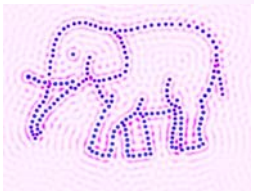
Lecture

E. Orlova

Determination of the image orientation

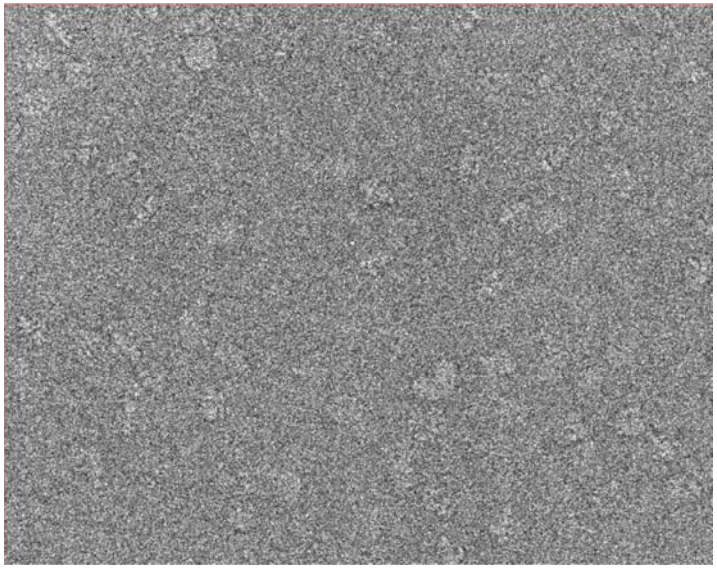
Images: shadow, projection
Euler angles
Methods of orientation determination:
Common lines in Fourier space
Common lines in real space
Free-align
Tomography
Projection matching



