CNRS
INSERM
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1) Short summary of general concepts

2) Single particle image processing and 3D reconstruction: pre-processing steps

→ **getting prepared for the practicals**

Bruno Klaholz 2017, ReNaFoBis, Oléron School
http://www.igbmc.fr
http://igbmc.fr/Klaholz

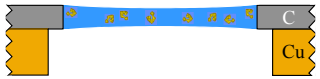
Electron microscopy: application examples - Summary

Negative staining	2D observation + 3D reconstruction
Spreading	2D observation only
Shadowing	2D observation only
Cryo-EM	(2D observation +) **3D reconstruction**
2D crystals	(2D observation +) **3D reconstruction**
Tomography of cellular structures	**(2D observation +) 3D reconstruction**
Freeze-fracture	2D observation only

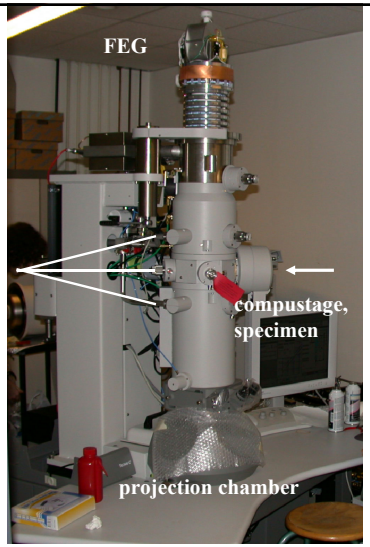
Sample preparation

A new research project – steps to cross for single particle cryo-EM:

- **functional studies**
- **purification and biophysical characterization of complexes (very important)**
- **optimization of the sample for imaging:**
  - **fast freezing (vitreous ice)**
  - **buffer composition**
  - **support type (holey carbon vs continuous carbon film)**
  - **concentration (~0.5 mg/ml)**
  - **ice thickness**
  - **absence of contamination**

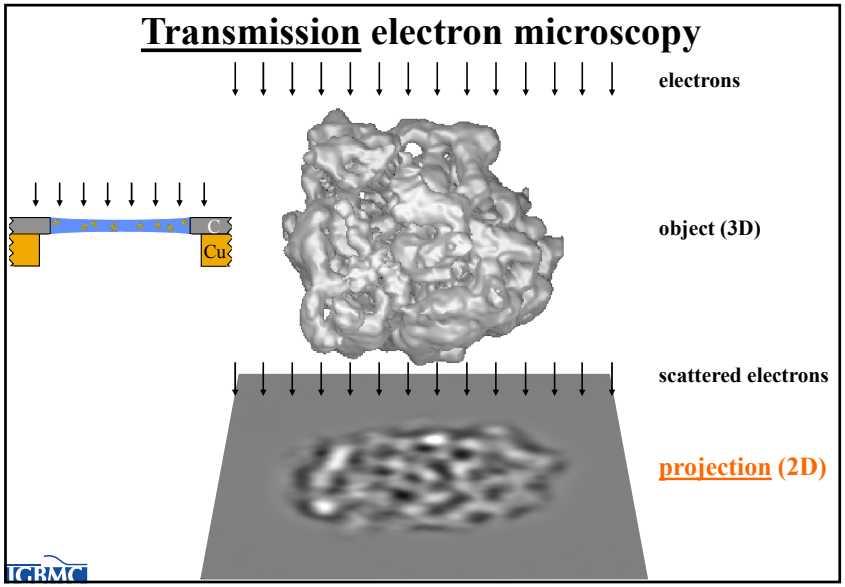


A transmission electron microscope (TEM)



Field emission gun (FEG) electron microscope (Tecnai20, IGBMC)

Transmission electron microscopy



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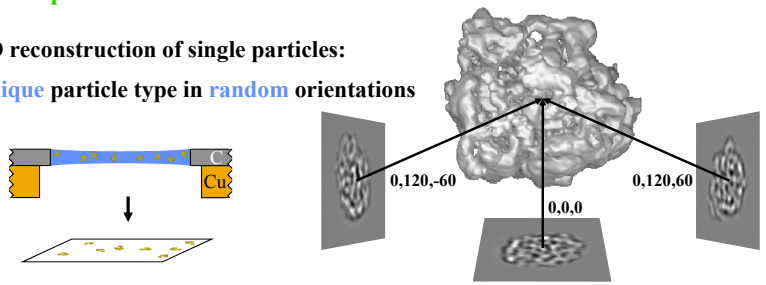
Structure determination by 3D reconstruction

Assumptions?

3D reconstruction of single particles:

unique particle type in random orientations



Reconstruction provides a 3D density map, i.e. a structure with all internal features

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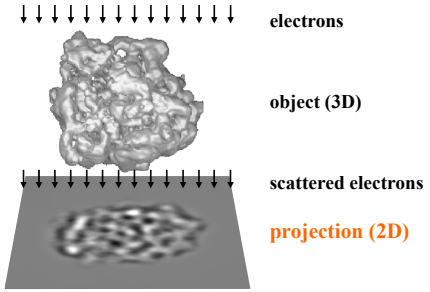
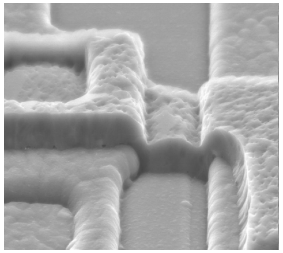
Transmission electron microscopy



All *internal* features in a 2D view!

Scanning electron microscopy



Only surface!

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sun rays: projections

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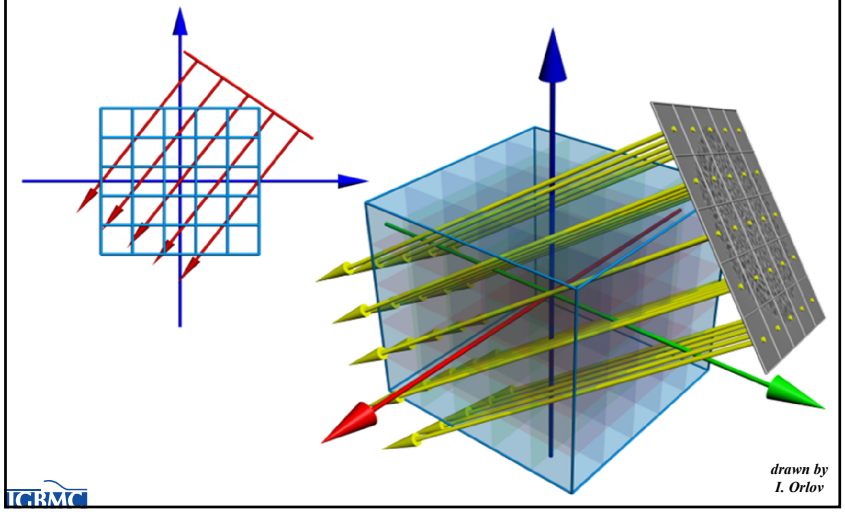
Back-Projections in 3D



drawn by I. Orlov

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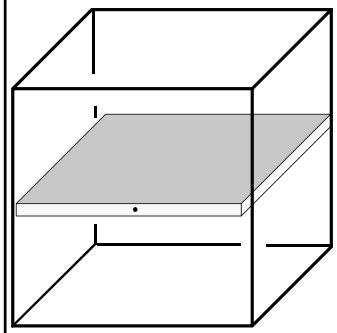
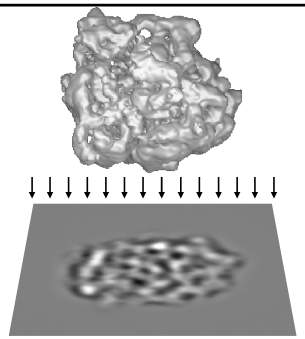
Theorem of the central section



reverse Fourier transform



Fourier transform

central section in Fourier space

Theorem of the Central Section:
The FT of a 2D projection corresponds to the central section of the 3D Fourier transform of the density (i.e. plane perpendicular to the direction of the projection).

