Getting the most from micron sized crystals

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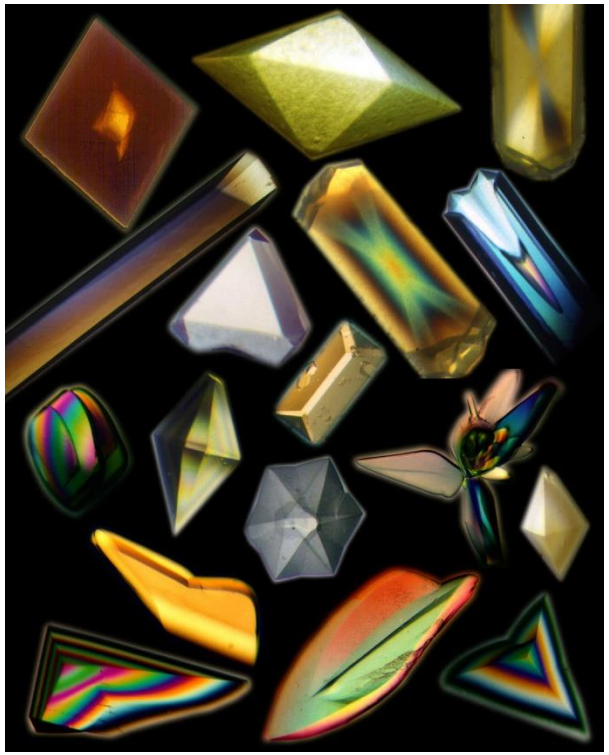
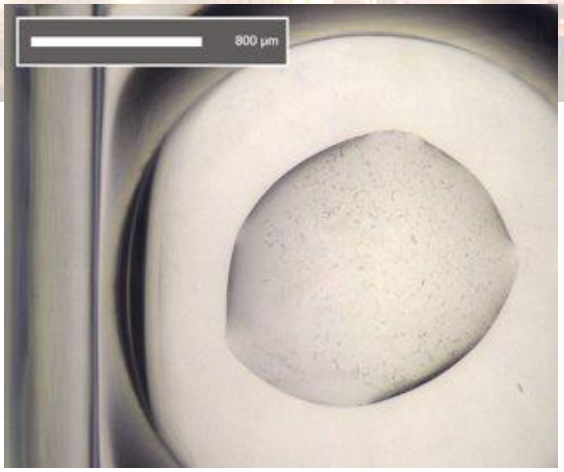
Introduction

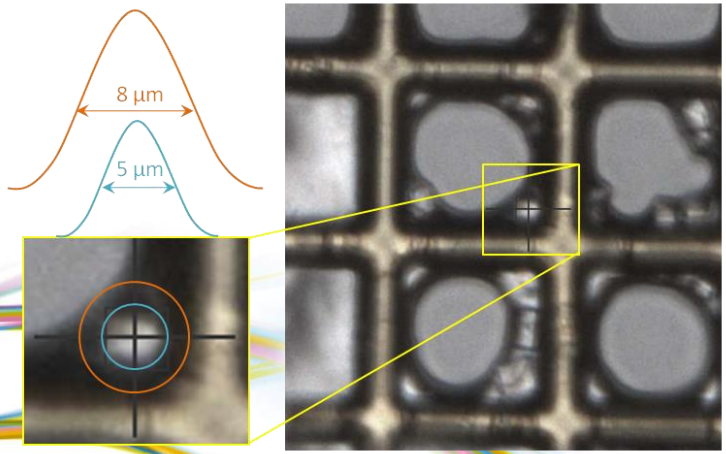
- X-ray crystallography remains the gold standard for the determination of macromolecular structure and protein substrate interactions
- Production of suitable crystals remains one of the largest bottlenecks
- Many crystallisation strategies have been developed
- Synchrotron beamlines have also improved to assist biochemists with their structural problems
- However, some complex proteins are difficult to crystallise and don’t form large uniform crystals

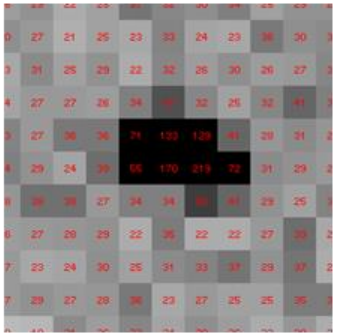


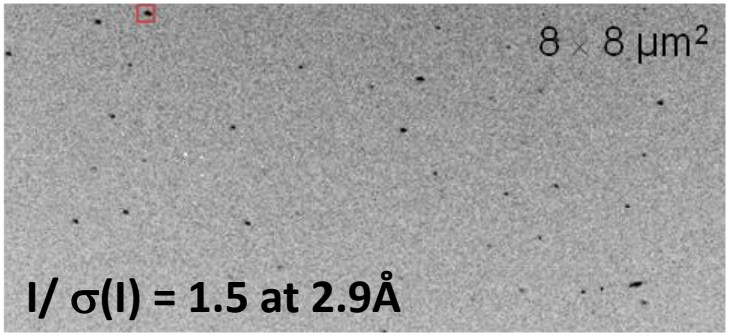
Introduction

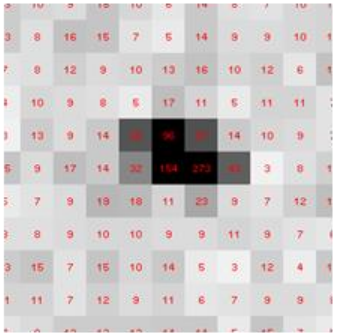
- Sometimes only small crystals are formed
- Can design ideal experiment to give optimal data quality

