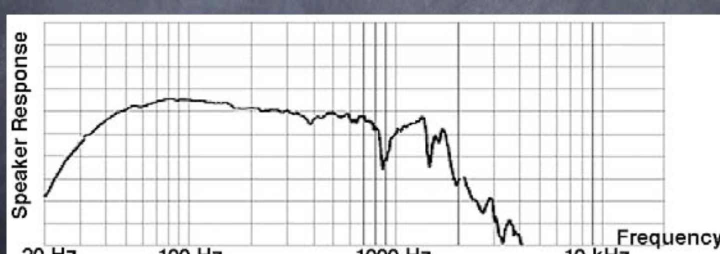
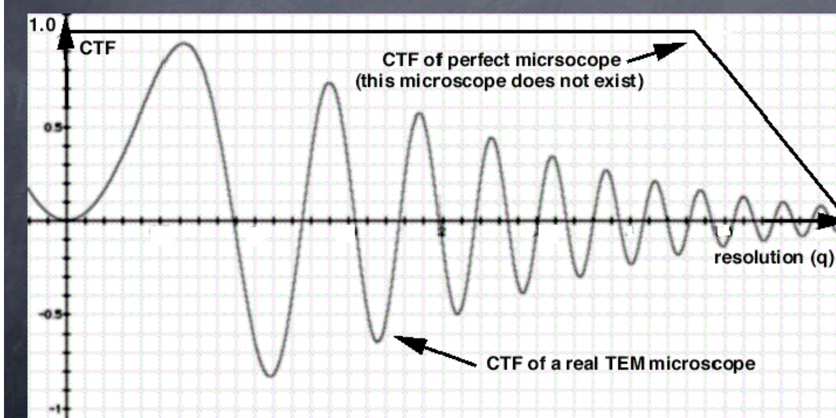
CTF

Henning Stahlberg, UC Davis

Transfer Function



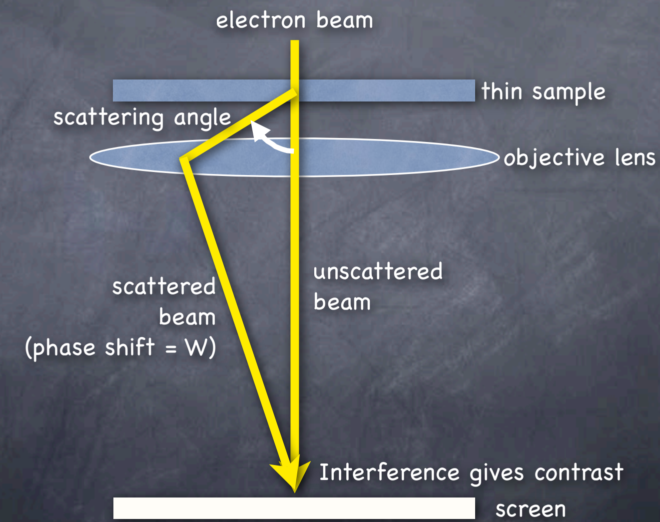
Transfer Function of a TEM



Otto Scherzer

(Mar. 9, 1909 - Nov. 15, 1982)

Contrast

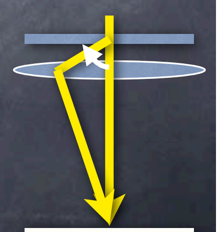


Scherzer Formula

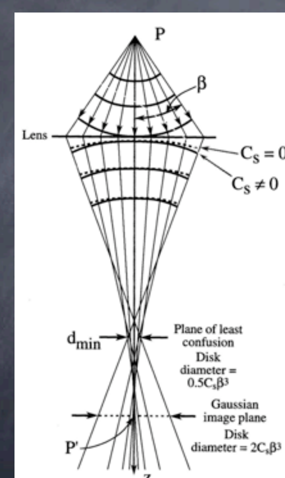
$$\gamma(\mathbf{u}) = \frac{2\pi W}{\lambda} = \frac{\pi}{2}\left[C_s \lambda^3 \mathbf{u}^4 - 2\Delta z \lambda \mathbf{u}^2\right]$$

$\sin(\gamma(u))$: phase contrast transfer function
$\cos(\gamma(u))$: amplitude contrast transfer function

u: scattering vector (≈scattering angle)
W: wave aberration
λ: electron wavelength
Δz: defocus
C_s: spherical abberation constant



Spherical Aberation



C_s

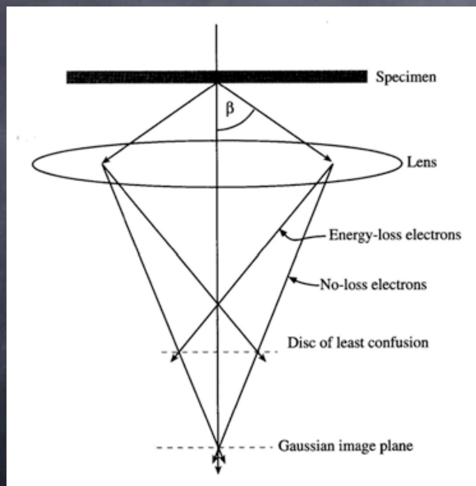
Limits resolution beyond ~ 2 Å

Electrons further from the axis are more strongly bent back towards the axis.

Or:

Electron waves further from the axis receive more phase shift.

Chromatic Aberation



C_c

Limits resolution beyond ~ 0.5 Å

Electrons of lower energy are bent more strongly than those of zero-loss energy.

CTF

CTF(u) = { A * cos(γ(u)) – sqrt(1–A^2) * sin(γ(u)) } * E(u)

$$\gamma(\mathbf{u}) = \frac{2\pi W}{\lambda} = \frac{\pi}{2}\left[C_s \lambda^3 \mathbf{u}^4 - 2\Delta z \lambda \mathbf{u}^2\right]$$

sin(γ(u)): phase contrast transfer function
cos(γ(u)): amplitude contrast transfer function

u: scattering vector (≈scattering angle)

A: Amplitude contrast fraction. (neg. stain: use 0.07)

Envelope functions

$E(\mathbf{u}) = E_s(\mathbf{u}) \cdot E_c(\mathbf{u}) \cdot E_d(\mathbf{u}) \cdot E_v(\mathbf{u}) \cdot E_D(\mathbf{u})$

with

$E_s(\mathbf{u})$: angular spread of the source

α = opening angle

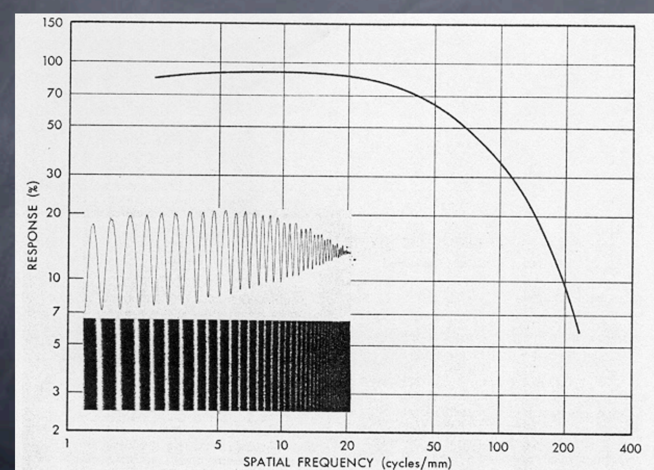
$$E_s(u) = exp[-(\frac{\pi\alpha}{\lambda})^2(\frac{\delta X(u)}{\delta u})^2] = exp[-(\frac{\pi\alpha}{\lambda})^2(C_s\lambda^3u^3 + \Delta f\lambda u)^2]$$

