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Central European Institute of Technology
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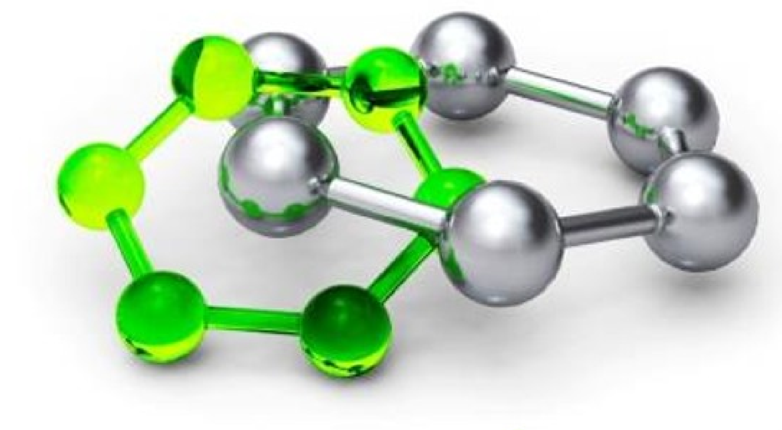
Image analysis II: 3D Reconstruction

C9940 3-Dimensional Transmission Electron Microscopy
S1007 Doing structural biology with the electron microscope

March 20, 2017



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OP Research and
Development for Innovation

Outline

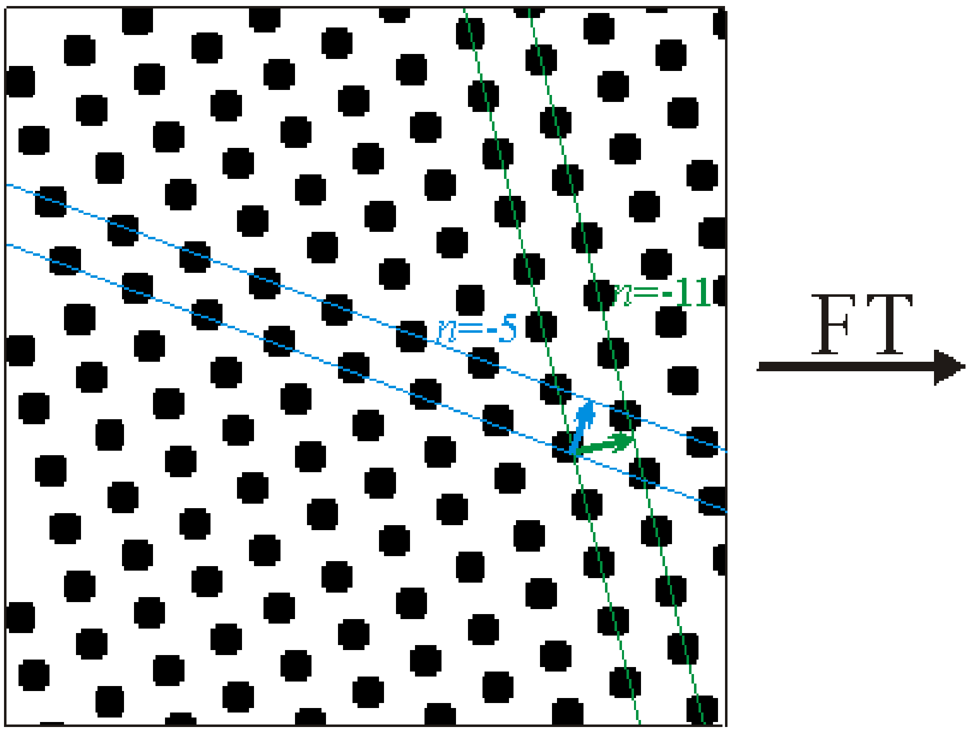
Image analysis II

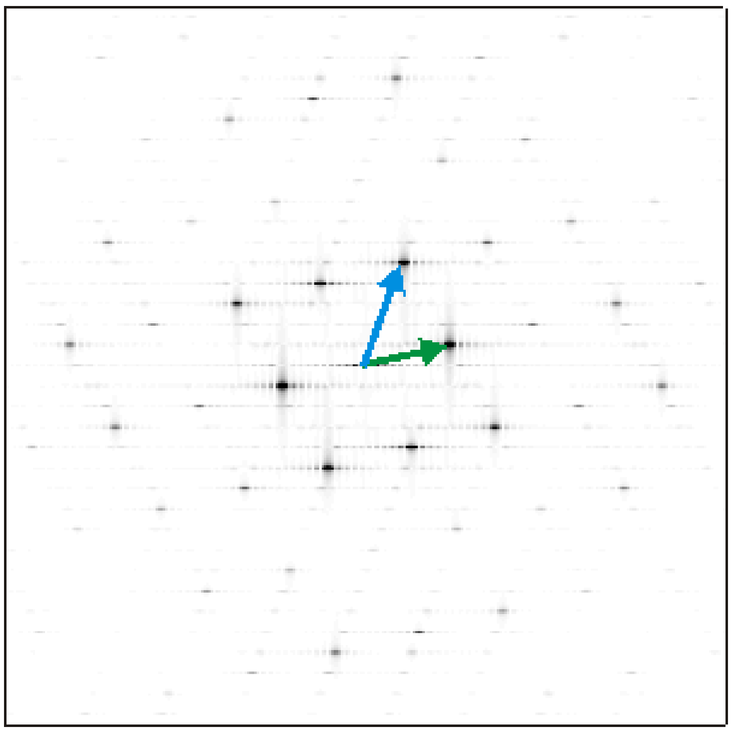
- 2D Fourier transforms

3D Reconstruction

- Principles
- Tomography
- Reference-based alignment
- Common lines
- RCT
- CTF-correction
- 3D classification

Some simple 2D Fourier transforms: a 2D lattice





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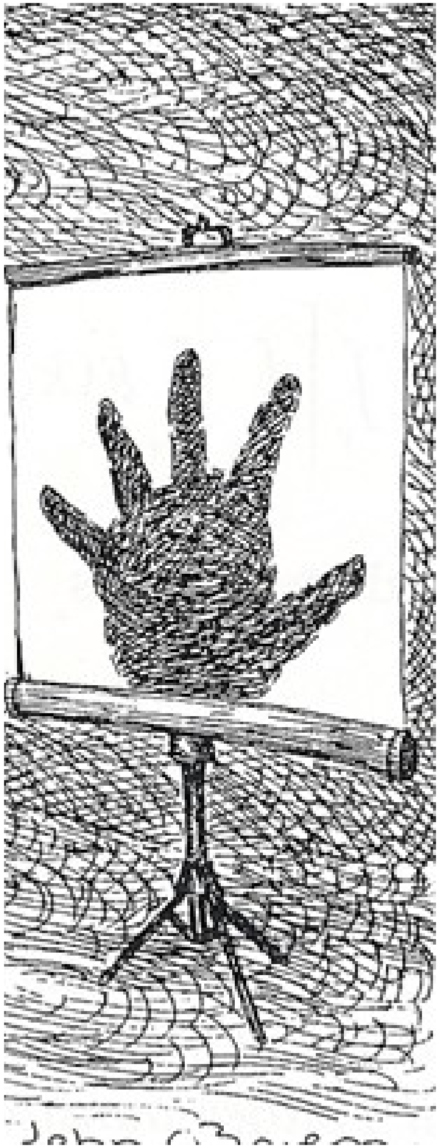
How do you go from 2D to 3D?



John O'Brien, 1991, *The New Yorker*

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What information do we need for 3D reconstruction?

1. different orientations
2. known orientations
3. many particles

What happens when we're missing views?

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

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

(more later...)

What information do we need for 3D reconstruction?

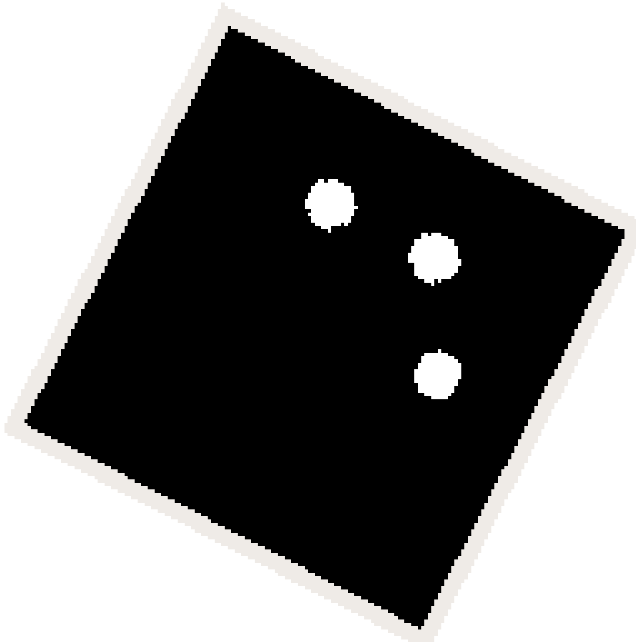
1. different orientations
2. known orientations
3. many particles

I have all of this information.
Now what?

There are two general categories of 3D reconstruction

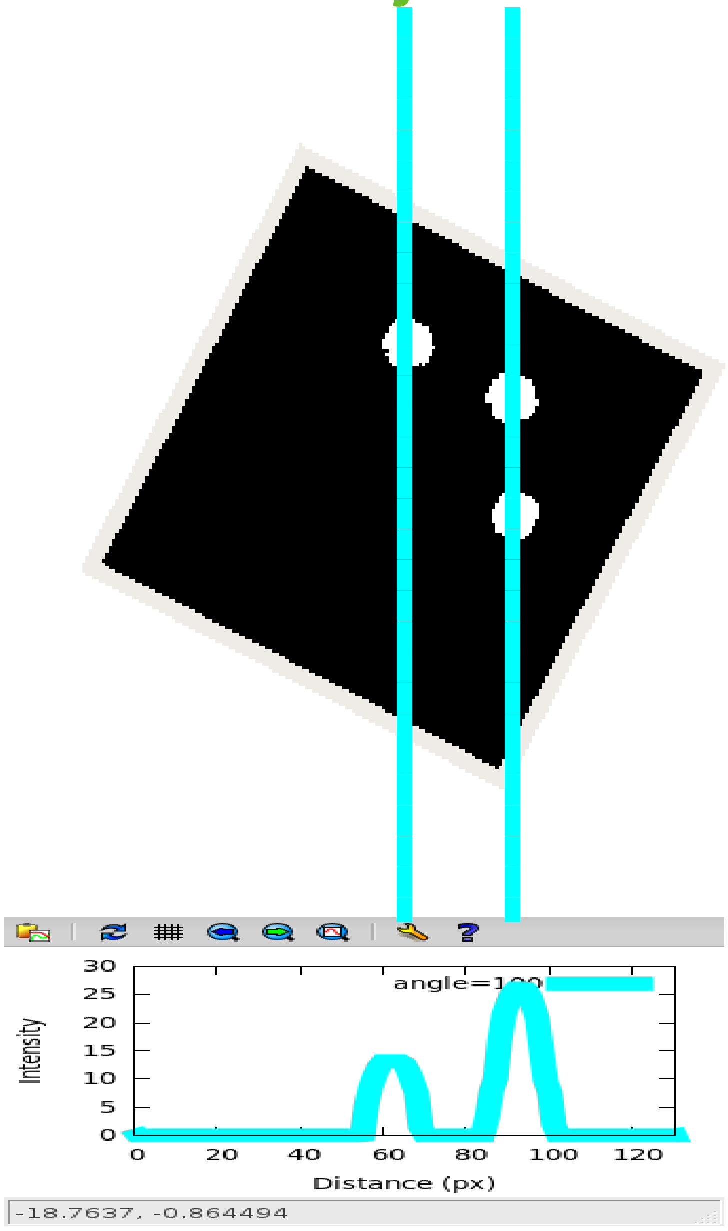
1. Real space
2. Fourier space

Reconstruction in real space

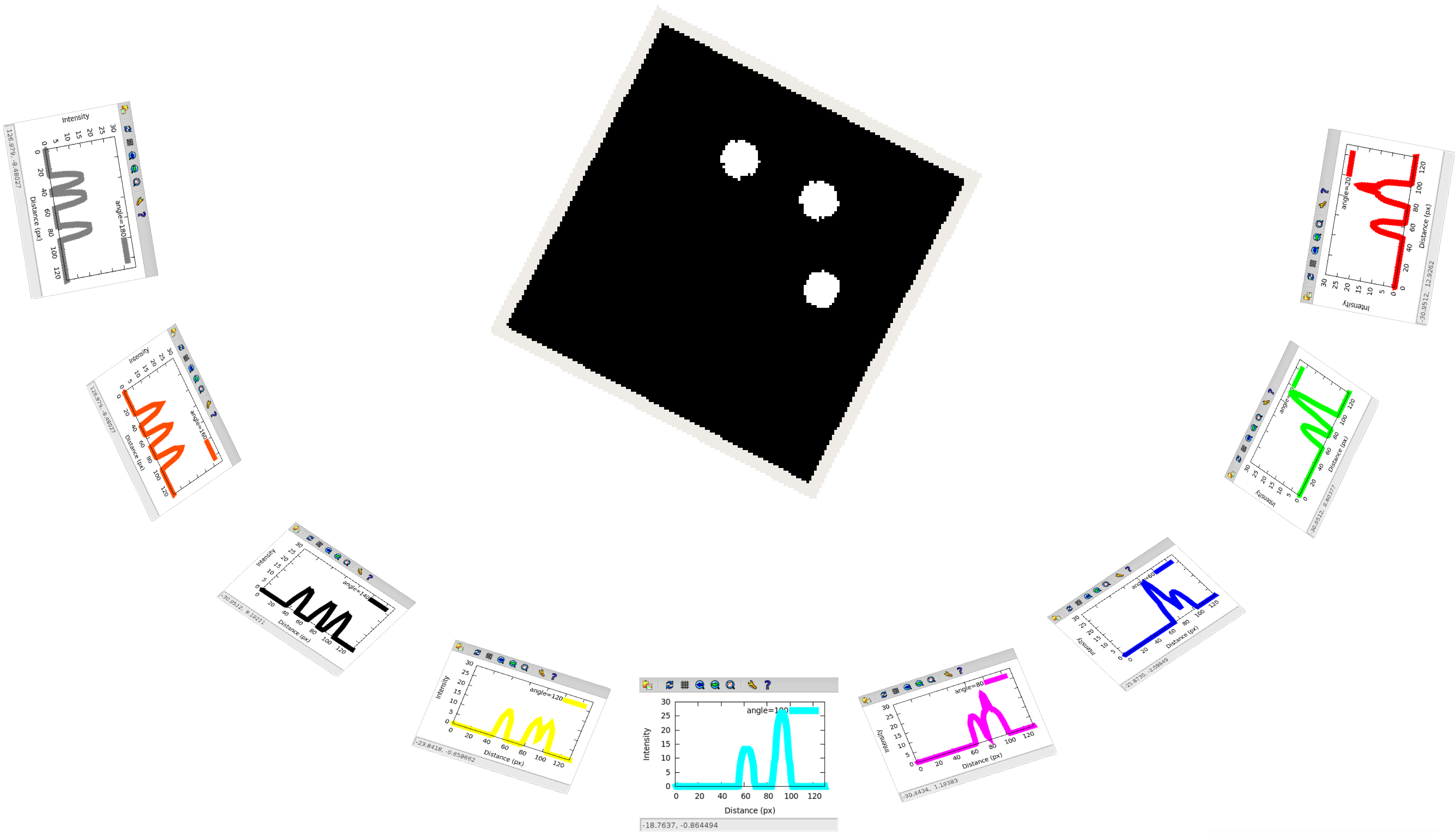


We are going to reconstruct a 2D object from 1D projections.
The principle is the similar to, but simpler than, reconstructing a 3D object from 2D projections.

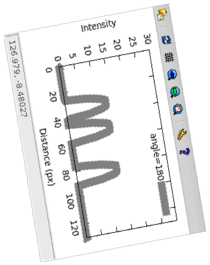
Projection of our 2D object

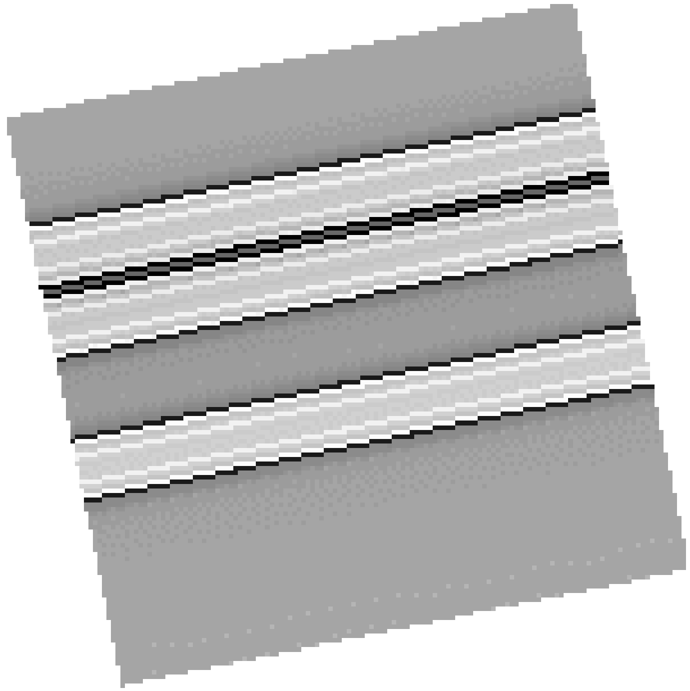


Now, project in several directions



Reconstruction is the inversion of projection





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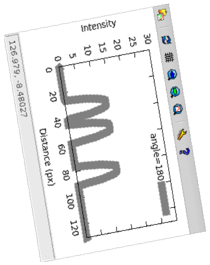

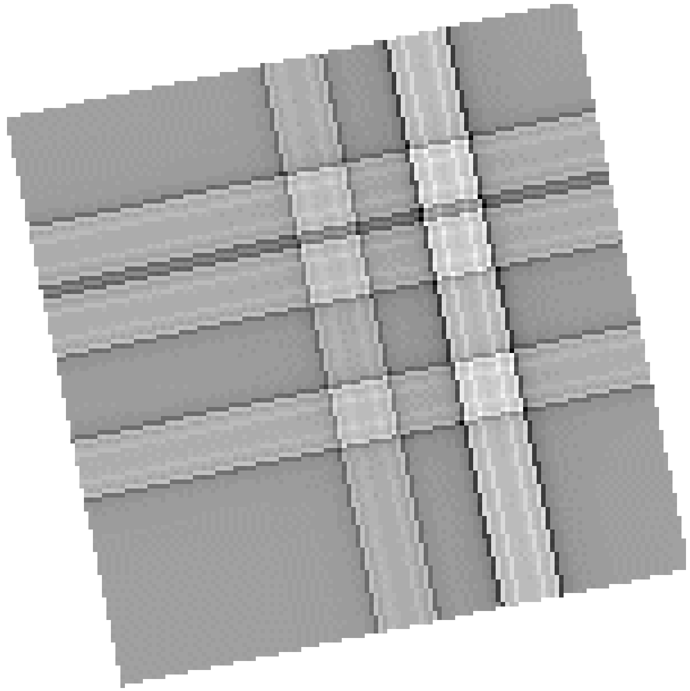

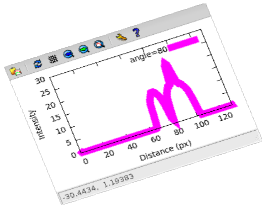

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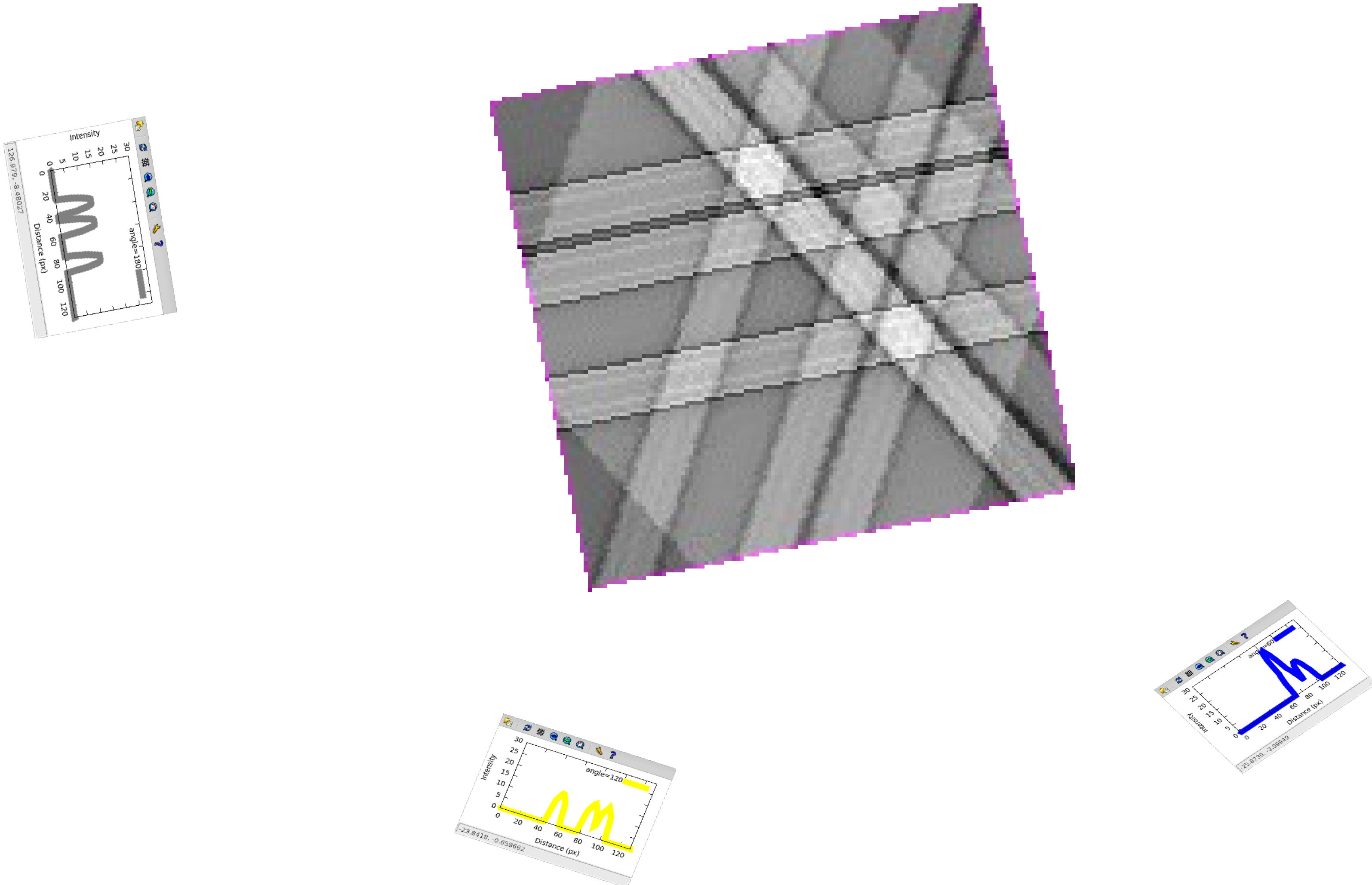

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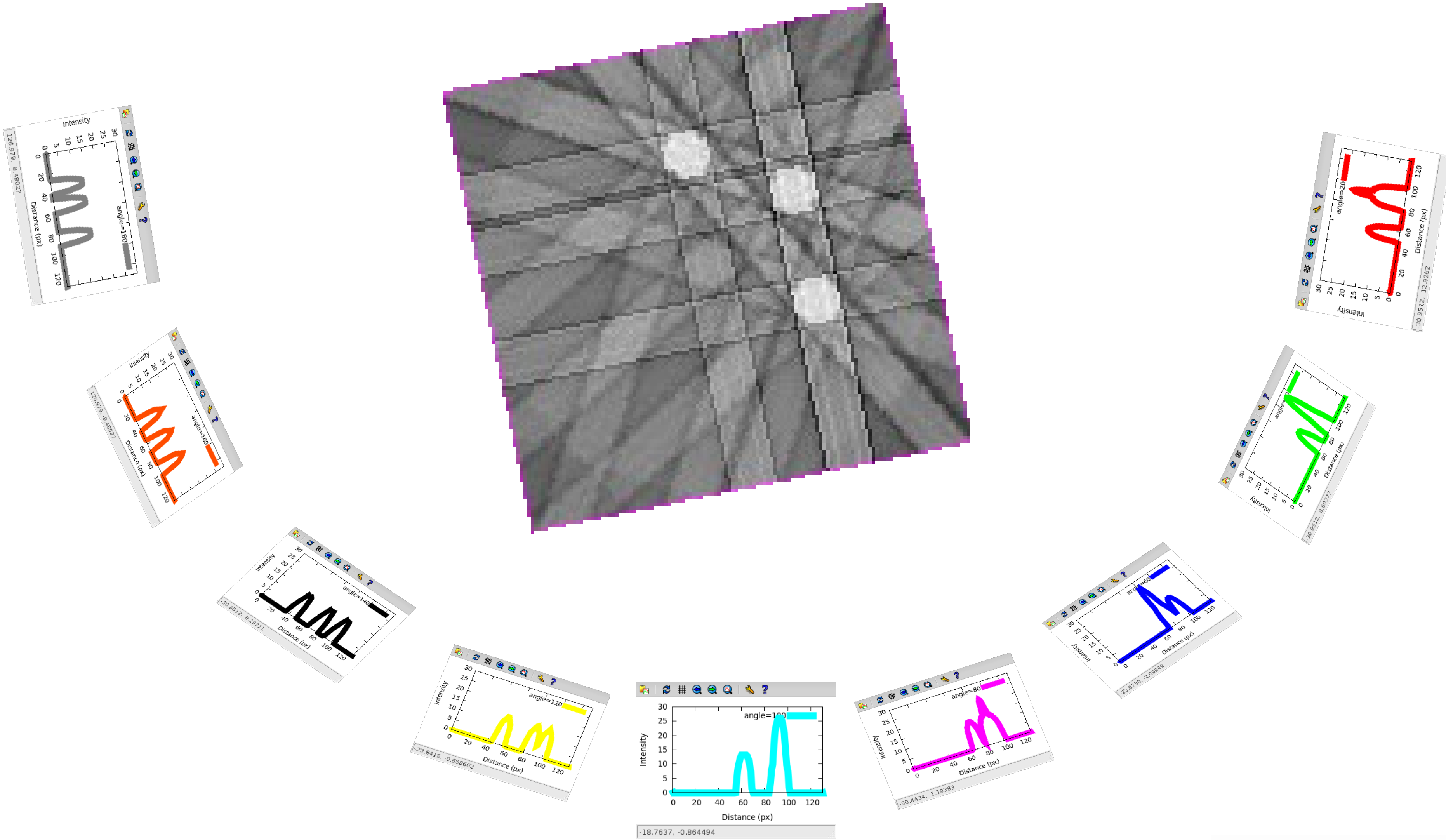

