Dose driven data collection to optimise your experiments

Better (starting) collection parameters

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

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Neil Paterson
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Murray Stuart
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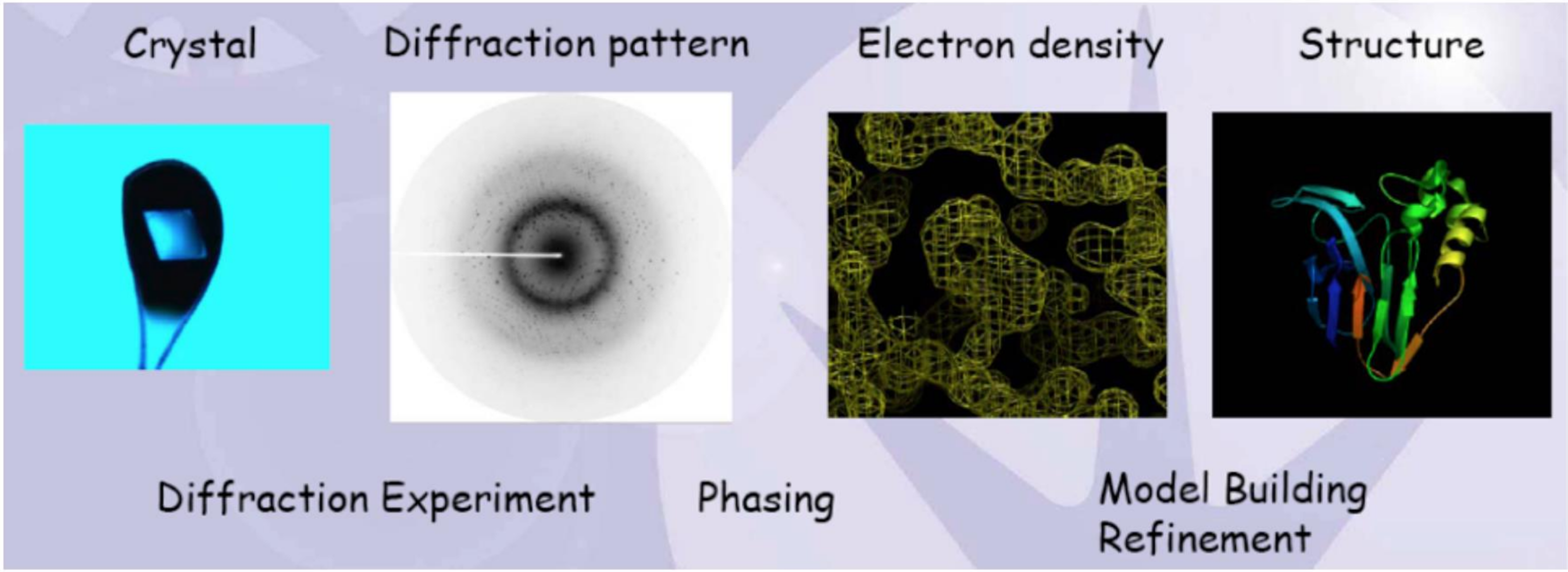
Tom Caradoc-Davies
[redacted] Andreas Förster <3
for slides & ideas

Kamel El Omari’s talk
“Basic Diffraction Theory”

Sofia Trampari talk
“Strategies for Photo conting detectors”

Ana Gonzalez’s talk
“Data processing”

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



Crystal

Diffraction pattern

Electron density

Structure

Diffraction Experiment

Phasing

Model Building
Refinement

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

REC TC: 00:00:00:00

Beamline day Mon 25th Nov 2024

Data collection is important!

This is the last experimental step

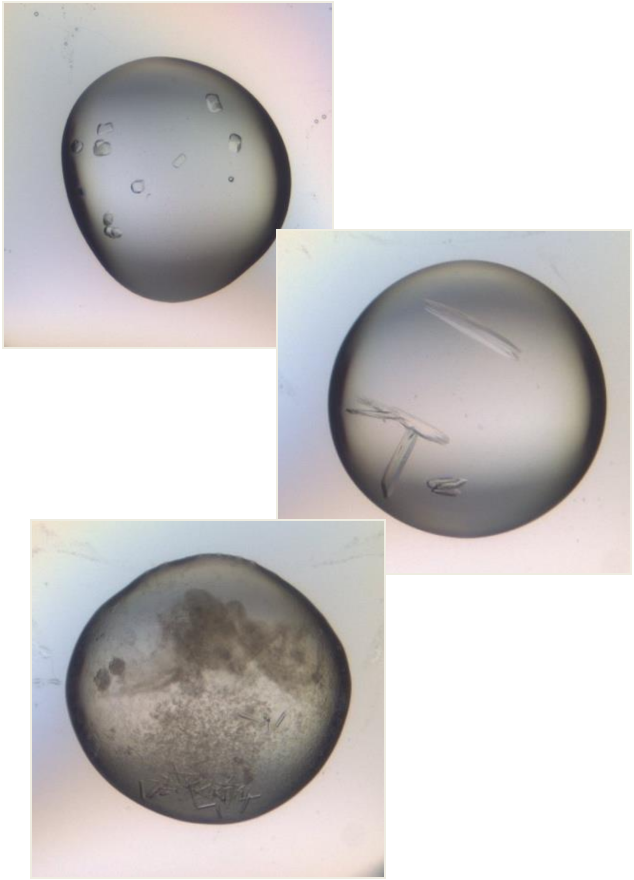
Time taken now to collect the best possible data can prevent pain and possible failure later on.

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

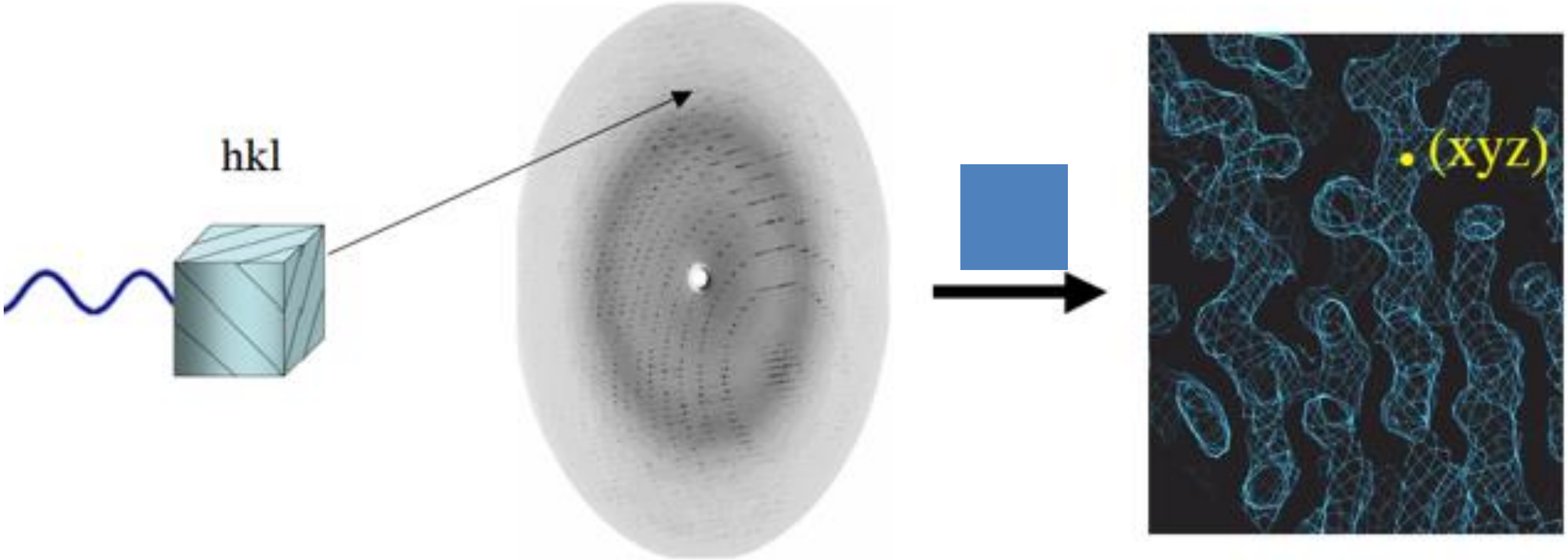
If the original data are poor then not much can be done about it later.


GIGO/RIRO paradigm

With fast sample changers, goniometers and detectors a lot of data can be collected very fast.
Quantity ≠ Quality



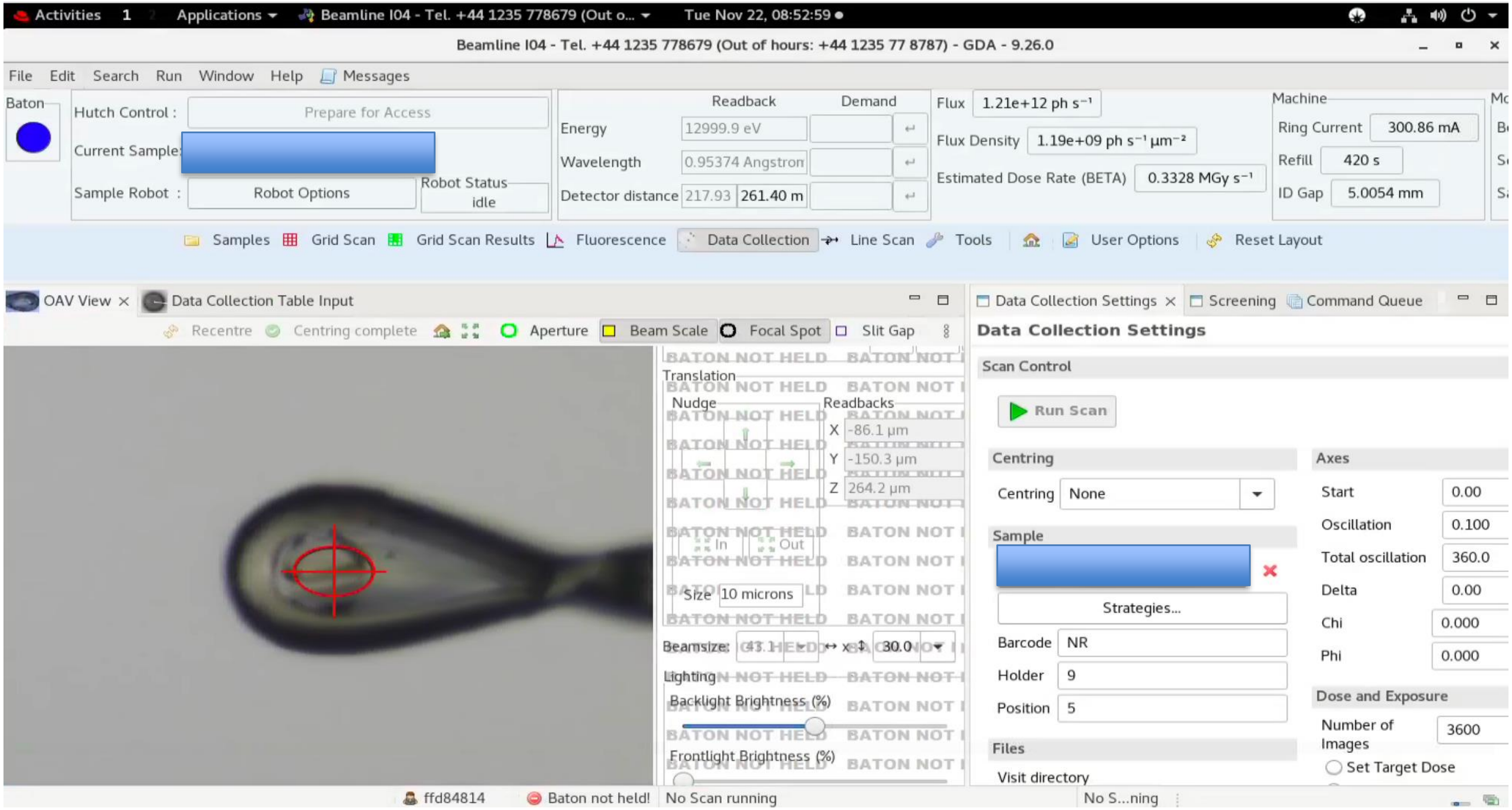
Data collection: the goal



- 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

Crystal alignment to beam

Data collection GUI



Beamline I04 - Tel. +44 1235 778679 (Out of hours: +44 1235 77 8787) - GDA - 9.26.0

Hutch Control : Prepare for Access

Current Sample:

Sample Robot : Robot Options

Robot Status: idle

	Readback	Demand
Energy	12999.9 eV	
Wavelength	0.95374 Angstrom	
Detector distance	217.93	261.40 m