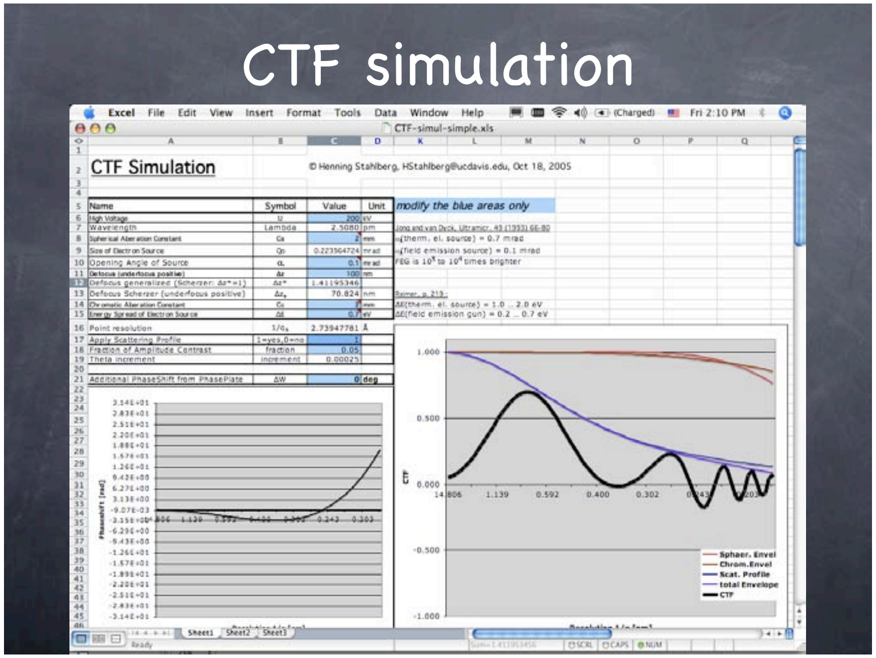
$E_c(\mathbf{u})$: chromatic aberration

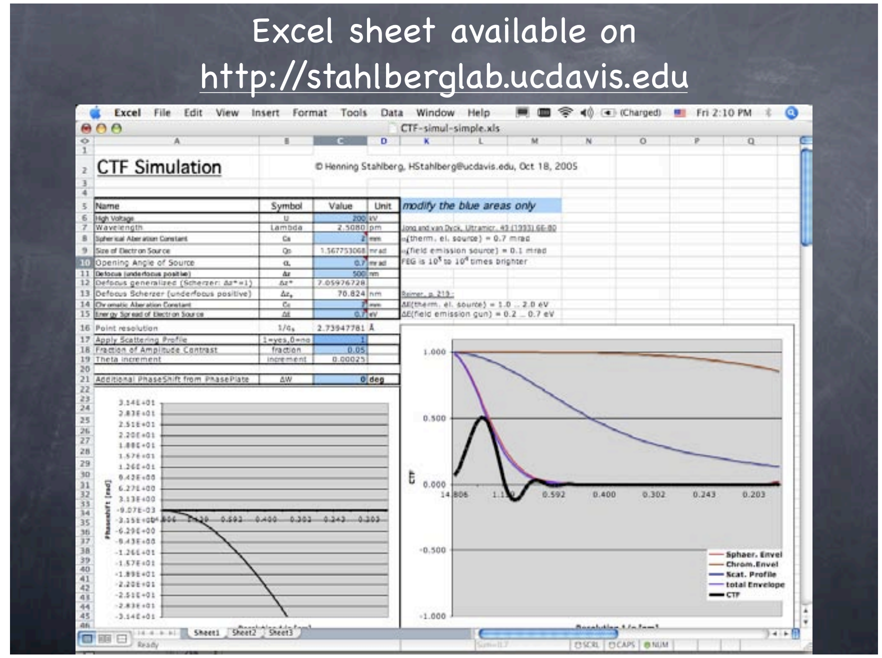
$$E_c(u) = exp[\frac{1}{2}(\pi\lambda\delta)^2u^4] \qquad \delta = C_c\sqrt{4(\frac{\Delta I_{obj}}{I_{obj}})^2 + (\frac{\Delta E}{V_{acc}})^2 + (\frac{\Delta V_{acc}}{V_{acc}})^2}$$

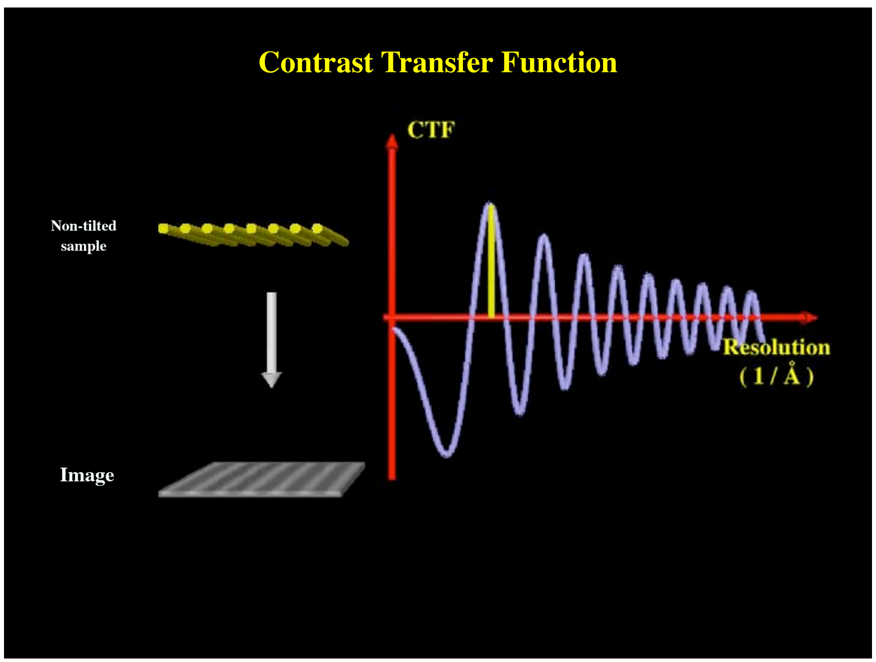
$E_d(\mathbf{u})$: specimen drift
$E_v(\mathbf{u})$: specimen vibration
$E_D(\mathbf{u}) = MTF_D(u)$: detector

