

# Tools and capabilities of the variable focus beamline I04

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## I04 team



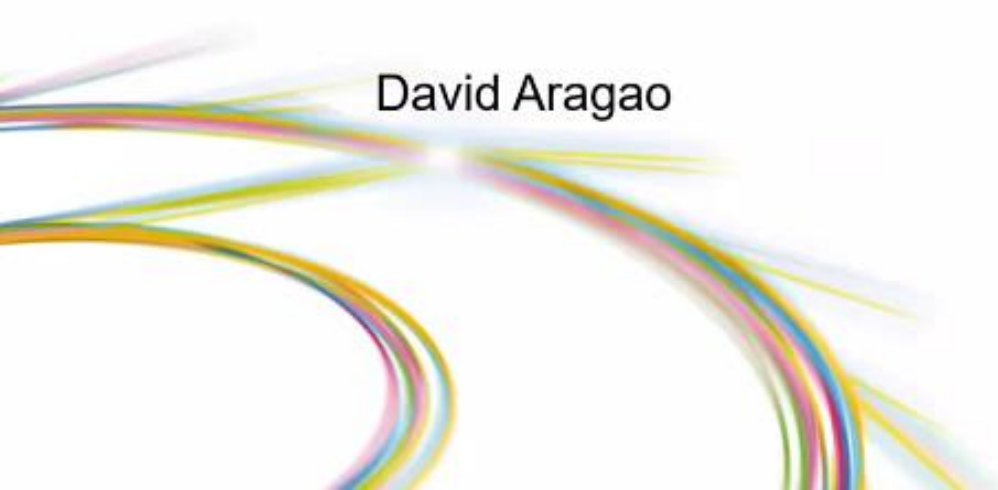
David Aragao



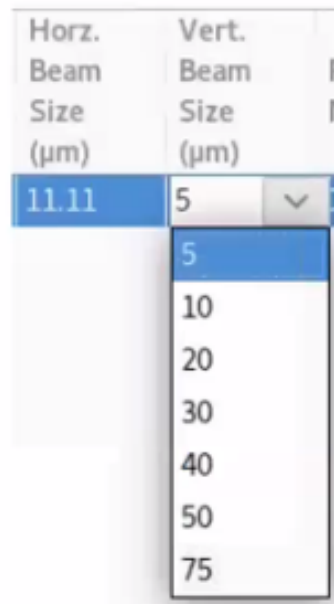
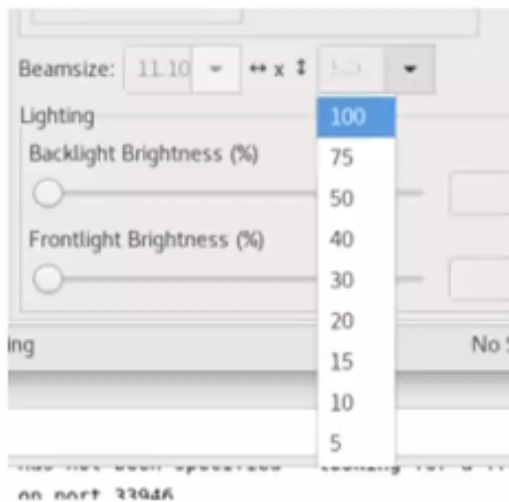
Pierpaolo Romano



