PAUL SCHERRER INSTITUT

PSI

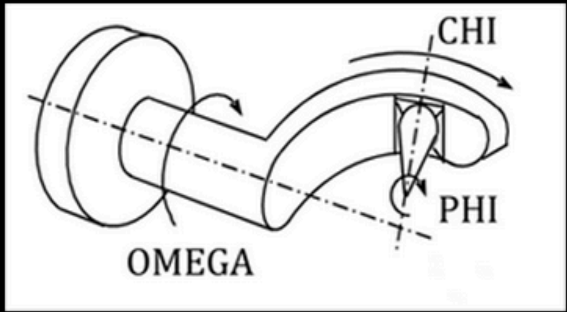
Workshop on Advanced Data Collection with Multi-Axis Goniometry

Wir schaffen Wissen – heute für morgen

Paul Scherrer Institut, Swiss Light Source

Meitian Wang

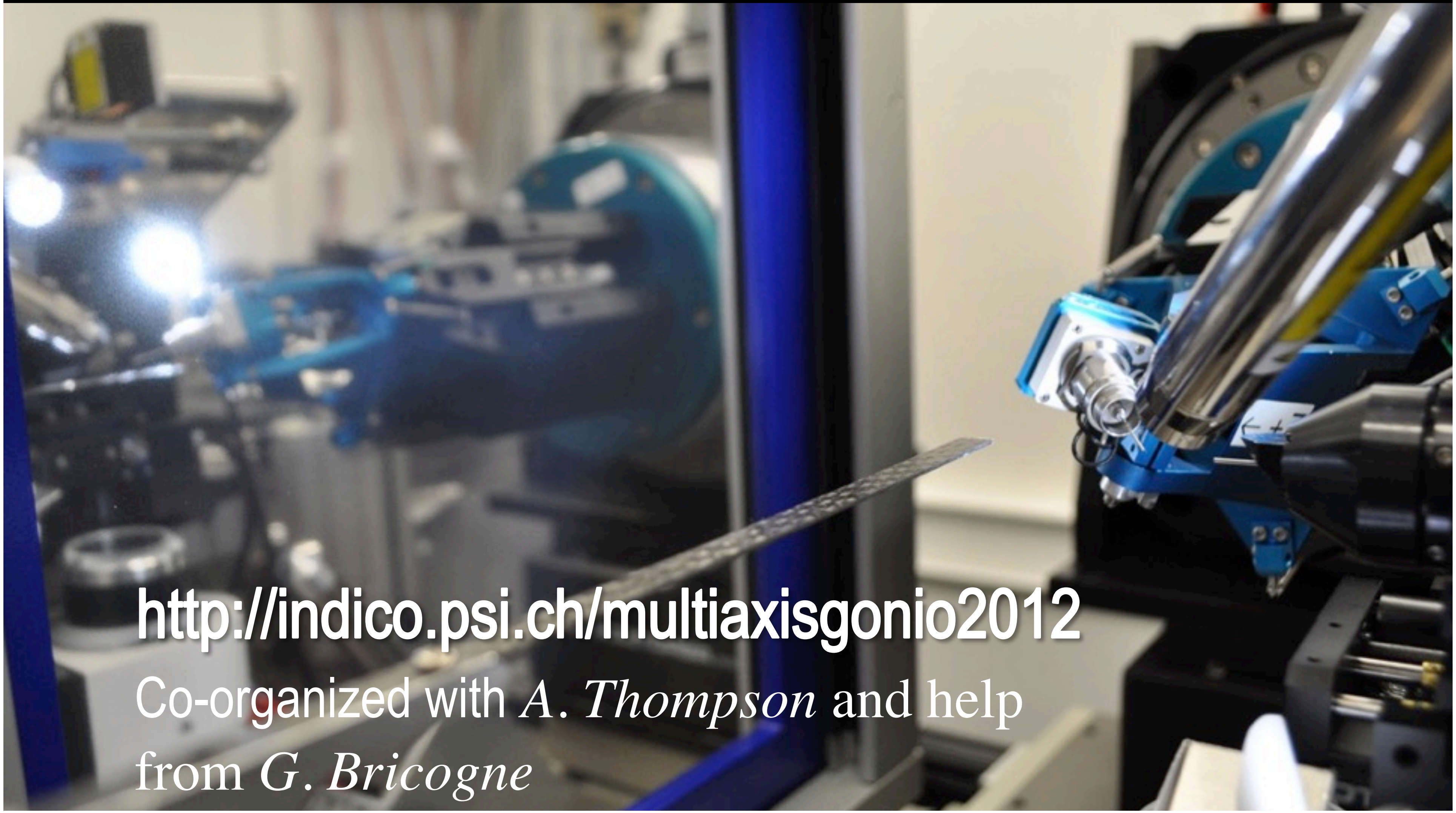
A Better Data Collection Strategy: You Can Always Use a Lower Dose



Workshop on Advanced Data Collection with Multi-Axis Goniometer and Single-Photon Counting Detector

6-8 November 2012 *Paul Scherrer Institut*

Europe/Zurich timezone



http://indico.psi.ch/multiaxisgonio2012

Co-organized with *A. Thompson* and help from *G. Bricogne*

Three PX Beamlines at the Swiss Light Source

Beamline	PXI (X06SA)	PXII (X10SA)	PXIII (X06DA)
Source	U19	U19	2.9T Superbend
Energy range	6.0 – 17.5 keV	6.5 – 20.0 keV	5.5 – 17.5 keV
Flux, phs/s (12.4 keV, focused beam)	2×10^{11} <–> 2×10^{12}	2×10^{12}	5×10^{11}
Beamsize, μm^2 (with focusing, slits)	**2 × 1 <–> 100 × 100 (fast beam size change)**	50 × 10 30 × 10, 20 × 10, 10 × 10	80 × 45 μm^2
Goniometer	Micro-diffractometer (SmarGon)		**Multi-axis, PRIGo (SmarGon)**
Detector	**EIGER 16M**	**PILATUS 6M**	**PILATUS 2M**
Data collection time	2 – 3 minutes		
Sample changer	IRELEC CATS		
Industrial usage	15%	50%	40%

Geometric Data Collection

Unique volume and unique reflections

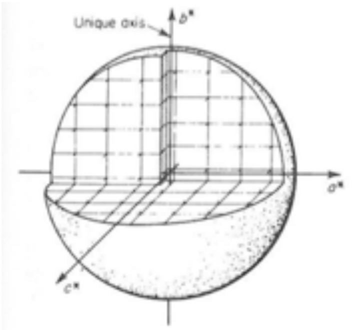


Figure 6.6. Unique volume in reciprocal space for a monoclinic crystal.

