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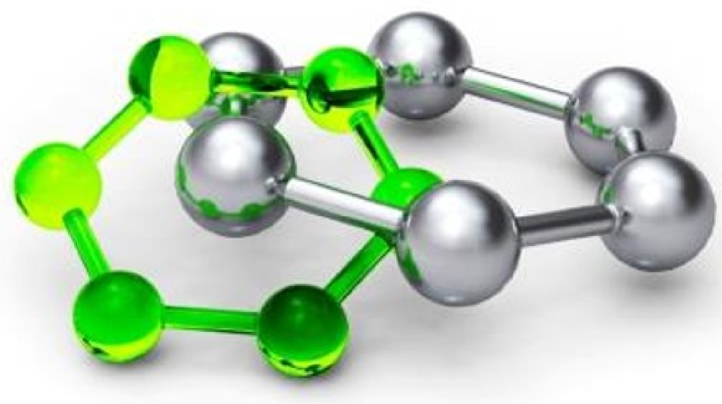
Image analysis III & 3D Reconstruction

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

March 23, 2015



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OP Research and
Development for Innovation

Outline

Image analysis III

- Still more Fourier transforms
    - Convolution
    - Step function
    - Power spectrum
    - Friedel's Law
- More orientation alignment
- More interpolation
- Classification

3D Reconstruction

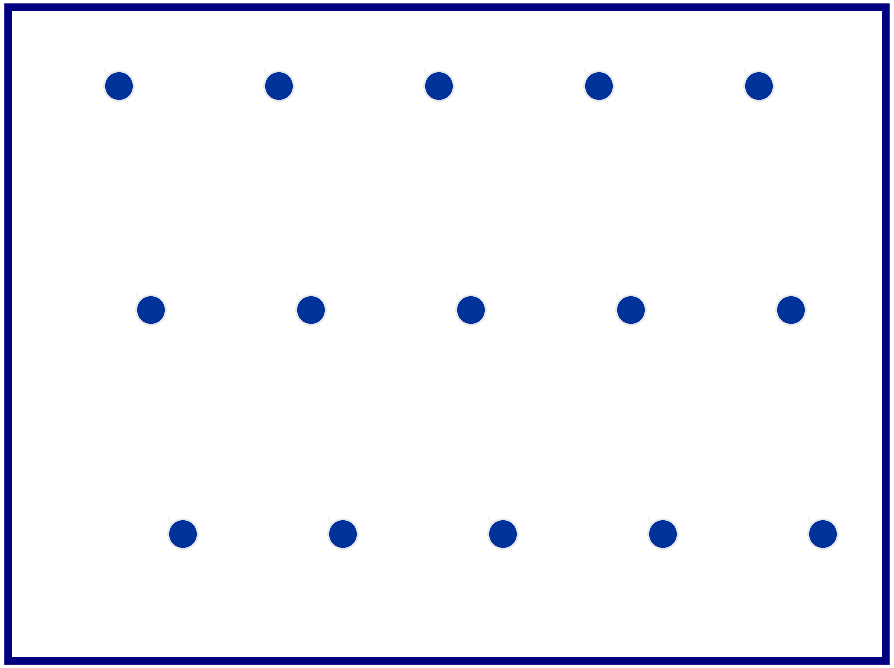
- Principles
- Reference-based alignment
- RCT

Current events:
Convolutions

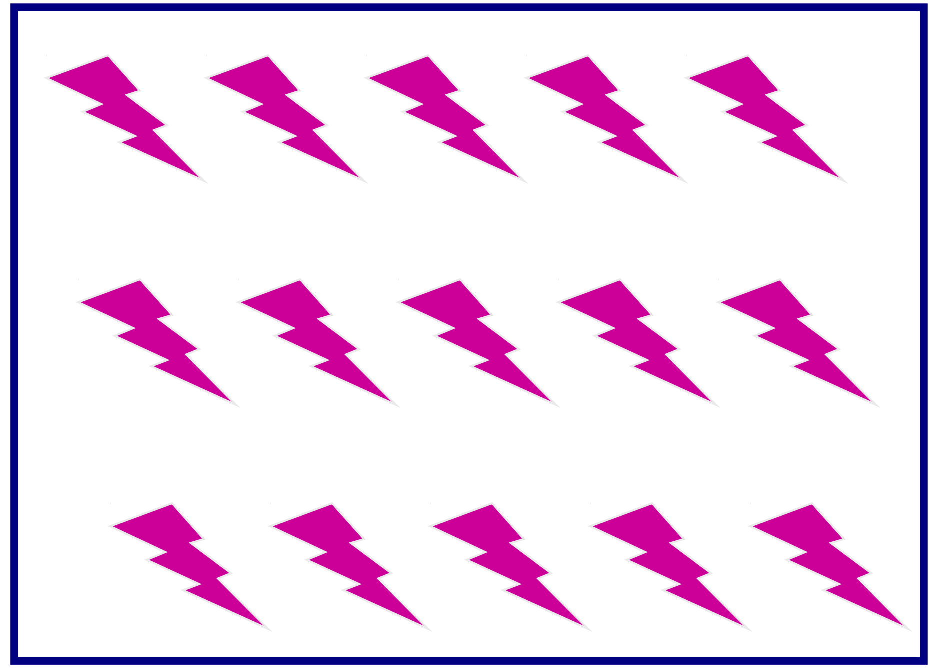
Convolution of a molecule with a lattice generates a crystal.

Notation: f(x)*l(x)

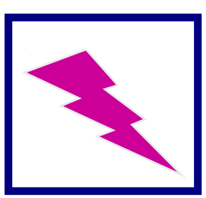
From David DeRosier



lattice = l(x)



Set a molecule down at every lattice point.

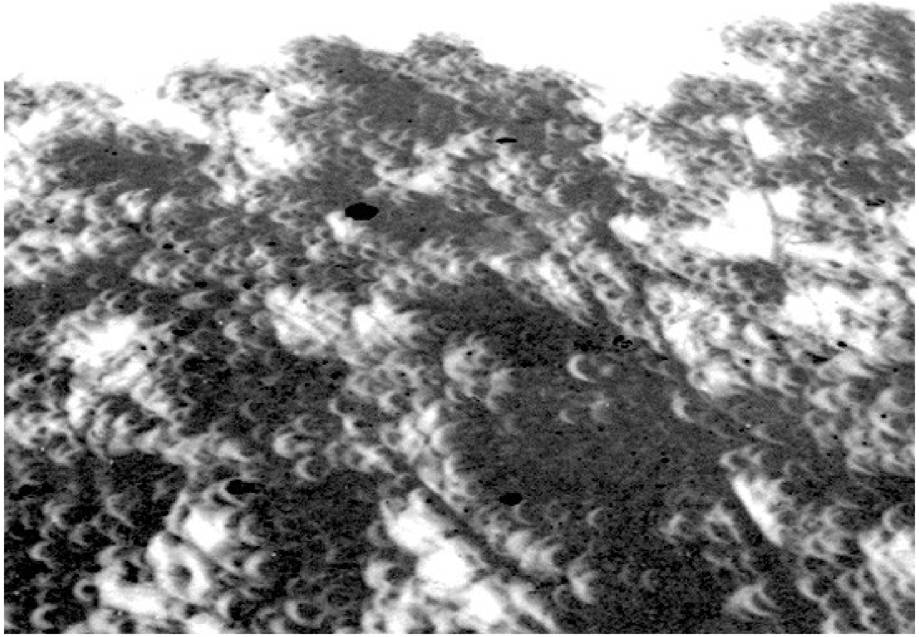


Molecule = f(x)

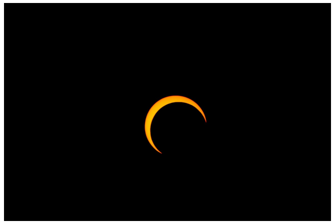
Convolution of a molecule with a lattice generates a crystal.

Notation: f(x)*l(x)

lattice = l(x)
(http://www.photos-public-domain.com)



http://www.symbolicmessengers.com



Molecule = f(x)
http://en.wikipedia.org

Set a molecule down at every lattice point.

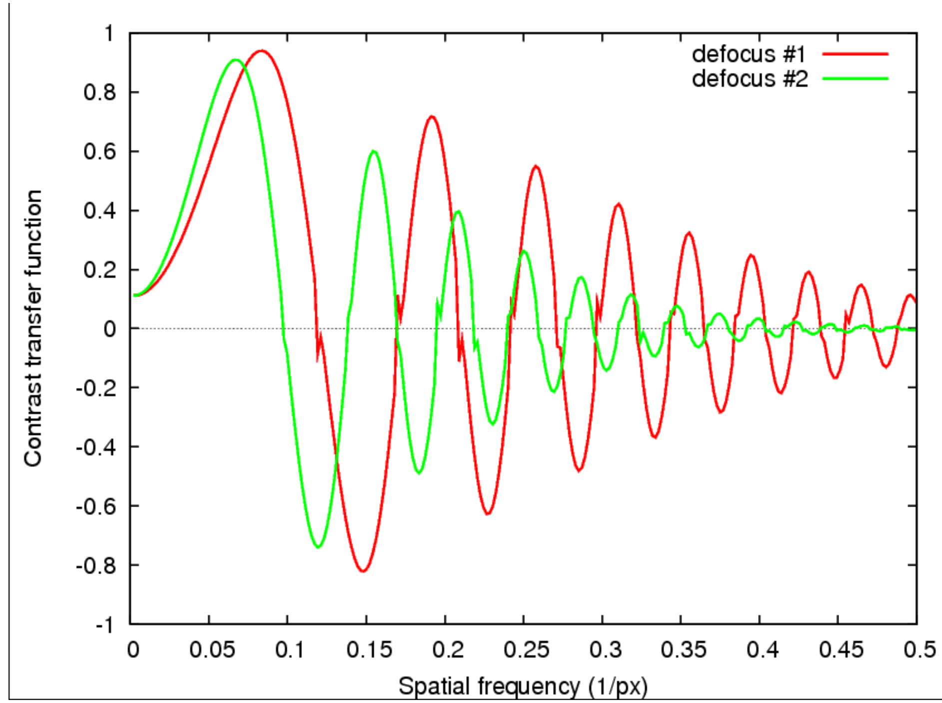
Cross-correlation vs. convolution

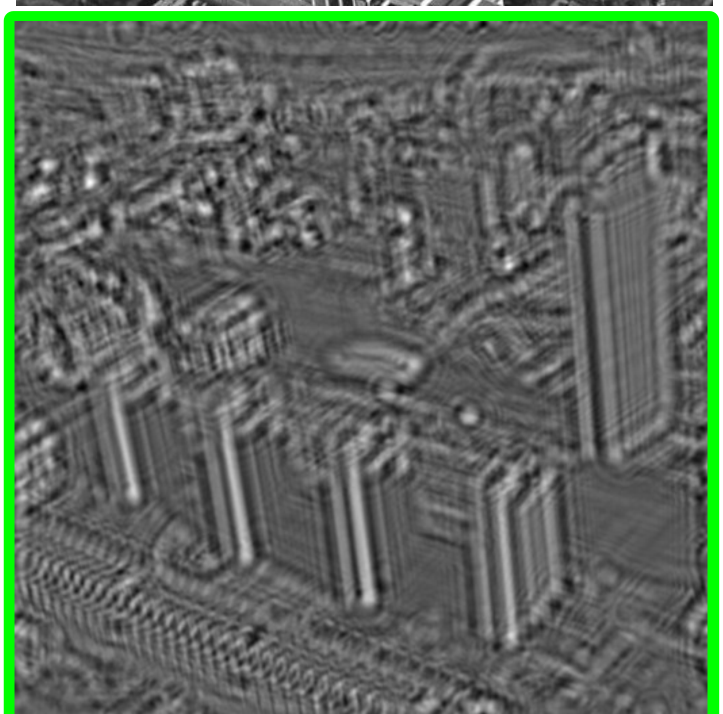
Complex conjugate:
If a Fourier coefficient *F(X)* has the form: a + bi
The complex conjugate *F*(X)* has the form: a - bi

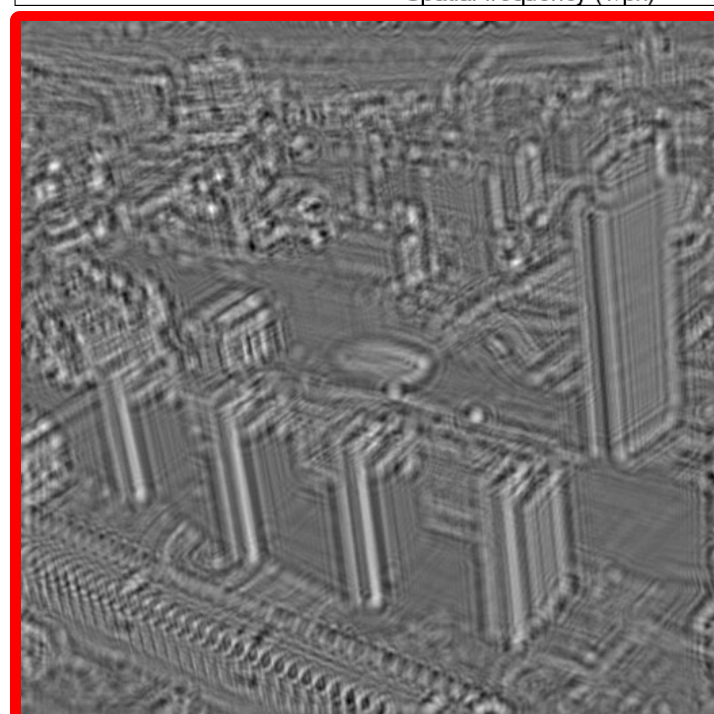
Cross-correlation: F*(X) G(X)

Convolution: F(X) G(X)

original

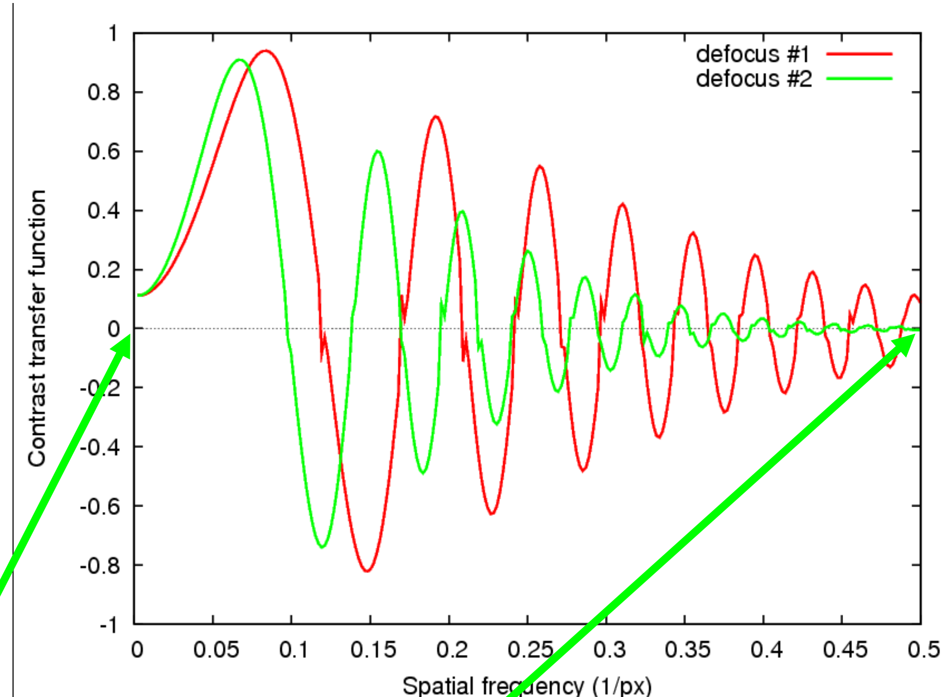

defocus #1
defocus #2
Contrast transfer function
Spatial frequency (1/px)
1
0.8
0.6
0.4
0.2
0
-0.2
-0.4
-0.6
-0.8
-1
0
0.05
0.1
0.15
0.2
0.25
0.3
0.35
0.4
0.45
0.5

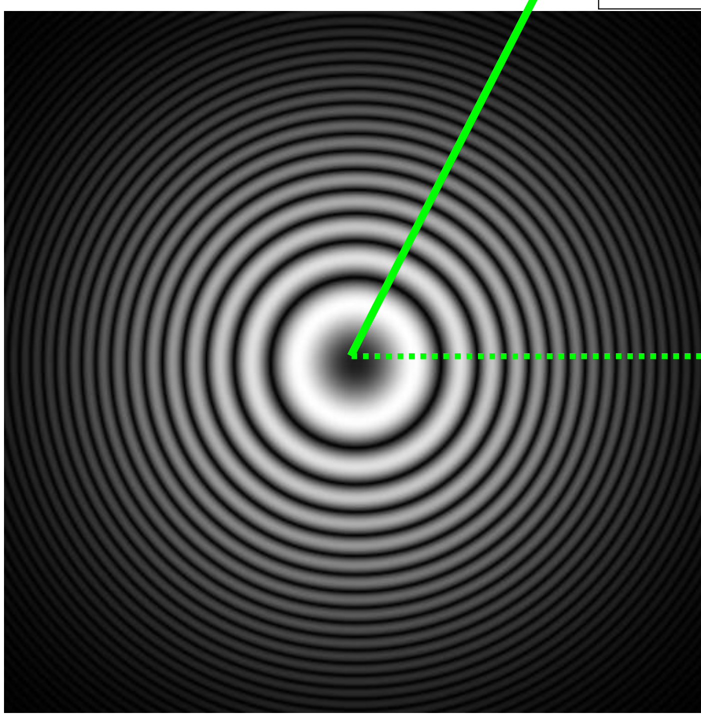




CTF

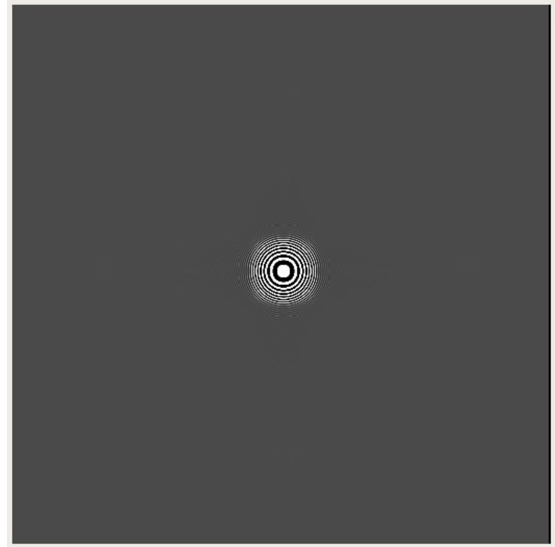
1D profile



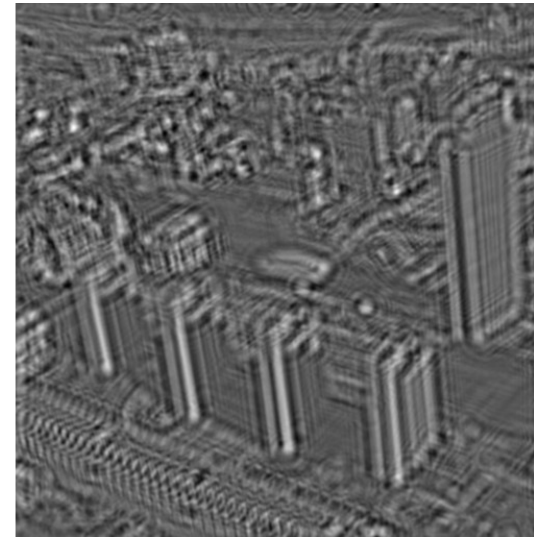


2D power spectrum
G(X)

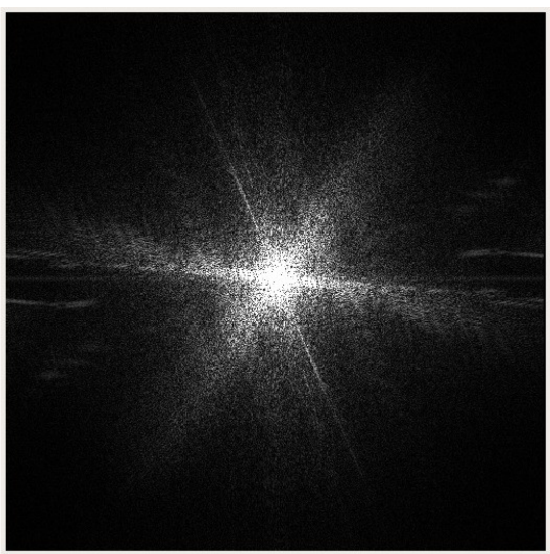
f(x)



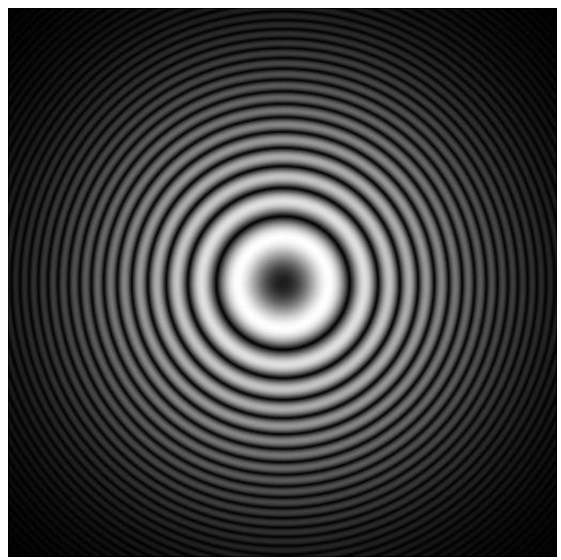
g(x)



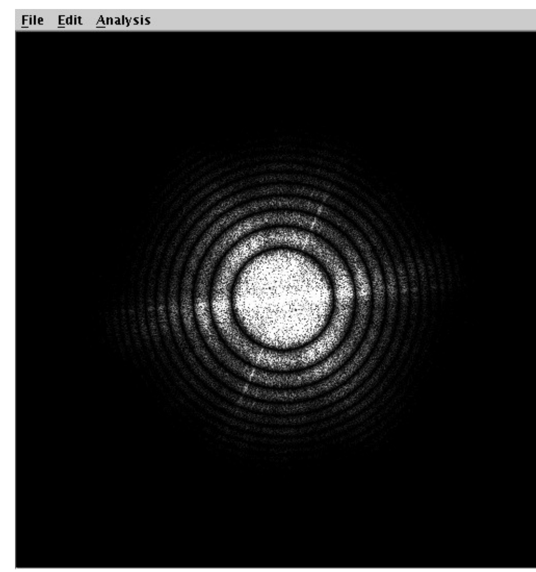
f(x) g(x)



F(X)

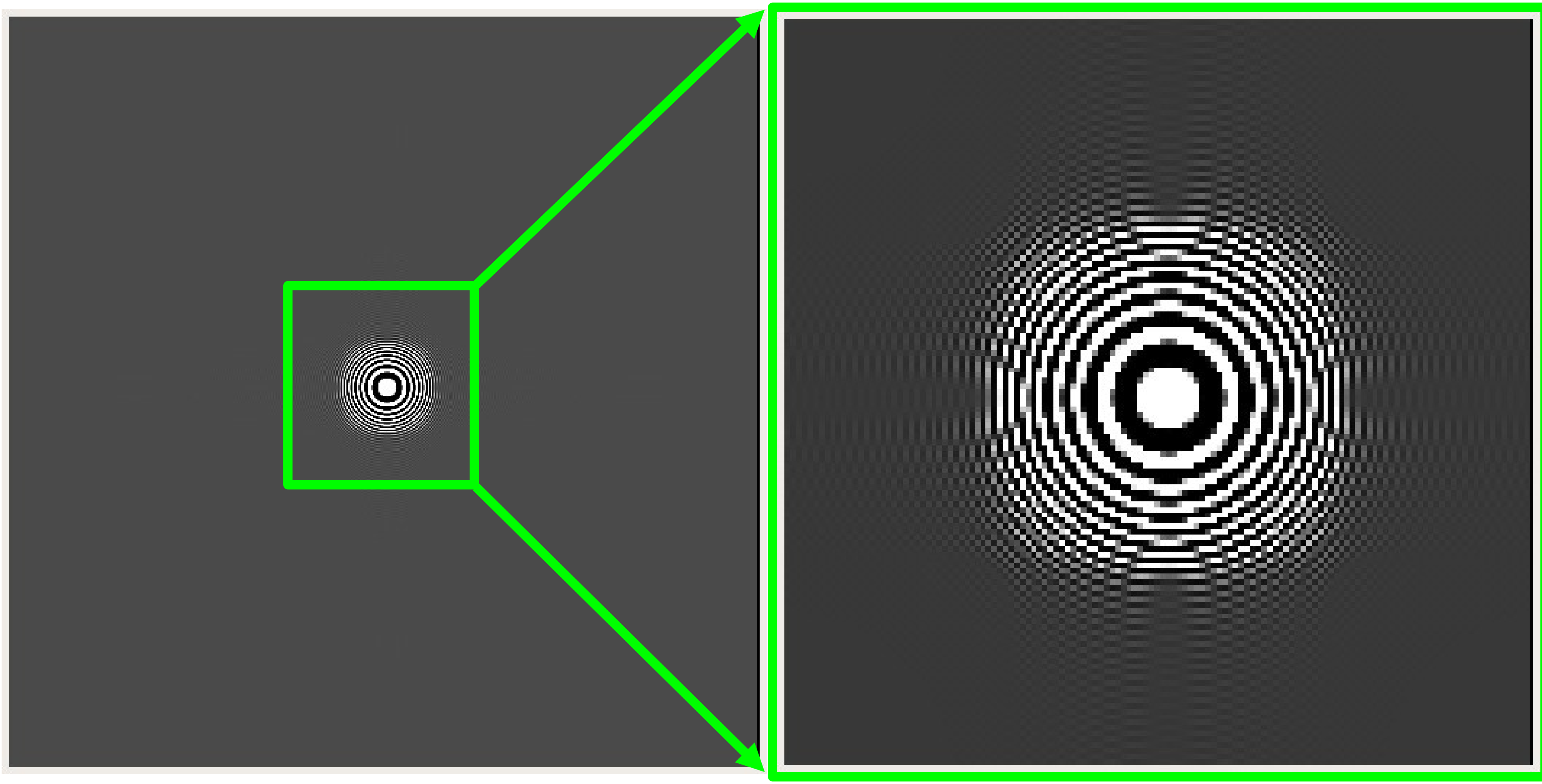


G(X)



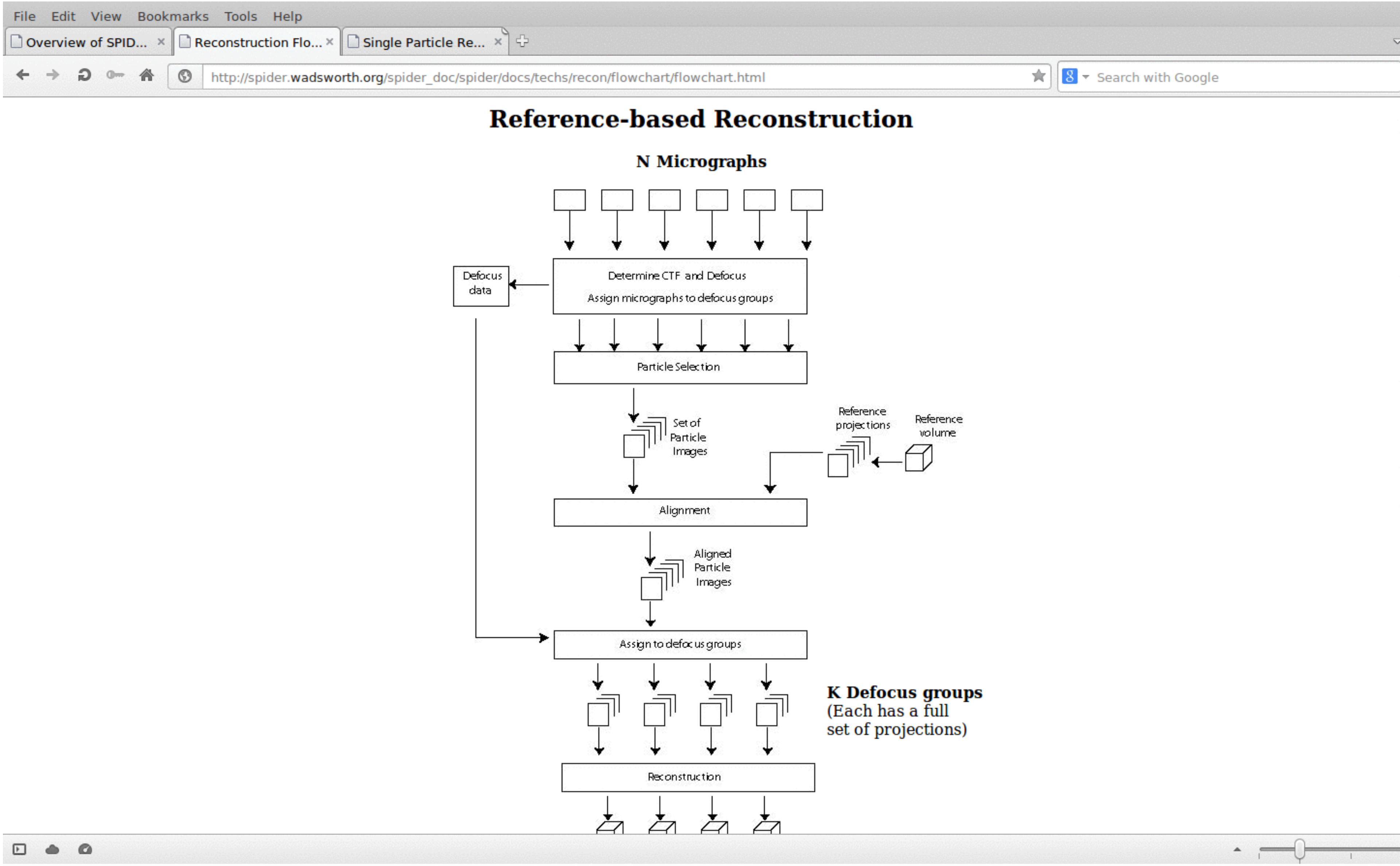
F(X) G(X)

Point spread function

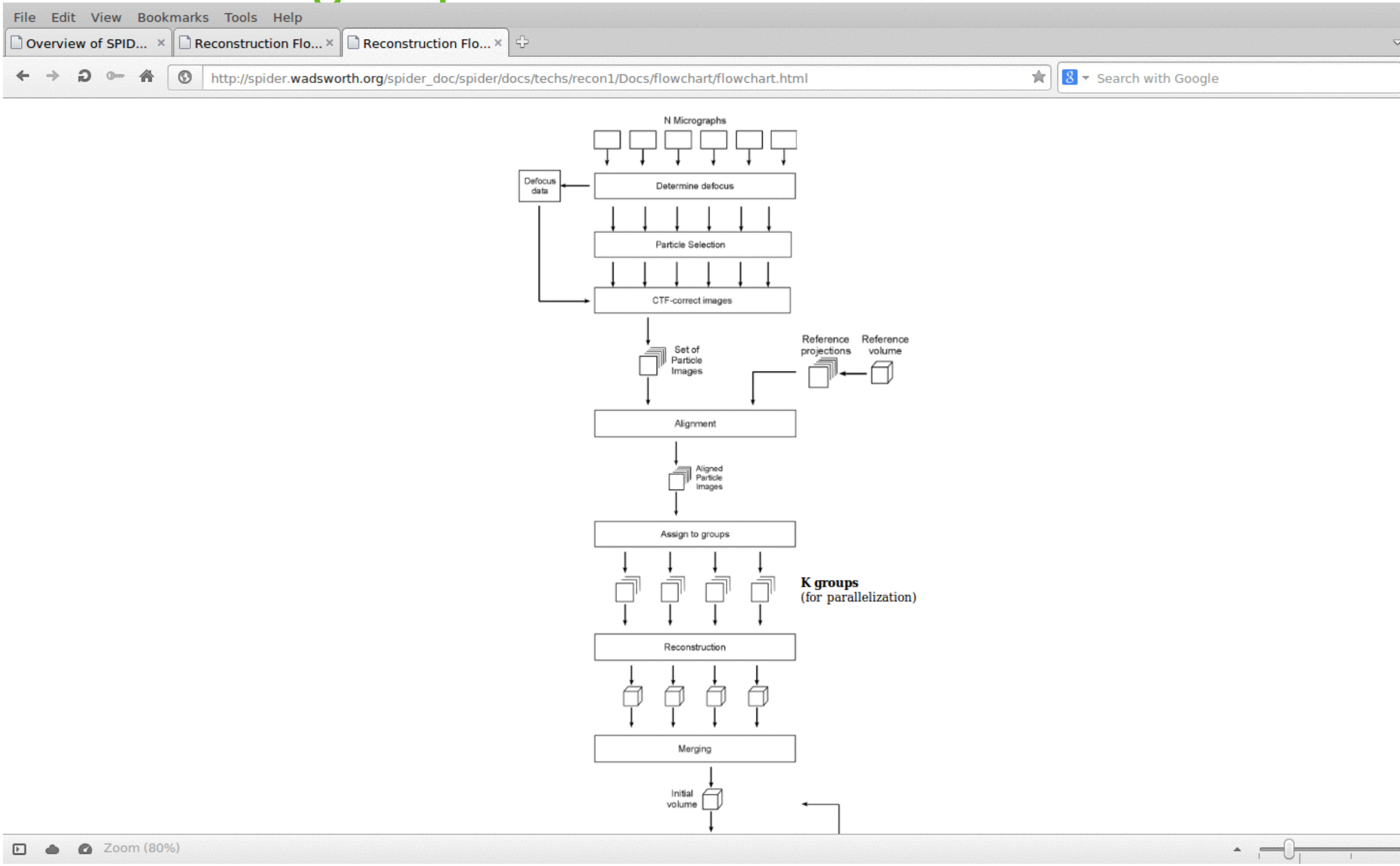


An ideal point spread function would be an infinitely-sharp point.

