# VMXm: Micro/nanofocus protein crystallography beamline at Diamond

Anna Warren
anna.warren@diamond.ac.uk

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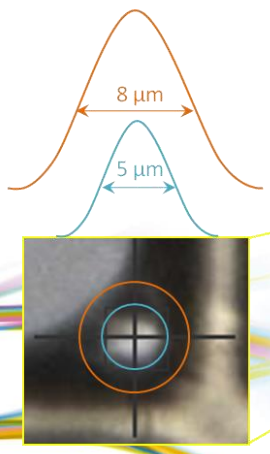
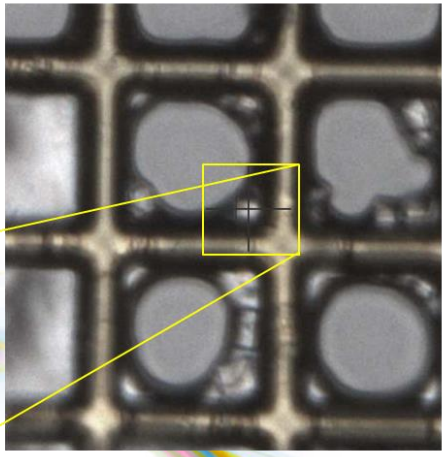
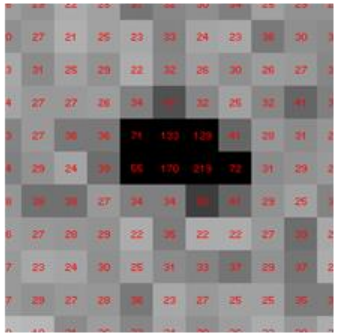
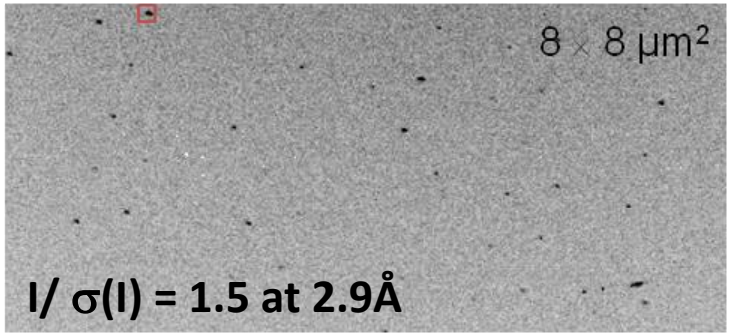
# Introduction

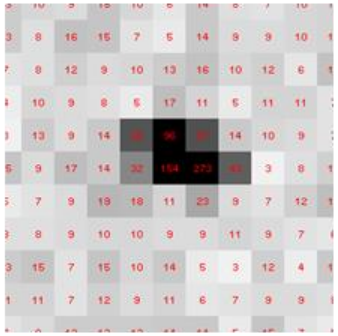
- Target proteins are getting more complex, leading to smaller and more disordered crystals
- Can design ideal experiment to give optimal data quality

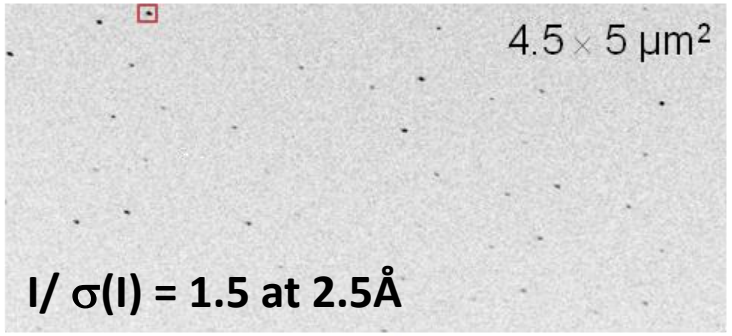









G. Evans, D. Axford and R. L. Owen (2011) *Acta Cryst.* **D67**, 261-270.

# Current Limits

- 2.2 Å data can be collected from 1 $\mu m^3$ crystals (~700 well diffracting crystals)
- 3 Å data can be collected from 5 $\mu m^3$ 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 $\mu m^3$ 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

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# 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)
- Interchangeable detectors:
  - Pilatus3 6M Si
  - Eiger2 X 9M CdTe

# 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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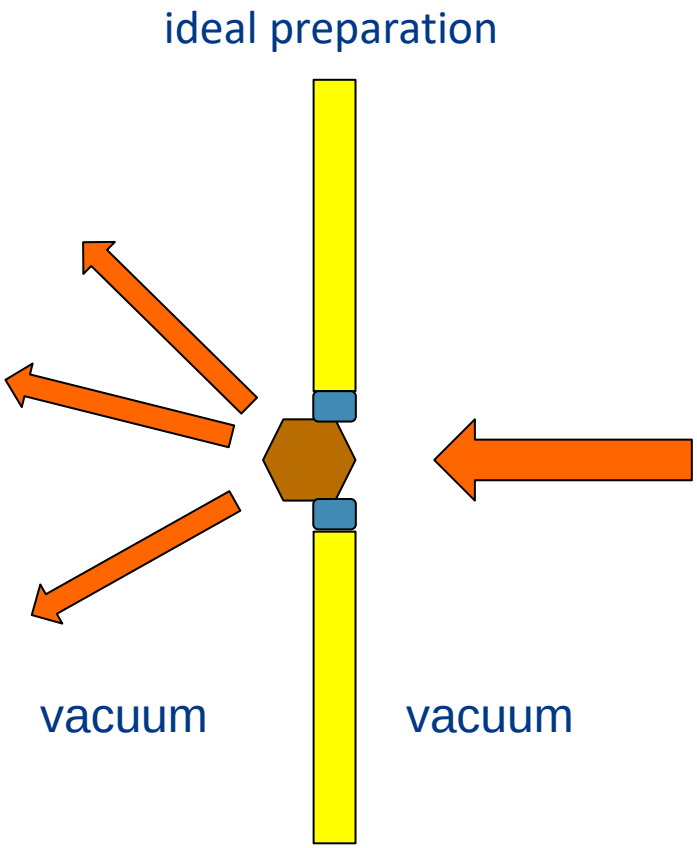
# Sample Preparation

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 cooling
- SEM for sample characterisation