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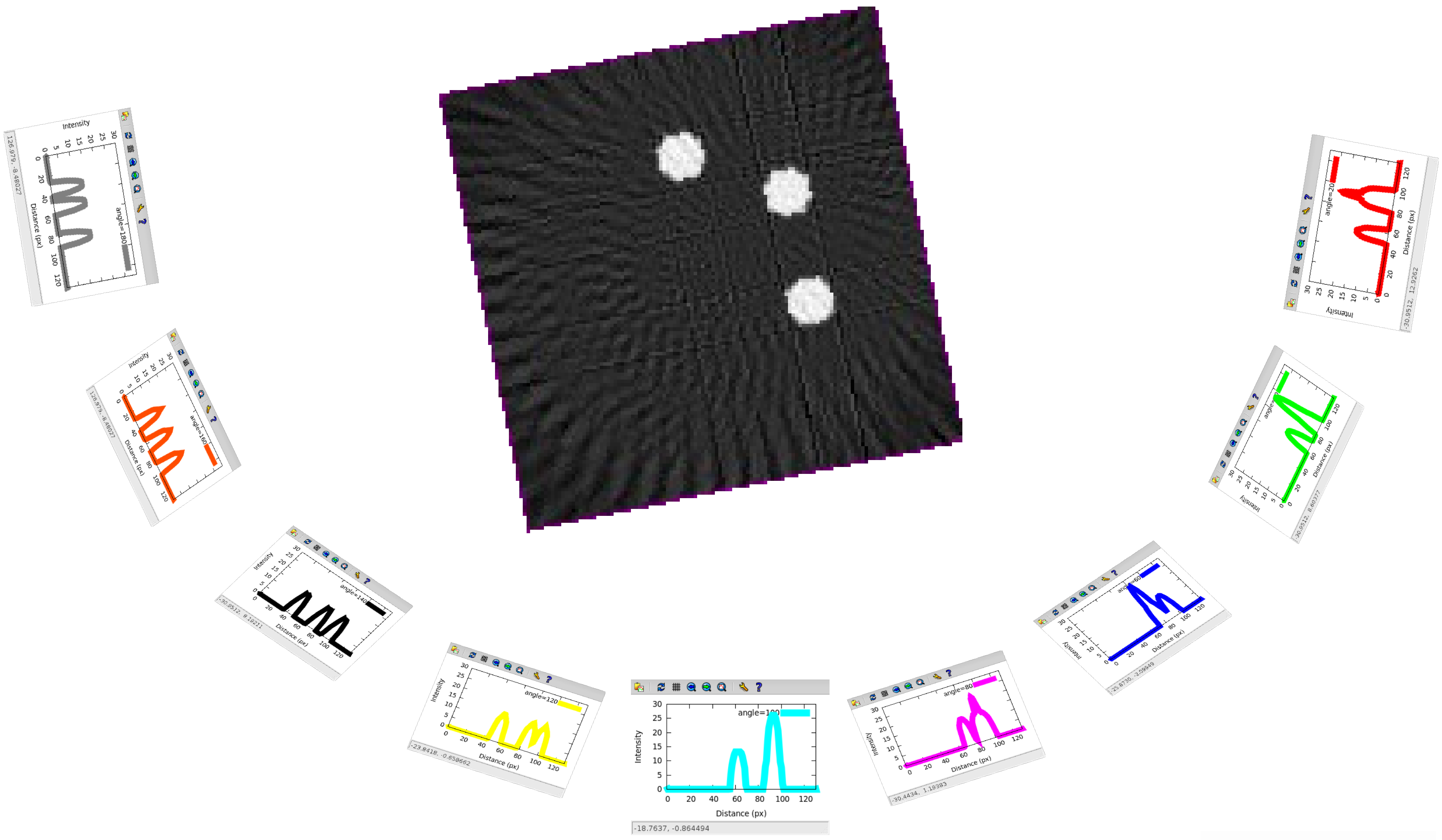


The reconstruction doesn't agree well with the projections. What can we do?

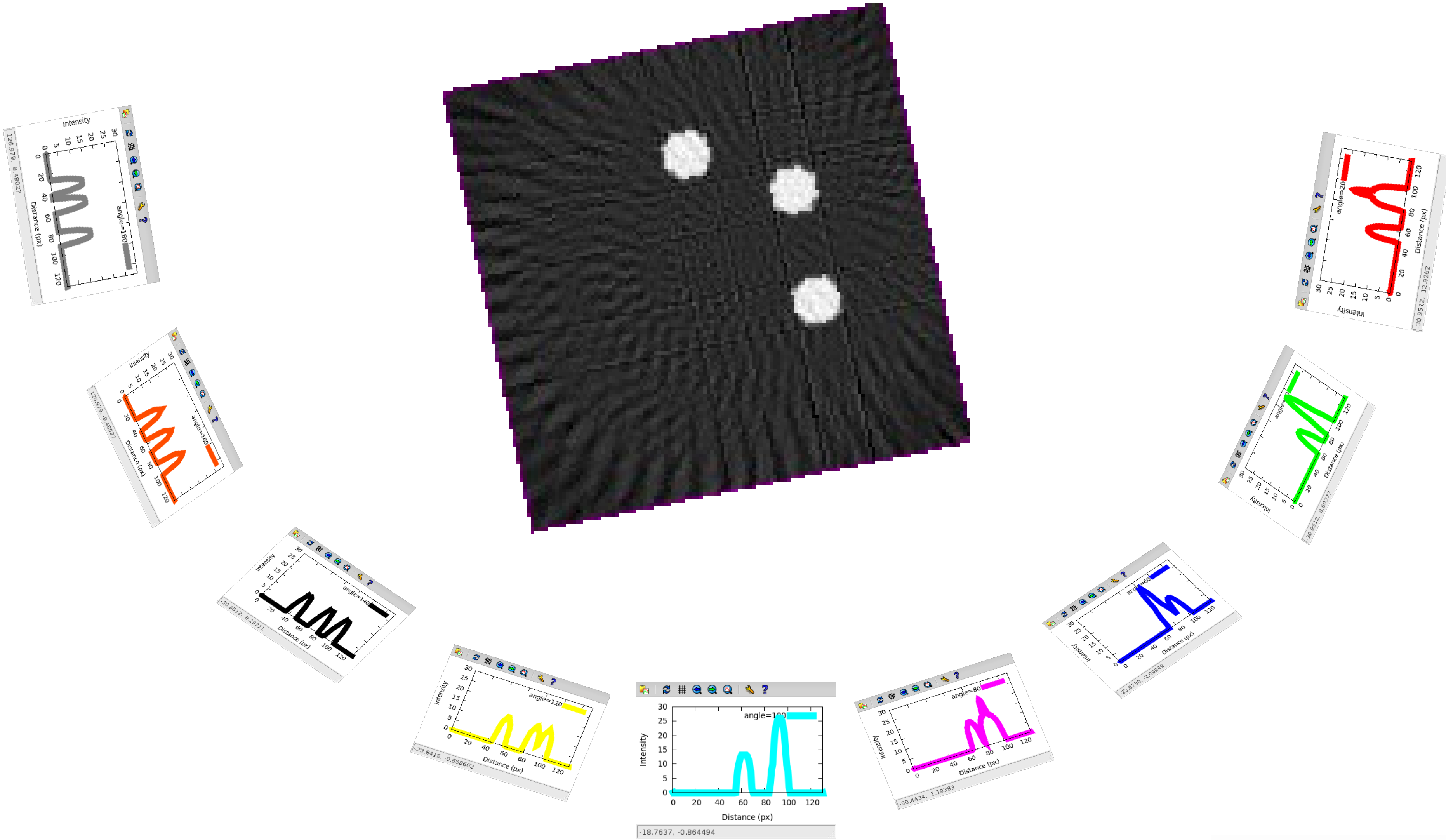
(one) ANSWER:
Simultaneous Iterative Reconstruction Technique

Simultaneous Iterative Reconstruction Technique

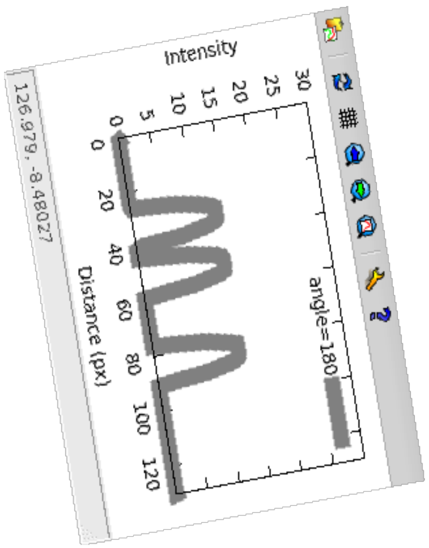
The idea:

- You compute re-projections of your model.
- Compare the re-projections to your experimental data.
    - There will be differences.
- You weight the differences by a fudge factor, λ.
- You adjust the model by the difference weighted by λ.
- Repeat.

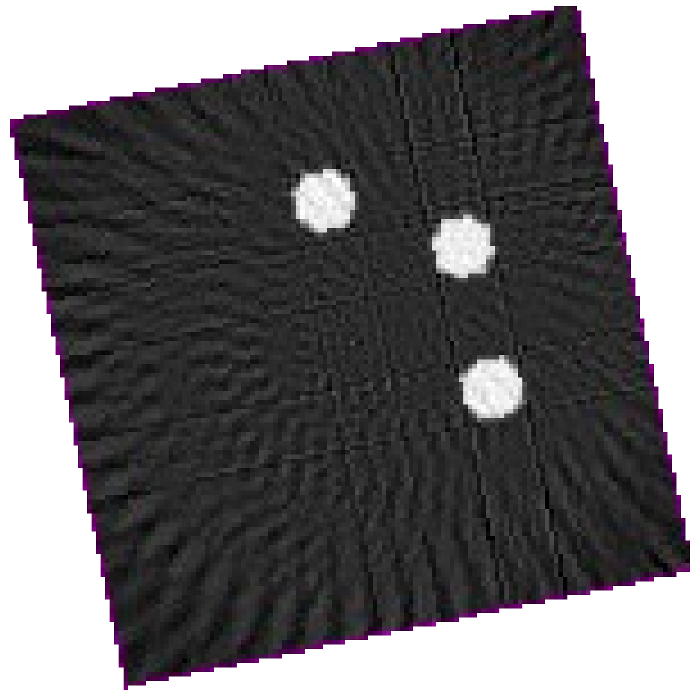
Simultaneous Iterative Reconstruction Technique



Simultaneous Iterative Reconstruction Technique



Experimental projection



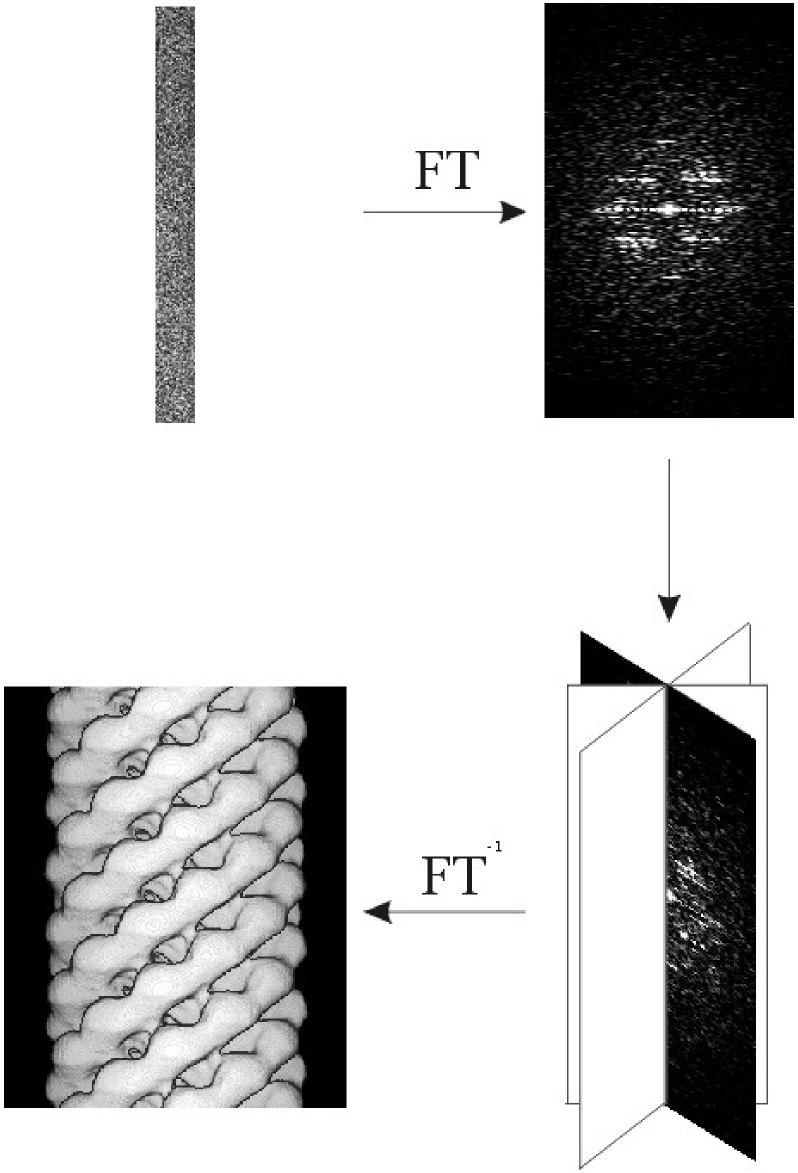
Model

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

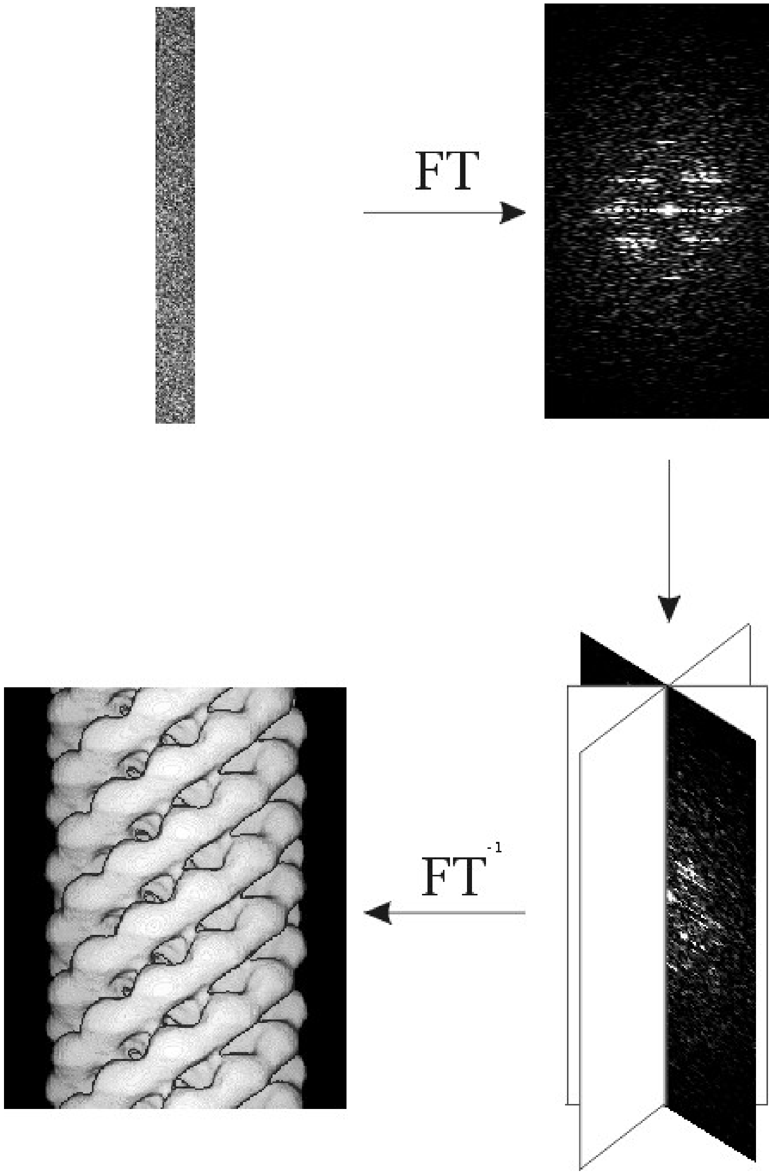
If we didn't down-weight by λ, we would overcompensate, and would amplify noise.

Reconstruction in Fourier space

Projection theorem
(or Central Section Theorem)



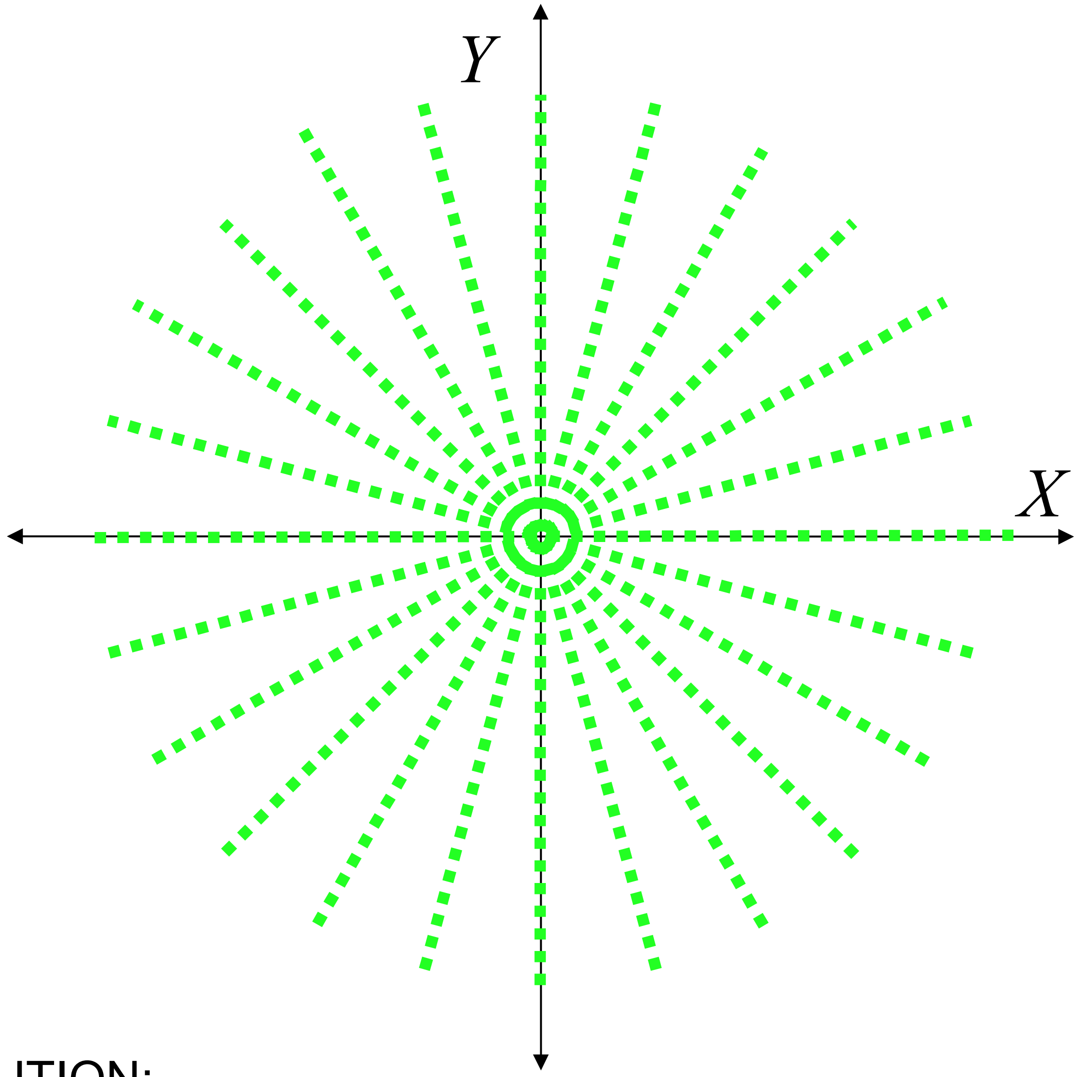
A central section through the 3D Fourier transform is the Fourier transform of the projection in that direction.



Projection theorem
(or 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.

Converting from polar to Cartesian coordinates



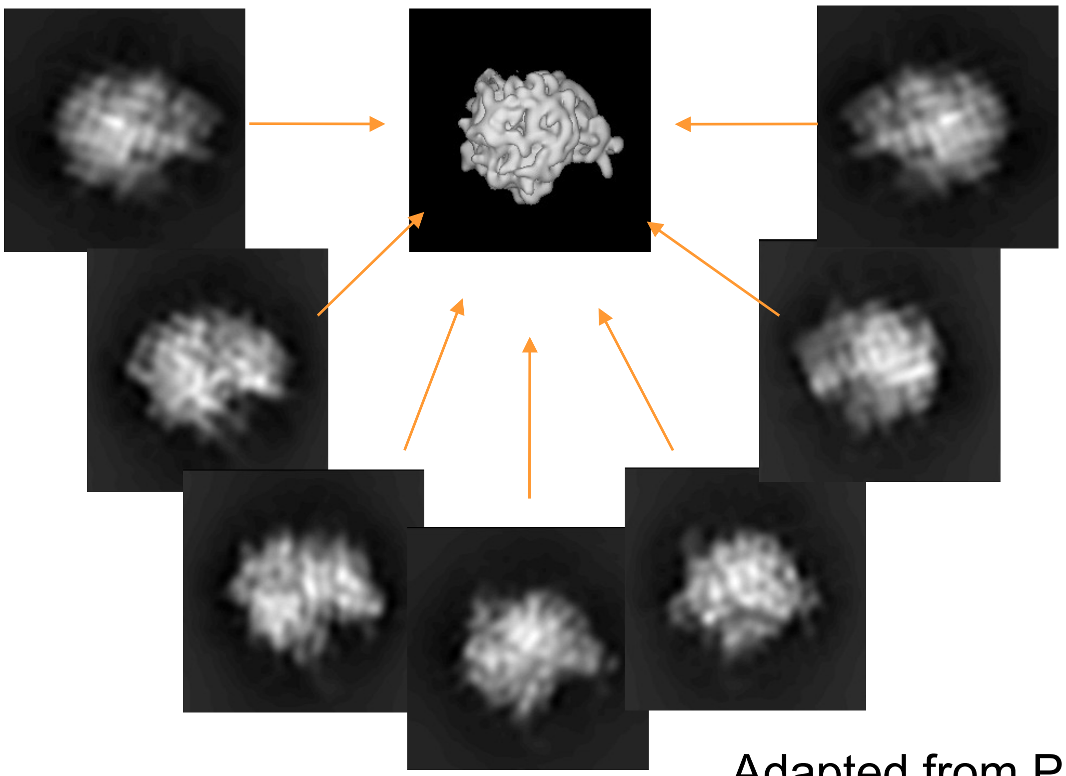
SOLUTION:

A simple weighting scheme is to divide the weight by the radius:

$r*$ weighting, or “r-weighted backprojection”

Going from 2D to 3D

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



Adapted from Pawel Penczek

How do we determine the last two Euler angles?

