Central European Institute of Technology
BRNO | CZECH REPUBLIC

FB820

Lecture 6

3DEM methods

Jiri Novacek

Content

- image formation, CTF, image filtering
- image alignment in 2D
- 3D reconstruction
- common lines
- random conical tilt

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Image formation

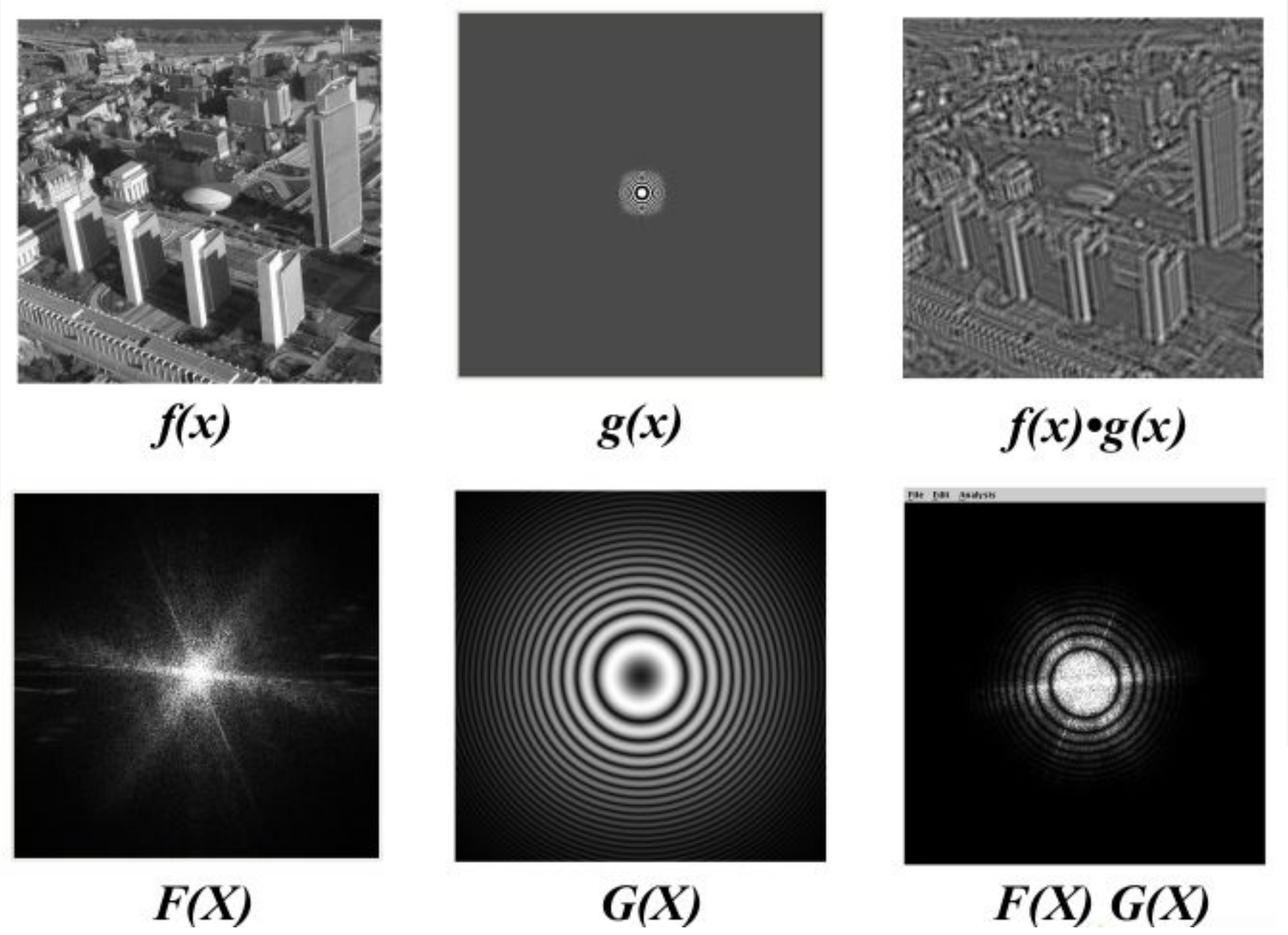
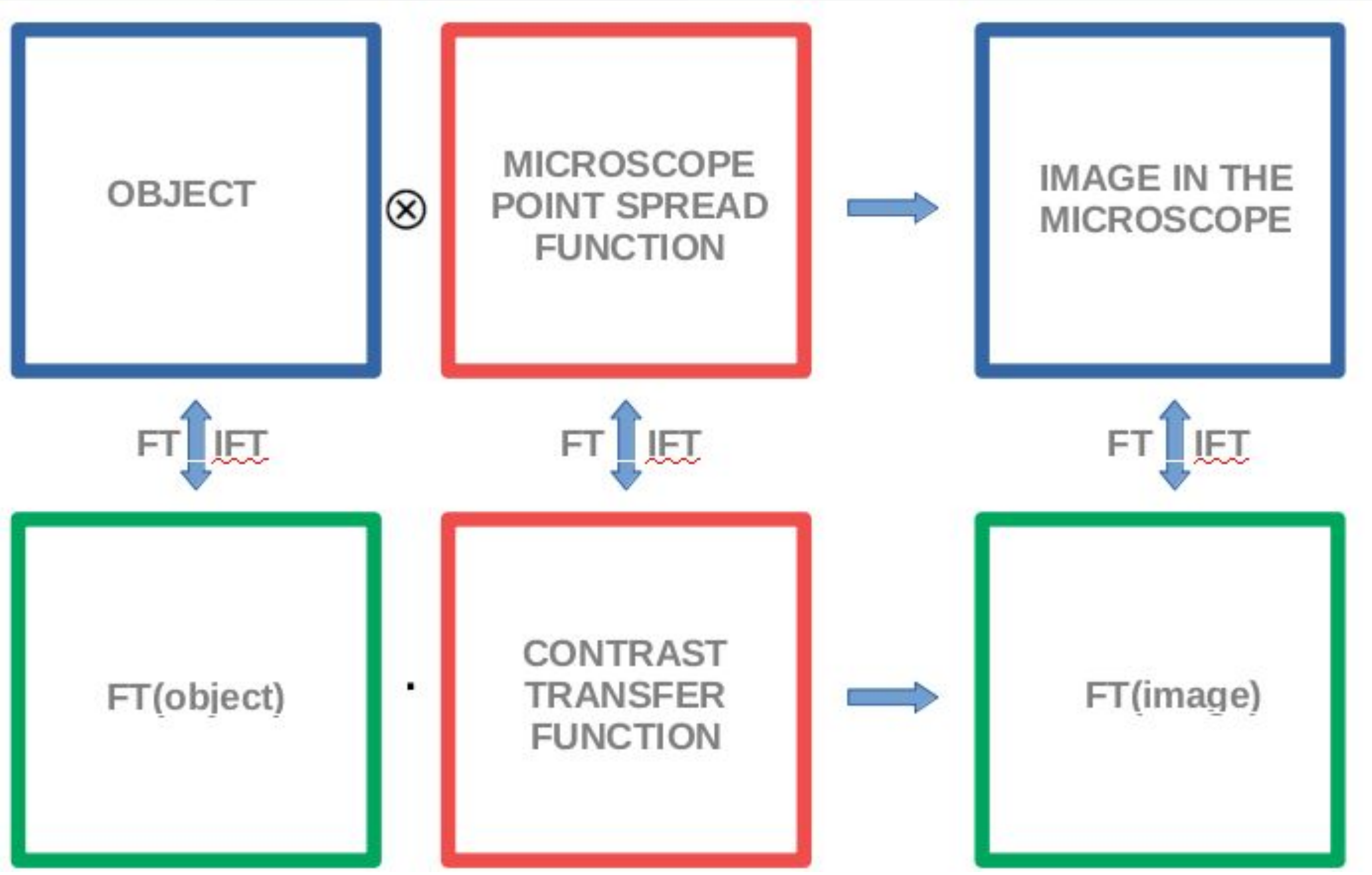


Image formation



Contrast transfer function

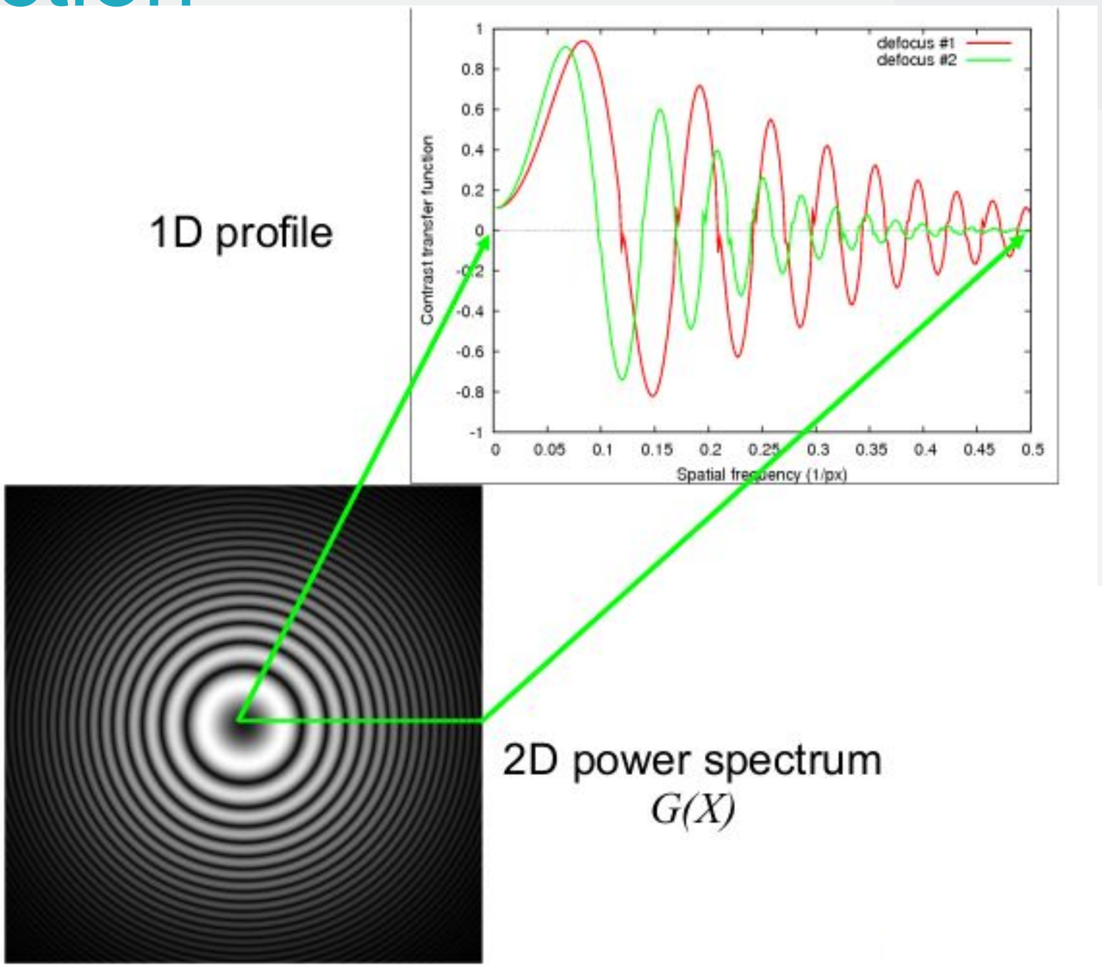
A – amplitude contrast

s – spatial frequency

Cs – spherical abberation

λ – electron wavelength

z – defocus



Contrast transfer function

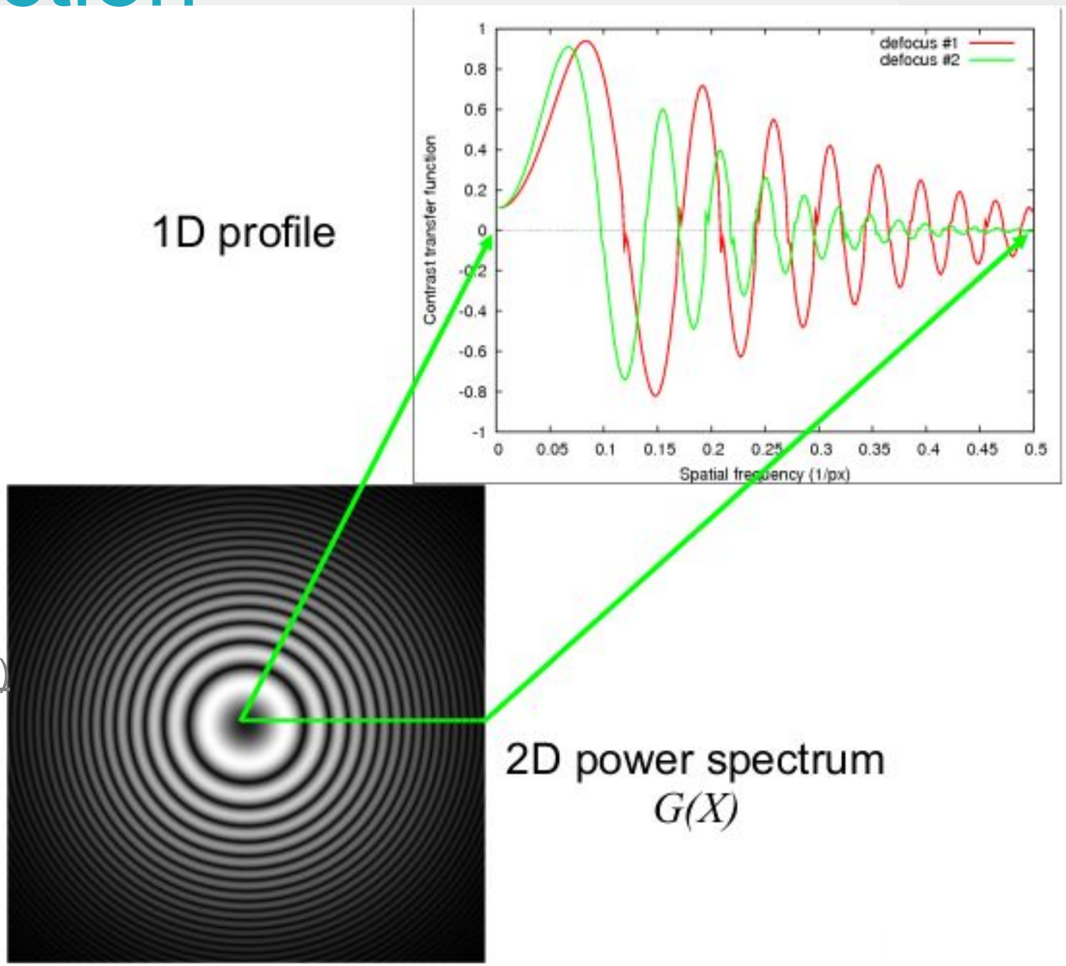
Envelope function

- Finite source size

- Energy spread (defocus)

- MTF of the camera

- Generic envelope (drift, charging, multiple scattering)



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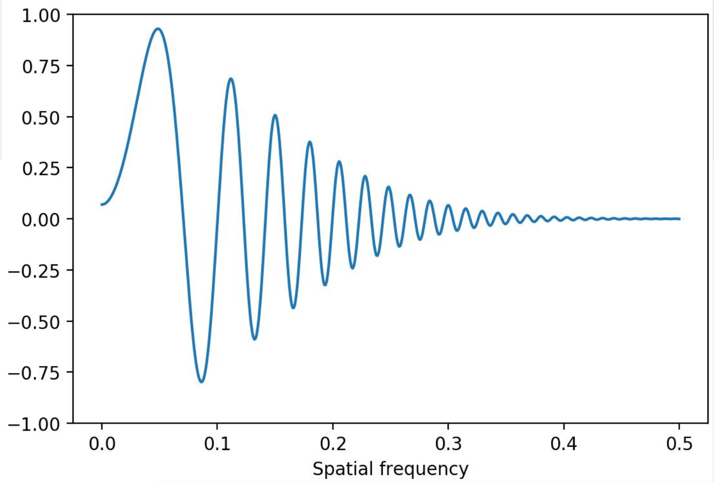
Contrast transfer function

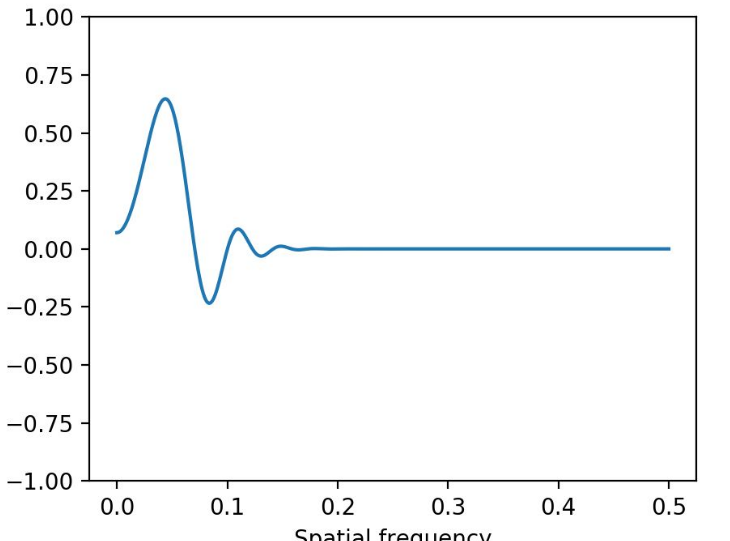
Envelope function

$$I(\mathbf{k}) = \underbrace{E_{pc}(k)E_{es}(k)E_f(k)E_g(k)}_{e^{-Bk^2}}H(k)\Phi(\mathbf{k}) + N(\mathbf{k}).$$

kV=300,ac=0.07,cs=2.7,z=-1,apix=1,B=30



kV=300,ac=0.07,cs=2.7,z=-1,apix=1,B=300



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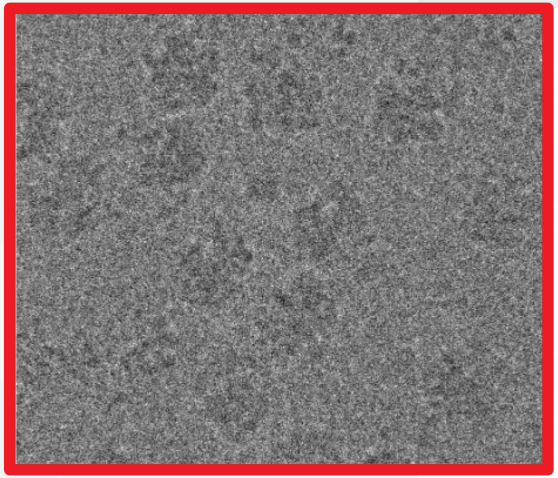
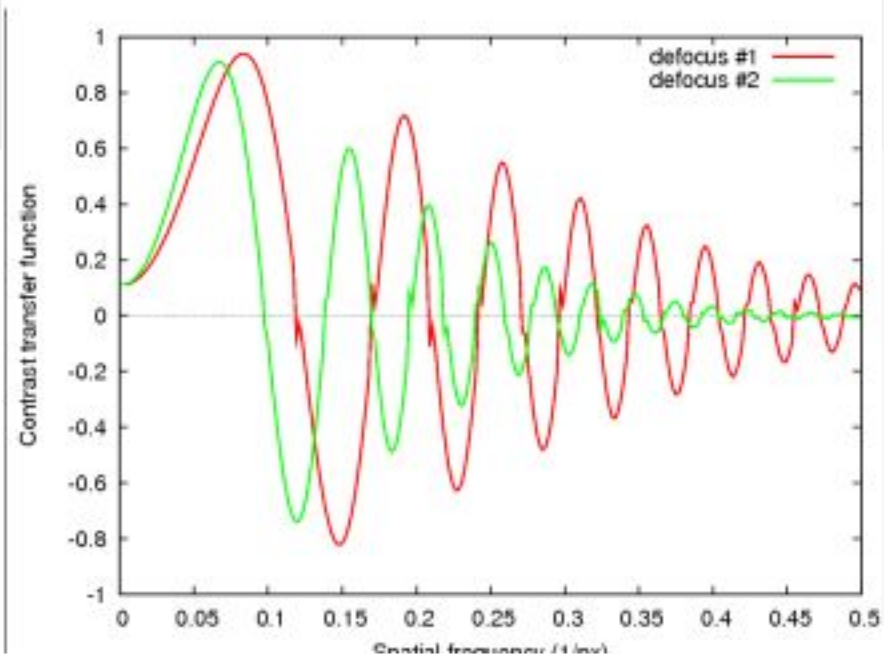
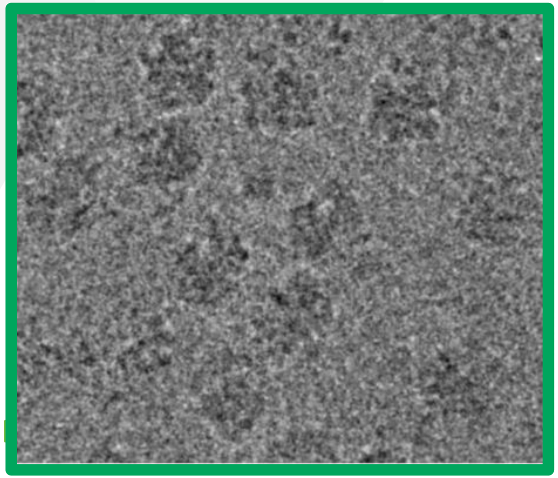
Contrast transfer function



Low defocus





High defocus

Image filtering

unfiltered image | lowpass filtered (50A) | lowpass filtered (250A)

