MX @ Diamond

Katherine McAuley
Diamond Light Source

Overview

Synchrotrons

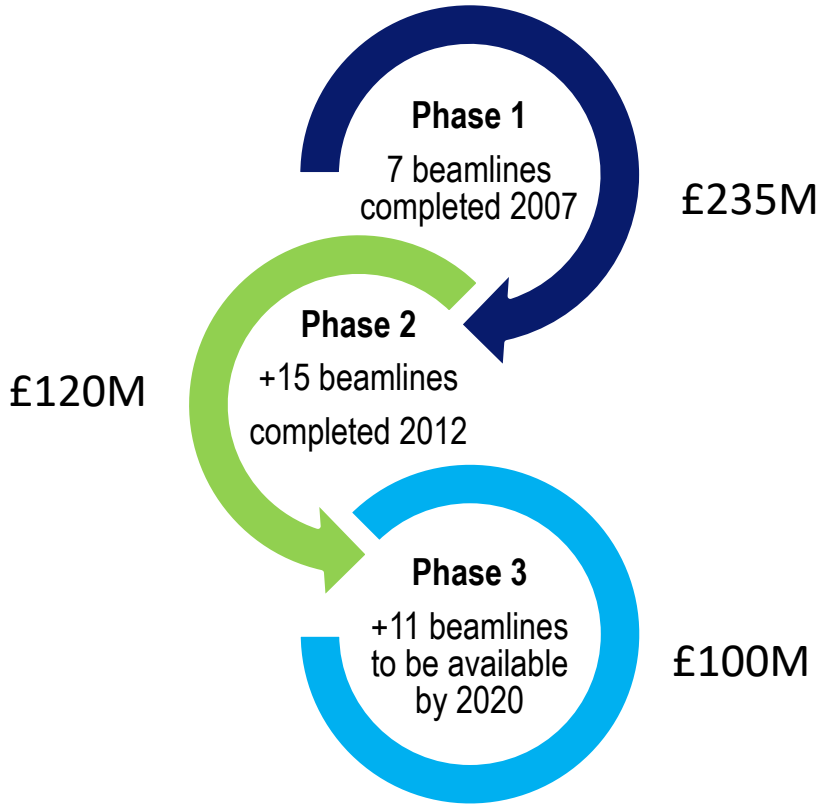
- Diamond facility
- How a synchrotron works
- Integrated structural biology

MX beamlines

- Components
- Techniques
- Details of Diamond MX beamlines

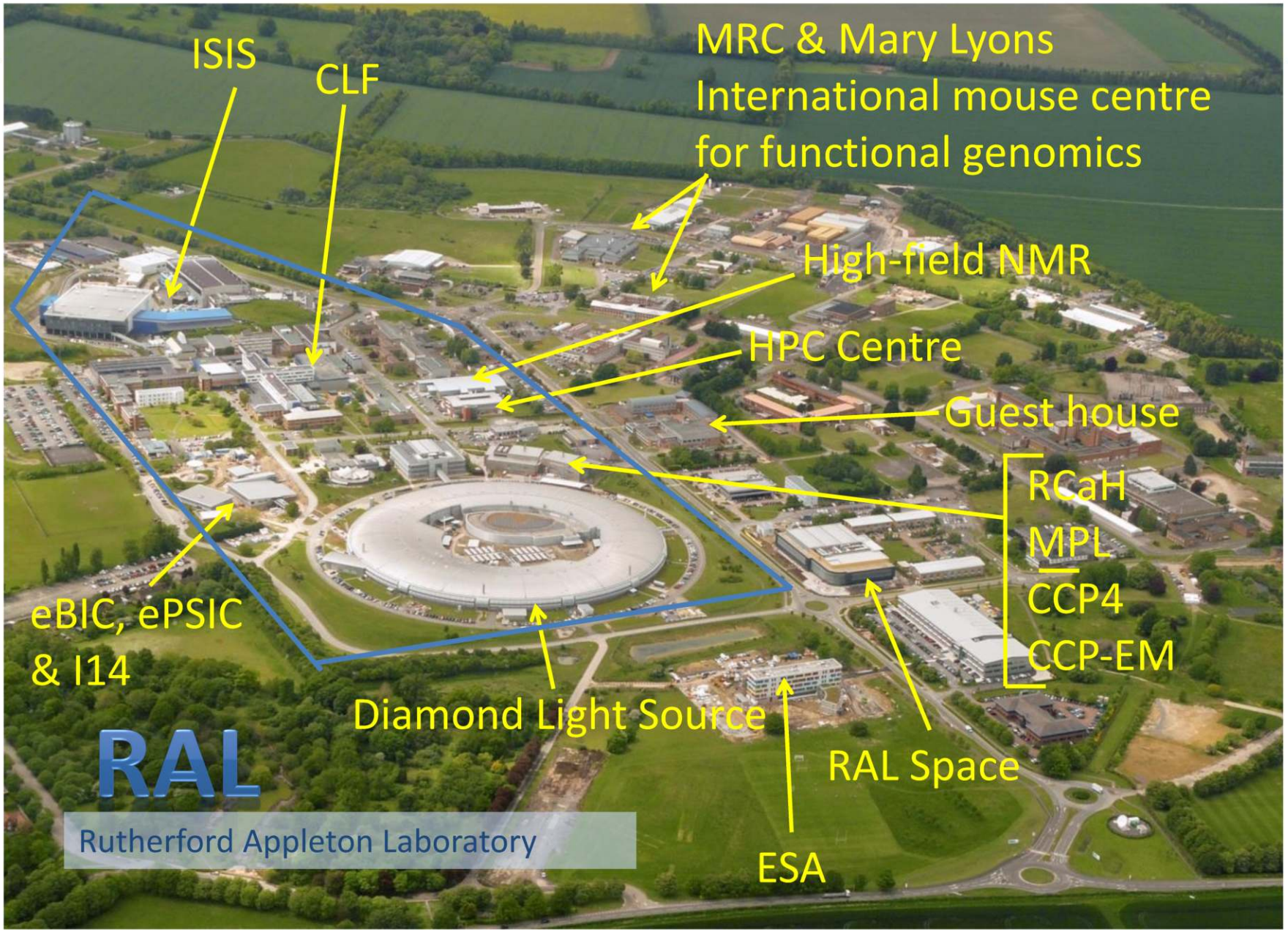
The Diamond Project

- Diamond is a private company formed as a joint venture between STFC (86%) and The Wellcome Trust (14%)
- All beamlines are owned and operated by Diamond



All areas of science covered except military and tobacco research

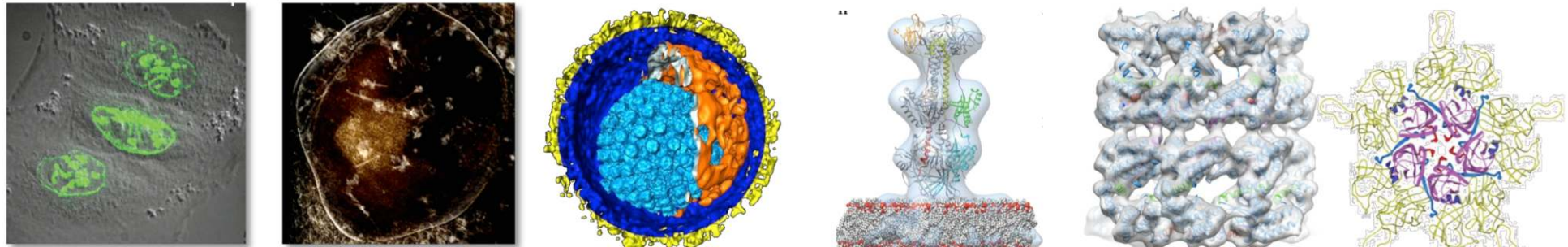
Harwell Campus



- The UK's national synchrotron facility, open from 2007 to researchers in universities and industry, and co-owned and funded by the UK government through STFC and the Wellcome Trust (86:14 ratio).
- A total of 12,000 registered users, and more than 100 companies paying for proprietary use.
- Currently in third phase of construction, due to finish in 2018 with 32 operational beamlines, plus 1 more in 2020 as well as a central facility for electron microscopy for the UK.
- First scientific users from 2007
- 2017 = 15 years of Diamond Project & 10 years of operations!!

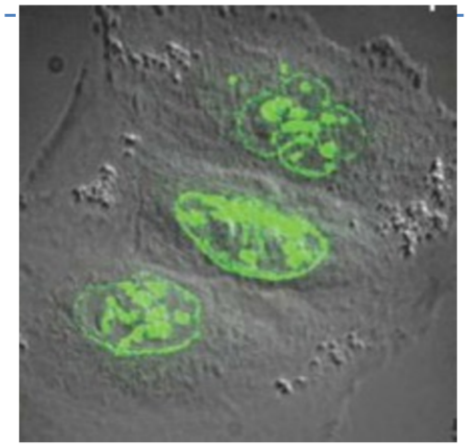
Imaging Single Cells to Molecules; an integrated approach at Harwell

- Provision of state of the art technologies for structural and cellular biology
- Challenge now is to understand how proteins function dynamically within
  - Multi-complex macromolecular assemblies
  - Cellular pathways
  - ...ultimately at the organism level
- **Requires a multifaceted approach across different resolutions scales:**
  - X-ray diffraction, SAXS, X-ray microscopy, X-ray spectroscopy, X-ray fluorescence, CD, IR imaging etc..
  - Harwell campus infrastructure – a unique resource: RC@H, PP-UK, CCP4, MPL@DLS, Central Laser Facility, ISIS...

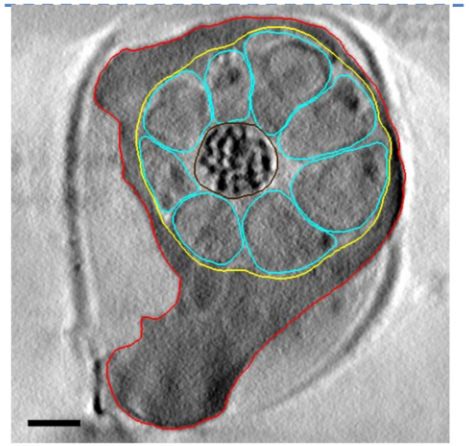


Integrated Structural biology

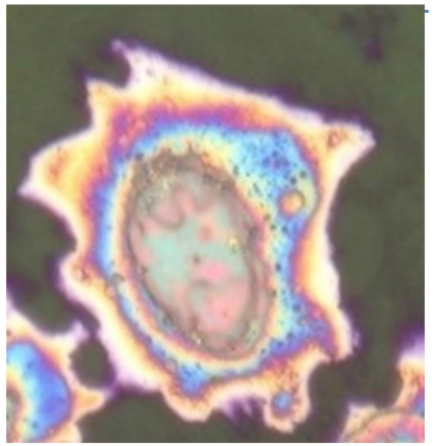
Increasing biological complexity and integrity



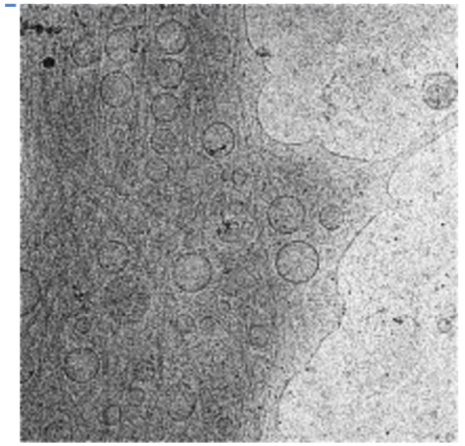
Fluorescence microscopy (B24/CLF)



B24 X-ray microscopy

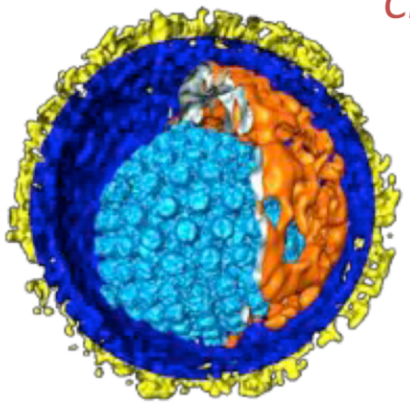


B22 - Infrared microspectroscopy

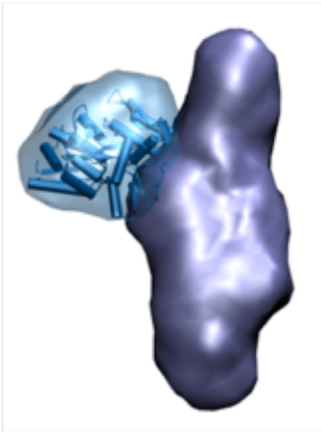


Cellular cryo-electron tomography

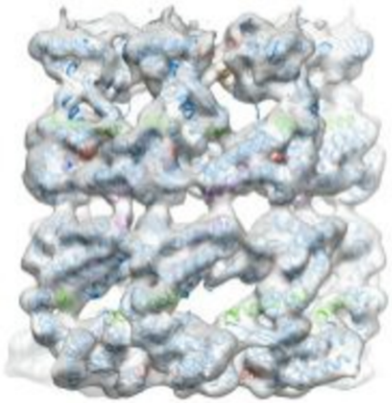
Cryo-CLXM
Cryo-CLEM



Cryo-electron tomography



B21 X-ray Solution Scattering

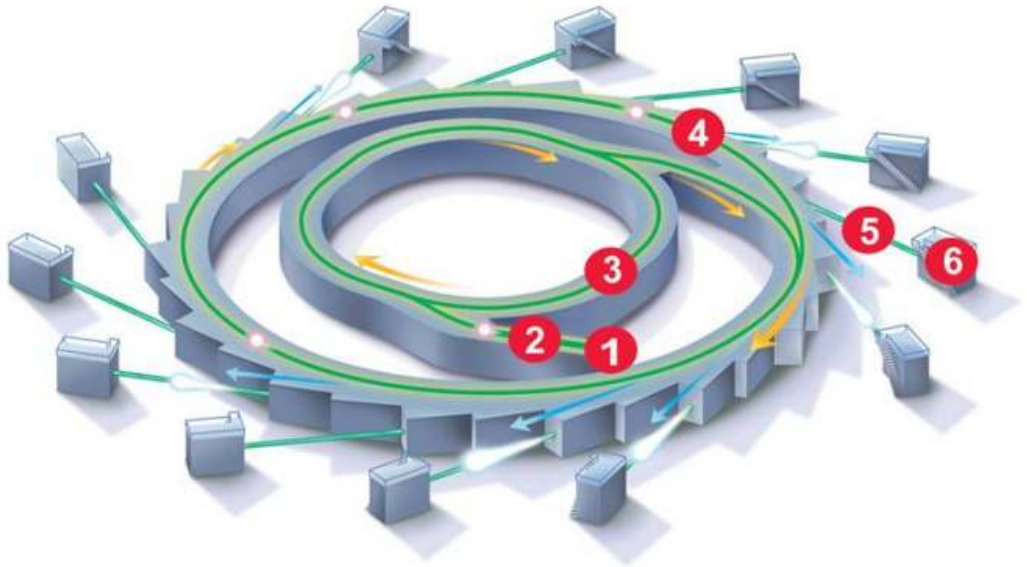


Single particle cryo EM

X-ray crystallography

Increasing resolution

Synchrotron - main components



1 Electron Gun: produces electrons

2 Linear Accelerator (LinAc): accelerates electrons to near the speed of light

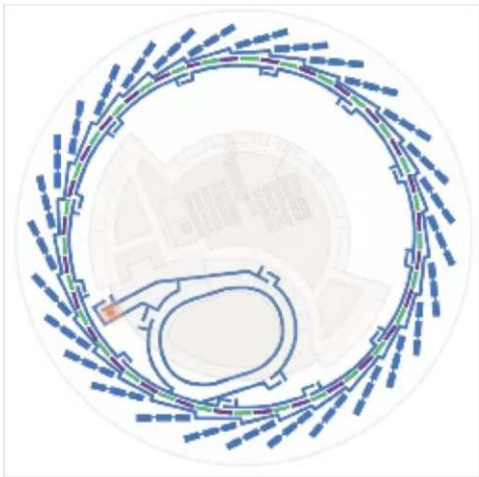
3 Booster Ring: further increases the energy of the electrons