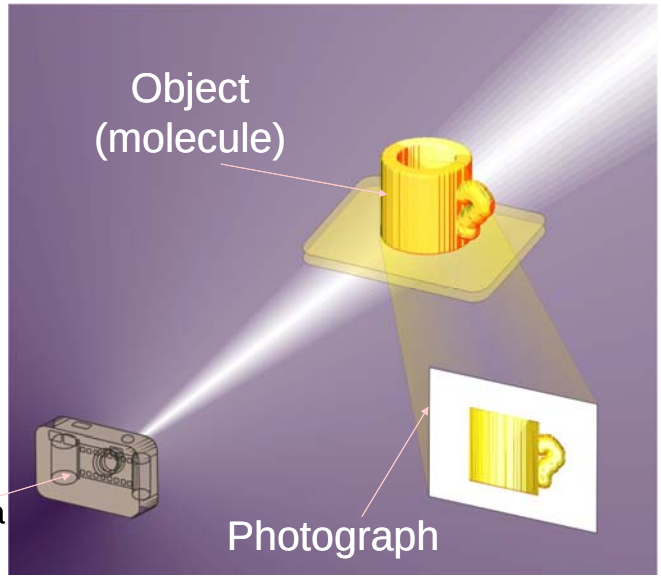
Practical Course

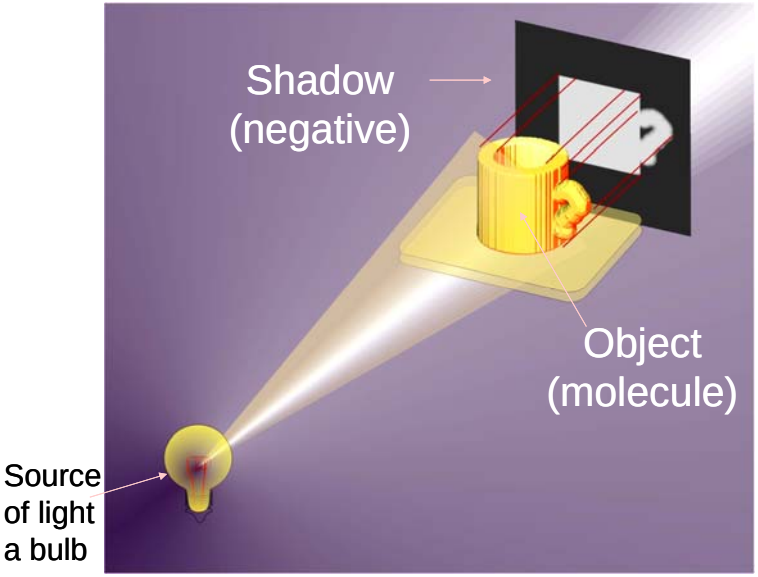
Birkbeck College
London

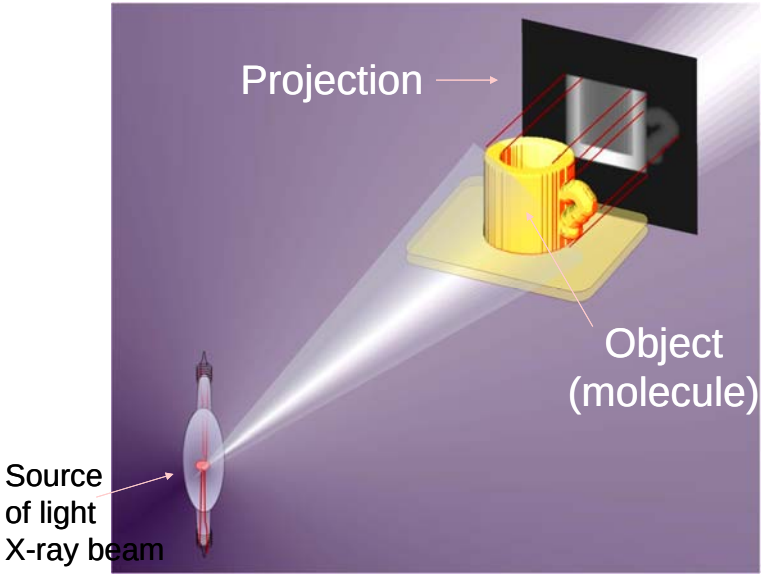
Images of molecules in vitreous ice

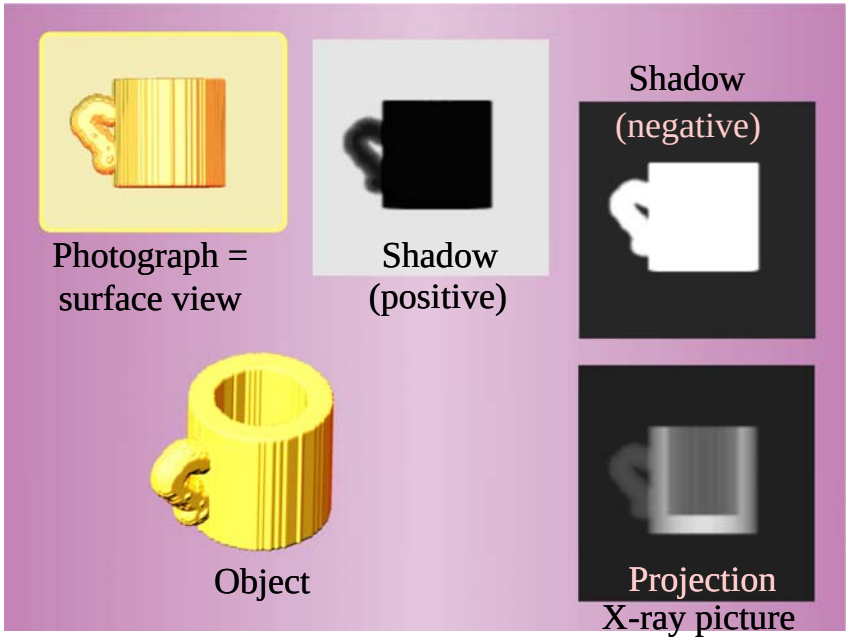


Images —??— Object
surface view,
shadow,
projection









3D object -> $\rho(x,y,z)$

Projection: 2D, 1D, Sinogram = ? = $\rho(x,y,z)$

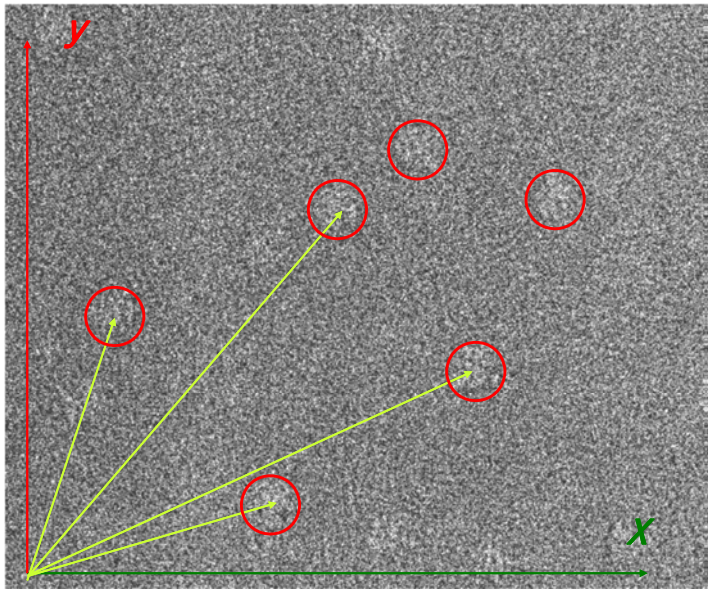
$$p(y,z) = \int \rho(x,y,z)dx$$

$p(y,z)$ – **2D projection**

$$l(y) = \int p(x,y)dx$$

$l(y)$ – **1D projection**

Ribosomes in vitreous ice – translational freedom

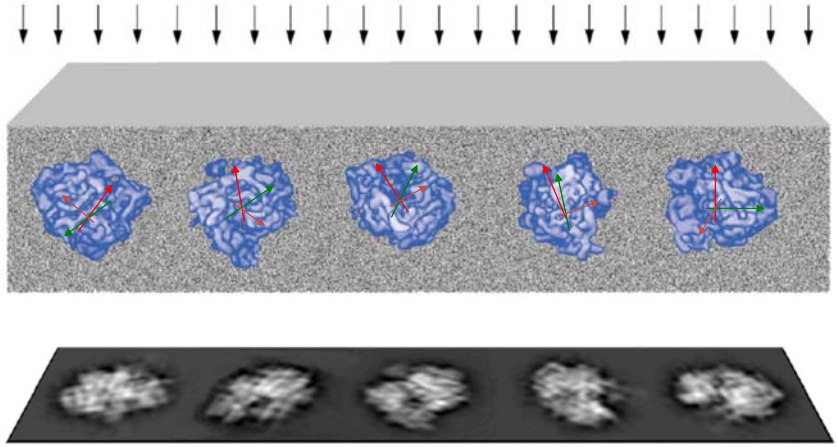


X and Y

2

Ribosomes in vitreous ice – rotational freedom

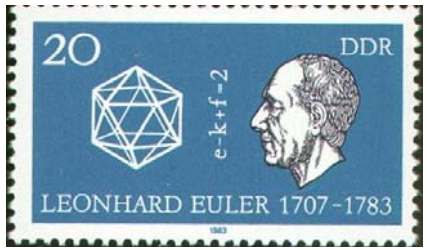
α, β, and γ → 3



J.Frank, *Annu. Rev. Biophys. Biomol. Struct.* 2002. 31:303–19

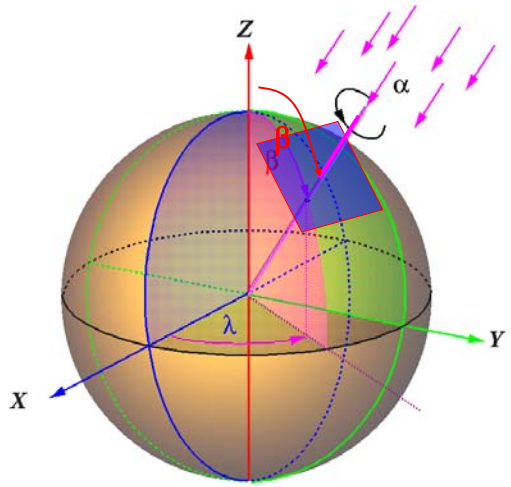
Leonhard Euler

(15 April 1707 – 18 September 1783) was a pioneering Swiss mathematician and physicist. He made important discoveries in fields as diverse as infinitesimal calculus and graph theory. He also introduced much of the modern mathematical terminology and notation, He is also renowned for his work in mechanics, fluid dynamics, optics, and astronomy.



Euler spent most of his adult life in St. Petersburg, Russia, and in Berlin, Prussia. He is the pre-eminent mathematician of the 18th century, and one of the greatest mathematicians ever. his collected works fill 60–80 quarto volumes

http://en.wikipedia.org/wiki/Leonhard_Euler



Euler Angles

α - rotation in the image plane (or around new Z axis)

β - rotation around X axis

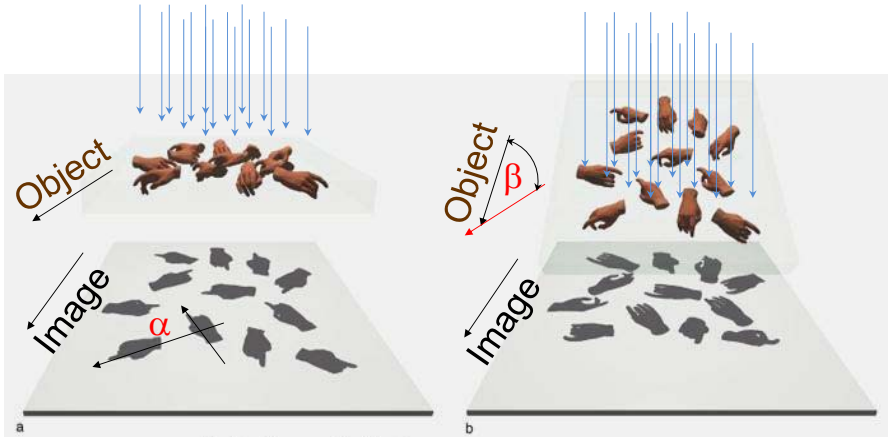
γ – rotation around old Z axis)

(IMAGIC)

Methods of orientation determination:

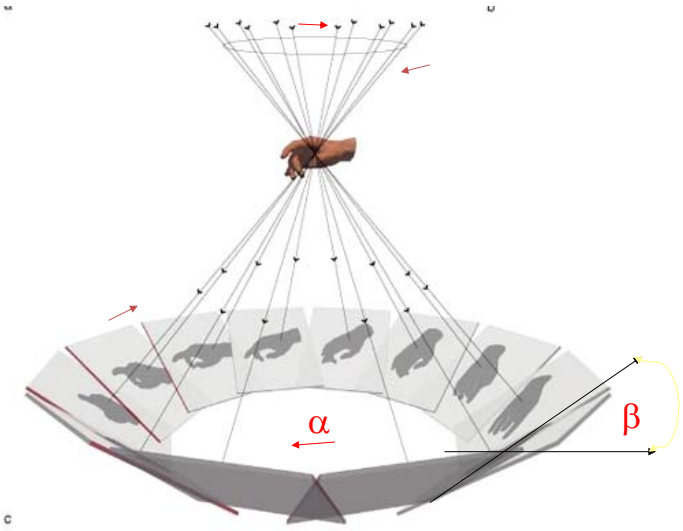
1. *Conical Tilt* – M.Radermacher, J. Frank
2. *Projection Matching* – P.Penczek, J.Frank
3. *Common Lines in Fourier Space* (viruses, phage tails) T.Crowther- MRC package + numerous modification. (S. Fuller)
4. *Common Lines in real space*
   Angular Reconstitution
   M. Van Heel
5. *Frealign -> projection matching in Fourier space*
   N. Grigorieff

