Lecture 7

Analysis of electron micrographs

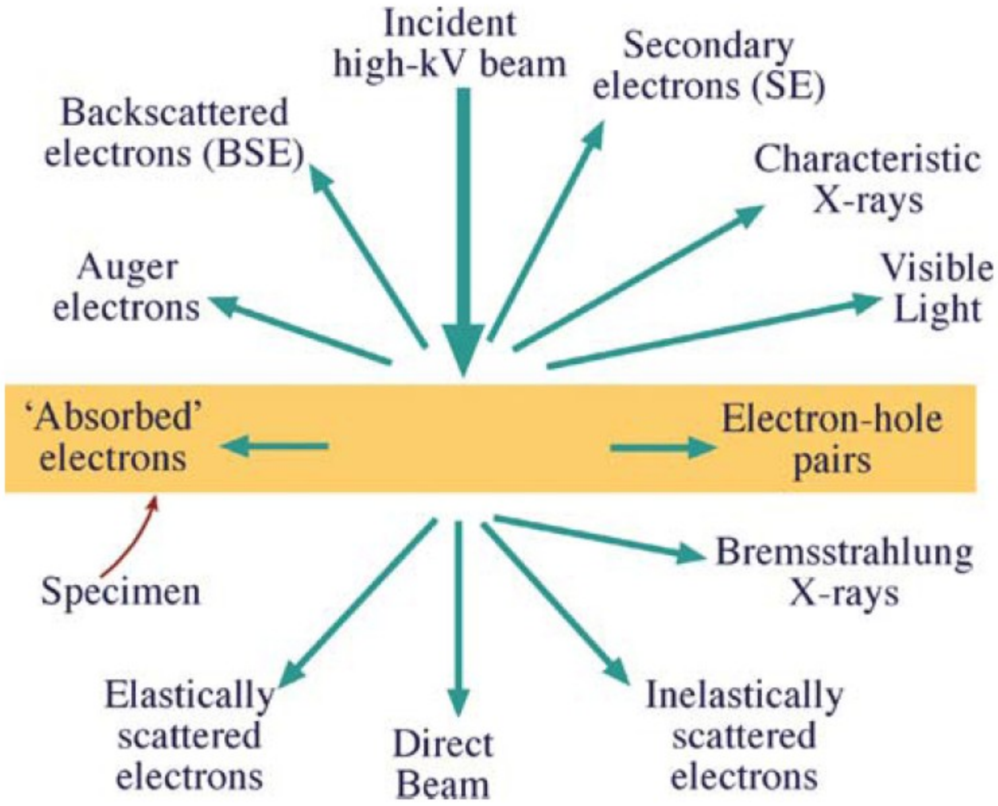
25th November 2020

Jiri Novacek

Content

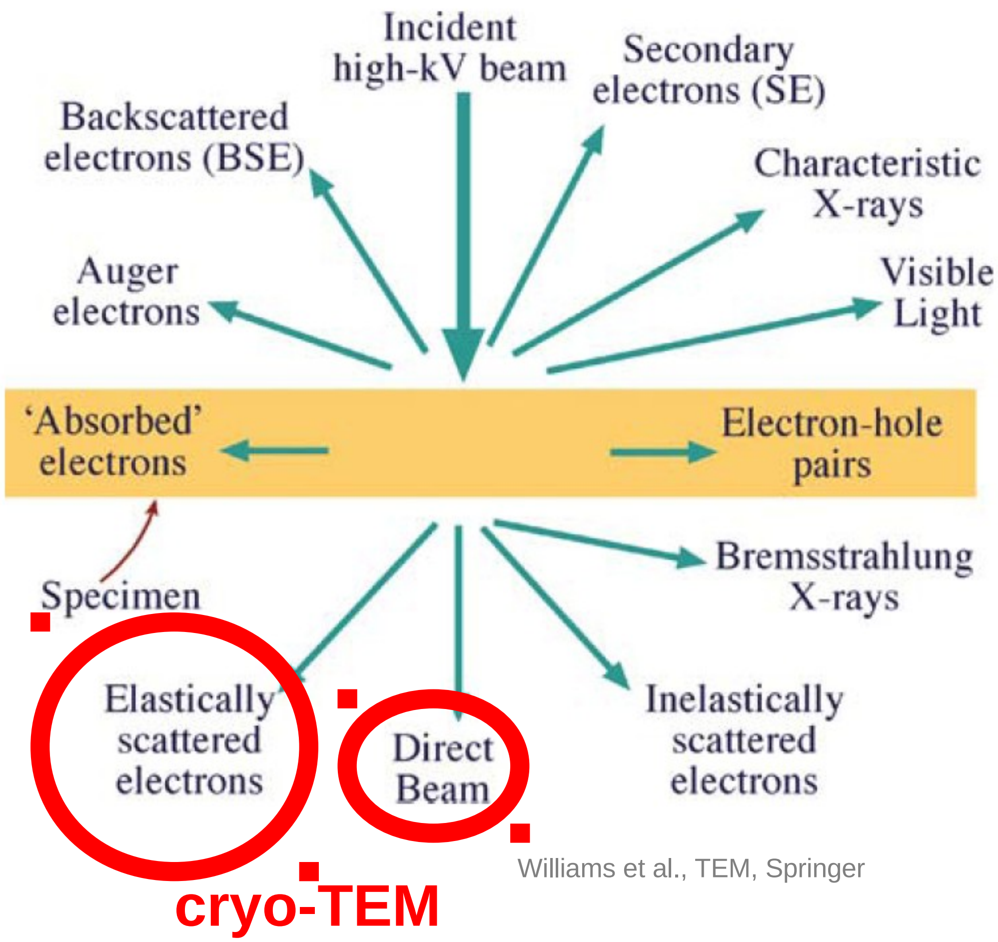
- interaction of electrons with matter, radiation damage
- data acquisition, image filtering
- projection theorem
- image averaging in 2D
- principal component analysis

Interaction of electrons with specimen

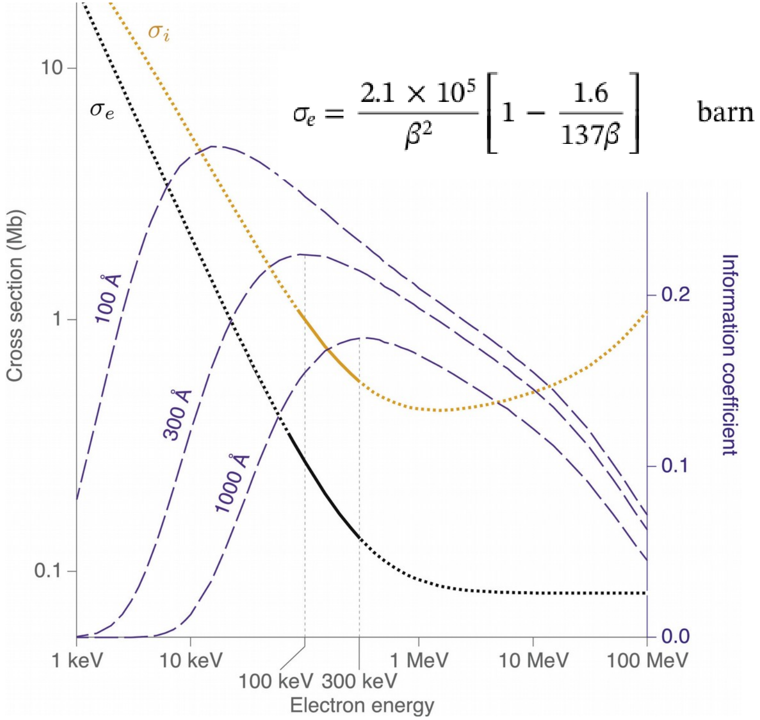


Williams et al., TEM, Springer

Interaction of electrons with specimen



Williams et al., TEM, Springer

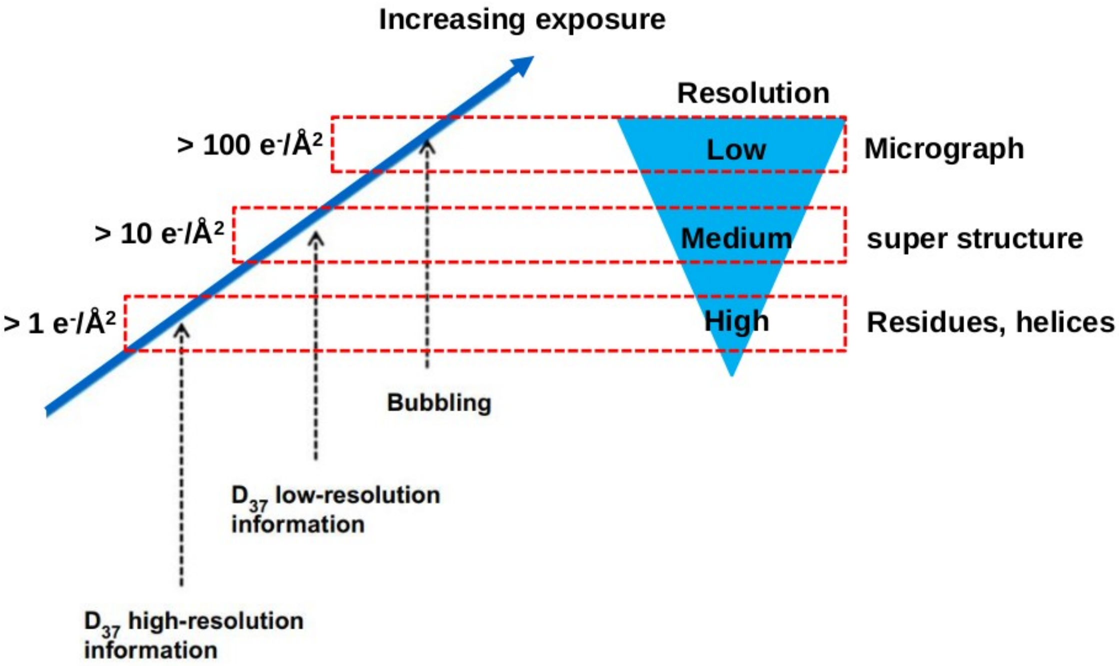


Peet et al., (2019) Ultramicroscopy

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

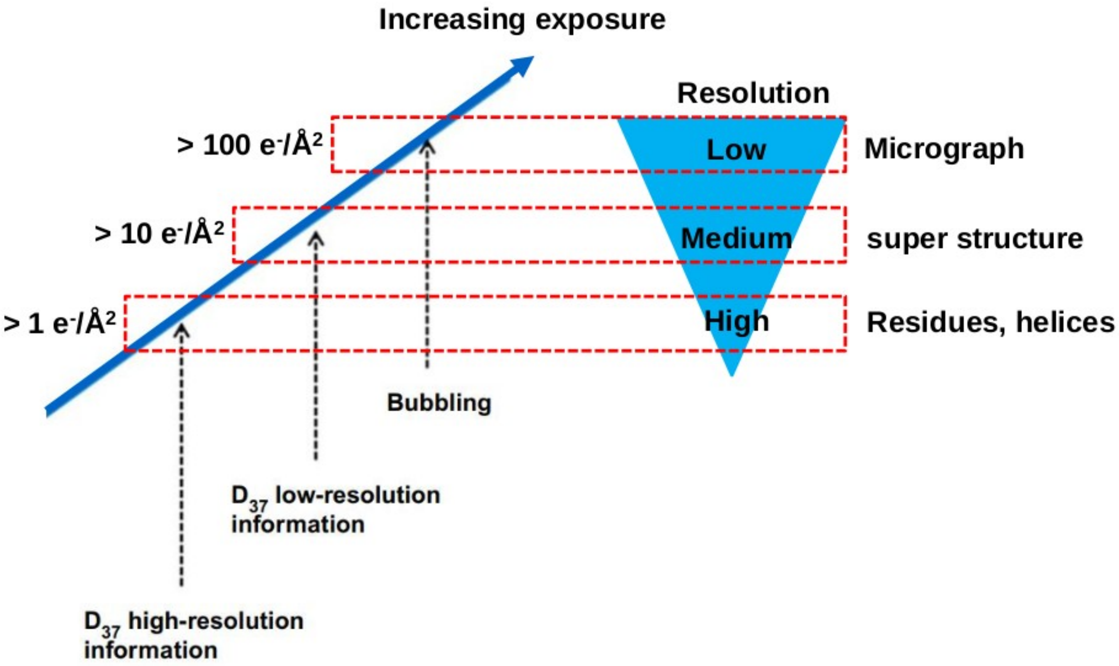
- mean free path of inelastic scattering in vitrified biological specimens: ~395nm

Radiation damage

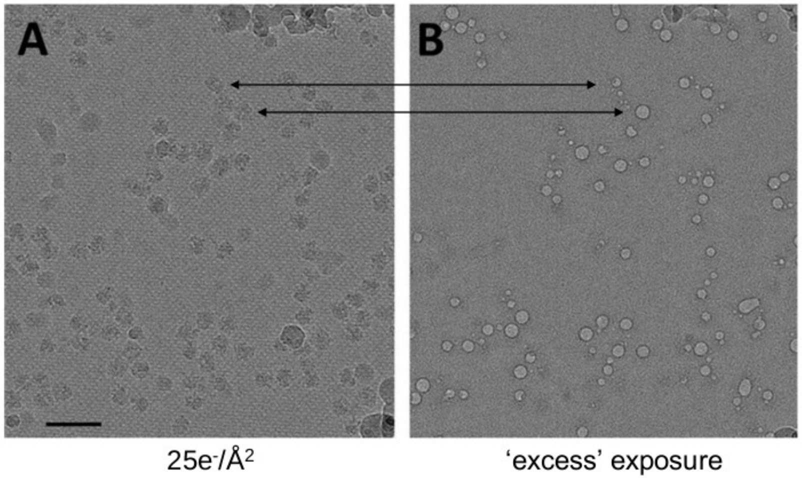


Glaser R. (2016), Meth. Enzym.

Radiation damage

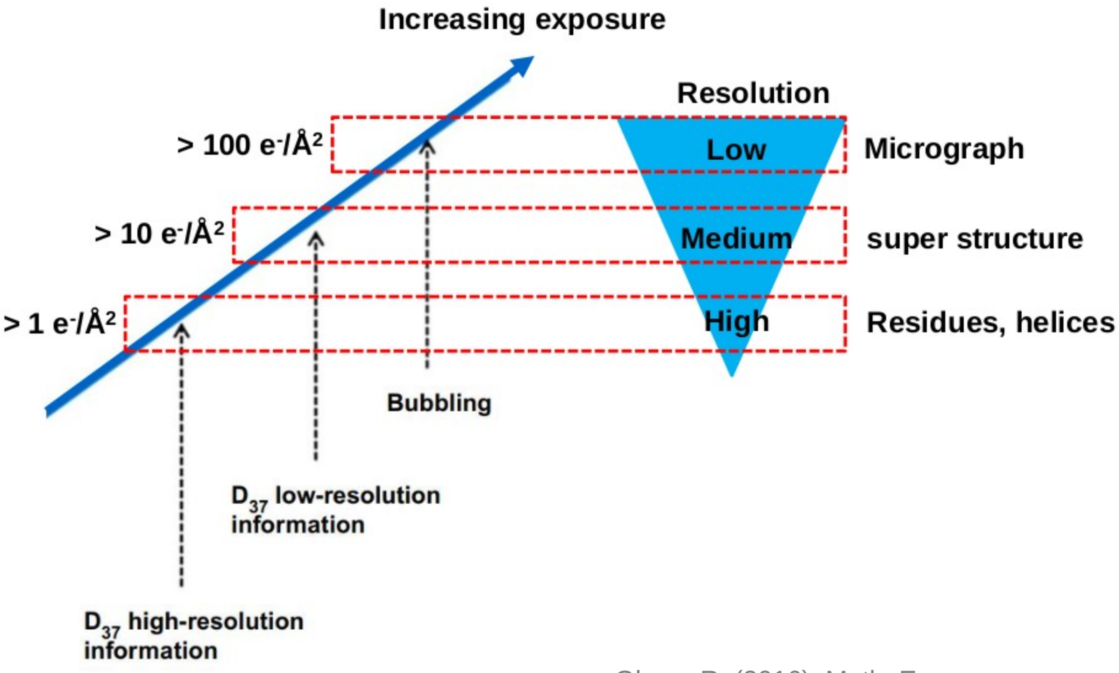


Glaser R. (2016), Meth. Enzym.

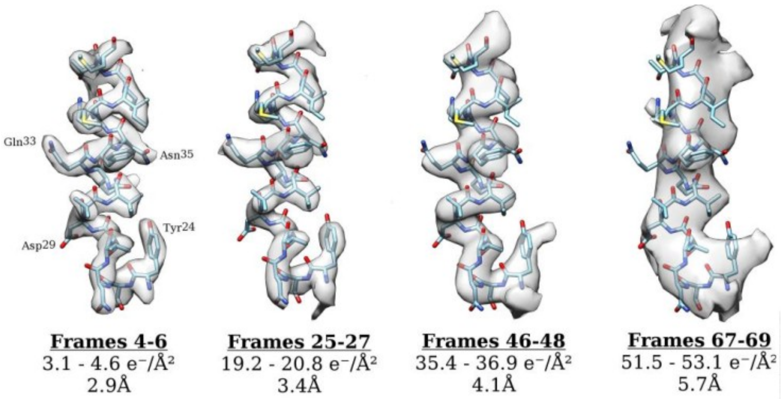


Glaser R. (2016), Meth. Enzym.

Radiation damage

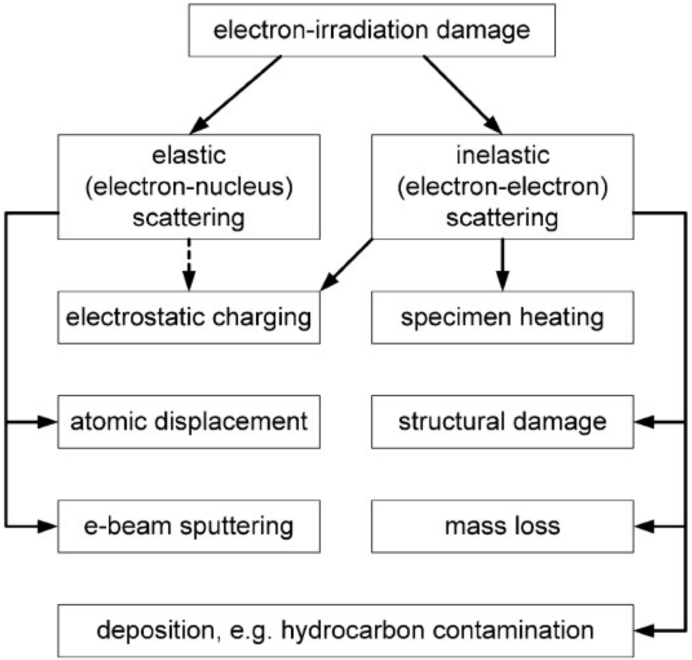


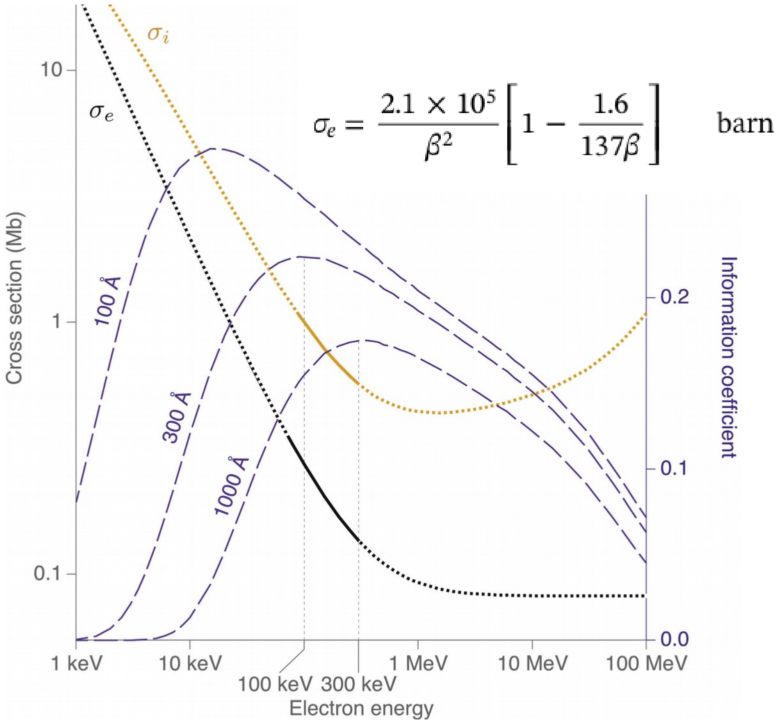
Glaser R. (2016), Meth. Enzym.