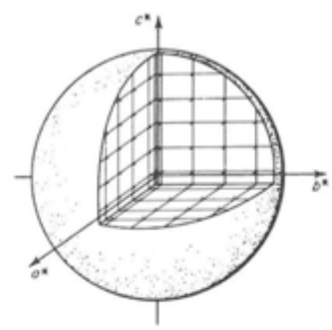
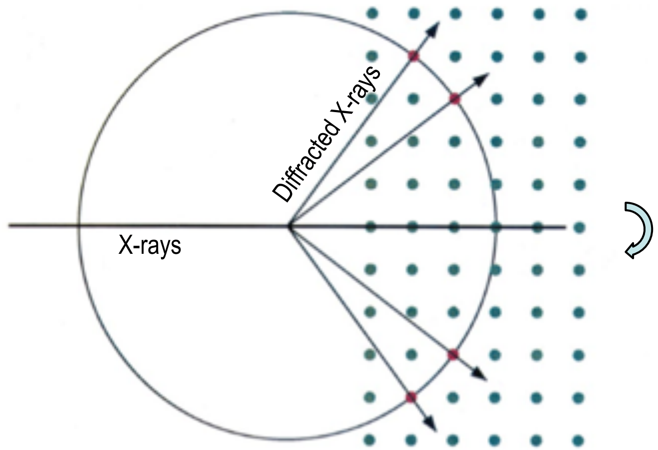


Figure 6.7. Unique volume in reciprocal space for an orthorhombic crystal.



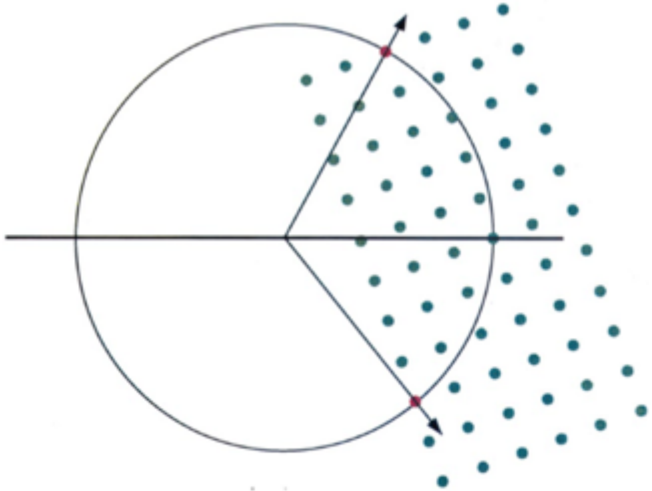


Table 1
Rotation range (°) required to collect a complete data set in different crystal classes.

The direction of the spindle axis is given in parentheses; *ac* means any vector in the *ac* plane.

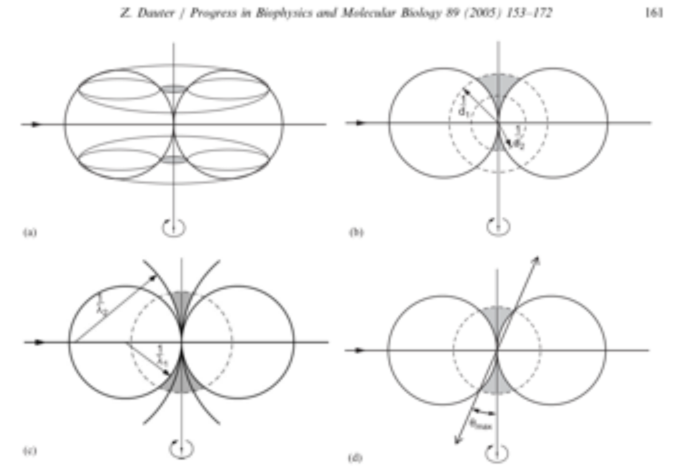
Point group	Native data	Anomalous data
1	180 (any)	180 + $2\theta_{max}$ (any)
2	180 (*b*); 90 (*ac*)	180 (*b*); 180 + $2\theta_{max}$ (*ac*)
222	90 (*ab* or *ac* or *bc*)	90 (*ab* or *ac* or *bc*)
4	90 (*c* or *ab*)	90 (*c*); 90 + θ_{max} (*ab*)
422	45 (*c*); 90 (*ab*)	45 (*c*); 90 (*ab*)
3	60 (*c*); 90 (*ab*)	60 + $2\theta_{max}$ (*c*); 90 + θ_{max} (*ab*)
32	30 (*c*); 90 (*ab*)	30 + θ_{max} (*c*); 90 (*ab*)
6	60 (*c*); 90 (*ab*)	60 (*c*); 90 + θ_{max} (*ab*)
622	30 (*c*); 90 (*ab*)	30 (*c*); 90 (*ab*)
23	~60	~70
432	~35	~45

Rotation method and rotation range

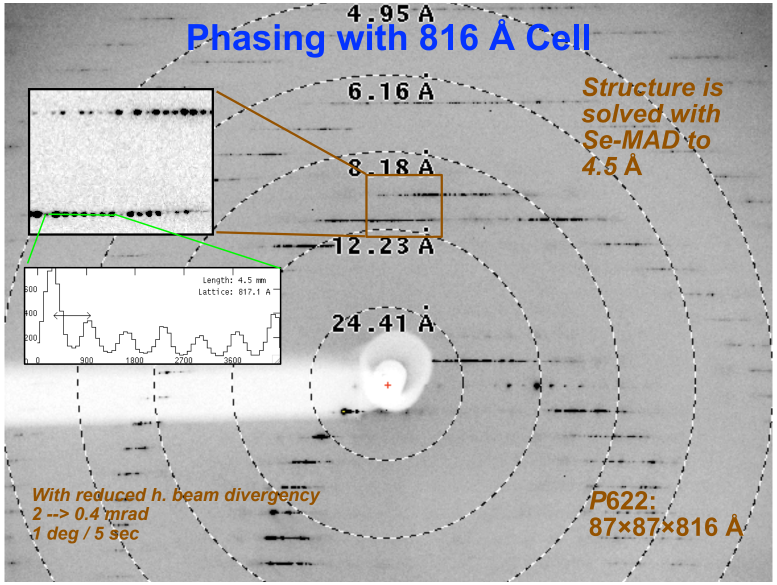
Stout and Jensen (1989), Dauter, *Acta Cryst. D***55,** 1703 (1999)

Geometric Data Collection with Multi-Axis Goniometry

- **Avoid blind region**

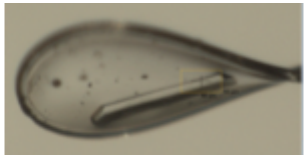


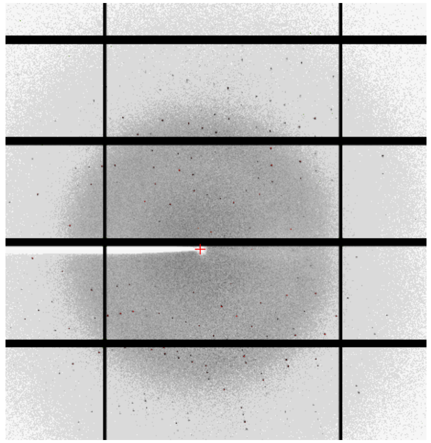
- **Align long unit-cell axis to avoid spots overlaps**



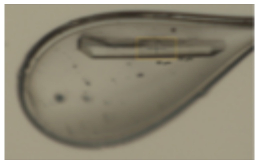
- **Align even-fold rotation axis to record Bijvoet pairs on the same image**

