VMXm: Micro/nanofocus protein crystallography beamline at Diamond

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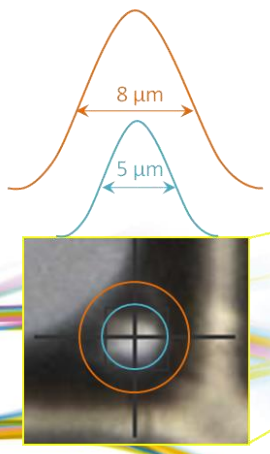
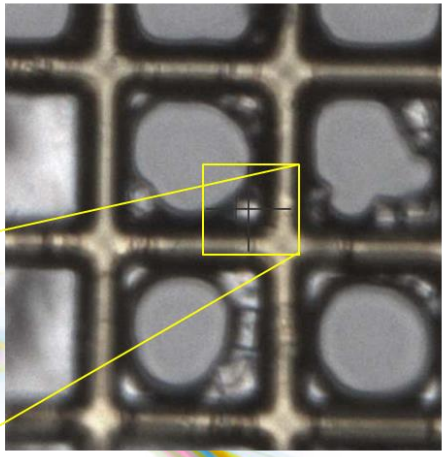
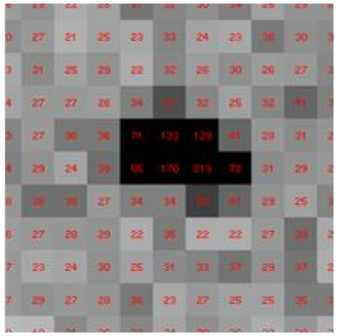
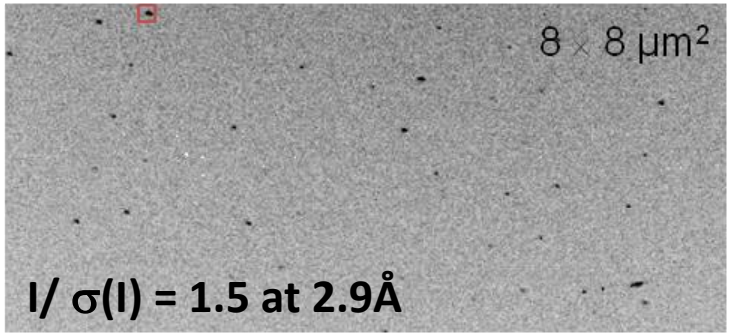
Introduction

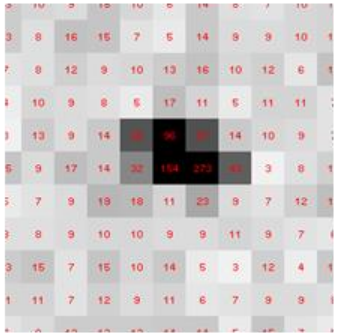
- Target proteins are getting more complex, leading to smaller and more disordered crystals
- Can design ideal experiment to give optimal data quality

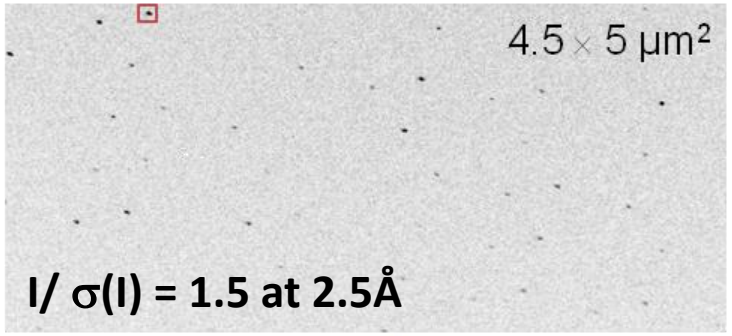












G. Evans, D. Axford and R. L. Owen (2011) *Acta Cryst.* **D67**, 261-270.

Current Limits

- 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

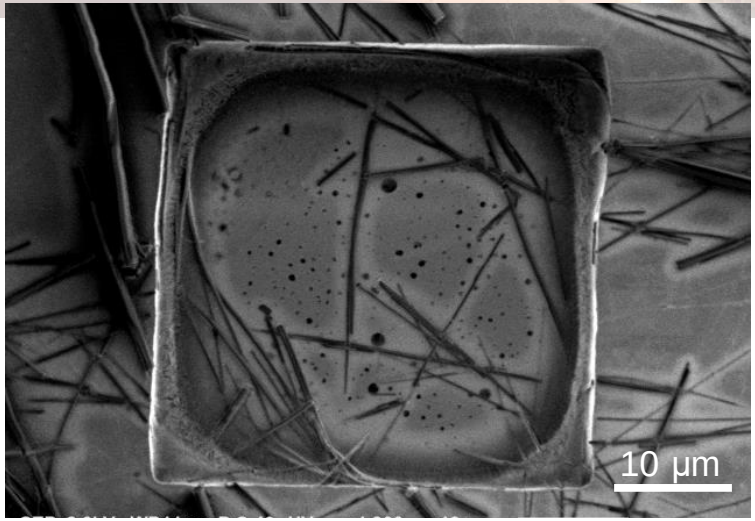
- Improve signal to noise by reducing background:
  - Sample environment under vacuum
  - Crystals mounted with minimal liquid
  - Reduce beamsize to match that of the crystal
- Standard rotation data collection on samples down to 500 nm
  - Alignment without the need for X-ray raster scanning
- Optimise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

