Science and Technology Facilities Council

Introduction to Image Processing

Matt Iadanza

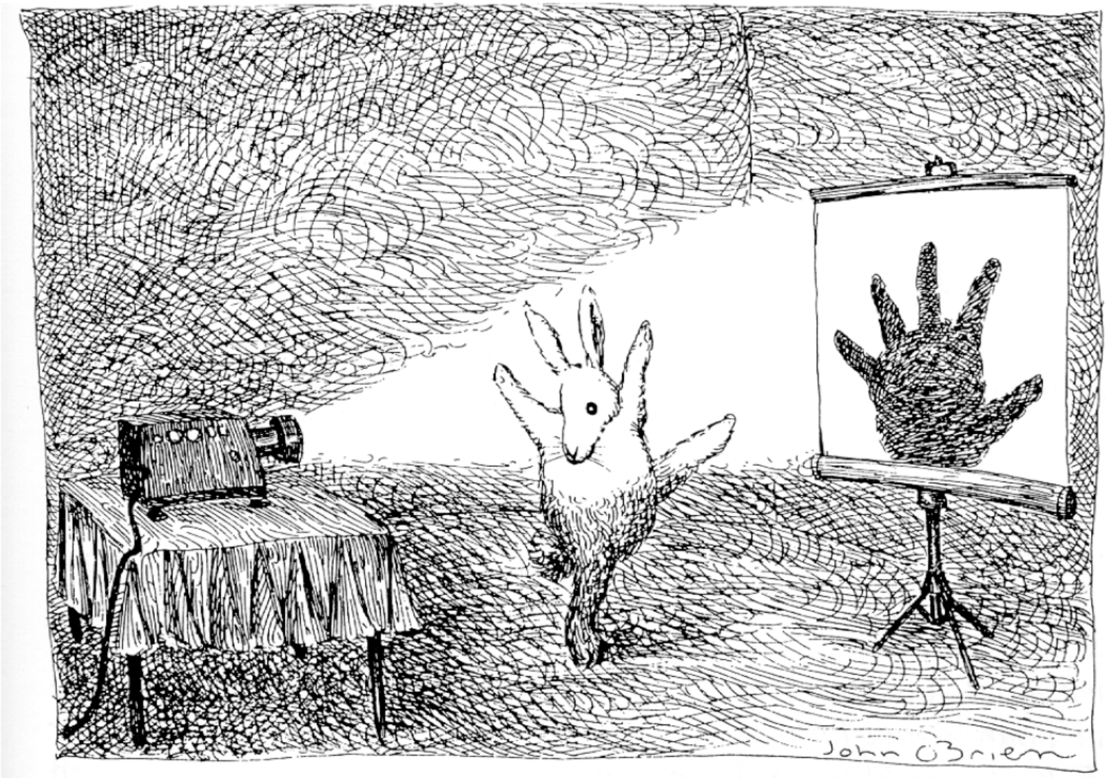
Many thanks to Bilal Qureshi and Juha Huiskonen for providing many images!

CCP-EM diamond

Overview

- Fourier transform
- Filters
- Convolution
- Point spread and contrast transfer functions
- Cross-correlation
- Central section theorem and 3D reconstruction
- Euler angles

2D images (projections vs. shadows/photos) of 3D objects



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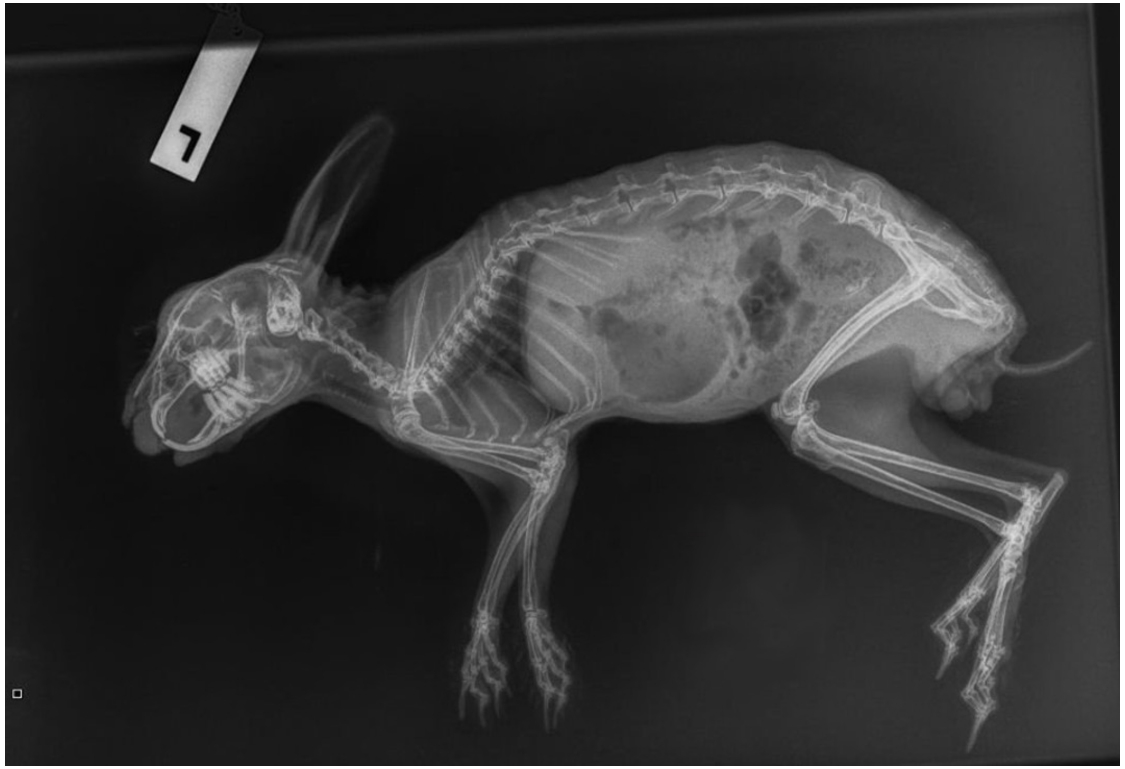


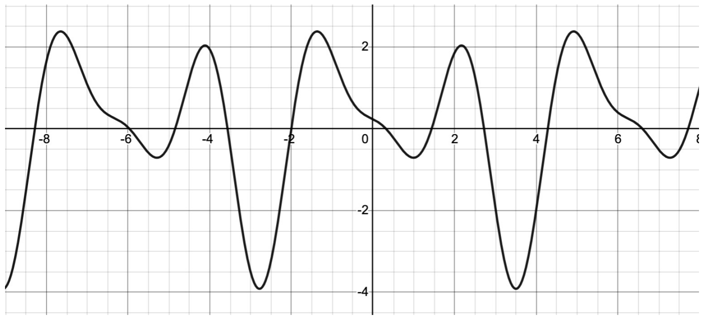
The Fourier Transform

Joseph Fourier
1768 - 1830

Fourier Transform and Fourier series

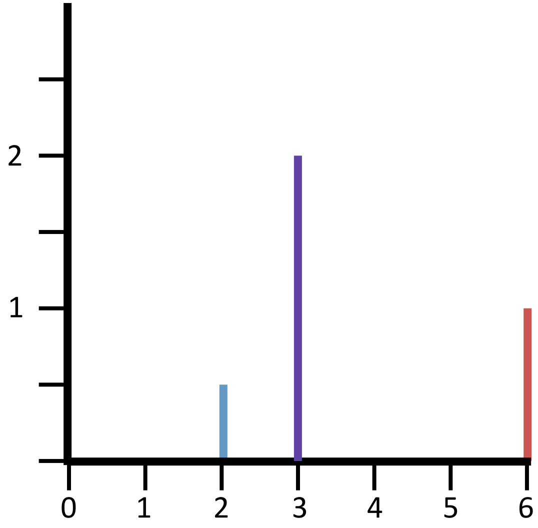
The Fourier transform decomposes a function of time into its constituent frequencies

Converts data from real space to Fourier space (AKA reciprocal space, frequency space)



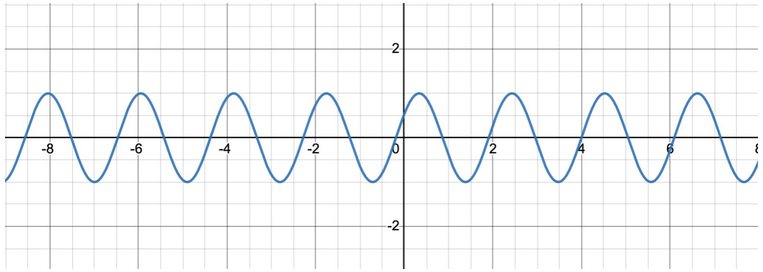
Fourier transform

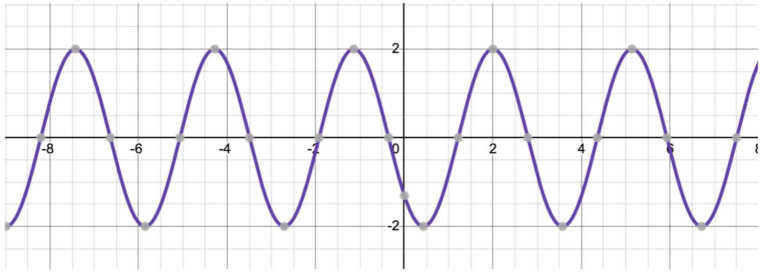
Inverse Fourier transform

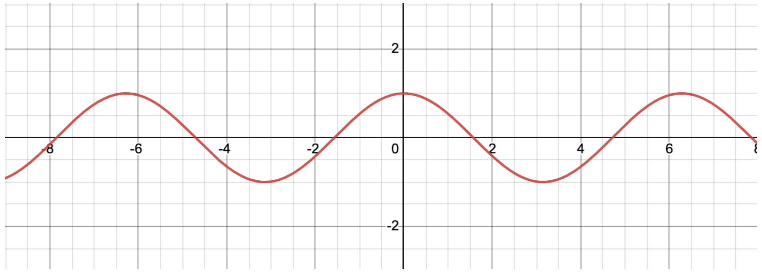


Real space data
$f(x)$

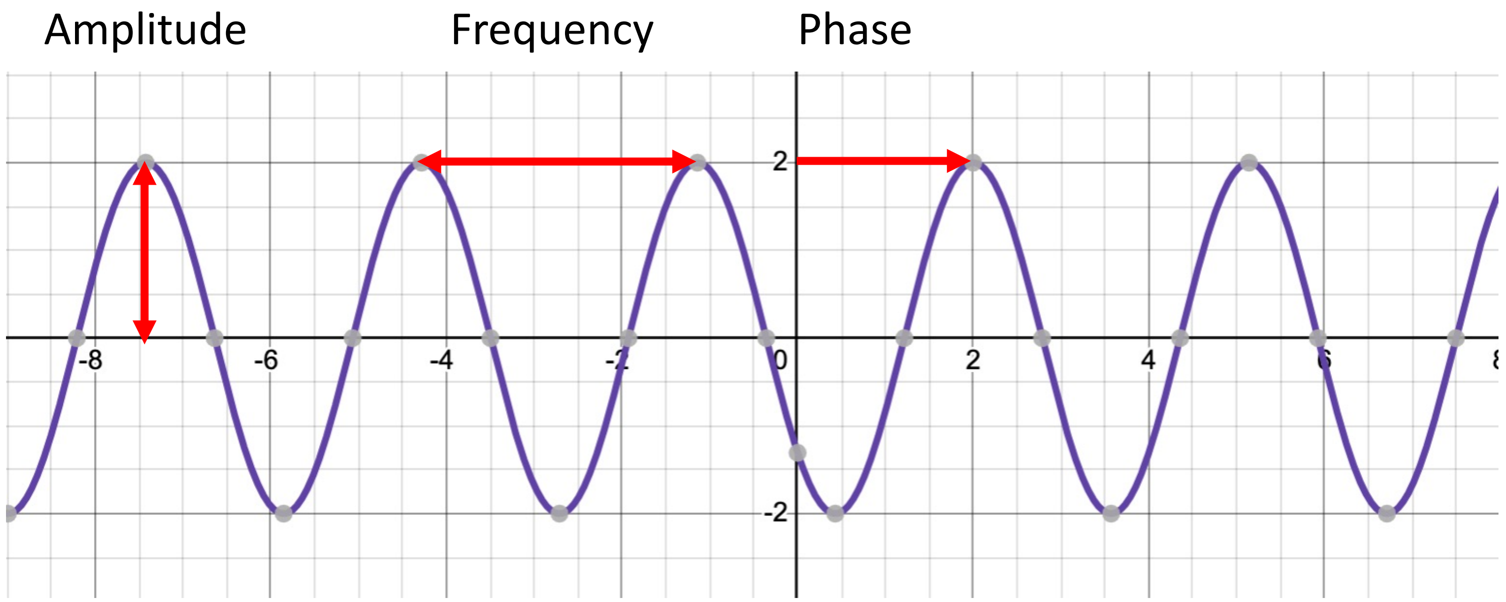
Fourier series
$F(u)$







Fourier components of a 1D wave have three pieces of info



Terminology

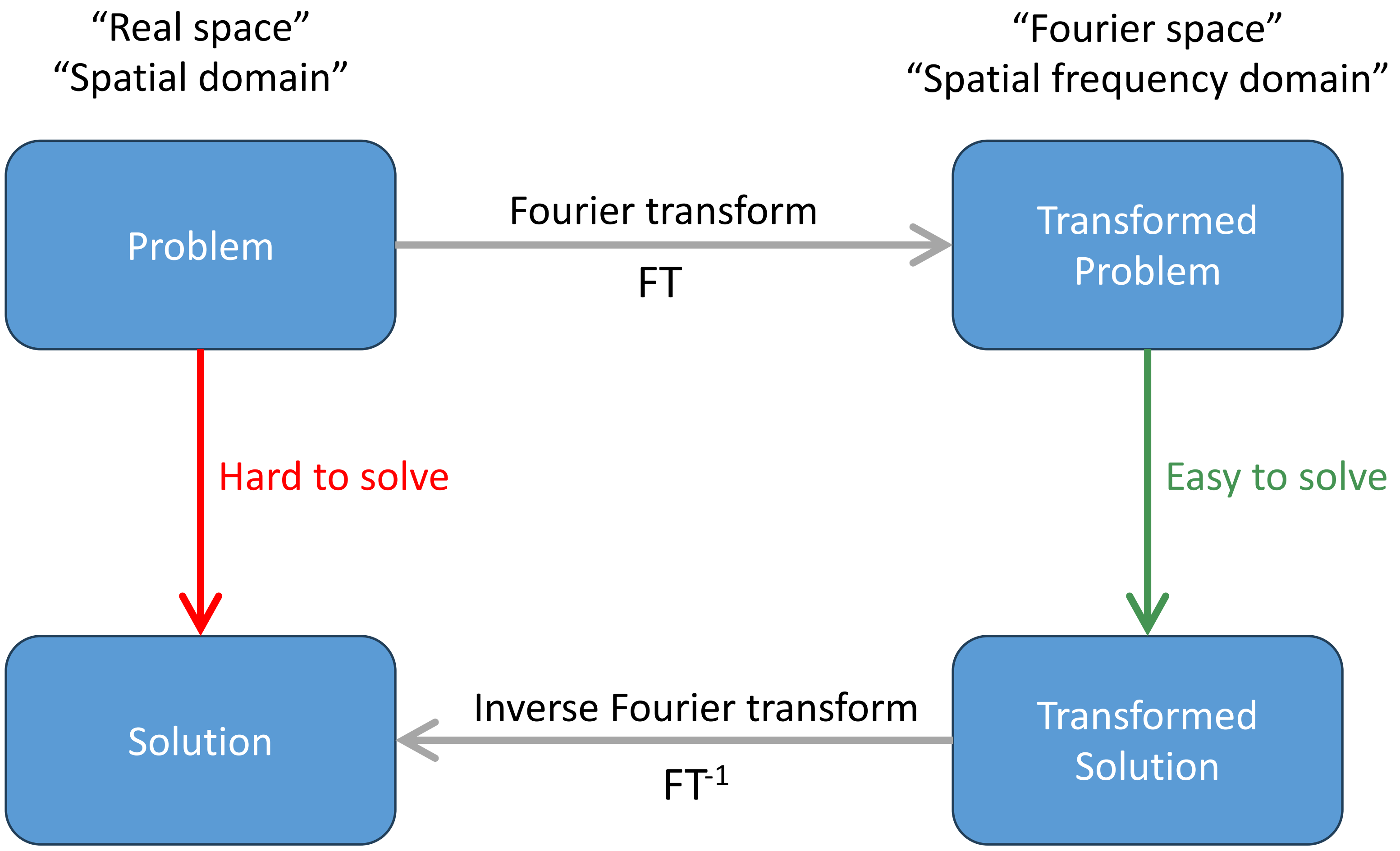
Fourier transform (**FT**) – Operates on continuous data (IE: wave equations)