Defocus groups



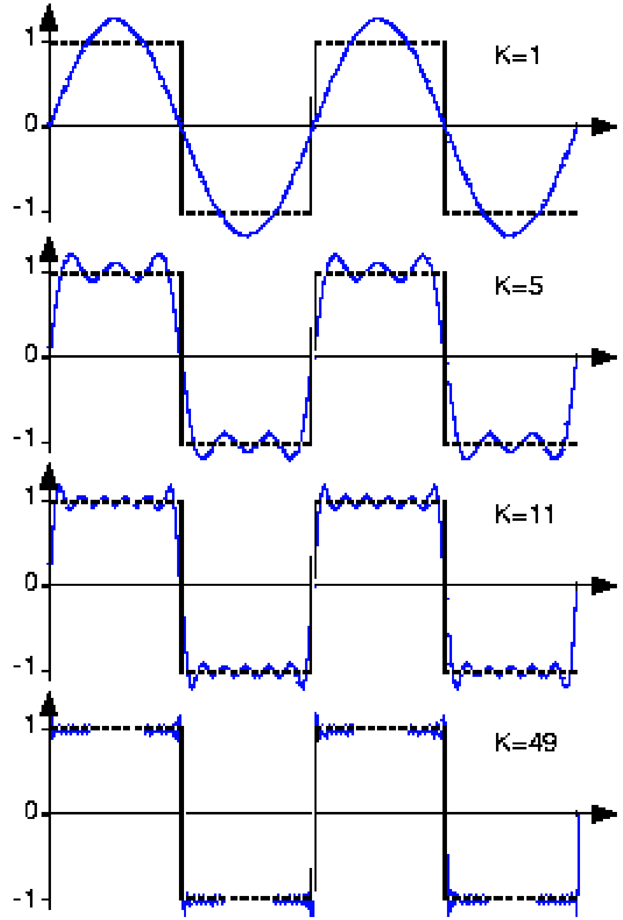
Defocus groups



Step function revisited

Fourier transforms: plot of step function

The higher the spatial frequencies (i.e., higher resolution) that are included, the more faithful the representation of the original function will be.



http://cnx.org

The power spectrum is the a real (as opposed to complex) map of the amplitudes of the Fourier transform

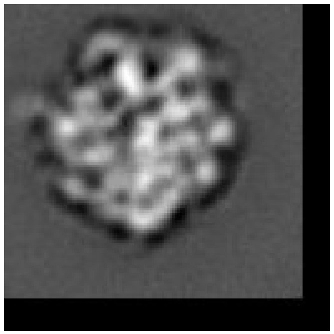


Image *f(x)*

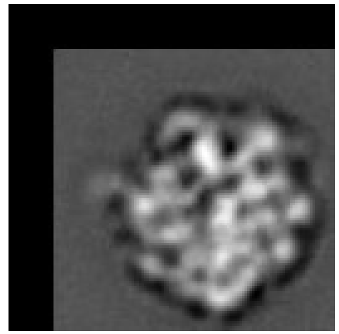
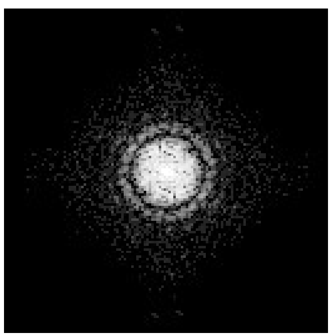
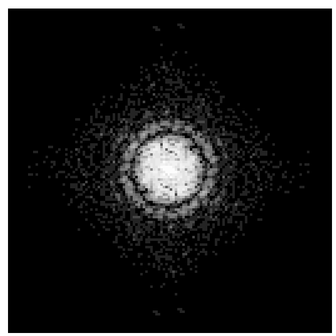


Image *g(x)*



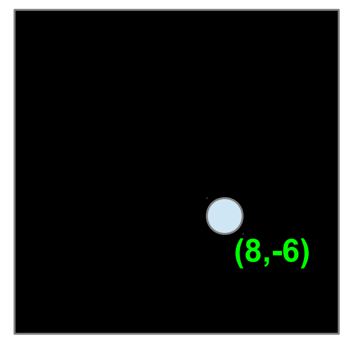
F.T. *F*(X)*
(complex conjugate)

x



F.T. *G(X)*

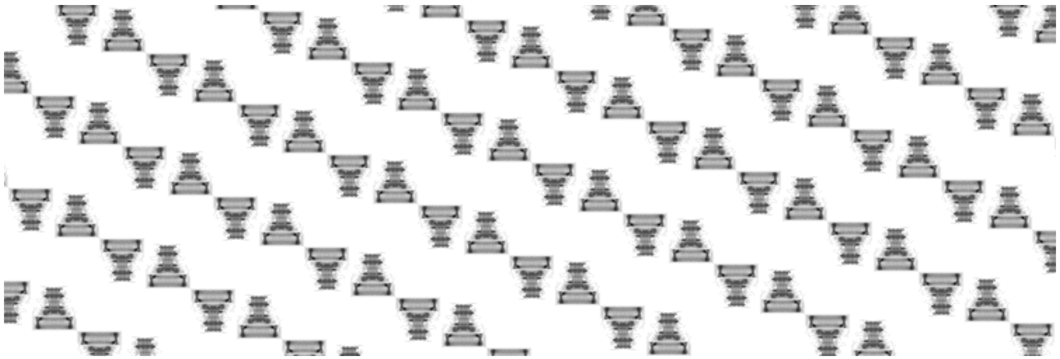
=



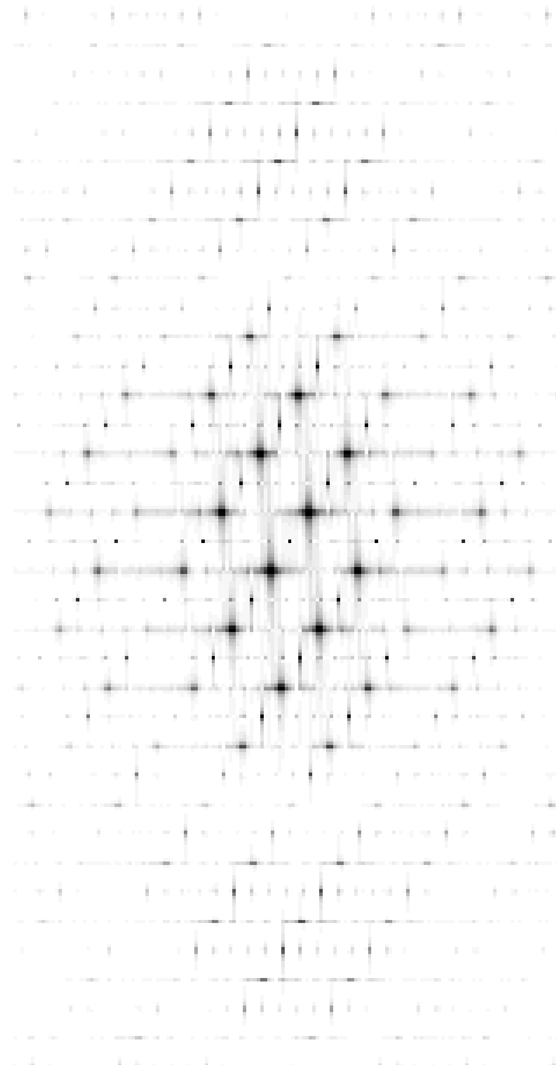
CCF

The position of the peak gives us the shifts that give the best match, *e.g.*, (8,-6).
It's more difficult to plot a 2D F.T. showing both amplitude and phase.

Fourier transform of a 2D crystal



h	k	Amp	Phase
0	0	500	0
1	-1	40	45
1	0	50	5
1	1	30	5
2	-2	2	54
2	-1	4	57



Power spectrum

QUESTION:
Why did I not list the Fourier data where h was negative?

h	k	Amp	Phase
0	0	500	0
1	-1	40	45
1	0	50	5
1	1	30	5
2	-2	2	54
2	-1	4	57

Friedel's Law

If the complex part of f(x) is zero, then

$$F(-X) = F^*(X)$$

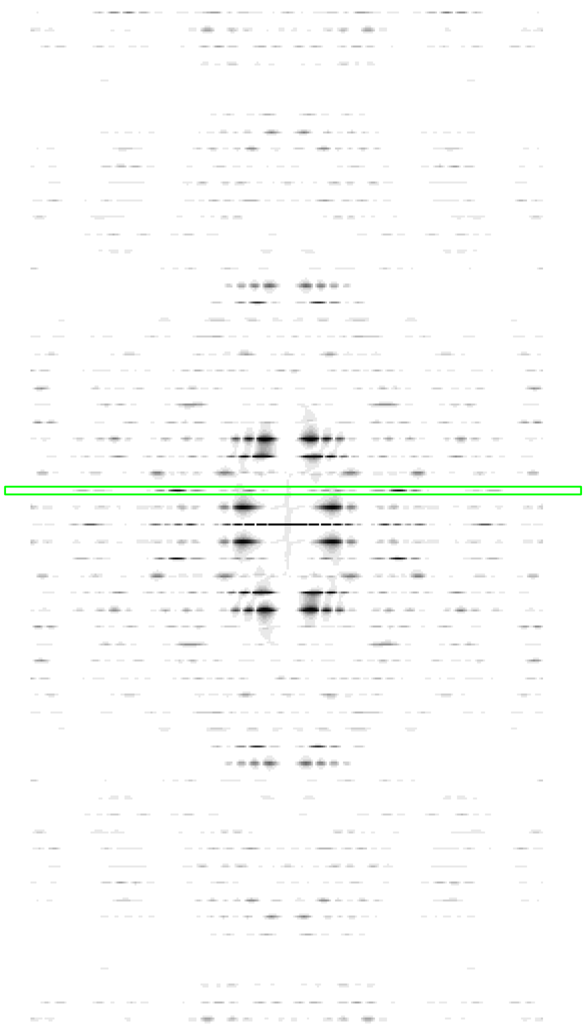
where * indicates the complex conjugate.

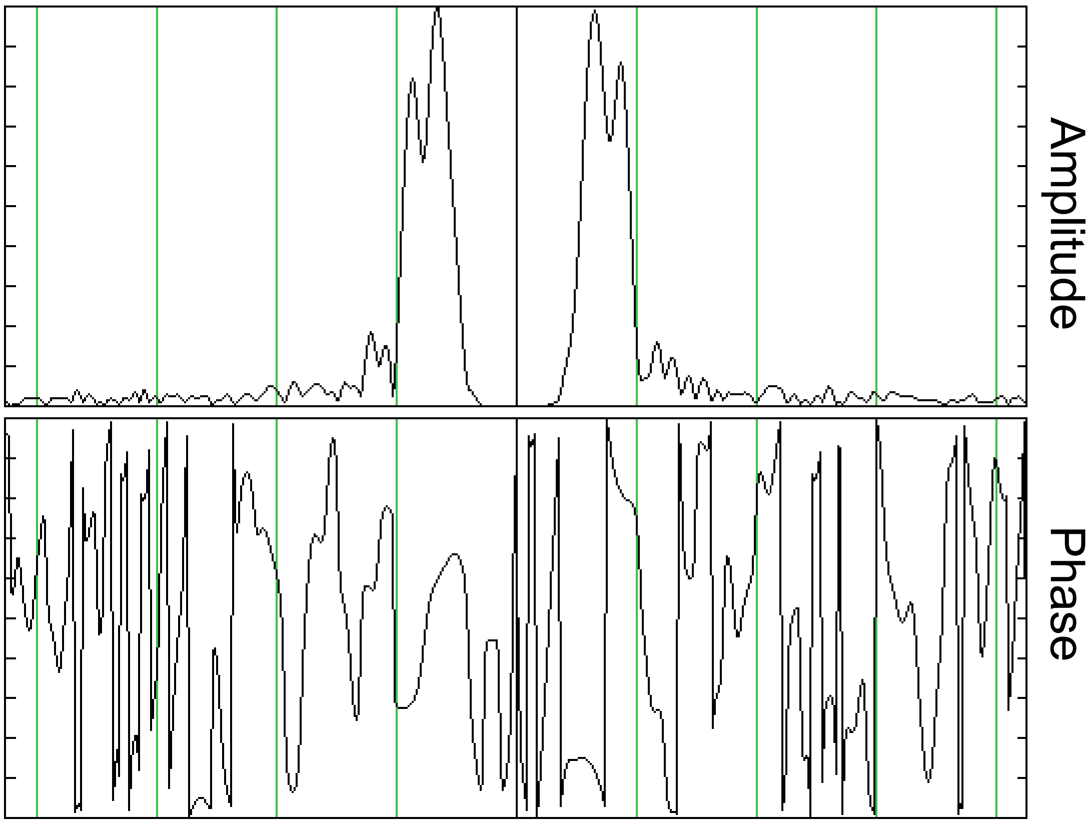
USE: Thus, centrosymmetrically related reflections have the same amplitude but opposite phases (Friedel's law).

When calculating a transform of an image, one only has to calculate half of it. The other half is related by Friedel's law.

From David DeRosier

2D Fourier transform of a helix





More orientational alignment

Orientation alignment

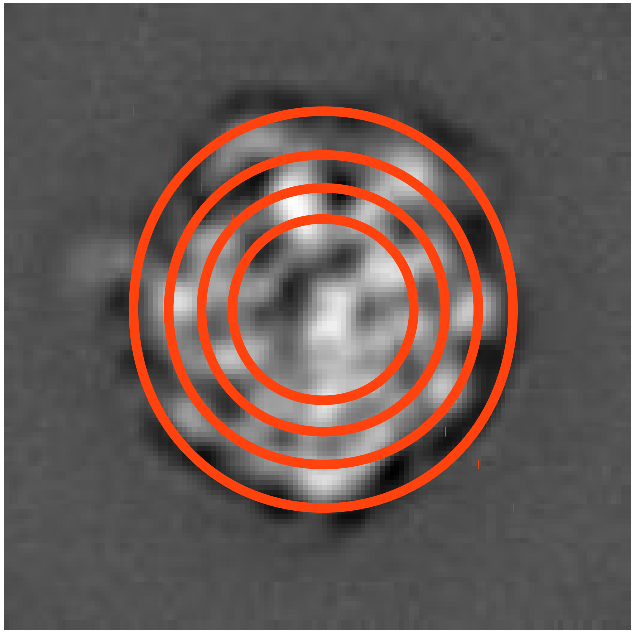


Image 1

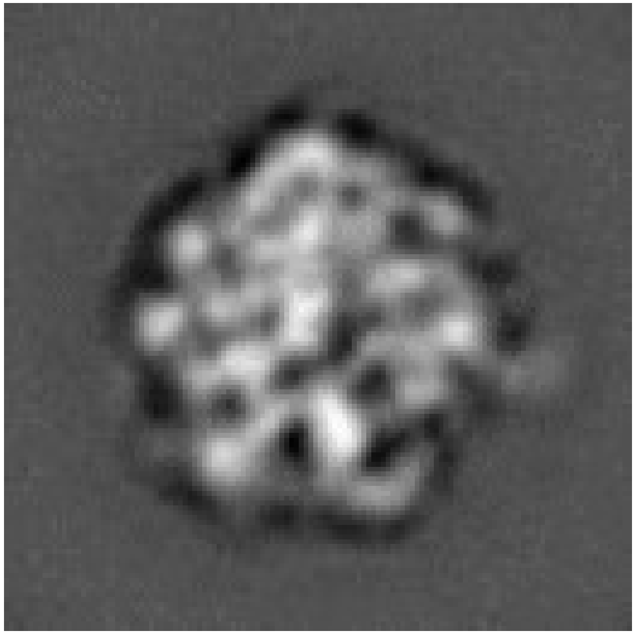


Image 2

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

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

Orientation alignment

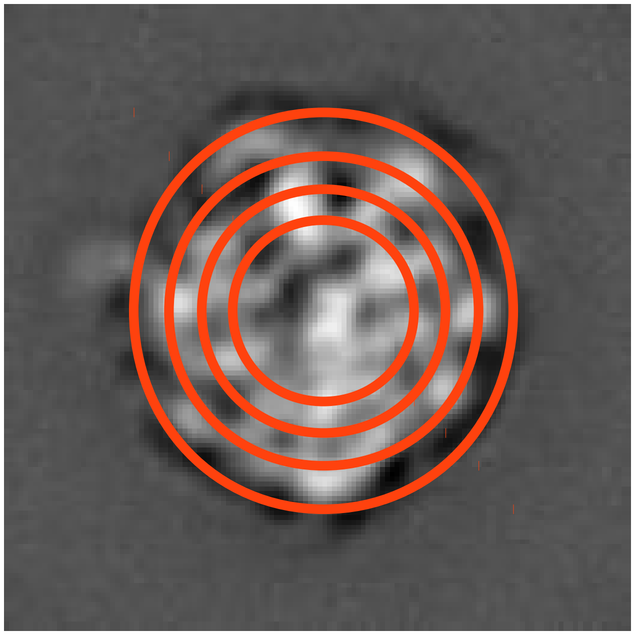


Image 1

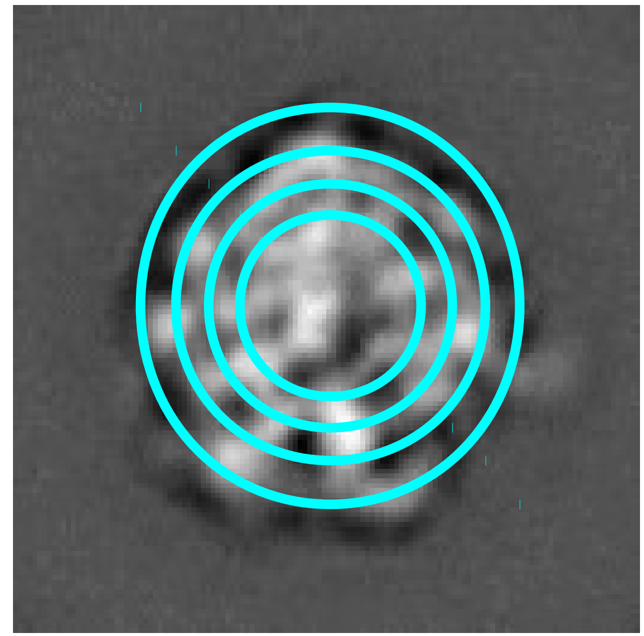
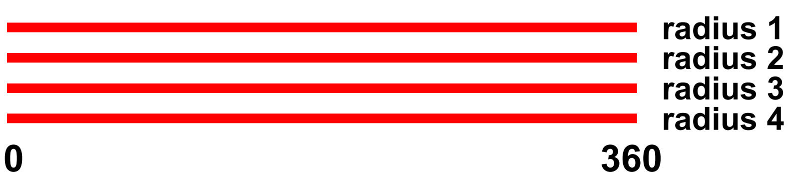
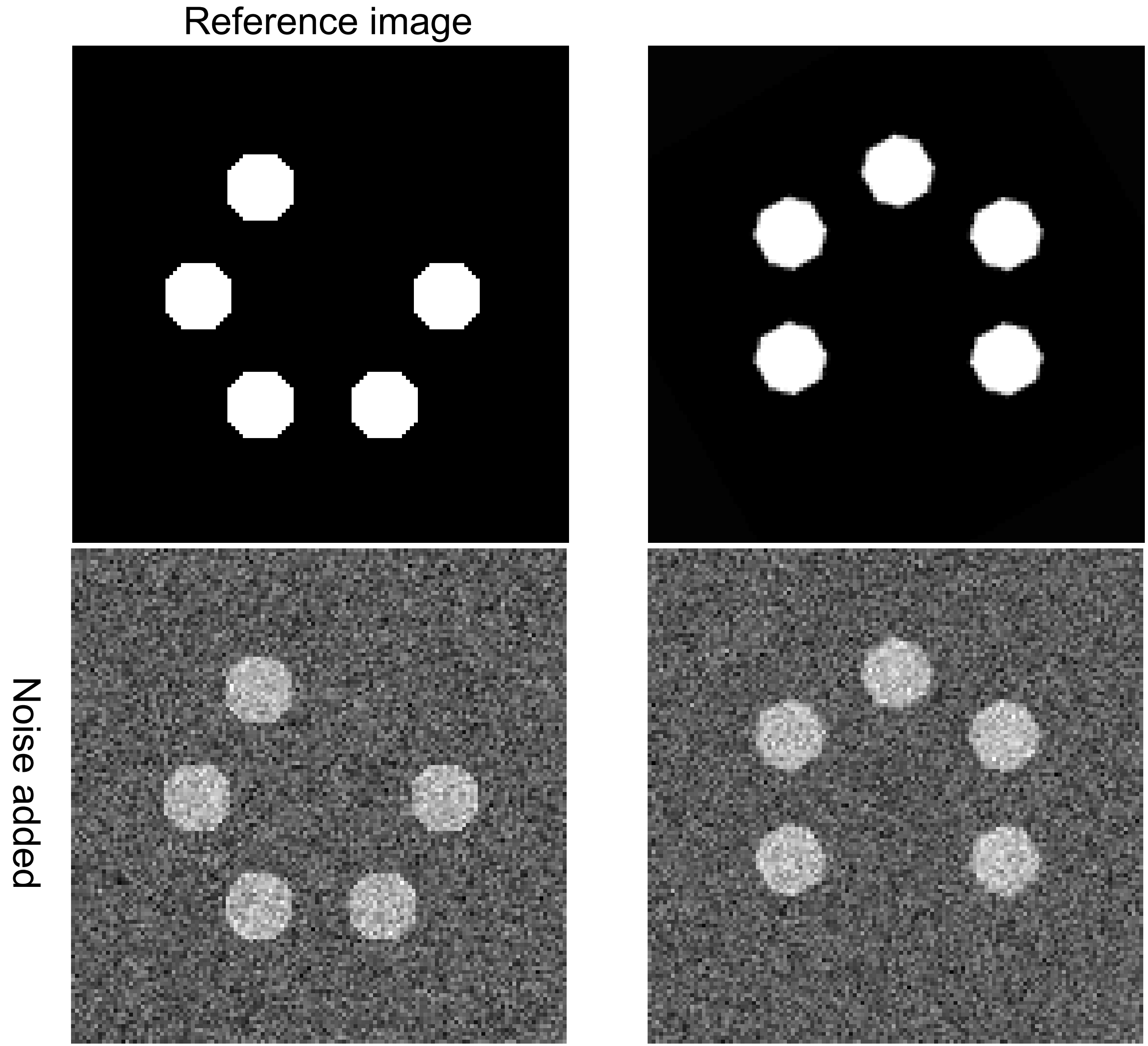


Image 2





Reference image
Noise added

Orientation alignment

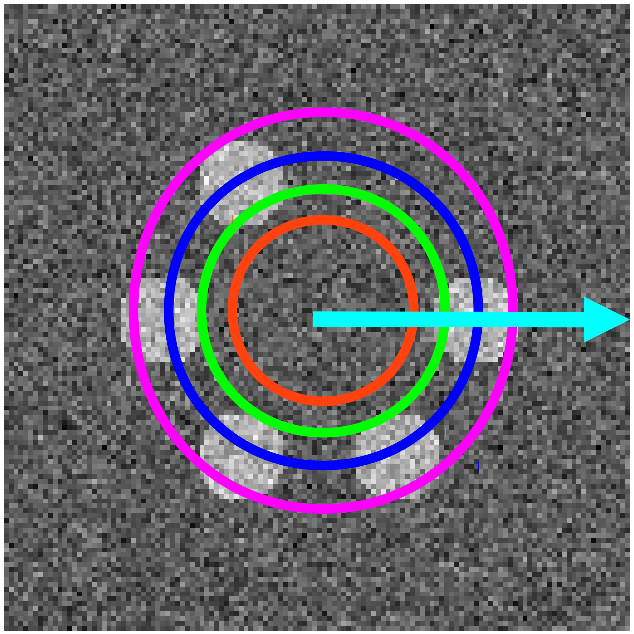


Image 1

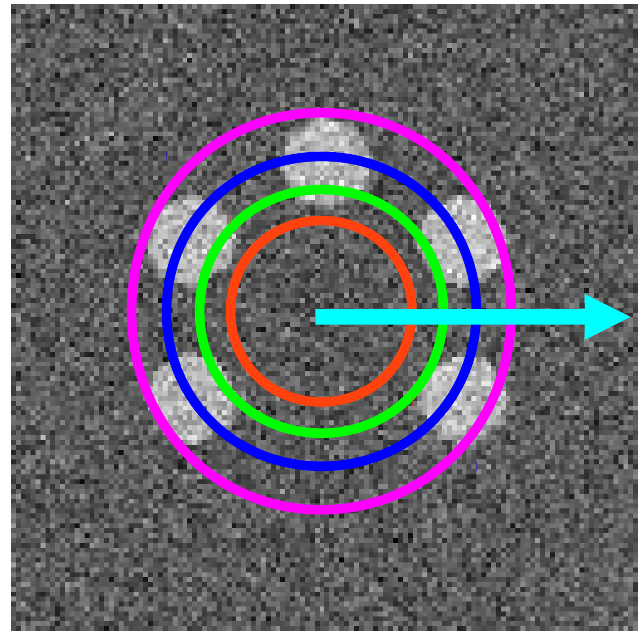
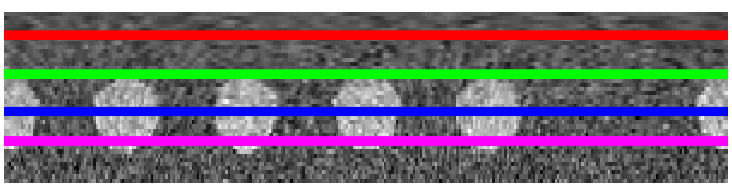
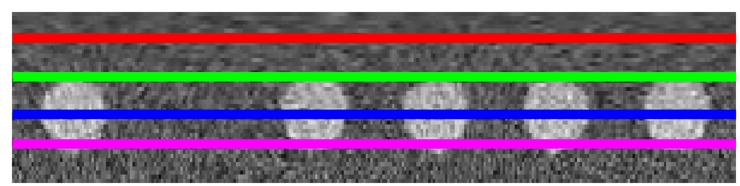


Image 2



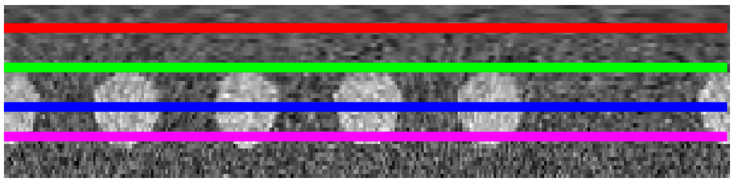
radius 1
radius 2
radius 3
radius 4



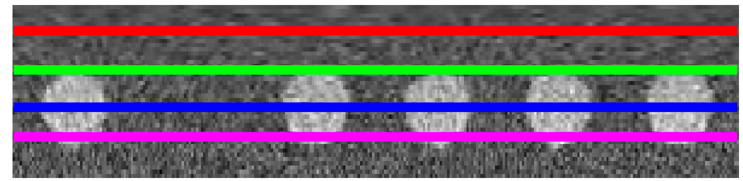
0 360 0 360

Polar representation

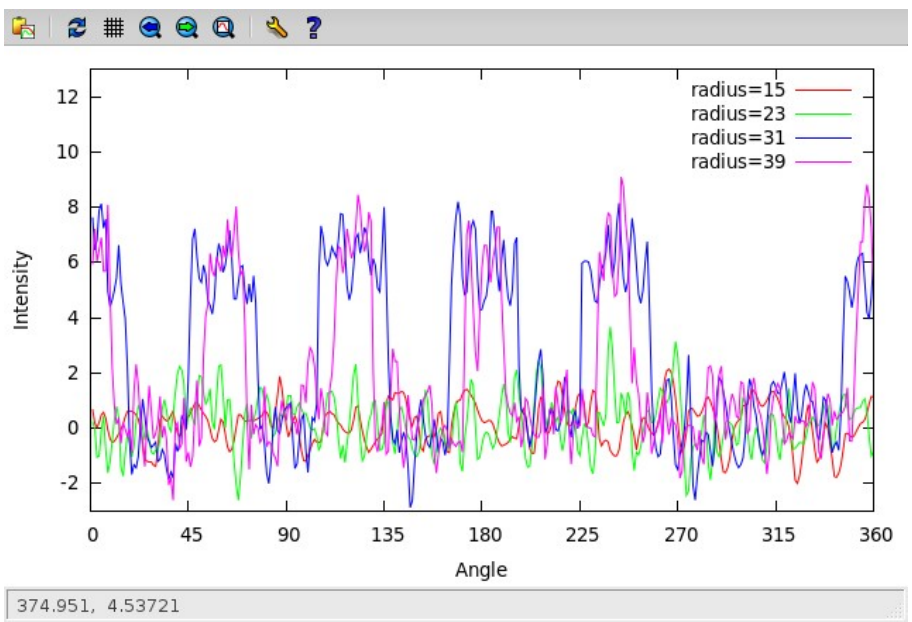
Orientation alignment

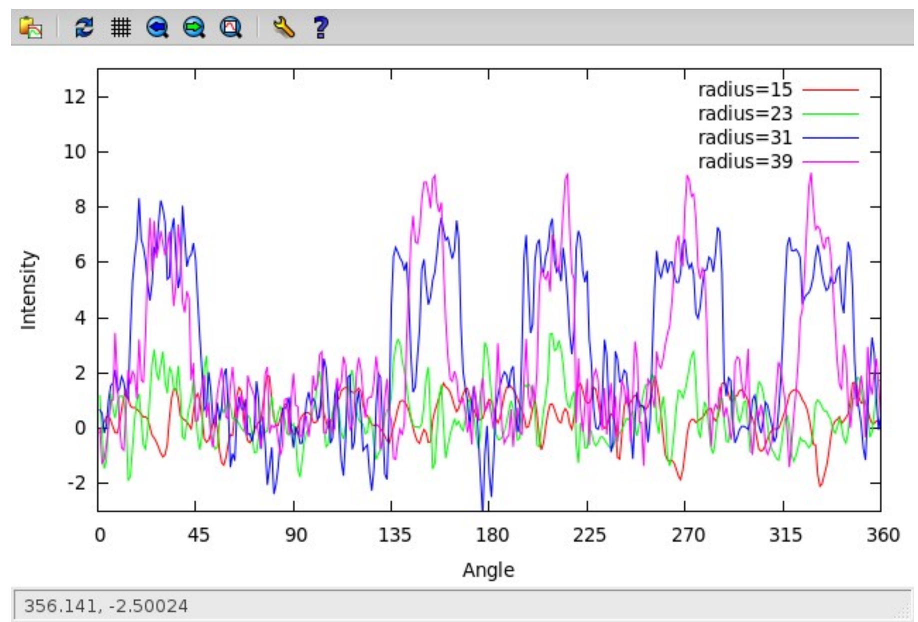


radius 1
radius 2
radius 3
radius 4

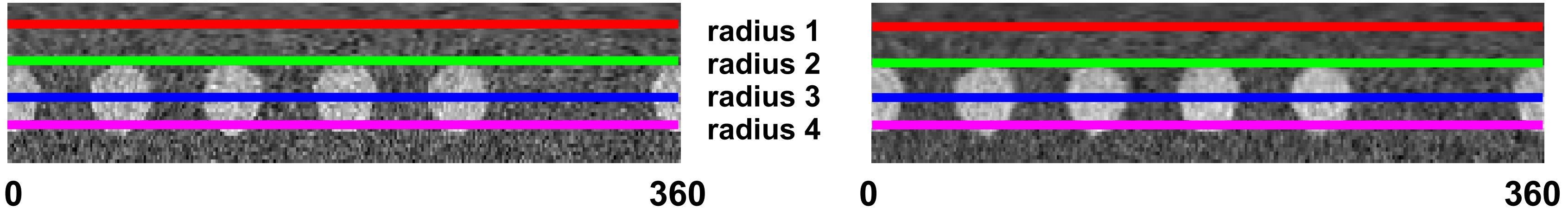


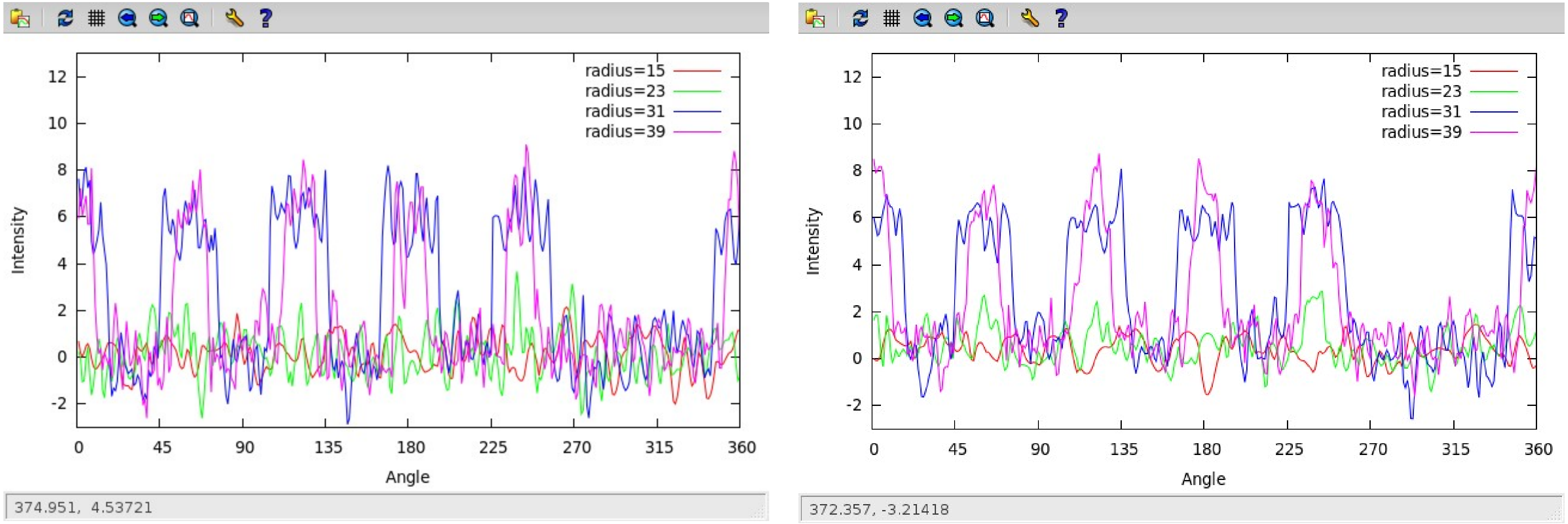
0 360 0 360





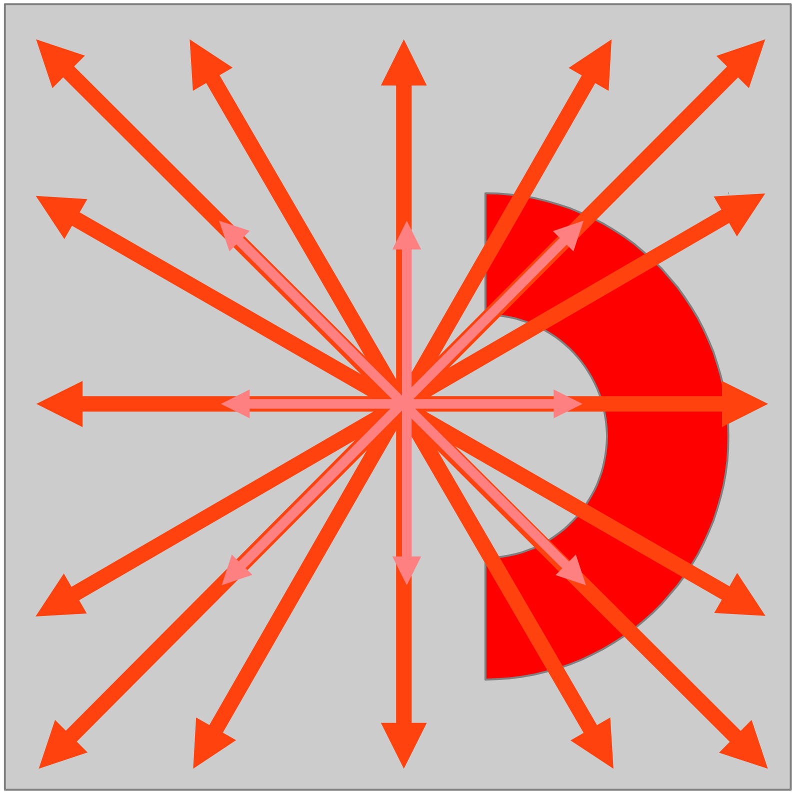
Orientation alignment: After rotation





Another strategy for translation and orientation alignment

Translational and orientation alignment are interdependent



Set of all shifts of up to 1 pixel

Set of all new shifts of up to 2 pixels

Shifts of (0, +/-1, +/-2) pixels results in 25 orientation searches.

The power spectrum is translationally invariant.
If we shift the object in real space,
the power spectrum is unchanged.

Cross-correlation function (CCF)

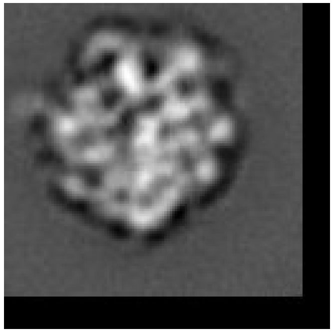


Image *f(x)*

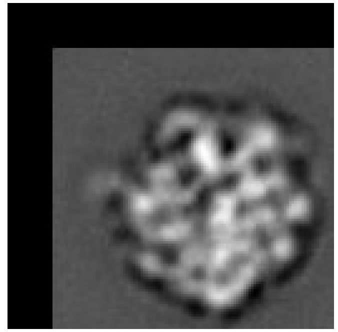
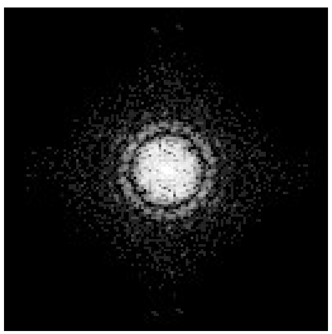
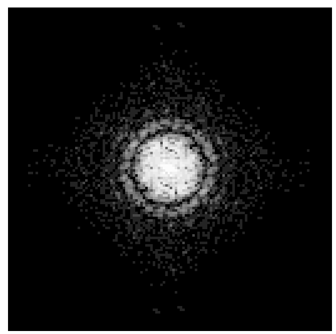


Image *g(x)*



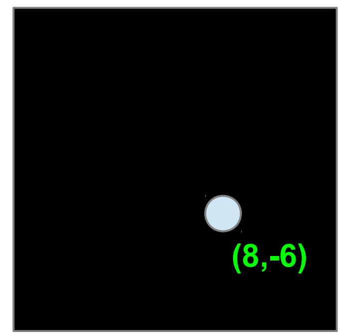
F.T. *F*(X)*
(complex conjugate)

x



F.T. *G(X)*

=



CCF

The position of the peak gives us the shifts that give the best match, *e.g.*, (8,-6).

