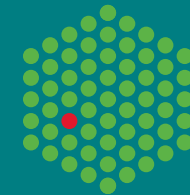


MASSIF1: fully automatic sample characterisation and data collection

Matthew W. Bowler and Didier Nurizzo

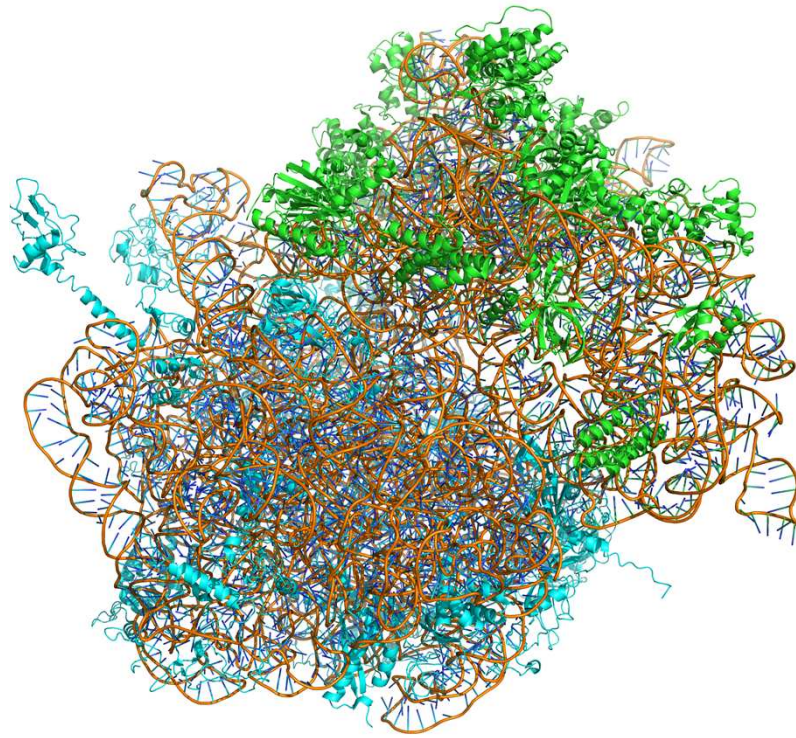


EMBL



Outline

- A short history of MASSIF
- Core technology 1: beams
- Core technology 2: robots
- Core technology 3: workflows
- The beamline so far...
- The future



Around 800 crystals
screened on ID14-1, 2 or 3

Data sets collected on ID14-4

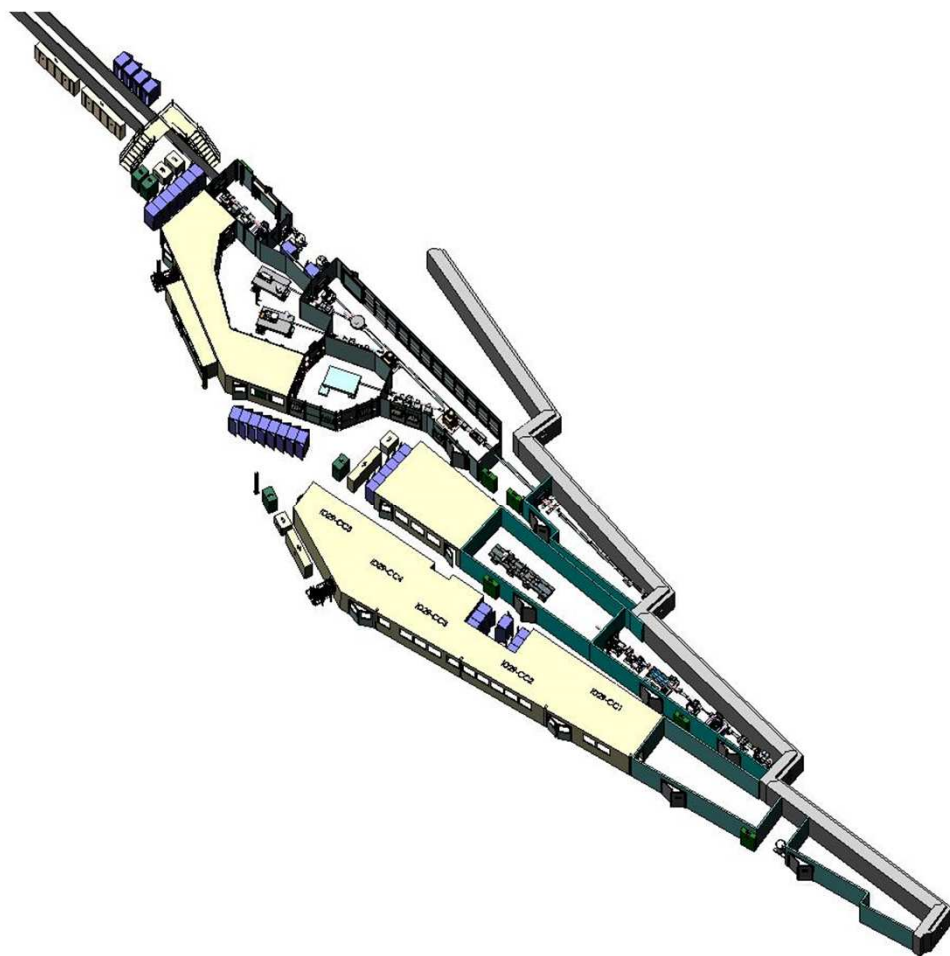


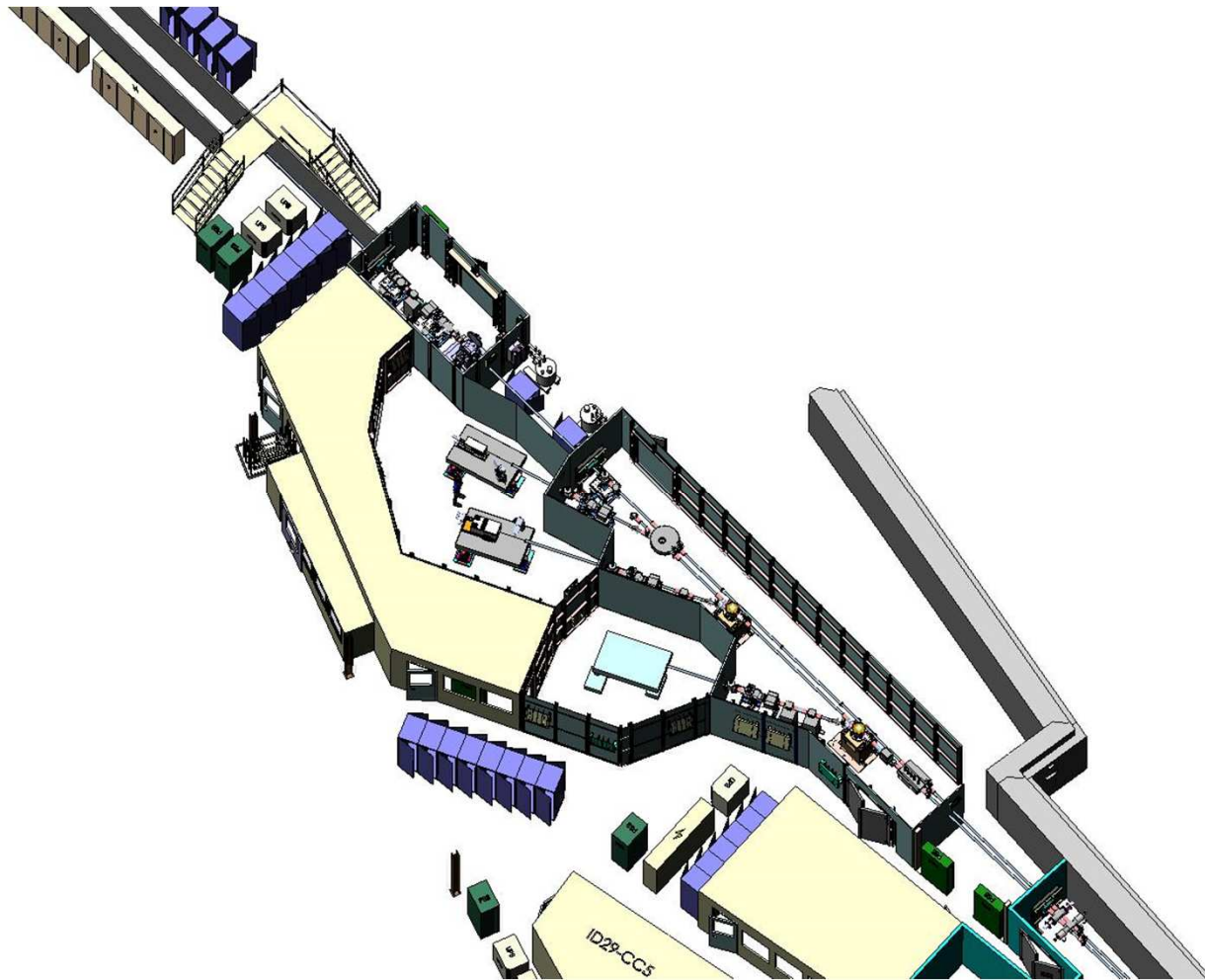
MASSIF

- Replacement for ID14
- 2 *independent* branches
- 2 beamlines to replace 14-1 and 2 – “large beam” with increased flux and stability
- Automation of sample screening
- High capacity sample changer
- 3rd beamline to be microfocus

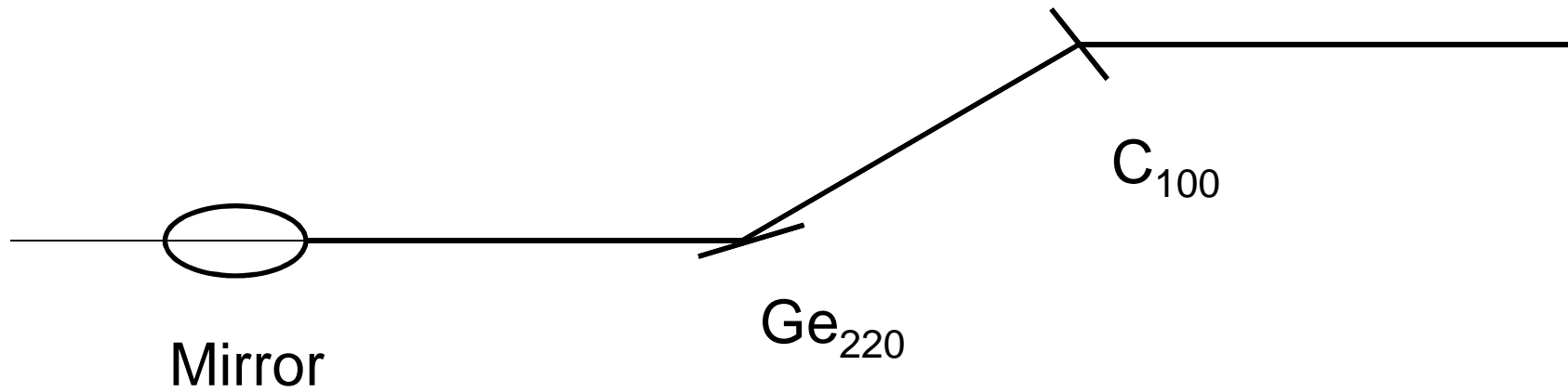
Core technology 1

Beams

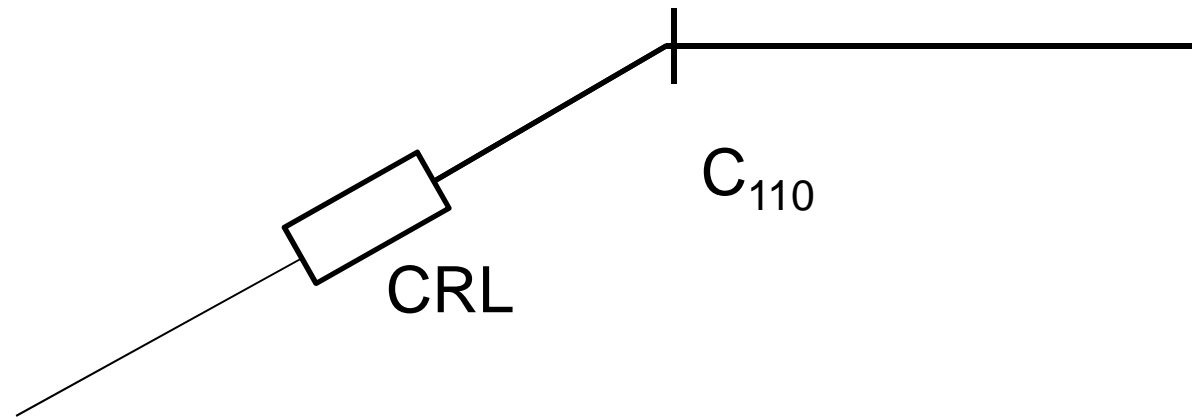




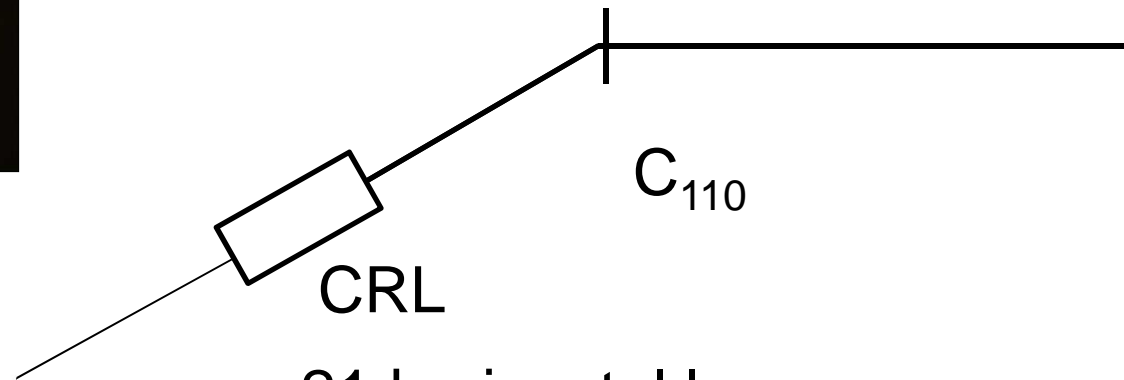
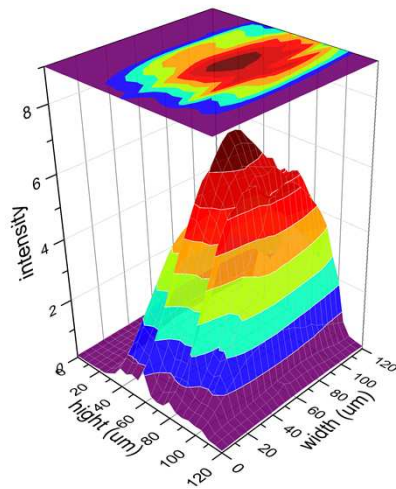
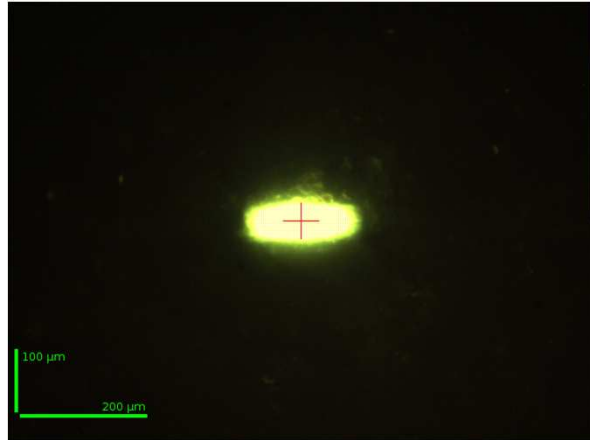
MASSIF1 v 14-2



