

Lecture 8

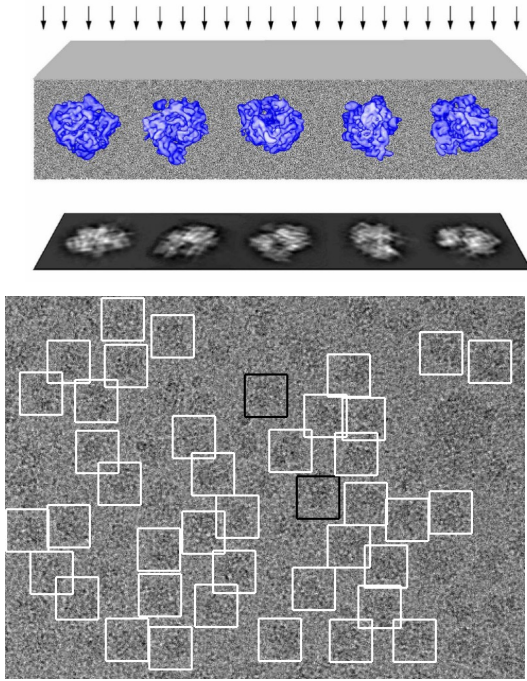
Methods for determination of 3D volumes from 2D experimental data

2nd December 2020
Jiri Novacek

Content

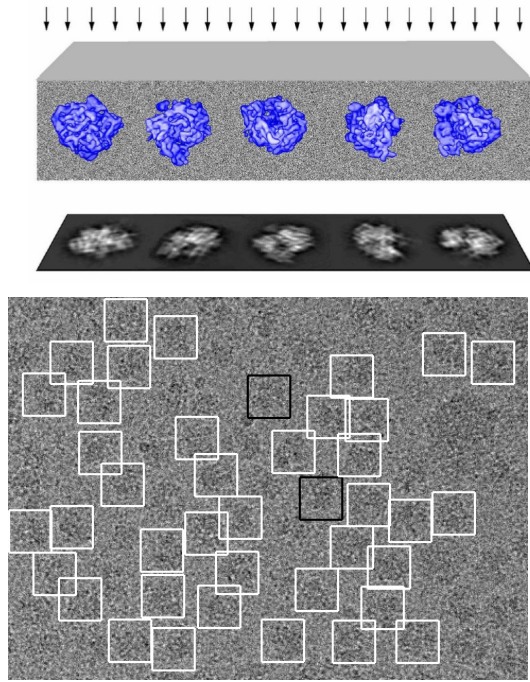
- principles
- electron tomography
- single particle analysis
- common lines
- random conical tilt

Revision



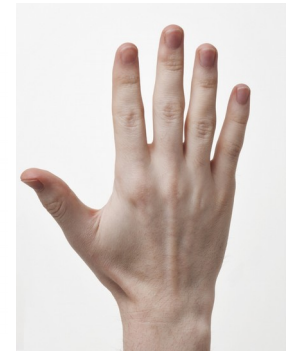
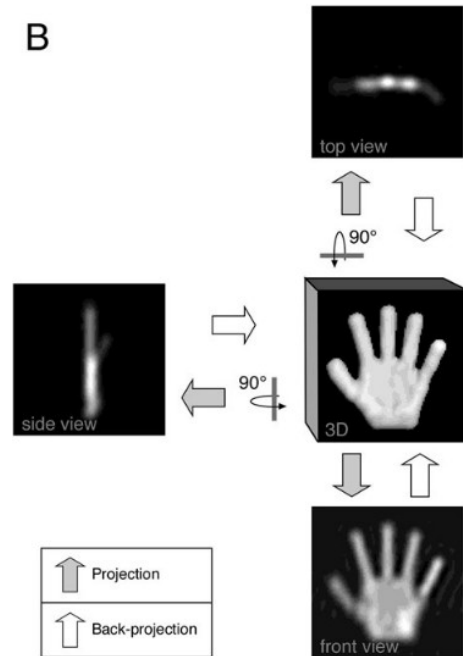
- 2D projections of an 3D object (handedness)
- high noise level (low sensitivity)
- convolution with microscope point spread functions

Revision

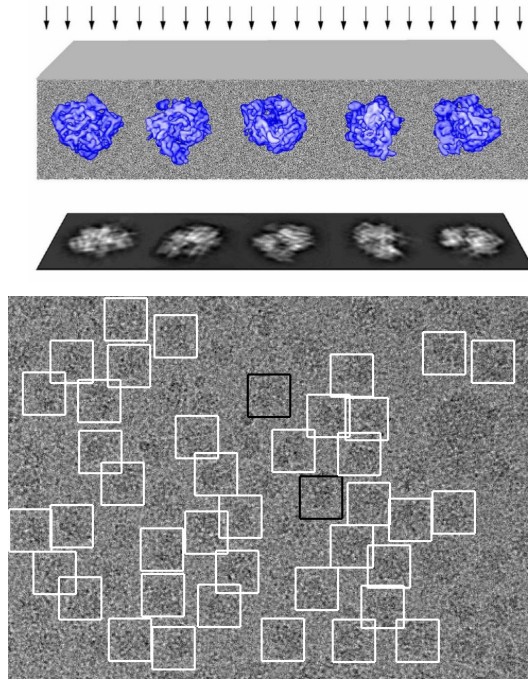


- 2D projections of an 3D object (handedness)
- high noise level (low sensitivity)
- convolution with microscope point spread functions

B

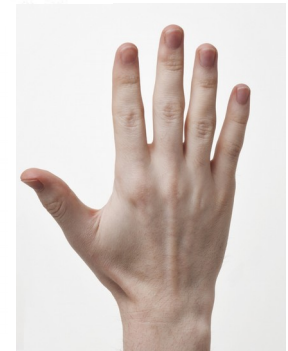
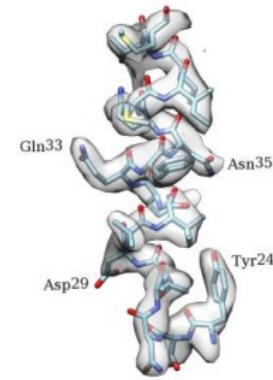
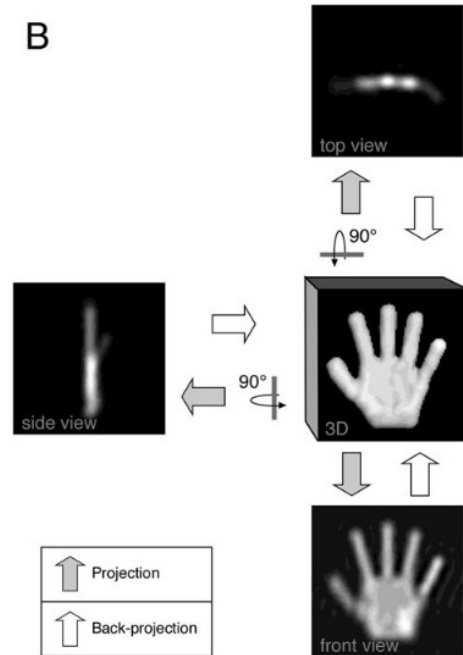


Revision

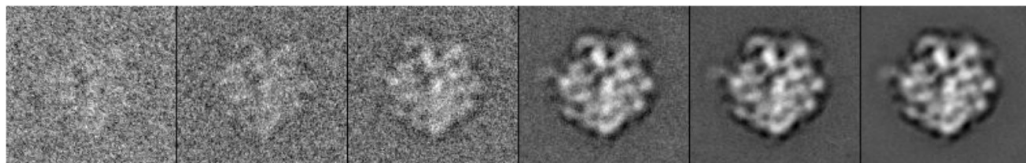
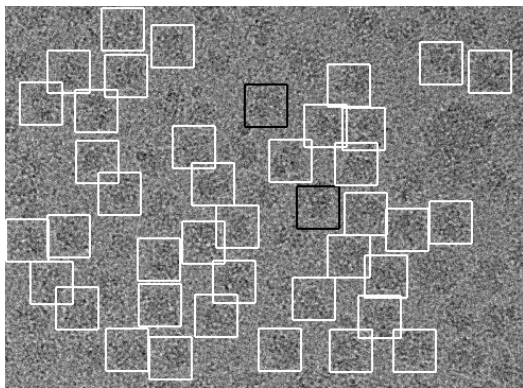
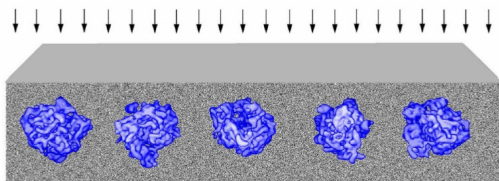


- 2D projections of an 3D object (handedness)
- high noise level (low sensitivity)
- convolution with microscope point spread functions

B



Revision



n=1

n=2

n=8

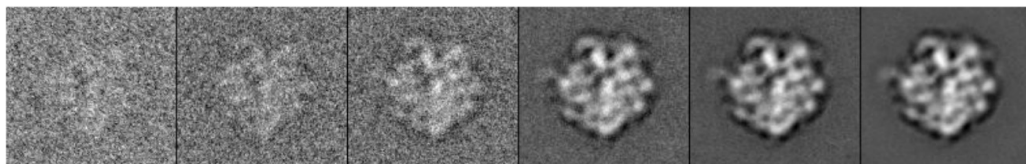
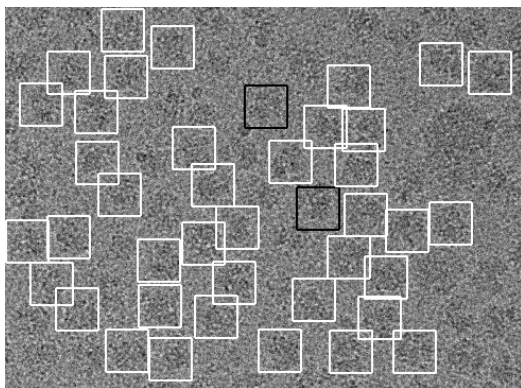
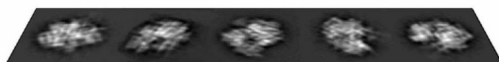
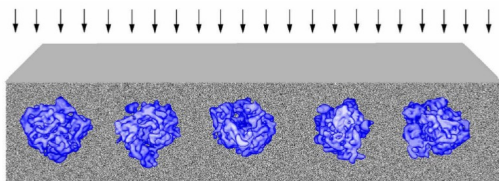
n=16

n=64

n=256

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

Revision



n=1

n=2

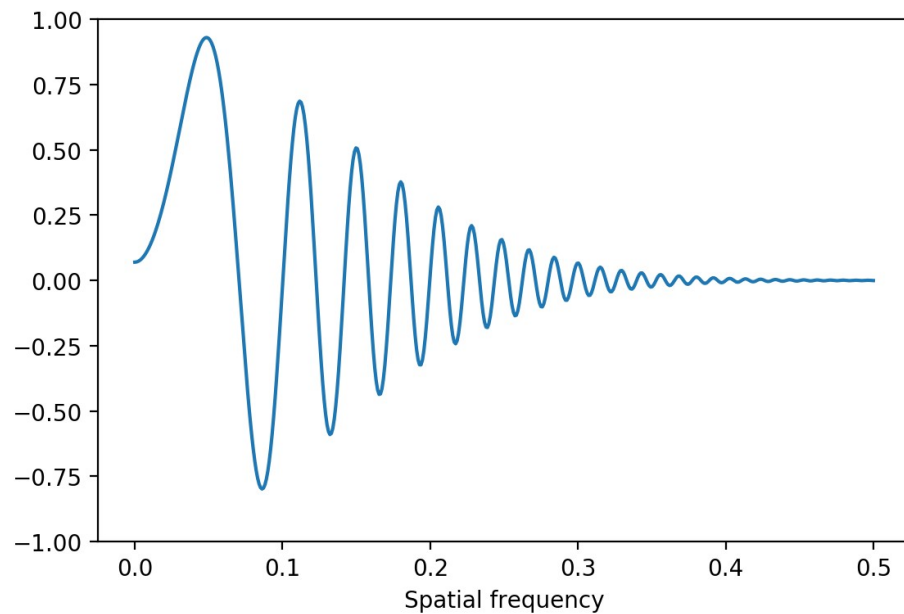
n=8

n=16

n=64

n=256

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



3D reconstruction

1. Different orientations
2. Known orientations
3. Many particles
4. CTF parameters



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

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

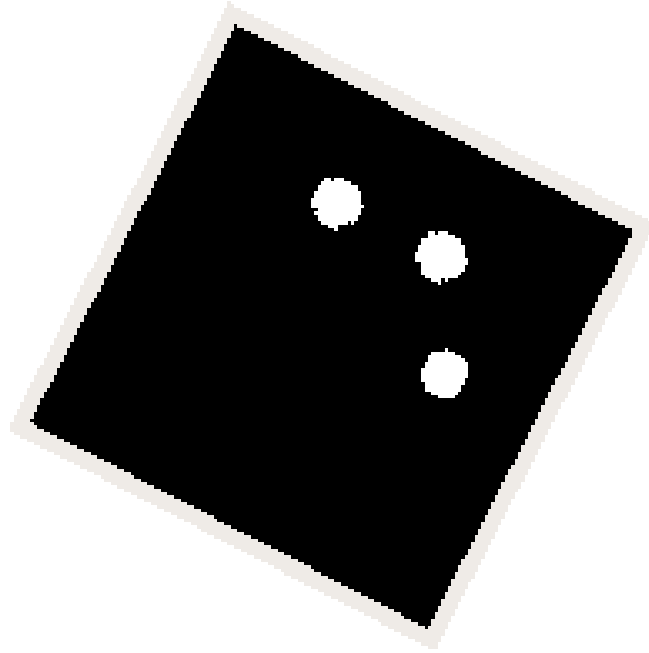
3D reconstruction

Two general ways for 3D reconstruction:

