

VMXi – Fully automated room-temperature MX



VMXi – Centrepiece of Diamond MX

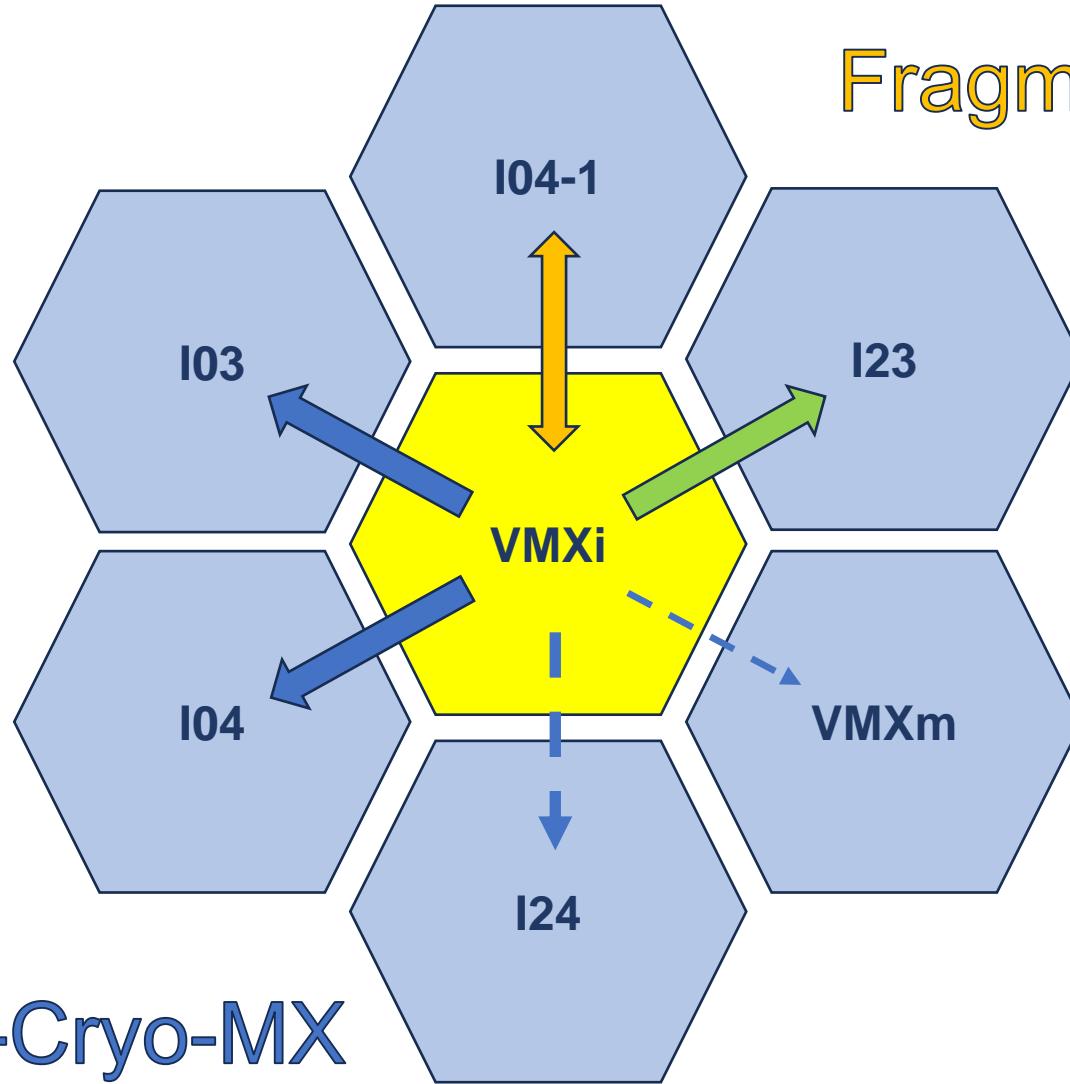
Flux 2×10^{13} ph/sec

16 KeV

Beam size $10 \times 10 \mu\text{m}$

Cryo-MX

Micro-Cryo-MX



Fragments / XChem

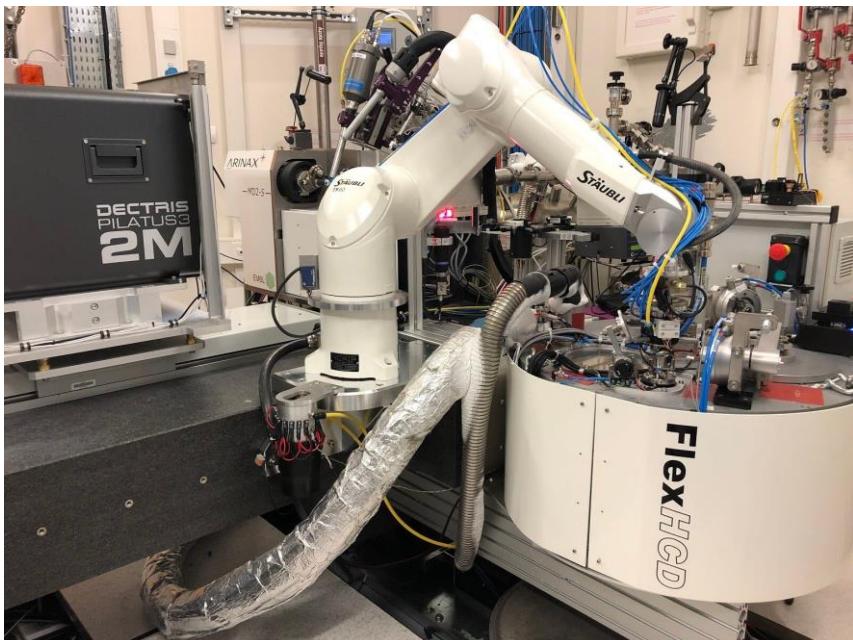
Phasing

Nano-Cryo-MX



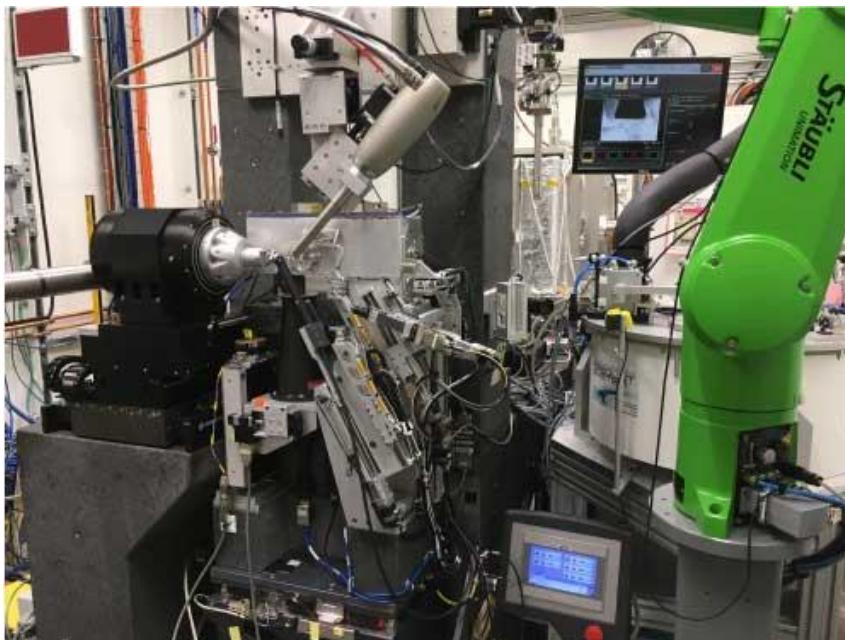
Automated MX beamlines

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.



MASSIF-1 - ESRF

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



AMX – NSLS-II

UDC at Diamond now extremely common

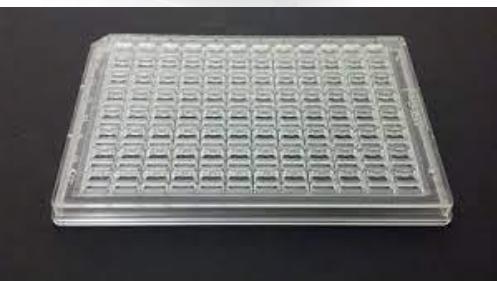
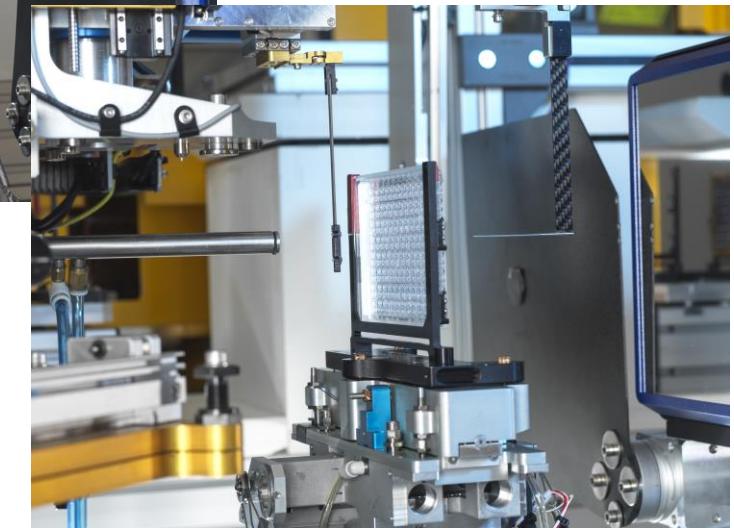
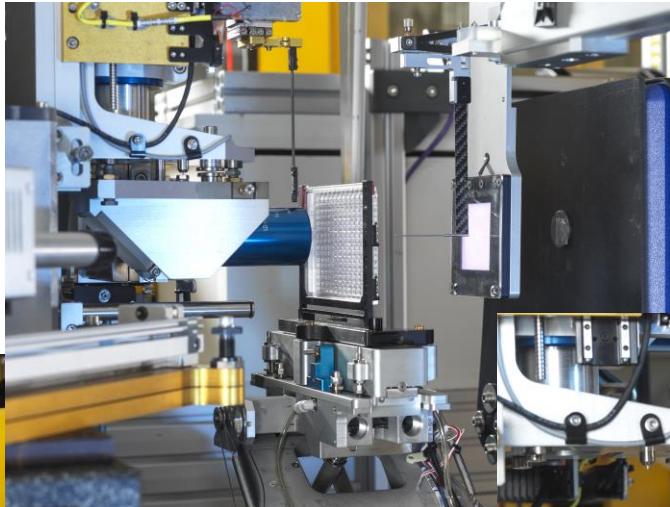
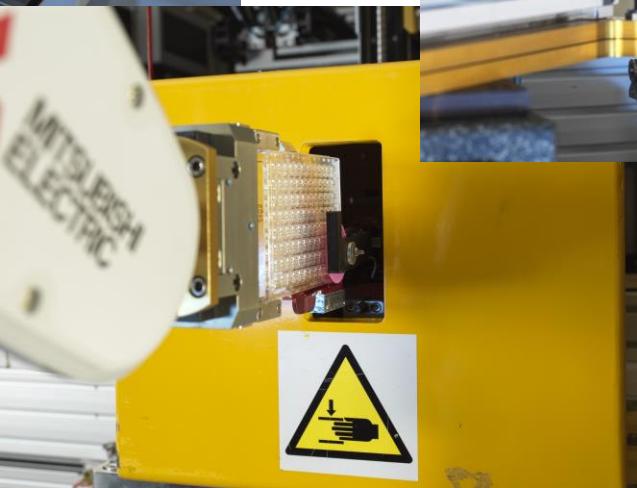
R. Bingel-Erlenmeyer,V. Olieric, et al. (2011). SLS Crystallization Platform at Beamline X06DA—A Fully Automated Pipeline Enabling *in Situ* X-ray Diffraction Screening, *Cryst. Growth Des.* **11**, 4, 916–923 .



