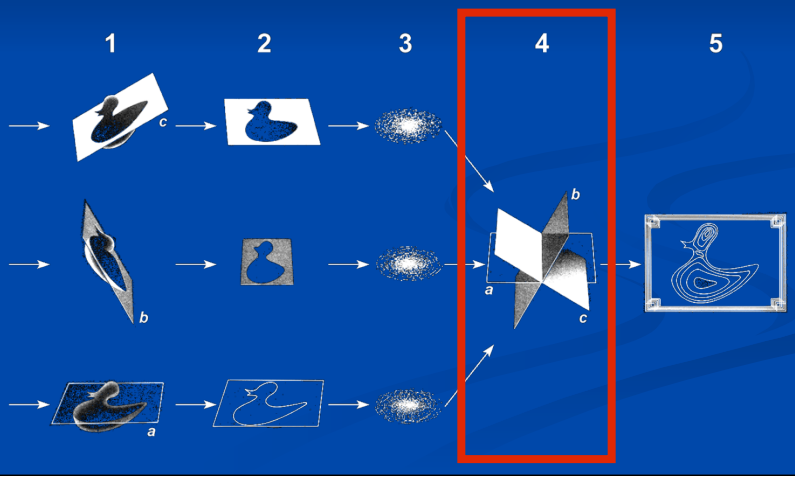
Merging with the MRC software

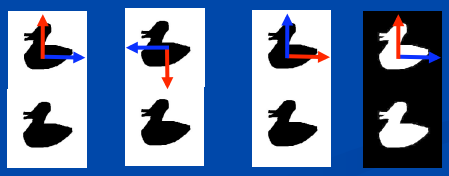
- Merging multiple images to common phase origin & scale
- Lattice line fitting



ORIGTILT program

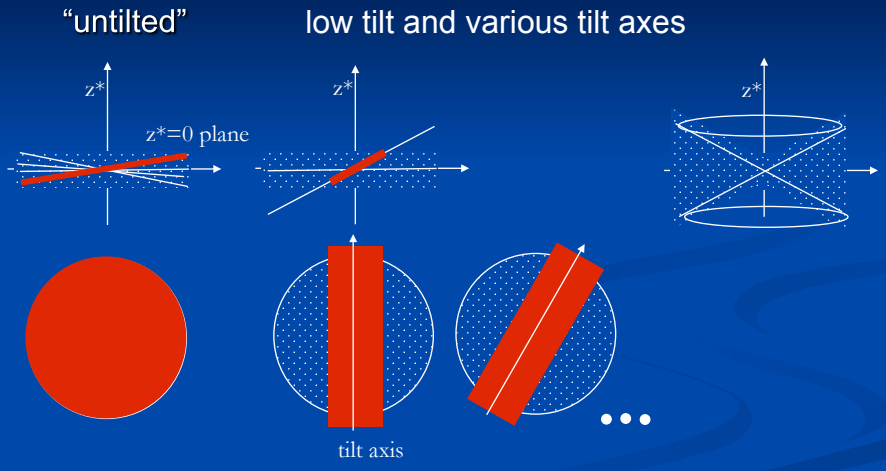
- Move the projection images (i.e., phase origins) to a common center and compatible orientation

- Reference
  - Symmetry special position
  - Last merged images on the fly
  - Last set of merged image
  - Last calculated 3D model

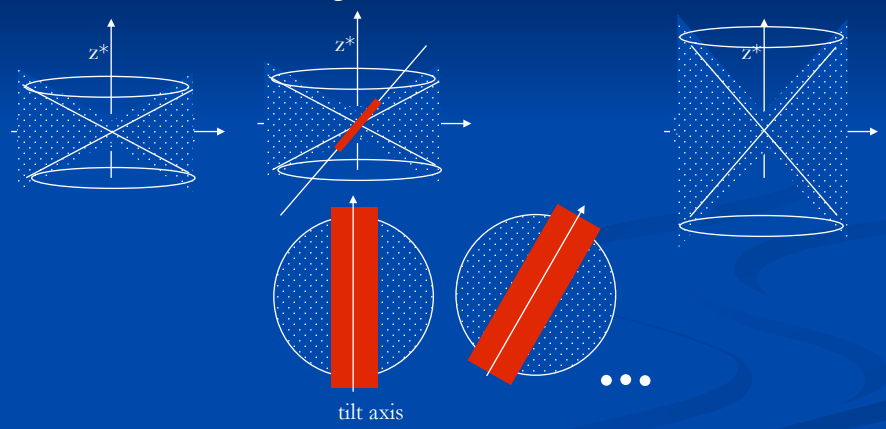


- Refine
  - Phase origin
  - Tilt geometry
  - Beam Tilt
- Standardize a, b axises
  - Rotation
  - Flip crystal upside down
  - Defocus inversion

