MX @ Diamond

Katherine McAuley
Diamond Light Source



Overview

Synchrotrons

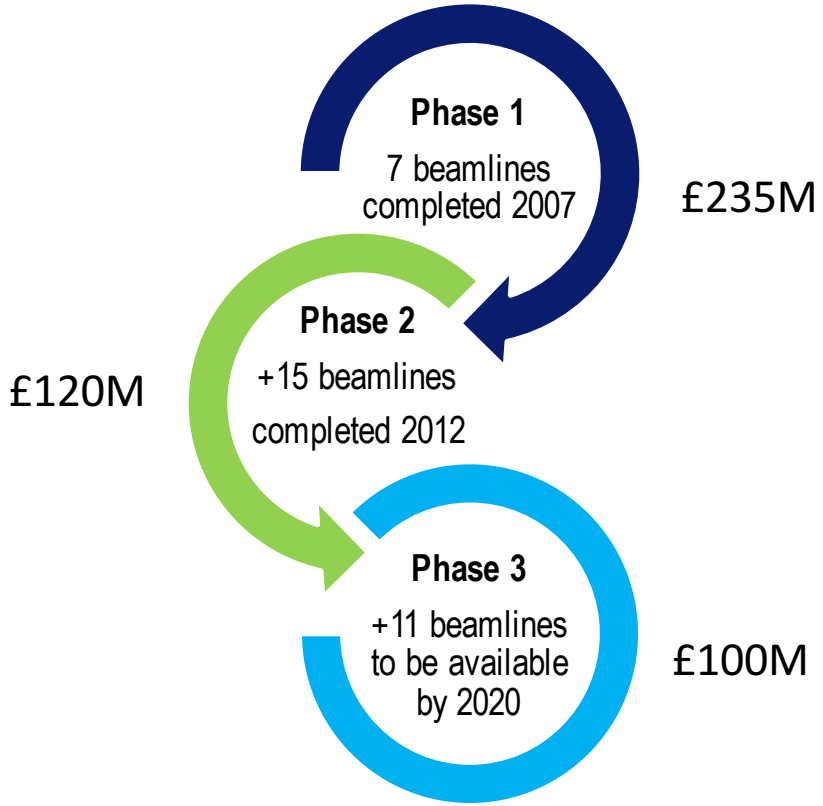
- Diamond facility
- How a synchrotron works
- Integrated structural biology

MX beamlines

- Components
- Techniques
- Details of Diamond MX beamlines

The Diamond Project

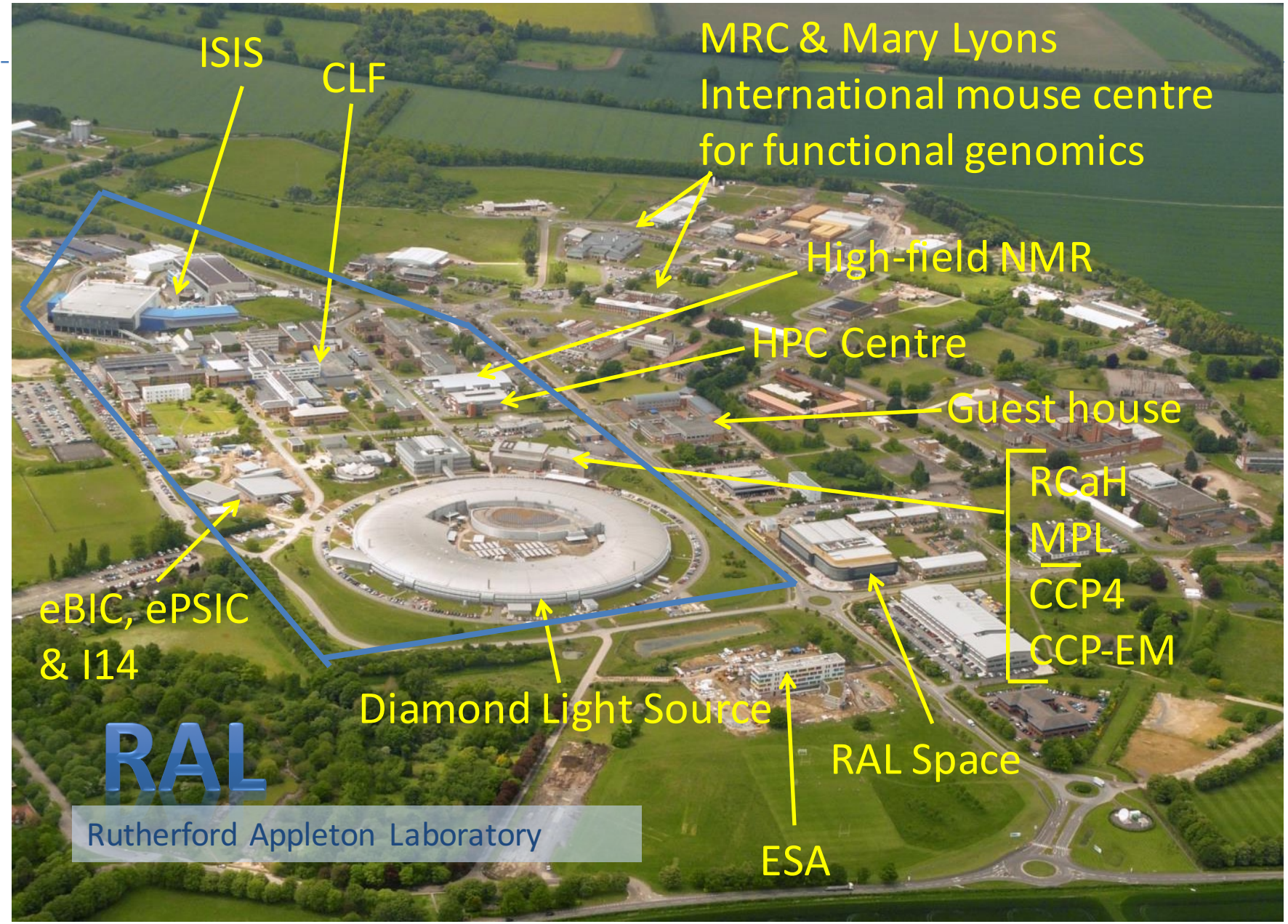
- Diamond is a private company formed as a joint venture between STFC (86%) and The Wellcome Trust (14%)
- All beamlines are owned and operated by Diamond





All areas of science covered except military and tobacco research

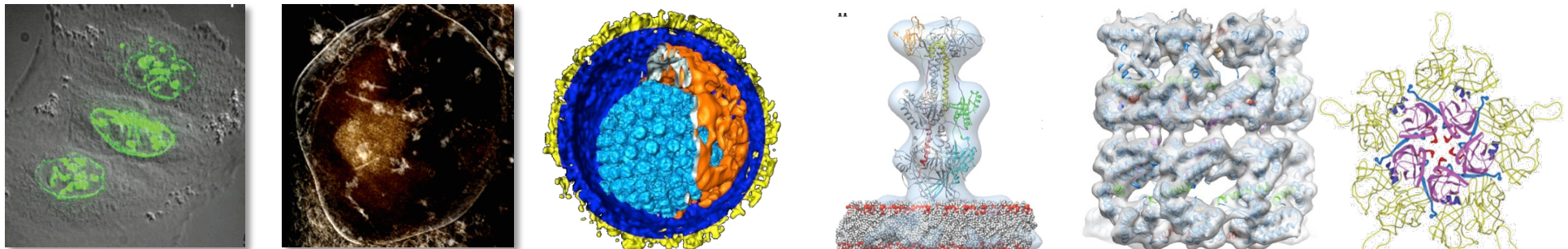
Harwell Campus



- 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!!

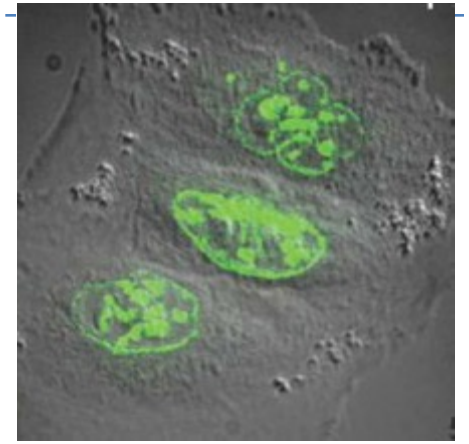
Imaging Single Cells to Molecules; an integrated approach at Harwell

- 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...

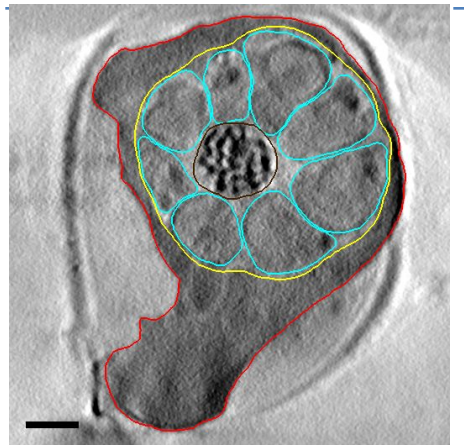


Integrated Structural biology

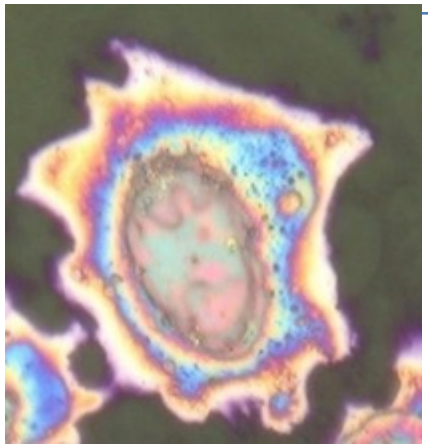
Increasing biological complexity and integrity



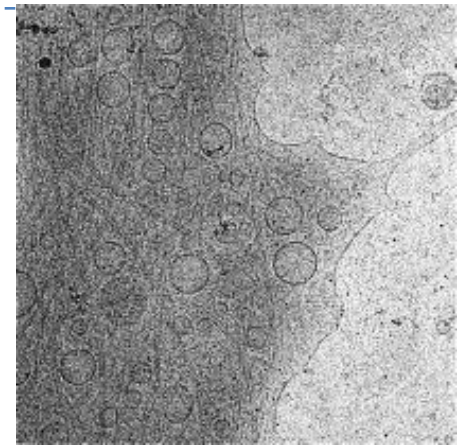
Fluorescence microscopy (B24/CLF)



B24 X-ray microscopy

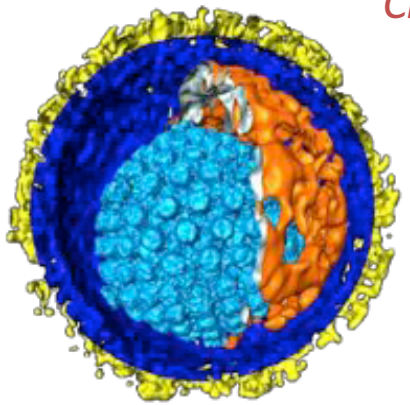


B22 - Infrared microspectroscopy

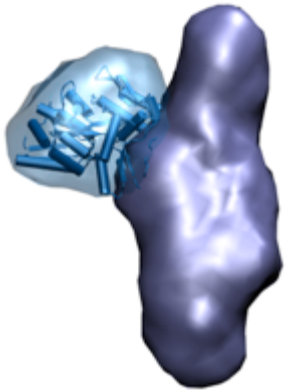


Cellular cryo-electron tomography

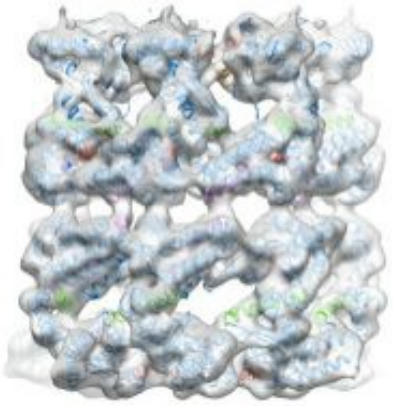
Cryo-CLXM
Cryo-CLEM



Cryo-electron tomography



B21 X-ray Solution Scattering



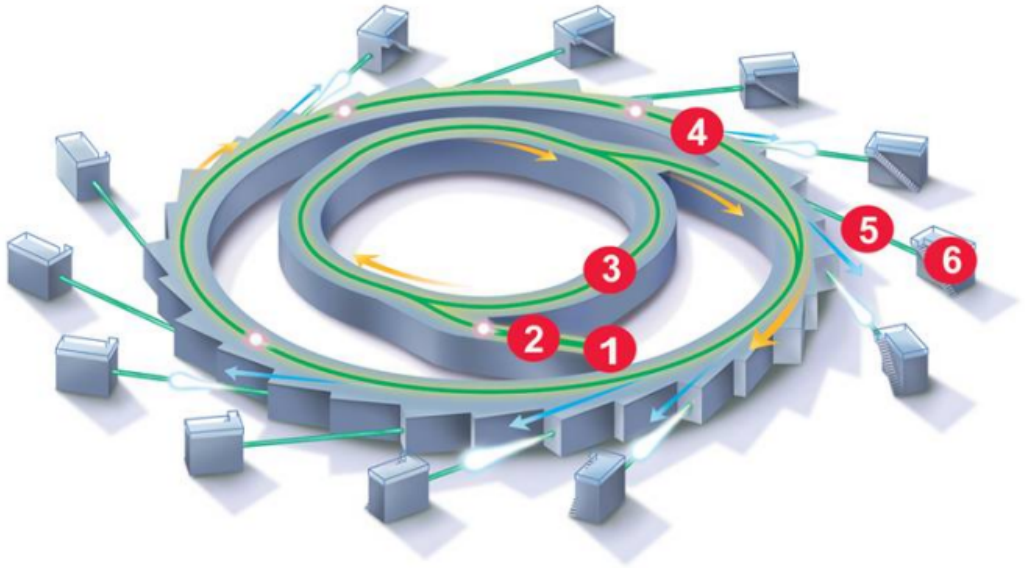
Single particle cryo EM



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

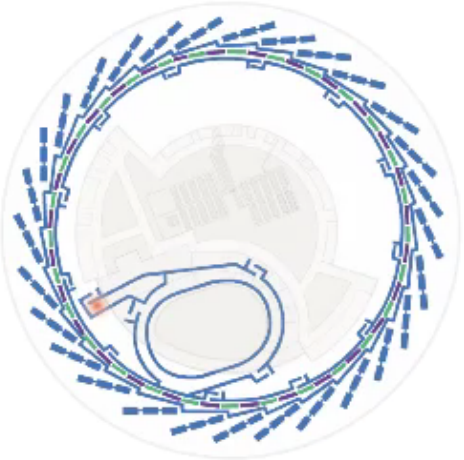
5 Beamline: set up of radiation for experiments

