Science and Technology Facilities Council

Introduction to image processing

Colin Palmer

Oxford WT DTP cryo-EM course

27 April 2021

CCP-EM

Many thanks to Bilal Qureshi and Juha Huiskonen for providing most of these slides

Overview

- Introduction
- Fourier transform

- Break

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

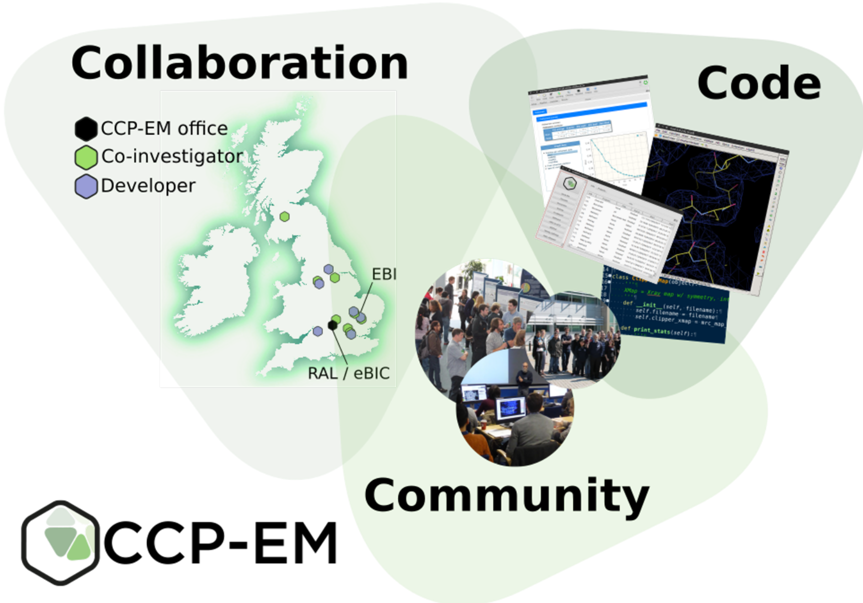
Science and Technology Facilities Council

Introduction – what is CCP-EM?

Collaborative **C**omputational **P**roject for **E**lectron cryo-**M**icroscopy

Aim: support users and developers in computational aspects of biological EM

Other CCP's too: CCP4, CCPN, CCPBioSim and more



Science and Technology Facilities Council

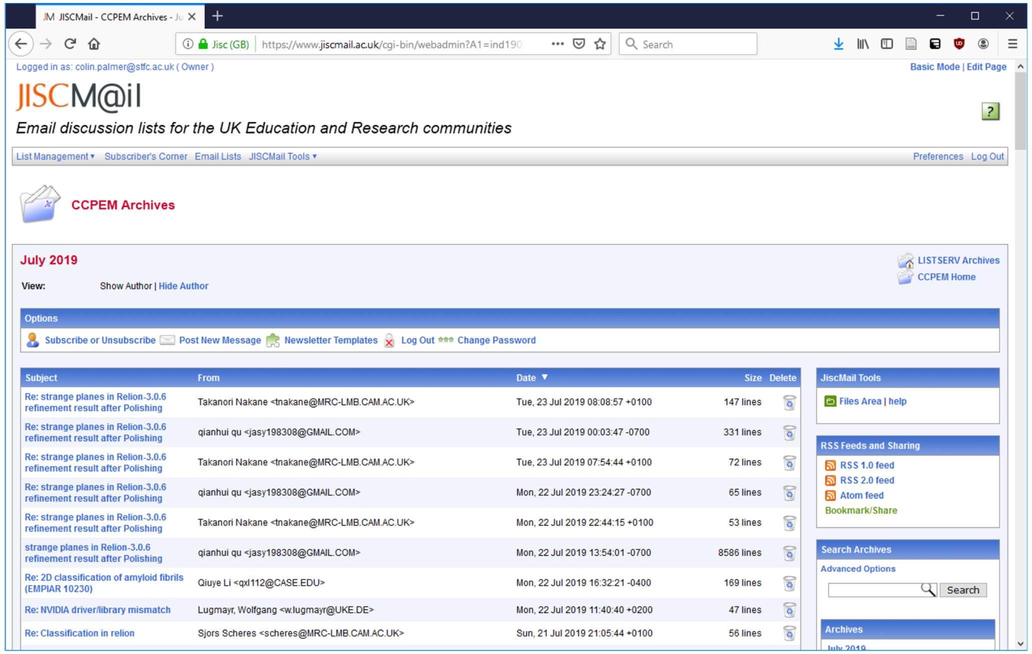
Science and Technology Facilities Council
Medical Research Council

wellcome

Introduction – what is CCP-EM?

When people say “CCP-EM,” they mean…

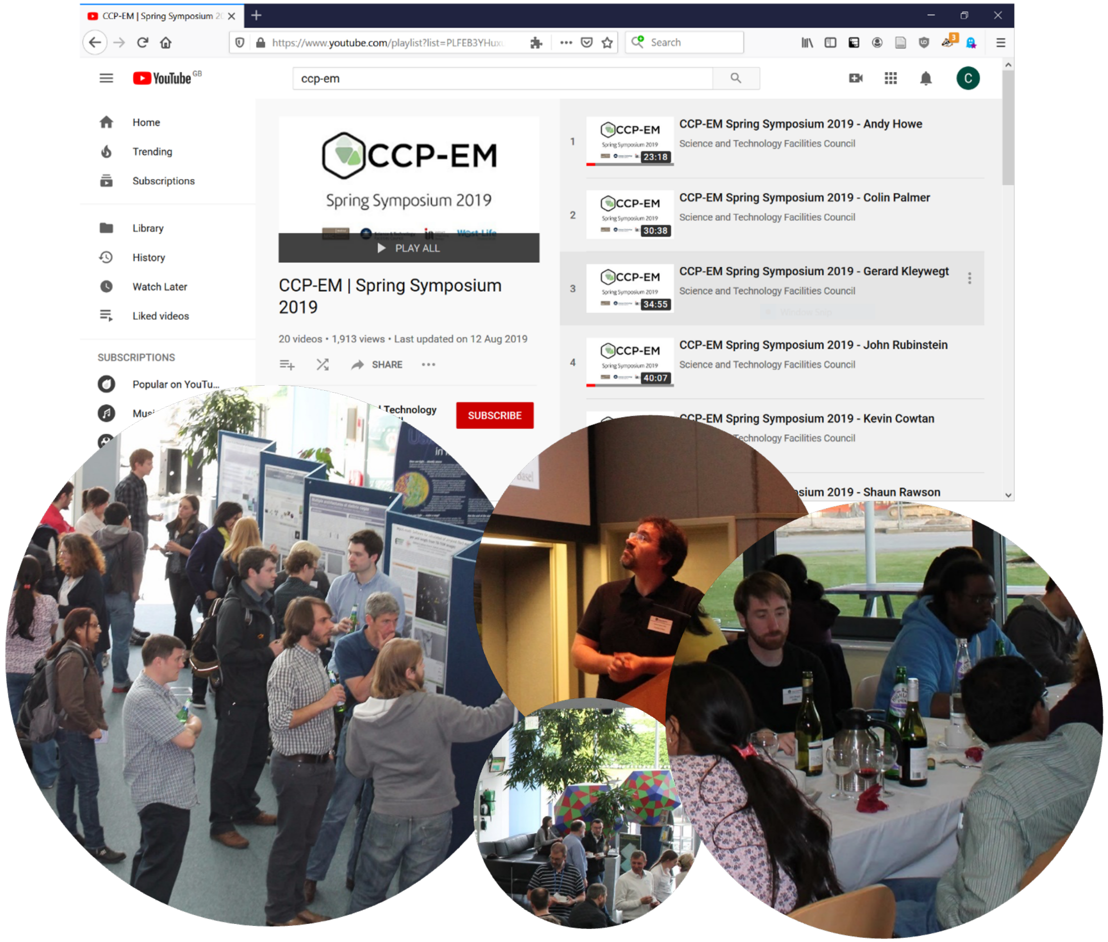
- Mailing list
    - https://www.jiscmail.ac.uk/CCPEM



Introduction – what is CCP-EM?

When people say “CCP-EM,” they mean…

- Mailing list
  - https://www.jiscmail.ac.uk/CCPEM
- Spring Symposium conference
  - Talks on YouTube – search “CCP-EM”

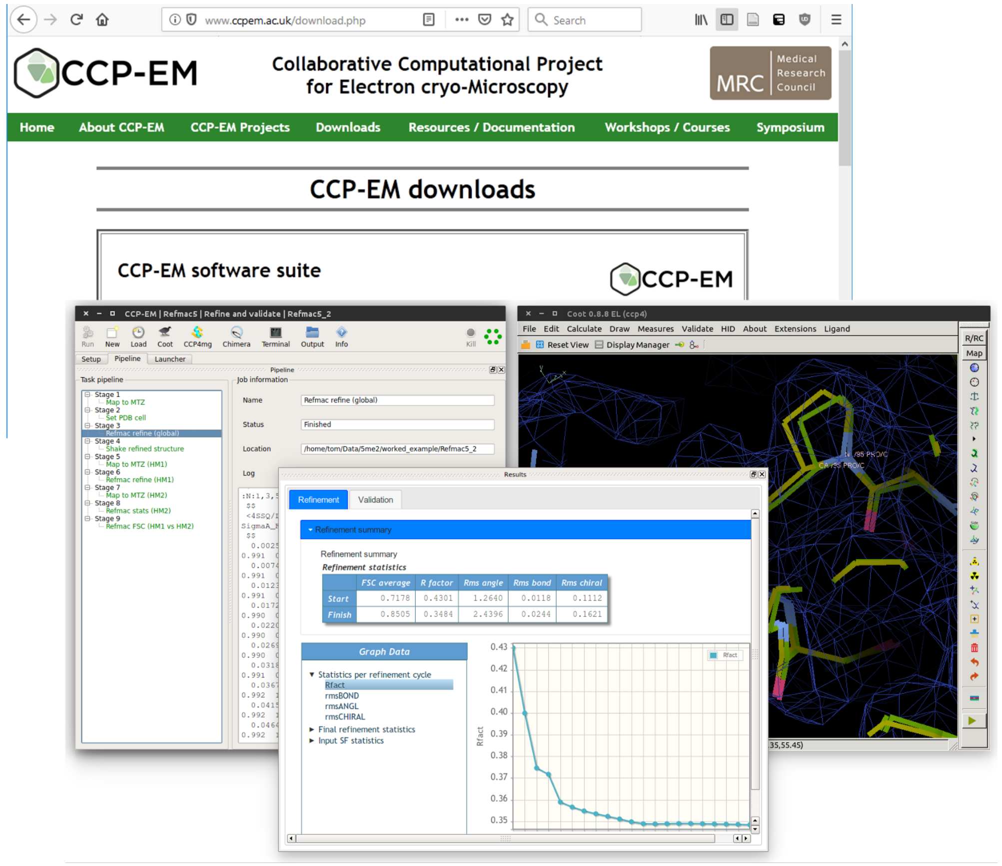


Science and Technology Facilities Council

Introduction – what is CCP-EM?

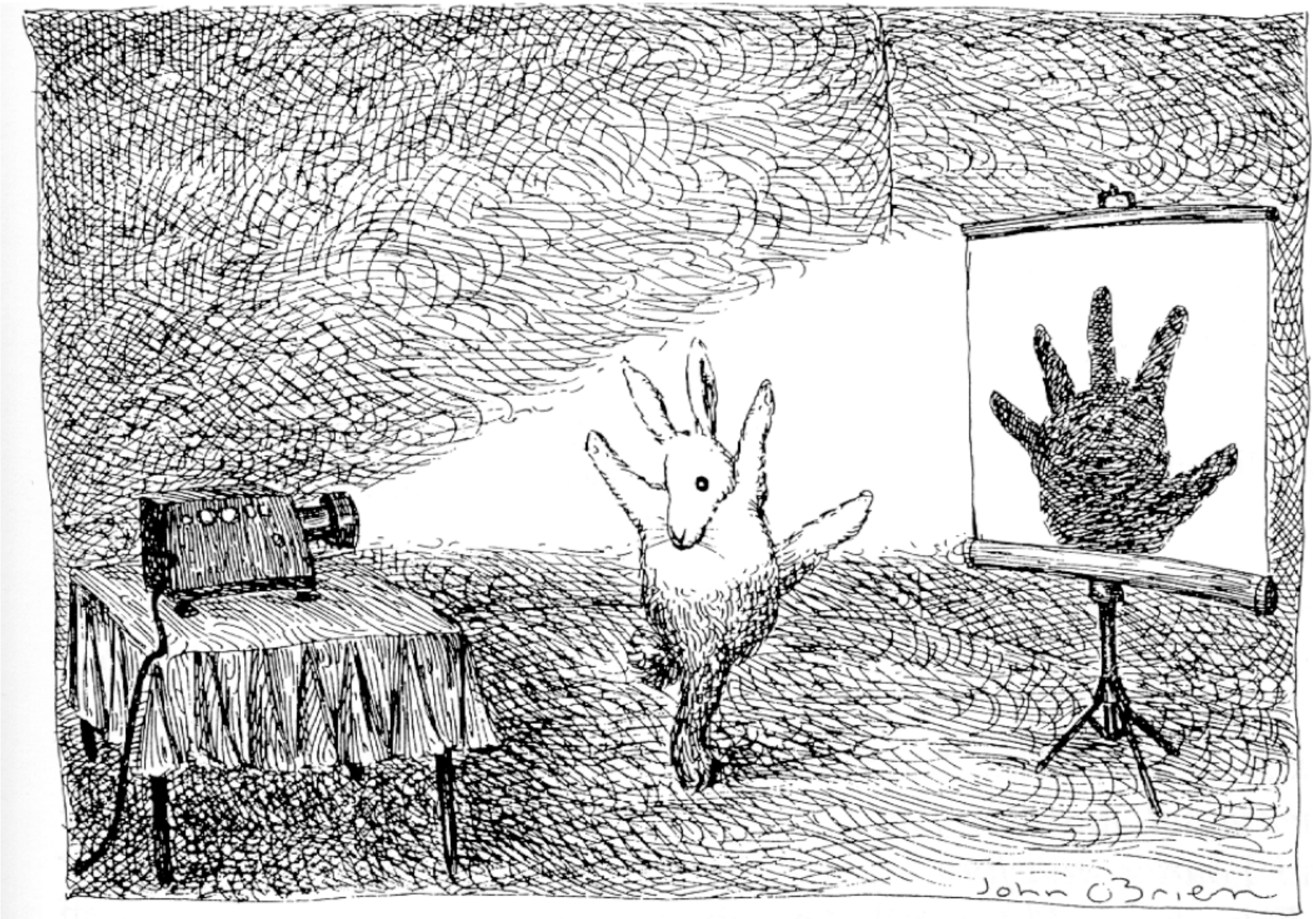
When people say "CCP-EM," they mean…

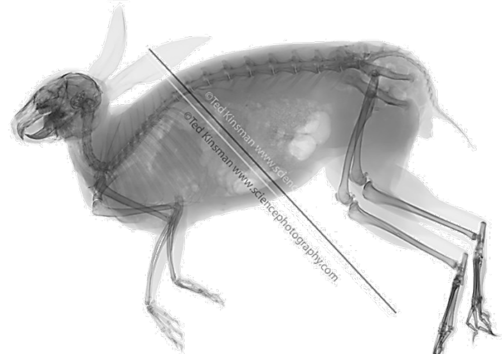
- Mailing list
  - https://www.jiscmail.ac.uk/CCPEM
- Spring Symposium conference
  - Talks on YouTube – search "CCP-EM"
- Software package
  - Tools for cryo-EM data processing
  - https://www.ccpem.ac.uk/download.php



