

Dose driven data collection to optimise your experiments

Better (starting) collection parameters

Towards the best data in a world of less experienced beamline users

Thanks to
Neil Paterson
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Daniela Stock
Murray Stuart
Meitian Wang
Tom Caradoc-Davies
Andreas Förster <3
for slides & ideas

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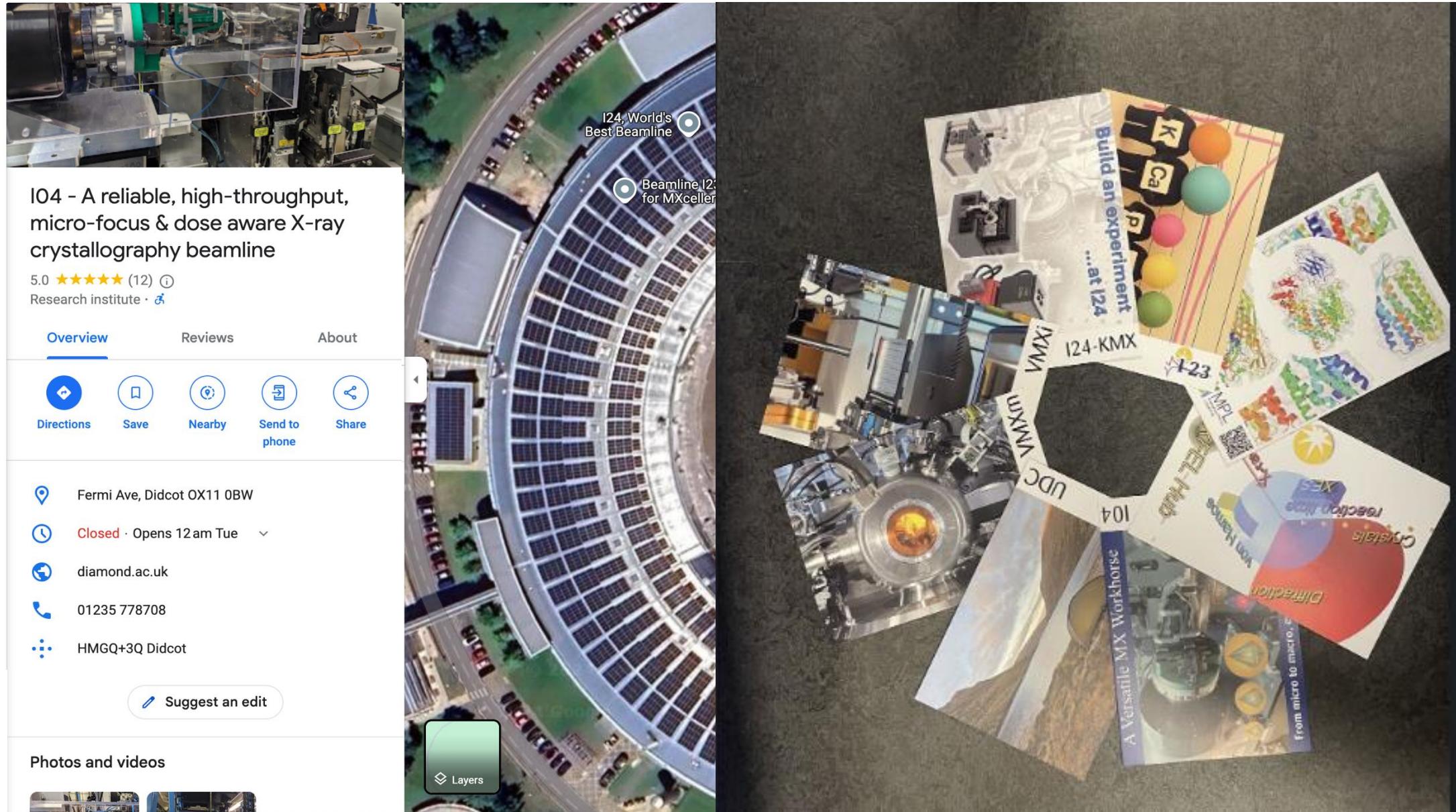
Mastodon: @aragaod@aus.social

Bluesky: <https://bsky.app/profile/aragaod.bsky.social>

ORCID: <https://orcid.org/0000-0002-6551-4657>



http://dls.MX



ASSUMPTIONS

- MX beamlines at a 3rd or 4th generation synchrotron source
- Fast pixel array detector
- Cryocooled samples
- Rotation method

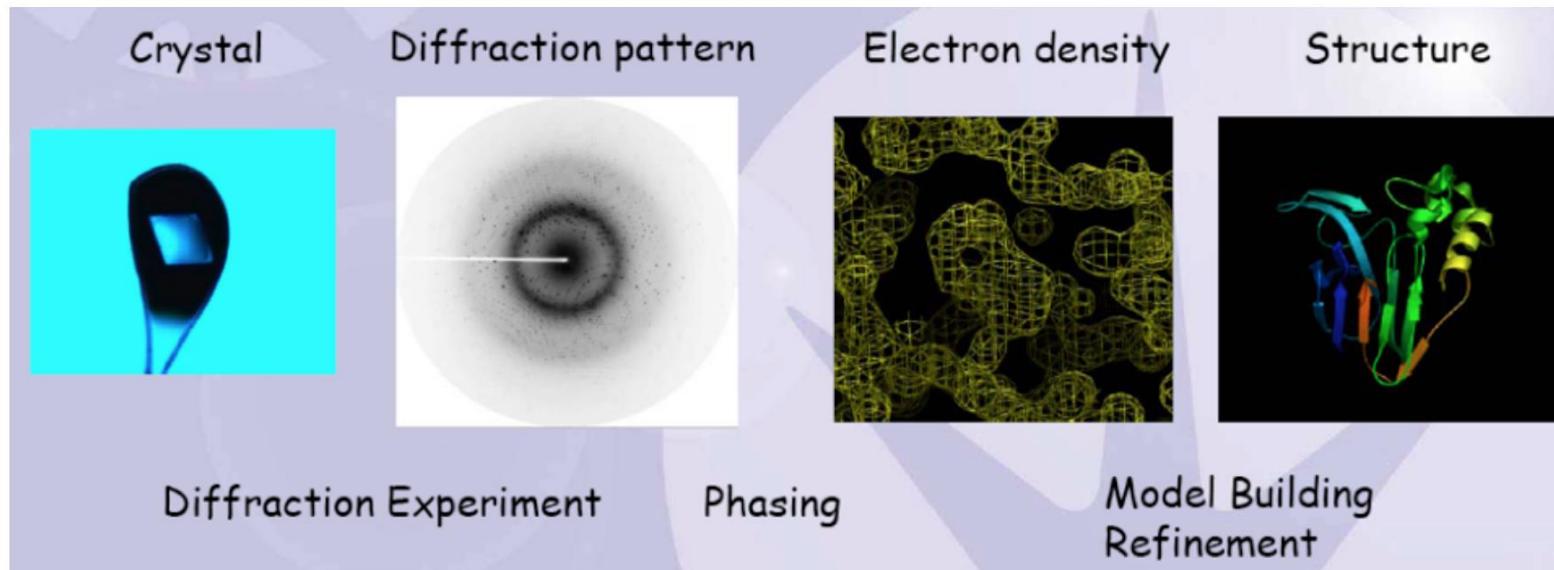
DISCLAIMER

- Not discussed
 - Room temperature data collection, *in situ* – VMXi@DLS – *Mentioned by Briony Yorke yesterday, more info ask Amy Thompson*
 - Ancillary methods (spectroscopy, humidity control, high pressure, etc.)
 - Serial methods – I24@DLS & VMXi@DLS & XFELhub@DLS – Talk yesterday Briony Yorke
 - Crystals in grids – See talk by VMXm@DLS staff – Monday talk Nanofocus Adam Crawshaw
 - Electron diffraction – Future HEXI@DLS – ask David Waterman
 - High energy (e.g. above 20 keV) – VMXm@DLS & I24@DLS – ask Adam Crawshaw or Sam Horrell
 - Very low energy (e.g. below 6 KeV) – I23@DLS – ask Sam Horrell

Kamel El Omari discuss the physics of diffraction

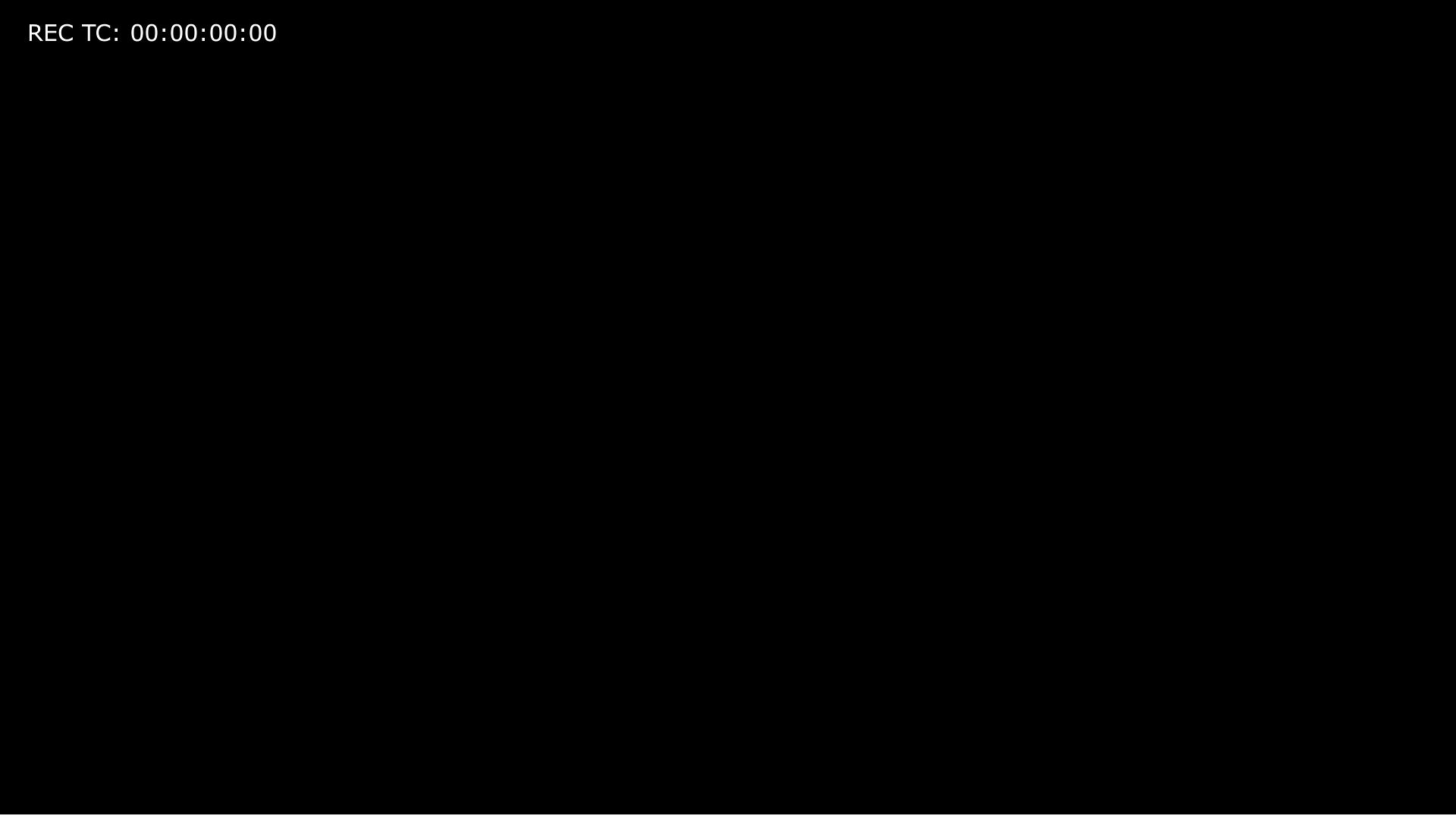
Ana Gonzalez- Data collection
Andrey Lebedev - Symmetry and space groups

$$\rho(xyz) = \frac{1}{V} \sum_{hkl} |F_{hkl}| \exp(i\alpha_{hkl}) \exp[2\pi i(hx + ky + lz)]$$



$$I(hkl) = I_0 \cdot \frac{\lambda^3}{\omega V_{cell}^2} \cdot V_{cr} \cdot L \cdot P \cdot T_r \cdot r_e^2 \cdot |F_{hkl}|^2$$

REC TC: 00:00:00:00



The diffraction experiment

DATA COLLECTION IS IMPORTANT!