Flux: 1.21e+12 ph s⁻¹

Flux Density: 1.19e+09 ph s⁻¹ µm⁻²

Estimated Dose Rate (BETA): 0.3328 MGy s⁻¹

Machine

Ring Current: 300.86 mA

Refill: 420 s

ID Gap: 5.0054 mm

Readbacks

X: -86.1 µm

Y: -150.3 µm

Z: 264.2 µm

Size: 10 microns

Beamsize: 43.1 ↔ x ↕ 30.0

Data Collection Settings

Centring

Centring: None

Sample

Barcode: NR

Holder: 9

Position: 5

Files

Visit directory

Axes

Start: 0.00

Oscillation: 0.100

Total oscillation: 360.0

Delta: 0.00

Chi: 0.000

Phi: 0.000

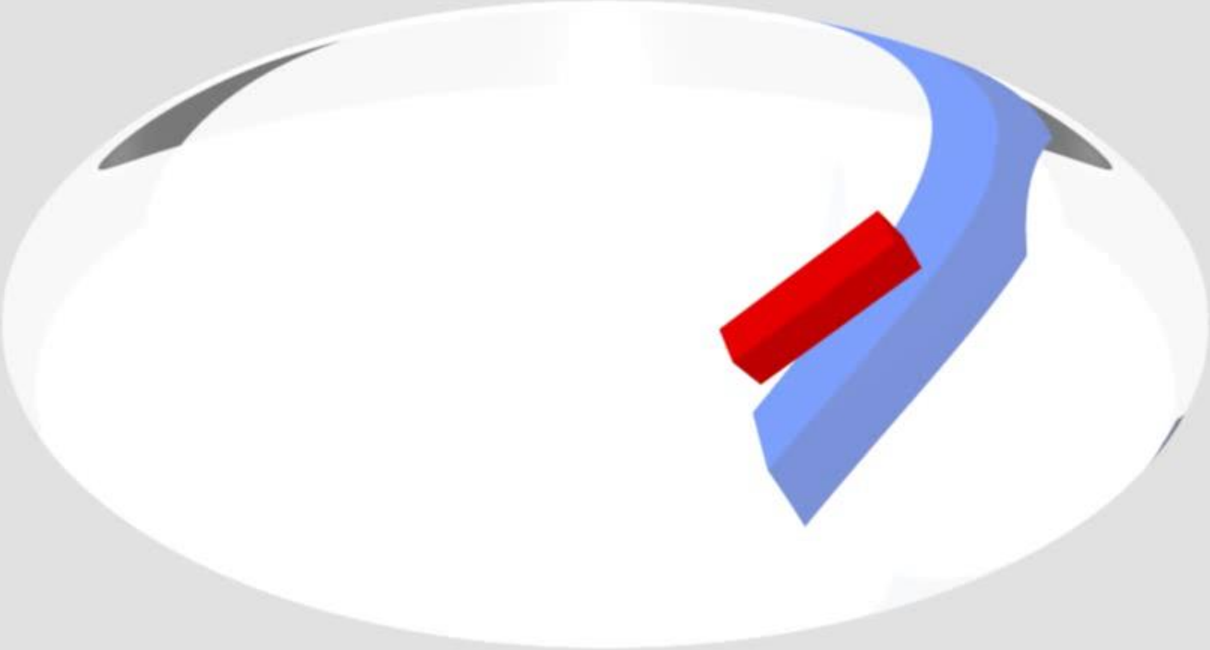
Dose and Exposure

Number of Images: 3600

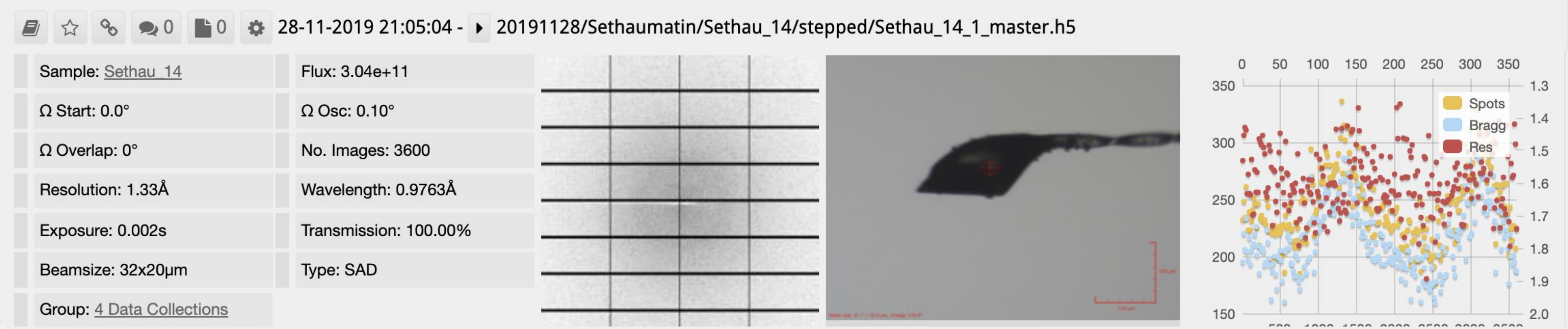
○ Set Target Dose

ffd84814 | Baton not held! | No Scan running | No S...ning

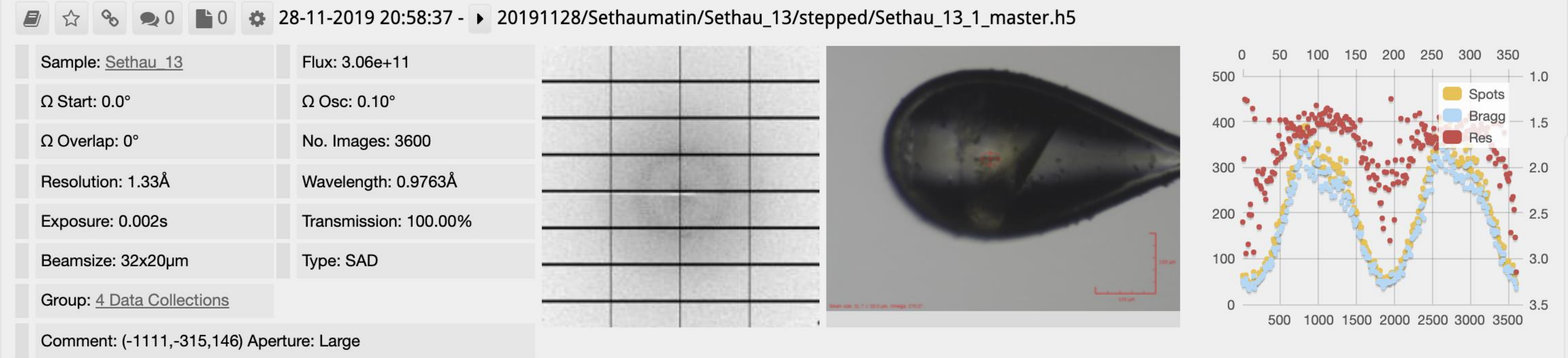
Good alignment is crucial



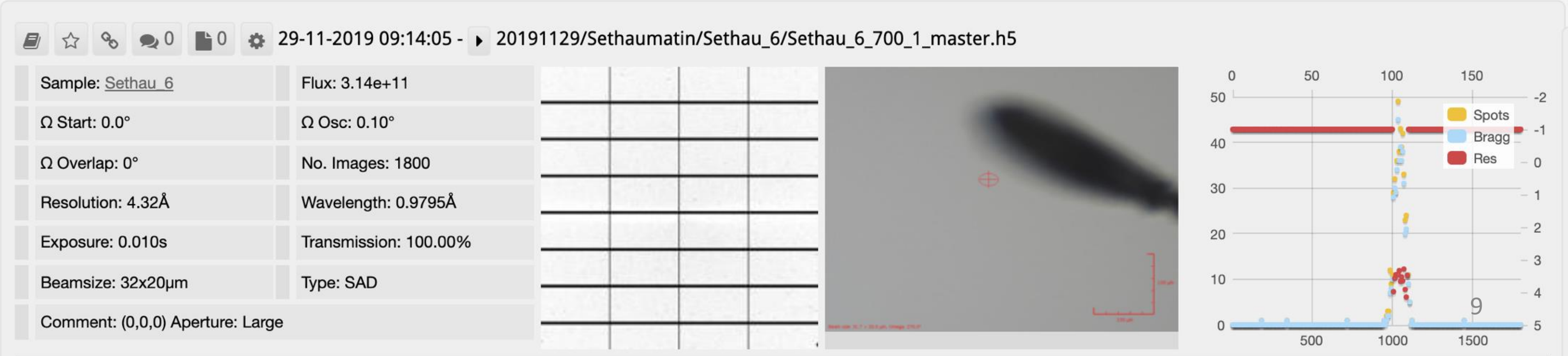
Data collection



Sample: Sethau_14
Ω Start: 0.0°
Ω Overlap: 0°
Resolution: 1.33Å
Exposure: 0.002s
Beamsize: 32x20µm
Group: 4 Data Collections
Flux: 3.04e+11
Ω Osc: 0.10°
No. Images: 3600
Wavelength: 0.9763Å
Transmission: 100.00%
Type: SAD

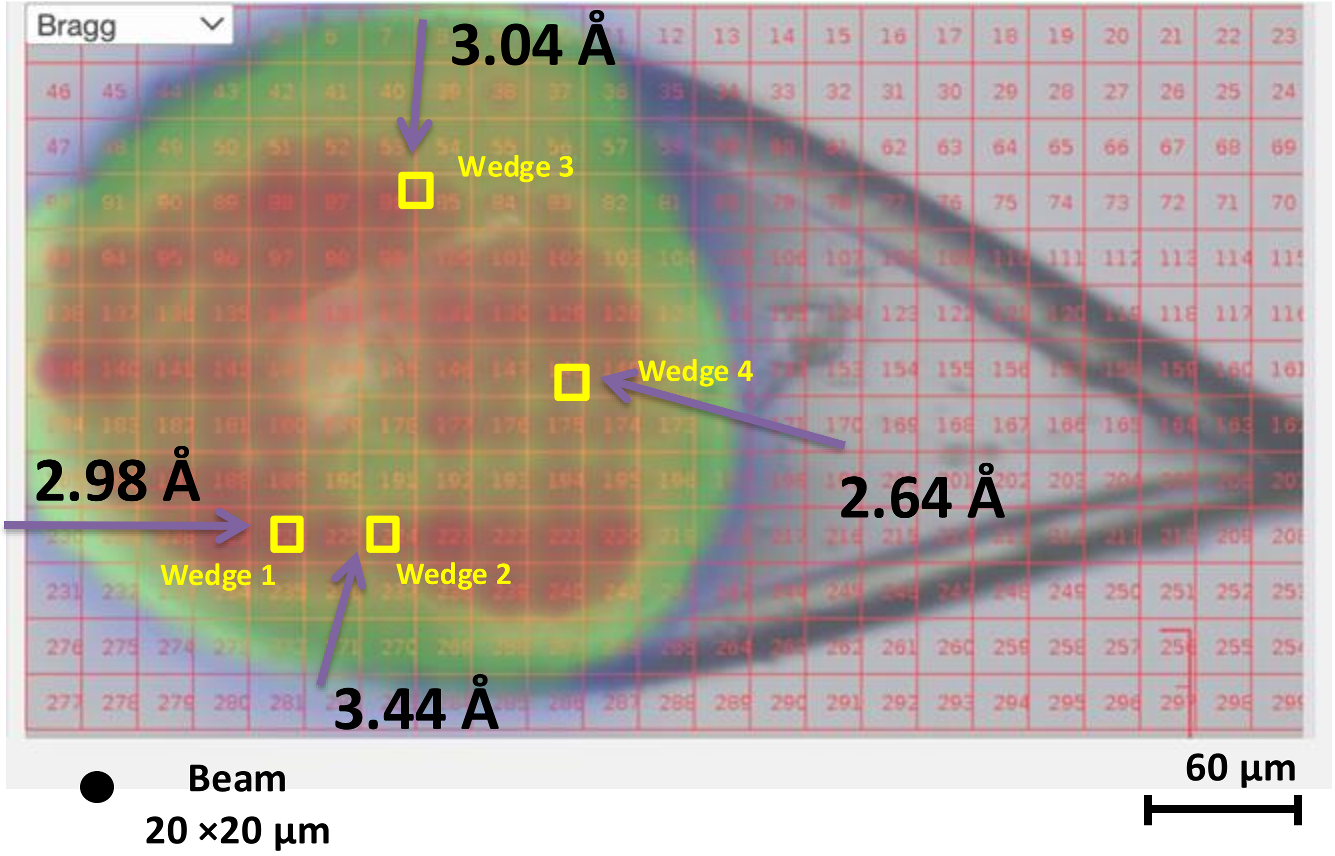


Sample: Sethau_13
Ω Start: 0.0°
Ω Overlap: 0°
Resolution: 1.33Å
Exposure: 0.002s
Beamsize: 32x20µm
Group: 4 Data Collections
Comment: (-1111,-315,146) Aperture: Large
Flux: 3.06e+11
Ω Osc: 0.10°
No. Images: 3600
Wavelength: 0.9763Å
Transmission: 100.00%
Type: SAD



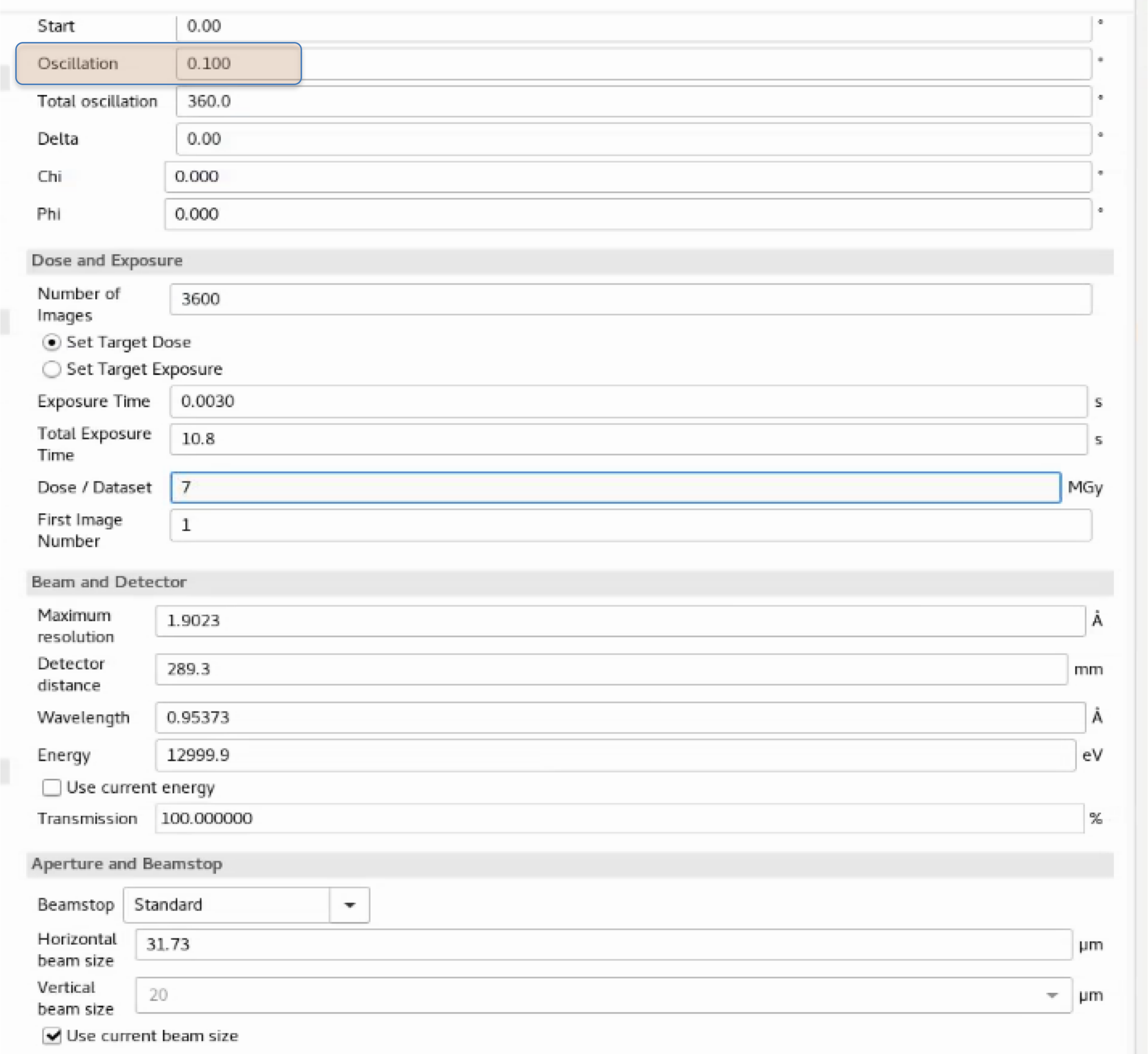
Sample: Sethau_6
Ω Start: 0.0°
Ω Overlap: 0°
Resolution: 4.32Å
Exposure: 0.010s
Beamsize: 32x20µm
Comment: (0,0,0) Aperture: Large
Flux: 3.14e+11
Ω Osc: 0.10°
No. Images: 1800
Wavelength: 0.9795Å
Transmission: 100.00%
Type: SAD

Even the same crystal can show a lot of variation…



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

Oscillation per frame



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	

● Set Target Dose
○ Set Target Exposure

Exposure Time	0.0030	s
Total Exposure Time	10.8	s
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

☐ Use current energy

Transmission	100.000000	%

Aperture and Beamstop

Beamstop	Standard	
Horizontal beam size	31.73	µm
Vertical beam size	20	µm

☑ Use current beam size

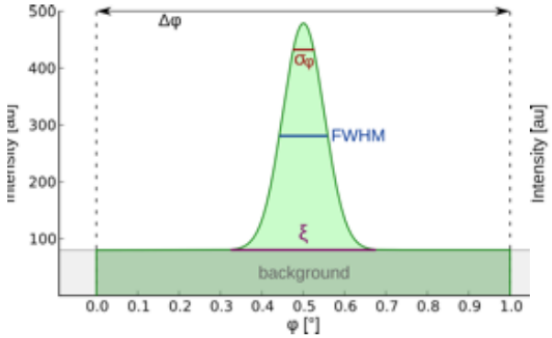
Random errors, counting statistics

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

$$I = N_p - N_b$$ **Signal is the difference**

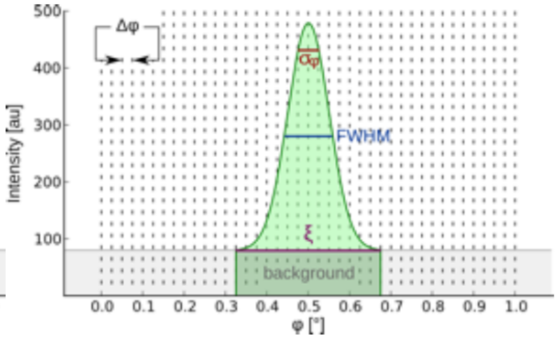
$$\sigma_I = (\sigma_p^2 + \sigma_b^2)^{1/2}$$

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



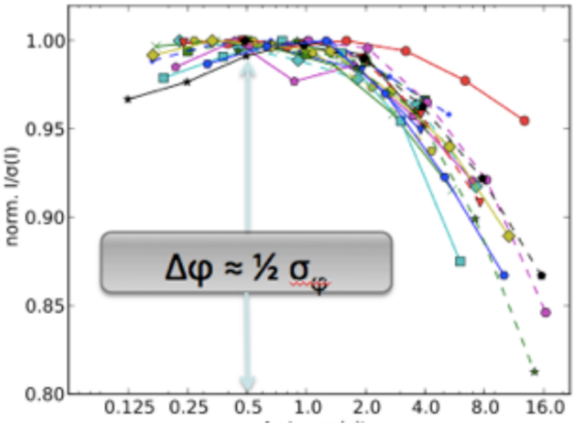
Wide φ-slicing

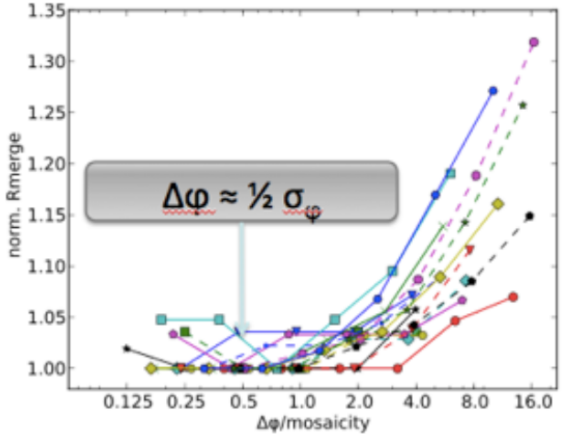
- Large $\Delta\varphi$ ($\Delta\varphi > \xi$)
- Large overlap of reflections and background along φ
- Few images



