Tools and capabilities of the variable focus beamline I04

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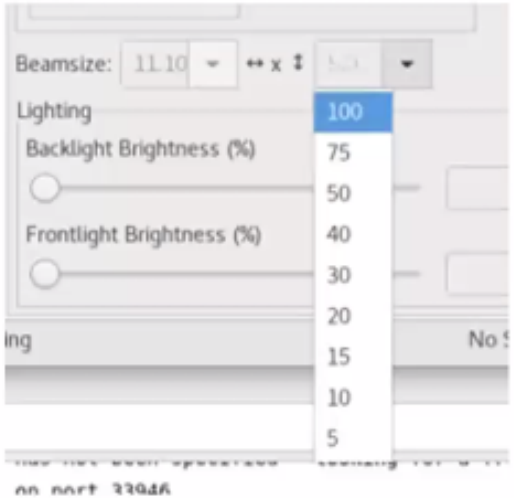
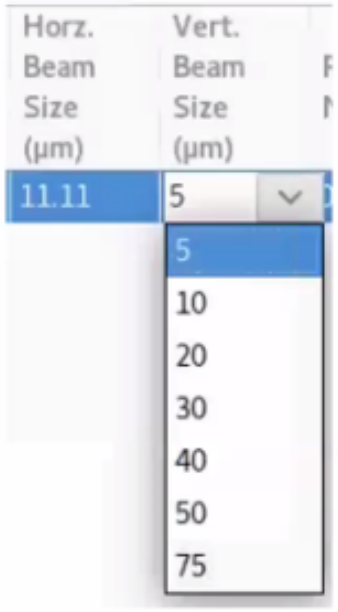
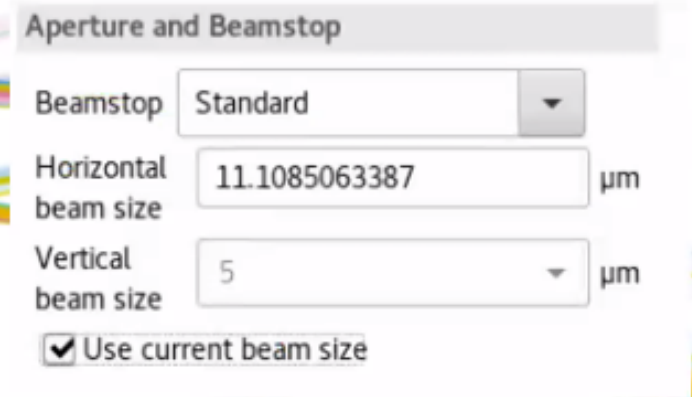
Choose your beam size

