VMXm: A new micro/nanofocus protein crystallography beamline at Diamond

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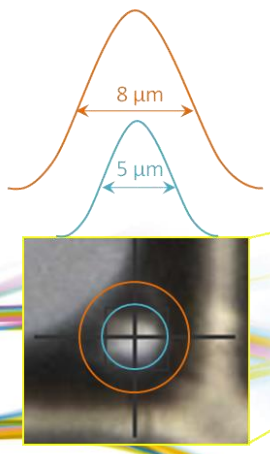
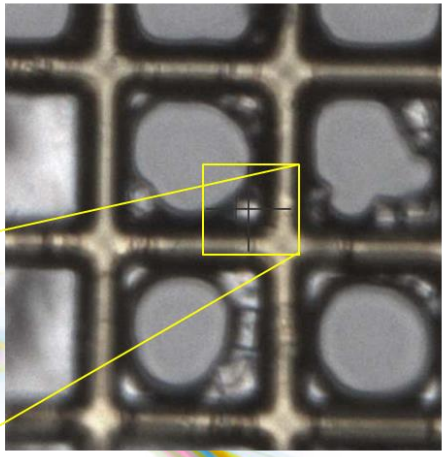
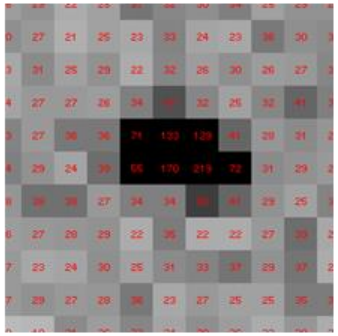
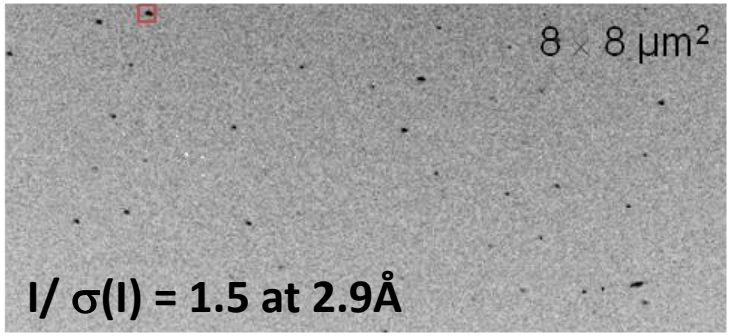
Introduction

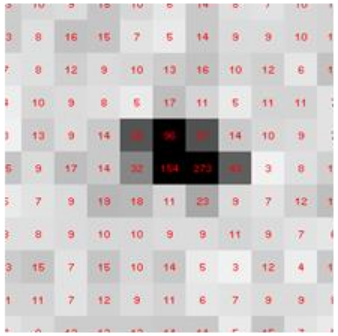
- Target proteins are getting more complex, leading to smaller and more disordered crystals
- Can design ideal experiment to give optimal data quality

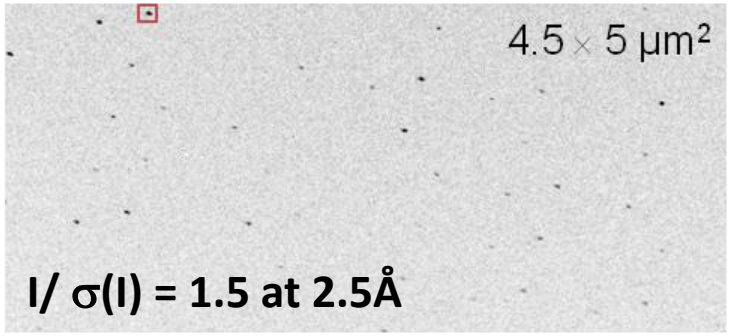












G. Evans, D. Axford and R. L. Owen (2011) *Acta Cryst.* **D67**, 261-270.

Current Limits

- 2.2 Å data can be collected from 1 μm^3 crystals (~700 well diffracting crystals)
- 3 Å data can be collected from 5 μm^3 membrane protein crystals grown in LCP (~35 crystals, grid scanned first for centring)
- From theoretical calculations a complete 2 Å dataset can be collected from a single 1 μm^3 lysozyme crystal (Holton and Frankel, 2010)
- Discrepancies between theory and experiment

Current Limits

- Dose tolerance of samples cannot be changed – Henderson/Garman limit fixed
- Reduce dose on sample to measure given data quality:
  - Reduce experimental background
  - Cleaner sample mounting
  - Improve analysis for weak and multicrystal data
  - Record rotation data to improve data quality
  - Visualization of micron and sub-micron crystals

VMXm Aims

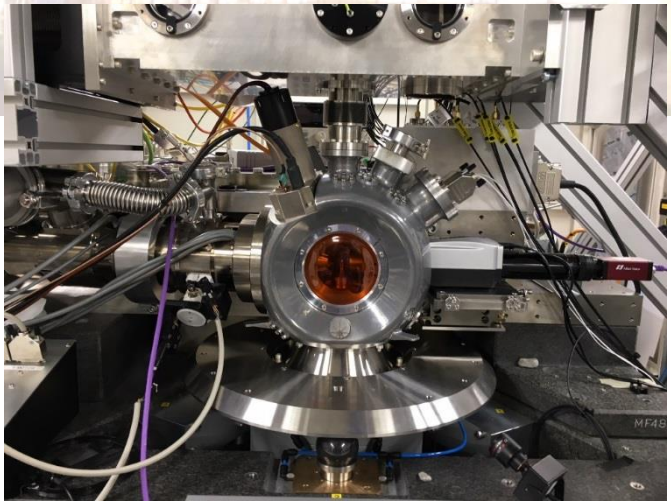
- Improve signal to noise by reducing background:
  - Sample environment under vacuum
  - Crystals mounted with minimal liquid
  - Reduce beamsize to match that of the crystal
- Standard rotation data collection on samples down to 500 nm
  - Alignment without the need for X-ray raster scanning
- Optimise sample alignment, sample cooling and data analysis for micron and sub-micron crystals
- Data collections using minimal amounts of sample

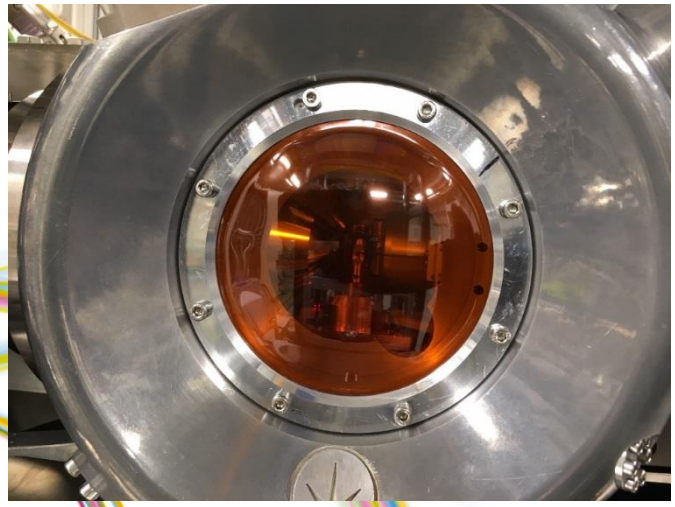
VMXm Specifications

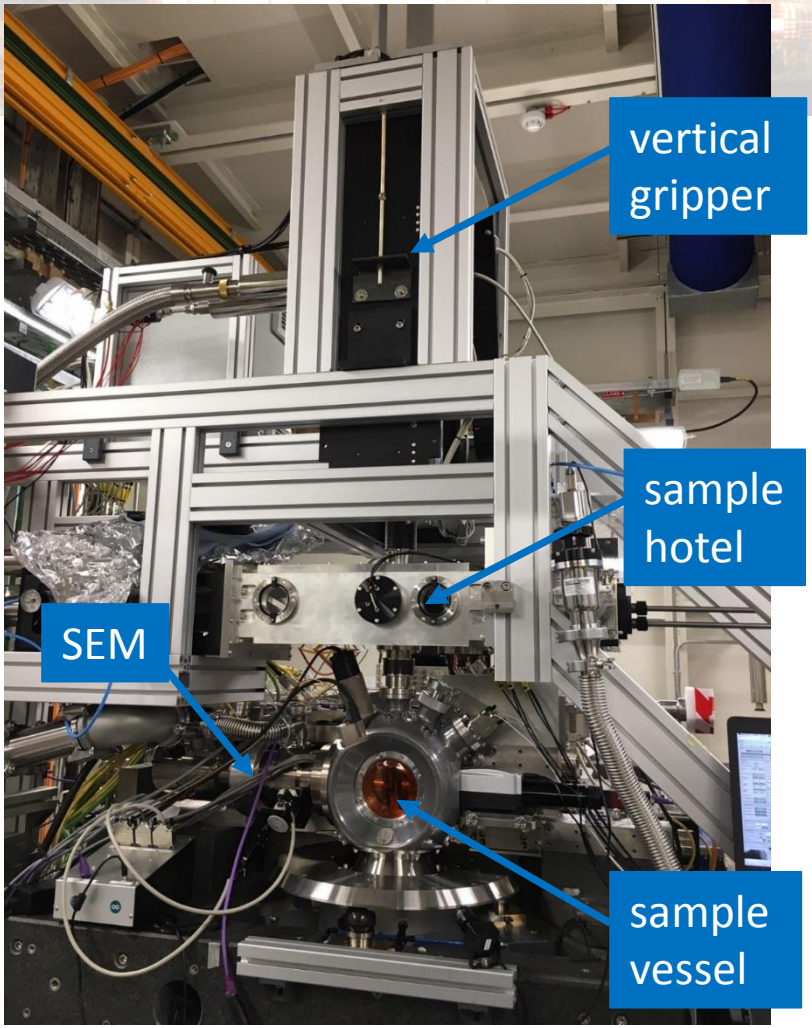
- 6 – 28 keV energy range
- 0.3 – 10 µm (v) & 0.5 – 5 µm (h)
- Flux
  - ~10^{13} ph/s in 0.3 × 5 µm (v x h)
  - >10^{11} ph/s 0.3 × 0.5 µm (v x h)
- initially monochromatic beam
- polychromatic beam as an upgrade path