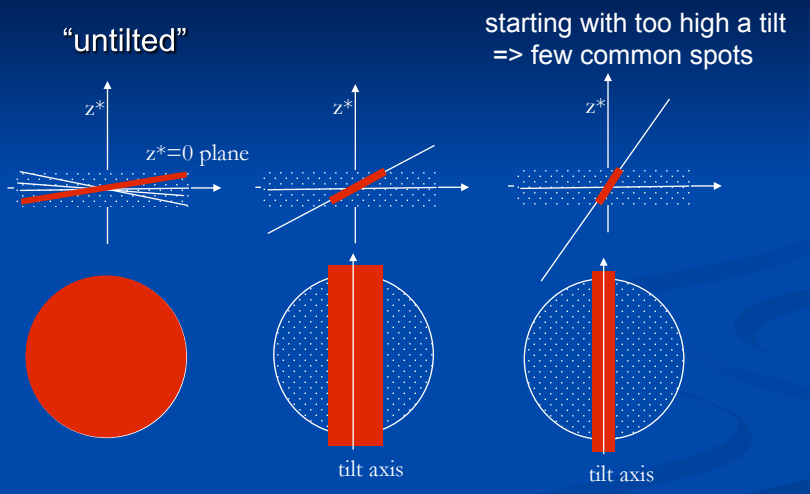
Accumulating merged images with tilts



Accumulating merged images with tilts



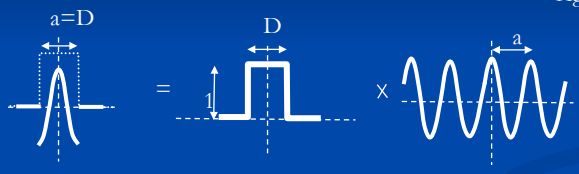
Accumulating merged images with tilts

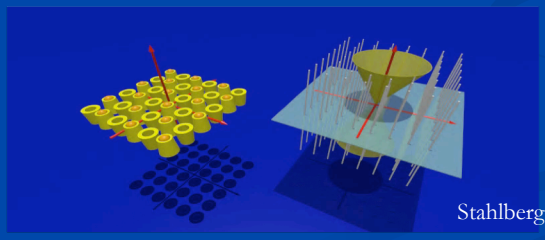


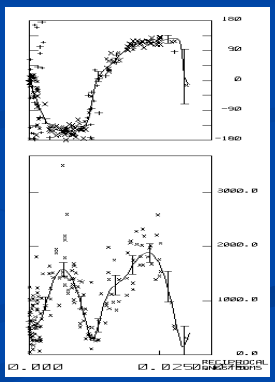
Lattice line fitting

- model fitting in the reciprocal space of the continuous line in the non-repeating (z*) direction based on the assumption that the density in the real space is limited by an envelop function