Cross-correlation function (CCF)

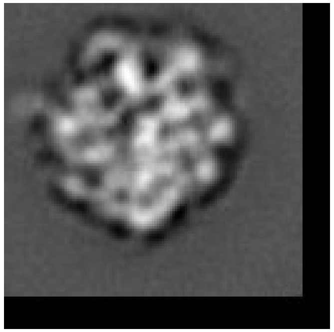


Image *f(x)*

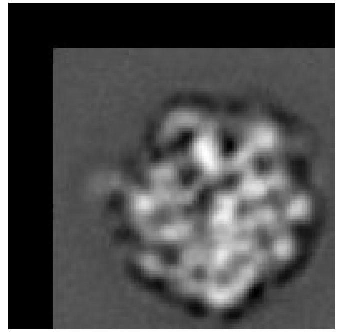
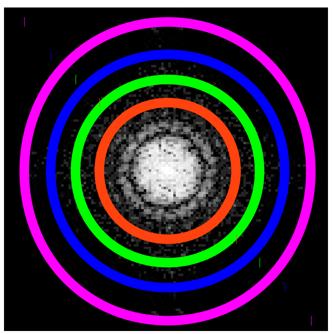
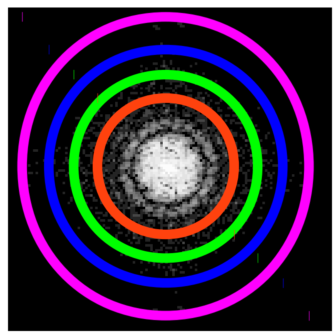


Image *g(x)*



F.T. *F*(X)*
(complex conjugate)

x



F.T. *G(X)*

=

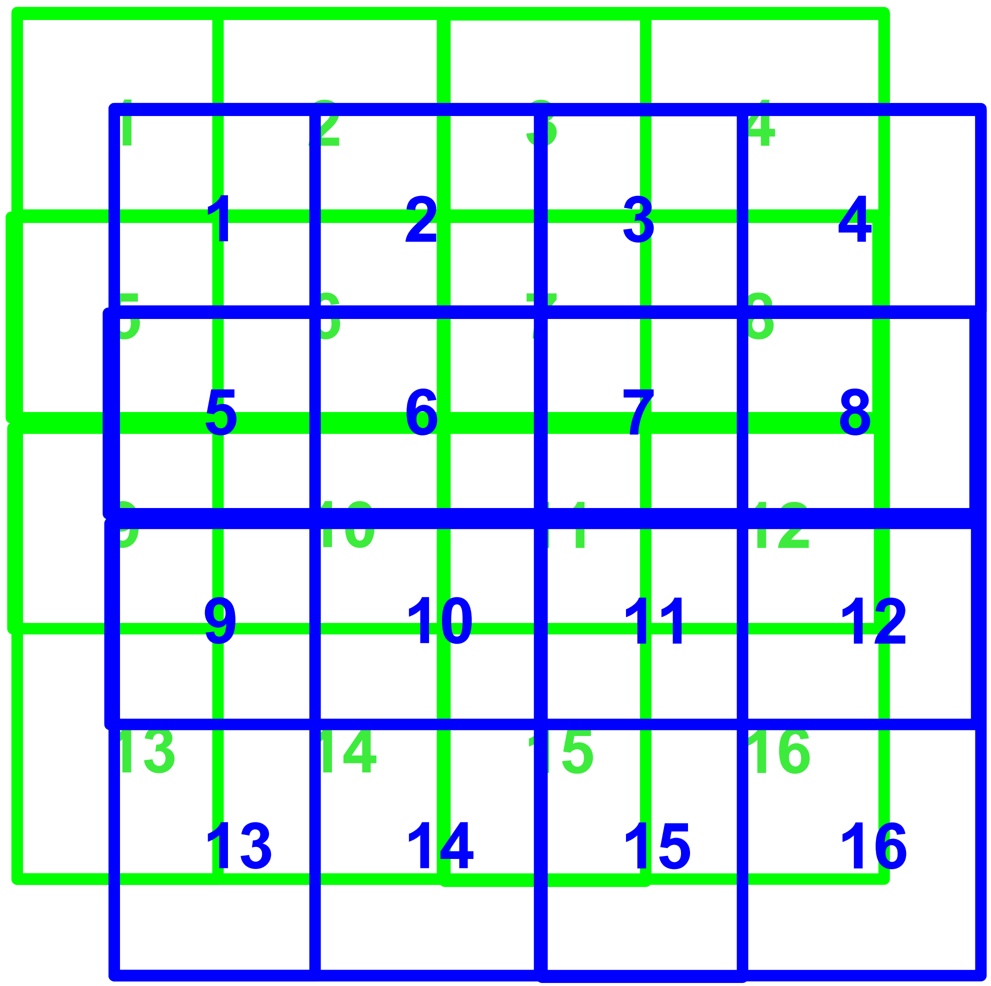
Series of
1D CCFs

Problems:
1. The power spectrum of a roughly round object is roughly round.
2. The amplitudes fall off quickly, so you don't have many rings of useful data.

More interpolation

Bammes... Chiu (2012) J. Struct. Biol.

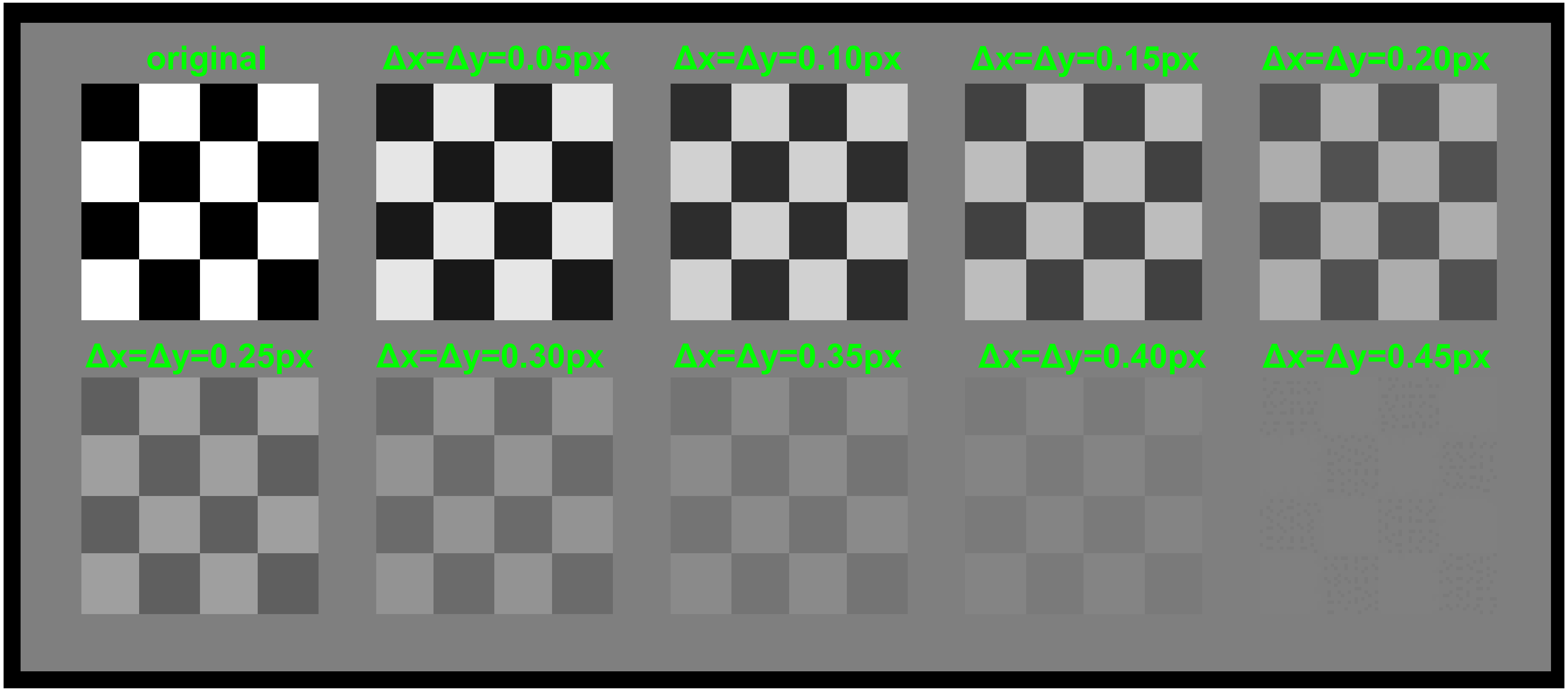
Shifts

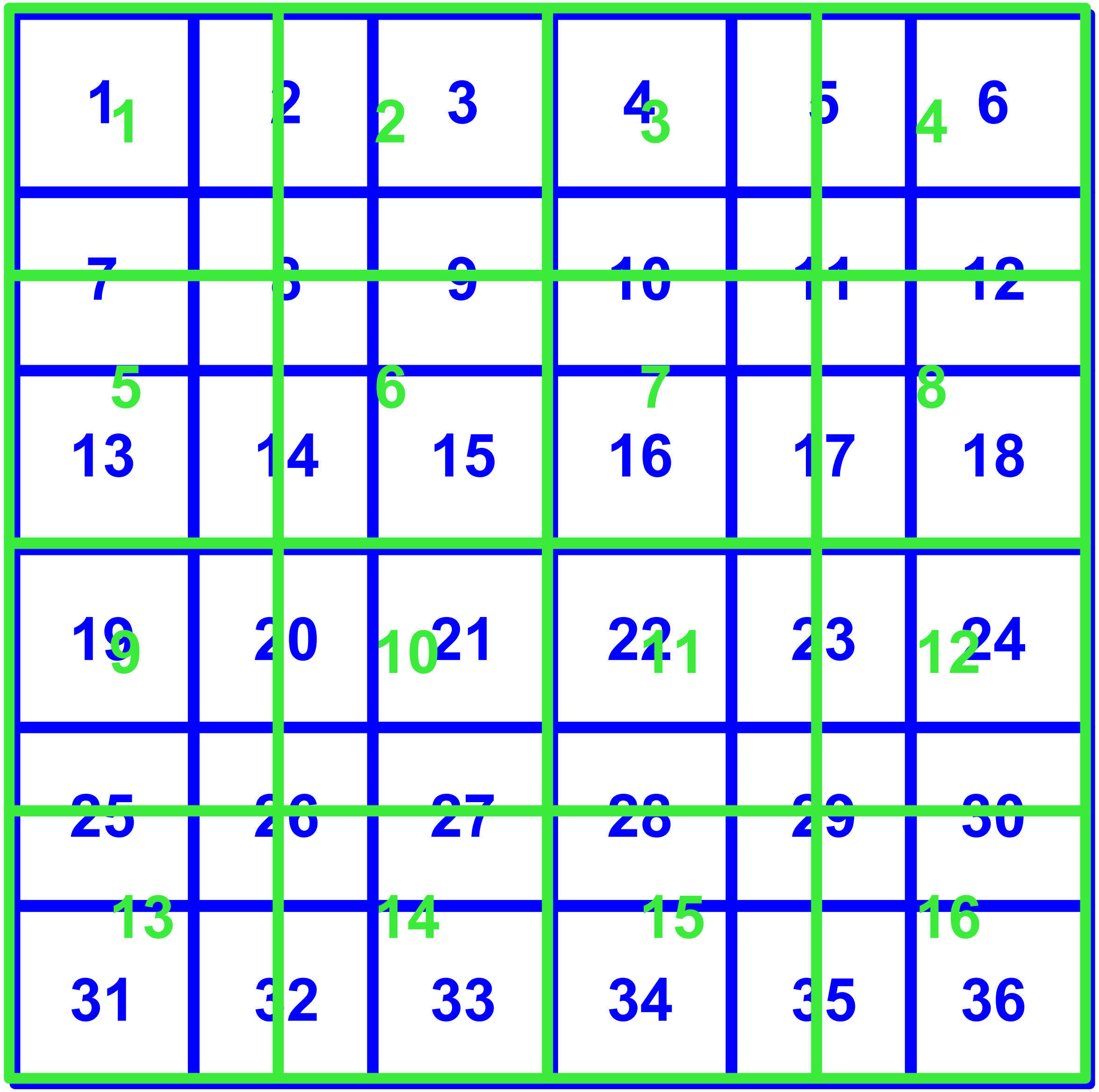


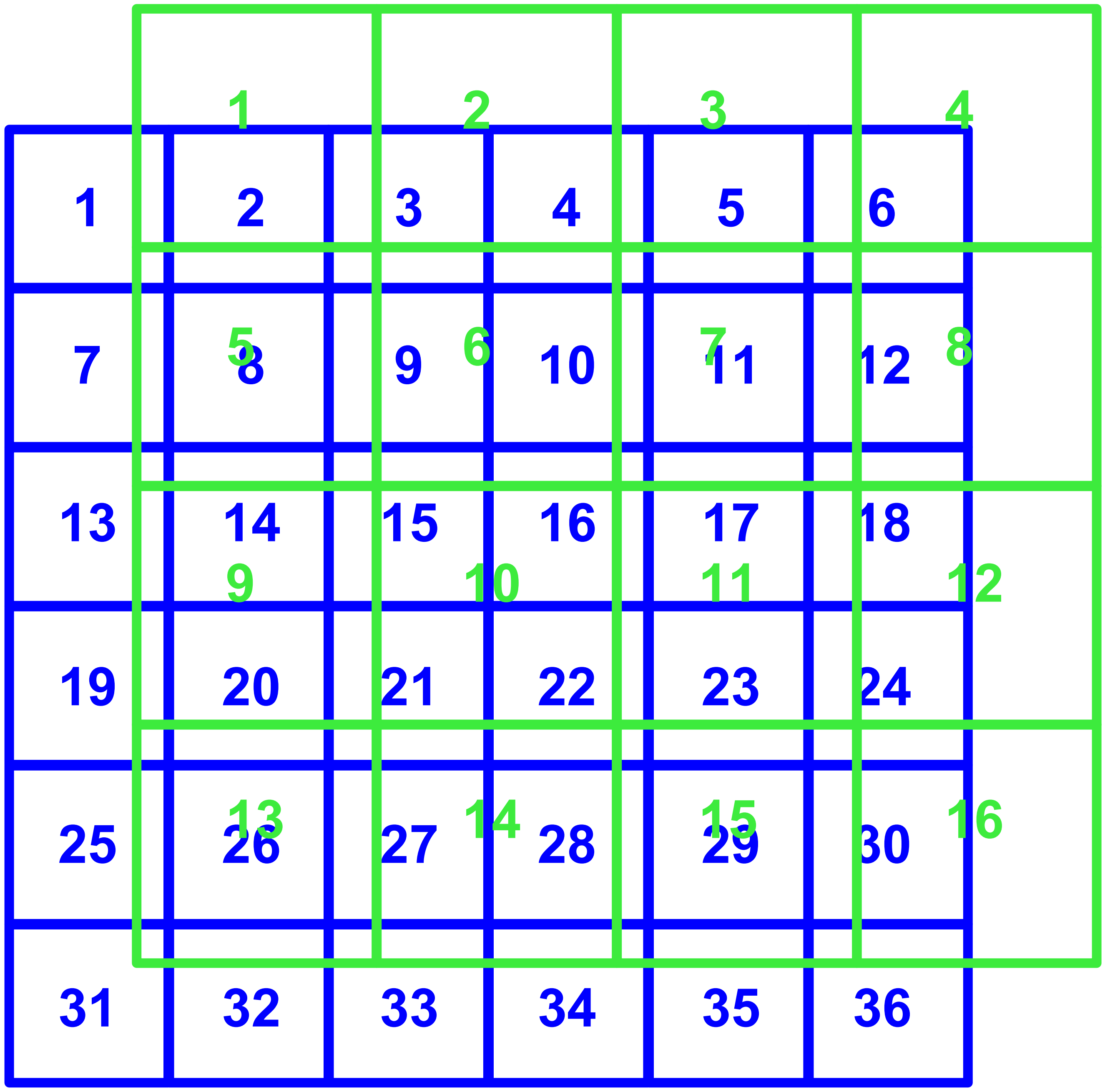
Suppose we shift the image in *x* & *y*.

The new pixels will be weighted averages of the old pixels.

Effect of shifts



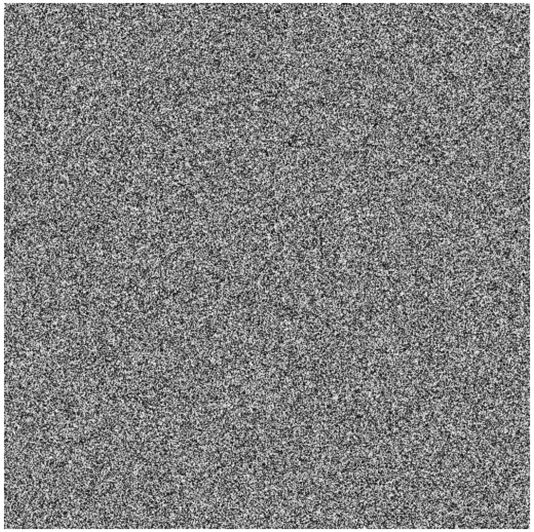

1 2 3 4 5 6
7 8 9 10 11 12
13 14 15 16 17 18
19 20 21 22 23 24
25 26 27 28 29 30
31 32 33 34 35 36
1 2 3 4
5 6 7 8
9 10 11 12
13 14 15 16


1 2 3 4 5 6
7 8 9 10 11 12
13 14 15 16 17 18
19 20 21 22 23 24
25 26 27 28 29 30
31 32 33 34 35 36
1 2 3 4
5 6 7 8
9 10 11 12
13 14 15 16

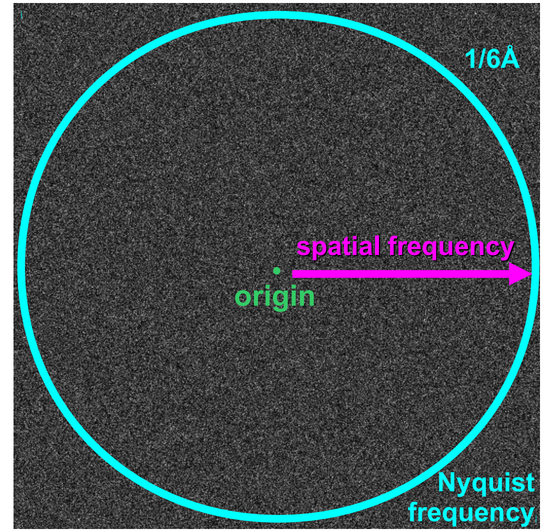
Questions

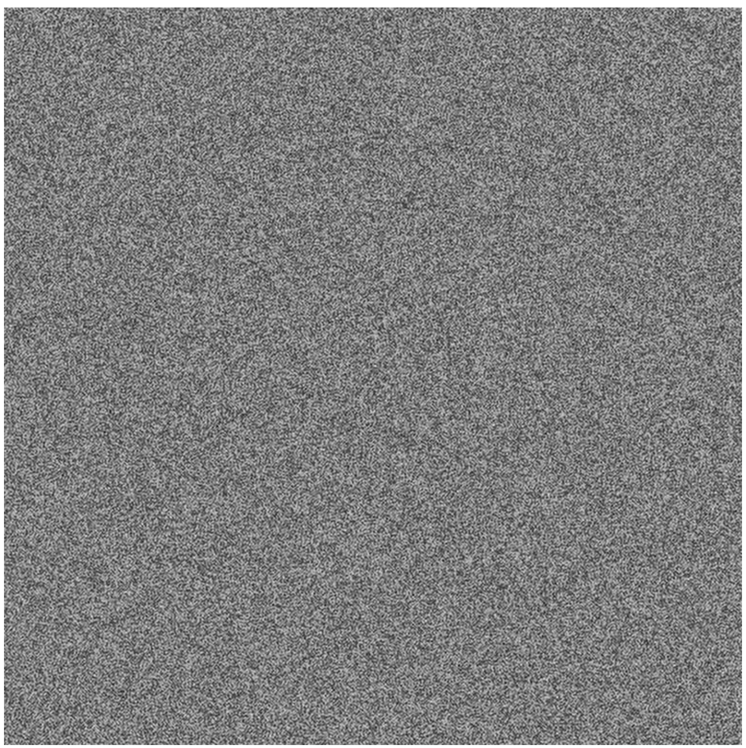
1) If the pixel size is 3 Å/px, what is the Nyquist frequency?
   - **ANSWER**: 1/6Å
2) If we oversample/upscale the image by a factor of 1.5X, what is the new pixel size?
   - **ANSWER**: 2 Å/px
3) What will be the new Nyquist frequency in the oversampled image?
   - **ANSWER**: 1/4Å

White noise

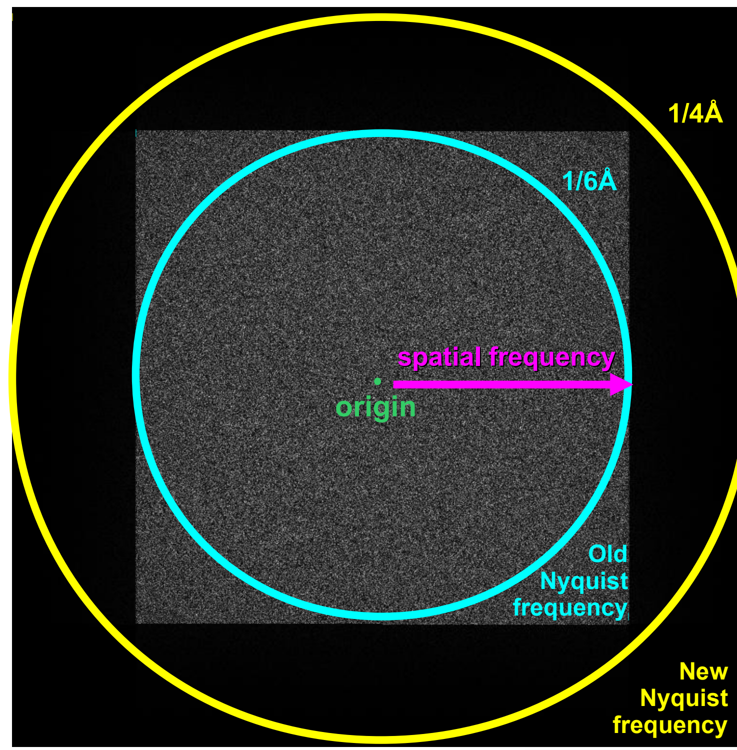


Power spectrum





Upscaled



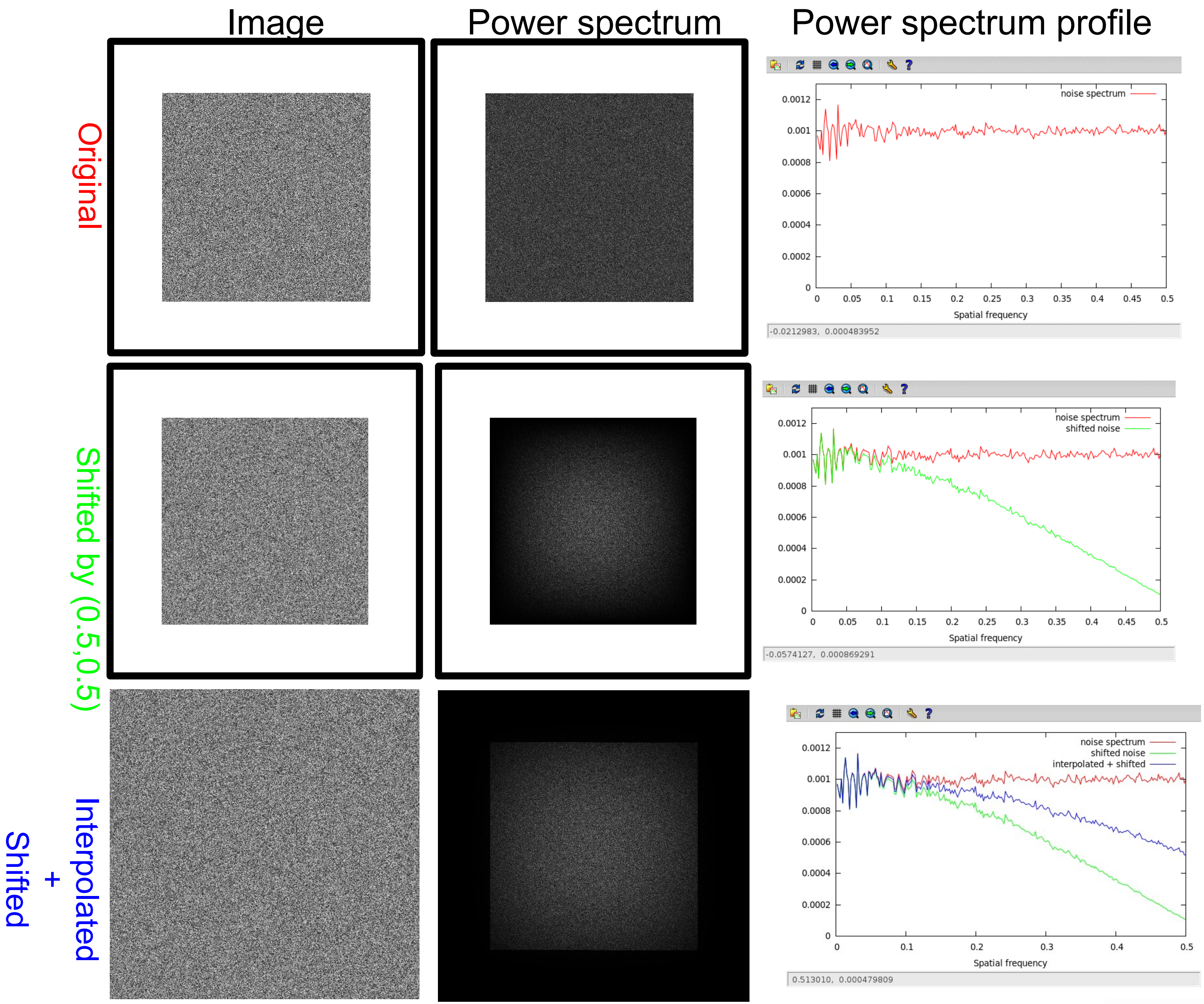
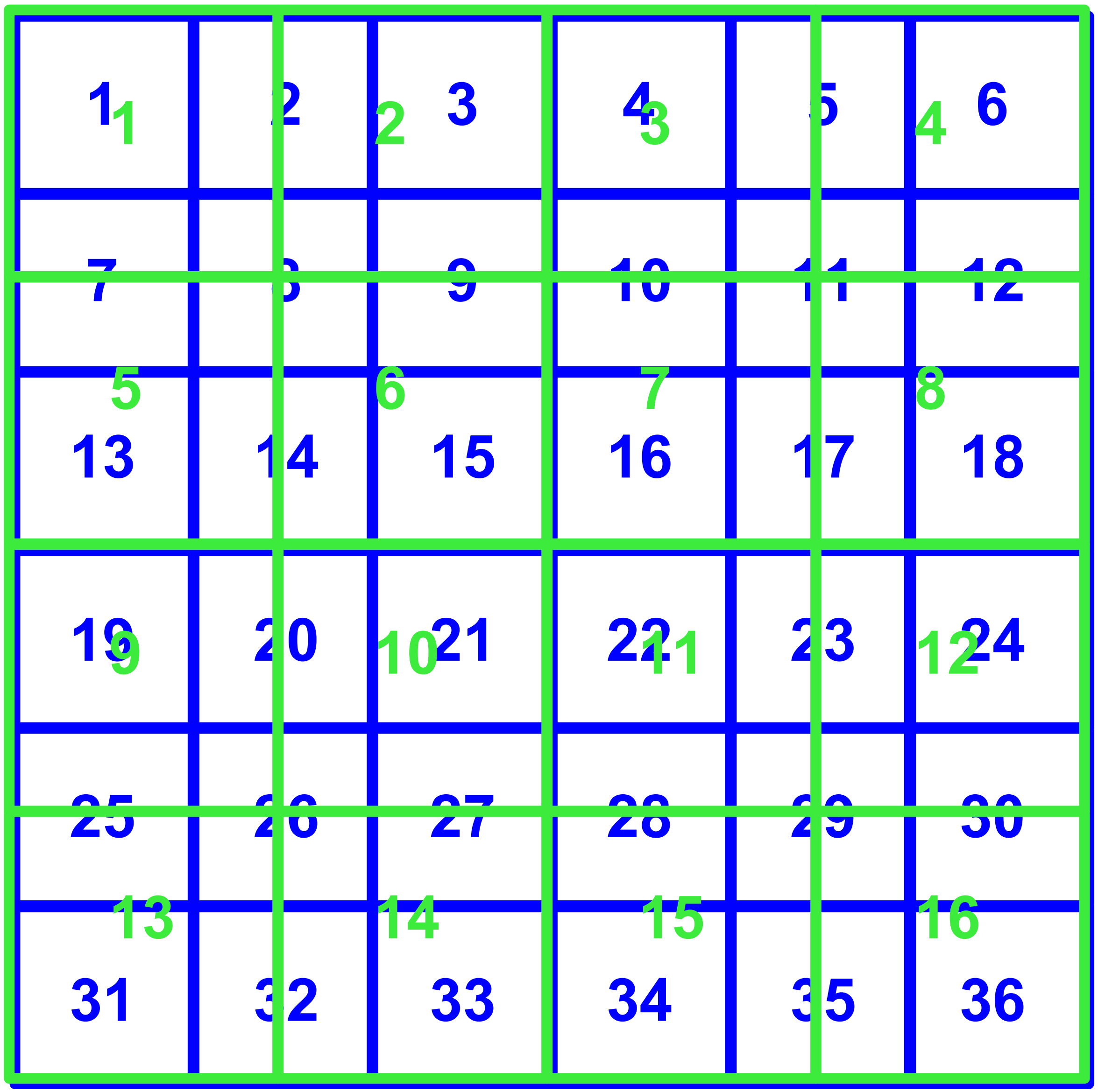


Image
Power spectrum
Power spectrum profile
Original
Shifted by (0.5,0.5)
Interpolated + Shifted
noise spectrum
shifted noise
interpolated + shifted
Spatial frequency
-0.0212983, 0.000483952
-0.0574127, 0.000869291
0.513010, 0.000479809


1 2 3 4 5 6
7 8 9 10 11 12
13 14 15 16 17 18
19 20 21 22 23 24
25 26 27 28 29 30
31 32 33 34 35 36
1 2 3 4
5 6 7 8
9 10 11 12
13 14 15 16

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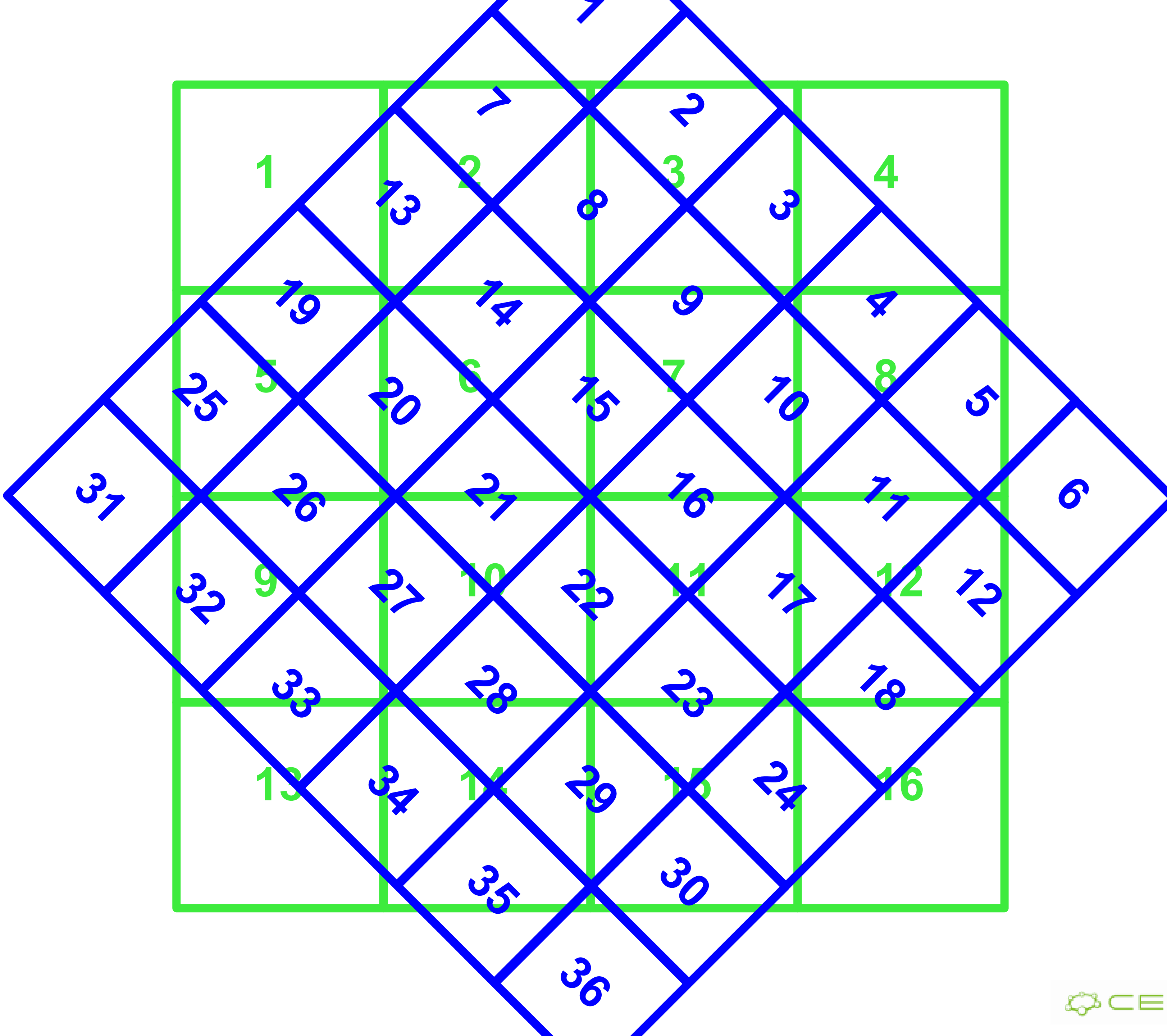


1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16

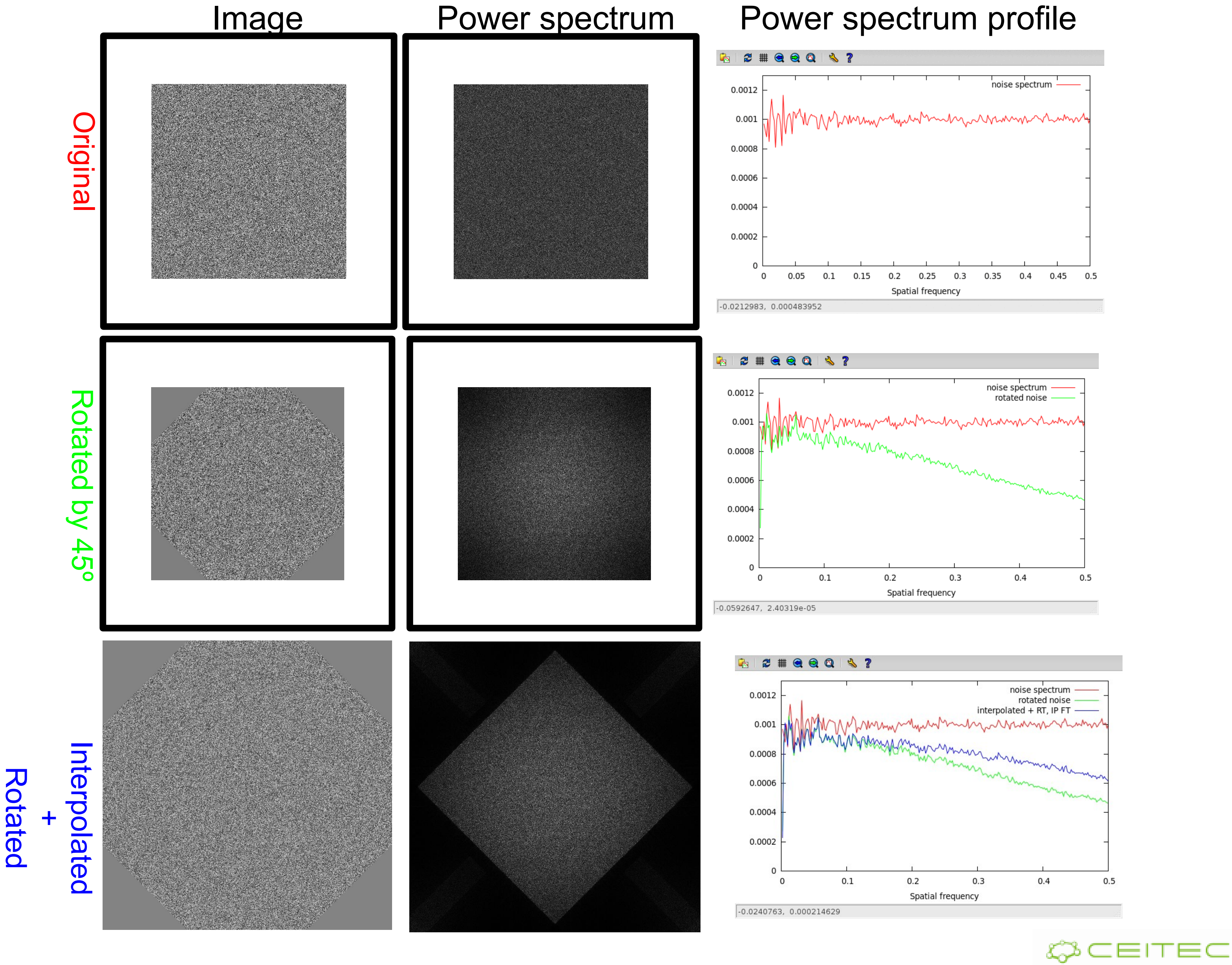


Image
Power spectrum
Power spectrum profile
Original
Rotated by 45°
Interpolated + Rotated
noise spectrum
rotated noise
interpolated + RT, IP FT
Spatial frequency
-0.0212983, 0.000483952
-0.0592647, 2.40319e-05
-0.0240763, 0.000214629
CEITEC

Conclusion:
You can do a little better by oversampling.

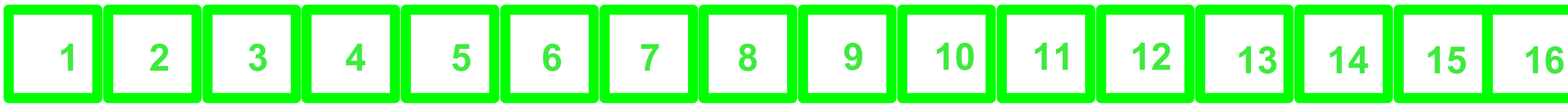
Bammes... Chiu (2012) J. Struct. Biol.