Parameters required for 3D reconstruction

Two translational:

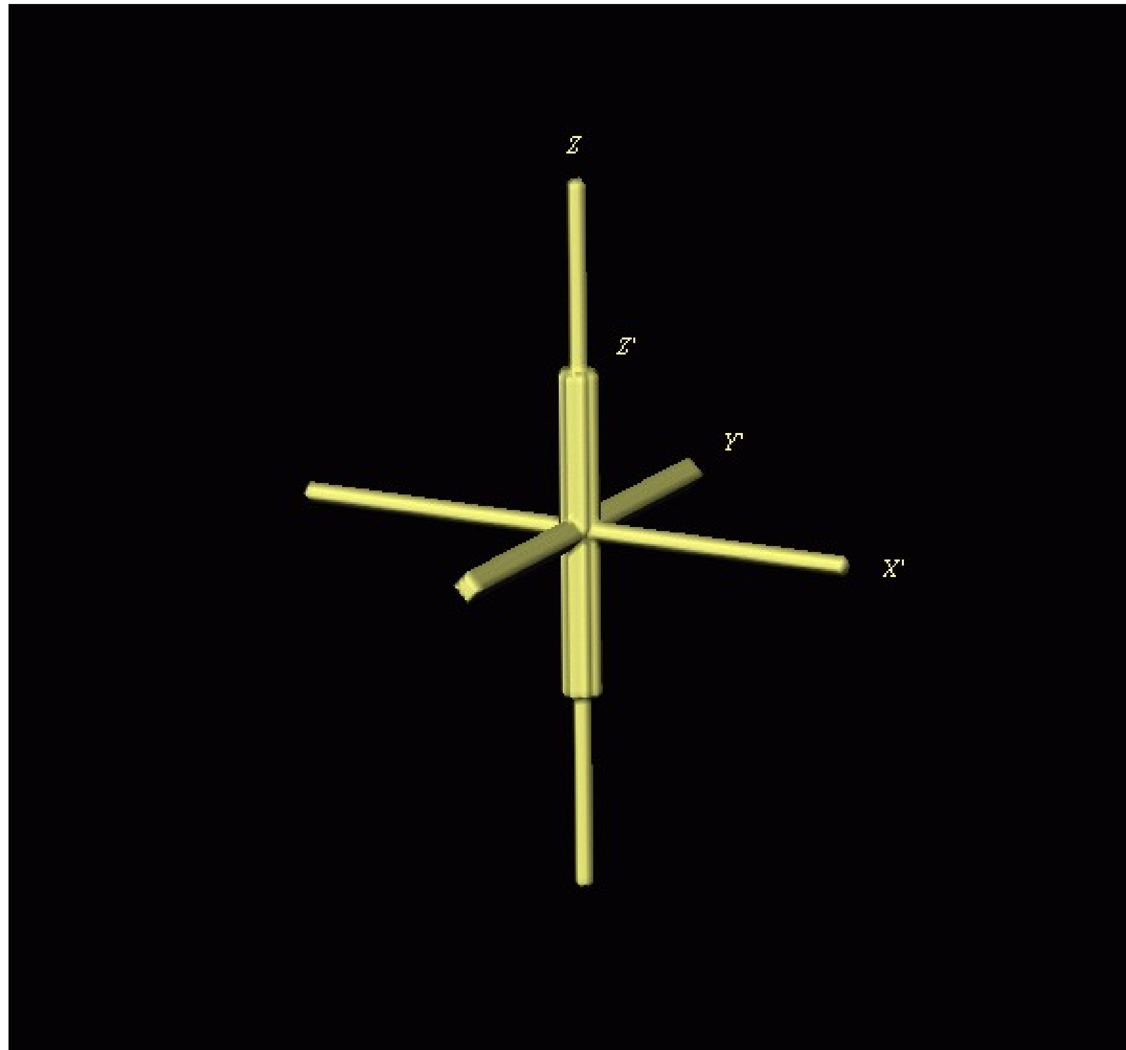
- ✓ *Δx*
- ✓ *Δy*

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

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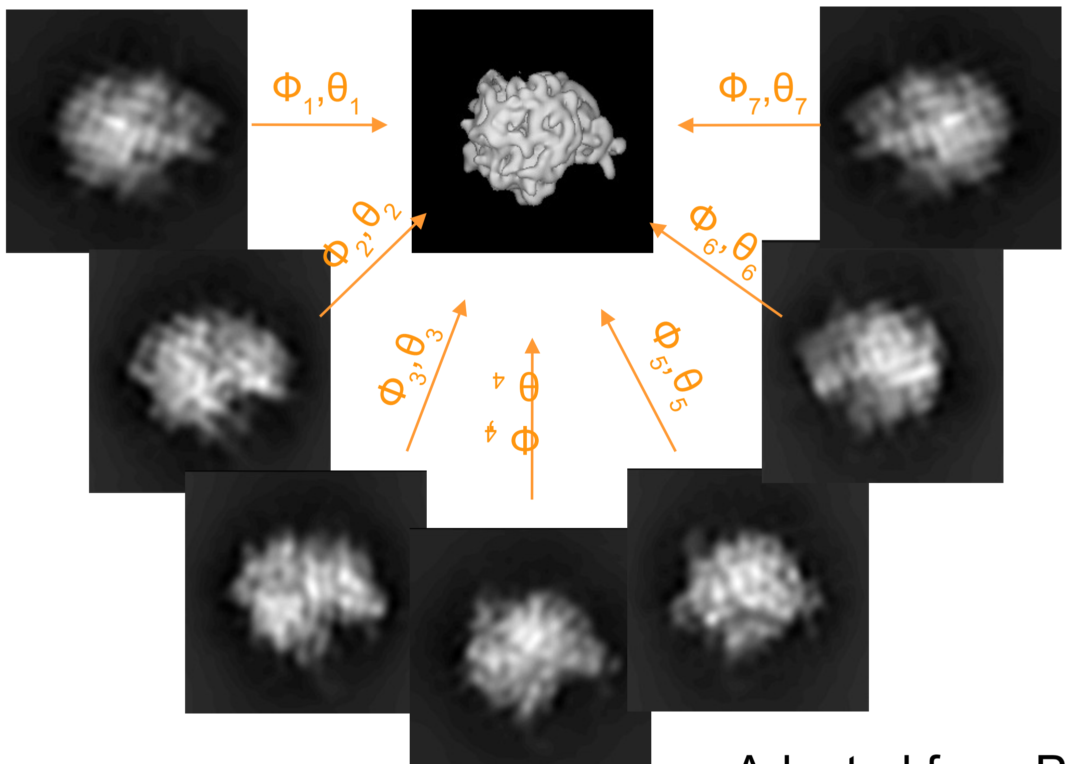

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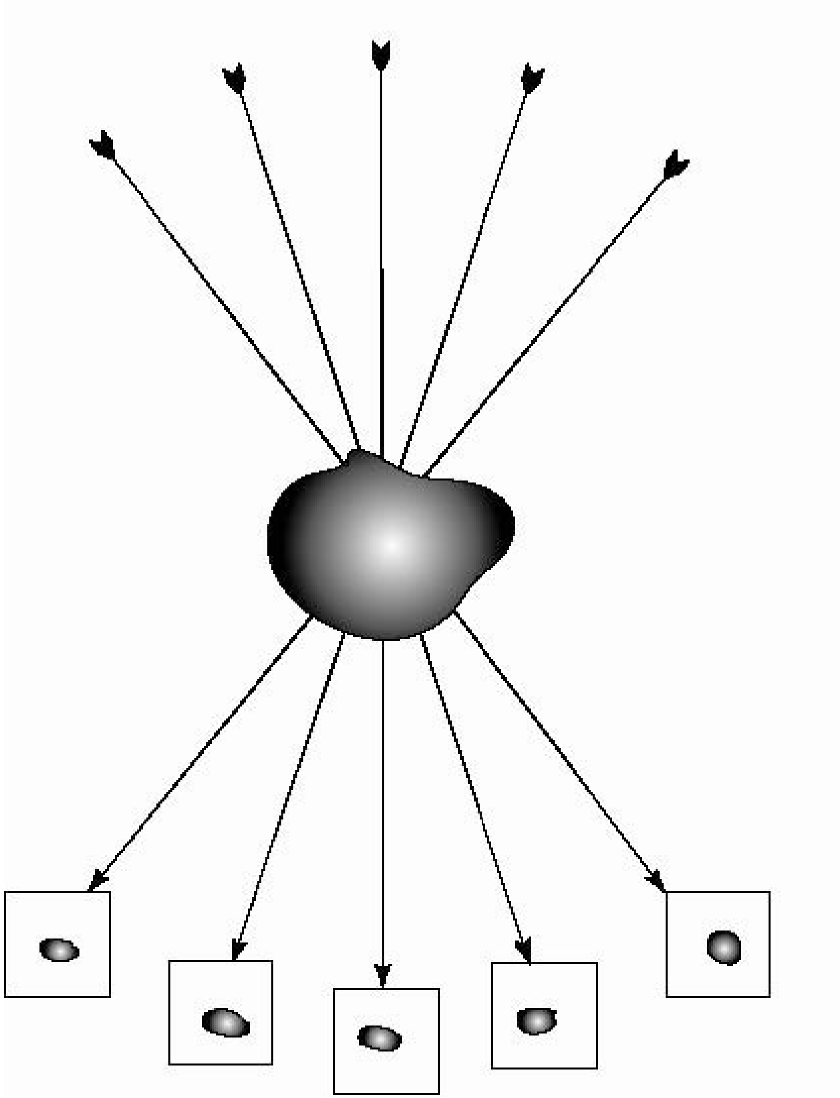
Tomography



From Ken Downing

We have:

- known orientations
- different views

BUT…

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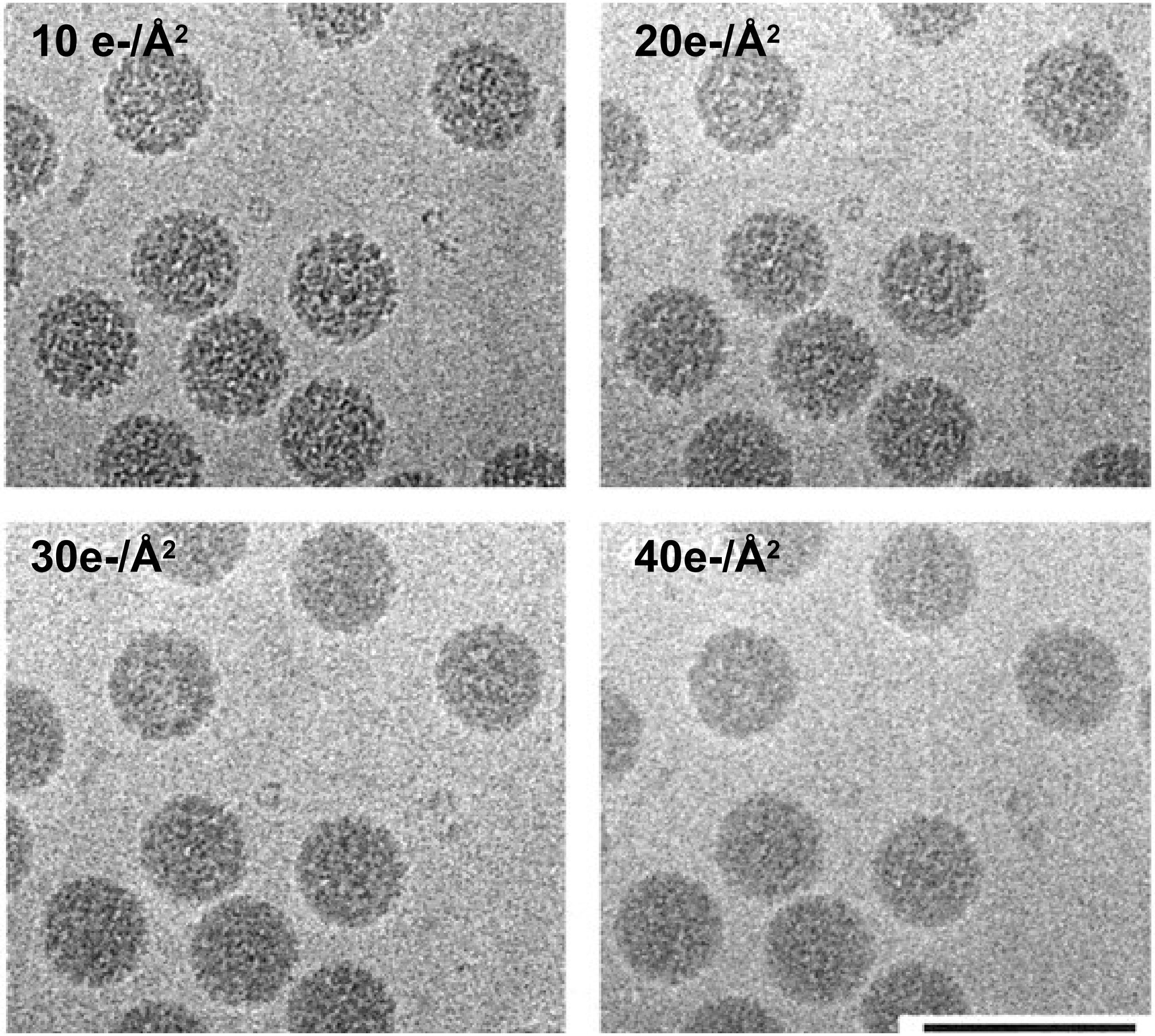
What happens when we image the sample?



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

We are destroying the sample as we image it.

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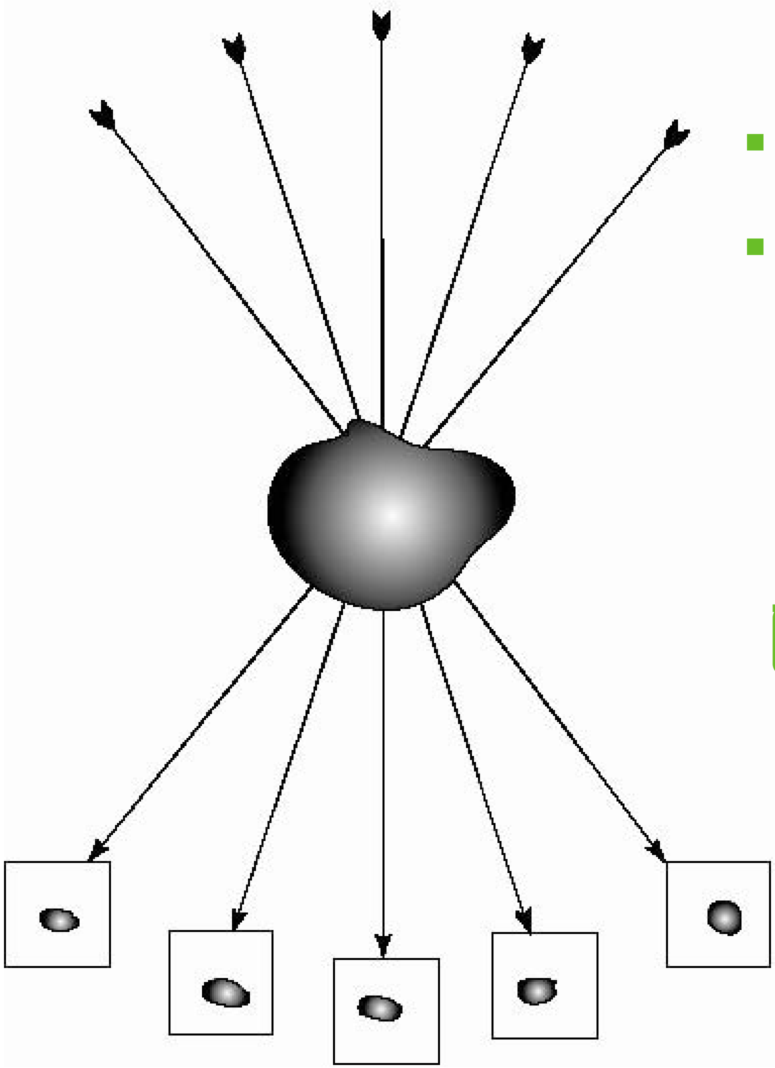
Consequences of repeated exposure



- Accumulated beam damage
- If number of views is limited, then distortions

Solution:

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

"Single-particle reconstruction"

From Ken Downing

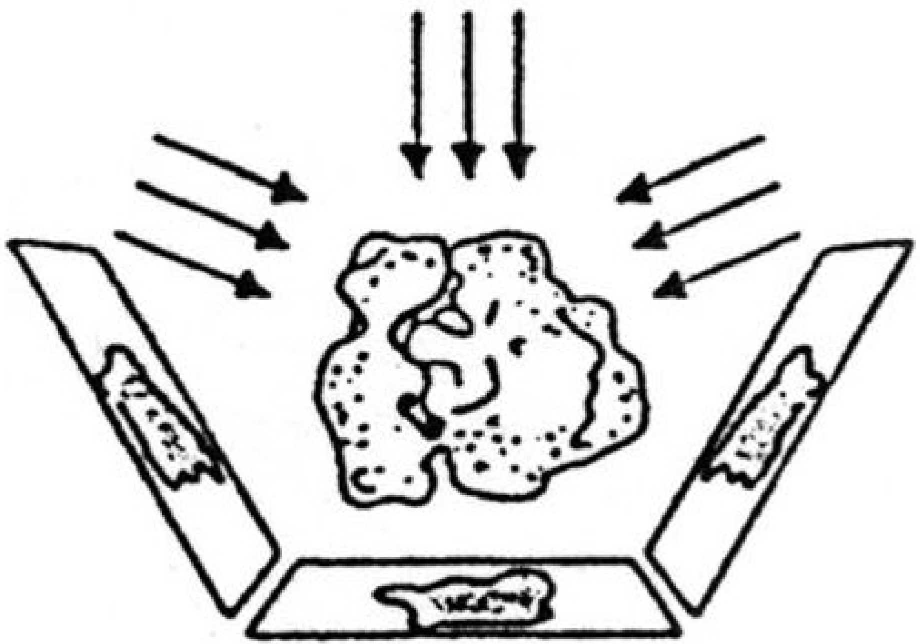
If we have many identical molecules,
and if we can determine the orientations,
we can use one exposure per molecule
and use these images in the reconstruction.

BUT:
Unlike in the tomographic case,
we don't know how the orientations
between the different 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.

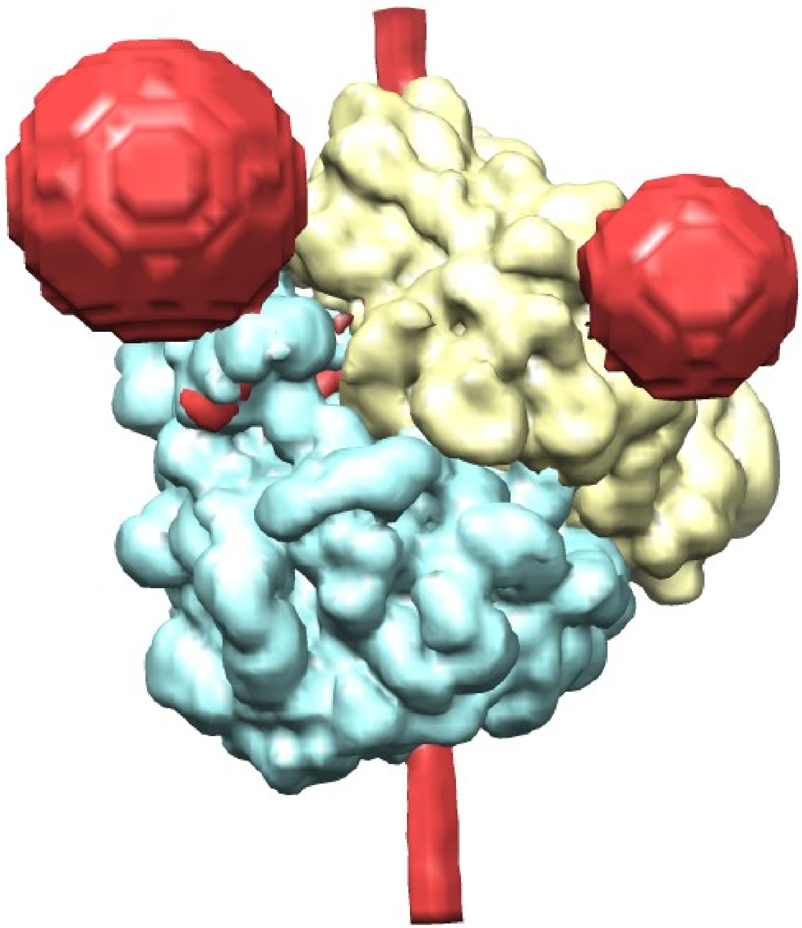
Step 1: Generation of projections of the reference.



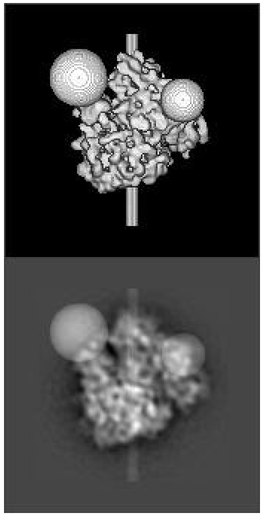
From Penczek *et al.* (1994), *Ultramicroscopy* **53**: 251-70.

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

The model



(The extra features helped determine handedness in noisy reconstructions.)



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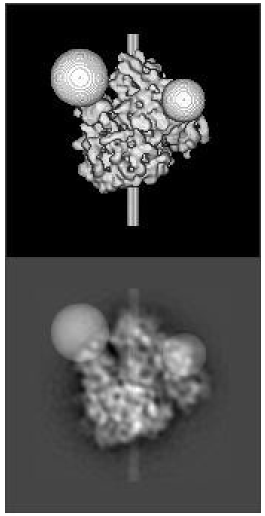

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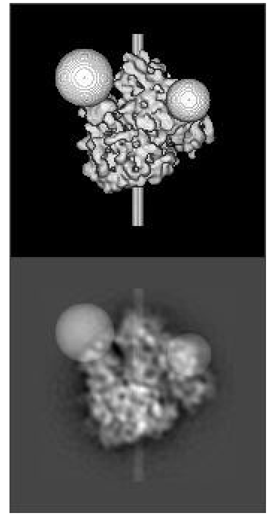

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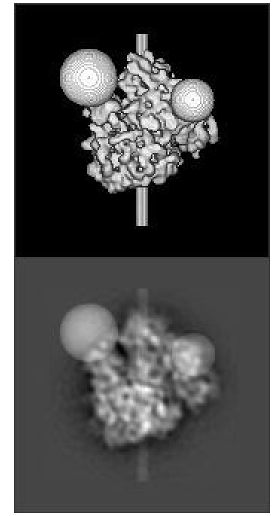

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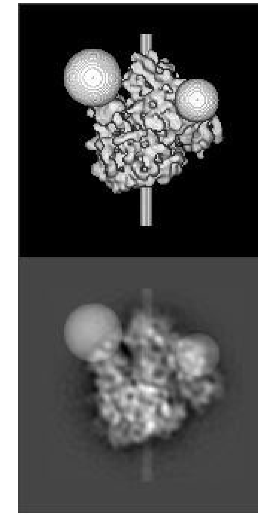

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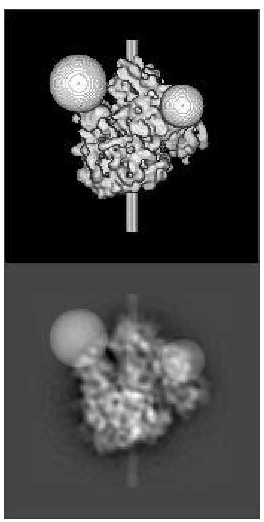

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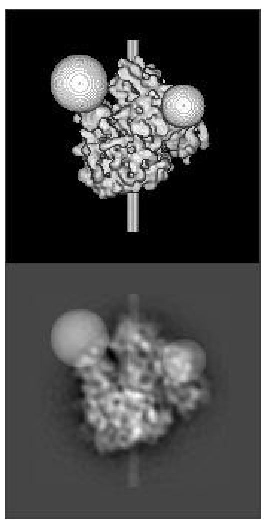

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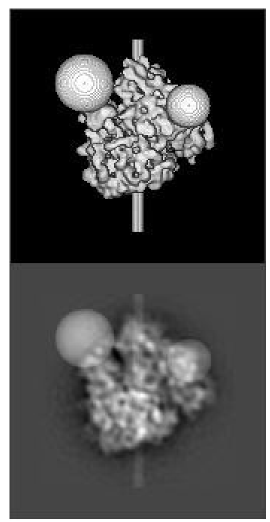

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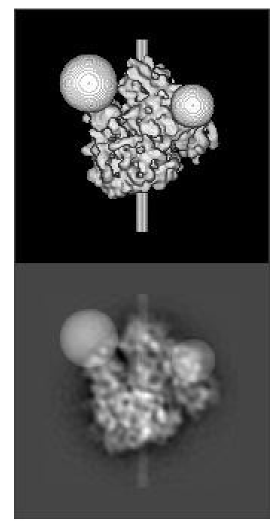

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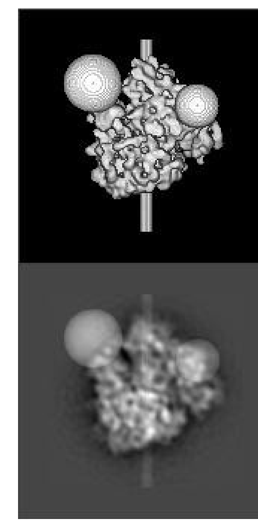

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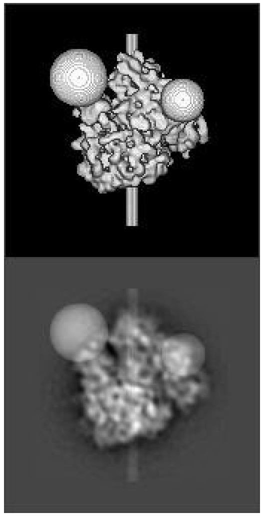

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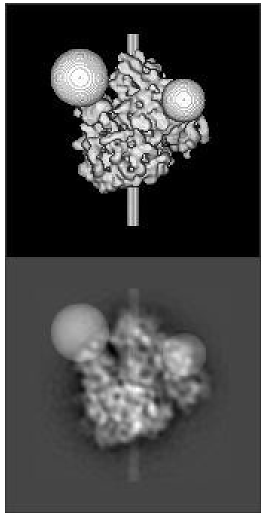

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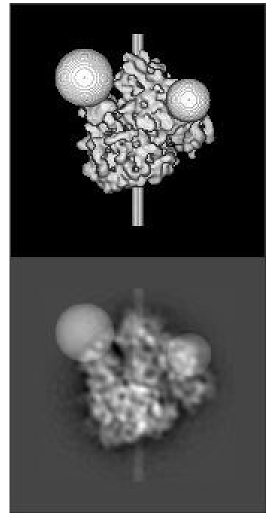

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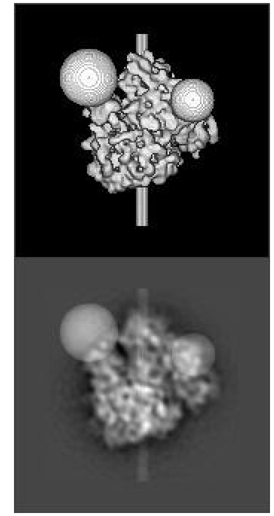