Grant. (2015), eLife

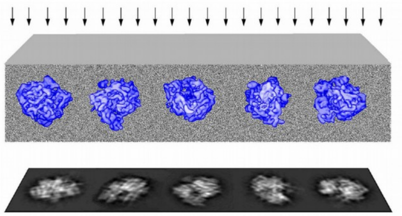
Interaction of electrons with specimen

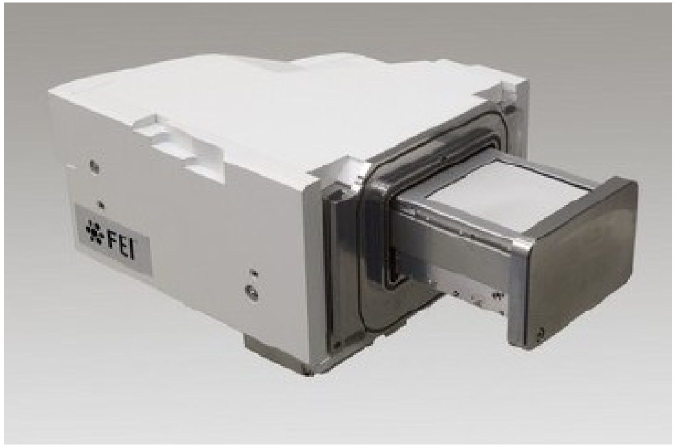


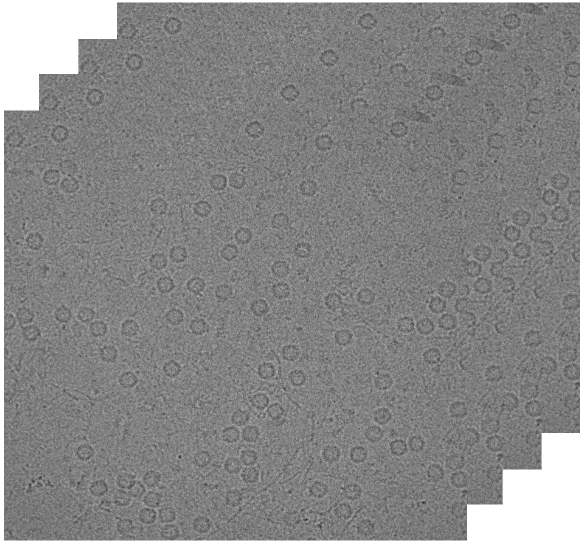


Peet et al., (2019) Ultramicroscopy

Data acquisition

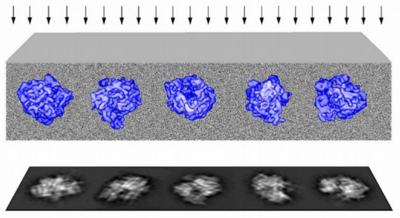


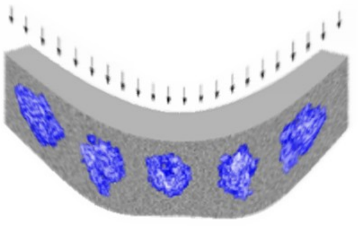




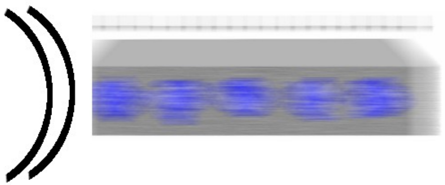
- data fom each position on the sample
  stored as a short movie
- compensation of sample radiation damage
- compensation of the sample motion
  during exposure

Data acquisition

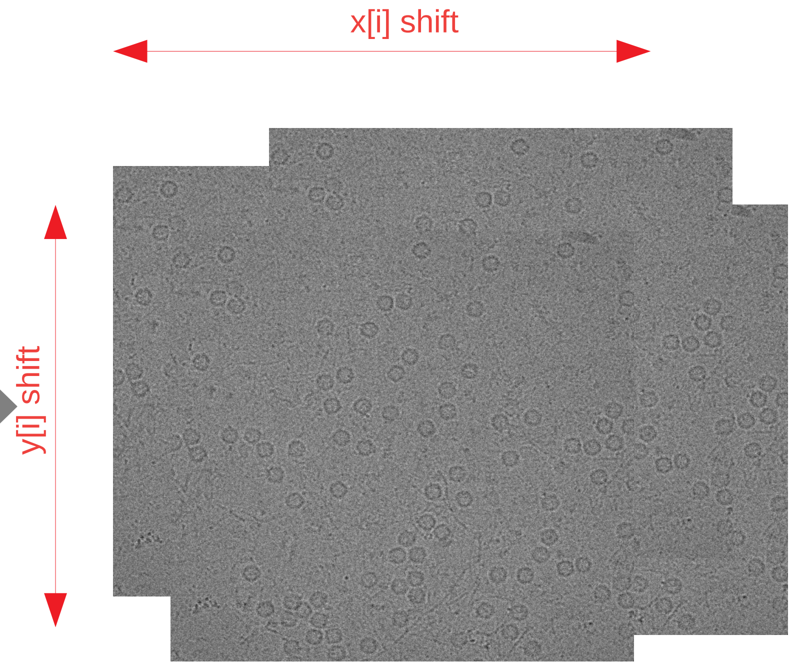




- beam induced motion
(sample geometry, local)



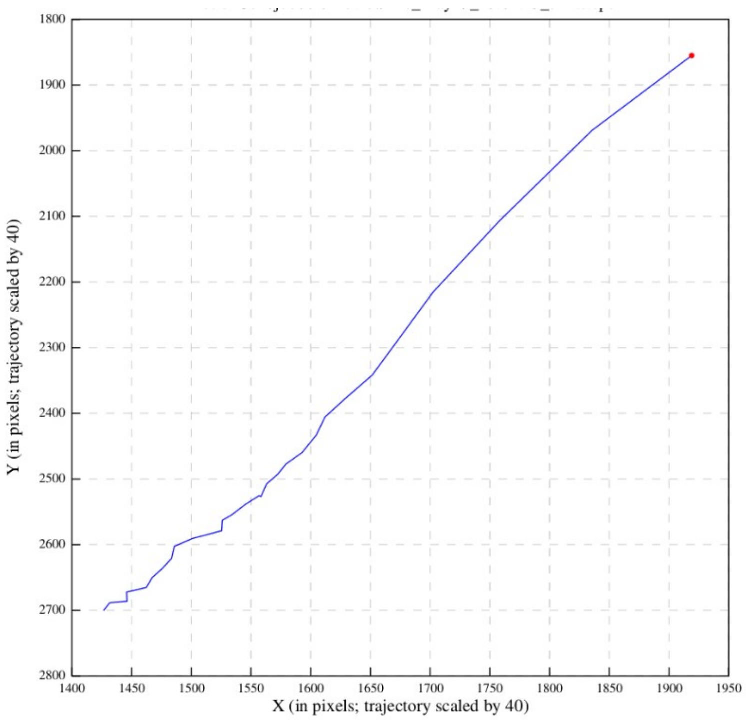
- drift, vibration
(external sources, global)



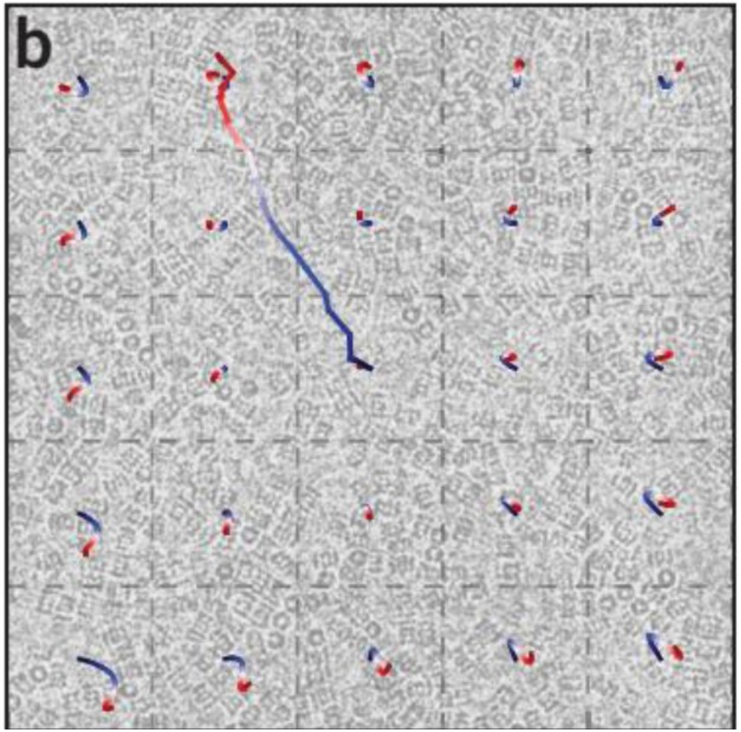
- data fom each position on the sample stored as a short movie
- compensation of sample radiation damage
- compensation of the sample motion during exposure

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



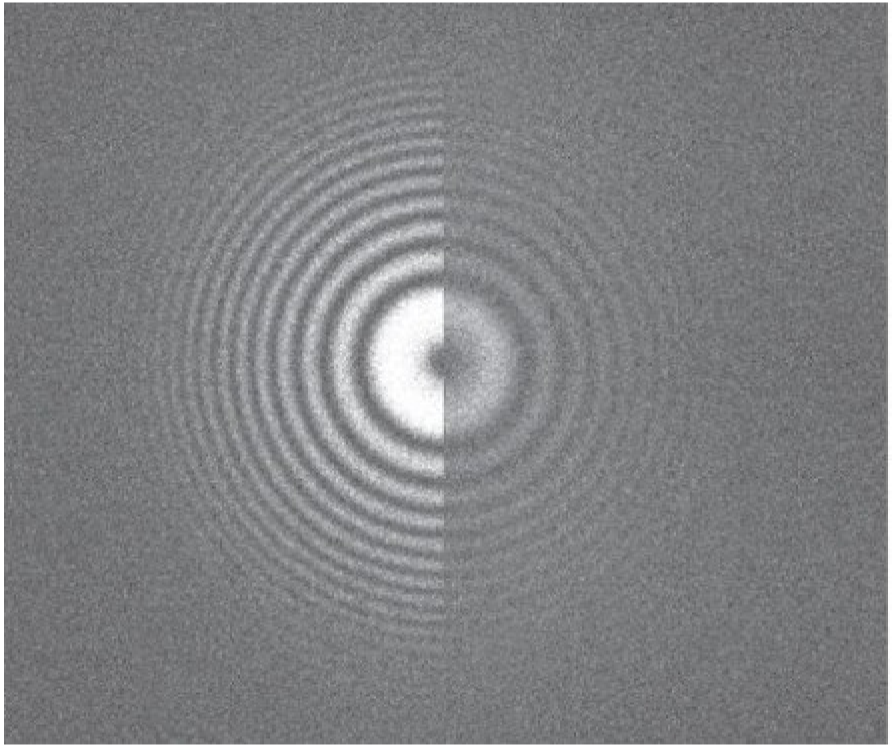
global motion



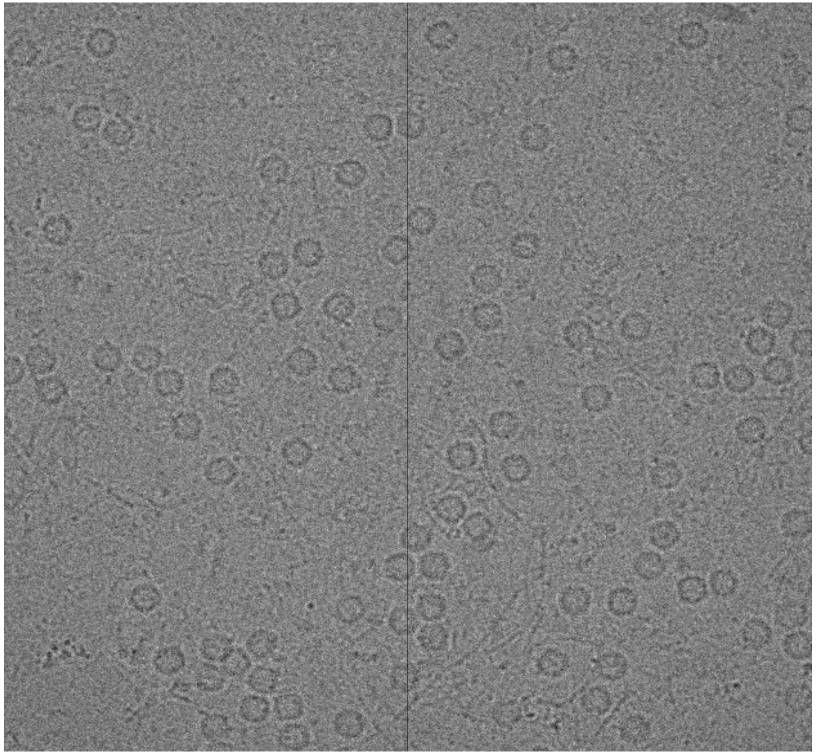
additional local motion

Data acquisition

- averaging of the movie into single image – increase S/N
- 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