Sample Environment

- *In vacuo* sample environment - reduce X-ray background to a minimum
- Cryo-stage - preserve sample *in vacuo*
- Standard on-axis optical microscope + SEM for sample visualization and alignment
- High stability goniometry - permit rotation data to be measured from micro and nanocrystals
- Serial data collection also possible







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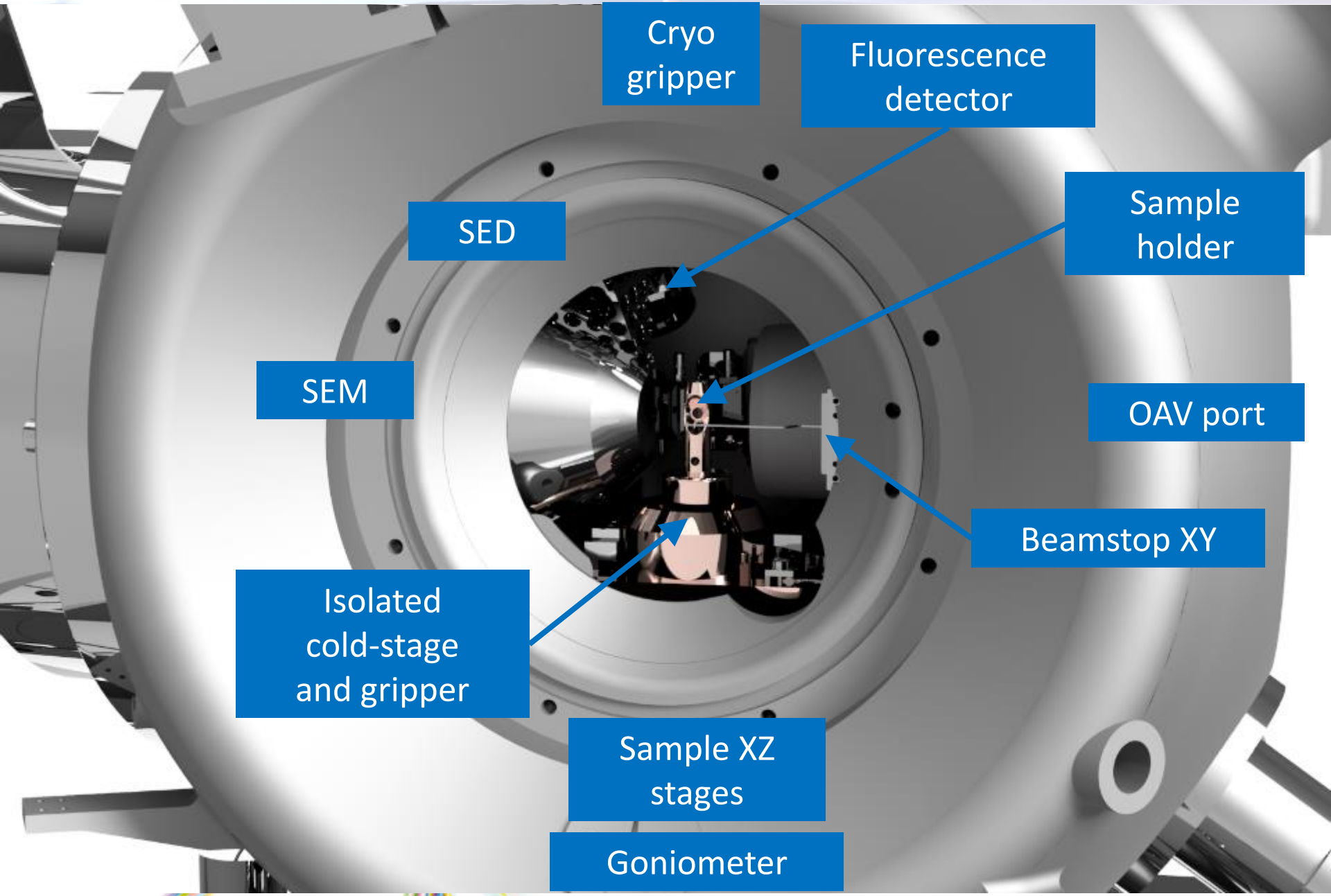
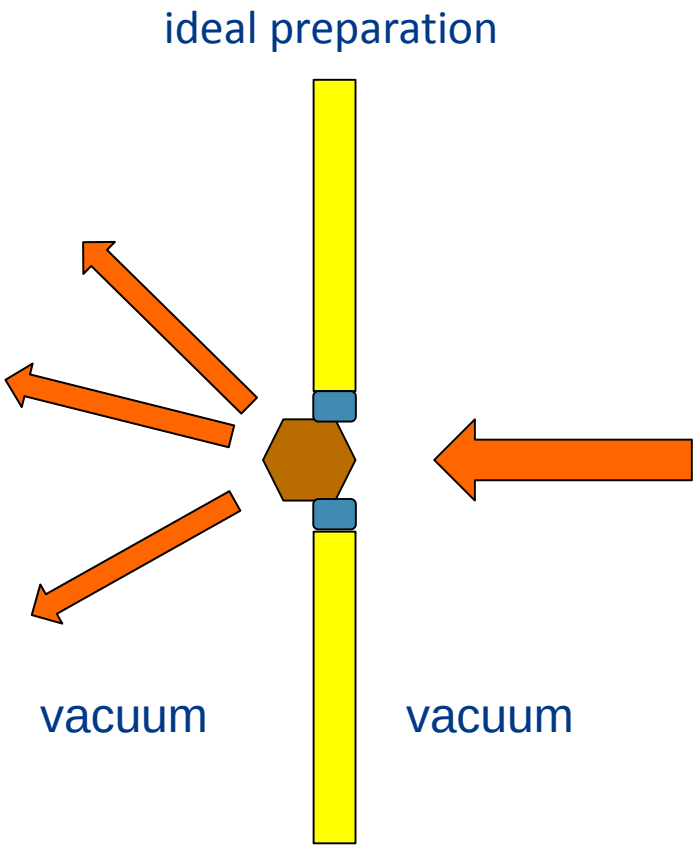

Cryo gripper
Fluorescence detector
SED
Sample holder
SEM
OAV port
Beamstop XY
Isolated cold-stage and gripper
Sample XZ stages
Goniometer

Sample Preparation

Ideal sample mounting:

- Reduce background to increase signal-noise:
  - Air path around sample
  - Sample mount
  - Solvent surrounding crystal
- Multiple crystals per mount
- Cryo-EM style sample preparation
  - Grids
  - Blotting
  - Plunge freezing
- SEM for sample characterisation



