

Image similarity measures, 2-D alignment,
3-D reconstruction algorithms
Houston, December 2008

Pawel A. Penczek

The University of Texas – Houston Medical School,
Department of Biochemistry and Molecular Biology,
6431 Fannin, MSB6.218, Houston, TX 77030, USA.

phone: (713) 500-5416

fax: (713) 500-0652

Pawel.A.Penczek@uth.tmc.edu



THE UNIVERSITY *of* TEXAS

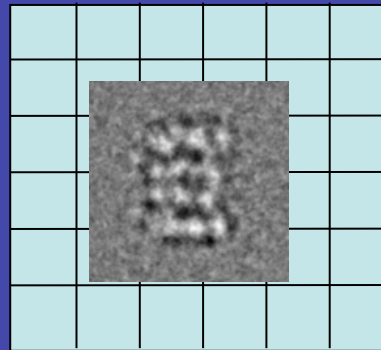
HEALTH SCIENCE CENTER AT HOUSTON

MEDICAL SCHOOL

Outline

- Measures of image similarity
- Resolution
- Image alignment (registration)
- 3-D reconstruction from projections

Alignment algorithms in cryo-EM



We need:

- a measure of similarity (discrepancy) between two images
- an accurate and efficient algorithm to find the orientation that would minimize the discrepancy between two images
- a method to align n images

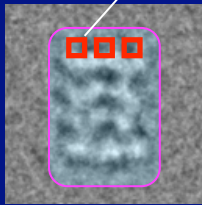
Similarity measures

- Euclidean distance
- Correlation coefficient
- Phase discrepancy

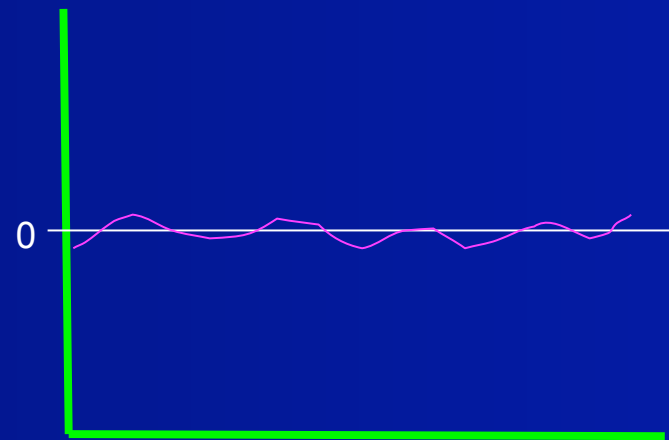
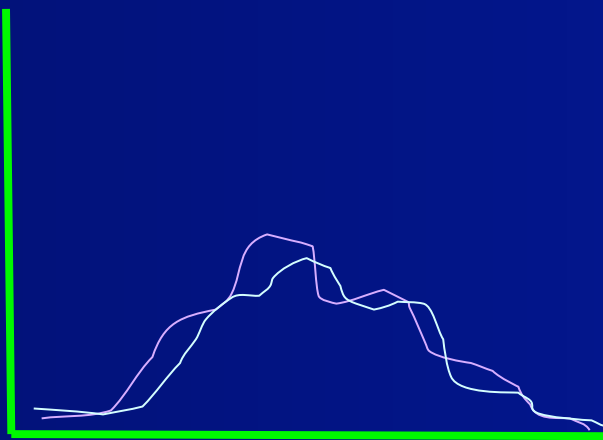
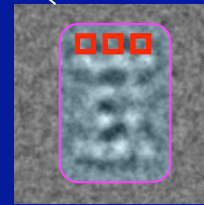
Euclidean distance

$$d_{xy}^2 = \sum (x - y)^2$$

x



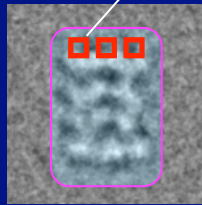
y



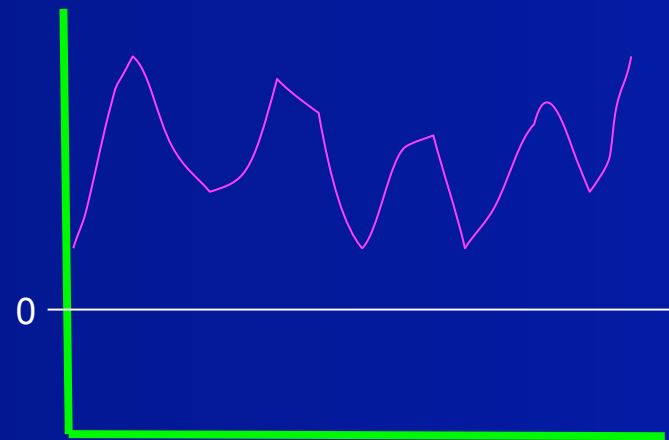
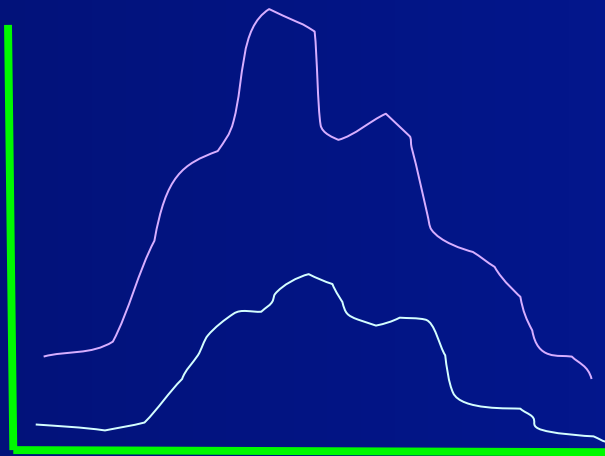
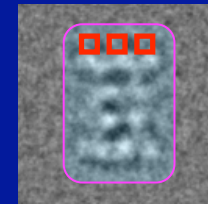
Euclidean distance depends on the scaling of images (both additive and multiplicative).

$$d_{xy}^2 = \sum (x - (ay + b))^2$$

x

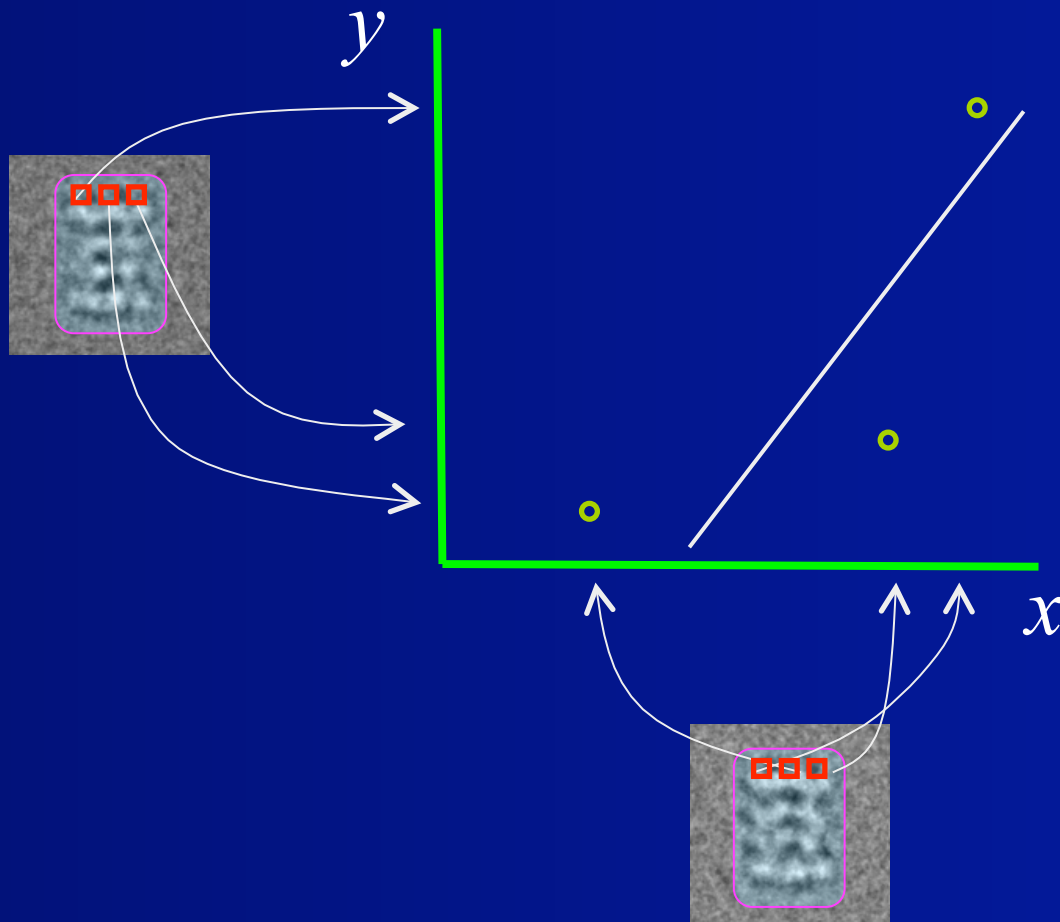


y



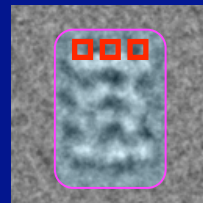
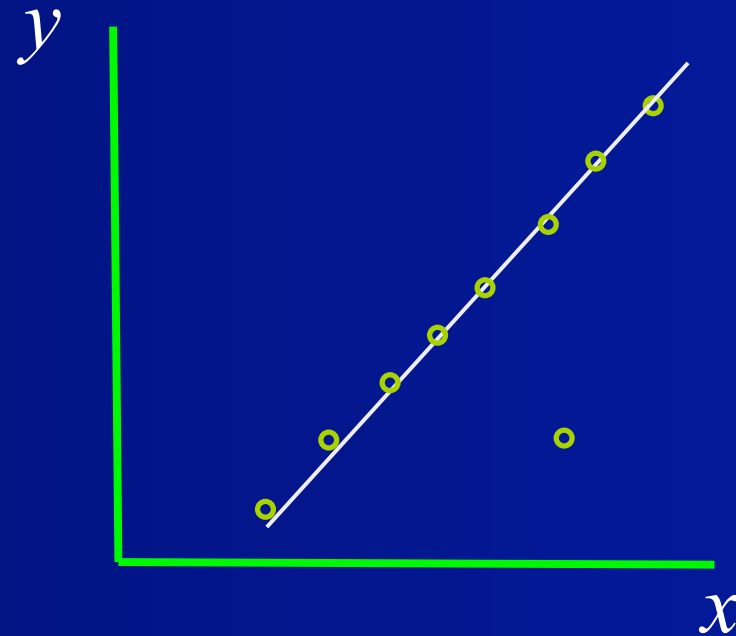
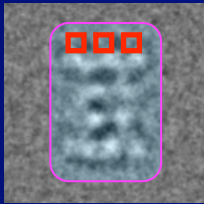
Correlation coefficient in image assessment