MASSIF1 v 14-2

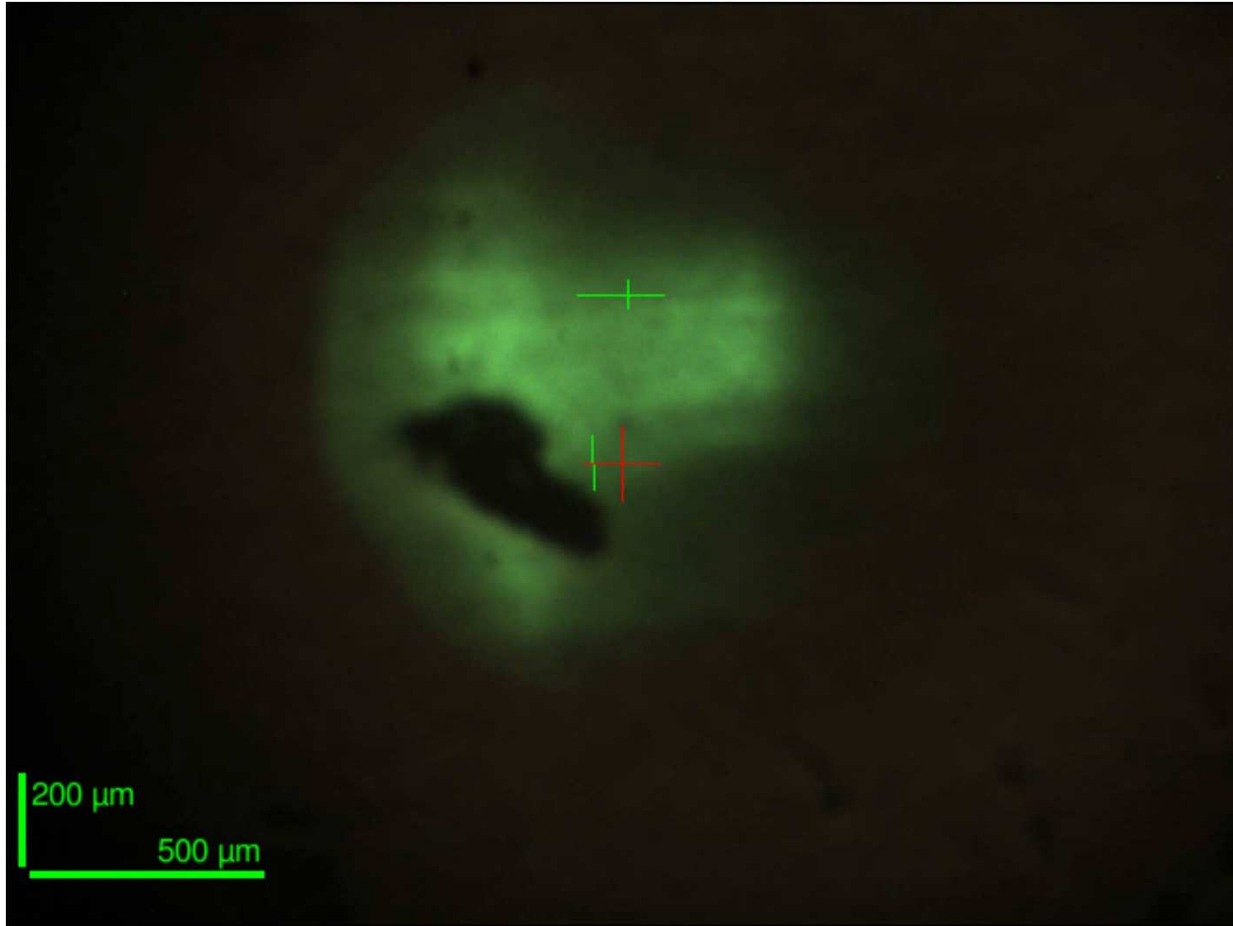


MASSIF1



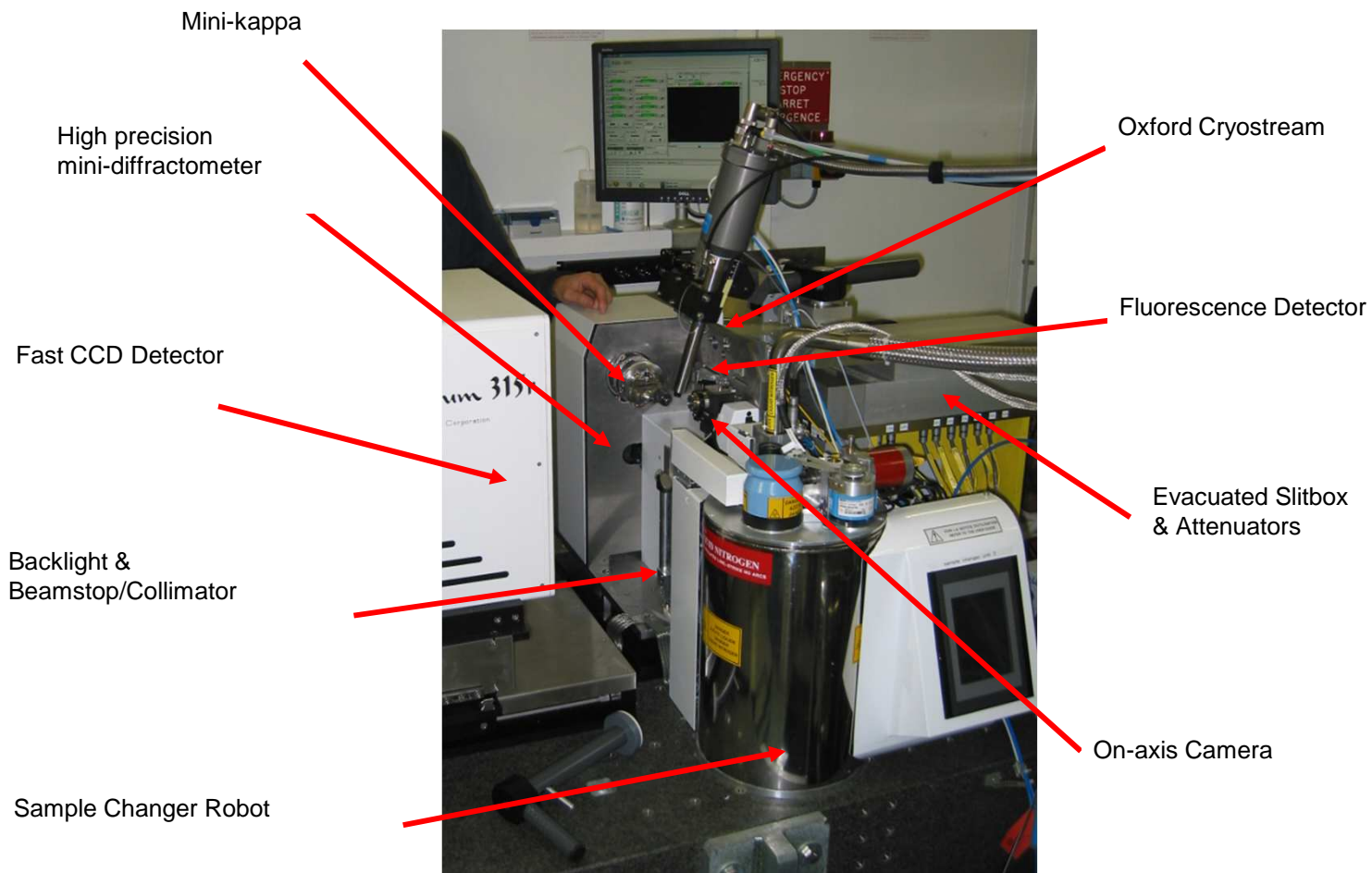
CRL
21 horizontal lenses
7 vertical lenses