This is the last experimental step

Investing time upfront in gathering the best data possible minimizes risks and increased probability of a successful outcome.

With fast robots, goniometers and detectors a lot of data can be collected very fast. Quantity ≠ Quality (*)

Attraction from the Abyss
Call of the void



The quality of the initial data sets the foundation for everything that follows. Prioritize data collection methods that ensure accuracy.

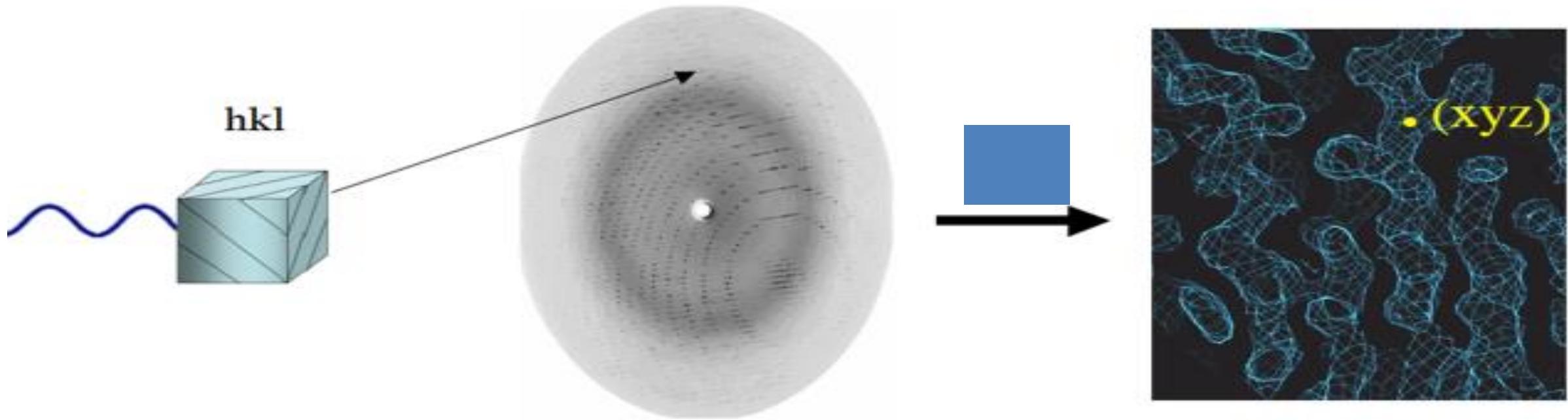
(*) "One good friend is better than a thousand poor ones." Indian Proverb

GIGO/RIRO paradigm



Garbage in, garbage out: Poor quality original data creates limitations for later analysis and can lead to misleading conclusions

Data collection: the goal



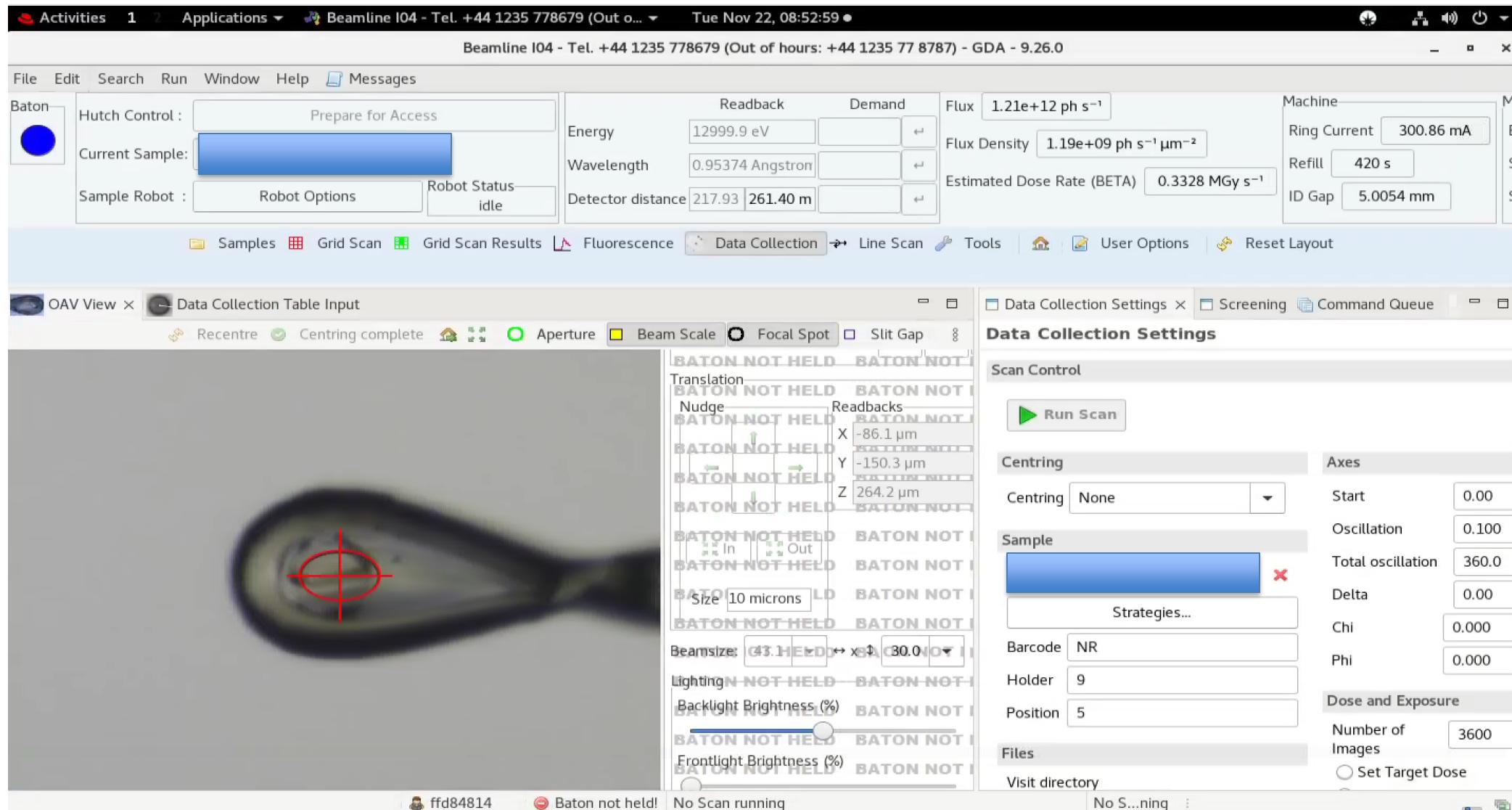
- record complete set of X-ray diffraction intensities from a crystal
- the better the data (high resolution, high completeness, low noise), the easier the following steps and the better refinement works
- like all previous steps this might need some optimisation and a lot of patience

Overview at data
collection time

The Master Checklist

- **Aligning correctly your crystal (Optically, using X-rays)**
 - Oscillation per frame
 - Total Oscillation
 - Multiple orientations
 - Detector distance / Maximum Resolution
 - Beam size / Crystal size
 - Energy selection
 - Exposure (total) / Dose
 - Transmission.
 - ~~Cryo vs Room Temperature~~

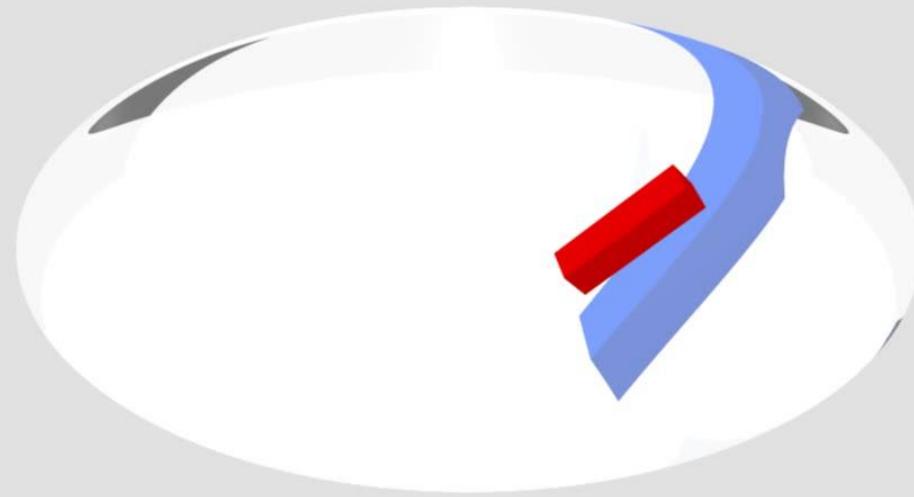
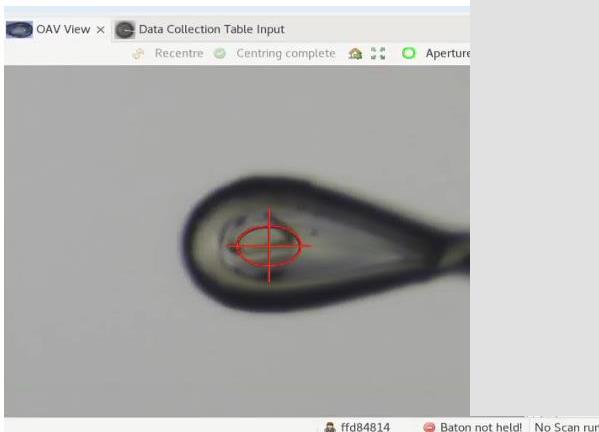
Optical Alignment (The Basics)