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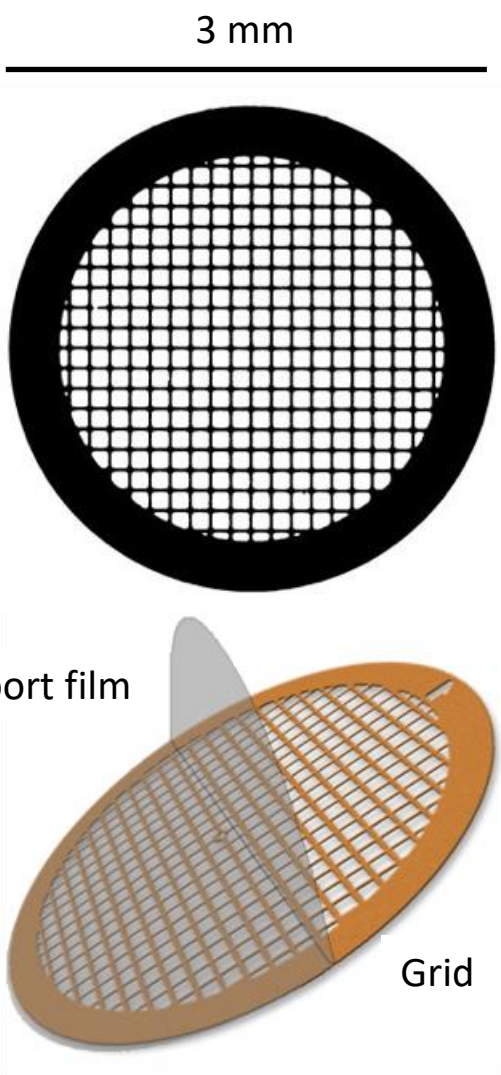
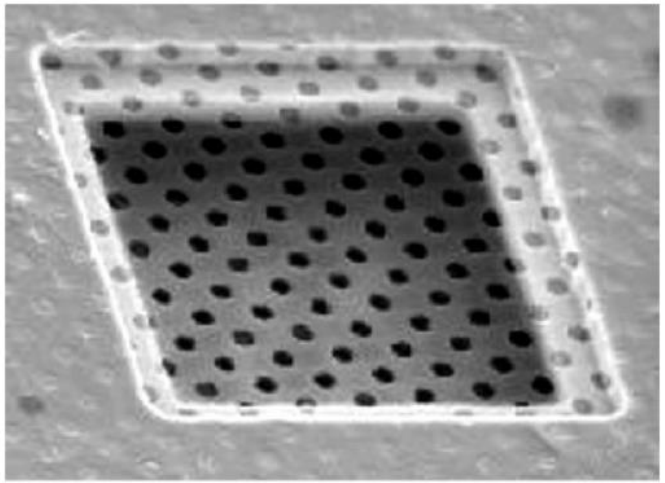
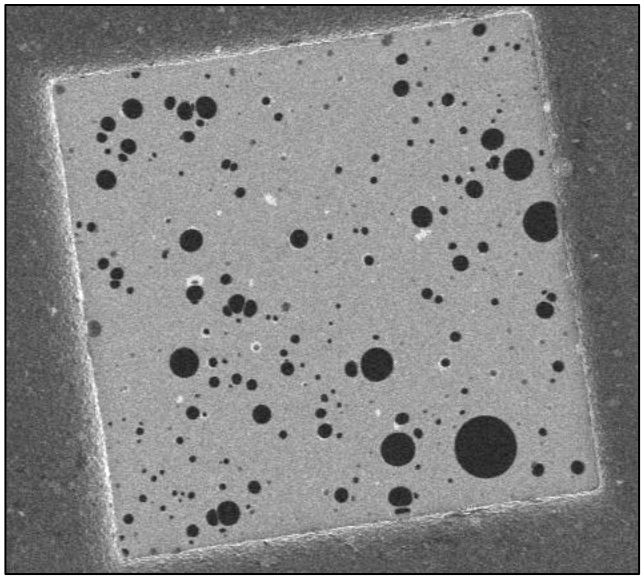
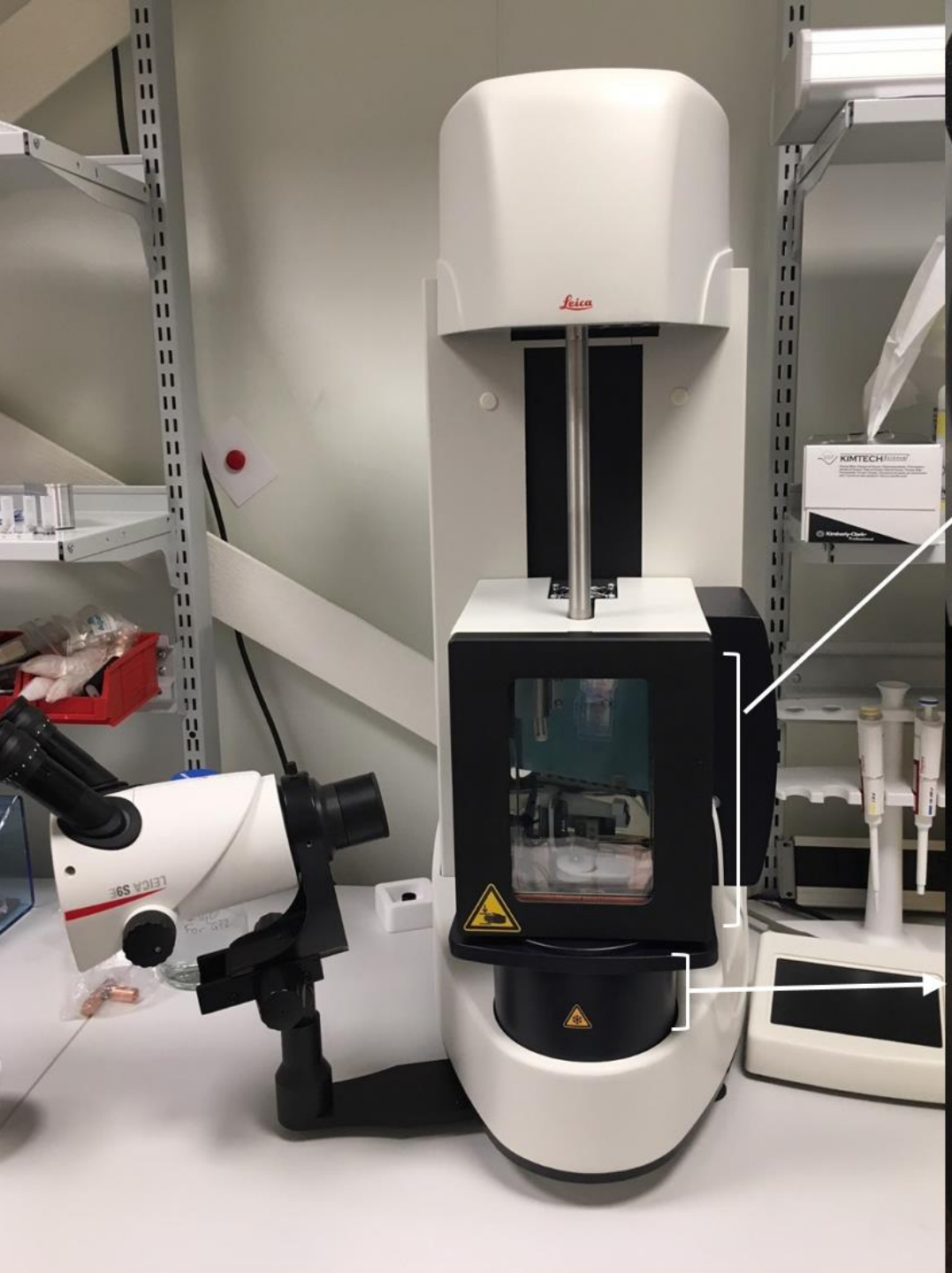
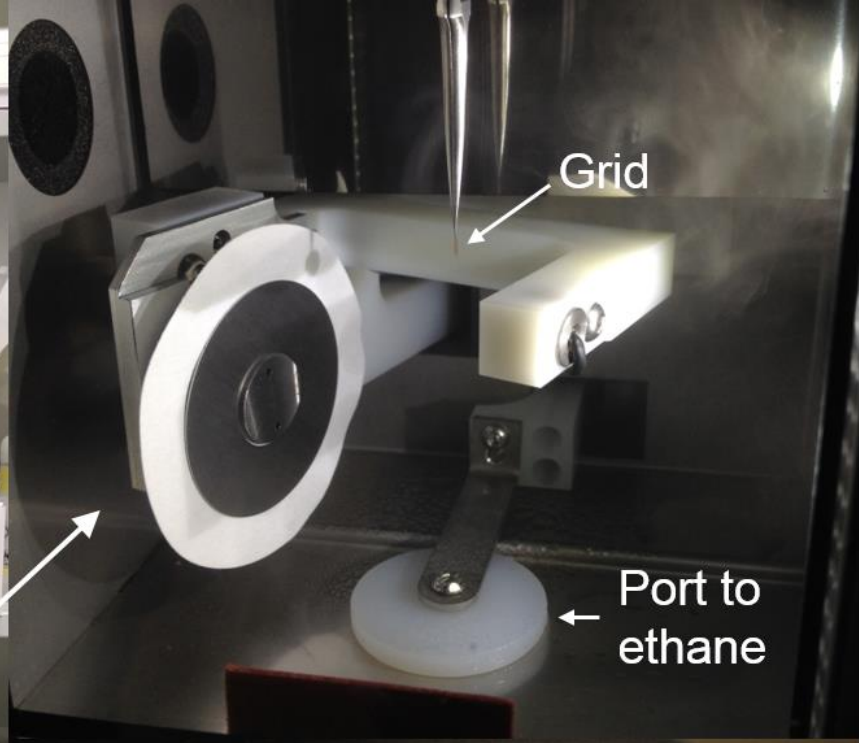
# Cryo-EM Grids