Science and Technology Facilities Council

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





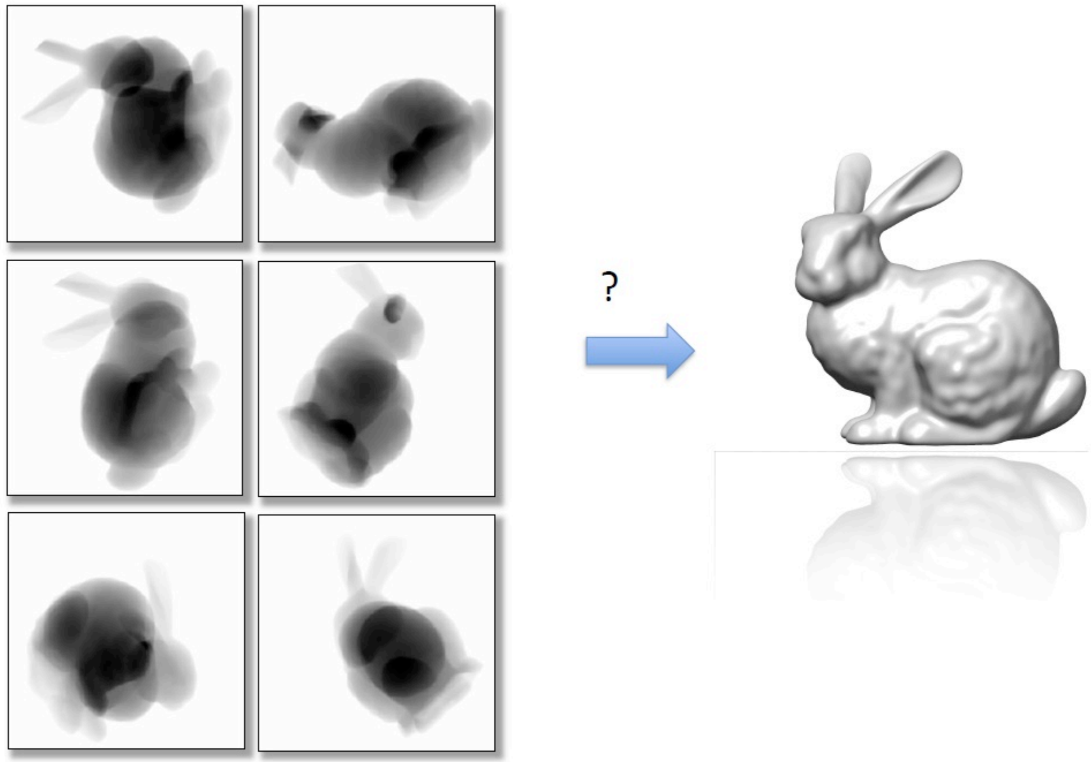
https://tedkinsman.photoshelter.com/image/I0000rQCSPSbNCQ0

https://dissolve.com/stock-photo/Normal-hand-ray-year-old-rights-managed-image/102-D943-144-684

Cartoon from The New Yorker

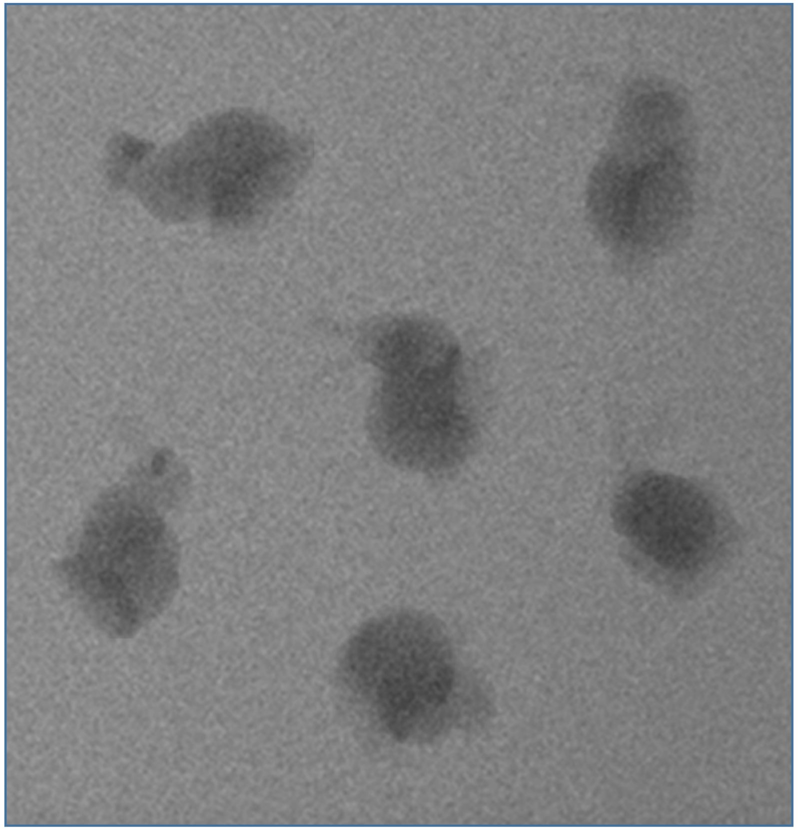
Juha Huiskonen & Bilal Qureshi

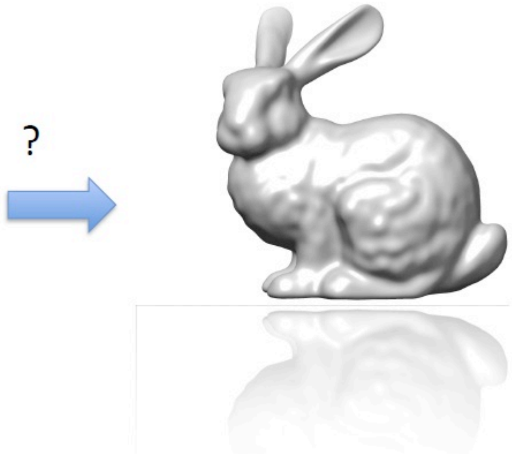
EM: Reconstruct 3D from 2D projections



Juha Huiskonen & Bilal Qureshi

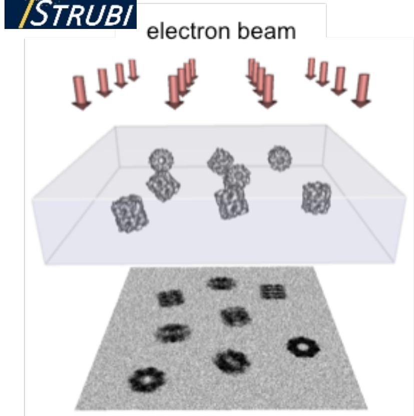
EM: Reconstruct 3D from 2D projections

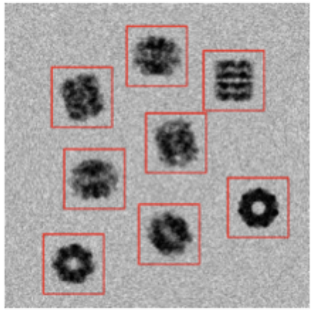


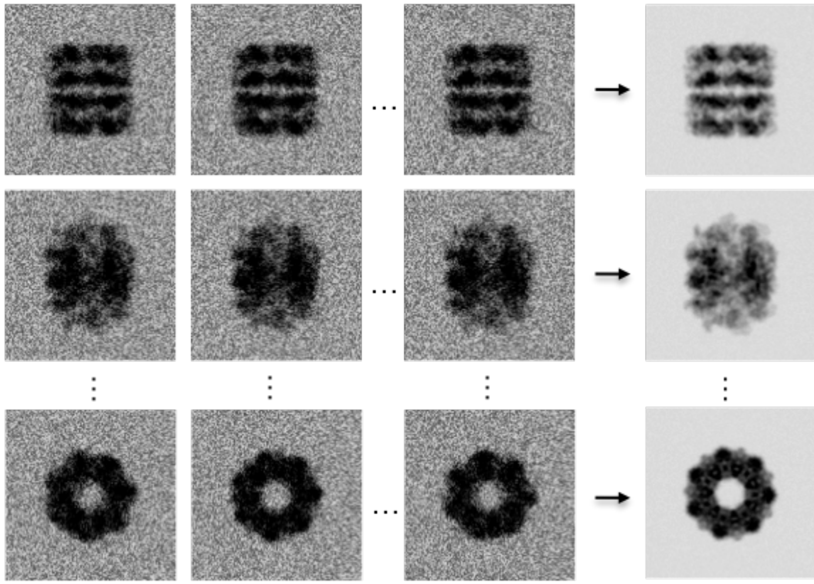


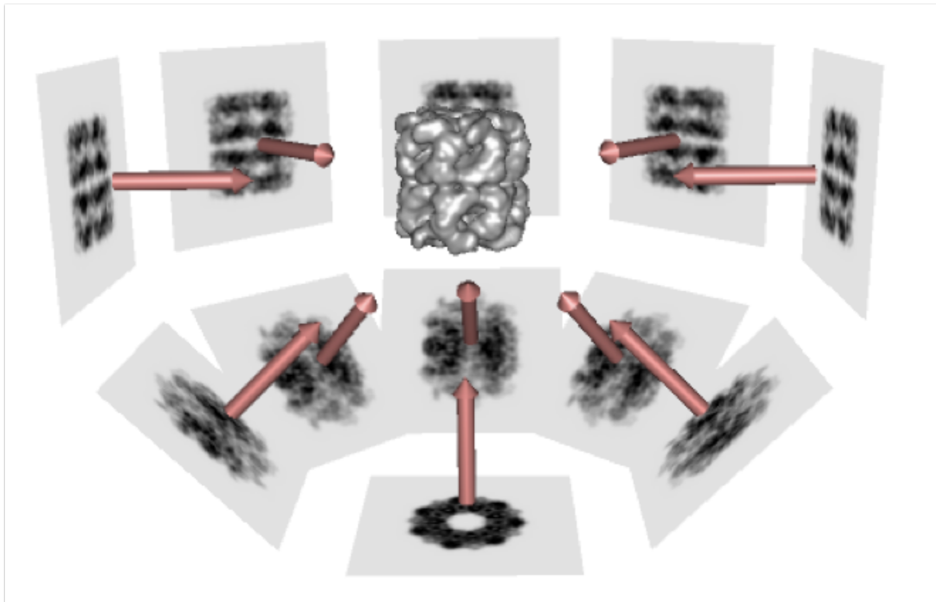
Juha Huiskonen & Bilal Qureshi

Single particle: reconstruct 3D from “back projected” 2D projections









https://people.csail.mit.edu/gdp/cryoem.html

Juha Huiskonen & Bilal Qureshi

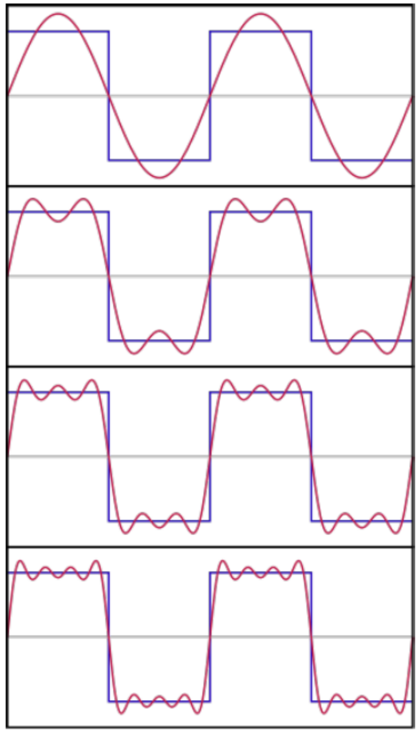
Fourier Transform

Juha Huiskonen & Bilal Qureshi

Fourier Transform and Fourier series

The Fourier transform decomposes a function of time (signal) into its constituent frequencies (Wikipedia)

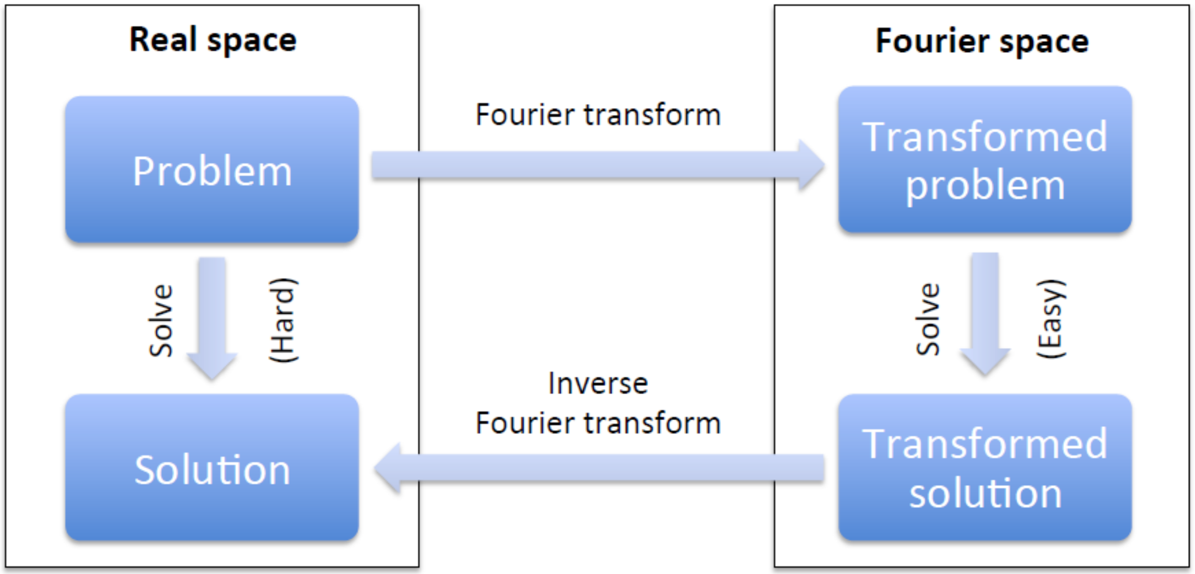
- Any signal can be represented as an infinite sum of sine and cosine functions!
- The process of finding these functions is called a Fourier transform
- In digital image processing: discrete Fourier transform



