

VMXi – Fully automated room-temperature MX

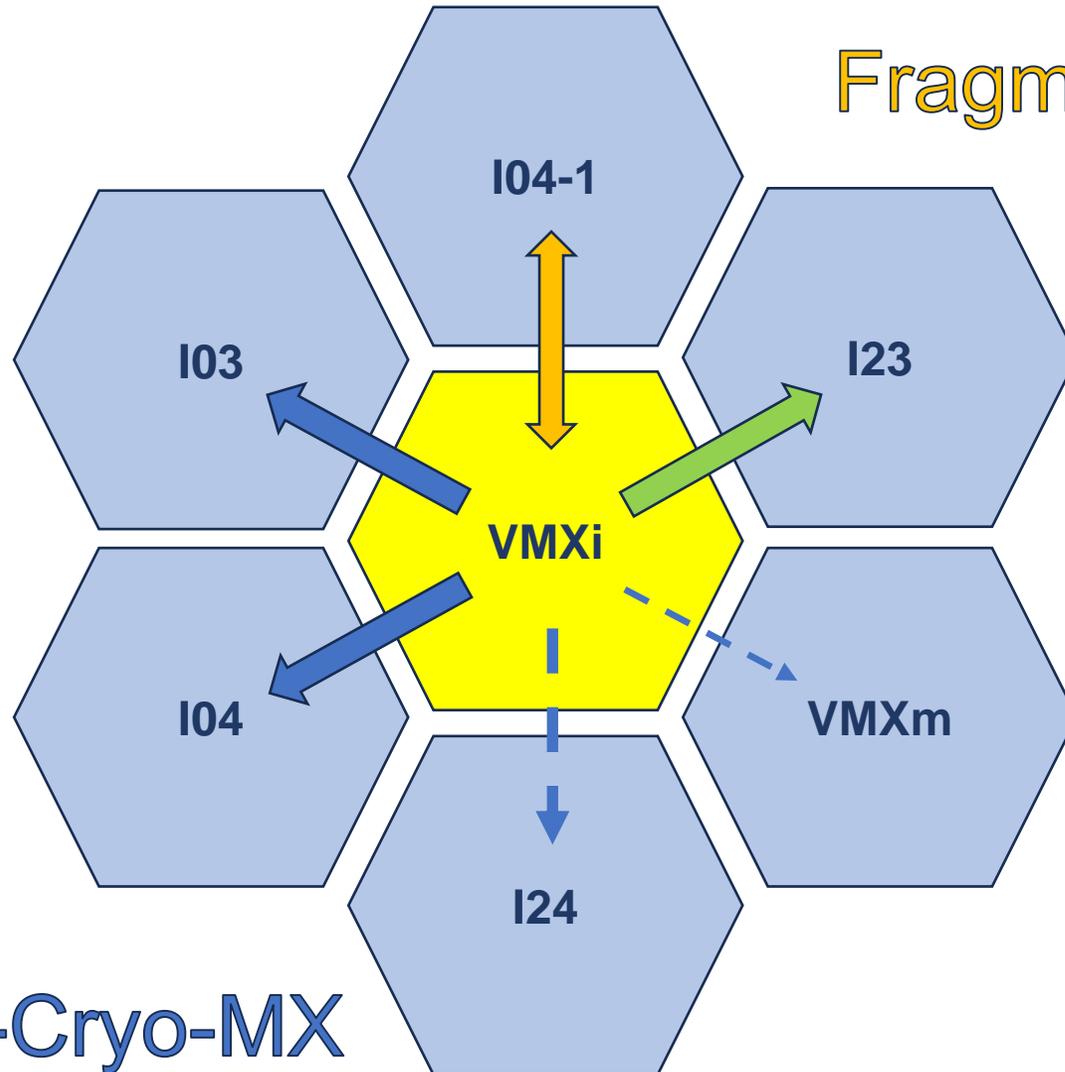


VMXi – Centrepiece of Diamond MX

Flux 2×10^{13} ph/sec
16 KeV
Beam size $10 \times 10 \mu\text{m}$

Cryo-MX

Micro-Cryo-MX



Fragments / XChem

Phasing

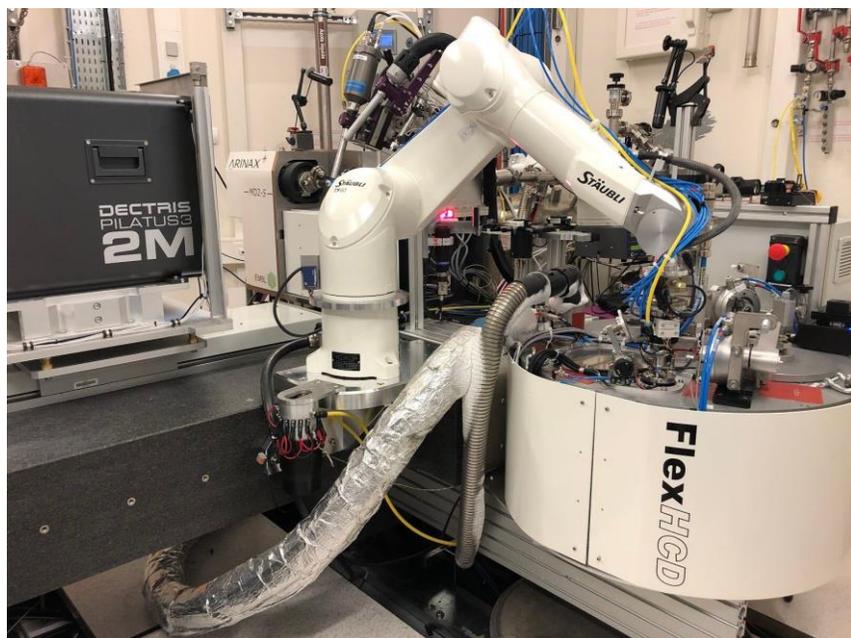
Nano-Cryo-MX

Automated MX beamlines

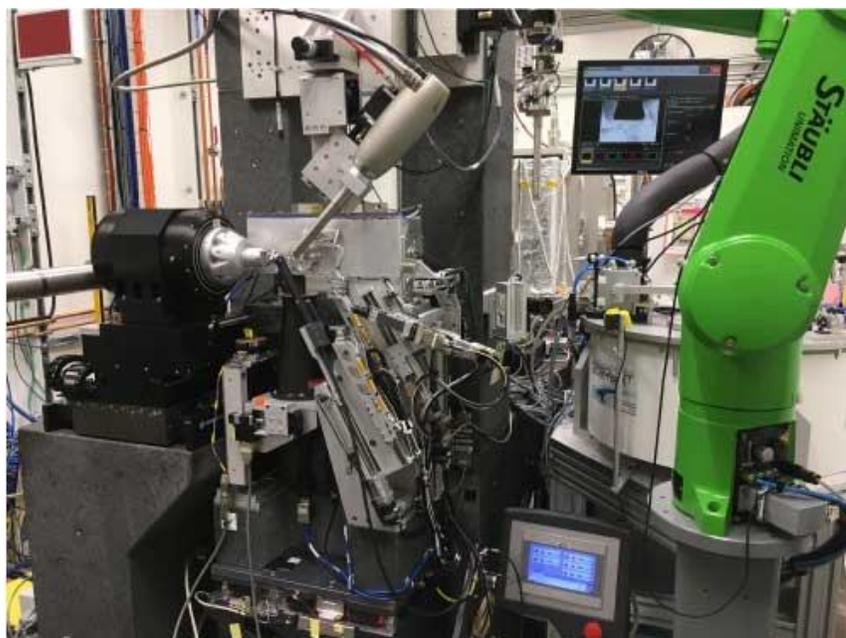
Bowler MW, Nurizzo D et al. (2015). MASSIF-1:A beamline dedicated to the fully automatic characterisation and data collection from crystals of biological macromolecules, *J. Synchrotron Rad.* **22**, 1540-1547.

M Fuchs, R Sweet et al. (2014). NSLS-II MX Beamlines FMX for Micro-crystallography & AMX for Highly Automated MX. *Acta Cryst.* **A70**, C1733.

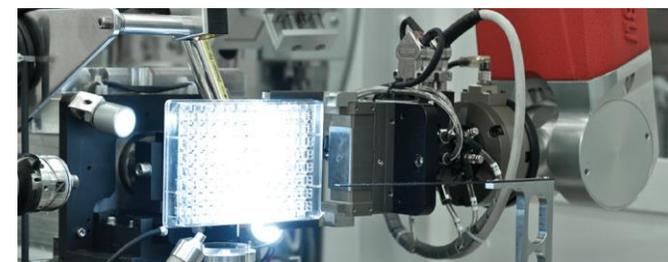
R. Bingel-Erlenmeyer, V. Olieric, et al. (2011). SLS Crystallization Platform at Beamline X06DA—A Fully Automated Pipeline Enabling *in Situ* X-ray Diffraction Screening, *Cryst. Growth Des.* **11**, 4, 916–923.



MASSIF-1 - ESRF



AMX – NSLS-II

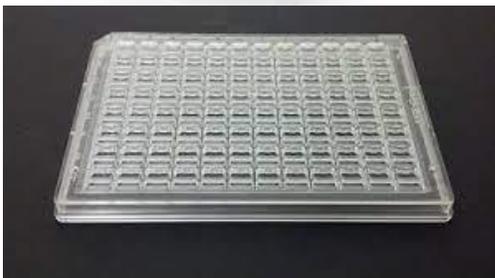
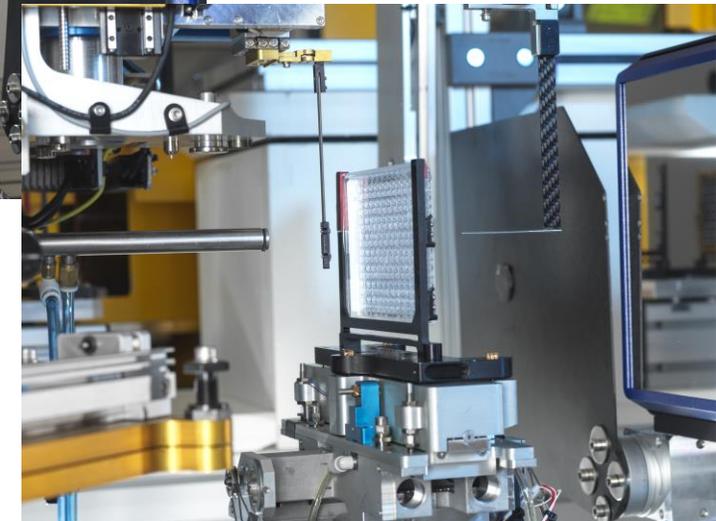
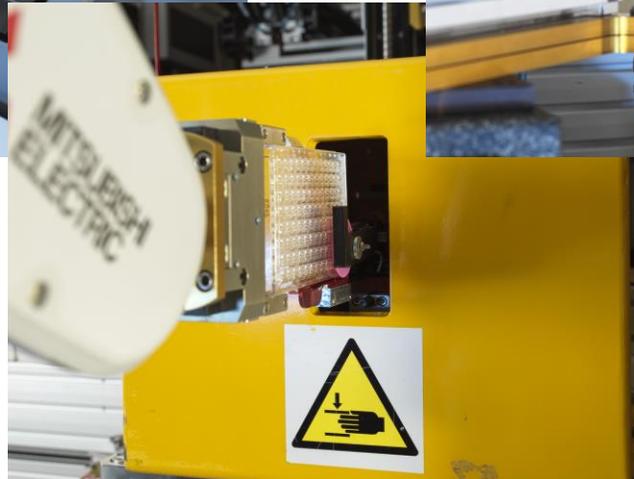
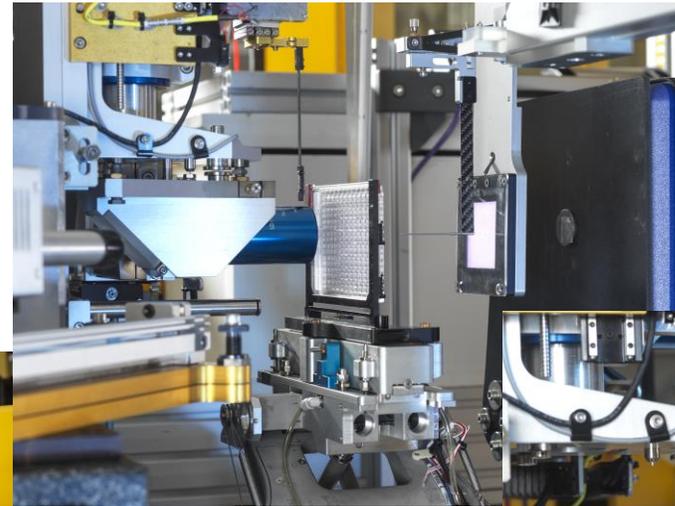
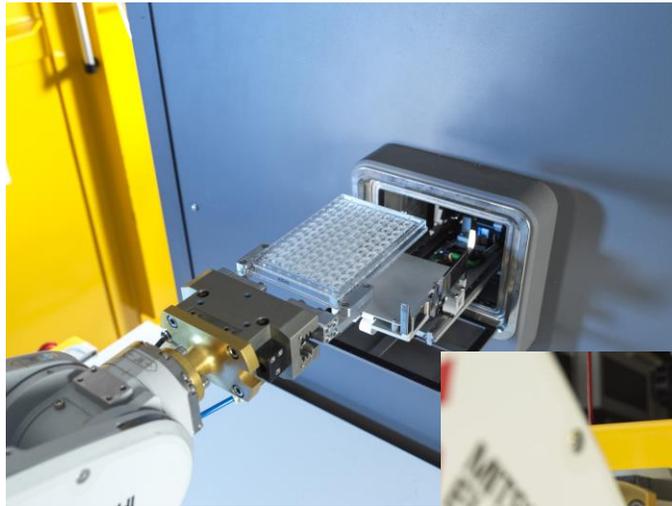