D. DeRosier & A. Klug, *Nature* 1968; R. G. Hart, *Science* 1968.

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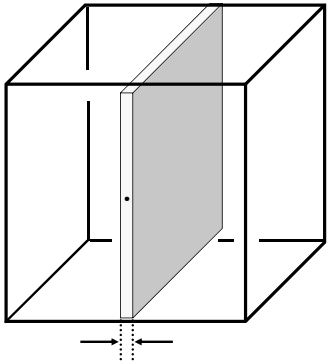
3D reconstruction by feeding in central sections in Fourier space



1/D, D particle size

(all representations in Fourier space)

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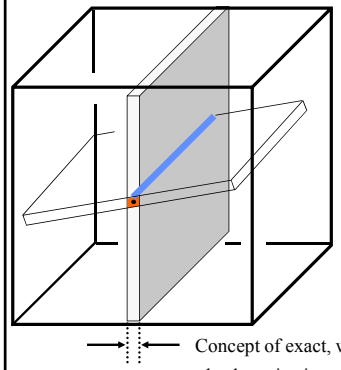
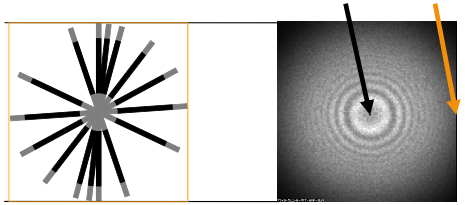
3D reconstruction by feeding in central sections in Fourier space



1/D

Reminder: power-spectrum:

low resolution **high resolution**
low frequency **high frequency**



(representation in 2D for simplification)

Concept of exact, weighted filtered back-projection (WBP) reconstruction:
- back-projection
- filtered (partial removal of low frequencies to reduce overlap of central sections)
- weighting (down-weighting preferential orientations)

(Harauz & van Heel, 1986; Radermacher *et al.*, 1986)

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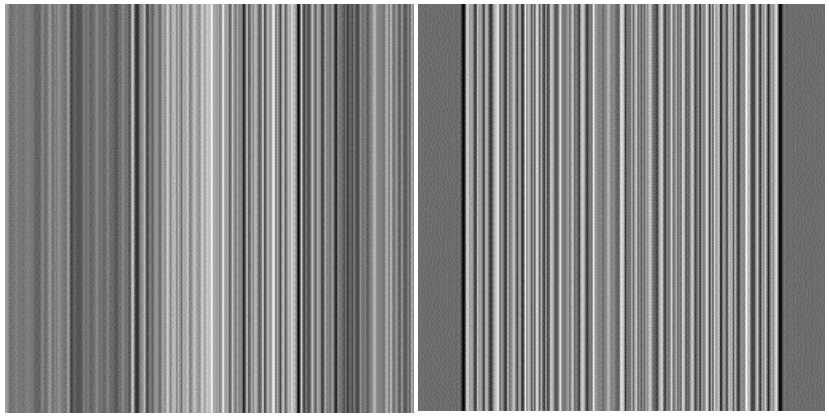

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I. Orlov

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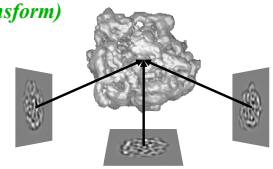
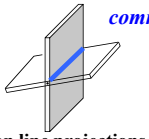
	Real space		Fourier space
map	3d density distribution	3D FFT	3D Fourier transform
	back-project / project		extract central section / insert
class average	2D projection	2D FFT	2D central section
	back-project / project to line		extract central line / insert
sinogram line (Radon transform)	1D projection	1D FFT	1D central line





common line

common line projections theorem
Theorem of the central section.

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	Real space		Fourier space
map	3d density distribution	3D FFT	3D Fourier transform

$$\rho(\mathbf{x}) = \frac{1}{V}\sum_{\mathbf{h}} \mathbf{F}(\mathbf{h}) \exp(-2\pi i \mathbf{h}\cdot\mathbf{x})$$

Diffraction pattern,

[h, k, l, phases]

Patterson (molecular replacement,

self correlation functions)

// auto-correlation function in imaging

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Single particle image processing and 3D reconstruction

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Single particle image processing and 3D reconstruction

I. Pre-processing

- **image acquisition (CCD/CMOS camera;** digitization of micrographs / negatives**)**
- **particle selection, « boxing »**
- **correction of the contrast transfer function**
- **band-pass filtering and normalisation of particle images**

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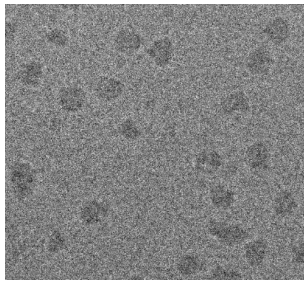
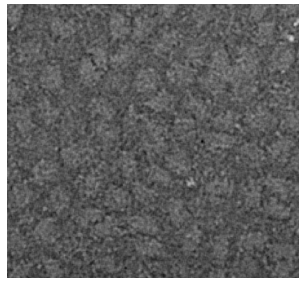
I. Pre-processing

- Digitization of micrographs (negatives); not needed if CCD/CMOS images



CCD image (positive contrast)



micrograph (negative contrast)

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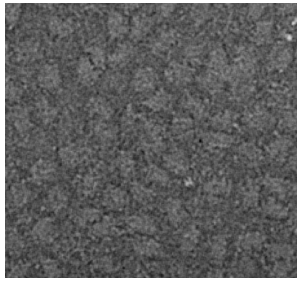
I. Pre-processing

- Digitization of micrographs (negatives)

high-resolution scanner (5000 dpi)



Sampling = Pixel size / Magnification

5 µm / 50 000 = 1 Å / pixel at specimen level

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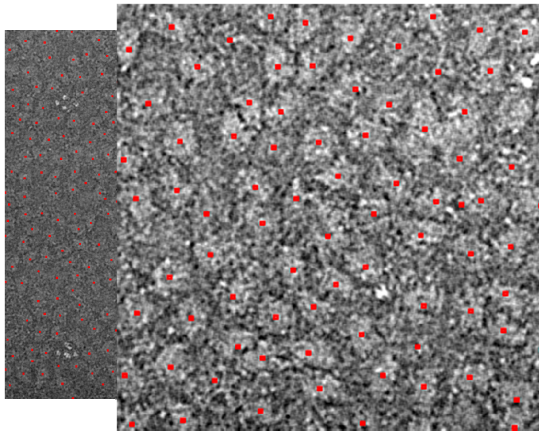
I. Pre-processing

- particle selection, « boxing »

What is important when selecting particles?

Proper centering!



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I. Pre-processing
- correction of the contrast transfer function

Background:

A cryo-TEM is a Phase Contrast Microscope

Frederik Zernike
Phase-contrast micr. (1930),
Nobel prize for physics 1956.

The electron microscope primarily is a phase contrast microscope.

Otto Scherzer formulated the contrast transfer theory for EM in 1949.

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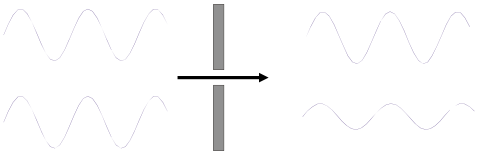
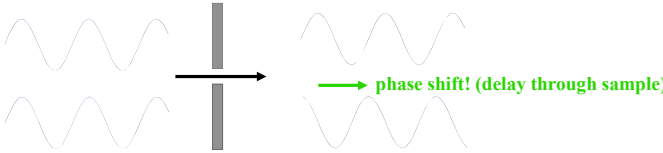
I. Pre-processing
- correction of the contrast transfer function

Amplitude contrast (inelastic scattering, absorption)



Phase contrast (elastic scattering)



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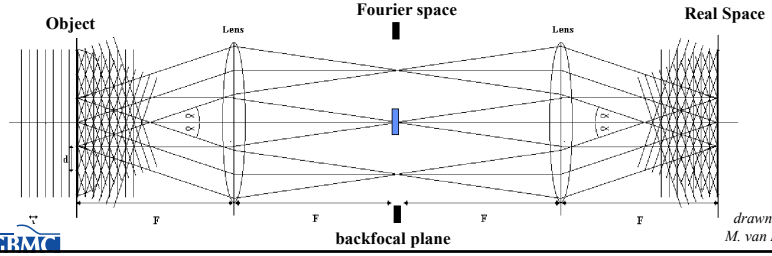
biological specimens are weak phase objects

I. Pre-processing
- correction of the contrast transfer function

Background:

Phase Contrast Microscope

- Small phase difference difficult to observe
- Limited to study of thin specimens (<1000Å)
- **Phase contrast is converted to amplitude contrast by defocusing the specimen**
- Same technique used in light microscopy to study unstained specimens
- Staining provides strong amplitude contrast but may affect the macromolecular structure



drawn by M. van Heel

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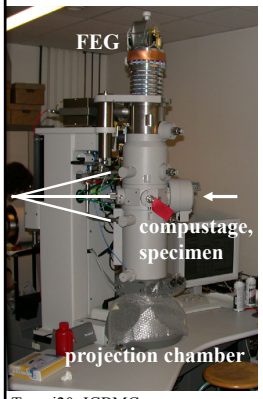
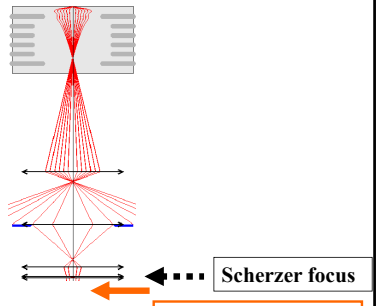