4 Storage Ring: maintains the electron energy and produces radiation

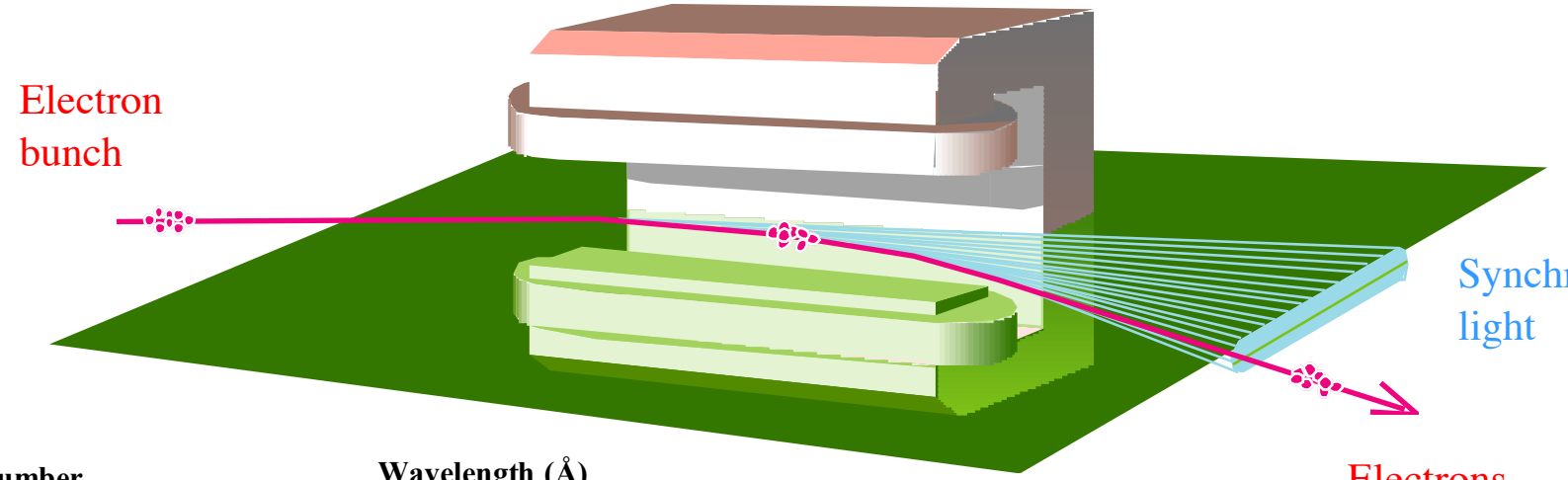
5 Beamline: set up of radiation for experiments

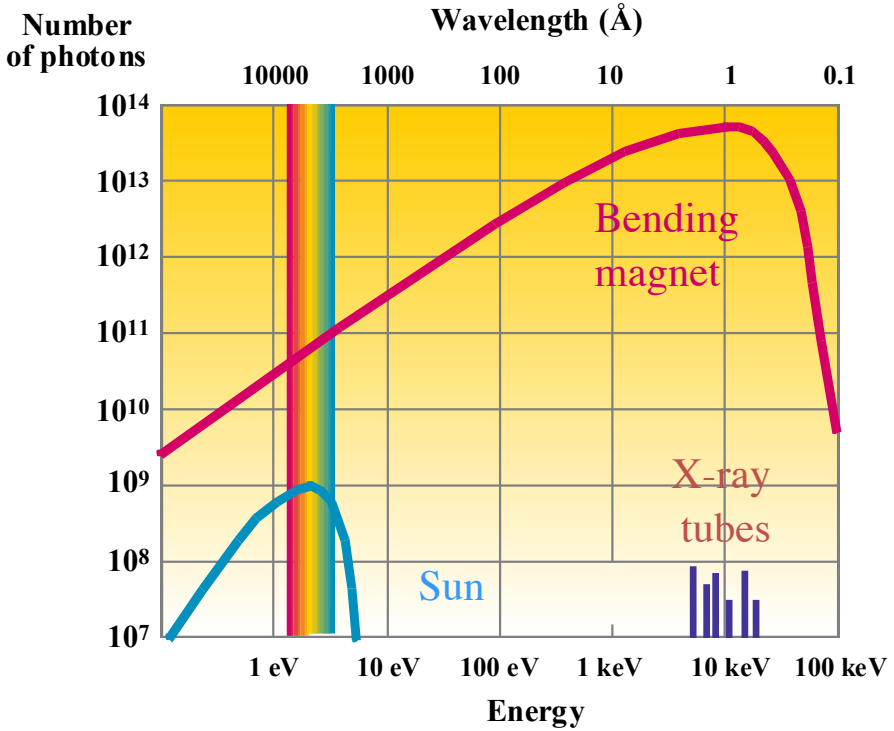
6 Experimental Hutch: User area

Views from inside the storage ring



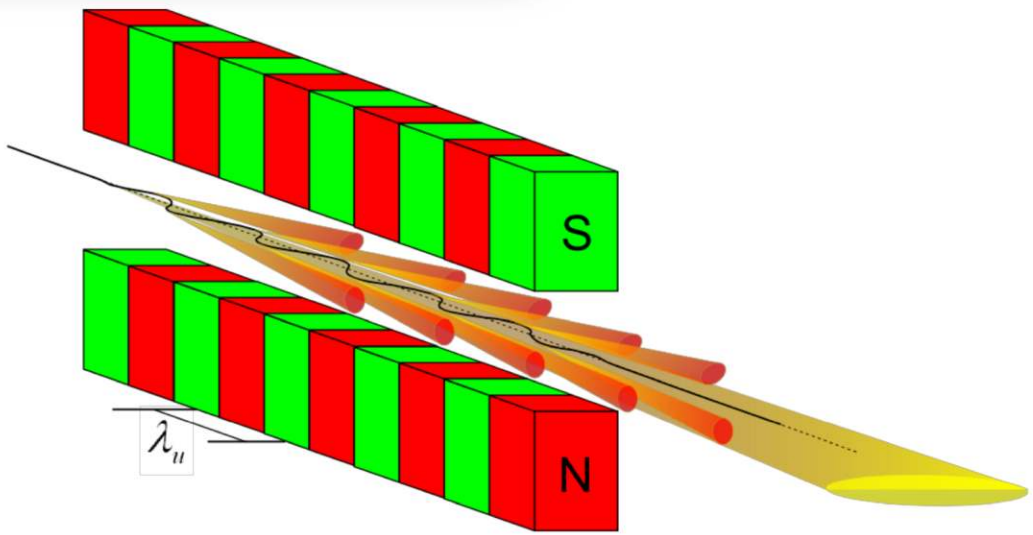
Bending Magnets curve the electron beam between adjacent straight sections and generate synchrotron light