- Real space
- Fourier space

3D reconstruction

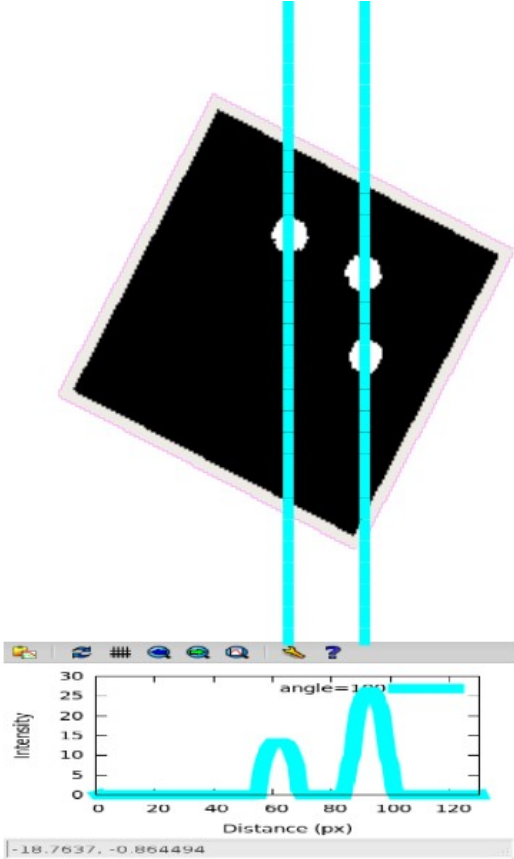
Real space reconstruction



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

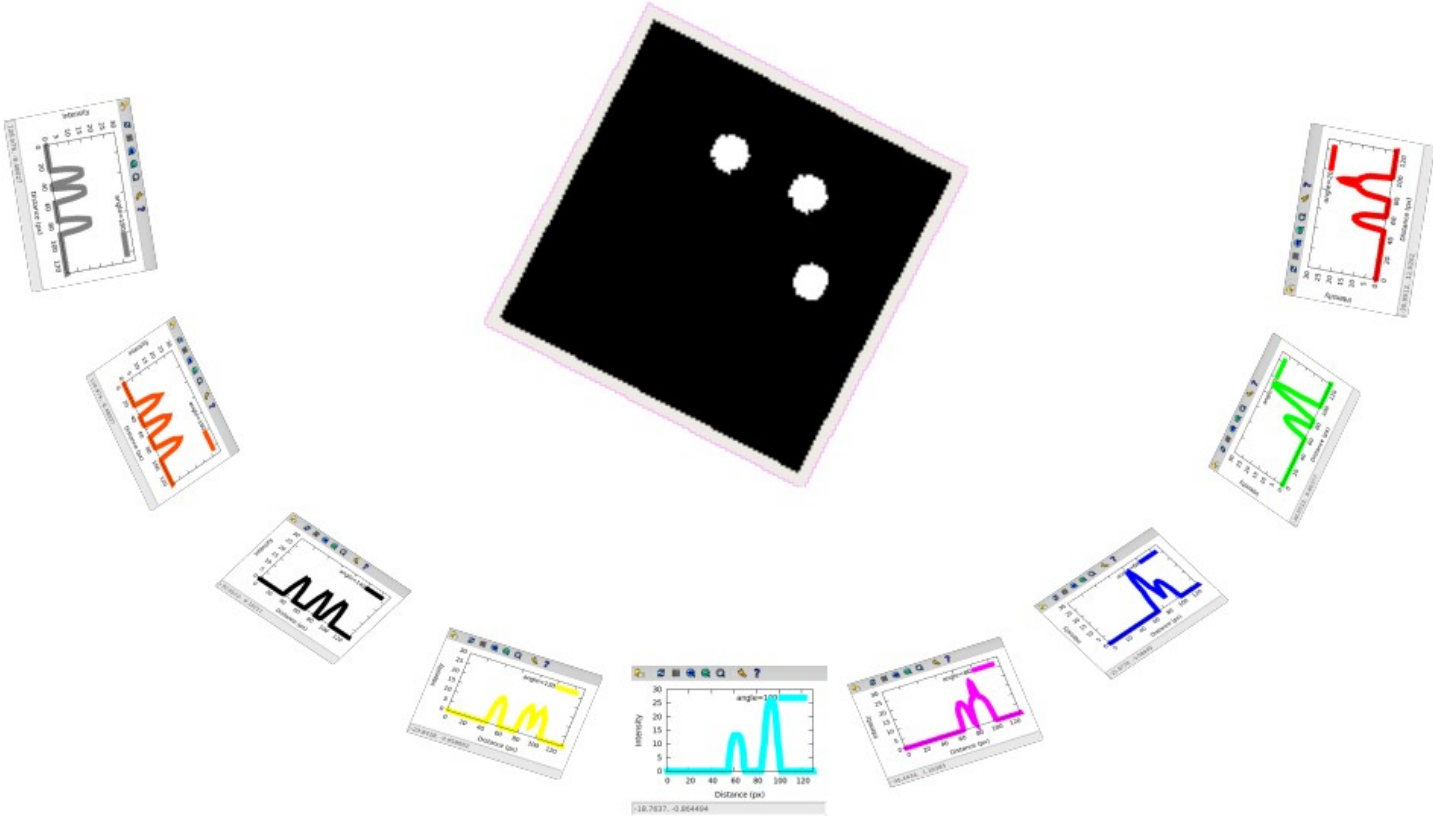
3D reconstruction

Real space reconstruction



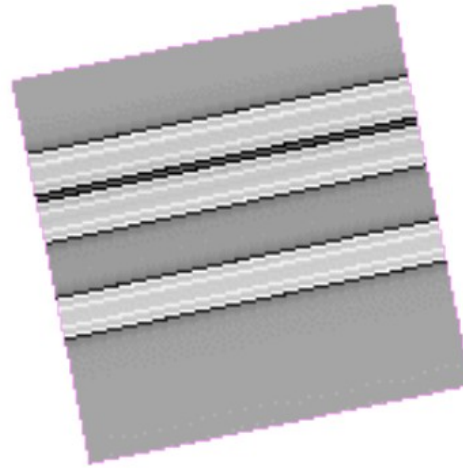
3D reconstruction

Real space reconstruction



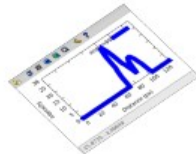
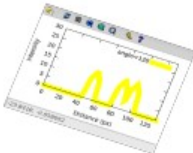
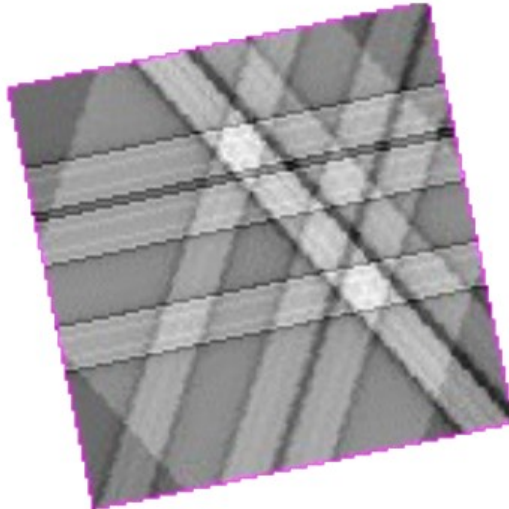
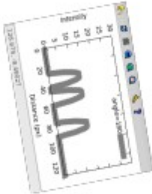
3D reconstruction