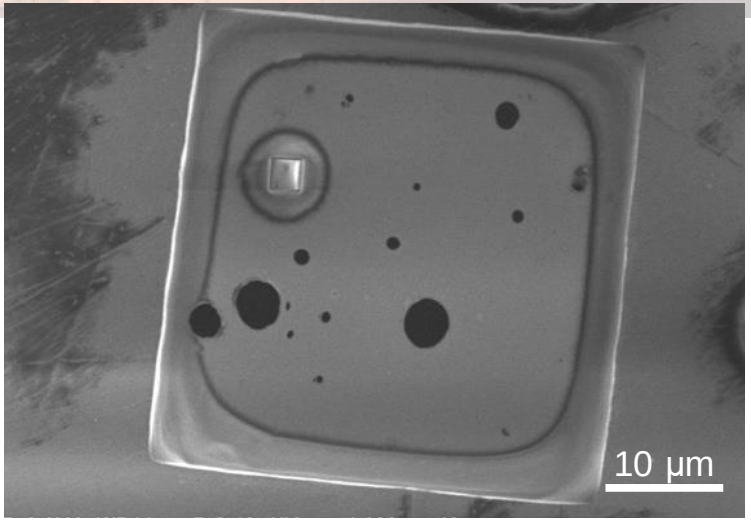
VMXm Specifications

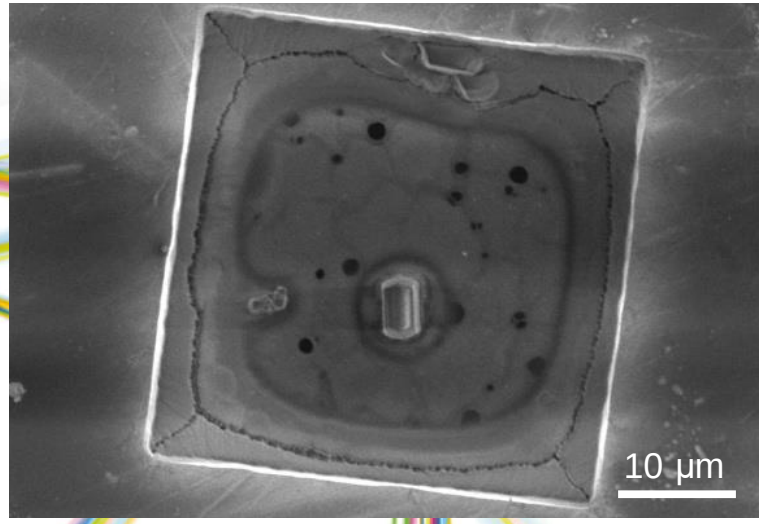
- 6 – 28 keV energy range
- 0.3 – 10 µm (v) & 0.5 – 5 µm (h)
- Flux @12.4 keV:
  - ~10^{12} ph/s in 0.3 × 3 µm (v x h)
  - >10^{11} ph/s 0.3 × 0.5 µm (v x h)
- Interchangeable detectors:
  - Pilatus3 6M Si
  - Eiger2 X 9M CdTe

