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Single-particle reconstruction

*With an emphasis on Random Conical Tilt in
SPIDER*

March 9th, 2015



EUROPEAN UNION
EUROPEAN REGIONAL DEVELOPMENT FUND
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**OP Research and
Development for Innovation**

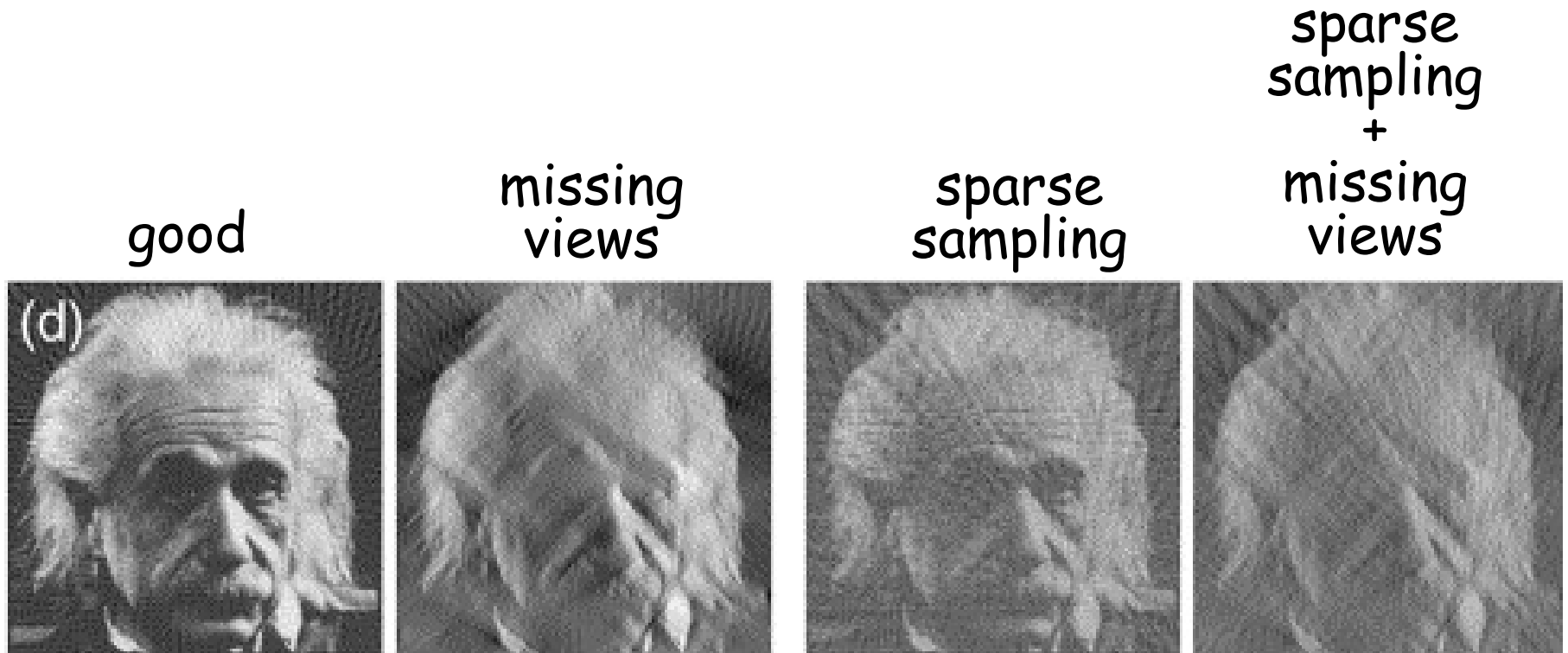


What information do we need for 3D reconstruction?

1. different orientations
2. known orientations

*What happens when we
don't have enough views?*

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 may need to explicitly tilt the microscope stage.
(more later...)

What information do we need for 3D reconstruction?

1. different orientations

2. known orientations

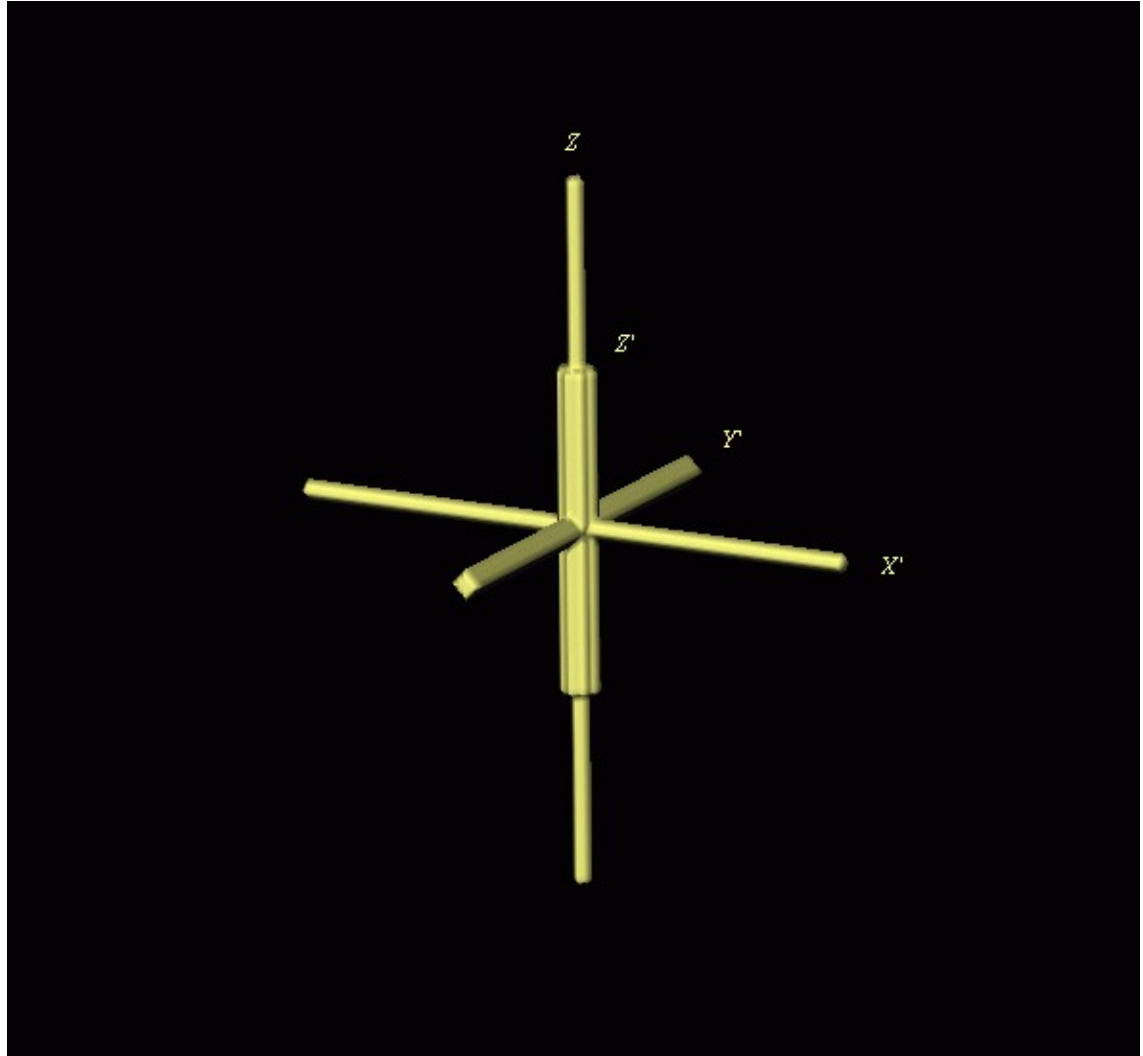
Required orientation parameters

Two translational:

- Δx
- Δy

Three orientational
(Euler angles):

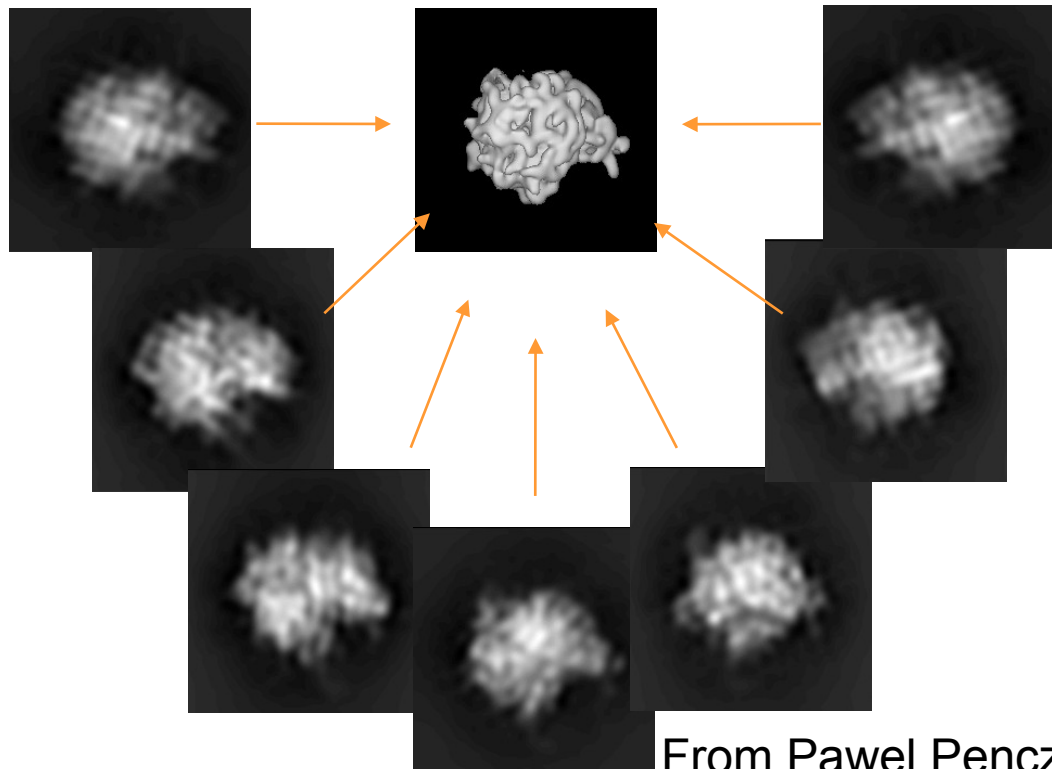
- phi (about z axis)
- theta (about y)
- psi (about new z)