- reconstruction is the inversion of projection



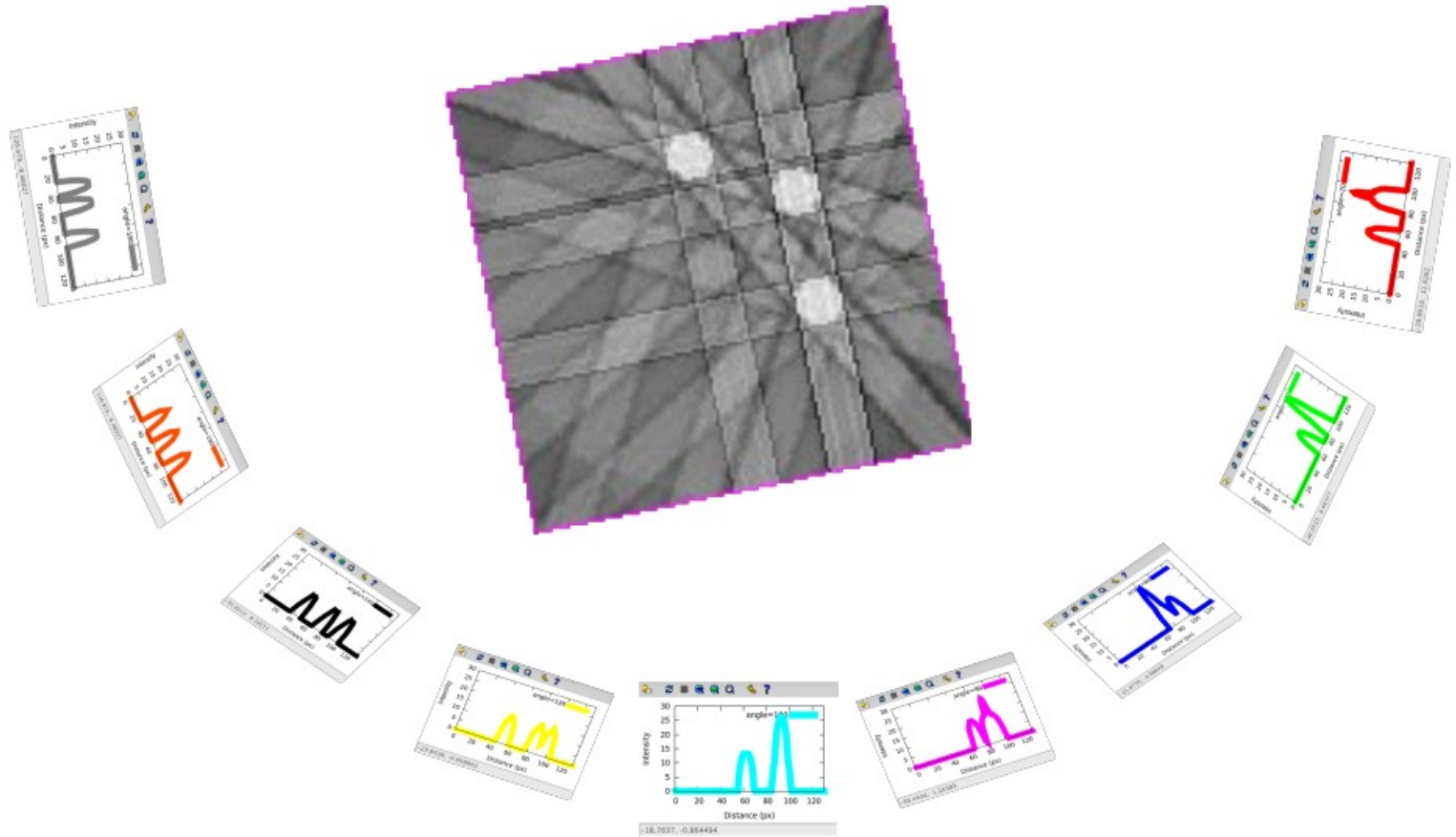
3D reconstruction

- reconstruction is the inversion of projection



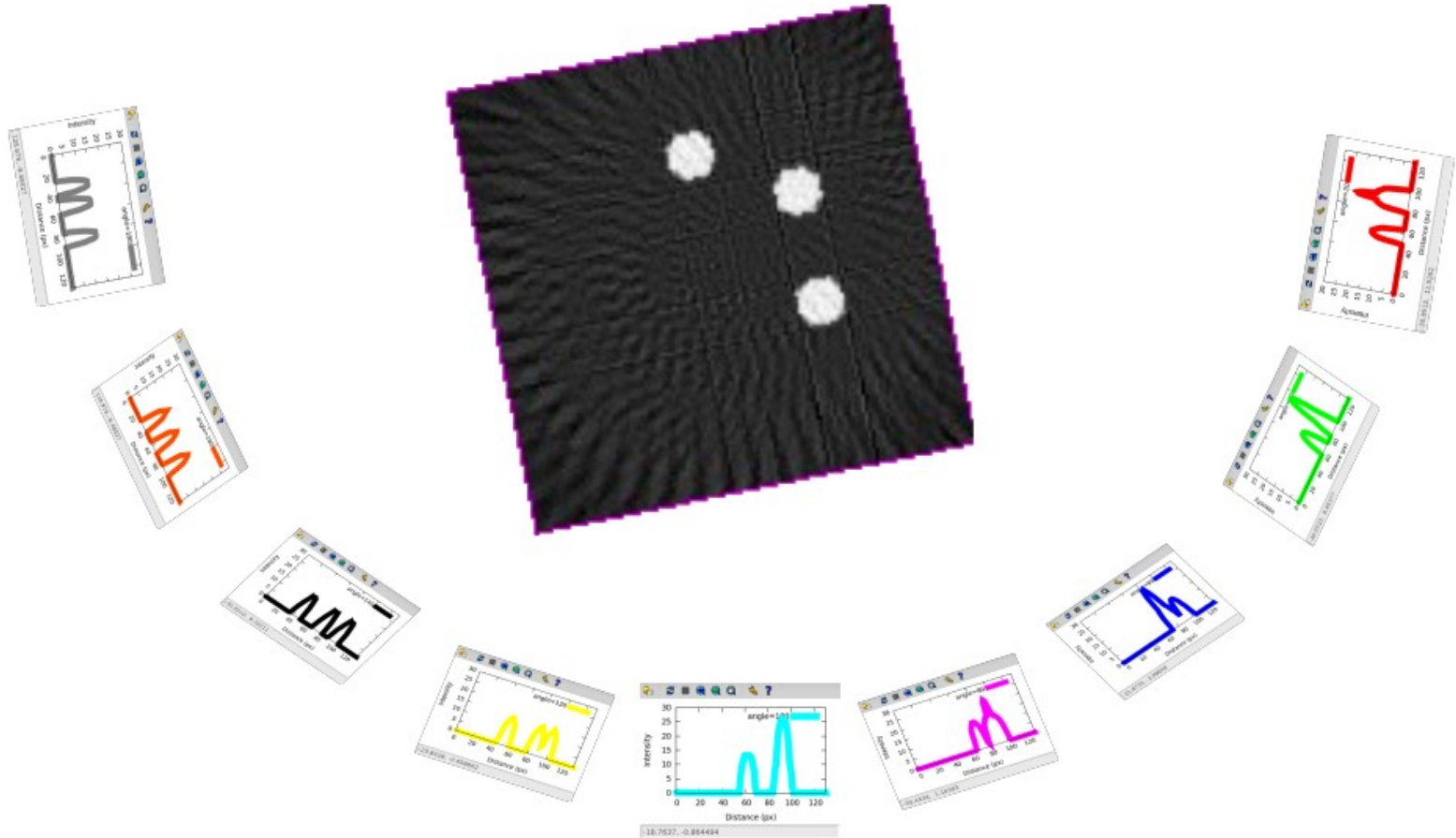
3D reconstruction

- reconstruction is the inversion of projection



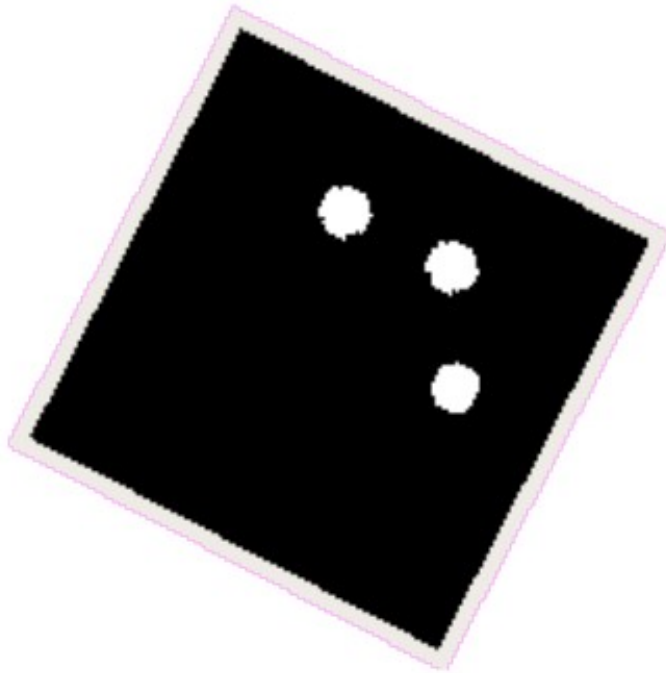
3D reconstruction

- reconstruction is the inversion of projection

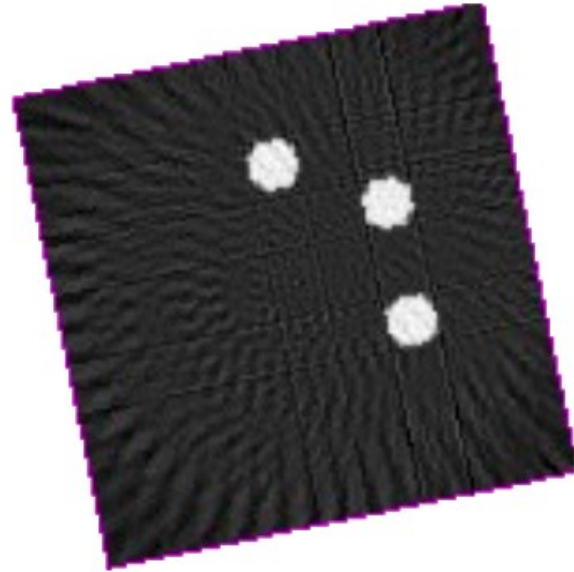


3D reconstruction

Original



Reconstructed



The reconstruction does not agree well with the projections

Potential solution: Simultaneous Iterative Reconstruction Technique

3D reconstruction

- simultaneous iterative reconstruction technique

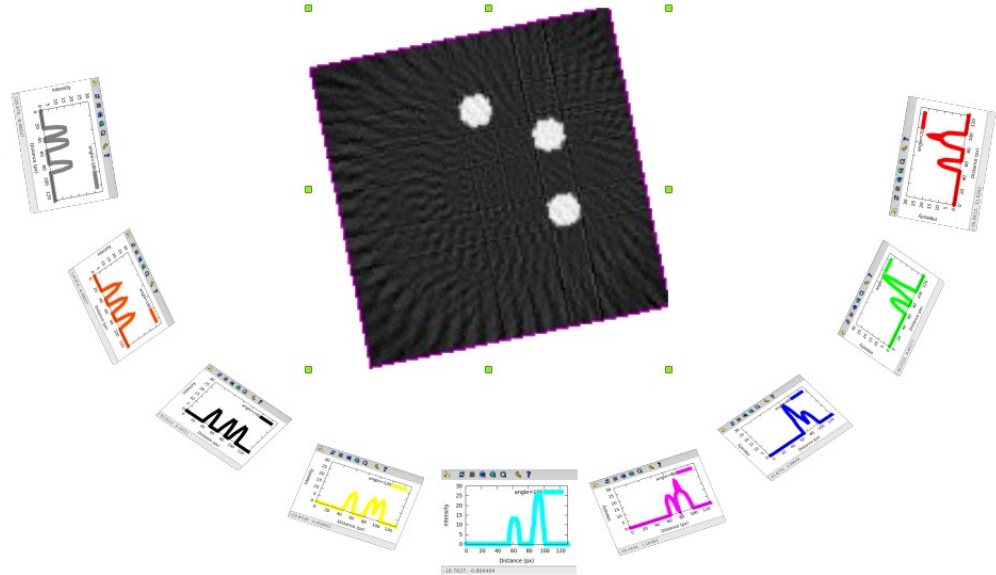
Compute re-projections of your model.

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

Weight the differences by a fudge factor, λ .

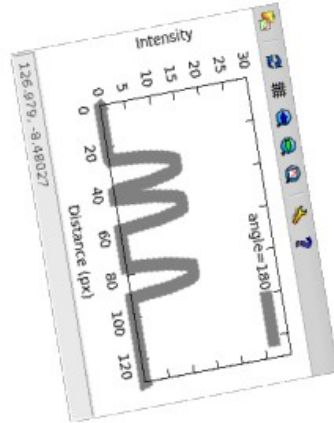
Adjust the model by the difference weighted by

Repeat

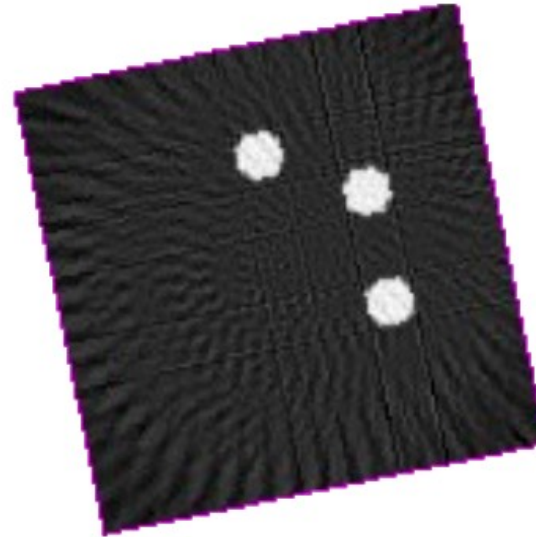


3D reconstruction

- simultaneous iterative reconstruction technique



Experimental projection



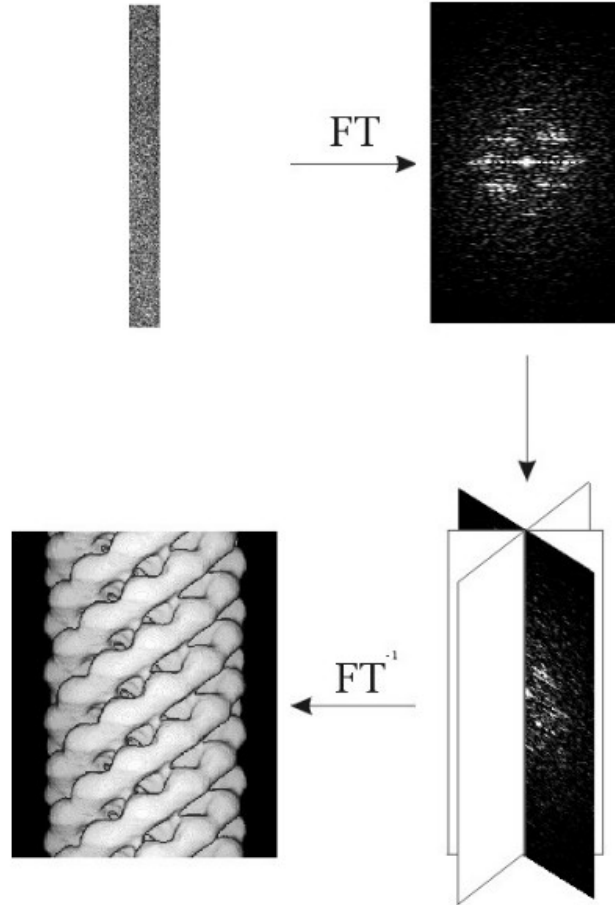
Model

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

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

3D reconstruction

Fourier space reconstruction

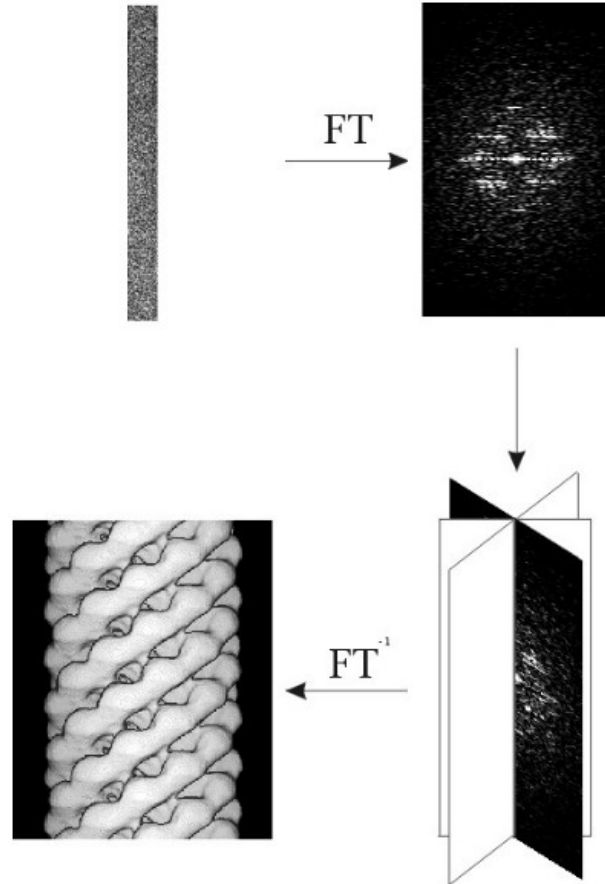


Projection theorem
Central section theorem

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

3D reconstruction

Fourier space reconstruction

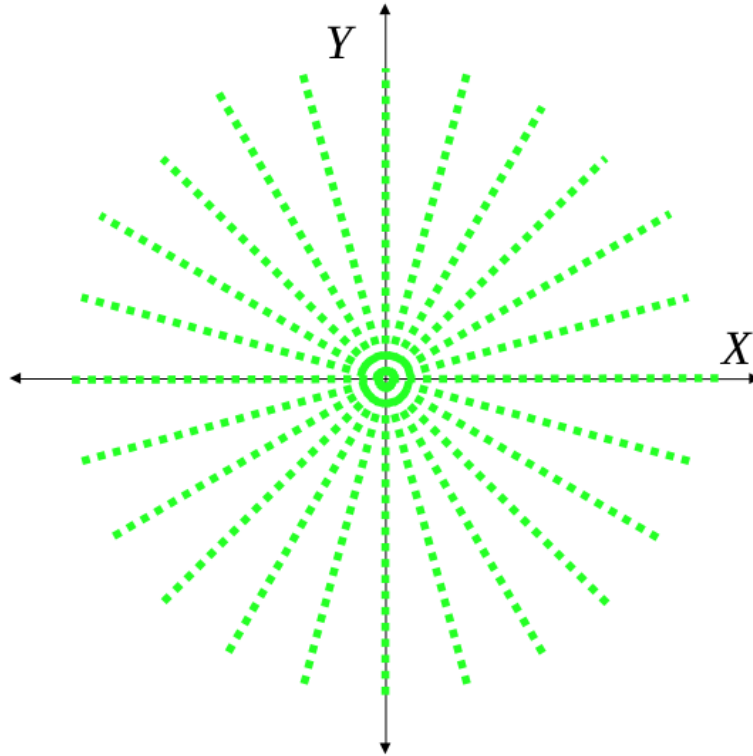


Projection theorem
Central section theorem

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

3D reconstruction

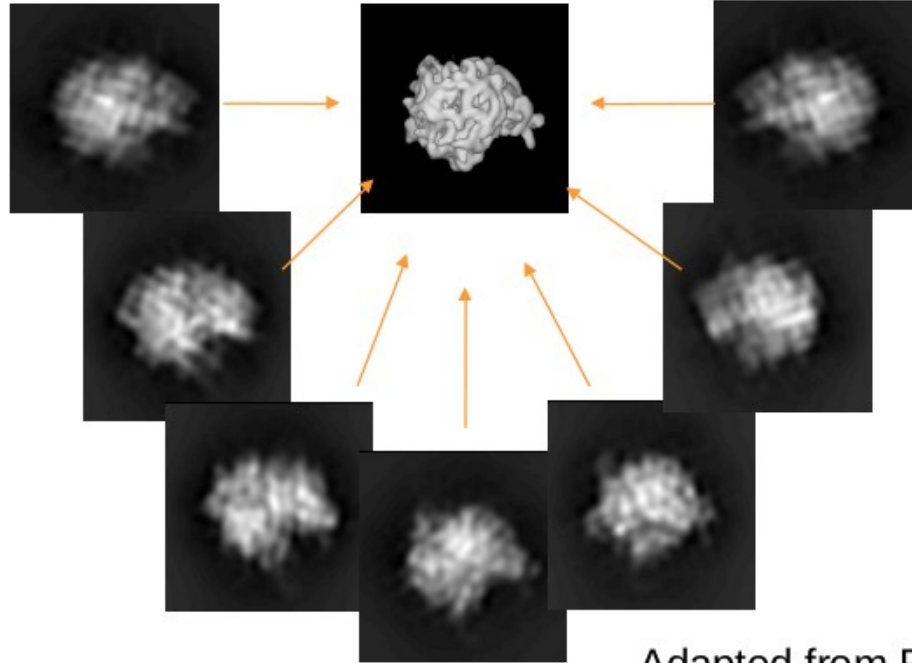
Converting from polar to Cartesian coordinates



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

3D reconstruction

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



Adapted from Pawel Penczek

3D reconstruction

1. Different orientations
2. Known orientations
3. Many particles
4. CTF parameters

Two translational:

▫ Δx

▫ Δy

Three orientational
(Euler angles):

