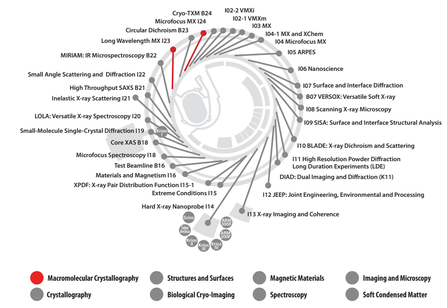


Macromolecular Crystallography

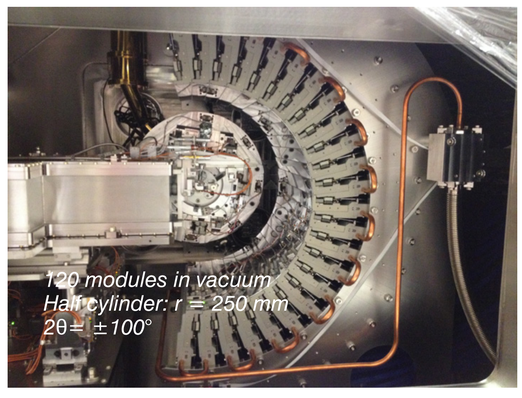
Diamond is a world leader in this field through the innovative and creative leadership of staff and its large user community. Together they move the goalposts of what is feasible for ‘conventional’ MX as well as developing techniques and beamlines that transform the offer for structural biology to the next level, enabling new experiments and methodologies which often impact the field on a global level.

I23: the unique Long Wavelength MX beamline

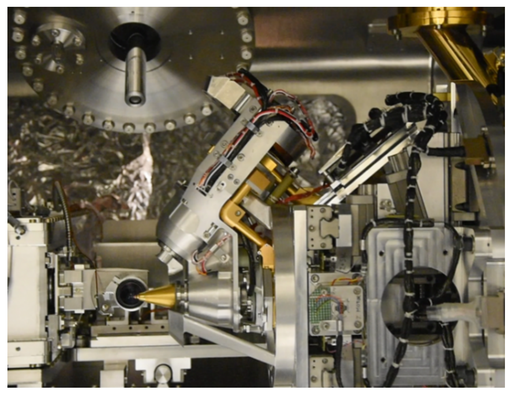
The long-wavelength macromolecular crystallography beamline I23 operating at wavelengths between 1.5 to 4 Å is a unique facility for solving the crystallographic phase problem, using the small anomalous signals from sulphur or phosphorus which are present in native protein or RNA/DNA crystals. This is of increased importance for projects where protein labelling to introduce anomalous scatters is not feasible. In addition, the beamline’s wavelength range provides access to the M-edges of elements, with huge anomalous signals offering new opportunities for phasing large molecular complexes.

The unique wavelength range also allows identification and location of lighter atoms of biological relevance such as Cl, K and Ca and assistance with low-resolution model building by locating P or S atom positions. In 2017, I23 entered an exciting phase in its development, with a concerted push to facilitate user access alongside ground-breaking experiments with invited user groups. General user access to this technical tour de force was initiated in 2017 by its first call for users via the peer review proposal system for experiments in 2018 with great interest shown by the user community. Ongoing developments will continue on I23 to explore and exploit its potential to the full – we are entering a whole new world of what is possible with in vacuum MX and the samples and technology that enable this.

End station



Pilatus 12M Detector



Multi-axis goniometer

The high absorption of long-wavelength X-rays by air imposes the necessity to conduct the diffraction experiments in vacuum. The entire sample environment, as well as the sample transfer system, is kept at vacuum levels of < 10-8 mbar. A 12 million pixels detector allows measurements of a large range of diffraction angles and a multi-axis goniometer is available for crystal alignment and orientation. A Strategy Design Program adapted to the geometry of the 12M Pilatus detector and to the kappa goniometer’s collision and shadowing maps for optimal data collection strategies is being developed by Global phasing.