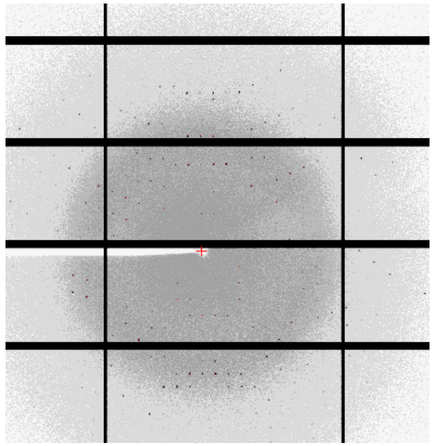
Misaligned:





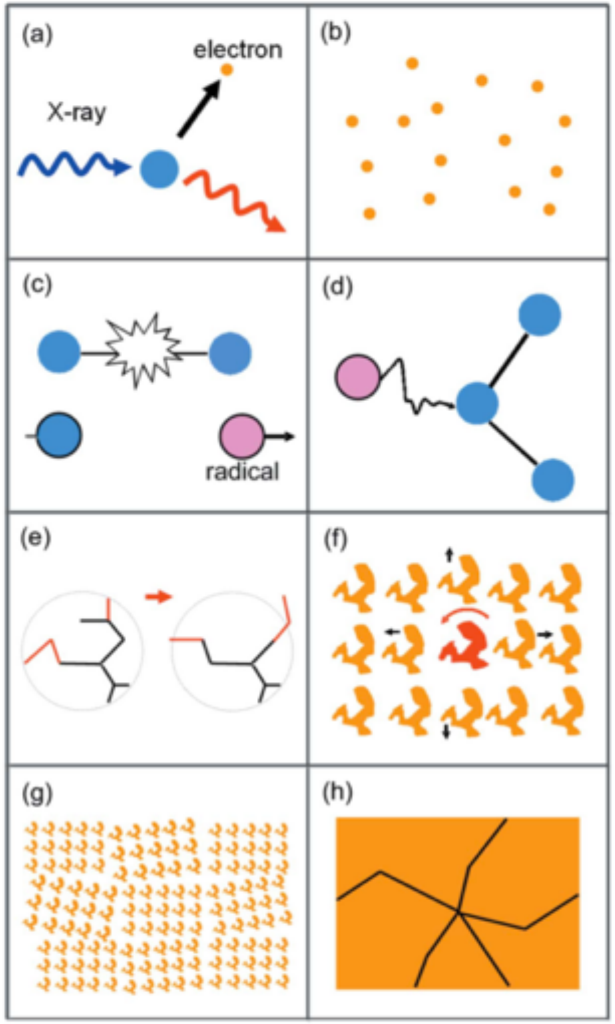
Aligned to twofold:





(XOAlign, Stratcalc)

Radiation Damage



M. Warkentin *et al. J. Synchrotron Rad.* **20,** 7 (2013)

Native data collection (20 MGy)

- Henderson, *Proc. R. Soc. B.* **241**, 6 (1990)
- Owen, *et al. Proc. Natl. Acad. Sci. USA*, **103**, 4912 (2006)

Experimental phasing (< 5 MGy)

- Holton, J. M. *J. Synchrotron Rad.* **14**, 51 (2007)
- Olieric, *et al.* *Acta Cryst.* **D63**, 759 (2007)

Rule of thumb

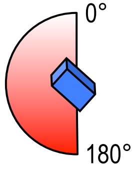
- Resolution dependency of 10 MGy / Å, Howells *et al. J. El. Spect. & Rel. Phen.* **170**, 4 (2009)
- Scaling *B* factor *vs.* dose of ~ 1 *B*-factor / MGy, Kmetko *et al. Acta Cryst.* **D62**, 1030 (2006)
- Does estimation, Holton, *J. Synchrotron Rad.* **16**, 133 (2009)

  $Dose = (t_{expo} \times flux) / (k_{dose} \times I_{H\text{-}beam} \times I_{B\text{-}beam})$

  $k_{dose} = 2000\lambda^{-2}$

"Traditional" Data Collection Strategy

High-dose low multiplicity data collection strategy
(180° data within dose limit)



Intensity Data Collection: Counting Statistics

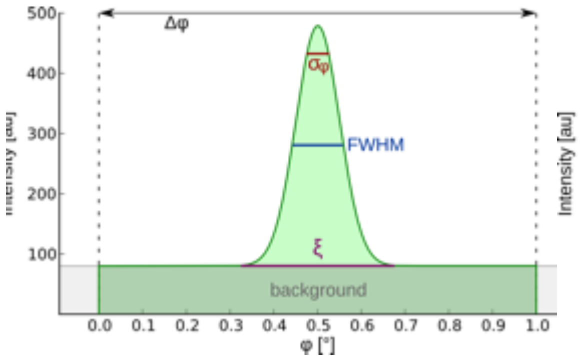
Random errors, counting statistics

$\sigma_{count} = N^{1/2}$

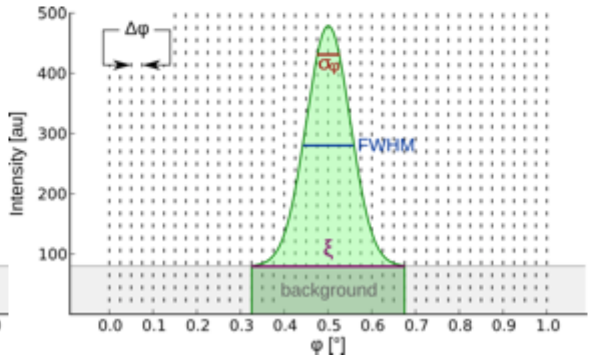
$I = N_p - N_b$ Signal is the difference

$\sigma_I = (\sigma_p{}^2 + \sigma_b{}^2)^{1/2}$

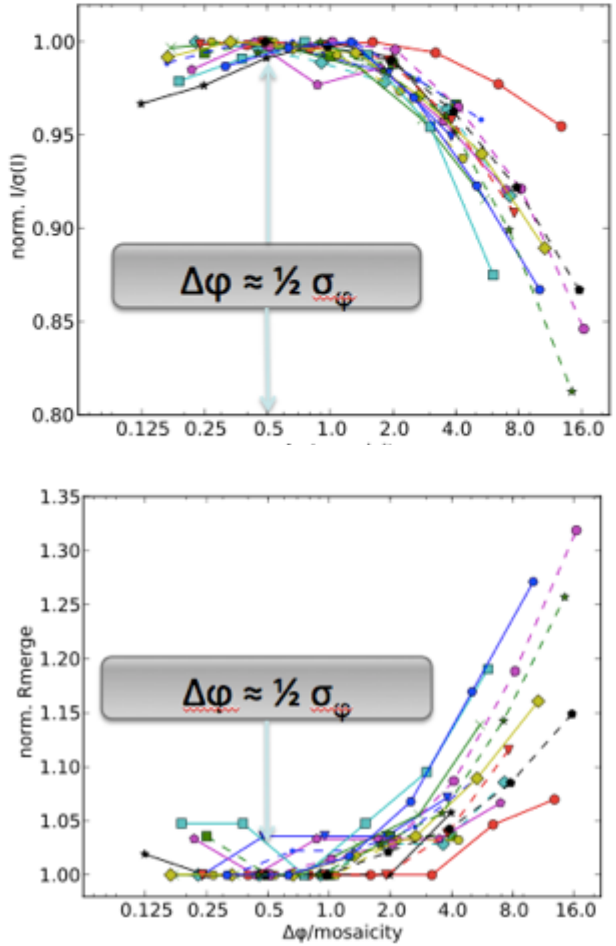
$\sigma_I = (N_p + N_b)^{1/2}$ Uncertainty is the sum