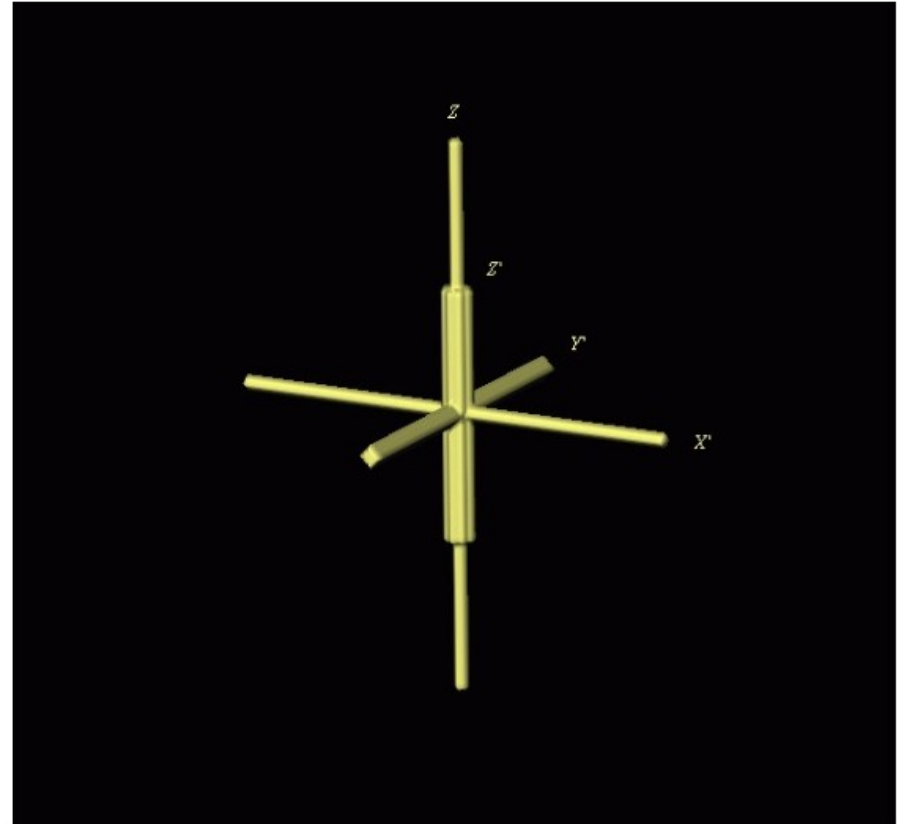
▫ phi (about z axis)

▫ theta (about y)

▫ psi (about new z)

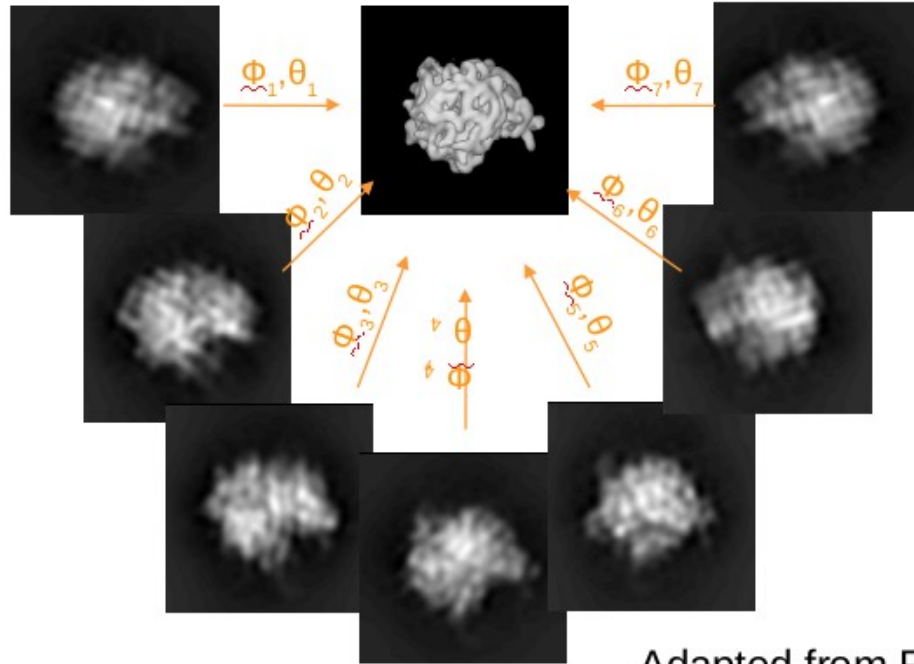
These are determined in 2D.

These are determined in 3D.



3D reconstruction

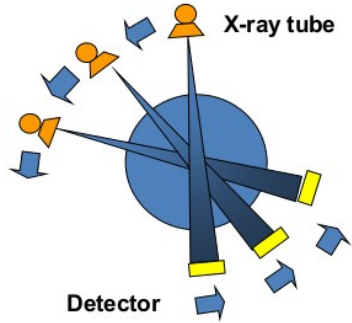
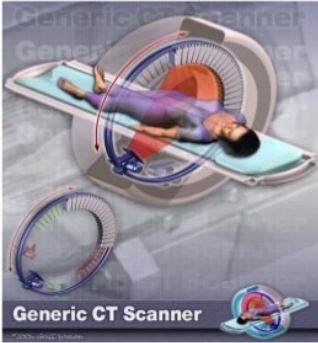
If you know the orientation angles for each image, you can compute a back-projection.



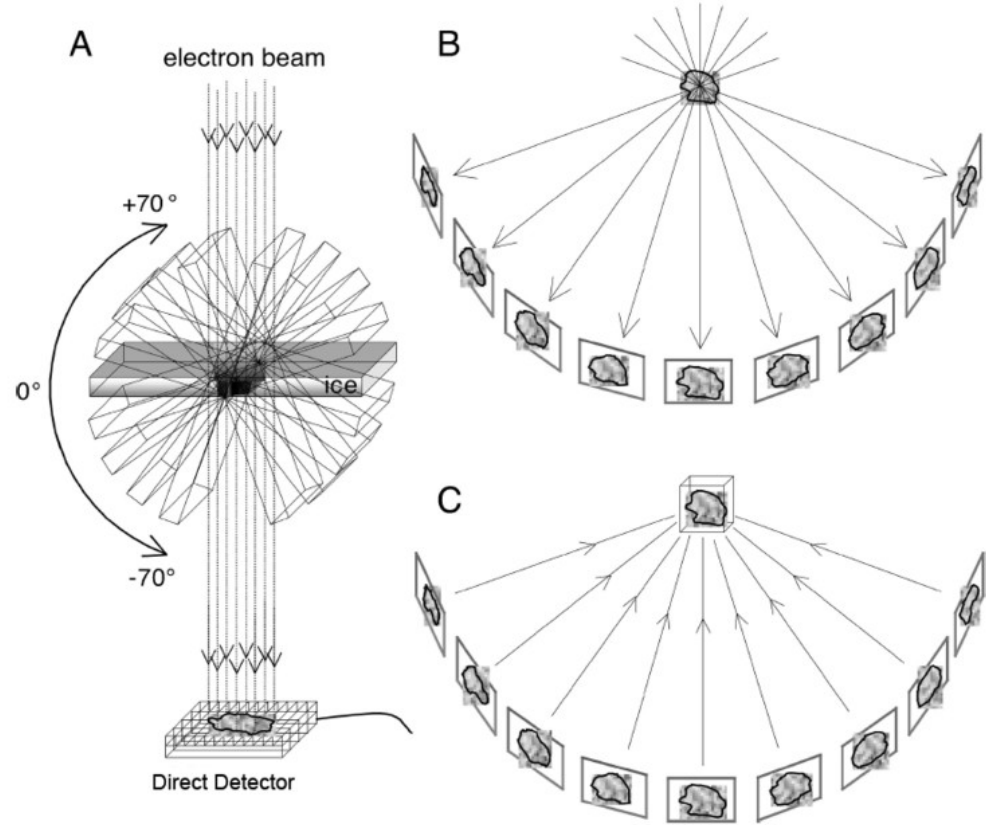
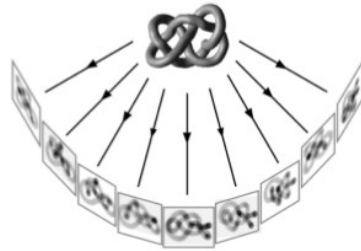
Adapted from Pawel Penczek

Tomography

Computer Tomography



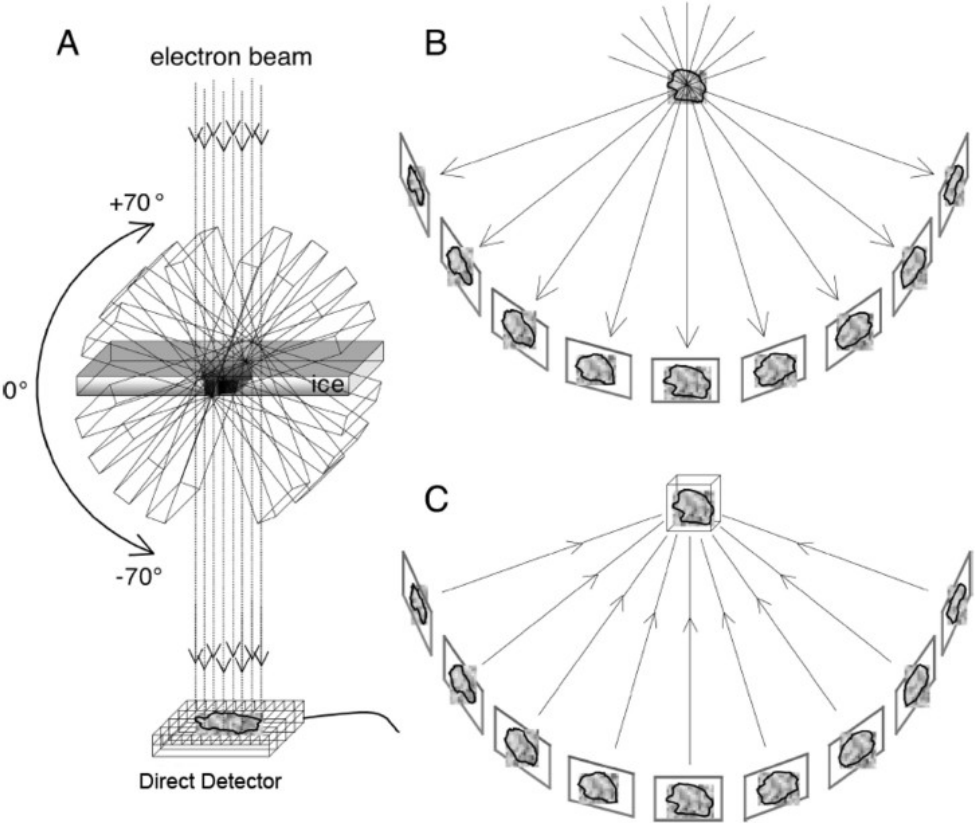
Electron Tomography



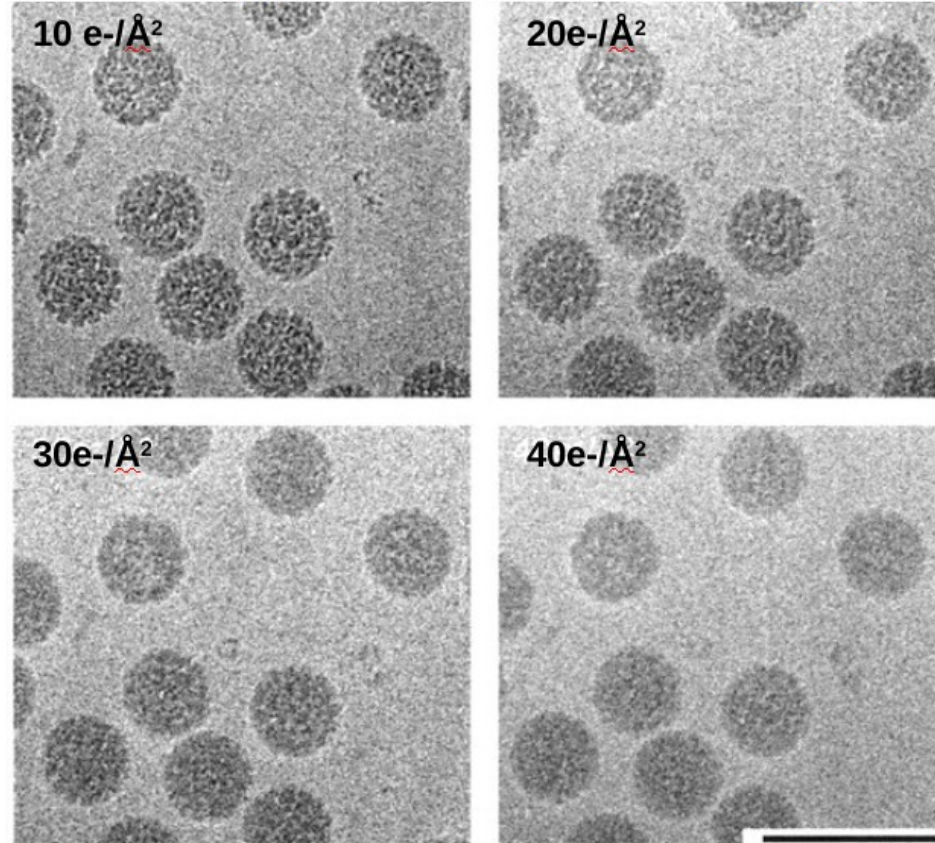
Tomography

We know orientations...

We have different view...



