Image Assessment

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Correlation coefficient

definition and properties

(Pearson's *r*)

$$r_{xy} = \frac{\langle (x - \langle x \rangle)(y - \langle y \rangle) \rangle}{\sigma_x \sigma_y} = \frac{\langle xy \rangle - \langle x \rangle \langle y \rangle}{\sigma_x \sigma_y}$$

$$\sigma_x^2 = \langle (x - \langle x \rangle)^2 \rangle = \langle x^2 \rangle - \langle x \rangle^2$$

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

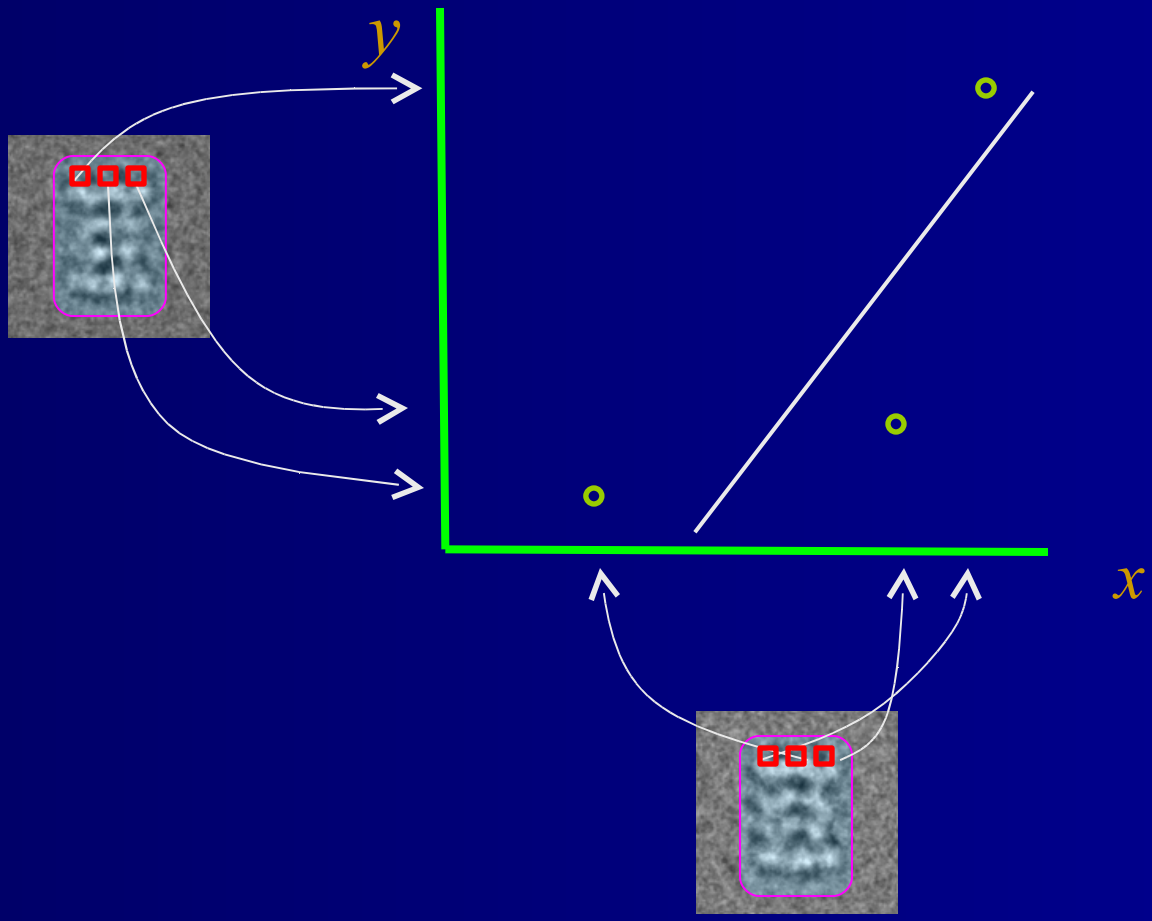
$$-1 \le r_{xy} \le 1$$

$$\sigma_x^2 = \frac{\sum x^2}{N} - m_x^2$$

$$m_x = \frac{\sum x}{N}$$

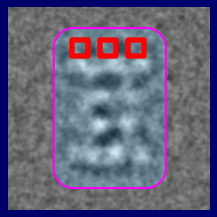
Correlation coefficient in image assessment

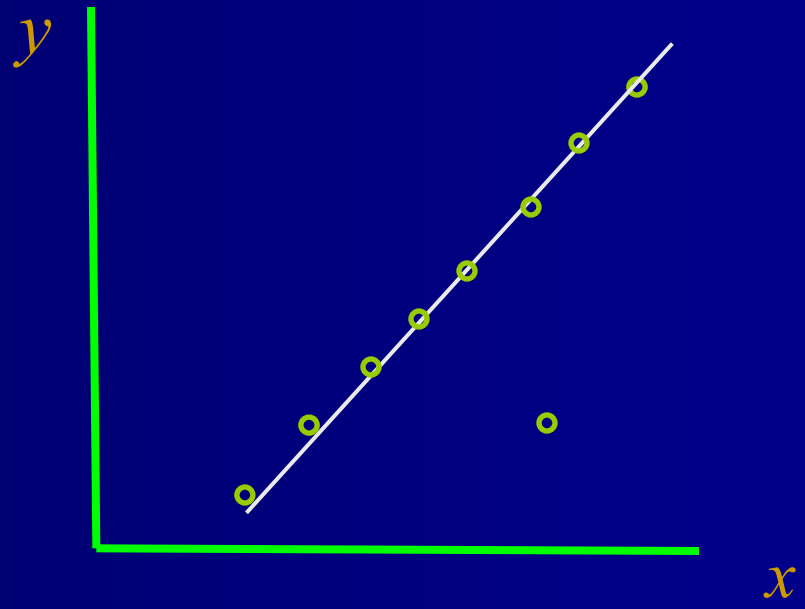
$$r_{xy} = \frac{\frac{1}{N}\sum xy - m_x m_y}{\sigma_x \sigma_y}$$



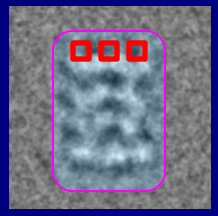
Correlation coefficient in image assessment

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



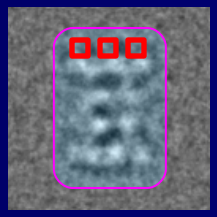


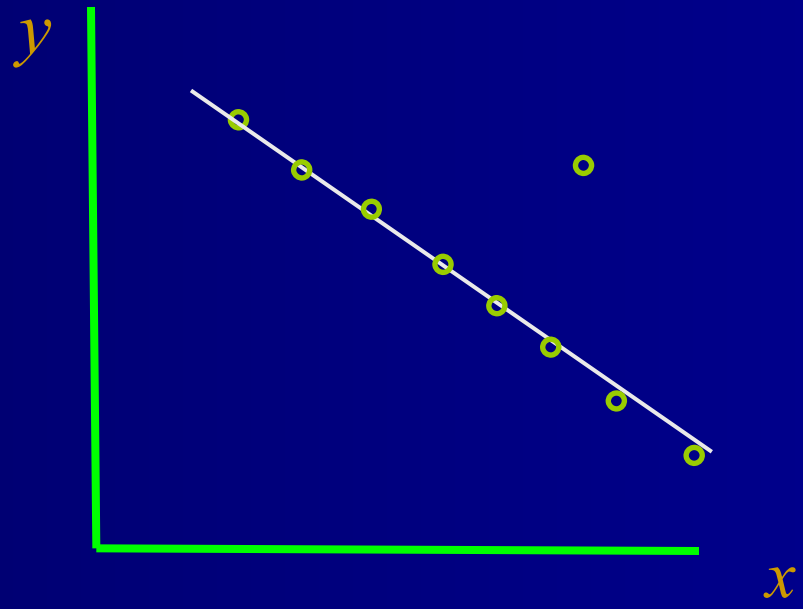
r=0.99



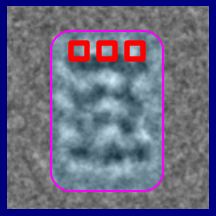
Correlation coefficient in image assessment