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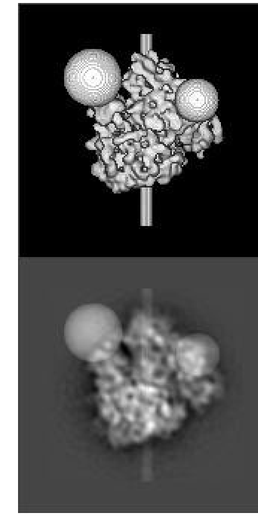

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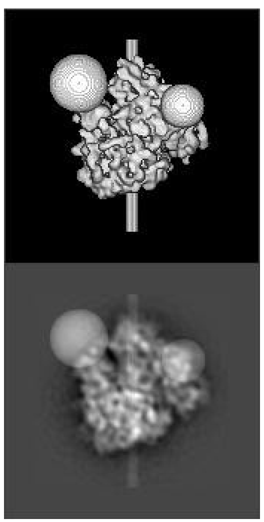

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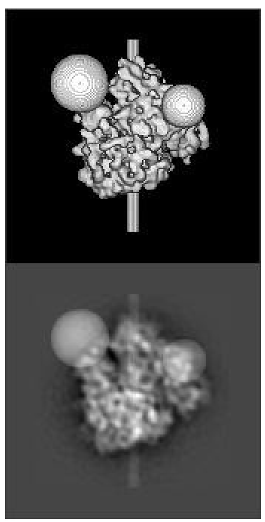

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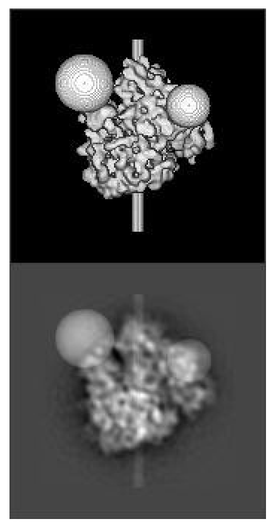

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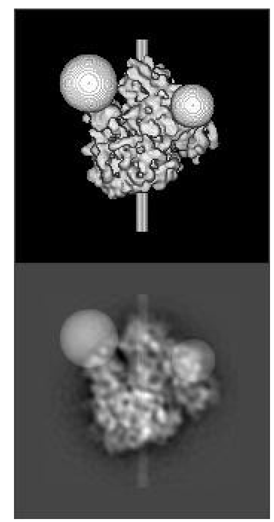

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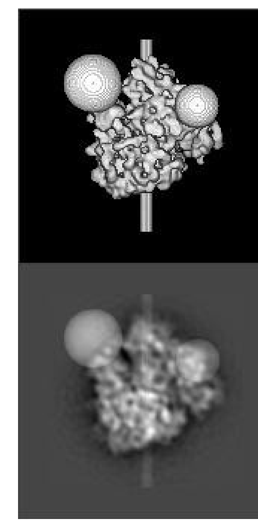

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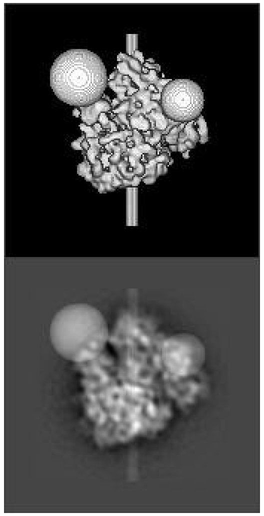

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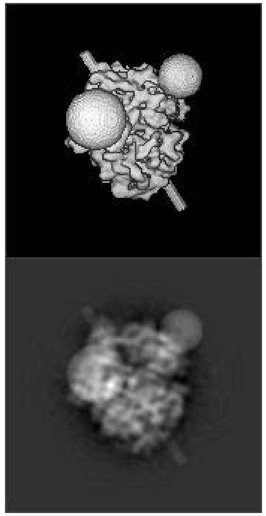

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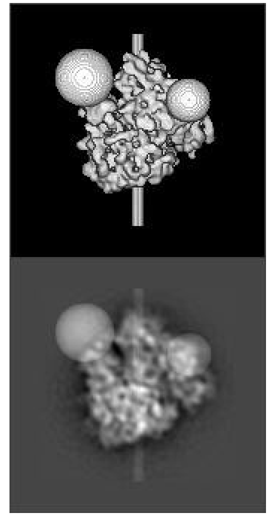

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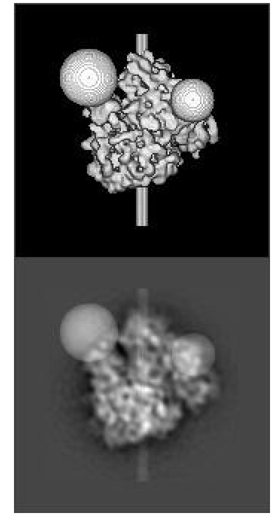

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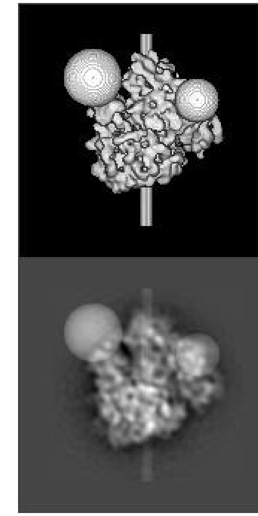

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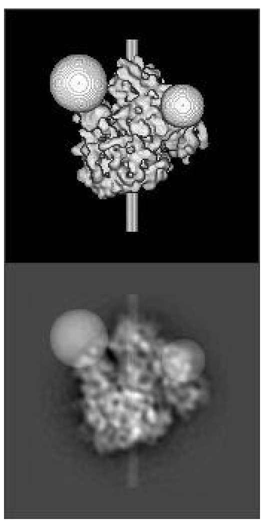

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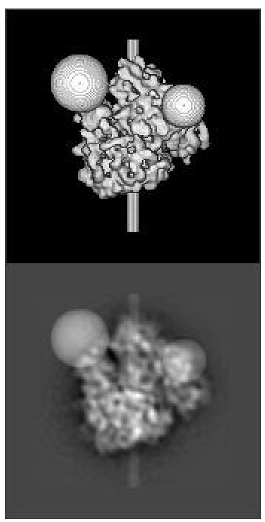

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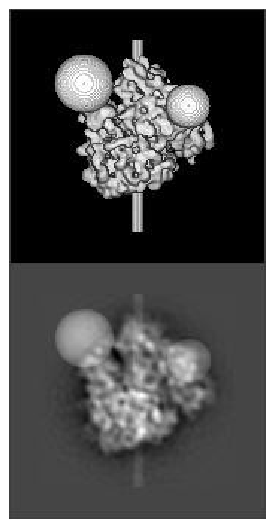

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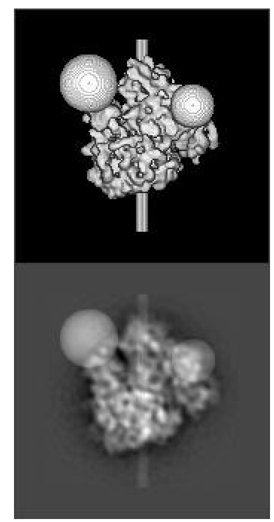

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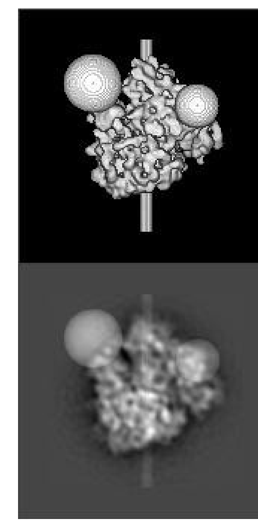

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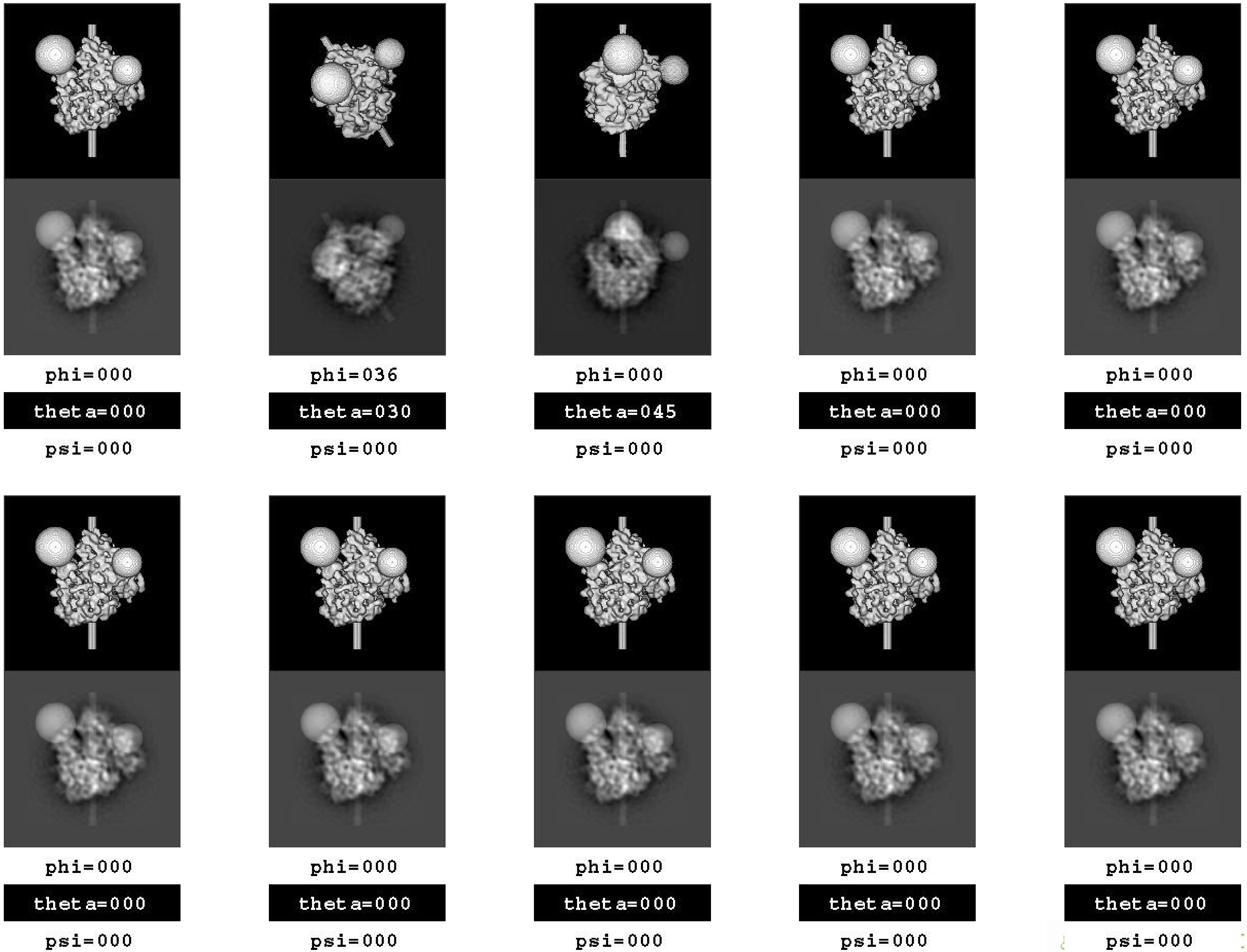
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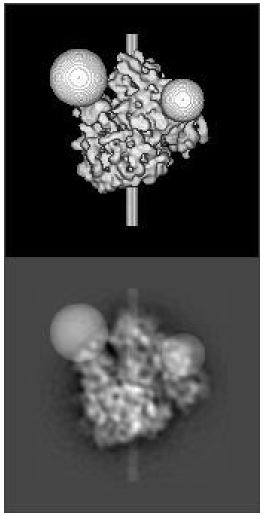

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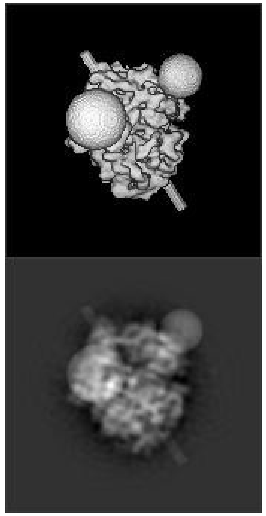

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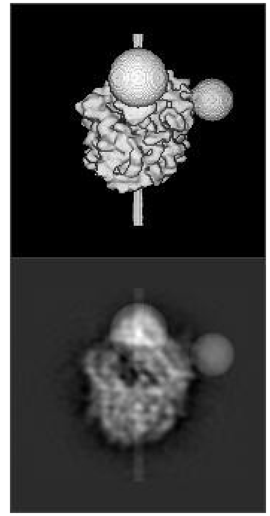

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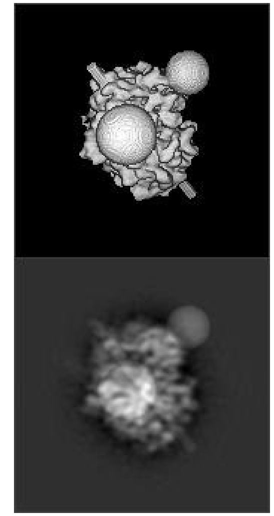

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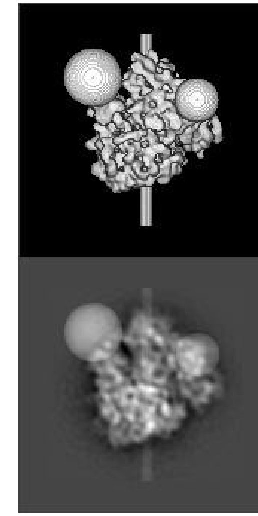

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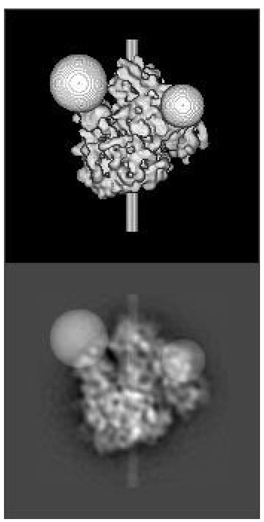

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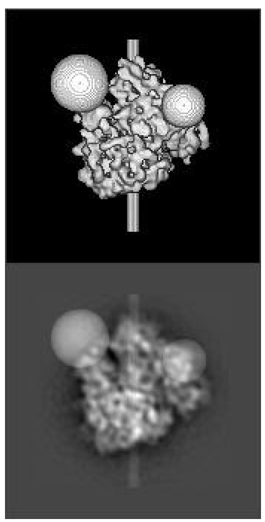

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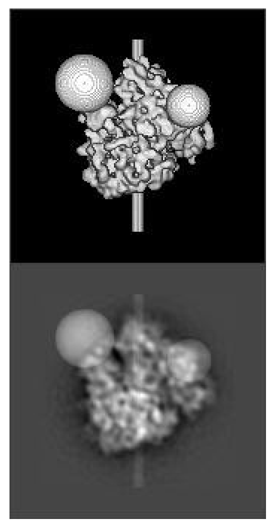

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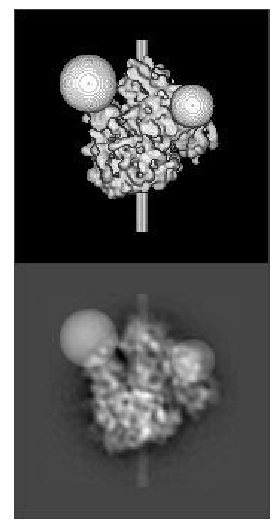

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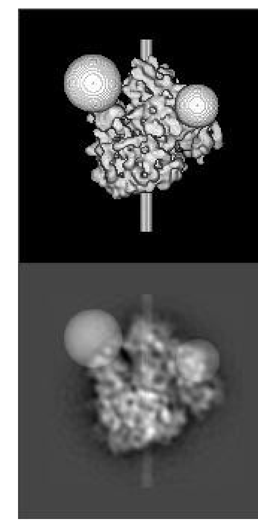

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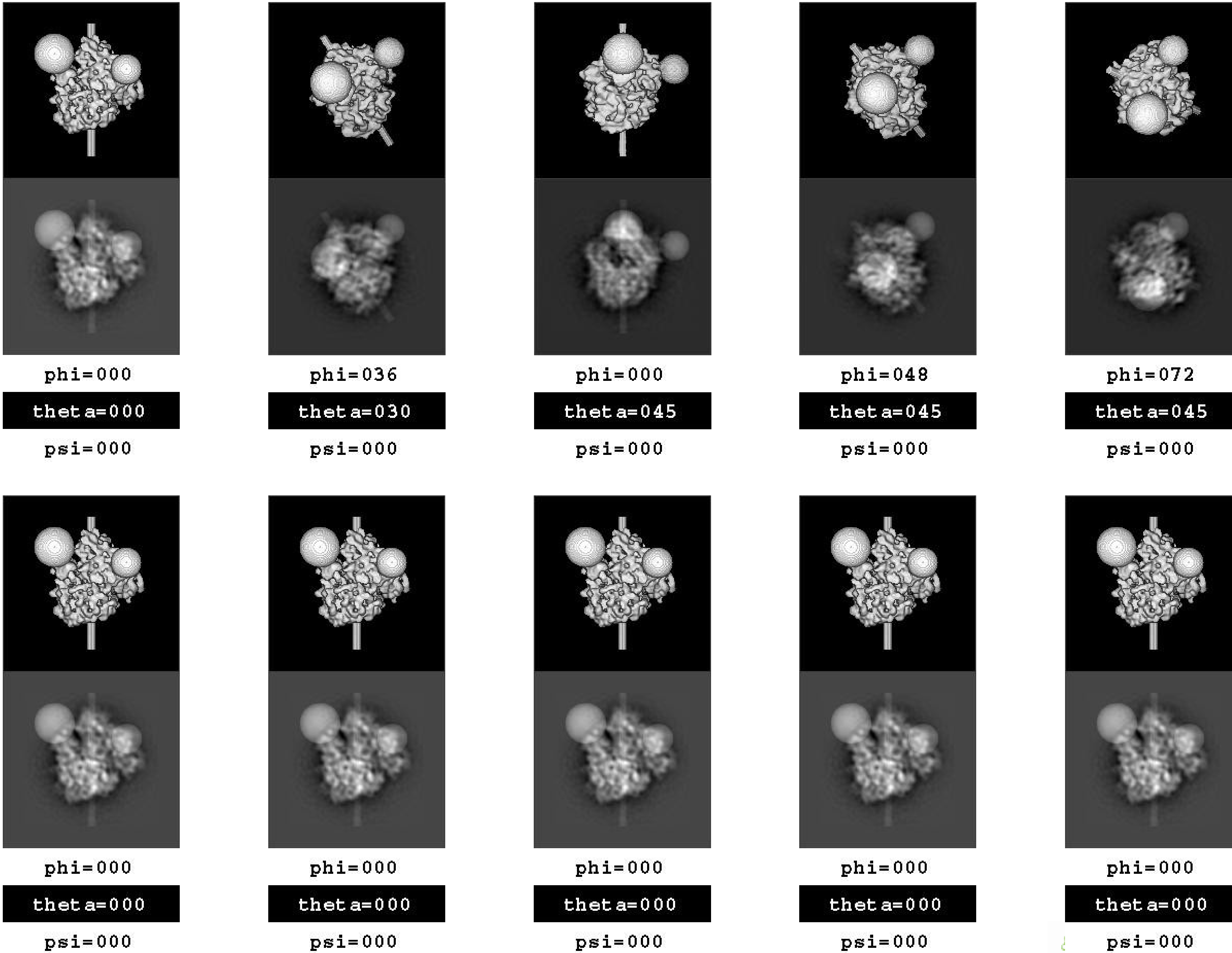
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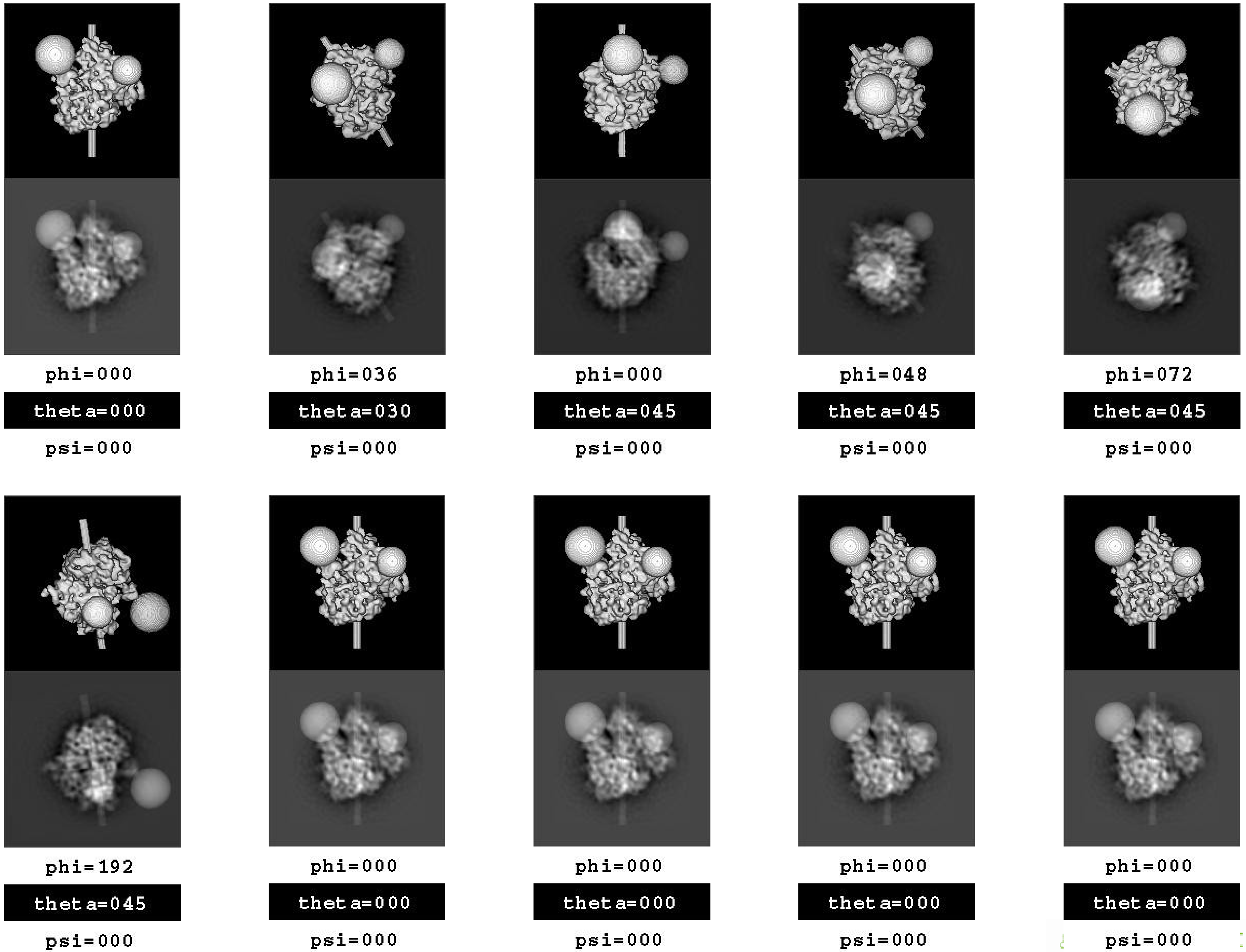
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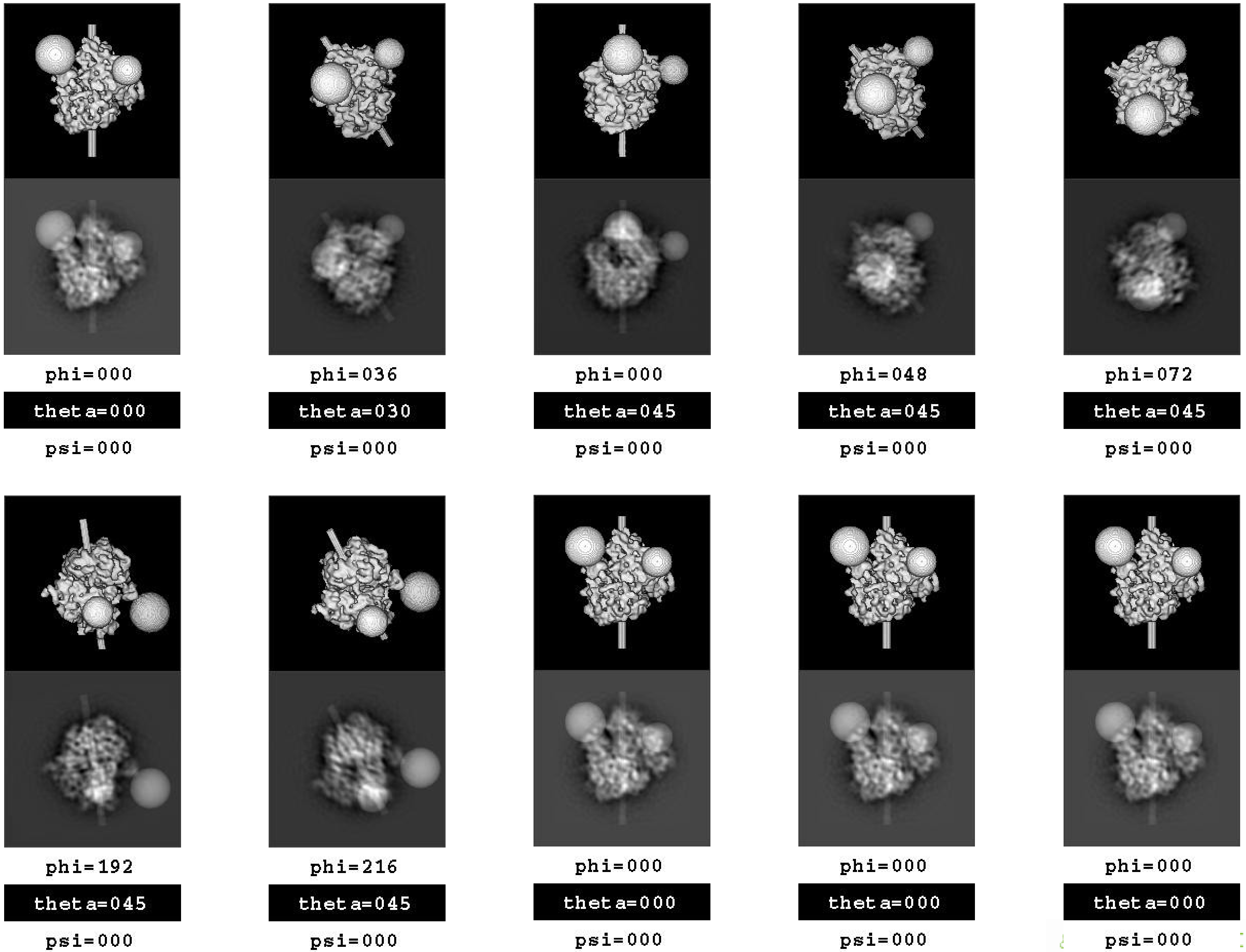