Tomography

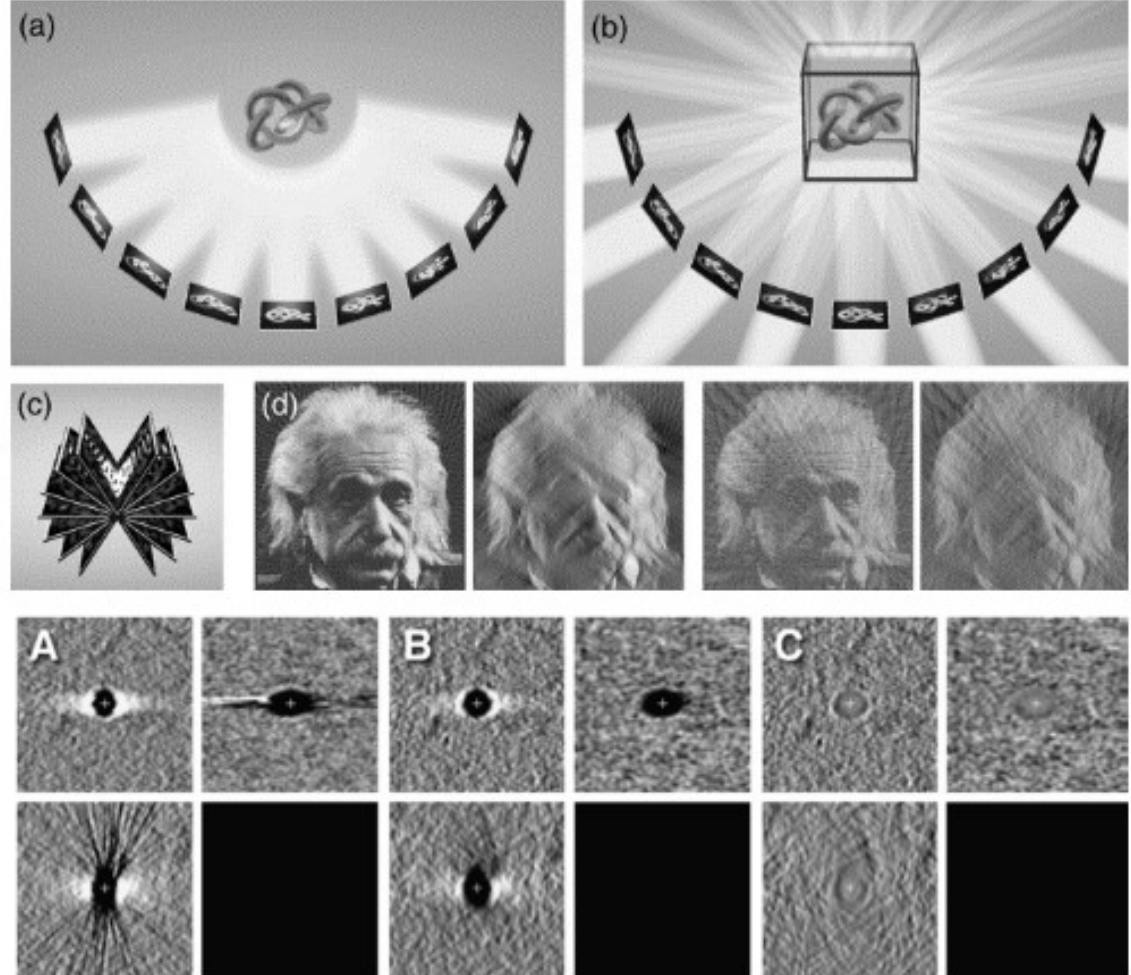


Baker et al. (1999) Microbiol. Mol. Biol. Rev. **63**: 862

We are destroying the sample as we image it

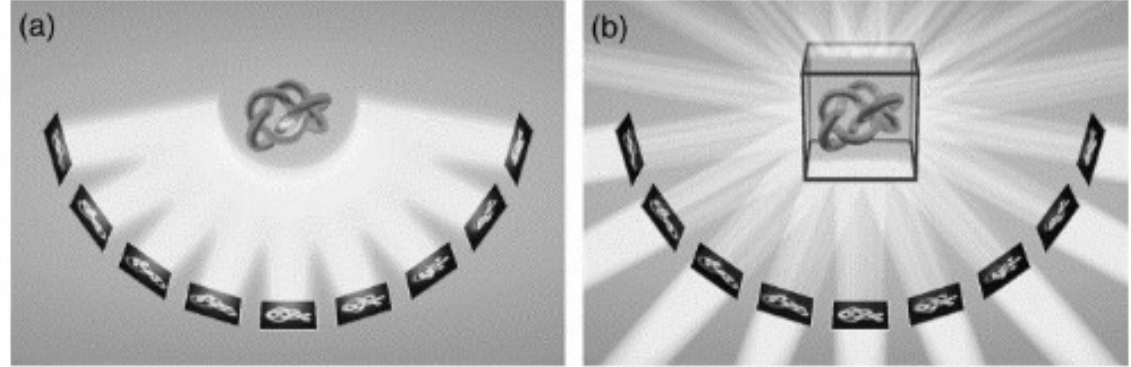
Tomography

Accumulated beam damage
If number of views is limited → image distortions



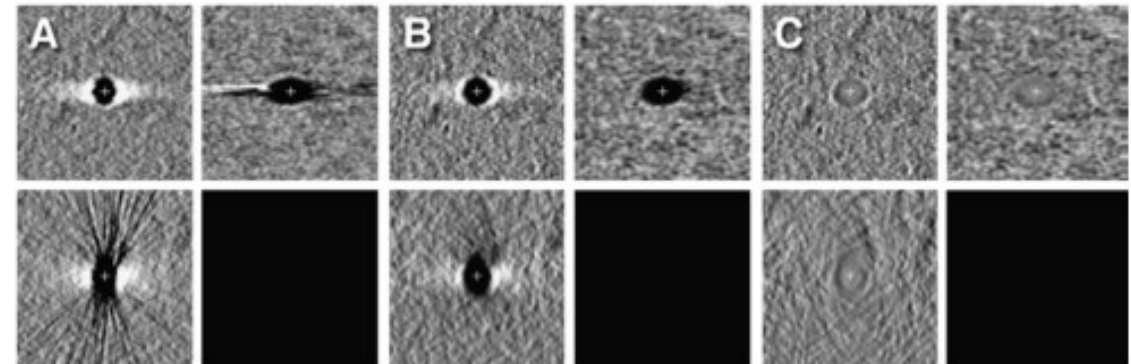
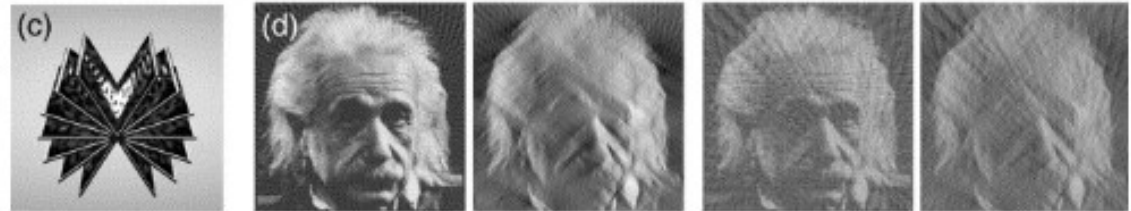
Tomography

Accumulated beam damage
If number of views is limited → image distortions



If we have many identical molecules and if we can determine their orientations, we can use one exposure per molecule and use the images in the reconstruction

→ single particle analysis

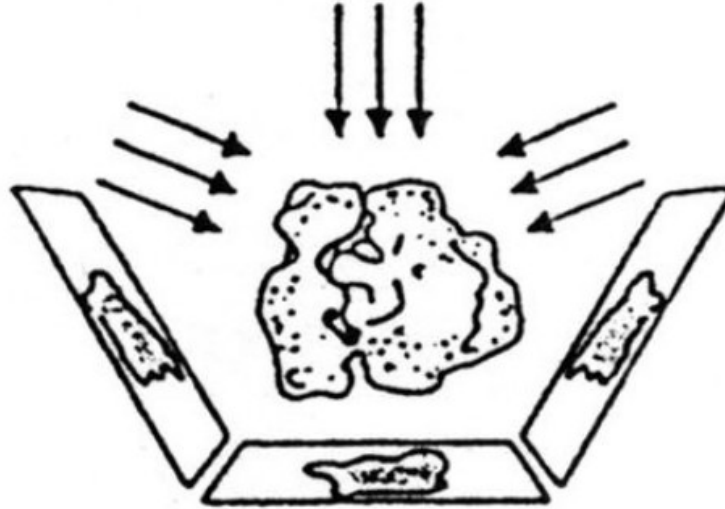


Single particle analysis

Unlike the tomography data, we do not know how the orientations between individual images are related
→ **reference based alignment**

You will record the direction of projection (the Euler angles), such that if you encounter an experimental image that resembles a reference projection, you will assign that reference projection's Euler angles to the experimental image.

Step 1: Generation of projections of the reference.



From Penczek et al. (1994), Ultramicroscopy **53**: 251-70.

Assumption: reference is similar enough to the sample that it can be used to determine orientation.

Single particle analysis

Unlike the tomography data, we do not know how the orientations between individual images are related
→ **reference based alignment**

Two translational:

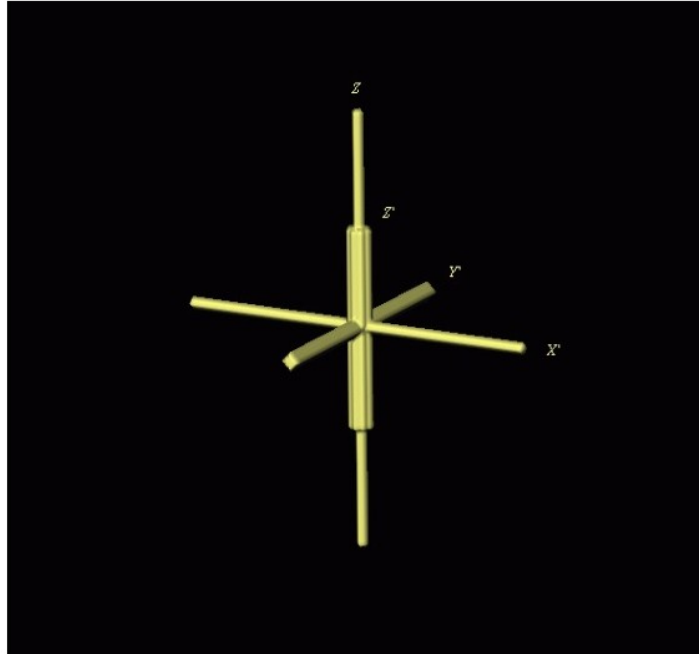
- Δx
- Δy

Three orientational (Euler angles):

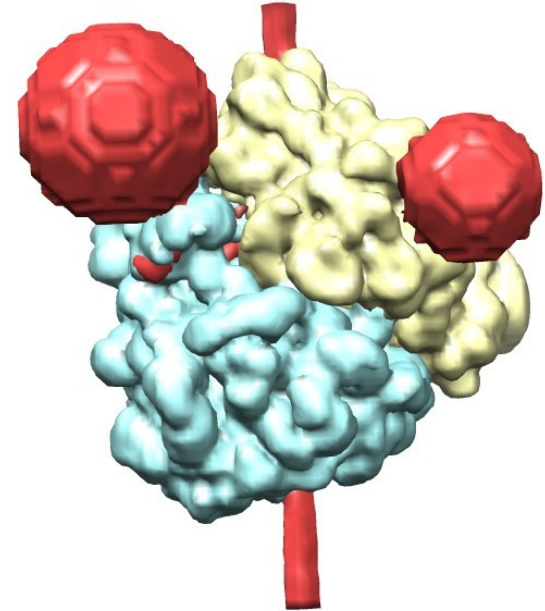
- phi (about z axis)
- theta (about y)
- psi (about new z)

These are determined in 2D.

These are determined in 3D.

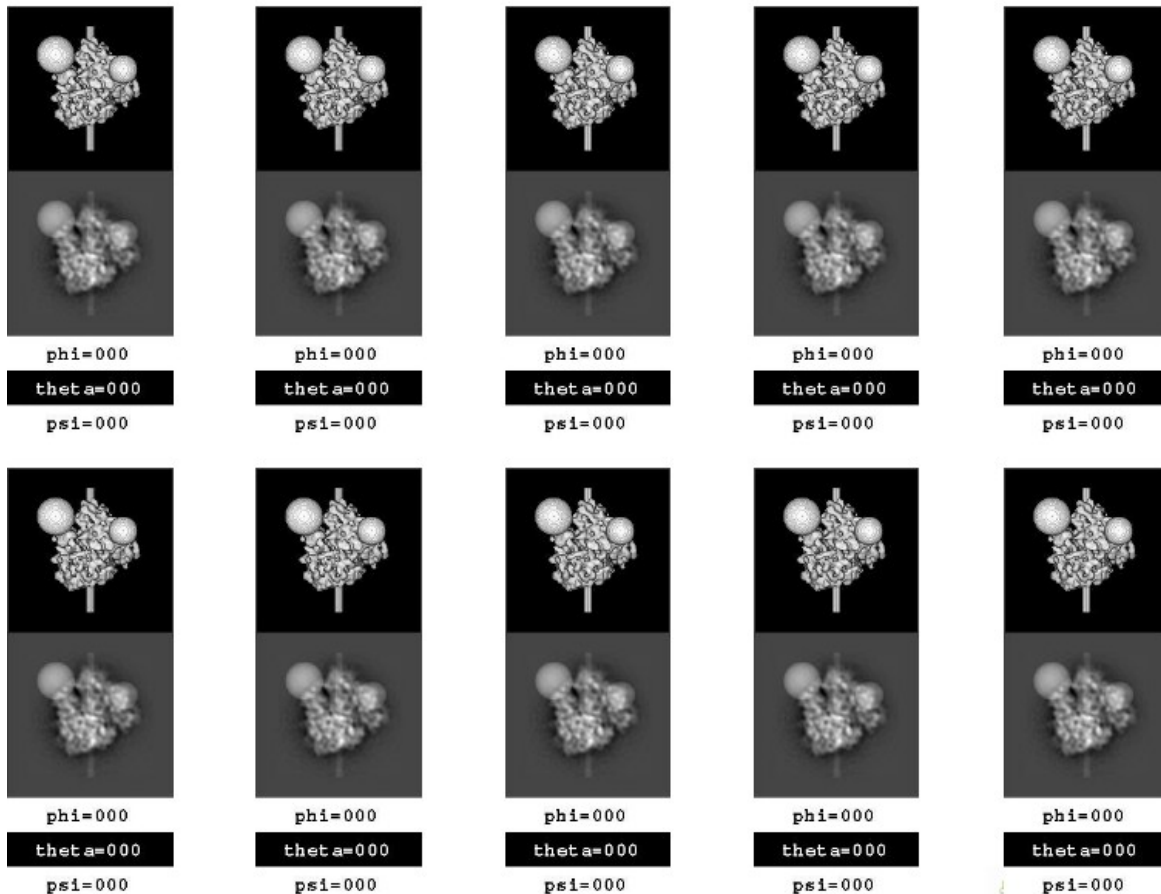


<http://www.wadsworth.org>



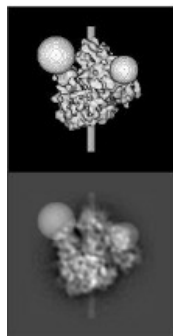
Single particle analysis

Projection of the reference at the defined angular step



Single particle analysis

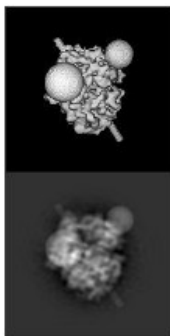
Projection of the reference at the defined angular step



phi=000

theta=000

psi=000



phi=036

theta=030

psi=000



phi=000

theta=000

psi=000



phi=000

theta=000

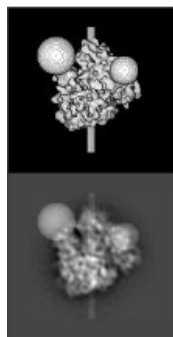
psi=000



phi=000

theta=000

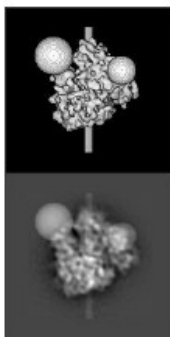
psi=000



phi=000

theta=000

psi=000



phi=000

theta=000

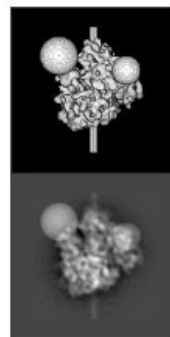
psi=000



phi=000

theta=000

psi=000



phi=000

theta=000

psi=000



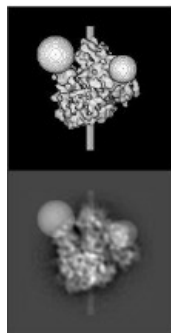
phi=000

theta=000

psi=000

Single particle analysis

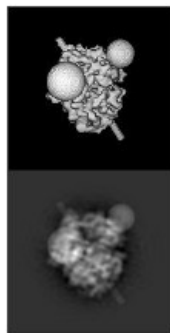
Projection of the reference at the defined angular step