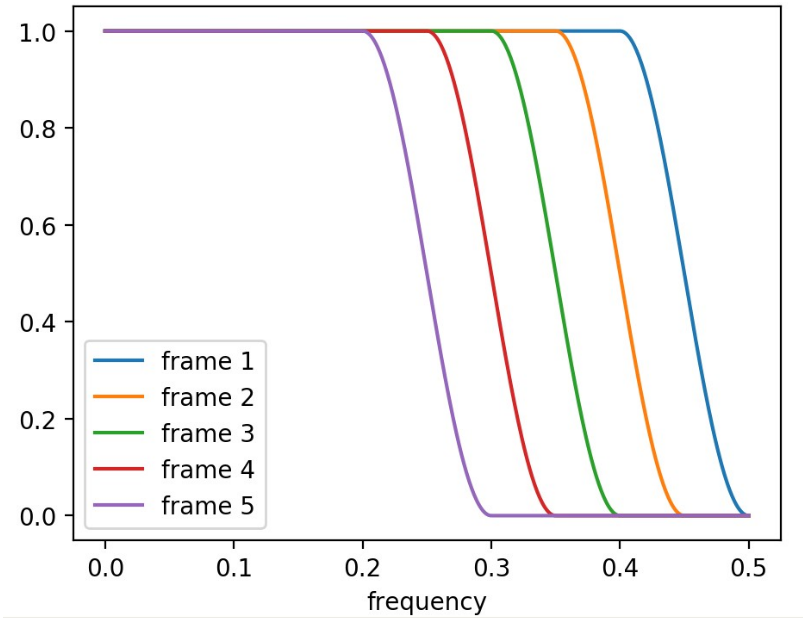
aligned image unaligned image

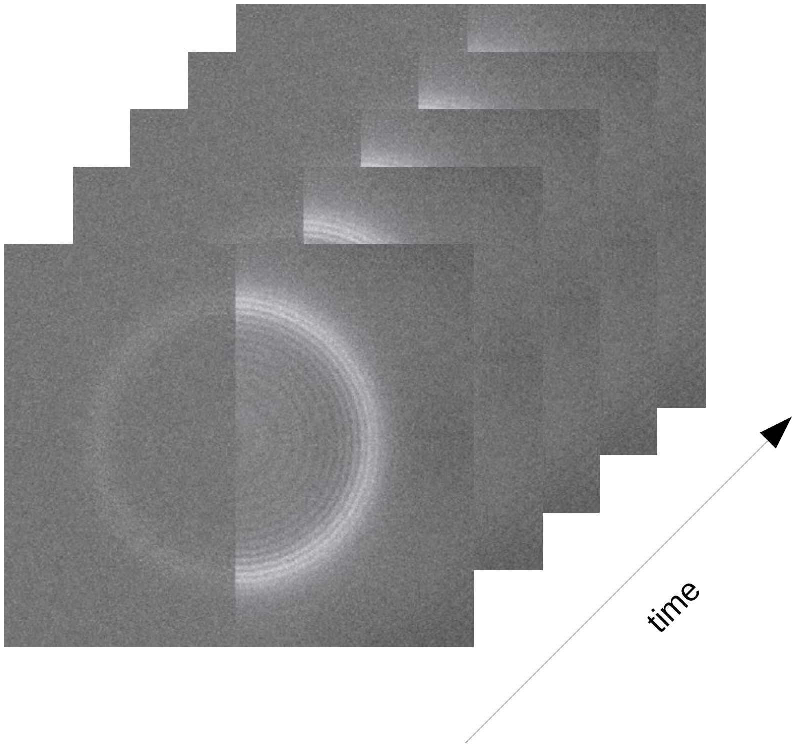


aligned image unaligned image

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





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

Image filtering

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

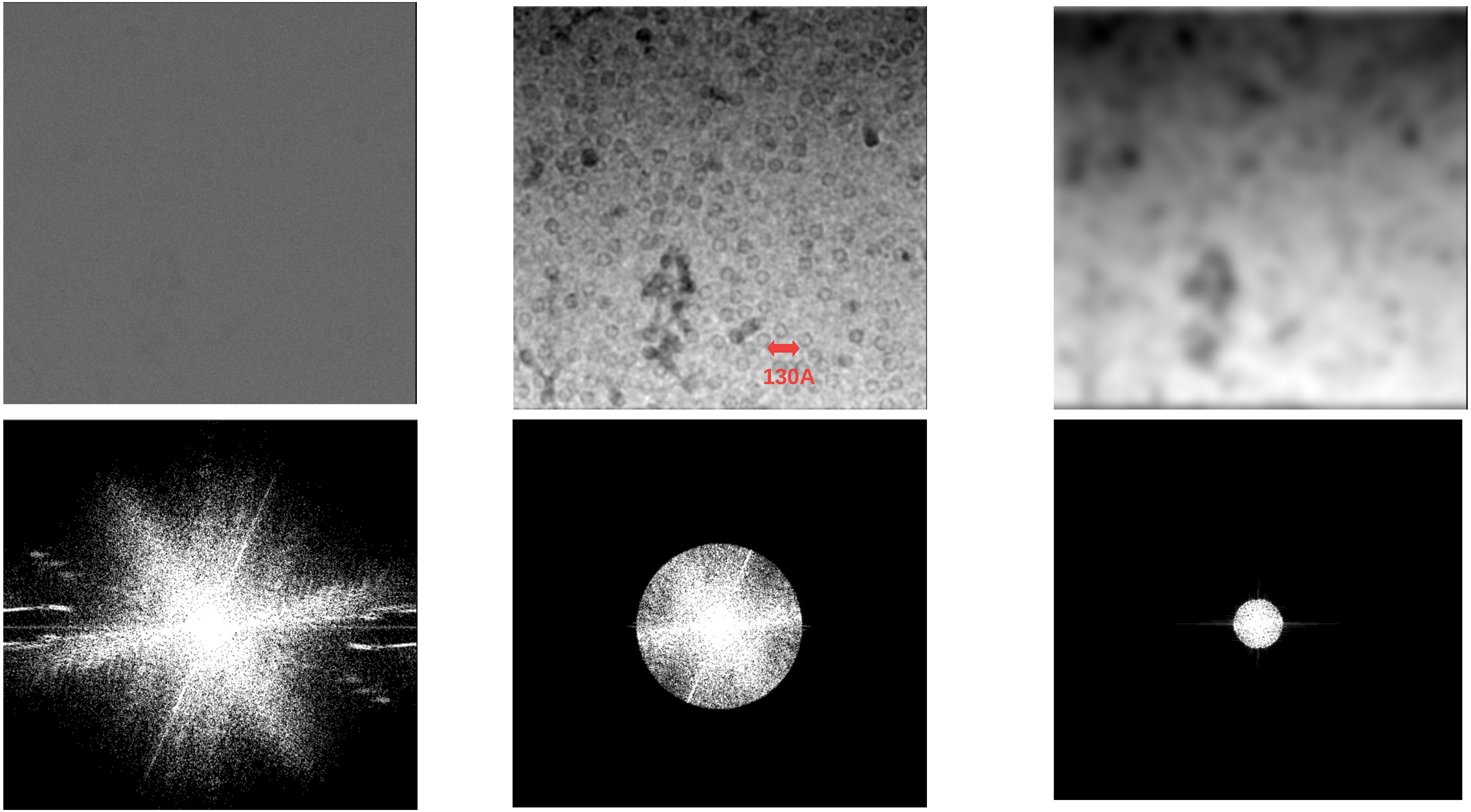
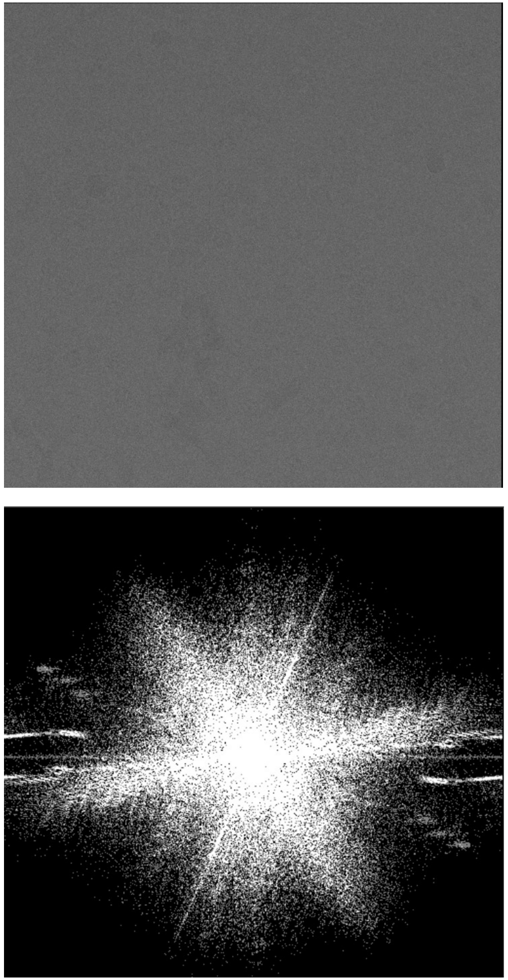
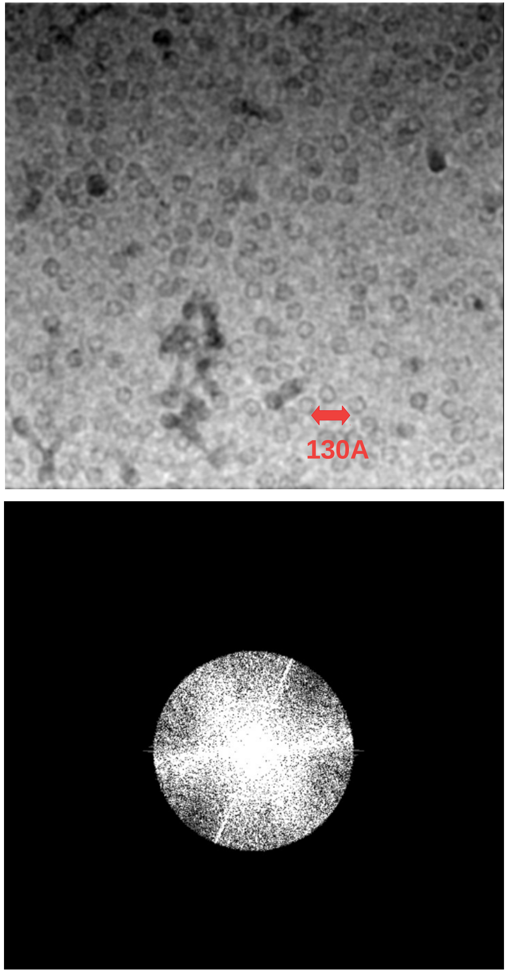


Image filtering

unfiltered image



lowpass filtered (50A)



bandpass filtered (1000,10A)

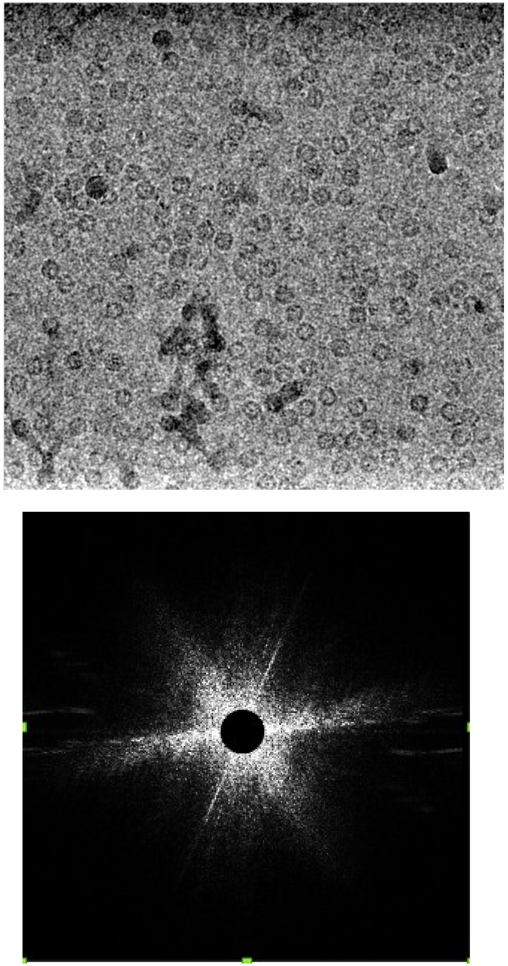
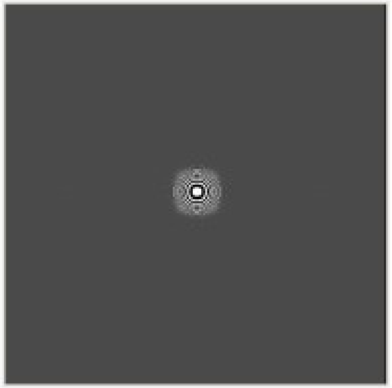
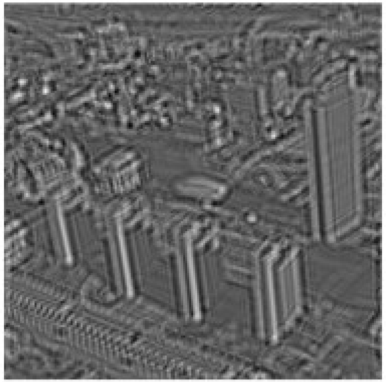


Image formation

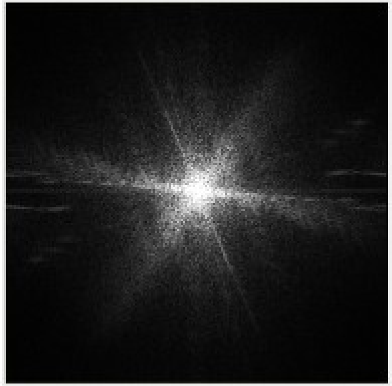
$f(x)$



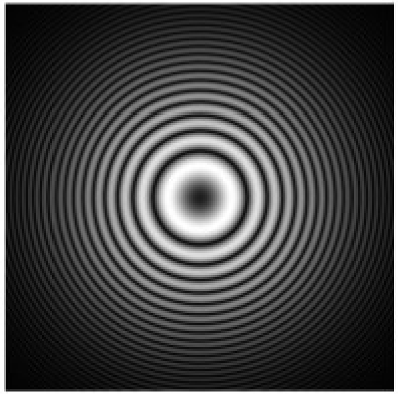
$g(x)$



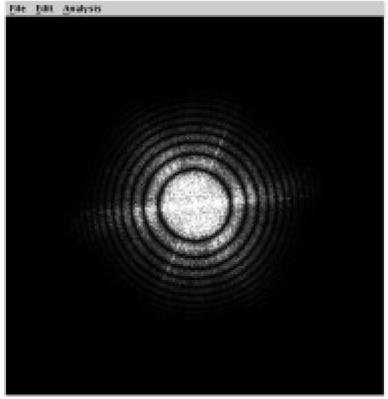
$f(x) \bullet g(x)$



$F(X)$

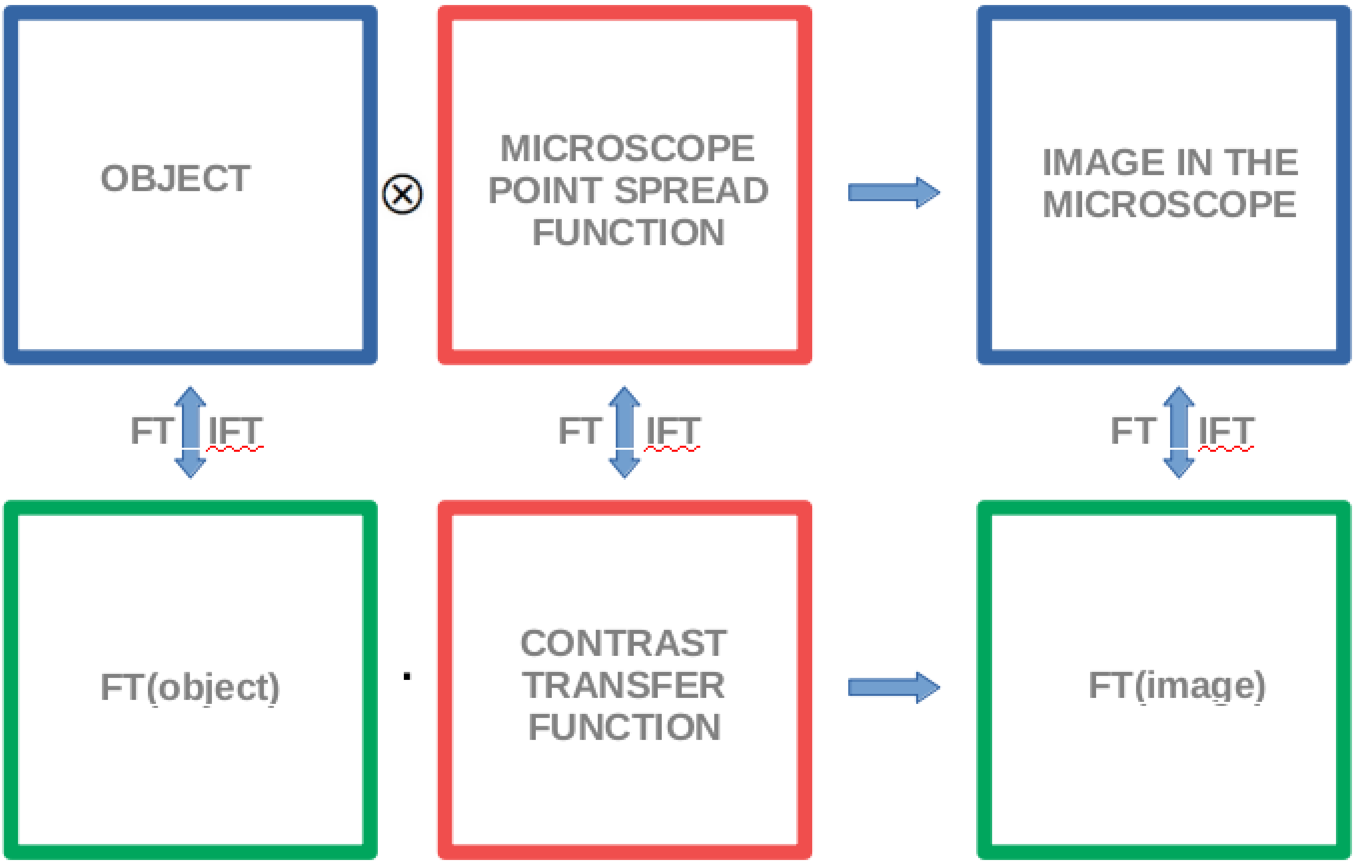


$G(X)$



$F(X)\ G(X)$

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

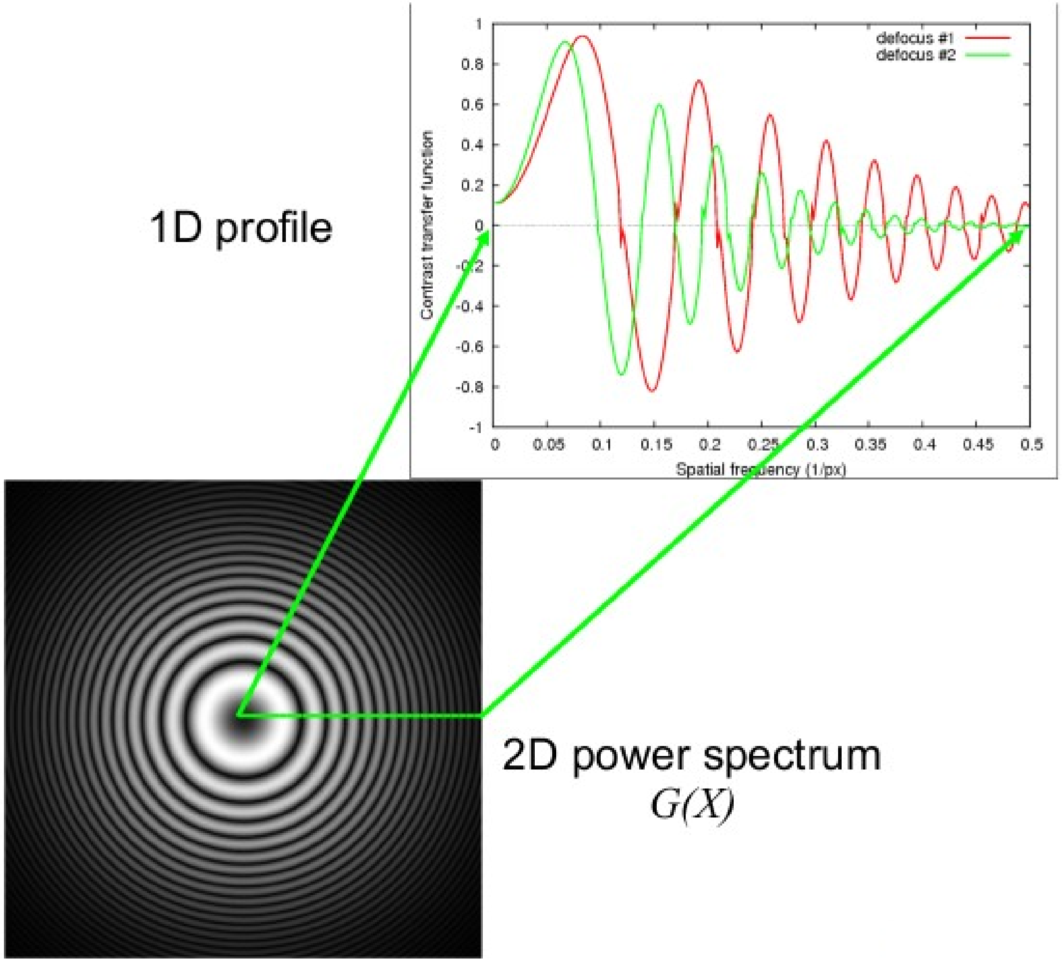
s – spatial frequency

Cs – spherical abberation

λ – electron wavelength

z – defocus

1D profile



2D power spectrum
G(X)

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)

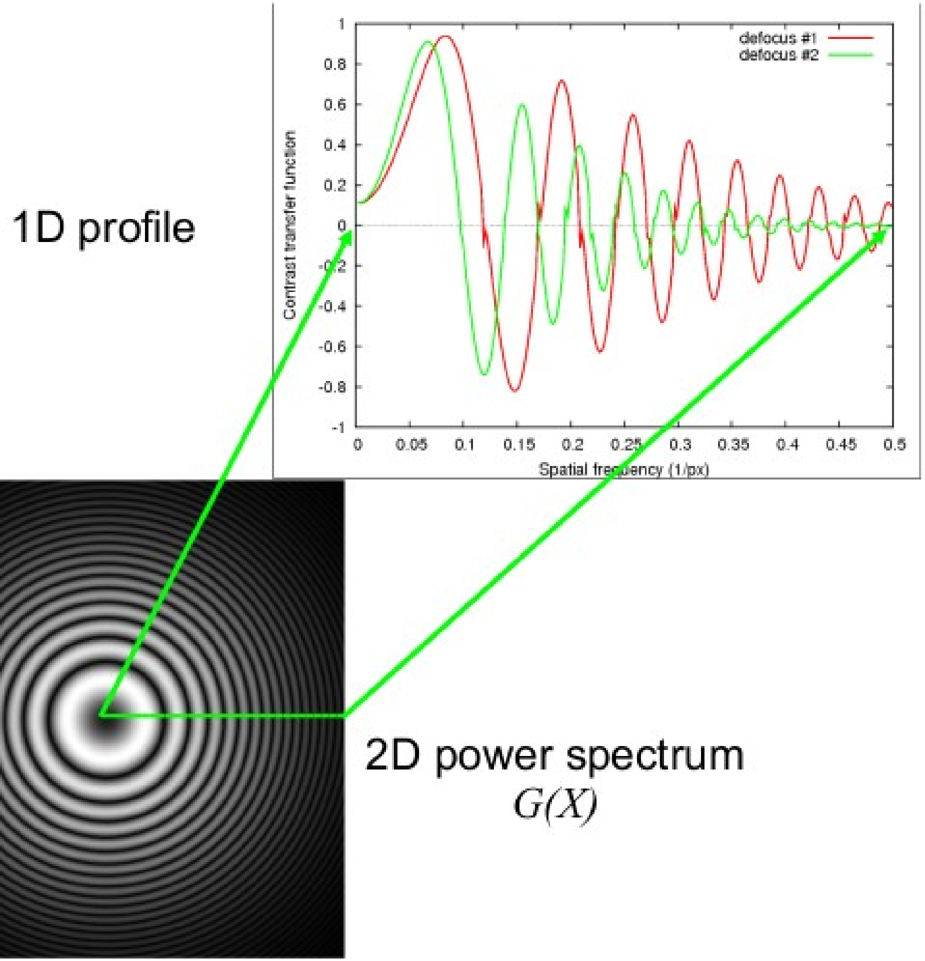
$$E_{es}(k) = \exp\left[-\frac{1}{16 \ln 2} \pi^2 \delta z^2 k^4 \lambda^2\right],$$

- MTF of the camera

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

- Generic envelope (drift, charging, multiple scattering)

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



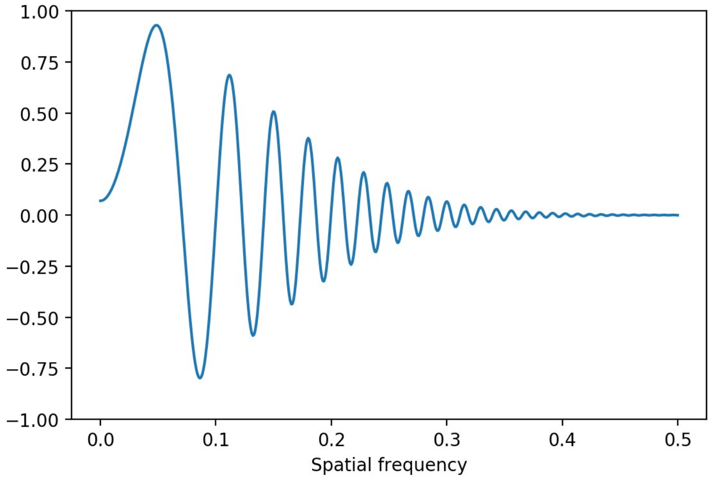
Contrast transfer function

Envelope function

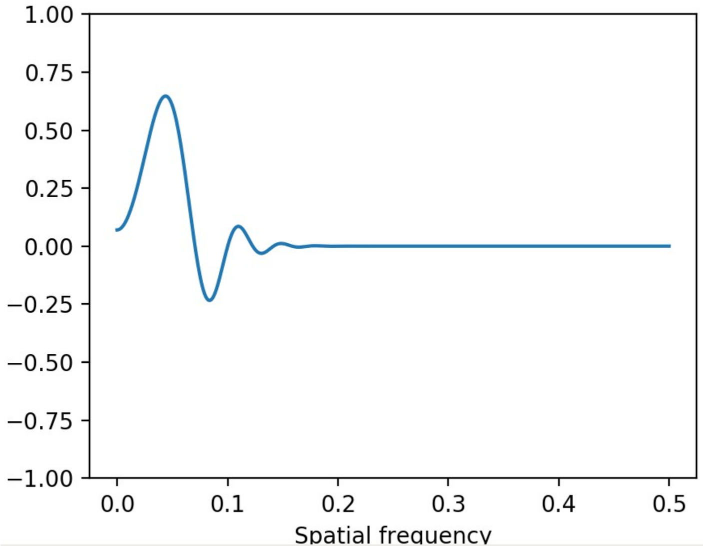
$$I(\mathbf{k}) = E_{pc}(k)E_{es}(k)E_f(k)E_g(k)H(k)\Phi(\mathbf{k}) + N(\mathbf{k}).$$