6 Experimental Hutch: User area

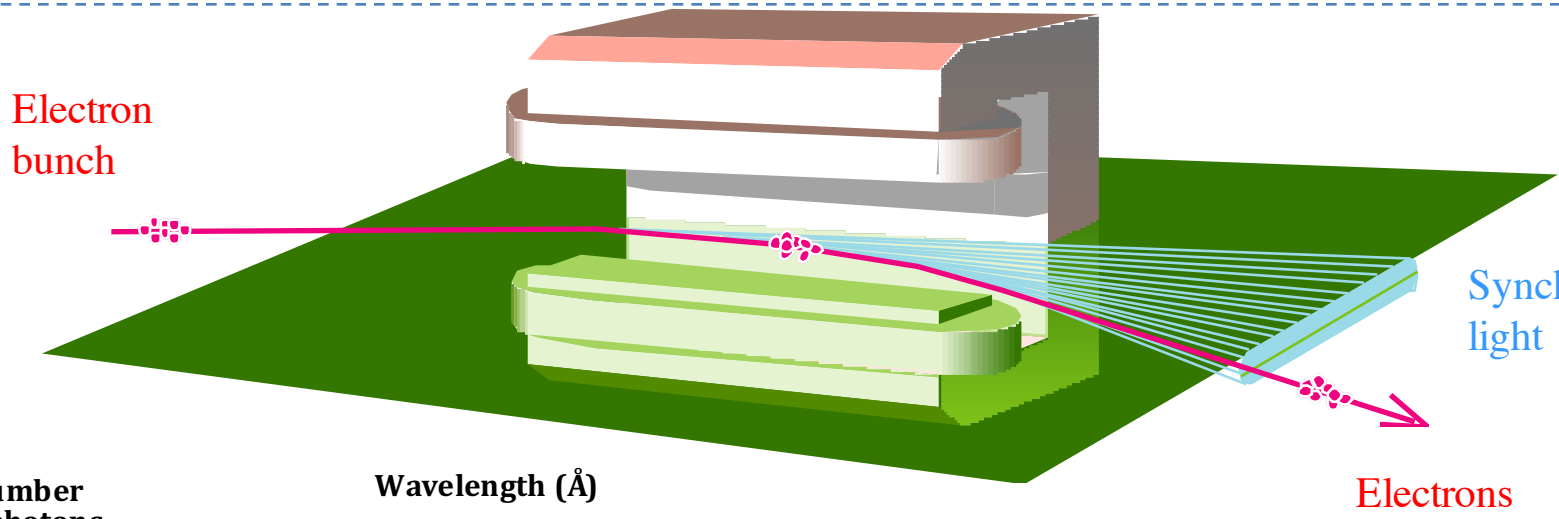
Views from inside the storage ring

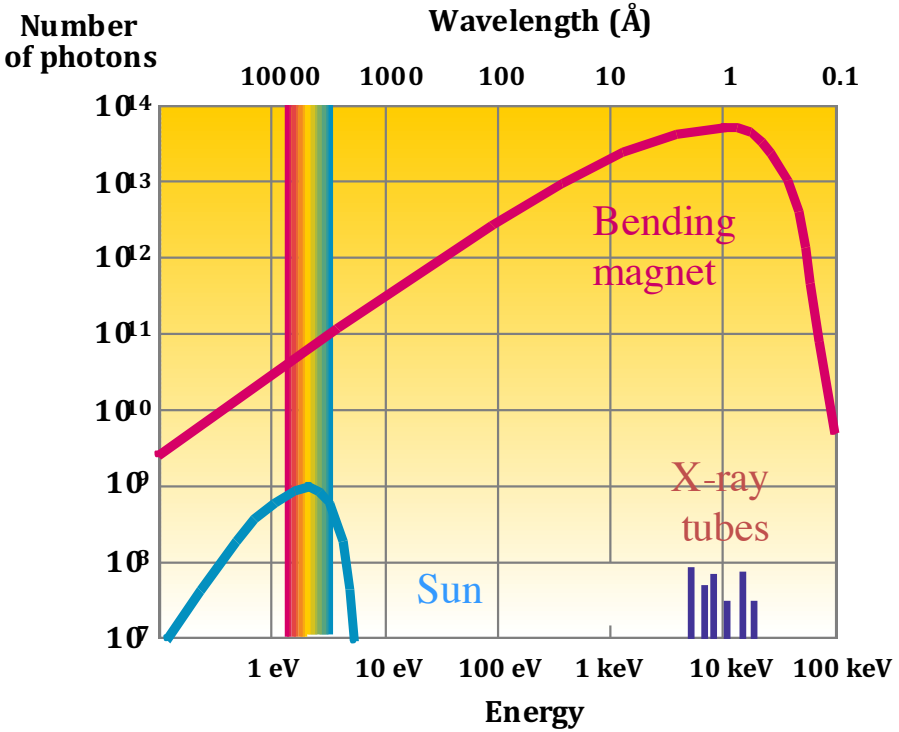






Bending Magnets curve the electron beam between adjacent straight sections and generate synchrotron light

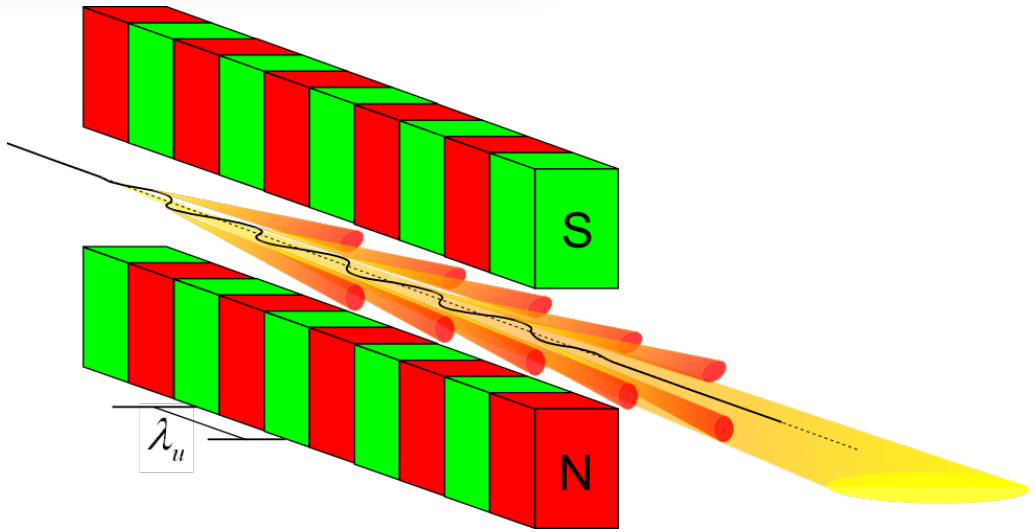


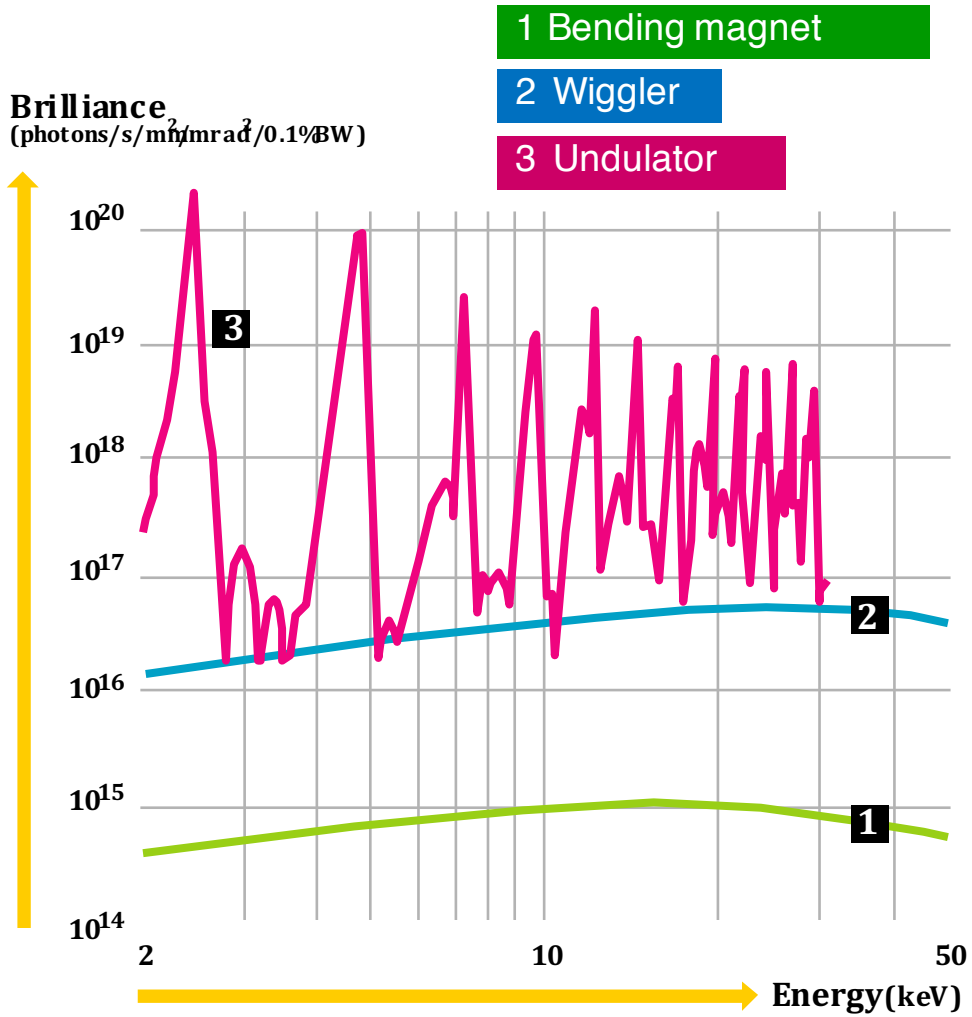


Emission spectrum

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



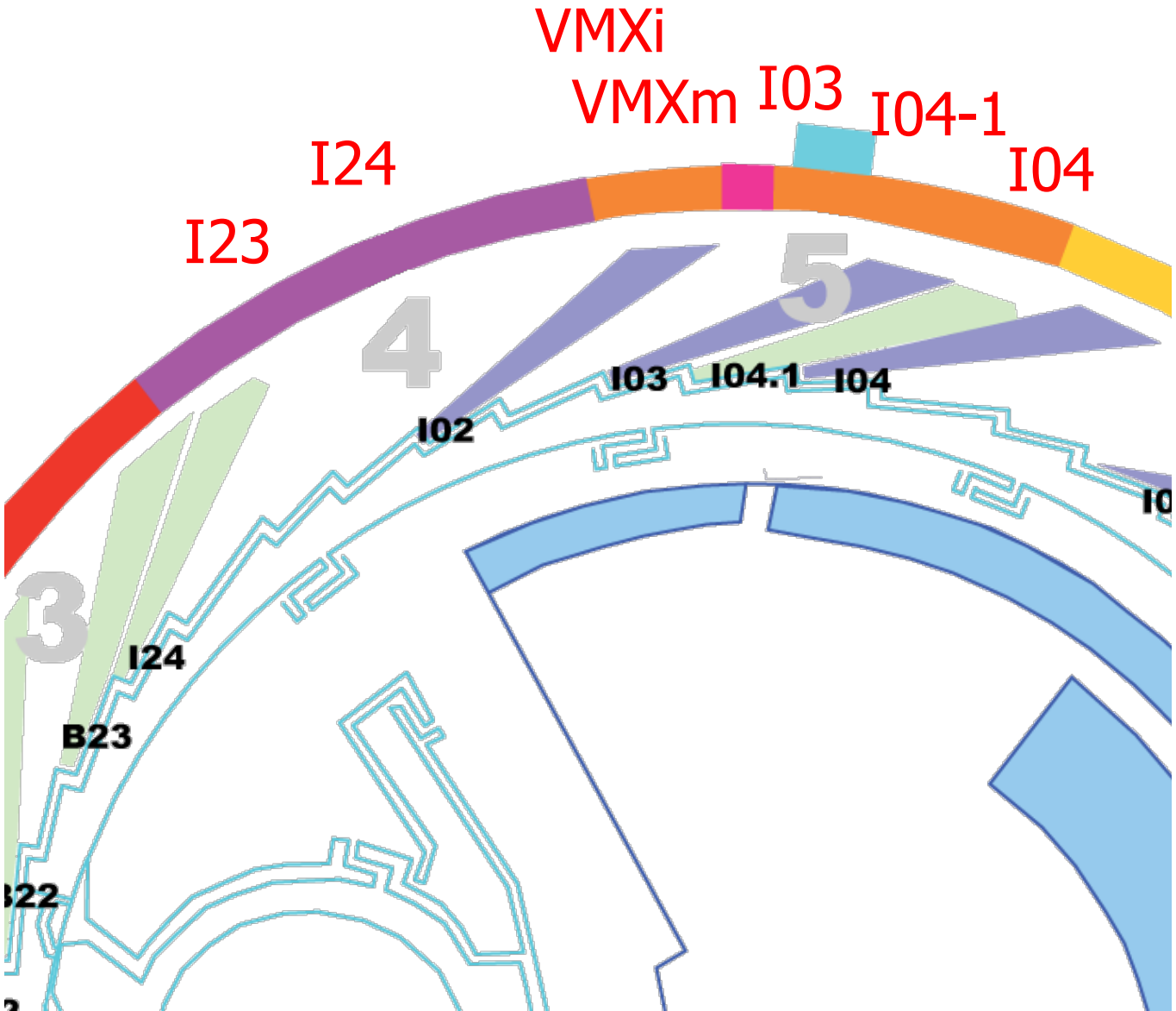
B24 Cryo-TXM
Microfocus MX I24
I02 MX: Macromolecular Crystallography
I03 MX
Circular Dichroism B23
I04-1 Monochromatic MX
Long Wavelength MX I23
I04 MX
Infrared Microspectroscopy B22
I05 ARPES
I06 Nanoscience
Non-Crystalline Diffraction I22
I07 Surface & Interface Diffraction
High throughput SAXS B21
B07 VERSOX: Versatile Soft X-ray Beamline
Inelastic X-ray Scattering I21
I08 Soft X-ray Microscopy
LOLA: X-ray Spectroscopy I20
I09 SISA: Surface & Interface Structural Analysis
Small Molecule Single Crystal Diffraction I19
Core EXAFS B18
I10 BLADE: X-ray Dichroism & Scattering
Microfocus Spectroscopy I18
I11 High Resolution Powder Diffraction
Test beamline B16
Materials & Magnetism I16
X-ray Pair Distribution I15-1
Extreme Conditions I15
JEEP: Joint Engineering, Environmental and Processing
Hard X-ray Nanoprobe for Complex Systems I14
I13 X-ray Imaging (LI) and Coherence (LC)