Discrete Fourier transform (**DFT**) – Operates on a discrete chuck of data (data points, an audio sample, an image)

Fast Fourier transform (**FFT**) – Efficient algorithm for doing a DFT

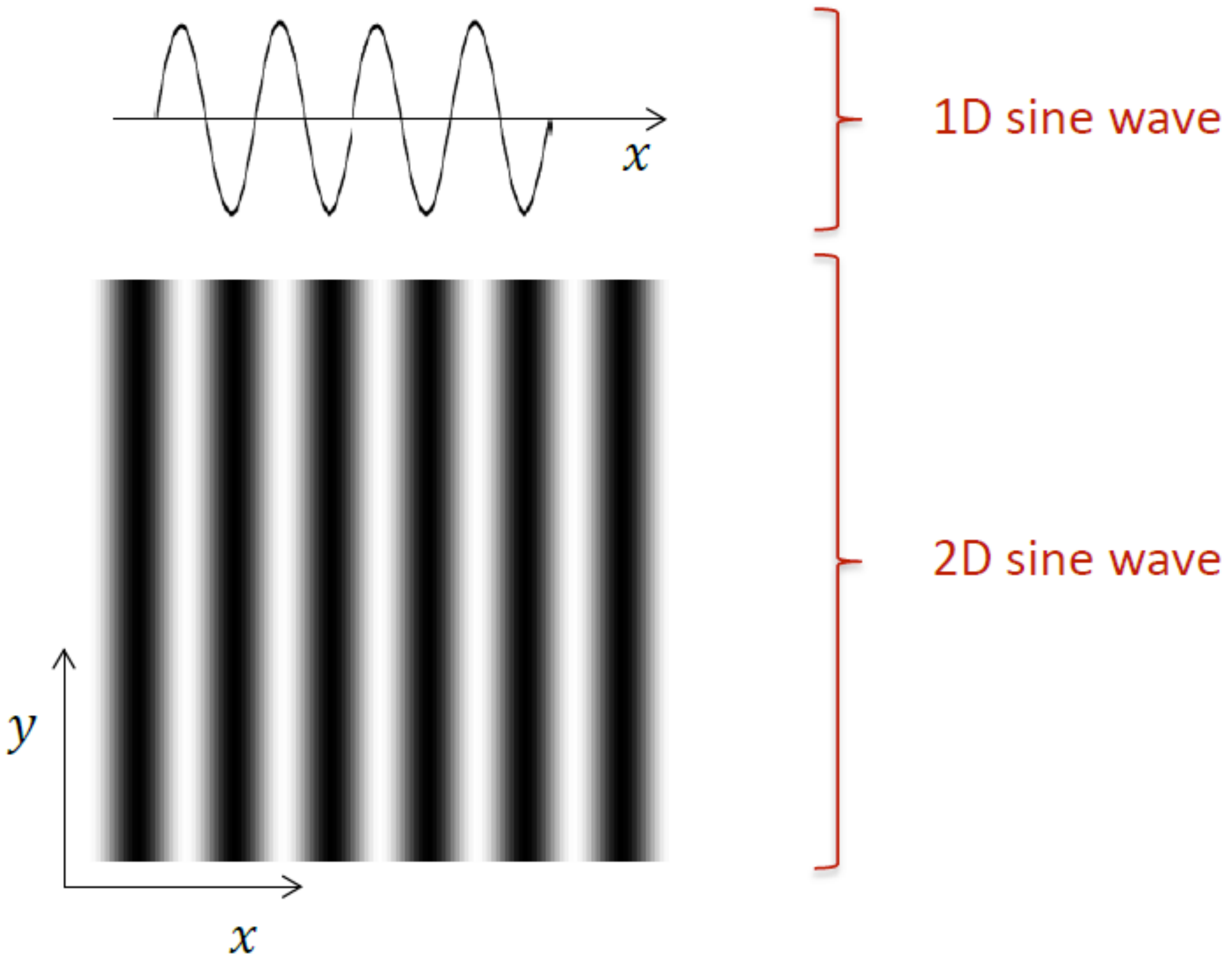
Fourier transform in image processing

A useful mathematical tool for image processing tasks

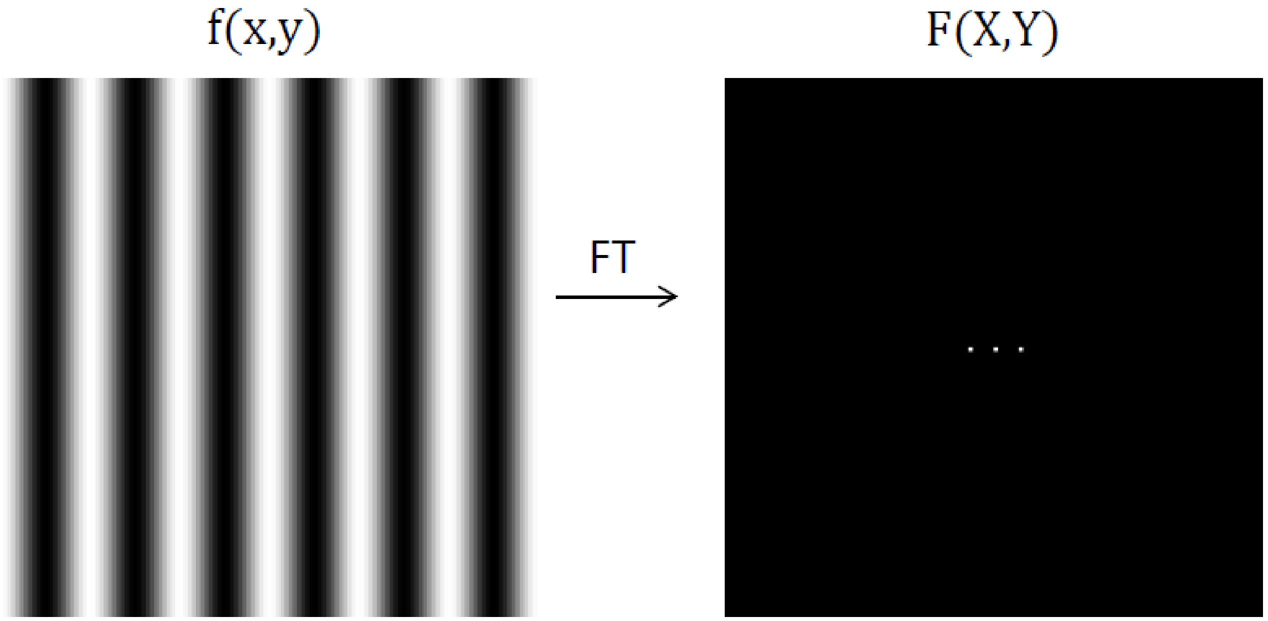


The Fourier transform in 2D

Fourier transform in 2D

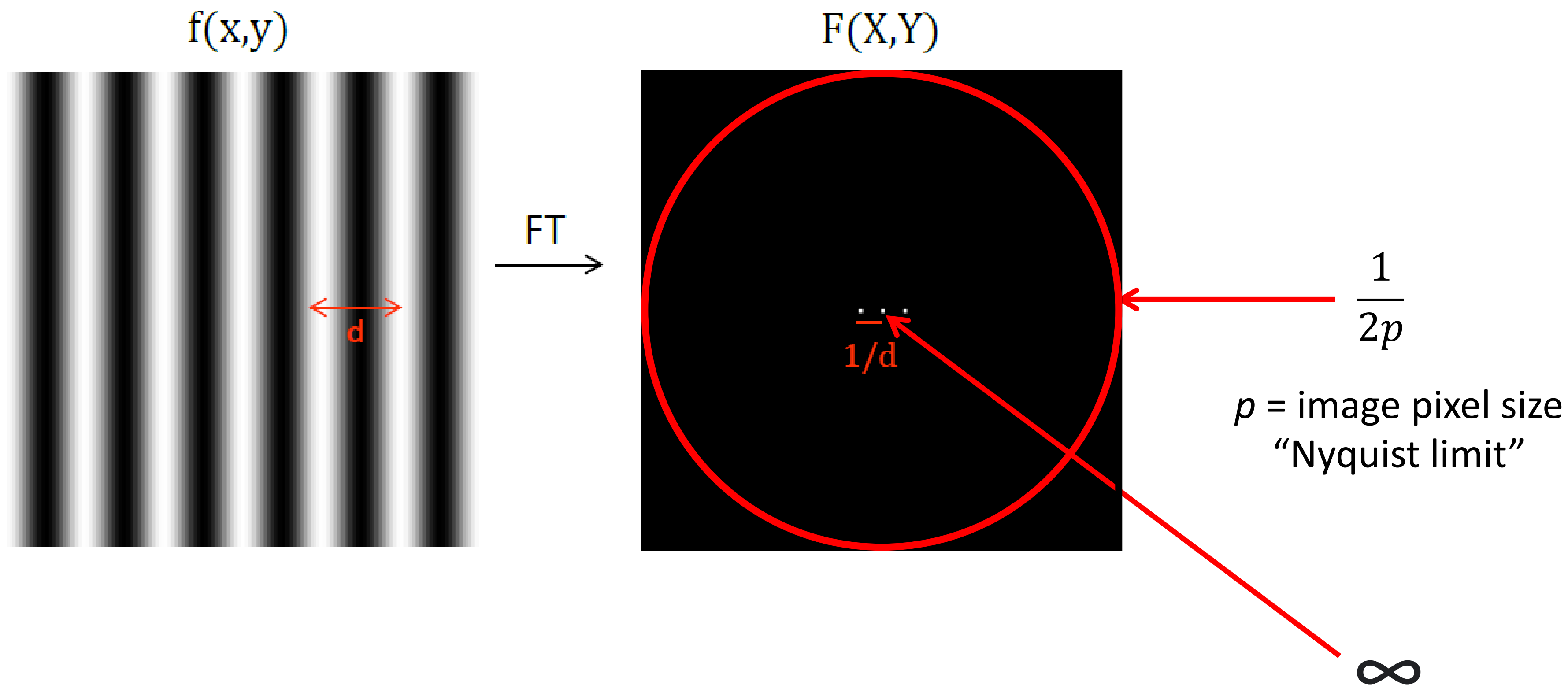


Fourier transform in 2D



Fourier transform in 2D

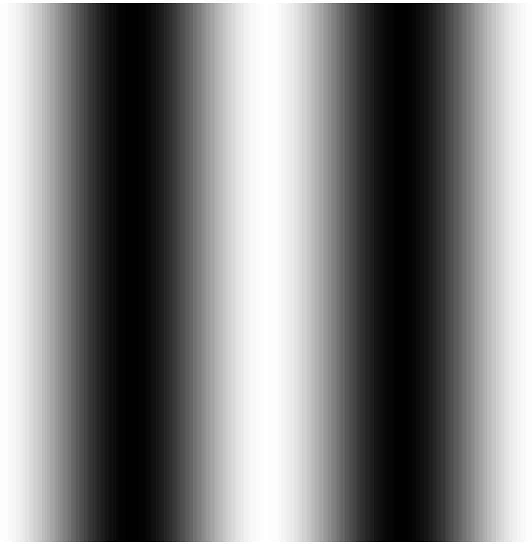
Frequency



Fourier transform in 2D

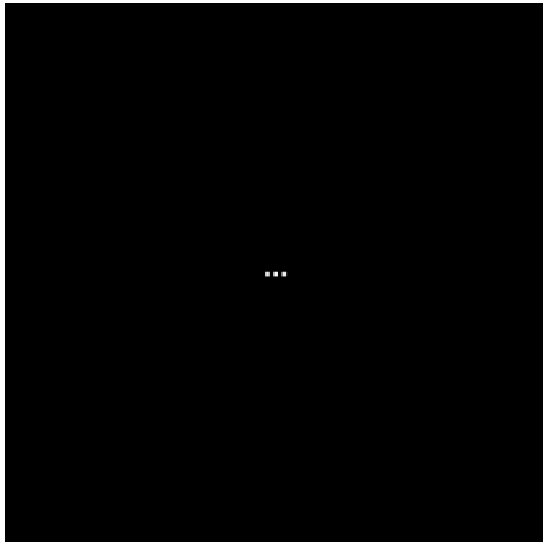
Frequency

f(x,y)



$\xrightarrow{FT}$

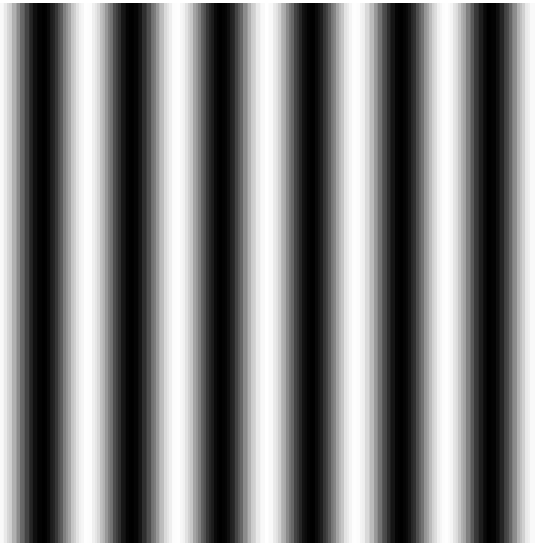
F(X,Y)



Fourier transform in 2D

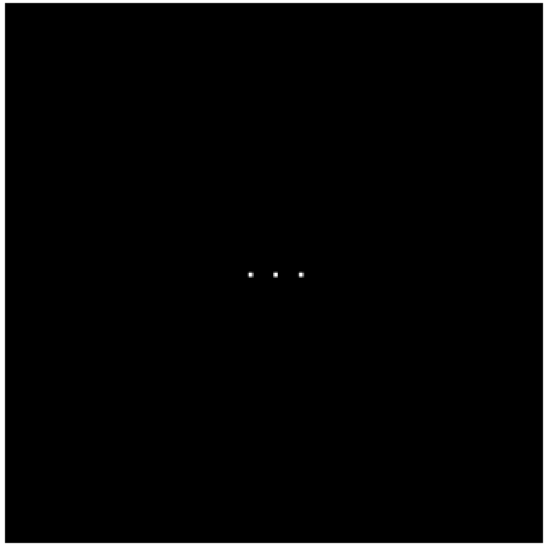
Frequency

f(x,y)



FT →

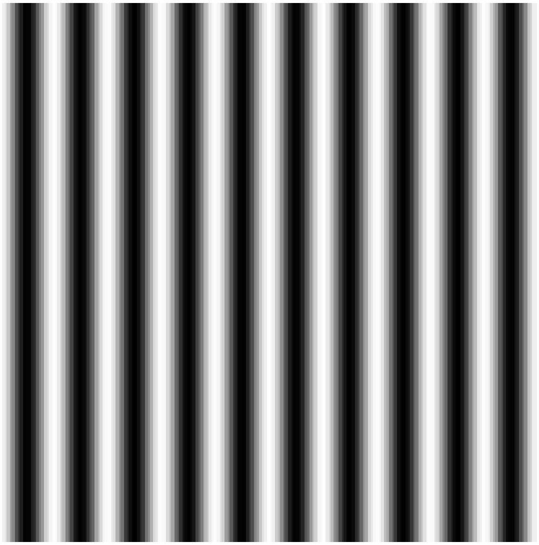
F(X,Y)