Size: 100 x 65 μm^2 FWHM
Actual flux: 3.1×10^{12} ph/s



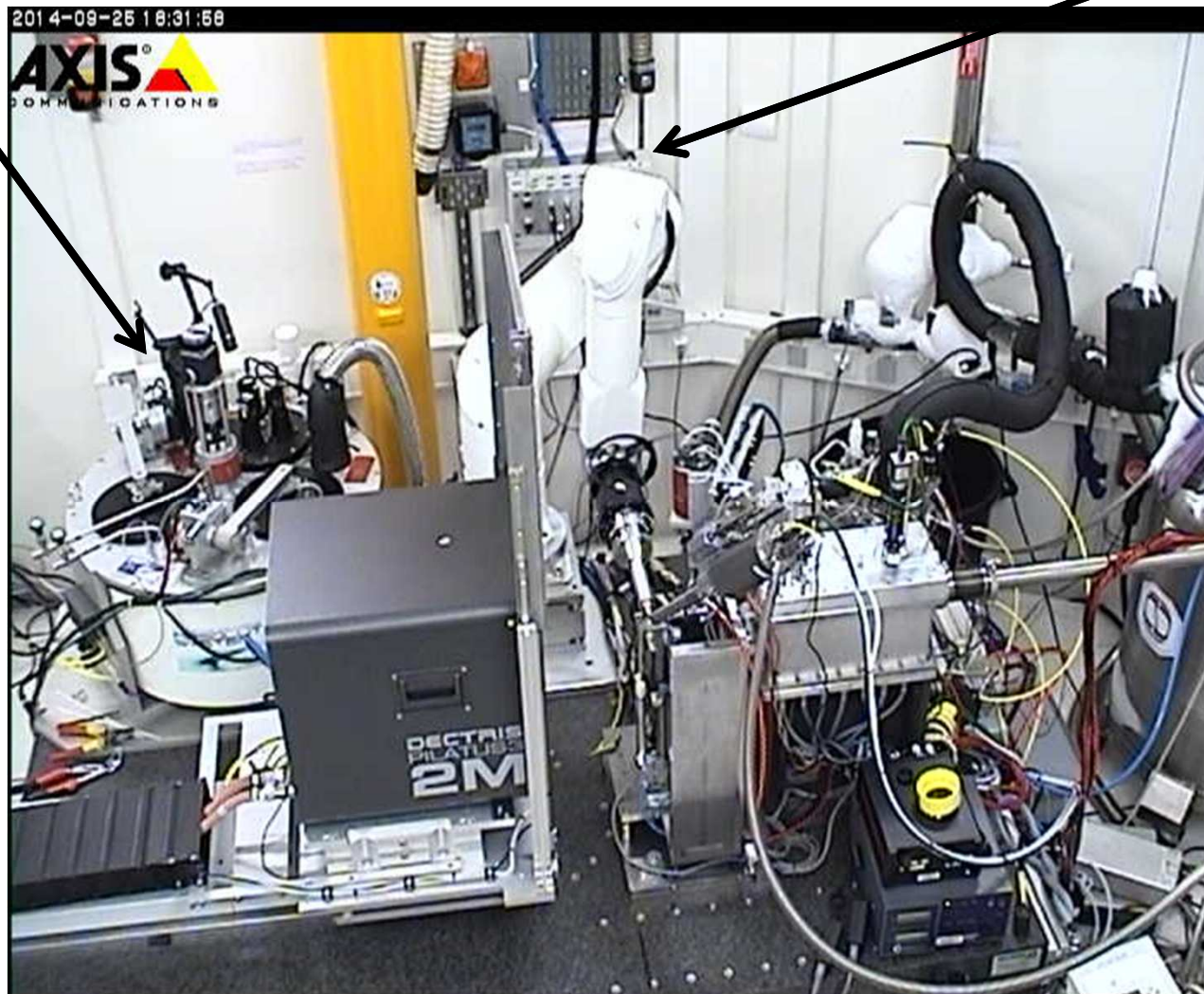
Core technology 2

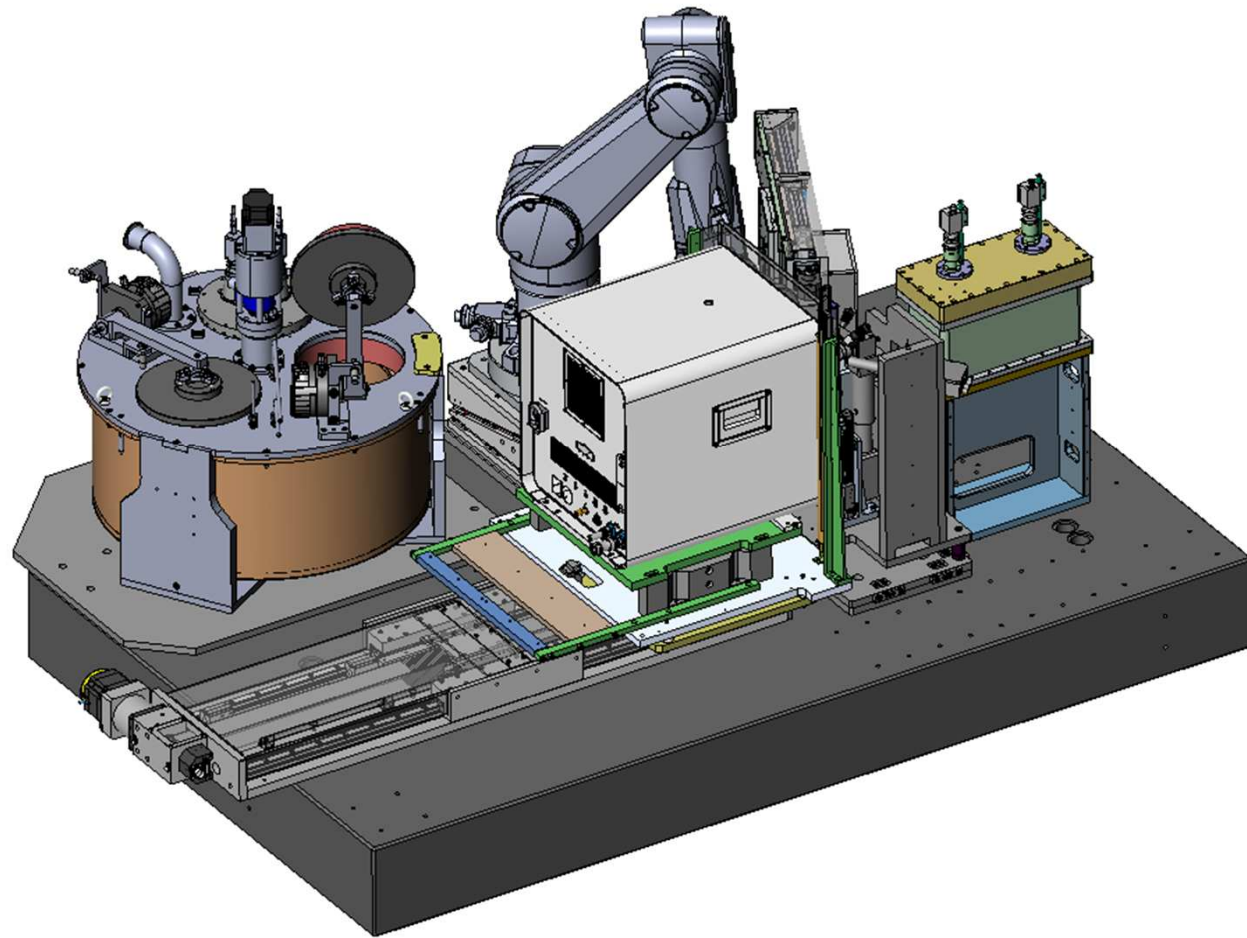
Robots

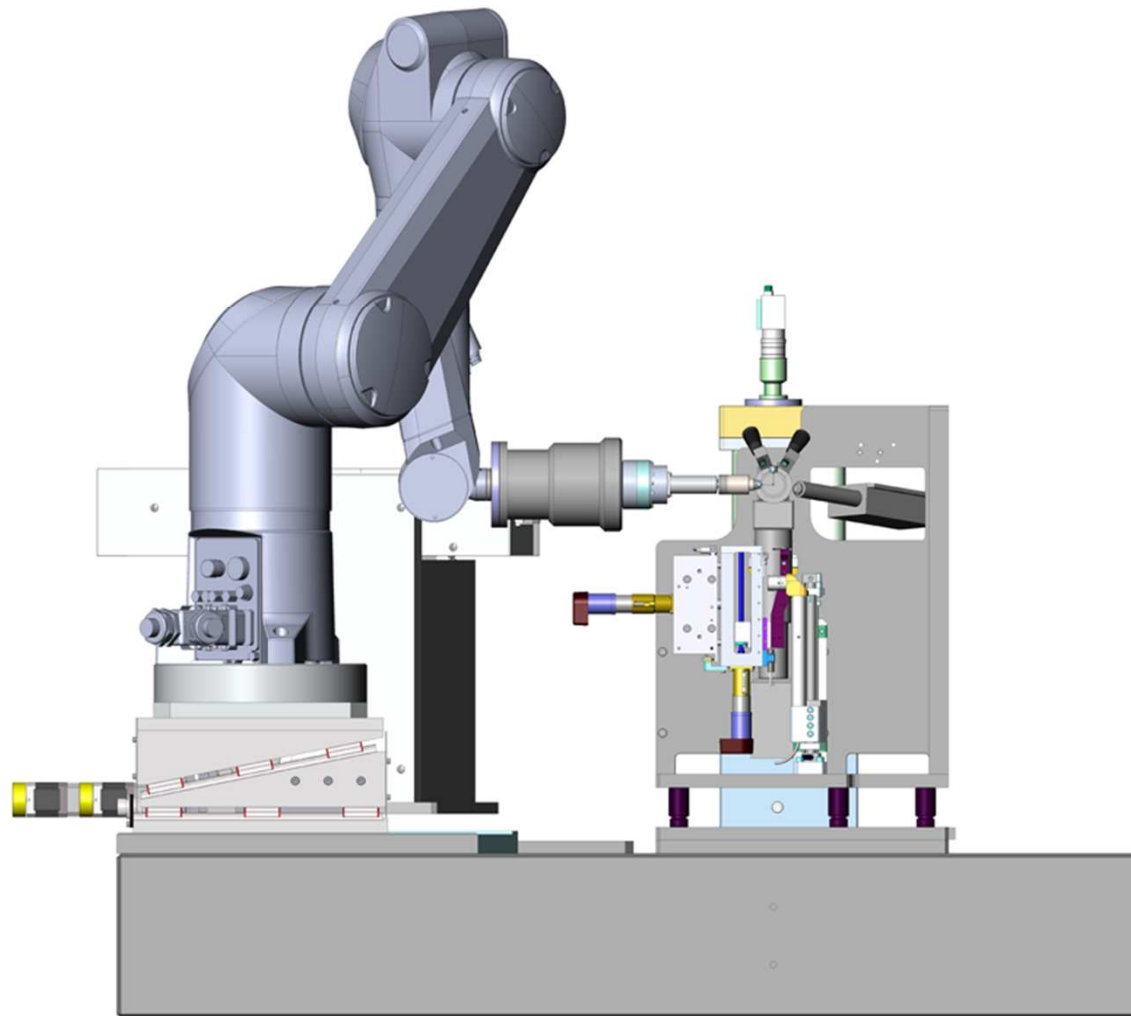


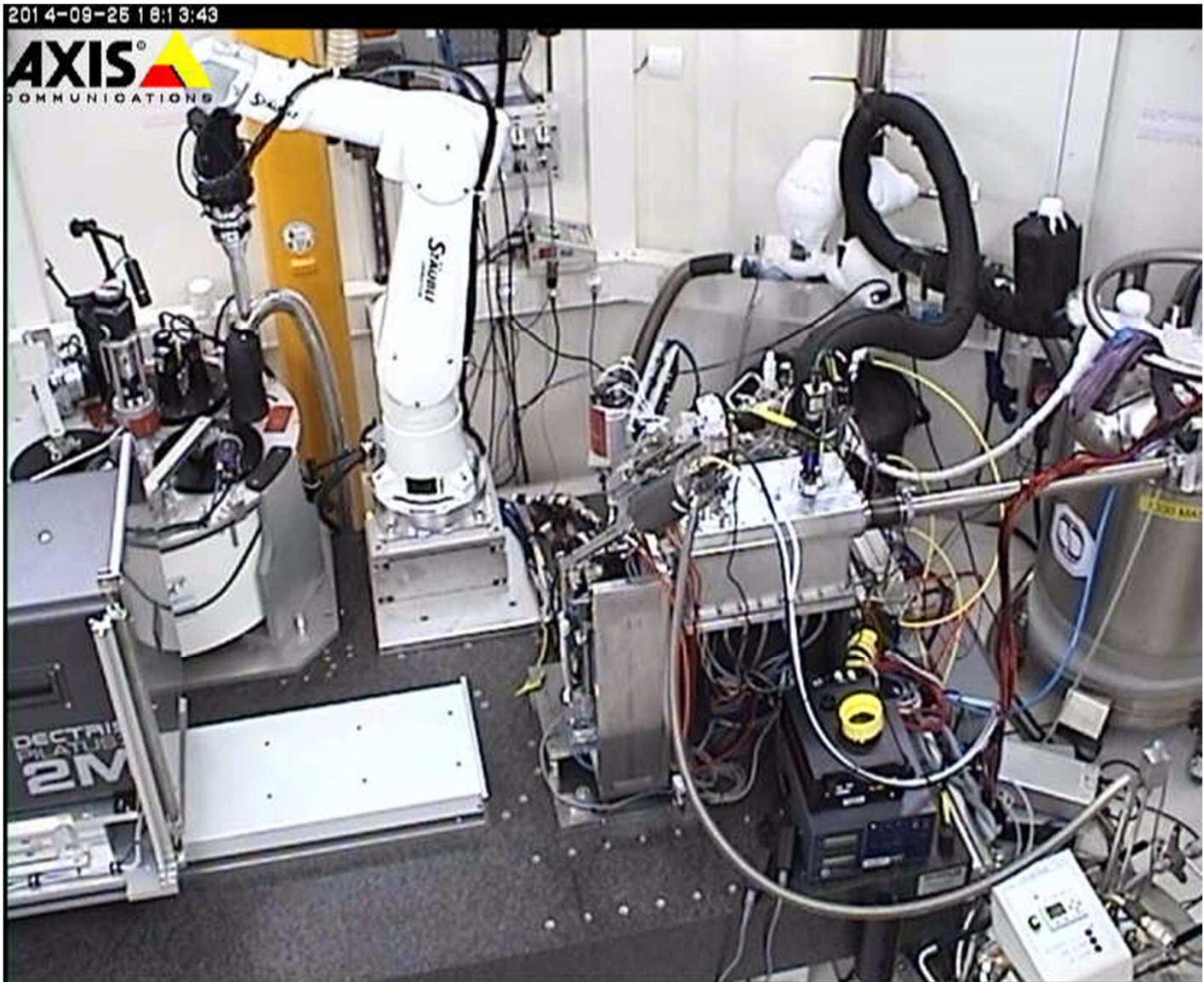
HCD dewar
240 sample
capacity

RoboDiff



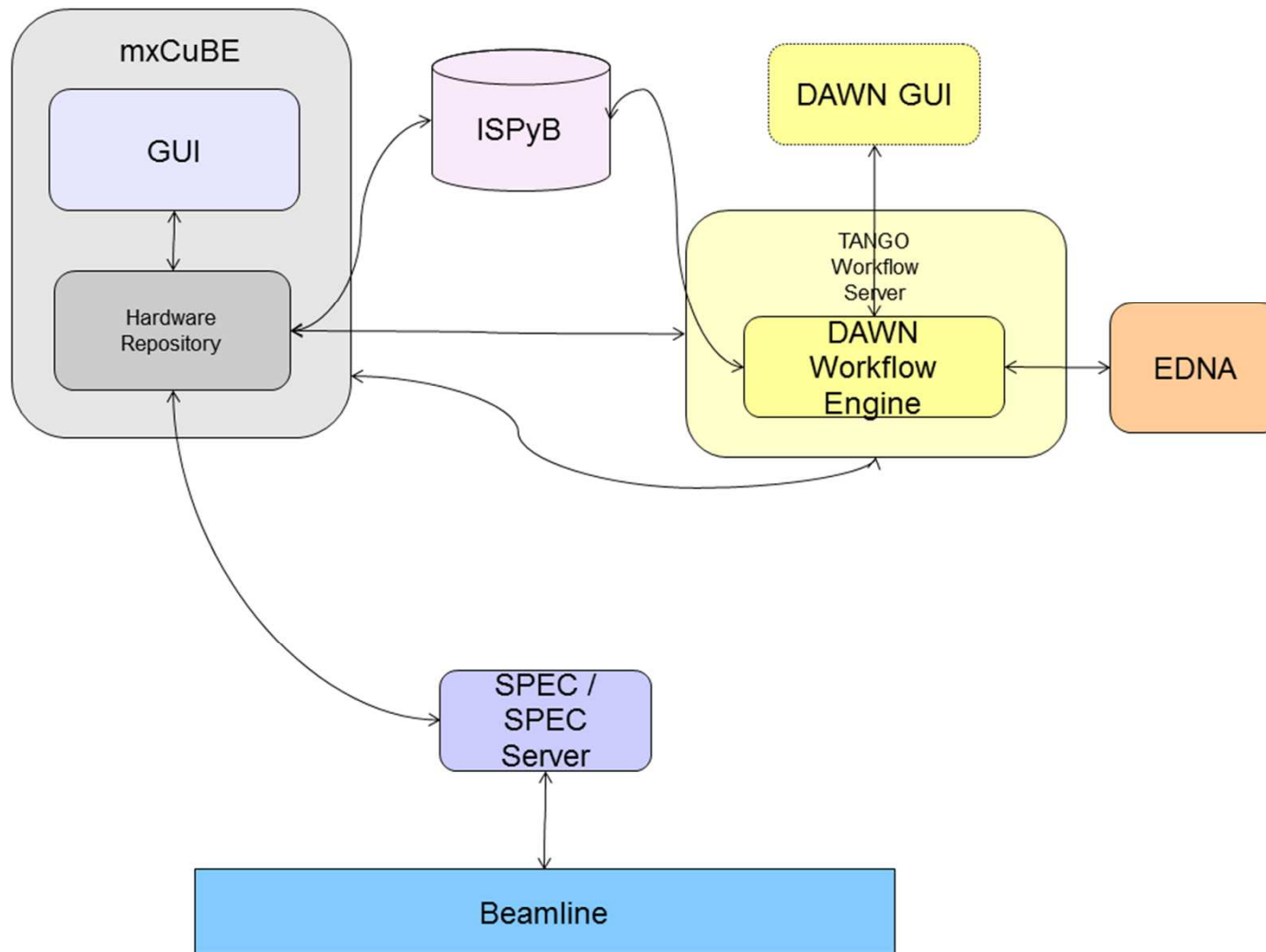


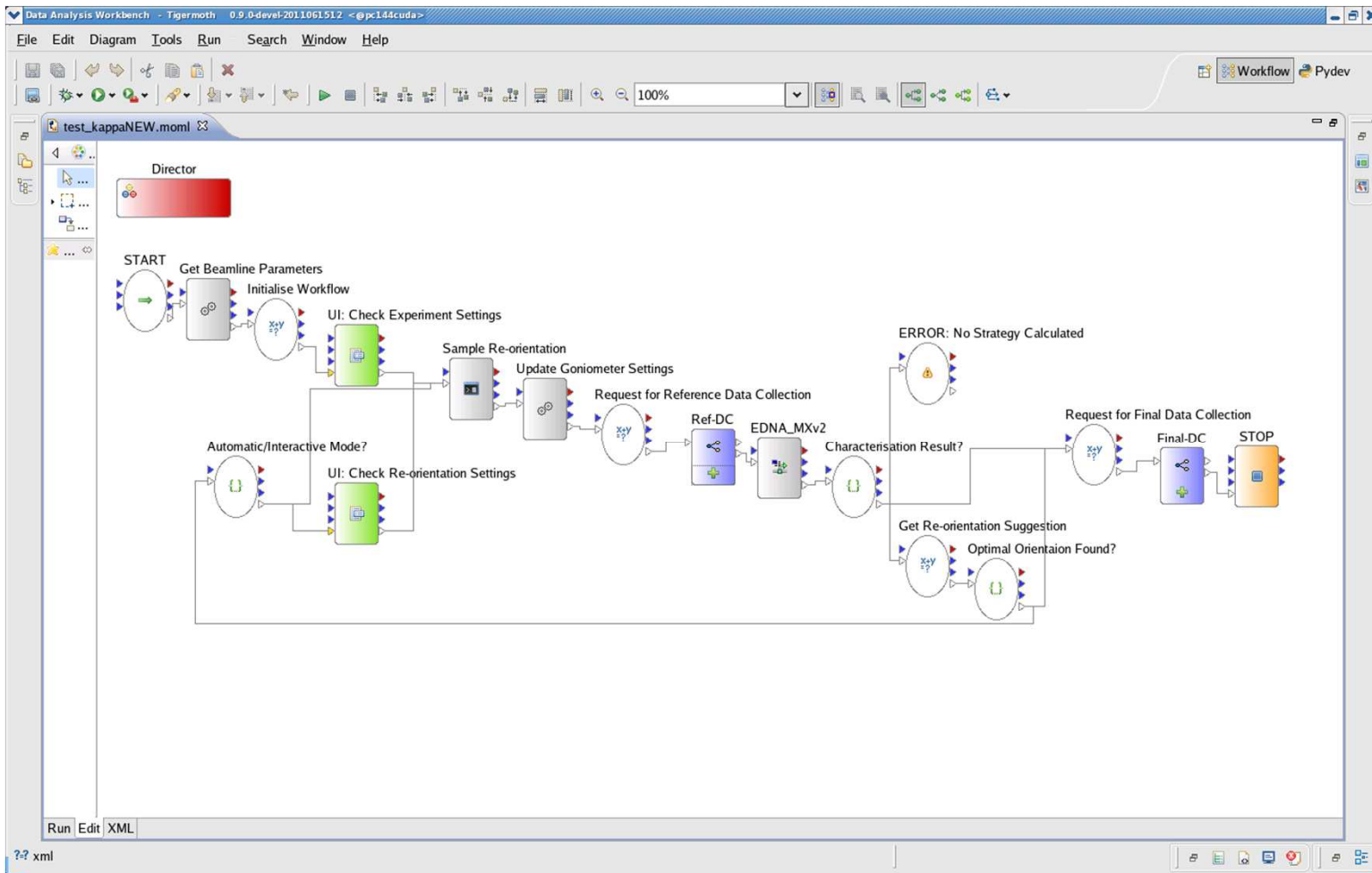




Core technology 3

Workflows





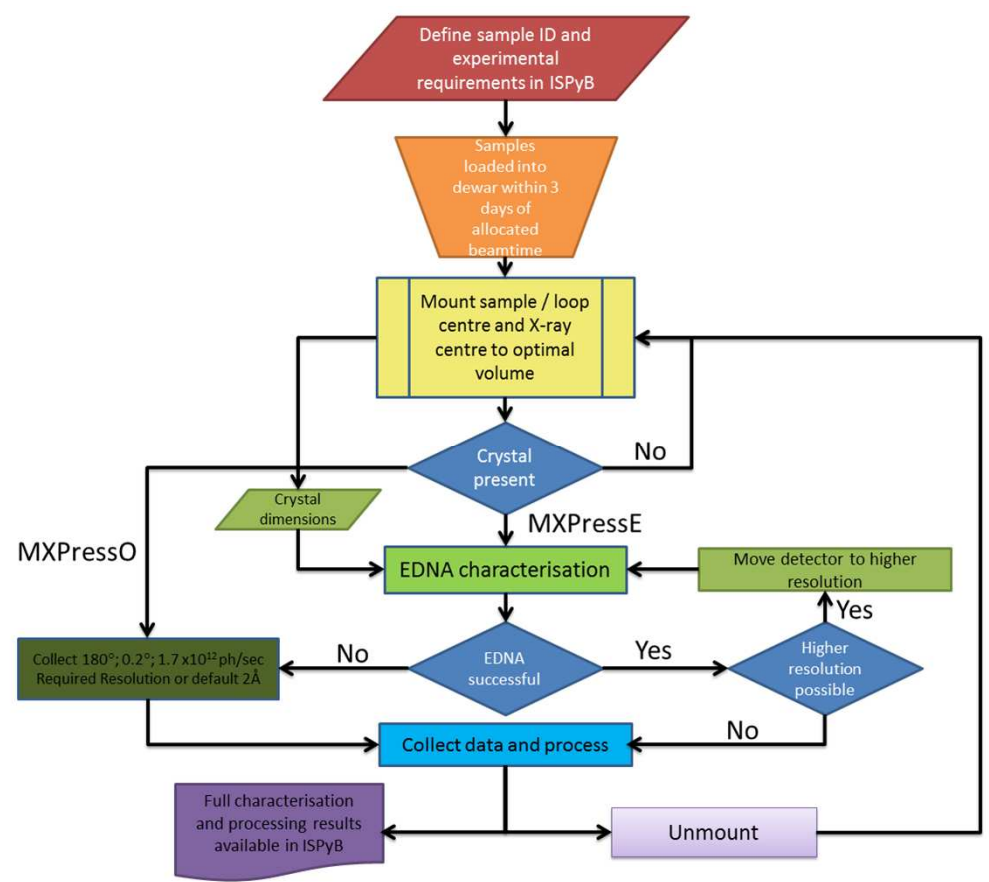
Dehydration workflow in MXCuBE

The screenshot displays the MXCuBE software interface for the mx-415 system. The main window is titled "mxCuBE (mx-415)" and includes a menu bar (File, Instrumentation, Help) and a toolbar (Collect, XRF spectrum, System, Feedback, Chat). The interface is divided into several panels:

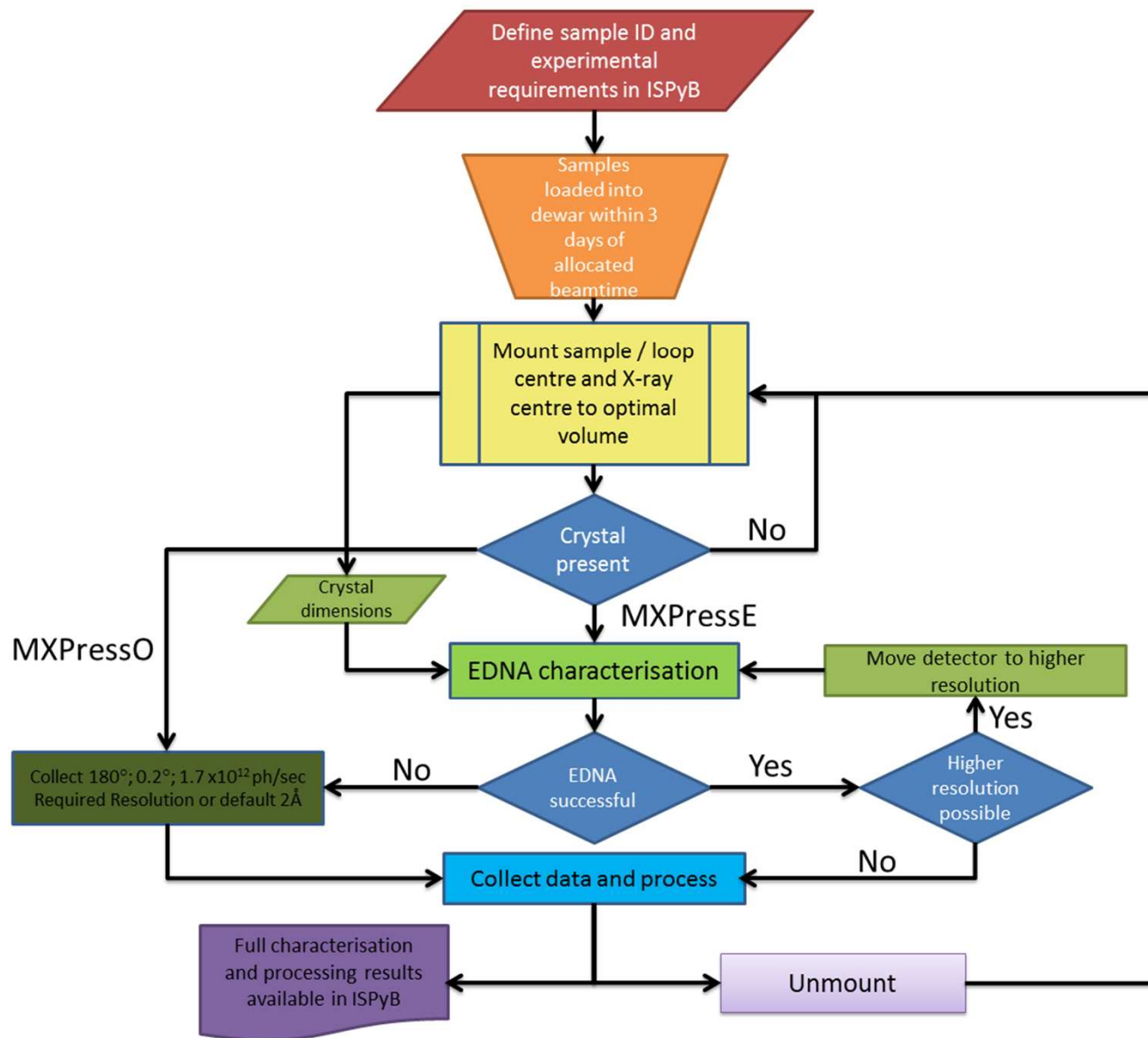
- User:** Shows the user "mx-415" and a "Logout" button.
- Sample list:** A list of sample IDs from 1:1 to 4:9, with 1:1 selected.
- Sample centring:** Contains controls for "Sample position" (Omega: 91.74, Kappa: 0.0, Phi: 0.0, Holder length: 21.852) and "Sample video" (Light: 0.0, Focus: 0.0, Front light: 3.0, Zoom: 1.0). A central video window shows a sample with a 200 µm and 500 µm scale bar.
- Collection method:** A panel with a red circle around the "Advanced" and "Workflow type" options. The "Workflow type" dropdown is open, showing options like "Burn Strategy", "Enhanced Characterisation", "Kappa Re-orientation", "Visual Re-orientation", "Line Scan", "Mesh Scan", "X-ray Centring", "Dehydration", and "Trouble shooting".
- Machine current:** Shows "192.7 mA" and "uniform multibunch".
- Safety shutter:** Status "closed".
- Fast shutter:** Status "closed".
- Beamstop:** Status "in".
- Current users:** Shows "My name: artemis2".

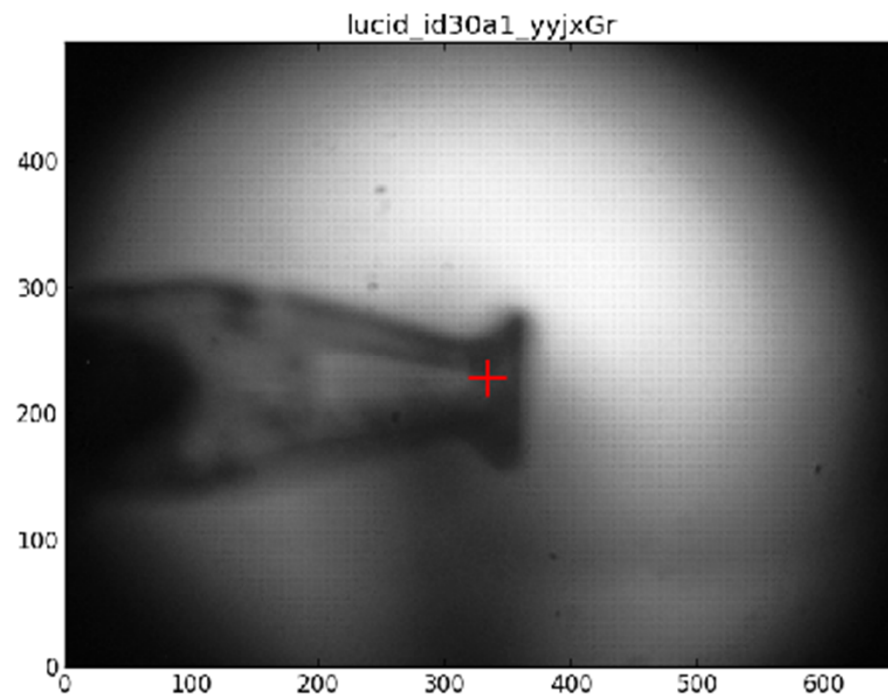
The bottom of the window shows a taskbar with the system tray, including the time "14:08" and the user name "artemis2".