Wide φ-slicing
- Large $\Delta\varphi$ ($\Delta\varphi > \xi$)
- Large overlap of reflections and background along φ
- Few images



Fine φ-slicing
- Small $\Delta\varphi$ ($\Delta\varphi << \xi$)
- Minimal overlap of reflections and background along φ
- Many images

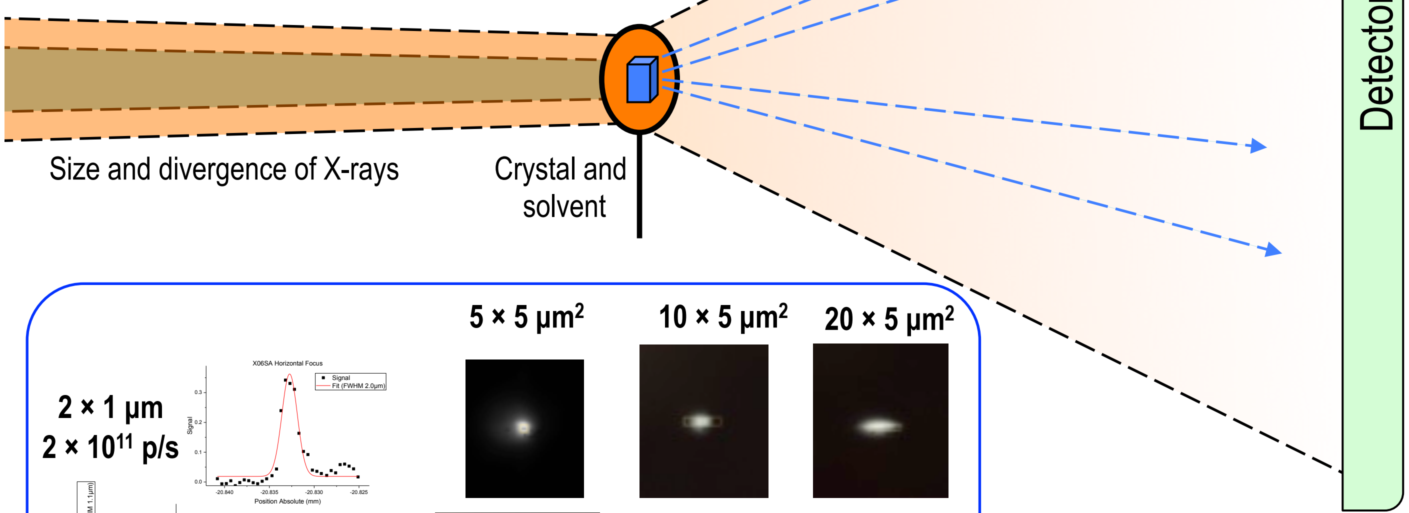


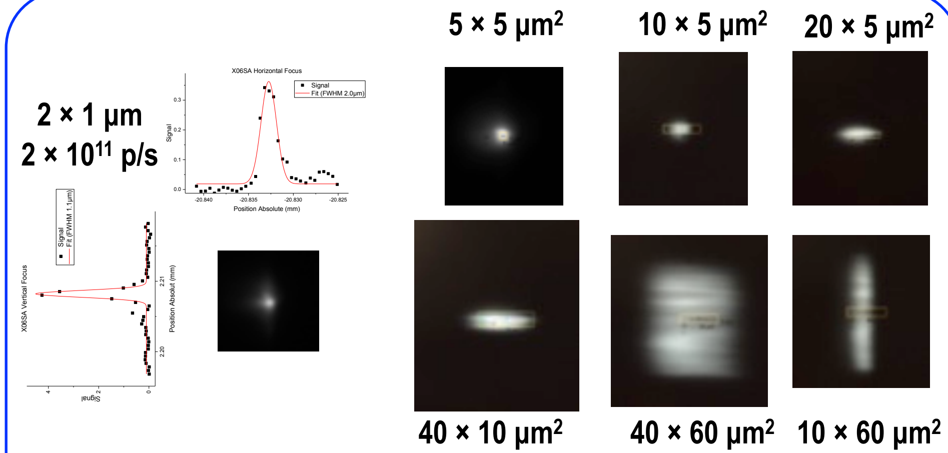
Fine-phi slicing data collection is enabled by the pixel array detector (PILATUS, EIGER), which has single-photon sensitivity and no readlout noise

Mueller, *et al*, *Acta Cryst*. (2012) **D68**, 42

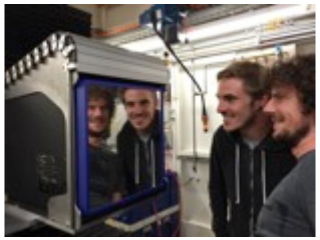
Intensity Data Collection: Reduce Background

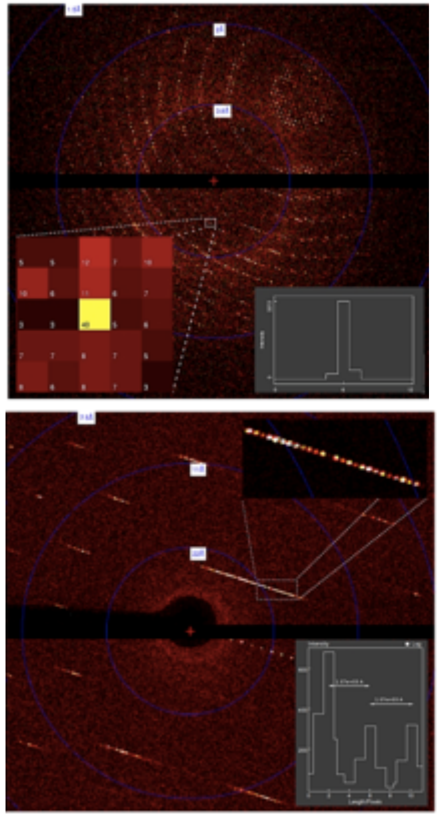
- Match beam size to crystal size
- Use best part of crystal
- Match detector distance to diffraction resolution
- Remove extra solvent
- Focus beam on detector





Variable beam size from 1 to 200 μm at beamline PXI, SLS





Spot resolution with EIGER 16M detector (75 μm pixel) at beamline PXI, SLS

Precision and Accuracy of the Intensity Measurement

- *Precision* of measurements tells us how much they differ *from each other*
- To increase *precision* → repetitive measurements (high multiplicity) within radiation damage limit
- *Accuracy* of measurements tells us how much they differ *from the truth*, but the truth is not known
- To improve *accuracy* → reduce systematic errors: beam stability, diffraction geometry, crystal orientation, absorption and variation, detector non-ideality, …
- <u>All data quality indicators measure precision, not accuracy!</u>