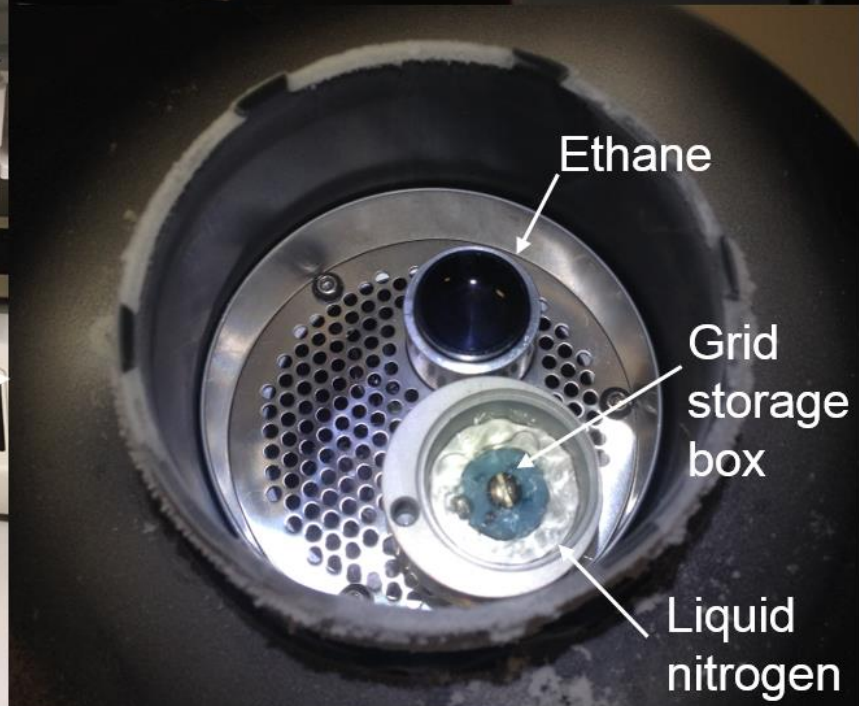


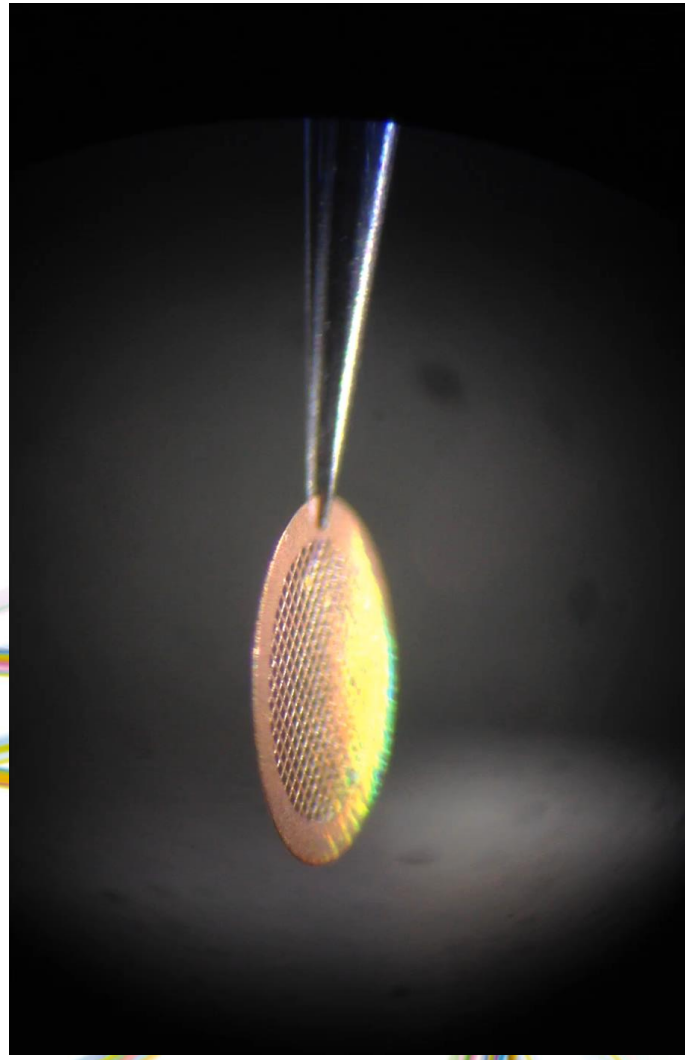


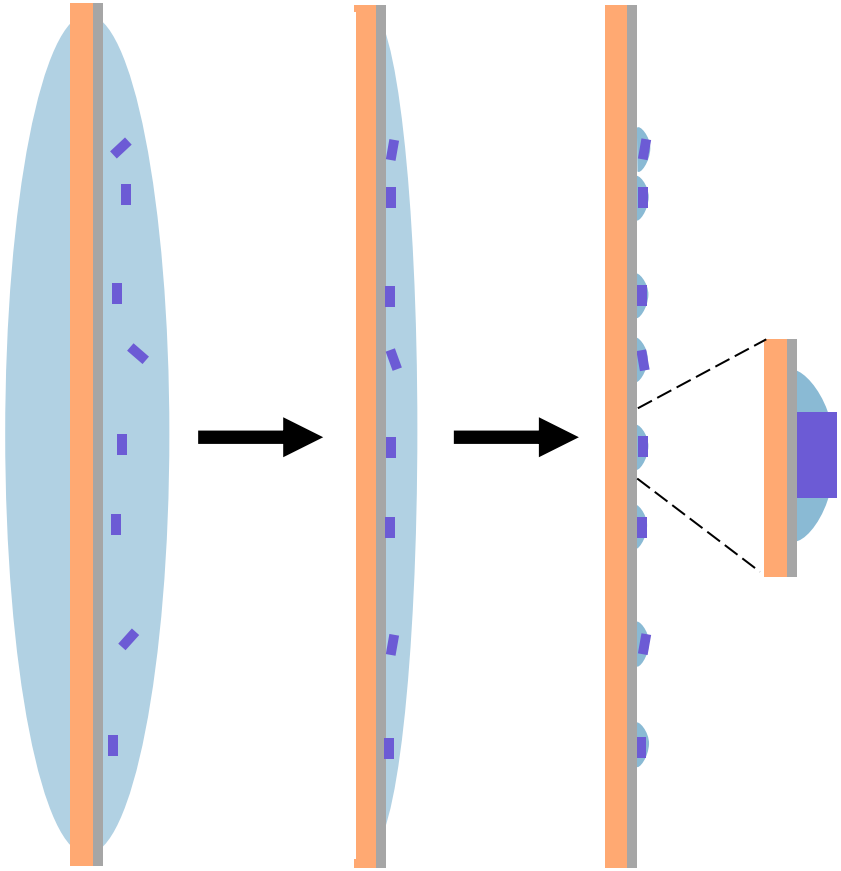

Leica
KIMTECH
Kimberly-Clark Professional
LEICA S9E


Grid
Port to ethane

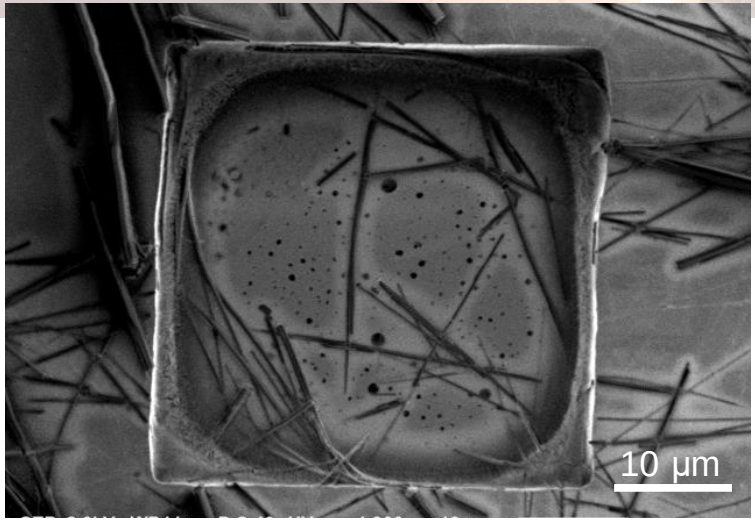

Ethane
Grid storage box
Liquid nitrogen

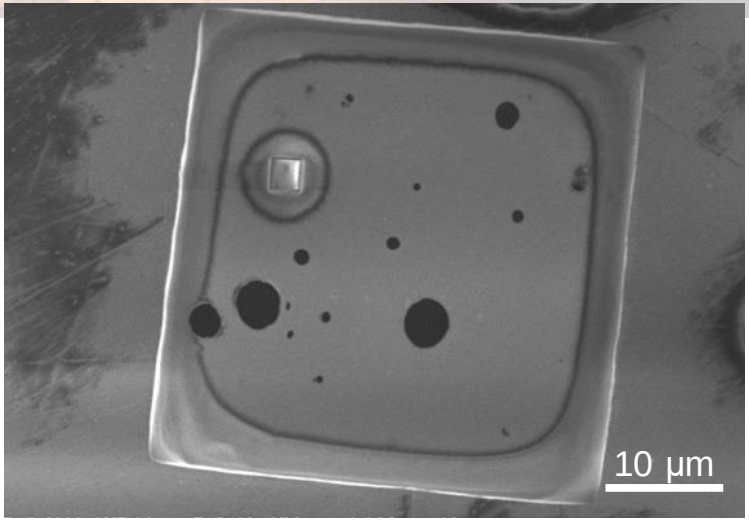