X06DA - PSI



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

SynchWeb

Container: 7259_thaum

	1	2	3	4	5	6	7	8	9	10	11	12
A	1	2	3	4	5	6	7	8	9	10	11	12
B	25	26	27	28	29	30	31	32	33	34	35	36
C	49	50	51	52	53	54	55	56	57	58	59	60
D	73	74	75	76	77	78	79	80	81	82	83	84
E	97	98	99	100	101	102	103	104	105	106	107	108
F	121	122	123	124	125	126	127	128	129	130	131	132
G	145	146	147	148	149	150	151	152	153	154	155	156
H	169	170	171	172	173	174	175	176	177	178	179	180

Score: 9 - 3D Crystals

Display

- Current Score
- Max Scores
- Data Status Rank By AP Resolution
- CHIMP Auto Scores Class: crystal

Inspections

05:32 14-11-2024 (+2.6d) +

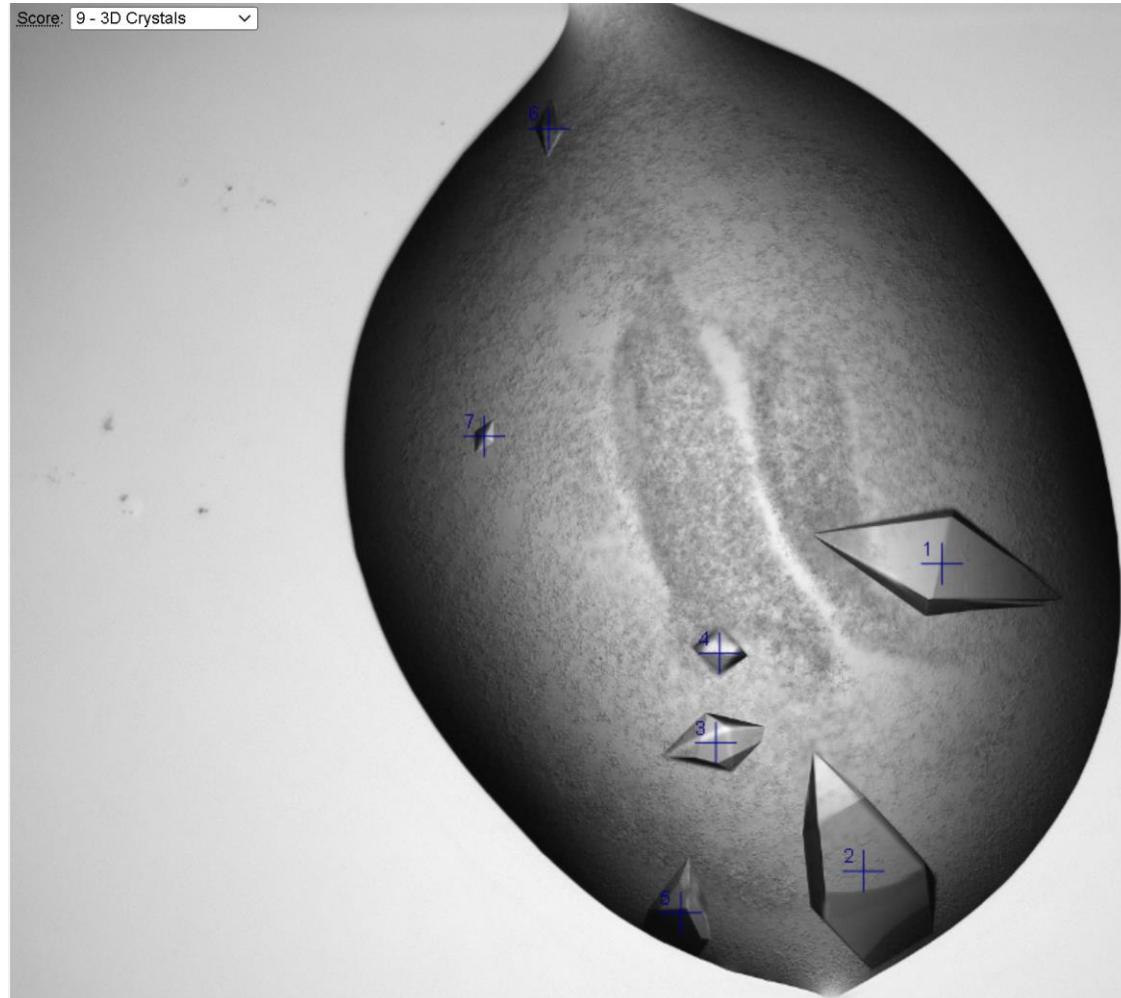
Movie

Gap: 0.5 s Play

Sample

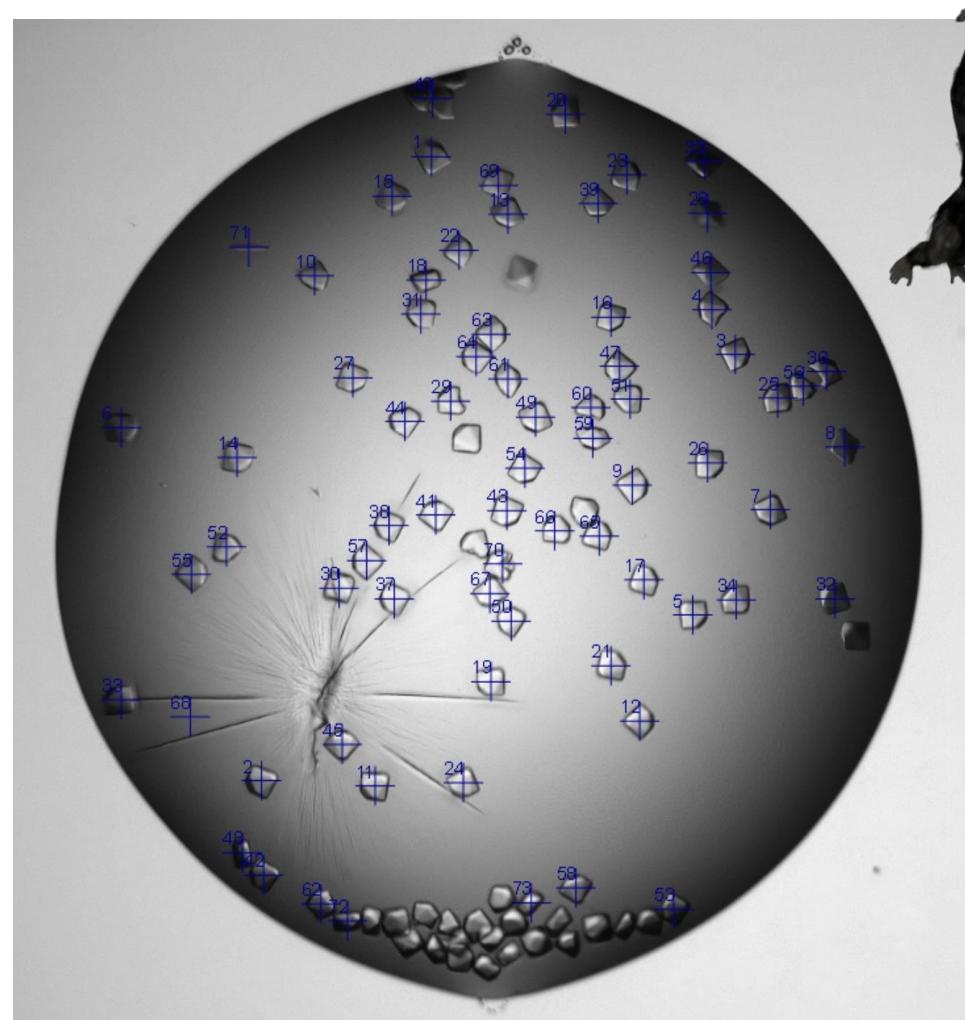
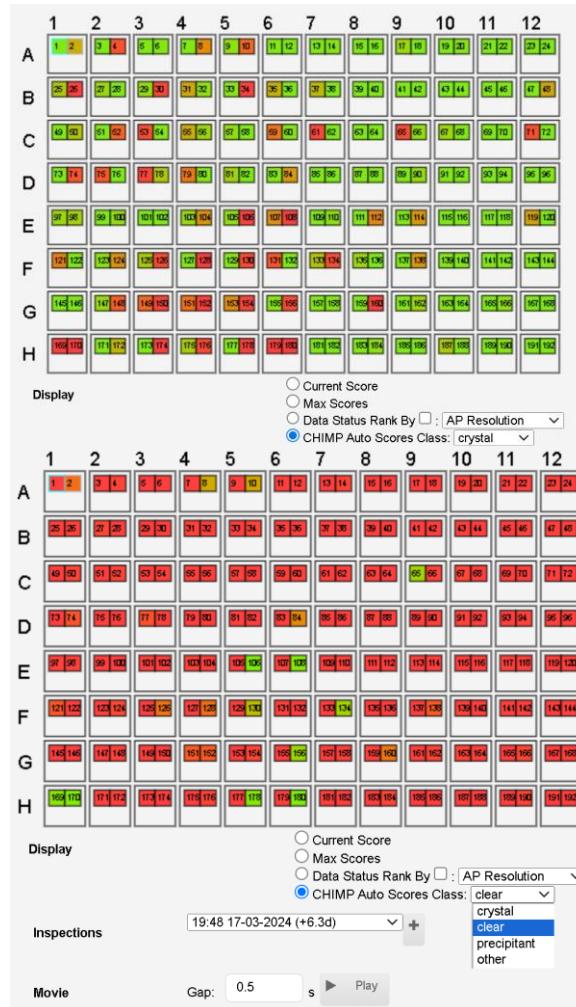
Protein TestThaumatin