$$r_{xy} = \frac{\frac{1}{N} \sum xy - m_x m_y}{\sigma_x \sigma_y}$$



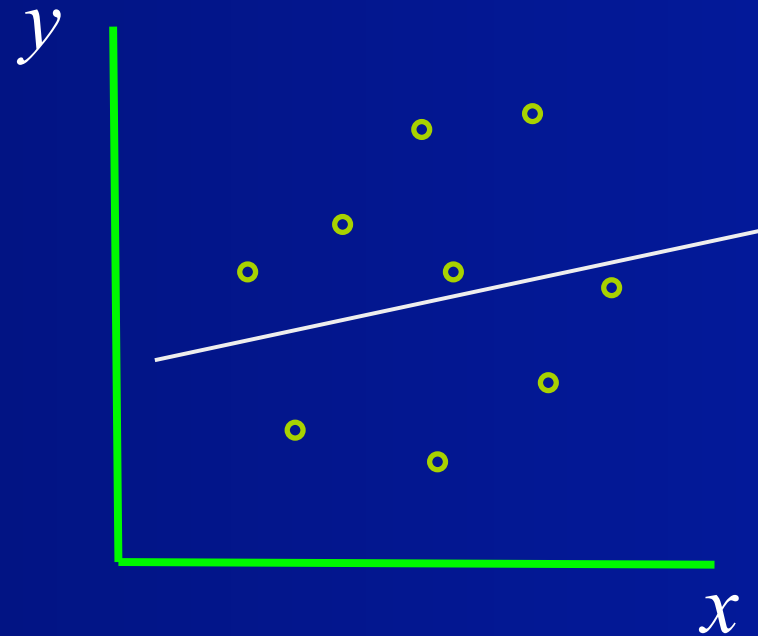
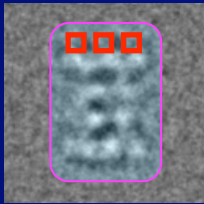
Correlation coefficient in image assessment

$$r_{xy} = \frac{\frac{1}{N} \sum xy - m_x m_y}{\sigma_x \sigma_y}$$

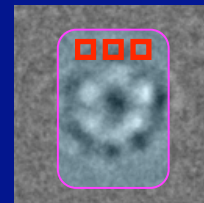


Correlation coefficient in image assessment

$$r_{xy} = \frac{\frac{1}{N} \sum xy - m_x m_y}{\sigma_x \sigma_y}$$



$r=0.11$



Correlation coefficient in image assessment

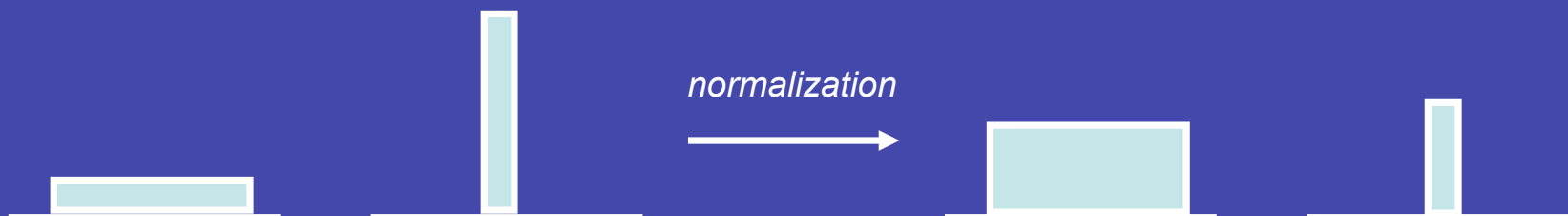
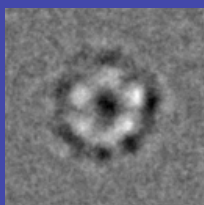
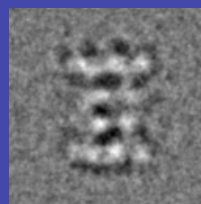
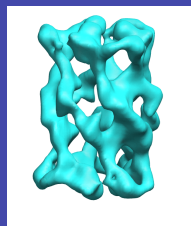
$$r_{xy} = \frac{\frac{1}{N} \sum xy - m_x m_y}{\sigma_x \sigma_y}$$

The value of correlation coefficient does not depend on linear scaling of images:

- the average is subtracted
- discrepancy is divided by the standard deviation

Correlation coefficient is tuned towards “shape” of objects.

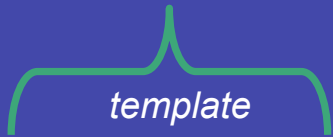
Problems

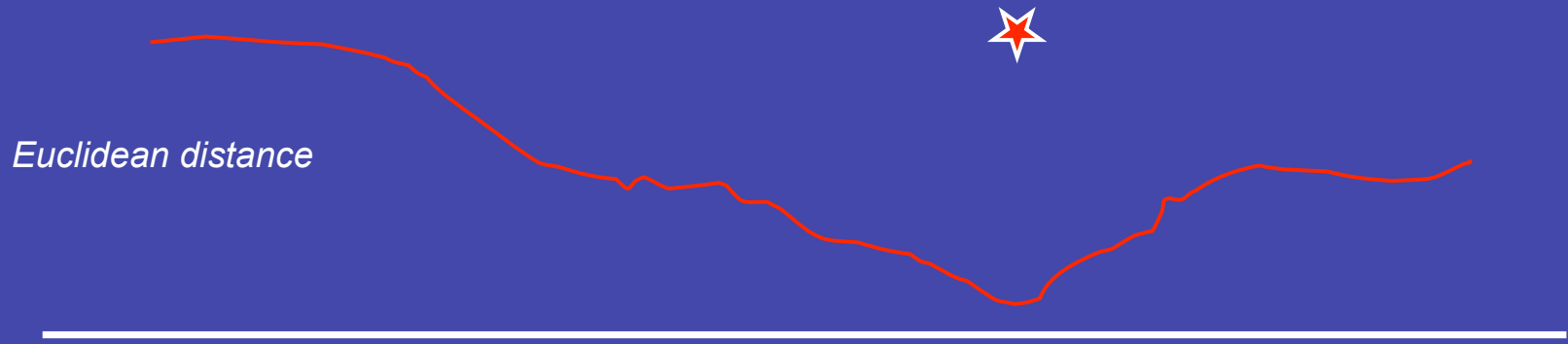


Correlation coefficient (with local normalization) makes sense for particle searches in unevenly illuminated micrograph fields....

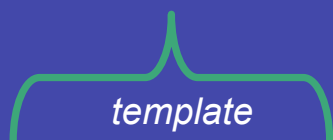


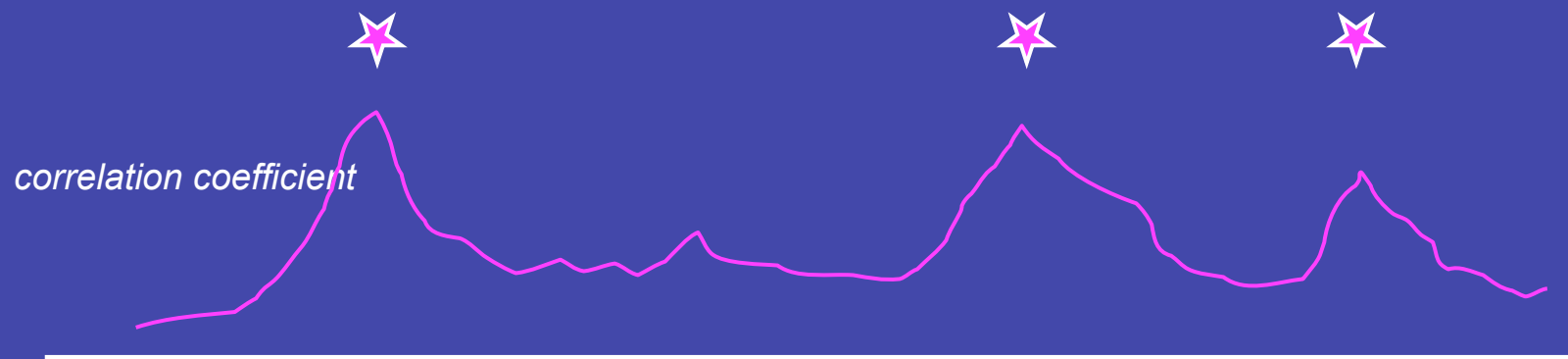
but it is a bad idea in projection matching or in 3-D docking!