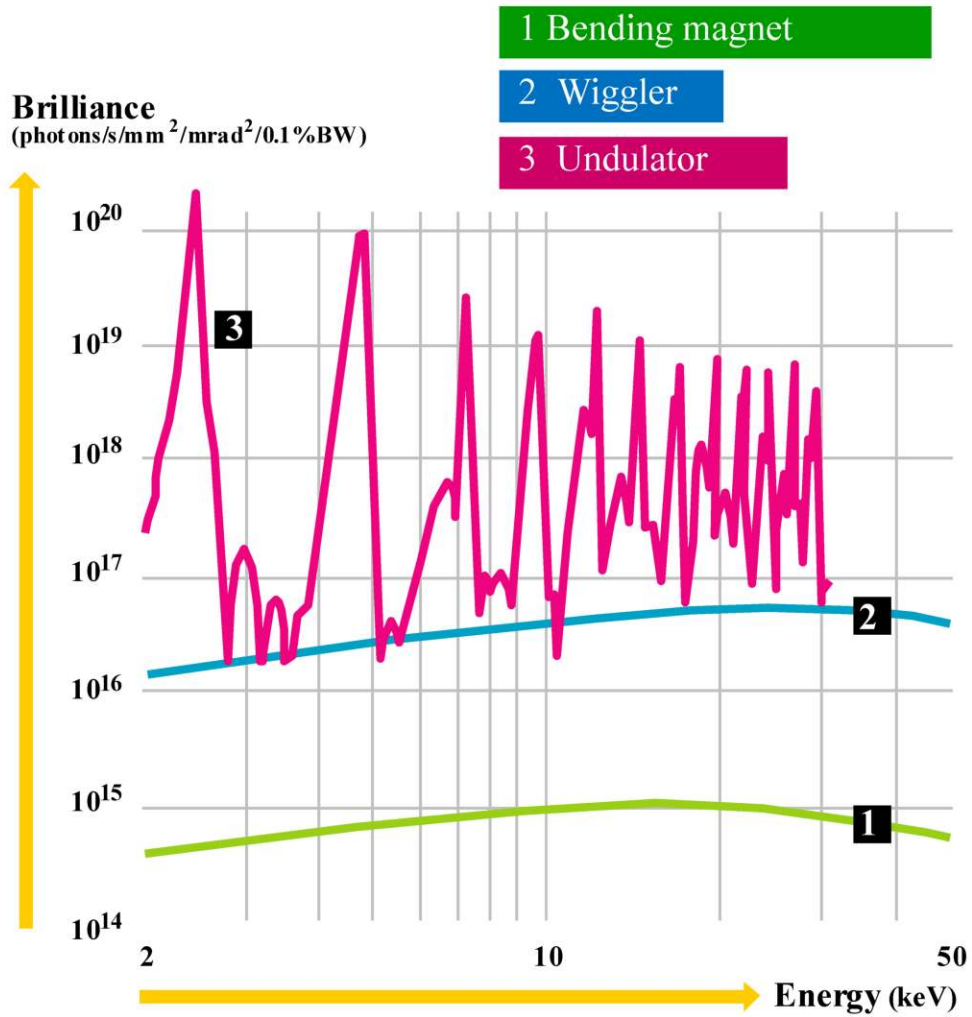




Emission spectrum

The MX beamlines at Diamond use insertion devices called undulators

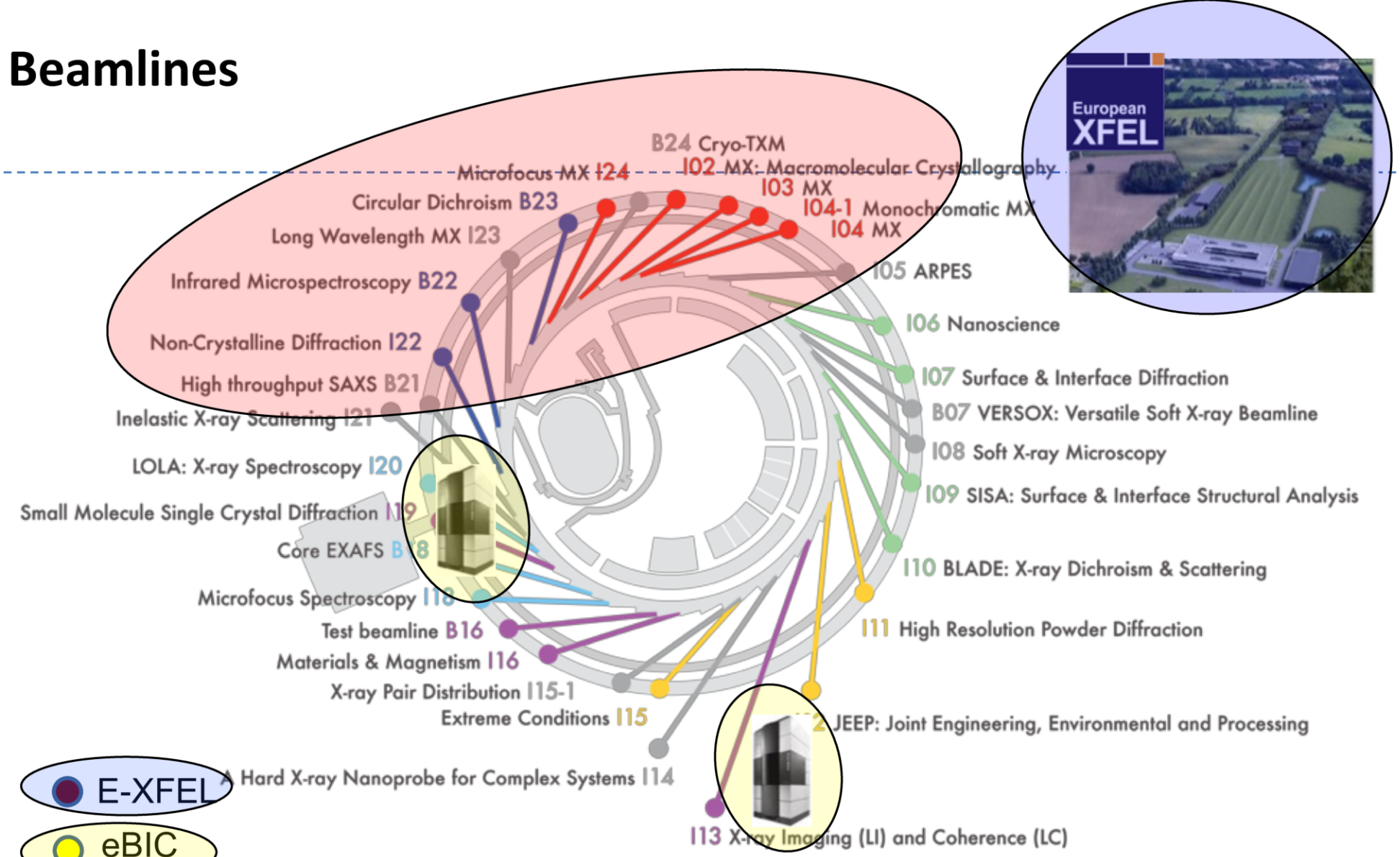




Introduction to MX beamlines

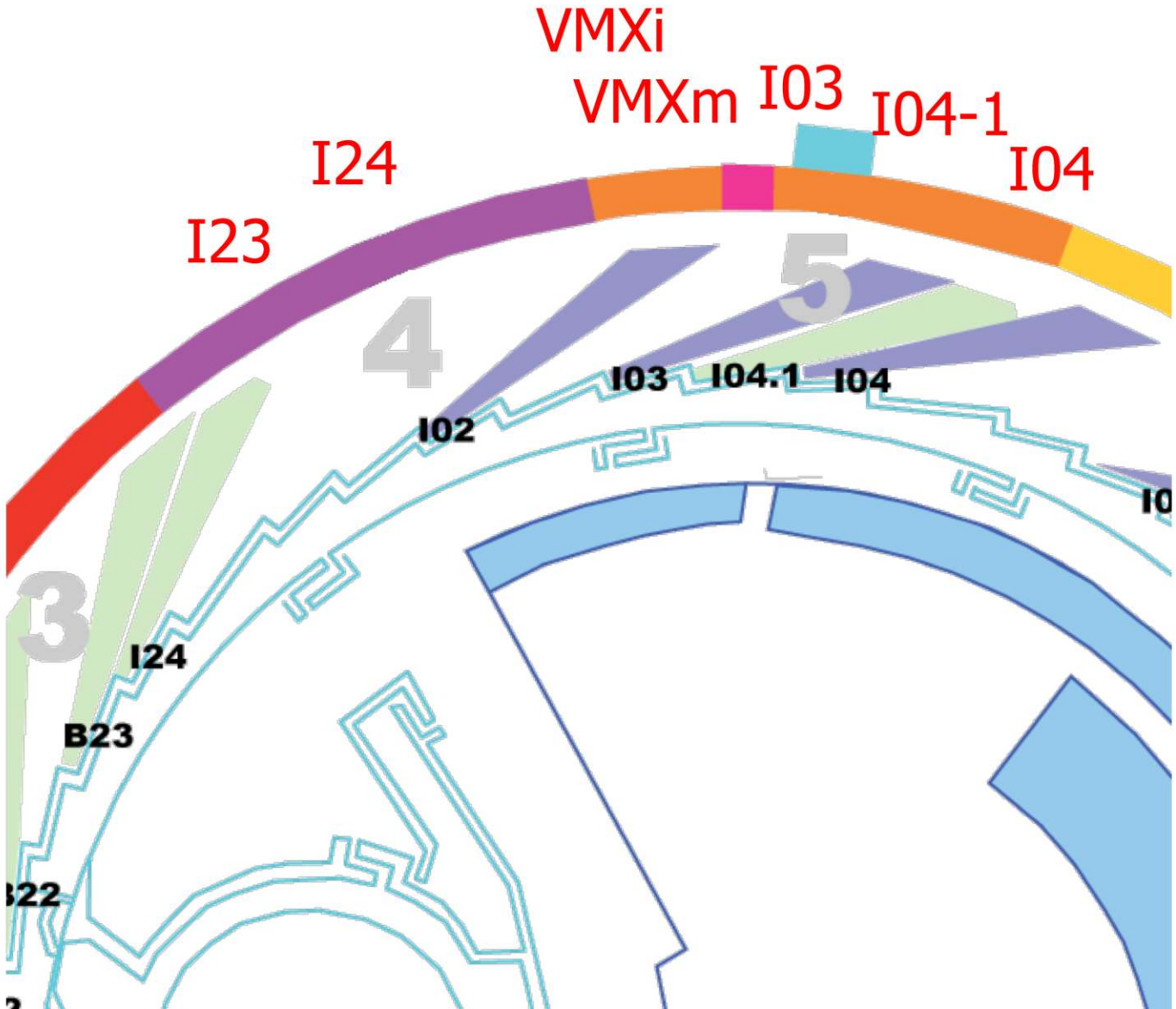
- The MX Village at DLS
- Why MX needs synchrotrons
- Components of a typical MX beamline

Diamond Beamlines

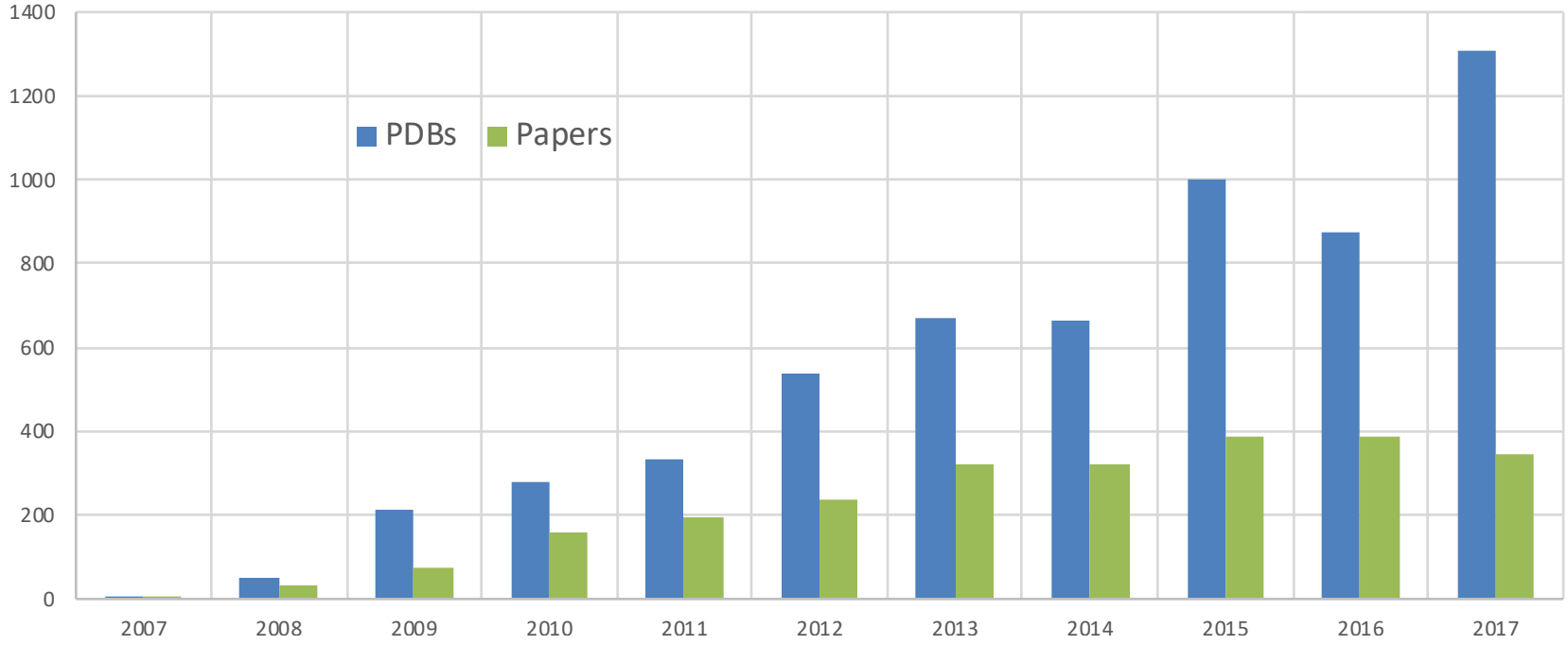


MX Village consists of 7 beamlines

- **Operational**
  - **Phase I**
    - Tuneable I03, I04
  - **Phase II**
    - Fixed λ I04-1
    - Tuneable microfocus I24
  - **Phase III**
    - Long λ I23
    - VMX *m* and *i*



MX Users @ DLS



- With 4 operational beamlines
  - ~50% of DLS users are from the MX community
  - ~55% of experimental sessions
  - ~35% accepted proposals are from EU applicants
- Proprietary and non-proprietary usage
- 2527 papers = ~38% DLS papers
- 6489 PDB depositions (at 13/7/18) and rising

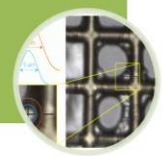
MX Beamline Capabilities

- PADs
- Automation
- Pipelines
- Serial Crystallography
- Access (rapid/freq)

High Throughput

- Tuneable
- Rapid
- Variable focus
- High flux
- *In situ*

Microfocus



- Tuneable beamlines
- I23 dedicated
- Smart data collection strategies
- Expert systems

Long Wavelength

- Screening
- Data collection
- CLIII
- VMXi

In situ

- Pipelines
- Kappa strategies
- Inverse beam
- Long wavelength

Experimental Phasing

- *In situ*
- Containment Level III
- Schedule 5

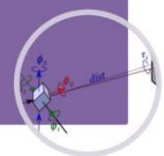
Biological Containment

- Humidity control
- Spectroscopy
- Tomography
- Dynamics
- Fluorescence

Complementary information

- Diffraction image processing for SR Electron and XFEL data
- Collaborative effort
- Led and managed by DLS

Dials Project



- Development of cloud computing
- Automated pipelines
- Supported and distributed programs worldwide

CCP4

- Experiment database
- Synchweb
- Synchlink – iOS app
- Mobile devices

Data Management

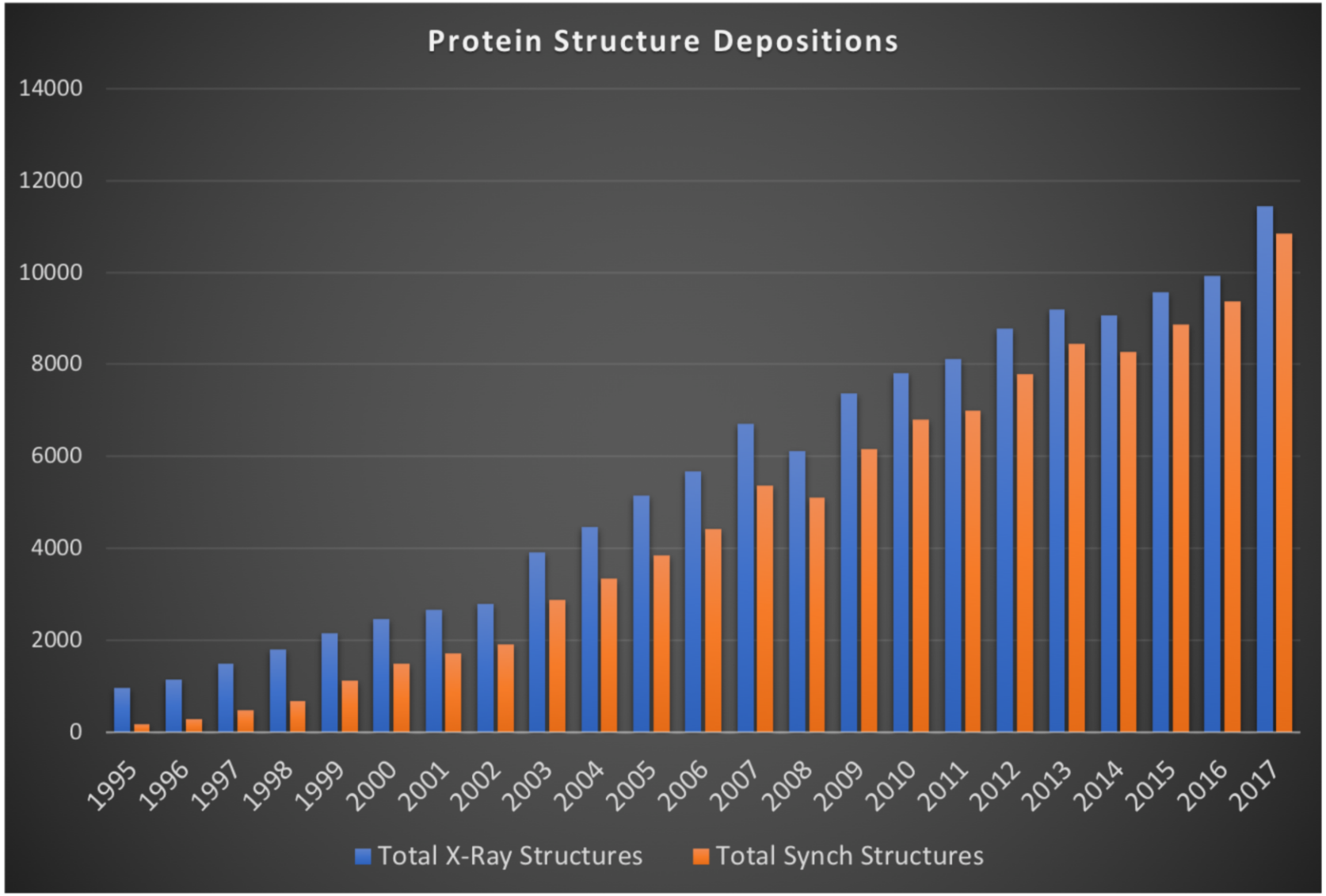
Why do we need a synchrotron for MX experiments?