From http://www.wadsworth.org/spider_doc/spider/docs/euler.html

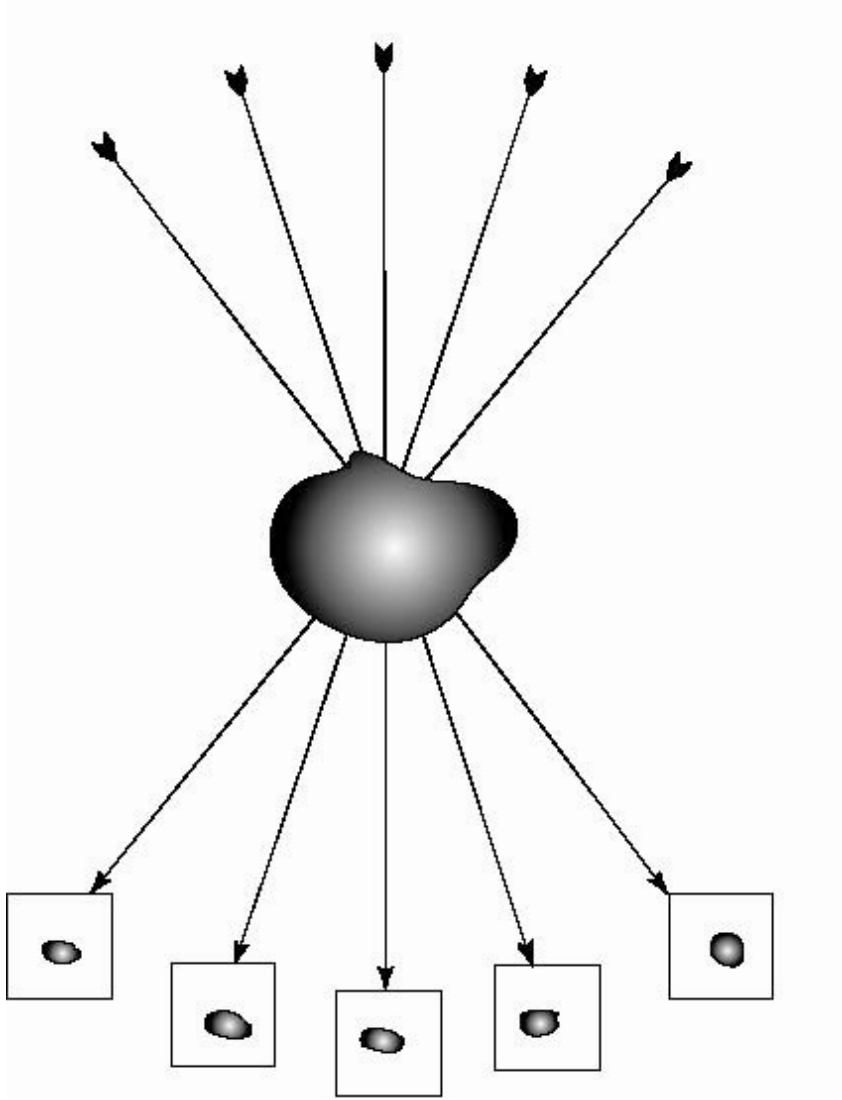
How do we use those orientation parameters?

Now that you know the Euler angles for each image, you can compute a back-projection.



*Getting different views:
Tomography vs. single-particle*

Tomography



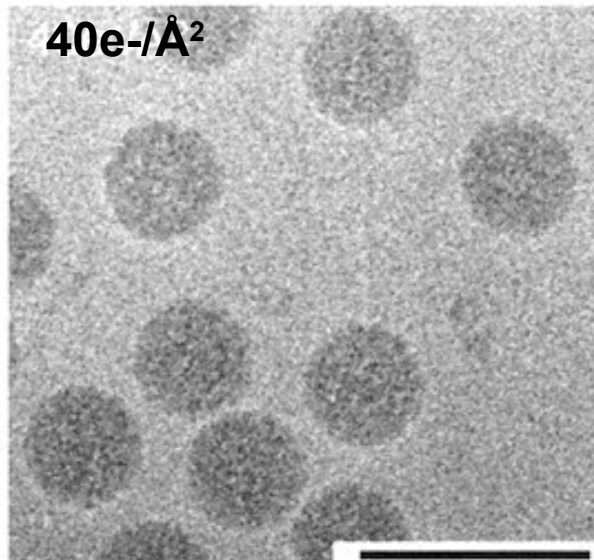
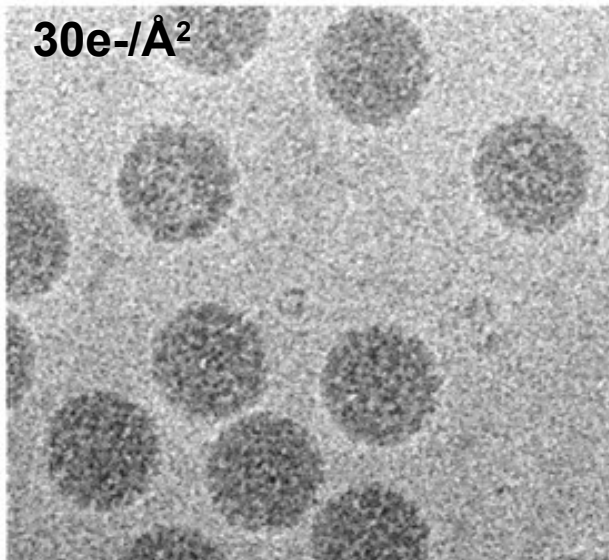
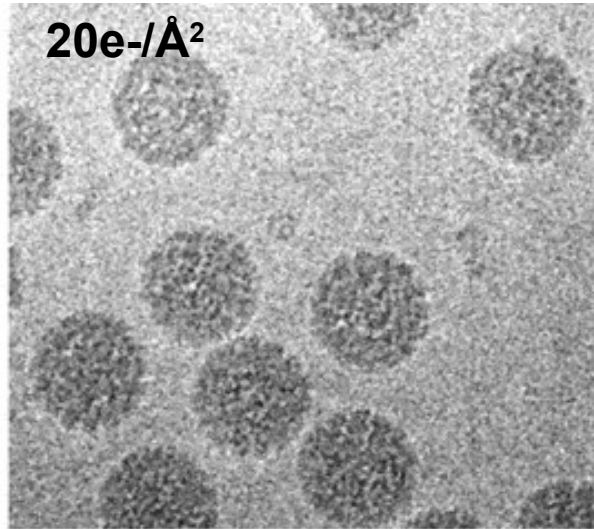
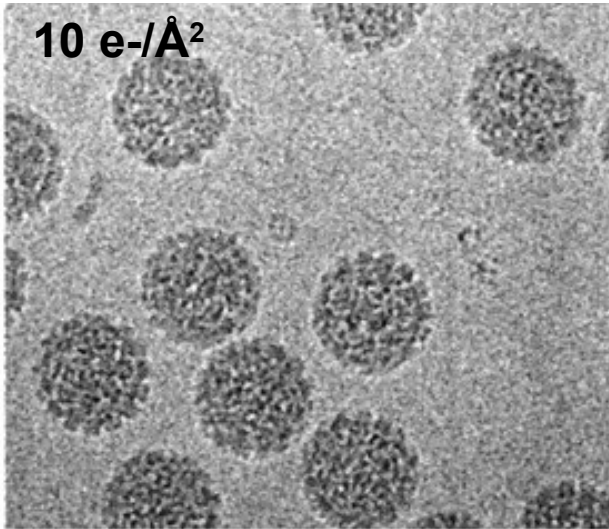
From Ken Downing

We have:

- known orientations
- different views

BUT...

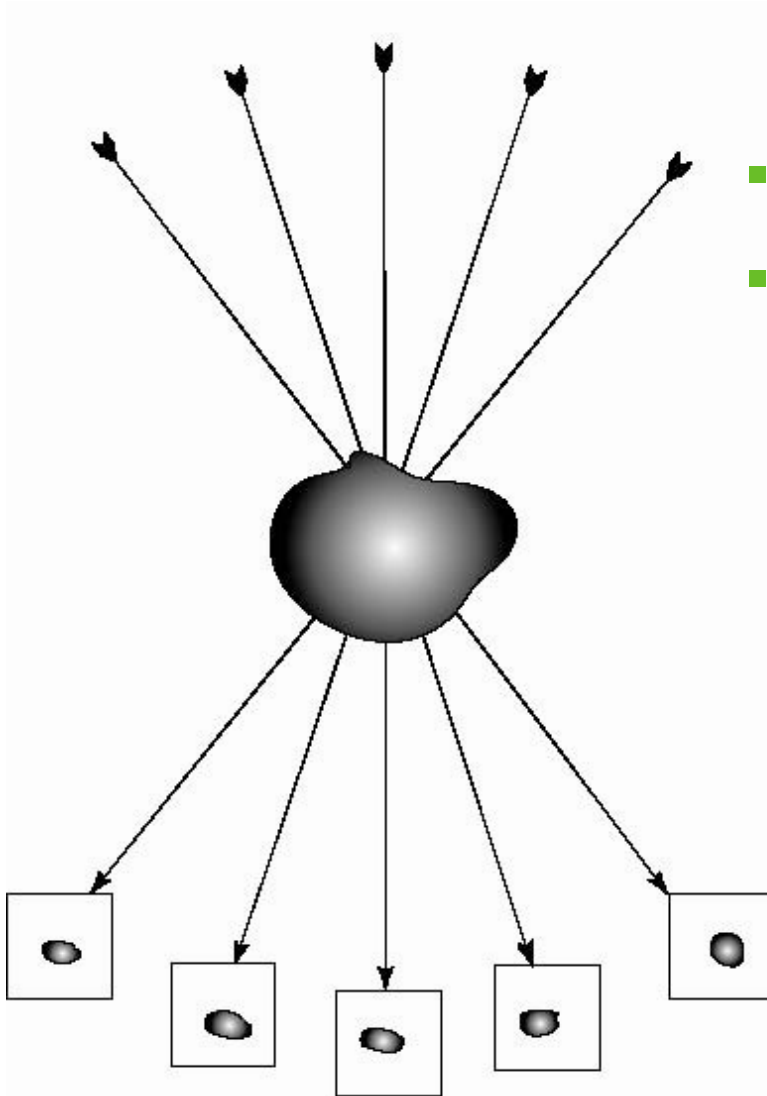
What happens when we image the sample?



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

We are destroying the sample as we image it.

