



Science and
Technology
Facilities Council

Introduction to image processing

Colin Palmer

Oxford WT DTP cryo-EM course

27 April 2021



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

Overview

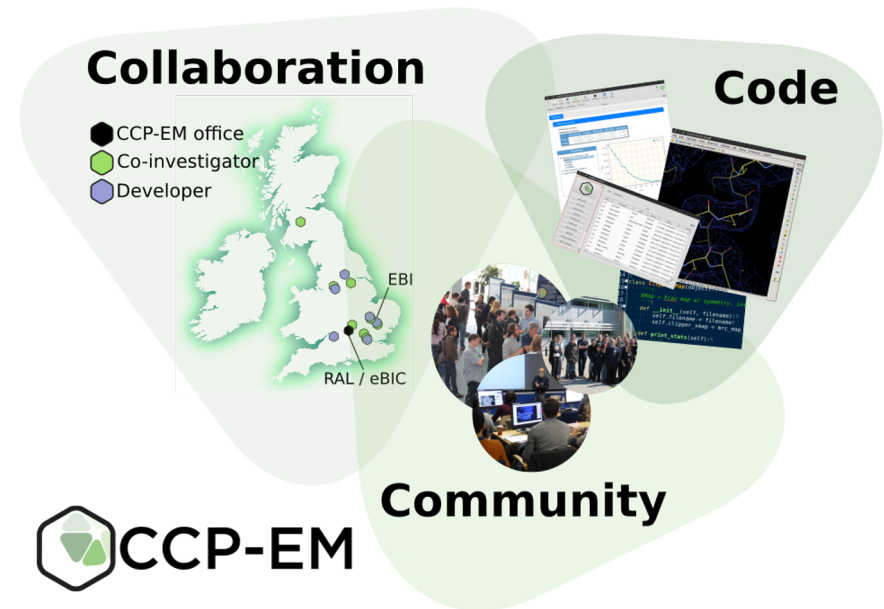
- Introduction
- Fourier transform
- Break
- Filters
- Convolution
- Point spread and contrast transfer functions
- Cross-correlation
- Central section theorem and 3D reconstruction
- Euler angles

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



Introduction – what is CCP-EM?

When people say “CCP-EM,” they mean...

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

The screenshot shows the JISCmail CCP-EM Archives web interface. The browser address bar displays the URL <https://www.jiscmail.ac.uk/cgi-bin/webadmin?A1=ind190>. The page header includes the JISCmail logo and the text "Email discussion lists for the UK Education and Research communities". Below the header, there are navigation links for "List Management", "Subscriber's Corner", "Email Lists", and "JISCmail Tools". The main content area is titled "CCPEM Archives" and shows a list of messages for July 2019. The list includes columns for Subject, From, Date, and Size. The messages are sorted by date, with the most recent at the top. The interface also includes options for "Subscribe or Unsubscribe", "Post New Message", "Newsletter Templates", "Log Out", and "Change Password". On the right side, there are sections for "JiscMail Tools" (Files Area | help), "RSS Feeds and Sharing" (RSS 1.0 feed, RSS 2.0 feed, Atom feed, Bookmark/Share), and "Search Archives" (Advanced Options, Search).

Subject	From	Date	Size	Delete
Re: strange planes in Relion-3.0.6 refinement result after Polishing	Takanoni Nakane <tnakane@MRC-LMB.CAM.AC.UK>	Tue, 23 Jul 2019 08:08:57 +0100	147 lines	
Re: strange planes in Relion-3.0.6 refinement result after Polishing	qianhui qu <jasy198308@GMAIL.COM>	Tue, 23 Jul 2019 00:03:47 -0700	331 lines	
Re: strange planes in Relion-3.0.6 refinement result after Polishing	Takanoni Nakane <tnakane@MRC-LMB.CAM.AC.UK>	Tue, 23 Jul 2019 07:54:44 +0100	72 lines	
Re: strange planes in Relion-3.0.6 refinement result after Polishing	qianhui qu <jasy198308@GMAIL.COM>	Mon, 22 Jul 2019 23:24:27 -0700	65 lines	
Re: strange planes in Relion-3.0.6 refinement result after Polishing	Takanoni Nakane <tnakane@MRC-LMB.CAM.AC.UK>	Mon, 22 Jul 2019 22:44:15 +0100	53 lines	
strange planes in Relion-3.0.6 refinement result after Polishing	qianhui qu <jasy198308@GMAIL.COM>	Mon, 22 Jul 2019 13:54:01 -0700	8586 lines	
Re: 2D classification of amyloid fibrils (EMPIAR 10230)	Oluye Li <qli112@CASE.EDU>	Mon, 22 Jul 2019 16:32:21 -0400	169 lines	
Re: NVIDIA driver/library mismatch	Lugmayr, Wolfgang <wlugmayr@UKE.DE>	Mon, 22 Jul 2019 11:40:40 +0200	47 lines	
Re: Classification in relion	Sjors Scheres <scheres@MRC-LMB.CAM.AC.UK>	Sun, 21 Jul 2019 21:05:44 +0100	56 lines	

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”



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>

The image shows a screenshot of the CCP-EM website and its software interface. The website, titled "Collaborative Computational Project for Electron cryo-Microscopy", features a navigation menu with links to Home, About CCP-EM, CCP-EM Projects, Downloads, Resources / Documentation, Workshops / Courses, and Symposium. Below the navigation is a section for "CCP-EM downloads" and "CCP-EM software suite".

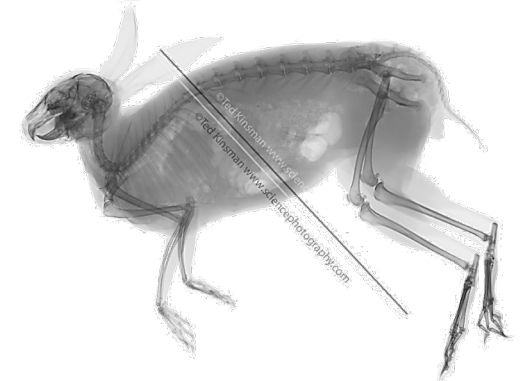
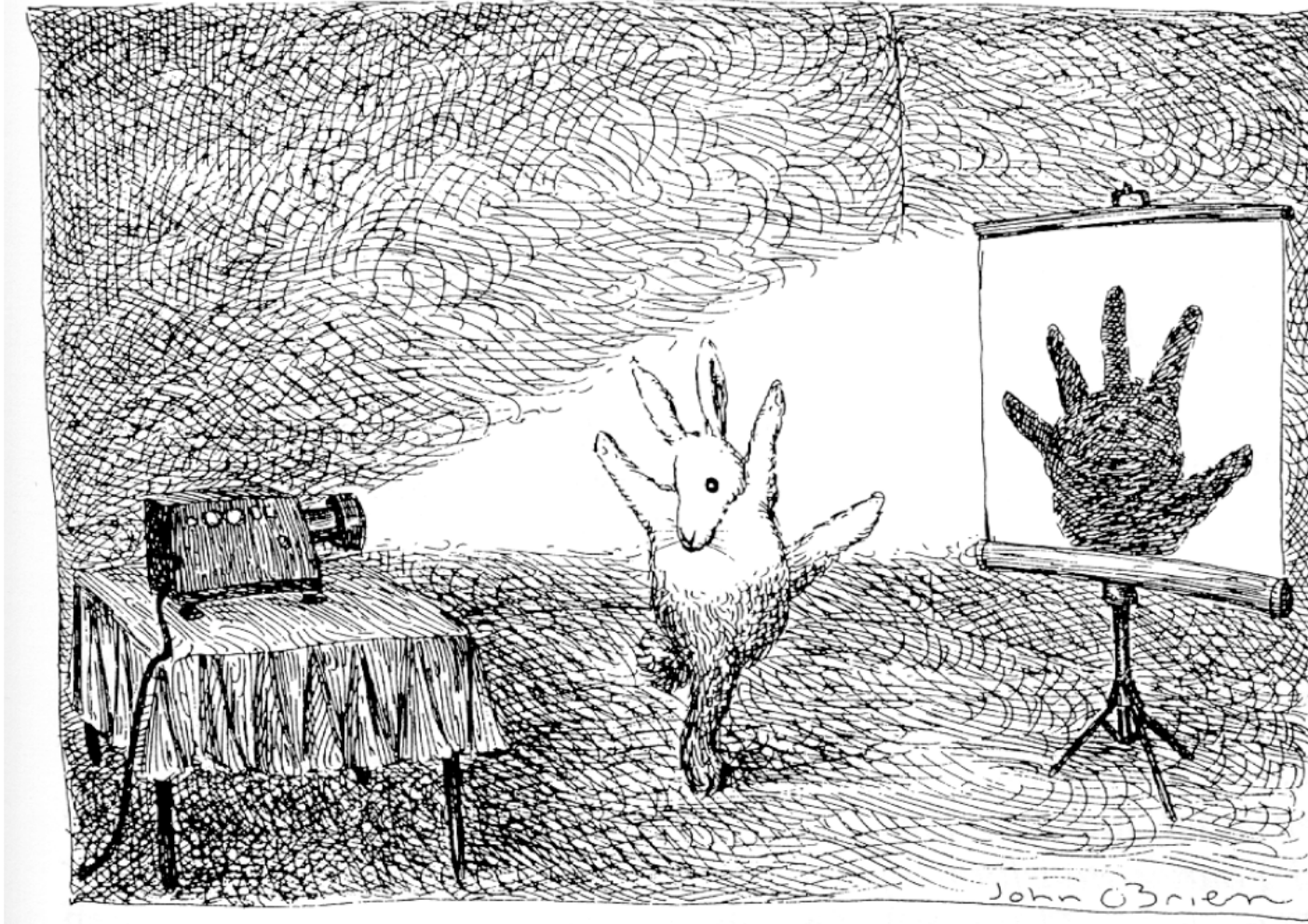
The software interface is a graphical user interface for cryo-EM data processing. It includes a "Task pipeline" window showing a sequence of steps: Stage 1 (Map to MTZ), Stage 2 (Set PDB cell), Stage 3 (Refmac refine (global)), Stage 4 (Shake refined structure), Stage 5 (Map to MTZ (HM1)), Stage 6 (Refmac refine (HM1)), Stage 7 (Map to MTZ (HM2)), Stage 8 (Refmac stats (HM2)), and Stage 9 (Refmac FSC (HM1 vs HM2)).

The "Results" window displays a "Refinement summary" table with the following data:

	FSC average	R factor	Rms single	Rms bond	Rms chiral
Start	0.7178	0.4301	1.2440	0.0115	0.1112
Finish	0.8505	0.3484	2.4396	0.0244	0.1621

Below the table is a "Graph Data" window showing a plot of "Refact" (Y-axis, ranging from 0.35 to 0.43) versus "Refact" (X-axis, ranging from 0.35 to 0.43). The plot shows a curve that starts at approximately (0.35, 0.43) and decreases to approximately (0.36, 0.35). The graph also includes a legend for "Refact" and a "Statistics per refinement cycle" section with sub-sections for "Final refinement statistics" and "Input SF statistics".

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



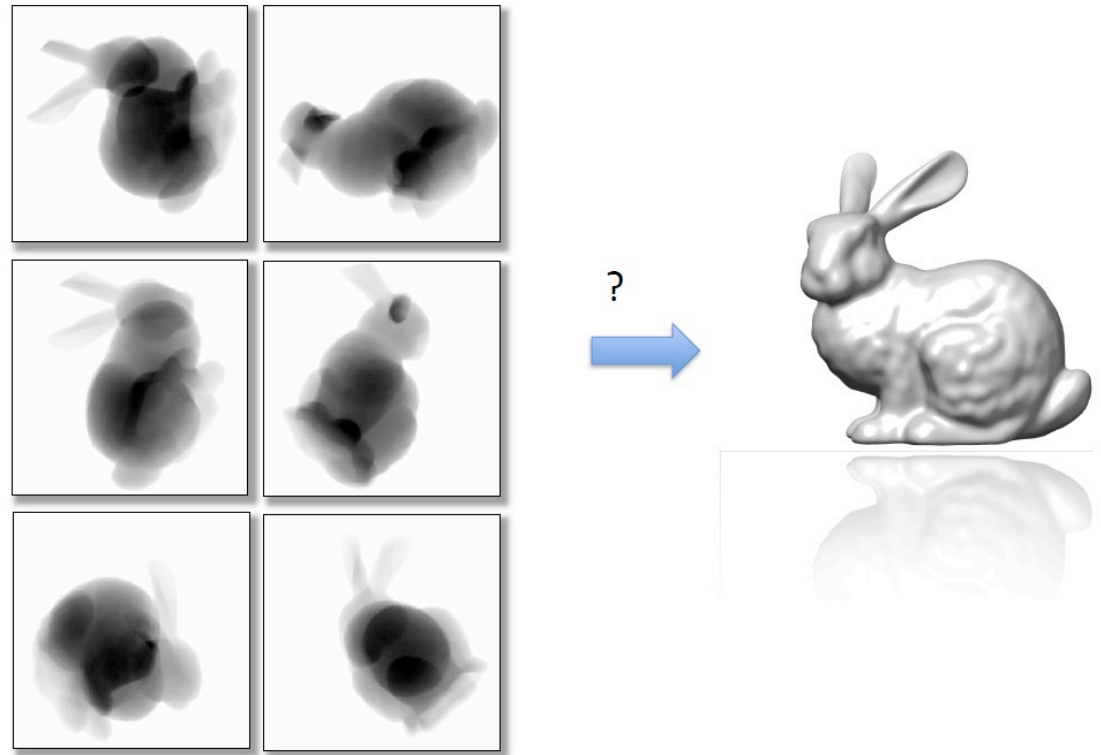
<https://tedkinsman.photoshelter.com/image/I0000rQCSPSbNCQO>