Classification

Multivariate data analysis (MDA)

1	2	3	4
5	6	7	8
9	10	11	12
13	14	15	16

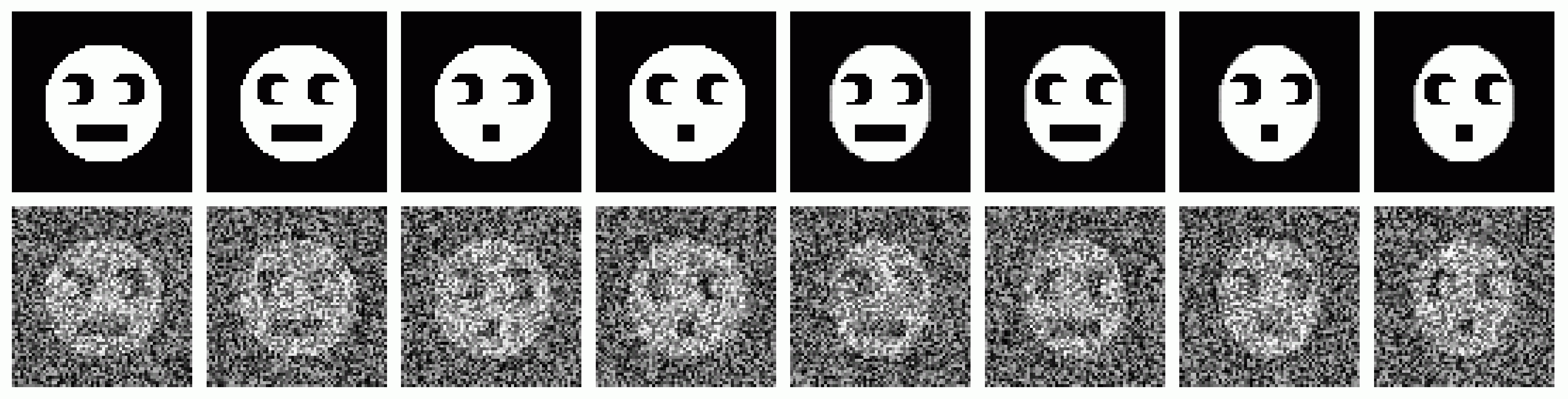
Multivariate data analysis (MDA), or Multivariate statistical analysis (MSA)



Our 16-pixel image can be reorganized into a 16-coordinate vector.

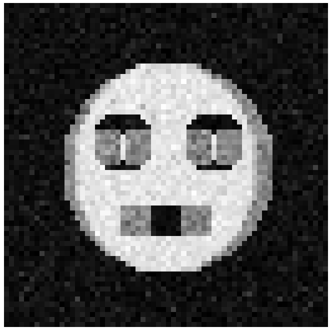
MDA: An example

8 classes of faces, 64x64 pixels



With noise added

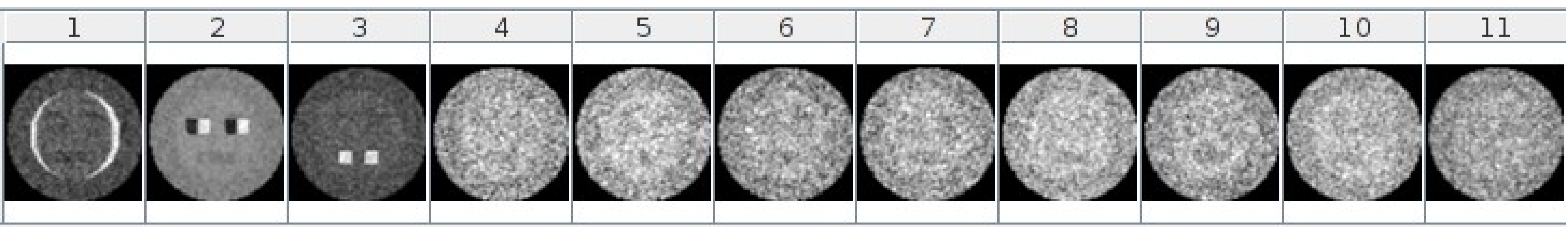
Average:



From http://spider.wadsworth.org/spider_doc/spider/docs/techs/classification/tutorial.html

Principal component analysis (PCA) or Correspondence analysis (CA)

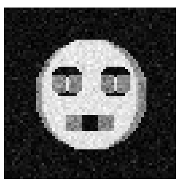
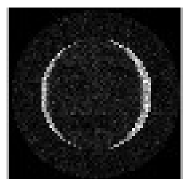
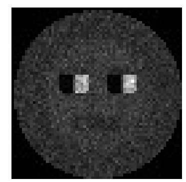
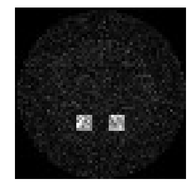
- For a 4096-pixel image, we will have a 4096x4096 covariance matrix.
- Row-reduction of the covariance matrix gives us “eigenvectors.”
    - The eigenvectors describe correlated variations in the data.
    - The eigenvectors have 64 elements and can be converted back into images, called “eigenimages.”
    - The first eigenvectors will account for the most variation. The later eigenvectors may only describe noise.
    - Linear combinations of these images will give us approximations of the classes that make up the data.



Eigenimages

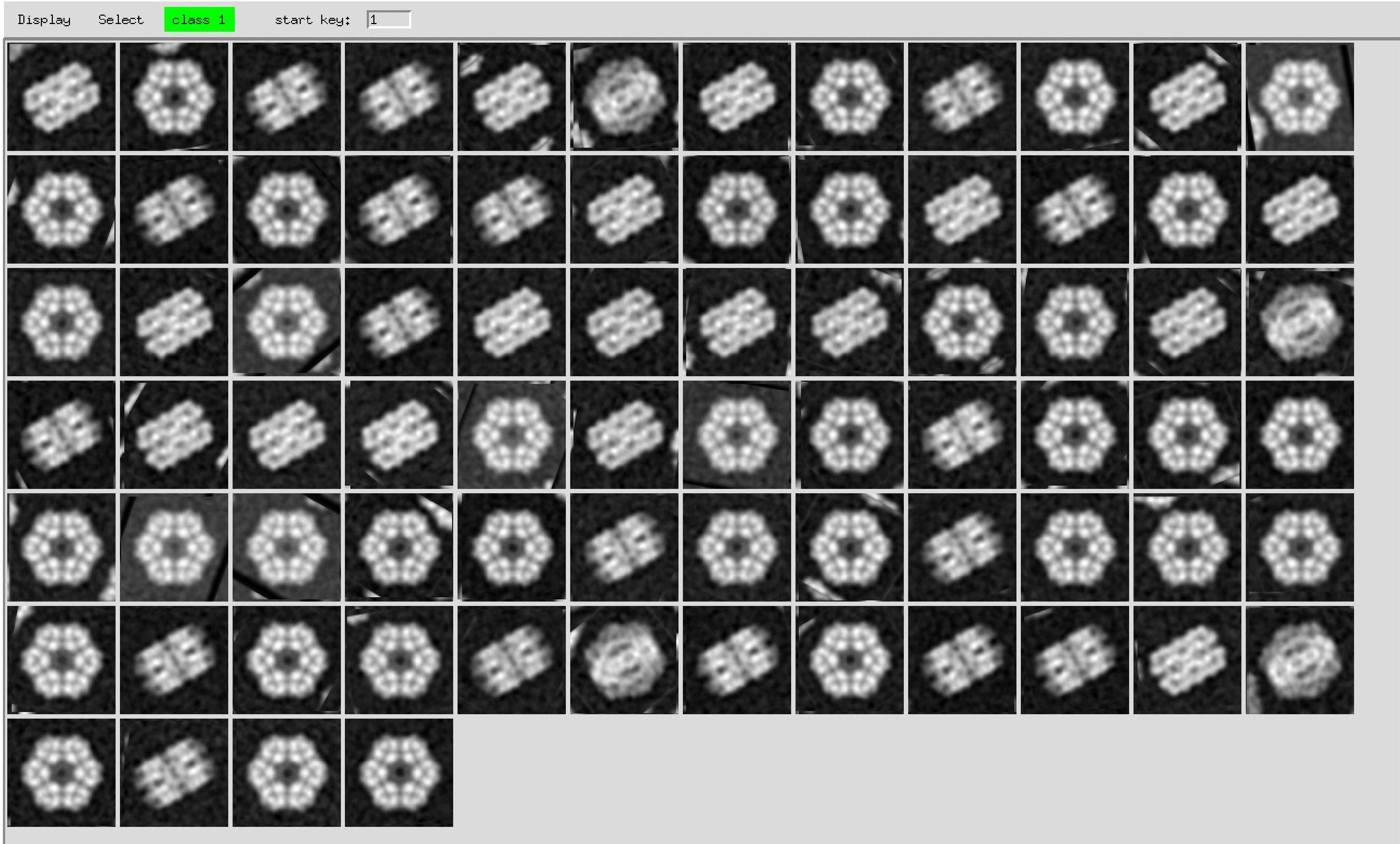
Reconstituted images

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

c_0  $+\ c_1$  $+\ c_2$  $+\ c_3$  $+\ \ldots$

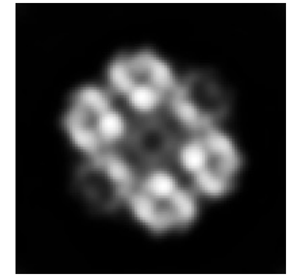
Average | Eigenimage #1 | Eigenimage #2 | Eigenimage #3

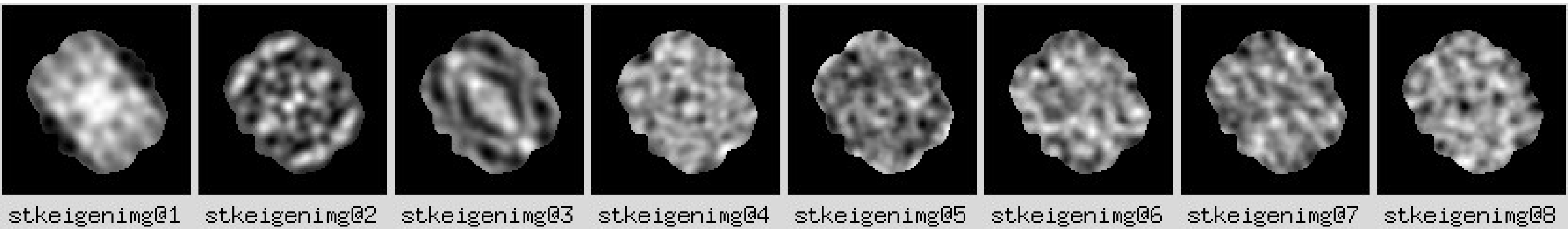
Another example: worm hemoglobin



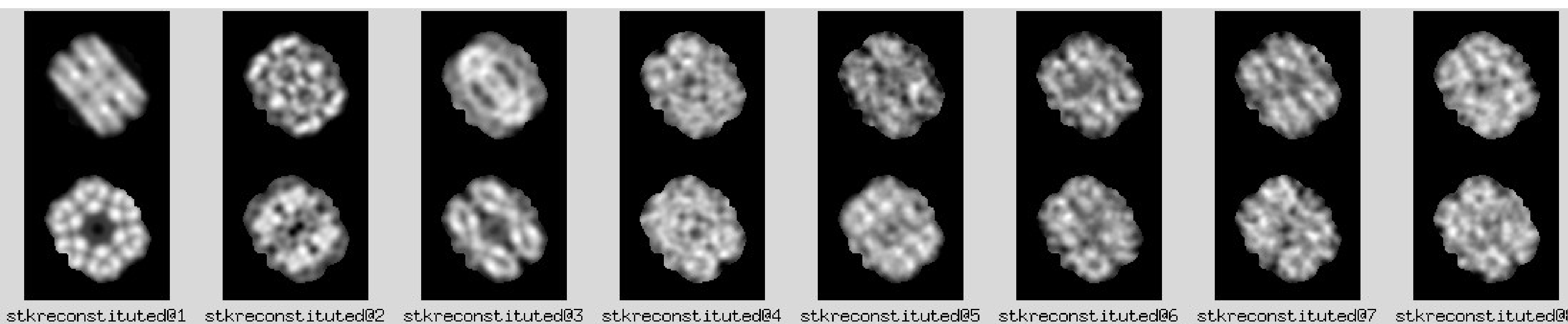
Phantom images of worm hemoglobin

PCA of worm hemoglobin

Average: 



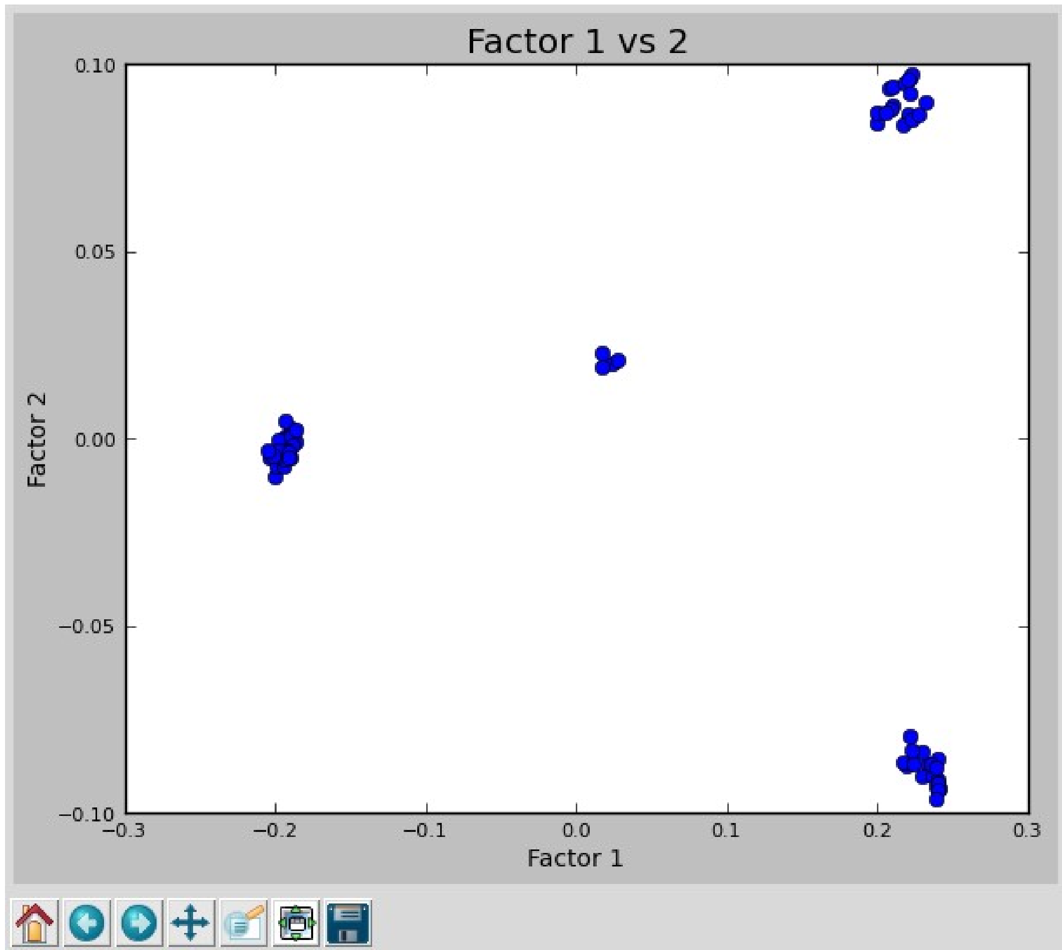
$+c_0$ $+c_1$ $+c_2$ $+c_3$ $+c_4$ $+c_5$



$-c_0$ $-c_1$ $-c_2$ $-c_3$ $-c_4$ $-c_5$

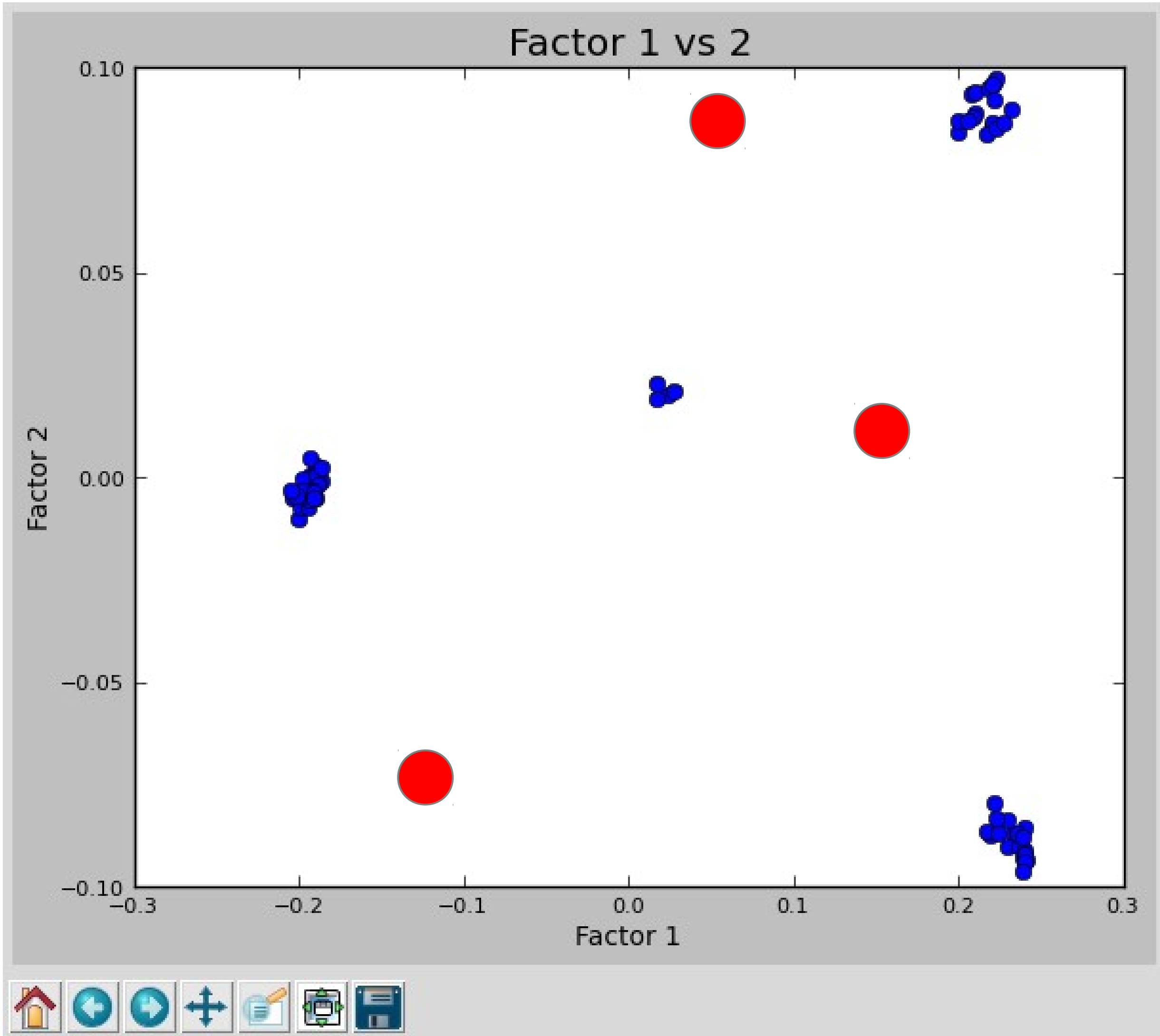
Classification

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16

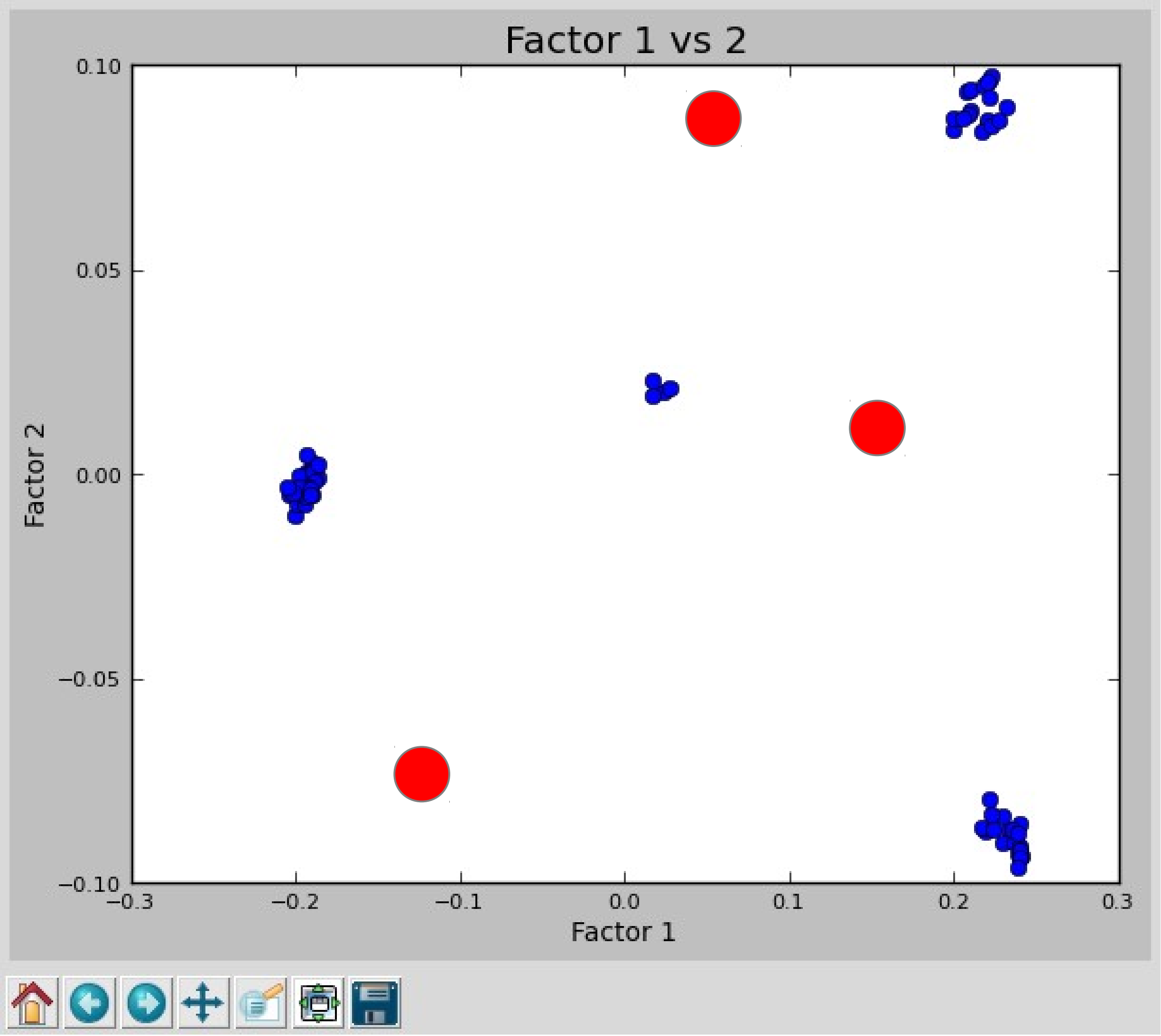


How do we categorize/classify the images?

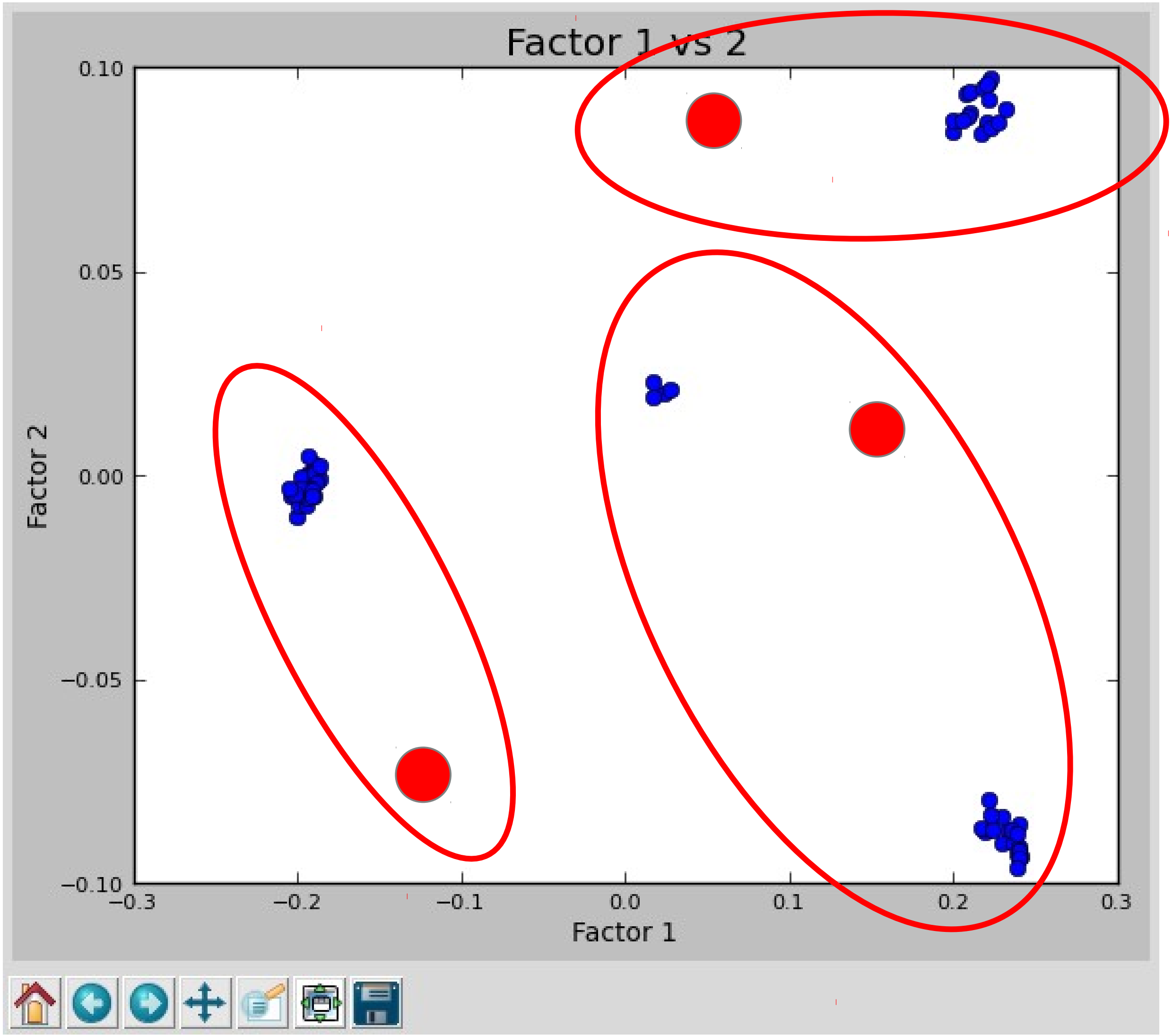
K-means classification



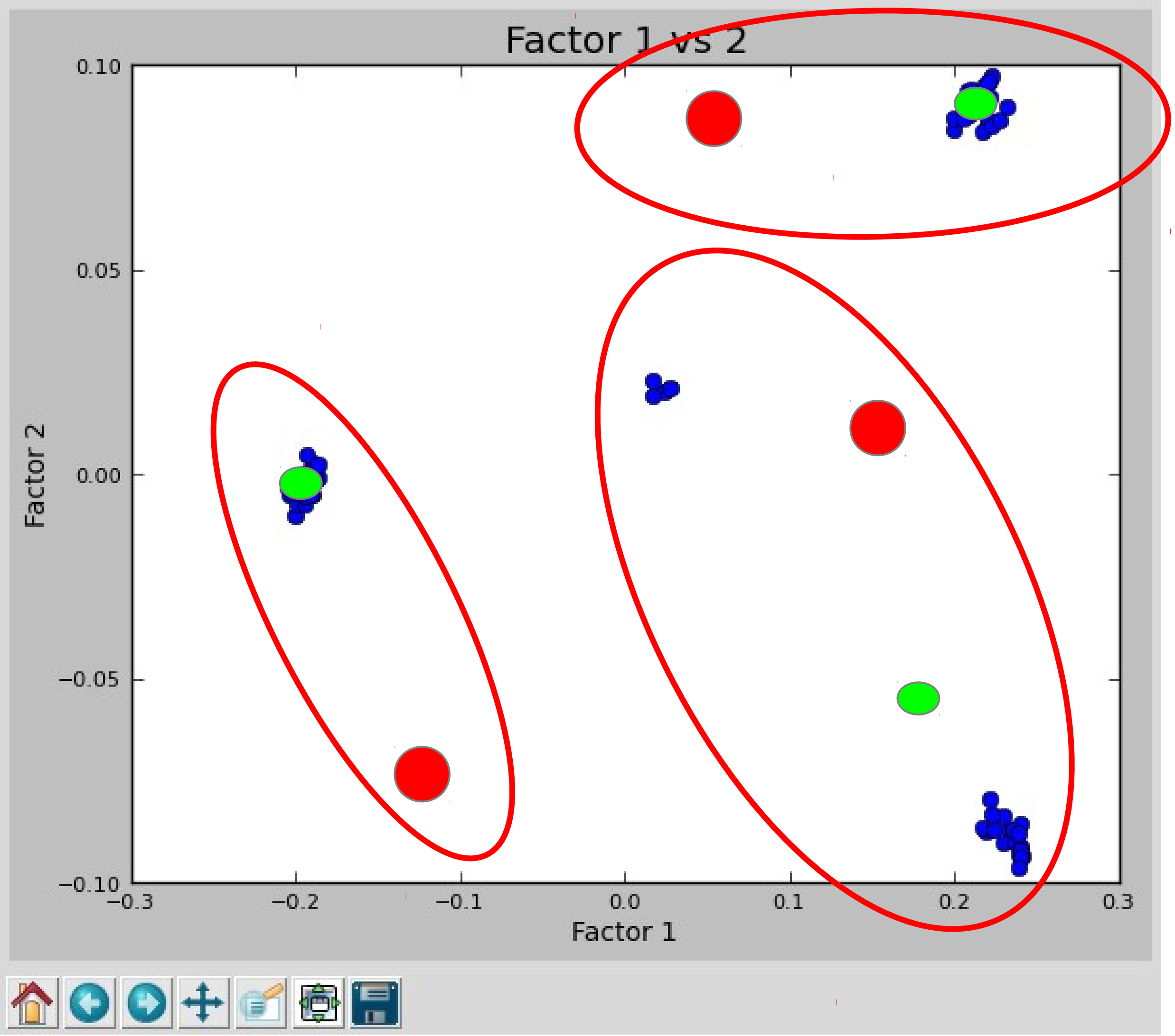
Diday's method of moving centers



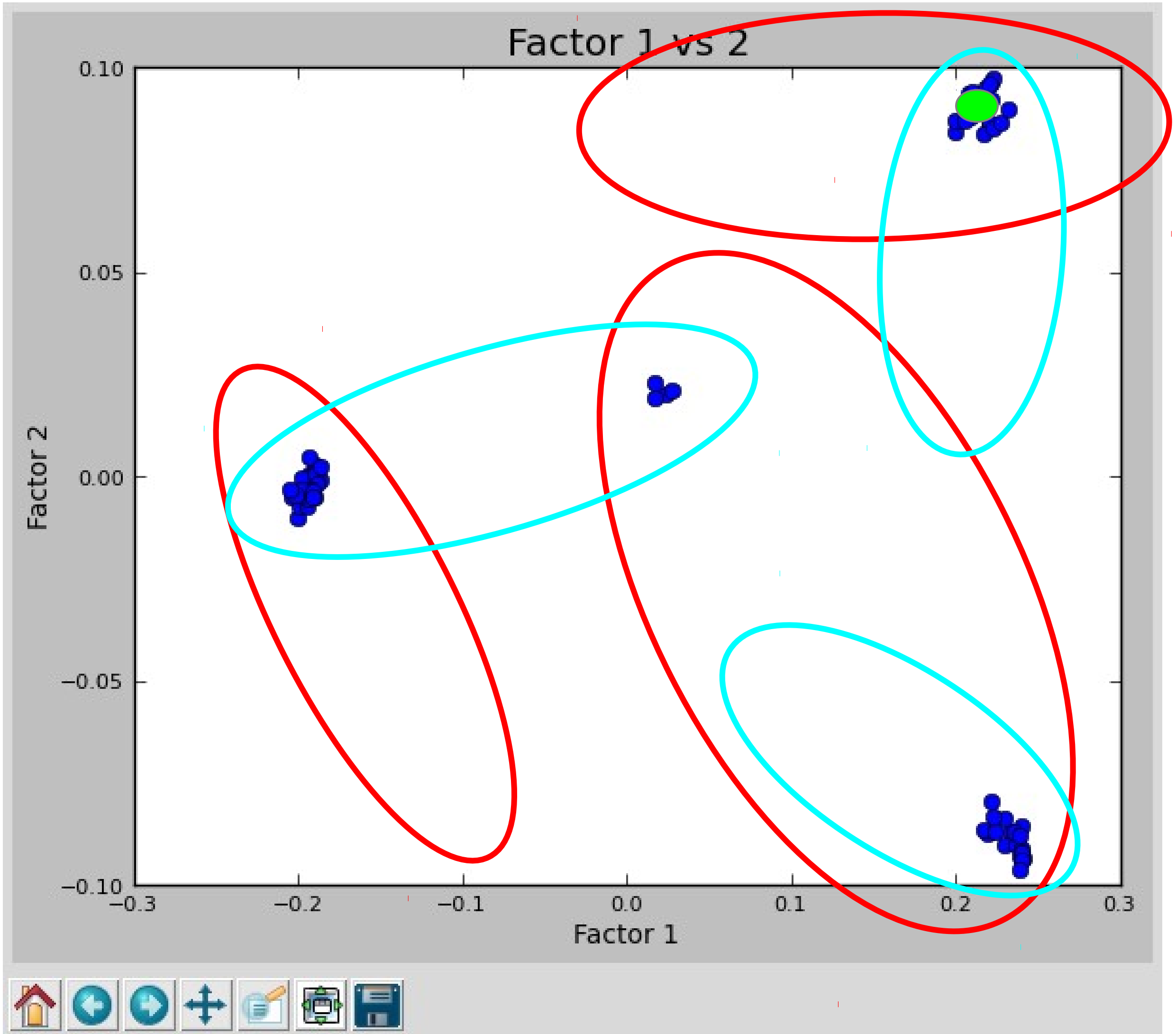
Diday's method of moving centers



Diday's method of moving centers



Diday's method of moving centers



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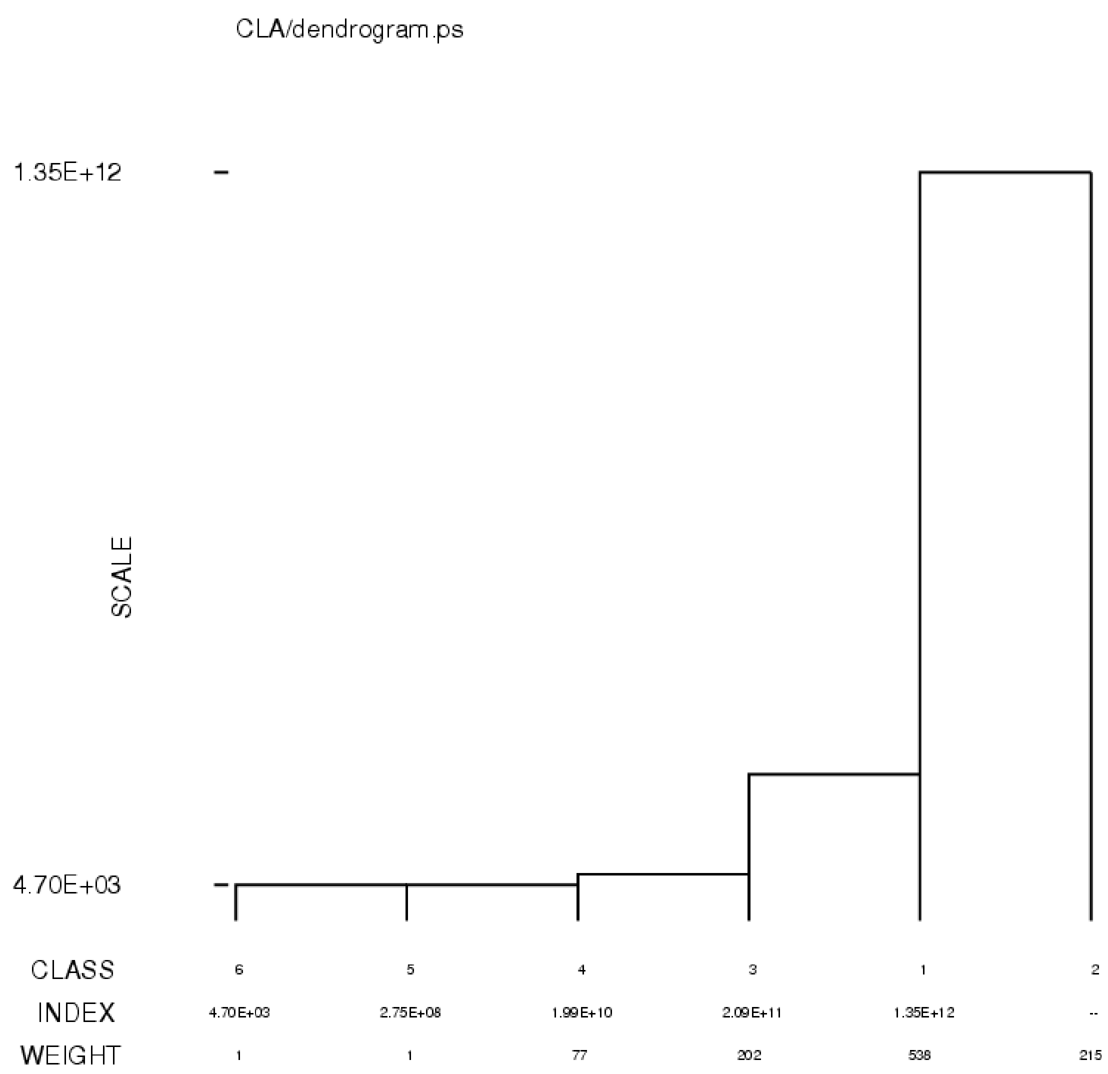
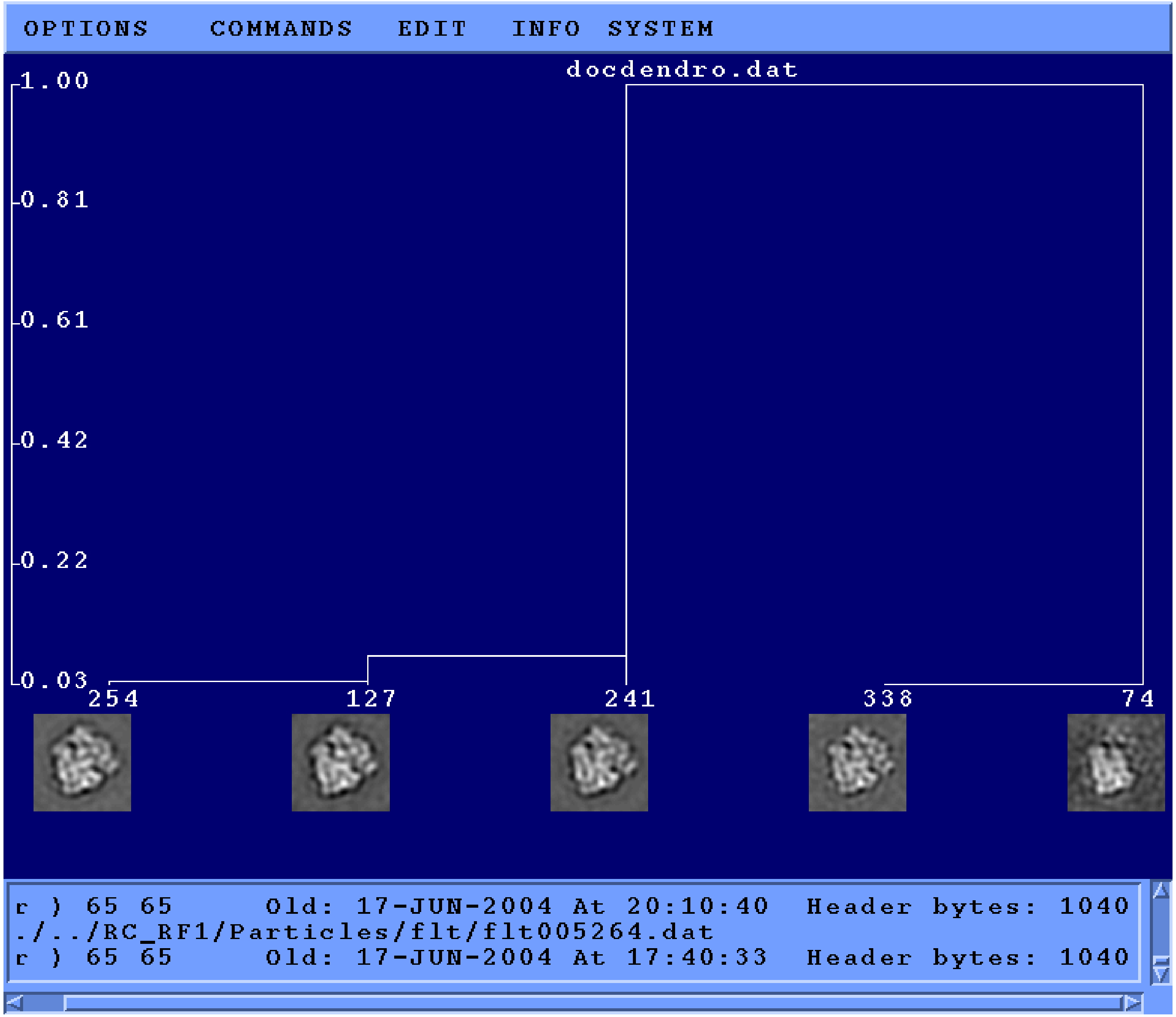
Dendrogram