phi=000

theta=000

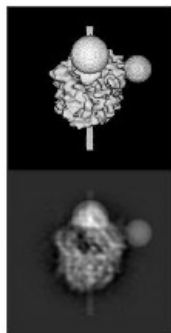
psi=000



phi=036

theta=030

psi=000



phi=000

theta=045

psi=000



phi=000

theta=000

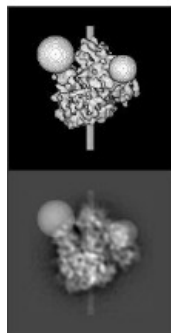
psi=000



phi=000

theta=000

psi=000



phi=000

theta=000

psi=000



phi=000

theta=000

psi=000



phi=000

theta=000

psi=000



phi=000

theta=000

psi=000



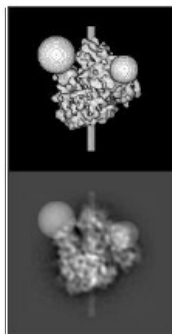
phi=000

theta=000

psi=000

Single particle analysis

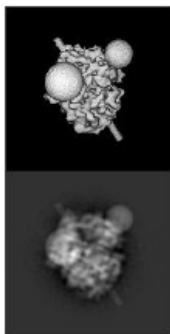
Projection of the reference at the defined angular step



phi=000

theta=000

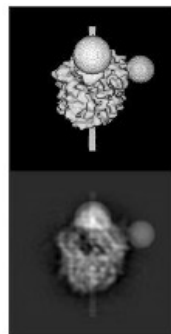
psi=000



phi=036

theta=030

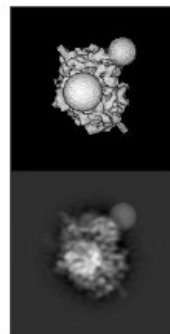
psi=000



phi=000

theta=045

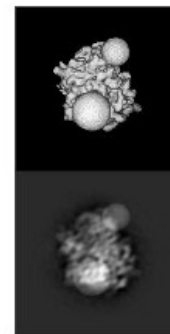
psi=000



phi=048

theta=045

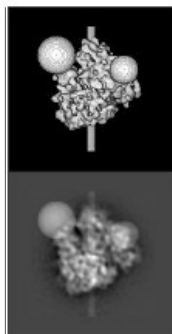
psi=000



phi=072

theta=045

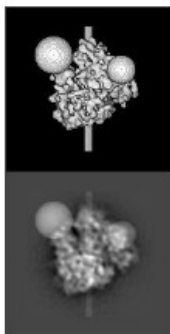
psi=000



phi=000

theta=000

psi=000



phi=000

theta=000

psi=000



phi=000

theta=000

psi=000



phi=000

theta=000

psi=000



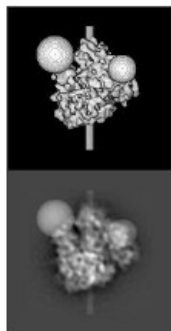
phi=000

theta=000

psi=000

Single particle analysis

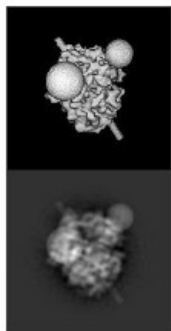
Projection of the reference at the defined angular step



phi=000

theta=000

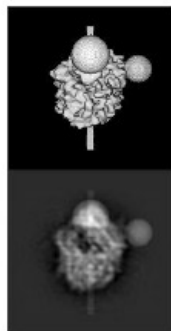
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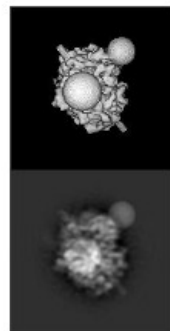
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phi=000

theta=045

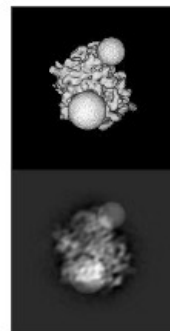
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phi=048

theta=045

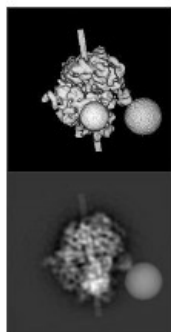
psi=000



phi=072

theta=045

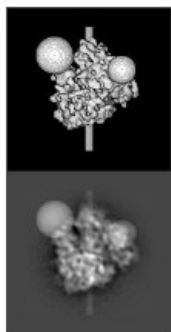
psi=000



phi=192

theta=045

psi=000



phi=000

theta=000

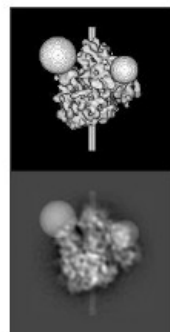
psi=000



phi=000

theta=000

psi=000



phi=000

theta=000

psi=000



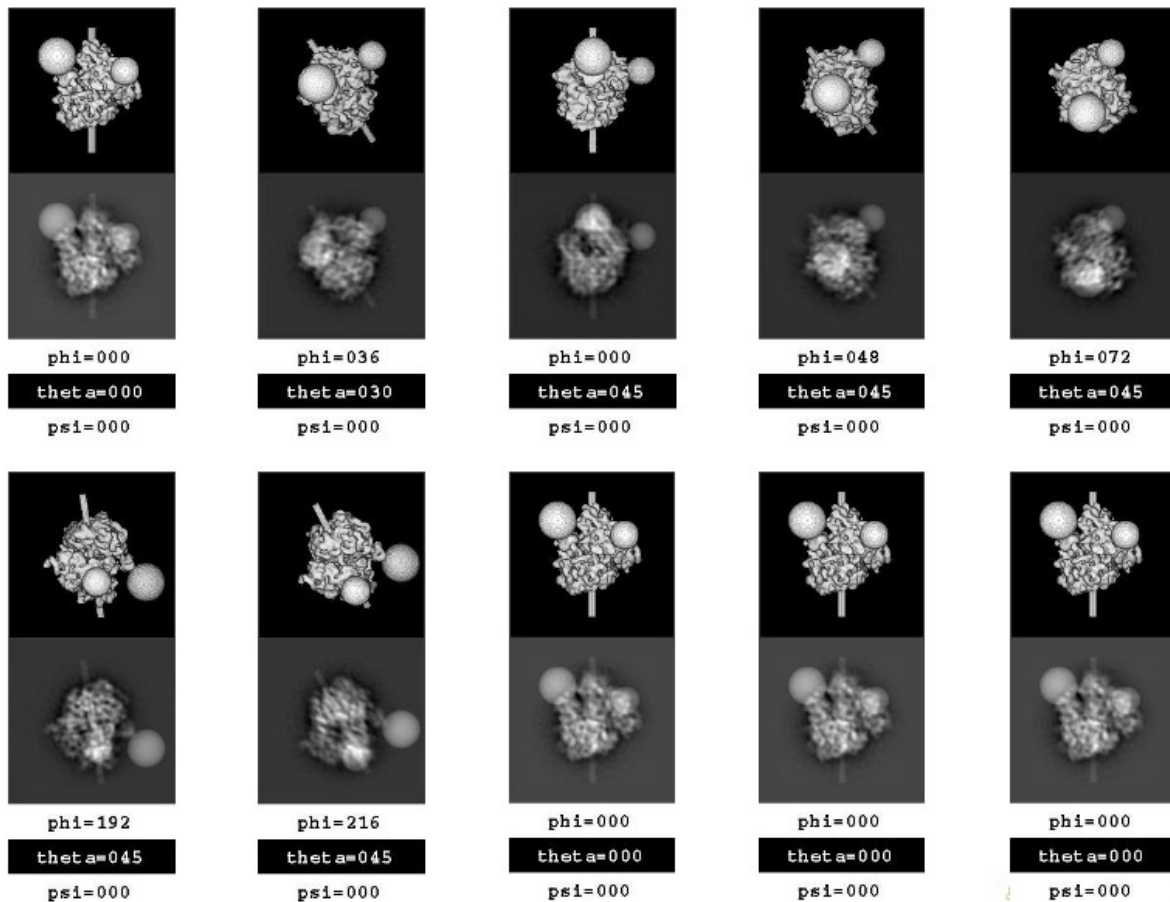
phi=000

theta=000

psi=000

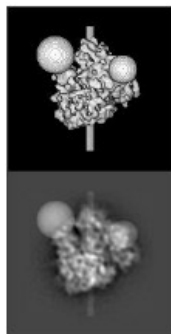
Single particle analysis

Projection of the reference at the defined angular step



Single particle analysis

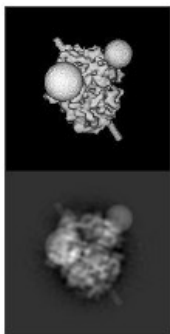
Projection of the reference at the defined angular step



phi=000

theta=000

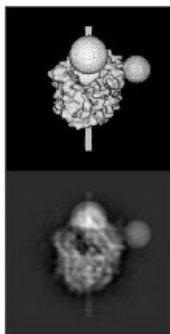
psi=000



phi=036

theta=030

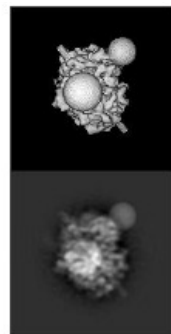
psi=000



phi=000

theta=045

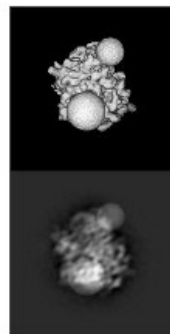
psi=000



phi=048

theta=045

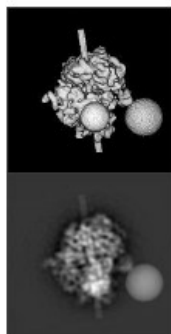
psi=000



phi=072

theta=045

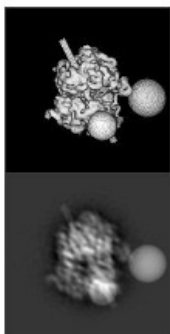
psi=000



phi=192

theta=045

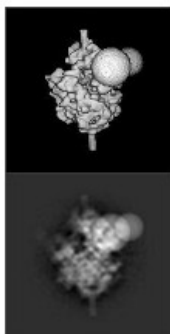
psi=000



phi=216

theta=045

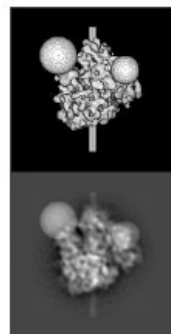
psi=000



phi=016

theta=075

psi=000



phi=000

theta=000

psi=000



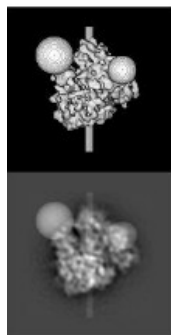
phi=000

theta=000

psi=000

Single particle analysis

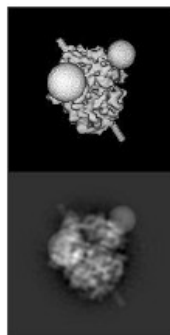
Projection of the reference at the defined angular step



phi=000

theta=000

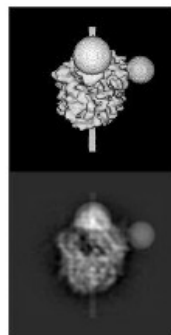
psi=000



phi=036

theta=030

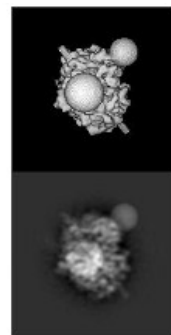
psi=000



phi=000

theta=045

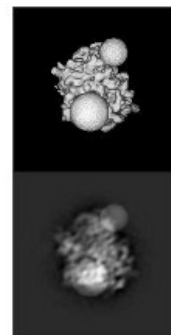
psi=000



phi=048

theta=045

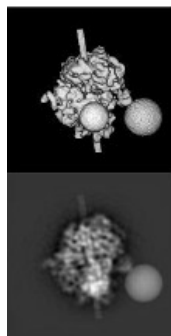
psi=000



phi=072

theta=045

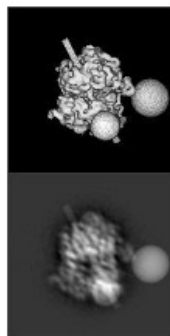
psi=000



phi=192

theta=045

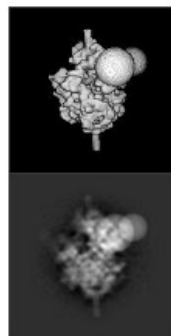
psi=000



phi=216

theta=045

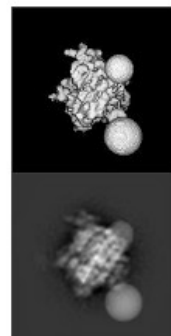
psi=000



phi=016

theta=075

psi=000



phi=115

theta=075

psi=000



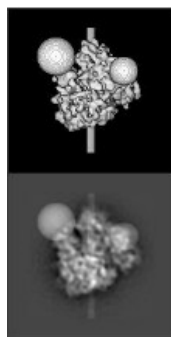
phi=000

theta=000

psi=000

Single particle analysis

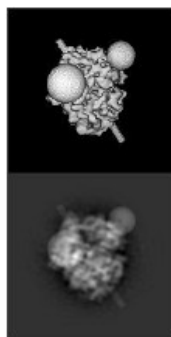
Projection of the reference at the defined angular step