Abundance Click to edit



- 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

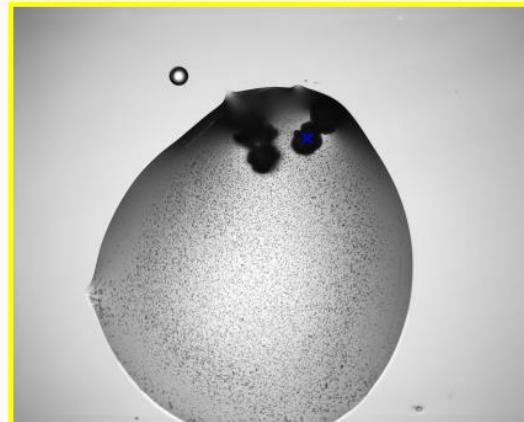
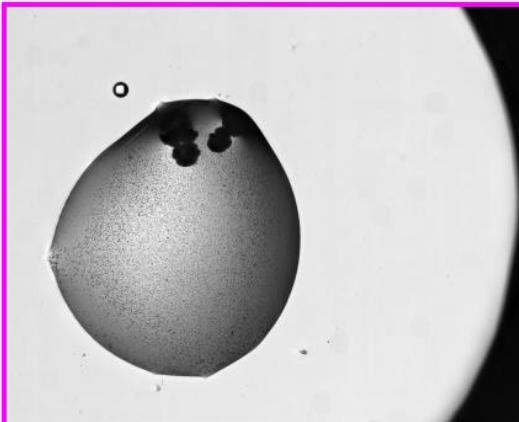
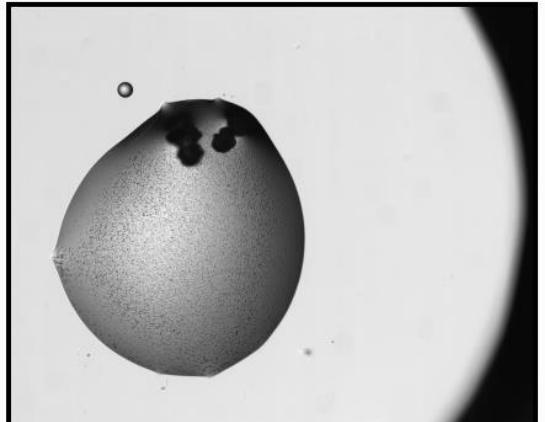
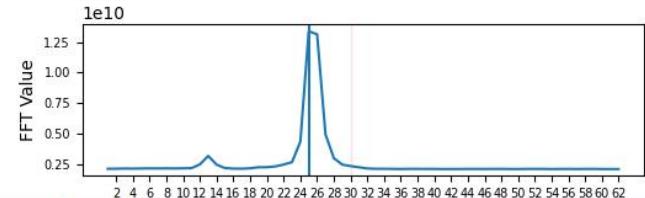
King, O.N.F., Levik, K.E. et al. (2024) CHiMP: deep-learning tools trained on protein crystallization micrographs to enable automation of experiments. *Acta Cryst. D80*: 744-764



CrystalMatch

Message	Timestamp	Duration
1 Matching Started	2024-11-14 10:52:48.346	0
2 FFT calculation finished	2024-11-14 10:52:53.993	5
3 Alignment Complete	2024-11-14 10:52:58.194	9
4 Crystal Match Complete	2024-11-14 10:52:58.198	9

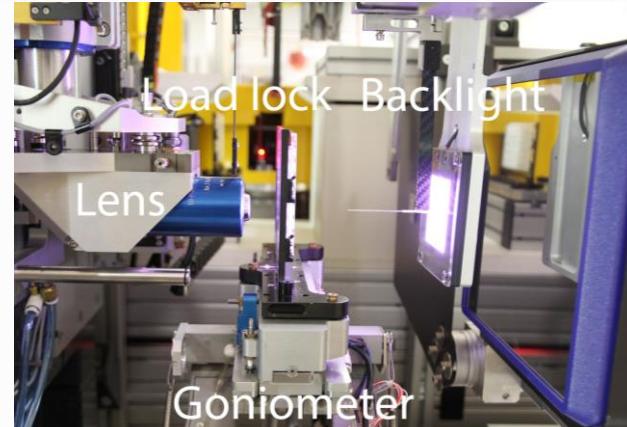
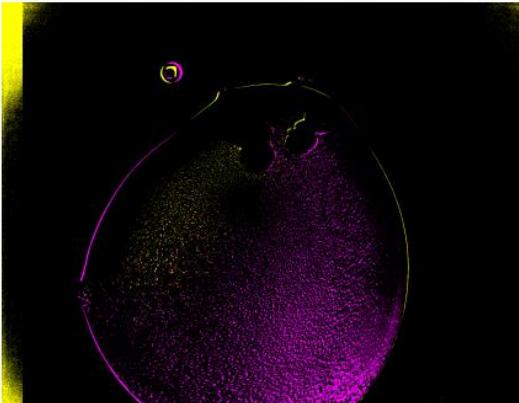
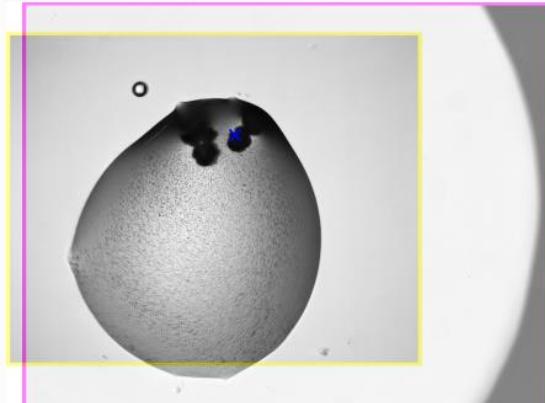
Alignment info
 Alignment score: 32.849610726007036
 Status: 2, Good Alignment
 Scale: 0.6349
 Transform coordinates: x: -84.0000, y: 157.0000



Best FFT: /dls/mx/data/nr27313/nr27313-389/VMXI-AB7324/well_102/zstack 20241114 105218/stack/stack Image 25.tif

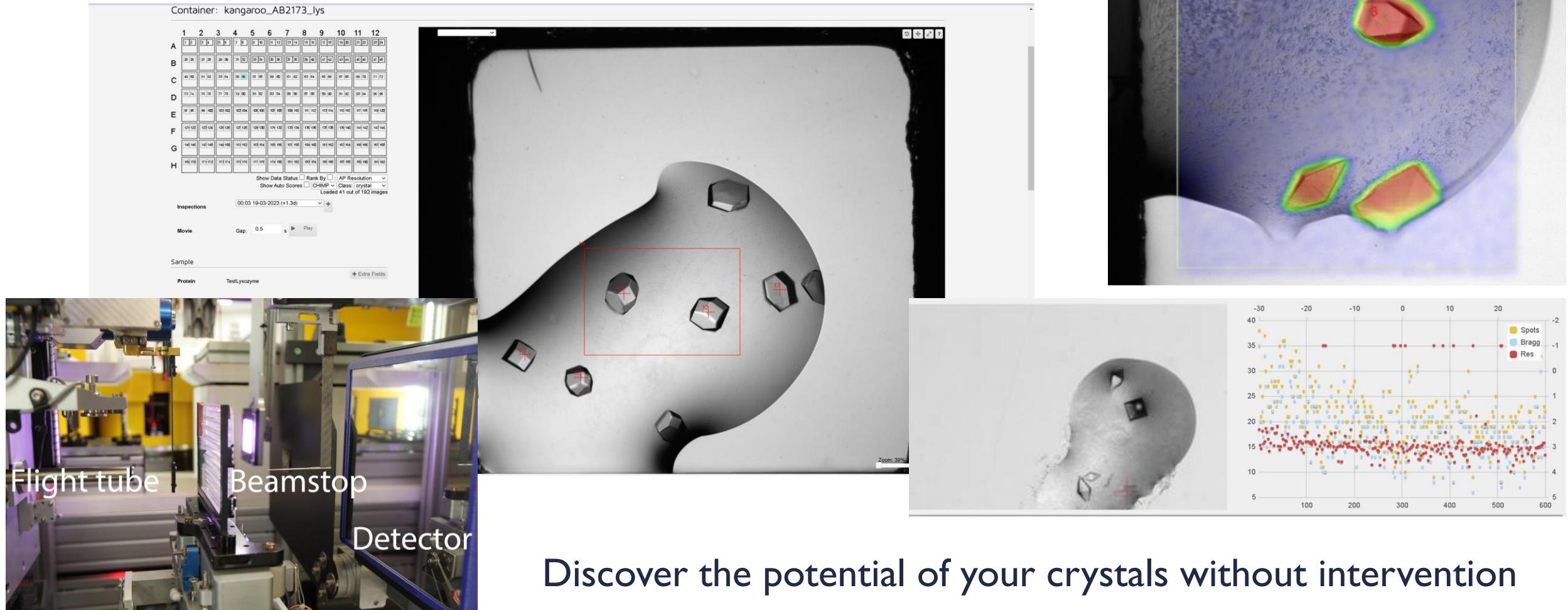
Beamline Processed: /dls/mx/data/nr27313/nr27313-389/VMXI-AB7324/well 102/zstack 20241114 105218/processed.tif

