

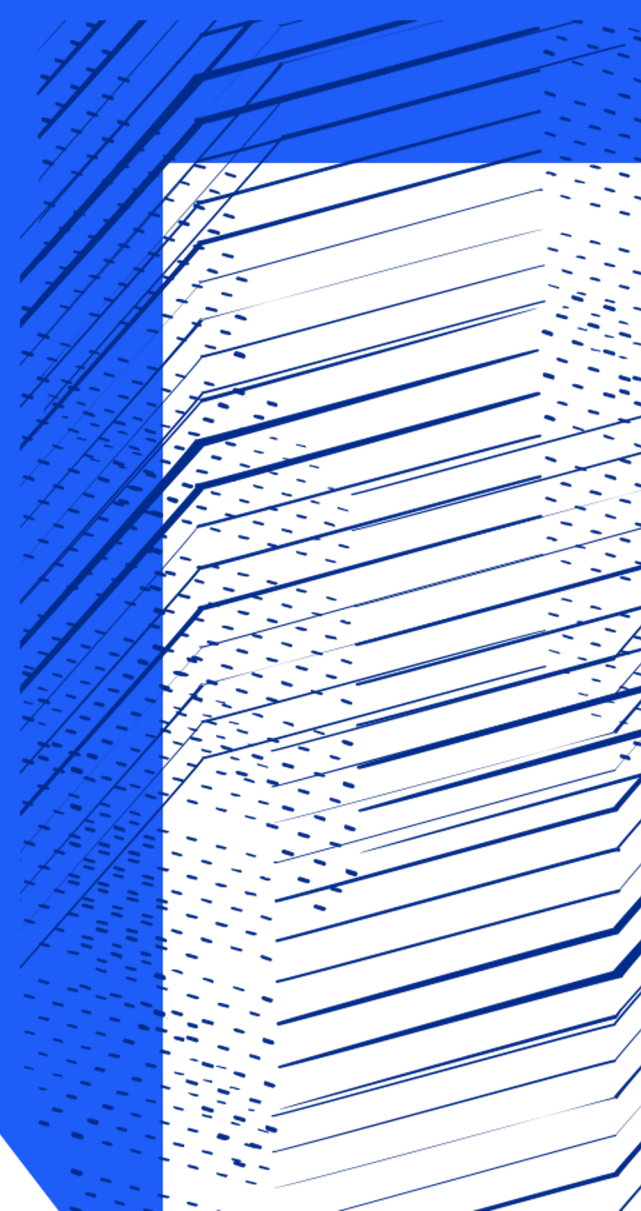


Science and
Technology
Facilities Council

Introduction to Image Processing

Matt Iadanza

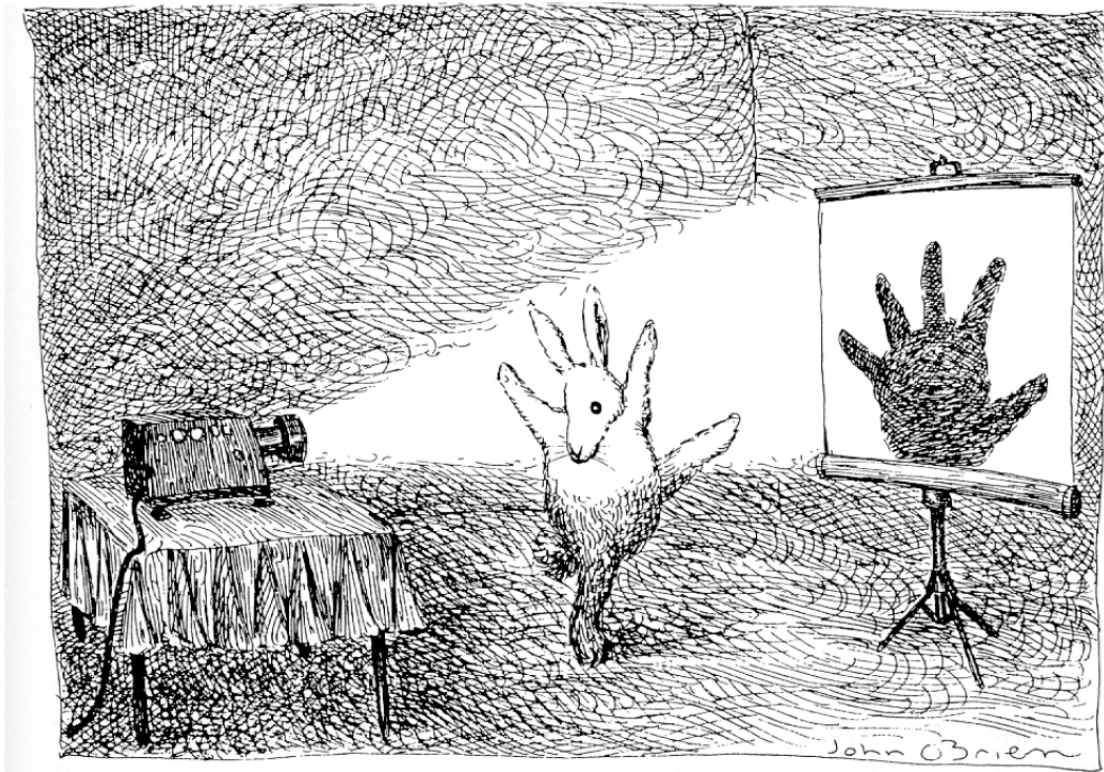
Many thanks to Bilal Qureshi
and Juha Huiskonen for
providing many images!



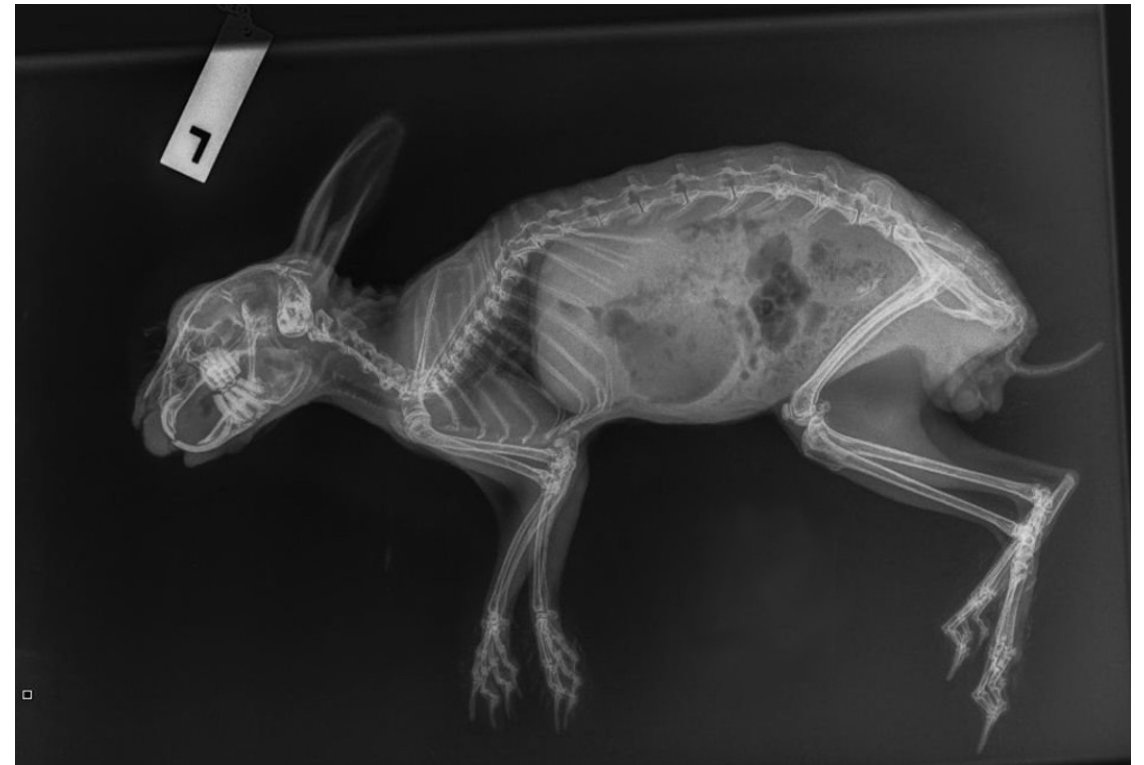
Overview

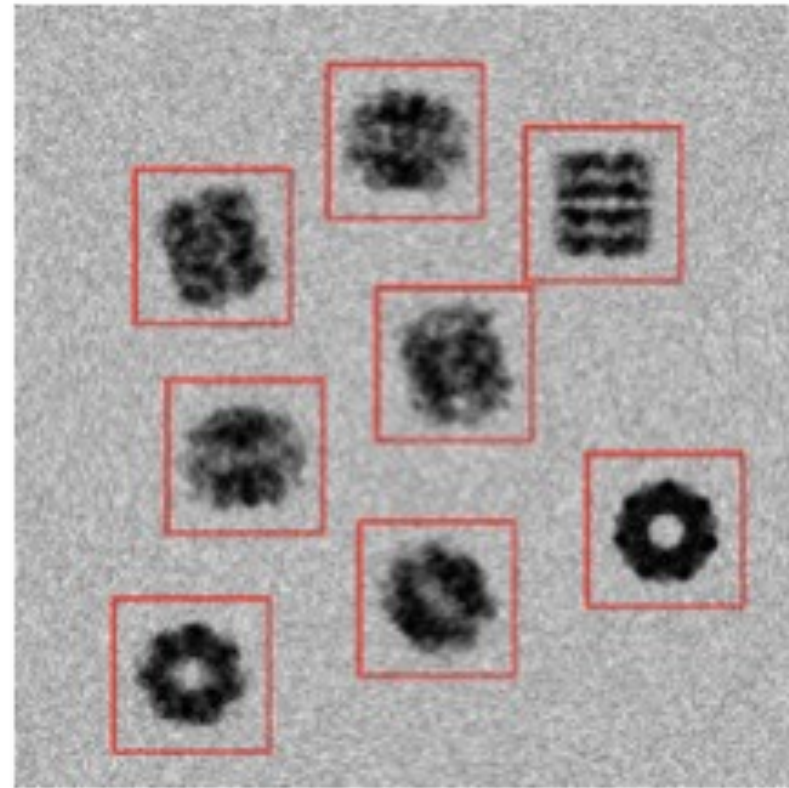
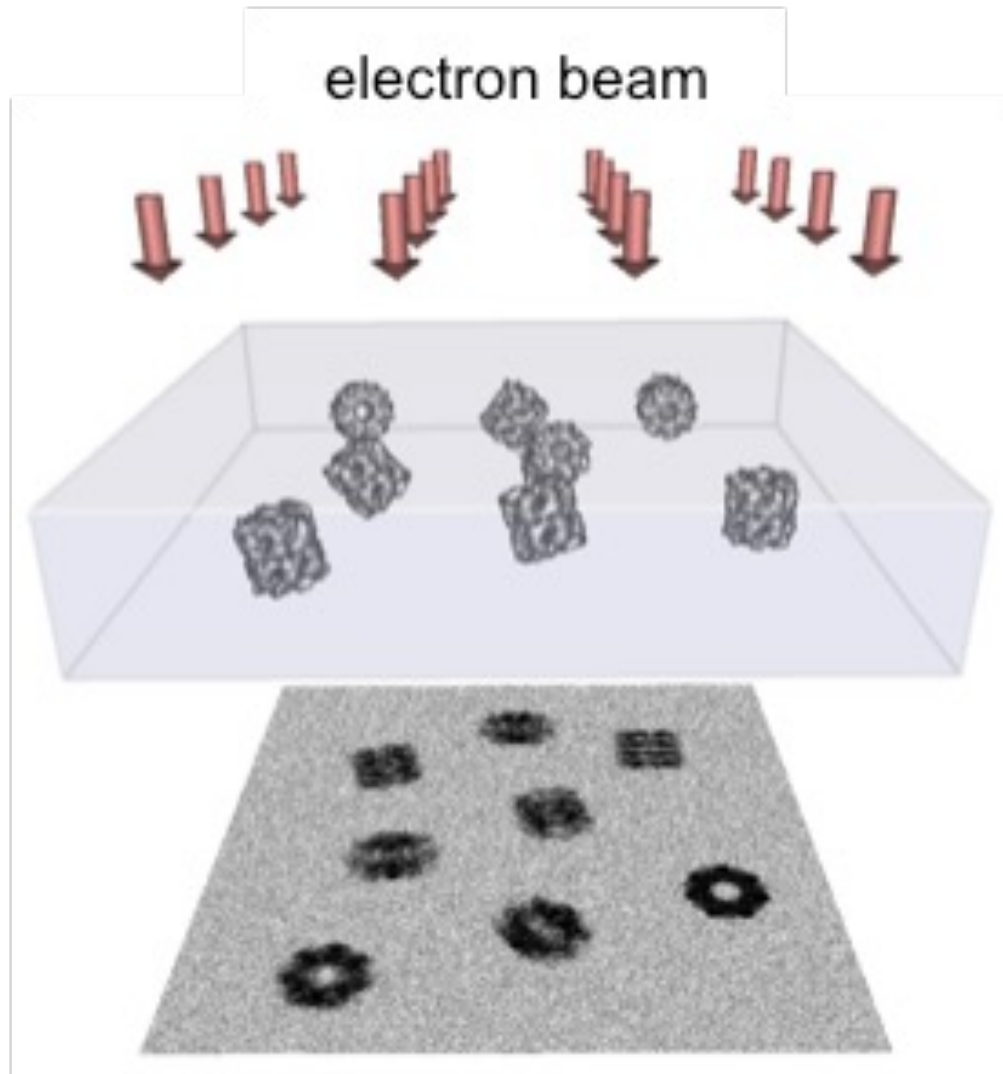
- Fourier transform
- Filters
- Convolution
- Point spread and contrast transfer functions
- Cross-correlation
- Central section theorem and 3D reconstruction
- Euler angles

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

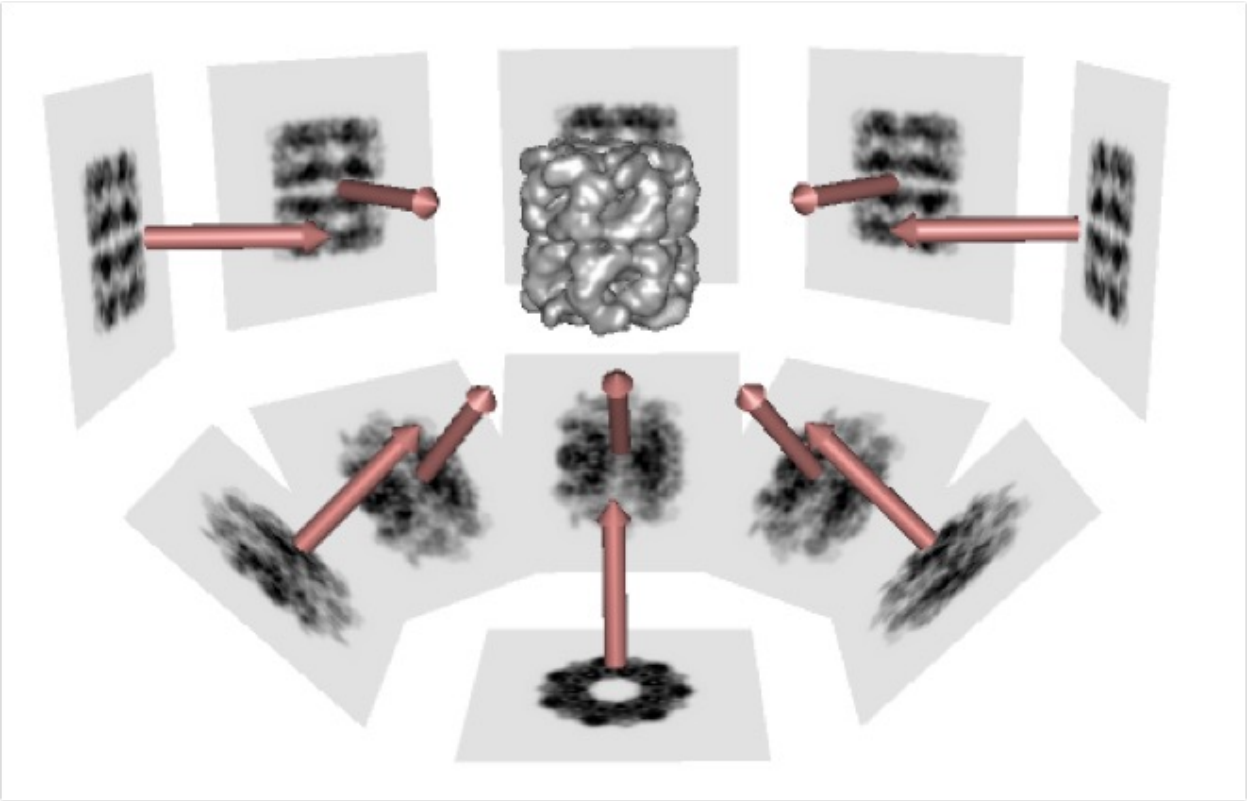
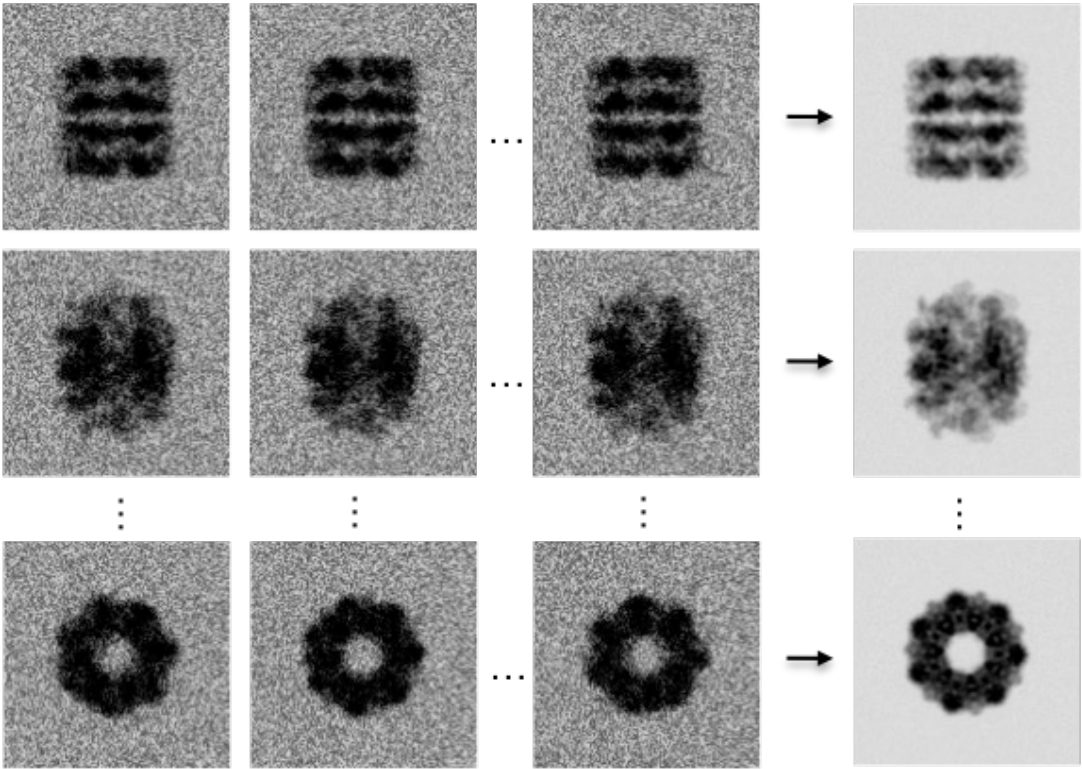