Tecnai20, IGBMC

electron source

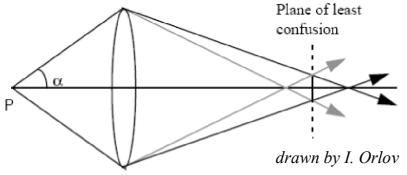
sample

image acquisition



Scherzer focus

defocus = under-focus (e.g. -2 µm)



drawn by I. Orlov

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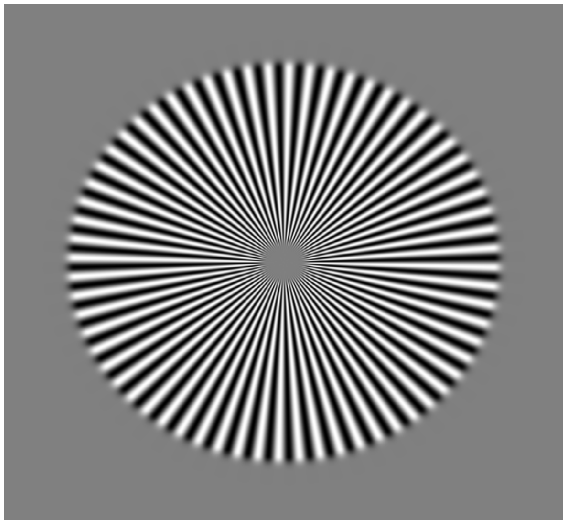
Siemens stars: a whole range of spacings / frequencies / resolutions in a single image



drawn by M. van Heel

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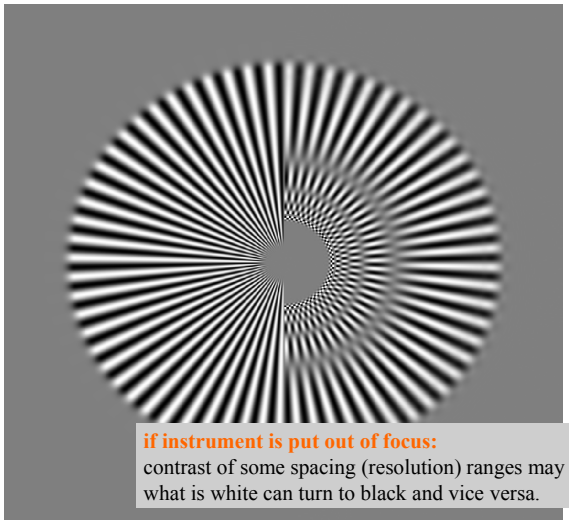
Calculated effect of an electron microscopical PhCTF on the image of a Siemens star



if instrument is put out of focus:
contrast of some spacing (resolution) ranges may be reversed, what is white can turn to black and vice versa.

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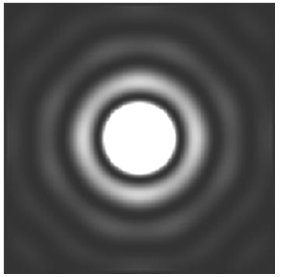
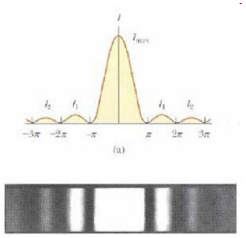
Contrast Transfer Function (CTF)

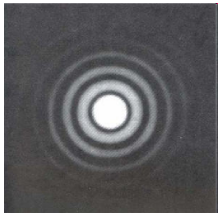
The phase contrast of heavy atoms theoretically is sufficient for imaging single atoms.

The image of a single atom will be a diffraction disc surrounded by diffraction fringes

→ contrast reversals.







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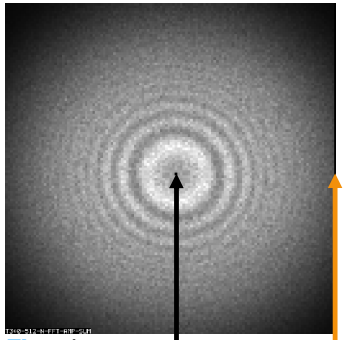
Analogy with X-ray diffraction (Fourier space!)

cryo-EM: powerspectrum



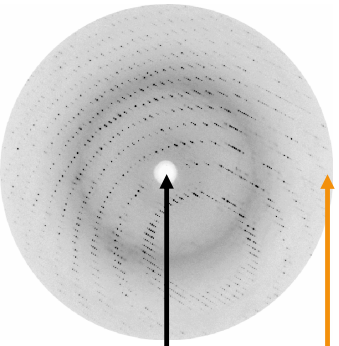
Thon rings (at zero crossing)

low resolution
low frequency

high resolution
high frequency

λ = ~ 0.01 Å

crystallography: diffraction pattern



low resolution

high resolution

λ = ~ 1 Å

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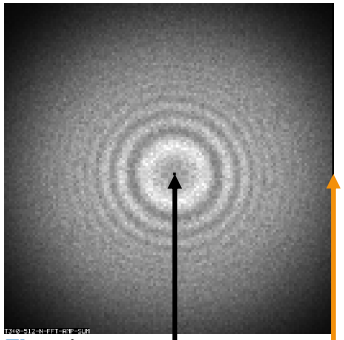
Powerspectrum = Fourier Transformation of the image

powerspectrum



Thon rings
(at zero crossing)

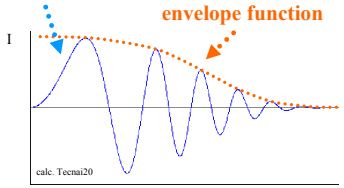
low resolution
low frequency

high resolution
high frequency

Profile of the intensity distribution

Contrast transfer function

envelope function



very low resolution difficult to measure

→ **averaging techniques**
to cover the complete resolution range

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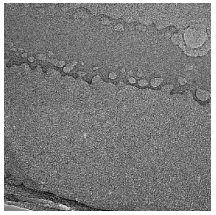
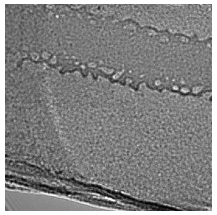
Defocusing: effect on contrast, power-spectrum and max. resolution

defocus -1 µm

defocus -10 µm

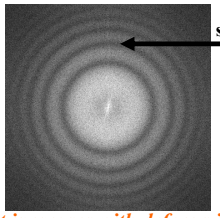
image



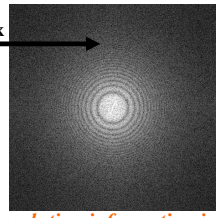


***contrast inversion* at the edge of the object**

power-spectrum



strong **weak**



Contrast increases with defocus increase, but high resolution information is reduced

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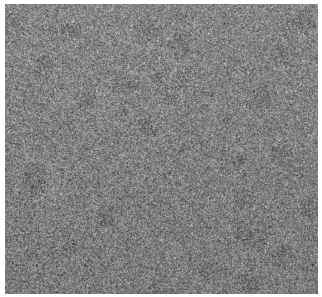
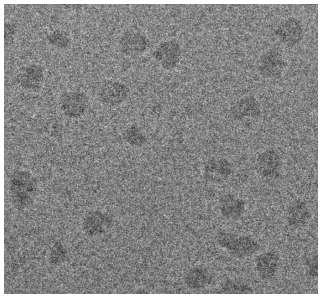
Images: continuous carbon area from holey carbon grid, cryo, Tecnai20, CCD 2Kx2K, 1024 pixel mode

Defocusing: effect on contrast, power-spectrum and max. resolution

Images of single particles:



defocus ~ -1.5 µm



defocus ~ -4 µm

***contrast inversion* at the edge of the object**

Contrast increases with defocus increase, but high resolution information is reduced

[comment: this contradiction is resolved with phase plates]

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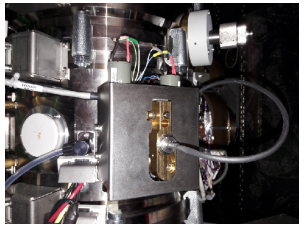
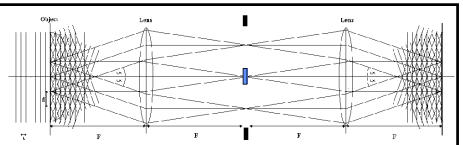
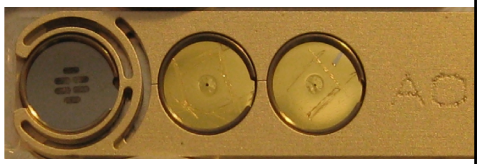


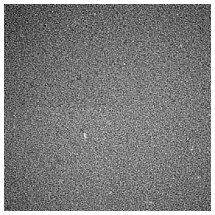




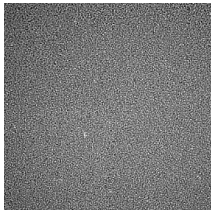
see pioneering work by
Danev & Nagayama, 2001 & 2006; Danev *et al.*, 2014; Khoshouei *et al.*, 2017; Danev *et al.*, 2017.

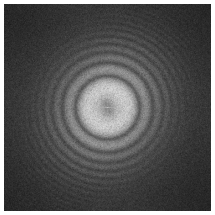
Typical imaging problems:

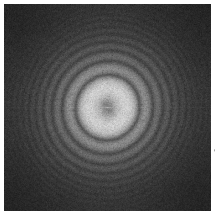
drift



astigmatism







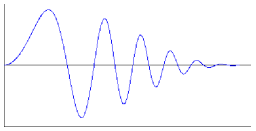
deformation

Images: continuous carbon area from holey carbon grid, cryo, Tecnai20, CCD 2Kx2K, 1024 pixel mode

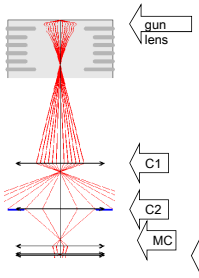
I. Pre-processing

- correction of the contrast transfer function

Weak-phase biological specimen → weak-contrast image



CTF function: $T(k) = - \sin (\pi/2\ C_s\ \lambda^3\ k^4 + \pi\ \Delta f\ \lambda\ k^2)$



CTF depends on parameters of imaging system:

- λ, wavelength defined by accelerating voltage (e.g. high tension 200 kV --> λ = 0.025 Å)
- ***Cs***, spherical aberration coefficient, determines the quality of objective lens
- other, constant parameters from electron microscope

} constant

- Δf, the **defocus value**, (offset from focus, "Scherzer" focus, underfocus: **focal point is below the sample**)
- k, spatial frequency

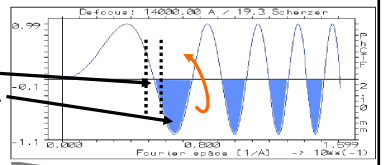
} variable parameter

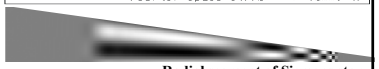
I. Pre-processing

- correction of the contrast transfer function (CTF)

No information transfer at the zero crossing;
CTF leads to contrast inversion within the image.