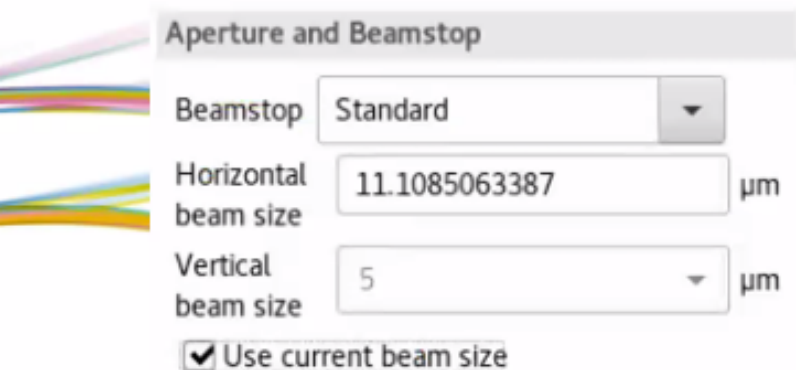
Marco Mazzorana



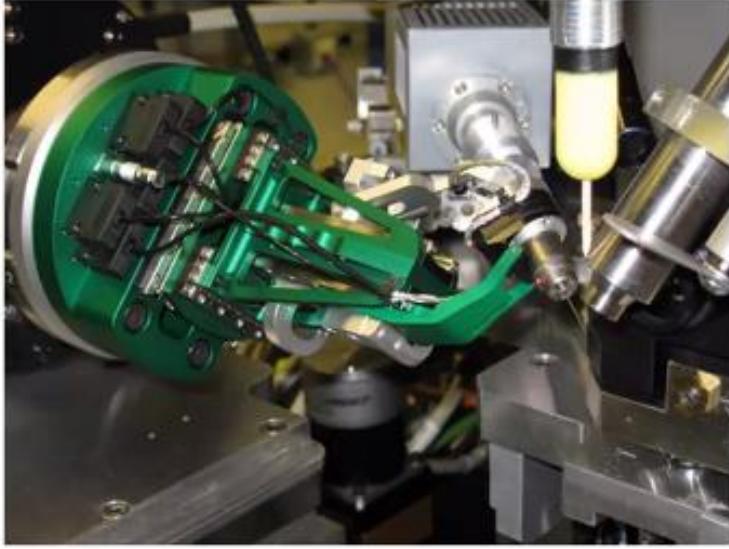
# Choose your beam size



- 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

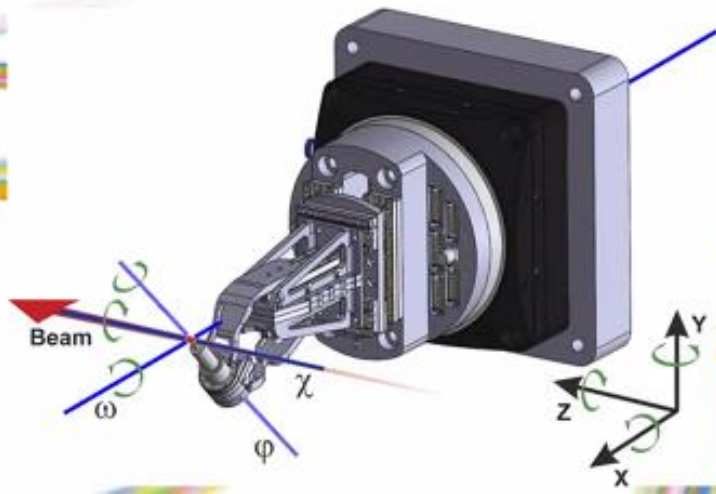


# SmarGon multi-axis goniometer

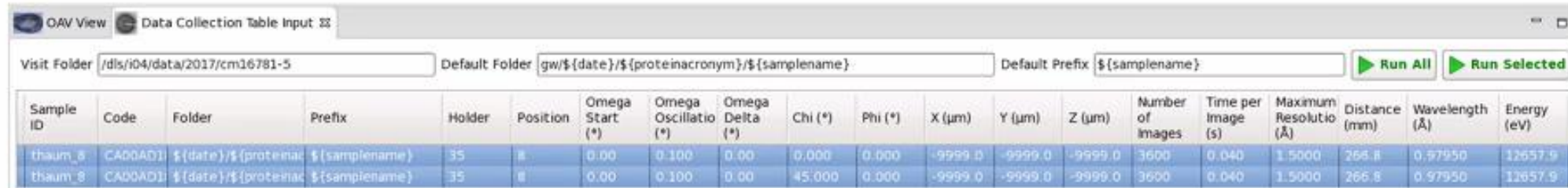


*104 performance achieved:  
SOC: omega:  $< 1 \mu\text{m}$ , chi  $< 3 \mu\text{m}$ , phi  $< 6 \mu\text{m}$*

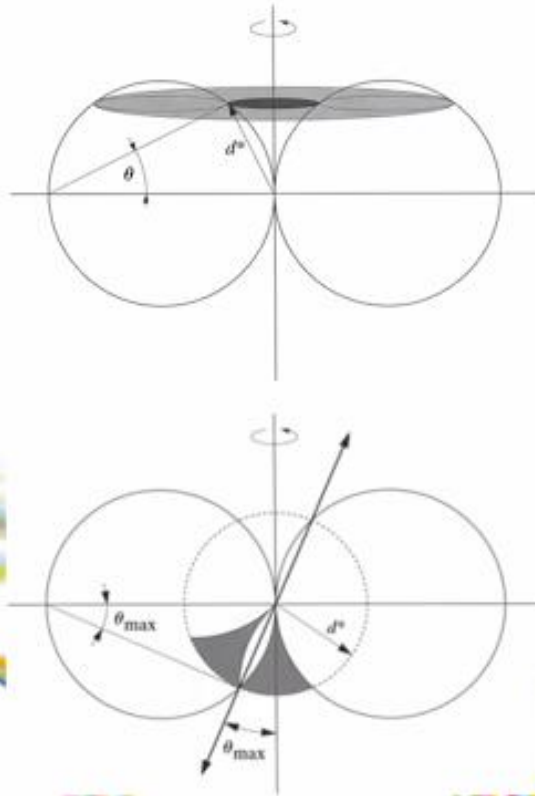
- crystal alignment/orientation
- get equivalent images from different crystals by reorienting them
- smart data collection strategies, better completeness of data
- reduced radiation damage by using smaller overall oscillation range
- help in point group determination
- comparing crystals in the same 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



