Resolution Estimation

For students of HI 6001-125

"Computational Structural Biology"

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http://biomachina.org/courses/structures/08.html

Resolution Limitations in EM

•The wavelength of the electrons (depends on the voltage: 100kV~0.037Å; 300kV~0.020Å)

•The quality of the electron optics (astigmatism, envelope functions)

•The underfocus setting. The resolution of the TEM is often defined as the first zero in the contrast transfer function (PCTF) at Scherzer (or optimum) defocus.

•Signal-to-Noise Ratio (SNR) level in the data

•Accuracy of the alignment / classification

Real Space Criteria

Clearly resolved

Rayleigh

Sparrow

Not resolved
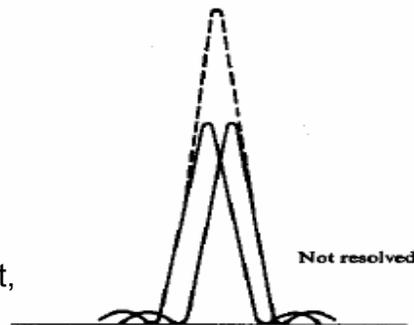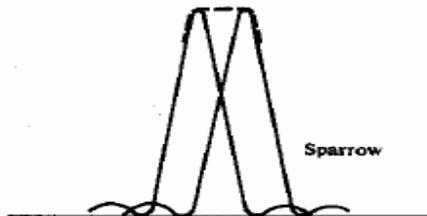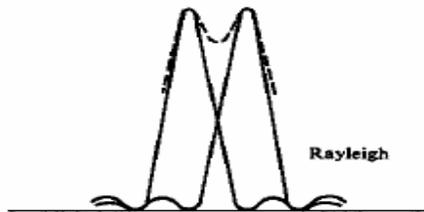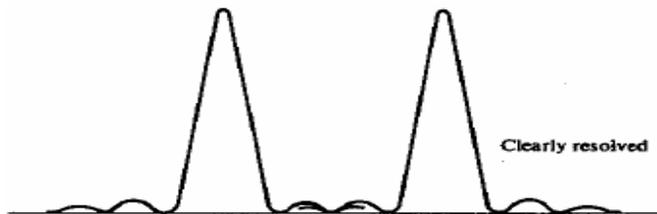
© Hecht, Optics, 4th ed.

According to the *Rayleigh criterion*, the angular resolution of a lens or mirror of diameter D is given by

$$\theta_{Rayleigh} = \frac{1.22\lambda}{D},$$

where λ is the wavelength of radiation. It follows from Fraunhofer diffraction around a circular aperture, and the value 1.22 is given by x_1/π, where $x_1 \approx 3.832$ is the first zero of the Bessel function of the first kind that forms part of the mathematical expression for the Airy Disk.

A more stringent criterion is the *Sparrow criterion*, where the Airy disks overlap more so that the first and second derivatives of the combined intensity pattern vanish:
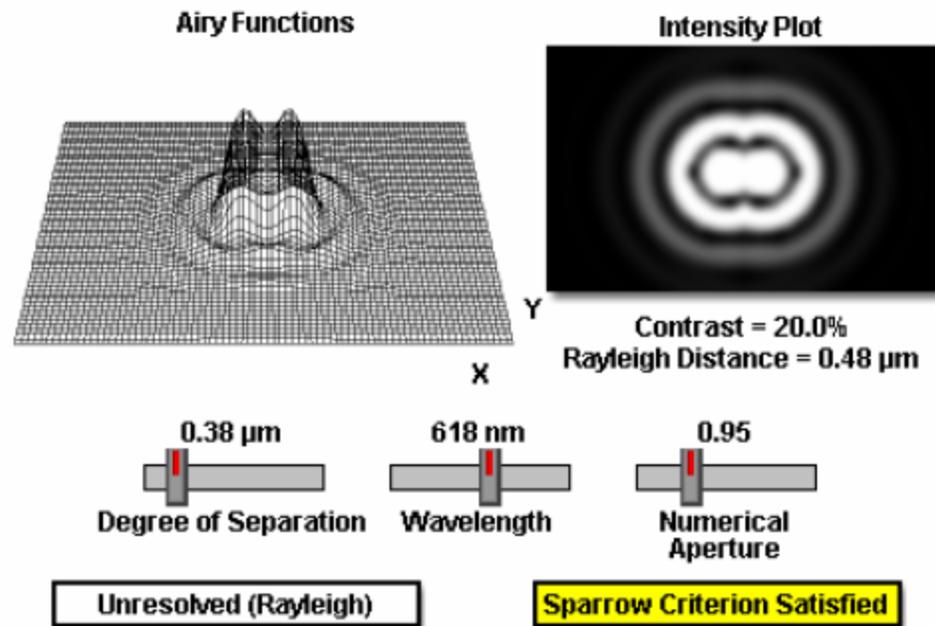
$$\theta_{Sparrow} = \frac{0.94\lambda}{D}.$$