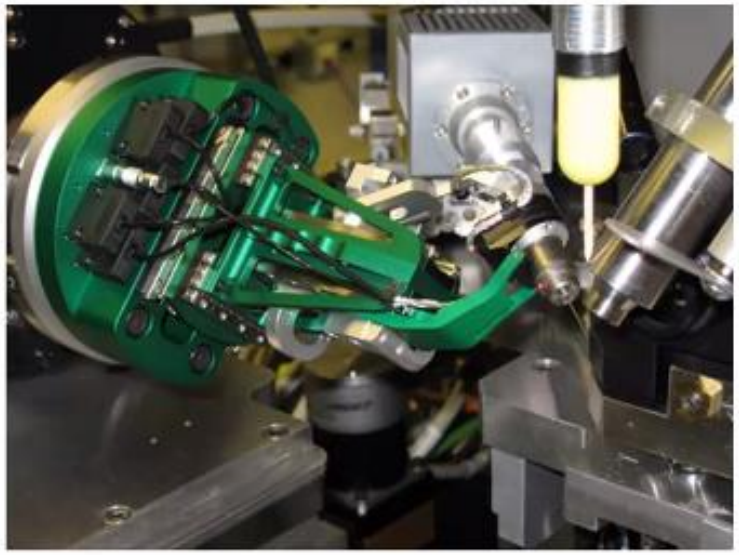




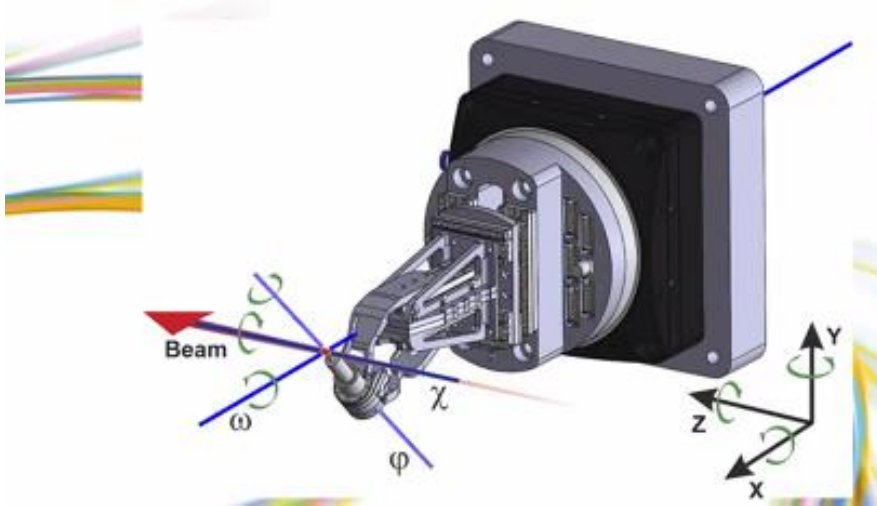


- Variable focus
- From microfocus to larger beam sizes
- Focus is achieved with lenses (no slitting down)
  - Flux relatively constant
- Allows to match beam size to crystal size
  - Needles, small rods, thin plates
  - Useful for line scans
  - Remember I/sigma
- Beware: flux density

SmarGon multi-axis goniometer



I04 performance achieved:
SOC: omega: < 1 µm, chi < 3 µm, phi < 6 µm



- crystal alignment/orientation
- get equivalent images from different crystals by reorienting them
- smart data collection strategies, better completeness of data
- reduced radiation damage by using smaller overall oscillation range
- help in point group determination
- comparing crystals in the same orientation
- optimised MAD data collection; Bijvoet pairs on the same frame
- improved high multiplicity SAD data collection protocols
- reducing/avoiding spot overlap, better spot separation (long unit cell problem)
- multiple crystal data collection: collect only what has not been collected before

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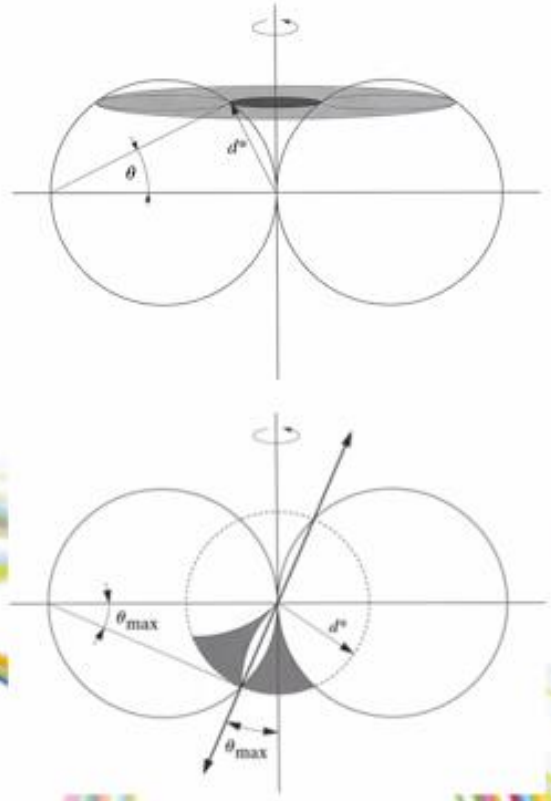
Complete data sets

OAV View | Data Collection Table Input

Visit Folder: /dls/i04/data/2017/cm16781-5 | Default Folder: gw/${date}/${proteinacronym}/${samplename} | Default Prefix: ${samplename} | Run All | Run Selected