Agard (1983) JMB 167,849-852

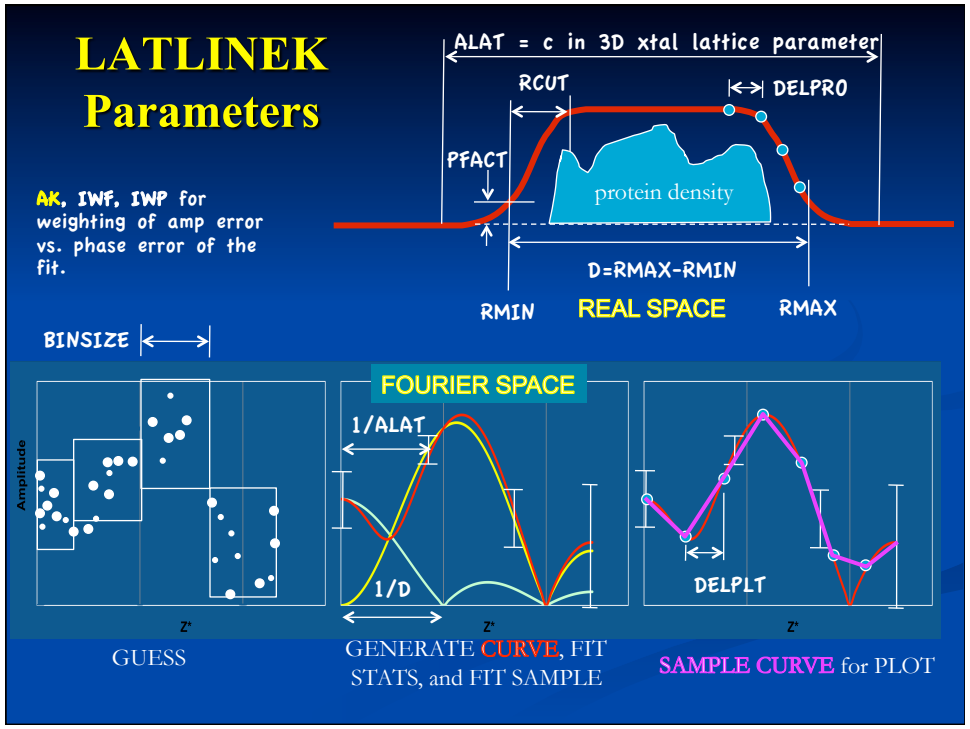






LATLINEK Parameters

AK, IWF, IWP for weighting of amp error vs. phase error of the fit.



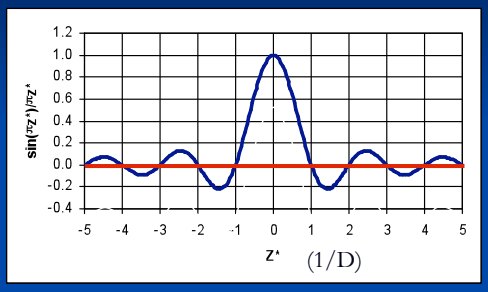
GUESS

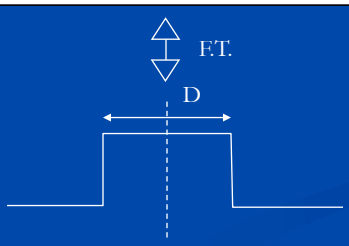
GENERATE CURVE, FIT STATS, and FIT SAMPLE