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



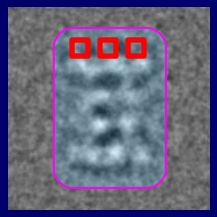


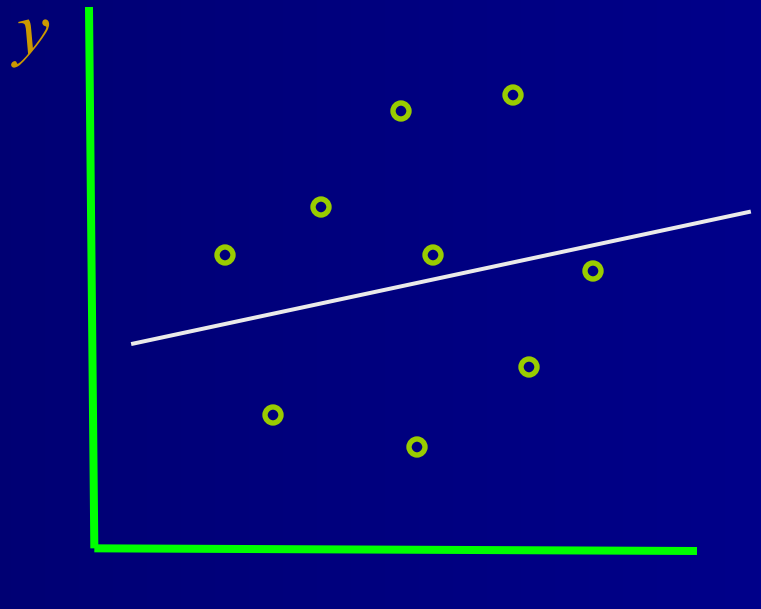
r=-0.99



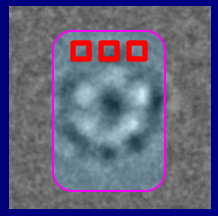
Correlation coefficient in image assessment

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





r=0.11



Correlation coefficient in image assessment

r^2 - proportion of the variance accounted for by the linear model

r		r^2
1.00	perfect linear relation	1.00
0.71		0.50
0.50		0.25
0.33		0.10
0.00	no linear relation	0.00
-1.0	inverted contrast	1.00

Correlation coefficient
statistical significance

r is calculated from a sample of n pairs of numbers

Fisher (1921):

$$z = \frac{1}{2}\log\frac{1+r}{1-r} \quad \in \quad N\left(\zeta = \frac{1}{2}\log\frac{1+\rho}{1-\rho}, 1/\sqrt{n-3}\right)$$

Type I error: rejection of the null hypothesis when is true.
The risk of Type I error is α, the *significance* level.
H_0 : $r = 0$.

Example: I calculated $r = 0.15$ with $n = 200$.
I can reject the null hypothesis on a 5% (0.14) significance level, but not on a 1% (0.18) significance level.
I can expect (tolerate) to be wrong 5 out of 100 times.

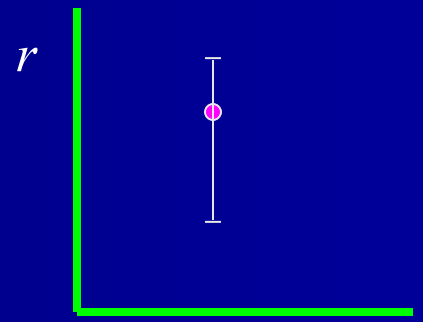
Correlation coefficient confidence interval

r is calculated from a sample of n pairs of numbers

$$z = \frac{1}{2}\log\frac{1+r}{1-r} \quad \in \quad N\left(\zeta = \frac{1}{2}\log\frac{1+\rho}{1-\rho} + \frac{\rho}{n},\ 1/\sqrt{n-3}\right)$$

Confidence intervals of z at 100(1-α)% are

$$z \pm q_{\alpha/2}\frac{1}{\sqrt{n-3}}$$



for $\alpha = 0.05, \quad q_{\alpha/2} = 1.96,$ so the confidence limits are $z \pm \frac{1.96}{\sqrt{n-3}}$

Confidence intervals of r : transform back using

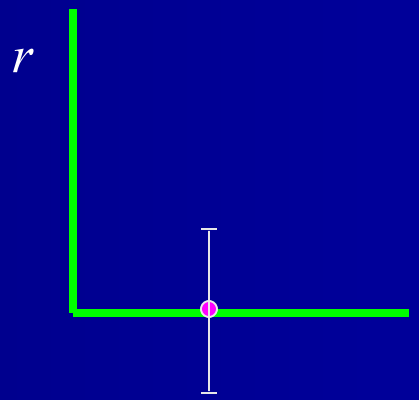
$$r = \frac{e^{2z}-1}{e^{2z}+1}$$

Correlation coefficient confidence interval

r is calculated from a sample of n pairs of numbers

If ρ=0, r has approximately normal distribution

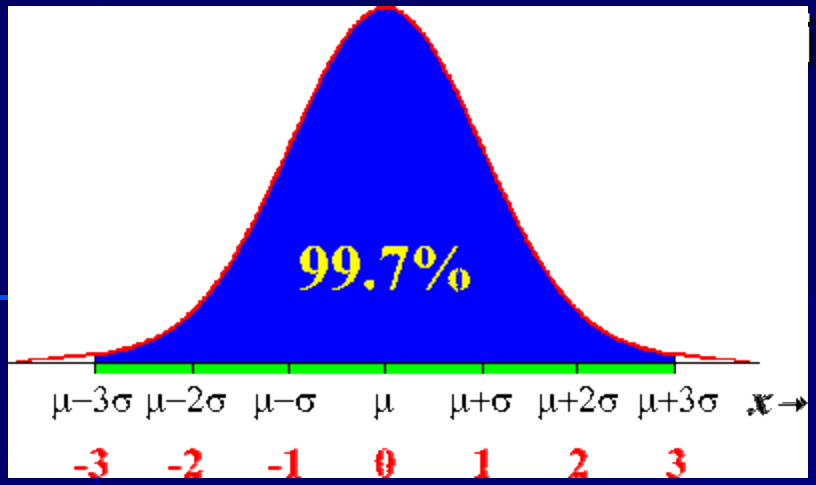
$$N\left(0, \frac{1}{\sqrt{n}}\right)$$



Confidence limits :

$$\pm q_{\alpha/2} \frac{1}{\sqrt{n}}$$

Correlation coefficient interval



$$z \pm q_{\alpha/2} \frac{1}{\sqrt{n-3}}$$

(1) 68% of observations fall within σ of μ.
(2) 95% of observations fall within 2σ of μ.
(3) 99.7% of observations fall within 3 σ of μ.

Physicists standard deviation σ	*Statisticians* significance level α
1.04	30%
1.96	5%
3.00	2.6%
3.09	2%
3.29	1%
5.00	0.00006%

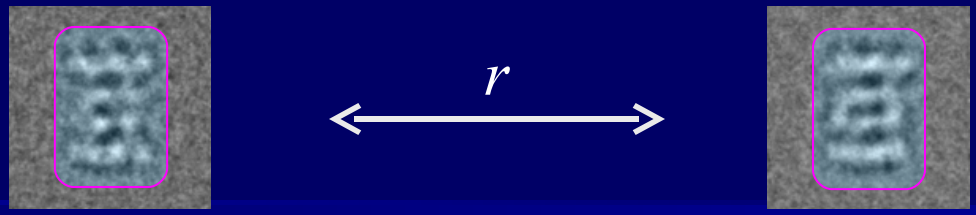
Signal-to-Noise Ratio (SNR)

$$\text{SNR} = \frac{\text{Power of signal}}{\text{Power of noise}} = \frac{\text{Variance of signal}}{\text{Variance of noise}}$$

means of signal and noise are both zero

Correlation coefficient

relation to Signal-to-Noise Ratio in the image



$$x_k = S_k + N_k \qquad x_k = S_k + M_k$$

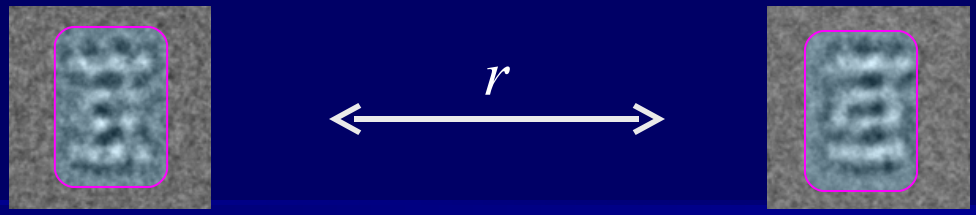
$$N_k,\ M_k \in N(0,\sigma), \qquad \langle N_k, N_l \rangle_{k \neq l} = 0, \qquad \langle N_k, M_l \rangle = 0, \qquad \langle N_k, S_l \rangle = 0, \qquad \sum_k S_k = 0,$$

$$r_{xy} = \frac{\sum xy}{\left(\sum x^2\right)^{1/2}\left(\sum y^2\right)^{1/2}}$$

$$\langle r_{xy} \rangle \cong \frac{\sum S_k^2}{\sum S_k^2 + \sum \sigma^2} = \frac{\frac{\sum S_k^2}{\sum \sigma^2}}{\frac{\sum S_k^2}{\sum \sigma^2} + 1} = \frac{SNR}{SNR + 1}$$