Conical tilt , more effective for negatively stained samples

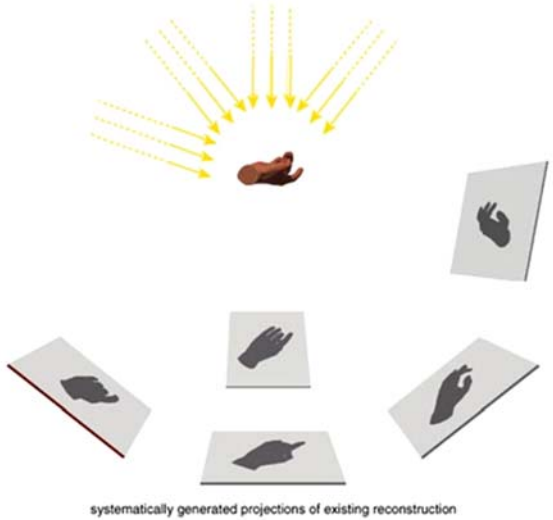


RADERMACHER M. Three-Dimensional Reconstruction of Single Particles From Random and Non random Tilt Series. *JOURNAL OF ELECTRON MICROSCOPY TECHNIQUE 9:359-394 (1988)*

Conical tilt

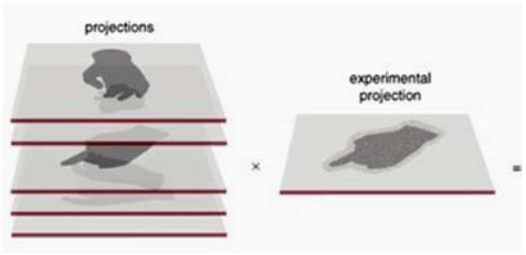


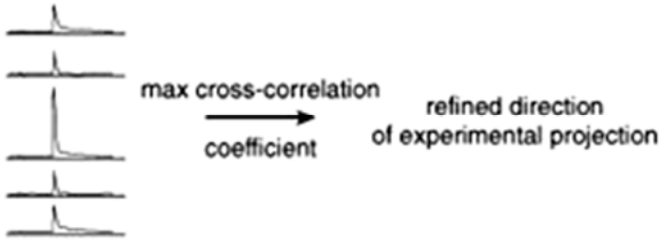
Projection Matching



P. Penczek & J. Frank

Projection Matching





P. Penczek & J. Frank

The section/projection theorem:

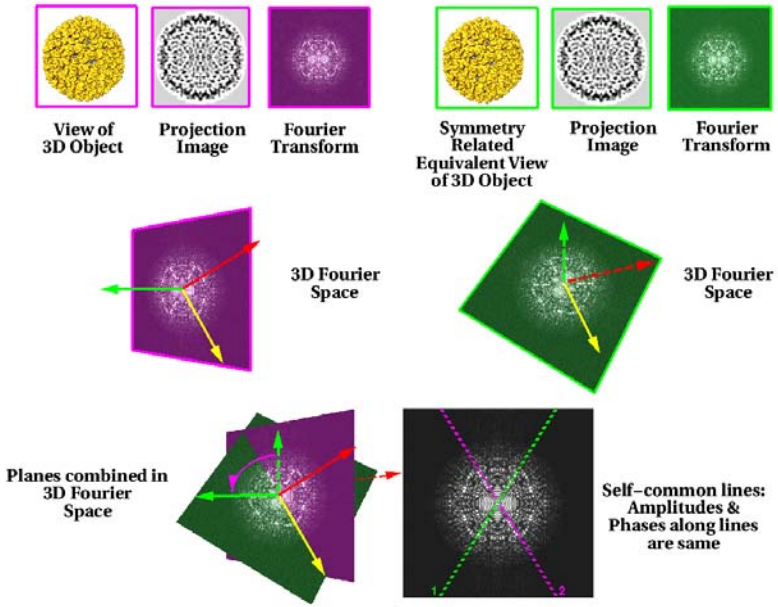
$$F\left[\int g(x,y,z)dx\right] = G(0,Y,Z)$$

USE:
The Fourier transform of a projection of a 3D object is equal to a central section of the 3D Fourier transform of the object.

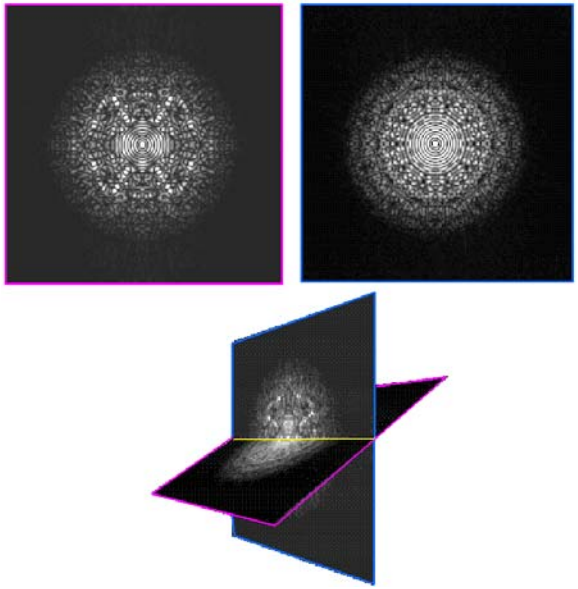
An electron micrograph is a projection of a 3D object.
Its transform provides one slice of the 3D transform of the 3D object.

By combining the transforms of different views, one builds up the 3D transform section by section. One then uses the Inversed Fourier Ttransform to convert the 3D transform into a 3D image.