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”

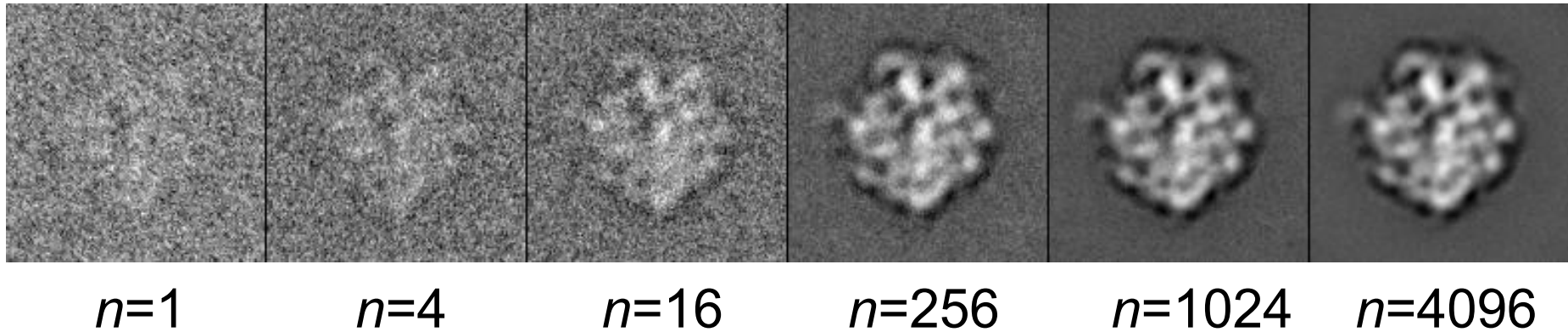
What information do we need for 3D reconstruction?

1. different orientations
2. known orientations

What information do we need for 3D reconstruction?

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

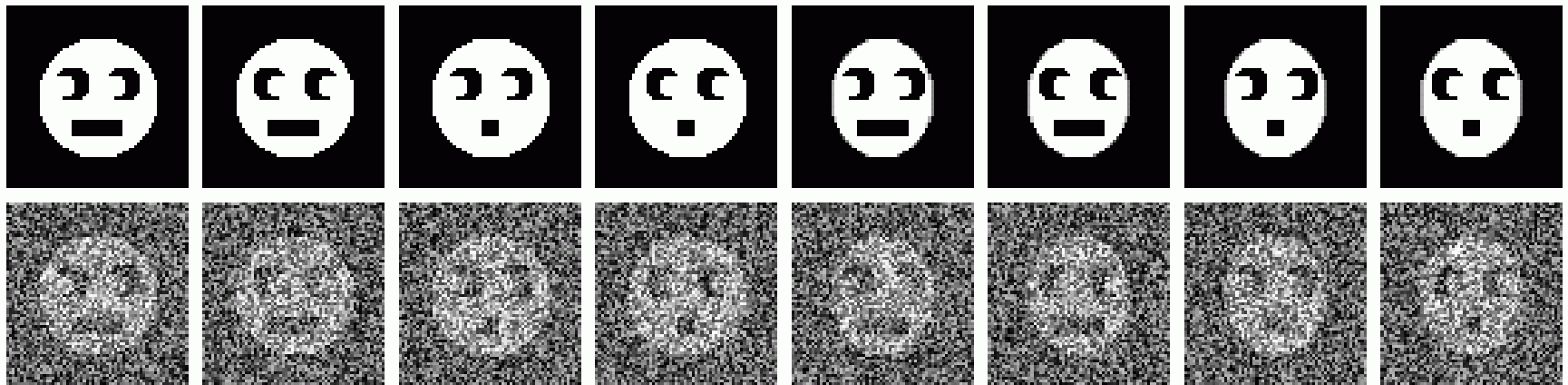
What happens as we include more particles?



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

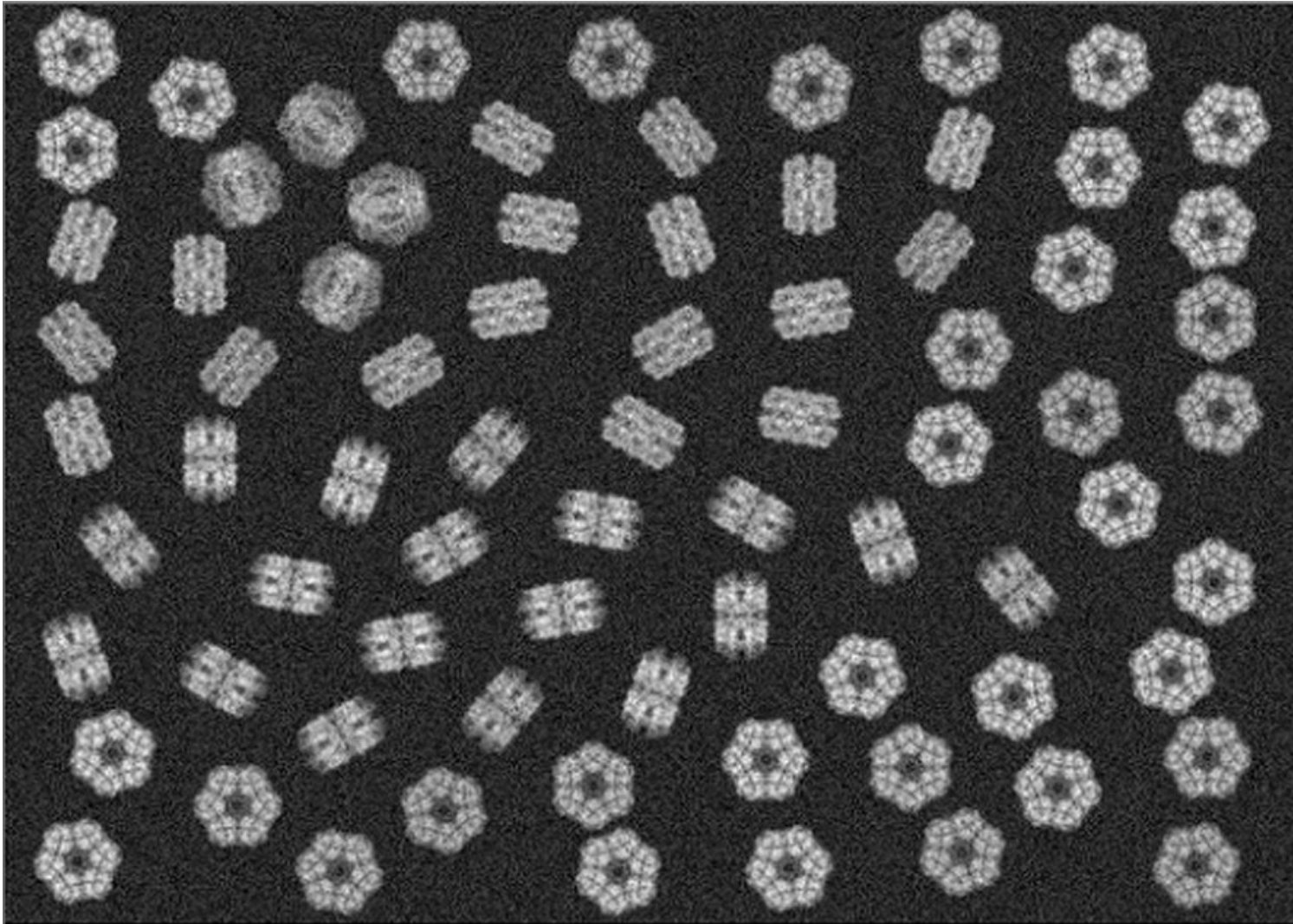
But wait...

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.



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

A more realistic (but still fake) example



From Nicolas Boisset
Synthetic images of worm hemoglobin
Shaikh *et al.*, (2008) *Nature Protocols* **3**: 1941-74.

What information do we need for 3D reconstruction?

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

What information do we need for 3D reconstruction?

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

Now we need to find the orientations for each particle.

How to determine orientation?

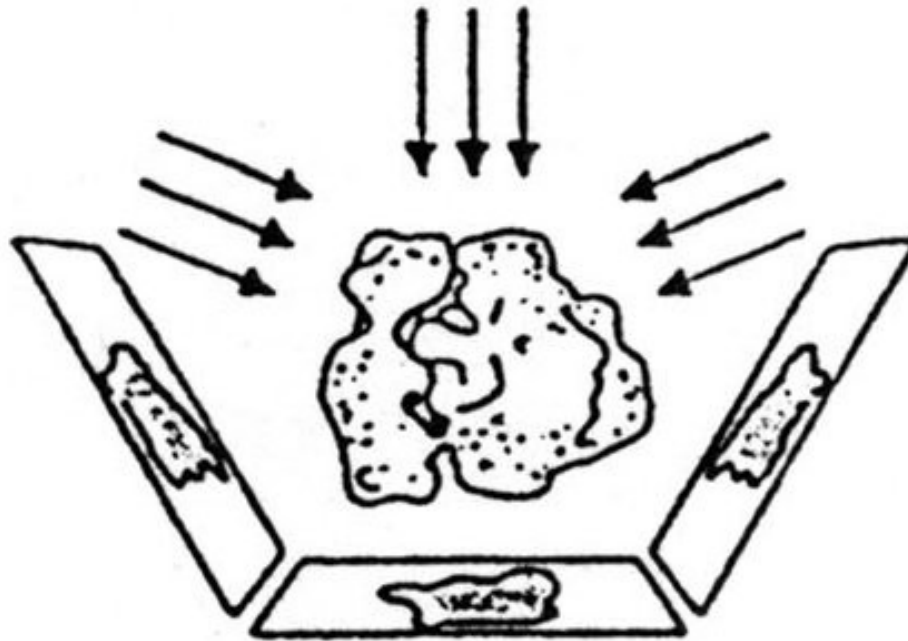
Two scenarios:

1. You have a reference.
2. You don't have a reference.

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.

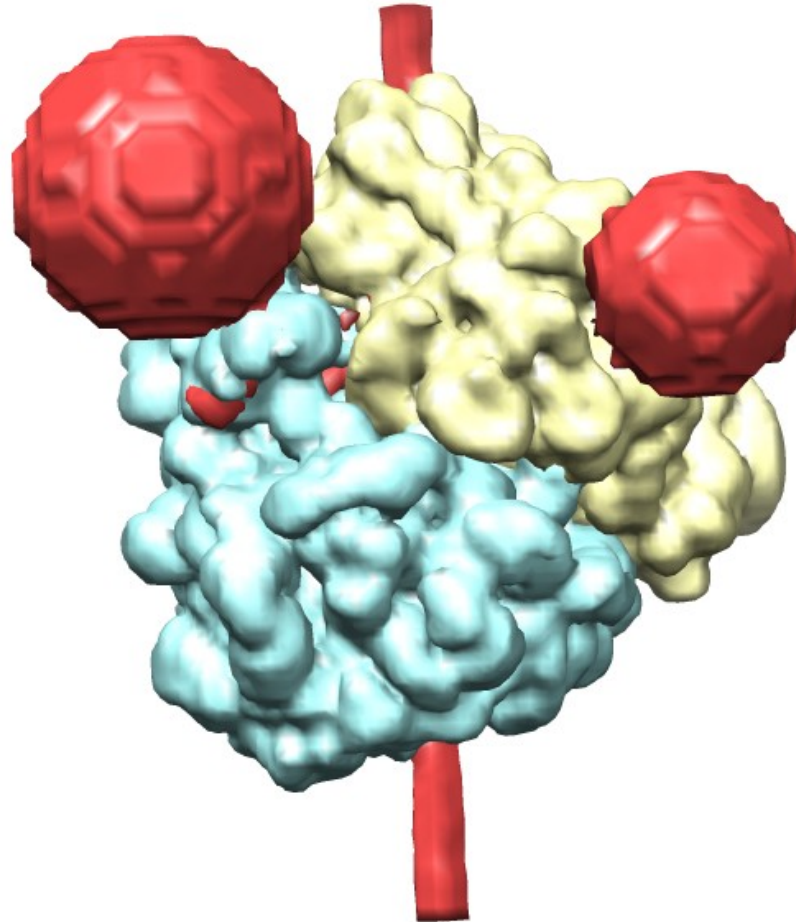
Step 1: Generation of projections of the reference.