# Complete data sets



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	CAD00AD1	\$(date)/\$(proteinac	\$(samplename)	35	II	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	CAD00AD1	\$(date)/\$(proteinac	\$(samplename)	35	II	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  $\theta_{\max}$  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

# 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

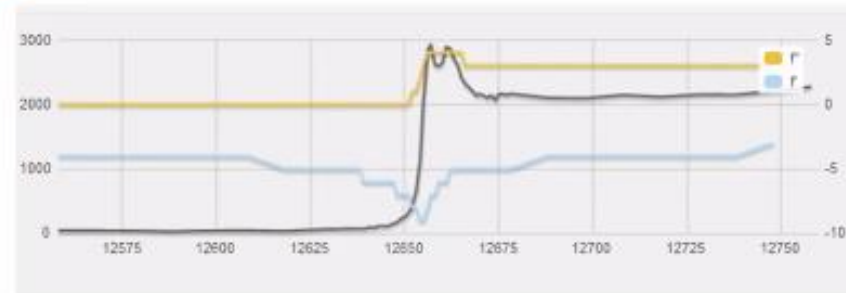
OAV View Data Collection Table Input

Visit Folder: /dls/i04/data/2018/cm19645-1 Default Folder: \${date}/\${proteinacronym}/\${samplename} Default Prefix: \${samplename} Run All Run Selected

Row Sel.	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_2				35	2	0.00	0.100	0.00	44.467	148.321	-9999.0	-9999.0	-9999.0	3600	0.040	1.5000	266.8	0.97950	12657.9
	thaum_2				35	2	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_2				35	2	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
	thaum_2				35	2	0.00	0.100	0.00	45.000	120.000	-9999.0	-9999.0	-9999.0	3600	0.040	1.5000	266.8	0.97950	12657.9
	thaum_2				35	2	0.00	0.100	0.00	45.000	240.000	-9999.0	-9999.0	-9999.0	3600	0.040	1.5000	266.8	0.97950	12657.9

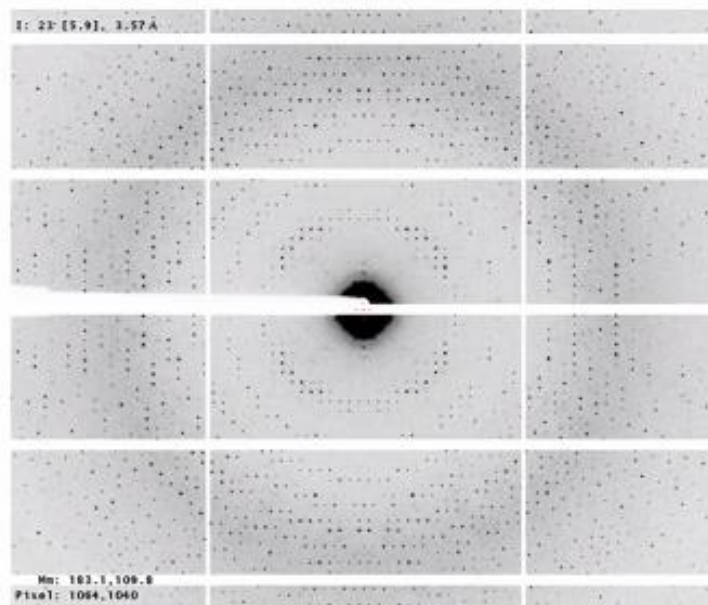
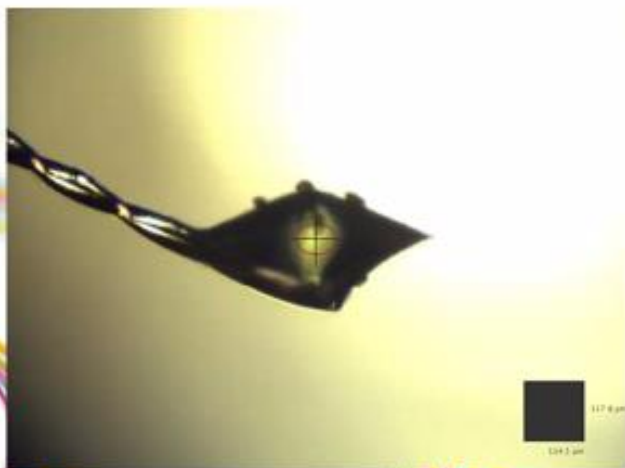
# MAD data collection