Compare these two values to the Full-Width Half-Max (FWHM) of the Airy function:

$$\theta_{FWHM} = \frac{1.03\lambda}{D}.$$

See http://scienceworld.wolfram.com/physics/FraunhoferDiffractionCircularAperture.html

Rayleigh vs. Sparrow Java Animation

Airy Functions

Intensity Plot

Contrast = 20.0%
Rayleigh Distance = 0.48 μm

0.38 μm
Degree of Separation

618 nm
Wavelength

0.95
Numerical Aperture

Unresolved (Rayleigh)

Sparrow Criterion Satisfied

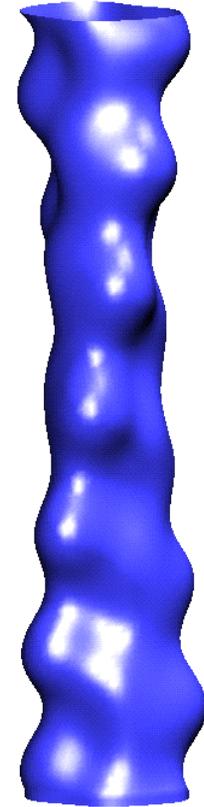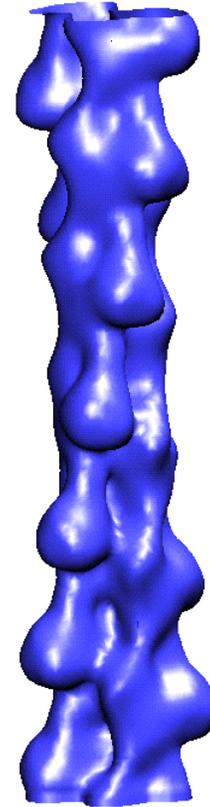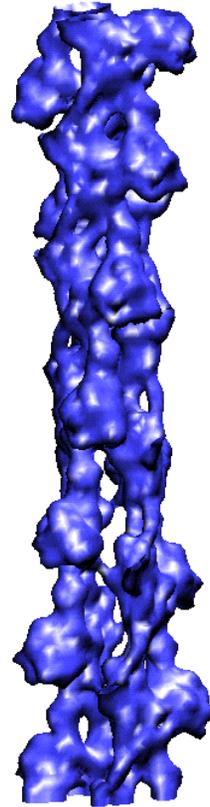http://www.olympusfluoview.com/java/resolution3d/

Resolution Lowering

Actin filament
(Holmes et al., 1990)

Convolution with
Gaussian:

$$G(r) = \exp\left(\frac{-3r^2}{2\sigma_{3D}^2}\right)$$

$2\sigma_{3D}$=0Å $2\sigma_{3D}$=10Å $2\sigma_{3D}$=20Å $2\sigma_{3D}$=40Å

Resolution Lowering

- Low-Pass Filtering = Convolution with a Gaussian

- Not a diffraction effect, but in analogy we can model the diffraction limited core of the Airy function as a Gaussian (here in 1 or 3 dimensions):

  - $G_{1D}(r) = C \exp(-r^2 / 2\sigma_{1D}^2)$
  - $G_{3D}(r) = C \exp(-3r^2 / 2\sigma_{3D}^2)$

  - Note: this way, σ corresponds to the **standard deviation** of the Gaussian:

$$\sigma^2 = \int \left(r - \hat{r}\right)^2 g(r) = \int r^2 g(r) - \hat{r}^2 \quad \textit{squared s.d. (variance), where:}$$

$$g(r) = G(r) \Big/ \left(\int G(r)\right) \quad \textit{probability (normalized density)}$$

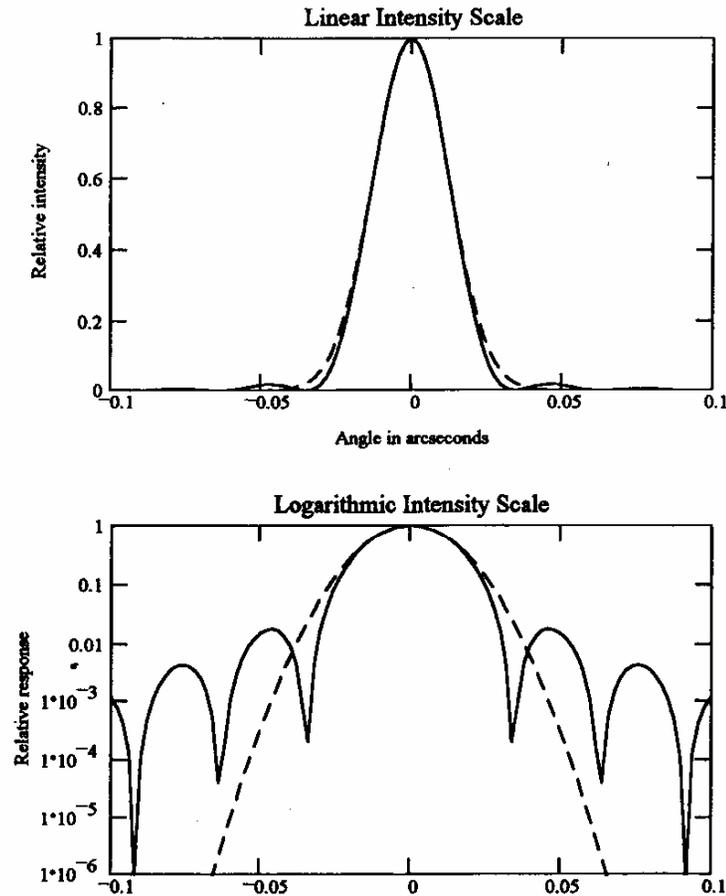$$\hat{r} = \int r \, g(r) \quad \textit{expectation value (here: } \hat{r}=0)$$
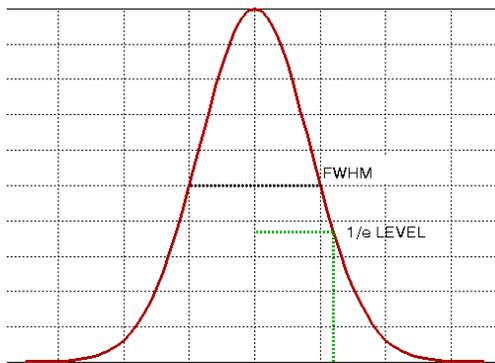
Full-Width Half-Max of Gaussian:
$FWHM = 2.355 \, \sigma_{1D}$
$FWHM = 1.360 \, \sigma_{3D}$

