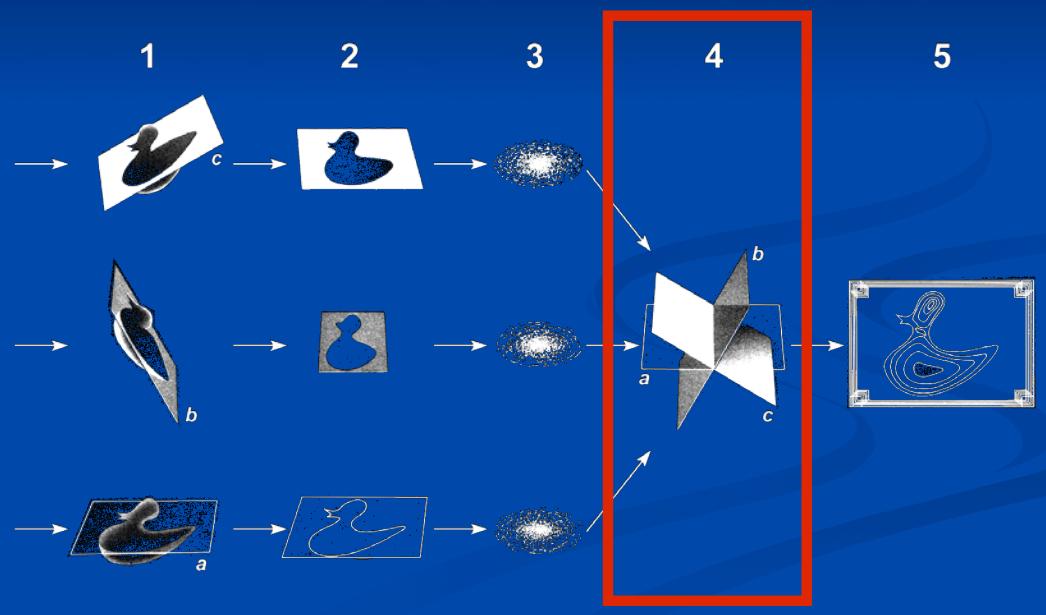
Merging with the MRC software

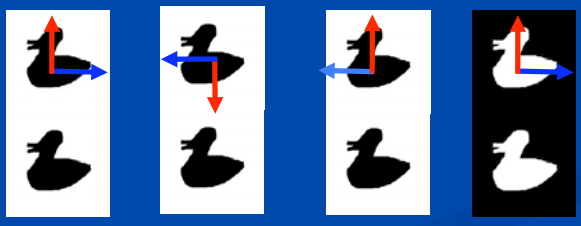
- Merging multiple images to common phase origin & scale
- Lattice line fitting
- Use the results to improve image acquisition/processing



ORIGTILT program

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

- Reference
  - Symmetry special position
  - Last set of merged image
  - Last calculated 3D model
- Refine
  - Phase origin
  - Tilt geometry
  - Beam Tilt

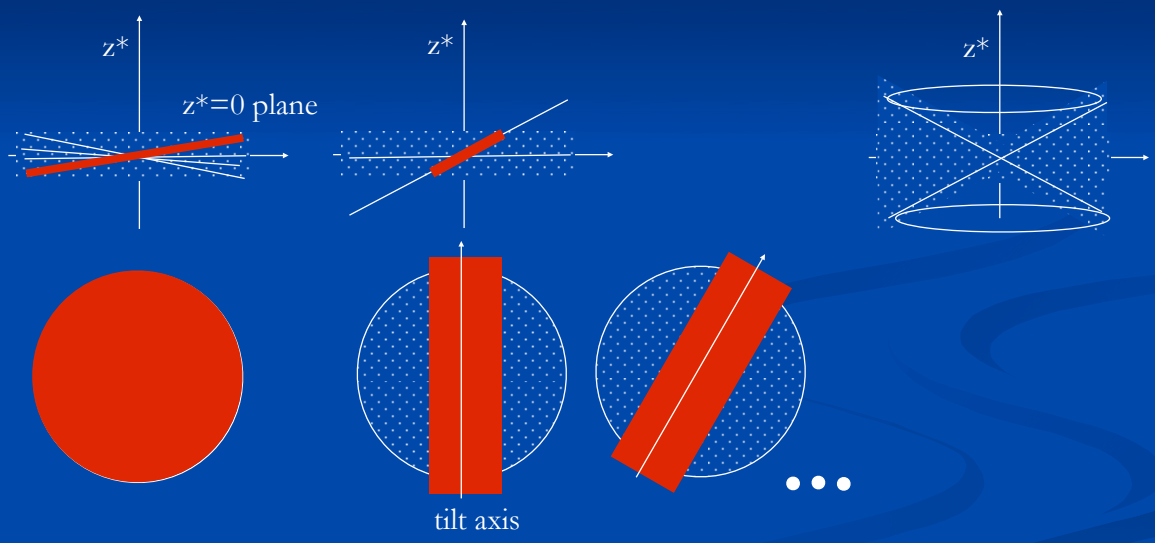


- Standardize a, b axises
  - Rotation
  - Flip crystal upside down
  - Defocus inversion