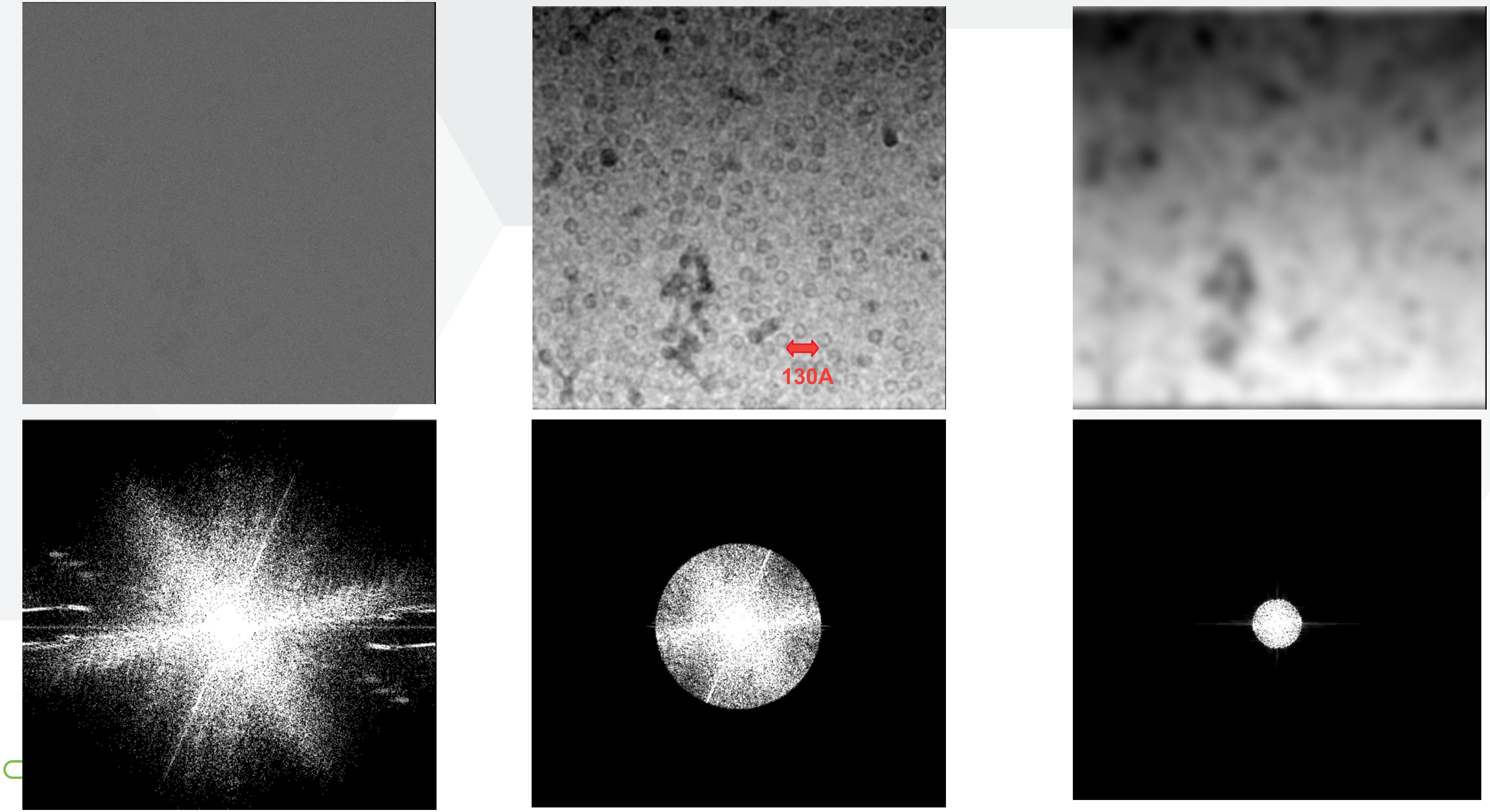
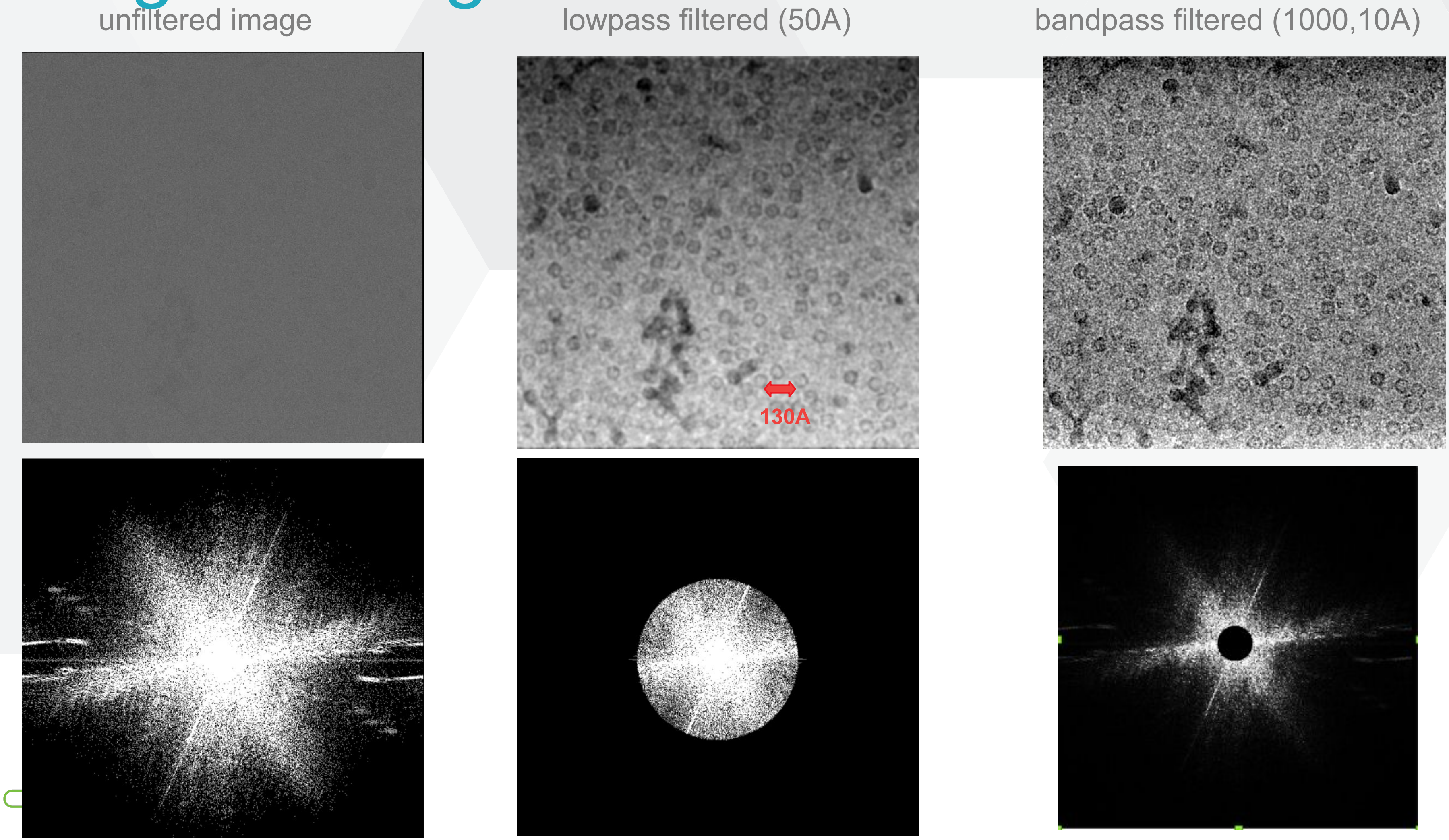
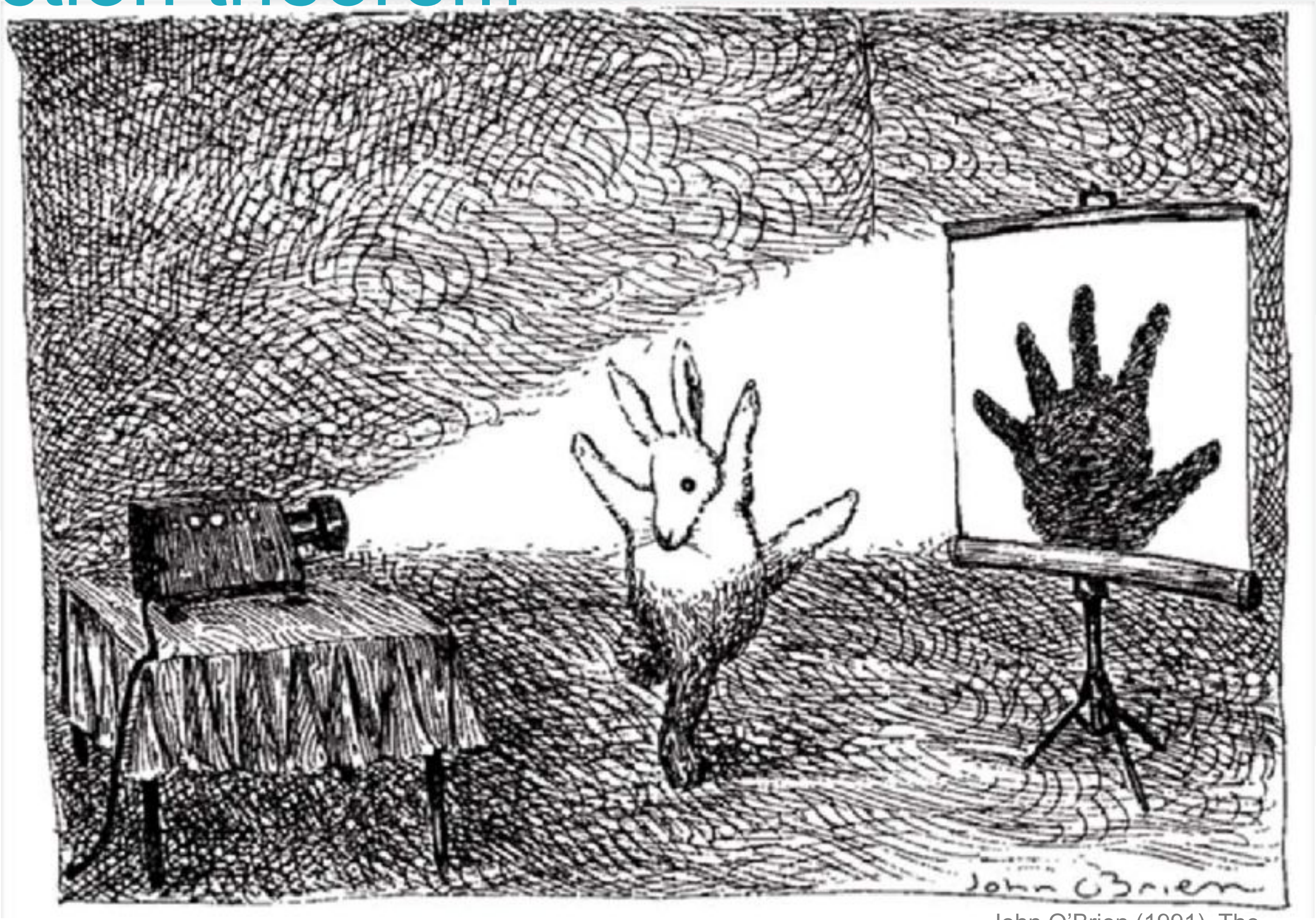


Image filtering



Projection theorem



John O'Brien (1991). The New Yorker

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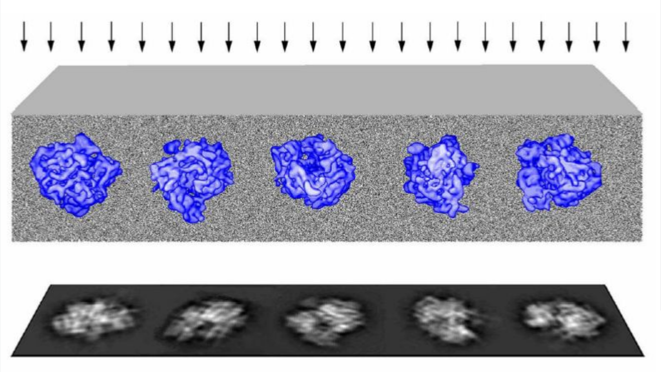
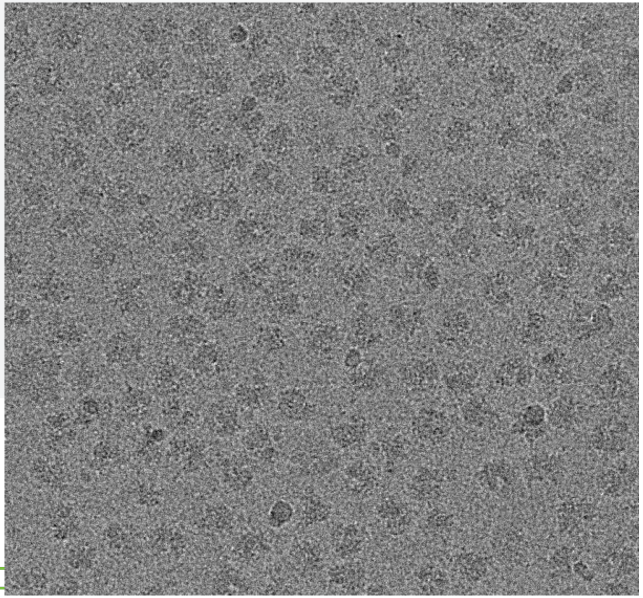
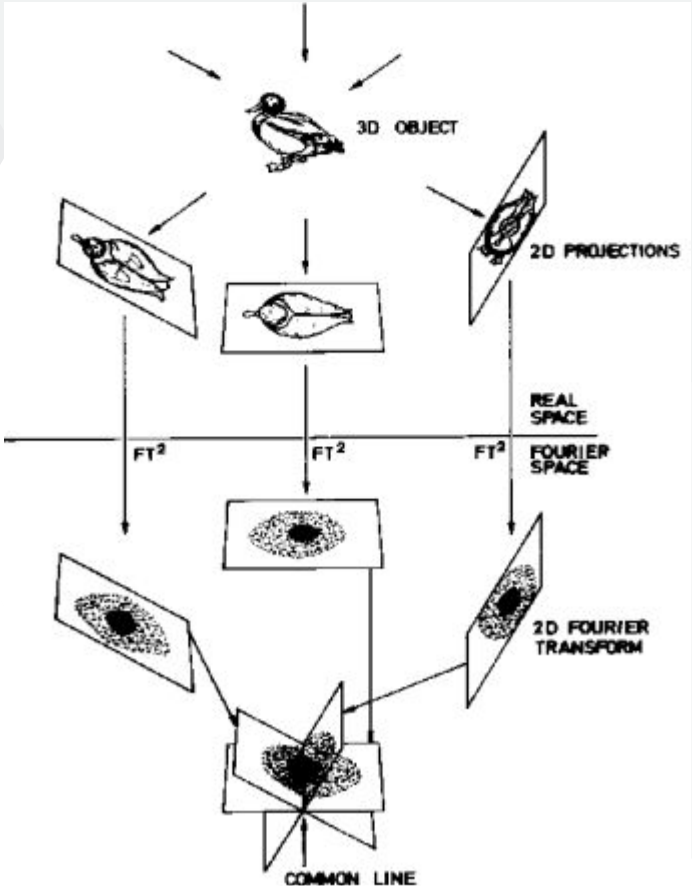
Projection theorem

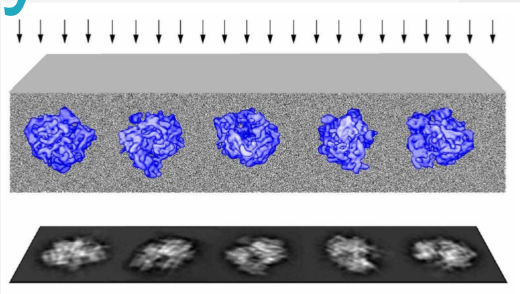


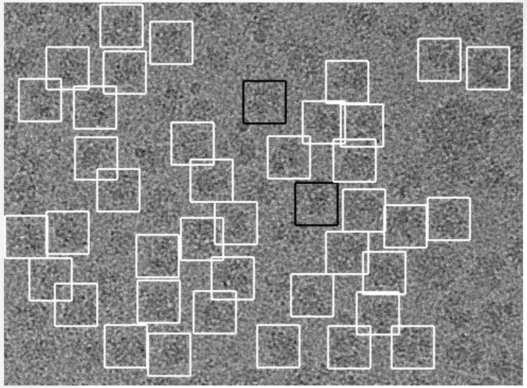




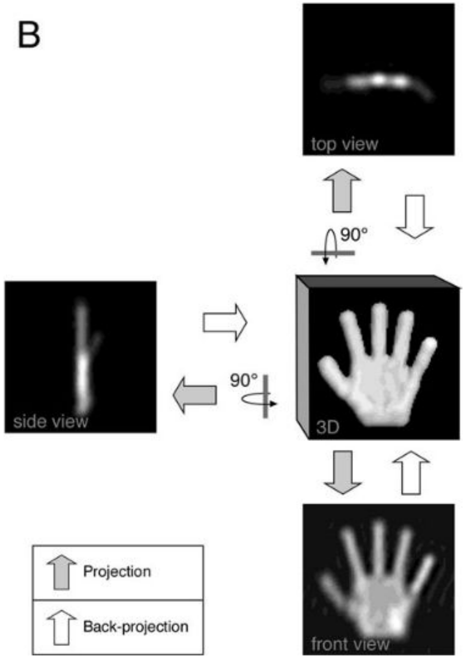
The 2D Fourier transform of the projection of a 3D density is a central section of the 3D Fourier transform of the density, perpendicular to the direction of projection.

cryo-TEM imaging





- 2D projections of an 3D object (handedness)
- high noise level (low sensitivity)
- convolution with microscope point spread functions



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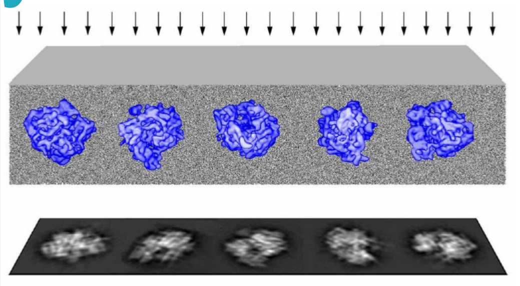
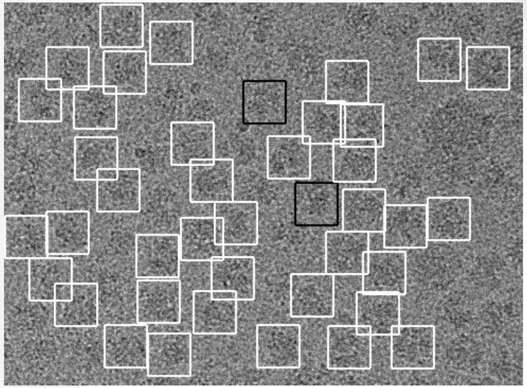
cryo-TEM imaging





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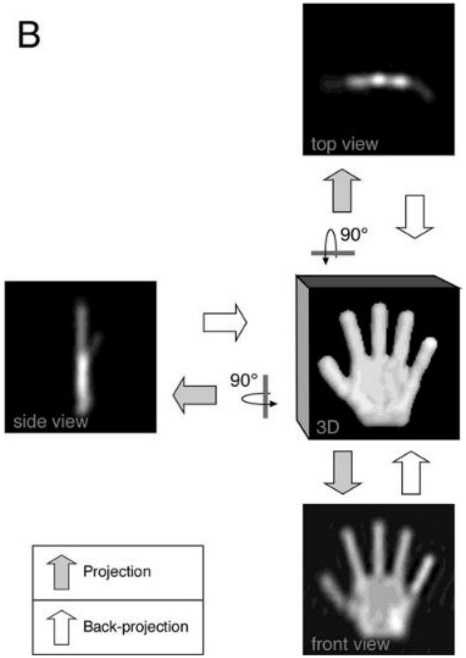

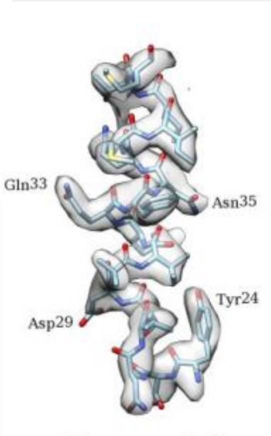

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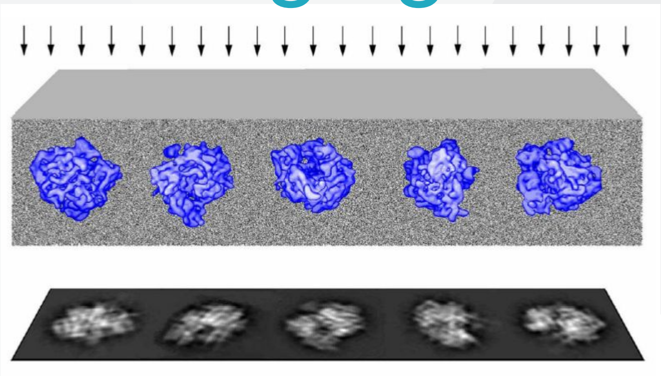
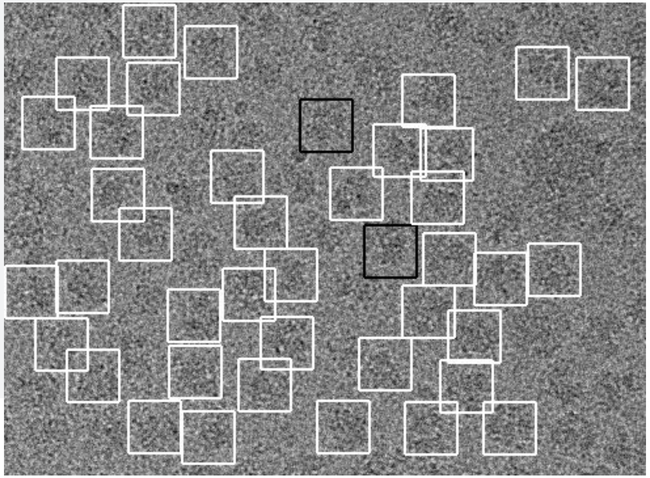
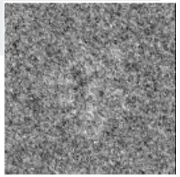
Averaging

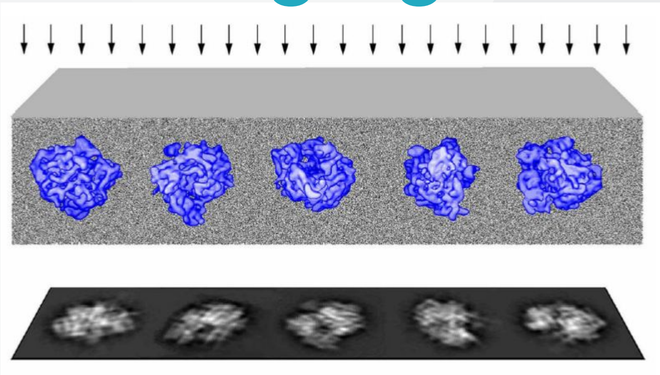


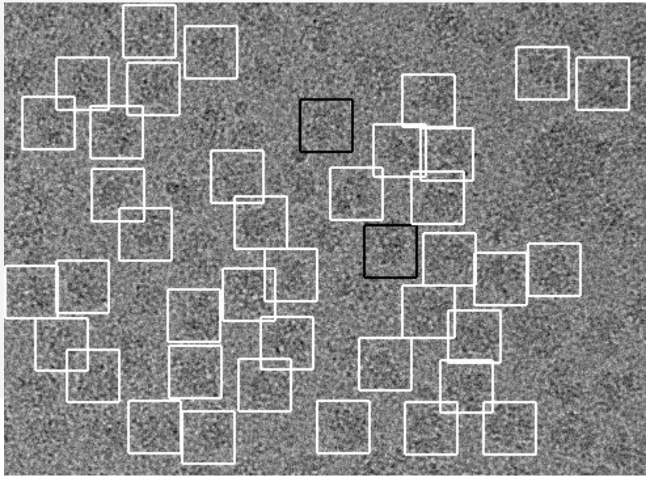


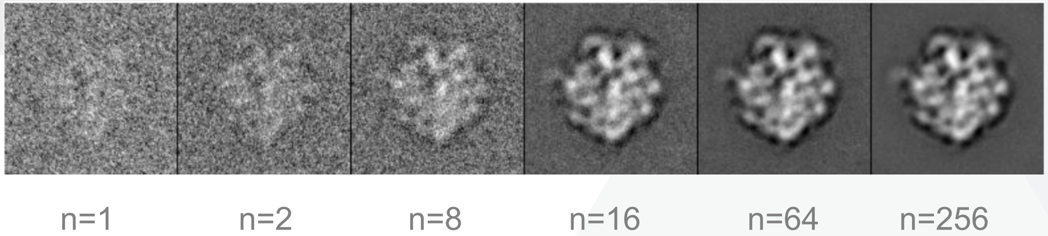


n=1

Averaging

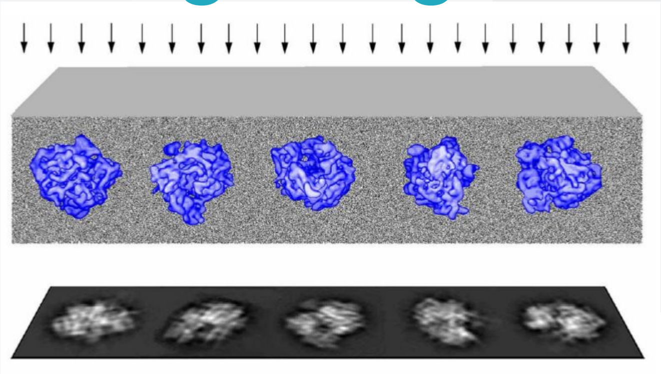


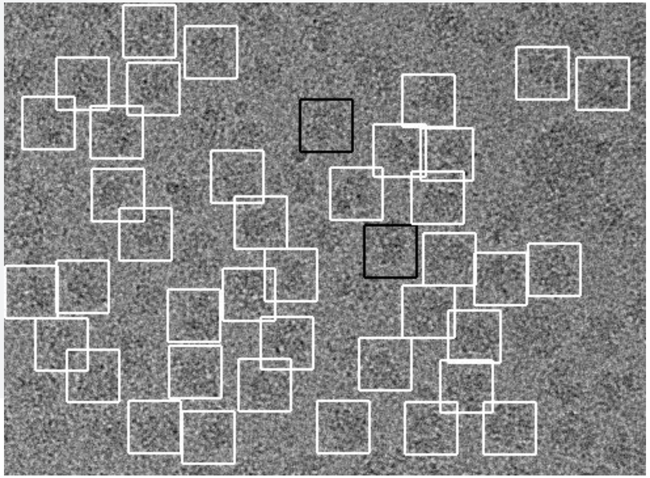




Signal to noise ratio increases with square-root of *n*

Image alignment in 2D





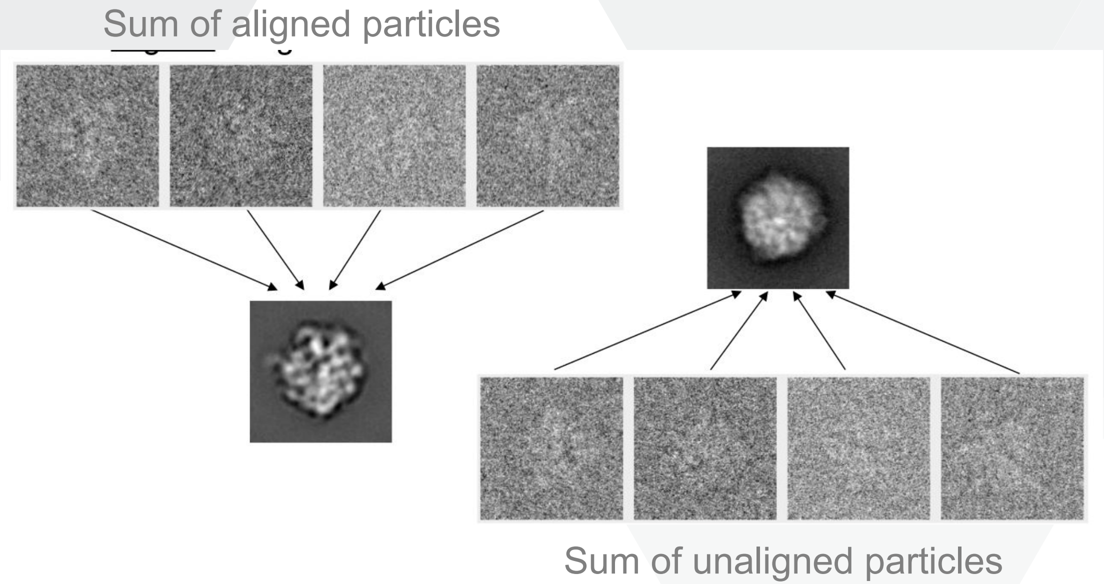
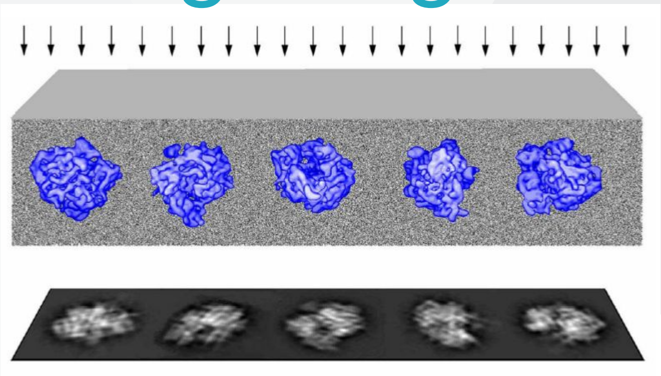
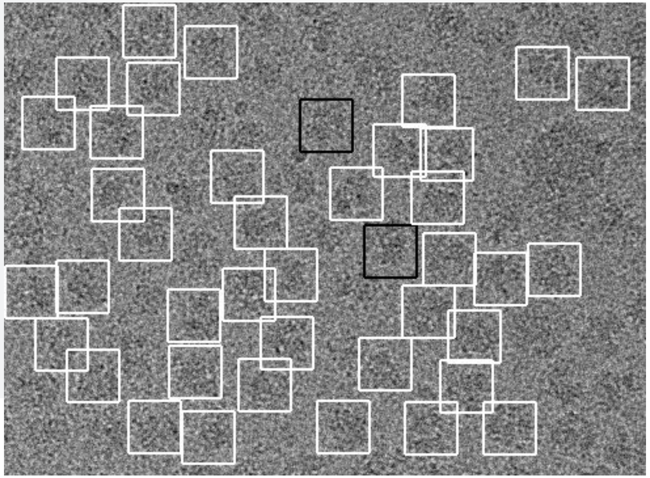
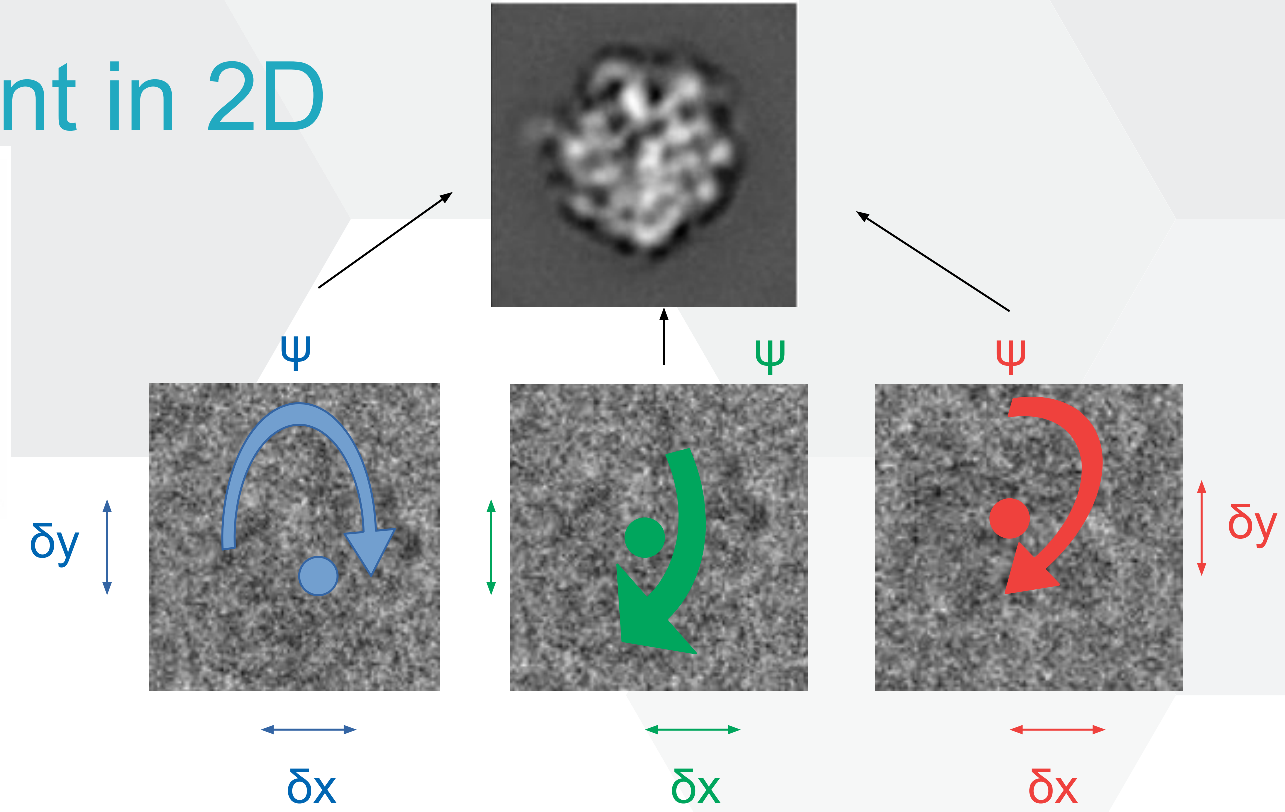