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

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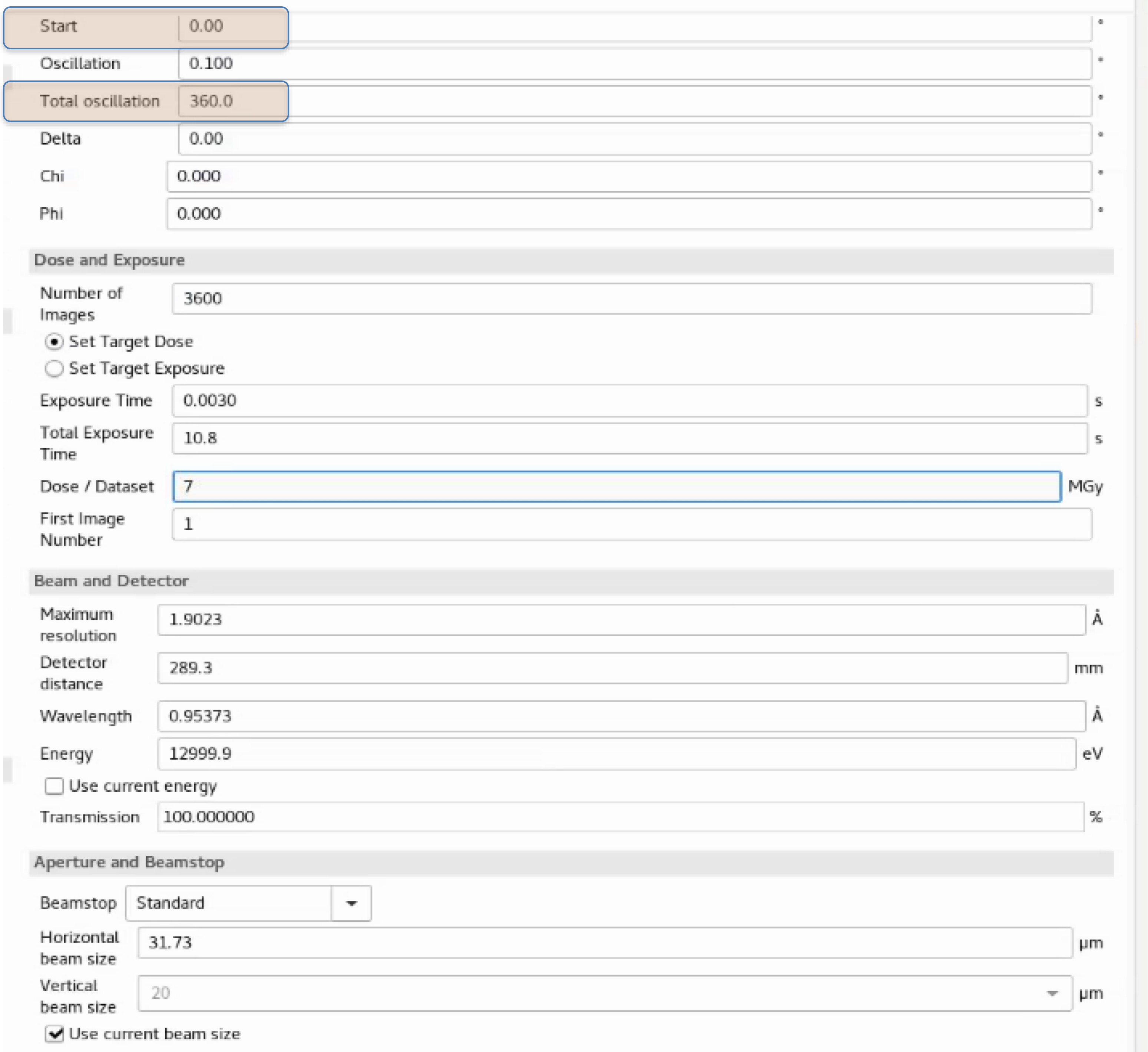
Mueller, *et al*, *Acta Cryst*. **D68**, 42 (2012)

Total oscillation & starting angle



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	

● Set Target Dose
○ Set Target Exposure

Exposure Time	0.0030	s
Total Exposure Time	10.8	s
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

☐ Use current energy

Transmission	100.000000	%

Aperture and Beamstop

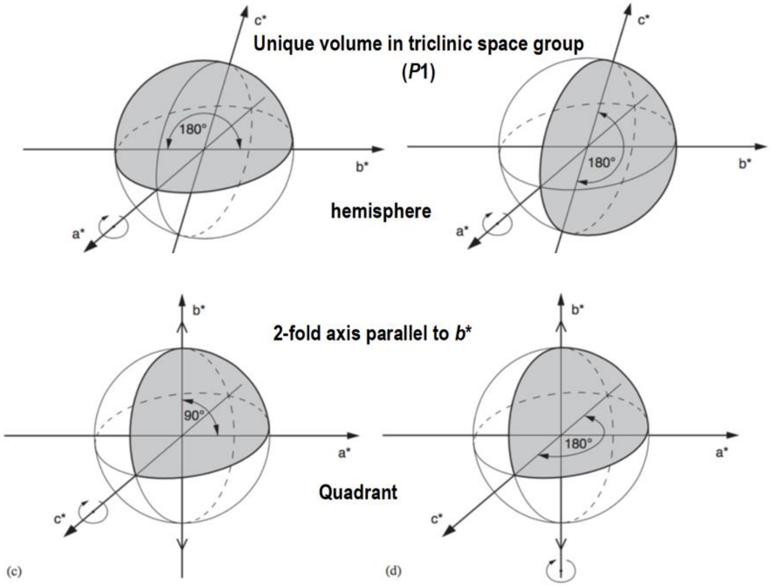
Beamstop	Standard	
Horizontal beam size	31.73	µm
Vertical beam size	20	µm

☑ Use current beam size

Completeness - crystal symmetry

Andrey Lebedev’s talk “Intro to space groups and symmetry” next Tues 26th Nov 2024

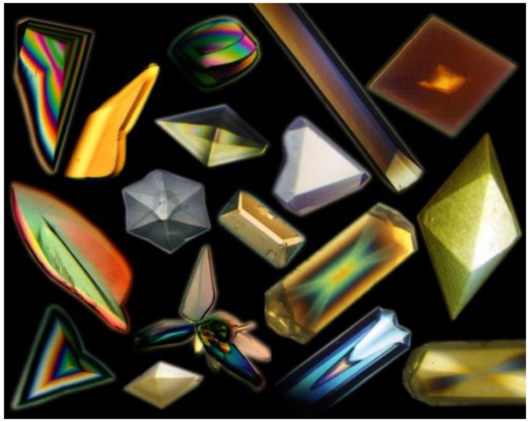
- reciprocal space symmetry = crystal symmetry plus
- Friedel’ s law : I (h k l) = I (-h -k -l)



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

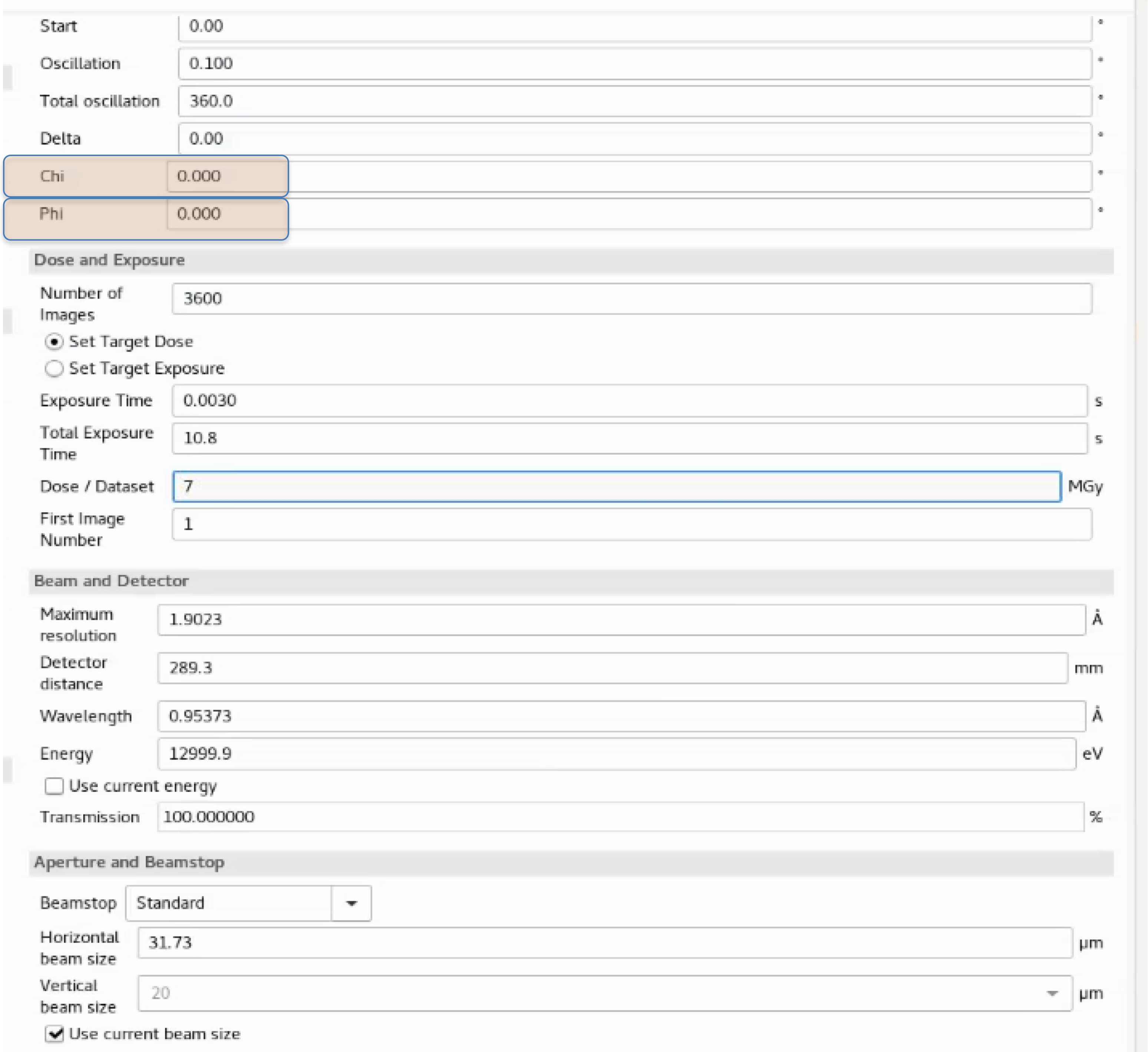
for N-fold symmetry **along** rotation axis, need 180/N degrees of data to obtain complete data set (assuming Friedel’ s law holds)

- Give yourself options (Radiation Damage) ← same dose in larger range
- Increase multiplicity ← Larger range more of same reflections
- It is not slower ← collect same total exposure (dose) in larger angle range
- Explore the crystal shape ← might be thicker, better spots, > crystals/twinning



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

Multi axis goniometry



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	

● Set Target Dose

○ Set Target Exposure

Exposure Time	0.0030	s
Total Exposure Time	10.8	s
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

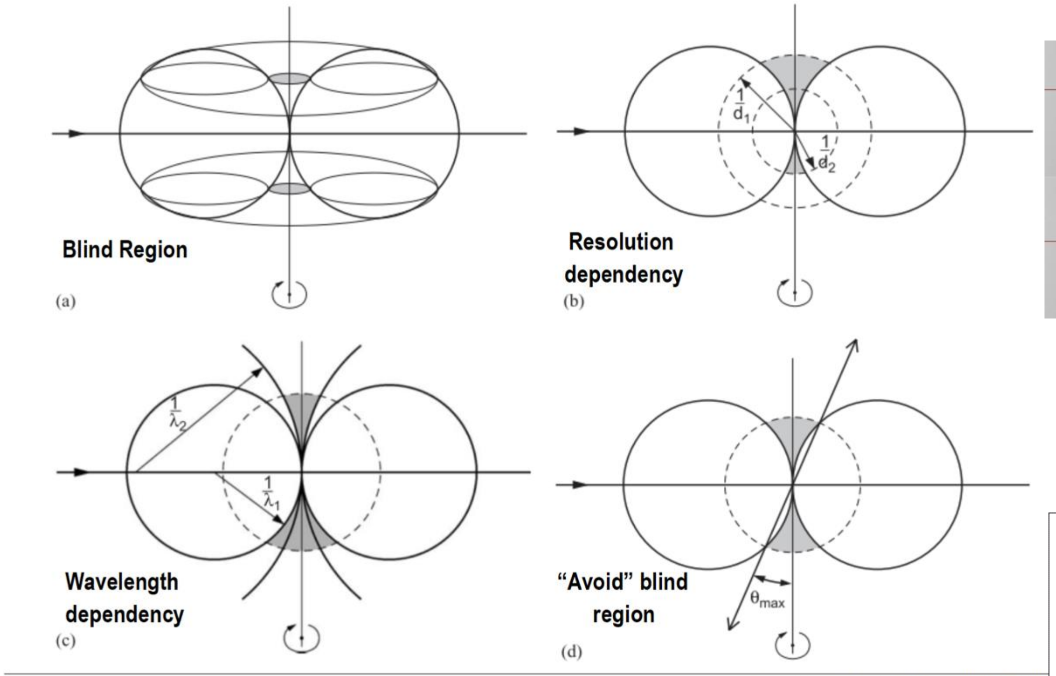
☐ Use current energy

Transmission	100.000000	%

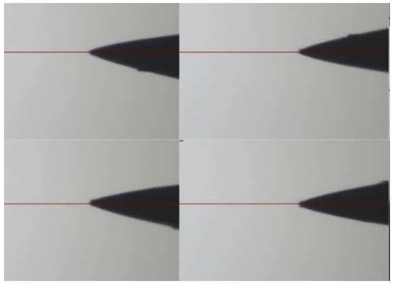
Aperture and Beamstop

Beamstop	Standard	
Horizontal beam size	31.73	µm
Vertical beam size	20	µm

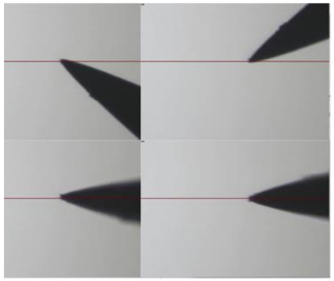
☑ Use current beam size



Dauter, *Prog. Biophys Mol. Bio.* **89**, 153 (2005)

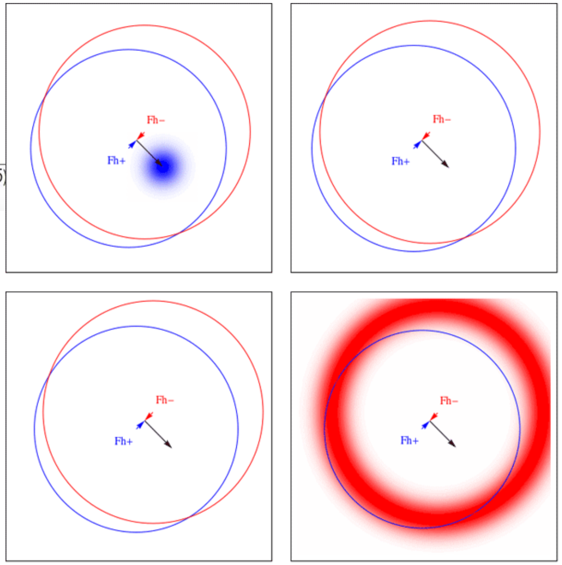


Chi=0 deg



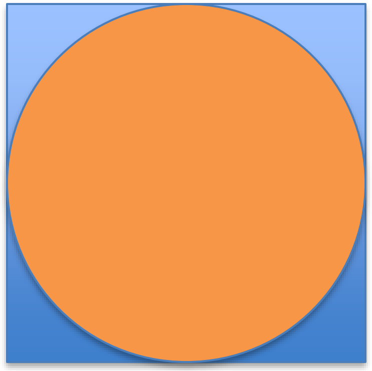
Chi=30 deg

Experimental Phasing

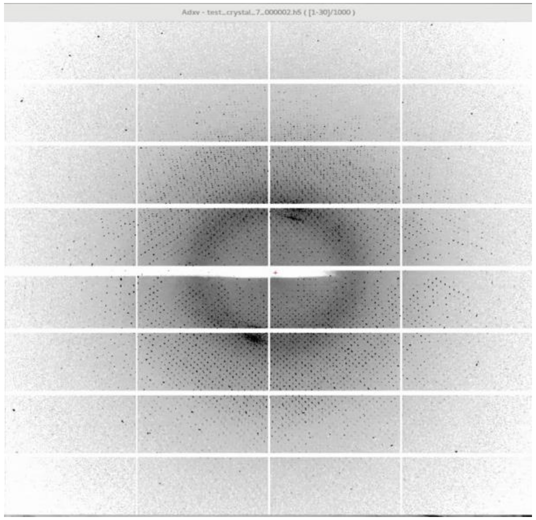


Picture courtesy of Acta Crystallogr D Biol Crystallogr. 2011 Apr 1; 67:338–344.