Sample ID	Code	Folder	Prefix	Holder	Position	Omega Start (°)	Omega Oscillatio (°)	Omega Delta (°)	Chi (°)	Phi (°)	X (µm)	Y (µm)	Z (µm)	Number of Images	Time per Image (s)	Maximum Resolutio (Å)	Distance (mm)	Wavelength (Å)	Energy (eV)
thaum_8	CA00AD1	${date}/${proteinac	${samplename}	35	8	0.00	0.100	0.00	0.000	0.000	-9999.0	-9999.0	-9999.0	3600	0.040	1.5000	266.8	0.97950	12657.9
thaum_8	CA00AD1	${date}/${proteinac	${samplename}	35	8	0.00	0.100	0.00	45.000	0.000	-9999.0	-9999.0	-9999.0	3600	0.040	1.5000	266.8	0.97950	12657.9



- particularly problematic for triclinic (*P* 1) samples
  - To collect missing reflections re-orient crystal by at least $2\theta_{max}$
- more pronounced with long wavelength radiation (increased curvature of Ewald sphere)
- high resolution reflections more affected than low resolution reflections
- Beware of nearly aligned crystals
  - skewing the axis by at least θ_{max} from the spindle axis ensures there will be no loss of completeness as a result of the blind region
- Option in GDA data collection table

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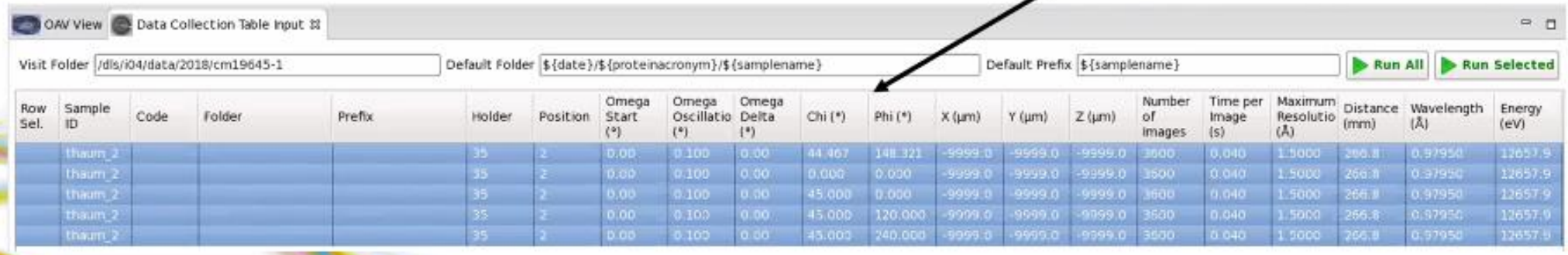
Multi-sweep SAD data

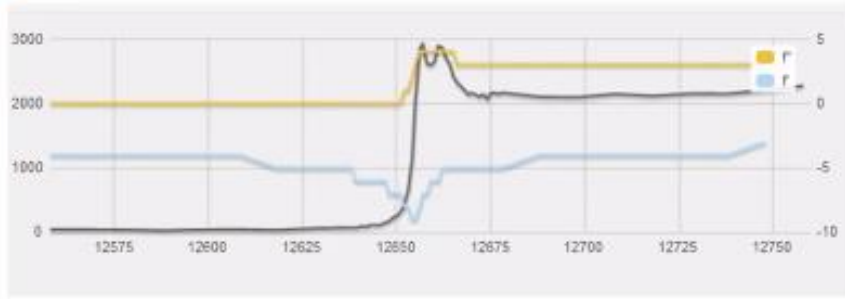
- target: high multiplicity
- collect at peak wavelength
- fluorescence scan
- single axis goniometer
  - rotate 360 deg and repeat
  - inverse beam
- multi-axis goniometer
  - use of different crystal orientations
  - aligned and random

Omega Start (°)	Omega Oscillatio (°)	Omega Delta (°)	Chi (°)	Phi (°)
0.00	0.100	0.00	44.467	148.321
0.00	0.100	0.00	0.000	0.000
0.00	0.100	0.00	45.000	0.000
0.00	0.100	0.00	45.000	120.000
0.00	0.100	0.00	45.000	240.000