# Sample Blotting

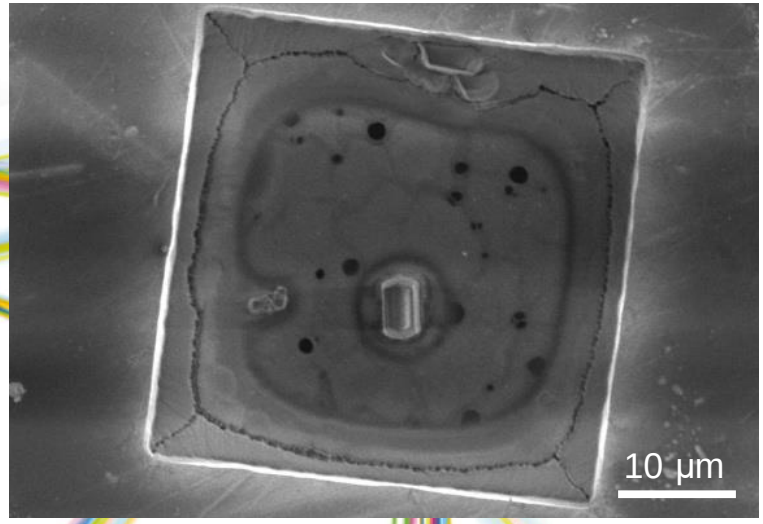


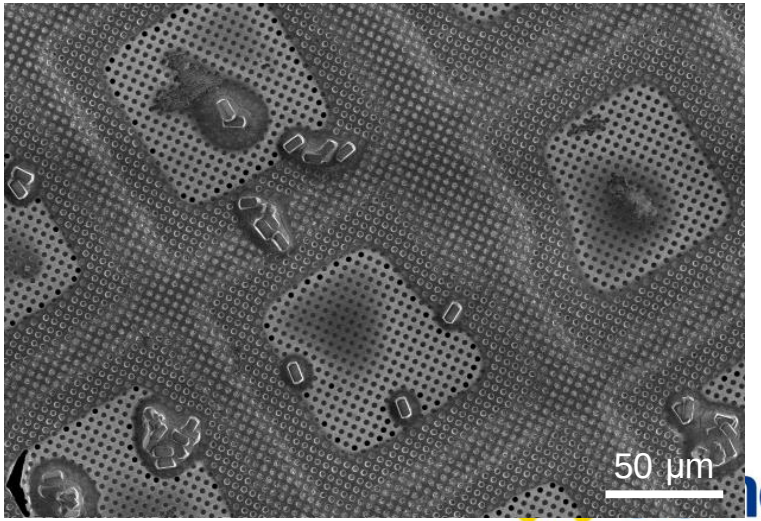


# Sample Characterisation

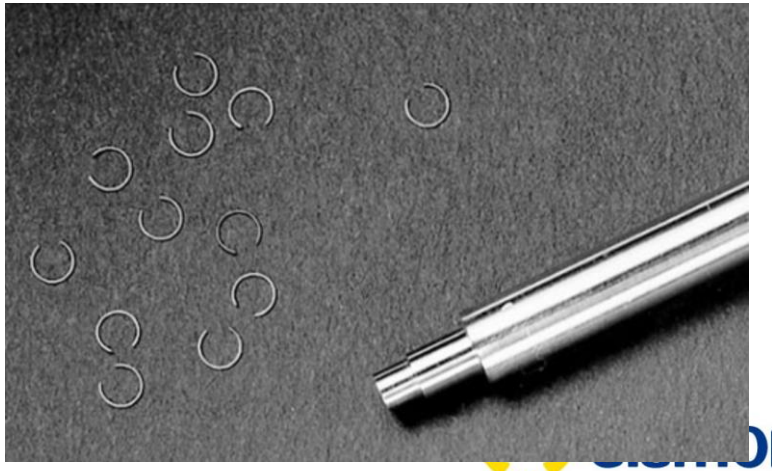


# Sample Mounting

# Sample Preparation Lab

# Beamline

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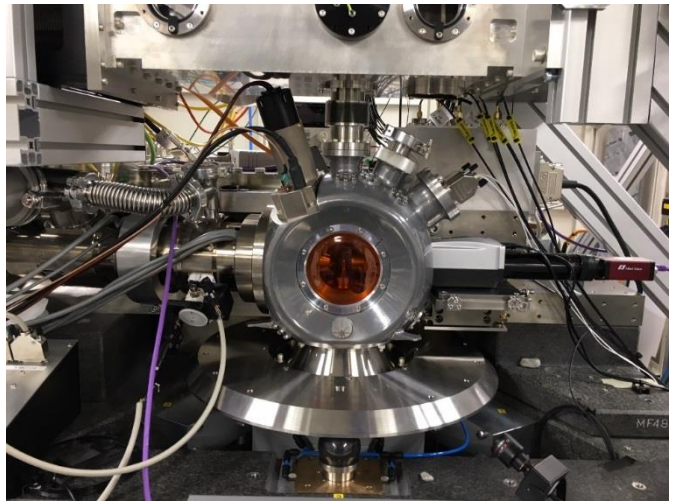
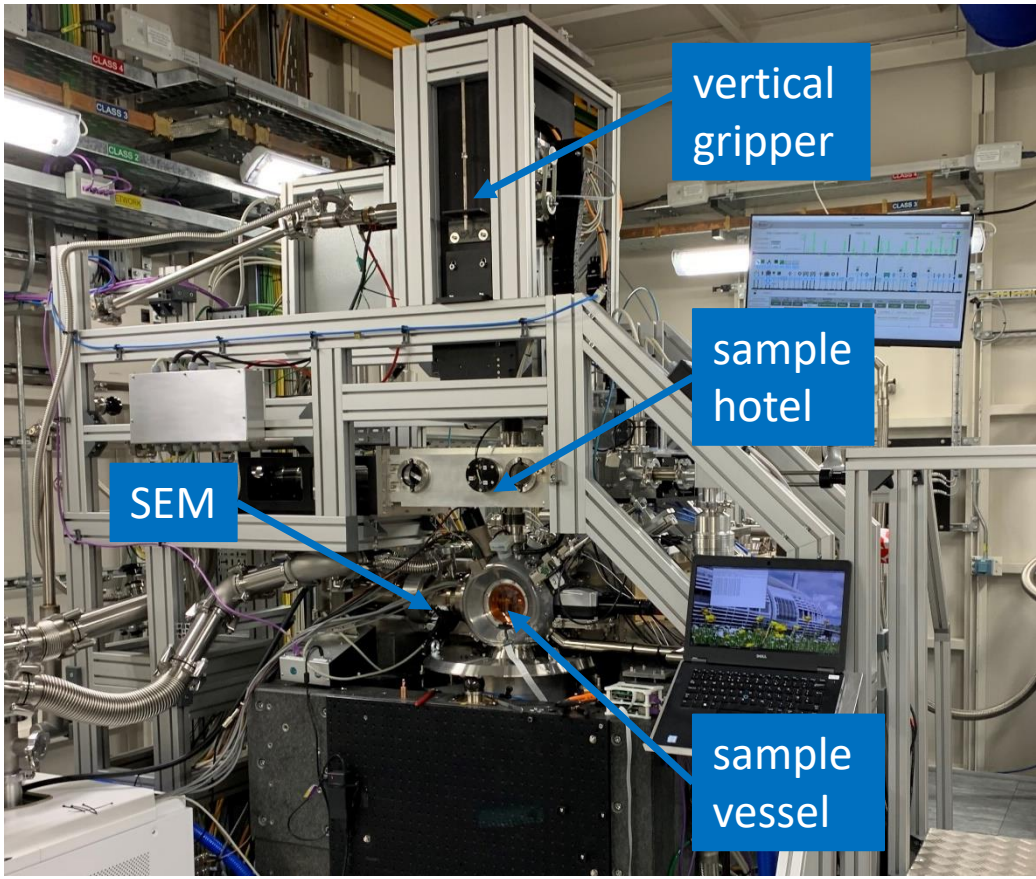
# Current Status