Correlation coefficient

relation to Signal-to-Noise Ratio in the image



$$\rho = \frac{SNR}{SNR + 1}$$

$$SNR = \frac{\rho}{1 - \rho}$$

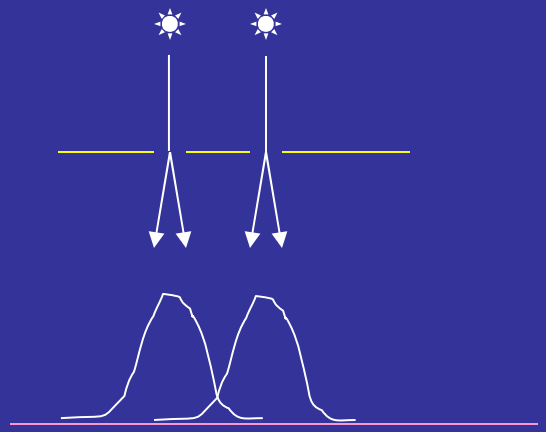
Correlation coefficient
properties

- Correlation coefficient is a measure of linear relationship between two variables
- The values of the correlation coefficient are between -1 and 1
- Correlation coefficient is invariant with respect to linear transformations of the data
- The value of the squared correlation coefficient corresponds to the proportion of the variance accounted for by the linear model
- For $\rho=0$, the distribution of the correlation coefficient is approximately normal with $\sigma=1/\mathrm{sqr}(n)$
- Using Fisher's transformation it is possible to calculate confidence intervals for any *r*
- Using correlation coefficient it is possible to calculate SNR in images

Fourier Shell Correlation

WHAT DOES IT HAVE TO DO WITH RESOLUTION?!?

Optical resolution



The resolution of a microscope objective is defined as the smallest distance between two points on a specimen that can still be distinguished as two separate entities.

Resolution is a somewhat subjective concept.

The theoretical limit of the resolution is set by the wavelength of the light source:

$$R = \text{const}\ \lambda$$

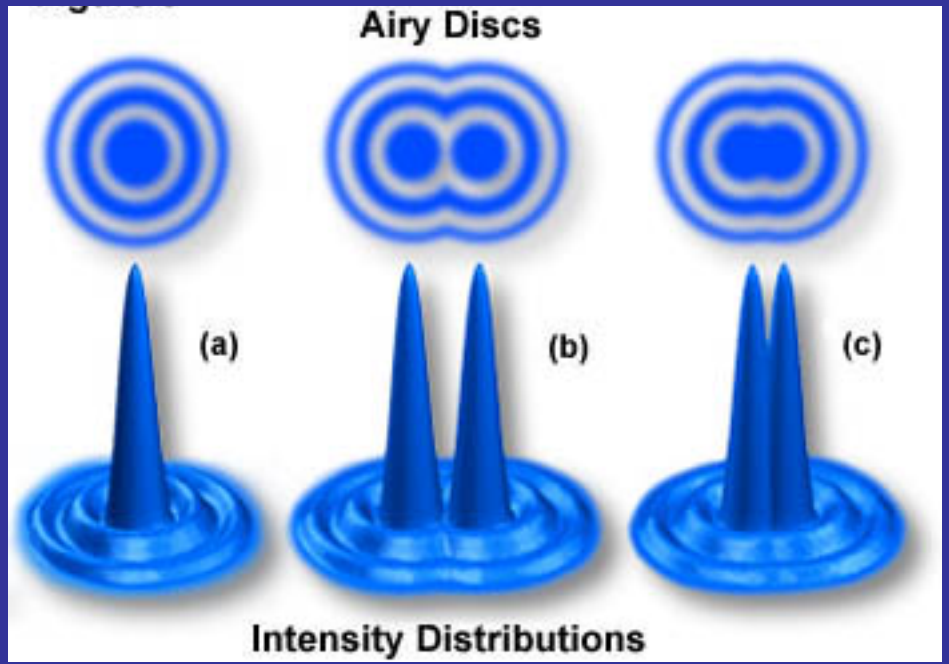
Optical resolution

Hypothetical ***Airy disk*** (a) consists of a diffraction pattern containing a central maximum (typically termed a zero'th order maximum) surrounded by concentric 1st, 2nd, 3rd, etc., order maxima of sequentially decreasing brightness that make up the intensity distribution.

If the separation between the two disks exceeds their radii (b), they are resolvable.

The limit at which two Airy disks can be resolved into separate entities is often called the ***Rayleigh criterion***.

When the center-to-center distance between the zero'th order maxima is less than the width of these maxima, the two disks are not individually resolvable by the Rayleigh criterion (c).



Resolution-limiting factors in electron microscopy

- The wavelength of the electrons
  (depends on the voltage: 100kV - 0.037 Å; 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

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

- In single particle reconstruction, there is no “external” standard by which the resolution of the results could be evaluated.
- Resolution measures in EM have to estimate “internal consistency” of the results.
- Unless an external standard is provided, objective estimation of the resolution in EM is not possible.

FRC - Fourier Ring Correlation

Saxton W.O. and W. Baumeister.
The correlation averaging of a regularly arranged bacterial cell envelope protein.
J. Microsc., **127**, 127-138 (1982).

FSC – Fourier Shell Correlation (3-D)

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)

2-D & 3-D

Q-factor

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 (Ed.: W. Baumeister).
Springer Verlag, Berlin (1980).

only 2-D

Fourier Ring Correlation

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

A. either:
1. Split (randomly) the data set of available images into halves;
2. Perform the alignment of each data set “independently”;

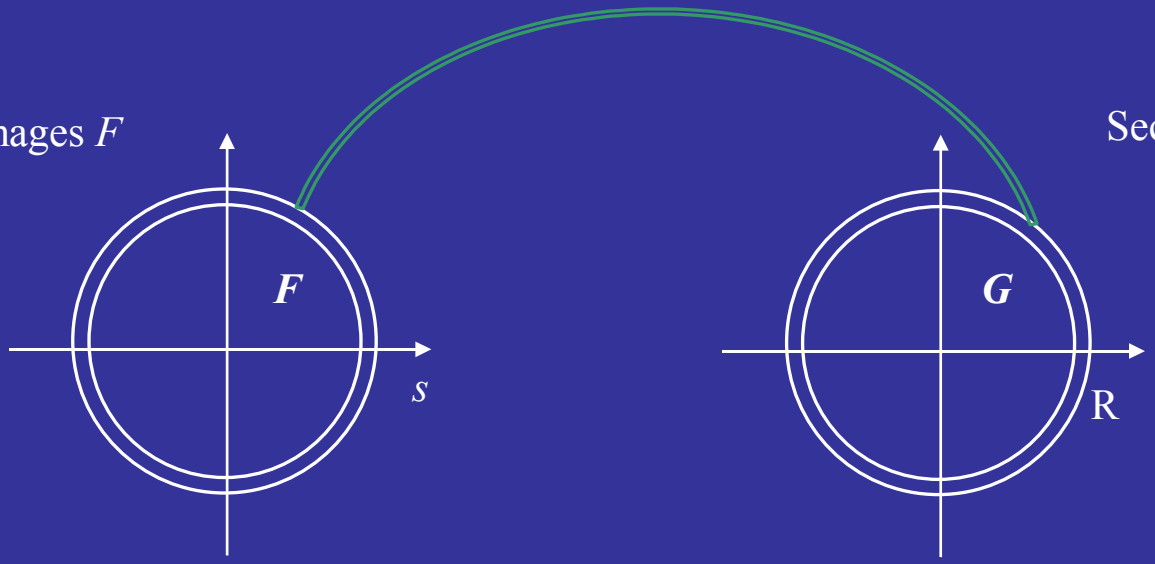
B. or:
1. Perform the alignment of the whole data set;
2. Split (randomly) the aligned data set into halves;
3. Calculate two averages (3-D reconstructions);
4. Compare the averages in Fourier space by calculating the FRC.

WARNINGS - method B valid *only* if the noise component in the data is independent (not aligned)
- the two sets in method A might not be as independent as one assumes.