E-XFEL
eBIC

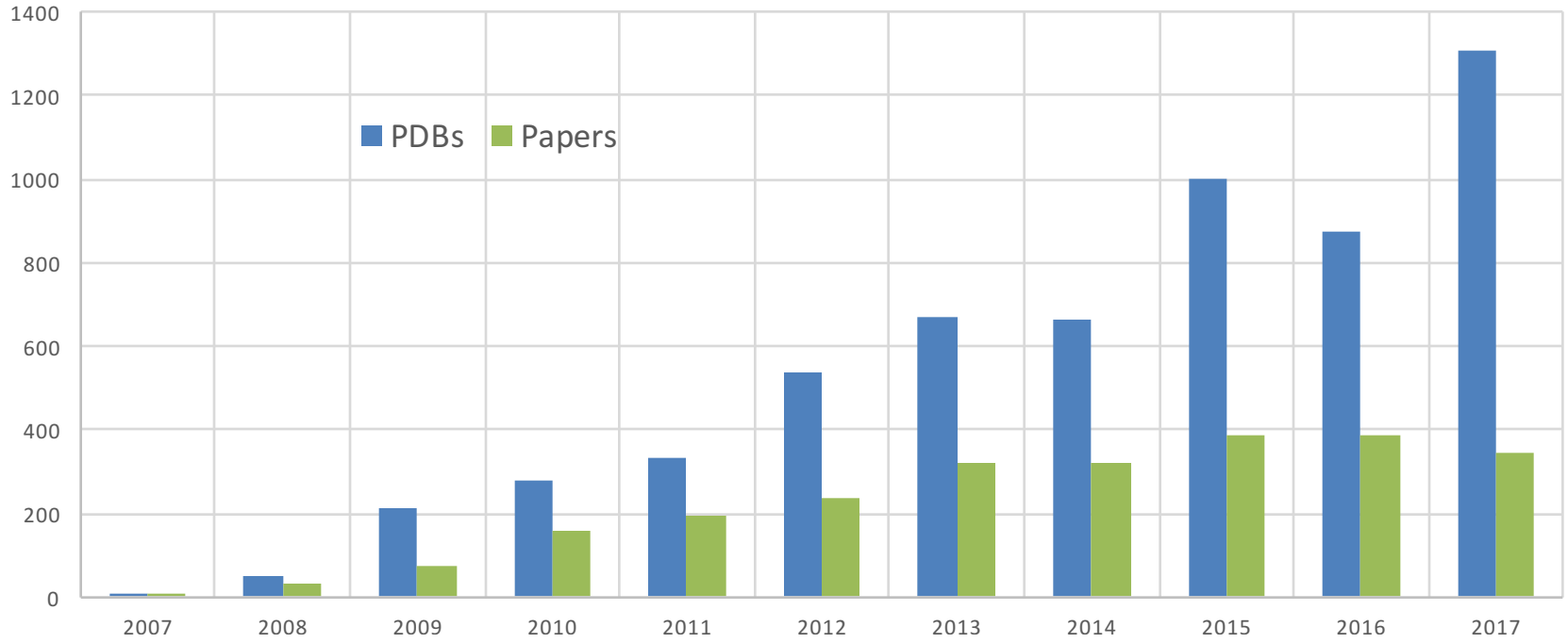
Macromolecular Crystallography
Soft Condensed Matter
Spectroscopy
Materials
Engineering & Environment
Surfaces & Interfaces

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

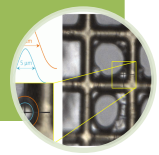
- PADs
- Automation
- Pipelines
- Serial Crystallography
- Access (rapid/freq)

High Throughput



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

Microfocus



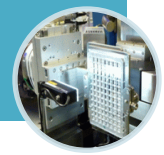
- Tuneable beamlines
- I23 dedicated
- Smart data collection strategies
- Expert systems

Long Wavelength



- Screening
- Data collection
- CLIII
- VMXi

In situ



- Pipelines
- Kappa strategies
- Inverse beam
- Long wavelength

Experimental Phasing



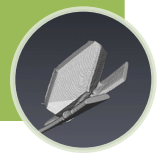
- *In situ*
- Containment Level III
- Schedule 5

Biological Containment



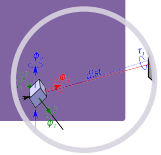
- Humidity control
- Spectroscopy
- Tomography
- Dynamics
- Fluorescence

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



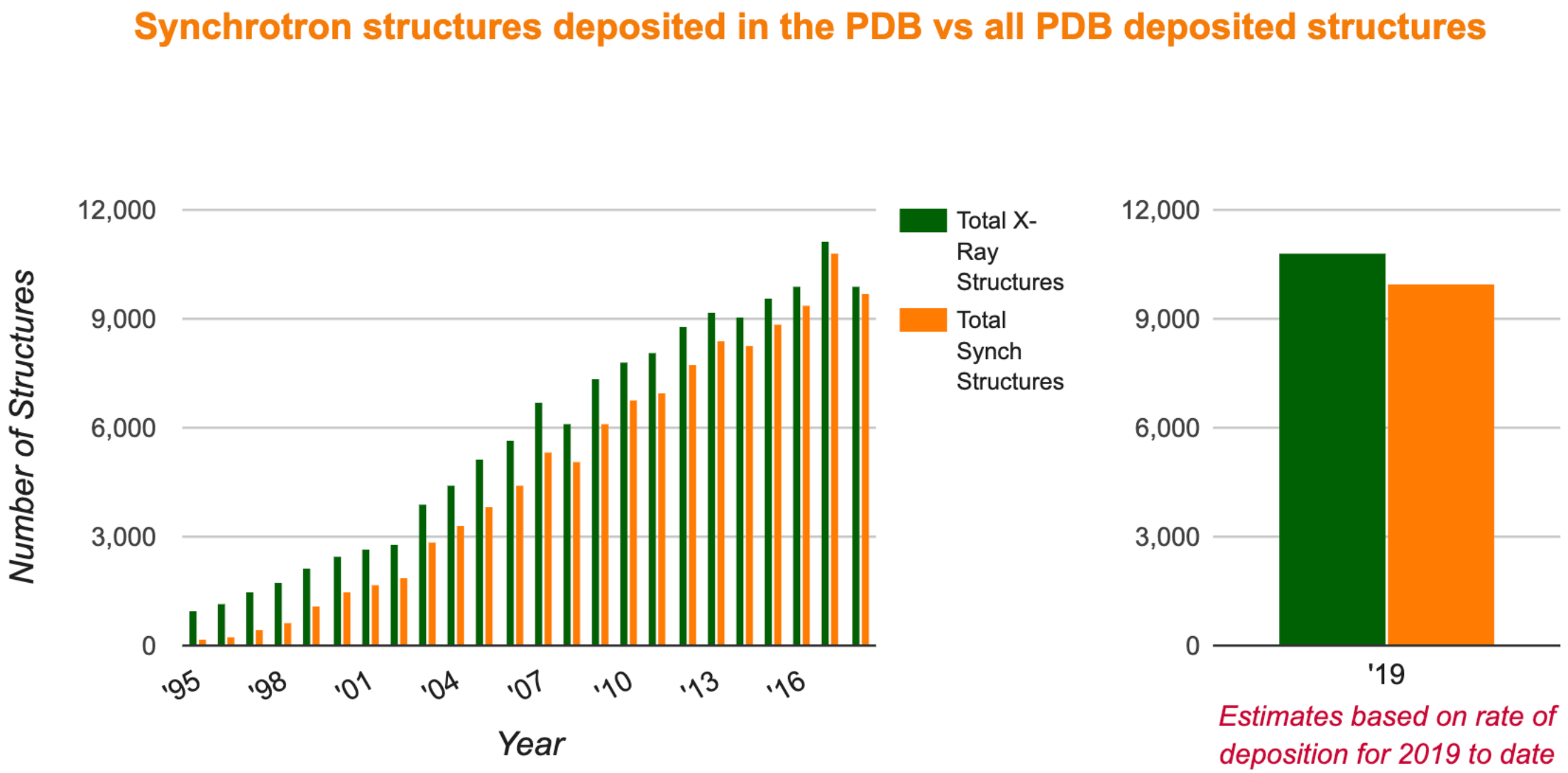
Why do we need a synchrotron for MX experiments?

- 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

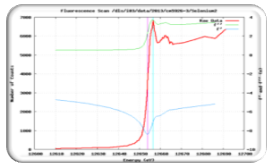
- Allows us to target more challenging samples

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

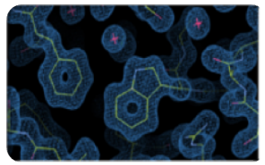


Reasons for using MX beamlines at a synchrotron



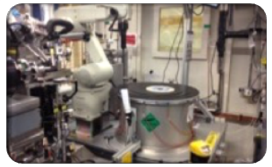
Phasing experiments

- Tune wavelength for SAD, MAD etc



High brilliance

- Good for weakly diffracting samples



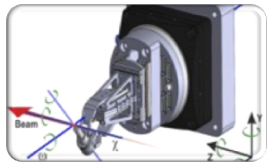
High throughput

- Fast robots & detectors



Special Conditions

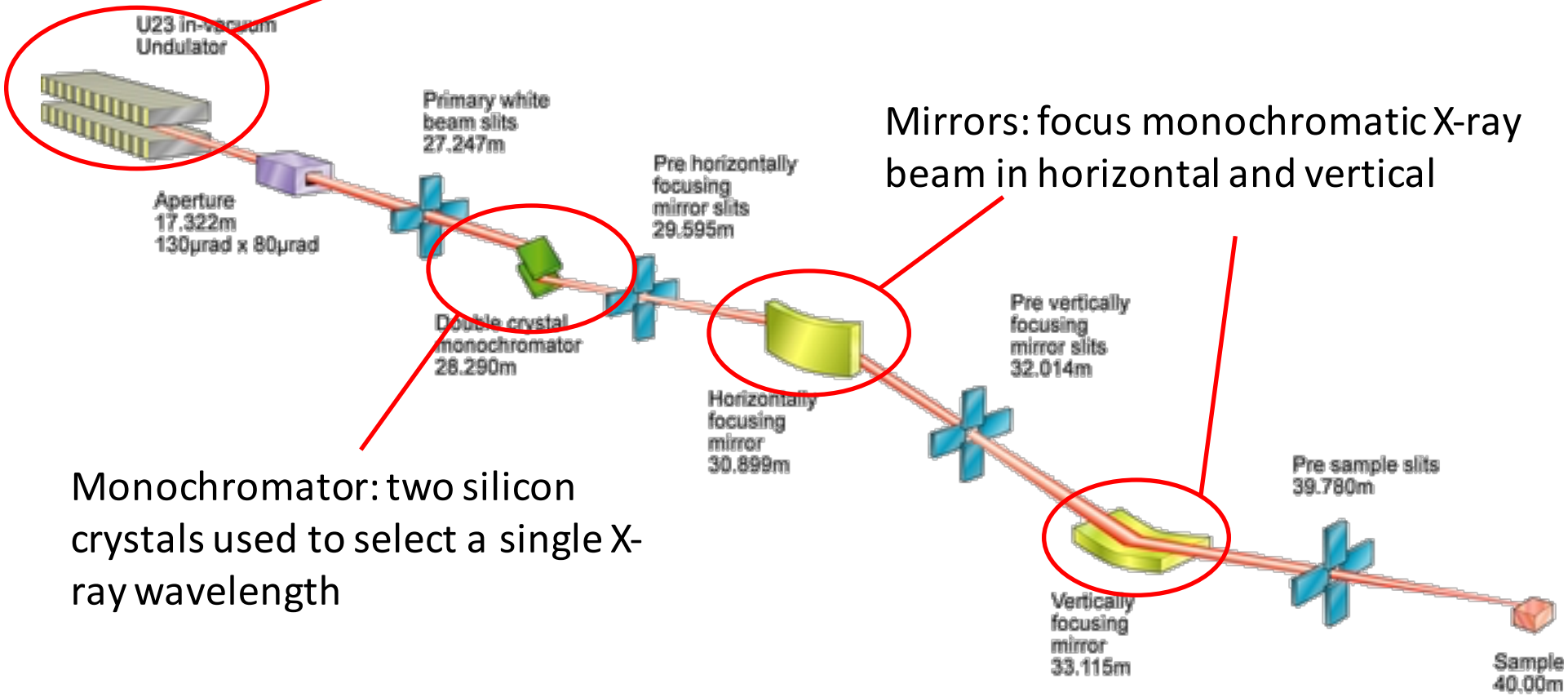
- *In situ,* HC1, fluorescence spectra etc



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

Properties of the X-ray beam

Monochromator

Typically use (111) reflection from Si crystals

Cryogenically cooled to liquid nitrogen temperature (~80 K) to improve thermal stability

Mirrors

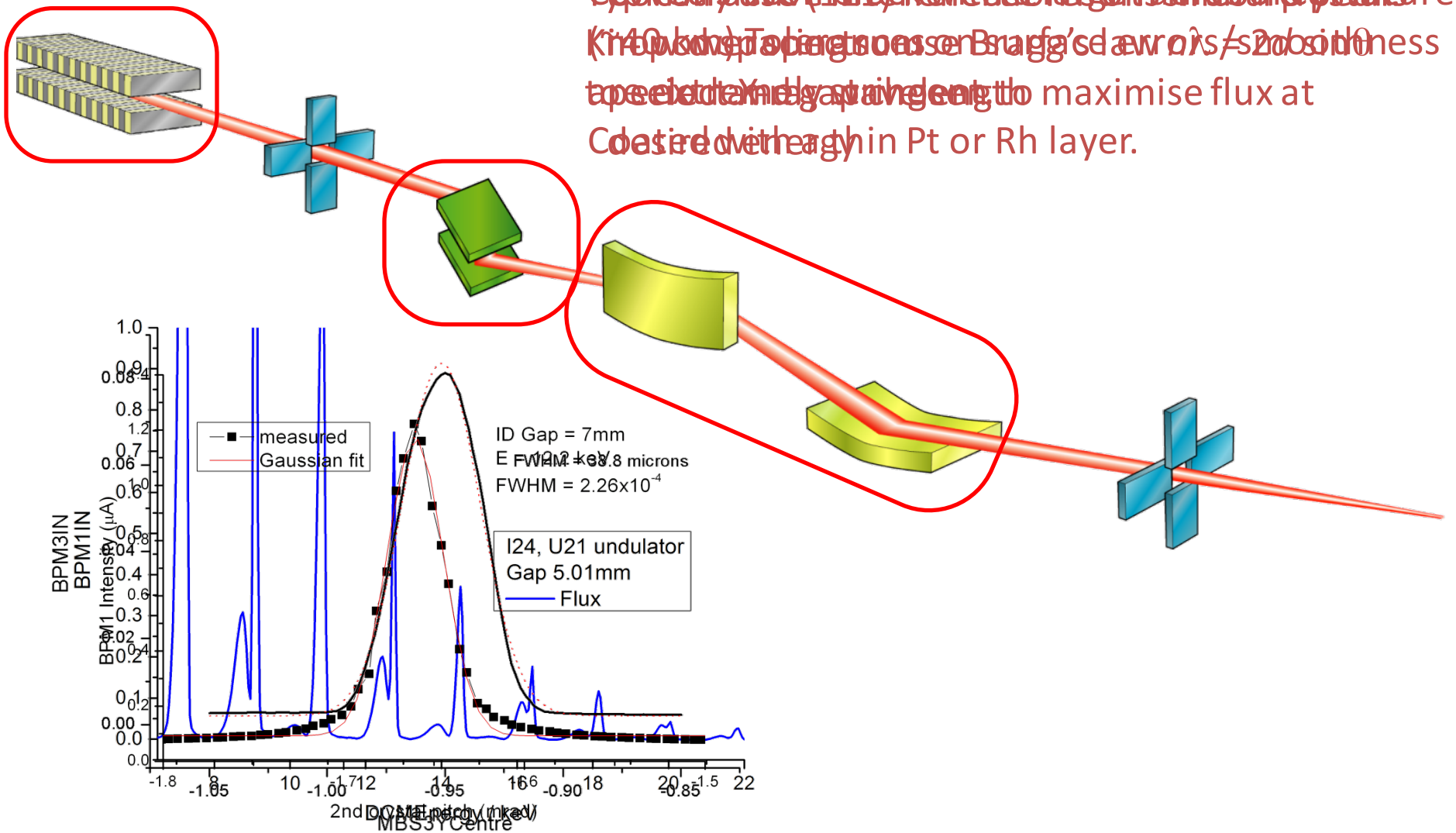
Typically use 1 to 3 mirrors to focus beam

