

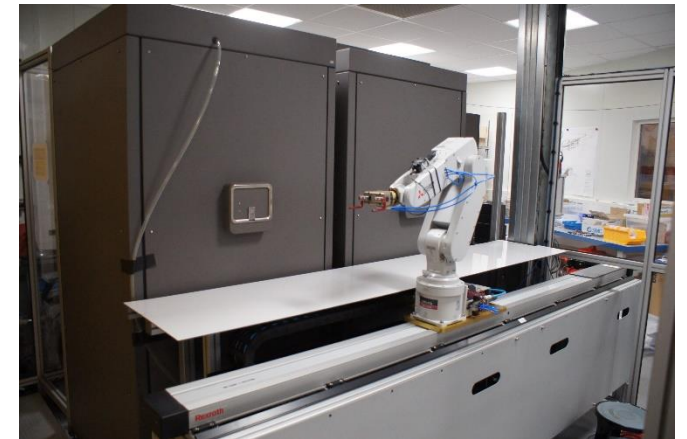
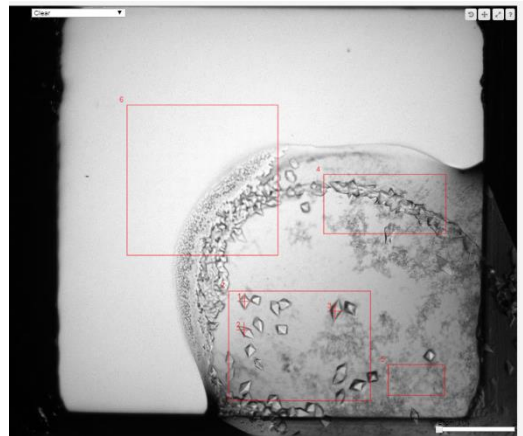
VMXi the only fully *in-situ* fully remote beamline

What the beamline does and how it could benefit your work

Juan Sanchez-Weatherby
James Sandy
Halina Mikolajek

What is VMXi?

- *In-situ*
- Fully automated
- Fully remote

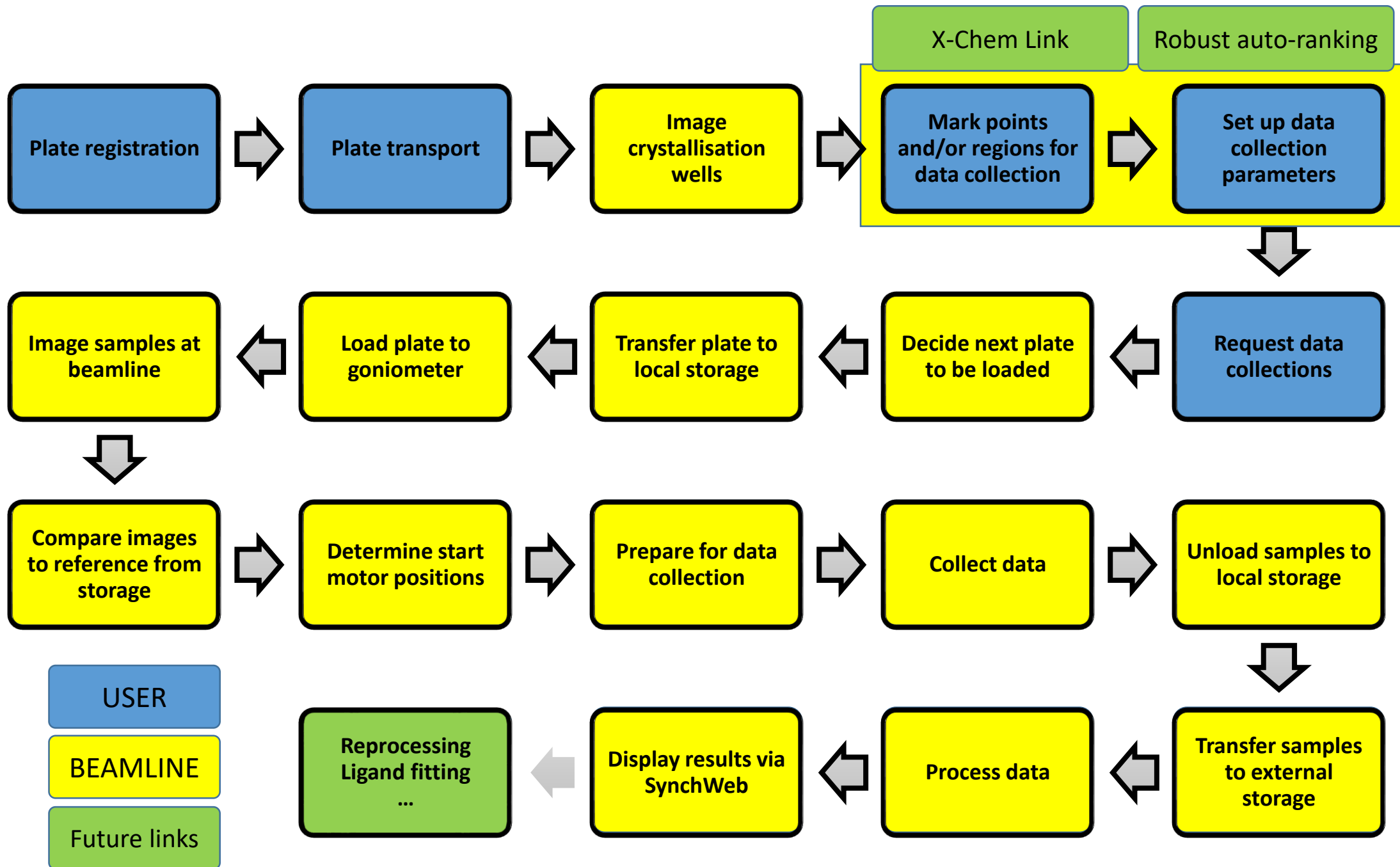


This beamline collects high quality RT datasets

- Determining the intrinsic quality of crystal without introducing external variables
 - Dehydration
 - Manual handling
 - Cold shock
 - Chemistry
- Rapidly solving structures
 - Speeds up the early crystal optimisation cycles (hit finding and optimisation)
 - From click to structure in minutes
 - Not requiring fishing, cooling, cryo-optimising transporting, loading, etc.
 - Making every sample count
 - Rapidly solve and visualise multiple ligand structures
- Samples that do not cryo-protect well
 - Complexes
 - Mb Proteins
 - Large crystals

Current beamline status

- Lab facility for crystallisation
- Fully automated handling of plates (*in-situ* plates and films)
- 20 °C soon 4 °C
- $\sim 10 \times 10 \text{ } \mu\text{m}^2$ beam size
- DMM monochromator $> 10^{14}$ phs/s (extremely high flux)
- Eiger2X 4M detector (500 Hz)
- Grid scans 500 Hz (100% beam)
- Omega scans -30° to 30° 560Hz (2-5% beam)
- Synchweb web interface



Paste from Spreadsheet

Shipment

Dewar

Container Type

Container Name

Barcode

Owner

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

Comments

Comment for the container

Requested Imager

Imager this container should go into

Imaging Schedule

Requested imaging schedule

Crystallisation Screen

Crystallisation screen that was used for this container

Sample

CCP4_TEST

Dewar1

CrystalQuickX ▼

CCP4_TEST

JUAN_TES_001

Juan Sanchez-Weatherby [You] ▼ Q View

VMXi 20c ▼

Fibonacci ▼ Q View

Ax-CP-CUSTOM-101 ▼

+ Plate + Column + Row x Plate x Column x Row x

+ Extra Fields

Protein

JEWGDH ▼

Abundance

Sample Name

NONSENS

Spacegroup

Comments

Components

Add:

No Components

	1	2	3	4	5	6	7	8	9	10	11	12
A	1	3	5	7	9	11	13	15	17	19	21	23
B	26	27	29	31	33	35	37	39	41	43	45	47
C	49	51	53	55	57	59	61	63	65	67	69	71
D	73	75	77	79	81	83	85	87	89	91	93	95
E	97	99	101	103	105	107	109	111	113	115	117	119
F	121	123	125	127	129	131	133	135	137	139	141	143
G	145	147	149	151	153	155	157	159	161	163	165	167
H	169	171	173	175	177	179	181	183	185	187	189	191

This page shows the contents of the selected container. Samples can be added and edited by clicking the pencil icon, and removed by clicking the x

	1	2	3	4	5	6	7	8	9	10	11	12
A	1 2	5 6	9 10	13 14	17 18	21 22	25 26	29 30	33 34	37 38	41 42	45 46
B	49 50	53 54	57 58	61 62	65 66	69 70	73 74	77 78	81 82	85 86	89 90	93 94
C	97 98	101 102	105 106	109 110	113 114	117 118	121 122	125 126	129 130	133 134	137 138	141 142
D	149 150	153 154	157 158	161 162	165 166	169 170	173 174	177 178	181 182	185 186	189 190	193 194
E	197 198	201 202	205 206	209 210	213 214	217 218	221 222	225 226	229 230	233 234	237 238	241 242
F	249 250	253 254	257 258	261 262	265 266	269 270	273 274	277 278	281 282	285 286	289 290	293 294
G	297 298	301 302	305 306	309 310	313 314	317 318	321 322	325 326	329 330	333 334	337 338	341 342
H	349 350	353 354	357 358	361 362	365 366	369 370	373 374	377 378	381 382	385 386	389 390	393 394

Show Data Status ☐ | Loaded 135 out of 192 images

Inspections

13:29 15-12-2017 (+0.0d) [Manual]

Movie

Gap: 0.1 s

Sample

Protein

TestThaumatococcus

Abundance

Click to edit

Components

No Components

Add:

Sample Name

C2d1_Thaum_Again

Spacegroup

Click to edit

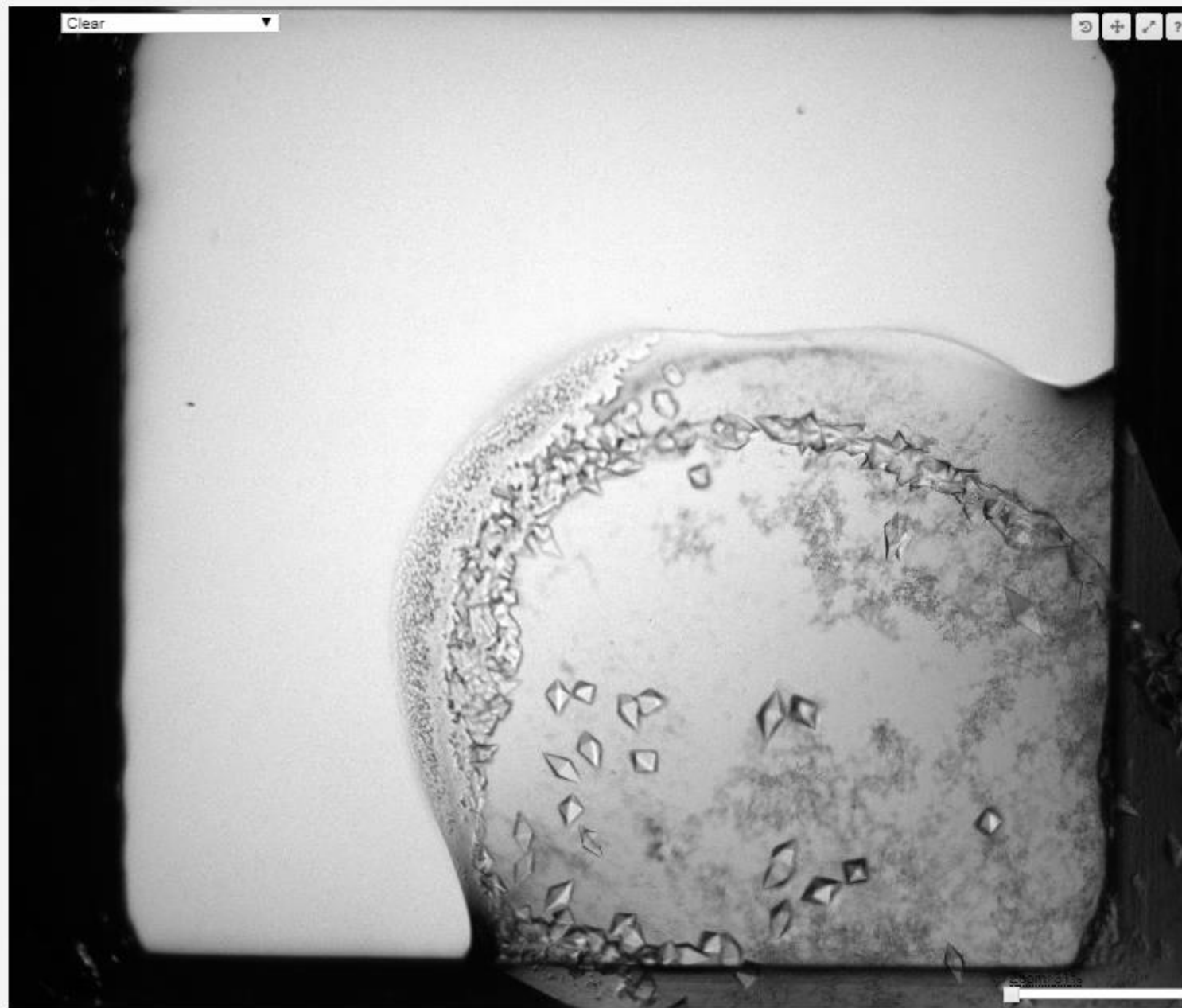
Comments

Click to edit



+0.0d

Marked Sub Samples



This page shows the contents of the selected container. Samples can be added and edited by clicking the pencil icon, and removed by clicking the x

	1	2	3	4	5	6	7	8	9	10	11	12
A	1.12	5.13	5.13	7.13	9.13	10.13	15.13	17.13	17.17	17.17	20.17	22.17
B	24.28	24.28	24.28	31.32	32.32	32.32	35.32	35.32	35.32	35.32	41.32	41.32
C	44.33	41.32	41.32	44.33	47.33	47.33	47.33	47.33	47.33	47.33	47.33	47.33
D	74.74	74.74	74.74	74.74	81.74	81.74	81.74	81.74	81.74	81.74	81.74	81.74
E	95.75	95.75	95.75	95.75	95.75	95.75	95.75	95.75	95.75	95.75	95.75	95.75
F	124.76	124.76	124.76	124.76	124.76	124.76	124.76	124.76	124.76	124.76	124.76	124.76
G	144.76	144.76	144.76	144.76	144.76	144.76	144.76	144.76	144.76	144.76	144.76	144.76
H	169.77	170.77	170.77	170.77	170.77	170.77	170.77	170.77	170.77	170.77	170.77	170.77

Show Data Status ☐ | Loaded 185 out of 192 images

Inspections

13:29 15-12-2017 (+0.0d) [Manual]

Movie

Gap: 0.1 s

Sample

Protein

TestThaumatococcus

Abundance

Click to edit

Components

No Components

Add:

Sample Name

C2d1_Thaum_Again

Spacegroup

Click to edit

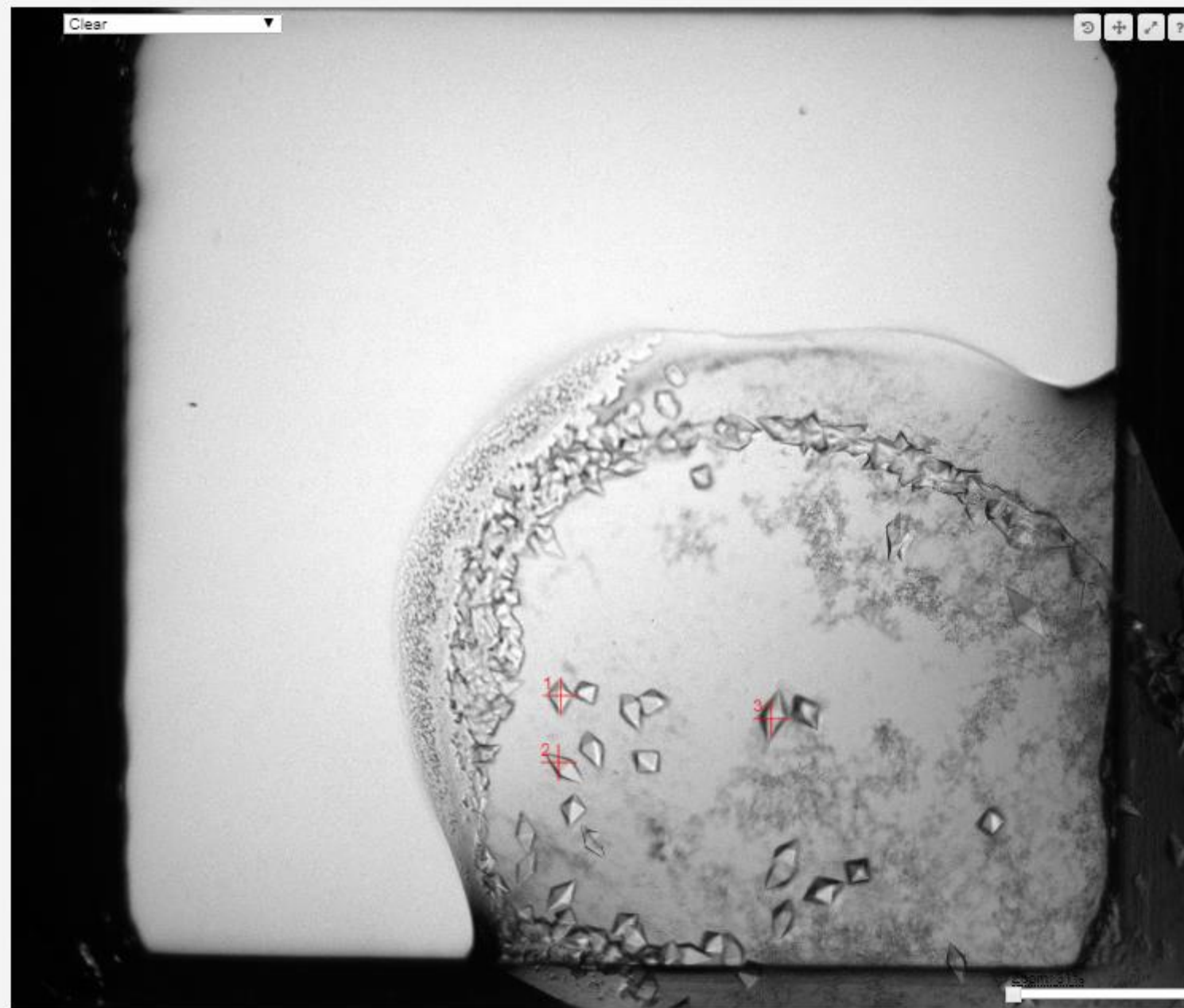
Comments

Click to edit



+0.0d

Marked Sub Samples



This page shows the contents of the selected container. Samples can be added and edited by clicking the pencil icon, and removed by clicking the x

	1	2	3	4	5	6	7	8	9	10	11	12
A	1.12	5.13	5.13	7.13	9.13	10.13	15.13	17.13	17.17	20.17	20.17	22.17
B	24.28	24.28	24.28	31.32	32.32	32.32	35.32	35.32	37.32	41.32	41.32	41.32
C	04.33	5.33	5.33	24.33	27.33	27.33	27.33	27.33	27.33	27.33	27.33	27.33
D	17.34	17.34	17.34	17.34	31.34	32.34	32.34	32.34	32.34	32.34	32.34	32.34
E	05.35	05.35	10.35	10.35	10.35	10.35	10.35	10.35	10.35	10.35	10.35	10.35
F	12.36	12.36	12.36	12.36	12.36	12.36	12.36	12.36	12.36	12.36	12.36	12.36
G	14.36	14.36	14.36	14.36	14.36	14.36	14.36	14.36	14.36	14.36	14.36	14.36
H	10.37	10.37	10.37	10.37	10.37	10.37	10.37	10.37	10.37	10.37	10.37	10.37

Show Data Status ☐

Inspections 13:29 15-12-2017 (+0.0d) [Manual]

Movie Gap: 0.1 s

Sample

Protein TestThaumatin

Abundance Click to edit

Components No Components

Add:

Sample Name C2d1_Thaum_Again

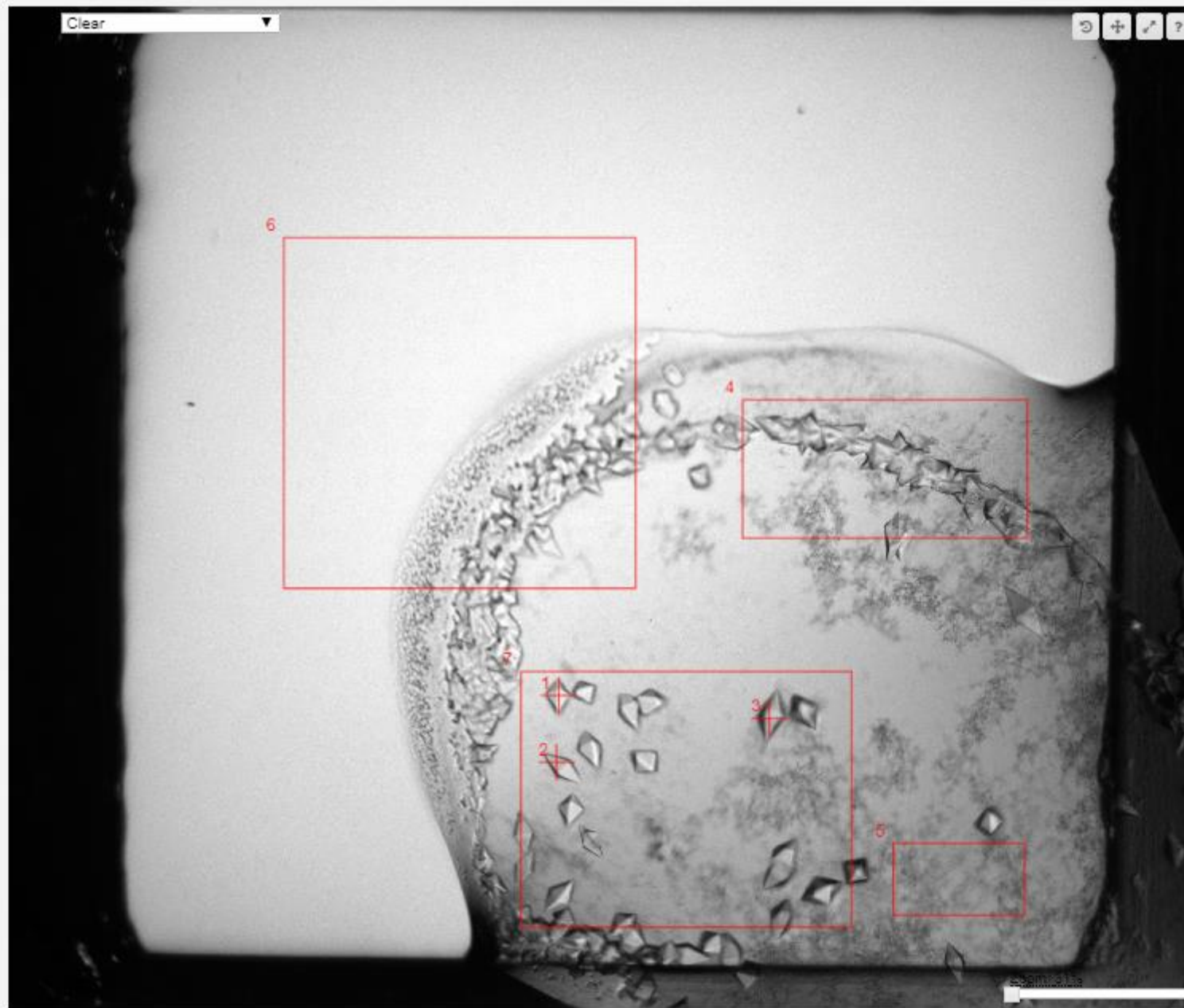
Spacegroup Click to edit

Comments Click to edit



+0.0d

Marked Sub Samples



#	Sample	Protein	Location	Type
51	A8d1_bob	TestThaumatococcus	A8d1	Region
52	A8d1_bob	TestThaumatococcus	A8d1	Region
53	F10d1_bob	TestThaumatococcus	F10d1	Region
54	A1d1_bob	TestThaumatococcus	A1d1	Region
55	A1d1_bob	TestThaumatococcus	A1d1	Region

5 ▾ Page < < 6 > >

Queued Samples

Point Region Invalid

#	Sample	Type
---	--------	------

51 ☐ A8d1_bob Region

55 ☐ A1d1_bob Point

Grid Scan ▾

Box Size 10 x 10

Ω Range 0

Energy 12658 ev

Transmission 20 %

Exposure 0.002 s

Resolution 2.8 Å

Beam Size 10 x 10

No. Images 600

Ω Start -30 °

Ω Range 0.1 °

Ω End 30 °

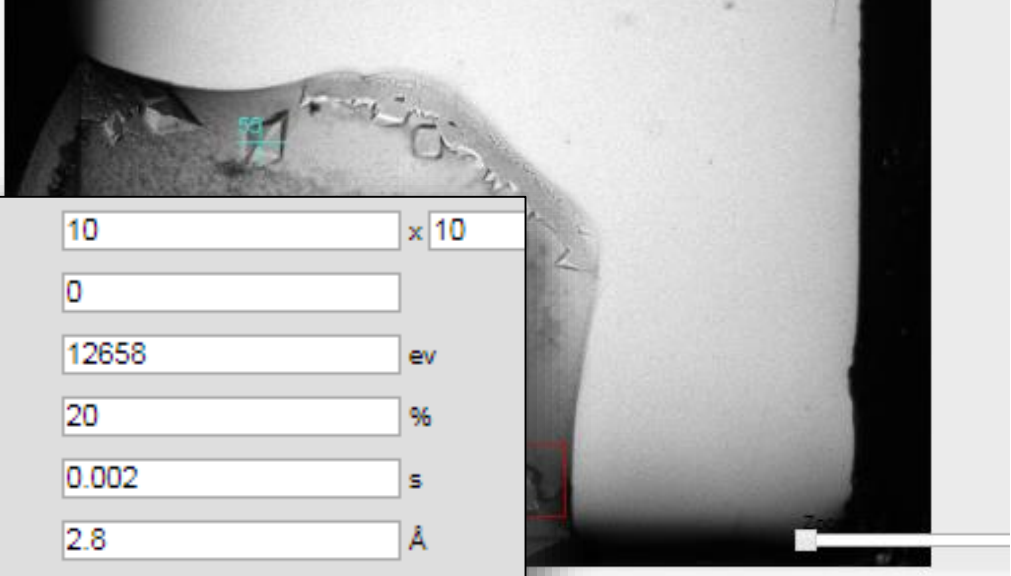
Energy 12658 ev

Transmission 100 %

Exposure 0.0014 s

Resolution 2.8 Å

Point Collection ▾

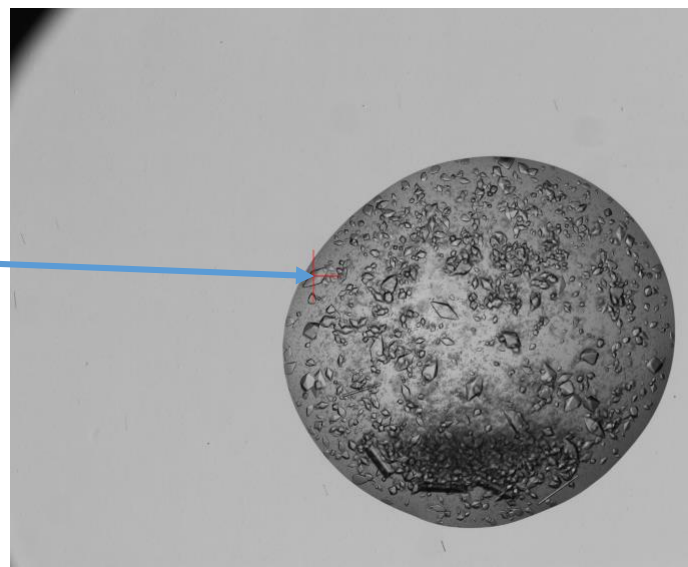
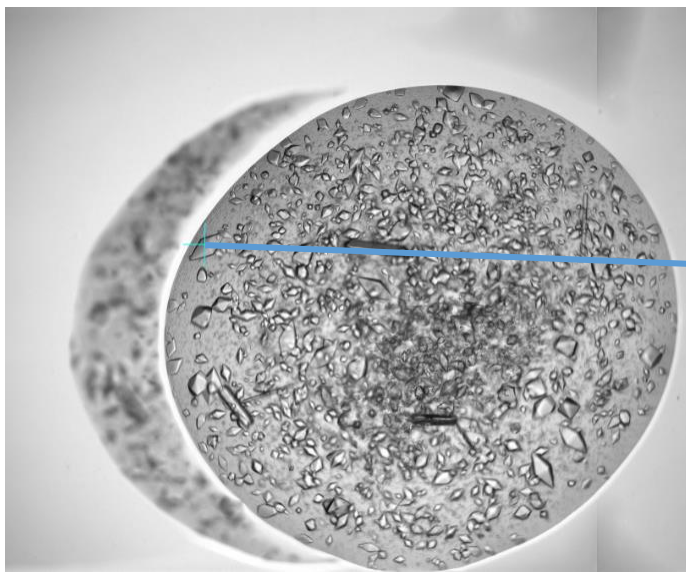
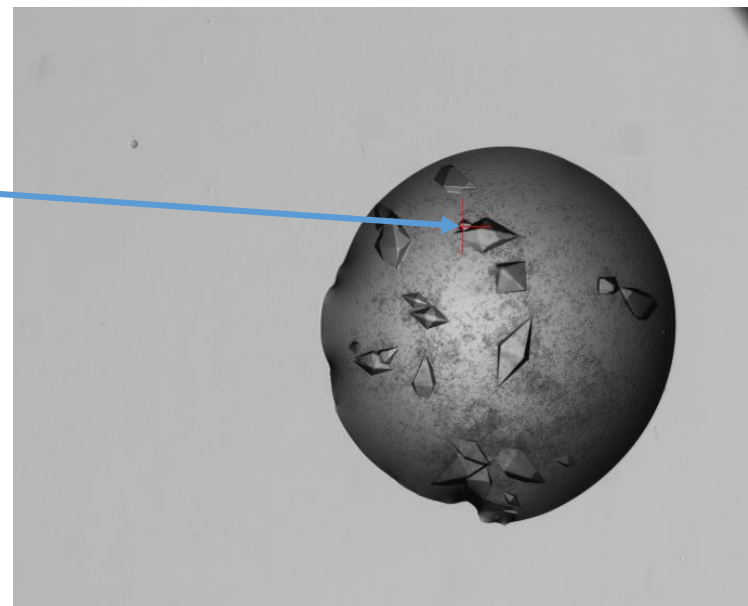
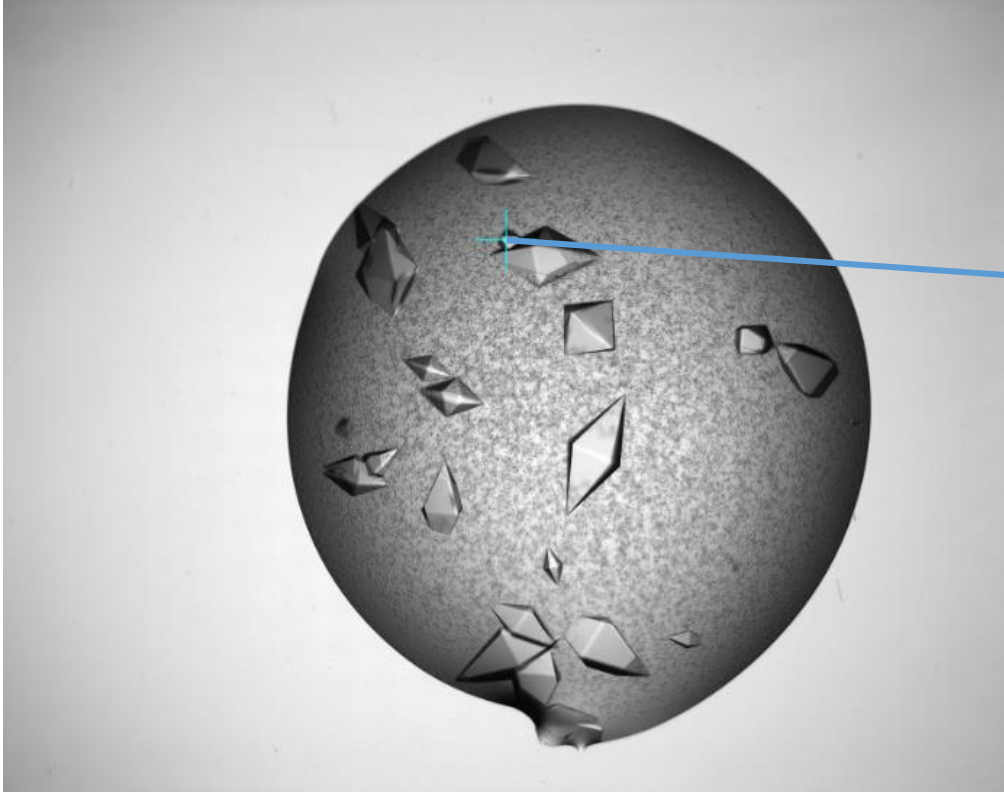