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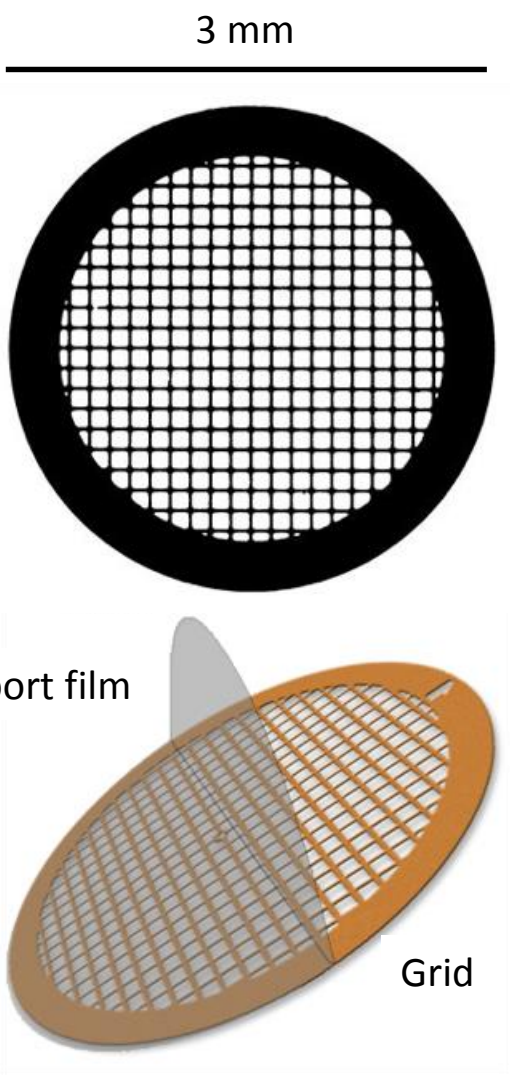
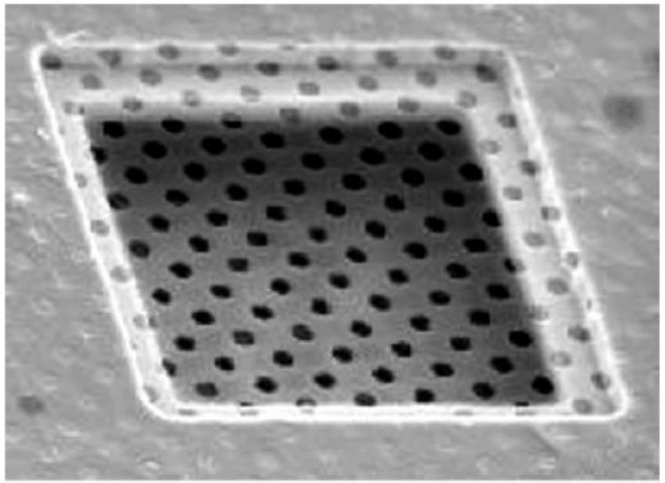
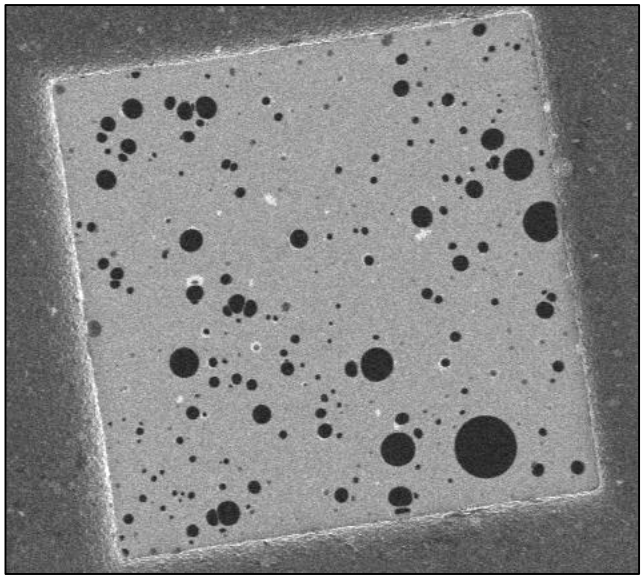
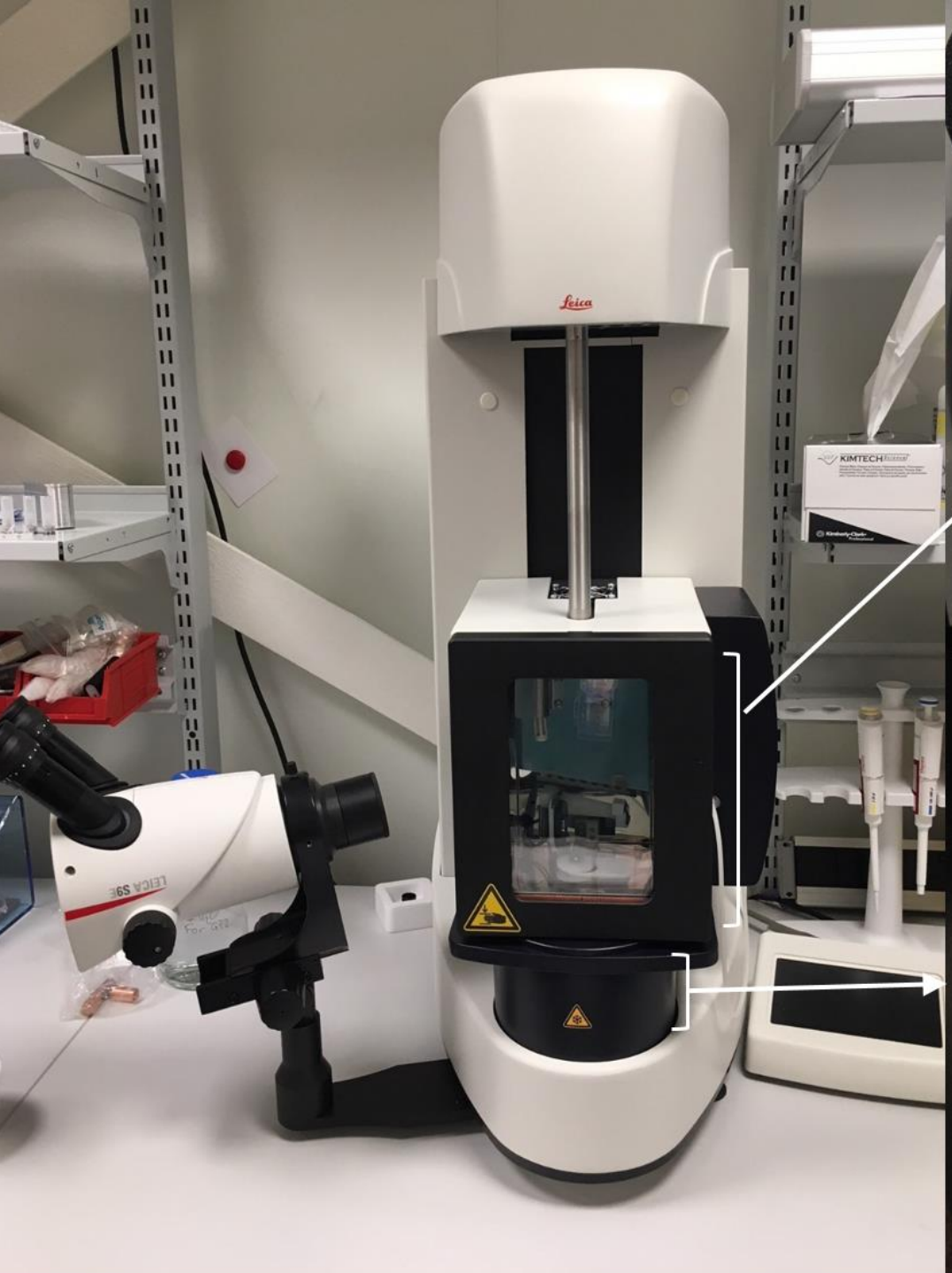
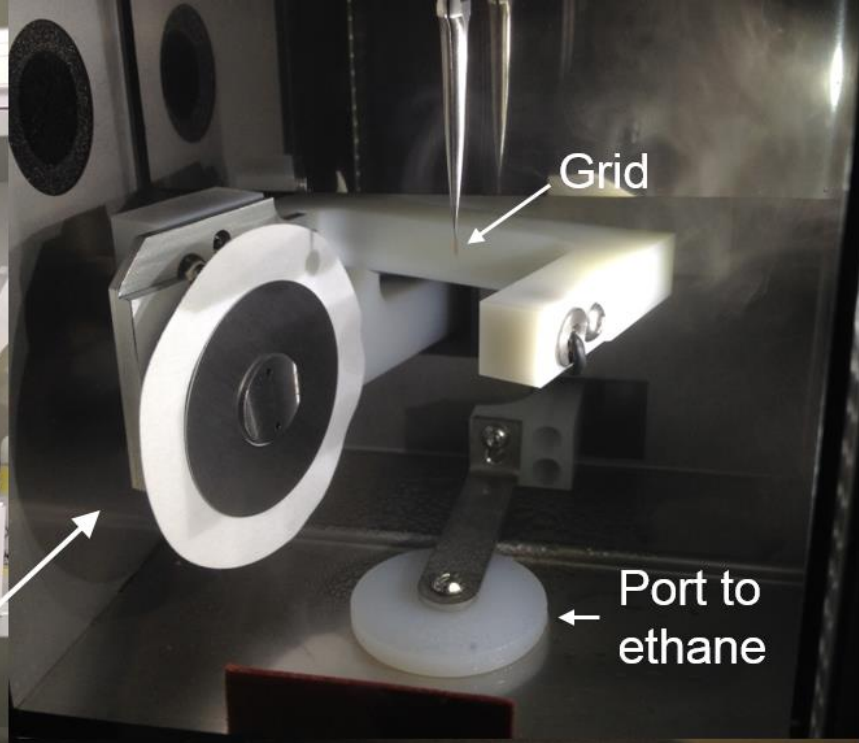
Cryo-EM Grids