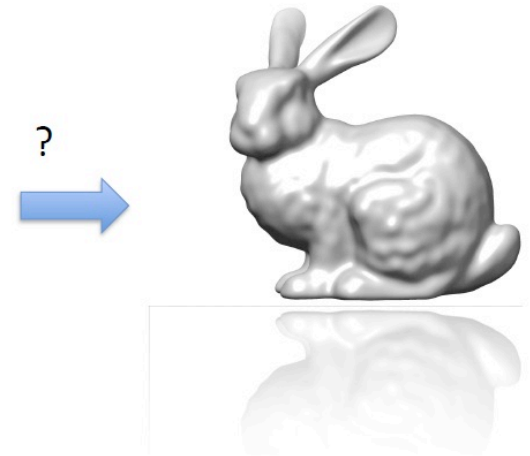
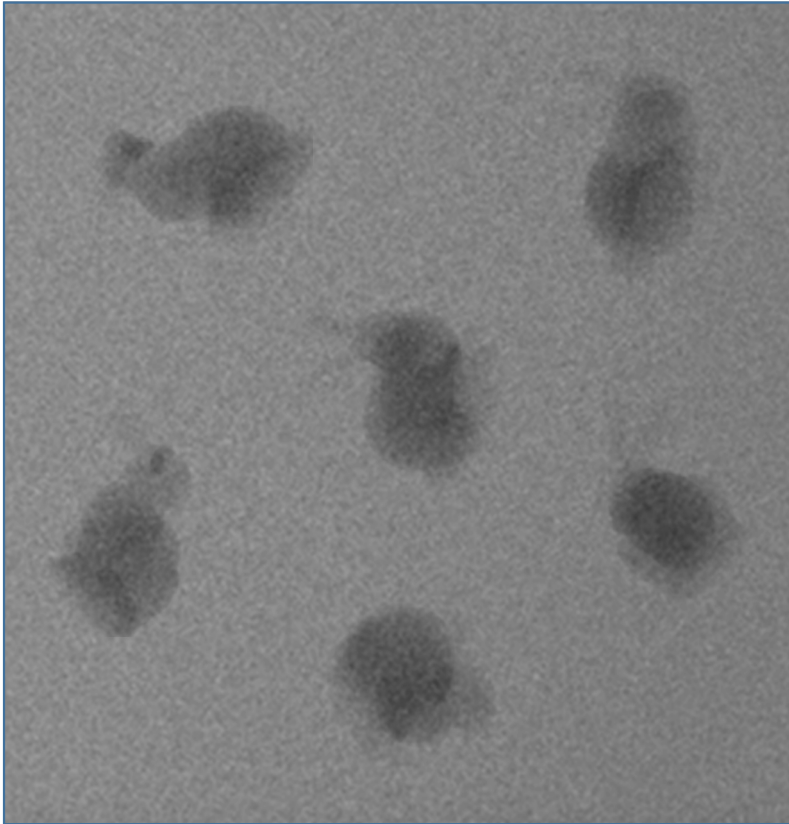
<https://dissolve.com/stock-photo/Normal-hand-ray-year-old-rights-managed-image/102-D943-144-684>

*Cartoon from The New Yorker
Juha Huiskonen & Bilal Qureshi*

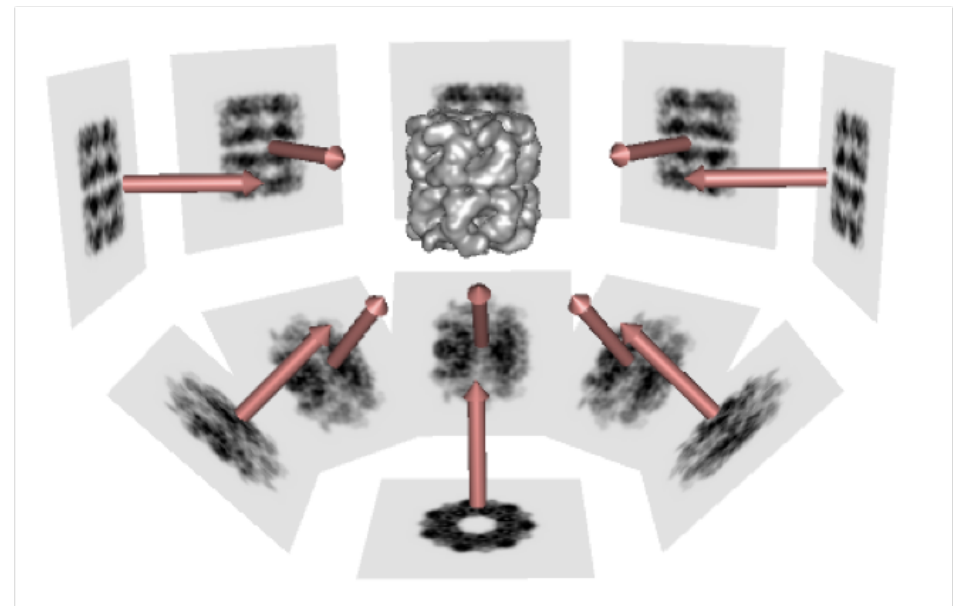
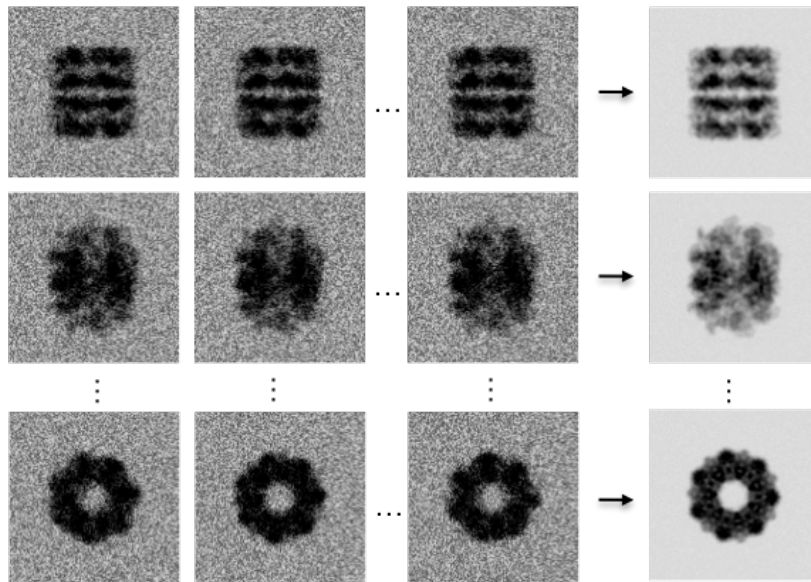
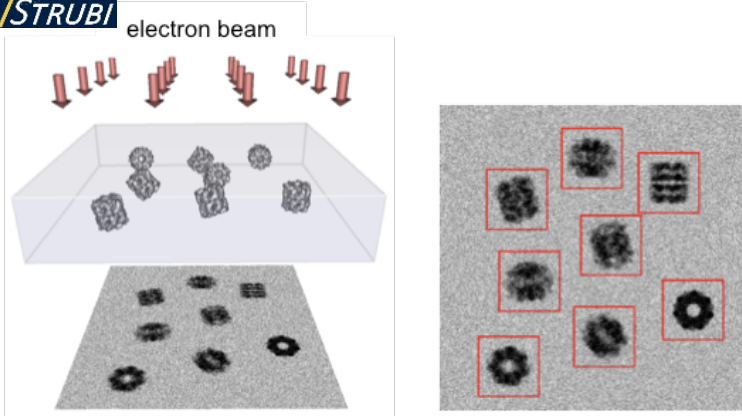
EM: Reconstruct 3D from 2D projections



EM: Reconstruct 3D from 2D projections



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





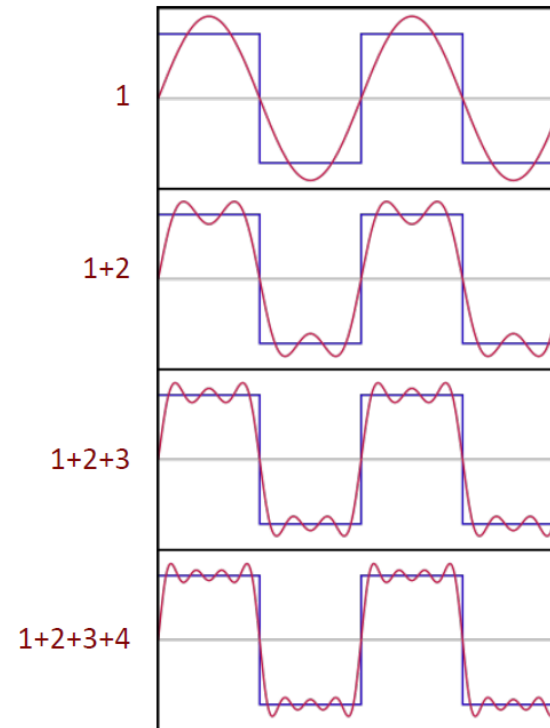
Fourier Transform

Juha Huiskonen & Bilal Qureshi

Fourier Transform and Fourier series

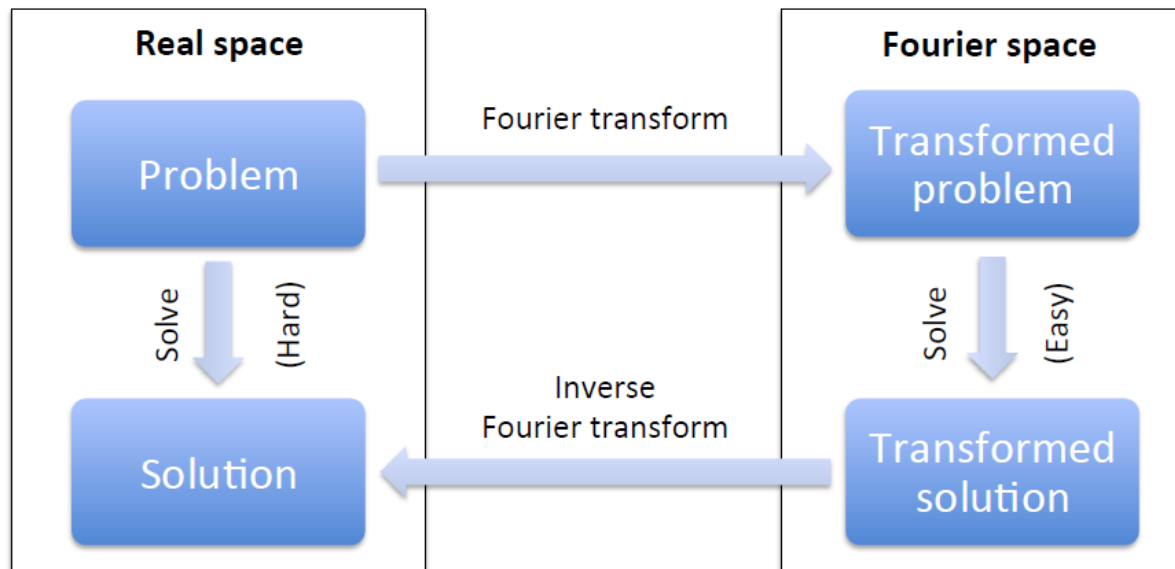
The Fourier transform decomposes a function of time (signal) into its constituent frequencies (Wikipedia)

- 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

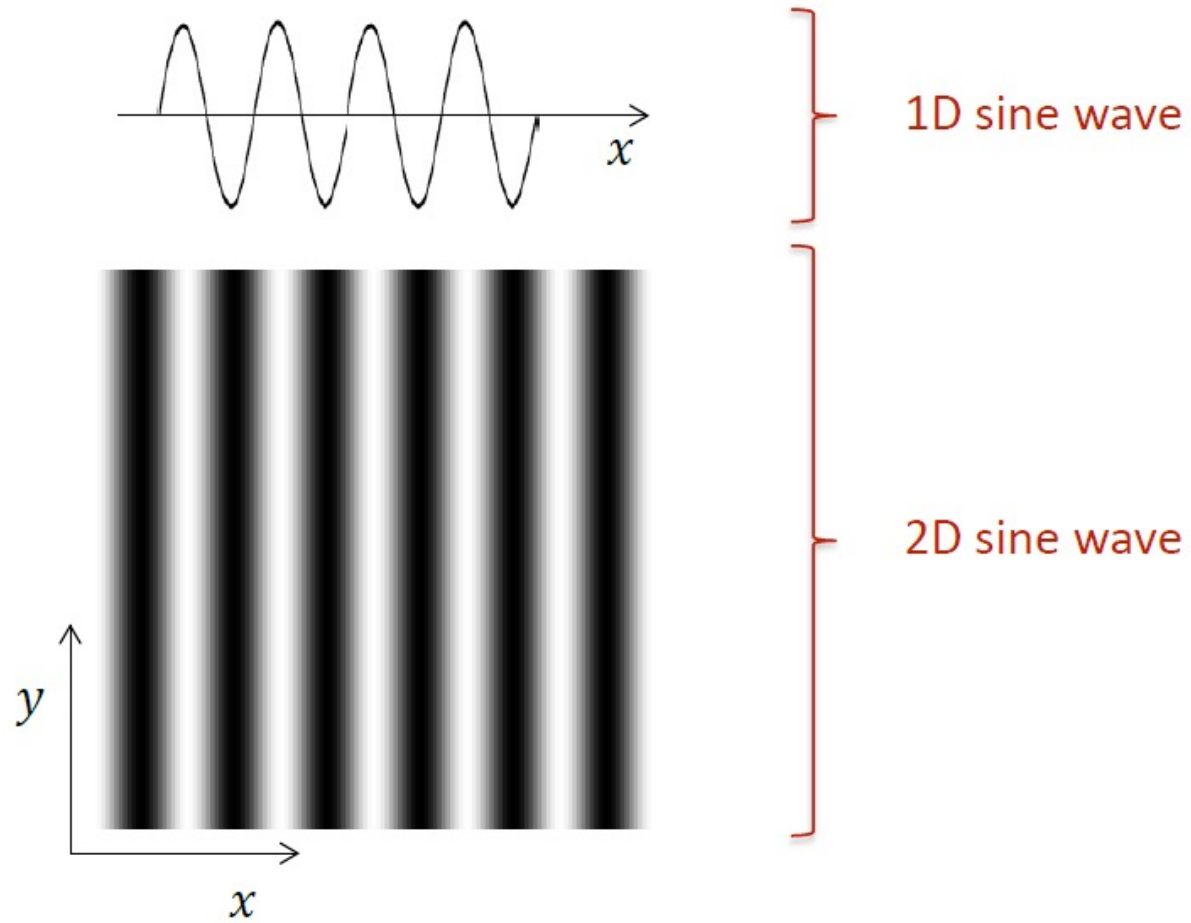


Fourier transform in image processing

- 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)