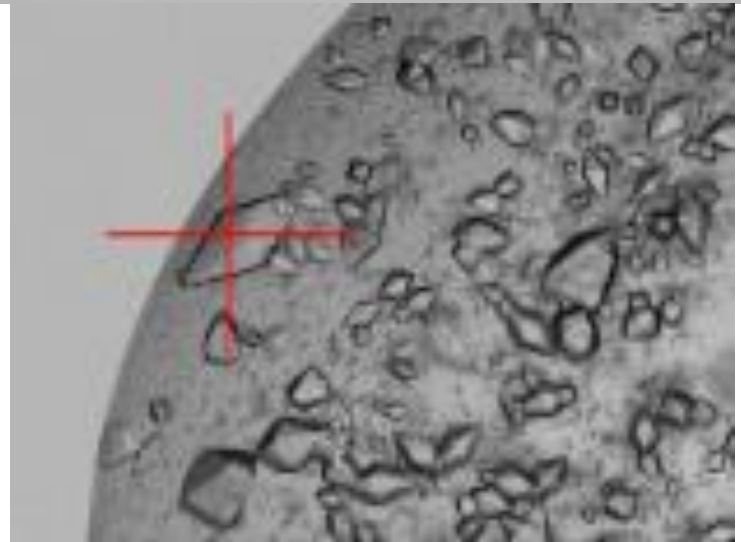
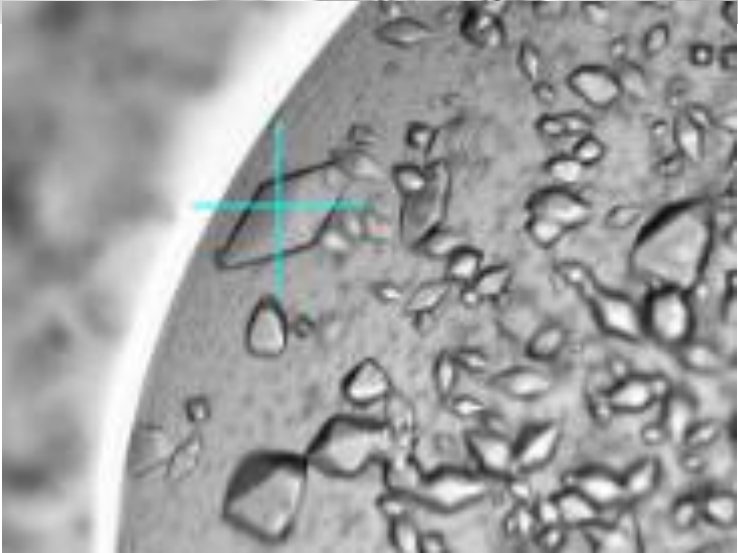
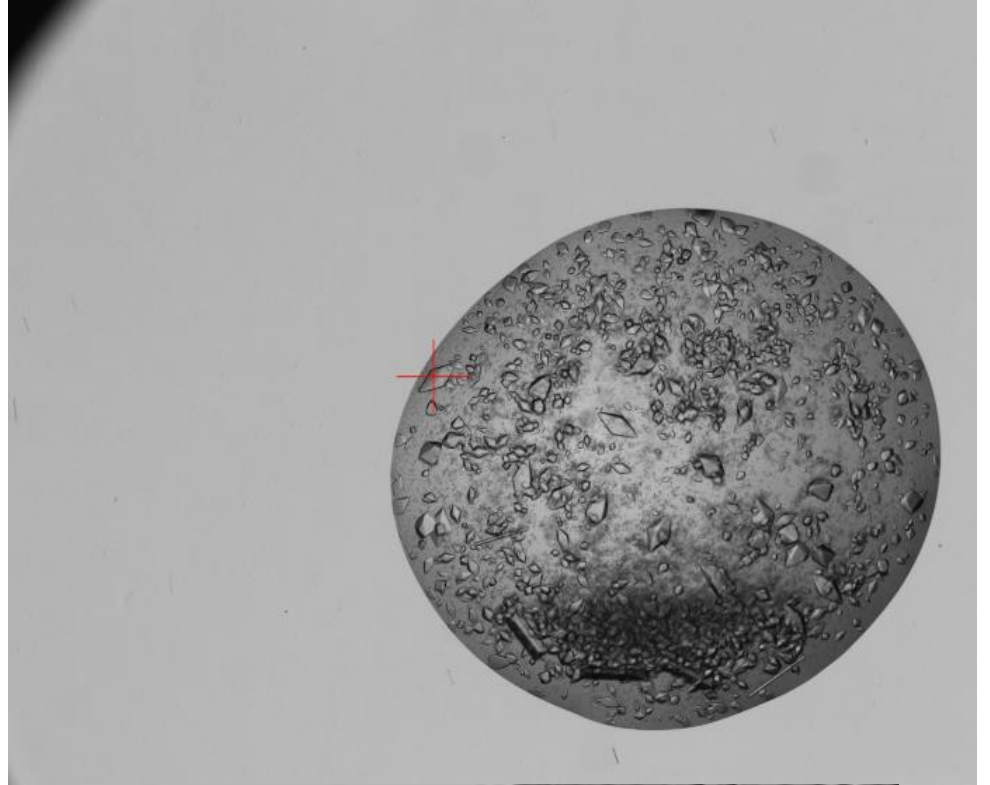
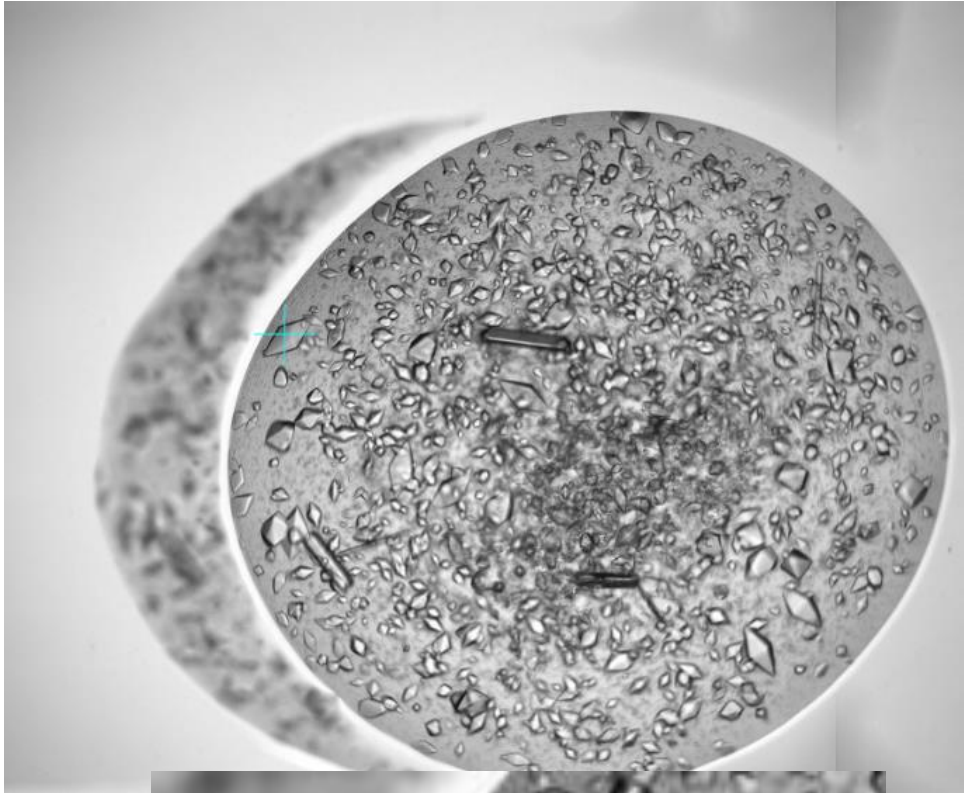
Presets: Point1 ▾

Valid

✓ + -

✓ + -





LIVE HISTORY

Start

Stop

Message	Timestamp	Duration
1 Matching Started	2020-12-09 22:41:15.290	0
2 FFT calculation finished	2020-12-09 22:41:29.200	13
3 Alignment Complete	2020-12-09 22:41:59.327	44
4 Crystal Match Complete	2020-12-09 22:41:59.453	44

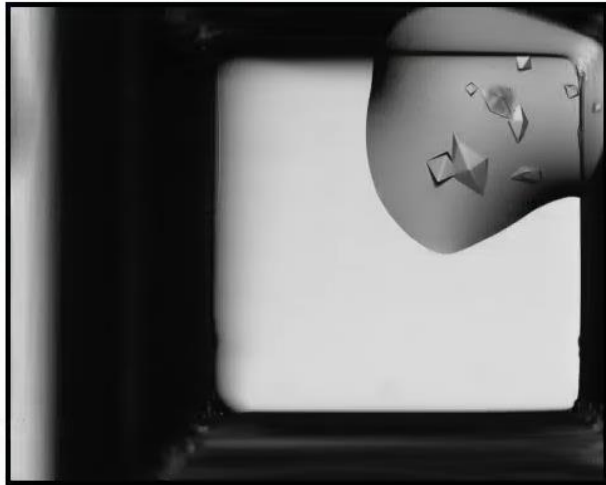
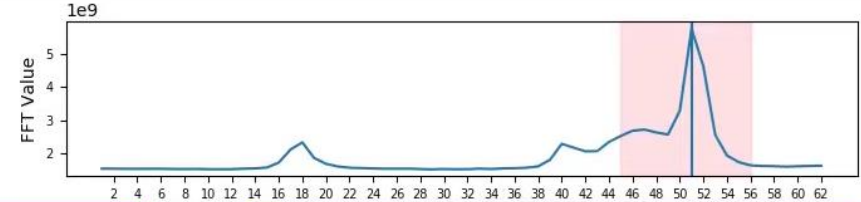
Alignment info

