Lecture 7

Analysis of electron micrographs

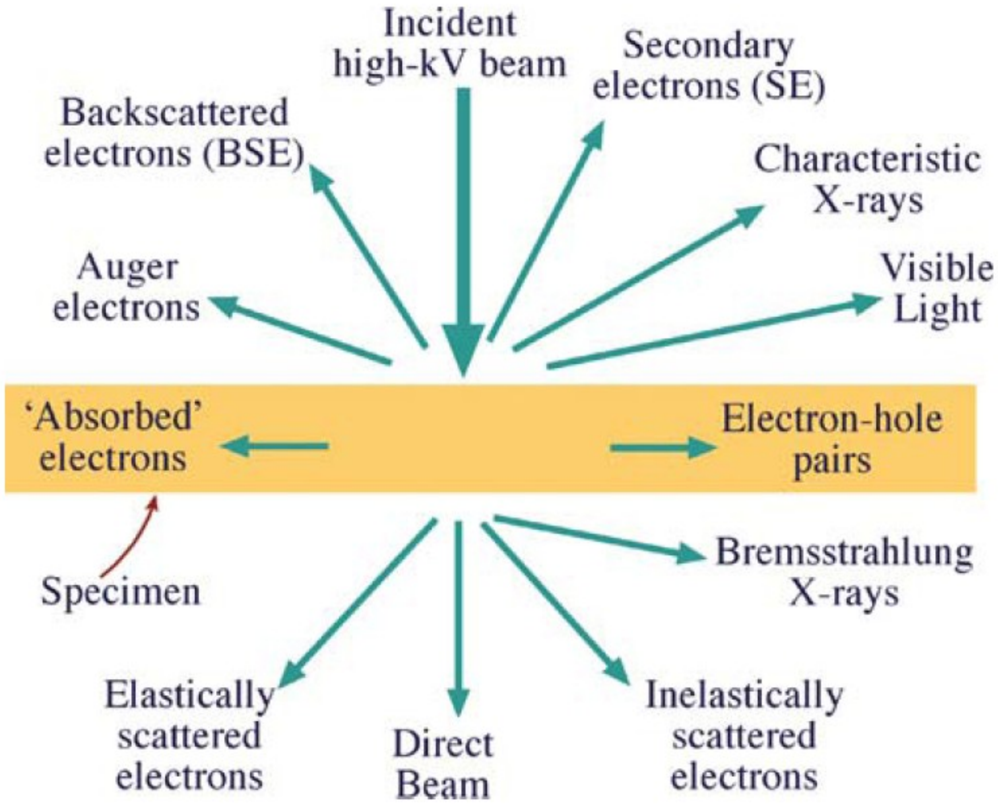
8th November 2022

Jiri Novacek

Content

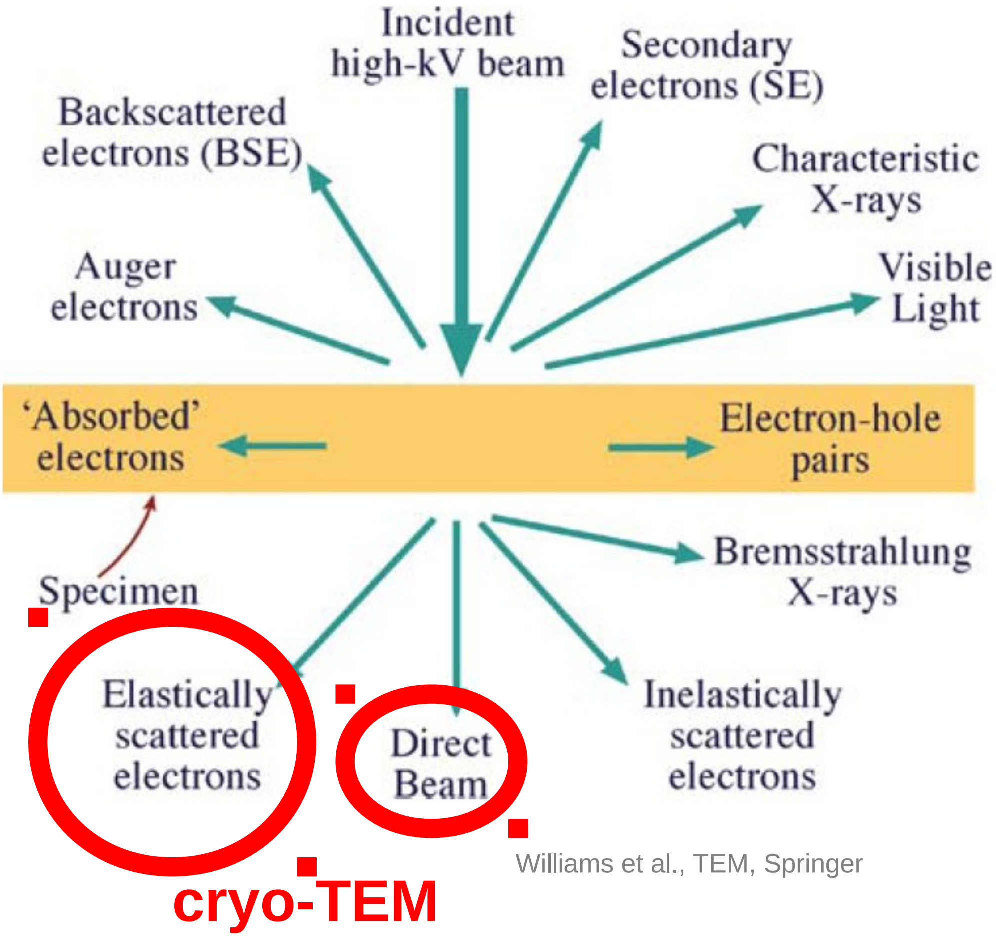
- interaction of electrons with matter, radiation damage
- data acquisition, image filtering
- projection theorem
- image averaging in 2D
- principal component analysis

Interaction of electrons with specimen

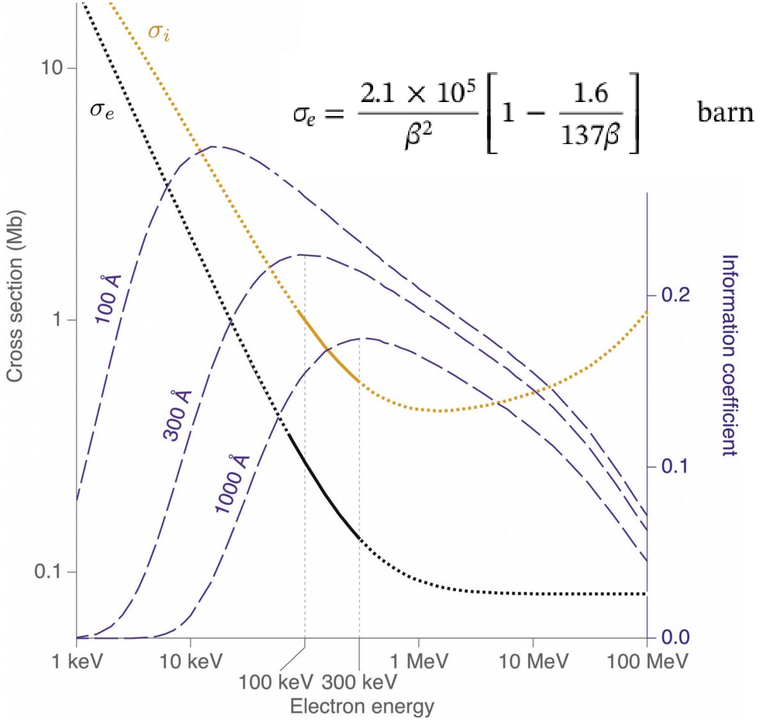


Williams et al., TEM, Springer

Interaction of electrons with specimen



Williams et al., TEM, Springer

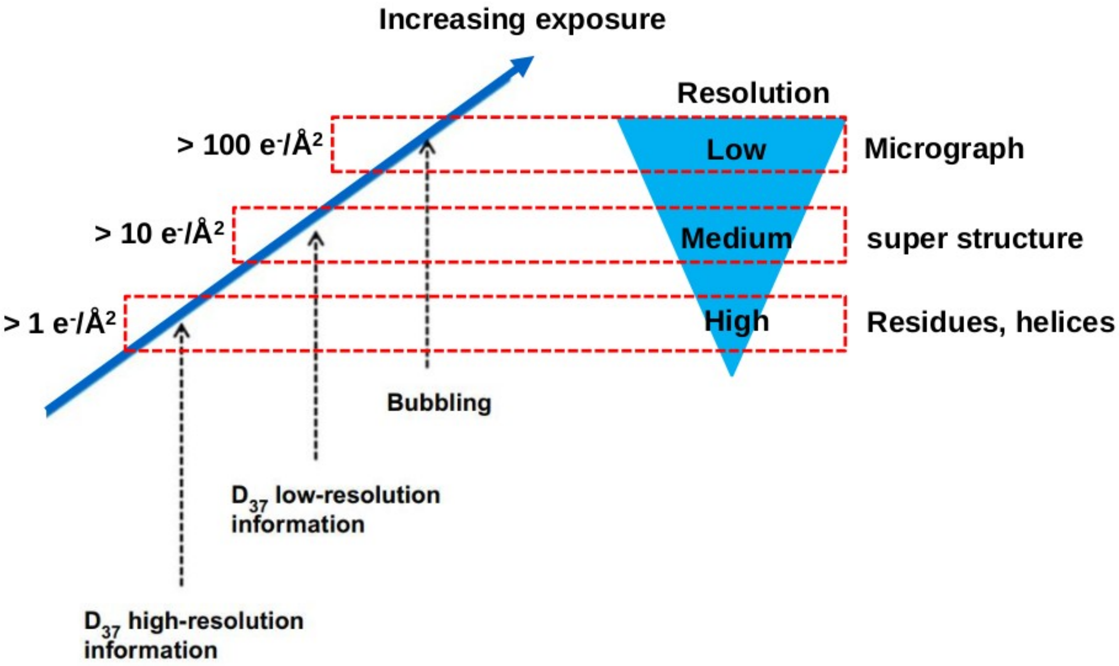


Peet et al., (2019) Ultramicroscopy

mean free path $\lambda = \frac{1}{\sigma_{total}} = \frac{A}{N_0 \sigma_{atom} \rho}$

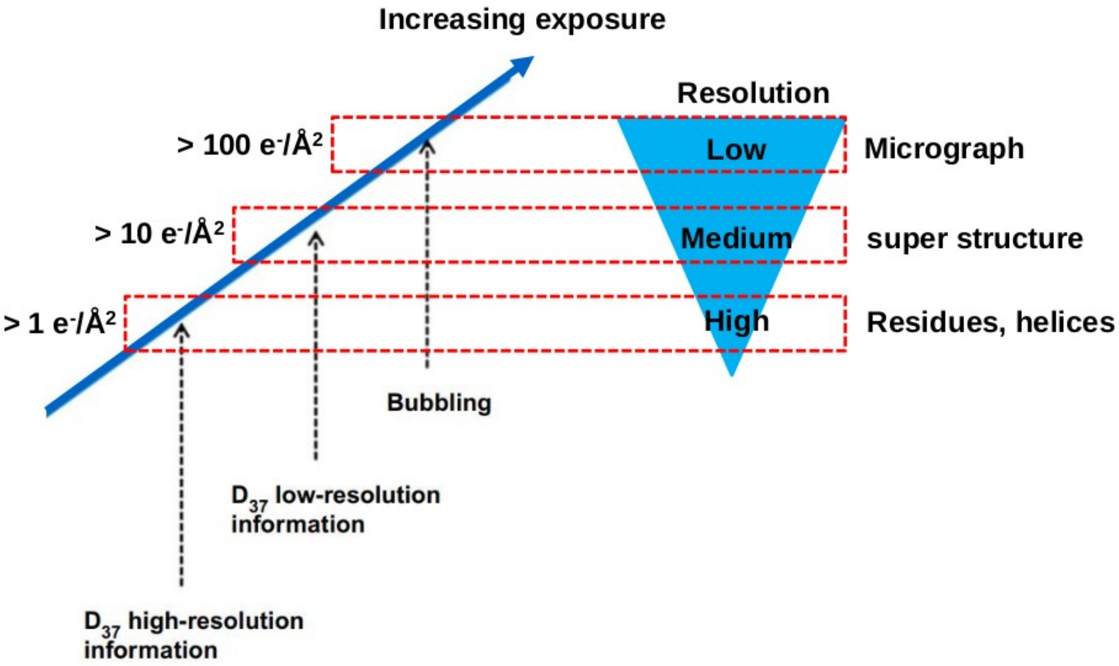
- mean free path of inelastic scattering in vitrified biological specimens: ~395nm

Radiation damage



Glaser R. (2016), Meth. Enzym.

Radiation damage



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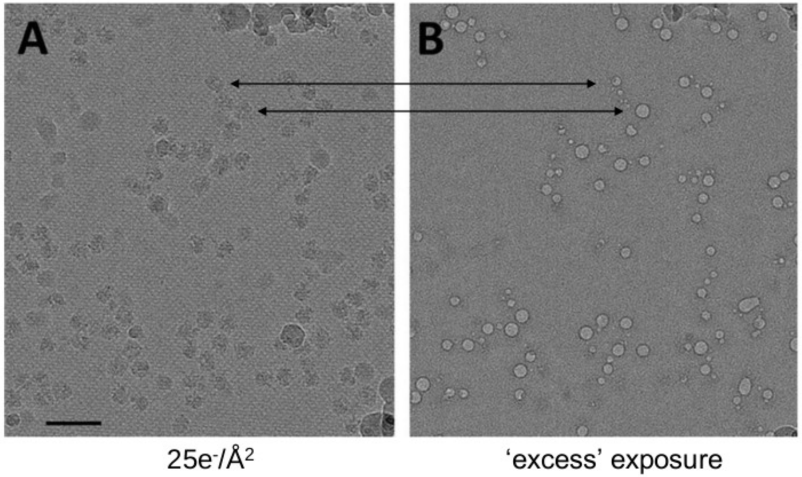

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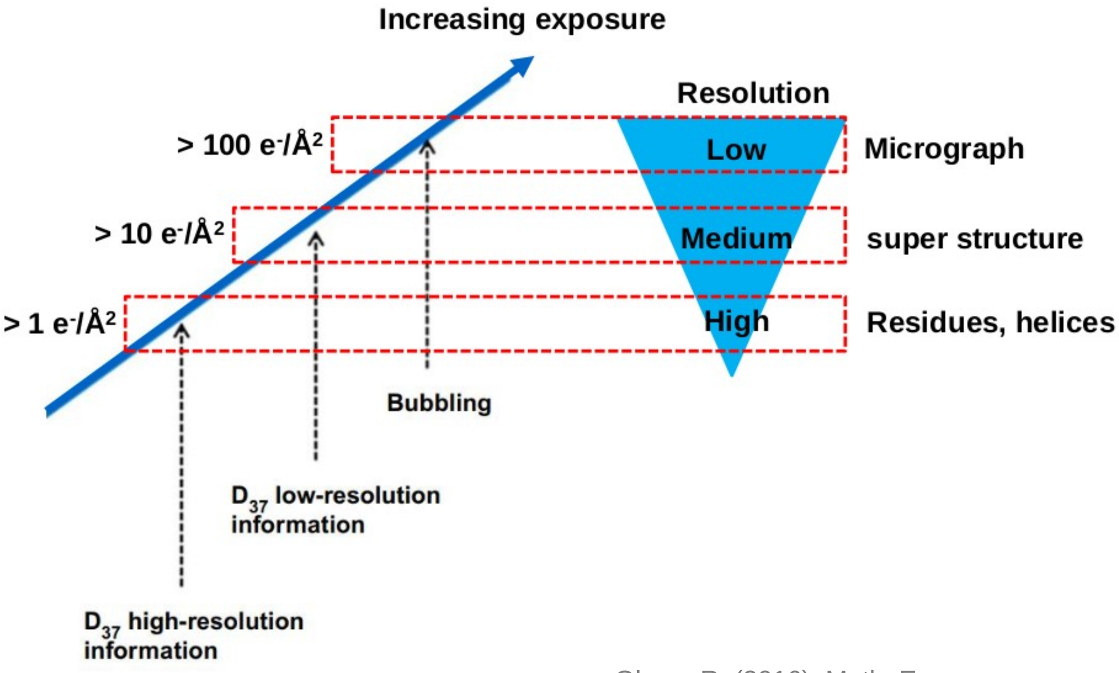
Radiation damage



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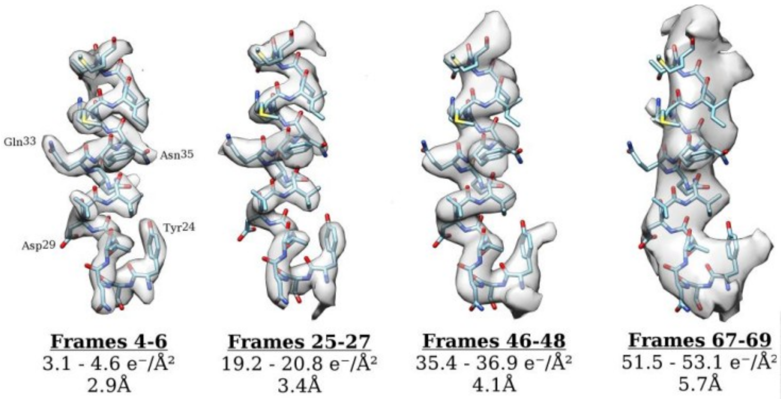
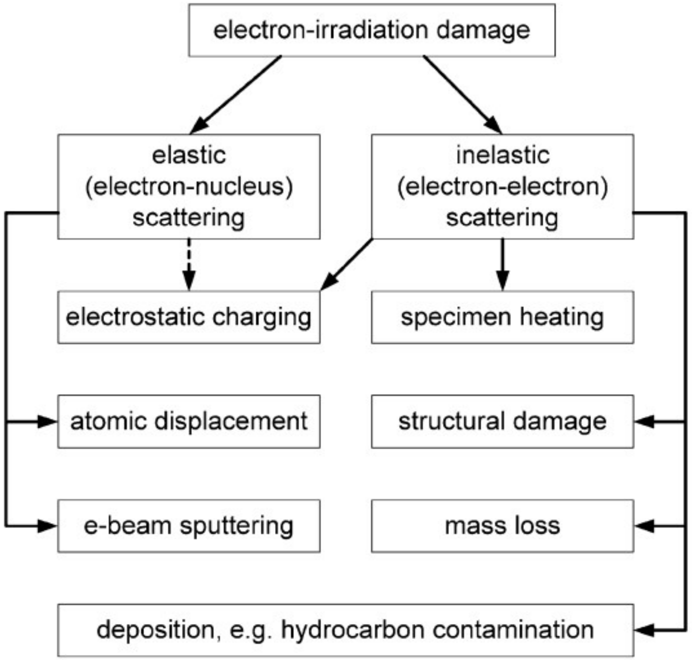
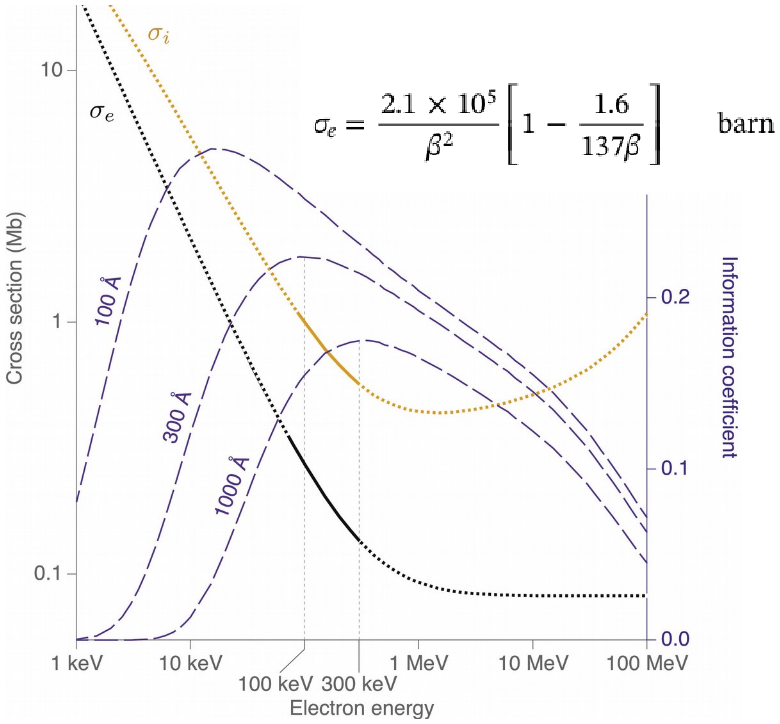


Grant. (2015), eLife

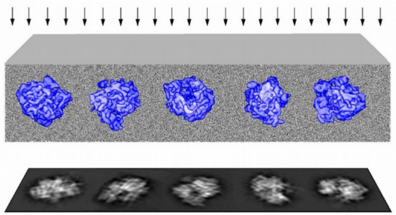
Interaction of electrons with specimen

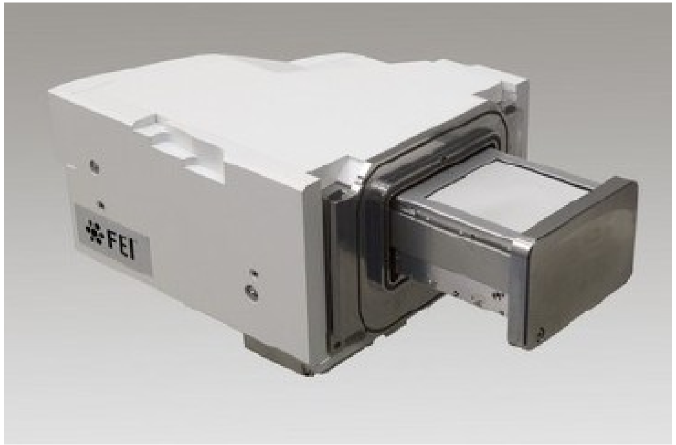


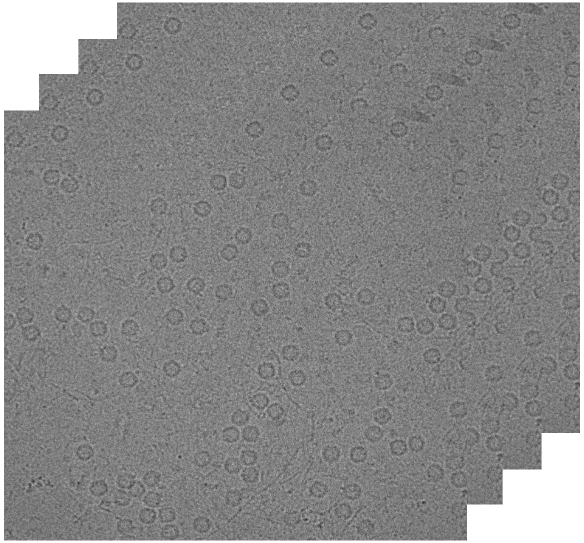


Peet et al., (2019) Ultramicroscopy

Data acquisition

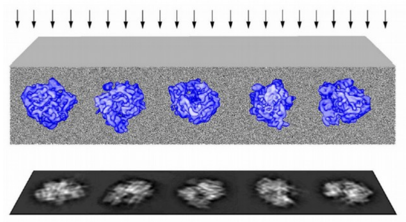


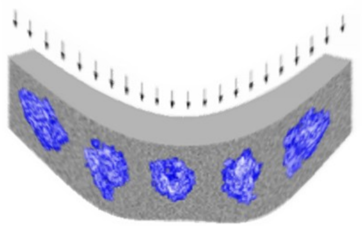


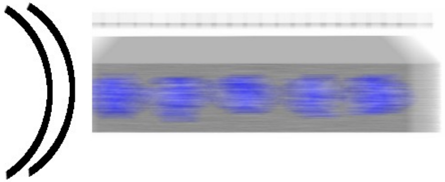


- data fom each position on the sample stored as a short movie
- compensation of sample radiation damage
- compensation of the sample motion during exposure