- Synchrotron light is
  - Tuneable
    - we can choose the wavelength of the X-rays. This is very important for phasing experiments
  - Highly brilliant
    - We can collect useable data from small, weakly diffracting crystals
    - The beam has a low divergence so spots are sharper and do not overlap

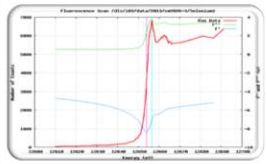
- Allows us to target more challenging samples

Brilliance = number of photons/second/mrad^2/mm^2/0.1% bandwidth

More than 80% X-ray structures deposited in PDB are from data collected at a synchrotron

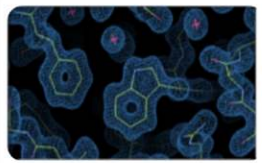


Reasons for using MX beamlines at a synchrotron



Phasing experiments

- Tune wavelength for SAD, MAD etc



High brilliance

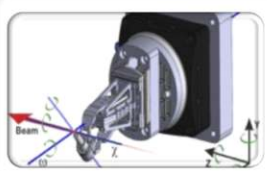
- Good for weakly diffracting samples

High throughput

- Fast robots & detectors

Special Conditions

- *In situ,* HC1 etc



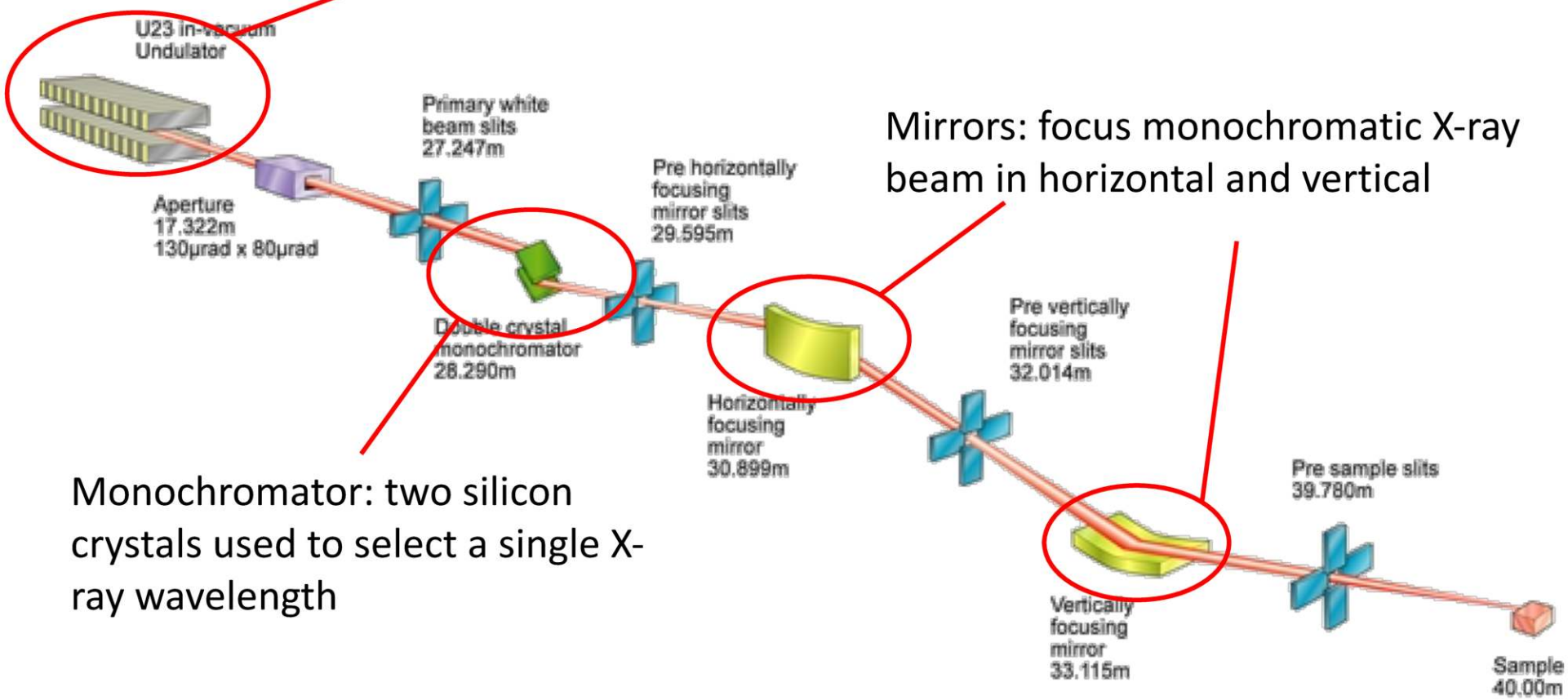
Multi-axis goniometry

This is a typical layout of a standard MX beamline

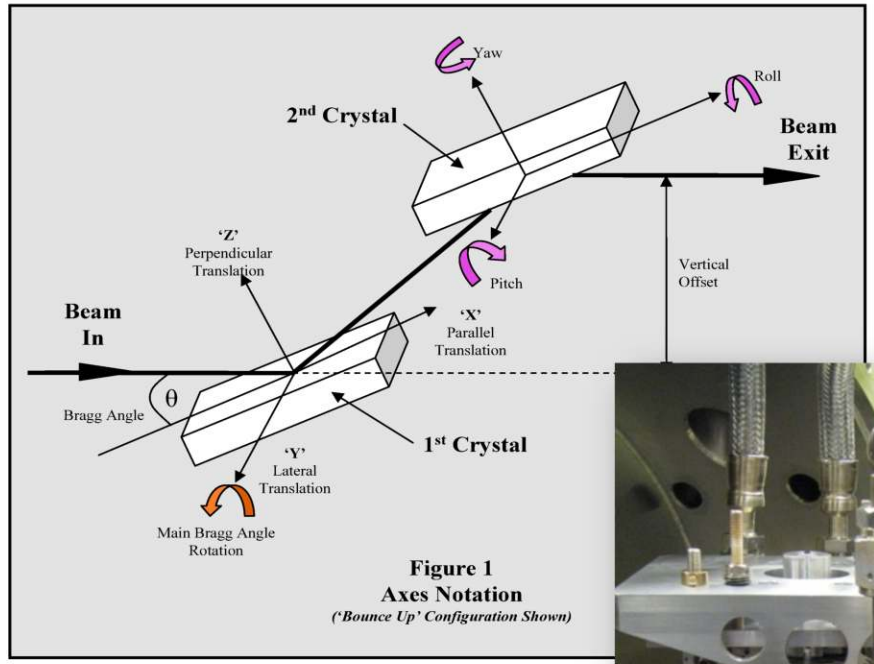
Source: most MX beamlines use an undulator. Provides an intense beam of X-rays

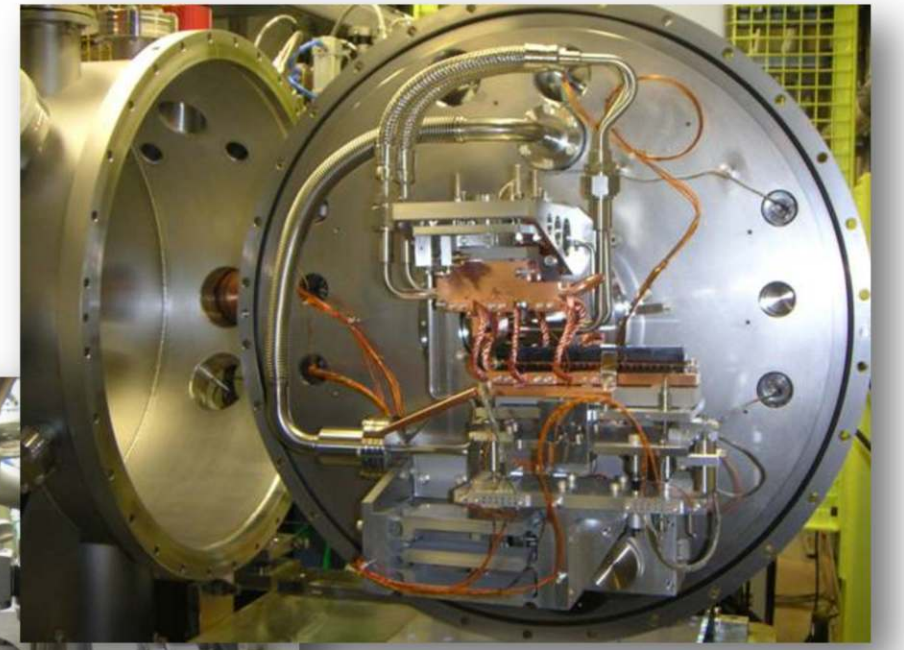
Mirrors: focus monochromatic X-ray beam in horizontal and vertical

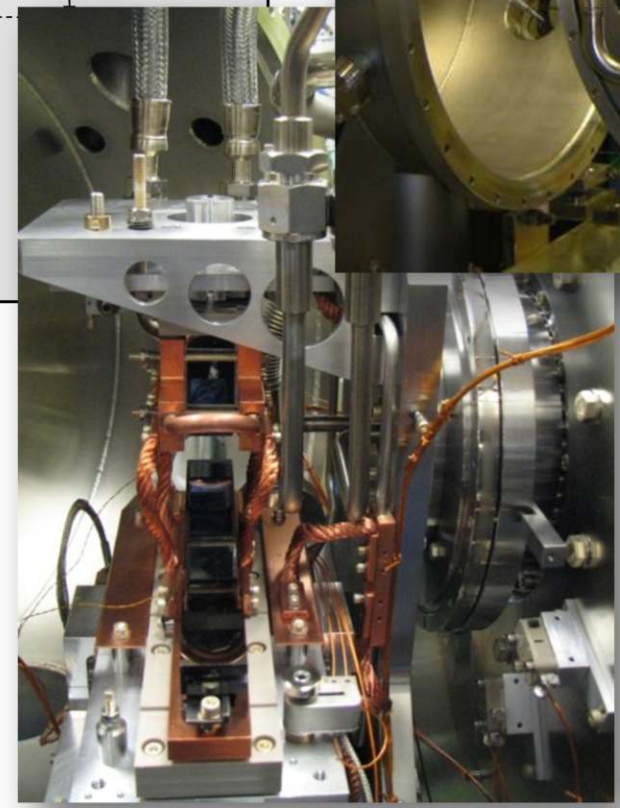
Monochromator: two silicon crystals used to select a single X-ray wavelength



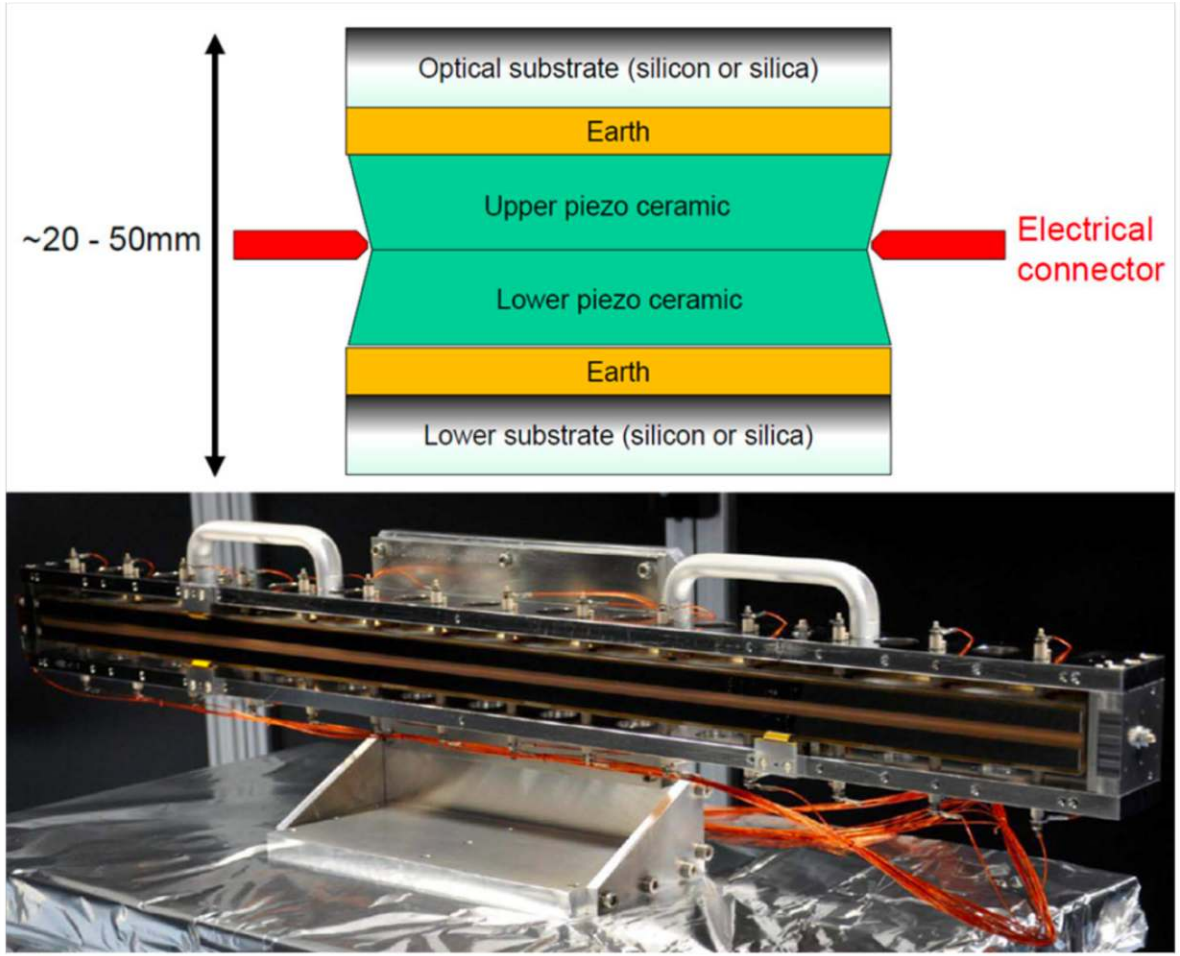
A double crystal monochromator is used to select the X-ray energy (wavelength)

