Micro and serial crystallography at Beamline I24

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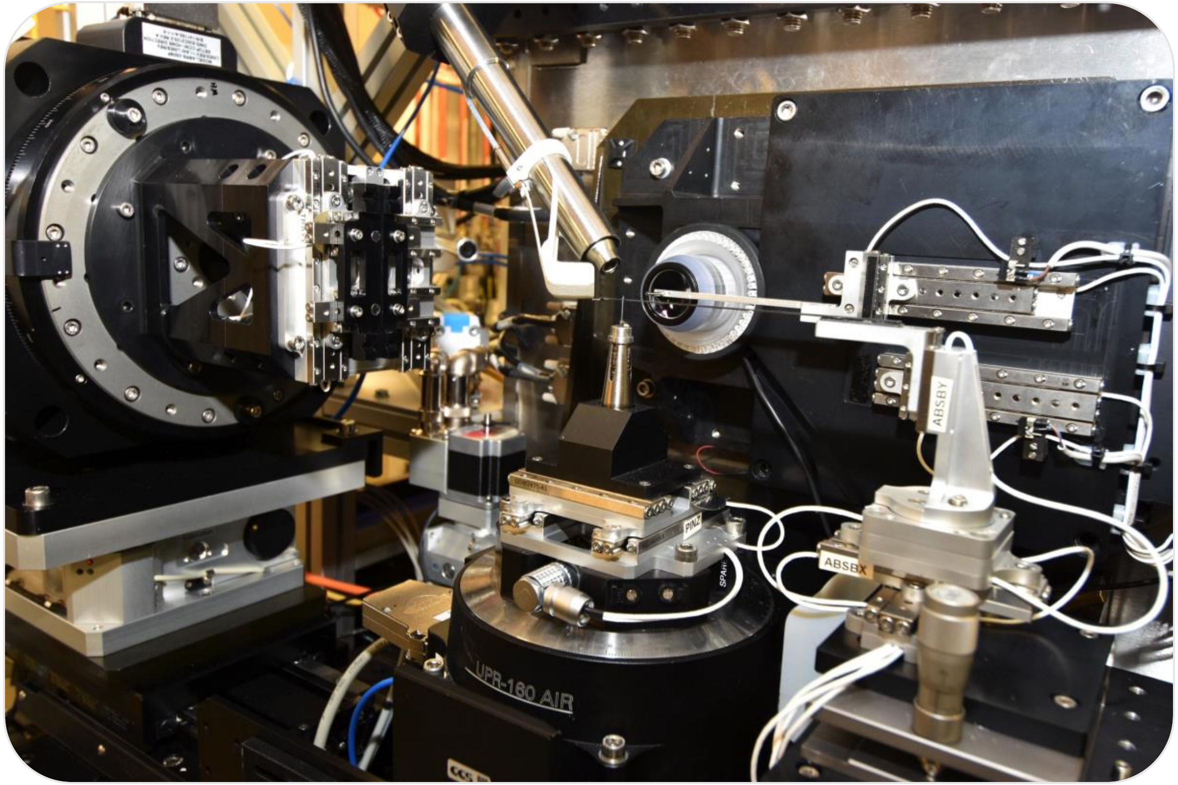
I24: Microfocus MX at DLS

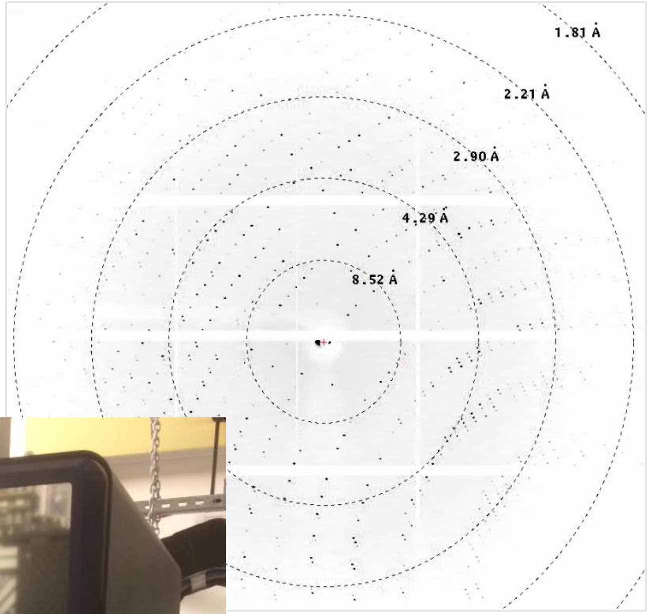
- Beamsize quickly variable from ~7×7 μm^2 to ~50×50 μm^2
- High flux (>5×10^{12} ph/s) and fully tuneable
- Silicon Pilatus or CdTe Eiger
  - Pick your energy 7 -25 keV…
- Conventional pin based MX or serial crystallography (SSX)
  - Modular sample environment

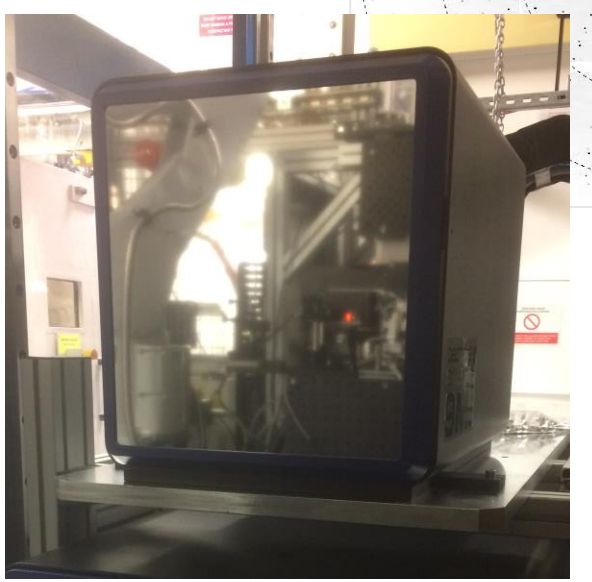


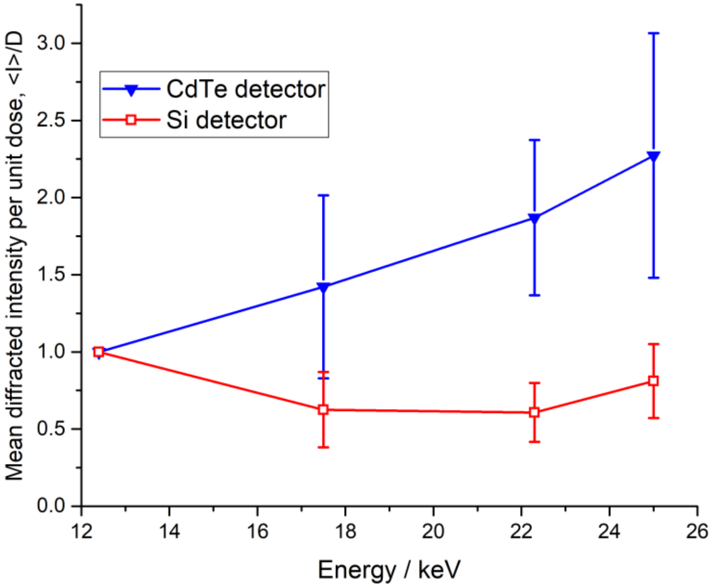
CdTe Eiger

a new detector for high energy MX

- Radiation damage is limiting in MX and crystals can only survive a finite dose before crystalline order is lost.
- This limiting dose is approximately constant for all proteins and viruses
- At higher X-ray energies it may be possible to collect more data from each crystal.
- A CdTe detector makes high energy data collection efficient and routine; default energy currently 20 keV.

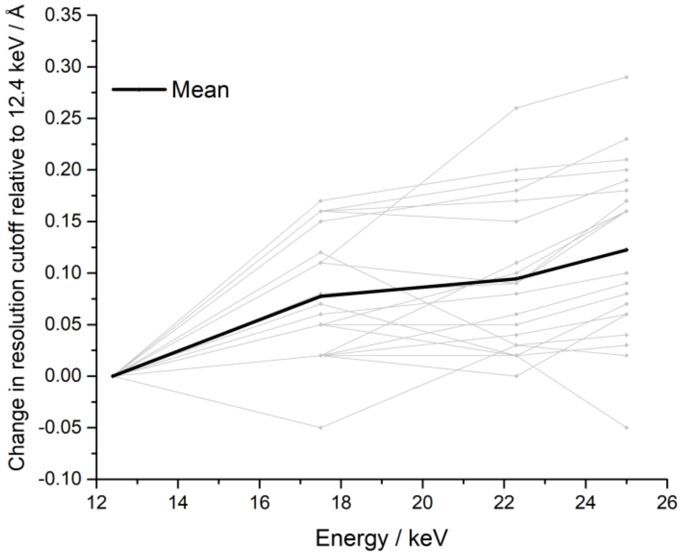




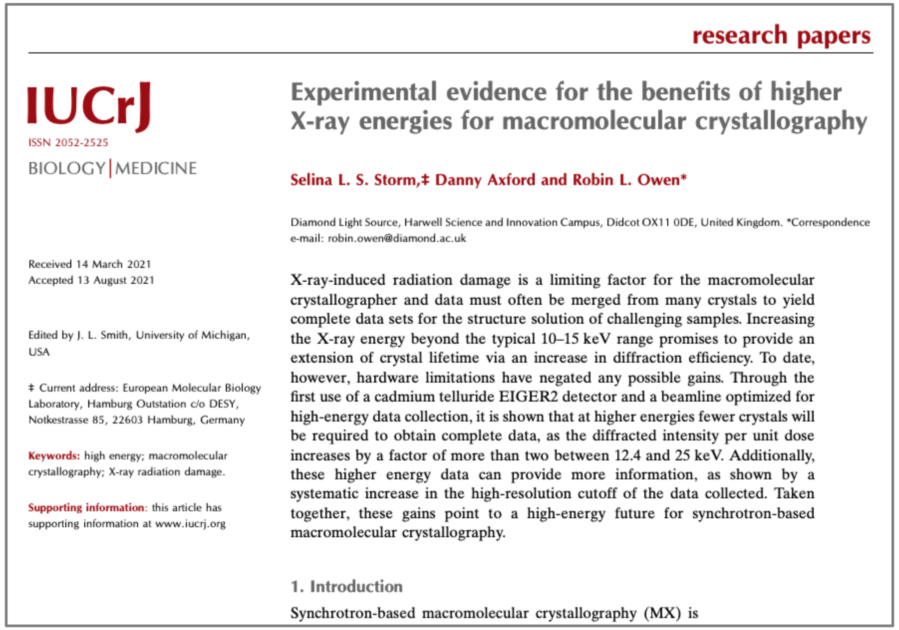


As the X-ray energy increases the diffracted intensity per unit absorbed dose increases significantly when using a CdTe based detector (blue line).

High energy data collection CdTe Eiger 9M



Data collected at higher energies are useful to a higher resolution even though the dose deposited in the crystal is the same in each case (mean change for twenty crystals shown left).



research papers

IUCrJ
ISSN 2052-2525
BIOLOGY | MEDICINE

Received 14 March 2021
Accepted 13 August 2021

Edited by J. L. Smith, University of Michigan, USA

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Keywords: high energy; macromolecular crystallography; X-ray radiation damage.

Supporting information: this article has supporting information at www.iucrj.org

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

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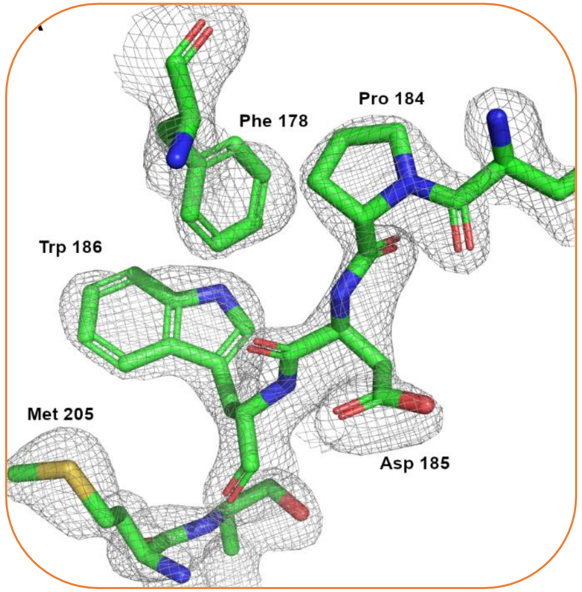
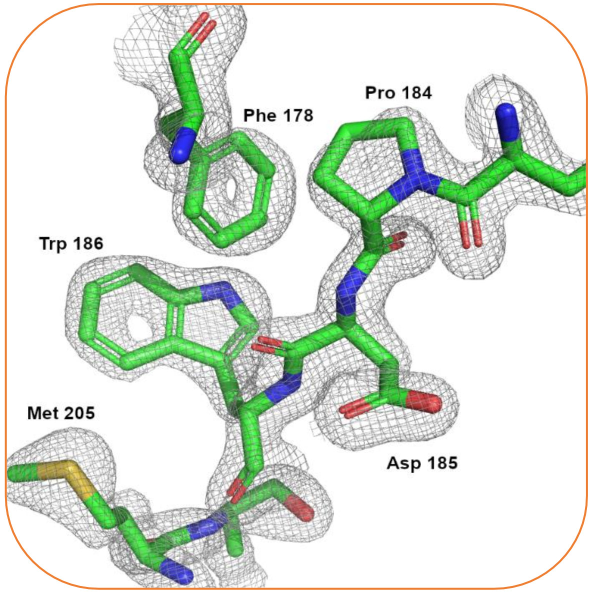
X-ray-induced radiation damage is a limiting factor for the macromolecular crystallographer and data must often be merged from many crystals to yield complete data sets for the structure solution of challenging samples. Increasing the X-ray energy beyond the typical 10–15 keV range promises to provide an extension of crystal lifetime via an increase in diffraction efficiency. To date, however, hardware limitations have negated any possible gains. Through the first use of a cadmium telluride EIGER2 detector and a beamline optimized for high-energy data collection, it is shown that at higher energies fewer crystals will be required to obtain complete data, as the diffracted intensity per unit dose increases by a factor of more than two between 12.4 and 25 keV. Additionally, these higher energy data can provide more information, as shown by a systematic increase in the high-resolution cutoff of the data collected. Taken together, these gains point to a high-energy future for synchrotron-based macromolecular crystallography.