Fourier transform in 2D

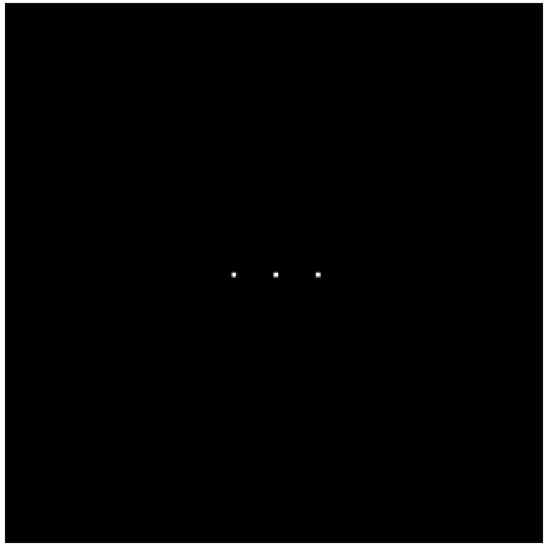
Frequency

f(x,y)



FT →

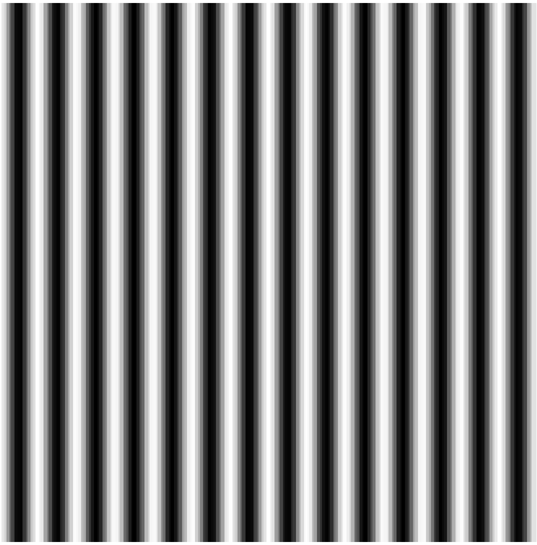
F(X,Y)



Fourier transform in 2D

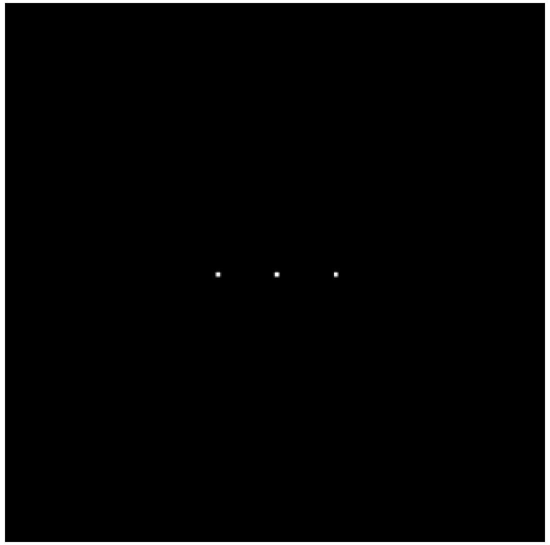
Frequency

f(x,y)



FT →

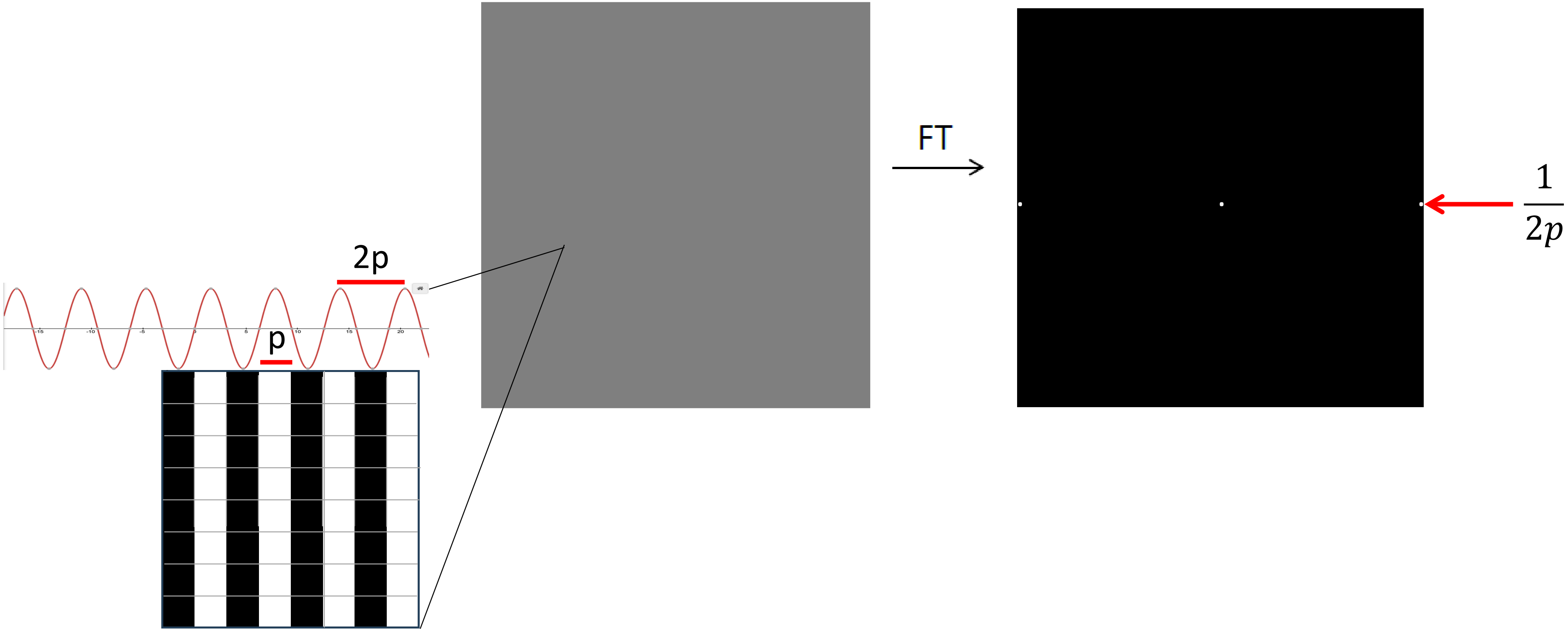
F(X,Y)



Fourier transform in 2D

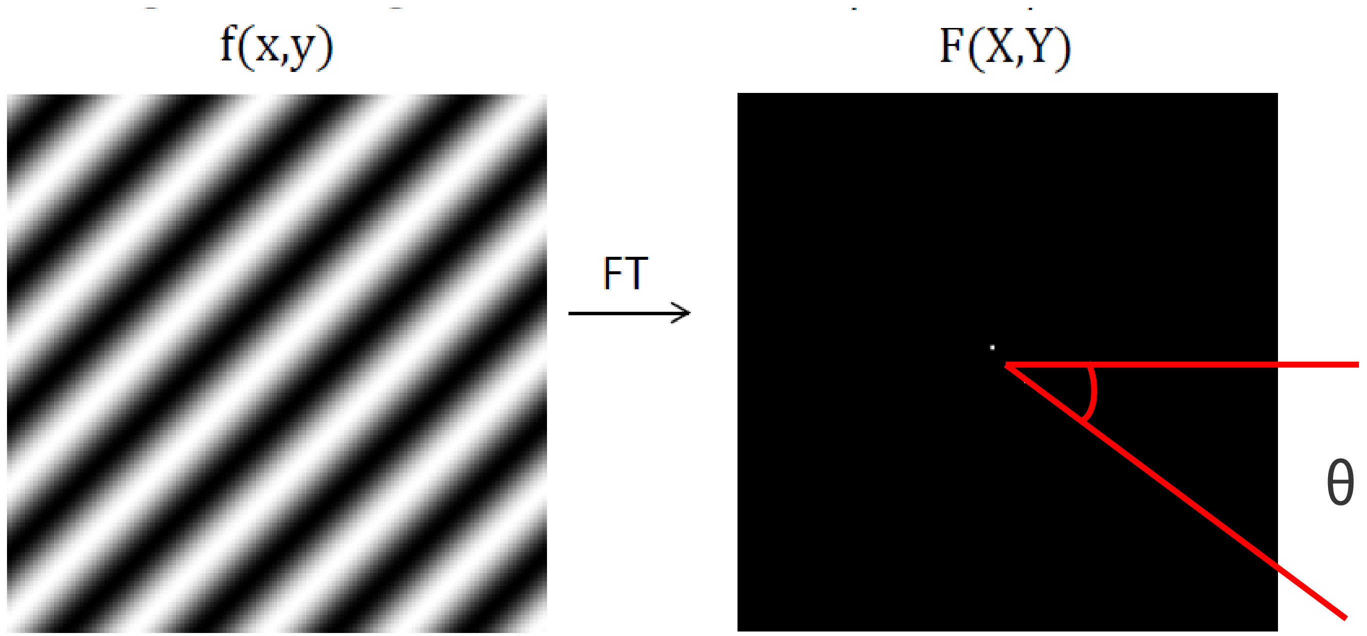
Nyquist-Shannon sampling theorem

Harry Nyquist
1884 - 1976



Fourier transform in 2D

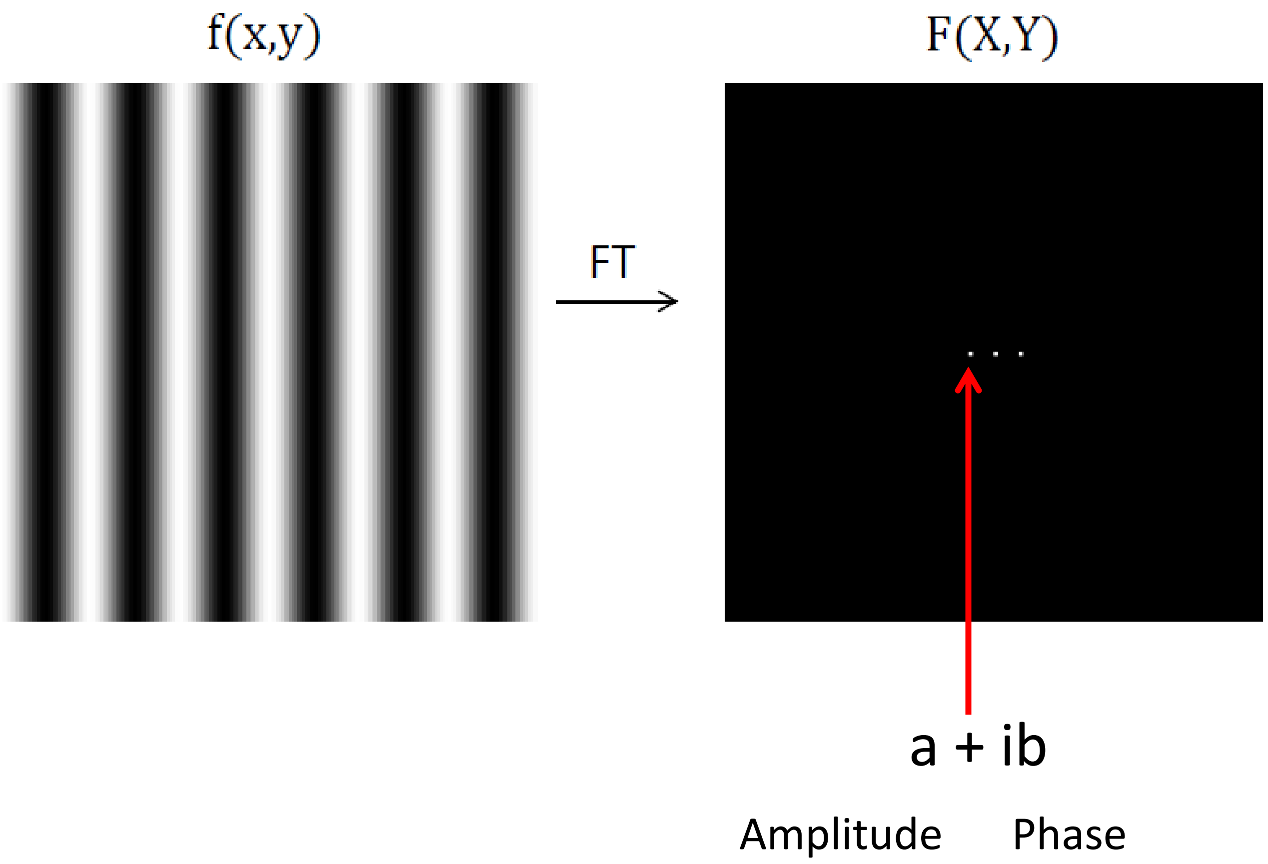
Orientation



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

Fourier transform in 2D

Amplitude & Phase

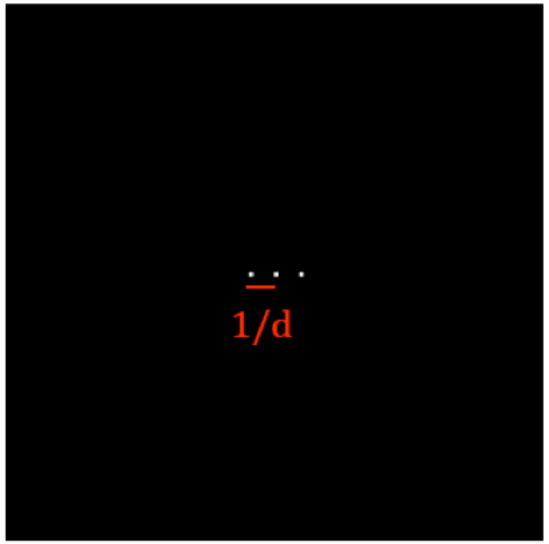


$i = \sqrt{-1}$

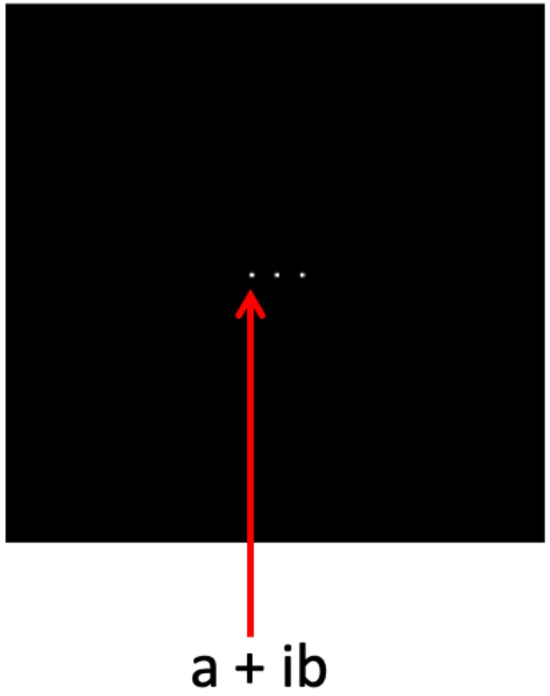
The value of each pixel in the Fourier transform is a complex number

Fourier transform in 2D

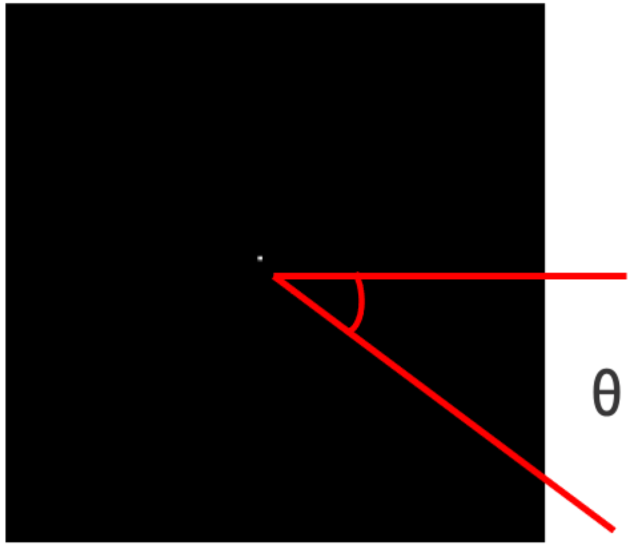
The Fourier transform of a 2D wave gives 4 attributes of each term in the Fourier series