The Problem of Refraction

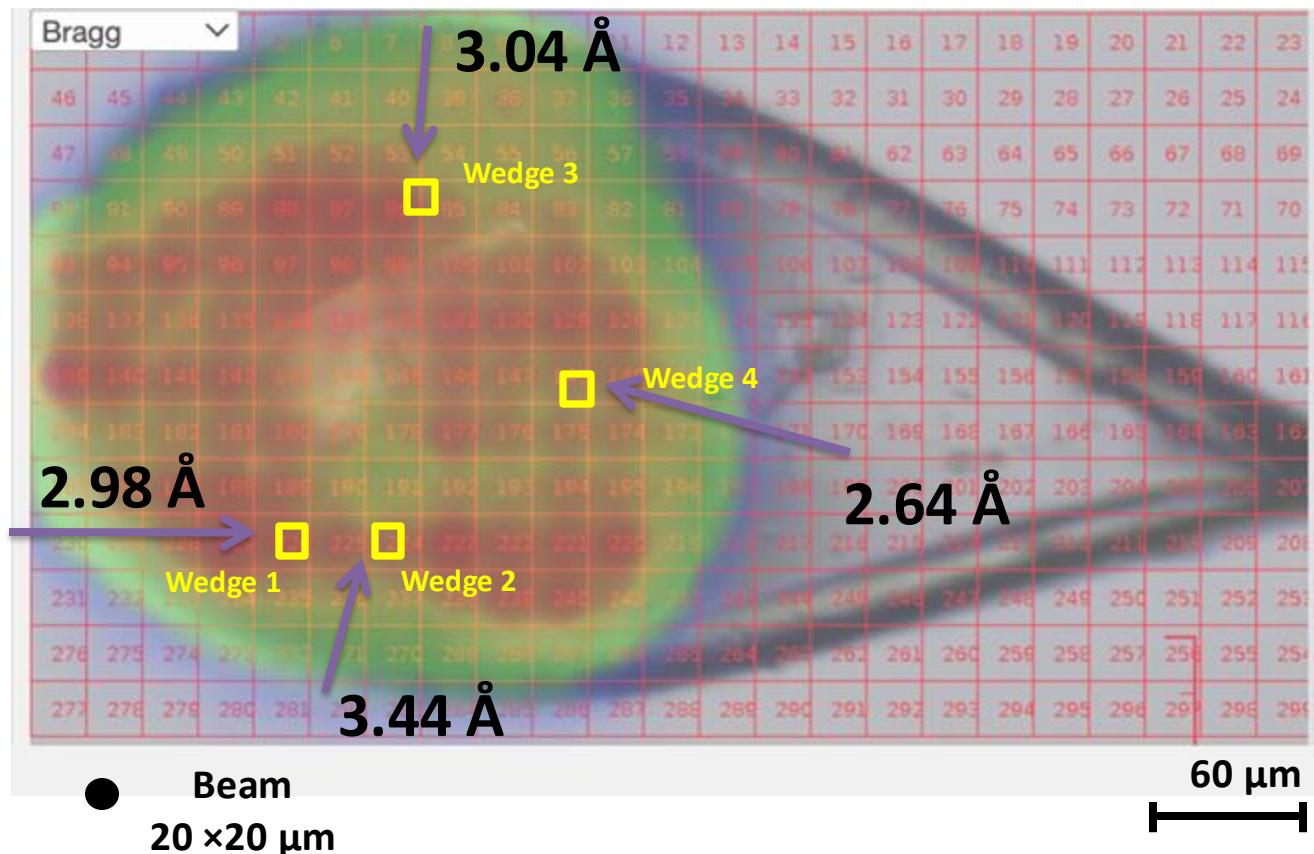
Unless beam is large and/or the crystal is very well visible in minimal liquid X-ray centring can be critically for a good alignment

Good alignment is crucial



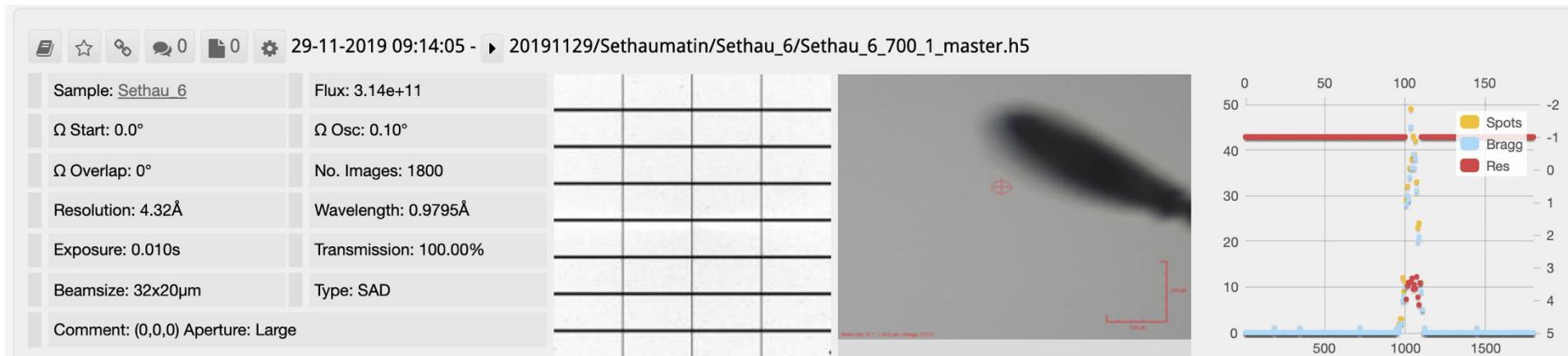
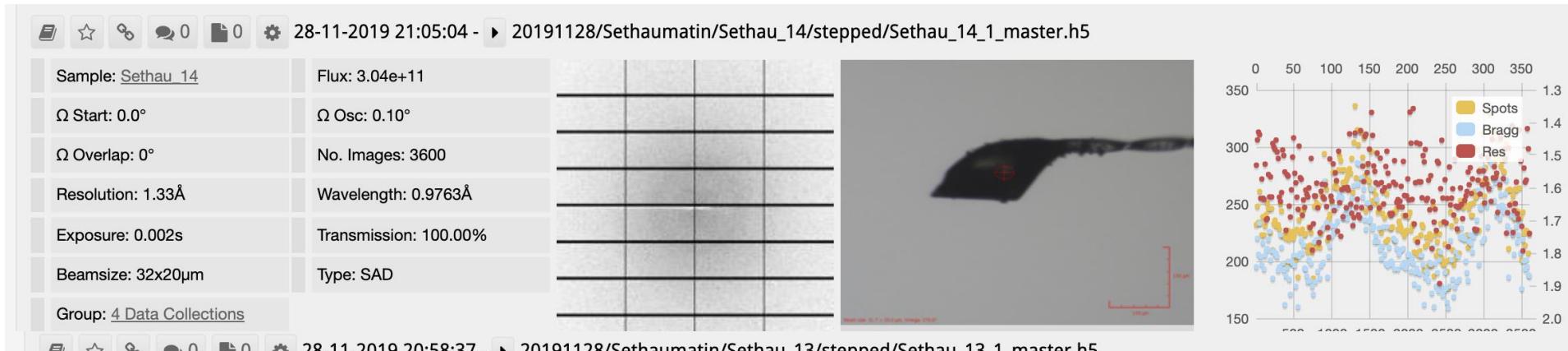
The Solution: X-ray Centring (Grid Scan)

Even the same crystal can show a lot of variation...



Sometimes worthwhile, sometimes not. Learn when to move on.

Verifying Alignment (ISPyB & Per Image Analysis)



The Master Checklist (Oscillation Focus)

- Aligning correctly your crystal (Optically, using X-rays)
- **Oscillation per frame**
- Total Oscillation
- Multiple orientations
- Detector distance / Maximum Resolution
- Beam size / Crystal size
- Energy selection
- Exposure (total)
- Transmission
- ~~Cryo vs Room Temperature~~

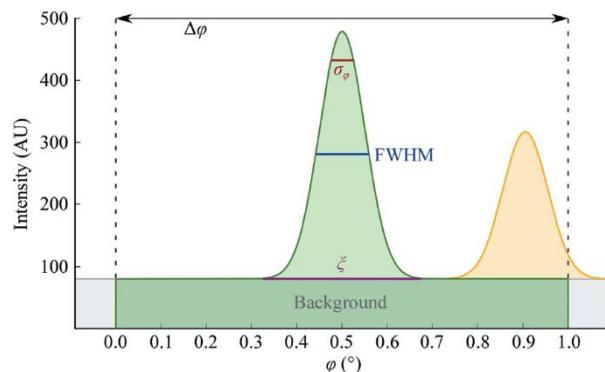
Oscillation Per Frame (Fine Slicing)

FINE SLICING

$$I = N_p - N_b$$

$$\sigma_{count} = \sqrt{N}$$

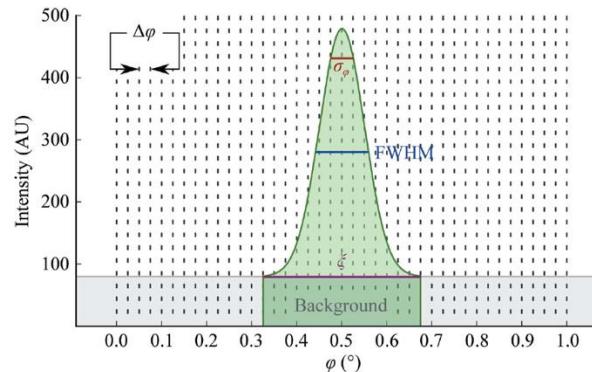
$$\sigma_I = \sqrt{(N_p + N_b)}$$



Wide omega slicing

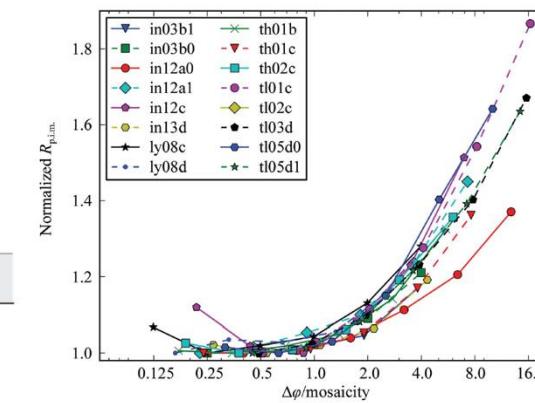
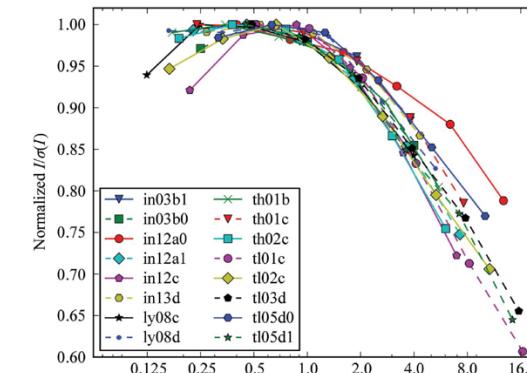
- Large rotation increment ($\Delta\omega > \xi$)
- Large overlap of reflections and background along ω
- Few images for complete data set

What oscillation to use?
0.1 degrees is a good compromise



Fine omega slicing

- Small rotation increment ($\Delta\omega \ll \xi$)
- Minimal overlap of reflections and background along ω
- Many images for complete data set



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

Pflugrath, *Acta Cryst.* (1999) D55, 1718

Pixel array detectors enable fine slicing

Practical Implementation (GDA Screenshot)