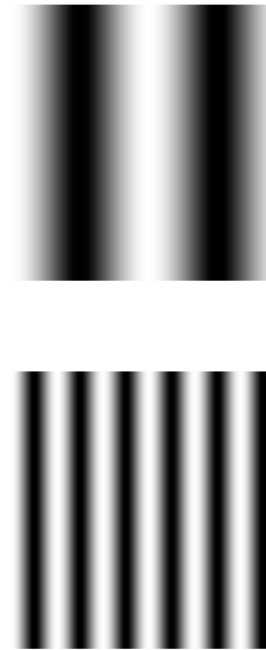


Fourier transform of images

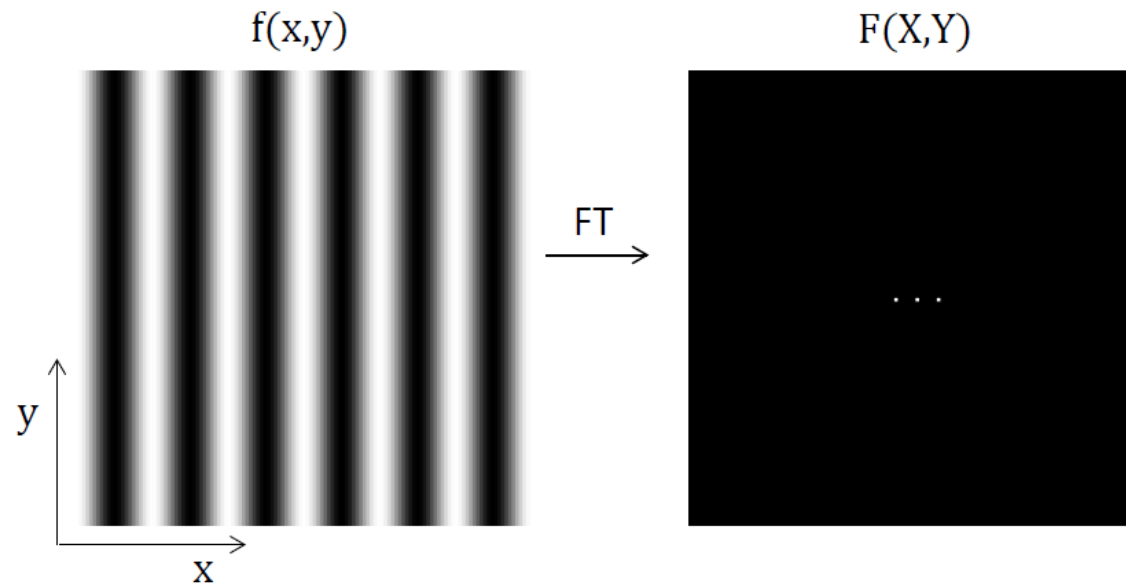


Fourier transform of images

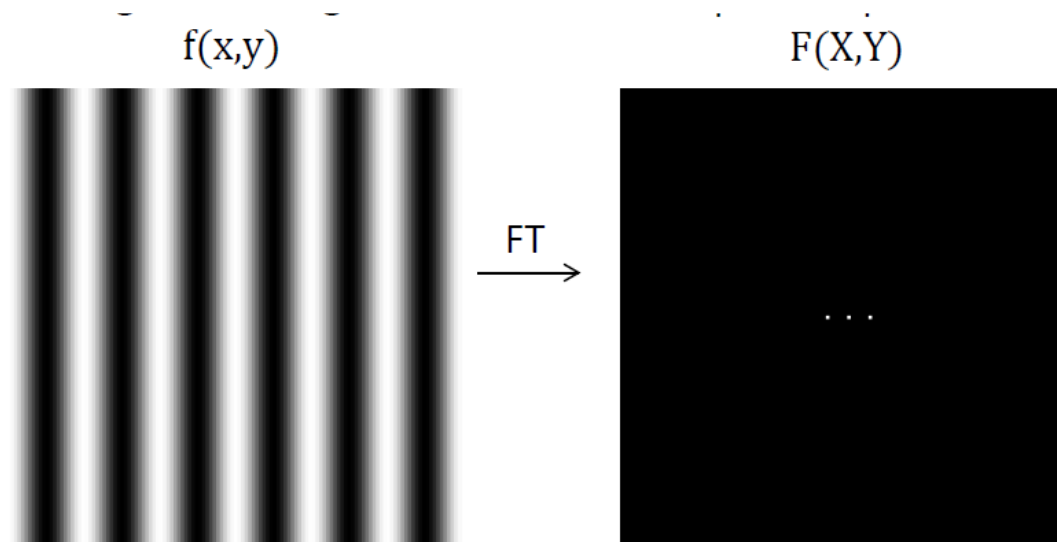
- Each sine function (Fourier term) encodes
 - the spatial **frequency**: the frequency across the image (here along the x-axis) with which the brightness modulates
 - the **magnitude** of the sine function (positive or negative): the difference between the dark and light areas in the image, negative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



Fourier transform of images

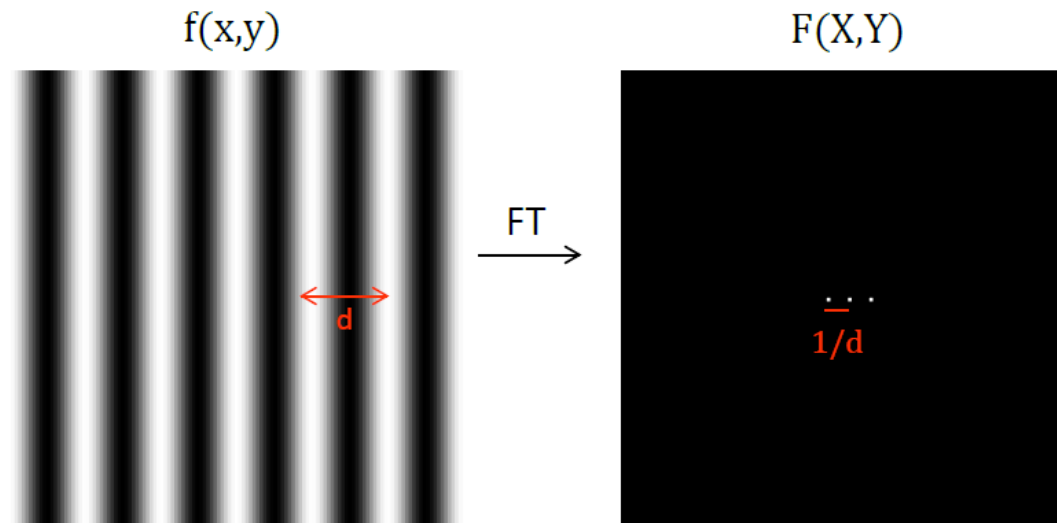


Fourier transform of images



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

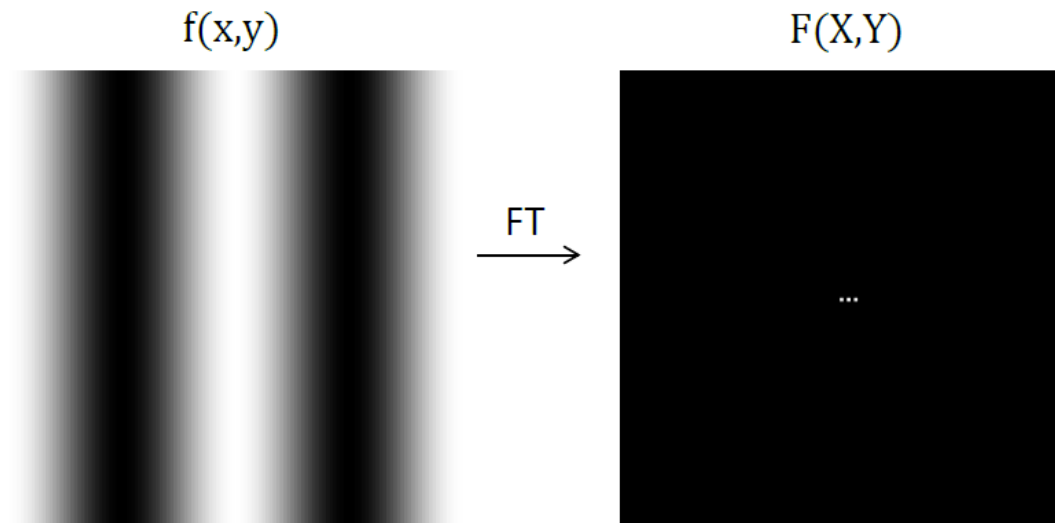
Fourier transform of images



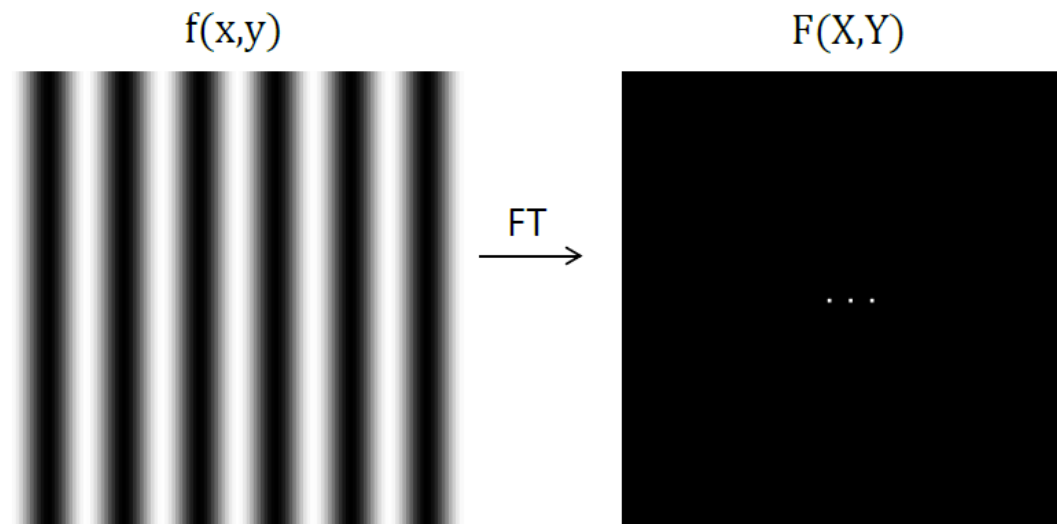
a brightness image with closely spaced features (high frequency information) will result in a amplitude spectrum with wide spacings