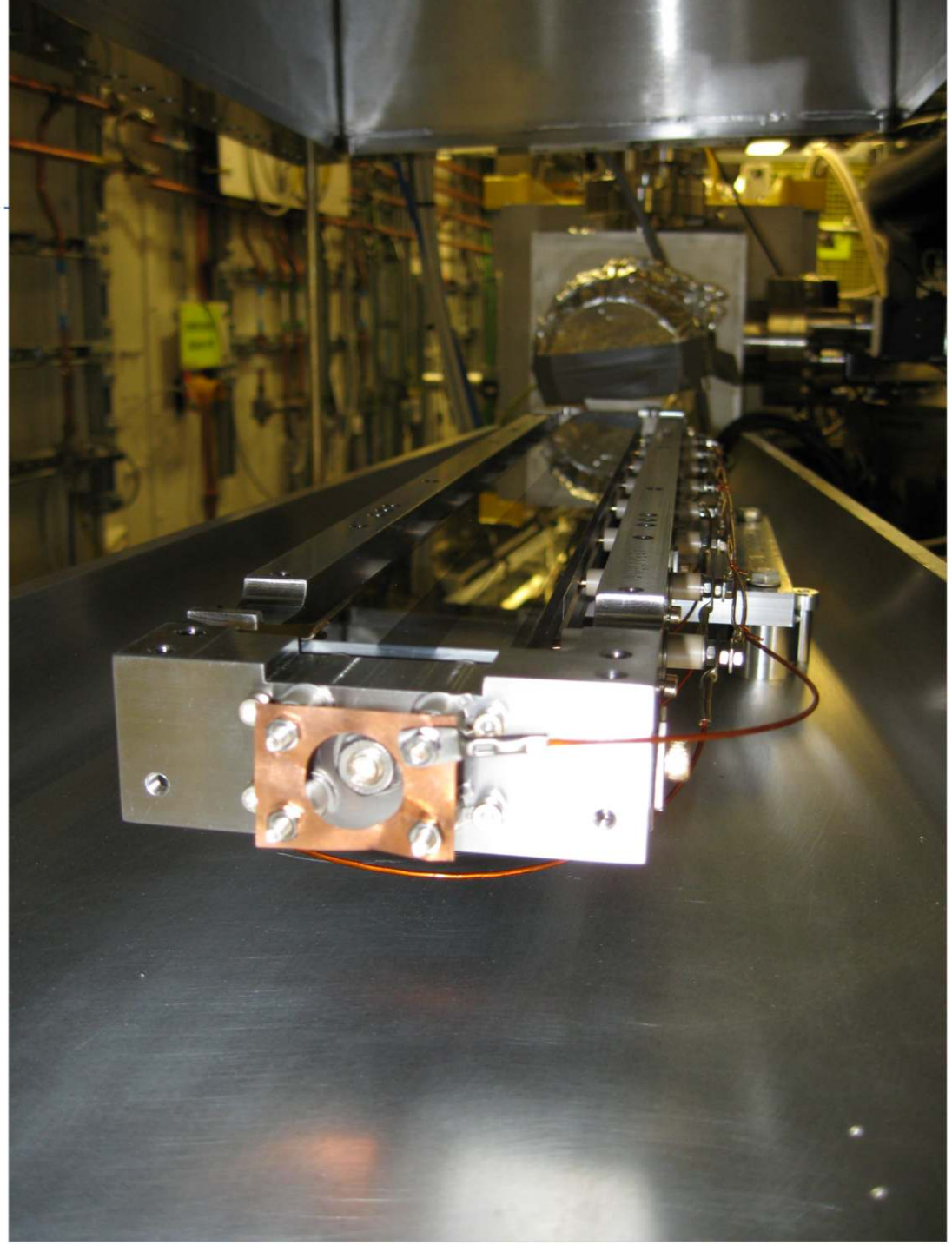




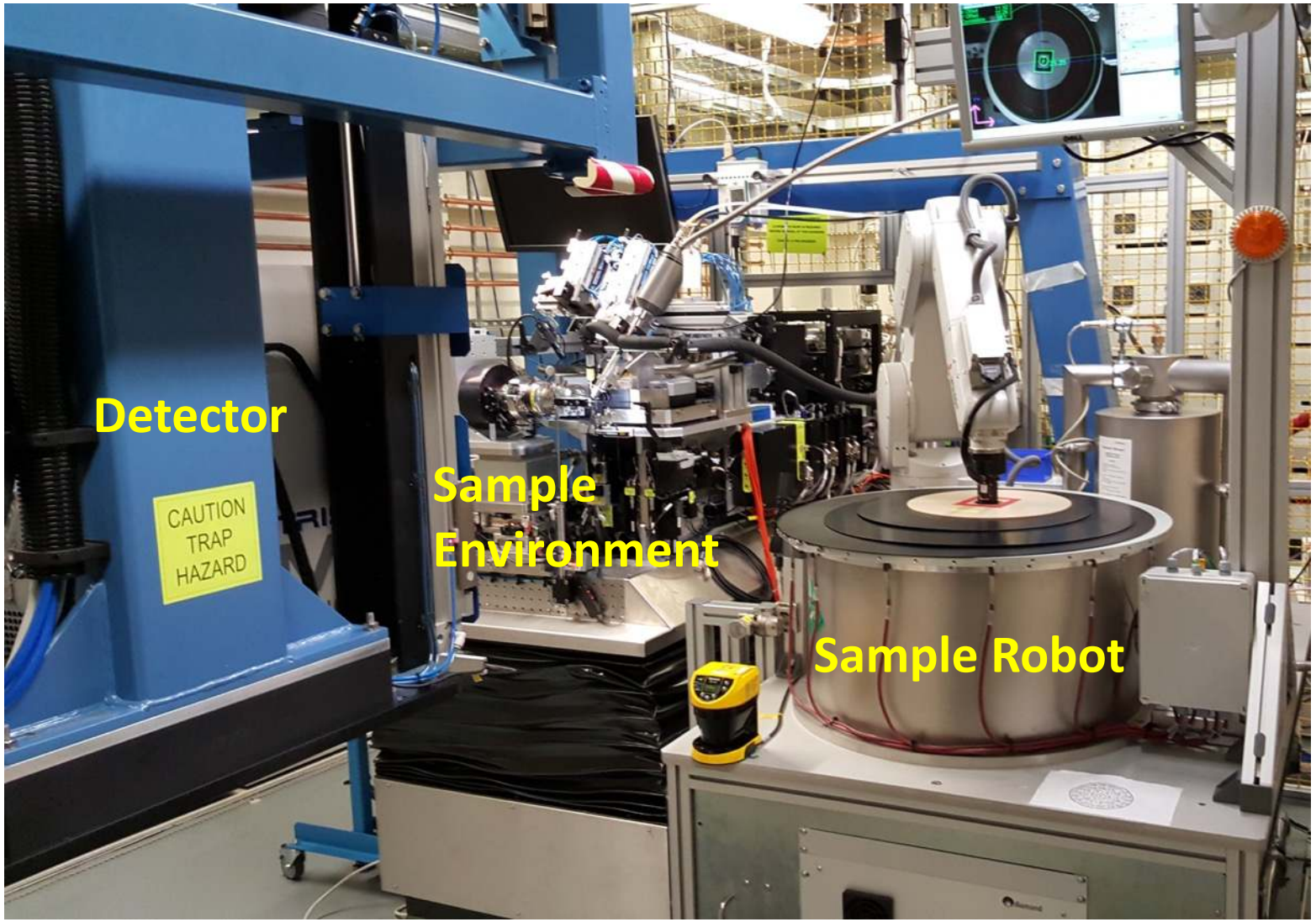


Bimorph focusing mirror

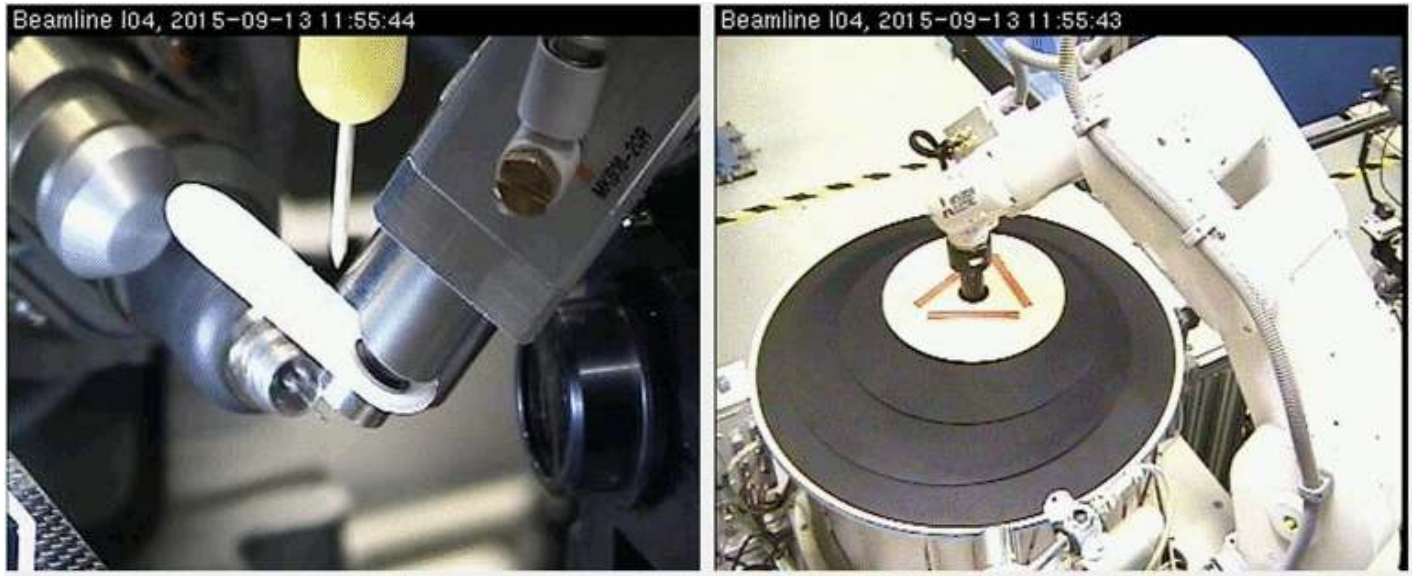




The experiment hutch contains beam conditioning elements, the sample environment and detector

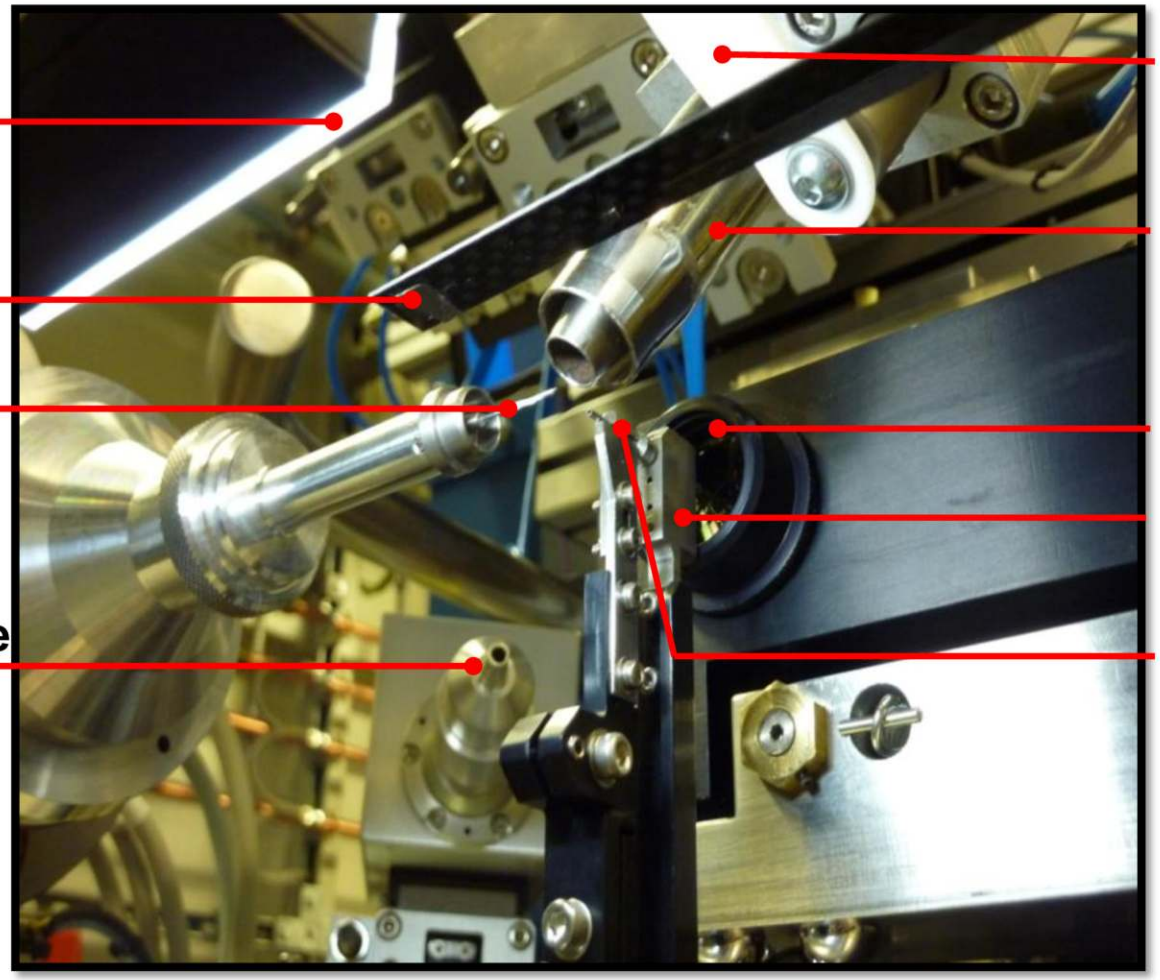


High-throughput of samples requires fast robotics



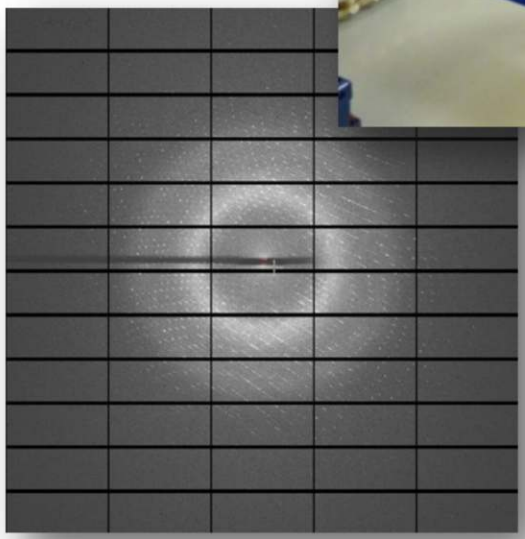
- BART robot system for exchanging cryocooled samples
- Holds nearly 600 sample holders at cryogenic temperatures
- Fast, reliable sample exchanges (12-18 s)