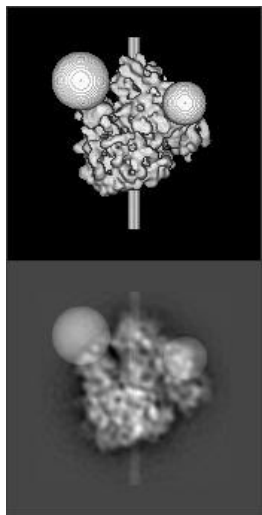
From Penczek *et al.* (1994), *Ultramicroscopy* **53**: 251-70.

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

The model



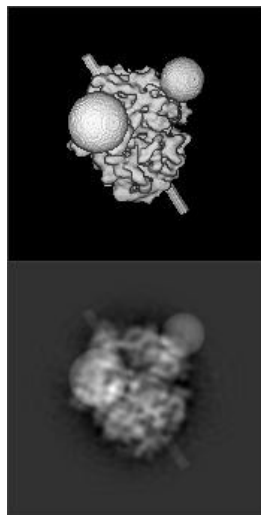
The extra features helped determine handedness in noisy reconstructions.



phi=000

theta=000

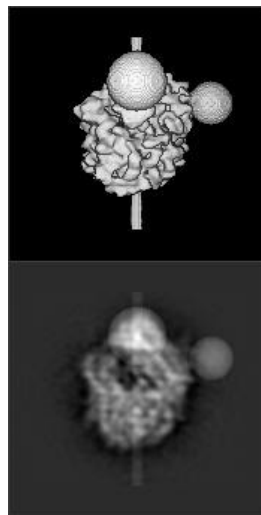
psi=000



phi=036

theta=030

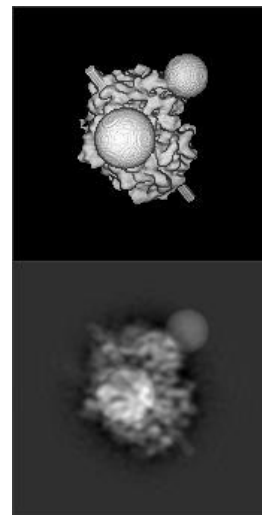
psi=000



phi=000

theta=045

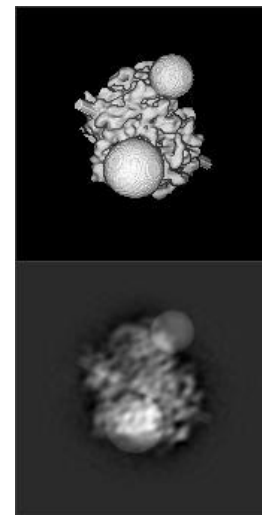
psi=000



phi=048

theta=045

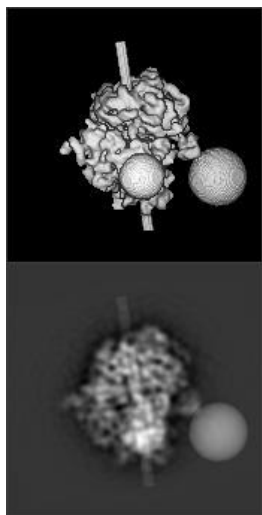
psi=000



phi=072

theta=045

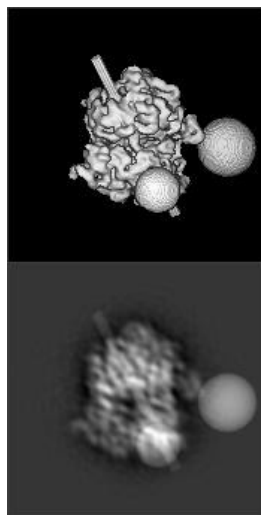
psi=000



phi=192

theta=045

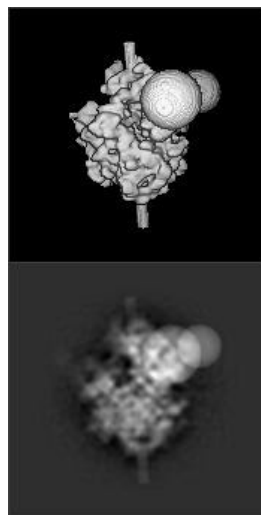
psi=000



phi=216

theta=045

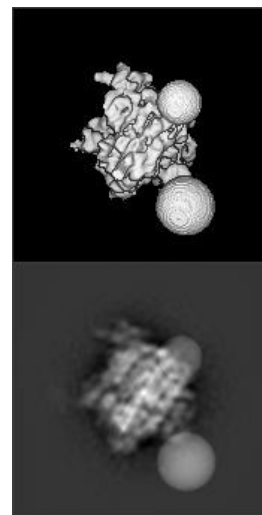
psi=000



phi=016

theta=075

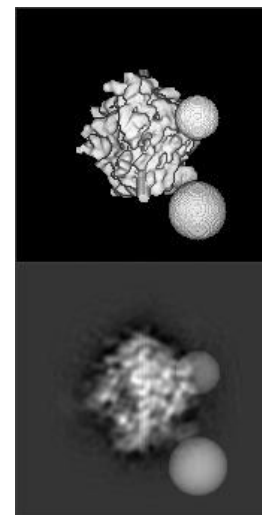
psi=000



phi=115

theta=075

psi=000

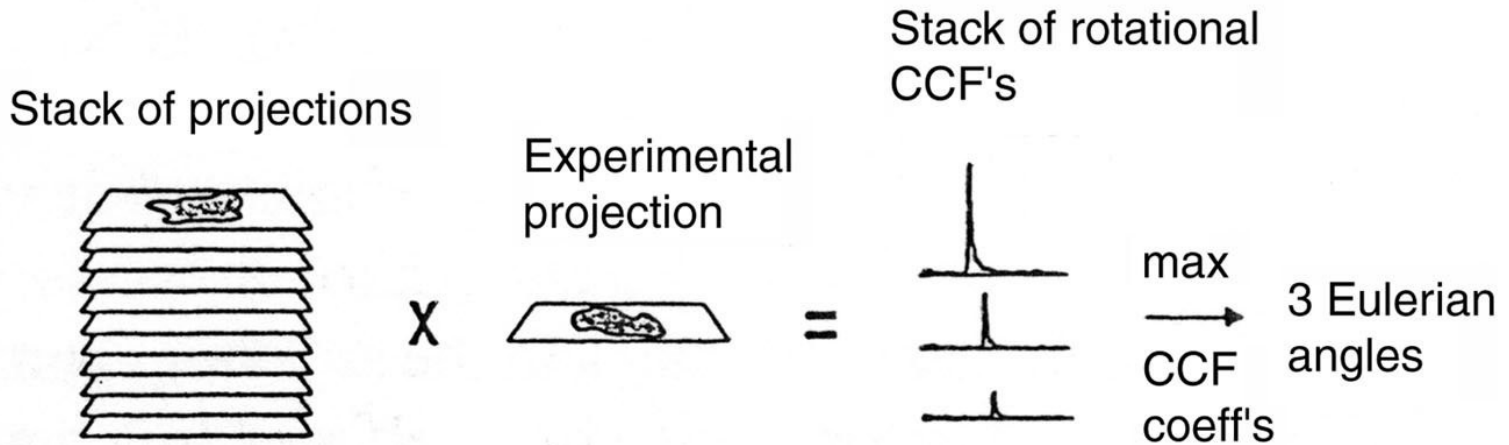


phi=131

theta=090

psi=000

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.

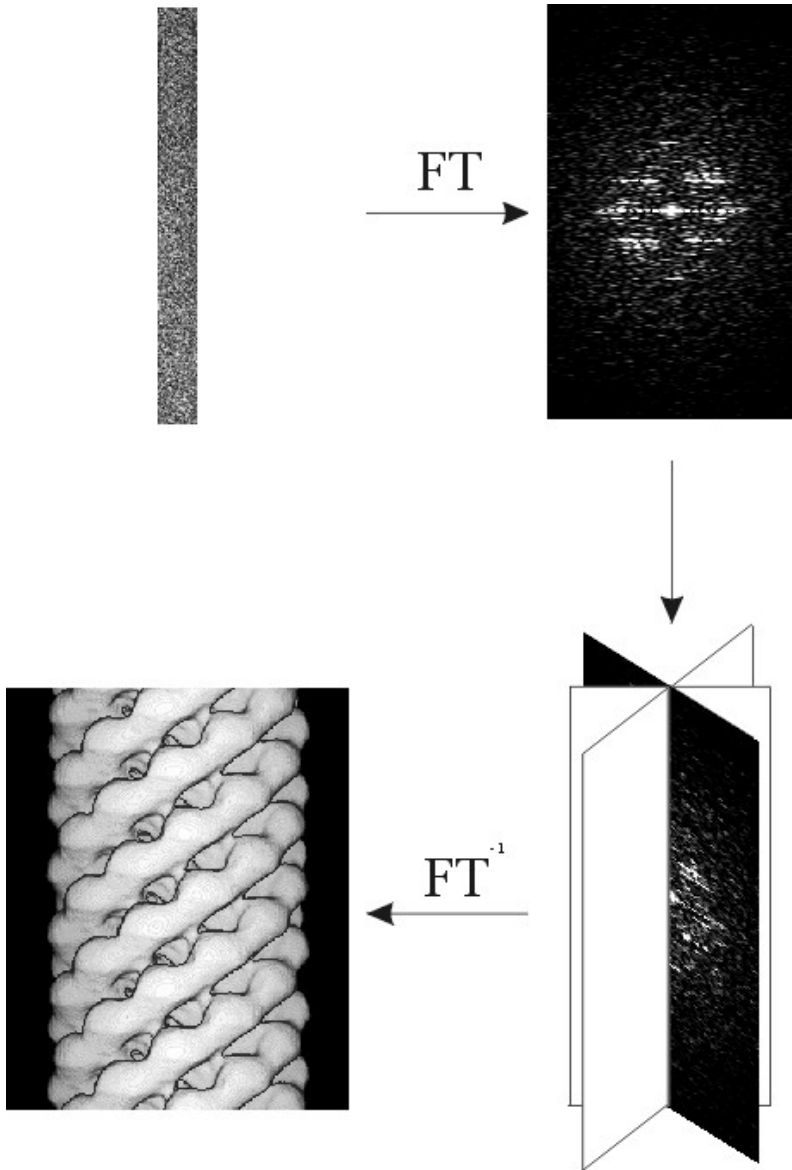
De novo reconstruction

If we don't have a reference reconstruction, how do we proceed?

