VMXm: Micro/nanofocus protein crystallography beamline at Diamond

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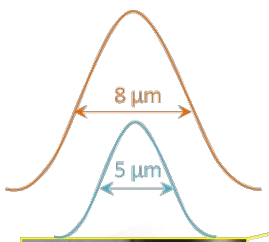
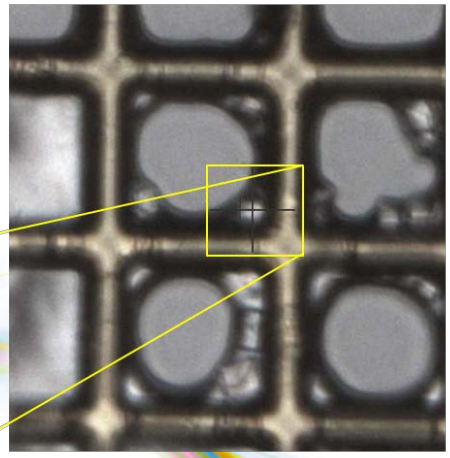
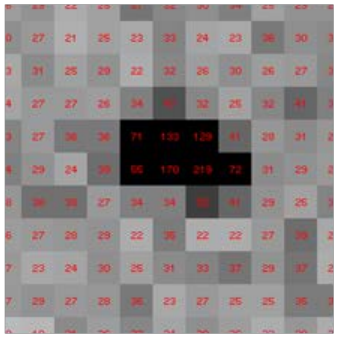
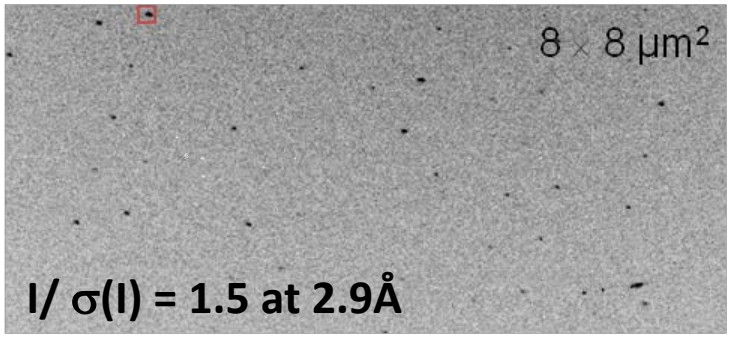
Introduction

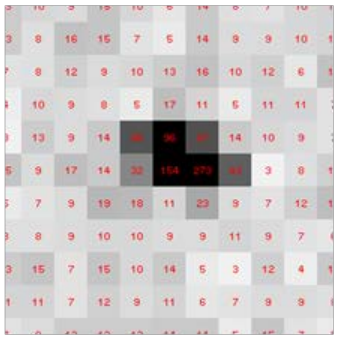
- Target proteins are getting more complex, leading to smaller and more disordered crystals
- Can design ideal experiment to give optimal data quality

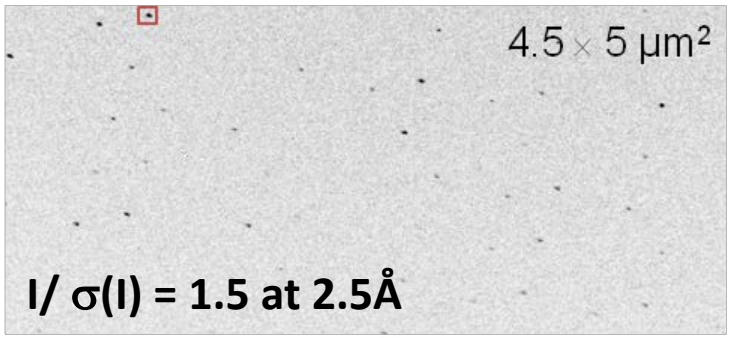












G. Evans, D. Axford and R. L. Owen (2011) *Acta Cryst.* **D67**, 261-270.

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
- Take advantage of photoelectron escape

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 aims to record high quality rotation diffraction data where both **size** and **number of crystals** are limited

VMXm Specifications

- 6 – 28 keV energy range
- 0.3 – 10 µm (v) & 0.5 – 5 µm (h)
- Flux
  - ~10^{12} ph/s in 0.3 × 5 µm (v x h)
  - >10^{11} ph/s 0.3 × 0.5 µm (v x h)
- Initially Pilatus 6M
- Upgrade to Eiger 9M CdTe imminent

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

Sample Preparation

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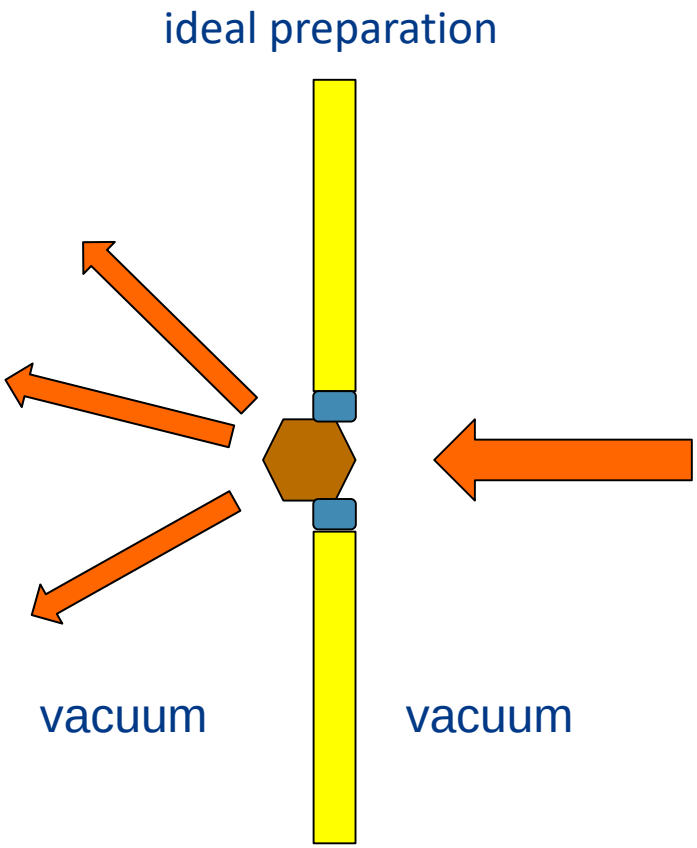
Sample Preparation