The sample environment for cryo crystallography

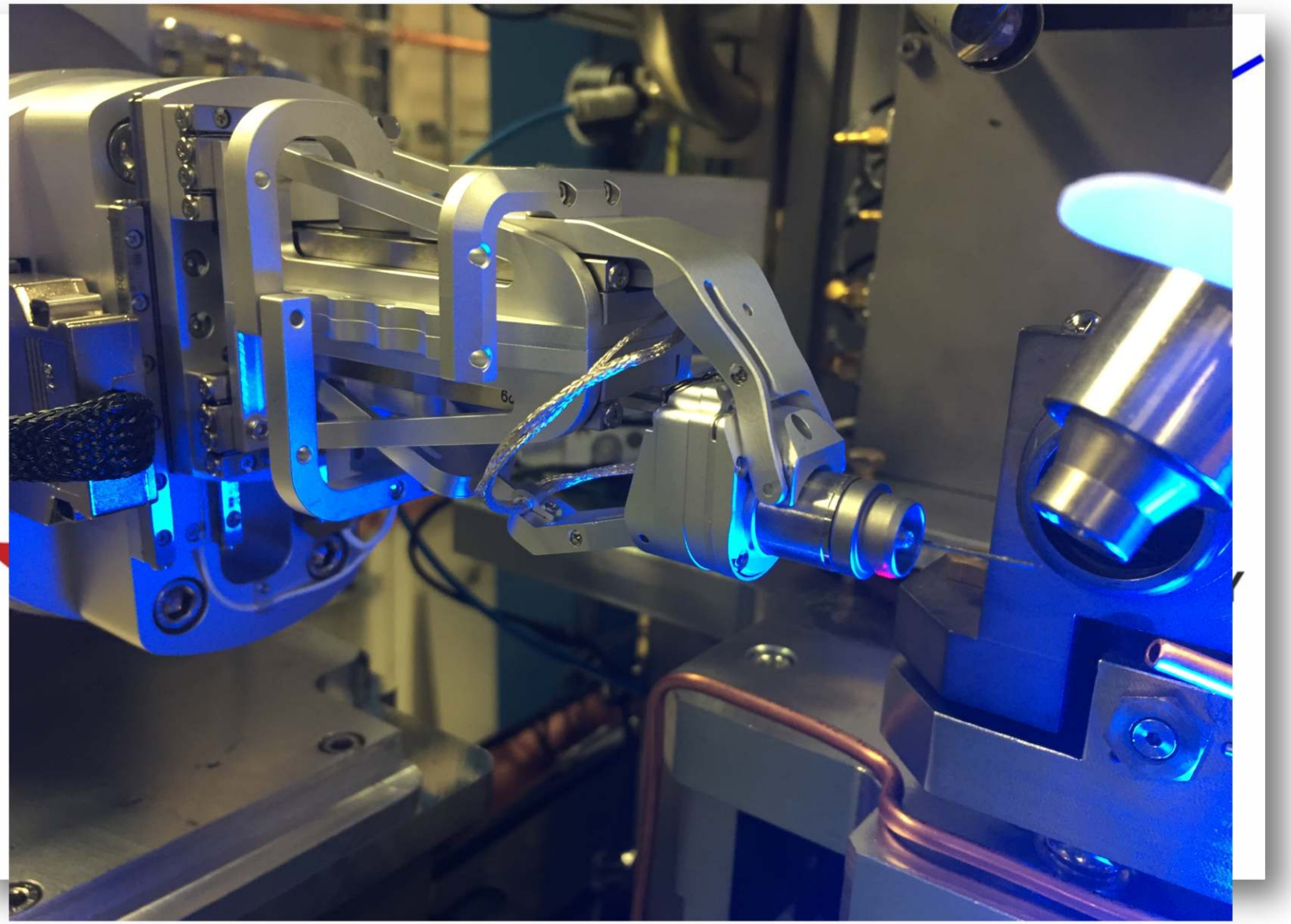


Fast pixel array detectors record the diffraction images

- Advantages
  - S/N much better than CCD
  - Higher throughput
  - Fine phi slicing – better data
- Pilatus detectors on I03, I04, I04-1, I23, I24
- Eiger2 detector on VMXi
- Eiger2 under commissioning for I03 and I04

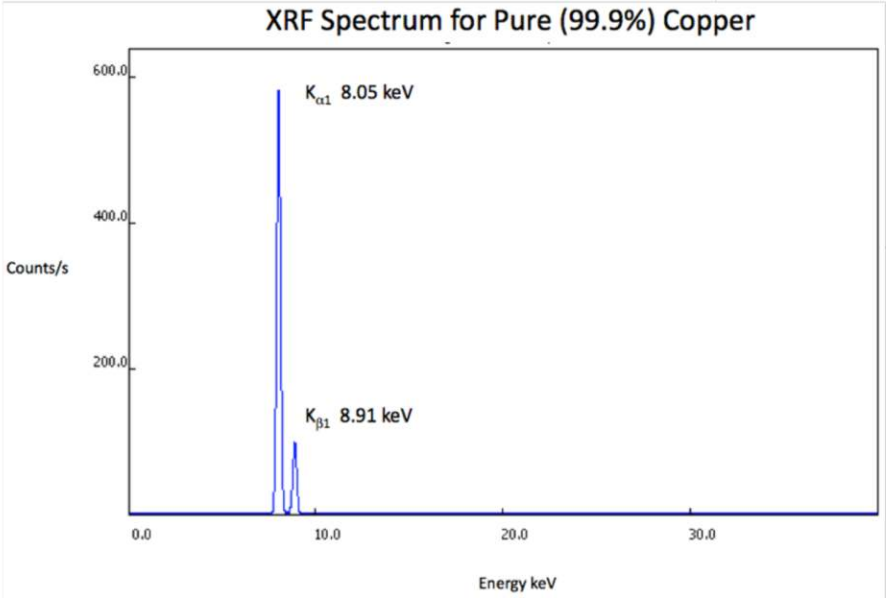


Crystal re-orientation – using multi-axis goniometry

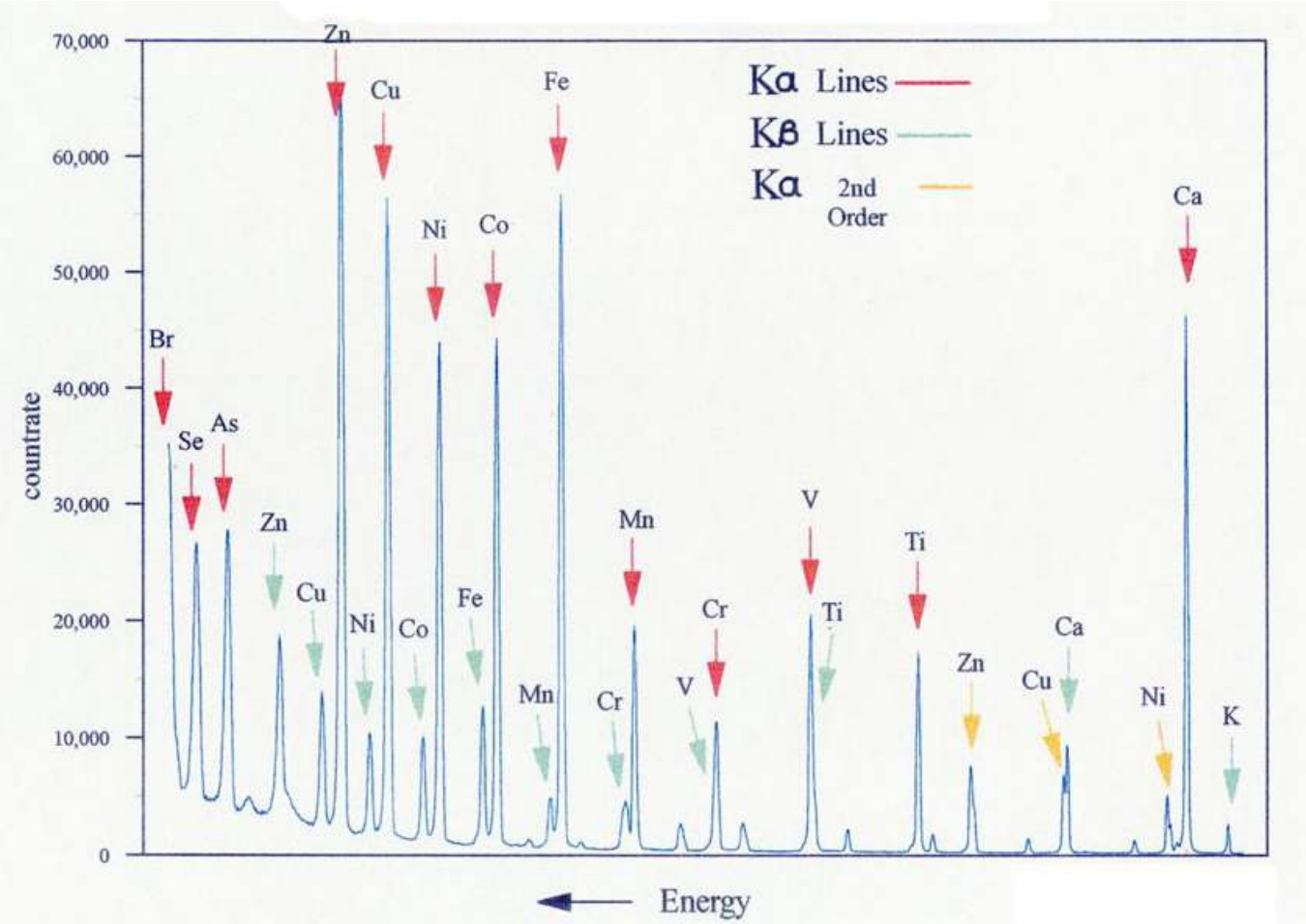


Fluorescence techniques - Metals in biology

- Metals are present in > 1/3 of all proteins
- Examples: Na, Mg, K, Ca, Mn, Fe, Co, Ni, Cu and Zn
- Examples of use
  - Metalloproteins and metalloenzymes
  - Electron-transfer proteins
  - Oxygen transport



Example fluorescence spectra



Example spectra

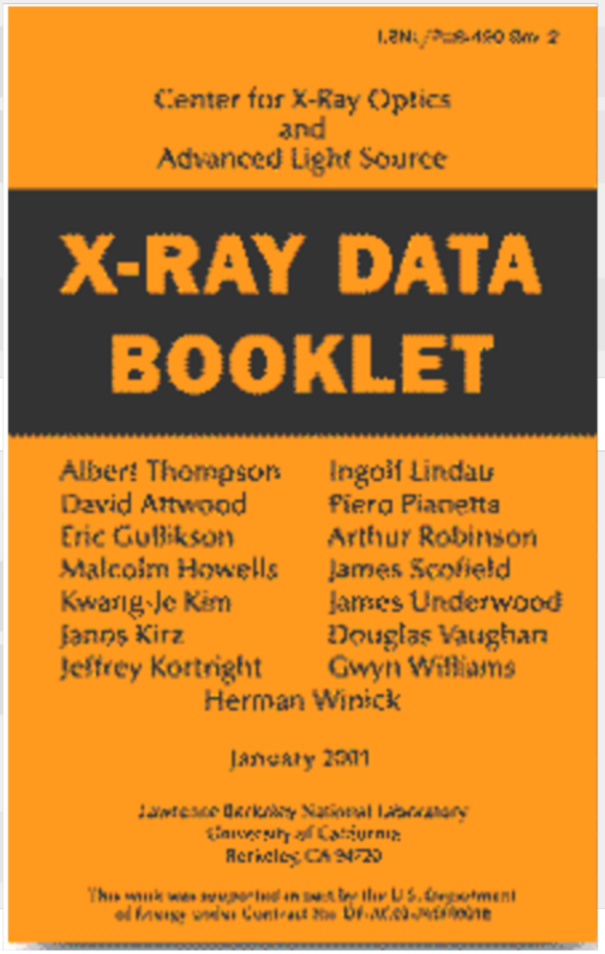
21-06-2018 15:31:35 - 20180621_15_31_35.mca