Fitting of Gaussian to Airy Profiles

A Gaussian profile with standard deviation $\sigma_{1D} = 0.44\ \lambda / D$ or $\sigma_{3D} = 0.76\ \lambda / D$ has the same width as the Airy function.

"Crystallographic" Resolution

[11] Resolution is a quantity in Fourier space and hence has dimension Å^{-1}. This Fourier-based resolution can be linked (see Appendix in Radermacher, 1988) to a "point-to-point" distance in real space by the Rayleigh criterion (see Born and Wolf, 1975). Rayleigh considered the diffraction-limited images of two points, which are Airy disks, each represented by the intensity distribution $[J_1(2\pi rR)/2\pi R]^2$, where J_1 is the first-order Bessel function, r is the radius, and R is the radius of the diffracting aperture. According to the criterion, the two points separated by distance d_0 are just resolved when the maximum of one Airy disk coincides with the minimum of the second Airy disk. This critical distance d_0 turns out to be $d_0 = 0.6/R$. If we interpret R as the radius of the circular domain within which Fourier terms contribute to the crystallographic Fourier synthesis ("crystallographic resolution"), then we can say that the Rayleigh point-to-point resolution, $1/d_0$, is 1.67 times the crystallographic resolution.

Colloquially, and somewhat confusingly, the real-space quantity 1/resolution is also often termed "resolution." Just to eliminate this confusion, we will use the term "resolution distance" when referring to the quantity 1/resolution. Hence, if we compare the distance d_0 and $1/R$ we arrive at the factor 0.6 (Radermacher, 1988): the point-to-point resolution distance according to Rayleigh is 0.6 times the inverse of the crystallographic resolution.

Chapter 3 in: Joachim Frank, Three-Dimensional Electron Microscopy of Macromolecular Assemblies (1996, Academic Press)

Example: Resolution Criteria

A "crystallographic resolution" of $(20Å)^{-1}$ corresponds to a point-to-point separation of:

- 12.2Å (Rayleigh)
- 9.4Å (Sparrow)

In both cases the Airy disk (or matched Gaussian) has a FWHM of 10.3Å.

In the Situs package an empirical FWHM for Gaussian convolution kernels is used that renders resolution-lowered maps similar to published resolution values of EM data:

Target resolution distance ≡ 2 σ_{3D} = *1.47 FWHM*

So a FWHM of 10.3Å in the above example corresponds to a target resolution distance of 15.1Å, which is closer to the Rayleigh criterion than to the (inverse) crystallographic resolution.

Example: Resolution Criteria

A "crystallographic resolution" of $(20\text{Å})^{-1}$ corresponds to a point-to-point separation of:

- 12.2Å (Rayleigh)
- 9.4Å (Sparrow)

In both cases the Airy disk (or matched Gaussian) has a FWHM of 10.3Å.

In EMAN, the functional form of the Gaussian real-space kernel is:
$$G_{3D}(r) = exp\ (-\pi^2 r^2\ /\ res^2)\ .$$

(*res* is defined in EMAN such that the Fourier transform of G_{3D}, which turns out to be $exp(-\ res^2\ k^2)$, is identical to 1/e)

Hence, it follows $\sigma_{3D} = \sqrt{\dfrac{3}{2}}\dfrac{1}{\pi} res \approx 0.39 res;\quad FWHM \approx 0.53 res.$

So a FWHM of 10.3Å in the above example corresponds to an EMAN resolution res=19.4Å, which is practically identical to the (inverse) crystallographic resolution.

Resolution Estimation in EM

• Many different resolution criteria used in 3D EM reconstructions.