Workflows link hardware and data analysis to collect optimal data faster than users allowing beamtime to be spent efficiently on more challenging stages of projects

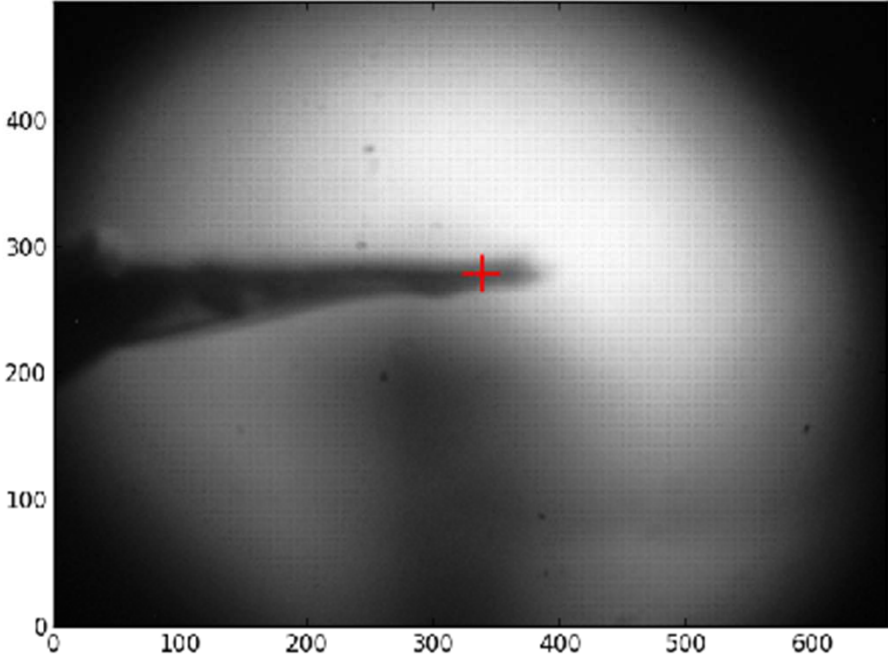


The MXPressE service

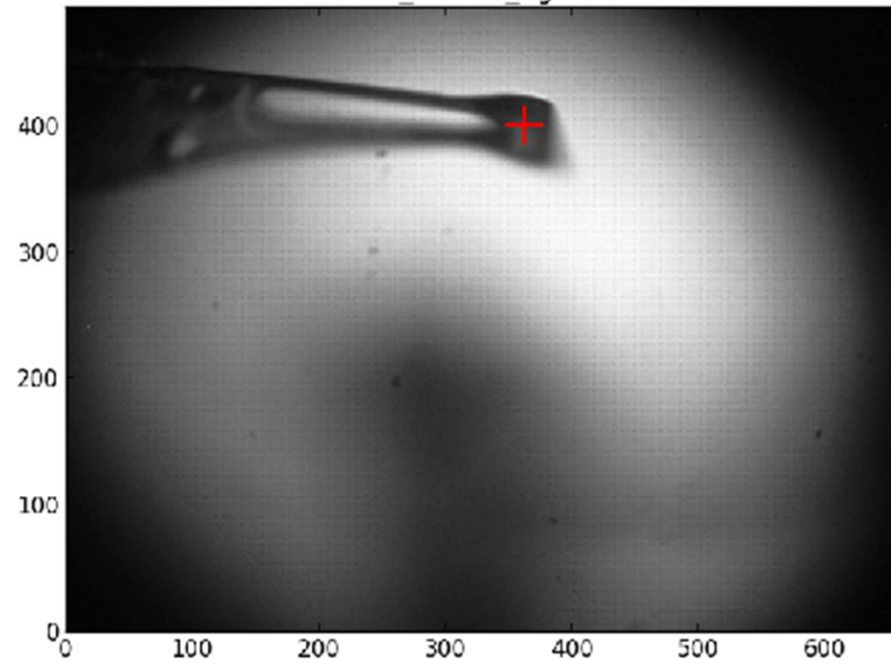


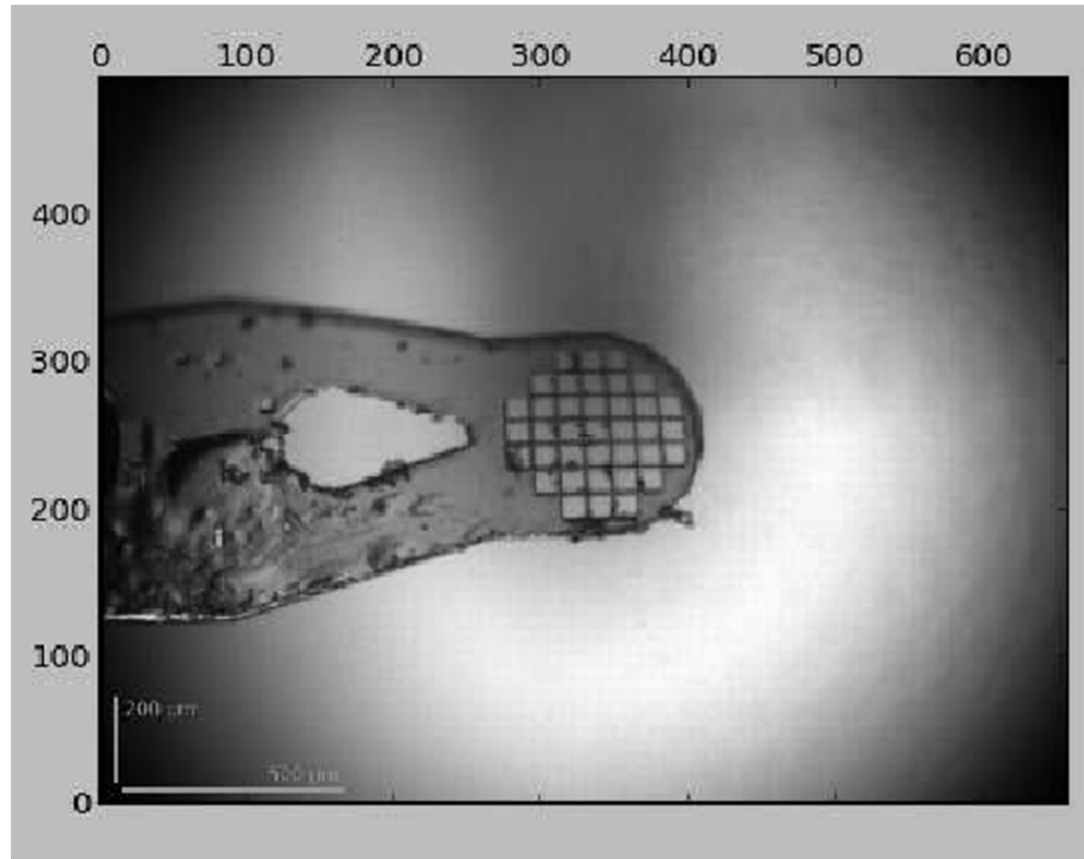


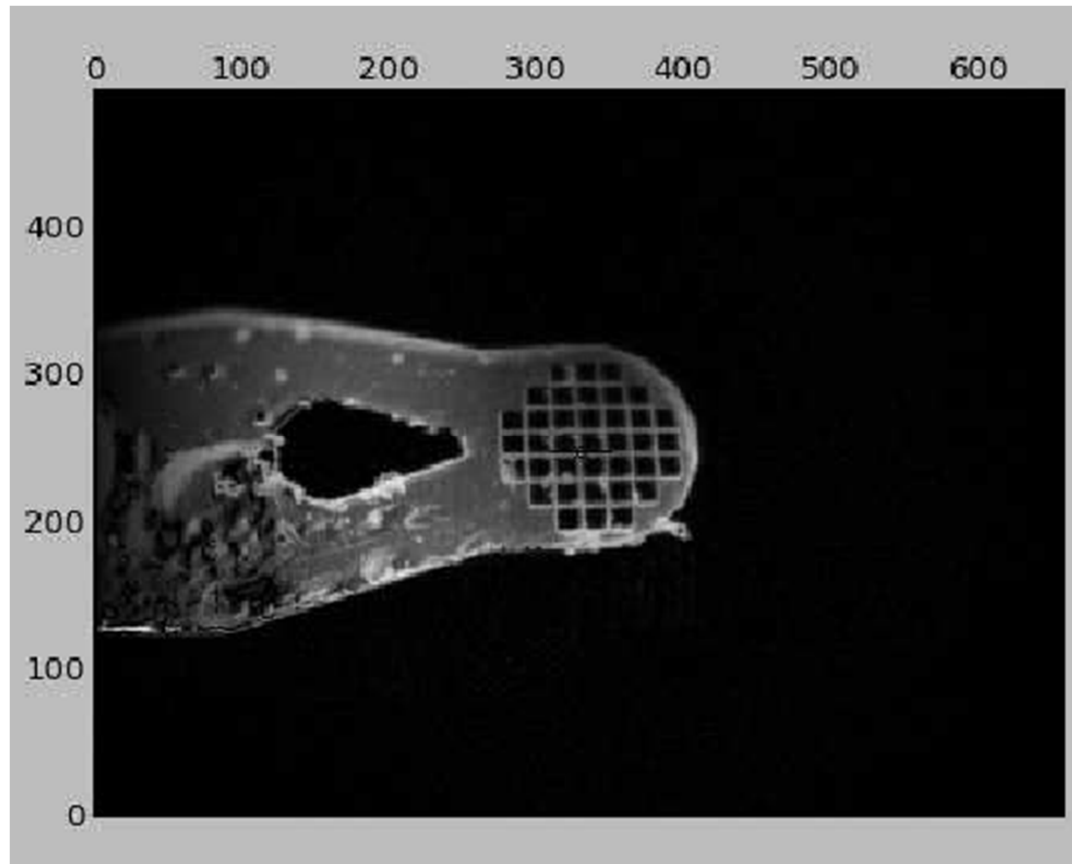
lucid id30a1 tXFbVi

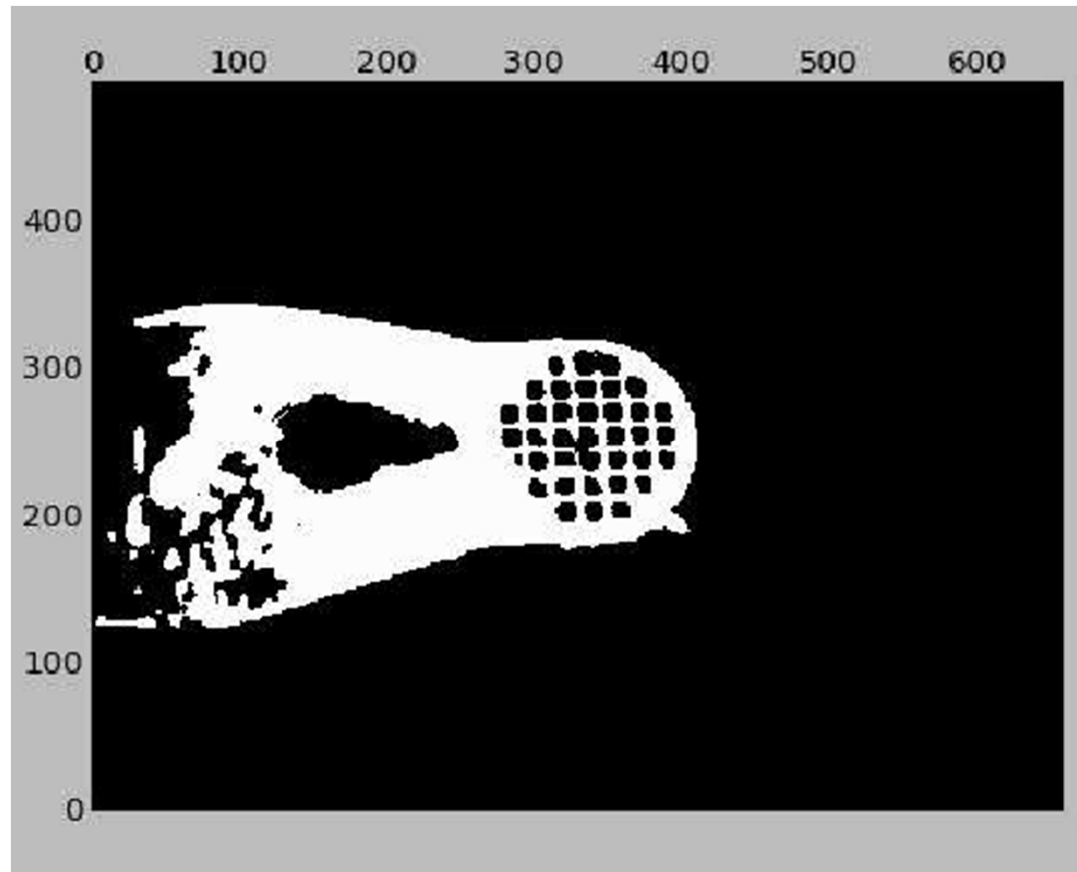


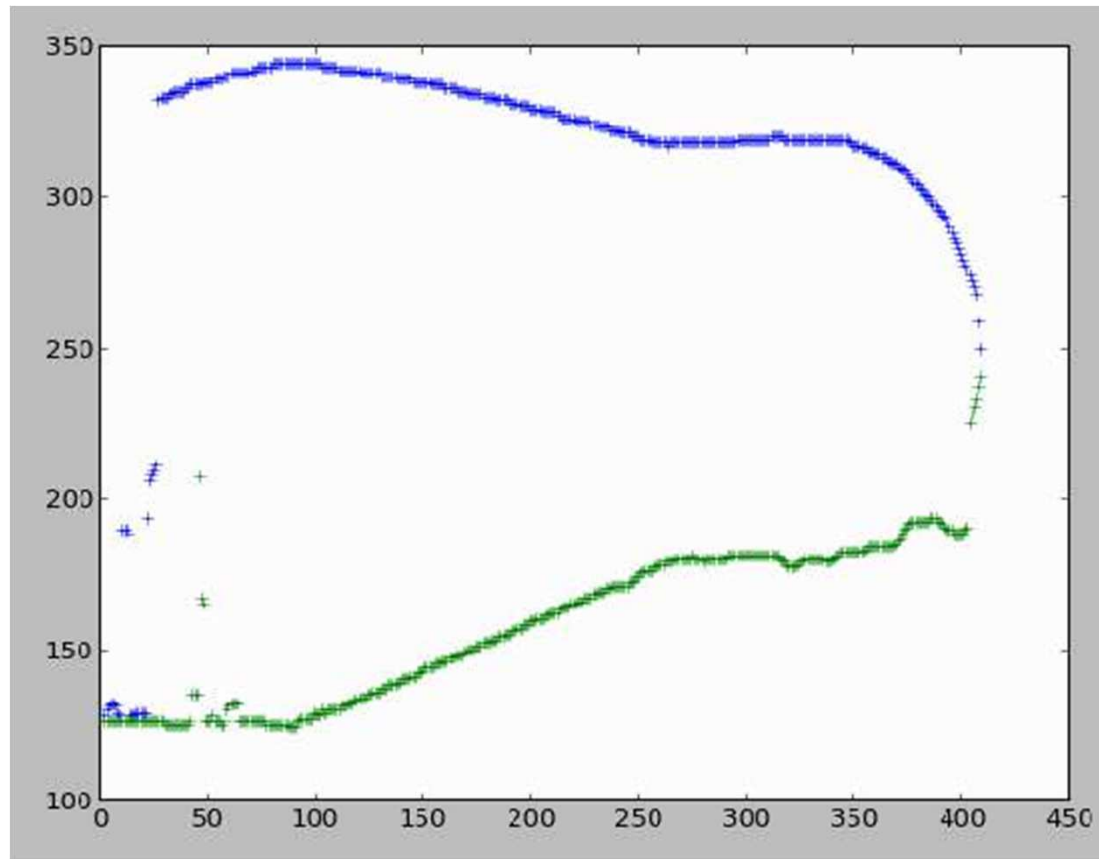
lucid_id30a1_5gW3rX

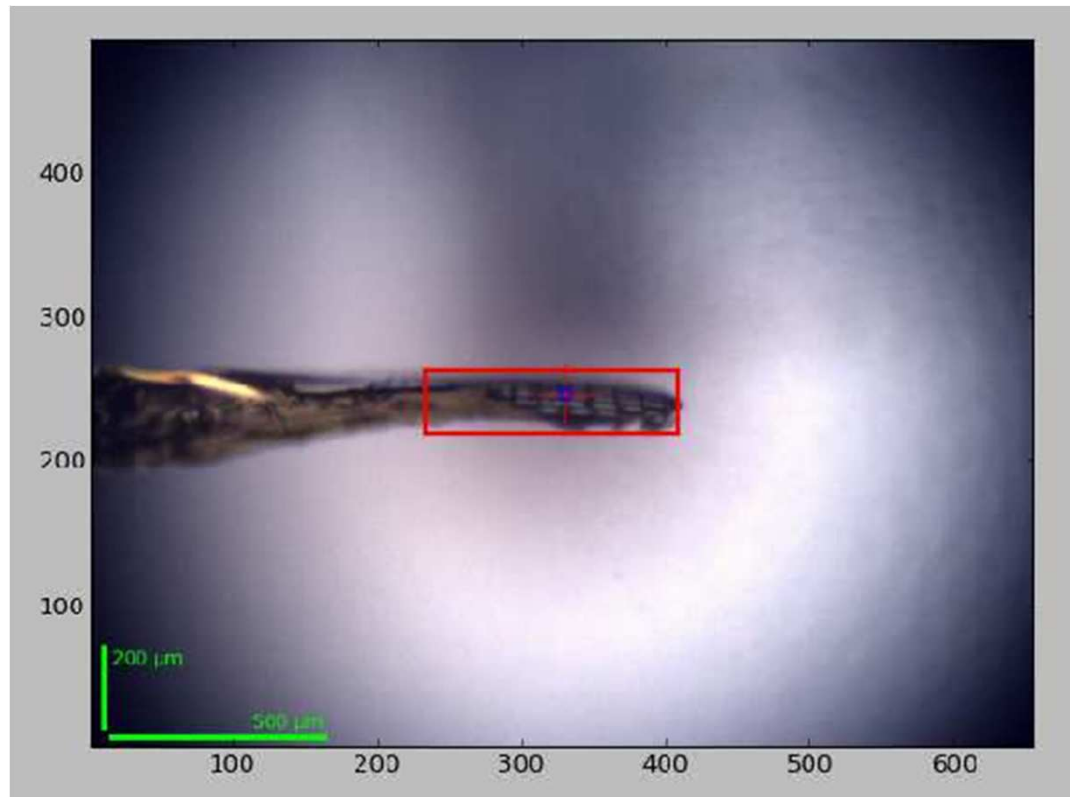