- Fourier space is also known as reciprocal space -

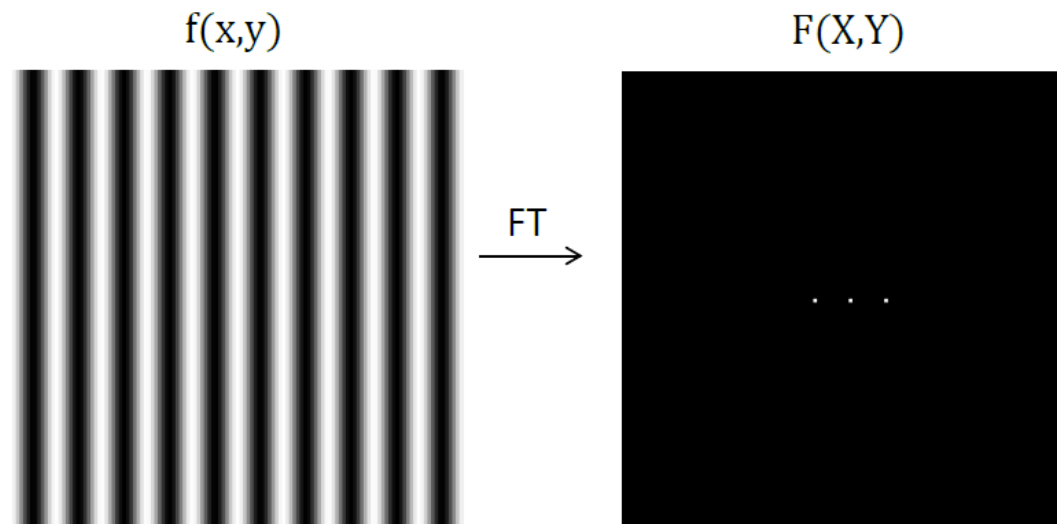
Fourier transform of images



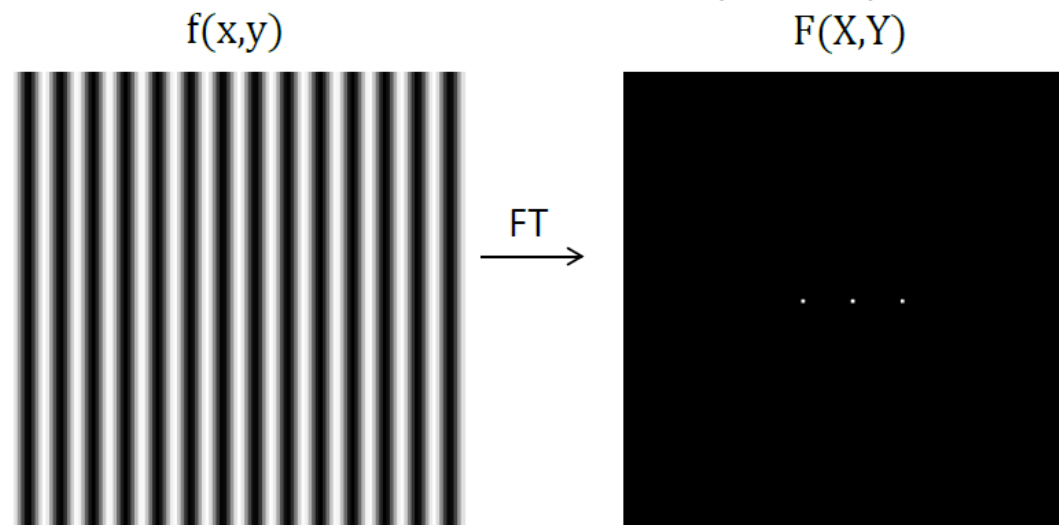
Fourier transform of images



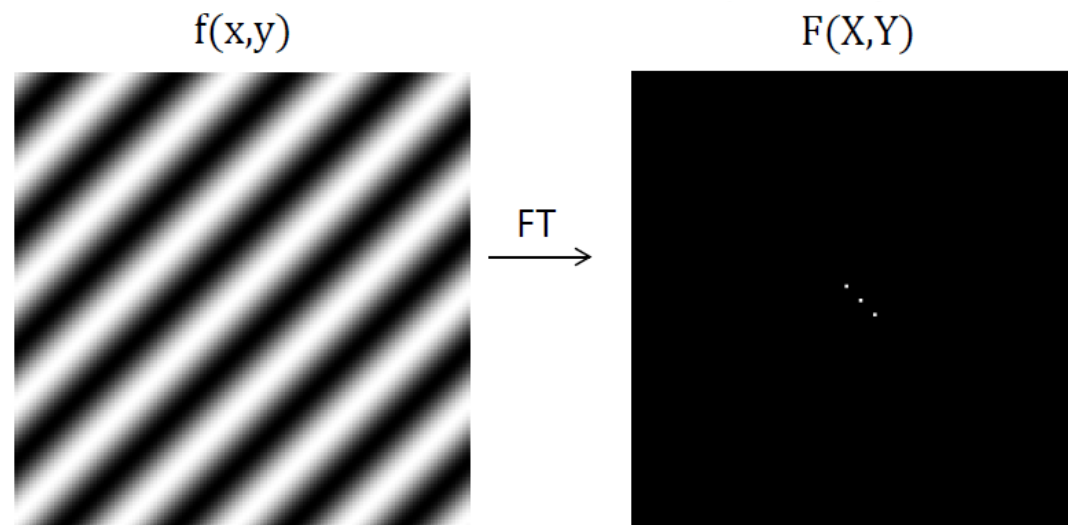
Fourier transform of images



Fourier transform of images



Fourier transform of images



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

Fourier transform of images

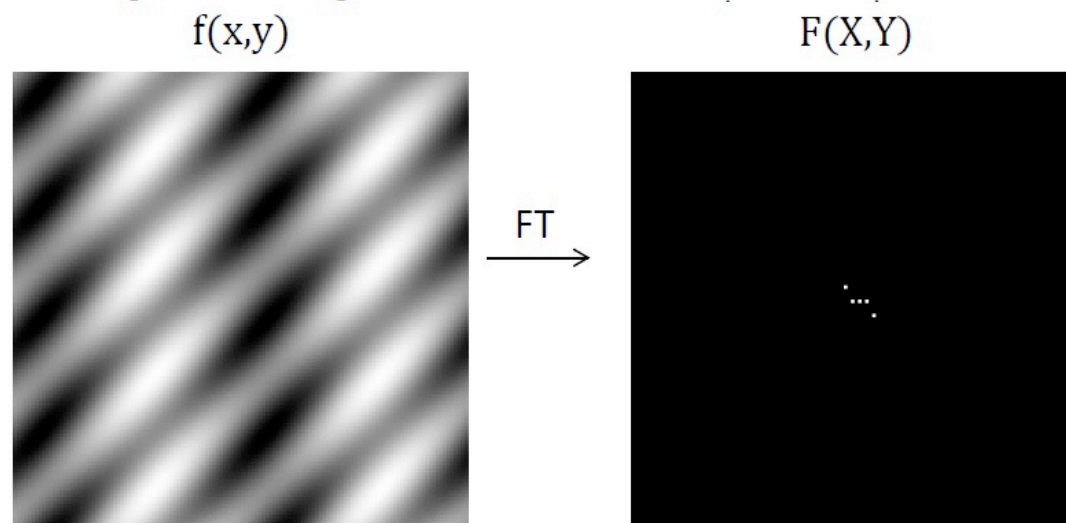
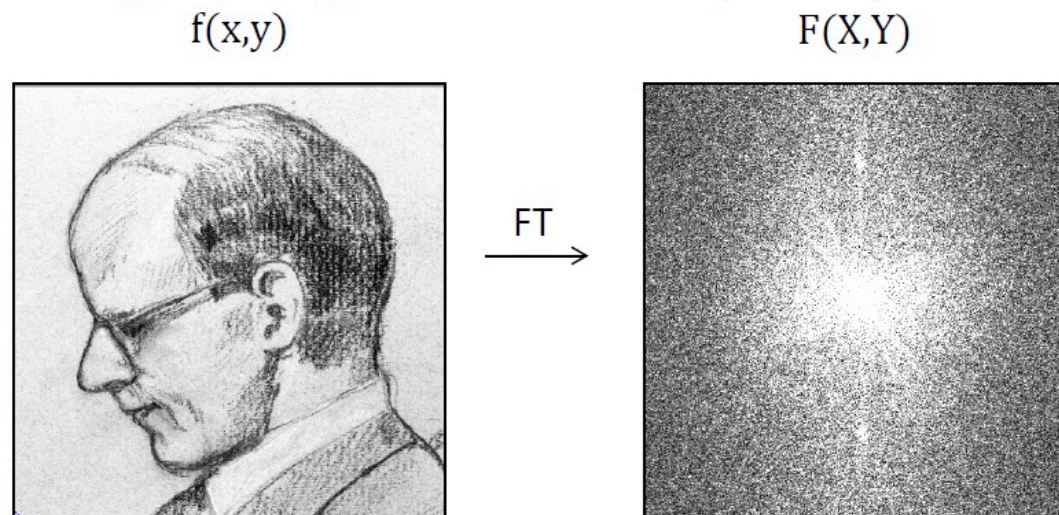


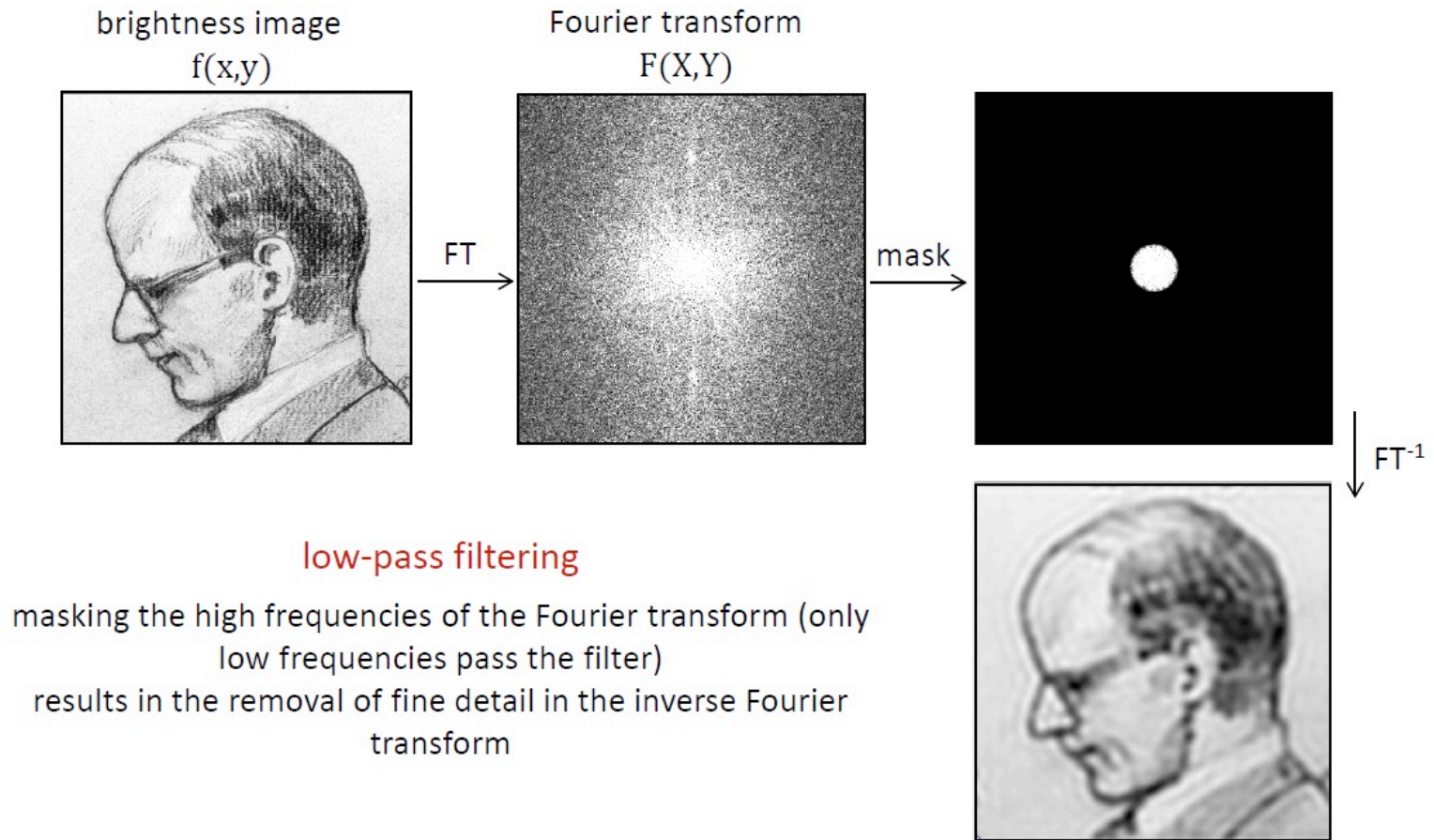
image formed by two sinusoidal components