© The New Yorker





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



The Fourier Transform

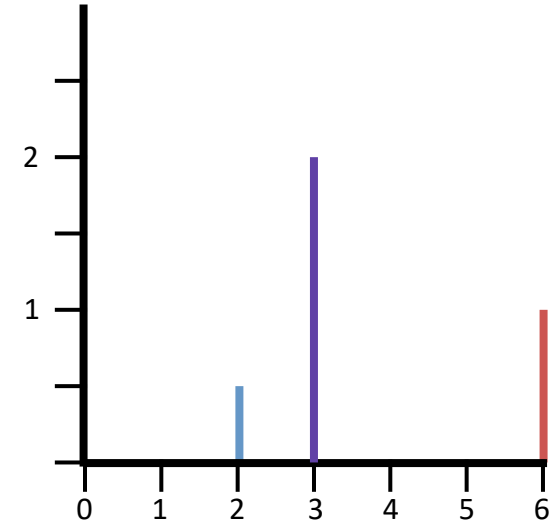
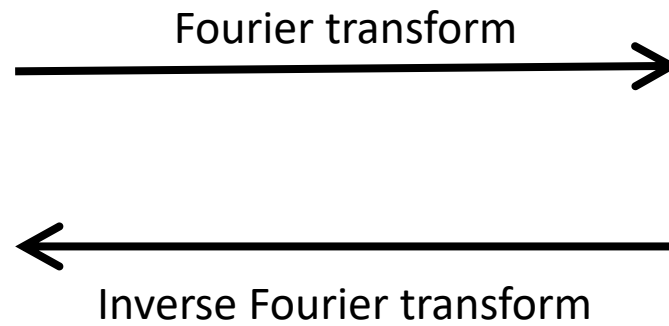
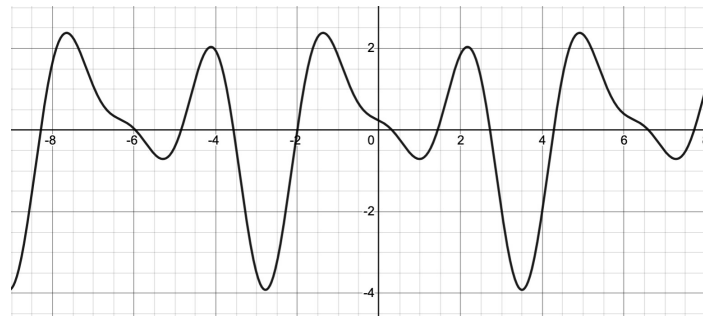


Joseph Fourier
1768 - 1830

Fourier Transform and Fourier series

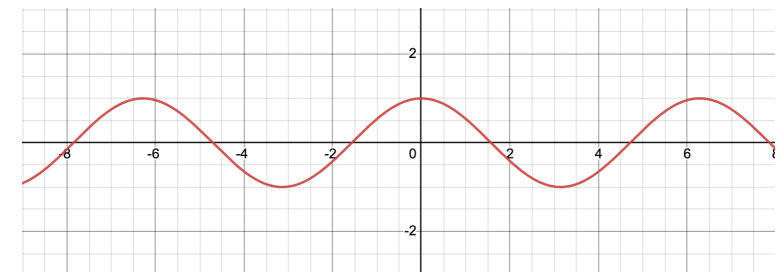
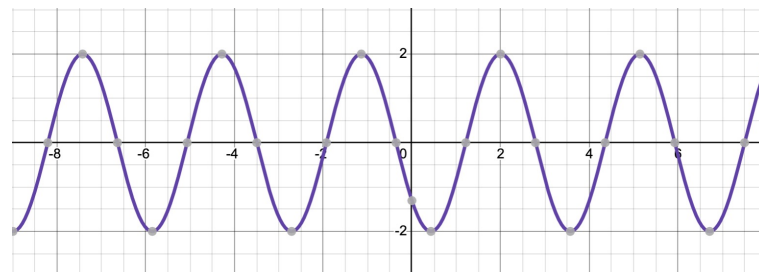
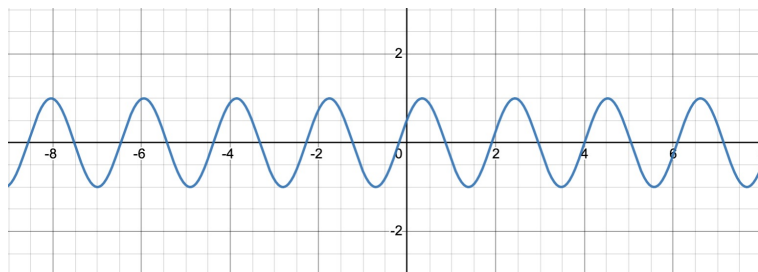
The Fourier transform decomposes a function of time into its constituent frequencies

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

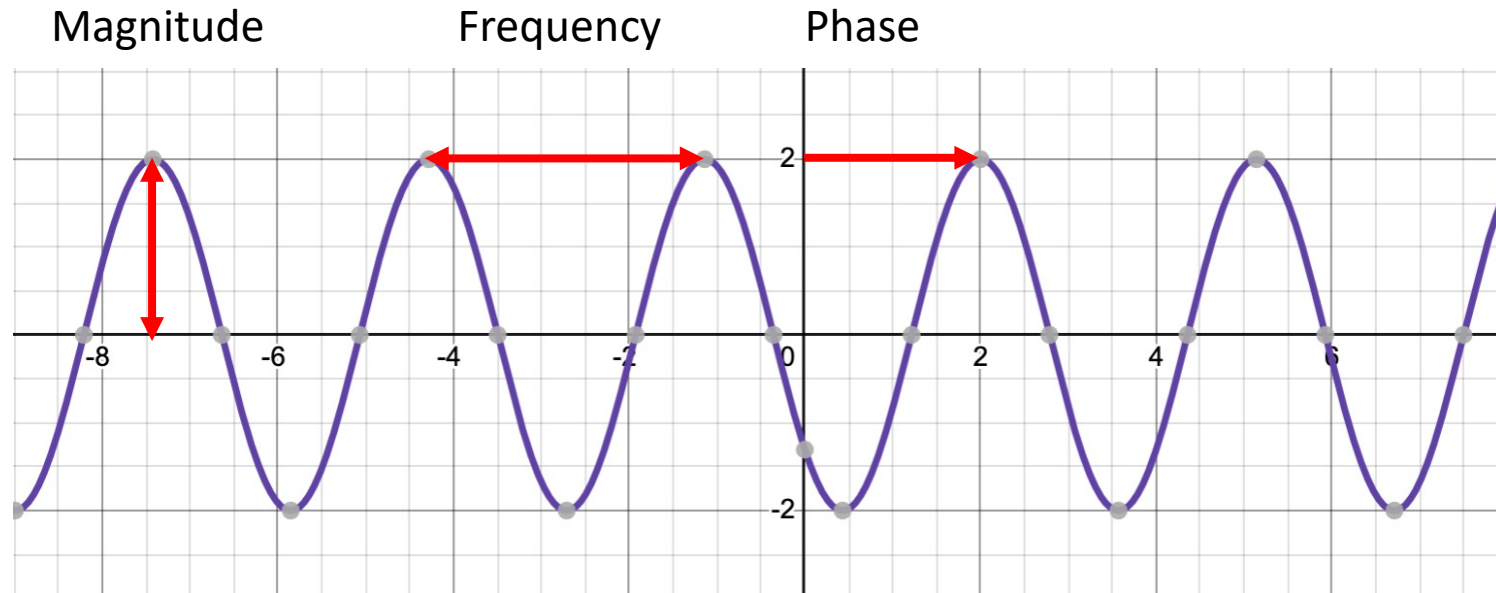


Real space data
 $f(x)$

Fourier series
 $F(u)$



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



Terminology

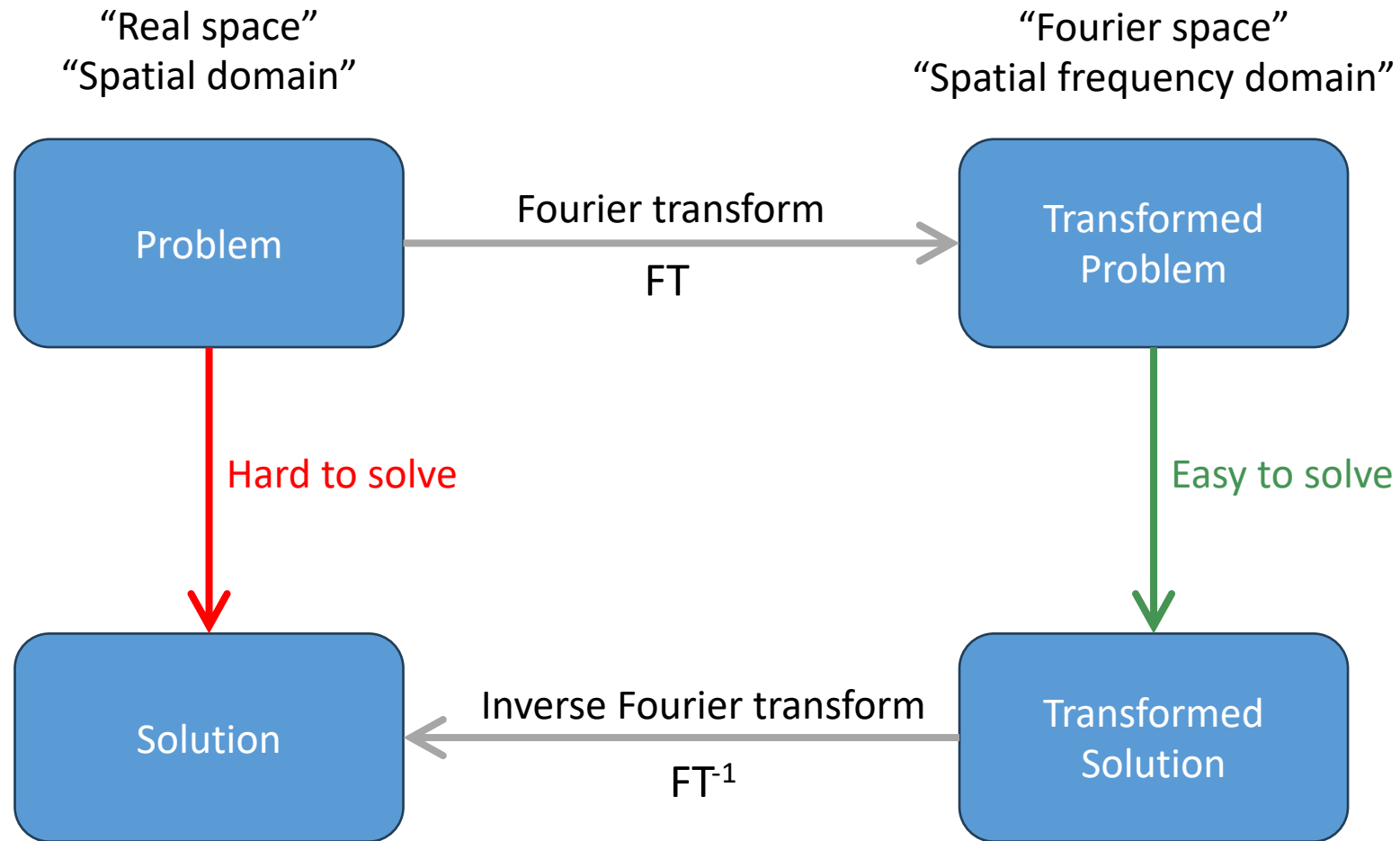
Fourier transform (**FT**) – Operates on continuous data (IE: wave equations)

Discrete Fourier transform (**DFT**) – Operates on a discrete chunk 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



Some problems are hard in real space and easy in frequency space

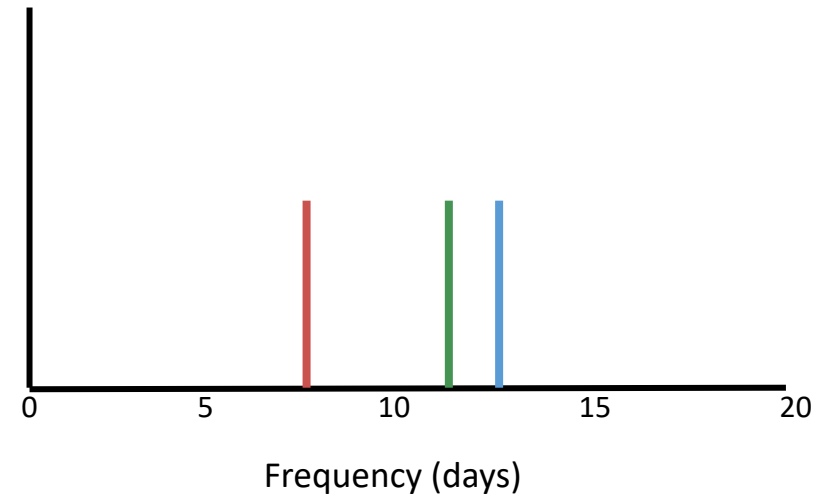
When should I take my web server down for maintenance?

Real space data

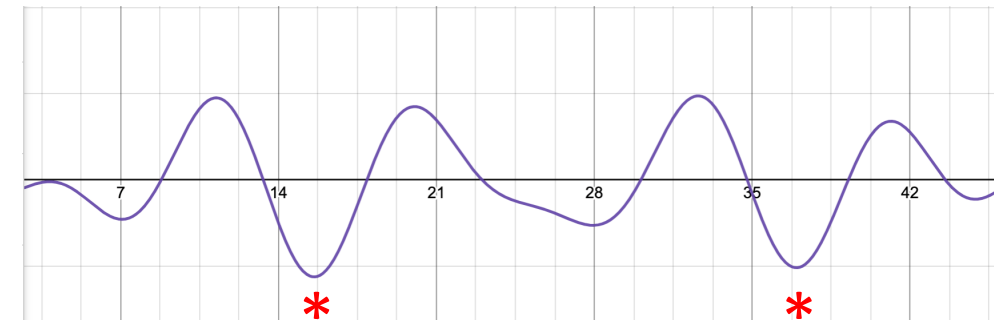
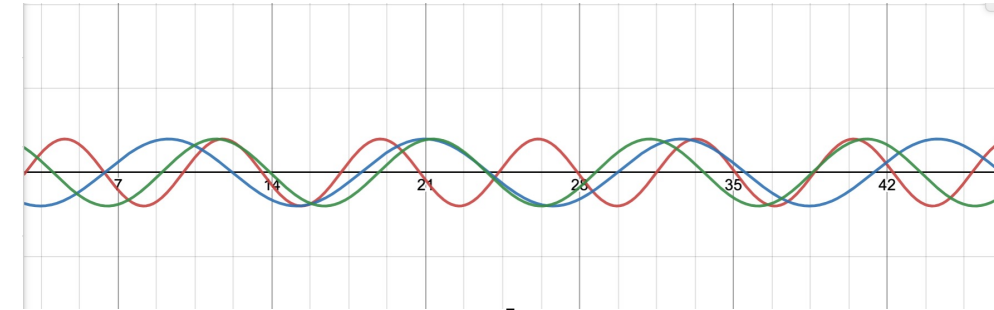
```
Logins:  
Tom: June 16, 2023  
Colin: June 17, 2023  
Tom: June 24, 2023  
Colin: June 30, 2023  
Tom: July 2, 2023  
Matt: July 4, 2023  
Tom: July 10, 2023  
Colin: July 13, 2023  
Matt: July 15, 2023  
Tom: July 18, 2023  
Matt: July 26, 2023  
Colin: July 26, 2023  
Tom: July 26, 2023  
Tom: August 3, 2023  
Matt: August 6, 2023  
Colin: August 8, 2023  
Tom: August 11, 2023  
Matt: August 17, 2023  
Tom: August 19, 2023  
Colin: August 21, 2023  
Matt: August 28, 2023  
Colin: September 3, 2023  
Matt: September 8, 2023  
Colin: September 16, 2023  
Matt: September 19, 2023  
Matt: September 30, 2023
```

Frequency space

| User | Frequency (days) | Phase (from Monday) |
|-------|------------------|---------------------|
| Matt | 11 | Mon +1 |
| Tom | 8 | Mon +4 |
| Colin | 13 | Mon +5 |