CLA/dendrogram.ps
1.35E+12
4.70E+03
SCALE
CLASS 6 5 4 3 1 2
INDEX 4.70E+03 2.75E+08 1.99E+10 2.09E+11 1.35E+12 --
WEIGHT 1 1 77 202 538 215

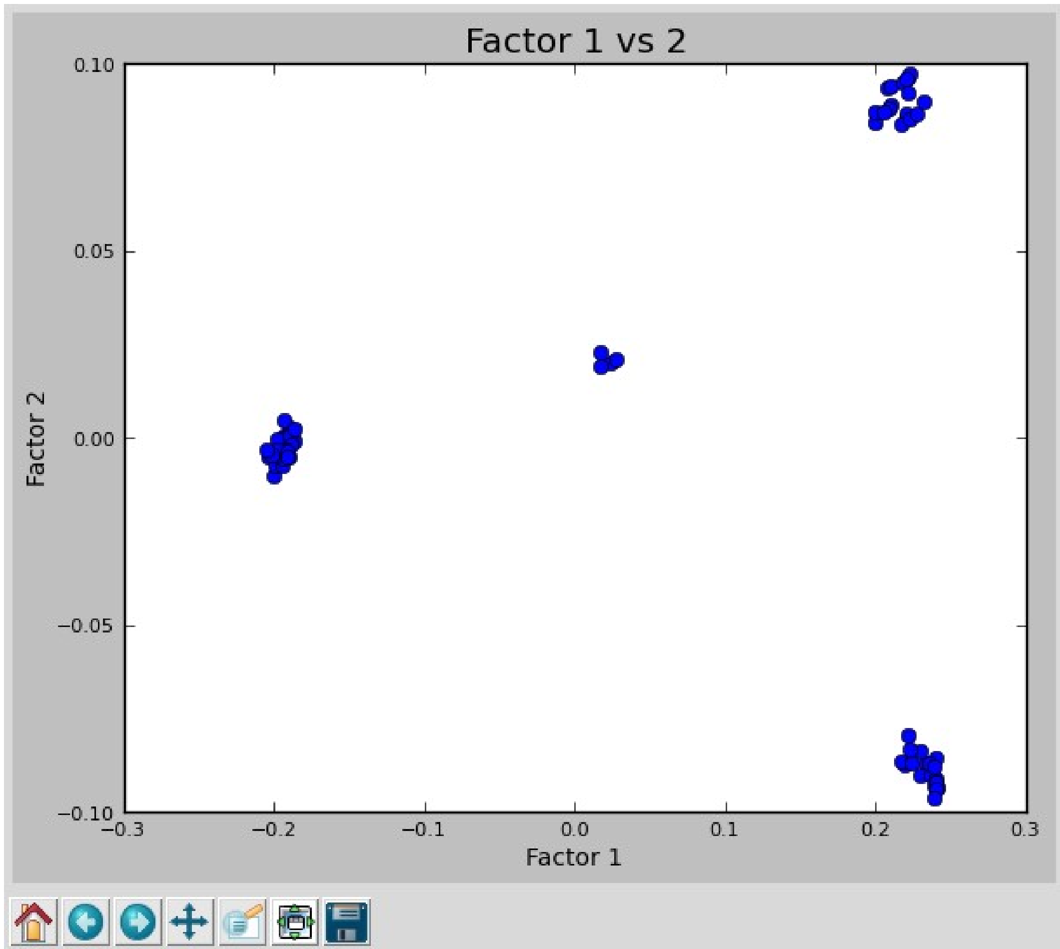
Dendrogram



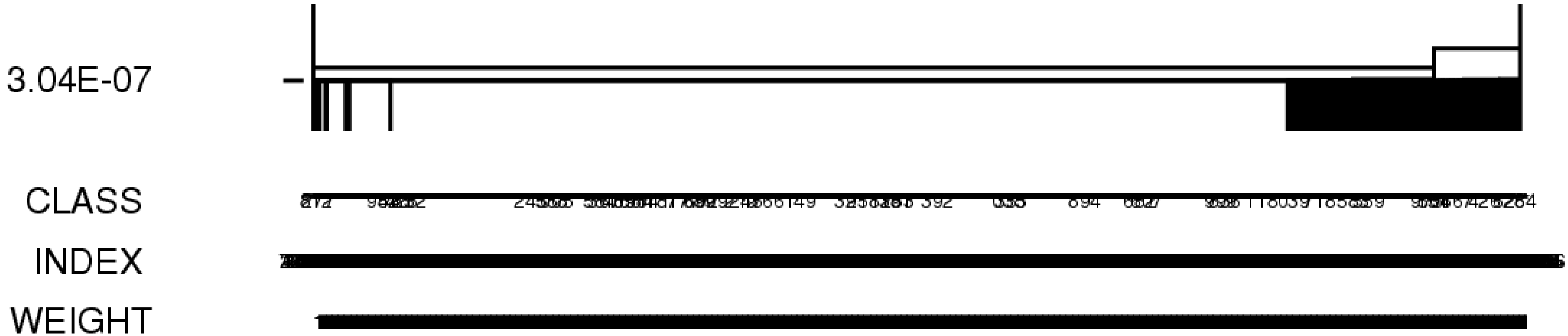
OPTIONS COMMANDS EDIT INFO SYSTEM
docdendro.dat
1.00
0.81
0.61
0.42
0.22
0.03
254
127
241
338
74
r) 65 65 Old: 17-JUN-2004 At 20:10:40 Header bytes: 1040
./../RC_RF1/Particles/flt/flt005264.dat
r) 65 65 Old: 17-JUN-2004 At 17:40:33 Header bytes: 1040

Hierarchical Ascendant Classification

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16

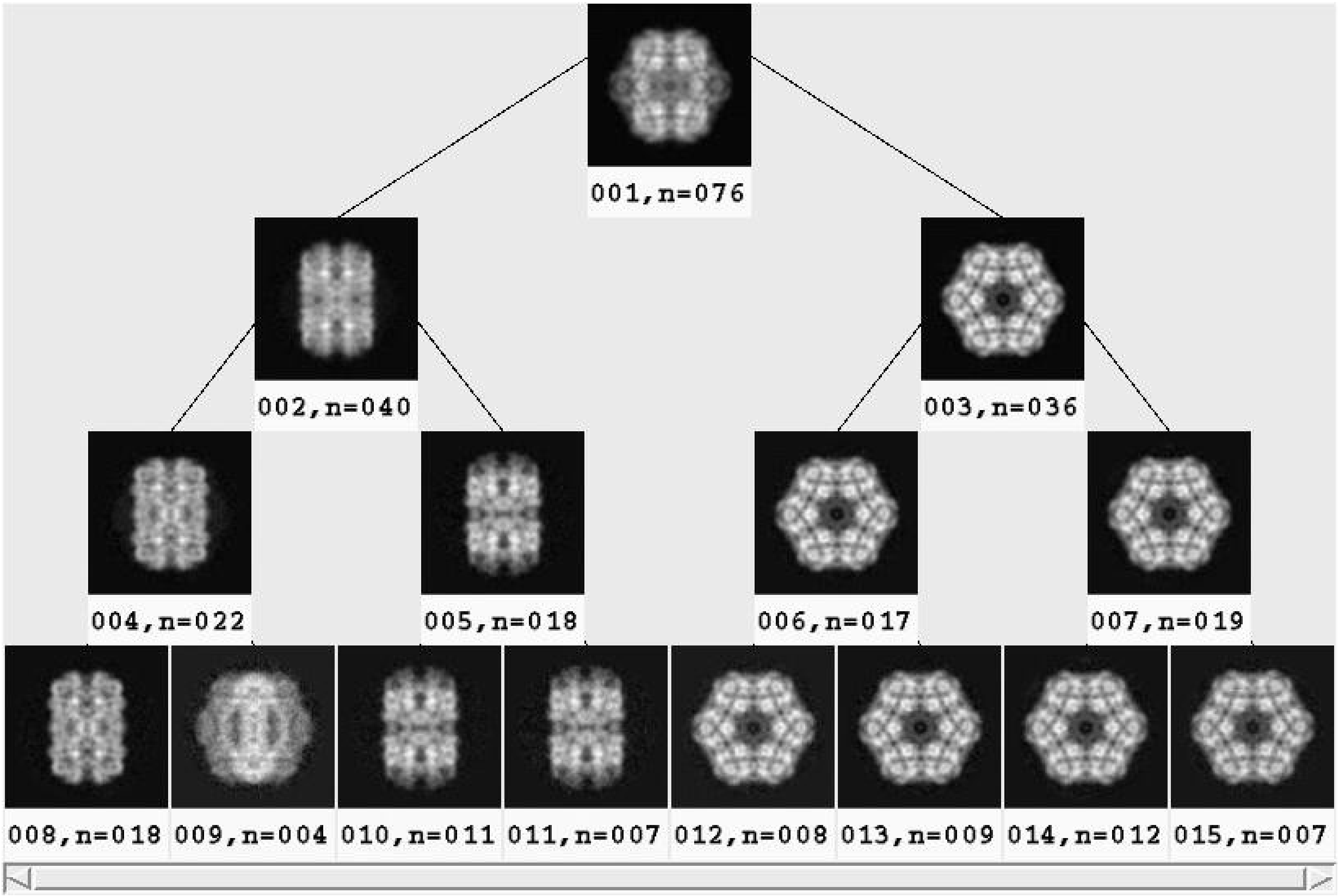


Hierarchical Ascendant Classification



All images are represented.
The dendrogram will be too heavily branched to interpret without truncation.

Binary-tree viewer



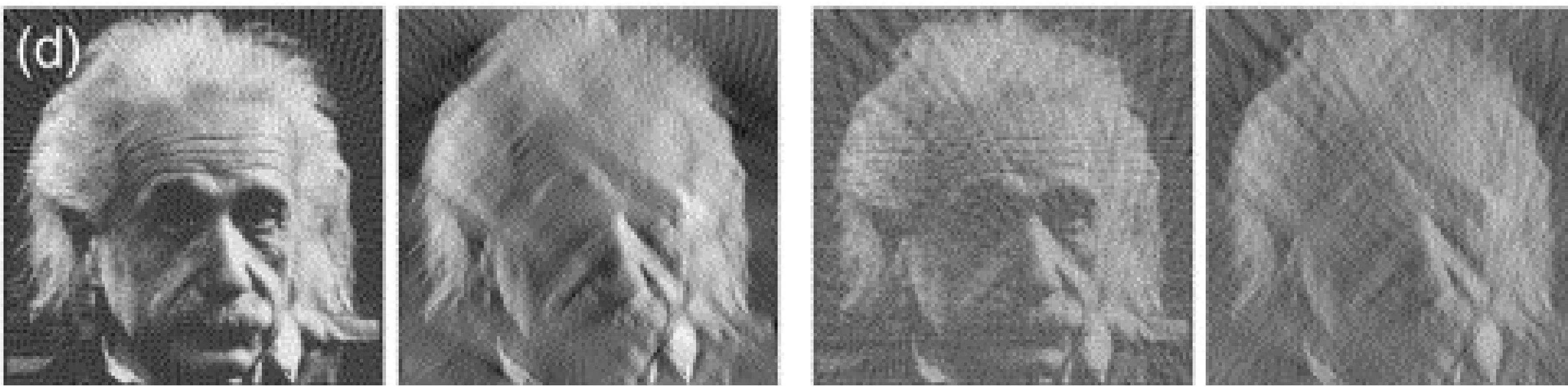
3D Reconstruction

What information do we need for 3D reconstruction?

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

What happens when we're missing views?

good | missing views | sparse sampling | sparse sampling + missing views

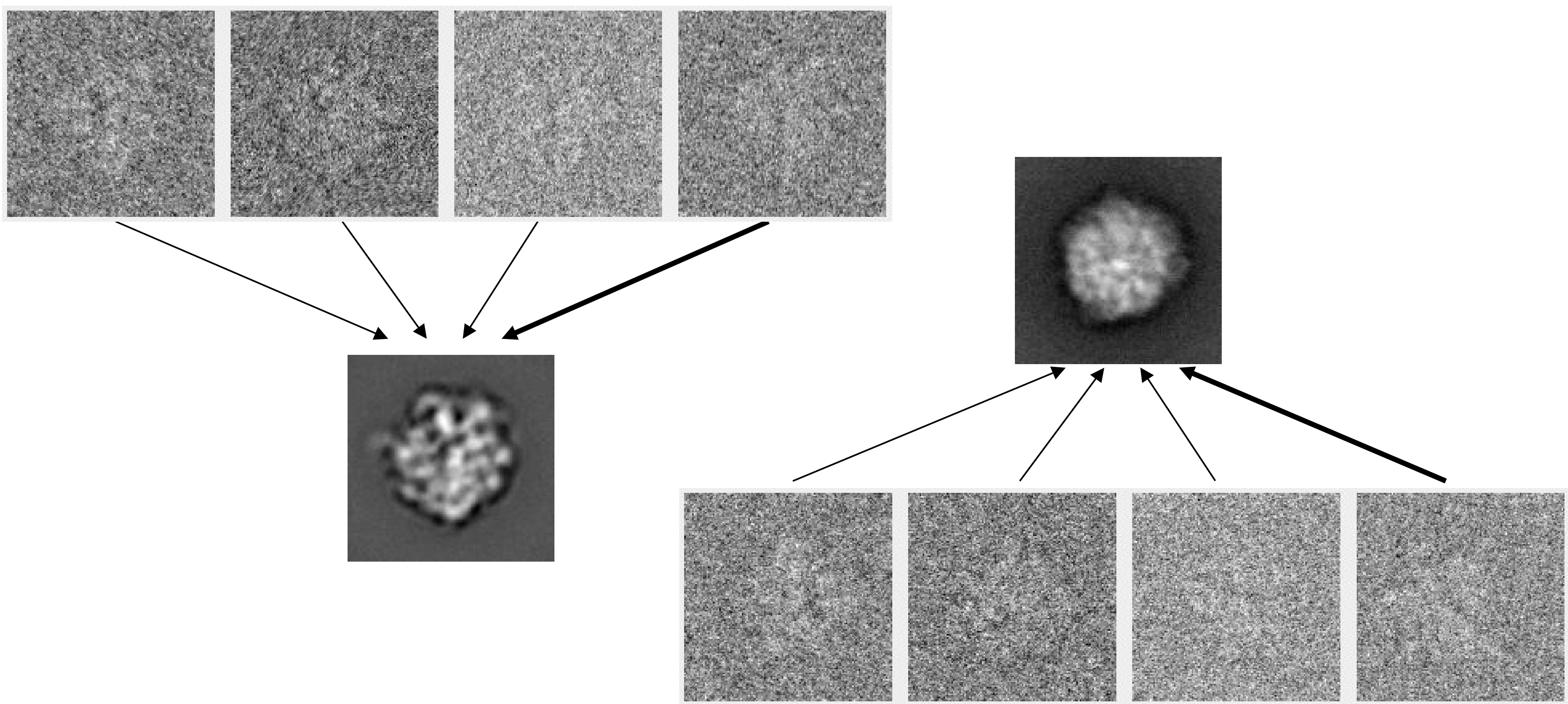


Baumeister et al. (1999), *Trends in Cell Biol.*, **9**: 81-5.

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

Why do we need orientation?

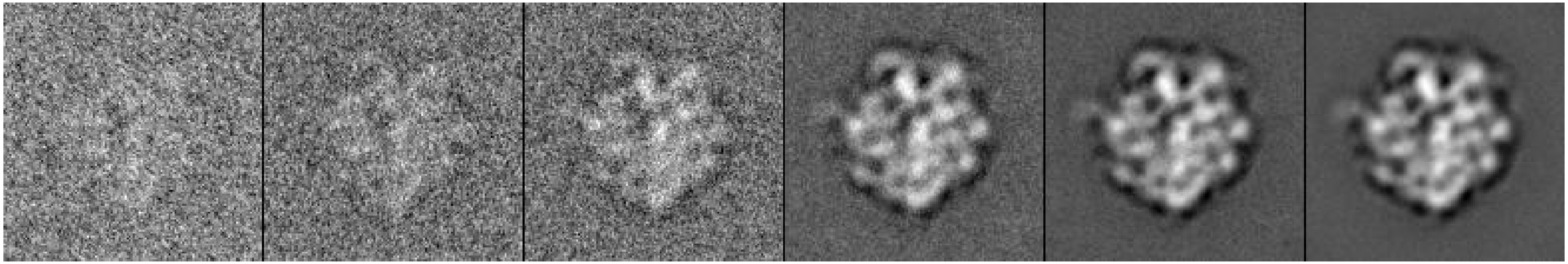
aligned images 1-4 of 4096 total



unaligned images 1-4 of 4096 total

This is a simple 2D case, but the effects are analogous in 3D.

What happens as we include more particles?



n=1 *n*=4 *n*=16 *n*=256 *n*=1024 *n*=4096

Signal-to-noise ratio increases with $\sqrt{n}$

What information do we need for 3D reconstruction?

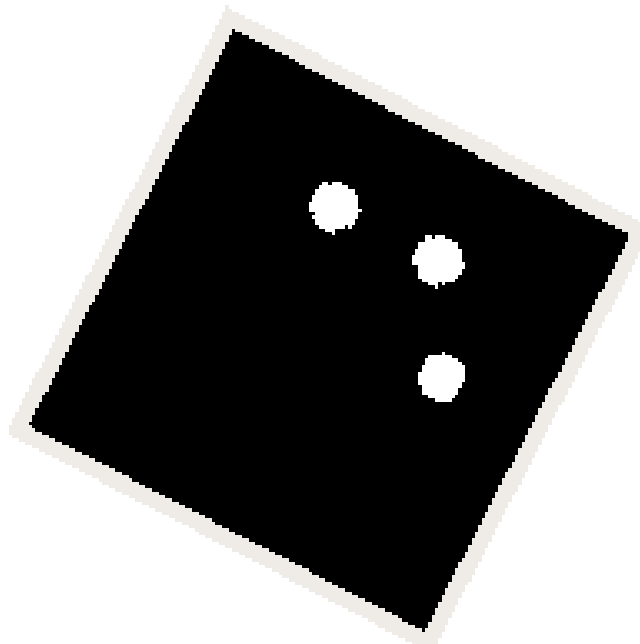
1. different orientations
2. known orientations
3. many particles

I have all of this information.
Now what?

There are two general categories of 3D reconstruction

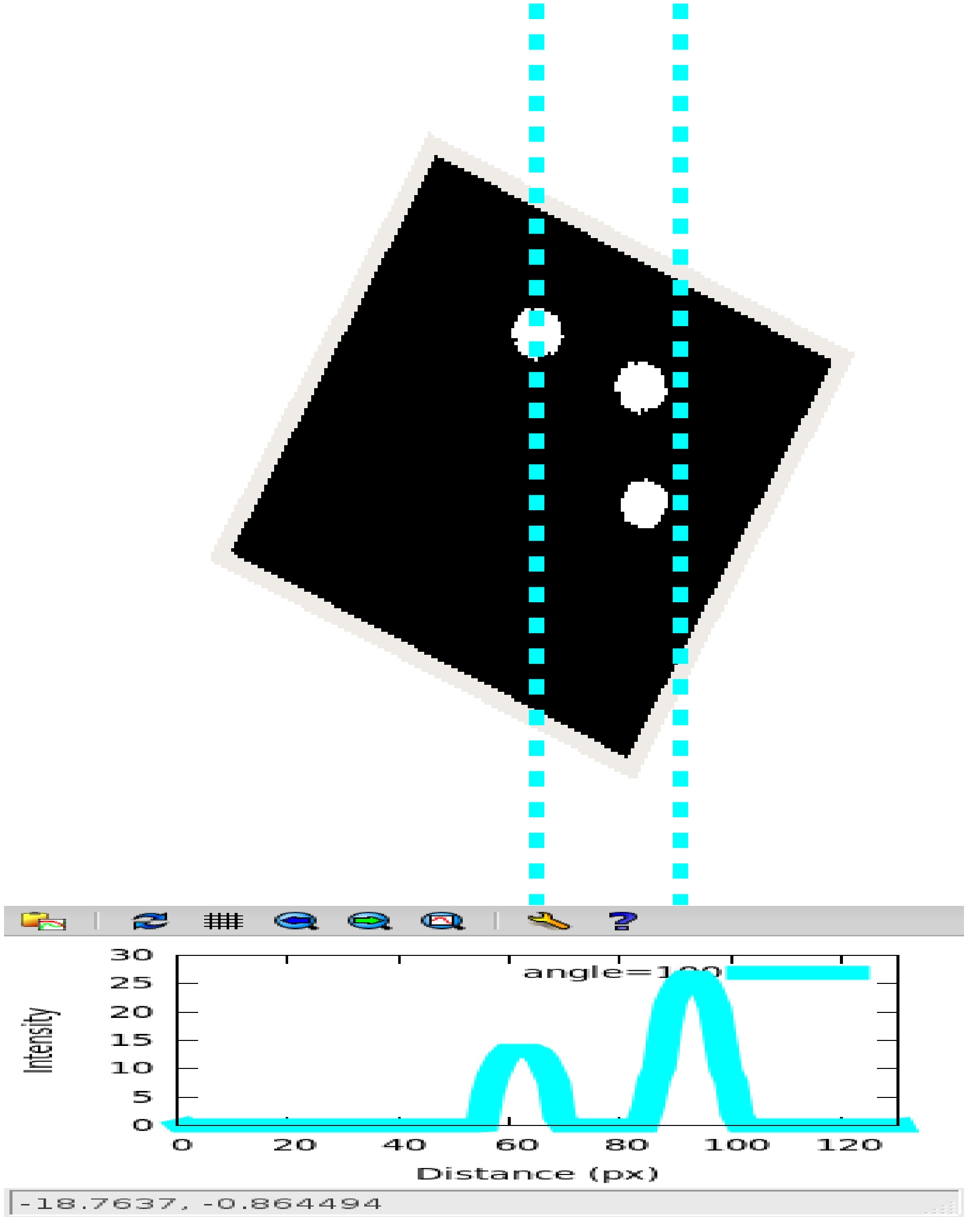
1. Real space
2. Fourier space

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



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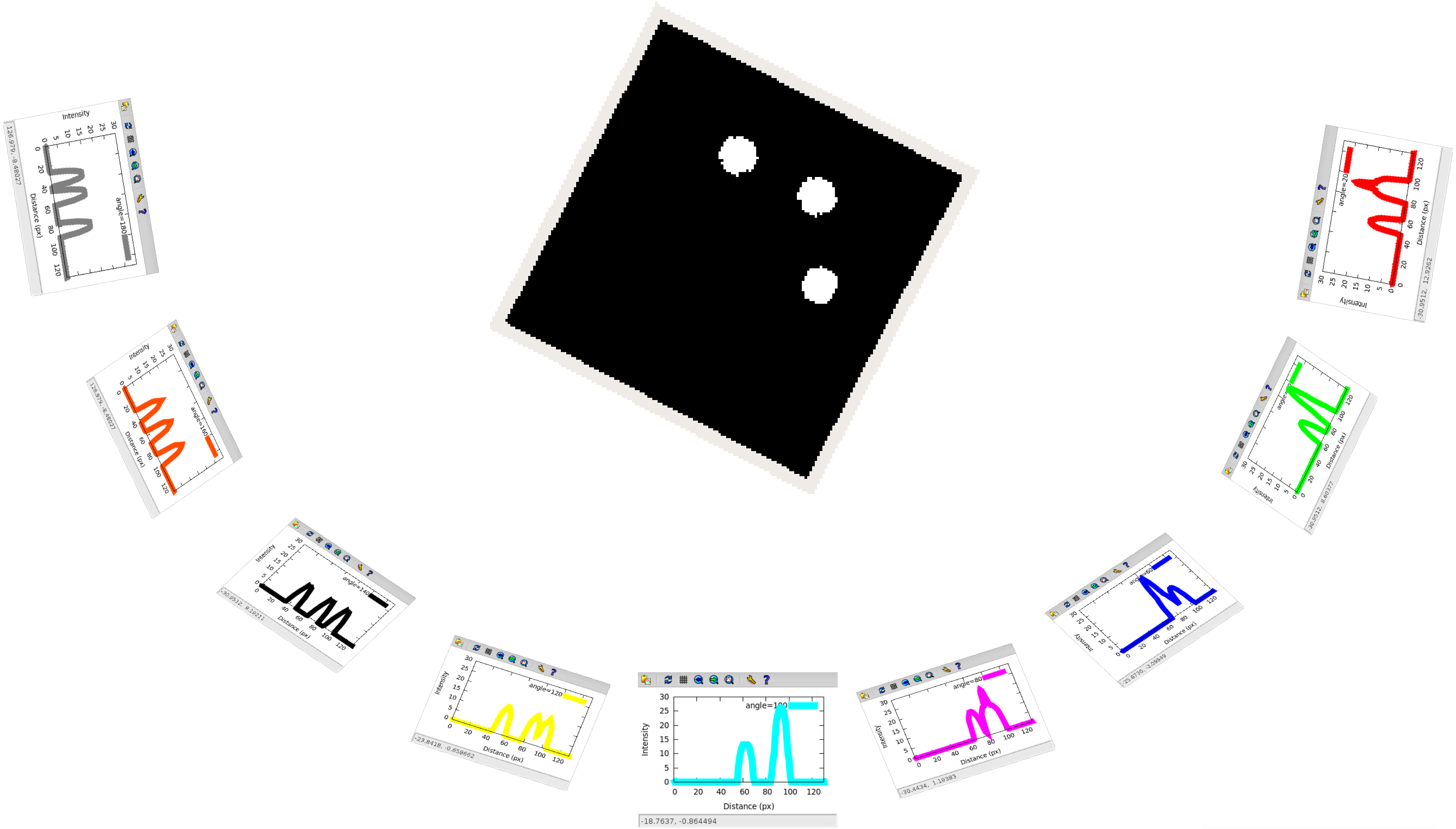
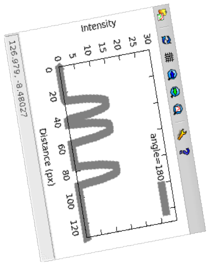
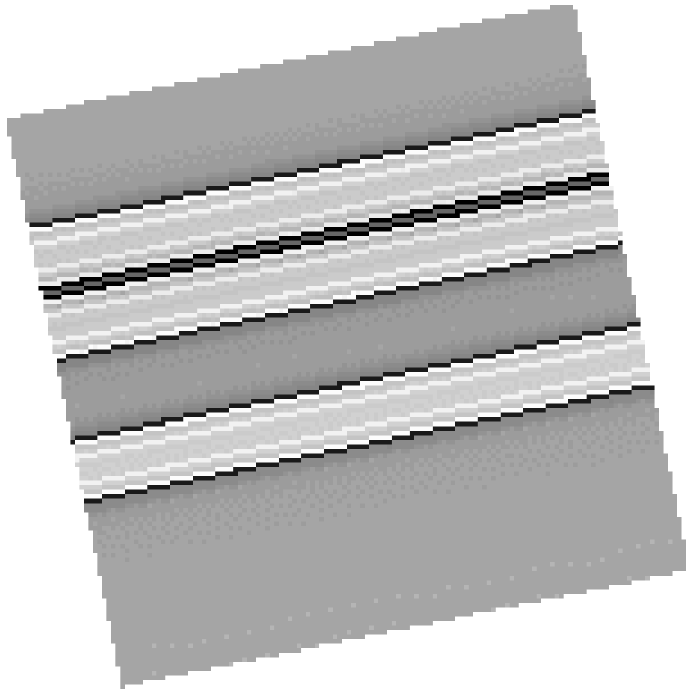


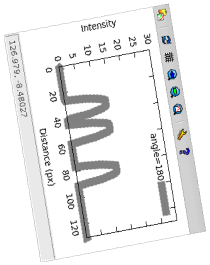
angle=180
angle=160
angle=140
angle=120
angle=100
angle=80
angle=60
angle=20
Intensity
Distance (px)
-18.7637, -0.864494
126.979, -8.48027
-30.9512, 8.18221
-23.8418, -0.658662
-30.4434, 1.19383
-30.9512, 12.9262

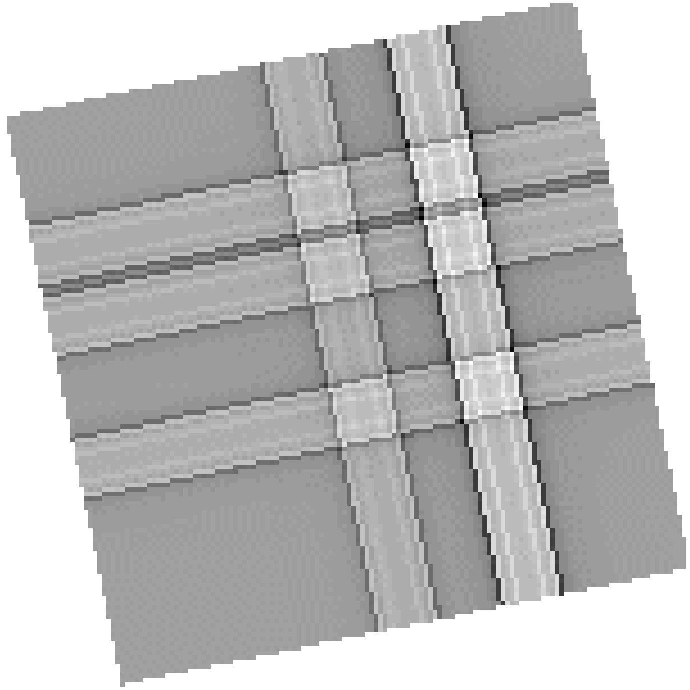
Reconstruction is the inversion of projection

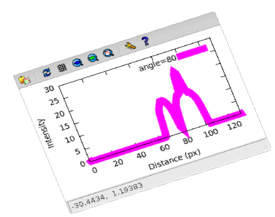




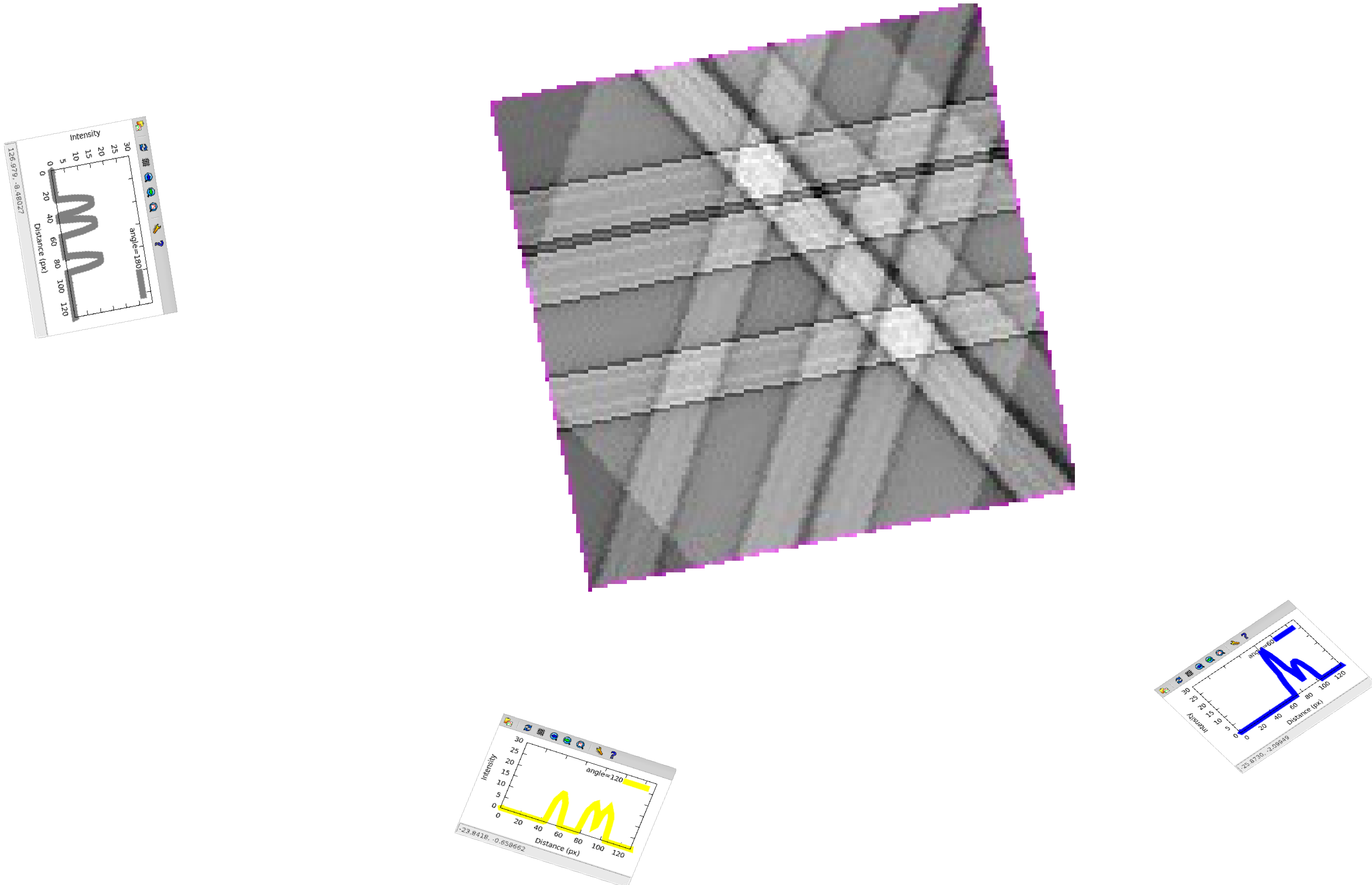
Reconstruction is the inversion of projection