Fourier transform of images

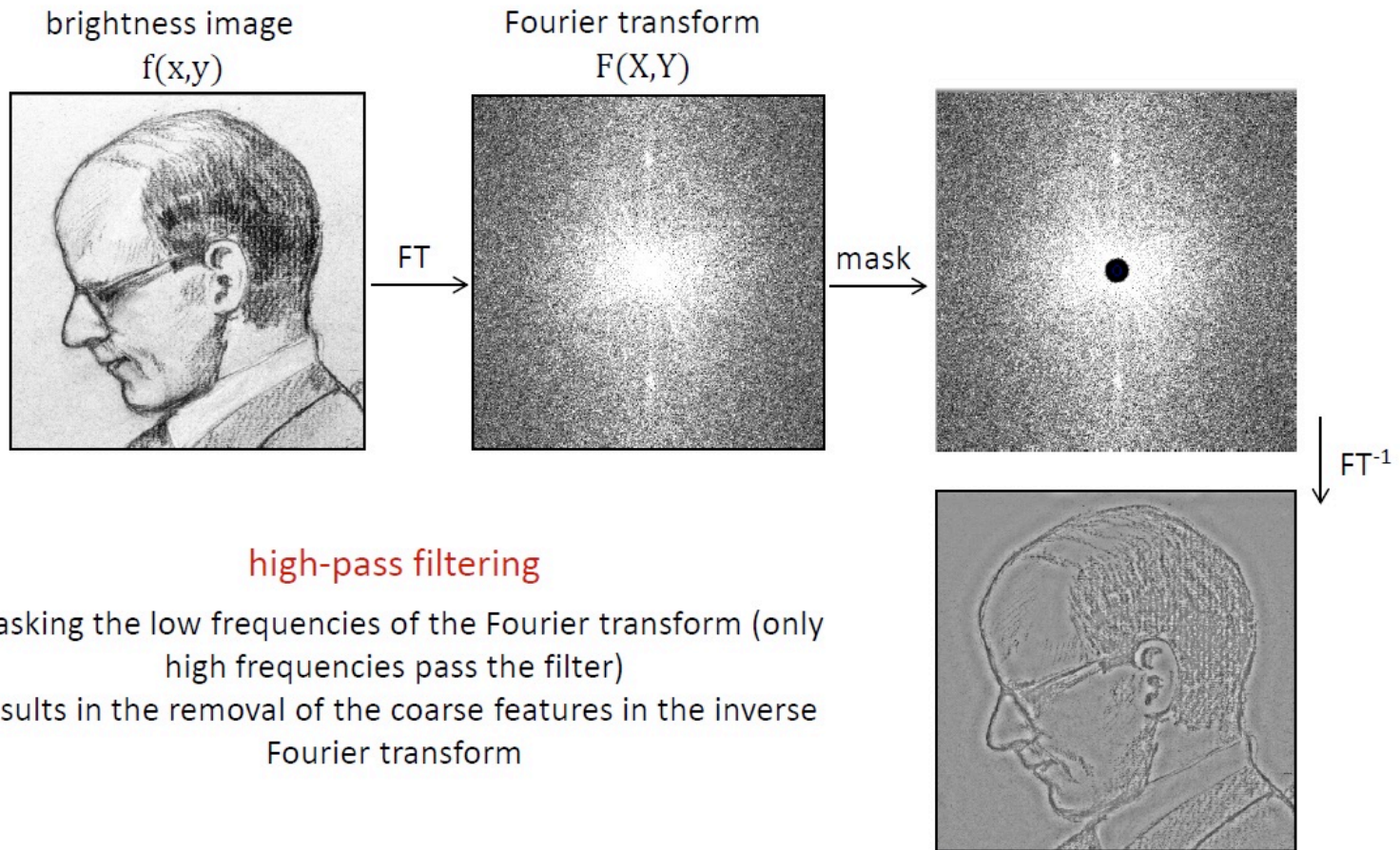


images formed by multiple sinusoidal components

Fourier transform of images



Fourier transform of images



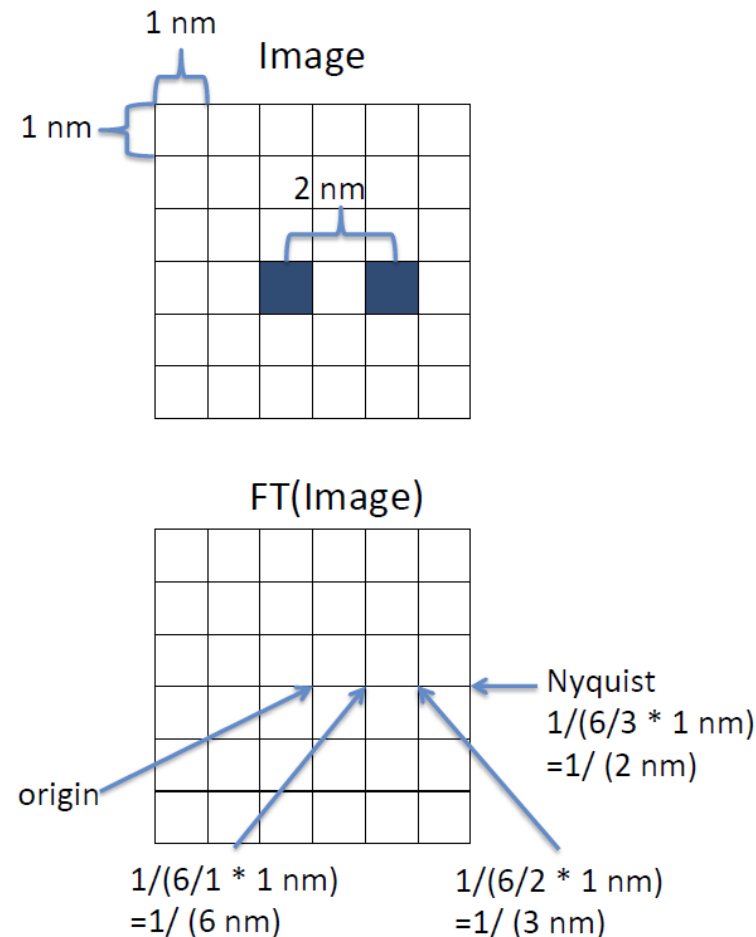
high-pass filtering

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

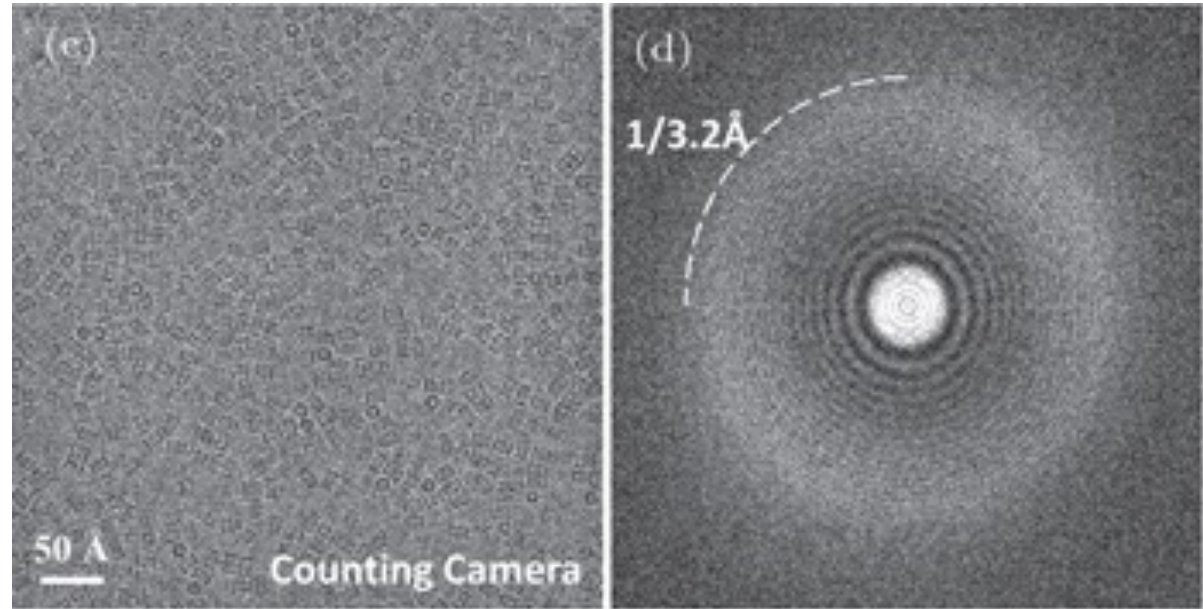
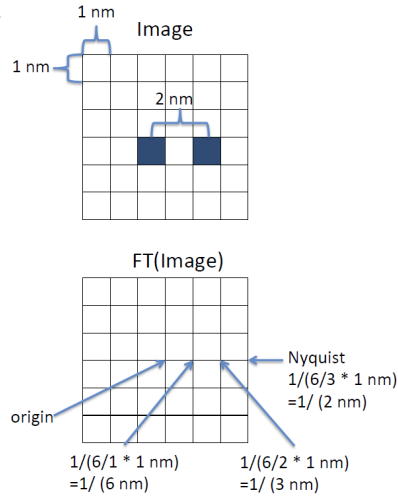
- The highest frequency that can be encoded in a digital image is called the Nyquist frequency
- In a Fourier transform of an image it is the edge of the transform
- It corresponds to the size of the digitized pixels and has the spatial frequency of $1/(2 \times \text{pixel})$
- Maximum resolution $2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



Fourier transform of images

Nyquist frequency

- The highest frequency that can be encoded in a digital image is called the Nyquist frequency
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- Maximum resolution 2x pixel size (here 2 nm)



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)*

$$\textit{rotated } F(u) = FT(\textit{rotated } f(x))$$

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

Break

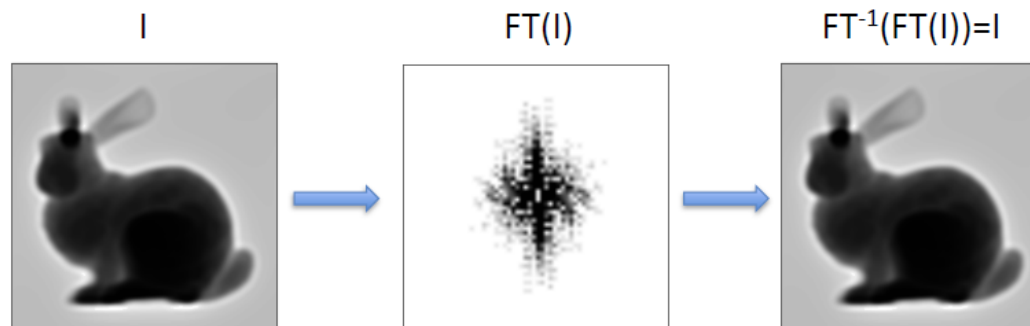
Any questions so far?



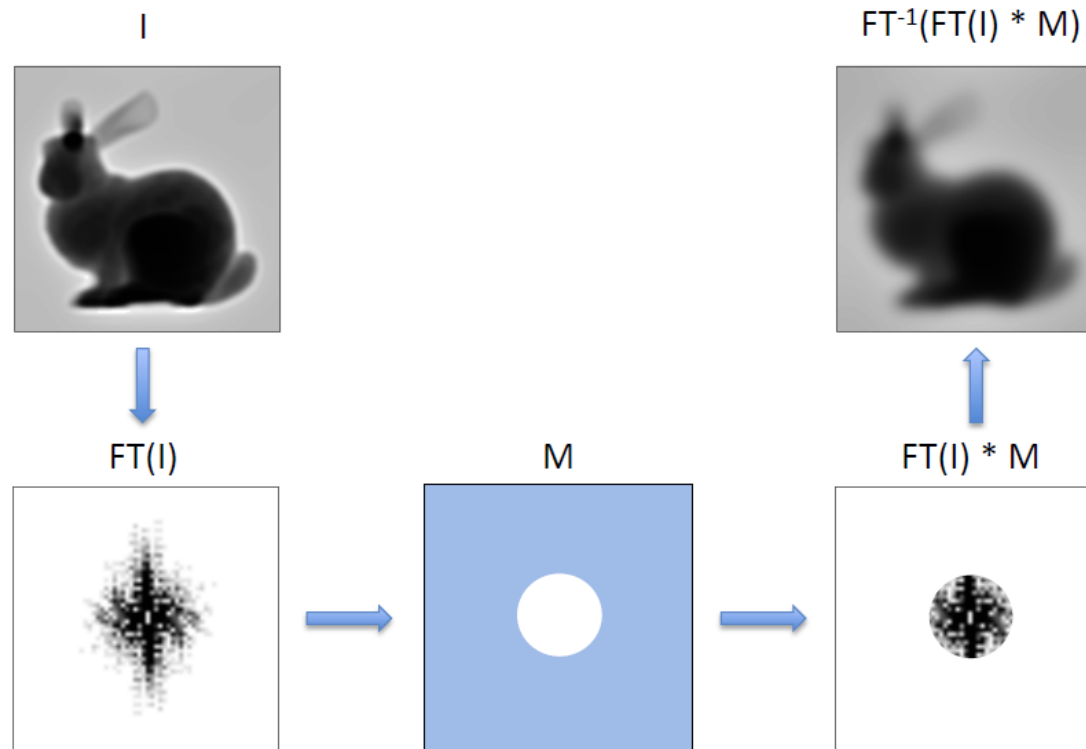
Fourier filters in image processing

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

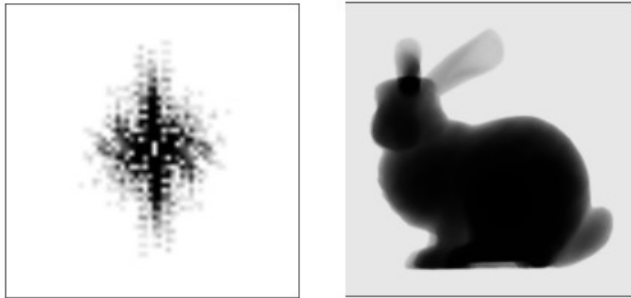


Fourier transform in filtering

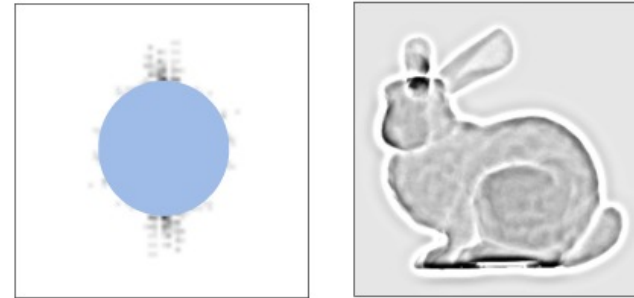


Fourier transform in filtering

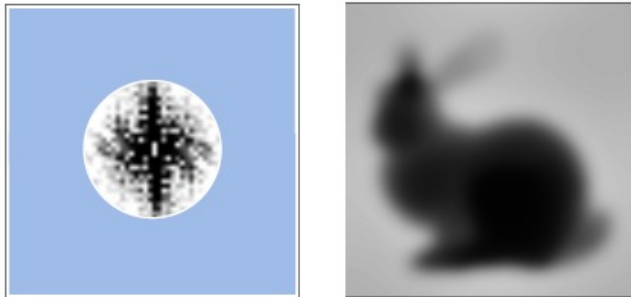
No filter



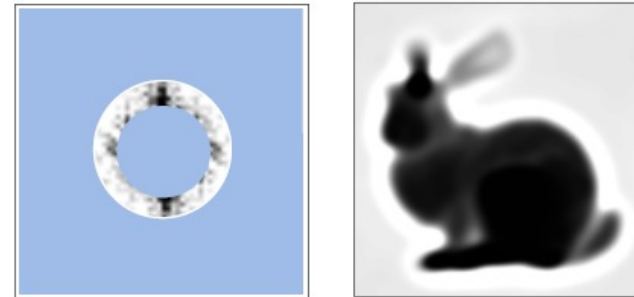
High pass filter



Low pass filter



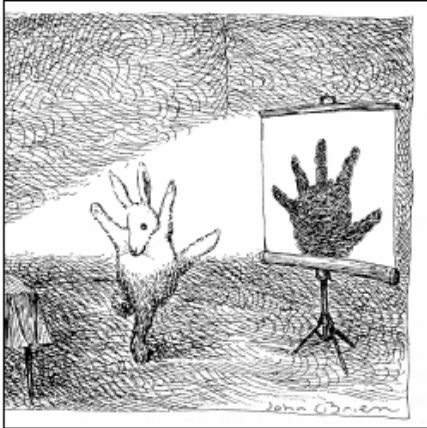
Band pass filter



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

Fourier transform in filtering

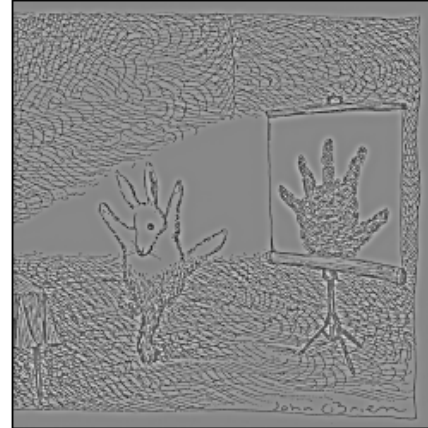
Original (512 pix)