Juha Huiskonen & Bilal Qureshi

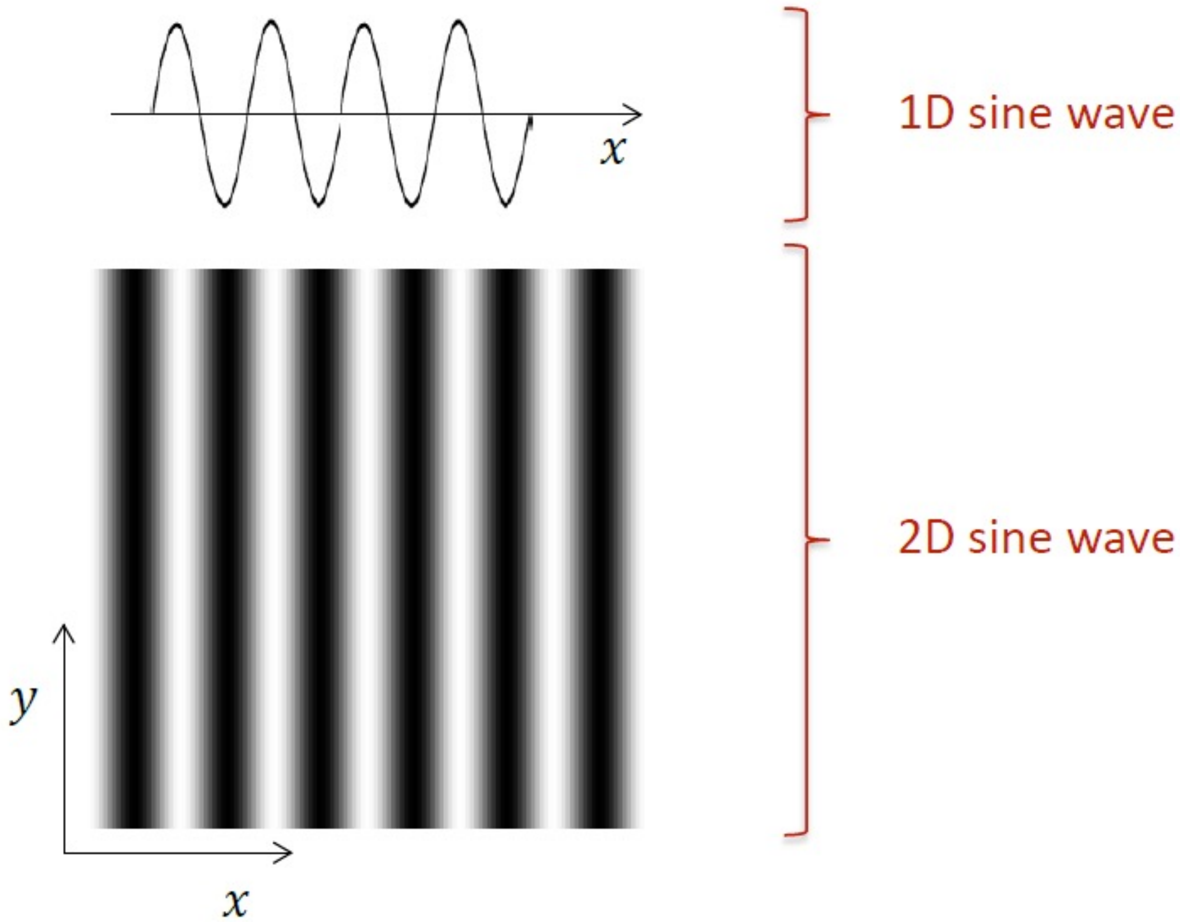
Fourier transform in image processing

- A useful mathematical tool that we can use for different image processing tasks
- “Real space” or “spatial domain” (nm) vs. “Fourier space” or “spatial frequency domain” (1 / nm)



Juha Huiskonen & Bilal Qureshi

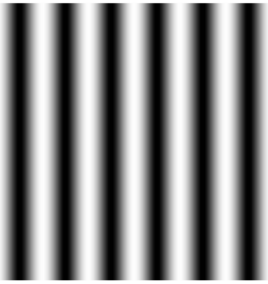
Fourier transform of images



Juha Huiskonen & Bilal Qureshi

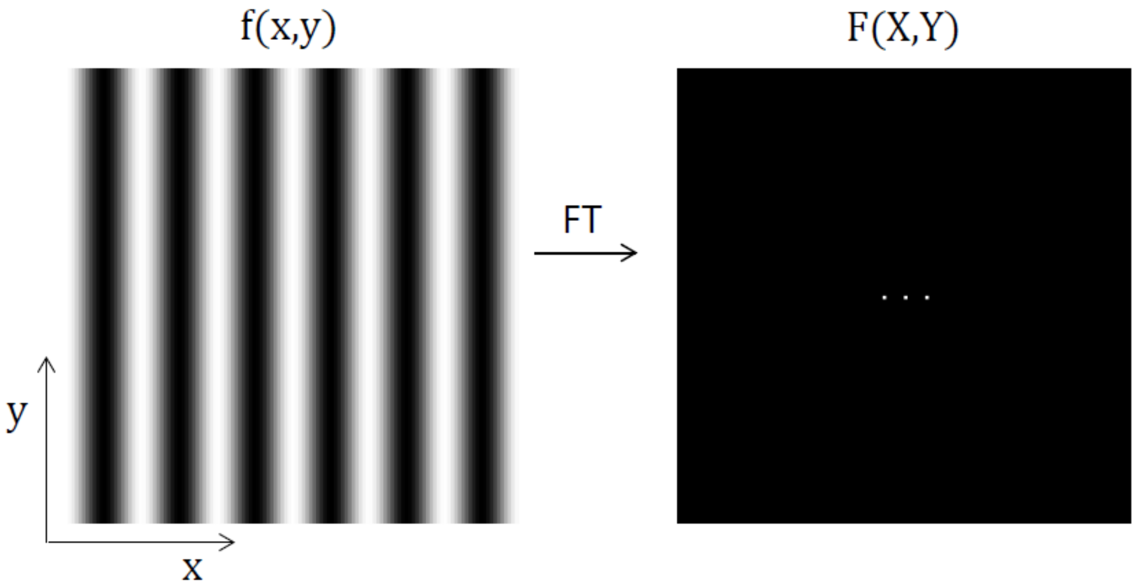
Fourier transform of images

- Each sine function (Fourier term) encodes
  - the spatial **frequency**: the frequency across the image (here along the x-axis) with which the brightness modulates
  - the **magnitude** of the sine function (positive or negative): the difference between the dark and light areas in the image, negative magnitude represents contrast reversal
  - the **phase**: represents how the sine wave is shifted to the left or right relative to the origin



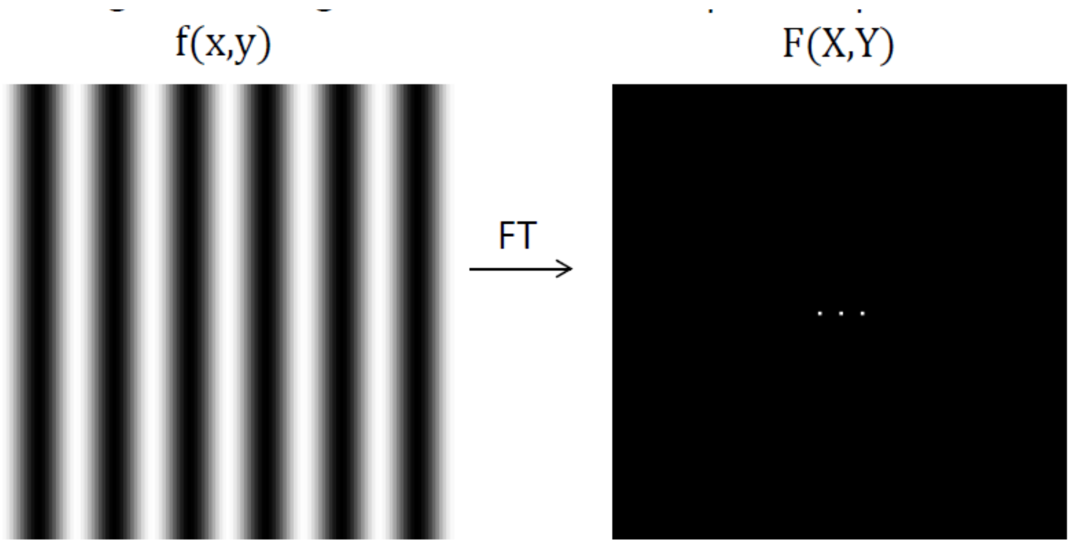
Juha Huiskonen & Bilal Qureshi

Fourier transform of images



Juha Huiskonen & Bilal Qureshi

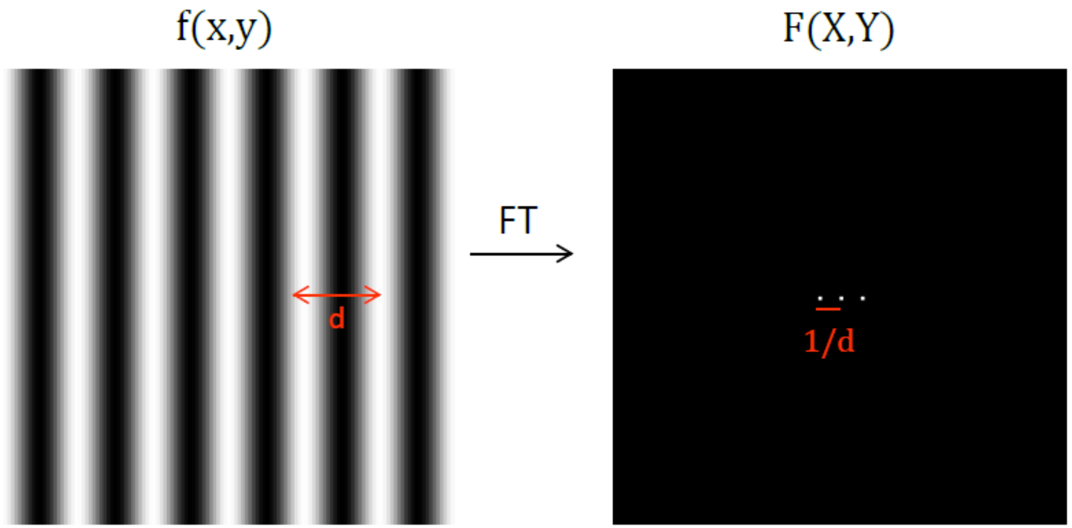
Fourier transform of images



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

Juha Huiskonen & Bilal Qureshi

Fourier transform of images

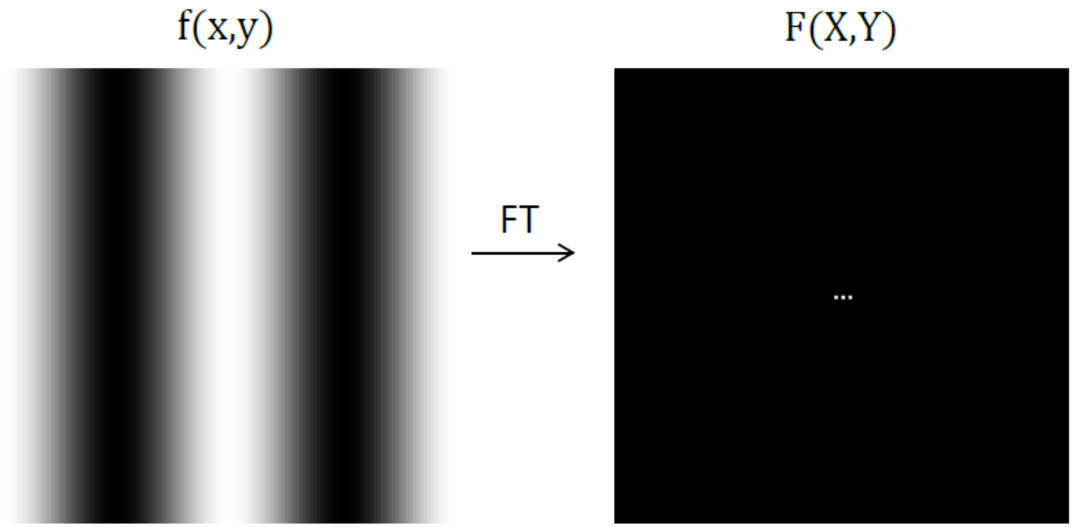


a brightness image with closely spaced features (high frequency information) will result in a amplitude spectrum with wide spacings