Data acquisition

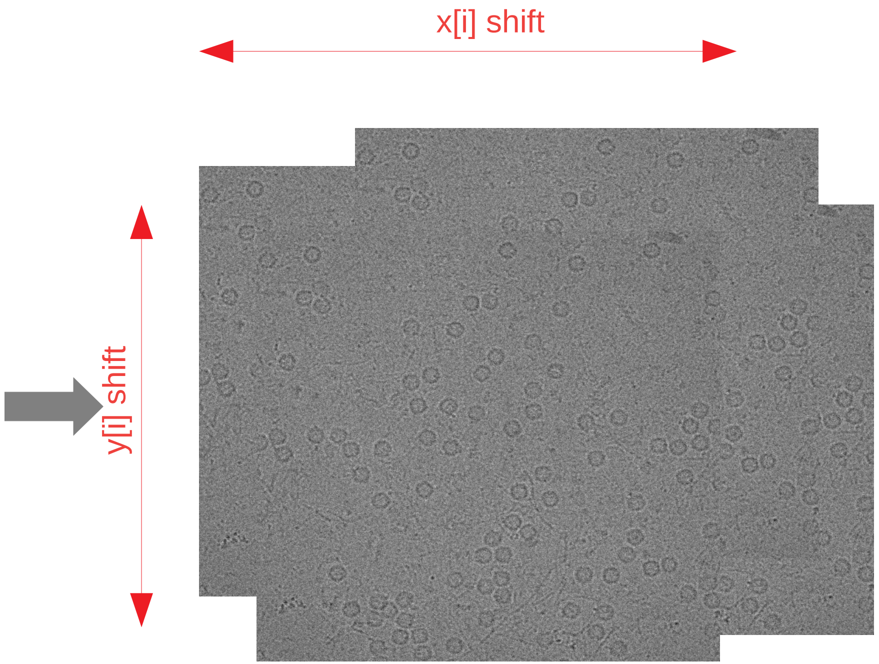






- beam induced motion
(sample geometry, local)

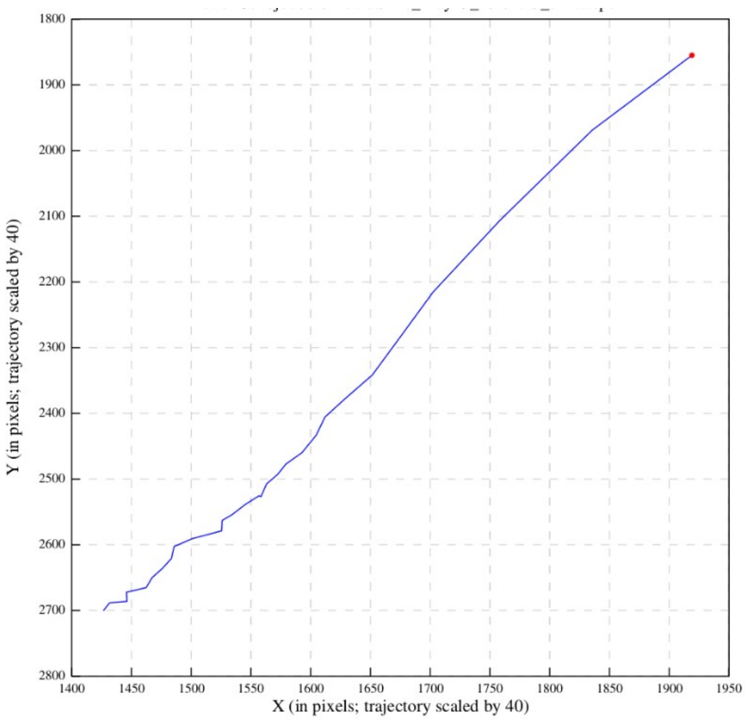
- drift, vibration
(external sources, global)



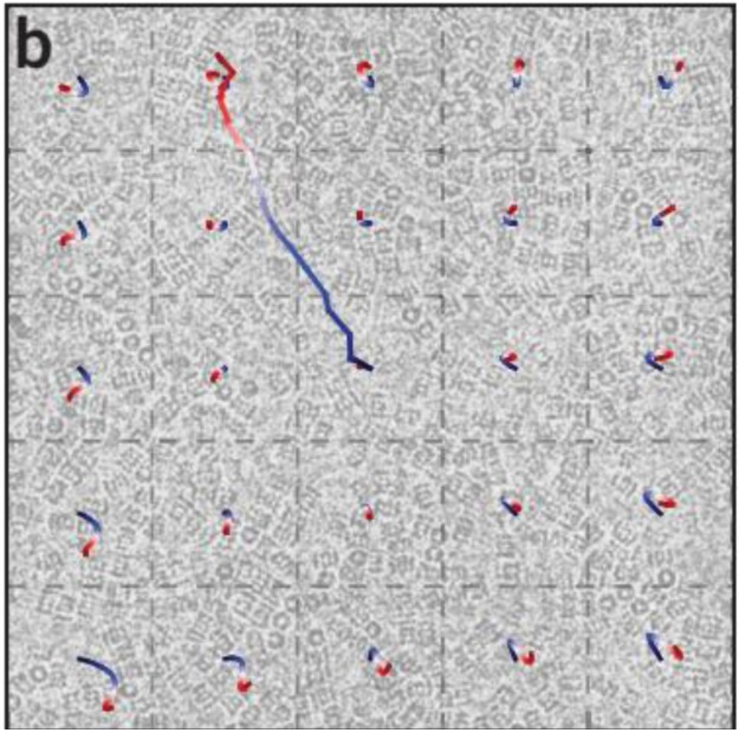
- data fom each position on the sample stored as a short movie
- compensation of sample radiation damage
- compensation of the sample motion during exposure

Data acquisition

- averaging of the movie into single image – increase S/N
- compensation for the global and local motion between the frames – minimize image blur, maximize high-res. Info
- dose-weighting – frame filtering based on acquired radiation damage



global motion



additional local motion

Data acquisition

- averaging of the movie into single image – increase S/N
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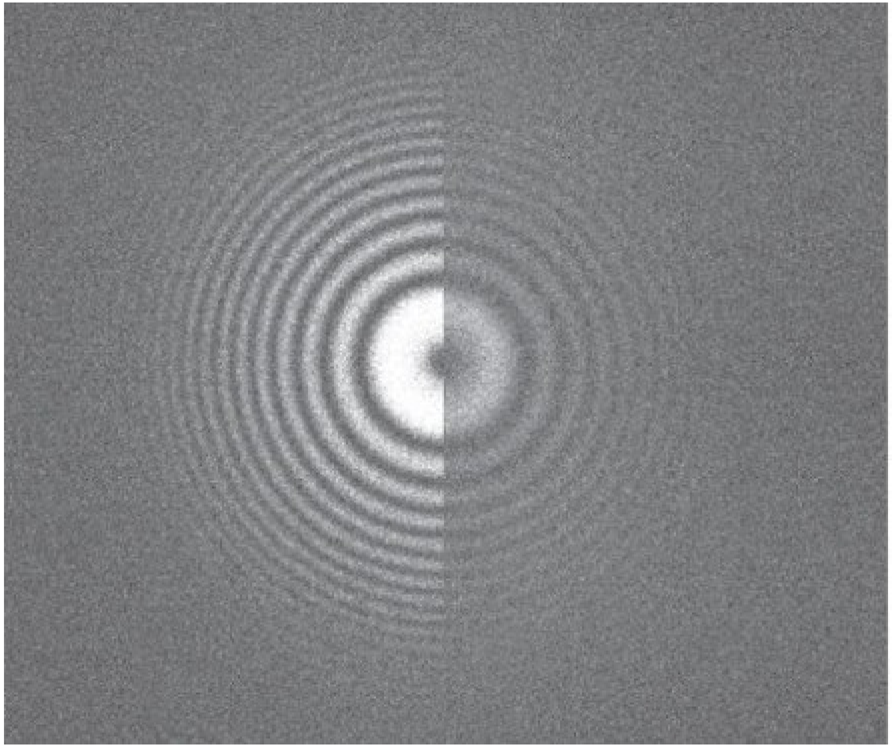
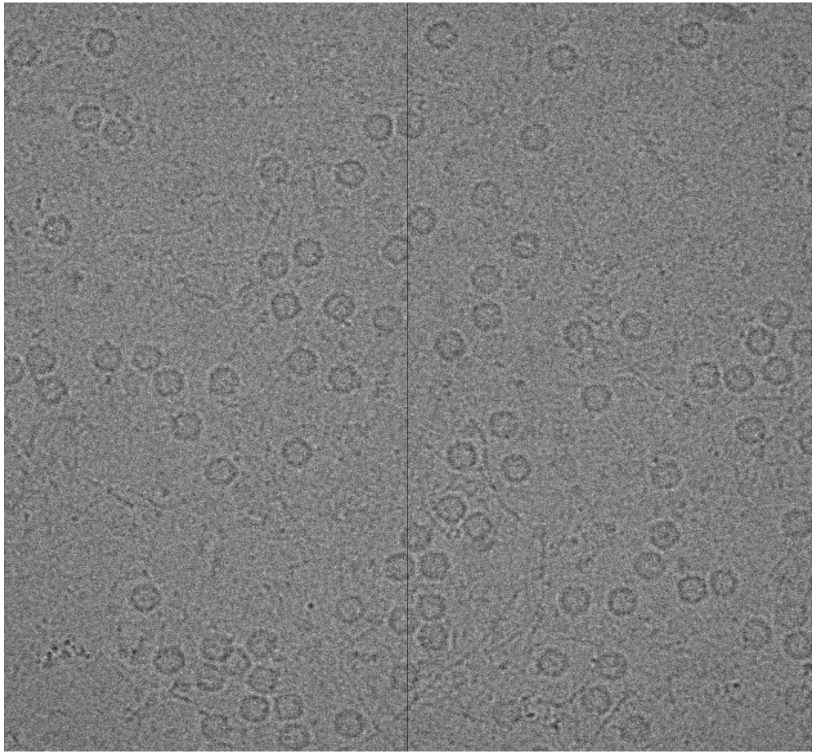
- dose-weighting – frame filtering based on acquired radiation damage



aligned image unaligned image



aligned image unaligned image

Data acquisition

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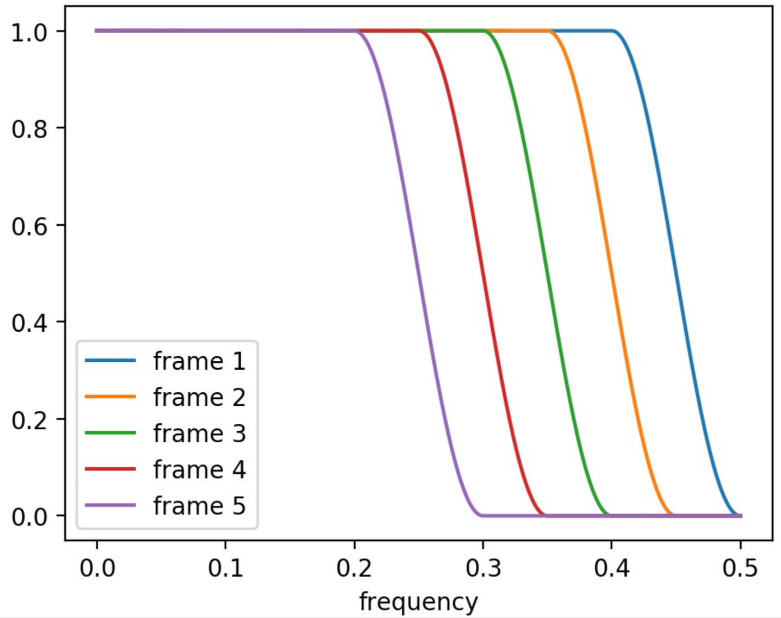
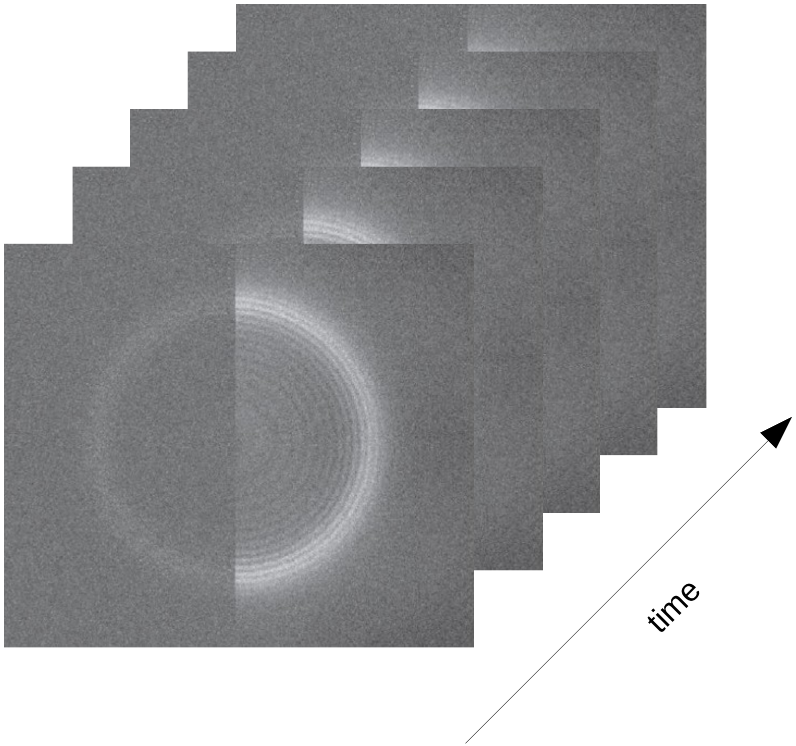




- application of adaptive per-frame low pass filter before averaging

Image filtering

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

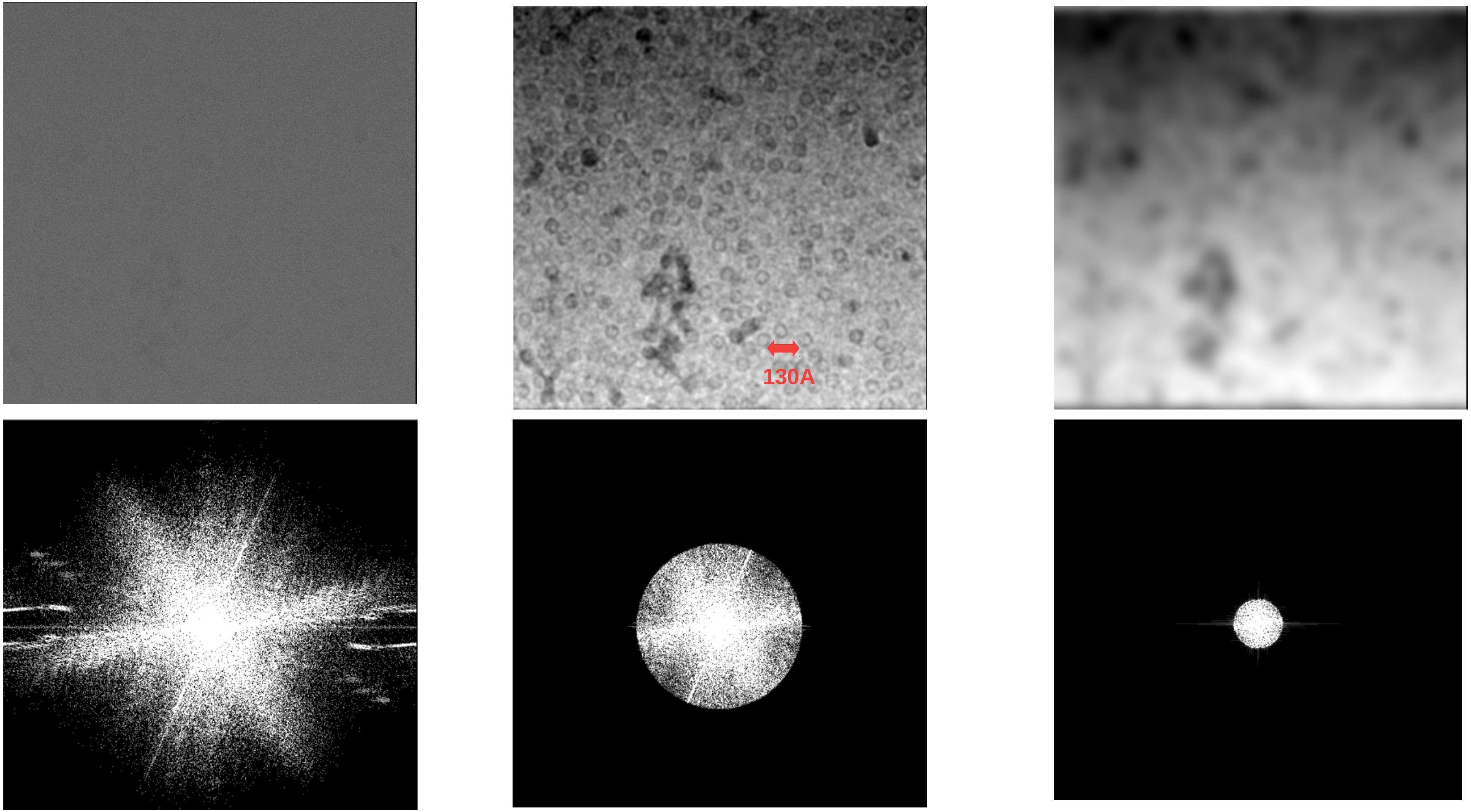


Image filtering

unfiltered image | lowpass filtered (50A) | bandpass filtered (1000,10A)

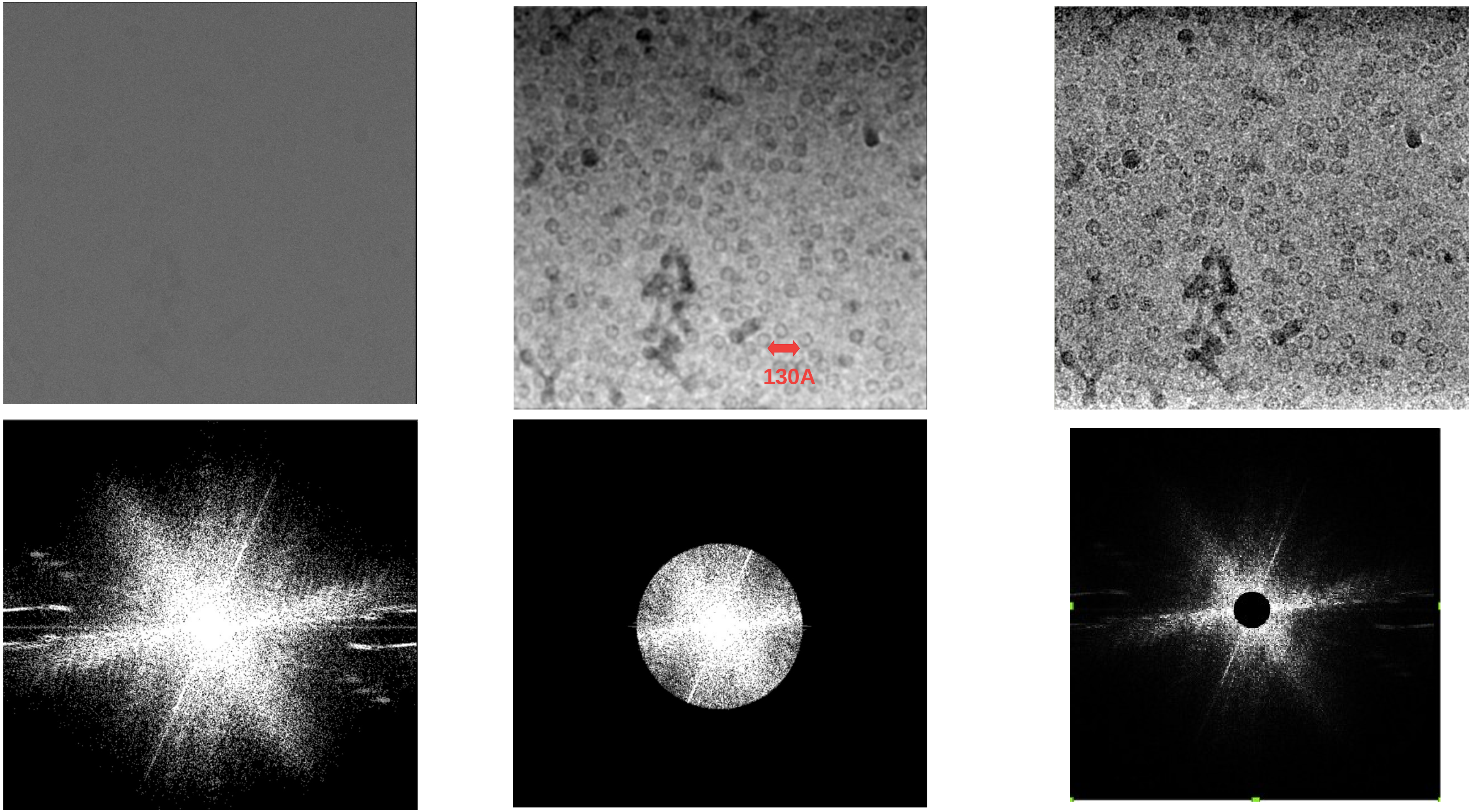
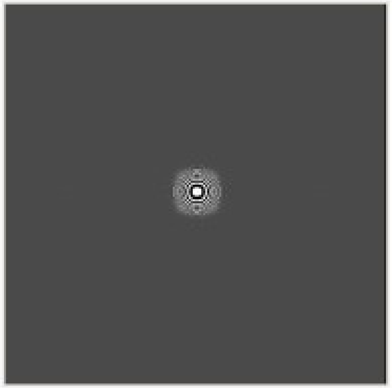
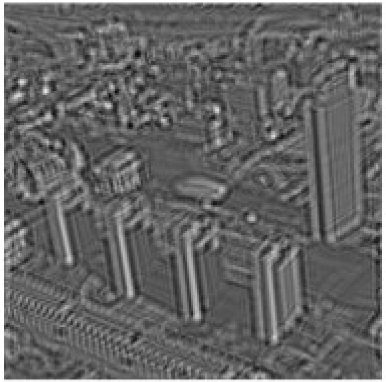