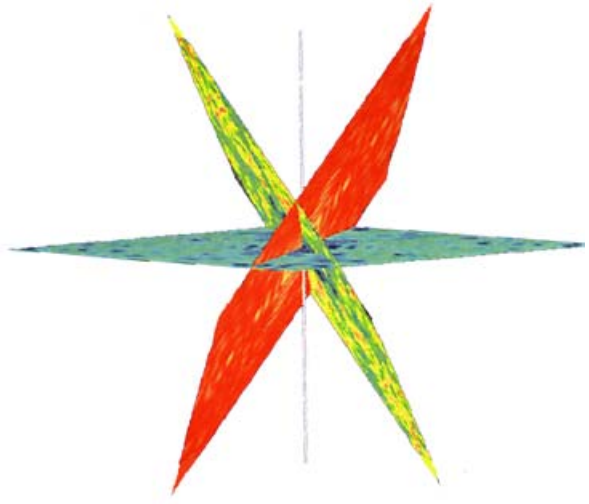
Common lines in Fourier space



Common lines in Fourier space

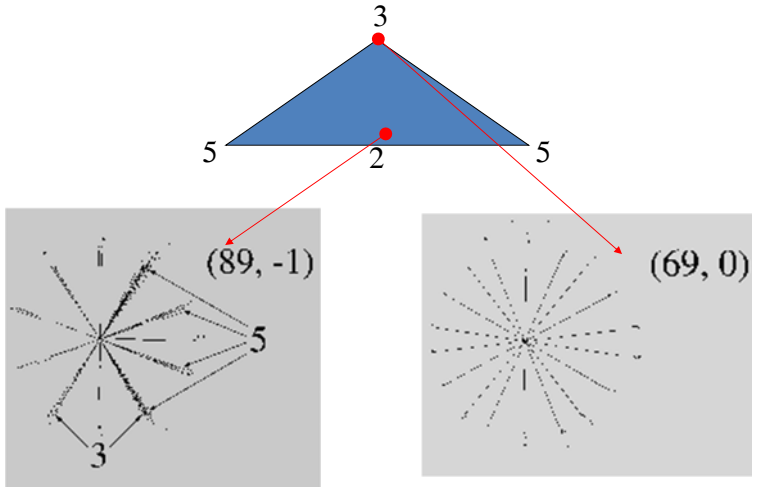


Common lines in Fourier space



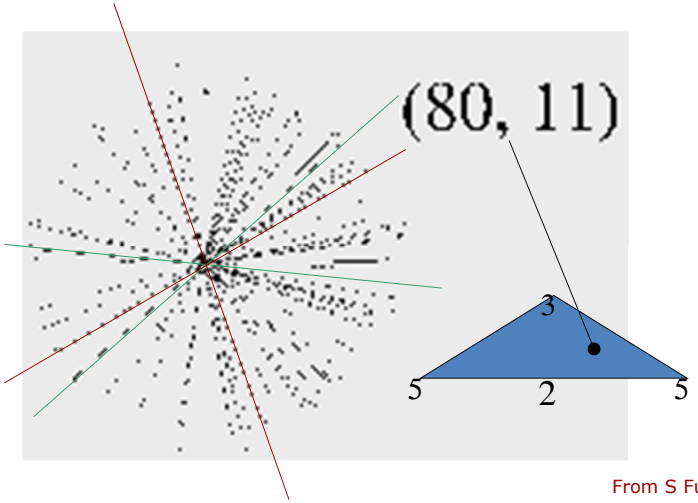
From S Fuller

Orientations near symmetry axes



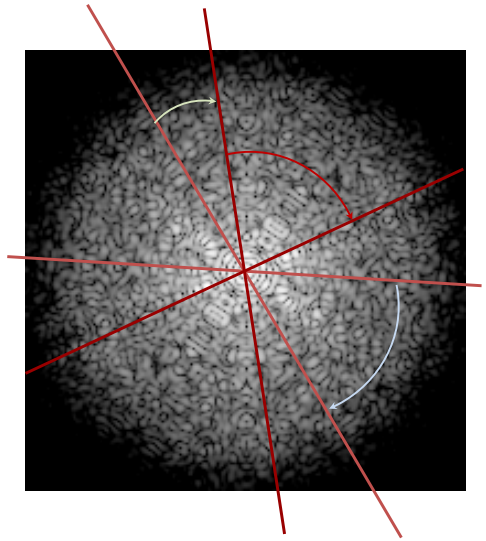
From S Fuller

An orientation away from symmetry axes



From S Fuller

Icosahedral symmetry, search for the common lines



Comparison of amplitudes and phases of radial lines, assessment of angles between them

Common lines in real space

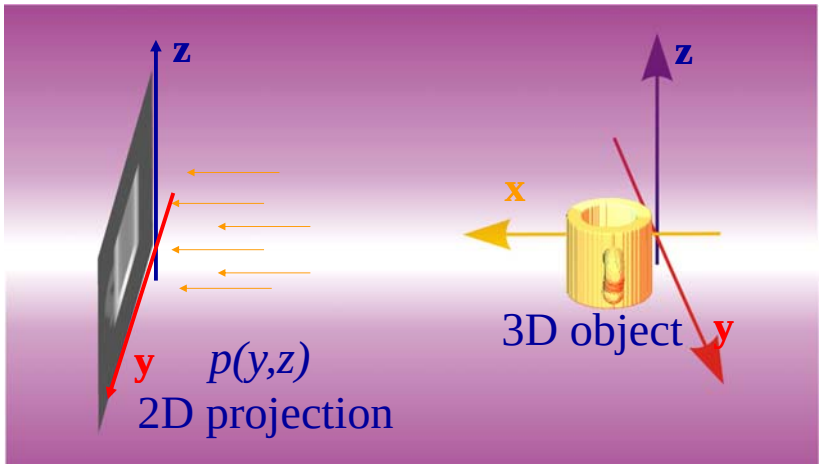
The angular reconstitution method is based on the "common-line projection" theorem:

Any two 2D projections of a 3D object have at least ONE common 1D line projection