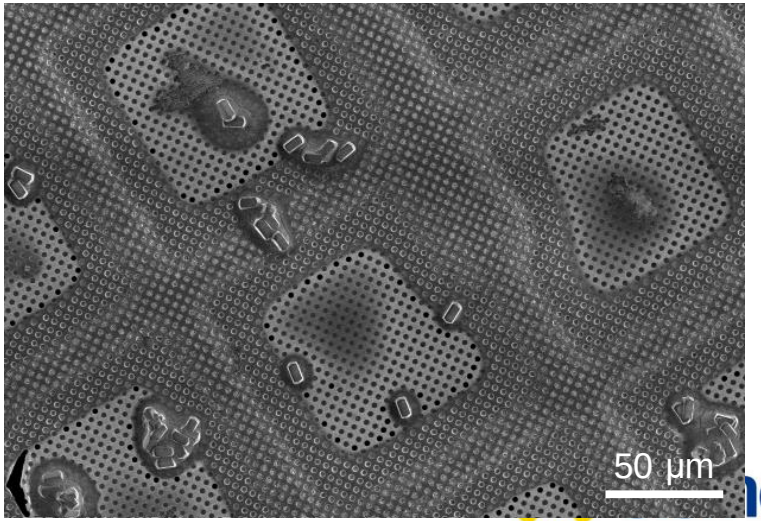
Sample Preparation Lab

Sample Characterisation

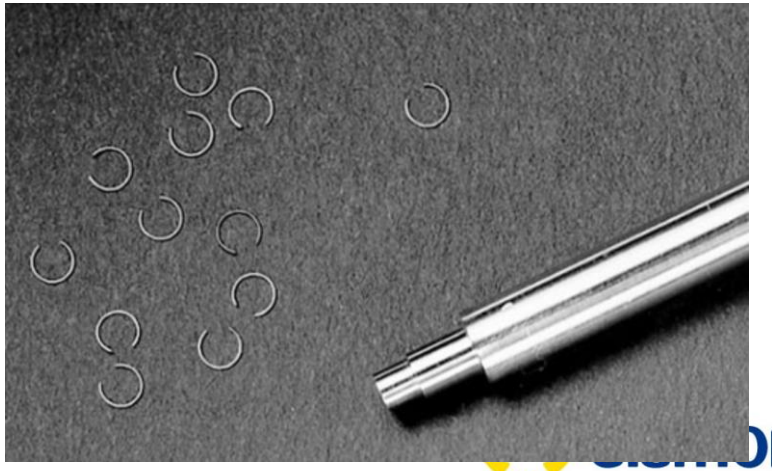








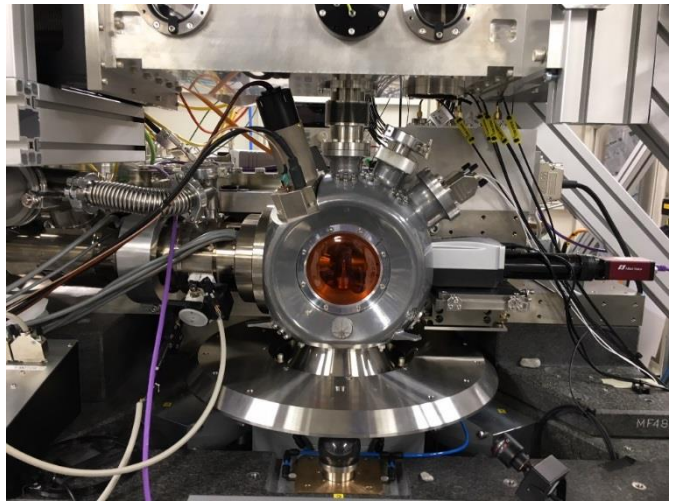
Sample Mounting

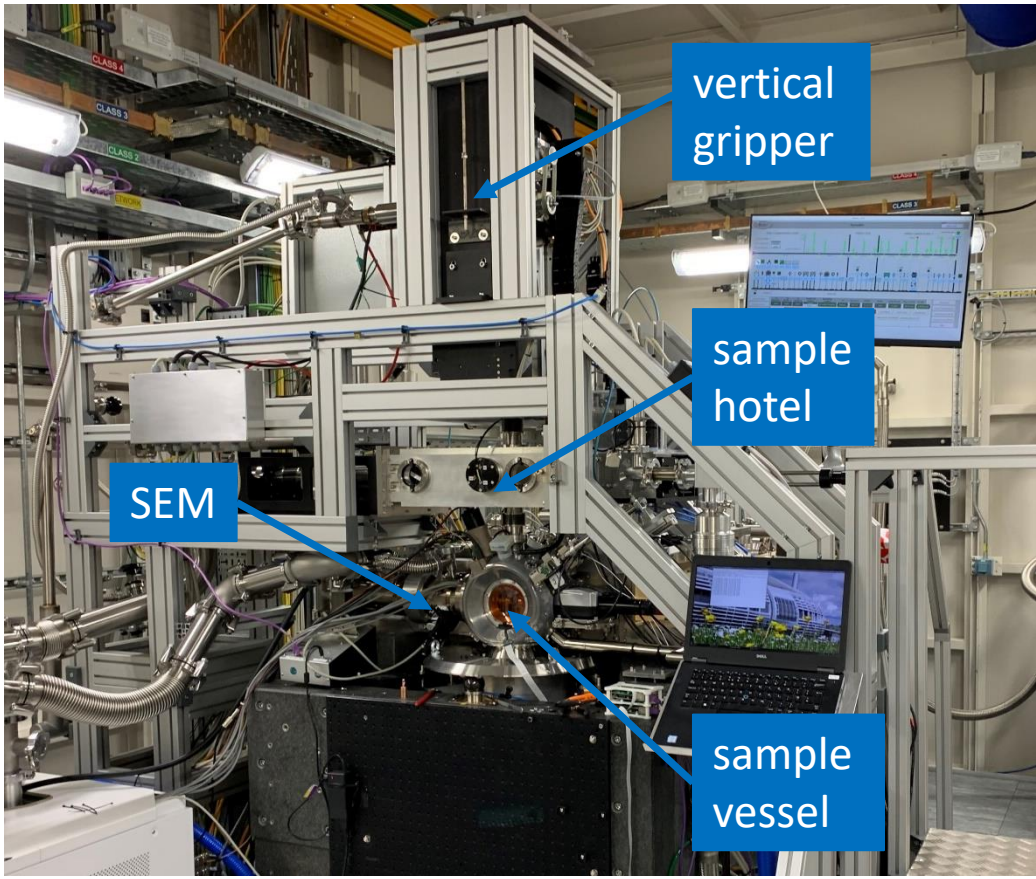


Sample Environment

- *In vacuo* sample environment - reduce X-ray background to a minimum
- *In vacuo* sample cooling to preserve samples
- Standard on-axis optical microscope + SEM for sample visualization
- High stability goniometry - permit rotation data to be measured from micro and nanocrystals