- 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: <i>Sst-Ithium_4</i>	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	



# Alignment from crystal screening

Strategies Mosflm: ✓ EDNA: ✓

Stepped transmission 1

Space Group	A	B	C	$\alpha$	$\beta$	$\gamma$
P4	78.77	78.77	36.88	90.00	90.00	90.00

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	$\alpha$	$\beta$	$\gamma$
P4	78.77	78.77	36.88	90.00	90.00	90.00

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	$\alpha$	$\beta$	$\gamma$
P4	78.78	78.78	36.95	90.00	90.00	90.00

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



# Know your dose

The screenshot displays the control software interface for a synchrotron beamline. A red box highlights the top-left corner, showing the following dose-related parameters:

- Flux:  $2.44e+11$  ph  $s^{-1}$
- Flux Density:  $5.60e+09$  ph  $s^{-1} \mu m^{-2}$
- Estimated Dose Rate (BETA):  $4.8323$  MGy  $s^{-1}$

The interface also shows various other settings and monitoring data:

- Machine: Ring Current: 271.19 mA, Beamline: idle, Refill: 511 s, D Gap: 5.3479 mm
- Monitoring: Script: [empty], Sample Control: uninitialised
- User feedback: Send feedback
- Data Collection Settings: Position, Files (Visit directory: /dls/i04/data/2021/cm28182-5, Folder: 20211215), Prefix, Run number: 0, Comment
- Beam and Detector: Maximum resolution: 1.5000 Å, Detector distance: 201.4 mm, Wavelength: 0.97949 Å, Energy: 12658.0 eV, Transmission: 100.000000 %
- Aperture and Beamstop: Beamstop: Standard, Horizontal beam size: 11.11  $\mu m$ , Vertical beam size: 5  $\mu m$
- Flux and Dose (beta version): Predicted Flux:  $2.647e+11$  ph  $s^{-1}$ , Average Absorption Coefficient: 0.185775, Calculated Dose / Dataset: 12.6356 MGy

- 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

# What's the goal?

<b>Aim</b>	<b>High priorities</b>	<b>Lower priority</b>	<b>Max Dose per dataset</b> (e.g. 360 degrees) For a ~2Å resolution dataset
Native data collection	High resolution (e.g < 1.8 Å) 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 Å), 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. <ul style="list-style-type: none"> <li>• searching for highest resolution native</li> <li>• ligand binding studies</li> </ul> Rapid feedback on a well characterised system from a small number of new crystals	Projects requiring manual input e.g. <ul style="list-style-type: none"> <li>• Selecting best region from a crystal with pathologies</li> <li>• Line or wedged scans</li> <li>• Fluorescence scans</li> </ul>
Standard SAD phasing experiments <ul style="list-style-type: none"> <li>• Try this first if you have sufficient crystals</li> </ul>	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 recipes 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

# Unattended data collection (UDC)

## Add New Shipment

Name

Name for the shipment

MyShipment20200608

Number of Dewars

Number of dewars to automatically create for this shipment

1

Facility Dewar Codes

Unique code for each dewar of the shipment.

No facility codes listed? Make sure they are [Registered](#) or [Migrated](#) from an old proposal

DLS-MX-9997

in session

-



Automated / Imager



Remote / Mail-in

Green

## Add New Container

Paste from Spreadsheet

Shipment

James\_Test

Dewar

Dewar1

Container Type

Puck

Container Name

I03-0001

Registered Container

Please select one

Priority Processing

Other data reduction pipelines will run on a lower priority queue

xia2/DIALS

Automated Collection



Owner

This user will be emailed with container updates. Check your email is up to date!

Mark Williams [You]

View

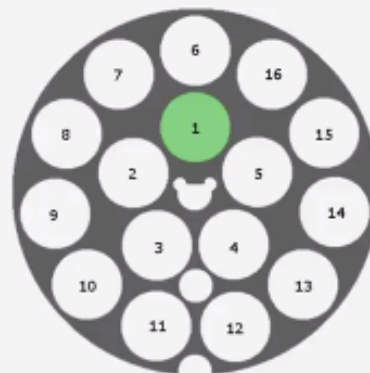
Comments

Comment for the container

Clone from Find Sample

Clear Puck

Assign Containers



Location	Protein Acronym	Name	Centering Method	Experiment Kind	Energy (eV)	Reduction Sensitivity	Comment	Anomalous	Required Files
1	TestFerritin	xia1_1	diffraction	native	12700				2.5
2	TestGlucosyltransferase								
3	TestInsulin								