1. Introduction

Synchrotron-based macromolecular crystallography (MX) is

12.4 keV,
1.9 Å resolution

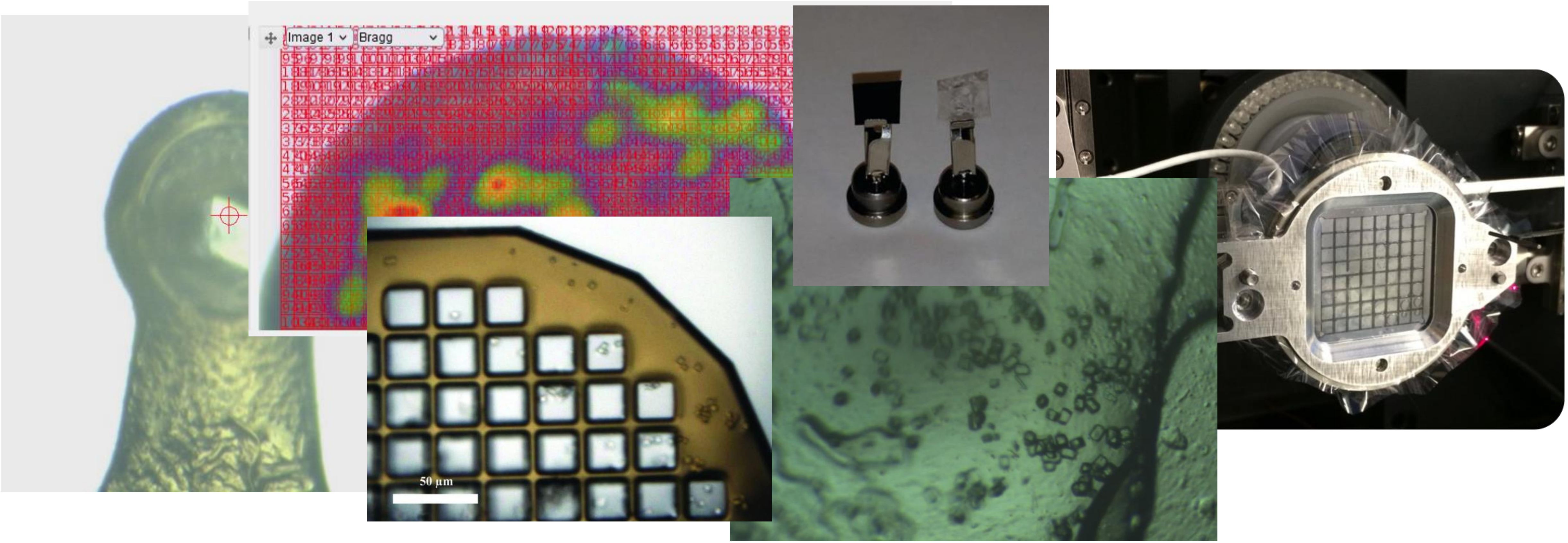




Same crystal 25 keV,
1.61 Å resolution

This suggests high energy collection should be more common-place.

The spectrum from single to serial crystallography



1 | 10s | 100s | 1000s

number of crystals →

← frames per crystal

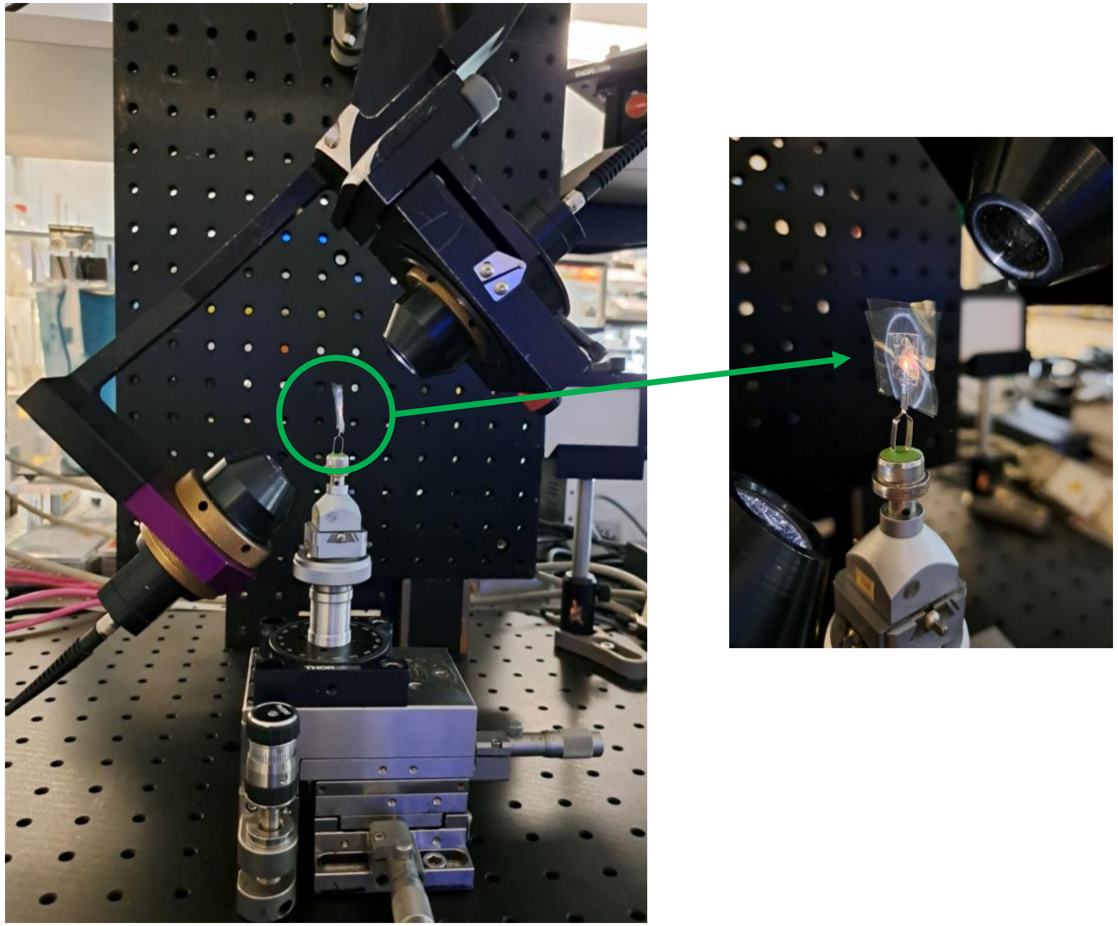
1000s | 100s | 10s | 1

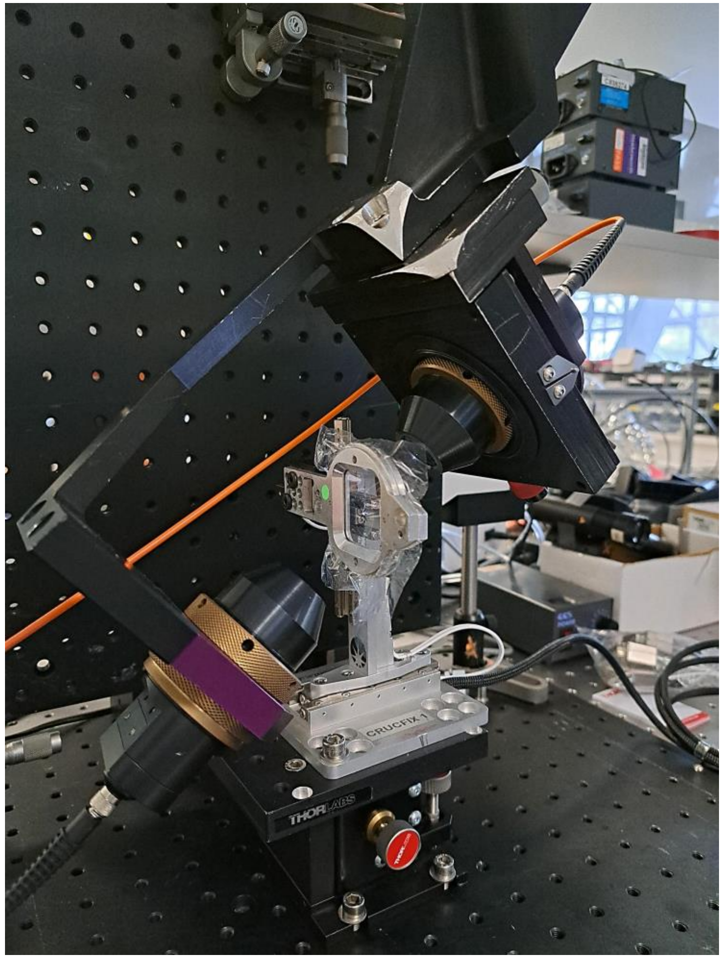
UV-Vis Microspectroscopy at I24

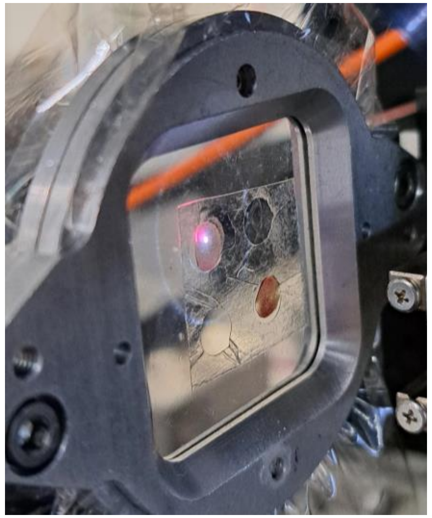
Off-line setup

- Room temperature data collection
- On thin films ('chipless' chip) mounted on loops or chip holders

- Deuterium-Tungsten Halogen or Xenon lamp
- Reflective objectives
- Optical fibres 50 – 600 µm diameter
- Spectrograph resolution better than 0.5 nm







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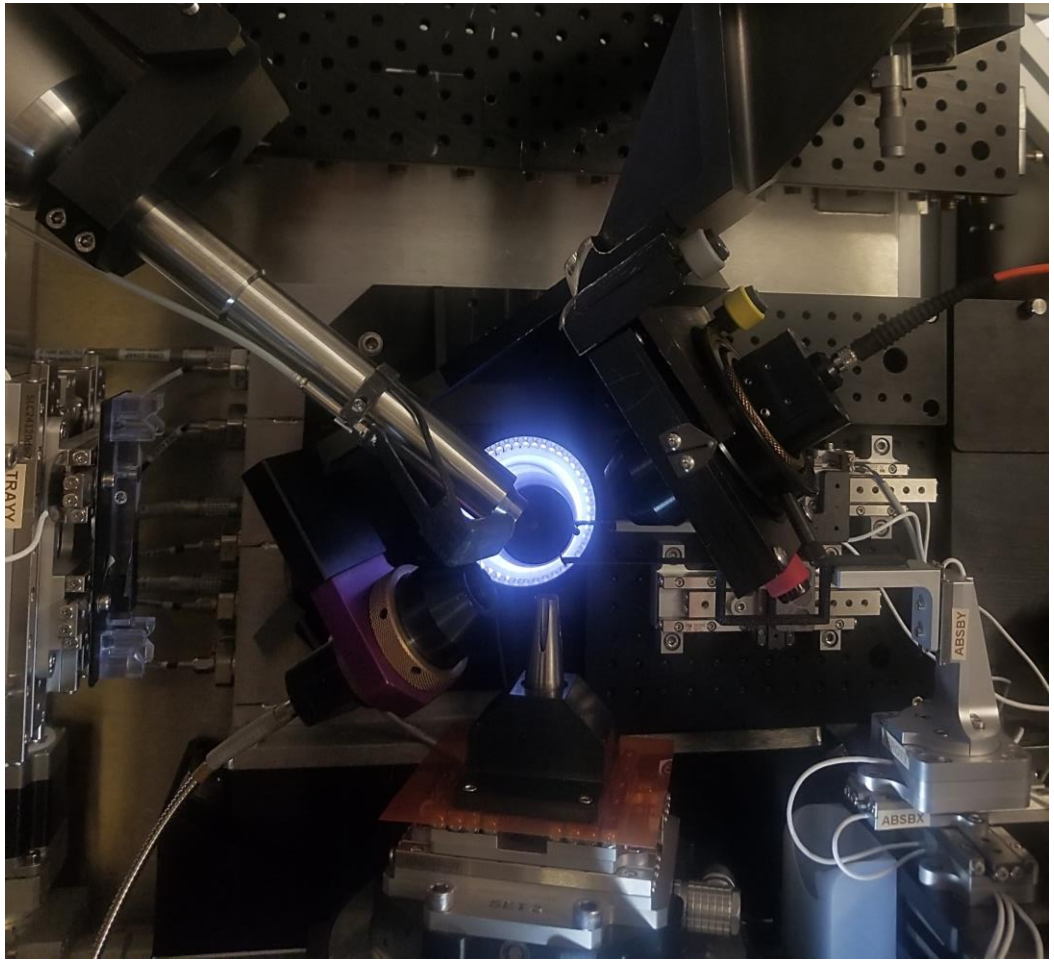
UV-Vis Microspectroscopy at I24