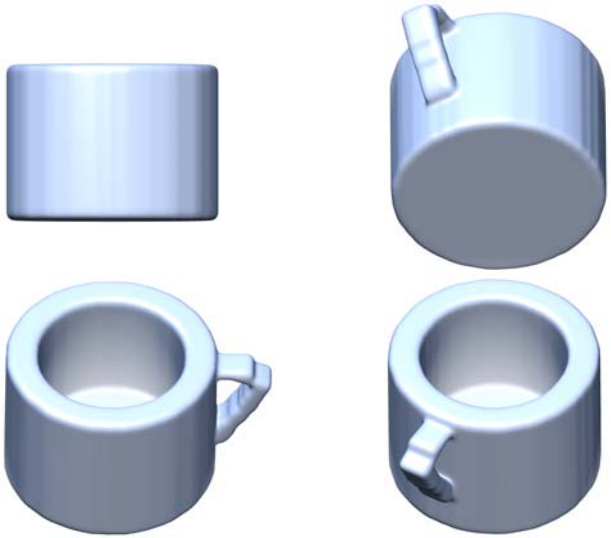
Common lines in real space

3D → **2D** → **1D** projection

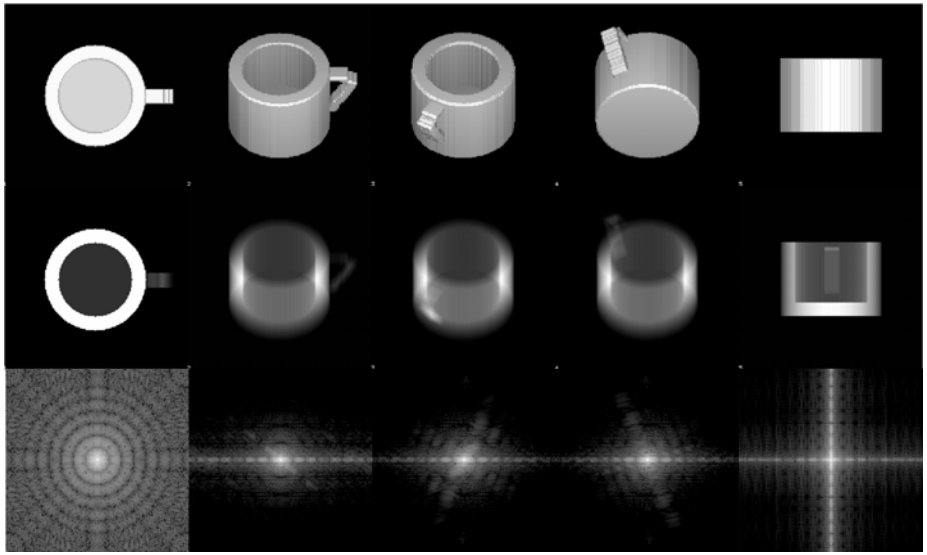
$$p(y,z) = \int \rho(x,y,z)dx$$



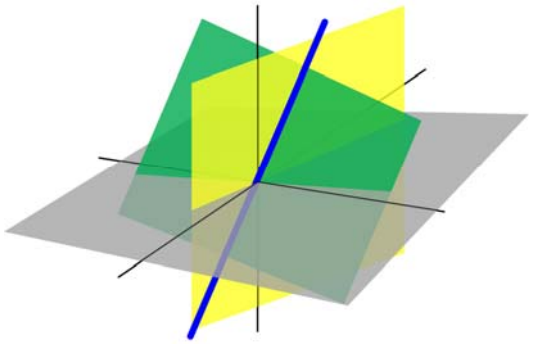
Common lines in real space



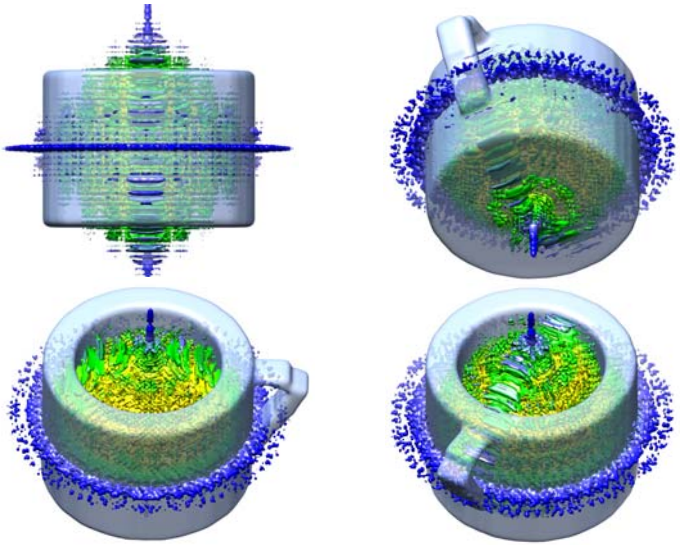
Common lines in real space



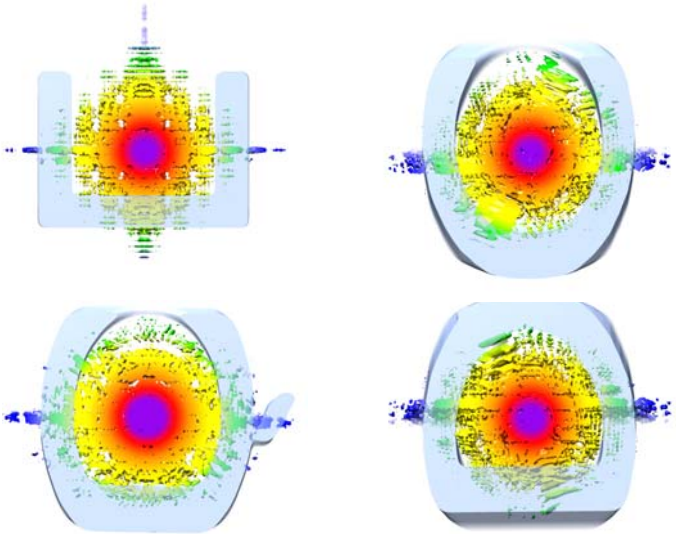
Common lines in real space



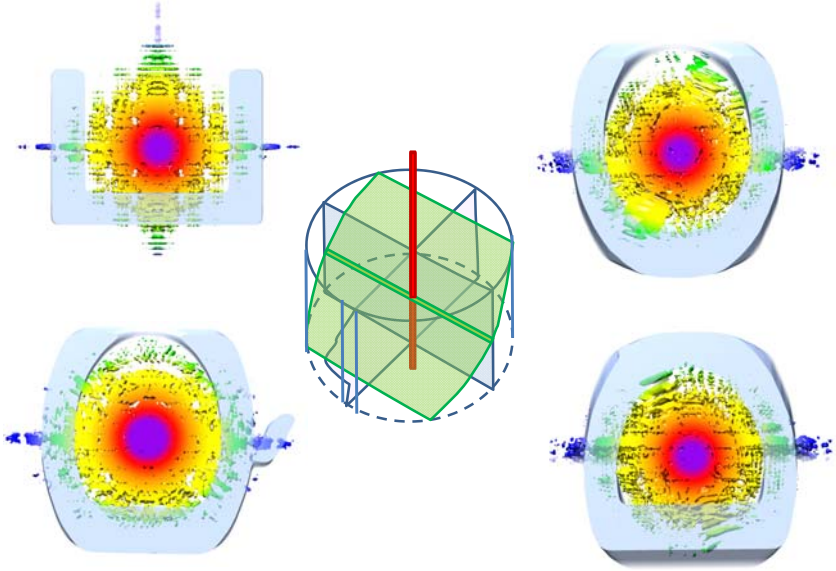
Common lines in R/F(ourier) space



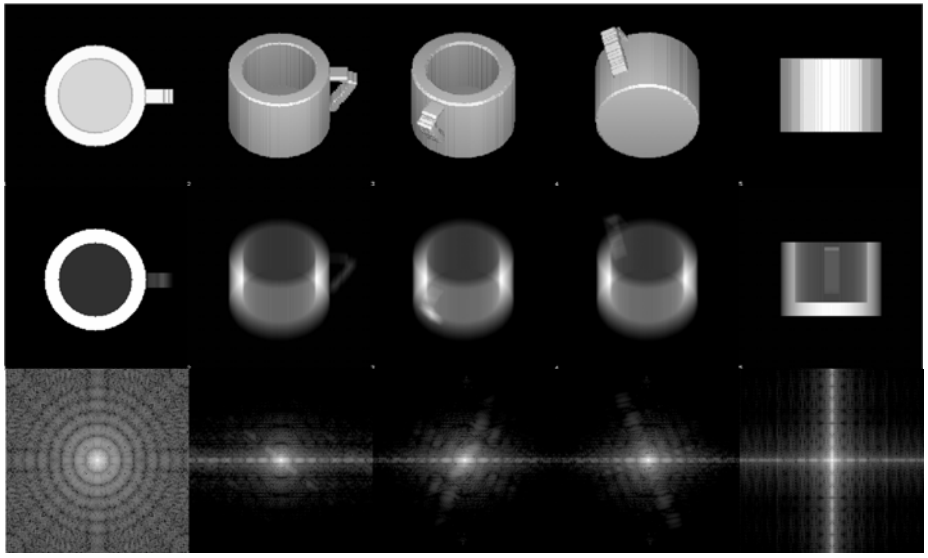
Common lines in R/F(ourier) space



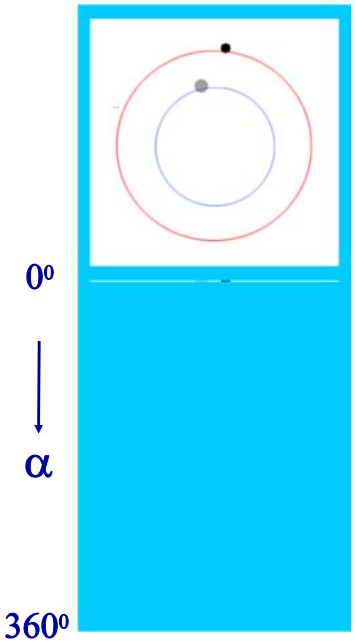