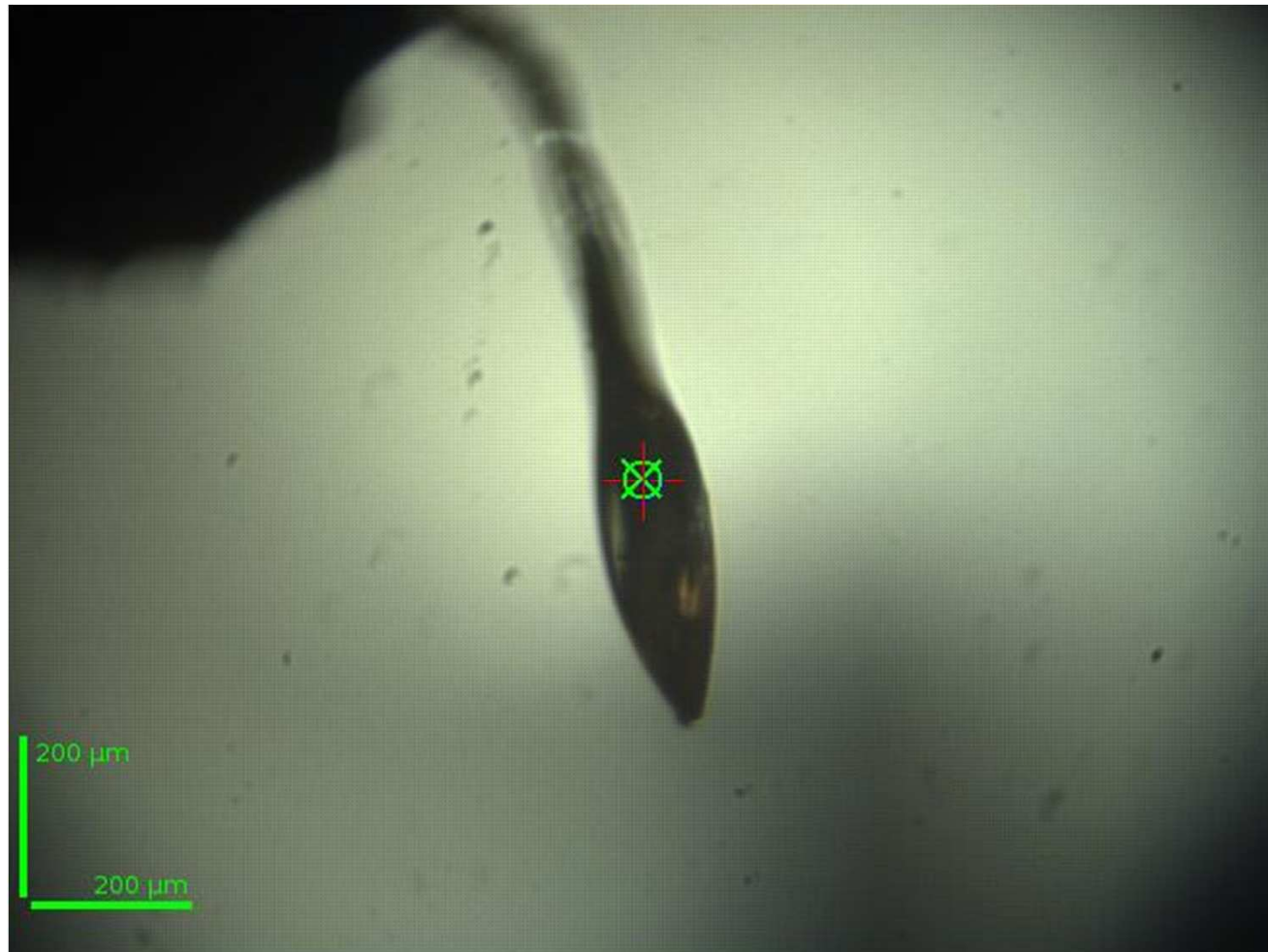


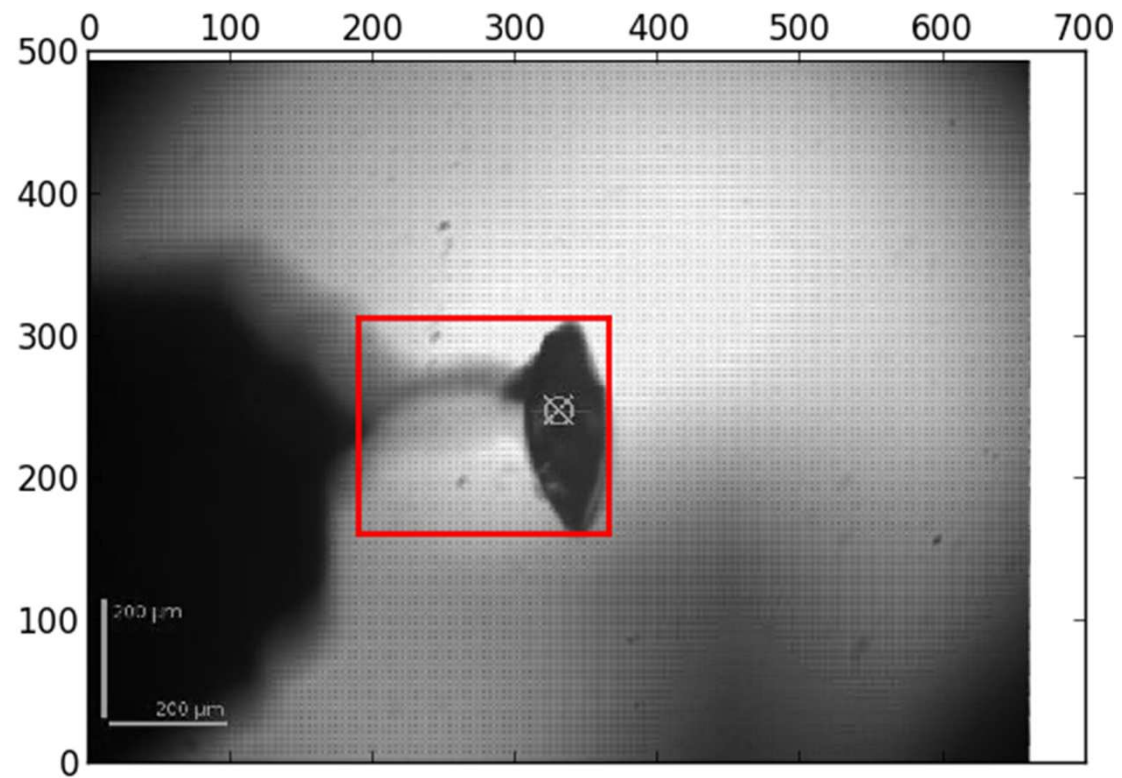


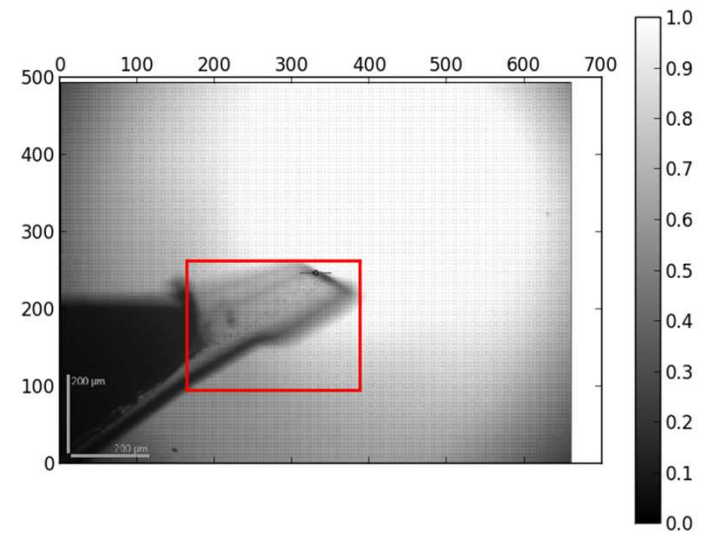
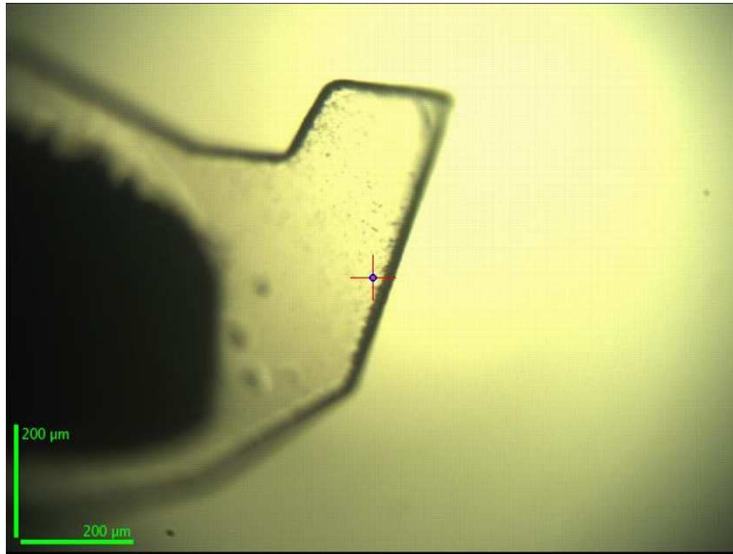
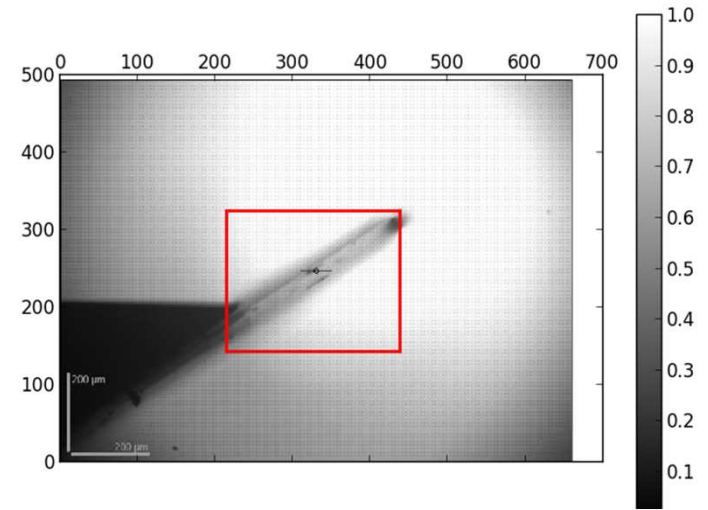
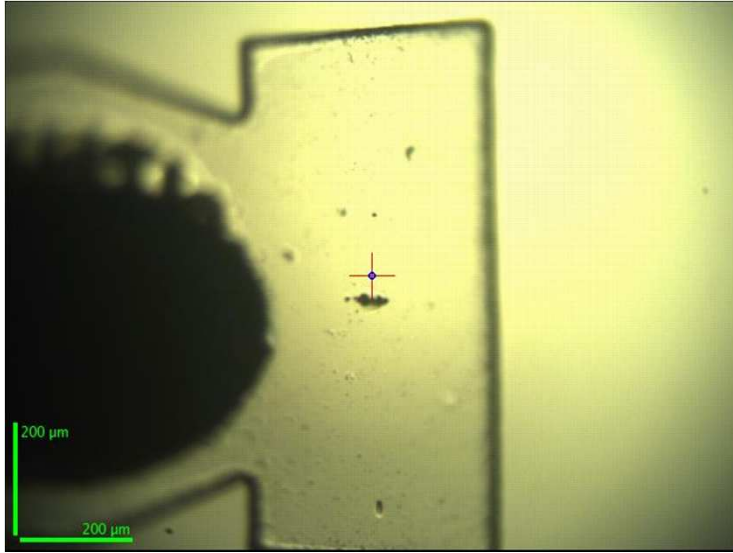


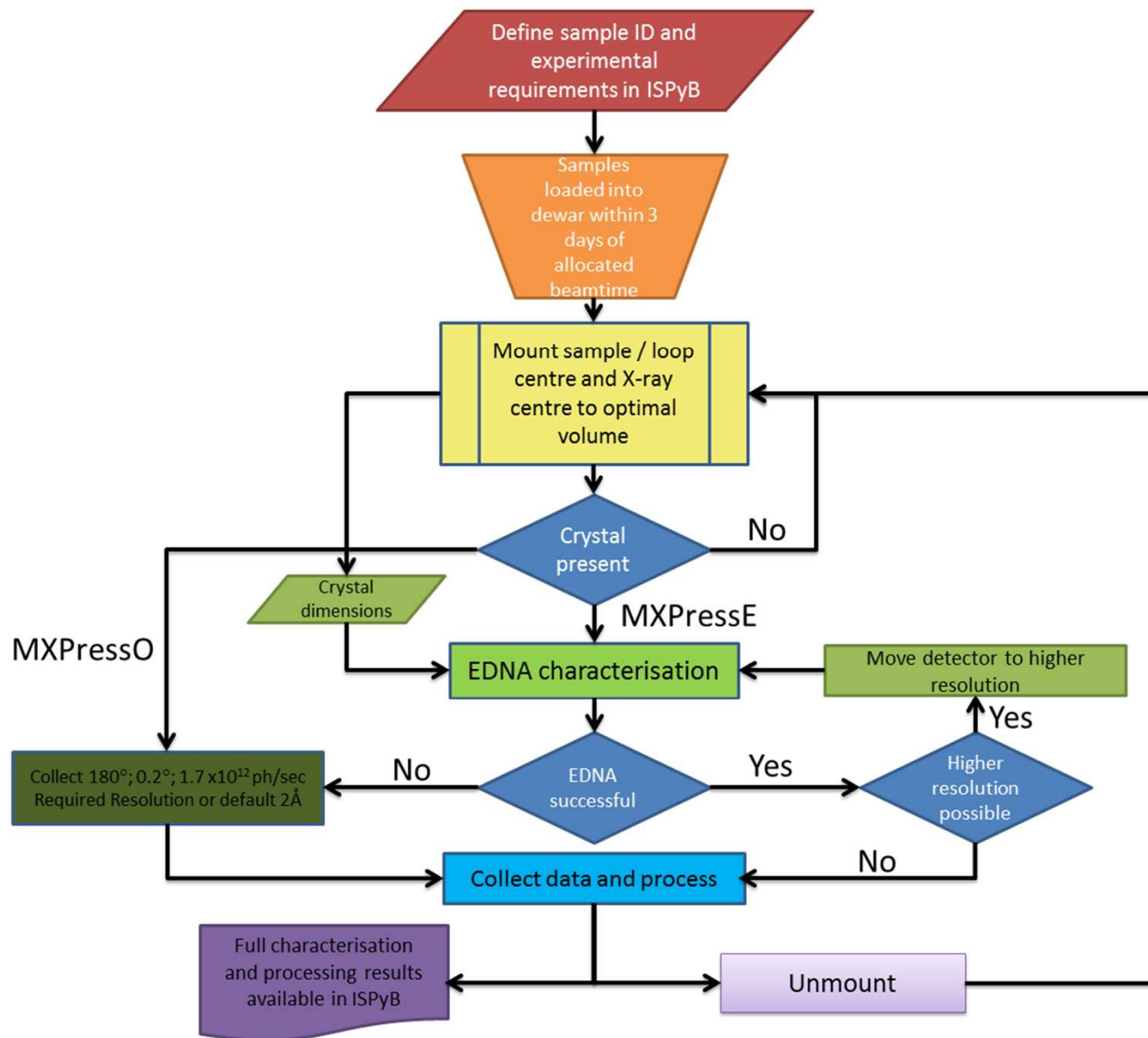




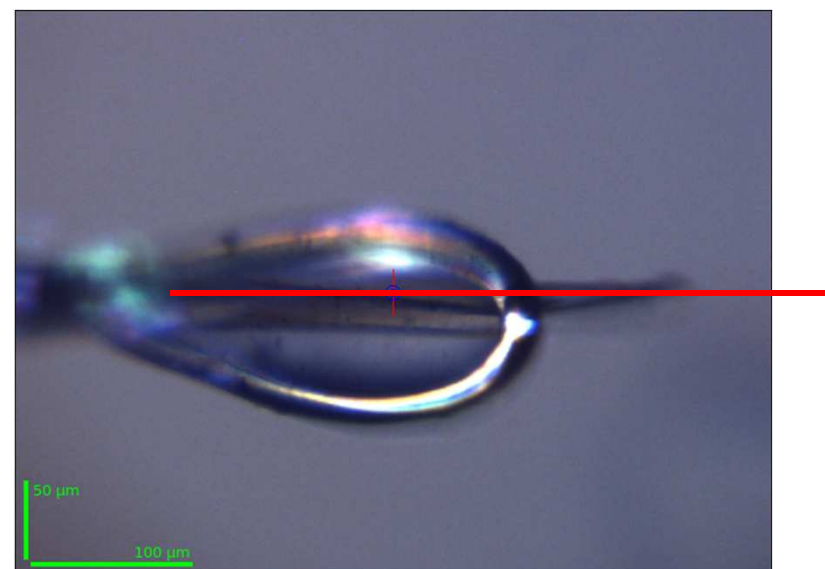
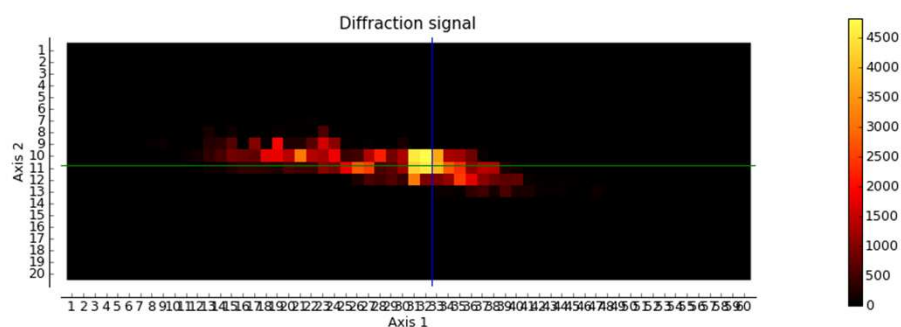
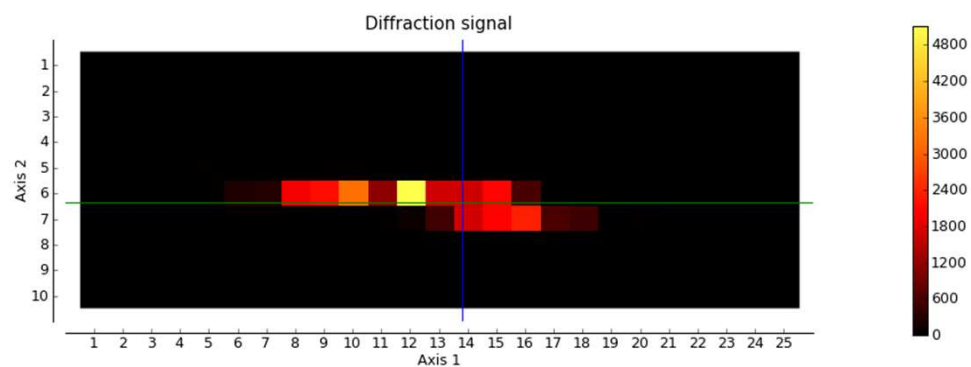




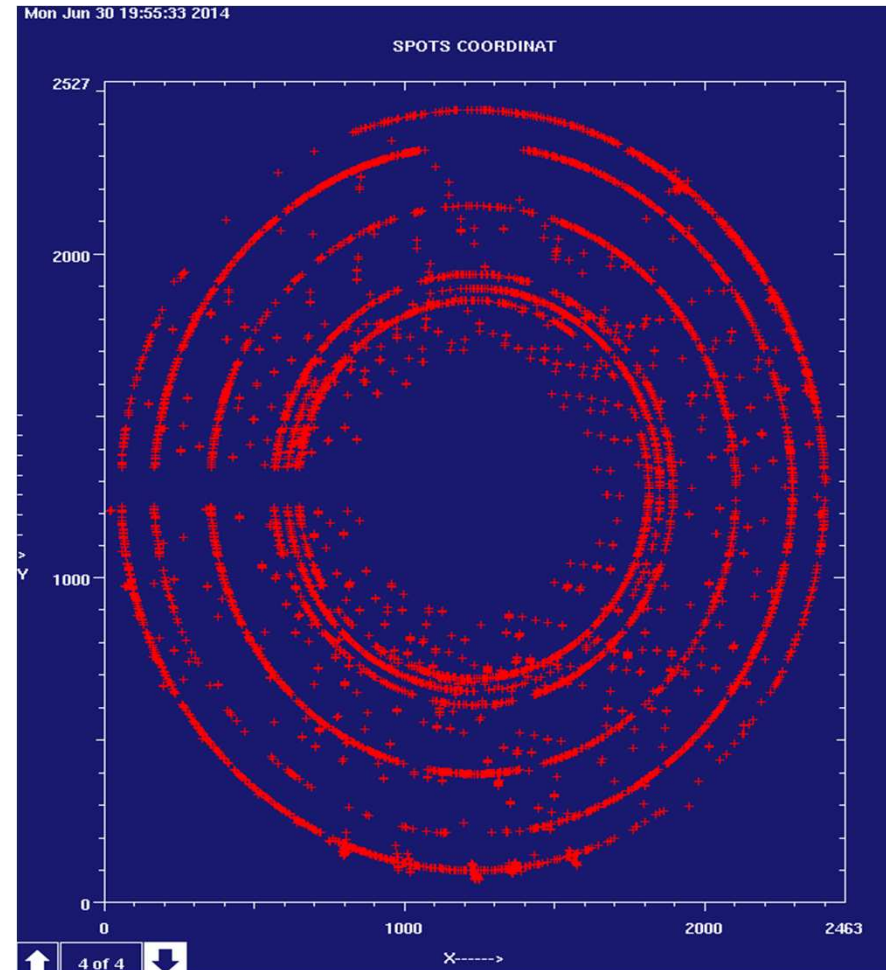
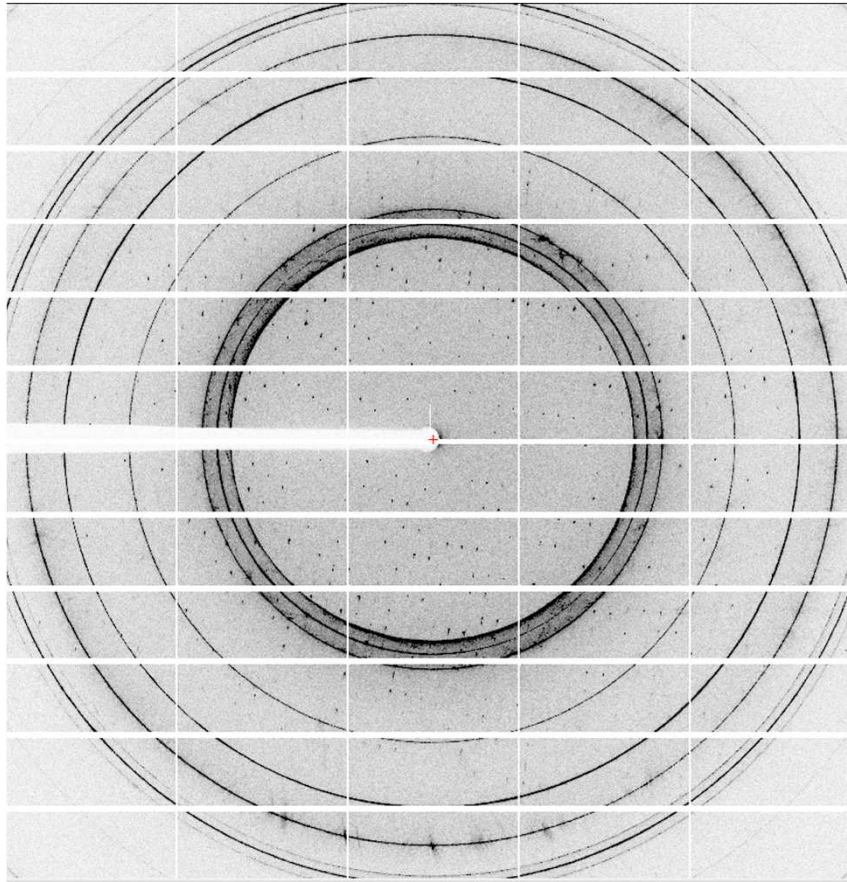




