Estimating Partialities in Serial Crystallography with EVAL

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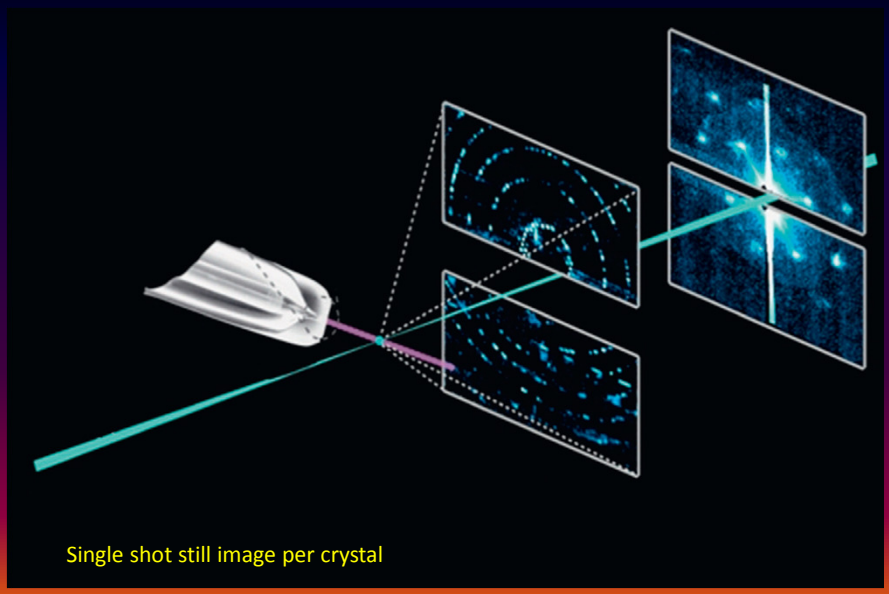
SFX experiment at LCLS



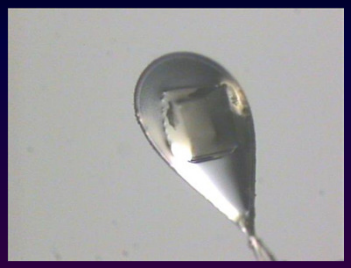
Why is still data collection so different from rotation data collection?

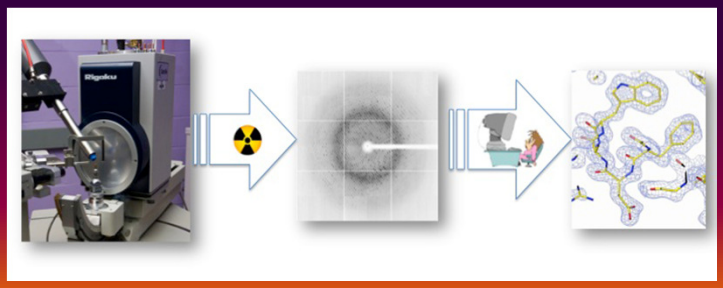
EVAL15

- Data integration software
- Prediction of reflection profiles from diffraction of crystals: ray tracing based on only a few physical parameters
- Use profiles in lsq-fit to get integrated intensity
- Adapted for treating still data

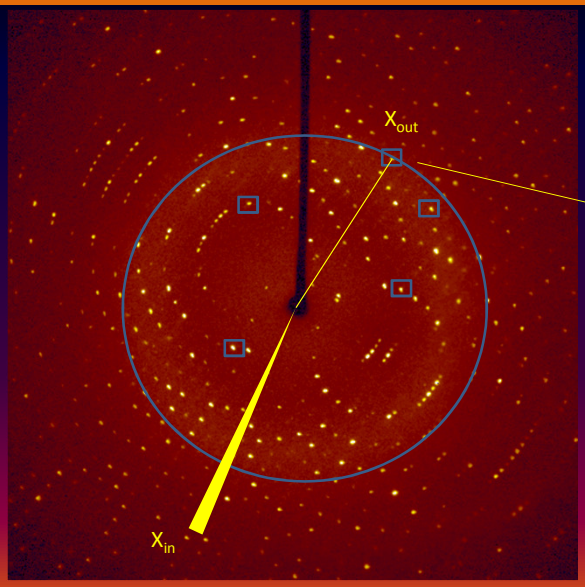
A.M.M. Schreurs, X. Xian and L.M.J. Kroon-Batenburg
EVAL15: a diffraction data integration method based on ab initio predicted profiles
J. Appl. Cryst. **43**, (2010) 70-82
L.M.J. Kroon-Batenburg, A.M.M. Schreurs, R.B.G. Ravelli, P.Gros, submitted.