Accumulating merged images with tilts

"untilted"

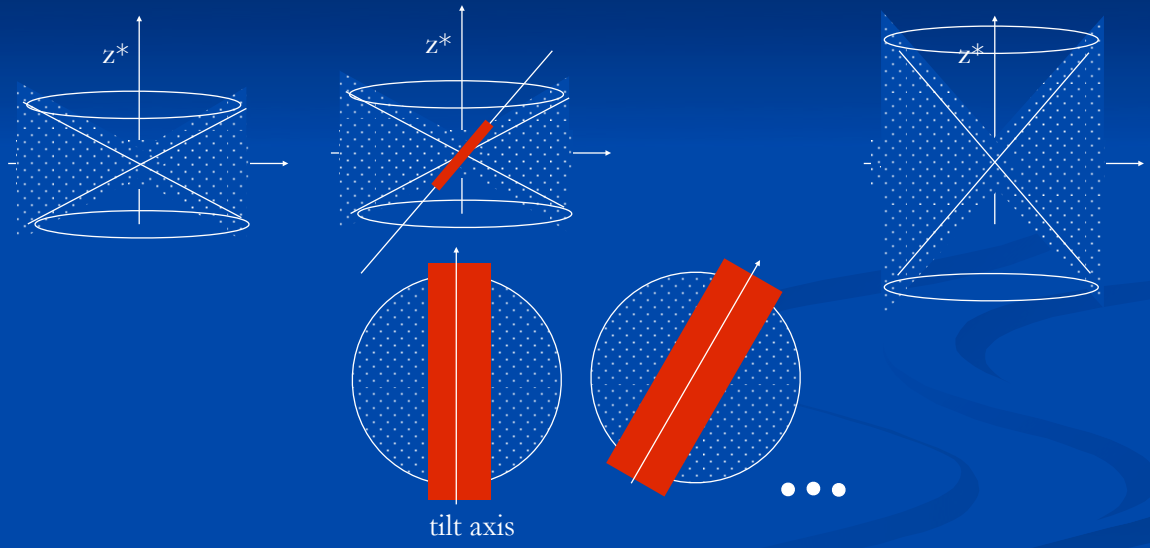
low tilt and various tilt axes



Accumulating merged images with tilts

low tilt

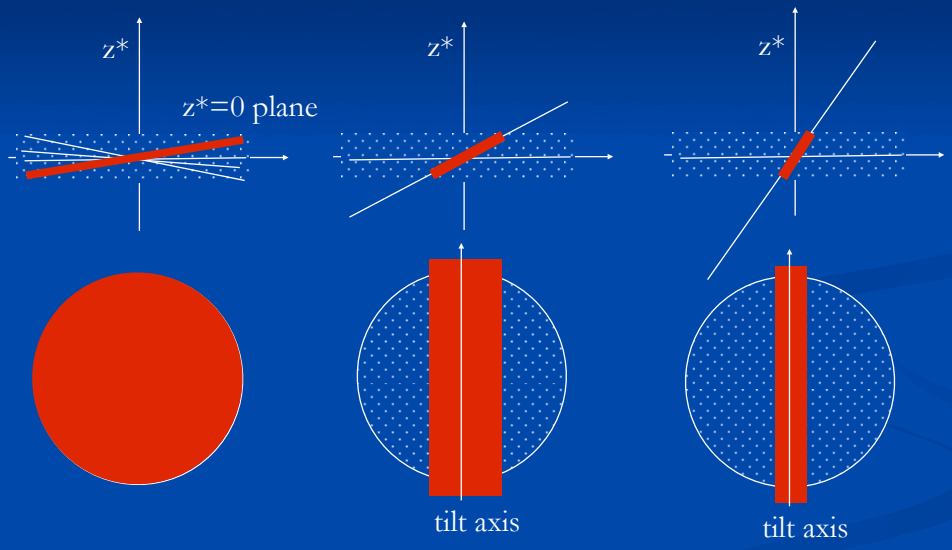
high tilt and various tilt axes



Accumulating merged images with tilts

"untilted"

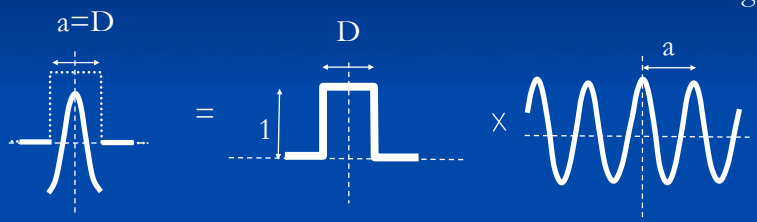
starting with too high a tilt
=> few common spots