Image formation

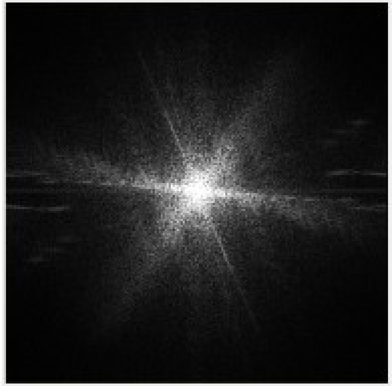
$f(x)$



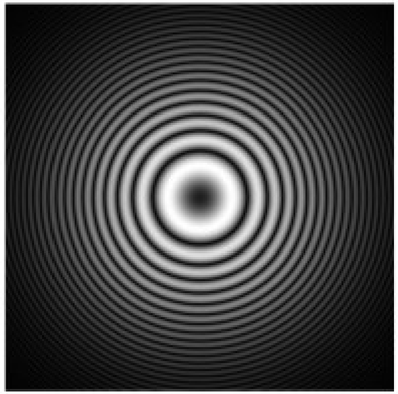
$g(x)$



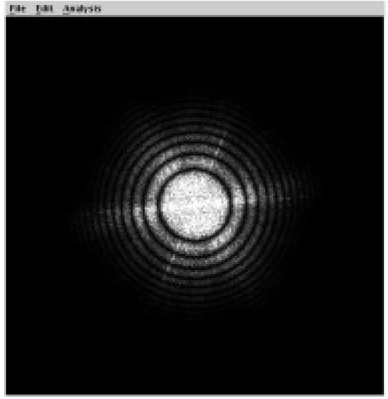
$f(x) \bullet g(x)$



$F(X)$

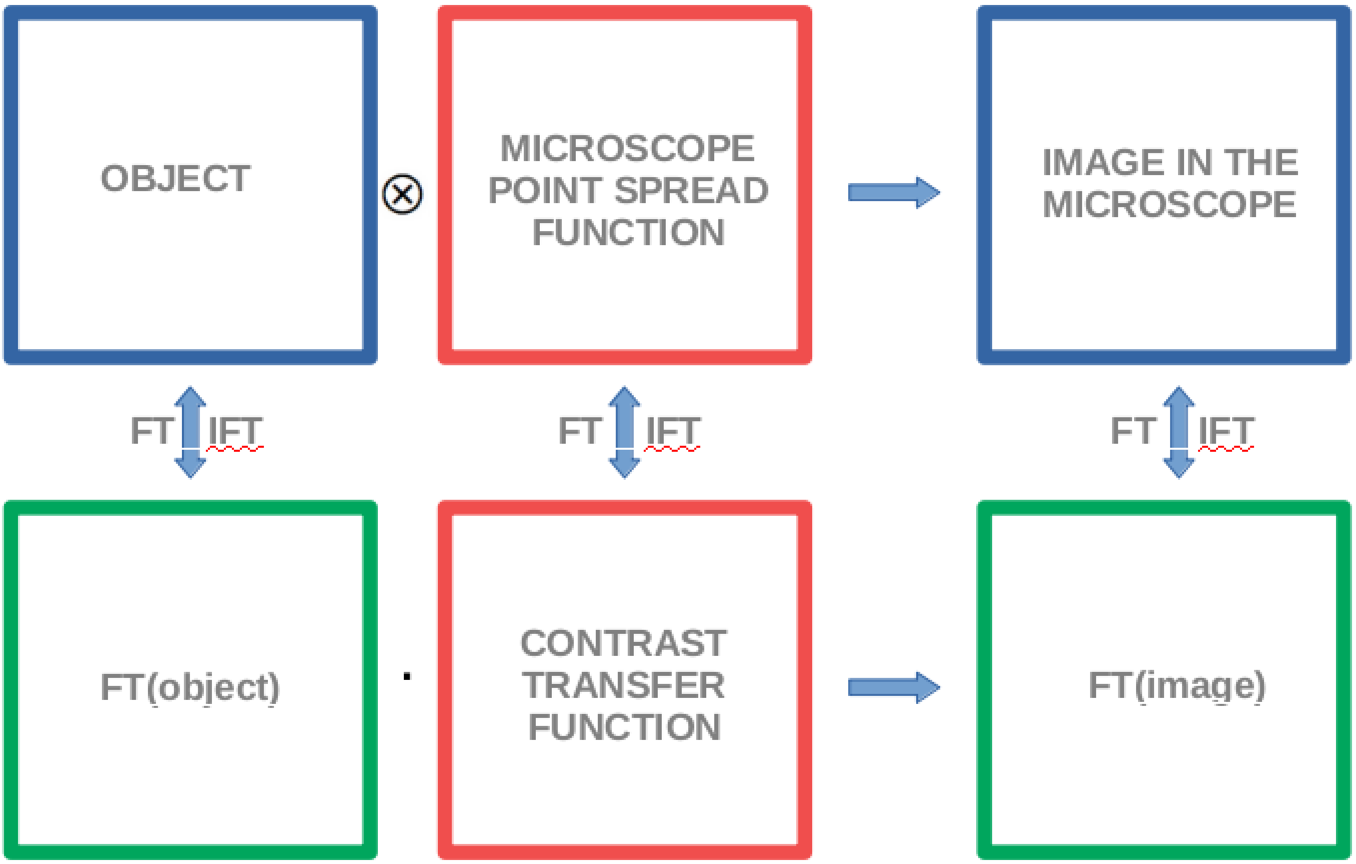


$G(X)$



$F(X)\ G(X)$

Image formation



Contrast transfer function

$$\mathrm{CTF}\ (\vec{s}) = -\sqrt{1-A^2}\cdot\sin(\gamma(\vec{s})) - A\cdot\cos(\gamma(\vec{s}))$$

$$\gamma(\vec{s}) = \gamma(s,\theta) = -\frac{\pi}{2}C_s\lambda^3 s^4 + \pi\lambda z(\theta)s^2$$

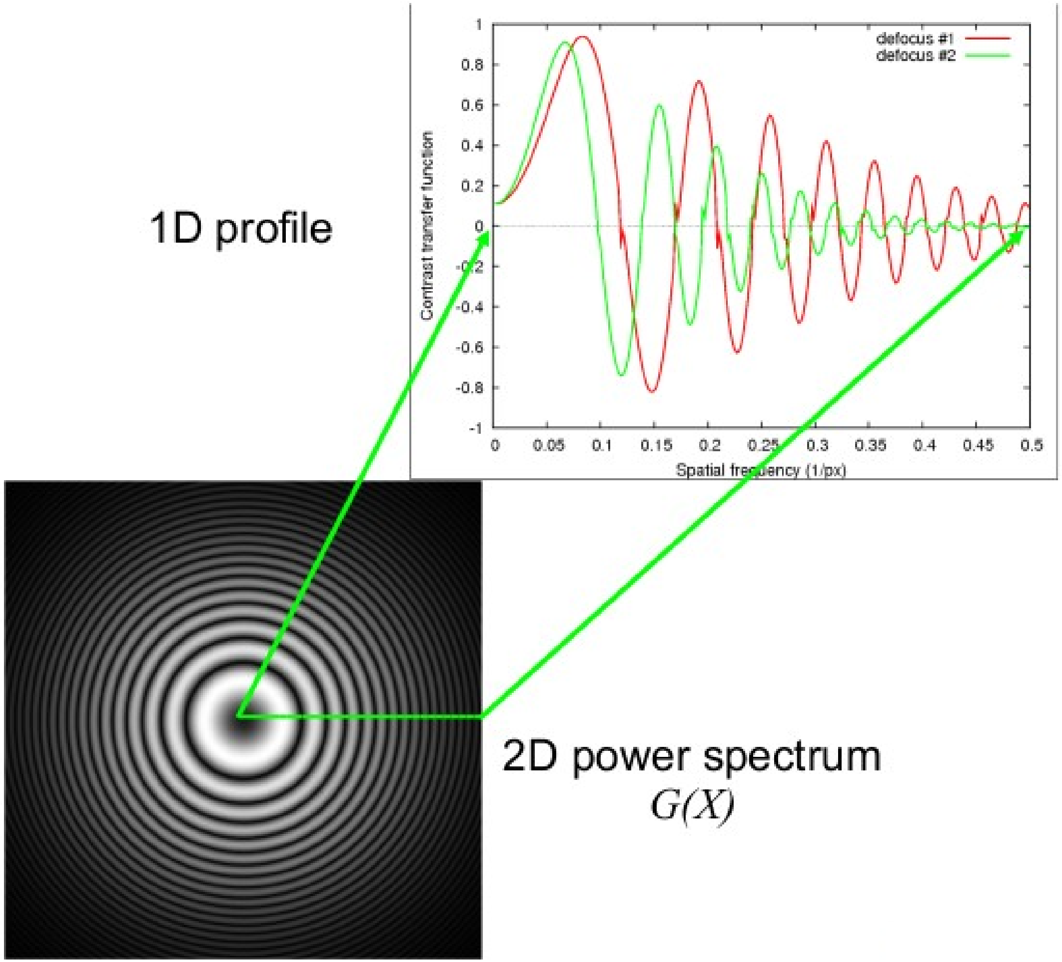
A – amplitude contrast

s – spatial frequency

Cs – spherical abberation

λ – electron wavelength

z – defocus



Contrast transfer function

Envelope function

- Finite source size

$$E_{pc}(k) = \exp\left[-\pi^2 q^2 (k^3 C_s \lambda^3 - \Delta z k \lambda)^2\right],$$

- Energy spread (defocus)

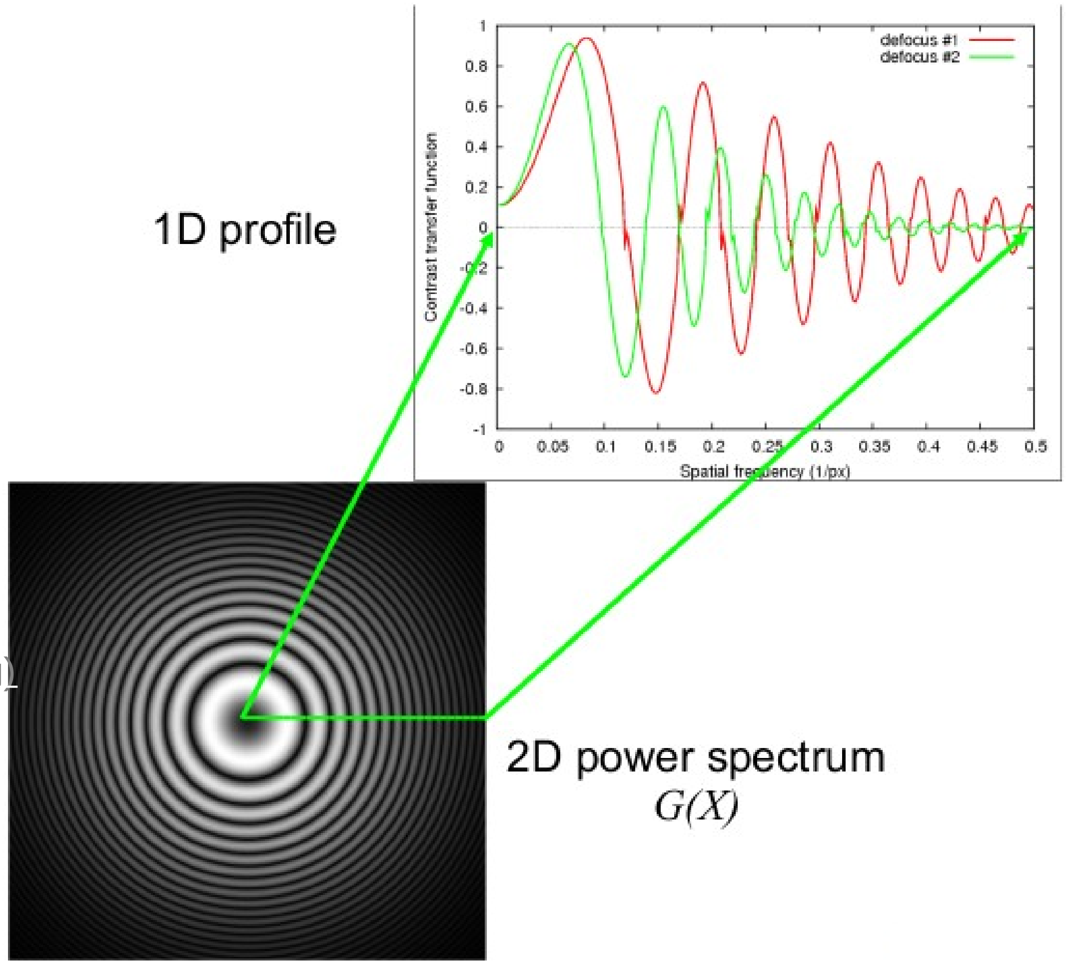
$$E_{es}(k) = \exp\left[-\frac{1}{16 \ln 2} \pi^2 \delta z^2 k^4 \lambda^2\right],$$

- MTF of the camera

$$E_f(k) = 1/[1 + (k/k_f)^2],$$

- Generic envelope (drift, charging, multiple scattering)

$$E_g(k) = \exp\left[-(k/k_g)^2\right],$$



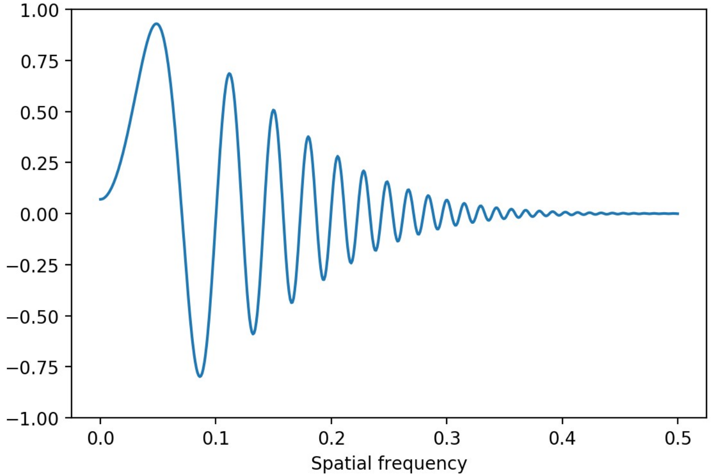
Contrast transfer function

Envelope function

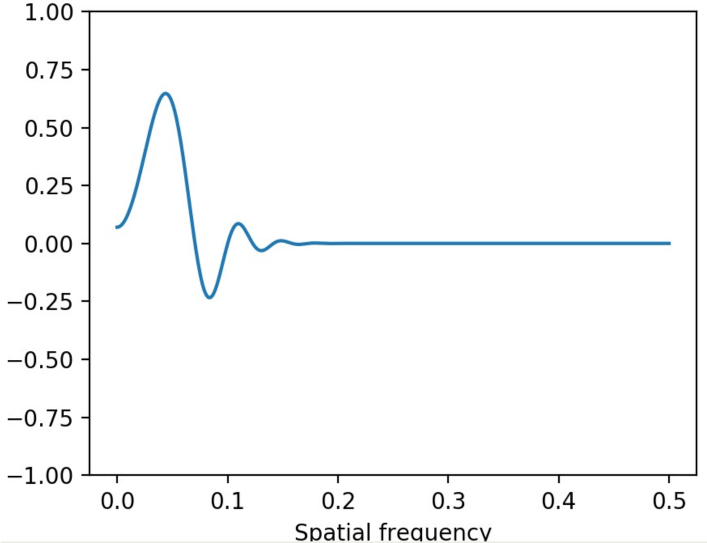
$$I(\mathbf{k}) = E_{pc}(k)E_{es}(k)E_f(k)E_g(k)H(k)\Phi(\mathbf{k}) + N(\mathbf{k}).$$

$$e^{-Bk^2}$$

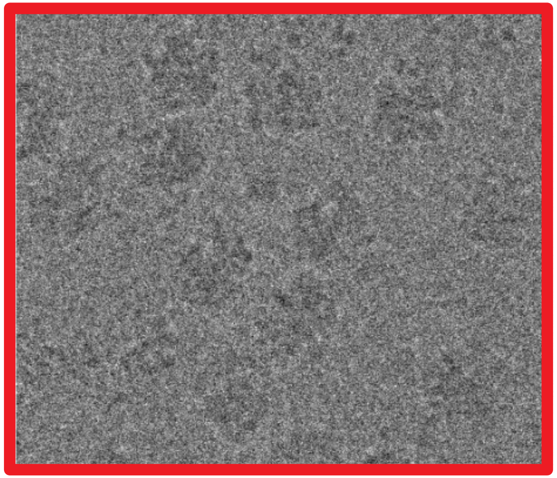
kV=300,ac=0.07,cs=2.7,z=-1,apix=1,B=30



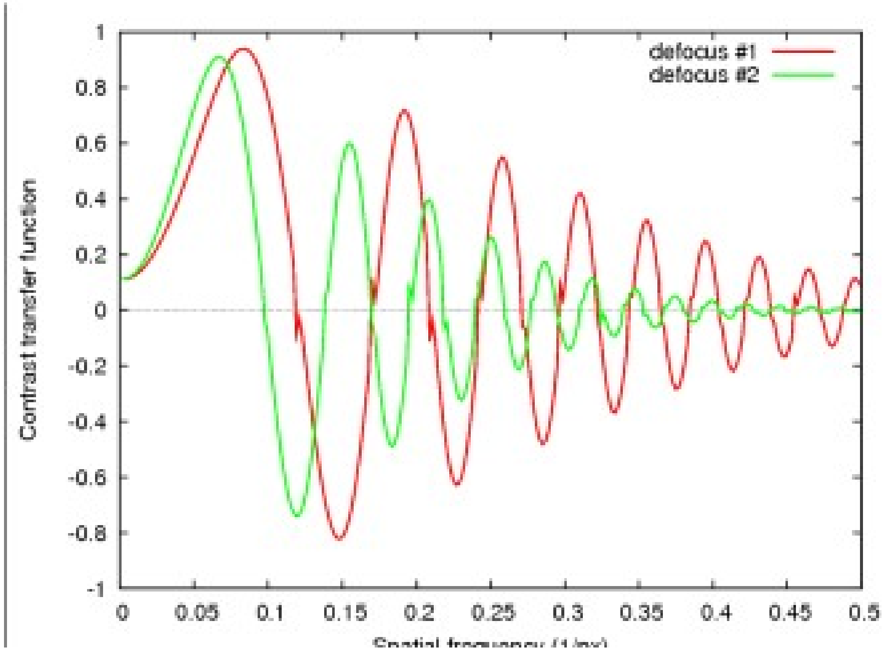
kV=300,ac=0.07,cs=2.7,z=-1,apix=1,B=300

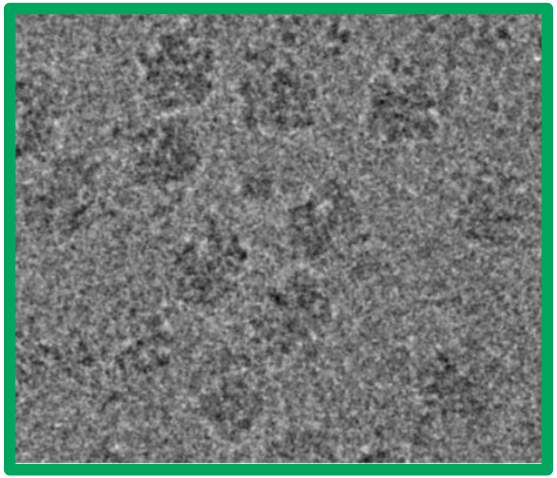


Contrast transfer function



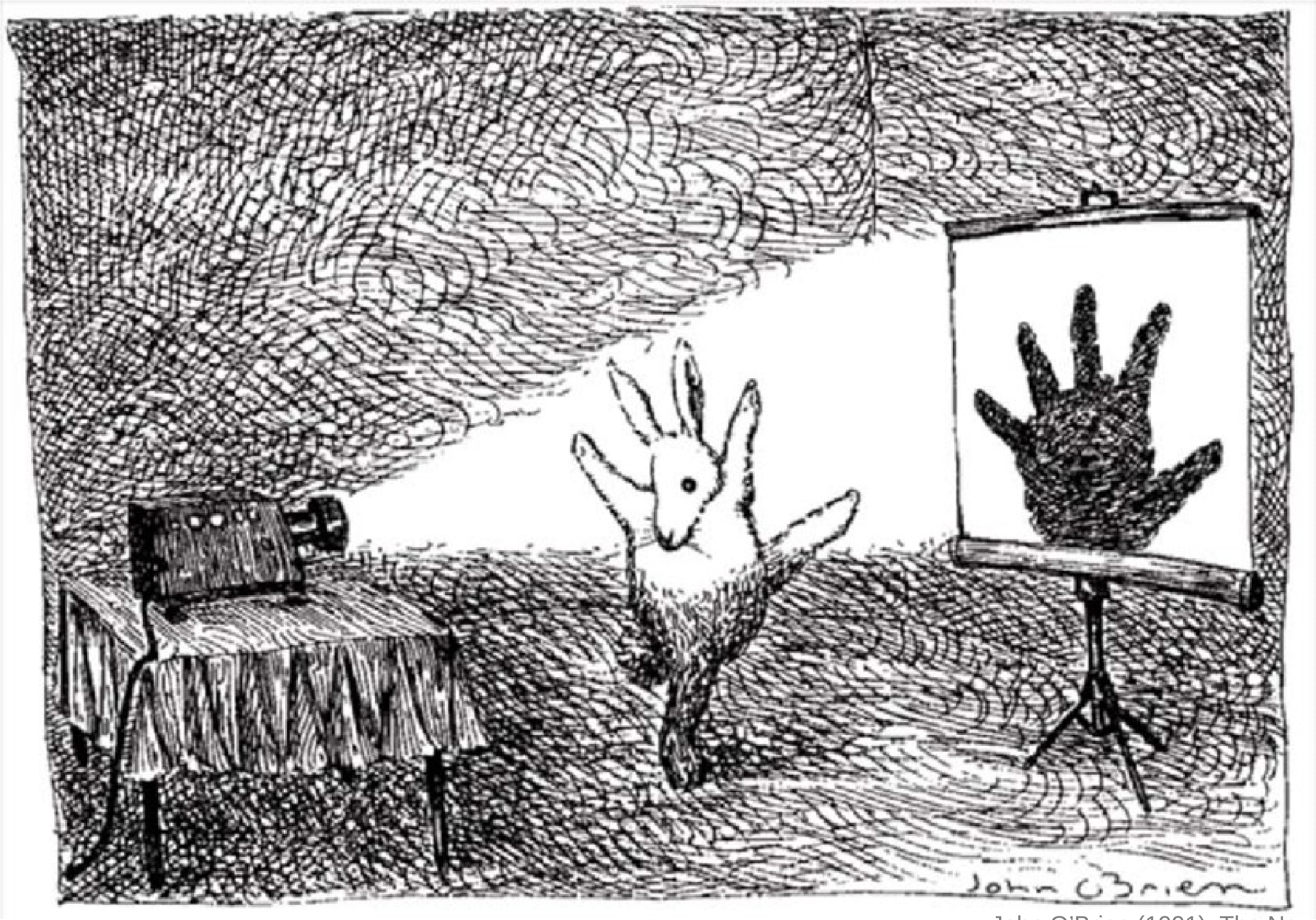
Low defocus





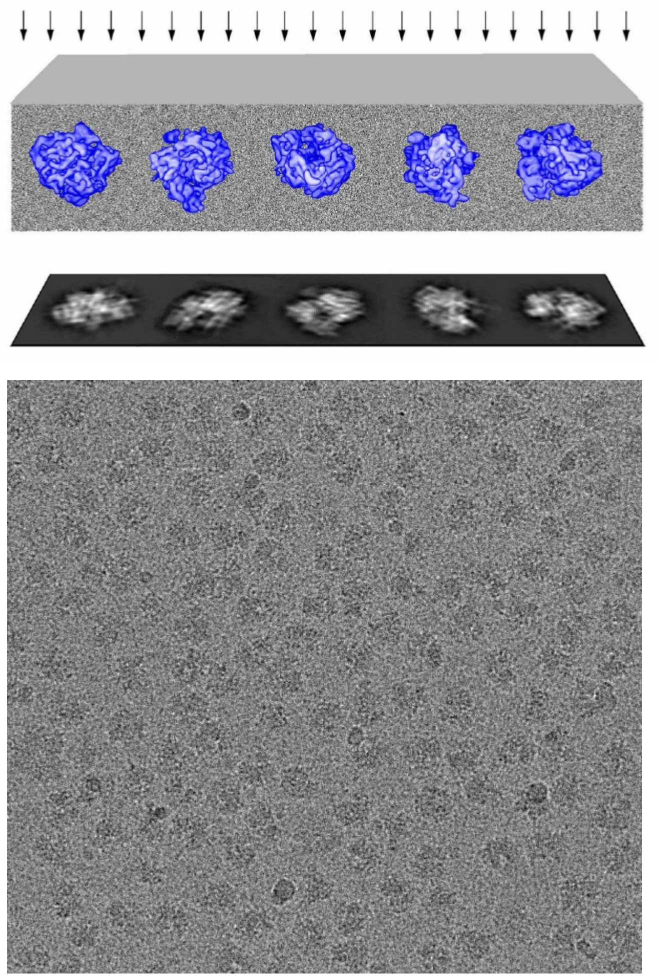
High defocus

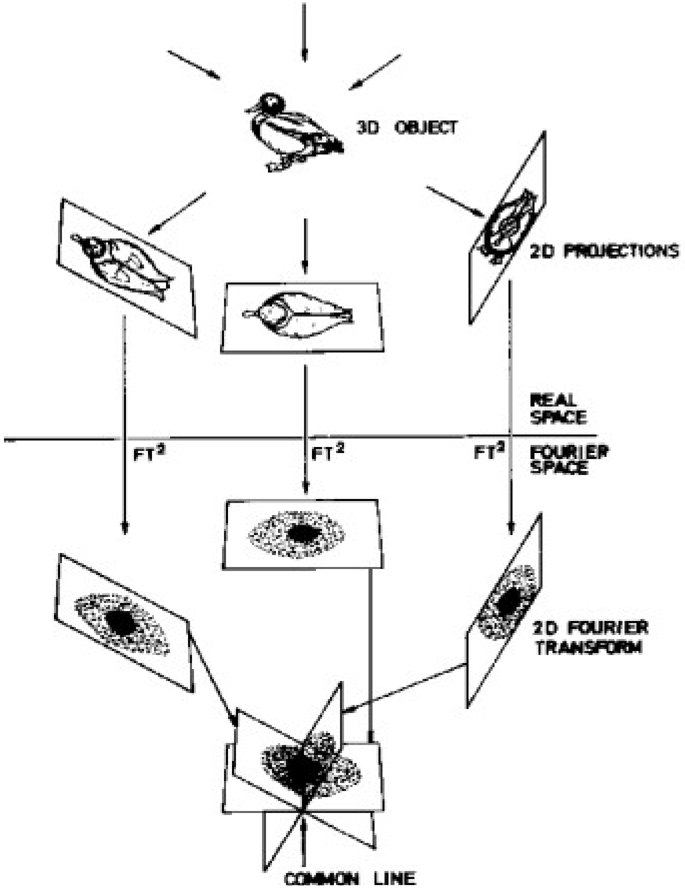
Projection theorem



John O’Brien (1991). The New Yorker

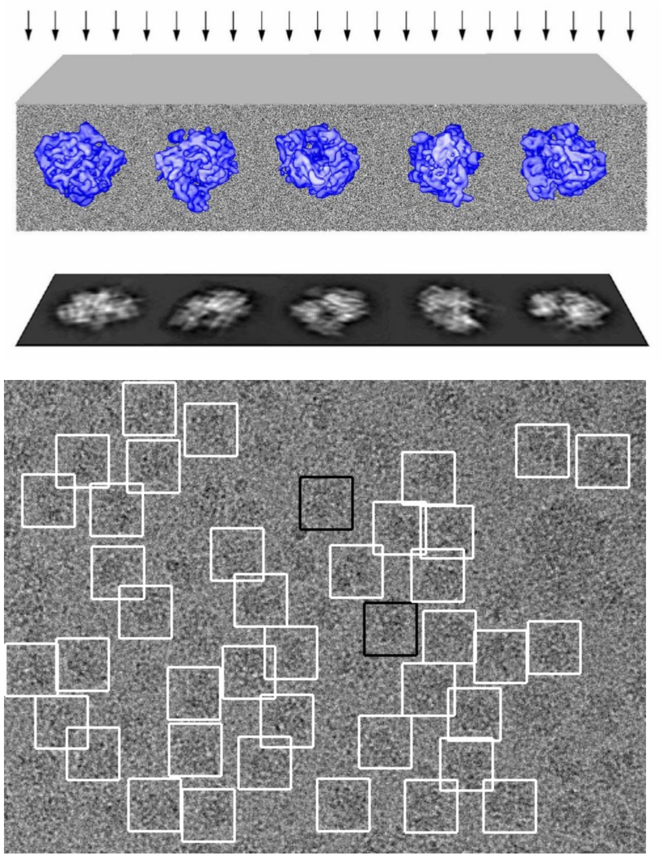
Projection theorem

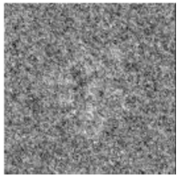




The 2D Fourier transform of the projection of a 3D density is a central section of the 3D Fourier transform of the density, perpendicular to the direction of projection.