Low pass (20 pix)



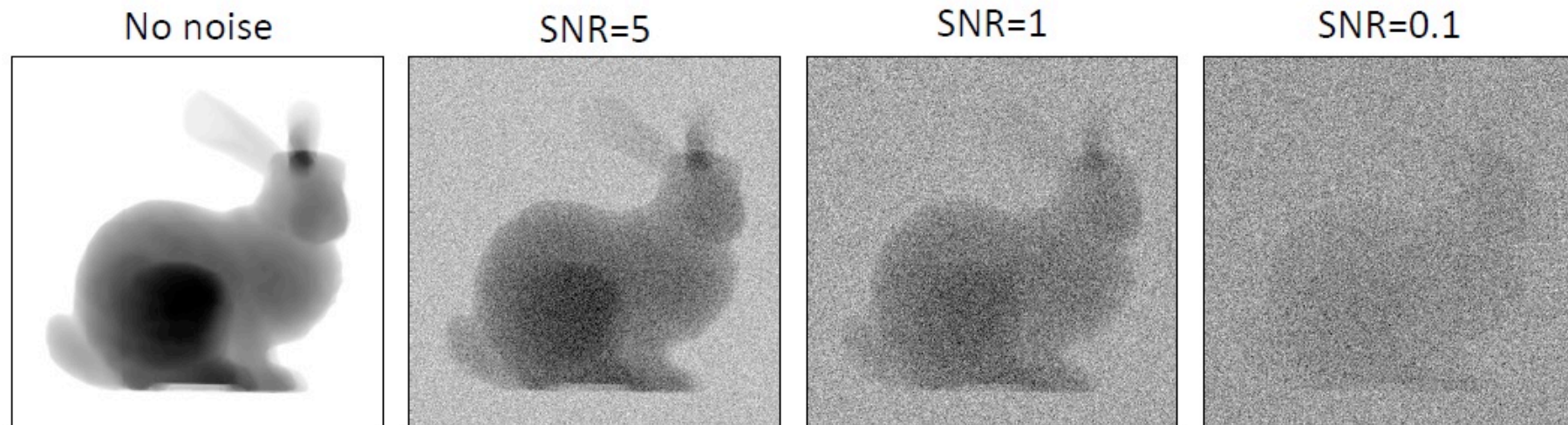
High pass (20 pix)



Band pass (10–30pix)



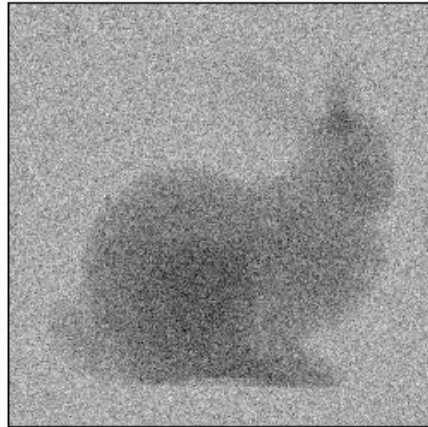
Images with Gaussian noise



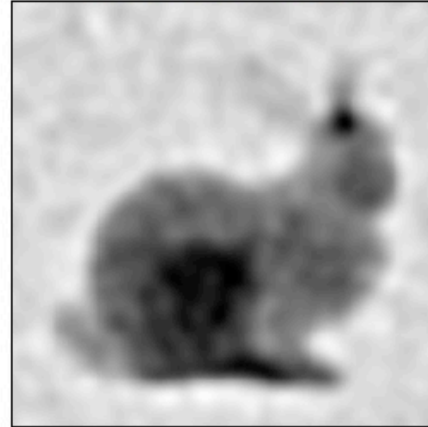
Signal-to-noise ratio (SNR) = contrast of the object / standard deviation of the noise

Use of low pass filter

SNR=1



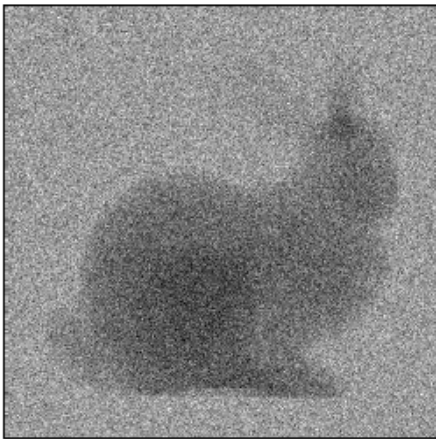
Low pass filter



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

Use of high pass filter

Original image



Gradient

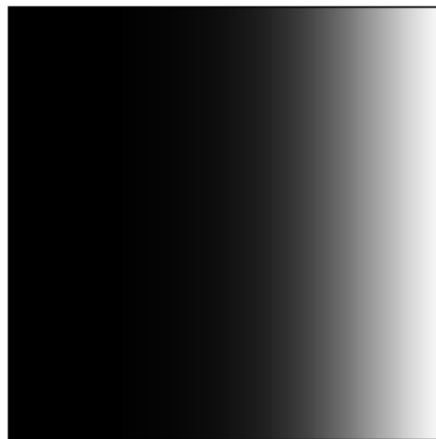
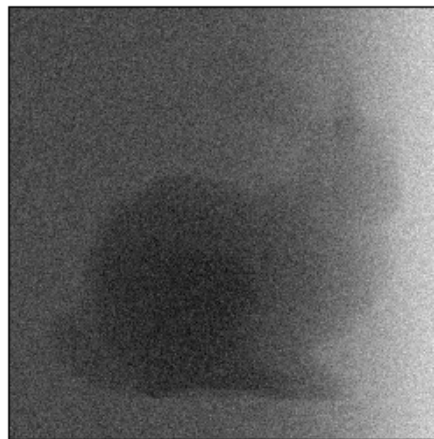
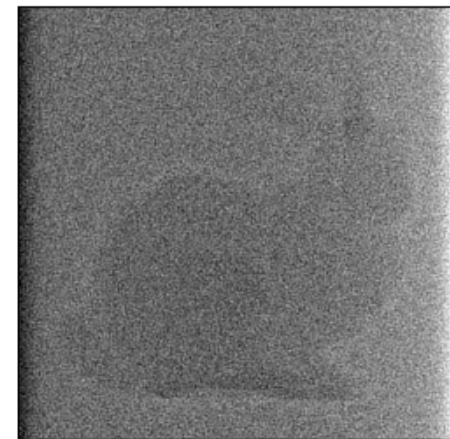


Image + gradient



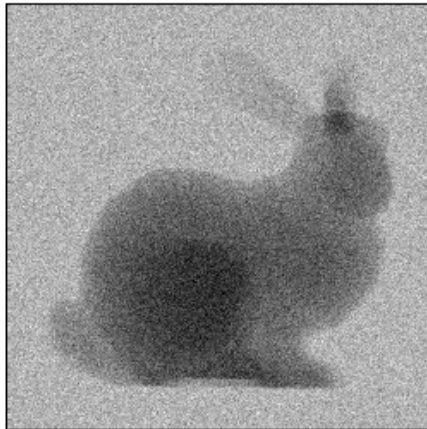
High-pass filter



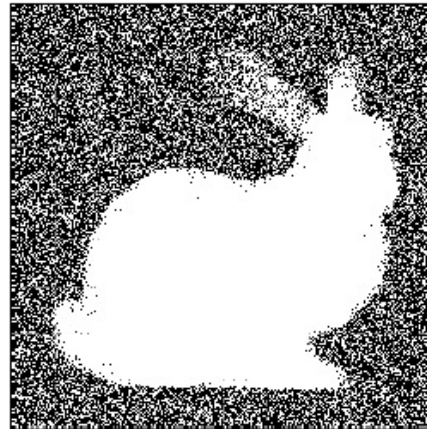
High pass filter can be used to remove low spatial frequency features, such as a density gradient.

Binary and morphological filters

Original image



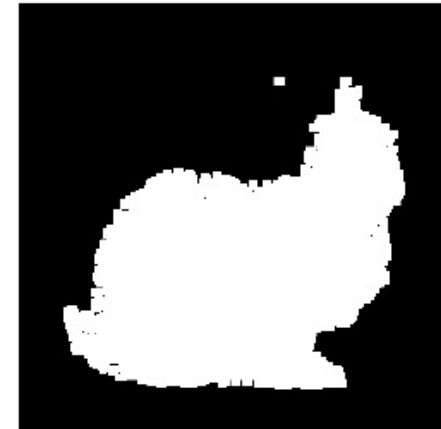
Binary mask



+ 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

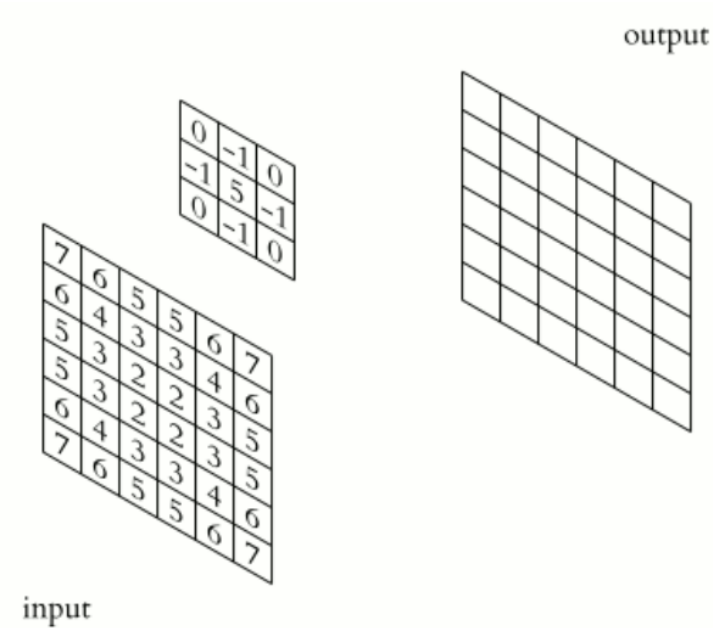
Dilation: if any value within a 3x3 kernel = 1: new value = 1