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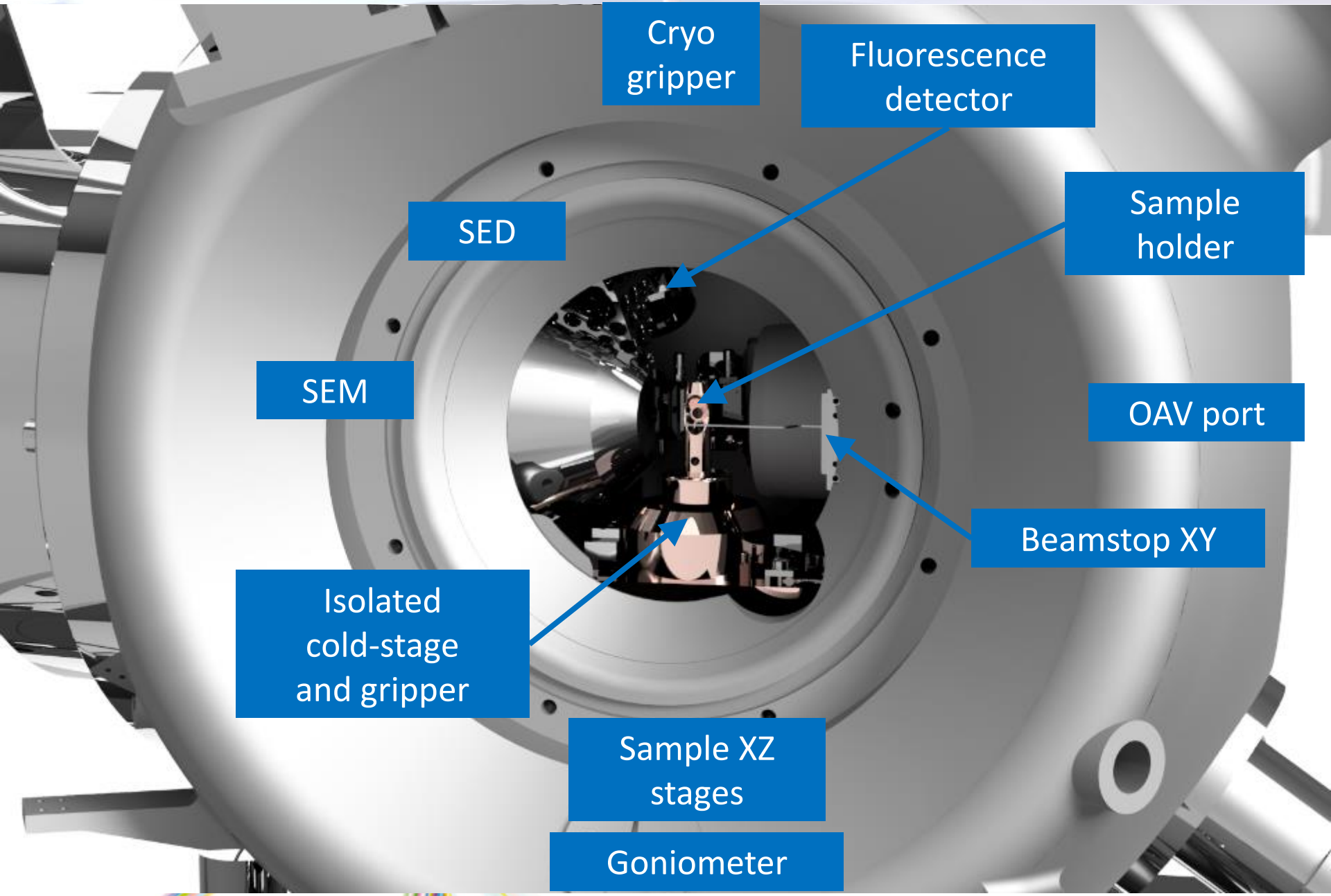
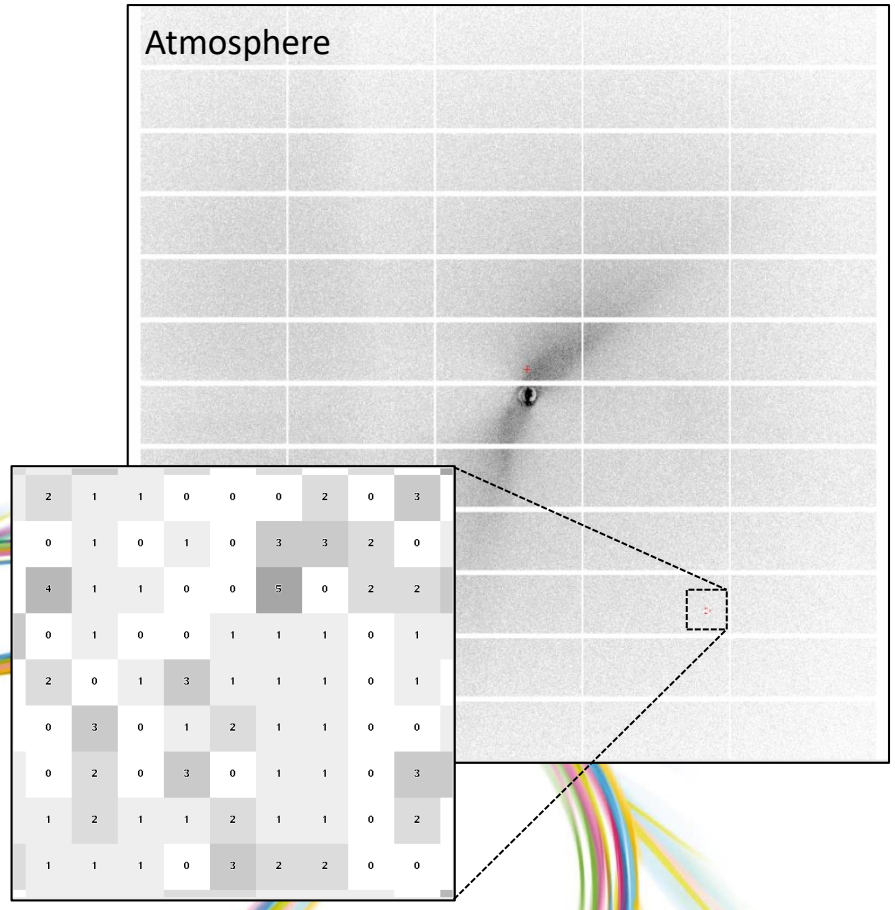
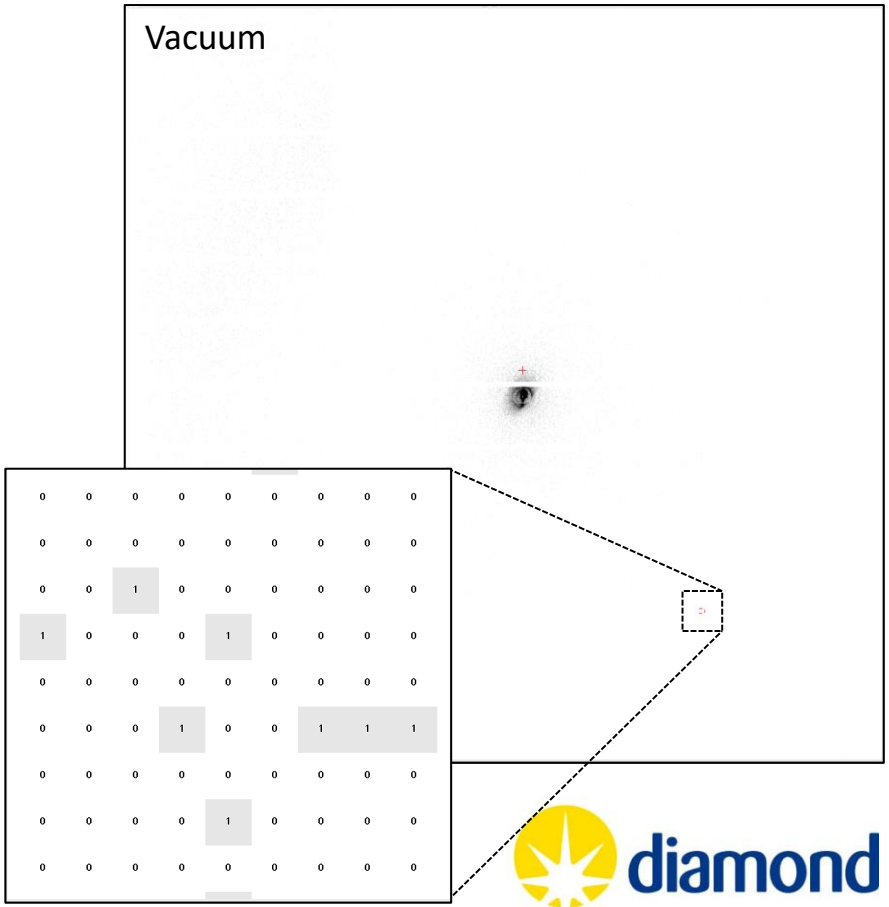