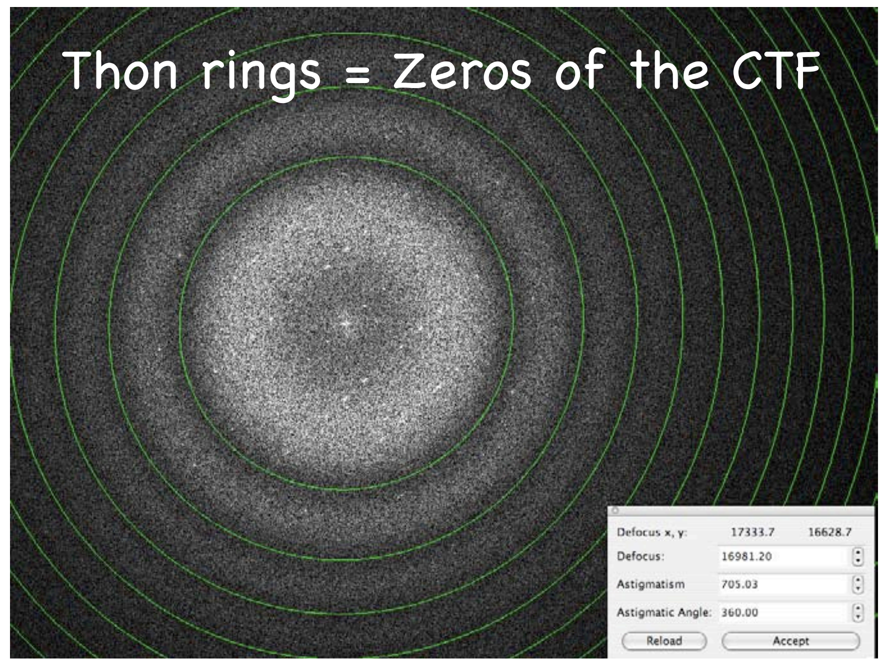
Modulation Transfer Function (MTF) of photographic film




CTF simulation
CTF Simulation
© Henning Stahlberg, HStahlberg@ucdavis.edu, Oct 18, 2005
modify the blue areas only

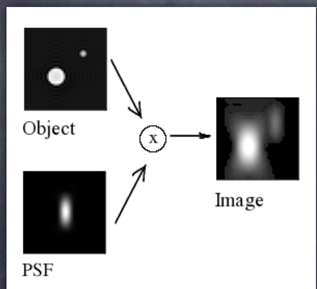

Excel sheet available on
http://stahlberglab.ucdavis.edu
CTF Simulation
© Henning Stahlberg, HStahlberg@ucdavis.edu, Oct 18, 2005
modify the blue areas only


Contrast Transfer Function
CTF
Non-tilted
sample
Image
Resolution
(1 / Å)


Thon rings = Zeros of the CTF
Defocus x, y: 17333.7 16628.7
Defocus: 16981.20
Astigmatism 705.03
Astigmatic Angle: 360.00
Reload
Accept

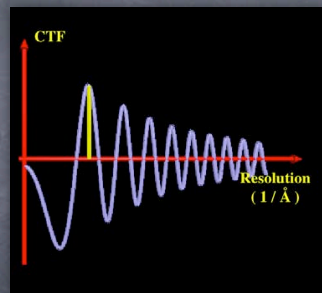
Real Space:
Point Spread Function

Object ⊗ PSF = Image



Fourier Space:
Contrast Transfer Function

FT(Object) • CTF = FT(Image)



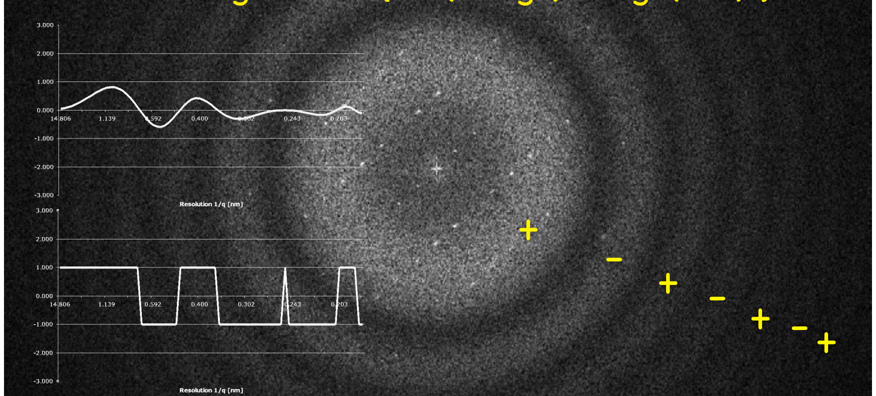
FT(PSF) ≈ CTF

PSF ≈ FT(CTF)

Can we correct the CTF ?

CTF correction: Phase flipping

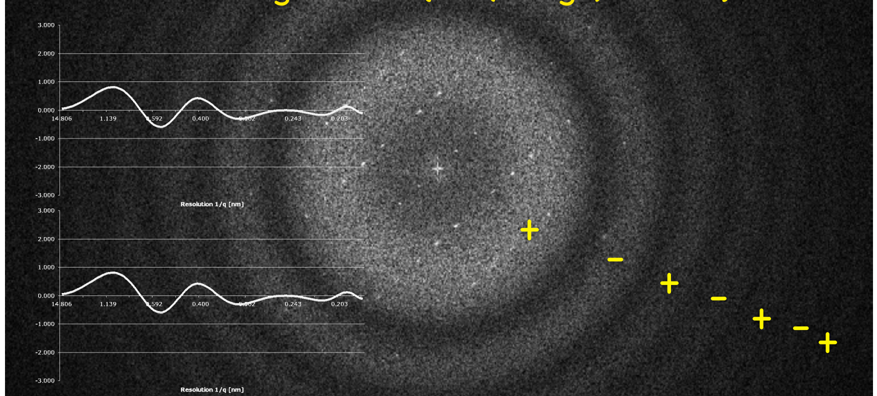
$$\text{NewImage} = FT^{-1}\{\, FT(\text{Image}) \bullet \text{sign}(CTF) \,\}$$



“Invert the phase behind every second Thon ring”

CTF correction: multiply by CTF

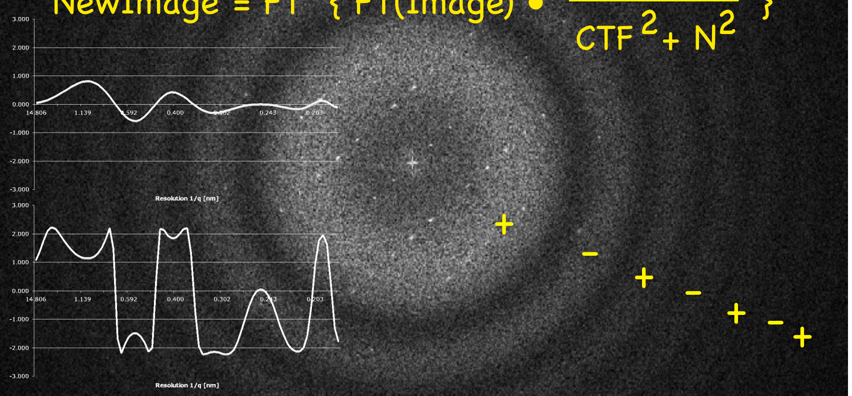
$$\text{NewImage} = FT^{-1}\{\, FT(\text{Image}) \bullet CTF \,\}$$



“Multiply by CTF, to increase S/N”

CTF correction: Wiener Filter

$$\text{NewImage} = FT^{-1}\left\{ FT(\text{Image}) \bullet \frac{CTF}{CTF^2 + N^2} \right\}$$



"Divide by the CTF (sort of...)"

Conclusions CTF

- The CTF defines the transfer of contrast from the sample onto the image.
- The PSF defines the impact on the image from a point in the sample. PSF = FFT(CTF).
- CTF needs to be fitted and corrected.
- CTF for tilted samples is a complicated and important story, which will be told by Ansgar Philippsen after the coffee break.