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

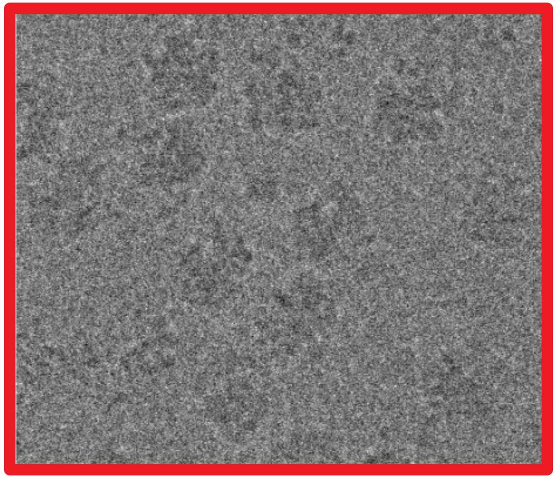
kV=300,ac=0.07,cs=2.7,z=-1,apix=1,B=30



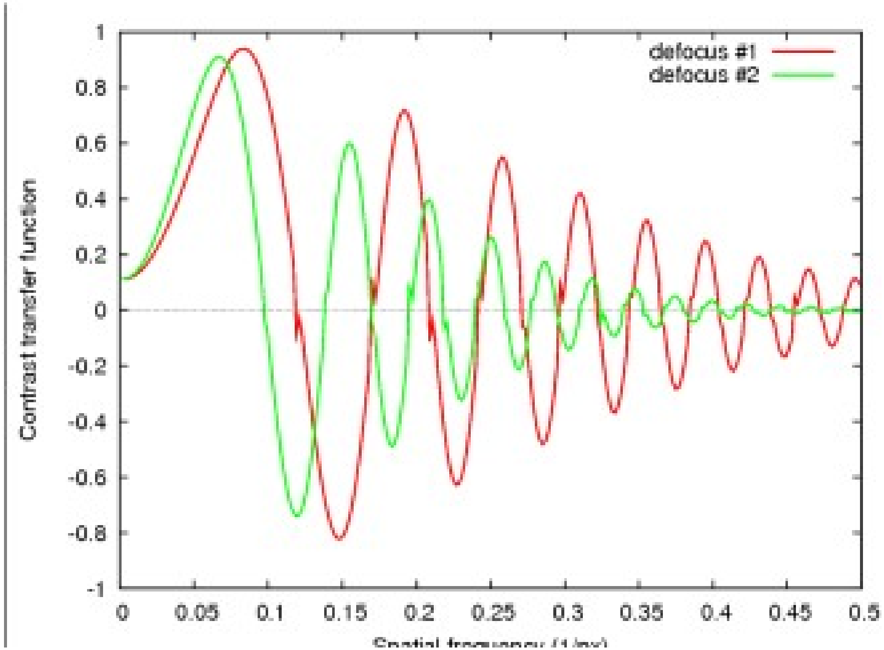
kV=300,ac=0.07,cs=2.7,z=-1,apix=1,B=300

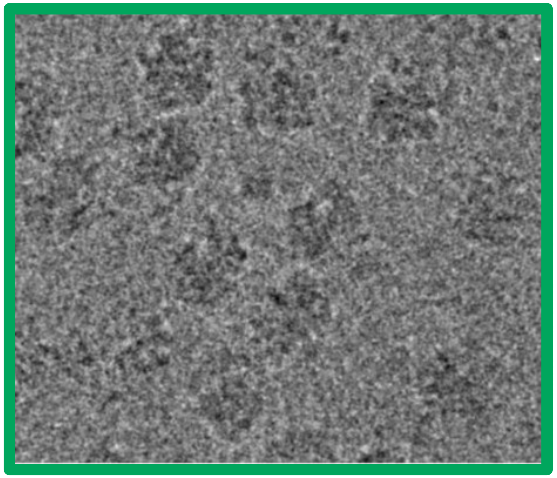


Contrast transfer function



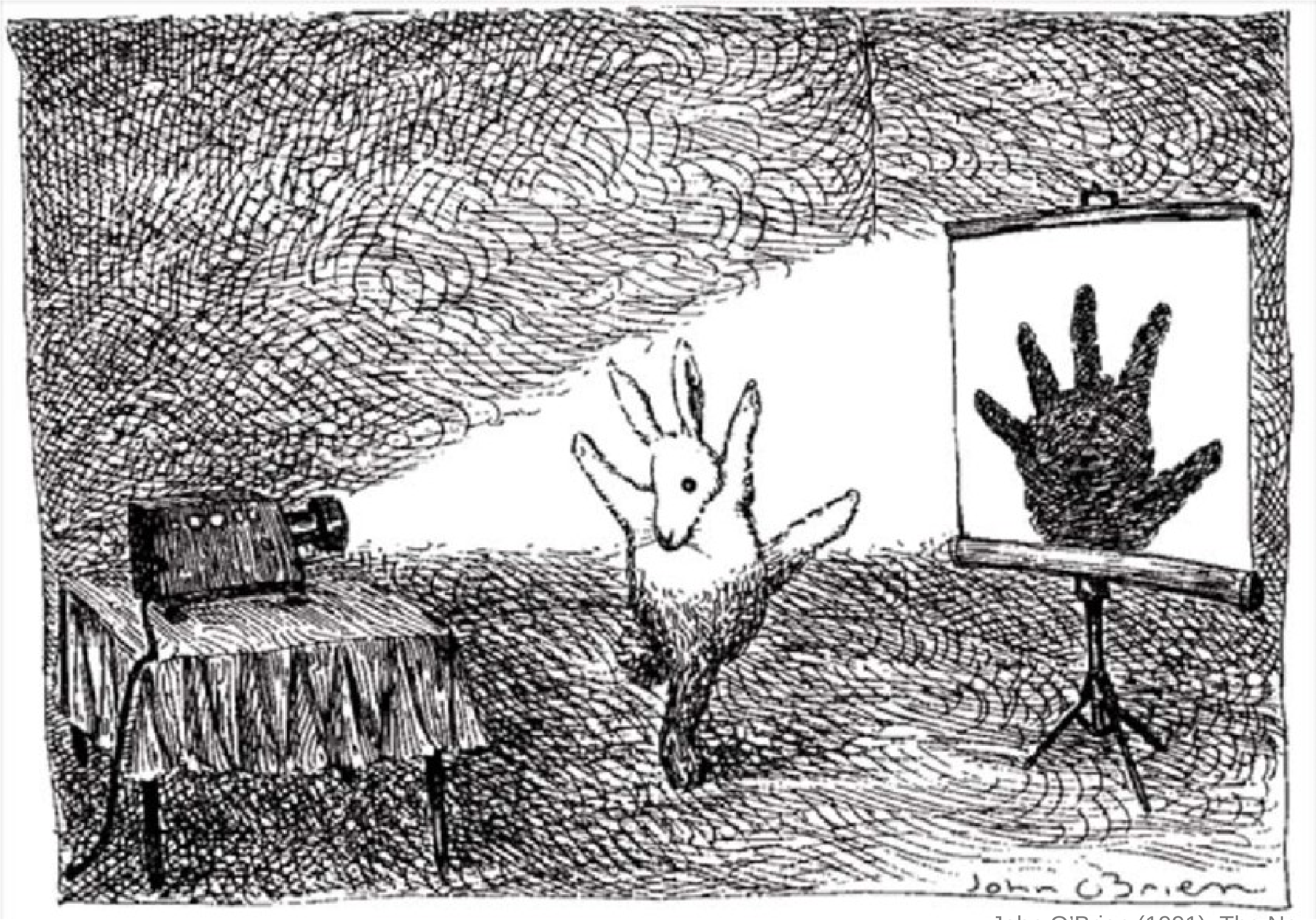
Low defocus





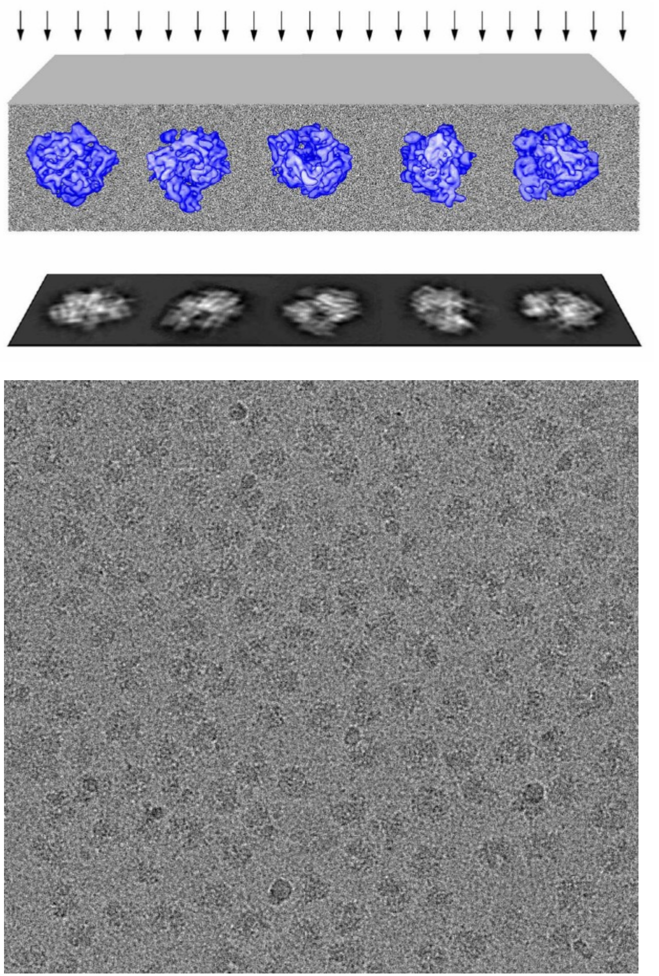
High defocus

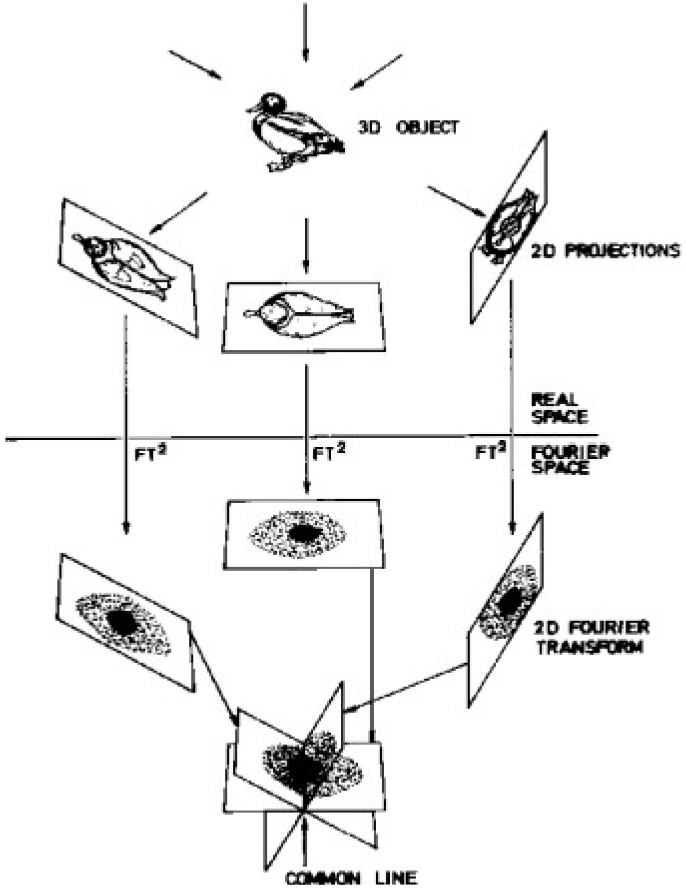
Projection theorem



John O'Brien (1991). The New Yorker

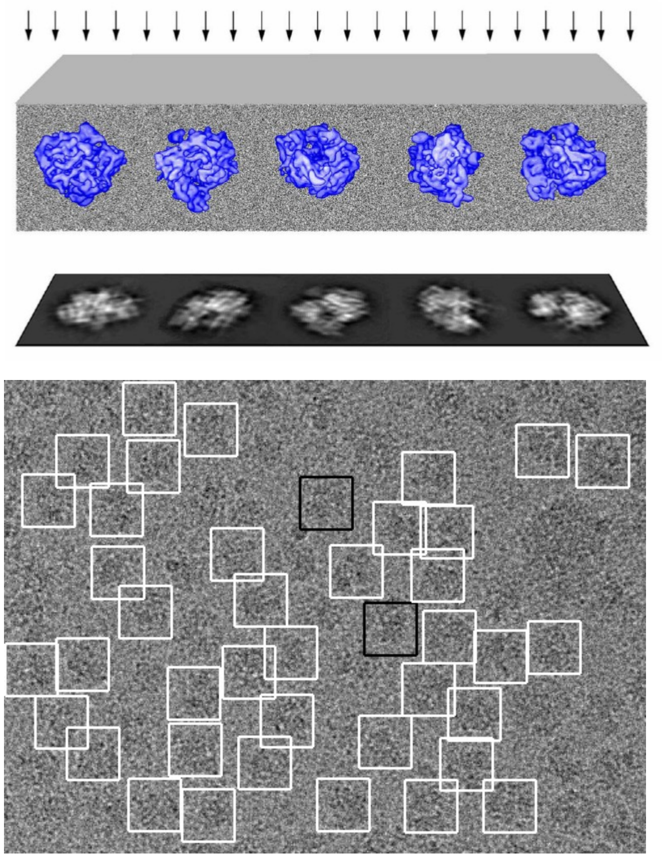
Projection theorem

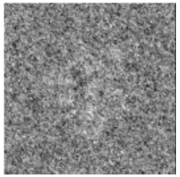




The 2D Fourier transform of the projection of a 3D density is a central section of the 3D Fourier transform of the density, perpendicular to the direction of projection.

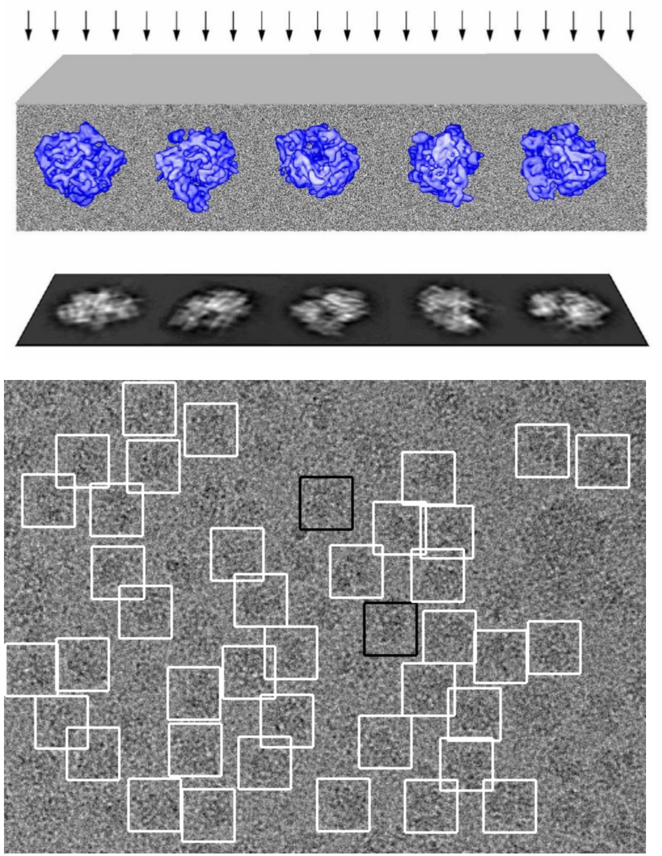
Particles (regions of interest)

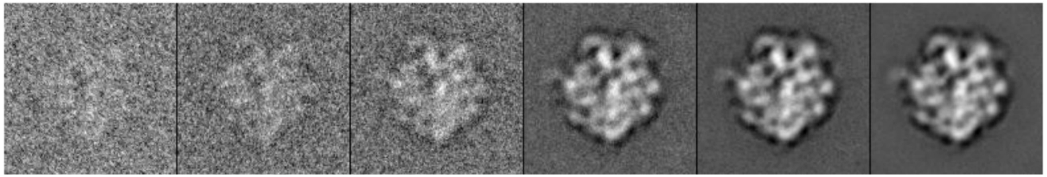




n=1

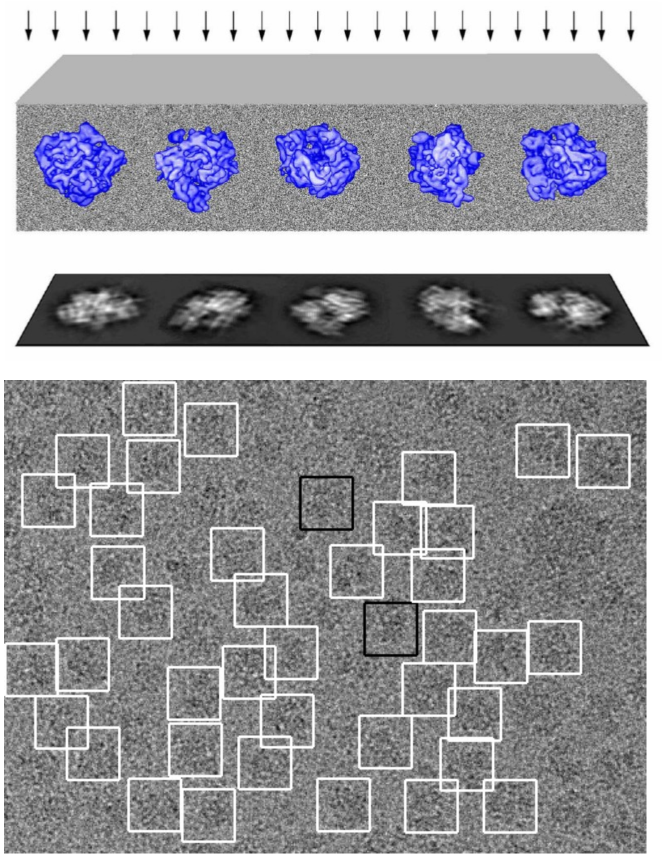
Particles (regions of interest)