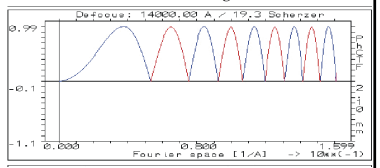
A





Radial segment of Siemens star

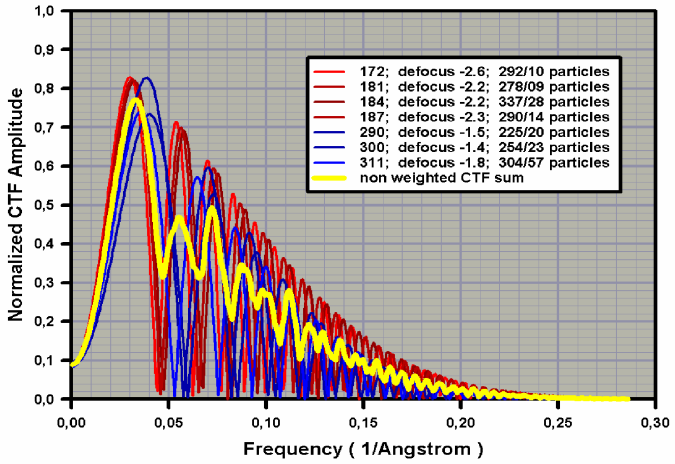
B



CTF-correction? By "phase flipping":

Therefore: data collection over range of defocus values,
e.g. under-focus -1.0 – 3.0 µm

Combination of powerspectra from different defocus images

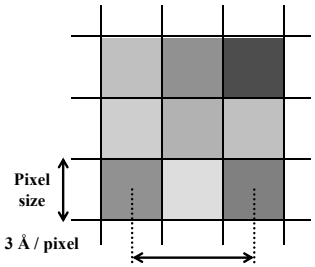


(spectra also take the envelope function into account)

I. Pre-processing

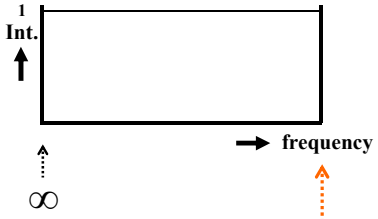
- band-pass filtering and normalisation of particle images

Grey values in pixels:



low resolution
low frequency

high resolution
high spacial frequency



Maximum resolution = 2 x pixel size = Nyquist-frequency

Nyquist-frequency
= 2 x sampling
e.g. 2 x 3 Å / pixel = 6 Å

***Sampling** = Pixel size / Magnification*

5 µm / 50 000 = 1 Å / pixel at specimen level

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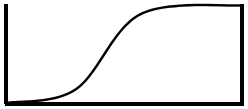
I. Pre-processing

- band-pass filtering and normalisation of particle images

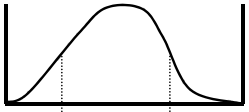
Combination of high-pass and low-pass filters:

low-pass

high-pass



band-pass



Removes:

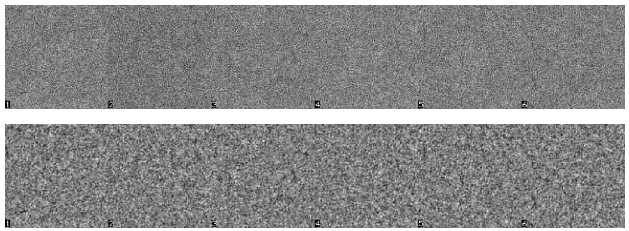
- low frequency contribution (scanner, etc.)
- high frequency noise

ICBMC

I. Pre-processing

- band-pass filtering and normalisation of particle images

Effect of bandpass filter:



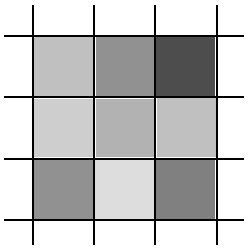
Removes:

- low frequency contribution (scanner, etc.)
- high frequency noise

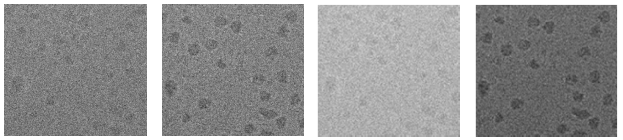
ICBMC

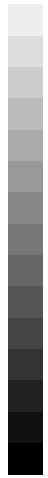
I. Pre-processing

- band-pass filtering and normalisation of particle images



zero floating of the images (average value becomes 0.0),
normalization of standard deviation (to 1)
[adjustment of histogram (stretching)],
mask the outside to zero:
i.e. scaling of dynamic range of images to obtain comparable grey values
→ important for multivariate statistical analysis (MSA) and classification





ICBMC

Single particle image processing and 3D reconstruction

I. Pre-processing

- **image acquisition (CCD/CMOS camera;** digitization of micrographs / negatives)
- **particle selection, « boxing »**
- **correction of the contrast transfer function**
- **band-pass filtering and normalisation of particle images**

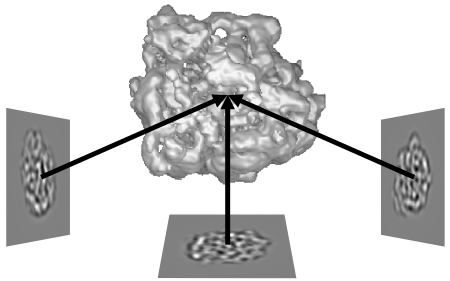
II. Structure determination

- **particle centering / alignments**
- **MSA (multivariate statistical analysis) + classification**
- **angle assignment**
  - **angular reconstitution**
  - **projection matching**
- **3D reconstruction (first map)**
- **structure refinement**
- **resolution assessment**
- **map interpretation; fitting of known structures, atomic model building**

details in next lectures

IGBMC

Single particle image processing and 3D reconstruction.



Concept of 3D reconstruction: back-projection (franc.: rétro-projection)
(requires to have **angles** assign beforehand)
Reconstruction provides a 3D density map, i.e. a **structure** with all internal features

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Some basic concepts of cryo-EM & 3D reconstruction

Correct terms are important (be precise and rigorous in science:-) :

By cryo-EM, we obtain:

- **a "3D reconstruction" (initial or refined)**
- **a "cryo-EM map" or "density"**
- **a "structure"**

technically:

- **back-projection**
- **angular reconstitution**
- **random conical tilt**
- **tilt series / tomogram**

NOT:

- **an "envelope" (would be SAXS or neg. stain. EM)**
- **a "volume", units would be Å^3 (e.g. volume of a pocket, volume x density = mol. mass)**
- **a "surface", units would be Å^2 (e.g. interaction surface between 2 proteins)**
- **a "model", would be a molecular model *fitted to* the map (crystallography/cryo-EM)**
  or a model *compatible with* SAXS data or NMR restraints;

other models: "homology model", "error model", "hypothetical model", "working model"

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Single particle image processing and 3D reconstruction

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- **map interpretation; fitting of known structures, atomic model building**

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Overview of the concept:
align – classify – reconstruct in 3D

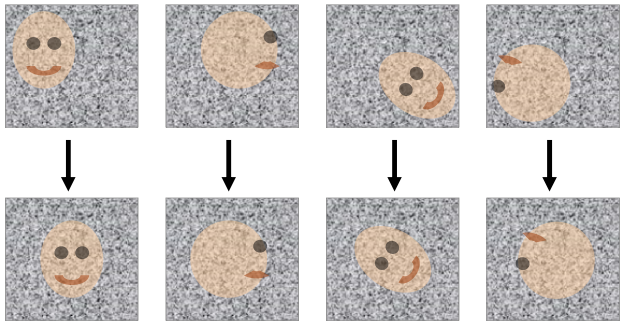
II. Structure determination

- particle centering / alignments

"reference-free" alignment (if structure unknown)
(or multiple reference alignment, if similar structure already known)

Translational Alignment

- Requires reference image(s) to align to



drawn by
A. Patwardhan

IGBMC

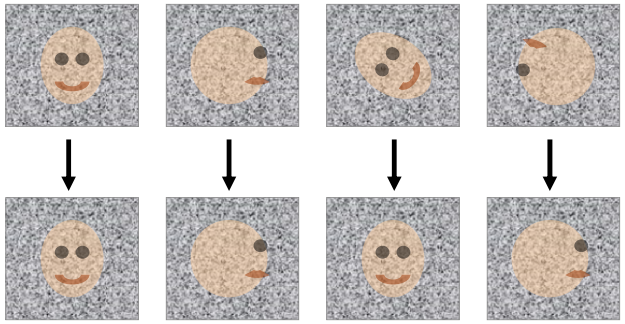
Overview of the concept:
align – classify – reconstruct in 3D