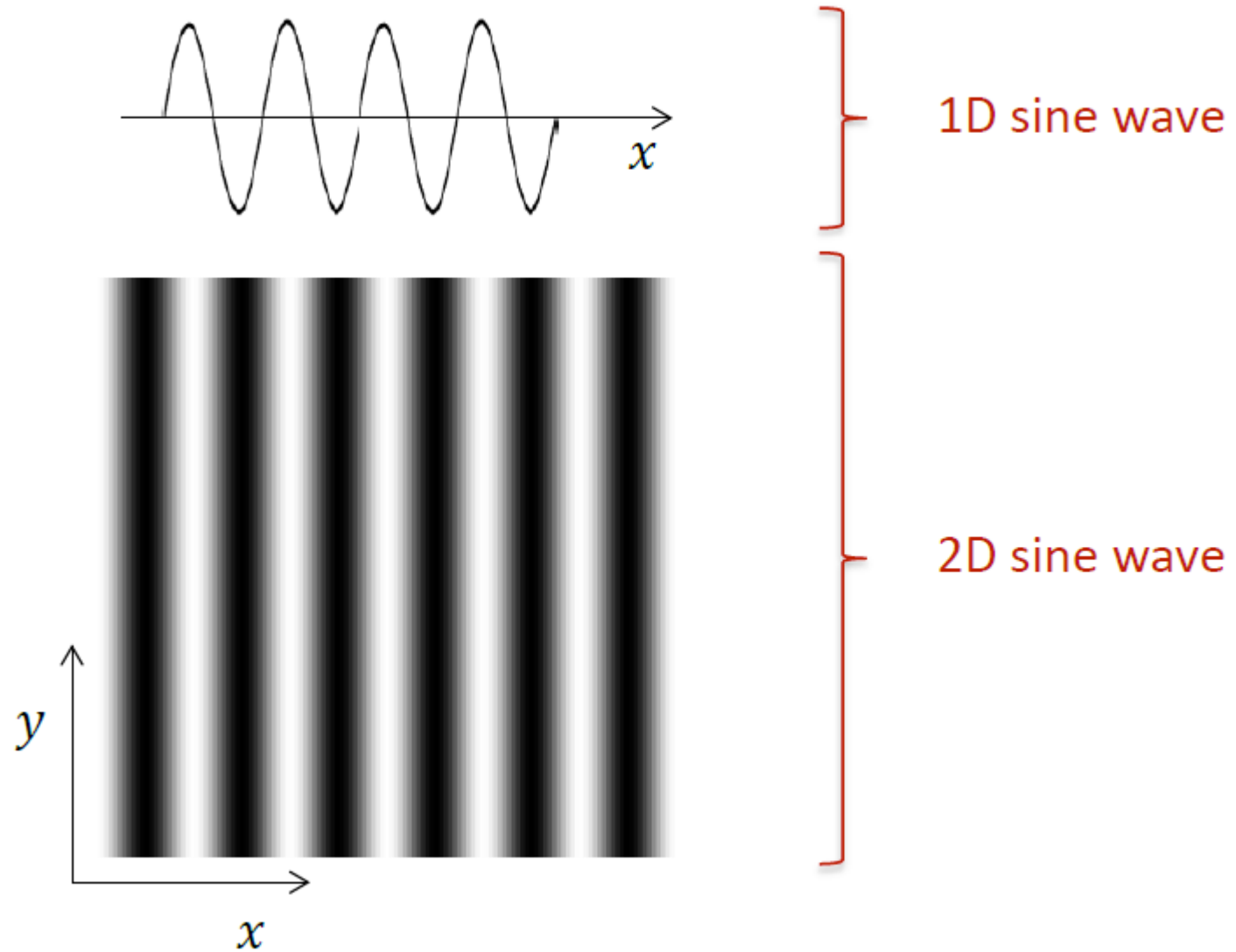


Real space predictions

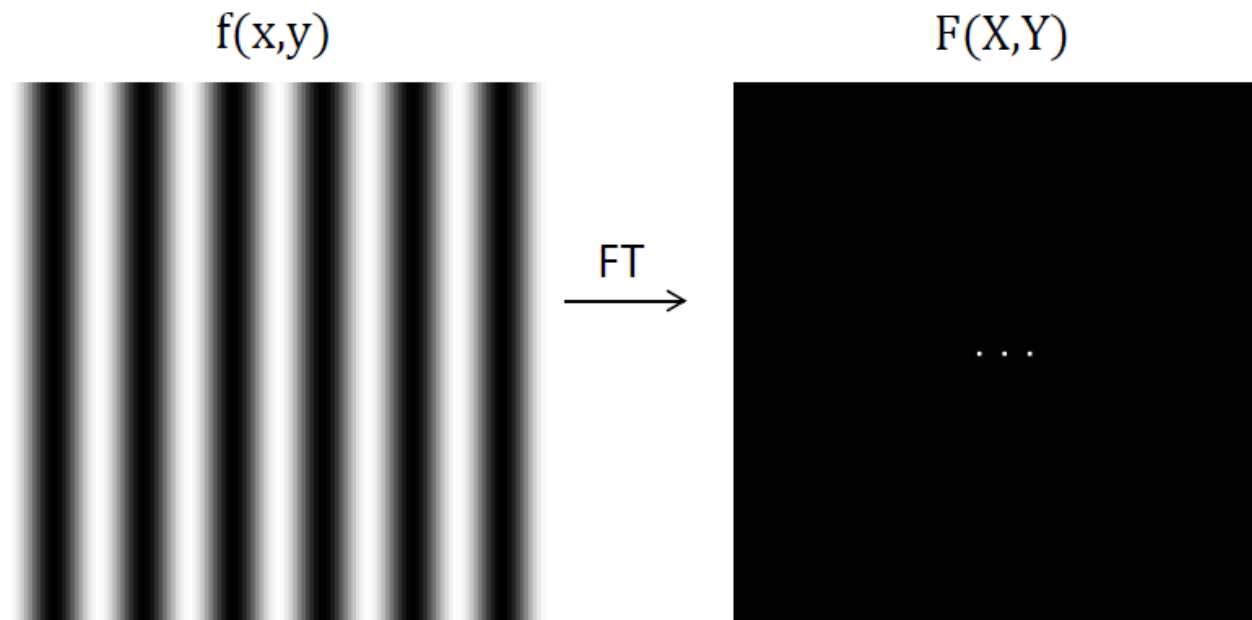


The Fourier transform in 2D

Fourier transform in 2D

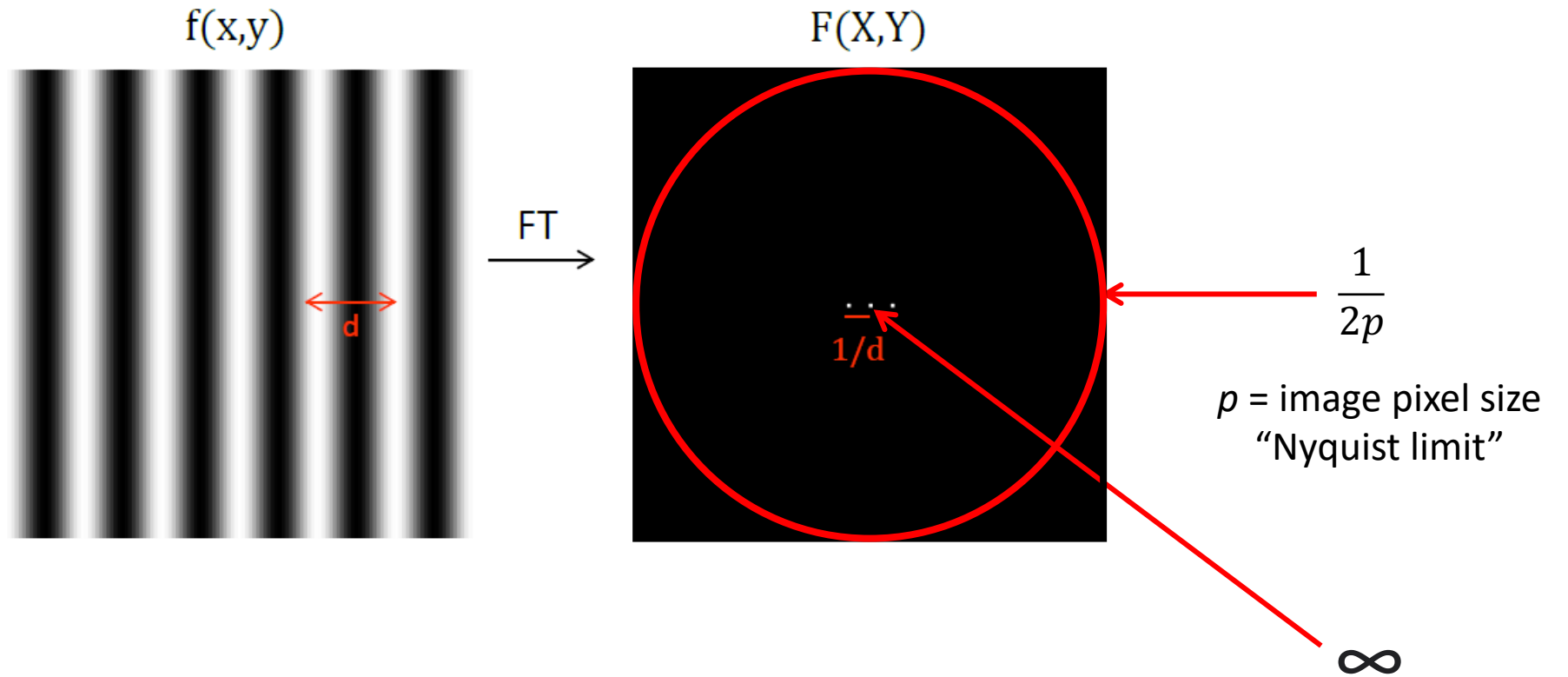


Fourier transform in 2D



Fourier transform in 2D

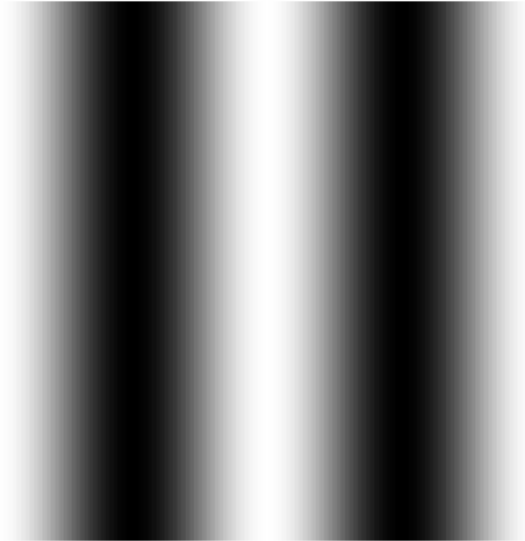
Frequency



Fourier transform in 2D

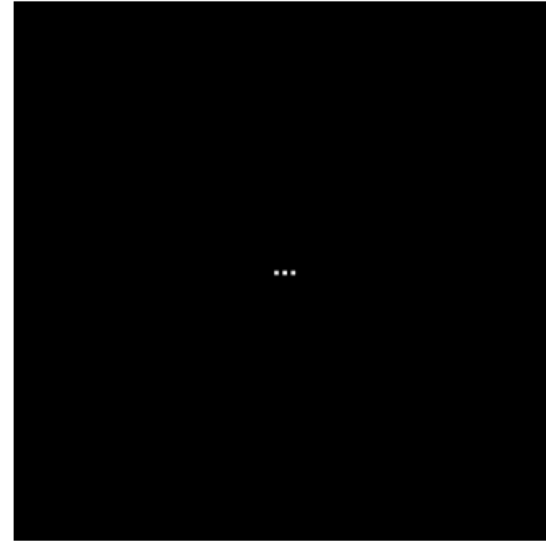
Frequency

$f(x,y)$



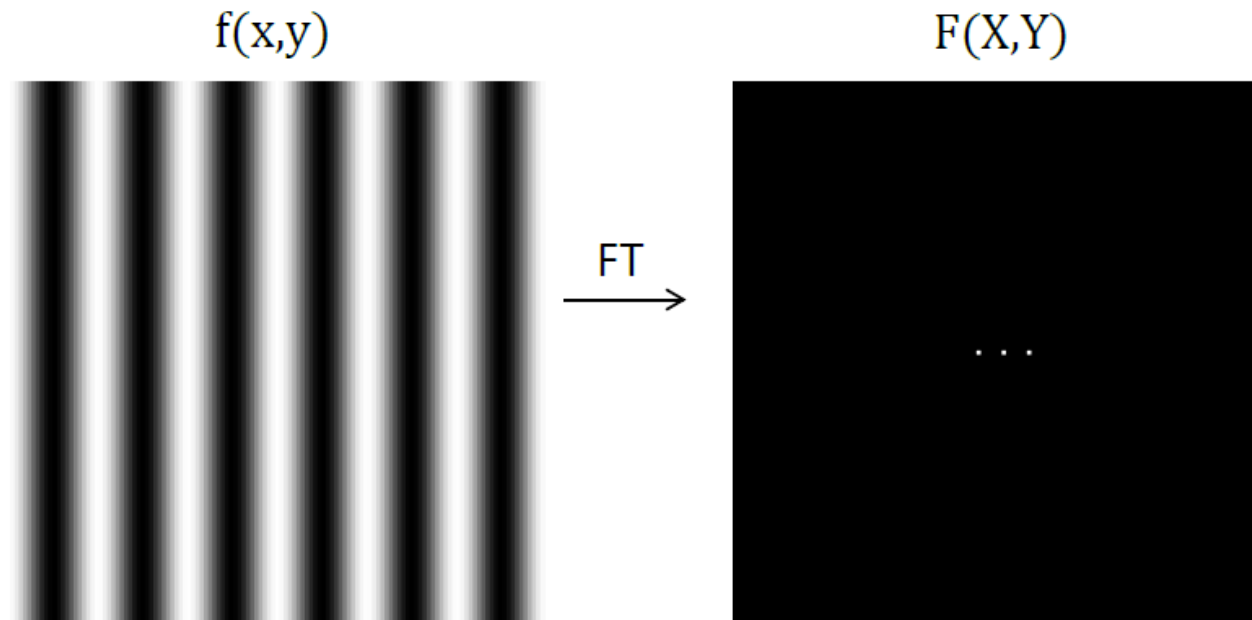
FT
→

$F(X,Y)$



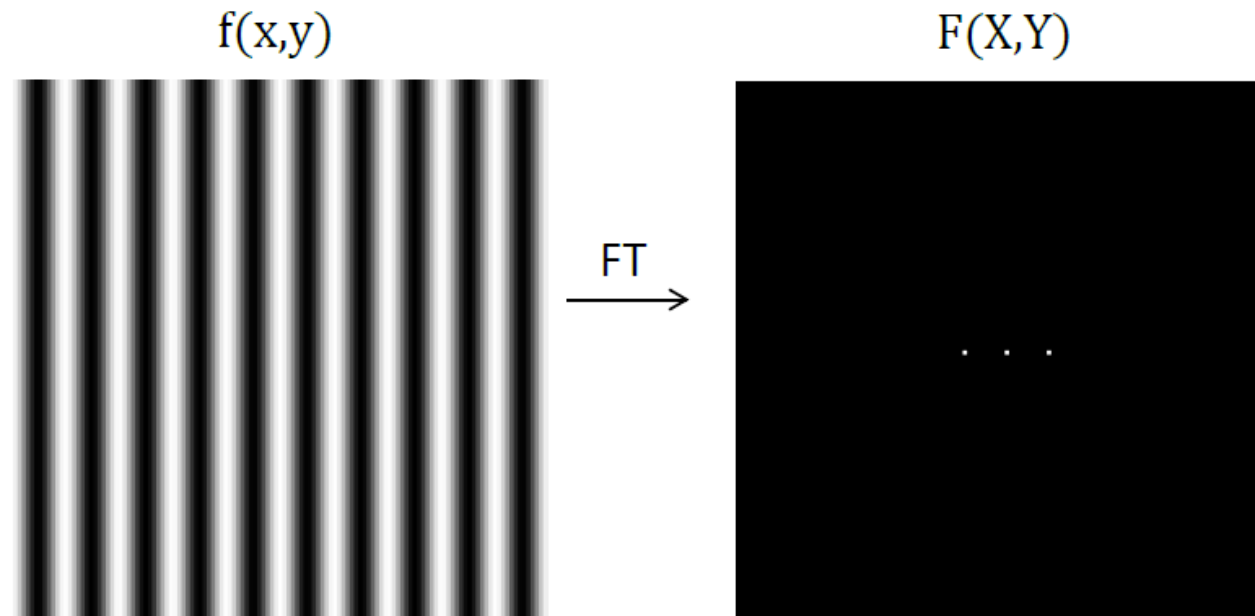
Fourier transform in 2D

Frequency



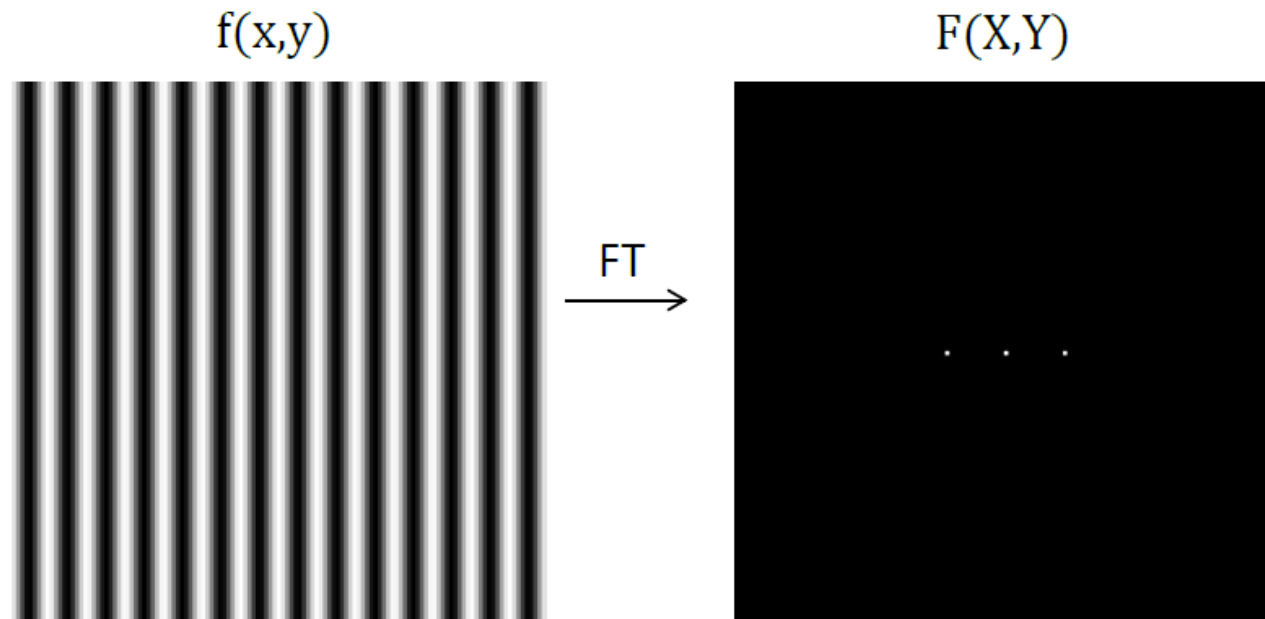
Fourier transform in 2D

Frequency



Fourier transform in 2D

Frequency

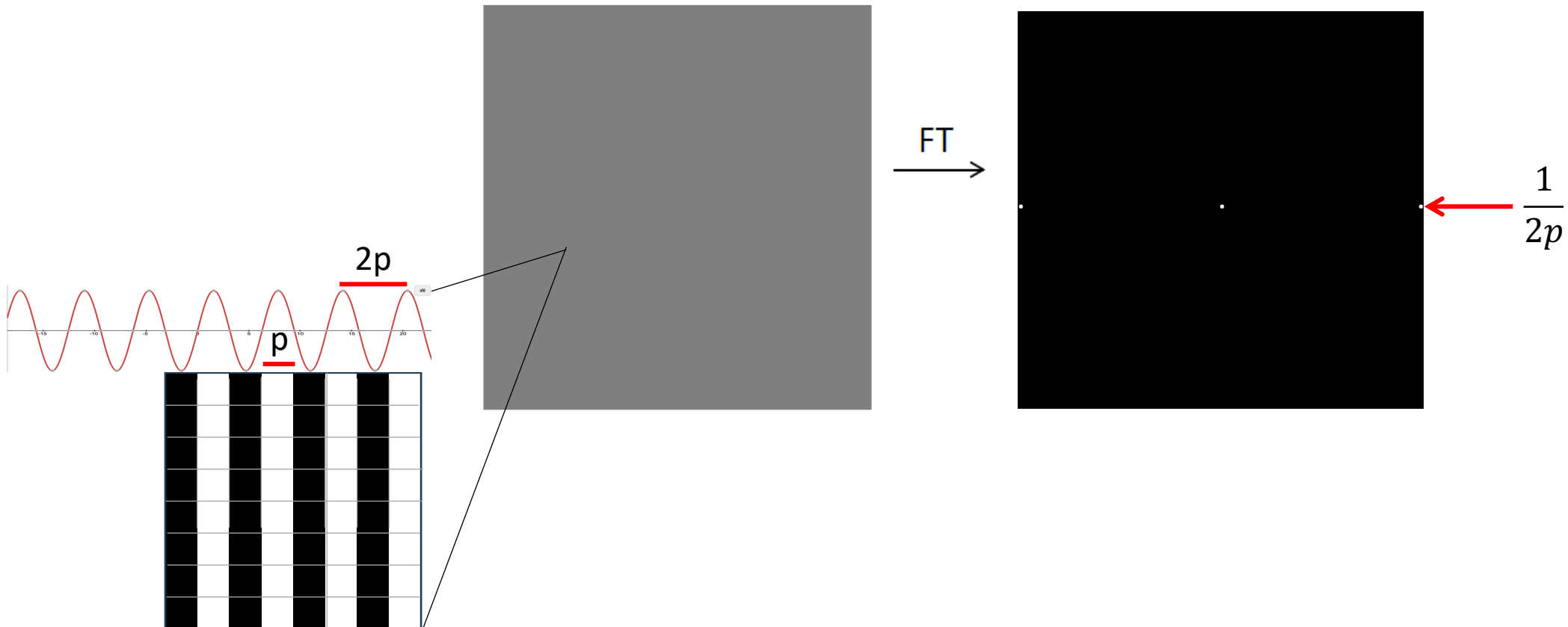