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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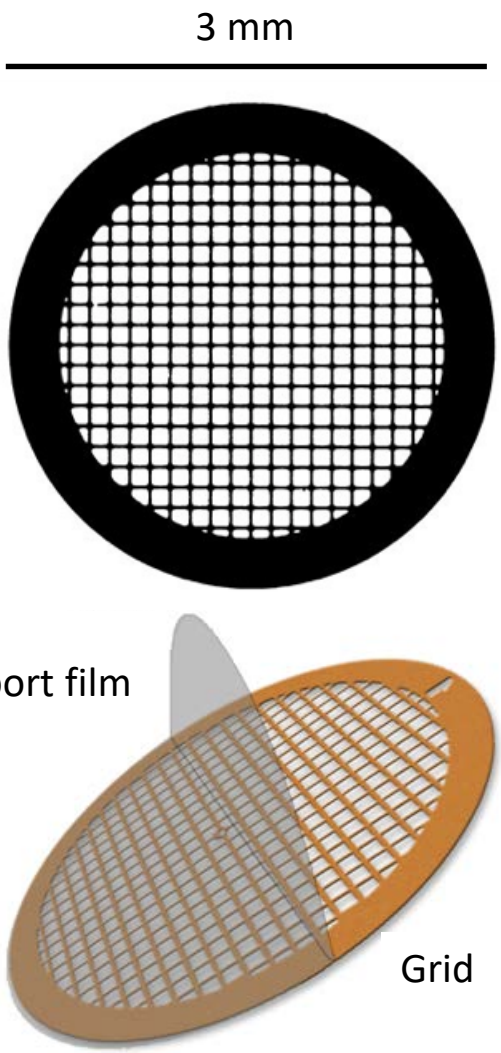
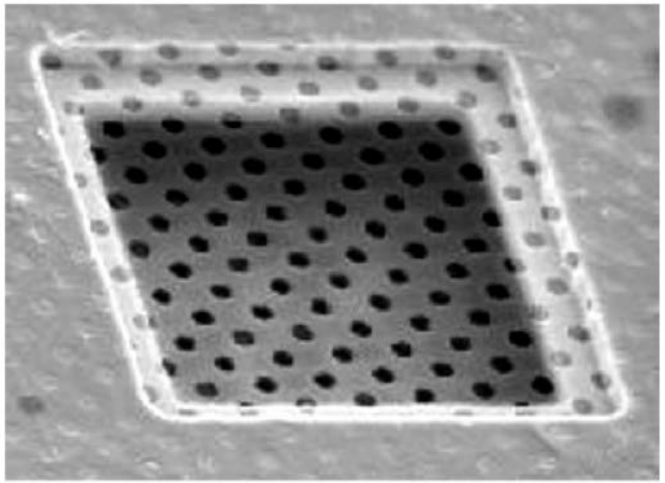
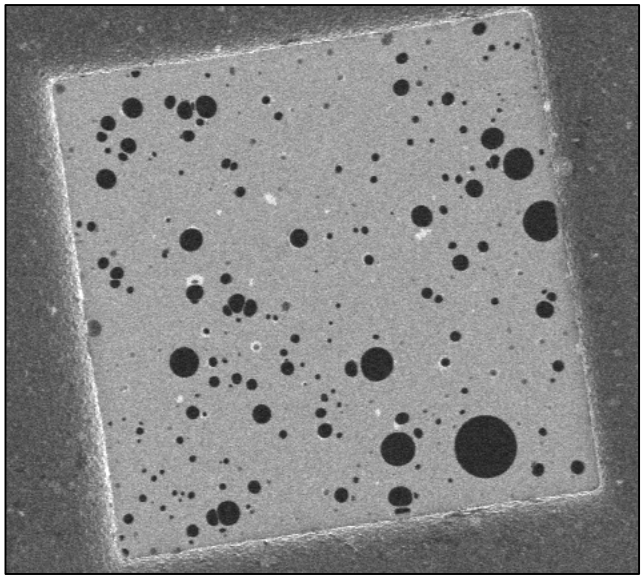
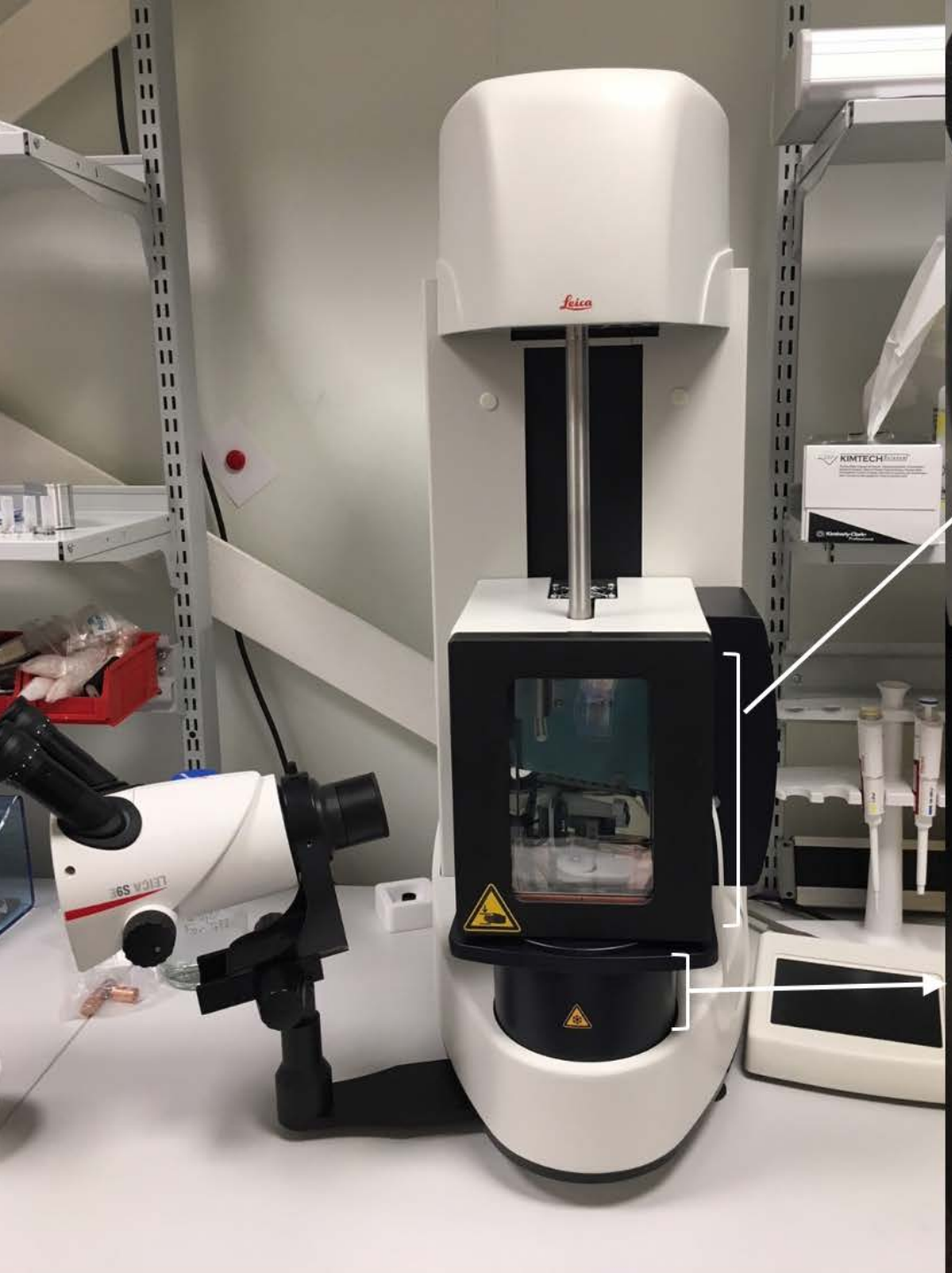
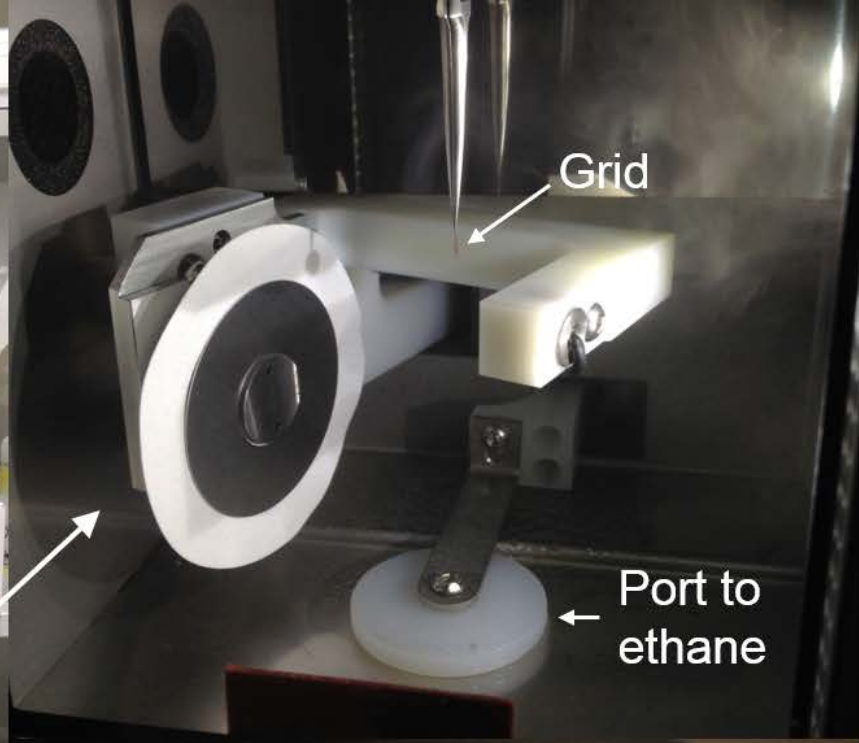
Cryo-EM Grids