Image alignment in 2D



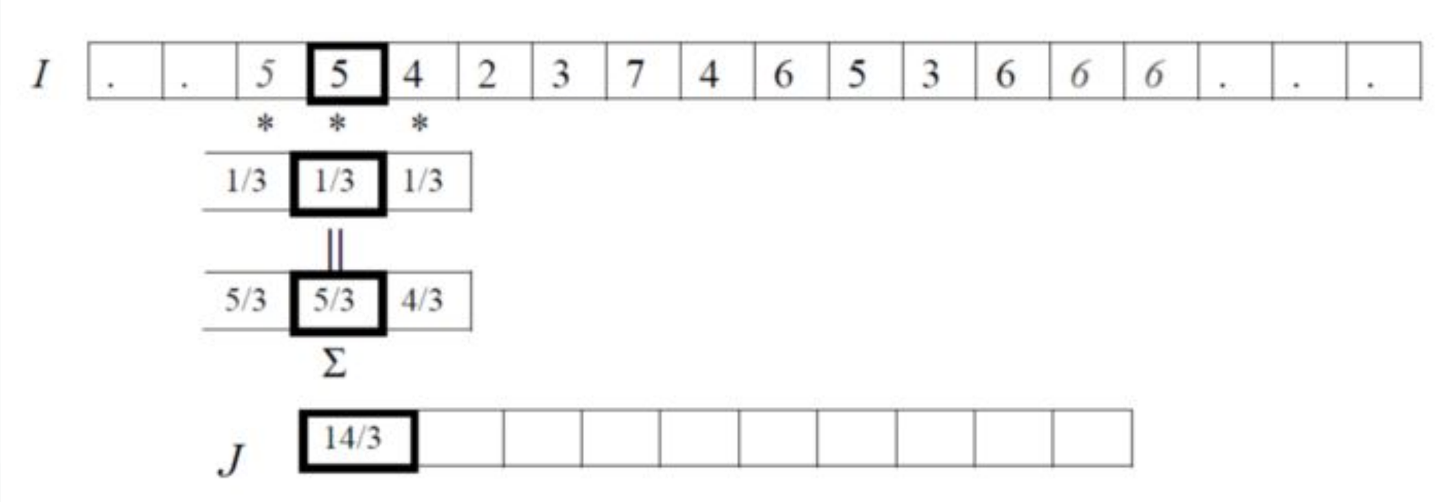




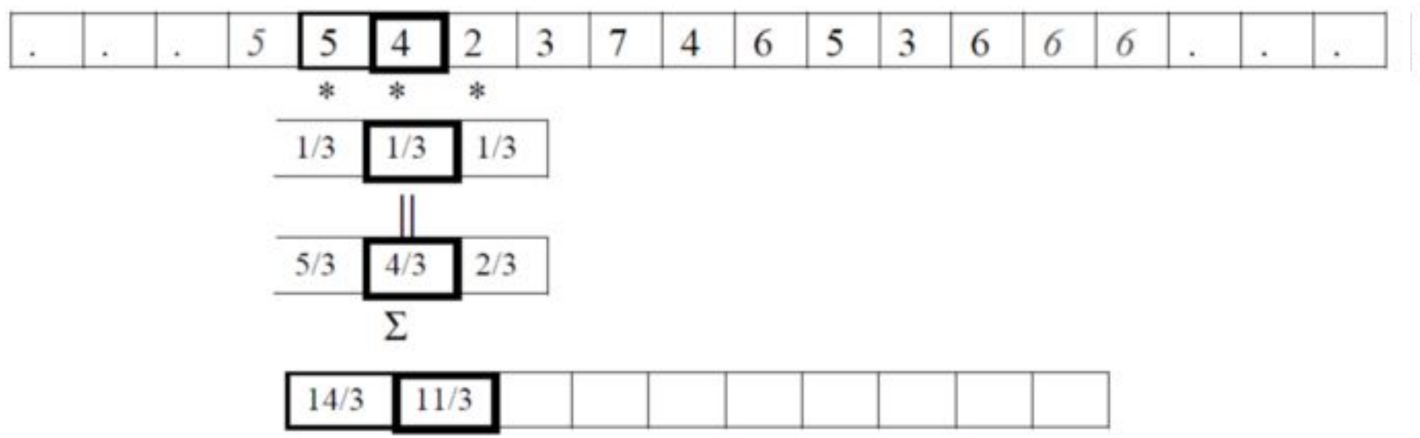
In order to align the particles in 2D, we need to determine three parameters:

- two translational
- one rotational (on of the Euler angles)

Cross correlation function in 1D



Cross correlation function in 1D



$$F \circ I(x) = \sum_{i=-N}^{N} F(i) I(x+i)$$

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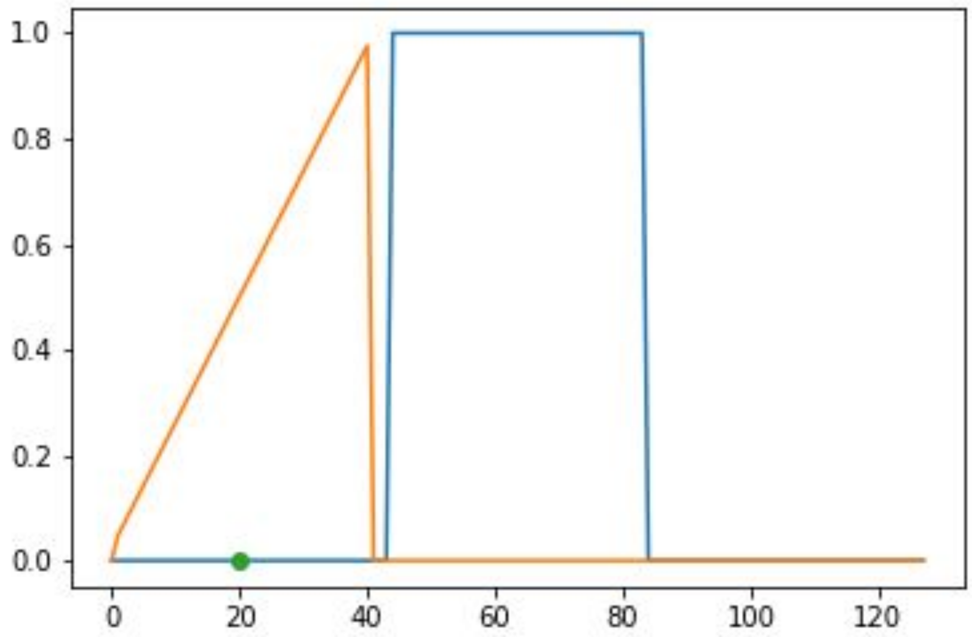
Cross correlation

- measure of similarity of two data series as a function of displacement of these functions
- in 2D optimal overlay of two images
- normalized cross-correlation – ccc = <-1,1>



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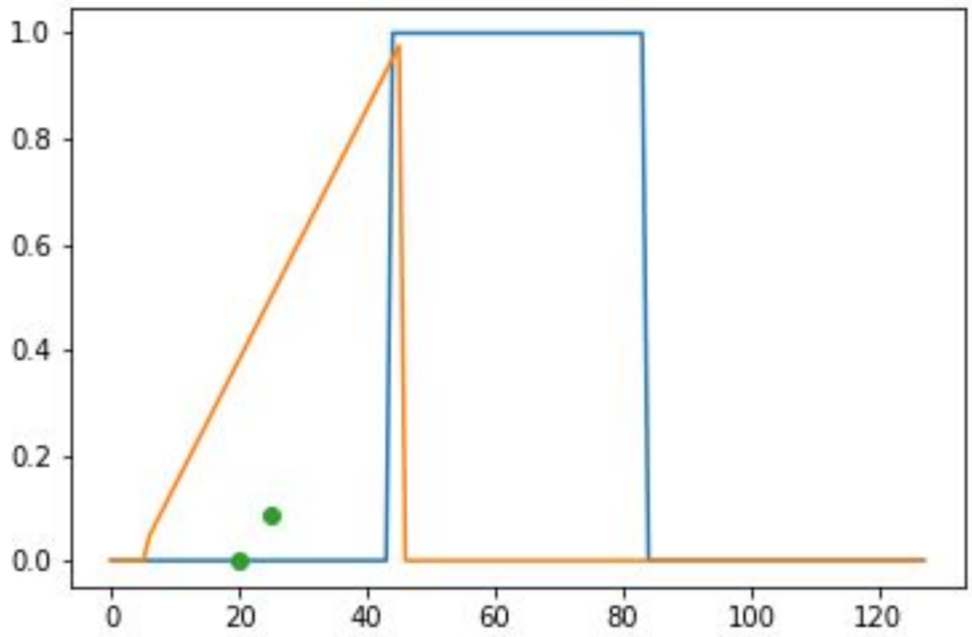
Cross correlation function in 1D



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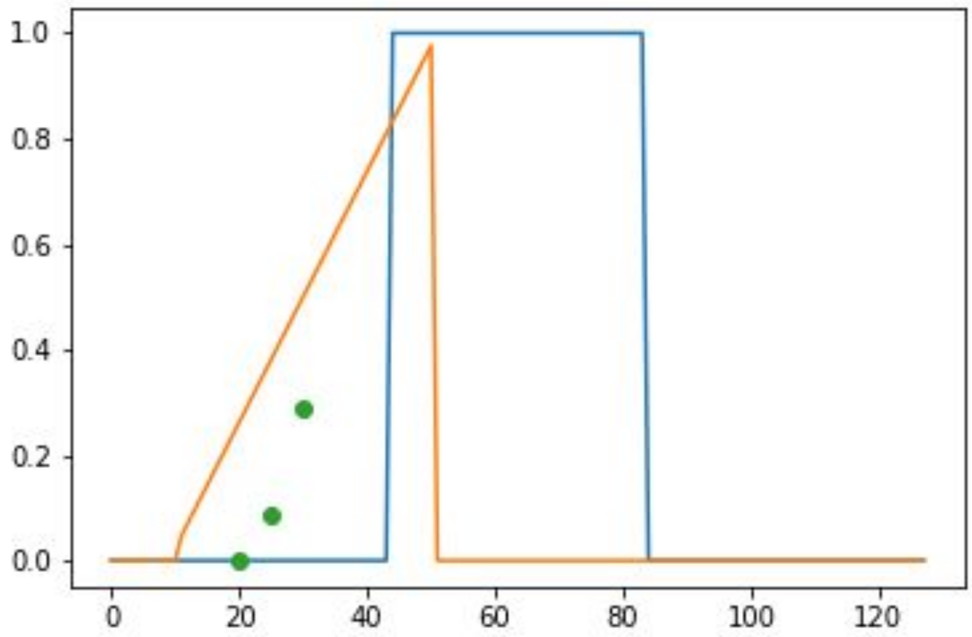
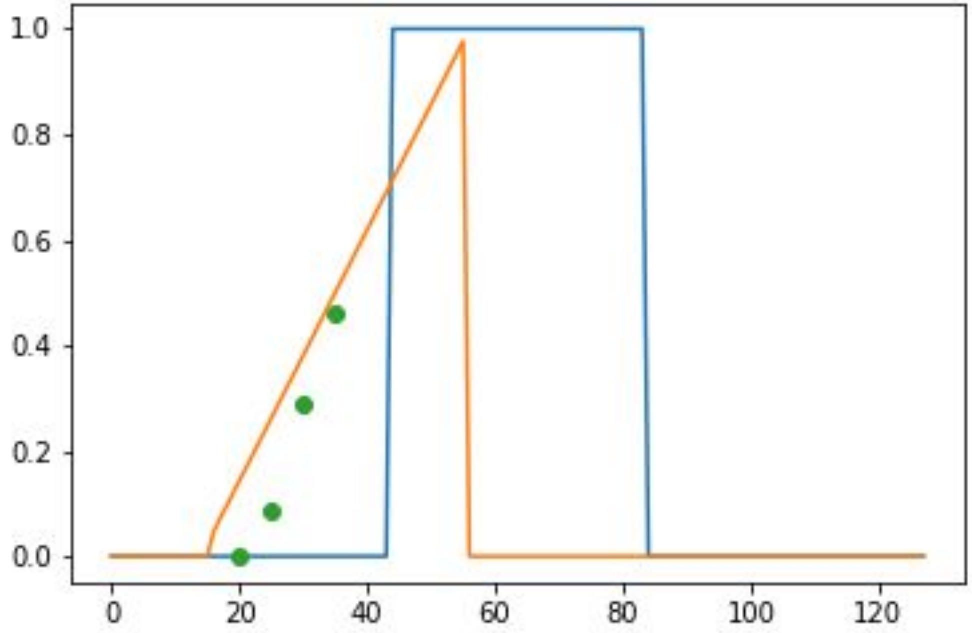
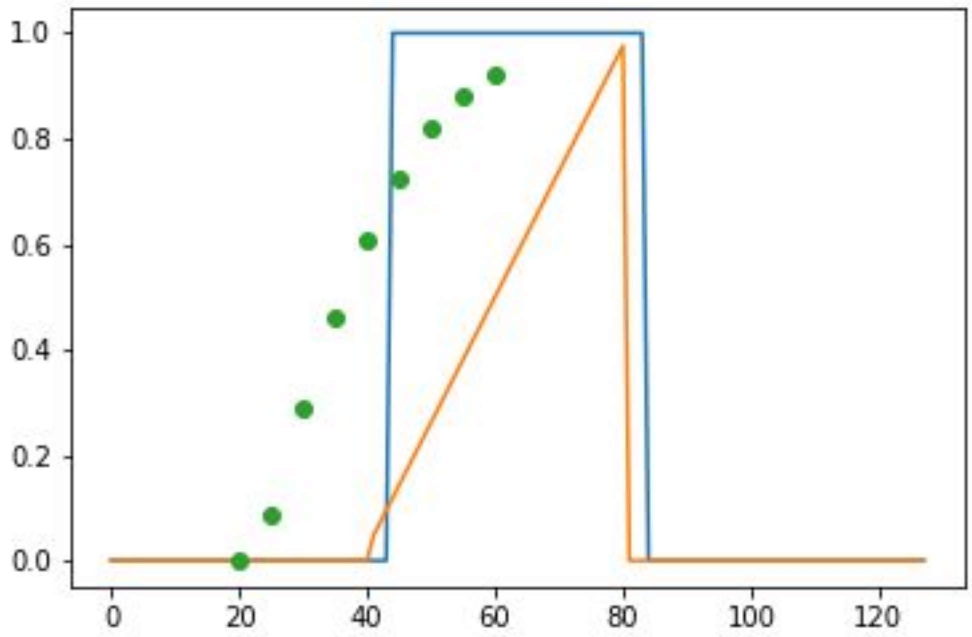
Cross correlation function in 1D



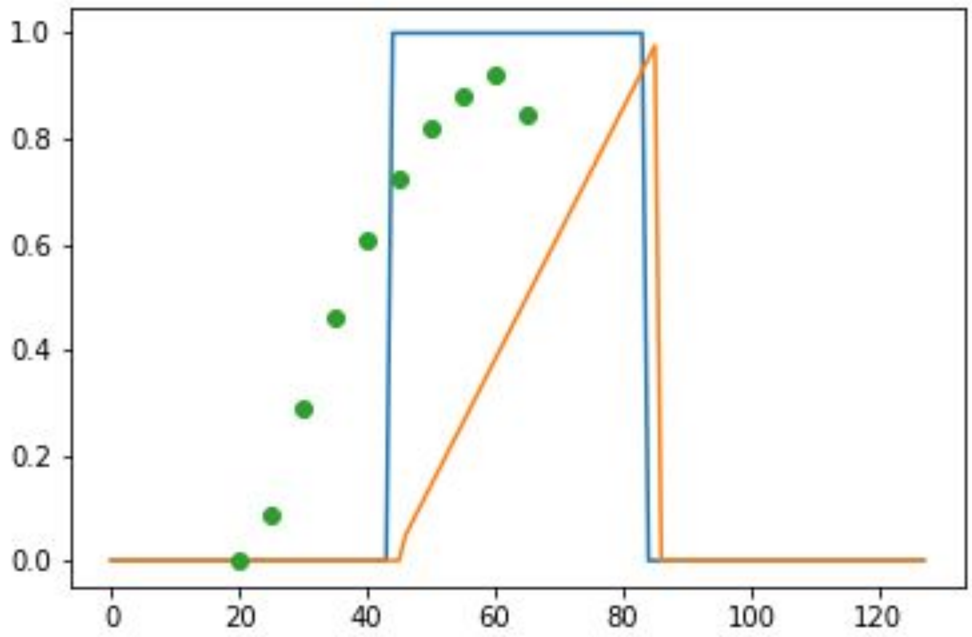
Cross correlation function in 1D



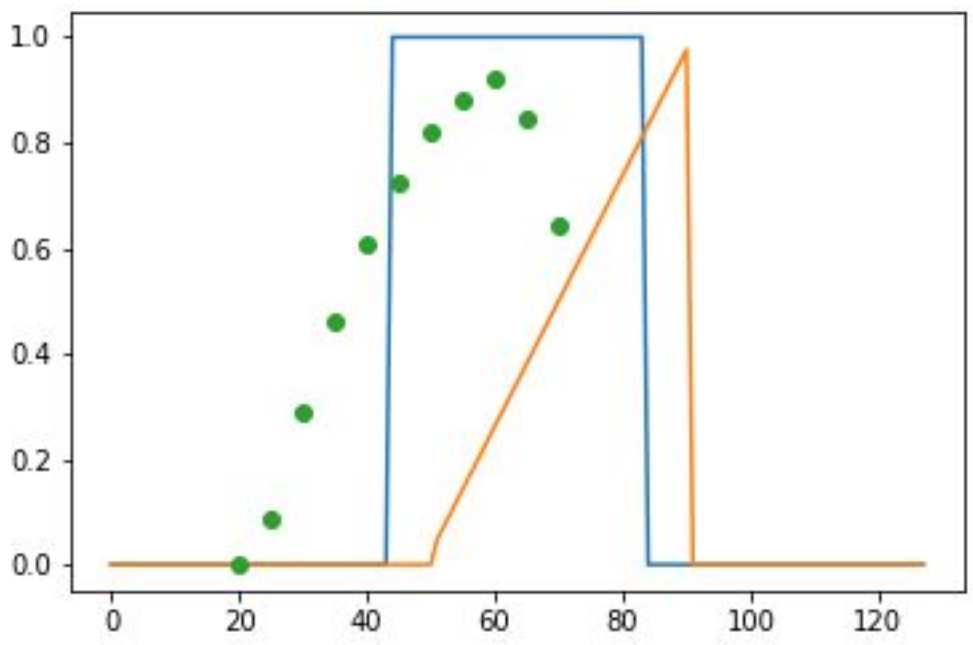
Cross correlation function in 1D



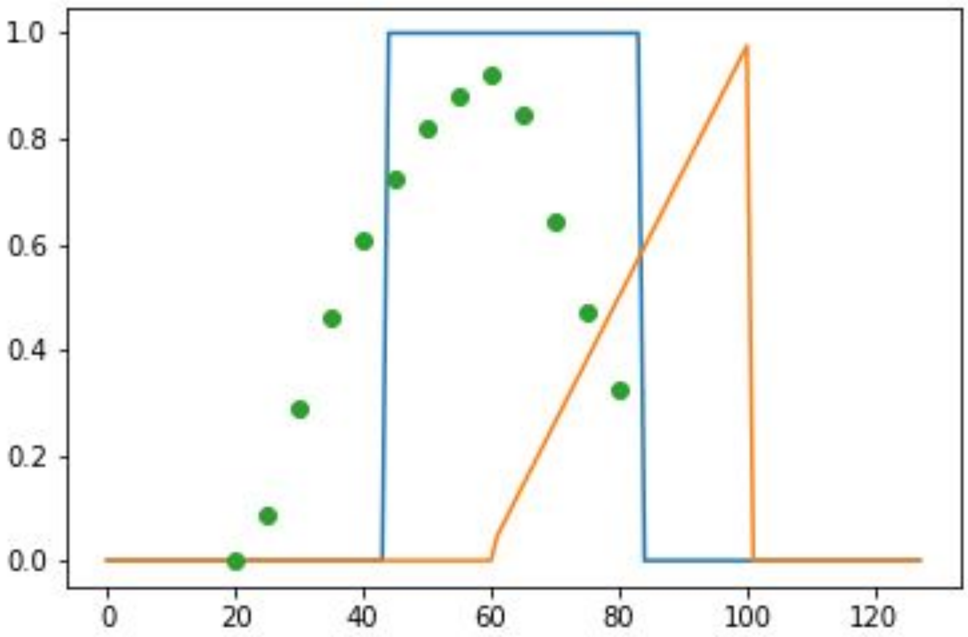
Cross correlation function in 1D



Cross correlation function in 1D



Cross correlation function in 1D



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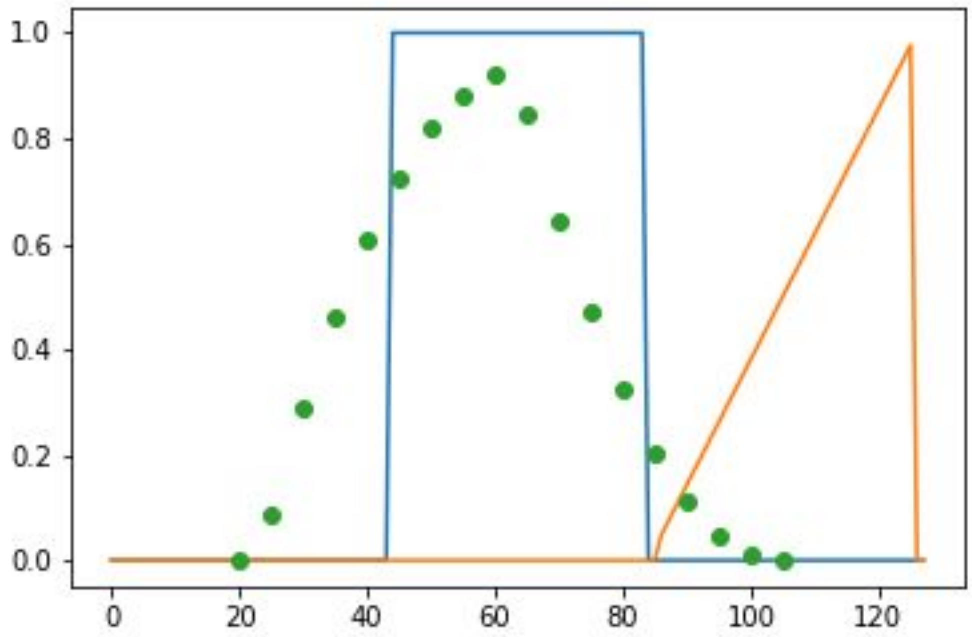
Cross correlation function in 1D