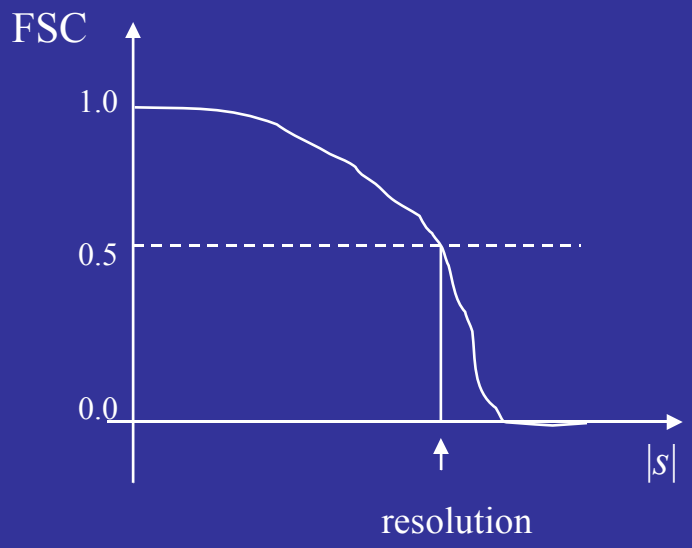
Fourier Shell Correlation
FSC

First set of images *F*

Second set of images *G*



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



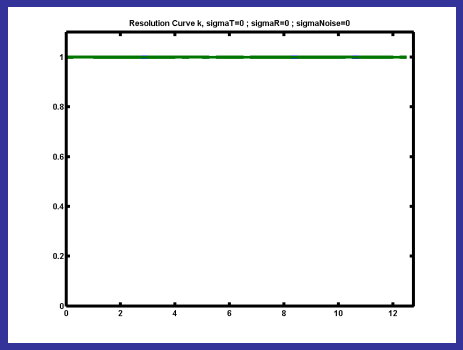
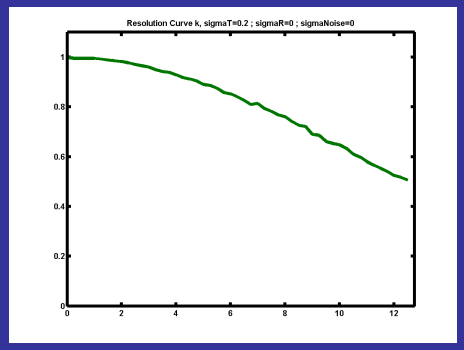
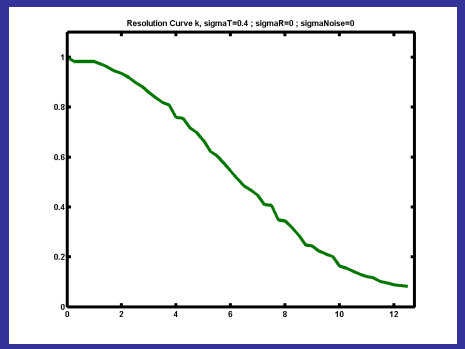
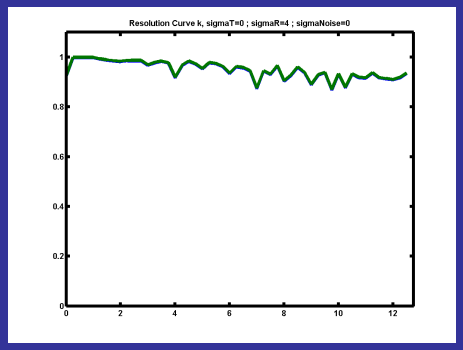
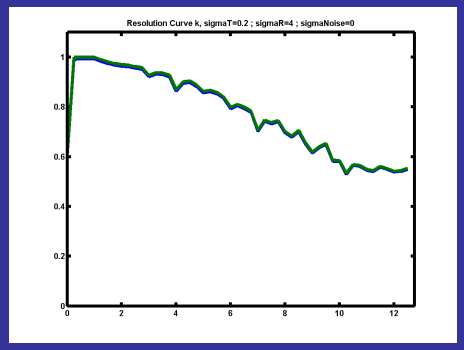
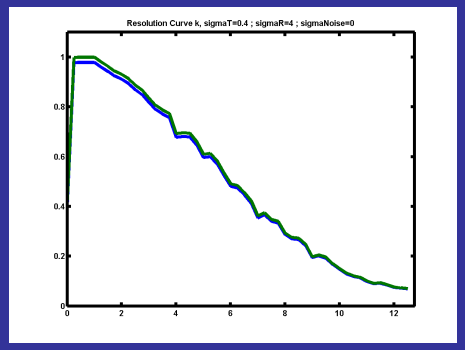
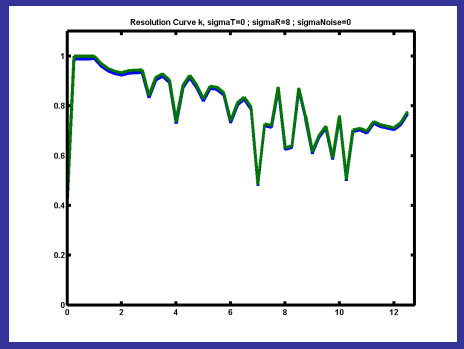
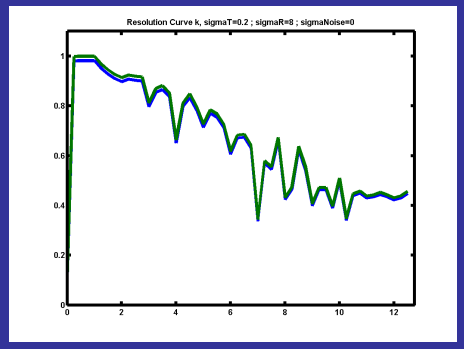
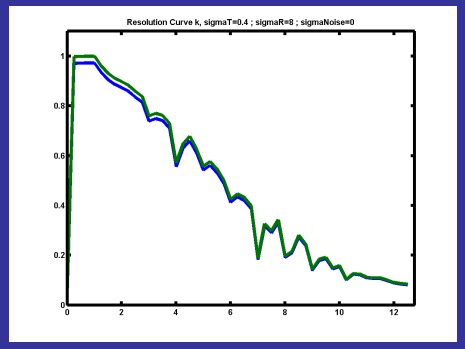
Fourier Shell Correlation

WHY DOES IT WORK?

FSC provides a measure of the Spectral Signal-to-Noise Ratio in the reconstruction.

FSC is directly related to alignment errors.

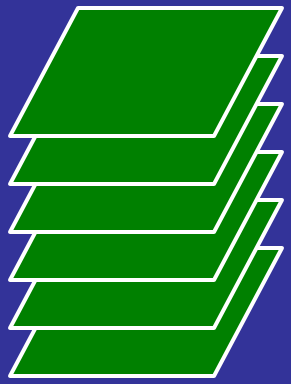
Impact of alignment errors on FRC curves

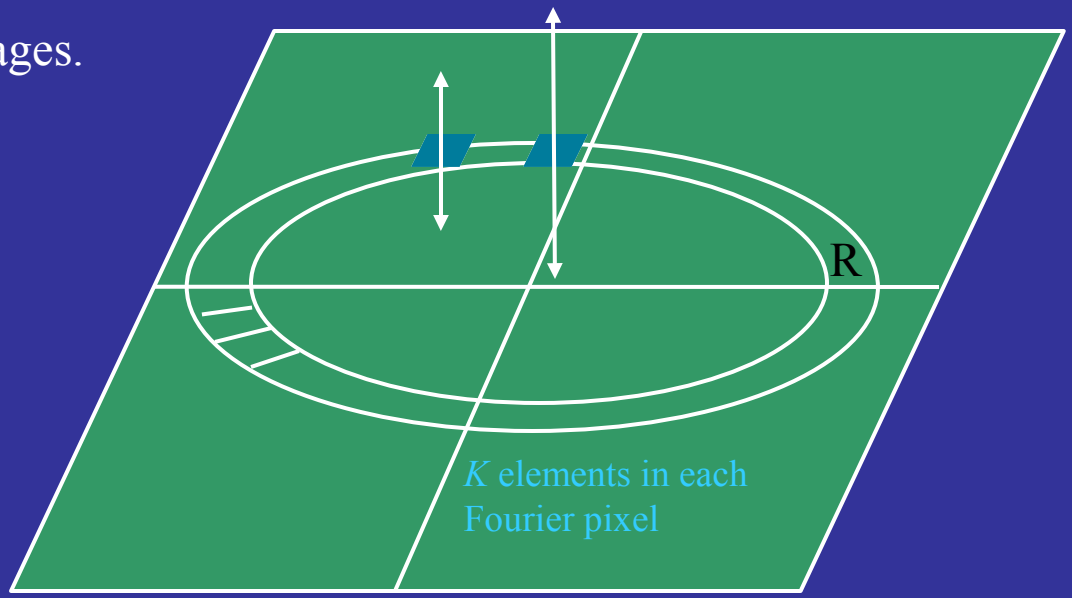
	σ_T=0	σ_T=0.2	σ_T=0.4
σ_R=0			
σ_R=4 degrees			
σ_R=8 degrees			

Baldwin, P.R. and Penczek P.A.: Estimating alignment errors in sets of 2-D images. *JSB* 150 211, 2005.

Spectral Signal-to-Noise Ratio (SSNR) in 2D

A set of Fourier transforms of 2D images.