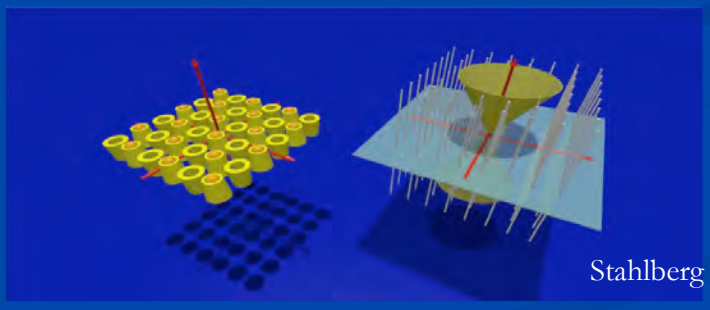


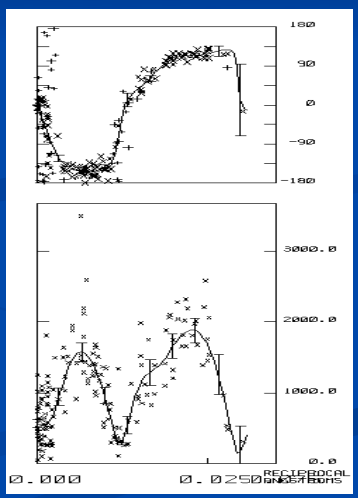
Lattice line fitting (LATLINEK)