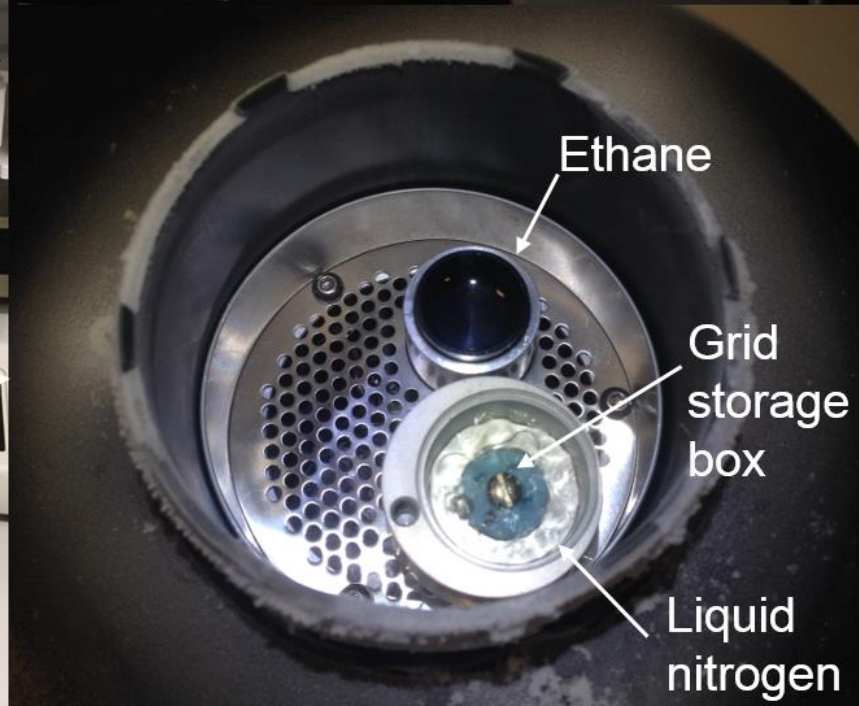




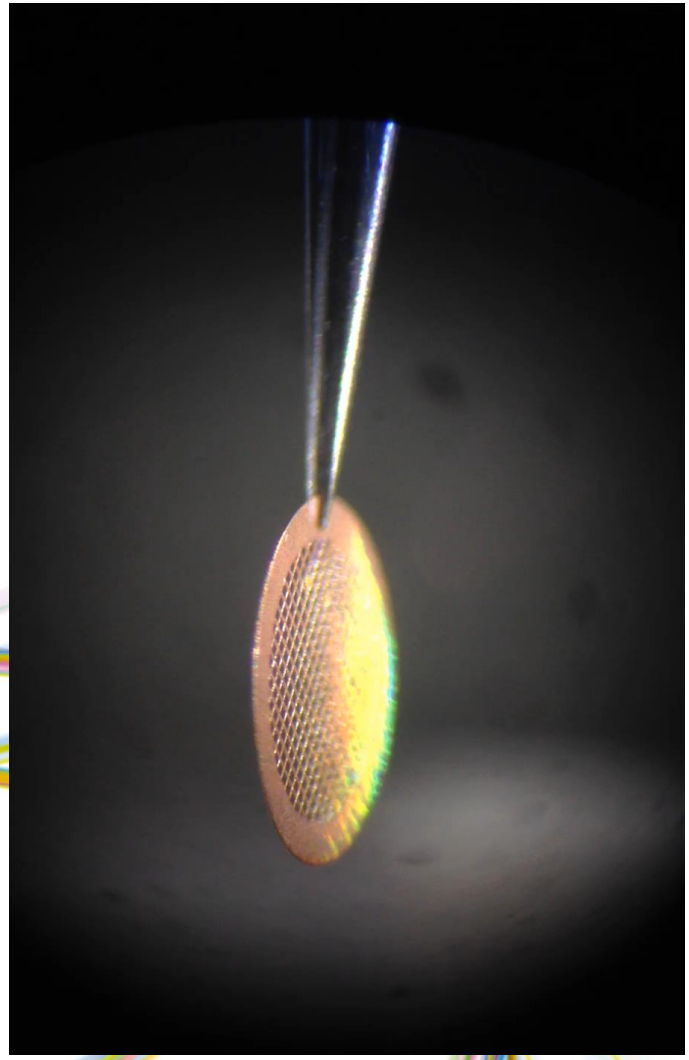


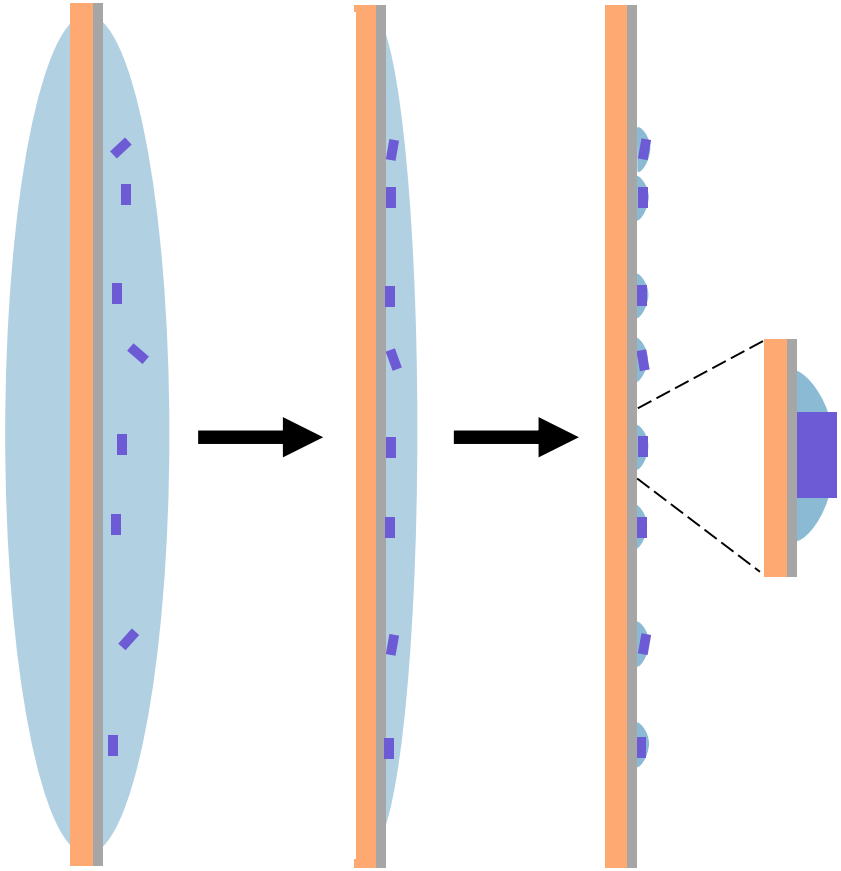

Leica
KIMTECH
Kimberly-Clark Professional
LEICA S9E


Grid
Port to ethane

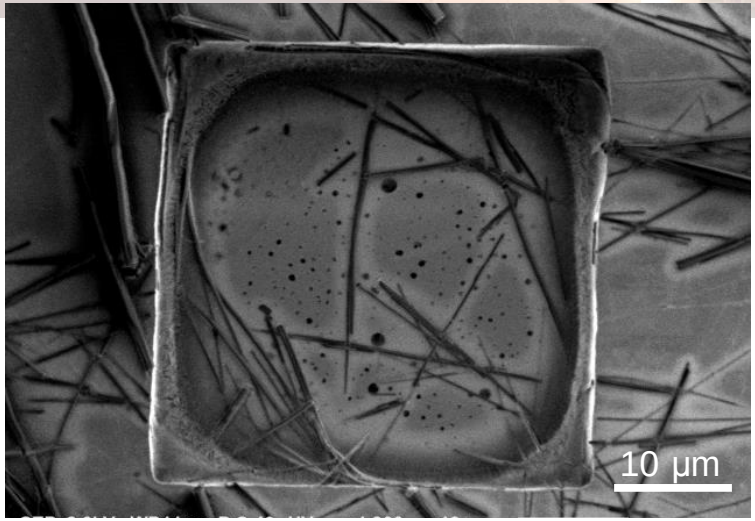

Ethane
Grid storage box
Liquid nitrogen

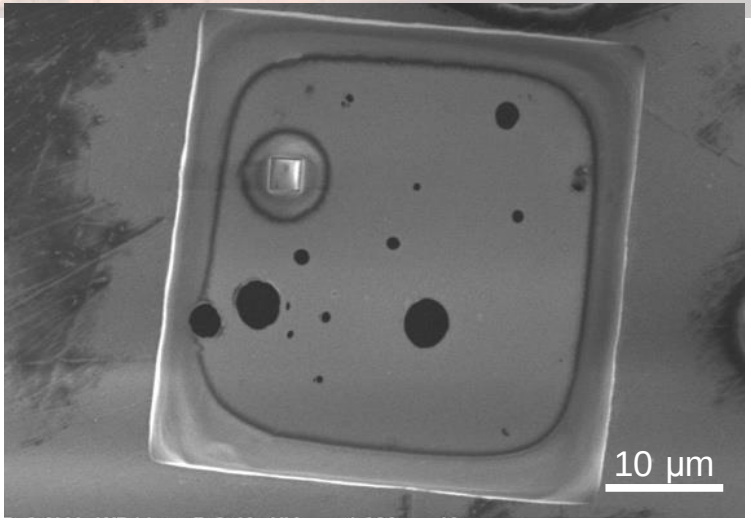
Sample Blotting

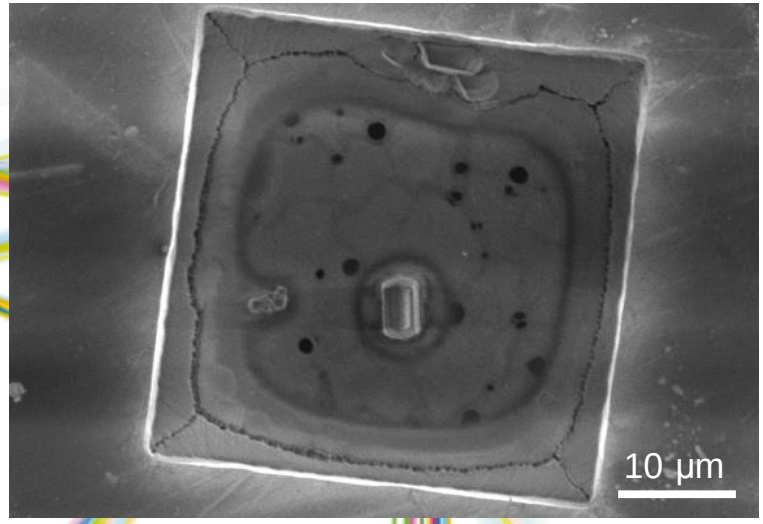


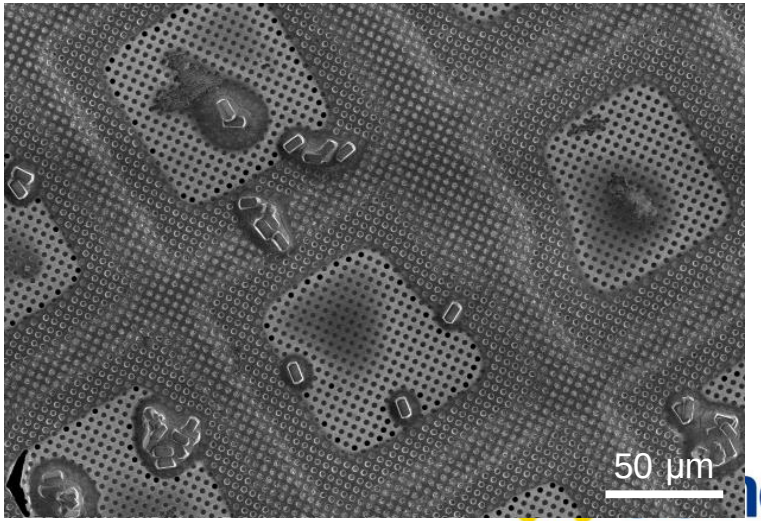


Sample Characterisation