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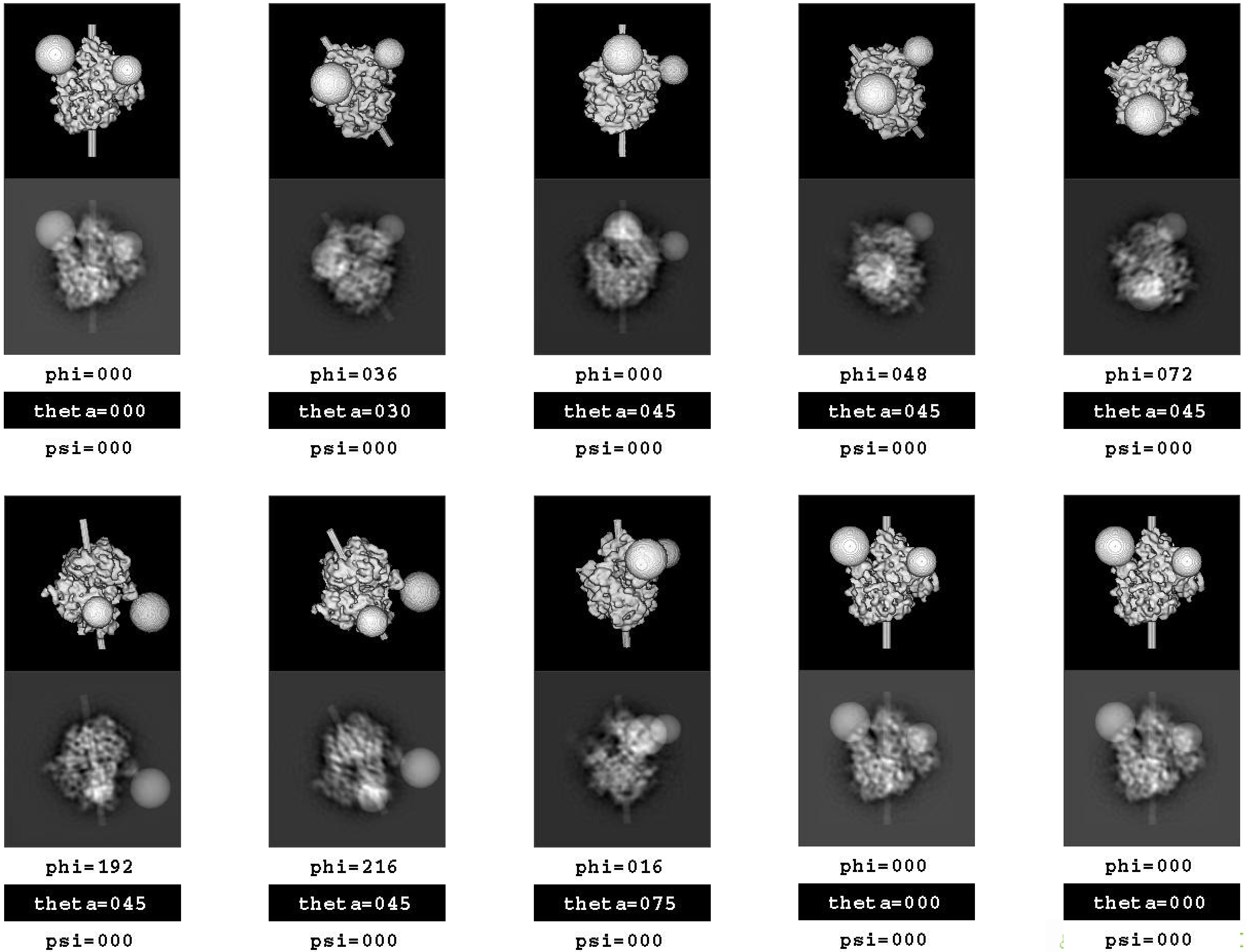
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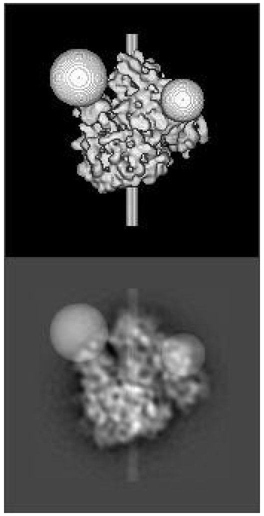

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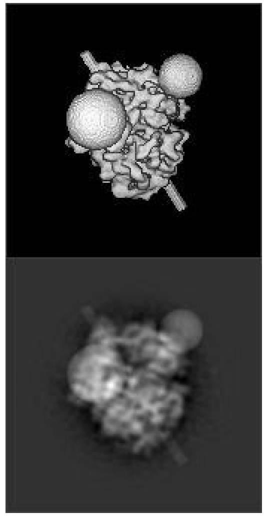

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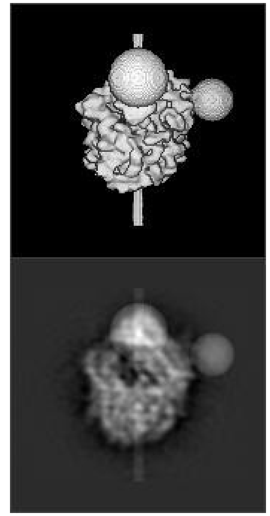

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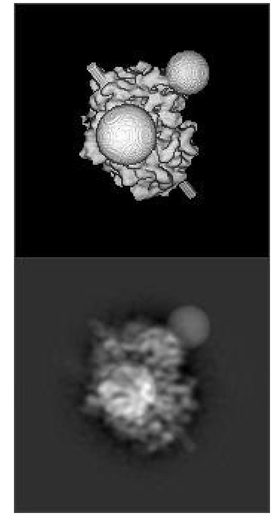

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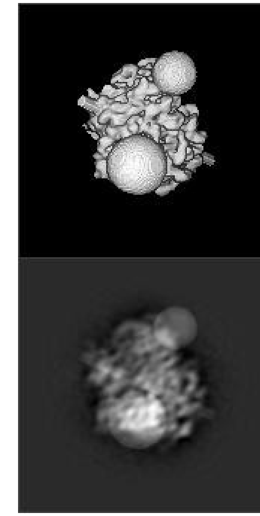

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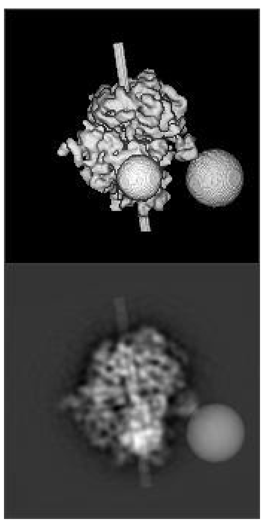

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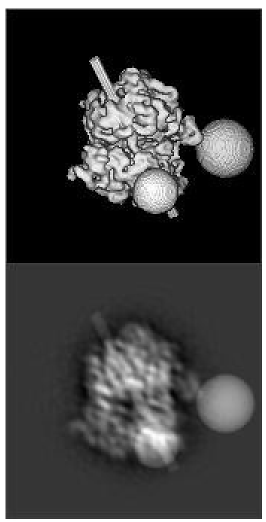

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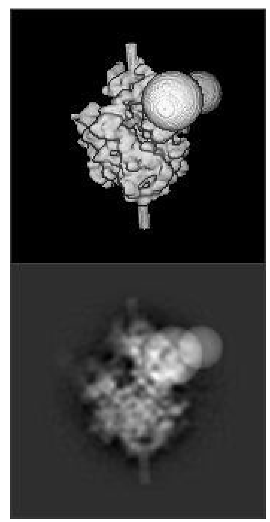

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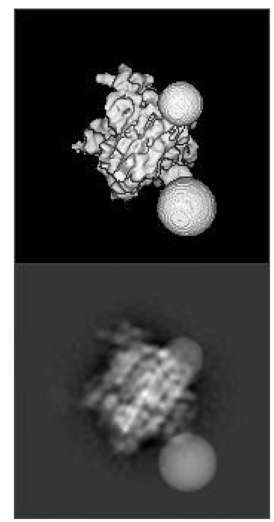
psi=000



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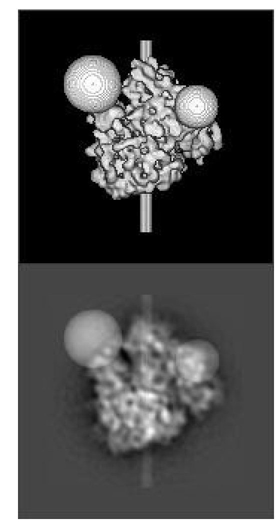

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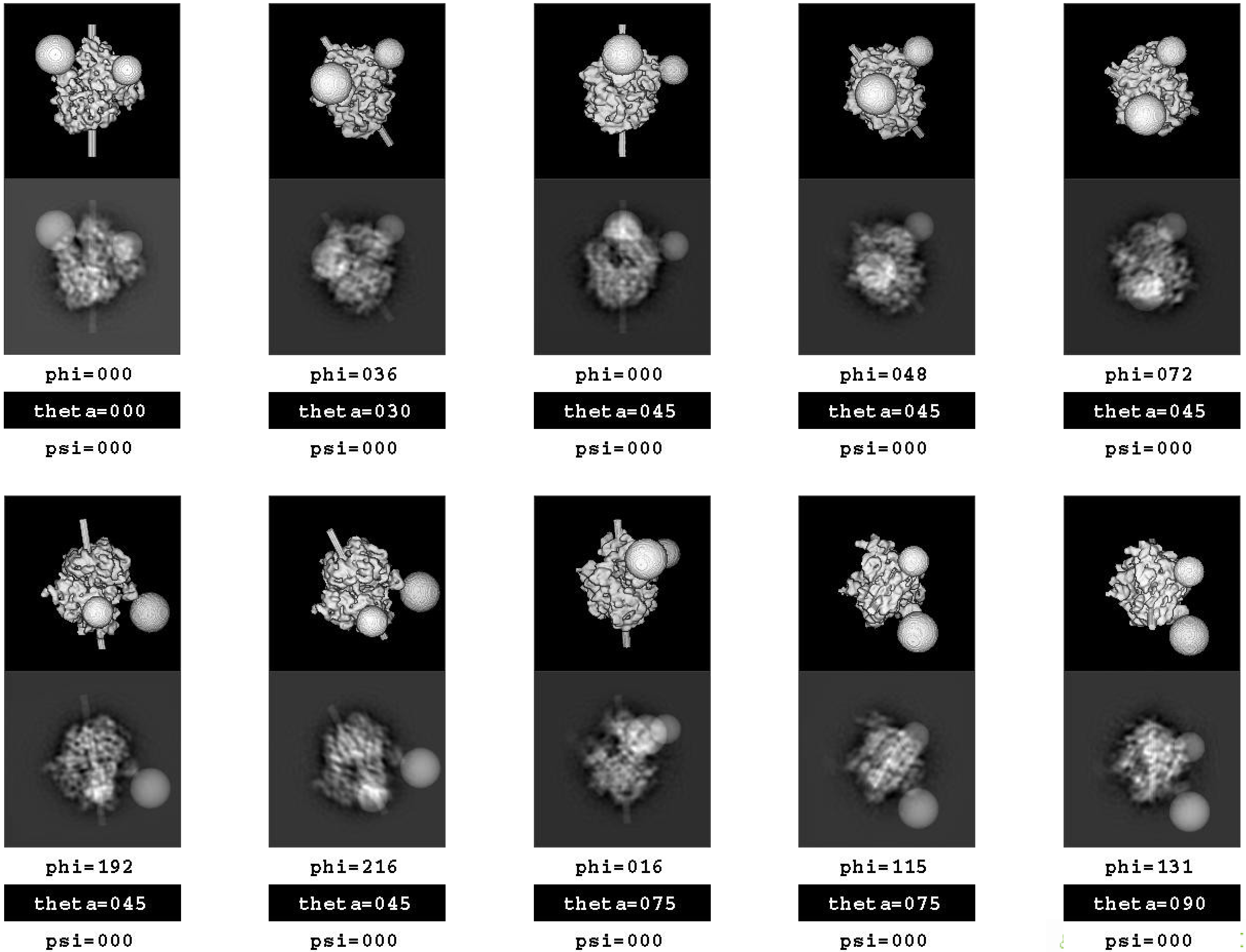
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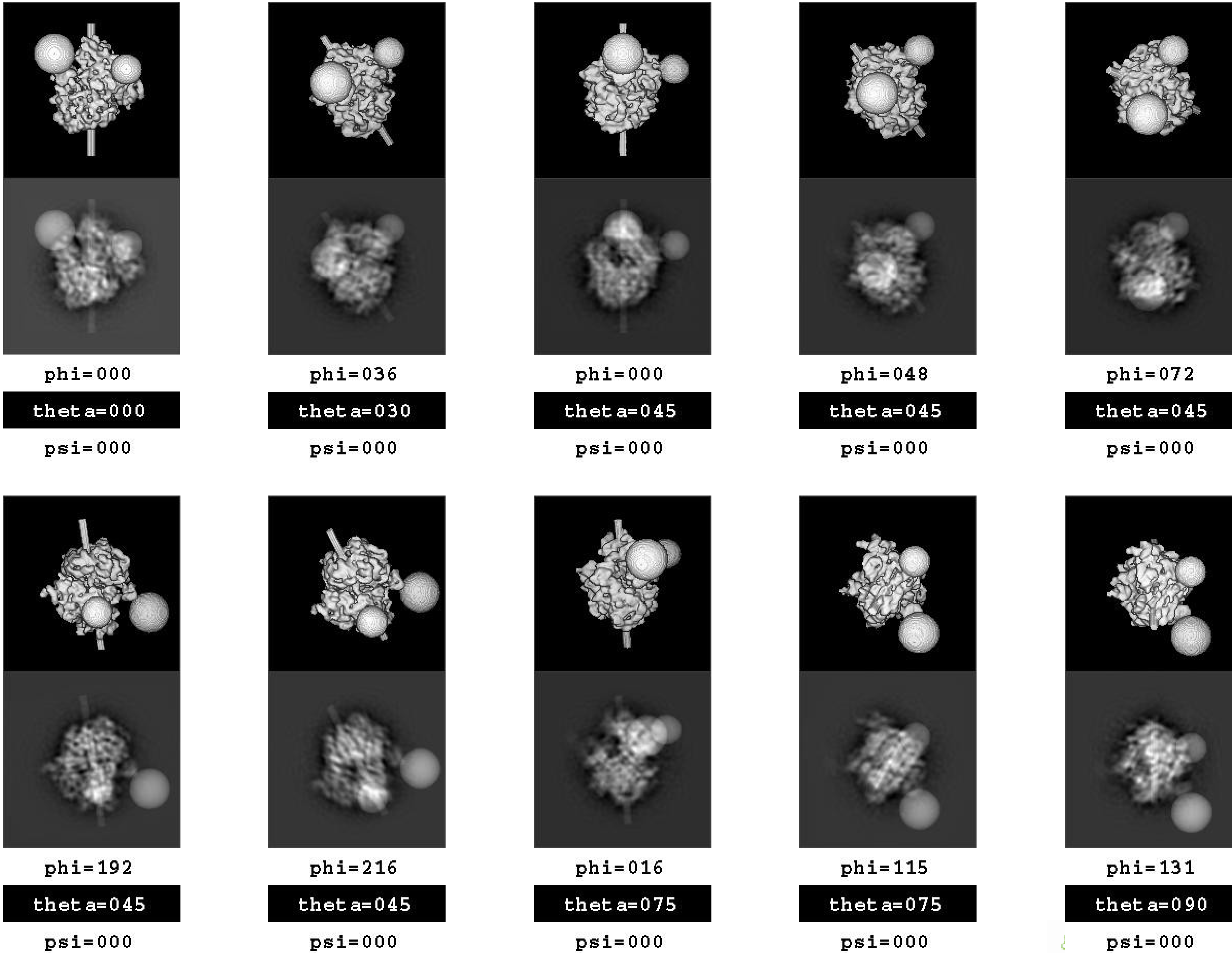
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phi=115
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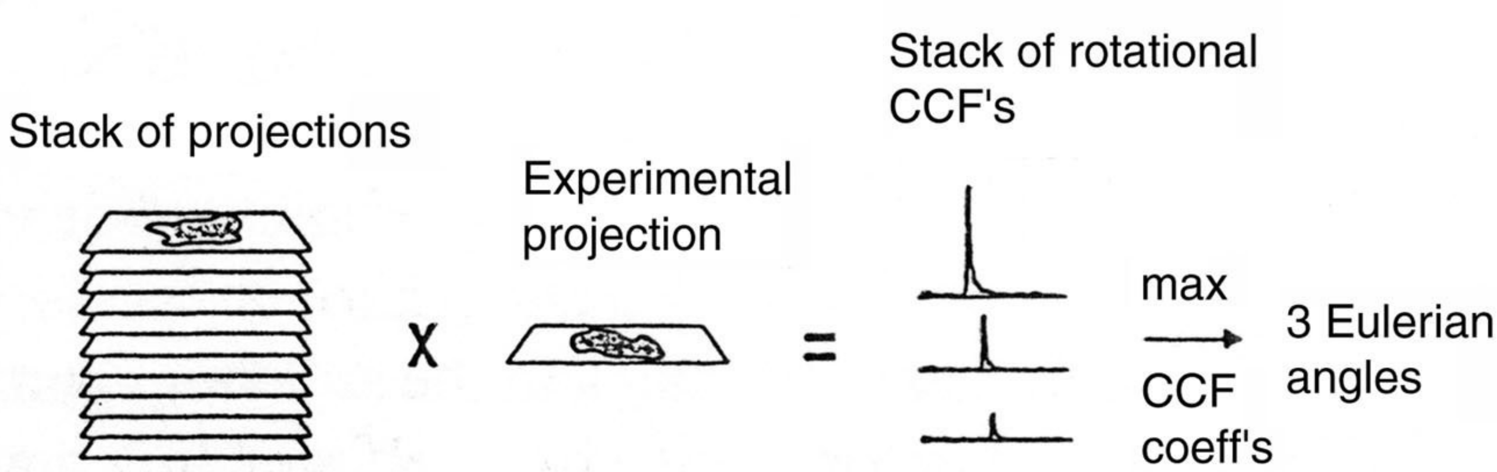
psi=000

Reference-based alignment



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

Steps:

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

Outline

Image analysis II

- 2D Fourier transforms

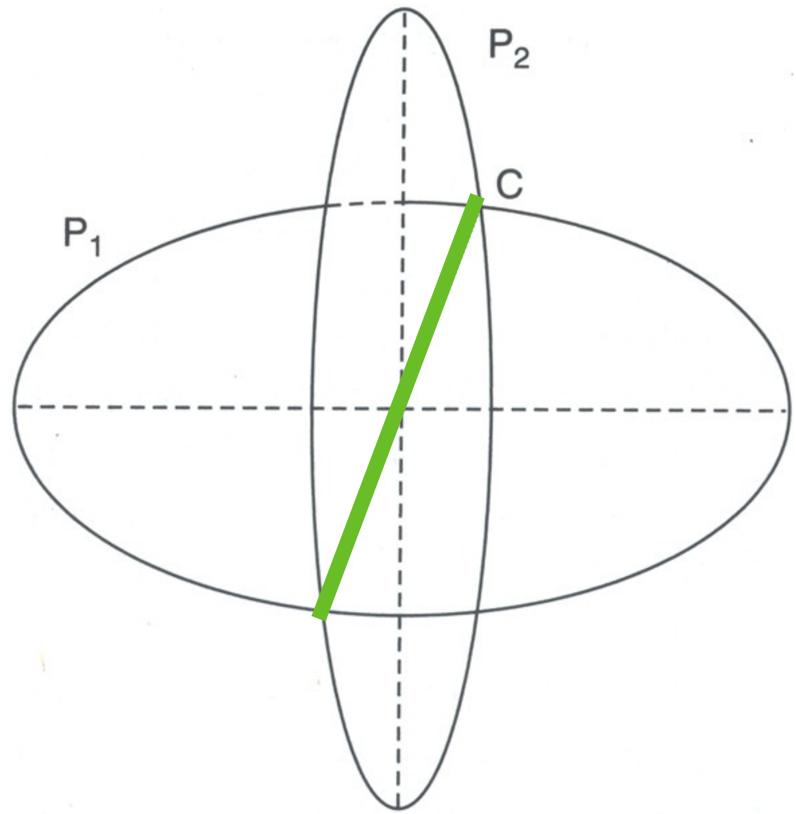
3D Reconstruction

- Principles
- Tomography
- Reference-based alignment
- Common lines
- RCT
- CTF-correction
- 3D classification

Common lines (or Angular Reconstitution)

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
(or Angular Reconstitution)

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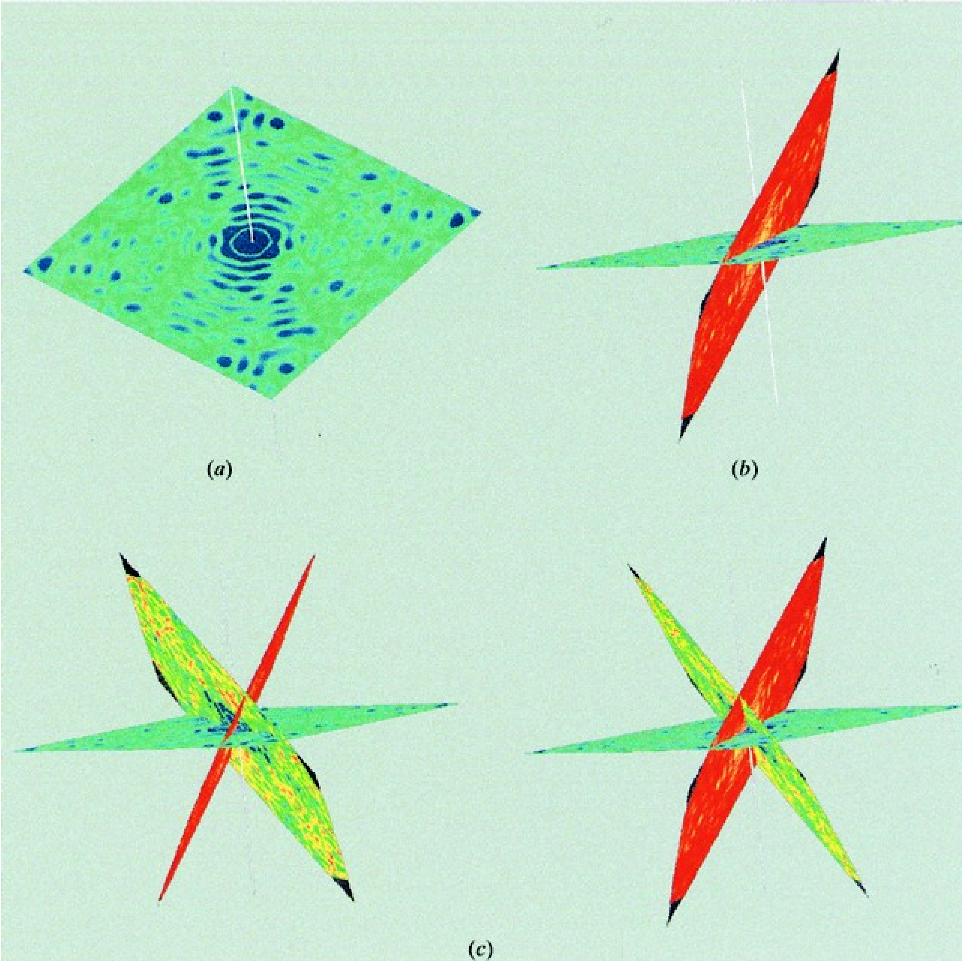

From Steve Fuller

Common lines: Problems

- Noise can lead to incorrect angles
  - Symmetry helps
- Handedness cannot be determined without additional information
  - Tilting
  - α-helices
- Assumes conformational homogeneity