1. Common lines
2. Random conical tilt

Brief summary of Fourier transforms

- A Fourier transform is an alternative representation of image or volumetric data.
- A Fourier transformation is a fully reversible mathematical transformation.



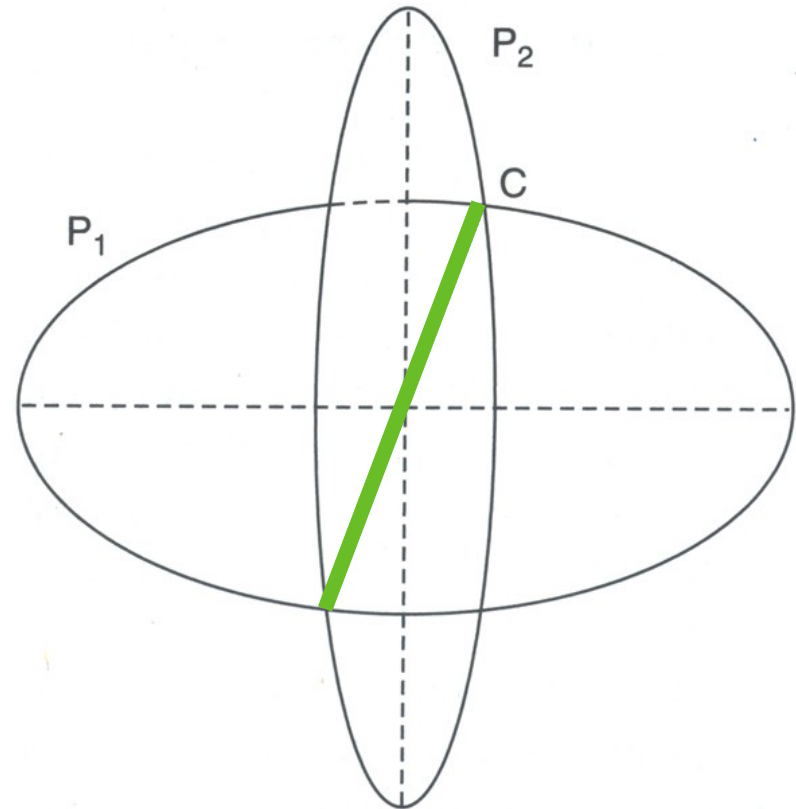
Projection theorem (or Central Section Theorem)

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

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.

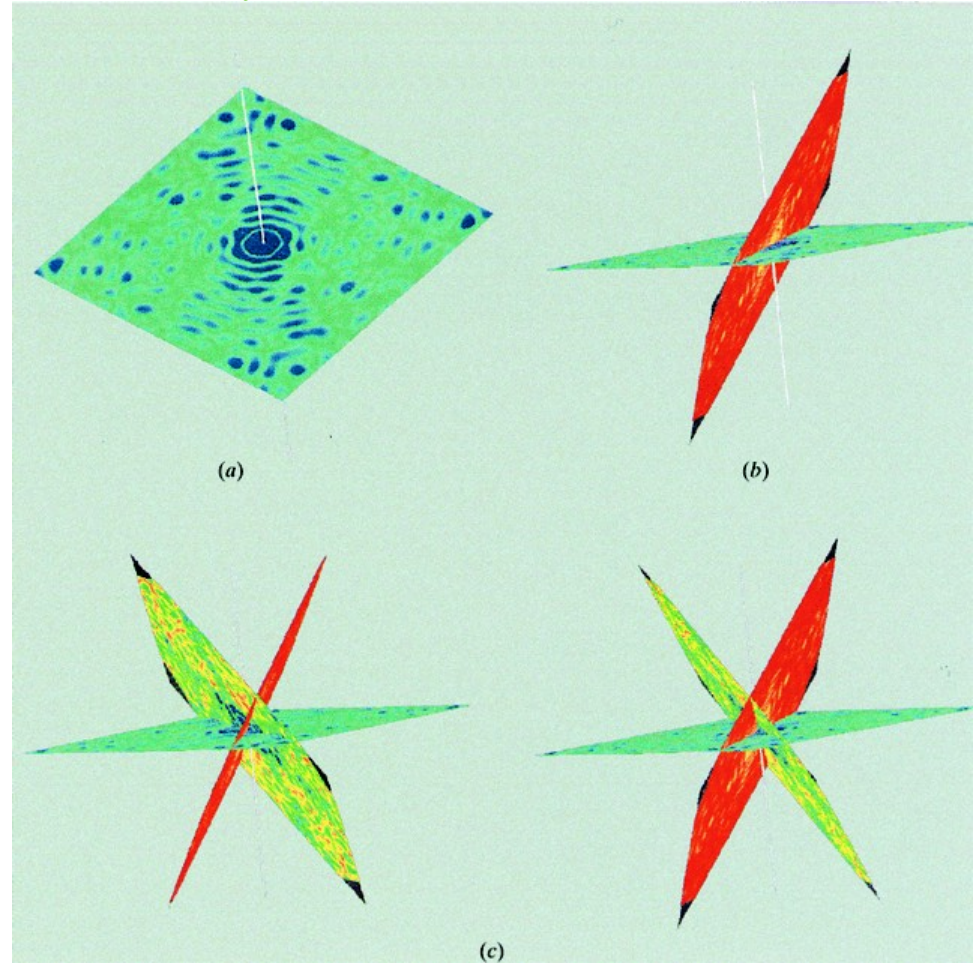


Frank, J. (2006) 3D Electron Microscopy of Macromolecular Assemblies

Common lines (or Angular Reconstitution)

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From Steve Fuller

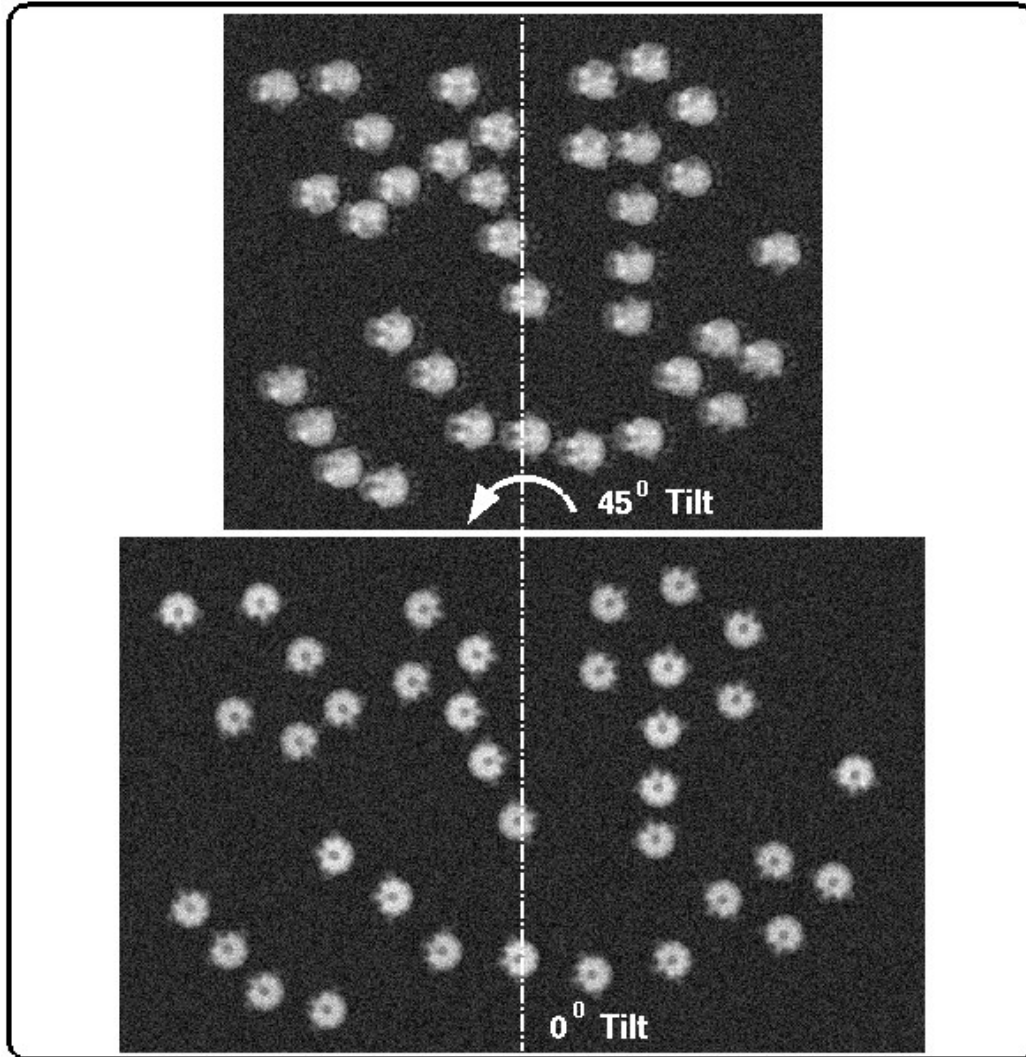
de novo reconstruction

If we don't have a reference reconstruction, how do we proceed?