Frequency



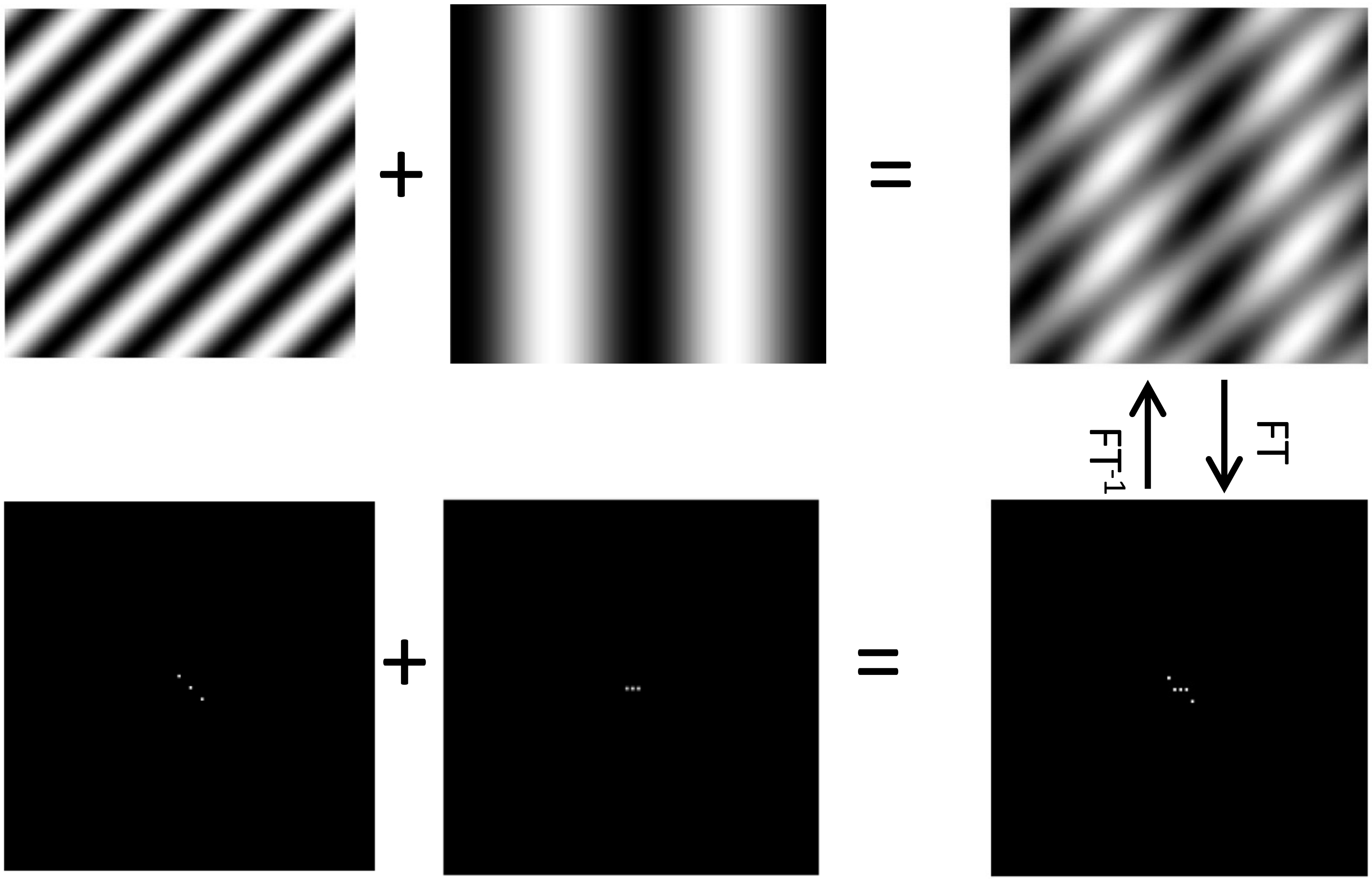
Amplitude and Phase



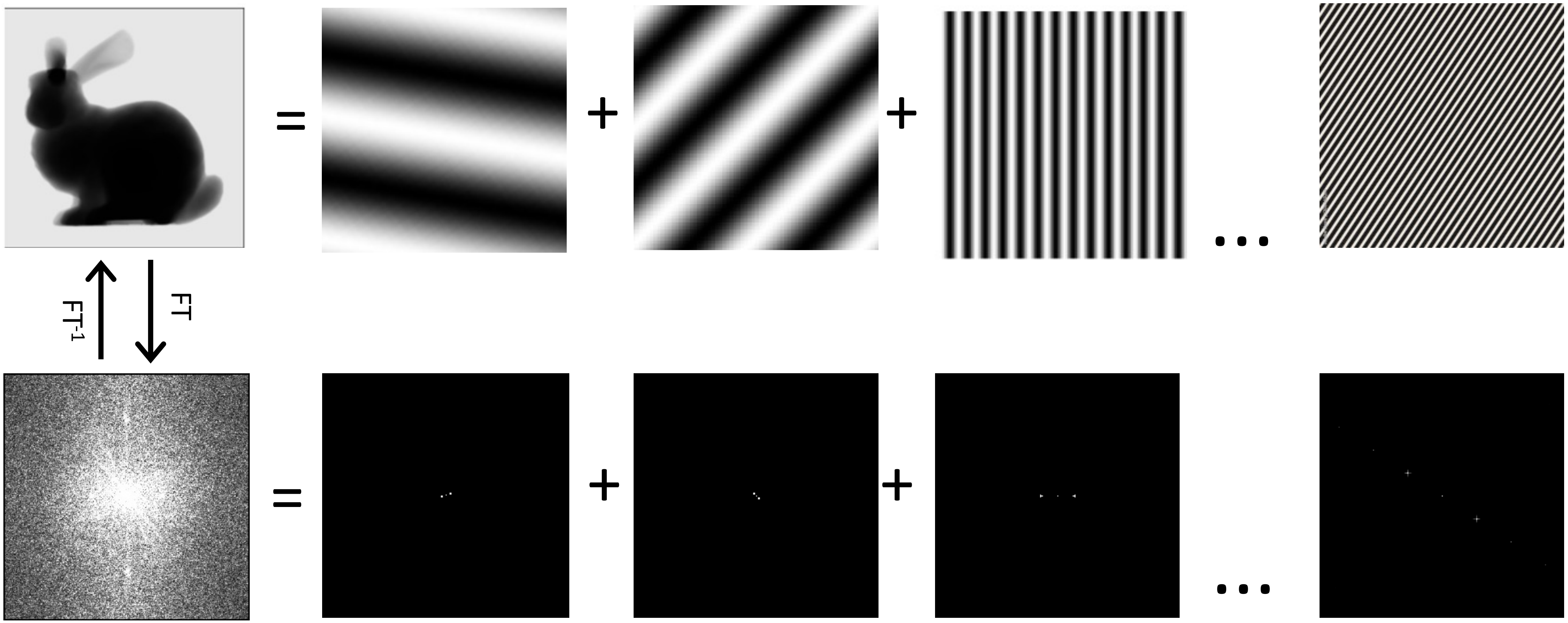
Orientation

Fourier transform of images

The FT of summed images is the sum of their Fourier transforms

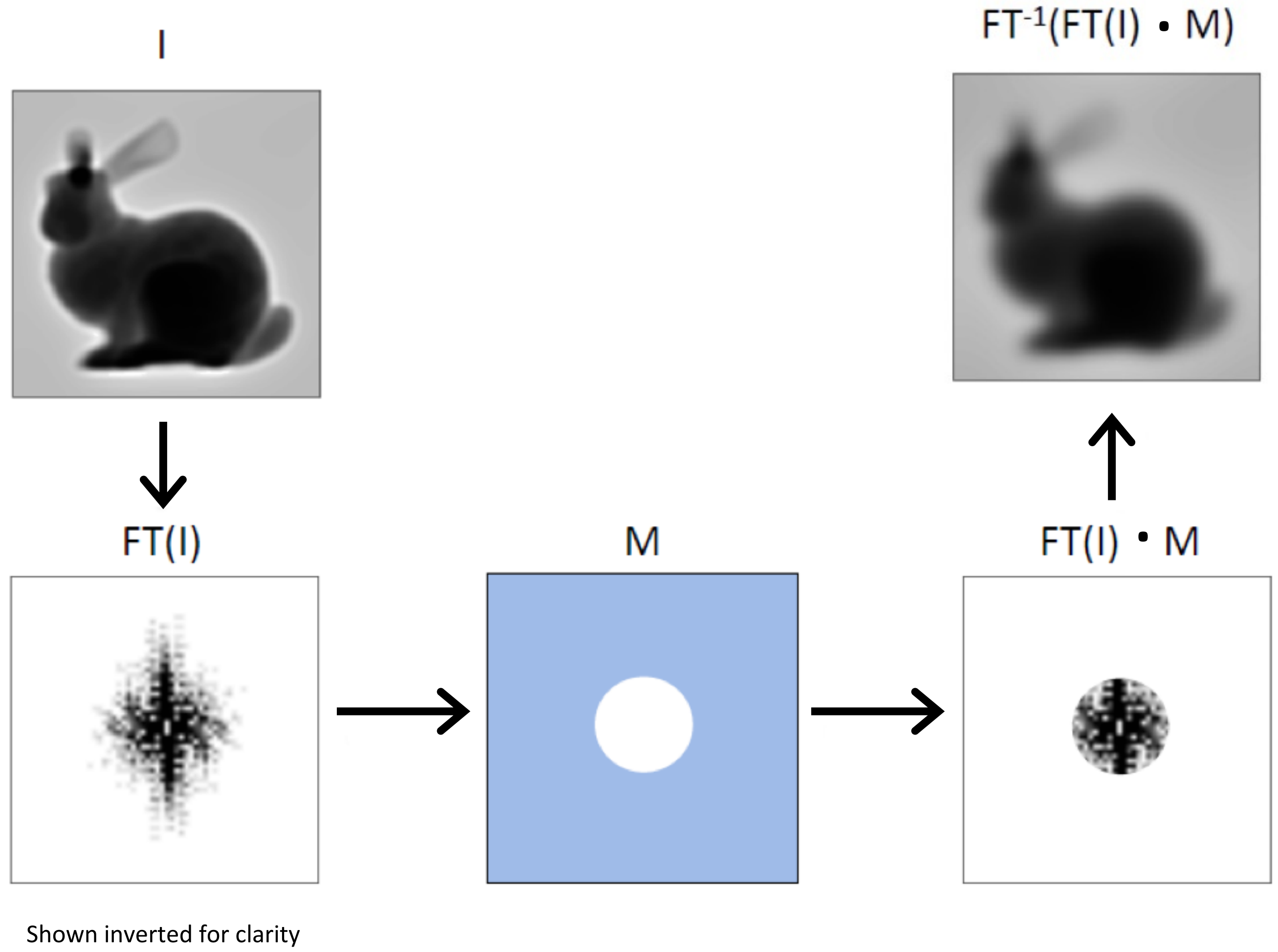


A 2D image is the sum of constituent waves

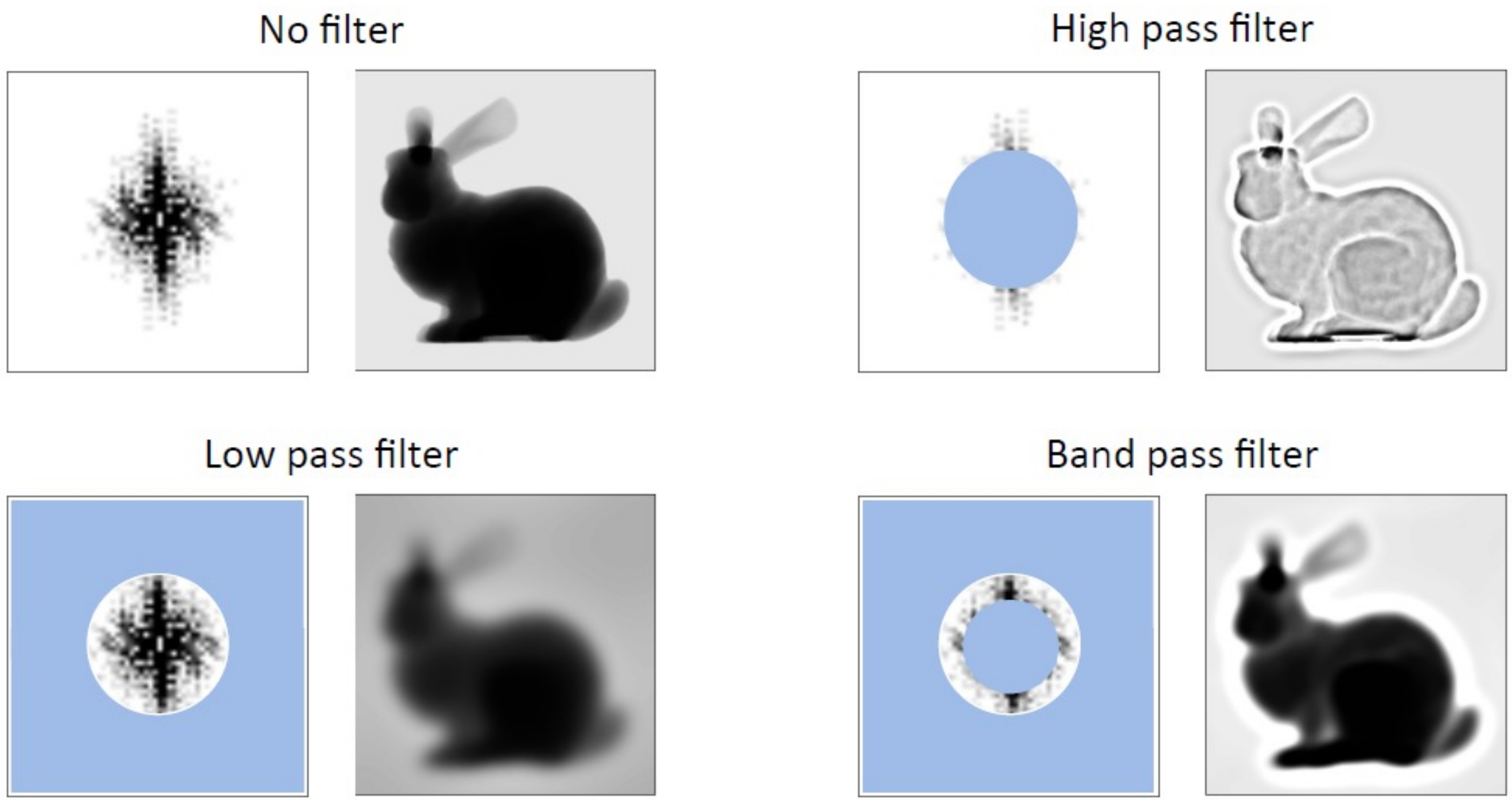


Using the Fourier transform in image processing

Fourier transform in filtering

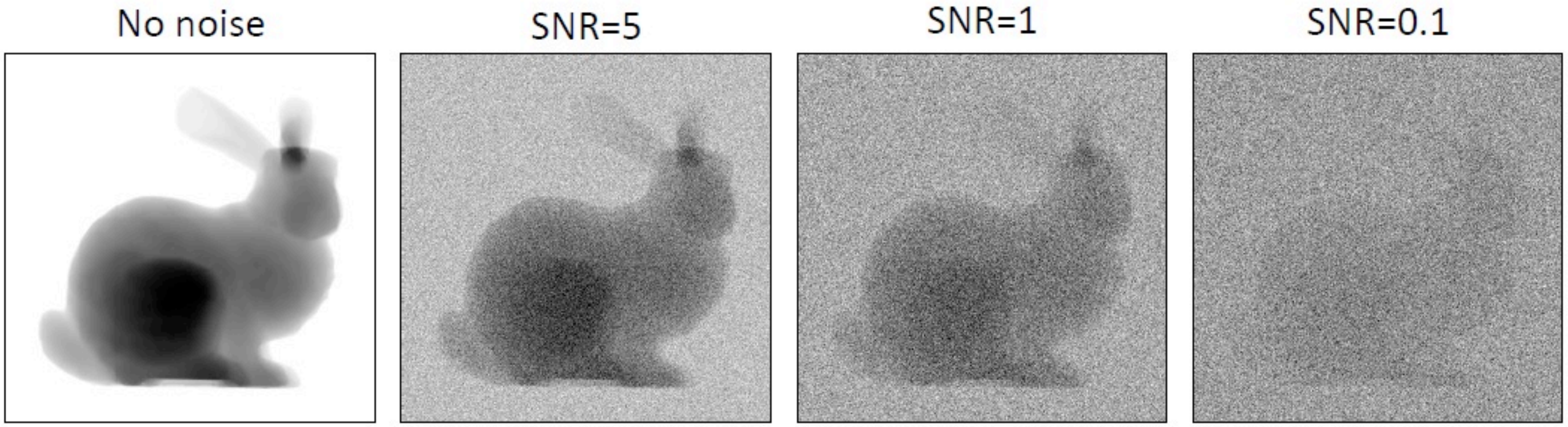


Fourier transform in filtering



Note: Masks shouldn't have sharp edges, instead they should have a Gaussian-shaped (or cosine-shaped) fall of from 1 to 0.