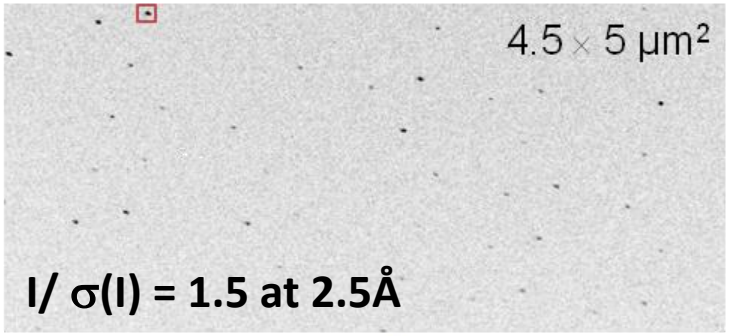












G. Evans, D. Axford and R. L. Owen (2011) *Acta Cryst.* **D67**, 261-270.

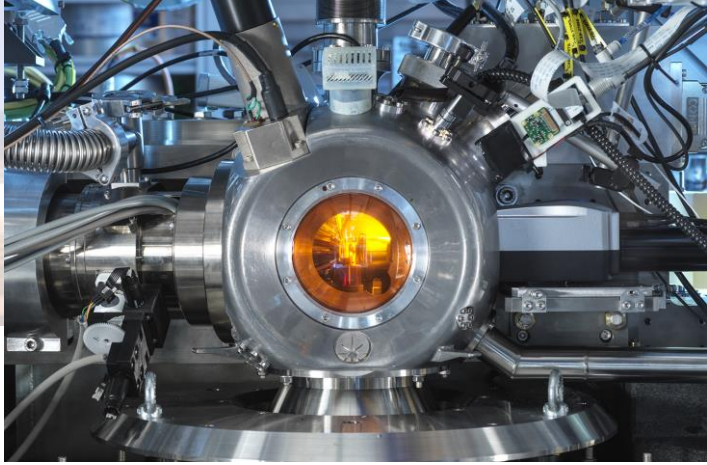
Previous Limits

- 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

Sample Preparation

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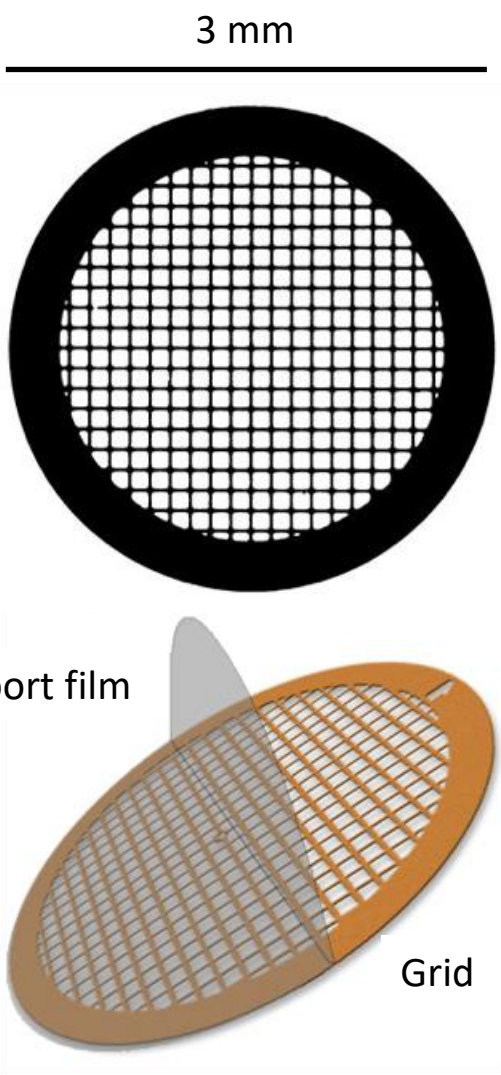
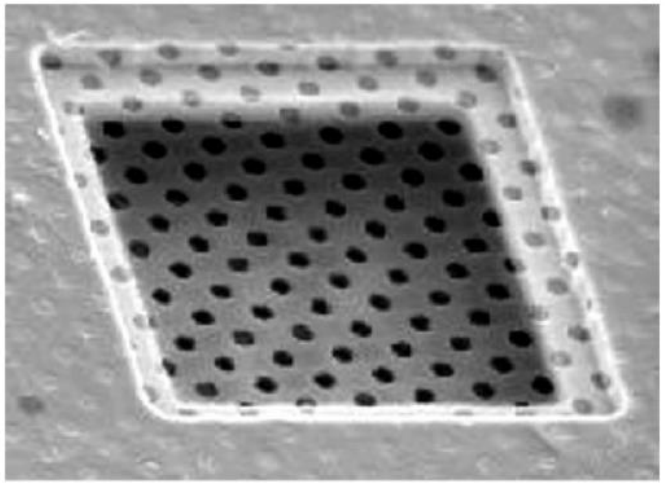
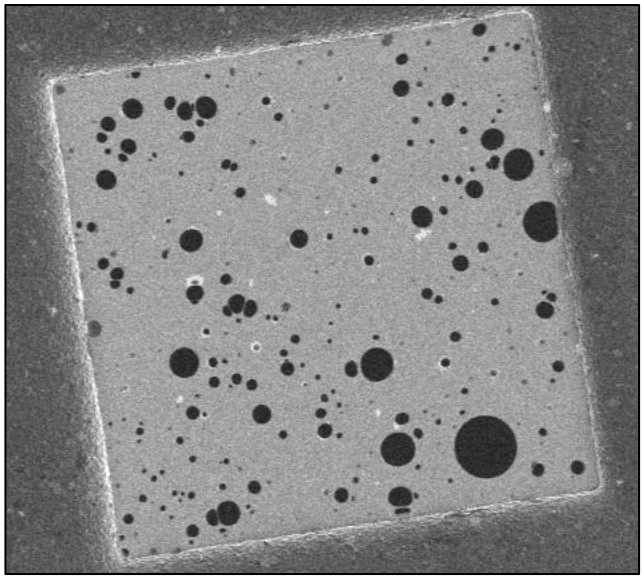
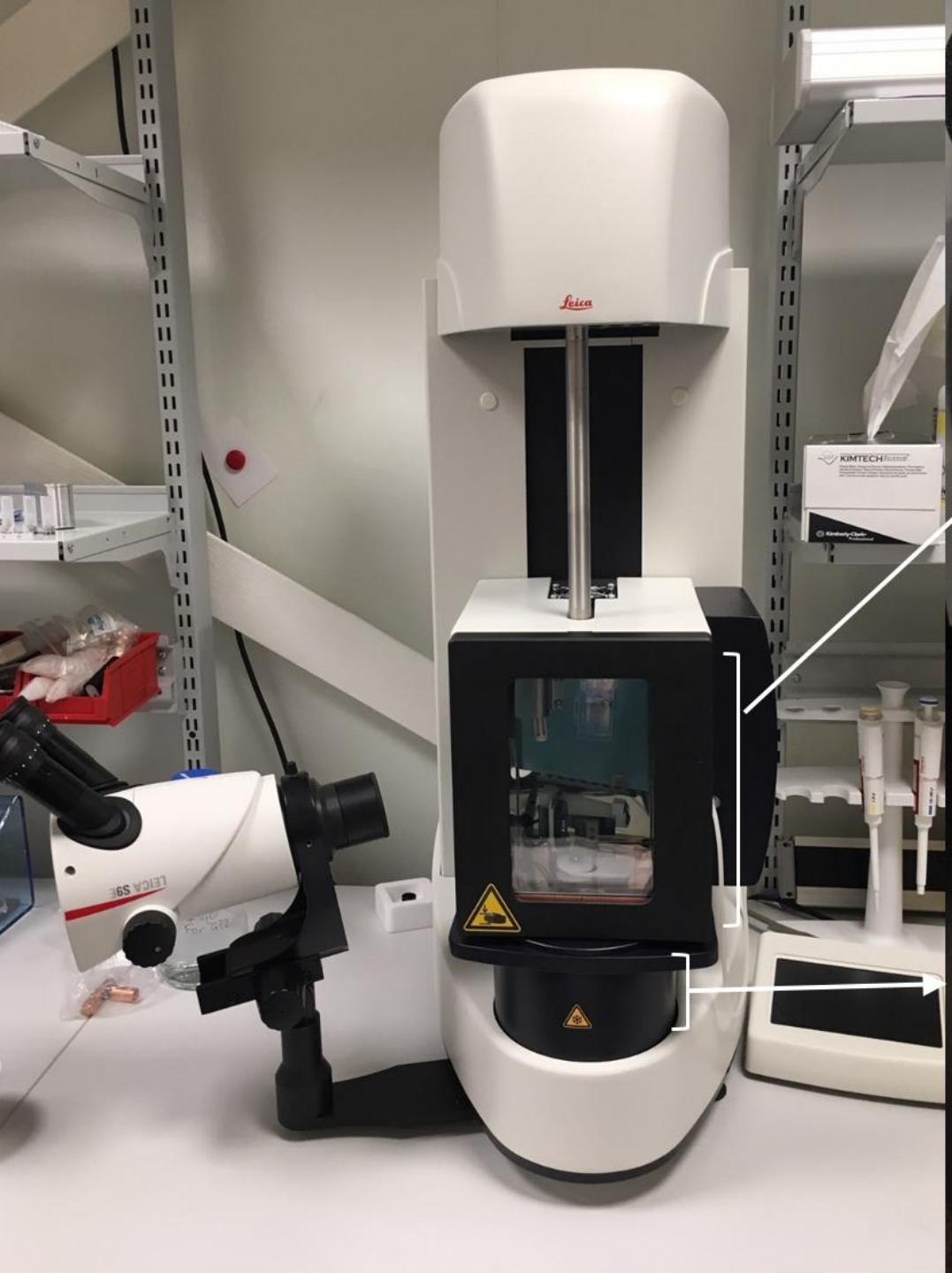
Sample Preparation Lab