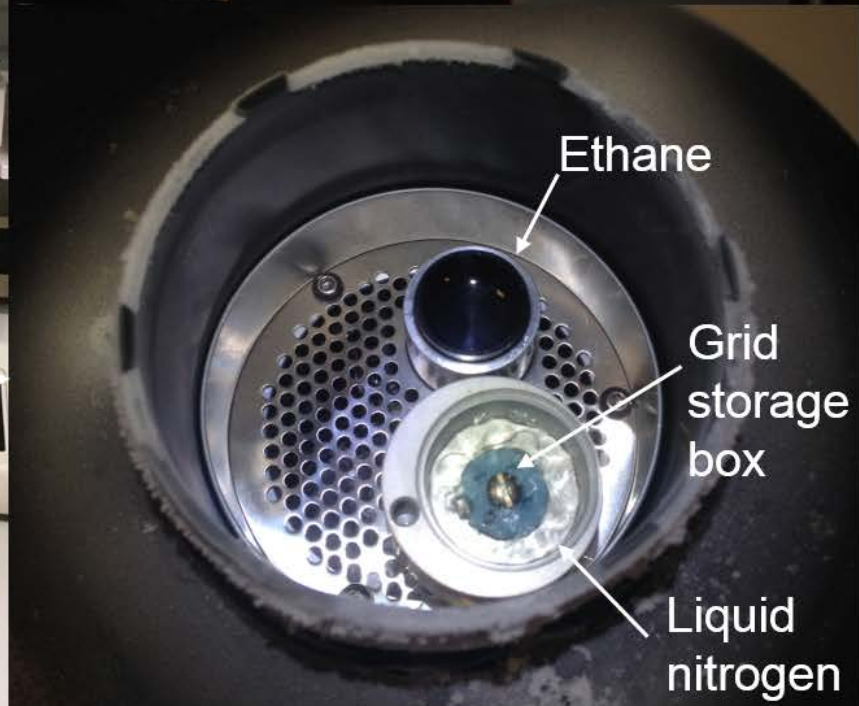




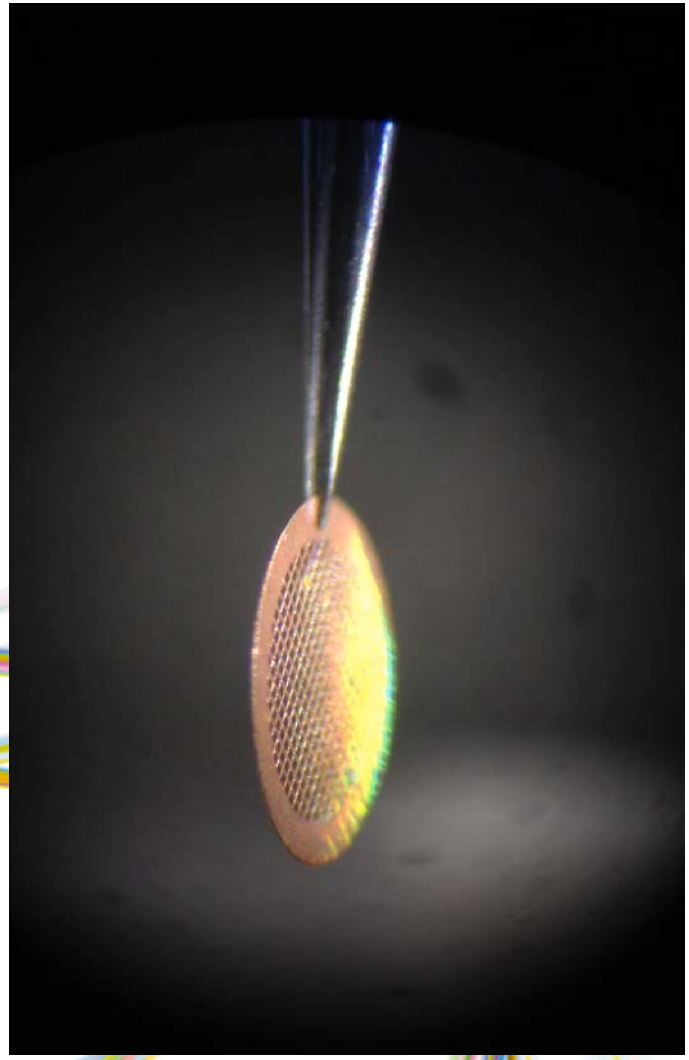


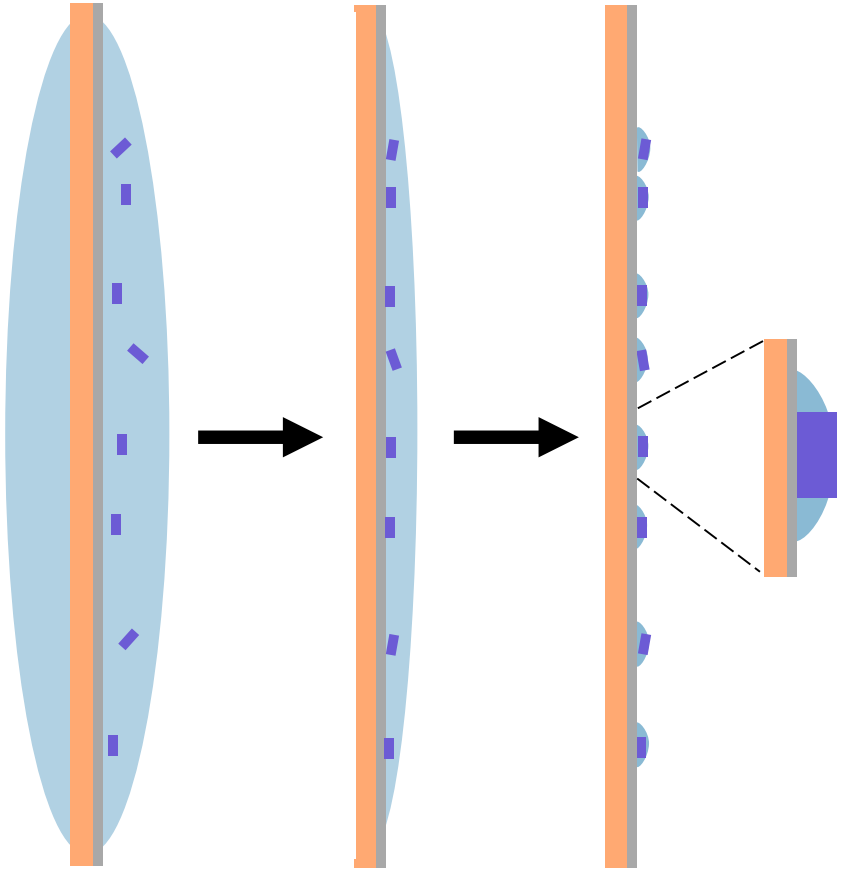

Leica


Grid
Port to ethane

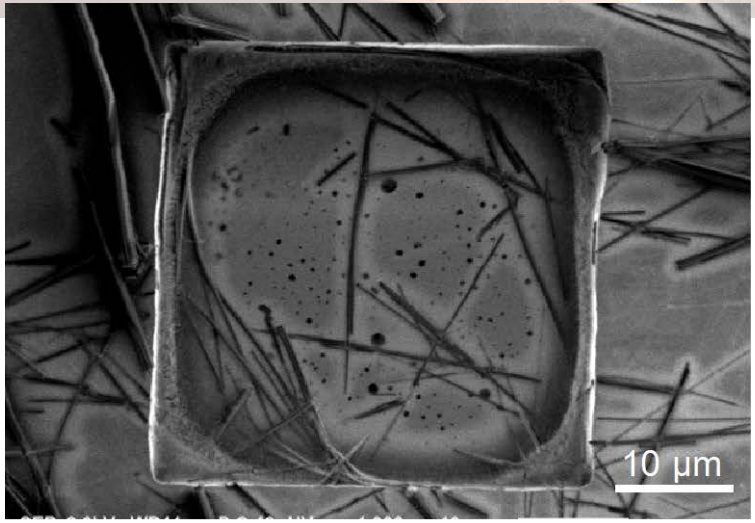

Ethane
Grid storage box
Liquid nitrogen

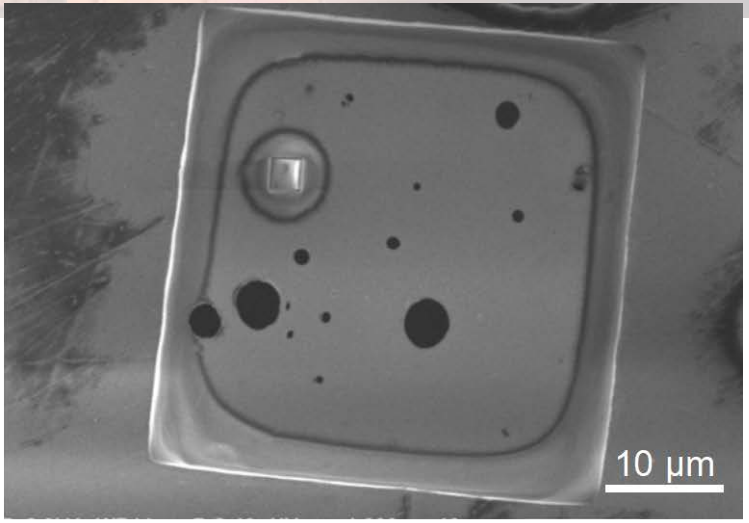
Sample Blotting

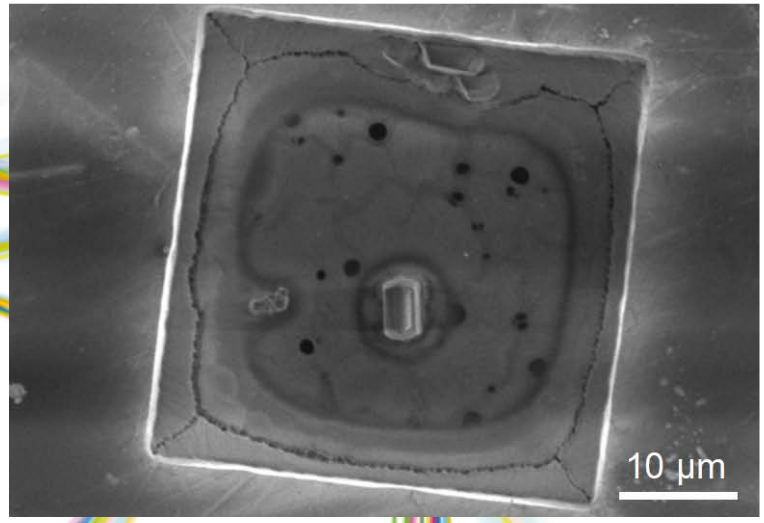


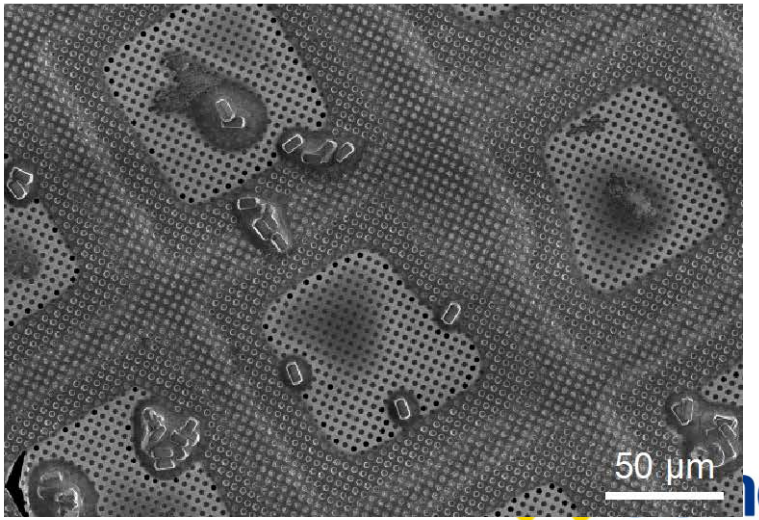


Sample Characterisation