Experimental Phasing, Airlie McCoy

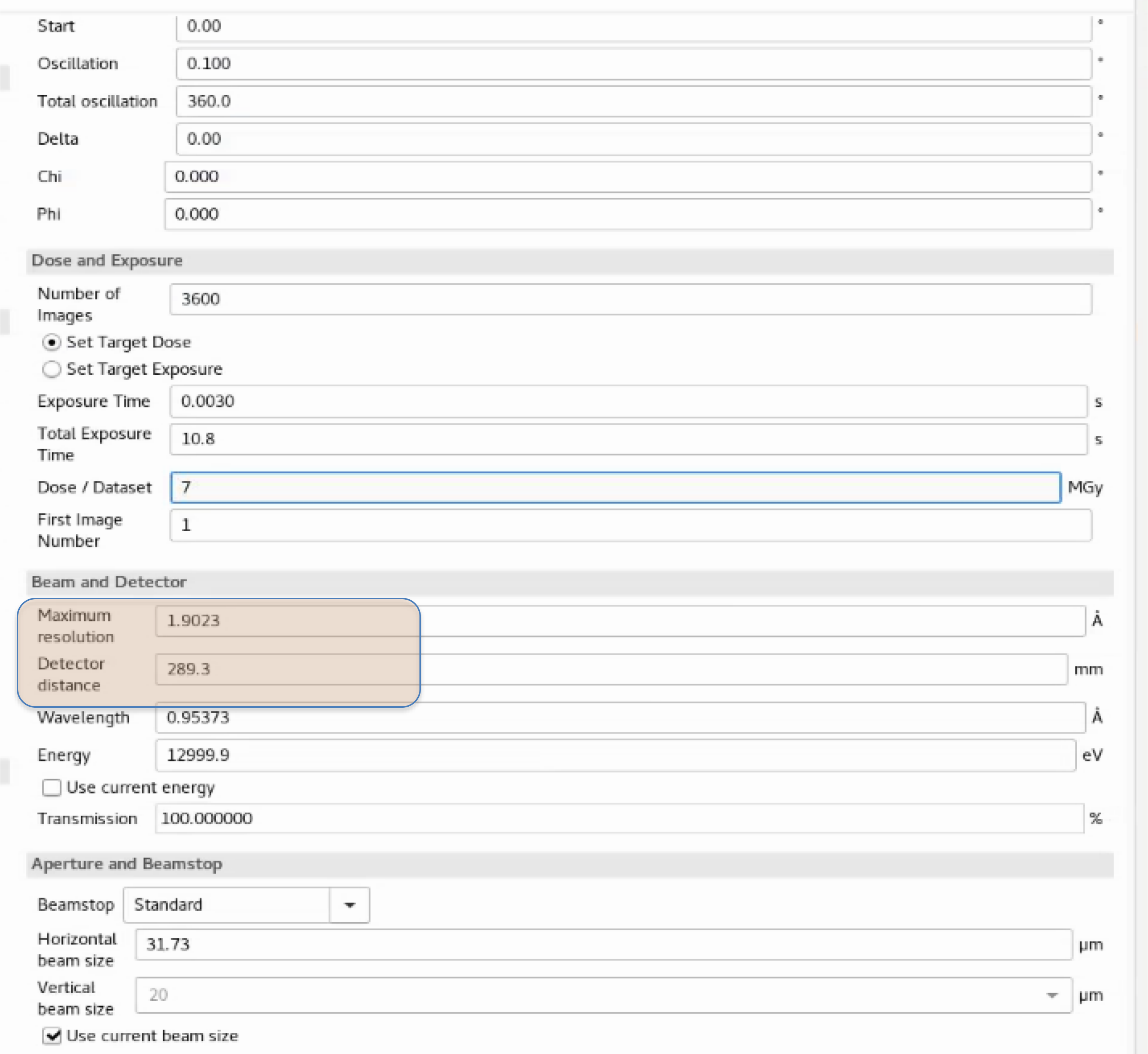


Corner vs Edge



Systematic error reduction
Detector module gaps

Detector distance / resolution



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

● Set Target Dose

○ Set Target Exposure

Exposure Time 0.0030 s

Total Exposure Time 10.8 s

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

☐ Use current energy

Transmission 100.000000 %

Aperture and Beamstop

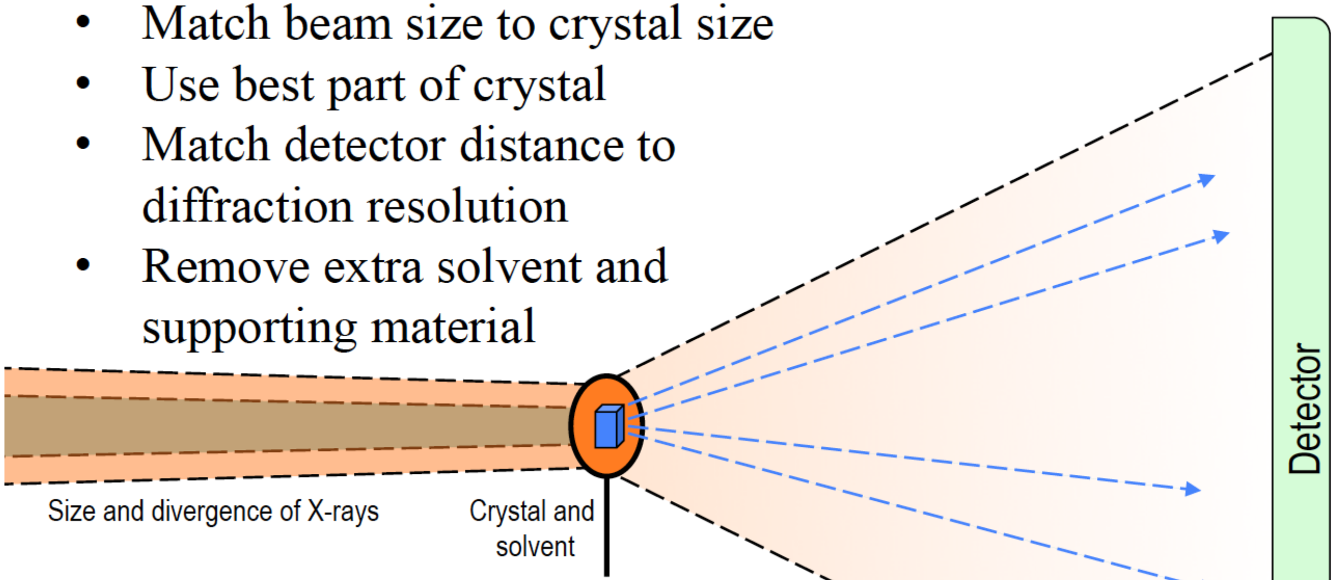
Beamstop Standard

Horizontal beam size 31.73 µm

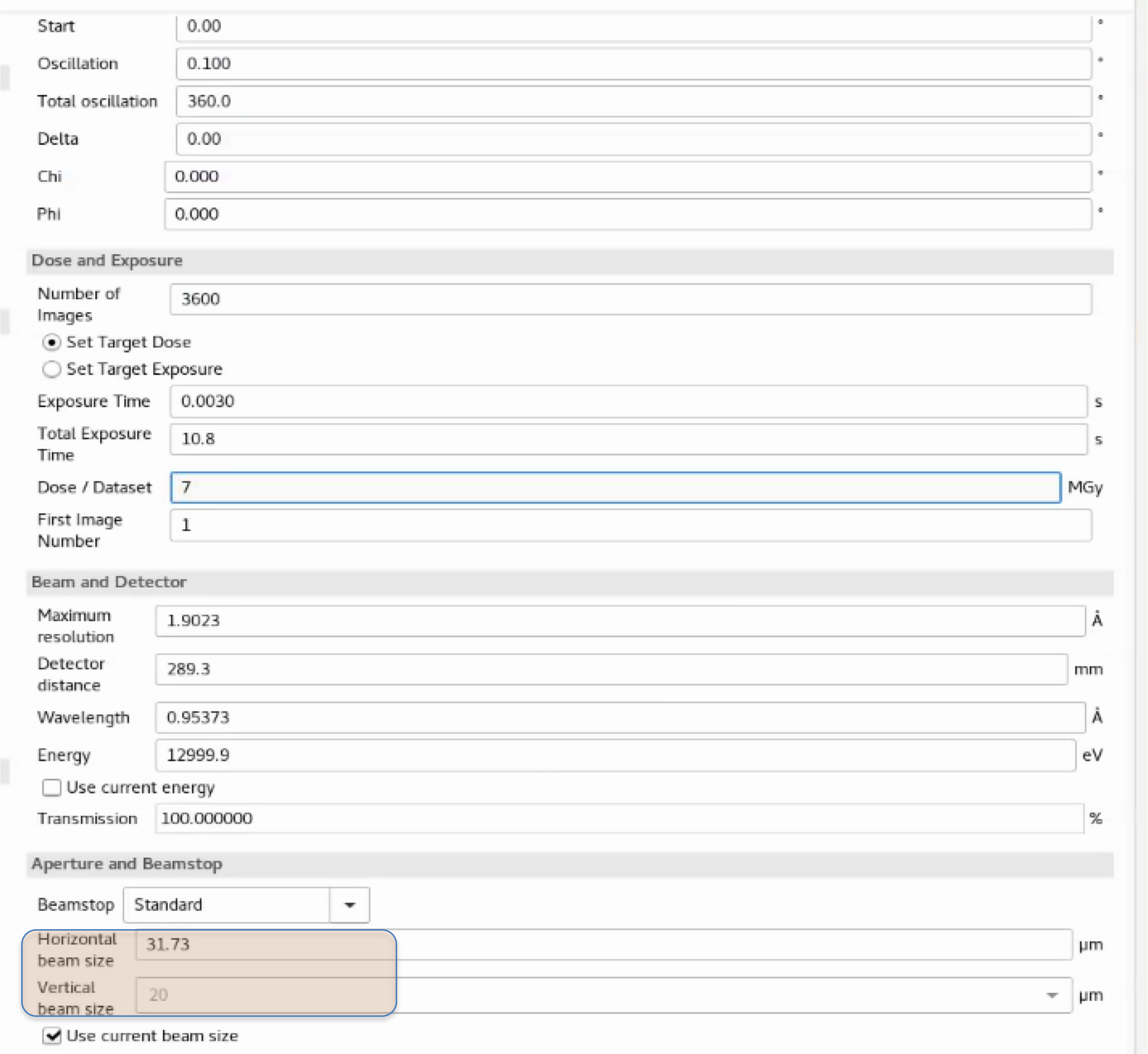
Vertical beam size 20 µm

☑ Use current beam size

- 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



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

● Set Target Dose

○ Set Target Exposure

Exposure Time: 0.0030 s

Total Exposure Time: 10.8 s

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

☐ Use current energy

Transmission: 100.000000 %

Aperture and Beamstop

Beamstop: Standard

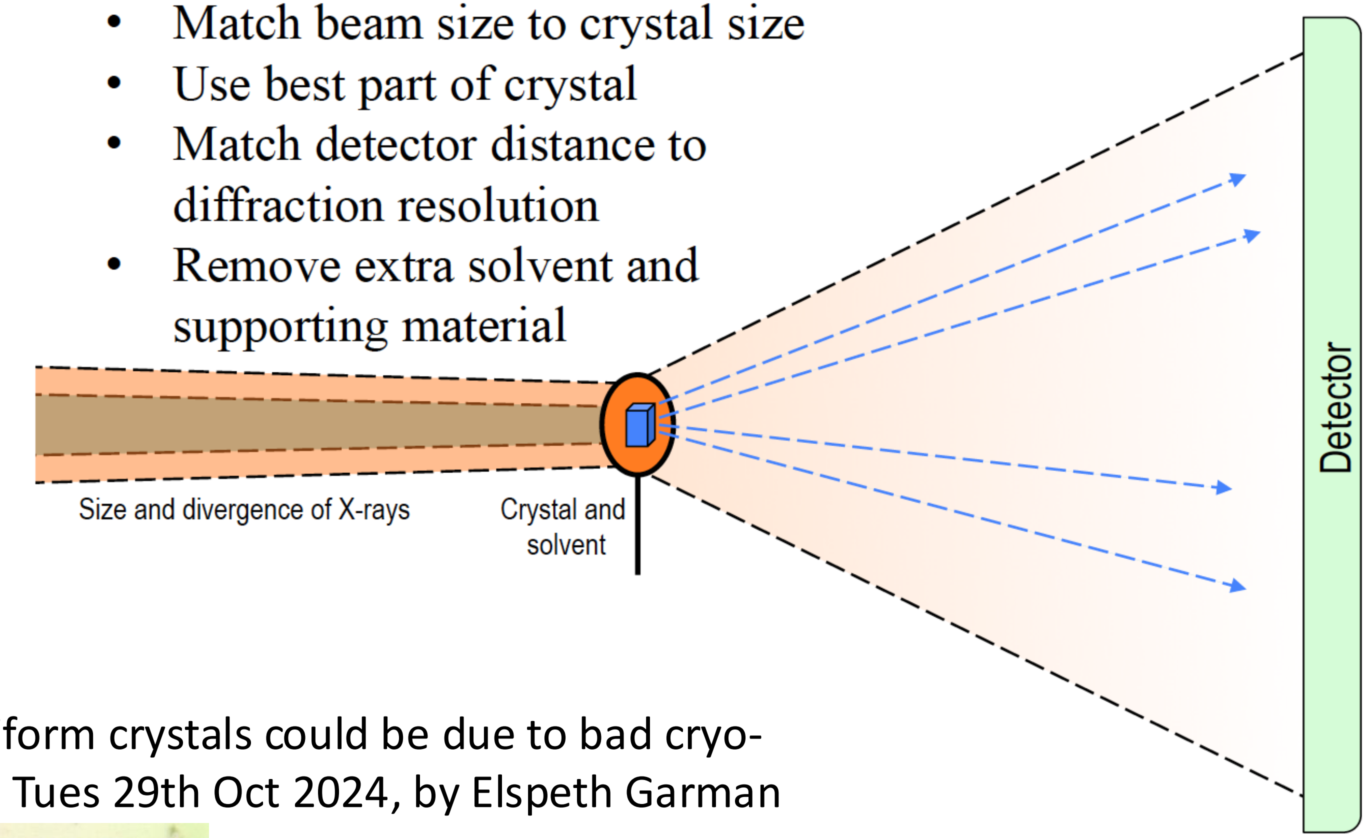
Horizontal beam size: 31.73 µm

Vertical beam size: 20 µm

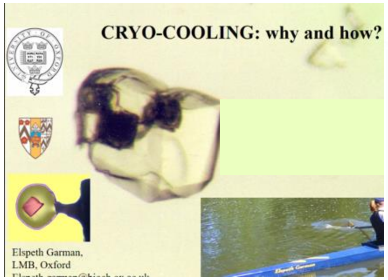
☑ Use current beam size

All about reducing background noise:
Ramona Duman's talk will talk about it using vacuum
"Anomalous Diffraction in 2024" on Wed 27th Nov 2024