Start	0.00
Oscillation	0.100
Total oscillation	360.0
Delta	0.00
Chi	0.000
Phi	0.000
Dose and Exposure	
Number of Images	3600
<input checked="" type="radio"/> Set Target Dose	
<input type="radio"/> Set Target Exposure	
Exposure Time	0.0030
Total Exposure Time	10.8
Dose / Dataset	7 MGy
First Image Number	1
Beam and Detector	
Maximum resolution	1.9023 Å
Detector distance	289.3 mm
Wavelength	0.95373 Å
Energy	12999.9 eV
<input type="checkbox"/> Use current energy	
Transmission	100.000000 %
Aperture and Beamstop	
Beamstop	Standard ▾
Horizontal beam size	31.73 μm
Vertical beam size	20 μm
<input checked="" type="checkbox"/> Use current beam size	

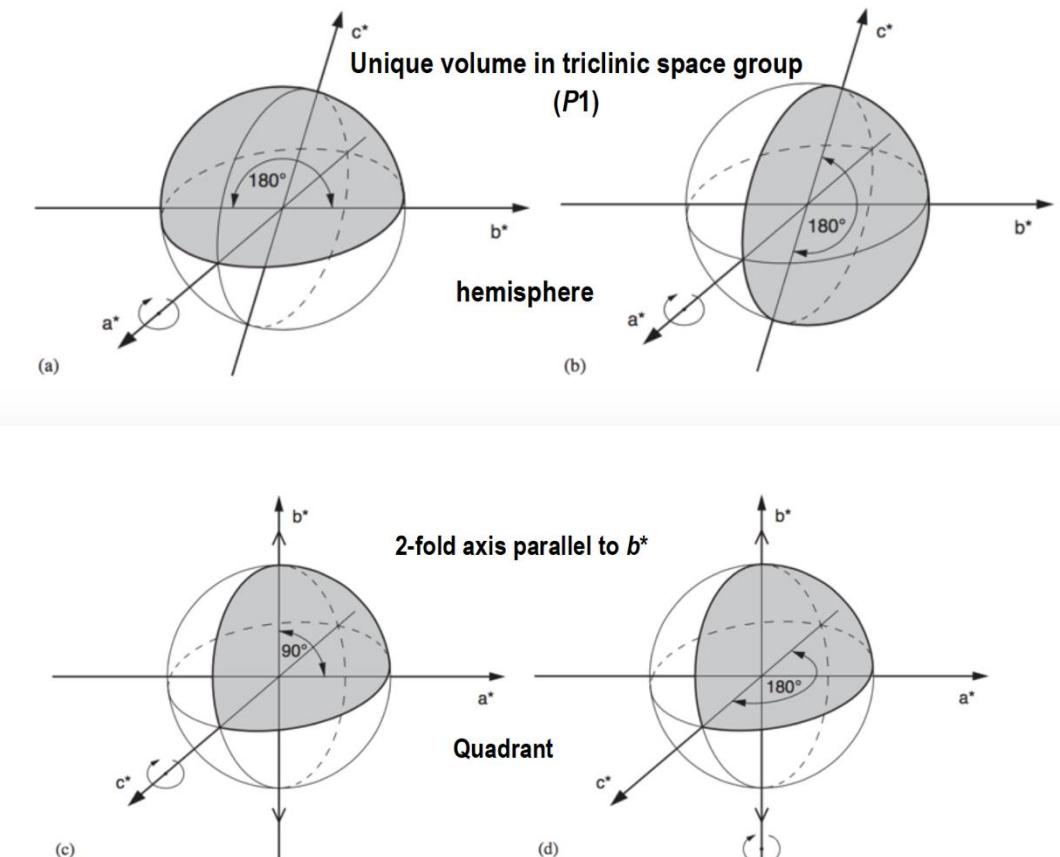
The Master Checklist (Total Oscillation)

- Aligning correctly your crystal (Optically, using X-rays)
- Oscillation per frame
- **Total Oscillation**
- Multiple orientations
- Detector distance / Maximum Resolution
- Beam size / Crystal size
- Energy selection
- Transmission
- Exposure (total)
- ~~Cryo vs Room Temperature~~

Completeness & Symmetry (The 360° Recommendation)

Andrey Lebedev talk Intro to space groups and symmetry

- reciprocal space symmetry = crystal symmetry plus
- Friedel's law : $I(h k l) = I(-h -k -l)$



thus even for P1 only 180° are needed to obtain complete data set of unique reflections - unless there is anomalous scattering

for N-fold symmetry along rotation axis, need 180/N degrees of data to obtain complete data set (assuming Friedel's law holds)

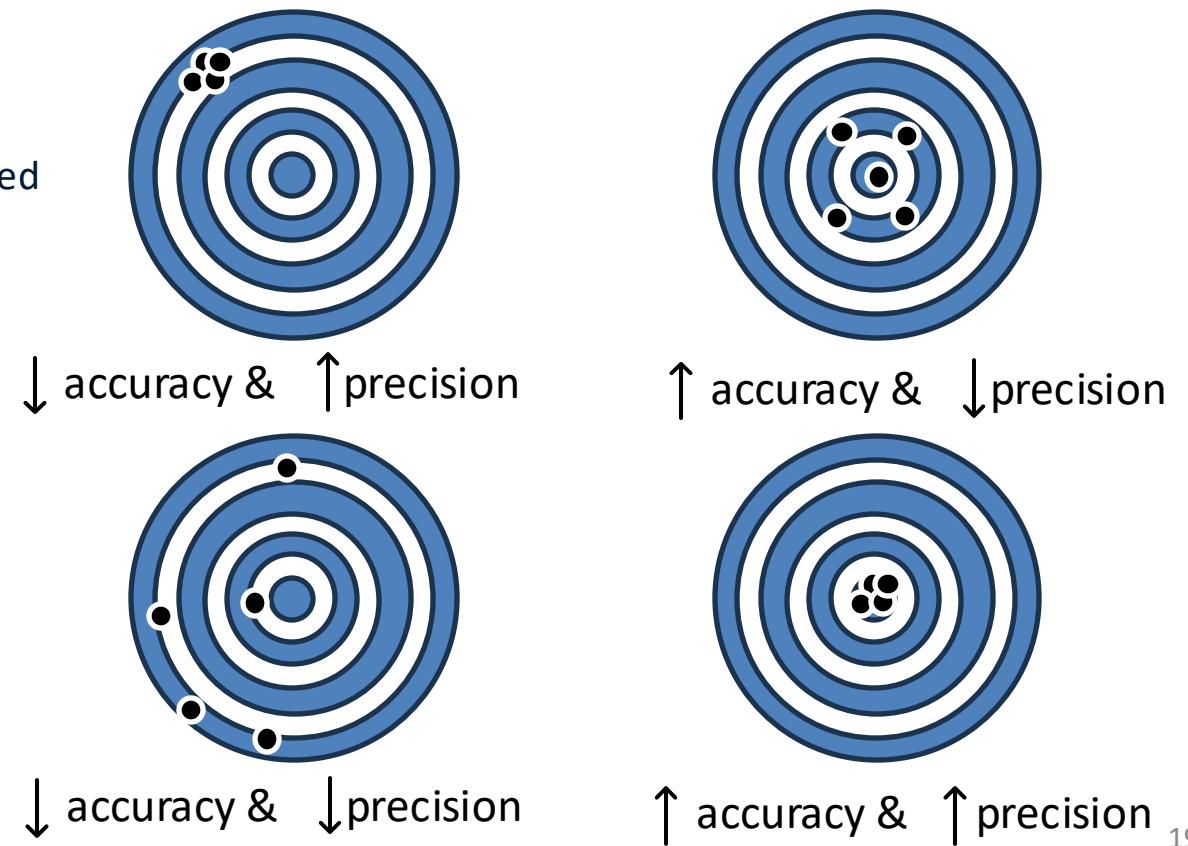
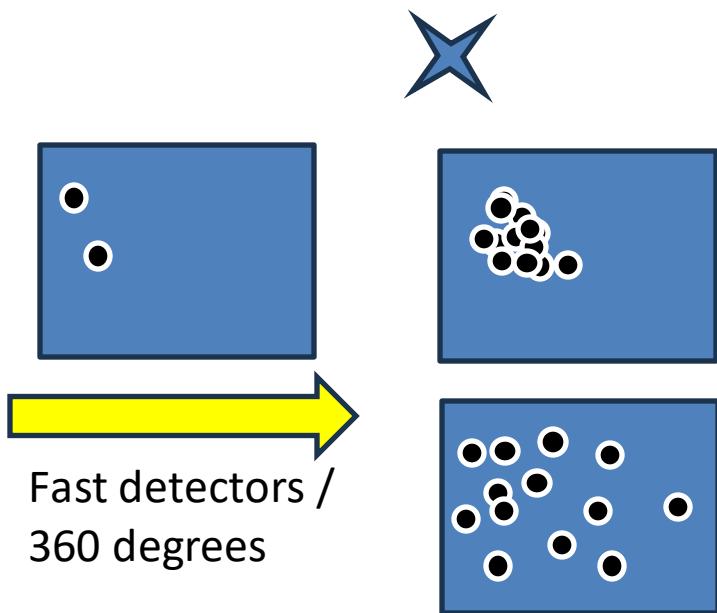


Crystals of proteins grown on the U.S. Space Shuttle or Russian Space Station, Mir.



Multiplicity

- **Accuracy:** How close the arrows are to the target or bullseye
- **Precision:** How consistently the arrows are hit, or how well they are grouped



The "Time" Misconception

- 90° @ 10s Total Exposure = 10s experiment.
- 180° @ 10s Total Exposure = 10s experiment.
- 360° @ 10s Total Exposure = 10s experiment.

Total Oscillation (The 360° Summary)

- Give yourself options (Radiation Damage) ← collect same dose in larger range
 - 10 MGy in 360 degrees results in 5 MGy in every 180 degrees of data
- Increase precision of the data ← increase your multiplicity by collecting at least a full rotation
 - Remember synchrotrons & beamlines are not perfect: beam moves, intensity varies, instrument has noise
 - Detectors are virtually noise free: no penalty to collect more frames with lower signal because signal adds up
- Guarantee always as complete data as possible
 - starting angle not an issue anymore, space group as critical for setting experiment anymore
- Collecting 10 MGy in 360 degrees takes the same (in fast detector) time as collecting 10 MGy in 180 degrees or 90 Degrees
 - no speed benefit of collecting smaller angle.
 - Dose/exposure is unrelated with oscillation but should be something defined by the crystal quality (& experimental aim, crystal composition etc).
- Explore the crystal shape
 - might be thicker, better spots, > crystals/twinning

Practical Implementation

Start	0.00
Oscillation	0.100
Total oscillation	360.0
Delta	0.00
Chi	0.000
Phi	0.000
Dose and Exposure	
Number of Images	3600
<input checked="" type="radio"/> Set Target Dose	
<input type="radio"/> Set Target Exposure	
Exposure Time	0.0030
Total Exposure Time	10.8
Dose / Dataset	7 MGy
First Image Number	1
Beam and Detector	
Maximum resolution	1.9023 Å
Detector distance	289.3 mm
Wavelength	0.95373 Å
Energy	12999.9 eV
<input type="checkbox"/> Use current energy	
Transmission	100.000000 %
Aperture and Beamstop	
Beamstop	Standard ▾
Horizontal beam size	31.73 μm
Vertical beam size	20 μm
<input checked="" type="checkbox"/> Use current beam size	

Multiple Orientations (The Blind Spot Exception)