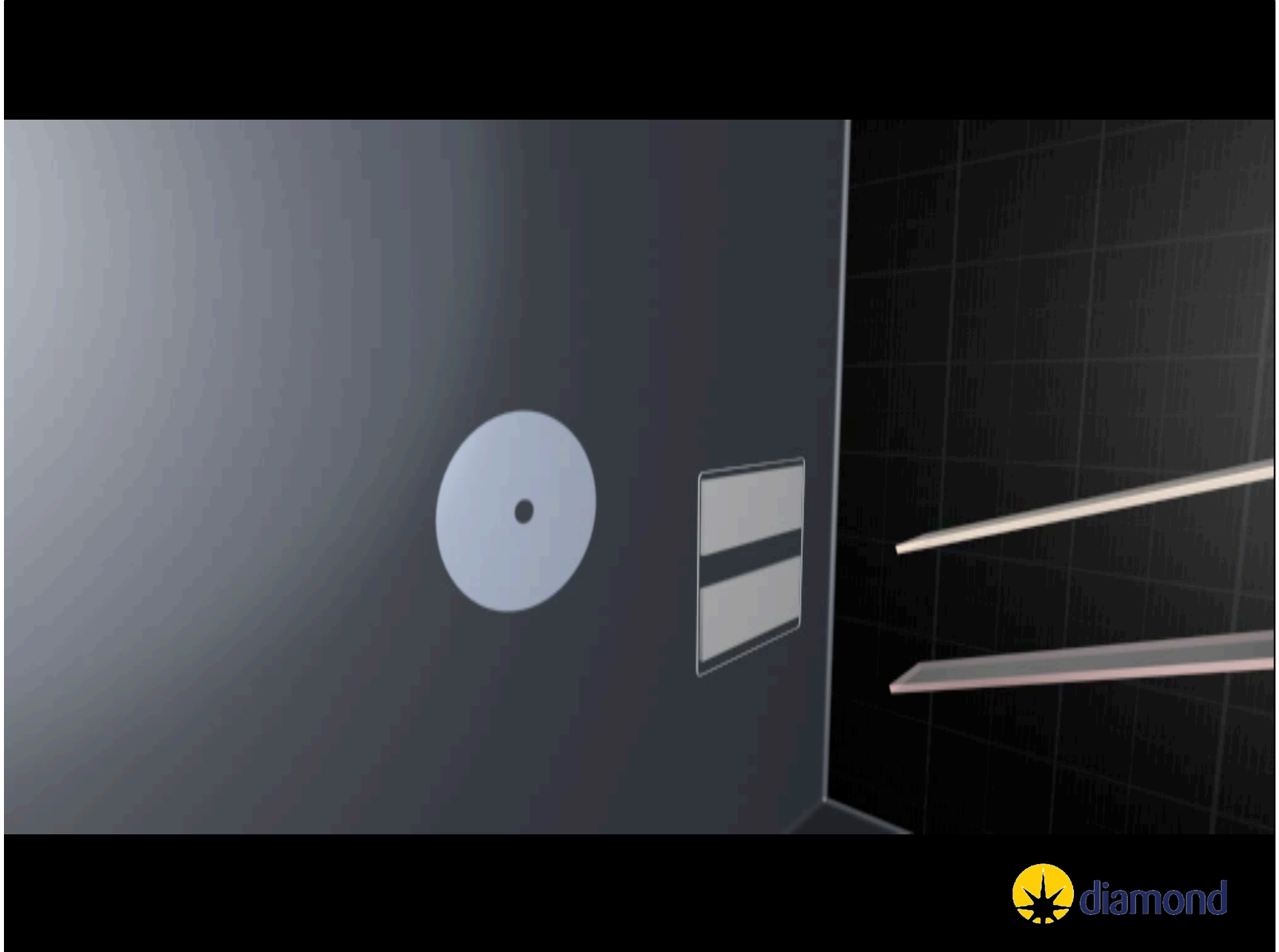
Coated with single layer of Pt or Rh

Undulator

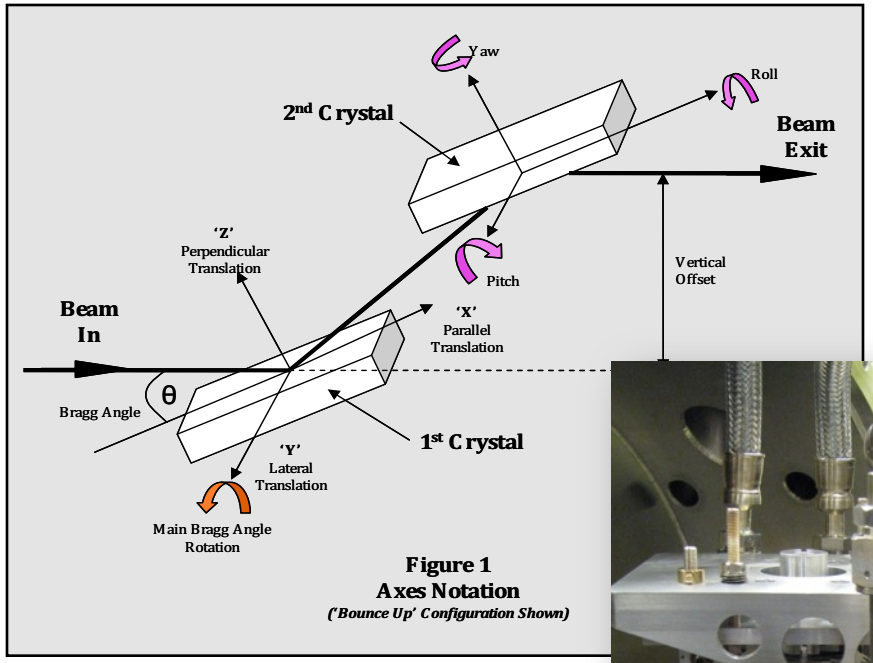
Typically use 1st harmonic of undulator

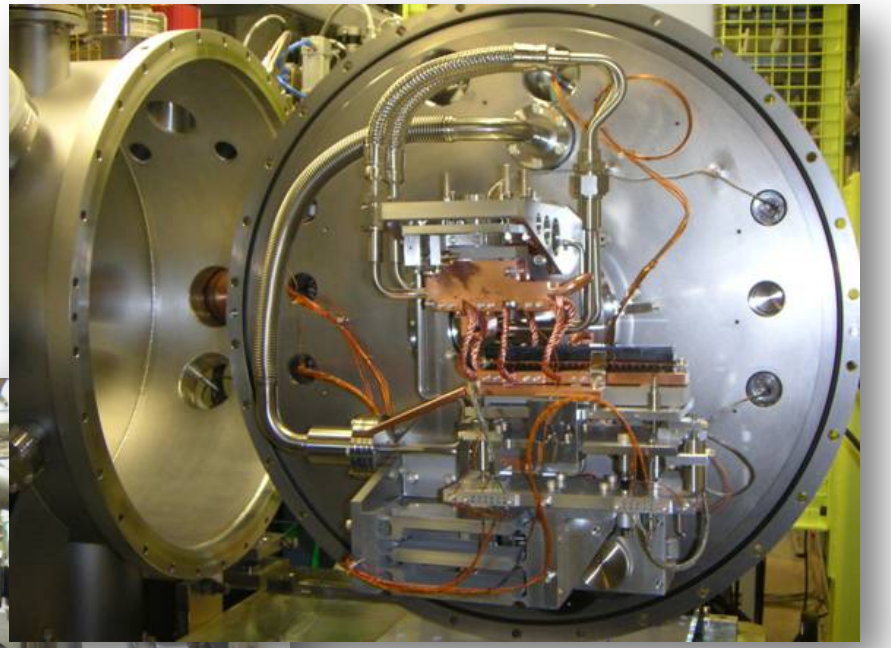
Tapered undulator gap to maximise flux at desired energy

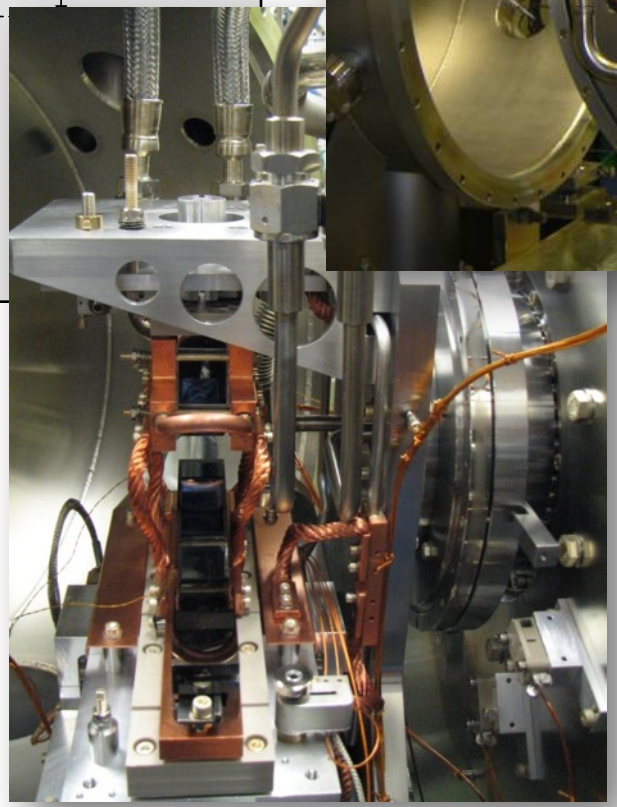



diamond

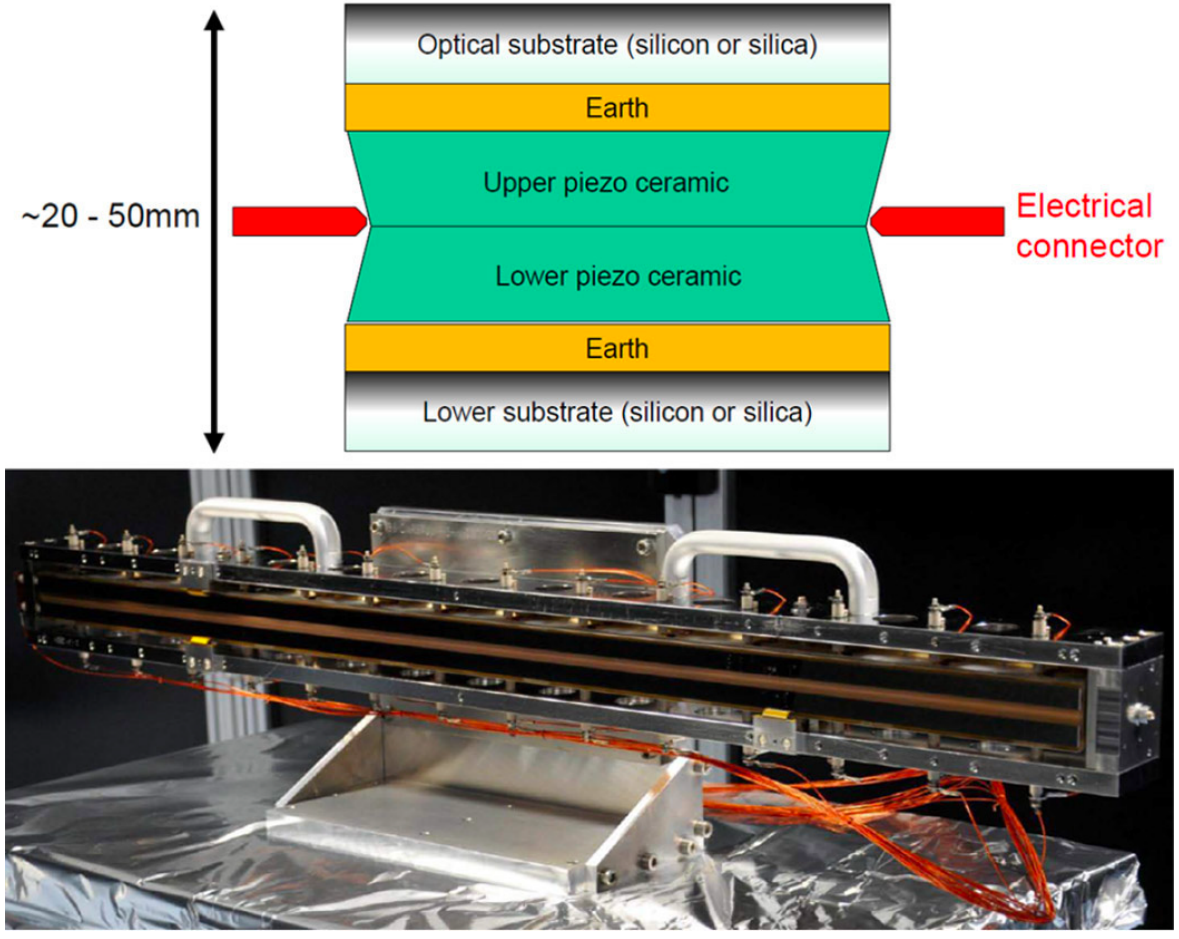
A double crystal monochromator is used to select the X-ray energy (wavelength)