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

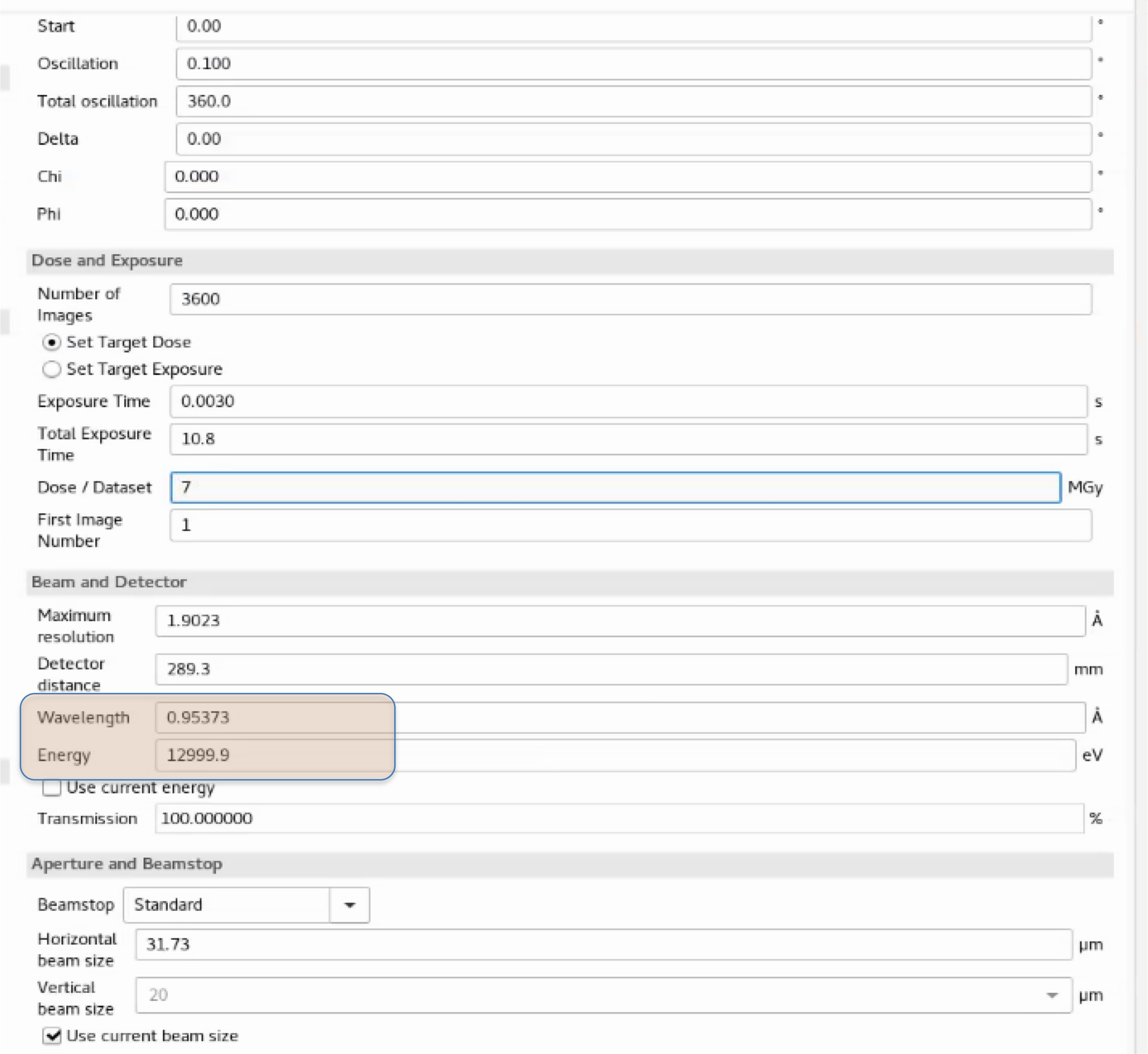


Non uniform crystals could be due to bad cryo-cooling: Tues 29th Oct 2024, by Elspeth Garman


CRYO-COOLING: why and how?

Elspeth Garman,
LMB, Oxford

Energy



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	

● Set Target Dose
○ Set Target Exposure

Exposure Time	0.0030	s
Total Exposure Time	10.8	s
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

☐ Use current energy

Transmission	100.000000	%

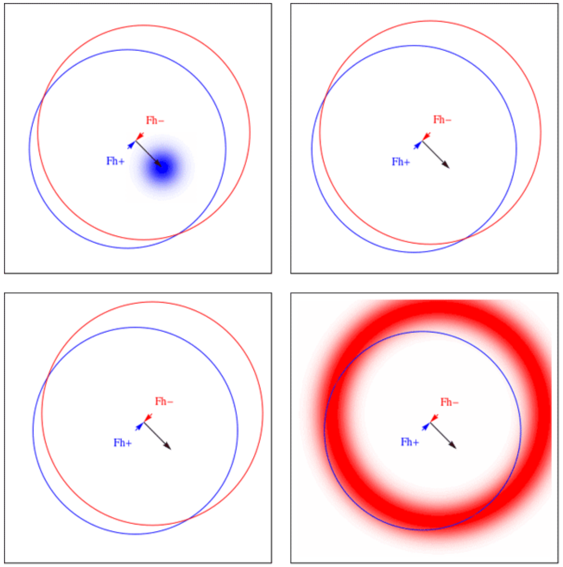
Aperture and Beamstop

Beamstop	Standard	
Horizontal beam size	31.73	µm
Vertical beam size	20	µm

☑ Use current beam size

Energy selection

Experimental Phasing



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

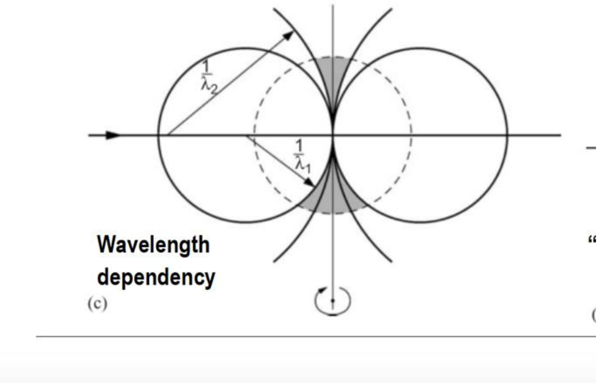
High resolution

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

Bragg's Law

https://www.diamond.ac.uk/Instruments/Mx/Common/Calculators.htr
(search for “useful calculators diamond”)

Blind region / Cuspid



Diffraction Theory by Kamel
Anomalous, by Ramona

Crystal life time / Radiation damage for small crystals
Sofia Trampari mentioned this before

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

Dose per dataset / Total Exposure

The problem: exposure per frame

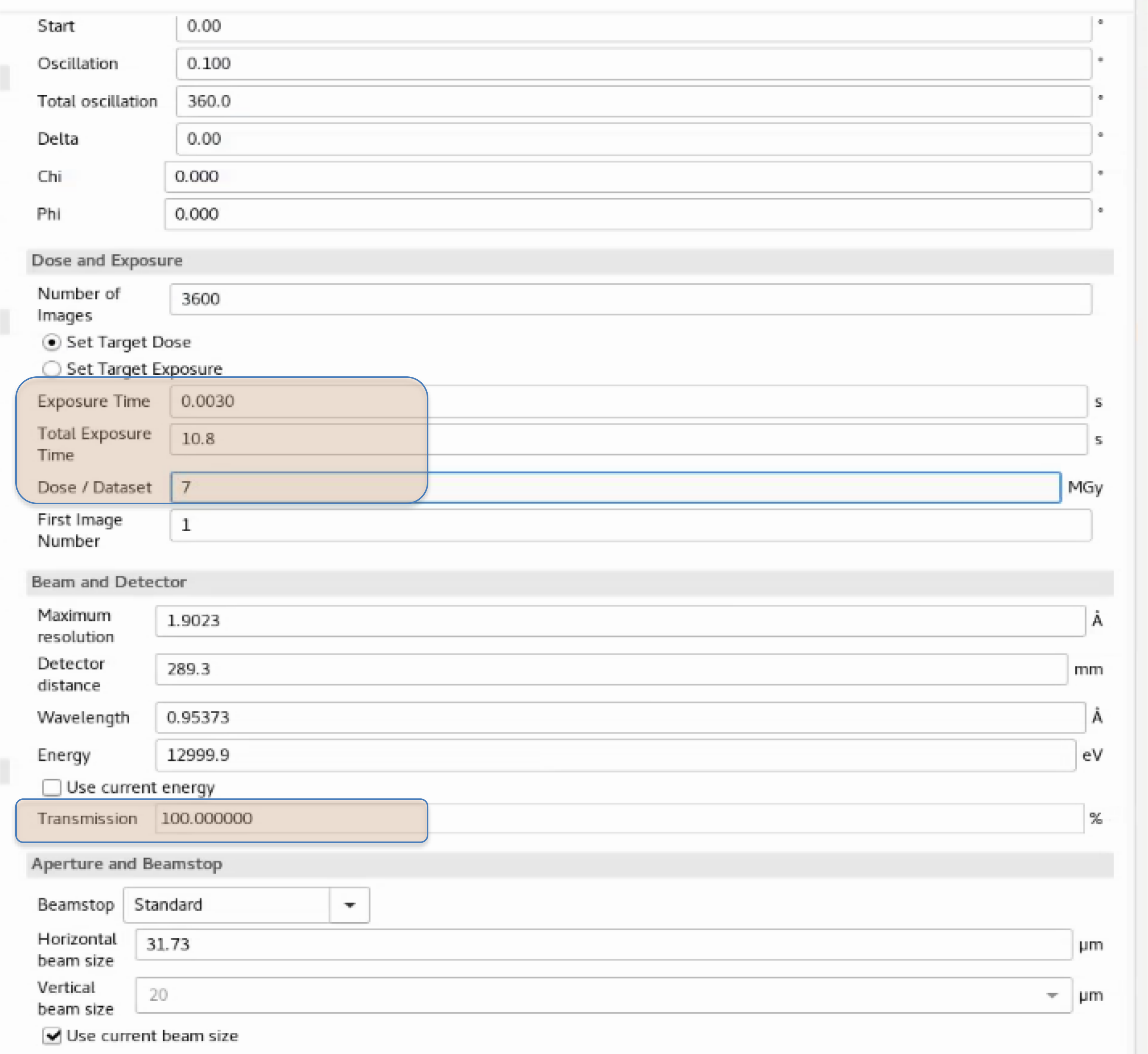
- Traditionally exposure per frame was a critical setting for a MX diffraction experiment. Is this still useful nowadays?

  - What does it mean when collecting 90º vs 120º, 180º or 360º degrees dataset?
    - Perpetuates the idea that to collect faster one should reduce the total oscillation range

  - What does it mean when slicing at 0.05º, 0.1º, 0.2º or 0.5º per oscillation?

  - Does it allow to compare / collect similar samples at different beamlines using similar experimental settings?

  - Can we really answer the typical user question: “Dear BL scientist, what is the typical exposure per frame I should use on this beamline?”