phi=000

theta=000

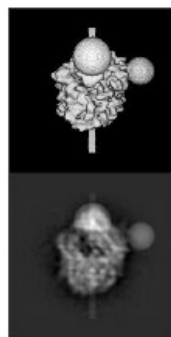
psi=000



phi=036

theta=030

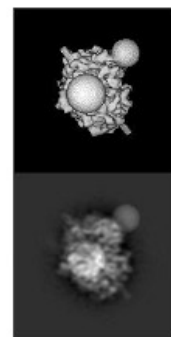
psi=000



phi=000

theta=045

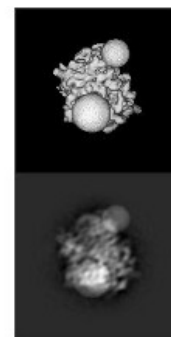
psi=000



phi=048

theta=045

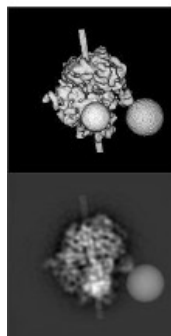
psi=000



phi=072

theta=045

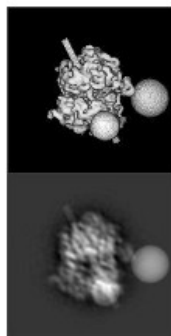
psi=000



phi=192

theta=045

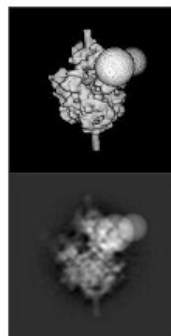
psi=000



phi=216

theta=045

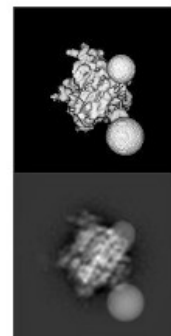
psi=000



phi=016

theta=075

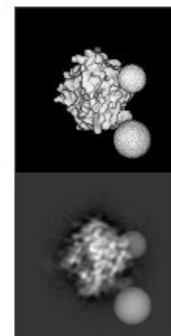
psi=000



phi=115

theta=075

psi=000

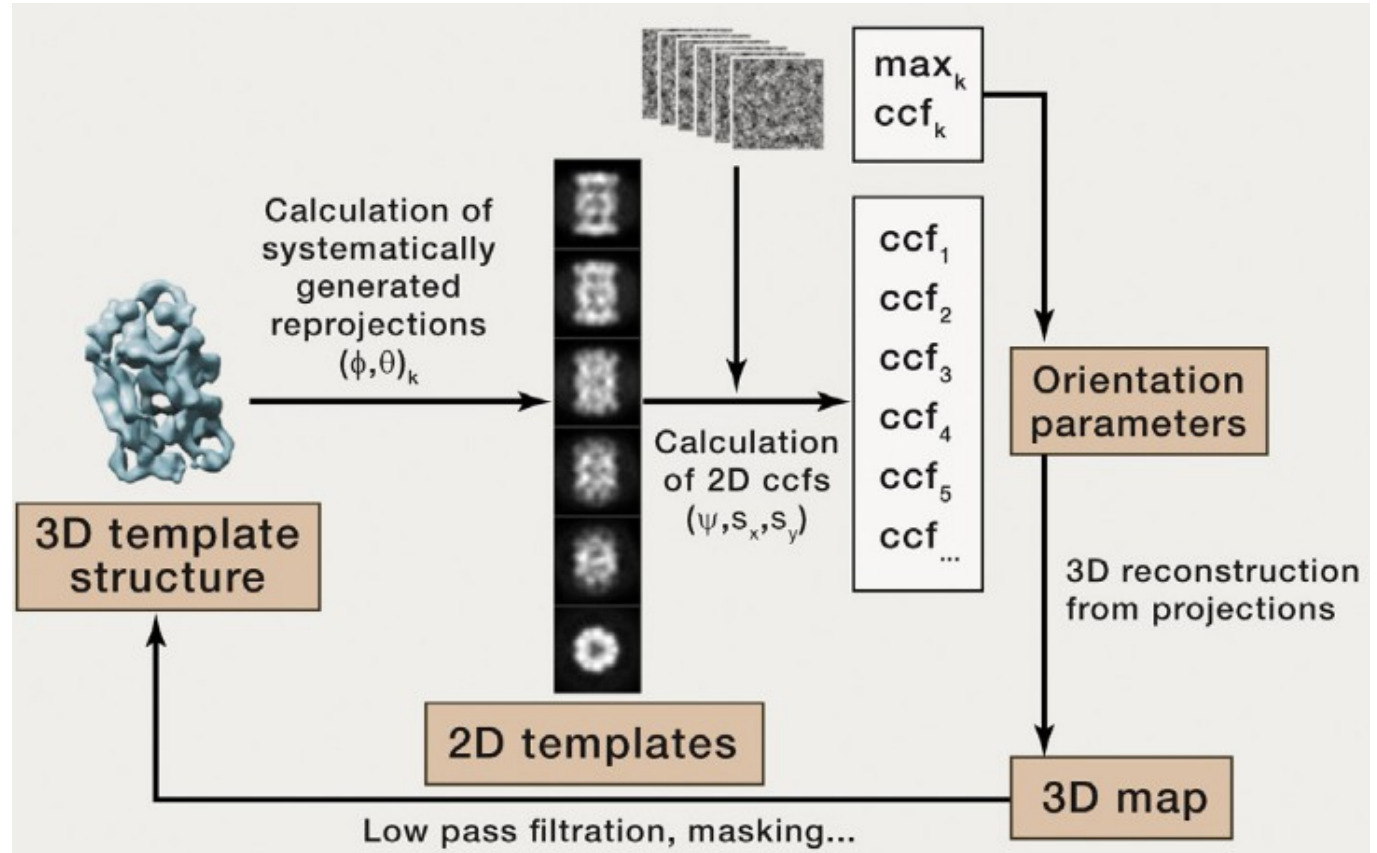


phi=131

theta=090

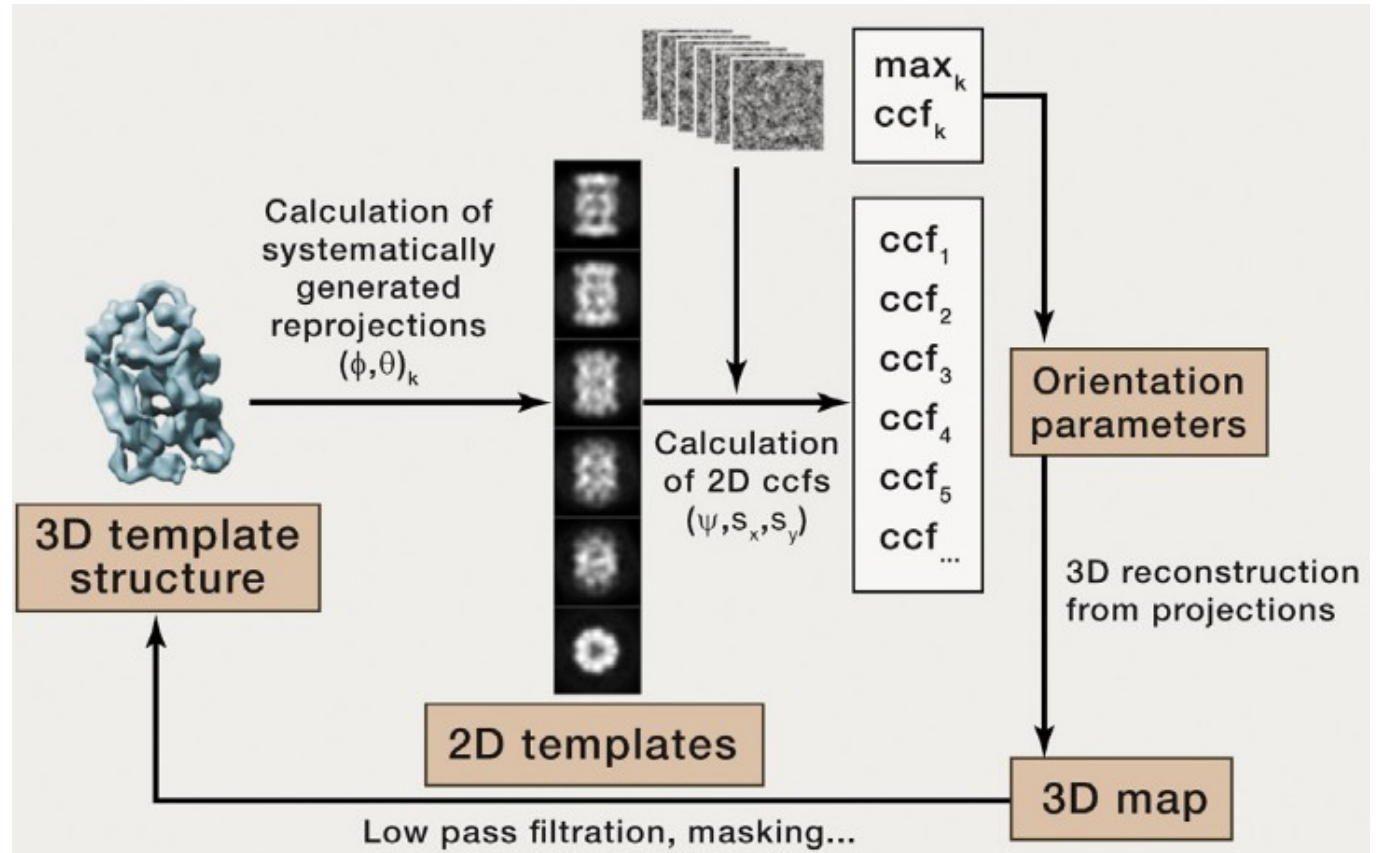
psi=000

Single particle analysis

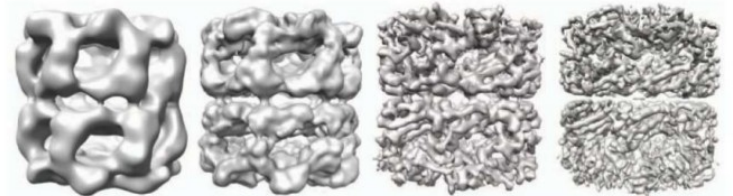


1. Compare the experimental images to all of the reference projections
2. Find the reference projection with which the experimental images match the best
3. Assign the Euler angles of that reference to the experimental image

Single particle analysis



4. Calculate a new reference
5. Project the new reference
6. Repeat from 1

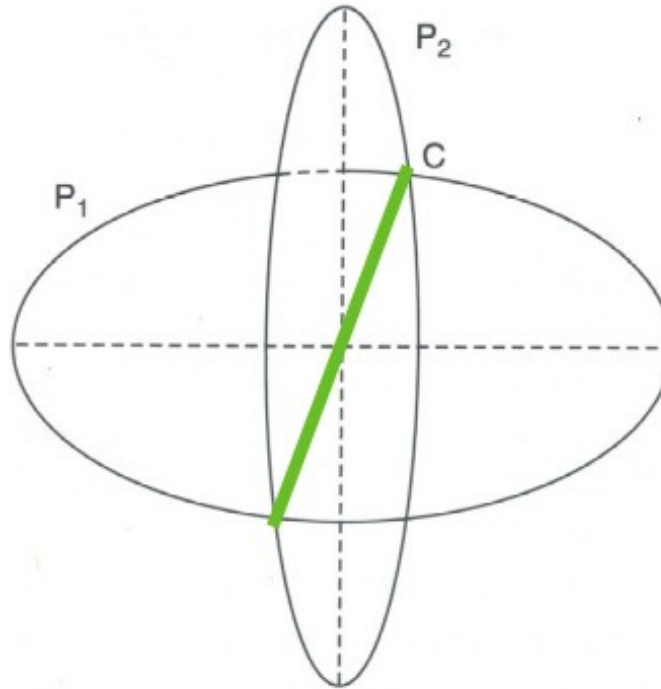


Common lines

Angular Reconstruction

Summary:

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



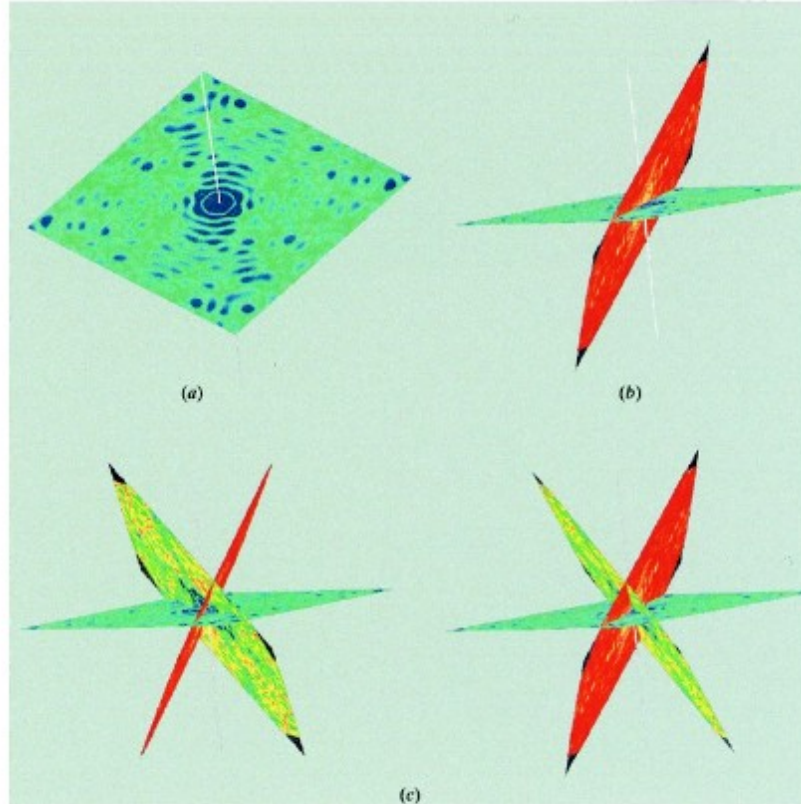
Frank, J. (2006) 3D Electron Microscopy of Macromolecular Assemblies