Alignment score: 18.64123051209382

Status: 2, Good Alignment

Scale: 0.6349

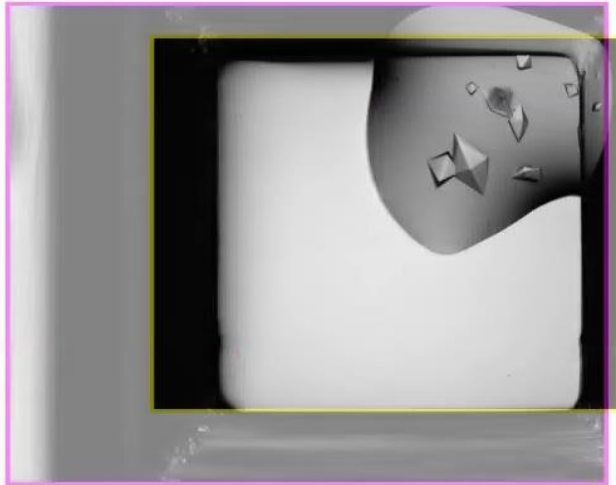
Transform coordinates: x: 667.0000, y: 152.0000



Best FFT: /dls/mx/data/nt24686/nt24686-72/VMXI-AB1396/well_6/zstack_20201209_224015/stack/stack_image_51.tif

Beamline processed: /dls/mx/data/nt24686/nt24686-72/VMXI-AB1396/well_6/zstack_20201209_224015/processed.tif

Formulatrix: /dls/mx/data/nt24686/nt24686-72/imaging/187946/65522/17768708.jpg

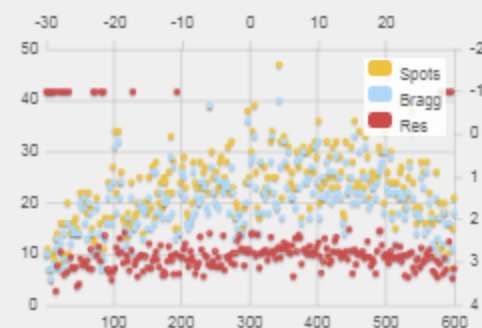
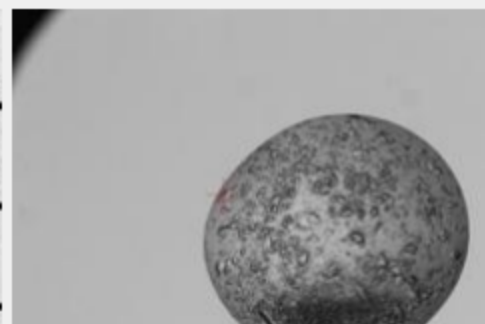
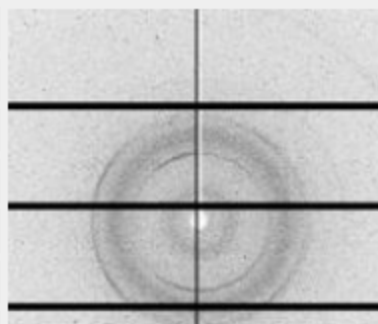


Overlap

Differences

12-12-2019 12:07:37 - VMXi-AB1165/well_61/images/image_42438_master.h5

Sample: C7d1_TestThaumatococcus
 Flux: NaN
 Ω Start: -30.0°
 Ω Osc: 0.10°
 Ω Overlap: 0°
 No. Images: 600
 Resolution: 2.45Å
 Wavelength: 0.9795Å
 Exposure: 0.002s
 Transmission: 5.00%
 Beamsize: 0x0µm
 Type: SAD
 Comment:



Auto Processing

Fast DP: ☒ Xia2/3dii: ☒ DIALS: ☒ Xia2/Multiplex: ☒ autoPROC: ☒

Type	Resolution	Spacegroup	Mn<4/sig(I)>	Rmeas Inner	Rmeas Outer	Completeness	Cell	Status
fast_dp	29.24 - 2.12	P 4 2 2	10.0	0.036	0.669	89.3	58.48 58.48 151.29 90.00 90.00 90.00	processing successful
xia2 dials	54.54 - 1.76	P 41 21 2	7.3	0.069	1.271	63.2	58.46 58.46 151.26 90.00 90.00 90.00	processing successful
xia2 3dii	46.27 - 1.99	P 41 21 2	8.4	0.061	0.718	83.2	58.48 58.48 151.29 90.00 90.00 90.00	processing successful
autoPROC	54.55 - 1.82	P 41 21 2	7.5	0.058	0.936	70.3	58.48 58.48 151.29 90.00 90.00 90.00	processing successful
autoPROC+STARANISO	54.55 - 1.95	P 41 21 2	8.6	0.053	0.680	84.4	58.48 58.48 151.29 90.00 90.00 90.00	processing successful

fast_dp xia2 dials xia2 3dii autoPROC autoPROC+STARANISO

Beam Centre	X	Y
Start	1,055.40	1,166.50
Refined	79.19	87.56
Δ	NaN	NaN

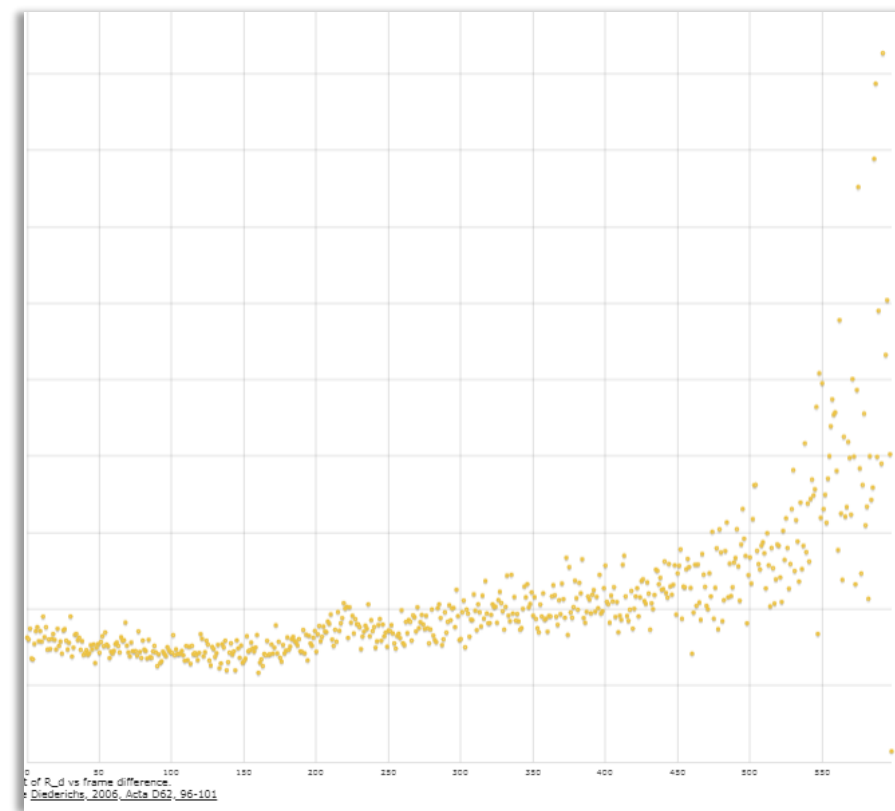
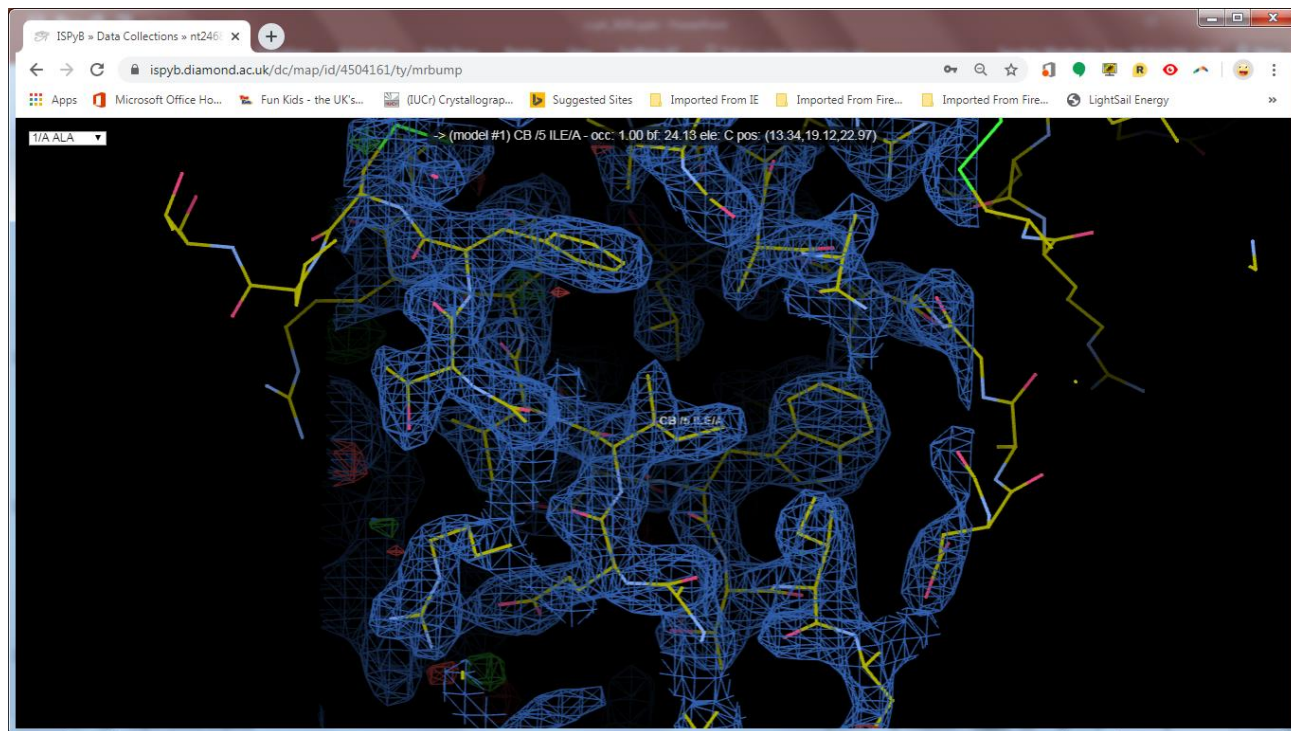
Space Group	A	B	C	α	β	γ
P 41 21 2	58.48	58.48	151.29	90.00	90.00	90.00