Cryo gripper
Fluorescence detector
SED
Sample holder
SEM
OAV port
Beamstop XY
Isolated cold-stage and gripper
Sample XZ stages
Goniometer

# Beamline Results

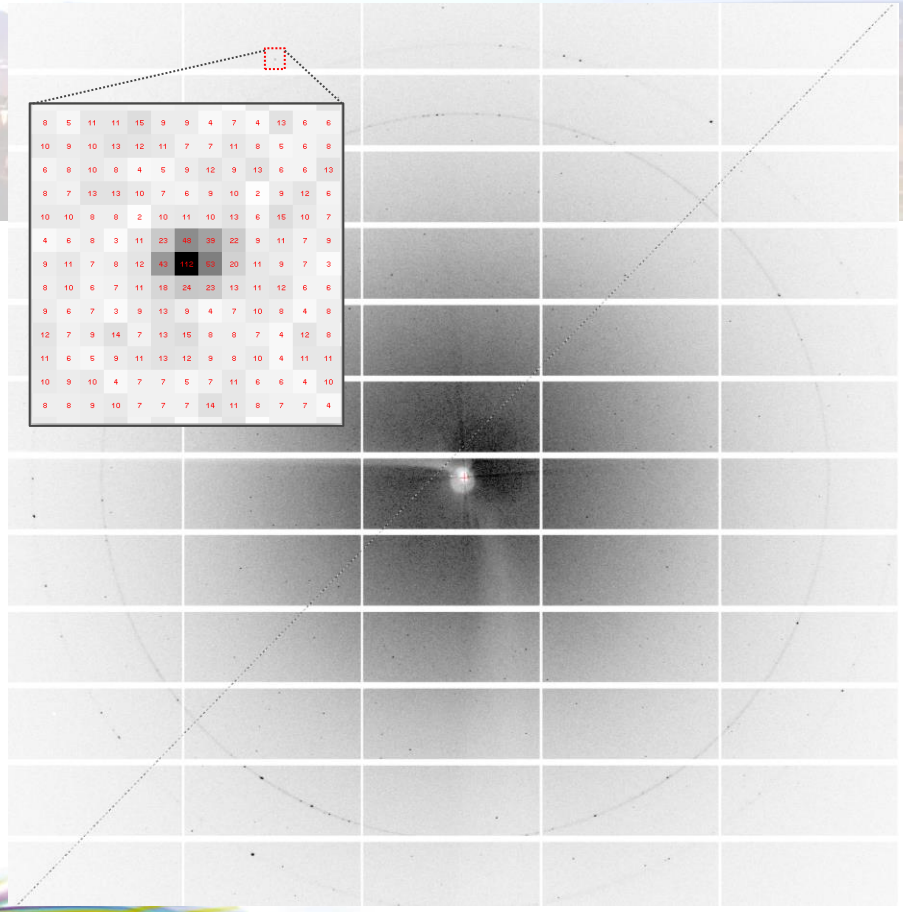
# Background Measurements

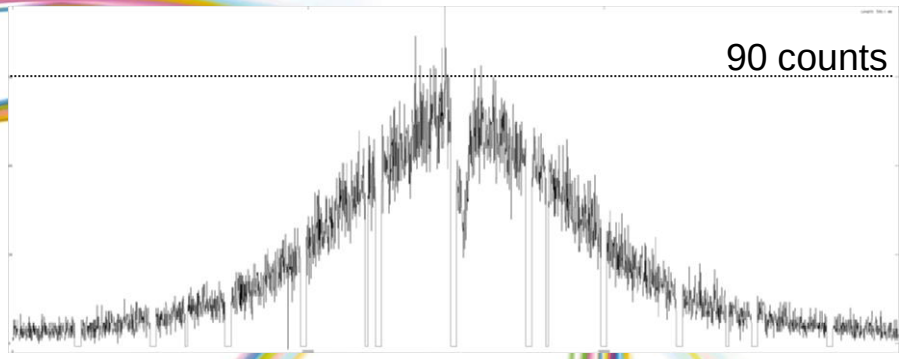
< 0.1 counts per pixel per second



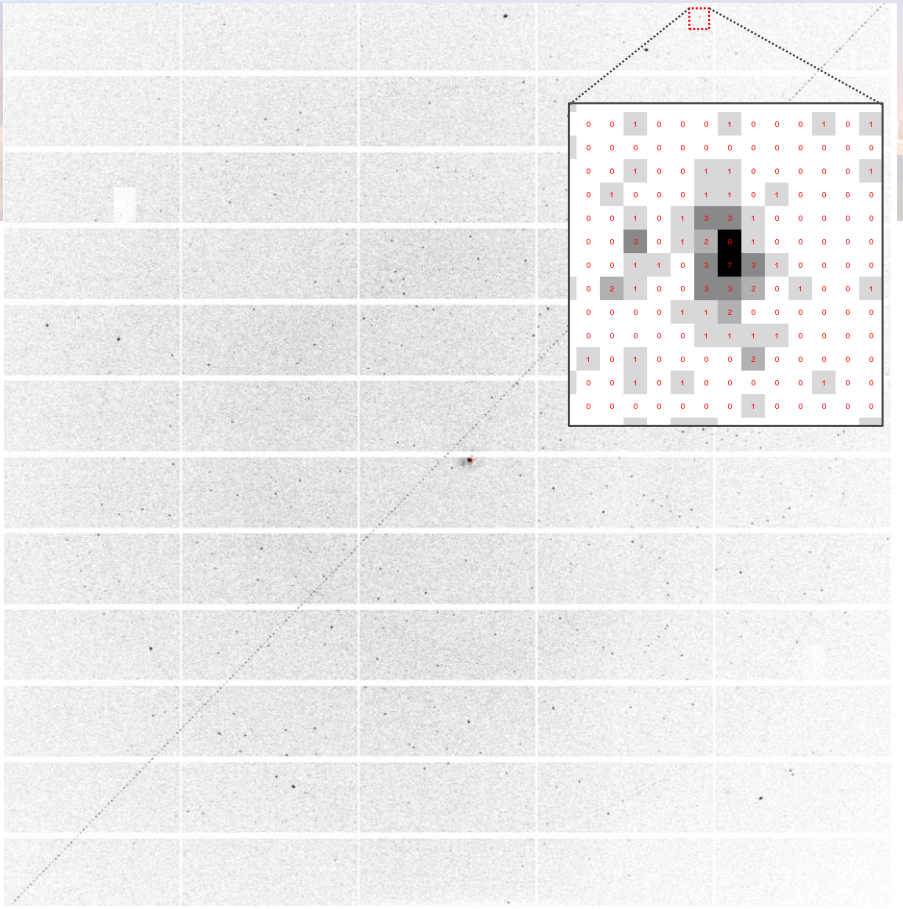



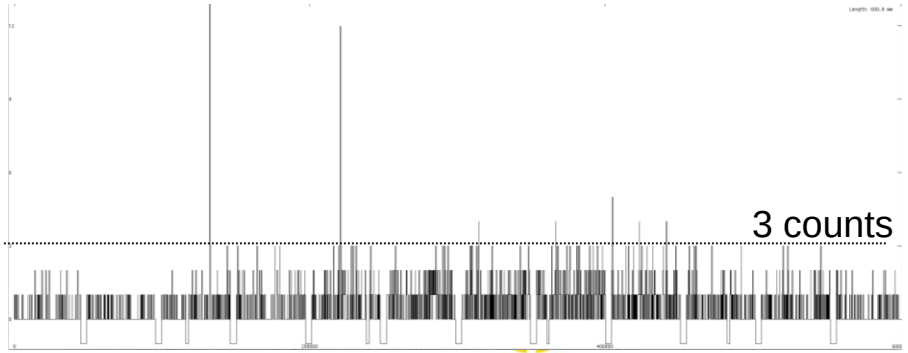