Beamline





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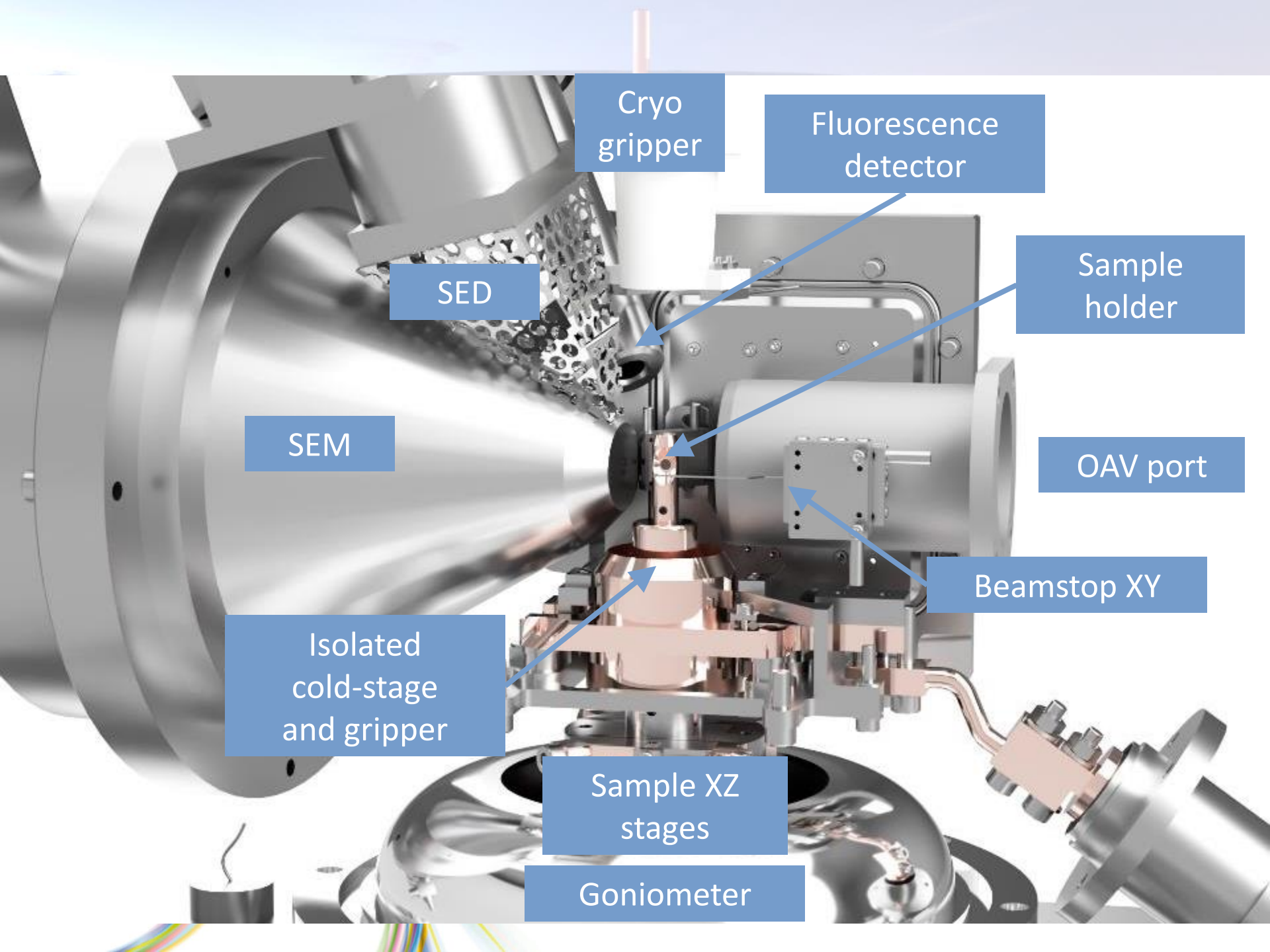