- Aligning correctly your crystal (Optically, using X-rays)
- Oscillation per frame
- Total oscillation
- **Multiple orientations – won't talk today**
- Detector distance / Maximum resolution
- Beam size / Crystal size
- Energy selection
- Transmission
- Exposure (total)
- ~~Cryo vs Room Temperature~~

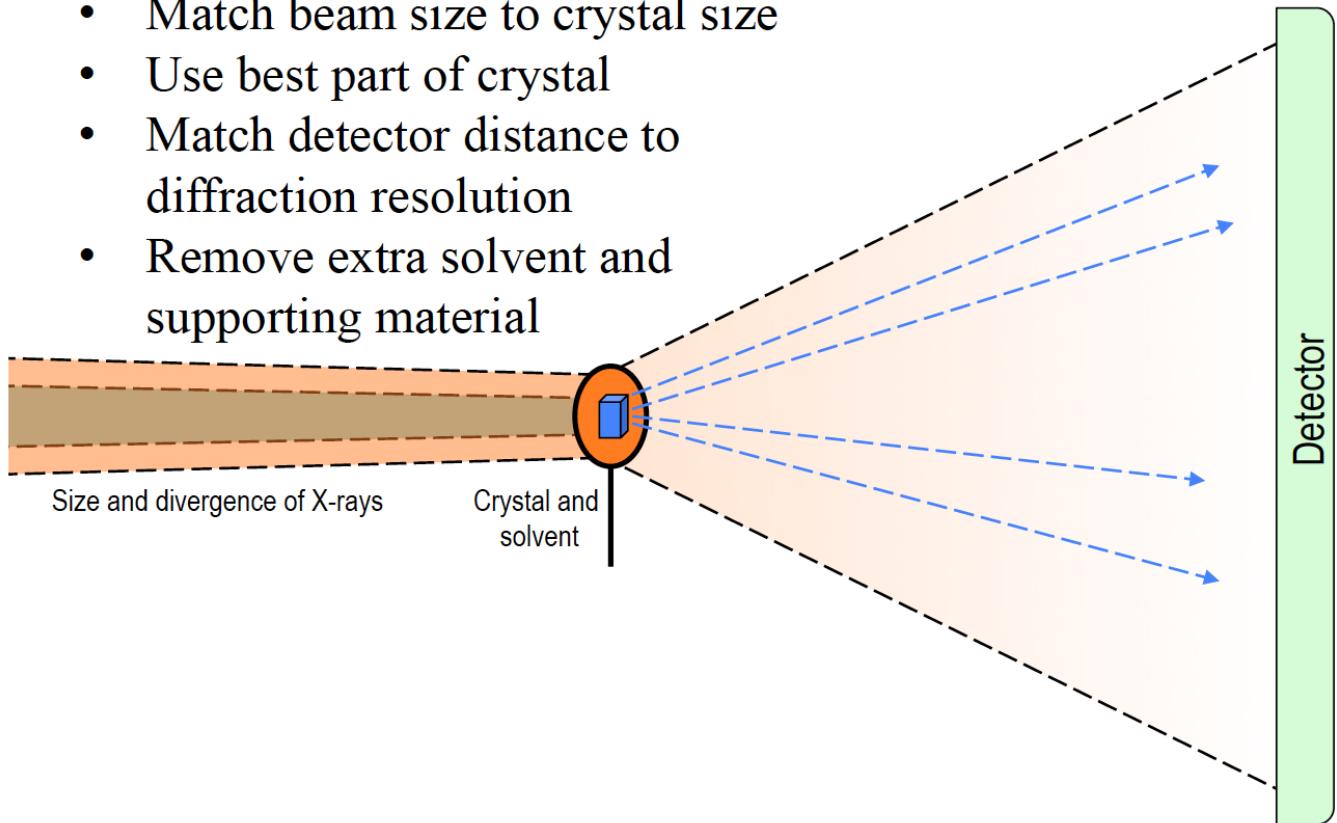
The Master Checklist (Distance & Beam)

- Aligning correctly your crystal (Optically, using X-rays)
- Oscillation per frame
- Total oscillation
- Multiple orientations
- **Detector distance / Maximum Resolution**
- **Beam size / Crystal size**
- Energy selection
- Transmission
- Exposure (total)
- ~~Cryo vs Room Temperature~~

Sample to detector distance

- Maximum resolution possible
- Signal drops \wedge^2 while background \wedge^3
- Overlaps (large unit cells)

- Match beam size to crystal size
- Use best part of crystal
- Match detector distance to diffraction resolution
- Remove extra solvent and supporting material



Beam size

- Match crystal size (keep same dose, not exposure)
- Smaller if large heterogenous crystal

Start	0.00
Oscillation	0.100
Total oscillation	360.0
Delta	0.00
Chi	0.000
Phi	0.000

Dose and Exposure

Number of Images

Set Target Dose
 Set Target Exposure

Exposure Time s

Total Exposure Time s

Dose / Dataset MGy

First Image Number

Beam and Detector

Maximum resolution Å

Detector distance mm

Wavelength Å

Energy eV

Use current energy

Transmission %

Aperture and Beamstop

Beamstop ▾

Horizontal beam size μm

Vertical beam size μm

Use current beam size

Start	0.00
Oscillation	0.100
Total oscillation	360.0
Delta	0.00
Chi	0.000
Phi	0.000

Dose and Exposure

Number of Images

Set Target Dose
 Set Target Exposure

Exposure Time s

Total Exposure Time s

Dose / Dataset MGy

First Image Number

Beam and Detector

Maximum resolution Å

Detector distance mm

Wavelength Å

Energy eV

Use current energy

Transmission %

Aperture and Beamstop

Beamstop ▾

Horizontal beam size µm

Vertical beam size µm

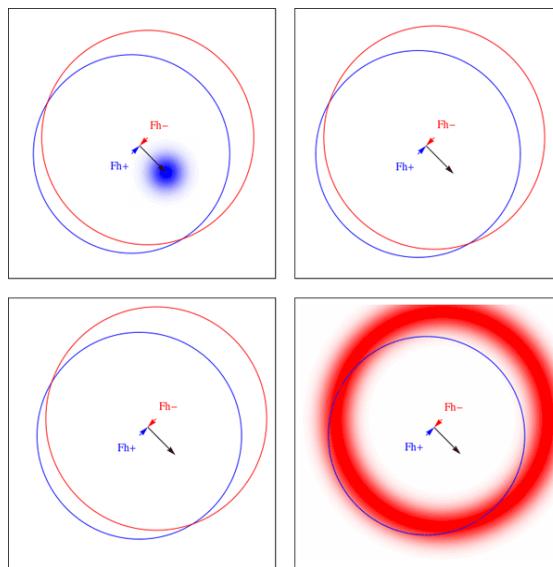
Use current beam size

The Master Checklist (Energy)

- Aligning correctly your crystal (Optically, using X-rays)
- Oscillation per frame
- Total oscillation
- Multiple orientations
- Detector distance / Maximum resolution
- Beam size / Crystal size
- **Energy selection**
- Transmission
- Exposure (total)
- ~~Cryo vs Room Temperature~~

Why Change Energy? (Theory)

Experimental Phasing



Picture courtesy of Acta Crystallogr D Biol
Crystallogr. 2011 Apr 1; 67:338–344.

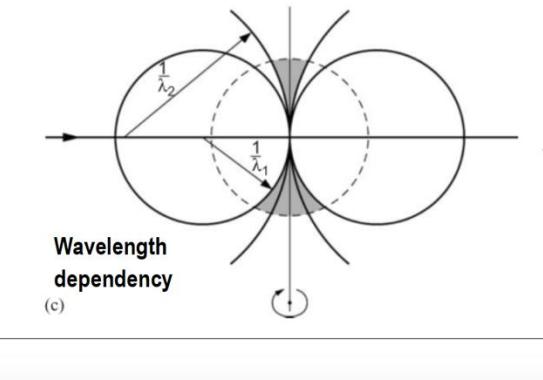
High resolution

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

Bragg's Law

<https://www.diamond.ac.uk/Instruments/Mx/Common/Calculators.htm>
(search for "useful calculators diamond")

Blind region / Cuspid



Diffraction Theory: This was covered by Kamel El Omari on Zoom Day 2 (last week).

Anomalous/Phasing: This will be covered during the on-site workshop on Day 3 (Wednesday 26th) by Ramona Duman ("Anomalous diffraction in 2025") and Ed Lowe ("The phase problem")

Crystal life time / Radiation damage for small crystals

Experimental evidence for the benefits of higher X-ray energies for macromolecular crystallography

Selina L. S. Storm,^a* Danny Axford^a and Robin L. Owen^{a*} IUCrJ 2021 Sep 9;8:896-904.

Most often: use the default energy that the beamline you using has been most optimised for

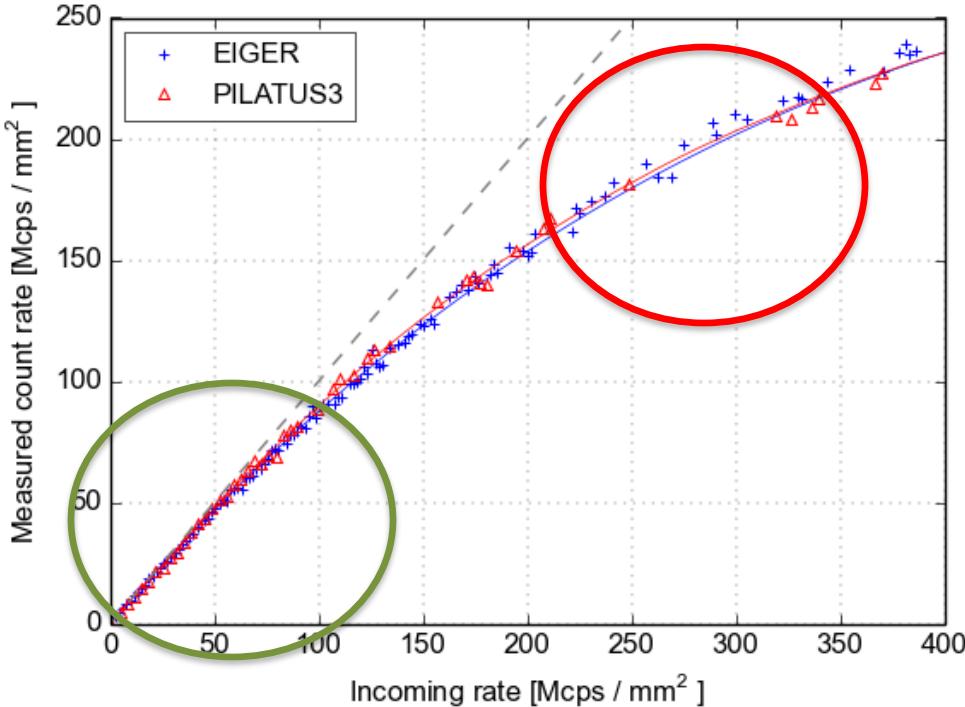
Practical Implementation

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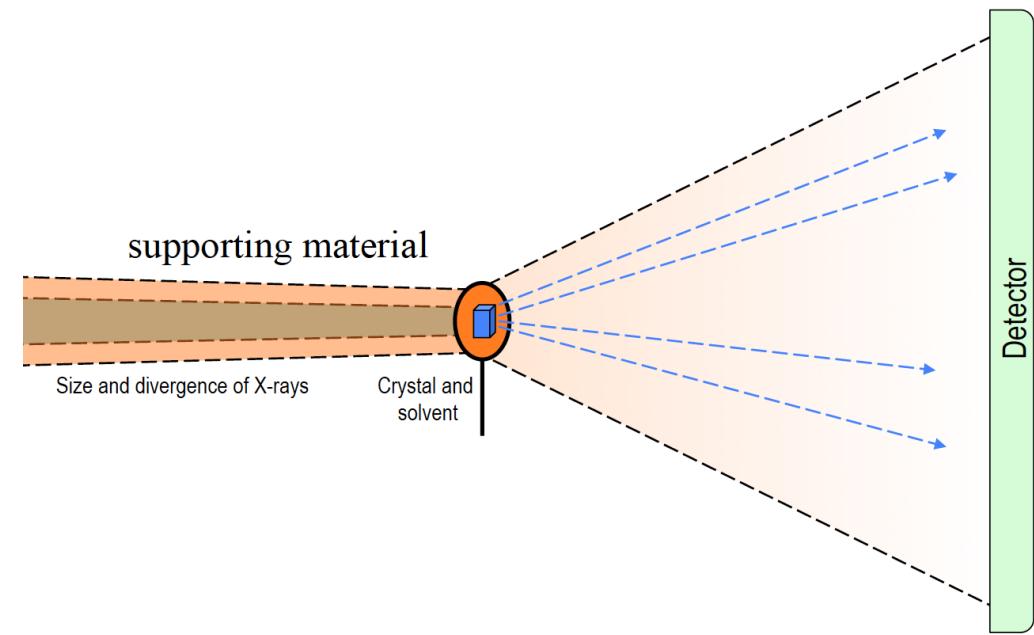
The Master Checklist (Transmission)