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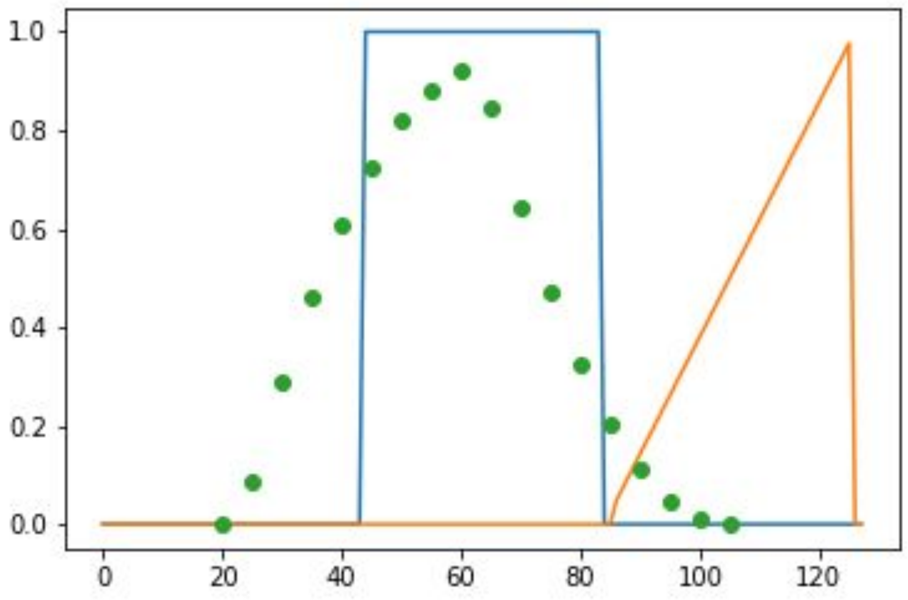
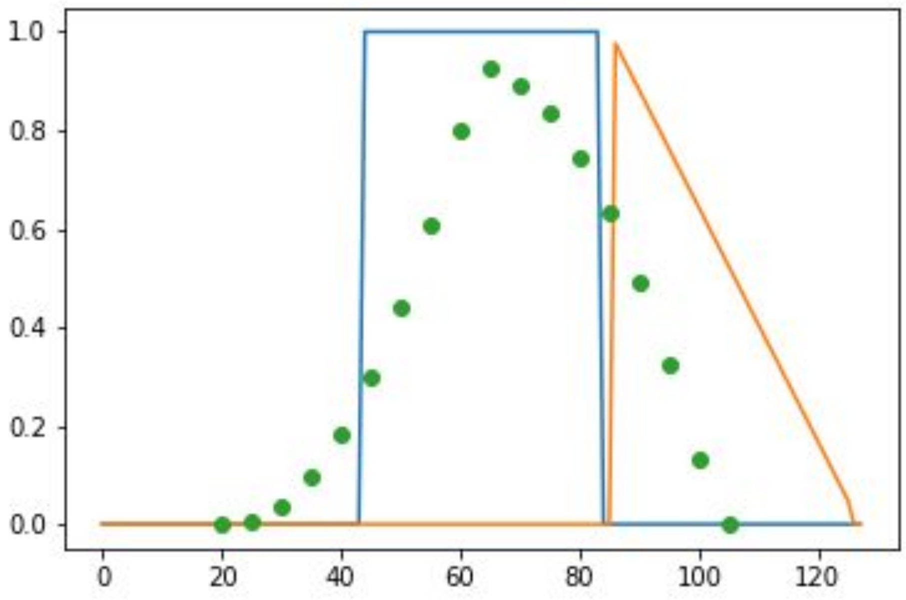
Cross correlation function in 1D

Cross-correlation



Convolution



Cross correlation function in 2D

Image alignment in 2D

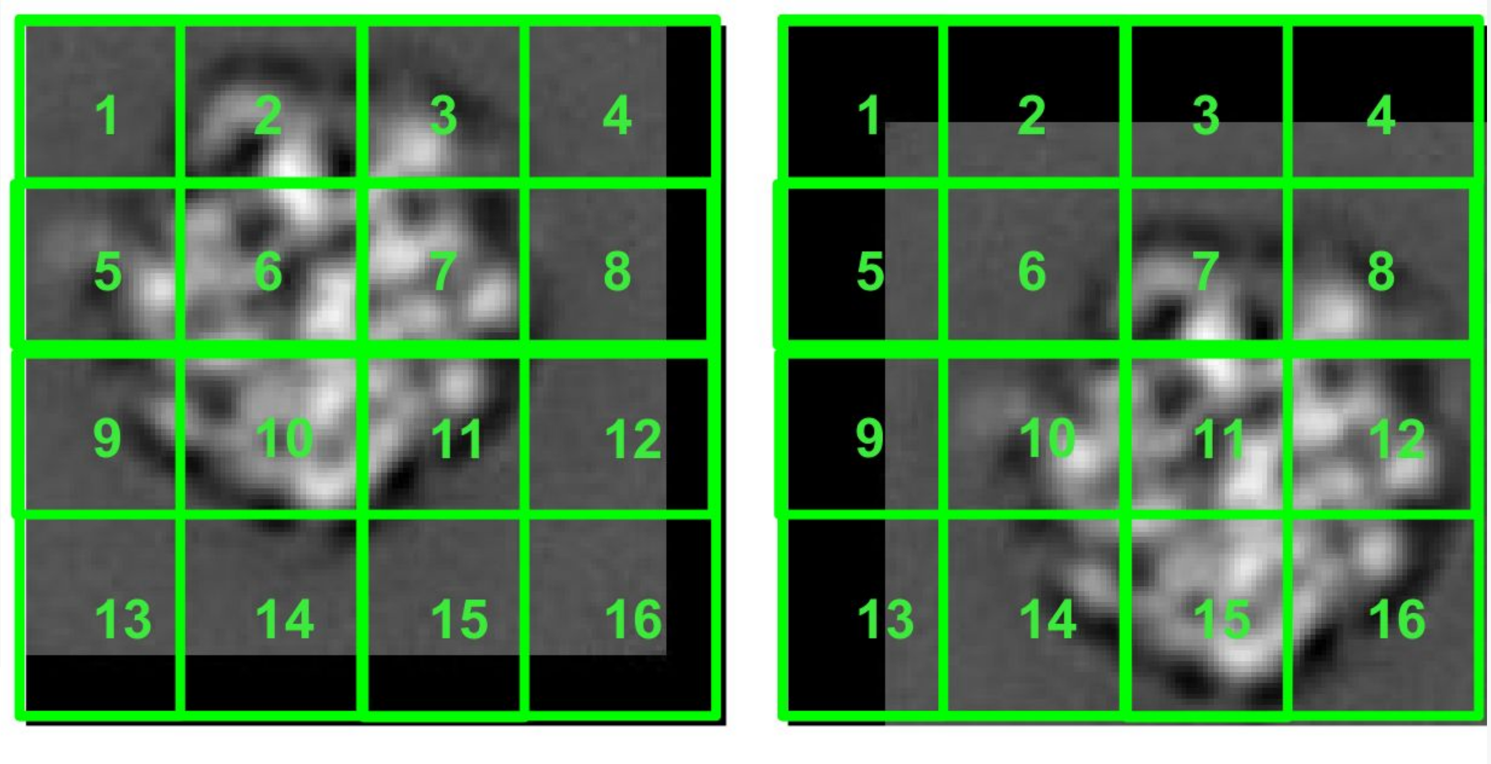
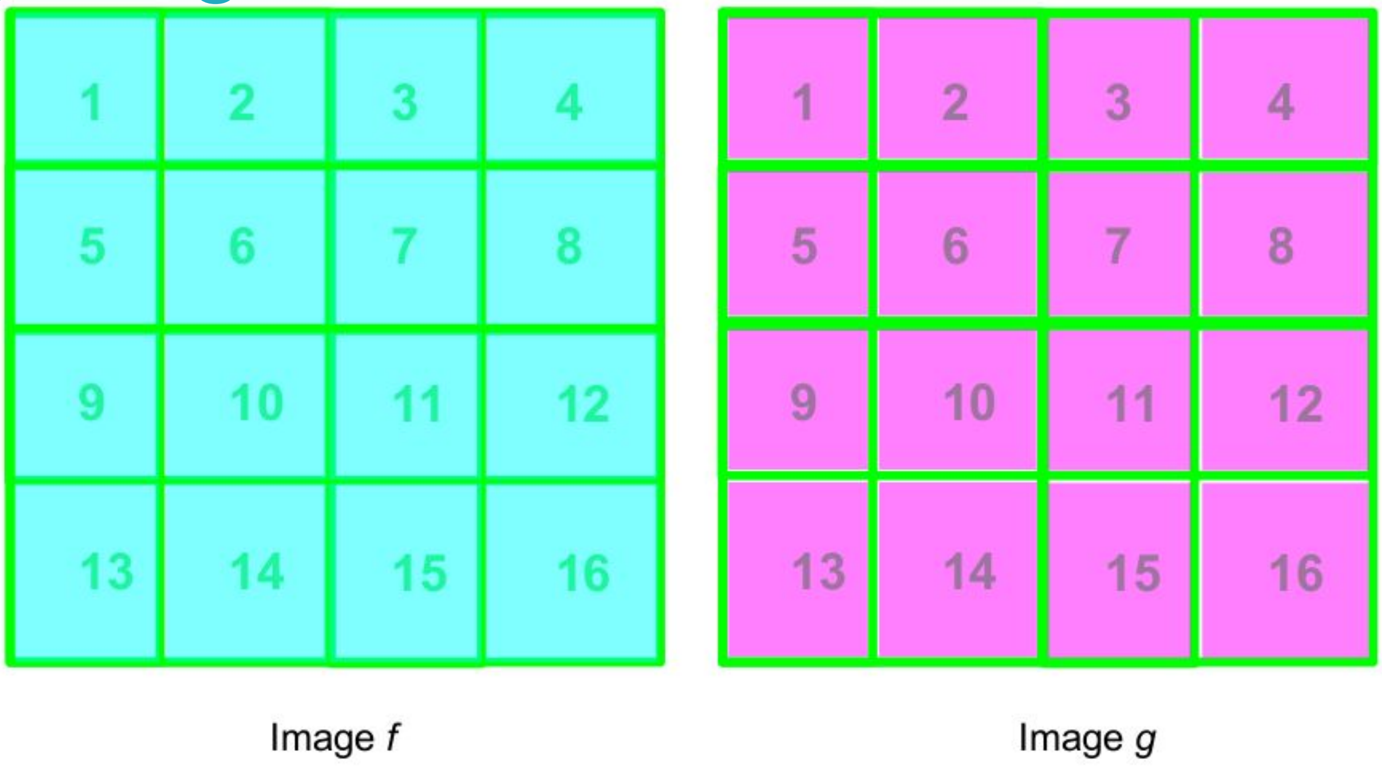


Image alignment in 2D



$$\text{Unnormalized CCC} = f_1g_1 + f_2g_2 + f_3g_3 + f_4g_4 + f_5g_5 + f_6g_6 + f_7g_7 + f_8g_8 + f_9g_9 + f_{10}g_{10} + f_{11}g_{11} + f_{12}g_{12} + f_{13}g_{13} + f_{14}g_{14} + f_{15}g_{15} + f_{16}g_{16}$$

Image alignment in 2D

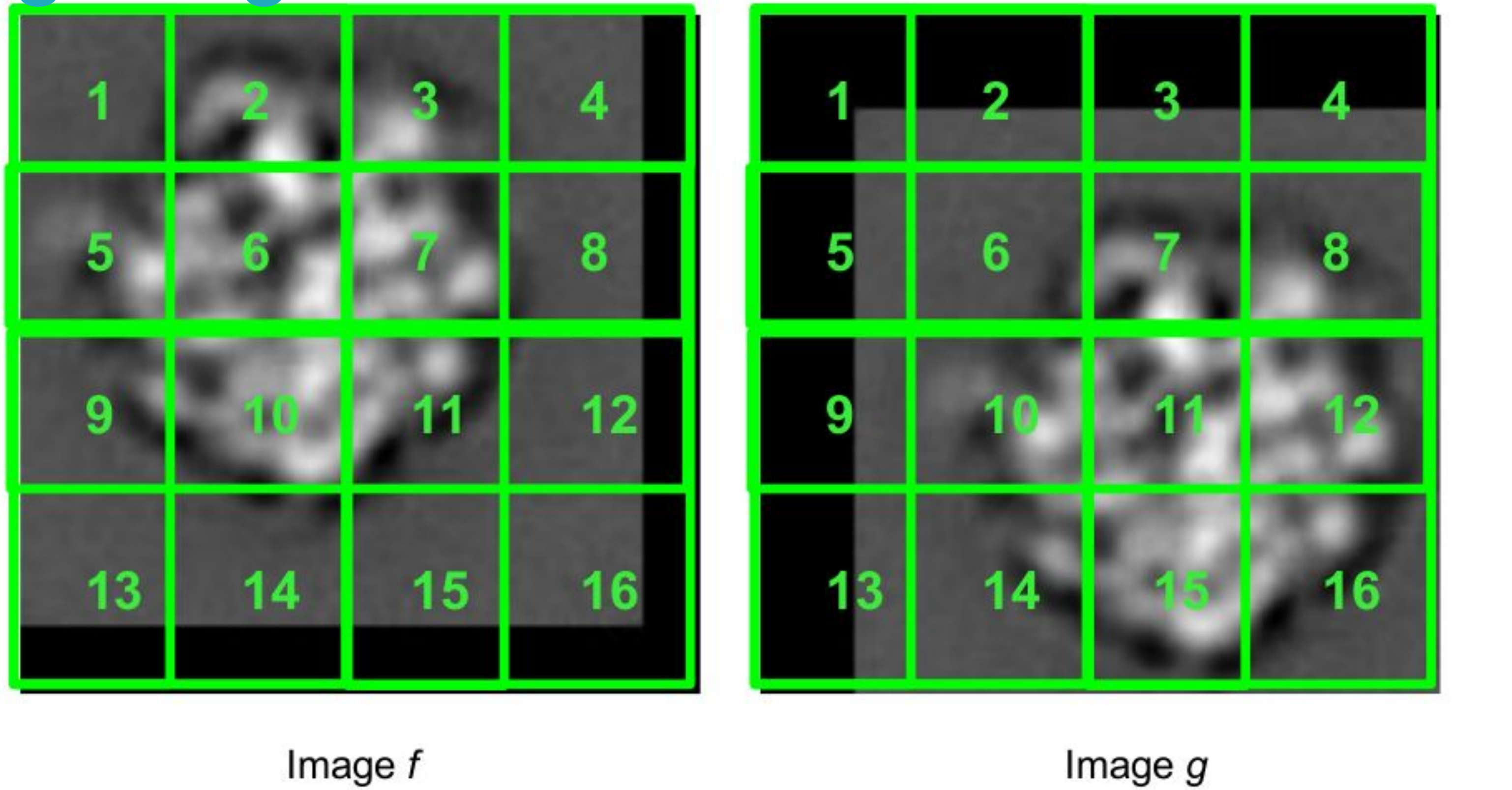


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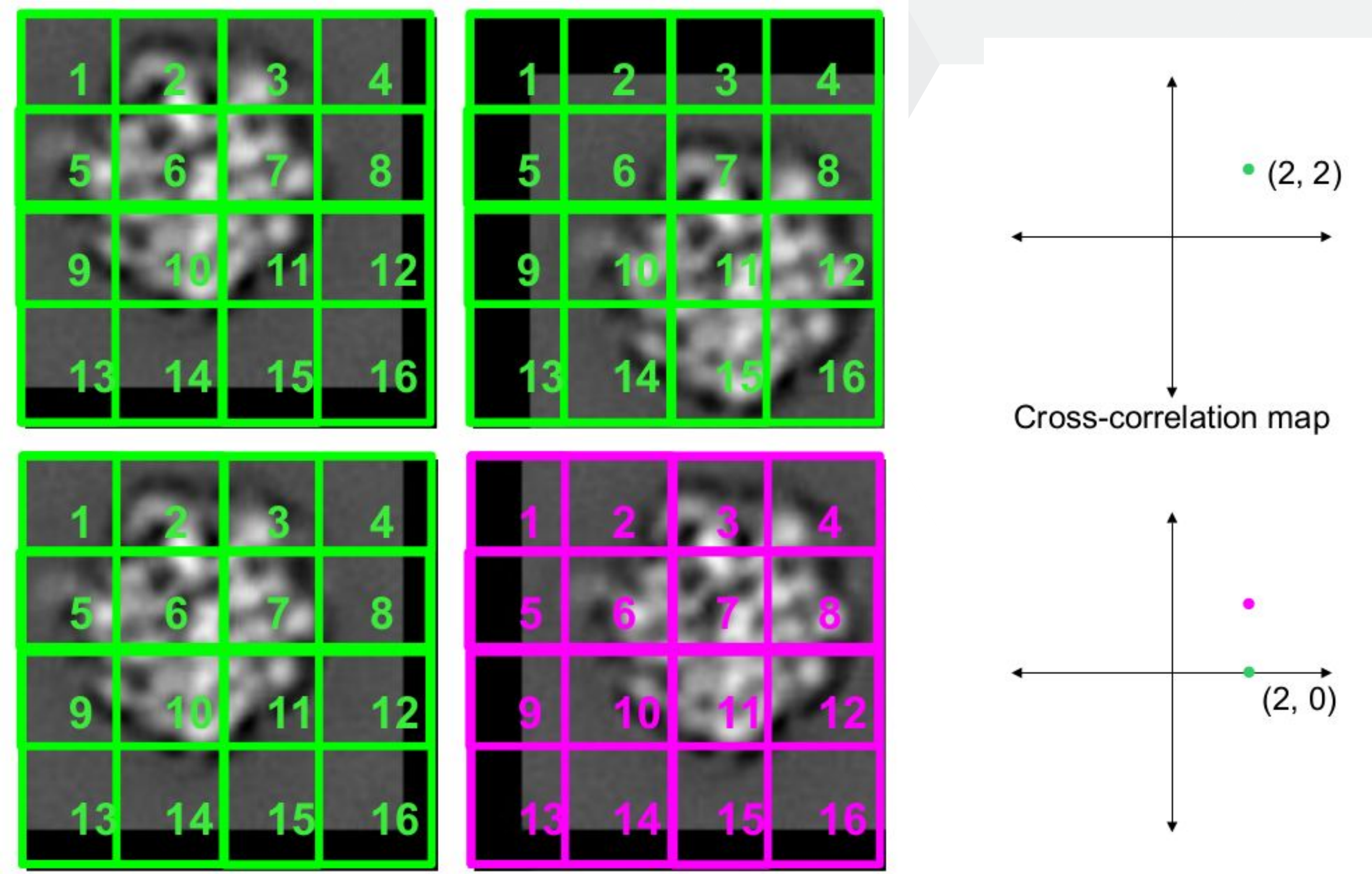
Image alignment in 2D



Cross-correlation in 2D

Convolution

FT(F I) = FT(F) . FT(I)

FT(F I) = FT(F)* . FT(I)

Convolution theorem

Cross-correlation in 2D

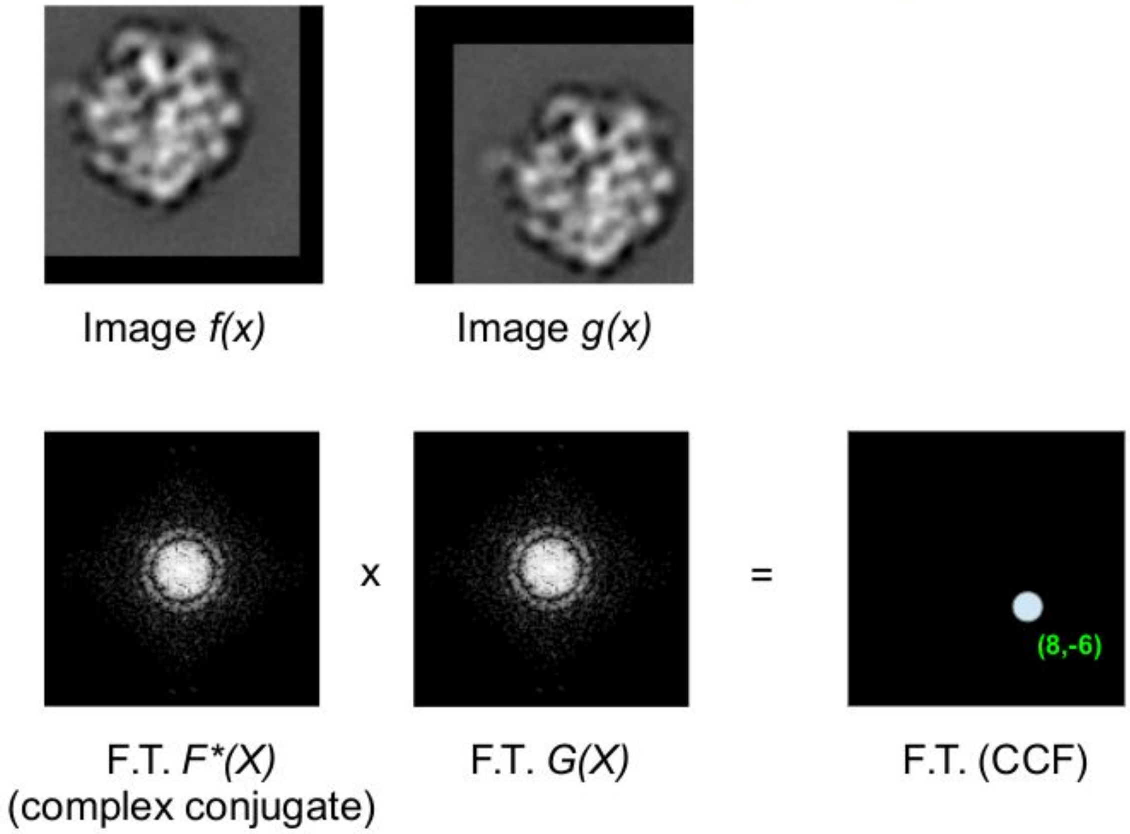


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Image alignment in 2D

Image rotation

- the images contain not only shift but also rotation
- cross-correlation - image sliding over the template (shift)
- (log)-polar transform → image transformation from cartesian to polar coordinates → rotational problem shifted to translational problem → utilization of similar approaches as for image shift determination

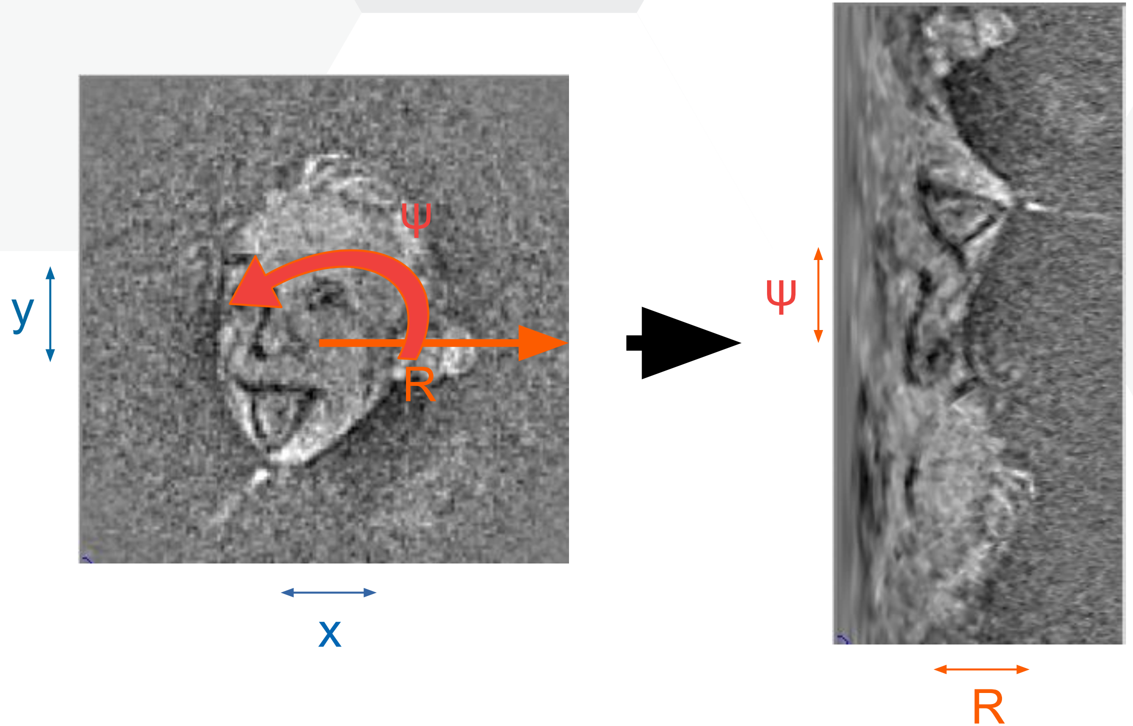

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Image alignment in 2D

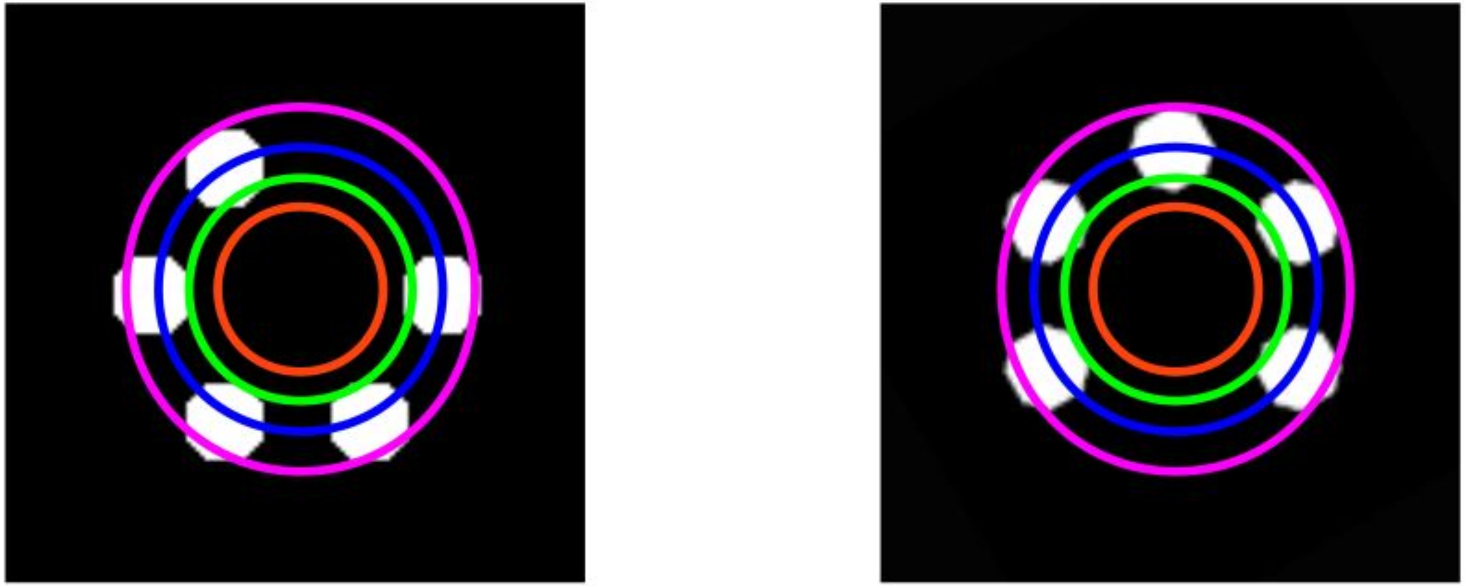

Image 1

Image 2

We take a series of rings from each image, unravel them, and compute a series of 1D cross-correlation functions.