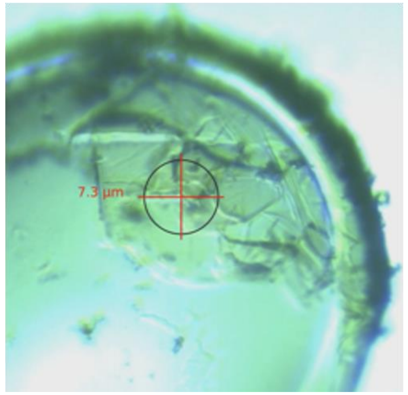
On-line setup

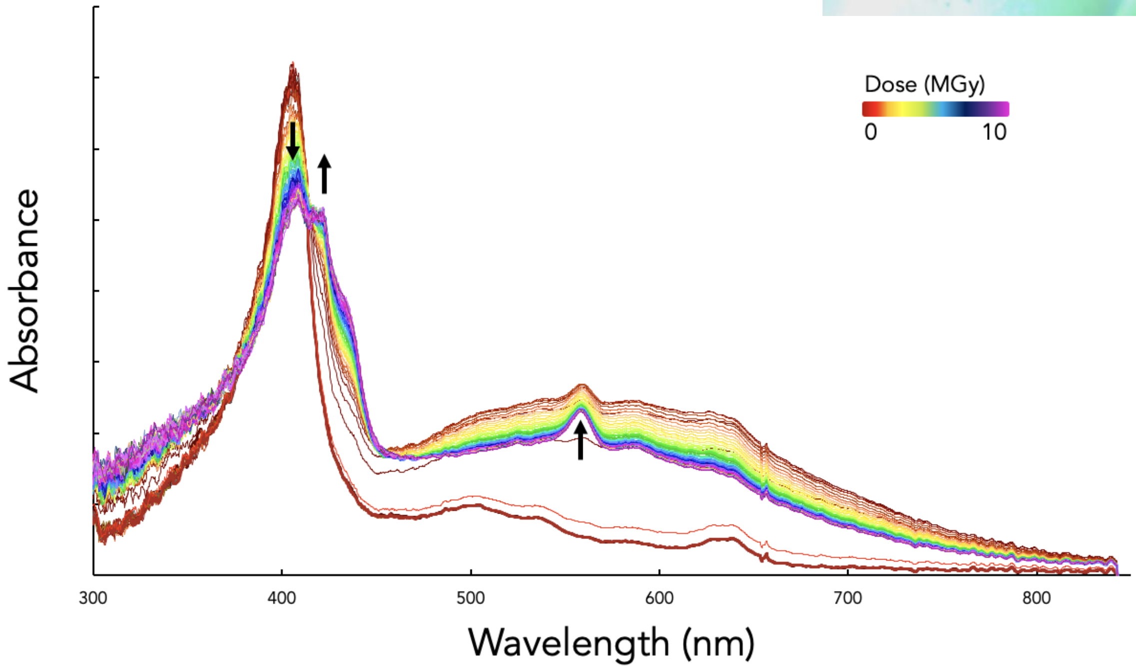
- Cryo and room temperature data collection
- Off-axis geometry (45°)



***Xc*TDO (Anna Bailey, XFEL Hub)**

- Cryo-cooled crystals
- Single crystal spectroscopy
- Kinetic series

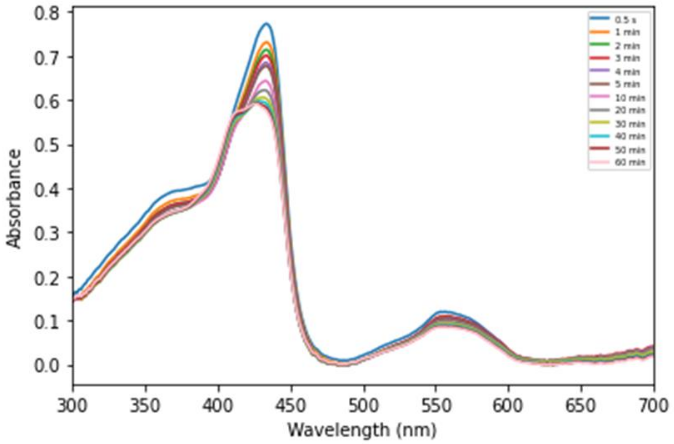




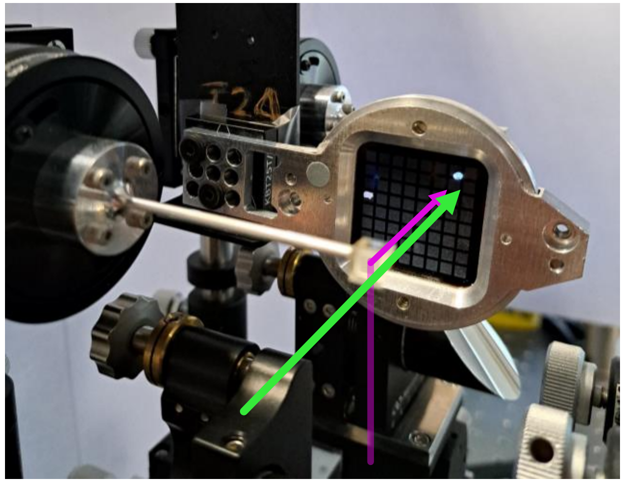
UV-Vis Microspectroscopy at I24

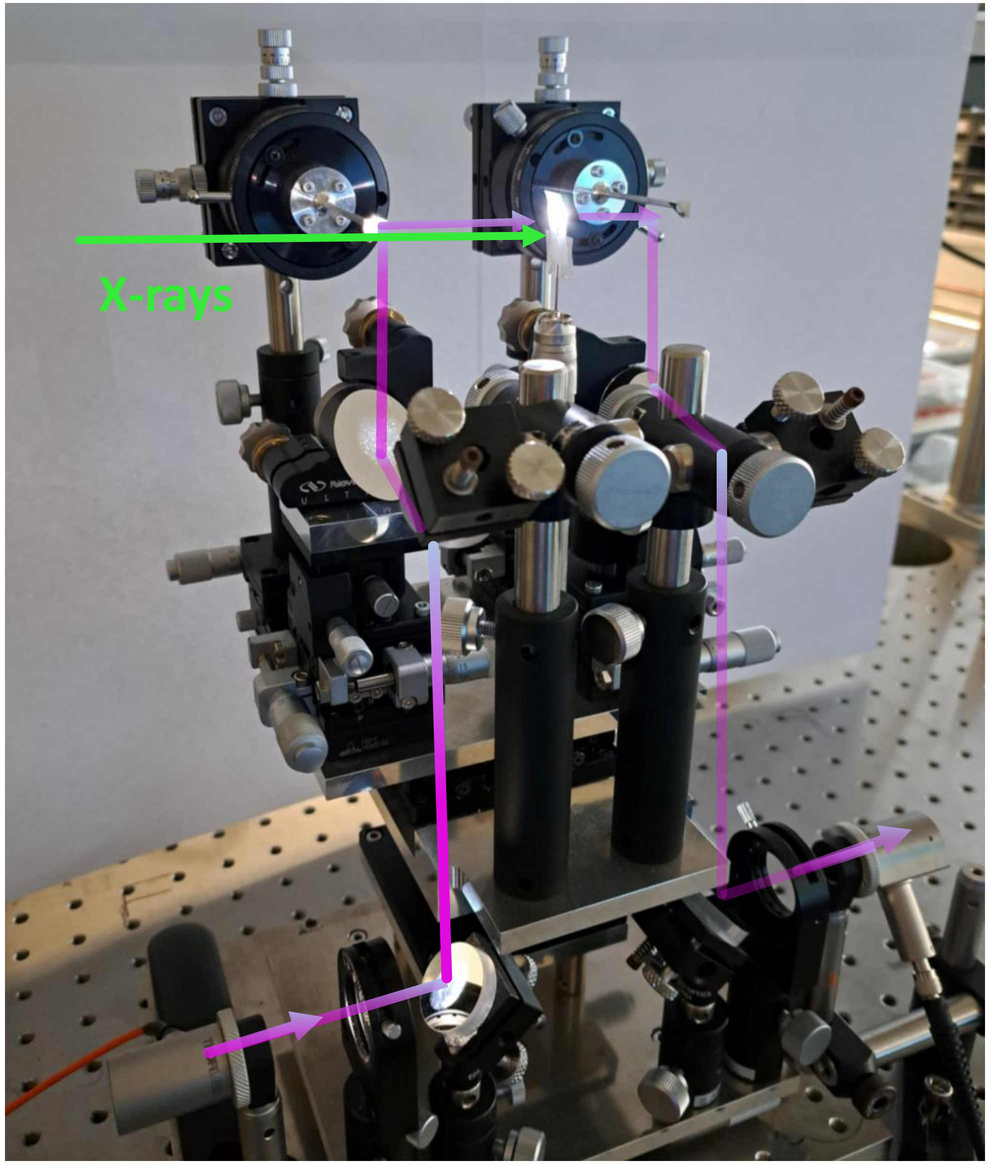
In development: **On-line spectroscopy + Fixed Target SSX (Sofia Jaho)**

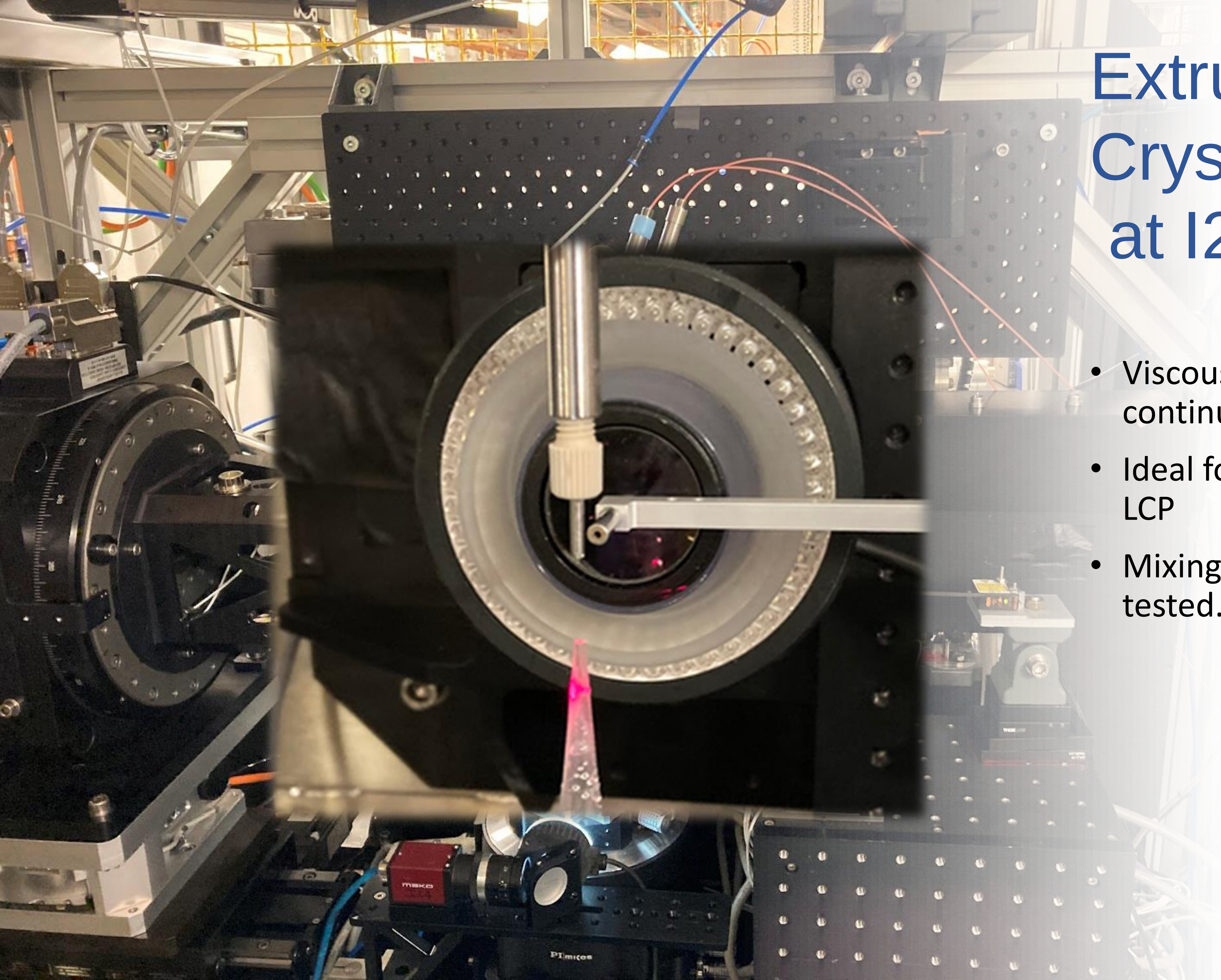
- On-axis geometry (< 5°) between X-rays and optical axis (Sakaguchi M., et al., J. Synchr. Rad., 2016, 23, 334-338)
- Time-resolved spectroscopy
- Spectral mapping of the chip (25600 positions)
- Python script for real-time spectral analysis