II. Structure determination

- particle centering / alignments

Rotational Alignment

- Requires reference image(s) to align to



drawn by
A. Patwardhan

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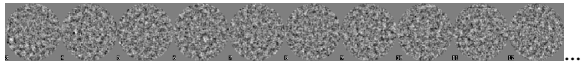
II. Structure determination

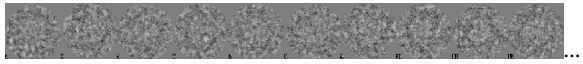
- particle centering / alignments

"reference-free" alignment (if structure unknown)
(or multiple reference alignment, if similar structure already known)



aligned to total sum of particles

provides centered particle images:



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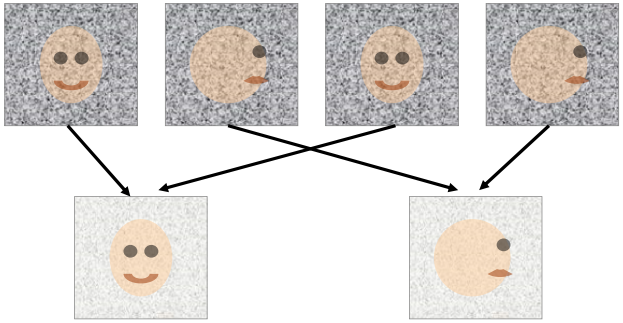
II. Structure determination *Overview of the concept: align – classify – reconstruct in 3D*

- MSA (multivariate statistical analysis) + classification

- Combine like views to improve signal to noise: **how?** **Requires statistical analysis of the pixel intensities**

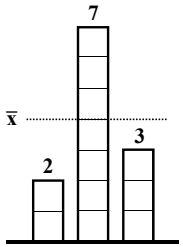


drawn by A. Patwardhan

ICBMC

Some "basic statistics"

example for 3 values (measurements)



n = 3

$\Sigma x_i = 2+7+3 = 12$

$\bar{x} = 1/n \Sigma x_i = 1/3 (2+7+3) = 4$

$\sigma^2 = 1/n \Sigma (x_i - \bar{x})^2 = 1/3 [(2-4)^2 + (7-4)^2 + (3-4)^2] = 14/3$ variance

$\sigma = \sqrt{1/n \Sigma (x_i - \bar{x})^2} = \sqrt{14/3} = 2.16$ standard deviation

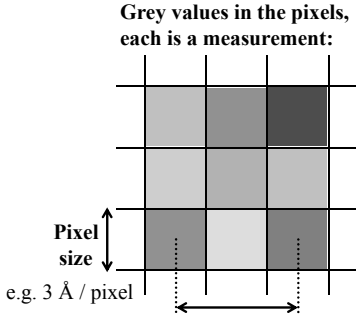
"how variable are the data"

"normalization"

ICBMC

Images are composed of pixels:

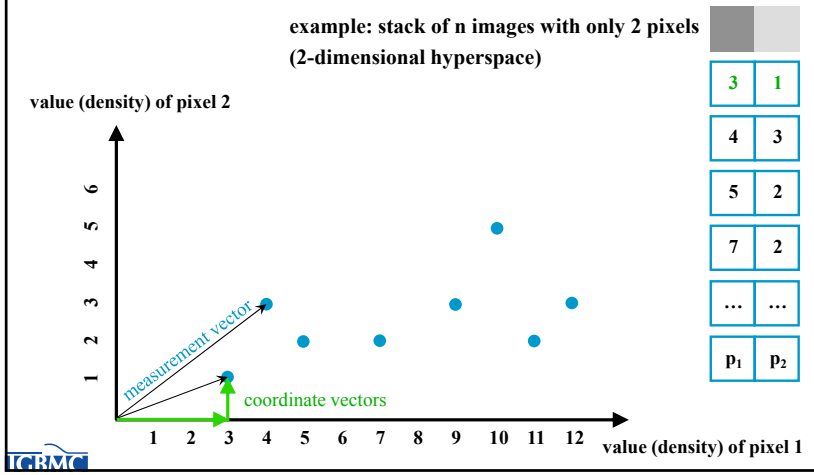
Grey values in the pixels, each is a measurement:



Maximum resolution = 2 x pixel size (**Nyquist frequency!**)

ICBMC

example: stack of n images with only 2 pixels (2-dimensional hyperspace)



3	1
4	3
5	2
7	2
…	…
p_1	p_2

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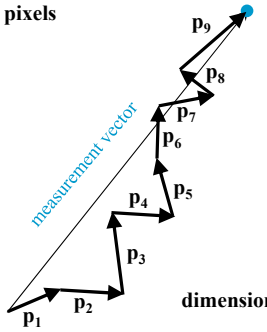
extension to more dimensions,
example: image with 3 x 3 pixels

p_1	p_2	p_3
p_4	p_5	p_6
p_7	p_8	p_9



dimensionality in image space: p^2

length of each coordinate vector ≙ intensity of pixel p_i

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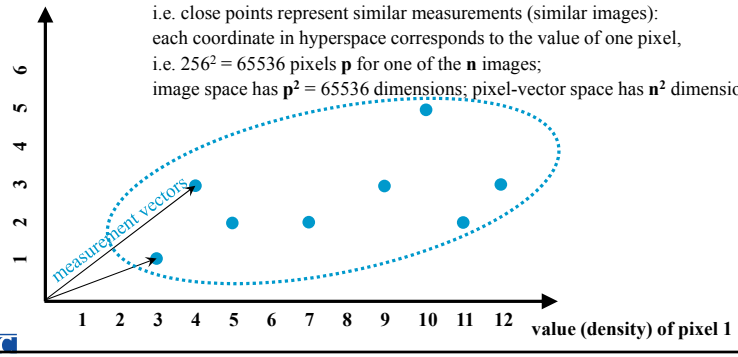
Large number of comparable measurements: stack of n images with the same size (e.g. 256x256)

Describe image pixels as a vector of numbers (individual pixel values / densities).
This vector is positioned in hyperspace:

→ each measurement corresponds to a point in hyperspace,
all measurements thus create a ***data cloud***.

Distance between points in hyperspace defines similarity,
i.e. close points represent similar measurements (similar images):
each coordinate in hyperspace corresponds to the value of one pixel,
i.e. $256^2 = 65536$ pixels **p** for one of the **n** images;
image space has $\mathbf{p^2} = 65536$ dimensions; pixel-vector space has $\mathbf{n^2}$ dimensions.



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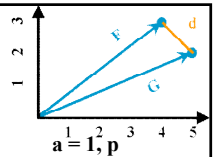
Similarity between two measurement vectors F and G:
inner product of the vectors (= correlation = covariance):



$$C_{FG} = 1/p \, \Sigma \, F_a \cdot G_a \qquad \text{covariance} \qquad a = 1, p$$

$$C_{FF} = 1/p \, \Sigma \, F_a \cdot F_a = 1/p \, \Sigma \, F_a^2 \qquad \text{variance} \qquad a = 1, p$$

Euclidian square distance: *e.g. unit cube:*

$d = \sqrt{1^2+1^2+1^2} = \sqrt{3}$

$$D^2_{FG} = \Sigma \, (F_a - G_a)^2$$
$$= \Sigma \, F_a^2 + \Sigma \, G_a^2 - 2 \, \Sigma \, F_a \cdot G_a \qquad a = 1, p$$

(variances in F and G) minus (2 · correlation between F and G):
short distance means high correlation

PCA metric (principal component analysis)

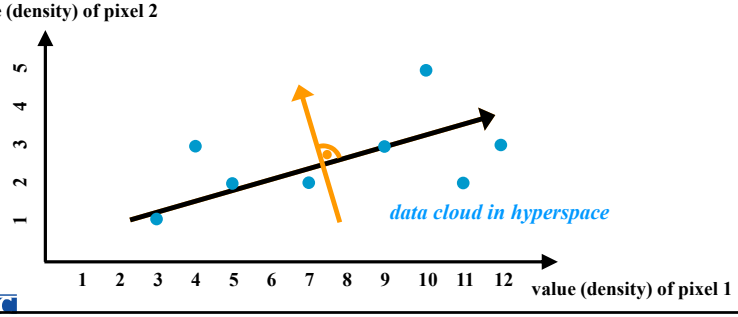
[for comparison: discrete correlation function: $C_{FG} = \Sigma \, F_a \cdot G_{(a-x)}$, with shift x;
cross-correlation coefficient CCC = $\Sigma \, F_a \cdot G_a \, / \, \mathrm{sqr} \, (\Sigma \, F_a^2 \cdot \Sigma G_a^2)$]

ICBMC

Aim of MSA: adapt the coordinate system of the hyperspace to the shape of the data cloud.

First axis of the rotated coordinate system will correspond to the largest elongation of the data cloud,
i.e. highest variance; the next (orthogonal) axis corresponds to the next-strongest variance.