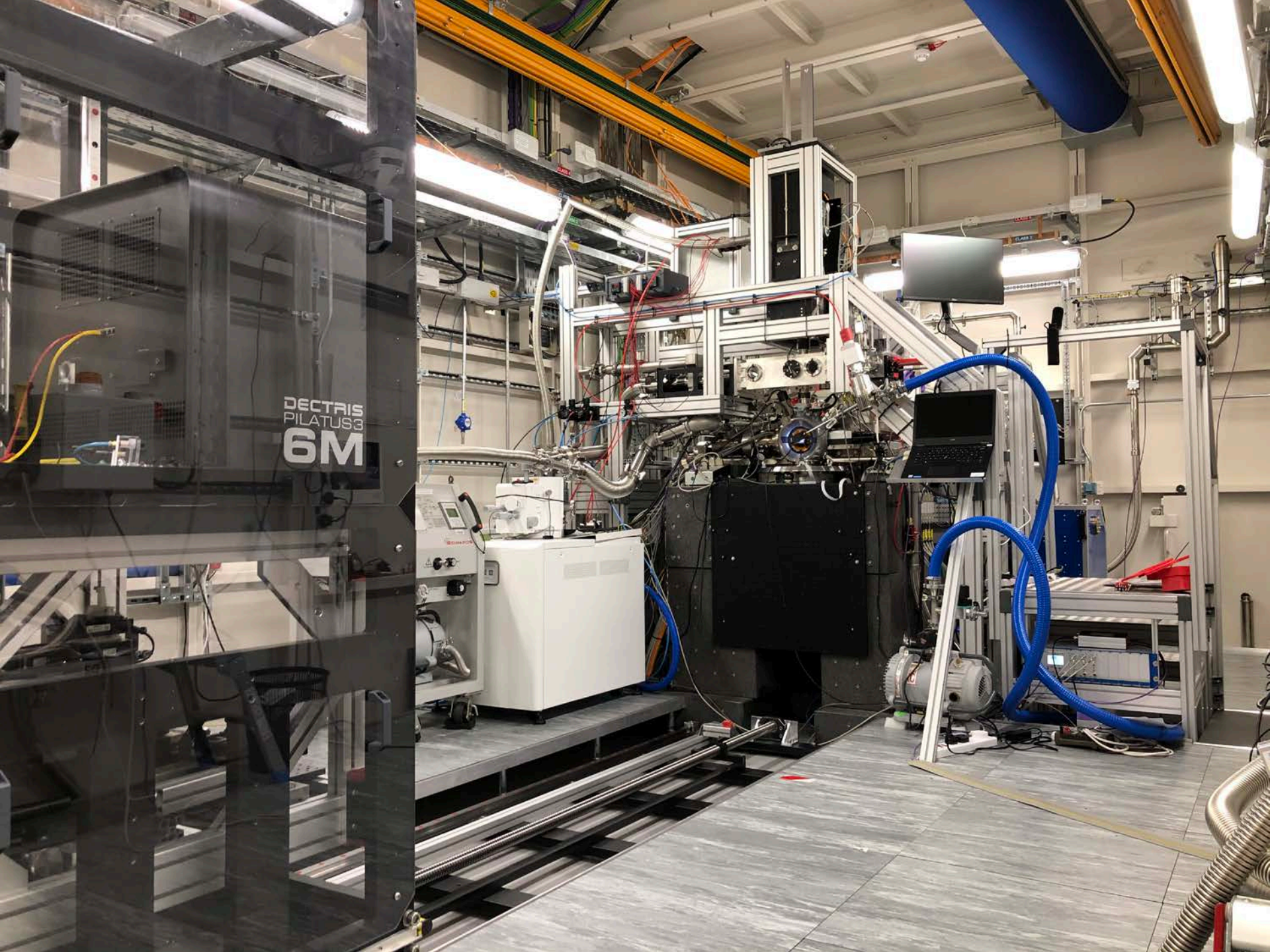


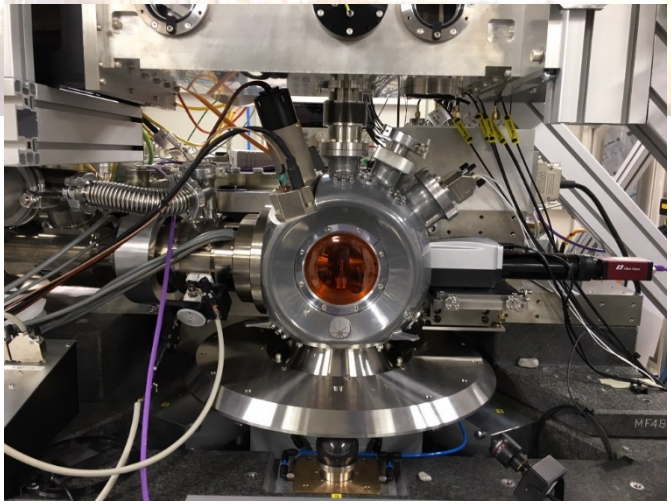


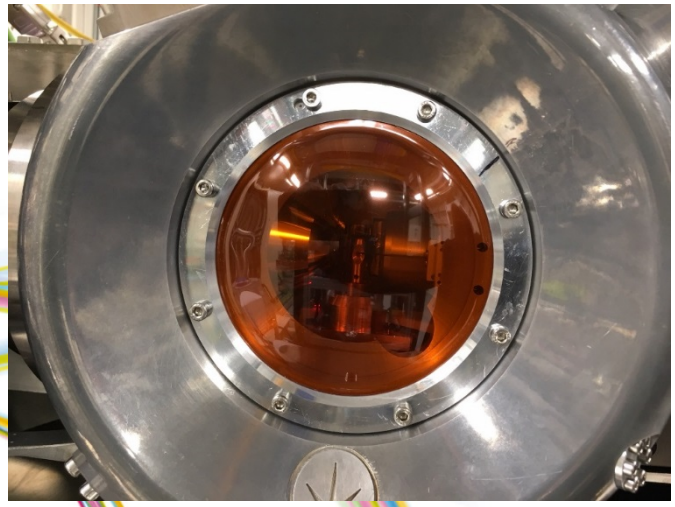


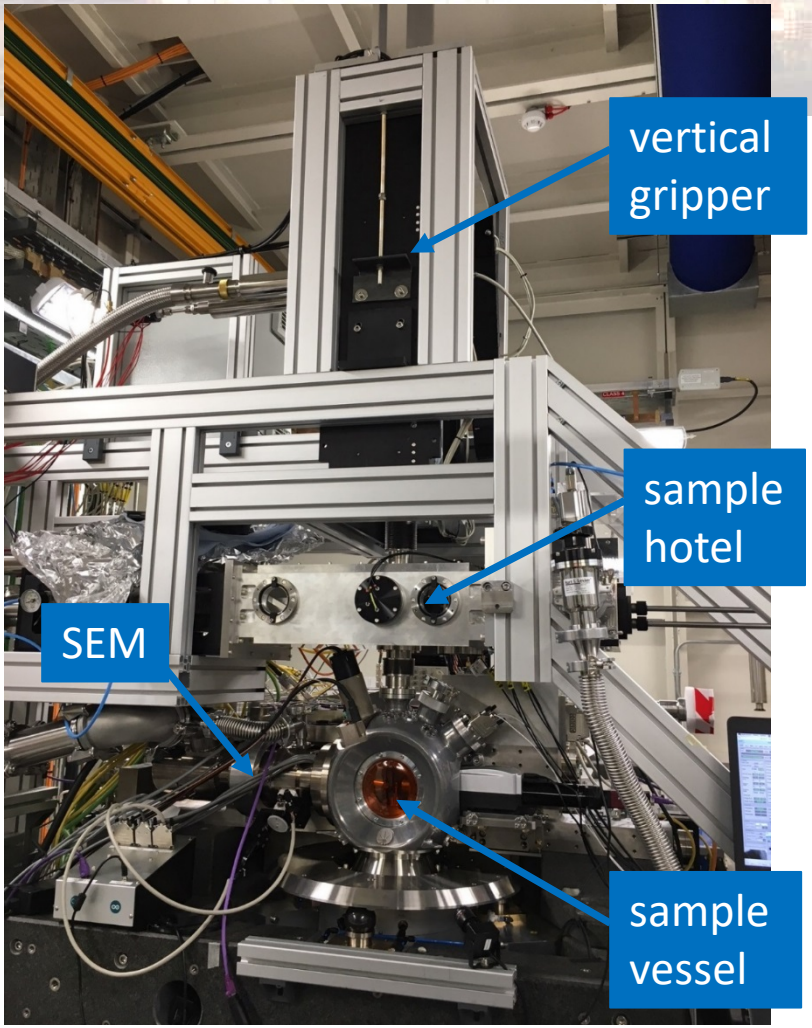


Sample Mounting


DECTRIS
PILATUS3
6M







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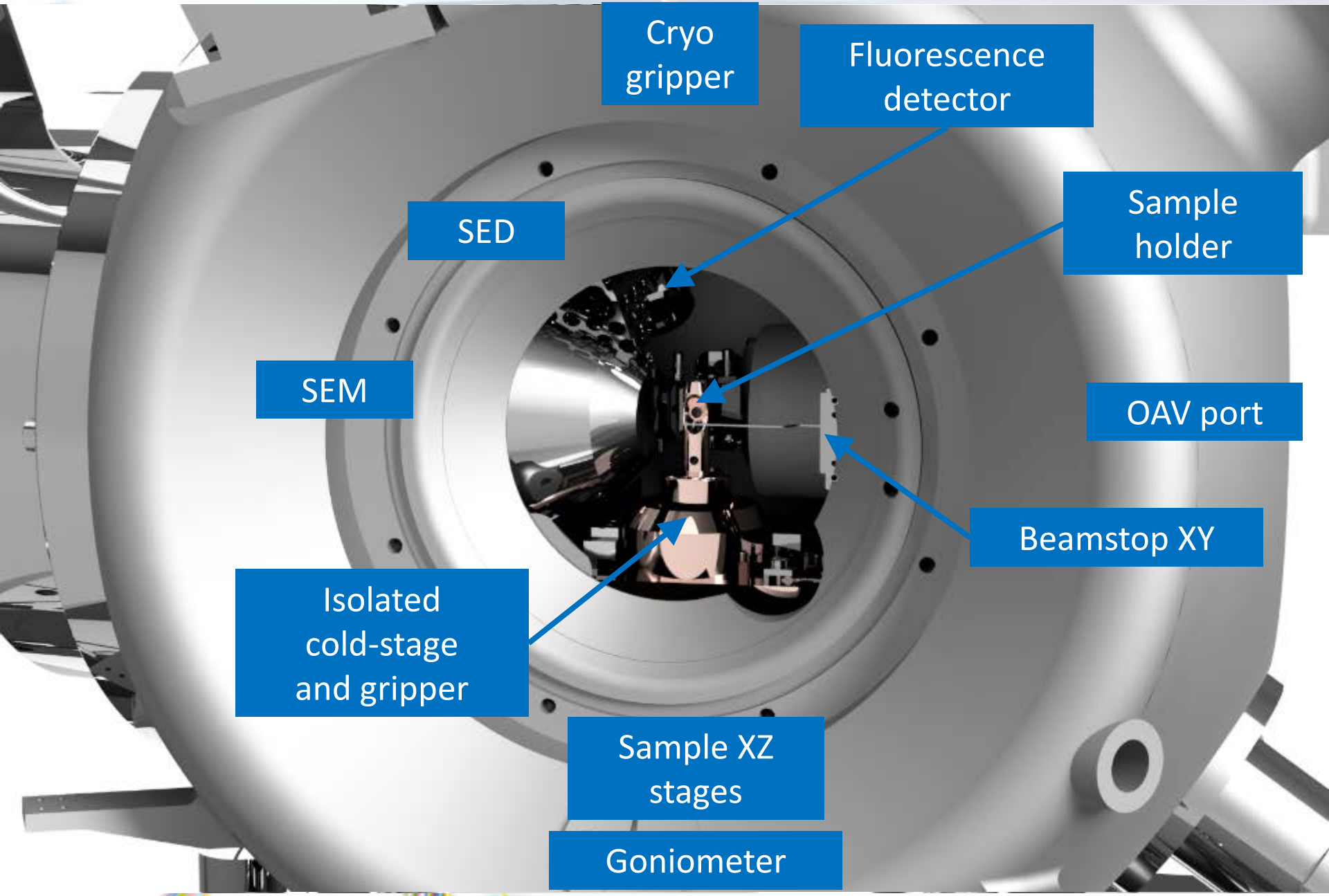
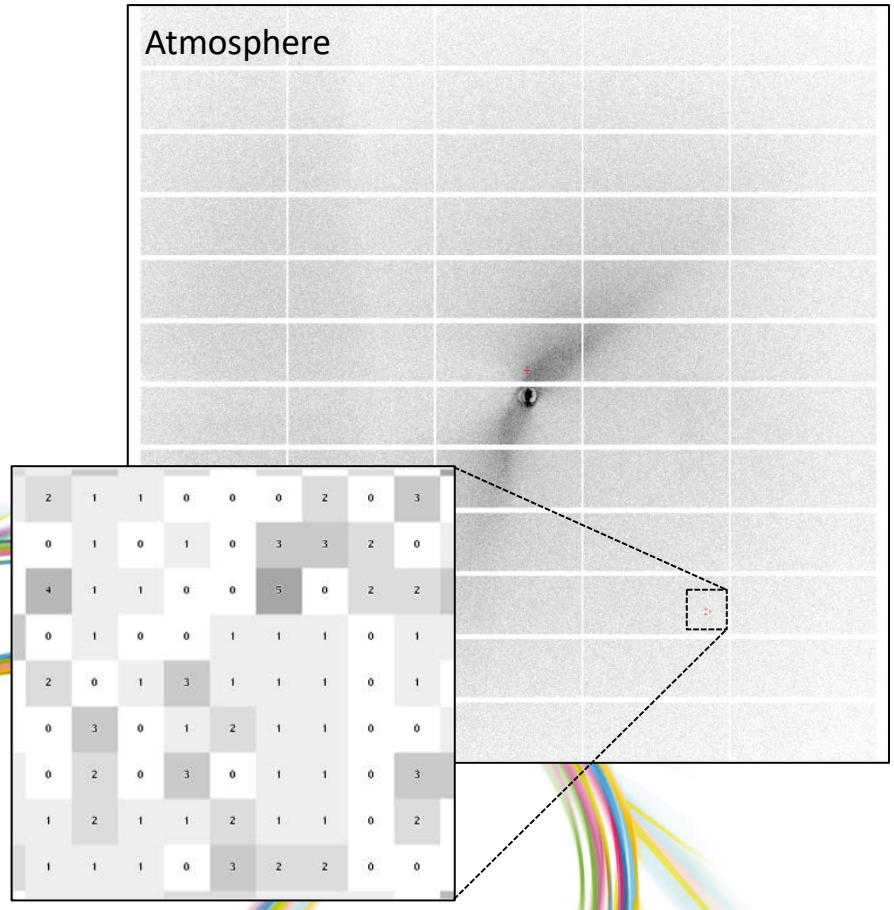
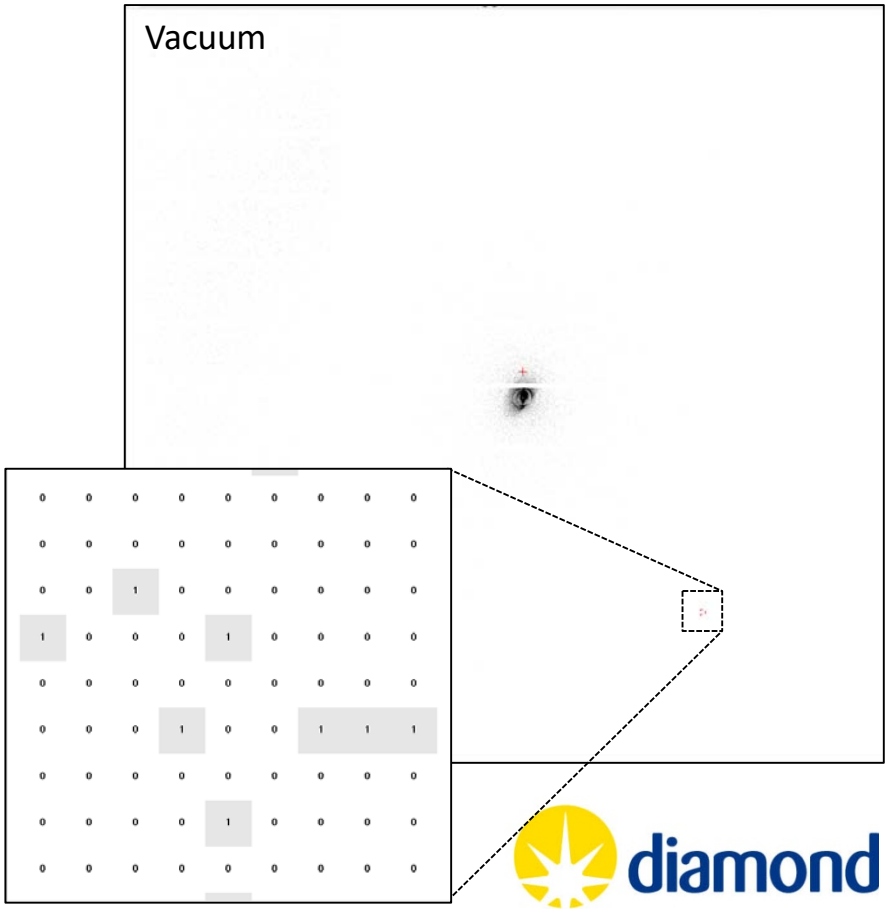