Myoglobin on ‘chipless’ chip
(kinetic series, oxygen diffusion)





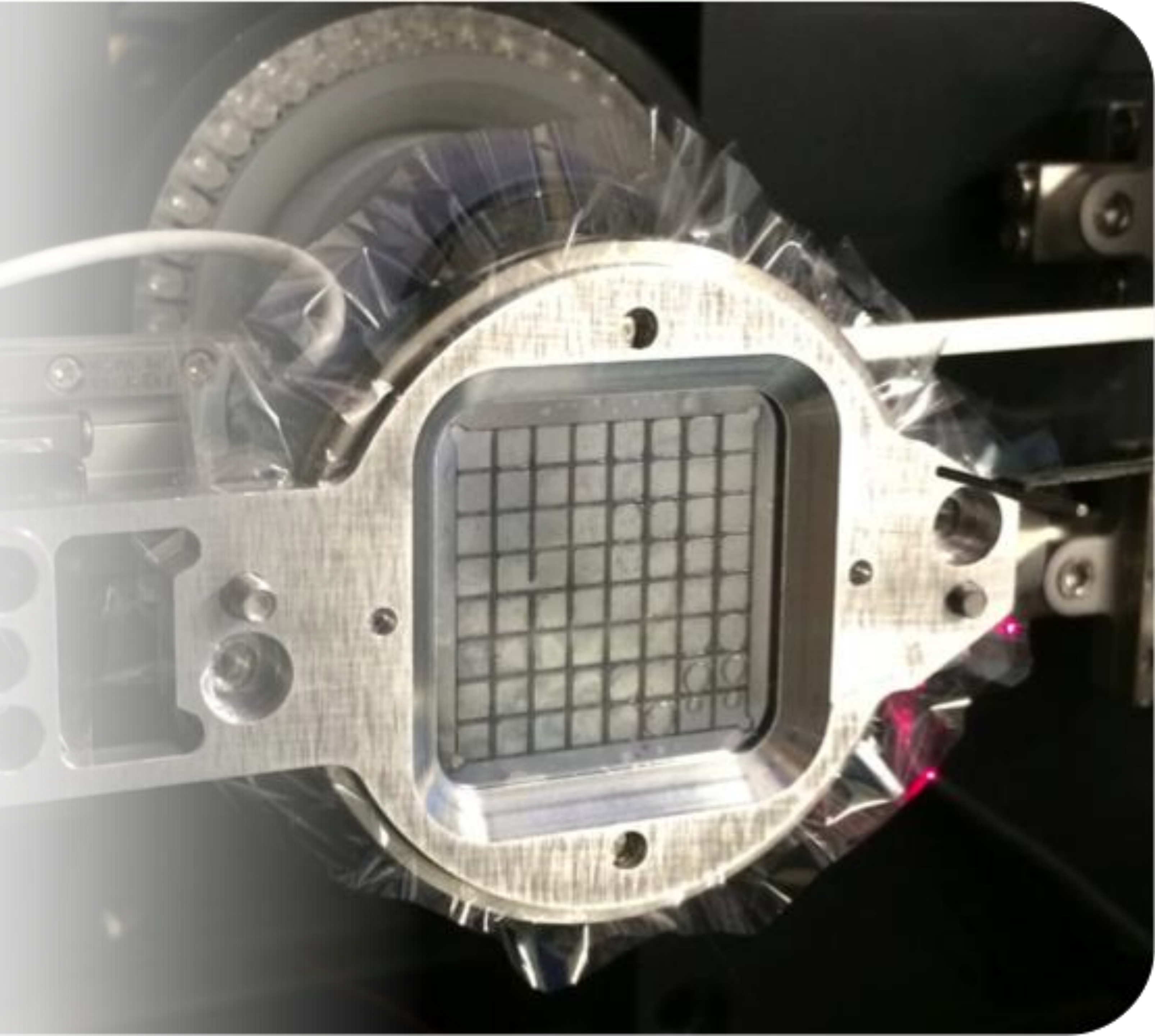


Extruder / jet serial Crystallography at I24

- Viscous extruder for 1D continuous sample delivery
- Ideal for membrane proteins in LCP
- Mixing system has also been tested.

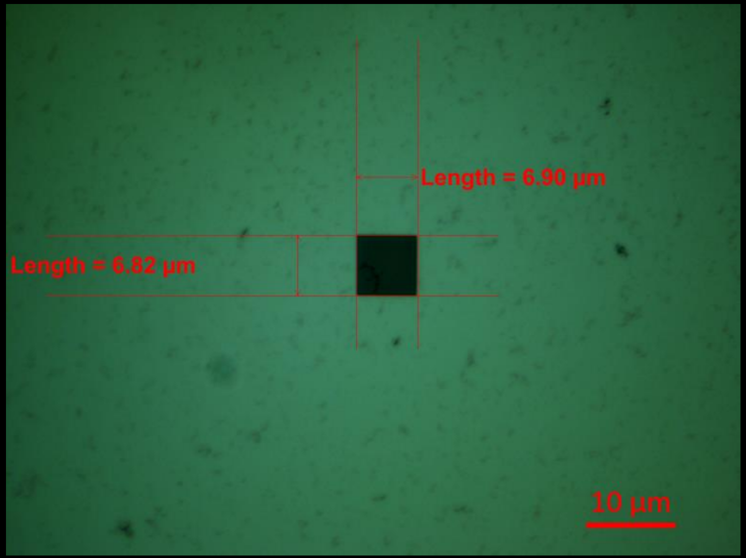
Fixed-Target serial Crystallography (FT-SSX) at I24

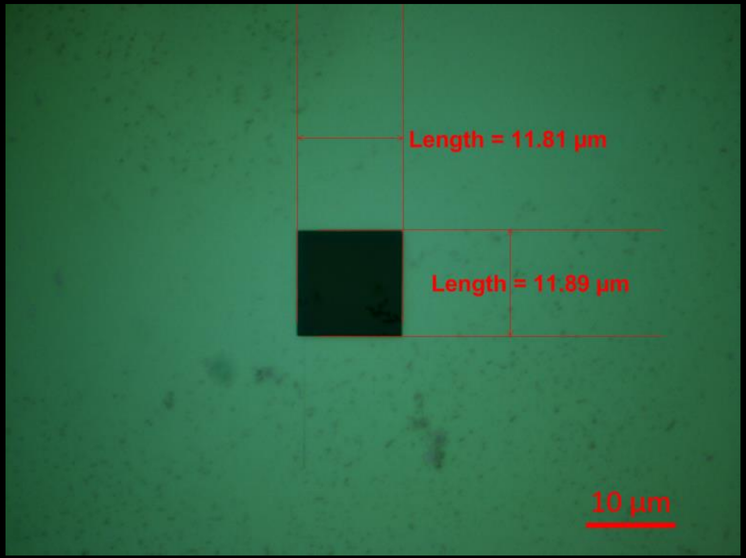
- Fixed target crystallography has become a specialism of I24
- Hardware is modular and can go to other beamlines and lightsources
- Typically, ~150uL of >10^6 crystal slurry from a batch method but can yield hit rates of >30%
- We can offer flexible alternative approaches during process of crystallization optimization for SSX.



Fixed targets

Instead load crystals onto a regular array
Silicon chips: robust, reusable, commercially available.