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

Image alignment in 2D



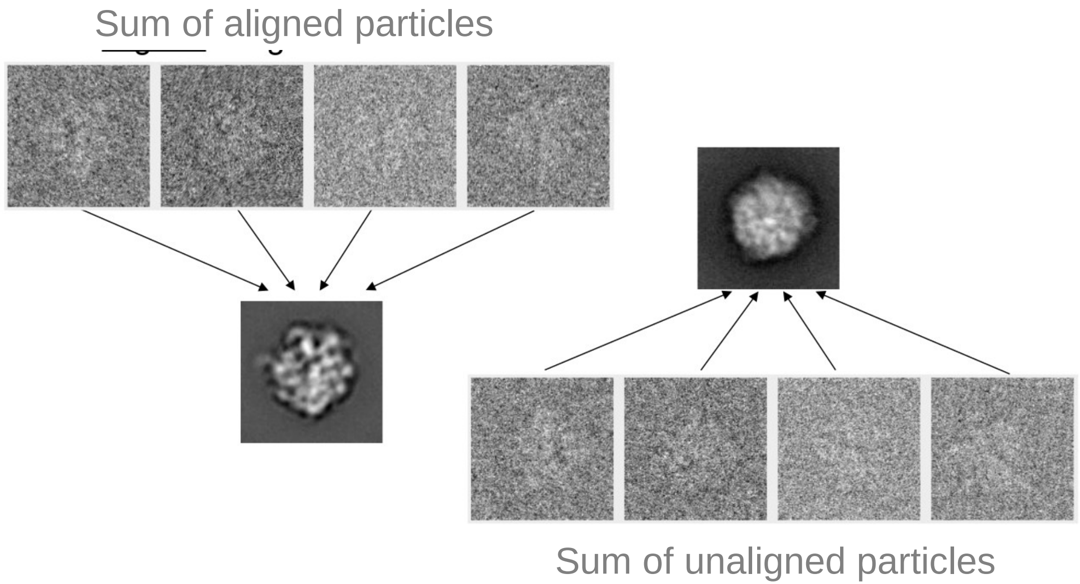
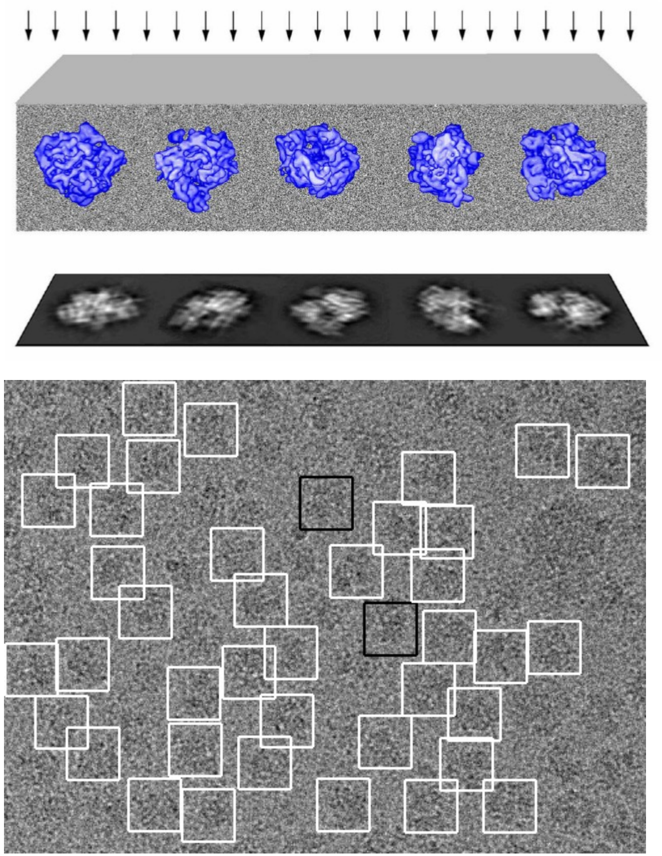
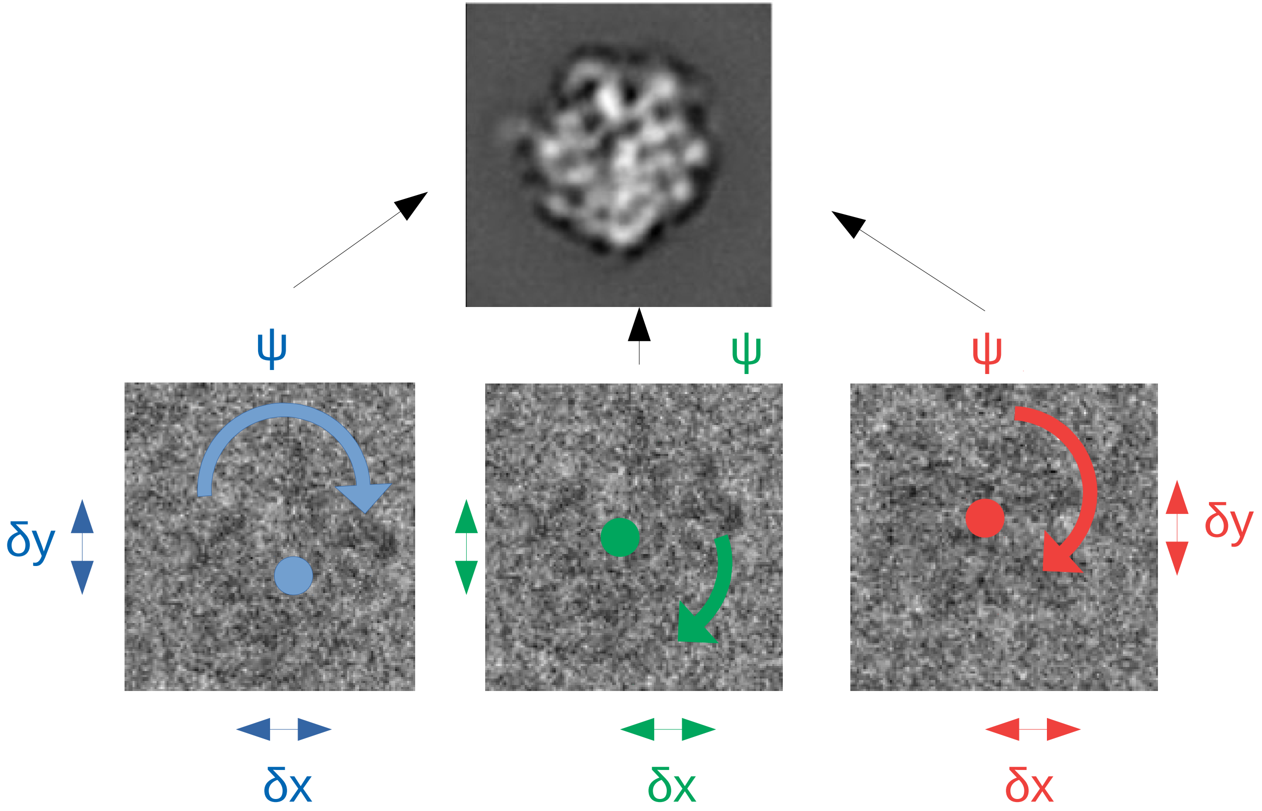


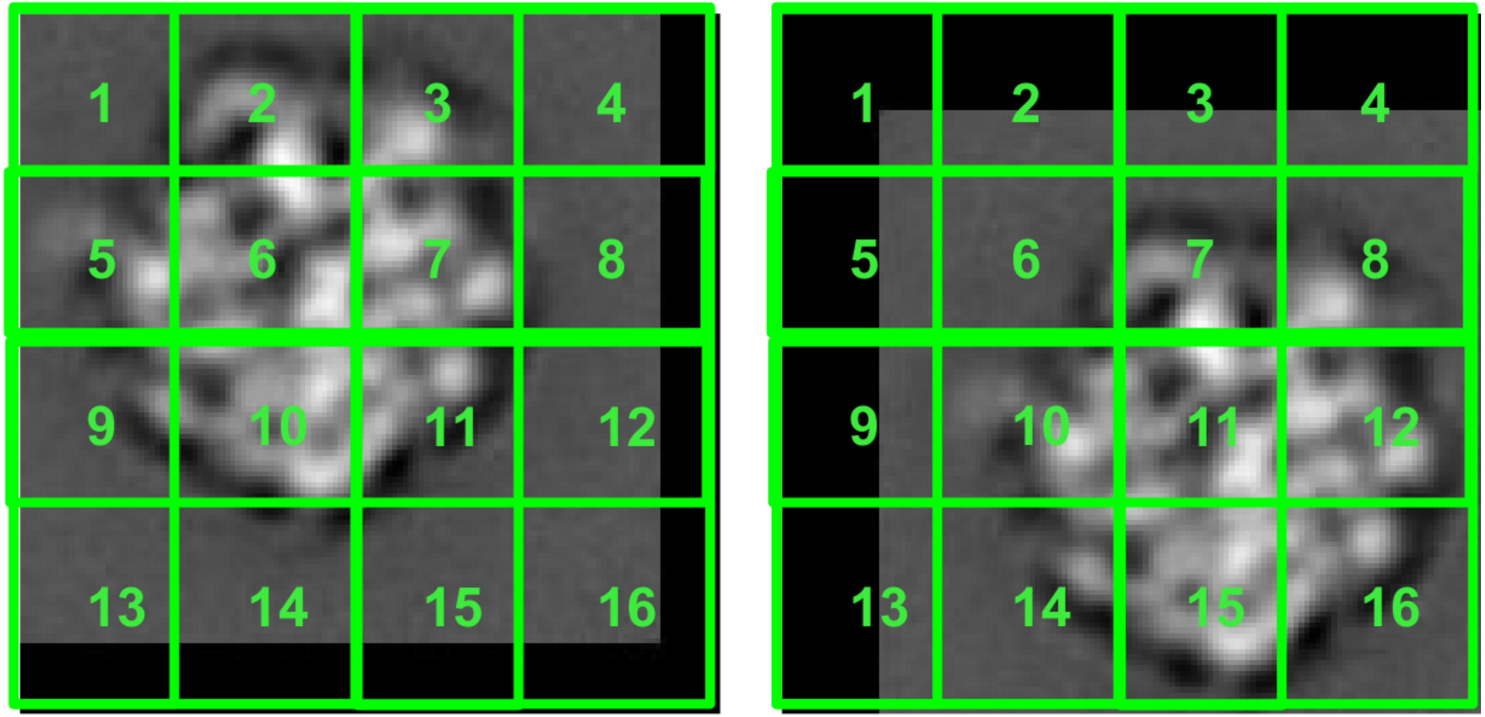
Image alignment in 2D





In order to align the particles in 2D, we need to determine three parameters:
- two translational
- one rotational (on of the Euler angles)

Image alignment in 2D



Cross correlation

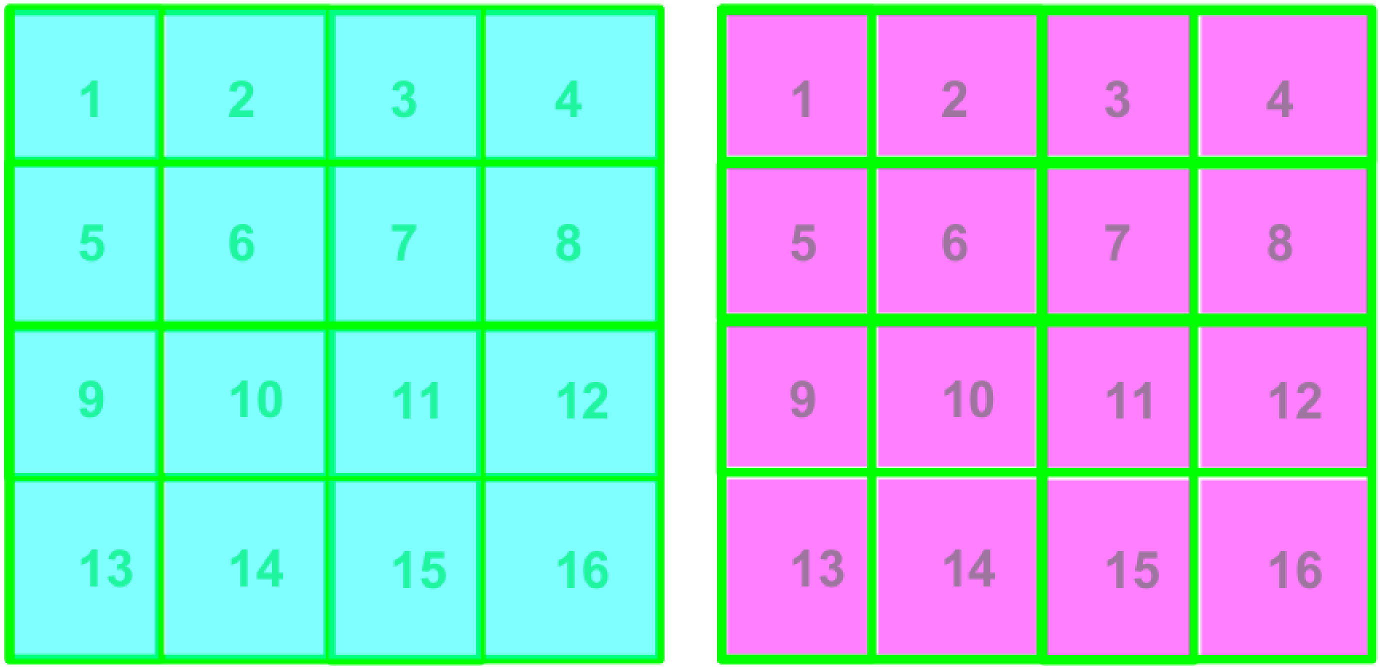


Image *f* Image *g*

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

Cross correlation

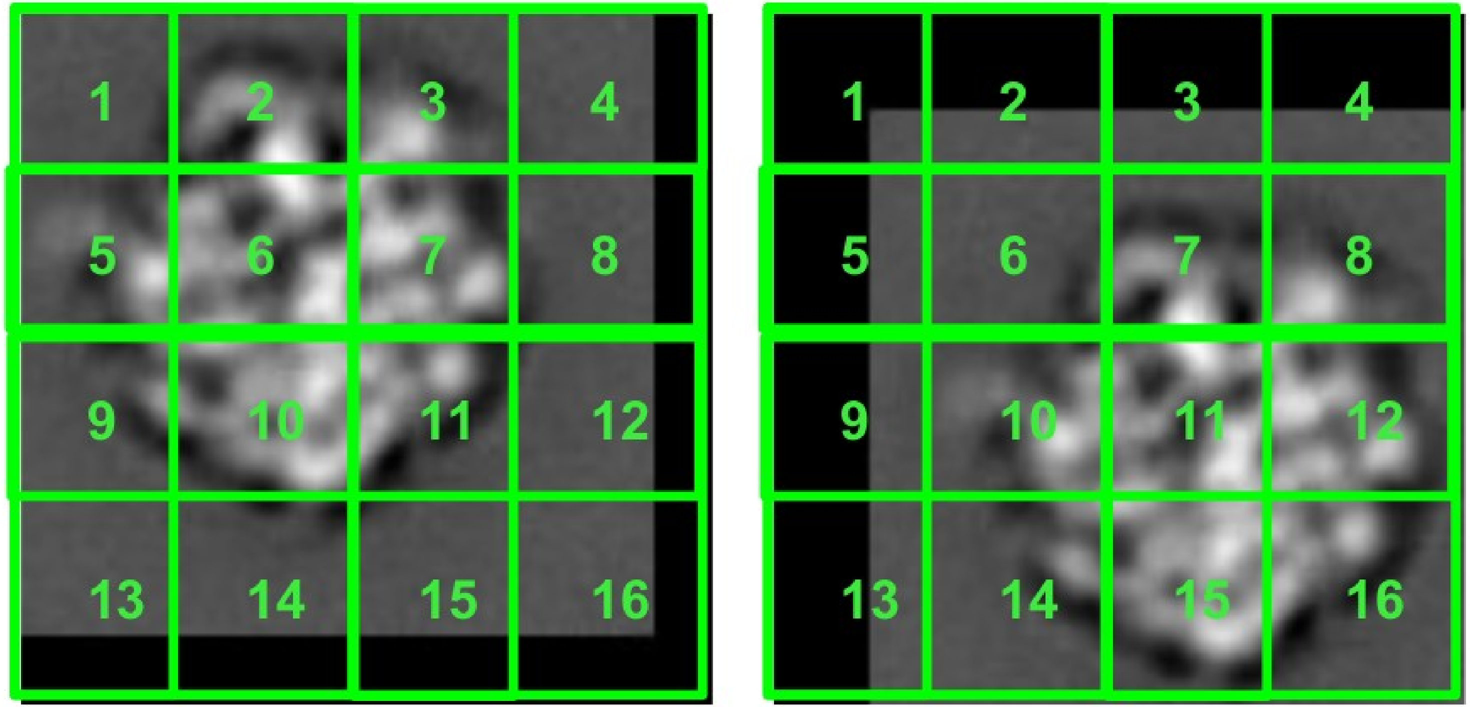


Image *f* Image *g*

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

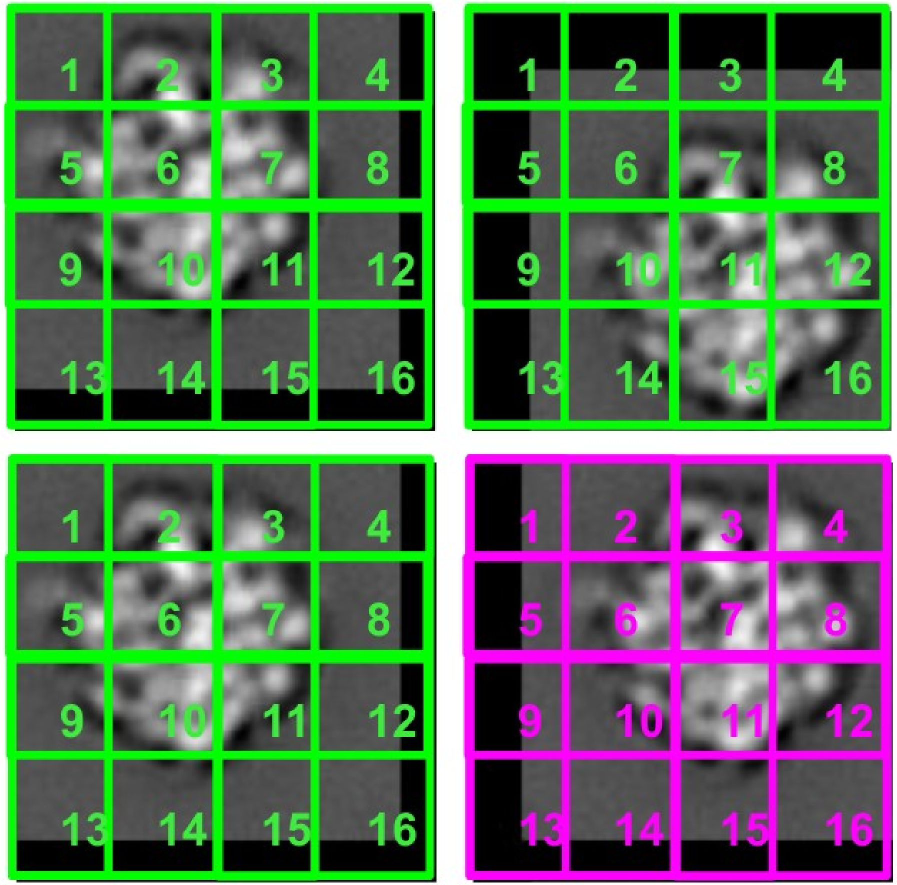
Cross correlation coefficient

Cross-correlation coefficient: $$\frac{\sum_{N=1}^{16} f(\vec{x})\, g(\vec{x})}{\sigma_f \sigma_g}$$