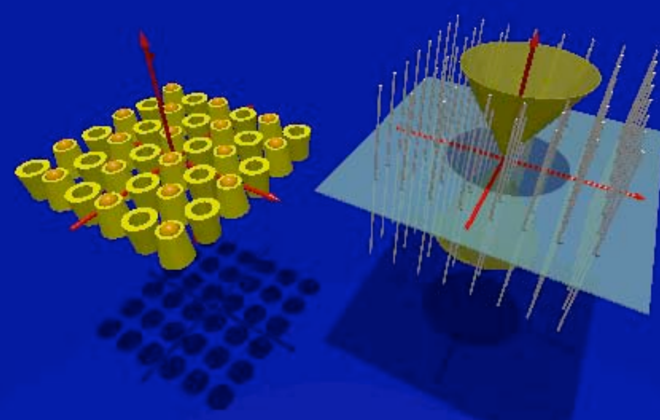
Tilt Geometry

Henning Stahlberg, UC Davis

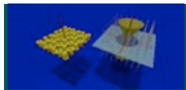
The Missing Cone

Real Space

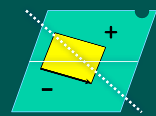
Fourier Space



Diffraction Spots in 2D
Lattice Lines in 3D



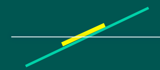
Tilt Geometry



Coordinate System of the Recorded Image

1. Where is the tilt axis? TLTAXIS: angle from X-axis to tilt-axis
2. How much tilt was there? TLTANG: tilt angle of sample
3. How is the crystal oriented? TLTAXA: angle from tilt-axis to A*

Coordinate System of the Sample



4. How much tilt was there? TANGL: tilt angle of sample
   (Same as TLTANG, but Sign dependent on:
   Is A above tilt axis? Sign of TLTAXA? Handedness of the lattice assignment?)*
5. How is the crystal oriented? TAXA: angle from tilt-axis to A* on sample
   (different than TLTAXA!)

Four ways to determine/refine tilt geometry:

- From defocus of negative (ctfsearch3)
- From lattice distortion (lattilt)
- From spot-splitting (ttrefine)
- From comparison with 3D dataset (origtiltd)

-------- Defocus values in 49 positions on image ----

12780	11990	10960	9900	9510	8430	7500
12610	10920	10800	9480	9330	8070	7090
11770	11230	10190	8890	8460	7390	6890
11400	10190	9480	**8685.35**	7770	6790	6590
11090	9630	9360	8590	7570	6730	5930
10590	9540	9260	7850	7240	6060	5670
10580	9460	8330	7310	6640	5930	5200

---------- Geometry calculation from ----------------------

	defocus (ctfsearch3)	lattice (lattilt)	spotsplit (ttrefine)	merging (origtiltd)
TLTAXIS =	63.1014	64.43140	64.4314	------
TLTANG =	42.7849	45.38935	45.3894	------
TLTAXA =	-87.7667	-87.76840	-87.7681	------
TANGL =	45.3900	45.38935	45.3894	46.998
TAXA =	-86.8216	-86.82402	-86.8236	-93.714

Determination and Refinement of the Tilt Geometry in 2dx

Determination and Refinement of the Tilt Geometry in 2dx

Defocus Gradient accross image

Rough Tilt Geometry, but absolute sign of tilt angle (TLTAXIS, TLTANGL)
No clue about crystal orientation (TAXA, TANGL)

Lattice Distortion

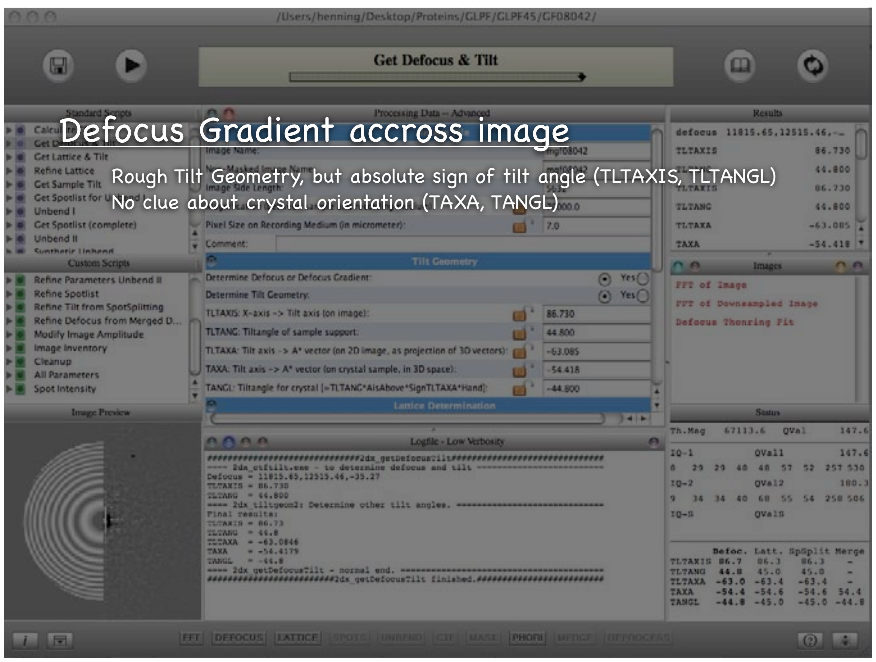
Precise Tilt Geometry if tilt larger than 25º,
but no clue about sign of tilt angle (sign taken from above)