Common lines in R/F(ourier) space



Common lines in real space



Common lines in real space



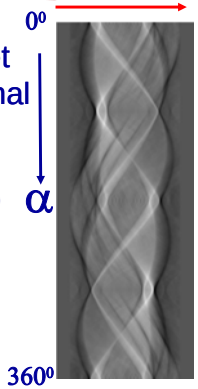
2D projection

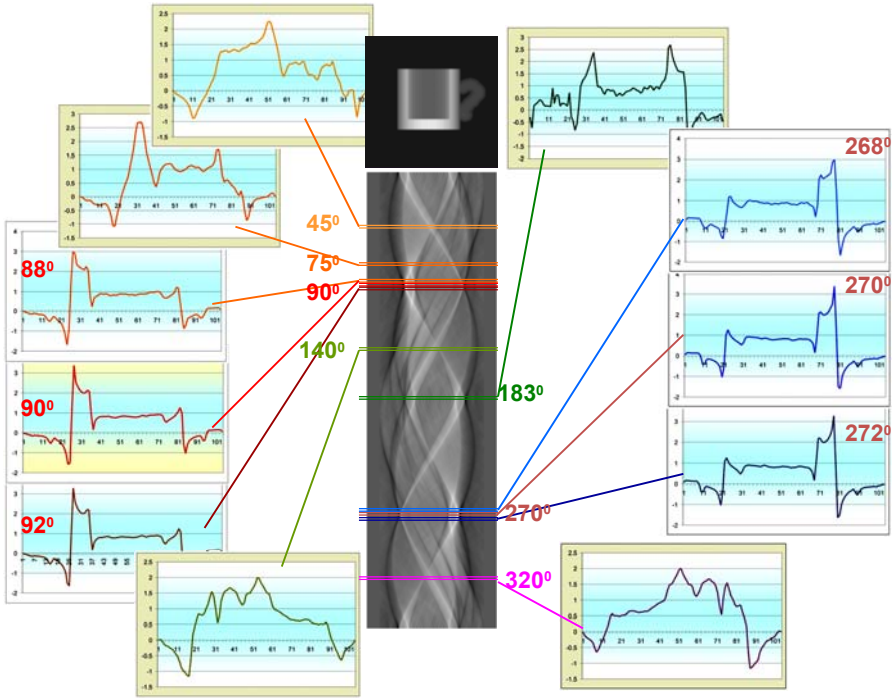
2D → 1D

y

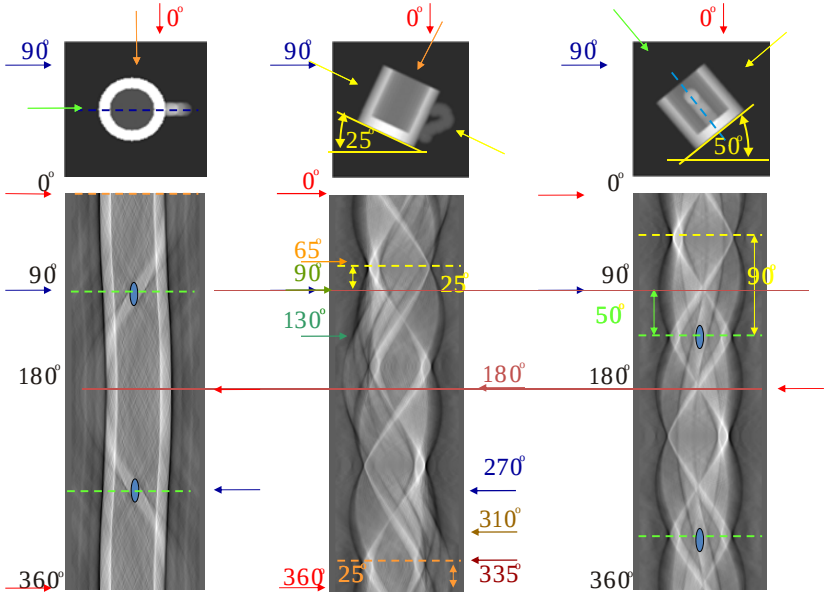
Sinogram is a set of one dimensional (line) projections

$0^0 \le a \le 360^0$





Projections > Sinograms



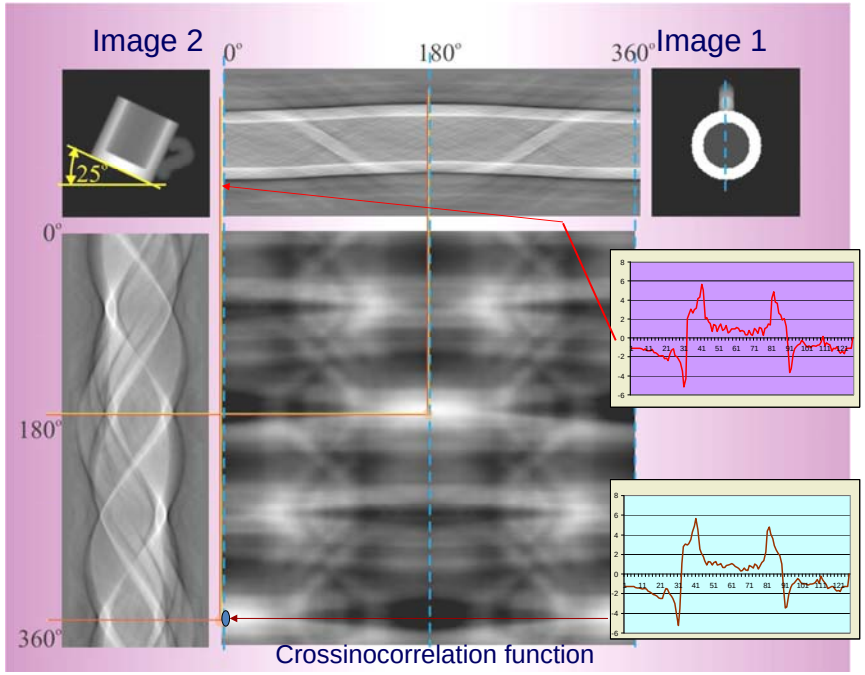


Image 2
0°
180°
360°
Image 1
25°
0°
180°
360°
Crossinocorrelation function

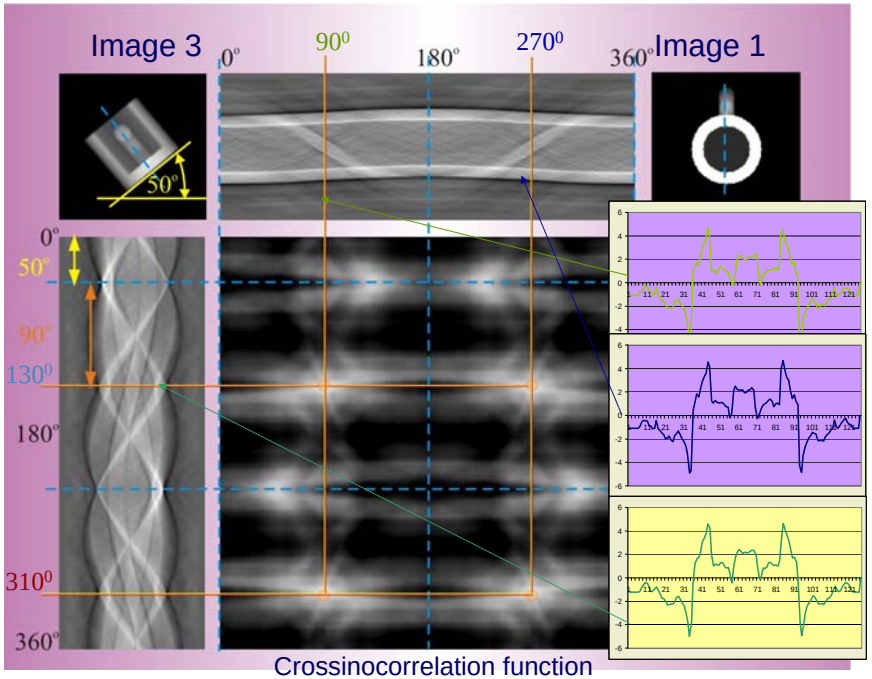


Image 3
0°
90°
180°
270°
360°
Image 1
50°
0°
50°
90°
130°
180°
310°
360°
Crossinocorrelation function