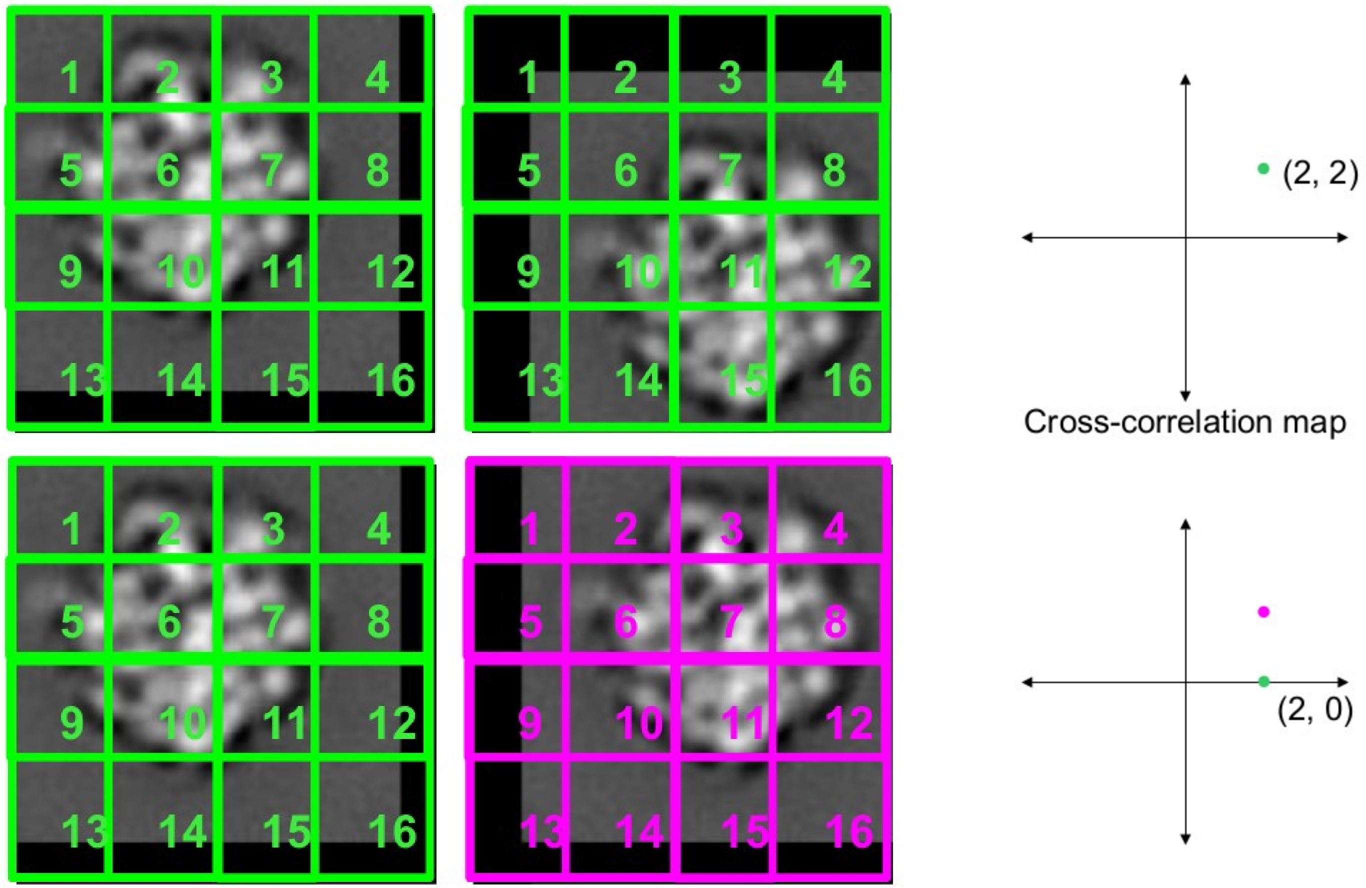
normalization

If the alignment is perfect, the cross-correlation coefficient will be 1

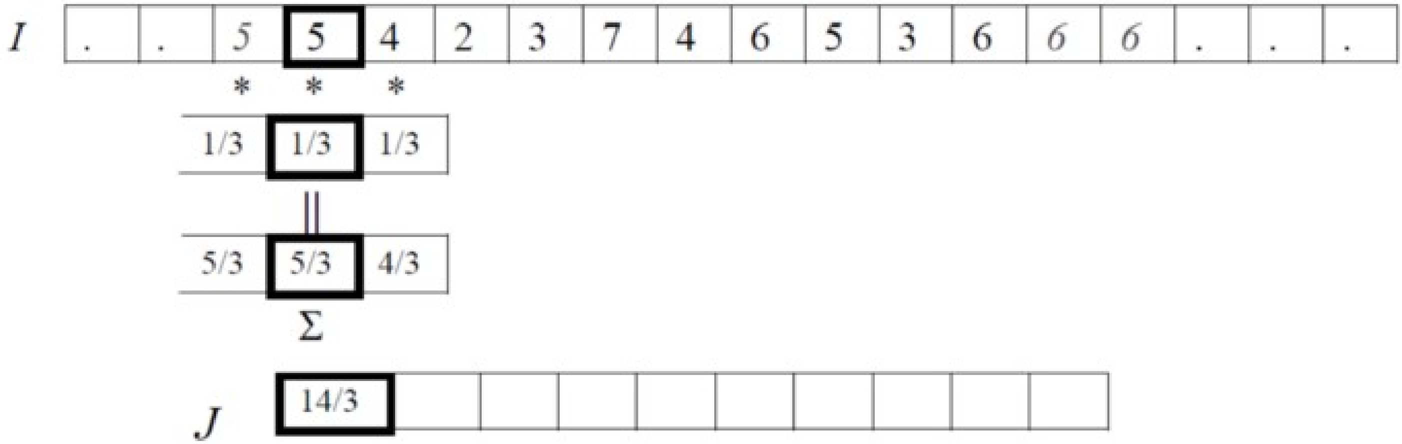
Cross correlation



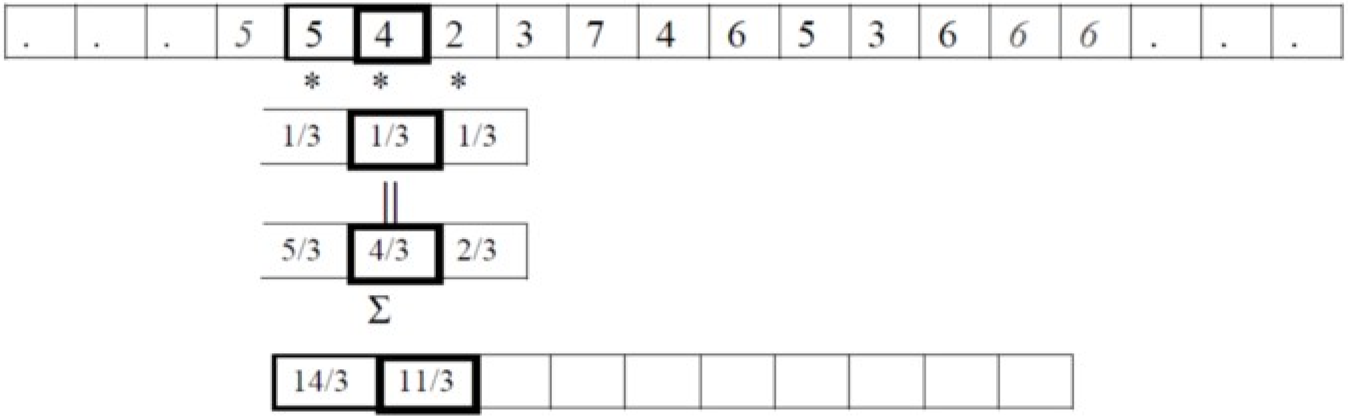
Cross correlation



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

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

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

Convolution

Cross correlation function in 2D

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

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

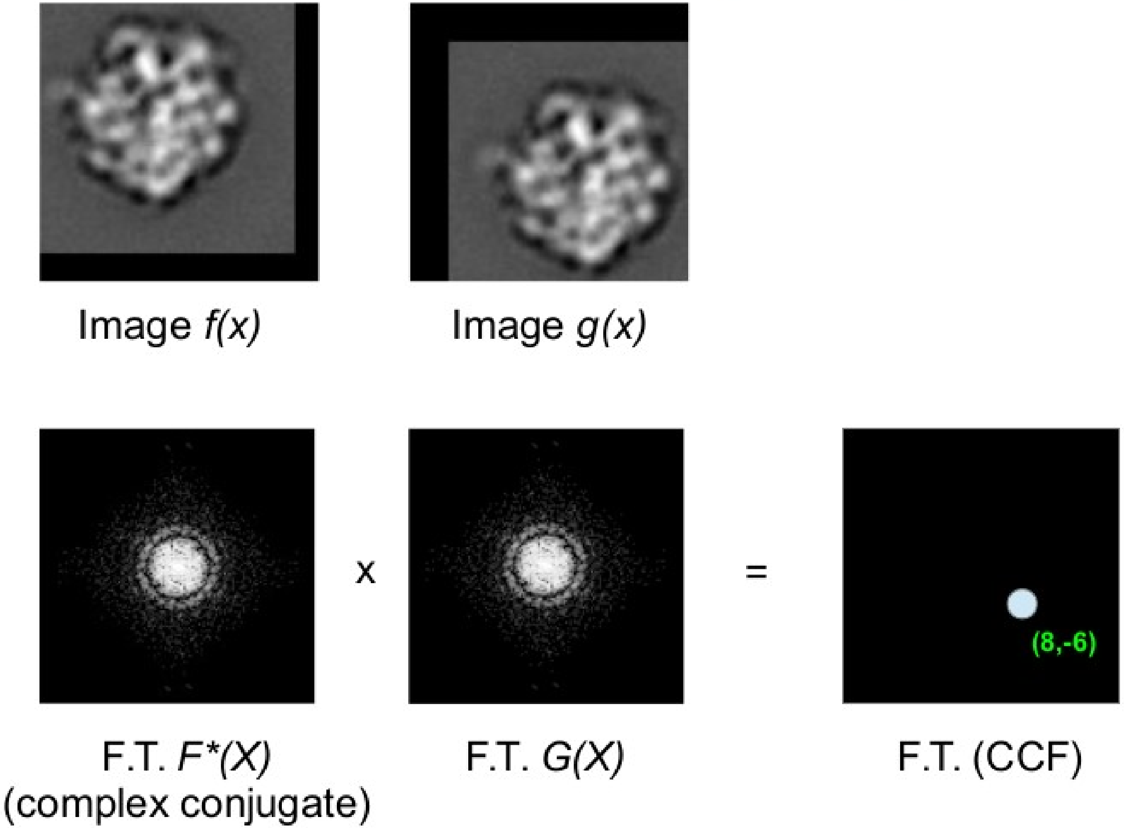
Convolution

$FT(F * I) = FT(F) \cdot FT(I)$

$FT(F \circ I) = FT(F)^* \cdot FT(I)$

Convolution theorem

Cross correlation function



Orientation alignment

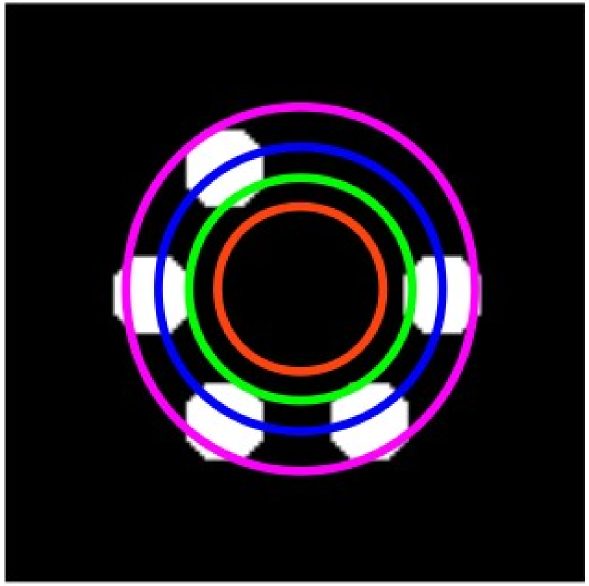


Image 1

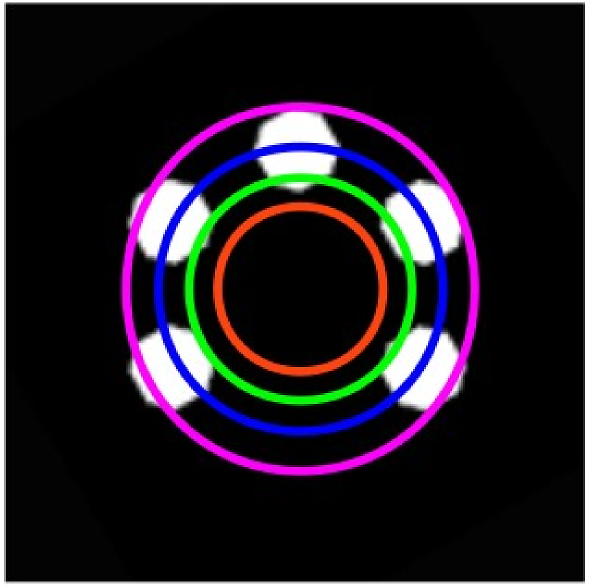
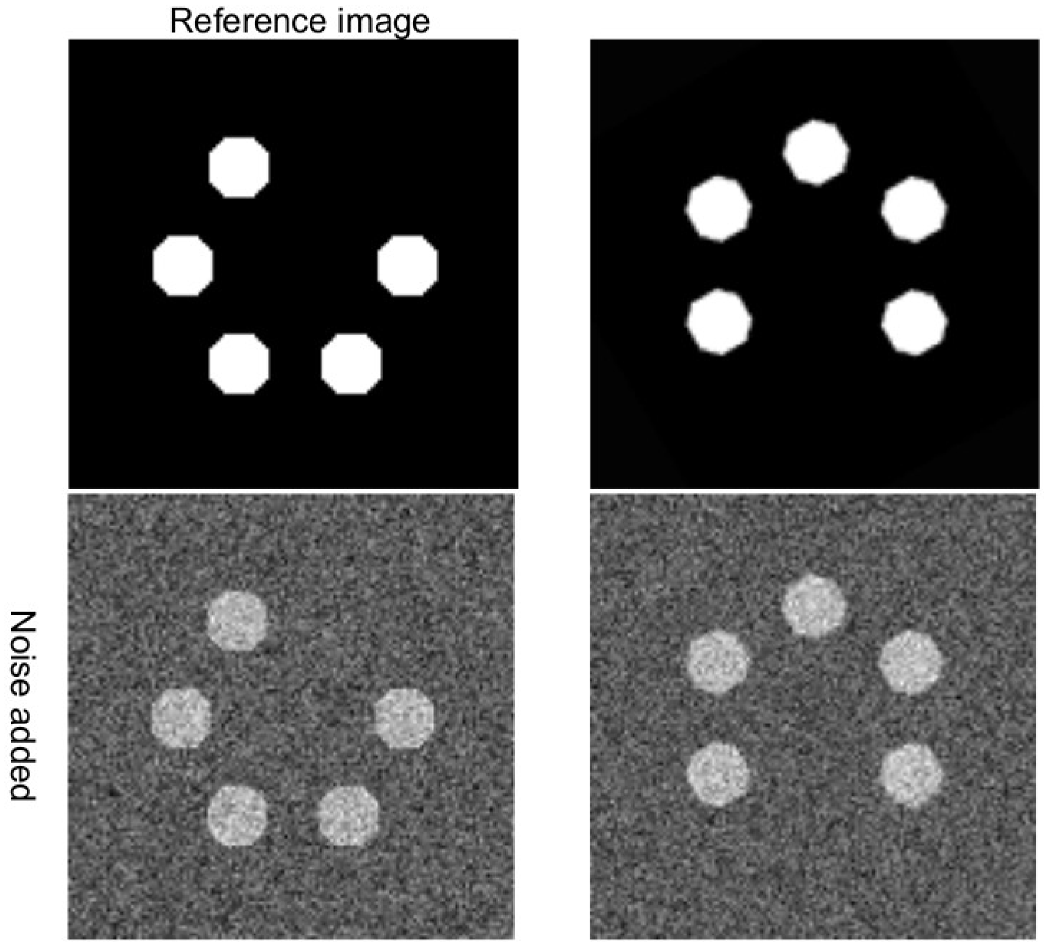


Image 2

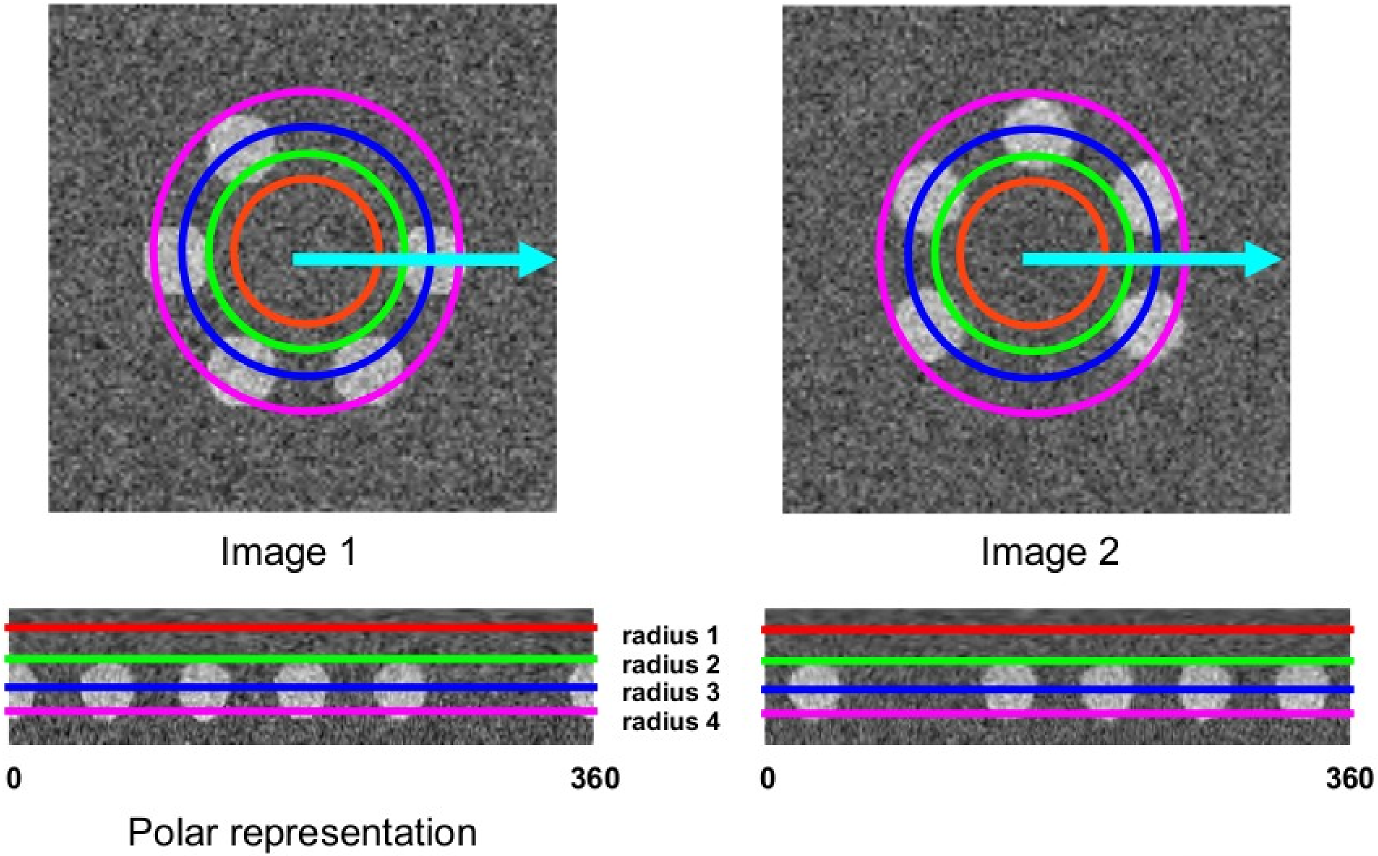
We take a series of rings from each image, unravel them,
and compute a series of 1D cross-correlation functions.