X06DA - PSI

UDC at Diamond now extremely common



Fully-automated room temperature MX



Sanchez-Weatherby J, Sandy, J, et al. (2019). VMXi: a fully automated, fully remote, high-flux *in situ* macromolecular crystallography beamline. [J Synchrotron Radiation 26\(1\):291-301](#)

H. Mikolajek, J. Sanchez-Weatherby, et al. (2023). Protein-to-structure pipeline for ambient-temperature *in situ* crystallography at VMXi. [IUCr 10: 420-429](#)

SynchWeb VMXi Interface



Container: 7259_thaum

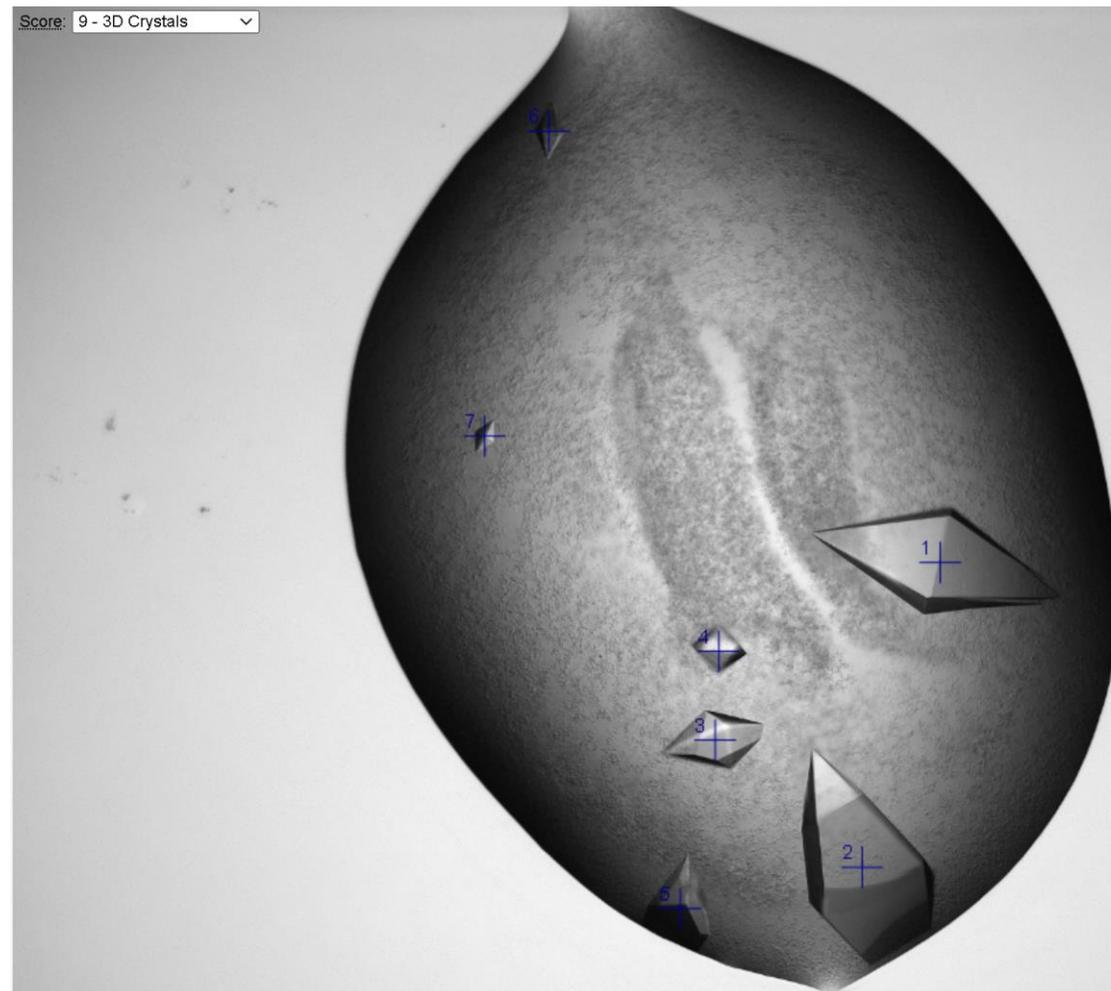
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B	25 26	27 28	29 30	31 32	33 34	35 36	37 38	39 40	41 42	43 44	45 46	47 48
C	49 50	51 52	53 54	55 56	57 58	59 60	61 62	63 64	65 66	67 68	69 70	71 72
D	73 74	75 76	77 78	79 80	81 82	83 84	85 86	87 88	89 90	91 92	93 94	95 96
E	97 98	99 100	101 102	103 104	105 106	107 108	109 110	111 112	113 114	115 116	117 118	119 120
F	121 122	123 124	125 126	127 128	129 130	131 132	133 134	135 136	137 138	139 140	141 142	143 144
G	145 146	147 148	149 150	151 152	153 154	155 156	157 158	159 160	161 162	163 164	165 166	167 168
H	169 170	171 172	173 174	175 176	177 178	179 180	181 182	183 184	185 186	187 188	189 190	191 192

Display
 Current Score
 Max Scores
 Data Status Rank By:
 CHIMP Auto Scores Class:

Inspections +

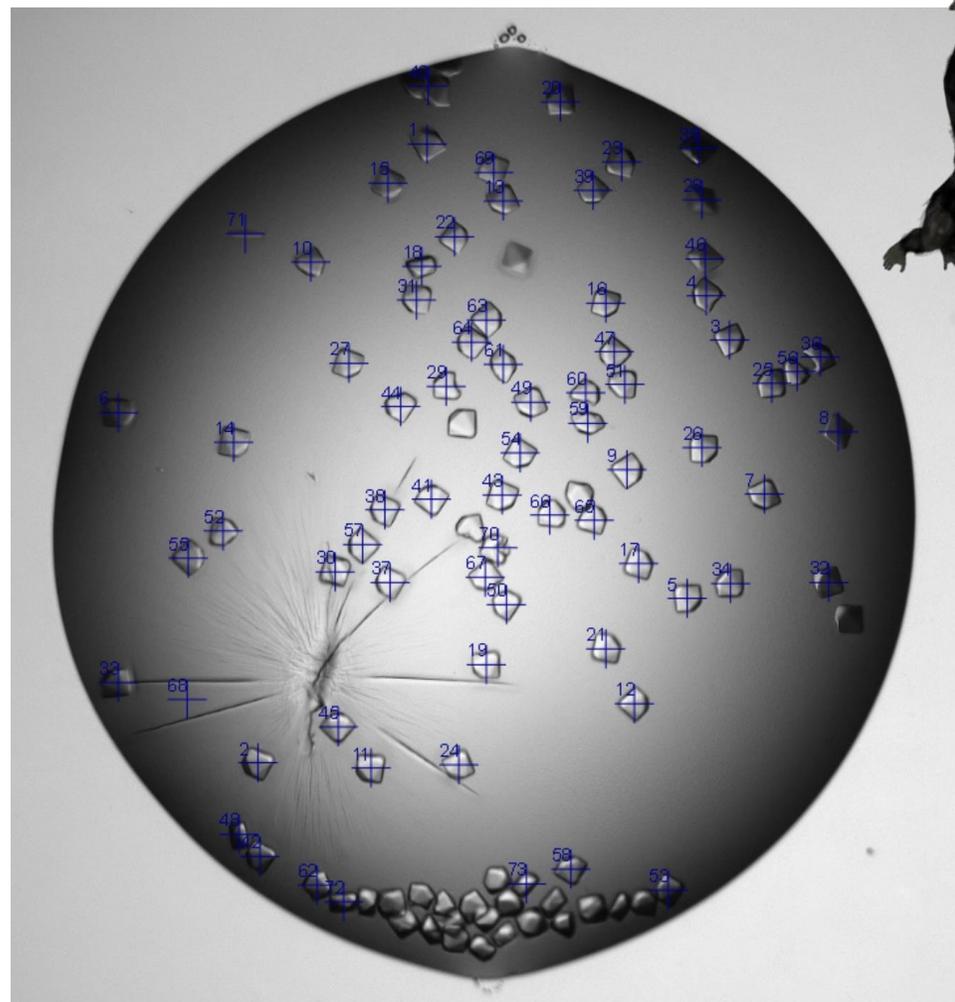
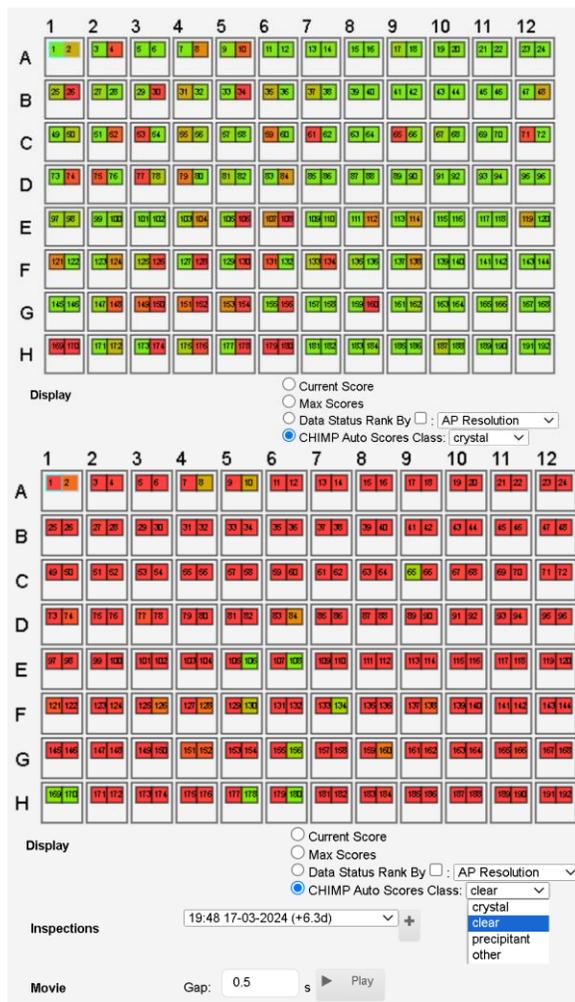
Movie Gap: s

Sample
Protein TestThaumatoin
Abundance



- Registration/management of experimental plates
- Allows rapid analysis of crystallisation experiment
- Manual/Auto scoring
- Simple selection of point/region(s)
- Data collection and analysis of results