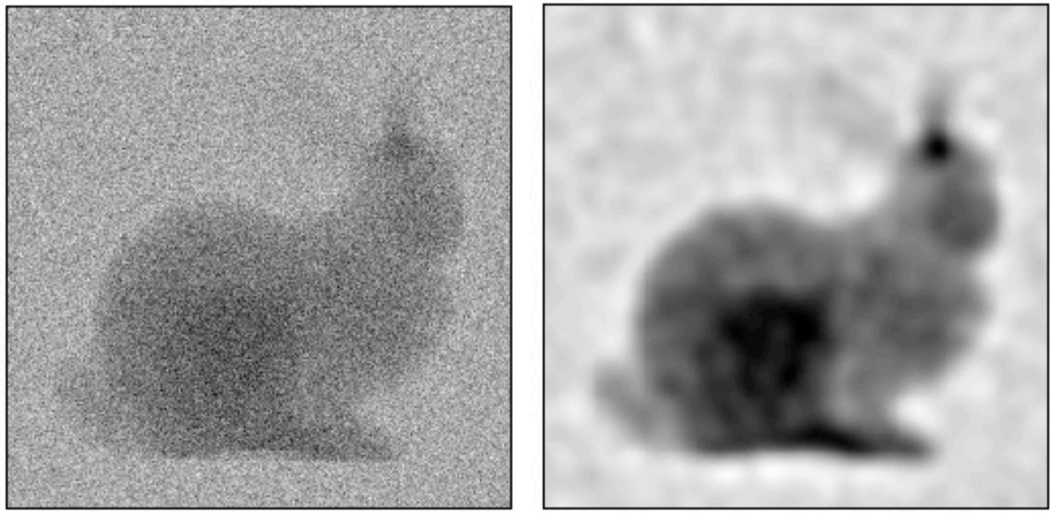
Applications of filtering



Signal-to-noise ratio (SNR) = contrast of the object / standard deviation of the noise

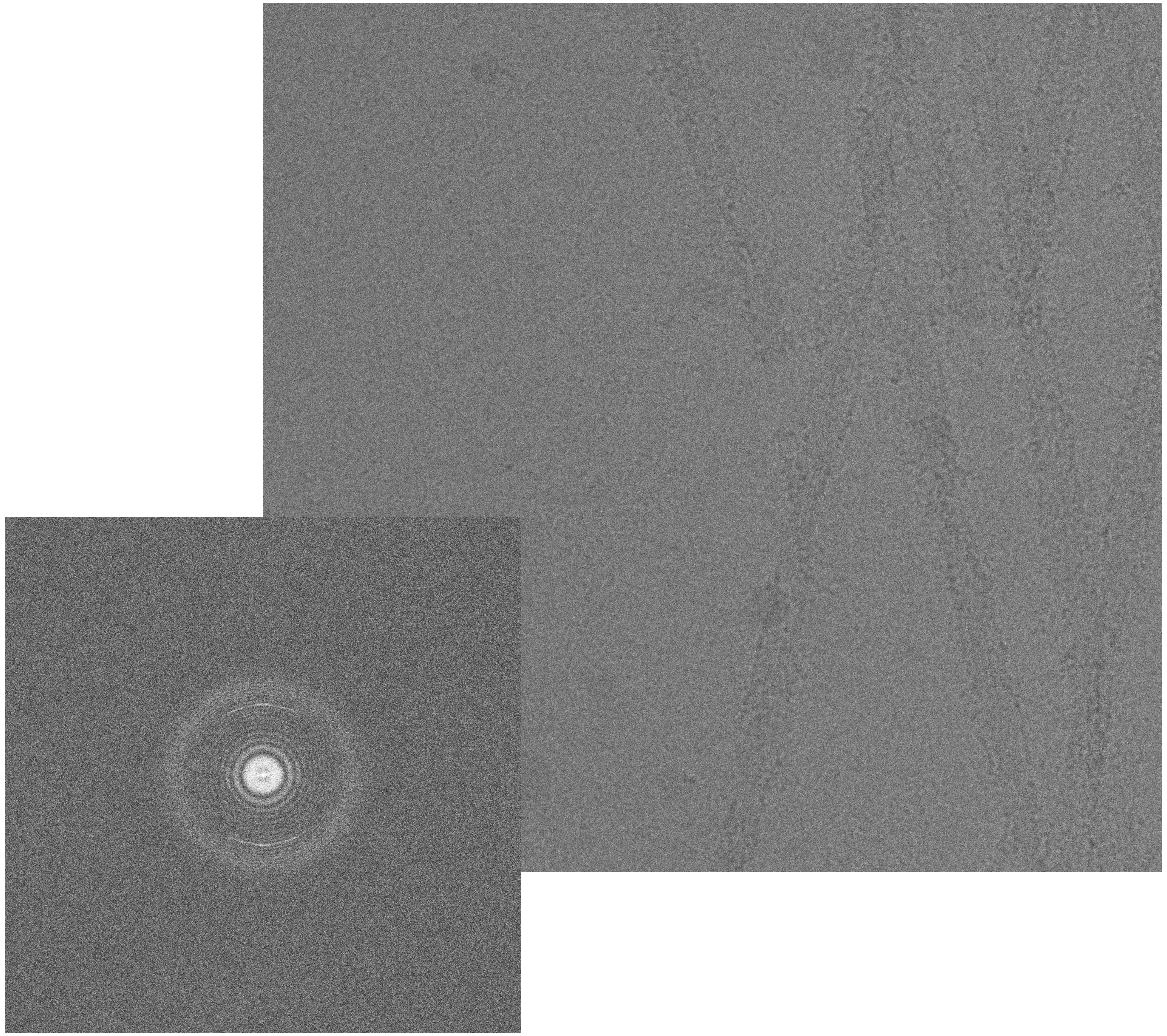
Use of low pass filter

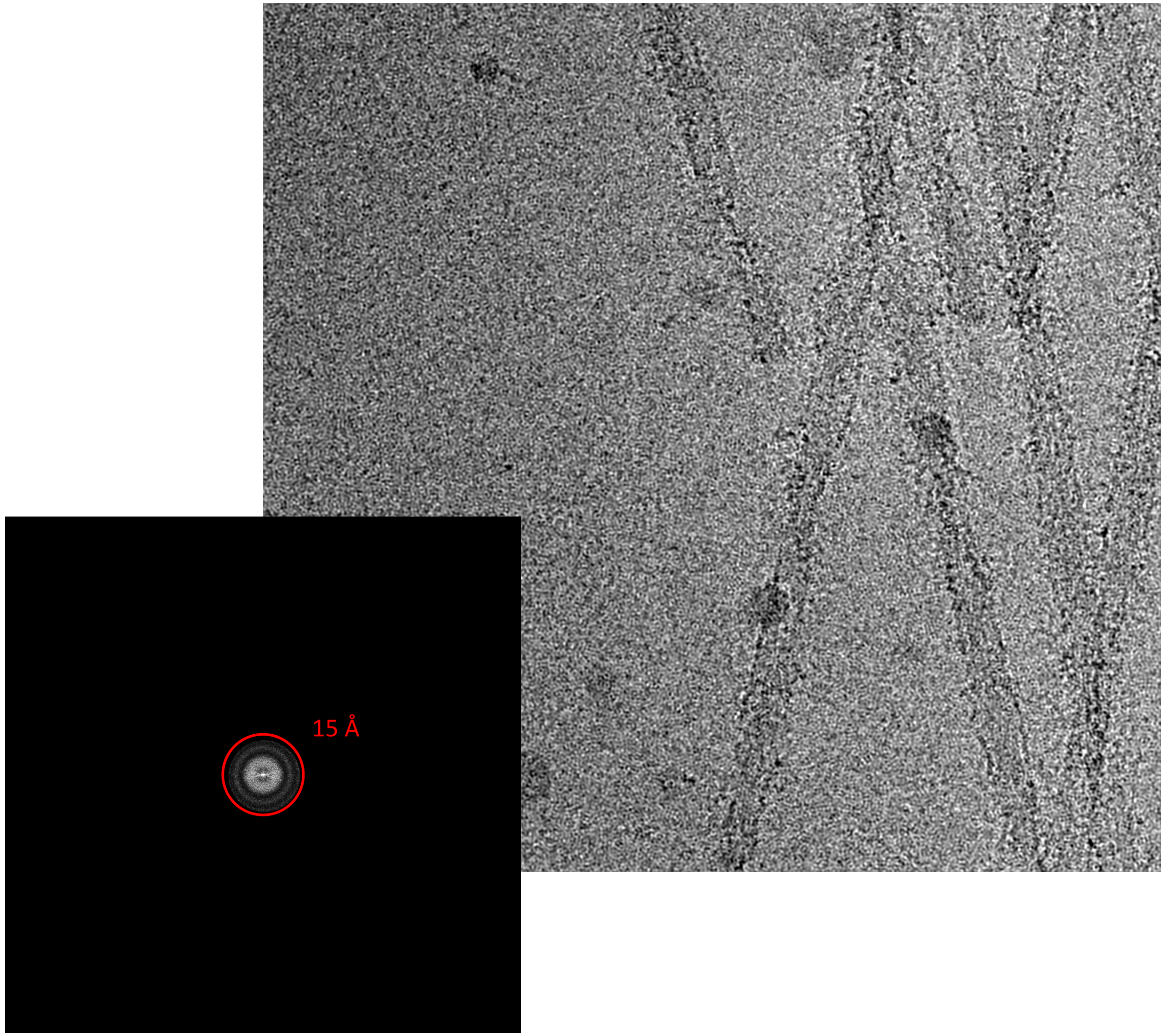
SNR=1 | Low pass filter



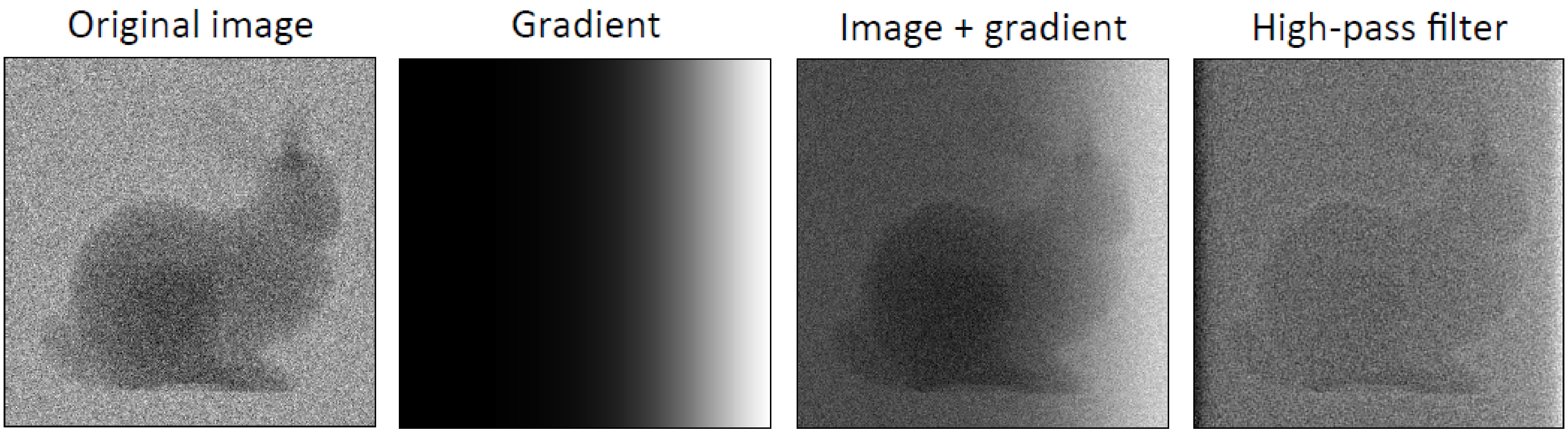
Low pass filter can be used to remove high spatial frequency noise. This makes the object easier to see.