- Aligning correctly your crystal (Optically, using X-rays)
- Oscillation per frame
- Total oscillation
- Multiple orientations
- Detector distance / Maximum resolution
- Beam size / Crystal size
- Energy selection
- **Transmission**
- Exposure (total)
- ~~Cryo vs Room Temperature~~

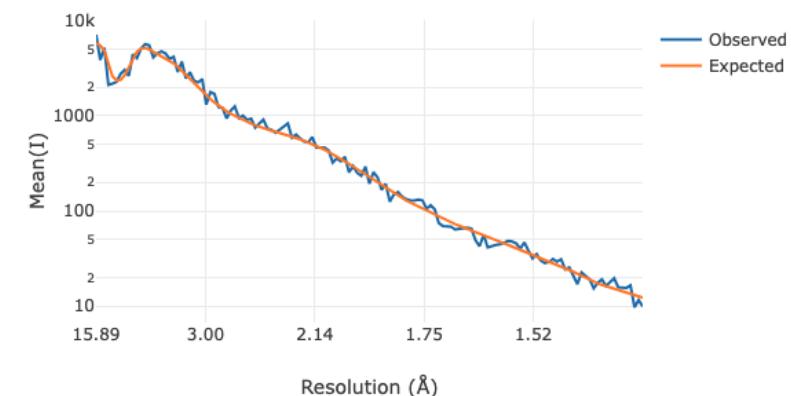
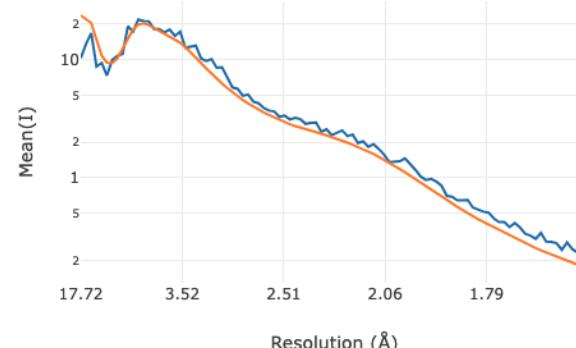
A Fundamental Constraint: Don't Overwhelm Your Detector



<https://www.dectris.com/en/features/features-eiger-r/outstanding-count-rate-performance/>



Wilson intensity plot



- R_{meas} and others is a measure of precision, not accuracy.
- Systematic errors can lead to low R_{meas} despite inaccurate data.
- Always consider multiple quality metrics to assess data quality.
- Be aware of potential sources of bias in your data collection and processing.

The Master Checklist (Exposure)

- Aligning correctly your crystal (Optically, using X-rays)
 - Oscillation per frame
 - Total oscillation
 - Multiple orientations
 - Detector distance / Maximum resolution
 - Beam size / Crystal size
 - Energy selection
 - Transmission
 - **Exposure (total)**
 - ~~Cryo vs Room Temperature~~
- Facility
 - Instrument (beamline)

The Old Way: A Focus on "Exposure Per Frame"

- A single, simple concept: The time the shutter was open for one image.
- Historically, it was our primary handle on the experiment.
- **The Problem:** This concept is now misleading and outdated (*)

$$\text{Total Dose} \approx \text{Flux} \times \left(\frac{\text{seconds}}{\text{frame}} \right) \times \text{Number of frames}$$

(*) At a synchrotron with very fast detectors where this number is typical a small decimal

Why "Exposure Per Frame" is Flawed

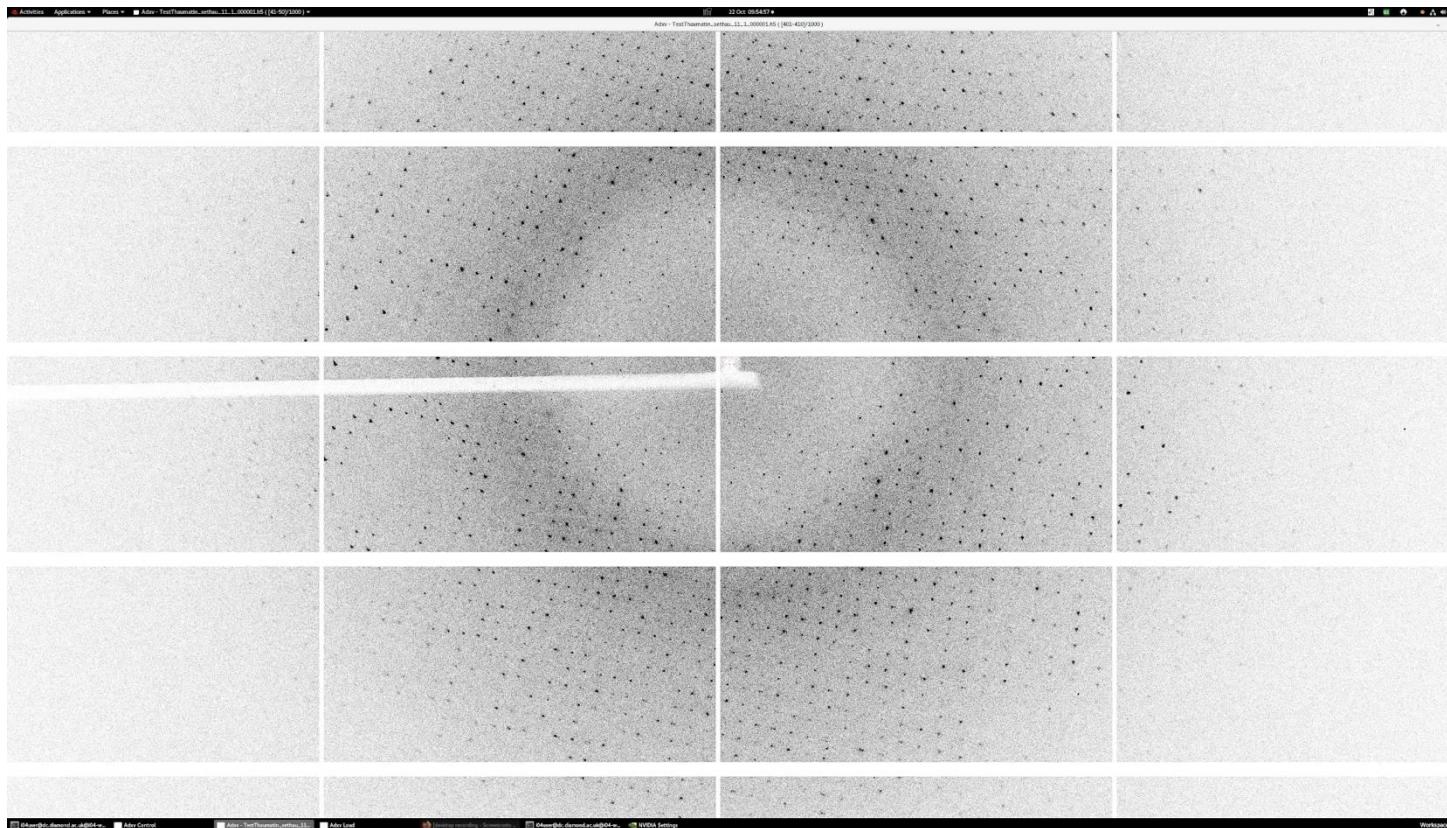
- What does it mean for a 90° vs. a 360° dataset?
- What does it mean when slicing at 0.1° vs. 0.5°?
- How can you compare experiments between different beamlines?

The user's question,

"What exposure should I use?", has no simple answer.

Modern Reality (Shutterless Mode)

- Modern detectors work like shooting a movie.
- The shutter opens only **once** for the entire scan.
- The detector records data continuously as the crystal rotates.
- Therefore, "exposure per frame" is a physically meaningless concept now.



Total Exposure (Better, But Not Enough)

- Is a 10-second exposure at one energy the same as at another?
- Is it the same for a tiny beam vs. a large beam?
- Is it the same today as on your last visit, when machine conditions might have changed?

Recommendation: Use total exposure or even better use a concept of Dose if you can

Dose aware data collection on the variable and microfocus macromolecular crystallography beamline I04 at Diamond Light Source. Ralf Flraig et al 2025 J. Phys.: Conf. Ser. 3010 012096. DOI [10.1088/1742-6596/3010/1/012096](https://doi.org/10.1088/1742-6596/3010/1/012096)

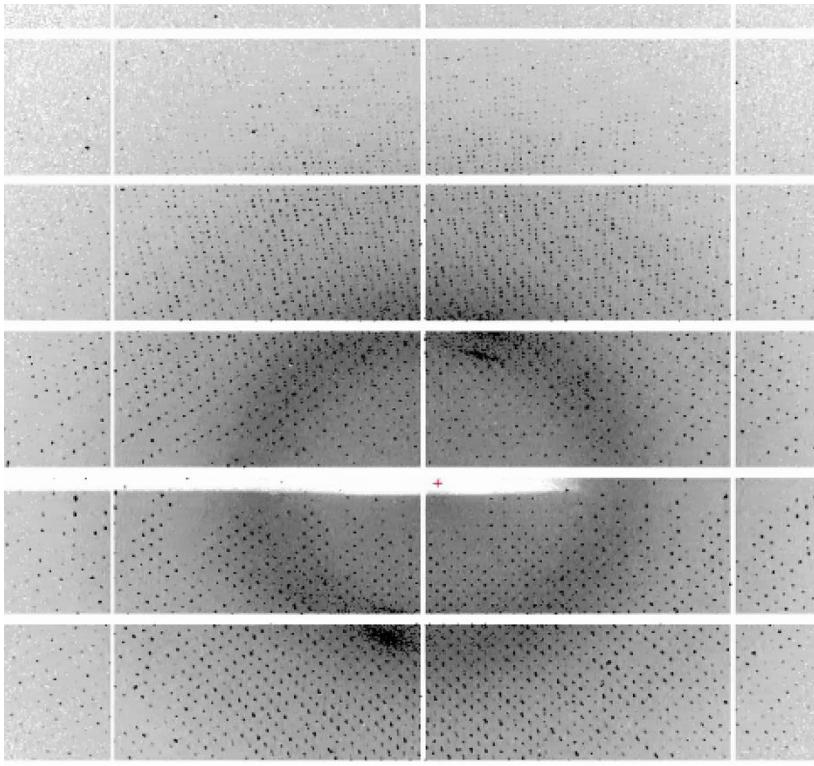
The Need for a Sample-Aware Metric

- "Exposure time" is clearly not enough.
- We need a metric that adapts to our specific crystal.
- The first question we must answer is:
"How good is my crystal?"
- This leads will be answered when we reach → **Screening** topic

Screening how &
when & why?