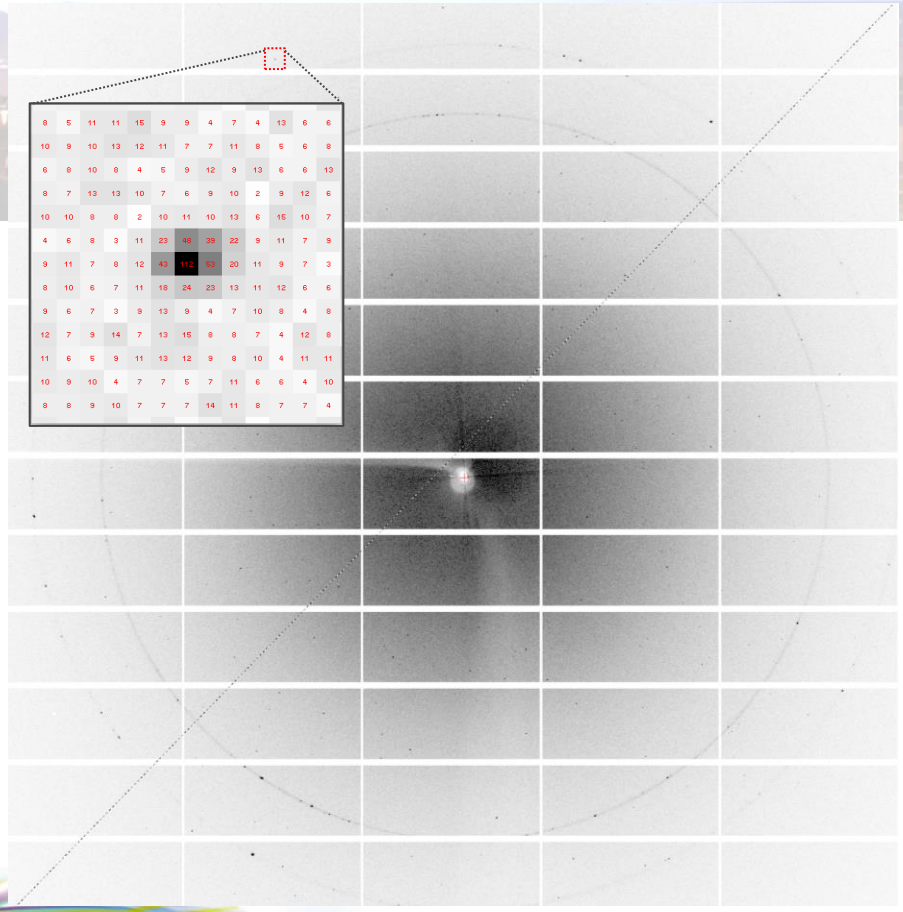
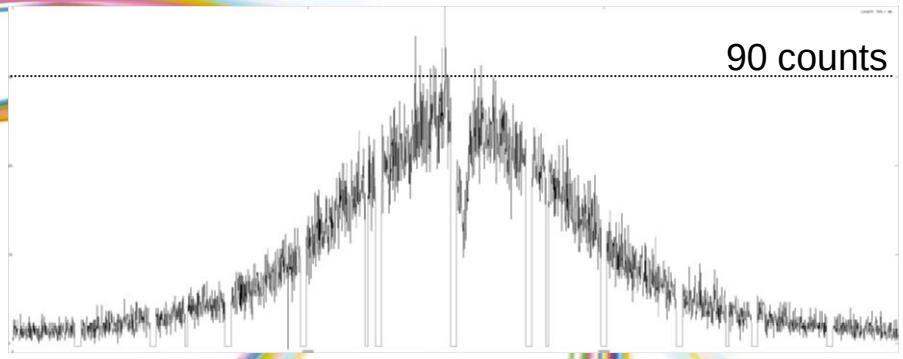
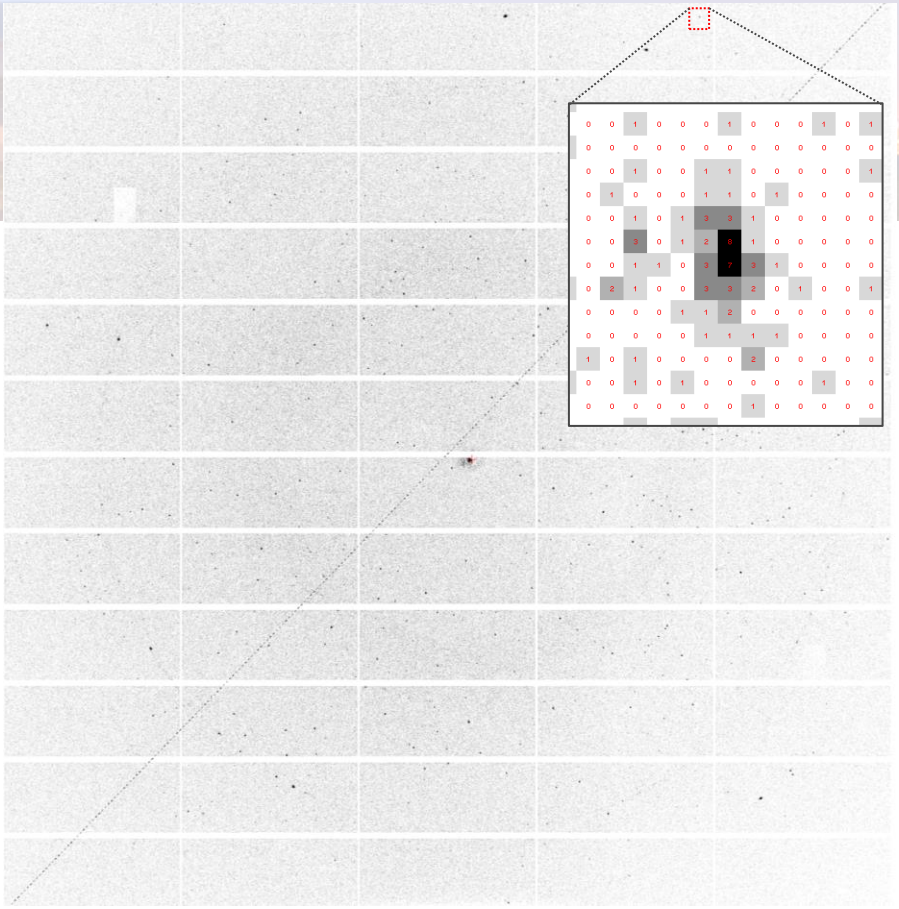

