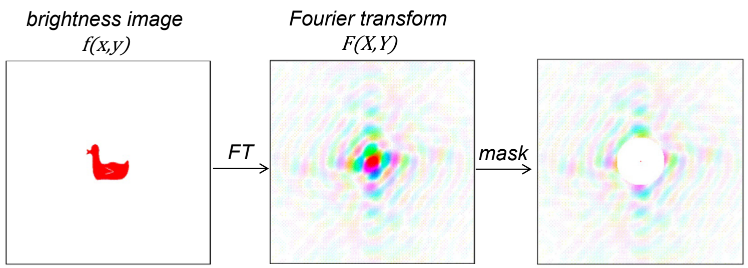


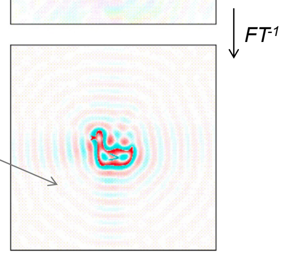
missing information

if a segment of data is missing in the Fourier transform, features perpendicular to that segment will be blurred in the inverse transform

- practical examples include: the “missing cone” in random conical tilt 3D reconstructions; the “missing wedge” in single tilt tomograms; distortions in 3D reconstructions due to incomplete information coverage in Fourier space -

Fourier transform of images





Caution: the inverse Fourier transform also includes the transform of the mask; a sharp mask applied in Fourier space will result in significant “ripple artefacts” in the inverse transform

- convolution of two functions -

Basic concepts of convolution

convolution of two functions: is the integral of their products after one function is reversed and shifted (by t)

Convolution of a function f(x) with a second function g(x):

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

Basic concepts of convolution

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Convolution of a function f(x) with a second function g(x):

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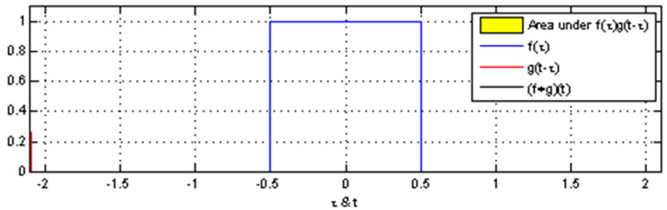
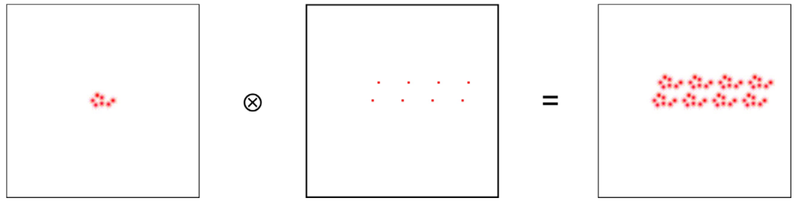


- *Illustration of the convolution of 2 identical box functions*
- *Convolution function spreads beyond boxes and is highest where 2 boxes overlap maximally (in this case at t=0)*

Basic concepts of convolution

the idea is combining 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



Basic concepts of convolution

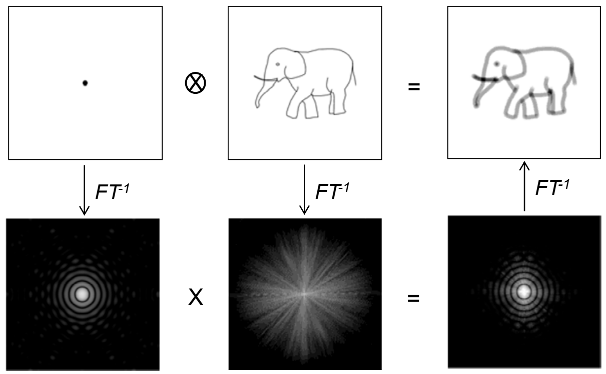
while the convolution of two functions can be directly calculated in real space, the calculation can be done much faster using Fourier transforms

$$f(x) * g(x) = F^{-1}(F(X) \times G(X))$$

the convolution of two functions in real space is identical to the inverse Fourier transform of the product of their Fourier transforms

Basic concepts of convolution

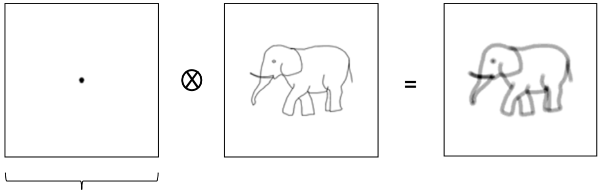
while the convolution of two functions can be directly calculated in real space, the calculation can be done much faster using Fourier transforms



Adapted from Elena Orlova slides

Basic concepts of convolution

- effect of a point spread function -



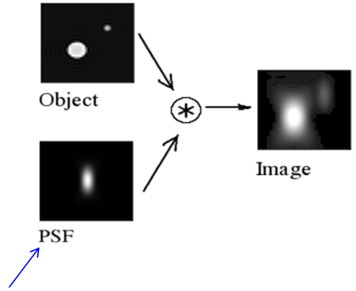
Point spread function - describes the response of an imaging system to a point source or point object (how an optical system sees a point)

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

Adapted from Elena Orlova slides

Basic concepts of convolution

- effect of a point spread function -



Point spread function from a confocal light microscope

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 (lecture 3)*
- *…*

Basic concepts of correlation

cross correlation between a function f(x) with a second function g(x):

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

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

- Fourier transforms are used in cross-correlation analysis as a method of rapid calculation
- calculation of the correlation function can be done using product of conjugate Fourier transforms

Basic concepts of correlation

cross correlation between a function f(x) with a second function g(x):

$$ccf = \frac{\int f(x)g(x-t)dx}{\sqrt{\int f^2(x)dx \int g^2(x)dx}}$$

normalised cross correlation function

- required for quantified comparisons between images -

Basic concepts of correlation

cross correlation between a function f(x) with a second function g(x):

$$ccf = \frac{\int f(x)g(x-t)dx}{\sqrt{\int f^2(x)dx \int g^2(x)dx}}$$

normalised cross correlation function

$ccf = 1$ *correlation of identical functions*

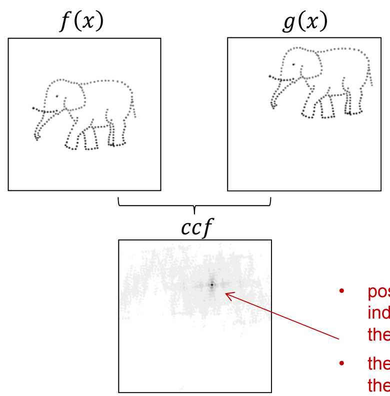
$ccf < 1$ *indicates different function distributions*

$ccf = 0$ *indicates no similarities between the two functions*

$ccf = -1$ *special case for a function correlated with that corresponding to its contrast reversal*

Basic concepts of correlation

- cross correlation of two images -



- position of the highest peak indicates the shift required for the best alignment
- the height of the peak shows their level of similarity

Adapted from Elena Orlova slides

Basic concepts of correlation

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

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