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

Collection at room temperature

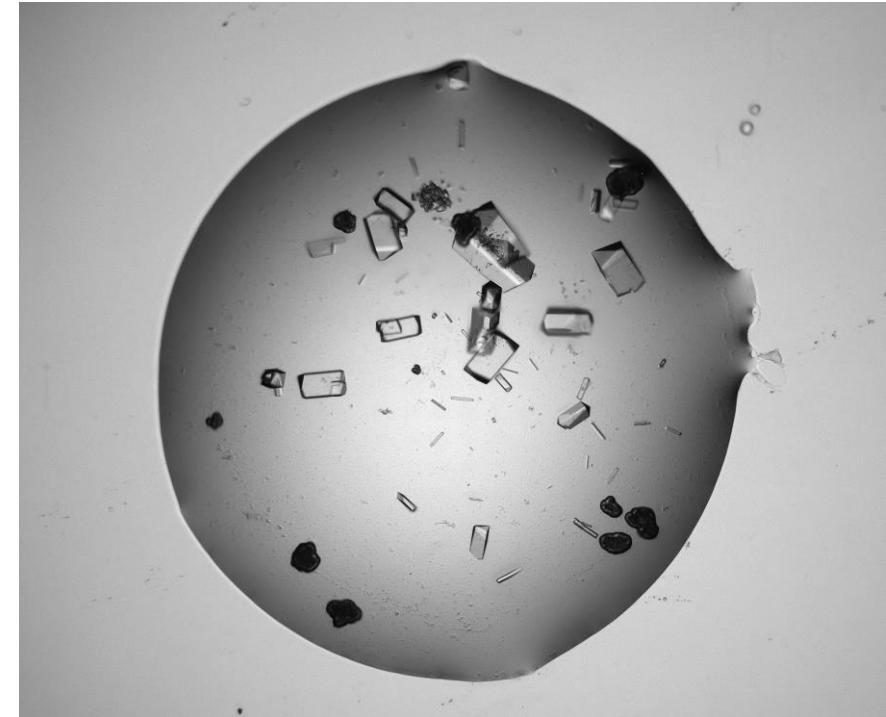
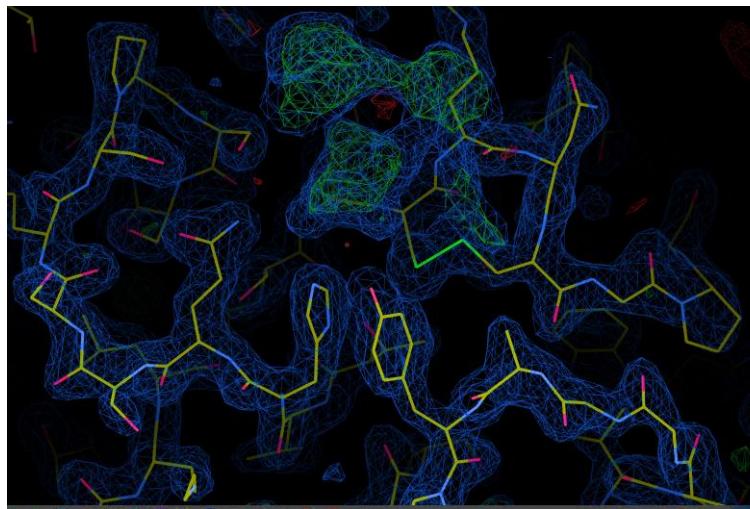


Sandy, J., Mikolajek, H., et al. (2024). Crystallization and *In Situ* Room Temperature Data Collection Using the Crystallization Facility at Harwell and Beamline VMXi, Diamond Light Source. *J. Vis. Exp.* (205), e65964.



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: 10x10μm	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 1 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 2 1	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

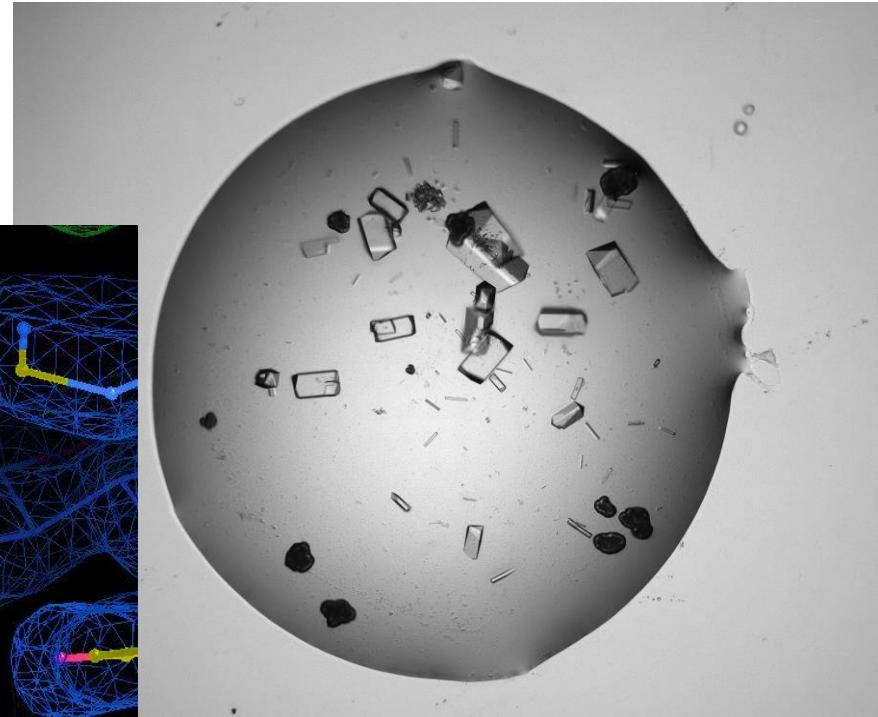
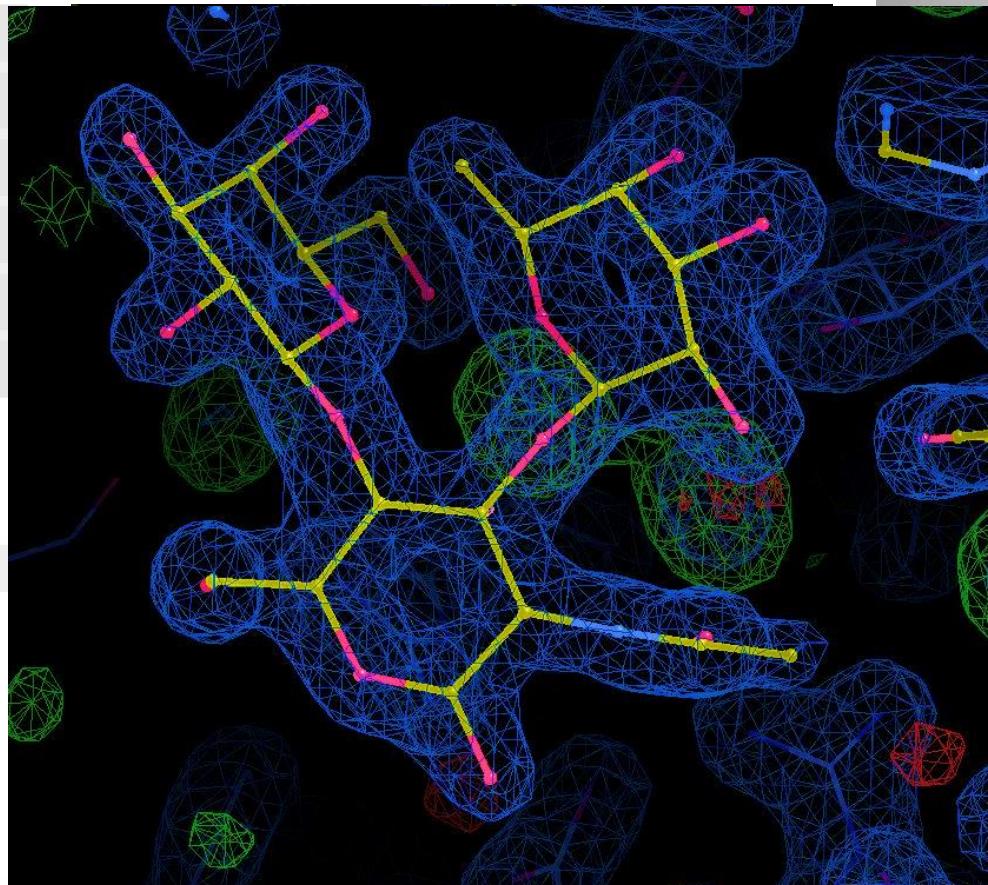
Each sweep - 60 degrees data in ~1 second

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: 10x10µm	Type: SAD

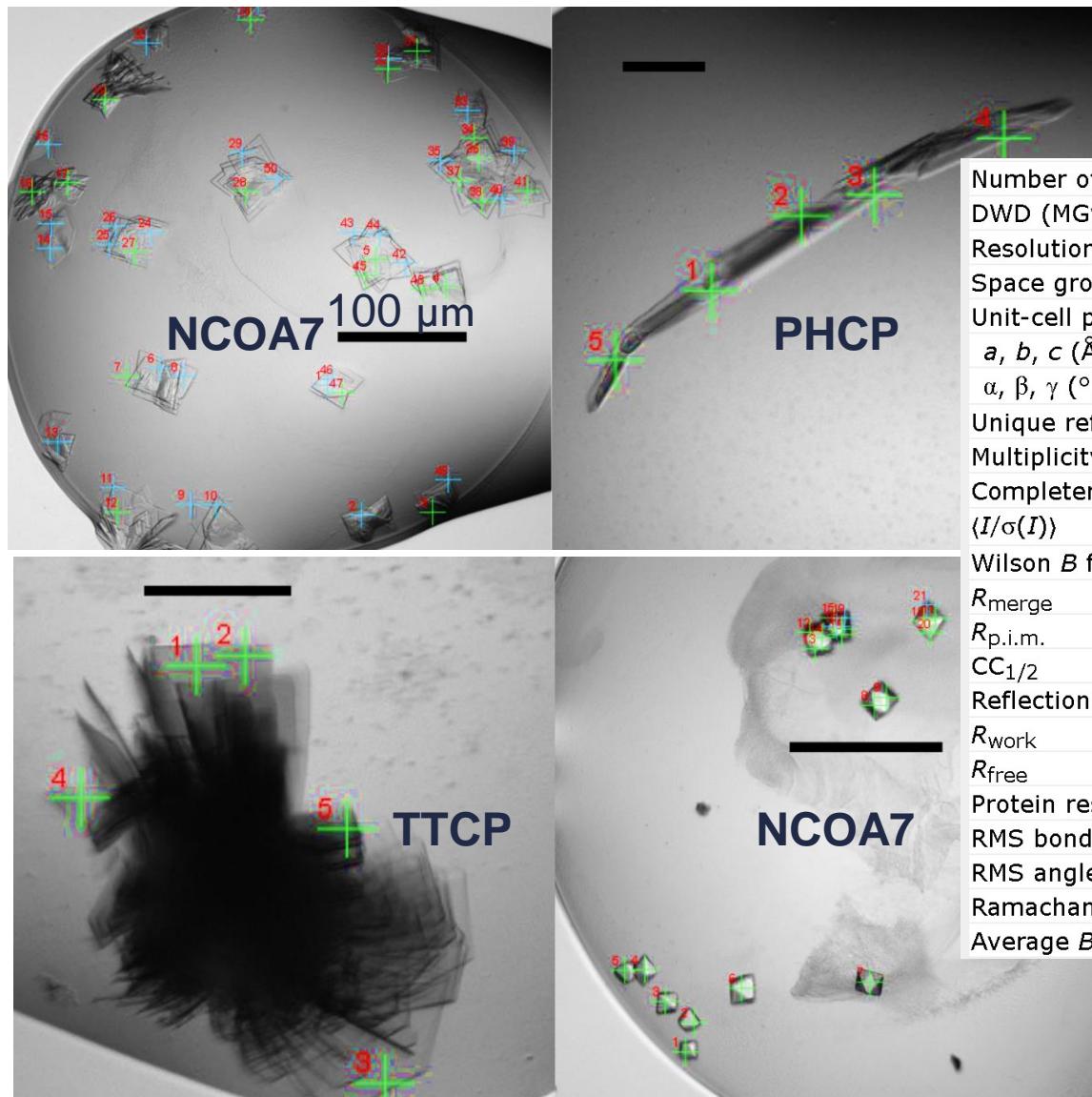
Multiple crystals selected

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
4x xia2.multiplex	70.44 - 1.91



Ligand clearly visible in fo-fc maps and was easily built into density – publication pending

Challenging crystals – ugly or tiny!