 *template*



but it is a bad idea in projection matching or in 3-D docking!

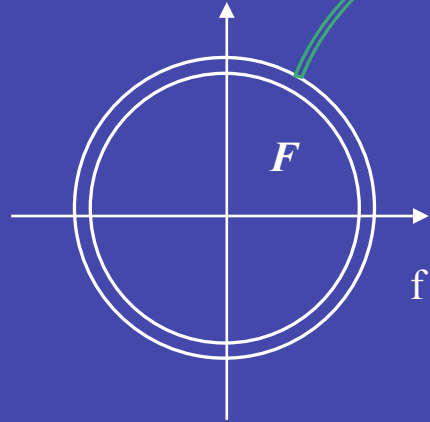
 *template*



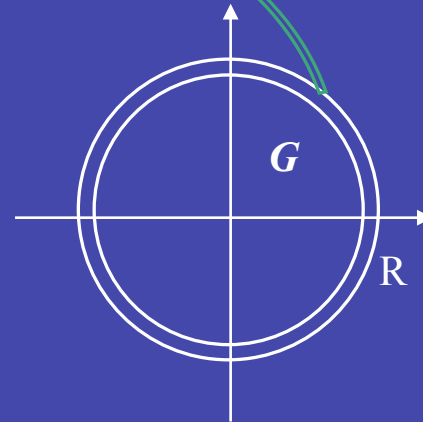
Fourier Shell Correlation (FSC)

(phase discrepancy)

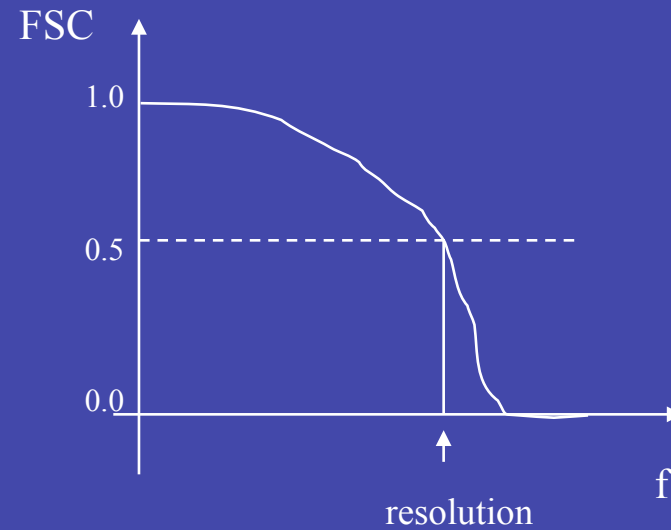
Fourier transform of image F



Fourier transform of image G



$$FSC(R) = \frac{\sum_{n \in R} F_n G_n^*}{\left\{ \left(\sum_{n \in R} |F_n|^2 \right) \left(\sum_{n \in R} |G_n|^2 \right) \right\}^{1/2}}$$



Relations between FSC and SSNR

$$SSNR = \frac{FSC}{1 - FSC}$$

$$FSC = \frac{SSNR}{SSNR + 1}$$

For large number of images $Variance(SSNR) \approx Variance(FSC)$

When FSC is calculated for a dataset split into halves:

$$SSNR = 2 \frac{FSC}{1 - FSC}$$

FSC is a biased estimate of SSNR.

For large number of images, the bias is negligible.

The expectation value of FRC is calculated as

$$E[FRC] \cong \frac{E\left[\sum_{n=1}^{N_R} F^n G^{n*}\right]}{E\left[\left\{\left(\sum_{n=1}^{N_R} |F^n|^2\right)\left(\sum_{n=1}^{N_R} |G^n|^2\right)\right\}^{1/2}\right]}$$

$$= \frac{\sum_n F_T^{n^2}}{\sum_n F_T^{n^2} + \sum_n \frac{1}{L} \sigma_N^{n^2}} = \frac{\sum_n \frac{F_T^{n^2}}{L \sigma_N^{n^2}}}{\sum_n \frac{F_T^{n^2}}{L \sigma_N^{n^2}} + 1} = \frac{SSNR}{SSNR + 1}$$

Resolution criteria
should be based on the SNR considerations

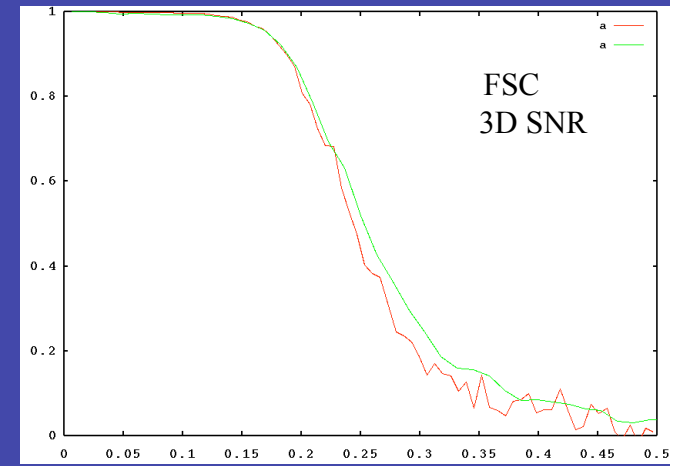
$$SSNR = 2 \frac{FSC}{1 - FSC}$$

Reasonable criterion: include only Fourier information that is above the noise level, i.e., $SSNR > 1$.

$$SSNR = 1 \Rightarrow FSC = 1/3 = 0.333$$

Another criterion: (3σ) include Fourier information that is significantly higher than zero, i.e., $SSNR > 0$.

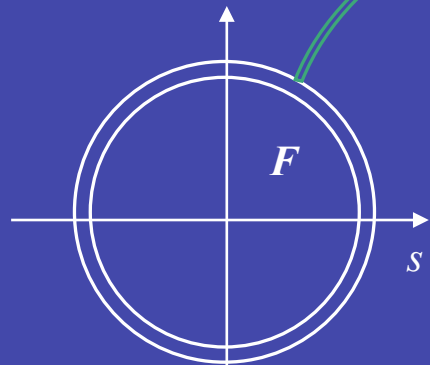
$$SSNR = 0 \Rightarrow FSC = 0$$



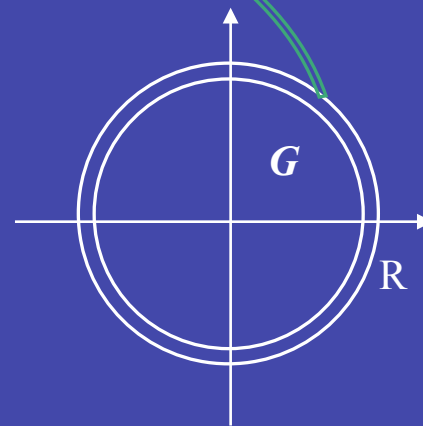
Cross-resolution

relation between FRC and SSNR

X-ray map F
(noise-free)



EM map G
(corrupted by noise and
other errors)

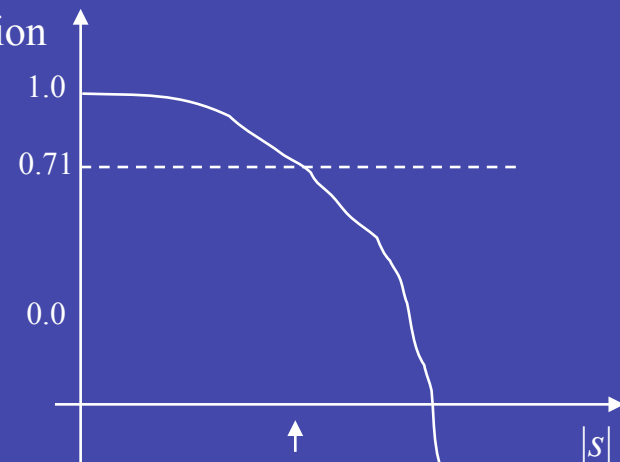


$$FSC(R) = \frac{\sum_{n \in R} F_n G_n^*}{\left\{ \left(\sum_{n \in R} |F_n|^2 \right) \left(\sum_{n \in R} |G_n|^2 \right) \right\}^{1/2}}$$

$$SSNR = \frac{FSC^2}{1 - FSC^2}$$

$$SSNR = 1 \quad \Rightarrow \quad FSC = \sqrt{\frac{1}{2}} = 0.71$$

Cross-resolution



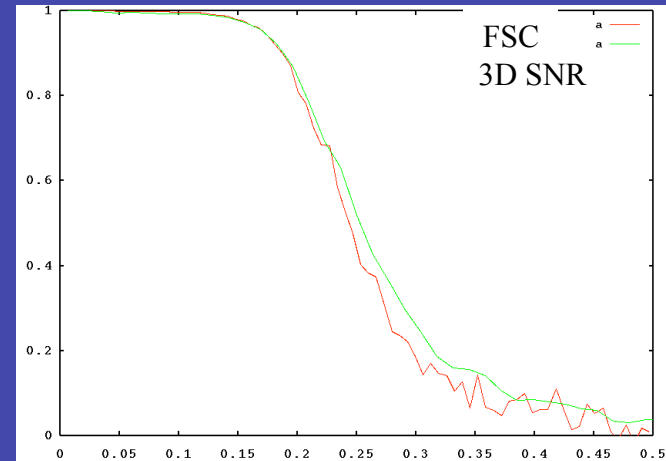
Resolution curve and optimum filtration

$$SSNR = 2 \frac{FSC}{1 - FSC}$$

Wiener filter:

$$G = \frac{SSNR}{SSNR + 1} F$$

$$G = 2 \frac{FSC}{FSC + 1} F$$



The *FSC* curve should be used for optimum filtration.