Diederichs, K. *Methods in Molecular Biology*, **1320**, 147 (2015)

Intensity Data Collection: Systematic Errors

Random errors, counting statistics

$\sigma_{count} = N^{1/2}$

$I = N_p - N_b$ Signal is the difference

$\sigma_I = (\sigma_p{}^2 + \sigma_b{}^2)^{1/2}$

$\sigma_I = (N_p + N_b)^{1/2}$ Uncertainty is the sum

Systematic errors, instrumental instability

$\sigma_{sys} = k_1 I$ (typical range of k_1 is 0.001 to 0.01)

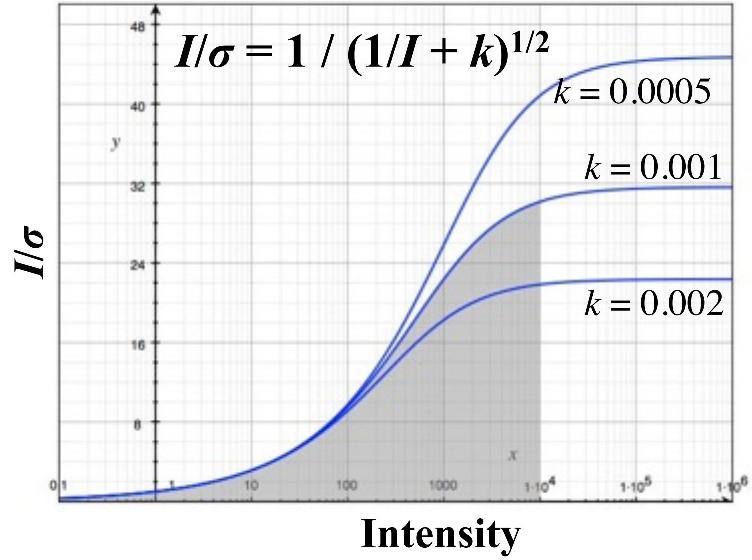
$\sigma = (\sigma_{count}{}^2 + \sigma_{sys}{}^2)^{1/2}$

$\sigma_{count} \sim I^{1/2}$; $\sigma_{\mathrm{sys}} \sim I$

$\sigma = (I + kI^2)^{1/2}$

$I/\sigma = I / (I + kI^2) = 1 / (1/I + k)^{1/2}$

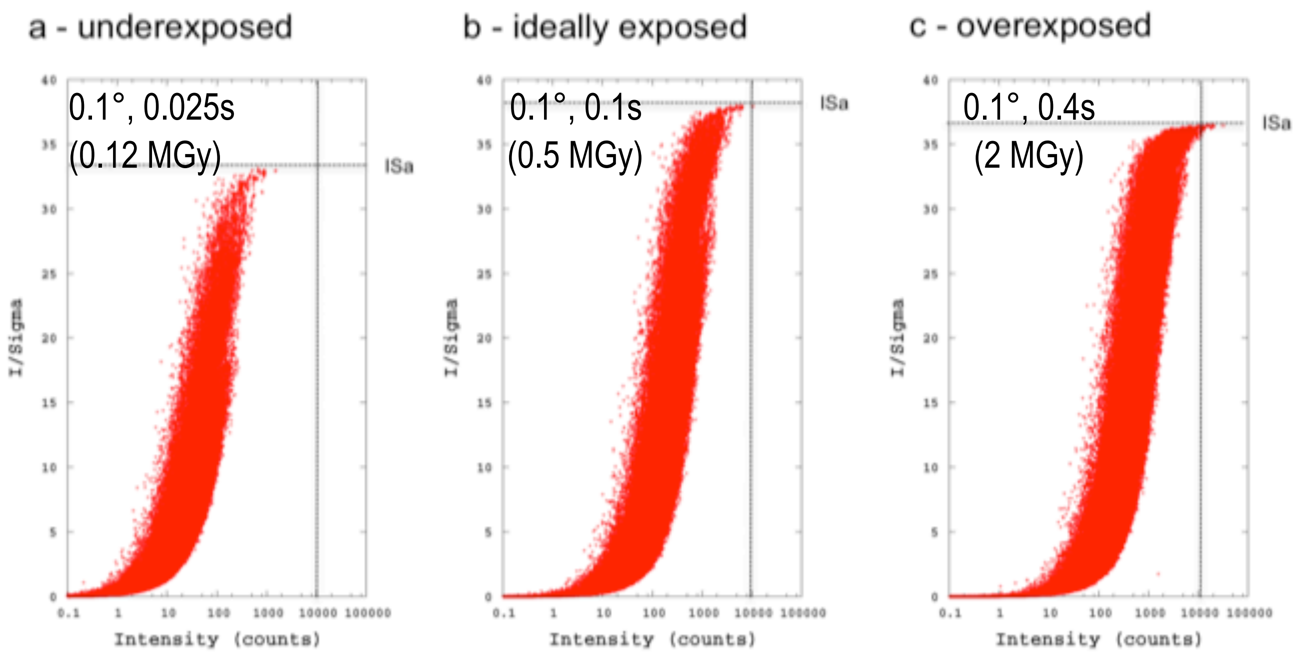
Asymptotic behavior of *I/σ* (*ISa*)



Achievable *I/σ* is limited by systematic errors, *ISa*

Diederichs, *Acta Cryst*. **D66,** 733 (2010)

Best exposure? You Can Always Use a Lower Dose



$(I/\sigma)^{asymptotic}$ plots of data sets of 180° collected at 6 keV with 1.5×10^{10} photons/s

- Achievable *I/σ* at low-med resolution (phasing) is limited by systematic error
- Achievable *I/σ* at high resolution (refinement) is limited by counting statistics
- Low *ISa* means high systematic error
- Good datasets have *ISa* > 30, bad ones < 10

Diederichs, *Acta Cryst*. **D66,** 733 (2010)