1. Common lines

2. Random conical tilt

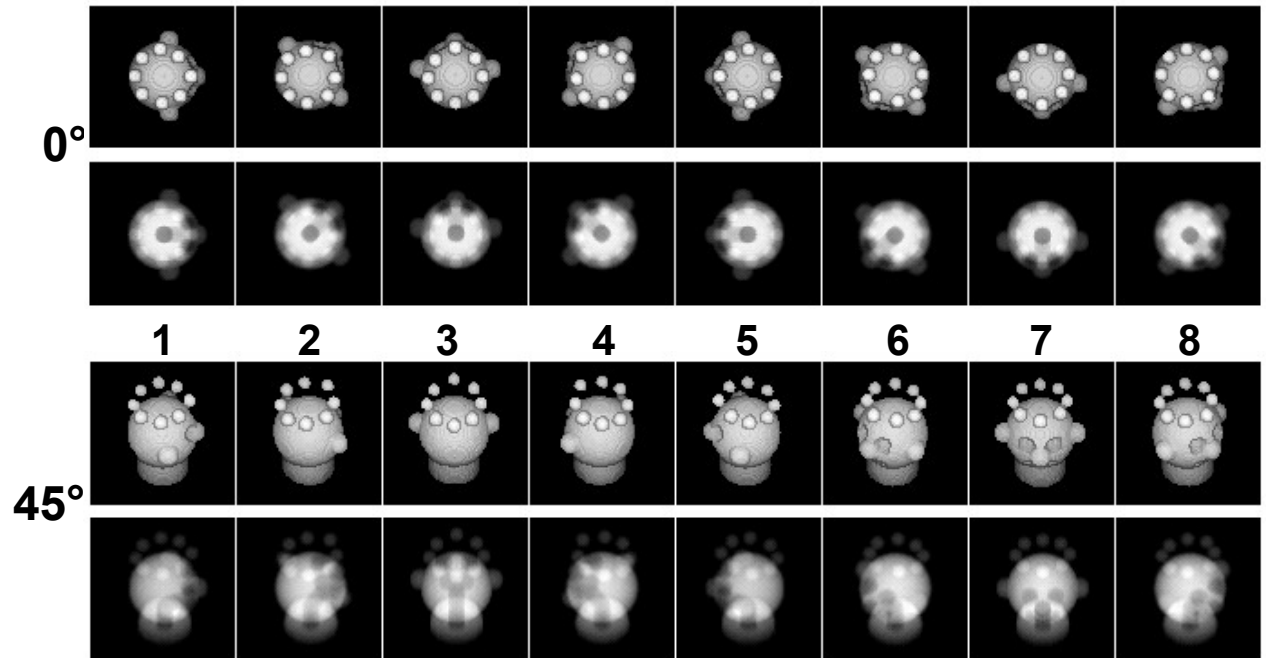
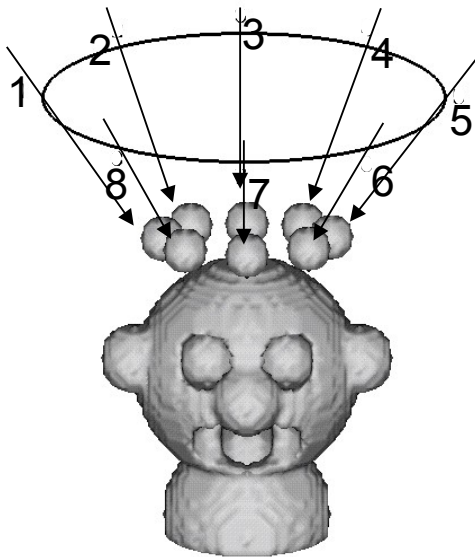
Random-conical tilt: Determination of Euler angles



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

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).

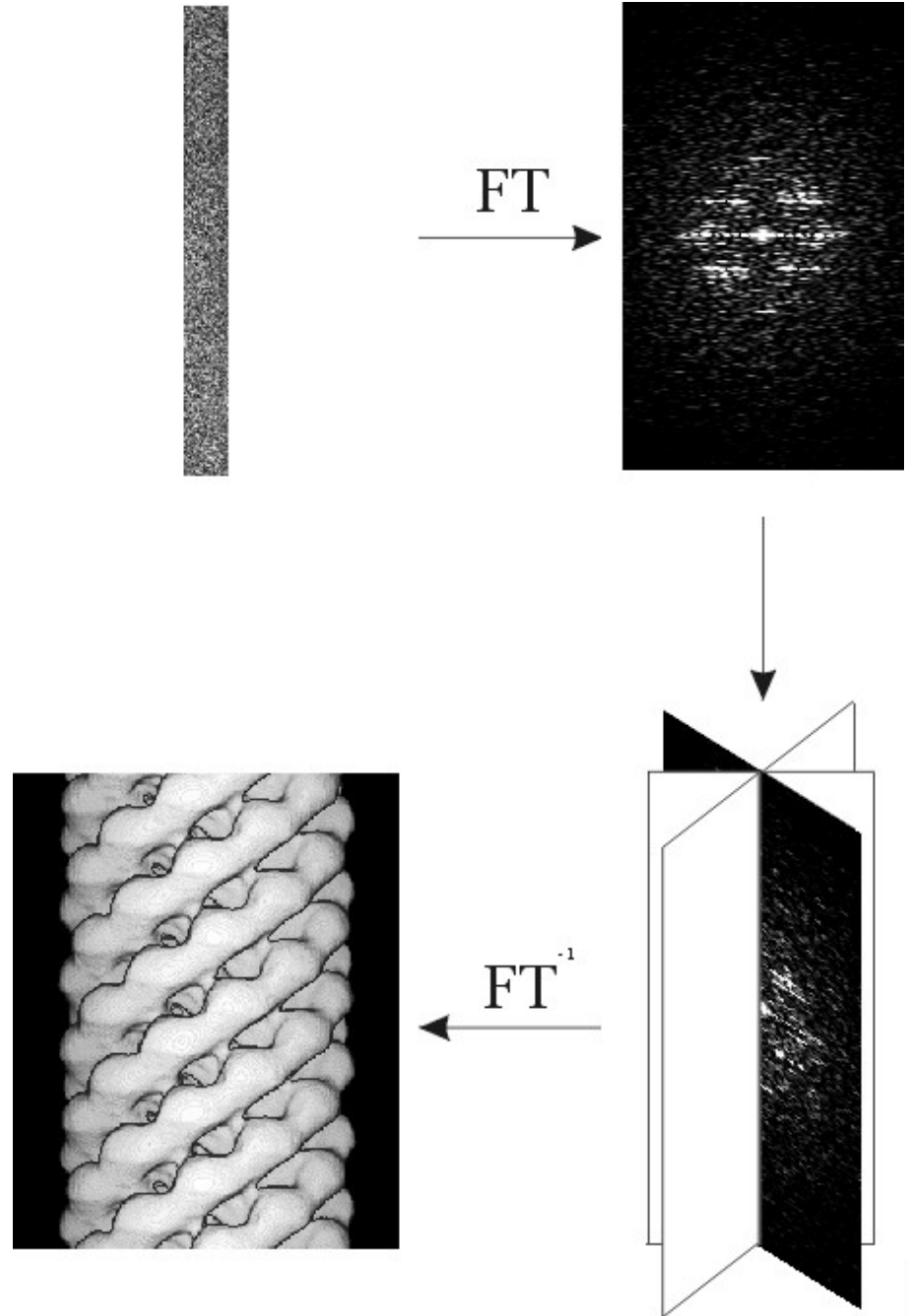
See movie rct-part1.avi

See movie rct-part2.avi

One problem though:

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

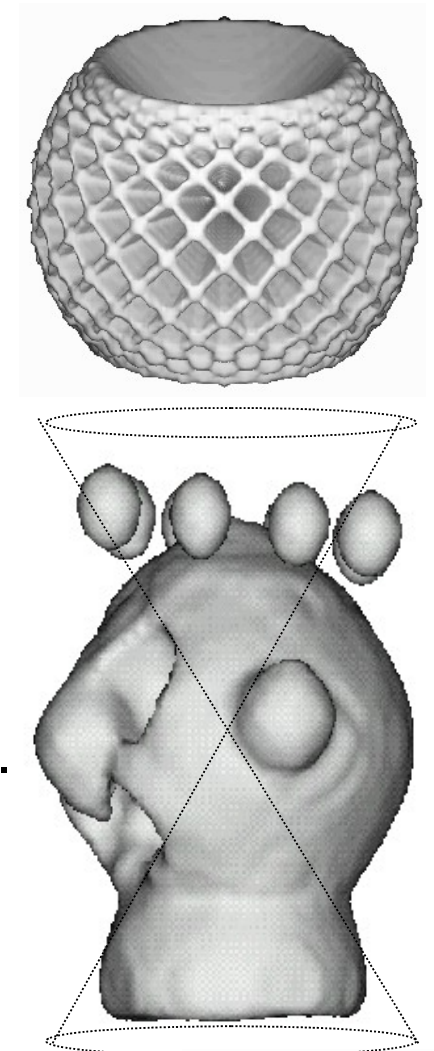
Projection theorem



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.

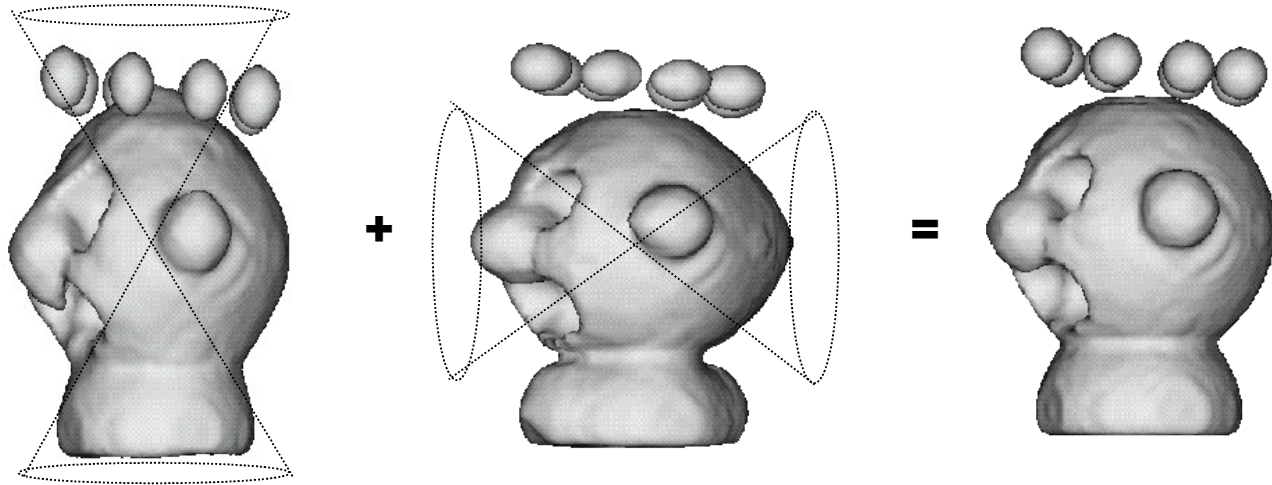


From Nicolas Boisset

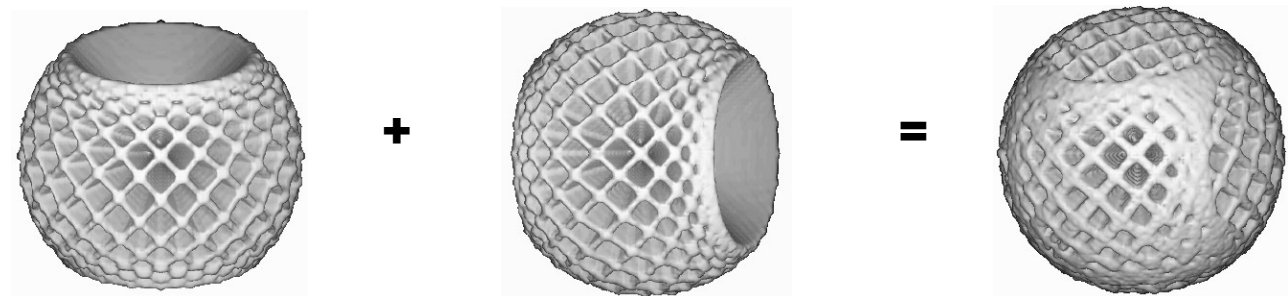
Random-conical tilt: Filling the missing cone

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

Reconstruction



Distribution
of orientation



From Nicolas Boisset

SPIDER

SPIDER

System for

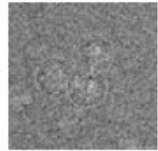
Processing

Image

Data from

Electron microscopy and

Related fields



[Random Info](#)
Future of EM
Software,
MRC Image
Stacks Harmful,
GPU's and Snake
Oil

[Documentation](#)

[Whats New](#)

[Availability](#)

[Download](#)

[Installation](#)

[Getting Started](#)

[User Guide](#)

[Tutorial](#)

SPIDER (System for Processing Image Data from Electron microscopy and Related fields) is an image processing system for electron microscopy.