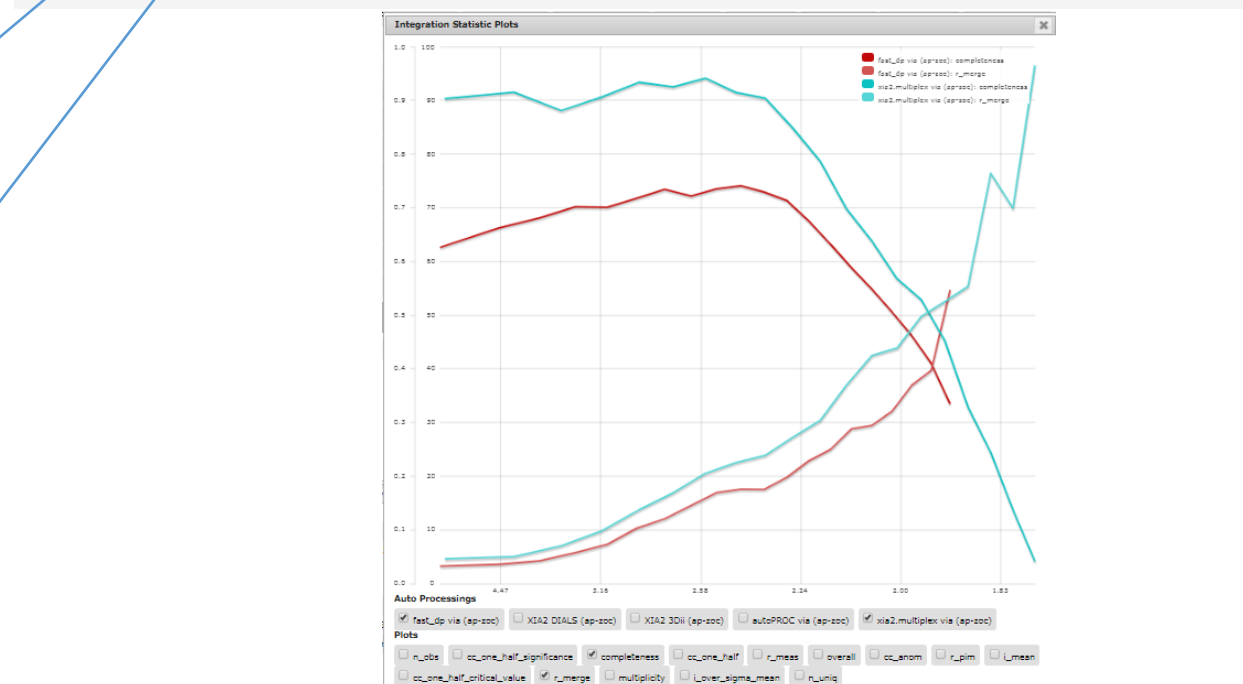
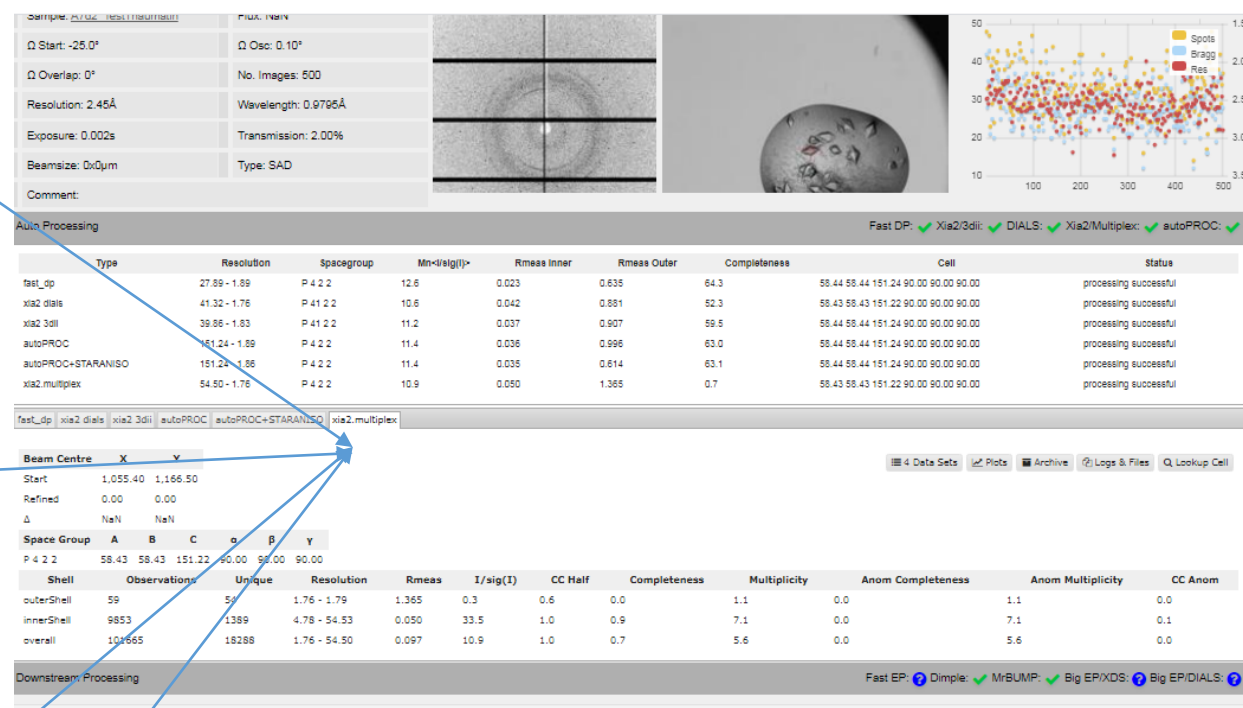
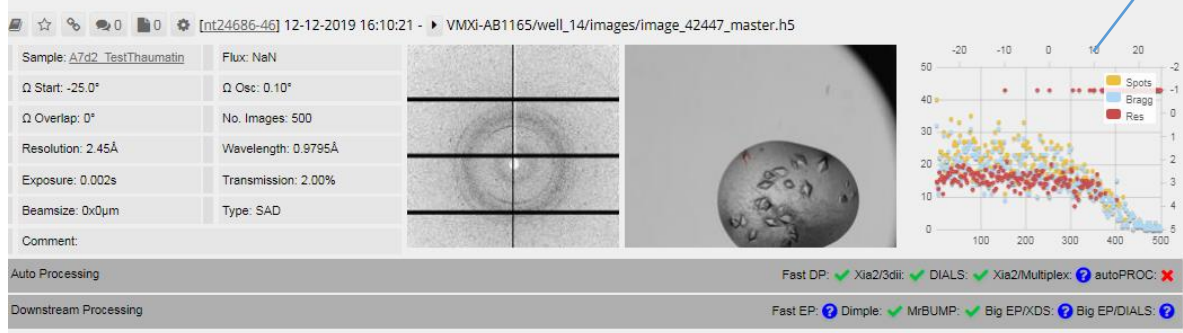
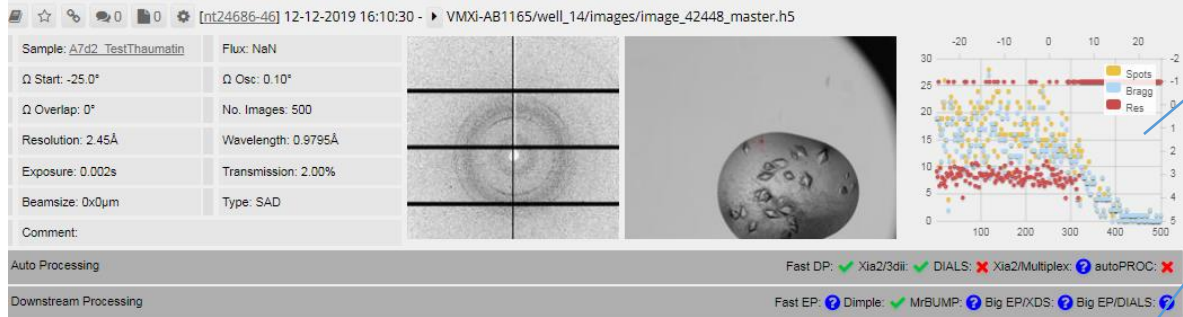
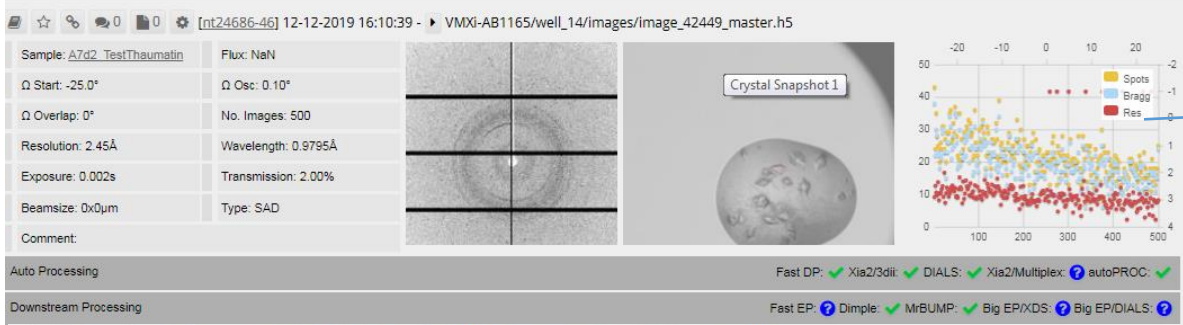
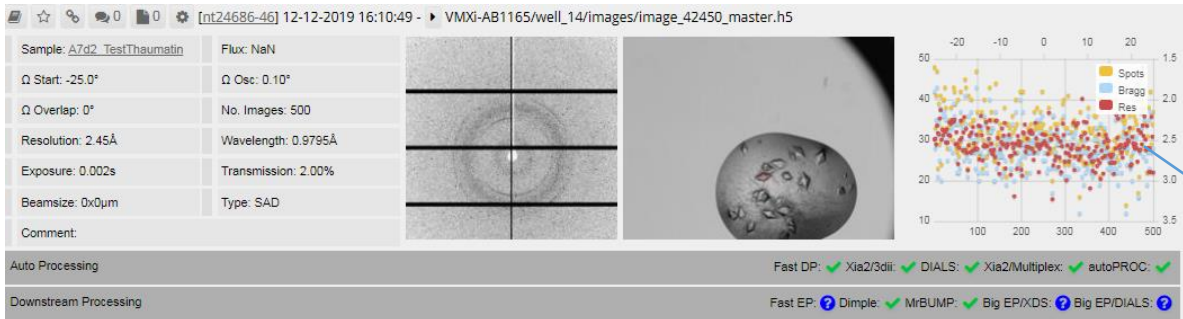
Shell	Observations	Unique	Resolution	Rmeas	I/sig(I)	CC Half	Completeness	Multiplicity	Anom Completeness	Anom Multiplicity	CC Anom
outerShell	921	426	1.99 - 2.02	0.718	1.1	0.6	46.3	2.2	24.2	1.5	0.1
innerShell	4102	1013	5.40 - 46.28	0.061	20.9	1.0	94.7	4.0	81.0	2.7	0.2
overall	61468	15663	1.99 - 46.27	0.125	8.4	1.0	83.2	3.9	70.0	2.3	0.2