- 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

Agard (1983) JMB 167,849-852

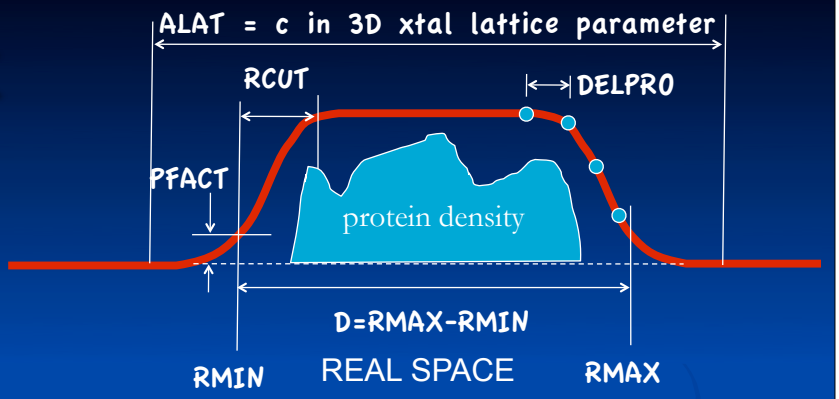


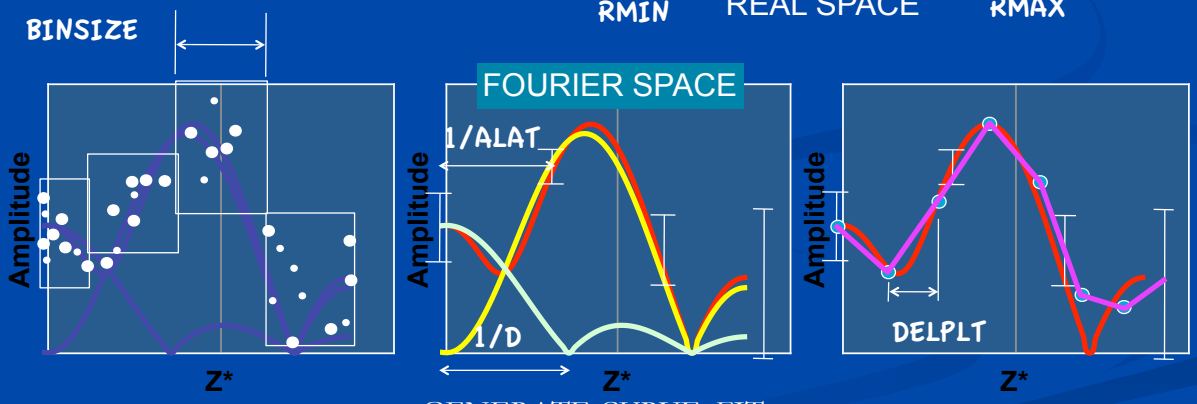




LATLINEK

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



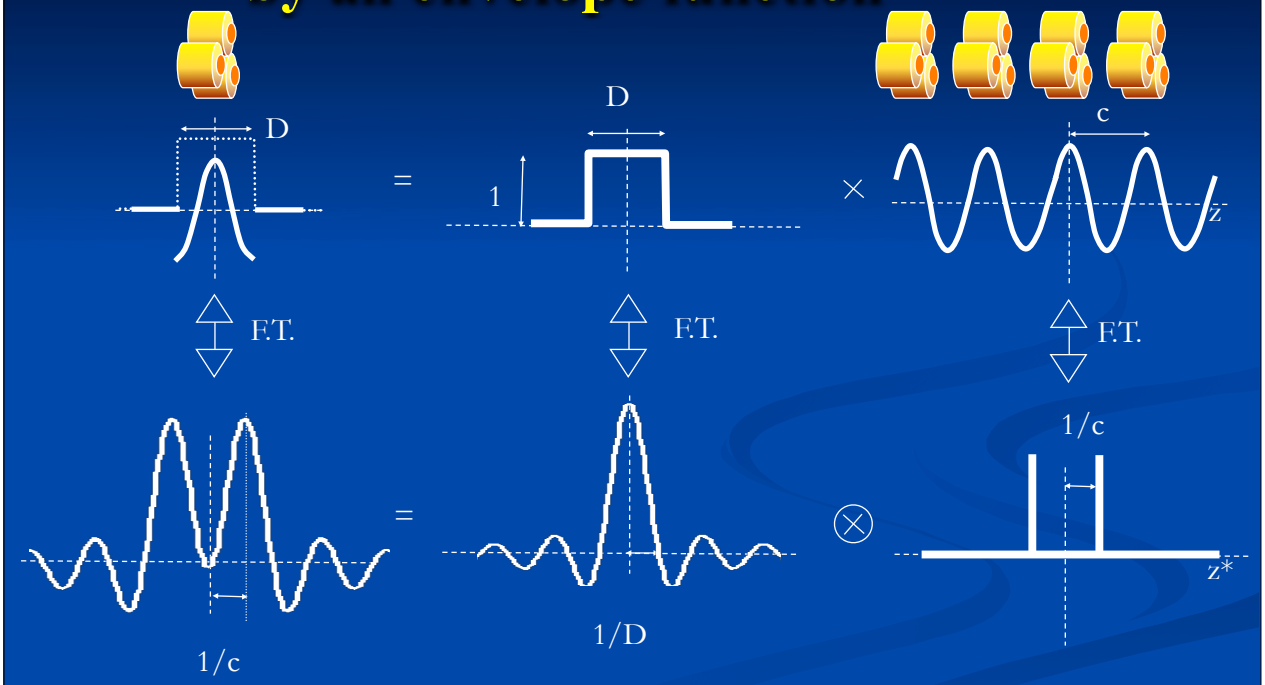


GUESS

GENERATE CURVE, FIT STATS, and FIT SAMPLE

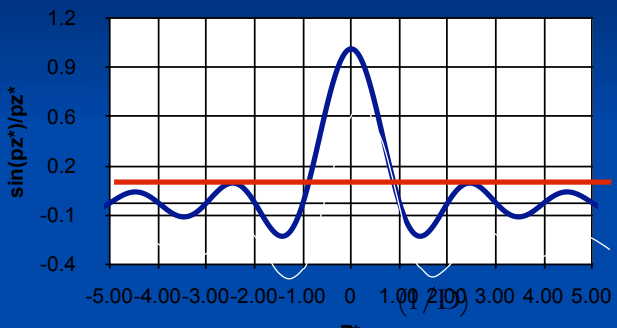
SAMPLE CURVE for PLOT

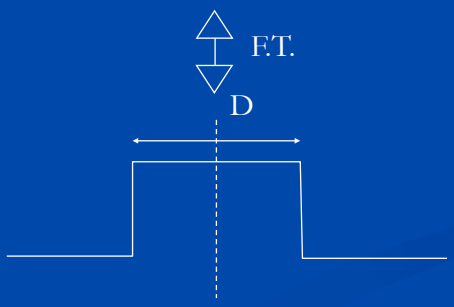
2D x'tal is 3D x'tal limited by an envelope function