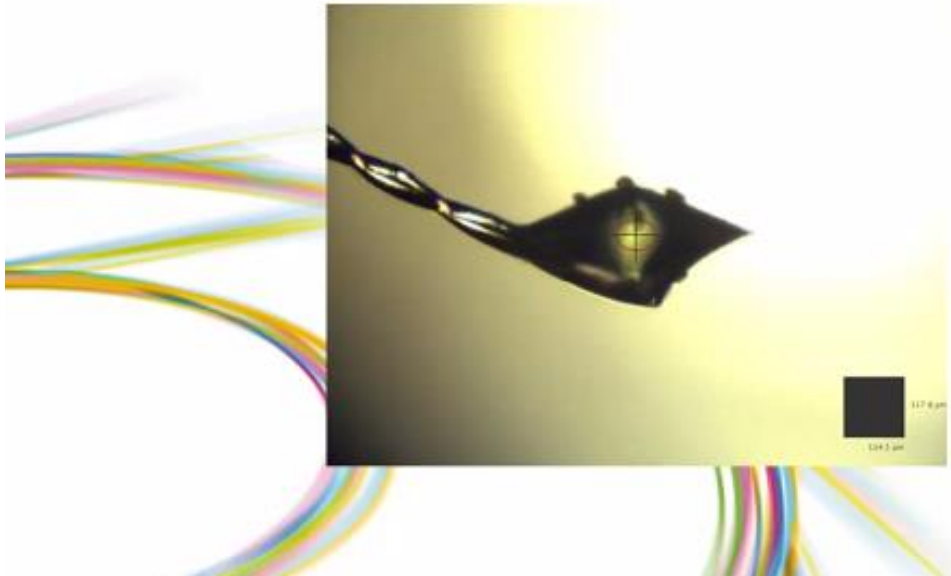
MAD data collection

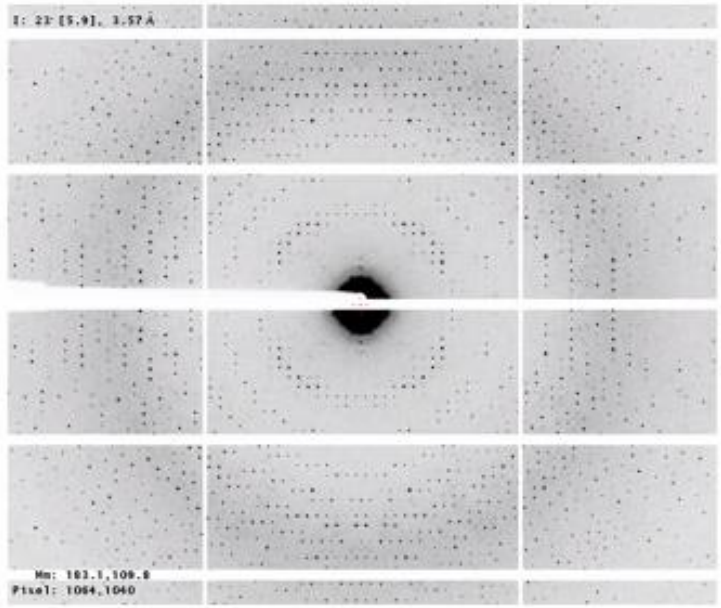
- 3 wavelengths: peak, inflection, remote
- fluorescence scan
- data as complete as possible
- single axis goniometer
  - wedged MAD (takes time)
- multi-axis goniometer
  - use of different crystal orientations
  - aligned crystal
  - Bijvoet pairs on the same frame
  - offset crystal to get better completeness



29-11-2018 15:28:00 - Se Edge Scan

Sample: Set-Thaum_4	Scan File: my_crystal.fluo
E(Peak): 12656.9eV (0.9796Å)	f': 4.92 / f": -7.66e
E(Inf): 12654.9eV (0.9797Å)	f': 3.53 / f": -9.66e
Exposure: 1s	Transmission: 1.00%
Beamsize: 31.72598x20µm	