Cryo-EM Grids





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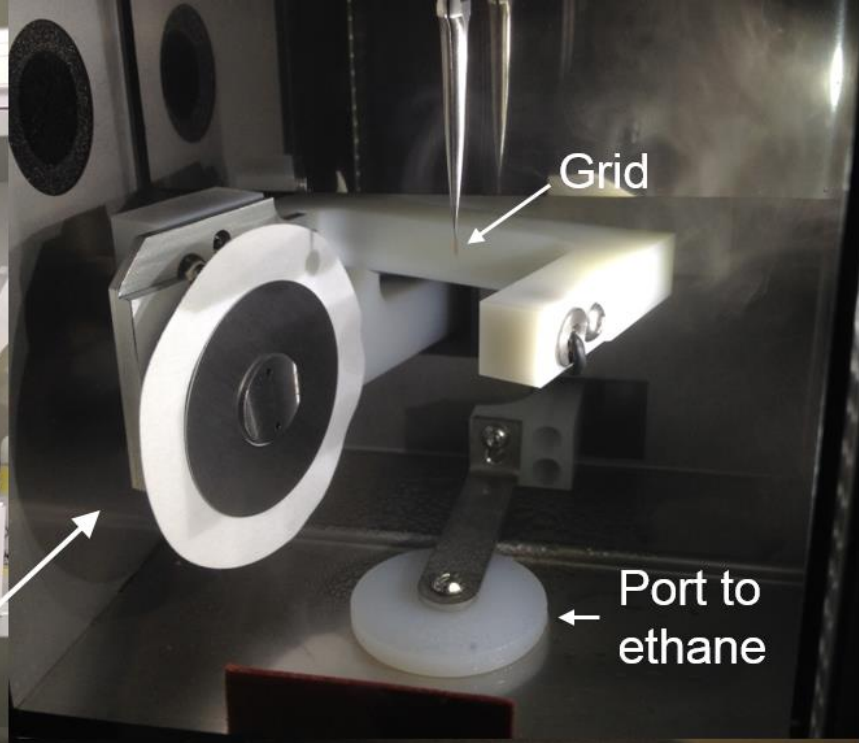
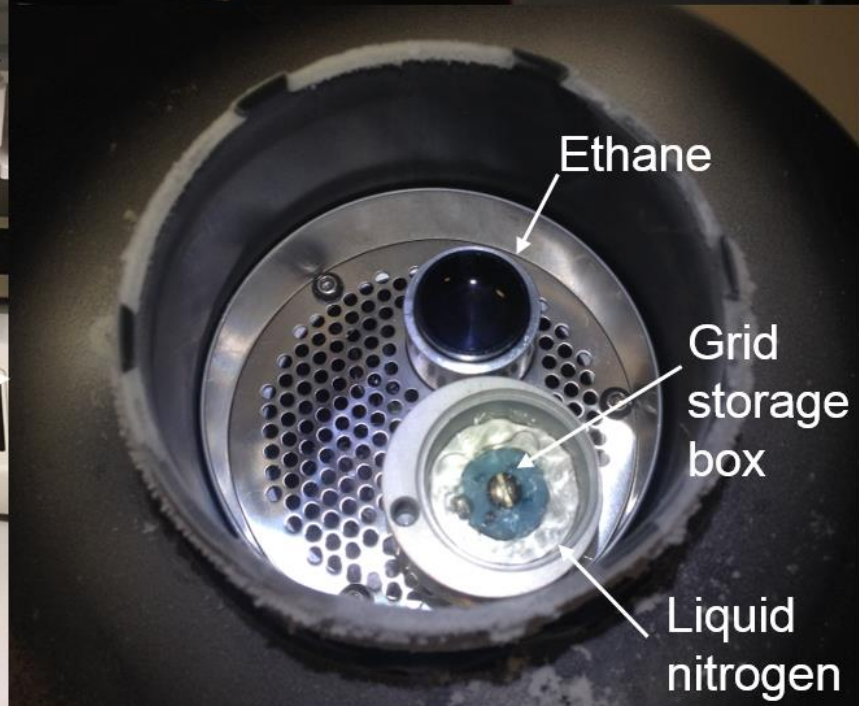
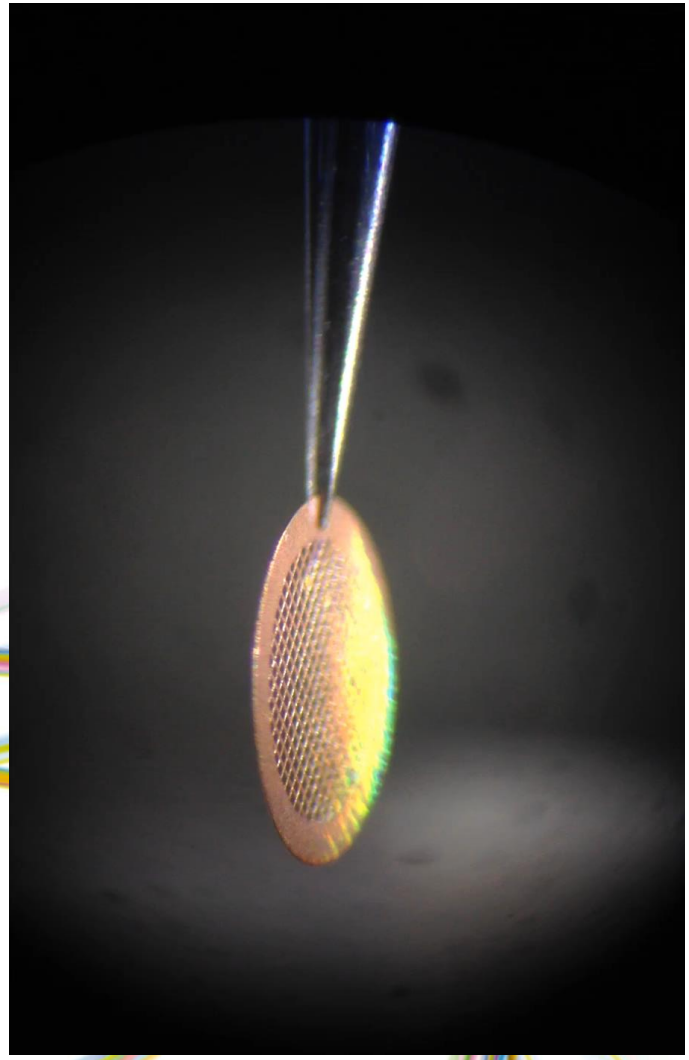
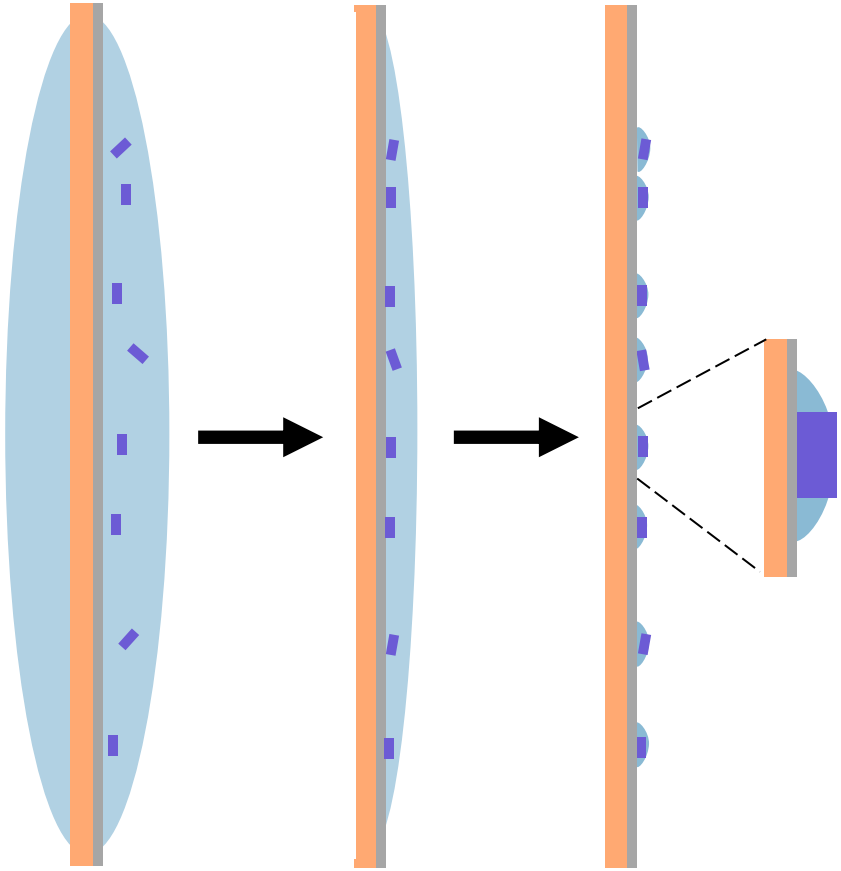
KIMTECH
Kimberly-Clark Professional
LEICA S9E