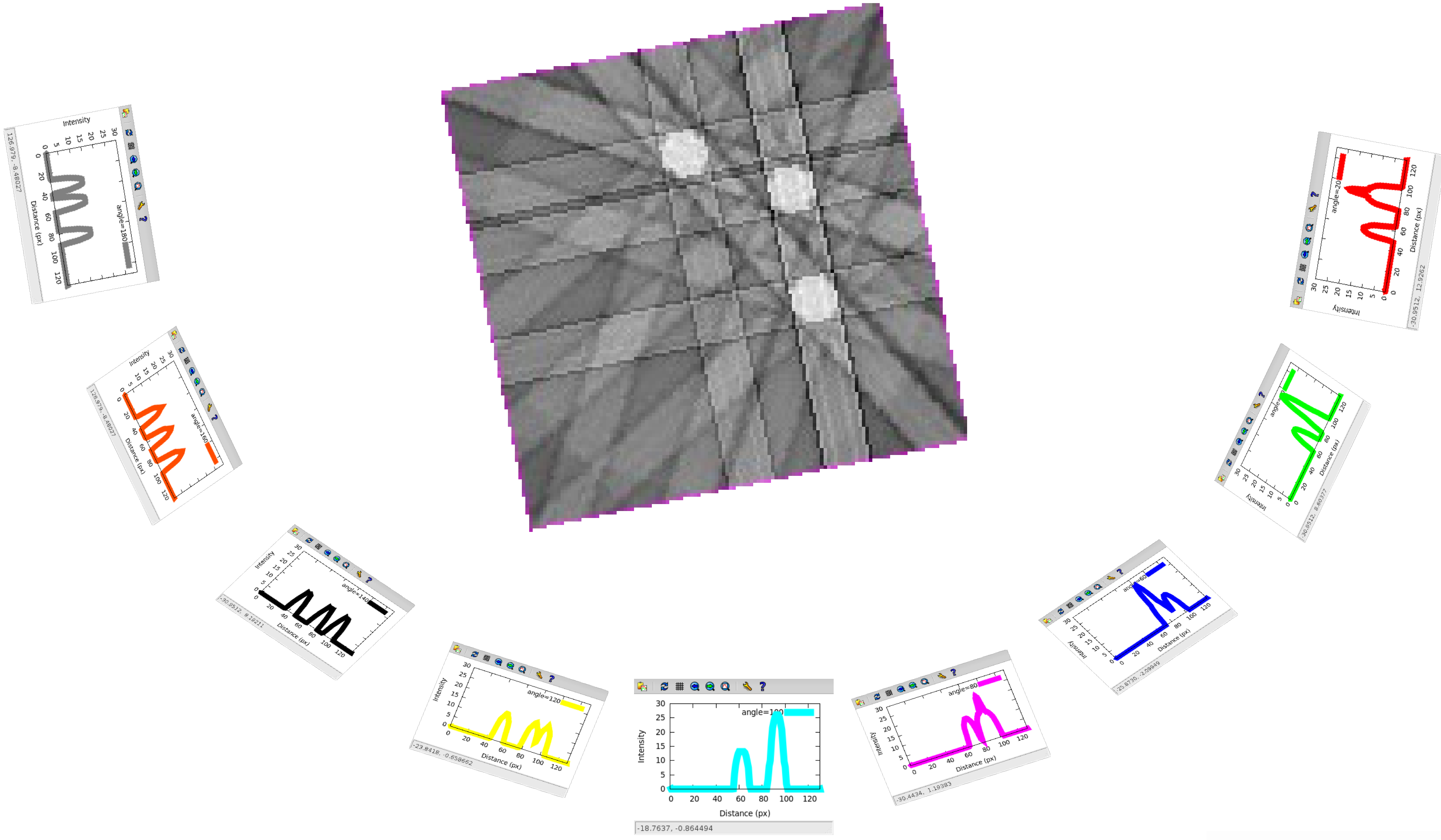




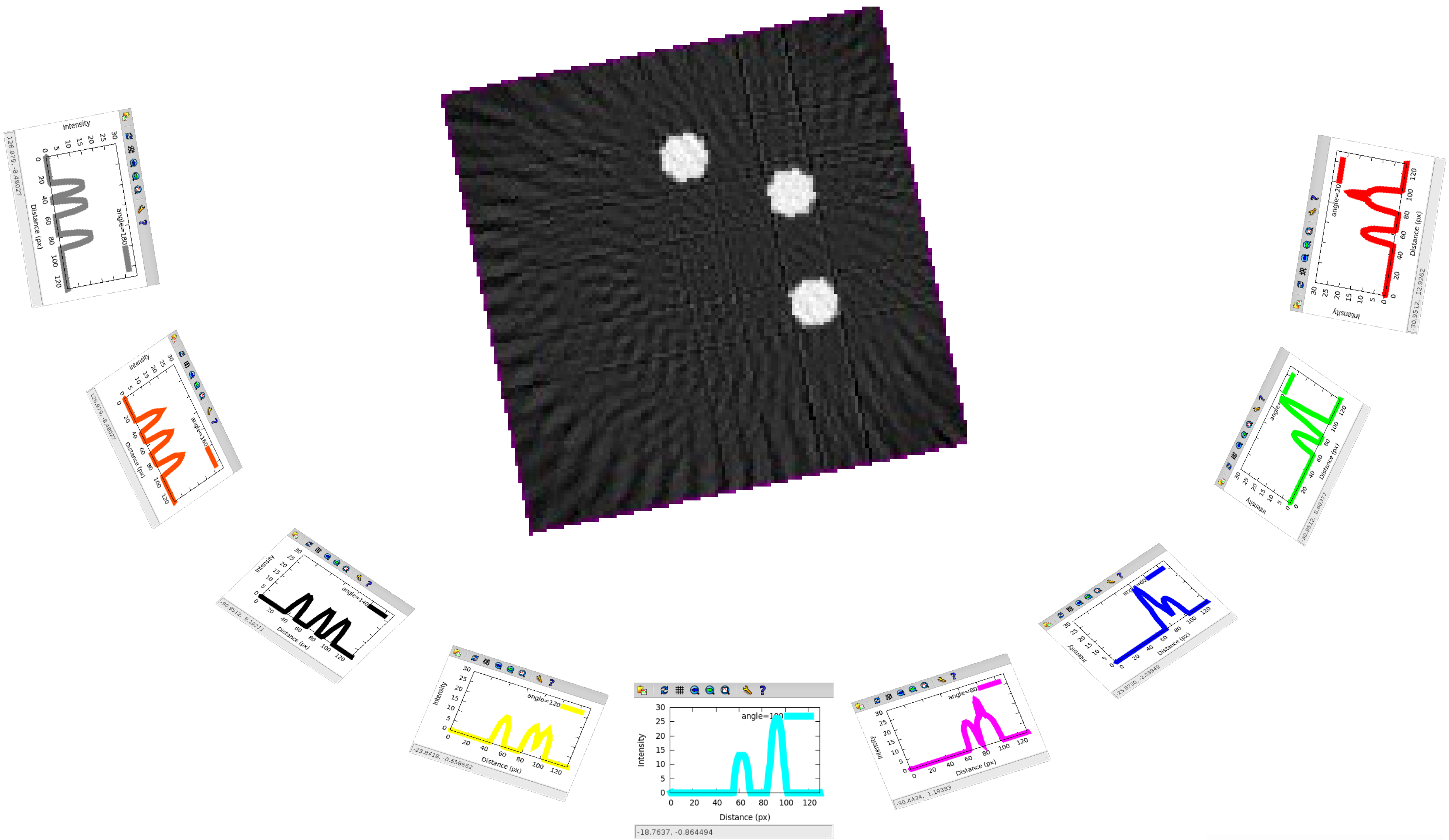
Reconstruction is the inversion of projection



Reconstruction is the inversion of projection



Reconstruction is the inversion of projection



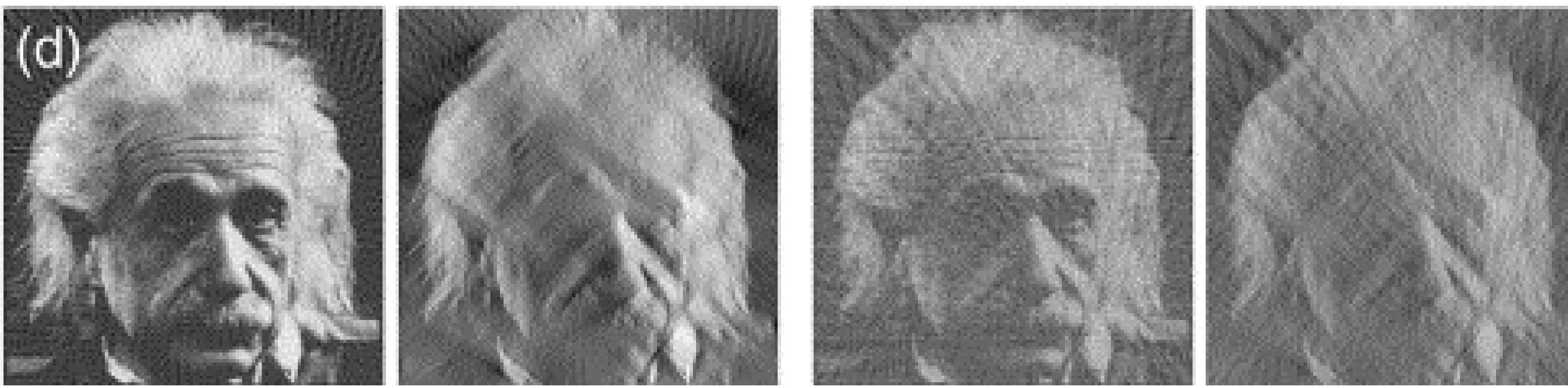
What happens when we're missing views?

good

missing views

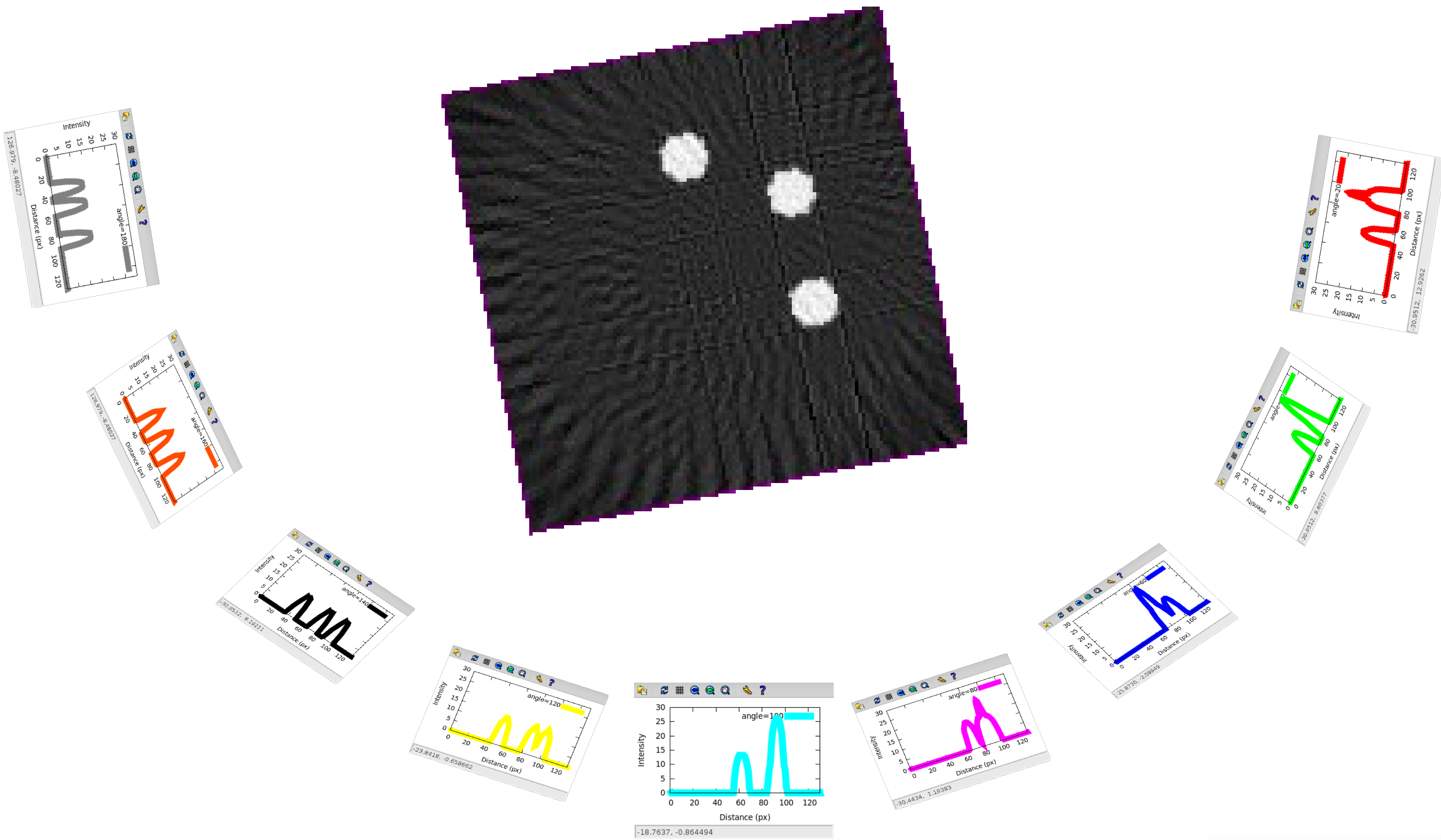
sparse sampling

sparse sampling + missing views



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

Simultaneous Iterative Reconstruction Technique



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

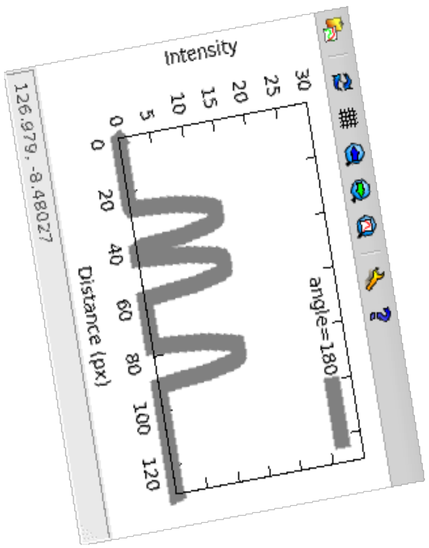
(one) ANSWER:
Simultaneous Iterative Reconstruction Technique

Simultaneous Iterative Reconstruction Technique

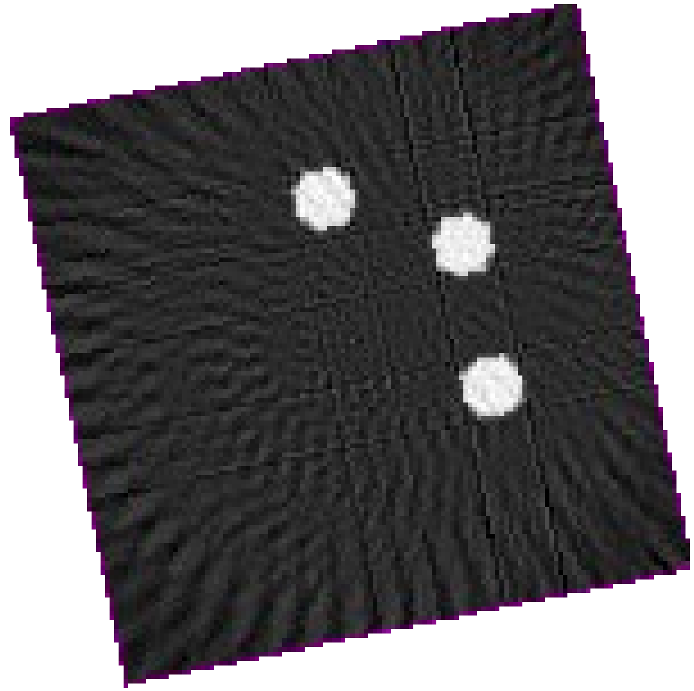
The idea:

- 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



Experimental projection



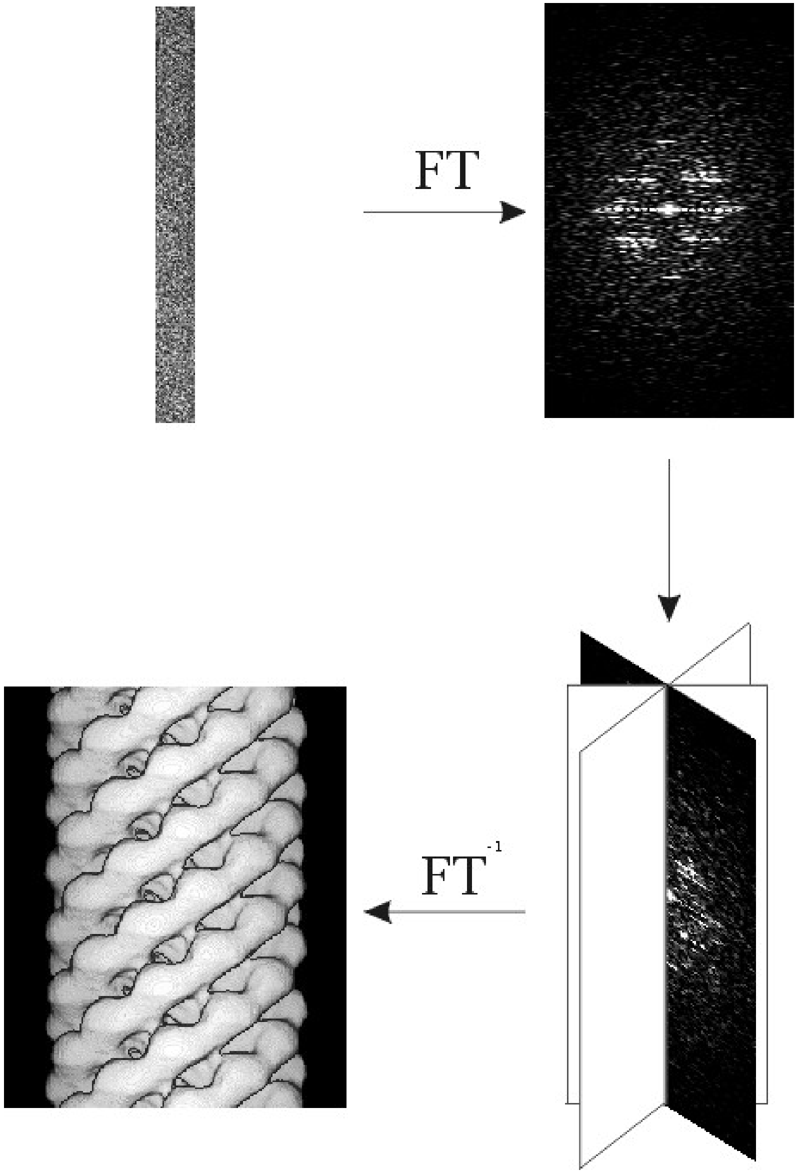
Model

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 over compensate, and would amplify noise.

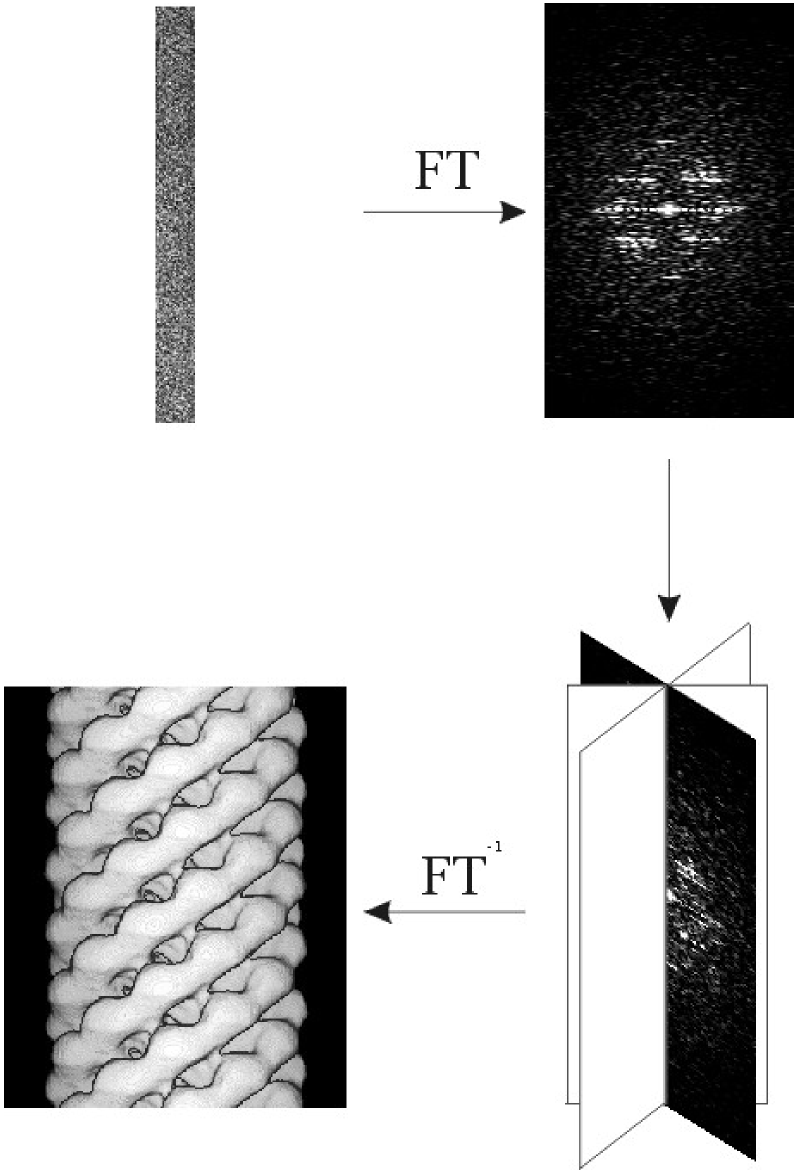
Reconstruction in Fourier space

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.

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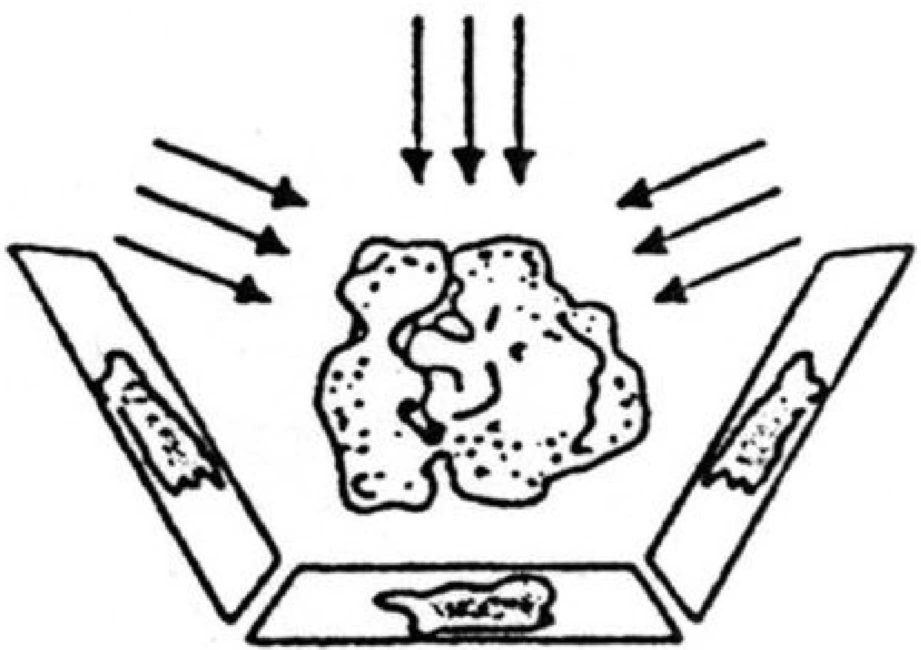
Reference-based alignment
(or projection-matching)

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.

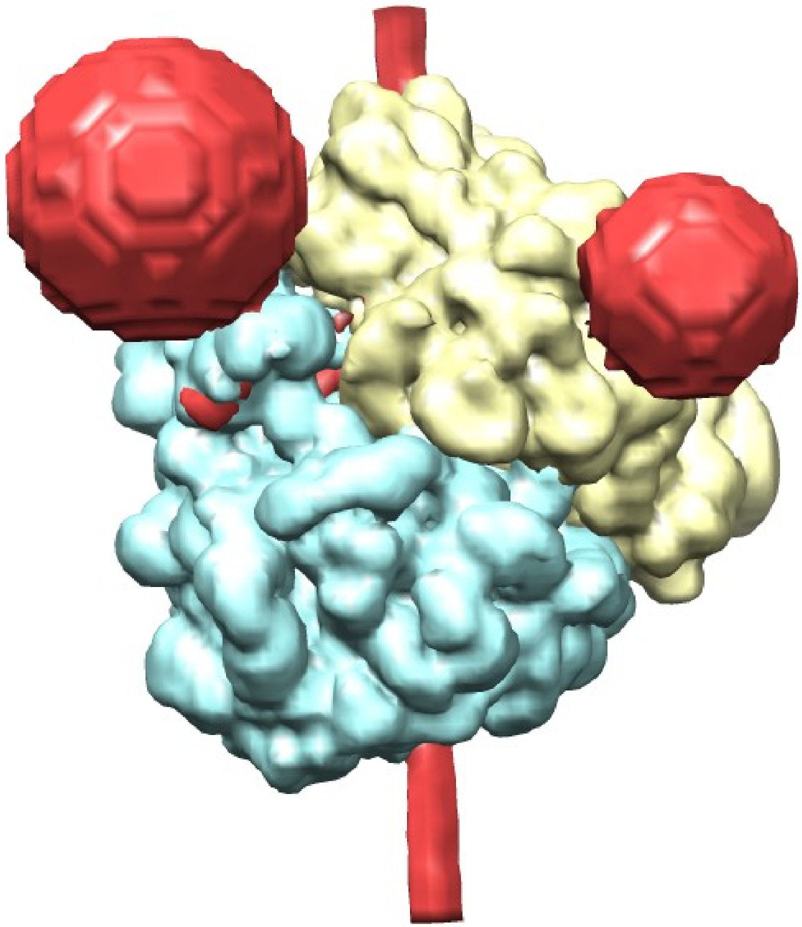
Step 1: Generation of projections of the reference.



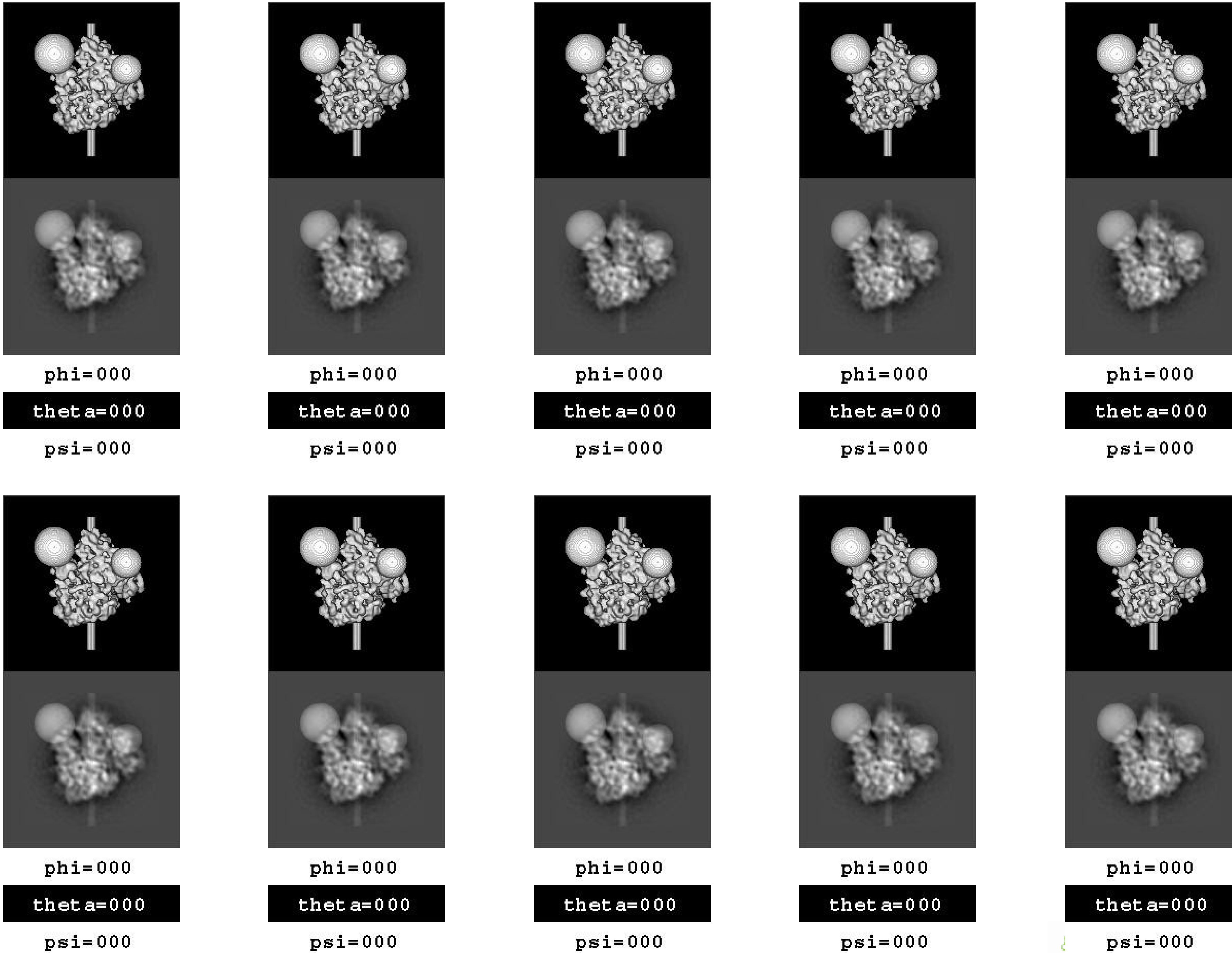
From Penczek *et al.* (1994), *Ultramicroscopy* **53**: 251-70.

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.)


phi=000
theta=000
psi=000
phi=000
theta=000
psi=000
phi=000
theta=000
psi=000
phi=000
theta=000
psi=000
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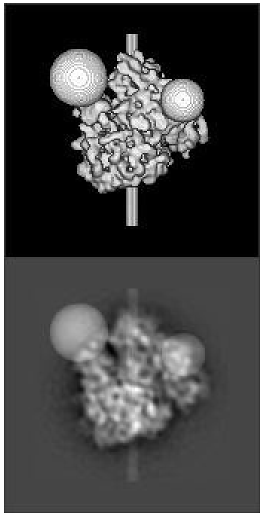
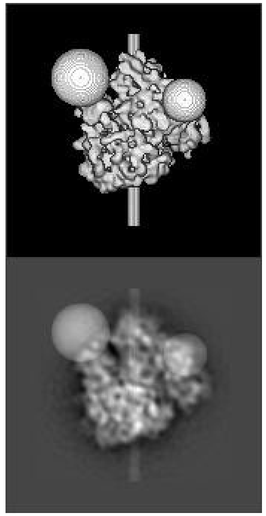
phi=000
theta=000
psi=000



phi=000
theta=000
psi=000



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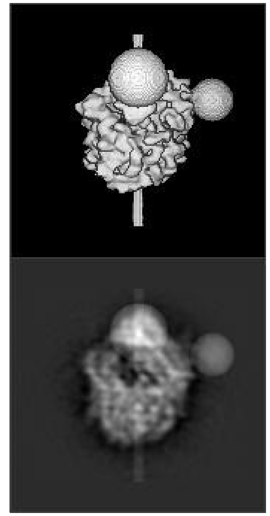

phi=000
theta=045
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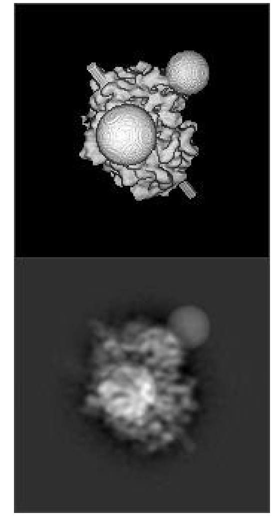

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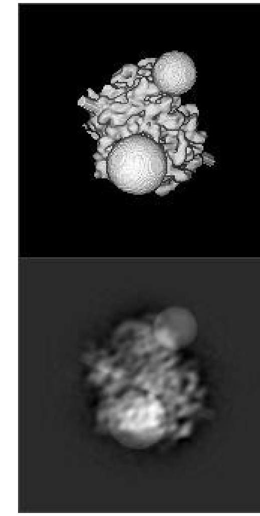

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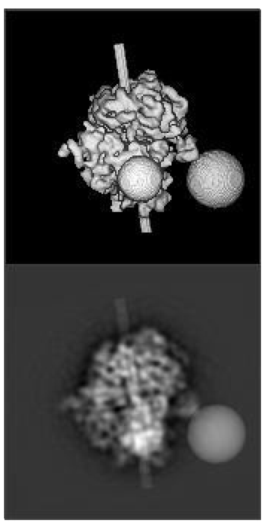
theta=045
psi=000



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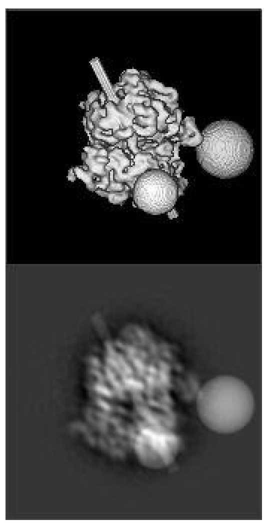
theta=045
psi=000



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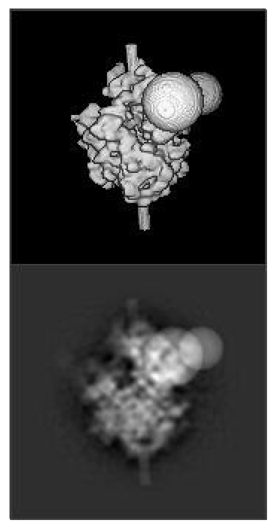
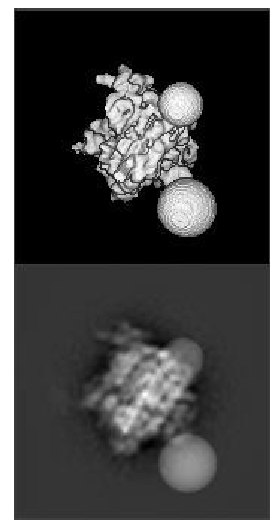
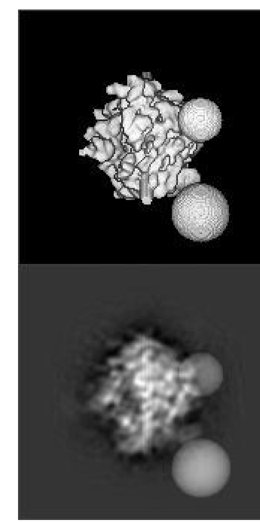
theta=045
psi=000