- Fourier space is also known as reciprocal space -

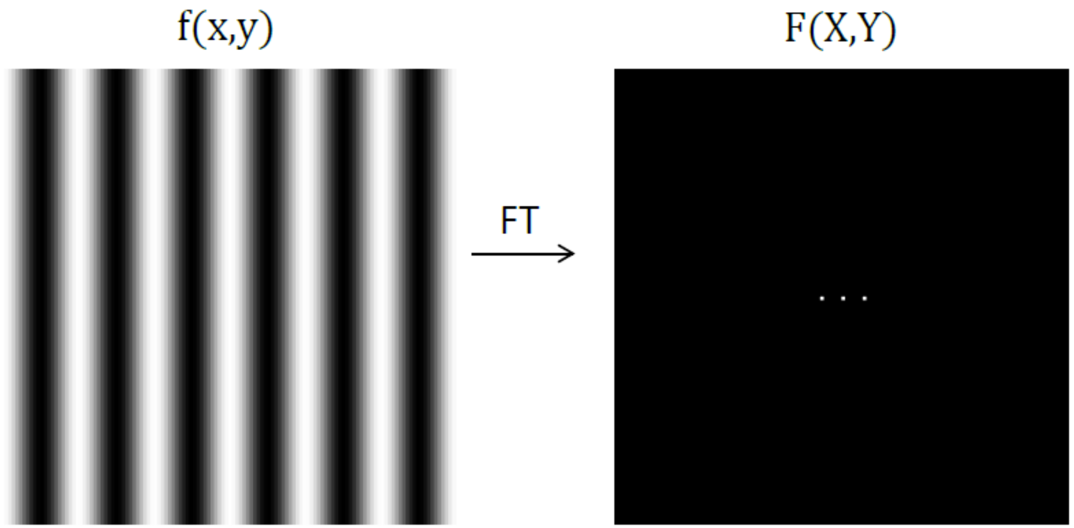
Juha Huiskonen & Bilal Qureshi

Fourier transform of images



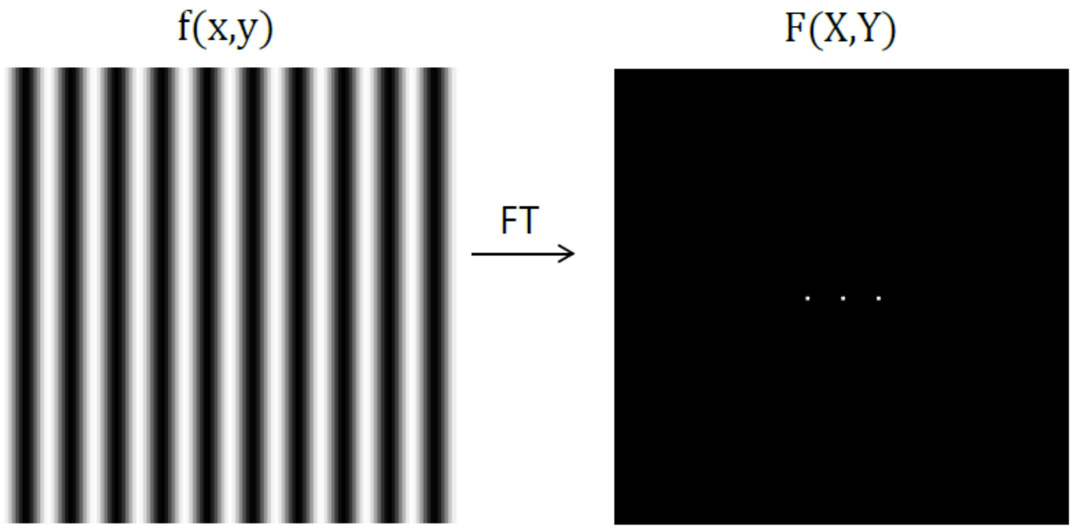
Juha Huiskonen & Bilal Qureshi

Fourier transform of images



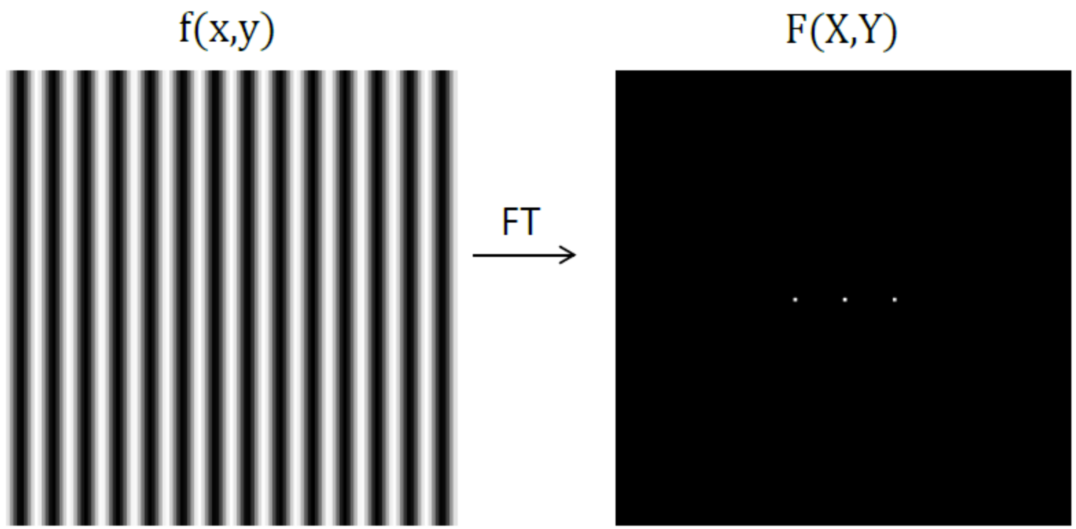
Juha Huiskonen & Bilal Qureshi

Fourier transform of images



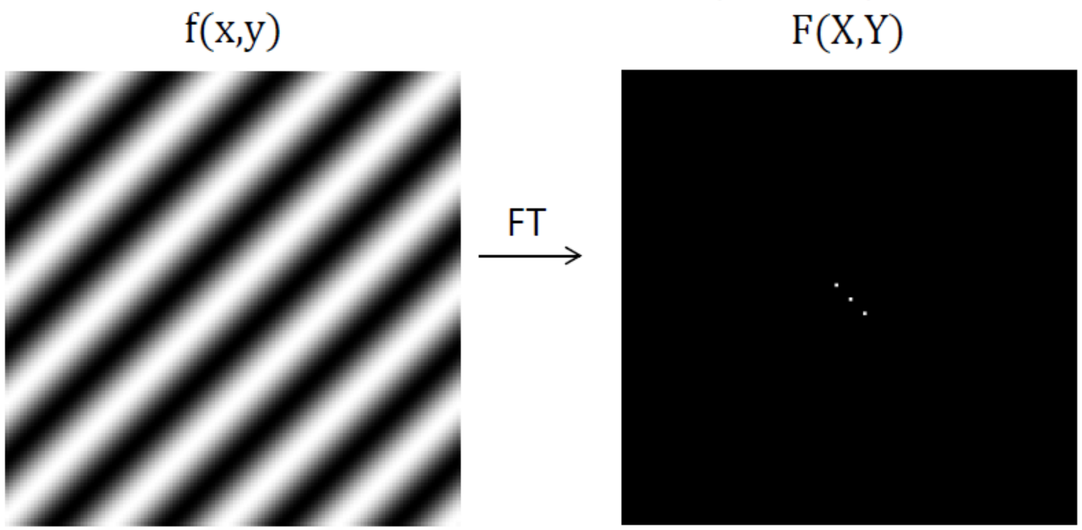
Juha Huiskonen & Bilal Qureshi

Fourier transform of images



Juha Huiskonen & Bilal Qureshi

Fourier transform of images



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

Juha Huiskonen & Bilal Qureshi

Fourier transform of images

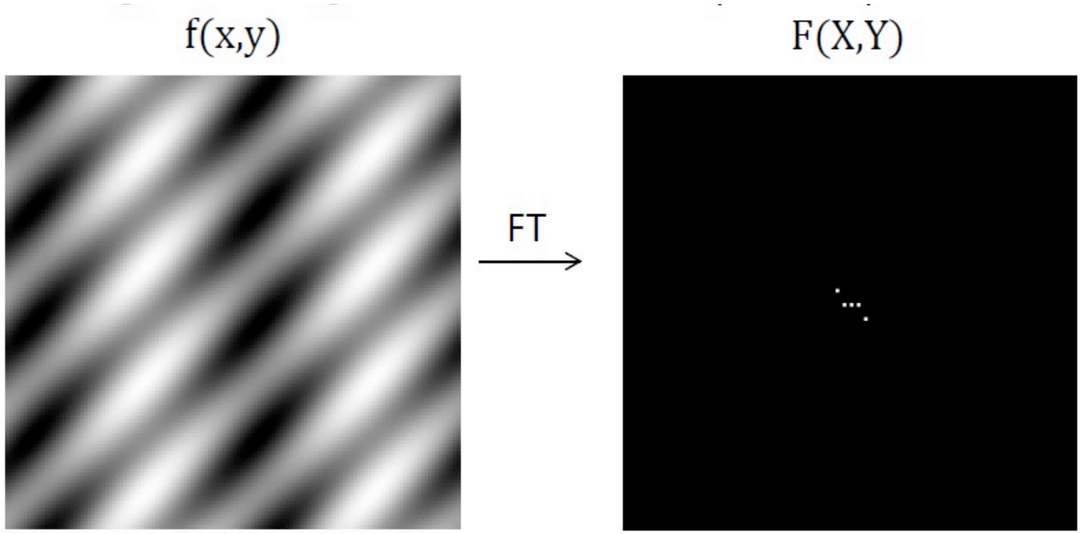
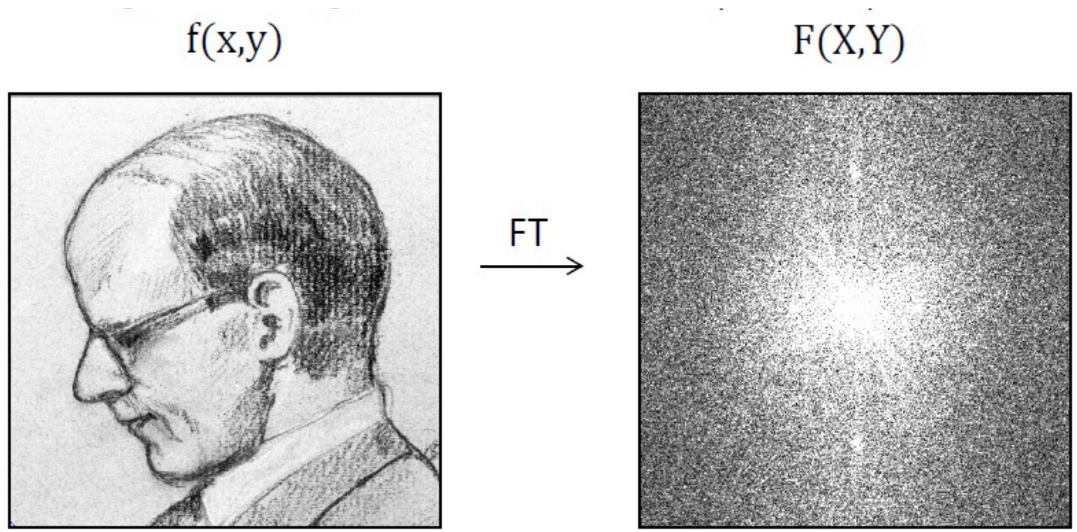


image formed by two sinusoidal components

Juha Huiskonen & Bilal Qureshi

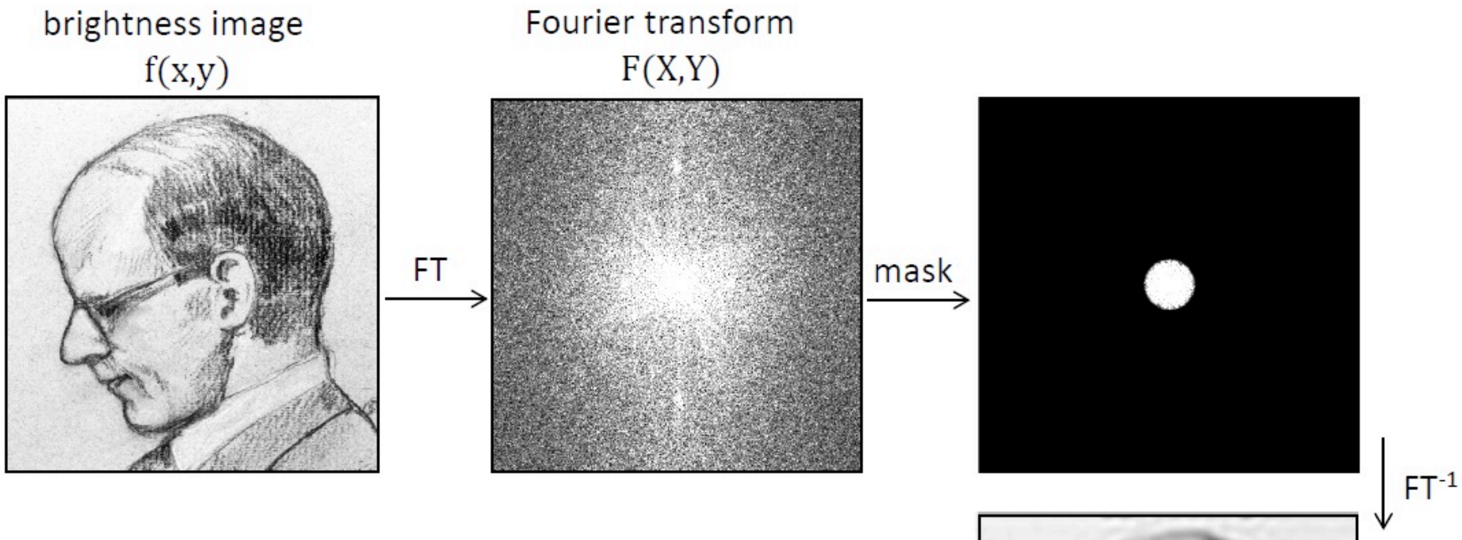
Fourier transform of images



images formed by multiple sinusoidal components

Juha Huiskonen & Bilal Qureshi

Fourier transform of images

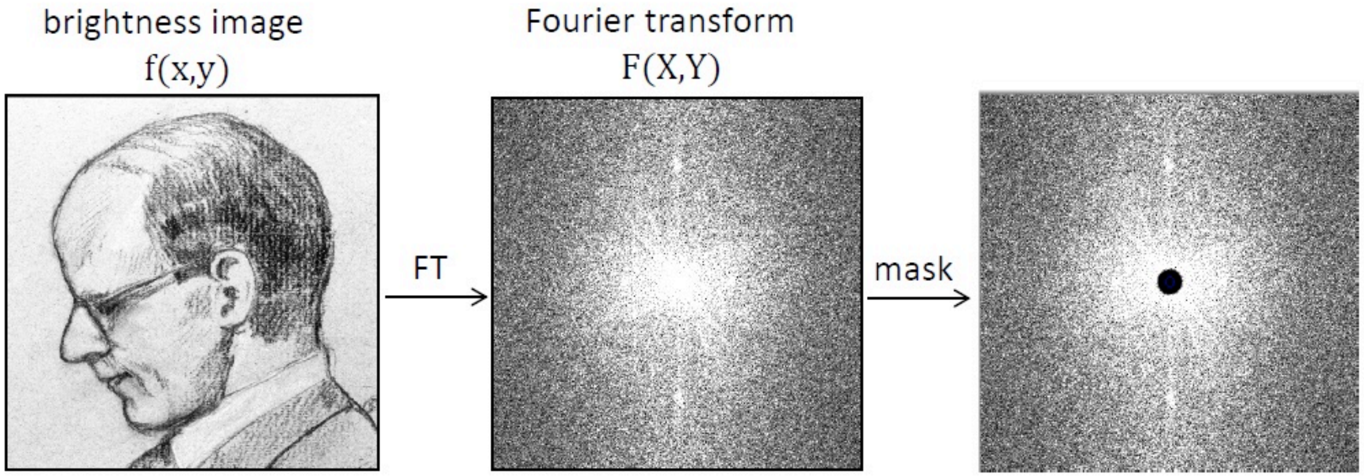


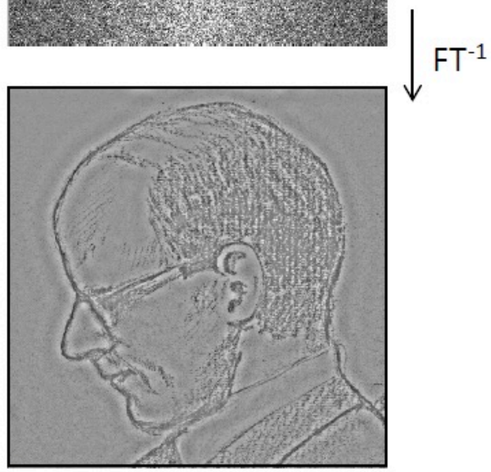
low-pass filtering

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

Juha Huiskonen & Bilal Qureshi

Fourier transform of images





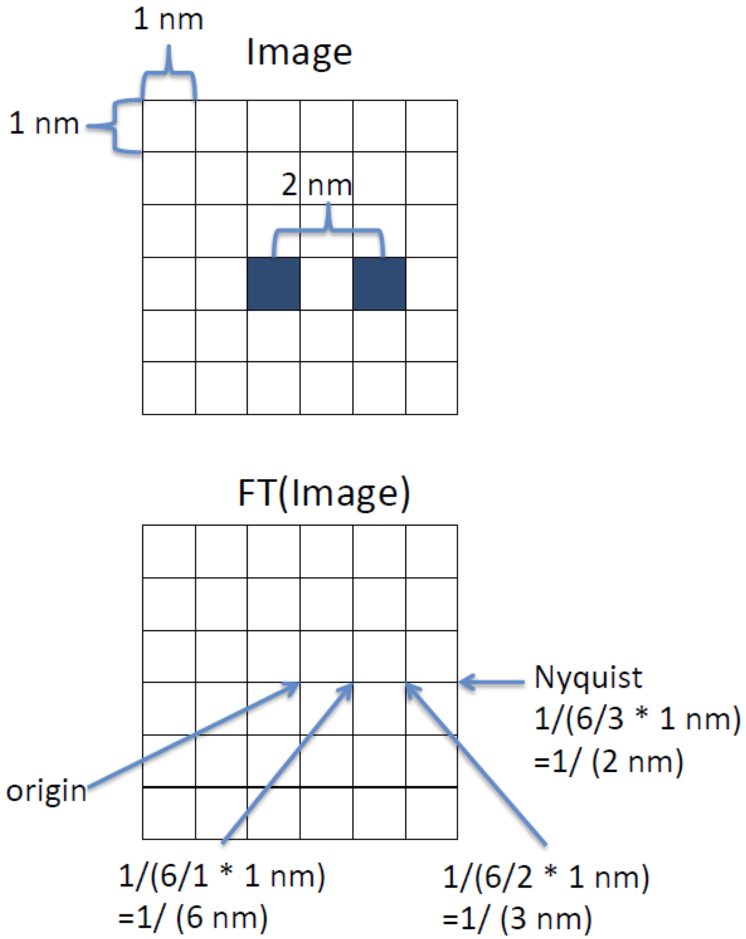
high-pass filtering

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

Nyquist frequency

- The highest frequency that can be encoded in a digital image is called the Nyquist frequency
- In a Fourier transform of an image it is the edge of the transform
- It corresponds to the size of the digitized pixels and has the spatial frequency of 1/(2 x pixel)
- Maximum resolution 2x pixel size (here 2 nm)