Crystallographic data collection

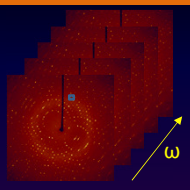




Indexing



0.5° rotation image



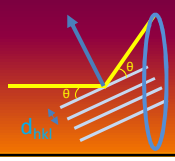
2θ from centroids

$\rightarrow d^*_{hkl}$

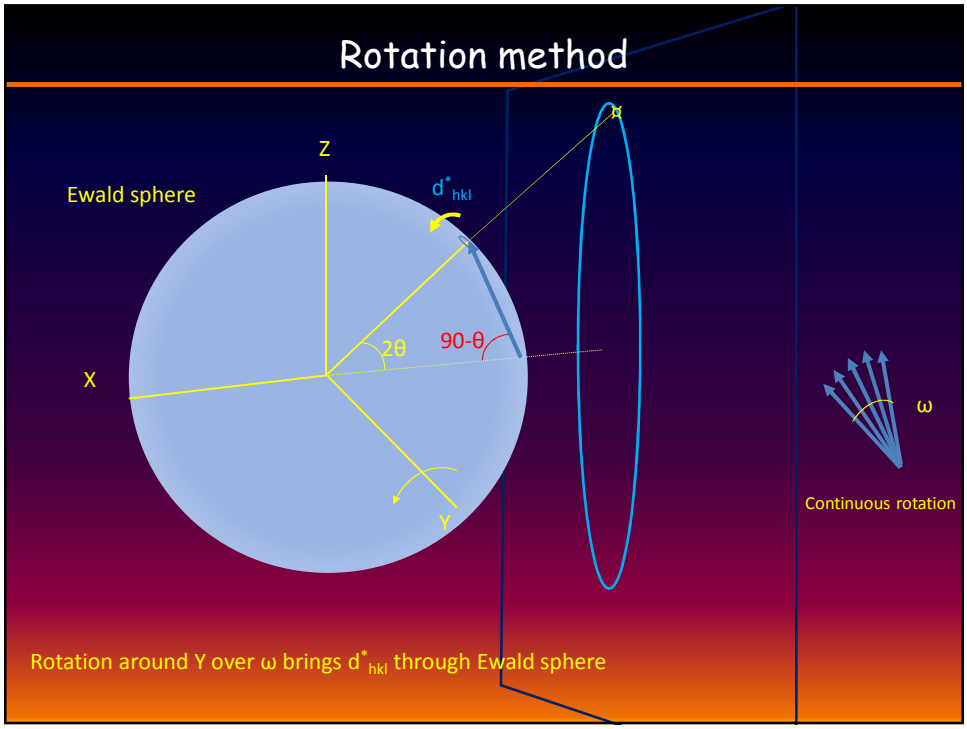
$\{\cdots, d^*_{hkl}, \cdots\}$

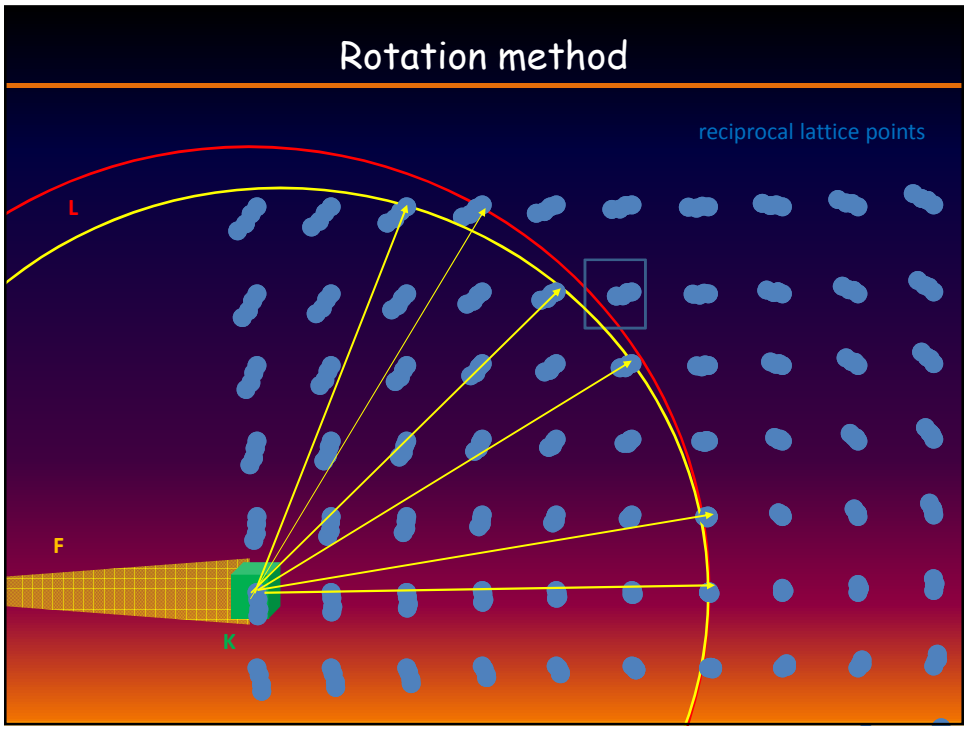
$a^*, b^*, c^*, \alpha^*, \beta^*, \gamma^*$