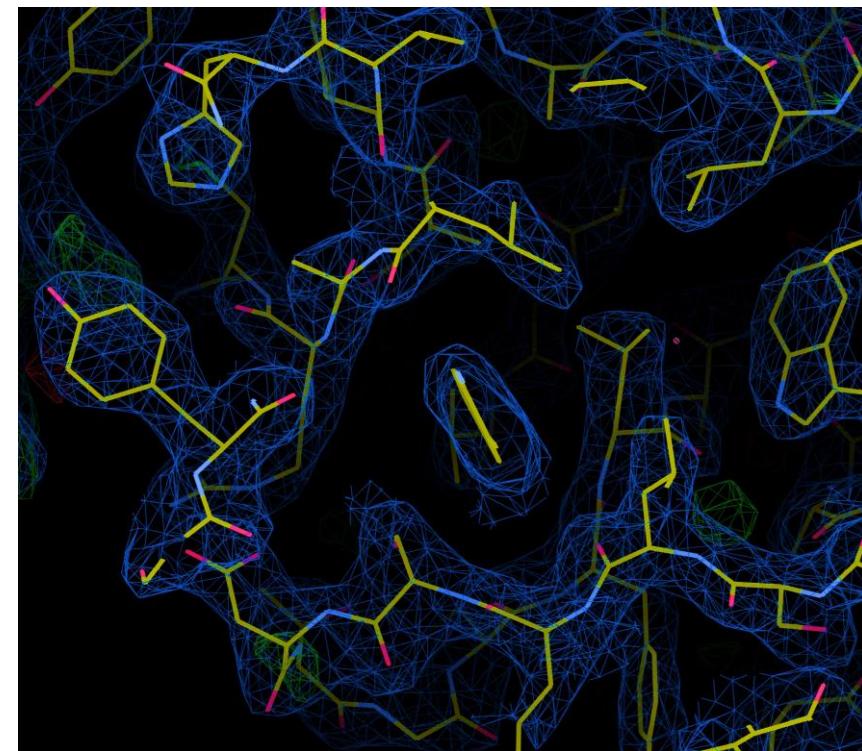
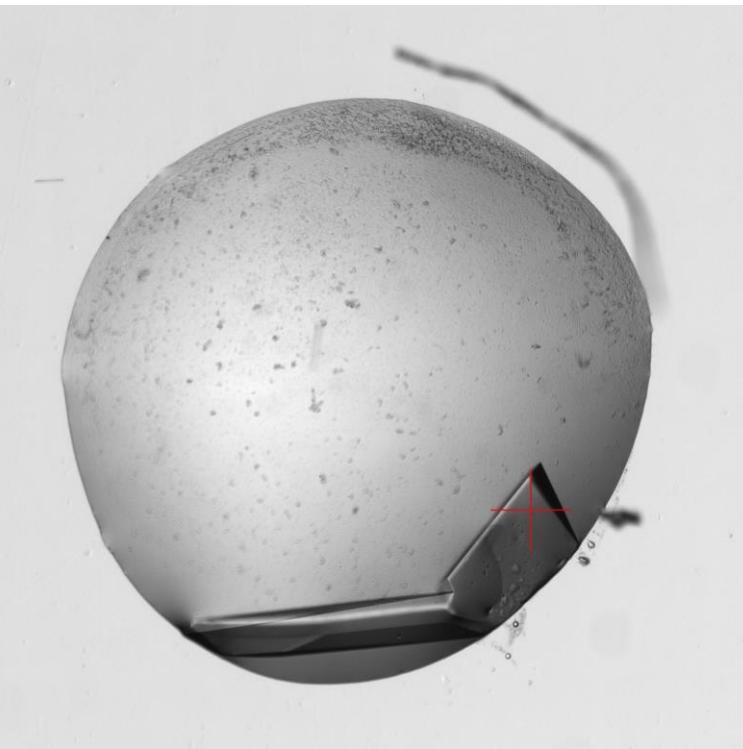
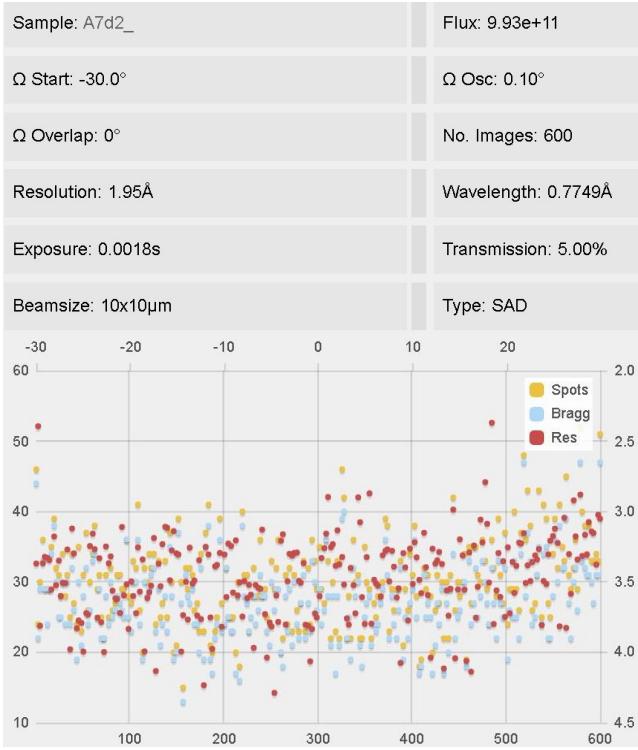
From thin irregular plate and needle-like objects, clusters of crystals, to tiny crystals, VMXi can deliver structures.

	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	P6 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
α , β , γ (°)	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> / σ (<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. *JUCr* 10: 420–429



Challenging crystals – stuck to plate!



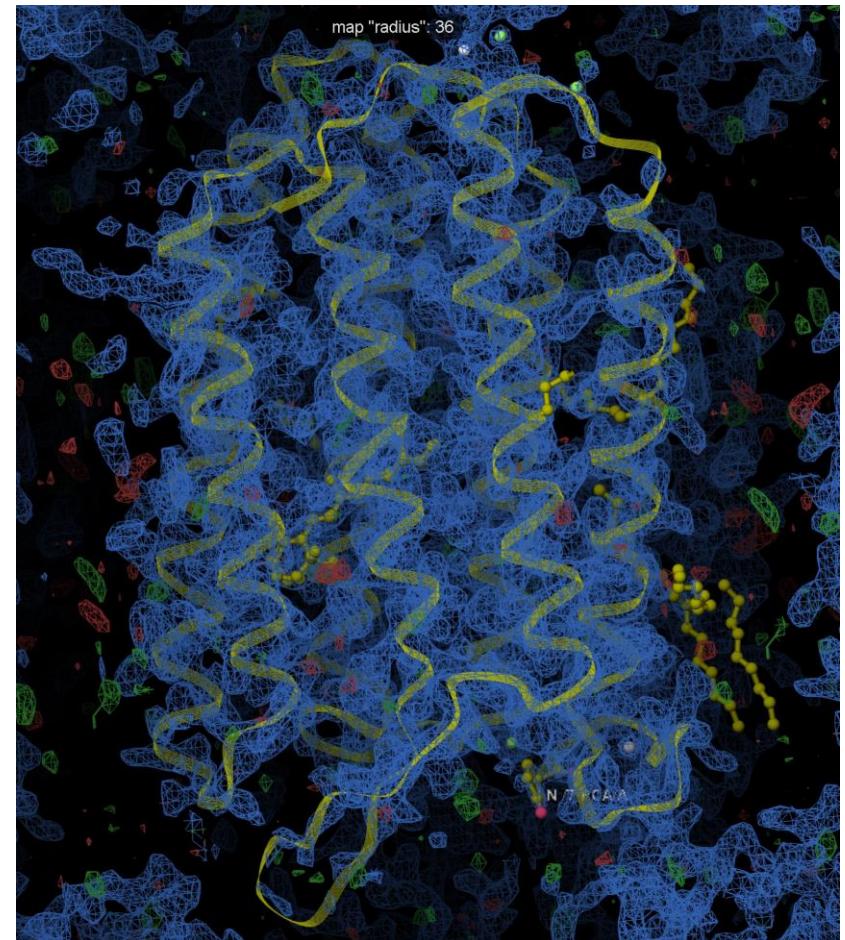
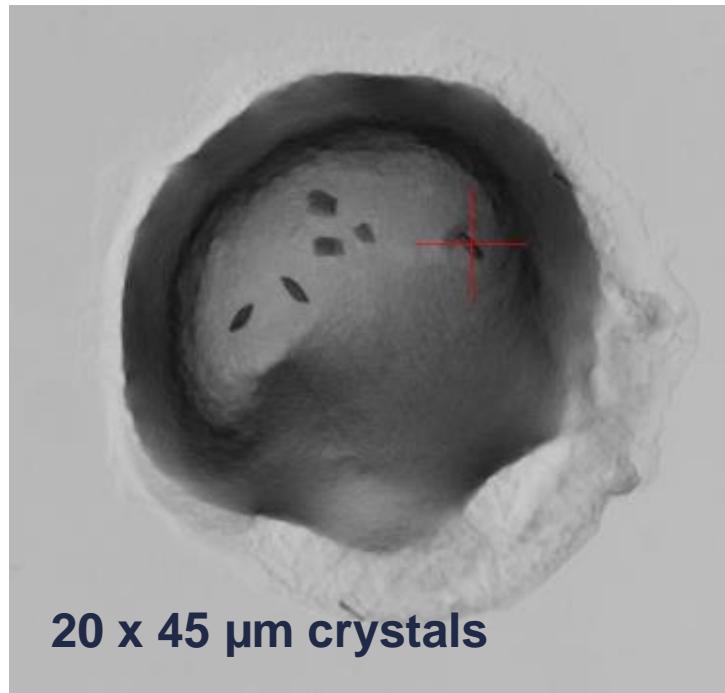
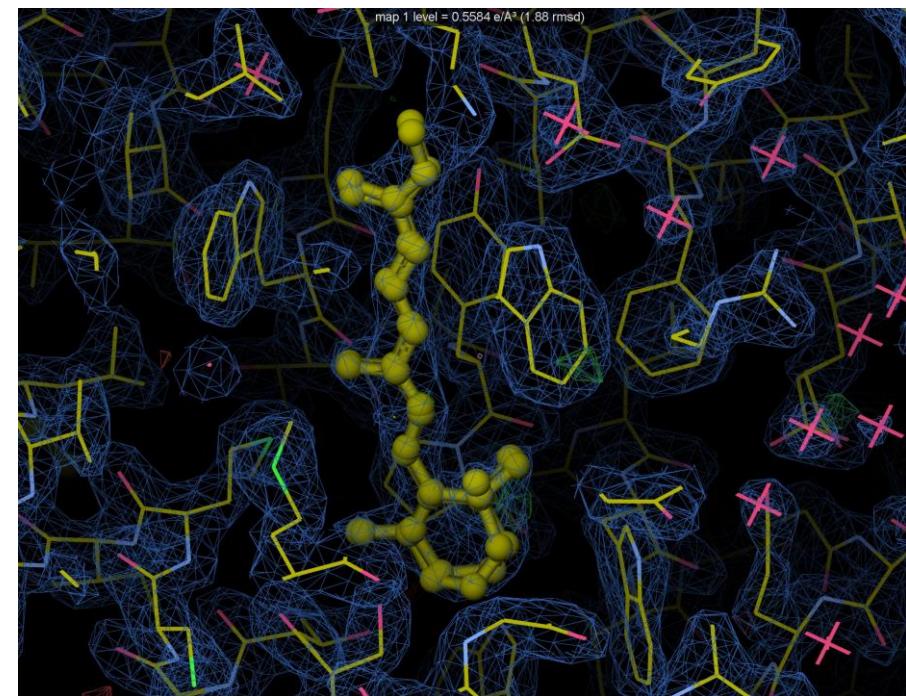
data	Resolution	Resolution I/sig(I)=2	Spacegroup	Mn<I/sig(I)>	Rmeas Inner	Rmeas Outer	Completeness
fast_dp	29.54 - 2.74	0.00	I 2 2 2	10.5	0.024	0.929	53.2
xia2_dials	68.88 - 2.38	2.66	I 2 2 2	9.7	0.044	2.295	54.0
xia2_3dii	74.85 - 2.44	2.76	I 2 2 2	7.0	0.043	2.114	53.7
autoPROC	68.86 - 2.51	2.71	I 2 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 Archaeorhodopsin-3 – from MPL group at DLS