Data reduction: use first few components that describe the strongest variations / main components;
reduction of dimensionality: e.g. 20 or 50 instead of p^2 (here 65536): gain factor **>1000**;
reduction of noise: by omitting higher components which describe only variance in noise;
main directions of variations: given by eigenvectors



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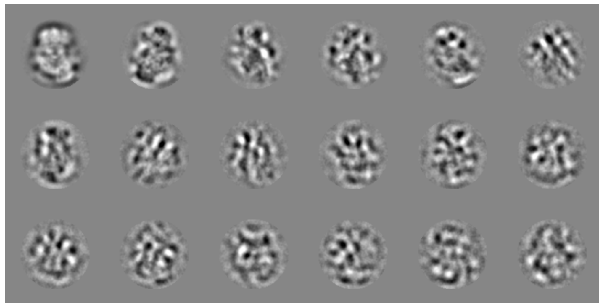
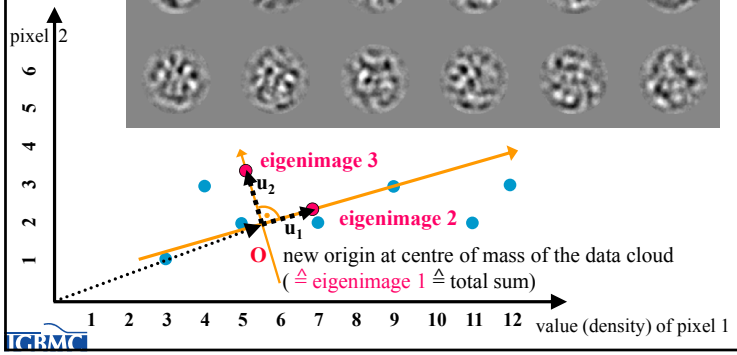
example: eigenimages





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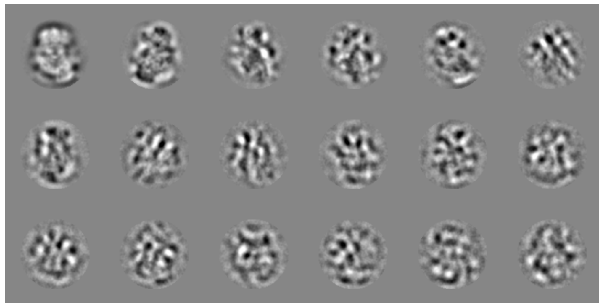
example: eigenimages



example: first 18 eigenimages of a data set

The entire data set can be reconstituted from a linear combination of the eigenimages, or simply approximated by a small subset of eigenimages (data reduction!)

"$a \cdot u_1 + b \cdot u_2 + \ldots$"

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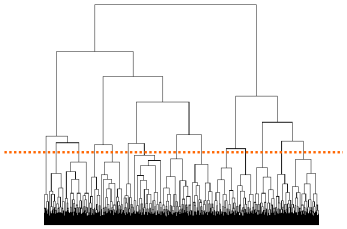
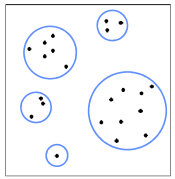
II. Structure determination
- MSA (multivariate statistical analysis) + classification

multivariate statistical analysis (MSA), related to principal component analysis:
data set can be represented as a linear combination of images, each describing
the highest differences within the data
→ data compression
→ images with statistically similar pixel intensity distribution can be grouped =
classified into groups of images describing similar views of the 3D object





hierarchical ascendant classification

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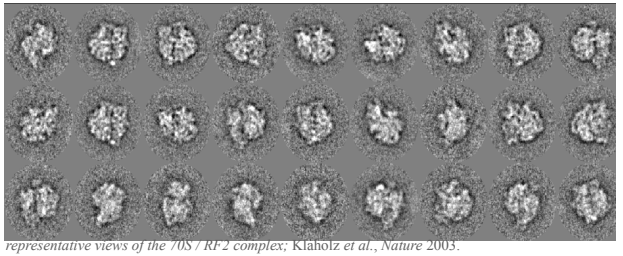
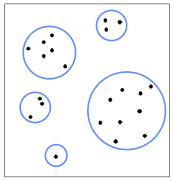
II. Structure determination
- MSA (multivariate statistical analysis) + classification

signal enhancement after
- classification by MSA
- hierarchical ascendant classification
- averaging of particles representing same views into class averages:



representative views of the 70S / RF2 complex; Klaholz *et al., Nature* 2003.



Average the images of each class

Typical class averages of ribosome particle images

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Correct terms are important:

A classification is based on a statistical analysis:

- multivariate statistical analysis (MSA) provides information on variance (variability) which serves to merge similar images into class averages (classes); is *independent* of a reference

- classes *are NOT*: the sum of images that correlate best with a reference (through a multi-reference alignment)

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Some references

- L. Borland & M. van Heel; Classification of image data in conjugate representation spaces, *J. Optic. Soc. Am*. A, 7 (1990) 601-610.
- M. van Heel *et al.;* Single-particle cryo electron microscopy: towards atomic resolution; Quart. Rev. Biophys. 33 (2000) 307-369.
- M. van Heel, Multivariate Statistical Classification of Noisy Images (Randomly Oriented Biological Macromolecules) *Ultramicroscopy* 13 (1984) 165-183.
- M. van Heel, Classification of very large electron microscopical image data sets, *Optik* 82 (1989) 114-126.
- E.R. Malinowski, *Factor Analysis in Chemistry*, 3rd ed. (2002)
- Benzécri J.-P. , L'Analyse des Données Vol 2, L'analyse des correspondances (1973-1980) Dunod Paris.
- Frank J: Three-Dimensional Electron Microscopy of Macromolecular Assemblies, Oxford University Press (2006).
- van Heel M, Frank J: Use of multivariate statistics in analyzing the images of biological macromolecules, *Ultramicroscopy* 6 (1981) 187-194.
- van Heel M: Multivariate Statistical Classification of Noisy Images (Randomly Oriented Biological Macromolecules), *Ultramicroscopy* 13 (1984a,)165-183.
- Ward JH: Hierarchical grouping to optimize an objective function. *J. Amer. Statist. Assoc*. 58 (1982) 236-244.

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What can we apply MSA to?

- 2D classification (reference-free alignment: only centered data, not rotationally aligned)

- alignment by classification (alignment against class averages or a typical eigenimage)

- analysis of symmetry (through symmetry in the eigenimages)

- local MSA (focus on an area with high structural variability)

- re-classification of class averages belonging to an object view

- size-classification (e.g. White et al., *J. Mol. Biol.* **336** (2004) 453-460).

- 3D classification of structures (separation of mixed particle populations): particles:3D-SC, sub-tomograms

- classification of powerspectra (sorting of defocus classes)

Important to do before MSA:

- normalisation

- filtering

- centered data (aligned if for structure refinement)

- define MSA area: MSA mask

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Determining structures of multiple conformational states in a single sample

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II. Structure determination

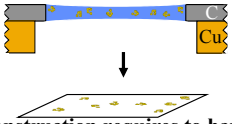
- angle assignment

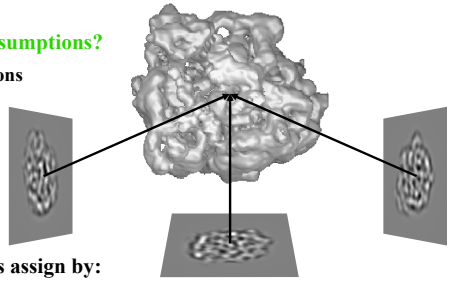
- angular reconstitution (in early stage of structure determination)

- projection matching (if structure already well refined): find best correlation between input image and reference images from 3D re-projections)

3D reconstruction of single particles: assumptions?

unique particle type in random orientations





Reconstruction requires to have angles assign by:

- angular reconstitution (in early stage of structure determination), or

- projection matching (if structure already refined; reference-dependent; bias), or

- maximum likelihood parameter assignment/refinement

ICRMC

Real space *Fourier space*

map 3d density distri ourier transform

back-project pr extract central section

insert

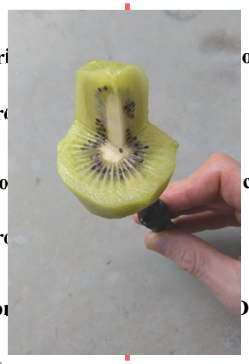
class average 2D projectio central section

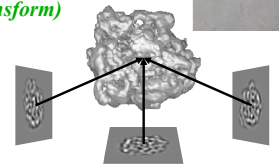
back-project pr extract central line

insert

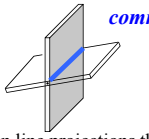
sinogram line 1D projectio central line

(Radon transform)





common line



common line projections theorem

Theorem of the central section.