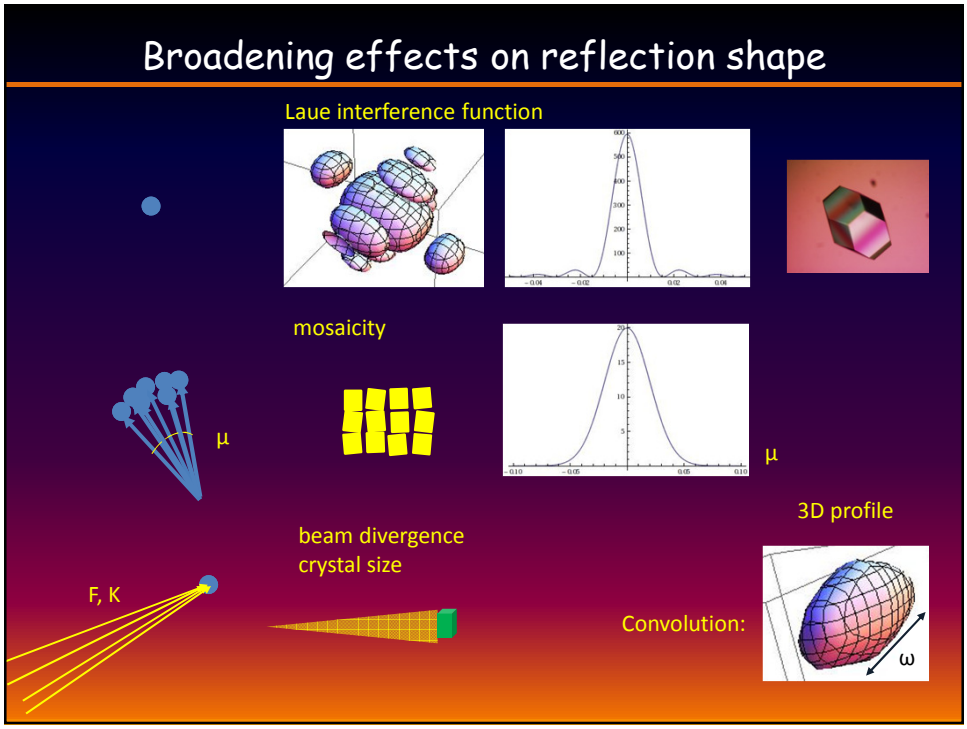
$R\phi_x, R\phi_y, R\phi_z$

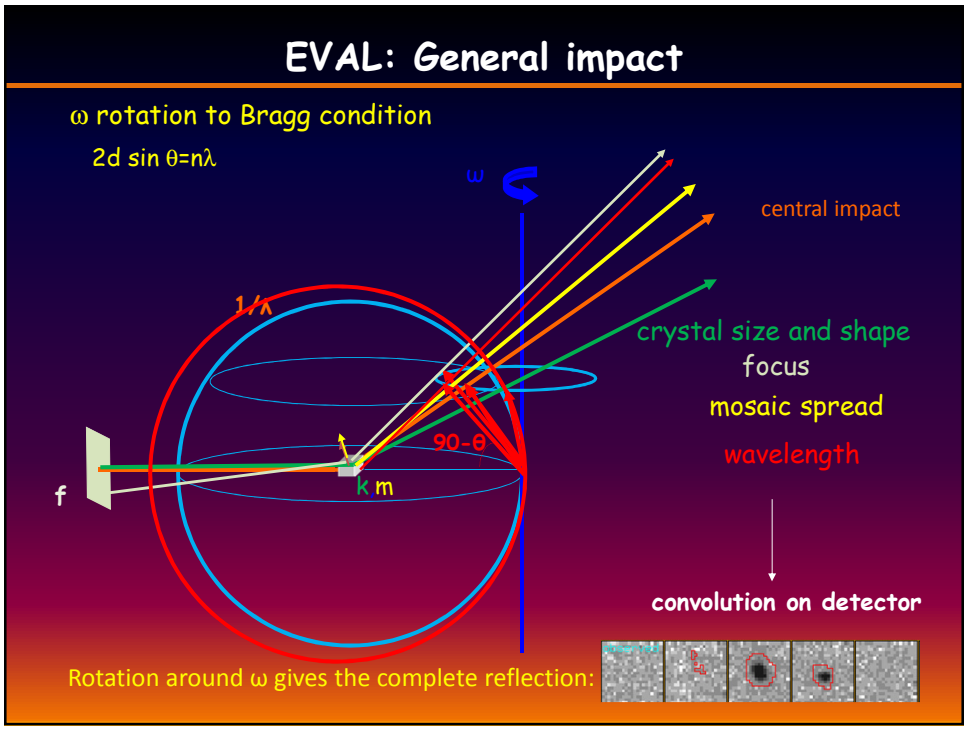


Bragg condition
$2d\sin\theta = n\lambda$

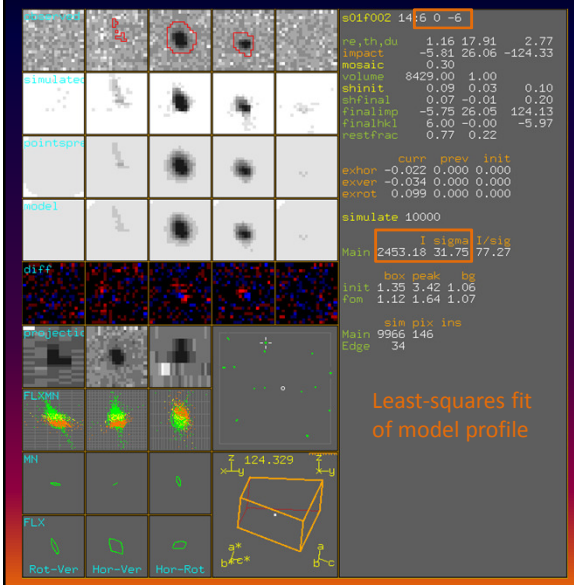

Rotation method
Z
Ewald sphere
d*hkl
2θ
90-θ
X
Y
ω
Continuous rotation
Rotation around Y over ω brings d*hkl through Ewald sphere


Rotation method
reciprocal lattice points
L
F
K


Broadening effects on reflection shape
Laue interference function
mosaicity
μ
μ
3D profile
beam divergence
crystal size
F, K
Convolution:
ω

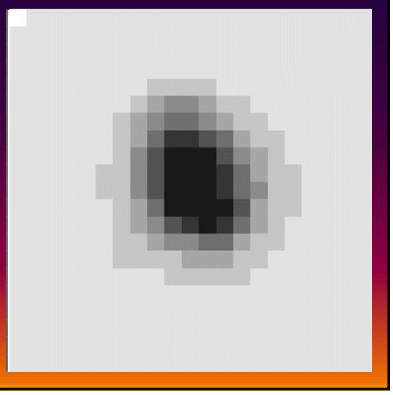

EVAL: General impact
ω rotation to Bragg condition