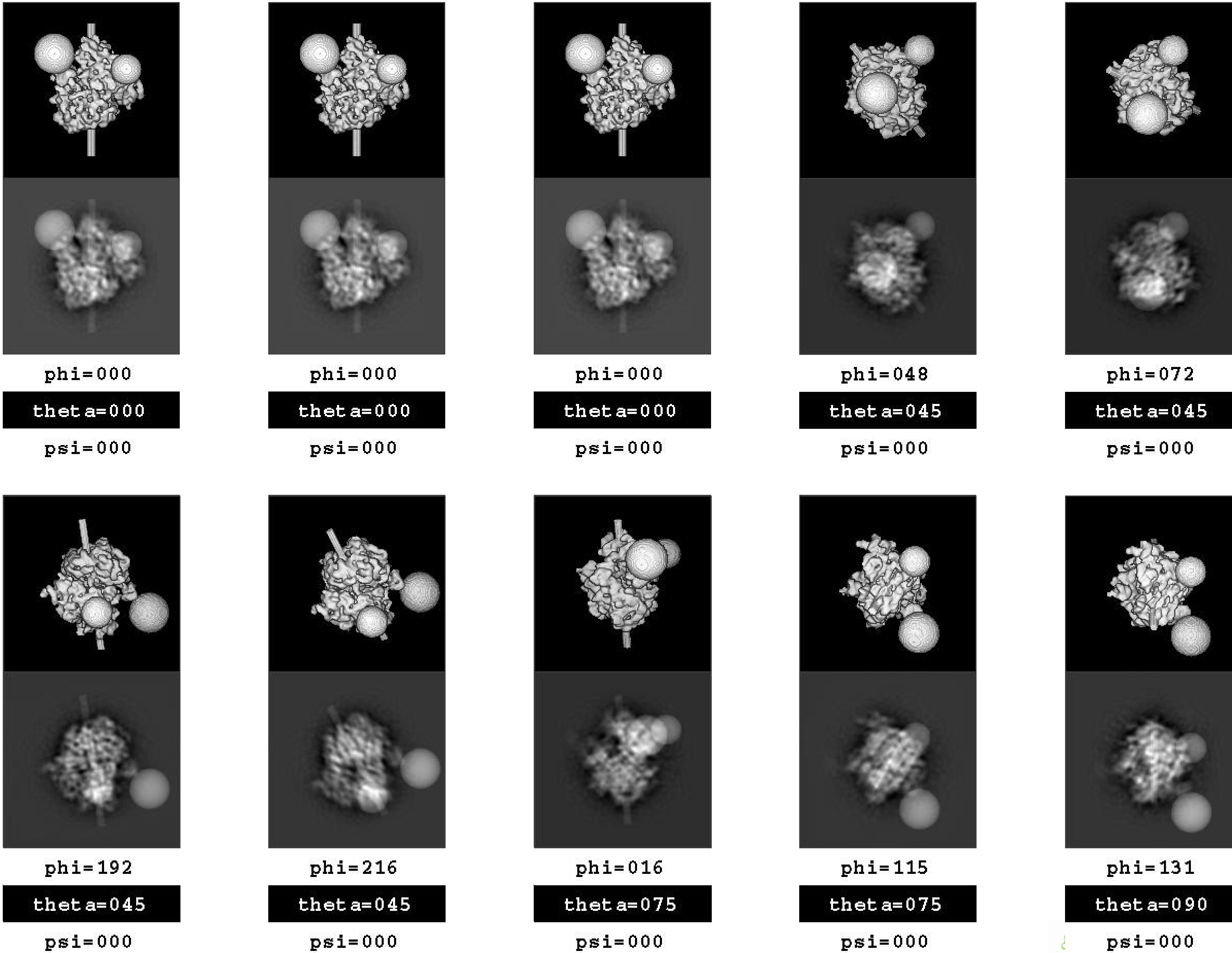
phi=016
theta=075
psi=000



phi=115
theta=075
psi=000



phi=131
theta=090
psi=000


phi=000
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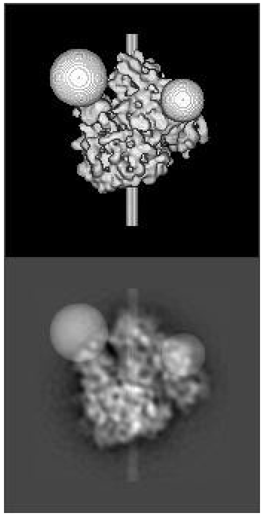
theta=090
psi=000



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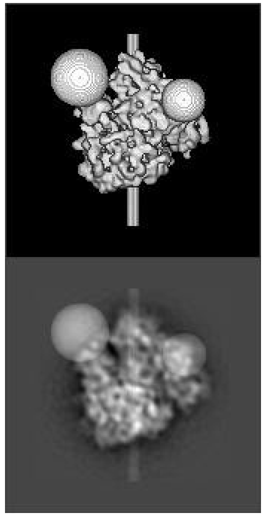

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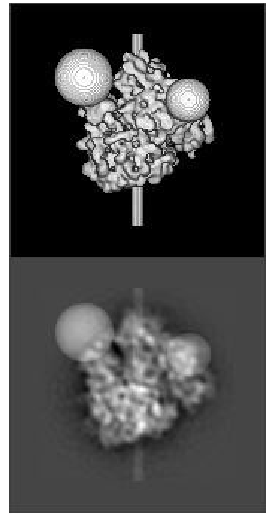

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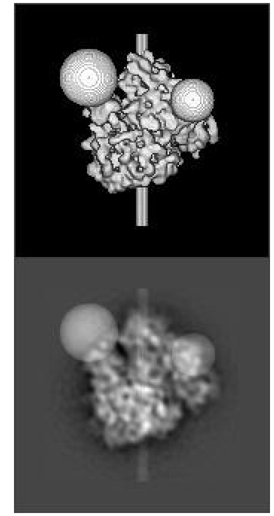

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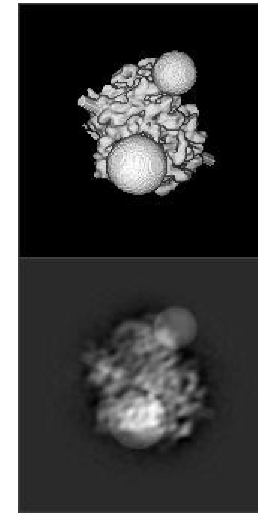

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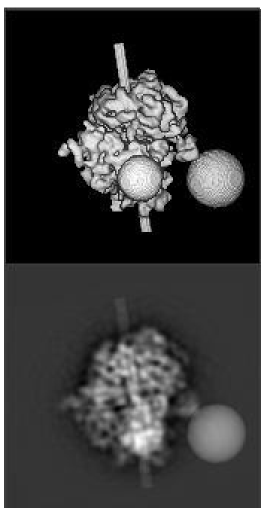

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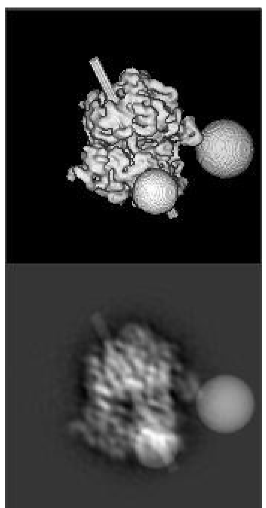
theta=045

psi=000



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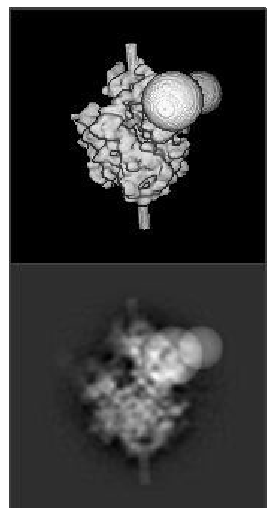
theta=045

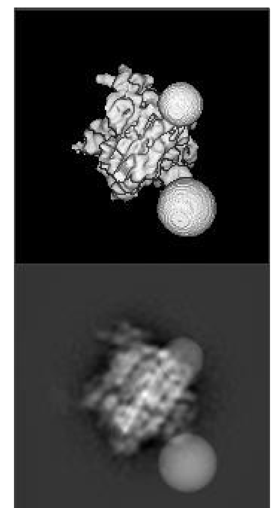
psi=000



phi=016

theta=075

psi=000



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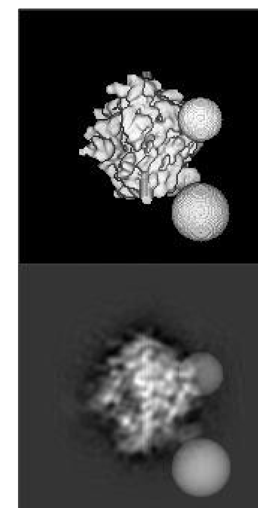
theta=075

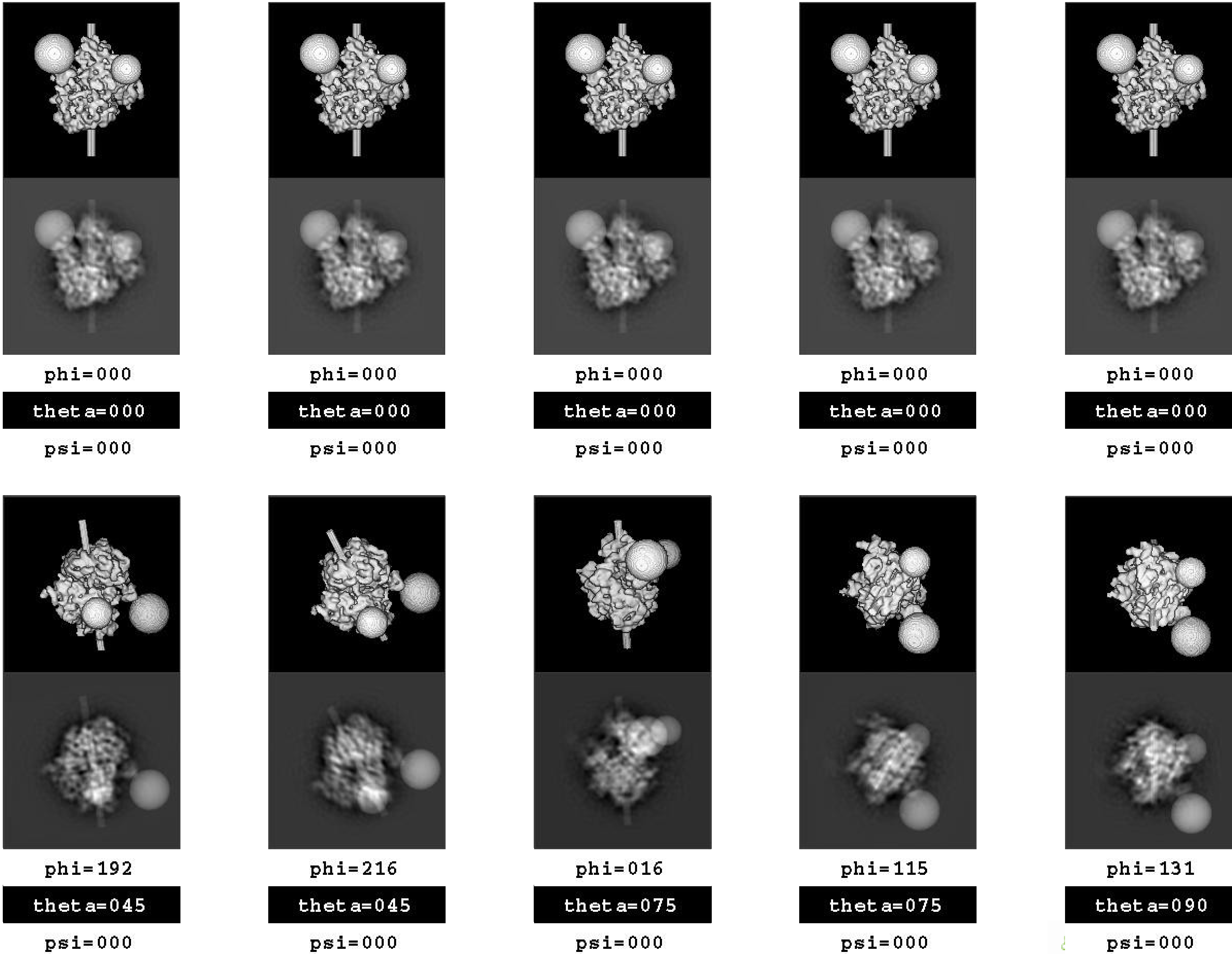
psi=000



phi=131

theta=090

psi=000



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psi=000
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psi=000
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psi=000
phi=216
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psi=000
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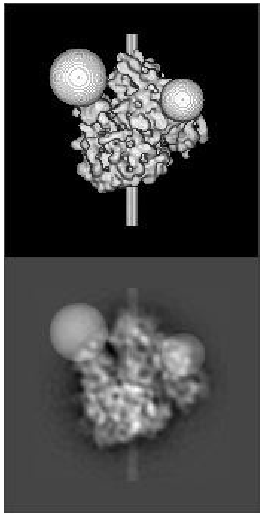
psi=000
phi=115
thet a=075
psi=000
phi=131
thet a=090
psi=000



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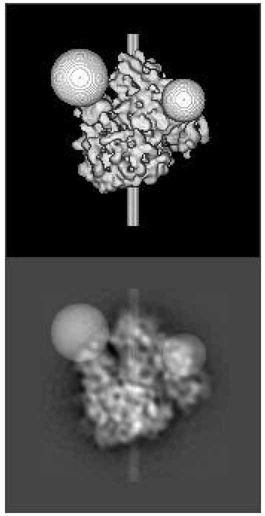

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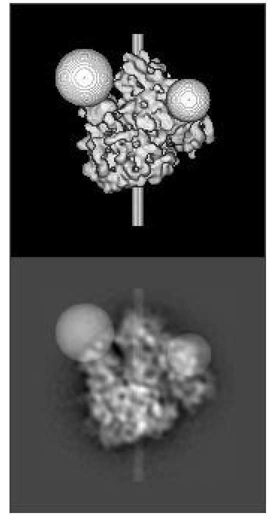

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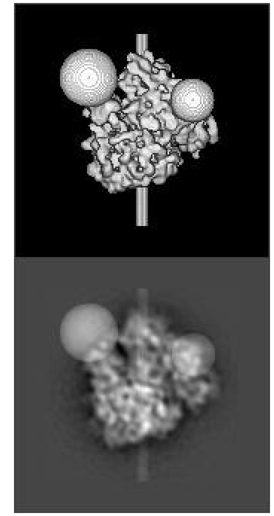

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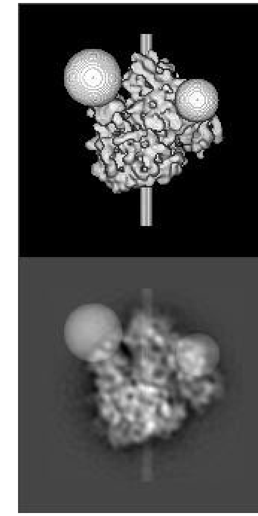

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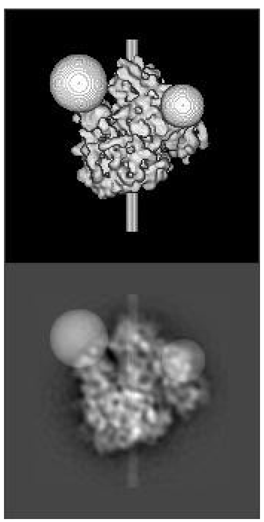

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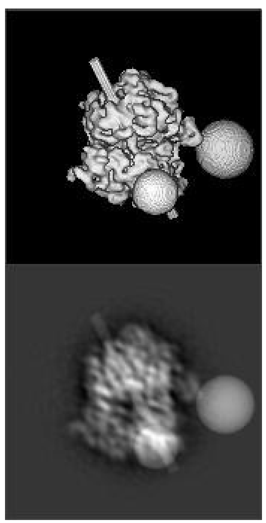

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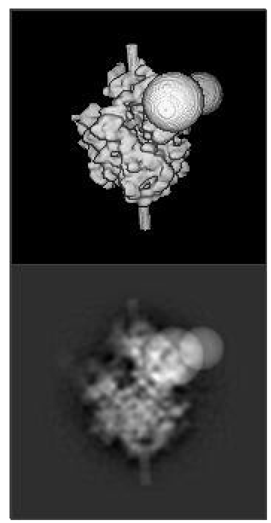

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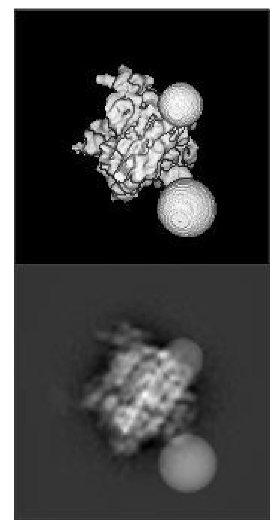
psi=000



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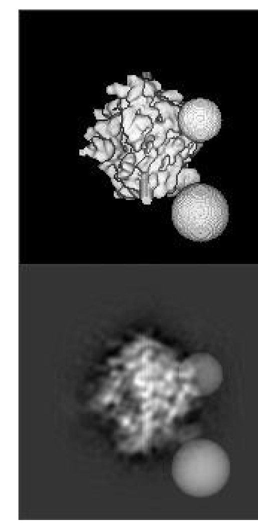
theta=075
psi=000



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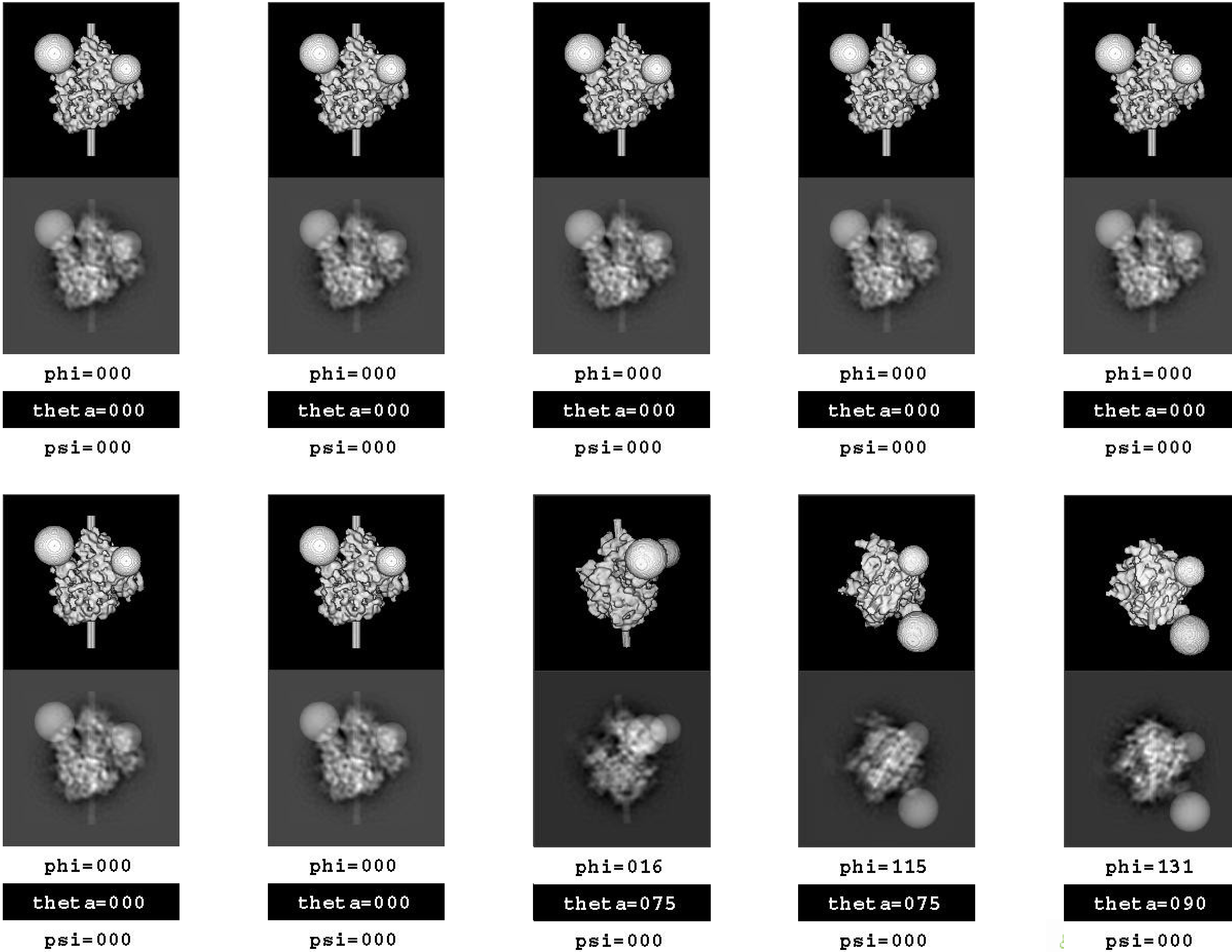
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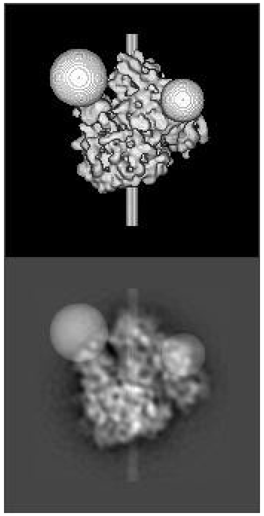
psi=000



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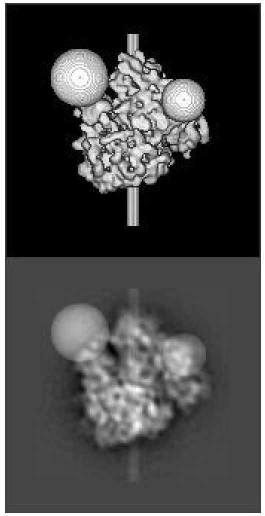

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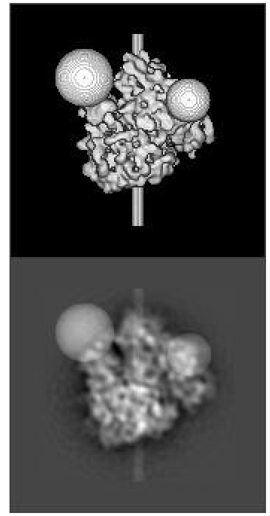

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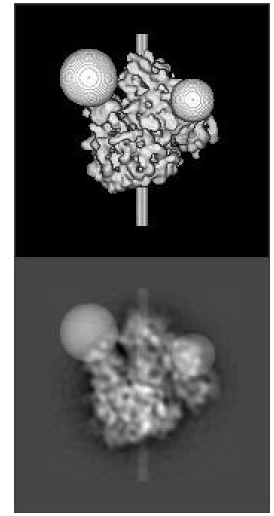

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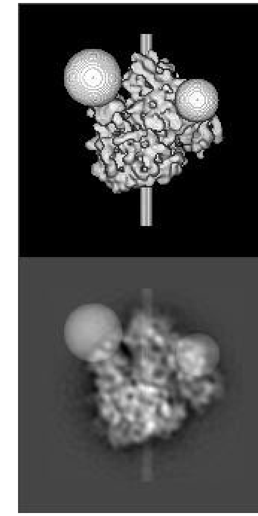

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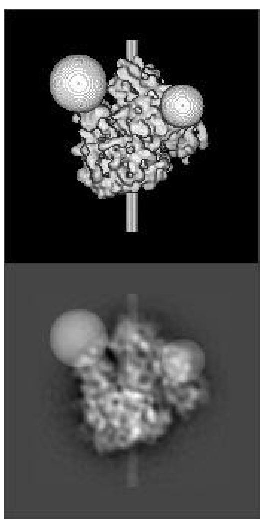

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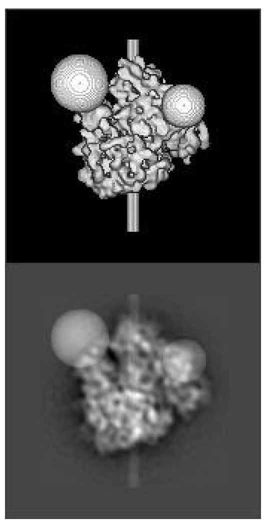

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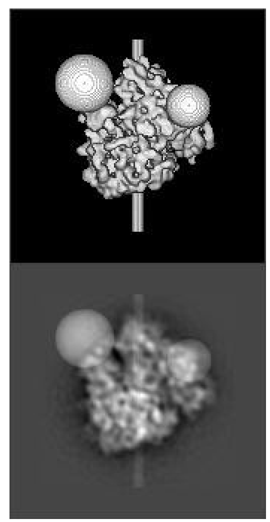

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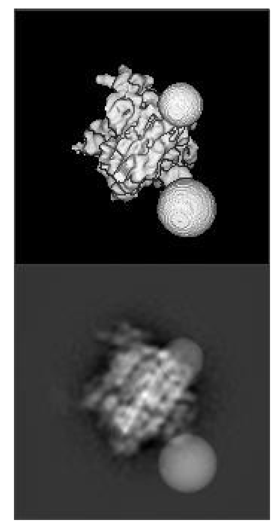

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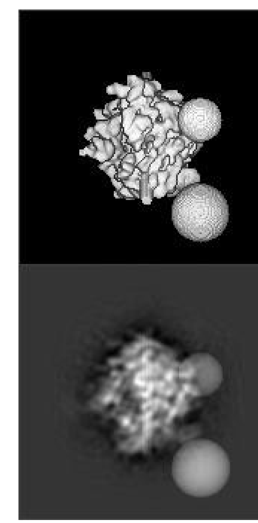

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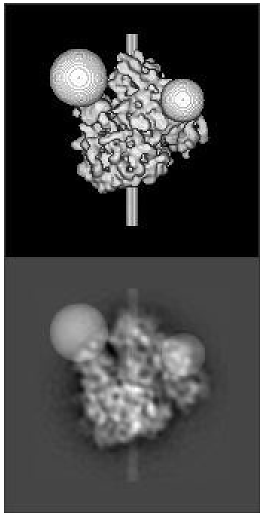

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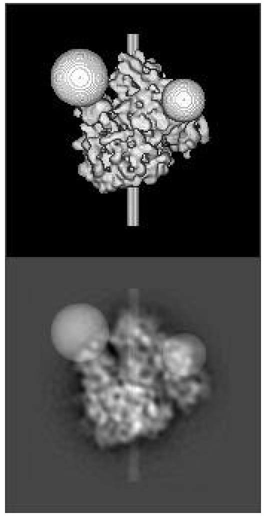

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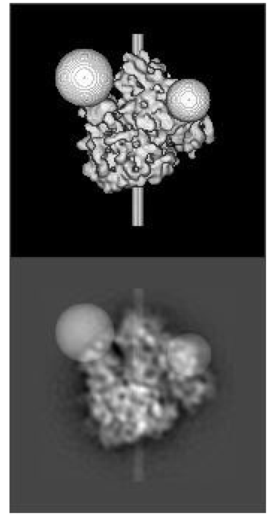

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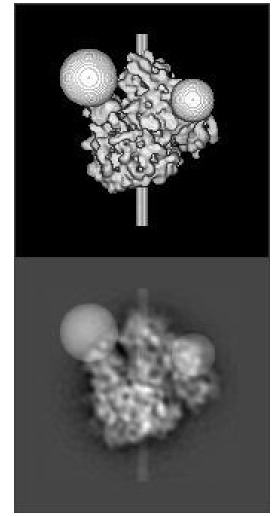

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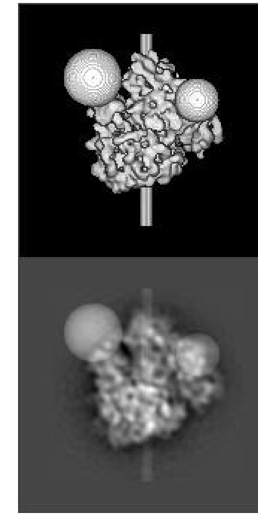

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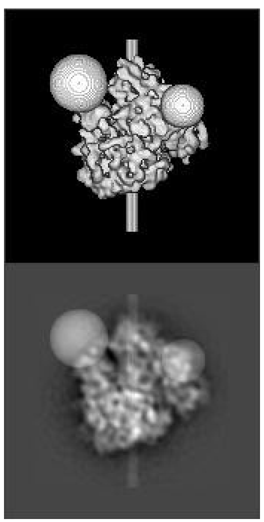

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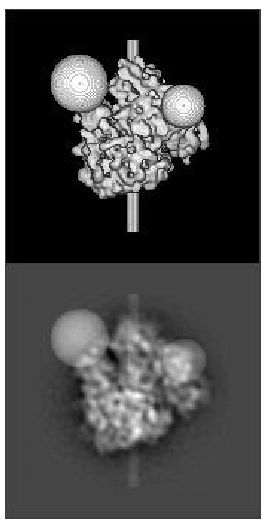

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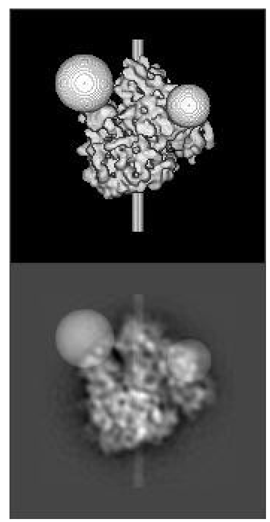

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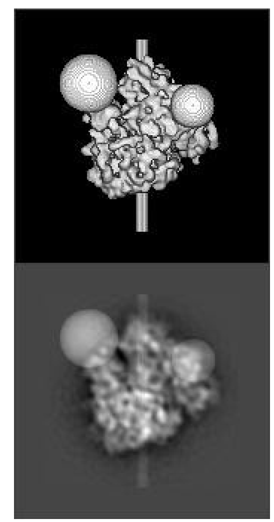

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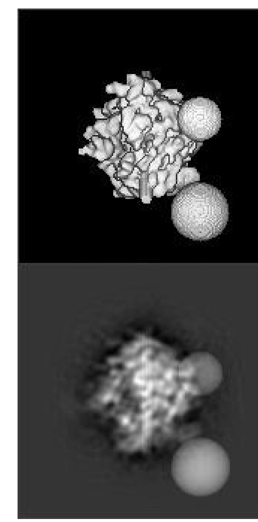

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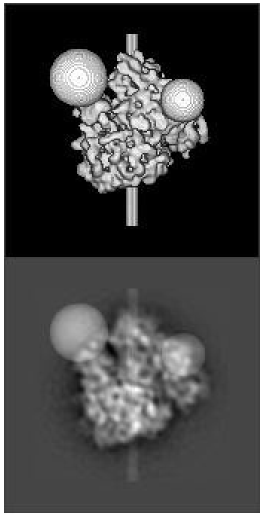

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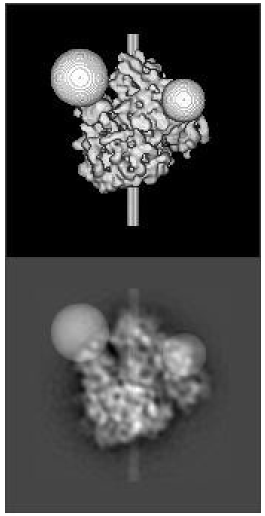

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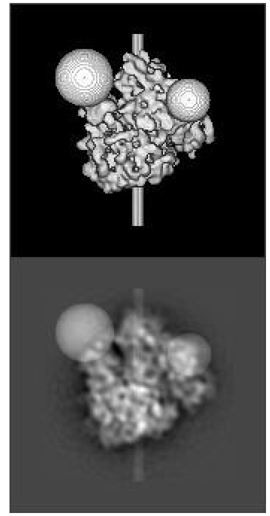

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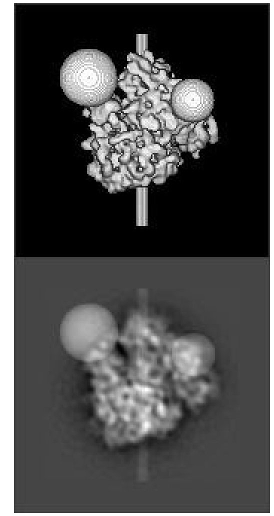

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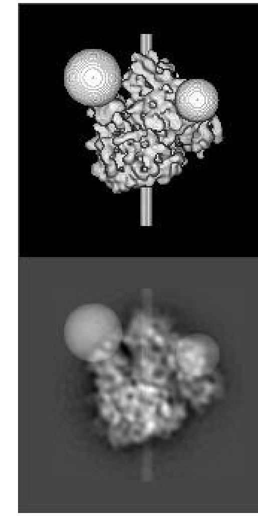

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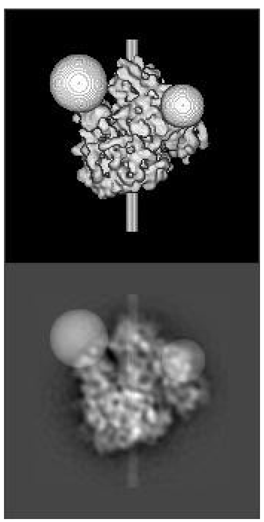

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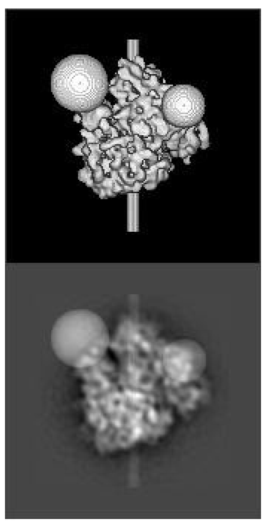

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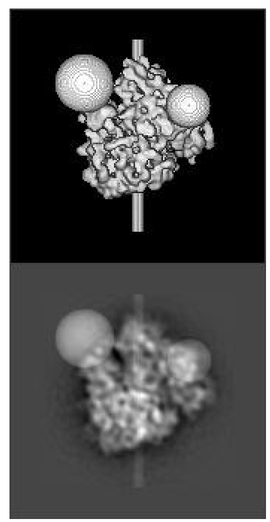

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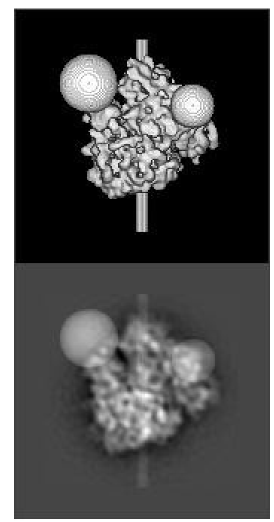

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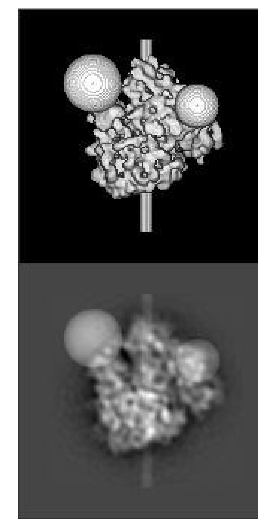

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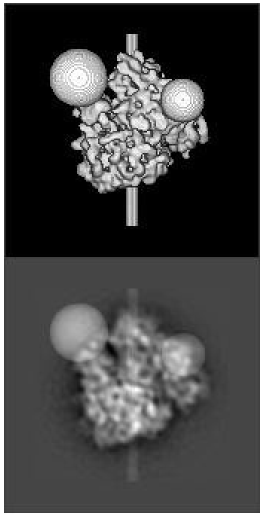

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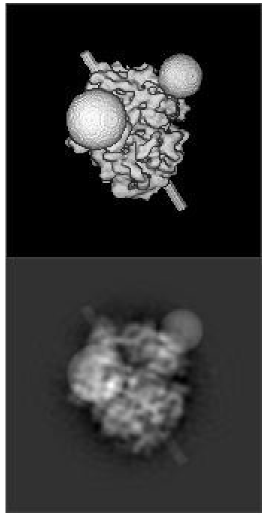

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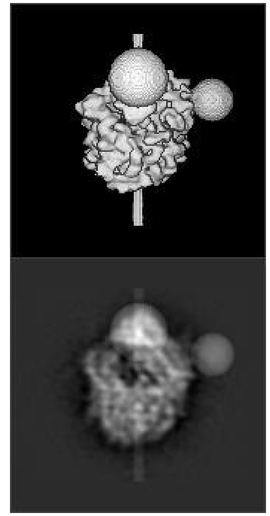
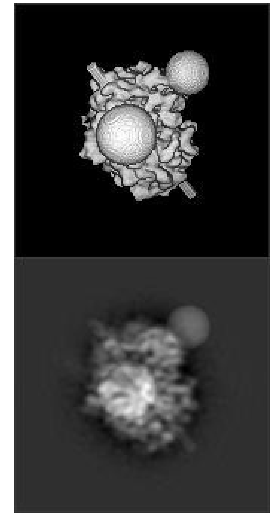
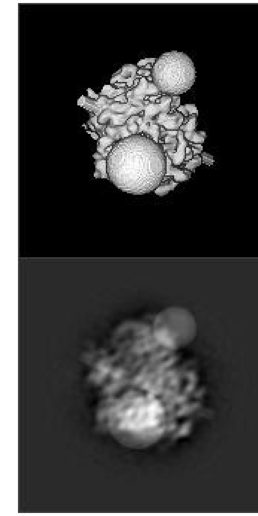