• In EM image processing there is no "external", objective standard by which the resolution of the results, e.g. of single particle analysis, could be evaluated, such as with the real-space distance criteria.

• Therefore, the resolution measures in EM have to estimate "internal consistency" of the results (cross-validation).

• Objective estimation of the resolution in EM is not possible without external standards (e.g. known structures).

2D and 3D Algorithms - Overview

FRC -Fourier Ring Correlation (3D: F. *Shell* C.)
•Saxton W.O. and W. Baumeister.
The correlation averaging of a regularly arranged bacterial cell envelope protein.
J. Microsc., **127**, 127-138 (1982).

DPR –Differential Phase Residual
•Frank J., A. Verschoor, M. Boublik.
Computer averaging of electron micrographs of 40S ribosomal subunits.
Science, **214**, 1353-1355 (1981).

SSNR –Spectral Signal-to-Noise Ratio
•Unser M., L.B. Trus, A.C. Steven.
A new resolution criterion based on spectral signal-to-noise ratios.
Ultramicroscopy, **23**, 39-52 (1987).
•Penczek, P. A.
Three-dimensional Spectral Signal-to-Noise Ratio for a class of reconstruction algorithms.
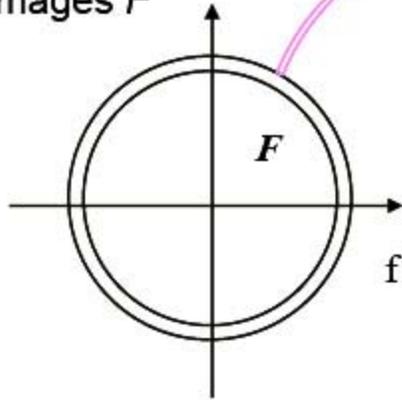J. Struct. Biol., **138**, 34-46 (2002)

Q-factor (2D only)
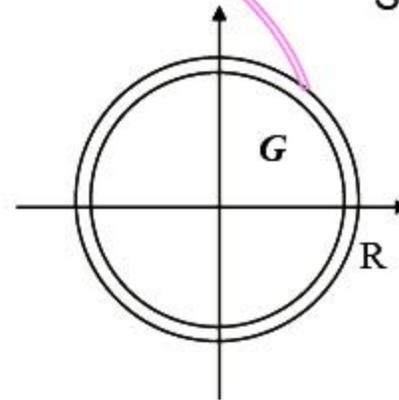•van Heel M. and J. Hollenberg.
The stretching of distorted images of two-dimensional crystals.
In:Proceedings in Life Science: Electron Microscopy at Molecular Dimensions.
Springer Verlag, Berlin (1980).
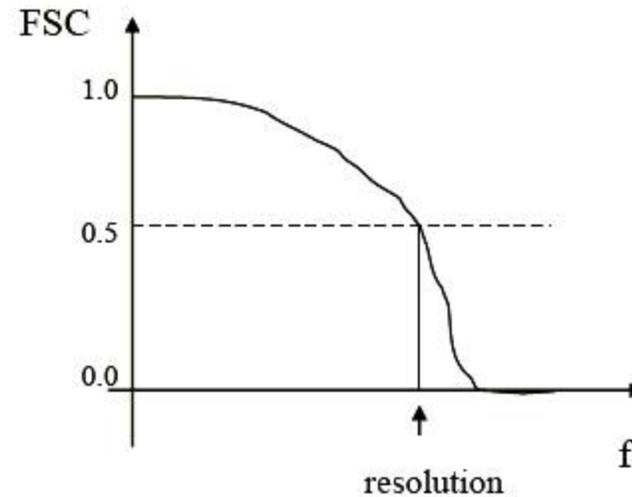
Fourier Shell Correlation

First set of images *F*

Second set of images *G*

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

Fourier Shell Correlation

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

A. either:
    1. Split (randomly) the data set of available images into halves;
   2. Perform the alignment of each data set "independently";
B. or:
    1. Perform the alignment of the whole data set;
    2. Split (randomly) the aligned data set into halves;
    3. Calculate two averages (3D reconstructions);
    4. Compare the averages in Fourier space by calculating the FRC.

Caveat: method B valid *only* if the noise component in the data is independent (not aligned); also the two sets in method A might not independent.