CHiMP – Crystal hits in my plates



- Based on MARCO algorithm
- Finds “objects” for users
- Up to 100 objects per drop marked
- Soon to be replaced by Chimp-Chomp – multithreaded update
- Developing new uses for this AI algorithm – ECHO, size of object etc

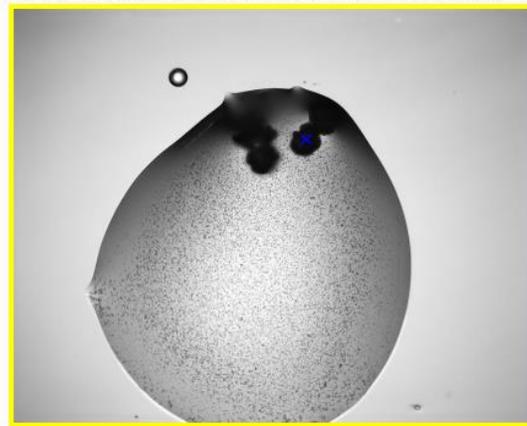
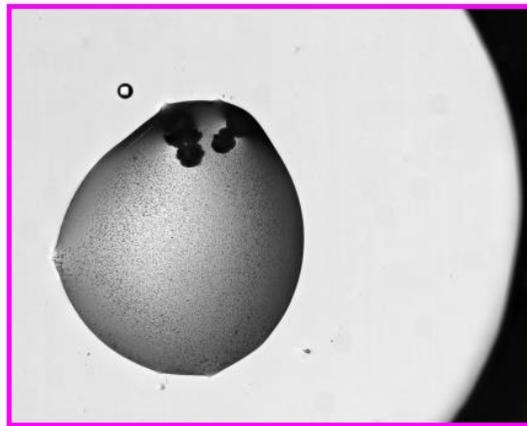
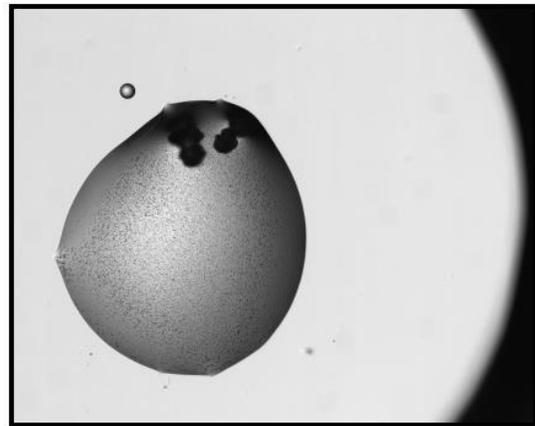
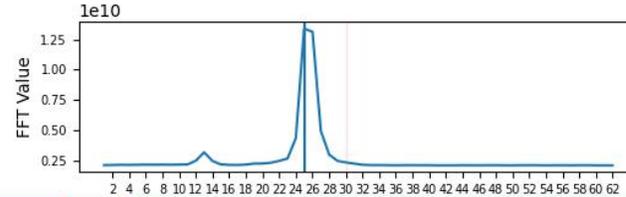
King, O.N.F., Levik, K.E. et al. (2024) CHiMP: deep-learning tools trained on protein crystallization micrographs to enable automation of experiments. *Acta Cryst. D80*: 744-764



Bruno AE, Charbonneau P, et al. (2018). Classification of crystallization outcomes using deep convolutional neural networks. *PLOS ONE* 13(6): e0198883

CrystalMatch

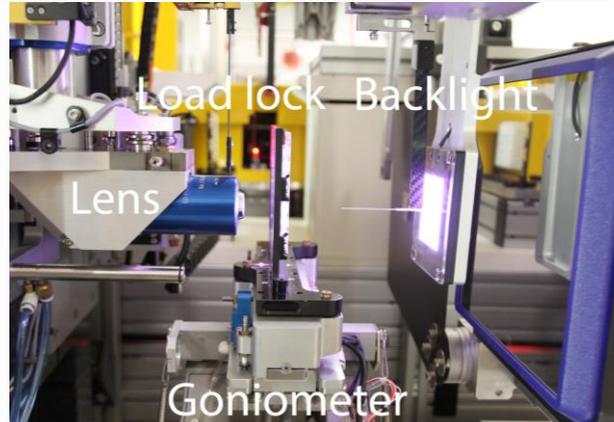
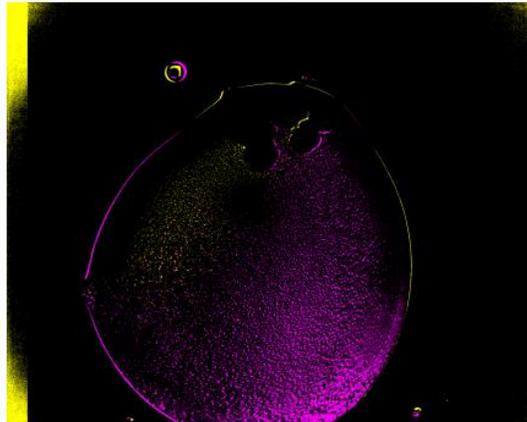
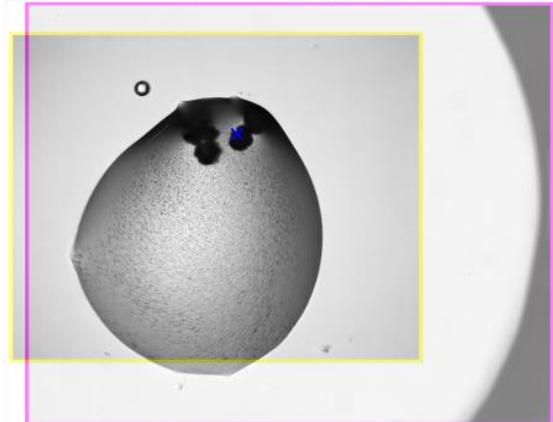
Message	Timestamp	Duration	Alignment info
1 Matching Started	2024-11-14 10:52:48.346	0	Alignment score: 32.849610726007036
2 FFT calculation finished	2024-11-14 10:52:53.993	5	Status: 2, Good Alignment
3 Alignment Complete	2024-11-14 10:52:58.194	9	Scale: 0.6349
4 Crystal Match Complete	2024-11-14 10:52:58.198	9	Transform coordinates: x: -84.0000, y: 157.0000



Best FFT: /dls/mx/data/nr27313/nr27313-389/VMXI-AB7324/well_102/zstack_20241114_105218/stack/stack image 25.tif

Beamline Processed: /dls/mx/data/nr27313/nr27313-389/VMXI-AB7324/well_102/zstack_20241114_105218/processed.tif

Formulatrix: /dls/mx/data/nr27313/nr27313-389/imaging/315839/151391/33692794.jpg

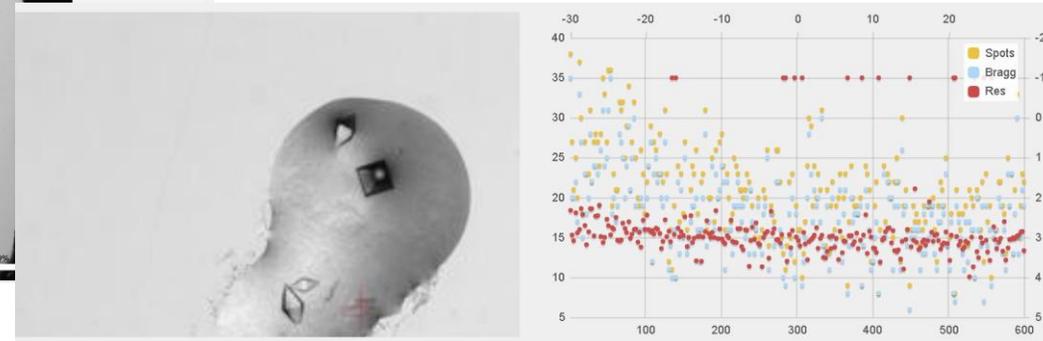
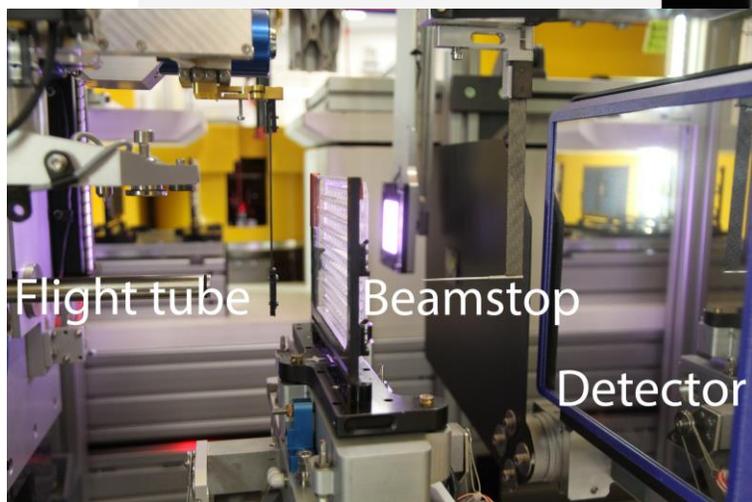
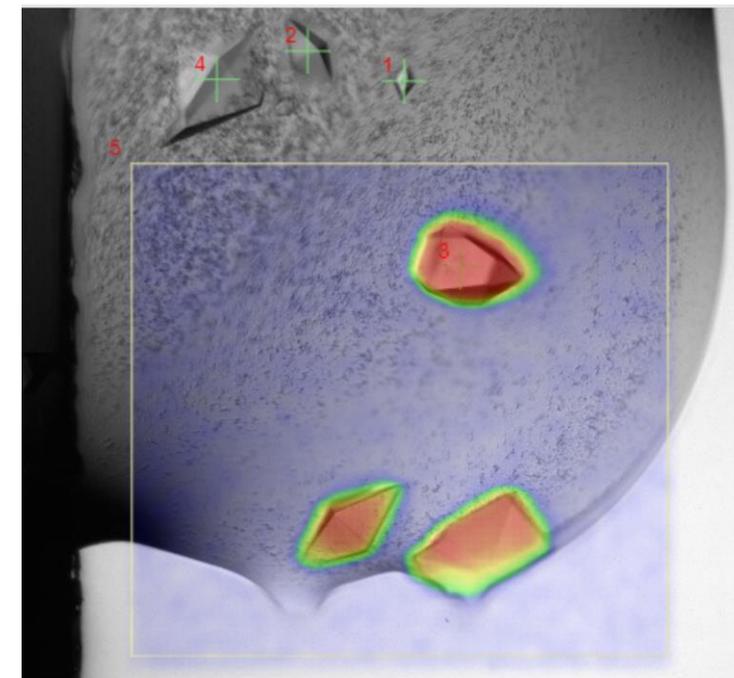
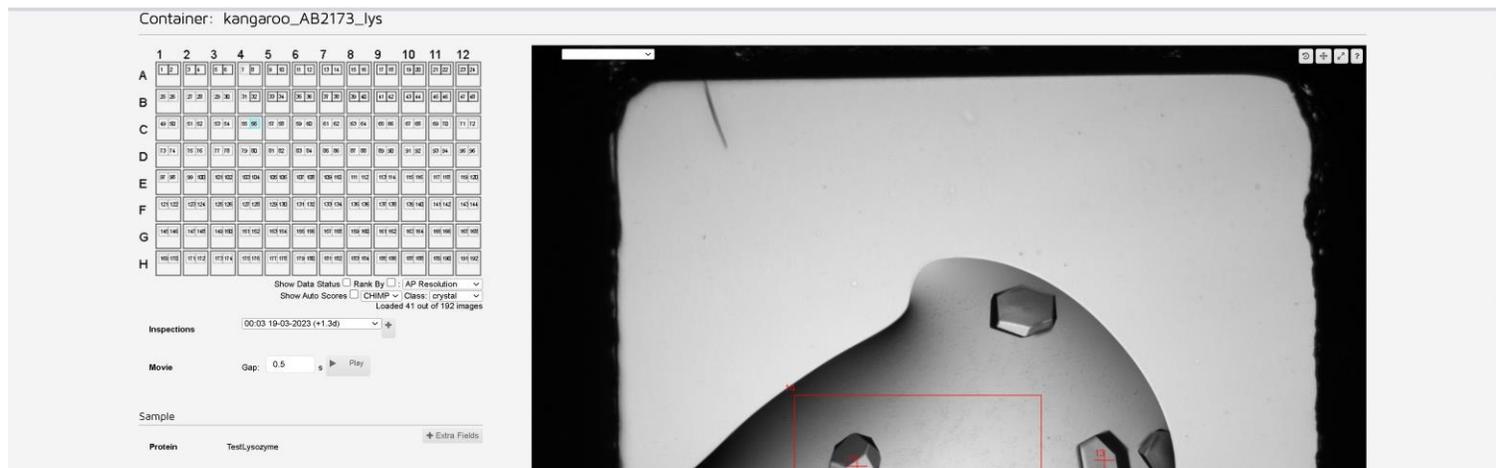