2d sin θ=nλ
ω
central impact
1/λ
crystal size and shape
focus
mosaic spread
wavelength
90-θ
f
k m
convolution on detector
Rotation around ω gives the complete reflection:

Simulating impacts by ray tracing: EVAL15 predicted profile



Least-squares fit of model profile

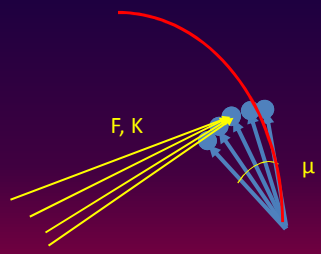
Profile parameters are usually:
beam size and divergence,
wavelength dispersion, detector pointspread,
crystal size, mosaic spread



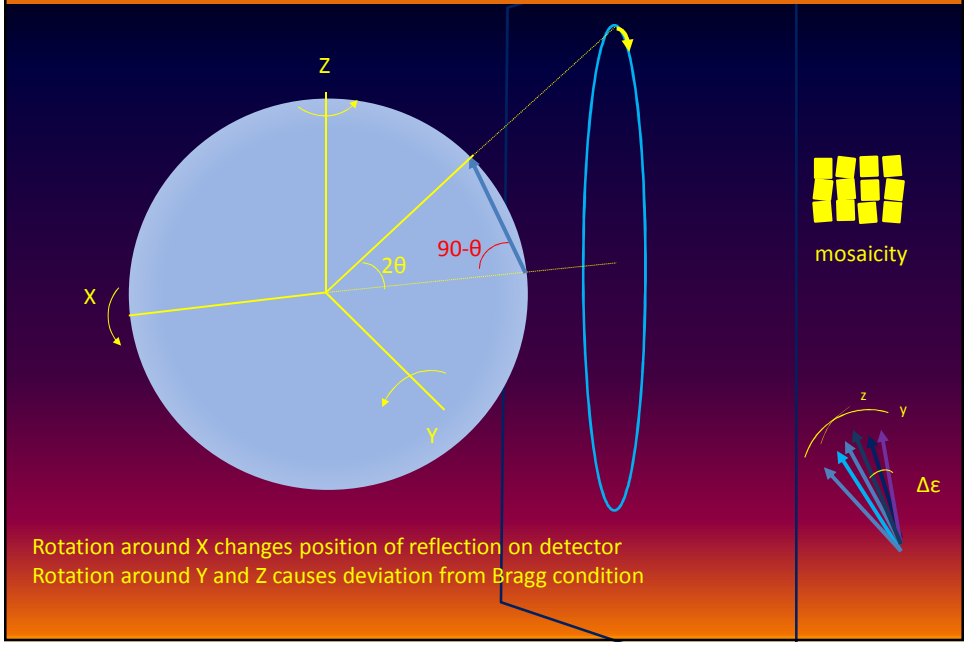
sucrose

Indexing of still images

1) Reflection positions do not correspond to centroids: unit cells are less accurate