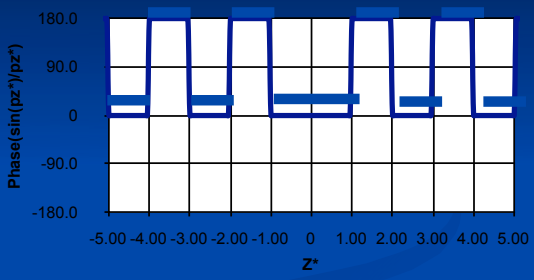
Sinc Function

- sinc(x)=sin(πx)/πx

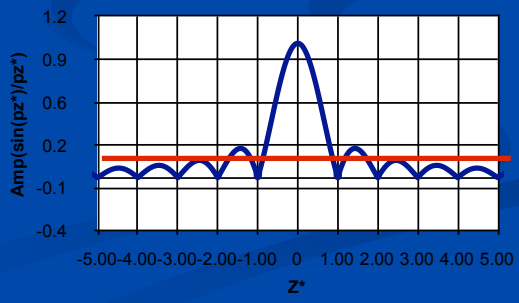




Phase



Amplitude

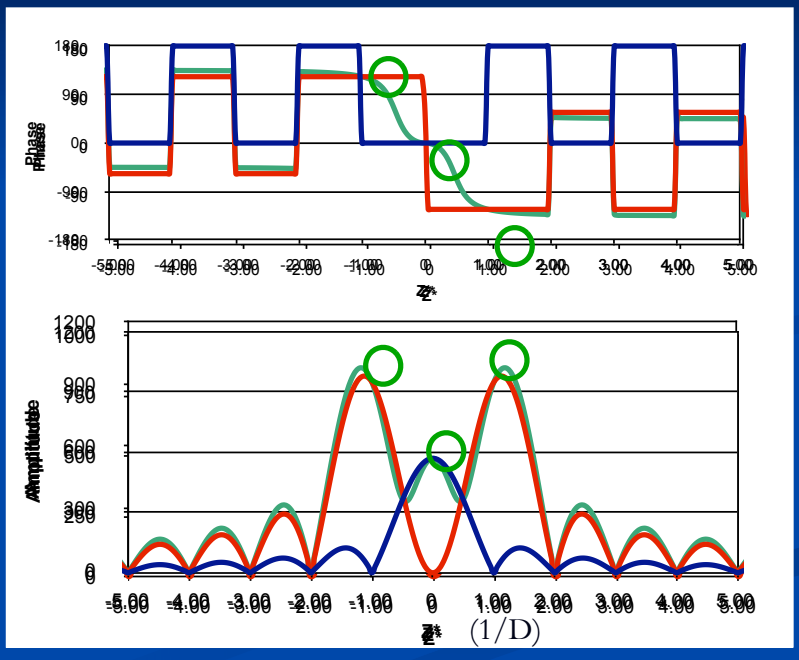


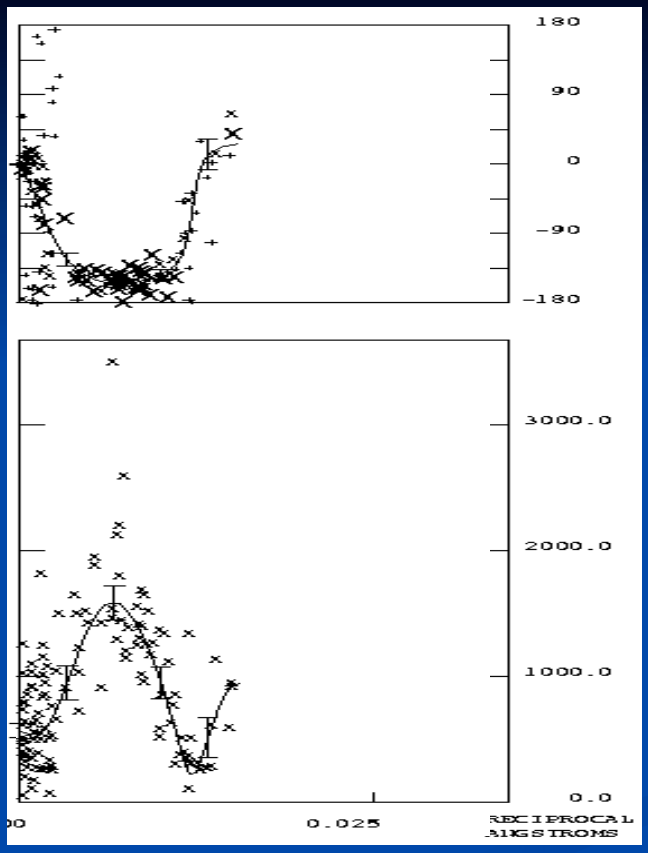
Whitaker-Shannon sampling theorem:

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

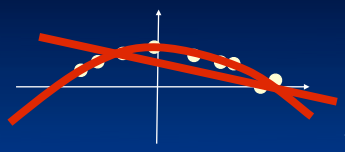
p6 crystal





- 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

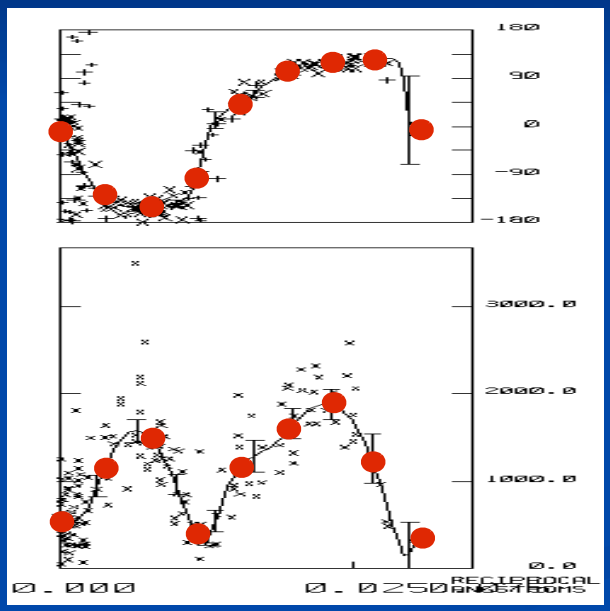
Importance of Defining



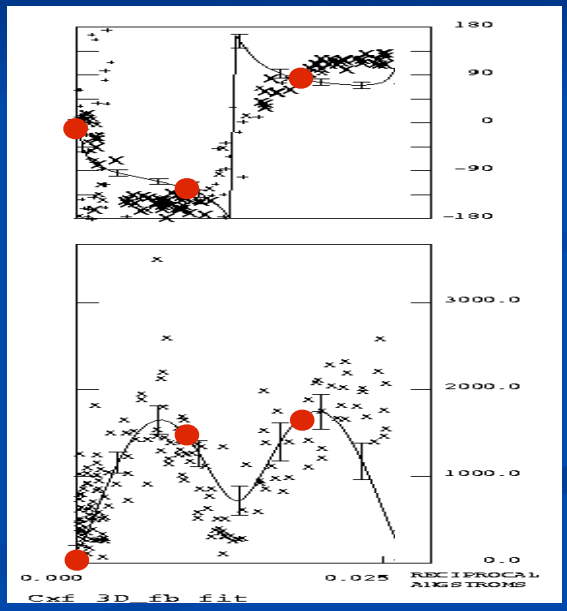
$y=a_0+a_1x$

$y=a_0+a_1x+a_2x^2$

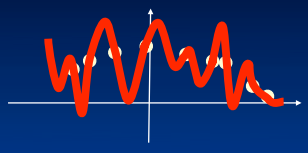
D=260 A



D=100 A



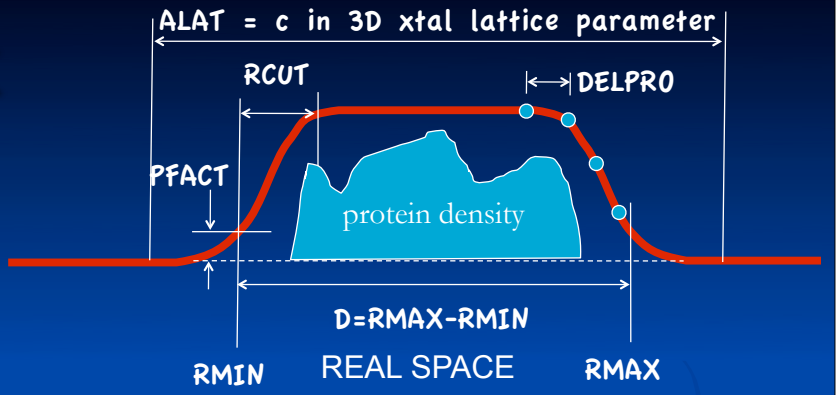
What happens then if D = 500 Å is used?

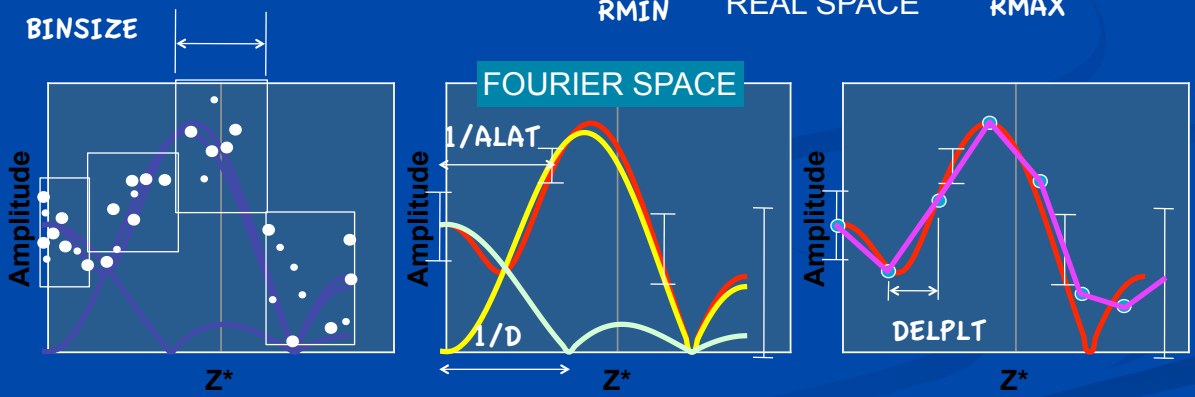


$y=a_0+a_1x+a_2x^2+a_3x^3+\ldots$

LATLINEK

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





GUESS

GENERATE CURVE, FIT STATS, and FIT SAMPLE

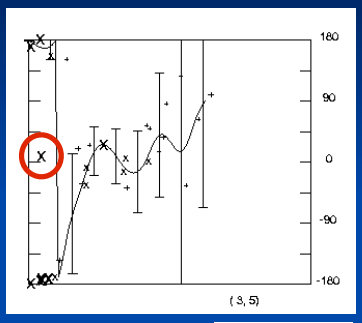
SAMPLE CURVE for PLOT

Evaluating the results and improving them w/ accumulated information

- Plots
  - general fit-> adjust the lattice line 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)

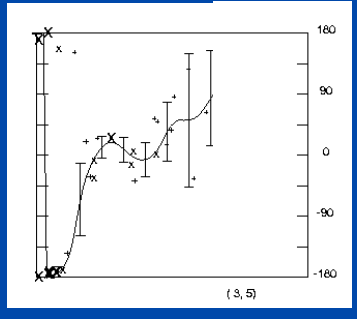
Improving statistics

- ctf (1 image 700 ->800 nm)