Fourier transform in 2D

Nyquist-Shannon sampling theorem

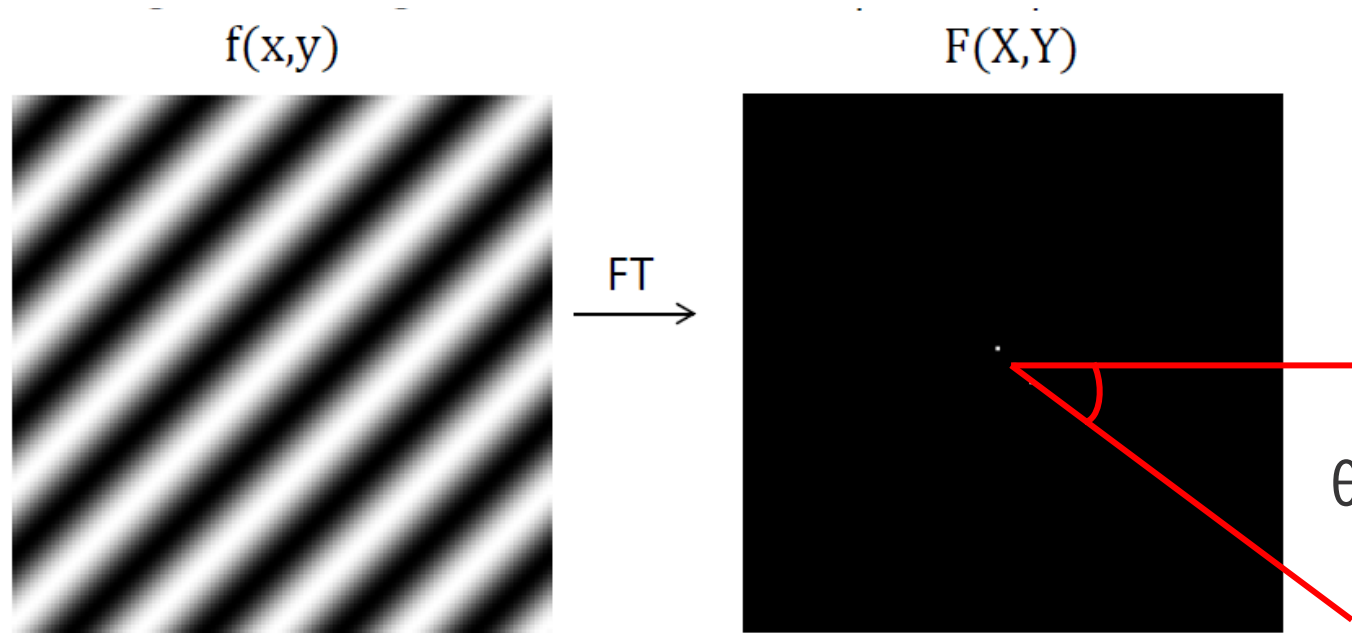


Harry Nyquist
1884 - 1976



Fourier transform in 2D

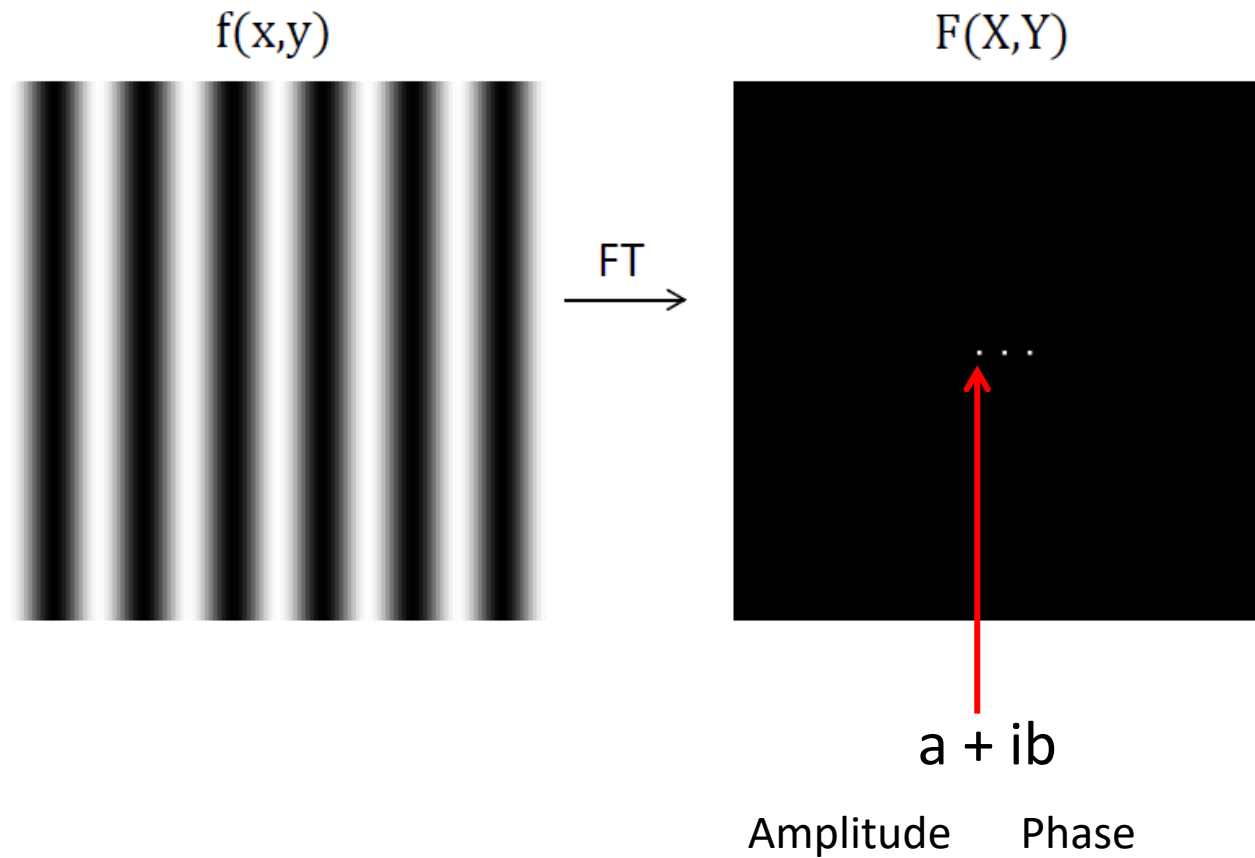
Orientation



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

Fourier transform in 2D

Amplitude & Phase

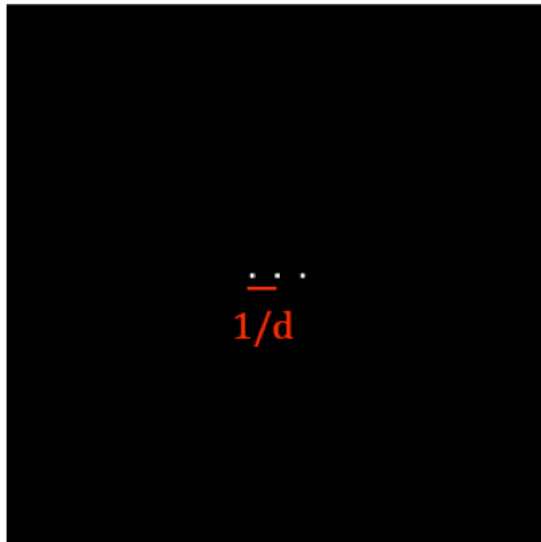


$$i = \sqrt{-1}$$

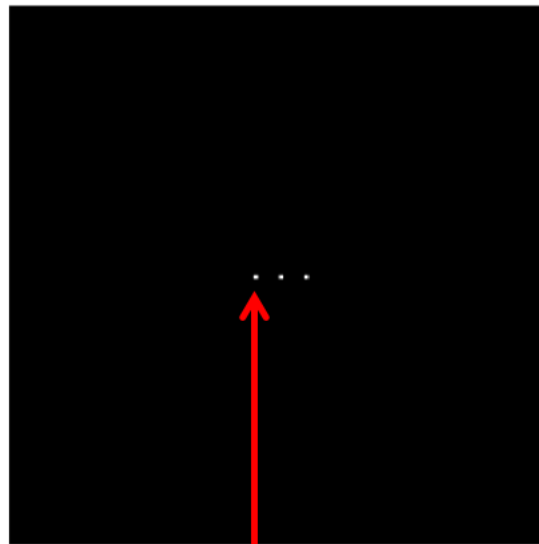
The value of each pixel in the Fourier transform is a complex number

Fourier transform in 2D

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



Frequency



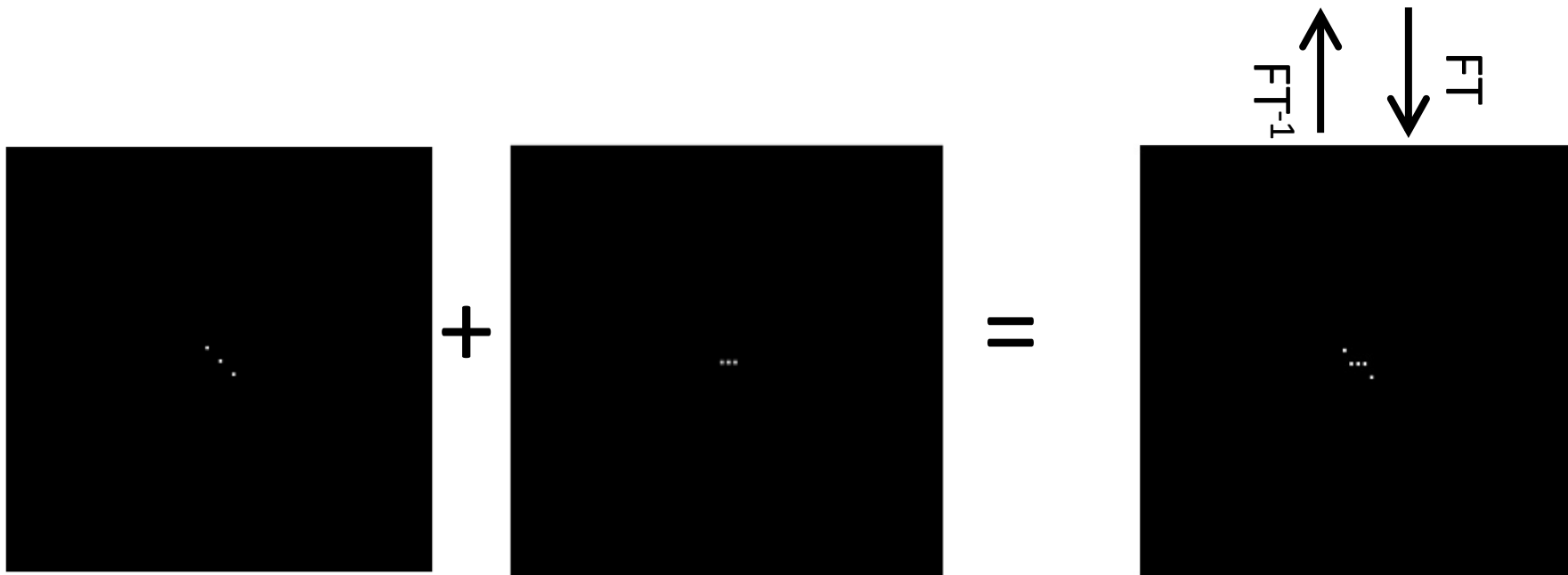
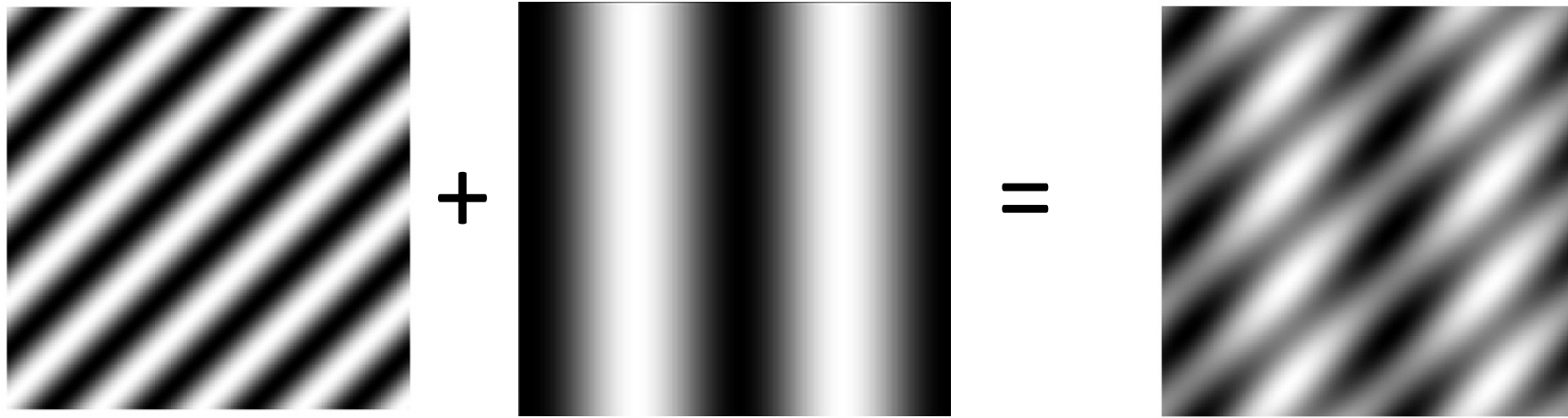
$a + ib$
Amplitude and Phase