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



Serial data collection

Diffraction-weighted dose (kGy)

HEWL (8 drops, PDB entry 8rge)

33

Resolution range (Å)

55.56–1.88 (1.95–1.88)

Space group

P4₃2₁2

a, b, c (Å)

78.57, 78.57, 37.77

α, β, γ (°)

90, 90, 90

No. of diffraction patterns merged

9891

Volume dispensed

1.6 µl [200 nl per drop]

Total reflections

1117460 (32281)

Unique reflections

10076 (973)

Multiplicity

110.9 (65.1)

Completeness (%)

99.27 (99.90)

Mean I/σ(I)

19.1 (1.2)

Wilson B factor (Å²)

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)

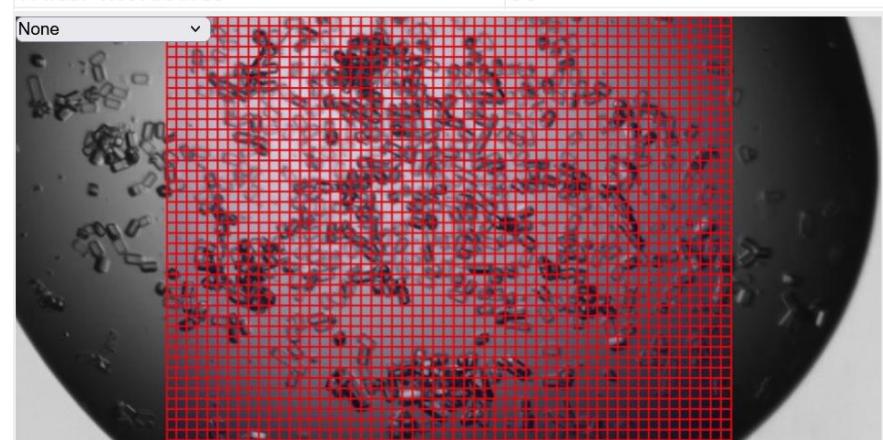
R_{free}

0.2305 (0.4288)

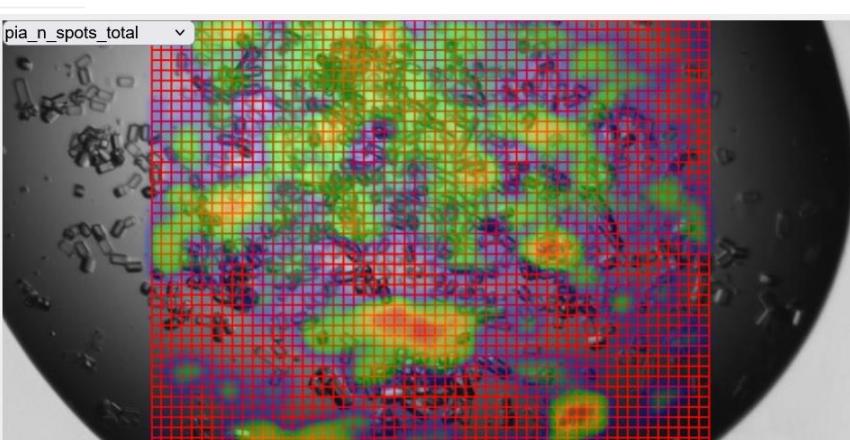
Water molecules

83

None



pia_n_spots_total



Sample: A11d2_

& Start: 0.0°

Resolution: 1.95 Å

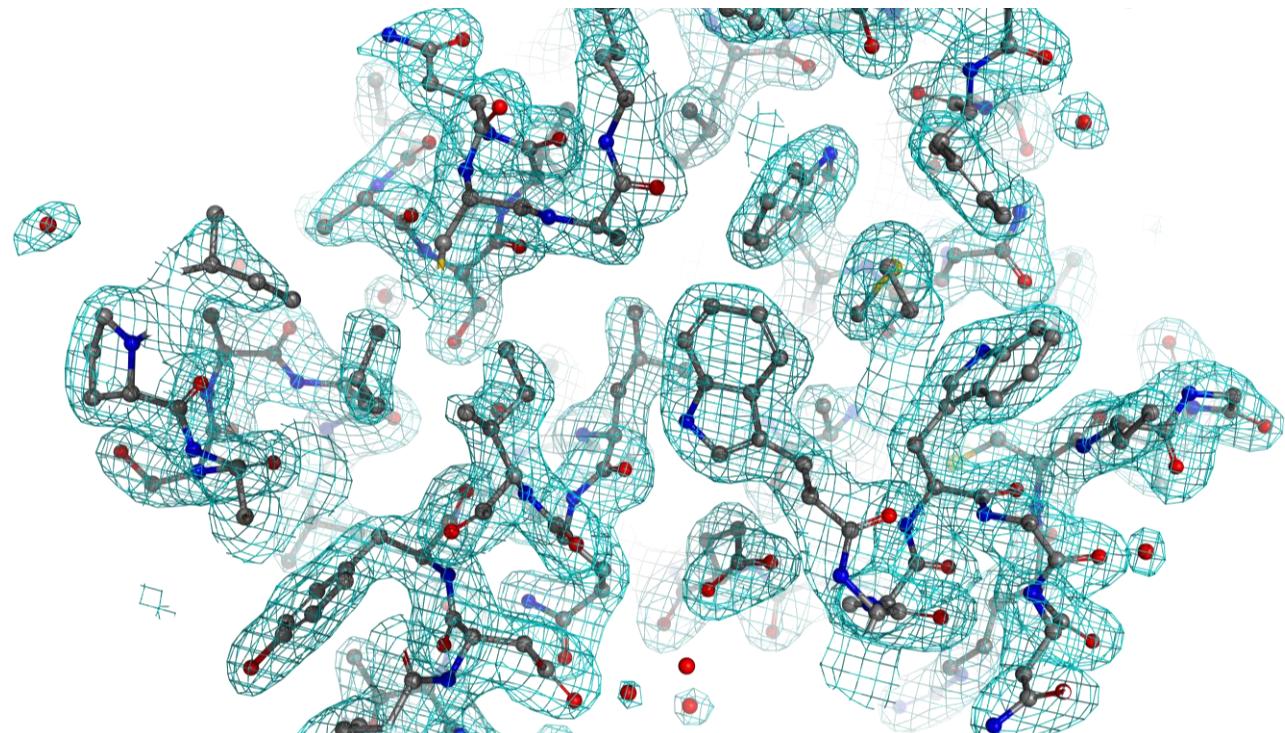
Wavelength: 0.7749 Å

Exposure: 0.0020s

Transmission: 100.00%

Beamsize: 10x10 µm

Boxsize: 10x10 µm



~3 mins / grid <30 mins total time

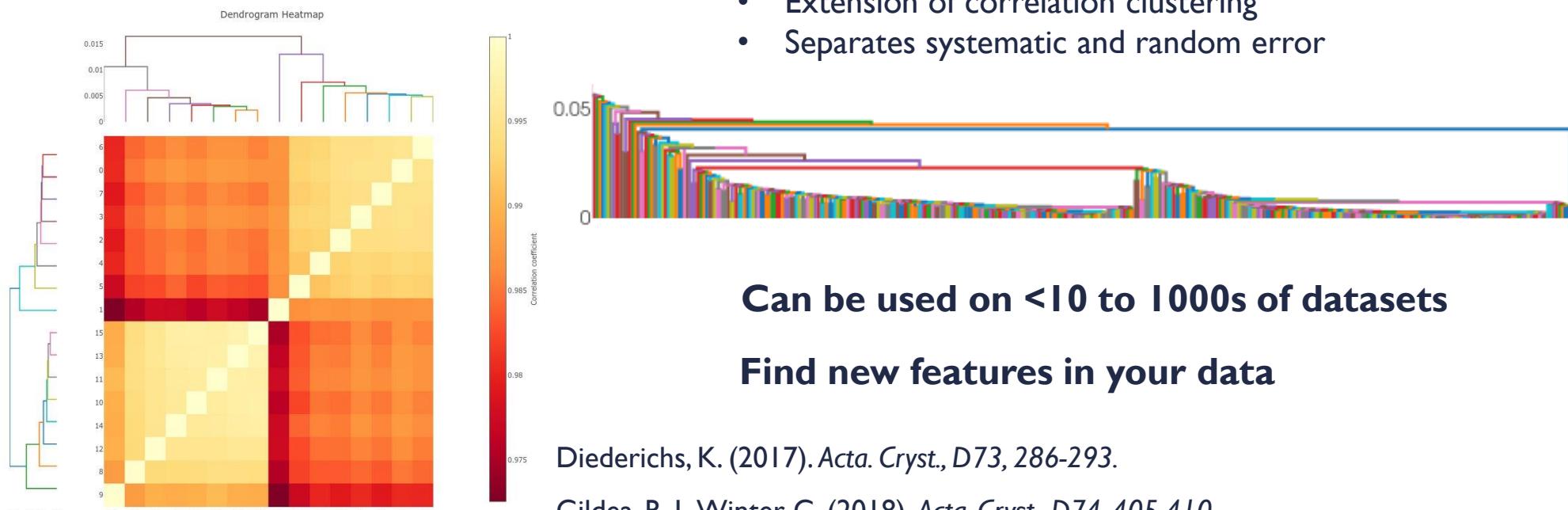
Thompson, A. J., Sanchez-Weatherby, J., et al. (2024). Efficient in situ screening of and data collection from microcrystals in crystallization plates. Acta Cryst. D80: 279–288