Real world example of low pass filtering



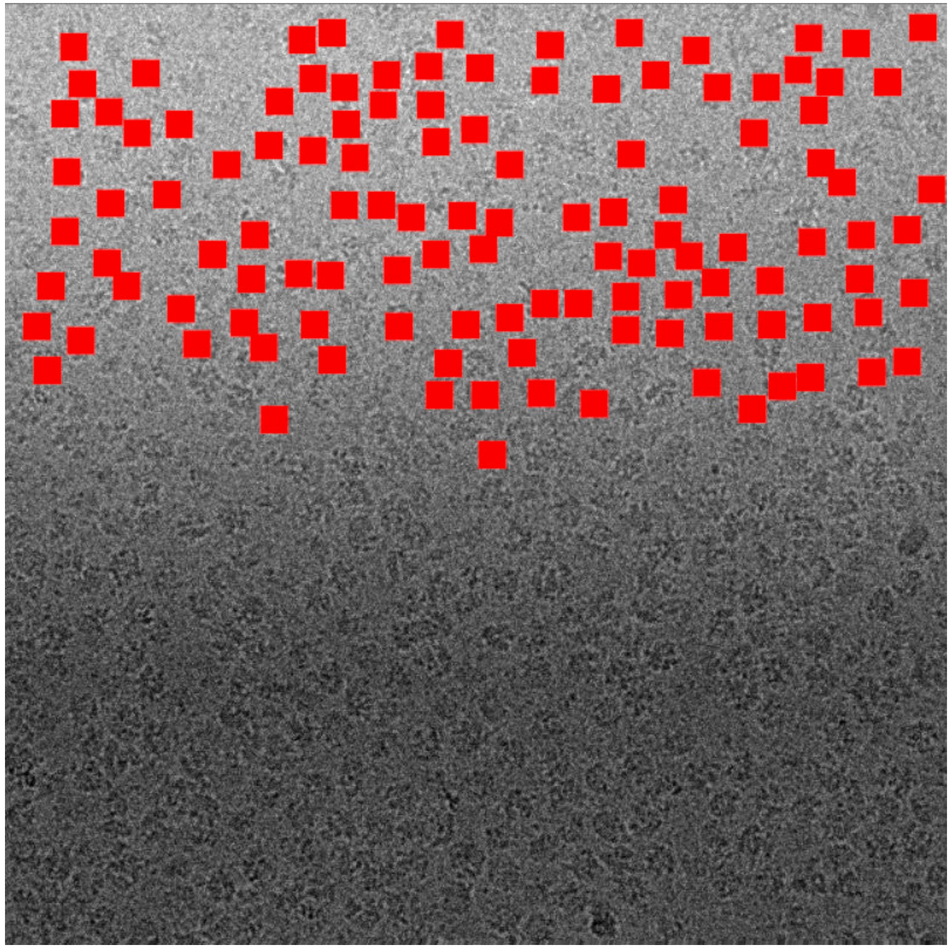


Use of high pass filter

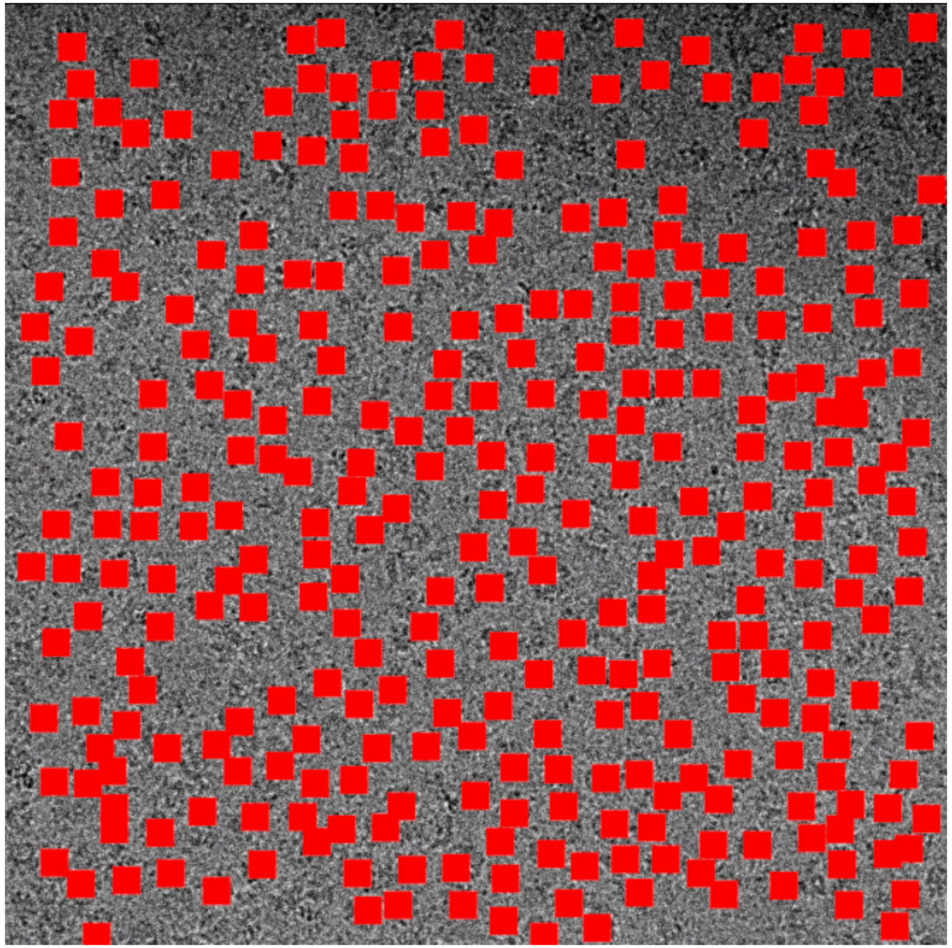


High pass filter can be used to remove low spatial frequency features, such as a density gradient.

Real world example of high pass filtering



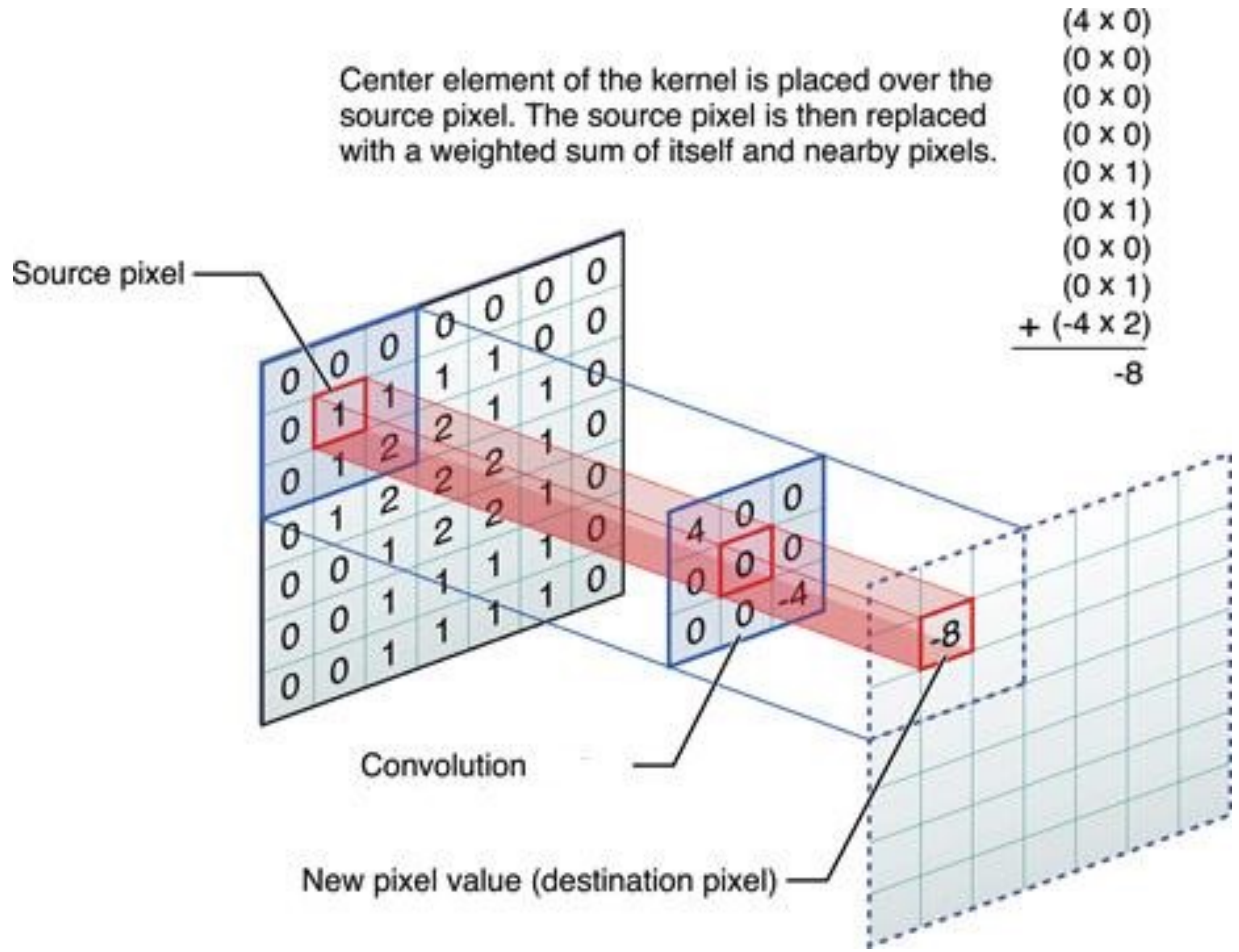
Ice thickness gradient



High pass filter $\frac{1}{325\ \text{Å}}$

Convolution in image formation and processing

Convolution in real space



Convolution in real space is computationally expensive!

convolution of a 2k x 2K image...

Kernal size	# calculations
9 x 9	648,000,000
55 x 55	10,952,000,000
1999 x 1999	31,968,000,000,000

Convolution in Fourier space is much easier

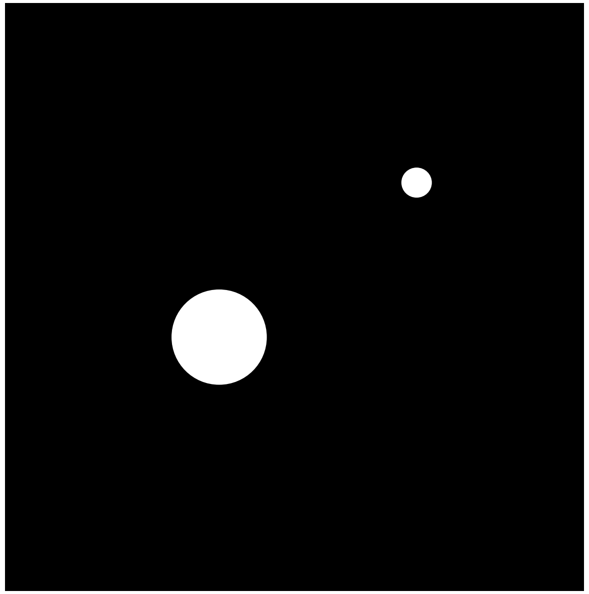
$$f(x) * g(x) = FT^{-1}[F(u) \cdot G(u)]$$

The point spread function

Convolution

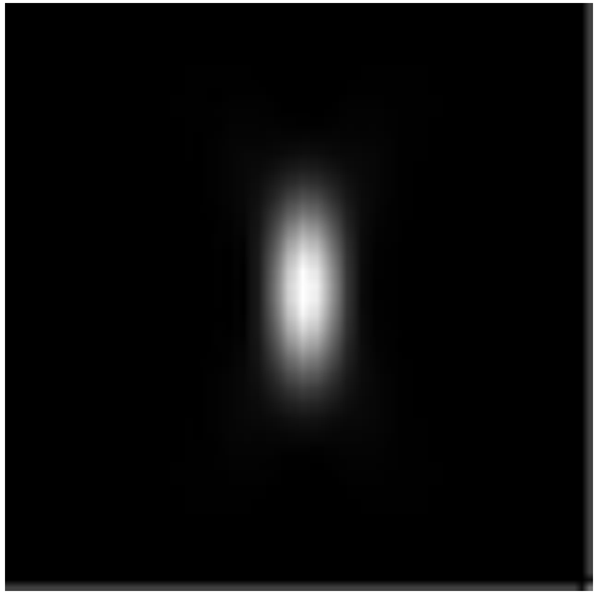
$$f(x) * g(x) = FT^{-1}[F(u) \cdot G(u)]$$

Actual object
$f(x)$



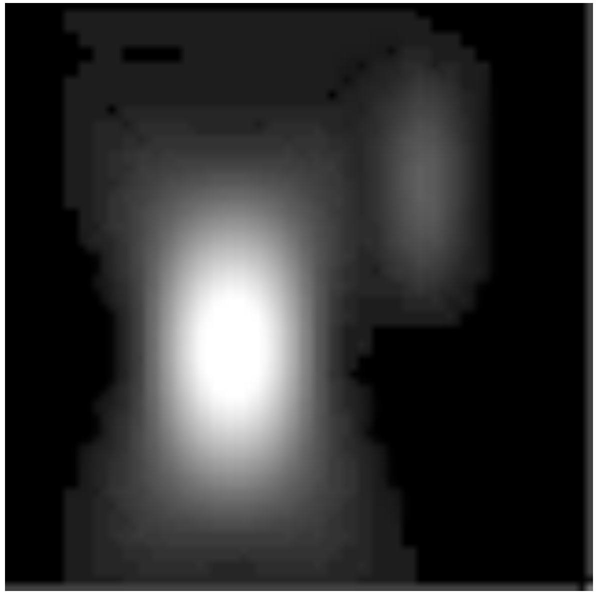
*

PSF
$g(x)$



=

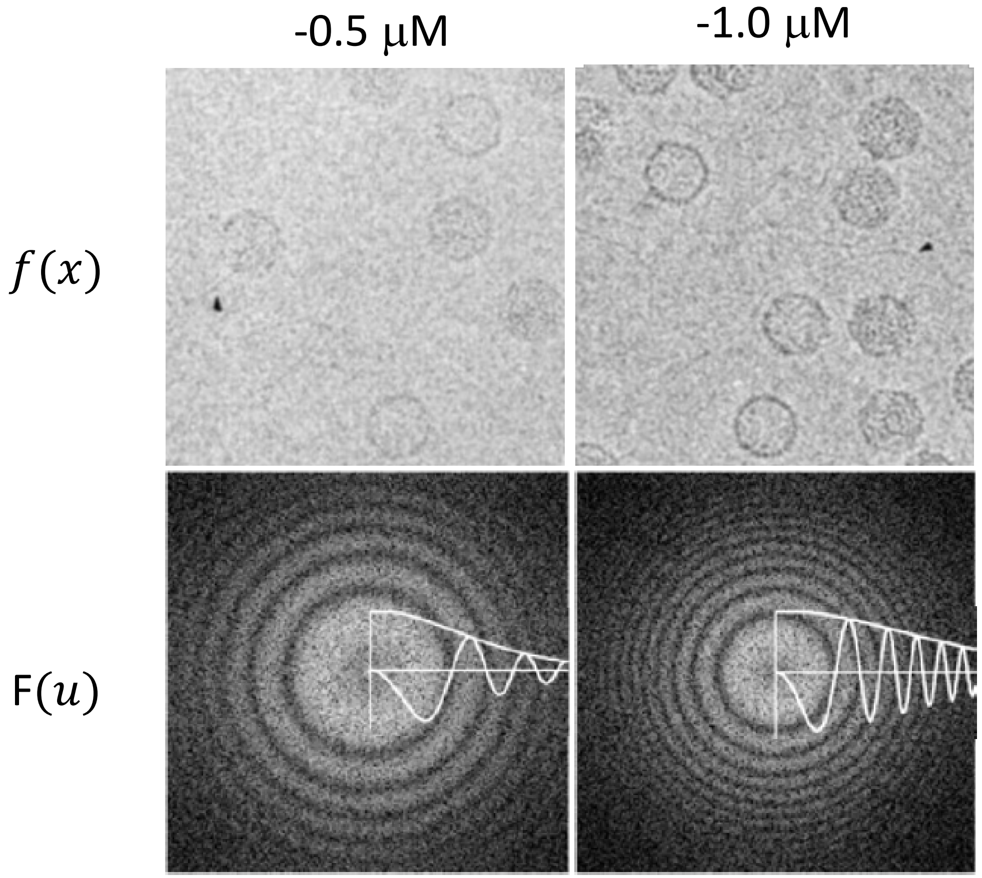
Image
$f(x) * g(x)$



$$f(x) */* g(x) = FT^{-1}\left[\frac{F(u)}{G(u)}\right]$$

Deconvolution

Defocus and Contrast Transfer Function

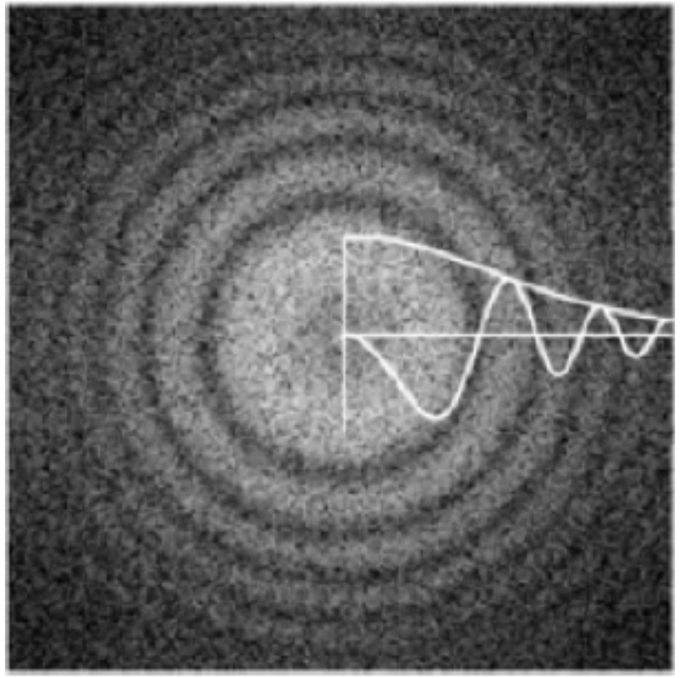


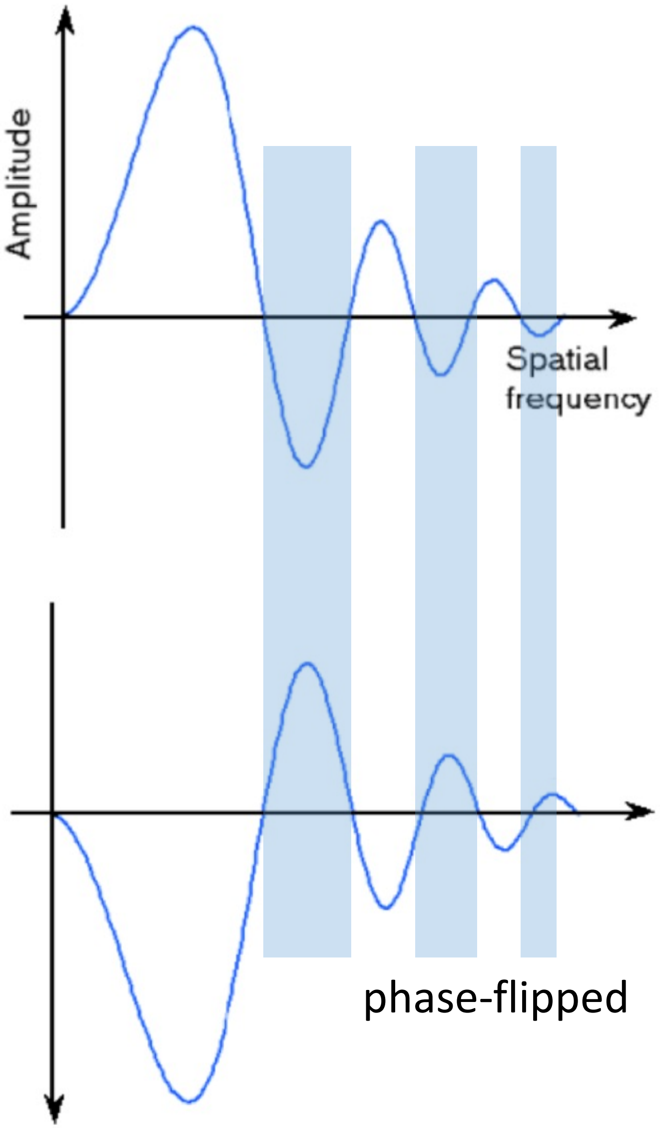
Contrast Transfer Function (CTF): The sum of all the effects of the imaging system