X-ray centring allows the full characterisation of multiple positions (upto 5600), selection of beam size and location of crystals

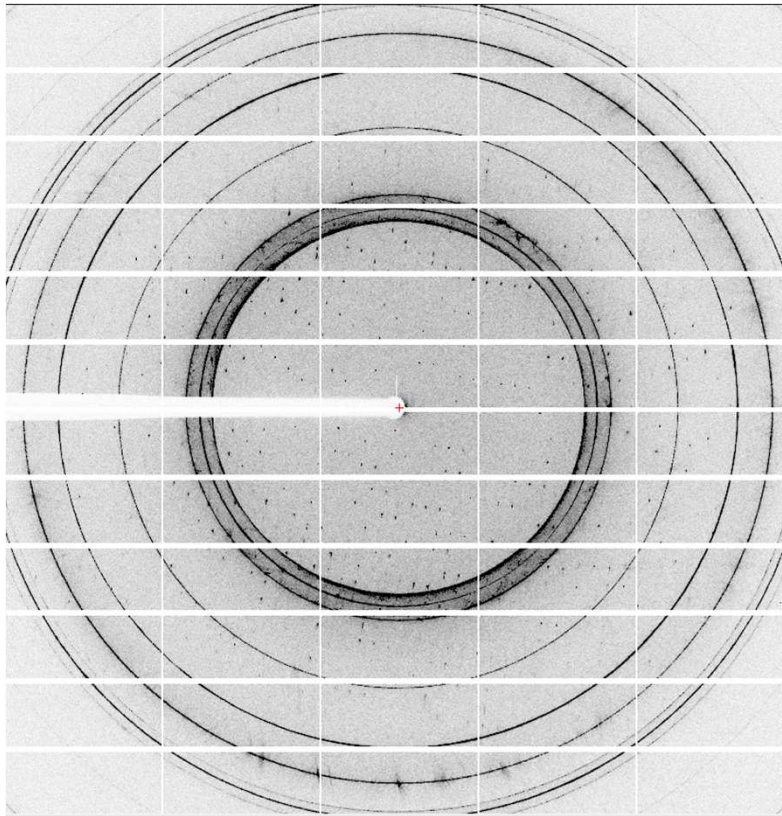


DOZOR identifies individual Bragg spots

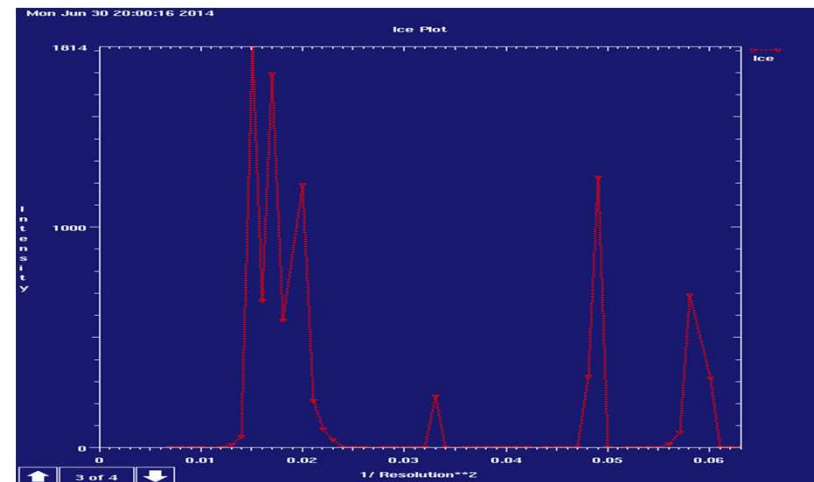
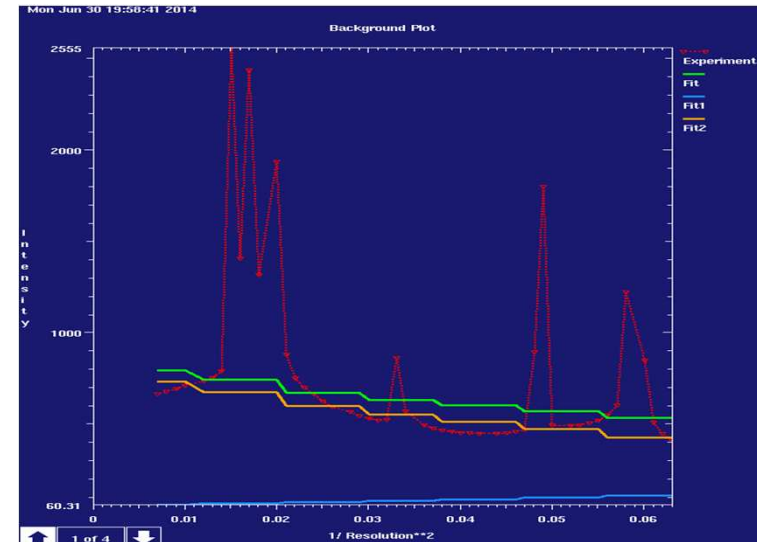


Sasha Popov

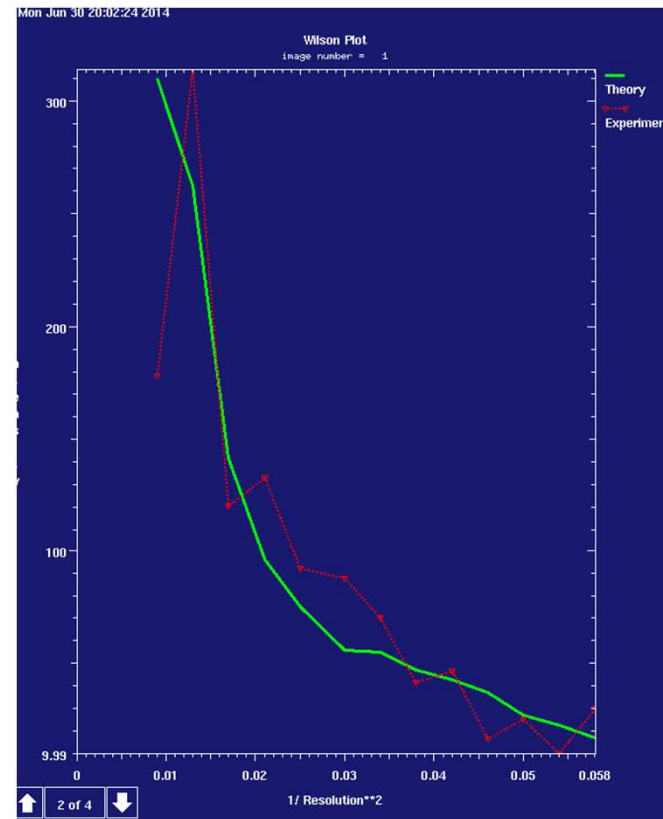
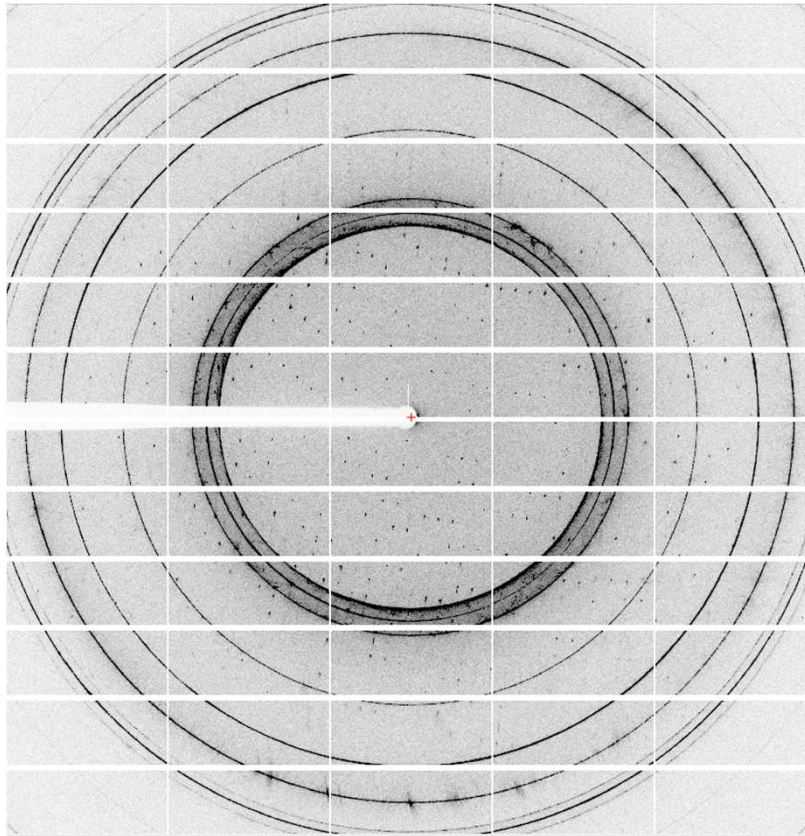
DOZOR - indication on the ice or salt diffraction contaminations



Sasha Popov

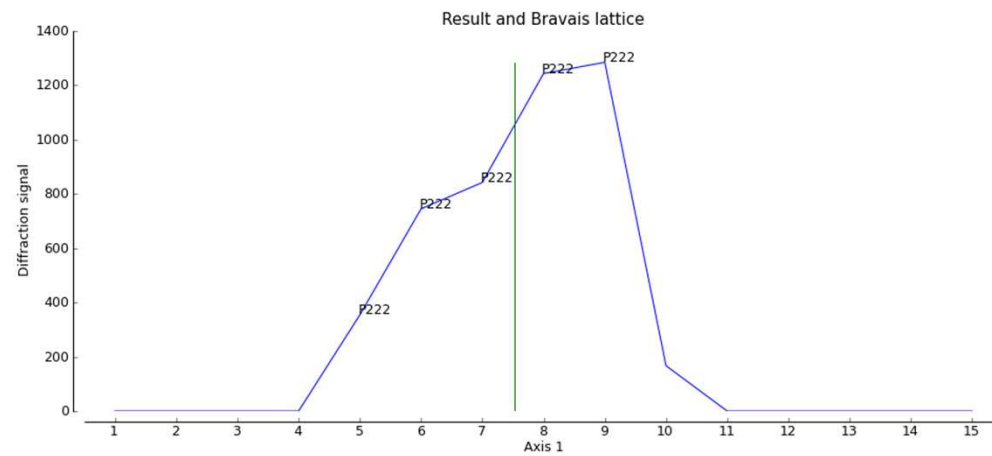
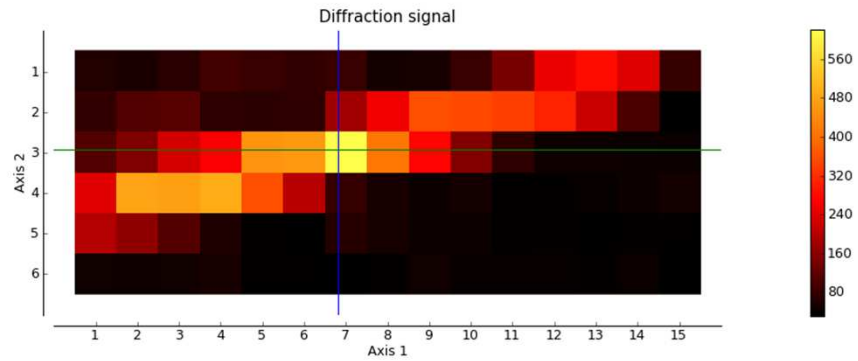


DOZOR approximates the experimental data by applying isotropic Debye-Waller factor and the standard protein Wilson plot pattern