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Alignment from crystal screening

Strategies — Mosflm: ✔ EDNA: ✔

Stepped transmission 1

Space Group	A	B	C	α	β	γ
P4	78.77	78.77	36.88	90.00	90.00	90.00

Lookup Cell

Strategy	Description	Axis	Axis Start	Axis Osc	Res (Å)	Ranking Res (Å)	Rel Trn (%)	Abs Trn (%)	Exposure (s)	No. Images
	Stepped transmission 1	omega	0	0.10	2.00	0.00	12.5	12.5	0.002	3600
	Stepped transmission 1	omega	0	0.10	2.00	0.00	25.0	25	0.002	3600
	Stepped transmission 1	omega	0	0.10	2.00	0.00	50.0	50	0.002	3600
	Stepped transmission 1	omega	0	0.10	2.00	0.00	100.0	100	0.002	3600

dials.align_crystal

Axes	Kappa	Chi	Phi
c* (4-fold)		29.59	86.27

mosflm

Space Group	A	B	C	α	β	γ
P4	78.77	78.77	36.88	90.00	90.00	90.00

Lookup Cell

Strategy	Description	Axis	Axis Start	Axis Osc	Res (Å)	Ranking Res (Å)	Rel Trn (%)	Abs Trn (%)	Exposure (s)	No. Images
MOSFLM anomalous Wedge1		omega	82	0.90	2.00	0.00	0.0	0	0.000	100
MOSFLM native Wedge1		omega	82	0.90	2.00	0.00	0.0	0	0.000	100

EDNA MXv1

Space Group	A	B	C	α	β	γ
P4	78.78	78.78	36.95	90.00	90.00	90.00

Lookup Cell

Strategy	Description	Axis	Axis Start	Axis Osc	Res (Å)	Ranking Res (Å)	Rel Trn (%)	Abs Trn (%)	Exposure (s)	No. Images
Strategy1 Wedge1	Standard Native Dataset Multiplicity=3 I/sig=2 Maxlifespan=50.0 s	Omega	52	0.25	3.32	3.32	100.0	100	0.135	316
Strategy3 Wedge1	High multiplicity Multiplicity=16 I/sig=2 Maxlifespan=50.0 s	Omega	0	0.25	3.32	3.32	100.0	100	0.030	1440
Strategy4 Wedge1	Gentle Multiplicity=2 I/sig=2 Maxlifespan=5.0 s	Omega	63	0.25	3.59	3.59	54.8	54.8	0.008	480