Still data collection

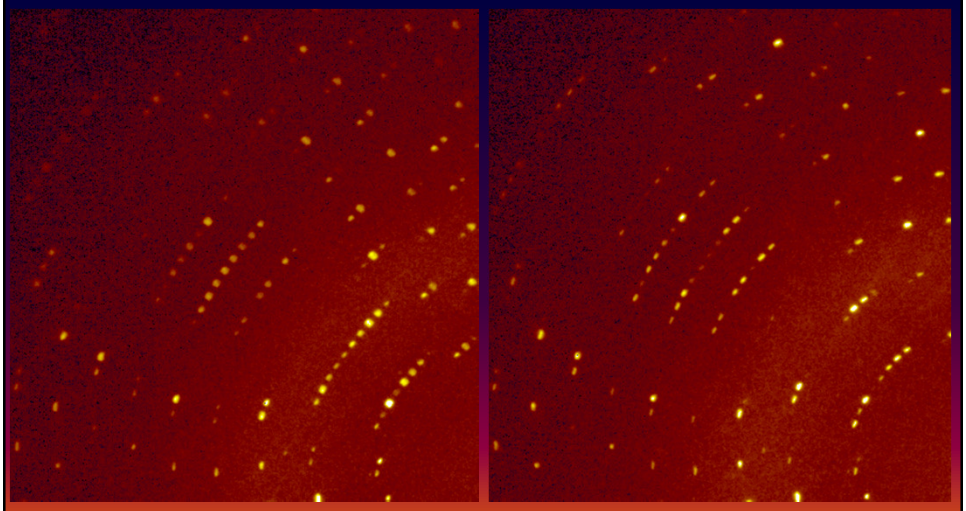


Rotation around X changes position of reflection on detector
Rotation around Y and Z causes deviation from Bragg condition

Rotation vs still images

Rotation 0.5°

still



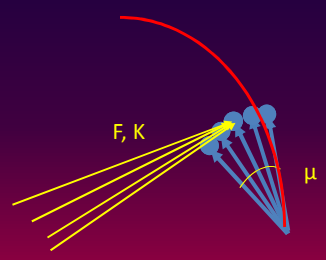
Zoomed in diffraction images for HEWL

Reflections on still images are only partially observed

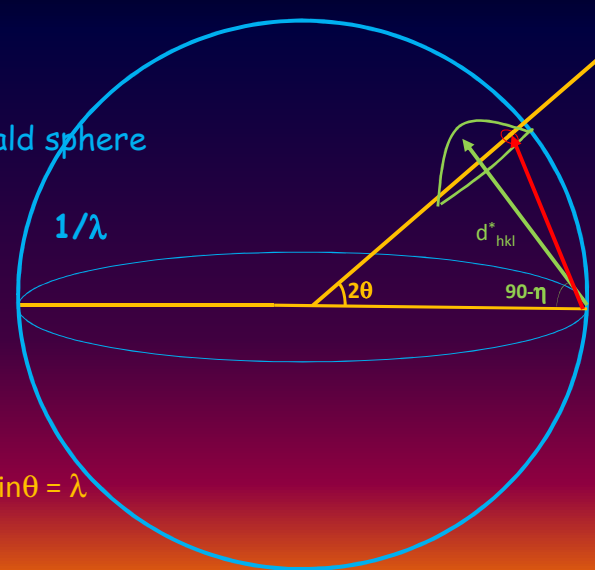
2) Reflections do not all have the same overlap with the Ewald sphere

$$I_i \propto I_0 VLpA\ P_i F_{hkl}^2$$

partiality



EVAL ray tracing of still images

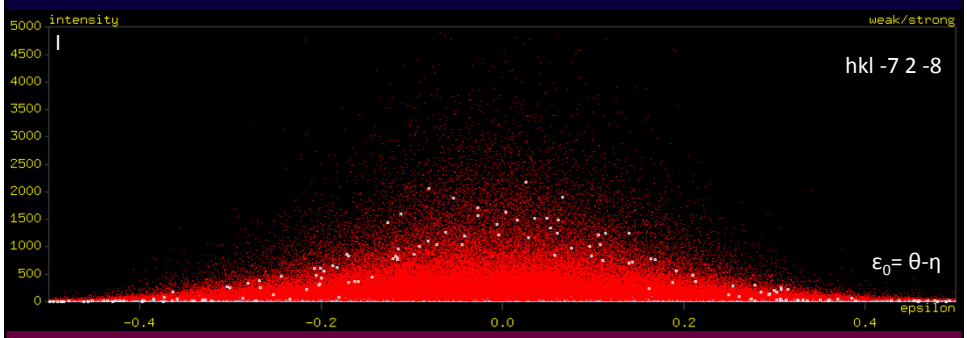


mosaic crystal

r.l. vectors from tail of mosaic distribution may be in Bragg condition

How much of the complete reflection is visible?