Cryo gripper
Fluorescence detector
SED
Sample holder
SEM
OAV port
Beamstop XY
Isolated cold-stage and gripper
Sample XZ stages
Goniometer

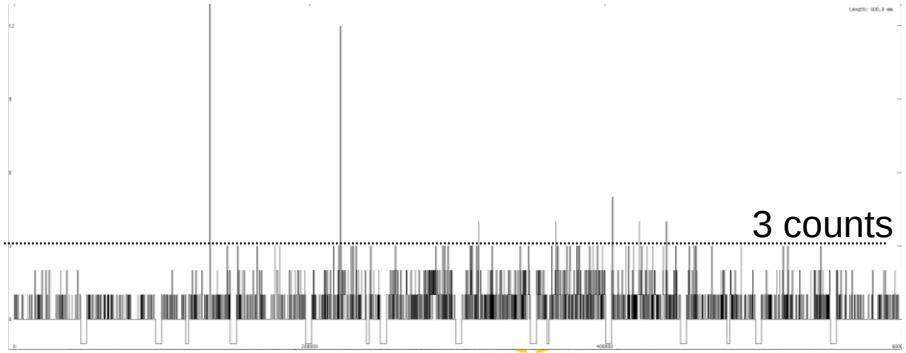
Standard MX





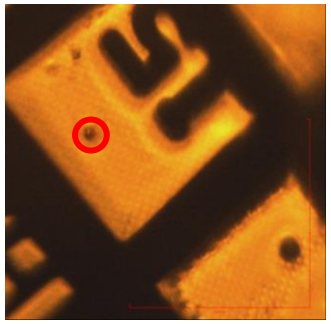
VMXm





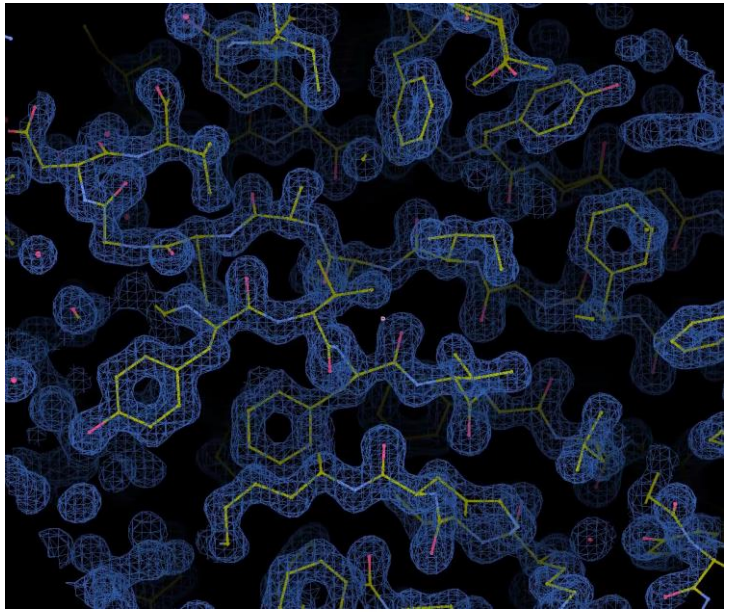
CPV Ld14 – Cytoplasmic polyhedrosis virus

- Spacegroup I23
- Unit cell a=b=c=103 Å
- Approximate crystal size 2 – 4 µm
- Data collected at 21.3keV – exploit photoelectron escape
- Eiger2 X CdTe 9M



Stat	Value
Resolution	72.94-1.38
N. Reflections all/free	37547/1920
R/Rfree	0.136/0.193
RMS dev	
Bonds	0.0141
Angles	1.806

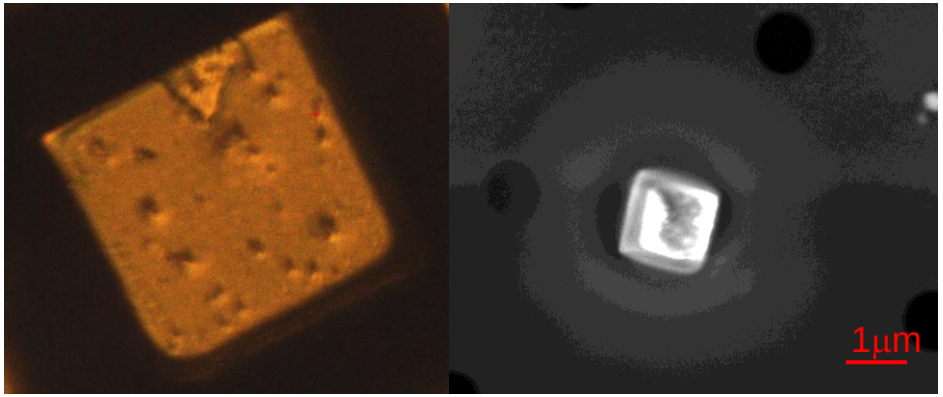
	Ji et al., I24	VMXm
Detector	Pilatus 6M Si	Eiger2 X CdTe 9M
Number of crystals	20	1
Energy keV	12.458 keV	21.3 keV
Resolution	72.5–1.91 (2.02-1.91)	72.9-1.30 (1.32-1.30)
Unique Reflection	12952(1045)	40616(1350)
Completeness (%)	92.3(52.1)	90.7(62.2)
R_{merge}	0.199(0.327)	0.284(0.881)
I/σI	8.4(1.8)	3.8(0.5)
CC1/2	??	0.933(0.407)
Beamsize	6 x 6 µm slit	3.6 x 3.6 µm