Cryo gripper
Fluorescence detector
SED
Sample holder
SEM
OAV port
Beamstop XY
Isolated cold-stage and gripper
Sample XZ stages
Goniometer

Beamline Results

Background Measurements

< 0.1 counts per pixel per second





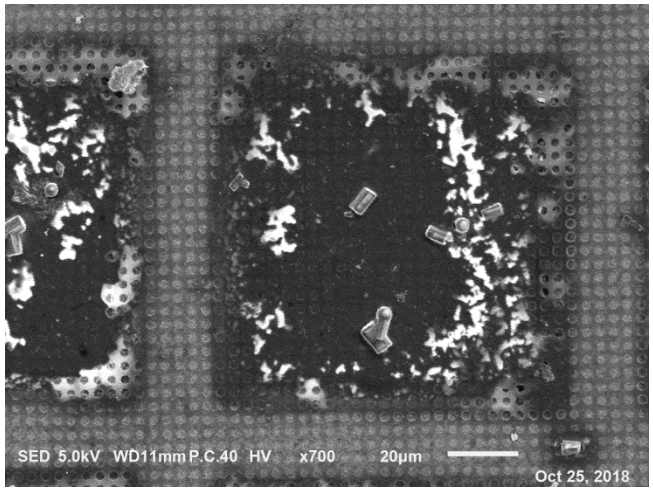
First User Experiments

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

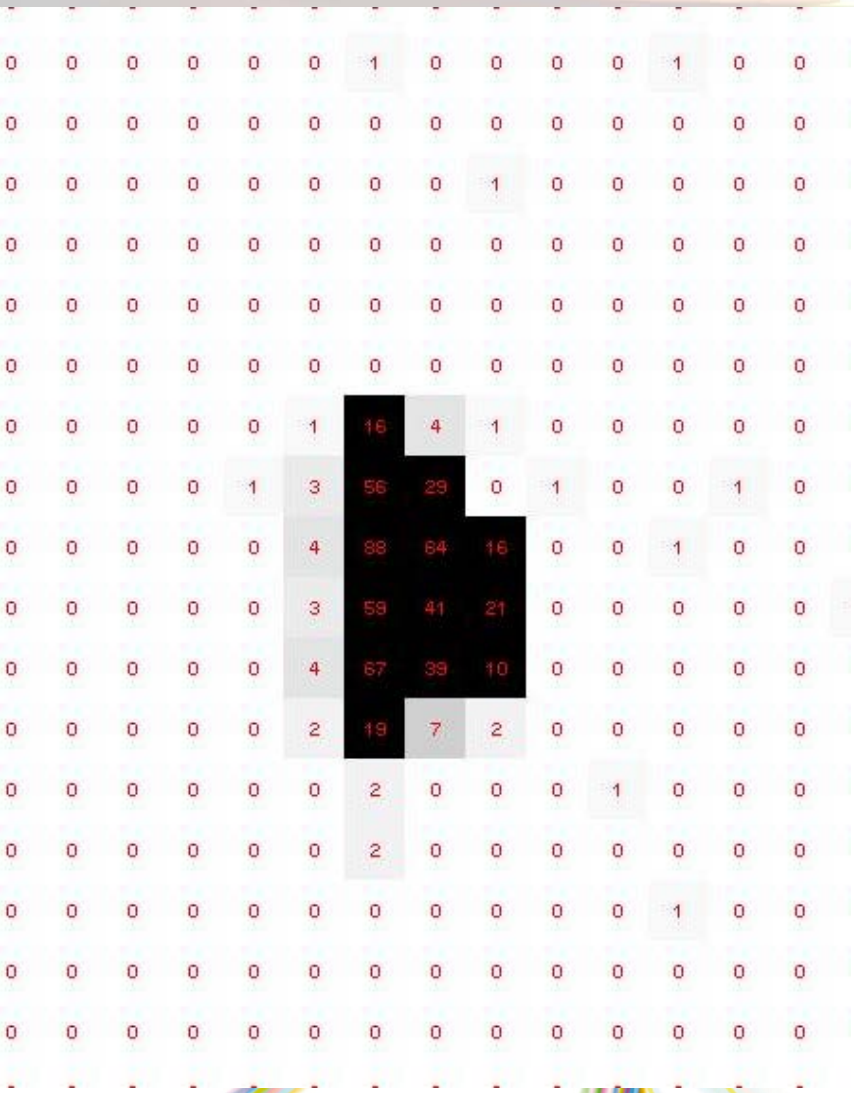
- FutA
  - ABC transporter subunit responsible for Fe uptake in cyanobacteria from oceanic biomass
  - Space group P21
  - Unit cell 39.2, 77.7, 47.7, 90.0, 97.9, 90.0
  - Approx. crystal size 5 - 10 µm
  - Measured ~5 – 10 degs per crystal