Shifts along these unraveled CCFs is equivalent to a rotation in Cartesian space.

Image alignment in 2D

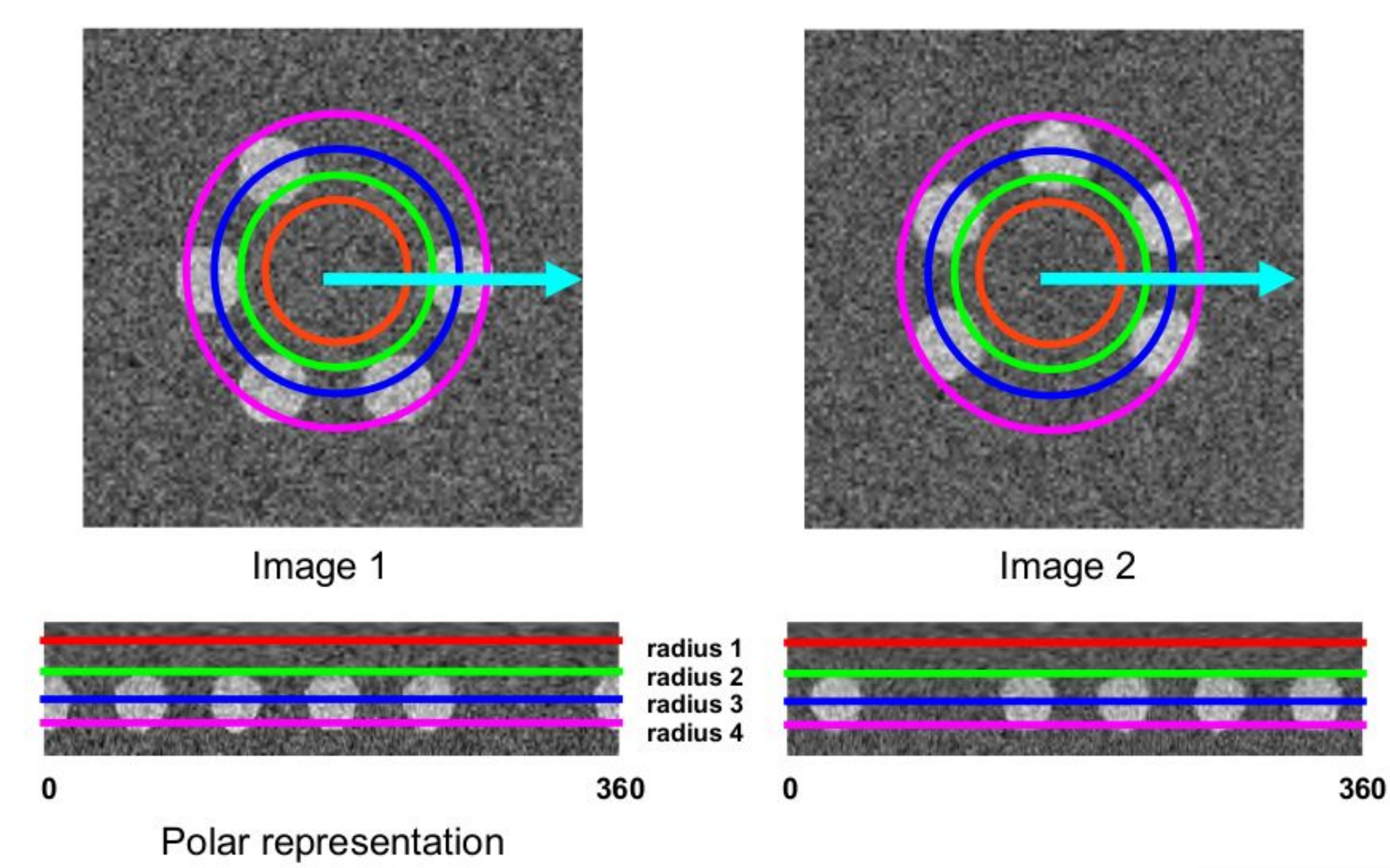

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Image alignment in 2D

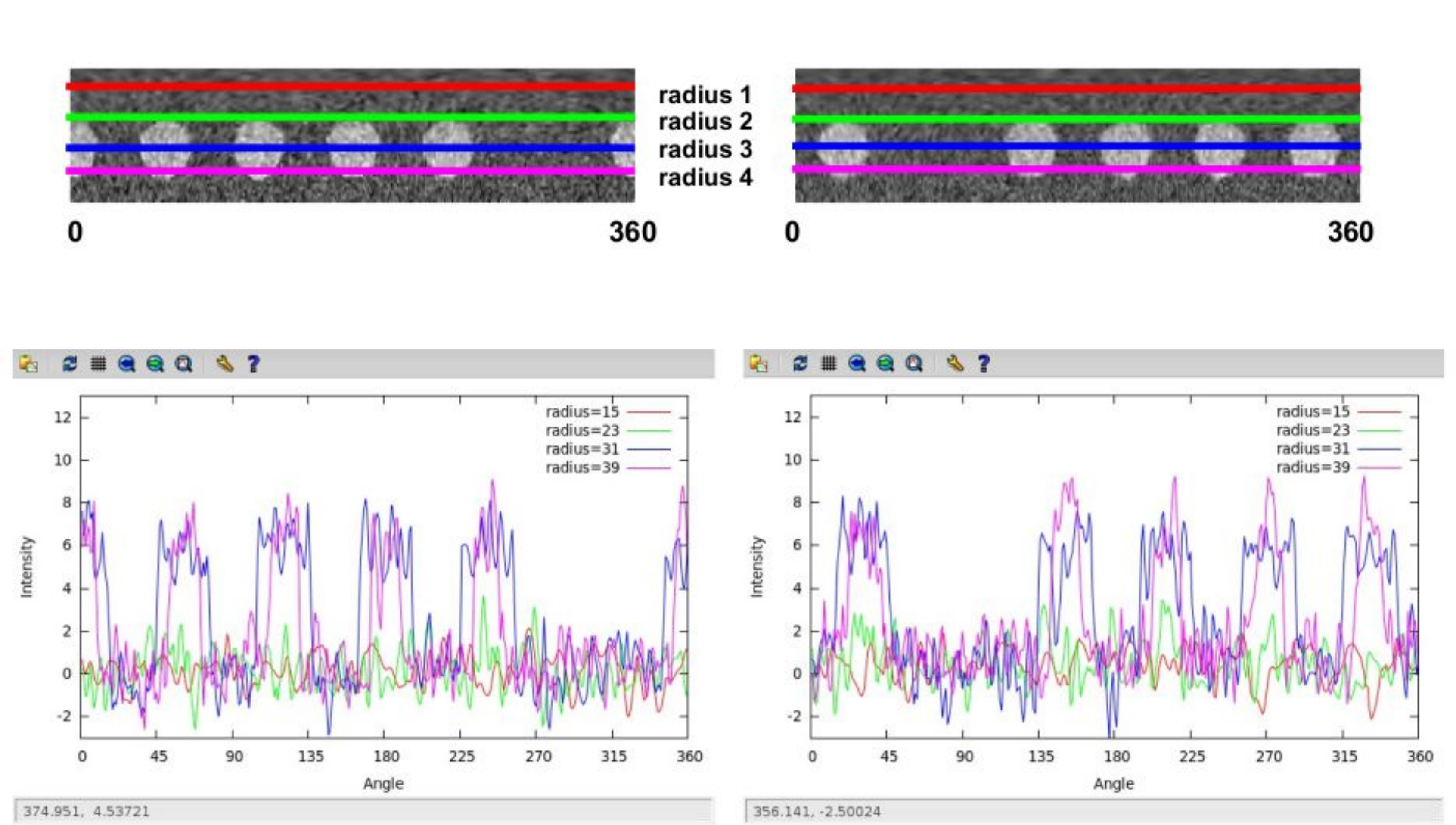


Image alignment in 2D

- after rotation

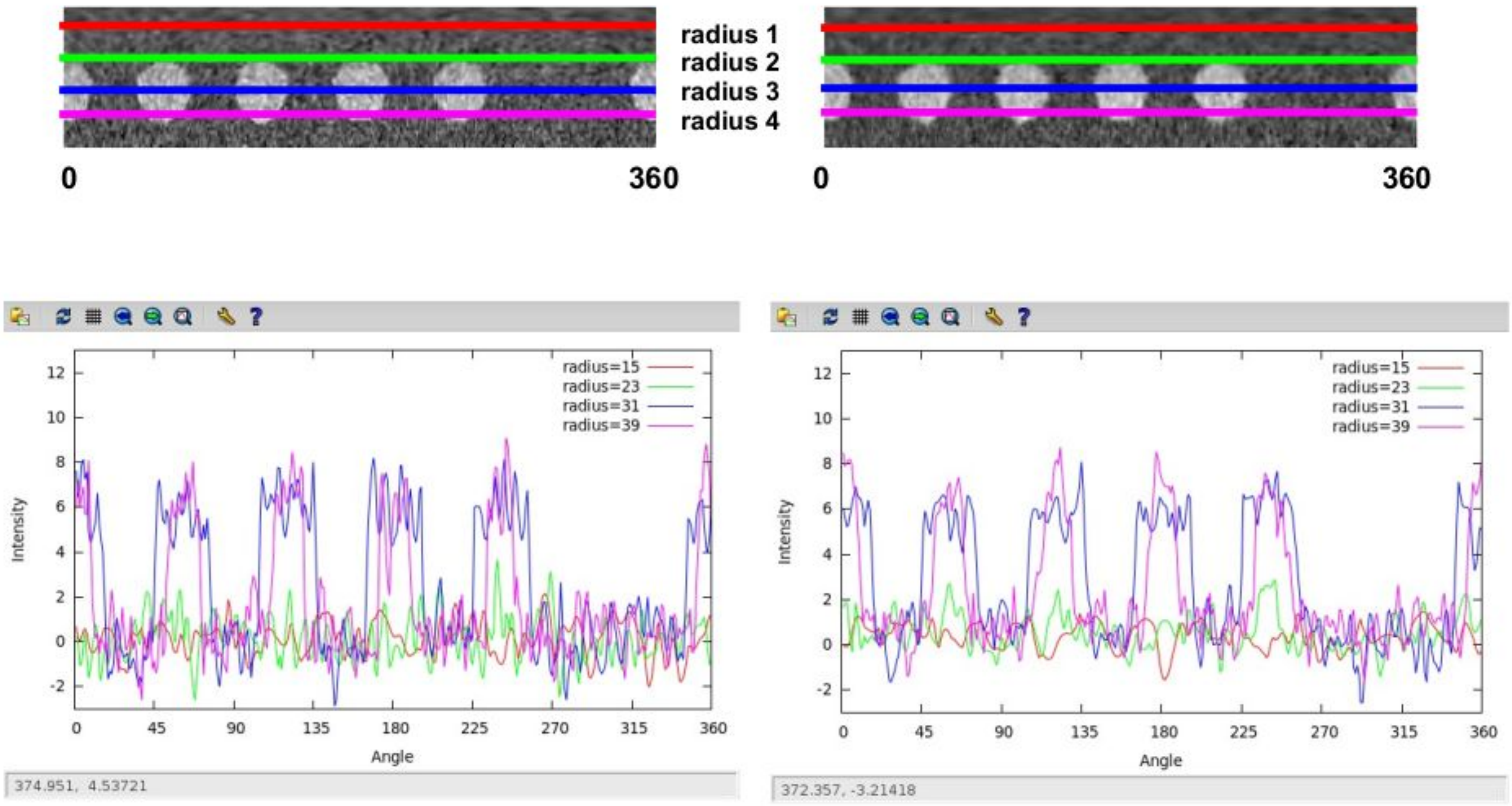
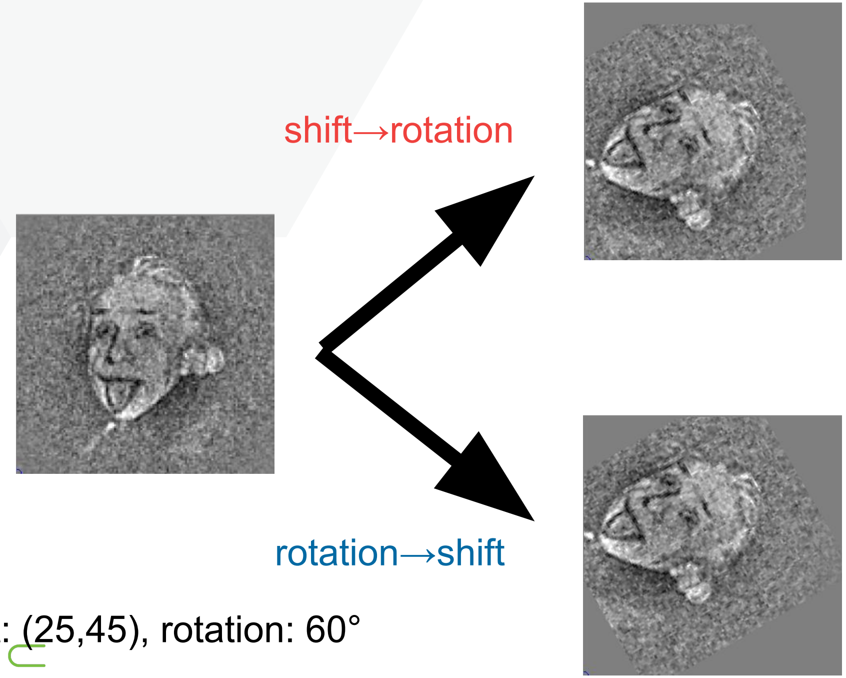


Image alignment in 2D

- rotation and translation are interdependent – (rot→trans) ≠ (trans→rot)
  => order of the operation matters



shift: (25,45), rotation: 60°

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Image alignment in 2D

- rotation and translation are interdependent – (rot→trans) ≠ (trans→rot)

- define reasonable range of shifts (e.g. (-2;+2)) and perform rotational alignment for each shifted image

Example: for the shift of +/-2 pixels in *x* and *y* → 25 alignment rotational alignments → each alignment results in optimal rotational alignment and *ccc* → compare *ccc* and select maximal *ccc* to determine the final shift and translation

=> increased complexity

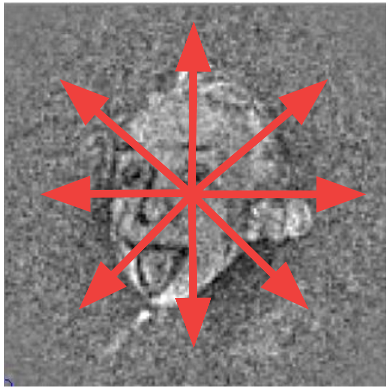
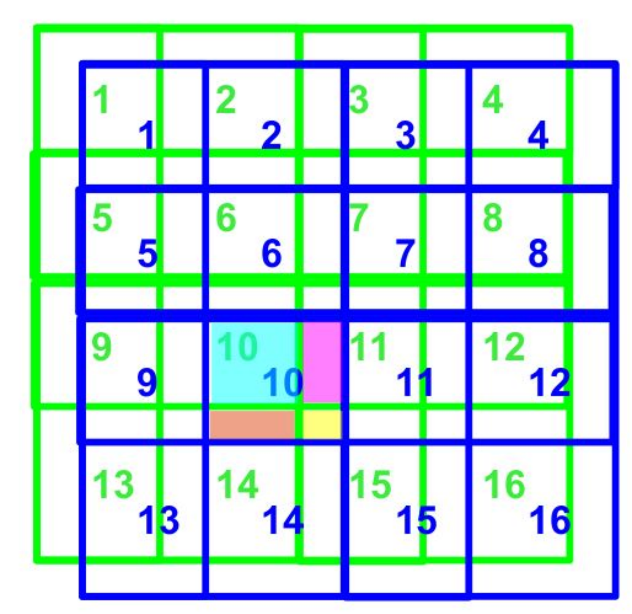


Image alignment in 2D

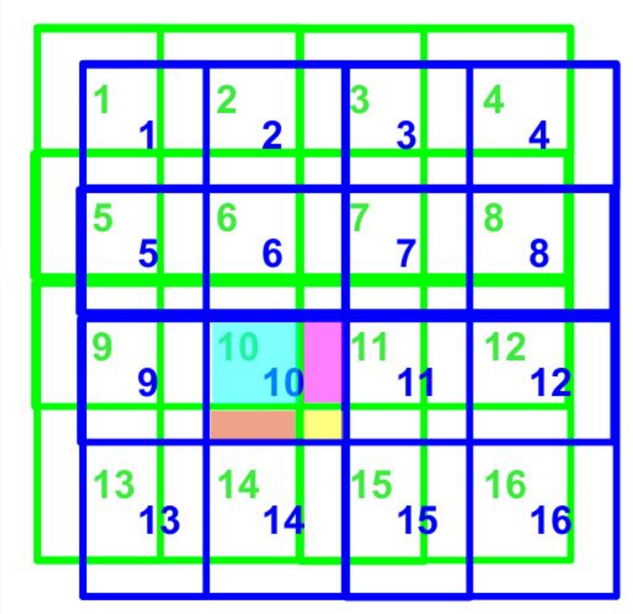
Interpolation



Suppose we shift the image in x & y.
The new pixels will be weighted averages of the old pixels.
The more the mix the pixels, the worse the result will be.

Image alignment in 2D

Interpolation



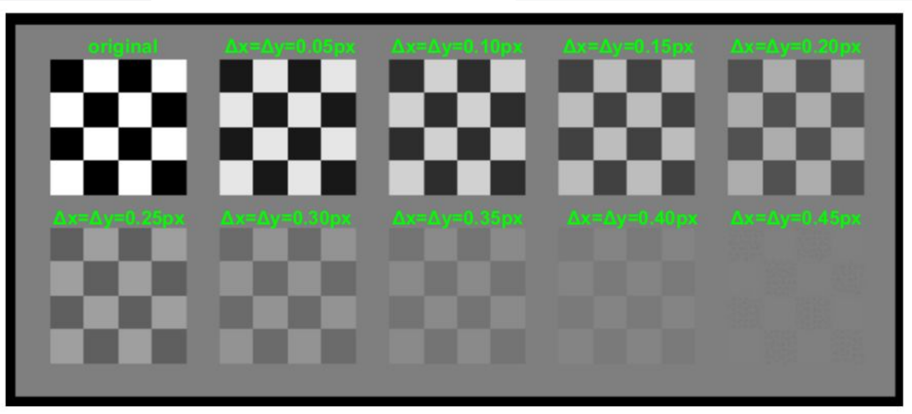


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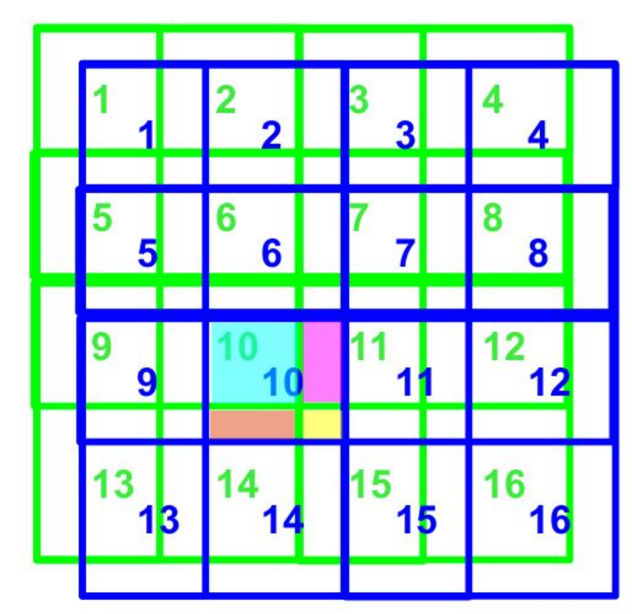
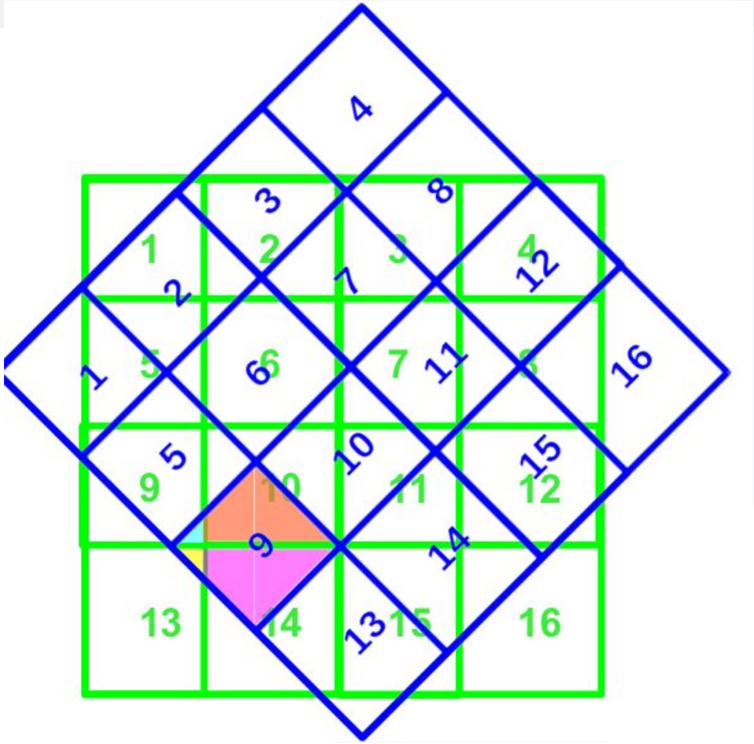
Image alignment in 2D

Shift



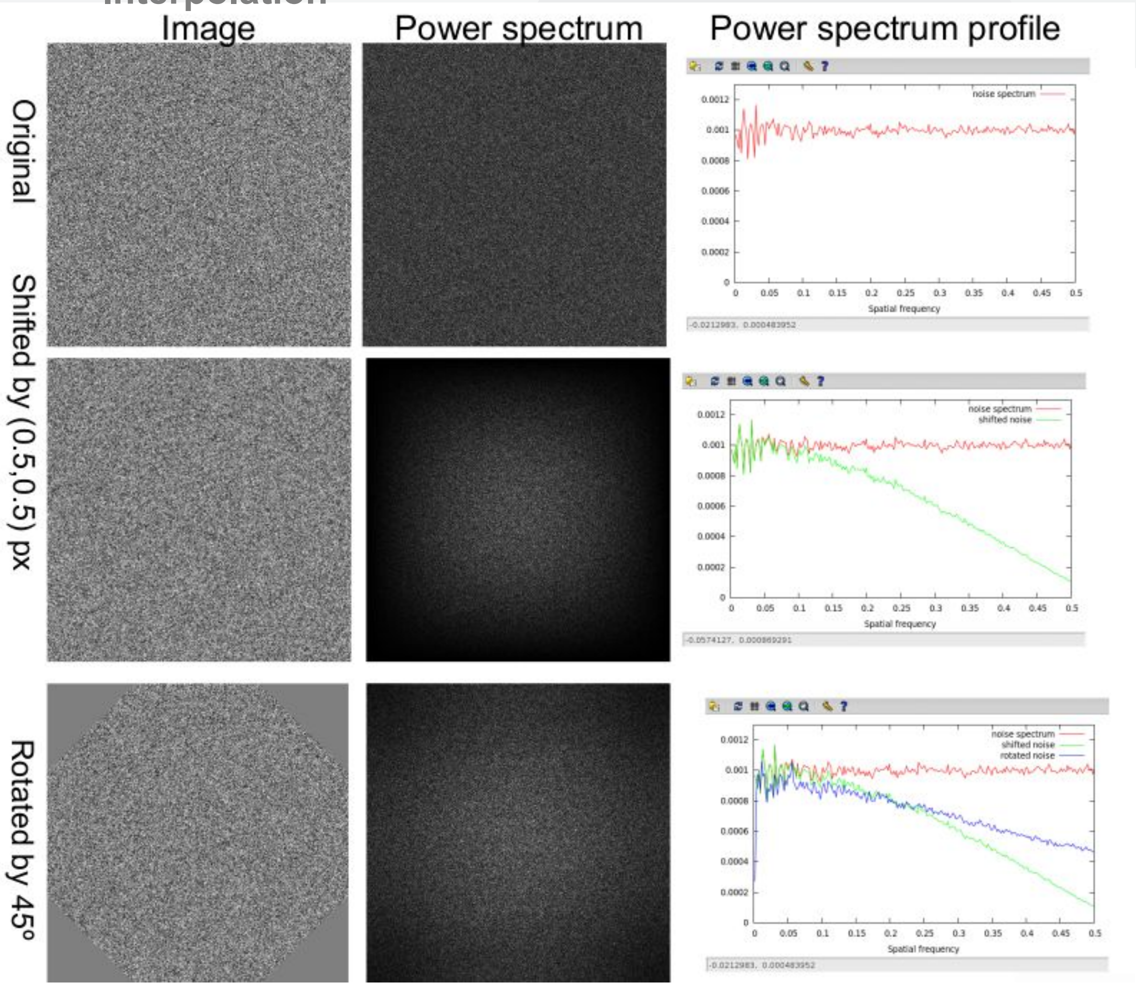
Rotation



Suppose we shift the image in x & y.
The new pixels will be weighted averages of the old pixels.
The more the mix the pixels, the worse the result will be.