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

● Set Target Dose

○ Set Target Exposure

Exposure Time 0.0030 s

Total Exposure Time 10.8 s

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

☐ Use current energy

Transmission 100.000000 %

Aperture and Beamstop

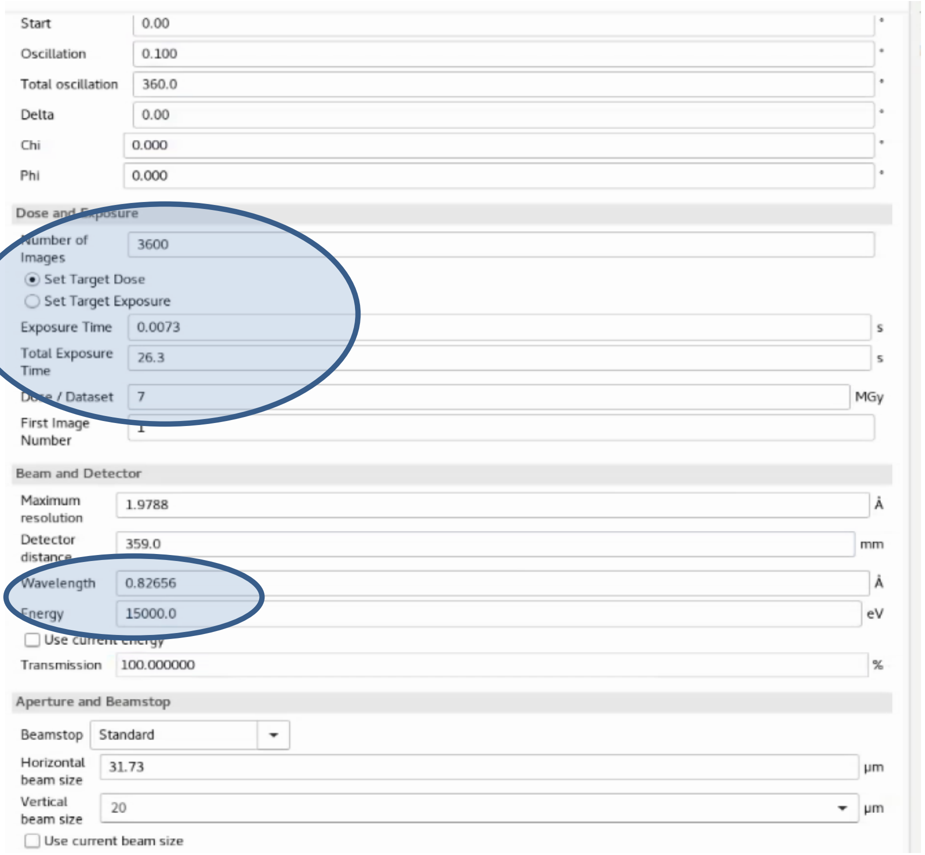
Beamstop Standard

Horizontal beam size 31.73 µm

Vertical beam size 20 µm

☑ Use current beam size

Energy selection- with fixed dose



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

● Set Target Dose

○ Set Target Exposure

Exposure Time 0.0073 s

Total Exposure Time 26.3 s

Dose / Dataset 7 MGy

First Image Number 1

Beam and Detector

Maximum resolution 1.9788 Å

Detector distance 359.0 mm

Wavelength 0.82656 Å

Energy 15000.0 eV

☐ Use current energy

Transmission 100.000000 %

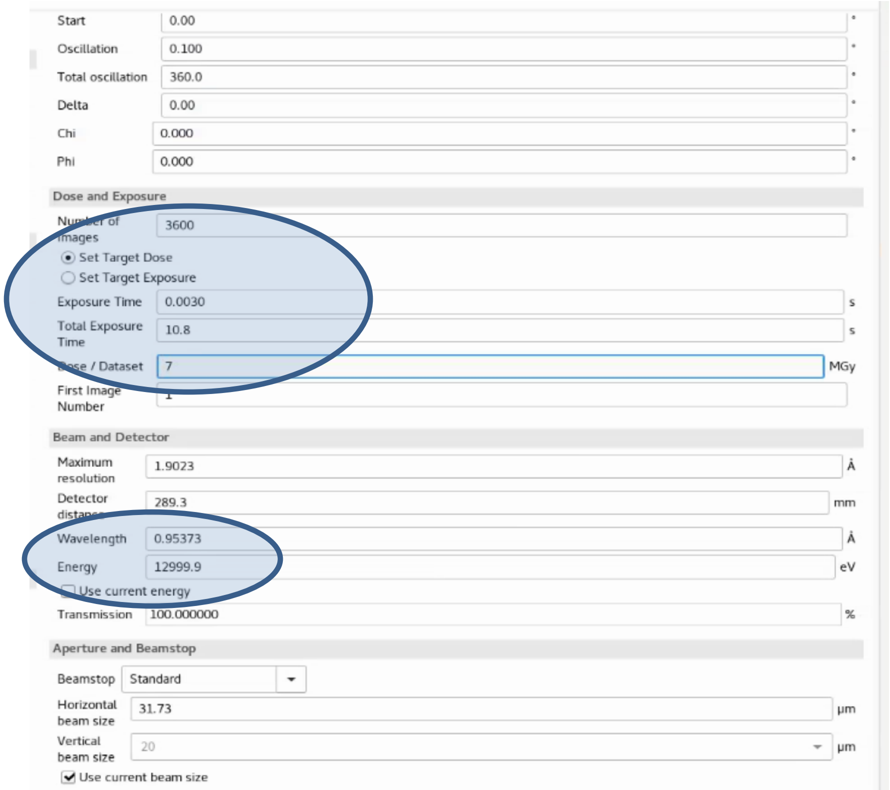
Aperture and Beamstop

Beamstop Standard

Horizontal beam size 31.73 μm

Vertical beam size 20 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 3600

● Set Target Dose

○ Set Target Exposure

Exposure Time 0.0030 s

Total Exposure Time 10.8 s

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

☐ Use current energy

Transmission 100.000000 %

Aperture and Beamstop

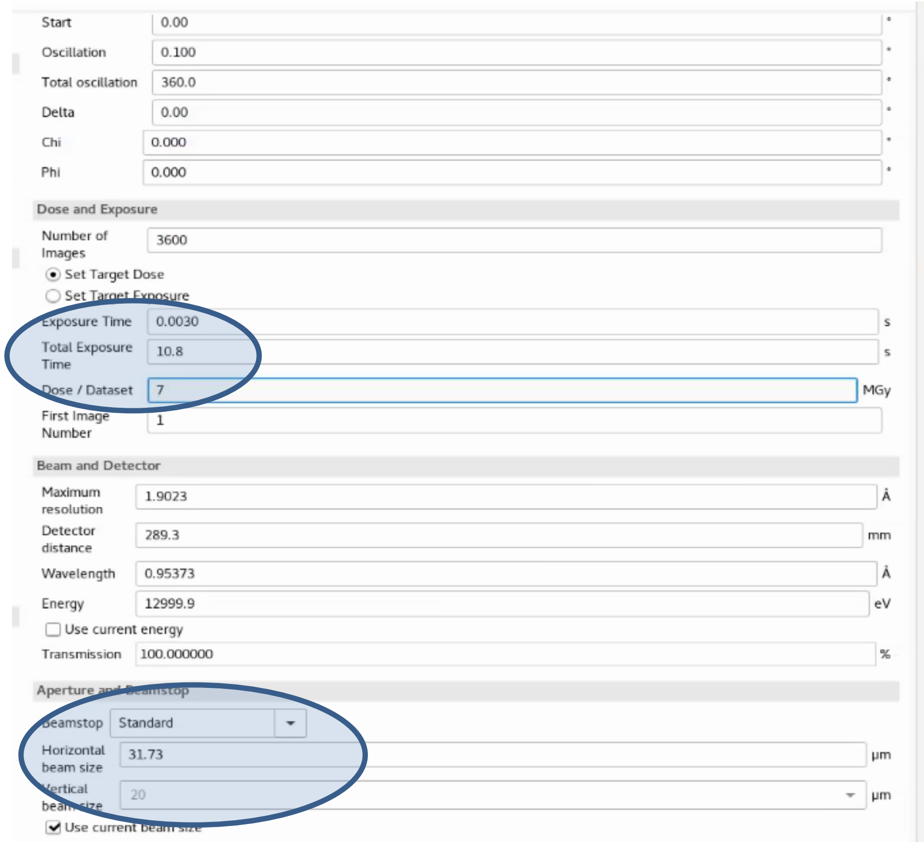
Beamstop Standard

Horizontal beam size 31.73 μm

Vertical beam size 20 μm

☑ Use current beam size

Beam size (focus) – with fixed dose



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

Dose and Exposure

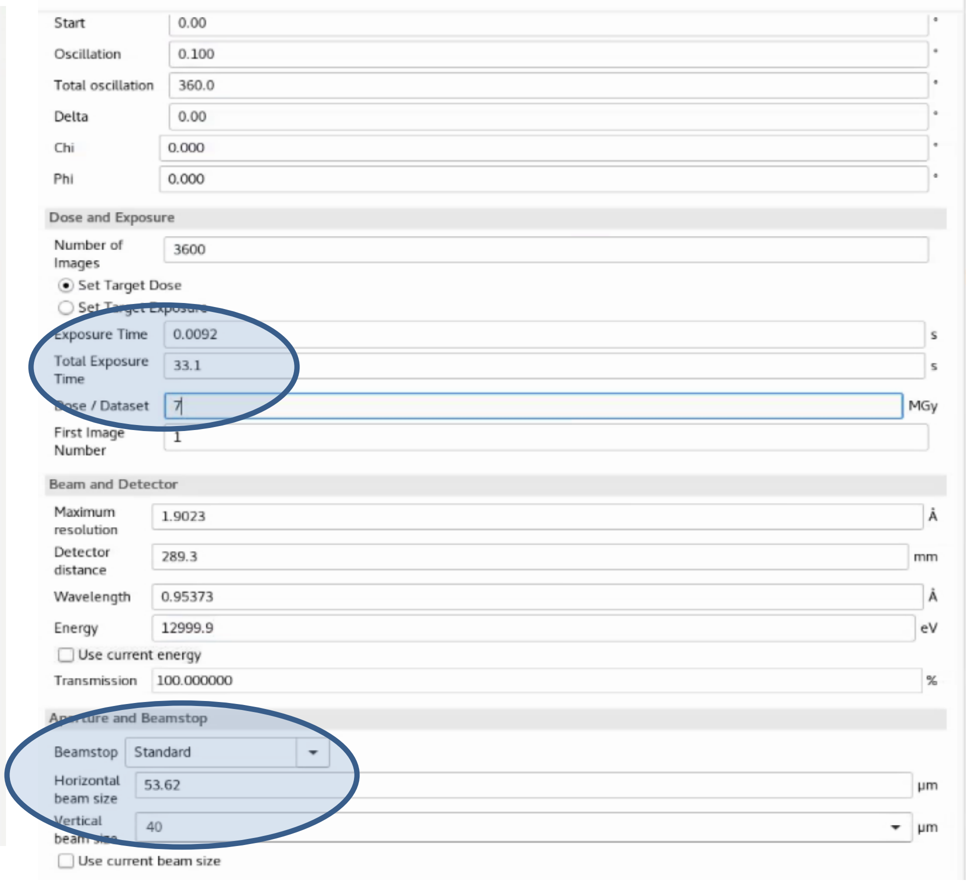
Number of Images: 3600
● Set Target Dose
○ Set Target Exposure
Exposure Time: 0.0030 s
Total Exposure Time: 10.8 s
Dose / Dataset: 7 MGy
First Image Number: 1

Beam and Detector

Maximum resolution: 1.9023 Å
Detector distance: 289.3 mm
Wavelength: 0.95373 Å
Energy: 12999.9 eV
☐ Use current energy
Transmission: 100.000000 %

Aperture and Beamstop

Beamstop: Standard
Horizontal beam size: 31.73 µm
Vertical beam size: 20 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: 3600
● Set Target Dose
○ Set Target Exposure
Exposure Time: 0.0092 s
Total Exposure Time: 33.1 s
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
☐ Use current energy
Transmission: 100.000000 %

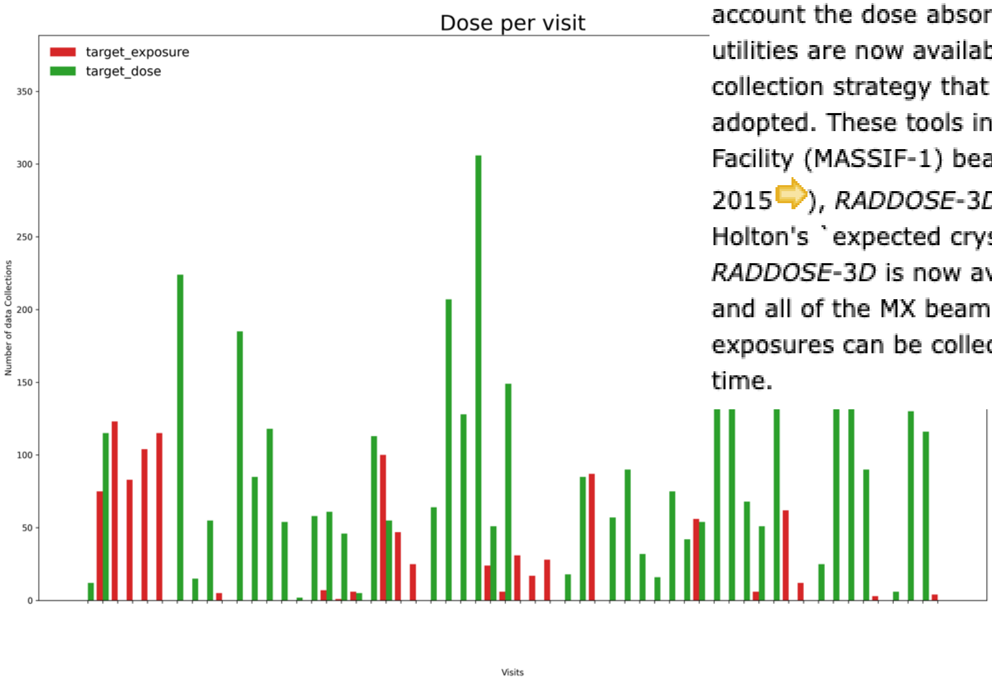
Aperture and Beamstop

Beamstop: Standard
Horizontal beam size: 53.62 µm
Vertical beam size: 40 µm
☐ Use current beam size

Advantages dose drive data collection

- Allows experiments that happen months apart to get comparable results (different flux?)
- Concentrate on the science not on what set parameters to use on their data collection
- Use of set target dose increasing year after year (green on the graph)

A very important method to avoid RD is to adopt a data-collection strategy that takes into account the dose absorbed by the samples using the characteristics of the beamline. Several utilities are now available to estimate the dose absorbed during a data collection so that a collection strategy that keeps the dose as low as possible for the whole data set can be adopted. These tools include *DOZOR* at the Massively Automated Sample Selection Integrated Facility (MASSIF-1) beamline of the European Synchrotron Radiation Facility (Svensson *et al.*, 2015), *RADDOSE-3D* (Zeldin, Gerstel *et al.*, 2013; Bury *et al.*, 2018) and James Holton's `expected crystal lifetime calculator' (https://bl831.als.lbl.gov/xtallife.html). In fact, *RADDOSE-3D* is now available on several beamlines (for example I04 at Diamond Light Source and all of the MX beamlines at the Stanford Synchrotron Radiation Lightsource) so that exposures can be collected for a specified absorbed dose value rather than for a specified time.



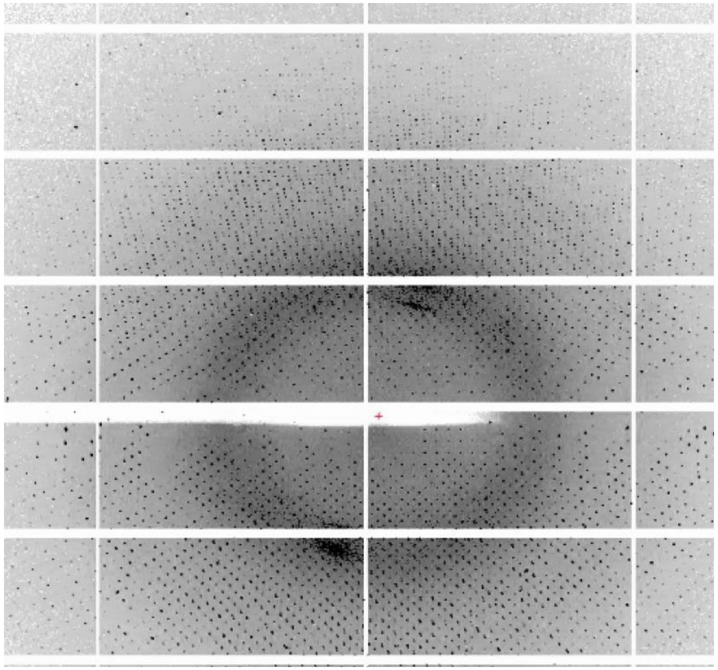
Shelley, K. L. & Garman, E. F. (2024). Acta Cryst. D80, 314-327.

Screening how & when

Screening and analysis

Determine crystal diffraction properties, orientation matrix and appropriate settings for data collection

Usually by collecting a small wedge of 15-20 degrees ideally at the thickest part of the crystal (or the old method of 3 images 45 degrees away from each other)



Important to check the images:

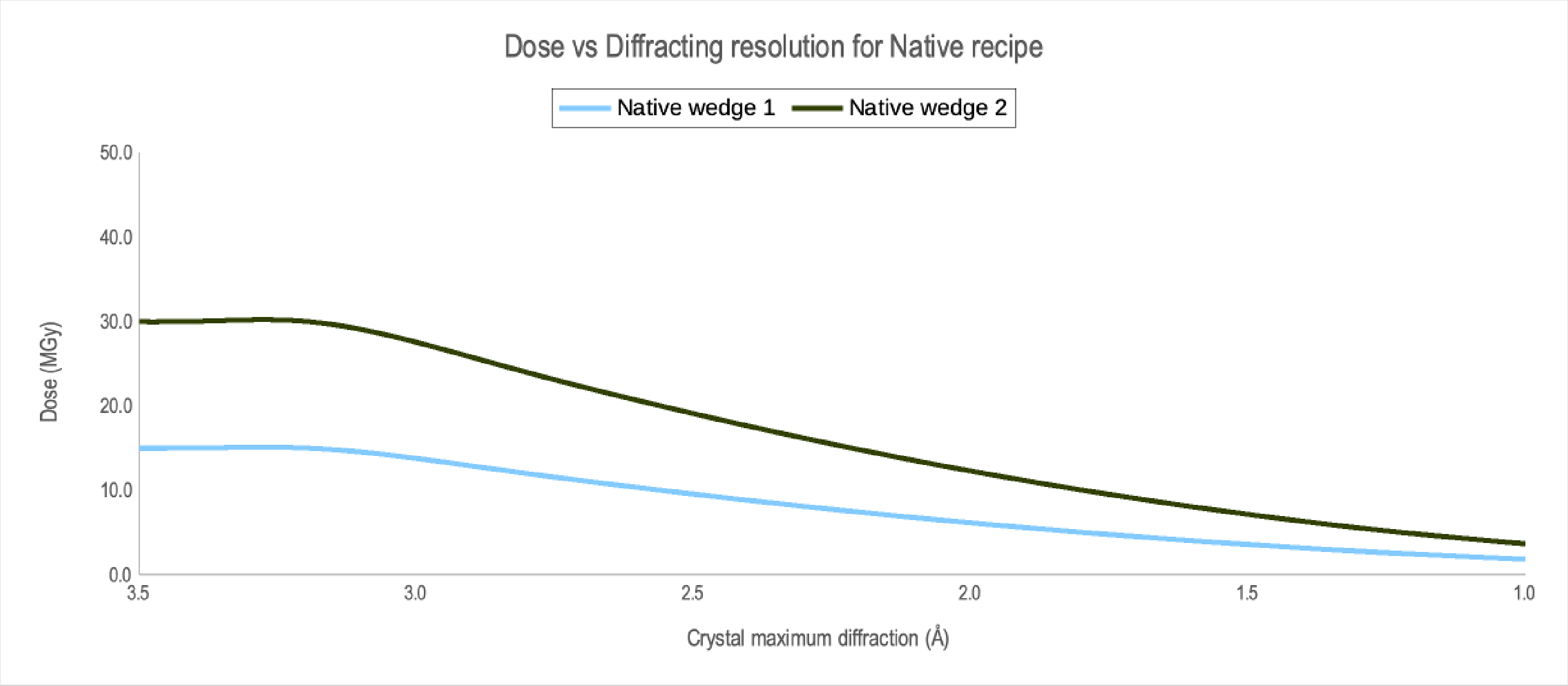
- Spot quality – circular, well spaced
- Resolution? Guides on
  - Detector distance
  - Dose/Exposure to deliver
- Autoindexing successful?