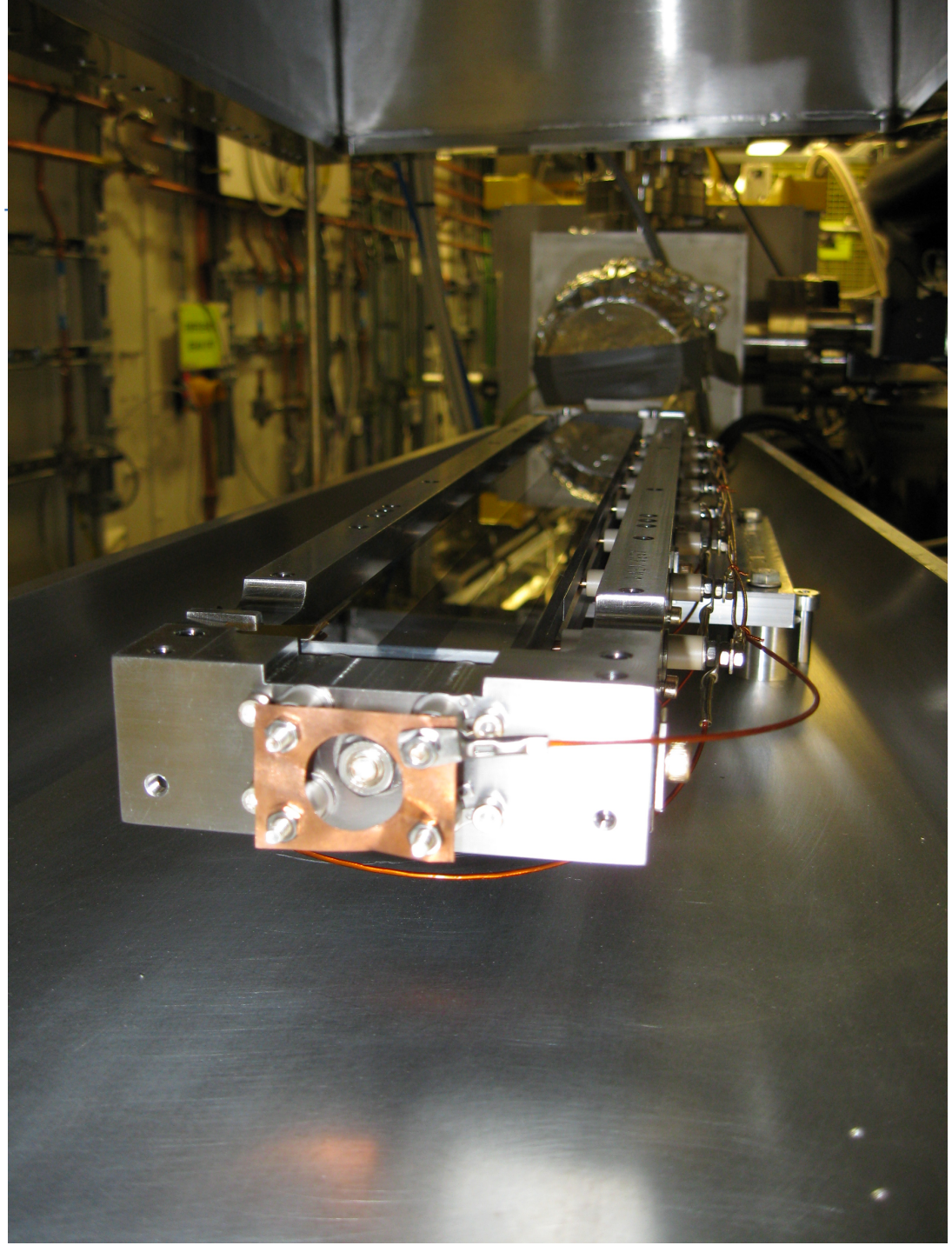




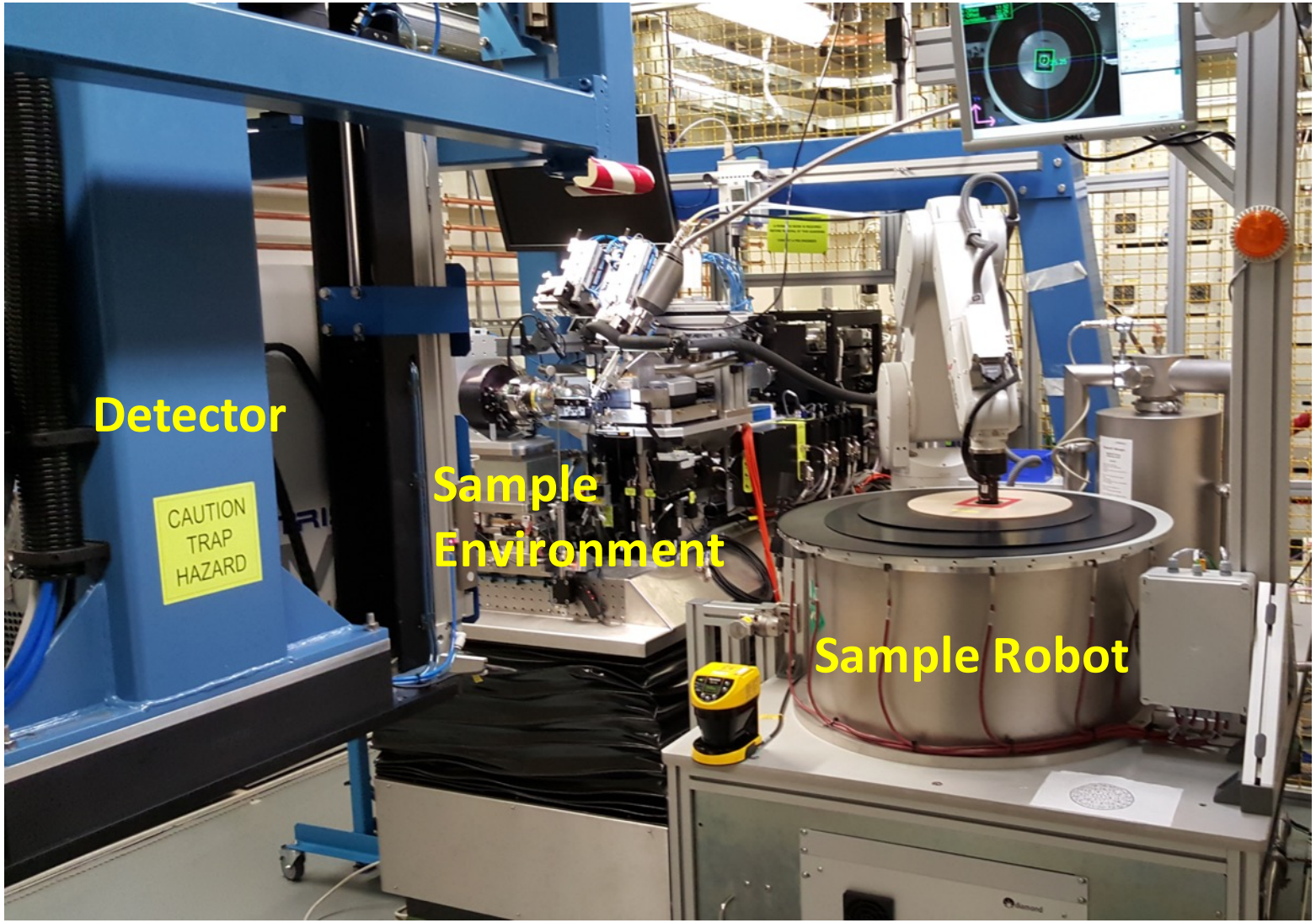


Bimorph focusing mirror

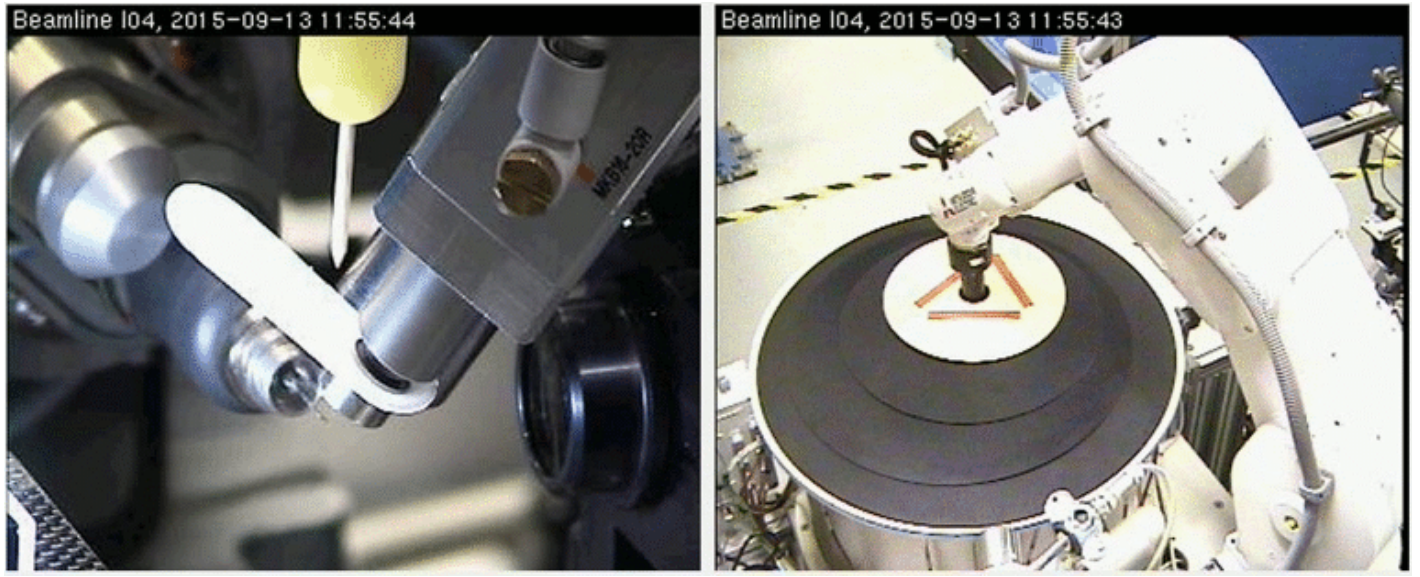




The experiment hutch contains beam conditioning elements, the sample environment and detector