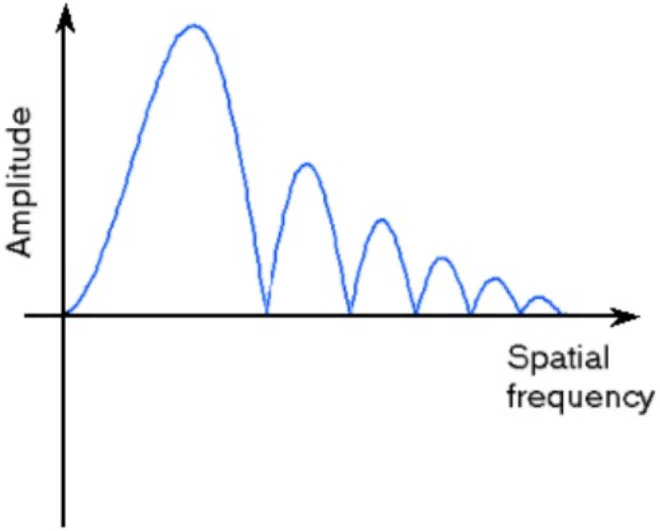
Defocus is applied to an image to increase contrast

The power spectrum (amplitudes of FT squared)
Rings ("Thon rings") are an effect of defocus

Images are ‘CTF corrected’ by phase flipping in Fourier space

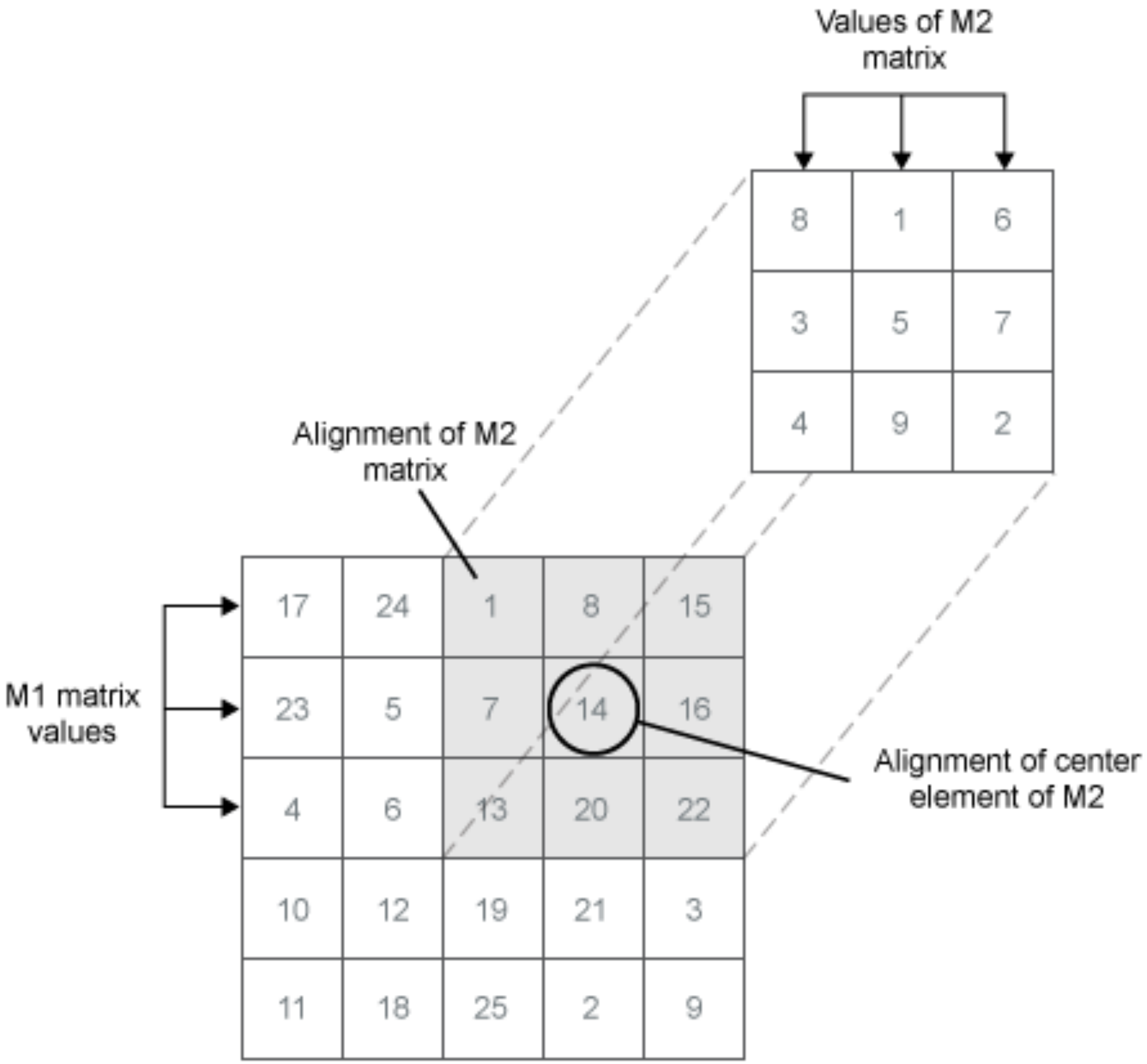






Cross-correlation and image alignment

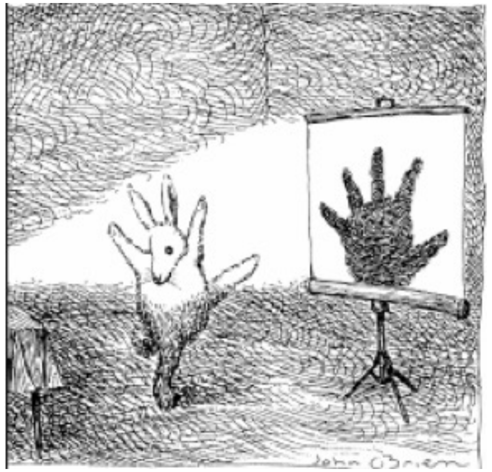
Cross-correlation



The cross-correlation for the indicated position is:

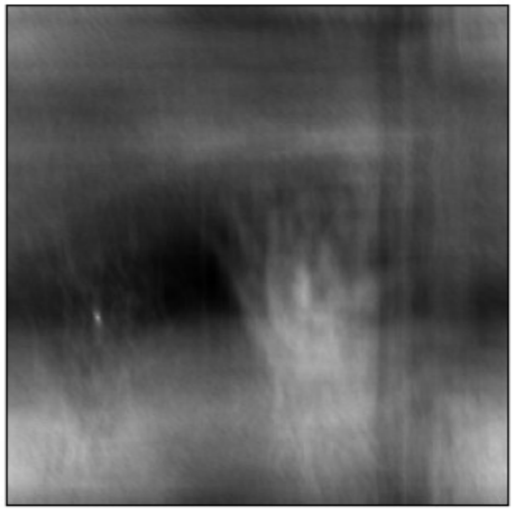
$(1 \cdot 8) + (8 \cdot 1) + (15 \cdot 6) + (7 \cdot 3) \ldots + (22 \cdot 2) = 585$

$f(x)$



g(x)

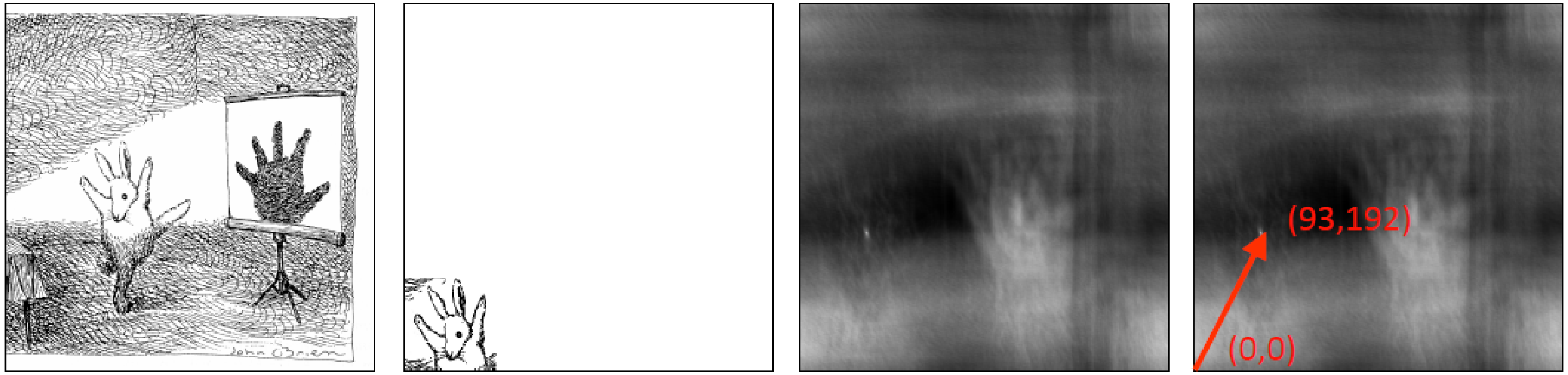
g$(x) \times f(x)$



Cross-correlation and image shifts

- Cross-correlation can be used to align two images (or to search all occurrences of a template image in the target image)
- Peaks in the cross correlation map define the locations
- When calculated in Fourier space, the two images must be of the same size. Here the template (originally 128 × 128) was ‘padded’ in a 512 × 512 box