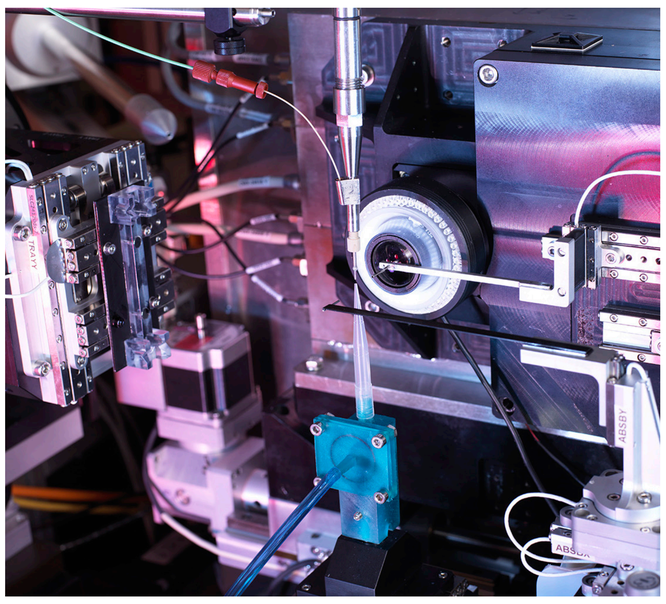
I24: Microfocus MX, a beamline well suited for Serial Synchrotron Crystallography

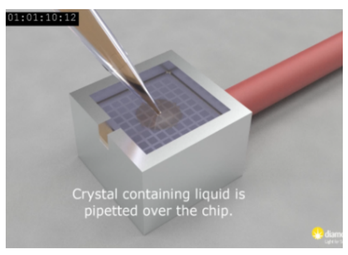
I24 is a tuneable microfocus beamline for MX. This beamline, operating since 2008, was completely rebuilt in 2015/ 2016 with a new dual goniometer end-station and new mirrors. I24 offers extremely high flux densities with the ability to investigate virus structure, membrane proteins, and microcrystals (crystals as small as 1.5 microns have been measured successfully). It combines versatile optics and the most sophisticated detectors available with the most advanced automation systems to enable sample location, data collection and analysis.

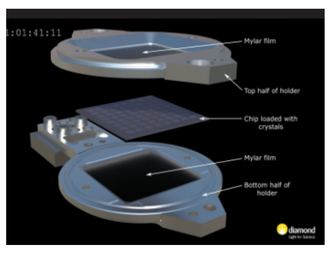
Modes availables:

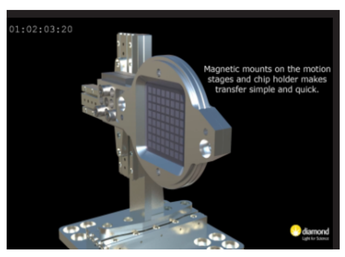
- Two standard modes of data collection cryo (SPINE pins) and *in situ*.
- Serial* (fixed target, thin film and LCP extruder) data collection.

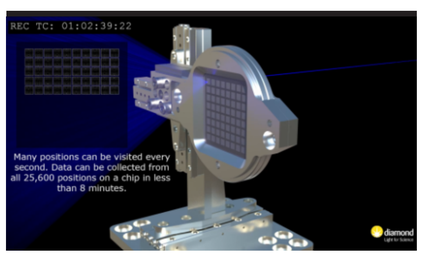
* Serial Synchrotron Crystallography (SSX) is an emerging method driven, in part, by the sample requirements imposed by X-ray Free Electron Lasers (XFELs). Serial techniques can also be exploited at synchrotrons, especially at microfocus beamlines, opening up many new opportunities. A number of approaches including fixed targets and extruders have been tried and tested at Diamond’s Microfocus MX beamline and are available for general users. Expertise at I24 is centred on Fixed Target Serial Synchrotron Crystallography (FT-SSX). FT-SSX is attractive as it potentially offers high hit-rates coupled with modest sample consumption. Furthermore, the same approach can be used almost without modification at both synchrotrons and XFELs.