Common lines

Angular Reconstruction

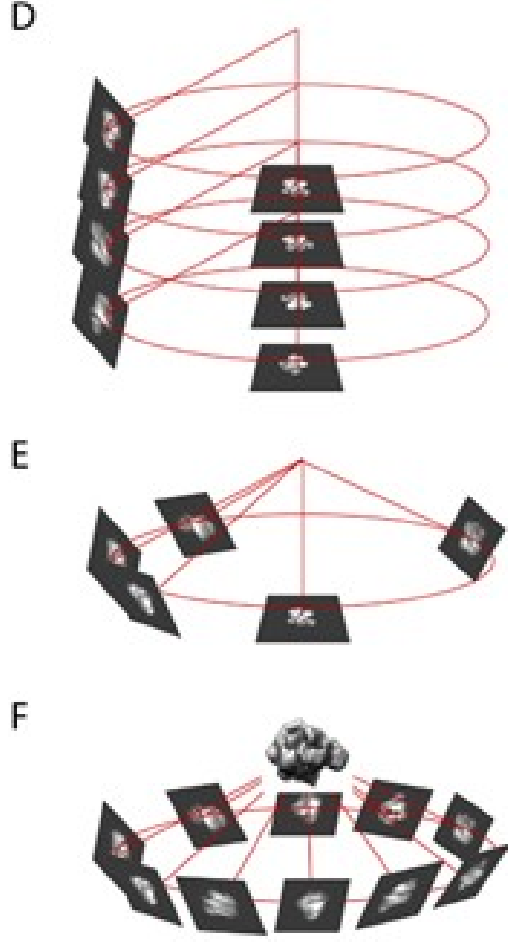
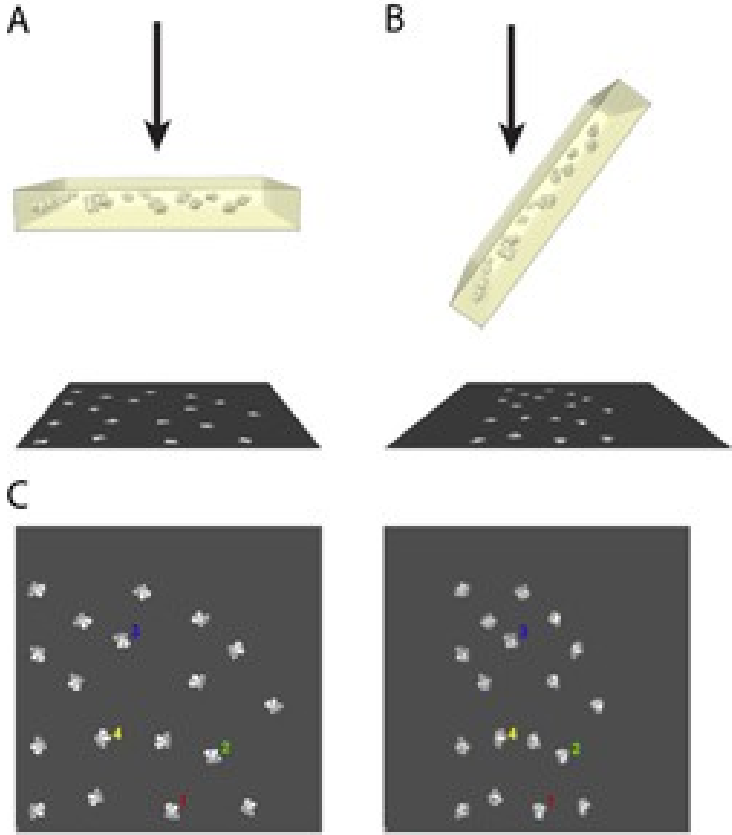
Summary:

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

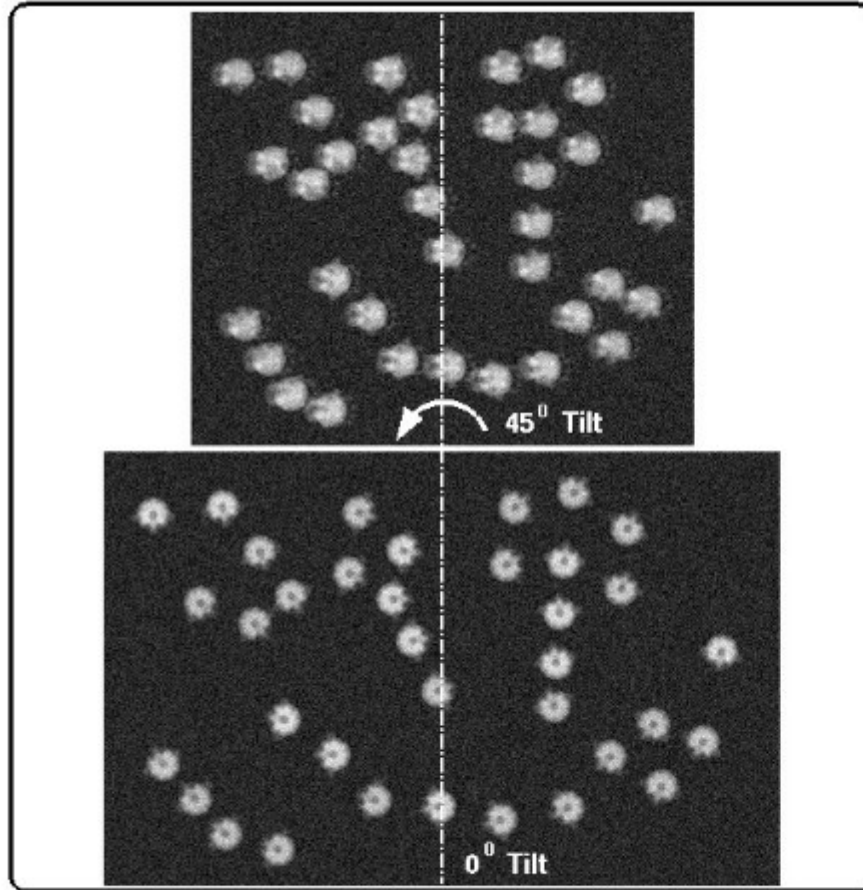
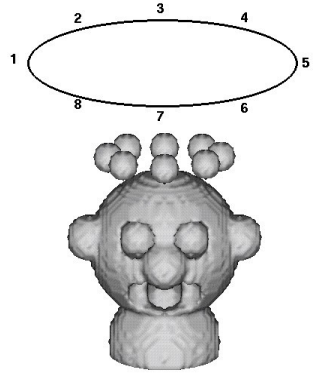


From Steve Fuller

Random conical tilt



Random conical tilt

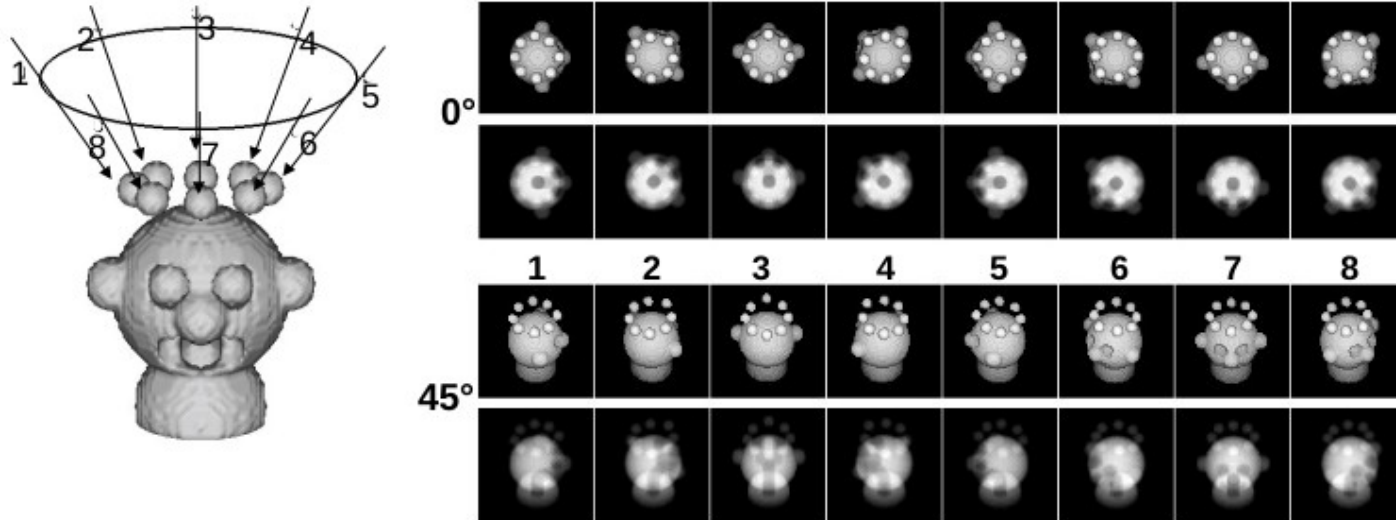


This scenario describes a worst case, when there is exactly one orientation in the 0° image. Since the in-plane angle varies, in the tilted image, we have different views available.

From Nicolas Boisset

Random conical tilt

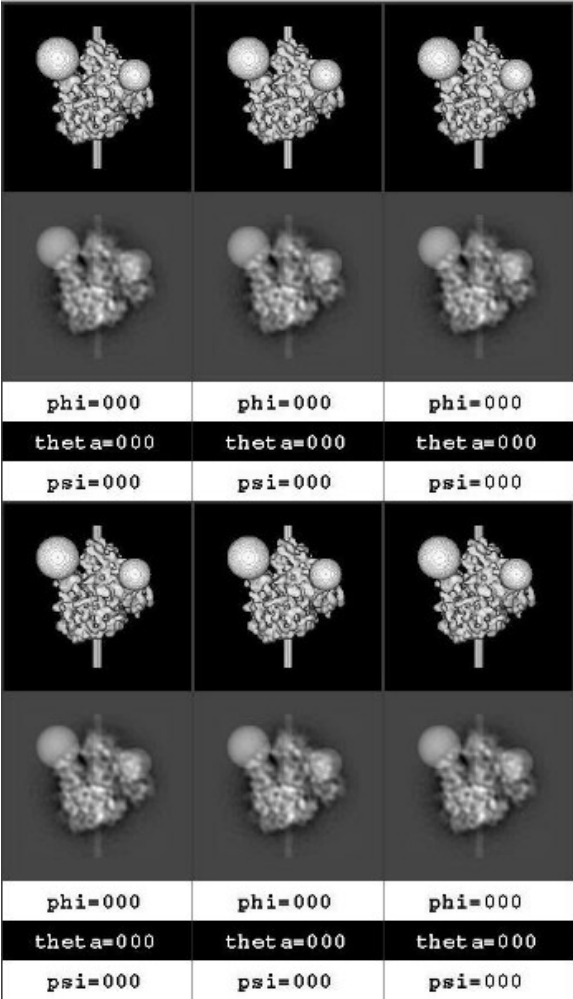
Two images are taken: one at 0° and one tilted at an angle of 45° .



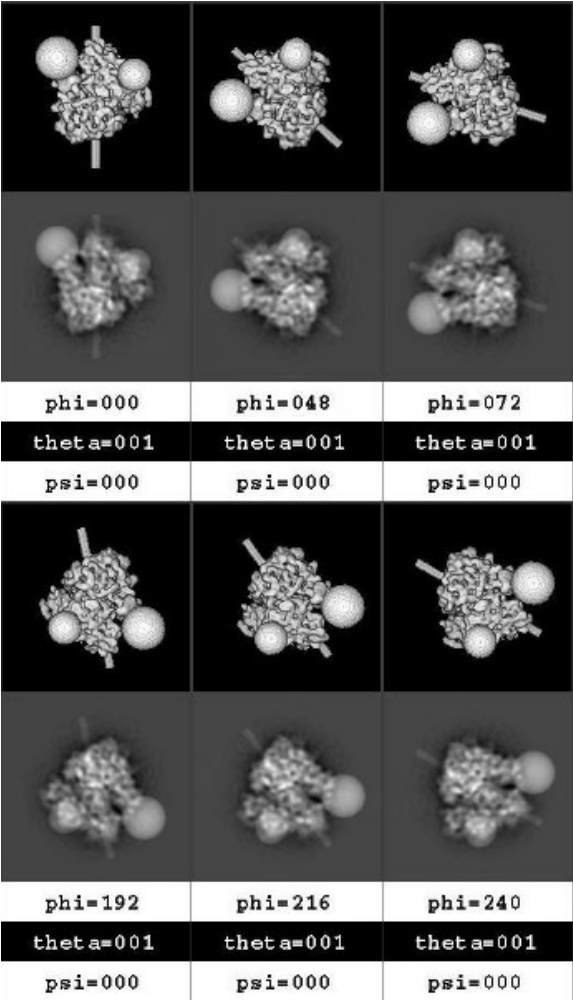
Radermacher, M., Wagenknecht, T., Verschoor, A. & Frank, J. Three-dimensional reconstruction from a single-exposure, random conical tilt series applied to the 50S ribosomal subunit of *Escherichia coli*. *J Microsc* **146**, 113-36 (1987).

From Nicolas Boisset

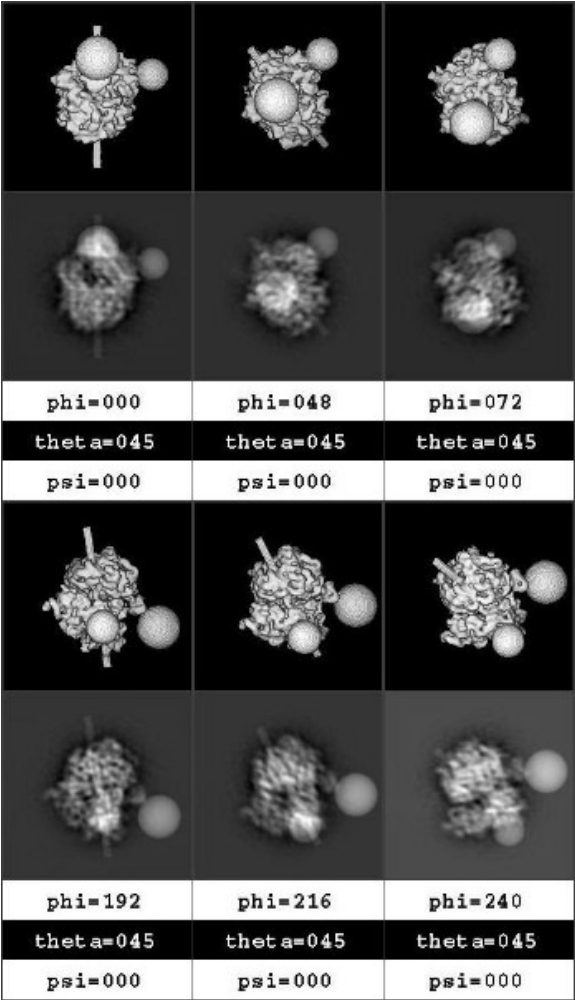
Random conical tilt



Random conical tilt



Random conical tilt

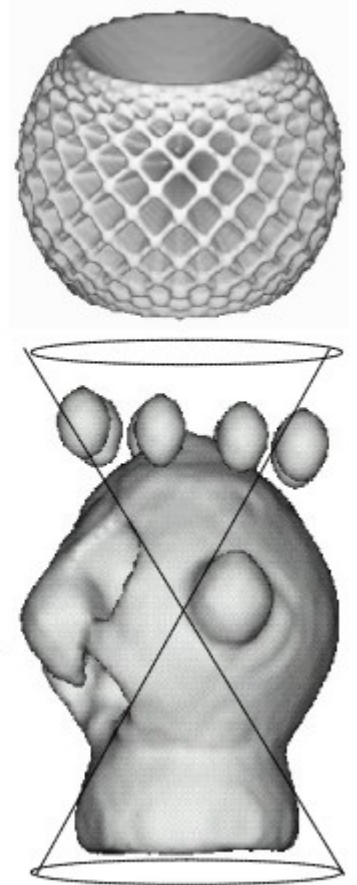


Random conical tilt

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

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

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



Random conical tilt

- filling the missing cone

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