Intensity distribution of reflections



Solution -> Monte Carlo integration -> F^2

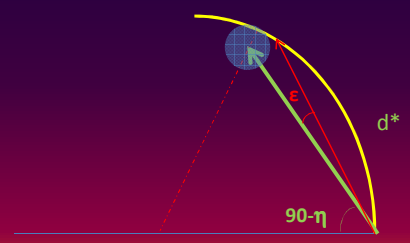
Kirian RA, Wang X, Weierstall U, Schmidt KE, Spence JC, Hunter M, Fromme P, White T, Chapman HN, Holton J., Opt Express. 18 (2010) 5713-5723. Femtosecond protein nanocrystallography-data analysis methods.

Challenge for ray tracing partiality estimation

Can the statistics of Monte Carlo integration be improved by partiality correction or can the required number of images be reduced?

Ray tracing for stills

In the ray tracing from F, L, K and M sample points, each d* vector should be given a finite intensity when it is close to the Ewald sphere



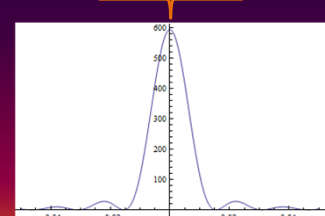
Off Bragg scattering

Interference function gives finite contribution to scattering for off-Bragg reciprocal lattice vectors

Angular deviation of Bragg angle ε= θ-η

$$I(\varepsilon) = I_0 F^2 p \left(\frac{e^2}{mc^2}\right)^2 N^2 \lambda^3 \frac{1}{2\sin^2\theta} dV \frac{1}{s} \underbrace{\frac{\sin^2(sB\varepsilon)}{(B\varepsilon)^2}}$$

B=2πd cosθ/λ



R. James & M.J. Buerger
Bragg scattering by stacks of s lattice planes separated by d
Reflectivity of planes in terms of Fresnel zones: amplitude ~ 1/sinθ

Still Lorentz factor

Integration over (uniform) ε distribution:

$$I = [I_0 F^2 p(\frac{e^2}{mc^2})^2 \lambda^3 N^2] \frac{1}{2\sin^2\theta} \frac{\pi}{B} = [..] \frac{1}{2\sin\theta\cos\theta}$$

Integrated intensity proportinal to the "still Lorentz-factor"

Implementation in EVAL: ray tracing

Each ray has contribution:

$$I(\varepsilon_i) = \frac{1}{2\sin^2\theta_\lambda} \frac{1}{s} \frac{\sin^2(sB\varepsilon_i)}{(B\varepsilon_i)^2}$$

$s=N_{cell}\cdot(|h|+|k|+|l|)$

- contains the still Lorentz factor
- Ncell is determined by the coherence length ~0.1 µm
- Ray tracing takes account of all broadening effects

Partiality = $\Sigma_i I_i / L_{still}$

Hen Egg White Lysozyme

Test data of stills with corresponding rotation data

Proteum home source : rotating anode Cu Kα radiation, Helios mirrors , Kappa goniometer, Platinum CCD detector

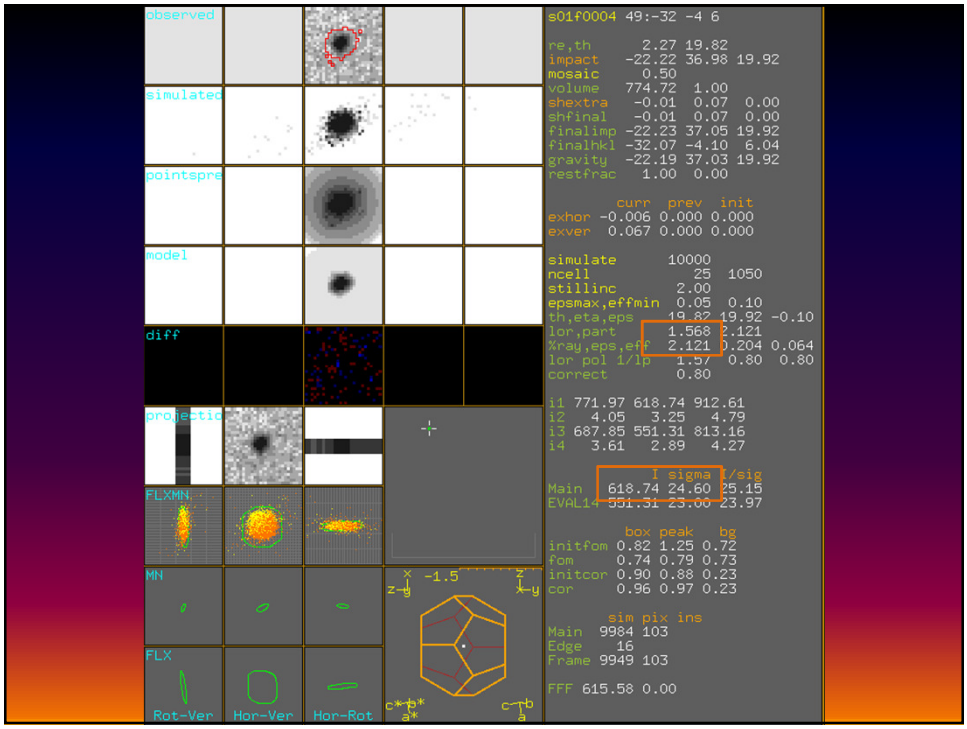
- 380 0.5 °oscillation scan around (horizontal) ϕ-axis — Rots
- 380 stills with 0.5° steps in the same orientations
- 494 stills with varying positions of ϕ,ω,κ