Signal vs. Noise

When we perform multiple measurements of the same phenomena, we equate the *"signal"* with the part of the measurement that remains the same between measurements, and we assume that the varying part of measurements is the *"noise"*.
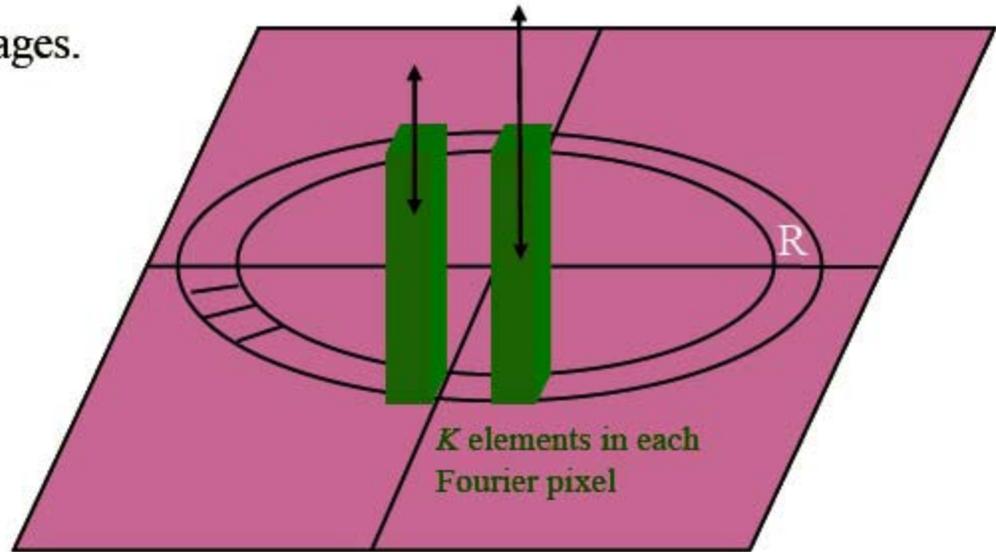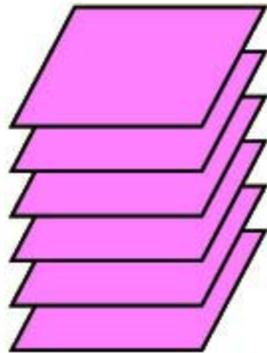

Sum (or average) = *"signal"*   Variance = *"noise"*
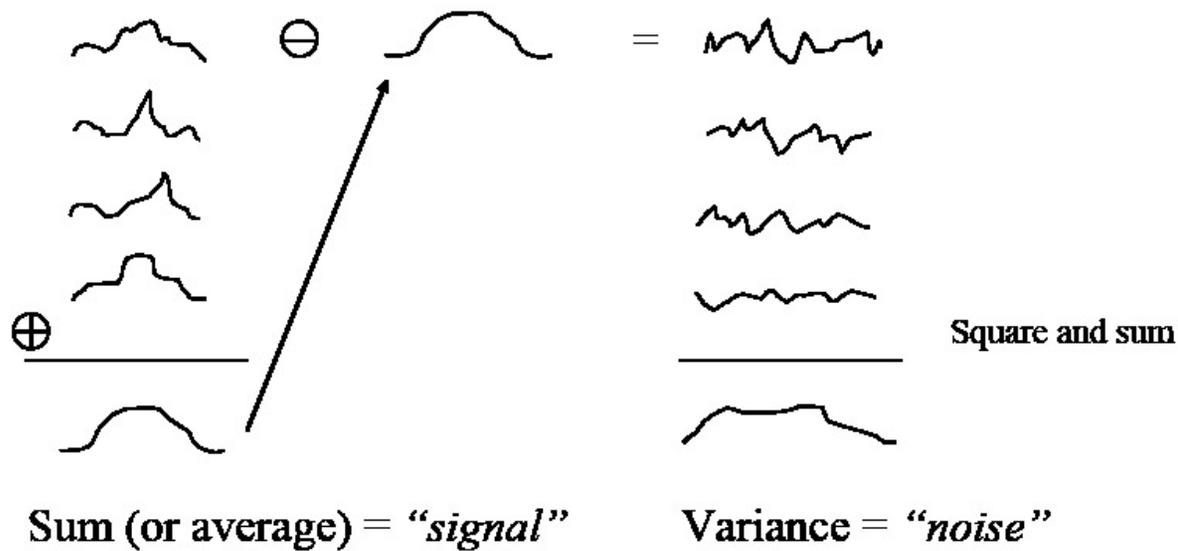
Signal to Noise Ratio (SNR) =

$$\frac{\text{Power of signal}}{\text{Power of noise}}$$

Spectral SNR (SSNR) in 2D

A set of Fourier transforms of 2D images.

K elements in each Fourier pixel

Calculate SSNR according to the equation:

$$SSNR(R) = \frac{\sum\limits_{n \in R} \left| \sum\limits_{k} F_{n,k} \right|^2}{\frac{K}{K-1} \sum\limits_{n \in R} \sum\limits_{k} \left| F_{n,k} - \langle F \rangle_n \right|^2} - 1$$

$$where \quad \langle F \rangle_n = \frac{1}{K} \sum\limits_{k} F_{n,k}$$

SSNR(R)

1.0

0   10   20   30   R
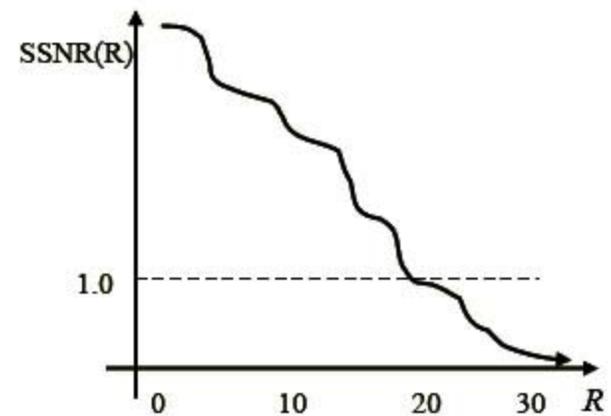
Relations between FSC and SSNR