Screening: What Are We Looking For?

Goal: Determine the crystal's diffraction properties before the main experiment.



Method: Collect a small wedge of data (e.g., 15-20 degrees). Ideally from the thickest part of your crystal.

Key Questions to Answer:

- How good are the spots?
- What is the maximum resolution?
- Did it auto-index correctly?

This information dictates your **detector distance** and **required dose**.

If evaluating by eye remember to “join” images to at least 1.0 degree rather than looking at fine sliced images. Max res: best to rely on data reduction programs and apply a buffer (e.g 0.5 Å) – huge dynamic range not possible to show on a screen easily

Maximum
diffraction &
dose/exposure

Dose

$$1 \text{ Gy} = 1 \frac{\text{J}}{\text{kg}}$$

The concept of dose refers to the amount of energy absorbed by a material per unit mass of that material.

The absorbed energy is determined by several factors, including the incident energy, flux, illuminated area (beam size), and the composition of the material.

Total exposure time (s)

Transmission (%)

Beamline energy (keV)

Photon flux (ph/s)

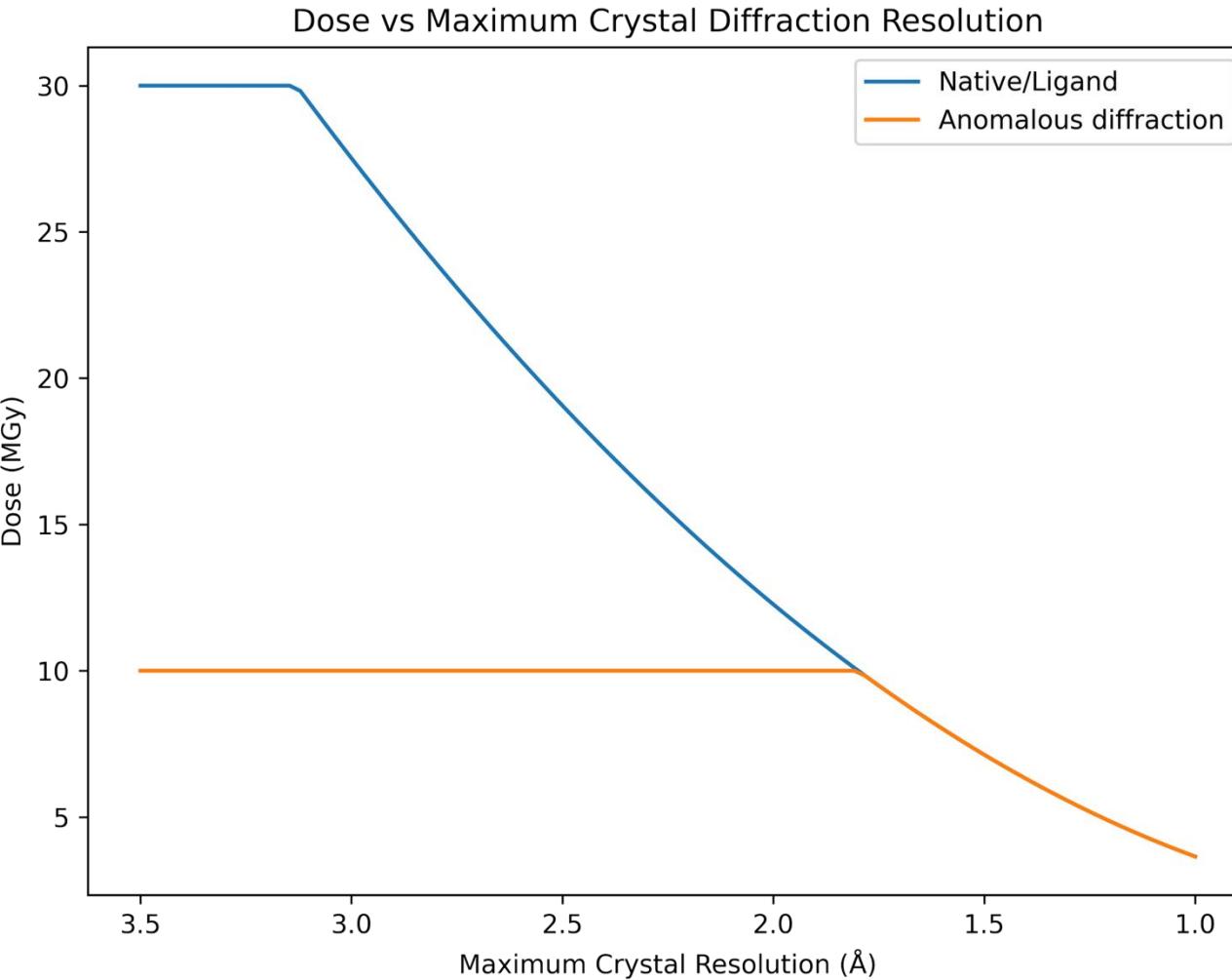
Beam size (VxH microns)

Sample info (sequence, unit cell, presence of HA, etc)



Dose

How Much Dose? Let Your Resolution Be Your Guide



- There is a relationship between the resolution a crystal diffracts to and the optimal dose it should receive.
- **General Rule:** Higher-resolution crystals require *less* dose.
- Lower-resolution crystals require *more* dose to maximize signal.
- This model is used by automated data collection systems at Diamond Light Source. (*)

Dose aware data collection on the variable and microfocus macromolecular crystallography beamline I04 at Diamond Light Source. Ralf Flraig et al 2025 J. Phys.: Conf. Ser. 3010 012096. DOI 10.1088/1742-6596/3010/1/012096

(*) Based and adapted from the discussion on “*Resolution and dose dependence of radiation damage in biomolecular systems*”. Atakisi, H., Conger, L., Moreau, D. W. & Thorne, R. E. (2019). *IUCrJ*, 6, 1040-1053.

<https://doi.org/10.1107/S2052252519008777>

expected crystal lifetime calculator

<https://bl831.als.lbl.gov/xtallife.html>

Raddose 3D

<https://github.com/GarmanGroup/RADDOSE-3D>

Beamline settings	Flux (ph/s)	Beam size (um)	Exposure (s)	Dose (MGy)	Dose rate (MGy/s)
A	3.8x10^12	100 x 100	~46	10	0.25
B	1.2x10^12	30x 20	~14	10	0.70
C	1.2x10^12	20x10	~5	10	2.00
D	1.0x10^13	80x15	~4	10	2.5
E	3.0x10^12	8x6	~0.5	10	20
F	3.0x10^12	20x20	~4	10	2.5

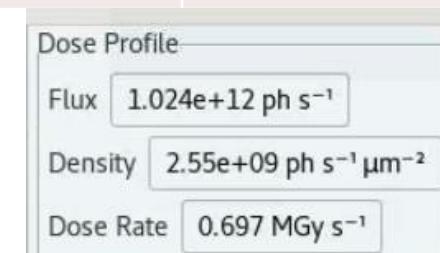
Assumptions:

Beamline energy at 0.9537 Å / 13.0 KeV

Crystal of the size of the beam

100% transmission

"standard" sample = no heavy metals (e.g. no Se, no As, average amount of S)



Dose references

10 MGy/A resolution:

Howells MR, Beetz T, Chapman HN, Cui C, Holton JM, Jacobsen CJ, Kirz J, Lima E, Marchesini S, Miao H, Sayre D, Shapiro DA, Spence JHC & Starodub D (2009). *J. Electron Spectrosc. Relat. Phenom.* **170**, 4-12.

1% non-isomorphism per MGy:

Banumathi S, Zwart PH, Ramagopal UA, Dauter M & Dauter Z (2004). *Acta Cryst. D* **60**, 1085-1093.

200 kGy for Room Temperature:

Warkentin M, Badeau R, Hopkins JB, Mulichak AM, Keefe LJ & Thorne RE (2012). *Acta Cryst. D* **68**, 124-133.

Barker AI, Southworth-Davies RJ, Paithankar KS, Carmichael I & Garman EF (2009). *J. Sync. Rad.* **16**, 205-216.

Blake CCF & Phillips DC (1962). pp. 183-191. Vienna: IAEA.

5 MGy for Se-Met:

Holton JM (2007). *J. Sync. Rad.* **14**, 51-72.

4 MGy for Hg-Cys:

Ramagopal UA, Dauter Z, Thirumuruh R, Fedorov E & Almo SC (2005). "Acta Cryst. D" **61**, 1289-1298.

2 MGy for Cys-Cys:

Murray JW & Garman EF (2002). *J. Sync. Rad.* **9**, 347-354.

500 kGy for Br-RNA:

Olieric V, Ennifar E, Meents A, Fleurant M, Besnard C, Pattison P, Schiltz M, Schulze-Briese C & Dumas P (2007). *Acta Cryst. D* **63**, 759-768.

500 kGy for Photosystem II:

Yano J, Kern J, Irrgang K-D, Latimer MJ, Bergmann U, Glatzel P, Pushkar Y, Biesiadka J, Loll B, Sauer K, Messinger J, Zouni A & Yachandra VK (2005). *PNAS USA* **102**, 12047-12052.

60 kGy for putidaredoxin:

Corbett MC, Latimer MJ, Poulos TL, Sevrioukova IF, Hodgson KO & Hedman B (2007). *Acta Cryst. D* **63**, 951-960.

60 kGy for bacteriorhodopsin:

Borshchevskiy V, Round E, Erofeev I, Weik M, Ishchenko A, Gushchin I, Mishin A, Willbold D, Buldt G & Gordeliy V (2014). *Acta Cryst. D* **70**, 2675-2685.

20 kGy for Fe reduction in myoglobin:

Radiat Phys Chem Oxf Engl 1993 **76**, 714-721.

rough rotisserie factor:

Holton JM (2009). "A beginner's guide to radiation damage", *J. Sync. Rad.* **16**, 133-142.

more accurate rotisserie factor calculations:

Zeldin OB, Brockhauser S, Bremridge J, Holton JM & Garman EF (2013). *PNAS USA* **110**, 20551-20556.