Grid
Port to ethane

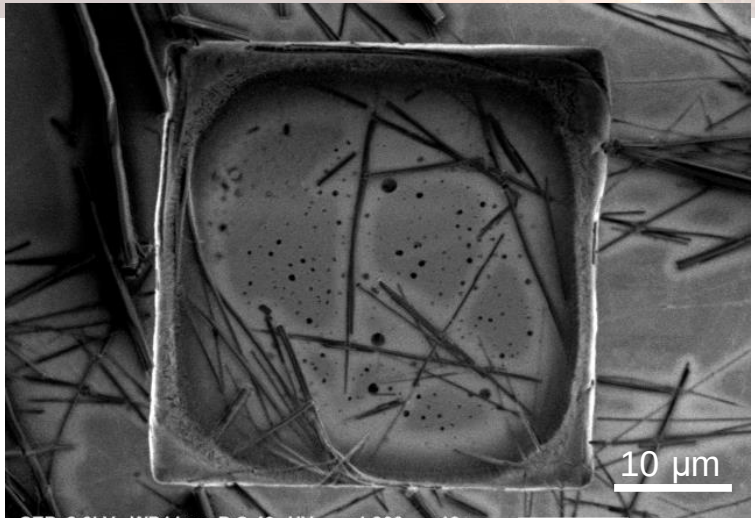

Ethane
Grid storage box
Liquid nitrogen

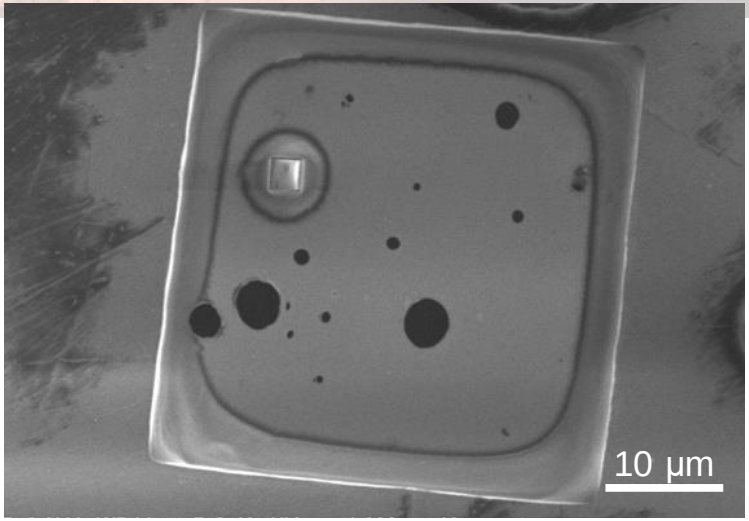
Sample Blotting

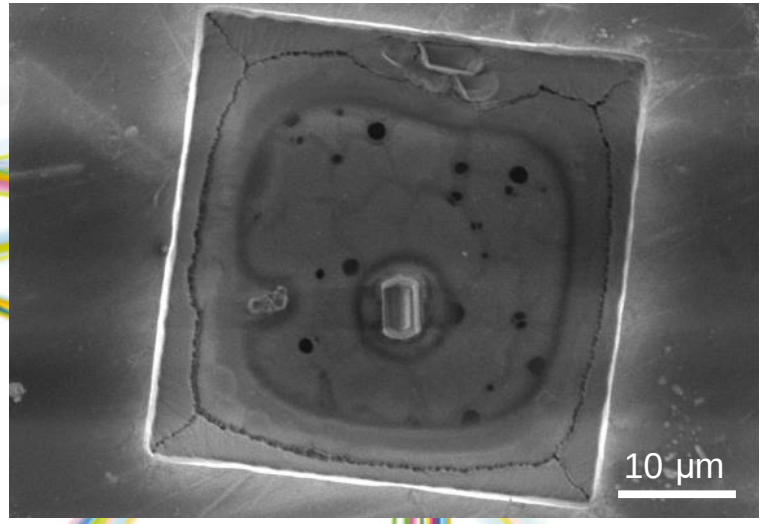


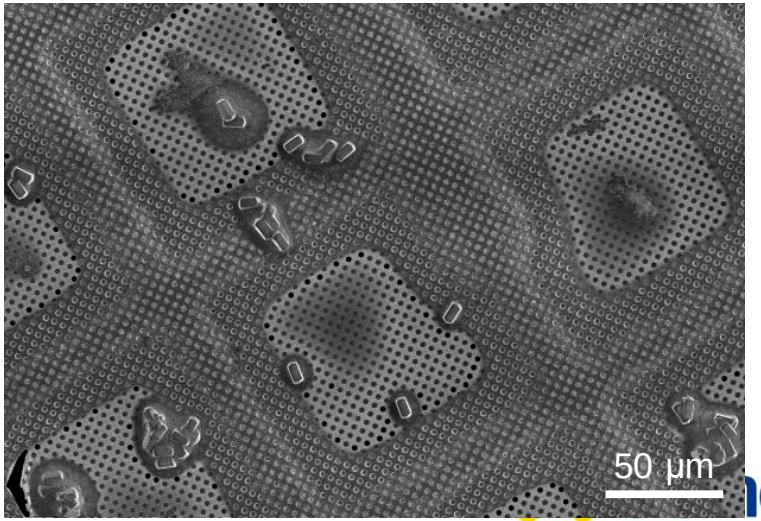


Sample Characterisation

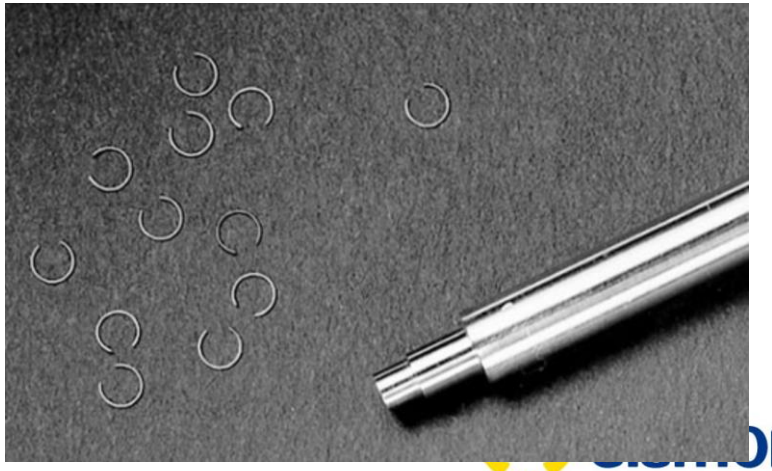








Sample Mounting