} Stills

Still frames:
- are indexed individually
- cell matrix is refined individually

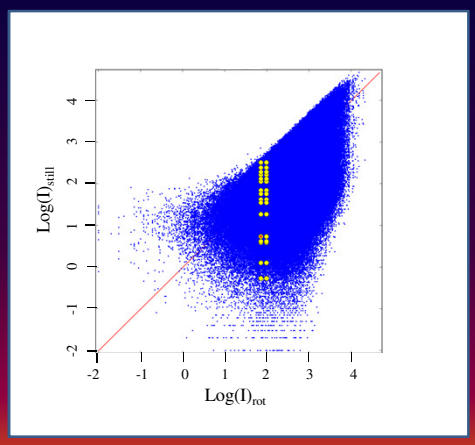
Data statistics: indexing

	EVAL Rotation	CrystFEL	EVAL	EVAL Post-refined
# images	380	774	774	
# peaks used for indexing	649	300-490[a]	289-300[a]	
# peaks used in refinement	101246		230759	396201
Cell a,b (Å)	78.666	78.735-79.453	78.679-79.119	78.719-79.049
c (Å)	36.819	36.819-37.481	36.706-36.989	36.810-36.980
orientation rms (°) a^*			0.102	0.103
b^*			0.108	0.095
c^*			0.107	0.111
Average residuals: hor, ver (mm) ε_0 (°)	0.0622 0.0390		0.0766 0.1496[b]	0.099 0.173[b]

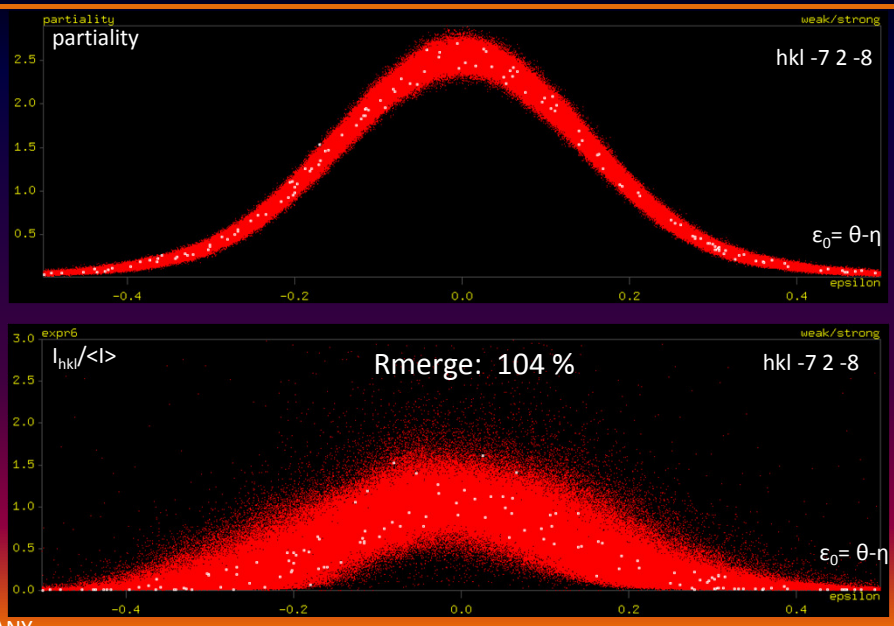


Comparison of still and rotation intensities

Lorentz correction applied



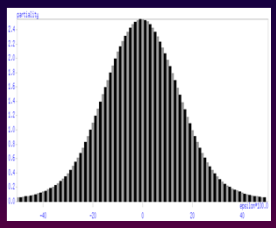
Partiality and relative intensities of equivalent reflections