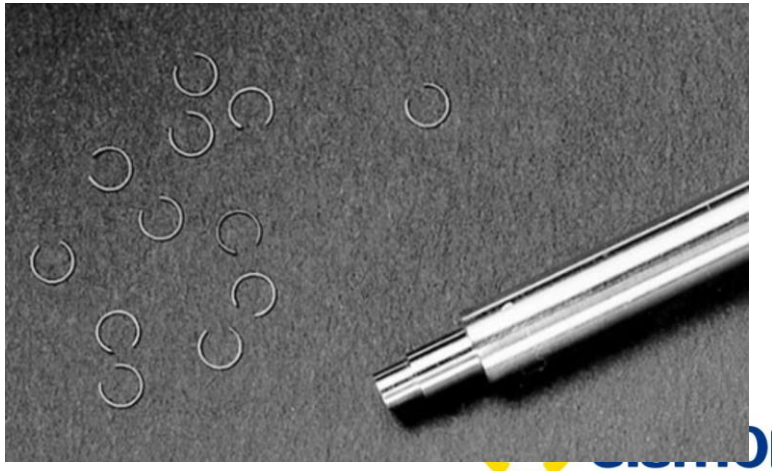






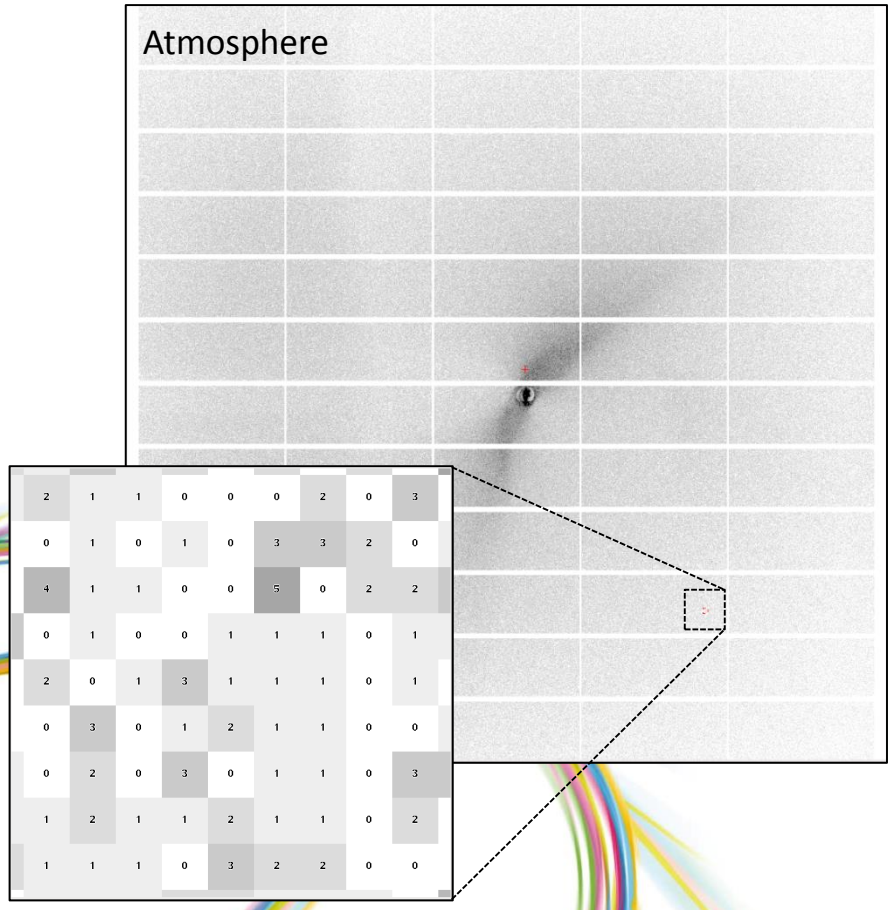


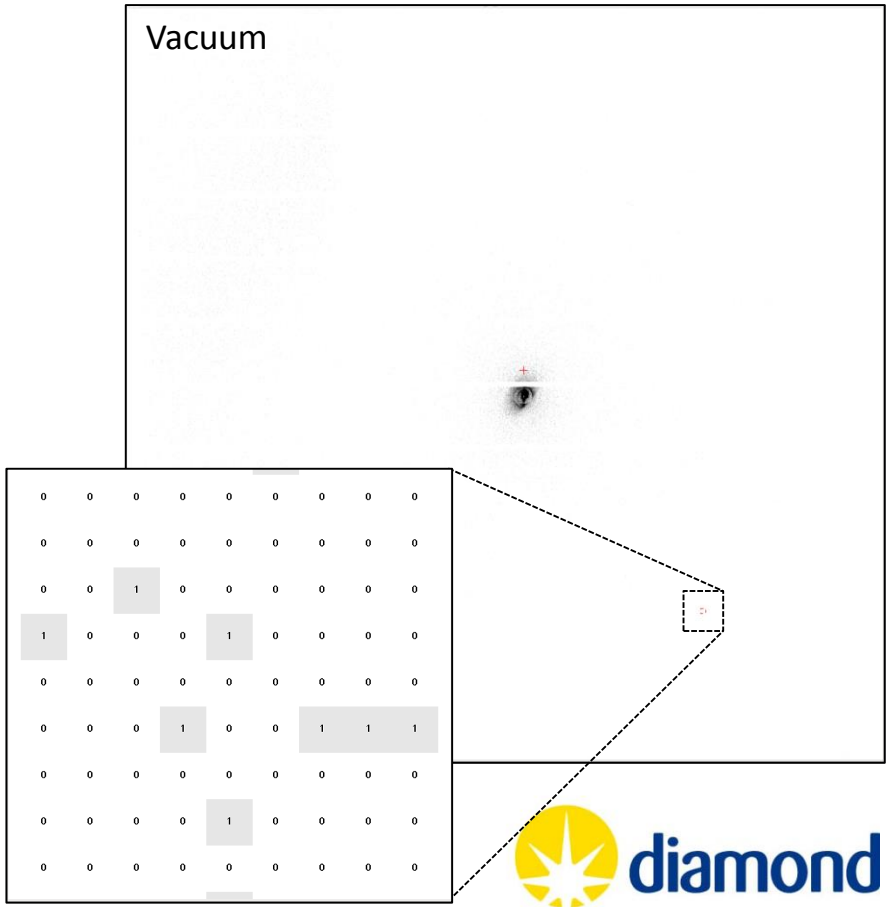
Sample Mounting



RT Measurements in Vacuum

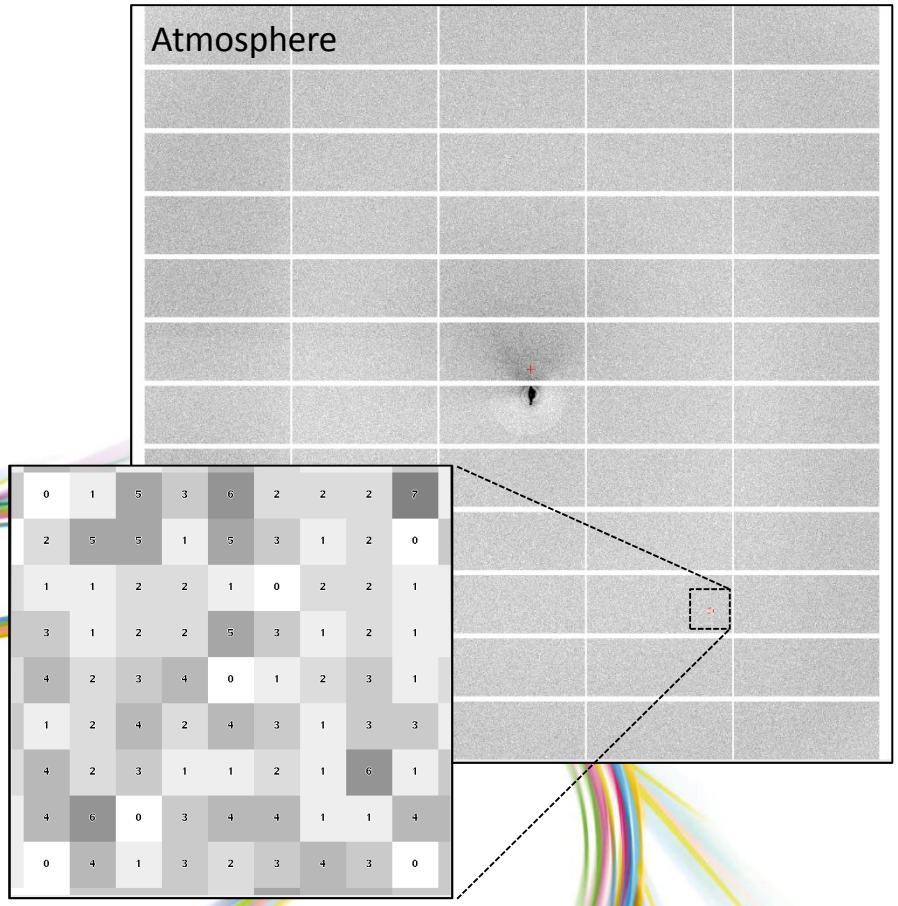
No sample
300 mA, 12.4 keV, 100 % transmission, 0.5s exposure

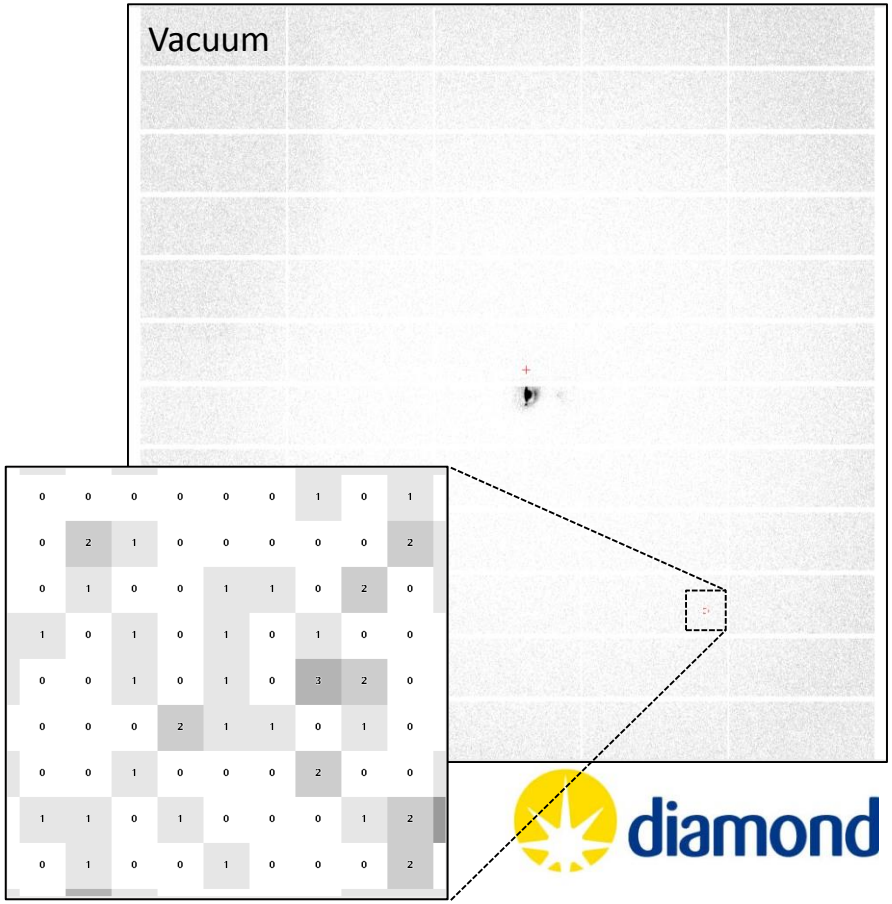




RT Measurements in Vacuum

Shot through SiN window containing 1M NaAc pH 3.0, 6% PEG 6000, 20% NaCl
500 nm windows x 2, ~10-20 um solvent thickness