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

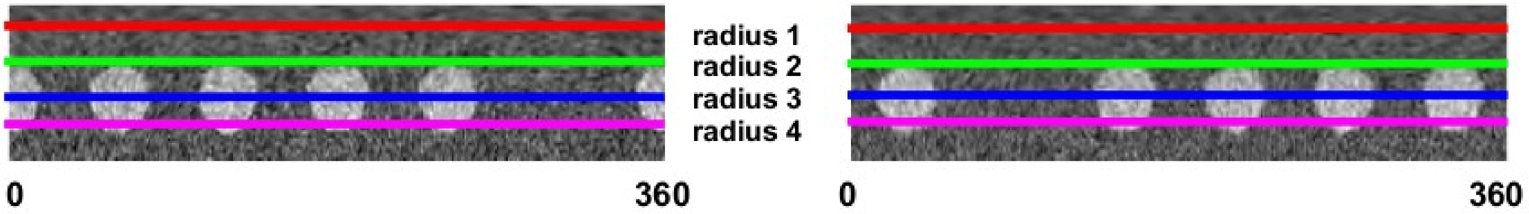
Orientation alignment

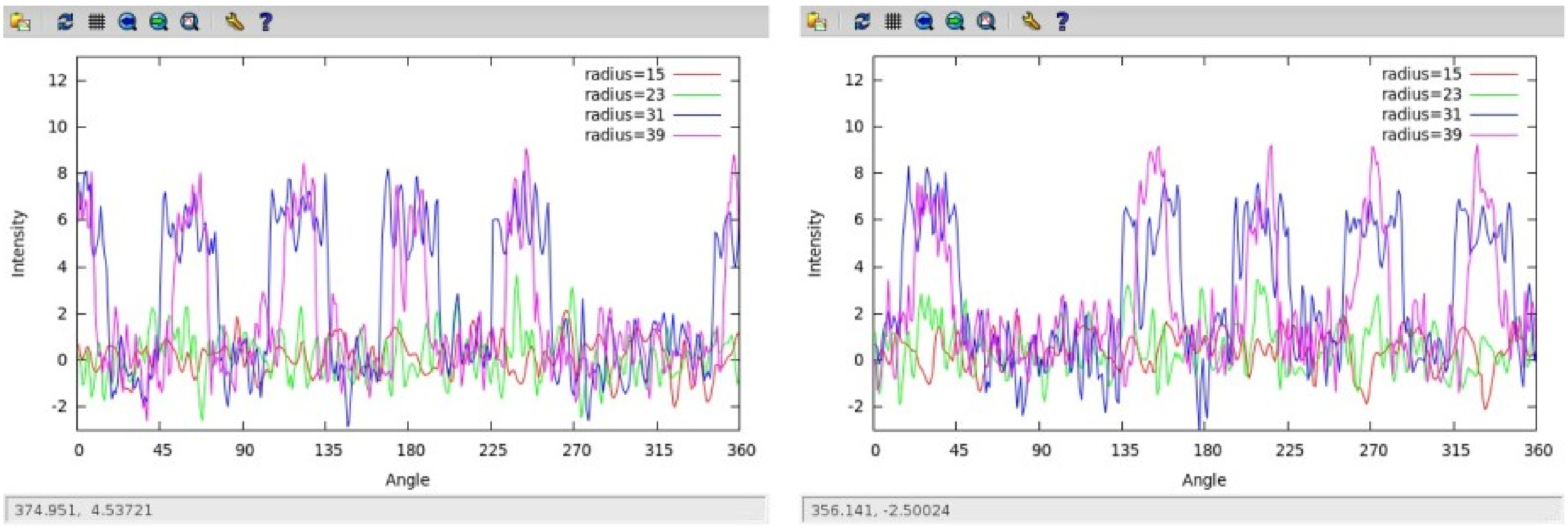


Orientation alignment



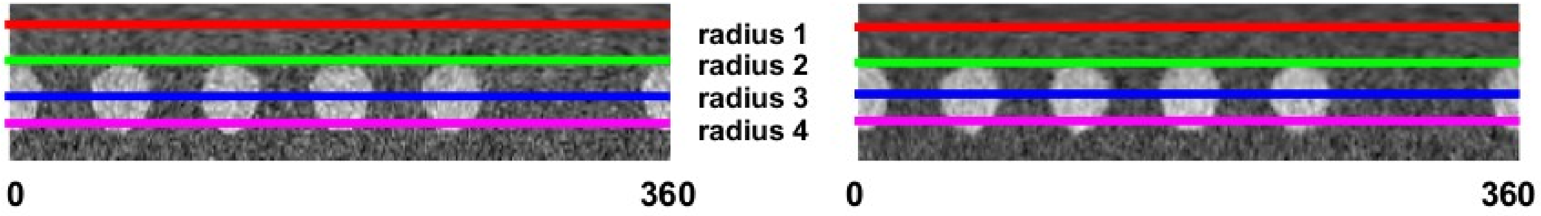
Orientation alignment

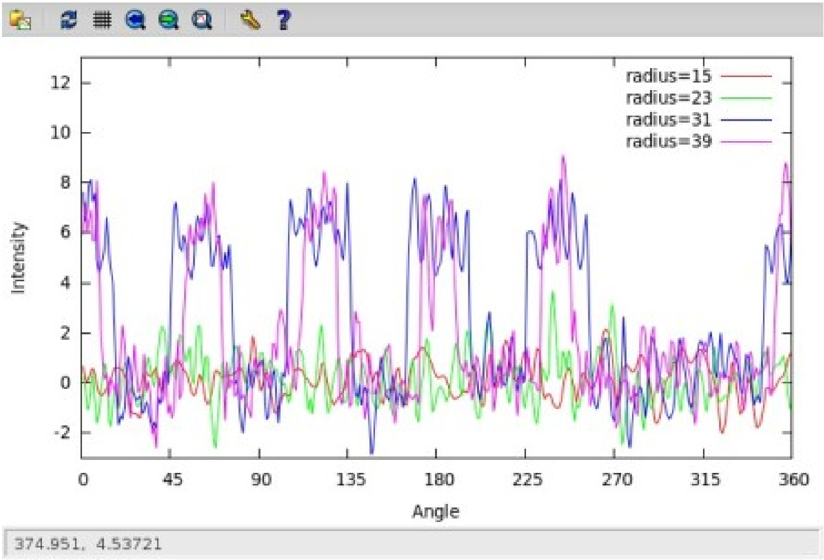




Orientation alignment

- after rotation





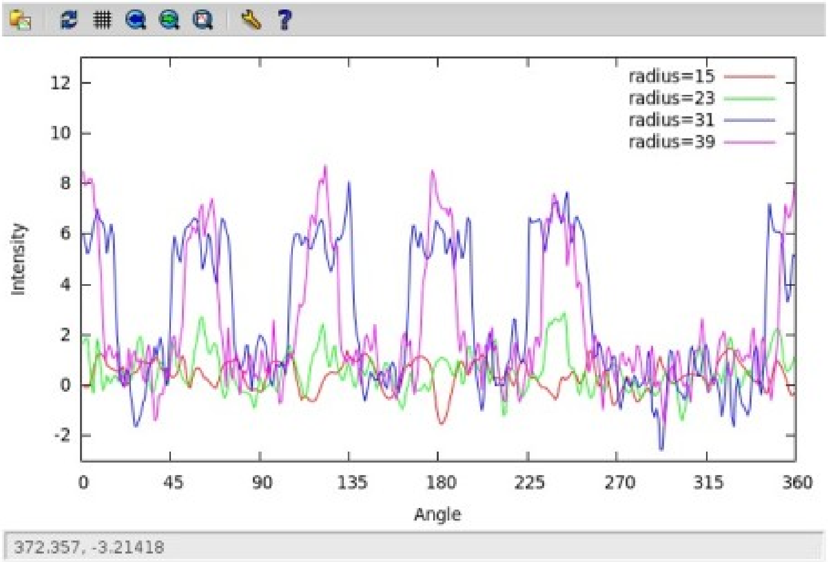
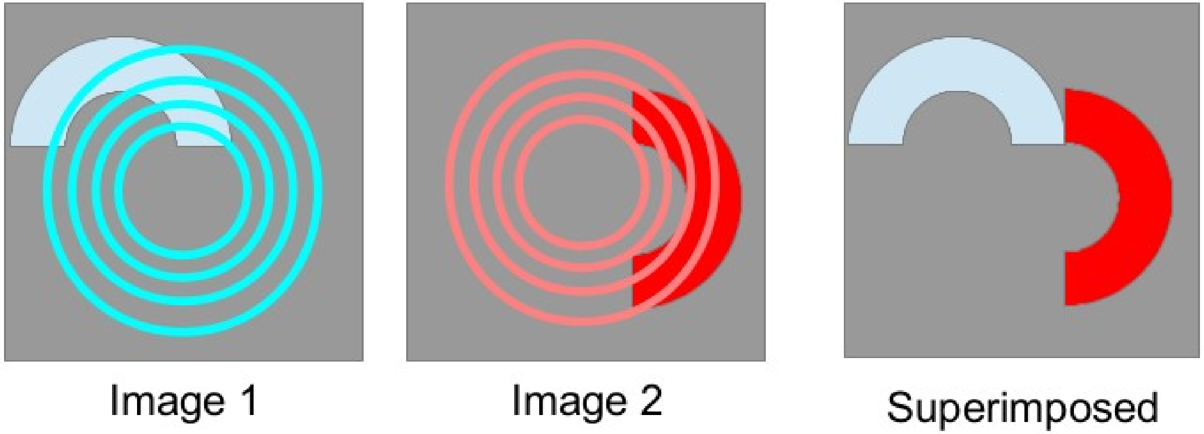


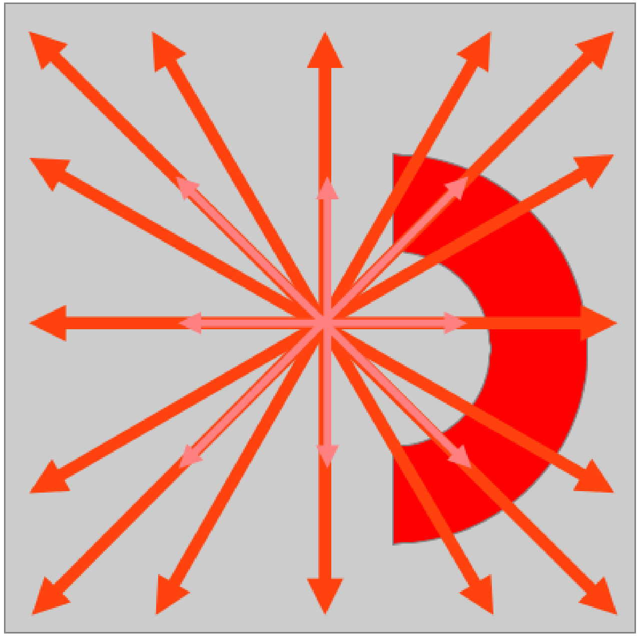
Image alignment in 2D



Translational and orientation alignment are interdependent

SOLUTION: You try a set of reasonable shifts, and perform separate orientation alignments for each.

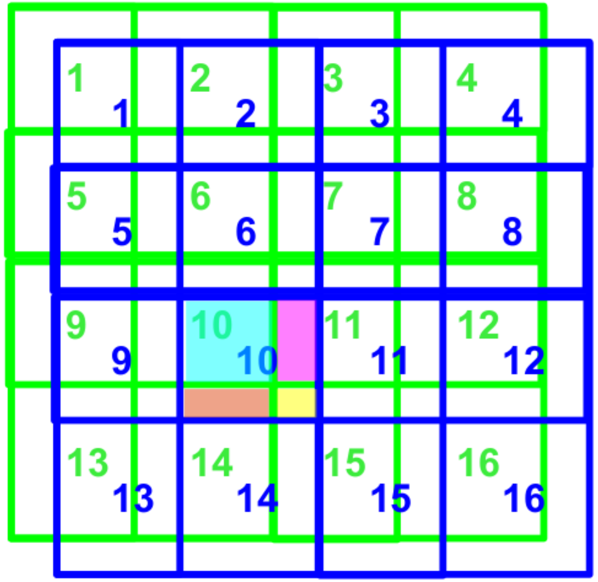
Image alignment in 2D



Set of all shifts of up to 1 pixel
Set of all new shifts of up to 2 pixels
Shifts of (0, +/-1, +/-2) pixels results in 25 orientation searches.

Interpolation

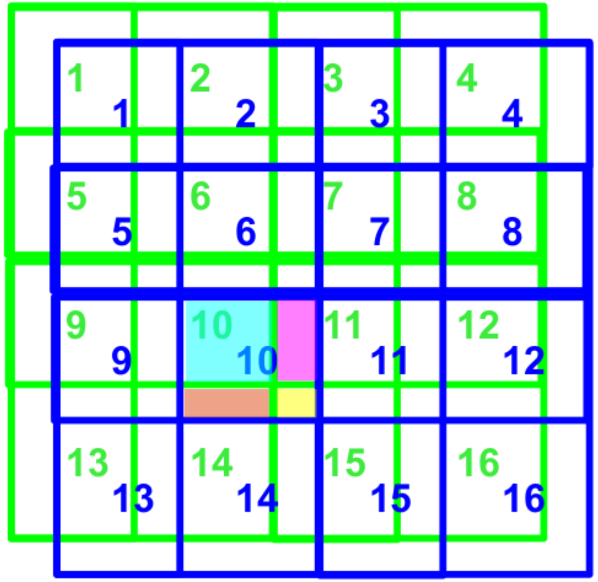
Shift

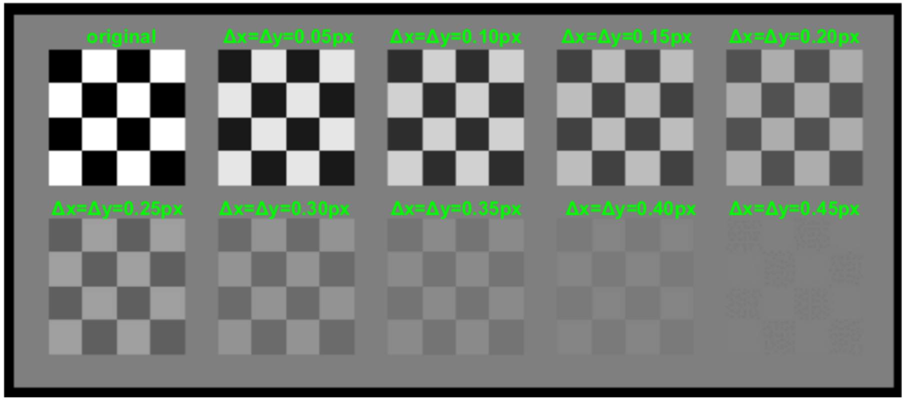


Suppose we shift the image in x & y.
The new pixels will be weighted averages of the old pixels.
The more the mix the pixels, the worse the result will be.

Interpolation

Shift

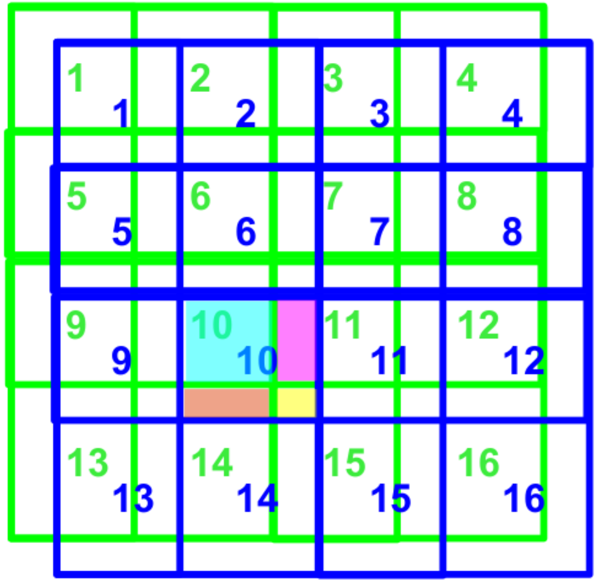




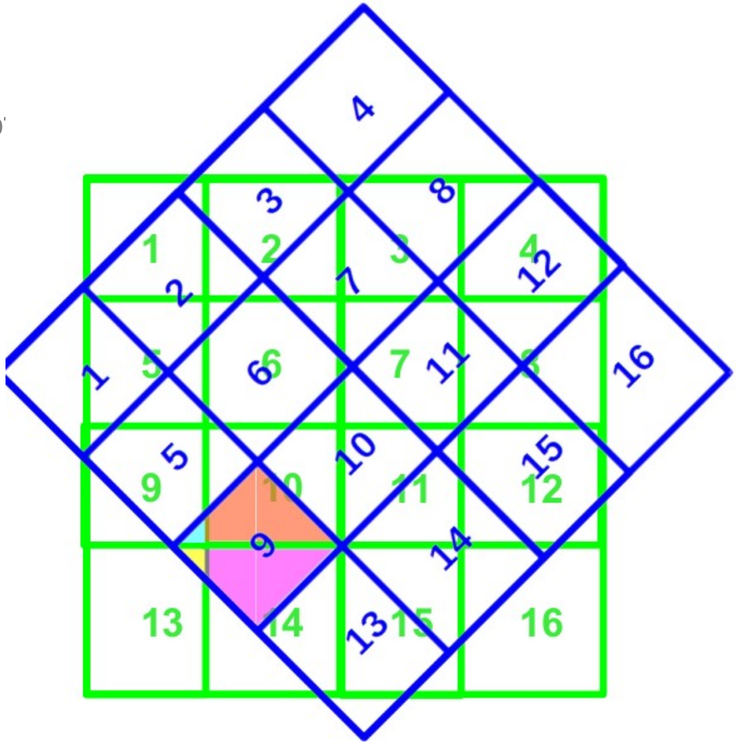
Suppose we shift the image in x & y.
The new pixels will be weighted averages of the old pixels.
The more the mix the pixels, the worse the result will be.