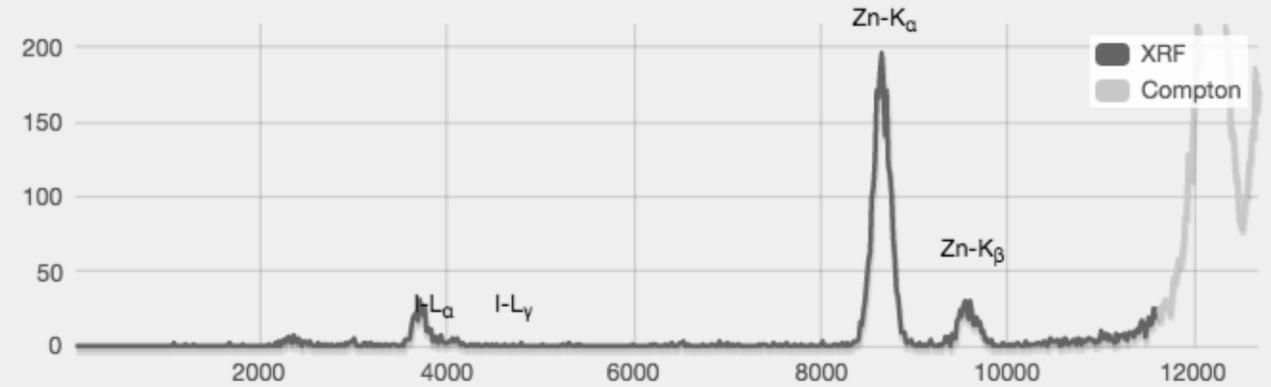
Sample: RNAP_1

Exposure: 1.00s

Beamsize: 20x20µm

Comment: null





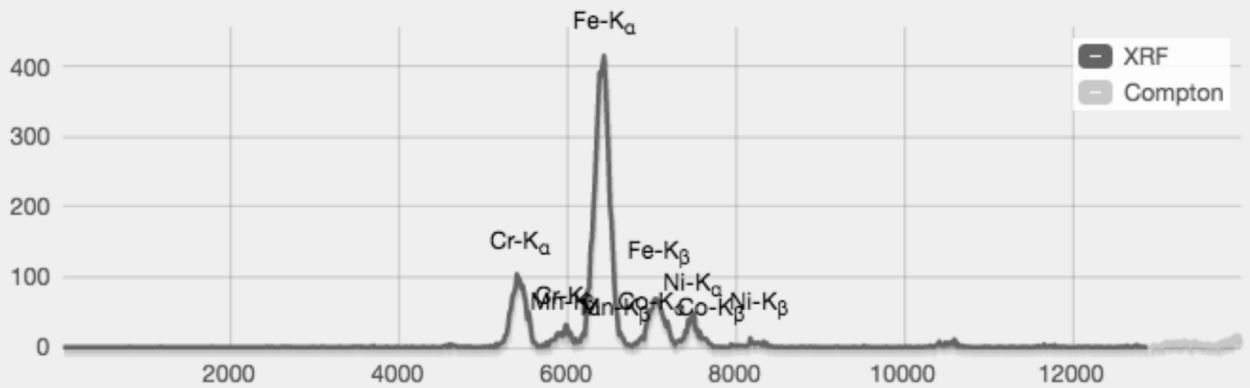
16-07-2018 16:34:48 - 201

Sample: n1642_4

Exposure: 1.00s

Beamsize: 80x20µm

Comment: Click to edit



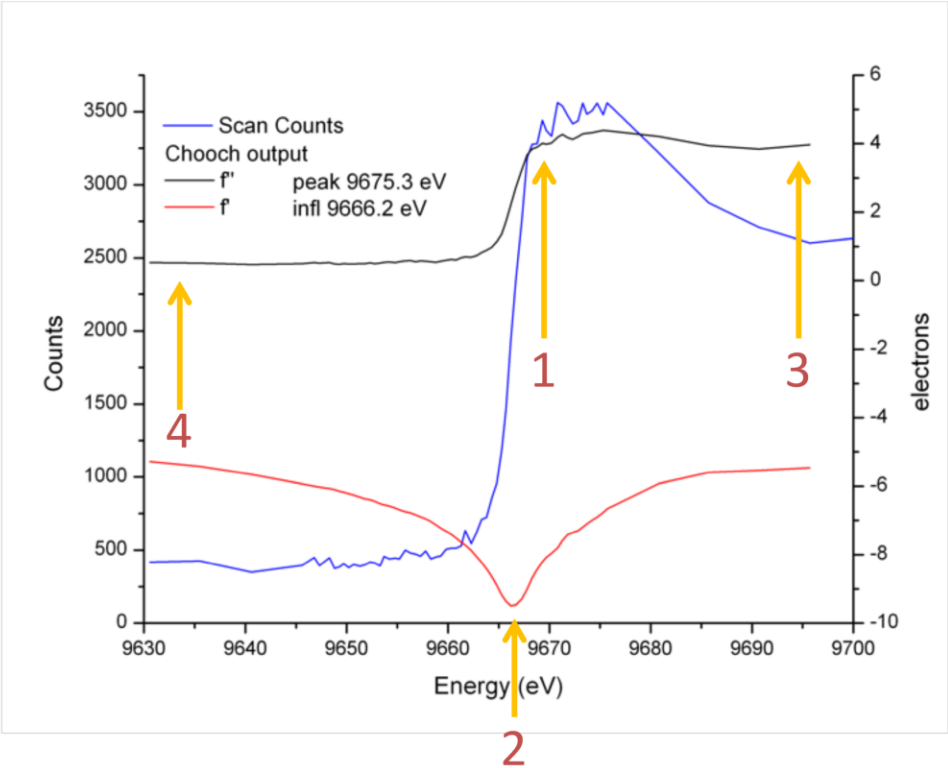
http://cxro.lbl.gov/x-ray-data-booklet

Fluorescence scans for phasing experiments

- Run a fluorescence scan to choose optimal energies for MAD or SAD experiments
- Energy (wavelength) is scanned around the expected absorption edge of the element of interest
- Fluorescence signal is measured as the energy is changed

Collecting anomalous data

The exact wavelength of the absorption edge can be determined by carrying out a fluorescence scan



1. Peak
   Maximal anomalous differences *ie* intensity difference between Friedel pairs
2. Inflexion
   Maximal dispersive differences *ie* the same reflection has a differing intensity at different wavelengths
3. High energy remote
4. Low energy remote

Note that heavy elements (Z > S) result in increased absorption and hence increased radiation damage/ reduced crystal lifetime. Absorption increases above the edge exacerbating the problem.

The MX beamlines - details

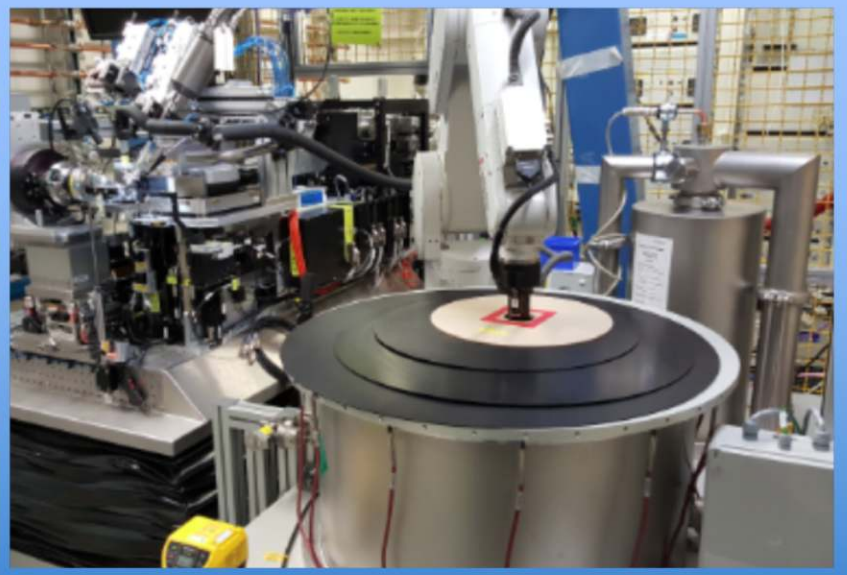
- High-throughput, I03 & I04
- Fragment screening I04-1, XChem
- Long-wavelength, I23
- Microfocus, I24
- VMXi and VMXm

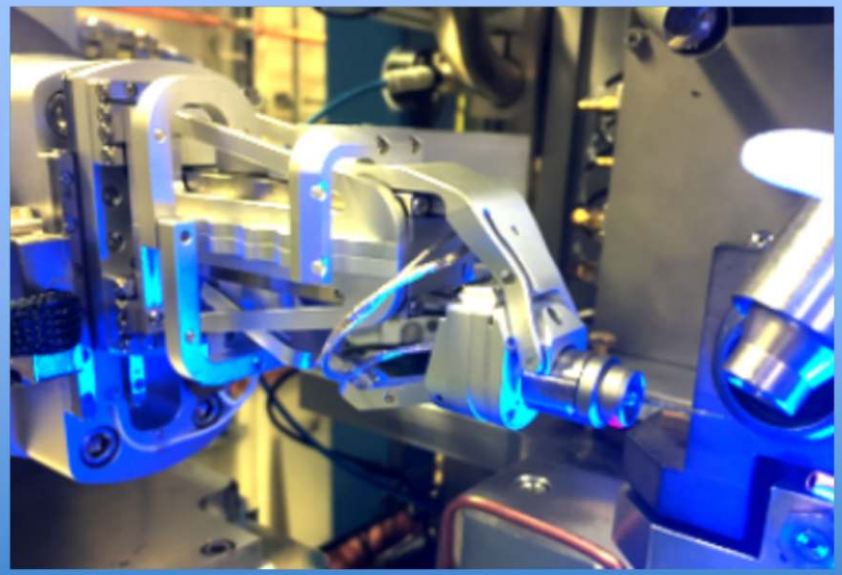
Automated single crystal experiment

I03 & I04 High-throughput MX

- I03
- Focus with mirrors
  - 100 um, 50 um, 20 um
- Pilatus3 6M (100 Hz)
- Multi-axis goniometry
- Biocontainment

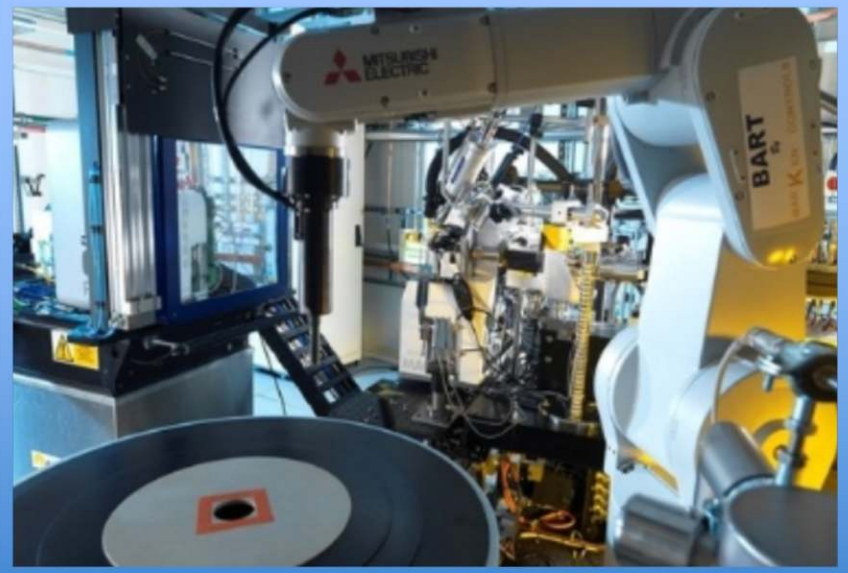
- I04
- Focus with CRLs
  - Variable beam sizes
- Pilatus 6M (25 Hz)
- Multi-axis goniometry





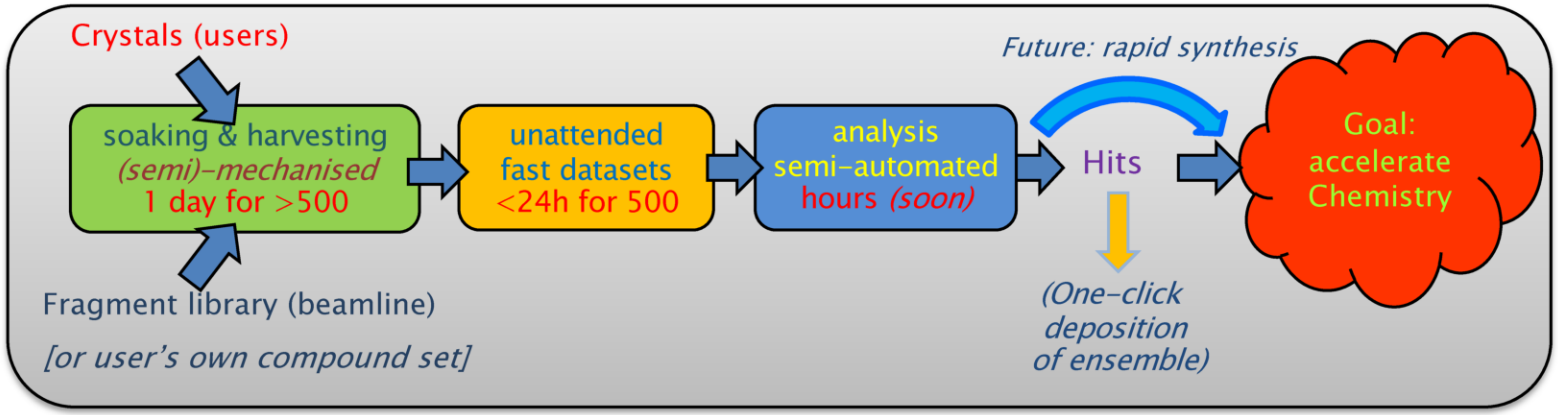
I04-1 Fixed Wavelength MX

- Fixed energy 0.92 A
- Pilatus 6M 25 Hz
- Beamsize 60 x 50 microns
- Low flux compared to the other MX beamlines



Xchem User Program @I04-1

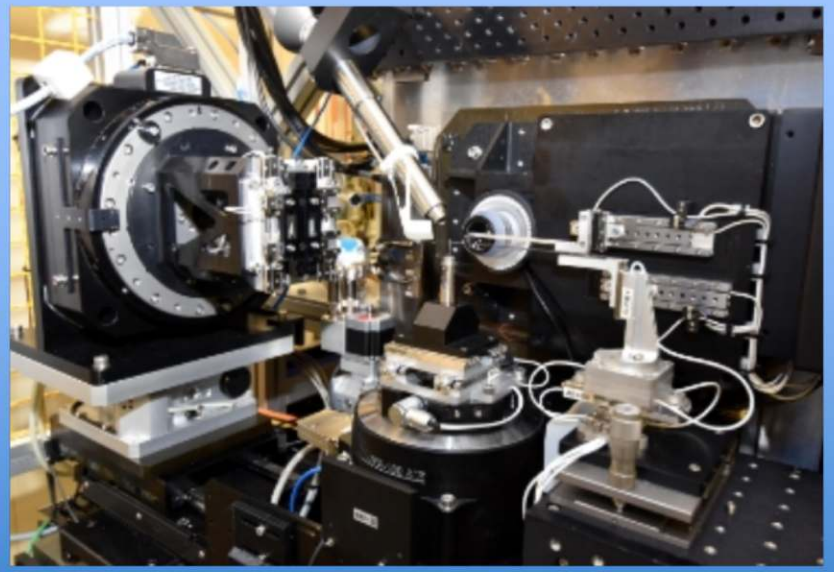
- A world-wide first facility: **fragment screening by crystal structure**
  - *User: bring crystals to beamline Beamline provides: advice (!!), fragment library, rapid soaking, data collection & analysis*
  - *Best case: 1 wk for whole experiment (500-1000 crystals) Capacity: >30 experiments/year;*