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

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

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

When FSC is calculated for a data set split into halves:

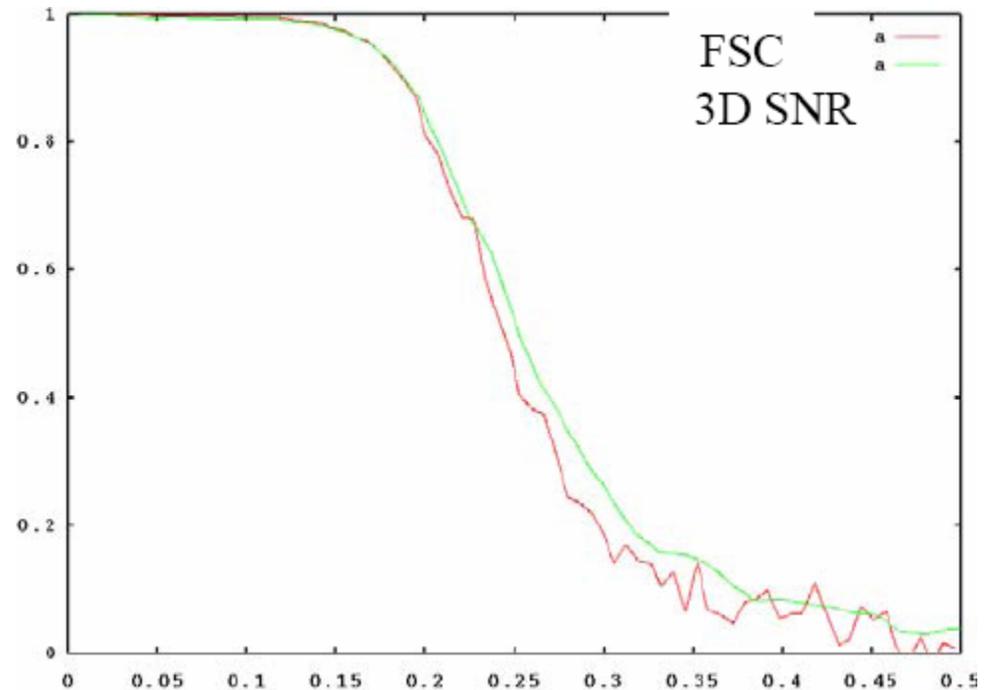$$SSNR = 2\frac{FSC}{1 - FSC}$$

FSC is a biased estimate of SSNR.
For large number of images, the bias is negligible.

Relations between FSC and SSNR

Reasonable criterion: include only Fourier information that is above the noise level, i.e., *SSNR*>1.
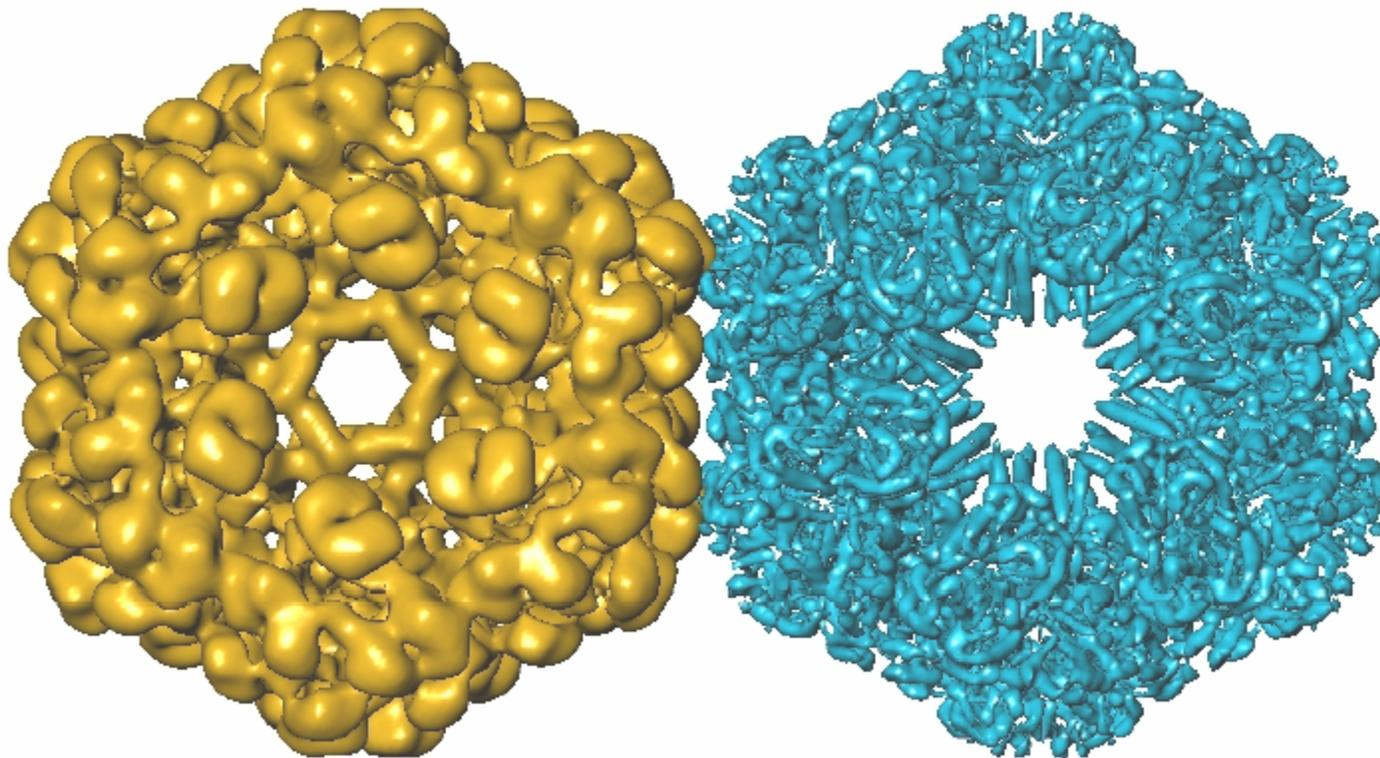*SSNR* = 1→
FSC = 1/3 = 0.333