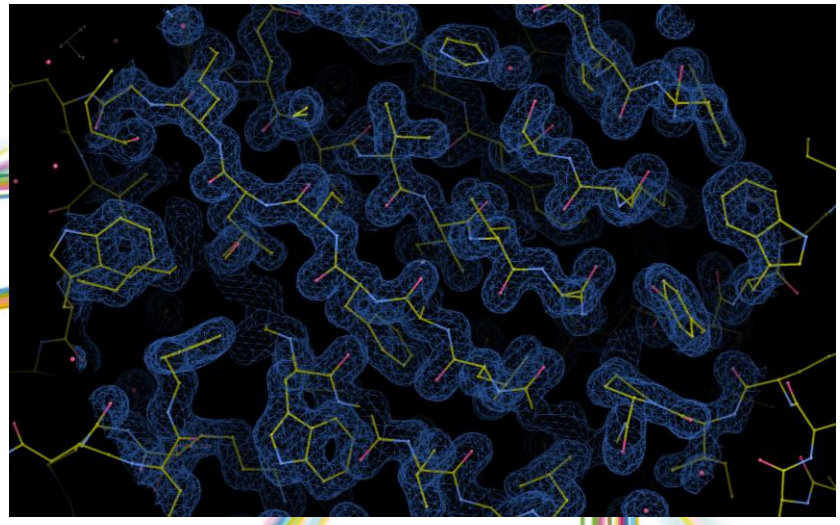


X. Ji et al. Journal of Structural Biology, 192, (2015), 88–99

CPV Us17 – 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
- Merged 17 datasets



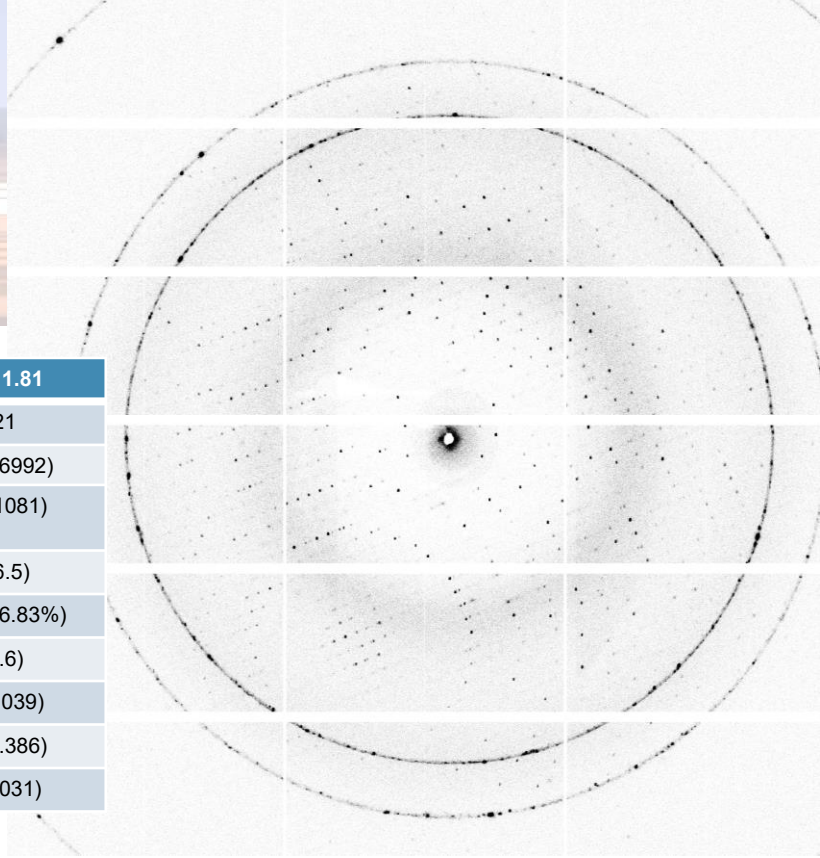


	VMXm	XFEL	I24
Number of crystals	13	5787	768
Resolution	74.28–1.46 (1.49-1.46)	28.3-1.75 (1.79-1.75)	74.16-2.20 (2.26-2.20)
Unique Reflection	32555(1664)	19096(1032)	9376(931)
Completeness (%)	97.2(100)	100(100)	99.9(100)
R_{merge}	9.265(-14.862)	-	0.665(0.000)
I/σI	6.7(0.8)	-	6.4(1.4)
CC1/2	0.469(0.268)	-	-