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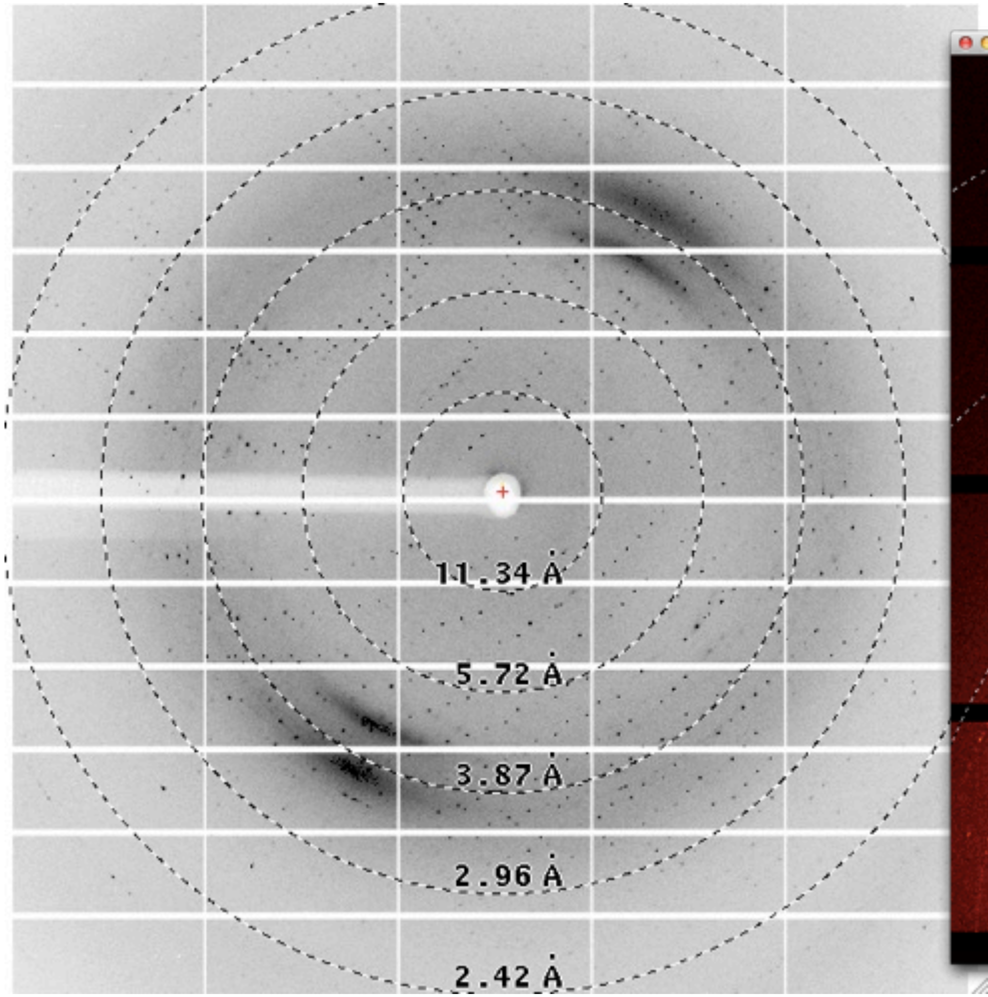
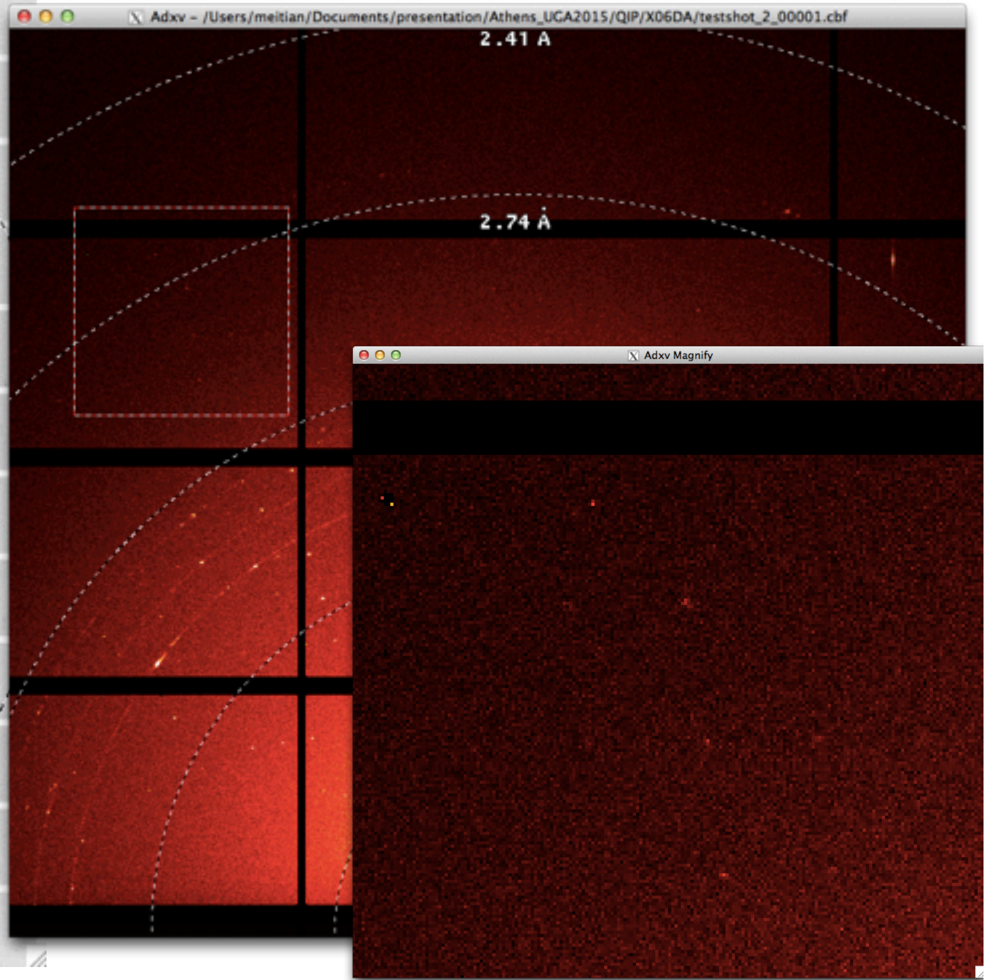
Lower Dose Example



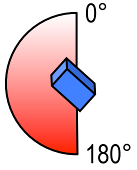


1 sec exposure with 1.5 × 10^{11} ph/sec
1° oscillation at beamline X10SA, SLS

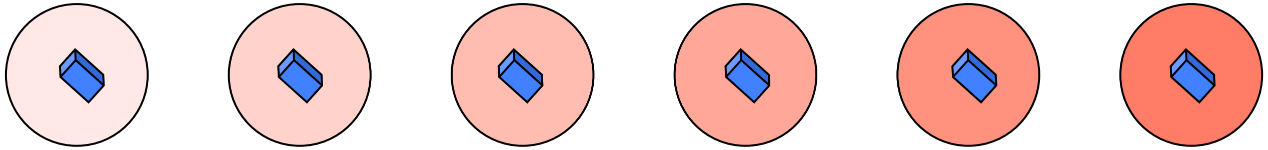
1 sec exposure with 1.5 × 10^{10} ph/sec
0.5° oscillation at beamline X06DA, SLS

Dose-slicing Data Collection Strategy

High-dose low multiplicity data collection strategy
(180° data within dose limit)