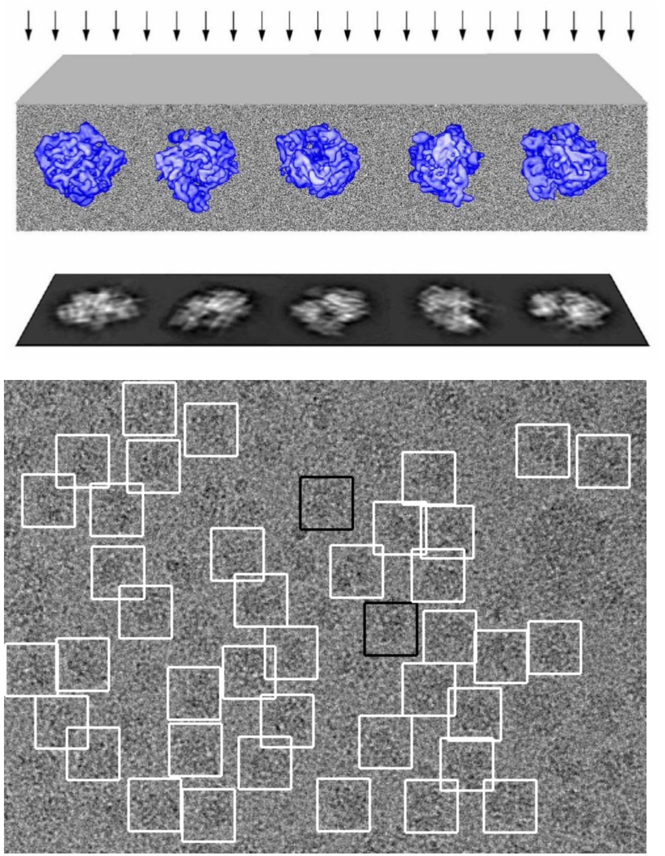
Particles (regions of interest)

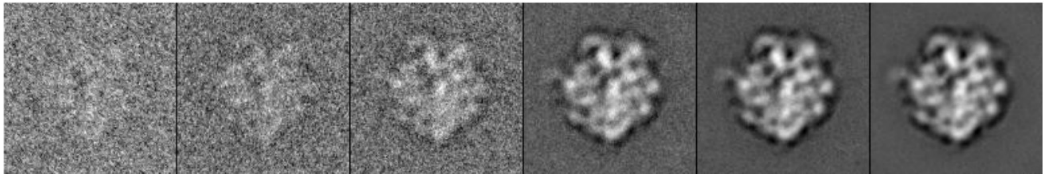




n=1

Particles (regions of interest)

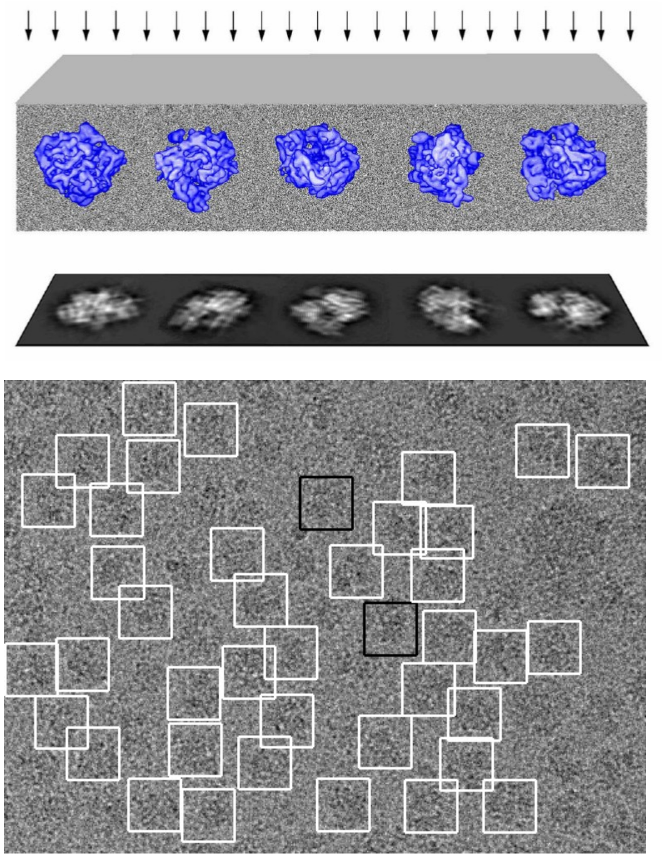




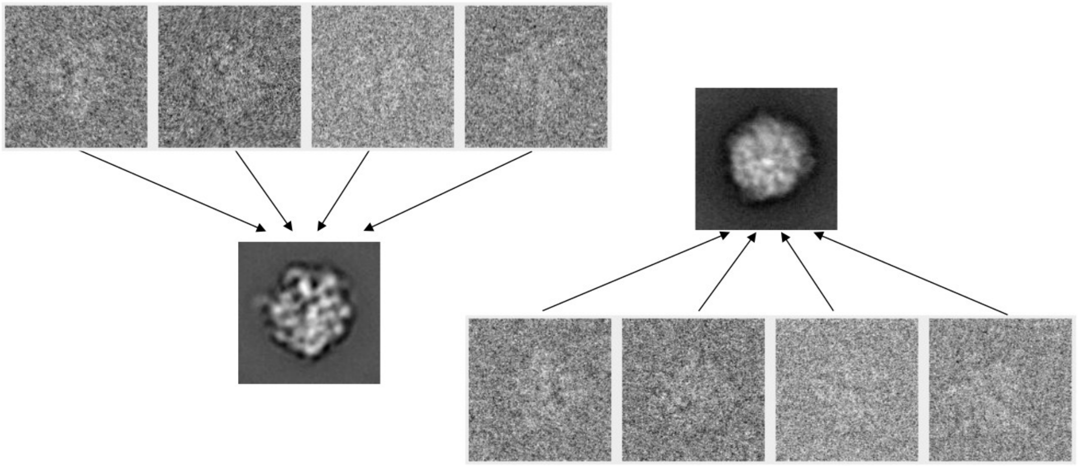
n=1 n=2 n=8 n=16 n=64 n=256

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

Image alignment in 2D

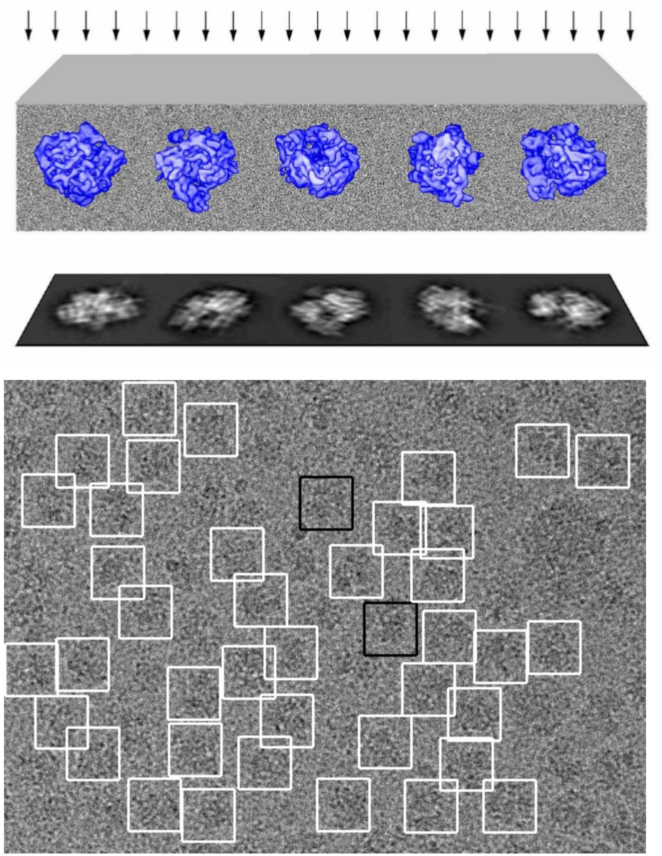


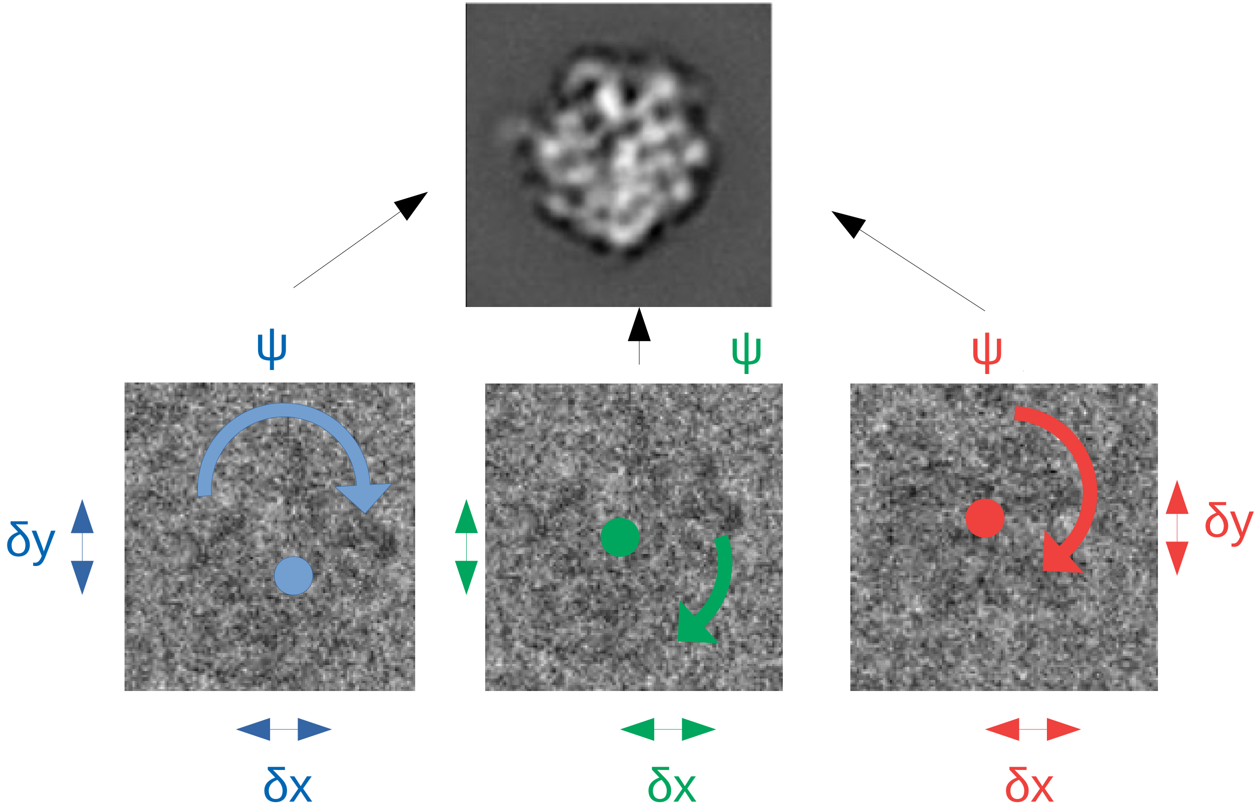
Sum of aligned particles



Sum of unaligned particles

Image alignment in 2D



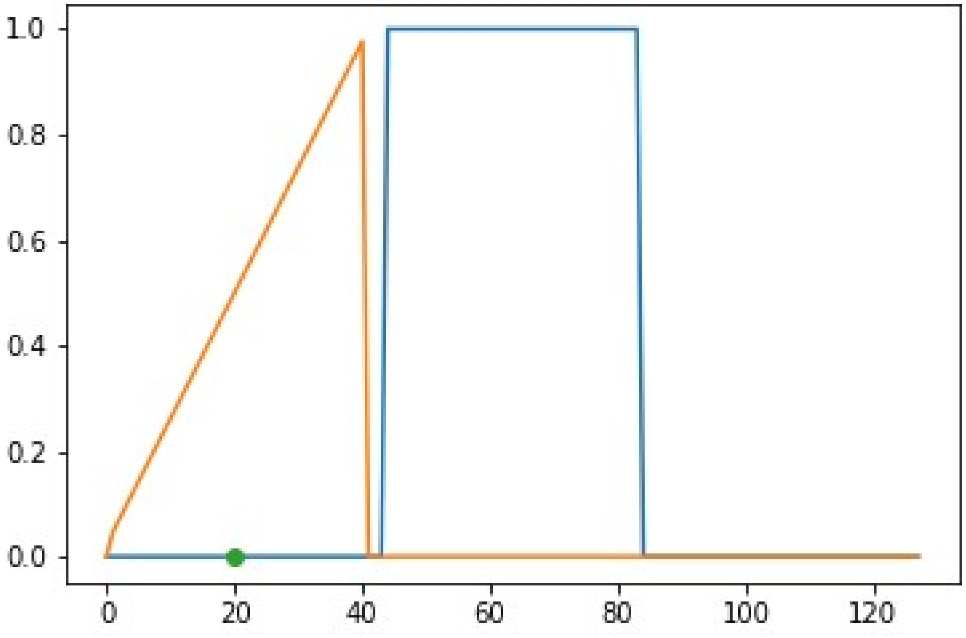


In order to align the particles in 2D, we need to determine three parameters:

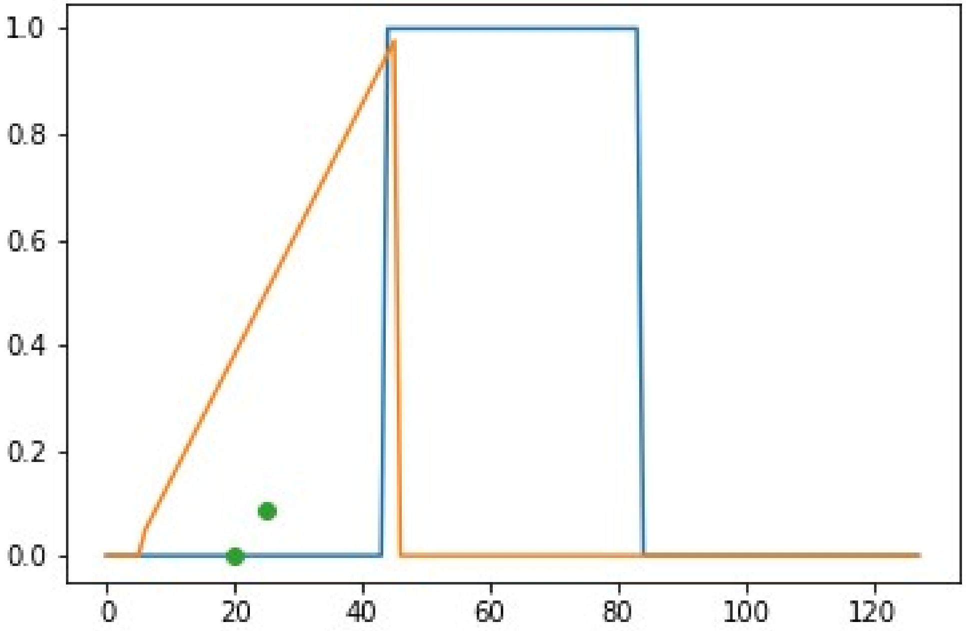
- two translational
- one rotational (on of the Euler angles)

Cross correlation

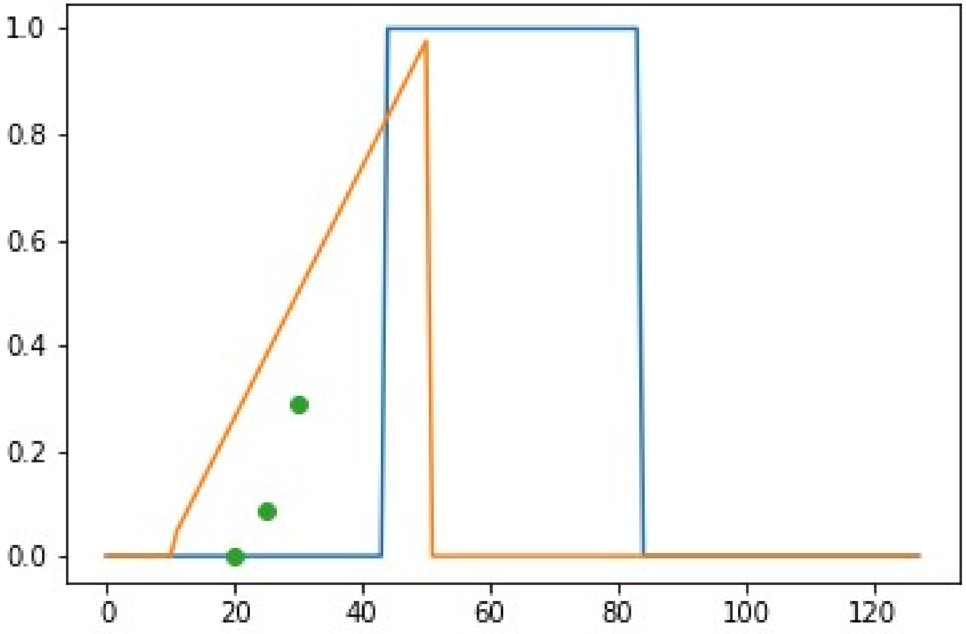
- measure of similarity of two data series as a function of displacement of these functions
- in 2D optimal overlay of two images
- normalized cross-correlation – ccc = <-1,1>