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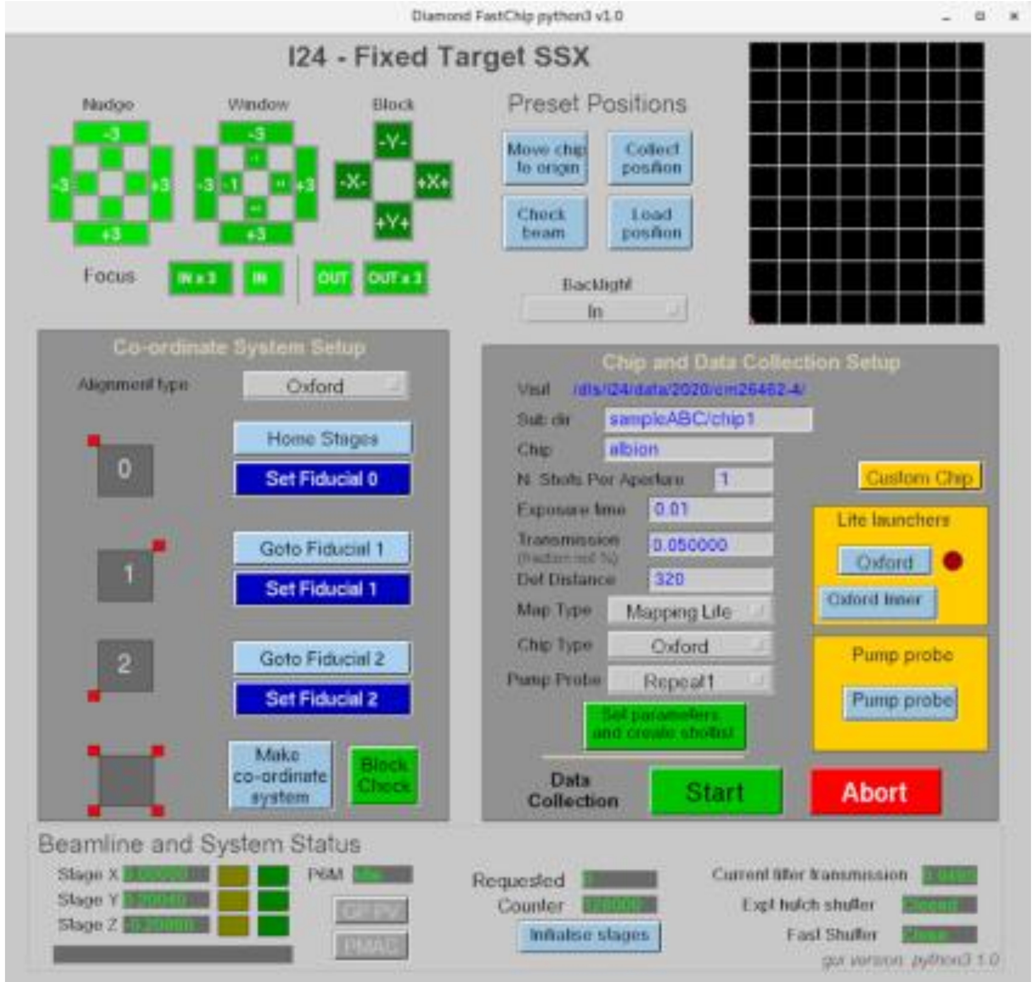
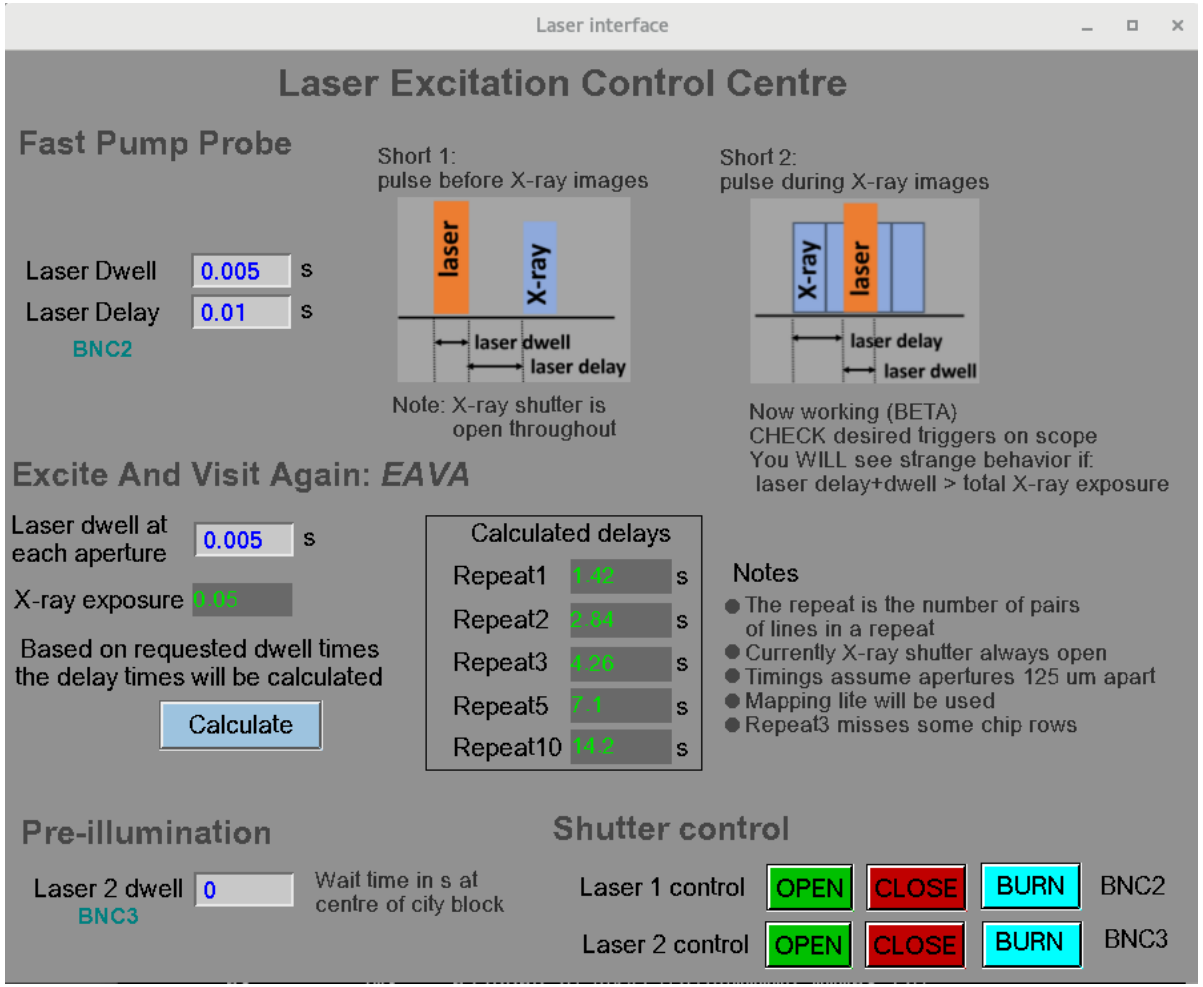
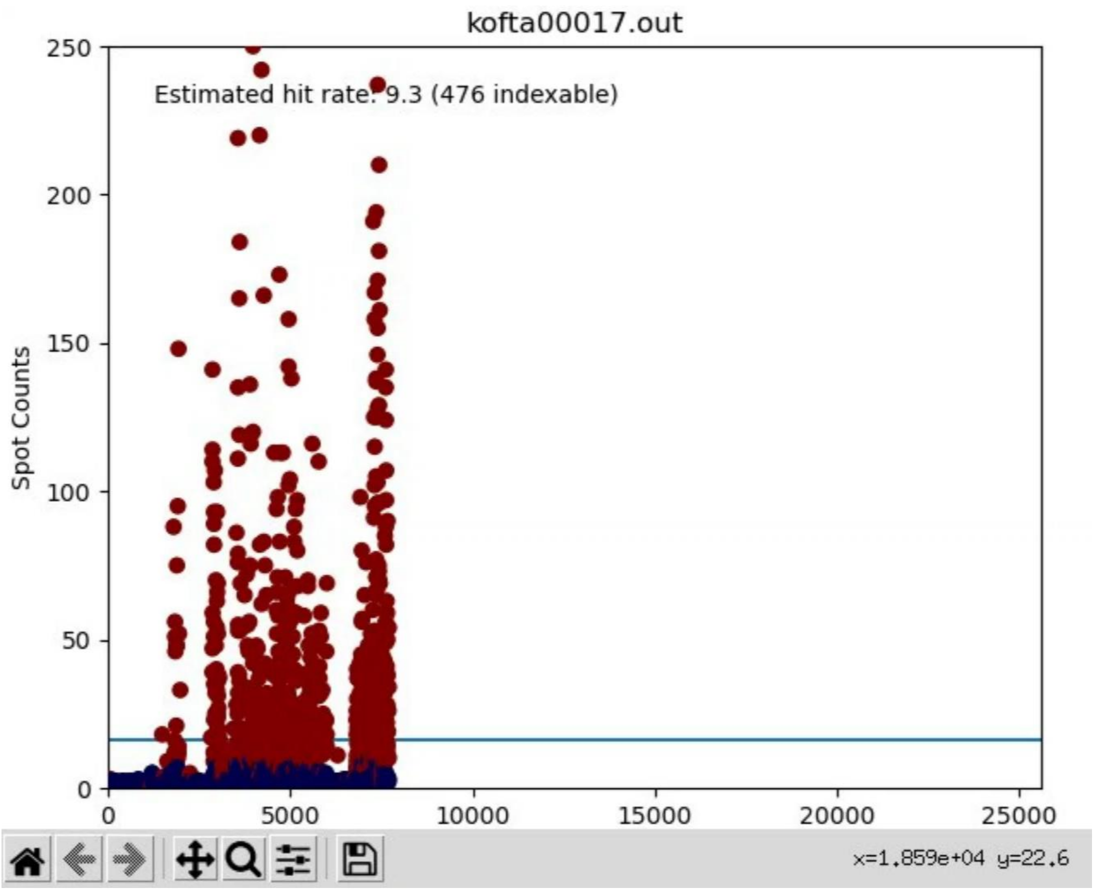
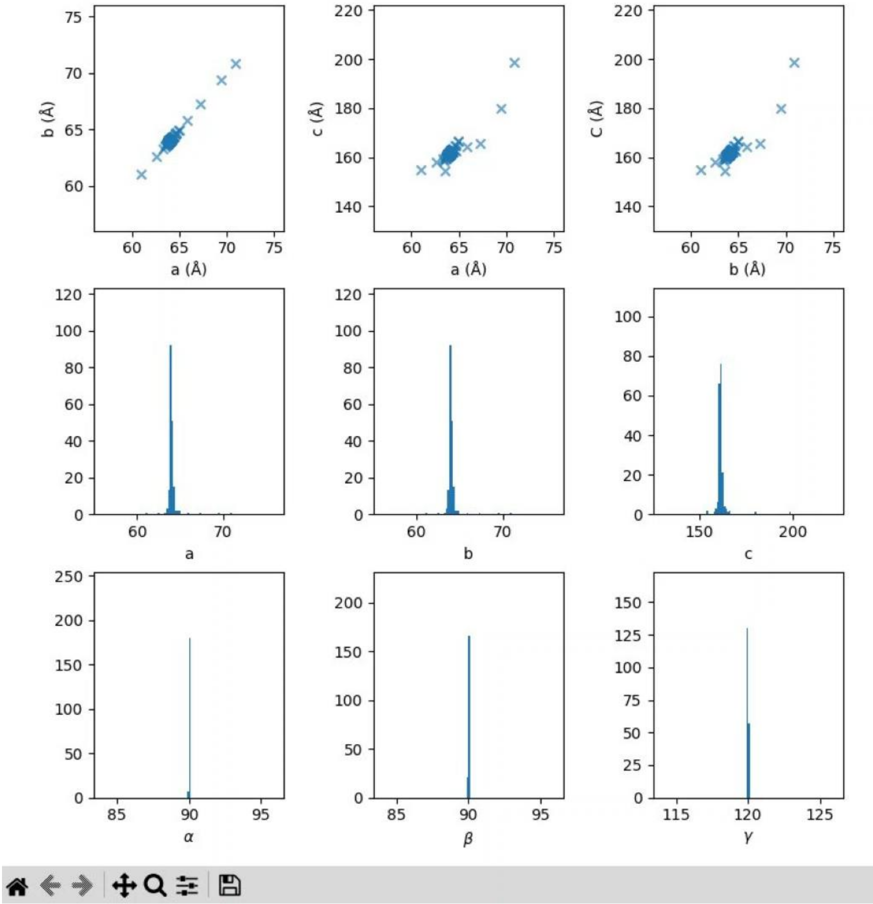
Software and UI developments





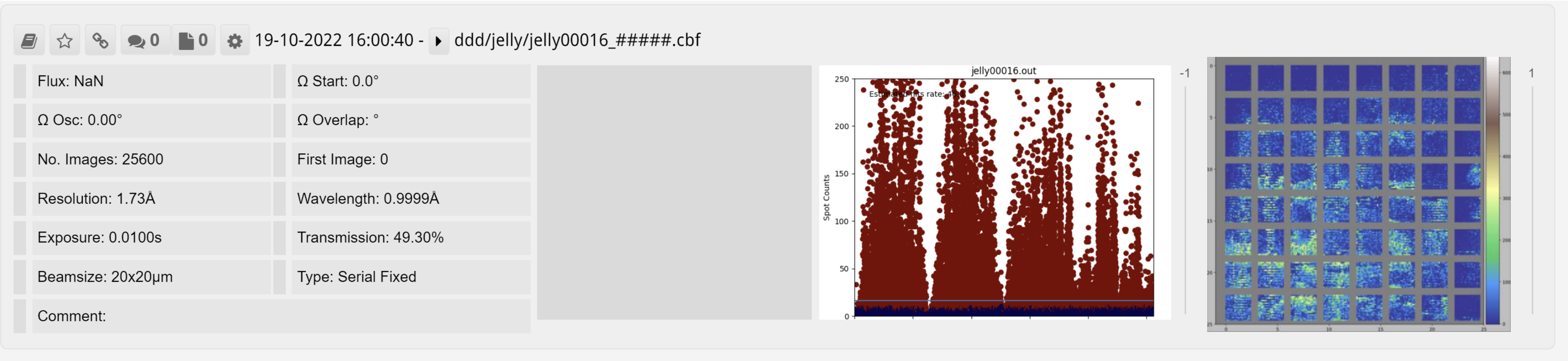
Serial Auto-processing Pipelines





Dials find spots and Index

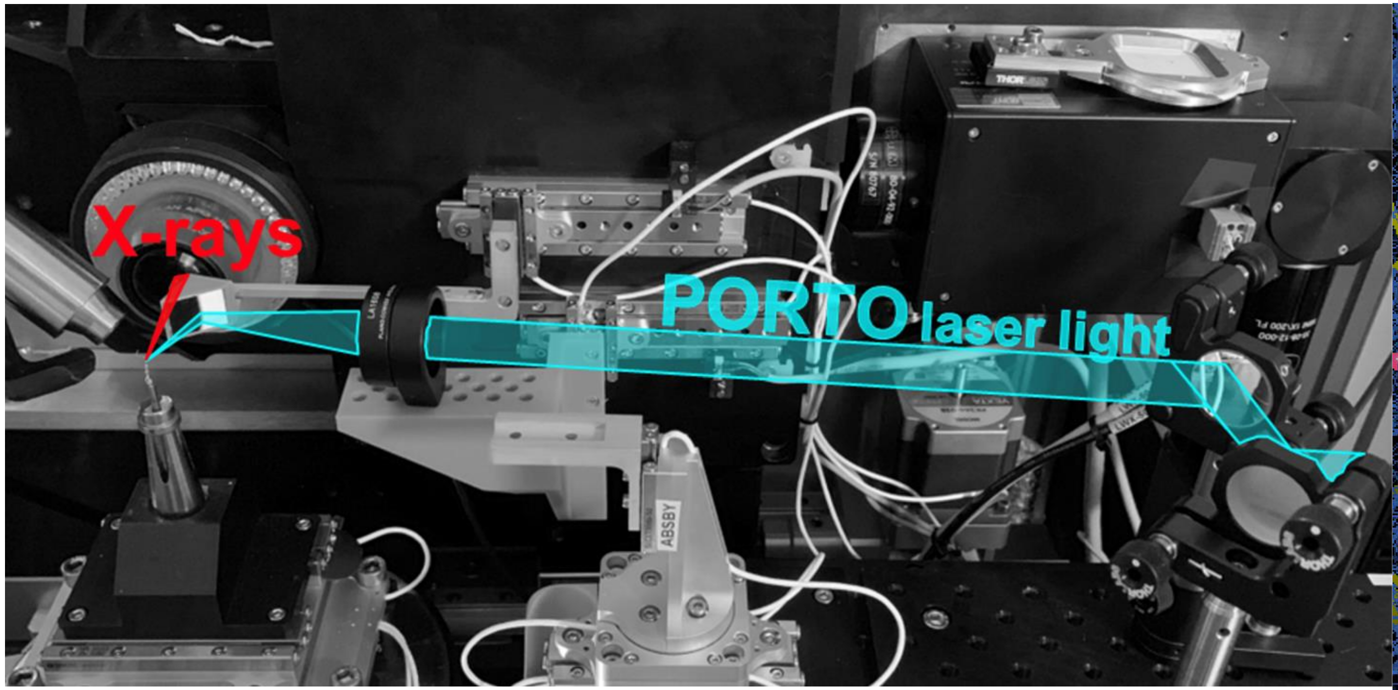
Serial auto-processing pipelines

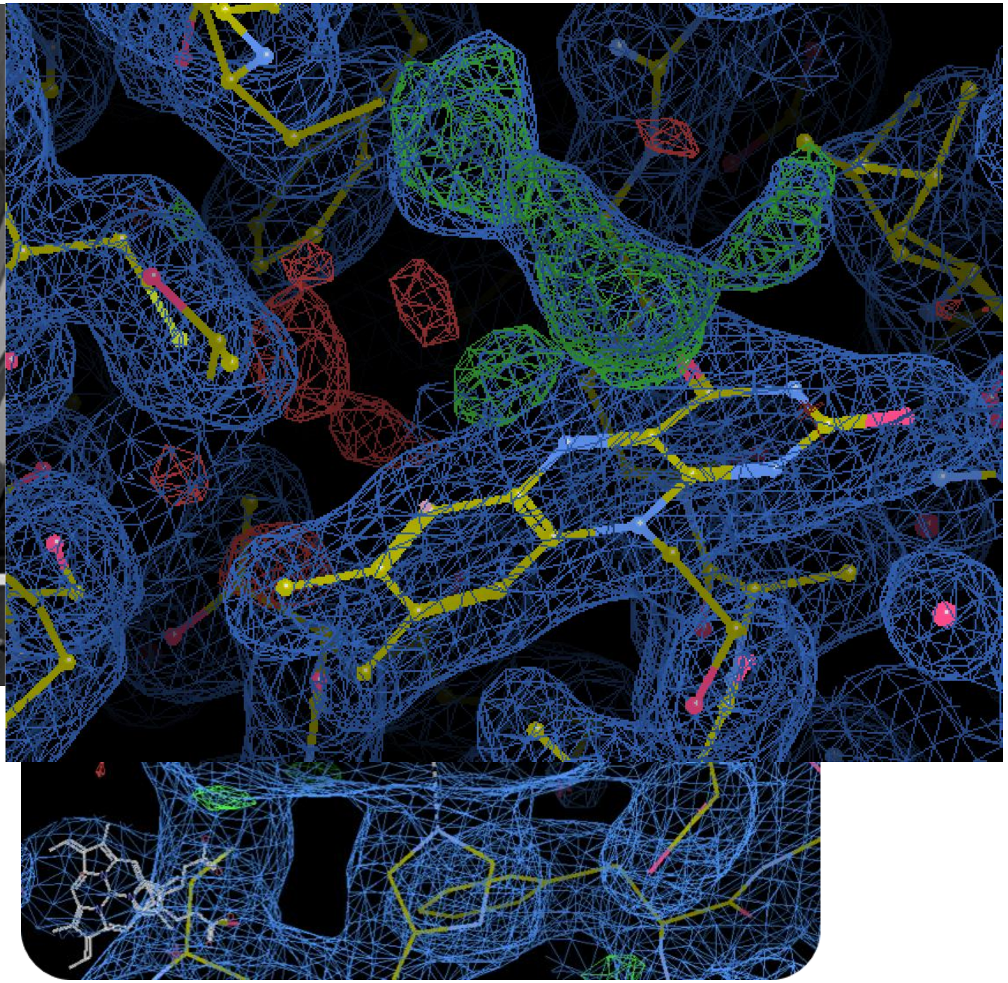


Winter, G. *J. Appl. Crystallogr.* (2010) **43**, 186-190
Gildea & Winter. *Acta Cryst.* (2018) **D74**, 405-410
Winter *et al*. *Acta Cryst*. (2018) **D74**, 85-97
Brewster et al. *Acta Cryst.* (2018) **74**, 877-894
Beilsten-Edmands *et al*. *Acta Cryst*. (2020) **D76**, 385-399
Winter *et al*. *Protein Sci* (2021) **31**, 232-250