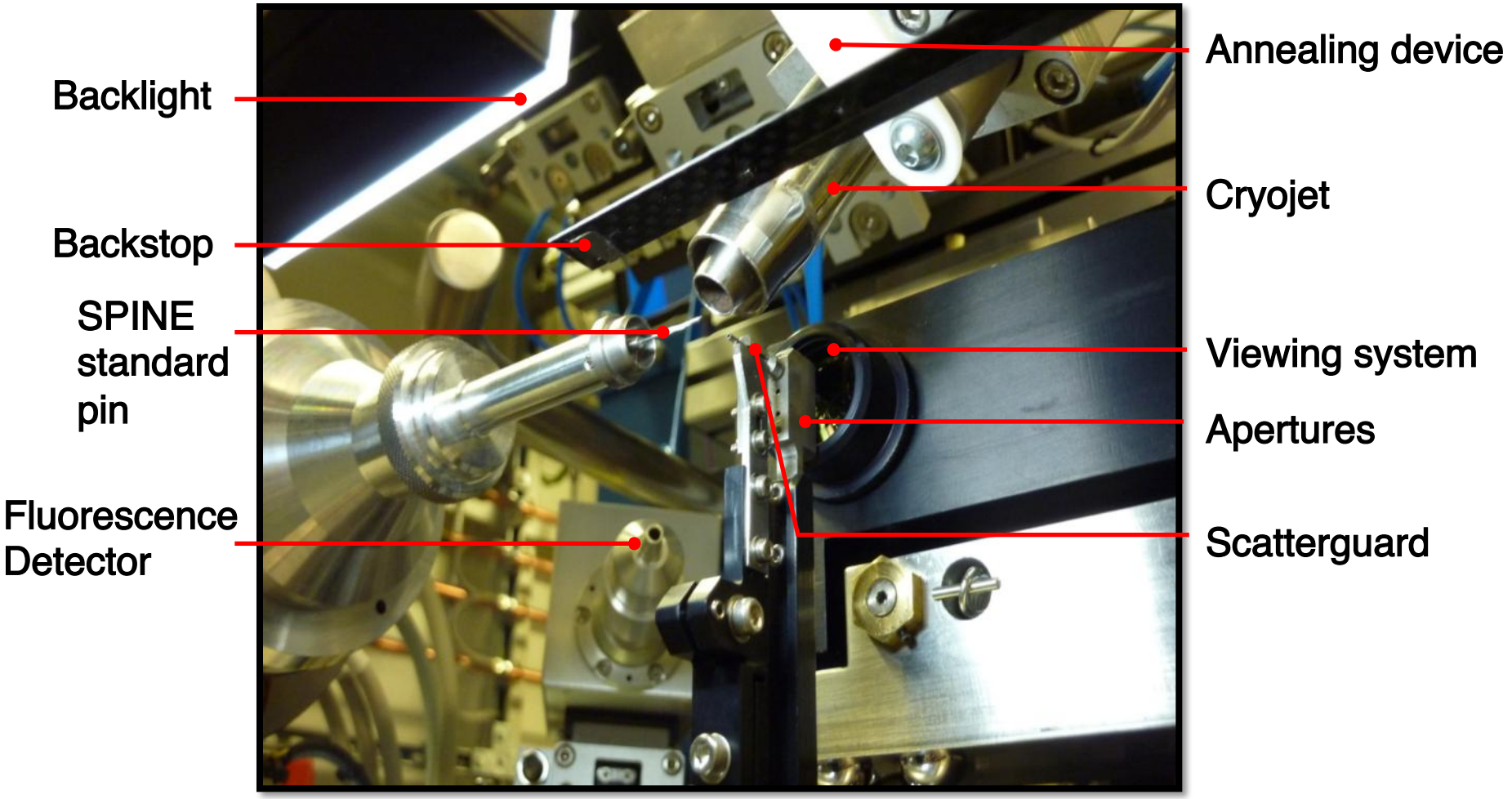


High-throughput of samples requires fast robotics



- 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

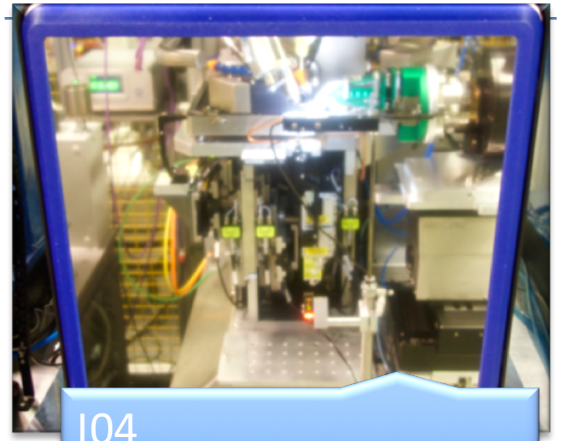


Detectors on the MX beamlines



I03

- Eiger2 XE 16M



I04

- Eiger2 XE 16M



I04-1

- Pilatus2 6M-F



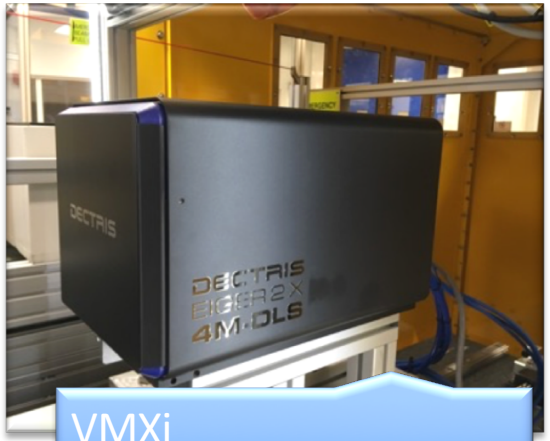
I23

- Pilatus2



I24

- Pilatus3 6M



VMXi

- Eiger2 X 4M



VMXm

- Pilatus3 6M

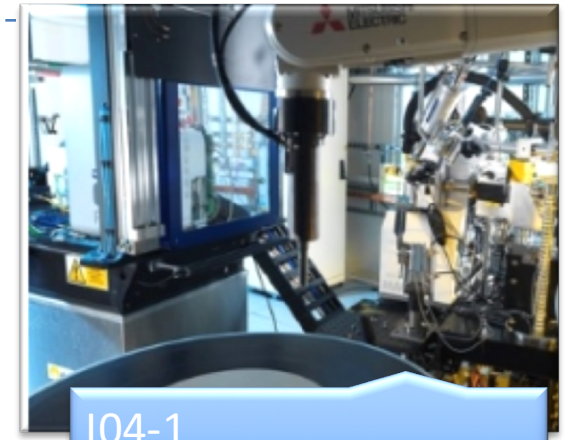
Detectors on the MX beamlines



I03
- Eiger2 XE 16M



I04
- Eiger2 XE 16M



I04-1
- Pilatus2 6M-F

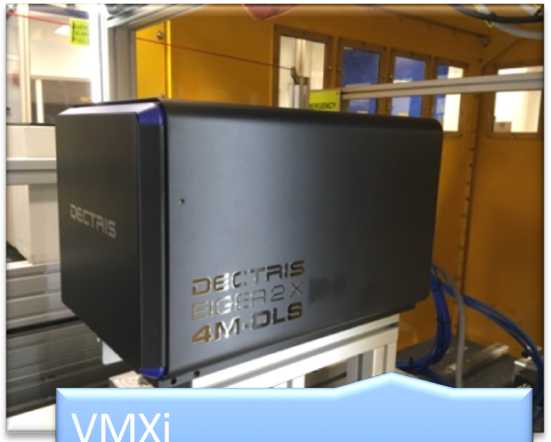


I23
- Pilatus2



CdTe Eiger 9M for 2020

I24
- Pilatus3 6M



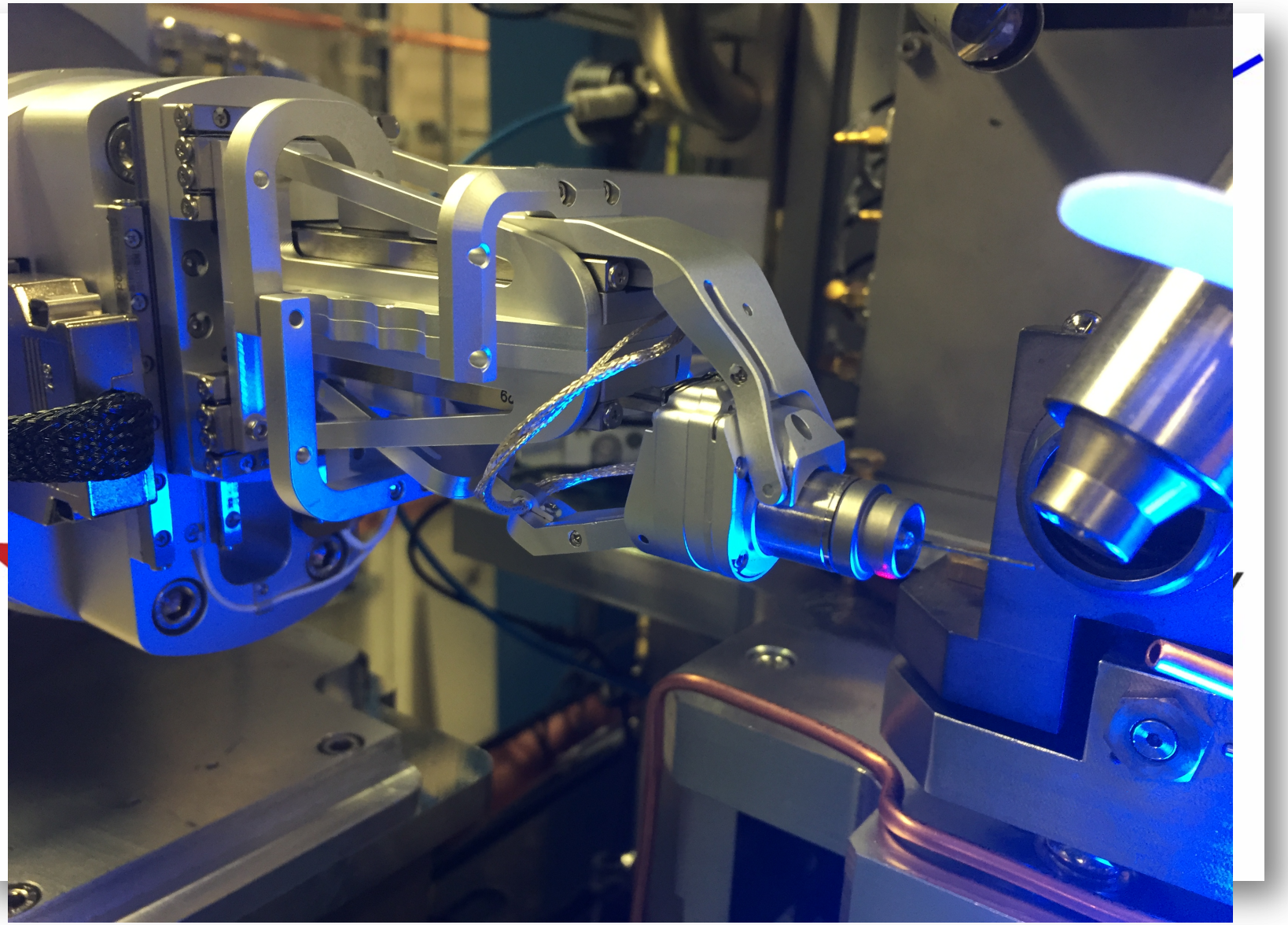
VMXi
- Eiger2 X 4M



CdTe Eiger 9M for 2020

VMXm
- Pilatus3 6M

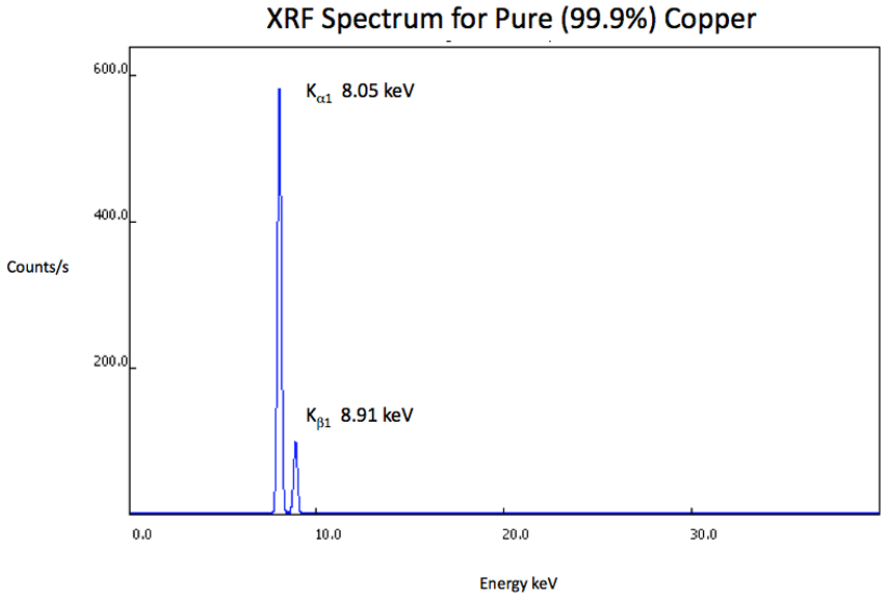
Crystal re-orientation – using multi-axis goniometry



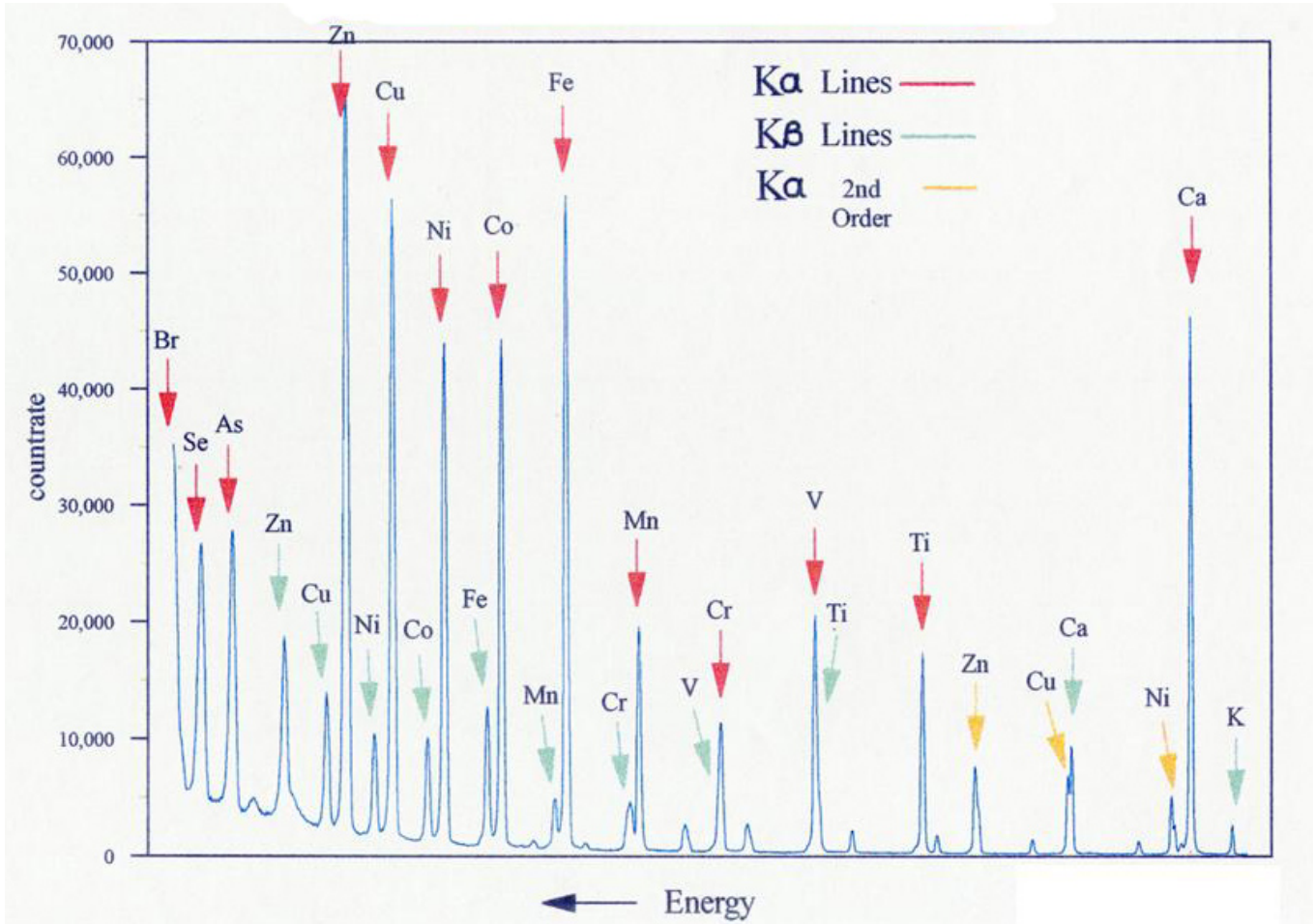
Fluorescence techniques - Metals in biology

- 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



By LinguisticDemographer at English Wikipedia - Transferred from en.wikipedia to Commons by Pieter Kuiper using CommonsHelper., Public Domain, https://commons.wikimedia.org/w/index.php?curid=6229332

Example spectra

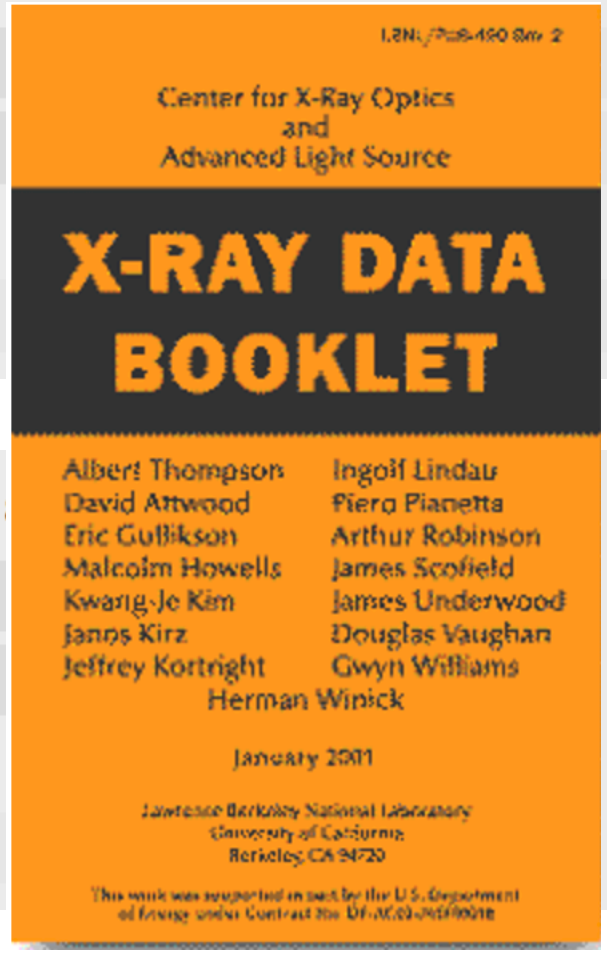
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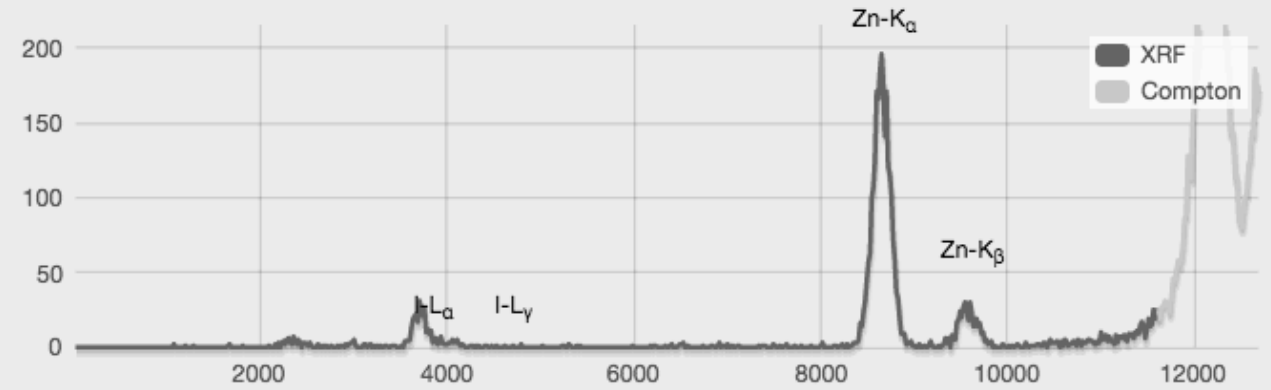
Sample: RNAP_1