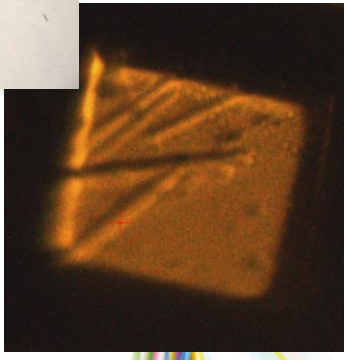
X. Ji et al. Journal of Structural Biology, 192, (2015), 88–99

Mpro ligand co-crystals

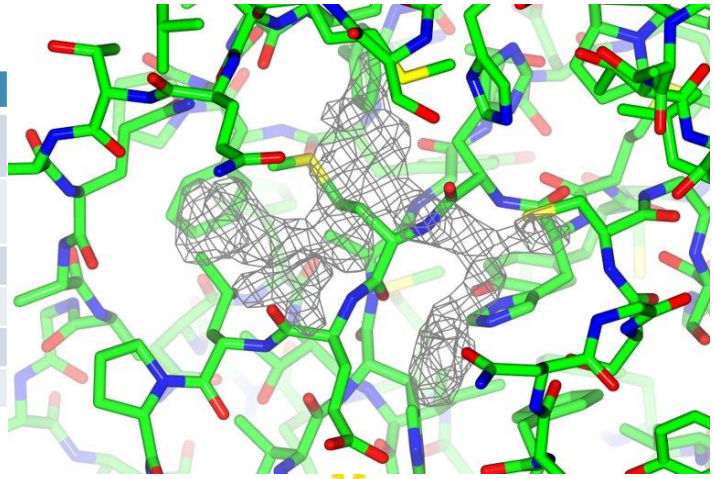
- Ligand absent from large crystal form
- Co-crystals with ligand of needle form grown
- Needle crystals (3-4 µm wide)
- 40° wedges of diffraction collected from 12 crystals
  - 3 x 3 µm beam at 21.3 KeV
- dials.multiplex used to combine the 12 datasets



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)



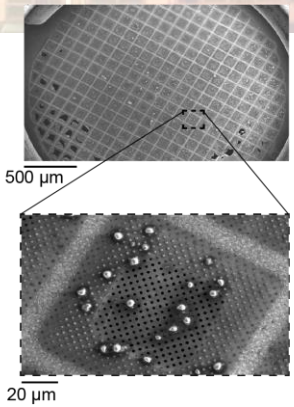
Stat	Value
Resolution	22.53 – 1.81
N. Reflections all/free	21520/1140
R/Rfree	0.19/0.24
RMS dev	
Bonds	0.01
Angles	1.459

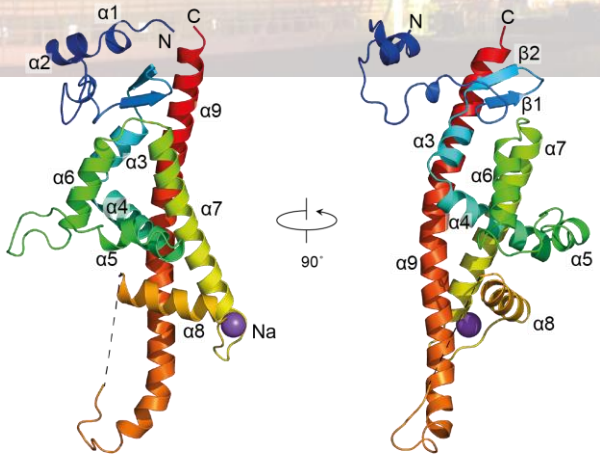


Crystals provided by David Owen

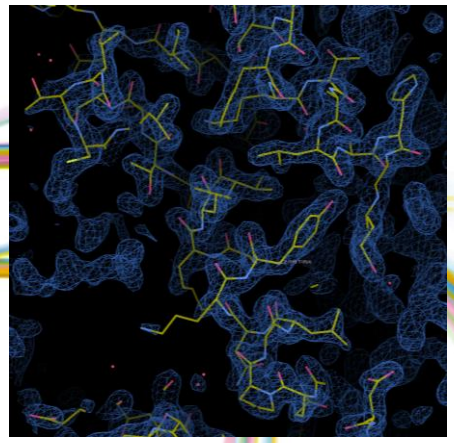
ToNV

- 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)

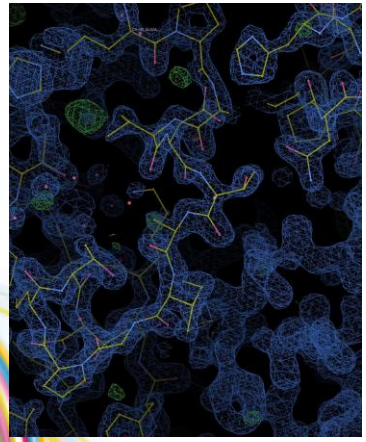




Se-Met to 1.86A



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

Jeremy Keown and Jonathan Grimes (STRUBI, Uni. of Oxford)
Adam Crawshaw, Richard Gildea, Jose Trincao (VMXm)

Final Remarks

- 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
- 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

Acknowledgements

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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

Gwyndaf Evans

Jose Trincao

Adam Crawshaw

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

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