# I24





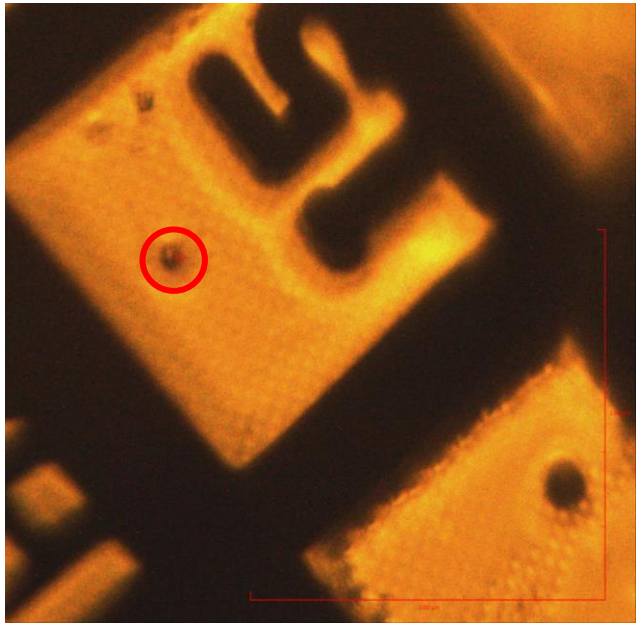

# VMXm





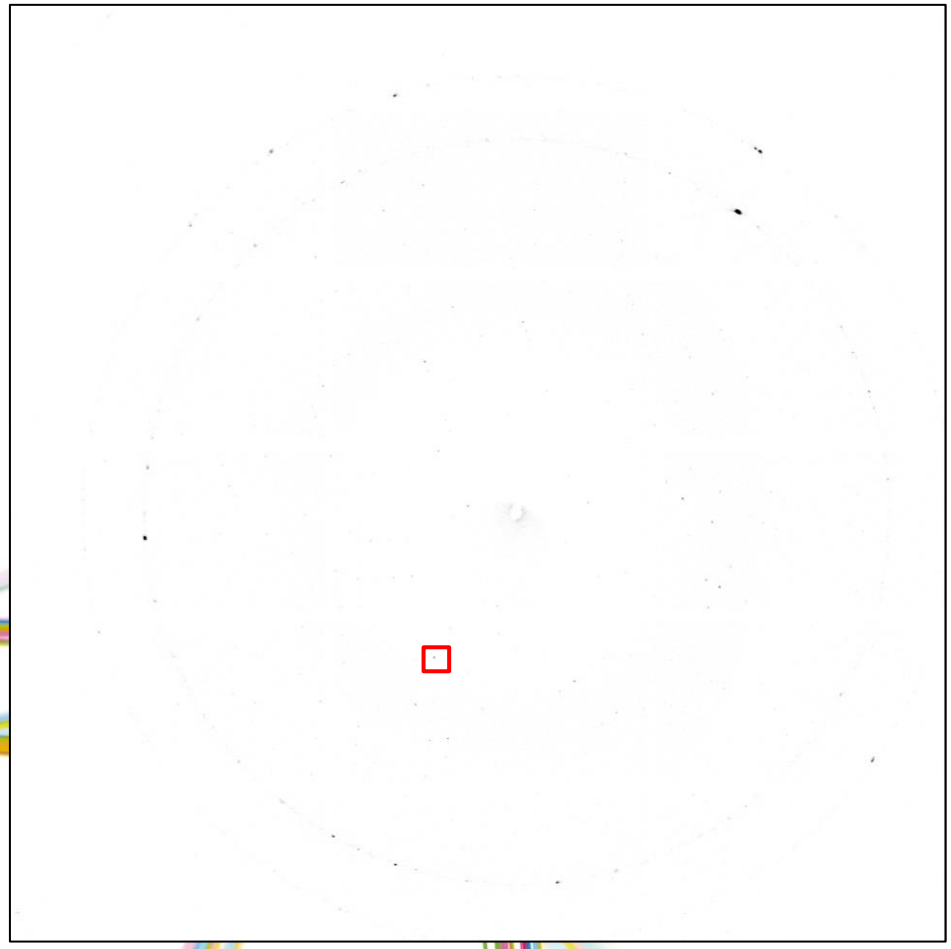
# CPV Ld14 – Cytoplasmic polyhedrosis virus

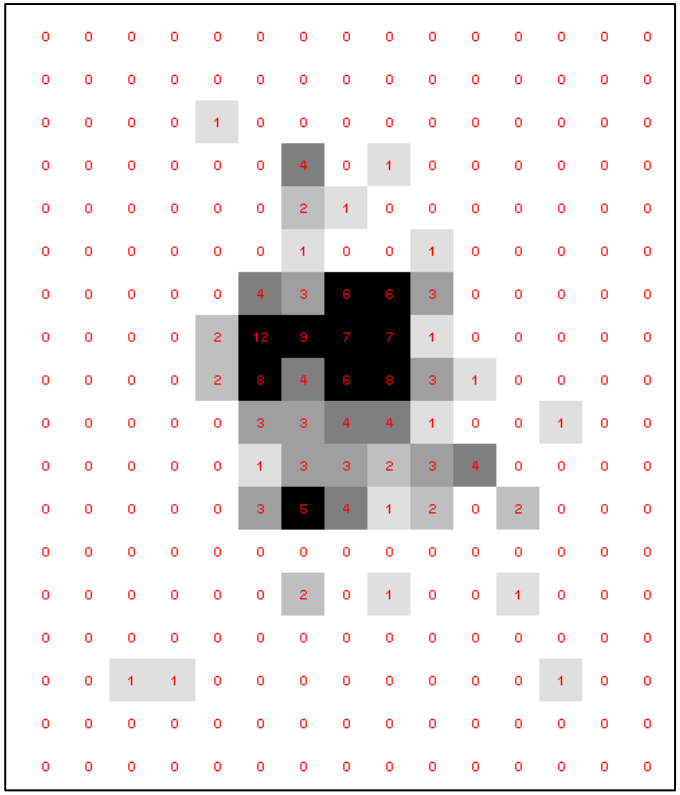
- Spacegroup I23
- Unit cell a=b=c=103 Å
- Approximate crystal size 2 – 4 µm
- Data collected at 21.3keV – exploit photoelectron escape
- Eiger2 X CdTe 9M