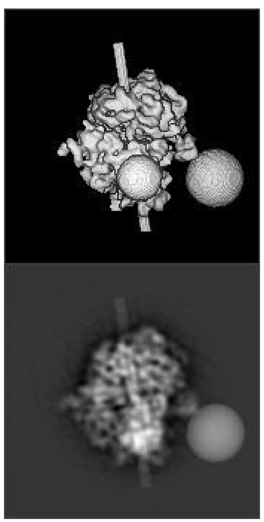
phi=000
theta=045
psi=000



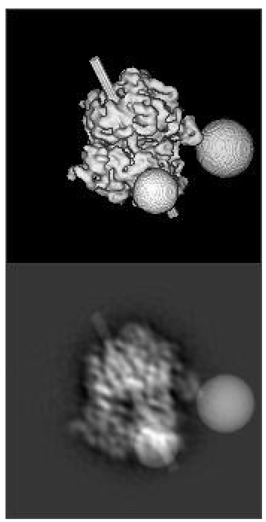
phi=048
theta=045
psi=000



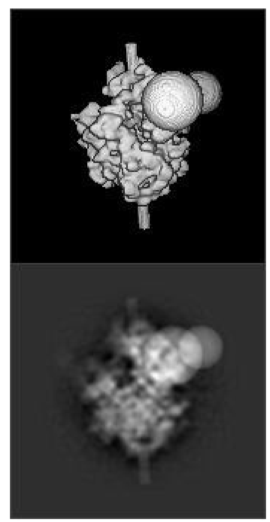
phi=072
theta=045
psi=000



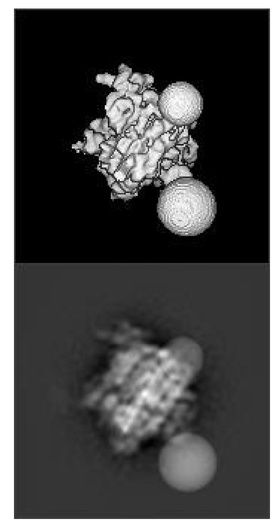
phi=192
theta=045
psi=000



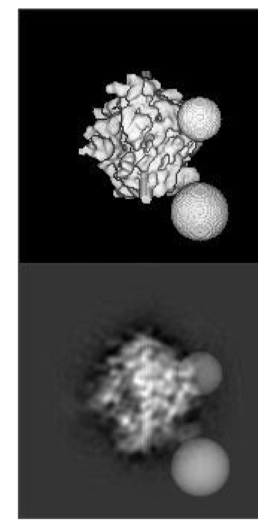
phi=216
theta=045
psi=000



phi=016
theta=075
psi=000

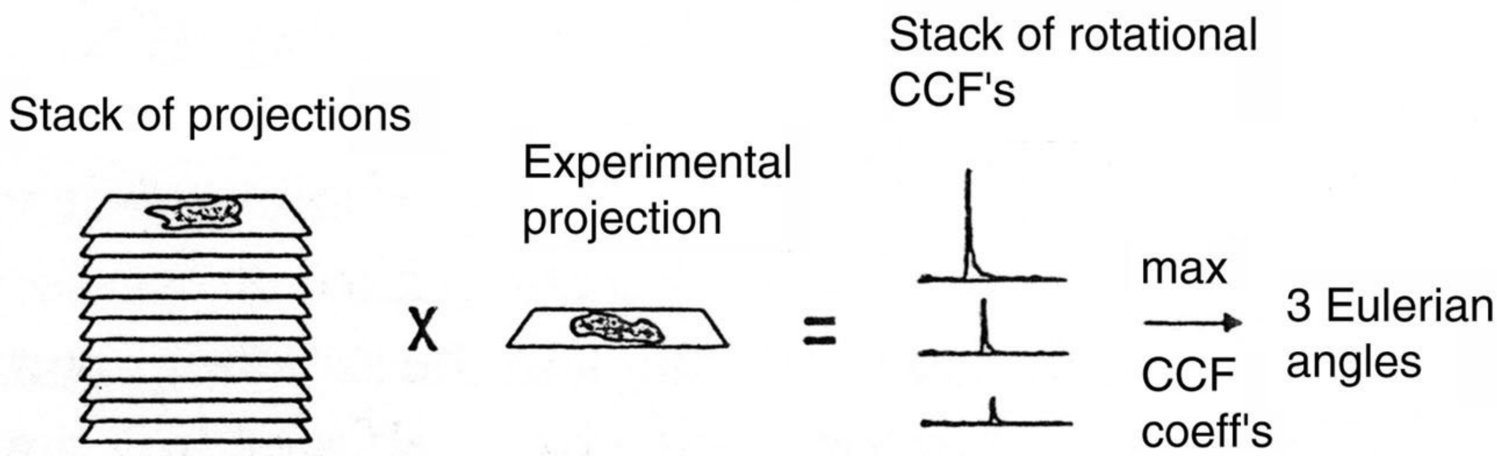


phi=115
theta=075
psi=000



phi=131
theta=090
psi=000

Reference-based alignment

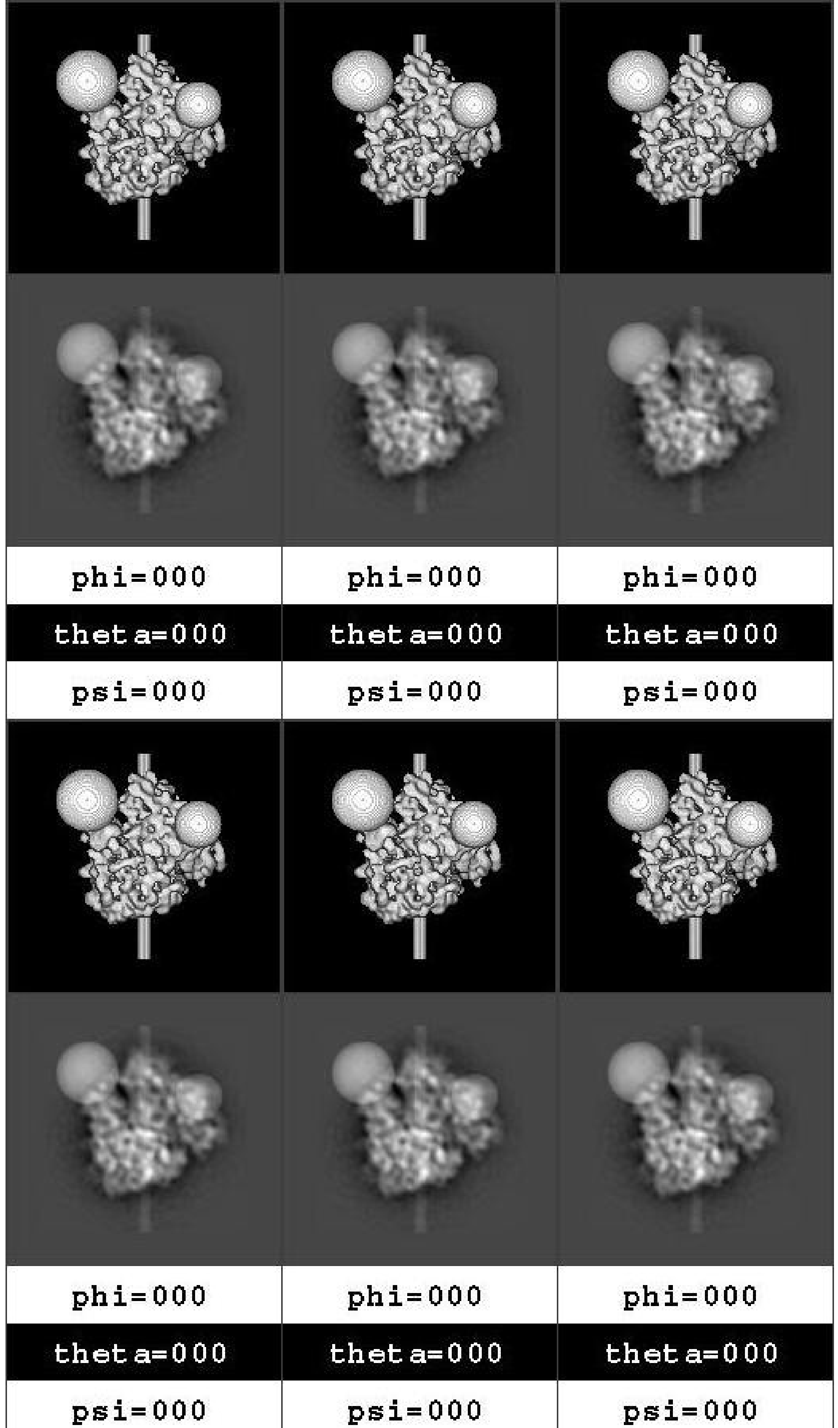


From Penczek *et al.* (1994), *Ultramicroscopy* **53**: 251-70.

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.

Random conical tilt


phi=000
theta=000
psi=000
phi=000
theta=000
psi=000
phi=000
theta=000
psi=000
phi=000
theta=000
psi=000
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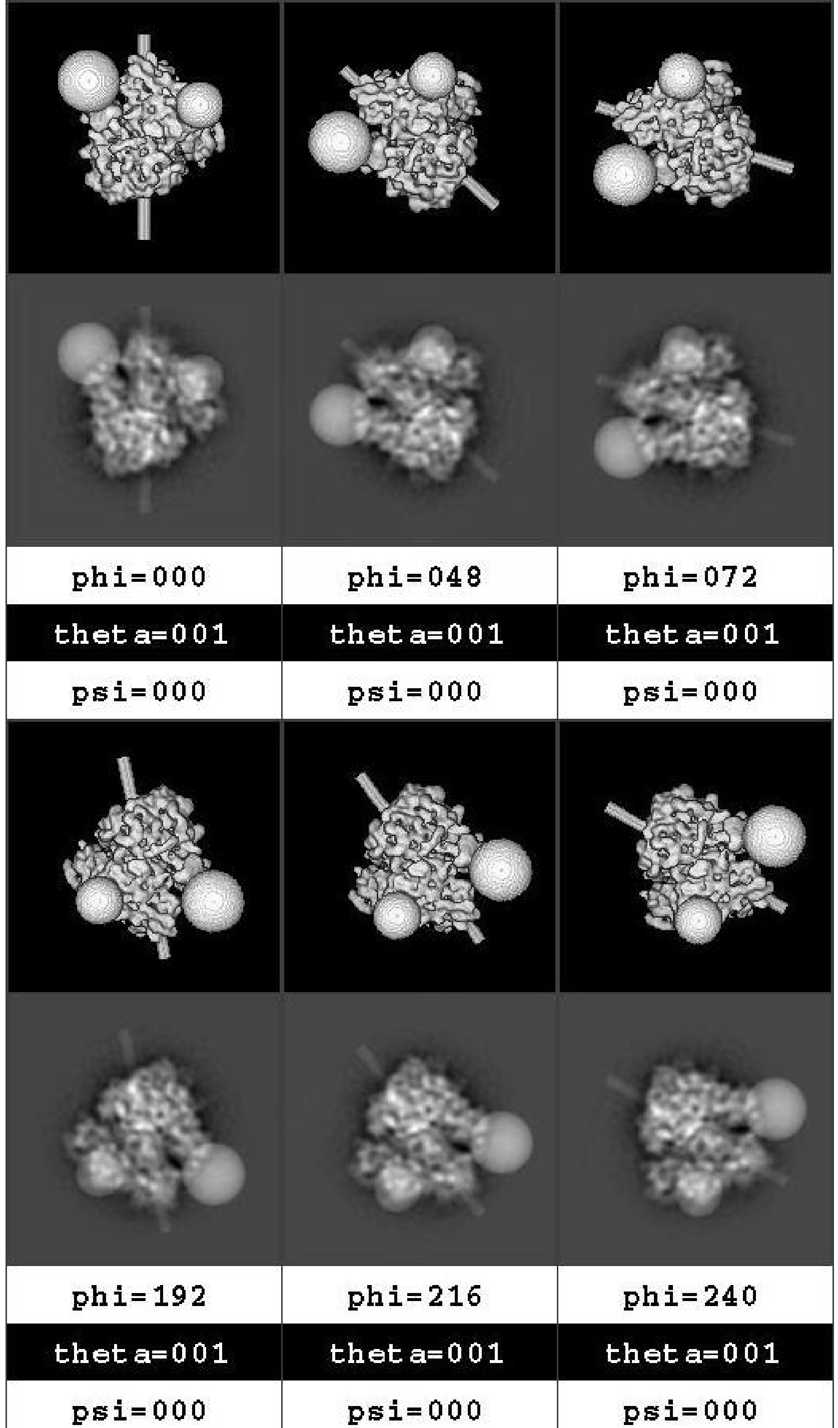
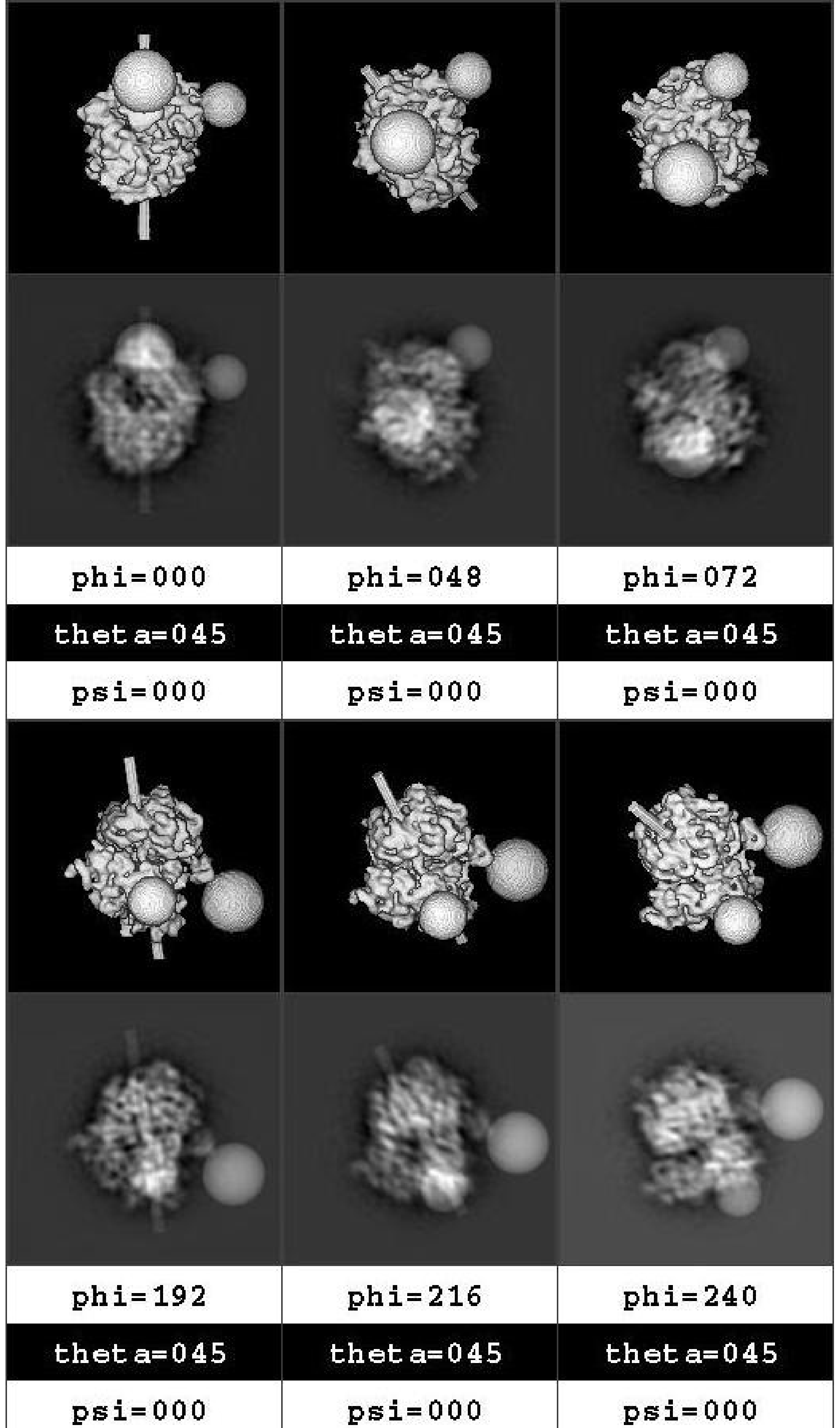
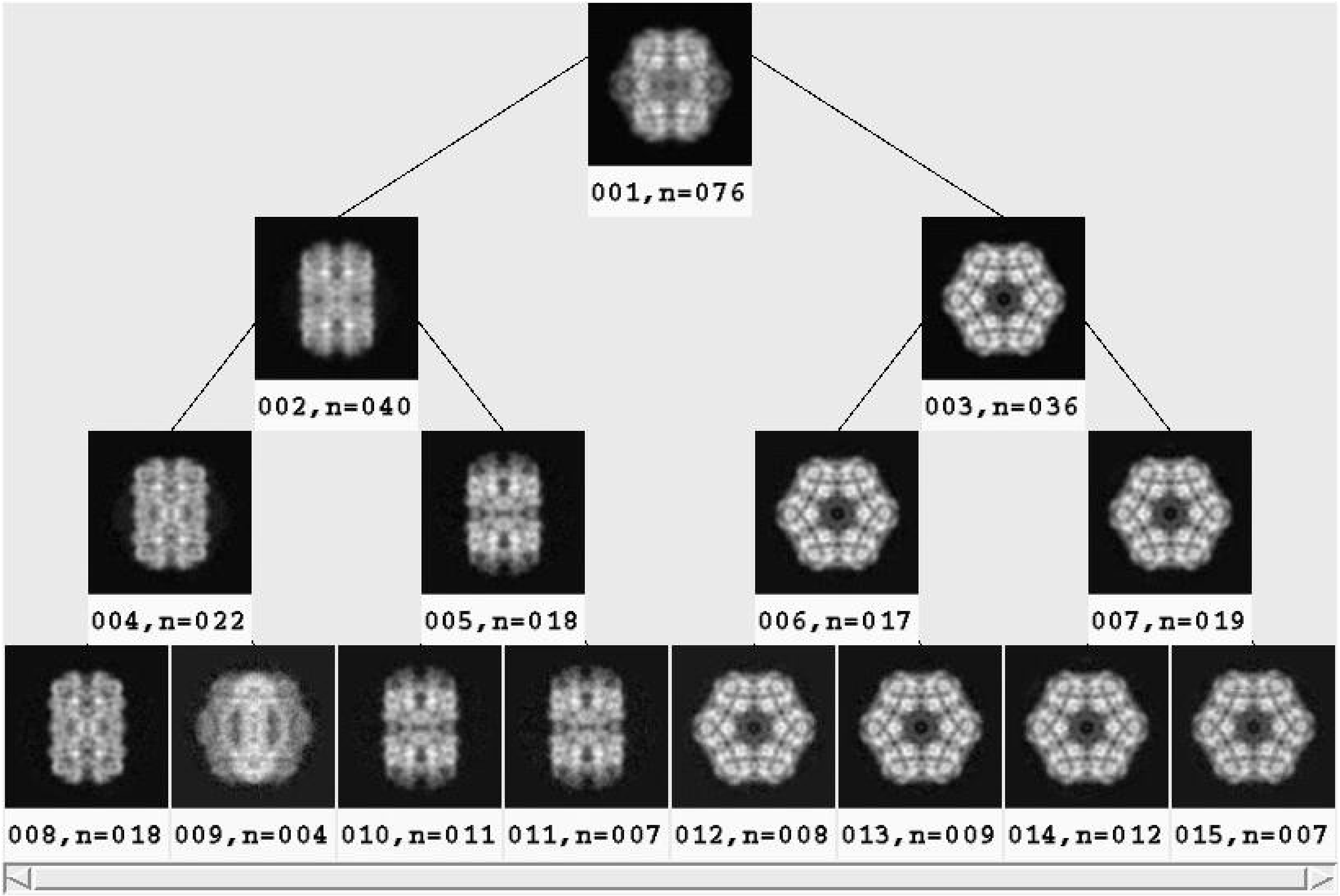
theta=000
psi=000
phi=000
theta=000
psi=000


phi=000
theta=001
psi=000
phi=048
theta=001
psi=000
phi=072
theta=001
psi=000
phi=192
theta=001
psi=000
phi=216
theta=001
psi=000
phi=240
theta=001
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=240
theta=045
psi=000

Binary-tree viewer

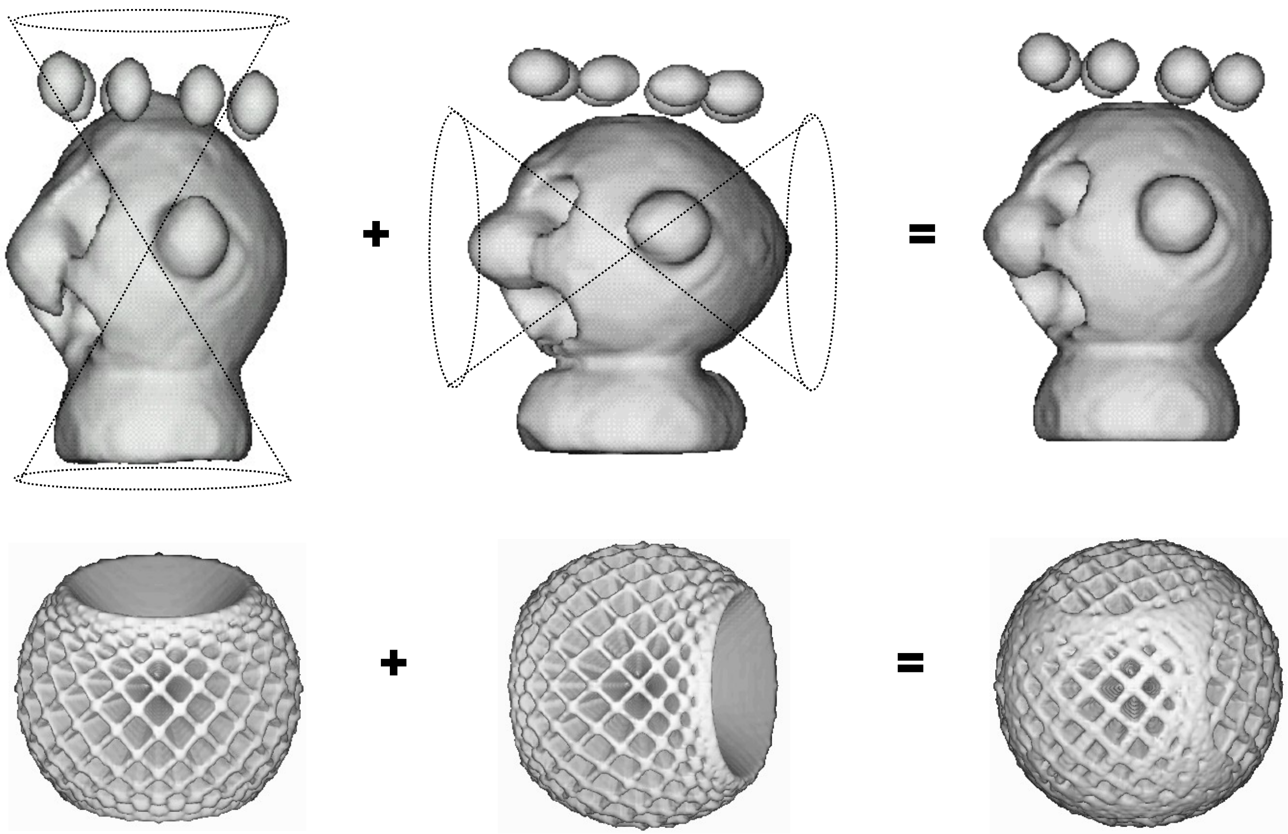


Random-conical tilt:
Filling the missing cone
Filling the missing cone

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

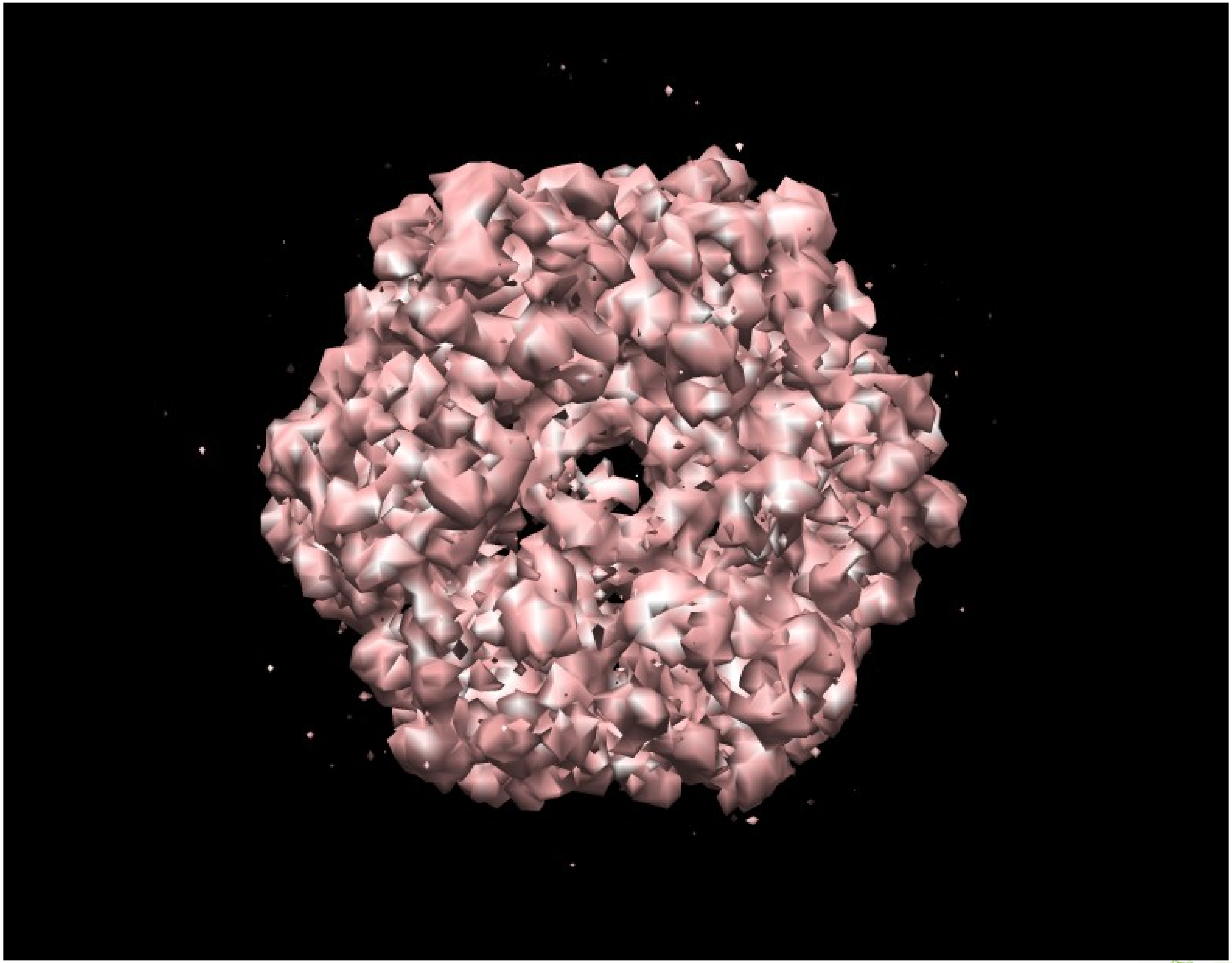
Reconstruction

Distribution of orientation

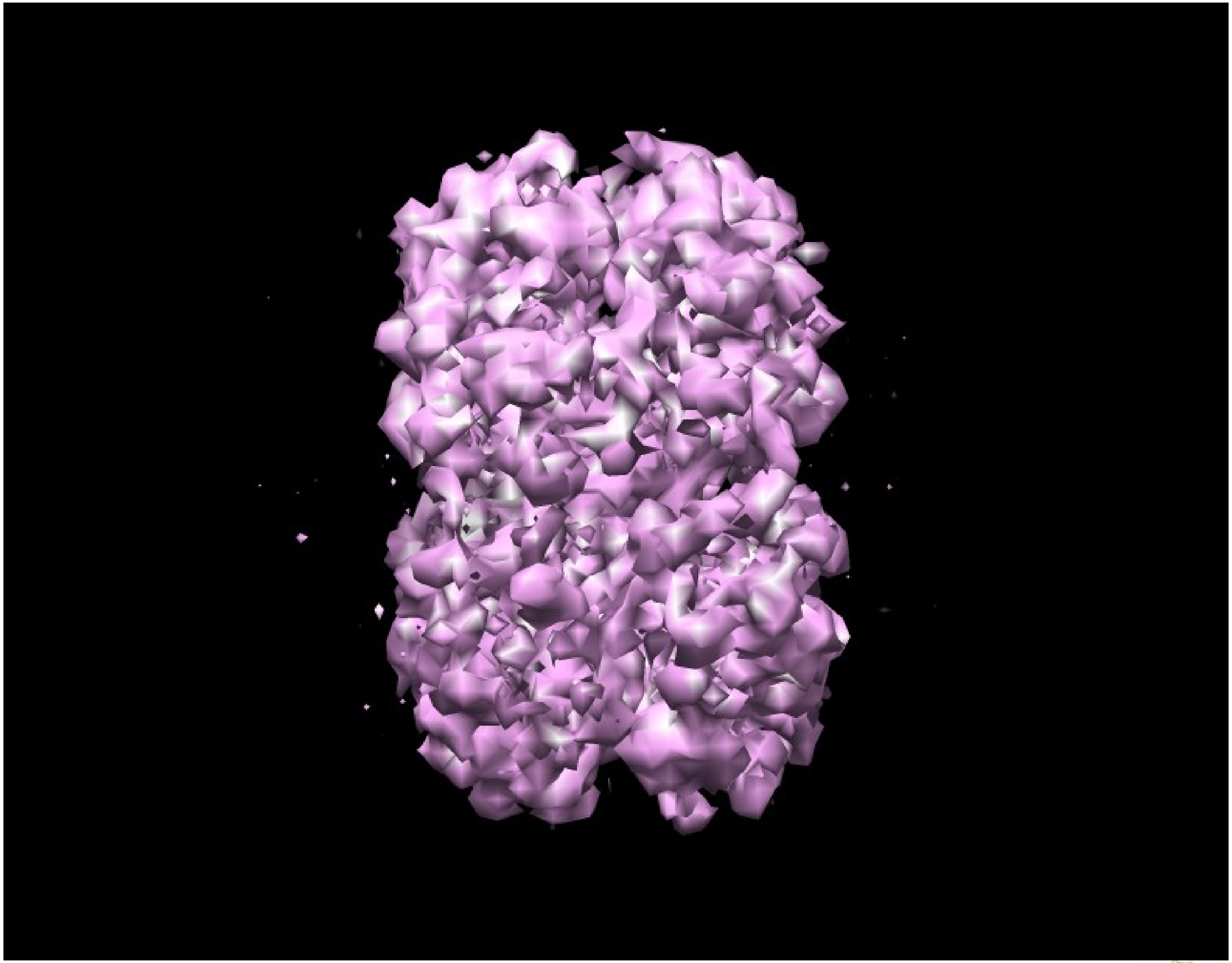


From Nicolas Boisset

Top view



Side view



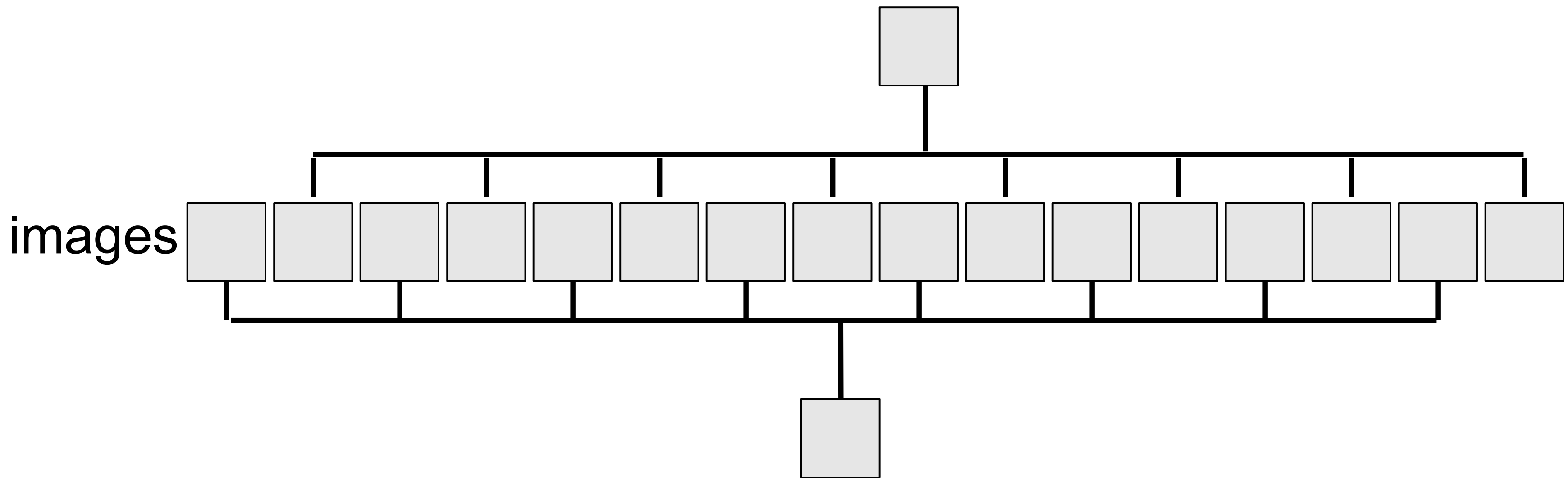
3D classification

Classification: Multi-reference alignment vs. Maximum likelihood (ML3D)

Multi-reference alignment:	ML3D
• Possible conformations must be known. • The combination of parameters (shift, rotation, class) is chosen from the highest correlation value.	• Possible conformations are not known. • The probability of the occurrence of the parameters (shift, rotation, class) is maximized.

Seeding ML3D classification

We split the data set into *K* classes at random.



There will be slight differences in the reconstructions. We will iteratively maximize the likelihood of a particle belonging to a particular class.

Thank you for your attention

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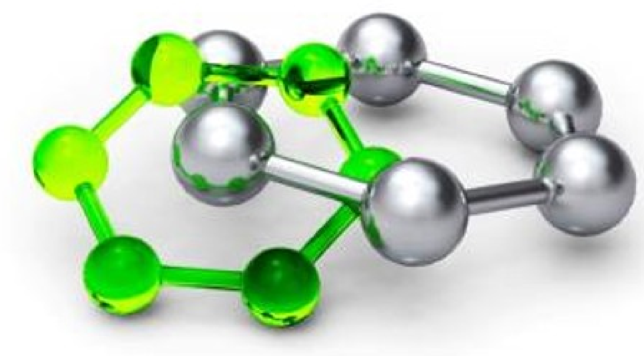

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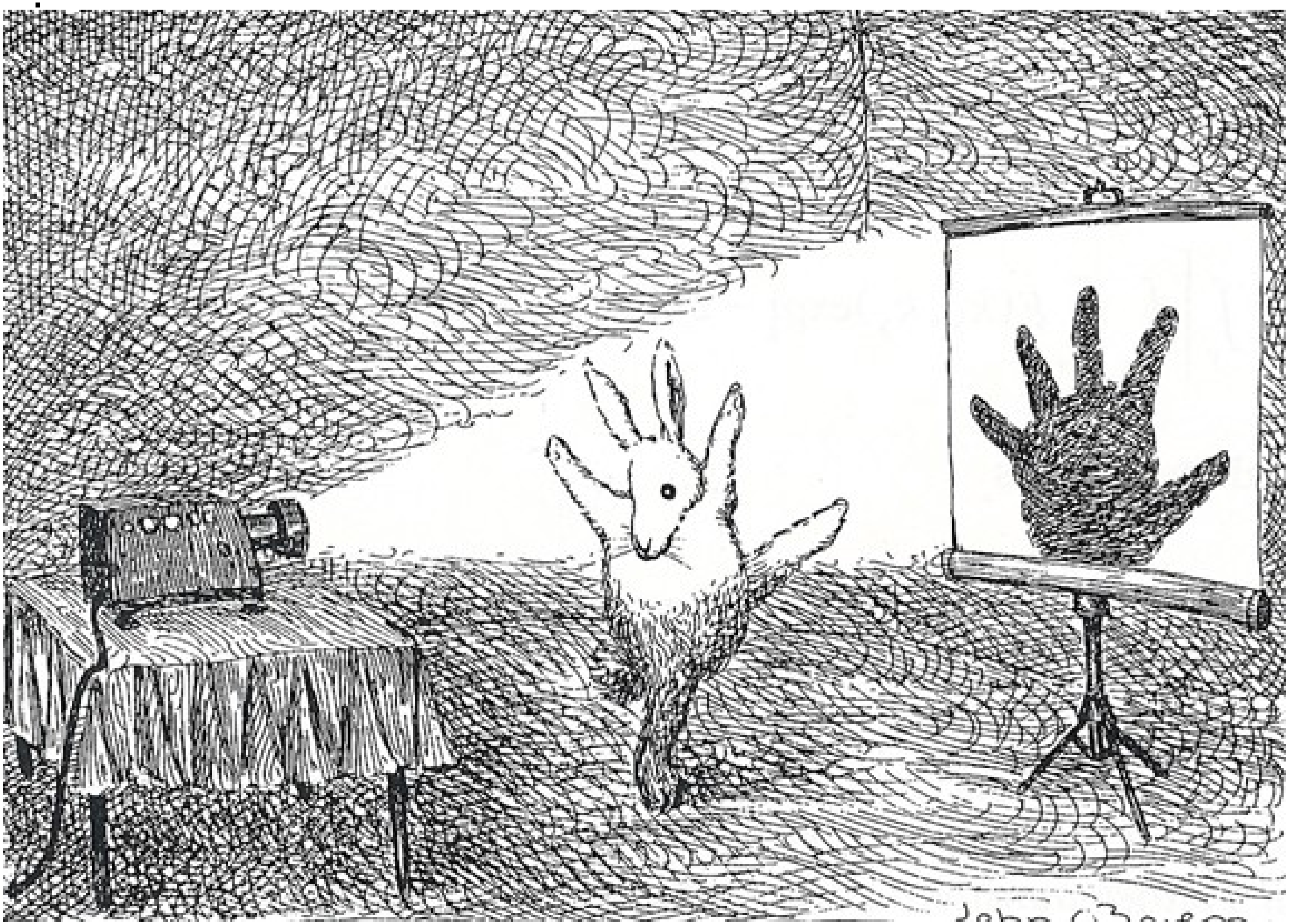
There isn't an unambiguous 3D structure if there's only one



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

What information do we need for 3D reconstruction?

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