Beamline

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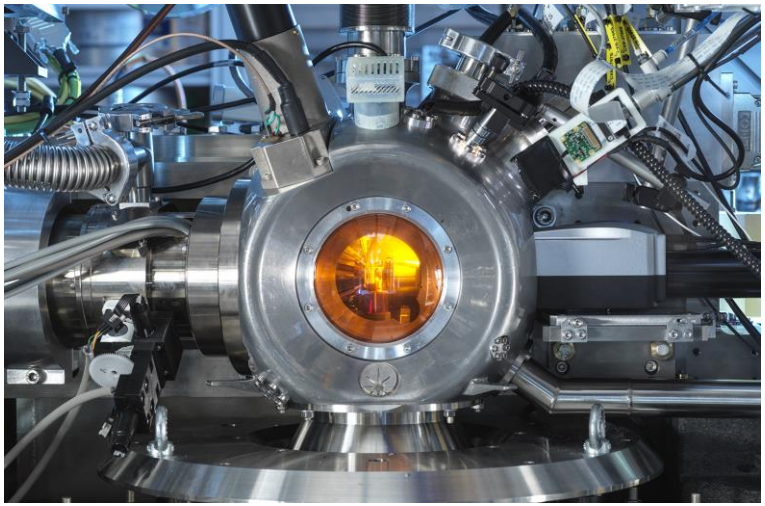
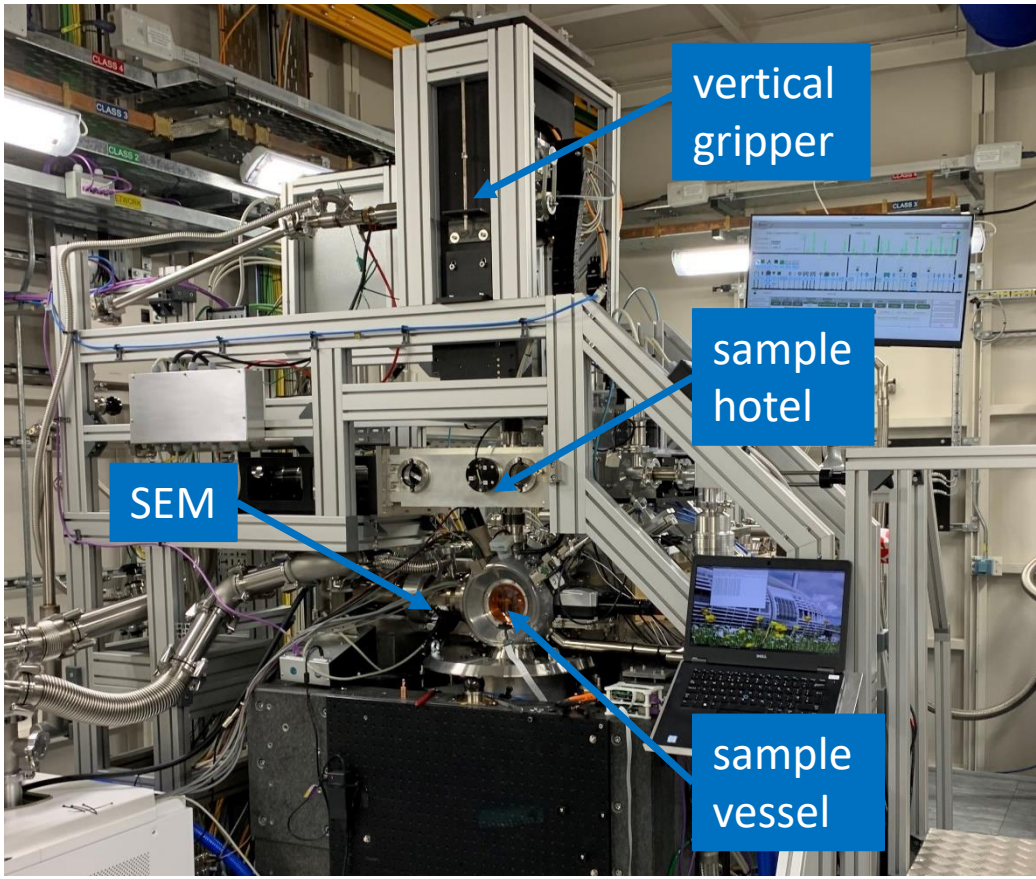
Beamline





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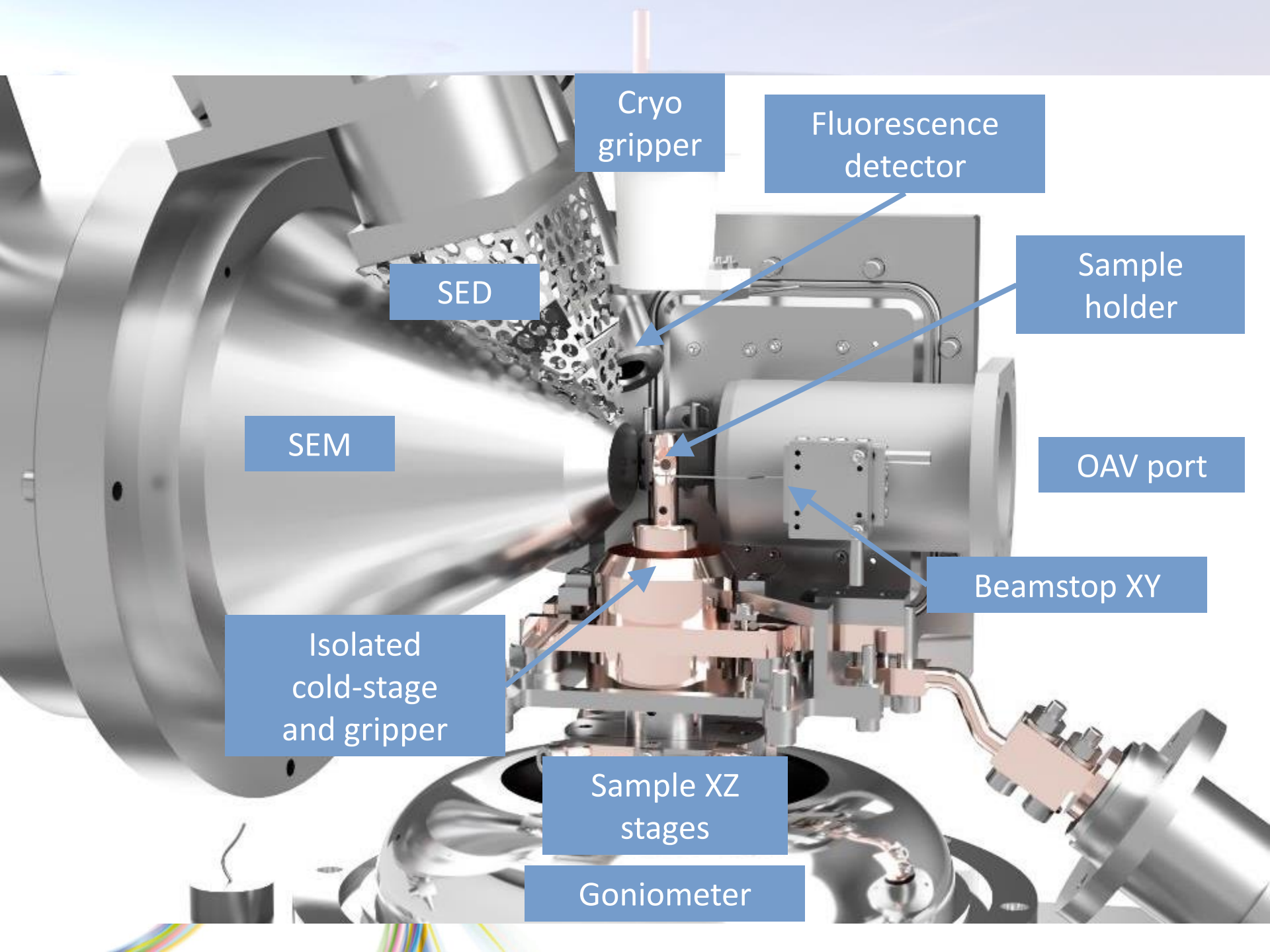
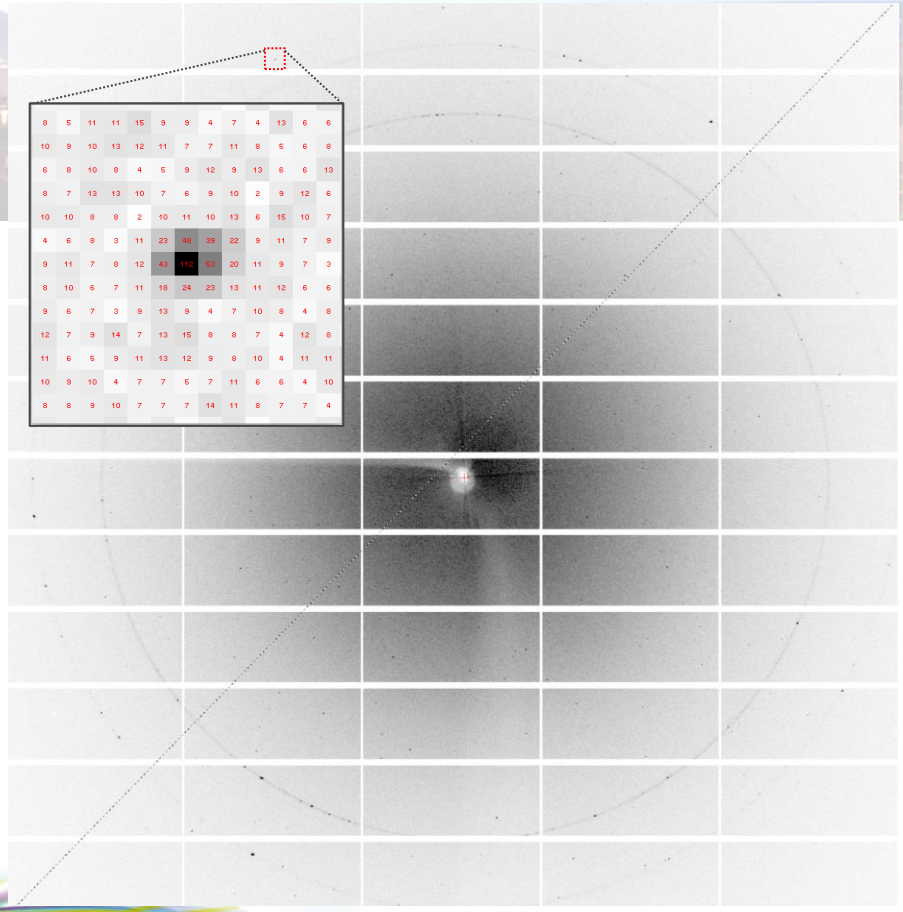
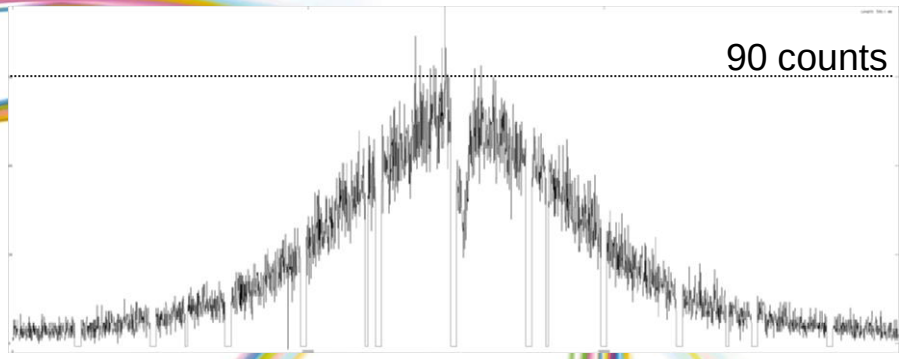