ANY

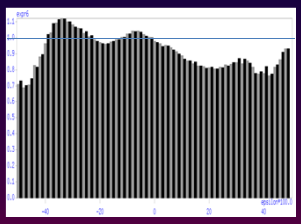
Averaged in ε bins

partiality



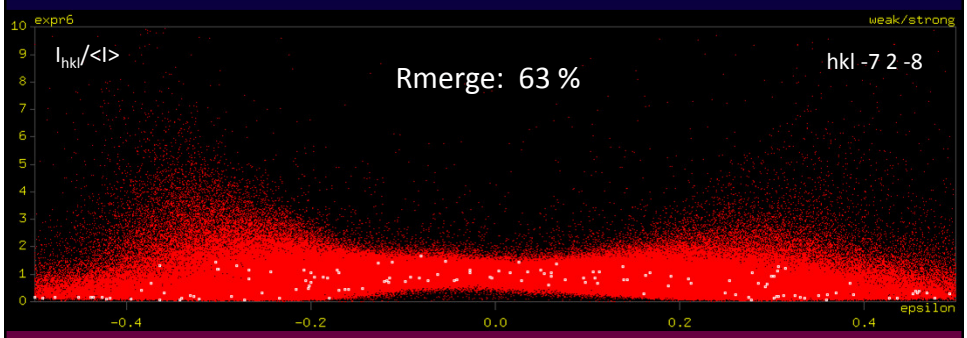
I_{hkl}/<I>

Corrected I_{hkl}/<I>



ε

Relative intensities after correction for partiality



$\varepsilon_0 = \theta - \eta$

Postrefinement: Rmerge 55.7%

Data statistics: quality

	EVAL Rotation	CrystFEL Monte Carlo	EVAL Monte Carlo	EVAL partiality corrected
# reflections	106021	733504	657782	657782
# unique[a]	8291		8352	8352
Multiplicity	12.8	88.4	78.8	78.8
Completeness (%)	100	100	99.2	99.2
$CC_{1/2}$ (%)	100	--	94.1 (88.6)	97.5 (90.9)
I/σ(I) merged	47.7 (20.3)	10.1 (5.7)	11.5(7.4)	15.1(9.0)
R_{int} (%)	3.8 (7.1)	--	104.9 (91.2)	63.8 (63.4)
Post-refinement				
I/σ(I) merged postrefinement				18.4 (10.8)
R_{int} (%) postrefinement				55.7 (55.1)

Comparison with rotation data

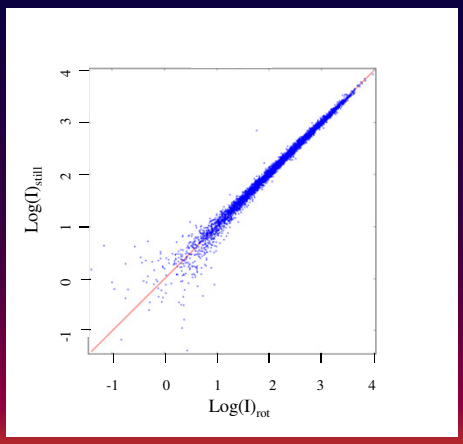
Same crystal and same exposure time

merged reflections

	CrystFEL Monte Carlo	EVAL Monte Carlo	EVAL Lorentz correction only	EVAL Partiality corrected	EVAL Partiality corrected post-refinement
R_{comp} (%)[a]	32.0	26.4	12.0	5.3	4.7
R on F (%)[b]	18.9	15.2	9.0	4.1	3.1

Comparison of still and rotation intensities

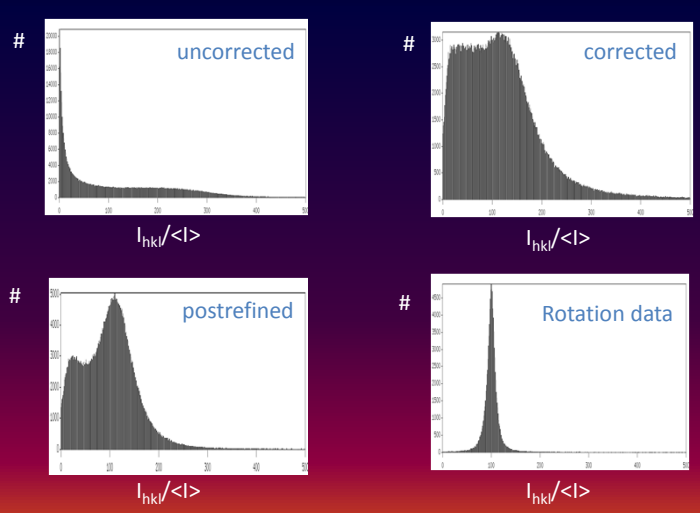
After partiality correction and merging



Comparison with rotation data

	EVAL	CrystFEL Monte Carlo	EVAL Monte Carlo	EVAL Lorentz correction only	EVAL Partiality corrected	EVAL Partiality corrected Post-refined
	Rotation	All stills	All stills	All stills	All stills	All stils
Refinement[a]						
R_{work} (%)	15.9	17.4	16.7	16.6	16.0	16.0
R_{free} (%)	19.8	21.4	20.2	20.2	20.0	20.2
<B> (Å^2)	13.8	25.0	20.6	11.8	13.2	13.4
Average anomalous densities[b](σ)						
# reflections[c]	6420	6614	6595	6571	6403	6406
SD_MET	13.3	3.2	4.2	4.6	6.3	7.2
SG_CYS	10.9	2.1	3.3	3.3	5.2	6.0
Cl^-	5.0	1.0	1.0	1.2	2.1	2.3
Na^+	1.8	--	0.8	0.8	1.5	1.5

Intensity distributions



Conclusions and plans

- EVAL ray tracing partiality prediction reduces R factors by 50%
- Statistical averaging (Monte Carlo) is very powerful
- Still data should be corrected for the still Lorentz factor
- Post-refinement improves partialities
- EVAL method can be used for many instrumentental and sample conditions
- Apply method to XFEL data of µm and nm sized crystals