ICRMC

II. Structure determination

- angle assignment

- angular reconstitution

sinogram = line-projection of the 2D image

(also called Radon transform)

amplitude-square-root filtered

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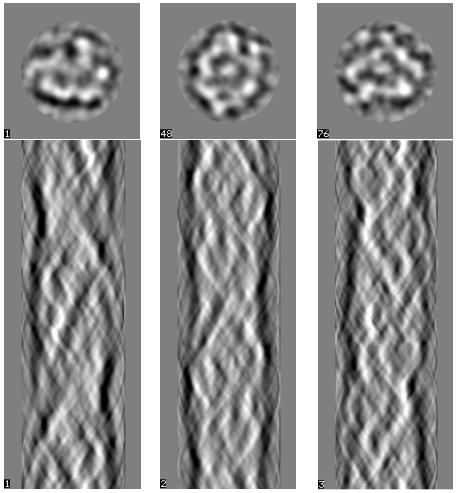
Select 3 clearly different views (here: class average numbers 1,48,76):



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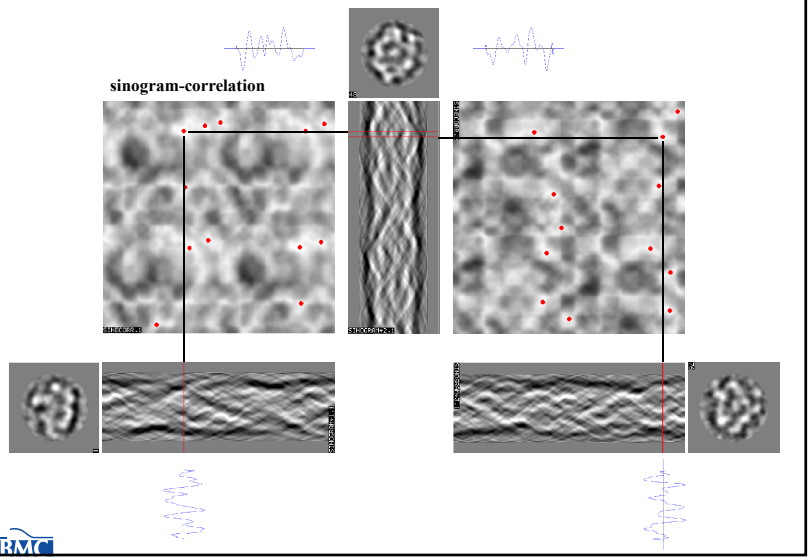
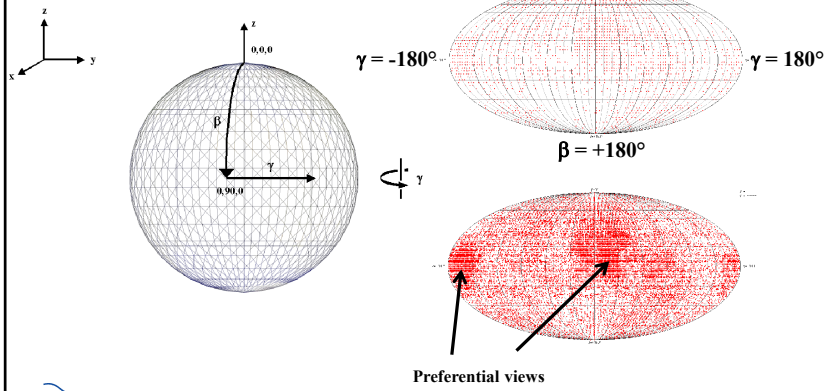


II. Structure determination
- angle assignment
- angular reconstitution

Particle angles plotted on sphere:



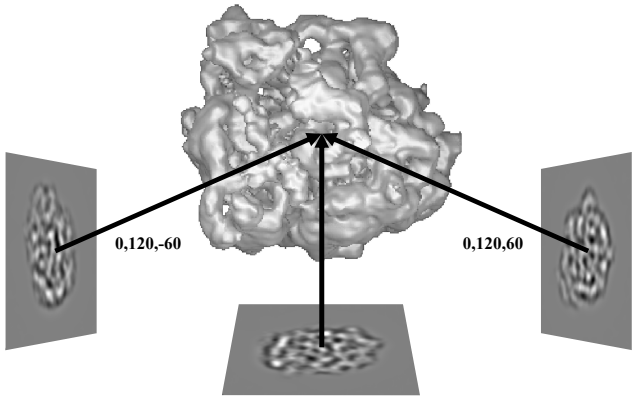
II. Structure determination
- angle assignment
- angular reconstitution

In case of *ab initio* structure determination by reference-free alignment and angular reconstitution:

Does not allow to determine handedness, requires either:

- random conical tilt (Radermacher *et al.*, J. Microsc. 1987)
- tomography
- phase residual error using a tilt pair (Rosenthal & Henderson, JMB 2003)
- fitting of crystal structures

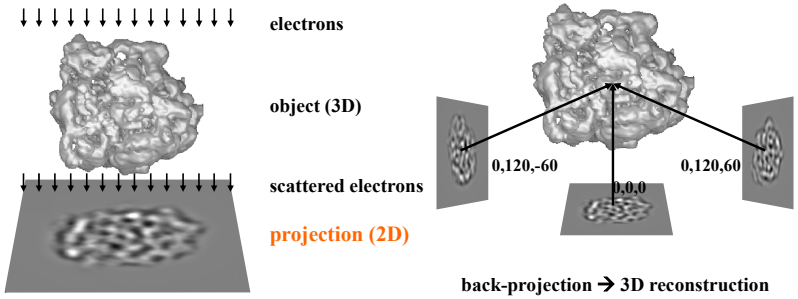
II. Structure determination
- 3D reconstruction



back-projection → 3D reconstruction

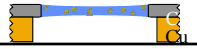
II. Structure determination
- 3D reconstruction

Transmission electron microscopy

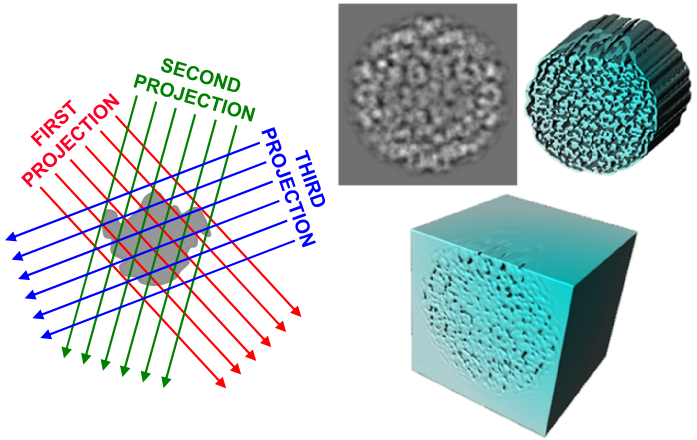


back-projection → 3D reconstruction

All *internal* features in a 2D view!

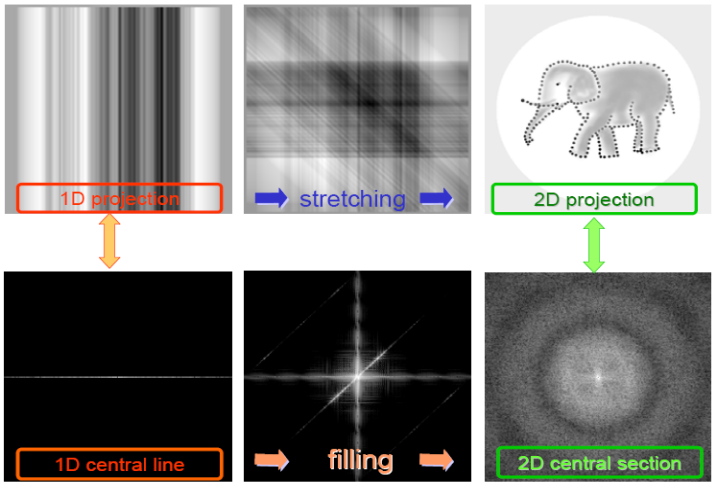


II. Structure determination
- 3D reconstruction



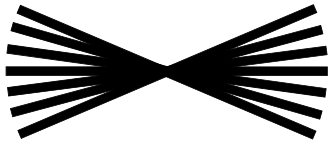
drawn by I. Orlov

3D Reconstruction in REAL and FOURIER Spaces



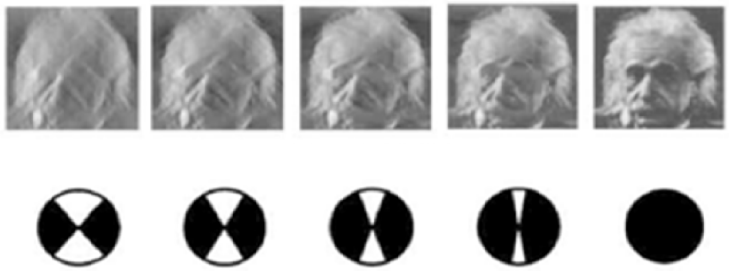
From E. Orlova EMBO course for Image Processing for Cryo Electron Microscopy, 2013

Back projection of individual images fills the reciprocal space by adding central sections:



central sections in Fourier space

Filling Fourier space



(example from tomography)

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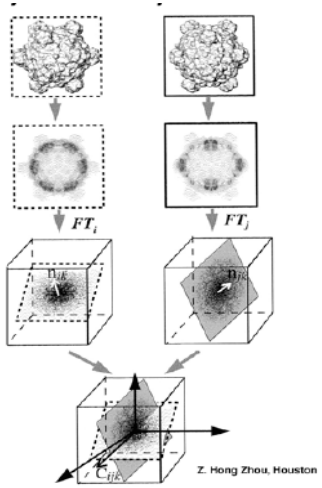
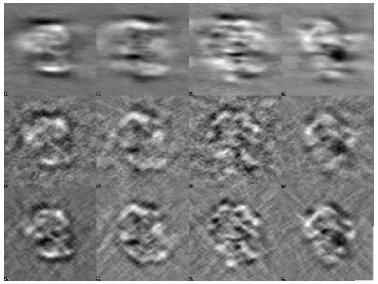
3D Reconstruction in REAL and FOURIER Spaces



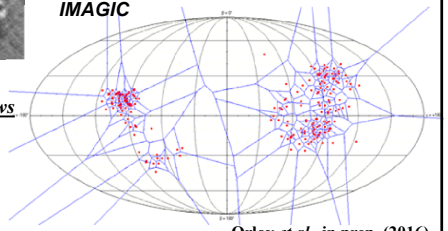
Importance of proper weighting in the case of preferential views



BKPR non-weighted ← **strong distortions/artifacts in 3D reconstruction**

BKPR weighted

IMAGIC



sections of 3D's calculated from 250 class averages with strong preferential views

Orlov *et al.*, in prep. (2016).