300 mA, 12.4 keV, 100% transmission, 0.5s exposure





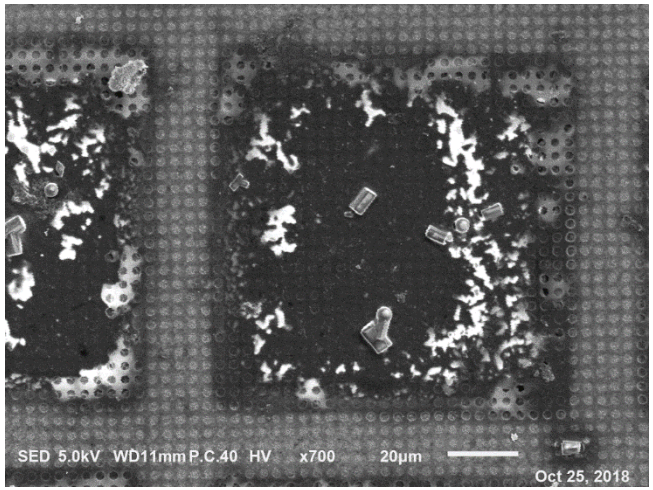
First User Experiments

- Dr. Ivo Tews and Rachel Bolton
  - Institute of Life Sciences, School of Biological Sciences, University of Southampton

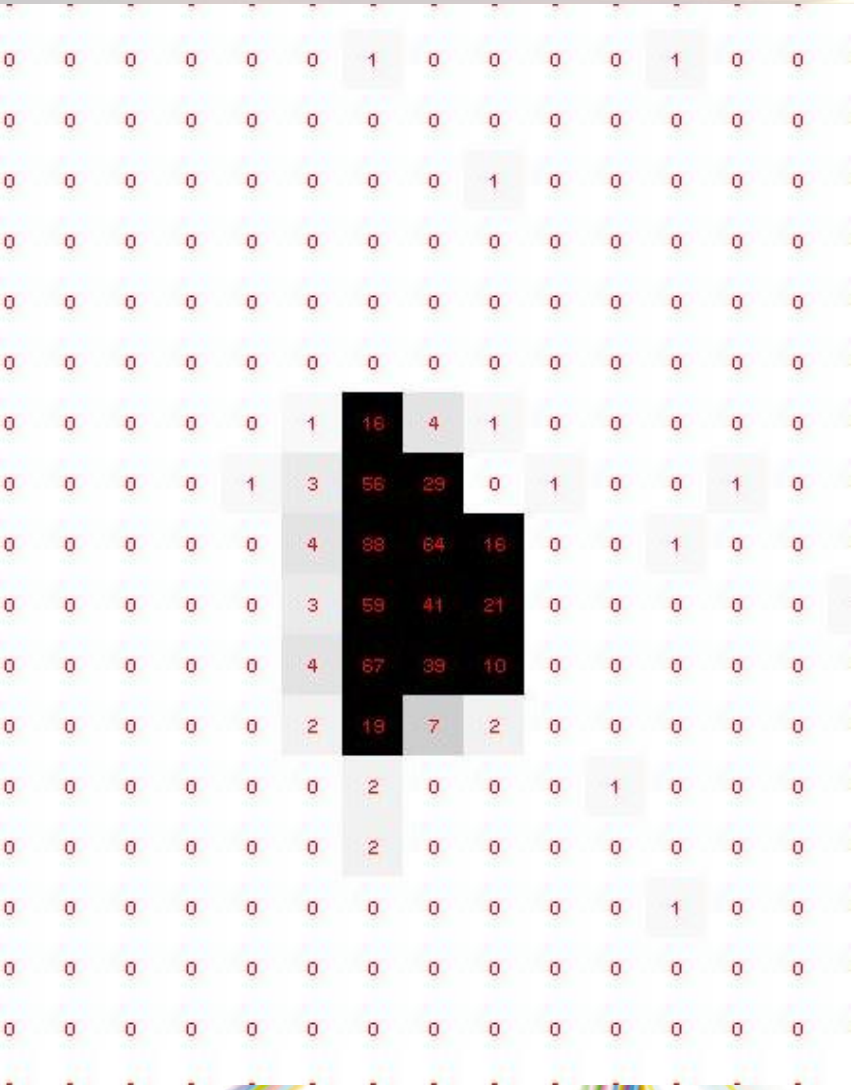
- FutA
  - Space group P21
  - Unit cell 39.2, 77.7, 47.7, 90.0, 97.9, 90.0
  - Approx. crystal size 5 - 10 µm
  - Measured ~5 – 10 degs per crystal