- 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

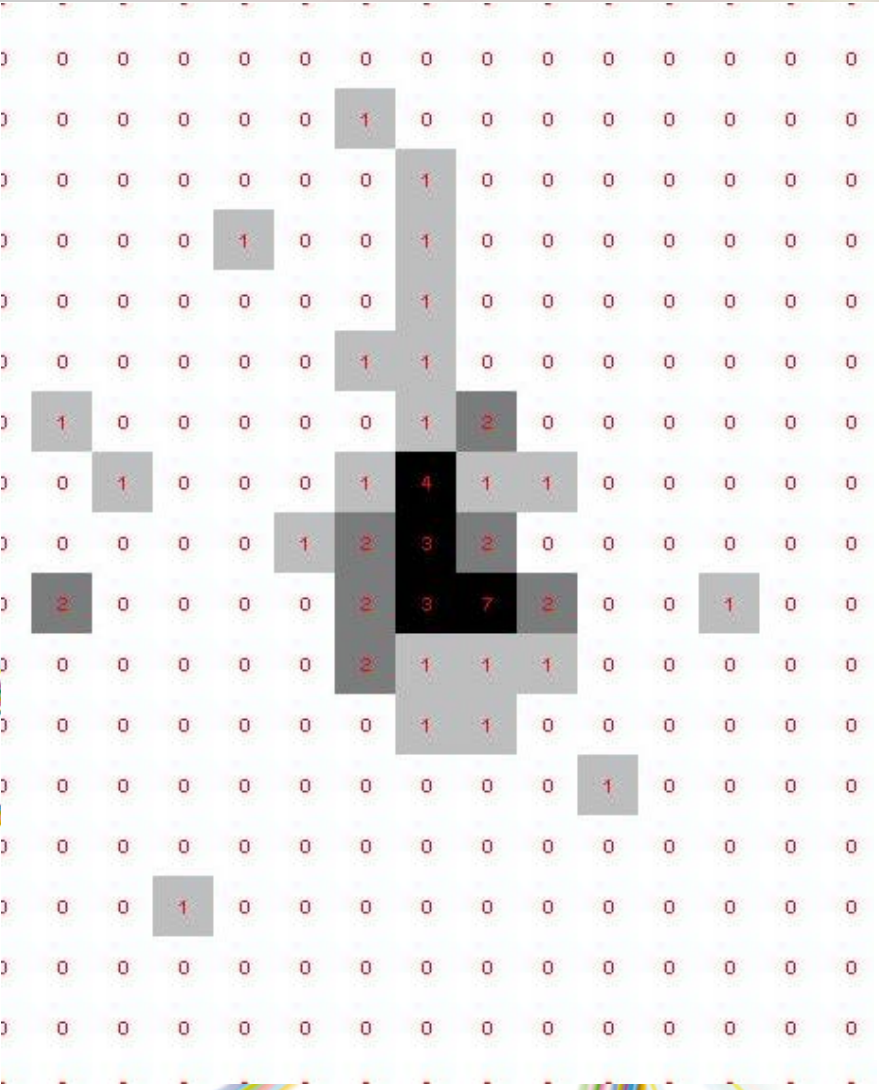


FutA: 12.423 keV; 0.1 s, 0.1 deg, 10 % transmission (~5x10^{10} ph/s)



11.45 Å

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



2.05 Å

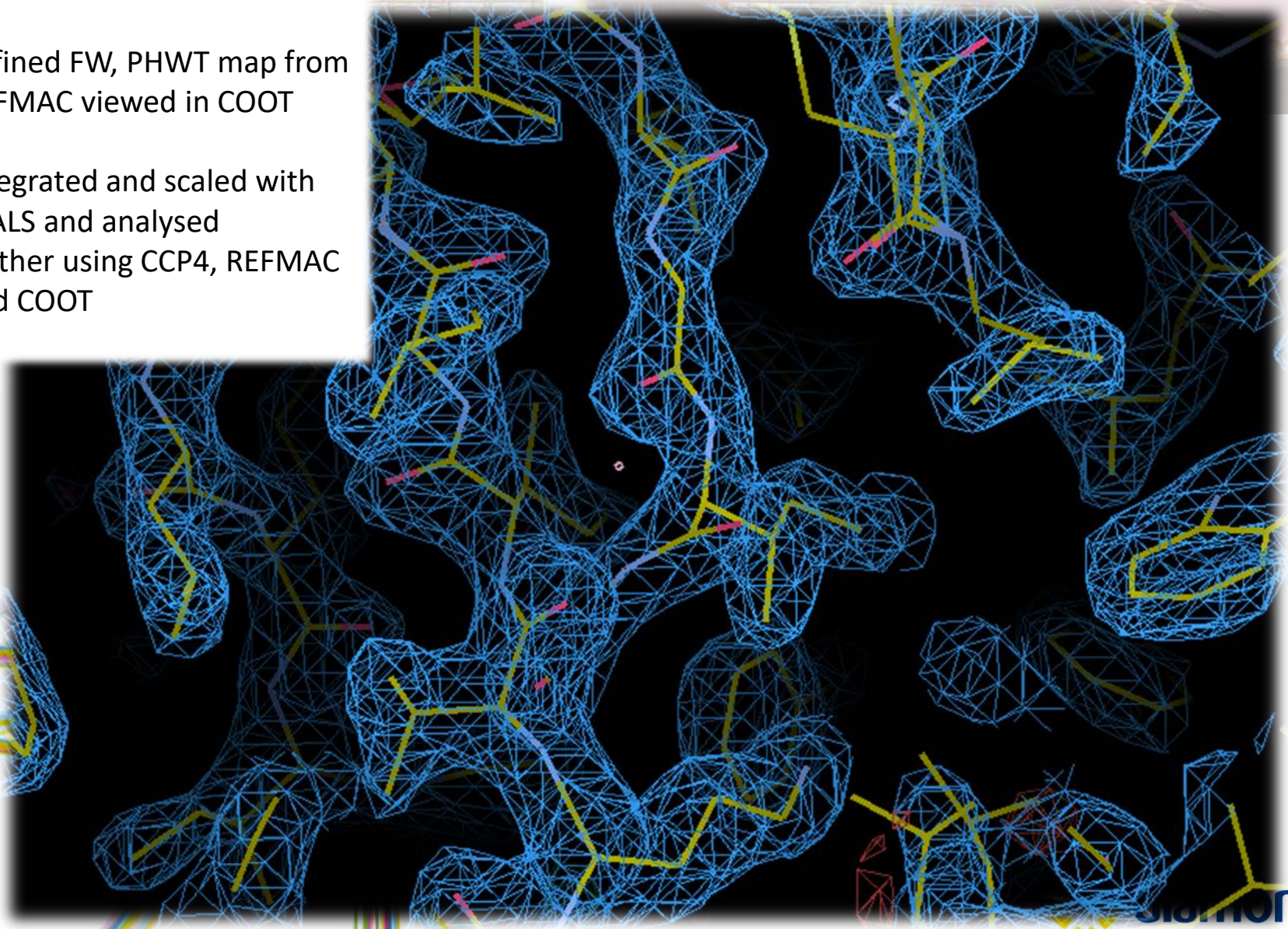
12 data sets

21 crystal lattices

90% complete data to 2.3 Å

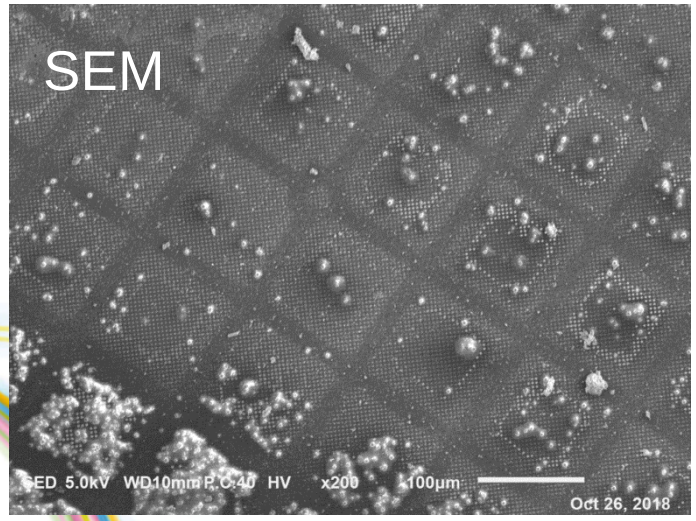
Refined FW, PHWT map from REFMAC viewed in COOT

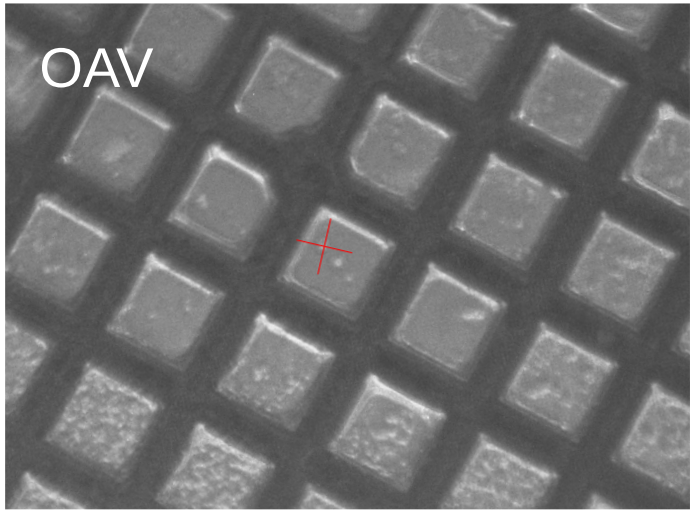
Integrated and scaled with DIALS and analysed further using CCP4, REFMAC and COOT



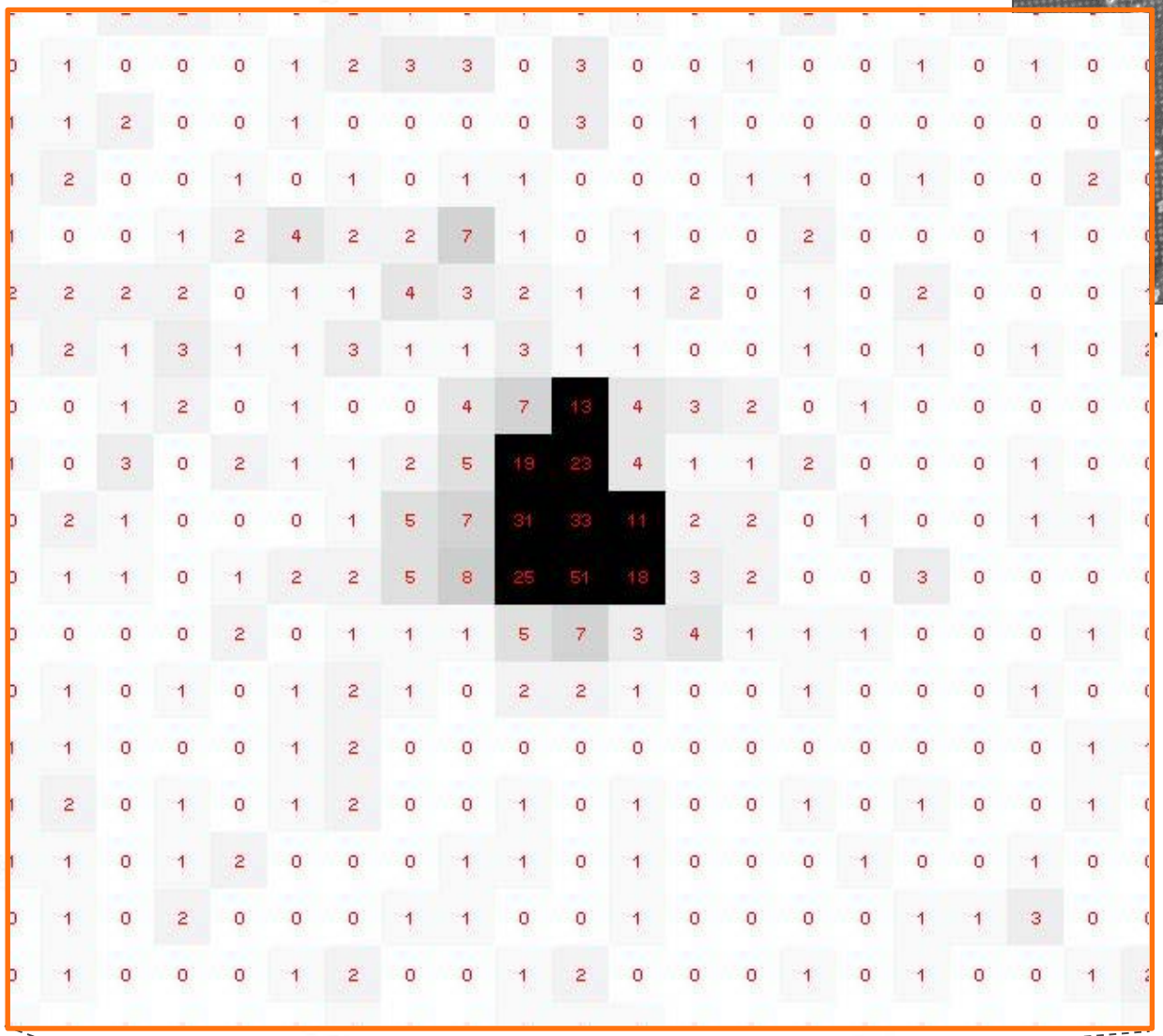
CPV Ld14 – Cytoplasmic polyhedrosis virus