Orientation

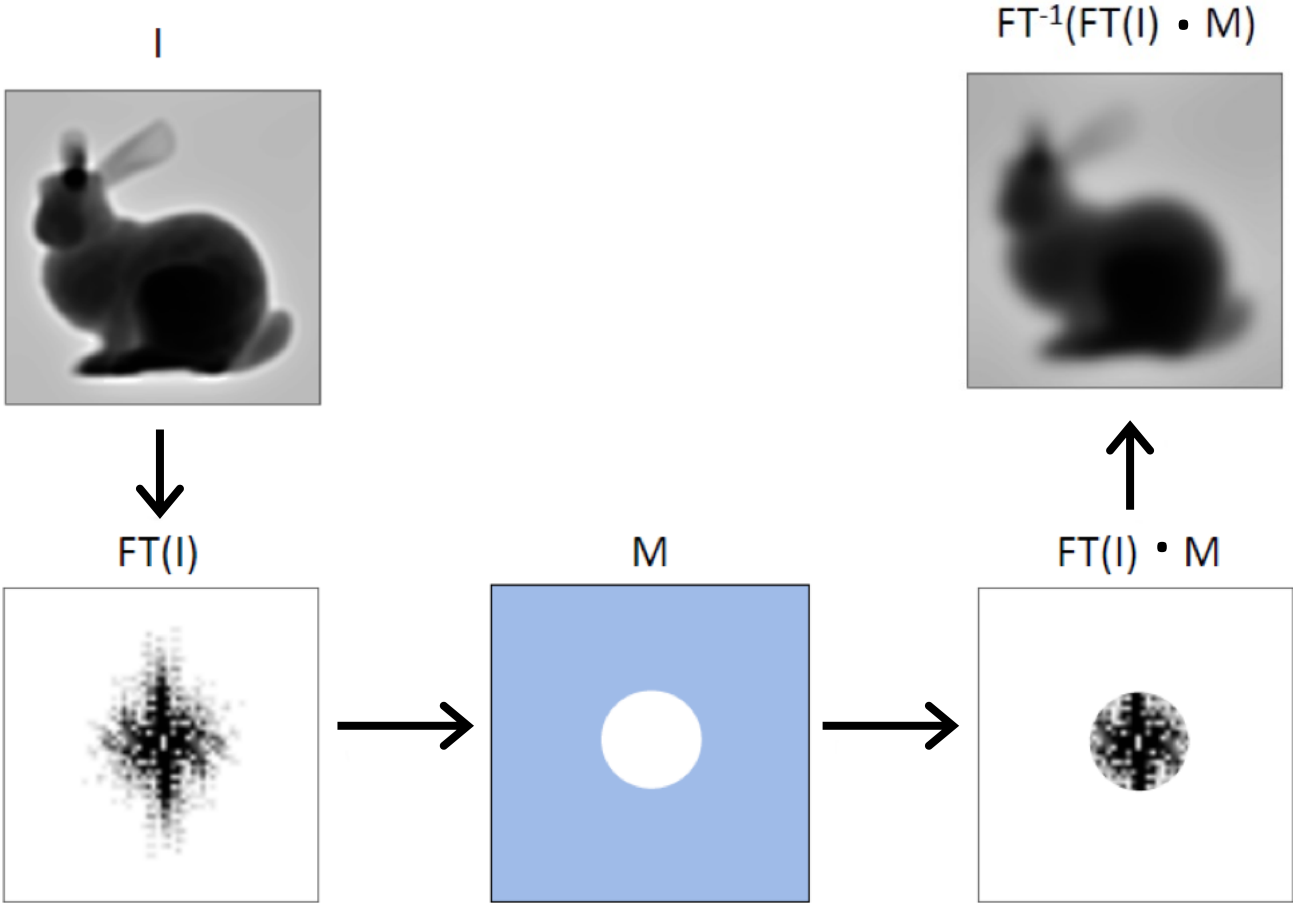
Fourier transform of images

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



Using the Fourier transform in image processing

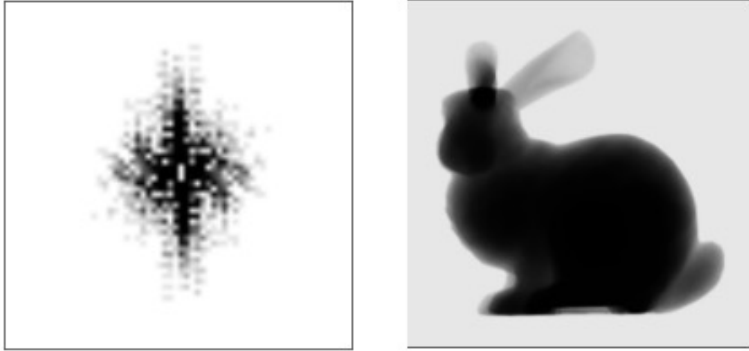
Fourier transform in filtering



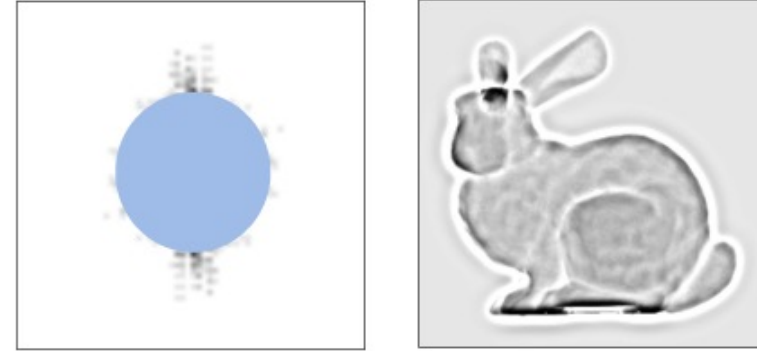
Shown inverted for clarity

Fourier transform in filtering

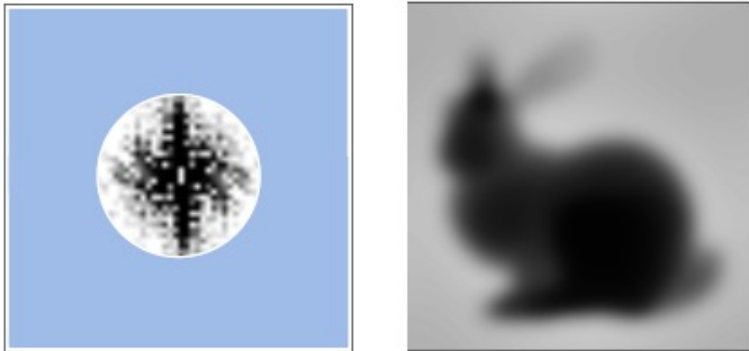
No filter



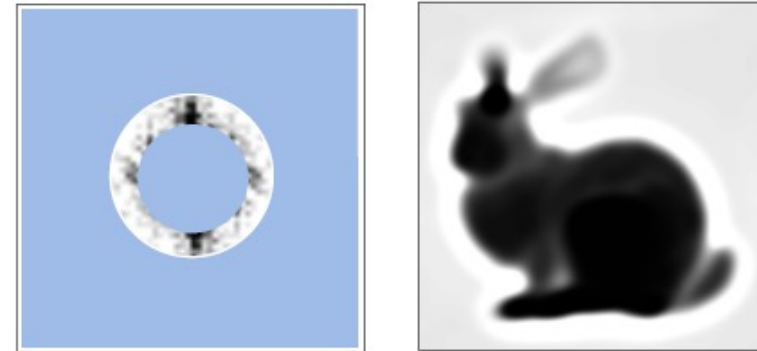
High pass filter



Low pass filter



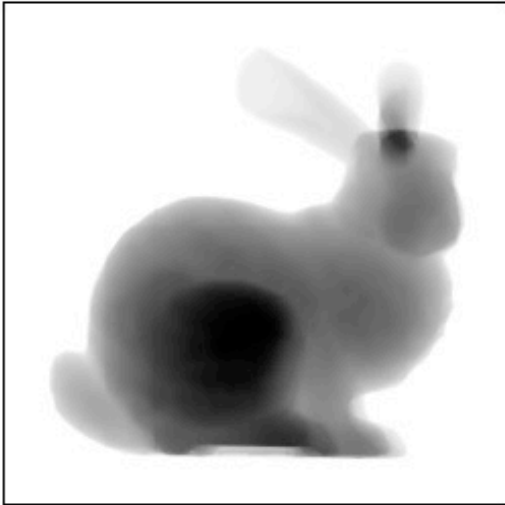
Band pass filter



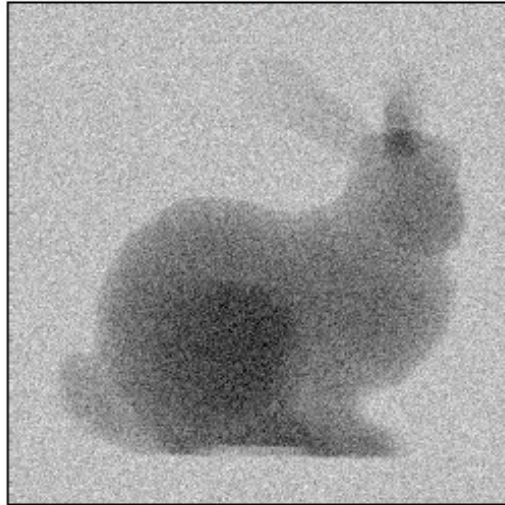
Note: Masks shouldn't have sharp edges, instead they should have a Gaussian-shaped (or cosine-shaped) fall off from 1 to 0.

Applications of filtering

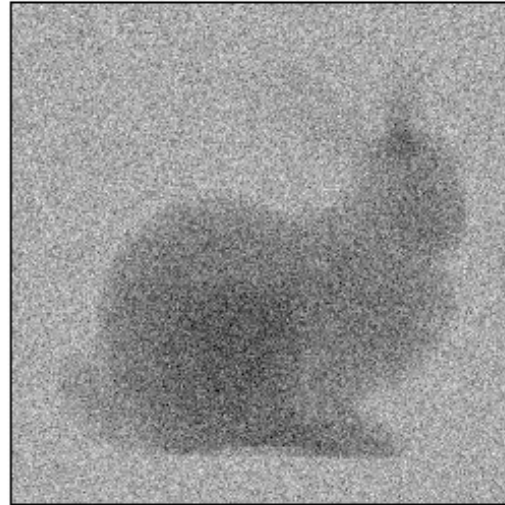
No noise



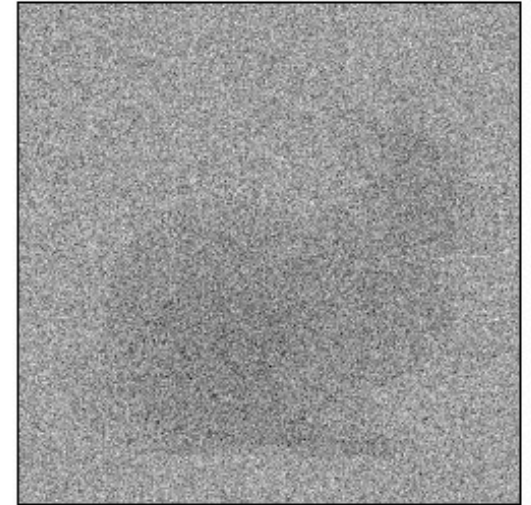
SNR=5



SNR=1



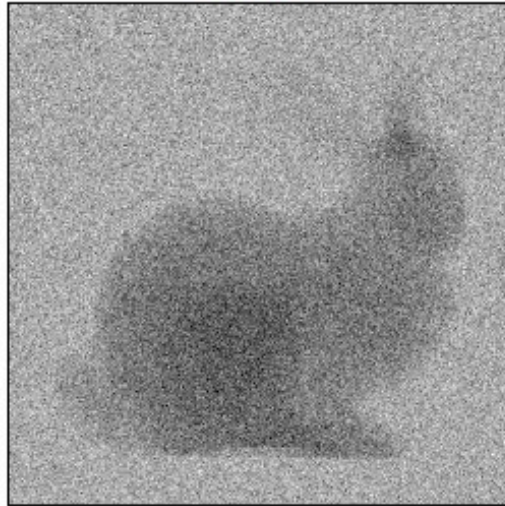
SNR=0.1



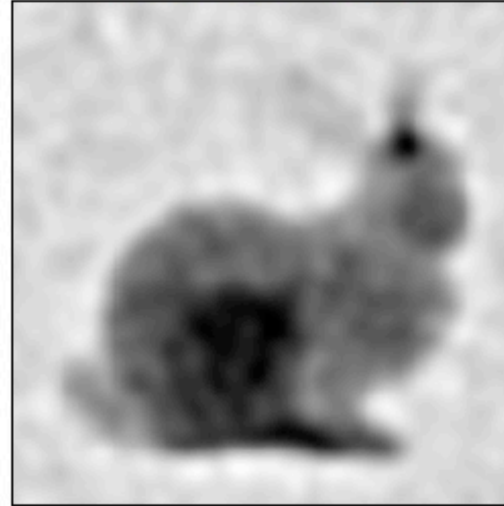
Signal-to-noise ratio (SNR) = contrast of the object / standard deviation of the noise

Use of low pass filter

SNR=1

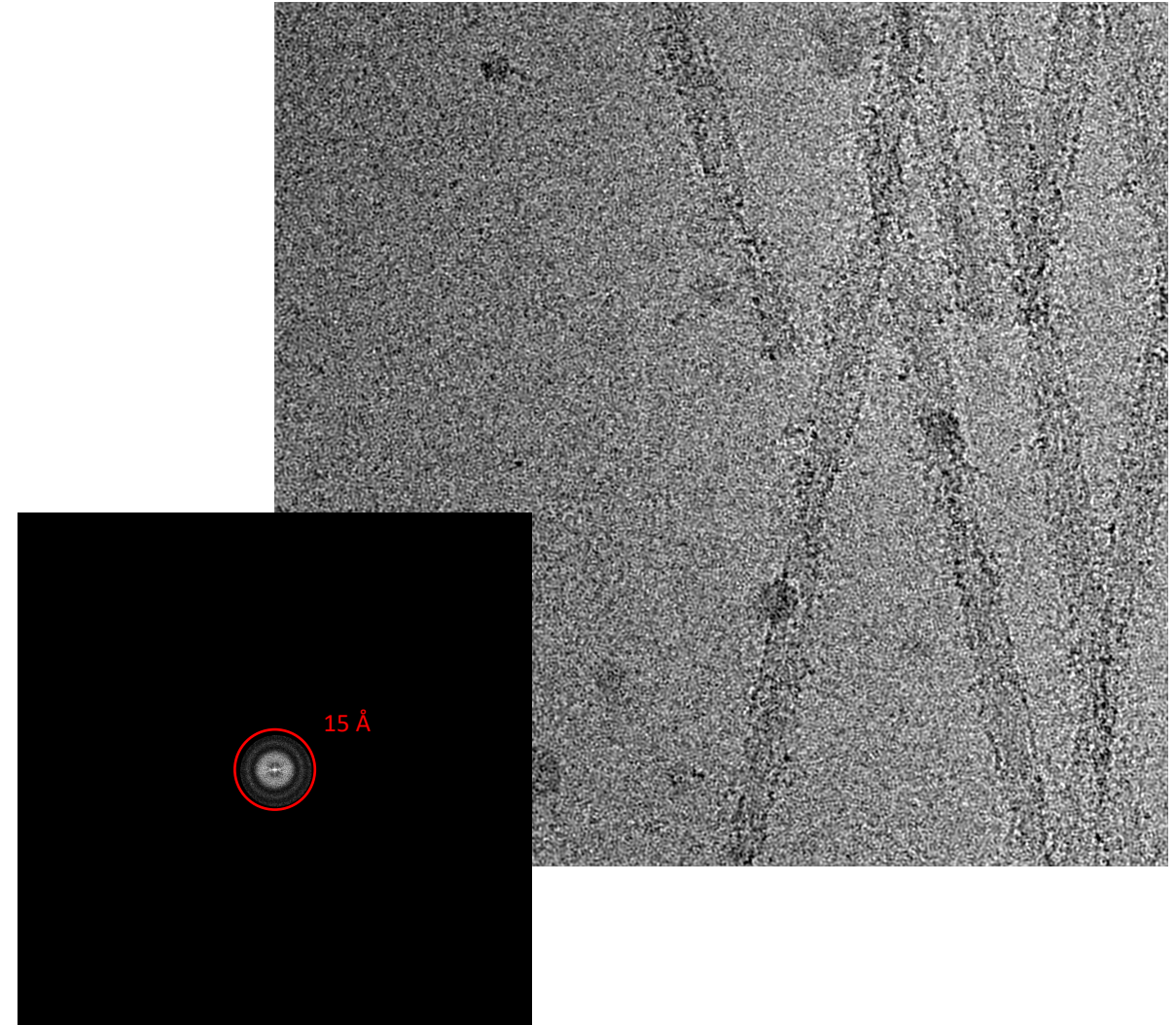
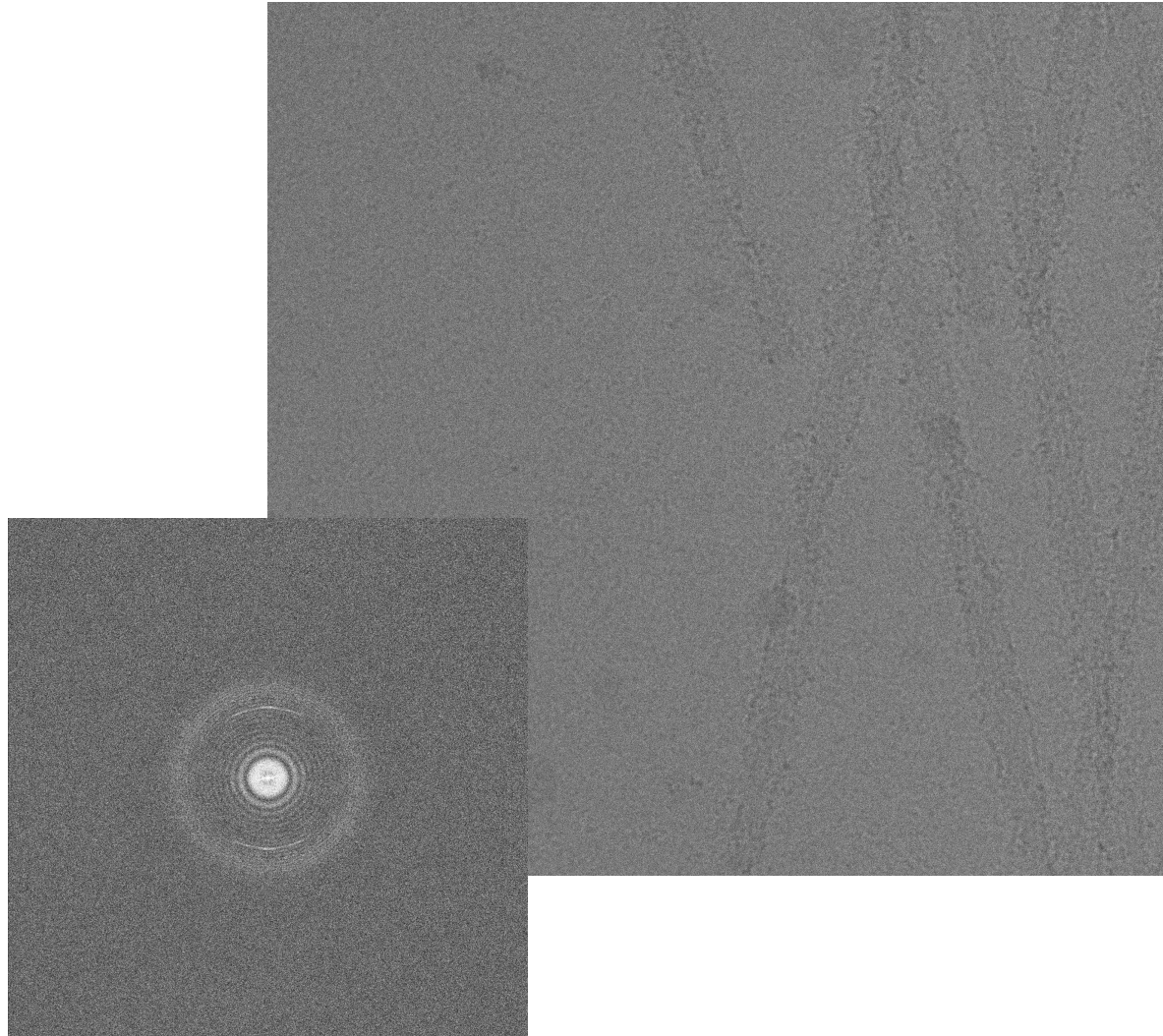


Low pass filter



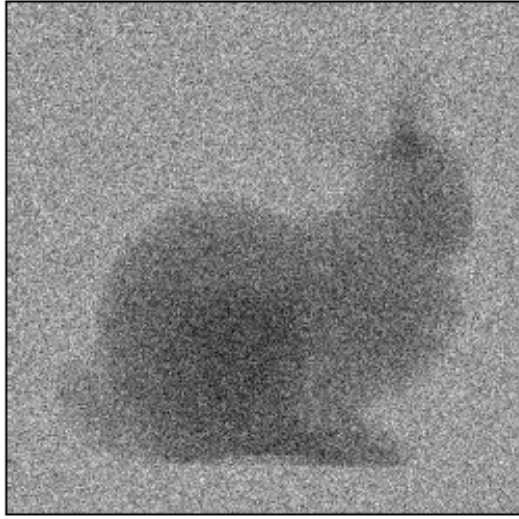
Low pass filter can be used to remove high spatial frequency noise. This makes the object easier to see.