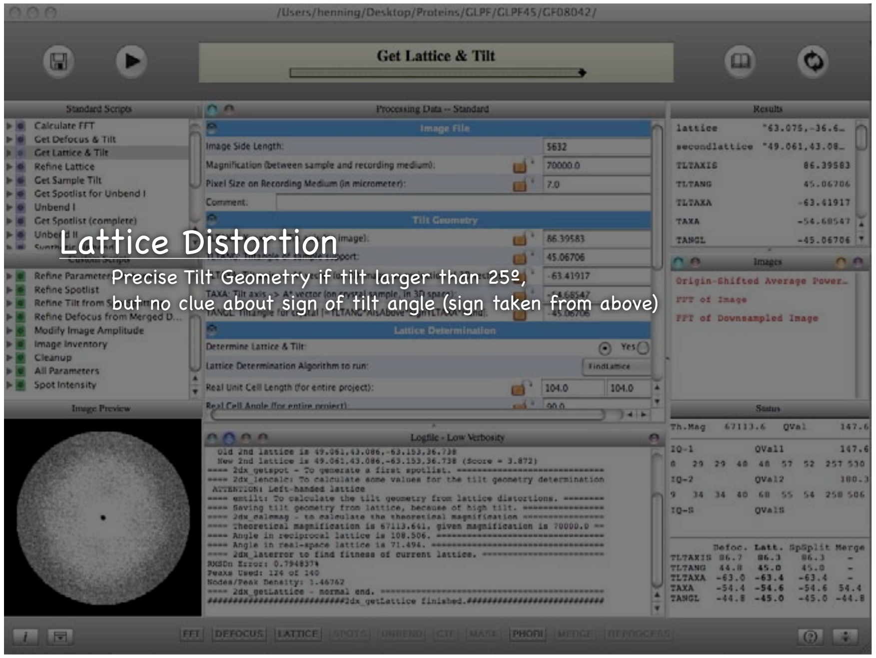
SpotSplitting

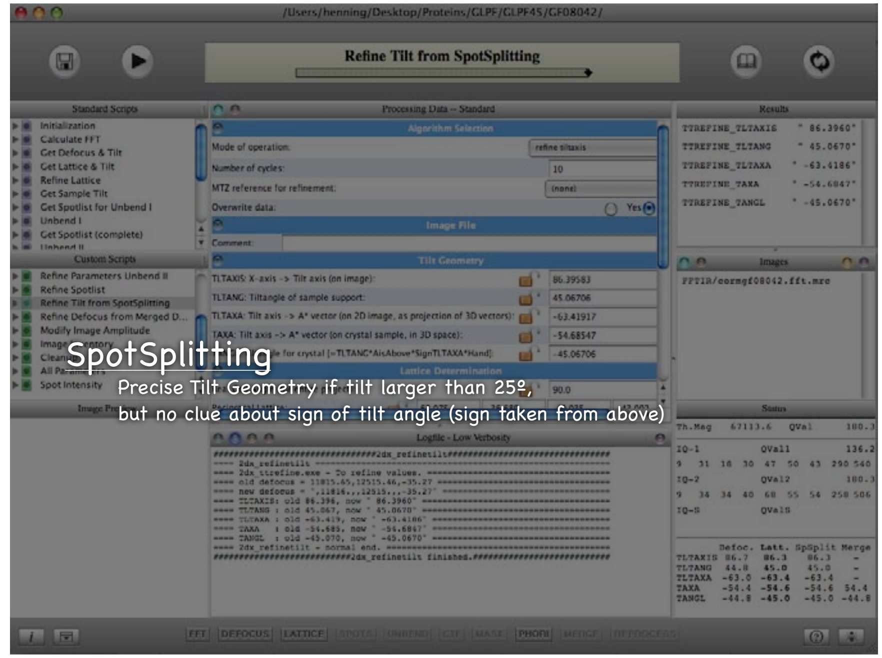
Precise Tilt Geometry if tilt larger than 25º,
but no clue about sign of tilt angle (sign taken from above)

3D Merging

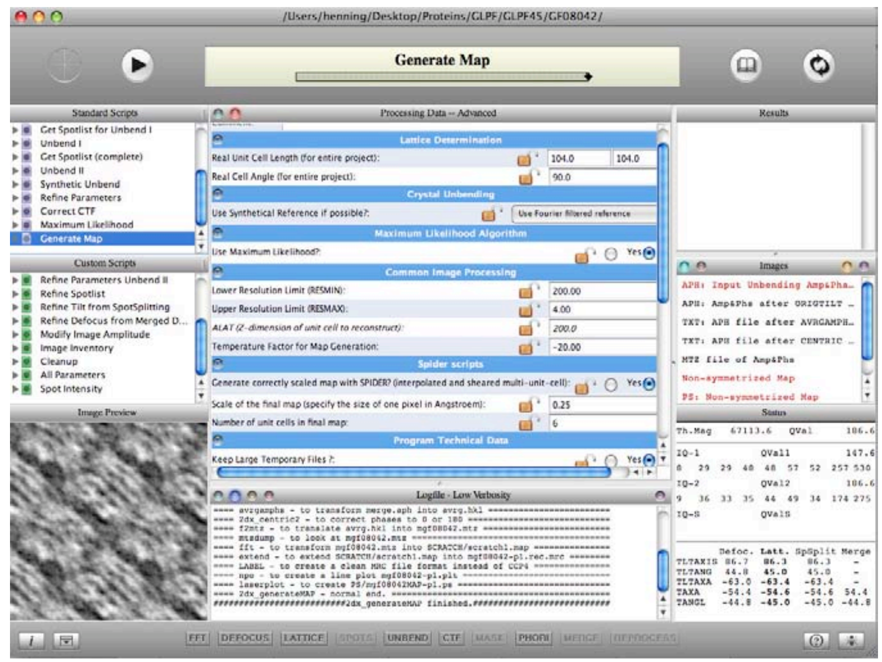
Precise Tilt Geometry for sample (TAXA, TANGL),
but no clue about carbon film orientation (TLTAXIS, TLTANGL)




/Users/henning/Desktop/Proteins/GLPF/GLPF45/GF08042/
Get Lattice & Tilt
Lattice Distortion
Precise Tilt Geometry if tilt larger than 25º,
but no clue about sign of tilt angle (sign taken from above)

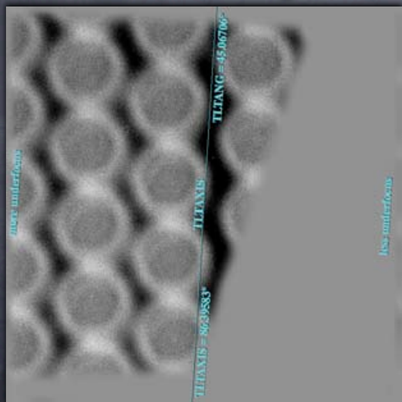

/Users/henning/Desktop/Proteins/GLPF/GLPF45/GF08042/
Refine Tilt from SpotSplitting
SpotSplitting
Precise Tilt Geometry if tilt larger than 25º,
but no clue about sign of tilt angle (sign taken from above)


Initialization
3D Merging
Precise Tilt Geometry for sample (TAXA, TANGL),
but no clue about carbon film orientation (TLTAXIS, TLTANGL)


/Users/henning/Desktop/Proteins/GLPF/GLPF45/GF08042/
Generate Map

Display of Tilt Geometry in 2dx

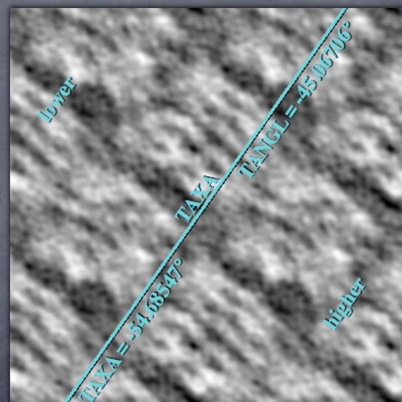
Sample Image



"T"

TLTAXIS, TLTANGL

Final Map



"Shift-T"

TAXA, TANGL

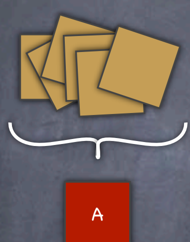
Maximum Likelihood

Henning Stahlberg, UC Davis

Xiangyan Zeng, FVSU, GA

with Niko Grigorieff, Brandeis Univ. and HHMI

Cross-Correlational Alignment and Averaging



X_i Particles

ϕ_i Alignment Parameters

A Average

$$A = \frac{1}{N}\sum_{i=1}^{N} X_i(\phi_i)$$

Align particles (e.g. via CCF), and then average them.