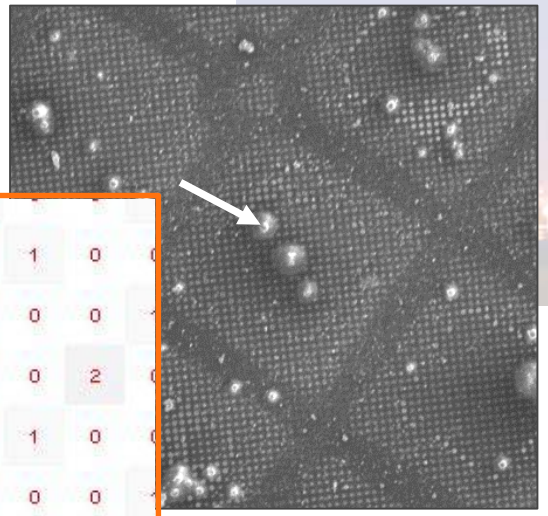
- Spacegroup I23
- Unit cell a=b=c=103 Å
- Approximate crystal size 2 – 4 µm





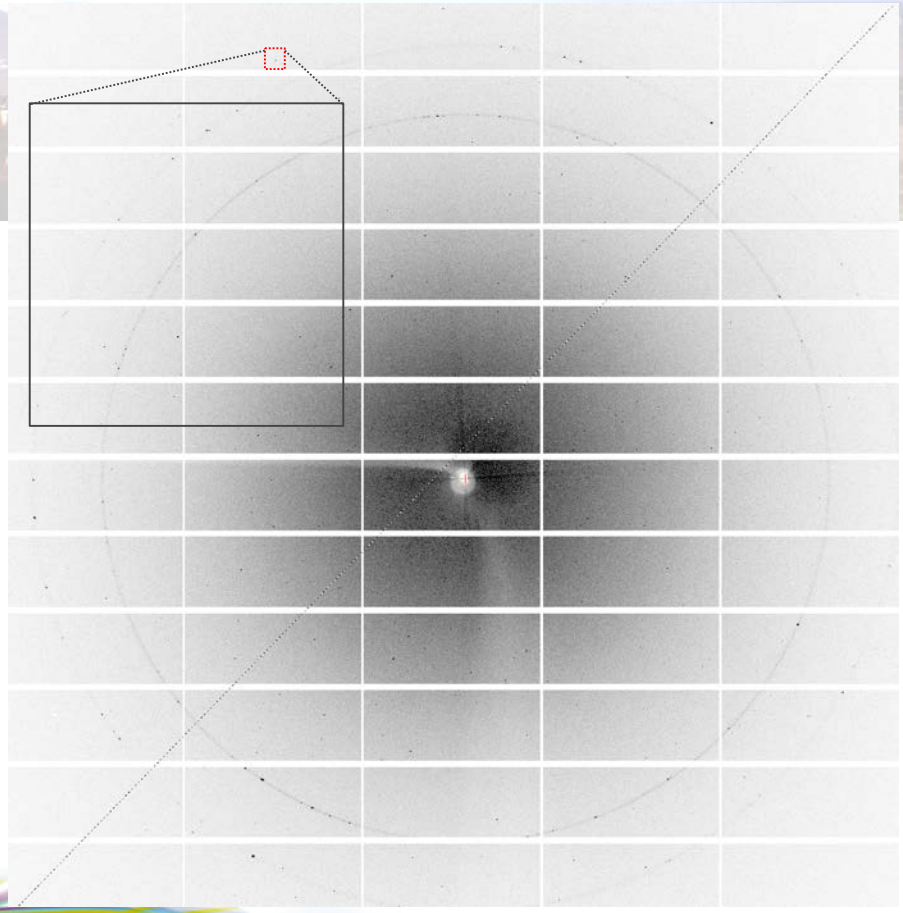
12.423 keV, 0.1 s, 0.1 deg, 100 % transmission (~5x10^{11} ph/s)

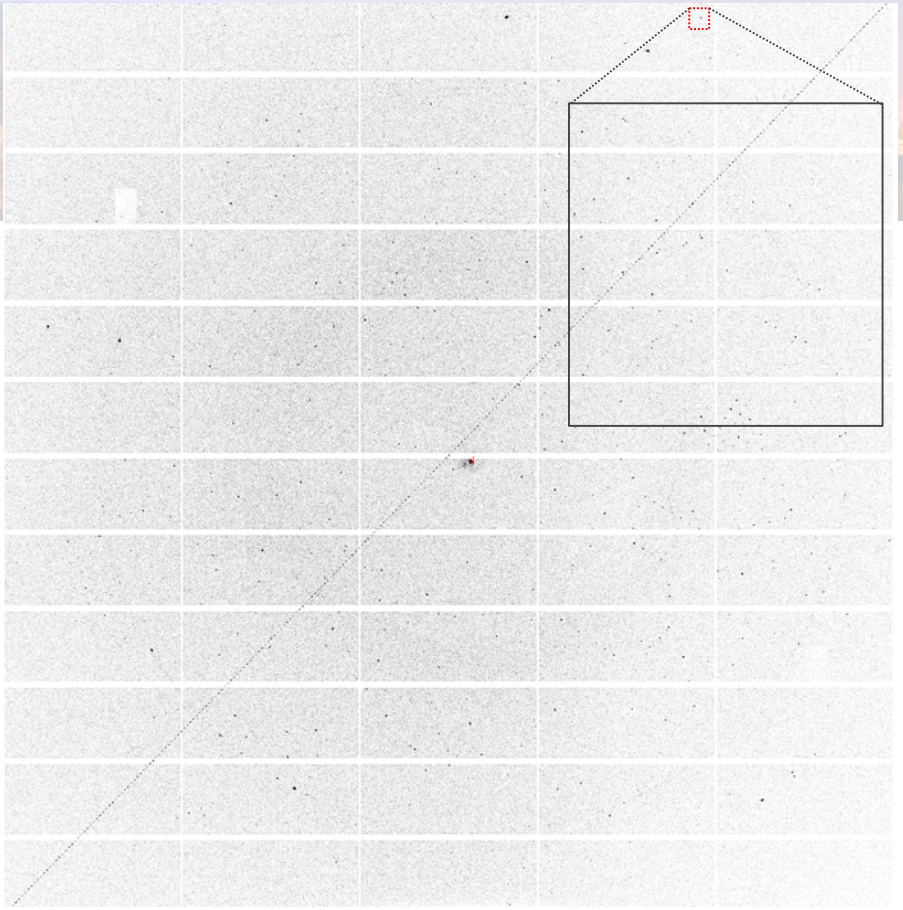


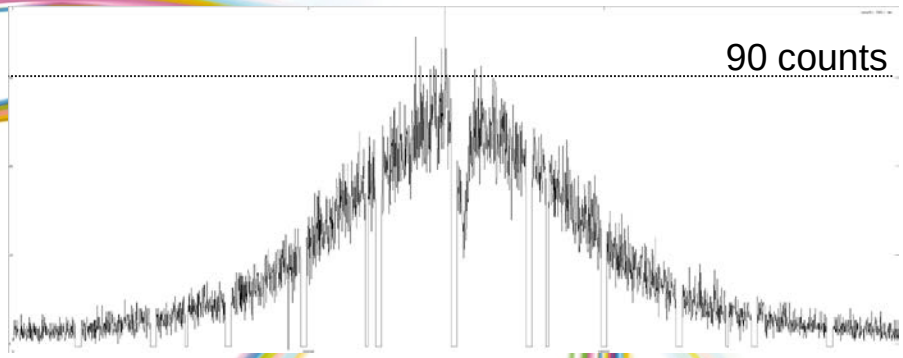


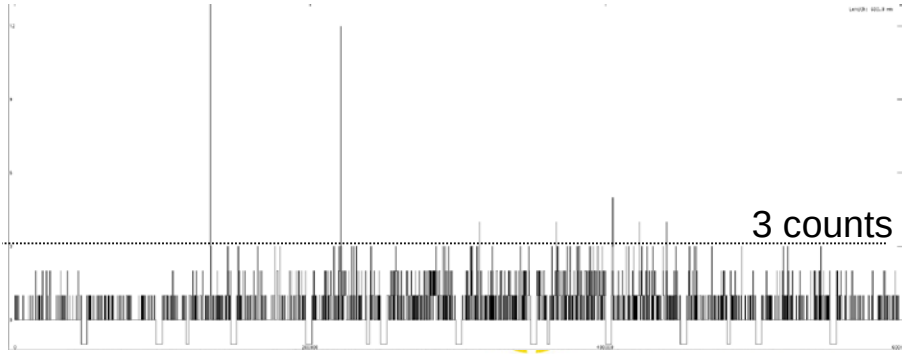
I24

VMXm









CPV Ld14 – Cytoplasmic polyhedrosis virus

27 Crystals

1.5 – 2.0° data per crystal

Summary of merging statistics

	Overall	Low resolution	High resolution
Resolution (Å)	36.57 - 1.50	36.58 - 4.07	1.53 - 1.50
Observations	116543	7779	1406
Unique reflections	25790	1405	833
Multiplicity	4.5	5.5	1.7
Completeness	87.23%	90.30%	57.21%
Mean I/sigma(I)	5.1	13.4	0.7
Rmerge	0.195	0.128	0.775
Rmeas	0.217	0.140	1.017
Rpim	0.088	0.053	0.646
CC1/2	0.969	0.970	0.319