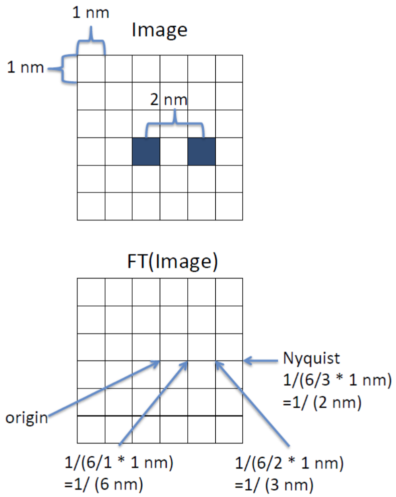


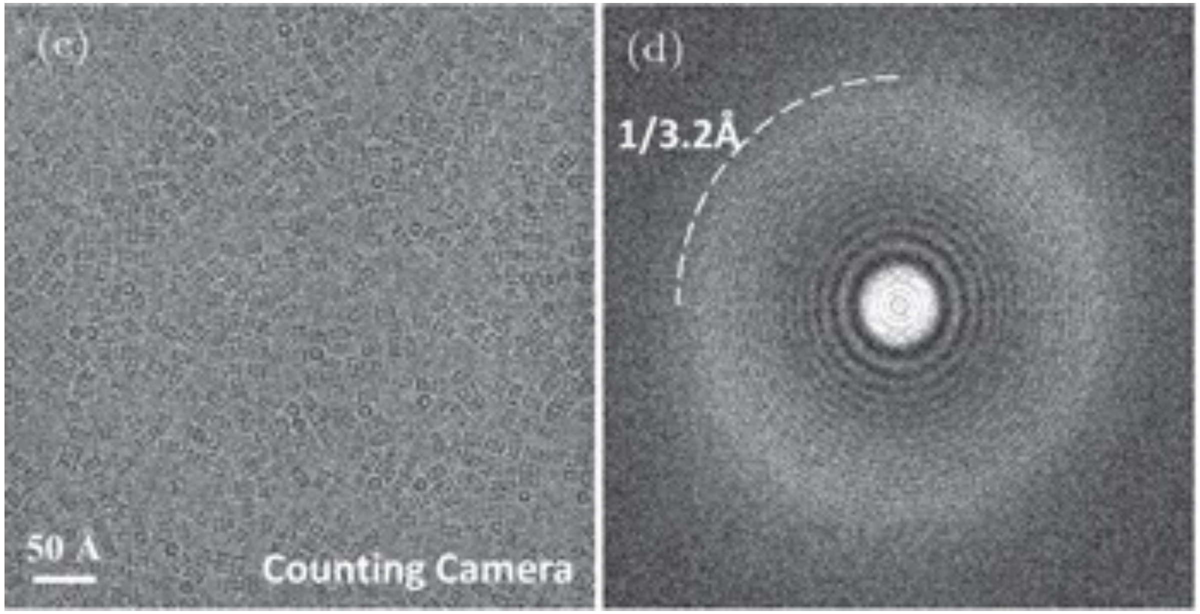
Juha Huiskonen & Bilal Qureshi

Fourier transform of images

Nyquist frequency

- The highest frequency that can be encoded in a digital image is called the Nyquist frequency
- In a Fourier transform of an image it is the edge of the transform
- It corresponds to the size of the digitized pixels and has the spatial frequency of 1/(2 x pixel)
- Maximum resolution 2x pixel size (here 2 nm)





Juha Huiskonen & Bilal Qureshi

Fourier transform – some properties

$$a * F(u) = FT(a * f(x))$$

If you put more contrast in the image, then the FFT's amplitude gets stronger.

$$F(u) + G(u) = FT(f(x) + g(x))$$

Adding two images f and g and calculating their FFT is like adding the FFTs F and G of them.

$$F(u/a) = FT(f(ax))$$

If you stretch an image by a, then you shorten the FFT by a. (===> reciprocity)

$$rotated \quad F(u) = FT(rotated \quad f(x))$$

If you rotate and image, then you also rotate its FFT.

Break

Any questions so far?

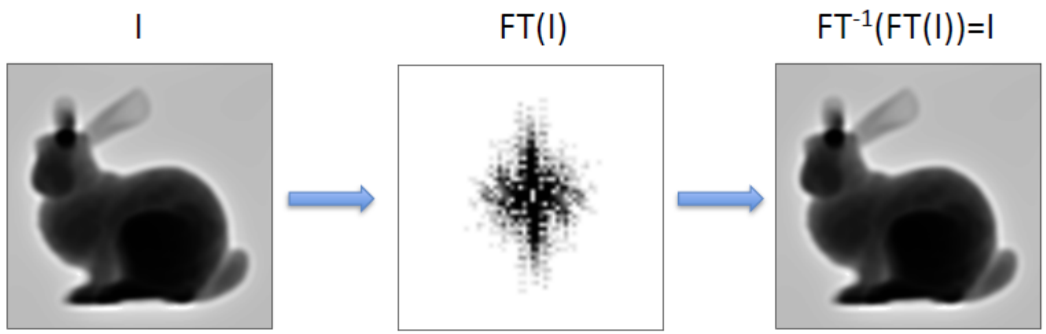
Science and
Technology
Facilities Council

Fourier filters in image processing

Juha Huiskonen & Bilal Qureshi

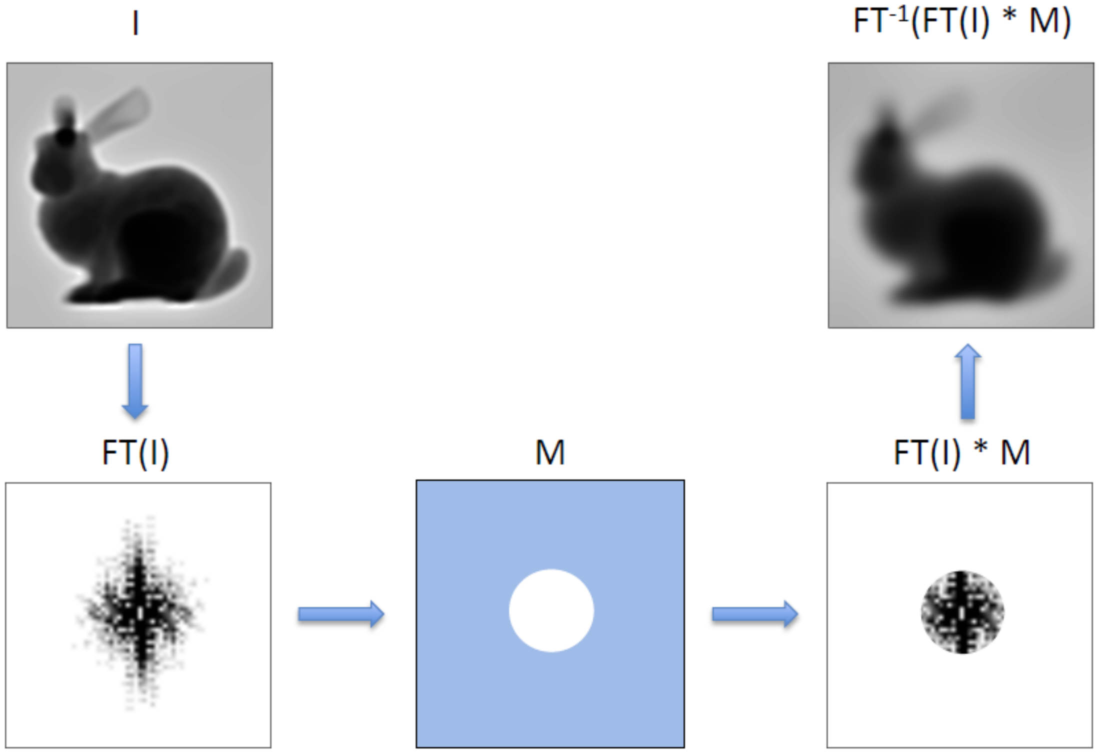
Fourier transform in image processing

- Operate in frequency domain
- Useful for removing certain spatial frequencies from the image
- Inverse Fourier transform gives the filtered image



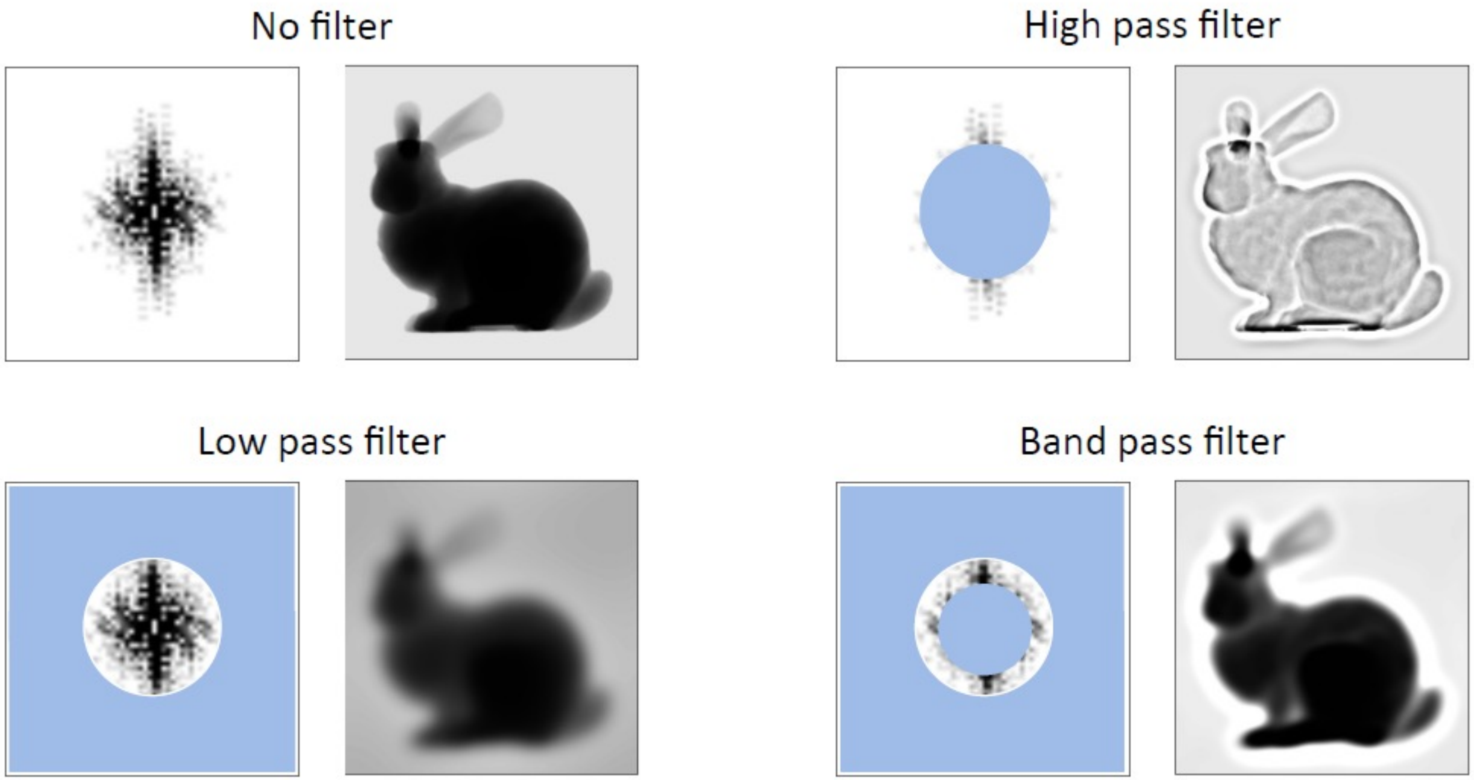
Juha Huiskonen & Bilal Qureshi

Fourier transform in filtering



Juha Huiskonen & Bilal Qureshi

Fourier transform in filtering

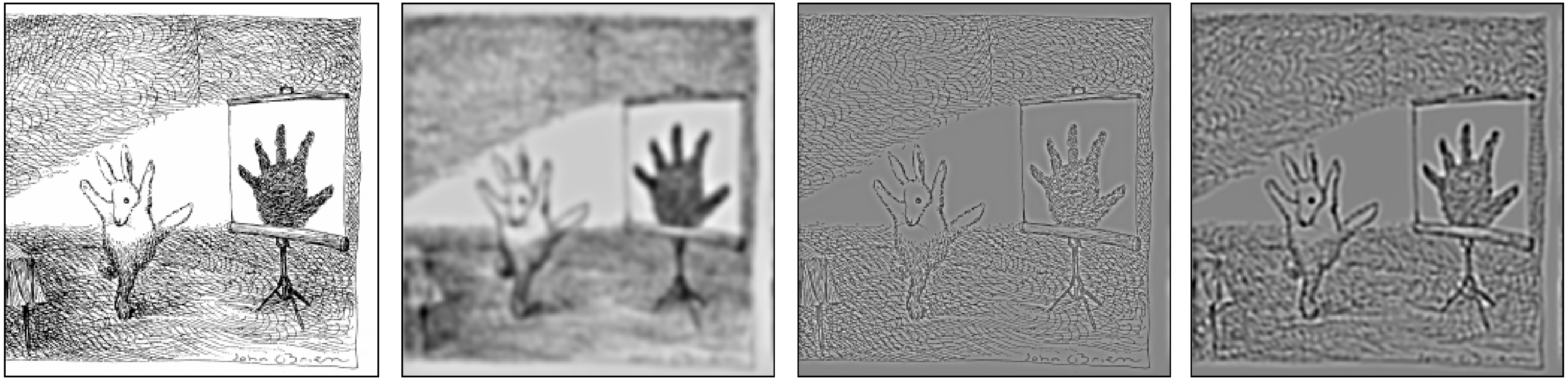


Note: Masks shouldn't have sharp edges, instead they should have a Gaussian-shaped (or cosine-shaped) fall of from 1 to 0.