- The first grid measured at cryo temperatures, in vacuum, using a 0.8 x 2.8µm X-ray beam produced beautiful diffraction

Grid measured after data collection
~ 7µm crystals in largest dimension

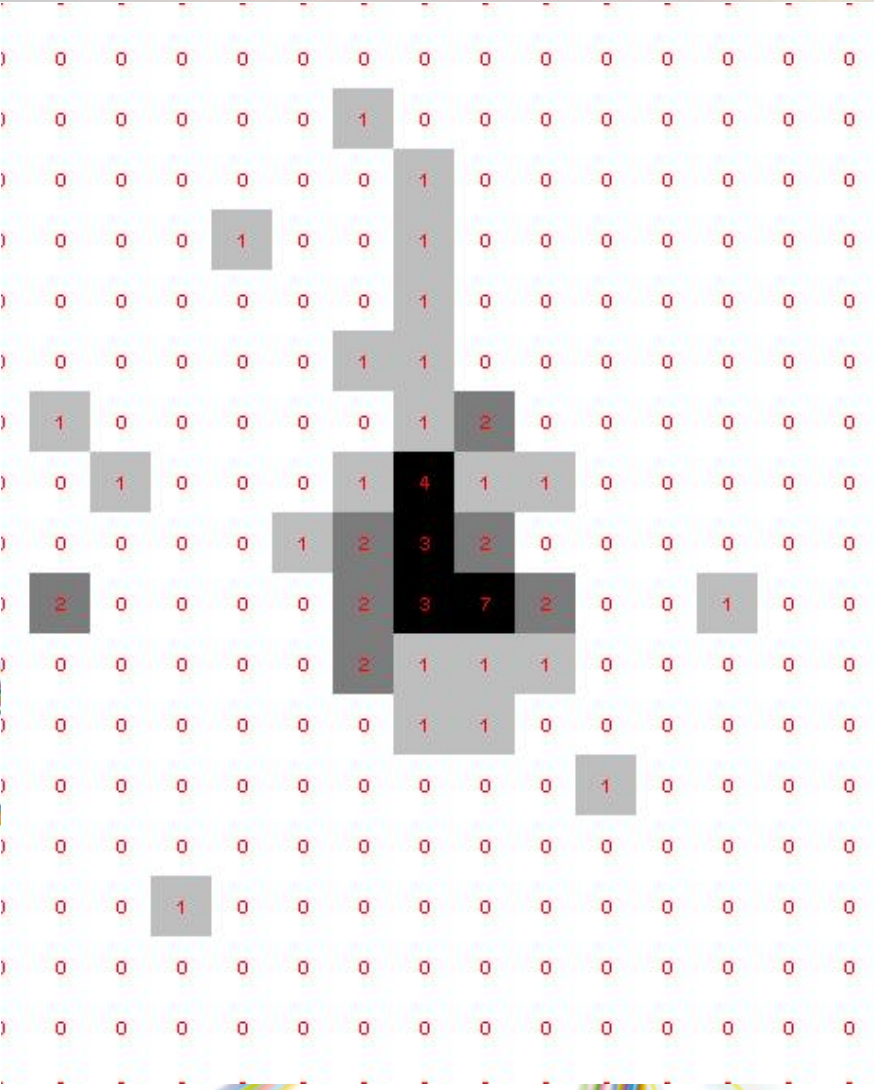


FutA: 12.423 keV; 0.1 s, 0.1 deg, 10 % transmission (~5x10^{10} ph/s)



11.45 Å

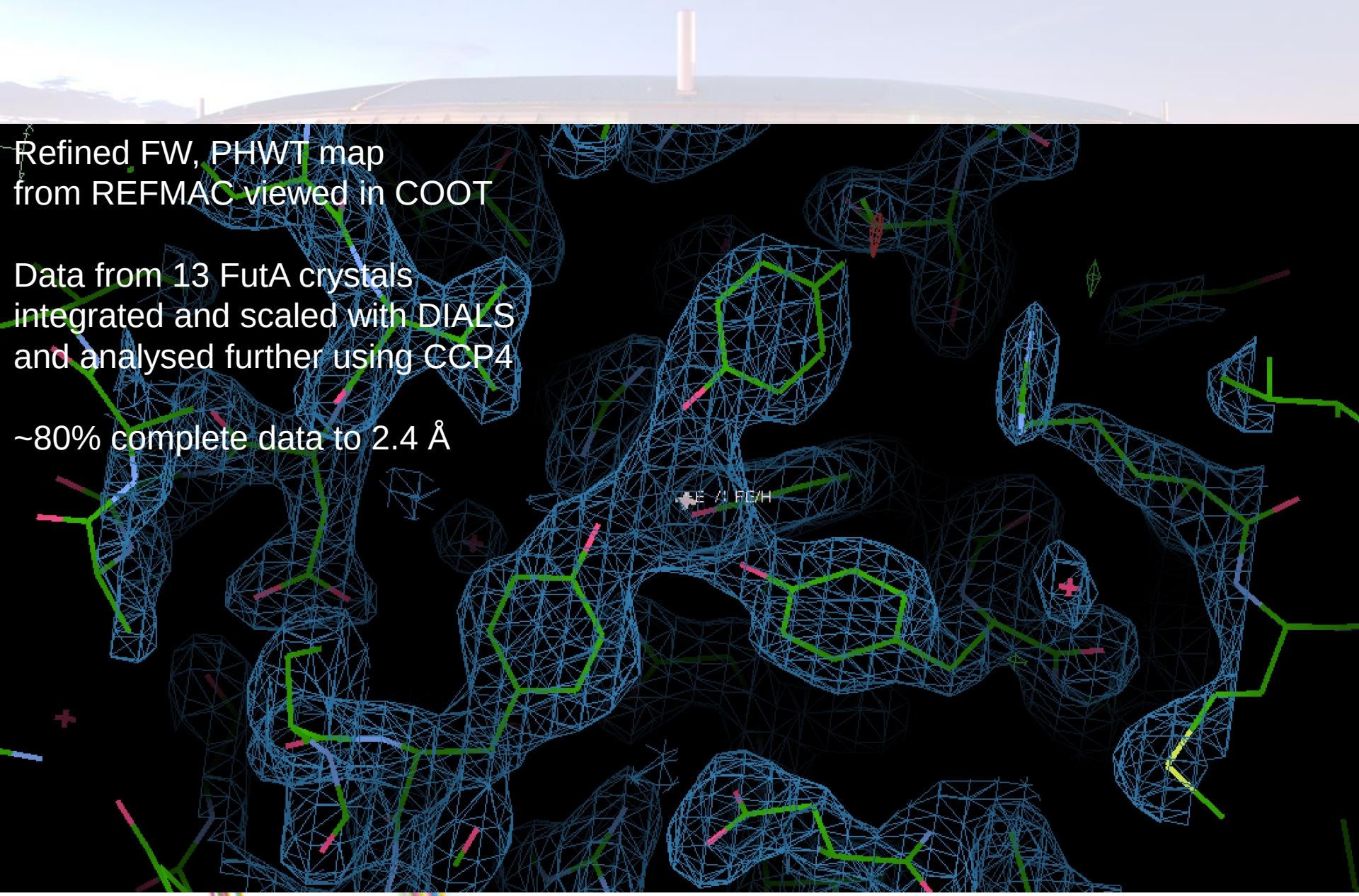
FutA: 12.423 keV; 0.1 s, 0.1 deg, 10 % transmission (~5x10^{10} ph/s)