Image alignment in 2D

Interpolation



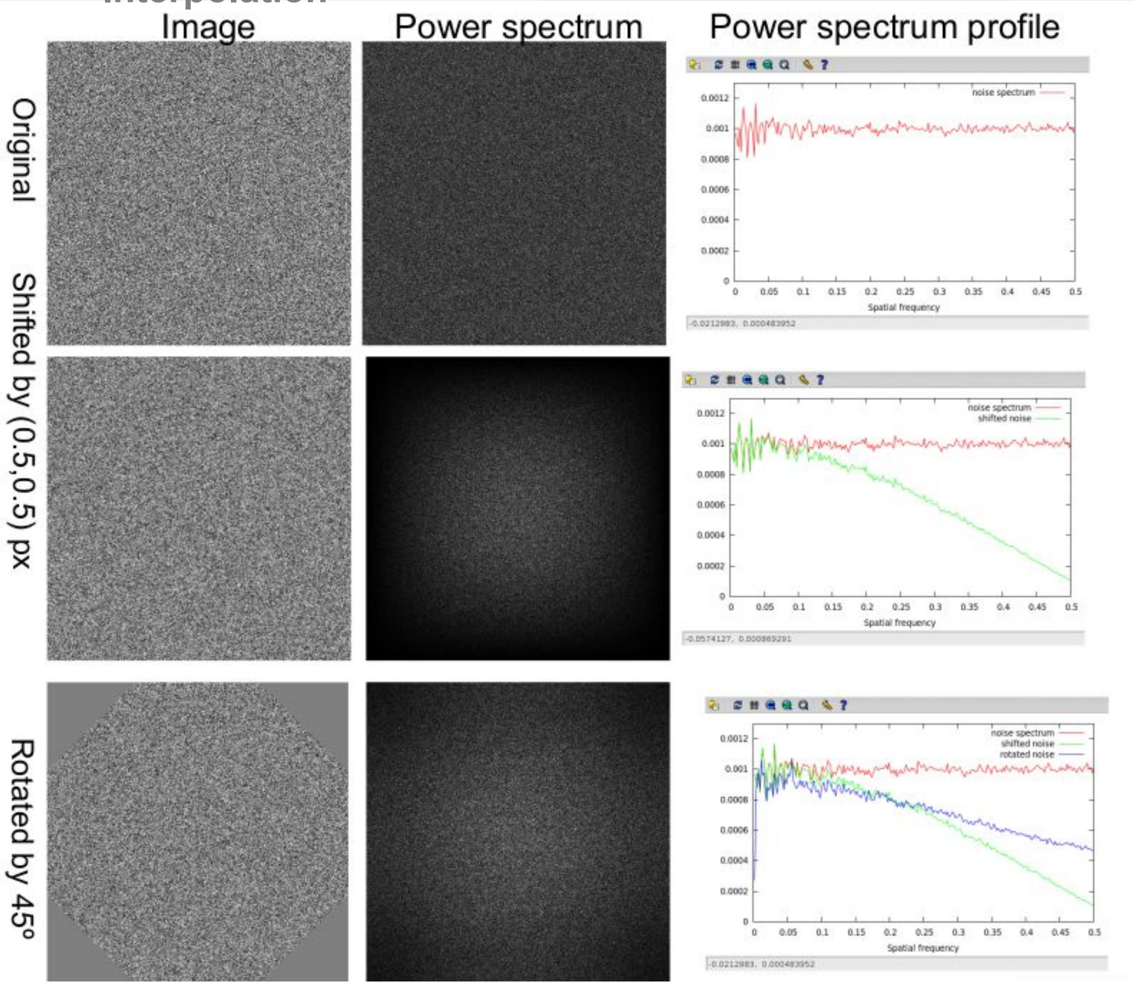
The Fourier transform of noise is noise

- “White” noise is evenly distributed in Fourier space

- “White” means that each pixel is independent

Image alignment in 2D

Interpolation



The Fourier transform of noise is noise

- "White" noise is evenly distributed in Fourier space

- "White" means that each pixel is independent

The degradation of the images means that we should minimize the number of interpolations.

Image alignment in 3D

Two translational:

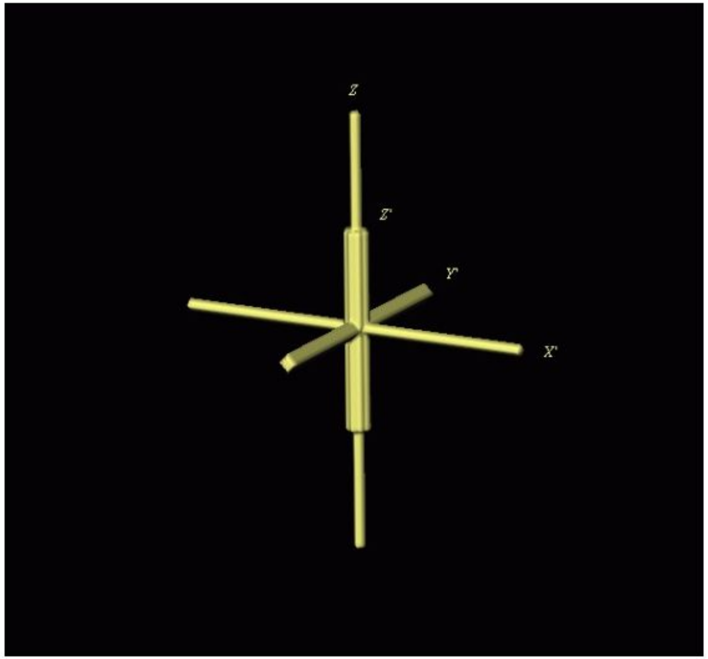
- Δx
- Δy

Three orientational (Euler angles):

- phi (about *z* axis)
- theta (about *y*)
- psi (about new *z*)

These are determined in 2D.

These are determined in 3D.



http://www.wadsworth.org

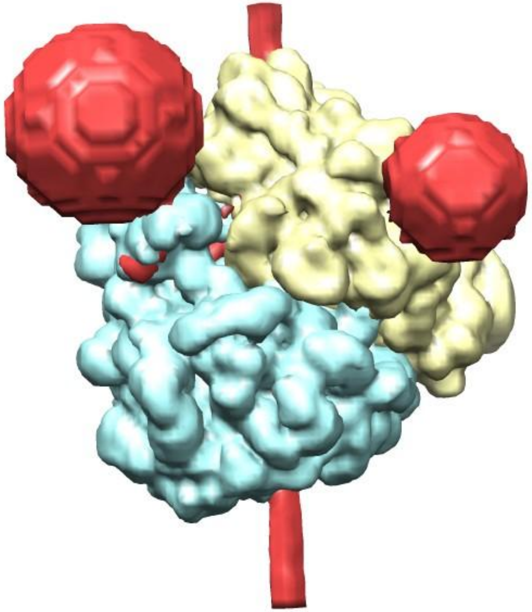


Image alignment in 3D

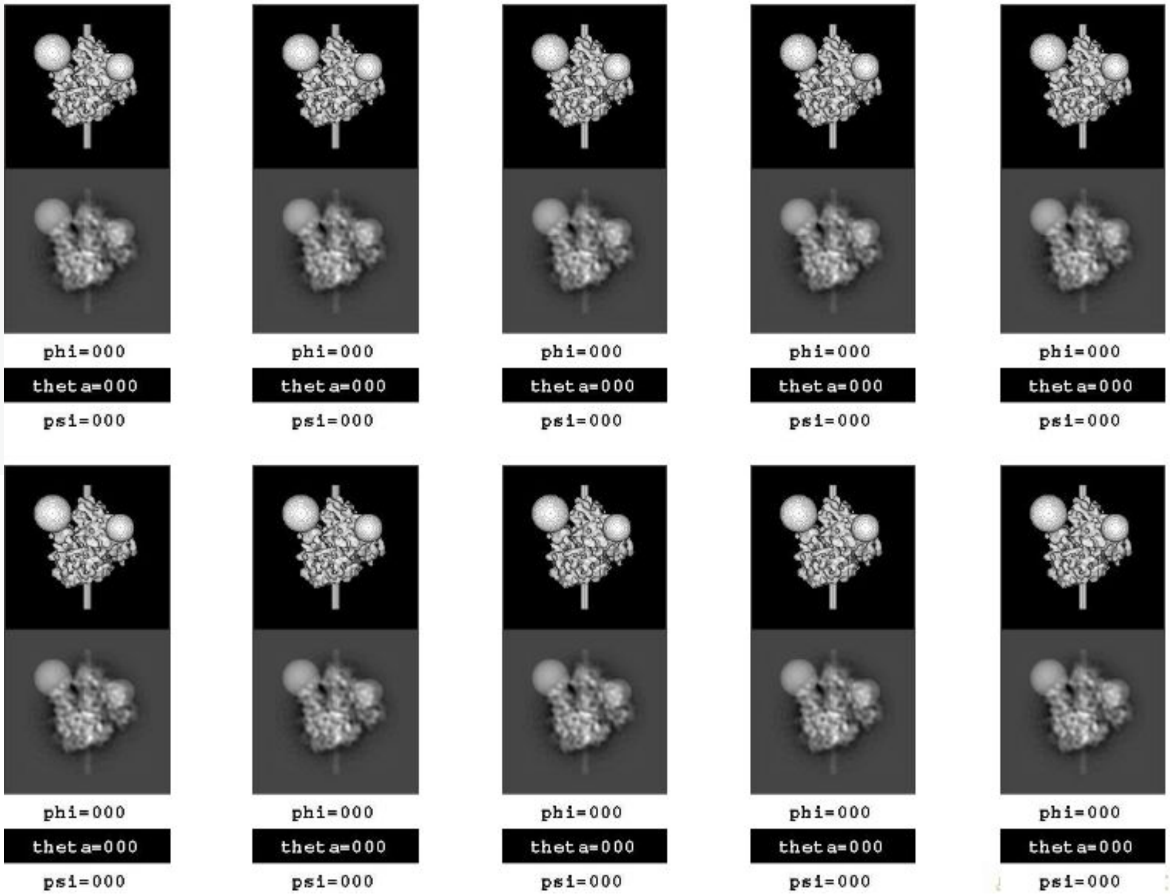
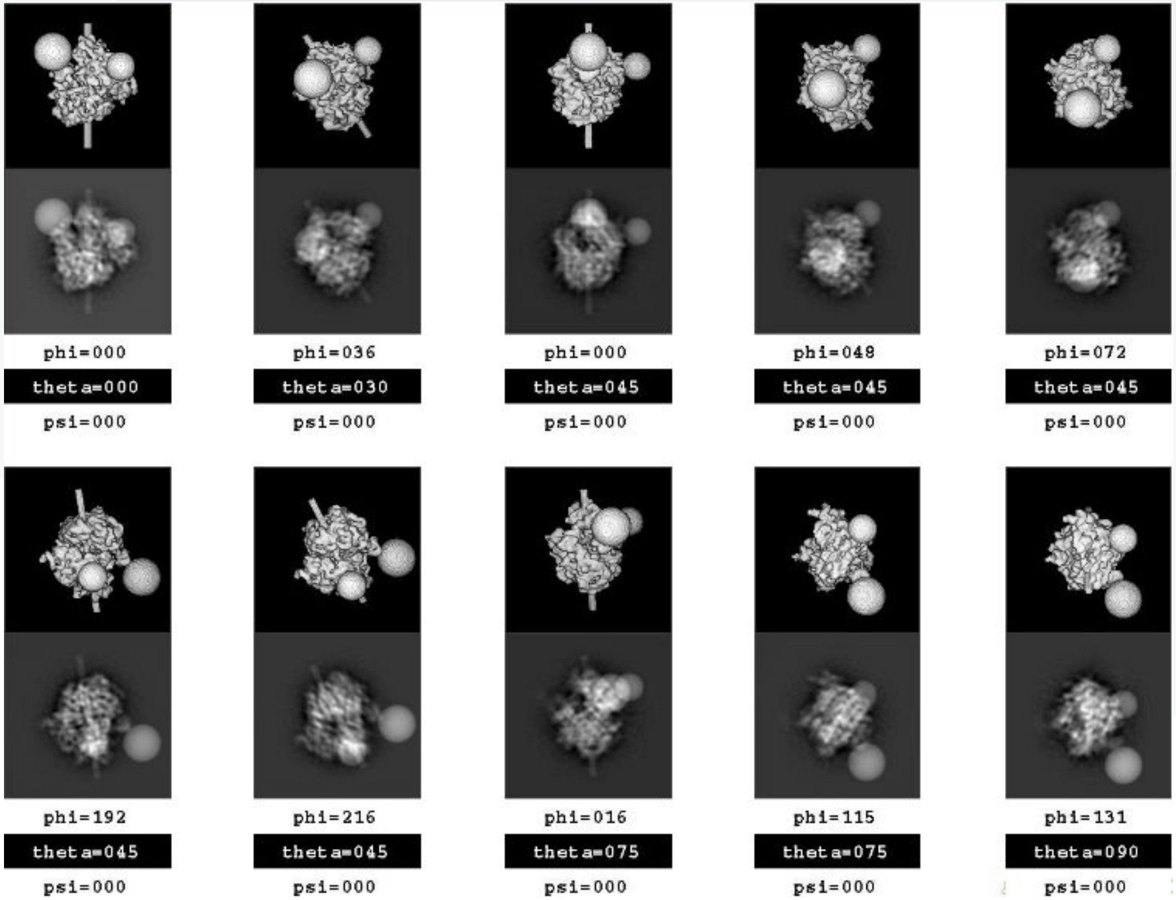


Image alignment in 3D



3D reconstruction

1. Different orientations

2. Known orientations

3. Many particles

4. CTF parameters

Baumeister et al. (1999), *Trends in Cell Biol.*, **9**: 81-5.

Your sample isn't guaranteed to adopt different orientations,
in which case you many need to explicitly tilt the microscope stage.

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3D reconstruction

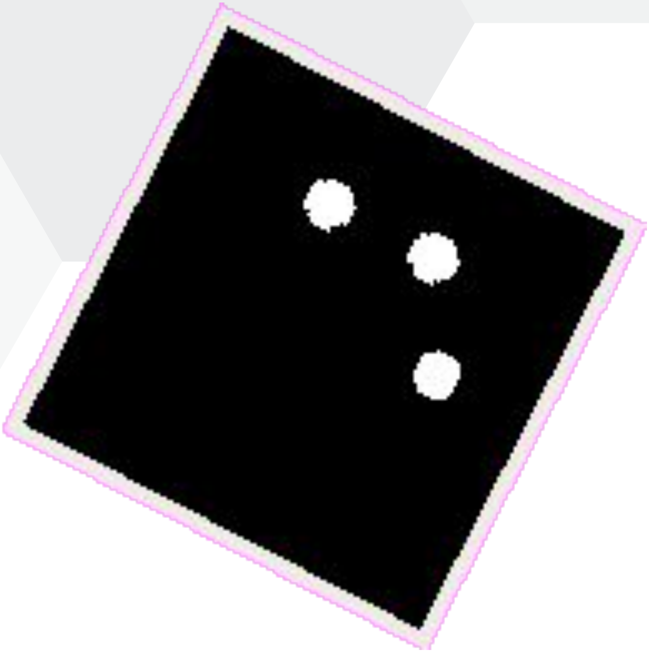
Two general ways for 3D reconstruction:

- Real space

- Fourier space

3D reconstruction

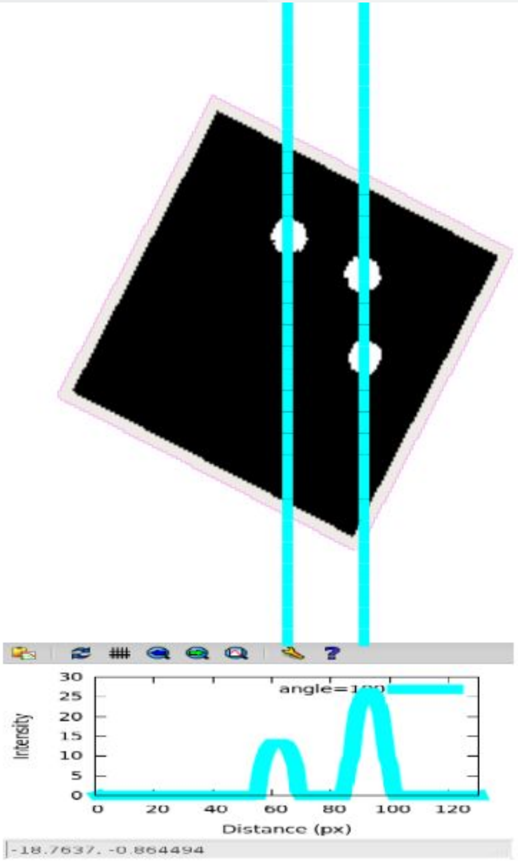
Real space reconstruction



We are going to reconstruct a 2D object from 1D projections. The principle is the similar to, but simpler than, reconstructing a 3D object from 2D projections.

3D reconstruction

Real space reconstruction



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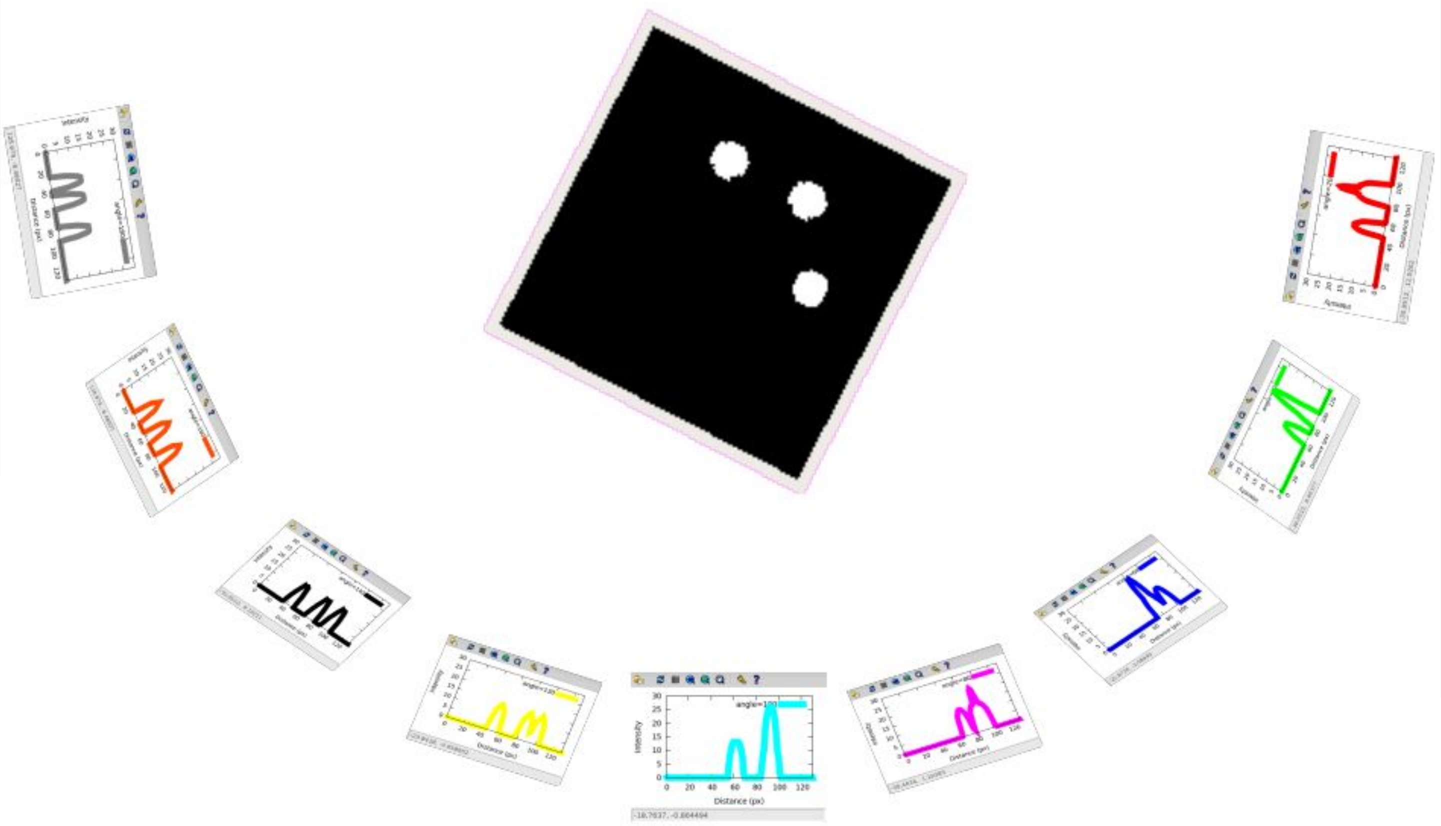
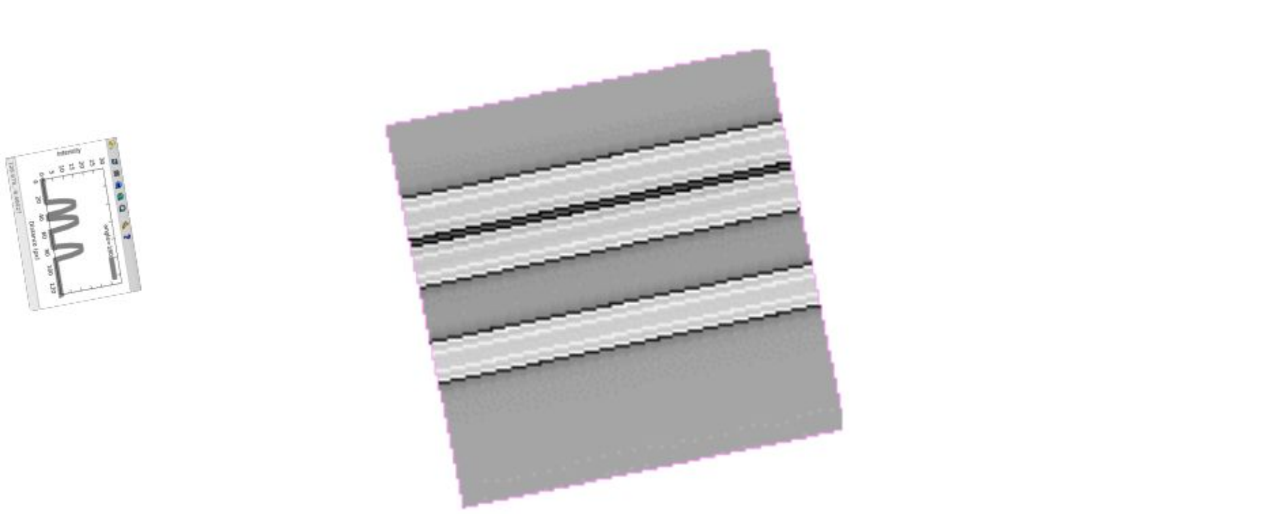
3D reconstruction