Juha Huiskonen & Bilal Qureshi

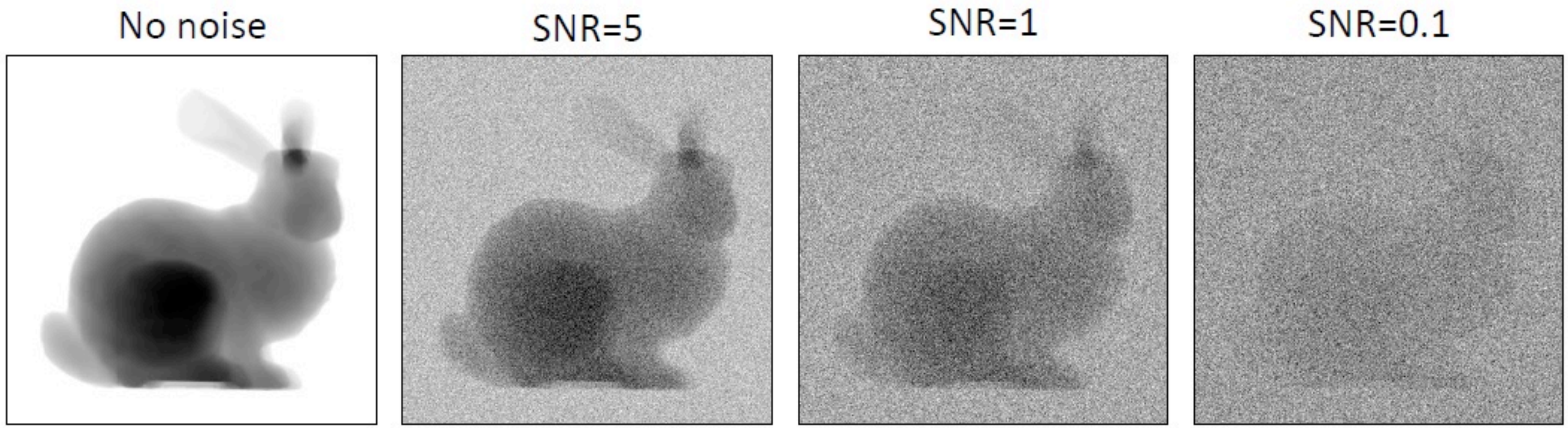
Fourier transform in filtering

Original (512 pix) | Low pass (20 pix) | High pass (20 pix) | Band pass (10–30pix)



Juha Huiskonen & Bilal Qureshi

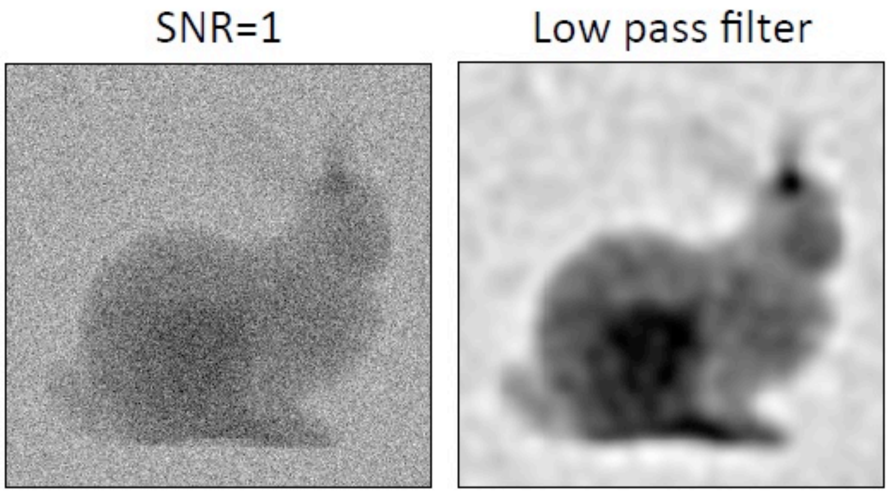
Images with Gaussian noise



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

Juha Huiskonen & Bilal Qureshi

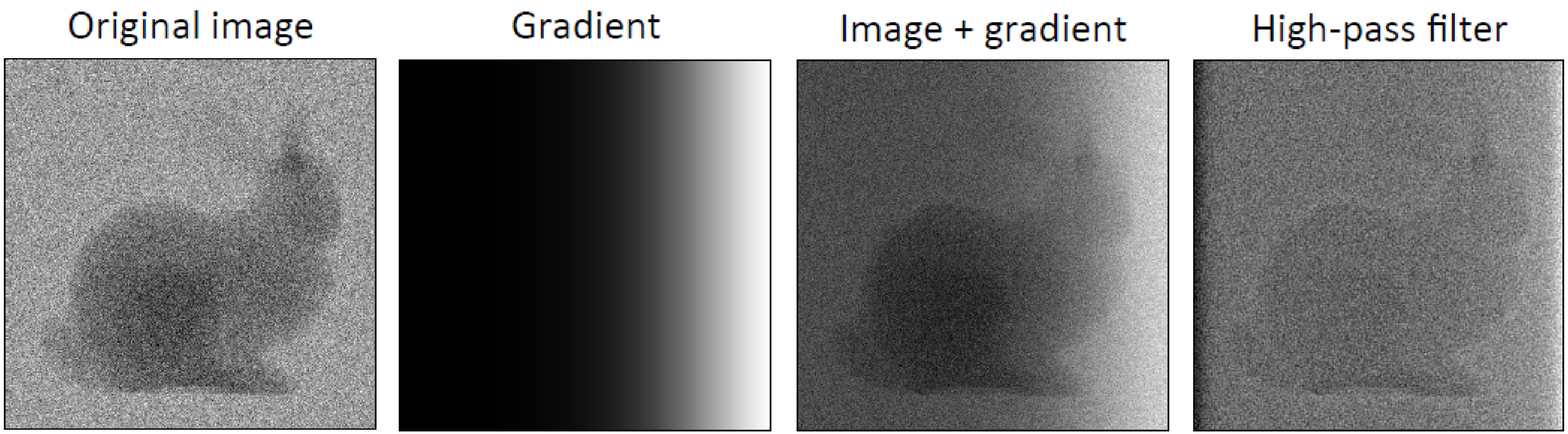
Use of low pass filter



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

Juha Huiskonen & Bilal Qureshi

Use of high pass filter

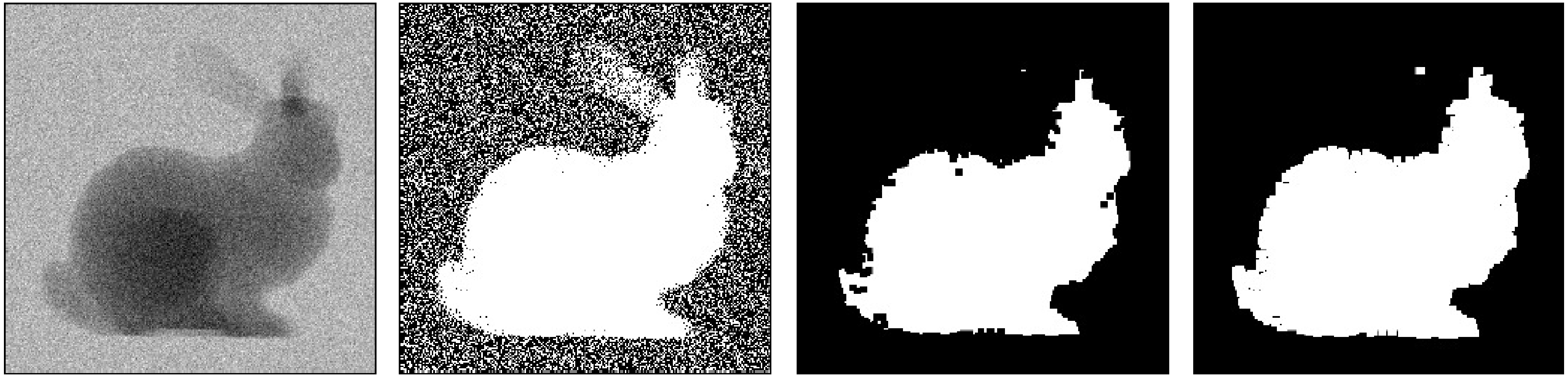


High pass filter can be used to remove low spatial frequency features, such as a density gradient.

Juha Huiskonen & Bilal Qureshi

Binary and morphological filters

Original image | Binary mask | + 2 x Erosion | + 2 x Dilation



Masks can be used to define the object. Value 1 = object | Value 0 = background

Binary mask: if value < threshold: new value = 1; else new value = 0
Erosion: if any value within a 3x3 kernel = 0: new value = 0
Dilation: if any value within a 3x3 kernel = 1: new value = 1

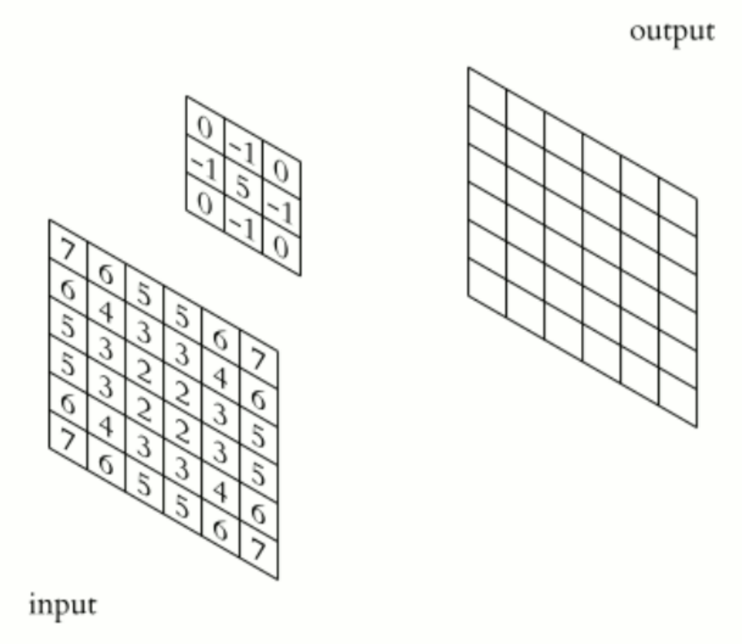
Juha Huiskonen & Bilal Qureshi

Convolution and convolution based filters in image processing

Juha Huiskonen & Bilal Qureshi

Convolution

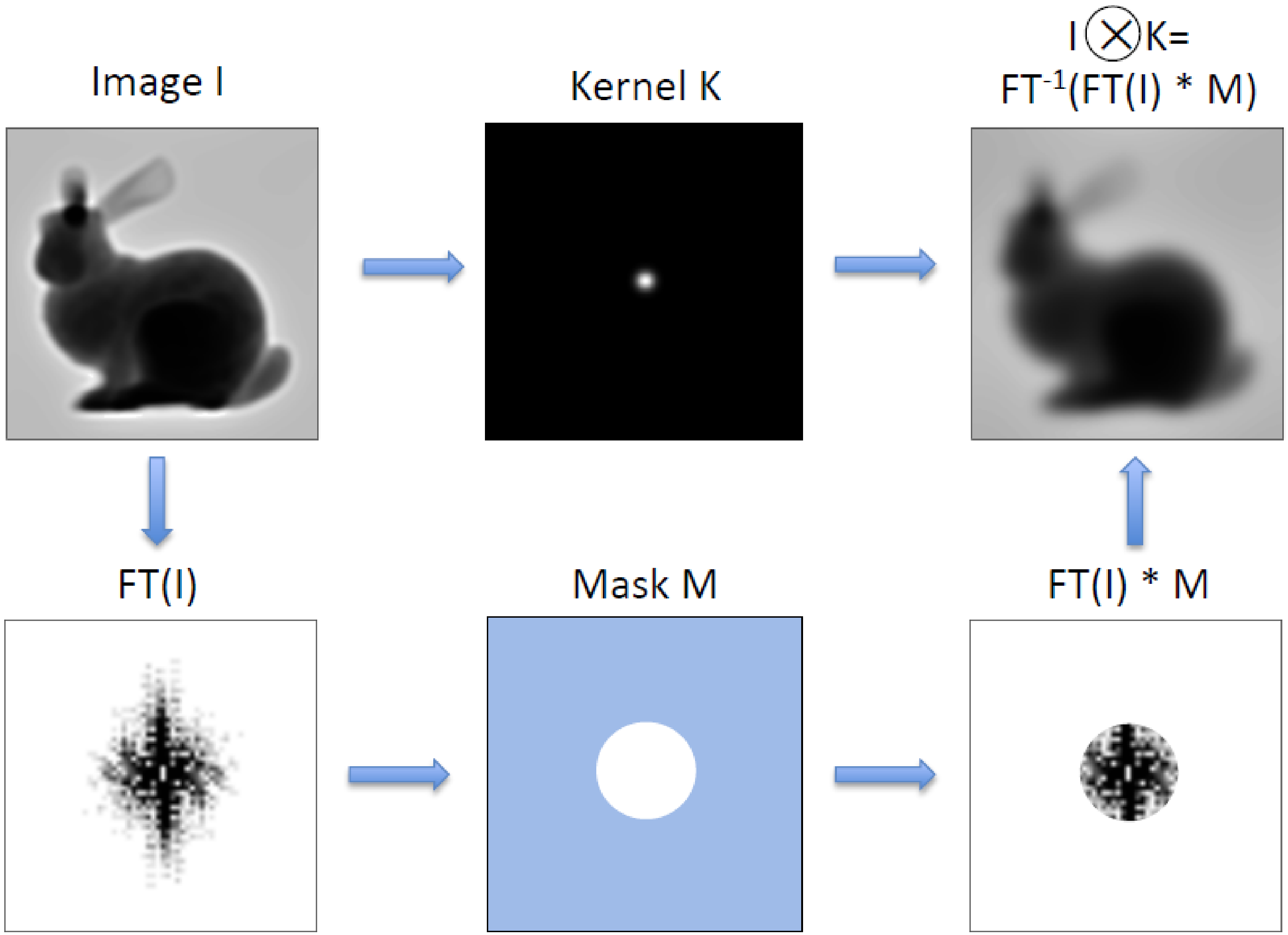
- Convolution is a mathematical operation that combines two signals into one
- The value of a given pixel in the output image is calculated by multiplying each kernel value by the corresponding input image pixel values



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

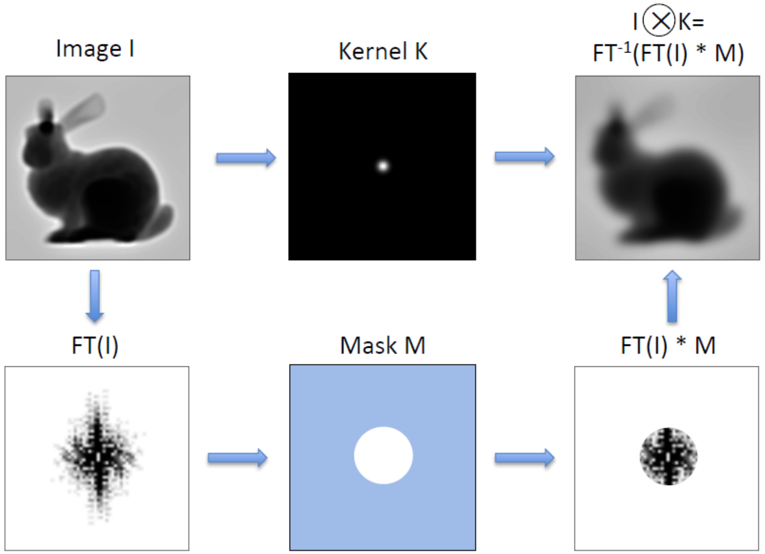
Convolution of f with g in real space is slow. It can be done much faster by multiplying their FFTs, and calculating the inverse FFT of the result.