Thus, the 'resolution' is given by the overall shape of the *FSC*, not by a single number.

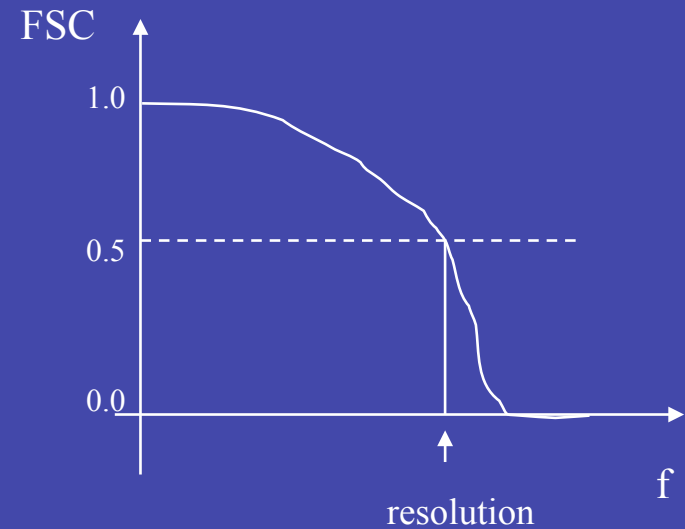
Fourier Shell Correlation (FSC)

properties when used as a similarity measure

- FSC yields a 1D curve, but the values can be added to yield one number, distance *dfsc*

$$dfsc = \int_0^{R_{\max}} FSC(R) dR$$

- dfsc* can be thought of as an Euclidean distance between high-passed images. Regretfully, filtering is adaptive, i.e., it varies from image to image, so results are difficult to compare.
- dfsc* will ignore weighting of amplitudes due to CTF.
- dfsc* is very sensitive to shapes of objects, so it will enhance edges in averages of aligned images creating appearance of 'high resolution'.
- dfsc* may work well for low-contrast images, such as tomograms.



$$FSC(R) = \frac{\sum_{n \in R} F_n G_n^*}{\left\{ \left(\sum_{n \in R} |F_n|^2 \right) \left(\sum_{n \in R} |G_n|^2 \right) \right\}^{1/2}}$$

Similarity measures

- *Correlation coefficient* and *Phase discrepancy* are popular as they are independent on scaling of the data (in EM difficult if not impossible to do properly) and they can be rapidly evaluated using FFT techniques.
- *Euclidean distance* is very sensitive to proper normalization of the data and small errors will yield entirely erroneous results. It is also more difficult to implement.

Normalizing projection images: a study of image normalizing procedures for single particle three-dimensional electron microscopy

C.O.S. Sorzano^a, L.G. de la Fraga^b, R. Clackdoyle^c, J.M. Carazo^{a,*}

^a Biocomputing Unit, National Center of Biotechnology (CSIC), Campus Universidad Autónoma, 28049 Cantoblanco, Madrid, Spain

^b Computer Science Section, Department of Electrical Engineering, CINVESTAV-IPN Av. Instituto Politécnico Nacional 2508, 07300 México DF, Mexico

^c Medical Imaging Research Laboratory, Department of Radiology, Utah University, CAMT 729 Arapeen Drive, Salt Lake City, UT 84108-1218, USA

$$N_0[I(x, y)] = I(x, y),$$

$$N_1[I(x, y)] = \frac{I(x, y) - \text{avg}[\text{bg}(I)]}{\text{avg}[\text{bg}(I)]},$$

$$N_2[I(x, y)] = \frac{I(x, y) - \text{avg}(I)}{\sqrt{\text{var}(I)}},$$

$$N_3[I(x, y)] = \frac{I(x, y) - \text{avg}[\text{bg}(I)]}{\sqrt{\text{var}[\text{bg}(I)]}},$$

$$N_4[I(x, y)] = \frac{I(x, y) - \text{avg}[\text{bg}(I)]}{\text{avg}(I) - \text{avg}[\text{bg}(I)]},$$

Poisson distribution of noise

Gaussian distribution of noise, will ruin relative amplitudes

Gaussian distribution of noise

?????

Arbitrary distribution of noise

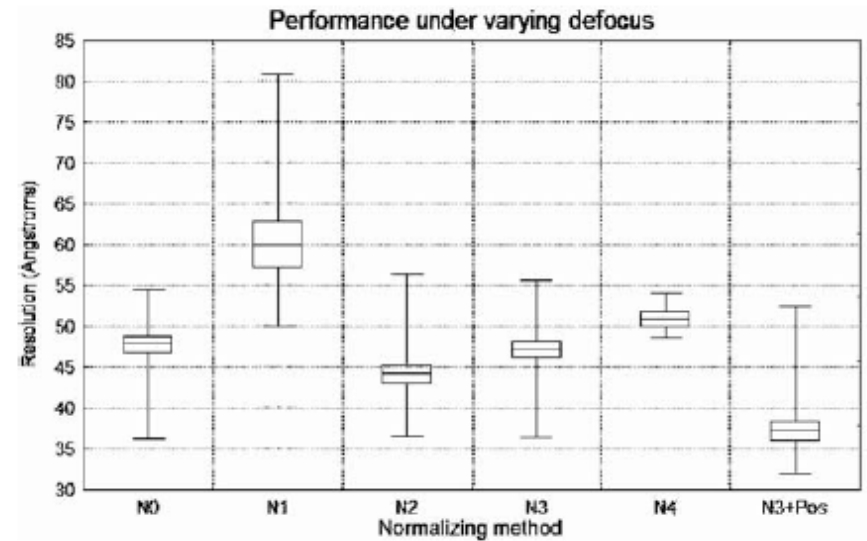
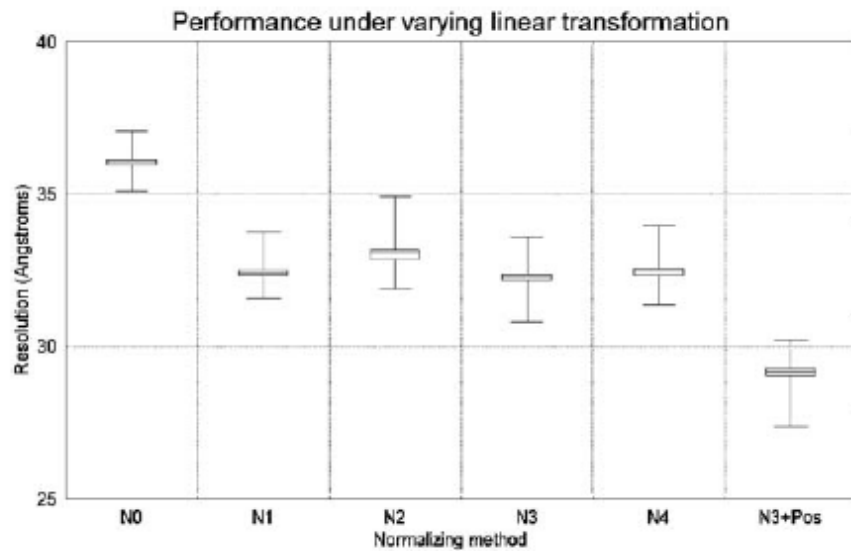
Match histogram of pixel values in the background to that of reference histogram derived from a representative area.

N. Boisset, P. Penczek, F. Pochon, J. Frank, J. Lamy, Three-dimensional architecture of human α 2-macroglobulin transformed with methylamine, J. Mol. Biol. 232 (1993) 522.

Data normalization in EM

no-CTF

N_0	N_1	N_2	N_3	N_4	$N_3 + pos$
50.8 ± 9.5	59.8 ± 9.4	44.1 ± 6.3	47.2 ± 5.6	47.6 ± 5.6	37.1 ± 6.0



Data normalization in EM

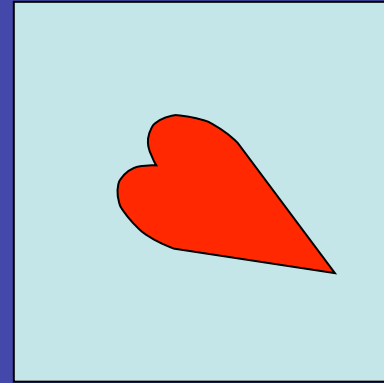
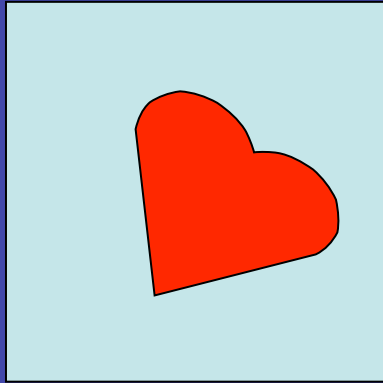
Renormalize the data after initial 3-D projection alignment parameters were found.

Compare 2-D data with reprojections of the structure.

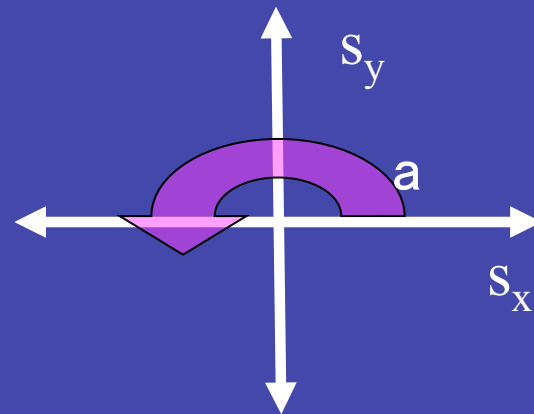
(Zhang and Penczek, 2008, Structure).