Using FSC to Cross-Validate EM Map
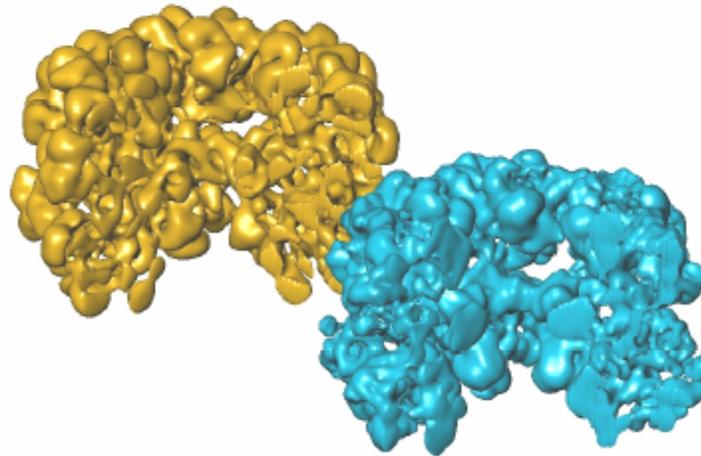

EM structure ⟷ FSC ⟷ X-ray crystallographic structure
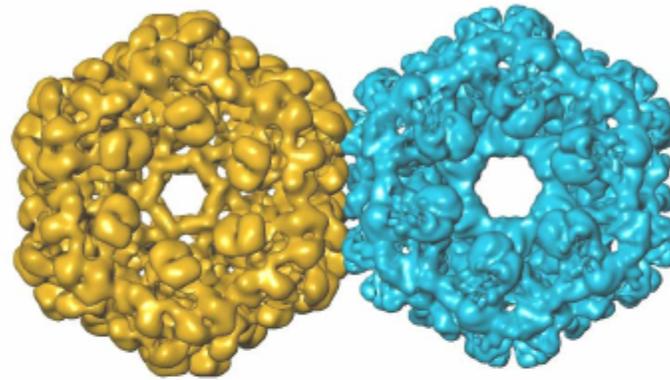electron density map, the voxel values are proportional to the Coulomb potentials of atoms
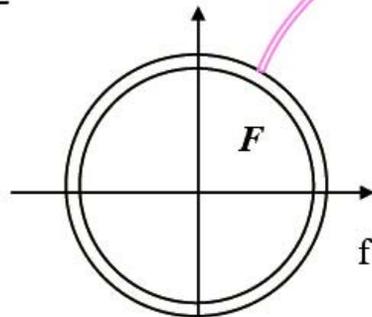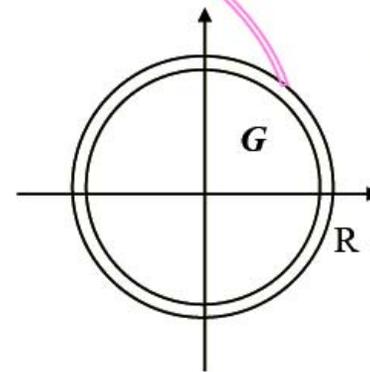
Using FSC to Cross-Validate EM Map

Using FSC to Cross-Validate EM Map

Cross-resolution:

X-ray map F (noise-free)
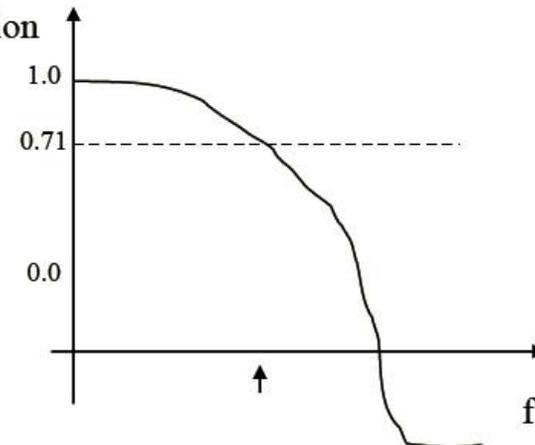
EM map G (corrupted by noise and other errors)

F

G

f

R

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

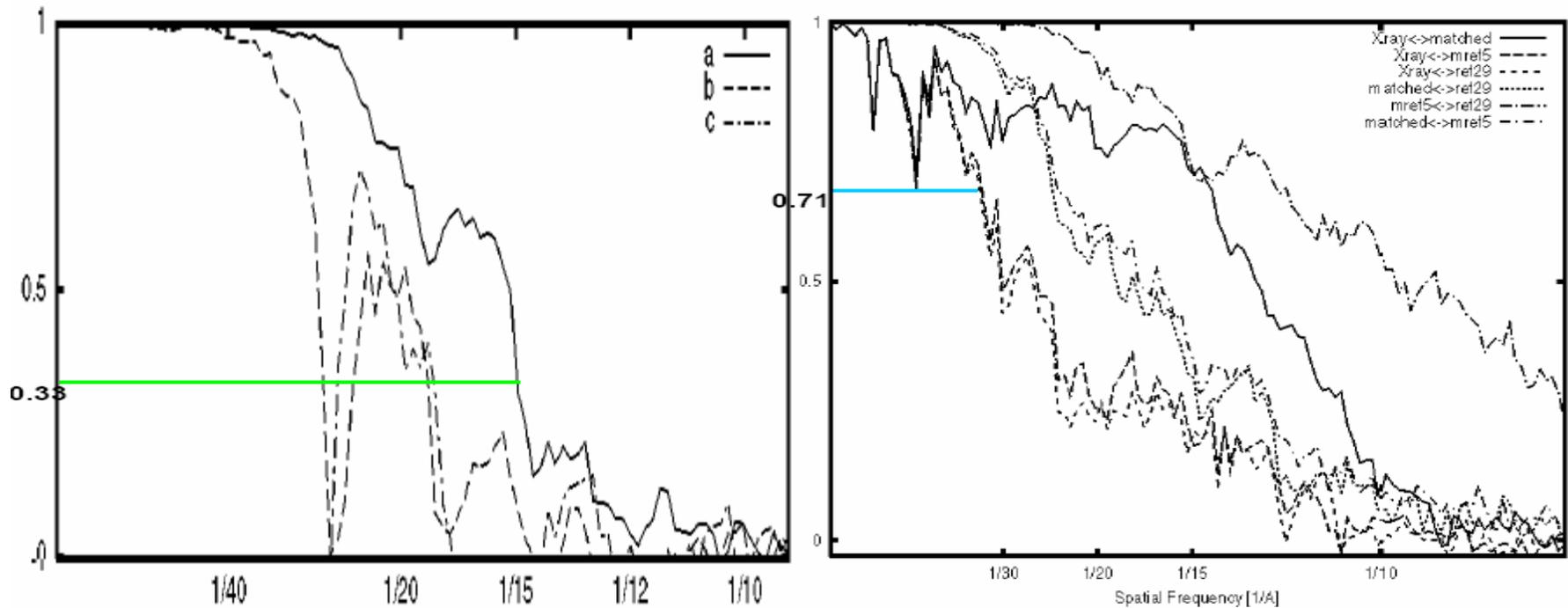Crossresolution

1.0

0.71

0.0

f

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

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

Using FSC to Cross-Validate EM Map

resolution *vs.* cross-resolution

Summary (Resolution Estimation)

The concept of optical resolution is not applicable to electron microscopy and single particle analysis.

• The resolution measures in EM estimate the "internal consistency" of the results. The outcome is prone to errors. The existing resolution measures cannot distinguish between "true" signal and the aligned (correlated) noise component in the data.

• FSC and SSNR are mathematically largely equivalent, although theSSNR-based estimate of the spectral signal to noise ratio has lower statistical uncertainty than the FSC-based estimate.

• The SSNR should be used whenever the number of the input projections is too small to make the division into halves possible (tomography).

• A reasonable resolution criterion should be based on the SSNR inthe data and set such that the Fourier coefficients with a dominant noise component are excluded from the final analysis. For example, SSNR=1 => FSC=0.333.

Resources

Textbook:

Chapter 5 in: Joachim Frank, Three-Dimensional Electron Microscopy of Macromolecular Assemblies (1996, Academic Press)

WWW:

- http://scienceworld.wolfram.com/physics/FraunhoferDiffractionCircularAperture.html
- http://www.olympusfluoview.com/java/resolution3d

Article:

Penczek, P. A., Three-dimensional Spectral Signal-to-Noise Ratio for a class of reconstruction algorithms. J. Struct. Biol., **138**, 34-46 (2002)