Serial Data-collection with Laser Triggering:

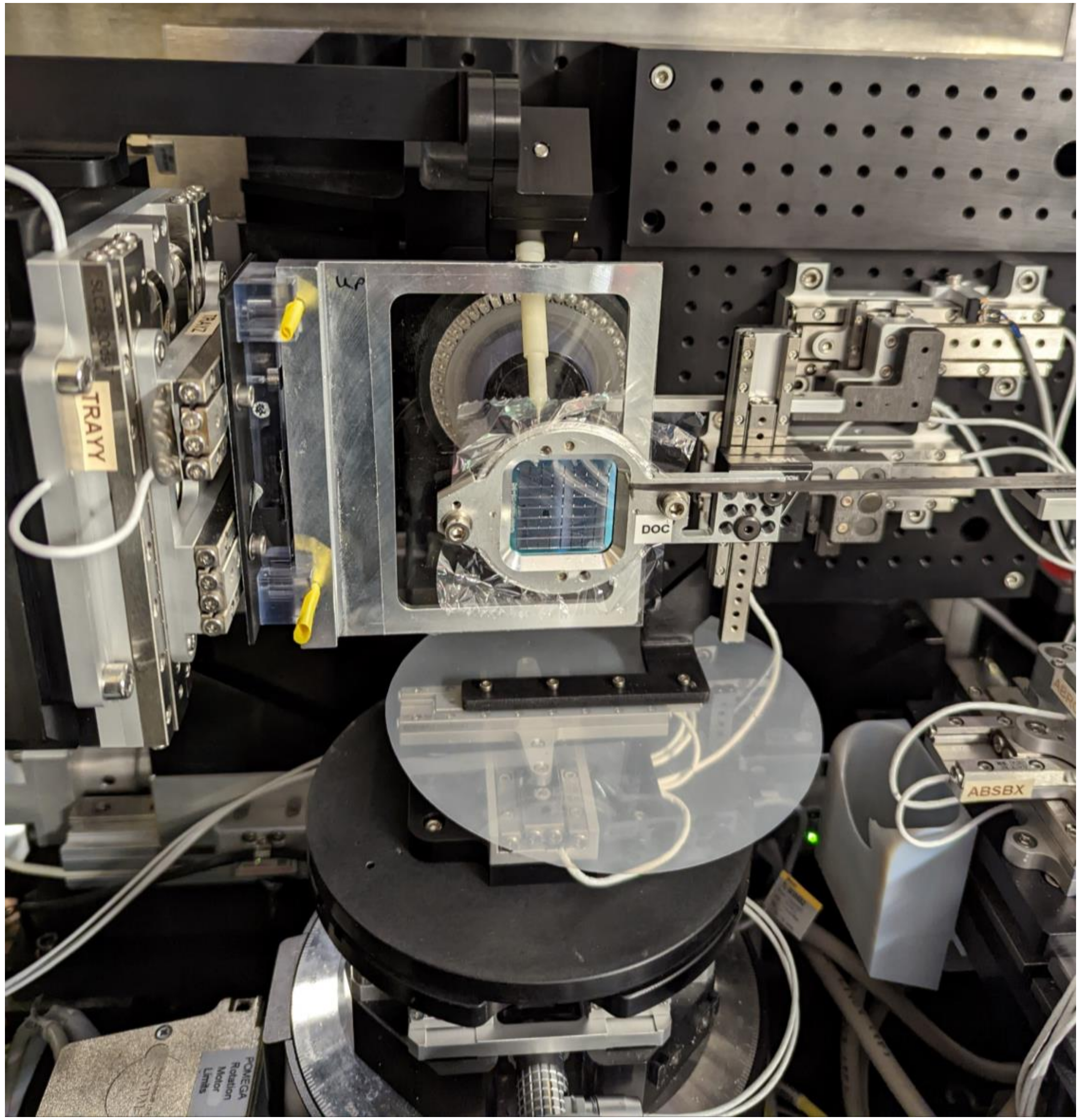
Using the PORTO tuneable femto-second laser light sensitive systems can be probed and interactions with photocaged-compounds tested.

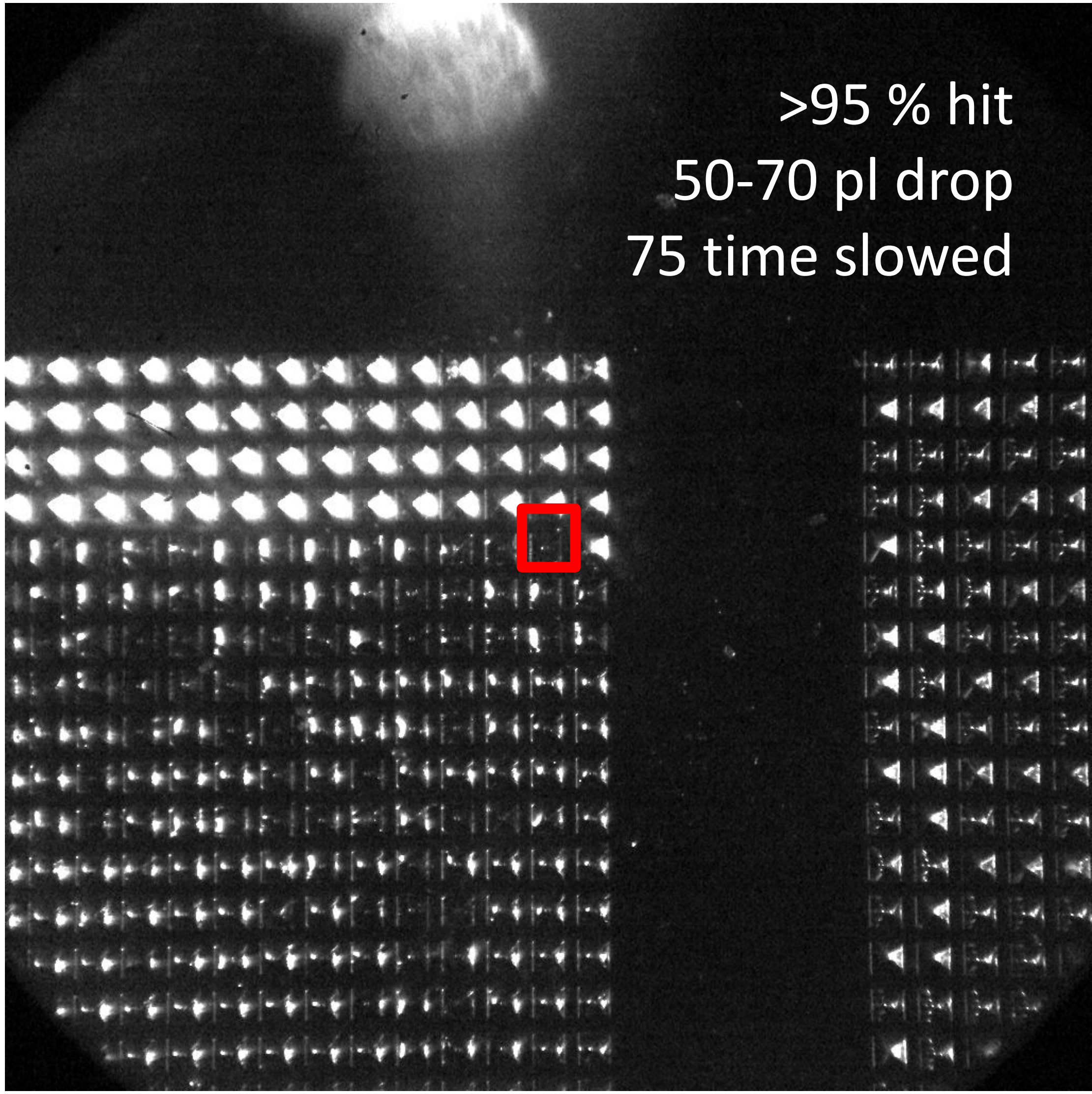




Substrate driven reactions:

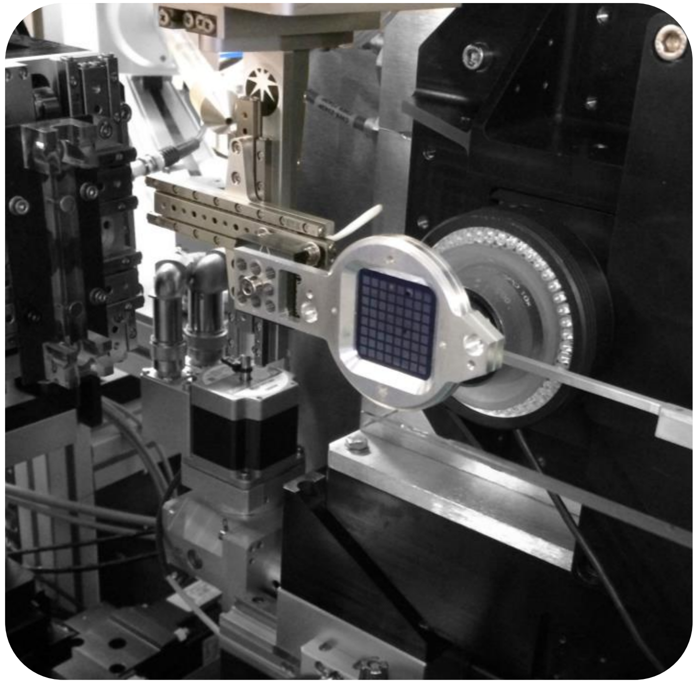
Rapid addition of pL volumes of substrate on a per-crystal basis.

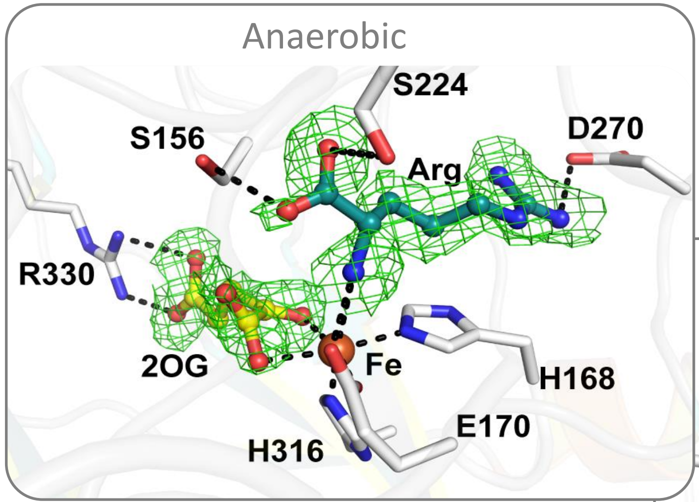


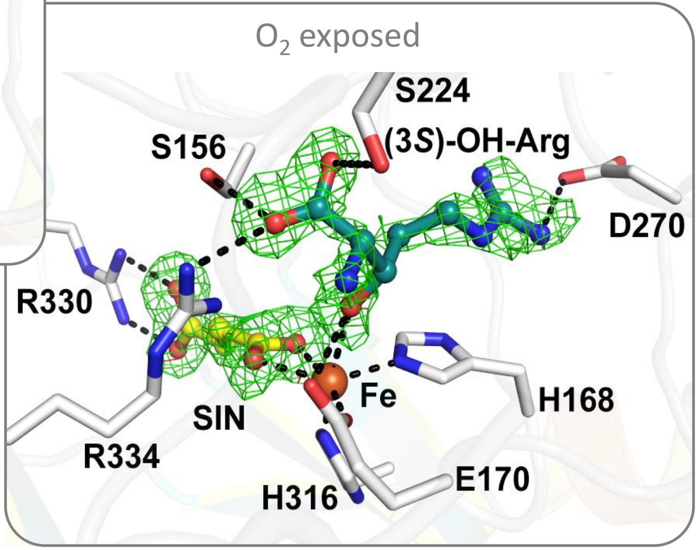


Data collection examples:

Controlled sample environments



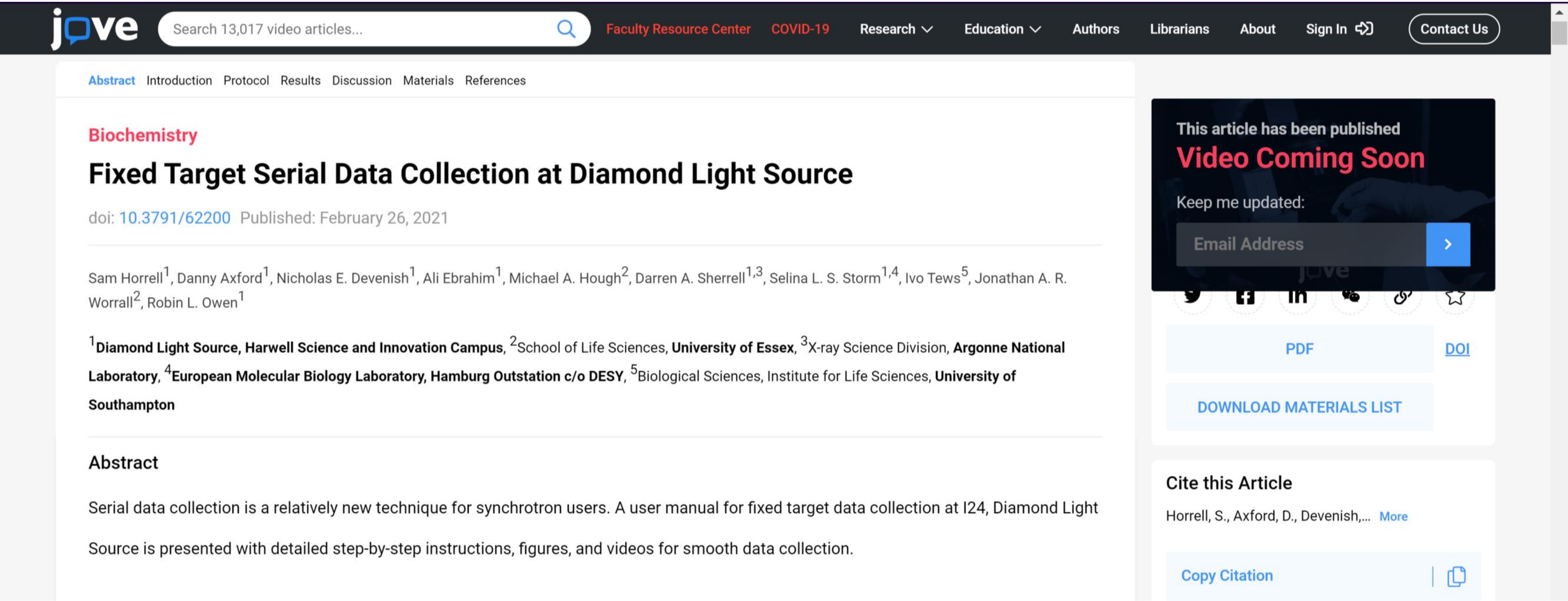




- Local environment control via preparation within glove-box in lab next to beamline
- For example, studying oxygen sensitive enzymes at room temperature

Anerobic and oxygen exposure structures of radiation sensitive oxygenase VioC
Rabe et al. IUCrJ (2020), **7**, 901-912

Serial data collection using Fixed Targets



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Abstract | Introduction | Protocol | Results | Discussion | Materials | References

Biochemistry

Fixed Target Serial Data Collection at Diamond Light Source

doi: 10.3791/62200 Published: February 26, 2021

Sam Horrell[1], Danny Axford[1], Nicholas E. Devenish[1], Ali Ebrahim[1], Michael A. Hough[2], Darren A. Sherrell[1,3], Selina L. S. Storm[1,4], Ivo Tews[5], Jonathan A. R. Worrall[2], Robin L. Owen[1]

[1]**Diamond Light Source, Harwell Science and Innovation Campus**, [2]School of Life Sciences, **University of Essex**, [3]X-ray Science Division, **Argonne National Laboratory**, [4]**European Molecular Biology Laboratory, Hamburg Outstation c/o DESY**, [5]Biological Sciences, Institute for Life Sciences, **University of Southampton**

Abstract

Serial data collection is a relatively new technique for synchrotron users. A user manual for fixed target data collection at I24, Diamond Light Source is presented with detailed step-by-step instructions, figures, and videos for smooth data collection.

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Horrell, S., Axford, D., Devenish,... More

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The future: I24 KMX

A series of upgrades allowing DLS-II to be fully exploited for microfocus MX and SSX

Key I24 KMX capabilities
Higher flux
- Monochromatic beam (**fully tuneable** ~8-30 keV)
- Pink beam (~13 and ~26 keV)
- Fast integrating detector

Smaller beam (retaining variable beamsize)
- 2×2 μm^2 to ~50×50 μm^2

Tools for driving **reactions in crystallo**
- *Light and substrate driven reactions*

Multiple forms of data recorded
- *Synchronised detector readout and data tagging*

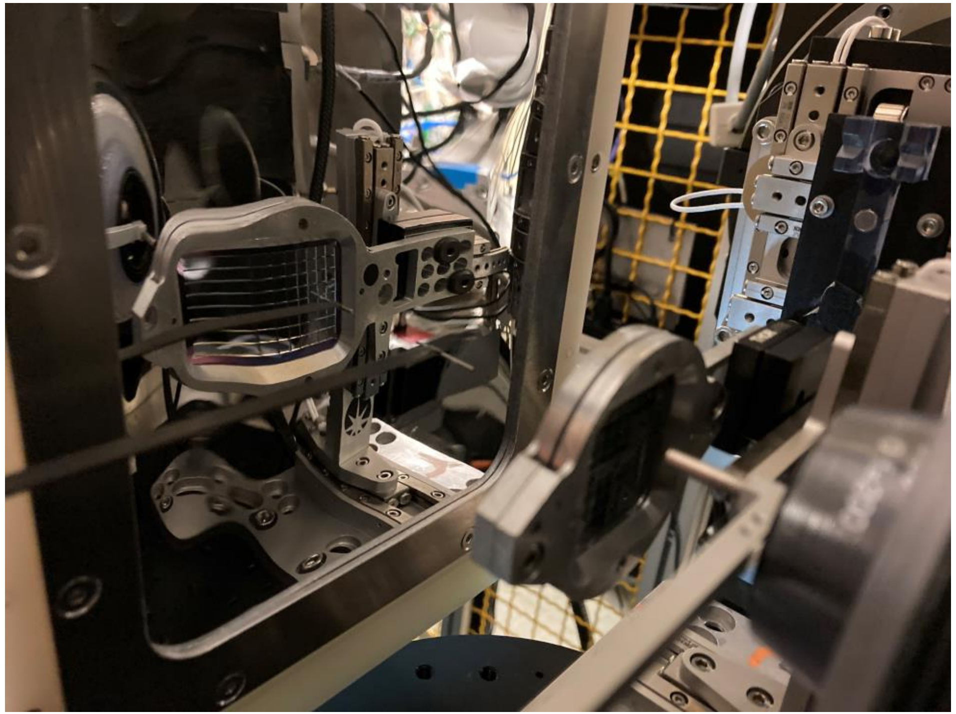
- **Adaptable** sample environment

Providing:

- Best possible **quality data** from small, poorly diffracting, or non-isomorphous crystals
- Access to dynamics in the **microsecond** time domain
- **Multi-modal data** (diffraction plus spectroscopy)
- Access for non-expert users and cases when sample supply is limited

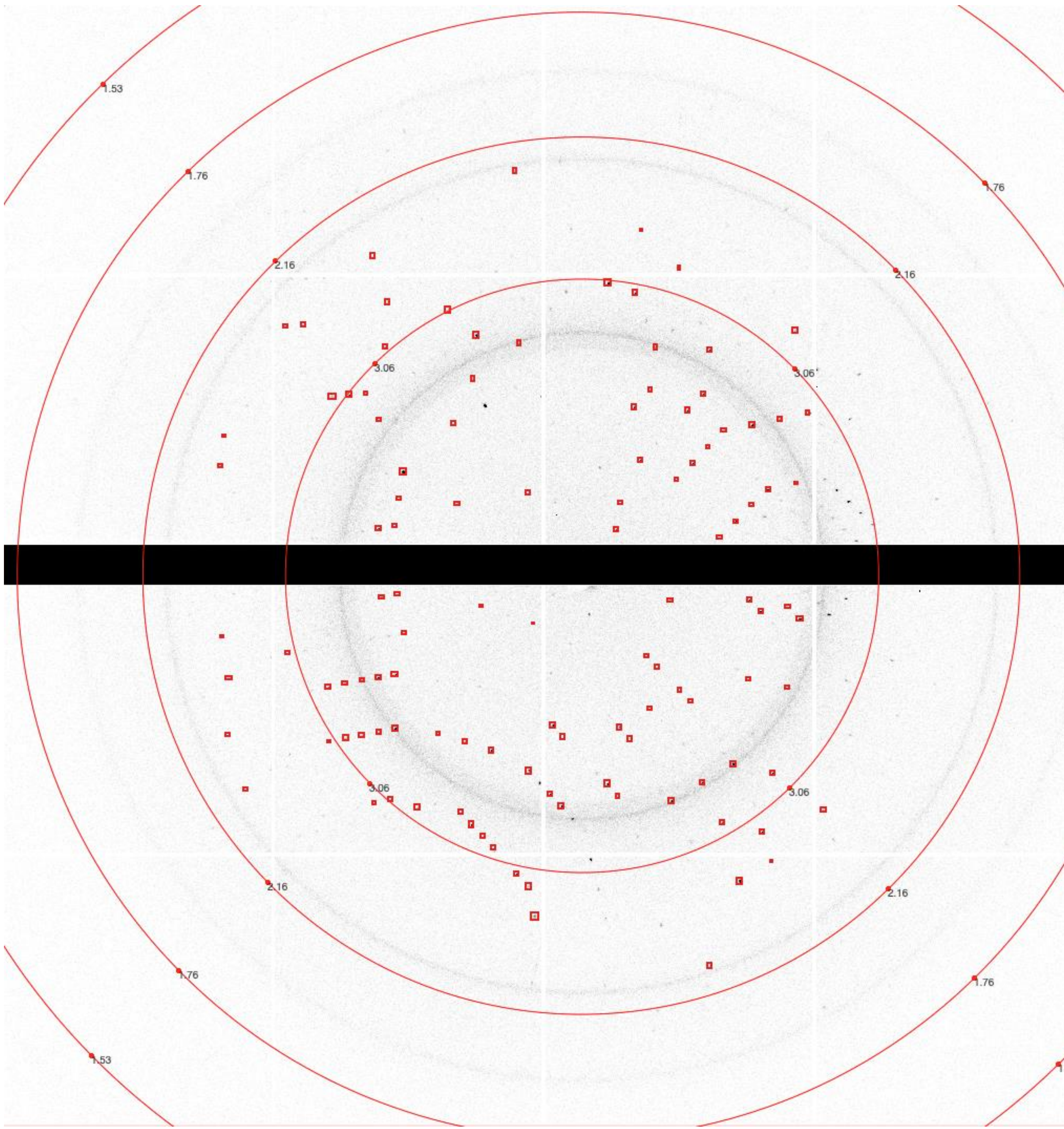
Jungfrau 1M test detector

- Only occupies middle third of housing (right)
- Physical distance 46mm, 63mm sample to active surface.