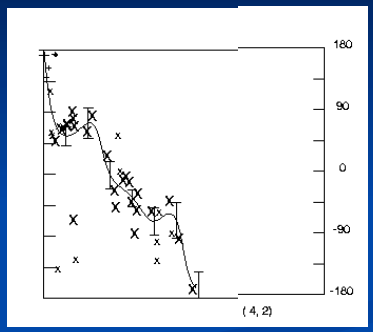
Weighted Phase Residual

16.4



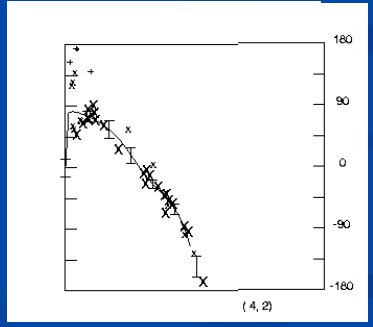
12.0

- tilt (3 images ΔTAXA 15 °)



Weighted Phase Residual

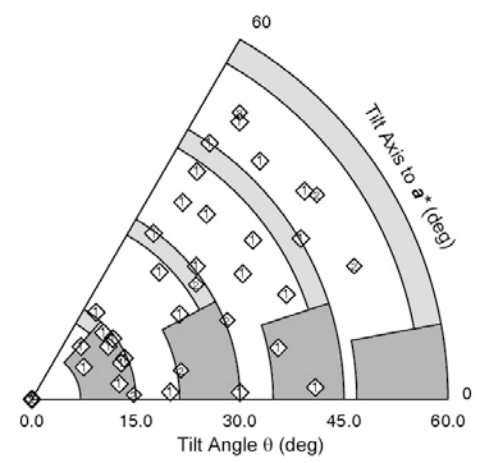
21.8



12.0

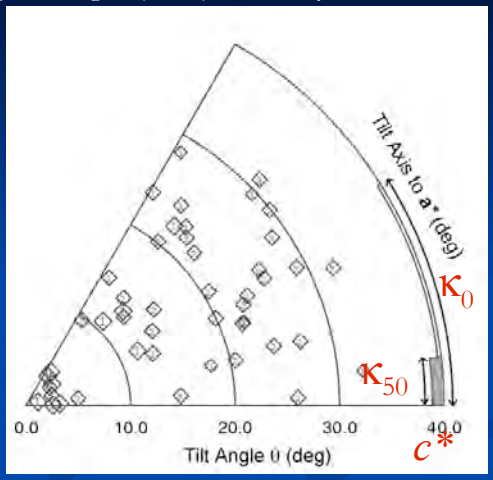
Tilt Geometry Distribution (PLTILT)

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)



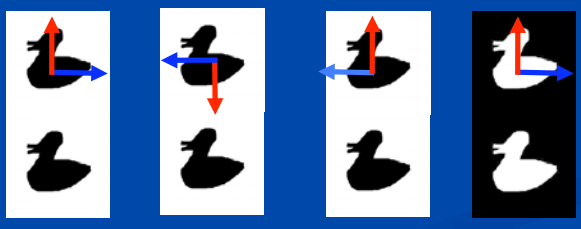
Resolution limit =7.5 Å
Two-sided plane group=p6
Lattice lines sampled at 1/300 Å^{-1}

Connexin 43 gap junction channels in vitreous ice

What we did in ORIGTILT program

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

- Reference
  - Symmetry special position
  - 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