- Calculates which images should be used to generate composite image
- Matches composite images from Formulatrix and beamline together to enable sample centring
- Outputs a series of X,Y,Z coordinates for each object selected by user

Overlap

Differences

Collection at room temperature



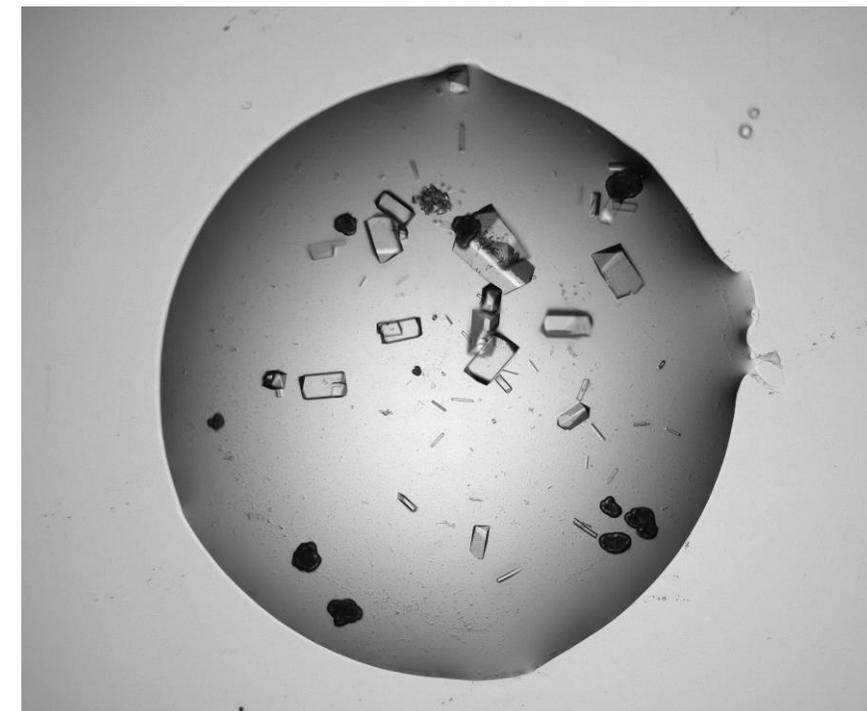
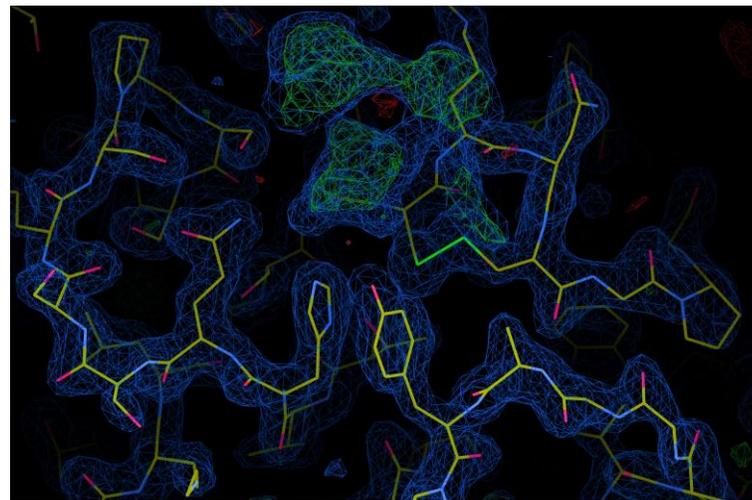
Discover the potential of your crystals without intervention

Sandy, J., Mikolajek, H., et al. (2024). Crystallization and *In Situ* Room Temperature Data Collection Using the Crystallization Facility at Harwell and Beamline VMXi, Diamond Light Source. *J. Vis. Exp.* (205), e65964.



Example VMXi data

Sample: B12d1_	Flux: 9.93e+11
Ω Start: -30.0°	Ω Osc: 0.10°
Ω Overlap: 0°	No. Images: 600
Resolution: 1.95Å	Wavelength: 0.7749Å
Exposure: 0.0018s	Transmission: 5.00%
Beamsize: 10x10 μ m	Type: SAD

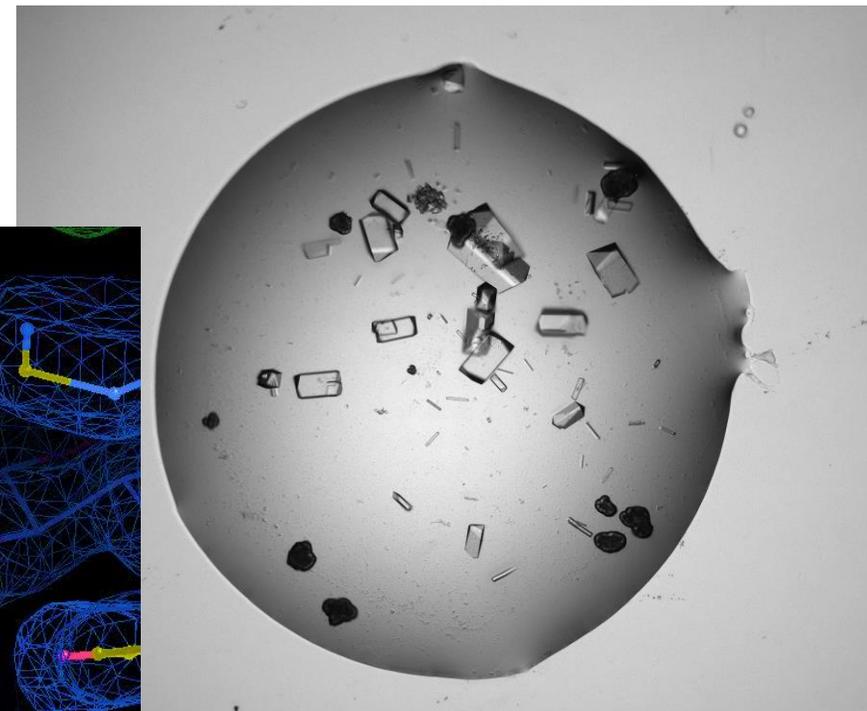


Multiple crystals selected

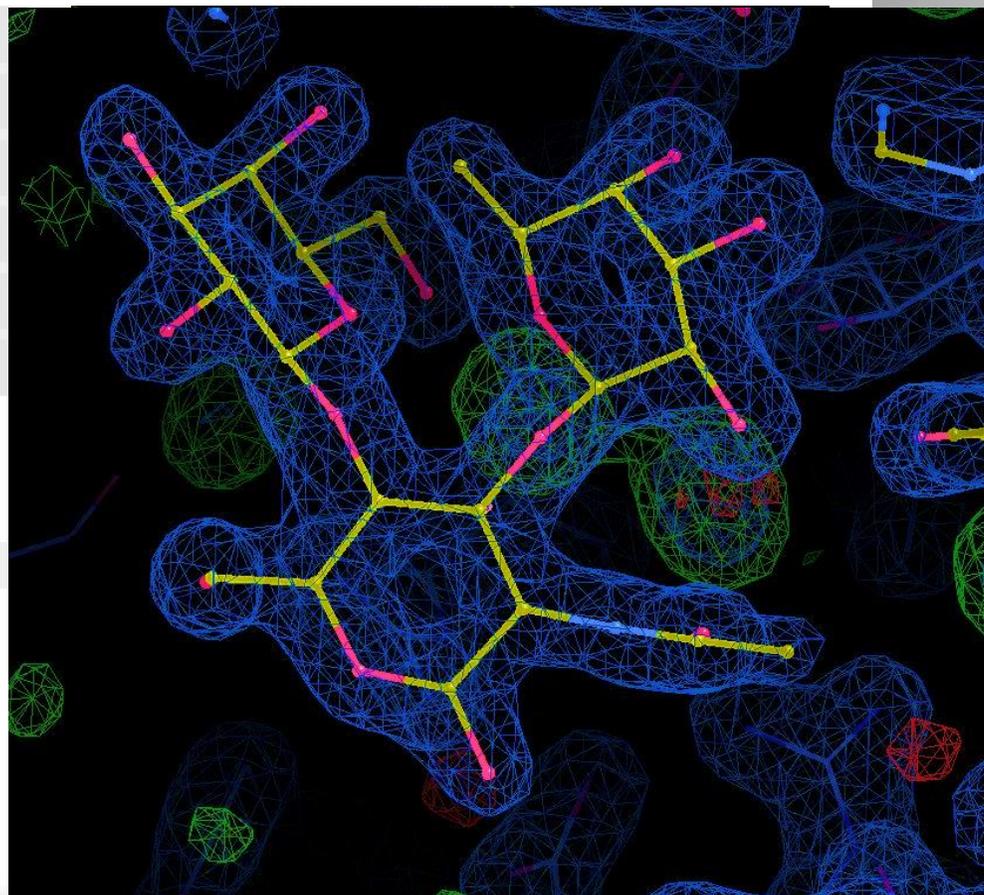
Type	Resolution	Resolution I/sig(I)=2	Spacegroup	Mn<I/sig(I)>	Rmeas Inner	Rmeas Outer	Completeness
xia2 dials	70.44 - 1.76	1.90	P 2 21 2	12.6	0.037	1.249	84.2
fast_dp	28.23 - 1.91	0.00	P 2 2 2	8.6	0.026	0.864	89.0
autoPROC	70.44 - 1.83	2.17	P 2 2 2	4.6	0.038	1.263	86.2
xia2 3dii	70.46 - 1.80	2.02	P 2 2 21	7.0	0.036	1.256	85.2
35x xia2.multiplex	70.44 - 1.69	0.00	P 21 21 21	14.6	0.196	167.079	100.0
autoPROC+STARANISO	70.44 - 1.80	0.00	P 2 2 2	5.6	0.036	0.630	81.1
4x xia2.multiplex	70.44 - 1.91	0.00	P 21 21 21	11.7	0.097	3.010	100.0

Each sweep - 60 degrees data in ~1 second

Example VMXi data



Sample: B12d1_	Flux: 9.93e+11
Ω Start: -30.0°	Ω Osc: 0.10°
Ω Overlap: 0°	No. Images: 600
Resolution: 1.95Å	Wavelength: 0.7749Å
Exposure: 0.0018s	Transmission: 5.00%
Beamsize: 10x10 μ m	Type: SAD



Multiple crystals selected

Type	Resolution
xia2 dials	70.44 - 1.76
fast_dp	28.23 - 1.91
autoPROC	70.44 - 1.83
xia2 3dii	70.46 - 1.80
35x xia2.multiplex	70.44 - 1.69
autoPROC+STARANISO	70.44 - 1.80