Real space reconstruction



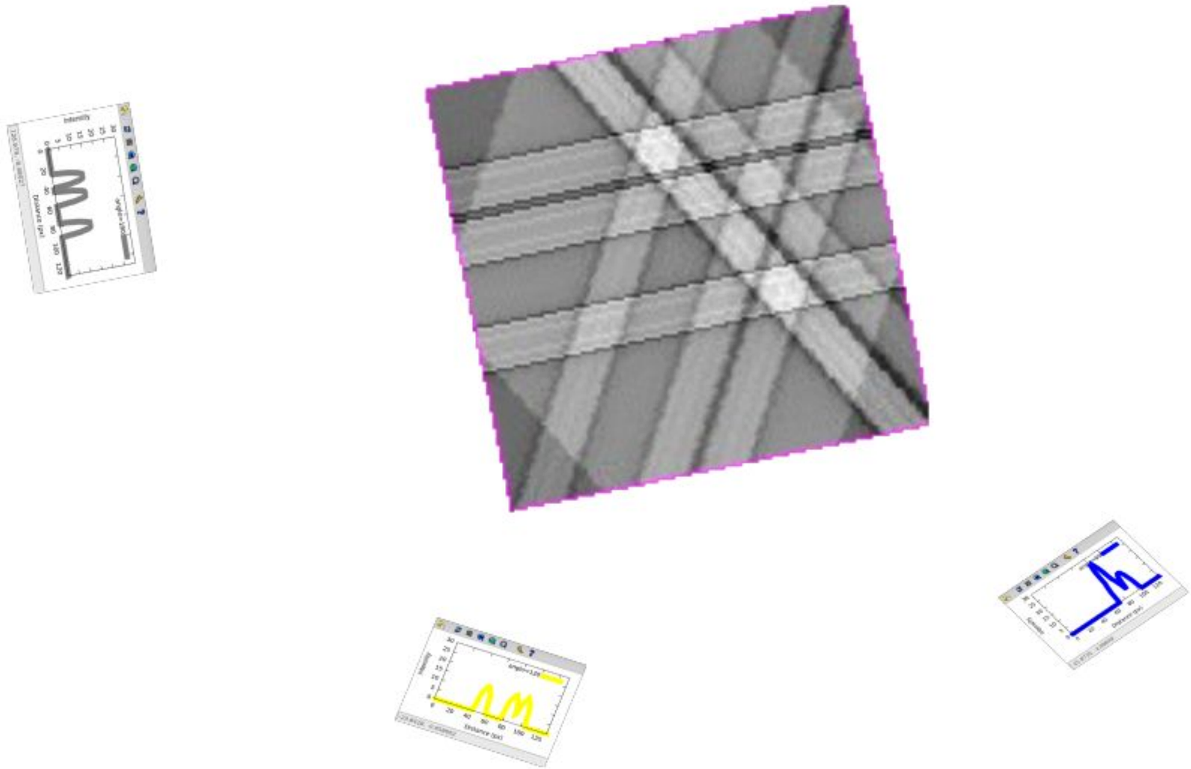
3D reconstruction

- reconstruction is the inversion of projection



3D reconstruction

- reconstruction is the inversion of projection



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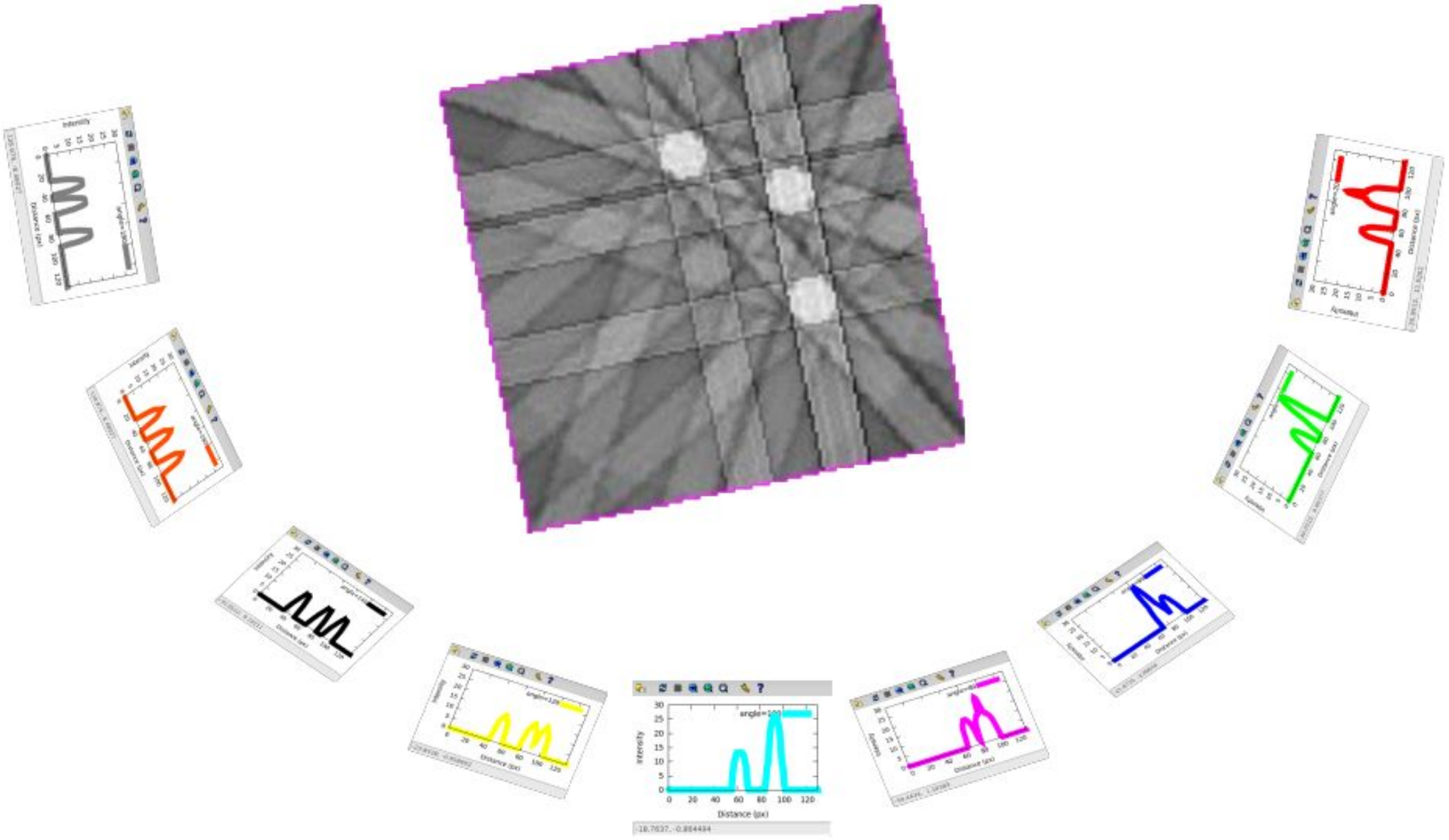
3D reconstruction

- reconstruction is the inversion of projection



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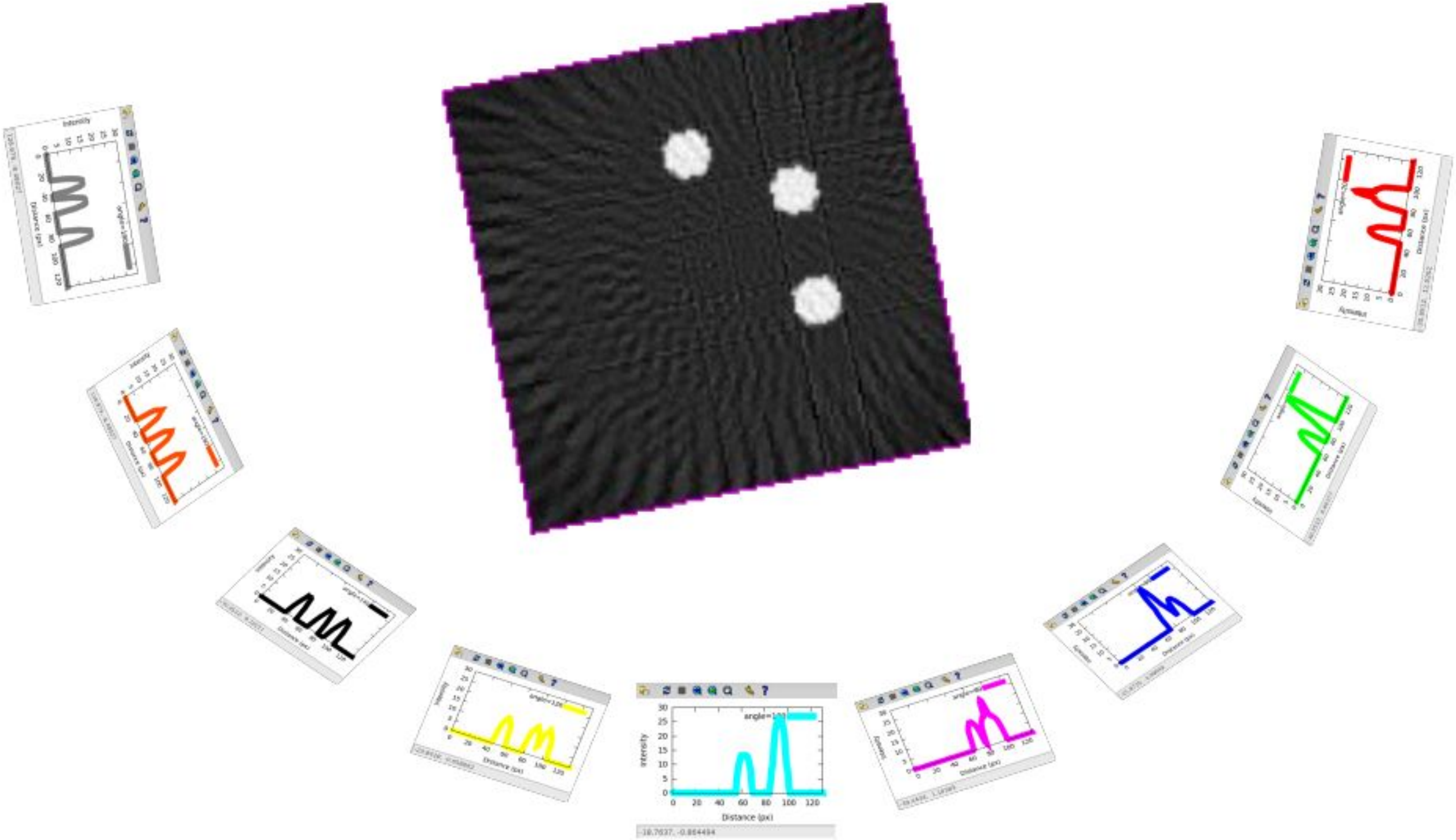
3D reconstruction

- reconstruction is the inversion of projection



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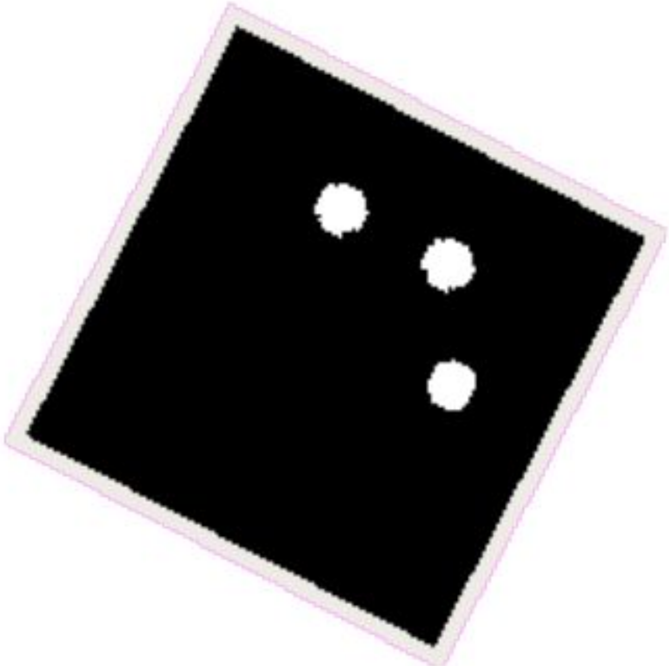
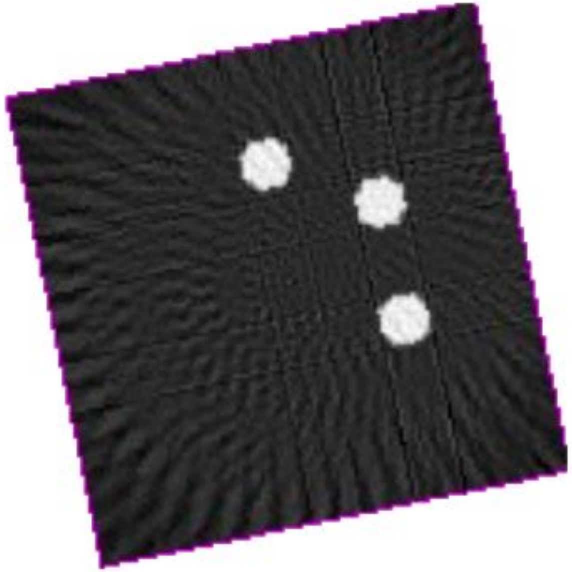
3D reconstruction

Original



Reconstructed



The reconstruction does not agree well with the projections

Potential solution: Simultaneous Iterative Reconstruction Technique

3D reconstruction

- simultaneous iterative reconstruction technique

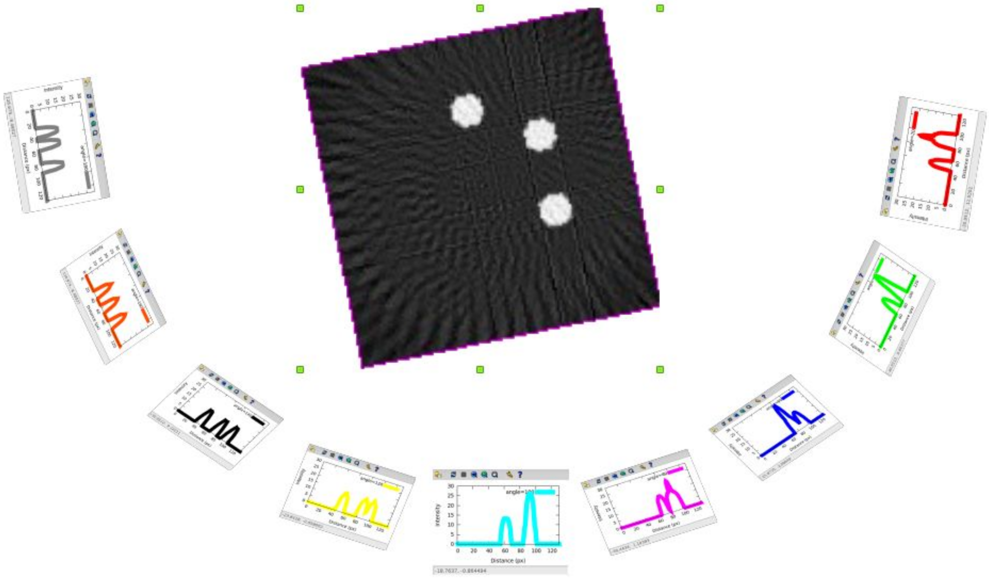
Compute re-projections of your model.

Compare the re-projections to your experimental data.
There will be differences.

Weight the differences by a fudge factor, λ.

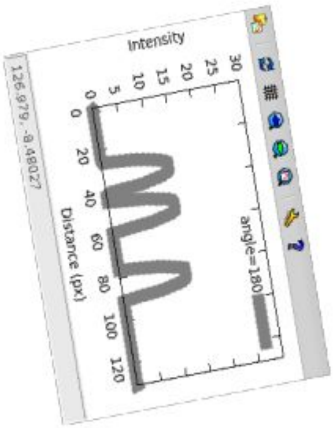
Adjust the model by the difference weighted by λ

Repeat

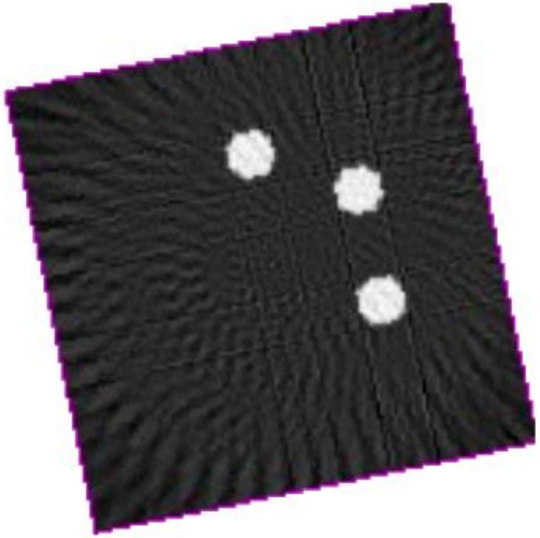


3D reconstruction

- simultaneous iterative reconstruction technique



Experimental projection



Model

Here, the differences (which will be down-weighted by λ) are the ripples in the background.

If we didn't down-weight by λ, we would overcompensate, and would amplify noise.

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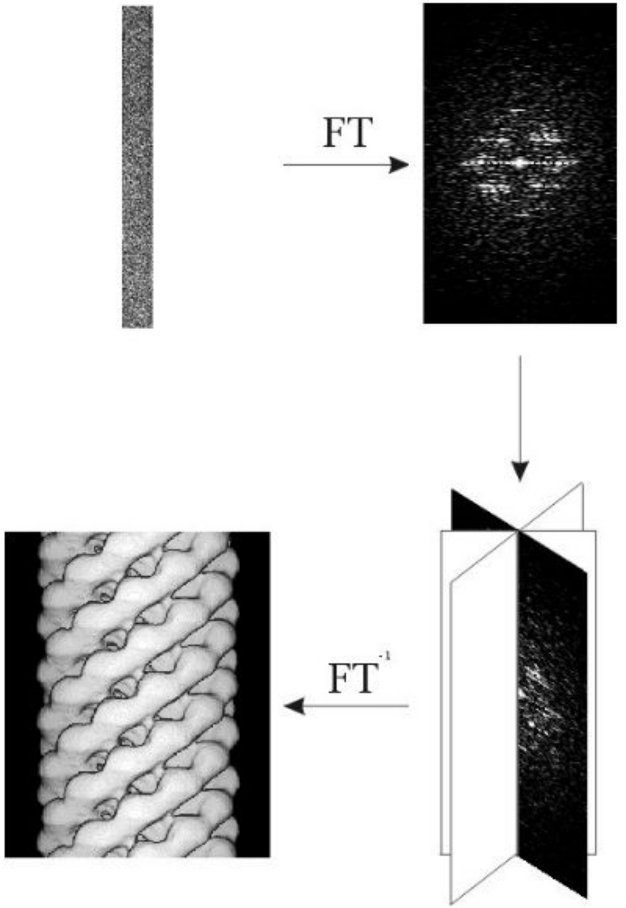
3D reconstruction

Fourier space reconstruction

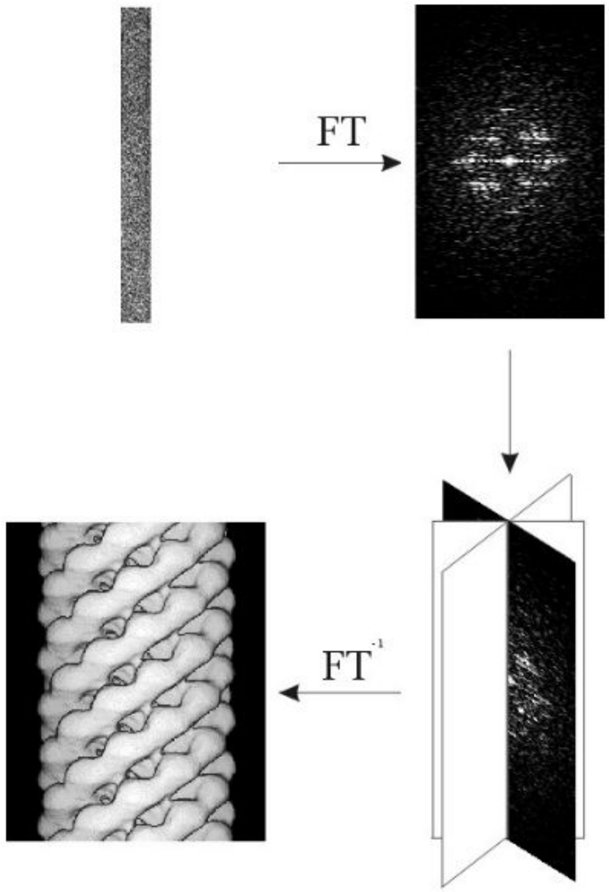


Projection theorem
Central section theorem

A central section through the 3D Fourier transform is the Fourier transform of the projection in that direction.

3D reconstruction

Fourier space reconstruction

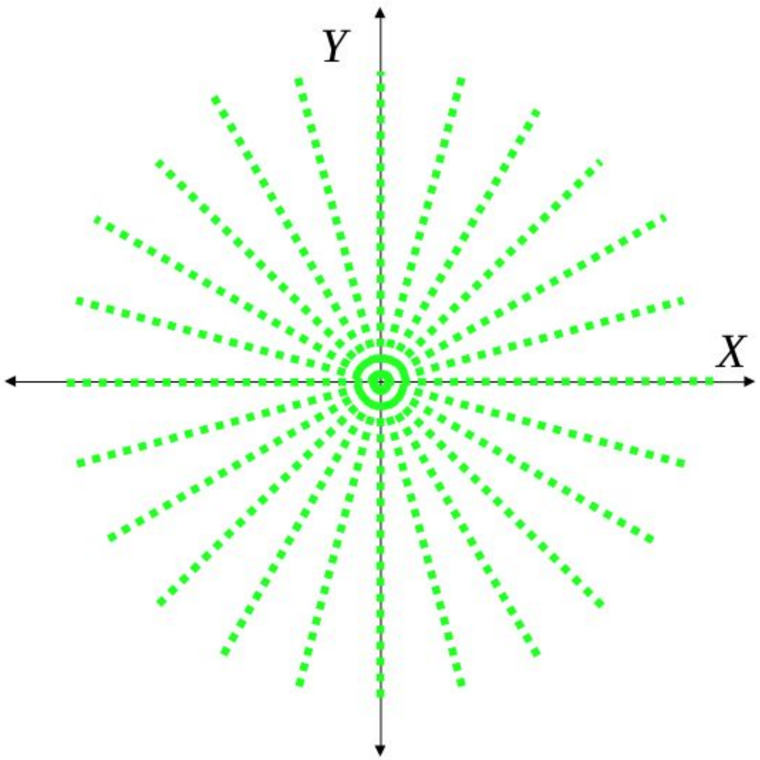


Projection theorem
Central section theorem

The disadvantage is that you have
To resample your central sections
from polar coordinates to
Cartesian space, i.e. interpolate.
There are new methods to better
Interpolate in Fourier space.

3D reconstruction

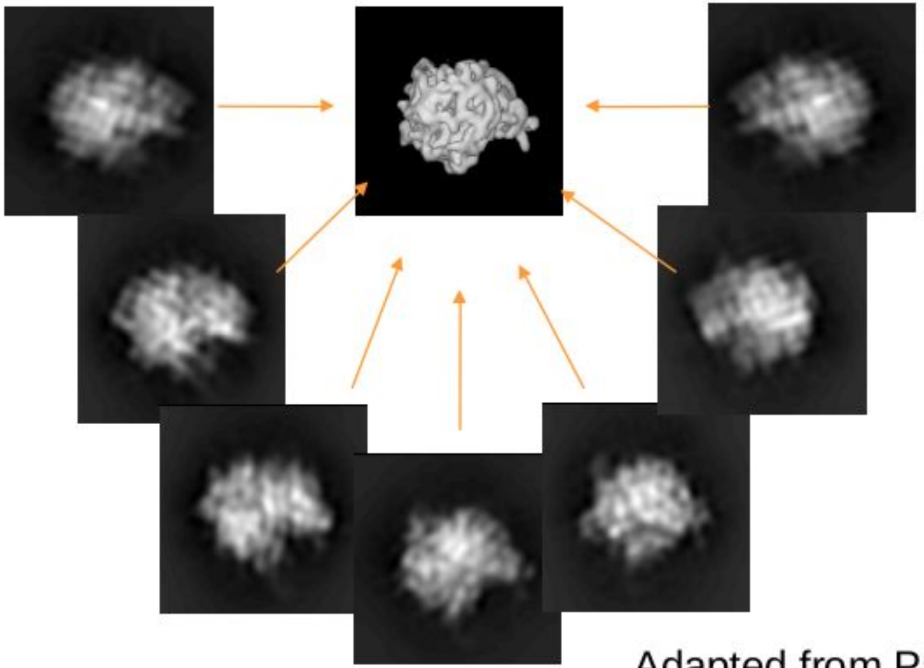
Converting from polar to Cartesian coordinates



A simple weighting scheme is to divide the weight by the radius:
*r** weighting, or “r-weighted backprojection”

3D reconstruction

If you know the orientation angles for each image,
you can compute a back-projection.