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

# CPV Ld14 – Cytoplasmic polyhedrosis virus



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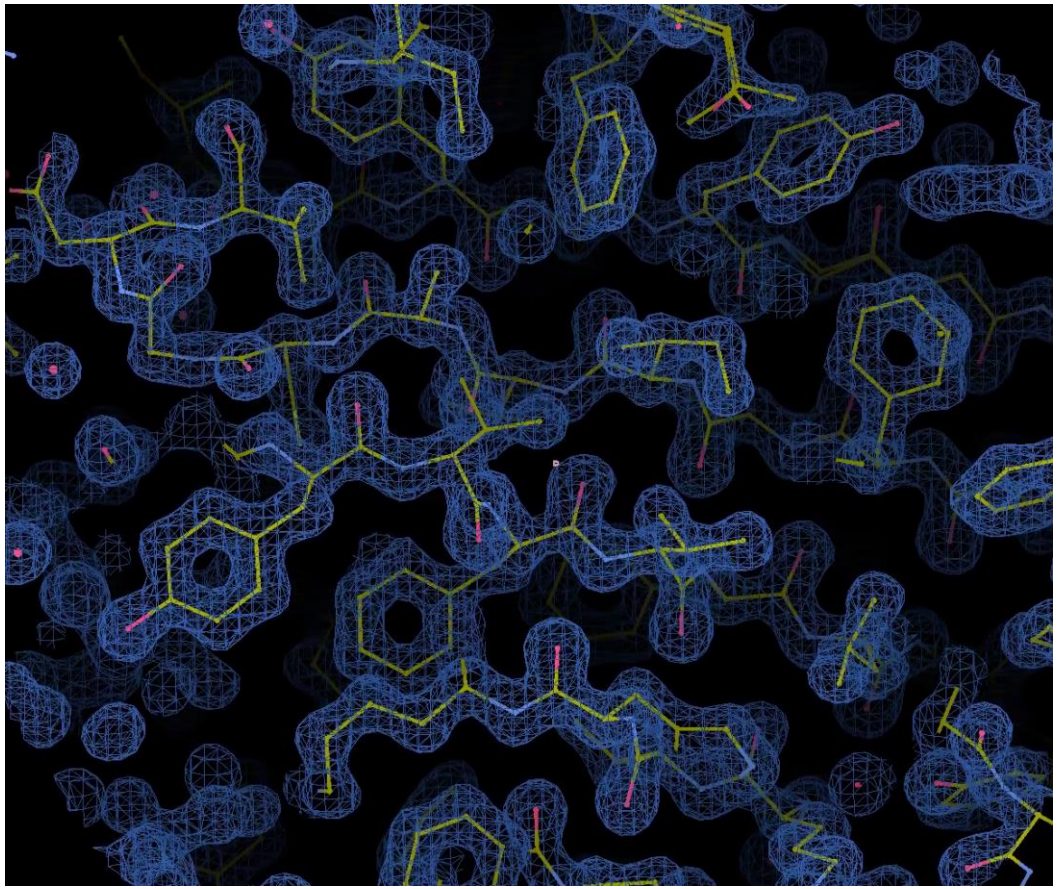
# CPV Ld14 – Cytoplasmic polyhedrosis virus

| | 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 Ld14 – Cytoplasmic polyhedrosis virus

| 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 |



# Membrane protein/LCP samples

Challenges of LCP:

- Viscous, hydrophobic - difficult to handle.
- Support films on cryoTEM grids are fragile.
- Generates significant background signal in X-ray diffraction experiments.

Changing the phase: Cubic → Sponge:

- Sponge has reduced viscosity - it is ‘pipettable’
- Maintains a hydrophobic environment.
- Smaller molecular weight precipitants can be used as ‘spongifying’ agents:
  - MPD, PEG200, PEG400

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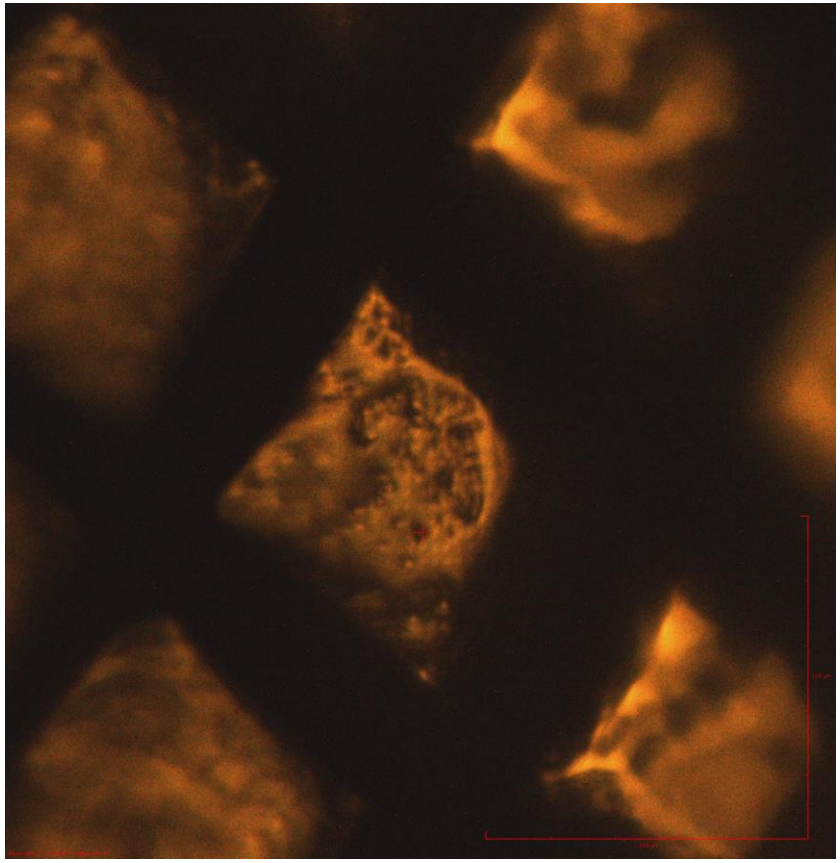
# FFAR1

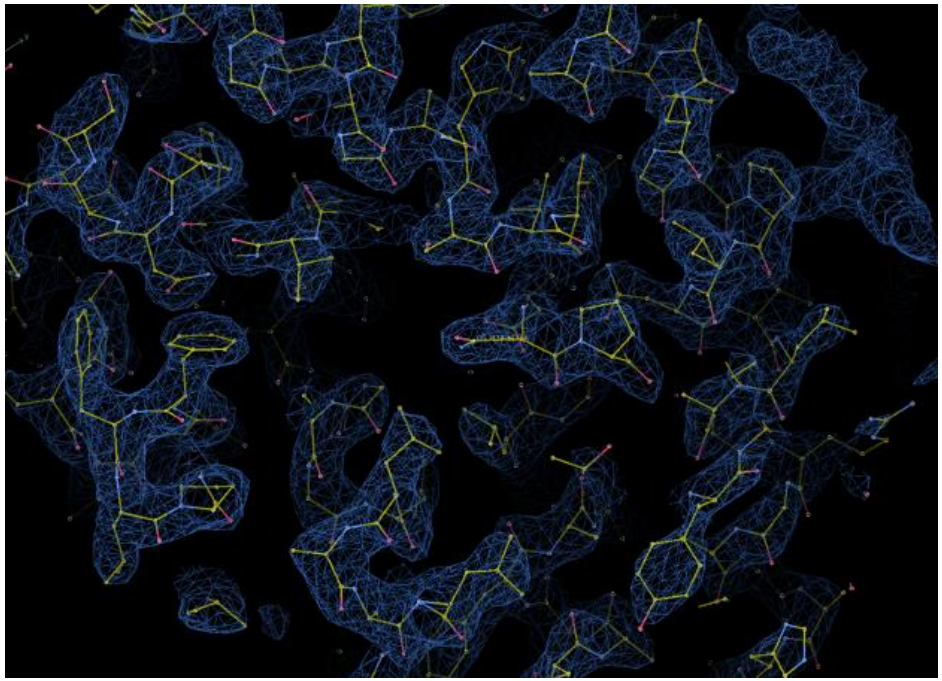
- GPCR membrane protein
- Grown in classic glass plate sandwich - generates crystals in variety of sizes
- Crystallisation solution including 10% MPD used to wash the bolus out from under the glass.
- Applied directly to grid mounted in plunge freezer as per soluble sample.



# FFAR1

- Data collected from 7 crystals at 21.3 or 19.5 KeV and merged.
- Completeness potentially affected by orientation and C2 spacegroup.

| | |
|---|---|
| Number of crystals | 7 |
| Energy (keV) | 21.3 or 19.5 |
| Resolution (Å) | 100.98 – 2.66 (2.71-2.66) |
| Spacegroup | C2 |
| Unique Reflections | 14118(737) |
| Completeness (%) | 76.1(78.6) |
| $R_{merge}$ | 0.558(2.842) |
| I/σI | 3.3(0.5) |
| CC1/2 | 0.913(0.155) |



| | |
|---|---|
| Resolution (Å) | 100.98-2.66 |
| No. reflections all/free | 14152/680 |
| R-factor/R-free | 0.248/0.293 |
| RMS Deviations | |
| Bonds (Å) | 0.0054 |
| Angles (°) | 1.460 |

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# Final Remarks

- VMXm aims to 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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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

**STRUBI, Oxford**

David Stuart
Geoff Sutton

**Membrane Protein Lab**

Andrew Quigley
Harish Cheruvara
James Birch


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# 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