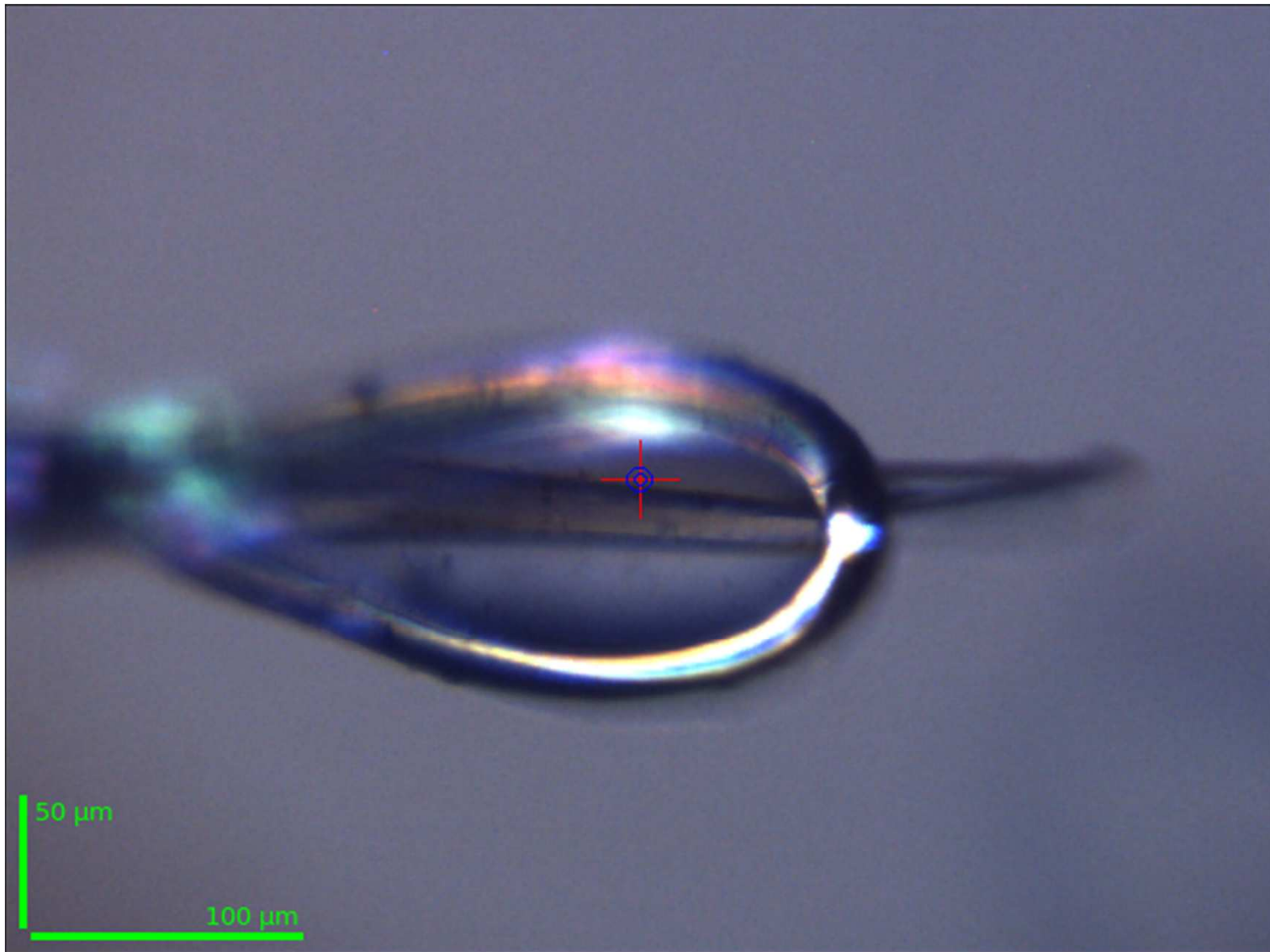


Sasha Popov

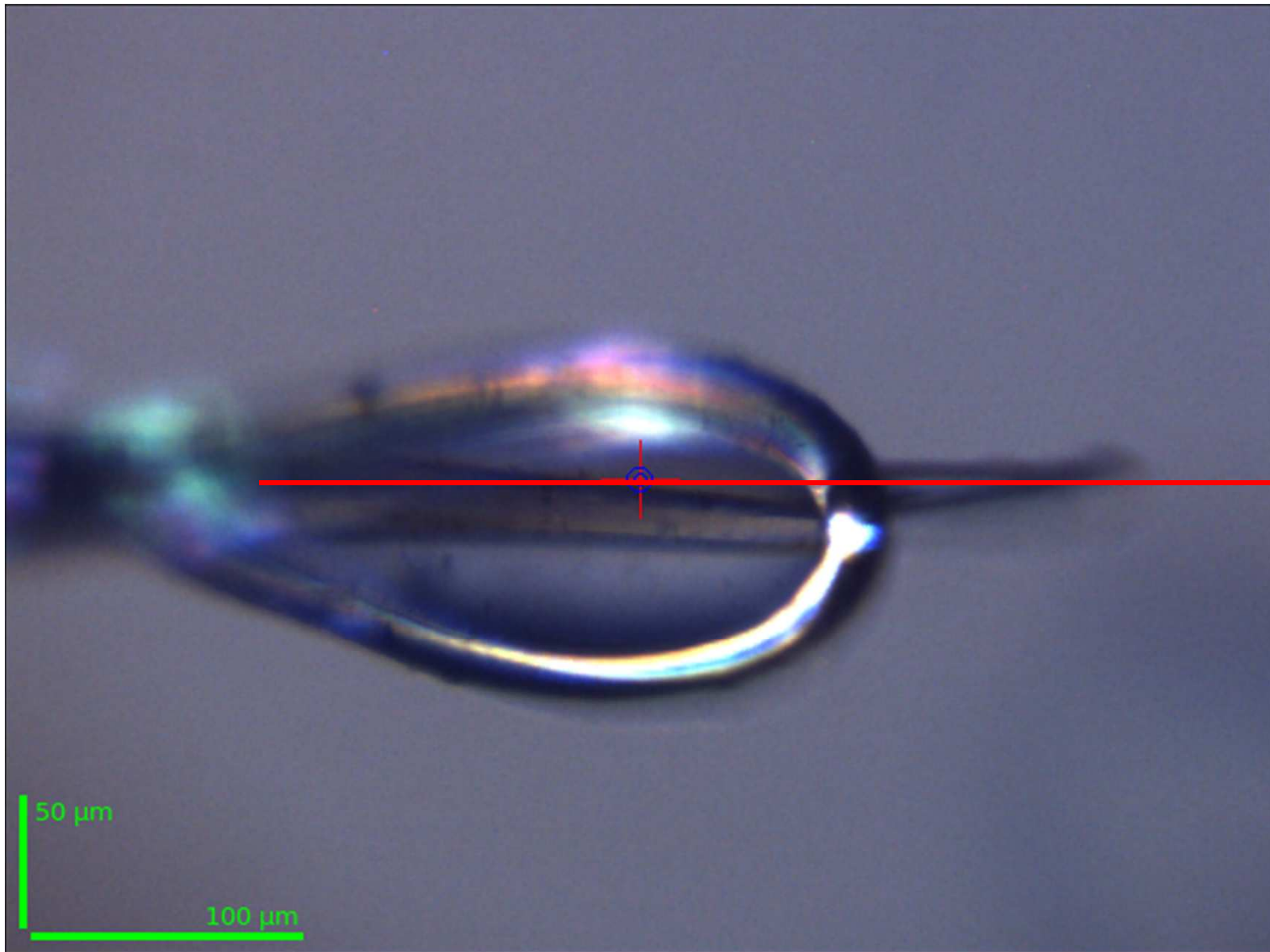
3D - autocentre



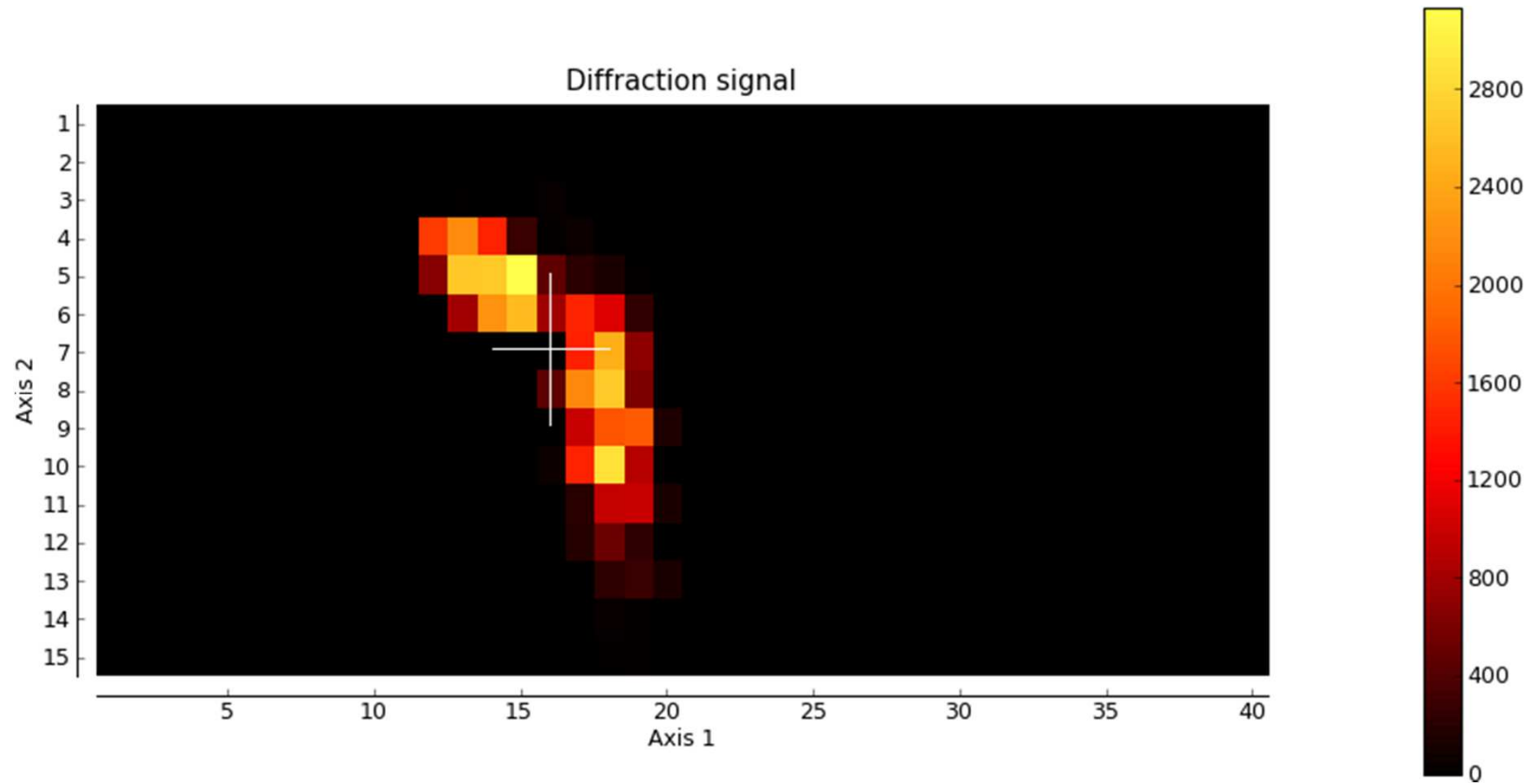
Olof Svensson and Matt Bowler



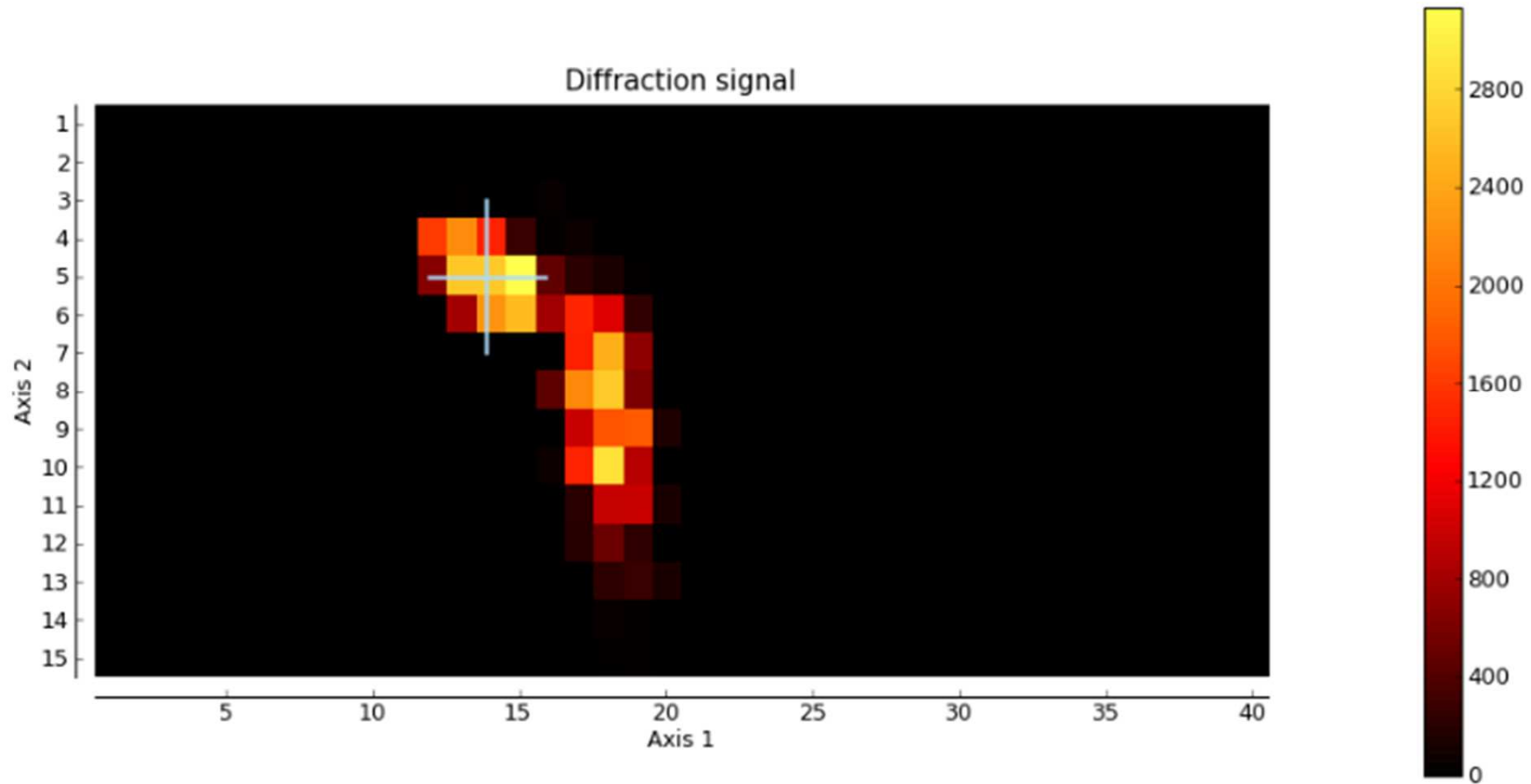


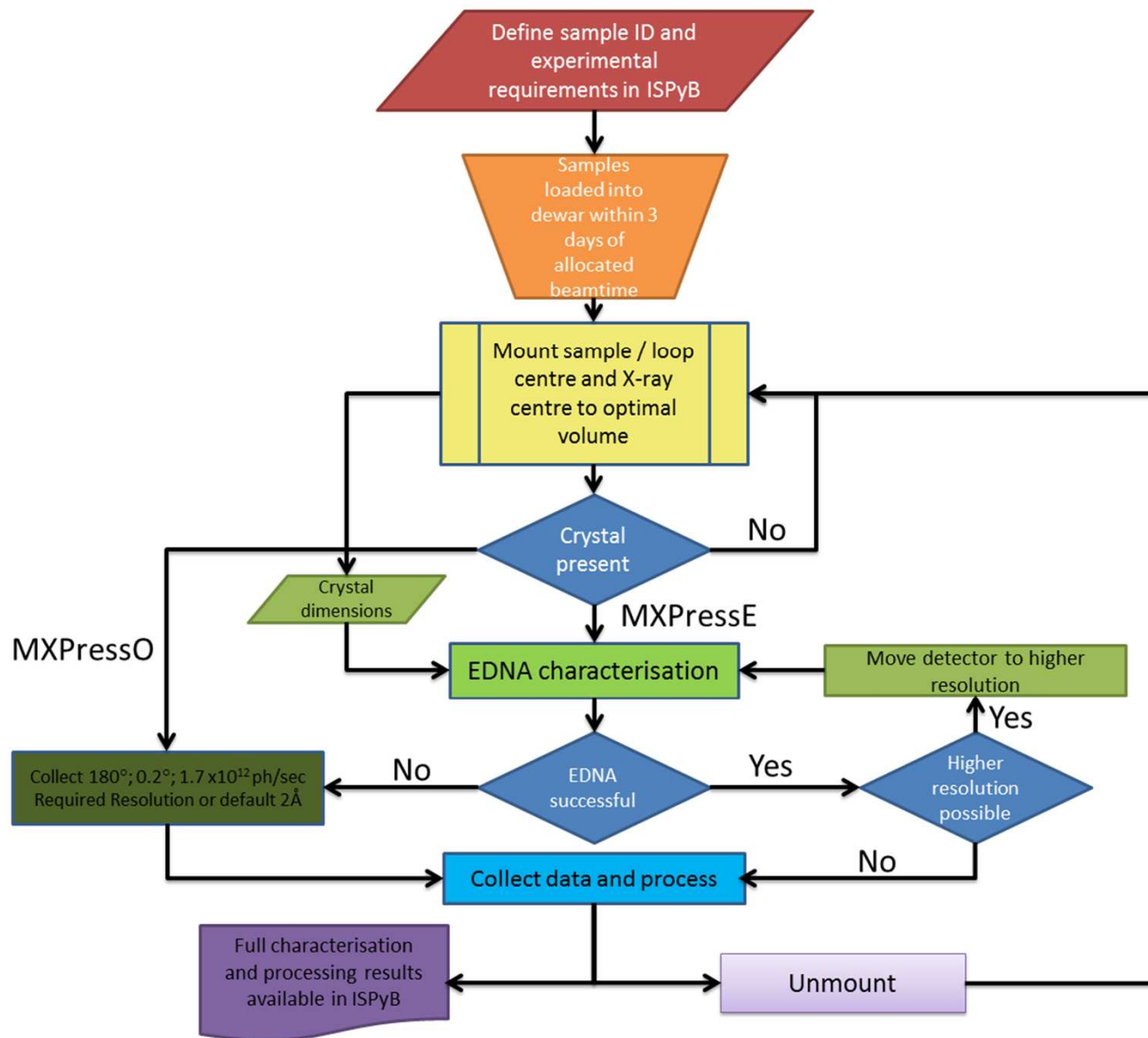


COG determination



COG determination





mxCuBE (opid-2.0)

File Instrumentation Help Expert mode

Collect XRF spectrum System Chat Feedback

User: opid-232 Group: [] Set Logout

Sample list
Mode: Sample changer Show SC details
Centring: Automatic Synch ISPyB

Sample centring
Sample position
Omega: 0.0 90 Kappa: 0.0 1.0 Phi: 0.0 0.1
Slit length: 21.5 0.001

Sample video
Light: 0.55 Focus: 0.499 0.001 Front light: 3.0 Zoom: 3

Collection method
Standard Collection
Characterisation
Helical Collection
Advanced
Workflow type: Enhanced characterisation

Data location
Folder: /data/d2/ehz/inhouse/opid232/20140129/RAW_DATA
Prefix: opid232
Run number: 1

Grid
Nb points

	points per line	steps (mm)	distance
Axis 1	2		
Axis 2	2		

Machine current: 196.4 mA
78 multibunch
04:16
Flux: +0.00 ph/s
Energy: 14.2037 keV
Wavelength: 0.871 Å
Resolution: Current: 1.999 A 180.66 mm
Move to: A
Transmission: Current: 42.41% Set to: Cryo: 100.0 K

Safety shutter: closed
Fast shutter: closed
Beamstop: out
Current users: [] Selecting gives control [] Allow timeout control Take control
My name: sybl

2014-01-29 16:42:49 Unloading
2014-01-29 16:43:15 Ready
2014-01-29 16:43:16 Ready

Taskbar: mxCuBE ISPyB Shell - Konsole jilapli 2.3 Temperature control
Front End & Vacuum mxCuBE (opid-232) jvacuum 2.0

System tray: 16:43 29/01/2014

Services offered to users on MASSIF1:

- MXPressL – load/optical centre/180°
- MXPressO - load/optical centre/X-ray centre/180°
- MXPressE - load/optical centre/X-ray centre/eEDNA strategy/collect
- MXScore - load/optical centre/X-ray centre/eEDNA strategy
- MXPressE_SAD - load/optical centre/X-ray centre/eEDNA SAD strategy/collect
- No restrictions on sample size, mount or quality
- All data are processed automatically with the option to pipeline to molecular replacement and ligand fitting with fragment structures uploaded to ISPyB

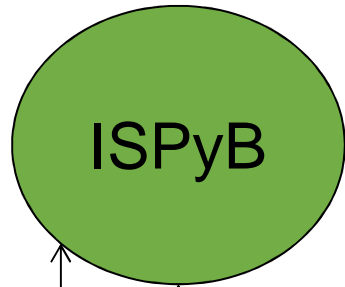
Average times per sample

- Number of samples depends on level of characterisation
- 150 to 400 samples per 24 hours
- 50 to 133 per shift

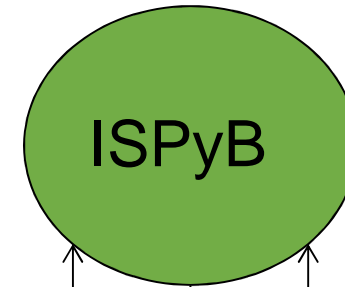
Average times per sample

Step	MXPressO	MXPressE	MXScore
100 image mesh scan	1'24"	1'38"	1'24"
15 image line scan	27"	23"	
EDNA characterisation	-	49"	49"
2 nd EDNA characterisation	-	54"	
Data collection	1'	1'18"	
Total workflow time	3'26"	5'20"	
Total time for sample	4'30"	6'27"	3'45"

Tracking / Identification



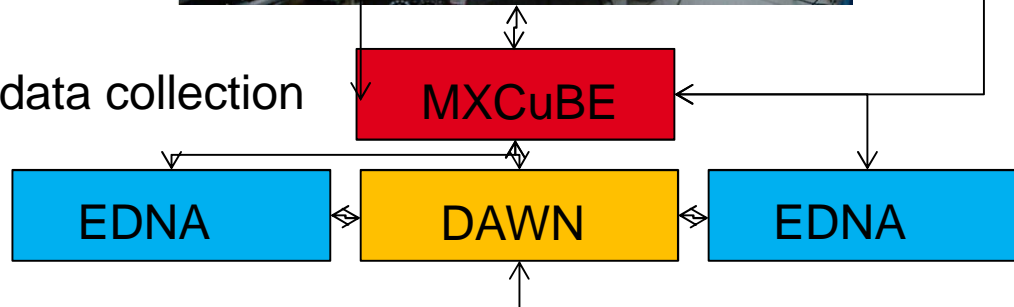
Tracking / Results



MASSIF1



Sample handling / data collection



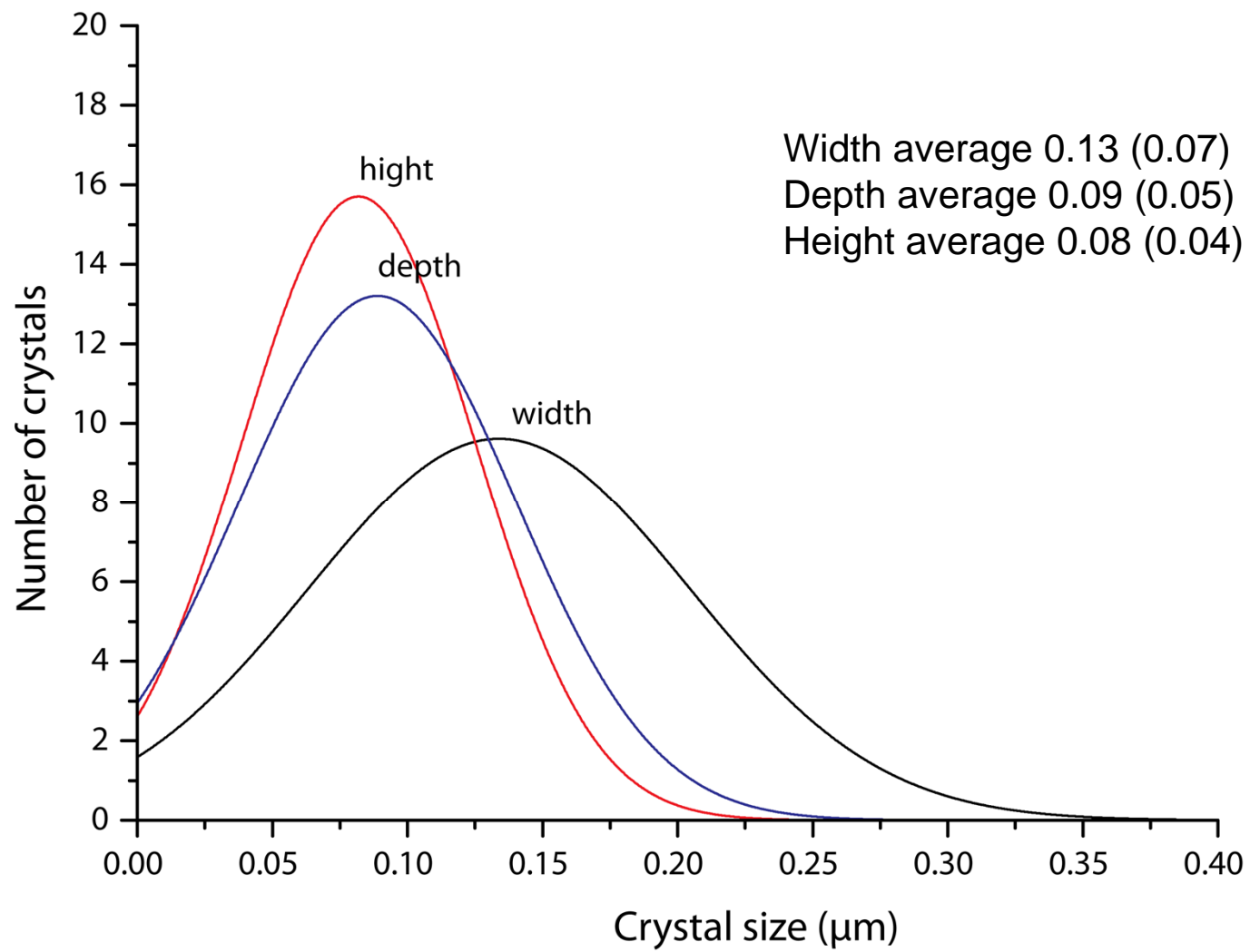
Detailed reports in ISPyB display all sample analysis