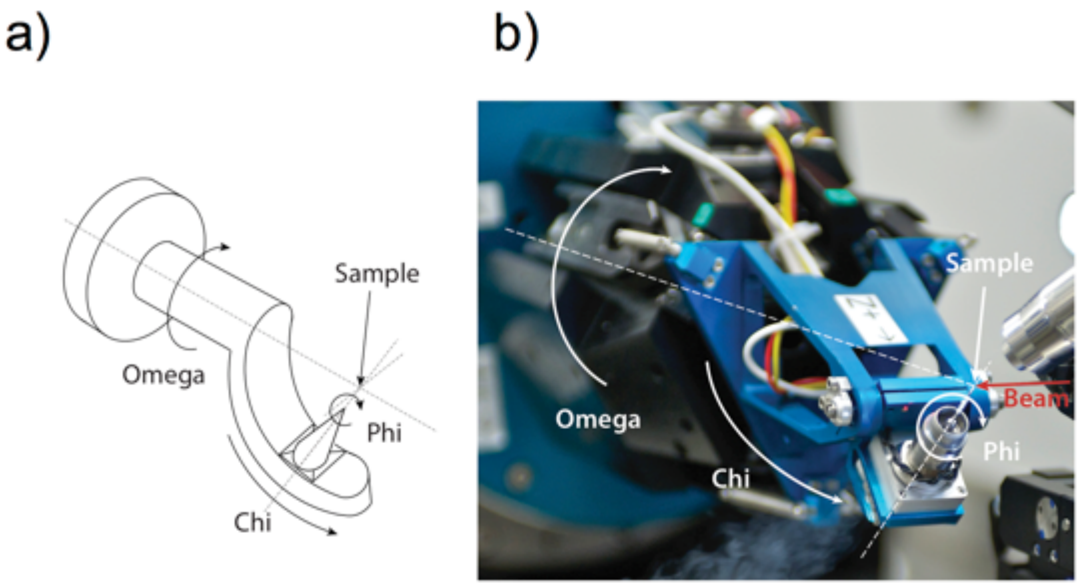


Dose-slicing high multiplicity data collection strategy, Liu *et al. Acta Cryst.* **A67**, 544, (2011)
(*n* × 360° data within dose limit)



Intensity Data Collection: Reduce Systematic Errors

Multi-orientation data collection with multi-axis goniometer



Manipulating crystal orientation changes

1) **Diffraction geometry**
2) **X-ray absorption path**
3) **Position of reflections on detector**

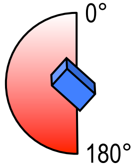
Sampling diffraction geometry, absorption path, and detector surface to average out systematic measurement errors

Waltersperger *et al. J. Synchrotron Rad.* **22**, 895 (2015).

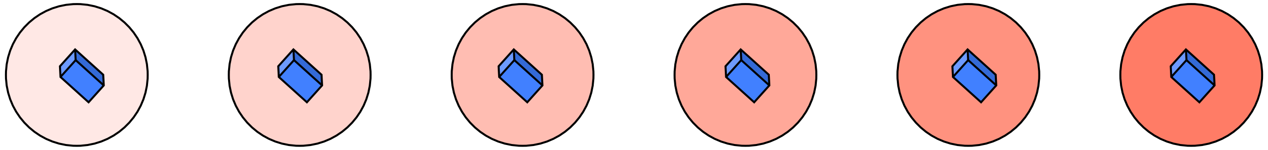
Weinert *et al. Nature Methods* **12,** 131 (2015)

Multi-Orientation Data Collection Strategy

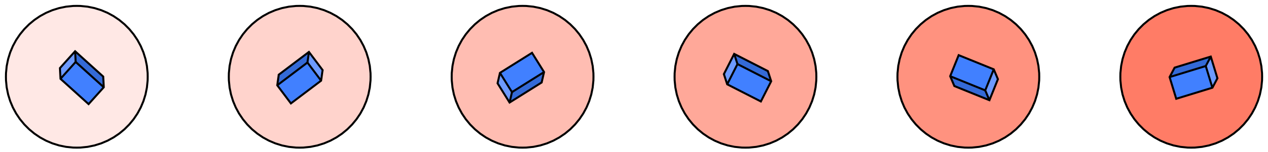
High-dose low multiplicity data collection strategy
(180° data within dose limit)



Dose-slicing high multiplicity data collection strategy, Liu *et al. Acta Cryst.* **A67**, 544, (2011)
(*n* × 360° data within dose limit)

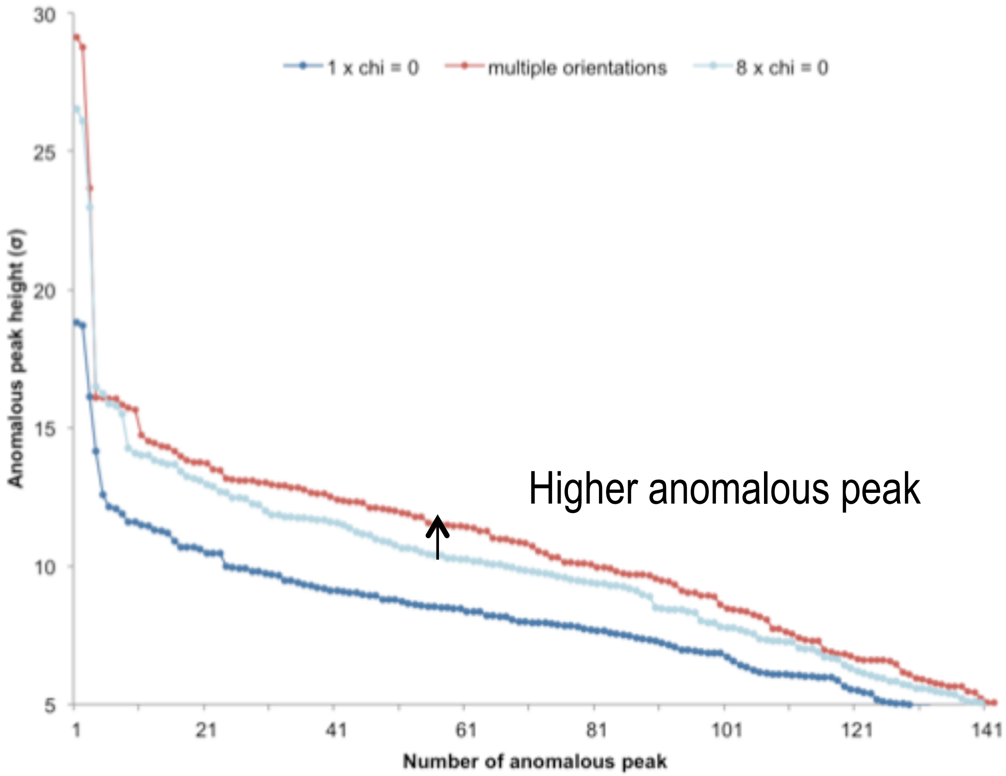


Multi-orientation high multiplicity data collection strategy, Weinert *et al. Nature Methods* **12**, 131, (2015)
(*n* × 360° data in multiple crystal orientation within dose limit)