Real world example of low pass filtering



Use of high pass filter

Original image



Gradient

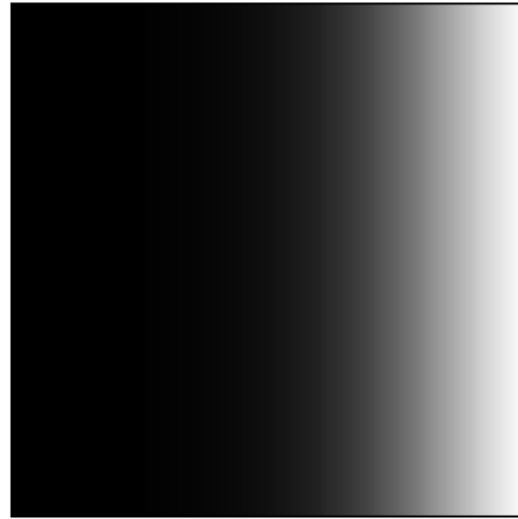
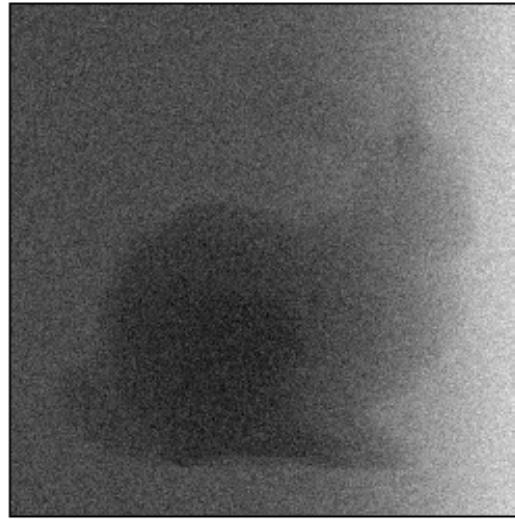
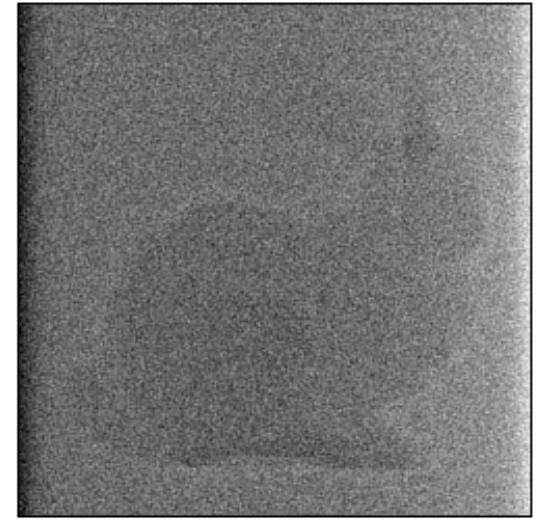


Image + gradient

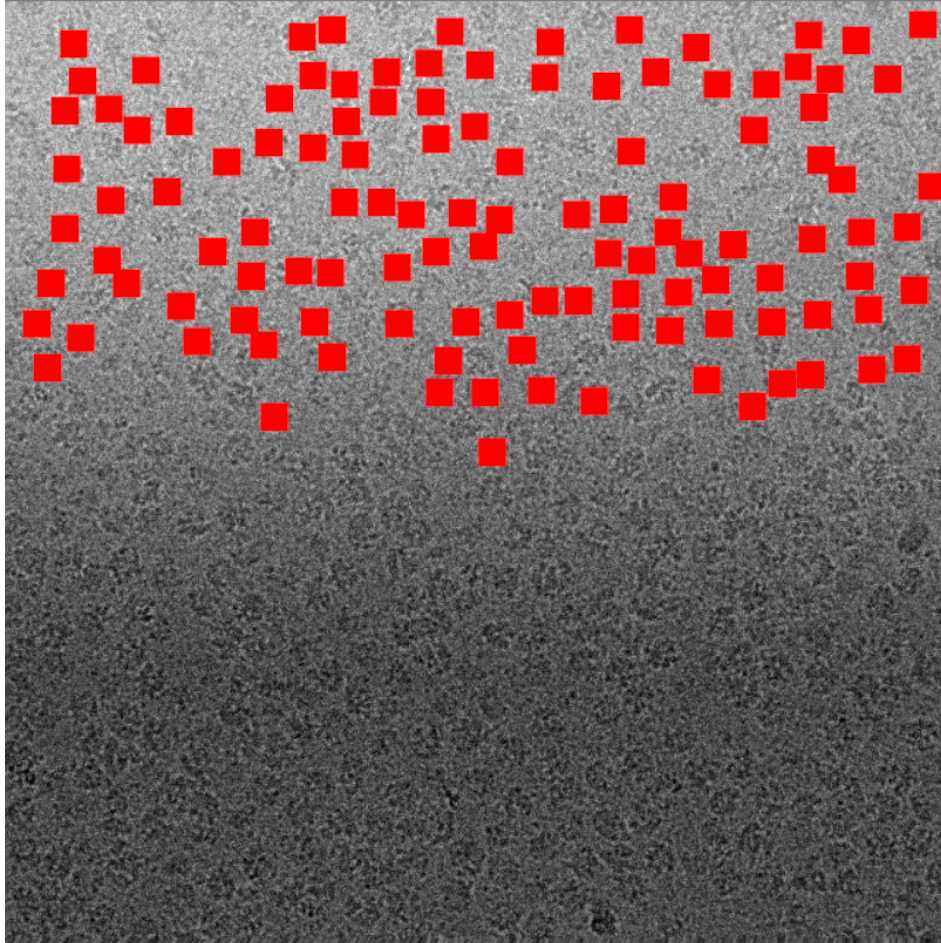


High-pass filter

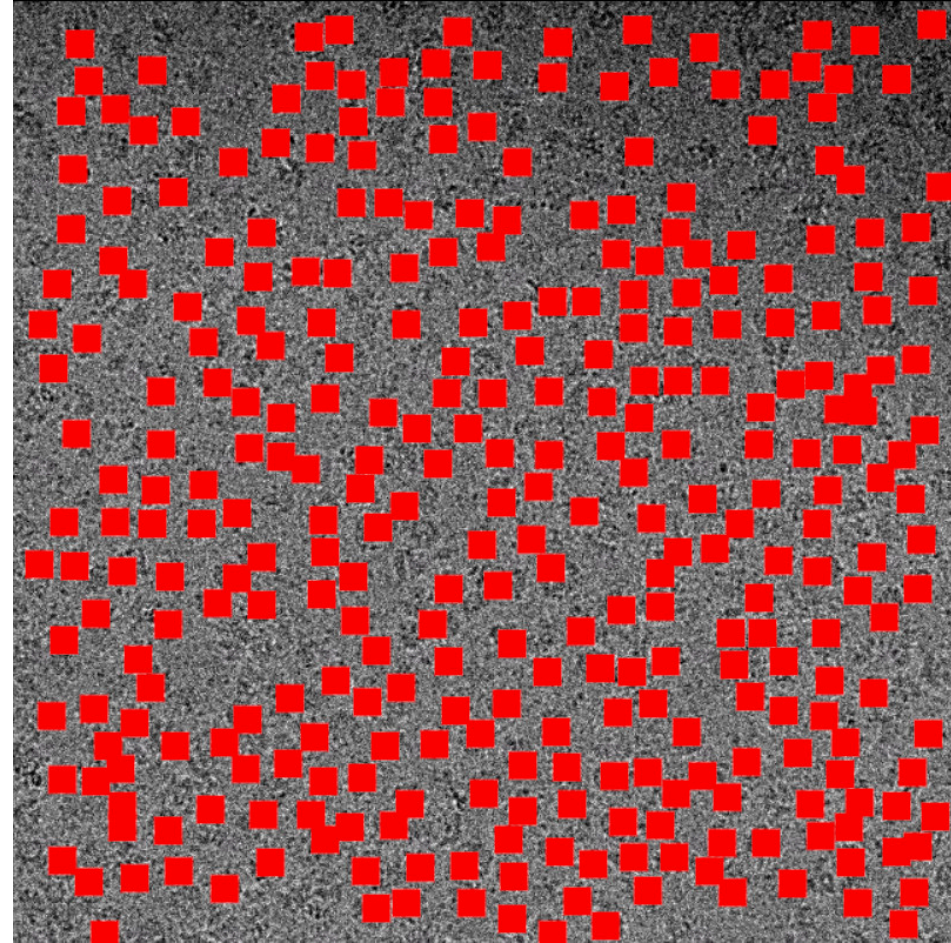


High pass filter can be used to remove low spatial frequency features, such as a density gradient.

Real world example of high pass filtering



Ice thickness gradient

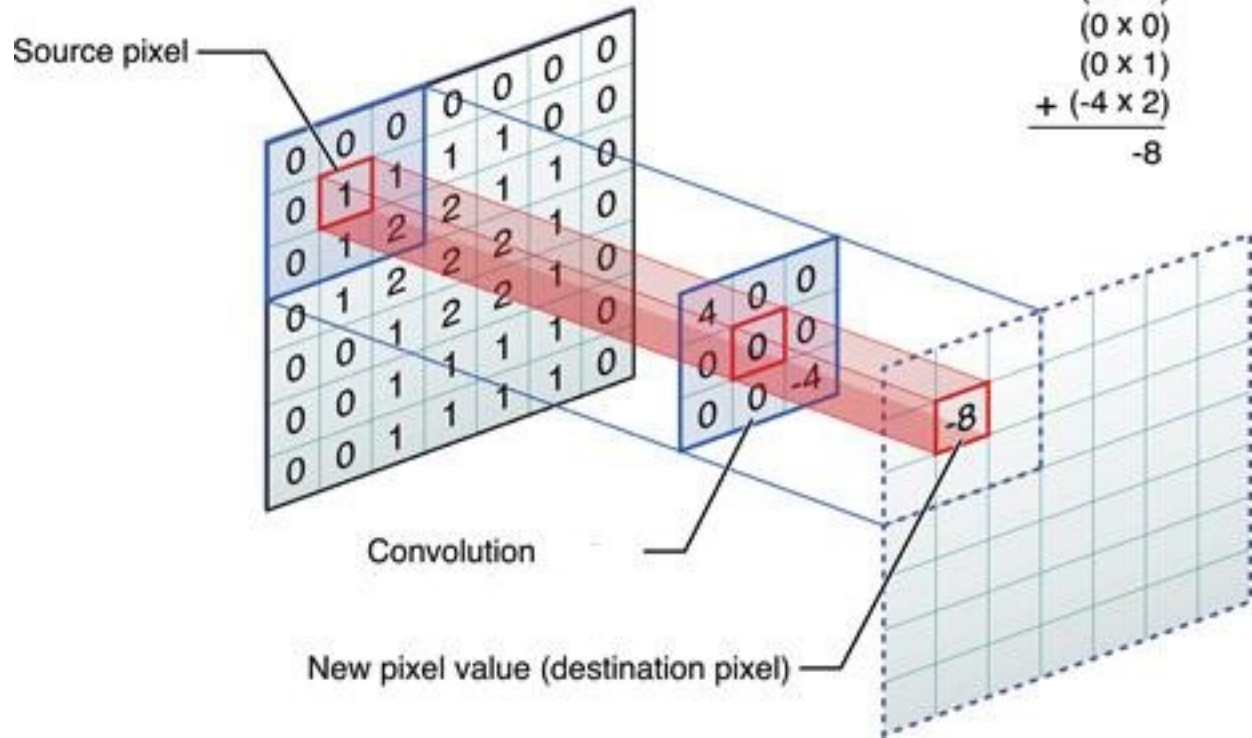


High pass filter $\frac{1}{325} \text{ \AA}$

Convolution in image formation and processing

Convolution in real space

Center element of the kernel is placed over the source pixel. The source pixel is then replaced with a weighted sum of itself and nearby pixels.



Convolution in real space is computationally expensive!

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

| Kernel 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

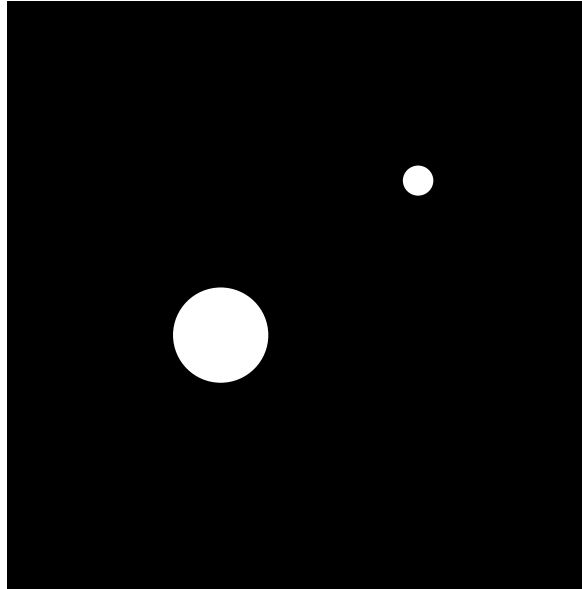
$$f(x) * g(x) = FT^{-1}[F(u) \cdot G(u)]$$