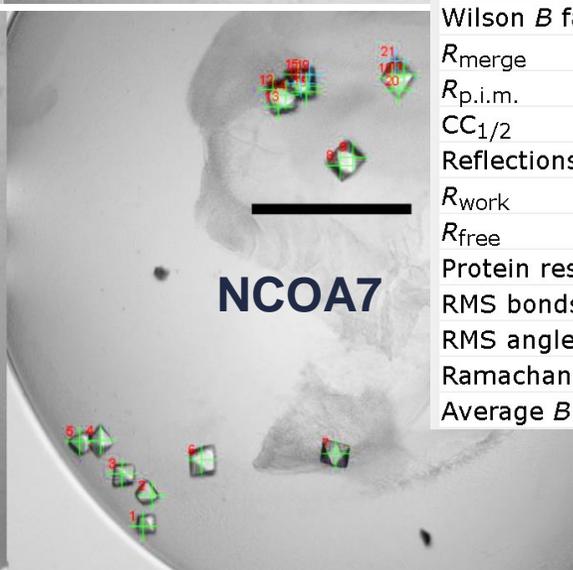
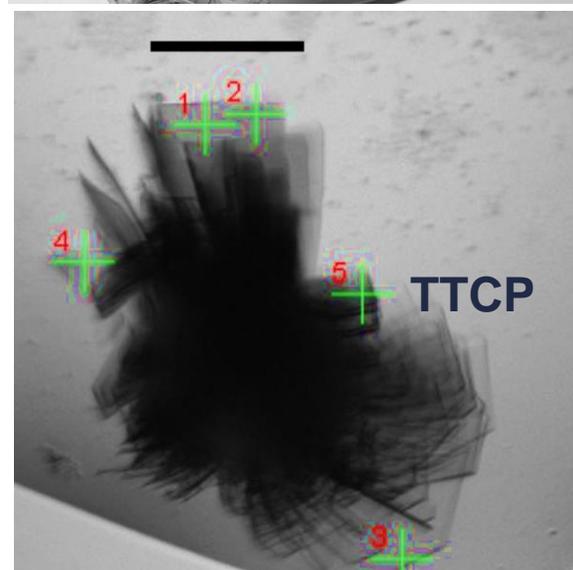
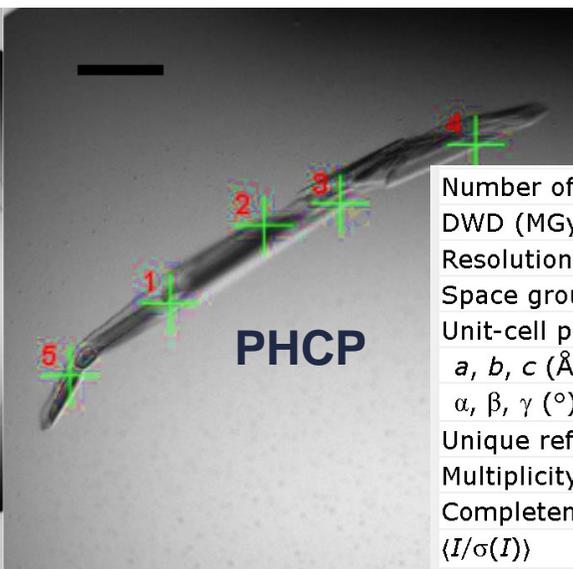
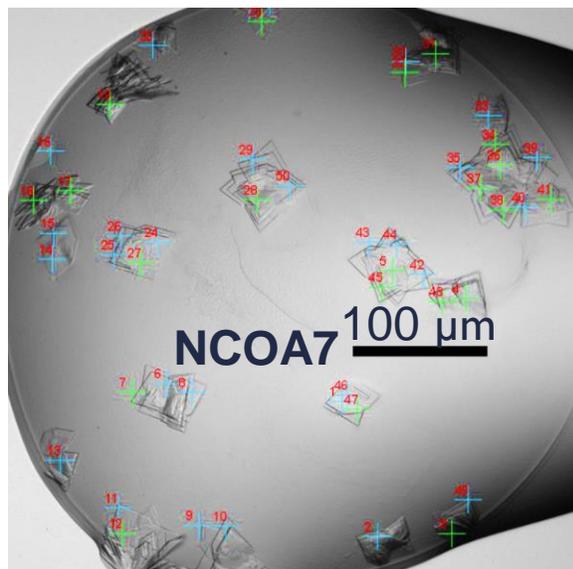
Rmeas Inner	Rmeas Outer	Completeness
0.037	1.249	84.2
0.026	0.864	89.0
0.038	1.263	86.2
0.036	1.256	85.2
0.196	167.079	100.0
0.036	0.630	81.1

4x xia2.multiplex	70.44 - 1.91	0.00	P 21 21 21	11.7	0.097	3.010	100.0
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Ligand clearly visible in fo-fc maps and was easily built into density – publication pending

Challenging crystals – ugly or tiny!

From thin irregular plate and needle-like objects, clusters of crystals, to tiny crystals, VMXi can deliver structures.

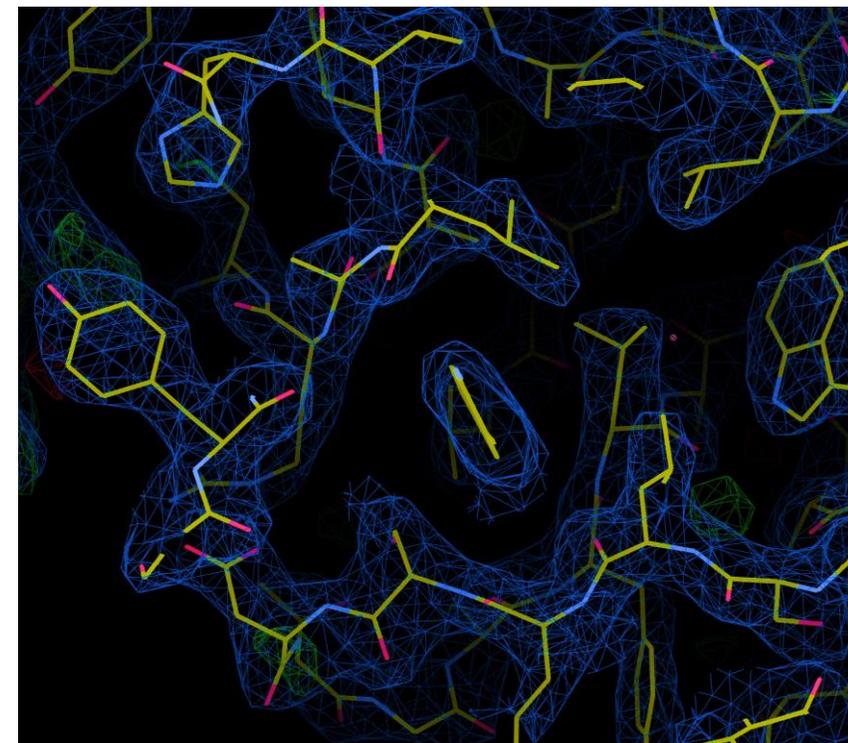
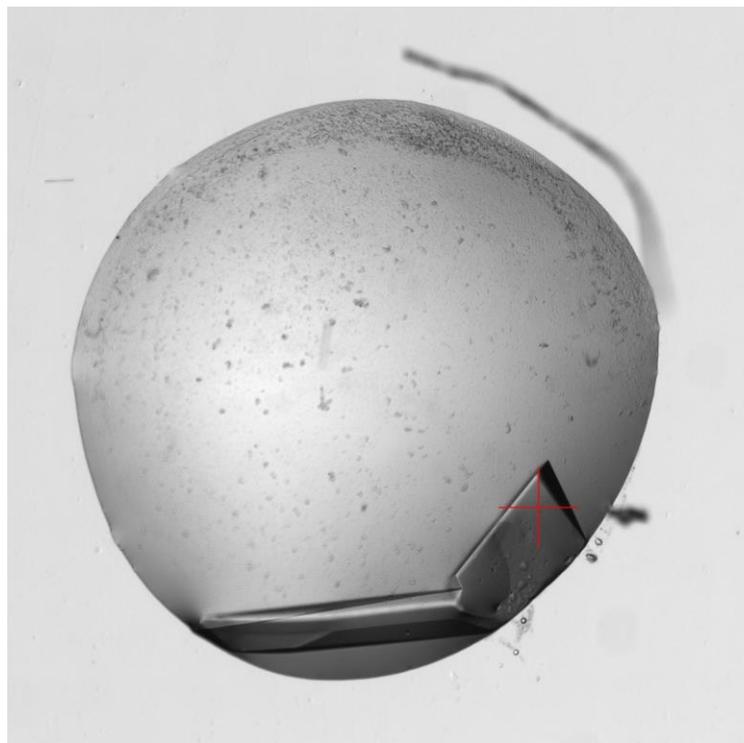
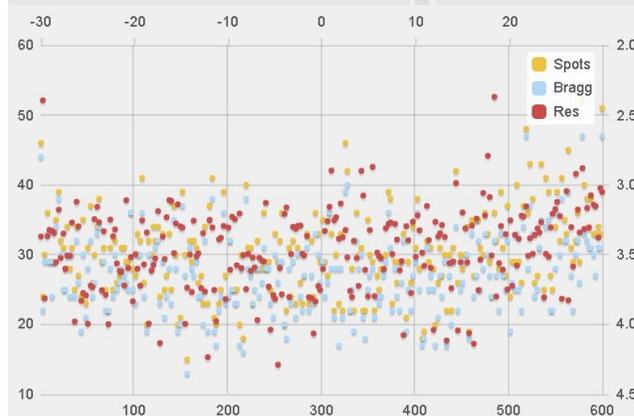


	NCOA7	PHCP	TTCP
Number of crystals	12	4	4
DWD (MGy)	1.01	1.05	1.37
Resolution range (Å)	46.38–2.36 (2.4–2.36)	56.03–1.88 (1.91–1.88)	35.12–1.75 (1.78–1.75)
Space group	C2	<i>P</i> 6 ₂ 22	C2
Unit-cell parameters			
<i>a</i> , <i>b</i> , <i>c</i> (Å)	100.0, 54.8 44.3	83.5, 83.5, 88.6	81.5, 39.0, 41.5
<i>α</i> , <i>β</i> , <i>γ</i> (°)	90, 119.5, 90	90, 90, 120	90, 97.2, 90
Unique reflections	8049 (420)	15440 (750)	12429 (490)
Multiplicity	13.9 (13.3)	22.3 (15.4)	4.4 (2.8)
Completeness (%)	92.55 (94.1)	100.0 (100.0)	93.9 (74.7)
<i>I</i> / <i>σ</i> (<i>I</i>)	5.7 (1.3)	7.9 (0.6)	9.9 (0.9)
Wilson <i>B</i> factor (Å ²)	30.8	19.50	15.38
<i>R</i> _{merge}	0.483 (2.335)	0.459 (5.496)	0.151 (1.171)
<i>R</i> _{p.i.m.}	0.13 (0.654)	0.093 (1.401)	0.076 (0.740)
CC _{1/2}	0.949 (0.261)	0.995 (0.258)	0.990 (0.364)
Reflections used in refinement	7958 (775)	15397 (777)	12425 (614)
<i>R</i> _{work}	0.196	0.180	0.163
<i>R</i> _{free}	0.259	0.203	0.197
Protein residues	163	135	133
RMS bonds (Å)	0.0090	0.0133	0.0118
RMS angles (°)	1.61	2.31	2.22
Ramachandran favoured (%)	95.7	99.2	100.0
Average <i>B</i> factor (Å ²)	26.8	33.0	25.0

H. Mikolajek, J. Sanchez-Weatherby, et al. (2023). Protein-to-structure pipeline for ambient-temperature *in situ* crystallography at VMXi. [IUCr 10: 420-429](https://doi.org/10.1107/IUCr.10.420-429)

Challenging crystals – stuck to plate!