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

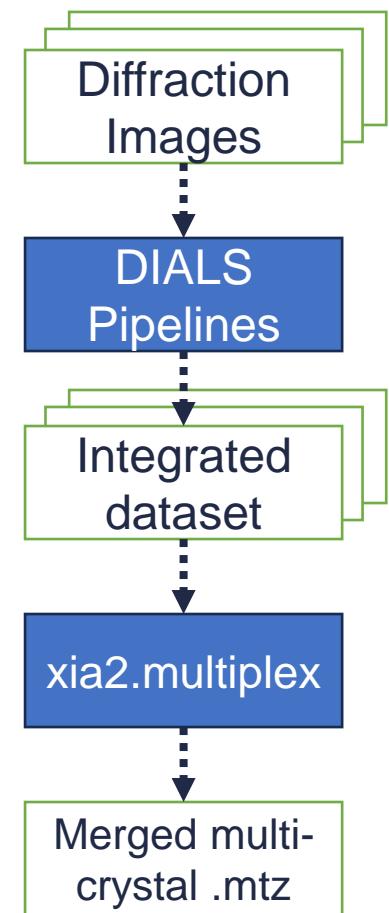
Can be used on <10 to 1000s of datasets

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.

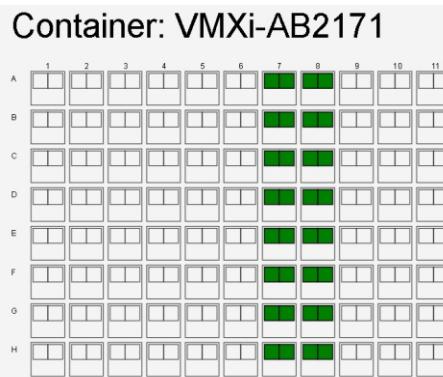
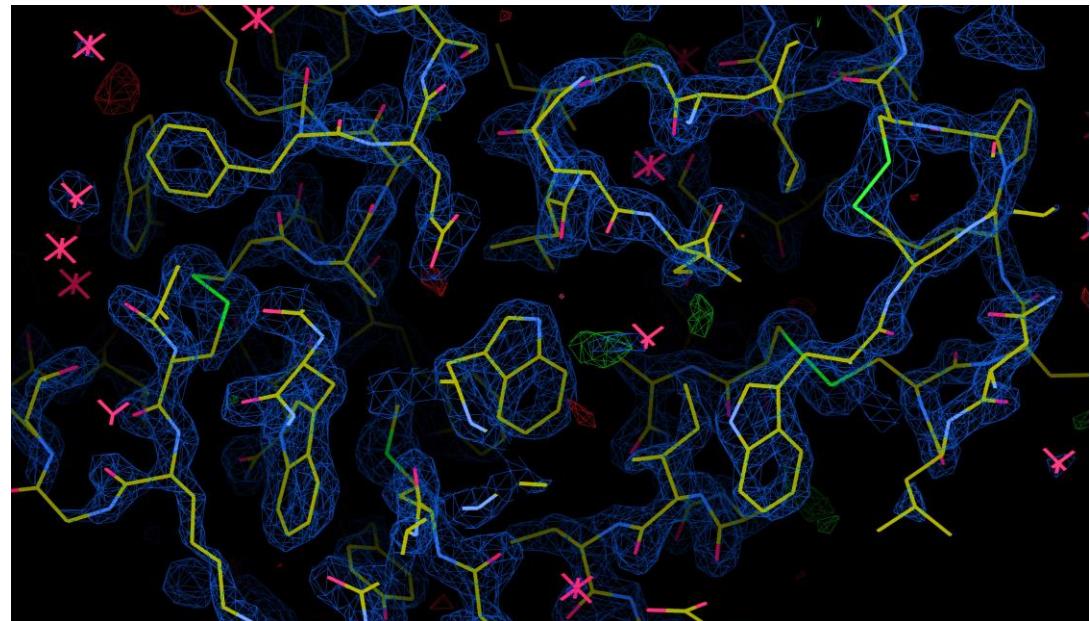
Auto-processing Pipeline



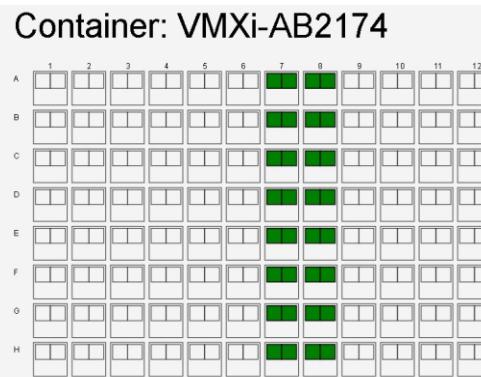
Sample groups

Taking xia2.multiplex to the next level

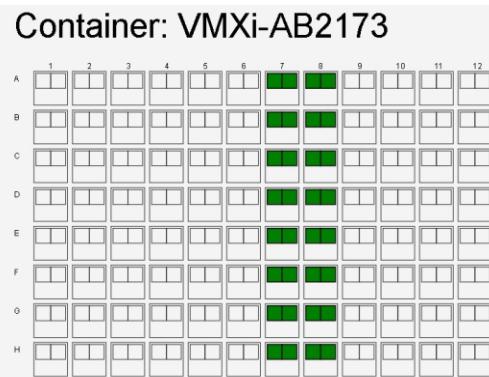
Type	Resolution
1 289x xia2.multiplex	1.51 - 78.89
3 292x xia2.multiplex	1.51 - 78.89



Shell	Observations
outerShell	94701
innerShell	1103776
overall	13894097



Unique	Resolution
913	1.51 - 1.54
1094	4.10 - 79.00
19187	1.51 - 78.89



Rmeas	I/sig(I)	CC Half	Completeness	Multiplicity
-14.456	0.1	0.3	100.0	103.7
0.180	252.5	1.0	100.0	1,008.9
0.938	44.6	1.0	100.0	724.1

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

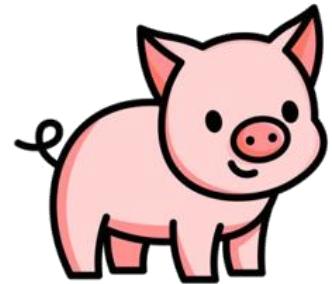
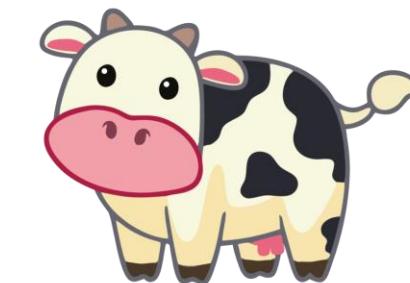


Clustering – Test Case



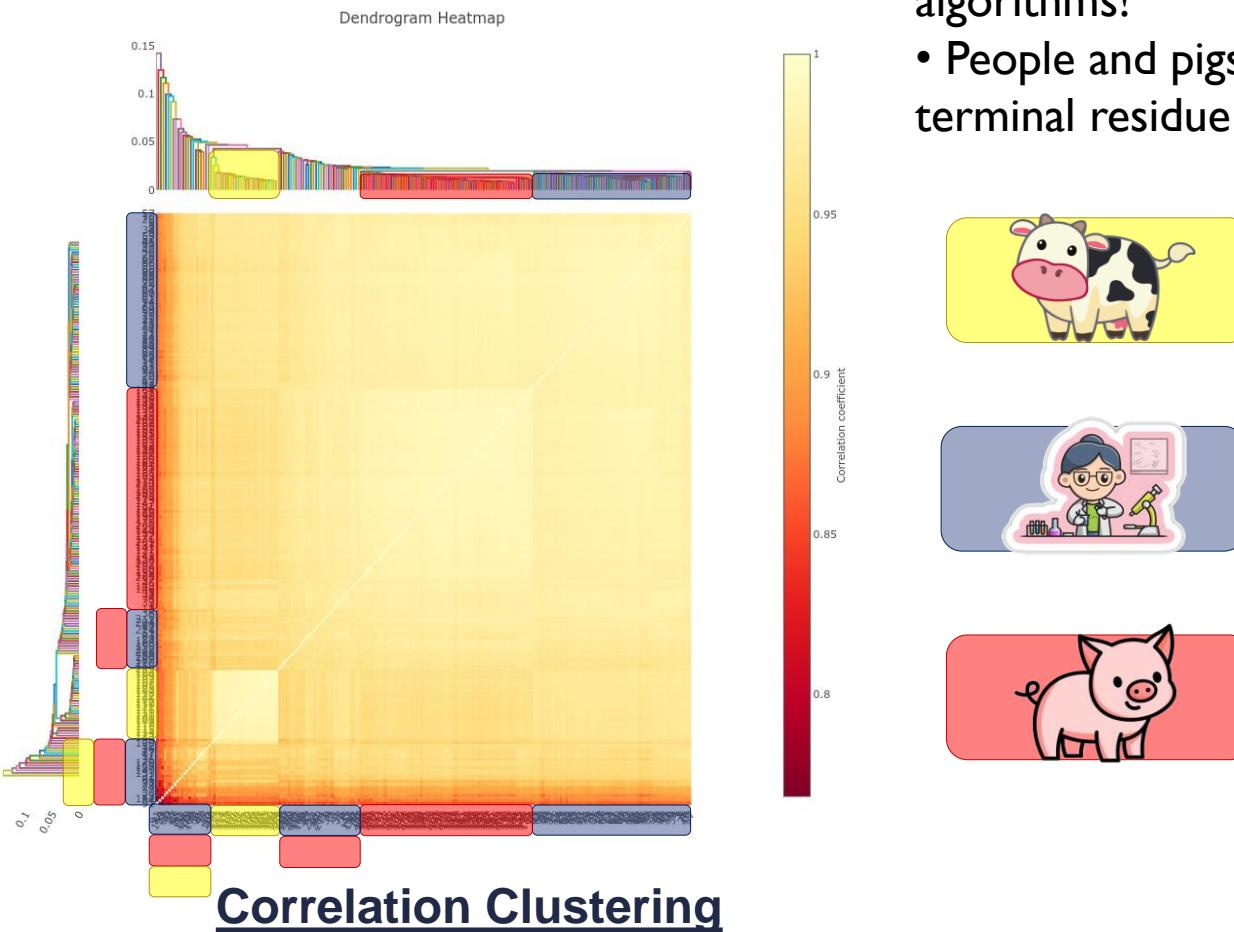
Human insulin, porcine insulin and bovine insulin differ subtly in their amino acid sequences and readily grow high-quality, cubic, isomorphous crystals