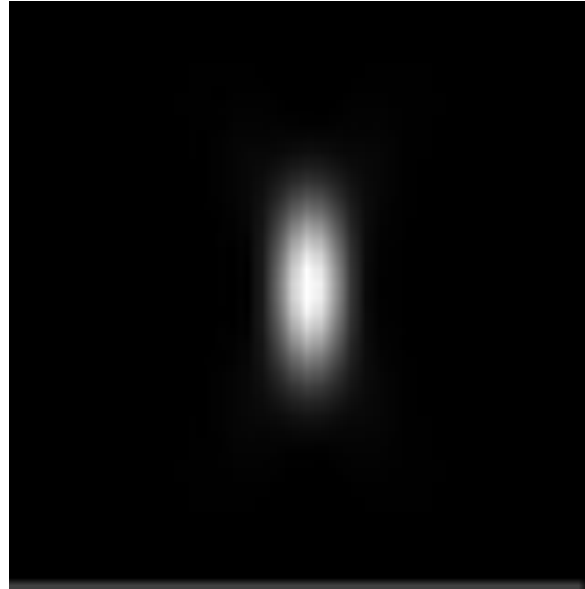
Actual object
 $f(x)$

PSF
 $g(x)$

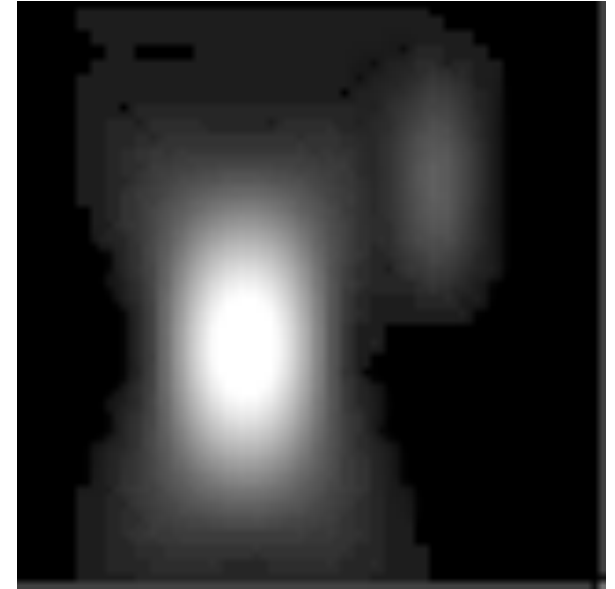
Image
 $f(x) * g(x)$



*



=



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

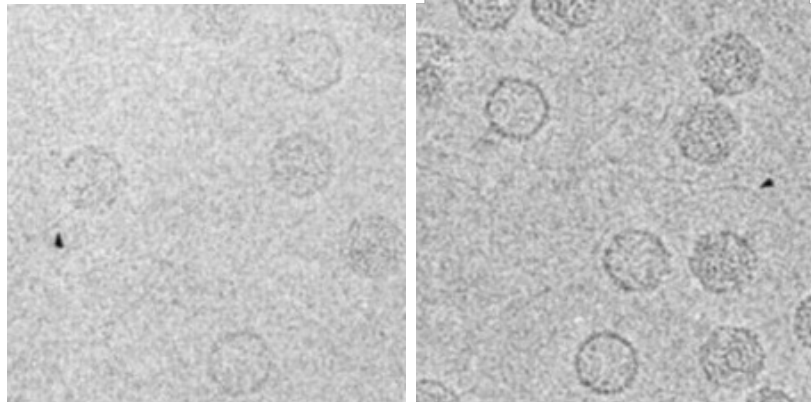
Deconvolution

Defocus and Contrast Transfer Function

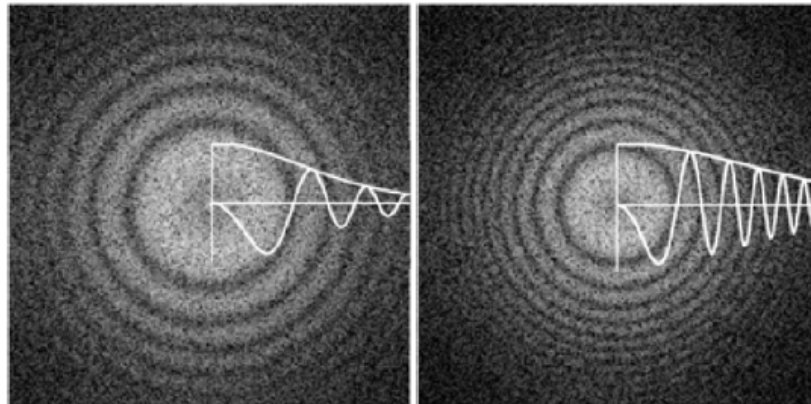
-0.5 μM

-1.0 μM

$f(x)$



$F(u)$

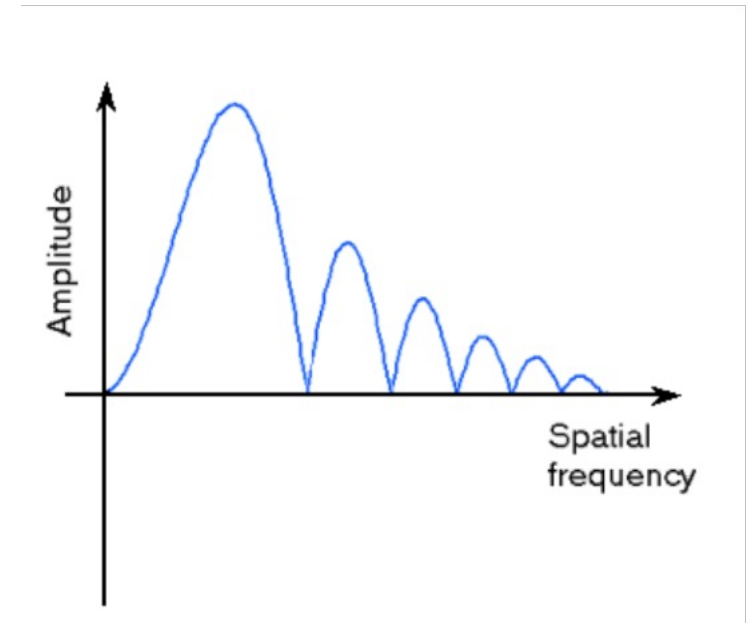
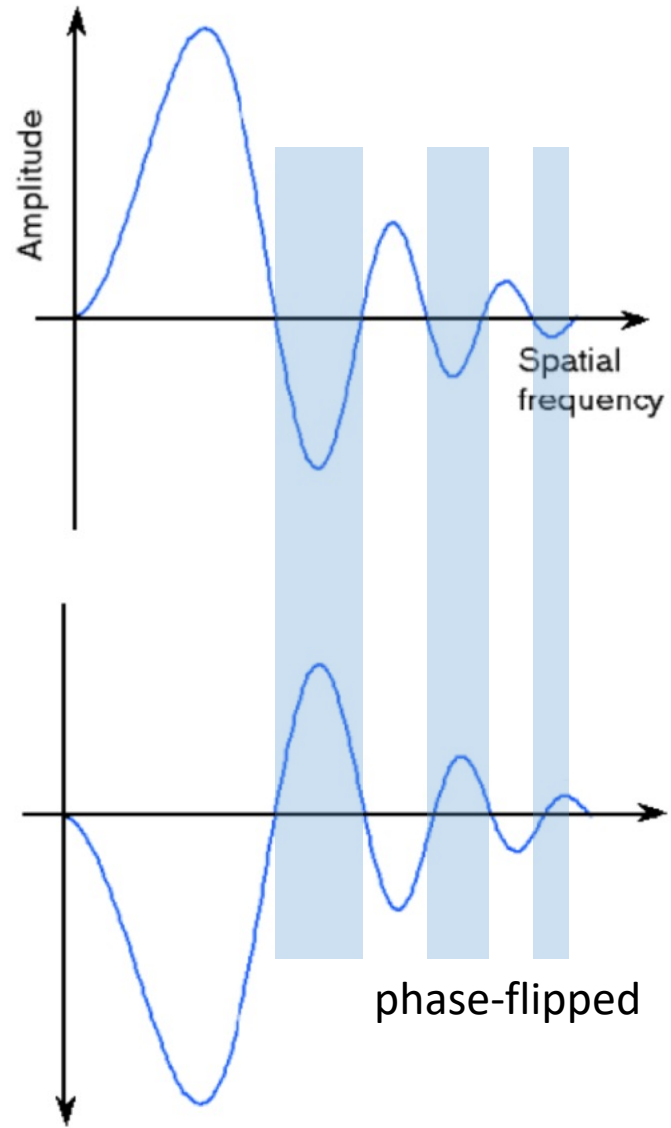
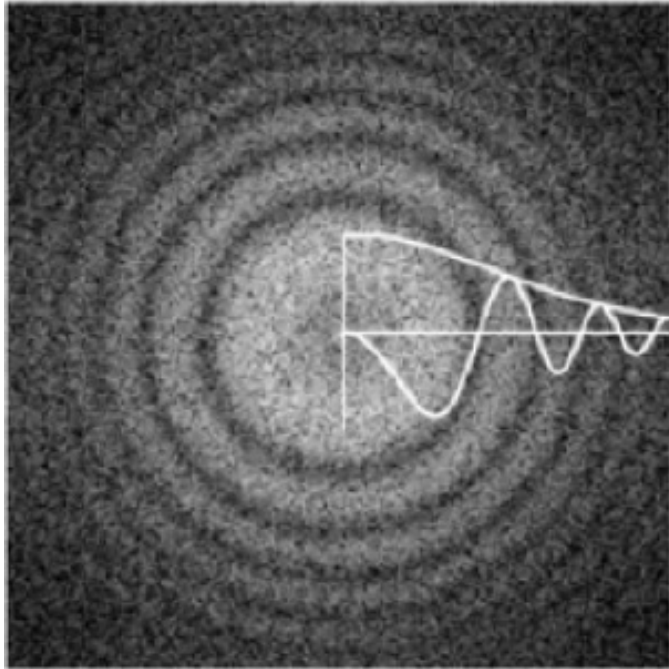


Contrast Transfer Function (CTF): The sum of all the effects of the imaging system

Defocus is applied to an image to increase contrast

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

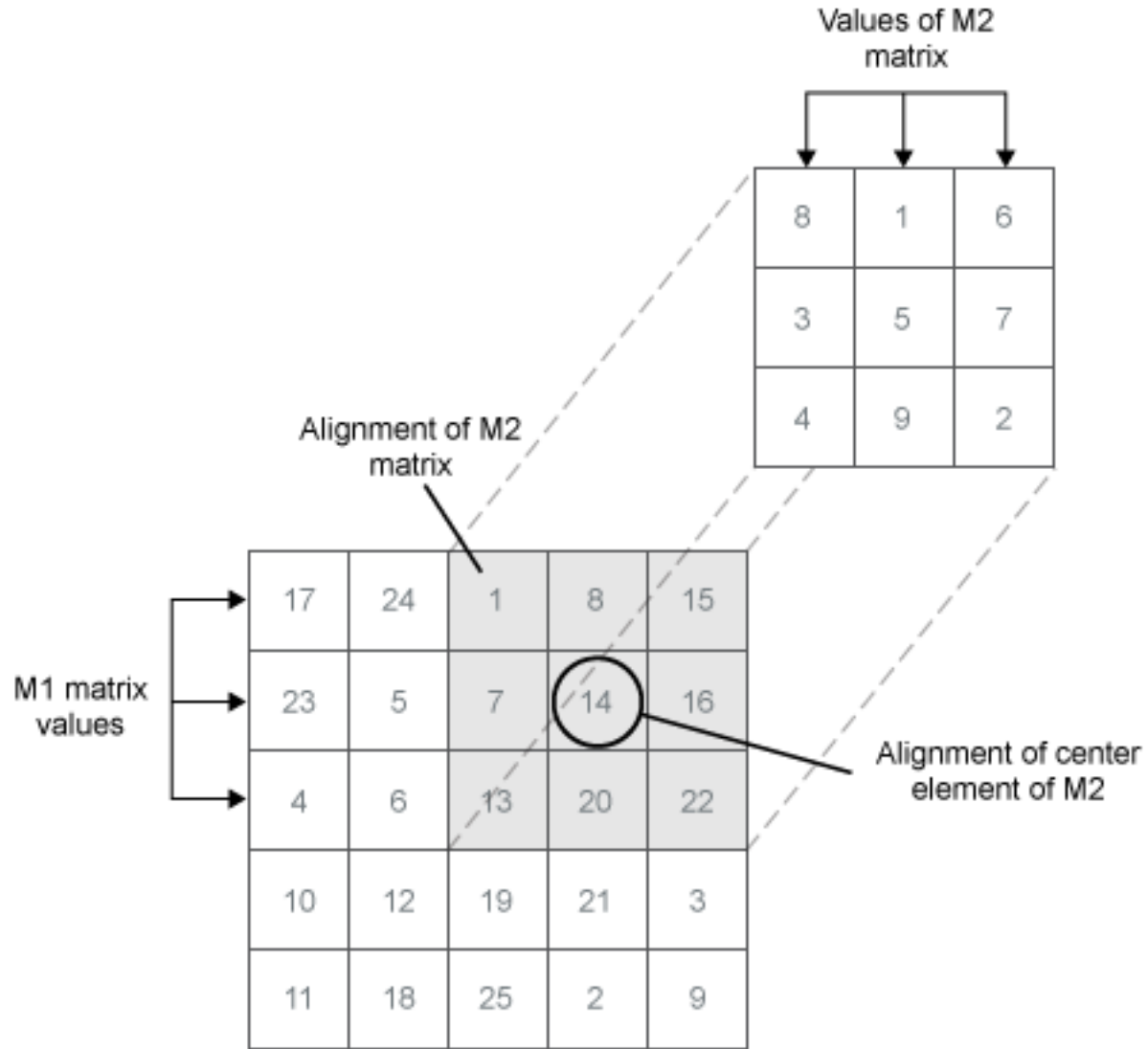
Images are 'CTF corrected' by phase flipping in Fourier space