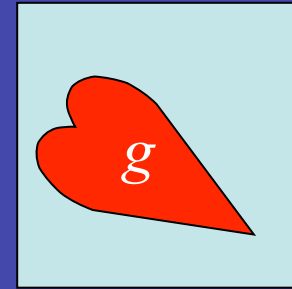
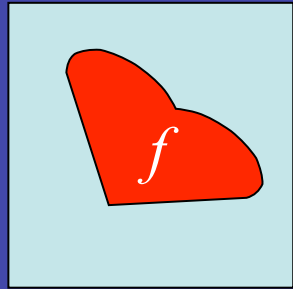
The alignment problem

Two 2D images:



Three degrees of freedom:





Two images are aligned if the least square discrepancy between them is minimized:

$$\int \left| f\left(\mathbf{x}; s_x, s_y, \alpha\right) - g(\mathbf{x}) \right|^2 d\mathbf{x} \rightarrow \min$$

$$\int |f|^2 d\mathbf{x} + \int |g|^2 d\mathbf{x} - 2 \int f\left(\mathbf{x}; s_x, s_y, \alpha\right) g(\mathbf{x}) dx \rightarrow \min$$

$$\text{const} + \text{const} - c\left(s_x, s_y, \alpha\right) \rightarrow \min$$

$$c\left(s_x, s_y, \alpha\right) \rightarrow \max$$

Cross-correlation

is used as a tool to align (bring into register) images that are in different orientations.

One of the images is shifted with respect to the other, reference image, and for each shift position the similarity between two images is calculated.

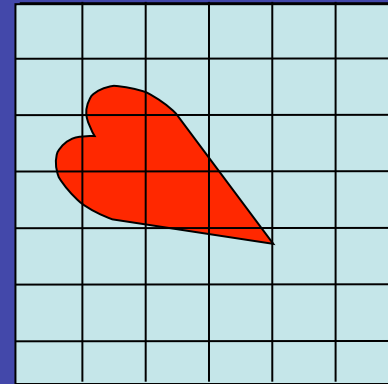
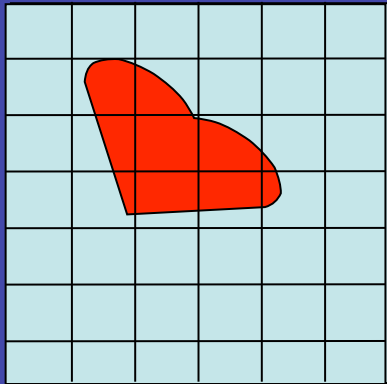
A set of similarity values as a function of image position is called **cross-correlation function (CCF)**.

The maximum of CCF indicates the best mutual orientation between a pair of images.



Alignment as a discrete problem

Two 2D images



Translation *or* rotation can be found effectively using the Fast Fourier Transform algorithm.

There is *no* fast method for finding both translation and rotation.

To find translation *and* rotation we can:

- use exhaustive search (explore all possible orientations)
- use gradient-based methods to move towards best orientation

Methods of 2-D alignment

- *Direct alignment in real space*
- *Direct alignment using 2-D FFT*
- *Sinograms*
- *Indirect alignment using autocorrelation function*
- *Alignment using resampling to polar coordinates*

2-D alignment

Resampling into polar coordinates
around systematically selected centers of the image.

L. Joyeux, P.A. Penczek / Ultramicroscopy 92 (2002) 33–46

35

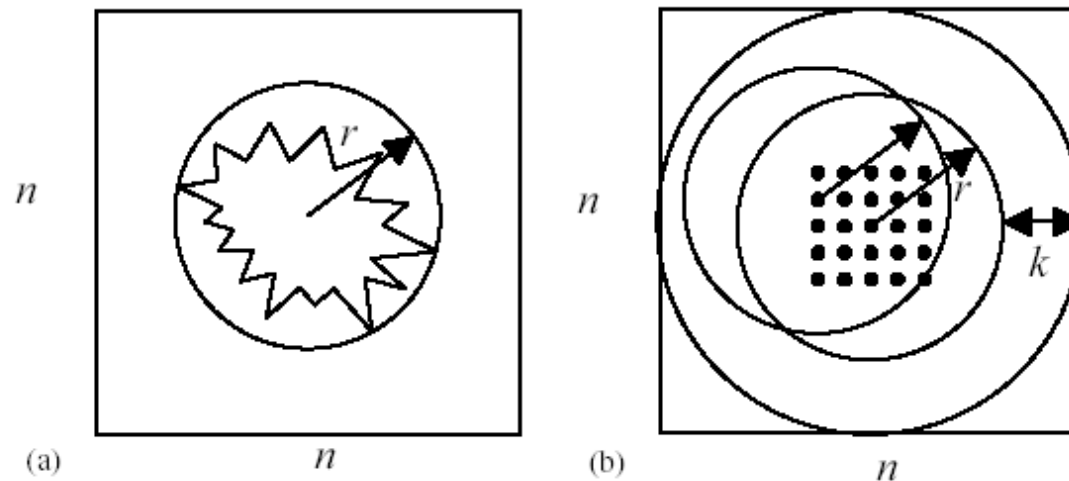
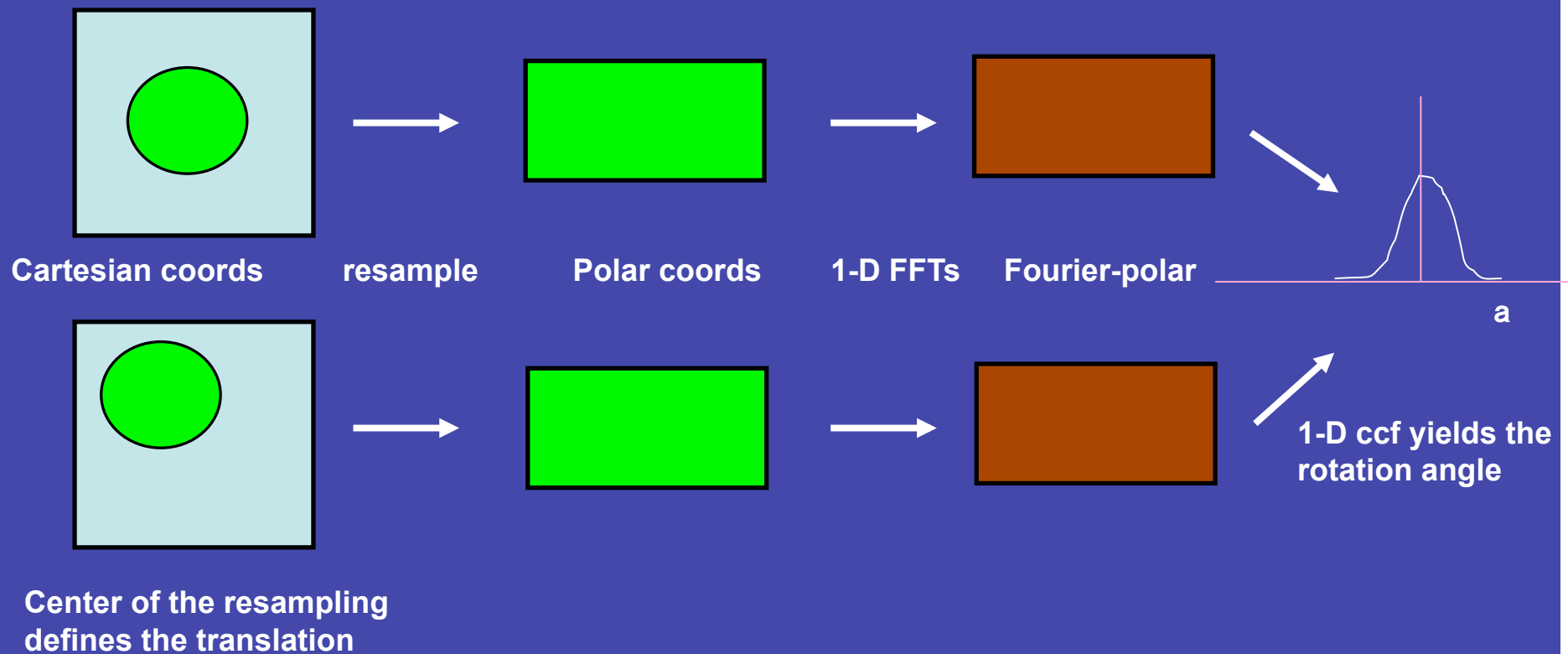