- News:
 - SPIDER is now an open source project maintained by unpaid volunteers. We invite contribution of code, documentation, and funding.
- Emphasis on:
 - 3D reconstruction
 - Averaging of [single particle](#) macromolecule specimens
 - [Multivariate statistical classification](#)
 - [Electron tomography](#).
- Features:
 - Interactive command line interface.
 - Hierarchical modular design for scripting.
 - Graphical User Interface, [Web](#), for visualizing and interacting with images.
 - [File format](#) interchangeable with other scientific imaging systems.
 - Extensive [documentation](#) of [operations](#) and [techniques](#).
 - Includes [source code](#).
 - Includes [PubSub](#) for use on clusters.
 - [Available](#) for Linux, OSX, and Aix. (OSX support will be discontinued in 2014.)
- History:
 - Originated by: [Joachim Frank](#). Available [since 1978](#).

<http://spider.wadsworth.org>

Interacting with SPIDER: The classic way

```
spiro nodupes/em2em 96> spider spi

  \_ '0 0' _/
  /_ xXXXx _/
  /_ xXXXx _/
  /_ /xxx\ _/
  /_ /xxx\ _/

SPIDER -- COPYRIGHT
HEALTH RESEARCH INC., ALBANY, NY.

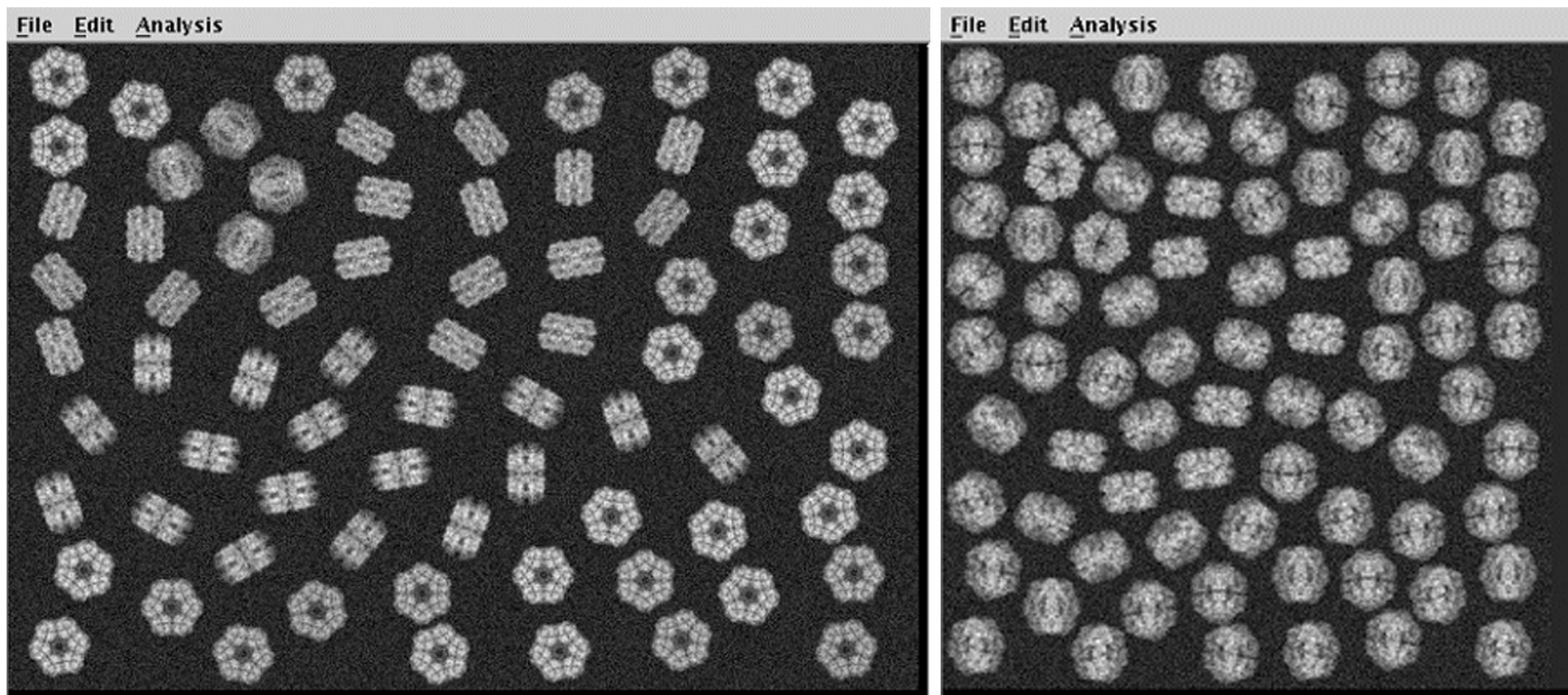
VERSION:  UNIX  21.13  ISSUED: 12/16/2013
DATE:      17-SEP-2014   AT  12:44:11

If SPIDER is useful, please cite:
Frank J, Radermacher M, Penczek P, Zhu J, Li Y, Ladjadj M, Leith A.
SPIDER and WEB: Processing and visualization of images in 3D electron
microscopy and related fields.  J. Struct. Biol. 1996; 116: 190-199.

Results file: results.spi.0
Running: /home/tapu/local/spider/bin/spider_linux_mp_intel64

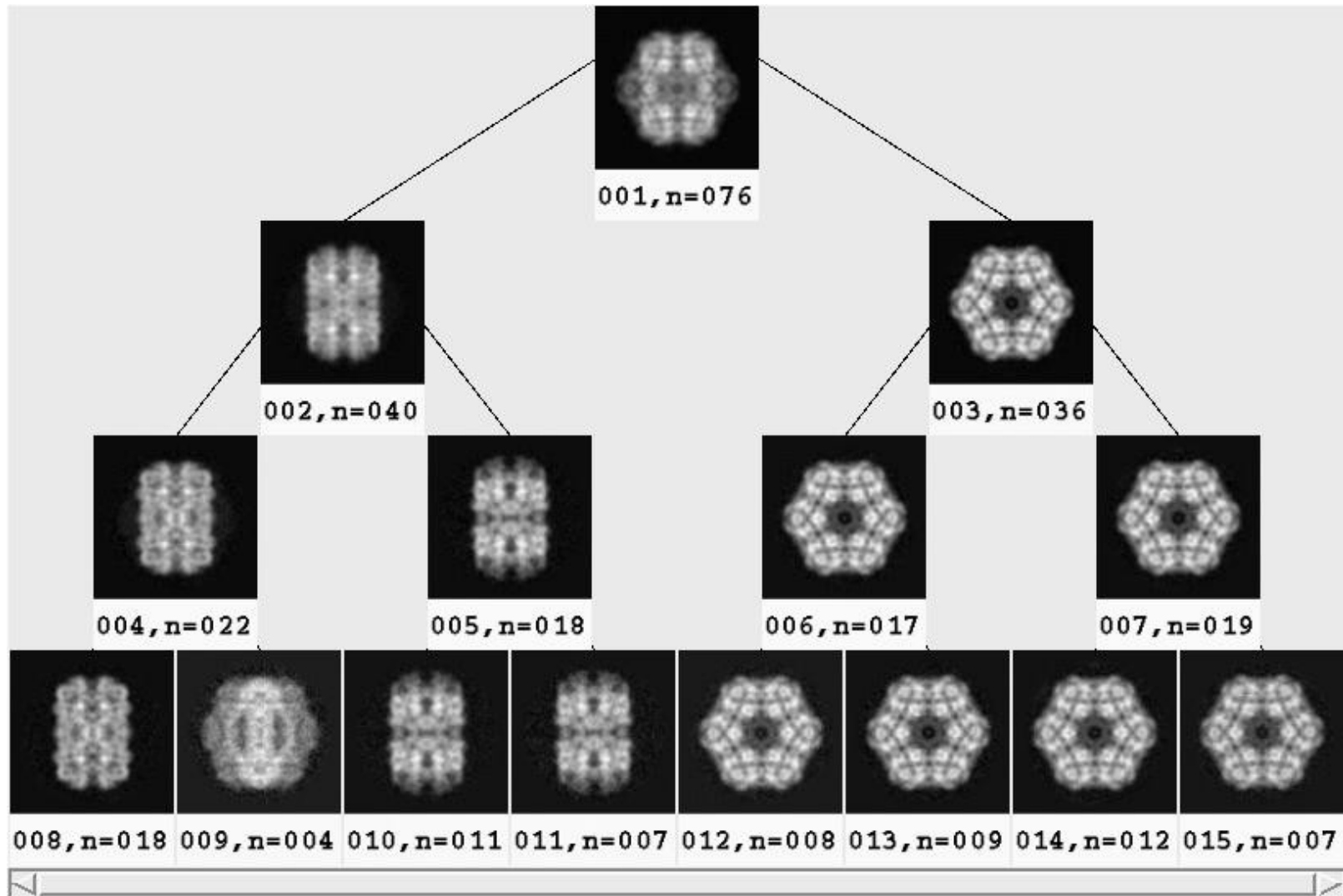
.OPERATION: WI
WI
.INPUT FILE: testing
testing
testing
(R )  230  230  CREATED 17-SEP-2014 AT 12:44:04  0 HEADER BYTES:  1840
.OUTPUT FILE: testwin
testwin
.X & Y DIMENSIONS: 128,128
      128   128
testwin
(R )  128  128  CREATED 17-SEP-2014 AT 12:44:59  N HEADER BYTES:  1024
.TOP LEFT COORDINATES: 52,52
      52   52
.OPERATION: [ ]
```