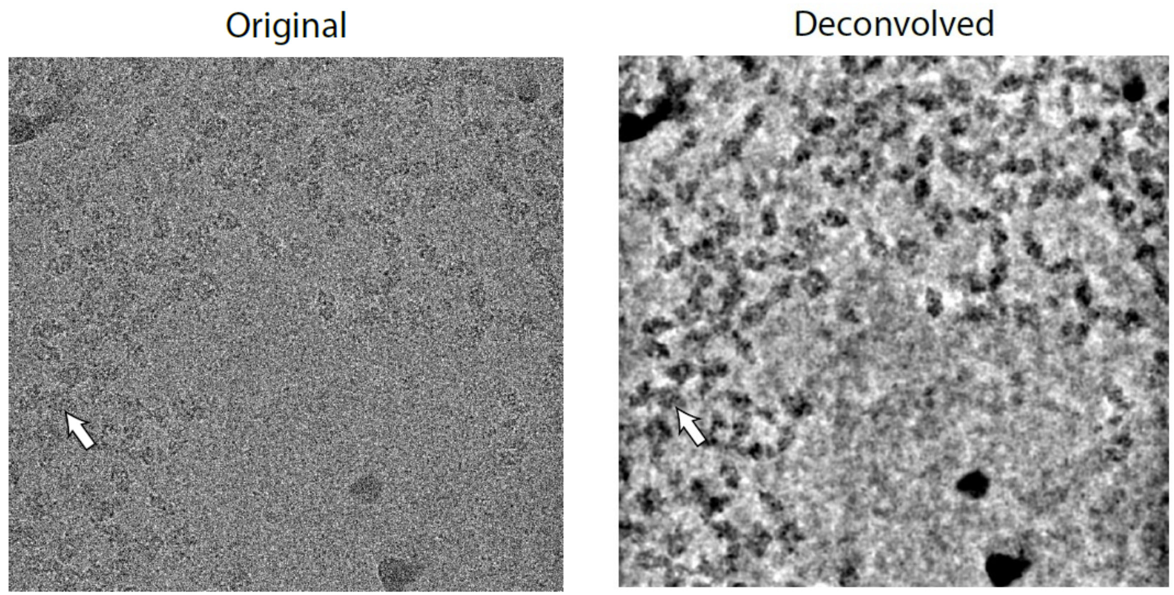
Fourier vs. Convolution based filtering



Juha Huiskonen & Bilal Qureshi

Fourier vs. Convolution based filtering





Juha Huiskonen & Bilal Qureshi

Correcting for effects of point spread function or contrast transfer function

Juha Huiskonen & Bilal Qureshi

Convolution and correction of Point Spread Function (PSF)

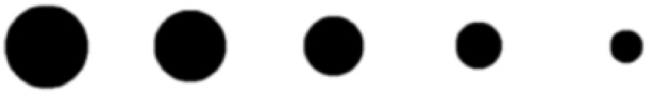
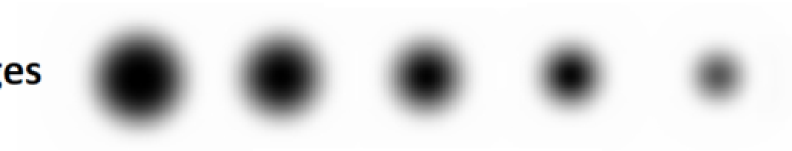
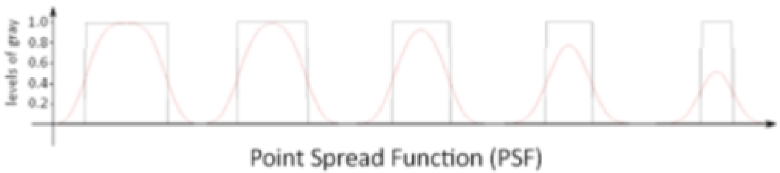
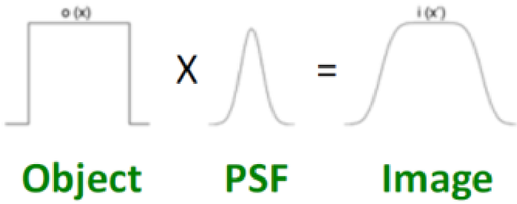
Objects 

Image = Original object x Point spread function (PSF)

Images 

PSF= a constant because of defects in EM





Convolution: Convolution is a mathematical operation on two functions (object and PSF) producing a third function (image) that is typically viewed as a modified version of one of the original functions (object)

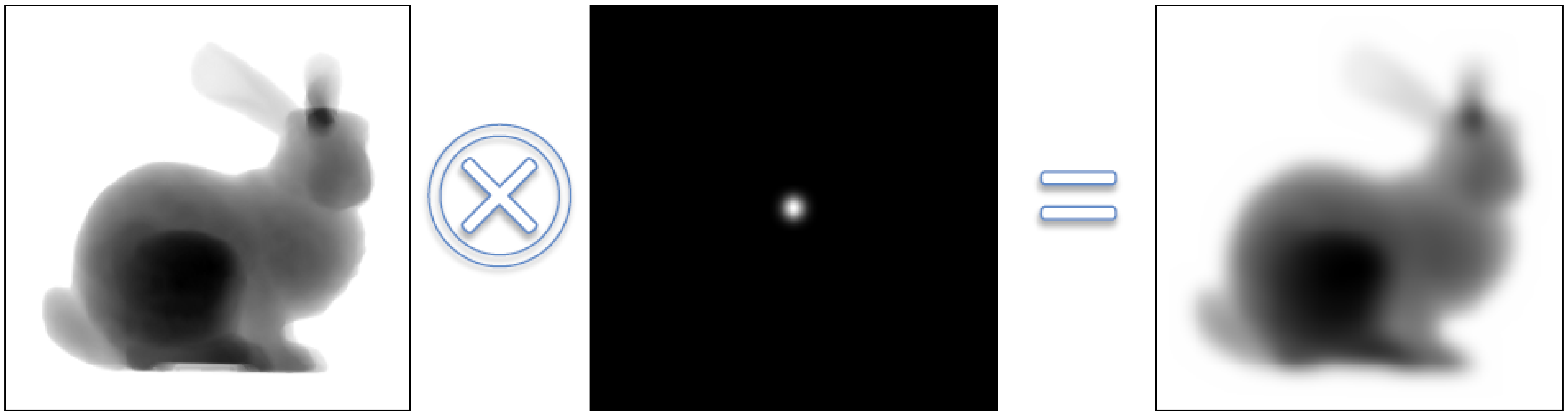
Inverse of convolution is deconvolution = image/PSF
If we know PSF, we can improve our images

Courtesy: Shashi Bhushan

Convolution and correction of Point Spread Function (PSF)

- In TEM the image we observe is said to equal an ideal image convolved by a point spread function (PSF) of the imaging system
- If PSF is known, the image can be deconvoluted
- The equivalent of PSF in Fourier space is called the Contrast Transfer Function (CTF)

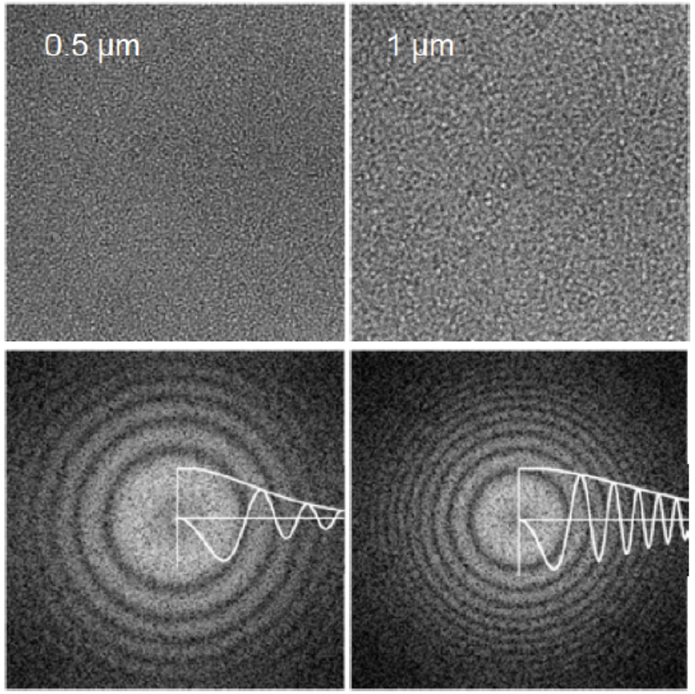
Ideal image | Point spread function | Observed image



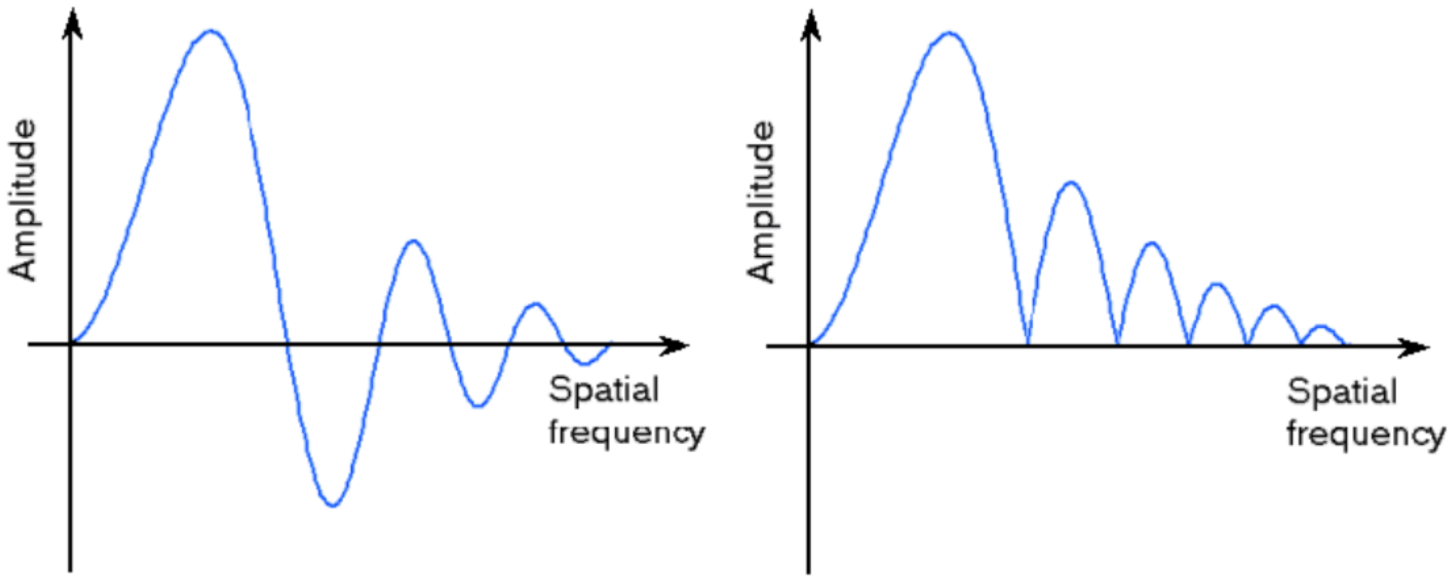
Juha Huiskonen & Bilal Qureshi

Estimating CTF from images

- The defocus in the recorded image can be different from the intended value
- Needs to be estimated from the images
- Power spectra (squared Fourier transforms) show the CTF peaks = Thon rings



Images are corrected by phase flipping in Fourier space



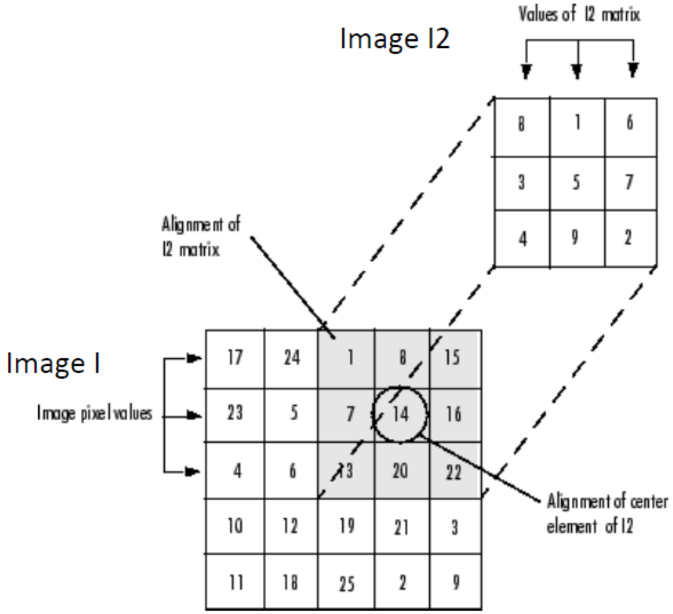
Juha Huiskonen & Bilal Qureshi

Cross-correlation and image alignment

Juha Huiskonen & Bilal Qureshi

Cross-correlation

- Cross-correlation measures the similarity of two images
- Values normalized between 0 and 1
- Auto-correlation: correlation of a image with itself