SAMPLE CURVE for PLOT

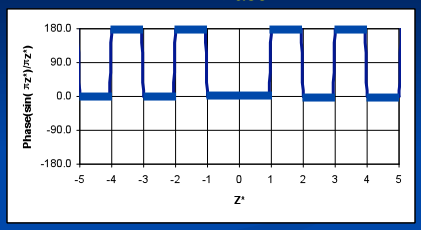
Sinc Function

- sinc(x)=sin(πx)/πx

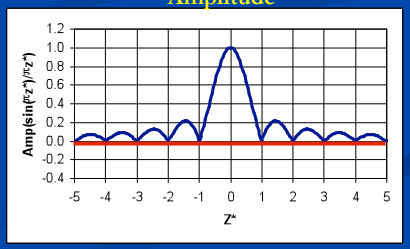




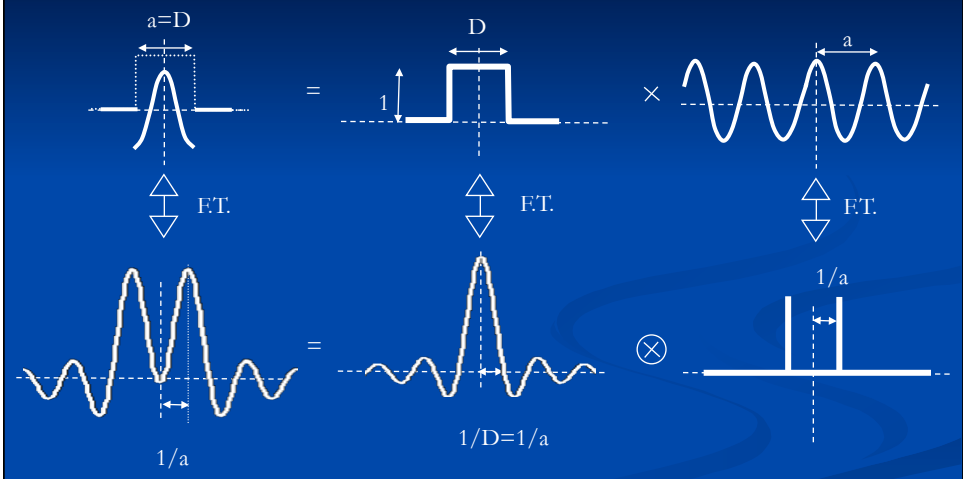
Phase



Amplitude



cosine wave limited by an rectangle pulse

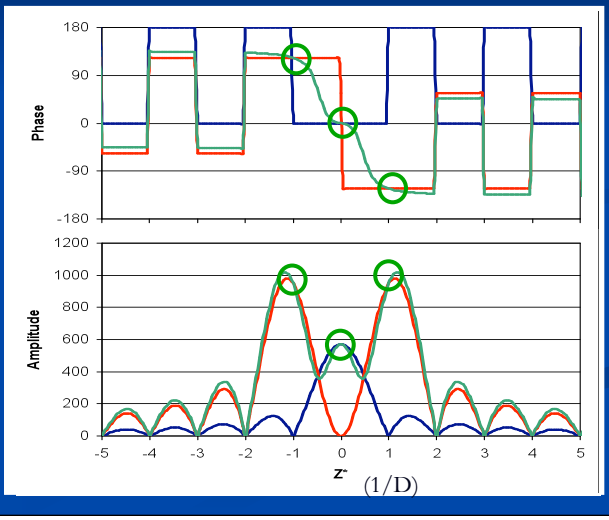


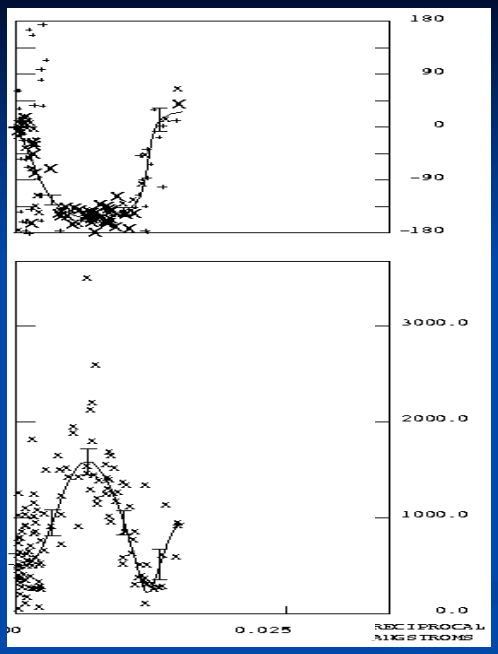
Whitaker-Shannon sampling theorem:

If the density is limited within an envelop of width D, its transform may be reconstructed by samples at 0,+/- 1/D, +/- 2/D,....convoluted using appropriate sinc functions.

- z*=0:
  570**sinc*(z*)
  ϕ=0
- z*=1/D:
  950**sinc*(z*)
  ϕ=-120
- z*=-1/D:
  950**sinc*(z*)
  ϕ=+120

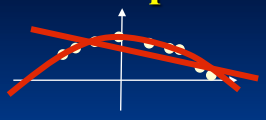
p6 crystal





- Whittaker-Shannon sampling theorem reduces the number of parameters in the fitting.
- The damping of amplitude as z* is away from its peak reduces its contribution to z*'s far away.
  - lack of high tilt data does not affect fit at low z* much

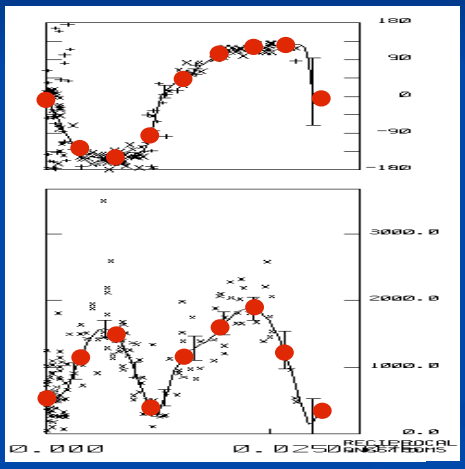
Importance of Defining Correct Envelope Function