Adapted from Pawel Penczek

3D reconstruction

1. Different orientations

2. Known orientations

3. Many particles

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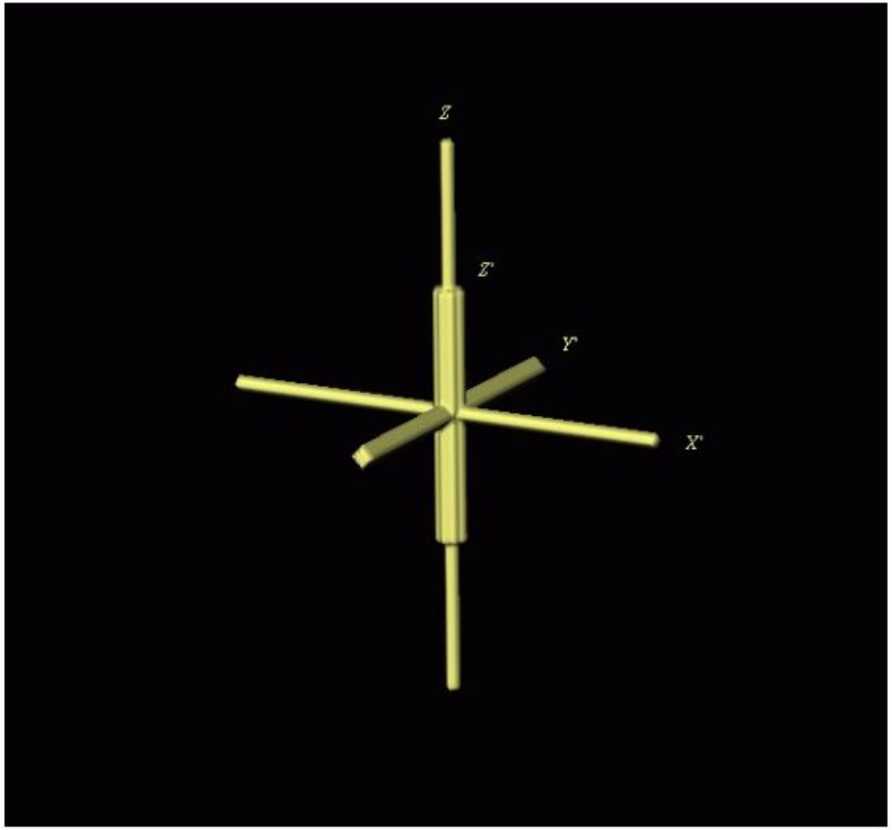

http://www.wadsworth.org

3D reconstruction

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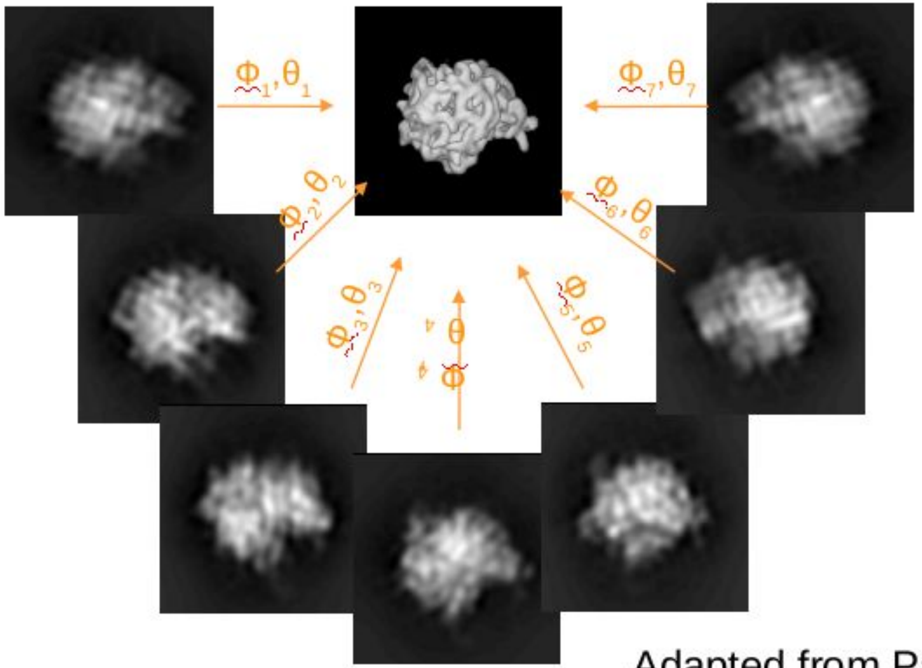

Adapted from Pawel Penczek

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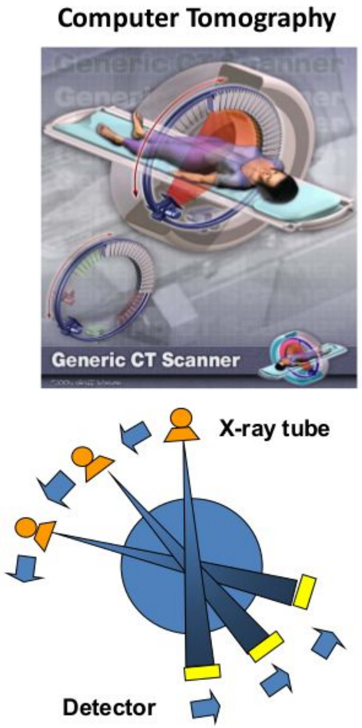
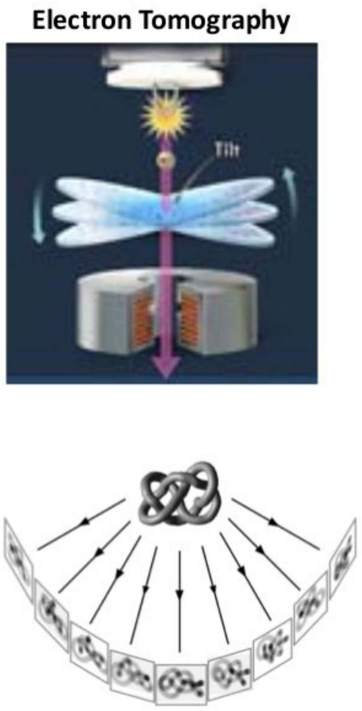
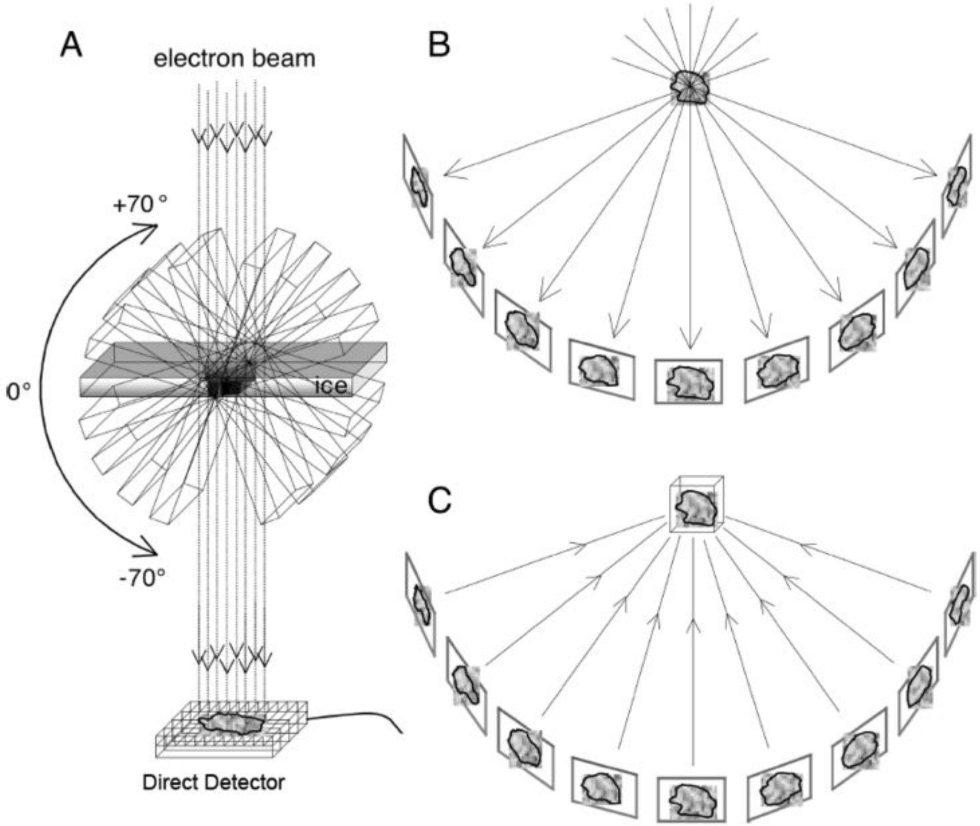
3D reconstruction

Tomography







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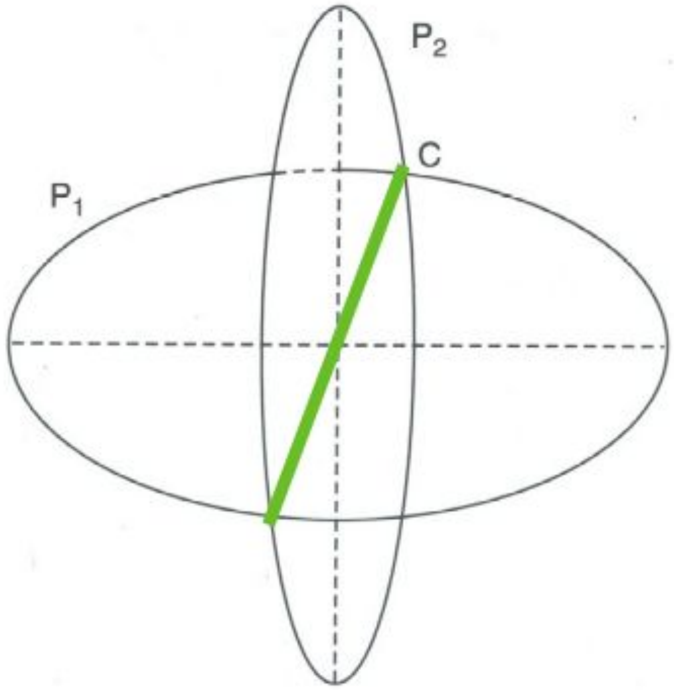
Common lines

Angular Reconstruction

Summary:

- A central section through the 3D Fourier transform is the Fourier transform of the projection in that direction
- Two central sections will intersect along a line through the origin of the 3D Fourier transform
- With two central sections, there is still one degree of freedom to relate the orientations, but a third projection (i.e., central section) will fix the relative orientations of all three.



Frank, J. (2006) 3D Electron Microscopy of Macromolecular Assemblies

Common lines

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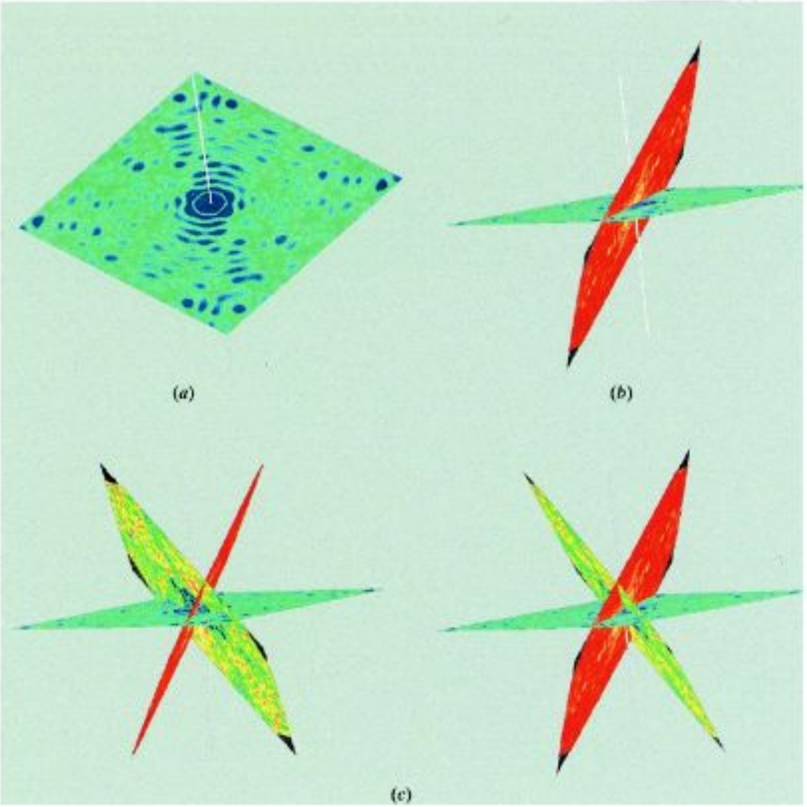
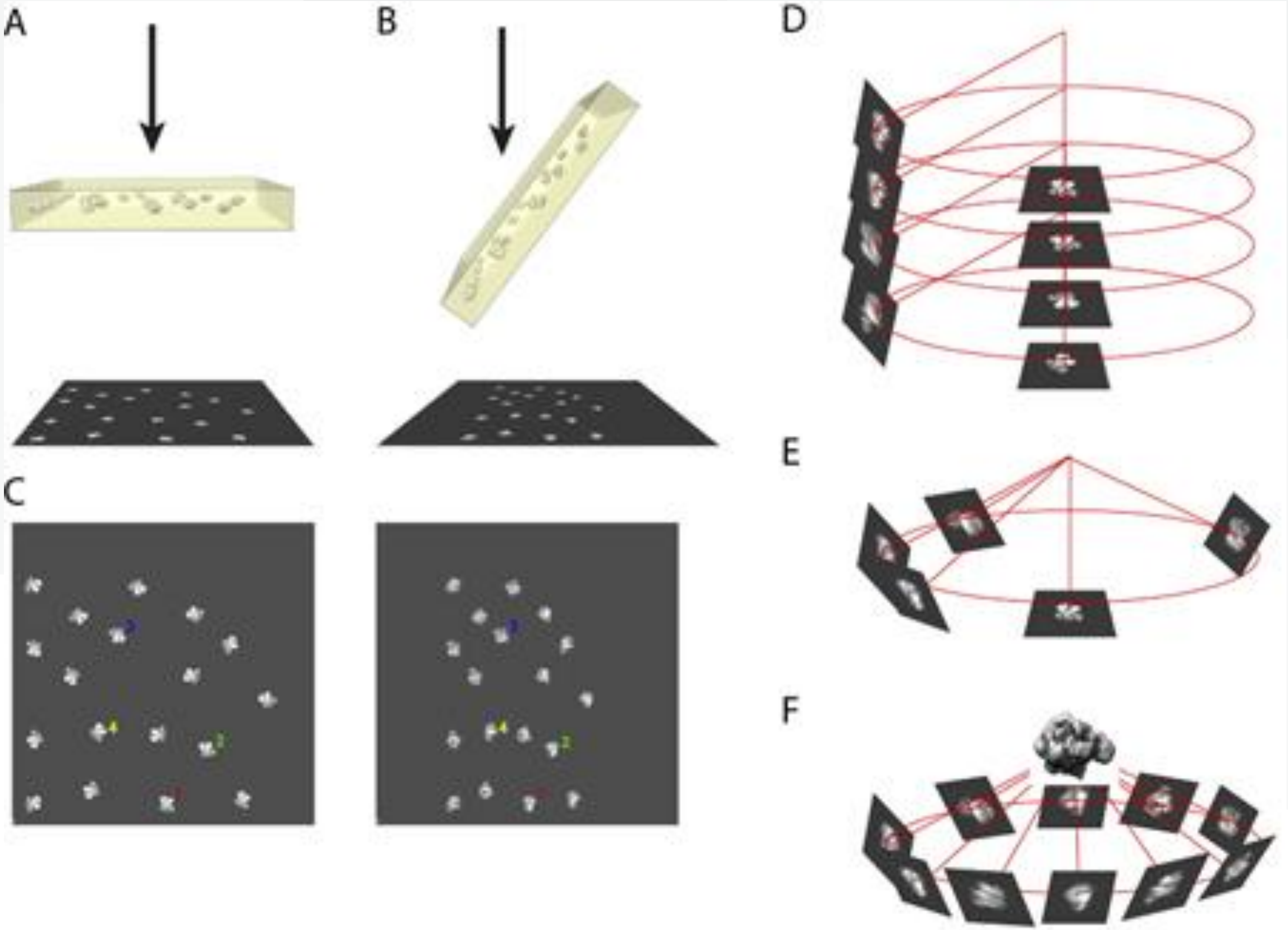
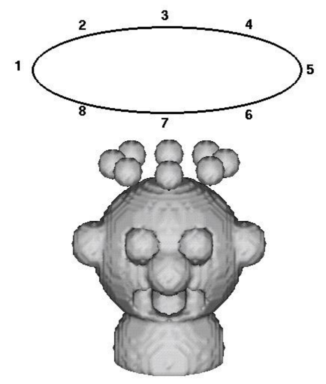


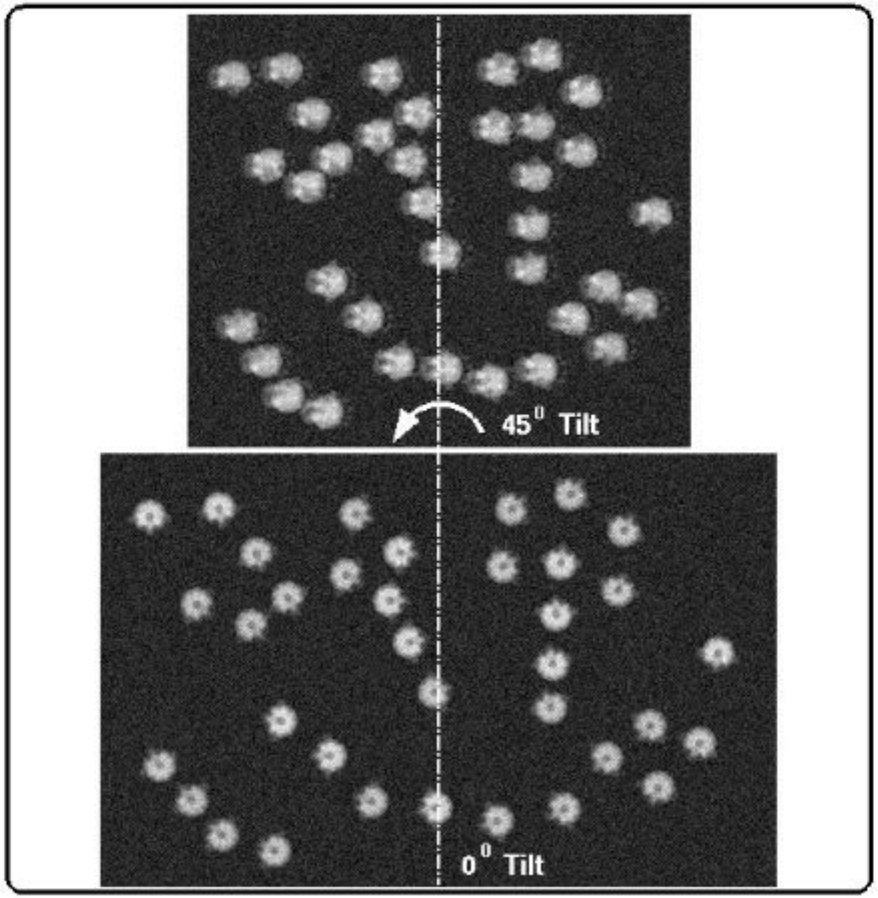
From Steve Fuller

Random conical tilt



Random conical tilt



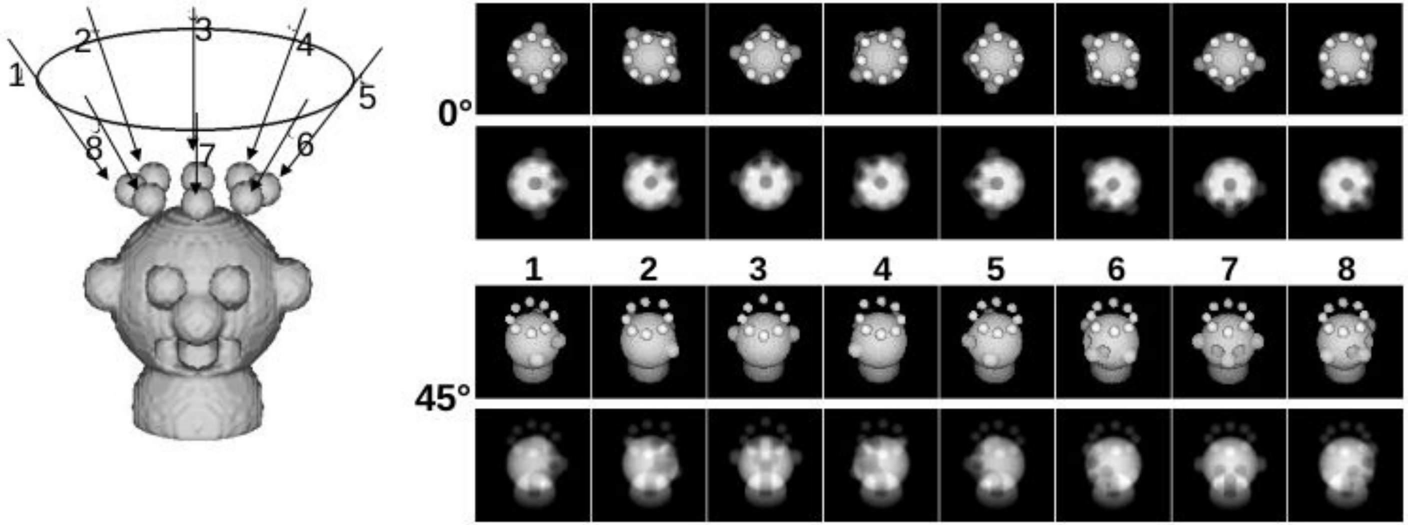


From Nicolas Boisset

This scenario describes a worst case, when there is exactly one orientation in the 0º image. Since the in-plane angle varies, in the tilted image, we have different views available.

Random conical tilt

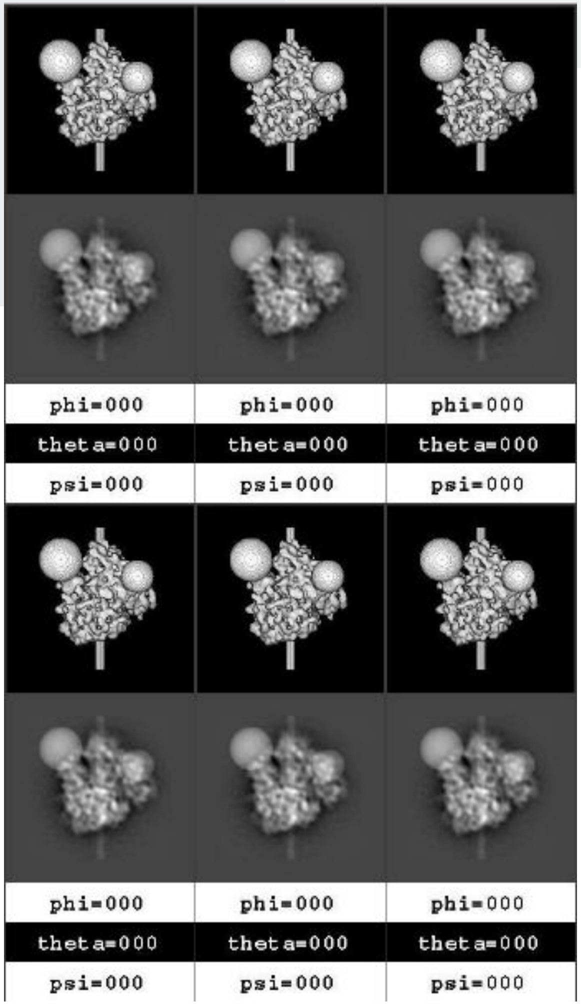
Two images are taken: one at 0° and one tilted at an angle of 45°.



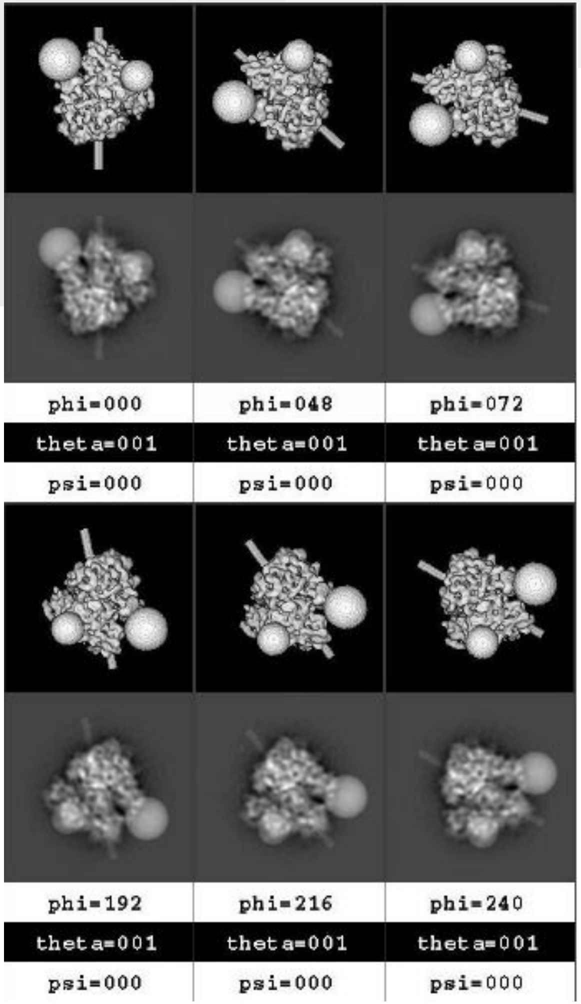
Radermacher, M., Wagenknecht, T., Verschoor, A. & Frank, J. Three-dimensional reconstruction from a single-exposure, random conical tilt series applied to the 50S ribosomal subunit of *Escherichia coli*. *J Microsc* **146**, 113-36 (1987).

From Nicolas Boisset

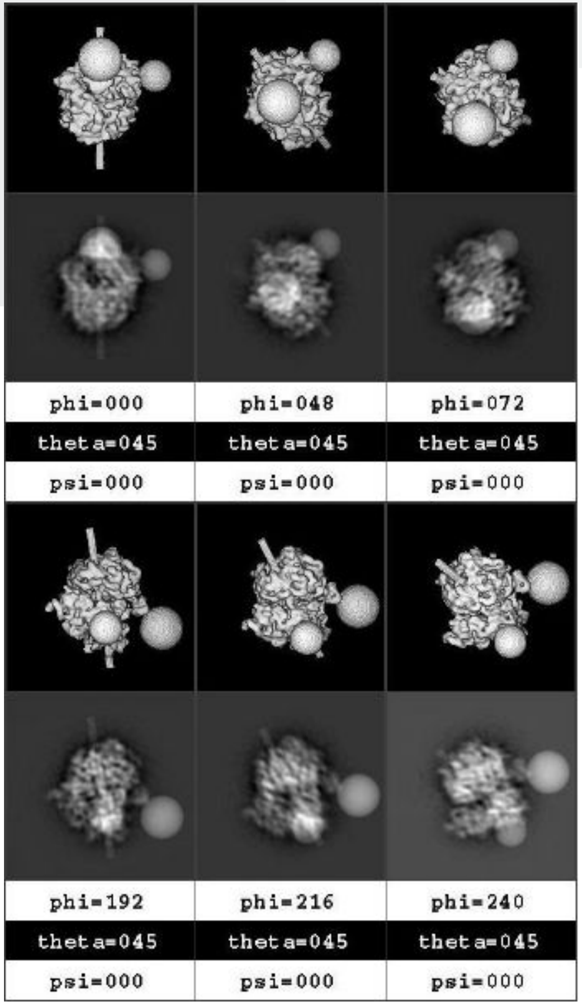
Random conical tilt



Random conical tilt



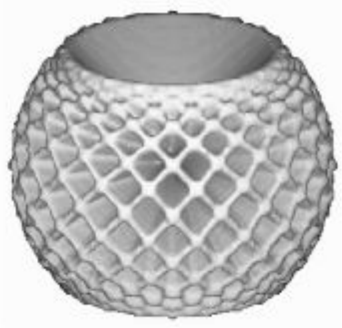
Random conical tilt



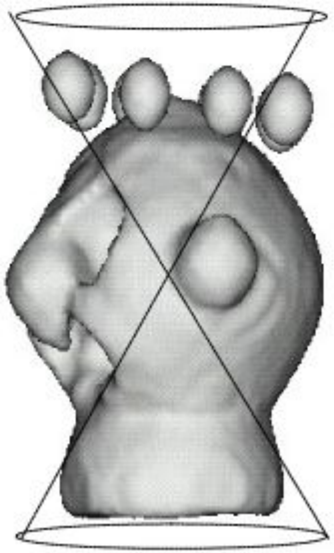
Random conical tilt

- we cannot tilt the stage to 90 deg → “missing cone”

Representation of the distribution of views, if we display a plane perpendicular to each projection direction



The missing information, in the shape of a cone, elongates features in the direction of the cone's axis.



Random conical tilt

- filling the missing cone

If there are multiple preferred orientations, or if there is symmetry that fills the missing cone, you can cover all orientations.