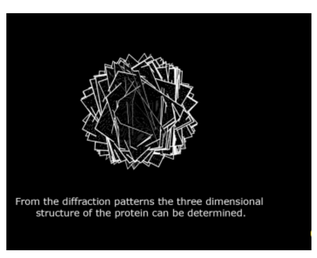


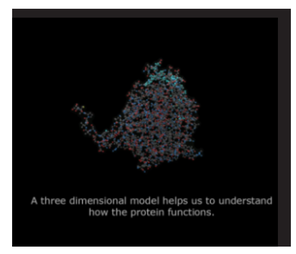




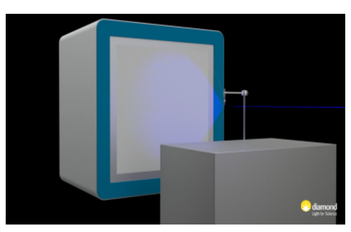


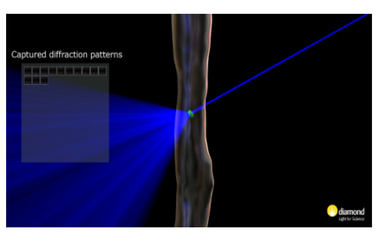


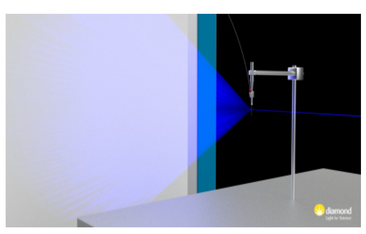


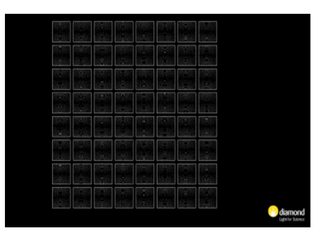


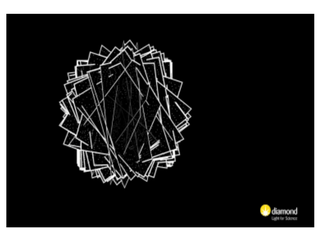
Fixed target SSX: In Fixed Target Serial Synchrotron Crystallography (FT-SSX), crystals are loaded onto a chip and protected by mylar layers (avoiding dehydration). They are then mounted in a holder which is moved quickly - 25,600 chip apertures in a few minutes - and with sub-micron accuracy through the X-ray beam. Data collected from many thousands of crystals are then merged to obtain a complete dataset from the protein under study and allow 3D structure determination.

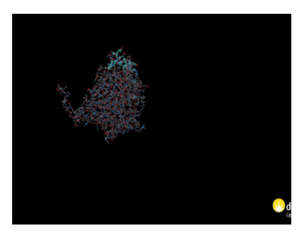












Lipidic Cubic Phase serial synchrotron crystallography (LCP-SSX), another serial approach, consists of crystallisation in a liquid crystalline phase called Lipidic Cubic Phase. The principle is to use an extruder with a continuously flow of crystals suspended in a high viscosity carrier through the X-ray beam. The 3D structure is determined by merging single frames from several thousand microcrystals injected through into the X-ray beam.

Some advantages of these challenging methods:

- Reduction of the time of exposure (potential radiation damage) for same quality of the data;
- High hit-rates coupled with modest sample consumption (thousands of crystals can be screened in a short time and with less than a milligram of protein);
- Providing a more native-like membrane environment for proteins for LCP;
- Well suited for time-resolved diffraction studies on the microsecond to millisecond timescale;
- Data collection possible at room temperature;
- Eliminates the need for difficult handling of individual crystals.