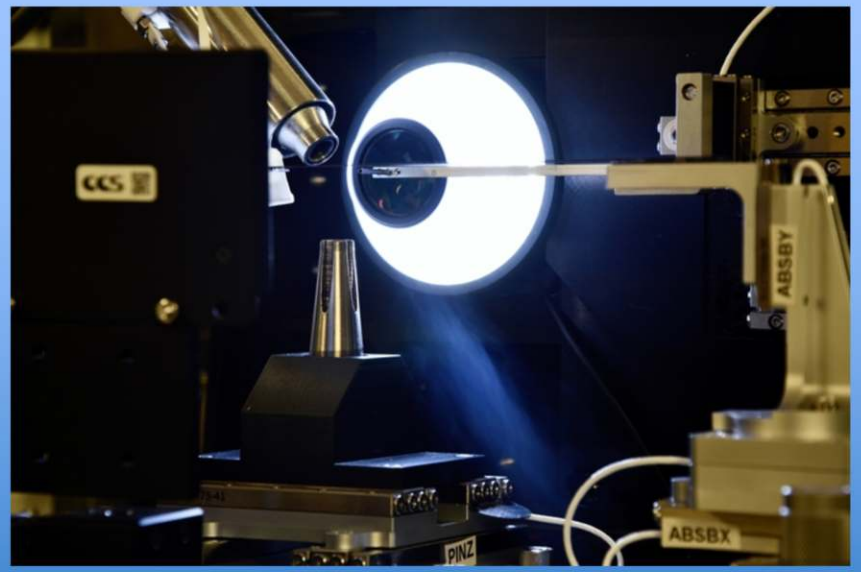


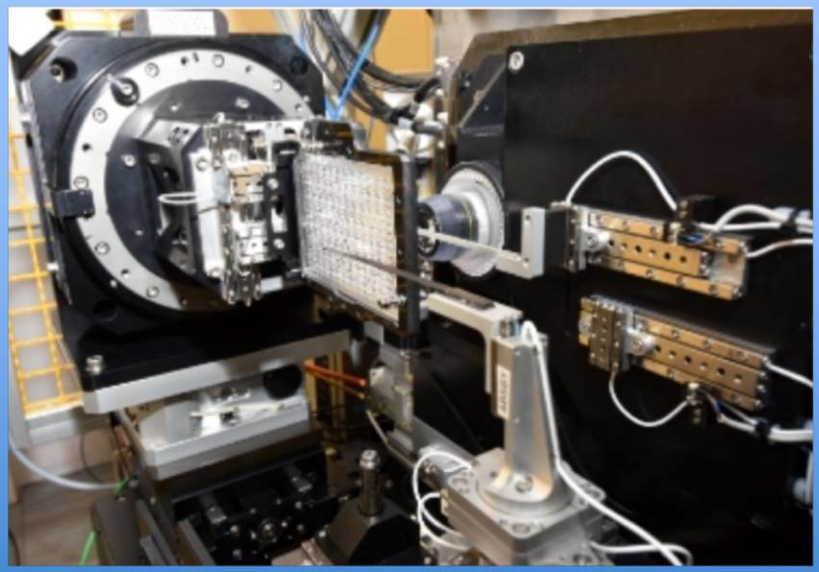
- New fragment library (DSPL) – 770 compounds. (Cox *et al*, Chem. Sci., 2016)
- New analysis tools deployed:
  - *PanDDA*: multi-dataset 3D background correction – highly sensitive ligand identification (Pearce et al Nat. Comms, 2017)
  - *XChemExplorer*: batch-wise analysis of 100s of datasets (Krojer et al Acta D 2017)

I24 Microfocus Beamline

- Beamsize: 5x5 - 50x40 (variable aspect ratio)
- Detector: Pilatus3 6M, 100 Hz
- Wavelength: 0.62Å - 1.77Å





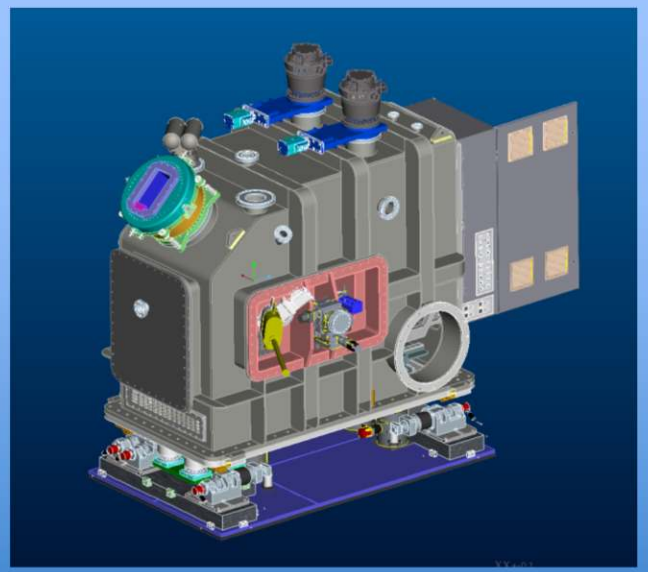


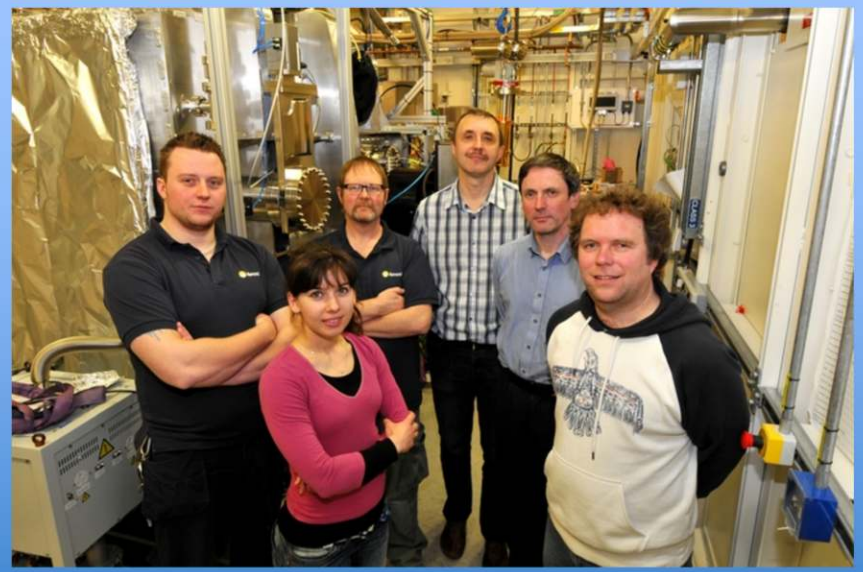
I23 Long wavelength MX

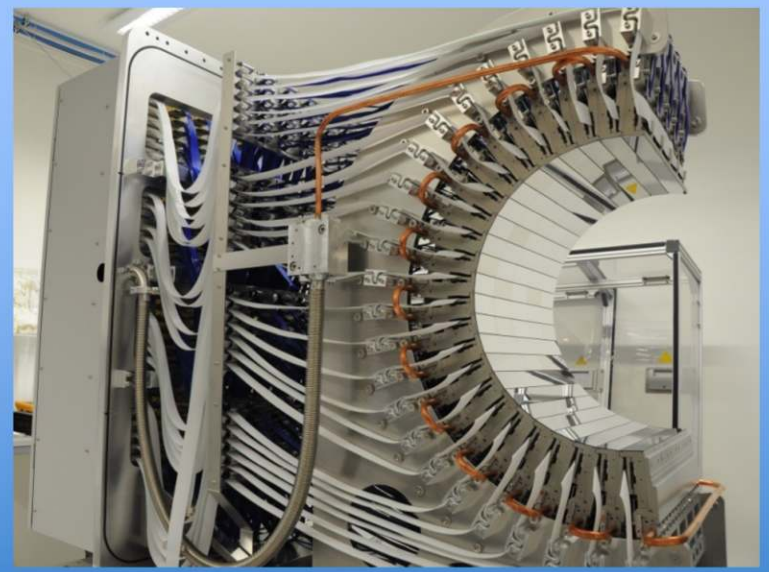
- I23 first MX beamline optimized for the long-wavelength region (1.5 – 4 Å).

- Provides a unique tool to fully exploit the potential of experimental phasing from native protein and DNA/RNA crystals.

- P12M detector
- Half cylinder, 250 mm radius

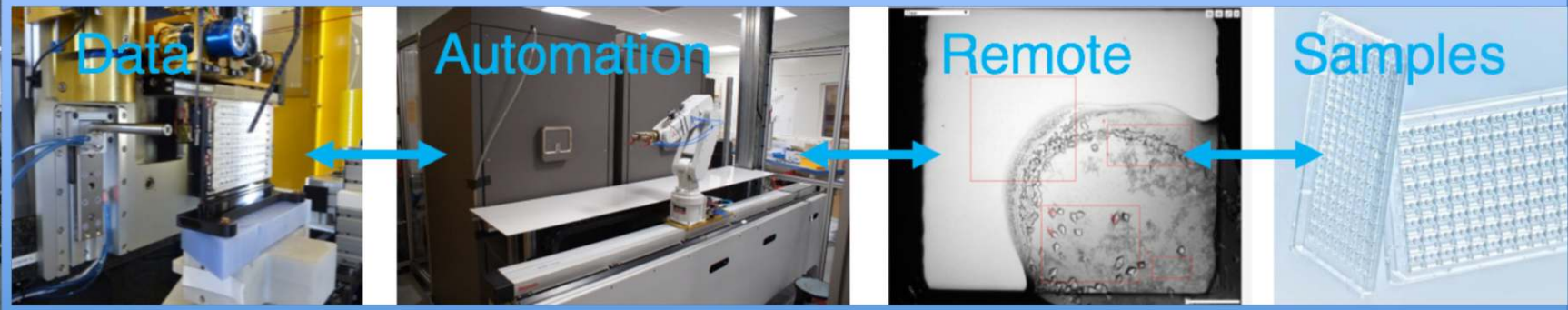






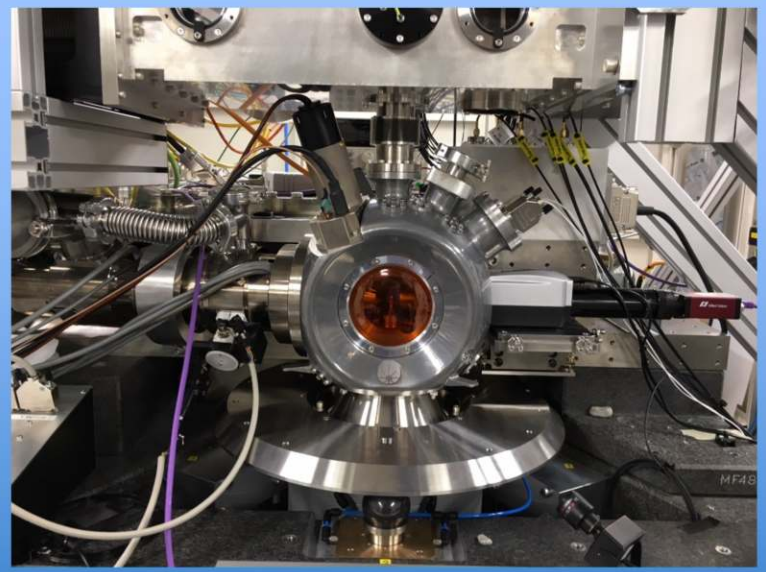
VMX$_i$ - dedicated to *in situ* (plate) crystallography

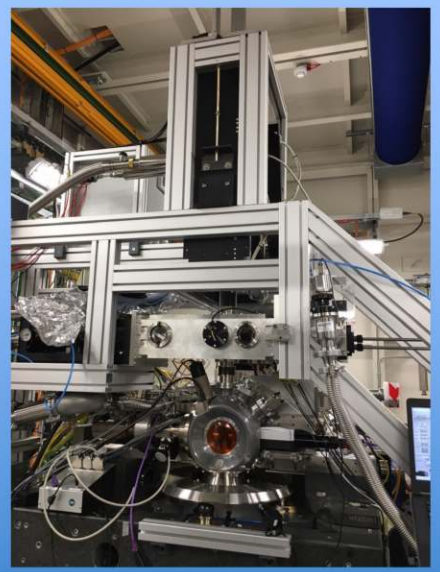
- Automated transfer of plates between storage and beamline
- Advanced imaging of drops (on-line and off-line)
- X-ray data collection and screening (automated or interactive)
- Tuneable microfocus beam (5x5 micron – 10-25 keV)
- Narrow or broad bandpass beam (2eV – 100eV)

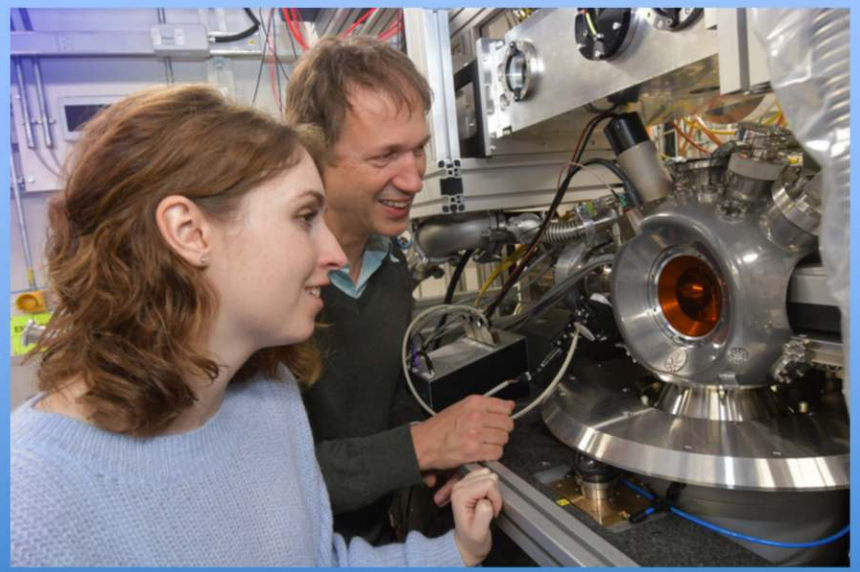


VMX_{micron} Sub micron variable focus

- < 500 nm beam size
- X-ray energies from 7 - 25 keV
- in vacuum sample environment
- SEM







The End

- With thanks to Diamond MX Group

The MX Beamline Teams

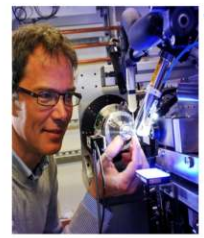
I03 – Katherine McAuley
Neil Paterson
Mark Williams

I04 – Ralf Flaig
Pierpaolo Romamo
Marco Mazzorana

I04-1/XChem – Frank von Delft
Jose Brandao-Neto
Patrick Collins
Romain Talon
Alice Douangamath
Anthony Aimon

I23 – Armin Wagner
Vitalily Mykhaylyk
Kamel el Omari
Ramona Duman

I24 – Robin Owen
Danny Axford
Selina Storm
John Beale



VMXi – *Vacancy*
Juan Sanchez-Weatherby
James Sandy
Halina Mikolajek

VMXm – Gwyndaf Evans
Jose Trincao
Anna Warren
Emma Beale
Adam Crawshaw

XFEL Hub – Allen Orville
Pierre Aller
Agata Butryn