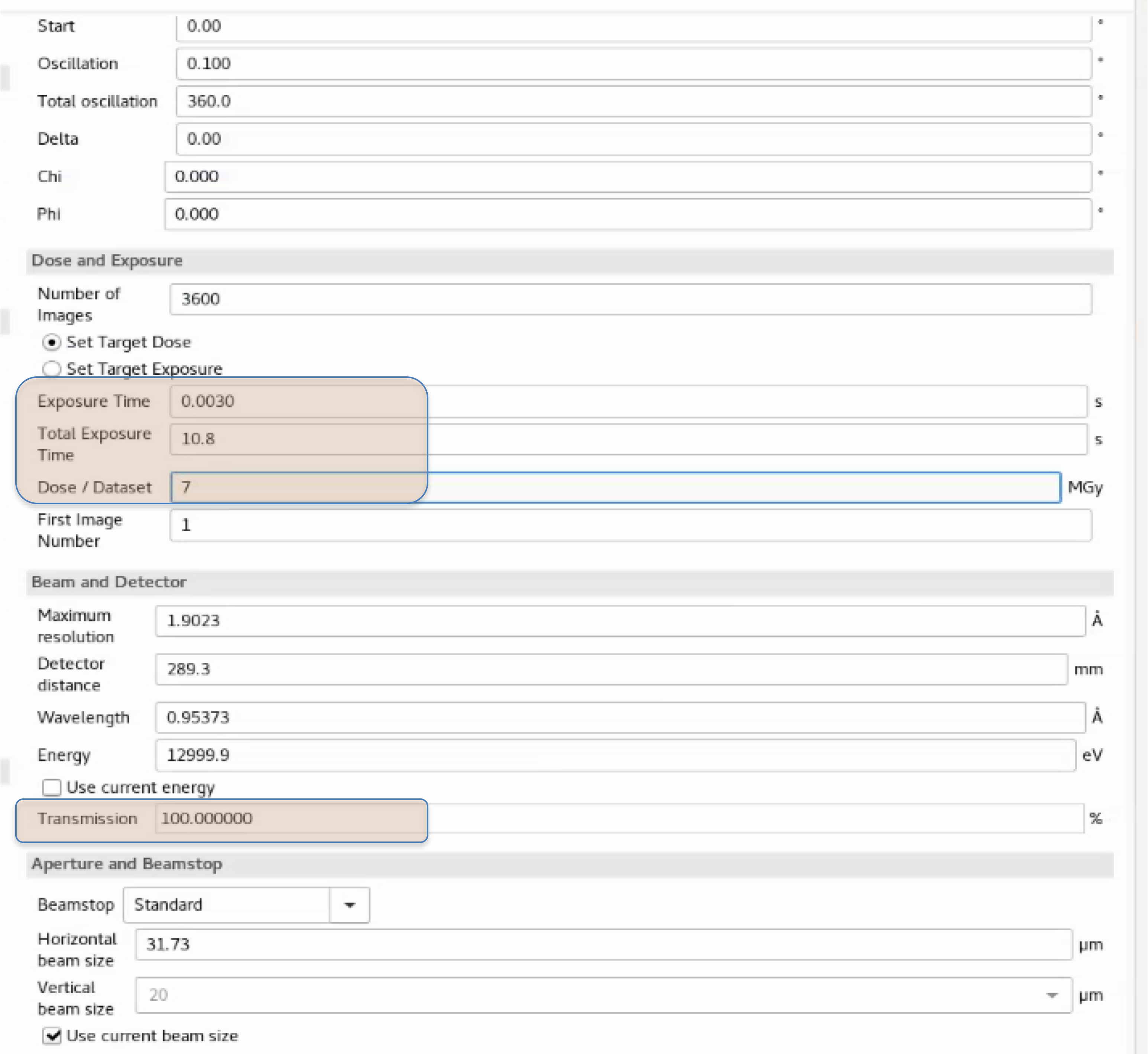
If evaluating by eye remember to “join” images to at least 1.0 degree rather than looking at fine sliced images. Max res: best to rely on data reduction programs and apply a buffer (e.g 0.5 A) – huge dynamic range not possible to show on a screen easily

Maximum diffraction & dose/exposure

Screening and analysis

Dose selection currently implemented in the Unattended Data collection at Diamond Light Source





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

● Set Target Dose

○ Set Target Exposure

Exposure Time 0.0030 s

Total Exposure Time 10.8 s

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

☐ Use current energy

Transmission 100.000000 %

Aperture and Beamstop

Beamstop Standard

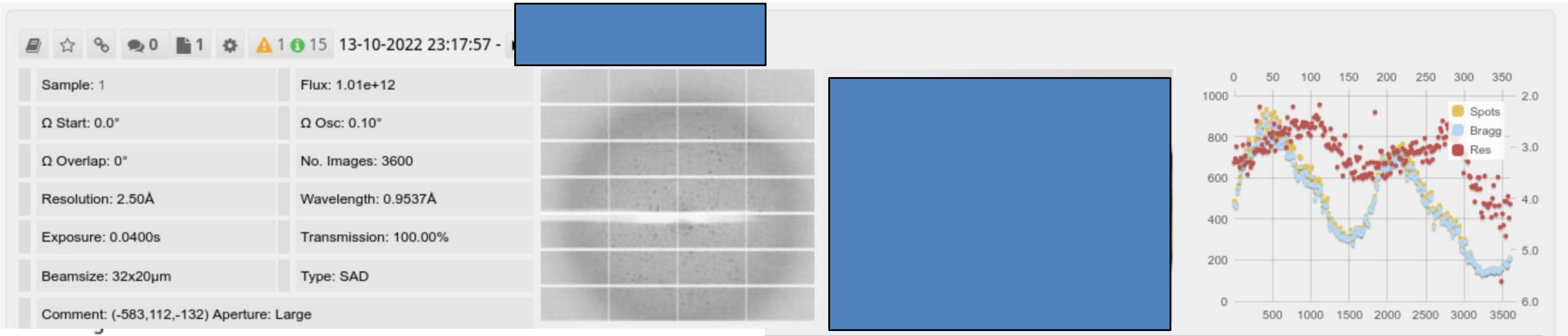
Horizontal beam size 31.73 µm

Vertical beam size 20 µm

☑ Use current beam size

What happens if you don't pay attention to dose?

Elsepth Garman's talk on radiation damage

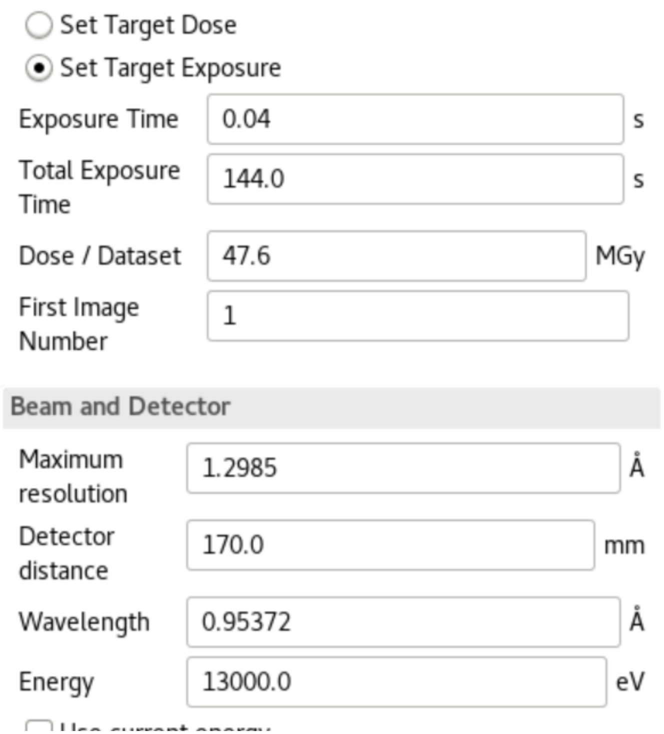


0 1 1 15 13-10-2022 23:17:57 - [redacted]

Sample: 1	Flux: 1.01e+12
Ω Start: 0.0°	Ω Osc: 0.10°
Ω Overlap: 0°	No. Images: 3600
Resolution: 2.50Å	Wavelength: 0.9537Å
Exposure: 0.0400s	Transmission: 100.00%
Beamsize: 32x20µm	Type: SAD

Comment: (-583,112,-132) Aperture: Large

[redacted]



○ Set Target Dose
● Set Target Exposure

Exposure Time	0.04	s
Total Exposure Time	144.0	s
Dose / Dataset	47.6	MGy
First Image Number	1	

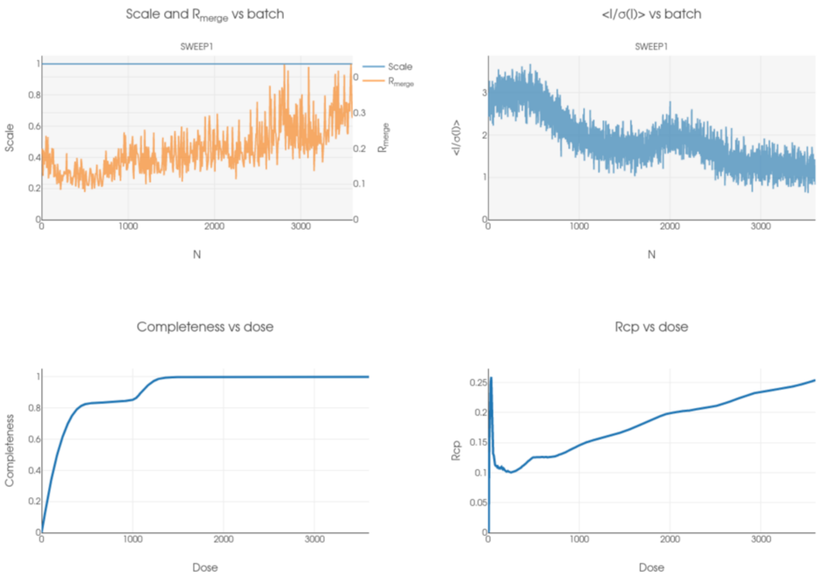
Beam and Detector

Maximum resolution	1.2985	Å
Detector distance	170.0	mm
Wavelength	0.95372	Å
Energy	13000.0	eV

☐ Use current energy

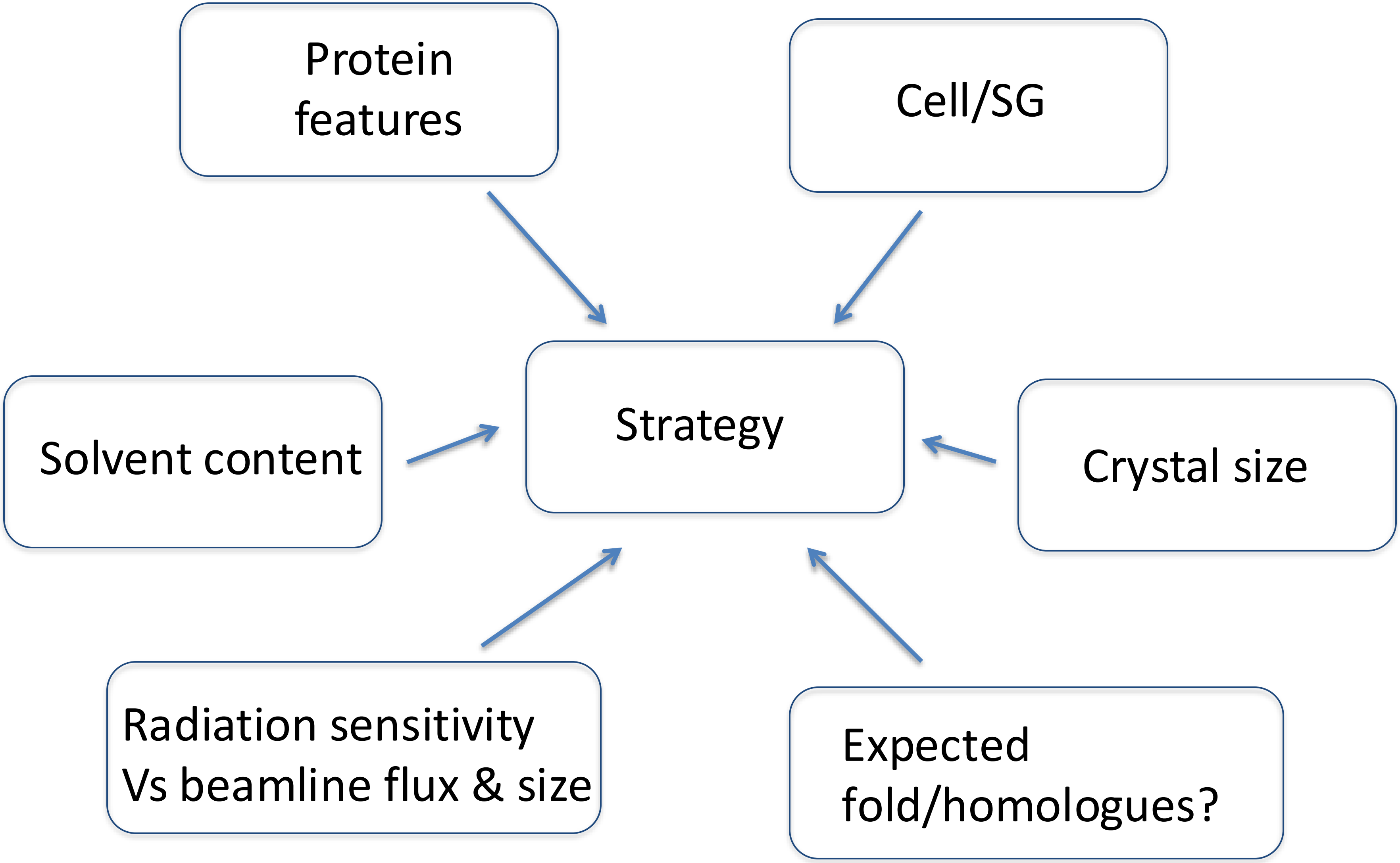
fast_dp: ✗ xia2 dials: ✓ xia2 3dii: ✗ autoPROC: ✓ xia2.multiplex: ✓ autoPROC+STARANISO: ✓

No processing results



Experimental aim

Preparation - Prior knowledge



Protein features

Cell/SG

Solvent content

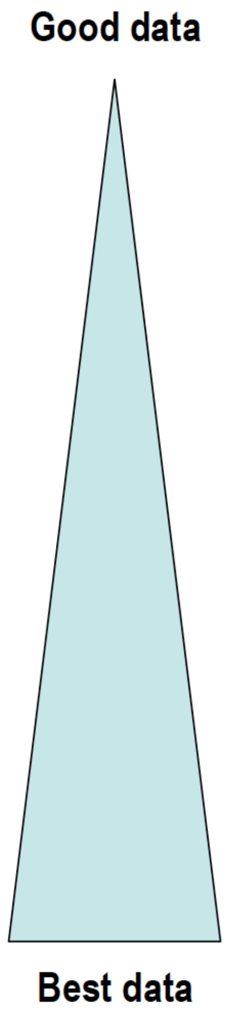
Strategy

Crystal size

Radiation sensitivity
Vs beamline flux & size

Expected fold/homologues?

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



Experimental aim?

1. Molecular Replacement

2. Native Data collection

3. Single wavelength anomalous diffraction

4. Multi-wavelength anomalous diffraction

1
2
3
4
5

Increased complexity

1. Did I get a different quaternary structure arrangement ?
2. Did I capture the structure of the active conformation?
3. Did I get the complex of the two proteins I am searching for? Is my structure different from previous published ones?
4. Did the ligand bind in the active site?
5. Can I propose a mechanism for an enzymatic reaction involving side chains X,Y,Z in the active centre?

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 A) 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 A), complete data. High throughput (FAST)	High redundancy	10 - 20 MGy (*)

Trying to collect everything at once will usually result in failure

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

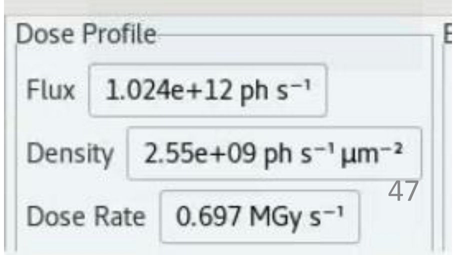
Planning for a particular instrument: Flux & beamsize

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 A / 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 Profile
Flux: 1.024e+12 ph s^{-1}
Density: 2.55e+09 ph s^{-1} μm^{-2}
Dose Rate: 0.697 MGy s^{-1}

Beam size and flux density

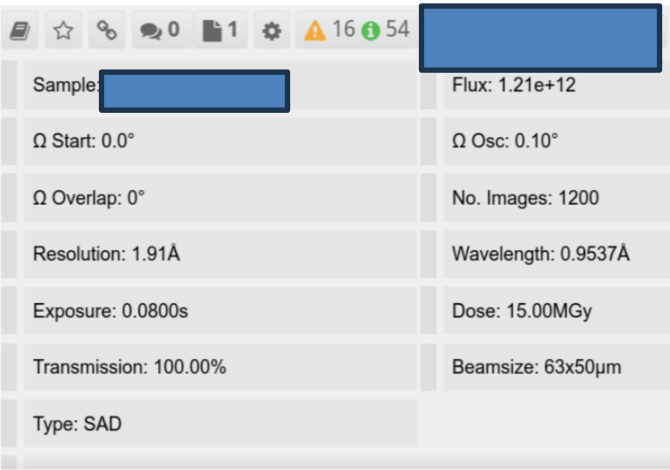
Be mindful of how the beamline generates a small beam size. Focusing (I04 +I24) can increase the flux density massively compared to apertures (I03)

Radiation damage is related to the flux density so small beam sizes on I04/I24 can kill a crystal very quickly.