The cross-correlation for the indicated position is:
1 * 8 + 8 * 1 + 6 * 15 + 7 * 3 + ... + 2 * 22 = 585

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

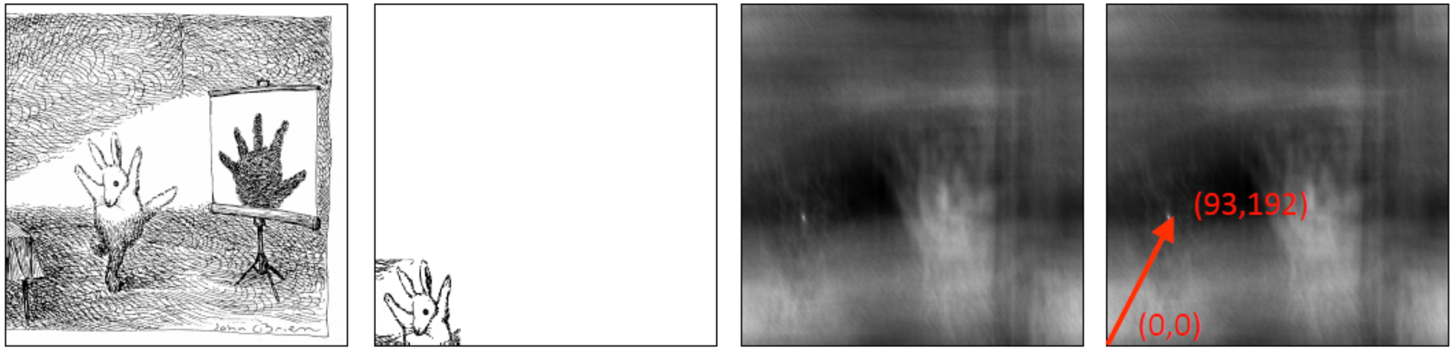
Cross-correlation of f with g in real space is slow. It can be done much faster by calculating their FFTs F and G, taking the complex conjugate of G, multiplying F with G*, and calculating the inverse FFT of the result.*

Juha Huiskonen & Bilal Qureshi

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

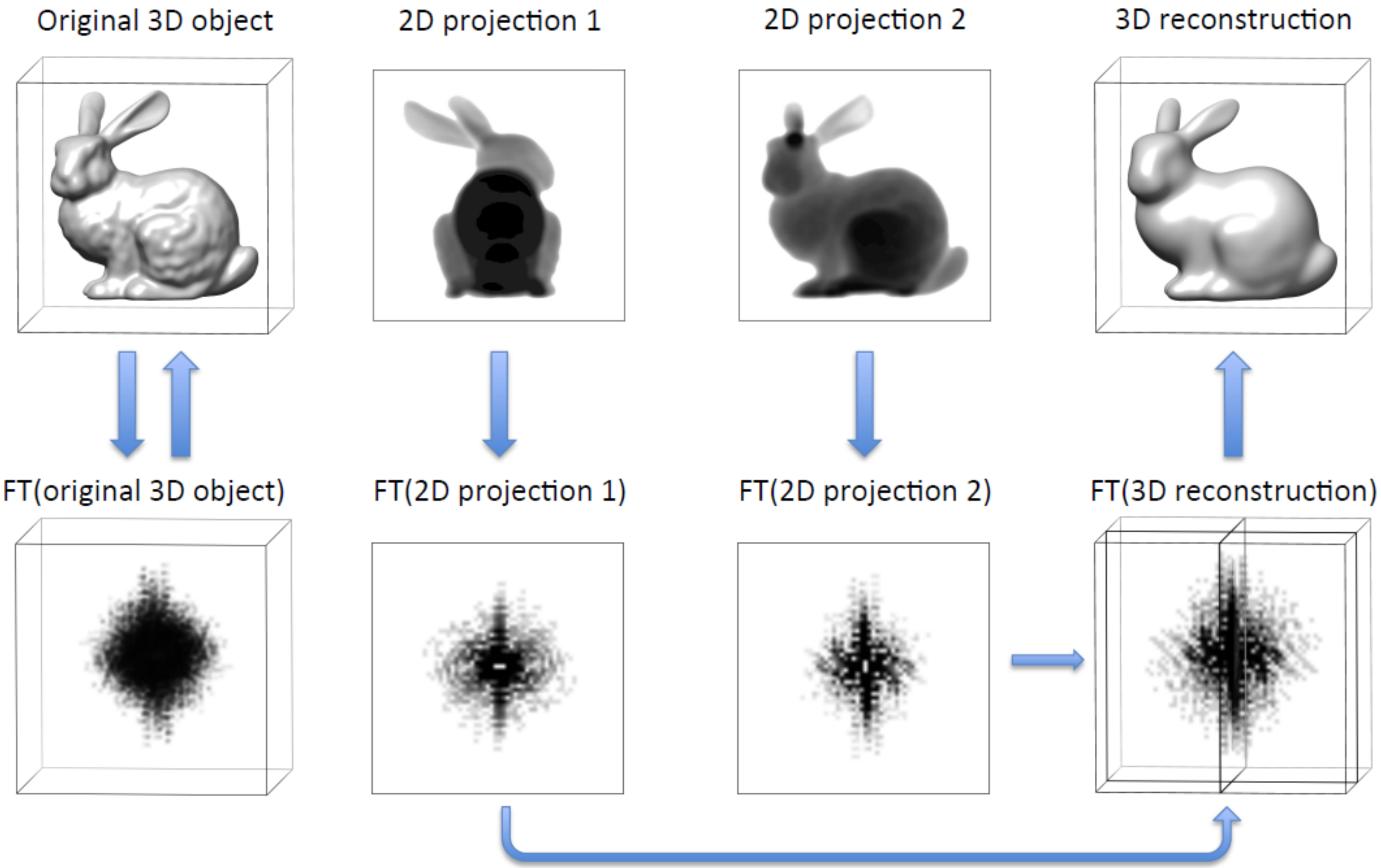
Target (512 × 512) | Template | Cross correlation map | Shift



From 2D to 3D: central section theorem and Euler angles

Juha Huiskonen & Bilal Qureshi

Central section theorem

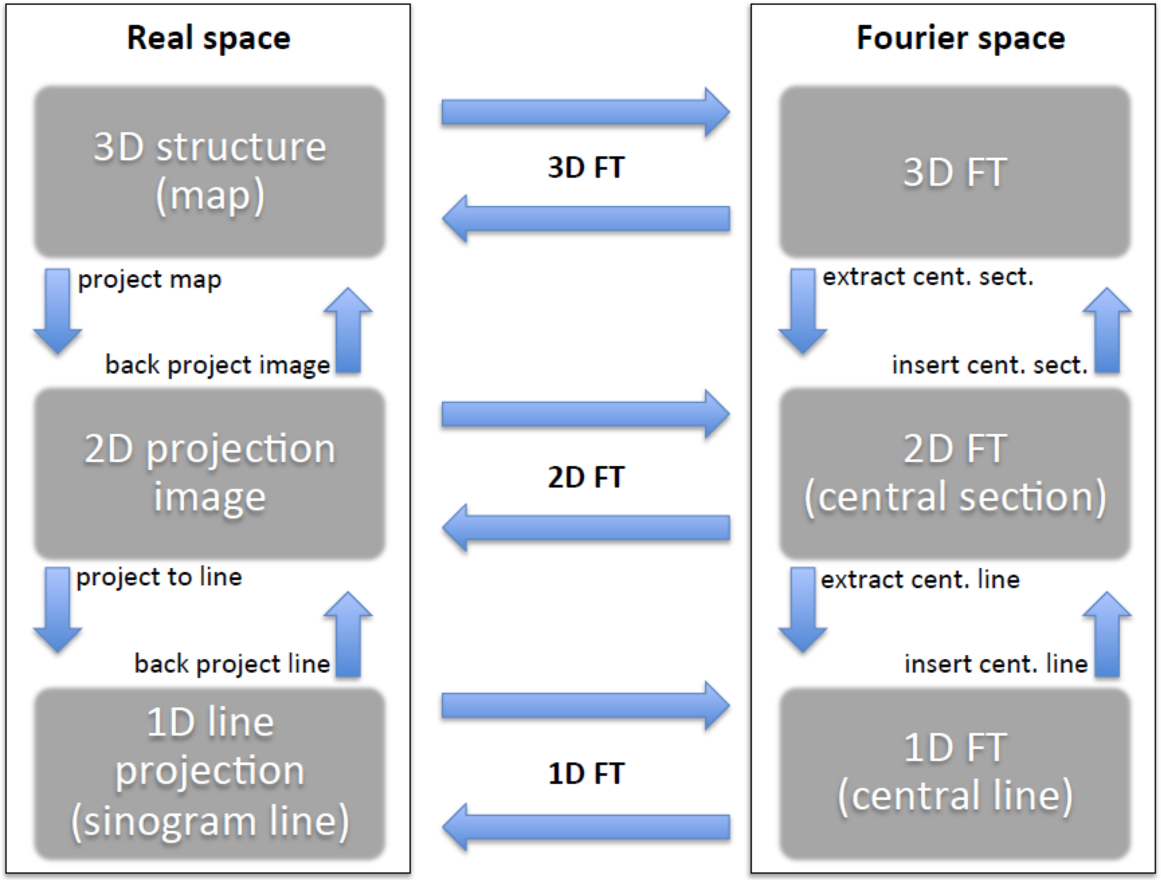


Juha Huiskonen & Bilal Qureshi

Central section theorem

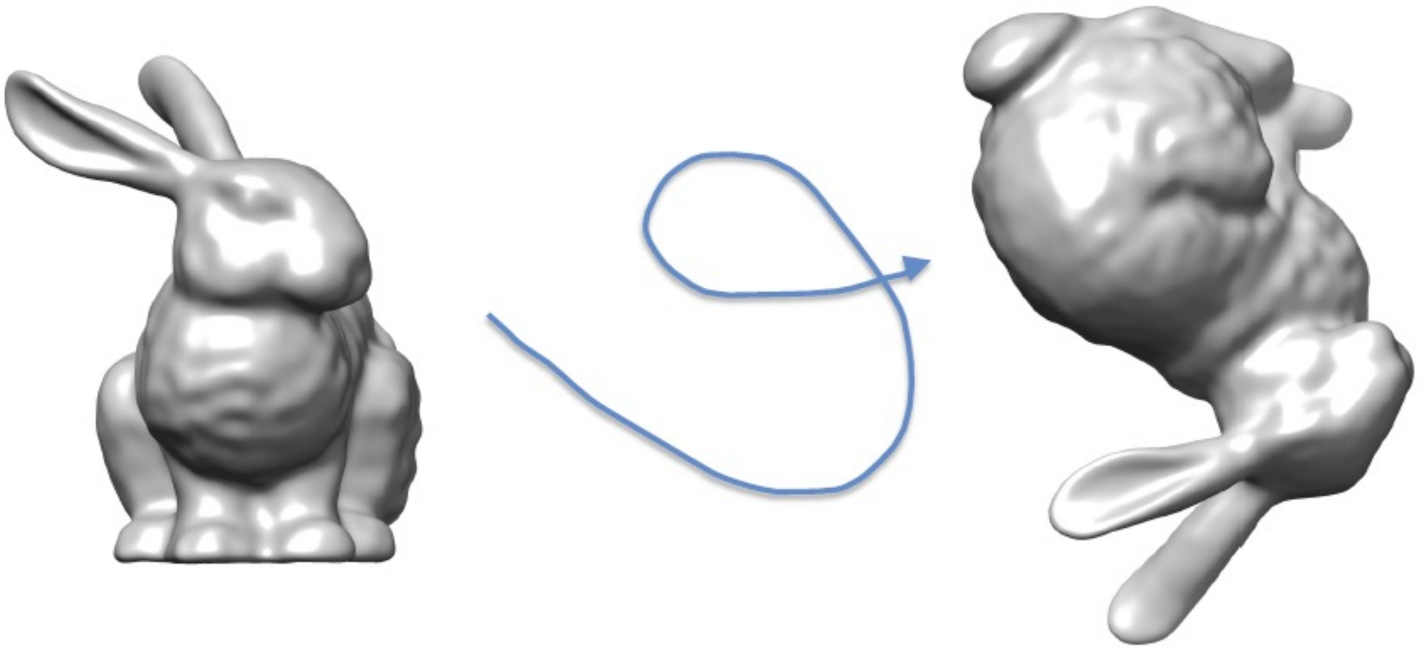
- A central section is a slice passing through the origin (center of the volume)
- The 2-dimensional Fourier transform of a projection of a 3-dimensional object is a central section of the 3-dimensional Fourier transform of the original object
- One can determine the 3-dimensional Fourier transform by sampling it on central sections obtained from 2-dimensional images of the specimen
- With enough central sections, the entire 3-dimensional transform can be determined
- Model (reconstruction) of the original 3-dimensional object can be calculated by an inverse Fourier transform operation on the estimated 3-dimensional Fourier transform
- More sections the more isotropic the resolution

Central section theorem – real space vs. FT



Juha Huiskonen & Bilal Qureshi

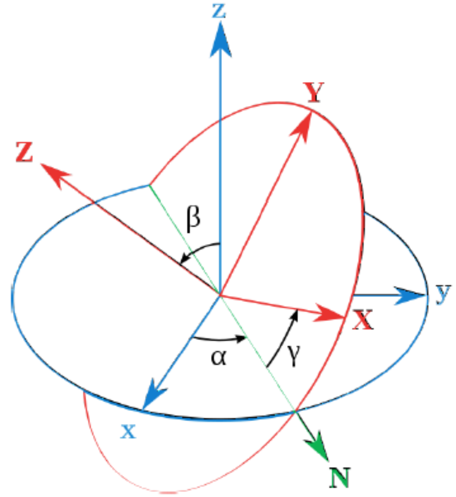
3D objects orientation in space

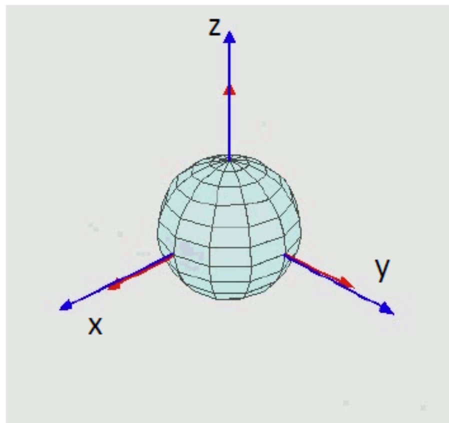


Juha Huiskonen & Bilal Qureshi

Euler angles

- Three parameters required to describe a rotation from (or to) a standard orientation
- α, β, and γ describe rotations around three axis
- The axis rotate together with the object!
- Here Z-X-Z rotation is illustrated





Juha Huiskonen & Bilal Qureshi

Summary

- Fourier transforms are used almost at every step of the cryo-EM image processing pipeline
- Many concepts can be described either in Fourier space or real space
- Cross-correlation is used to compare and align images
- Central section theorem is the idea behind 3D reconstruction
- Objects orientation (or view direction) can be described with 3 angles