Cryo gripper
Fluorescence detector
SED
Sample holder
SEM
OAV port
Beamstop XY
Isolated cold-stage and gripper
Sample XZ stages
Goniometer

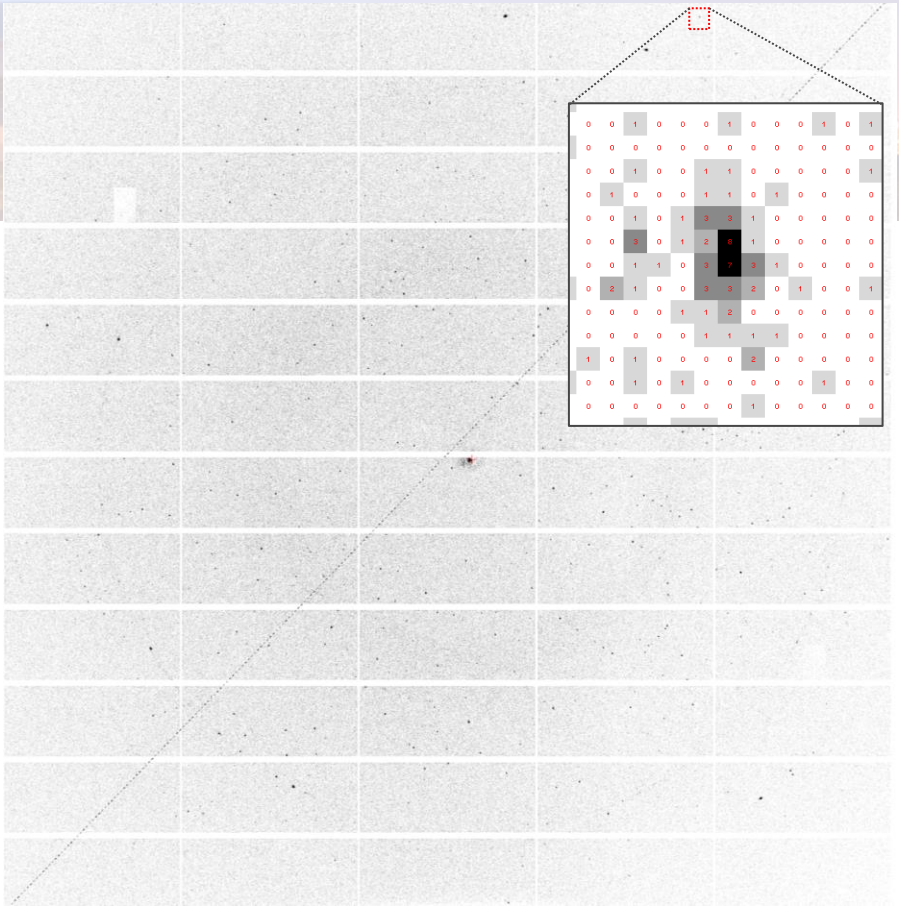
Beamline Results

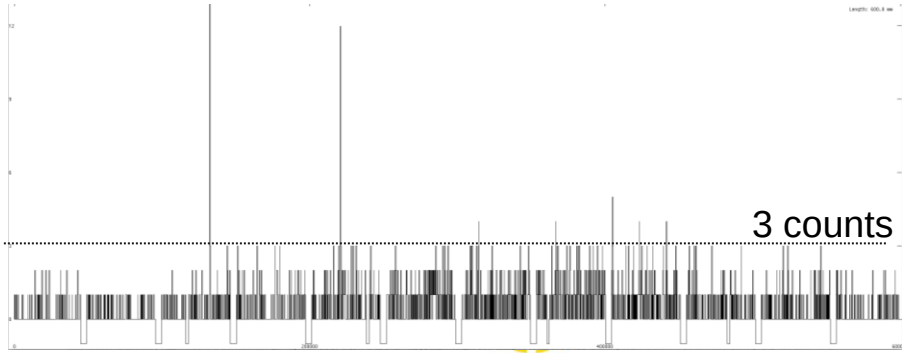
Standard in air MX





VMXm *in vacuo*

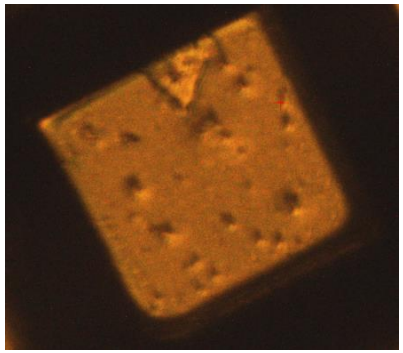


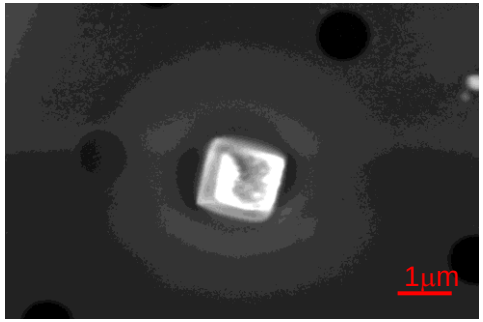


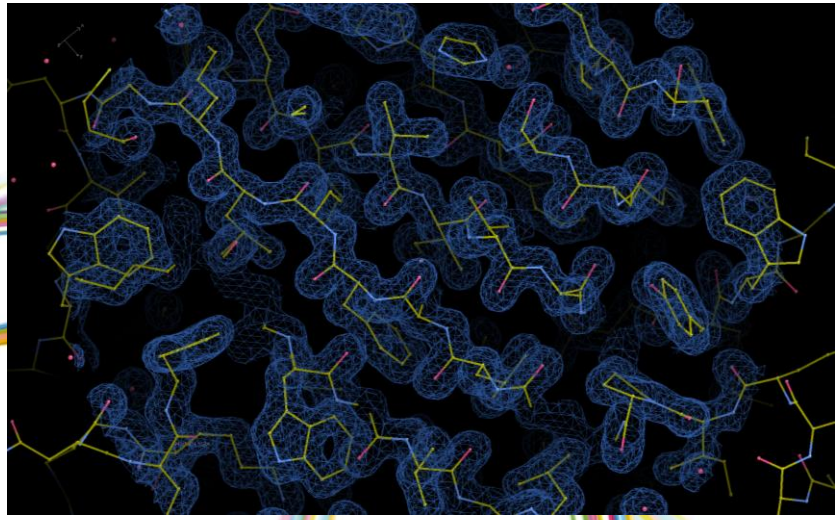
CPV Us17 – Cytoplasmic polyhedrosis virus

Previously measured on I24 and at CXI instrument @LCLS. Initially solved my MR using LCLS data to 1.75 Å and then extended with better XFEL data analysis to 1.46 Å.

- Cytoplasmic polyhedrosis virus
- Spacegroup I23
- Unit cell a=b=c=105 Å
- Approximate crystal size 1-2 µm
- Data collected at 21.3keV
- Eiger2 X CdTe 9M





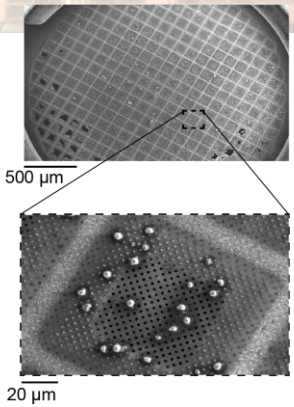


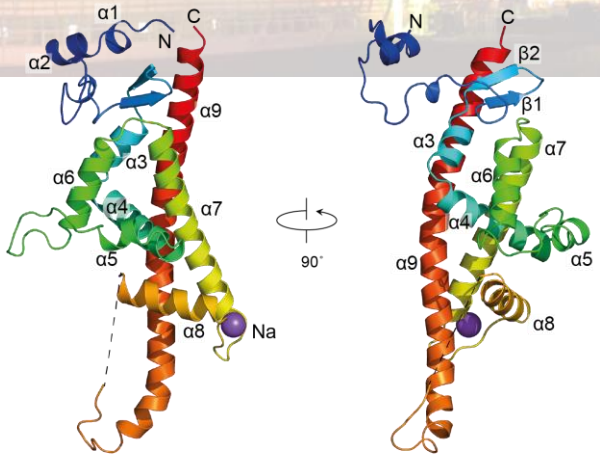
	VMXm	XFEL	I24
Number of crystals	14	6537	768
Resolution	74.2–1.45 (1.48-1.45)	25.0-1.46 (1.485-1.46)	74.16-2.20 (2.26-2.20)
Unique Reflection	33145(1699)	34369 (-)	9376(931)