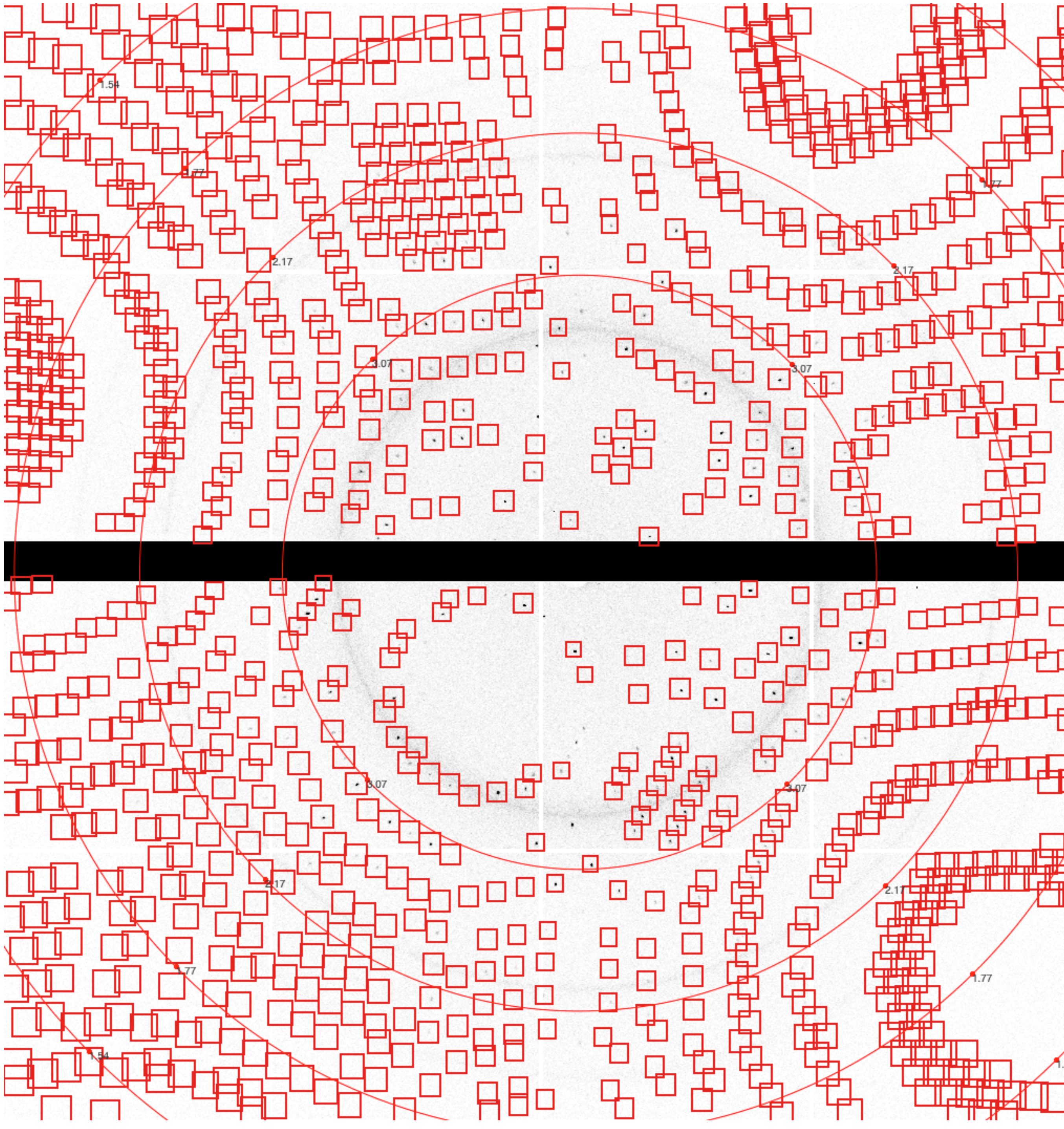
Optimised geometry

- Beam centre ~ middle of image
- Detector distance allows ~2Å at 12.4 keV
- Complete rotation possible < 1s



Optimised geometry

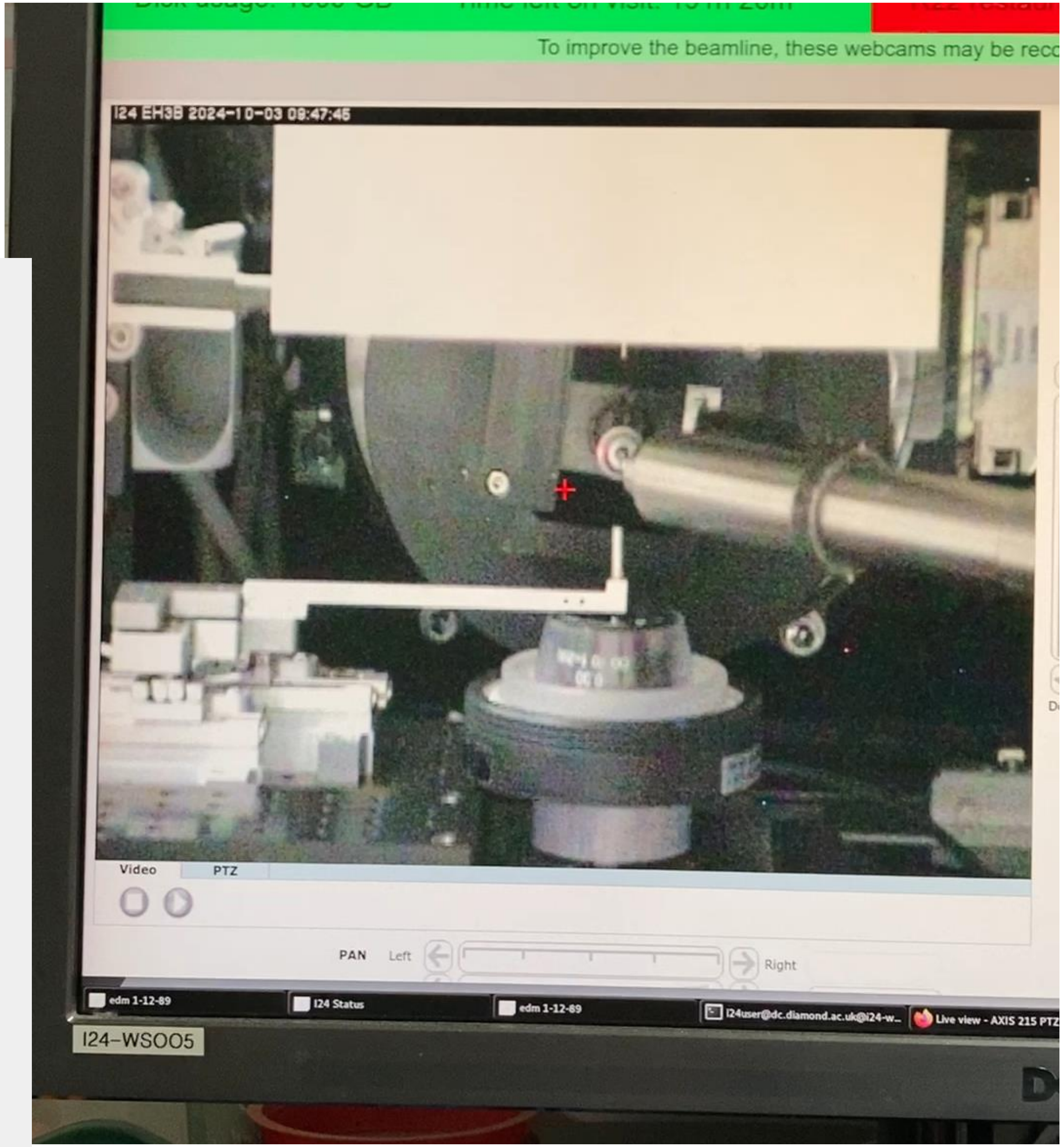
- Divergent beam at i24 - detector at 63mm means the spots are small
- Data process out of the box with DIALS
- Good data possible if small unit cell (e.g. insulin)



Fast rotation

- Up to 2000Hz, 400 deg per second

	Overall	Low	High
High resolution limit	1.65	4.48	1.65
Low resolution limit	55.18	55.21	1.68
Completeness	89.3	100.0	16.7
Multiplicity	16.0	50.8	2.3
I/sigma	27.2	88.1	1.6
Rmerge(I)	0.067	0.053	0.480
Rmerge(I+/-)	0.065	0.053	0.474
Rmeas(I)	0.068	0.054	0.646
Rmeas(I+/-)	0.068	0.054	0.657
Rpim(I)	0.013	0.008	0.427
Rpim(I+/-)	0.017	0.010	0.453
CC half	1.000	0.999	0.647
Anomalous completeness	72.3	100.0	1.6
Anomalous multiplicity	9.2	28.7	2.1
Anomalous correlation	0.165	0.272	-0.517
Anomalous slope	1.183		
dF/F	0.047		
dI/s(dI)	1.021		
Total observations	137979	26671	181
Total unique	8619	525	80



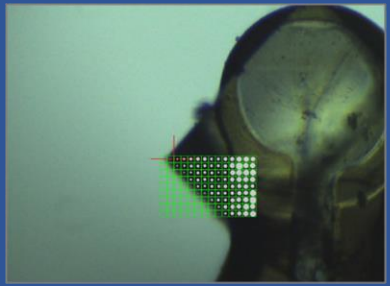
Fast rotation

- Optimising collection further improves statistics.

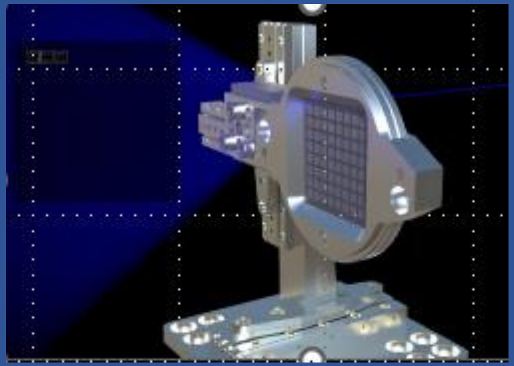
	Suggested	Low	High	Overall
High resolution limit	1.51	4.11	1.51	1.37
Low resolution limit	55.90	55.93	1.54	55.90
Completeness	99.8	100.0	97.6	91.9
Multiplicity	31.1	35.4	9.0	26.0
I/sigma	22.0	107.3	0.2	17.8
Rmerge(I)	0.087	0.034	3.324	0.088
Rmerge(I+/-)	0.086	0.033	3.096	0.087
Rmeas(I)	0.088	0.035	3.527	0.089
Rmeas(I+/-)	0.088	0.034	3.489	0.089
Rpim(I)	0.015	0.006	1.139	0.015
Rpim(I+/-)	0.020	0.008	1.551	0.021
CC half	0.999	0.998	0.310	0.999
Anomalous completeness	99.7	100.0	97.0	88.7
Anomalous multiplicity	16.2	19.7	4.7	13.8
Anomalous correlation	0.147	0.506	0.068	0.097
Anomalous slope	0.518			
dF/F	0.044			
dI/s(dI)	0.517			
Total observations	402403	24504	5572	415206
Total unique	12937	693	617	15955

Summary

A wide variety of experiments are possible at I24 now



Semi-automated multi-crystal collection & merging



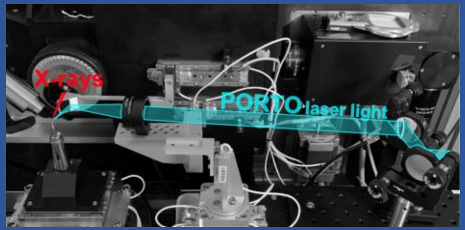
100K pin-based data collection

in situ data collection

Substrate triggered reactions

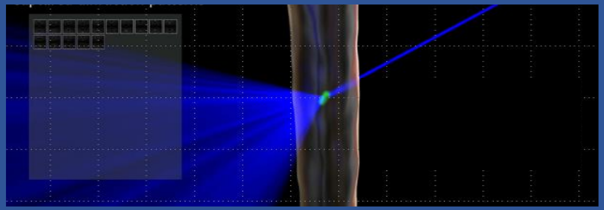
LCP extruder

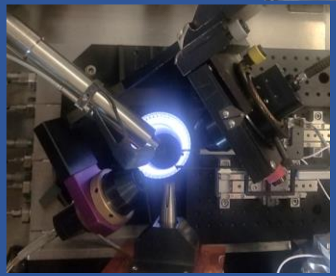
Fixed Target SSX



Thin film RT data collection

Light triggered reactions





In situ UV-Vis spectroscopy

New experiments and developments can be driven by you…!

Acknowledgements

I24 Team

- **Robin Owen**
- **Sofia Jaho**
- **Danny Axford**
- **Do-Heon Gu**

XFEL Hub

- **Allen Orville**
- **Pierre Aller**

and others…

DIALS Team

- **Graeme Winter**
- **Nick Devenish**
- **James Beilsten-Edmands**

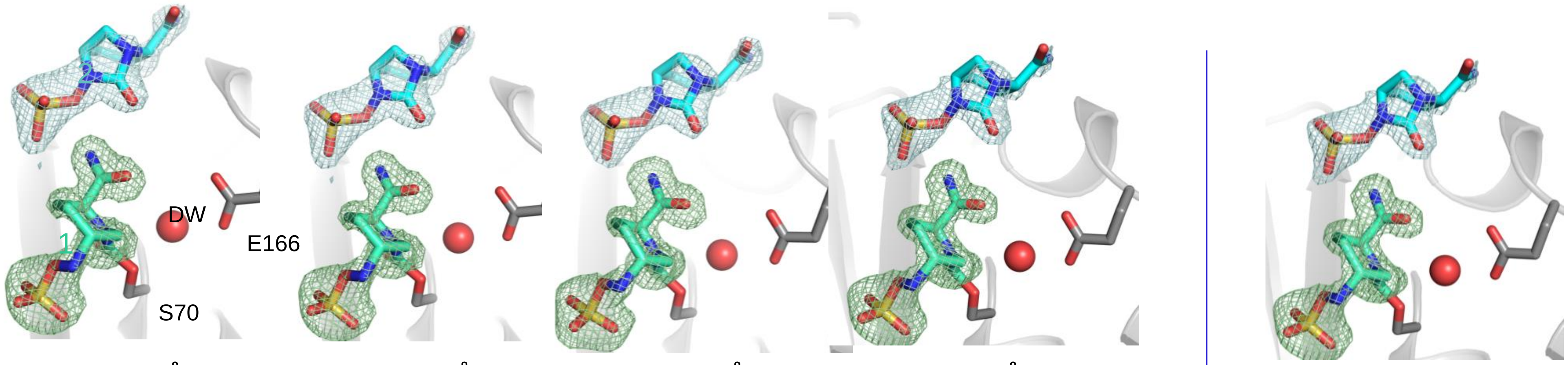
Data acq. software

- **Noemi Frisina**

Data collection example:

Substrate driven reactions

Formation of β-lactamase acyl-enzyme complex after 10 ms
Spencer Group – University of Bristol



10 ms 1.9 Å   30 ms 1.9 Å   60 ms 1.9 Å   100 ms 1.9 Å   560 ms 1.65 Å

F_o– F_c OMIT electron density maps displayed at 3σ

University of BRISTOL