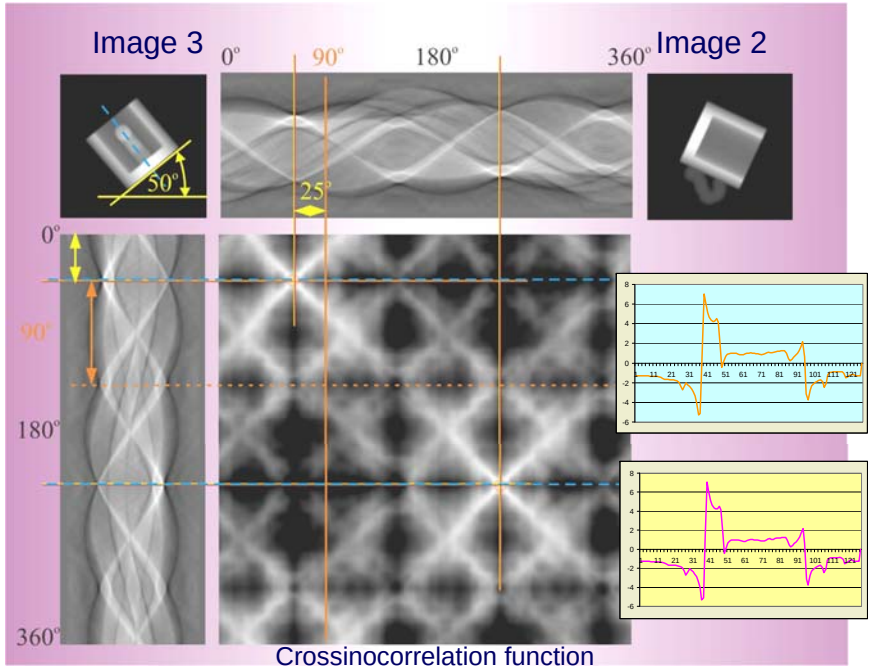
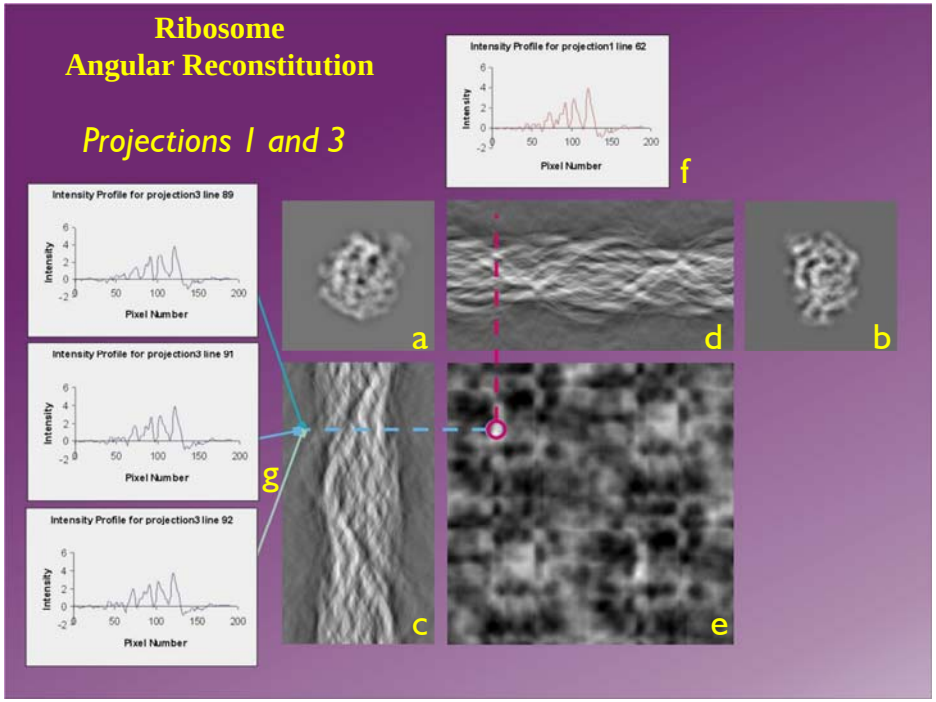
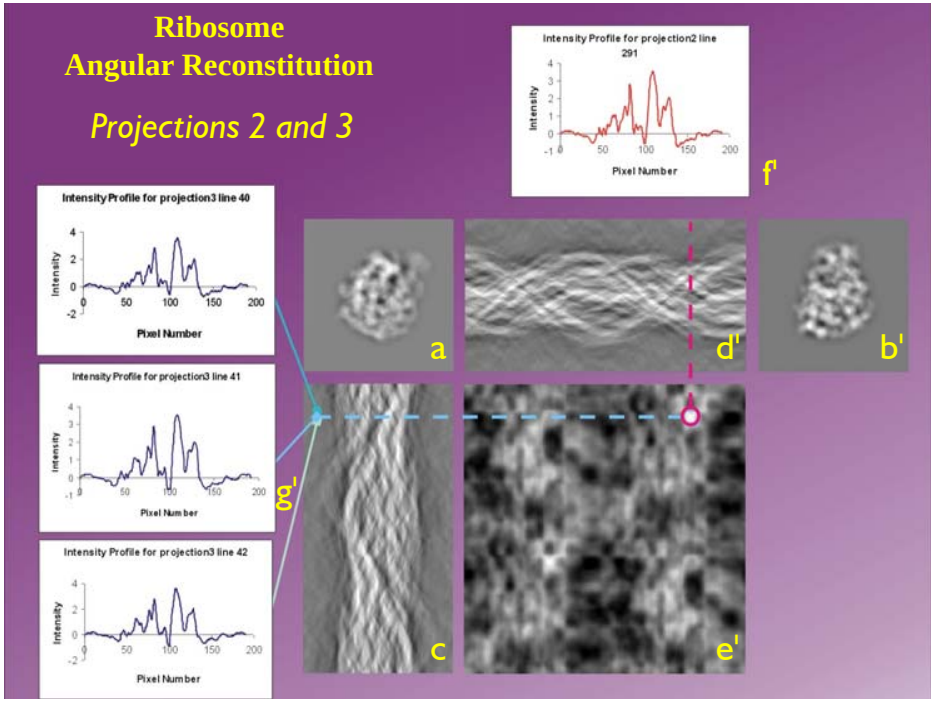
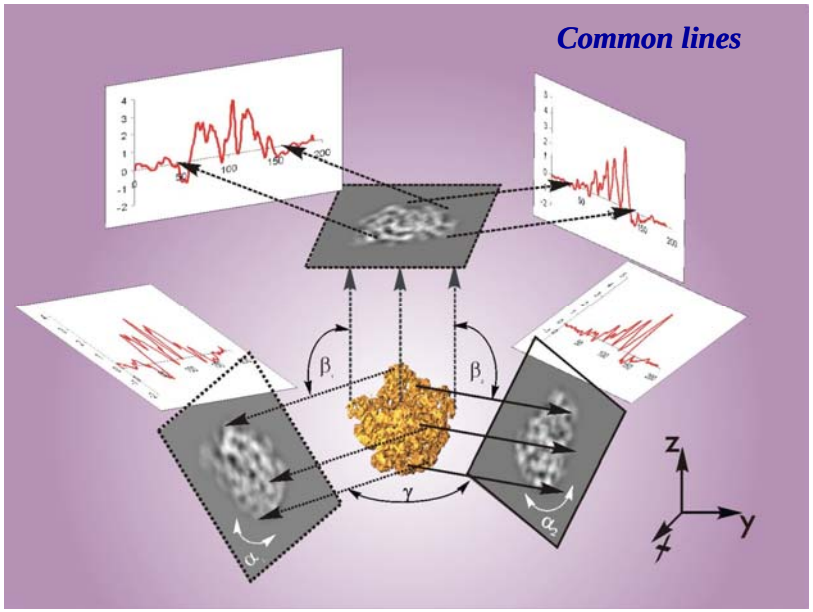


Image 3
0°
90°
180°
360°
Image 2
50°
25°
0°
90°
180°
360°
Crossinocorrelation function

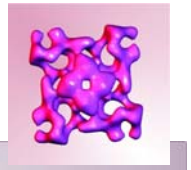

Ribosome
Angular Reconstitution
Projections 1 and 3
Intensity Profile for projection1 line 62
Intensity
Pixel Number
f
Intensity Profile for projection3 line 89
Intensity
Pixel Number
a
d
b
Intensity Profile for projection3 line 91
Intensity
Pixel Number
g
Intensity Profile for projection3 line 92
Intensity
Pixel Number
c
e

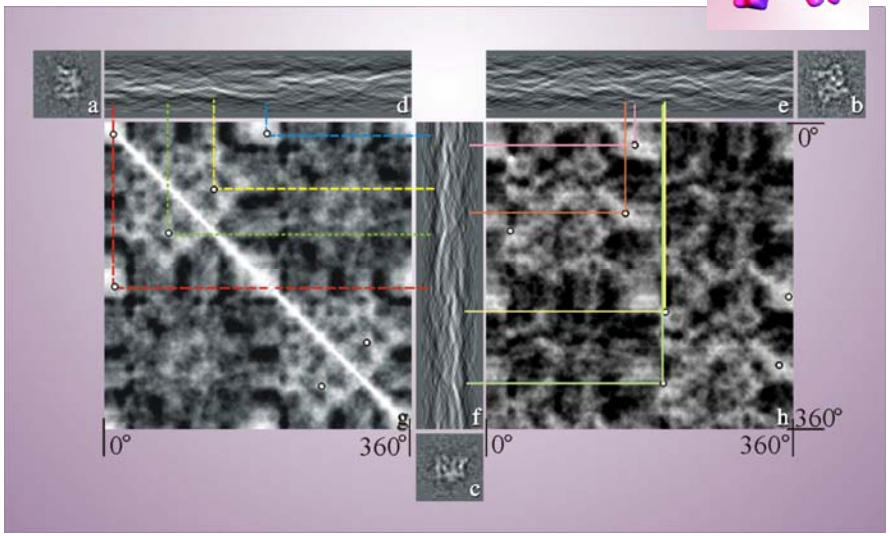


ANGULAR RECONSTITUTION



Calcium release channel RyR1





Icosahedral symmetry

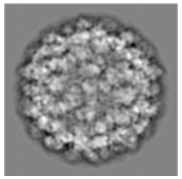
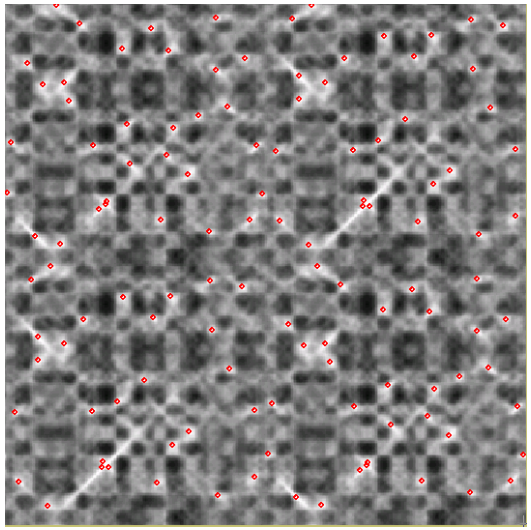