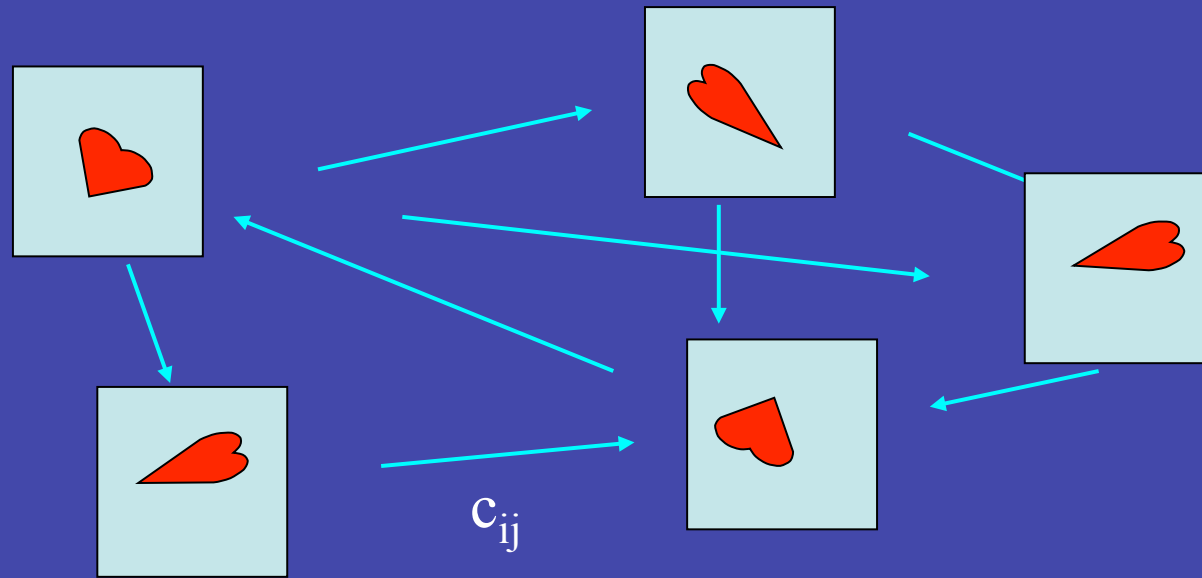
Fig. 1. The geometrical constraints of the 2D alignment problem. (a) The reference particle view is placed within a square image frame $n \times n$ pixels and its size is such that it can be bounded by a circle with a radius no larger than $r = n/2$. (b) The particle view, which size is bounded by the same radius as the reference view, can be located within a circle centered on discrete locations within the image frame, such that the maximum translation is $k = n/2 - r$. The number of possible translations is $(2k + 1)^2 = l$.

2-D alignment

Resampling into polar coordinates around systematically selected centers of the image.



Alignment of n images



The distances between all pairs of images have to be minimized simultaneously.

$$\sum_{k=1}^{n-1} \sum_{l=k+1}^n \int |f_k(\mathbf{x}; s_x^k, s_y^k, \alpha^k) - f_l(\mathbf{x}; s_x^l, s_y^l, \alpha^l)|^2 d\mathbf{x} \rightarrow \min$$

Methodology of 2D alignment

Types of alignment problems:

- A reference image is known or can be easily approximated, and there is only one particle orientation (with possible small variations). This case will be called *Reference-based alignment*.
- A small number of reference images are known or can be easily approximated, and their number is known, and particle orientations are well defined (with possible small variations). This case will also be called *Reference-based alignment*.
- An approximation of a reference image is known and there is only one particle orientation (with possible small variations). This case will be called *Alignment with the reference refinement*.
- Reference images are not known, and there is no clear groupings in the data set. This case will be called *Reference-free alignment*.
- Reference images are not known, but the data set can in principle be divided into a number (unknown) of homogeneous classes. This case will be called *Multireference alignment*.

Methods of 2-D alignment of n images

Various iterative schemes have been proposed, including Maximum Likelihood method.

No matter what the claim of the author might be and whether the author realizes it or not,

all methods of 2D alignment of n images try to circumvent the problem

that there is no algorithm that would guarantee the optimum alignment of n images.

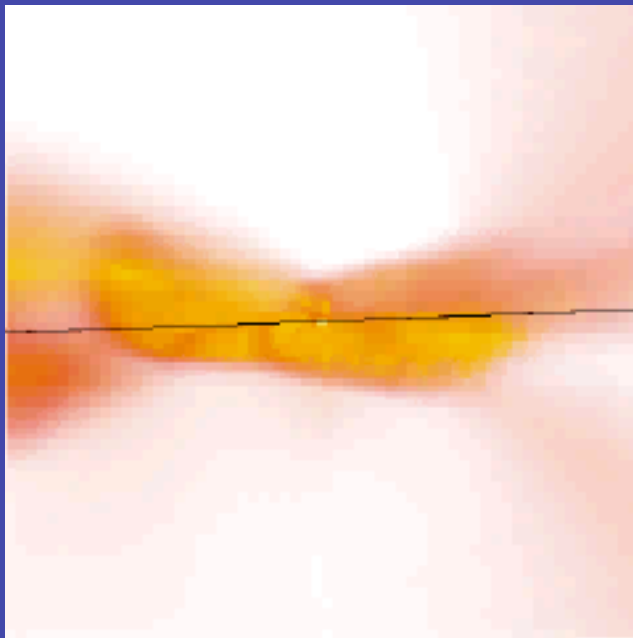
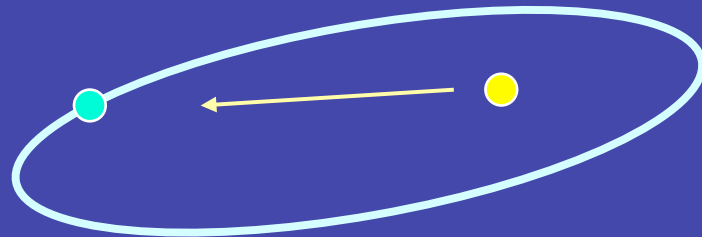
Tomography

historical background

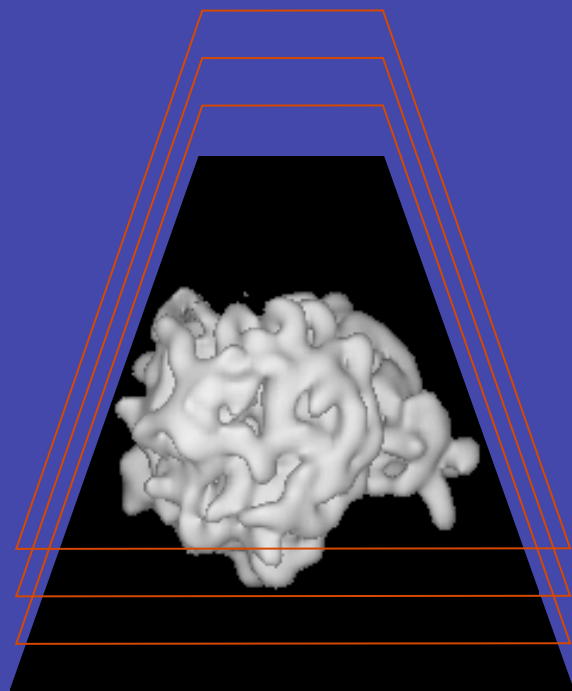
- 1956 - Bracewell reconstructed sun spots from multiple views of the Sun from the Earth.
- 1967 - Medical Research Council Laboratory, Cambridge, England: Aaron Klug and grad student David DeRosier reconstructed three-dimensional structures of a virus capsid (icosahedral symmetry).
- 1968 – W. Hoppe (Germany) proposed three-dimensional high resolution electron microscopy of non-periodic biological structures.
- 1972 - British engineer Godfrey Hounsfield of EMI Laboratories, England, and independently South African born physicist Allan Cormack of Tufts University, Massachusetts, invented CAT (Computed Axial Tomography) scanner. Tomography is from the Greek word *tomos* meaning "slice" or "section" and *graphia* meaning "describing".
- 1990 – *J. Frank & P. Penczek determined first cryo structure of the 70S ribosome using single particle approach and tomographic principle.*

Inner heliospheric plasma density

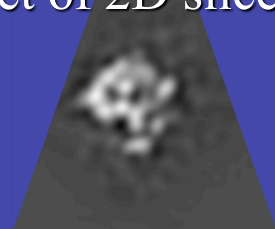
(to 1.5 times the distance of the Earth from the Sun).