2.05 Å

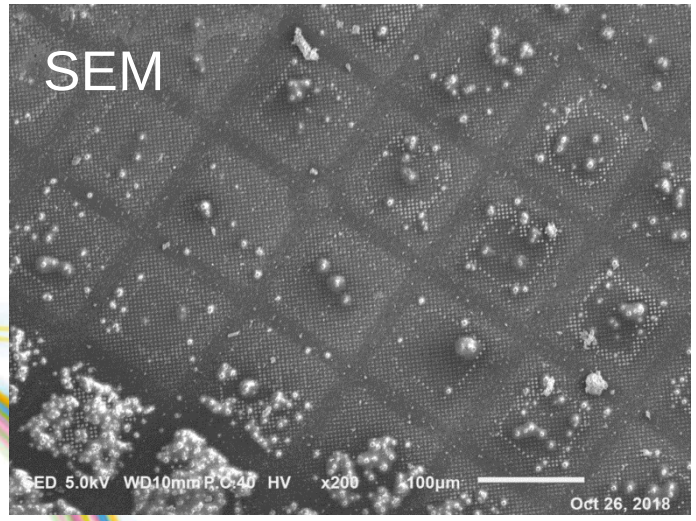
Merging statistics by resolution bin

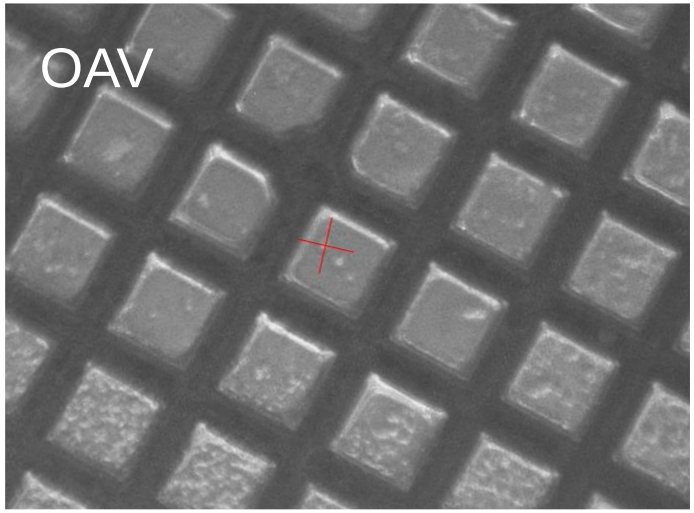
d_max	d_min	n_obs	n_uniq	mult	comp	<I>	<I/sI>	r_merge	r_meas	r_pim	cc1/2	cc_anom
29.97	5.13	2413	927	2.60	78.89	86.2	46.5	0.057	0.069	0.038	0.991	0.155
5.13	4.07	2463	946	2.60	81.13	99.9	39.8	0.075	0.091	0.050	0.975	0.269
4.07	3.56	2454	937	2.62	80.92	77.7	32.0	0.077	0.094	0.051	0.980	0.112
3.56	3.23	2461	920	2.67	80.28	49.7	24.2	0.100	0.122	0.066	0.967	0.196
3.23	3.00	2521	938	2.69	82.64	32.0	18.2	0.113	0.136	0.073	0.966	-0.049
3.00	2.83	2511	914	2.75	80.04	21.3	14.7	0.125	0.150	0.080	0.955	-0.008
2.83	2.68	2663	950	2.80	81.97	16.6	12.5	0.153	0.184	0.098	0.913	0.019
2.68	2.57	2243	874	2.57	75.93	12.4	10.5	0.168	0.201	0.107	0.923	-0.604
2.57	2.47	2041	814	2.51	72.23	11.2	9.3	0.173	0.208	0.111	0.935	0.433
2.47	2.38	1699	769	2.21	66.24	9.6	8.3	0.181	0.221	0.123	0.916	0.556
2.38	2.31	1333	686	1.94	60.76	8.7	7.2	0.191	0.238	0.138	0.917	0.618
2.31	2.24	1114	628	1.77	54.47	7.4	6.2	0.224	0.289	0.180	0.866	-0.923
2.24	2.18	856	530	1.62	47.36	6.3	5.3	0.201	0.261	0.163	0.906	0.000
2.18	2.13	729	509	1.43	44.34	5.1	4.0	0.249	0.327	0.208	0.776	0.615
2.13	2.08	561	410	1.37	36.51	5.9	3.4	0.296	0.405	0.274	0.549	0.000
2.08	2.04	445	352	1.26	29.93	4.0	3.0	0.198	0.272	0.186	0.874	0.000
2.04	2.00	345	289	1.19	25.92	2.9	2.4	0.245	0.344	0.241	0.732	0.000
2.00	1.96	246	210	1.17	18.21	2.4	2.2	0.113	0.159	0.113	0.957	0.000
1.96	1.92	156	134	1.16	11.80	1.9	2.0	0.108	0.153	0.108	0.951	0.000
1.92	1.89	76	66	1.15	5.86	1.6	1.5	0.439	0.620	0.439	0.994	0.000


Refined FW, PHWT map
from REFMAC viewed in COOT
Data from 13 FutA crystals
integrated and scaled with DIALS
and analysed further using CCP4
~80% complete data to 2.4 Å

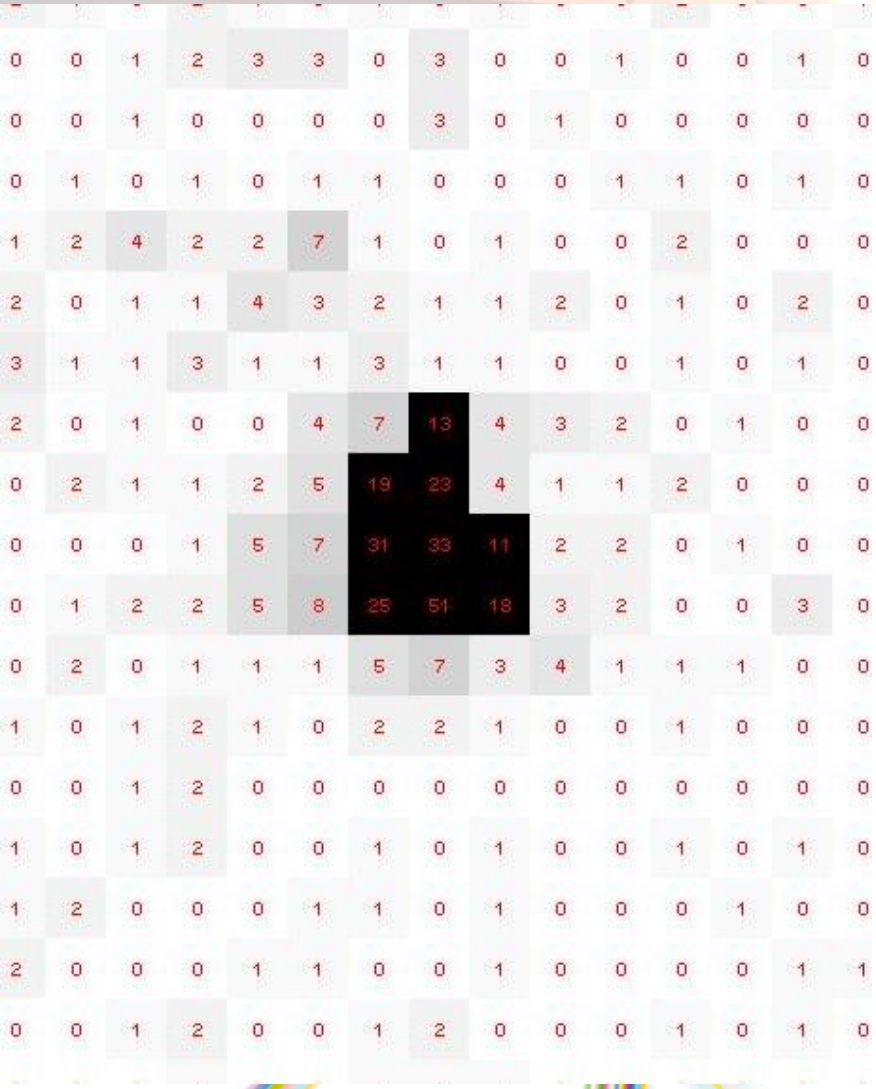
CPV Ld14

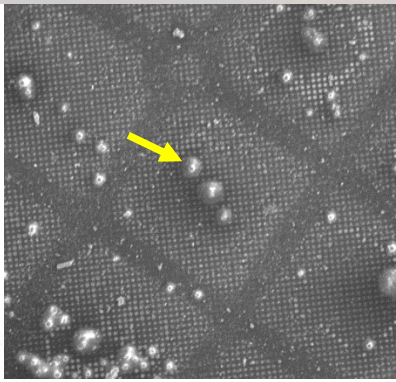
- Spacegroup I23
- Unit cell a=103 Å
- Approx crystal size 3 – 4 µm





CPV Ld14: 12.423 keV; 0.1 s, 0.1 deg, 100 % transmission (~5x10^{11} ph/s)





- 1.74 Å

Acknowledgements

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Richard Parr
Tony Gardner

Leo Cahill
Kevin Wilkinson
Alan Nash
Steve Buxcey
Paul Benson's IFM Team

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Brian Nutter
Ben Kemp

Victoria Lawson
Sonia Moon

Mark Lunnon
Dave Butler
Adam Taylor
Adam Prescott
Leon Adams

Simon Lay
Hugo Shiers
Guenther Rehm
Chris Bloomer

David Laundy
Lucia Alianelli
John Sutter
Simon Alcock
Kawal Sahwney

Gwyndaf Evans

Jose Trincao

Emma Beale

Adam Crawshaw