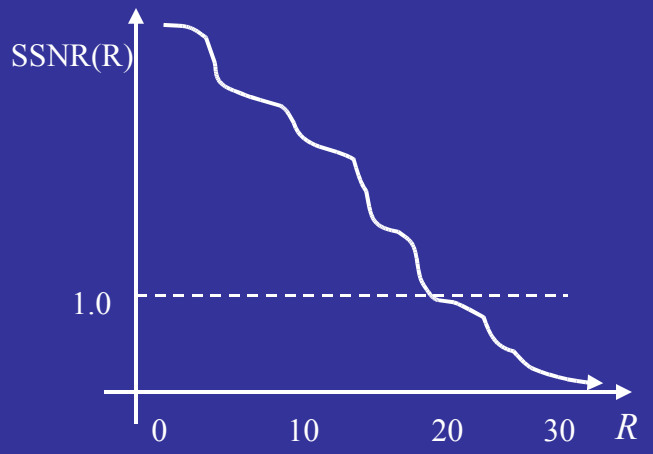




Calculate SSNR according to the equation:

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

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



Relations between FSC and SSNR

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

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.

The expectation value of FRC is calculated as

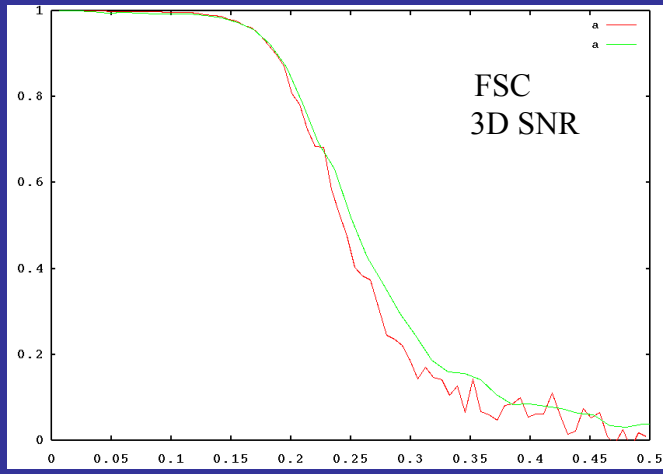
$$E[\mathrm{FRC}] \cong \frac{E\left[\sum_{n=1}^{n_R} F^n G^{n^*}\right]}{E\left[\left\{\left(\sum_{n=1}^{n_R} |F^n|^2\right)\left(\sum_{n=1}^{n_R} |G^n|^2\right)\right\}^{1/2}\right]}$$

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

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 => *FSC*=1/3=0.333

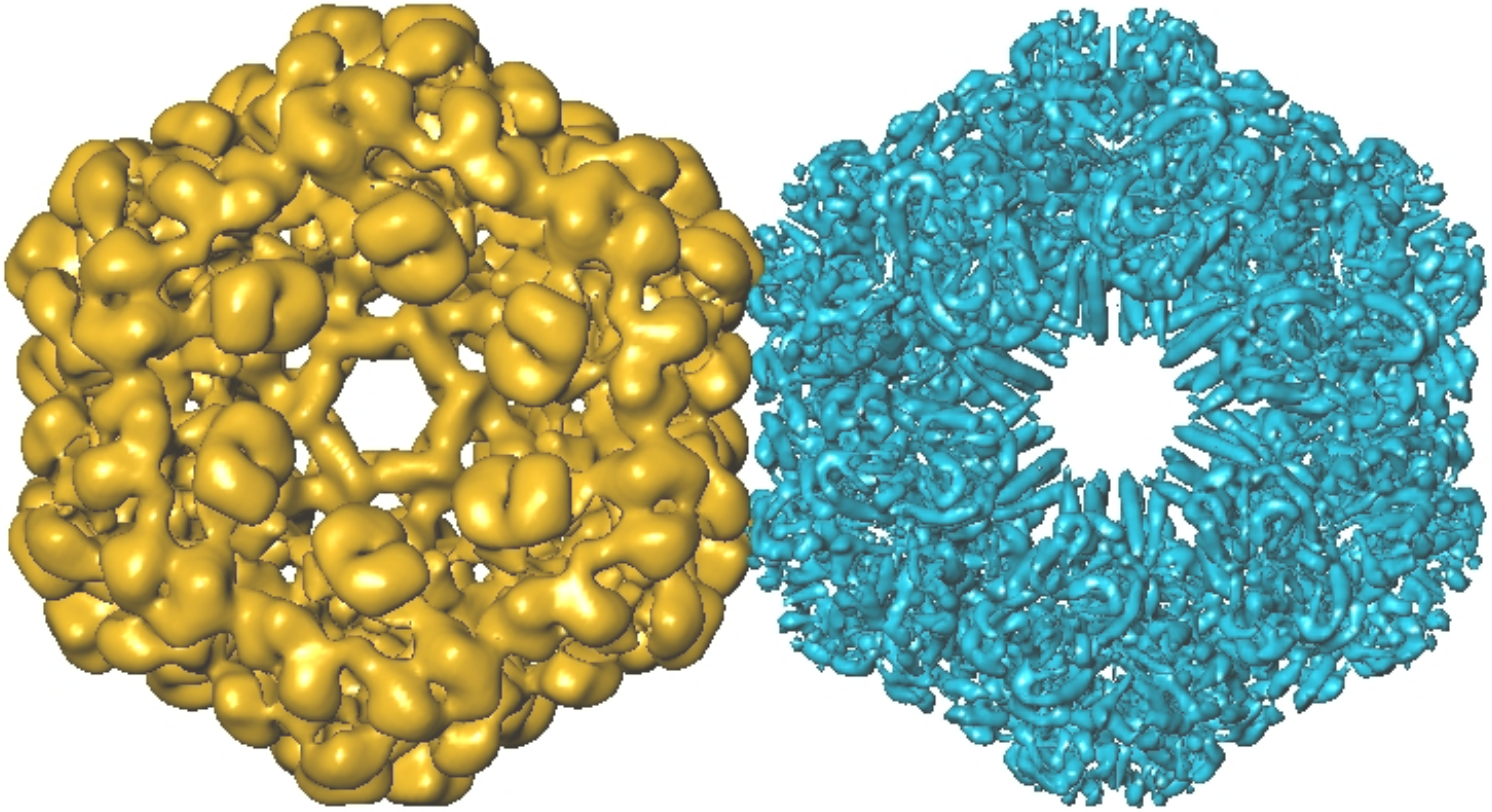
Another criterion: (3σ) include Fourier information that is significantly higher than zero, i.e., *SSNR*>0.
SSNR=0 => *FSC*=0

FSC can be used to cross-validate EM results

(**crossresolution**)

EM structure ⟷ **FSC** X-ray crystallographic structure

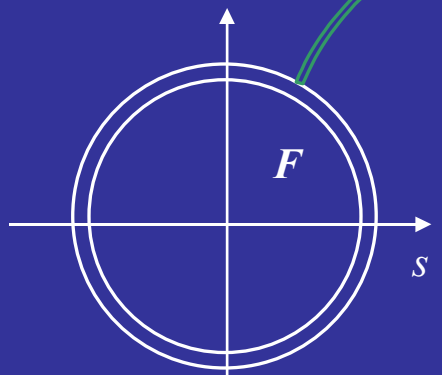
electron density map, the voxel values are proportional to the Coulomb potentials of atoms



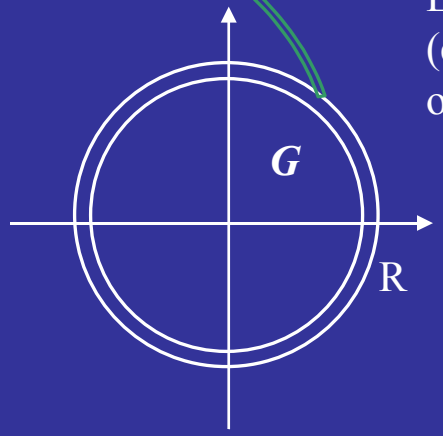
Cross-resolution

relation between FRC and SSNR

X-ray map F
(noise-free)

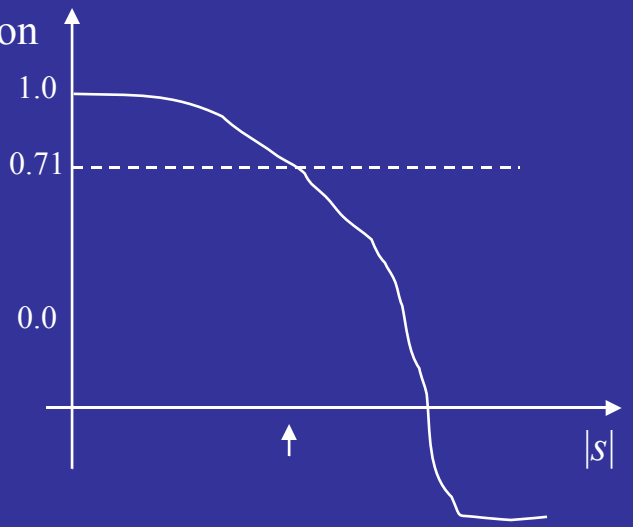


EM map G
(corrupted by noise and other errors)