Target (512 × 512) | Template | Cross correlation map | Shift

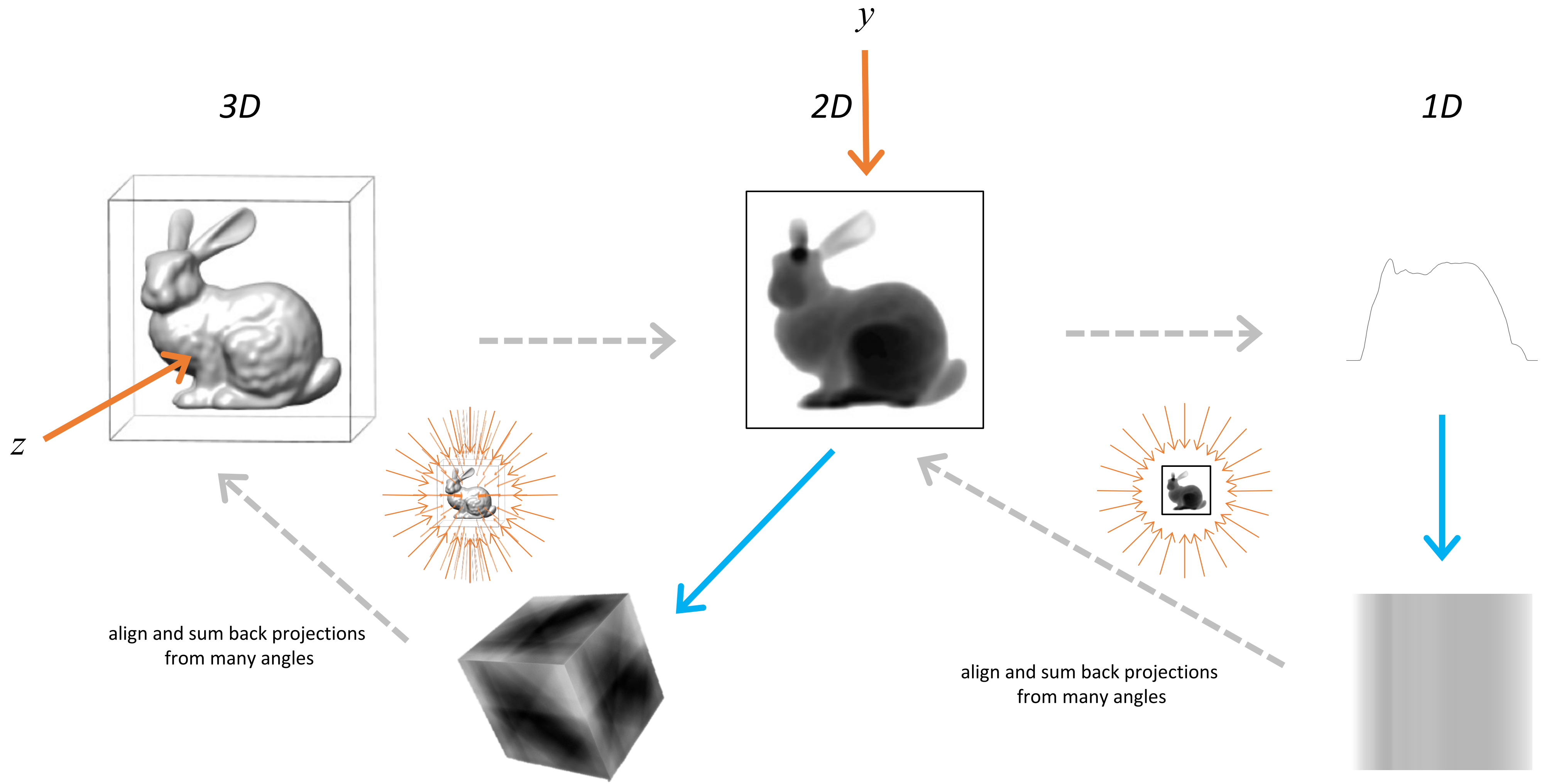


$g(x) \times f(x) = FT^{-1}[F(u) \cdot G^*(u)]$

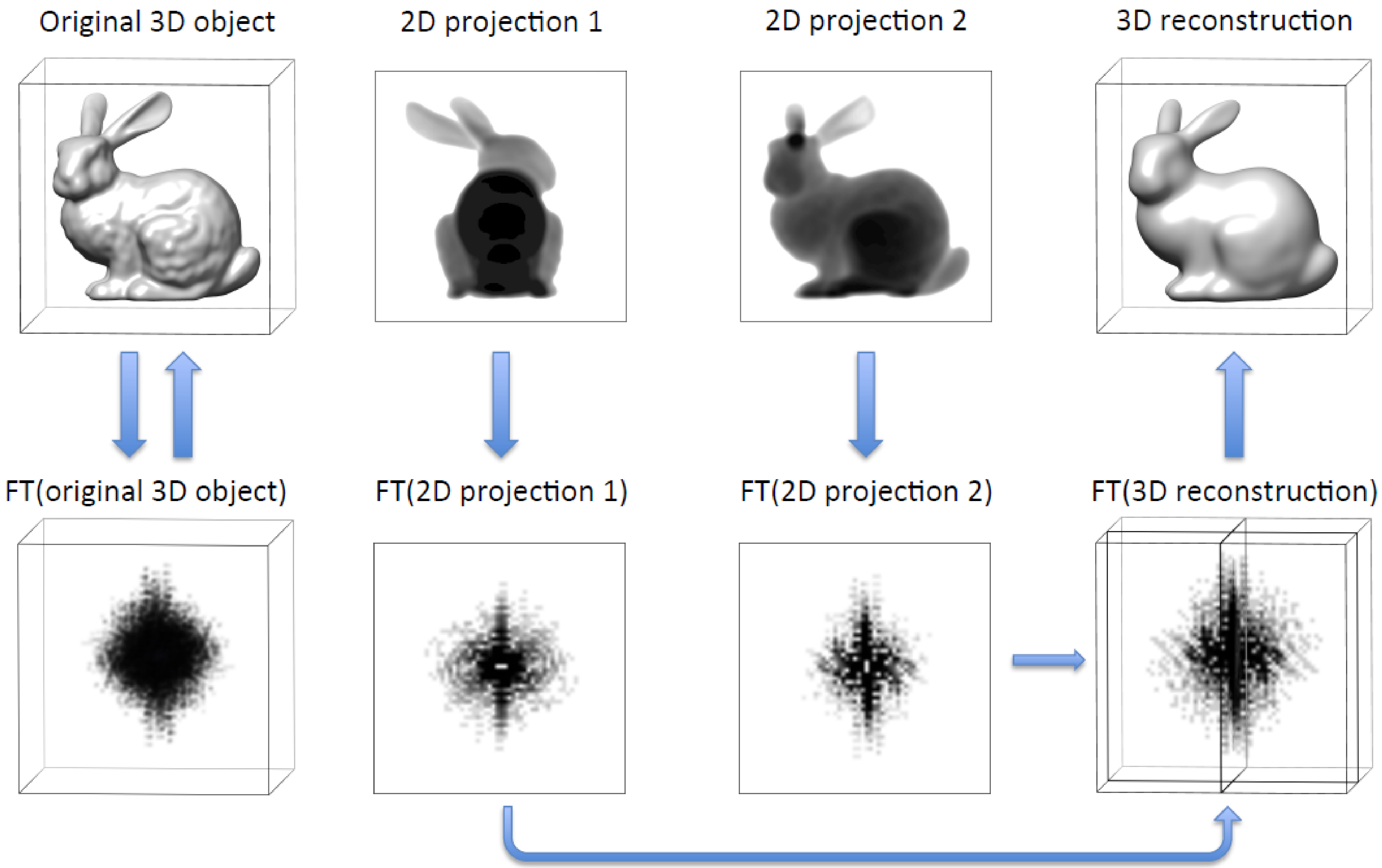
$G^*(u)$ = complex conjugate of $G(u)$

From 2D to 3D: Central Section Theorem and Euler angles

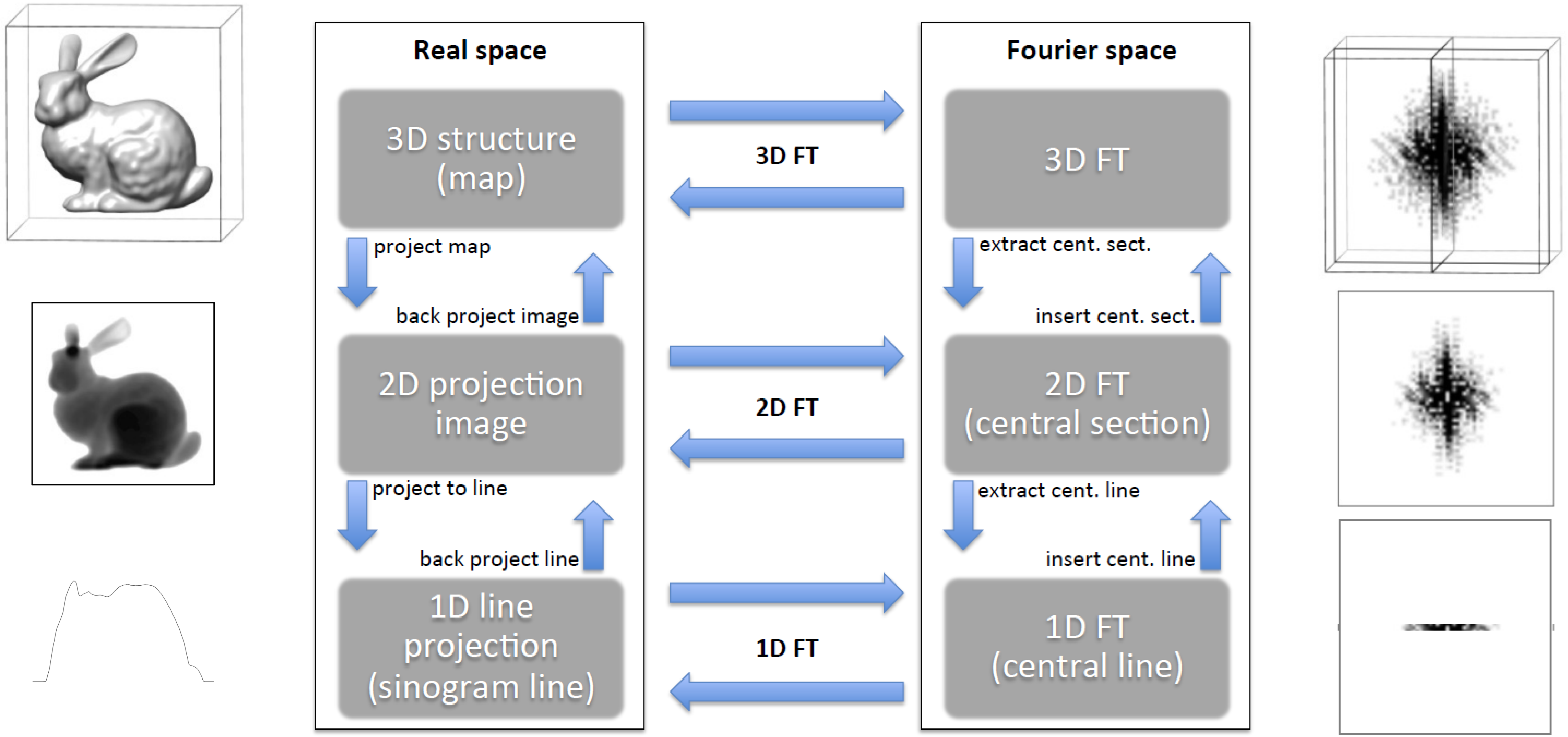
Projection and back projection in real space



Central section theorem



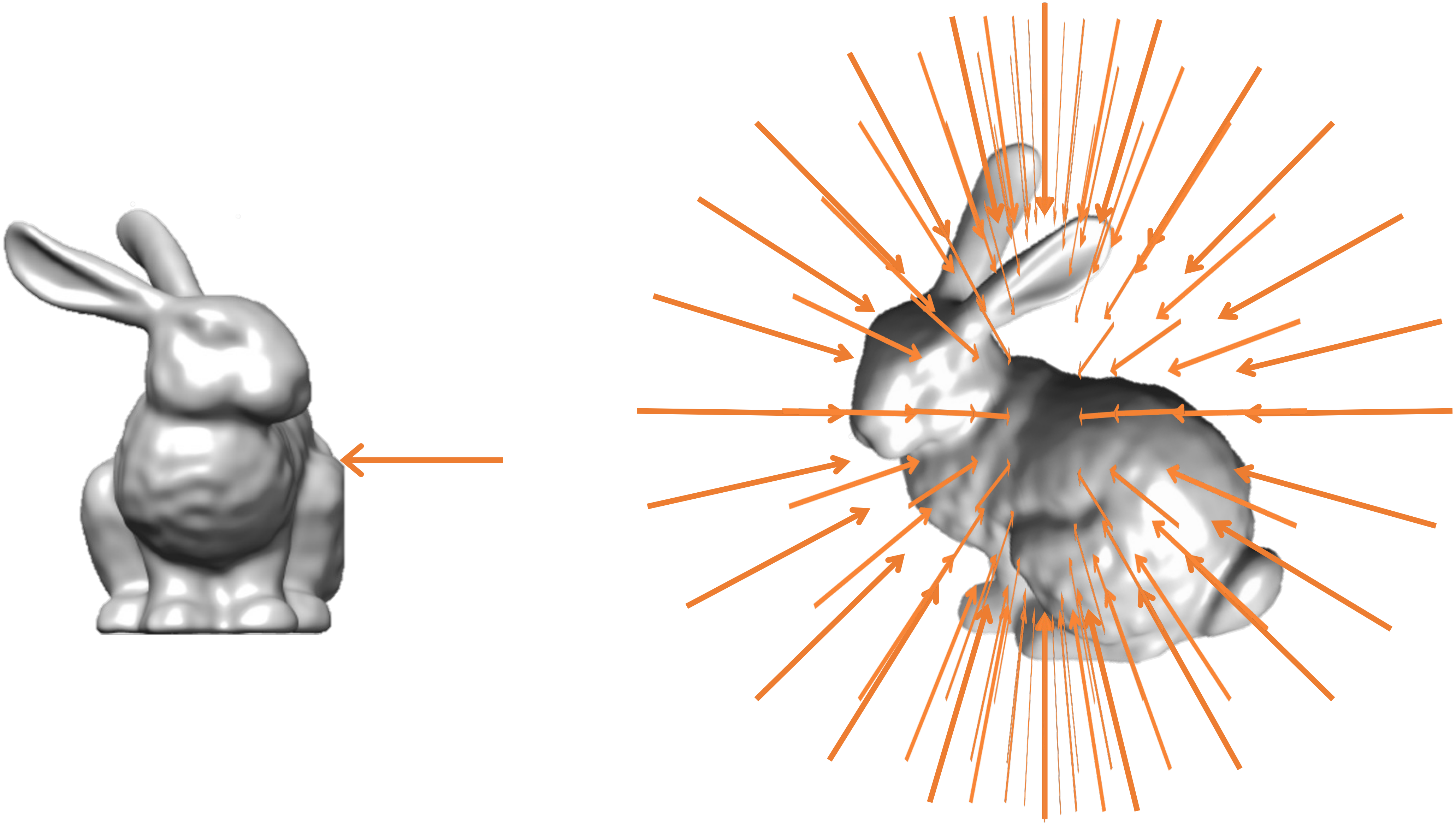
Central section theorem – real space vs. FT



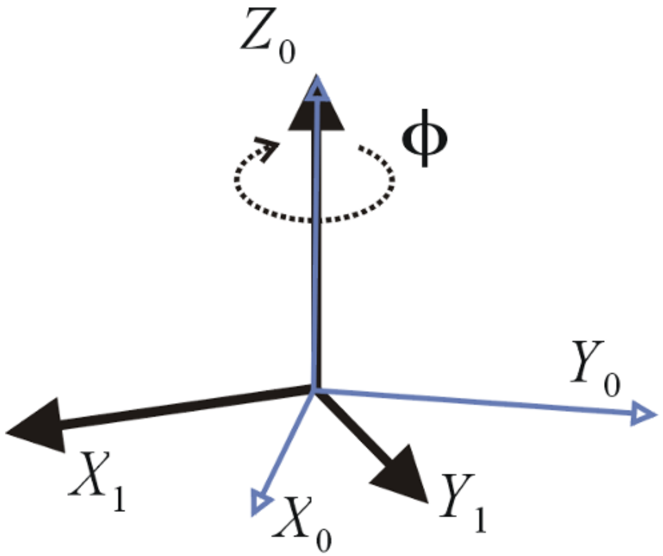
Central section theorem summary

- A central section is a slice passing through the origin (center of the volume)
- The 2D Fourier transform of a projection is a central section of the 3D Fourier transform
- The 3D Fourier transform can be recreated with enough samples from 2D projections of the specimen at different orientations
- The real space reconstruction of the object can be calculated by the inverse Fourier transform of the 3D Fourier transform

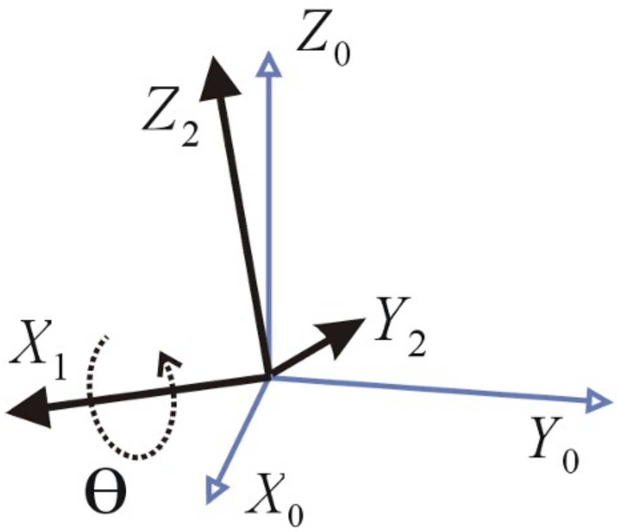
3D objects orientation in space



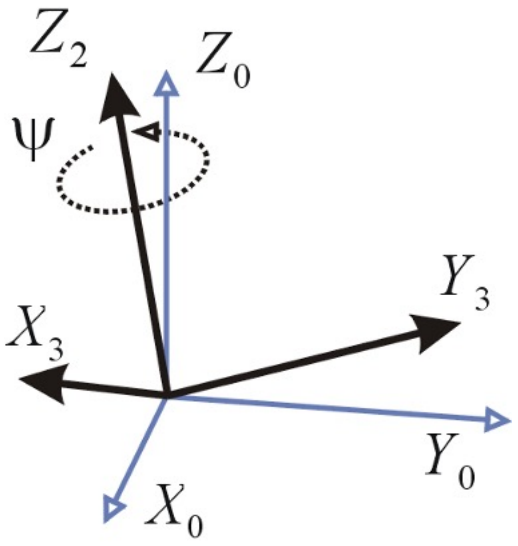
Euler angles ZXZ convention



clockwise around the Z axis
phi
Φ
rotation (rot)



counterclockwise around the new X axis
theta
θ
tilt



counterclockwise around the new Z axis
psi
ψ
psi

Other Euler angle conventions (ZYZ) are sometimes used – be aware of your Euler angle convention

Summary

Fourier transforms are used at almost every step of cryoEM image processing

The Fourier transform and inverse Fourier transform allow us to move between real space space and Fourier space

Many image processing tasks are faster and easier in Fourier space

Error introduced by the imaging process can be corrected in Fourier space
- Deconvolution of the PSF
- Phase flipping to correct defocus induced oscillations

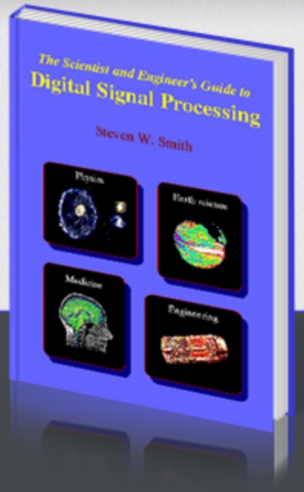
Cross correlation can be used to align images or find a template in an image

A 3D object can be recreated from 2D projections
- Aligning and summing back projections in real space
- Recreating the 3D Fourier transform from central sections

Objects' orientation in 3D space can be described with 3 Euler angles.
- ZXZ convention is generally used in cryoEM
- Be aware of your Euler angle conventions

The Scientist and Engineer's Guide to Digital Signal Processing

By Steven W. Smith, Ph.D.



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