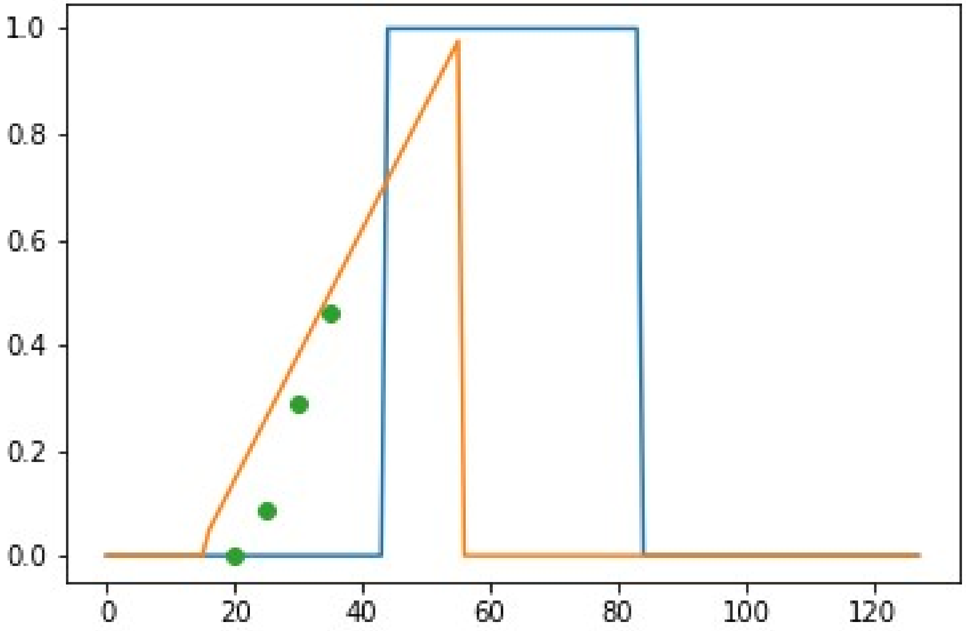
Cross correlation function in 1D



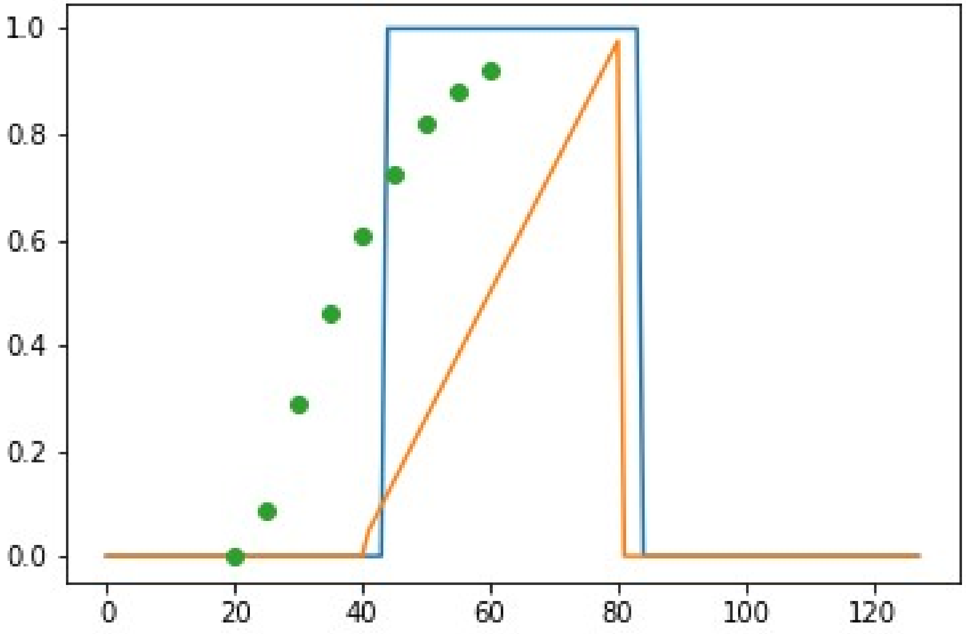
Cross correlation function in 1D



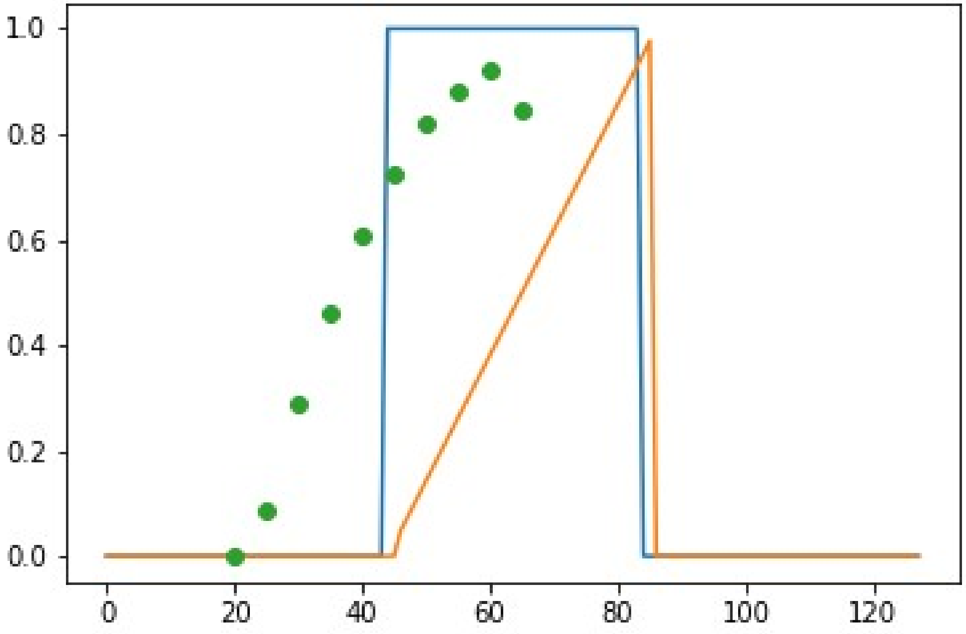
Cross correlation function in 1D



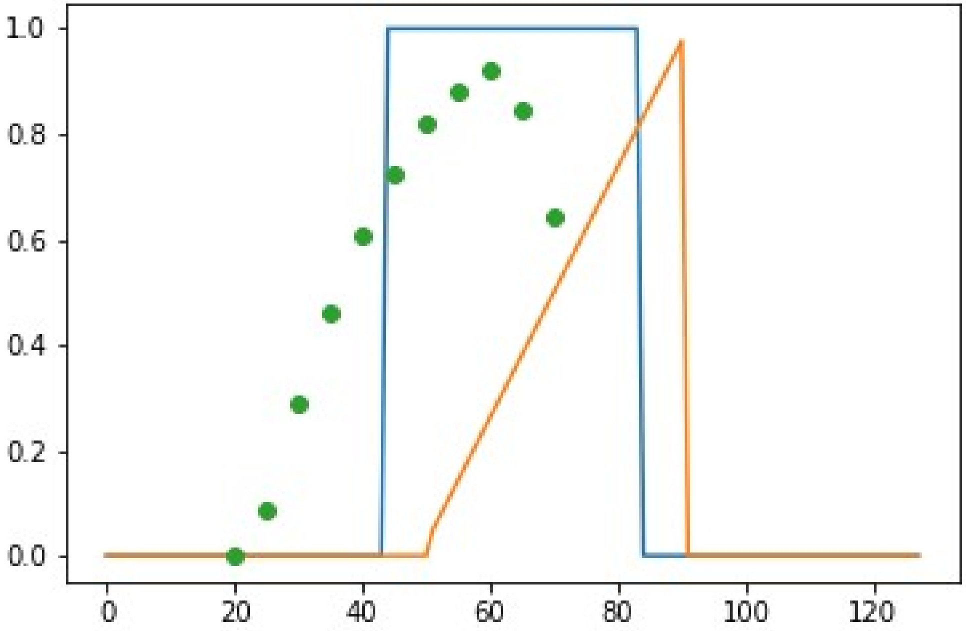
Cross correlation function in 1D



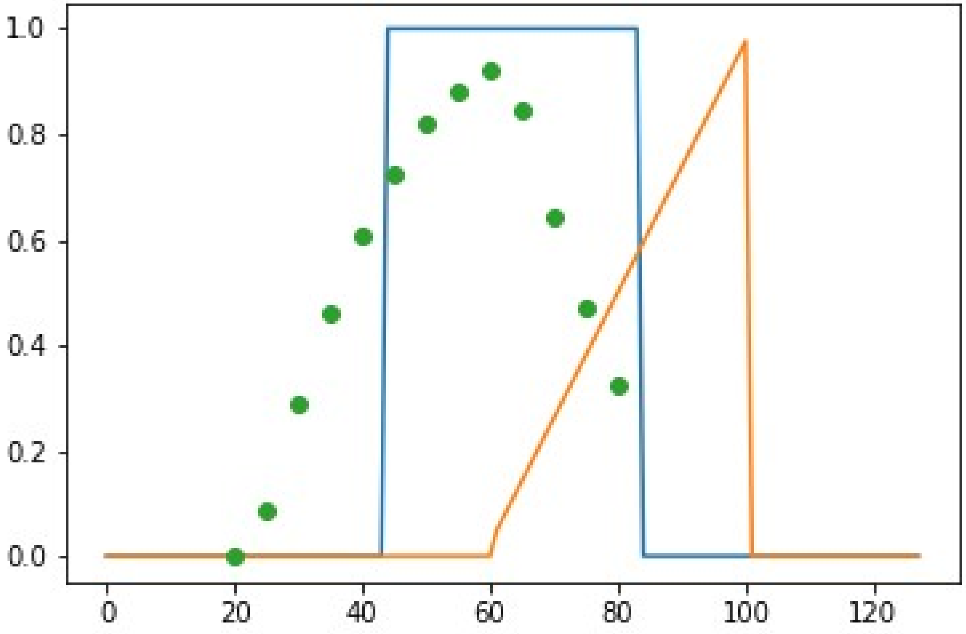
Cross correlation function in 1D



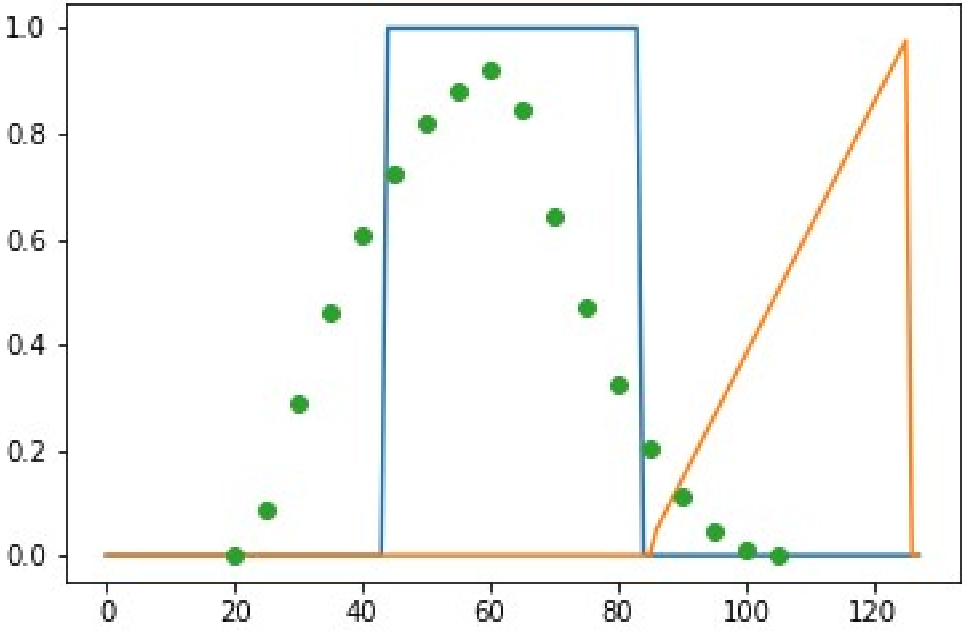
Cross correlation function in 1D



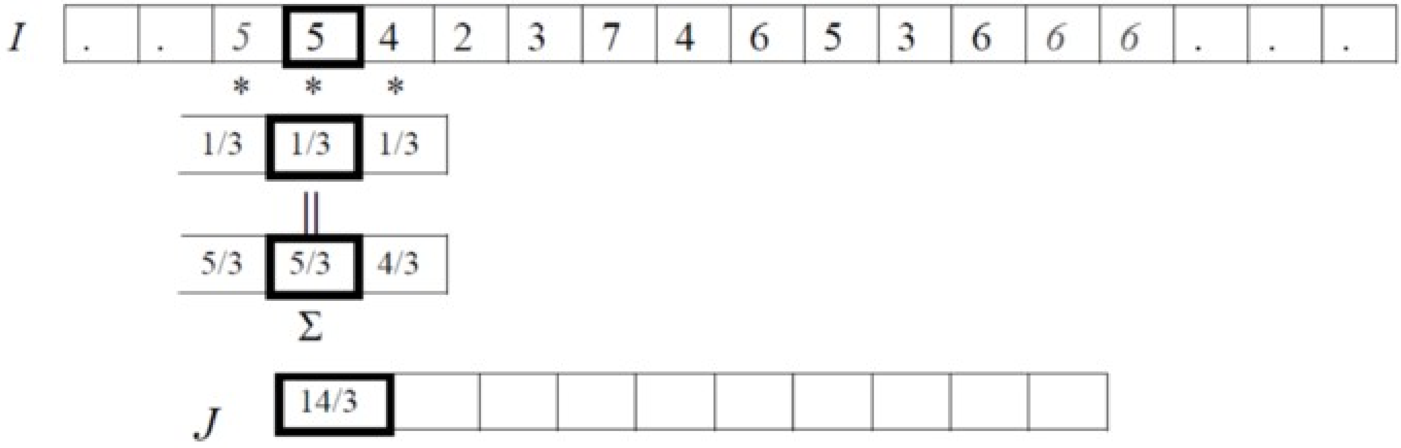
Cross correlation function in 1D



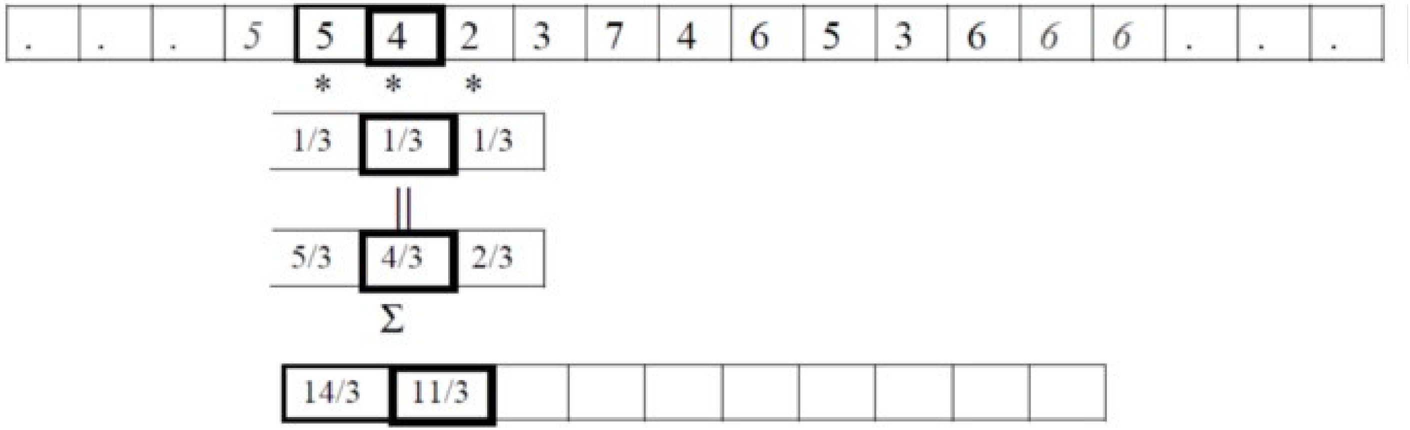
Cross correlation function in 1D



Cross correlation function in 1D



Cross correlation function in 1D



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

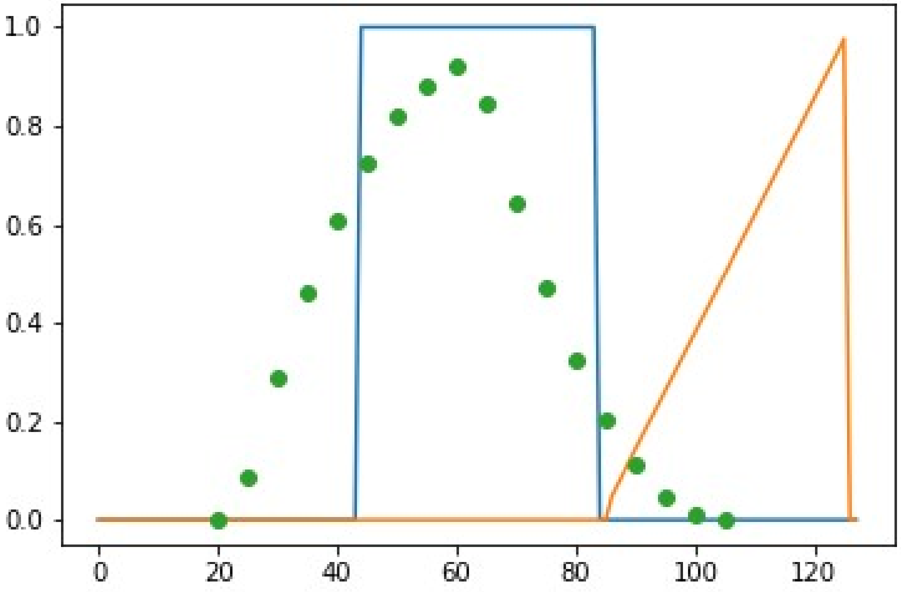
Cross correlation function in 2D

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

Cross correlation function in 2D

Cross-correlation

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



Convolution

$$F * I(x,y) = \sum_{j=-N}^{N} \sum_{i=-N}^{N} F(i,j) I(x-i, y-j)$$

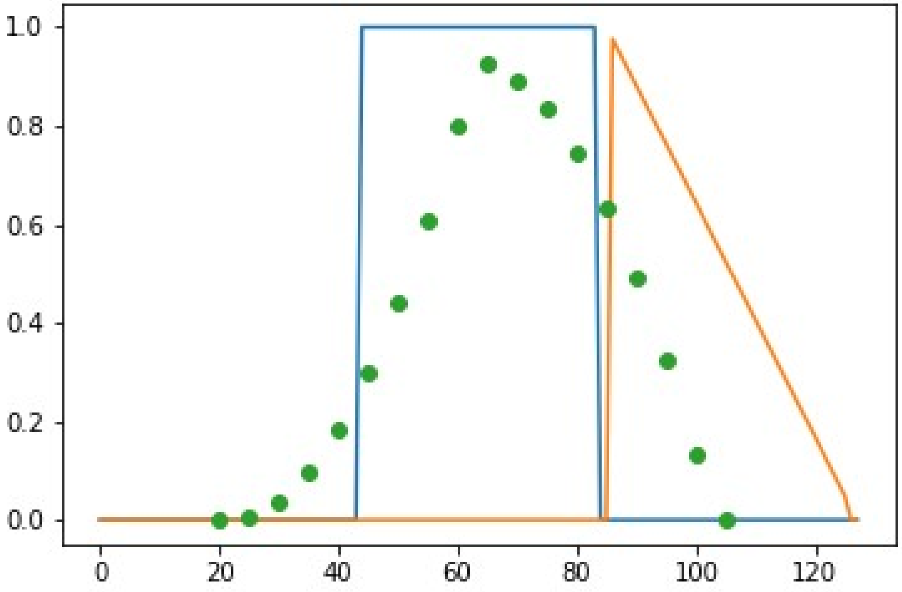
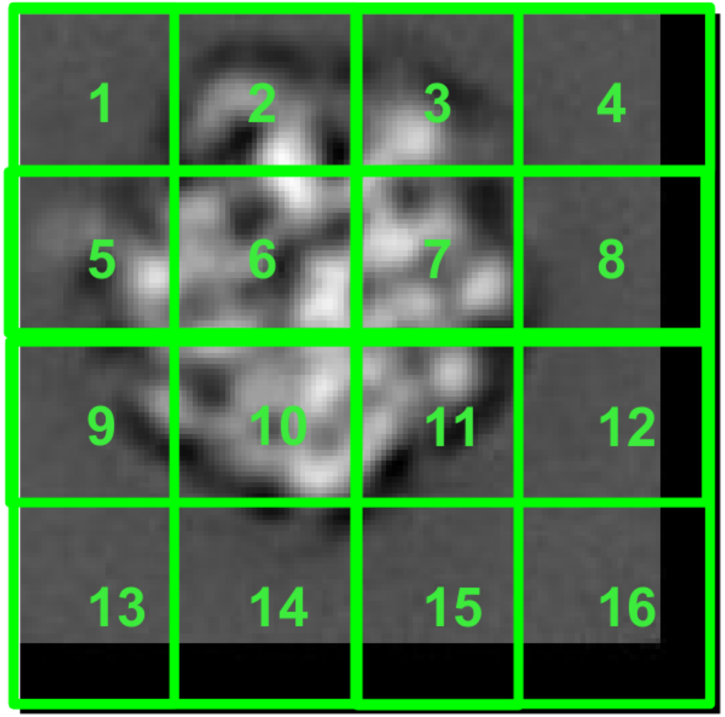
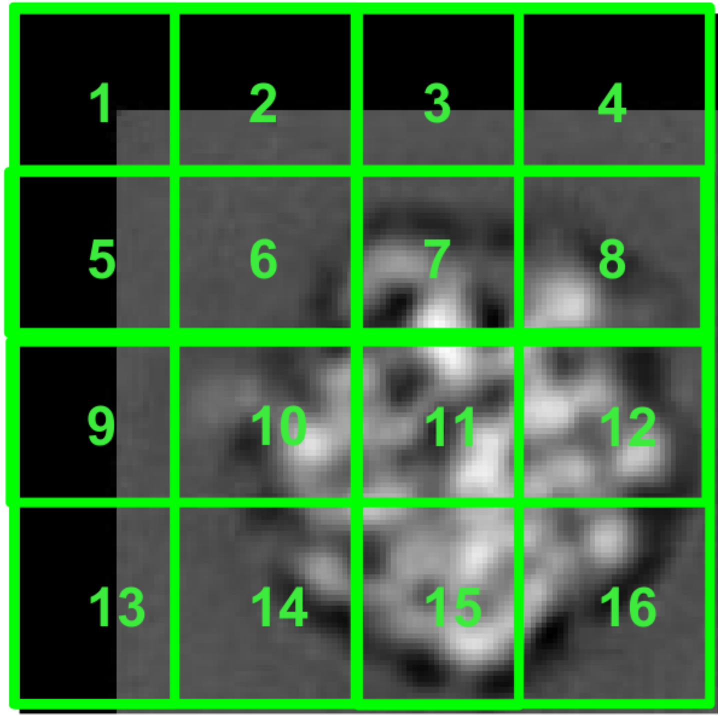