XOalign

Axes	Kappa	Chi	Phi
[(c*, b*), (c*, a*)]		29.95	86.79

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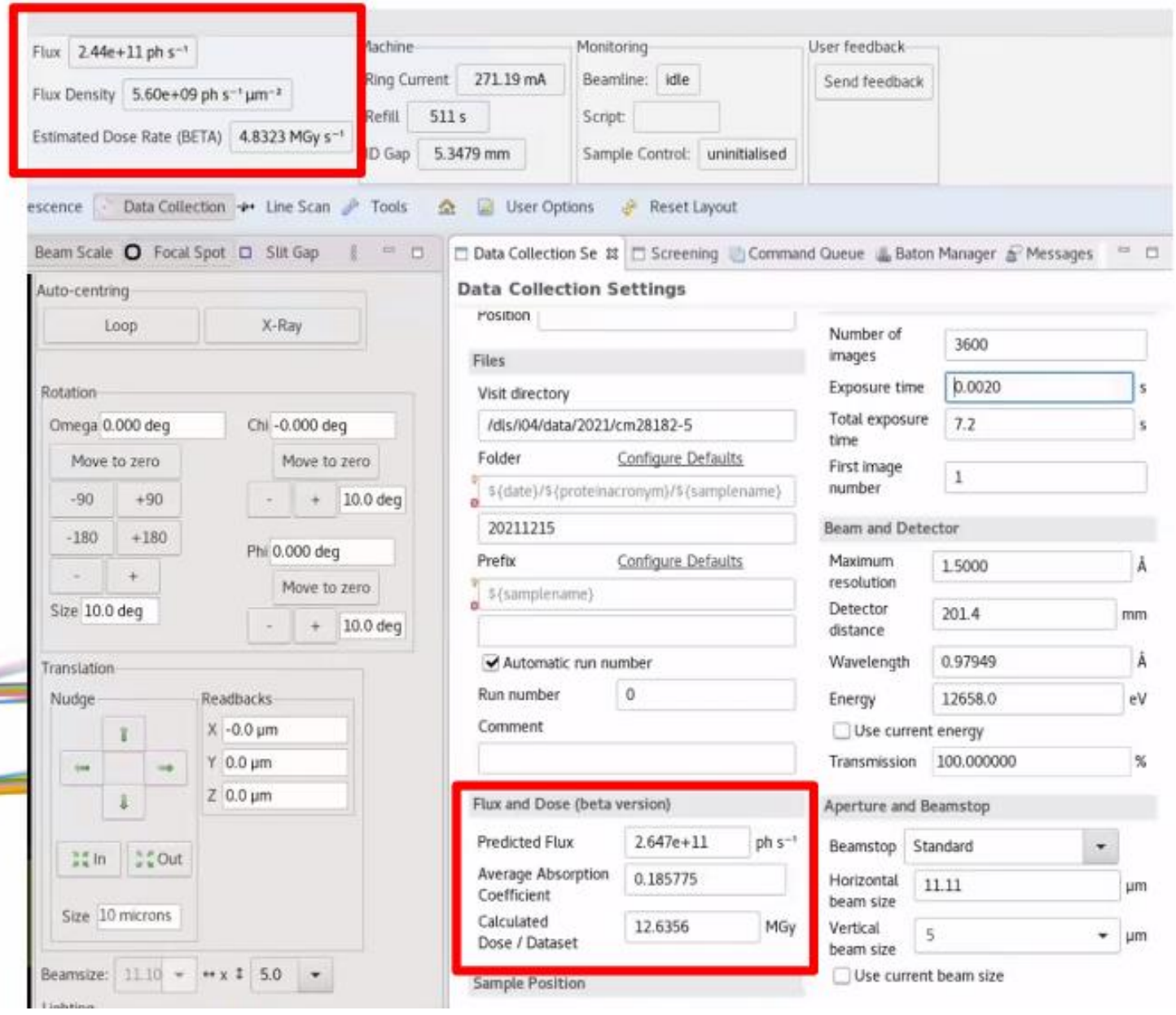
Know your dose



- Dose dependent on
  - Flux
  - Energy
  - Beam size/shape
  - Exposure time
  - Sample composition
  - Crystal size
- Raddose3D dose calculation implemented for interactive and UDC sessions
- Room temperature/serial is different
- Future work
  - Dial dose instead of exposure
  - Use user-provided sample information
  - GUI guidance/warnings
  - Roll-out to other beamlines

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What's the goal?

Aim	High priorities	Lower priority	Max Dose per dataset (e.g. 360 degrees) For a ~2A resolution dataset
Native data collection	High resolution (e.g < 1.8 A) Complete data	High redundancy	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

Unattended data collection (UDC)

When to use UDC	When not to use UDC
Single crystals	Split crystals, multiple lattices
Projects requiring data collections from many crystals e.g. • searching for highest resolution native • ligand binding studies Rapid feedback on a well characterised system from a small number of new crystals	Projects requiring manual input e.g. • Selecting best region from a crystal with pathologies • Line or wedged scans • Fluorescence scans
Standard SAD phasing experiments • Try this first if you have sufficient crystals	Phasing from limited number of crystals Complex multicrystal phasing experiments
Energy range: 7-18KeV	Energies outside this range are supported in remote access

- Rapid scheduling
- No user input required during beam time
- Optimised standardised data collection protocols
- All UDC recipies now fully implemented on I04

Experiment kind	Protocol	Samples/hr
Native	Prioritises completeness/resolution and collects 2 x 360° sweeps, 1st at chi=0 and 2nd at chi=30	20
Phasing	Two 360° sweeps are collected using different chi/phi values at your chosen energy, using a lower transmission to maximise anomalous multiplicity and completeness.	20
Ligand	Prioritises speed of data collection and collects a single sweep, 360° dataset	25
Stepped	Prioritises resolution and conducts a stepped transmission scan (see below)	10