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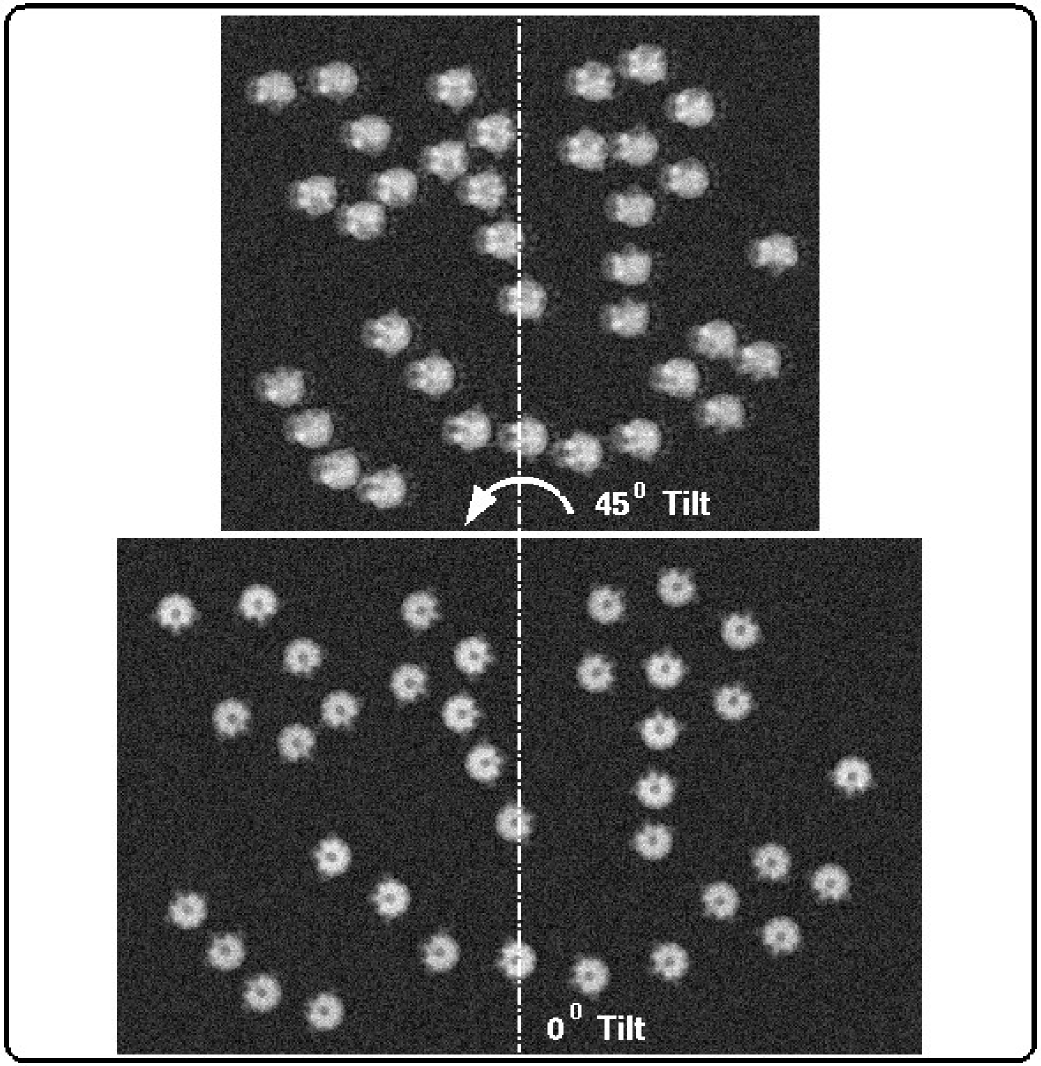
Random-conical tilt:
Determination of Euler angles

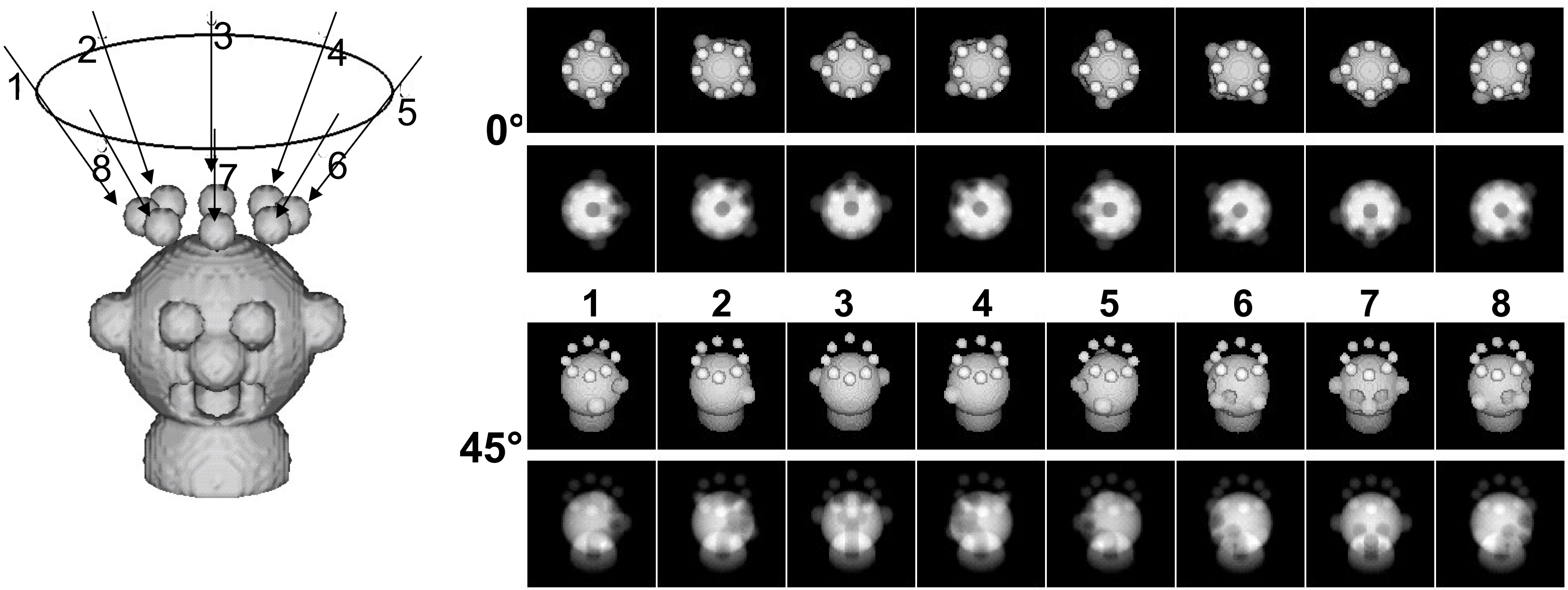


This scenario describes a worst case, when there is exactly one orientation in the 0º image. Since the in-plane angle varies, in the tilted image, we have different views available.

From Nicolas Boisset

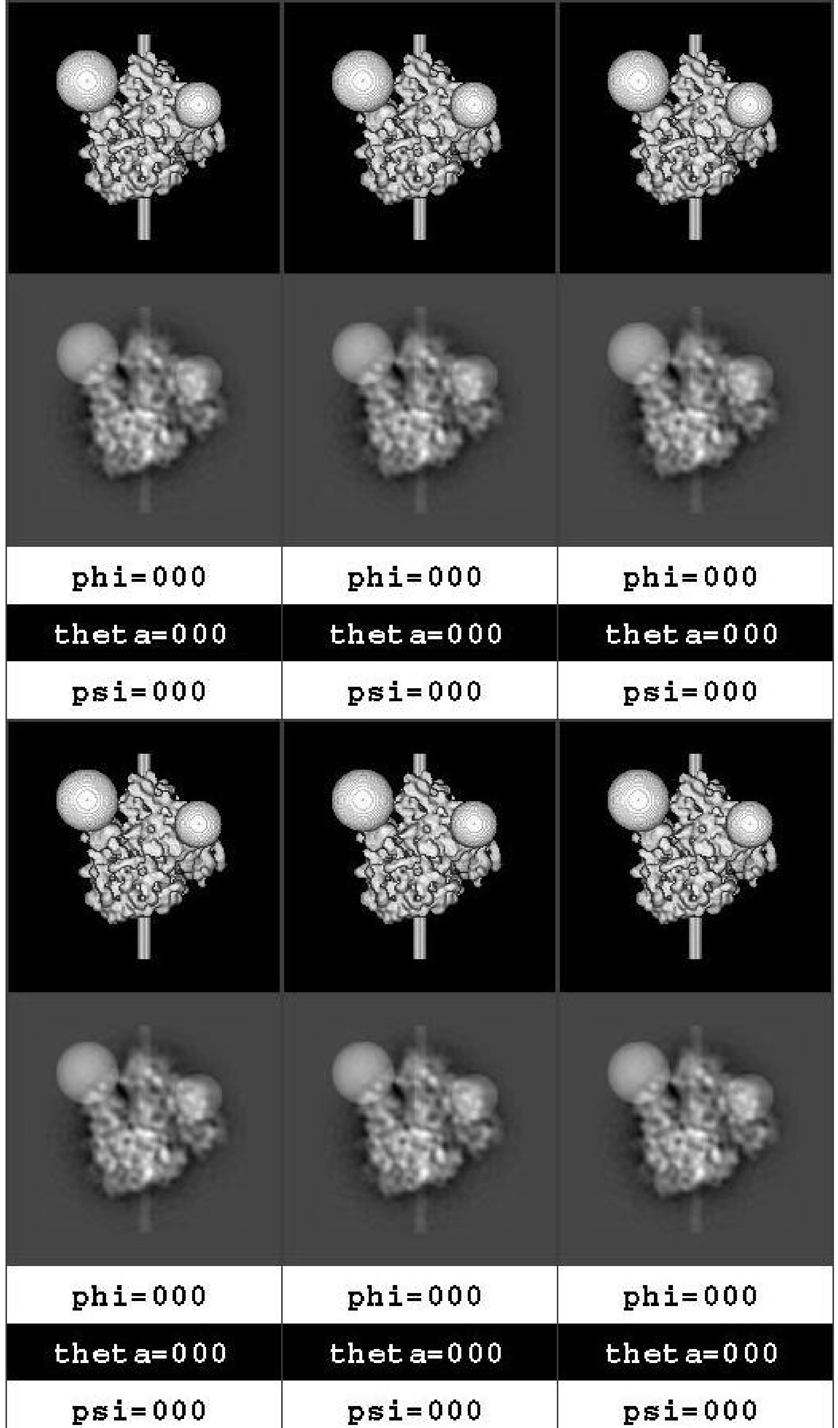
Random-conical tilt: Geometry

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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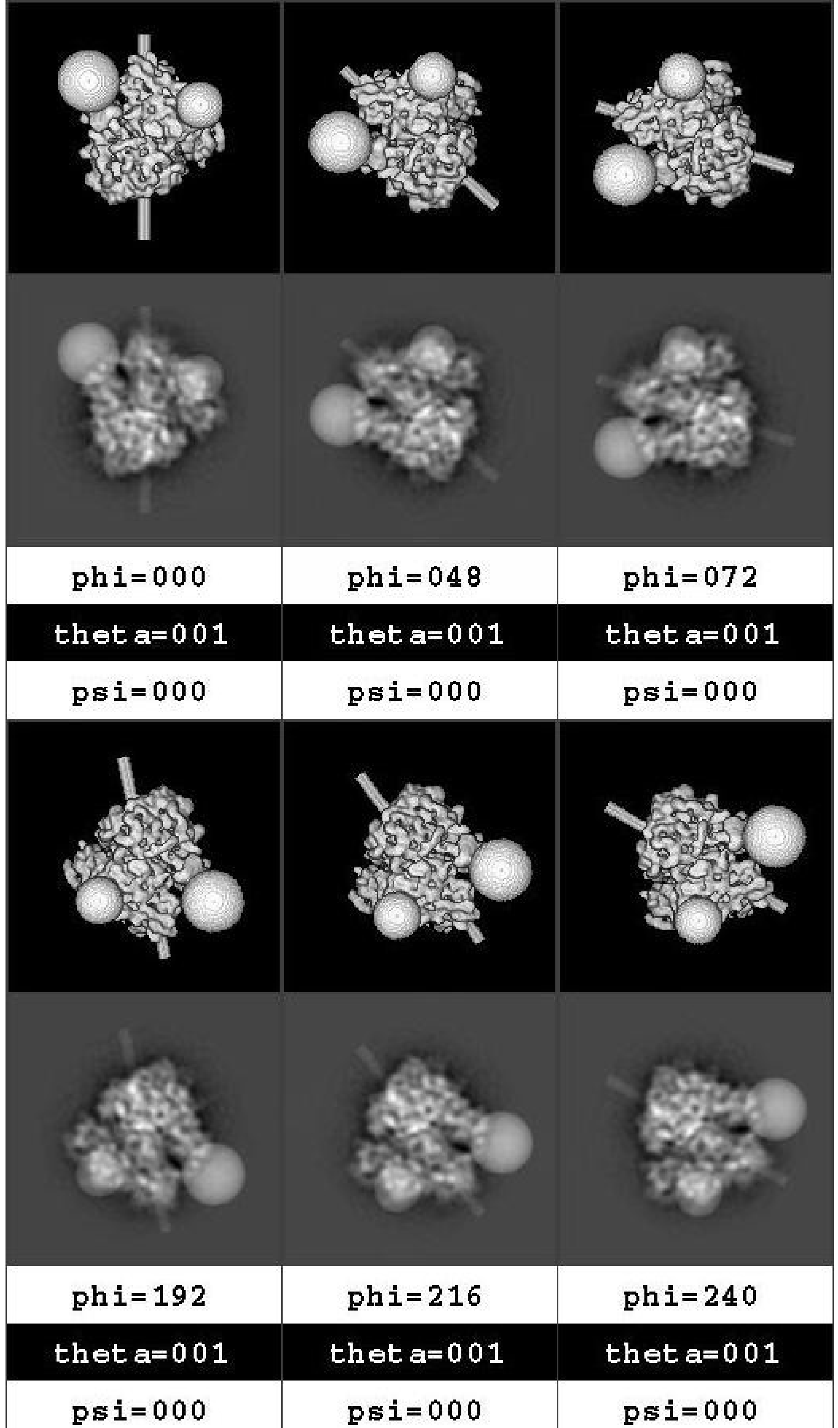

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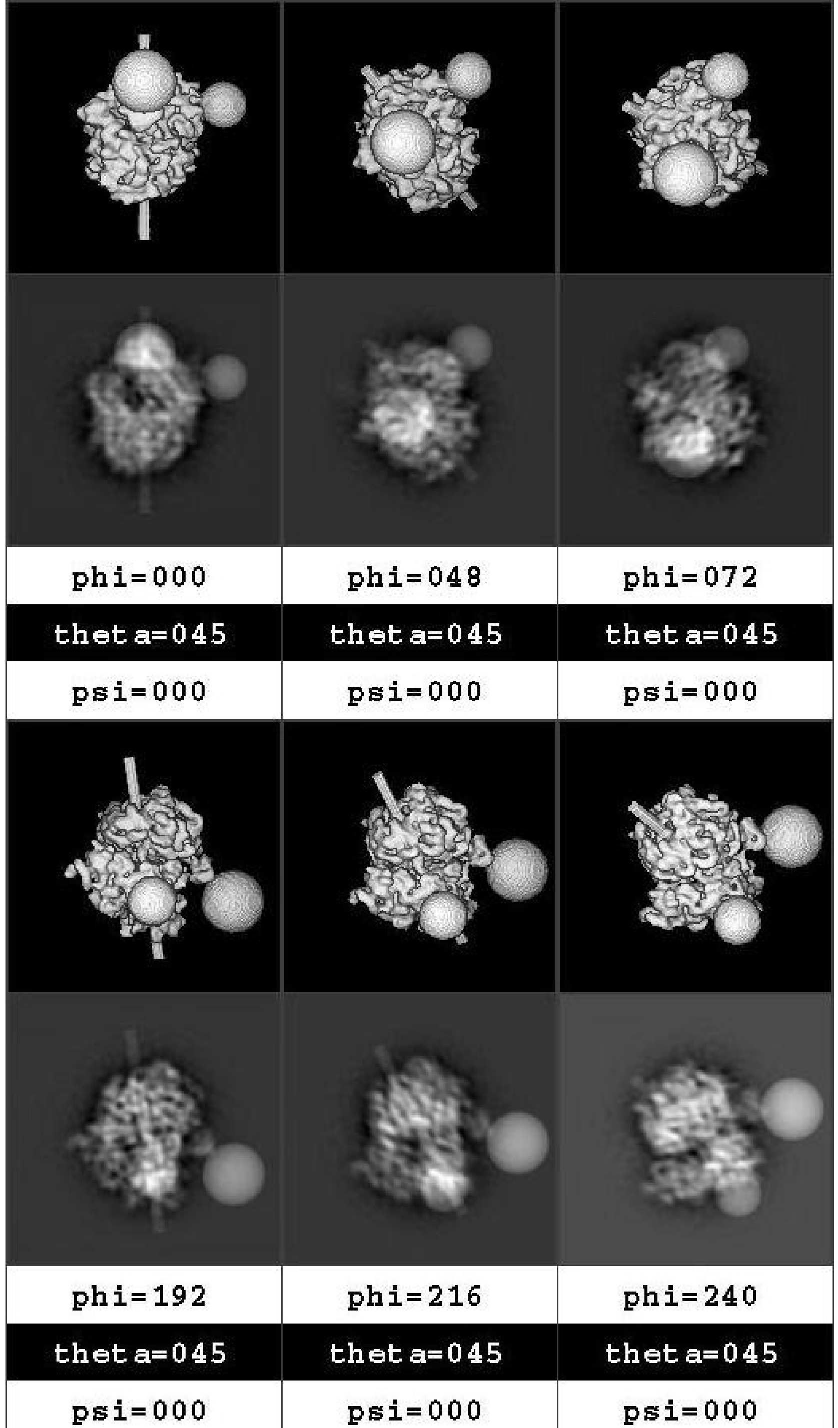
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psi=000
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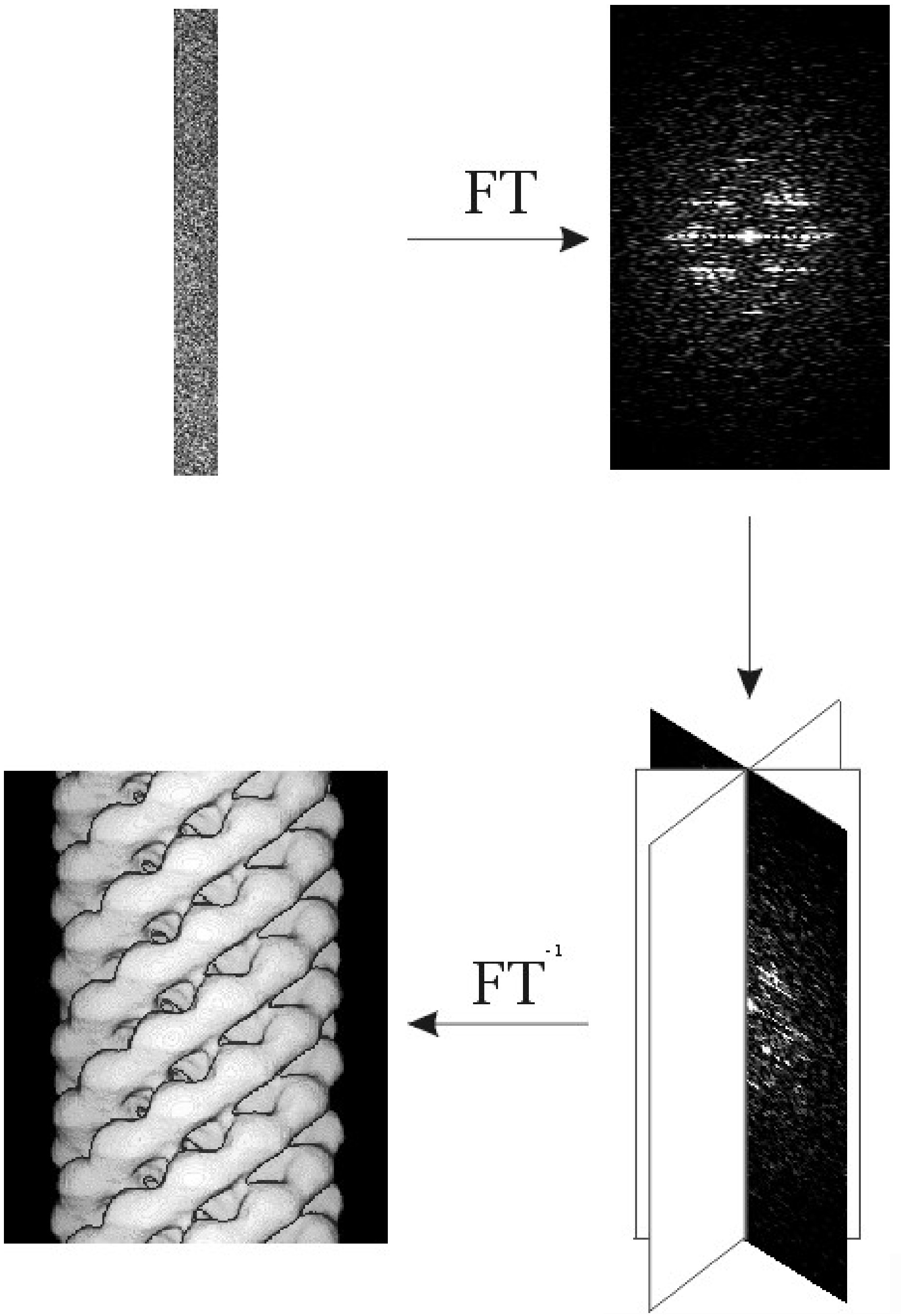
theta=045
psi=000

One problem though:

We can't tilt the stage all the way to 90 degrees.

Review:

Projection theorem

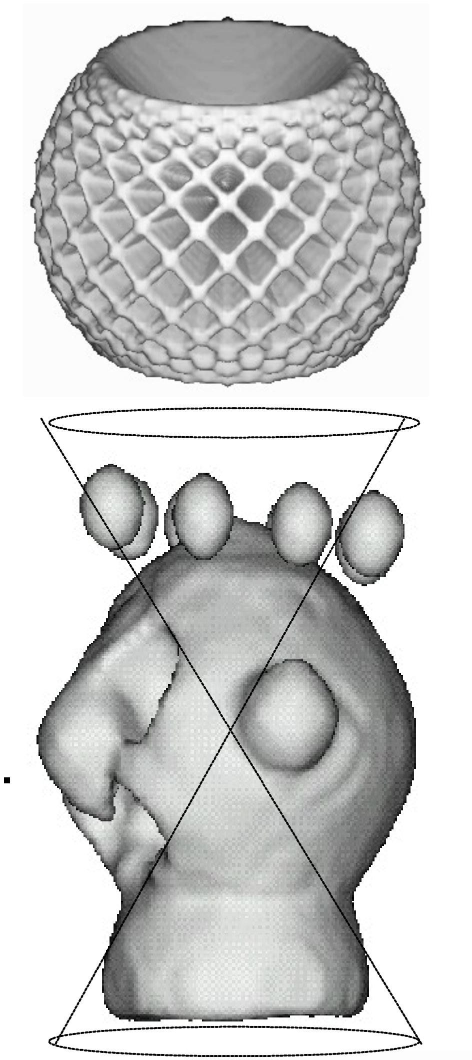


Random-conical tilt:

The "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.