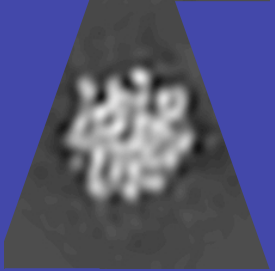
3D structure



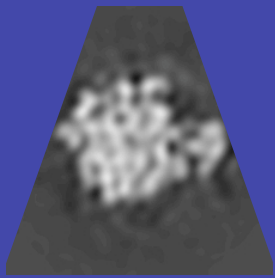
Set of 2D slices



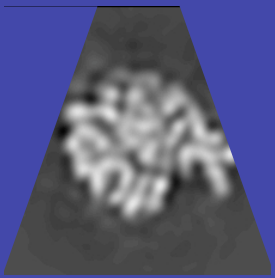
+



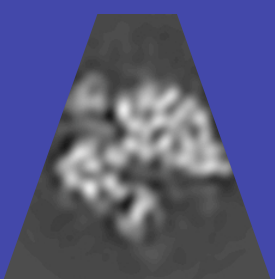
+



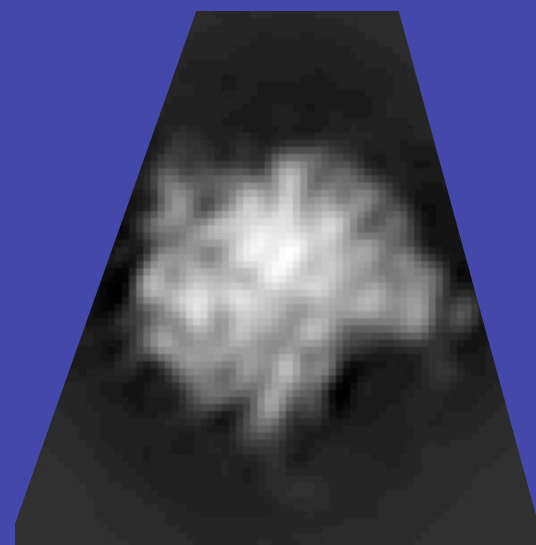
+



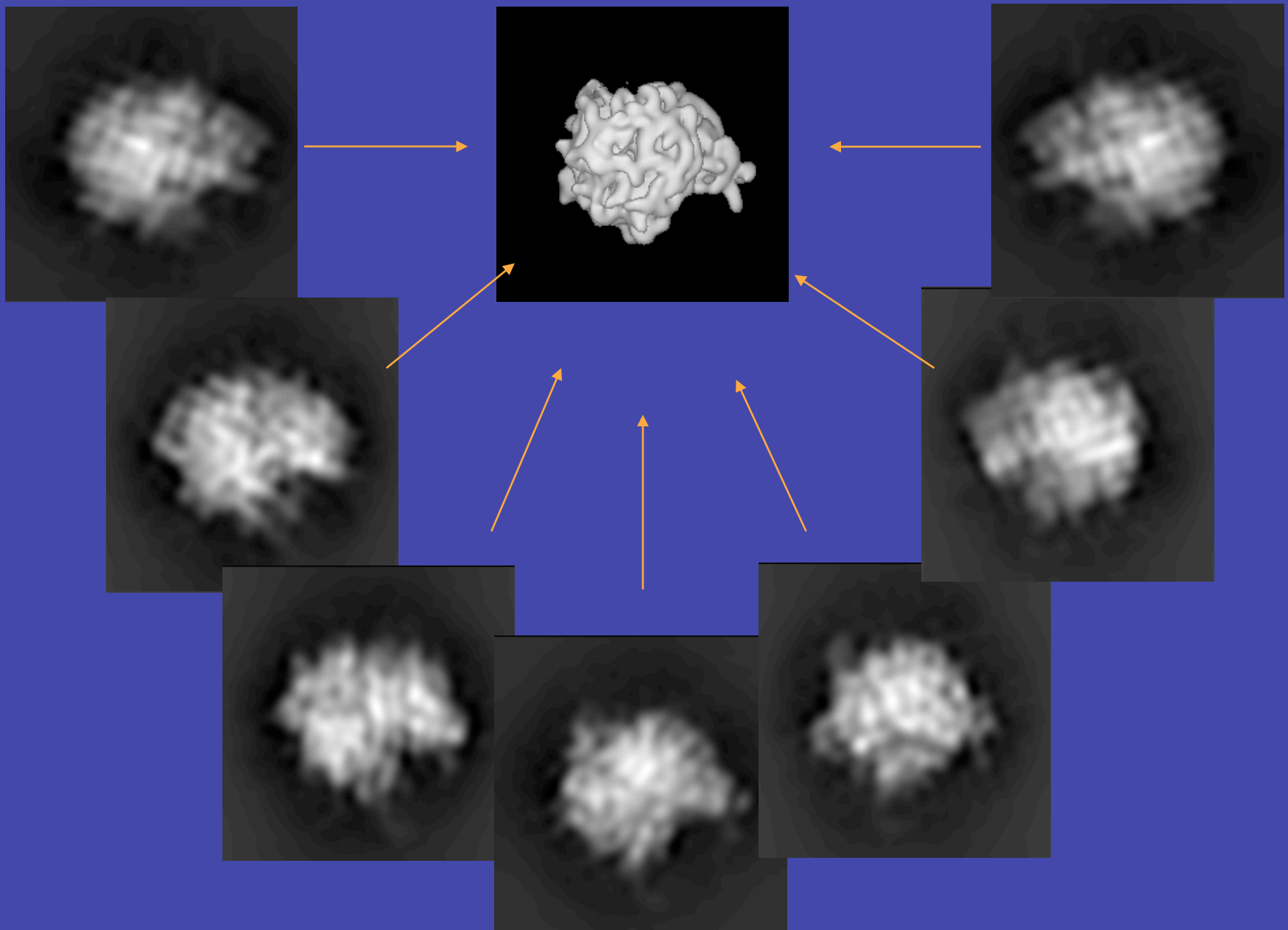
+



To project a 3D structure is to add densities in slices.



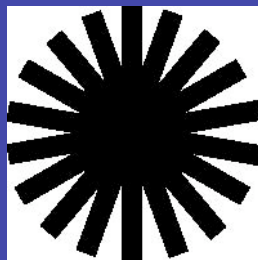
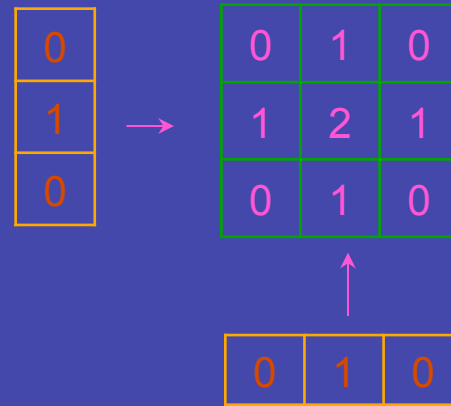
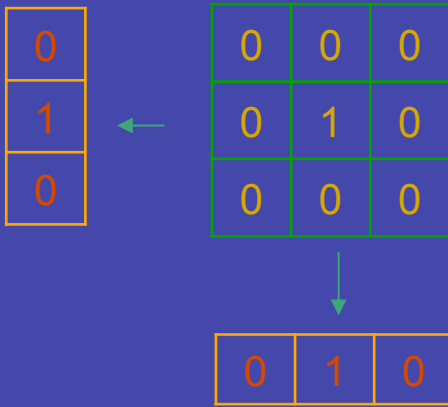
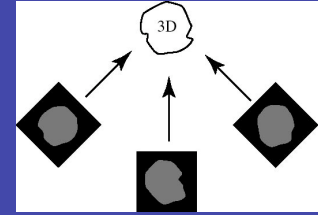
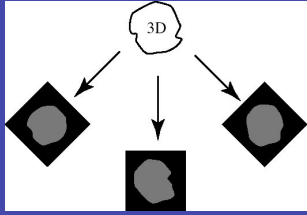
Projection



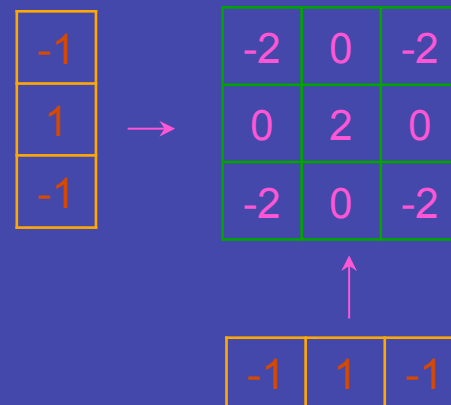
3D reconstruction (Back Projection)

Full range

Mechanism of projection-backprojection

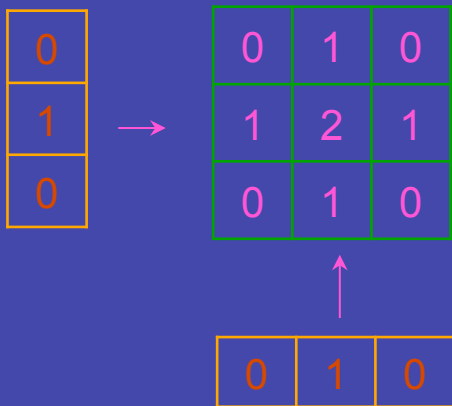


r^* weighting

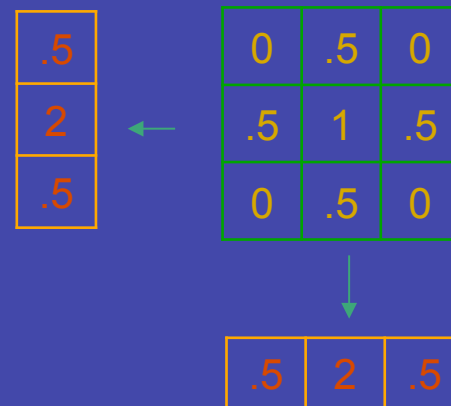


Iterative algebraic reconstruction (SIRT)

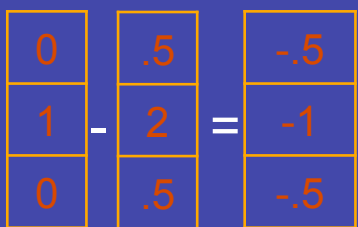
1. backproject



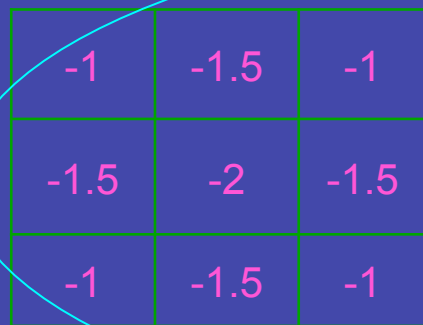
2. project



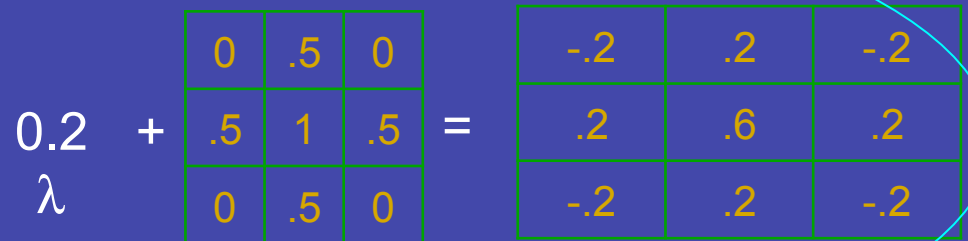
3. calculate errors between original data and projected structure



4. backproject errors



5. correct the current structure



Repeat steps 2-5



3D reconstruction algorithm can be considered the most important element of the single particle reconstruction process