Exposure: 1.00s

Beamsize: 20x20µm

Comment: null





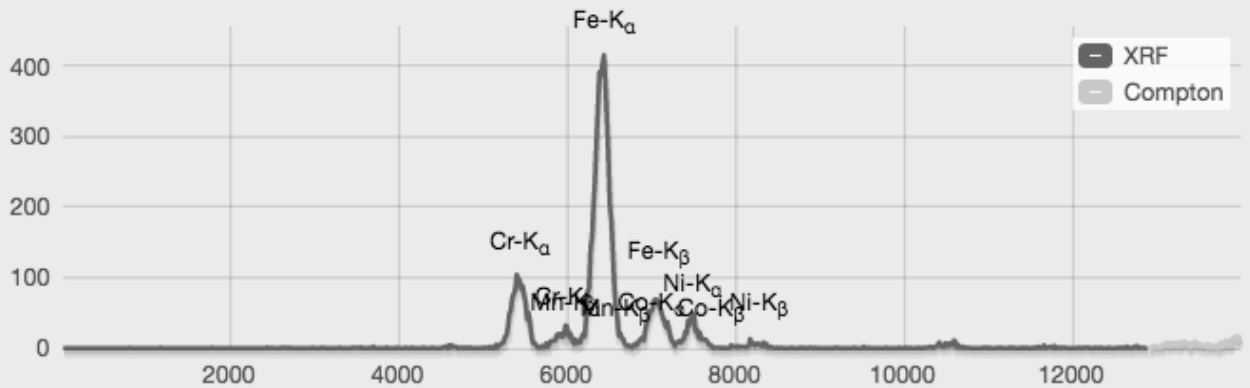
16-07-2018 16:34:48 - 201

Sample: n1642_4

Exposure: 1.00s

Beamsize: 80x20µm

Comment: Click to edit



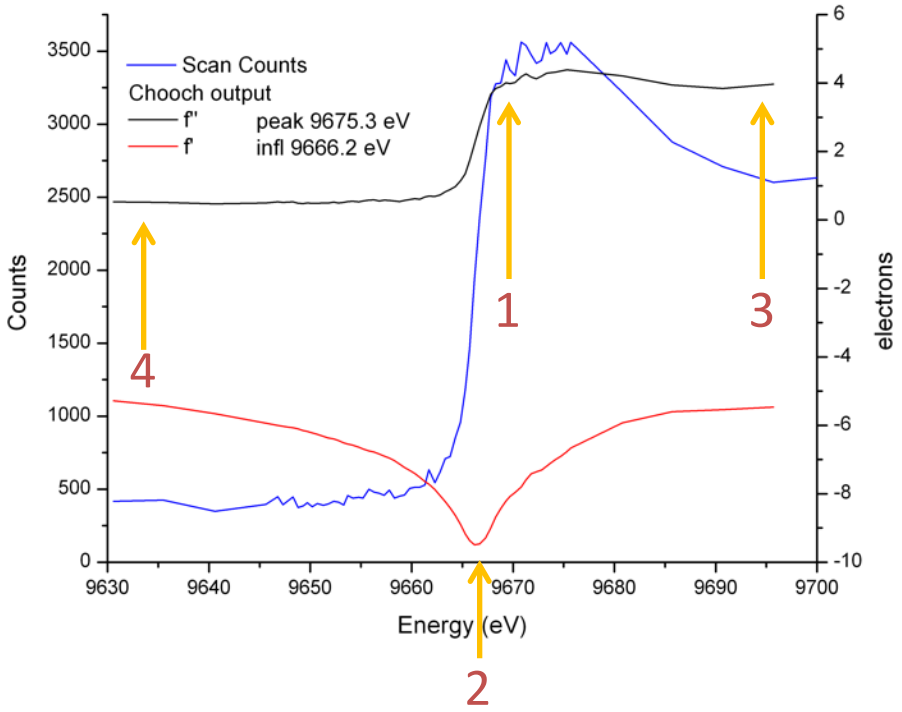
http://cxro.lbl.gov/x-ray-data-booklet

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

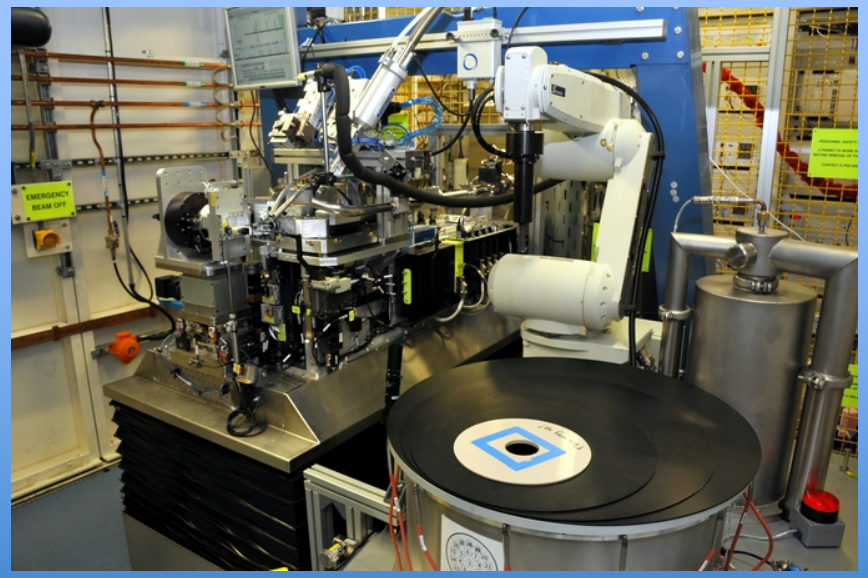
- High-throughput, I03 & I04
- Fragment screening I04-1, XChem
- Long-wavelength, I23
- Microfocus, I24
- VMXi and VMXm

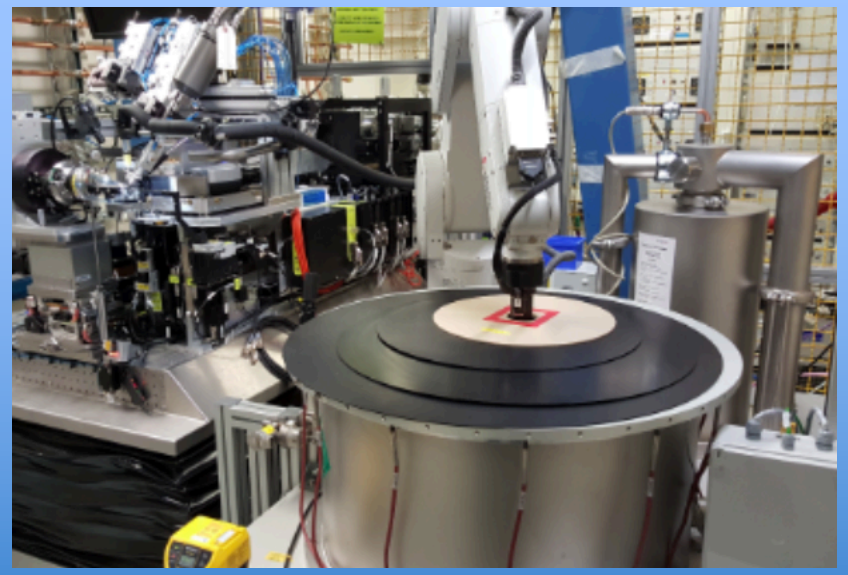
Automated single crystal experiment

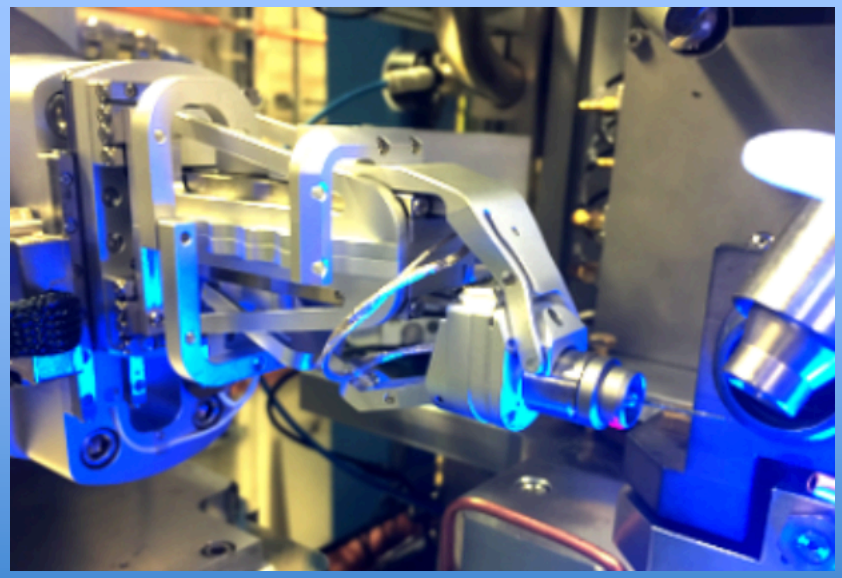
I03 & I04 High-throughput MX

- I03
- Focus with mirrors
  - 100 um, 50 um, 20 um
- Eiger2 XE 16M (500 Hz)
- Multi-axis goniometry
- Biocontainment

- I04
- Focus with CRLs
  - Variable beam sizes, inc microfocus
- Eiger2 XE 16M (500 Hz)
- Multi-axis goniometry

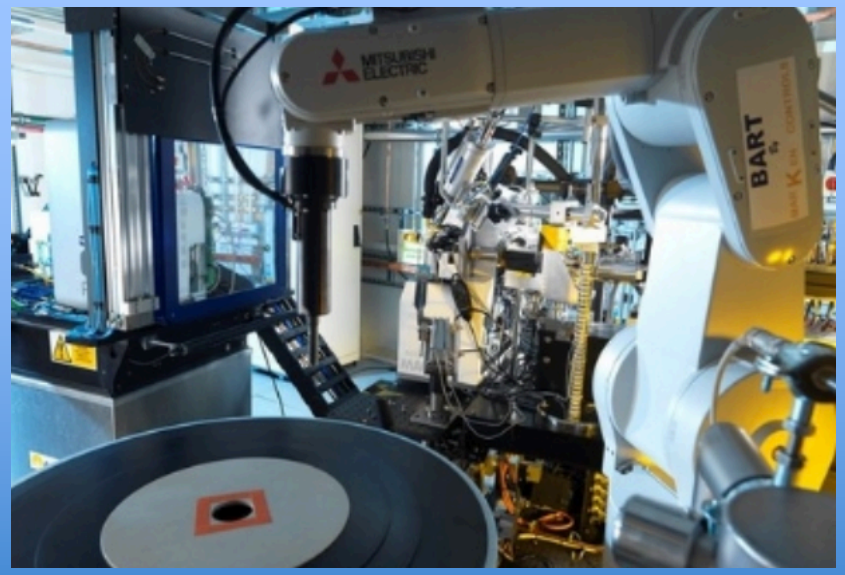






I04-1 Fixed Wavelength MX

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

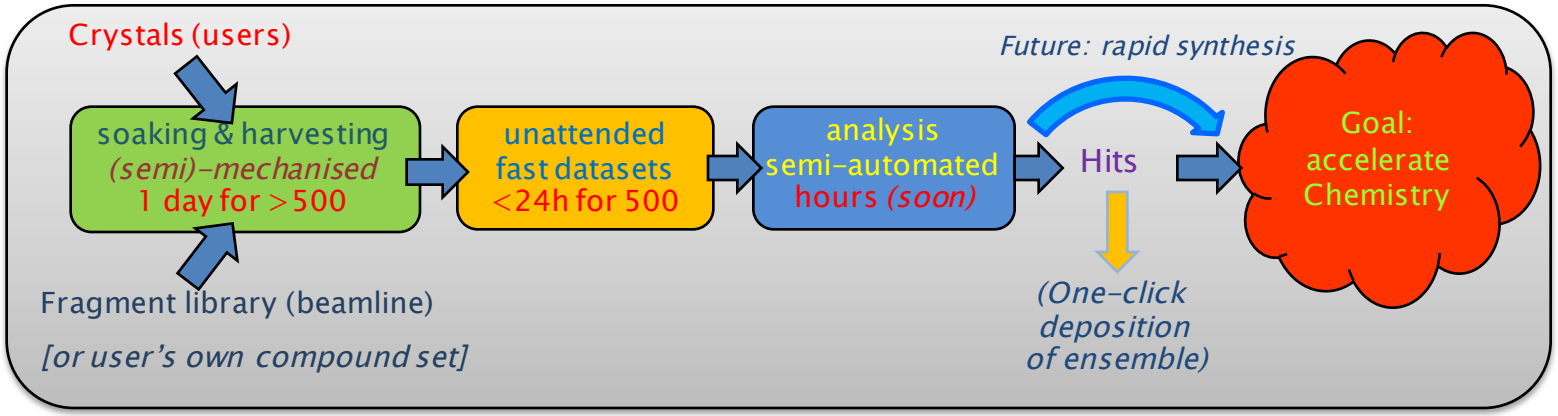






Xchem User Program @I04-1

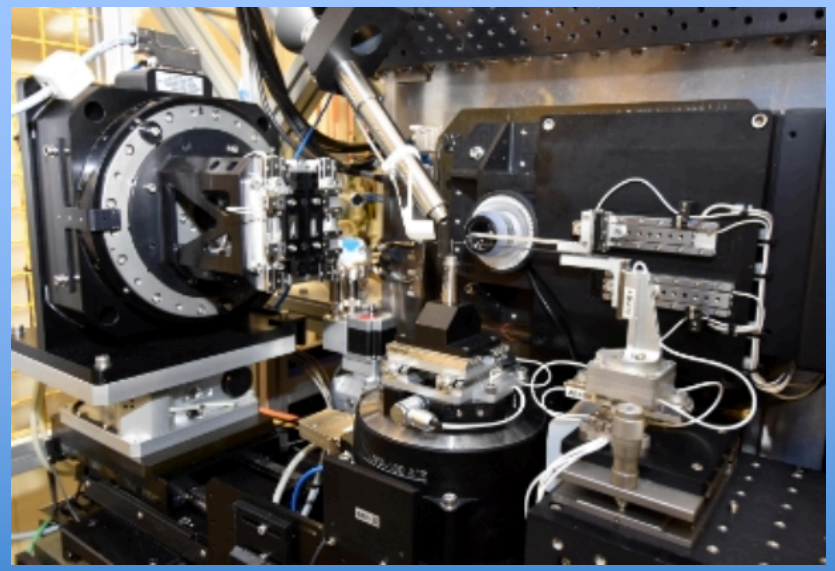
- A world-wide first facility: **fragment screening by crystal structure**
  - *User: bring crystals to beamline Beamline provides: advice (!!), fragment library, rapid soaking, data collection & analysis*
  - *Best case: 1 wk for whole experiment (500-1000 crystals) Capacity: >30 experiments/year;*