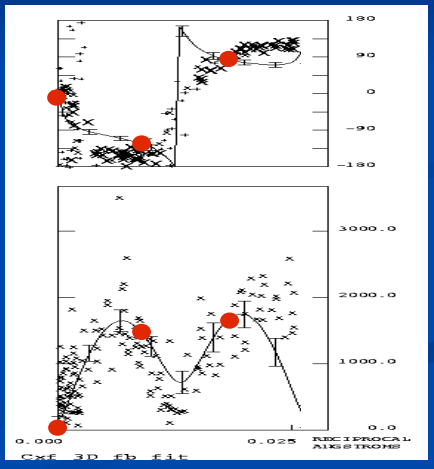
$y=a_0+a_1x$

$y=a_0+a_1x+a_2x^2$

D=260 A

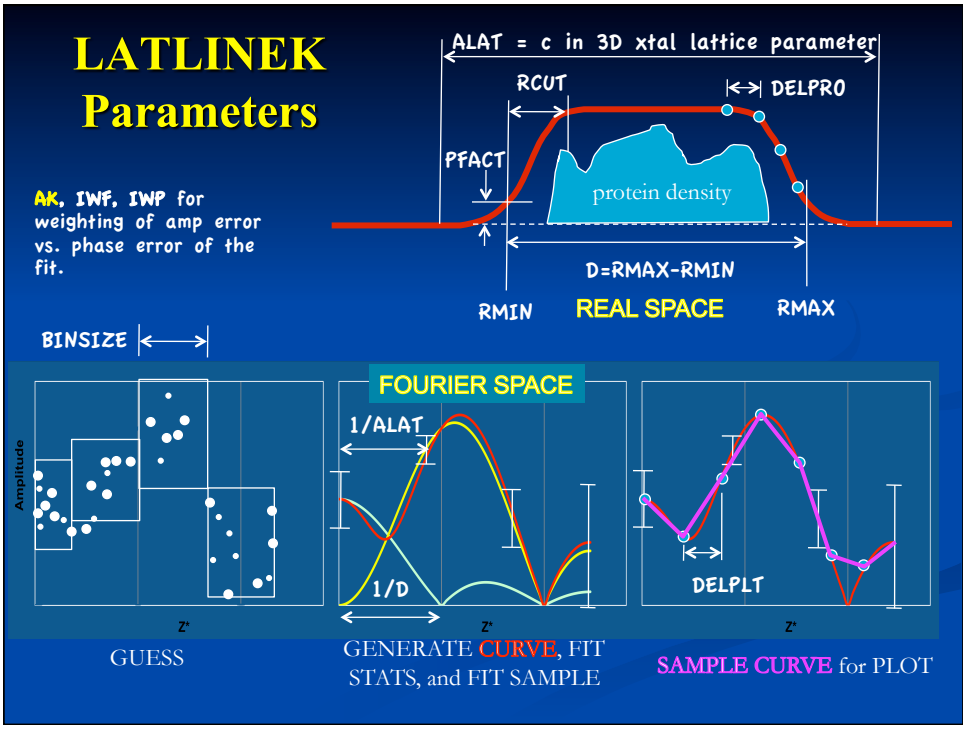


D=100 A



LATLINEK Parameters

AK, IWF, IWP for weighting of amp error vs. phase error of the fit.

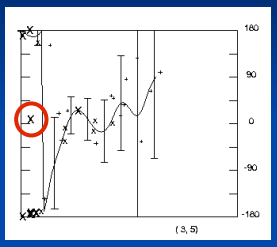


Evaluating the results

- Plots
  - general fit-> adjust the model
  - wrong defocus, tilt geometry->refinement
  - missing region-> better tilt geometry distribution
- Statistics:
  - Overall weighted R-factor (%)=== 0.304
    - (< 40% good)
  - Overall weighted phase residual (deg)= 17.6
    - (<20 deg very good)

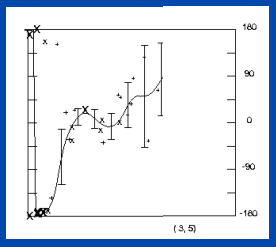
Improving statistics

- ctf (1 image 700 ->800 nm)