Sample: A7d2_	Flux: 9.93e+11
Ω Start: -30.0°	Ω Osc: 0.10°
Ω Overlap: 0°	No. Images: 600
Resolution: 1.95Å	Wavelength: 0.7749Å
Exposure: 0.0018s	Transmission: 5.00%
Beamsize: 10x10 μ m	Type: SAD



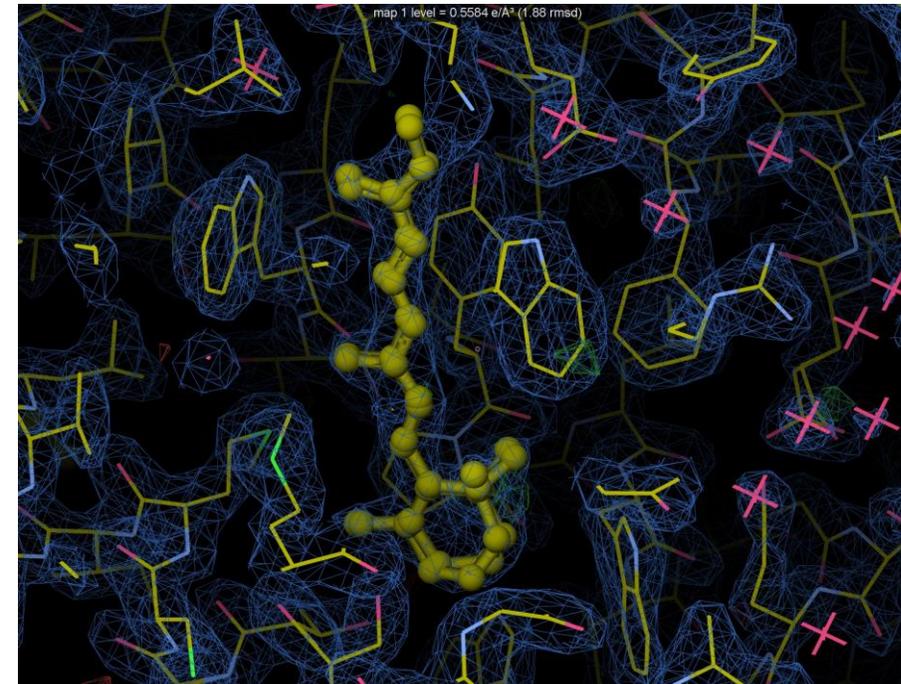
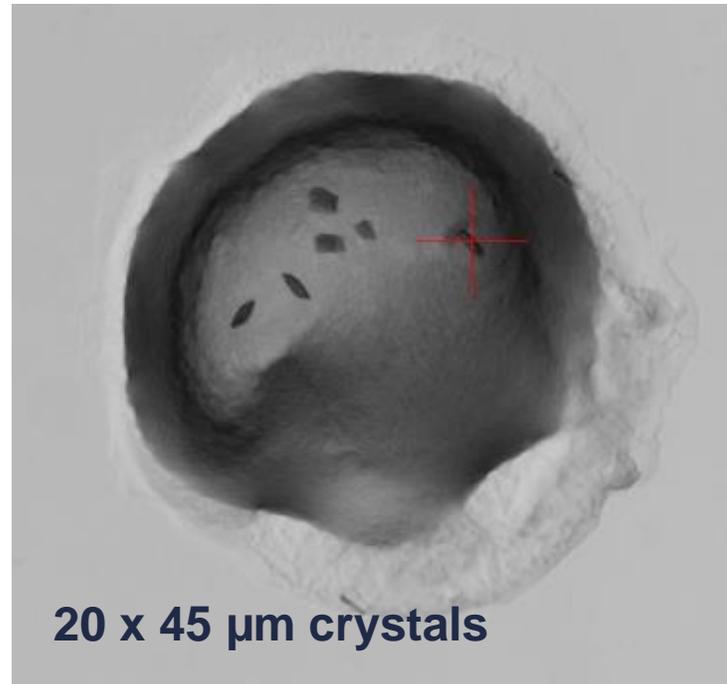
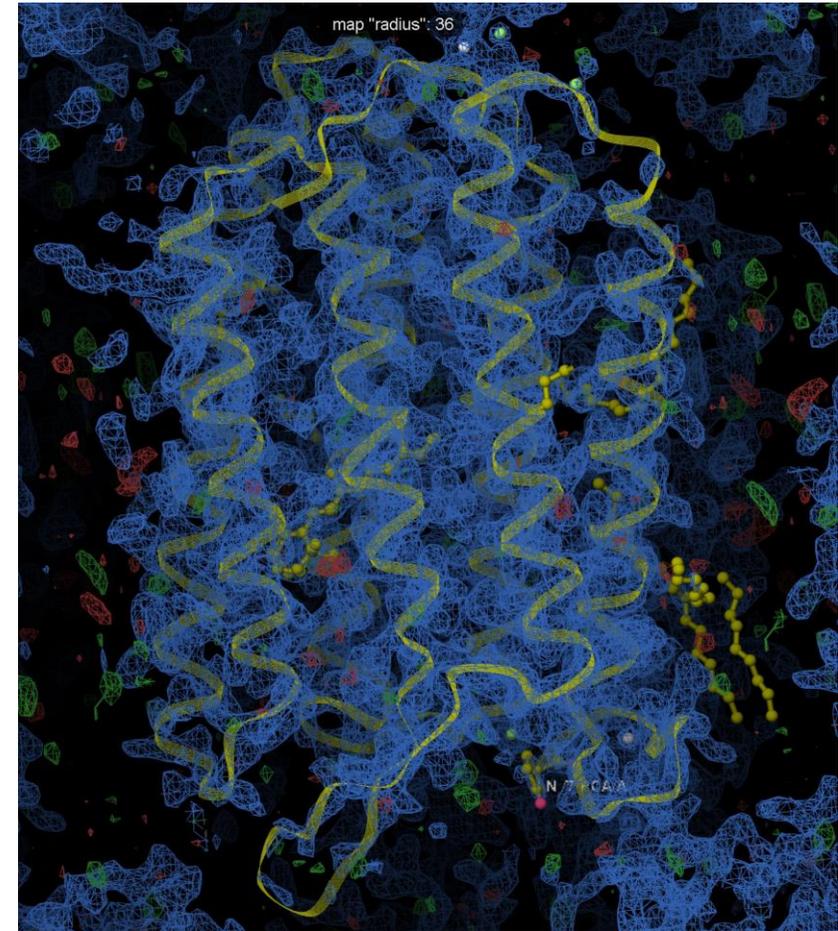
data	Resolution	Resolution I/sig(I)=2	Spacegroup	Mn<I/sig(I)>	Rmeas Inner	Rmeas Outer	Completeness
fast_dp	29.54 - 2.74	0.00	I 2 2 2	10.5	0.024	0.929	53.2
xia2 dials	68.88 - 2.38	2.66	I 2 2 2	9.7	0.044	2.295	54.0
xia2 3dii	74.85 - 2.44	2.76	I 2 2 2	7.0	0.043	2.114	53.7
autoPROC	68.86 - 2.51	2.71	I 2 2 2	6.9	0.046	2.291	53.4
2x xia2.multiplex	68.88 - 2.31	0.00	I 2 2 2	9.6	0.052	2.222	94.0
12x xia2.multiplex	74.86 - 2.17	0.00	I 2 2 2	11.6	0.075	15.069	100.0
autoPROC+STARANISO	74.83 - 2.22	0.00	I 2 2 2	7.9	0.045	1.030	56.9

First membrane protein structure

6 x 20° oscillation (<2.2 secs exposure) - 2Å structure

Ground state structure of Archaerhodopsin-3 – from MPL group at DLS

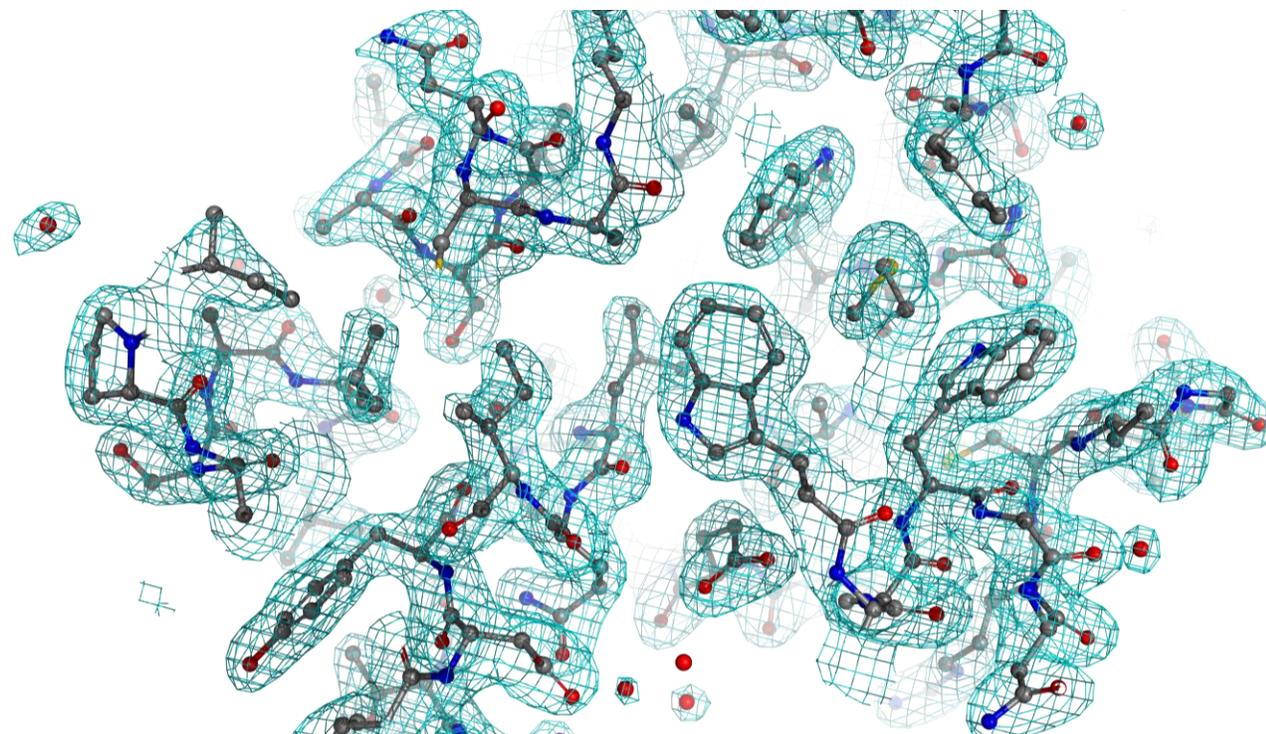
Collected data in MiteGen In Situ-1™ tray and film sandwich on VMXi



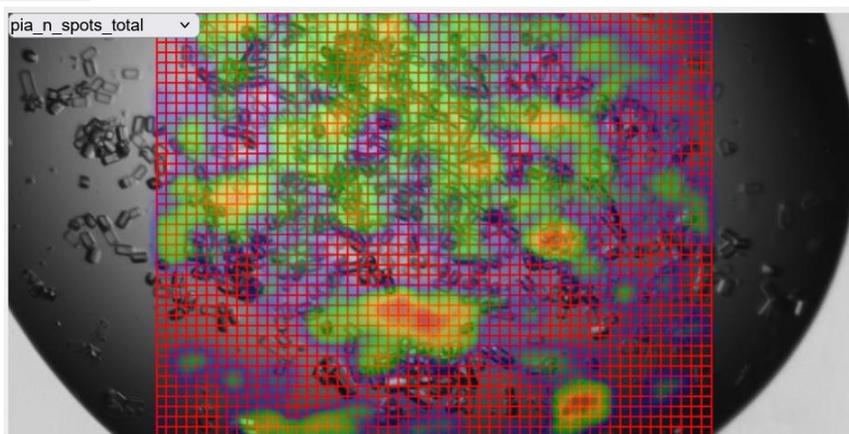
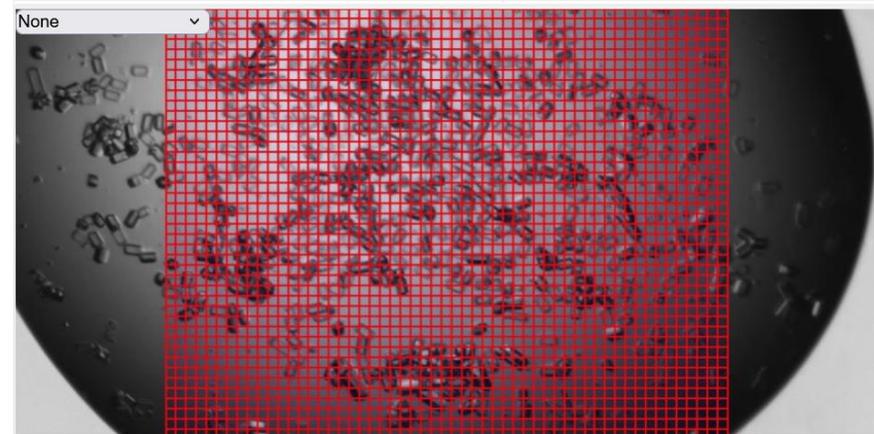
Serial data collection

HEWL (8 drops, PDB entry 8rge)