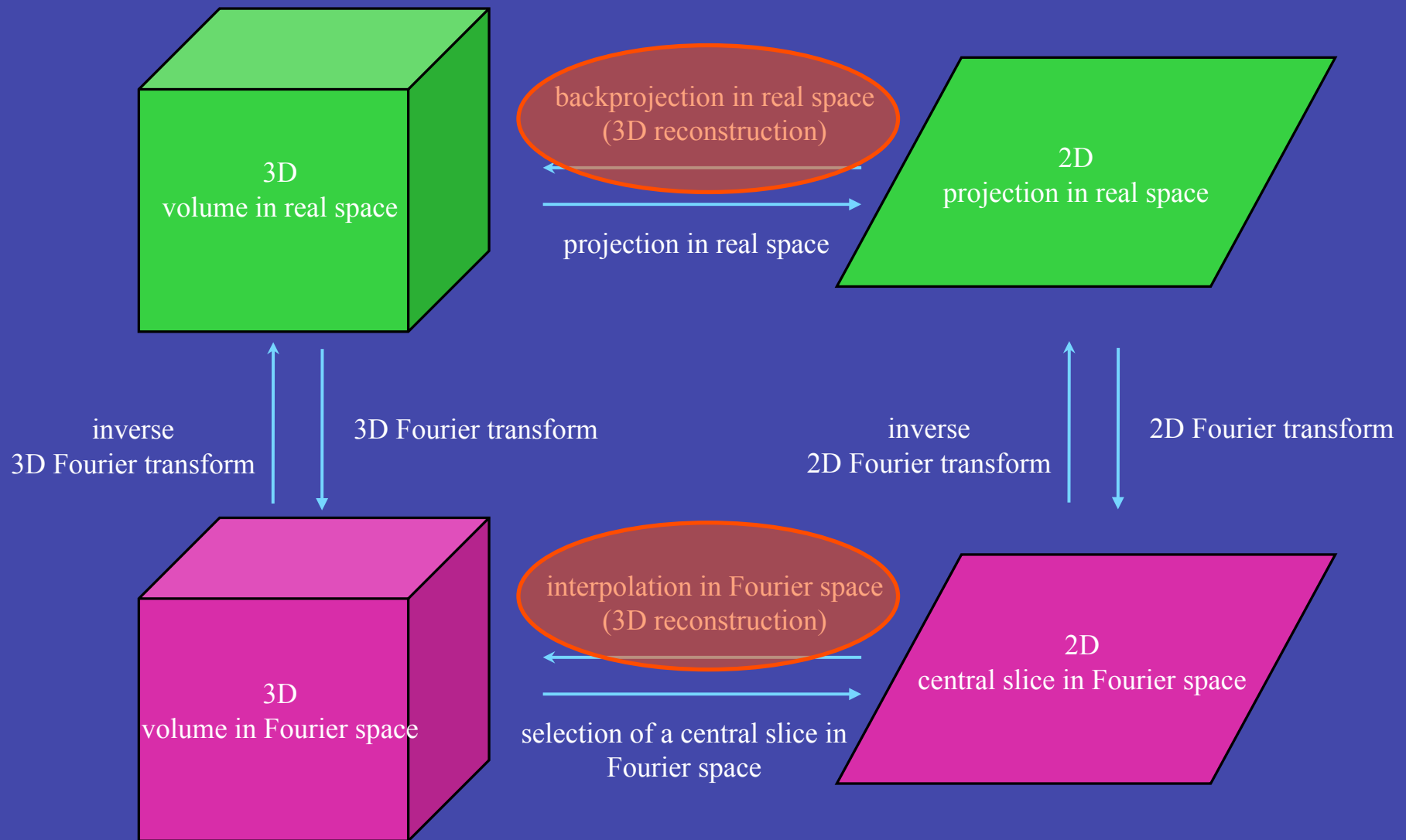
Many steps of the process are best understood in terms of the 3D reconstruction problem:

- construction of an initial model
- refinement of the structure
- resolution estimation

The problem of 3D reconstruction from projections in EM is substantially different from the problem of “classical” tomography:

- data collection geometry cannot be controlled (random distribution of projection directions)
- extremely uneven distribution of projection directions, in many cases resulting in gaps in Fourier space
- extremely low SNR
- large errors in orientation parameters, both random and systematic, in principle the 3D reconstruction should be a part of orientation refinement procedure
- number of projection data much larger than the linear size of projections

Tomography (reconstruction from projections).



Difficult inverse problems, exact inversion does not exist!

Taxonomy of 3-D reconstruction methods

	Direct (solution obtained after one scan through the data)	Iterative (the structure is "improved" iteratively)
Algebraic	<p>Direct solution of the system of equations defined by the projection matrix.</p> <p>Singular Value Decomposition (SVD)</p> <p><i>Due to the size of the matrix not used in 3-D.</i></p> <p><i>CTF makes the problem hopelessly complicated</i></p>	<p>1. Algebraic Reconstruction Technique (ART)</p> <p>2. Simultaneous Iterative Reconstruction Technique (SIRT)</p> <p><i>Very good results, very slow.</i></p> <p><i>CTF easily incorporated</i></p>
Filtered backprojection (Fourier space filtration)	<p>1. General Weighted Backprojection (Radermacher)</p> <p>2. Exact Filter (Harauz & van Heel)</p> <p>Require construction of a weighting function in Fourier space – no exact formula exists.</p> <p><i>Reasonably fast, reasonably accurate.</i></p> <p><i>CTF cannot be incorporated</i></p>	Not used.
Direct Fourier inversion (Fourier space interpolation)	<p>Gridding algorithm (Penczek)</p> <p>Requires full coverage of Fourier space by projection data.</p> <p><i>The most accurate method, fast.</i></p> <p><i>CTF easily incorporated.</i></p> <p><i>Very fast, but simple interpolation schemes give very poor results.</i></p>	Not used.

If iterative algorithms are slow and inconvenient, why would we want to use them?

- The quality of SIRT results surpasses the quality of results of other methods, particularly of those based on Fourier transform. Least disturbing artifacts.
- SIRT algorithms perform better in “extreme” situations, such as uneven distribution of projections, incomplete projections (“missing cone”, “missing wedge”), reconstruction from few directions.
- SIRT algorithms are flexible. It is possible to incorporate additional constraints (positivity, limited spatial support), *a priori* knowledge, CTF correction....

Summary

- ❖ Cryo-EM and single particle reconstruction rely on the tomographic effect in the electron microscope.
- ❖ There is no unique solution to the problem of recovering the 3D structure from the finite set of its 2D projections.
- ❖ The quality and speed of 3D reconstruction algorithms differ. Generally, the speed and quality are inversely proportional. Depending on the data set (presence and level of noise, errors, gaps in angular coverage) some algorithms perform better than others.

SIRT: (Gilbert, 1972; Penczek *et al.*, 1992; Penczek *et al.*, 1997; Zhu *et al.*, 1997)

ART: (Gordon *et al.*, 1970; Marabini *et al.*, 1998)

General Weighting Back-Projection: (Radermacher, 1992; Radermacher *et al.*, 1986)

Exact Weighting Back-Projection: (Harauz and van Heel, 1986)

Direct Fourier inversion: (Penczek *et al.*, 2004, Zhang and Penczek, 2008)

1. Gilbert, H., 1972. Iterative methods for the three-dimensional reconstruction of an object from projections. *Journal of Theoretical Biology* 36, 105-117.
2. Gordon, R., Bender, R., Herman, G. T., 1970. Algebraic reconstruction techniques (ART) for three-dimensional electron microscopy and x-ray photography. *Journal of Theoretical Biology* 29, 471-81.
3. Harauz, G., van Heel, M., 1986. Exact filters for general geometry three dimensional reconstruction. *Optik* 73, 146-156.
4. Marabini, R., Herman, G. T., Carazo, J. M., 1998. 3D reconstruction in electron microscopy using ART with smooth spherically symmetric volume elements (blobs). *Ultramicroscopy* 72, 53-65.
5. Penczek, P., Radermacher, M., Frank, J., 1992. Three-dimensional reconstruction of single particles embedded in ice. *Ultramicroscopy* 40, 33-53.
6. Penczek, P. A., Renka, R., Schomberg, H., 2004. Gridding-based direct Fourier inversion of the three-dimensional ray transform. *J. Opt. Soc. Am. A* 21, 499-509.
7. Penczek, P. A., Zhu, J., Schröder, R., Frank, J., 1997. Three-dimensional reconstruction with contrast transfer function compensation from defocus series. *Scanning Microscopy Supplement* 11, 1-10.
8. Radermacher, M. (1992) Weighted back-projection methods, *in* J. Frank (Ed.), *Electron Tomography*, pp. 91-115, Plenum, New York.
9. Radermacher, M., Wagenknecht, T., Verschoor, A., Frank, J., 1986. A new 3-D reconstruction scheme applied to the 50S ribosomal subunit of *E. coli*. *Journal of Microscopy* 141, RP1-RP2.
10. Zhu, J., Penczek, P. A., Schröder, R., Frank, J., 1997. Three-dimensional reconstruction with contrast transfer function correction from energy-filtered cryoelectron micrographs: procedure and application to the 70S *Escherichia coli* ribosome. *J. Struct. Biol* 118, 197-219.

Recommended reading

- Penczek, P.A., 2008. Single Particle Reconstruction, in: U. Shmueli, (Ed.), International Tables for Crystallography, Vol.B, 2008.
- Vainshtein, B.K., and P.A. Penczek, 2008. Three-dimensional reconstruction, in: U. Shmueli, (Ed.), International Tables for Crystallography, Vol.B, 2008.

Thank you!