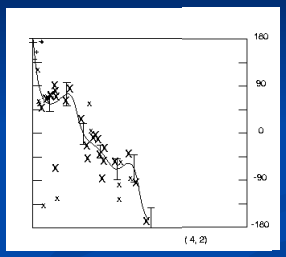
Weighted Phase Residual

16.4



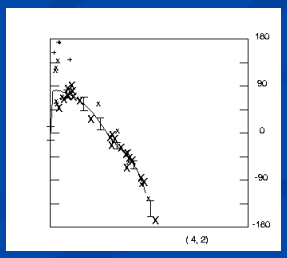
12.0

- tilt (3 images ΔTAXA 15 º)



Weighted Phase Residual

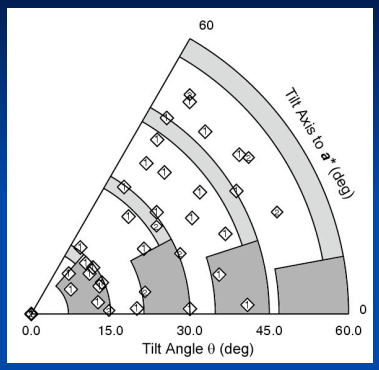
21.8



12.0

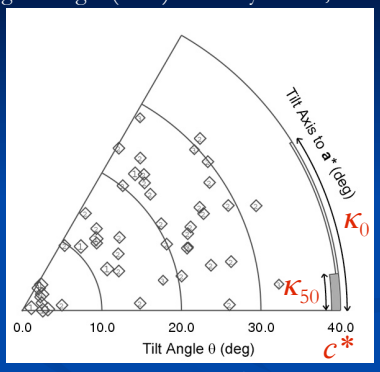
Tilt Geometry Distribution

Cheng & Yeager (2004) Acta Cryst. A60,351-354



Resolution limit = 15 Å
Two-sided plane group = p6
Lattice lines sampled at 1/110 Å^{-1}

Negatively-stained Moloney murine leukemia virus capsid protein crystals (Ganser et al. (2003) *EMBO J.* **22**, 2886-2892)



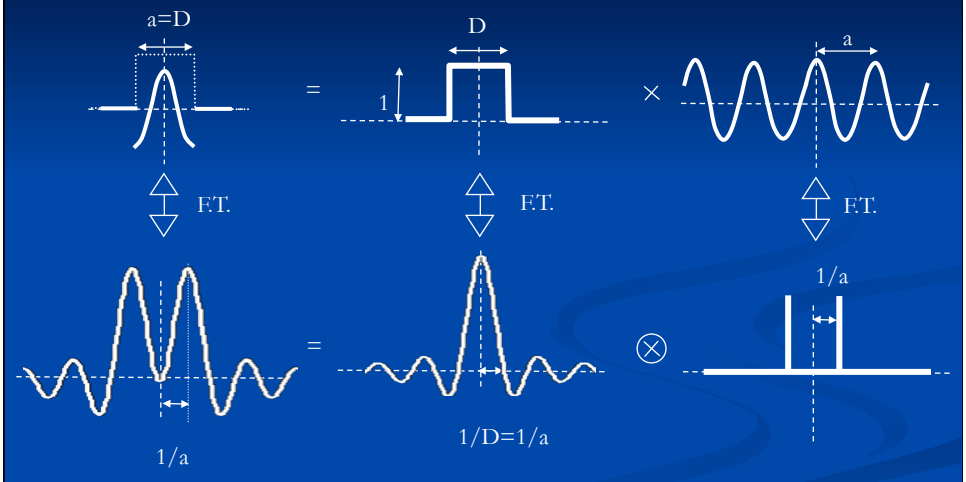
Resolution limit =7.5 Å
Two-sided plane group=p6
Lattice lines sampled at 1/300 Å^{-1}

Connexin 43 gap junction channels in vitreous ice

General Workflow-Feed back

- Acquire images
- Select images
- Increase signal-to-noise ratio of the ordered information.
- Extract spot amplitudes/phases from FT of the images.
- Determine defocus
- For untilted crystal- determine crystal symmetry
- For tilted crystal- determine tilt geometry
- Merging multiple images to common phase origin & scale
- Lattice line fitting
- Generate density map

Single layer crystal



Double layer crystal