MAKETRAN program

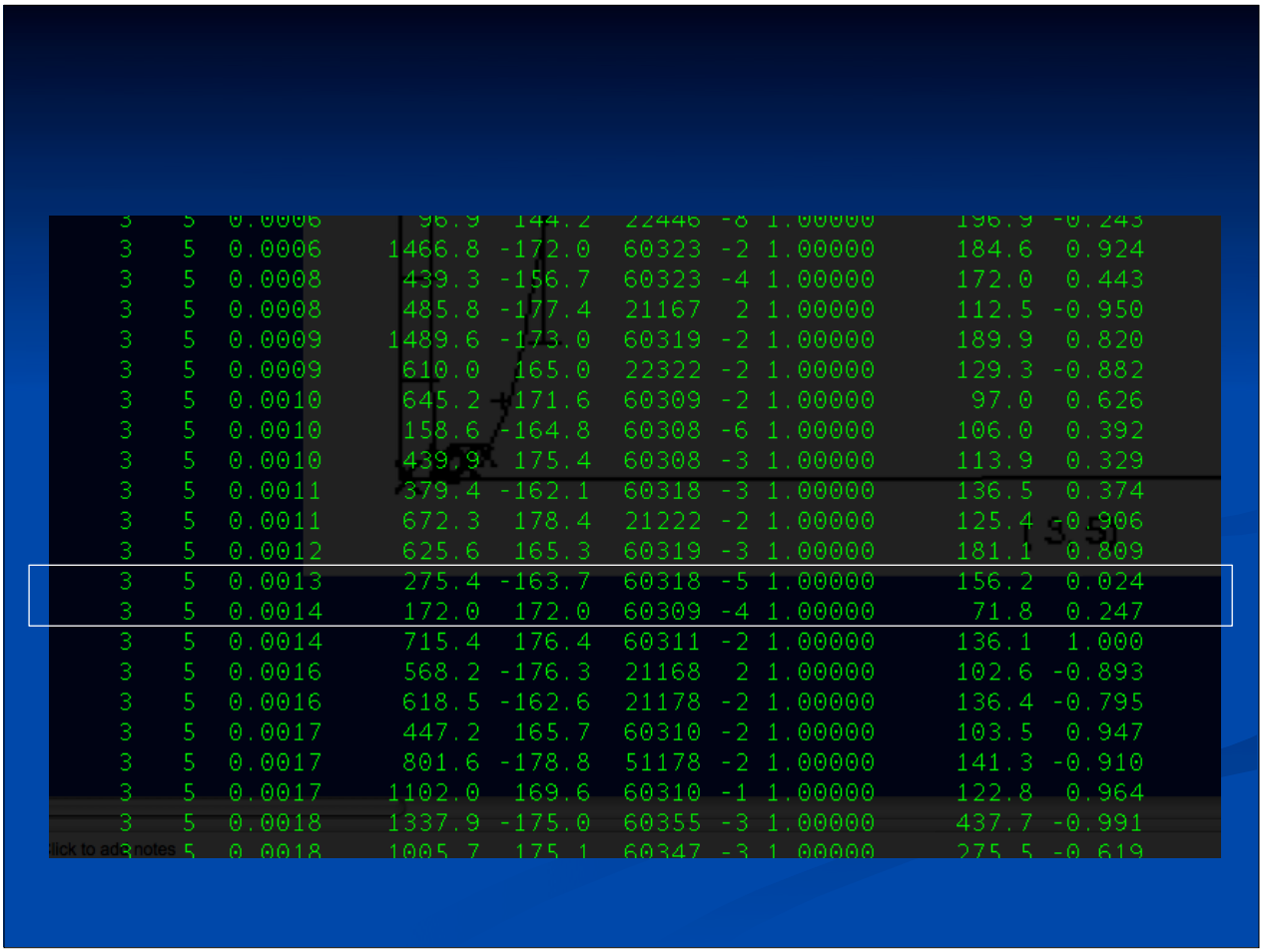
- Make synthetic image using all refined parameters to be used as reference for unbend images.

Kunji et. al. (2000) PNAS 97, 4637-4642

- 3D model (structure factors)
- Image information
  - Crystal orientation
  - Image Dimension / Pixel Size
  - CTF
  - Phase origin
  - Tilt geometry
  - Beam Tilt
  - Rotation
  - Flip crystal upside down
  - Defocus inversion

Feed back to General Workflow

- 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



3	5	0.0006	96.9	144.2	22446	-8	1.00000	196.9	-0.243
3	5	0.0006	1466.8	-172.0	60323	-2	1.00000	184.6	0.924
3	5	0.0008	439.3	-156.7	60323	-4	1.00000	172.0	0.443
3	5	0.0008	485.8	-177.4	21167	2	1.00000	112.5	-0.950
3	5	0.0009	1489.6	-173.0	60319	-2	1.00000	189.9	0.820
3	5	0.0009	610.0	165.0	22322	-2	1.00000	129.3	-0.882
3	5	0.0010	645.2	171.6	60309	-2	1.00000	97.0	0.626
3	5	0.0010	158.6	-164.8	60308	-6	1.00000	106.0	0.392
3	5	0.0010	439.9	175.4	60308	-3	1.00000	113.9	0.329
3	5	0.0011	379.4	-162.1	60318	-3	1.00000	136.5	0.374
3	5	0.0011	672.3	178.4	21222	-2	1.00000	125.4	-0.906
3	5	0.0012	625.6	165.3	60319	-3	1.00000	181.1	0.809
3	5	0.0013	275.4	-163.7	60318	-5	1.00000	156.2	0.024
3	5	0.0014	172.0	172.0	60309	-4	1.00000	71.8	0.247
3	5	0.0014	715.4	176.4	60311	-2	1.00000	136.1	1.000
3	5	0.0016	568.2	-176.3	21168	2	1.00000	102.6	-0.893
3	5	0.0016	618.5	-162.6	21178	-2	1.00000	136.4	-0.795
3	5	0.0017	447.2	165.7	60310	-2	1.00000	103.5	0.947
3	5	0.0017	801.6	-178.8	51178	-2	1.00000	141.3	-0.910
3	5	0.0017	1102.0	169.6	60310	-1	1.00000	122.8	0.964
3	5	0.0018	1337.9	-175.0	60355	-3	1.00000	437.7	-0.991
3	5	0.0018	1005.7	175.1	60347	-3	1.00000	275.5	-0.619

Click to add notes