E.g. 50x50 μm^2 beam will deliver around 45x less dose than 9x6 μm^2 beam on I24

So match beam to crystal, but attenuate when required

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



[redacted]

Sample: [redacted]

Flux: 1.21e+12

Ω Start: 0.0°

Ω Osc: 0.10°

Ω Overlap: 0°

No. Images: 1200

Resolution: 1.91Å

Wavelength: 0.9537Å

Exposure: 0.0800s

Dose: 15.00MGy

Transmission: 100.00%

Beamsize: 63x50µm

Type: SAD

[redacted]

Sample: [redacted]

Flux: 9.64e+11

Ω Start: 0.0°

Ω Osc: 0.10°

Ω Overlap: 0°

No. Images: 1200

Resolution: 1.91Å

Wavelength: 0.9537Å

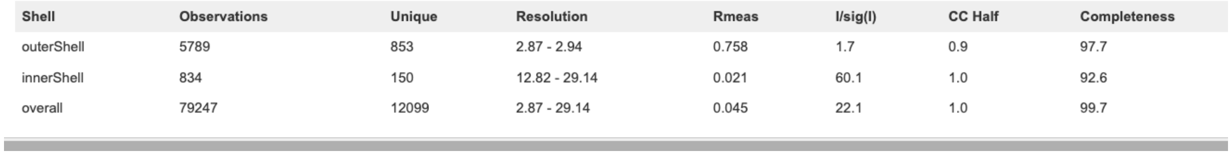
Exposure: 0.0800s

Dose: 194.70MGy

Transmission: 100.00%

Beamsize: 19x10µm

Type: SAD

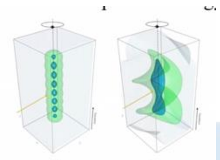


Shell	Observations	Unique	Resolution	Rmeas	I/sig(I)	CC Half	Completeness
outerShell	5789	853	2.87 - 2.94	0.758	1.7	0.9	97.7
innerShell	834	150	12.82 - 29.14	0.021	60.1	1.0	92.6
overall	79247	12099	2.87 - 29.14	0.045	22.1	1.0	99.7

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

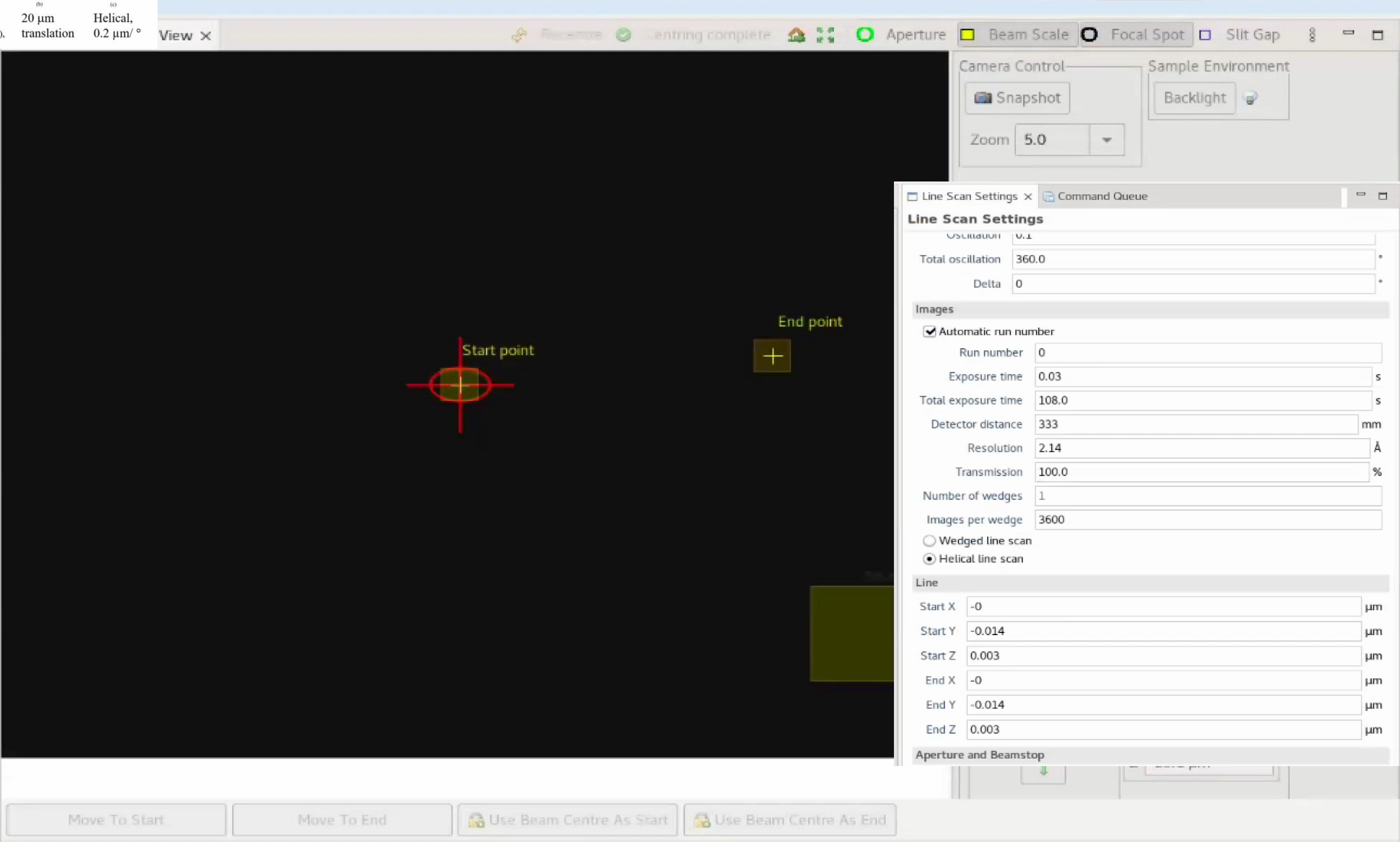
Downstream Processing

Sample with Line scan



20 µm translation

Helical, 0.2 µm/ °



Zoom 5.0

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

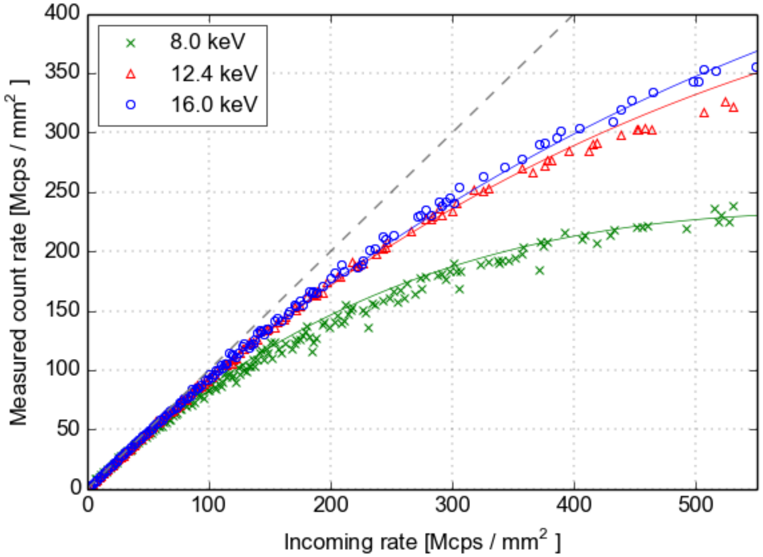
End Y -0.014 µm

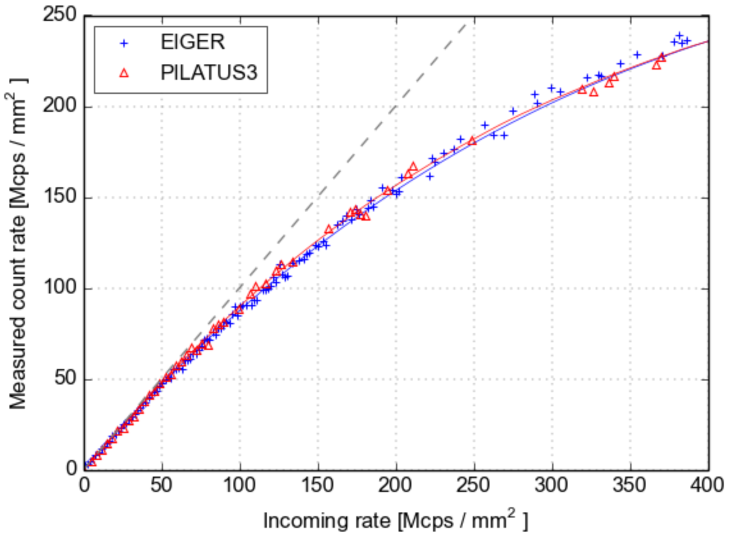
End Z 0.003 µm

Aperture and Beamstop

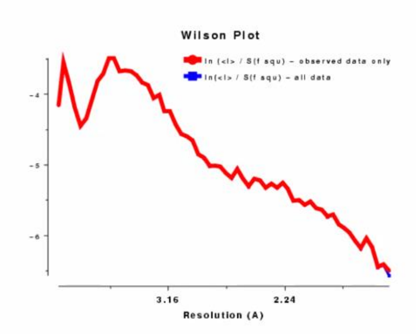
High flux → count rate corrections on Eiger detectors

Count rate performance in EIGER & PILATUS detectors



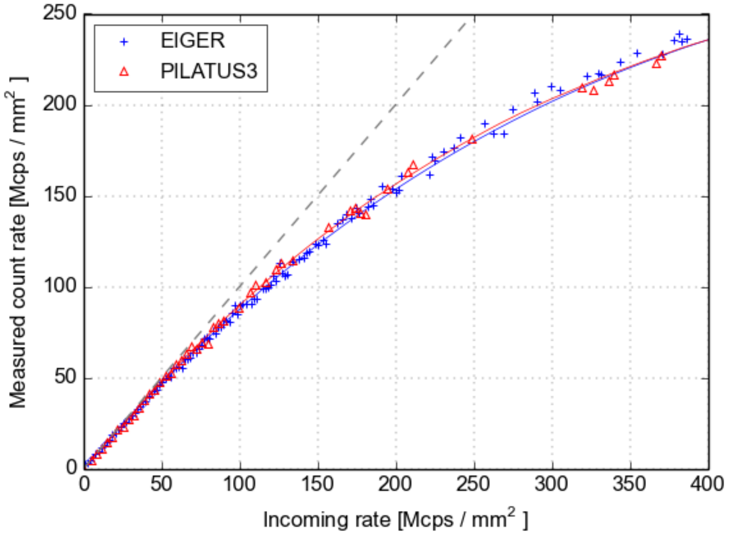


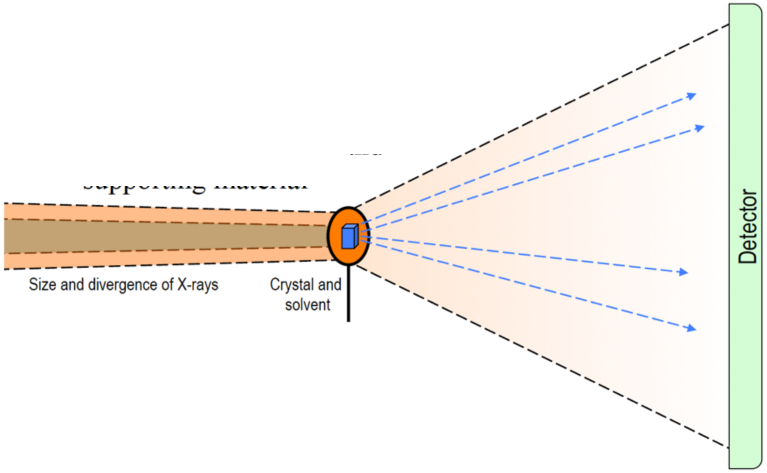
https://www.dectris.com/en/features/features-eiger-r/outstanding-count-rate-performance/



Wilson Plot
Ana Gonzalez talk “Data processing”

Count rate performance in EIGER & PILATUS detectors





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

Spreading the dose over large total angle range helps with this because each frame will be collected at lower transmission (lower flux rate ph/s)

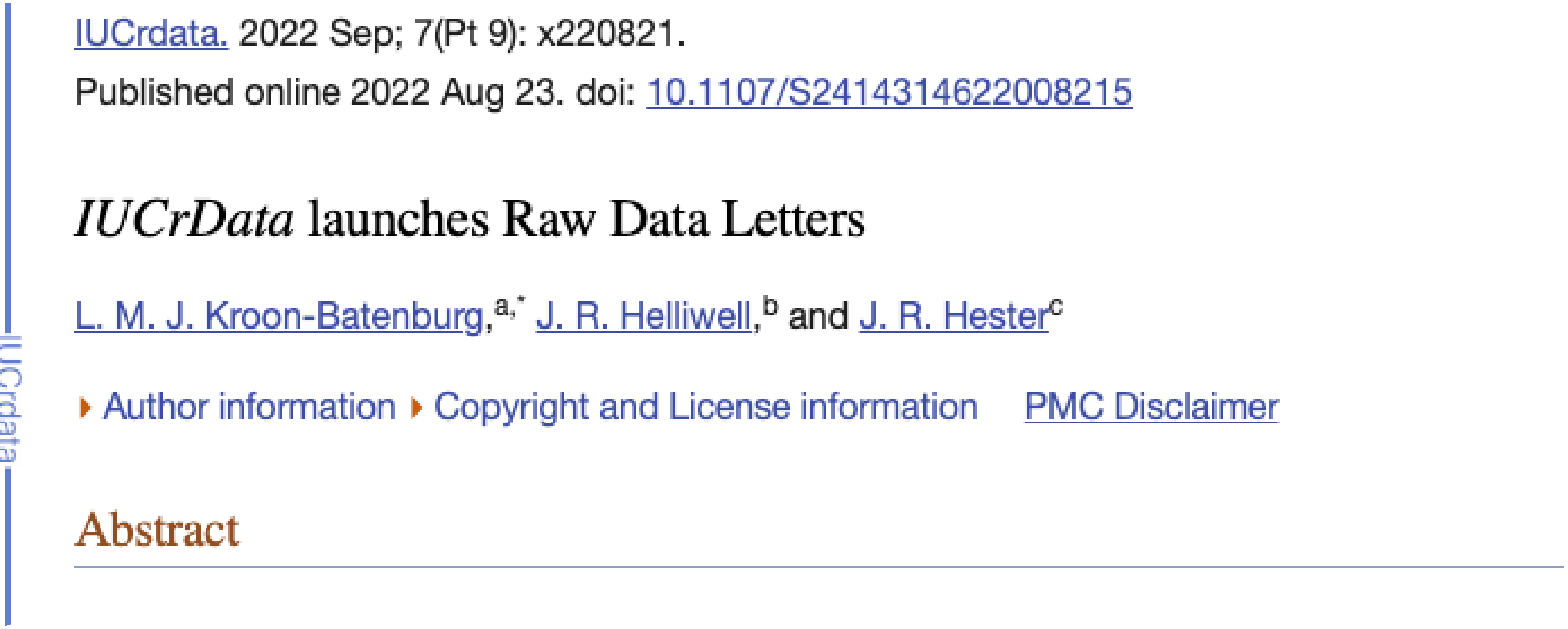
Data collection - think!

- 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)



IUCrdata. 2022 Sep; 7(Pt 9): x220821.
Published online 2022 Aug 23. doi: 10.1107/S2414314622008215

IUCrData launches Raw Data Letters

L. M. J. Kroon-Batenburg,[a,*] J. R. Helliwell,[b] and J. R. Hester[c]

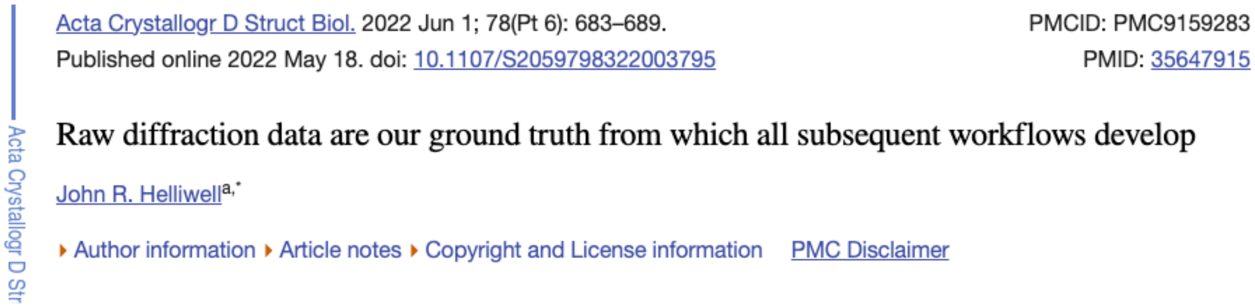
▸ Author information ▸ Copyright and License information PMC Disclaimer

Abstract

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

- Zenodo? others

Consider making your raw data public!



Acta Crystallogr D Struct Biol. 2022 Jun 1; 78(Pt 6): 683–689.
Published online 2022 May 18. doi: 10.1107/S2059798322003795

PMCID: PMC9159283
PMID: 35647915

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

John R. Helliwell[a,*]

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