Interpolation

Shift

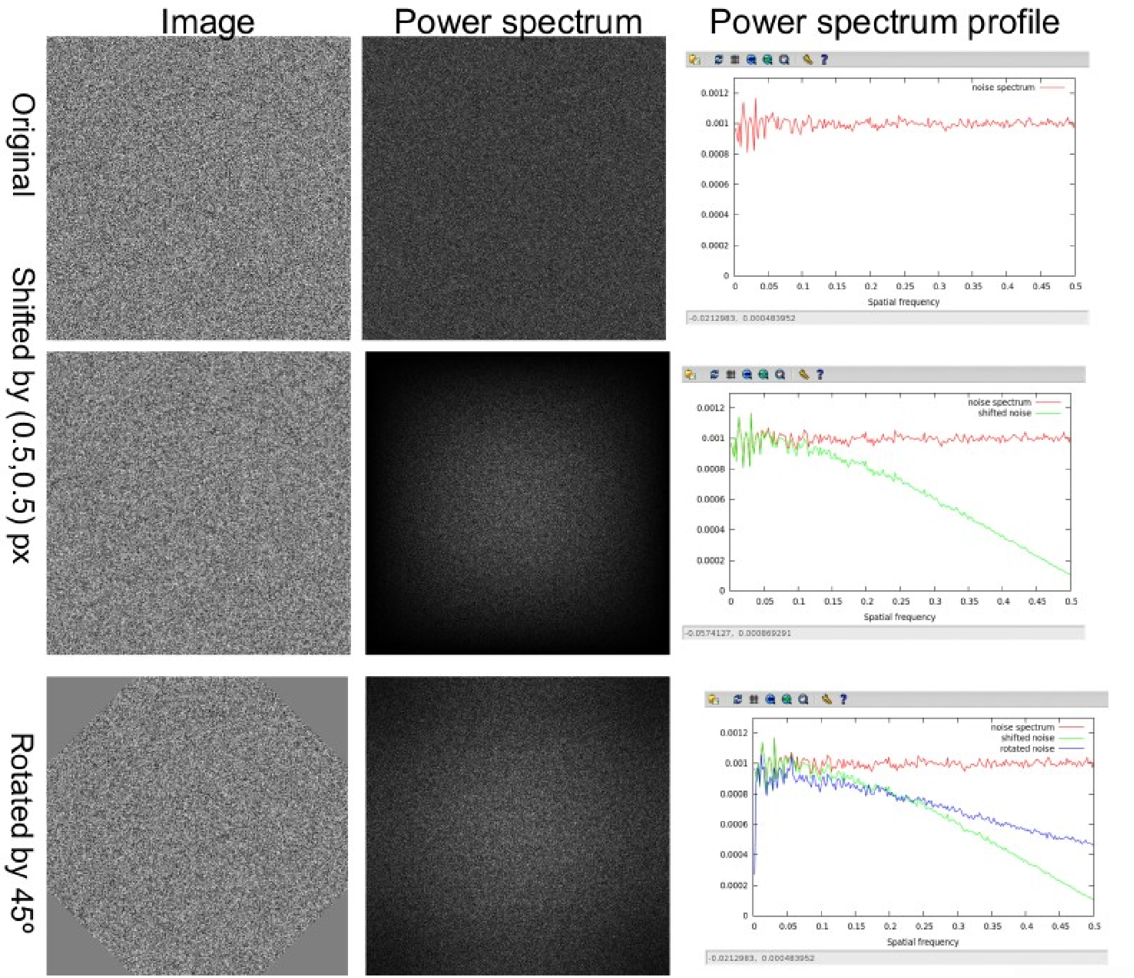


Ro



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

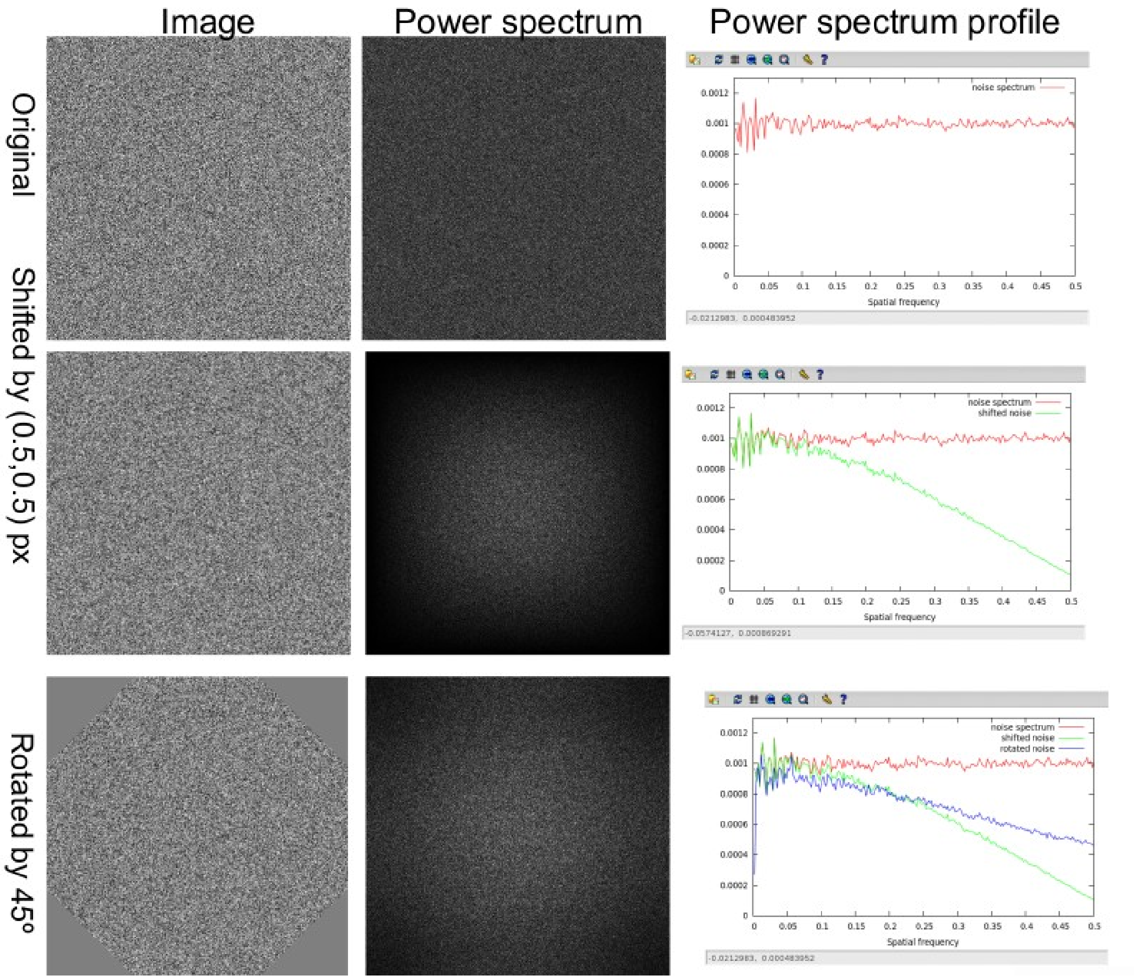


The Fourier transform of noise is noise

- “White” noise is evenly distributed in Fourier space

- “White” means that each pixel is independent

Interpolation

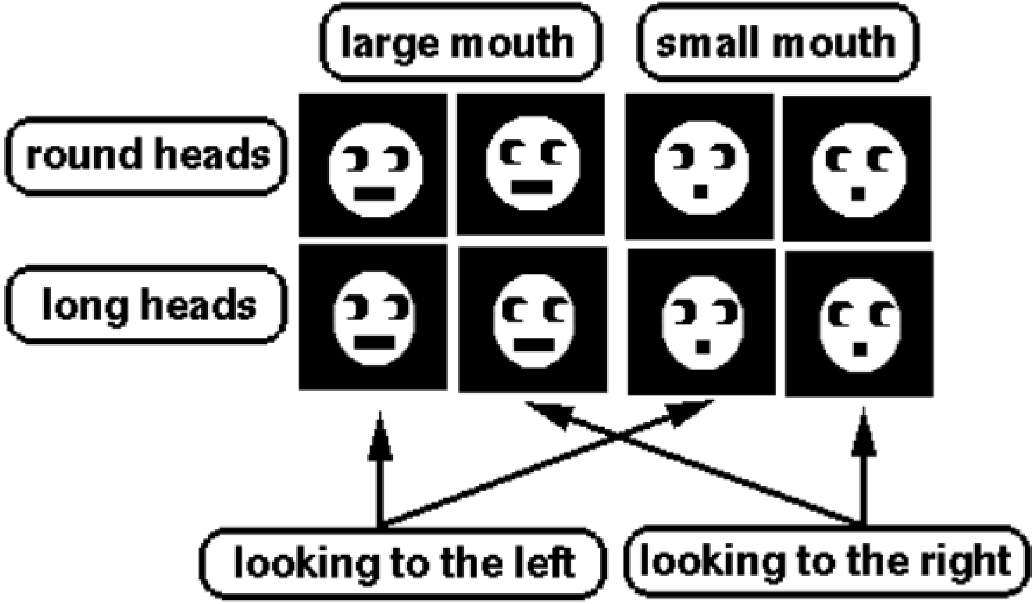


The Fourier transform of noise is noise

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

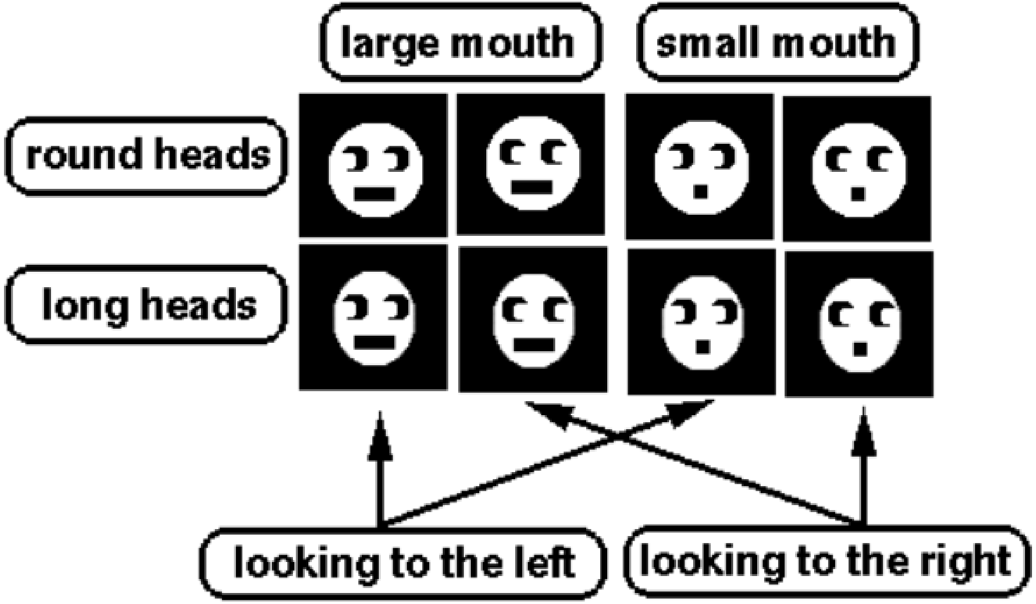
The degradation of the images means that we should minimize the number of interpolations.

Classification



Frank (1996), J.Microscopy

Classification



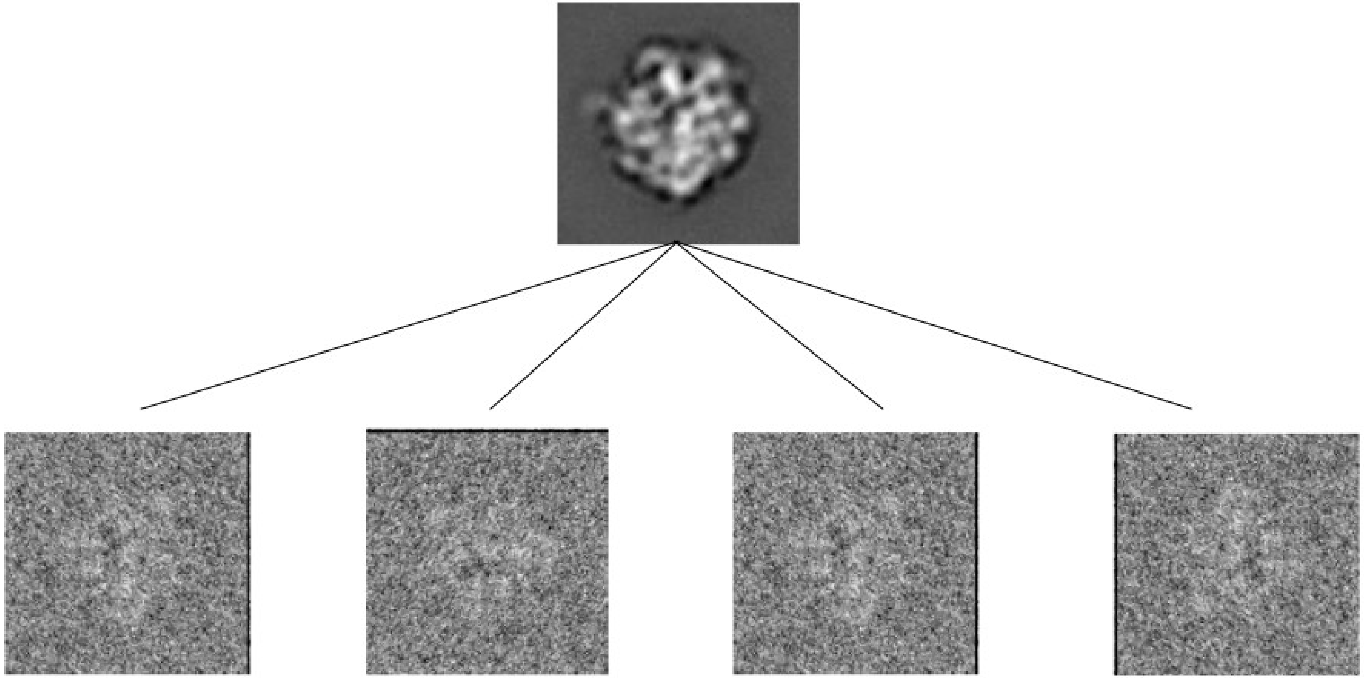
Frank (1996), J.Microscopy

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

Classification

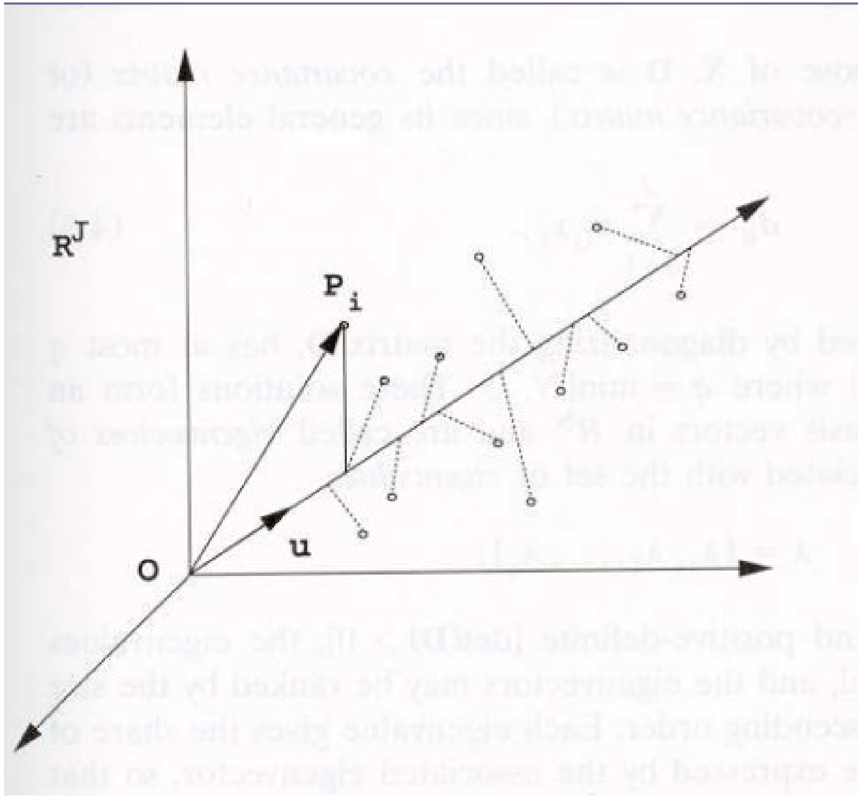
Reference based alignment



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.

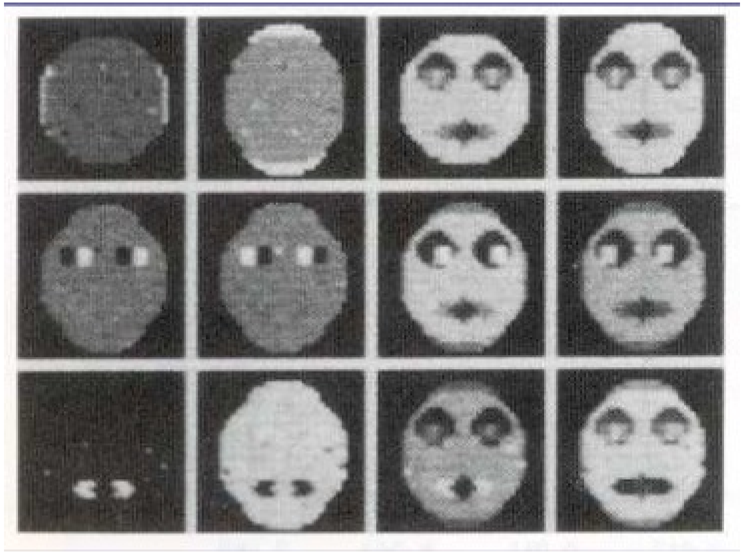


eigenvectors

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

- find new coordinate system tailored to the data

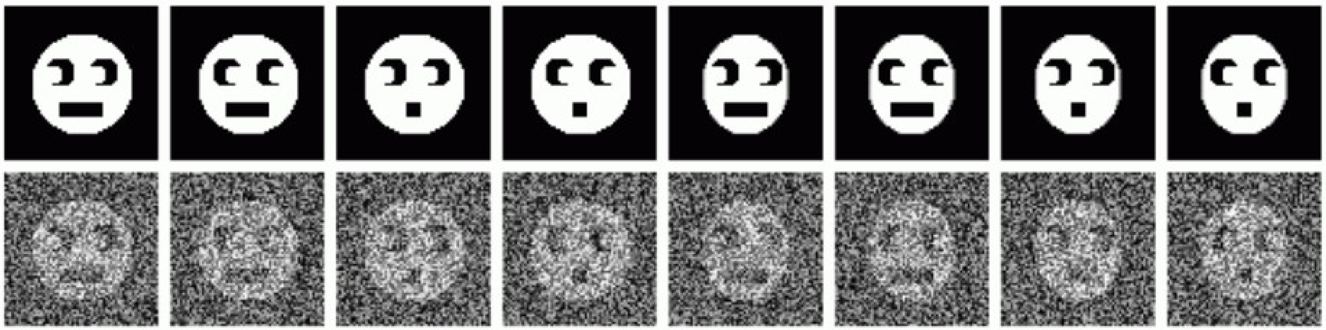
- find a space with reduced dimensionality for the representation of the objects. This greatly simplifies classification.



eigenimages

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

8 classes of faces, 64x64 pixels



With noise added

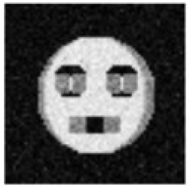
Average:

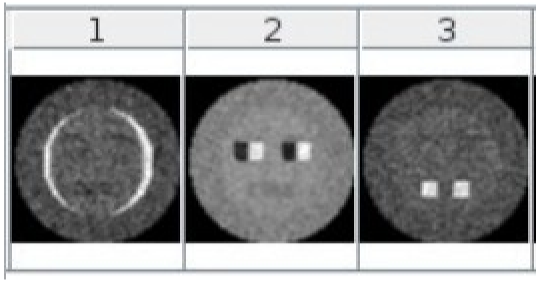
Principle component analysis (PCA), Correspondence analysis (CA)

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

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

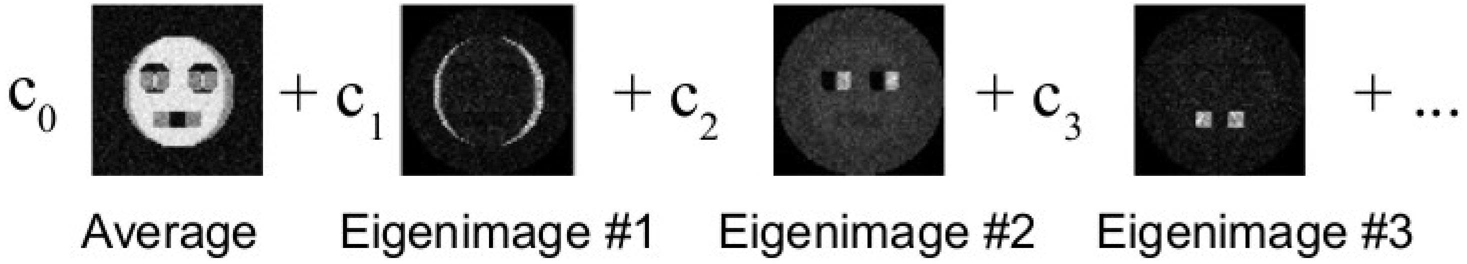
- The eigenvectors describe correlated variations in the data.
- The eigenvectors have 4096 elements and can beconverted back into images, called “eigenimages.”
- The first eigenvectors will account for the most variation. The later eigenvectors may only describe noise.
- Linear combinations of these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

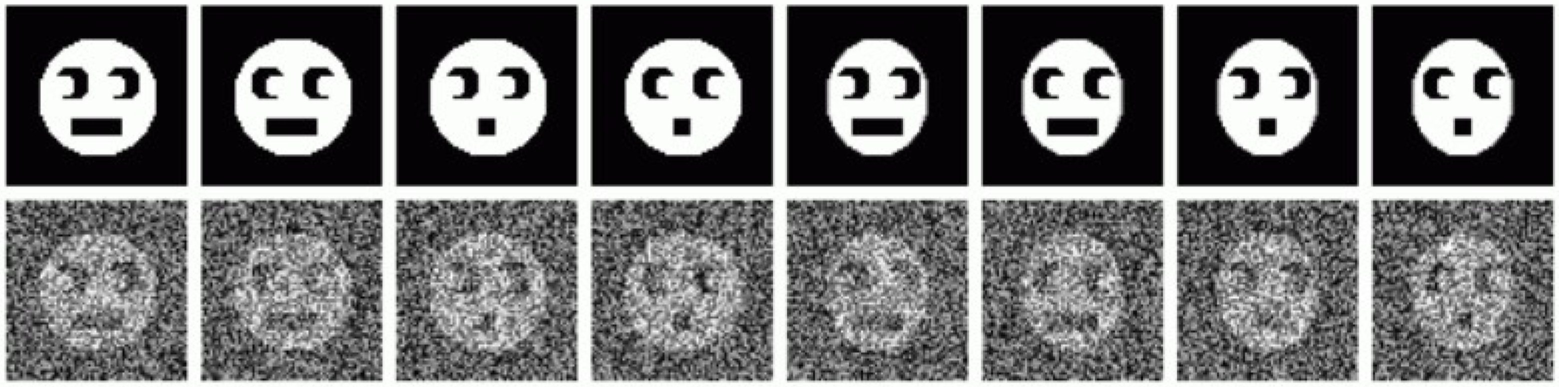




eigenimages

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





Linear combinations of these images will give us approximations of the classes that make up the data.