Image alignment in 2D





Cross correlation

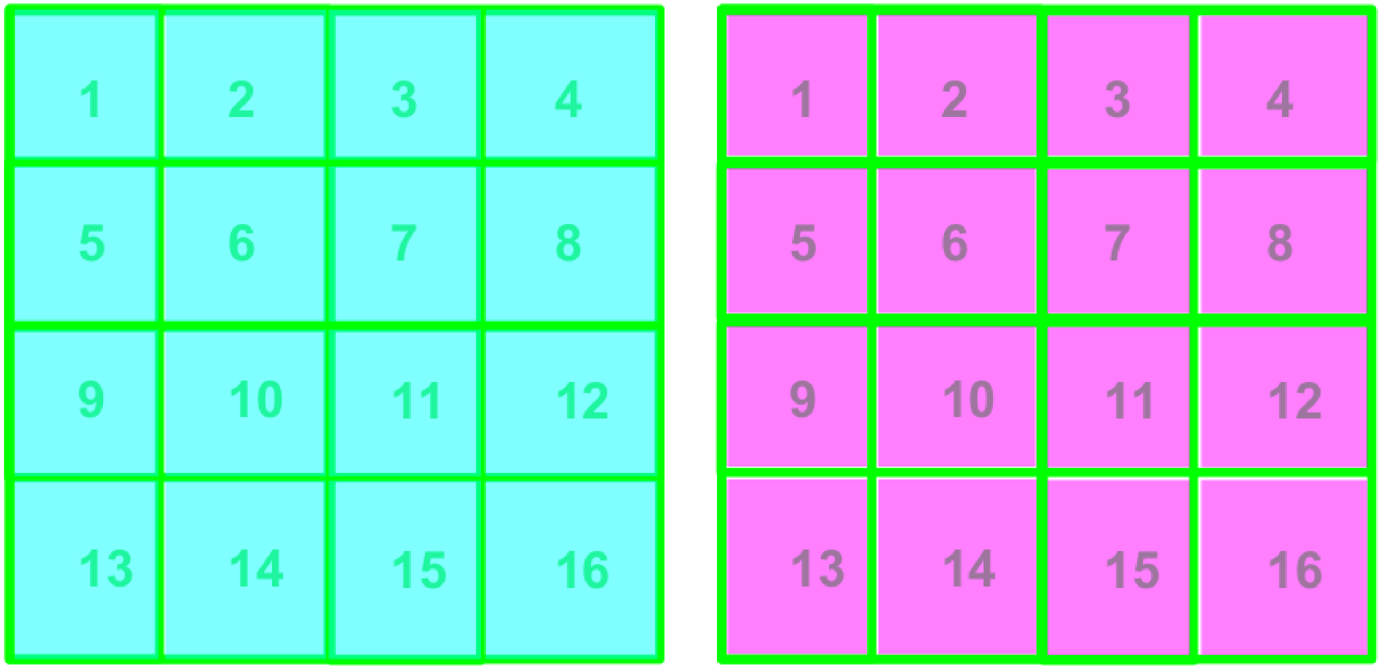


Image f Image g

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

Cross correlation

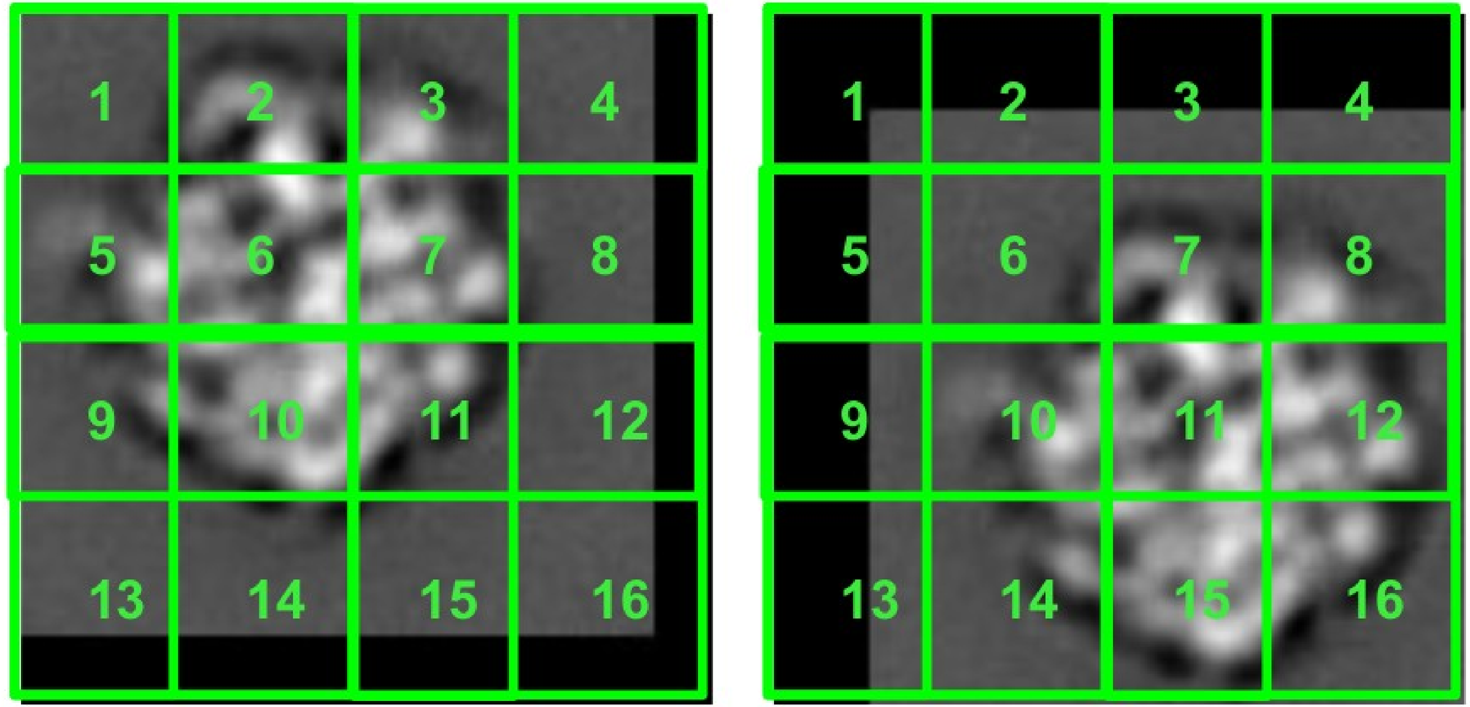


Image f Image g

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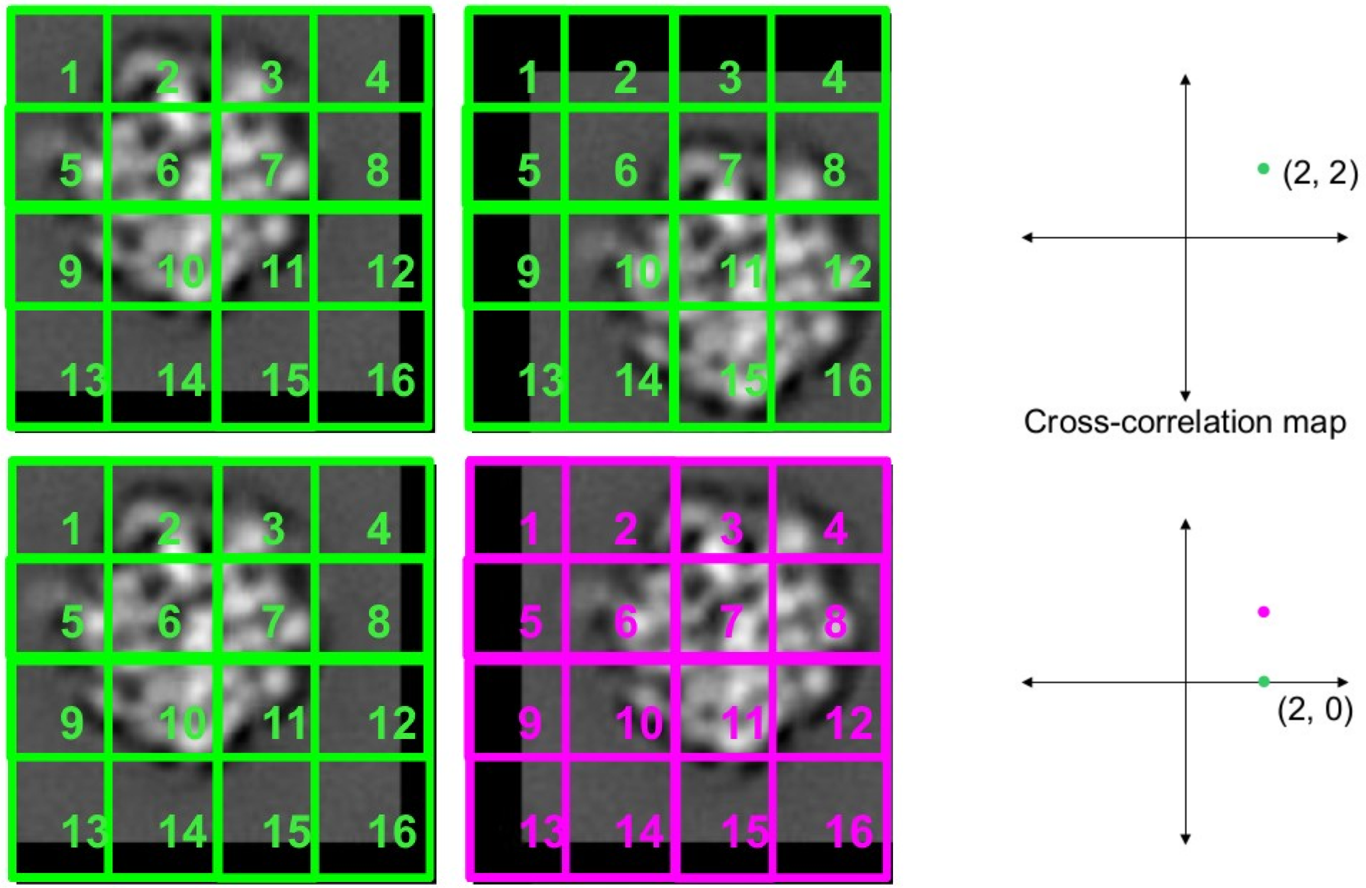
Cross correlation



Cross correlation function in 2D

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

$$F * I(x,y) = \sum_{j=-N}^{N} \sum_{i=-N}^{N} F(i,j) I(x-i, y-j)$$

Convolution

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

FT(F ∘ I) = FT(F)* . FT(I)

Convolution theorem

Cross correlation function

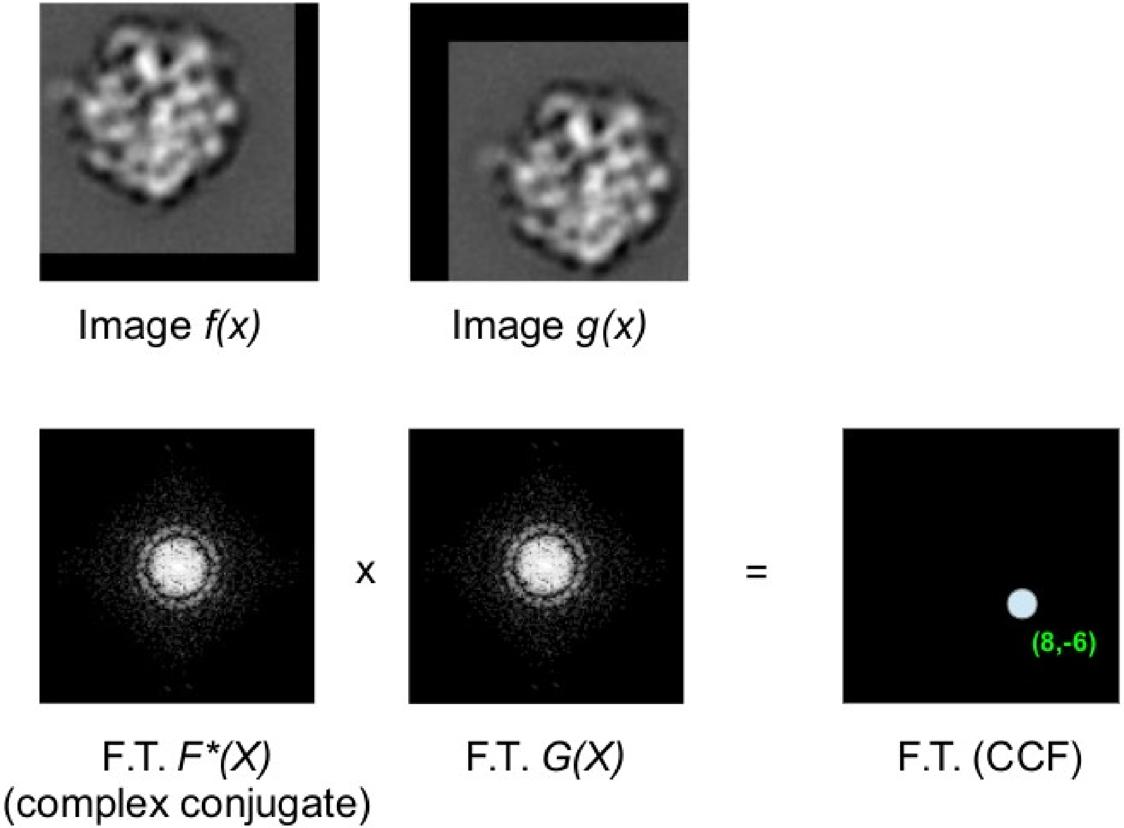
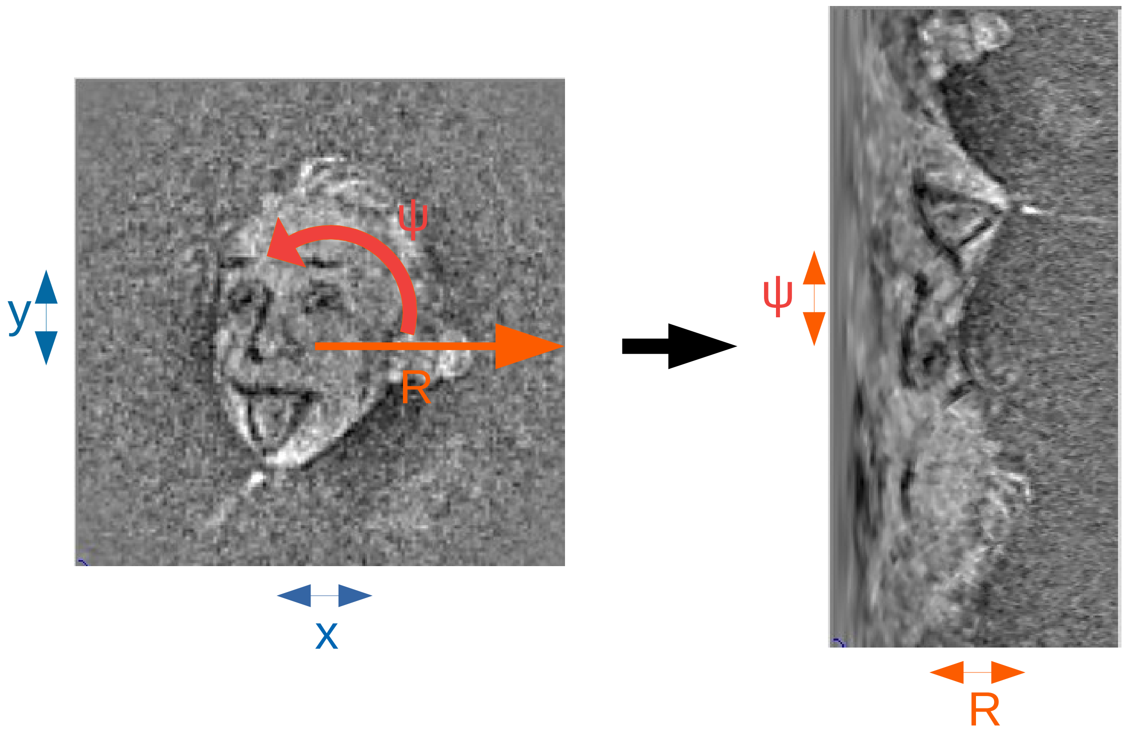


Image rotation

- 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



Orientation alignment

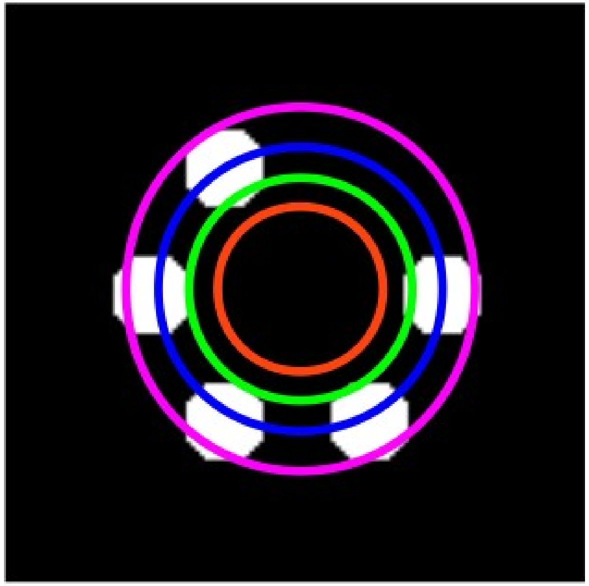


Image 1

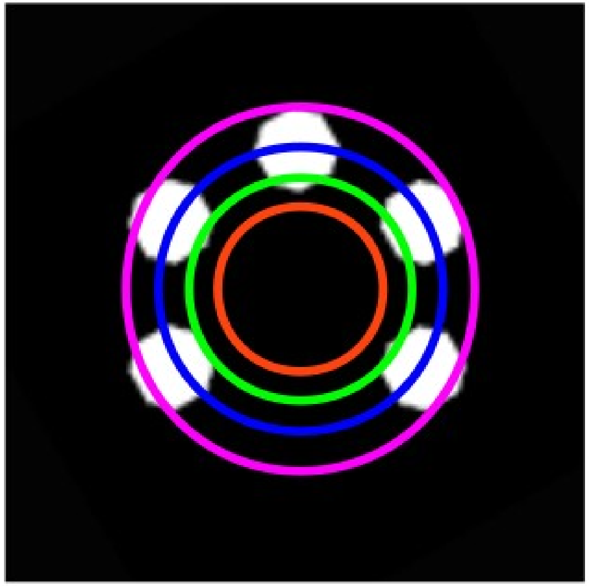
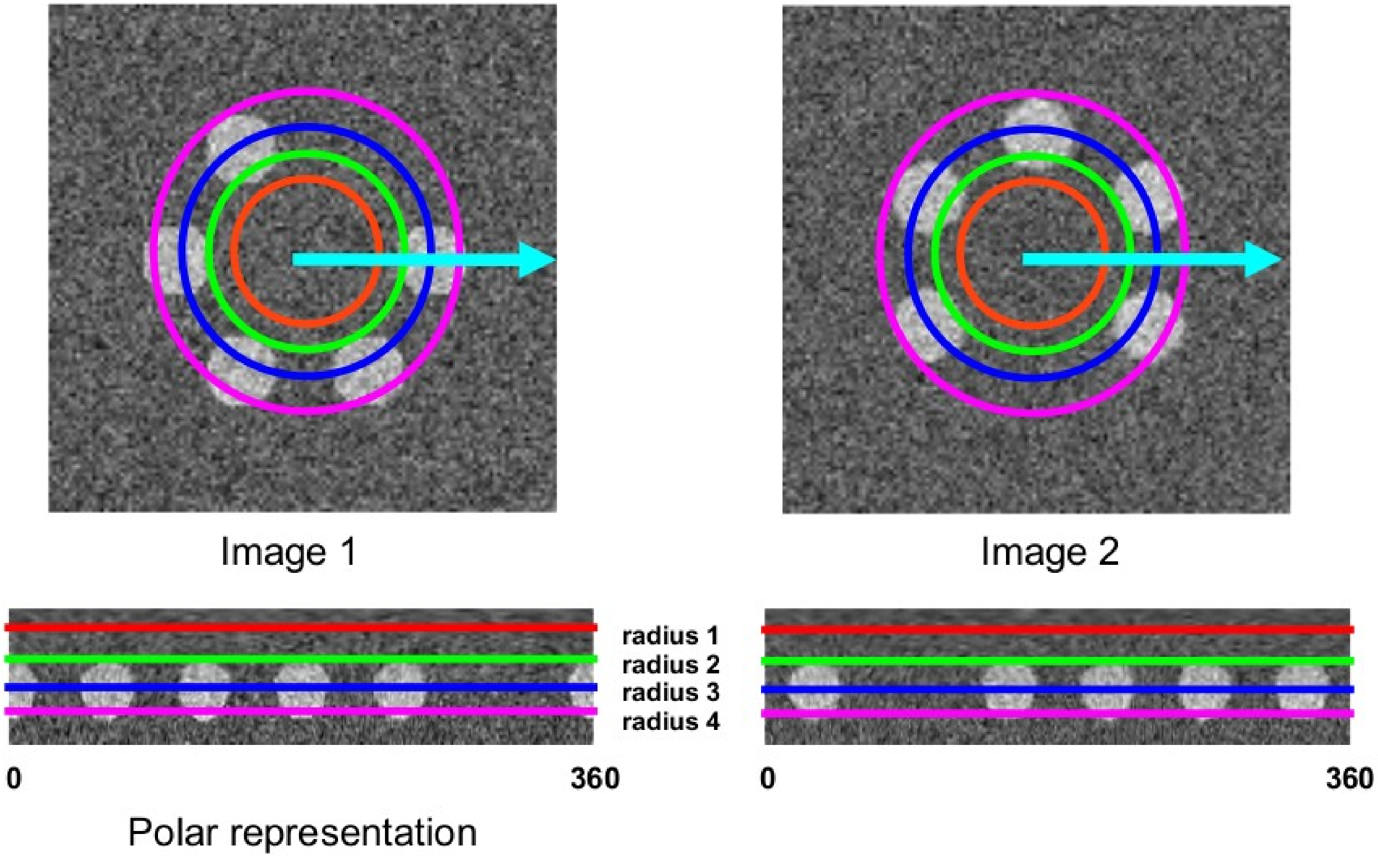


Image 2

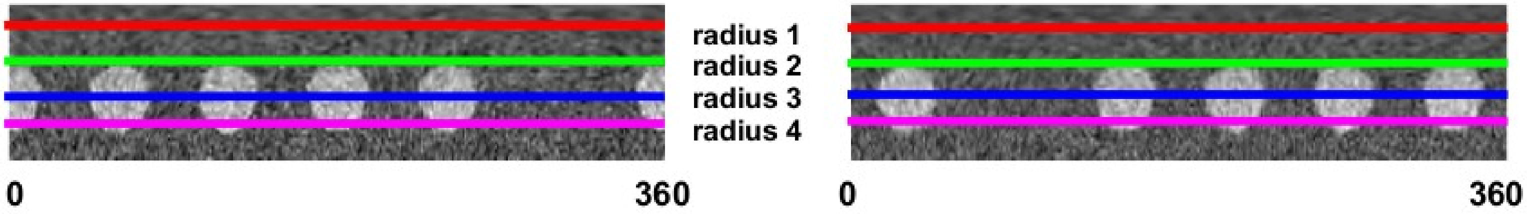
We take a series of rings from each image, unravel them,
and compute a series of 1D cross-correlation functions.

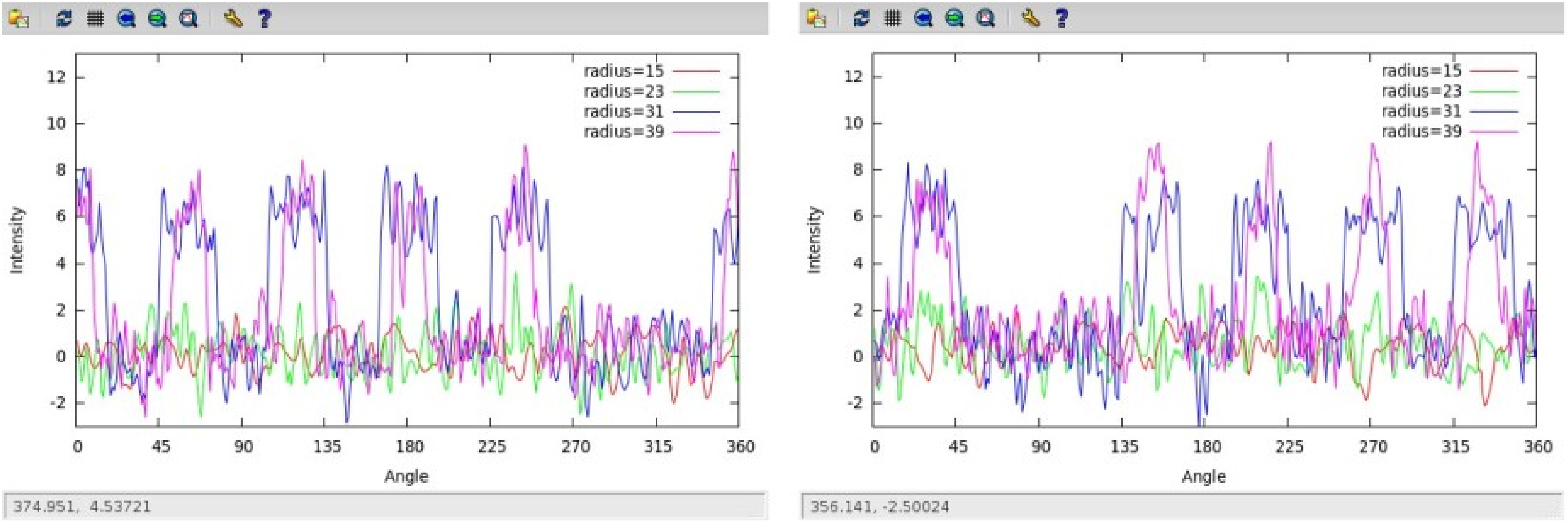
Shifts along these unraveled CCFs is equivalent to a rotation in Cartesian space.