Completeness (%)	97.2(100)	99.5(92.8)	99.9(100)
R_{meas} (%)	89.1(668.1)	19.0(71.4)	0.665(0.000)*
I/σI	9.2(1.1)	3.2 (-)	6.4(1.4)
CC1/2	0.986(0.302)	0.999(0.331)	--
R_{work}/R_{free} (%)	15.2/16.6	11.1/15.8	14.7/19.9

X. Ji et al. Journal of Structural Biology, 192, (2015), 88–99.
Ginn, H. M. et al. (2015). *Acta Cryst. D*, *71,* 1400-1410.

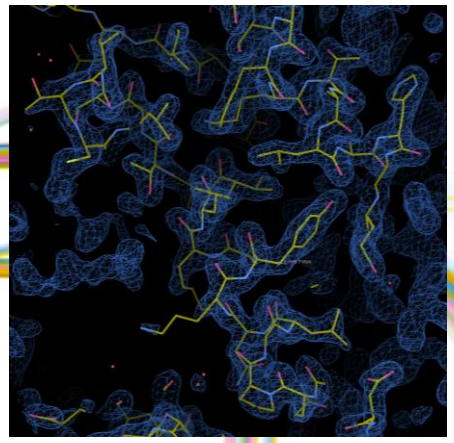
ToNV

- Polyhedra protein from a nudivirus
- Self-assembles into a dense lattice around new viral particles
- 3 x Met engineered into WT clone to allow SeMet protein
- $P3_121$ a=53.5 Å, c=105.2 Å, ɣ=120°
- Solvent content 21%
- Crystals ~ 5 – 7 µm (SeMet); 3 – 5 µm (WT)

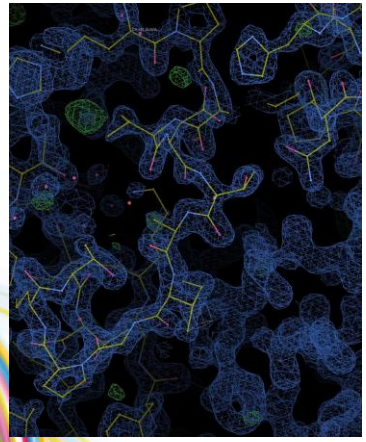




Se-Met to 1.86A



WT to 1.7A

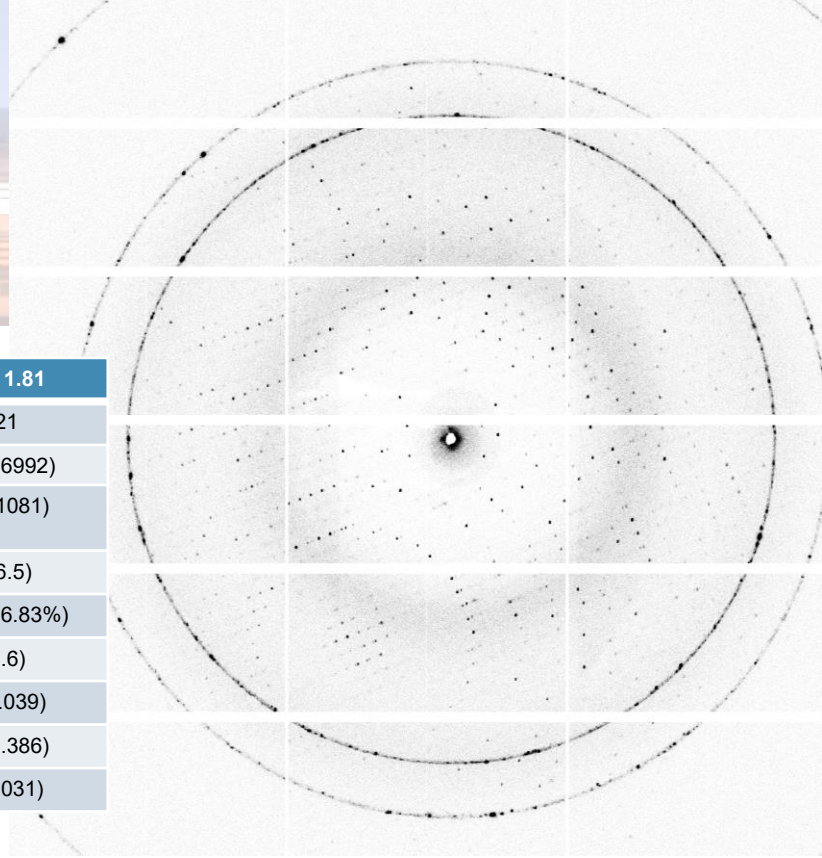


	Se-Met TonV on VMXm
Detector	Eiger2 X CdTe 9M
Number of crystals	67
Energy keV	12.67 keV
Resolution	105.0-1.86 (1.90-1.86)
Unique Reflection	13841(244)
Completeness (%)	91.3(32.0)
R_{merge}	0.288(1.342)
I/σI	17.7(0.7)
CC1/2	0.971(0.133)
Beamsize	3.6 x 3.6 µm