Diffraction-weighted dose (kGy)	33	Sample: A11d2_
Resolution range (Å)	55.56–1.88 (1.95–1.88)	& Start: 0.0°
Space group	$P4_32_12$	Resolution: 1.95Å
a, b, c (Å)	78.57, 78.57, 37.77	Wavelength: 0.7749Å
α, β, γ (°)	90, 90, 90	Exposure: 0.0020s
No. of diffraction patterns merged	9891	Transmission: 100.00%
Volume dispensed	1.6 μ l [200 nl per drop]	Beamsize: 10x10 μ m
Total reflections	1117460 (32281)	Boxsize: 10x10 μ m
Unique reflections	10076 (973)	
Multiplicity	110.9 (65.1)	
Completeness (%)	99.27 (99.90)	
Mean $I/\sigma(I)$	19.1 (1.2)	
Wilson B factor (Å ²)	31.36	
R_{split}	0.083 (1.143)	
$CC_{1/2}$	0.997 (0.398)	
Reflections used in refinement	10002 (972)	
R_{work}	0.1925 (0.2832)	
R_{free}	0.2305 (0.4288)	
Water molecules	83	



~3 mins / grid <30 mins total time



Thompson, A. J., Sanchez-Weatherby, J., et al. (2024). Efficient in situ screening of and data collection from microcrystals in crystallization plates. *Acta Cryst. D80*: 279-288

Multiplex

Processes datasets seamlessly and quickly to deliver improved completeness and resolution

After consistent symmetry determination using `dials.cosym`, there are three types of clustering available in `xia2.multiplex`:

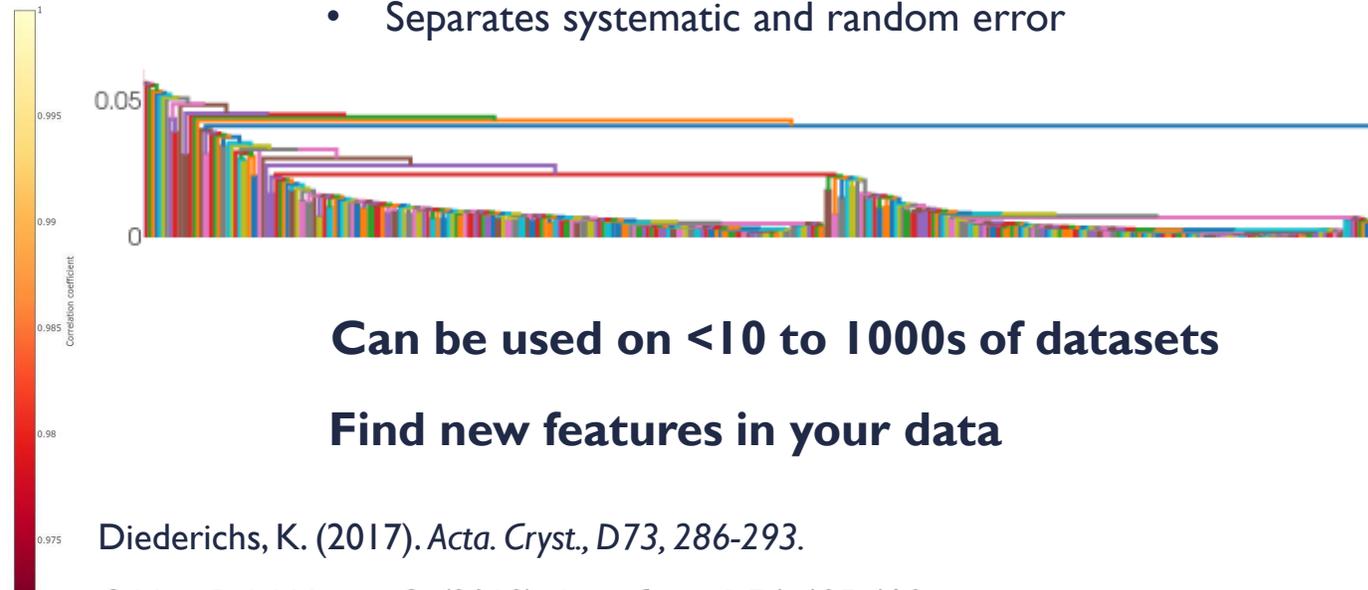
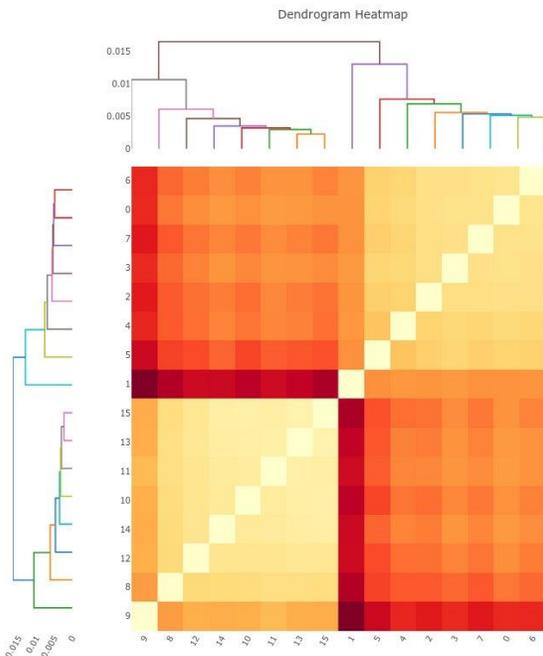
1. Unit Cell Clustering
2. Correlation Clustering (intensity-based)
3. Cosine Angle Clustering (intensity-based)

Correlation Clustering

- Comparison of pairwise correlation coefficients
- Confused by different dataset qualities (random error)

Cosine Angle Clustering

- Extension of correlation clustering
- Separates systematic and random error



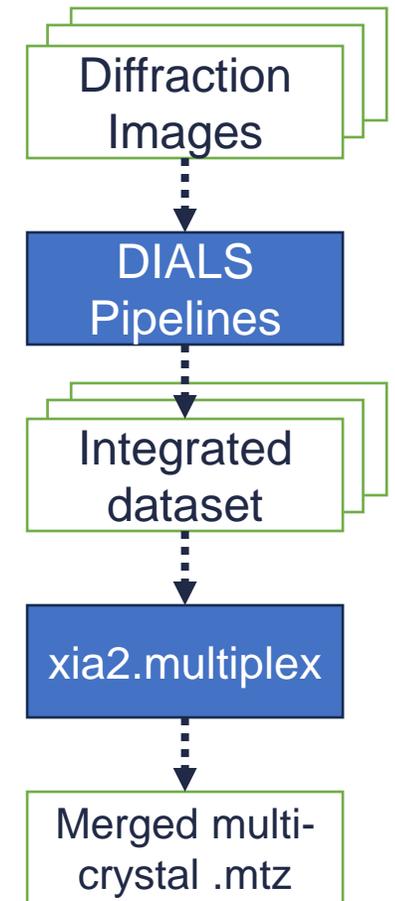
Can be used on <10 to 1000s of datasets

Find new features in your data

Diederichs, K. (2017). *Acta Cryst.*, D73, 286-293.

Gildea, R. J., Winter, G. (2018). *Acta Cryst.*, D74, 405-410.

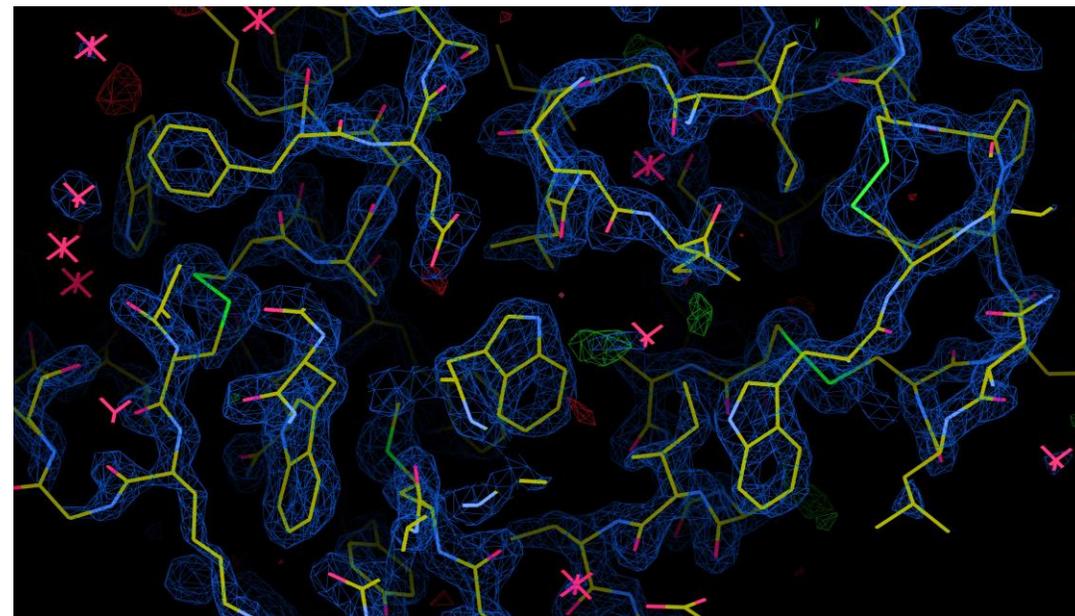
Auto-processing Pipeline



Sample groups

Taking xia2.multiplex to the next level

Type	Resolution
1 289x xia2.multiplex	1.51 - 78.89
3 292x xia2.multiplex	1.51 - 78.89



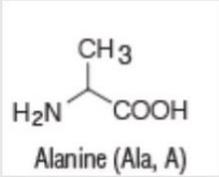
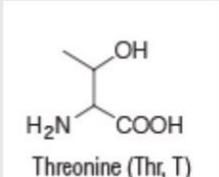
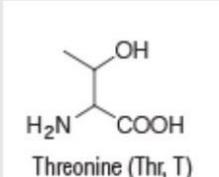
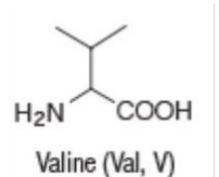
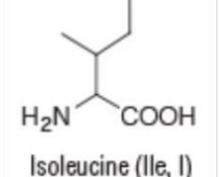
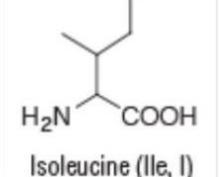
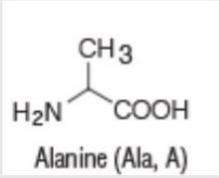
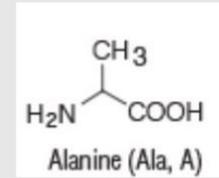
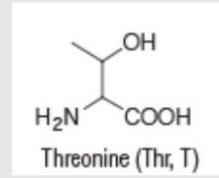
Container: VMXi-AB2171		Container: VMXi-AB2174		Container: VMXi-AB2173			Container: VMXi-AB2172	
Shell	Observations	Unique	Resolution	Rmeas	I/sig(I)	CC Half	Completeness	Multiplicity
outerShell	94701	913	1.51 - 1.54	-14.456	0.1	0.3	100.0	103.7
innerShell	1103776	1094	4.10 - 79.00	0.180	252.5	1.0	100.0	1,008.9
overall	13894097	19187	1.51 - 78.89	0.938	44.6	1.0	100.0	724.1

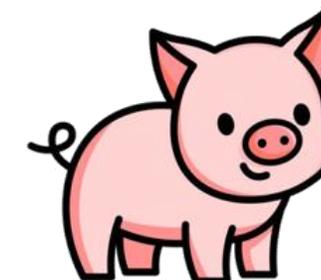
Analysis of different chemical conditions, dehydration experiments, fragment/ligand-binding

Clustering – Test Case



Human insulin, porcine insulin and bovine insulin differ subtly in their amino acid sequences and readily grow high-quality, cubic, isomorphous crystals