Maximum-Likelihood Processing

$$\Theta = (A, \sigma, \xi)$$

Θ Model Parameters

A Structure

σ Std.dev. of Gaussian noise

ξ Std.dev. of distribution of alignment parameters

A

X_i Observed Particles

$$X_i = A(-\phi_i) + \sigma R_i$$

Gaussian Noise R of distribution σ

Find model parameters
(structure and distributions
for alignment and noise),
**so that the given set of images has the
highest likelihood of being photographed.**

$$L(\Theta) = \sum_{i=1}^{N} \ln P(X_i|\Theta)$$

Goal: Maximize L

Sigworth, JSB 122 (1998)

Zeng, JSB 160 (2007)

$\Theta = (A, \sigma, \xi)$

Maximum-Likelihood Processing

$$L(\Theta) = \sum_{i=1}^{N} \ln P(X_i|\Theta)$$

Goal: Maximize L

$$L(\mathbf{X}|\Theta) = \sum_{i=1}^{N} \ln \int \gamma_i(\phi, \Theta) d\phi,$$

where

$$\gamma_i(\phi, \Theta) = P(X_i|\phi, \Theta) f(\phi|\Theta).$$

$$P(X_i|\phi, \Theta) = \left(\frac{1}{\sigma\sqrt{2\pi}}\right)^M \exp\left[-\frac{|X_i(\phi) - A|^2}{2\sigma^2}\right]$$

P: Probability density for all images under distribution parameters and alignment parameters

$$f(\phi|\Theta) = \left(\frac{1}{\sqrt{2\pi}}\right)^3 \frac{1}{\sigma_x\sigma_y\sigma_\alpha} \times \exp\left[-\frac{(q_x - \xi_x)^2}{2\sigma_x^2} - \frac{(q_y - \xi_y)^2}{2\sigma_y^2} - \frac{(q_\alpha - \xi_\alpha)^2}{2\sigma_\alpha^2}\right]$$

f: Probability density of all distribution parameters under alignment parameters

Find model parameters
(structure and distributions
for alignment and noise),
so that the given set of images has the highest likelihood of being photographed.

Sigworth, JSB 122 (1998)

Zeng, JSB 160 (2007)

$\Theta = (A, \sigma, \xi)$

Maximum-Likelihood Processing

$$L(\Theta) = \sum_{i=1}^{N} \ln P(X_i|\Theta)$$

Goal: Maximize L

$$\frac{\partial L}{\partial a_j} = 0$$

Goal: Find where derivative of L is zero

with:

$$\frac{\partial L}{\partial a_j} = \sum_{i=1}^{N} \frac{\int \left(\frac{X_{ij}(\phi) - a_j}{\sigma^2}\right) \gamma_i(\phi; \Theta)\, d\phi}{\int \gamma_i(\phi; \Theta)\, d\phi} = 0$$

Solution:

$$A^{(n+1)} = \frac{1}{N}\sum_{i=1}^{N} \frac{\int X_i(\phi)\gamma_i(\phi; \Theta^{(n)})\, d\phi}{\int \gamma_i(\phi; \Theta)^{(n)})\, d\phi}$$

Sigworth, JSB 122 (1998)

Zeng, JSB 160 (2007)

$\Theta = (A, \sigma, \xi)$

Maximum-Likelihood Processing

$$L(\Theta) = \sum_{i=1}^{N} \ln P(X_i|\Theta)$$

Goal: Maximize L

$$\frac{\partial L}{\partial a_j} = 0$$

Solution:

Structure A

$$A^{(n+1)} = \frac{1}{N}\sum_{i=1}^{N} \frac{\int X_i(\phi)\gamma_i(\phi, \Theta^{(n)}) d\phi}{\int \gamma_i(\phi, \Theta^{(n)}) d\phi},$$

Noise distribution

$$\sigma^{(n+1)} = \sqrt{\frac{1}{NM}\sum_{i=1}^{N} \frac{\int |X_i(\phi) - A^{(n)}|^2 \gamma_i(\phi, \Theta^{(n)}) d\phi}{\int \gamma_i(\phi, \Theta^{(n)}) d\phi}},$$

Alignment center
(x,y,rotation)

$$\xi_x^{(n+1)} = \frac{1}{N}\sum_{i=1}^{N} \frac{\int q_x \gamma_i(\phi, \Theta^{(n)}) d\phi}{\int \gamma_i(\phi, \Theta^{(n)}) d\phi}$$

$$\xi_y^{(n+1)} = \frac{1}{N}\sum_{i=1}^{N} \frac{\int q_y \gamma_i(\phi, \Theta^{(n)}) d\phi}{\int \gamma_i(\phi, \Theta^{(n)}) d\phi}$$

$$\xi_\alpha^{(n+1)} = \frac{1}{N}\sum_{i=1}^{N} \frac{\int q_\alpha \gamma_i(\phi, \Theta^{(n)}) d\phi}{\int \gamma_i(\phi, \Theta^{(n)}) d\phi},$$

Alignment distribution
(x,y,rotation)

$$\sigma_x^{(n+1)} = \sqrt{\frac{1}{2N}\sum_{i=1}^{N} \frac{\int \left[\left(q_x - \xi_x^{(n)}\right)^2\right] \gamma_i(\phi, \Theta^{(n)}) d\phi}{\int \gamma_i(\phi, \Theta^{(n)}) d\phi}}$$

$$\sigma_y^{(n+1)} = \sqrt{\frac{1}{2N}\sum_{i=1}^{N} \frac{\int \left[\left(q_y - \xi_y^{(n)}\right)^2\right] \gamma_i(\phi, \Theta^{(n)}) d\phi}{\int \gamma_i(\phi, \Theta^{(n)}) d\phi}}$$

$$\sigma_\alpha^{(n+1)} = \sqrt{\frac{1}{N}\sum_{i=1}^{N} \frac{\int \left(q_\alpha - \xi_\alpha^{(n)}\right)^2 \gamma_i(\phi, \Theta^{(n)}) d\phi}{\int \gamma_i(\phi, \Theta^{(n)}) d\phi}}.$$

Sigworth, JSB 122 (1998)

Zeng, JSB 160 (2007)

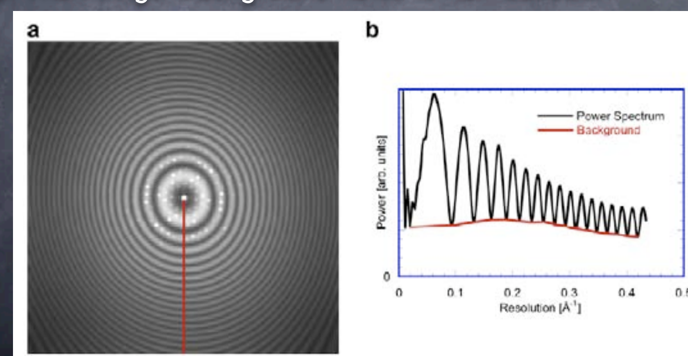
$\Theta = (A, \sigma, \xi)$

Maximum-Likelihood Processing

Assumption: Distributions are Gaussian

But: Distributions in TEM images aren't.

e.g.: Thon rings: Background noise is not Gaussian



Sigworth, JSB 122 (1998)

Zeng, JSB 160 (2007)

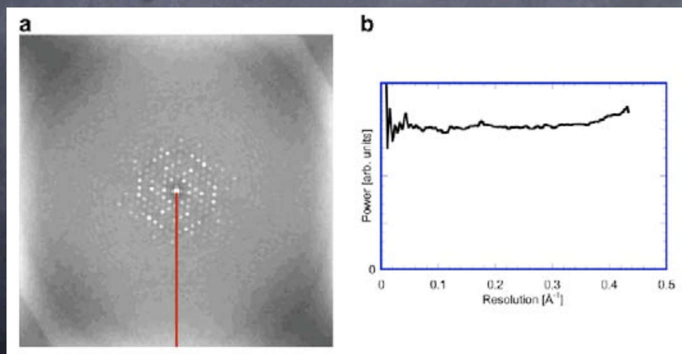
$\Theta = (A, \sigma, \xi)$

Maximum-Likelihood Processing

Assumption: Distributions are Gaussian

But: Distributions in TEM images aren't.