Orientation alignment



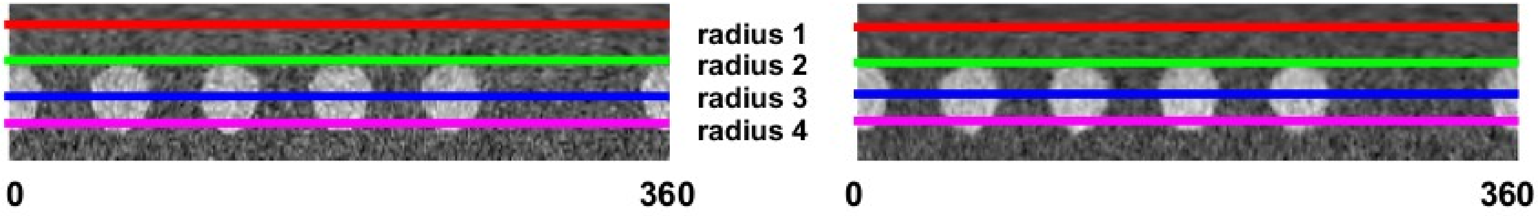
Orientation alignment

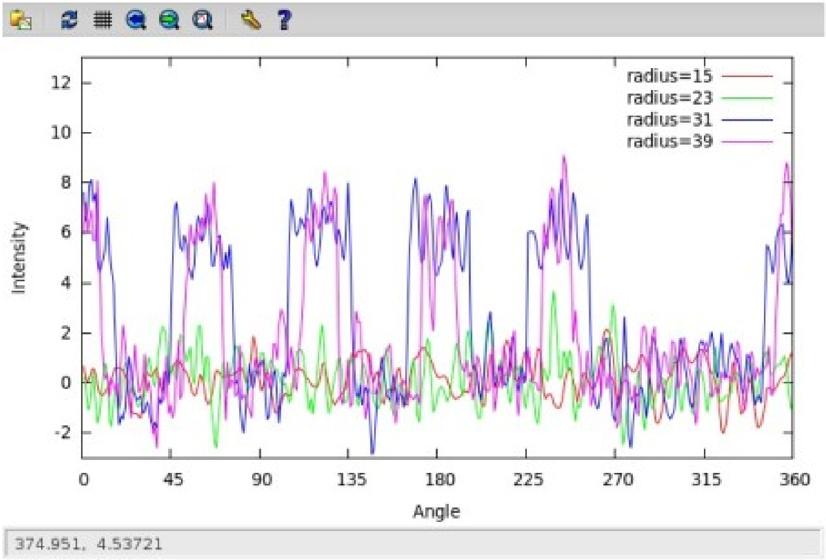




Orientation alignment

- after rotation





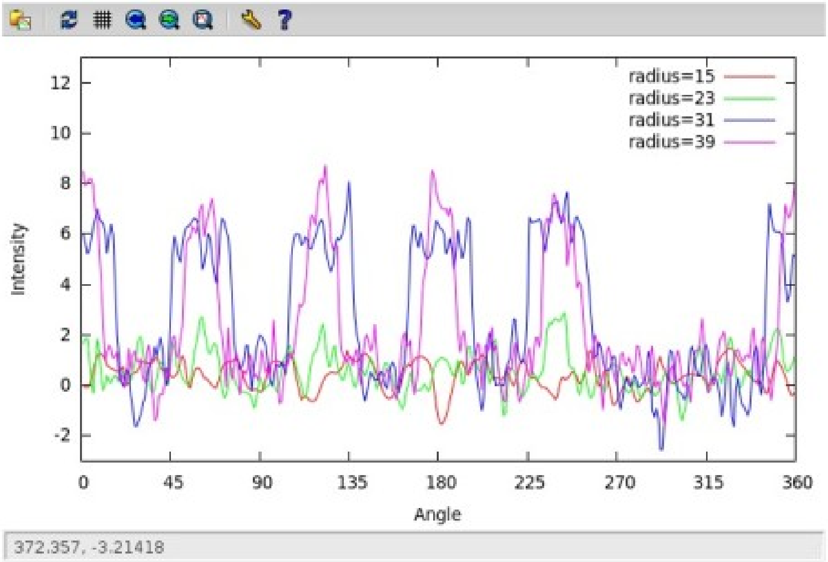


Image alignment in 2D

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

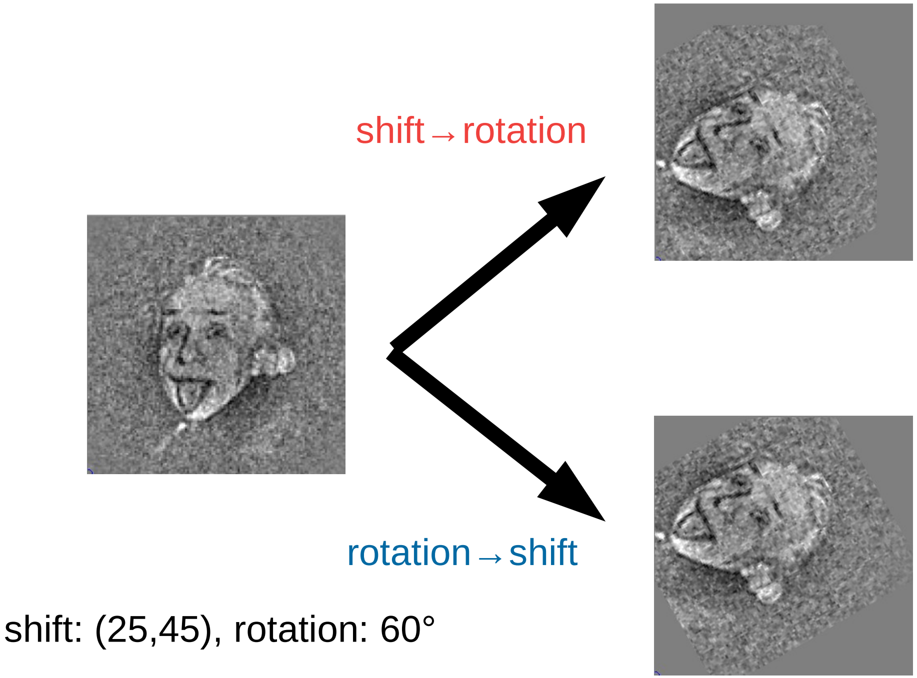


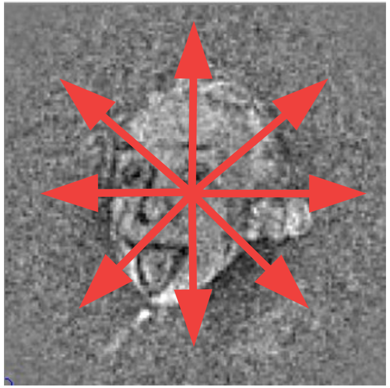
Image alignment in 2D

- 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

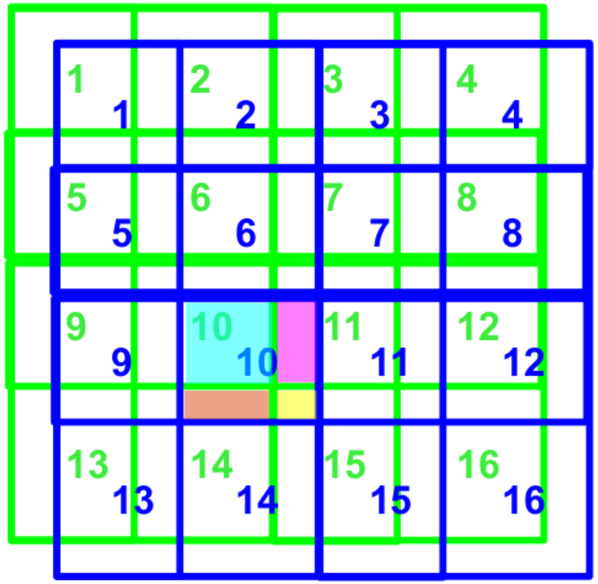
Example: for the shift of +/-2 pixels in *x* and *y* → 25 alignment rotational alignments → each alignment results in optimal rotational alignment and *ccc* → compare *ccc* and select maximal *ccc* to determine the final shift and translation

=> increased complexity



Interpolation

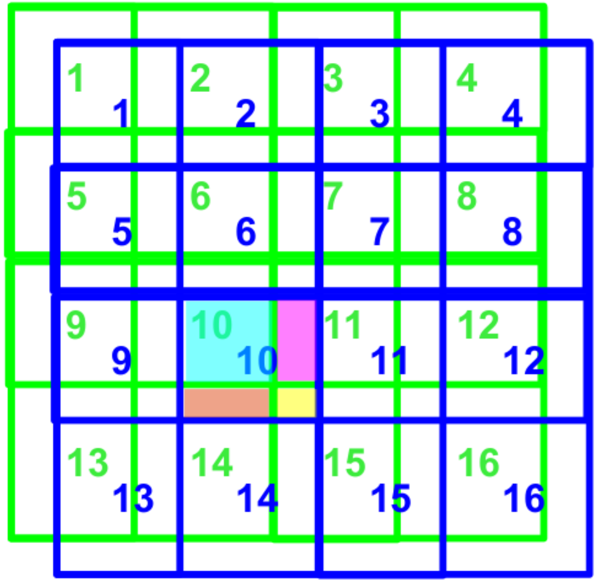
Shift

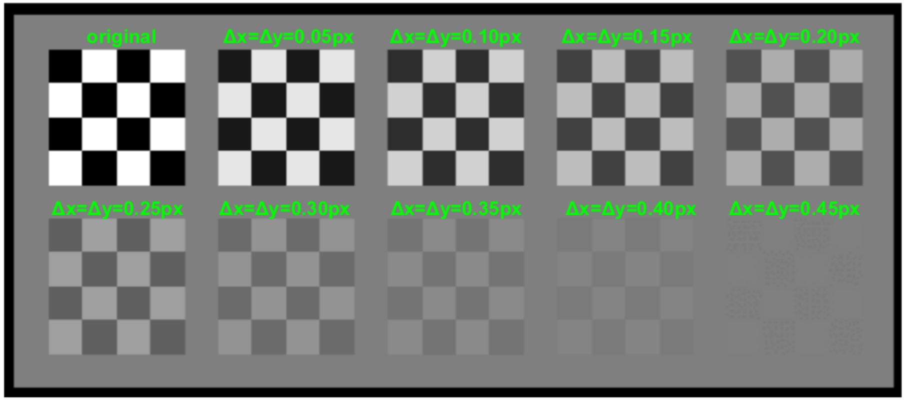


Suppose we shift the image in x & y.
The new pixels will be weighted averages of the old pixels.
The more the mix the pixels, the worse the result will be.

Interpolation

Shift





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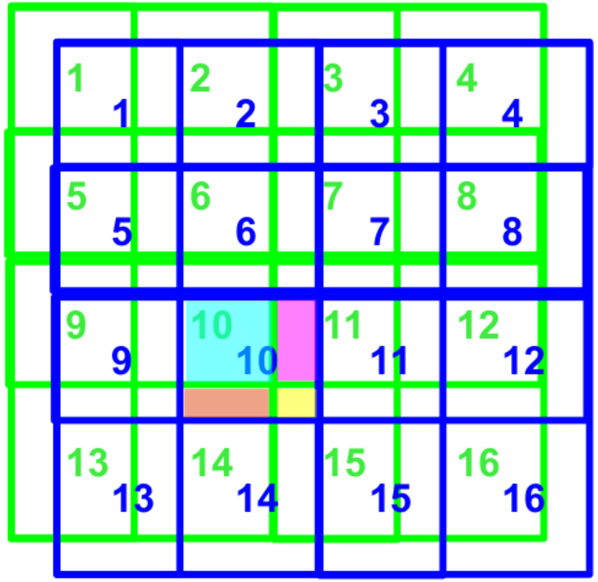
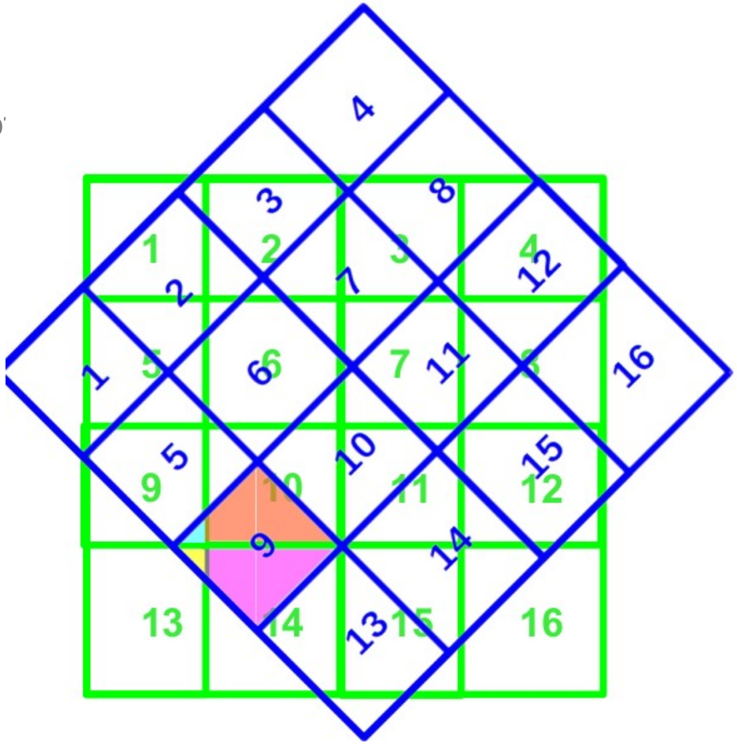


Ro



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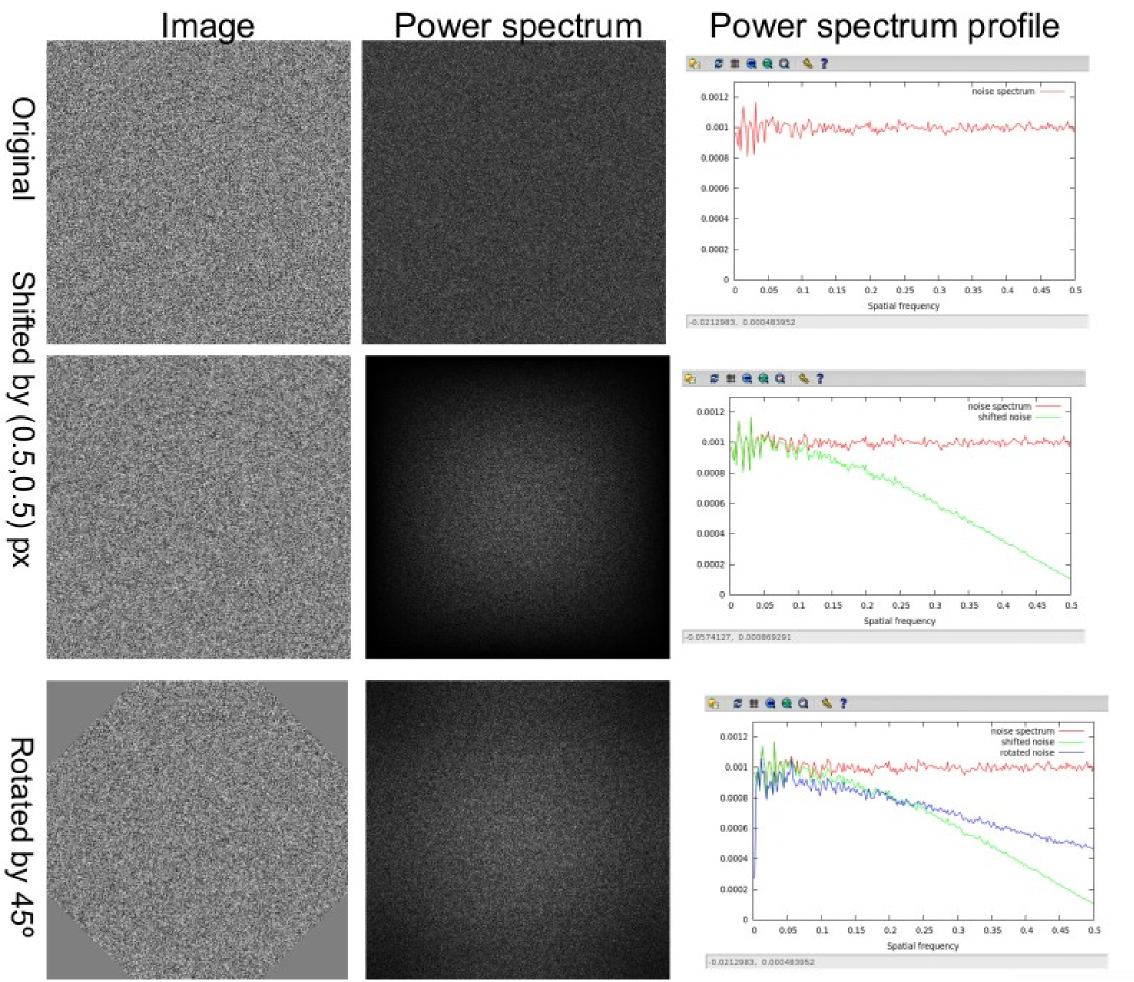
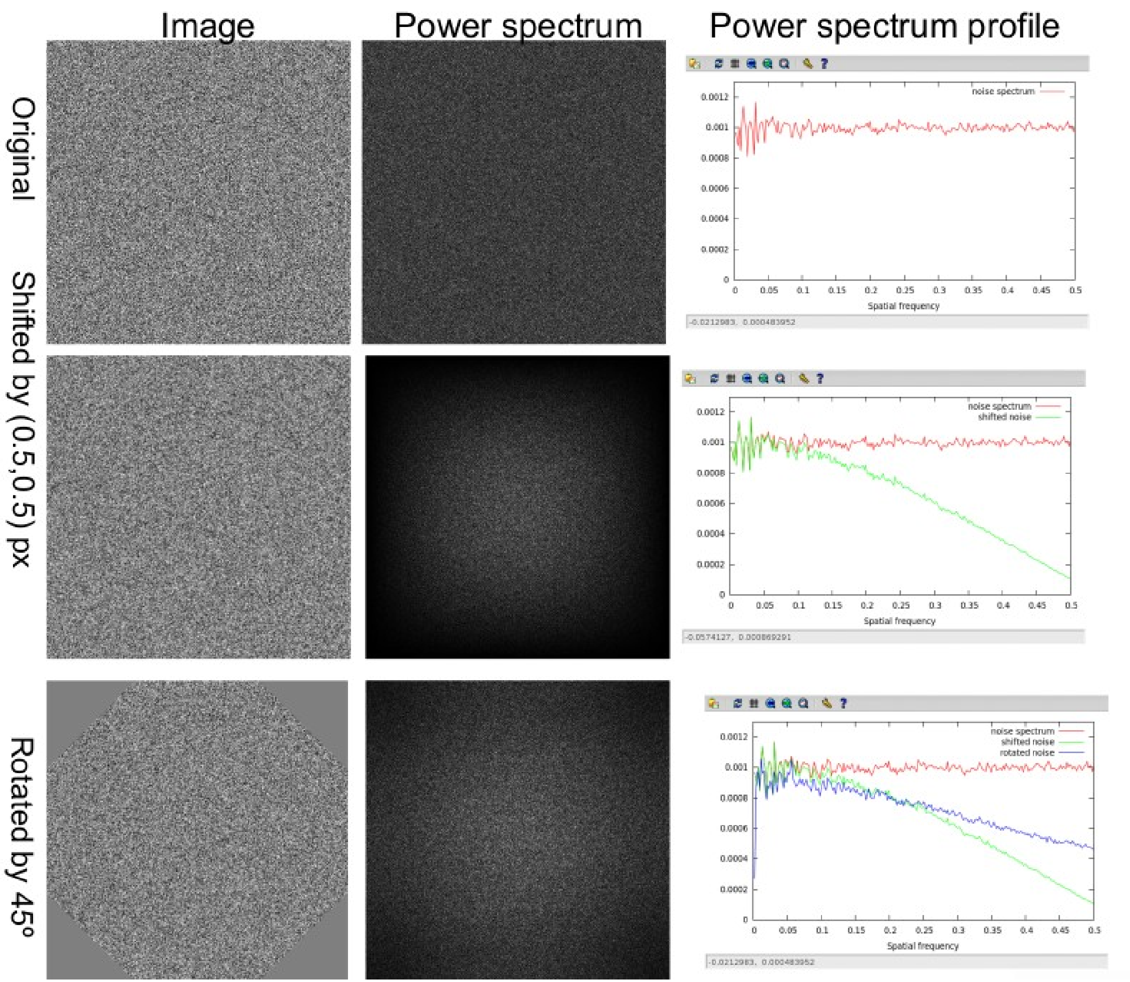


The Fourier transform of noise is noise

- “White” noise is evenly distributed in Fourier space
- “White” means that each pixel is independent

Interpolation



The Fourier transform of noise is noise

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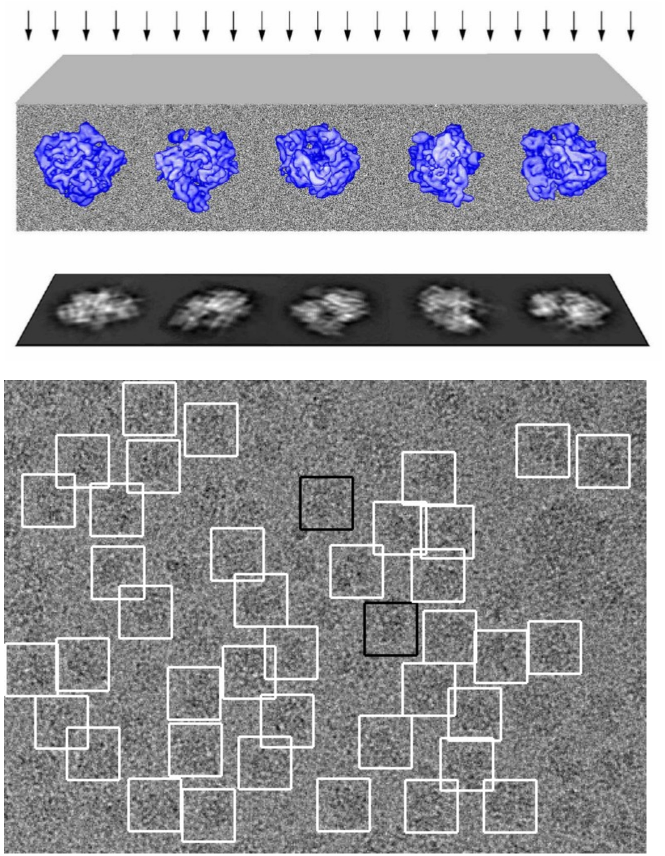
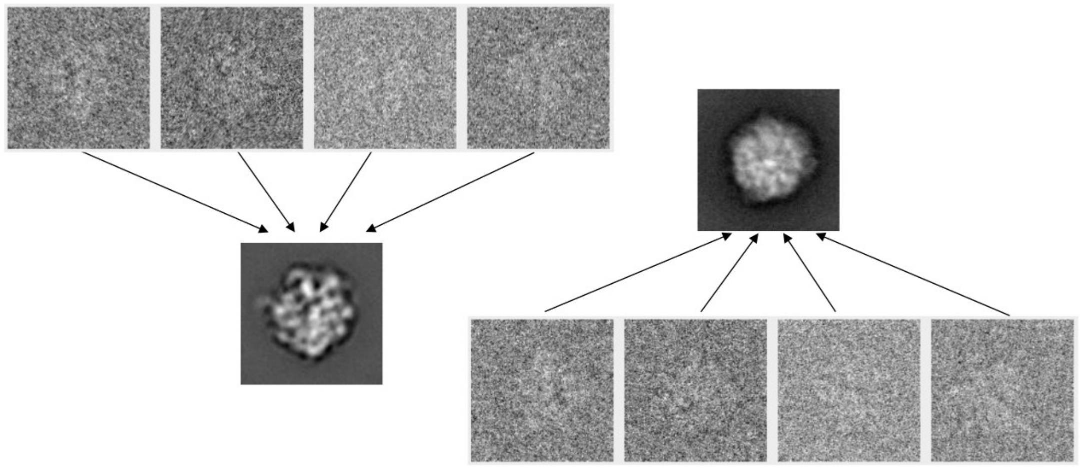
The degradation of the images means that we should minimize the number of interpolations.