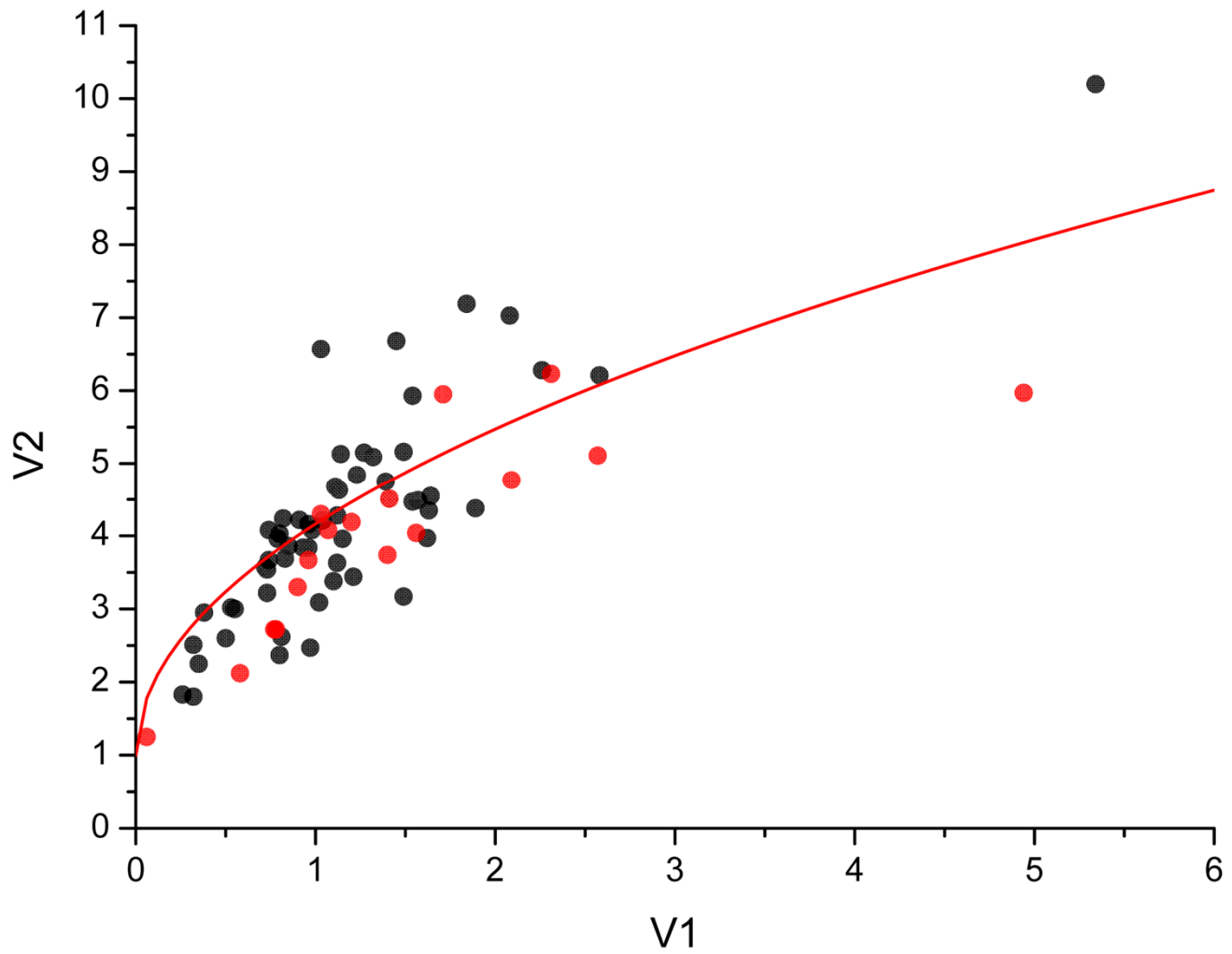
Session										
Experiment Type	Image Prefix	Run#	Start time	Parameters	Comments	Image Thumbnail	Crystal snapshot	Graph	Second Graph	Results
MXPressE	opid291	1	10:13:37 30-04-2014	Nb tot images: 1402 Nb images: 1402 Exp. time: 0.04 s Phi range: 0.10 ° Flux: 2.74E11 photons/sec Detector resolution: 2.18 Å Transmission: 6.94 Wavelength: 0.976 Å Total expo time: 45.88 s						MXPressE EDNA dp GrenADES fp GrenADES pp Space Group: P 21 21 21 Completeness:
MXPressE	opid291	1	10:07:02 30-04-2014	Nb tot images: 1062 Nb images: 1062 Exp. time: 0.04 s Phi range: 0.20 ° Flux: 3.32E12 photons/sec Detector resolution: 2.00 Å Transmission: 100.00 Wavelength: 0.976 Å Total expo time: 33.30 s						MXPressE EDNA dp GrenADES fp GrenADES pp Space Group: P 21 21 21 Completeness:
MXPressE	opid291	1	10:00:32 30-04-2014	Nb tot images: 1062 Nb images: 1062 Exp. time: 0.04 s Phi range: 0.20 ° Flux: 3.35E12 photons/sec Detector resolution: 2.00 Å Transmission: 100.00 Wavelength: 0.976 Å Total expo time: 33.30 s						MXPressE EDNA dp GrenADES fp GrenADES pp Space Group: P 21 21 21 Completeness:
MXPressE	opid291	1	09:53:43 30-04-2014	Nb tot images: 1282 Nb images: 1282 Exp. time: 0.04 s Phi range: 0.10 ° Flux: 2.85E11 photons/sec Detector resolution: 2.27 Å Transmission: 6.94 Wavelength: 0.976 Å Total expo time: 41.44 s						MXPressE EDNA dp GrenADES fp GrenADES pp Space Group: P 21 21 21 Completeness:
MXPressE	opid291	1	09:45:54 30-04-2014	Nb tot images: 1502 Nb images: 1502 Exp. time: 0.04 s Phi range: 0.10 ° Flux: 1.36E12 photons/sec Detector resolution: 2.44 Å Transmission: 38.26 Wavelength: 0.976 Å Total expo time: 49.58 s						MXPressE EDNA dp GrenADES fp GrenADES pp Space Group: P 21 21 2 Completeness:
Characterization	ref-p38_EDNA	1	09:38:21 30-04-2014	Nb images: 2 Exp. time: 0.04 s Phi range: 1.00 ° Flux: 5.47E11 photons/sec Detector resolution: 2.00 Å Transmission: 14.64 Wavelength: 0.976 Å Total expo time: 0.07 s						Indexing Strategy Space Group: Unit Cell a, b, c: alpha, beta, gamma: Mosaicity: Ranking Resolution:

ISPyB also allows to define required resolutions, forced space groups, services required and PDB and SMILES upload for downstream processing

The difference measurements make.....

	Default Crystal Size (0.1 x 0.1 x 0.1 mm ³)	Measured Crystal Size (0.124 x 0.1 x 0.043 mm ³)
Flux	1 x 10 ¹² ph/s	1 x 10 ¹² ph/s
Dose Rate	8.1 x 10 ⁰⁵ Gy/s	8.4 x 10 ⁰⁵ G/s
Time to reach Henderson Limit	25 s	24 s
Dose	4.9 MGy	15.3 MGy
Exposure Time	54 s	65 s
Oscillation Range	141 – 276	162 - 292
Resolution	2.3	2.16





How does it work practically?

- Shifts allocated to BAG as usual
- Book a slot on the MASSIF1 google calendar with BAG responsible
- A form + invitation will be sent
- Send crystals for slot
- Samples will be processed within 3 working beam days of slot
- All sample info **MUST** be entered in ISPyB

Google Recherche dans l'agenda

Agenda Aujourd'hui novembre 2014

Jour Semaine Mois 4 jours Mon planning Plus

	lun.	mar.	mer.	jeu.	ven.	sam.	dim.
novembre 2014	27	28	29	30	31	1 nov.	2
	HC1c on FIP MX-1652 BRACHER HC1c on ID23-1 EVENING	MDT Machine Dedicated Time	Not available HC1c Josan	Not available HC1c on ID23-2	Not available HC1b on BM14 need my help		
	3	4	5	6	7	8	9
	MX-1575 POGENBERG (~100 samples)	MDT Machine Dedicated Time	MX-1589 MAPELLI		MX-1575 POGENBERG (~50 samples)		
	10	11	12	13	14	15	16
	MX-1631 PHAN (50 samples max.)	MDT Machine Dedicated Time	MX-1638 CRONIN (100) MX-1638-DAVISON-test 100 samples				
	17	18	19	20	21	22	23
		MDT Machine Dedicated Time	MX-1644 Andersson 100 samples Mxpress - Steph - FX29MX5 - 3 pucks	MX-1648 KAJANDAR (80)			
	24	25	26	27	28	29	30
	MXPRESS TESTS - 100 samples	MDT Machine Dedicated Time			MX-1583 GARCIA-SAEZ (150-200)		

Mes agendas: Matthew Bowler, ESRF Sch... Matthew Bowler, HC1 calendar

Autres agendas: Météo, Numéros de semaine, Jours fériés en France

12:03 05/11/2014

- ▶ Create
 - Shipment
 - Puck
 - CSV upload
- ▶ View
 - Shipments
 - Dewars
- ▶ Search
 - Shipment
 - Dewar

Shipment

Name: EMPTY

Creation date: 01-10-2013

Status: opened

[Back to this Shipment](#)

Upload Shipment from Excel file

Help



dewar1

CA280 CA281

Save Reset Change sample name automatically

Sample Position	Protein Acronym (*)	Sample Name (*)	PinBarcode	Space Group	Pre-Observe resolution	Needed resolution	Oscillation Range	Experiment Type	Unit Cell a	Unit Cell b	Unit Cell c	Unit Cell alpha	Unit Cell beta	Unit Cell gamma	Comments
1	A-TM - P21212	samp01		P21212	0.0	0.0	0.0	OSC	0.0	0.0	0.0	0.0	0.0	0.0	
2	A-TM - P21212	samp02		P21212	0.0	0.0	0.0	OSC	0.0	0.0	0.0	0.0	0.0	0.0	
3	A315S - Undefined	test01		Undefined	0.0		0.0	OSC							
4	A-TM - I4122	crystal21		I4122	0	0	0	OSC	0	0	0	0	0	0	
5								OSC							
6								OSC							
7								OSC							
-								---							

Diffraction Plan entry	Comment
`observedResolution`	Limit of previously observed diffraction, the detector will be placed at this distance for mesh scans if specified (default 2 Å)
`minimalResolution`	Minimum required resolution, if specified crystals that diffract below this limit will not have data collected, for MXPressO this will be the resolution set for data collection.
`preferredBeamSize`	Aperture requirement, default scanning is made with a 50 micron beam with the aperture for data collection chosen by the size of homogenous diffraction areas.
`forcedSpaceGroup`	Use to force data collection strategies and autoprocessing to use this known space group
Experiment Type	MXPressO / E / SAD / Score
Various data collection parameters	Radiation sensitivity / Completeness / Redundancy

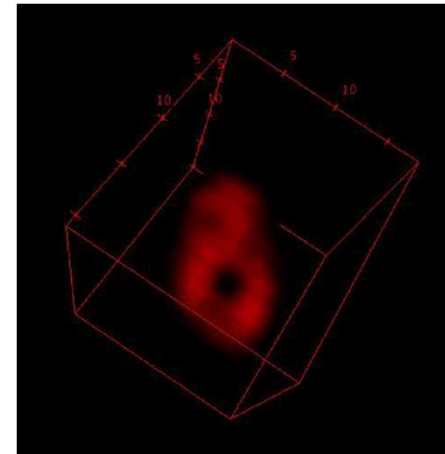
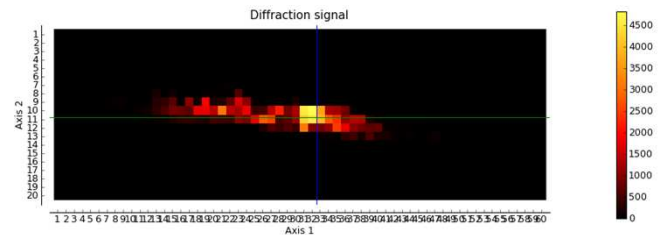
The beamline so far.....

- Over 4000 samples processed so far.....
- Over 20 BAGs
- Many cases of higher resolution obtained
- Bernhard Rupp:

"...and while we do not have any really acceptable crystals, it is quite reassuring that the programs actually found a crystal we missed on the manual run"

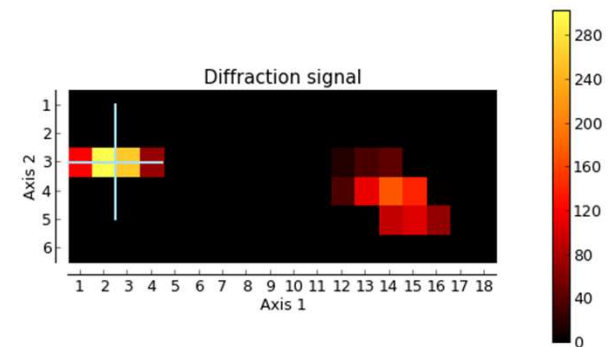
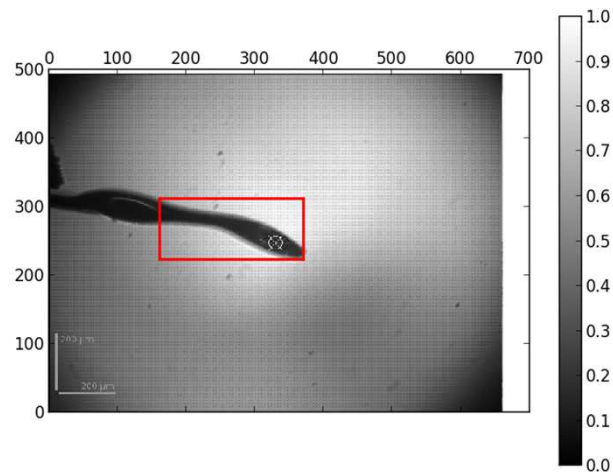
MASSIF1

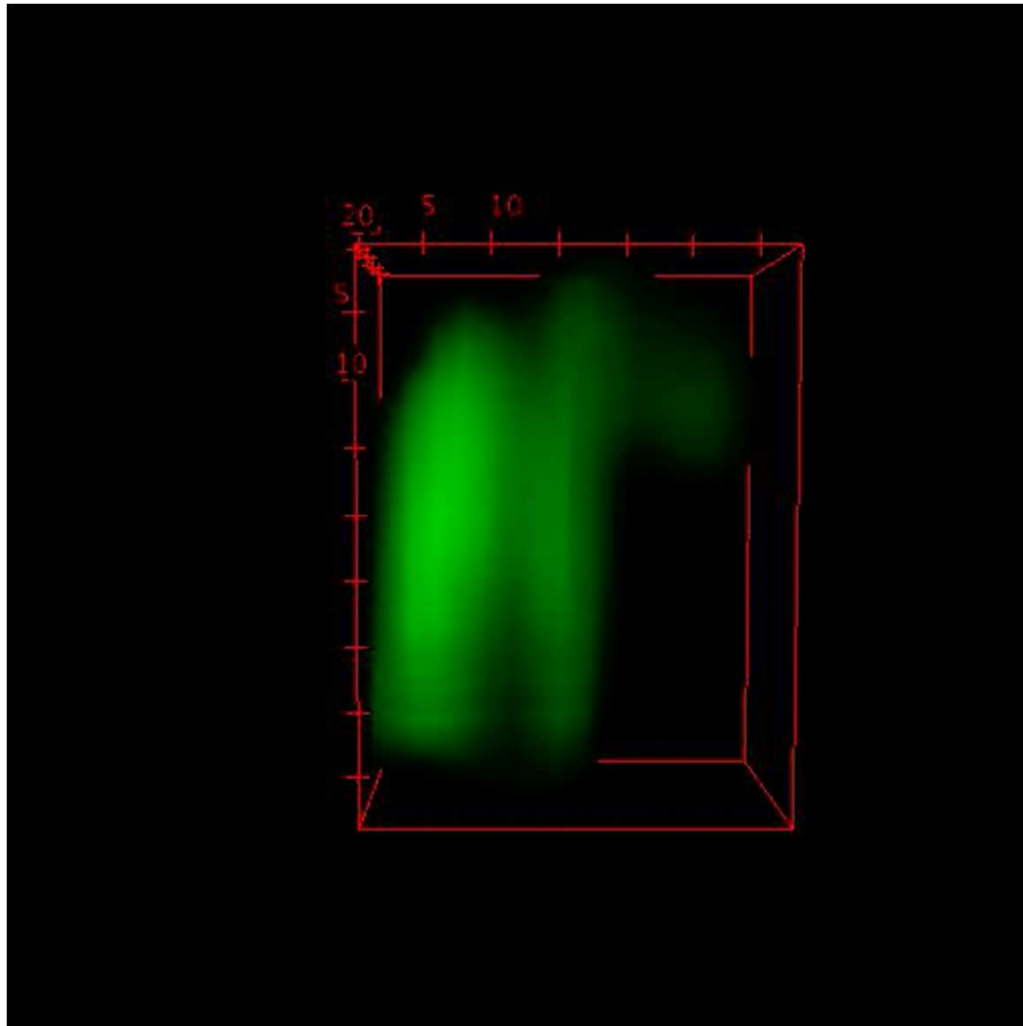
- Open to user samples September 2014
- Automatic sample characterisation and data collection only
- Fastest rate 300 samples / 24 hours
- Workflows define optimal crystal volumes (5600 positions) and data collection strategies
- Highest impact on projects at **early stages** (*screening*) and **late stages** (*multiple data collections for mutants / drug fragments*)



To come in 2015:

- Ligand fitting
- Full experiment design in ISPyB
- Multiple crystals
- Full 3D characterisation of crystals







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