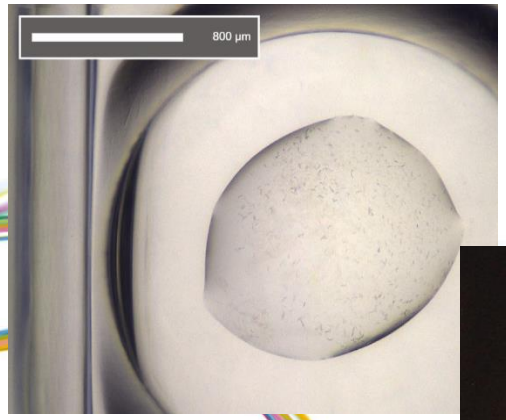
Keown, J.R. *et al. Nat Commun.* (2023), **14**, 4160.

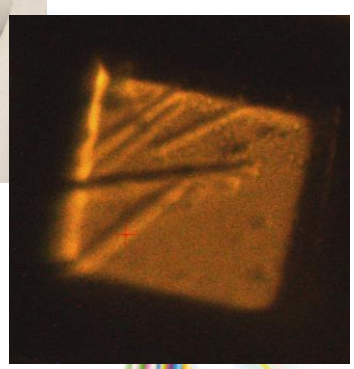
Mpro ligand co-crystals

- Ligand absent from large crystal form
- Co-crystals with ligand of needle form grown
- Needle crystals (3-4 µm wide)
- 40° wedges of diffraction collected from 12 crystals
  - 3 x 3 µm beam at 21.3 keV
- dials.multiplex used to combine the 12 datasets

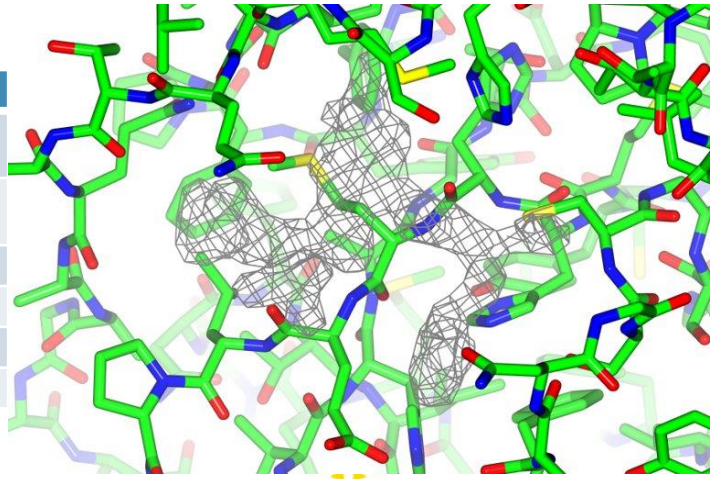


Resolution (Å)	22.53 – 1.81
Wavelength (Å)	0.5821
Observations	371253 (6992)
Unique Observations	23222 (1081)
Multiplicity	16.0 (6.5)
Completeness	81.34% (76.83%)
Mean I/σI	6.2 (0.6)
Rmeas	0.511(4.039)
Rpim	0.110 (1.386)
CC1/2	0.98 (0.031)



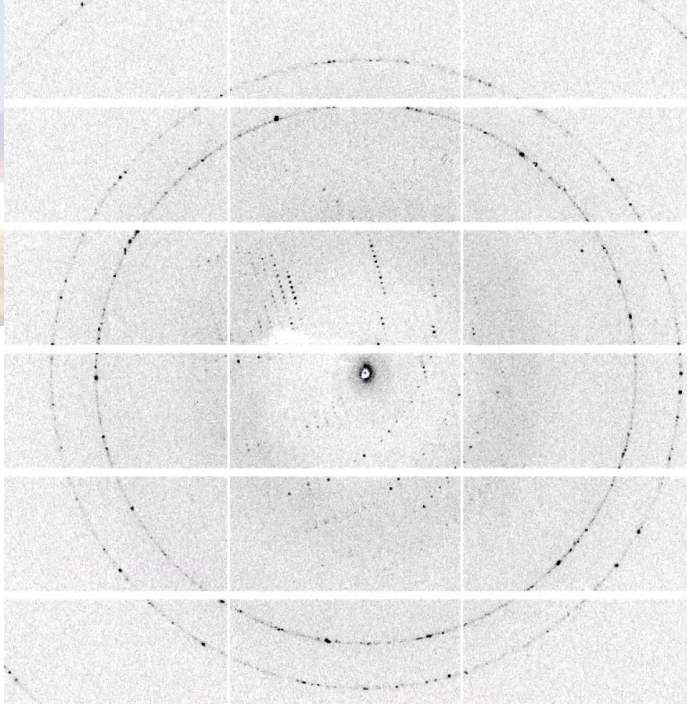


Stat	Value
Resolution	22.53 – 1.81
N. Reflections all/free	21520/1140
R/Rfree	0.19/0.24
RMS dev	
Bonds	0.01
Angles	1.459



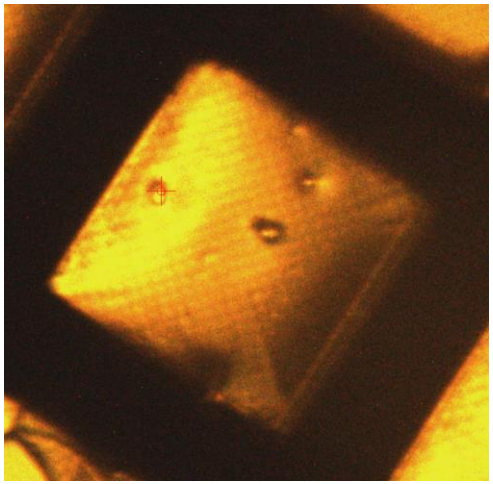
L. Brewitz et al. J. Med. Chem., 66, (2023), 2663-2680.

Membrane protein - FFAR1



- GPCR membrane protein – crystals ~5 µm
- Grown in classic glass plate sandwich in LCP – highly viscous, adds significant background scatter
- Crystallisation solution including 12% MPD used to wash the bolus out from under the glass
- Applied directly to grid mounted in plunge freezer as per soluble sample

	Overall	Low resolution	High resolution
Resolution (Å)	53.79 - 2.27	53.81 - 6.16	2.31 - 2.27
Observations	297971	14118	15053
Unique reflections	30700	1593	1512
Multiplicity	9.7	8.9	10.0
Completeness	99.96%	99.44%	100.00%
Mean I/σ(I)	6.3	26.3	0.6
R_{merge}	0.285	0.106	4.440
R_{meas}	0.302	0.113	4.687
R_{pim}	0.096	0.036	1.455
$CC_{½}$	0.990	0.993	0.277



Andrew Quigley and Rebecca Parr (MPL)

MPL Membrane Protein Laboratory

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Current status

- VMXm can record high quality diffraction data where both size and number of crystals are limited
- Exploiting low background, sample mounting, high energy and photoelectron escape to optimize experiment

User programme:

- Current commissioning call will continue until further notice
- Sample size envelope is broad – not just micron sized
  - Even though beam size is smaller we can sensibly prepare grids for the beamline with larger crystals
- Send VMXm staff pictures of sample!
  - This will help us advise on suitability and approach
- Drops with low numbers of crystals are OK! Do not need high concentration of crystals!
  - Access is relatively light touch at present requiring a brief scientific justification

VMXm Team

Gwyndaf Evans

Jose Trincao

Adam Crawshaw

Contact a member of the VMXm team if interested to know more or use the beamline

For the DLS-CCP4 Workshop data collection day:
Monday 27th November

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