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

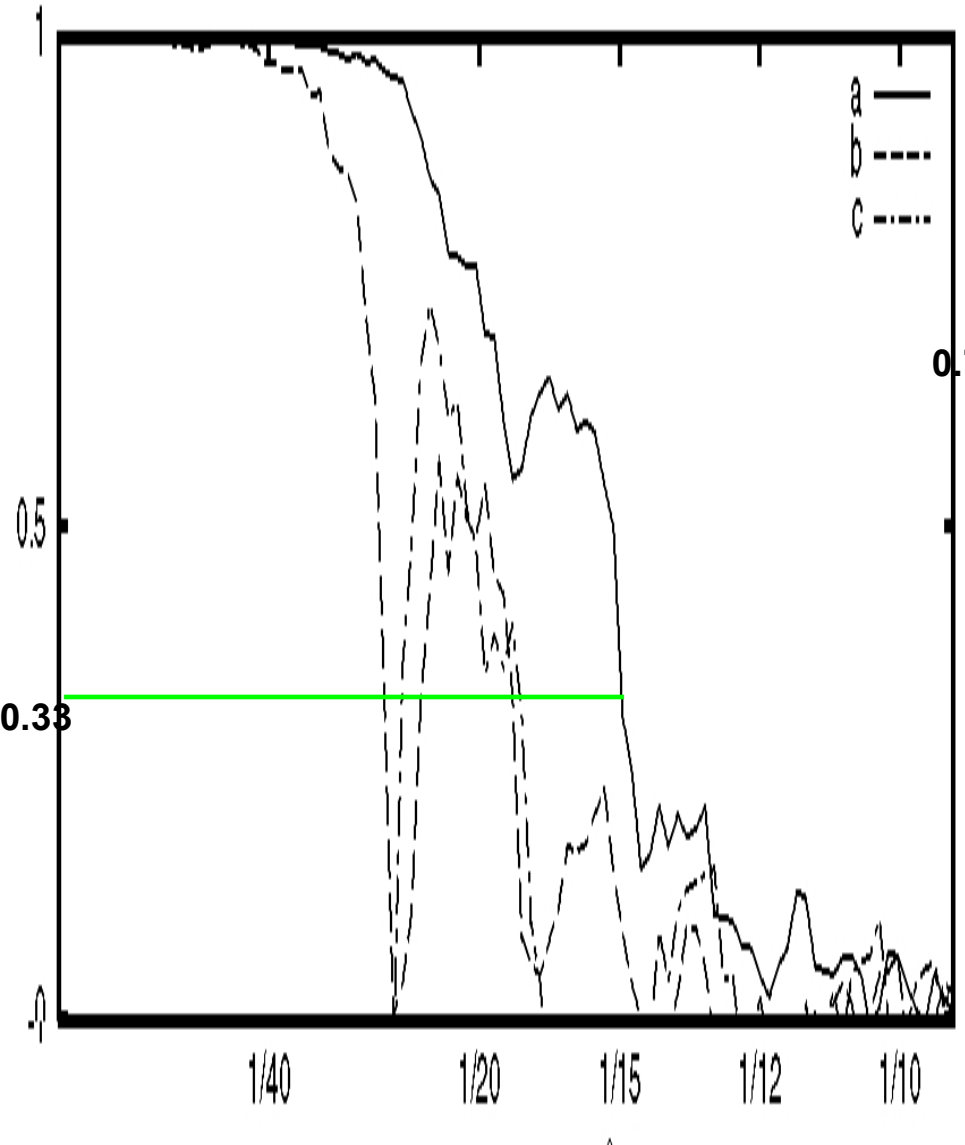
Cross-resolution

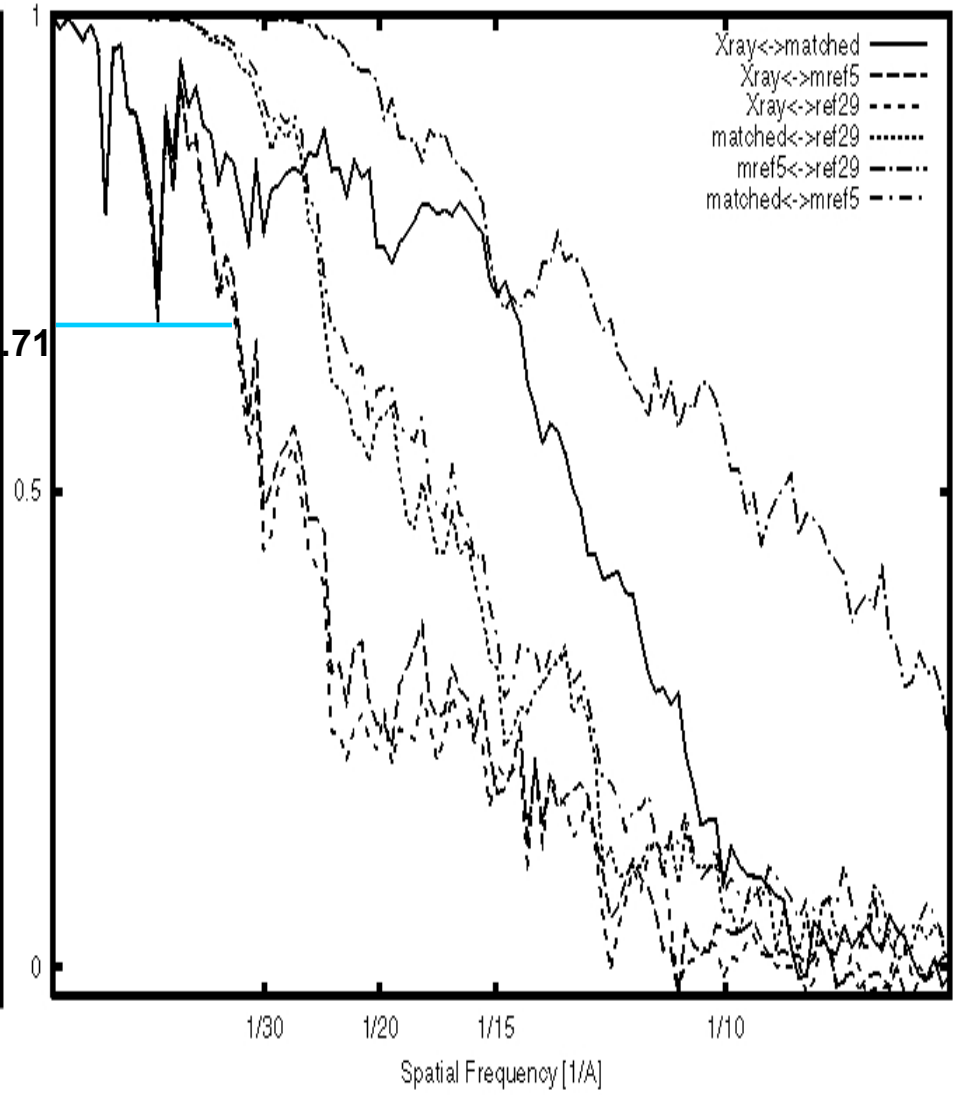


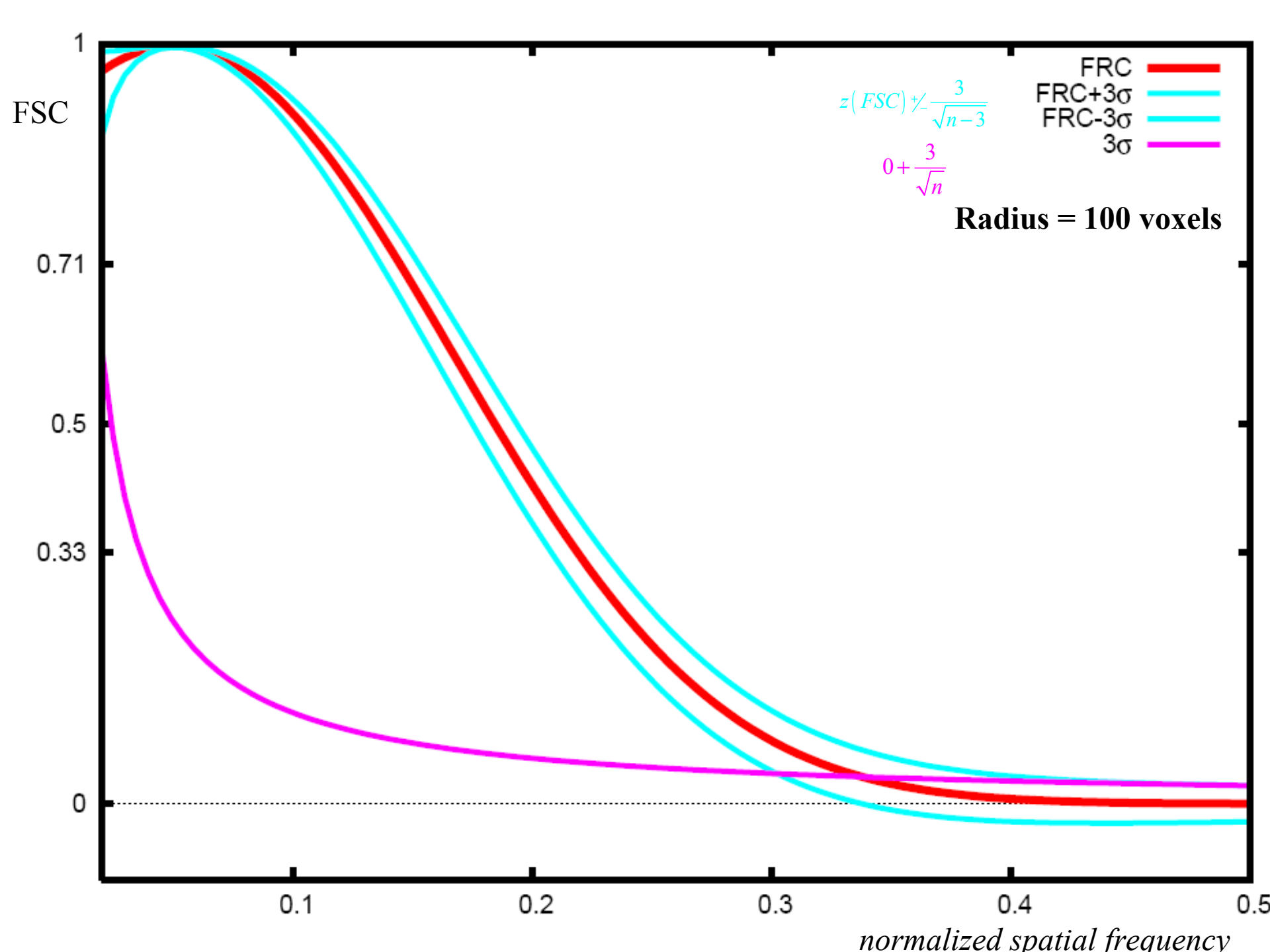
$$SSNR = \frac{FSC^2}{1 - FSC^2}$$

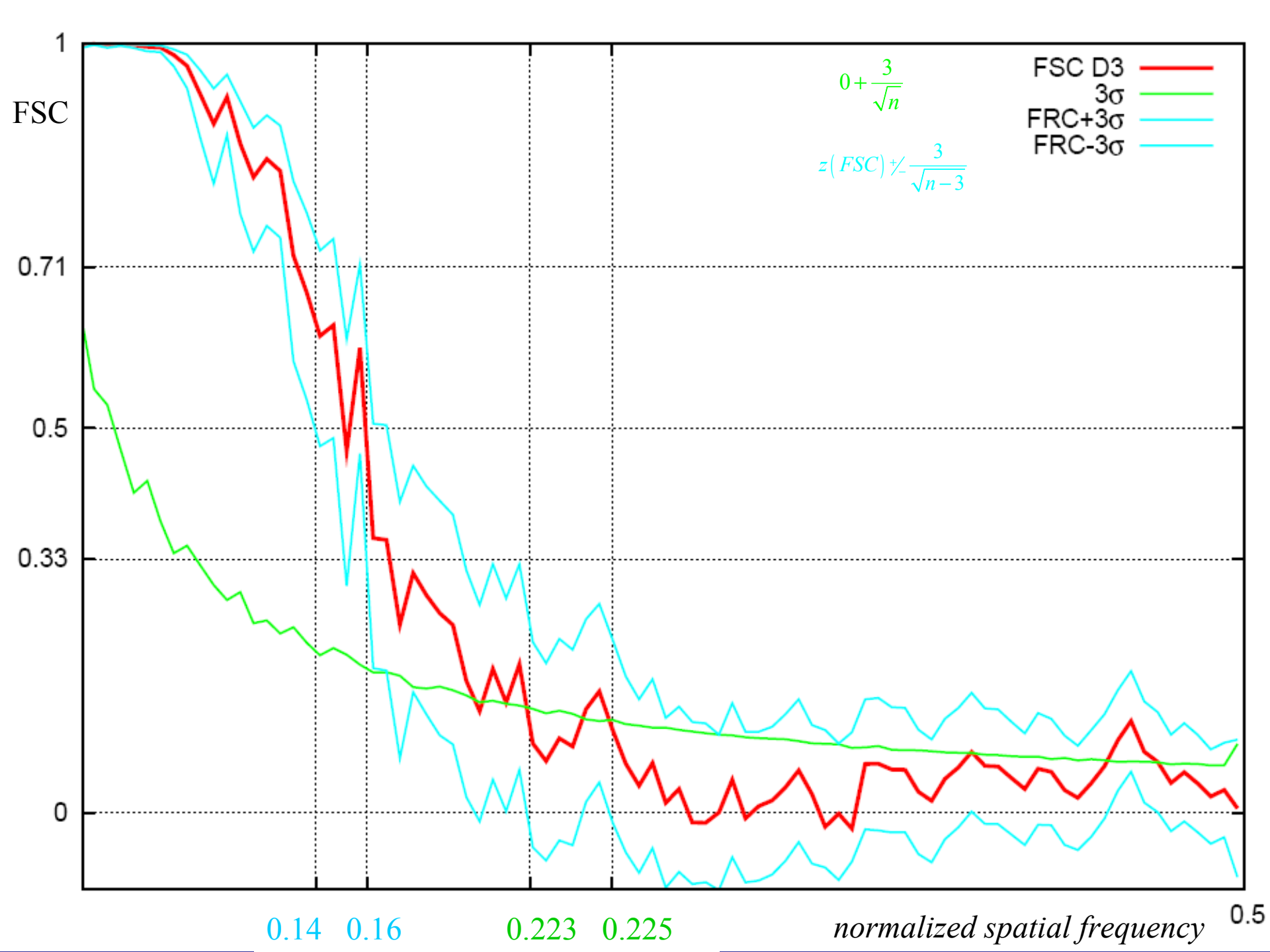
$$SSNR = 1 \quad \Rightarrow \quad FSC = \sqrt{1/2} = 0.71$$

Resolution *versus* cross-resolution



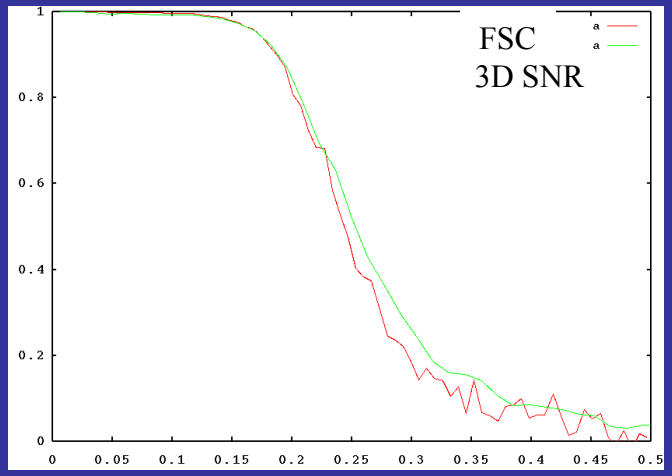



FSC
1
0.71
0.5
0.33
0
0.1
0.2
0.3
0.4
0.5
normalized spatial frequency
FRC
FRC+3σ
FRC-3σ
3σ
$z(FSC) +/- \frac{3}{\sqrt{n-3}}$
$0 + \frac{3}{\sqrt{n}}$
Radius = 100 voxels


1
FSC
0.71
0.5
0.33
0
$0+\frac{3}{\sqrt{n}}$
$z(FSC)\pm\frac{3}{\sqrt{n-3}}$
FSC D3
3σ
FRC+3σ
FRC-3σ
0.14
0.16
0.223
0.225
0.5
normalized spatial frequency

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.