Image 1



Crossinocorrelation function

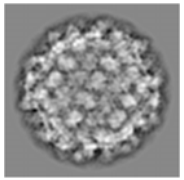
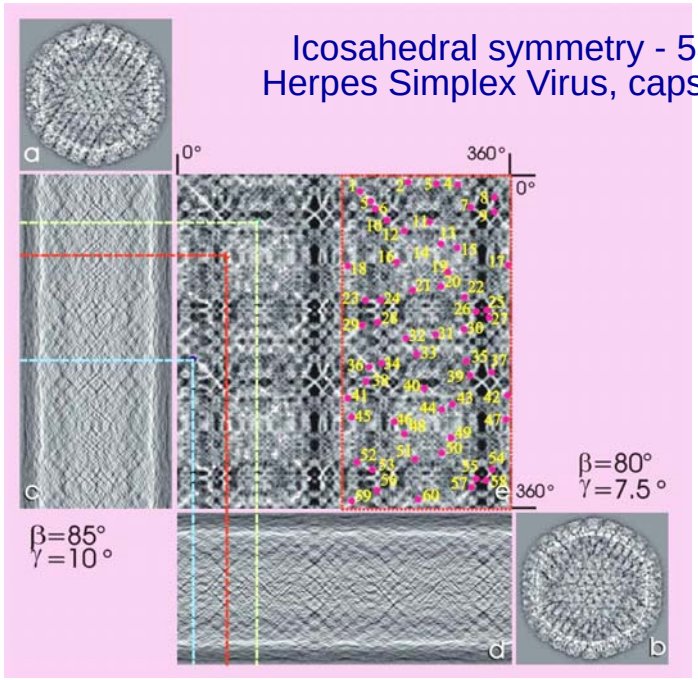
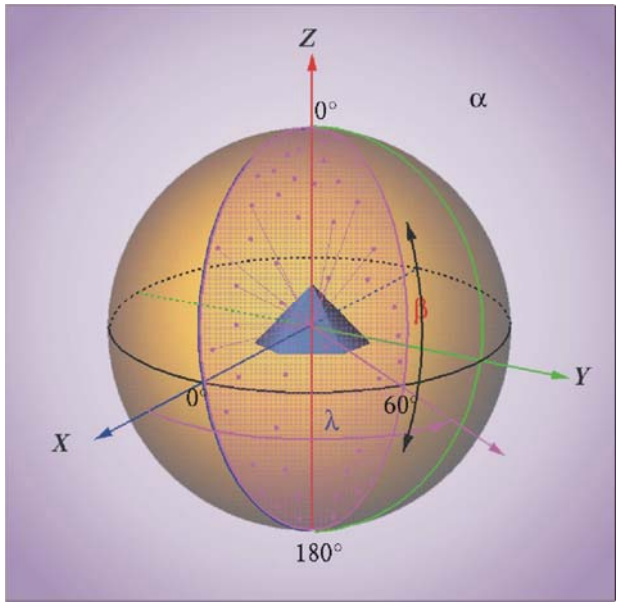


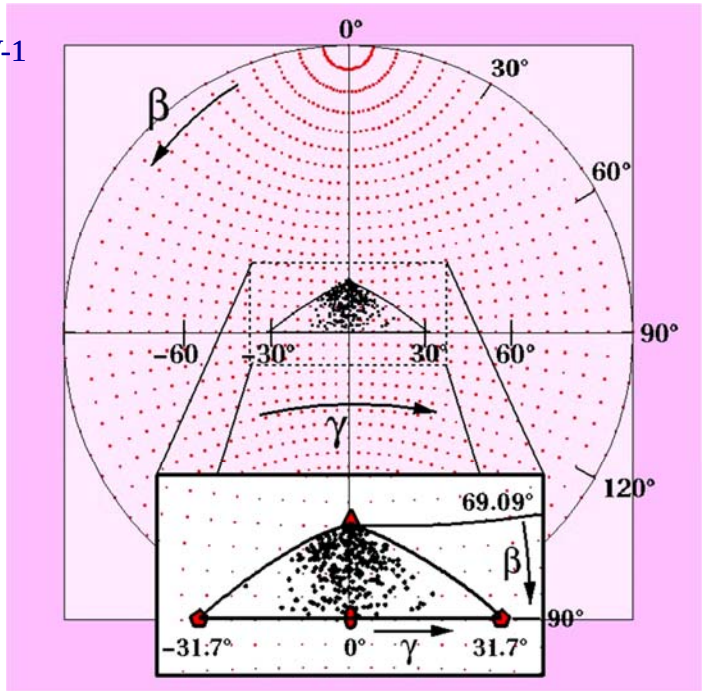
Image 2



Distribution of characteristic views - C6



HSV-1



Recommendations!!!

1. Don’t start from a symmetrical view.
2. Try to start from the general (common) view, then the second can be symmetrical.
3. Try to use as many DIFFERENT views as possible, ideally perpendicular to each other.
4. Do not use a sequence of similar views, the program will be confused!
5. If the standard deviation of peak heights starts to increase, it means that the process is starting to diverge.
   You will not get a reasonable solution.
6. Keep eye on the program: the long projections should have a consistent orientation, the PRINCIPLE of the BRICK can not be violated.

FREALIGN
Fourier Reconstruction and ALIGNment

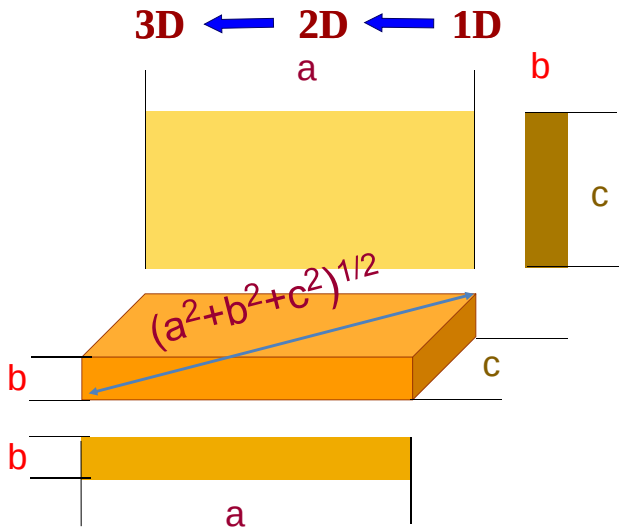
Iterations of alternating 3D reconstruction, using the improved alignment parameters, and realignment of the particles based on the updated 3D structure, are performed until the alignment parameters and 3D structure remain constant.

FREALIGN: image data (particle image stack, 3D reference reconstruction) and particle parameters (Euler angles, x, y translation, magnification, defocus and astigmatism, arc cosine of the correlation coefficient).

The algorithms in FREALIGN also introduced an efficient procedure for refining 3D structures by working entirely in Fourier space.

N. Grigorieff / Journal of Structural Biology 157 (2007) 117–125

The principle of the BRICK



Methods of orientation determination:

1. **Conical tilt. It is more efficient for negatively stained samples with a preferable orientation.**
   Missed cone
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2. **Angular reconstitution. It requires good signal/noise ratio, needs classification**
3. **Common lines in Fourier space and PFT are mostly used at analysis of particles with icosahedral symmetry**
4. **Projection matching, the most popular technique, requires an initial model.**

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