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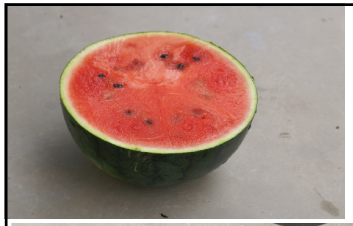
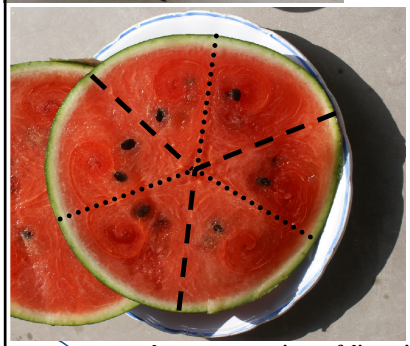
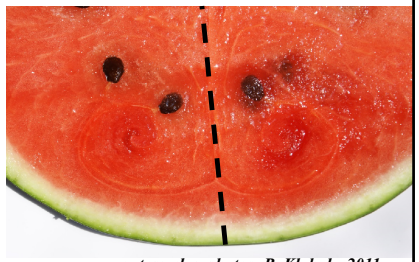
symmetries in nature…







pseudo-symmetry: trimer of dimers!

watermelon photos: B. Klaholz, 2011

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Point group symmetries of biological objects (e.g. used in single particle reconstructions)

C_n

80S ribosome, Khatter *et al.*, C_1

SPP1 bacteriophage Orlova *et al.*, *EMBO J* 2003. C_{13}

D_n

D_8

8-fold axis

2-fold axis

Chaperonine complex Zhang *et al.*, *Structure* 2011

T

O

I

http://csi.chemie.tu-darmstadt.de

Practical Example – Icosahedral Reconstruction

small asymmetric unit

other example:

Careful: imposition of inexplicit symmetry can happen…

Icosahedral Triangulation Number

The T-number is calculated by
(1) applying a grid to the surface of the virus with coordinates *h* and *k*,
(2) counting the number of steps between successive pentagons on the virus surface,
(3) applying the formula: $T = h^2 + h \cdot k + k^2$

gives the number of structural units per face, = number of subunits in the asymmetric unit

Pentamer Hexamer

T = 1

T =	3	7	13	16	21	25
(h,k) =	(1,1)	(2,1)	(3,1)	(4,0)	(4,1)	(5,0)

Hexamer Pentamer

: Subunit

always 12 pentamers,
but variable hexamer insertion

Multi-symmetric polyhedra: Goldberg, Tôhoku Math., 1937.

Concept of quasi-equivalence: Caspar & Klug, 1962.

k

h

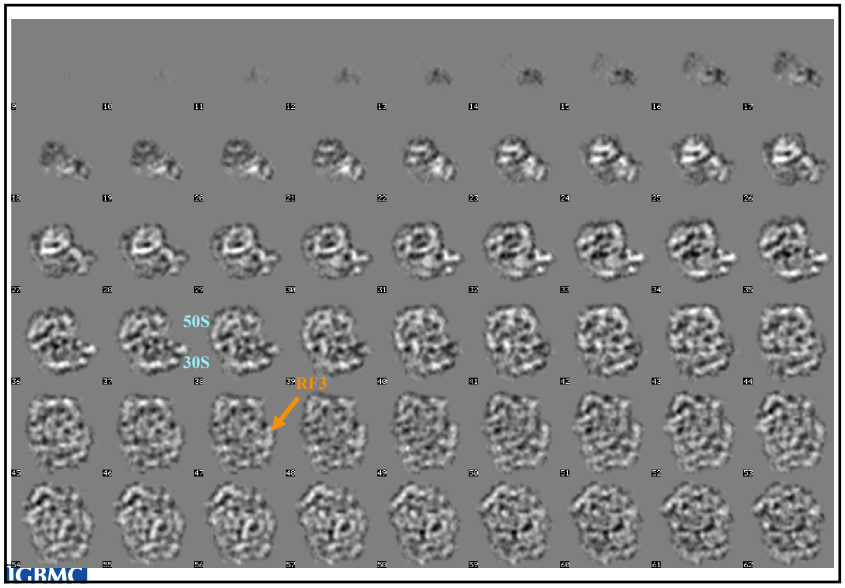
http://viralzone.expasy.org/all_by_protein/1057.html

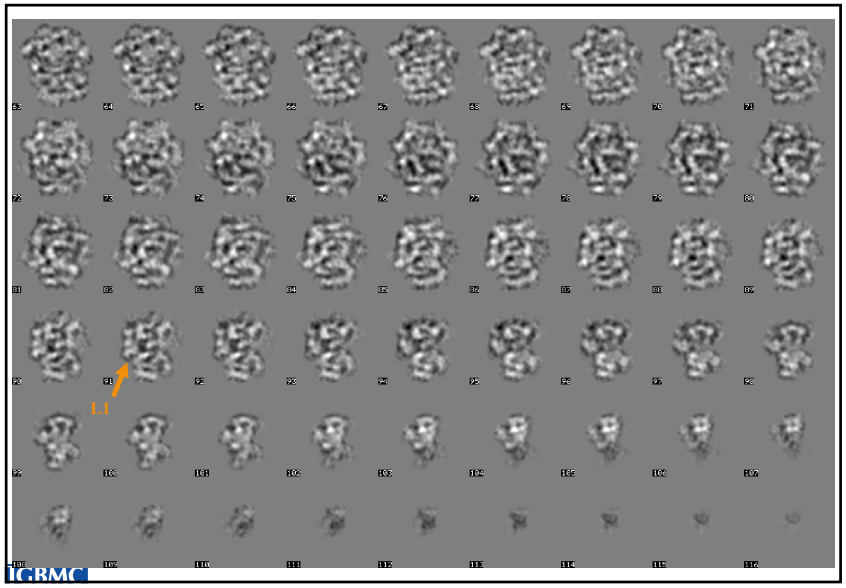
The two integers h and k describe the number of hexamers (h+k-1) one has to "walk over" to get from one pentamer to an adjacent pentamer.

see also Johnson & Speir, *JMB* 1997. Mannige & Brooks, PLoS One, 2010

II. Structure determination
- 3D reconstruction

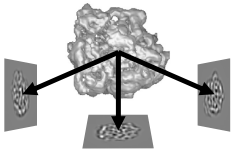
Representing 3D structures as consecutive sections through the 3D structure:

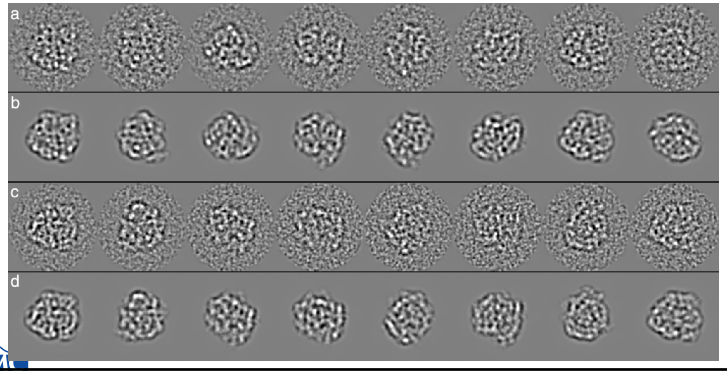




II. Structure determination
- 3D reconstruction

cross-validation of angle assignment and image quality by comparison with re-projections according to the same angles





II. Structure determination
- structure refinement

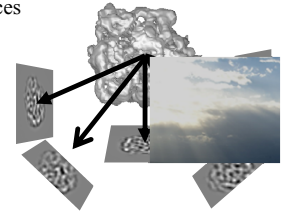
iteration

- centering/alignment
- variance analysis + classification
- angle assignment
- angular reconstitution → 3d-reconstruction
- reprojections = new references

"phase" the particles by aligning/classifying them

- improve quality of angle assignment

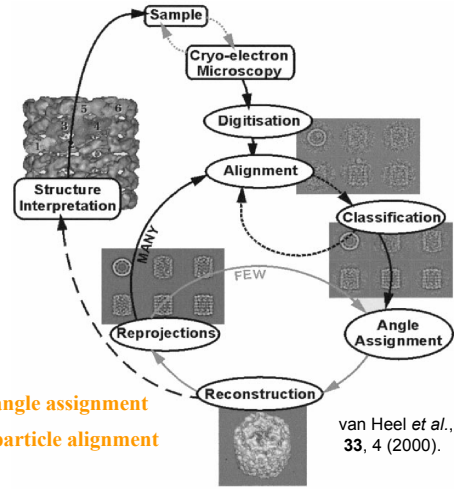
- improve quality of particle alignment



equally distributed forward-projections
(re-projections)

II. Structure determination

- overview



- improve quality of angle assignment

- improve quality of particle alignment

van Heel *et al.*, Quart. Rev. Biophys. **33**, 4 (2000).

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