Downstream Processing

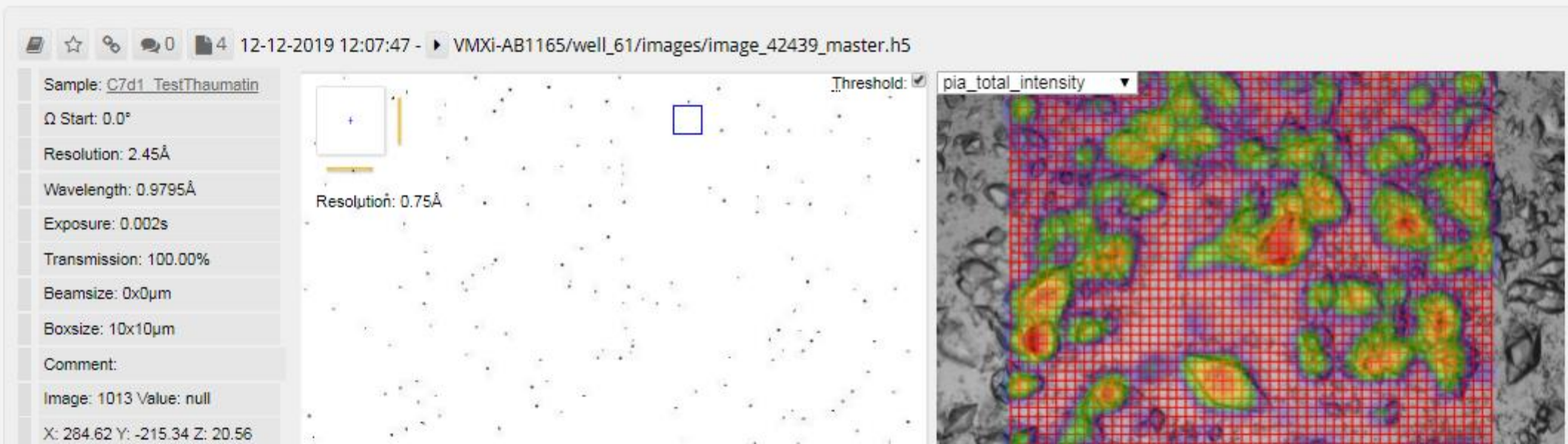
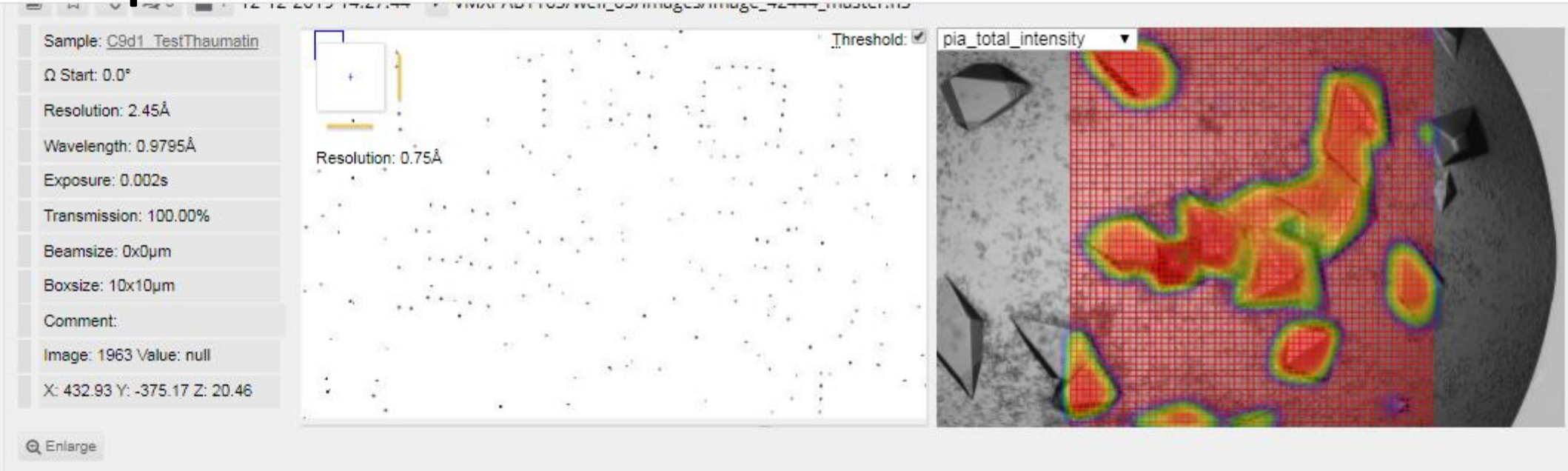
Fast EP: ☒ Dimple: ☒ MrBUMP: ☒ Big EP/XDS: ☒ Big EP/DIALS: ☒

ac.uk/cell/a/58.48/b/58.48/c/151.29/a/90.00/be/90.00/ga/90.00





Examples



Protein	Plate	No. of Datasets on Plate	Visit	Best Res.	Image	Well Number
SDZ-MPRO	VMXi-AB1362	72	mx27088-18	2.28	image_45854_master.h5	73
MPRO	VMXi-AB1361	19	mx27088-19	2.53	image_45894_master.h5	54
PLPRO	VMXi-AB1359	235	mx27088-21	2.23	image_45900_master.h5	54
PLPRO	VMXi-AB1356	214	mx27088-24	3.18	image_45396_master.h5	105

Covid-19 @ VMXi July 2020

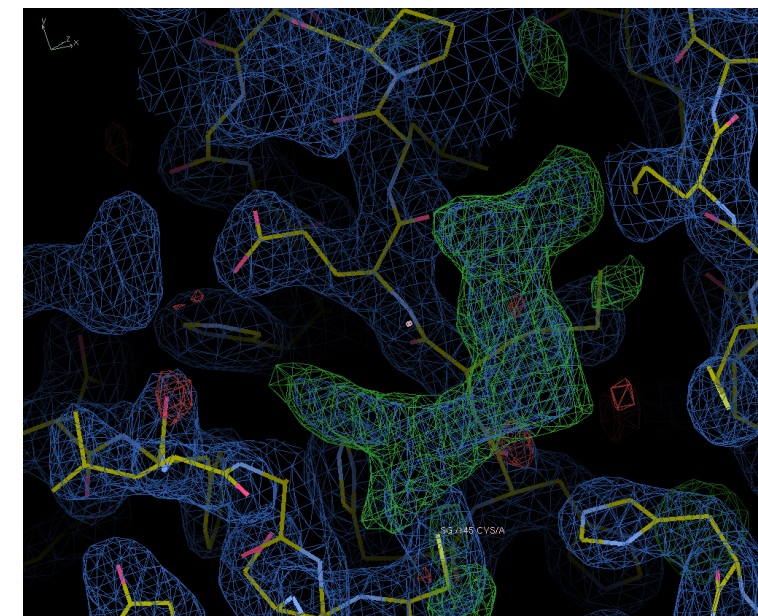
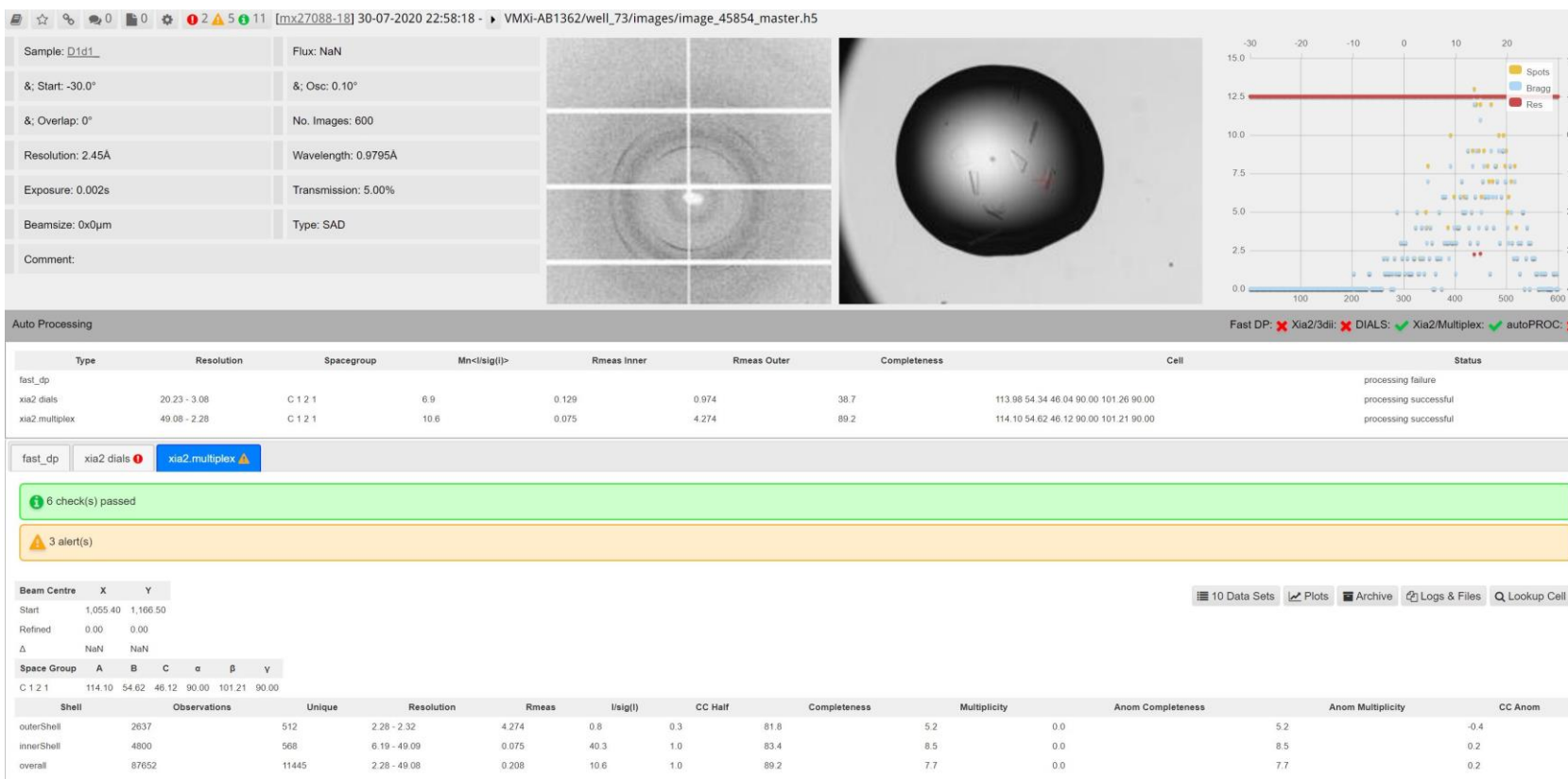
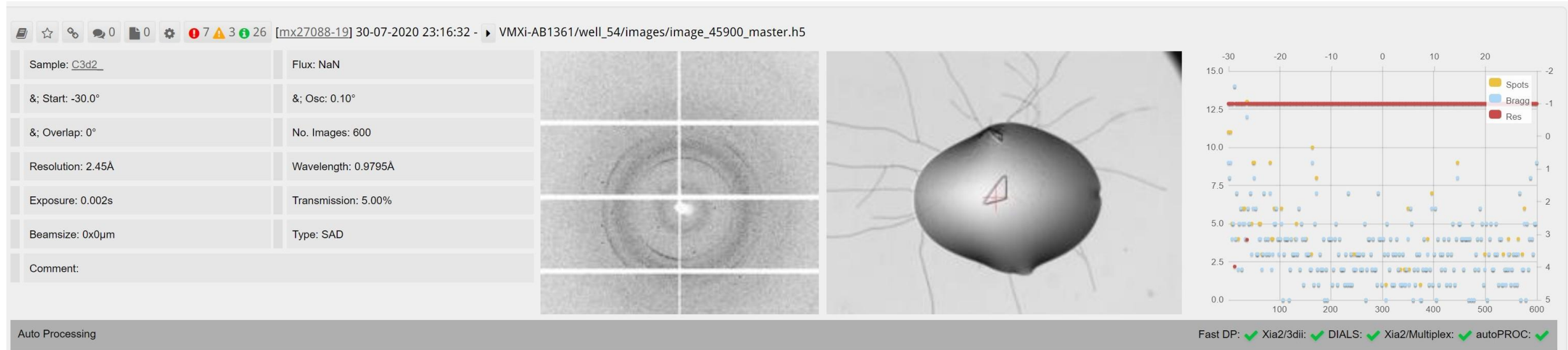


Figure kindly provided by David Owen

Datasets from this drop gave rise to a 2.28 Å structure of MPRO with a ligand (SDZ) bound – each sweep of data were collected in ~1 second, using 5% beam transmission collected using a DMM monochromator on an Eiger 2X 4M detector.