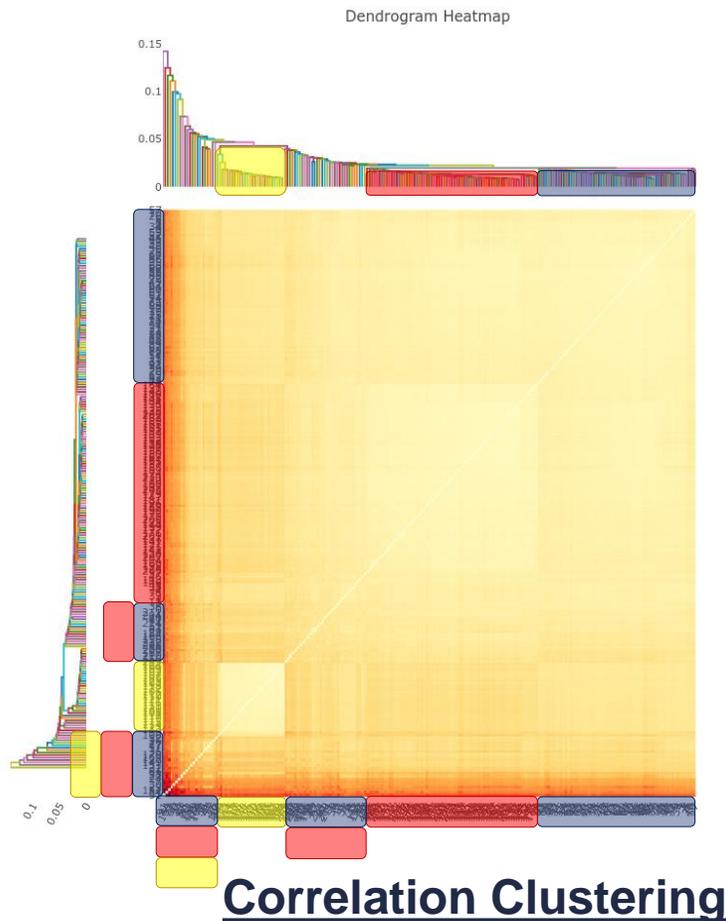
	Cows	Pigs	People
Chain A Residue 8	 Alanine (Ala, A)	 Threonine (Thr, T)	 Threonine (Thr, T)
Chain A Residue 10	 Valine (Val, V)	 Isoleucine (Ile, I)	 Isoleucine (Ile, I)
Chain B Residue 30 (terminus)	 Alanine (Ala, A)	 Alanine (Ala, A)	 Threonine (Thr, T)



Clustering – Test Case



- All three species are distinguishable at room temperature with new algorithms!
- People and pigs mix in the dendrogram before cows – consequence of terminal residue position being less well defined at room temperature



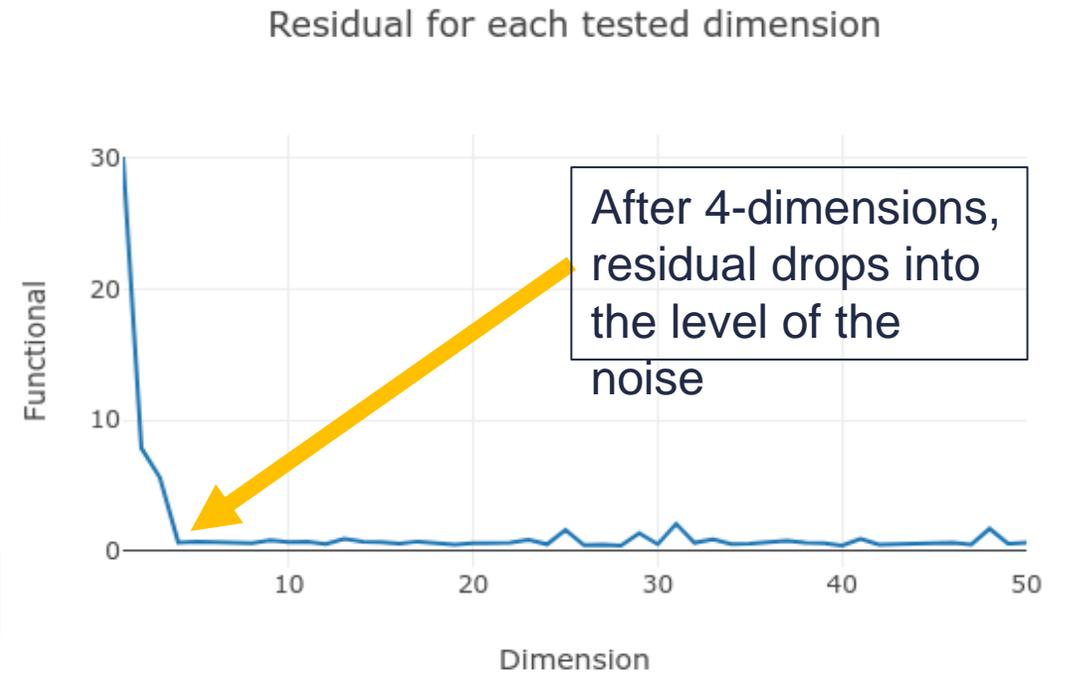
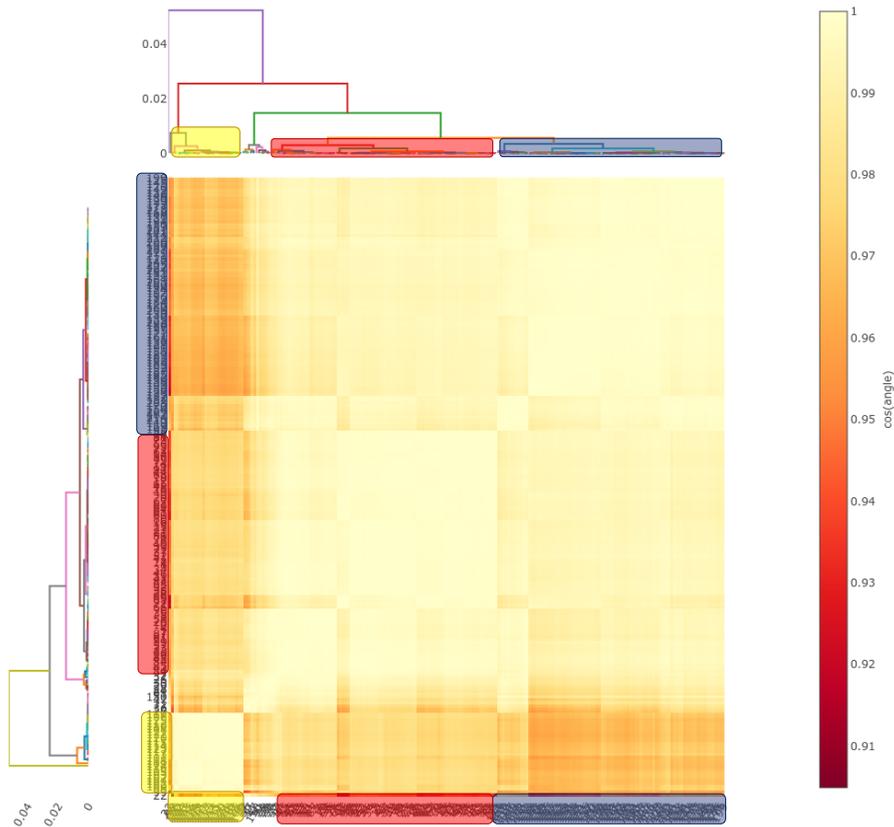
Clustering in this manner not particularly easy to separate – try other options

Clustering – Test Case



Multiple clusters due to different samples and poor data quality

Dendrogram Heatmap



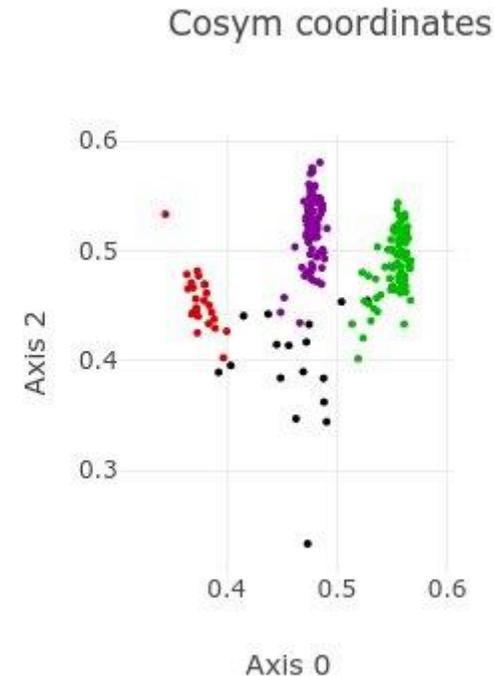
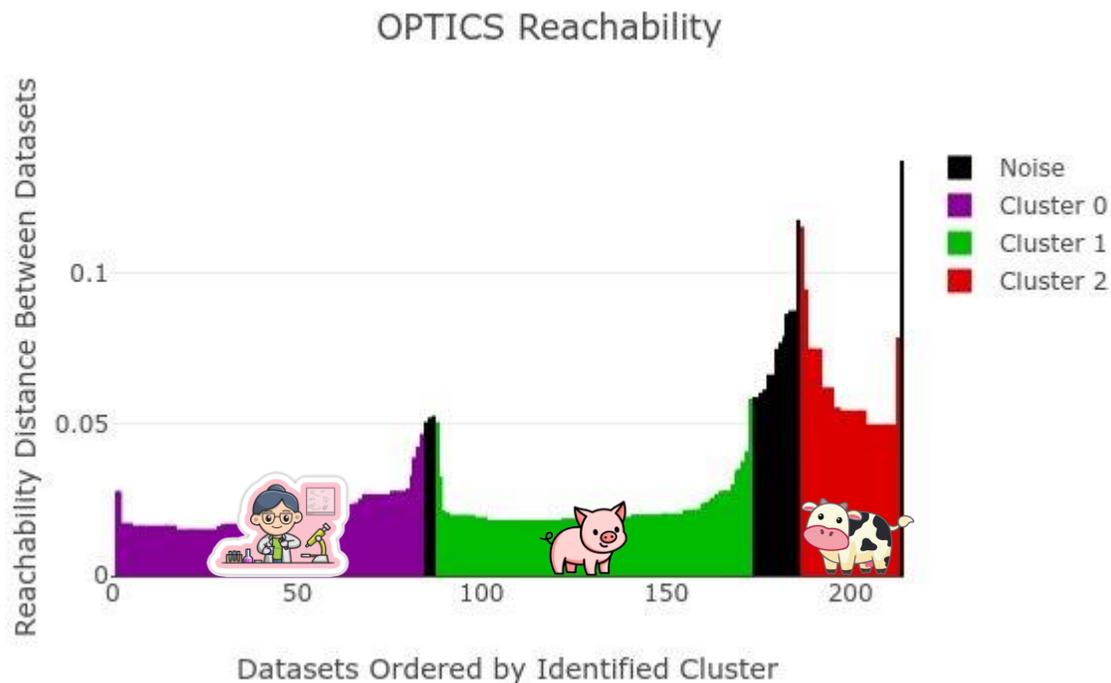
4D Cosine Angle Clustering

Clustering – Test Case



OPTICS is a density-based algorithm that automatically defines clusters with spatial relations (which we have calculated using cosine-angle clustering!)

- Requires no global density parameter (can detect clusters of varying density)
- Does not assume any specific number of clusters
- Can detect noise (does not require every dataset to be a part of a cluster)



Peaks between clusters are used to easily separate samples

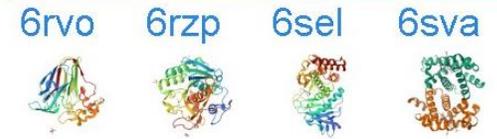
Processed data files for each cluster are automatically outputted for users

4D clustering has well-separated cows, pigs and people clusters, and an outlier group of datasets are identified

Summary

- Collection of fully-automated room temperature data *in situ* works and delivers excellent quality data
- The VMXi beamline can help with many aspects of the crystallisation experiment from initial hits to more advanced studies
- Improvements in data processing are enabling new science

2019 (4)



2022 (5)



2023 (5)



2024 (1)



Acknowledgements

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Electrical and Mechanical
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Electrical and Mechanical
Technicians

PLC and Motions Teams

Controls Team



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Olly King - CHiMP

Diamond GDA Team

MX Data Analysis Team

ISPyB Development Team

Diamond Users

Want to try VMXi? Contact us – VMXi@diamond.ac.uk