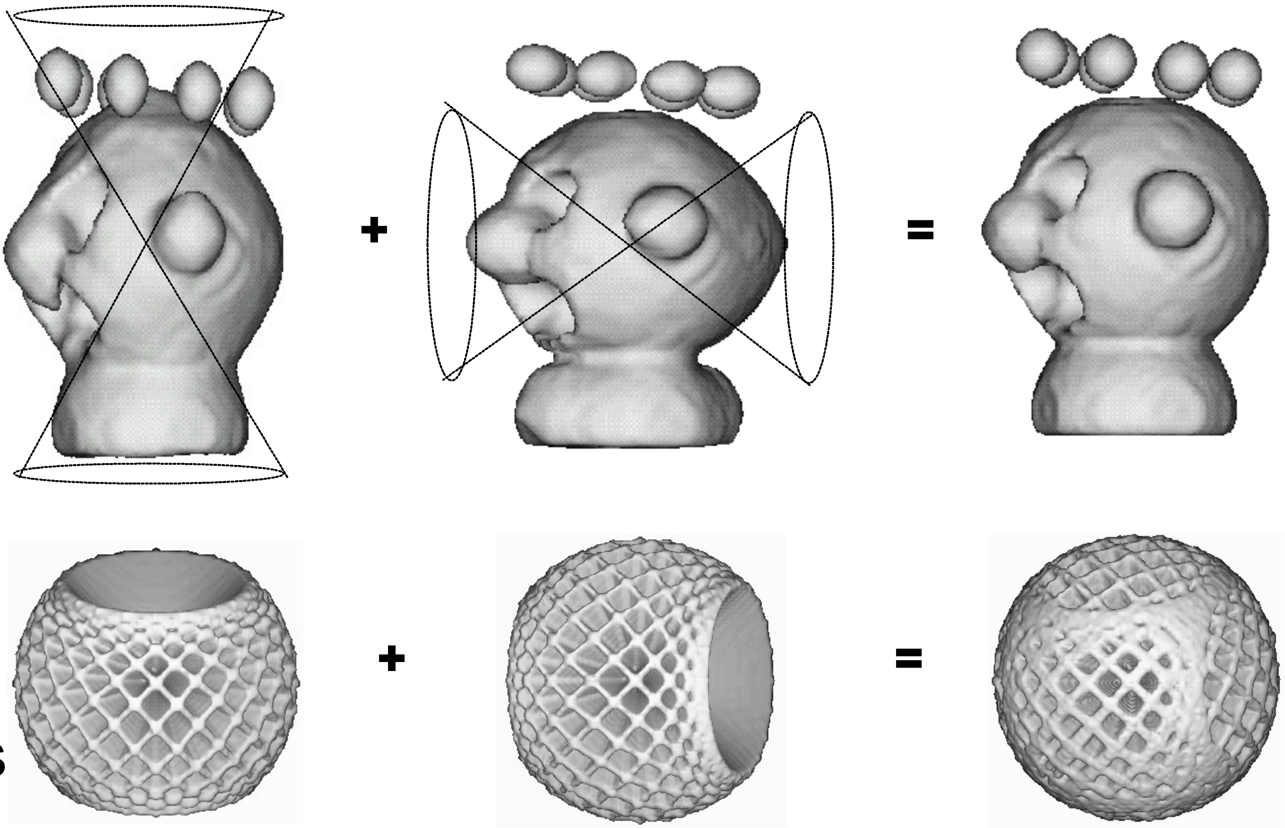
From Nicolas Boisset

Random-conical tilt:
Filling the missing cone

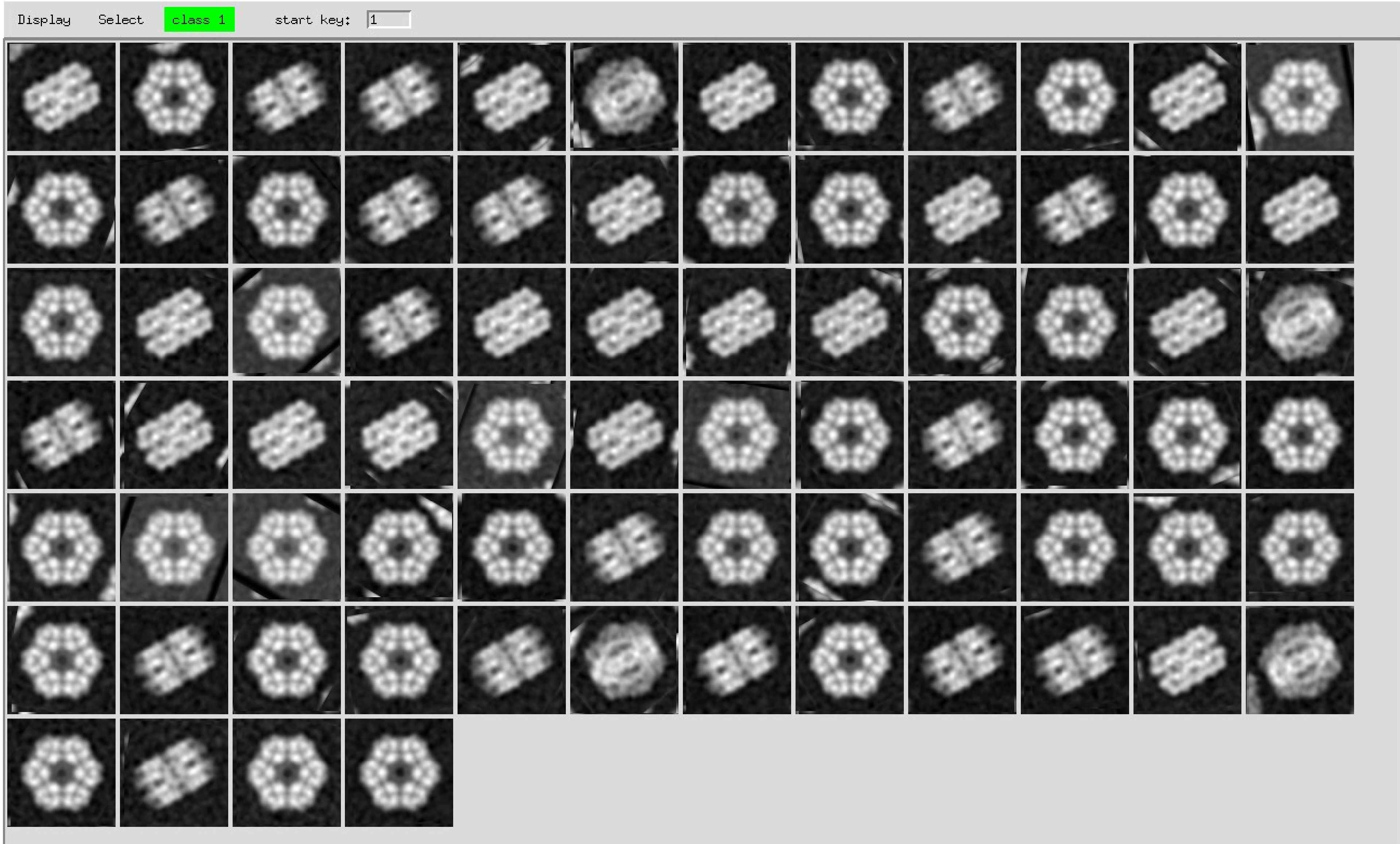
If there are multiple preferred orientations, or if there is symmetry that fills the missing cone, you can cover all orientations.

Reconstruction

Distribution of orientations



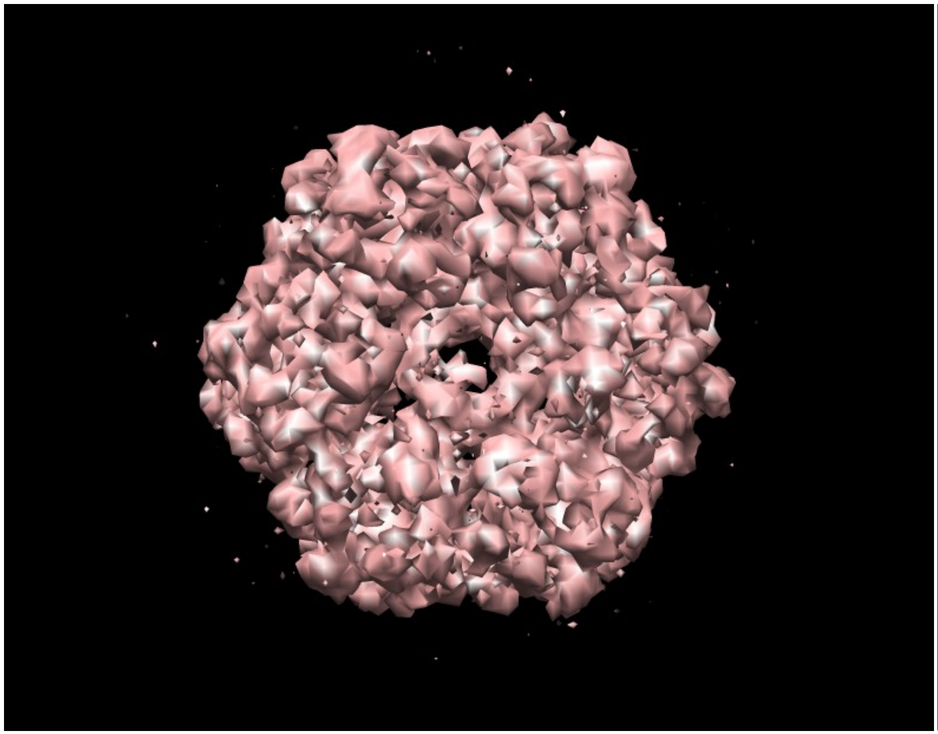
From Nicolas Boisset

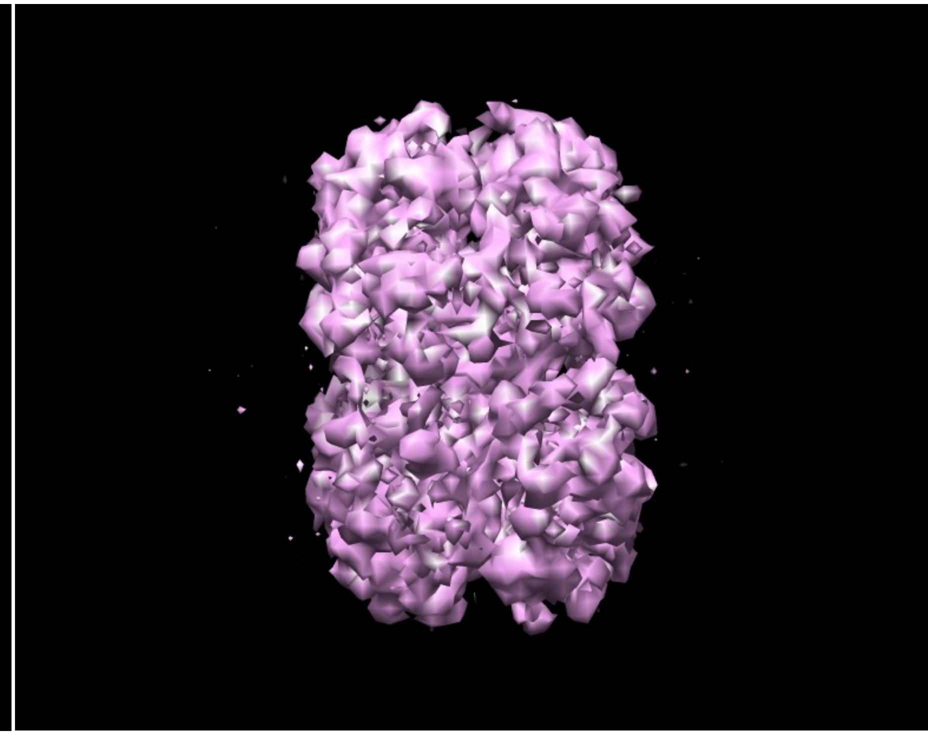


Phantom images of worm hemoglobin

CEITEC

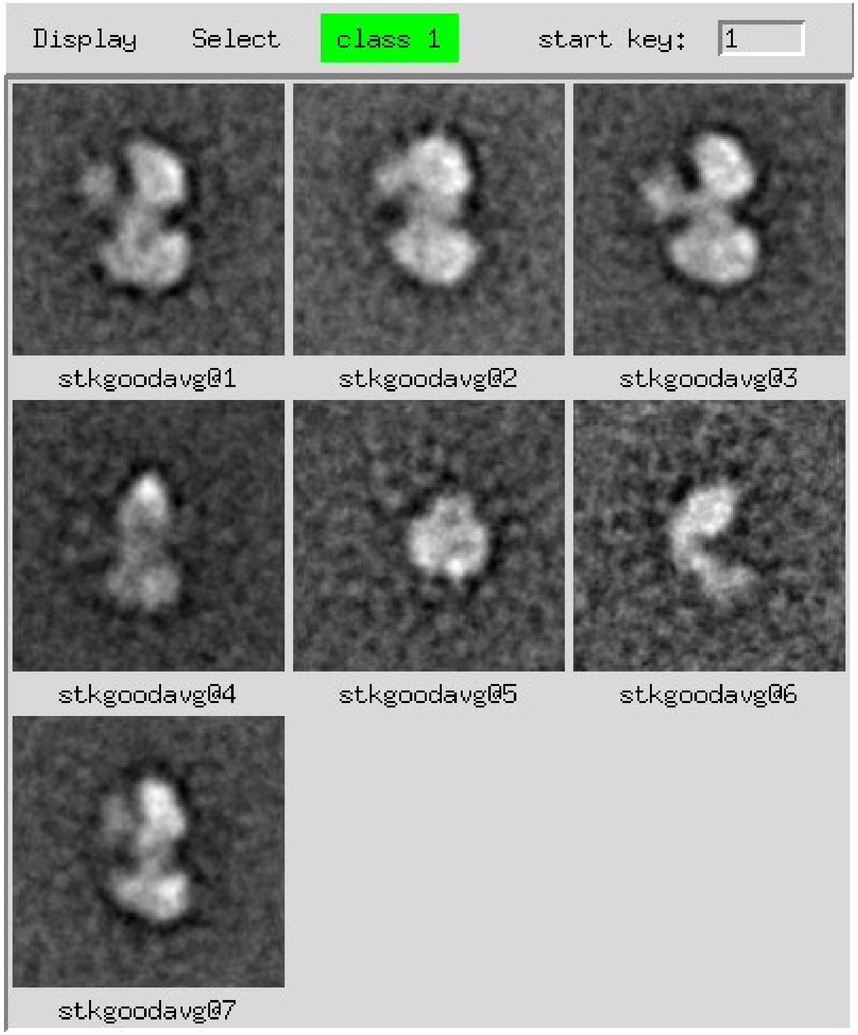
We compute a separate reconstruction for each class

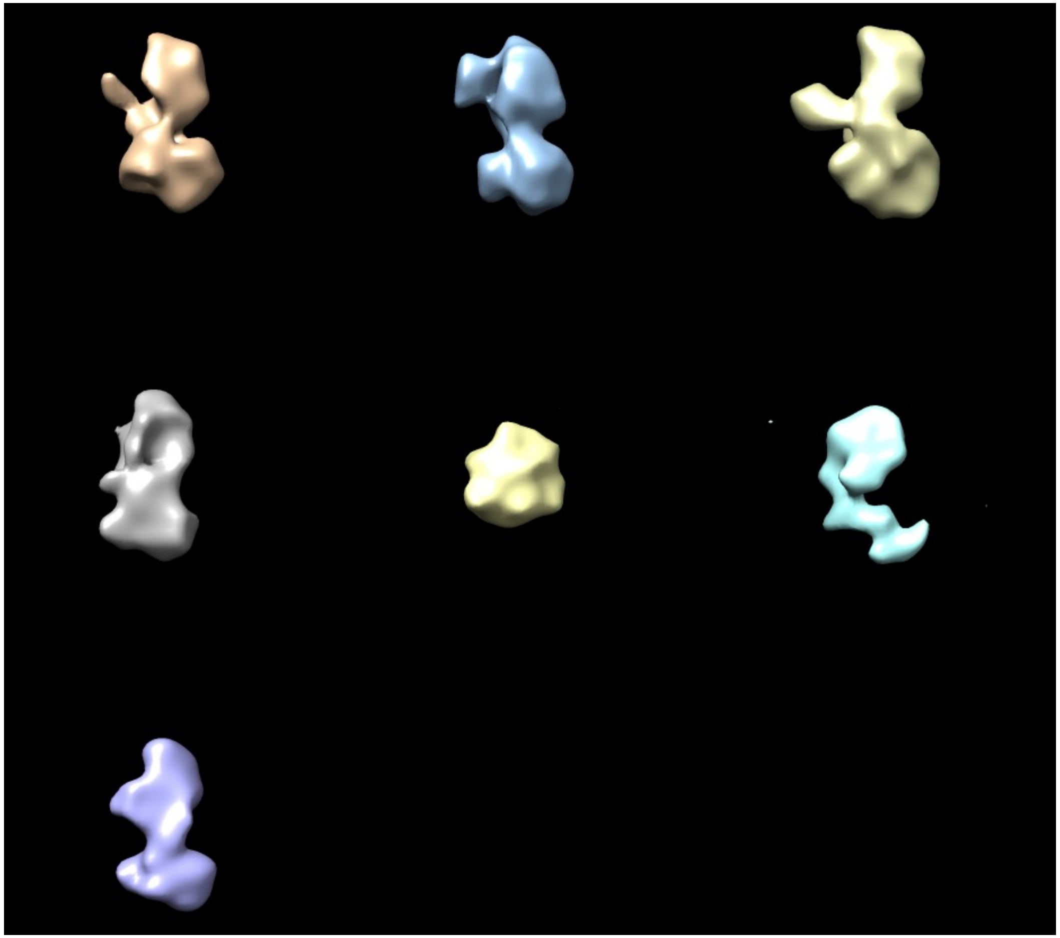




<u>IF</u> the classes simply correspond to different orientations, you can combine them, and boost the signal-to-noise.

Helicase G40P





If the classes correspond to different conformations, then you have to keep them as separate reconstructions.

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More properties of Fourier transforms: Convolutions

Why might two images in a data set look different?

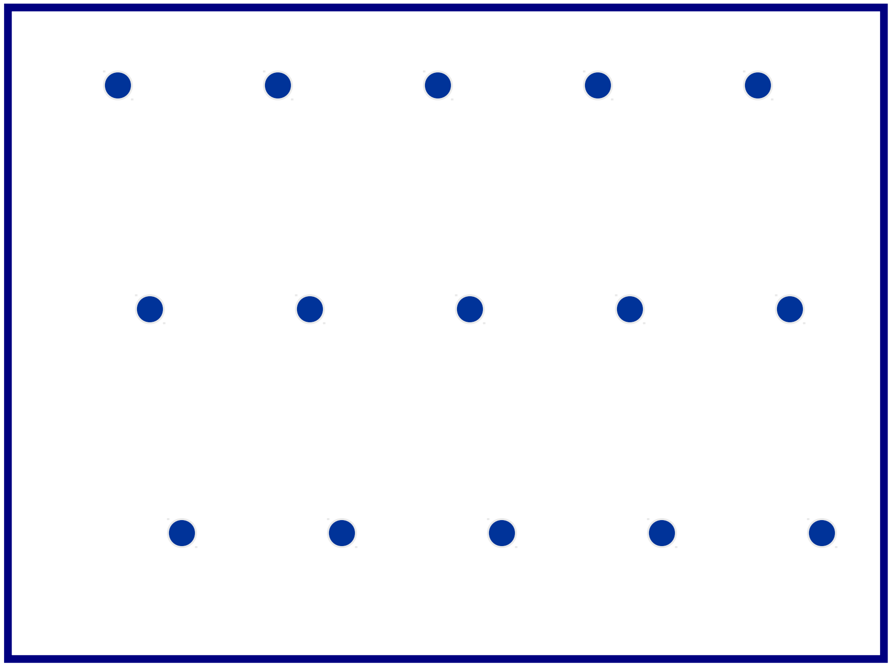
- different sample
- different magnification
- different illumination
- different orientations
- different defocus
- different conformations

- better biochemistry
- better microscopy
- normalization
- determine angles
- CTF correction
- Classification

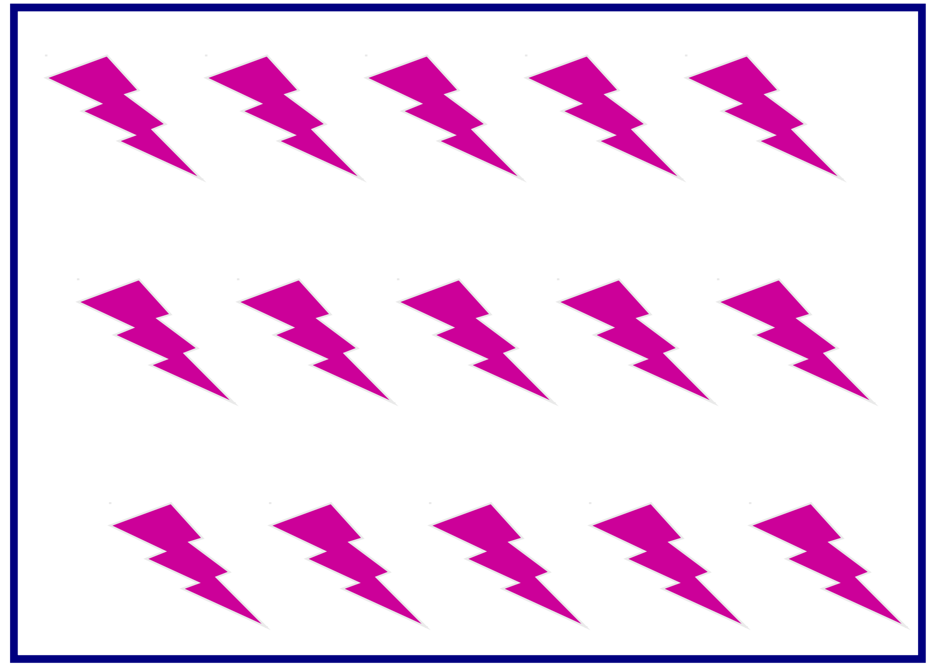
Convolution of a molecule with a lattice generates a crystal.

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

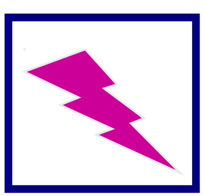
Adapted from David DeRosier



lattice: $f(x)$



Set a molecule down at every lattice point.



Molecule $g(x)$

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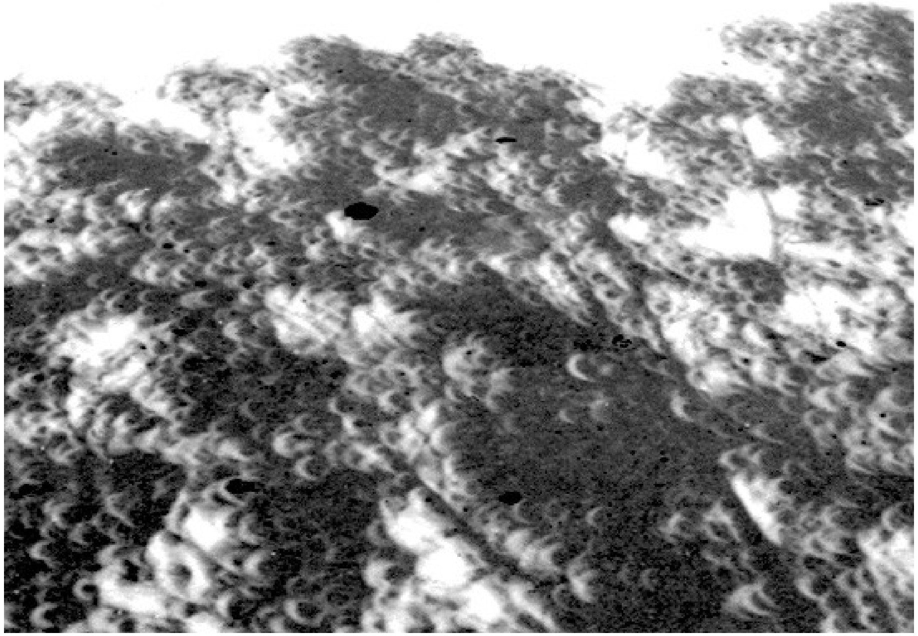
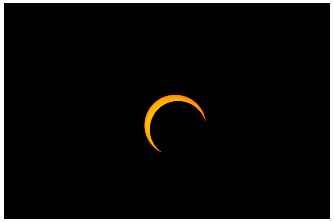
Convolution in real life

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

lattice: $f(x)$
http://www.photos-public-domain.com



http://www.symbolicmessengers.com



Molecule: $g(x)$
http://en.wikipedia.org

Set a molecule down at every lattice point.

CEITEC

Cross-correlation vs. convolution

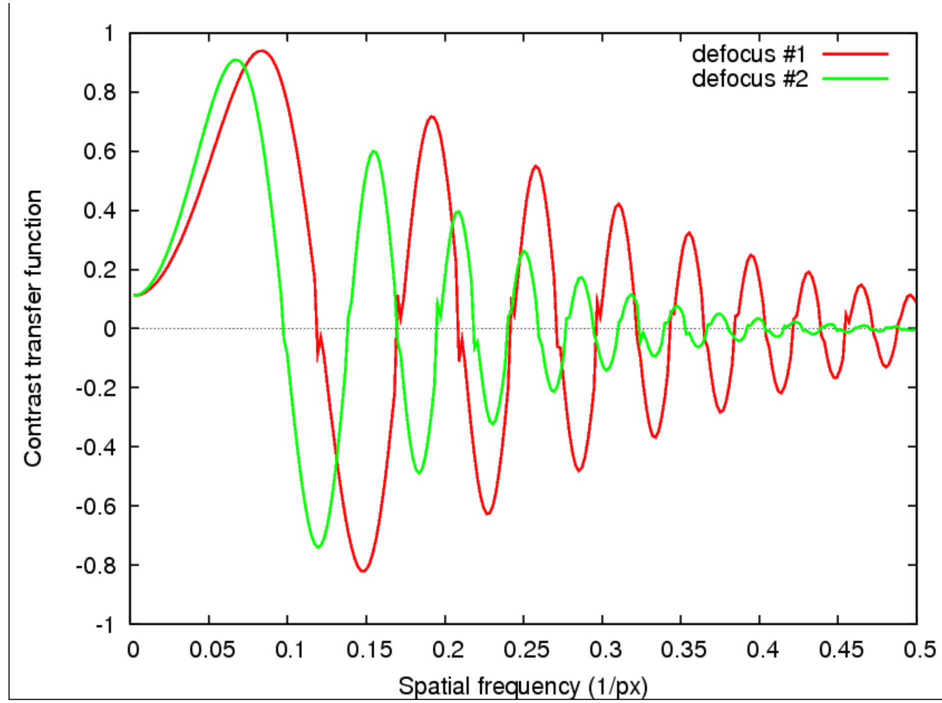
Complex conjugate:
If a Fourier coefficient $F(X)$ has the form: $a + bi$
The complex conjugate $F^*(X)$ has the form: $a - bi$