Convolution and convolution based filters in image processing

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

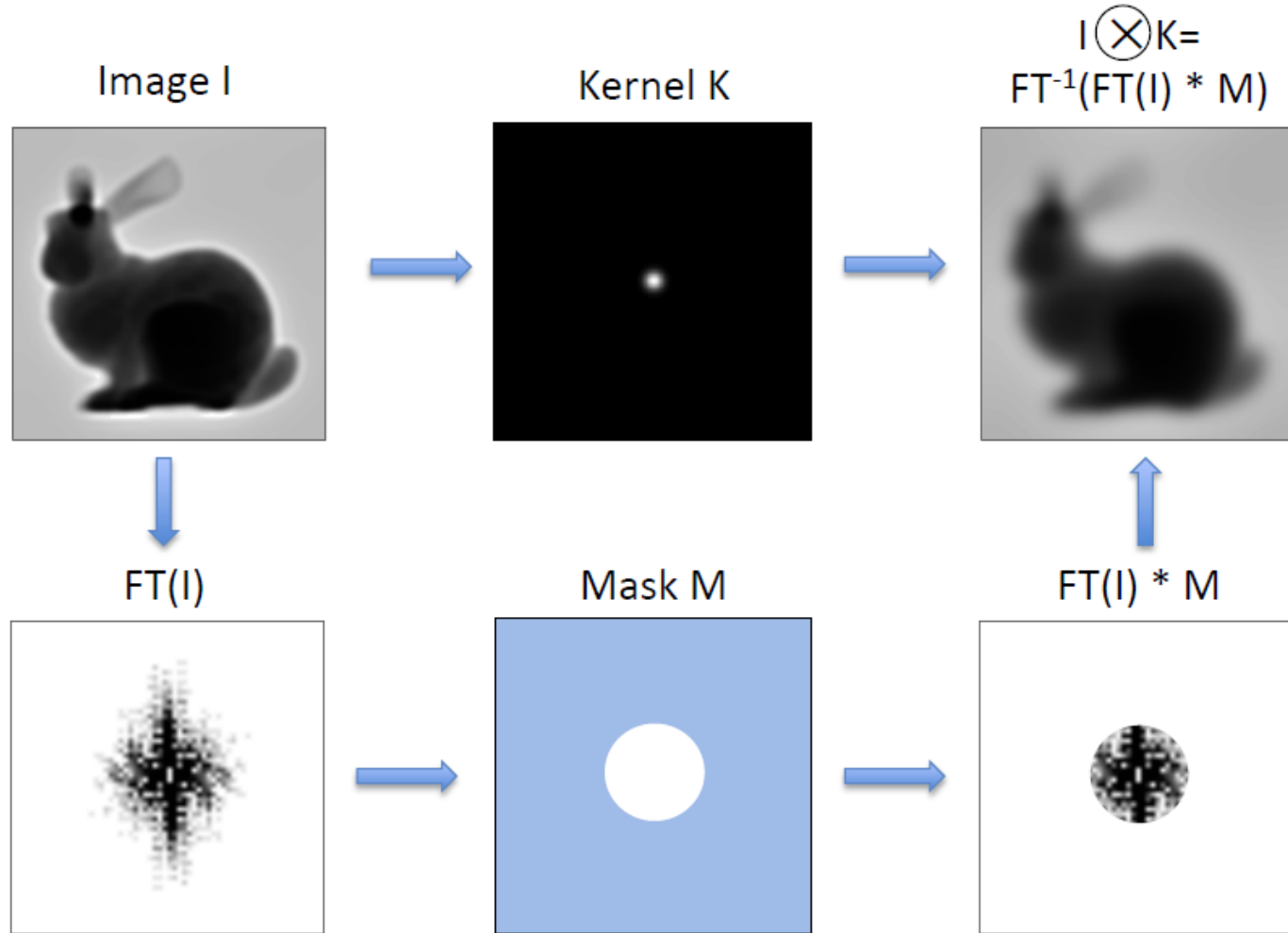


$$f(x) \otimes g(x) = FT^{-1}[F(u) \cdot G(u)]$$

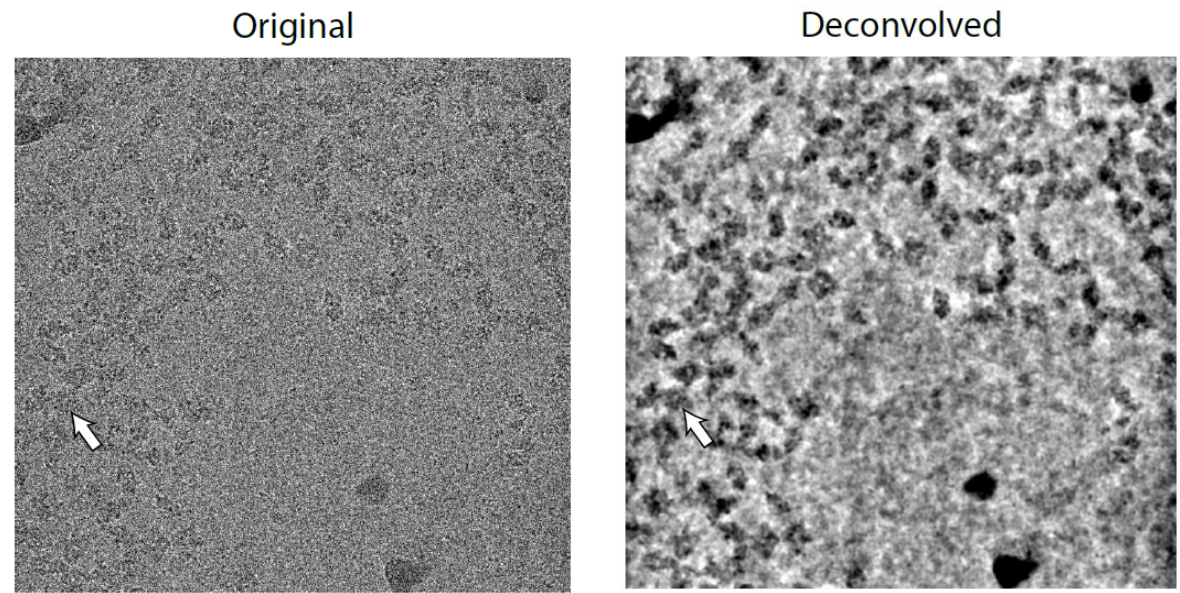
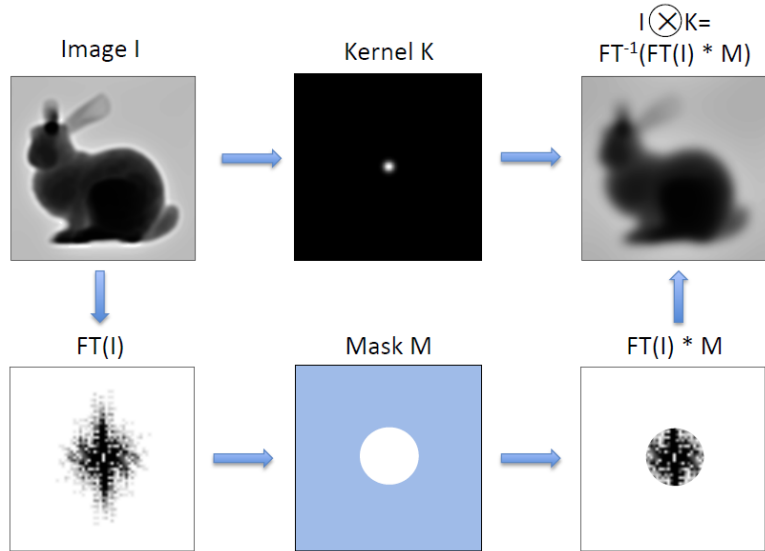
Convolution of f with g in real space is slow. It can be done much faster by multiplying their FFTs, and calculating the inverse FFT of the result.

Juha Huiskonen & Bilal Qureshi

Fourier vs. Convolution based filtering



Fourier vs. Convolution based filtering





Correcting for effects of point spread function or contrast transfer function

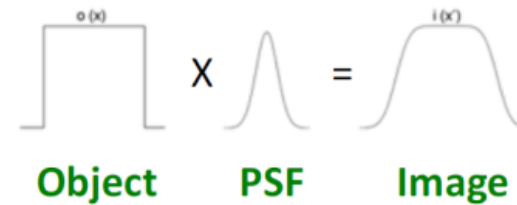
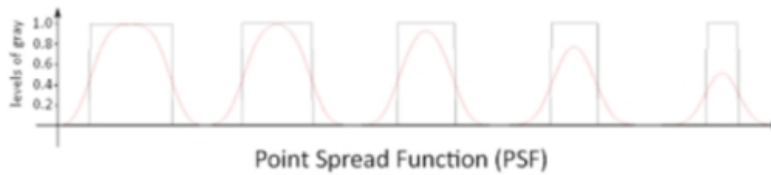
Convolution and correction of Point Spread Function (PSF)



Image = Original object x Point spread function (PSF)



PSF= a constant because of defects in EM

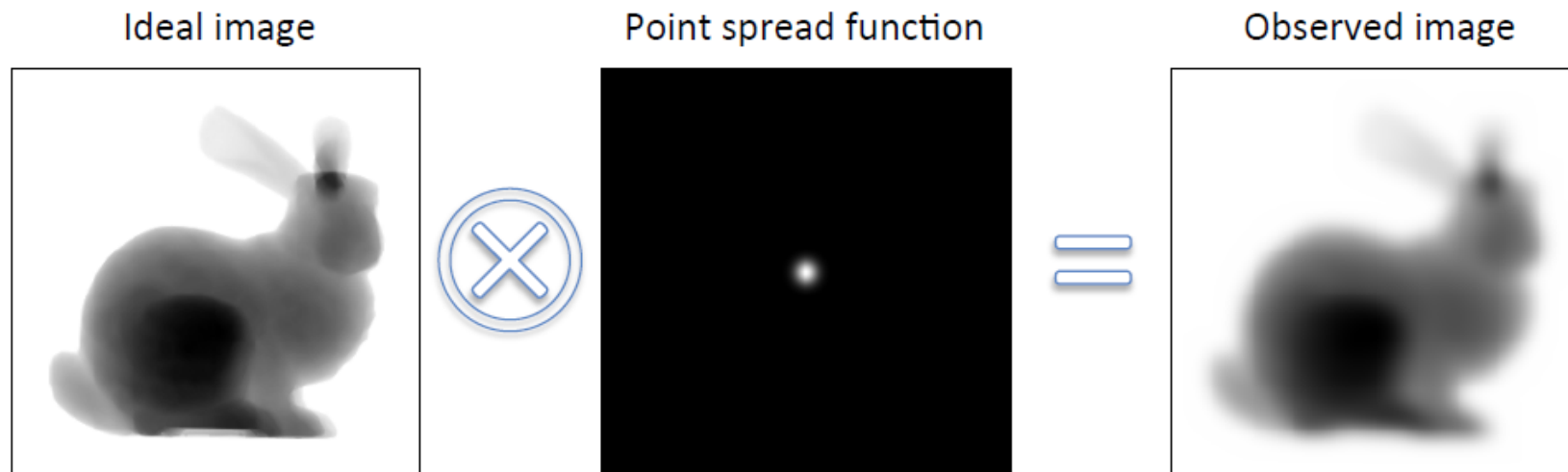


Convolution: Convolution is a mathematical operation on two functions (object and PSF) producing a third function (image) that is typically viewed as a modified version of one of the original functions (object)

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

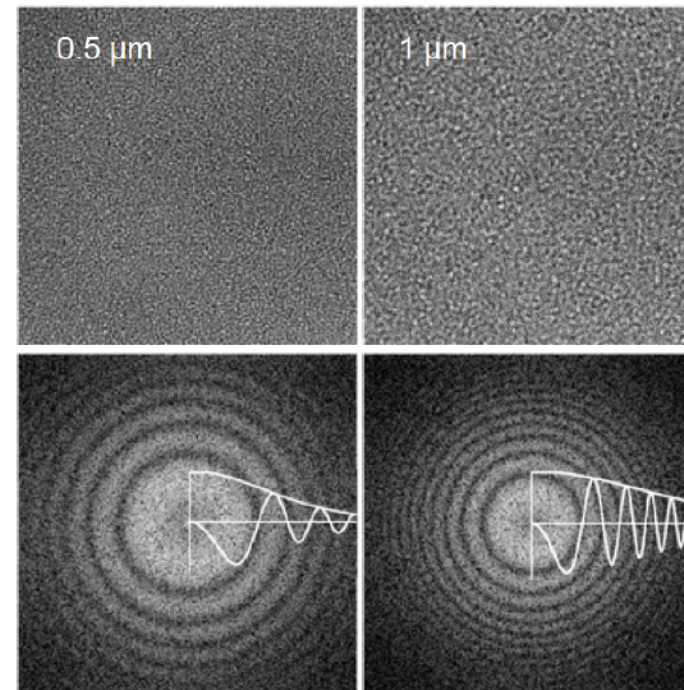
Convolution and correction of Point Spread Function (PSF)

- 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)

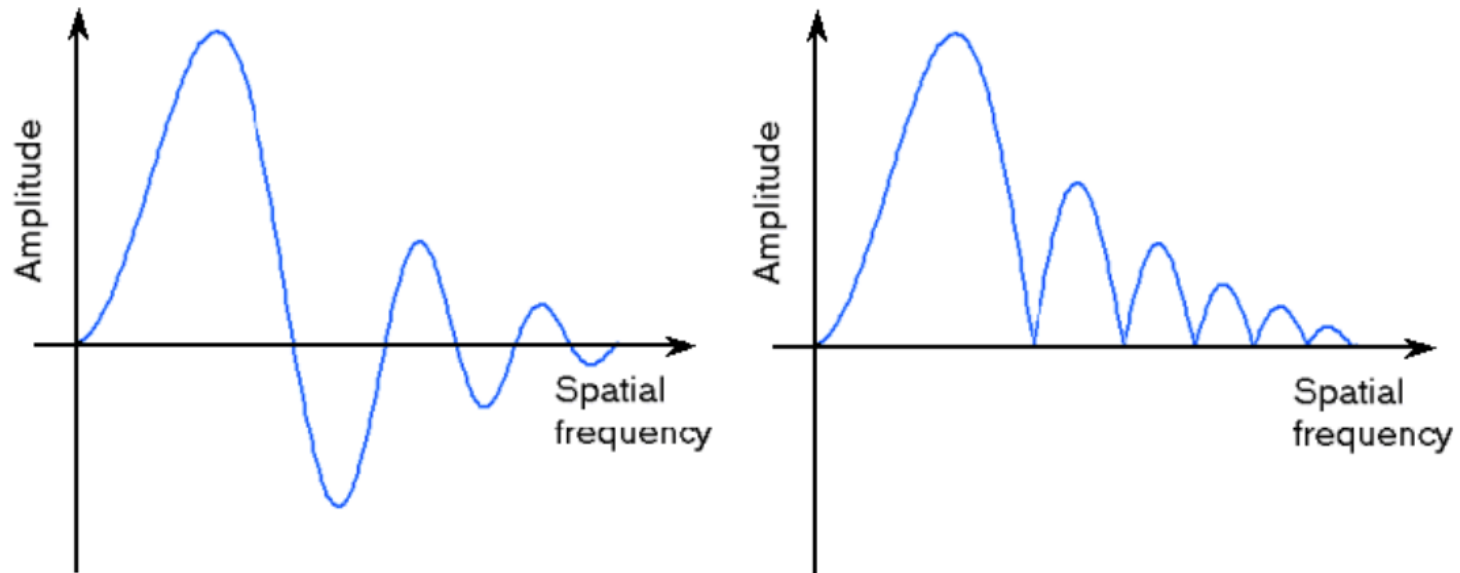


Estimating CTF from images

- 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

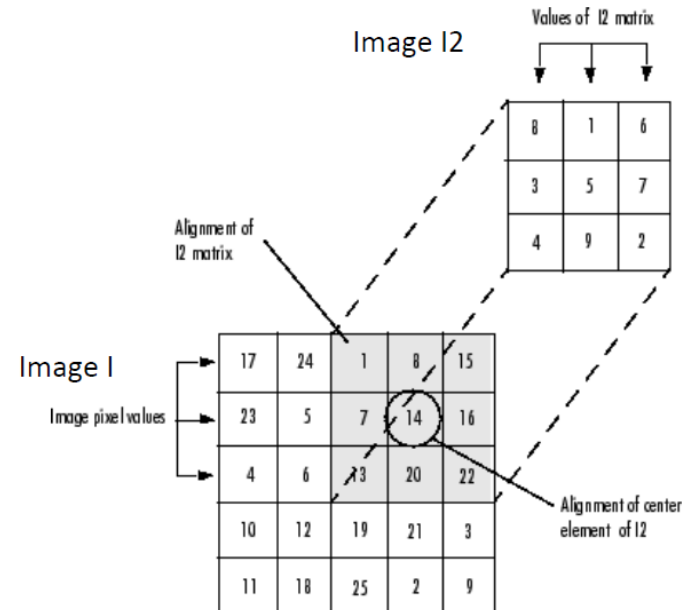




Cross-correlation and image alignment

Cross-correlation

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



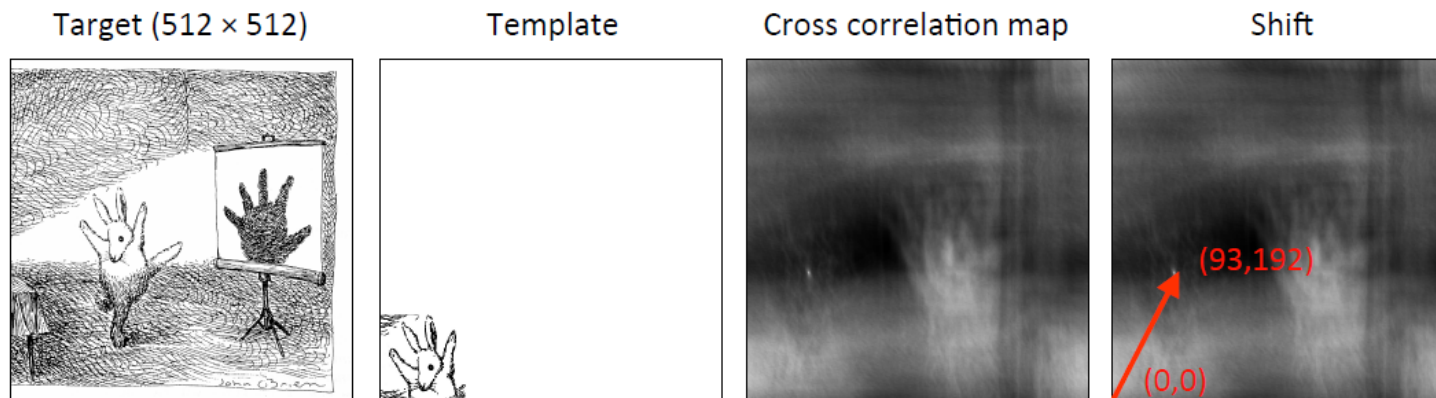
The cross-correlation for the indicated position is:
 $1 * 8 + 8 * 1 + 6 * 15 + 7 * 3 + \dots + 2 * 22 = 585$