Dickerson JL, McCubbin PTN, Brooks-Bartlett JC, Garman EF. Doses for X-ray and electron diffraction: New features in RADDOS-3D including intensity decay models. *Protein Science*. 2024; 33(7):e5005. <https://doi.org/10.1002/pro.5005>

Start	0.00
Oscillation	0.100
Total oscillation	360.0
Delta	0.00
Chi	0.000
Phi	0.000

Raddose 3D

<https://github.com/GarmanGroup/RADDOSE-3D>

Dose and Exposure

Number of Images	3600
<input checked="" type="radio"/> Set Target Dose	
<input type="radio"/> Set Target Exposure	
Exposure Time	0.0030
Total Exposure Time	10.8
Dose / Dataset	7 MGy
First Image Number	1

Beam and Detector

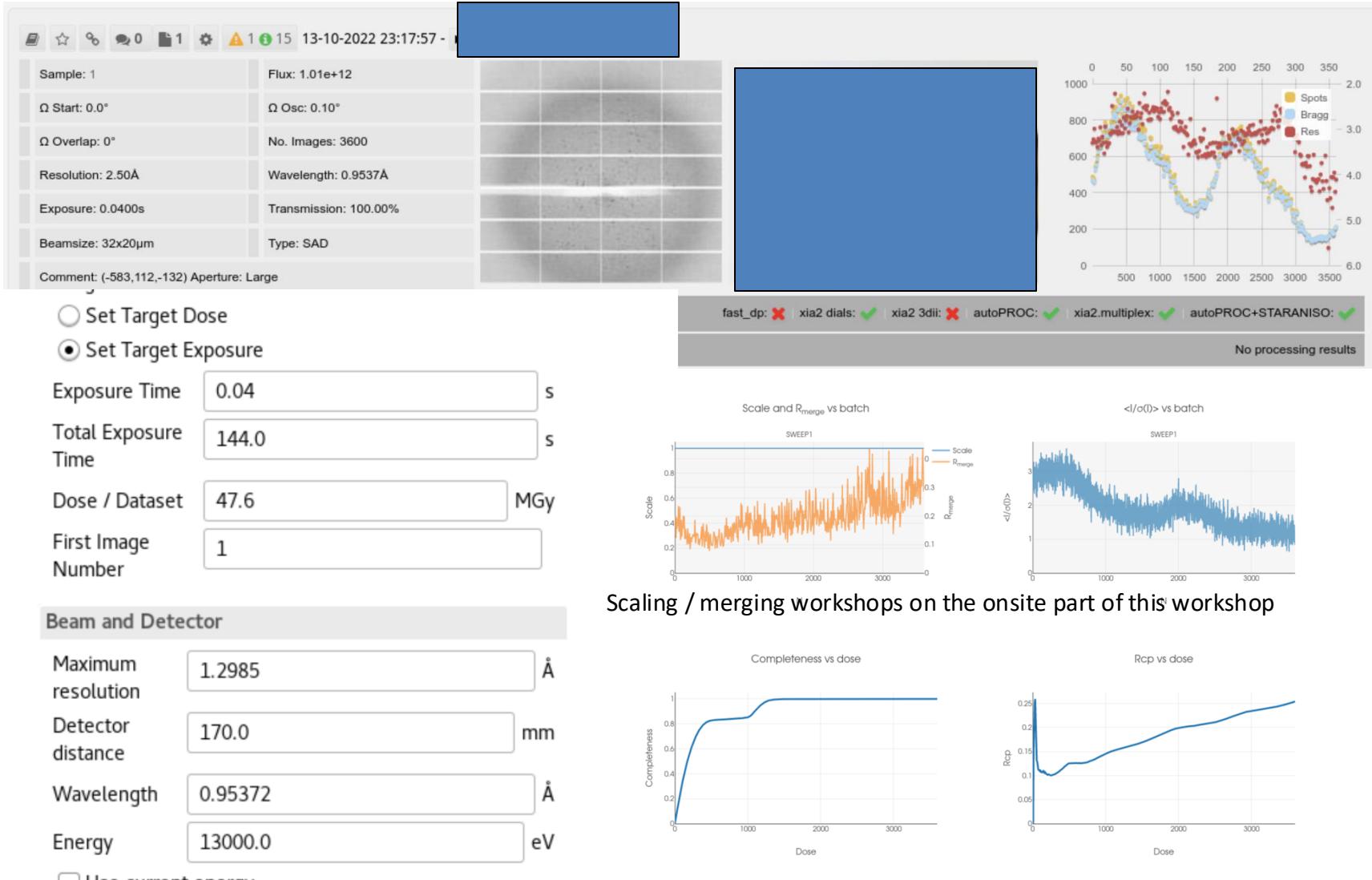
Maximum resolution	1.9023 Å
Detector distance	289.3 mm
Wavelength	0.95373 Å
Energy	12999.9 eV
<input type="checkbox"/> Use current energy	
Transmission	100.000000 %

Aperture and Beamstop

Beamstop	Standard
Horizontal beam size	31.73 μm
Vertical beam size	20 μm
<input checked="" type="checkbox"/> Use current beam size	

What happens if you ignore this? What happens if you just blast the crystal?

Elsepth
Garman's talk
on radiation
damage



Scaling / merging workshops on the onsite part of this workshop

Beam matching crystal size? Yes, but keep dose the Same

The image displays two side-by-side software interfaces, likely from the CCP4 suite, comparing experimental parameters and processing statistics for two different beam conditions.

Left Interface (Left Column):

- Sample: [REDACTED]
- Flux: 1.21e+12
- Ω Start: 0.0°
- Ω Osc: 0.10°
- Ω Overlap: 0°
- No. Images: 1200
- Resolution: 1.91 Å
- Wavelength: 0.9537 Å
- Exposure: 0.0800s
- Dose: 15.00 MGy
- Transmission: 100.00%
- Beamsize: 63x50 μm
- Type: SAD

Right Interface (Right Column):

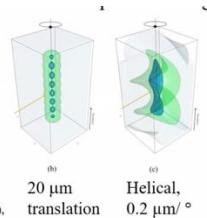
- Sample: [REDACTED]
- Flux: 9.64e+11
- Ω Start: 0.0°
- Ω Osc: 0.10°
- Ω Overlap: 0°
- No. Images: 1200
- Resolution: 1.91 Å
- Wavelength: 0.9537 Å
- Exposure: 0.0800s
- Dose: 194.70 MGy
- Transmission: 100.00%
- Beamsize: 19x10 μm
- Type: SAD

Processing Statistics (Bottom Tables):

Shell	Observations	Unique	Resolution	Rmeas	I/sig(I)	CC Half	Completeness
outerShell	5789	853	2.87 - 2.94	0.758	1.7	0.9	97.7
innerShell	834	150	12.82 - 29.14	0.021	60.1	1.0	92.6
overall	79247	12099	2.87 - 29.14	0.045	22.1	1.0	99.7

Shell	Observations	Unique	Resolution	Rmeas	I/sig(I)	CC Half	Completeness
outerShell	4364	700	3.12 - 3.20	0.752	1.4	0.8	99.4
innerShell	639	118	13.95 - 29.29	0.034	36.9	1.0	90.3
overall	61431	9560	3.12 - 29.29	0.062	16.1	1.0	99.7

Downstream Processing



Sample with Line scan

Samples Grid Scan Grid Scan Results Fluorescence Data Collection Line Scan Tools Use

View X

Resource Centring complete Aperture Beam Scale Focal Spot Slit Gap

Camera Control Sample Environment

Snapshot Backlight

Zoom 5.0

Line Scan Settings Command Queue

Line Scan Settings

Oscillation 0.1 Total oscillation 360.0 Delta 0

Images

Automatic run number

Run number 0 Exposure time 0.03 s Total exposure time 108.0 s Detector distance 333 mm Resolution 2.14 Å Transmission 100.0 % Number of wedges 1 Images per wedge 3600

Wedged line scan Helical line scan

Line

Start X -0 μm Start Y -0.014 μm Start Z 0.003 μm End X -0 μm End Y -0.014 μm End Z 0.003 μm

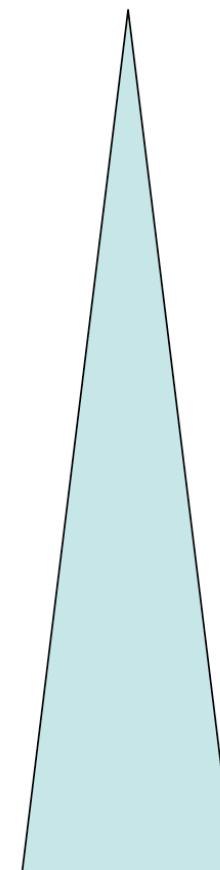
Aperture and Beamstop

Move To Start Move To End Use Beam Centre As Start Use Beam Centre As End

Experimental aim

- Molecular substitution
- Molecular replacement
- Isomorphous replacement (Hg, Pt)
- MAD/SAD phasing
- Native-SAD phasing
(Sulfur-SAD)

Good data



Best data

What's the goal?

Aim	High priorities	Lower priority	Max Dose per dataset (e.g. per 120, 180 or 360 degrees)
Native data collection	High resolution (e.g < 1.8 Å) Complete data Minimise radiation damage particular for high resolution data.	High redundancy Speed	~20 MGy (*)
Multi-wavelength anomalous dispersion (MAD) Single Anomalous dispersion (SAD)	Accurate, complete and highly redundant Very little radiation damage Choice of wavelength	High resolution data	1 - 5 MGy (heavy atom will increase dose for same exposure)
Sulphur SAD	As above + extra high redundancy (40-200fold) if not on I23 beamline		0.3-0.5 MGy
Molecular replacement	Completeness at low resolution (< 3Å) Good quality low resolution	High resolution data High redundancy	~20 MGy (*)
Ligand/mutation	Medium resolution (e.g. 2 Å), complete data. High throughput (FAST)	High redundancy	10 - 20 MGy (*)

Trying to collect everything at once will usually result in failure

(*For a ~2.5Å or worse diffracting crystal per Diamond UDC recipe

Data collection - think!



- before starting data collection make sure xtal is properly centered and does not “walk” out of beam
- consider dose (exposure time, attenuation, energy), wedge angle, detector distance... before it is too late!

If you forget almost all, what should you retain for a good data collection?

There is still confusion in the user community which has led to some incorrect common belief that exposure time is a default data collection parameter. Contrary to this belief, the key constraint is the dose we delivery to the sample, not data collection time or total rotation angle.

- Good sample prep
 - Good crystal, well cryo-cooled, good mount,
- Starting angle
 - any unless collecting less than 180 in P1, or other SG
- Total oscillation angle
 - 360 unless merging data from multiple crystals
- Oscillation per frame
 - 0.1 deg unless good reason (i.e, mosaicity, overlaps, background, etc)
- Energy
 - default, unless good reason (i.e, experimental phasing, high resolution, etc)
- Good crystal centring
 - x-ray centring, optically if beam is big but not if crystal is big
- Dose / indirectly exposure (*)
 - Use dose not simple exposure, relate the dose to diffracting resolution of your crystal (diffracting better / higher resolution? Lesser dose)

(*) RADDOSSE-3D has an online server <https://raddose.se/> and we recommend you use it to plan before a beamline session

IUCrData launches Raw Data Letters

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Abstract

Experiences with MX data reuse at Diamond

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FAIR data are data which meet principles of findability, accessibility, interoperability, and reusability

- Zenodo? others

Consider making your raw data public!

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PMCID: PMC9159283

PMID: [35647915](https://pubmed.ncbi.nlm.nih.gov/35647915/)

Raw diffraction data are our ground truth from which all subsequent workflows develop

[John R. Helliwell^{a,*}](#)

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Questions?