27 Crystals

1.5 – 2.0° data per crystal

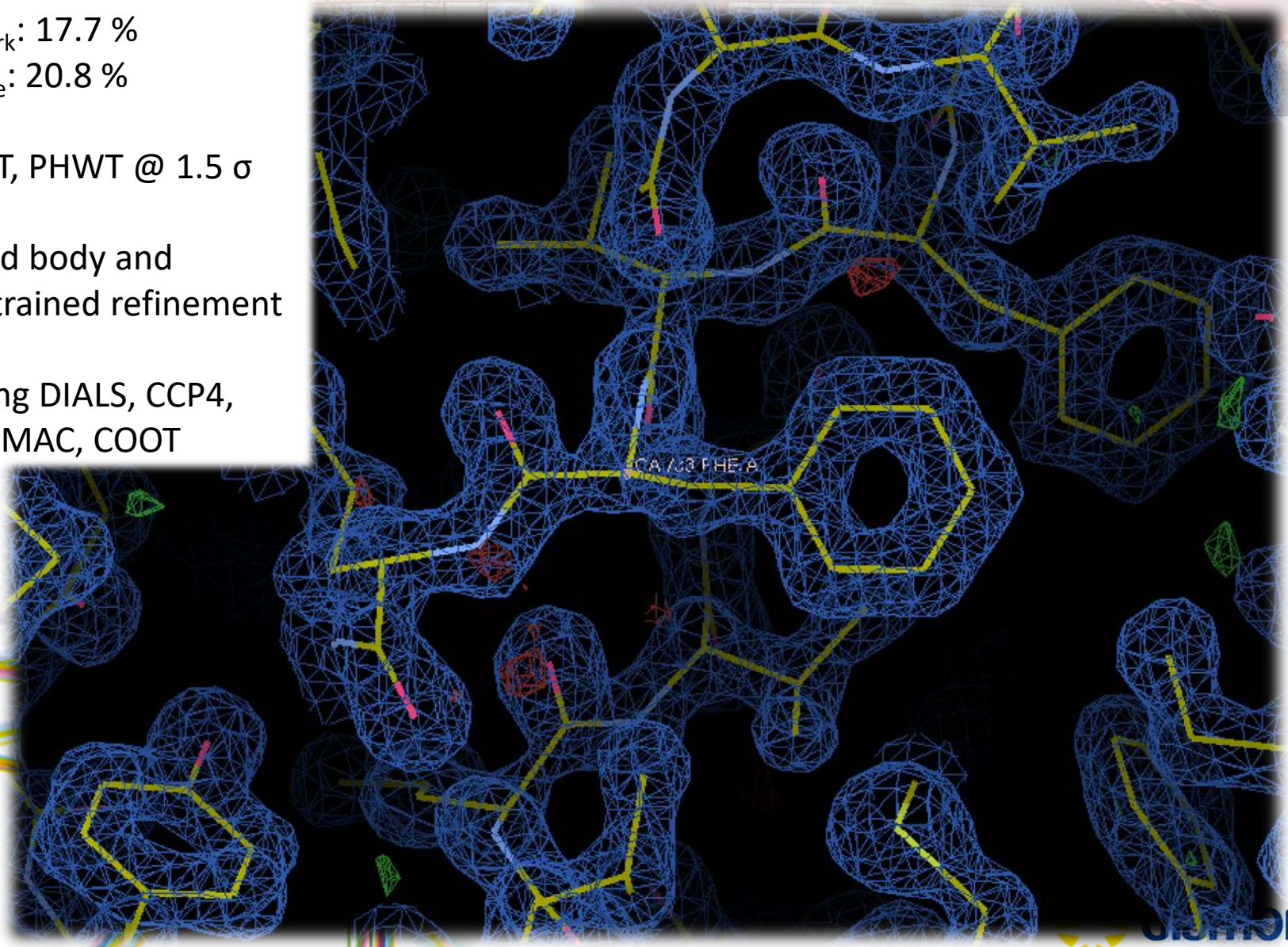
73 - 1.5 Å

R_{work}: 17.7 %
R_{free}: 20.8 %

FWT, PHWT @ 1.5 σ

Rigid body and restrained refinement

Using DIALS, CCP4, REFMAC, COOT



1 Crystal

20° data from single crystal

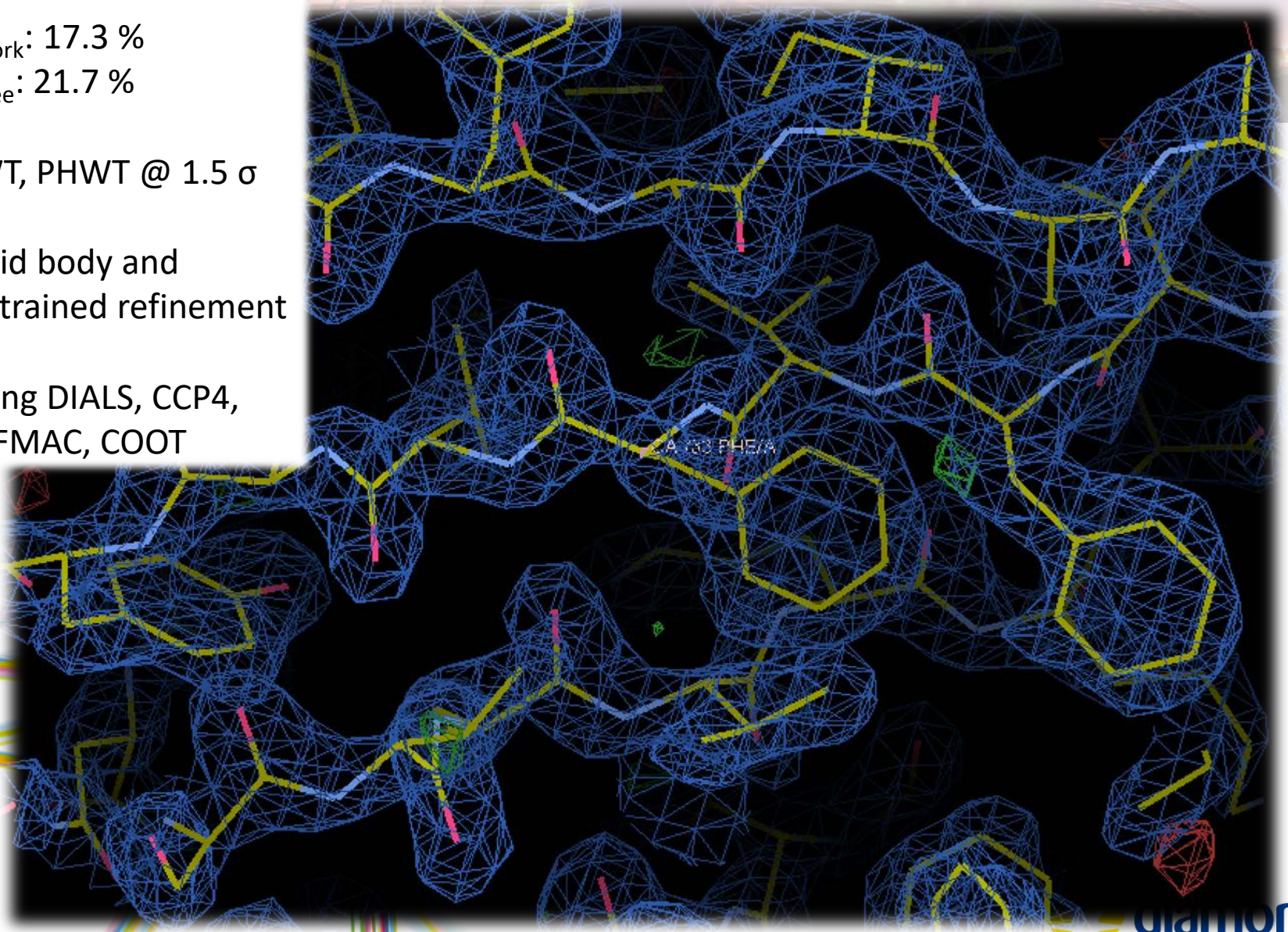
25 - 2.2 Å

R_{work}: 17.3 %
R_{free}: 21.7 %

FWT, PHWT @ 1.5 σ

Rigid body and restrained refinement

Using DIALS, CCP4, REFMAC, COOT



Final Remarks

- VMXm aims to record high quality diffraction data where both size and number of crystals are limited
- Successfully demonstrated the VMXm principal of operation
  - Rotation data recorded from crystals a few microns in size
  - Crystals mounted on cryoEM grids in vacuum at cryo-temperatures
- Commissioning of Eiger 9M CdTe
  - Switching between Pilatus and Eiger for optimal experiments
- More routine diffraction experiments with external users

Acknowledgements

Graham Duller
Andy Stallwood
Richard Parr
Tony Gardner
Dan Magrath
Marcell Nagy

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

Andy Foster
James O'Hea
Brian Nutter
Ben Kemp

Victoria Lawson
Neil Smith
Paul Coe

Mark Lunnon
Dave Butler
Adam Taylor
Adam Prescott
Leon Adams
Thomas Hartrampf

Simon Lay
Hugo Shiers
Guenther Rehm
Chris Bloomer
Sandira Gayadeen

David Laundy
Lucia Alianelli
John Sutter
Simon Alcock
Kawal Sahwney

Sonia Moon

VMXm

Gwyndaf Evans
Jose Trincao
Adam Crawshaw
Emma Beale

Southampton University

Ivo Tews
Rachel Bolton

STRUBI, Oxford

David Stuart
Geoff Sutton

diamond

We need you!

- Do you have suitable crystals with dimensions <10 um?
- Beamline staff will assist with sample preparation and data collection
- Please contact us to discuss:
  - anna.warren@diamond.ac.uk