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

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.*

Cross-correlation and image shifts

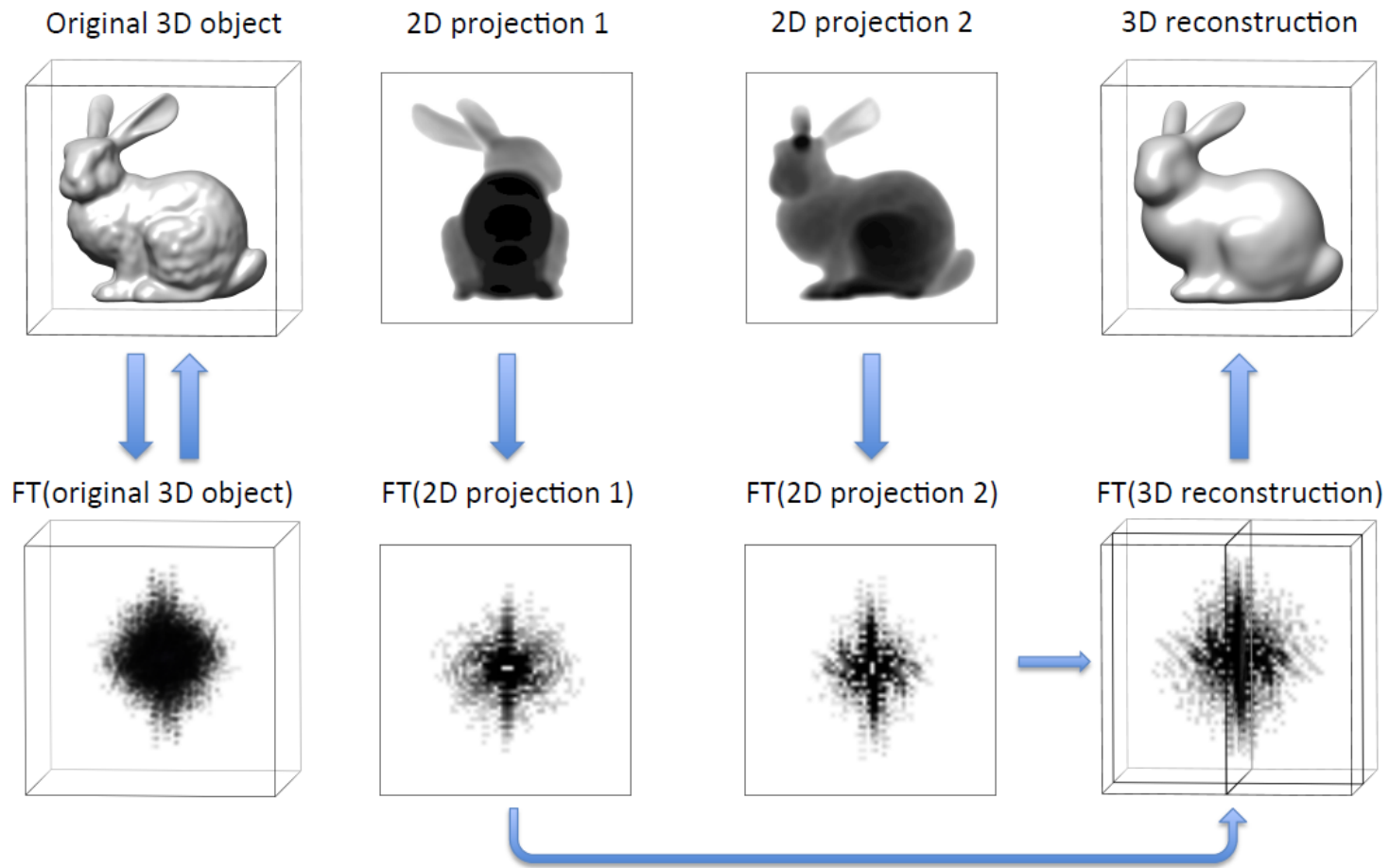
- Cross-correlation can be used to align two images (or to search all occurrences of a template image in the target image)
- Peaks in the cross correlation map define the locations
- When calculated in Fourier space, the two images must be of the same size. Here the template (originally 128×128) was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





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

Central section theorem



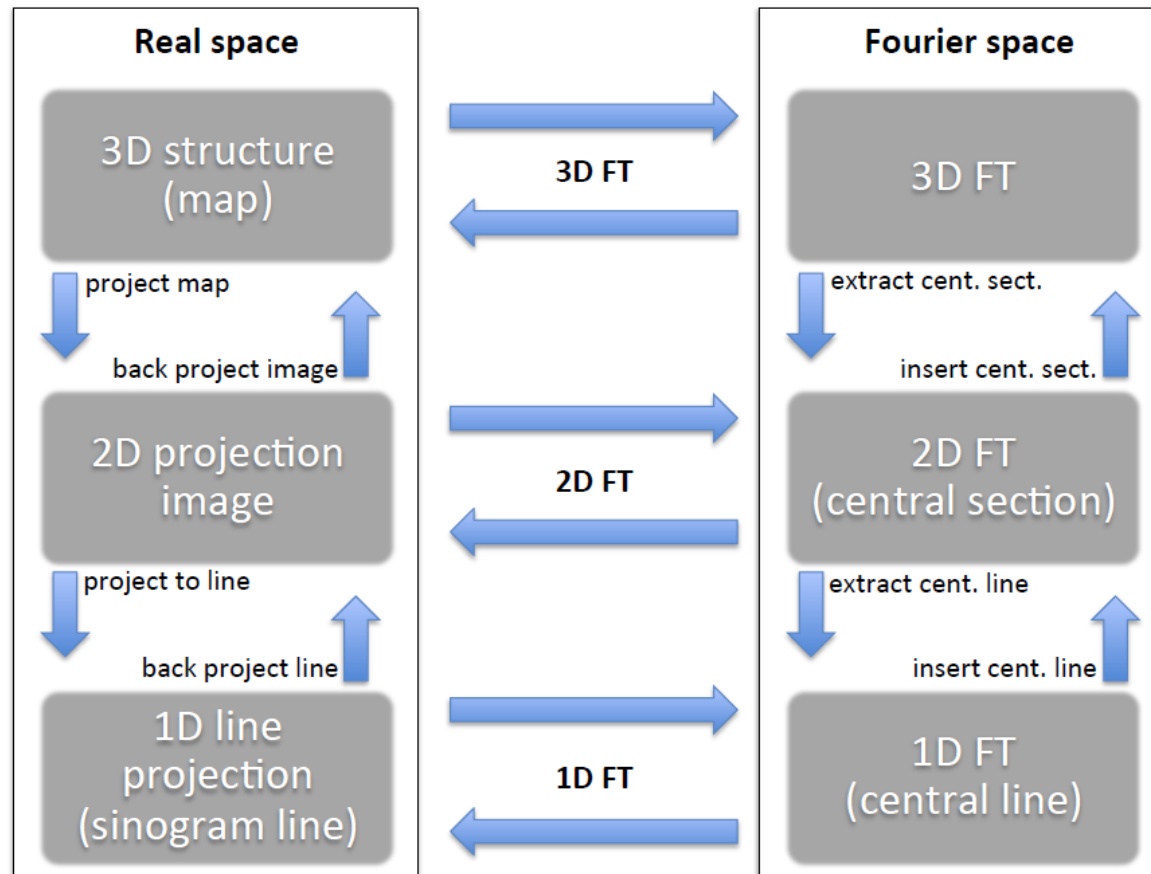


Central section theorem

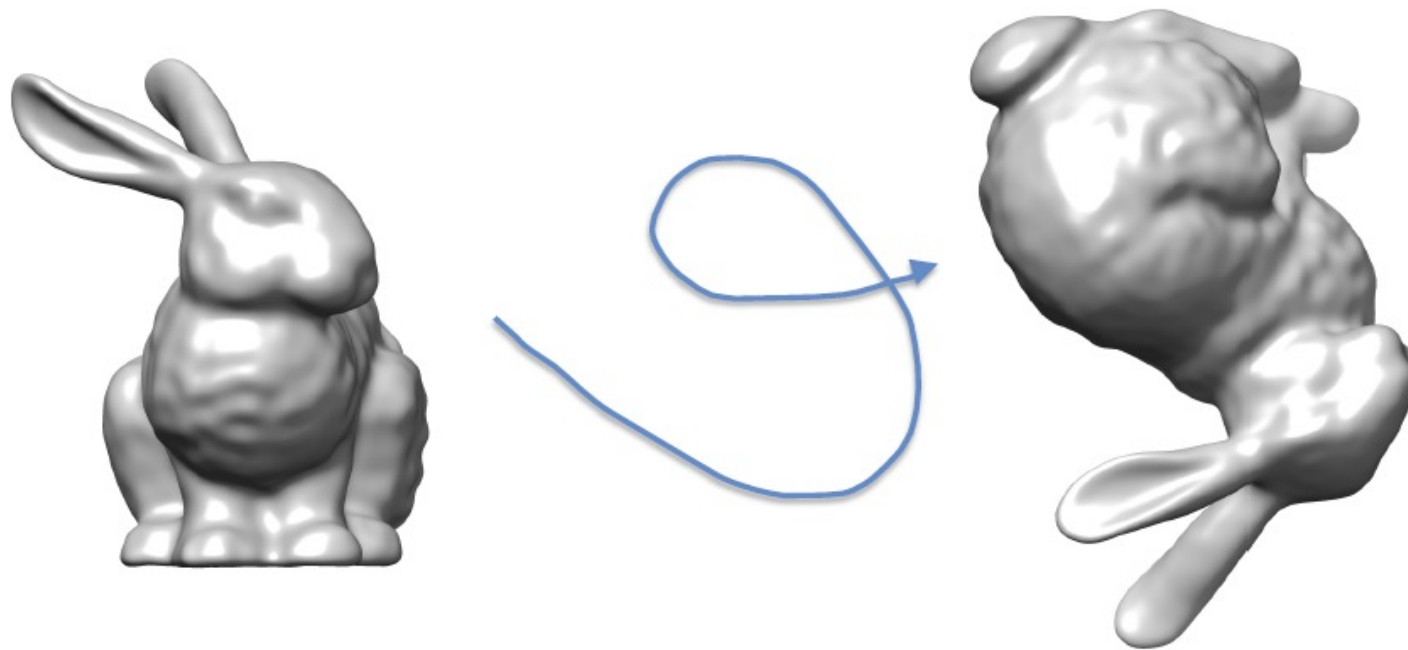


- A central section is a slice passing through the origin (center of the volume)
- The 2-dimensional Fourier transform of a projection of a 3-dimensional object is a central section of the 3-dimensional Fourier transform of the original object
- One can determine the 3-dimensional Fourier transform by 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

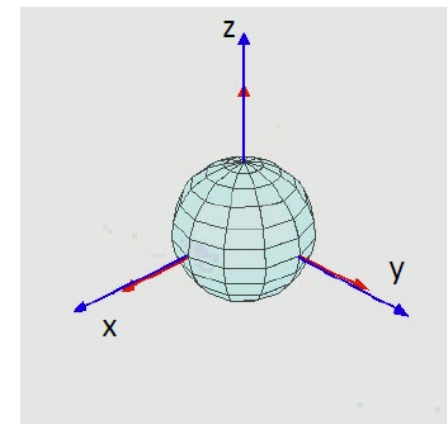
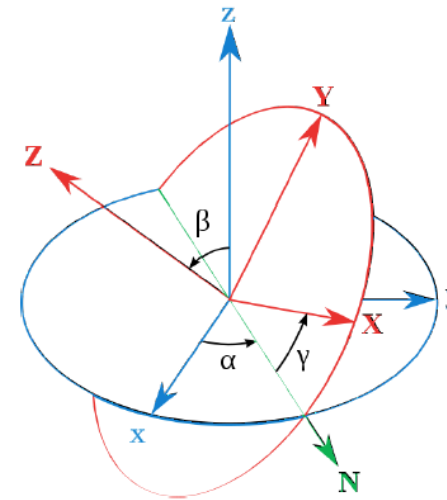


3D objects orientation in space



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





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