Noise whitening:

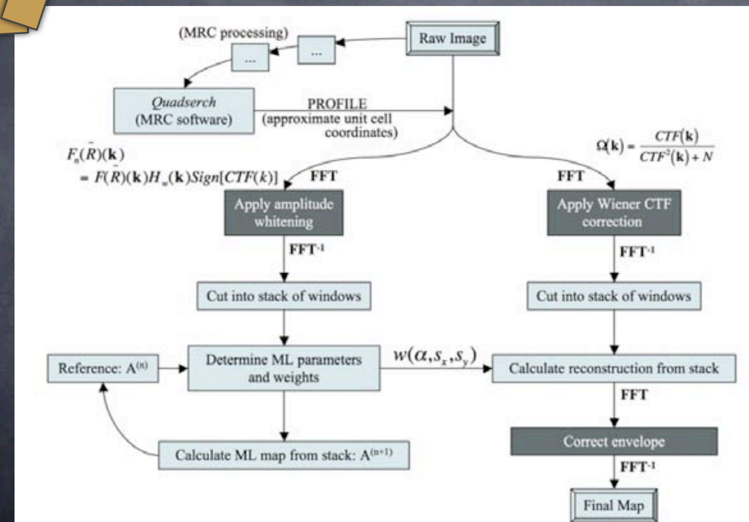


Sigworth, JSB 122 (1998)

Zeng, JSB 160 (2007)

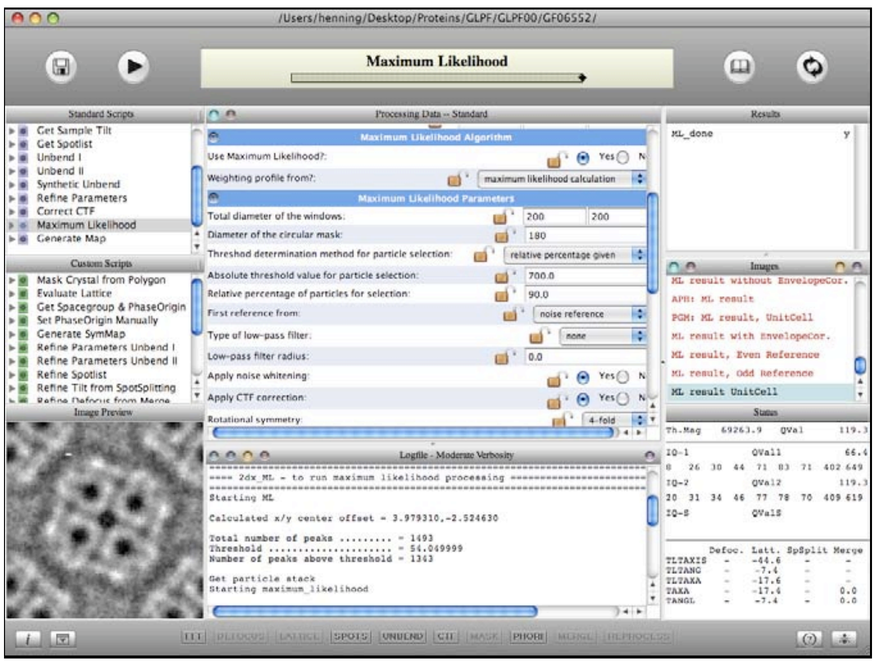
$\Theta = (A, \sigma, \xi)$

Maximum-Likelihood Processing

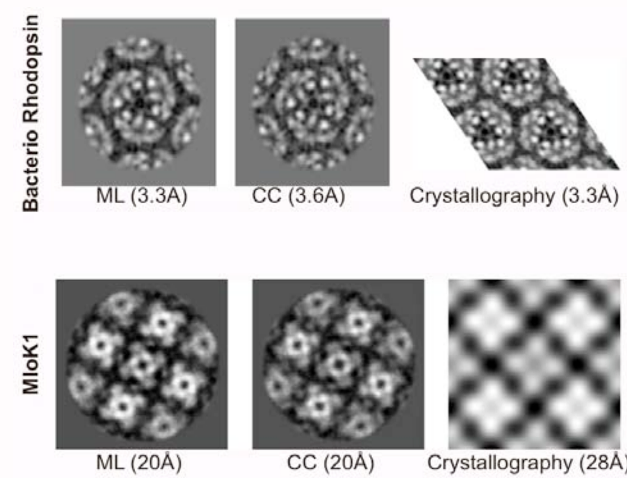


Sigworth, JSB 122 (1998)

Zeng, JSB 160 (2007)



A maximum likelihood approach to 2D crystals

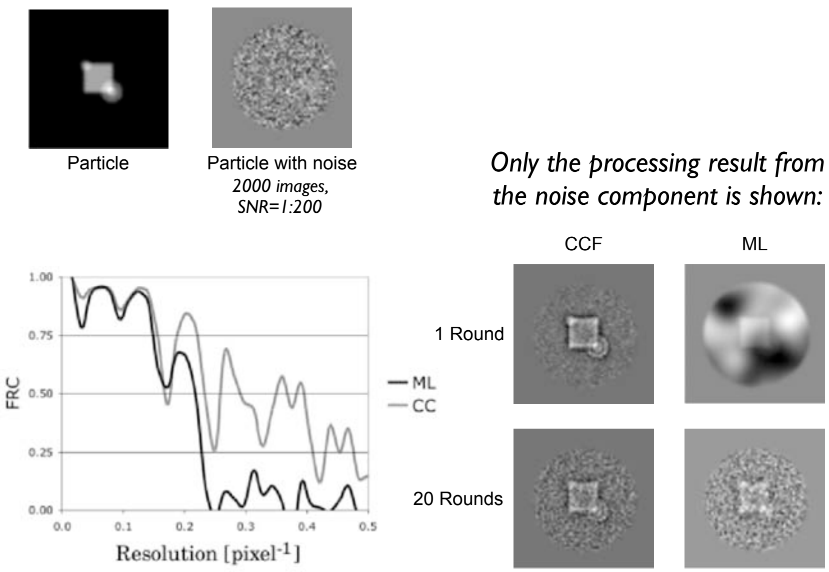


44

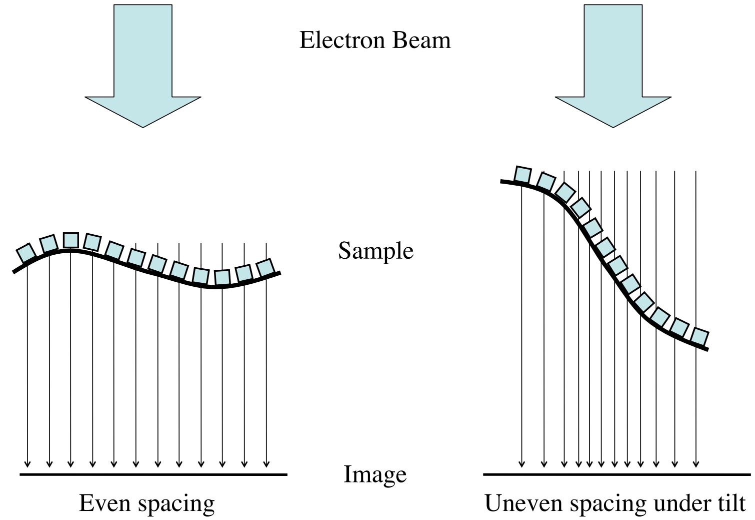
with Niko Grigorieff, Brandeis

Zeng et al., *J. Struct. Biol.* (2007)