Classification



Sum of aligned particles



Sum of unaligned particles

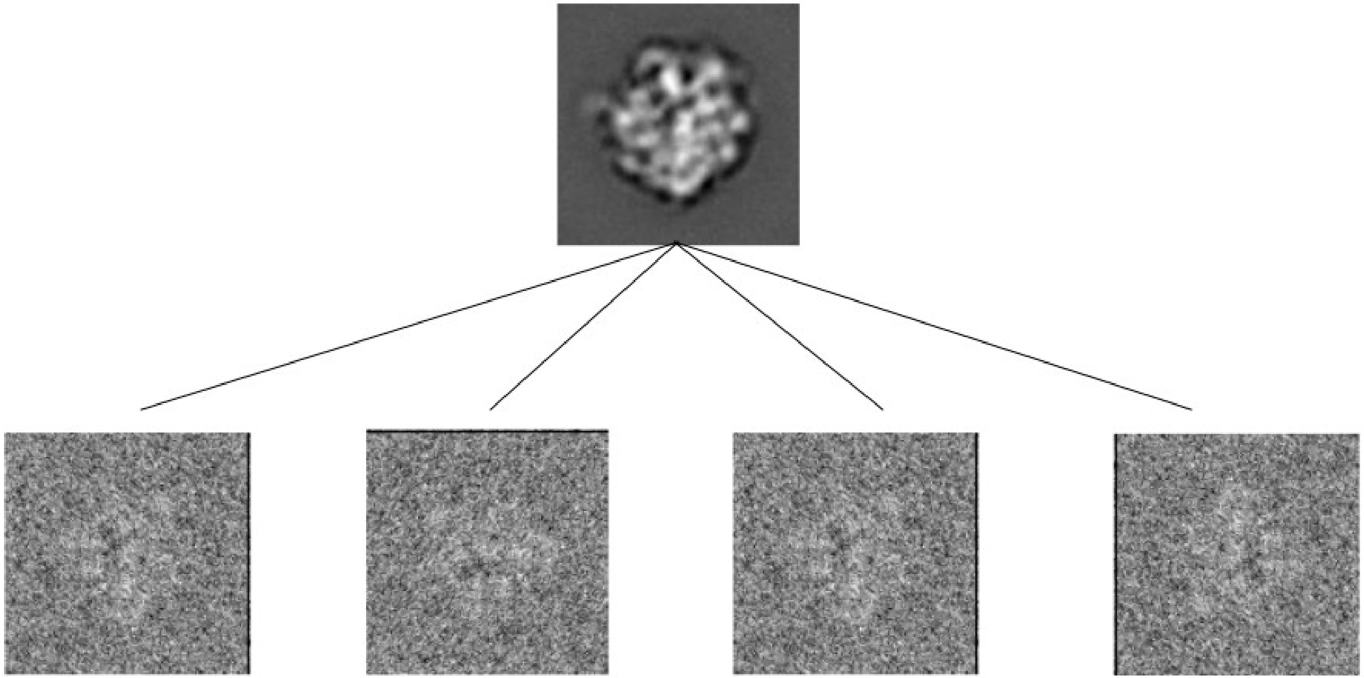
- inherently low signal-to-noise
- to estimate the sample quality – summarize same projection images to increase signal-to-noise to evaluate data quality

Classification

Classification methods are divided into those that are “supervised” and those which are “unsupervised”:

- Supervised: divide or categorize according to similarity with “template” or “reference” (e.g. projection matching)

- Unsupervised: divide according to intrinsic properties (e.g. find classes of projections representing the same view)



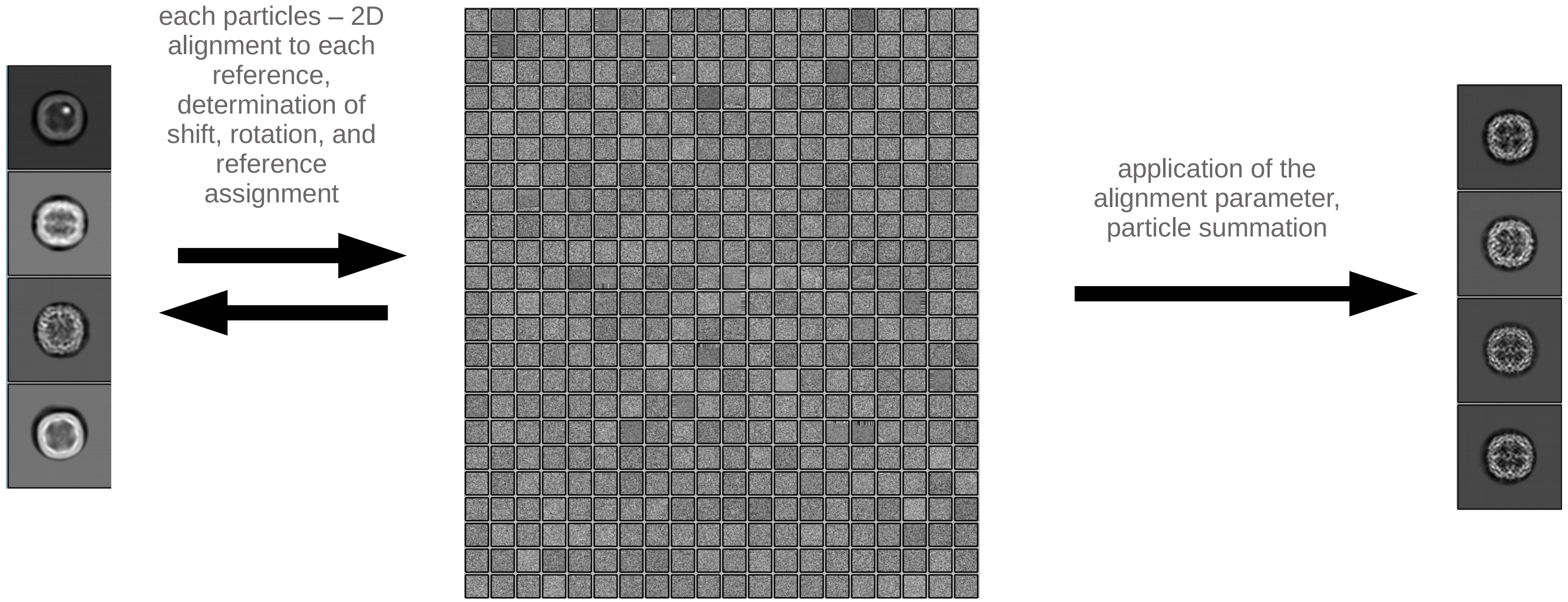
Classification

Supervised – reference based methods

- the reference images to which the experimental data are aligned are known

- the number or references determines the number of classes (input parameter – potential bias if the number is too low)

- assignment quality score – e.g. cross-correlation coefficient



reference images | experimental data (ROIs/particles) | average after alignment

Classification

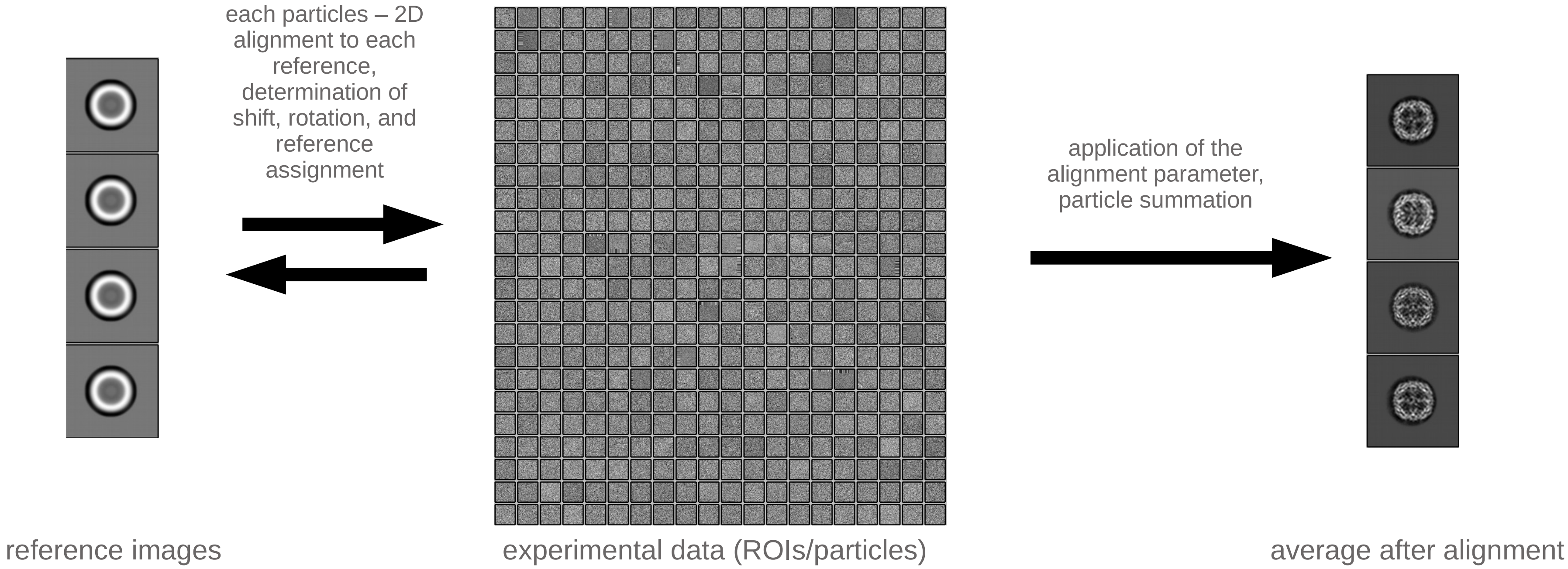
unsupervised – reference-free methods

- the reference for the image alignment not required

- the number of classes/references – required parameter (input parameter – potential bias if the number is too low)

- the initial reference are calculated by summation of a random subset of unaligned particles

- usually iterate refinement of the class assignment and alignment parameters



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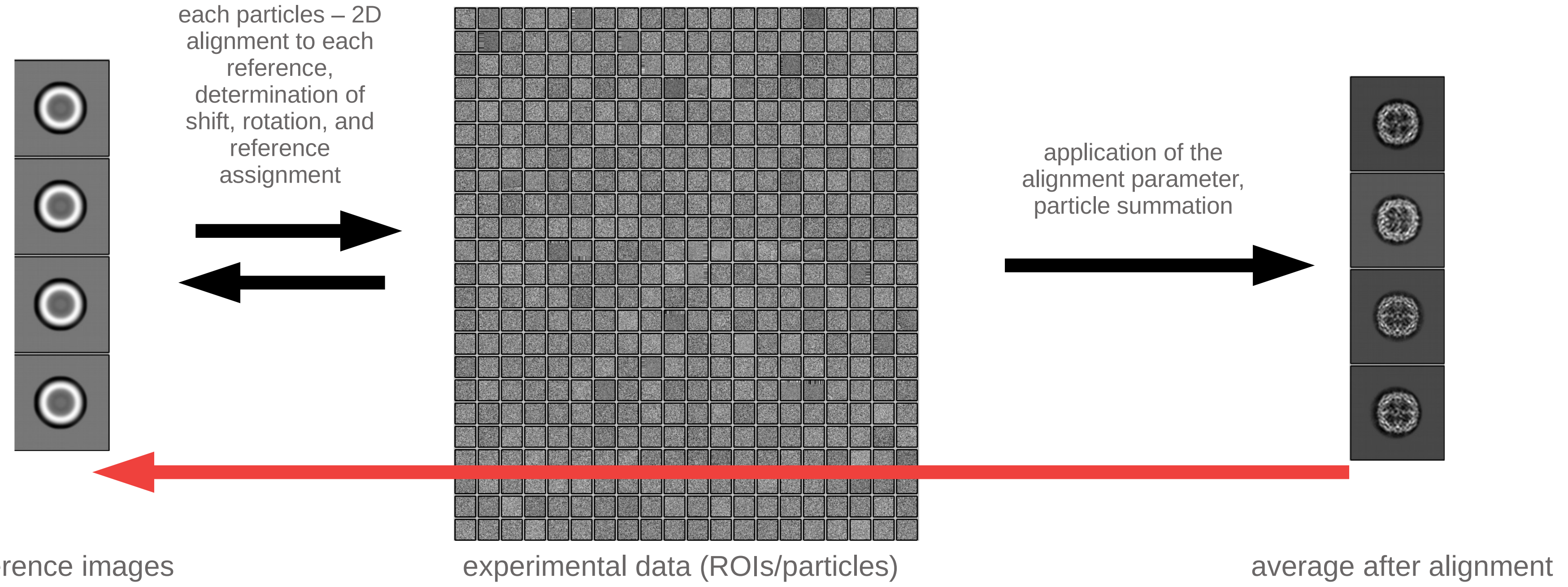


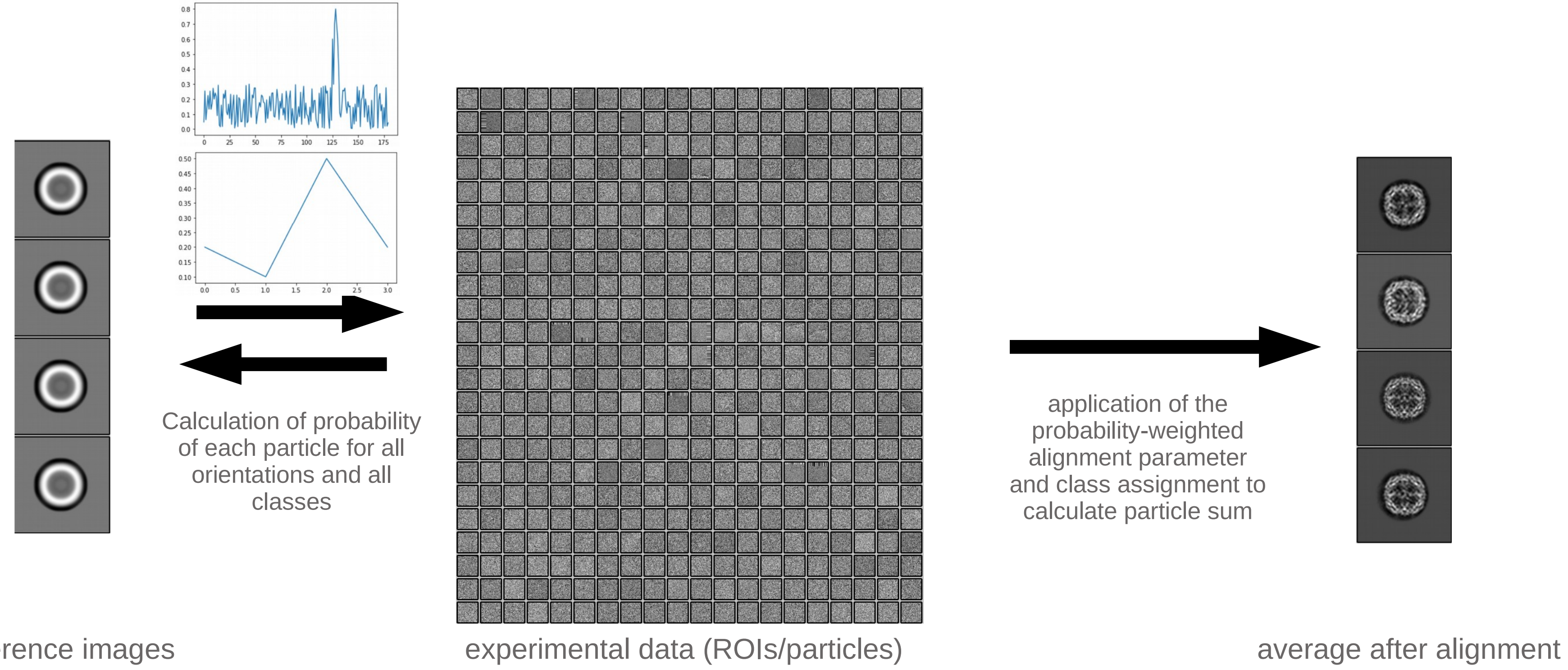
Classification

unsupervised – reference-free methods

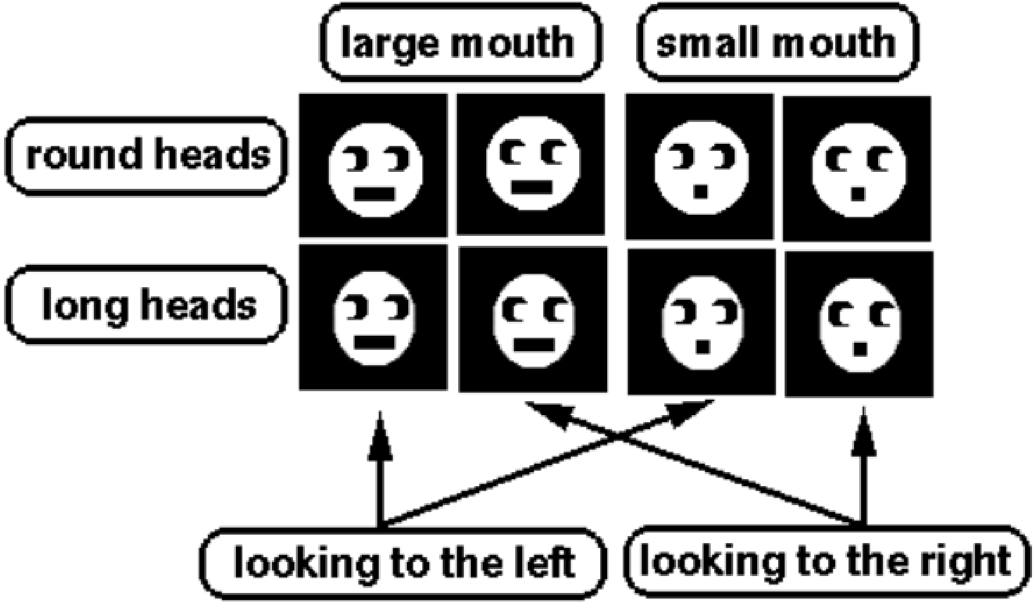
- utilization of different assignment quality score than ccc – e.g. Bayessian approaches – maximum likelyhood estim.

$$P(\Theta|X,Y) \propto P(X|\Theta,Y)P(\Theta|Y)$$

- for each orientation and class – calculate probability for a particle – use this probability when calculating particle sum



Classification

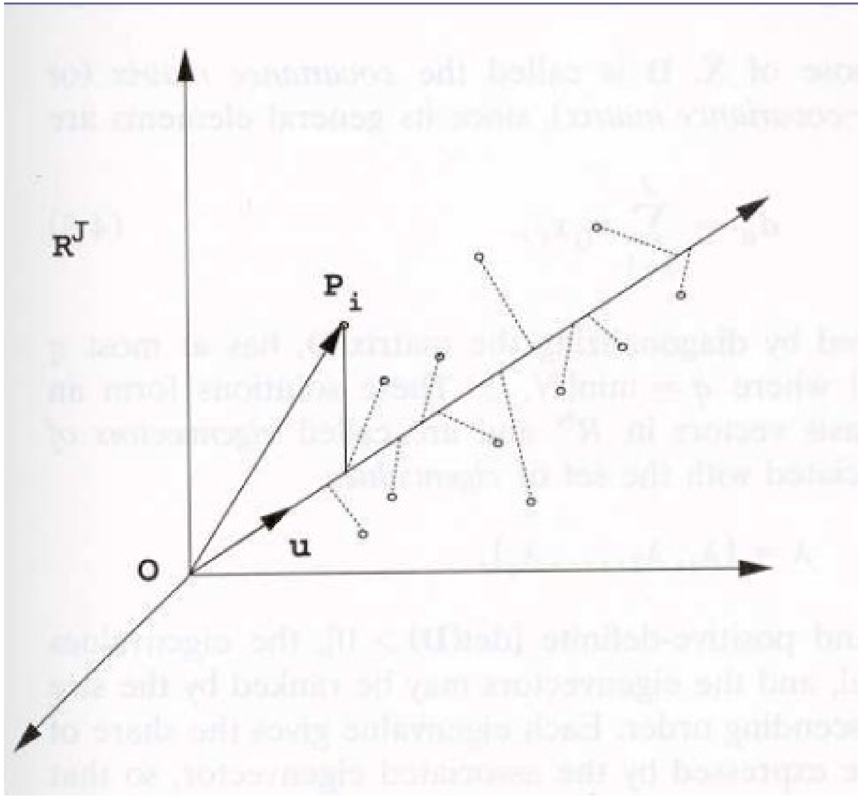


Frank (1996), J.Microscopy

Multivariate data analysis (MDA) or multivariate statistical analysis (MSA)

- find new coordinate system tailored to the data

- find a space with reduced dimensionality for the representation of the objects. This greatly simplifies classification.



eigenvectors

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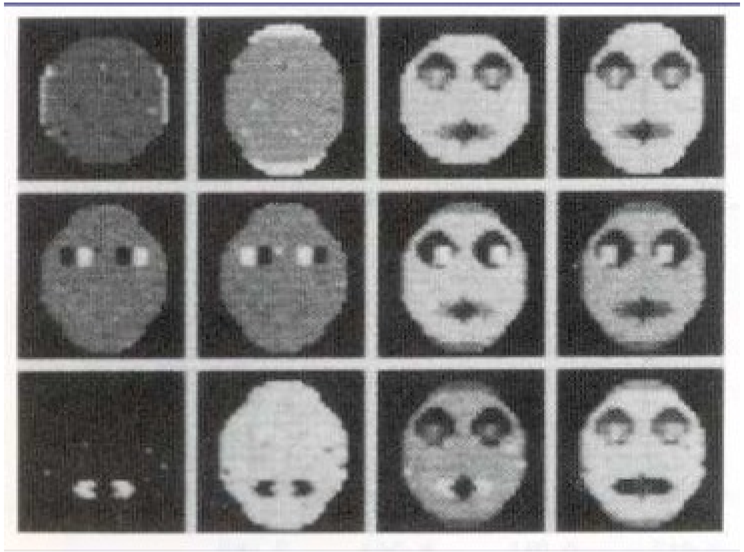
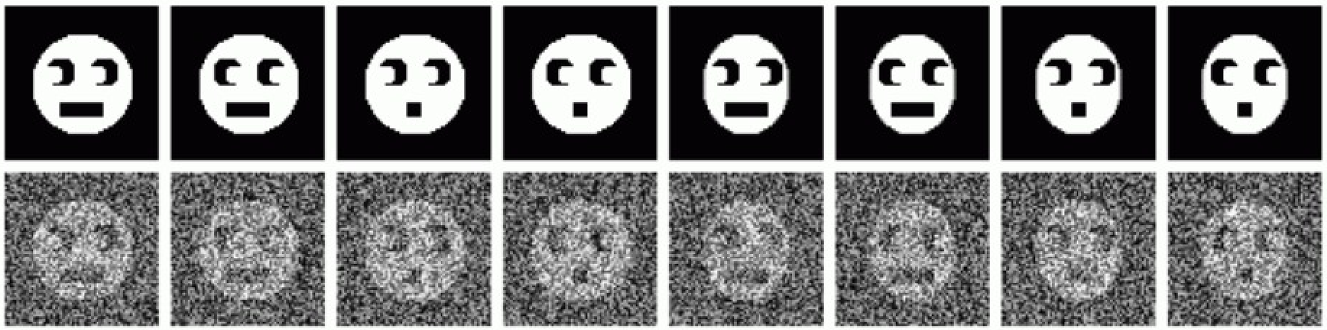


eigenimages

Principle component analysis (PCA), Correspondence analysis (CA)

8 classes of faces, 64x64 pixels



With noise added

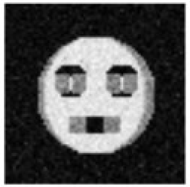
Average:

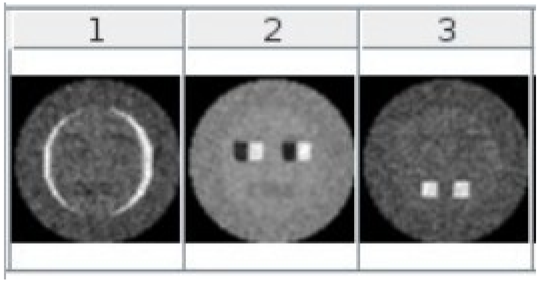
Principle component analysis (PCA), Correspondence analysis (CA)

For a 4096-pixel image, we will have a 4096x4096 covariance matrix.

Row-reduction of the covariance matrix gives us “eigenvectors.”

- The eigenvectors describe correlated variations in the data.
- The eigenvectors have 4096 elements and can beconverted back into images, called “eigenimages.”
- The first eigenvectors will account for the most variation. The later eigenvectors may only describe noise.
- Linear combinations of these images will give us approximations of the classes that make up the data.





eigenimages

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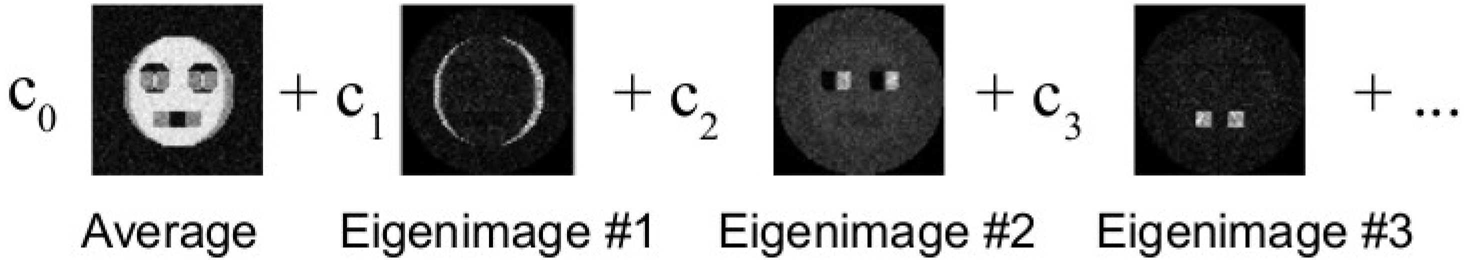

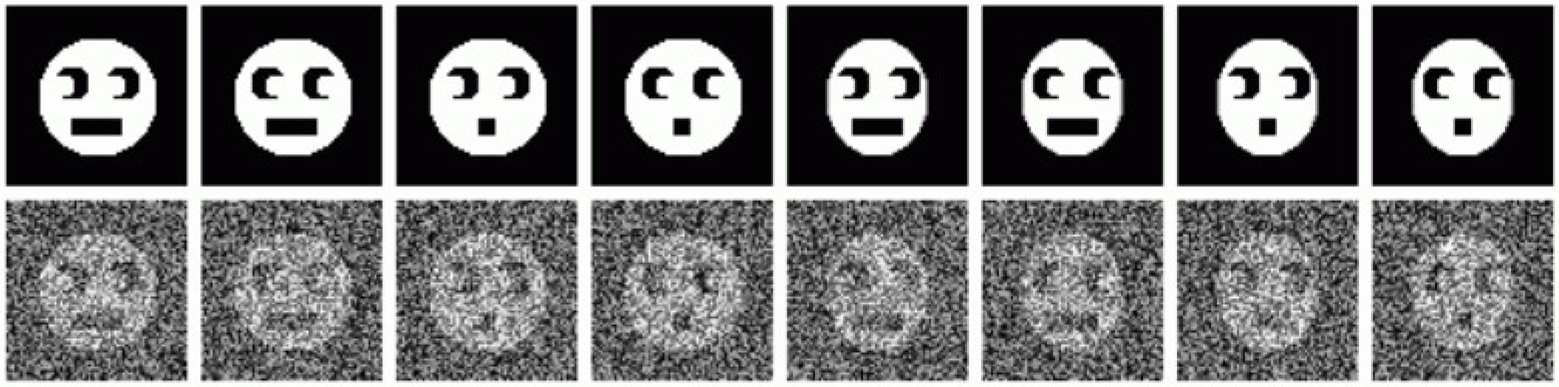

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