FSC – known problems

1. Number of independent voxels in Fourier space *n*

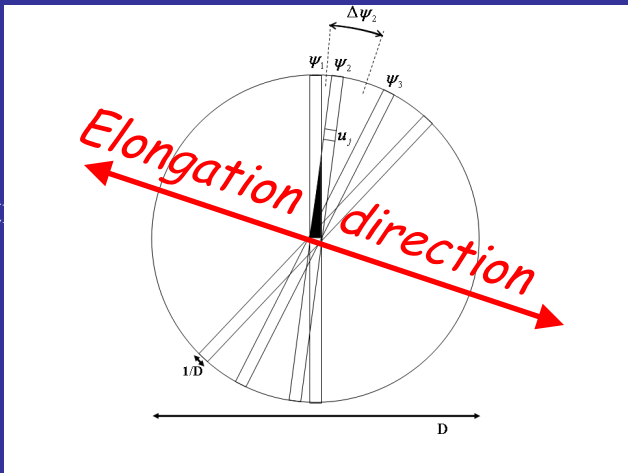
   Interpolation, scanning (image processing)
   Masking in real space (=convolution in F.space)
   Dependence on the box size
   Reduction of *n* due to symmetry (not included in the code)

   a. Oversample (pixel size 0.35 of that corresponding to expected resolution)
   b. Do not mask (particularly complicated shapes)
   c. Adjust box size
   d. Use higher cut-off (0.5)

2. Overabundand projections or gaps in angular coverage

   FSC curve impressive, but the structure visibly distorted (elongat

   Use 3D SSNR to check the anisortopy of resolution



3. Alignment of noise

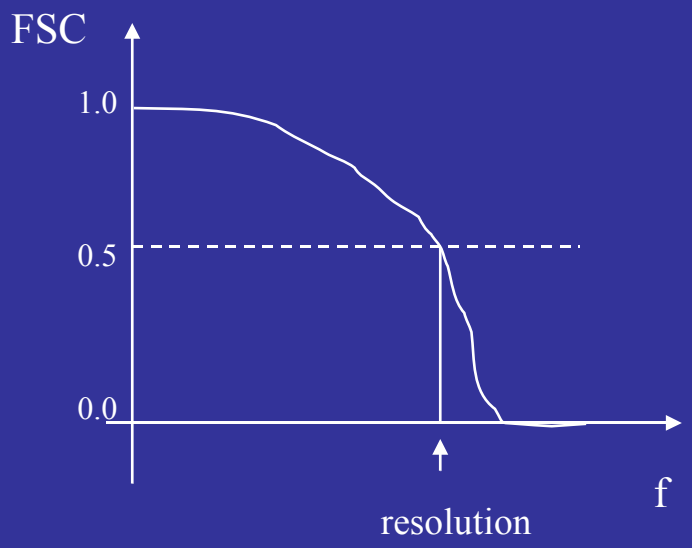
   a. Split dataset into halves and align independently

   b. Use matched filters

4. Not applicable when the number of projections is small (tomography!)

Examples of resolution curves

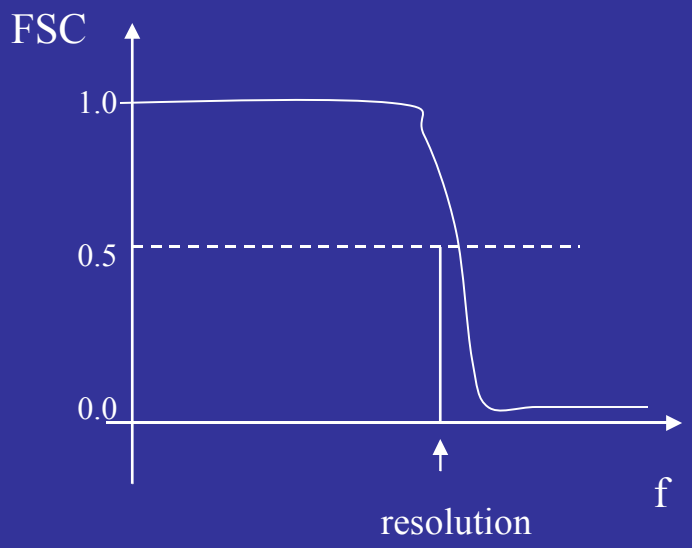
Healthy:



In low frequencies remains one, followed by a semi-Gaussian fall-off, drops to zero at around 2/3 of maximum frequency, in high frequencies oscillates around zero.

Examples of resolution curves

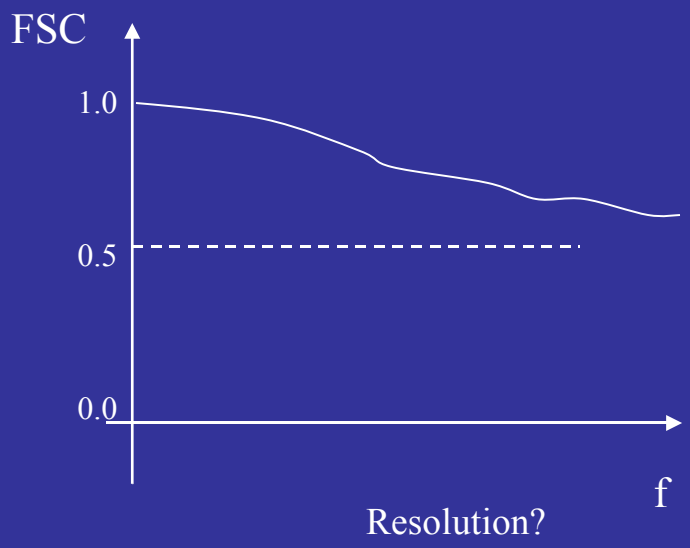
Unhealthy:



"Rectangular": in low frequencies remains one, followed by a sharp drop, in high frequencies oscillates around zero.
A combination of alignment of the noise and a sharp filtration during the alignment procedure. The result is fake.

Examples of resolution curves

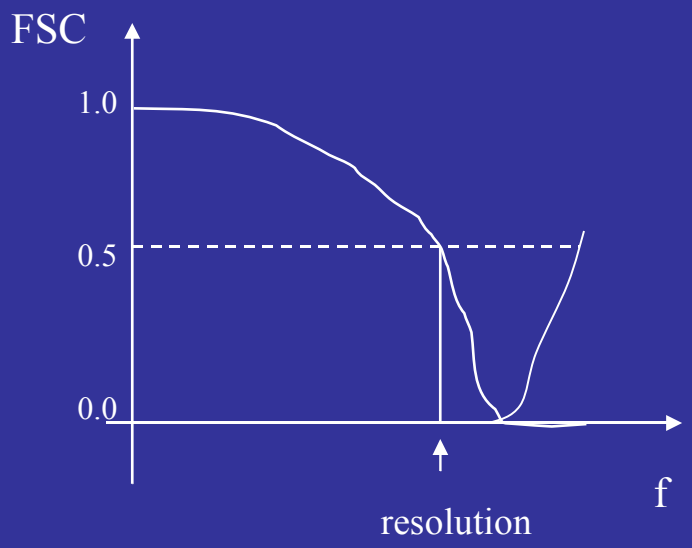
Unhealthy:



FSC never drops to zero in the whole frequency range.
The noise component in the data was aligned.
Non-linear operations applied (masking, thresholding).
The result is fake.
In rare cases it could mean that the data was severely undersampled (very large pixel size).

Examples of resolution curves

Unhealthy:

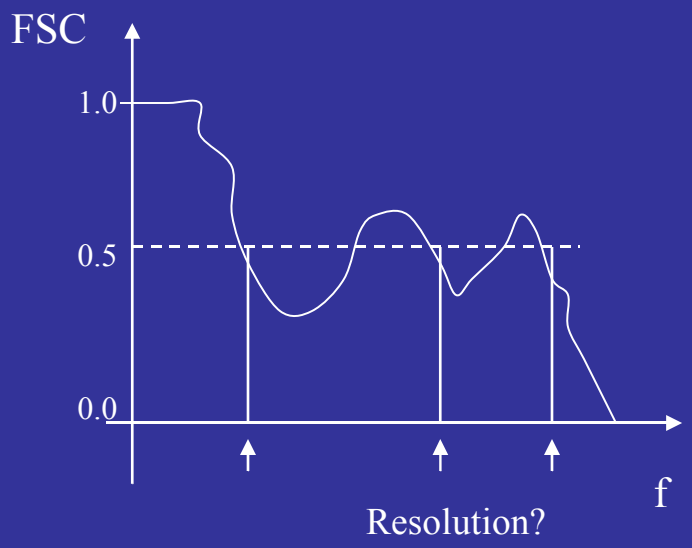


After it drops to zero, increases in high frequencies oscillation.

Data was low-passed filtered; errors in image processing code, mainly in interpolation; all images were rotated by the same angle.

Examples of resolution curves

Unhealthy:



FSC oscillates around 0.5.

The data is dominated by one subset with the same defocus value or there is only one defocus group. It is not incorrect per se, but unclear what is the resolution. Also, will result in artifacts.

Summary

- The concept of optical resolution is not applicable to electron microscopy and single particle reconstruction.
- 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 the SSNR-based estimate of the spectral signal to noise ratio has lower statistical uncertainty than the FSC-based estimate.
- A reasonable resolution criterion should be based on the SSNR in the data and set such that the Fourier coefficients that are dominate by noise are excluded from the final analysis. For example, SSNR=1 => FSC=0.333.
- Confidence interval for FSC curves can be given if he number of independent voxels in the reconstruction could be known.
- The shape of the FSC curve defines an optimum filter for the average/reconstruction.