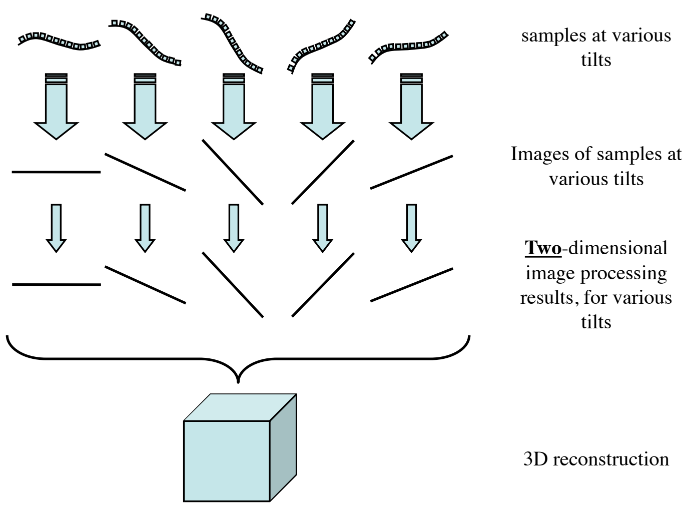
Maximum Likelihood is not entirely free of reference bias



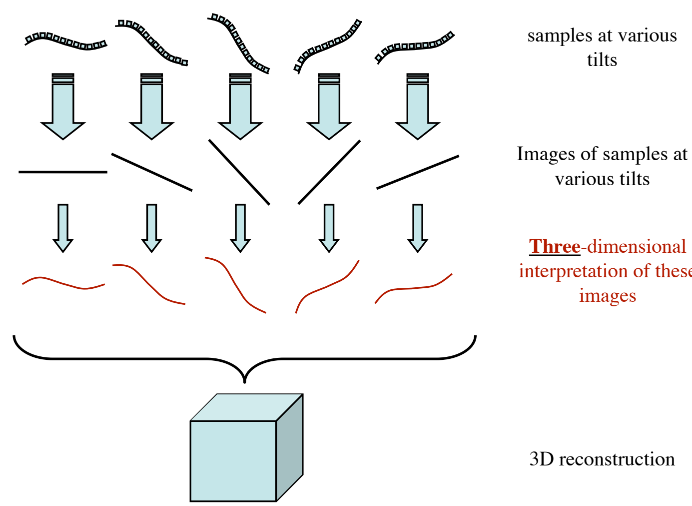
Badly prepared crystals are not flat.



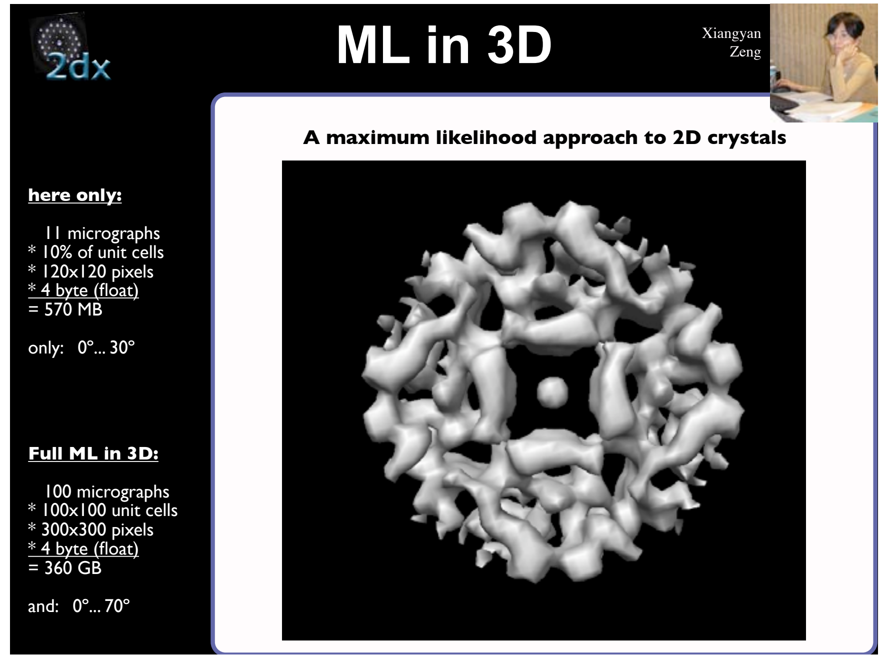
Badly prepared crystals are not flat.
This leads to resolution loss.



Badly prepared crystals are not flat.
This should not lead to resolution loss.




ML in 3D
2dx_merge File Edit Options View Select
Wed 10:07 AM
Maximum Likelihood
Standard Scripts
Initialization
Merge Once
Refine Once
Merge & Refine (iterative)
Generate Image Maps
Final Merge
Generate Merged Map
(Re-)Process all images
Custom Scripts
Maximum Likelihood
Modify Image Parameter
Copy Merged Dataset
Generate Map from Saved D...
Display Maps
Refresh Databases
Custom Script 1
Custom Script 2
Custom Script 3
Image Preview
Merge Directory TAXA TANGL PhaOri Change
GF06693_30b 30.94 -30.35 -1.20,1.80
GF06694_30b 38.57 31.19 -1.20,-0.30
GLPF45
GF08005 6.60 46.97 1.20,-12.30
GF08013 45.94 46.09 -6.00,-11.40
Processing Data -- Standard
Maximum Likelihood Algorithm
Weighting profile from?: maximum likelihood calculation
Maximum Likelihood Parameters
Total diameter of the windows: 120 120
Inner image diameter of the windows: 120 120
Diameter of the circular mask: 100
Threshold determination method for particle selection: relative percentage given
Absolute threshold value for particle selection: 700.0
Relative percentage of particles for selection: 90.0
First reference from: noise reference
Type of low-pass filter: none
Low-pass filter radius: 0.0
Logfile - Low Verbosity
Results
Parameter Value
Images


2dx
ML in 3D
Xiangyan
Zeng
A maximum likelihood approach to 2D crystals
here only:
11 micrographs
* 10% of unit cells
* 120x120 pixels
* 4 byte (float)
= 570 MB
only: 0°... 30°
Full ML in 3D:
100 micrographs
* 100x100 unit cells
* 300x300 pixels
* 4 byte (float)
= 360 GB
and: 0°... 70°


2dx.org
stahlberglab.ucdavis.edu
Acknowledgements
Expression
Purification
2D Crystallization
Sample Prep
Imaging
Image Processing
Model Building
MloK1:
Crina Nimigean
ClC-ec1:
Joe Mindell
Yoshinori Fujiyoshi
HIO:
John Spence
ML:
Niko Grigorieff
James Buban
David Carlson
Po-Lin Chiu
Hui-Ting Chou
Lenin Dominguez
Sumeet Hayer
Bryant Gipson
Mike Nielsen
Matthew Pagel
Ludovic Renault
Funding:
NSF-Bio CAREER,
NIH NHLBI R01GM081653,
NIH NIGMS Protein Structure Initiative.