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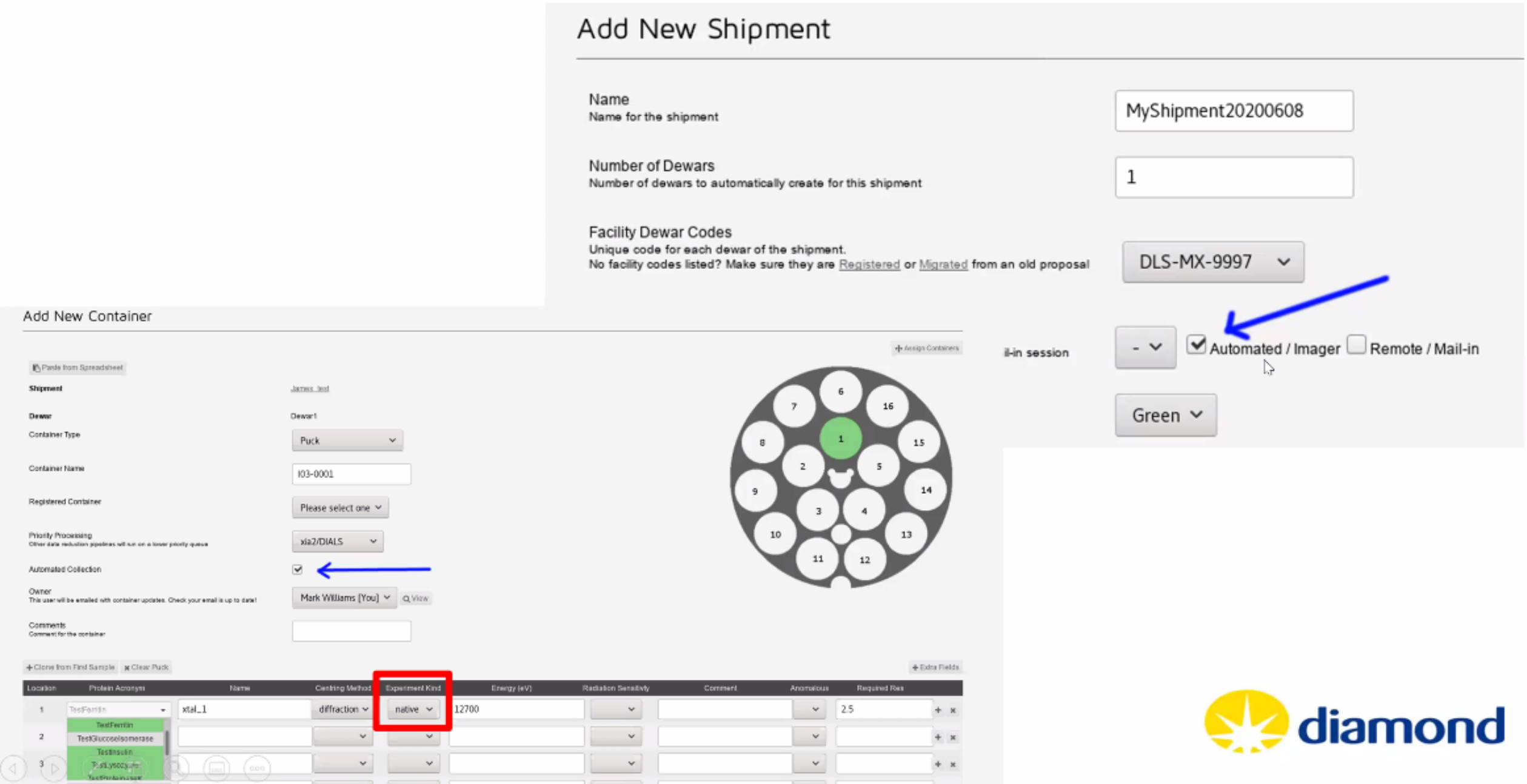
Unattended data collection (UDC)



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