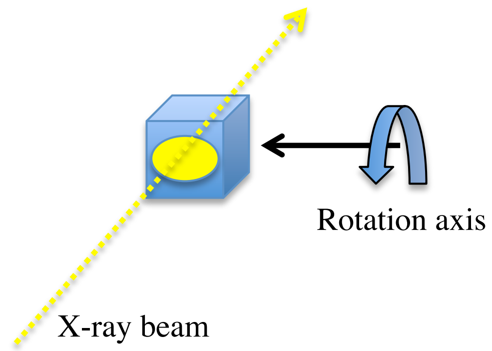
Advantage of Multi-Orientation Strategy

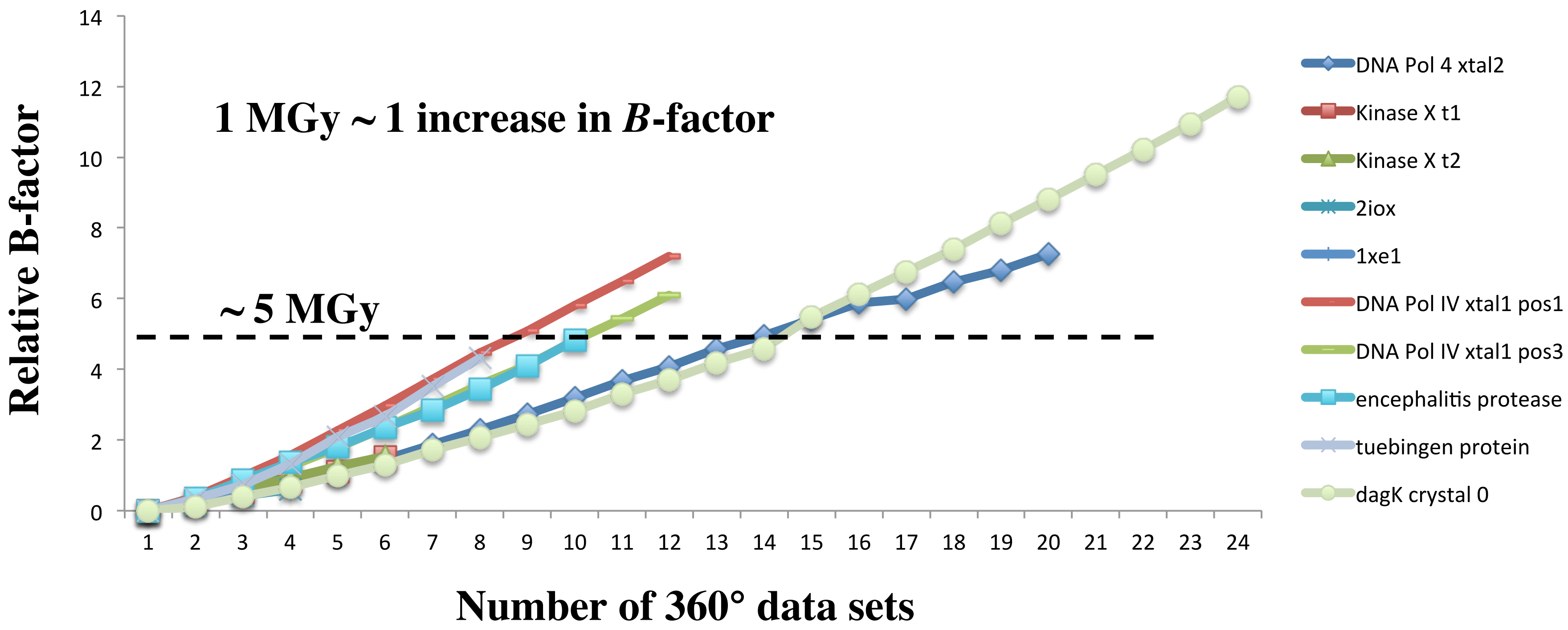
Sample	Dataset	Orientation, *chi*	Time	Native-SAD
Position 1	0.1° / 0.1 s, 720°	0°, 10°, 20°, 30°	48 min	✔
Position 2	0.1° / 0.1 s, 720°	0° (*i.e.* one orientation)	48 min	✖



Low-Dose Strategy Allows Radiation Damage Control

Crystal size 100 × 100 × 100 μm³
X-ray beam size 90 (h) × 45 (v) μm³
6 keV, 1.5 × 10^{10} ph/sec
➔ about 0.5 MGy/360 sec, 360° data

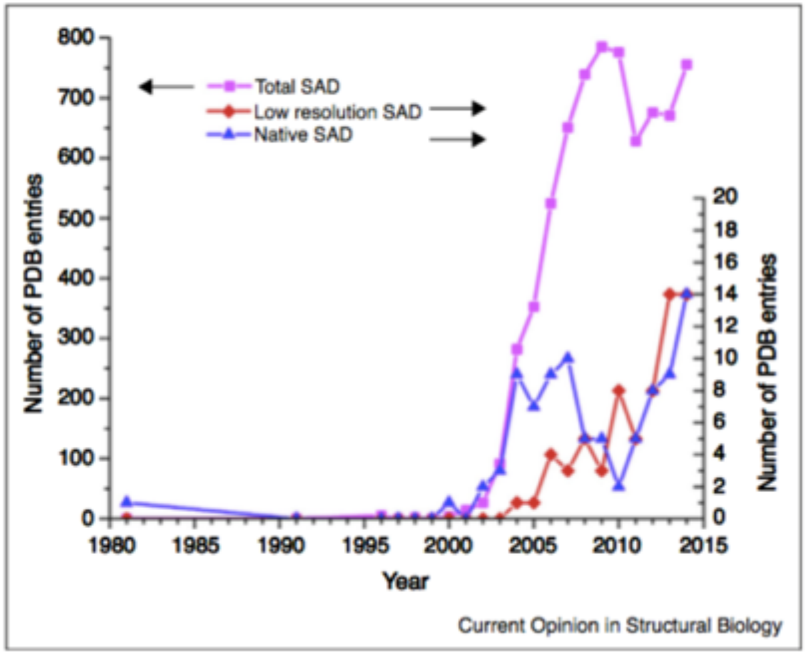




Application in Experimental Phasing

- Phasing by anomalous dispersion has been and still is by far the most popular *de novo* structure determination method

Figure 1



SAD structures in the PDB. PDB entries are as of 31 December 2014. *De novo* low-resolution SAD structures as defined here have $d_{min} \geq 3.5$ Å. *De novo* native-SAD structures as defined here have no preceding PDB deposits and have not contained atoms heavier than atomic number 20.

Liu & Hendrickson Curr. Opin. Struc. Bio. **34,** 99 (2015)

Current challenges

- Low-res SAD with $d > 3.5$ Å
- Native-SAD with $Z < 20$ (Vincent Olieric's talk in Session II)

Conclusion

Distributing tolerable X-ray does into multiple data sets measured at different crystal orientation can improve accuracy of intensity measurement

Low-dose, high-multiplicity, multi-orientation data collection strategy helps in abstracting weak anomalous signals for experimental phasing

Development in synchrotron beamline X-ray optics, multi-axis goniometer and pixel array detector has changed MX data collection strategies

Acknowledgement

X06SA micro-focusing upgrade

Clemens Schulze-Briese, Claude Pradervand, Roman Schneider, James Leuenberger, and MX group

EIGER 16M

Schweizerische Eidgenossenschaft
Confédération suisse
Confederazione Svizzera
Confederaziun svizra

Kommission für Technologie und Innovation KTI

Stefan Brandstetter, Clemens Schulze-Briese, Andreas Förster, Oliver Bunk, Arnau Casanas, and MX group

PRIGo/SmarGon/Native-SAD

Tobias Weinert, Vincent Olieric, Sandro Waltersperger, Claude Pradervand,, Ezequiel Panepucci

Data processing

Kay Diederichs

Global Phasing Ltd.

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Thank you for your attention!