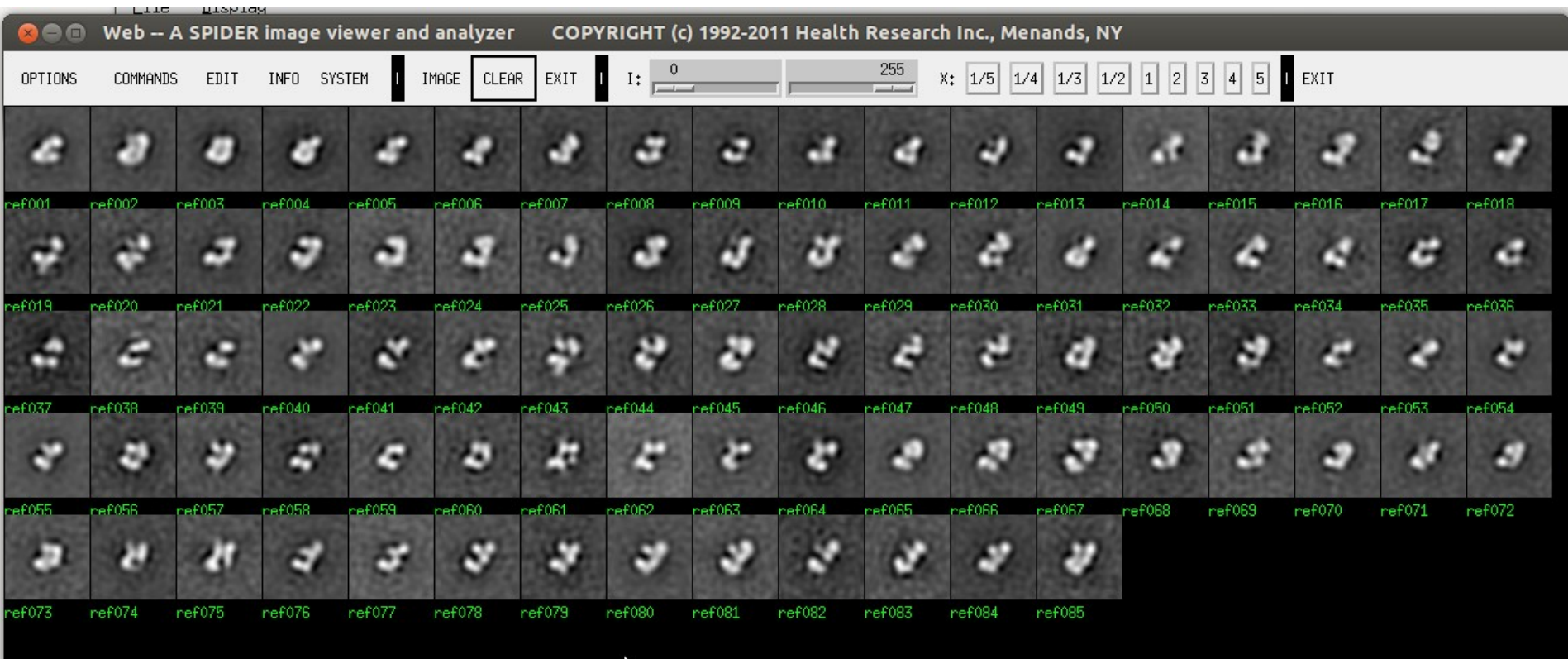
Tilt-pair selection



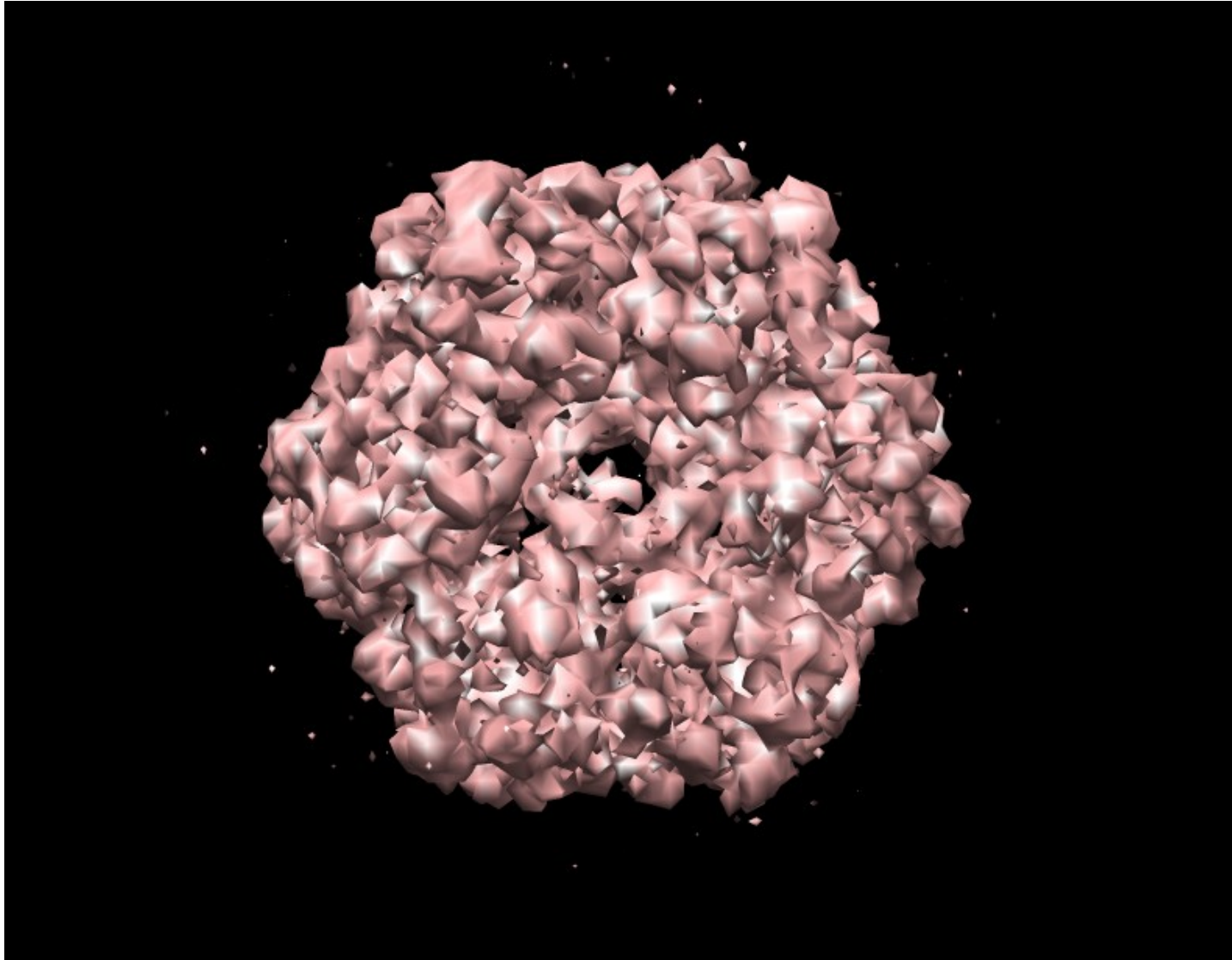
From Nicolas Boisset
Synthetic images of worm hemoglobin
Shaikh *et al.*, (2008) *Nature Protocols* **3**: 1941-74.

Classification of 0° images

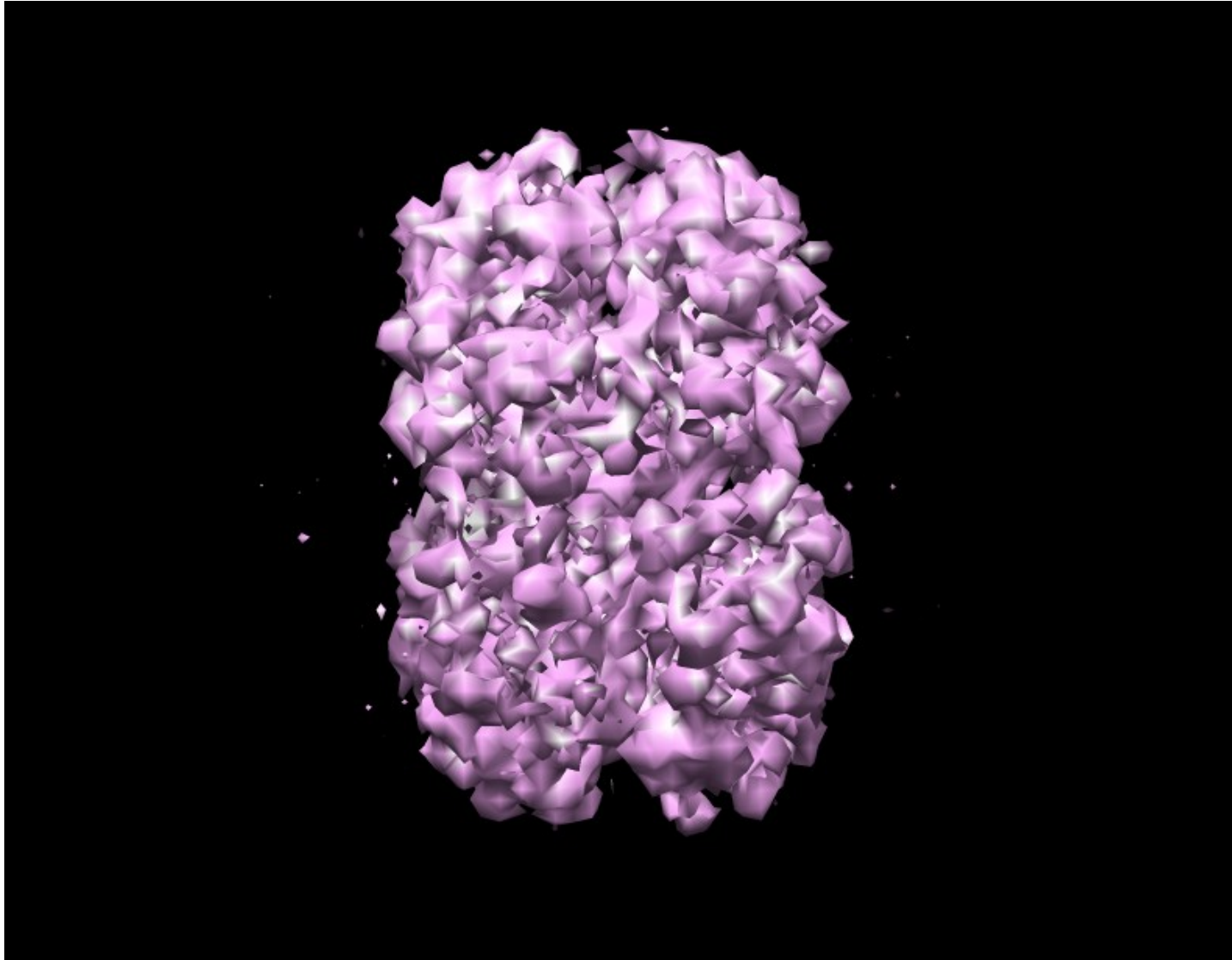




Worm hemoglobin (phantom data)



Worm hemoglobin (side view)



Thank you for your attention



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