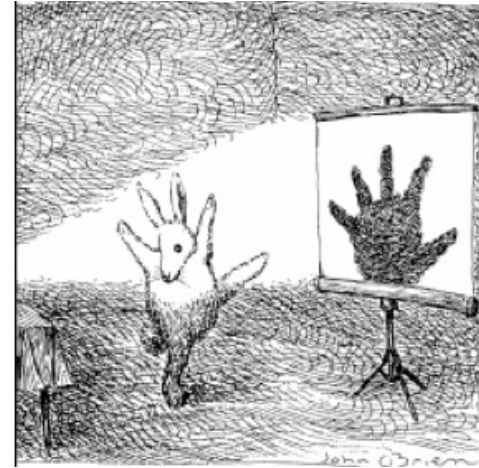
Cross-correlation and image alignment

Cross-correlation

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



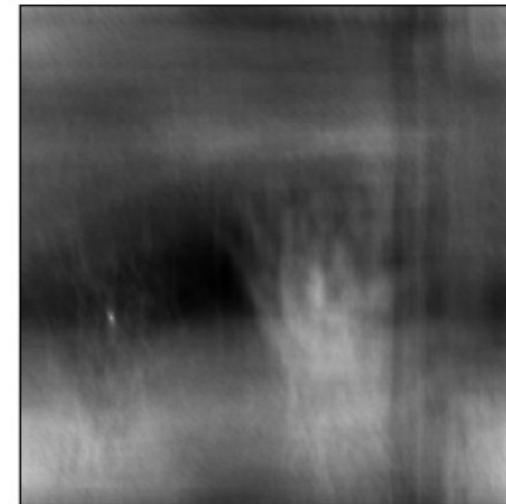
$f(x)$



$g(x)$

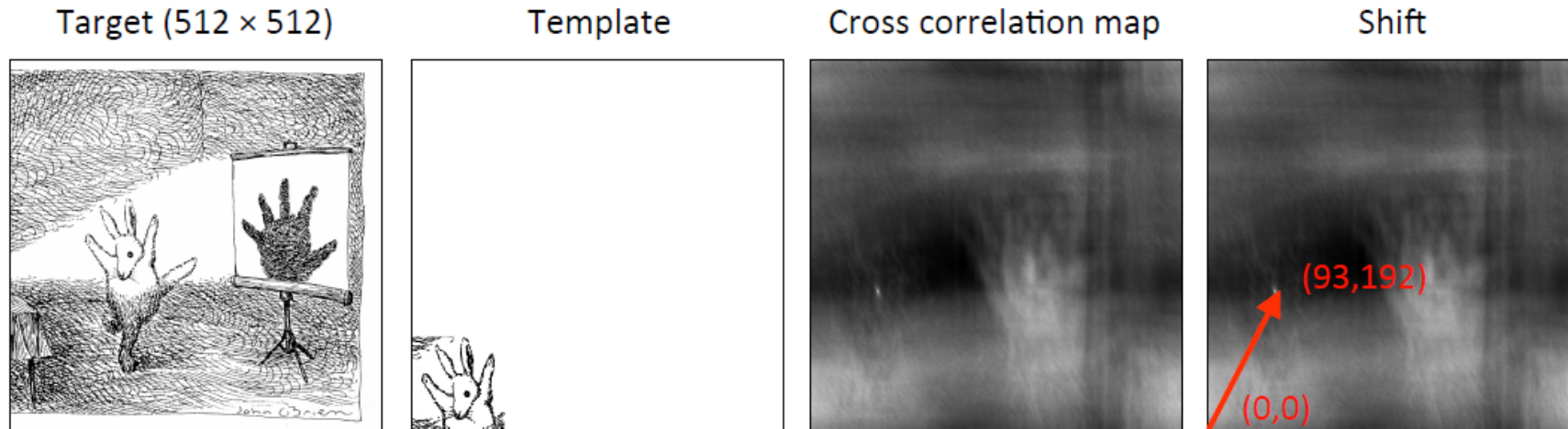


$g(x) \times f(x)$



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

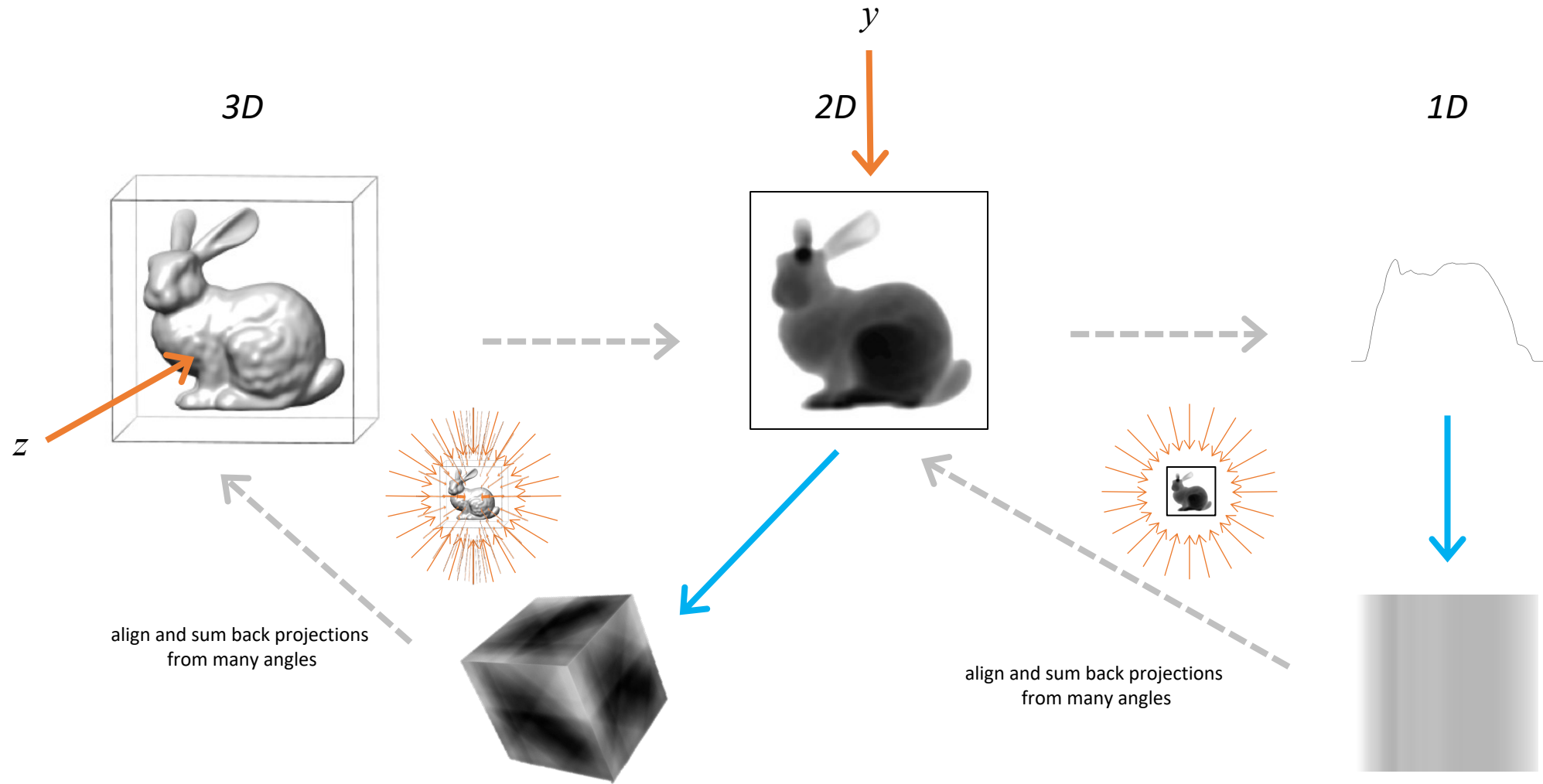


$$g(x) \times f(x) = FT^{-1}[F(u) \cdot G^*(u)]$$

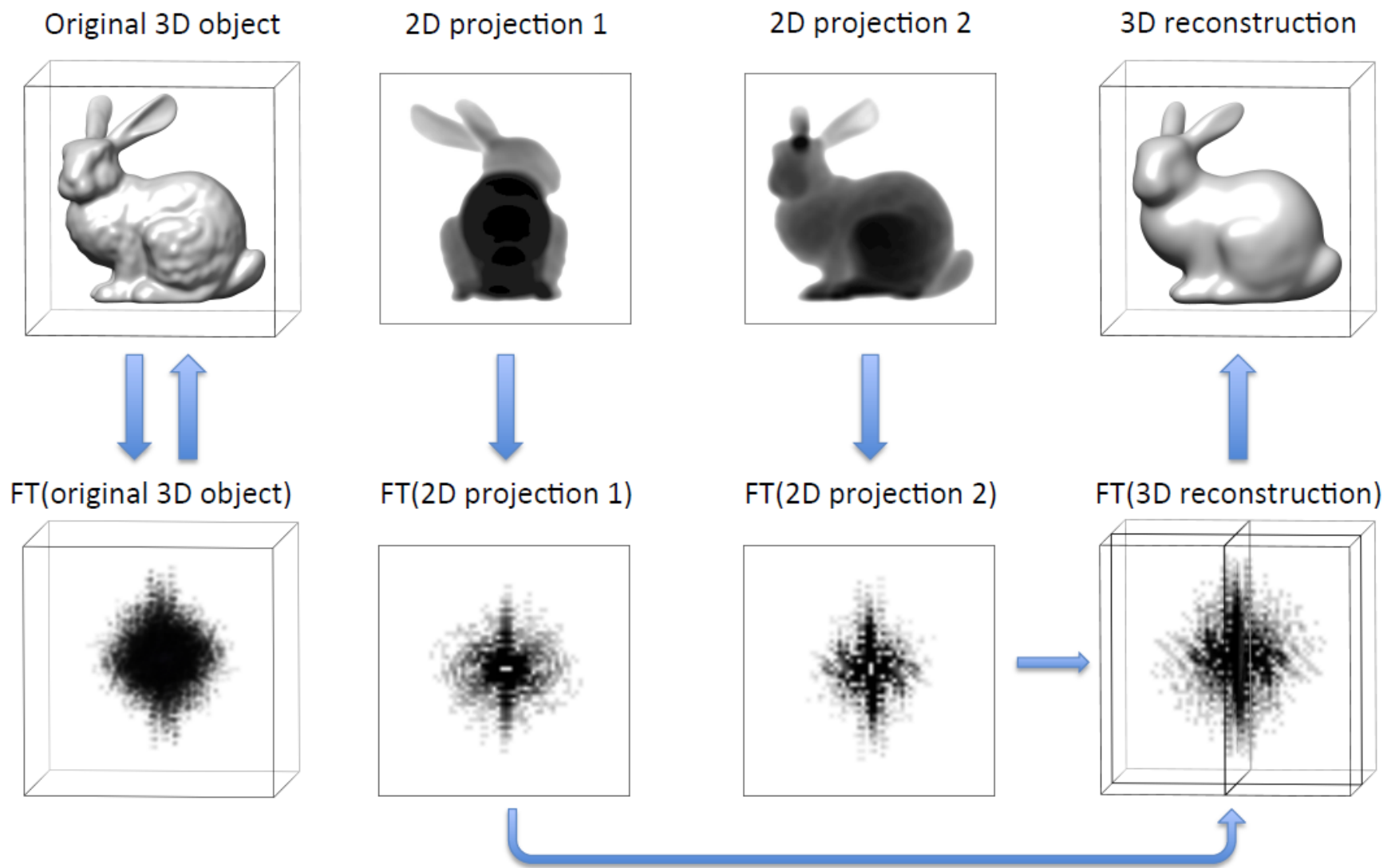
$$G^*(u) = \text{complex conjugate of } G(u)$$

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

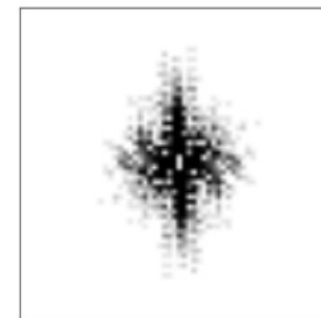
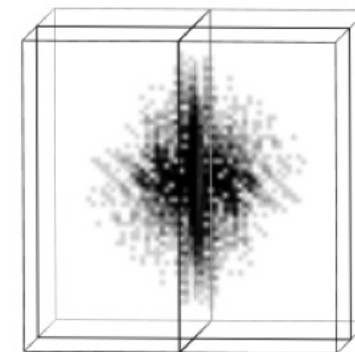
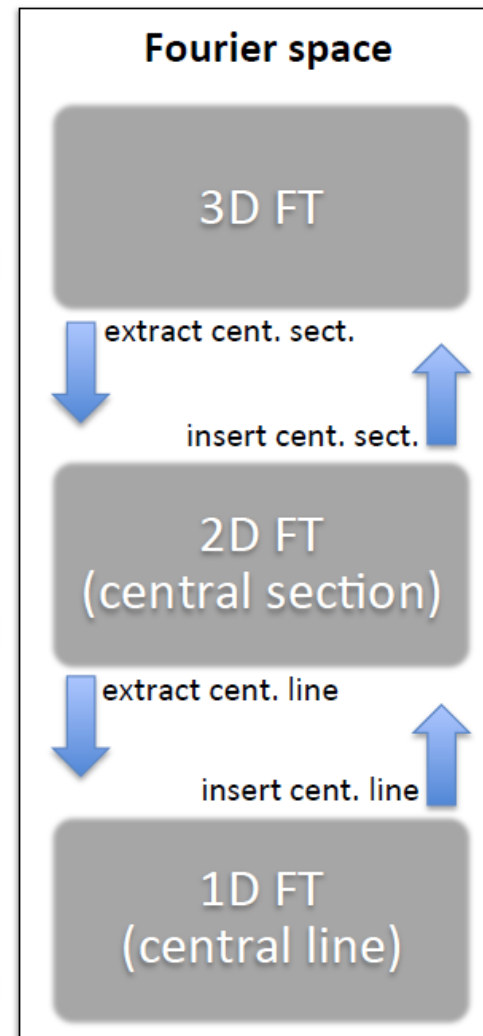
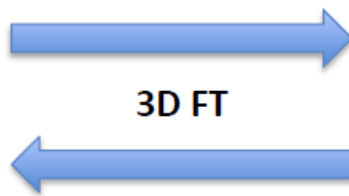
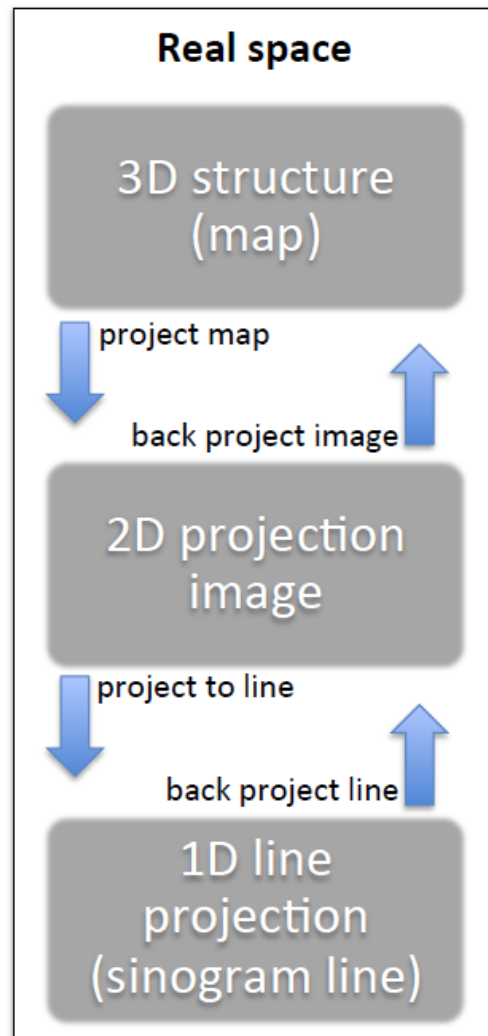
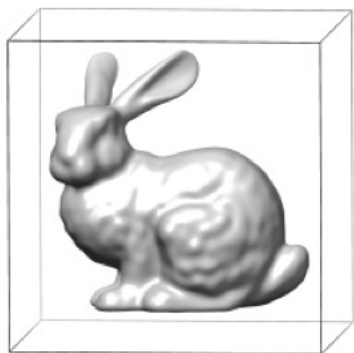
Projection and back projection in real space



Central section theorem



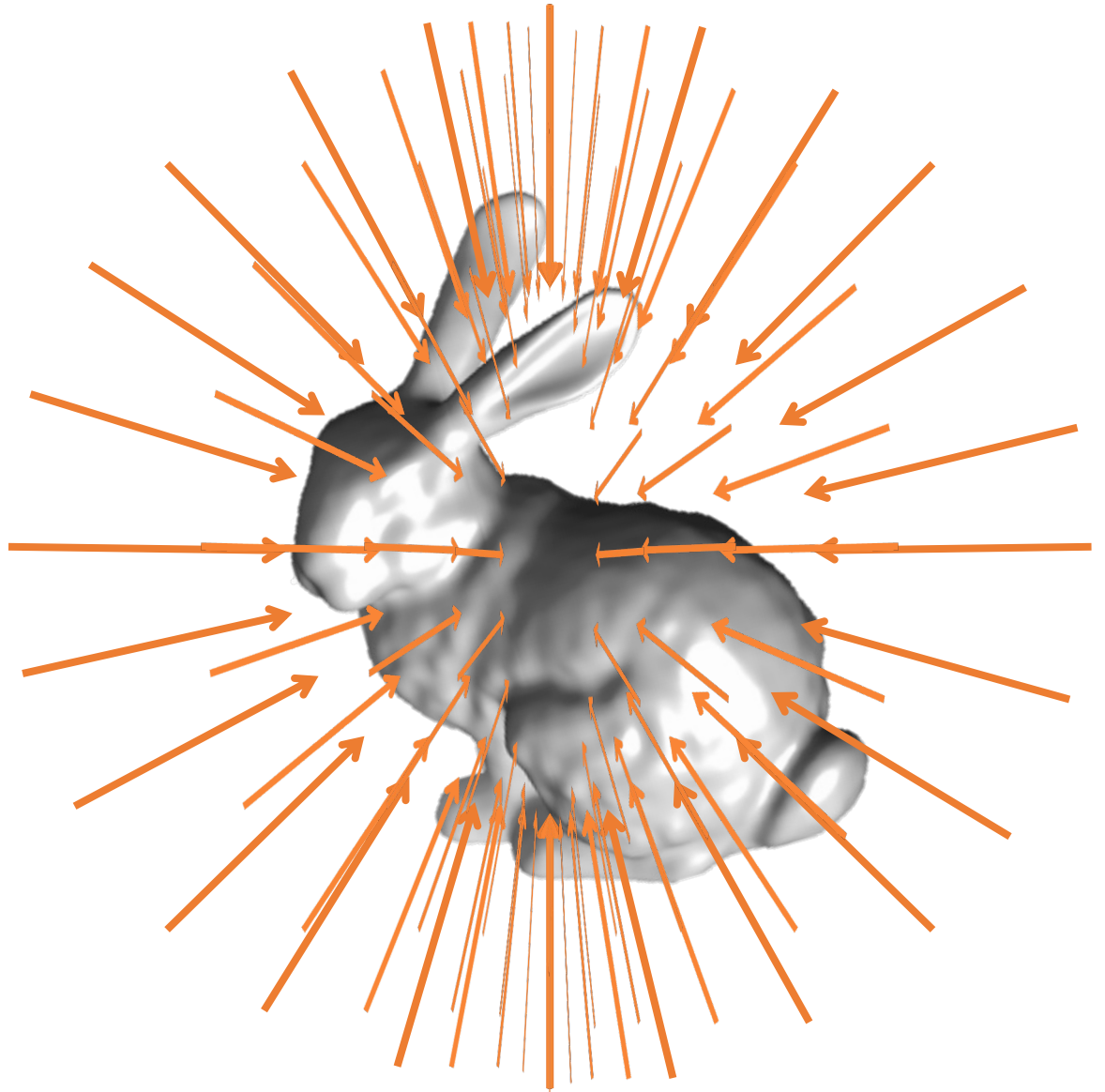
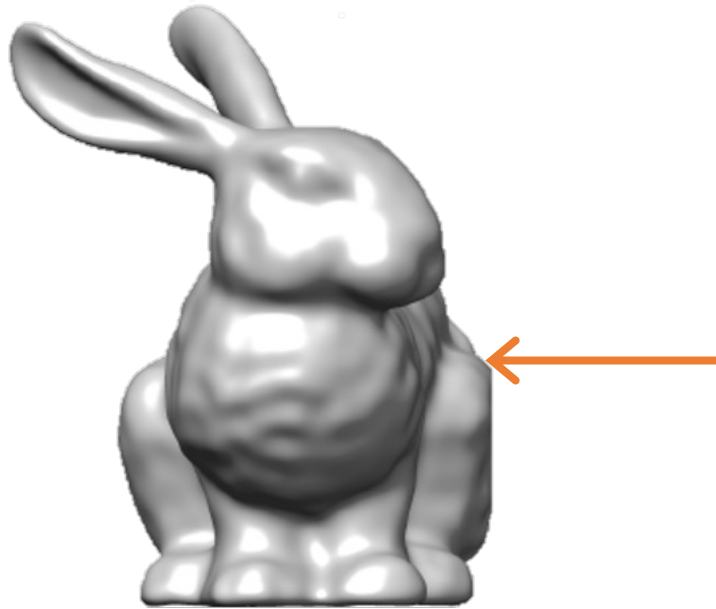
Central section theorem – real space vs. FT



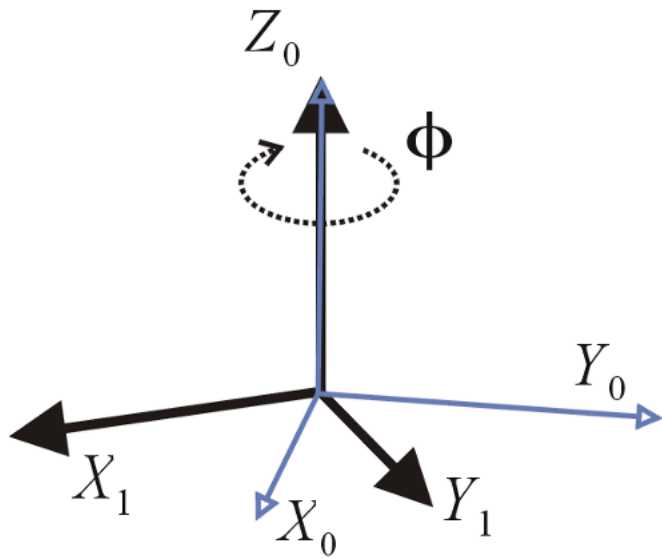
Central section theorem summary

- 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

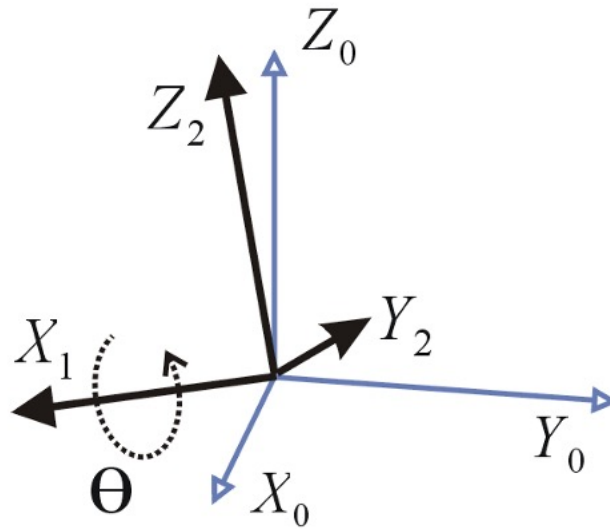
3D objects orientation in space



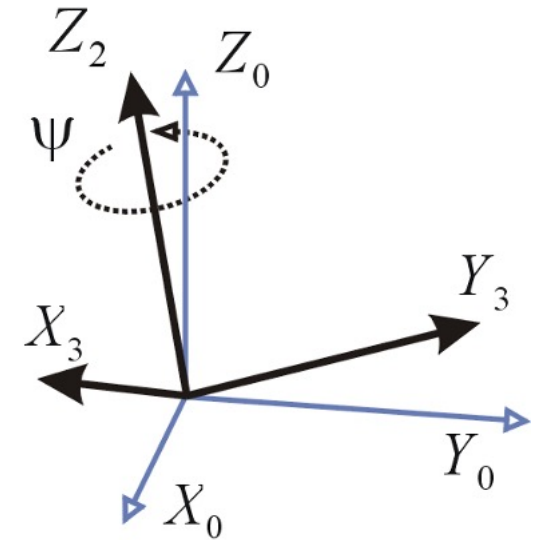
Euler angles ZXZ convention



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

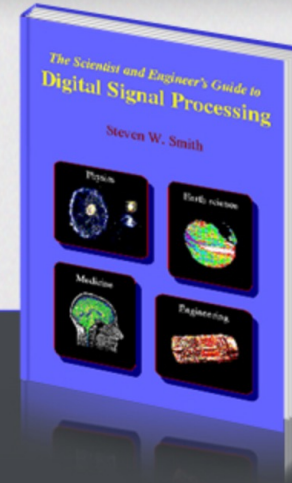
The Scientist and Engineer's Guide to Digital Signal Processing

By Steven W. Smith, Ph.D.

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