Cows	Pigs	People
Chain A Residue 8 <chem>NC(C(=O)O)C</chem> Alanine (Ala, A)	<chem>NC(CC(O)C(=O)O)C</chem> Threonine (Thr, T)	<chem>NC(CC(O)C(=O)O)C</chem> Threonine (Thr, T)
Chain A Residue 10 <chem>NC(C(C)C(=O)O)C</chem> Valine (Val, V)	<chem>NC(C(C)CC(=O)O)C</chem> Isoleucine (Ile, I)	<chem>NC(C(C)CC(=O)O)C</chem> Isoleucine (Ile, I)
Chain B Residue 30 (terminus) <chem>NC(C(=O)O)C</chem> Alanine (Ala, A)	<chem>NC(C(=O)O)C</chem> Alanine (Ala, A)	<chem>NC(CC(O)C(=O)O)C</chem> Threonine (Thr, T)





Clustering – Test Case



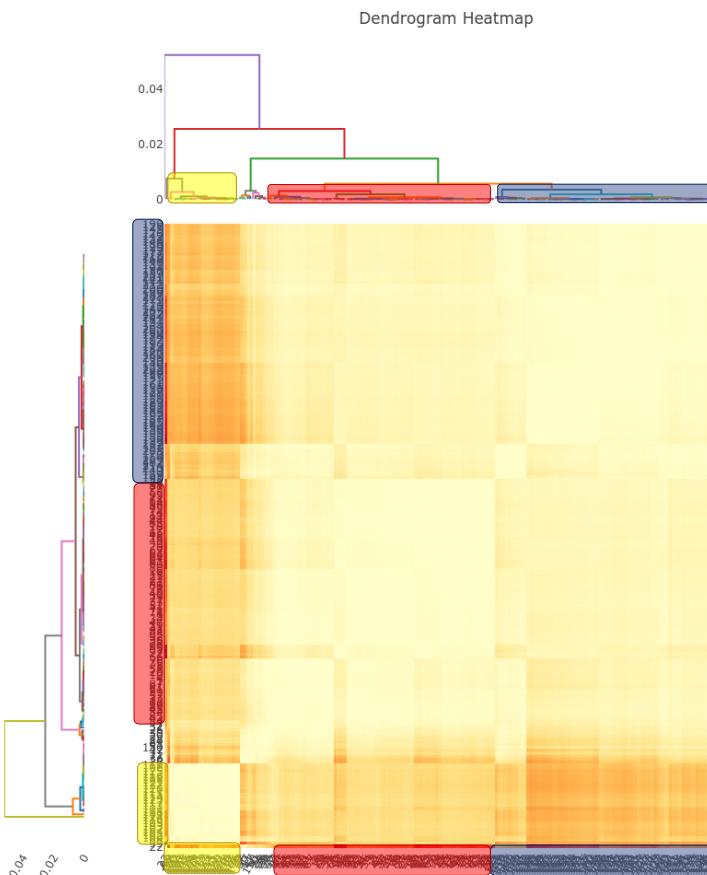
- All three species are distinguishable at room temperature with new algorithms!
- People and pigs mix in the dendrogram before cows – consequence of terminal residue position being less well defined at room temperature



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

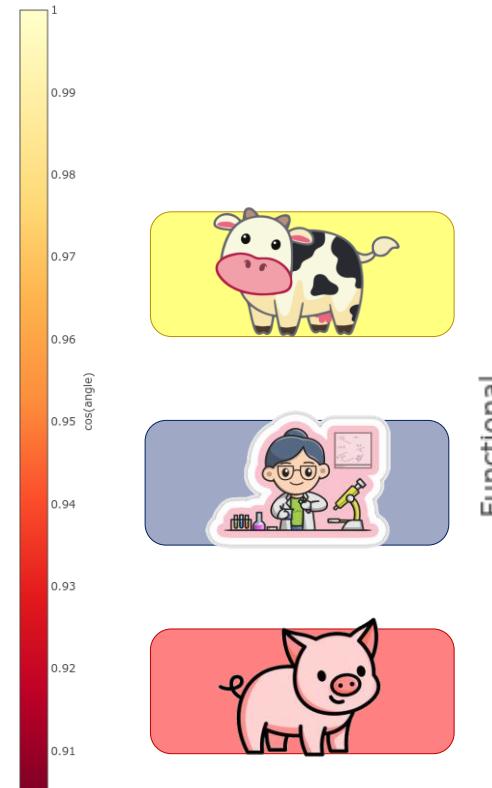


Clustering – Test Case

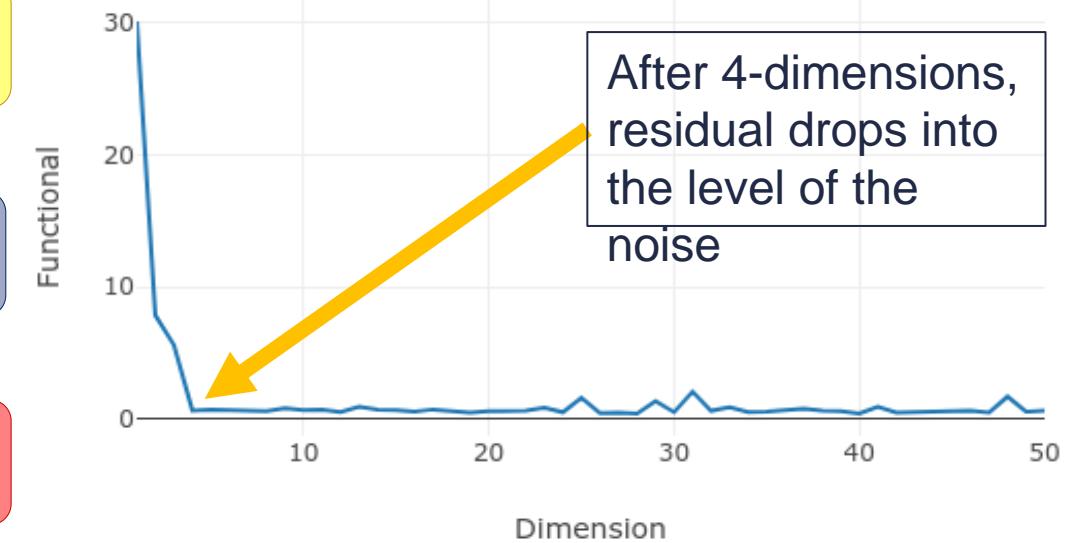


4D Cosine Angle
Clustering

Multiple clusters due to different samples and poor data quality



Residual for each tested dimension

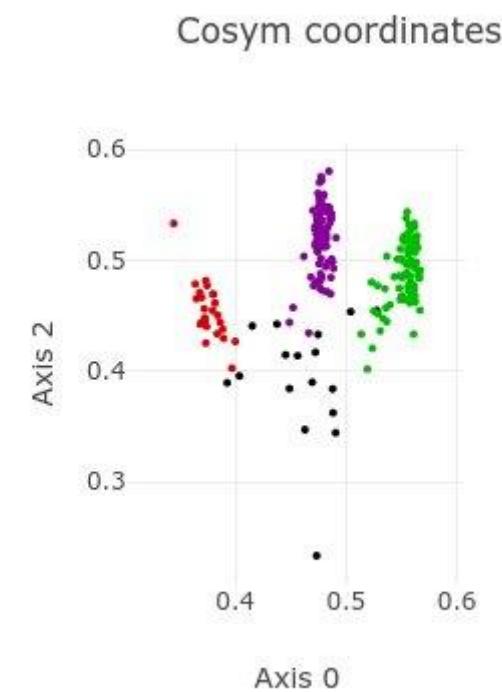
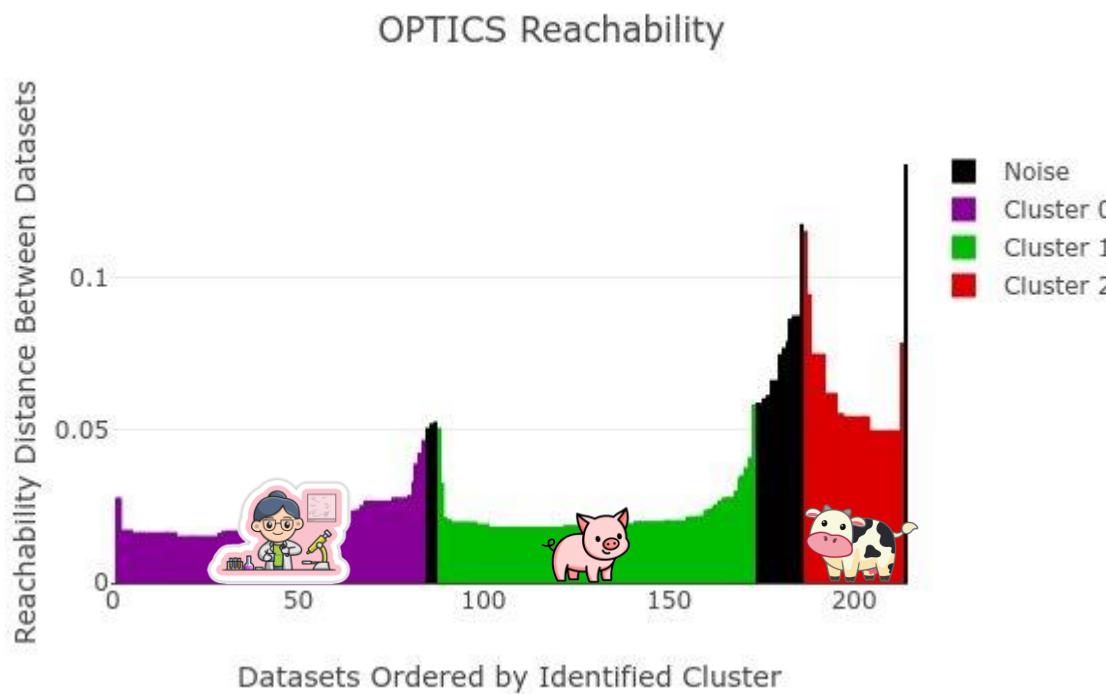




Clustering – Test Case

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)

6rvo 6rzp 6sel 6sva



2022 (5)

7zck 8a9d 8ar9 8brk 8brl



2023 (5)

8cif 8rge 8rgs 8rgw 8rgy



2024 (1)

9bkq



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Controls Team



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



Olly King - CHiMP

Diamond GDA Team

MX Data Analysis Team

ISPyB Development Team

Diamond Users