Protein	Plate	No. of Datasets on Plate	Visit	Best Res. (Å)	Image	Well Number
SDZ-MPRO	VMXi-AB1362	72	mx27088-18	2.28	image_45854_master.h5	73
MPRO	VMXi-AB1361	19	mx27088-19	2.53	image_45894_master.h5	54
PLPRO	VMXi-AB1359	235	mx27088-21	2.23	image_45900_master.h5	54
PLPRO	VMXi-AB1356	214	mx27088-24	3.18	image_45396_master.h5	105

Covid-19 @ VMXi July 2020



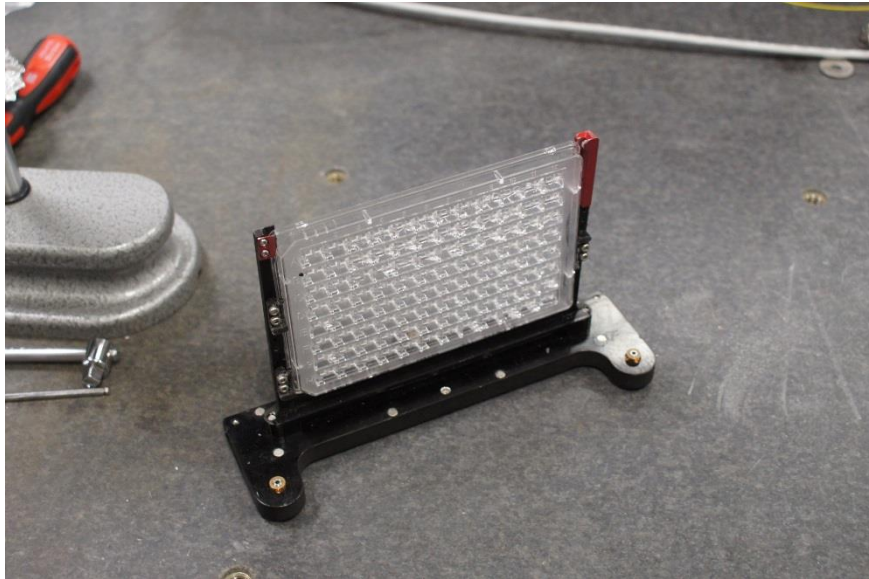
Example PLPro Dataset collected in ~ 1 second using 5% beam on VMXi. Data from multiple collections were automatically merged to generate a dataset to 2.23 Å.

530 datasets collected in just over four hours (with no human interaction) following marking of samples.

Plates moved from Formulatrix storage units to the beamline, data were collected and plates safely returned post collection.

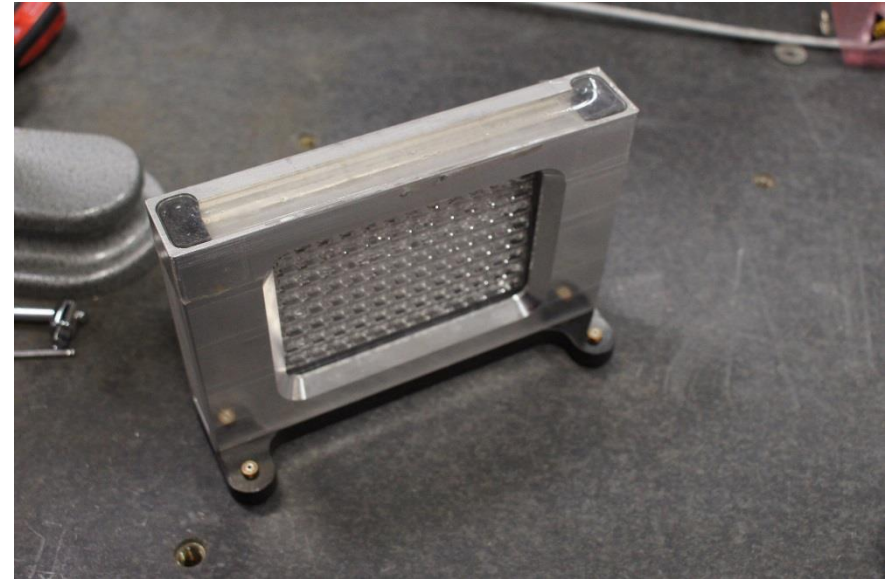
Future updates (4C plate handling)

Present 20°C



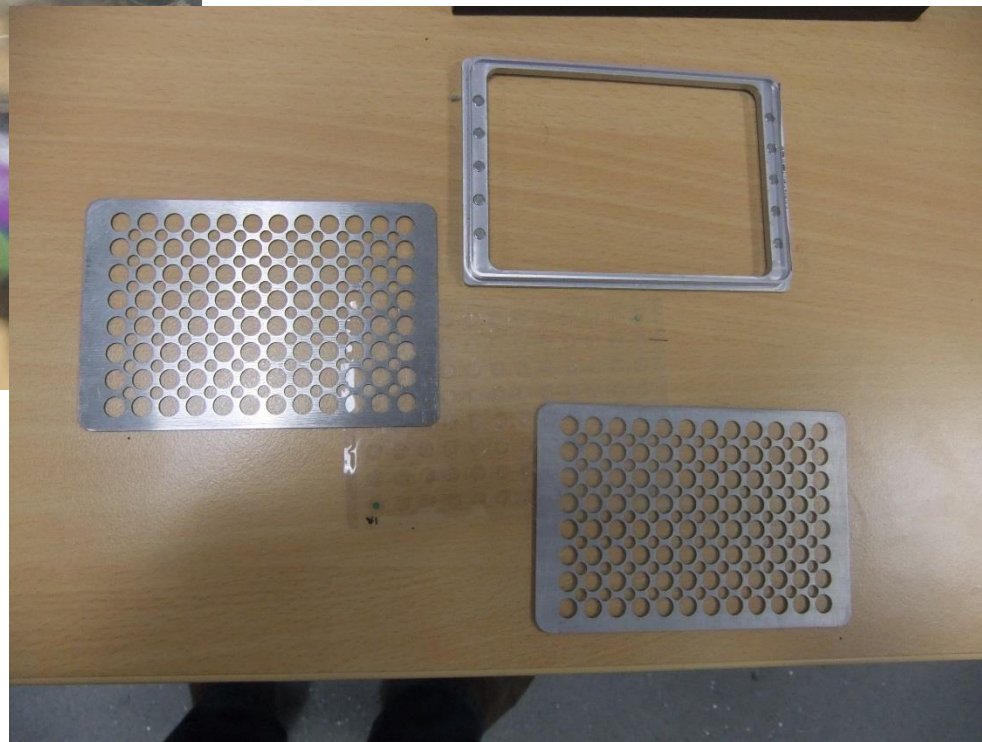
Tested substantially and working reliably

Future 4°C



Based on passive cooling using water
Should maintain a plate ~4°C for 20-30 minutes
Requires major software improvements and testing

Future updates (Film crystallography)



What to remember about VMXi



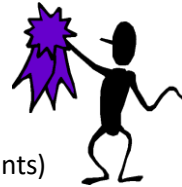
- The beamline is fully automated:

- Easy and fast to use
- “All” you need is a plate or protein
- From click to structure in minutes
- Not requiring fishing, cooling, cryo-optimising transporting, loading, etc.
- Most data should auto process (even for multiple datasets)
- Making every sample count



- Uses:

- Good for optimising crystals
- Good for fast turnaround of structures
- Good for collecting multiple states
- Good for binding studies (ligands/fragments)



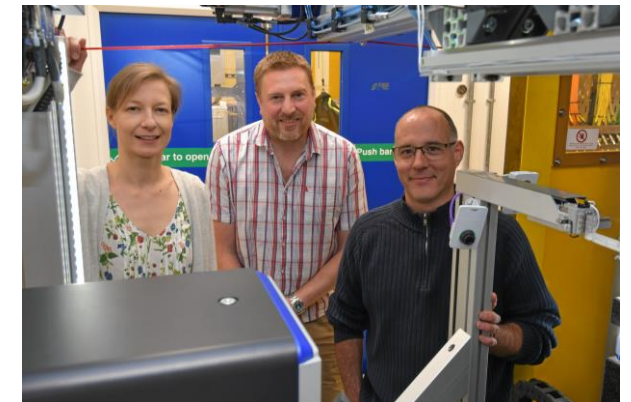
- Data:

- Data is “un-altered” by cryocooling and of good quality
- Potentially you may find new biologically relevant states
- Caution with radiation damage
- Will require multiple crystals



- Future

- X-Chem integration
- More software automation (crystal finding, etc)
- 4°C samples
- Films
- Serial Microcrystal automatic processing



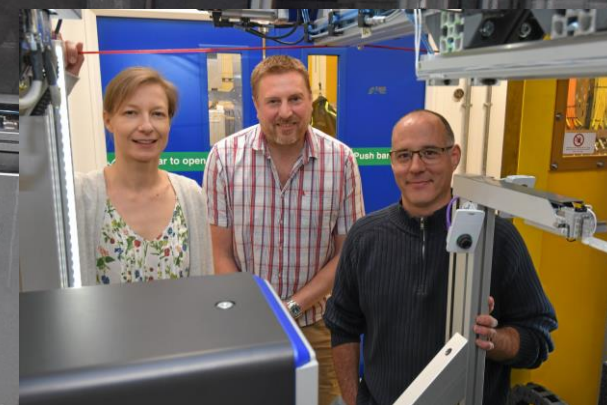
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