Cross-correlation: $F^*(X)\ G(X)$

Convolution: $F(X)\ G(X)$

Remember:
$f(x)$, $g(x)$ are real-space functions
$F(X)$, $G(X)$ are Fourier-space functions

original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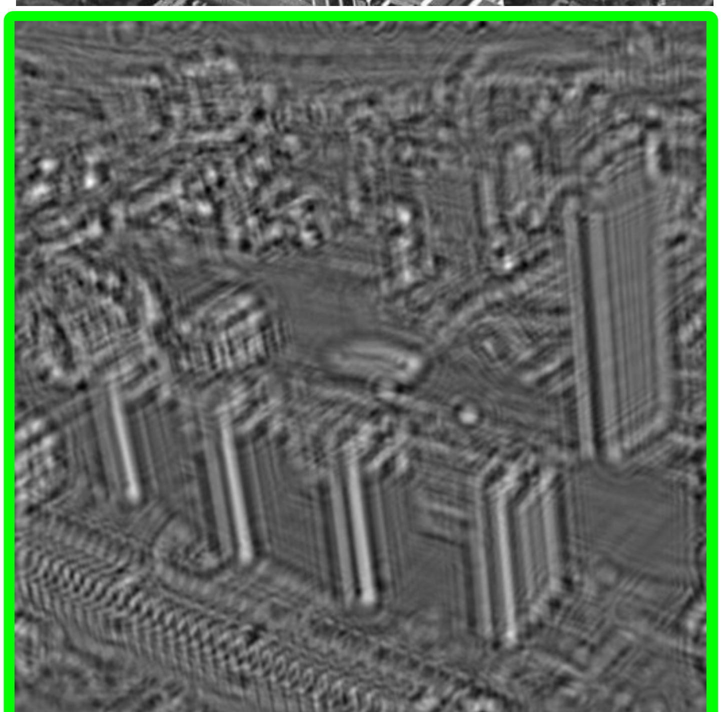
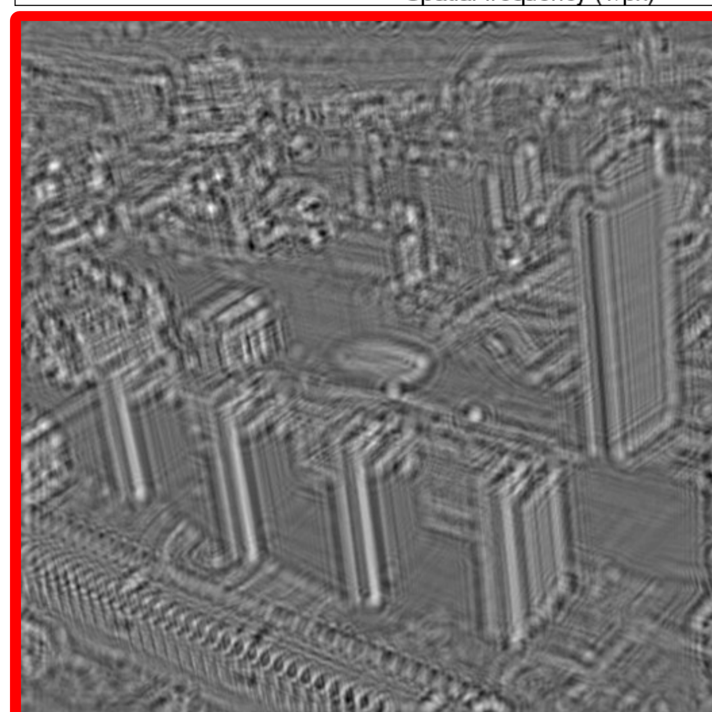
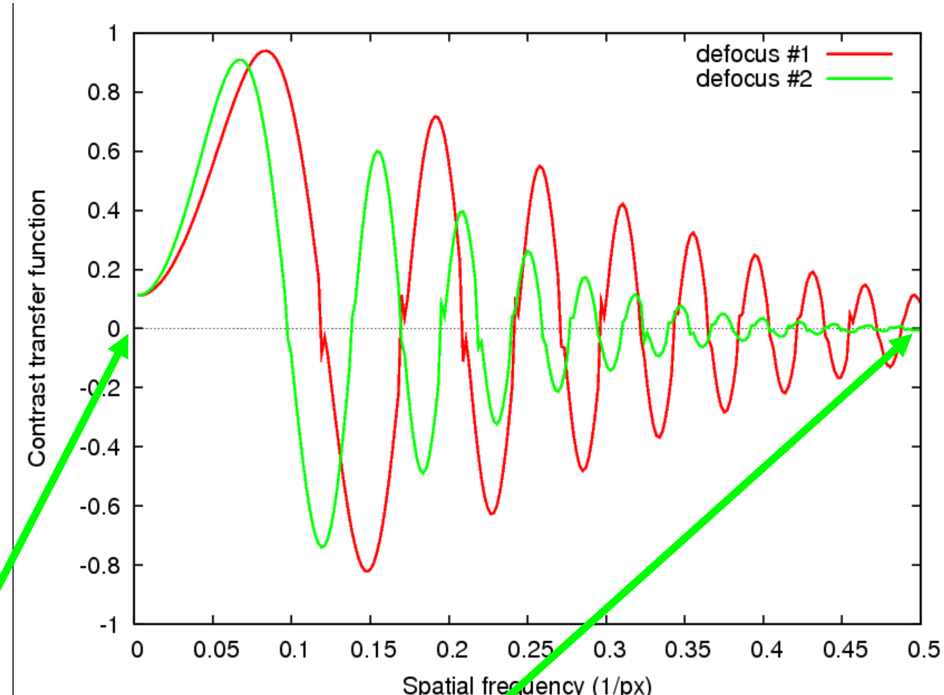
0.45
0.5

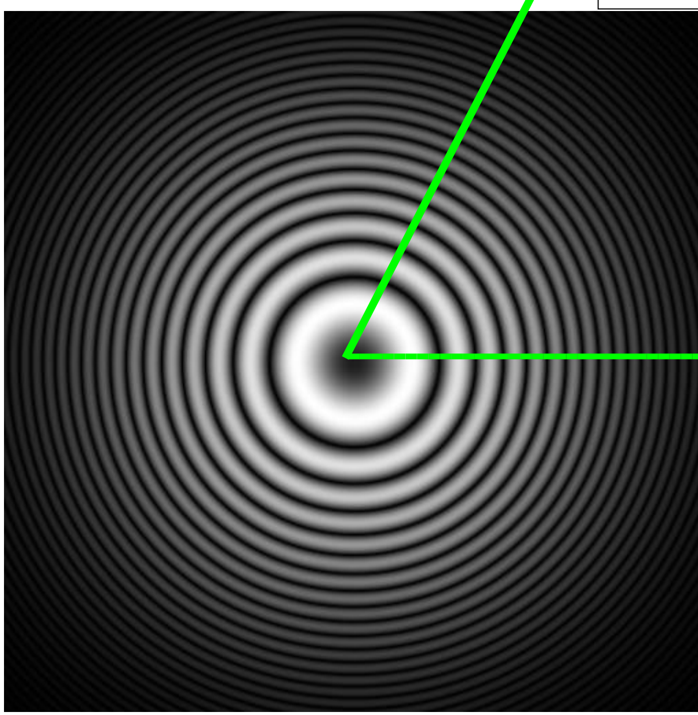




CTF

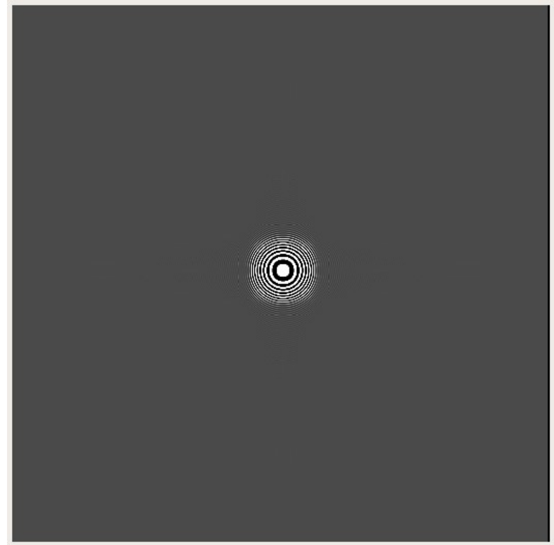
1D profile



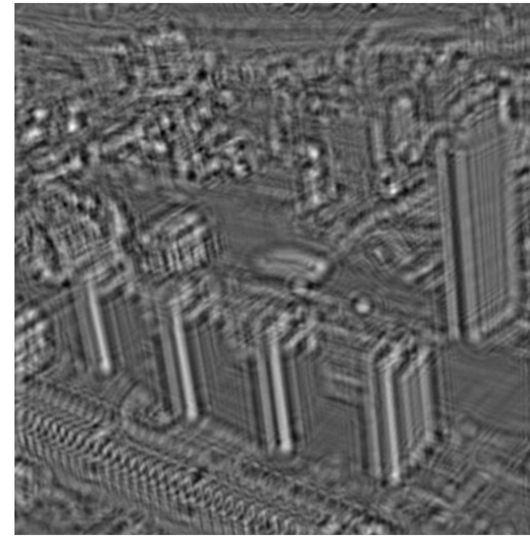


2D power spectrum
$G(X)$

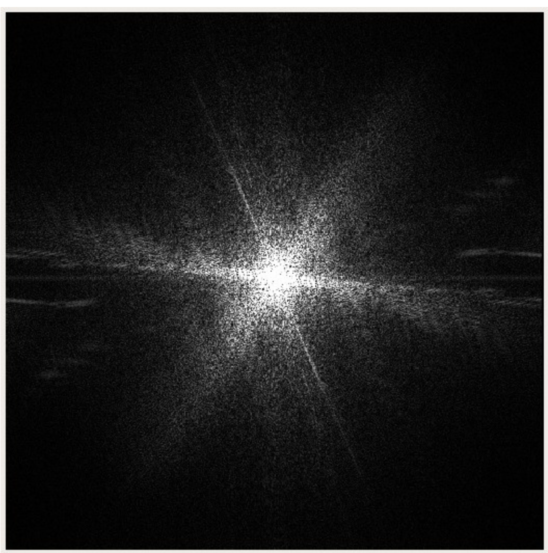
f(x)



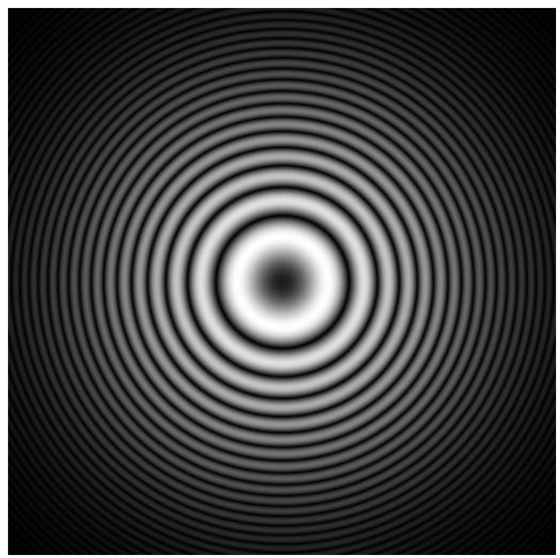
g(x)



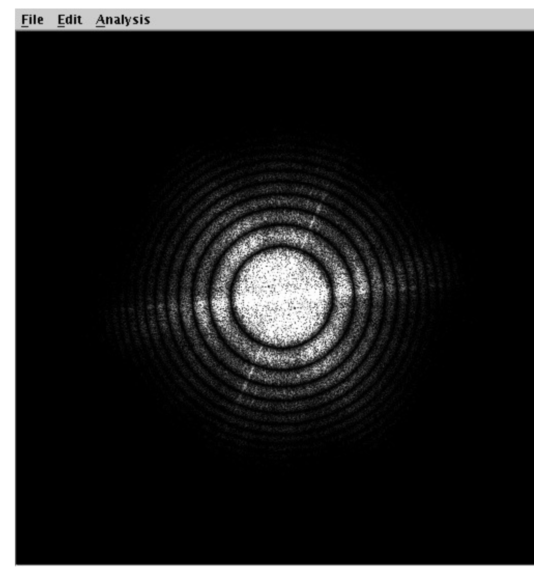
f(x)•g(x)



F(X)

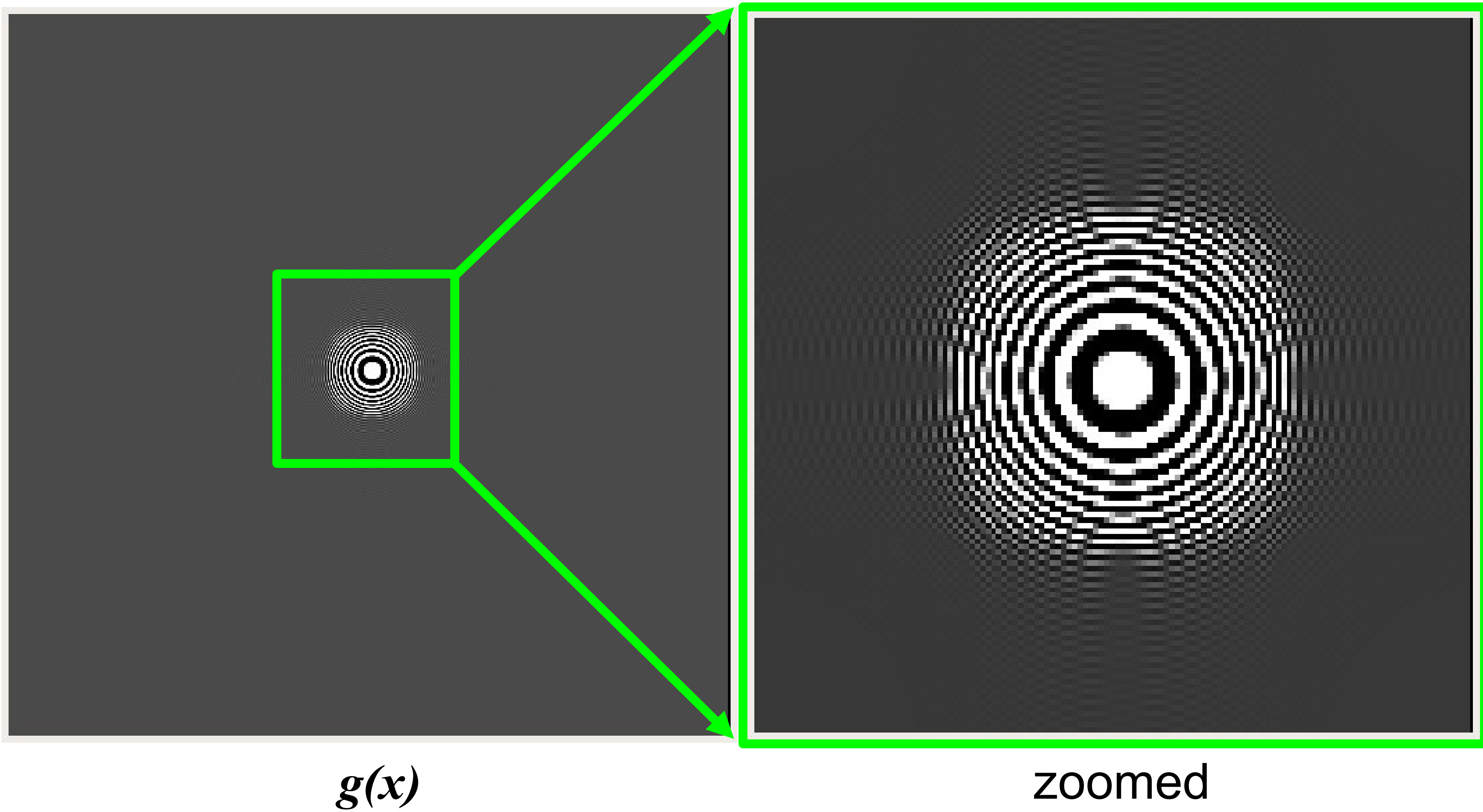


G(X)

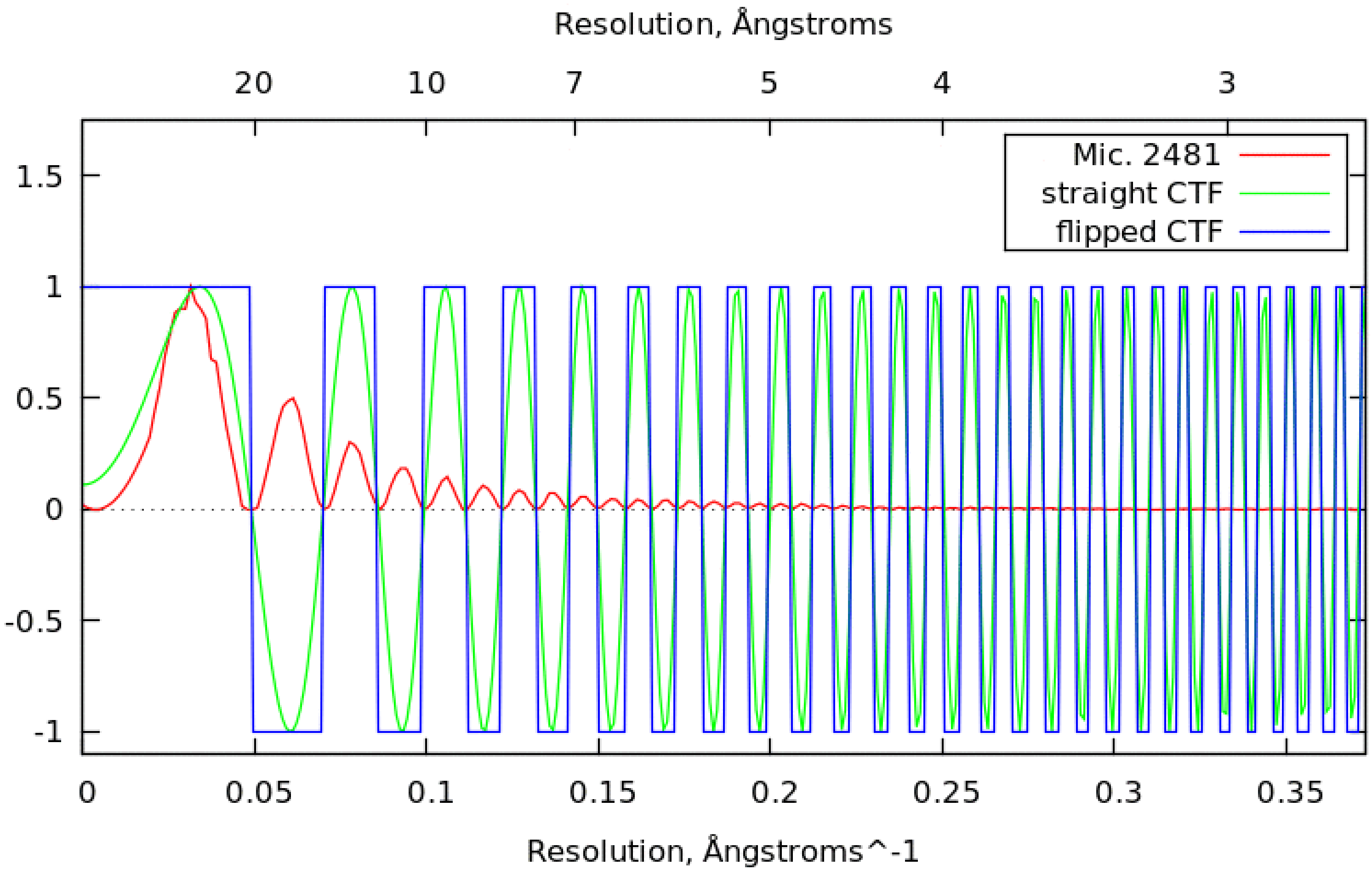


F(X) G(X)

Point spread function



An ideal point spread function would be an infinitely-sharp point.



Red: Power-spectrum profile calculated from experimental image
Green: Fitted, theoretical power-spectrum profile
Blue: Phase-only correction profile

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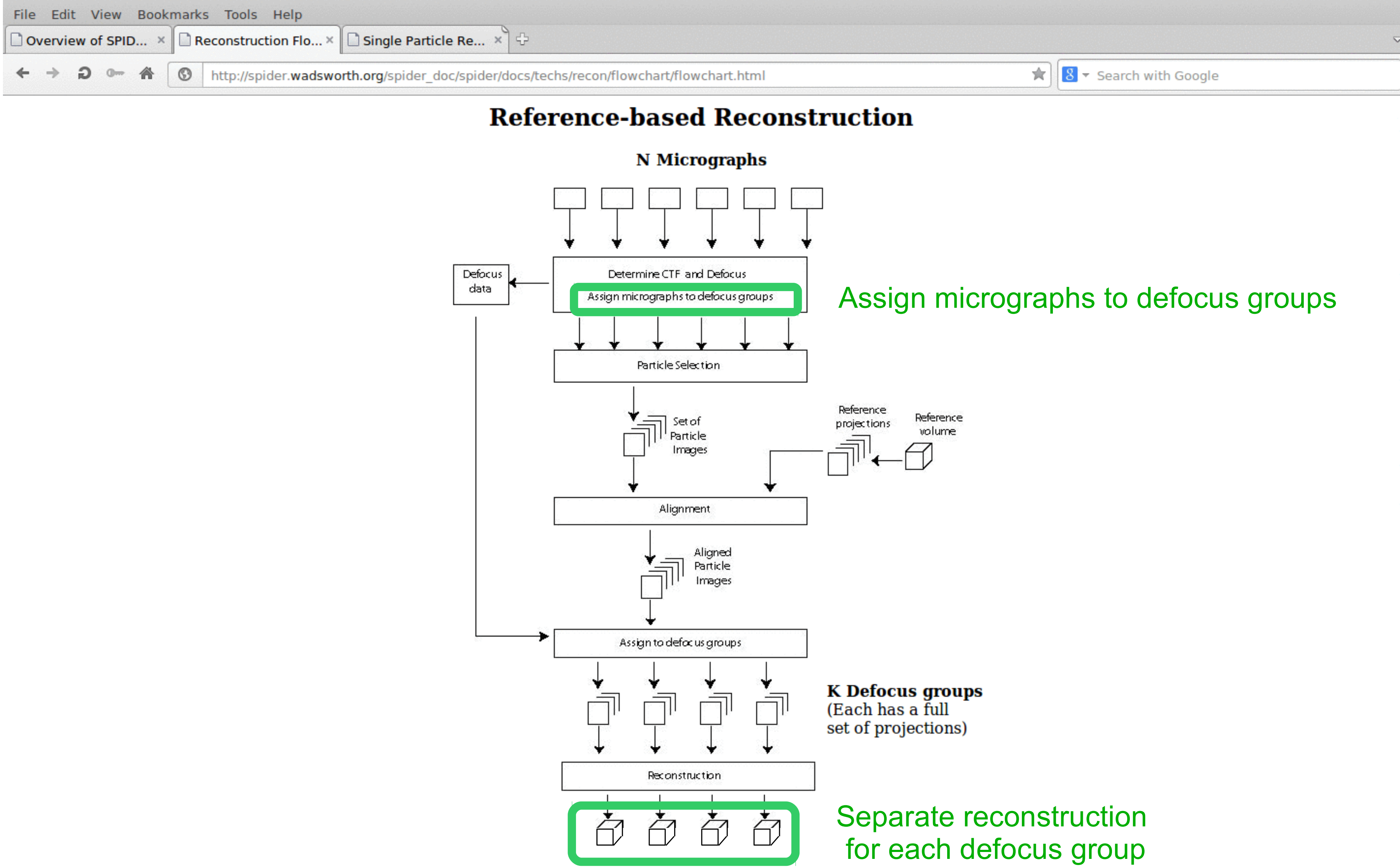
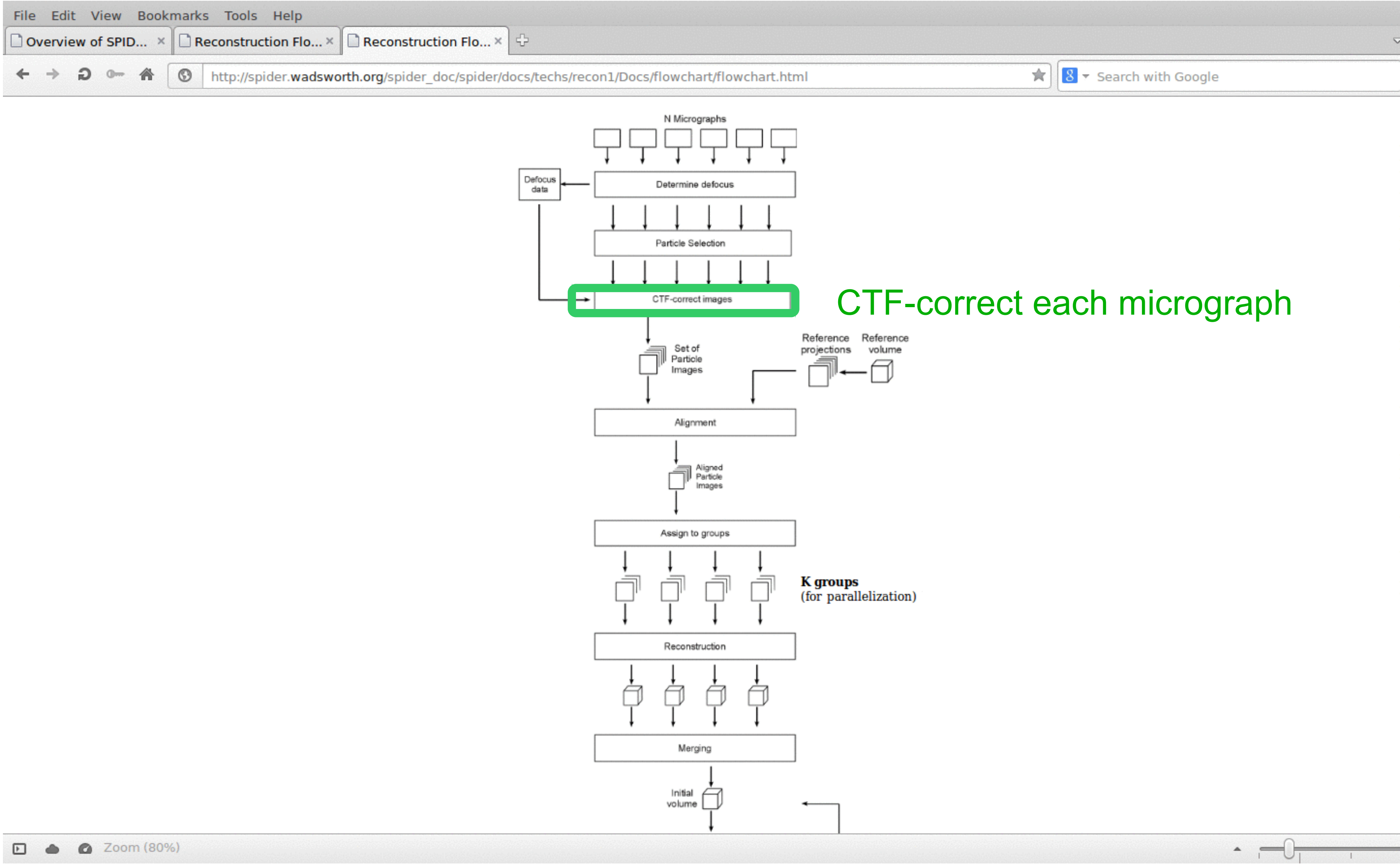
Defocus groups: CTF correction in 3D



Assign micrographs to defocus groups

Separate reconstruction for each defocus group

CTF-correction of micrographs in 2D



Why might two images in a data set look different?

- different molecule
- different magnification
- different illumination
- different defocus
- different orientations
- different conformations

- better biochemistry
- better microscopy
- normalization
- CTF correction
- determine angles
- Classification

Thank you for your attention

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