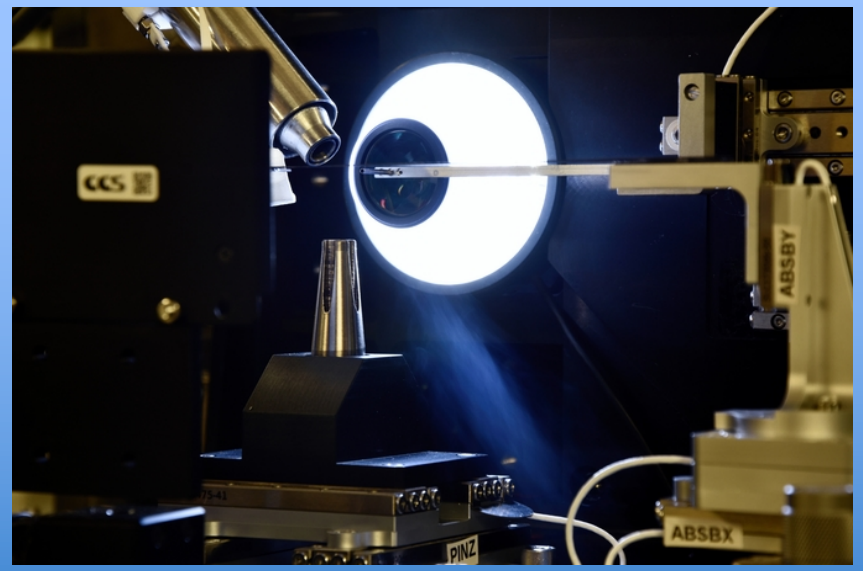


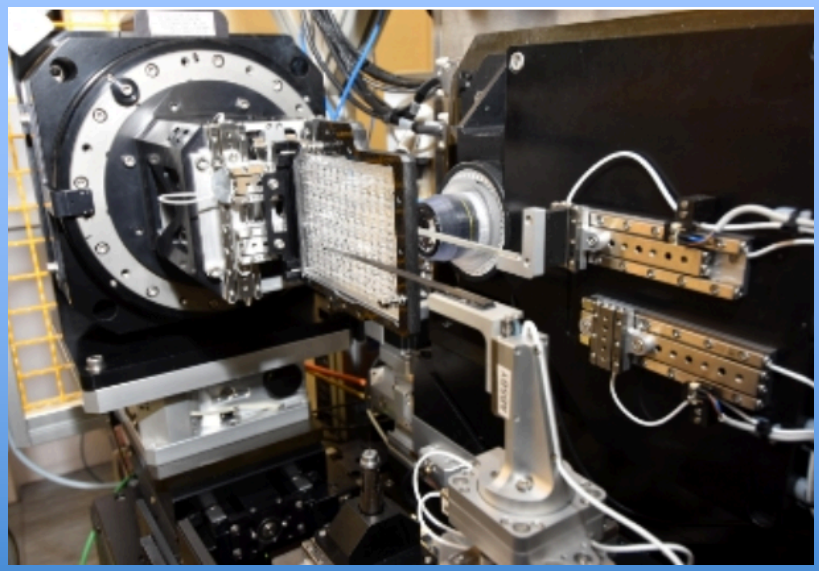
- New fragment library (DSPL) – 770 compounds. (Cox *et al*, Chem. Sci., 2016)
- New analysis tools deployed:
  - *PanDDA*: multi-dataset 3D background correction – highly sensitive ligand identification (Pearce et al Nat. Comms, 2017)
  - *XChemExplorer*: batch-wise analysis of 100s of datasets (Krojer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

I24 Microfocus Beamline

- Beamsize: 5x5 - 50x40 (variable aspect ratio)
- Detector: Pilatus3 6M, 100 Hz
- Wavelength: 0.62Å - 1.77Å
- Dual goniometer end-station – easy swap between *in situ* and cryo samples

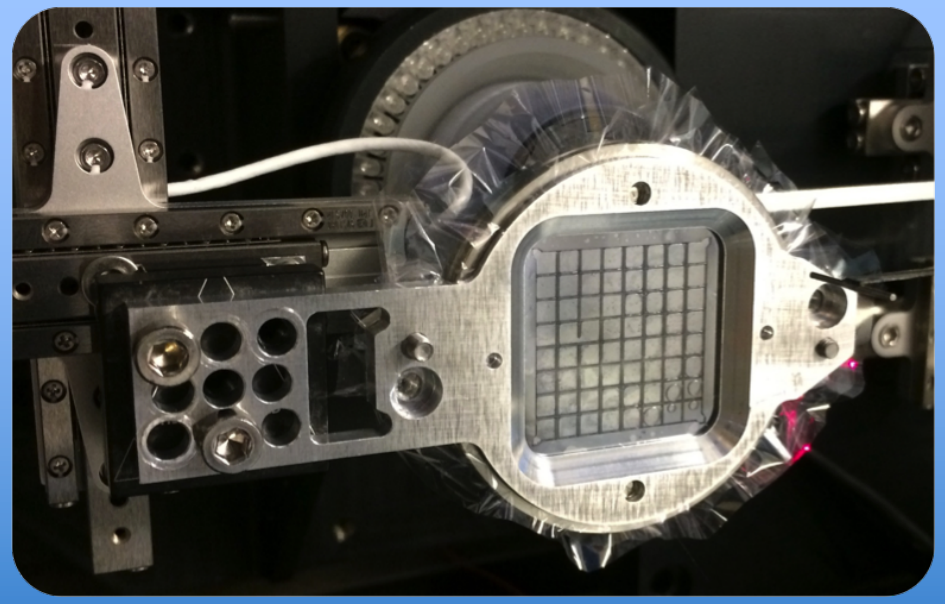


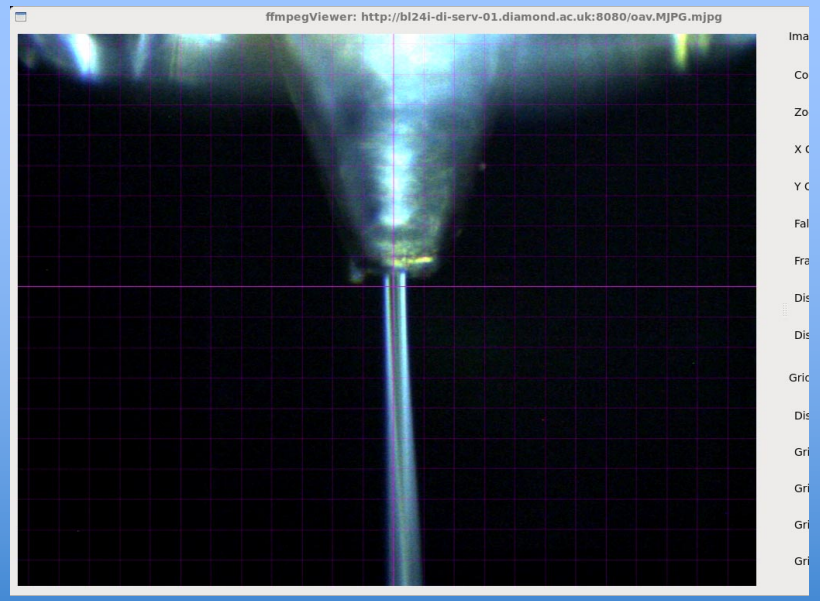




SSX at I24

- Serial Synchrotron Crystallography (SSX) available on request
  - Fixed target serial crystallography
  - LCP extruder
  - Thin film data collection
  - https://www.diamond.ac.uk/Instruments/Mx/I24/I24-serial.html





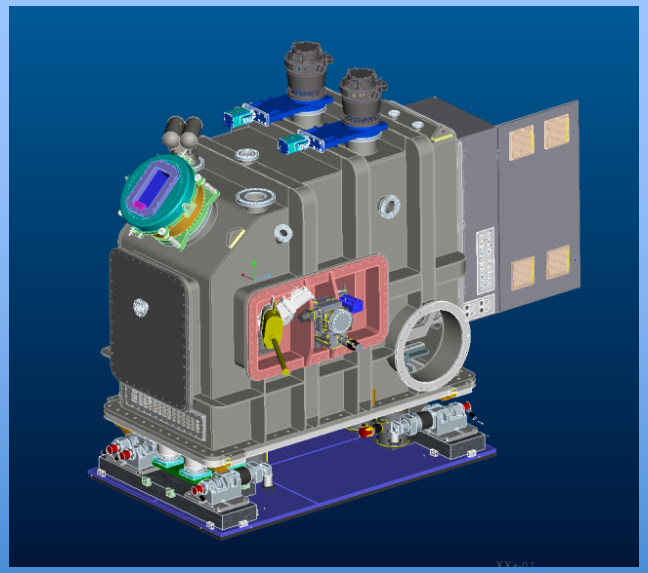


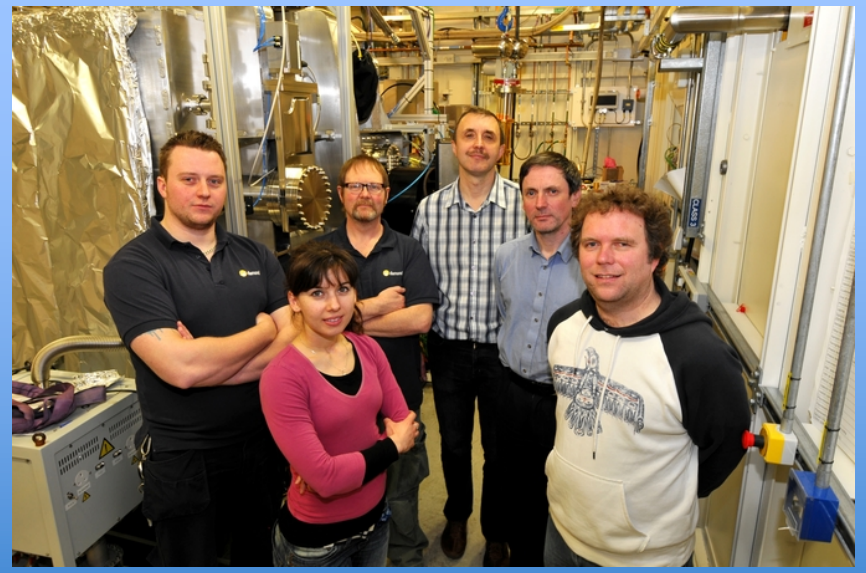
I23 Long wavelength MX

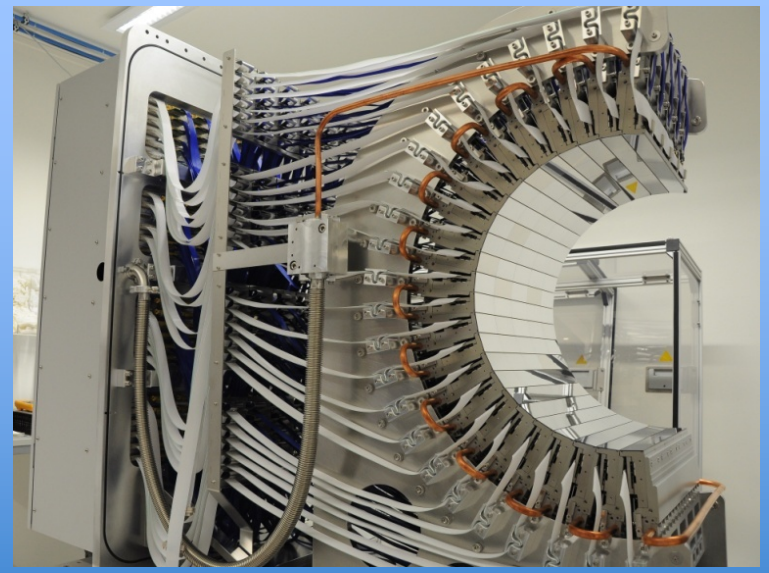
- I23 first MX beamline optimized for the long-wavelength region (1.5 – 4 Å).

- 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



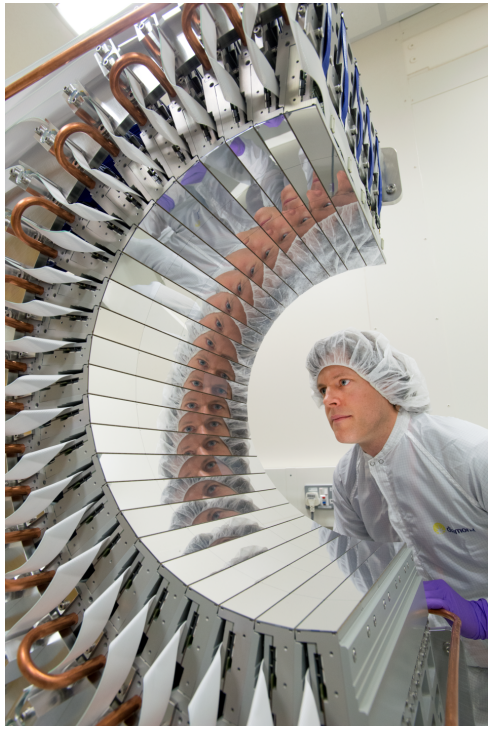




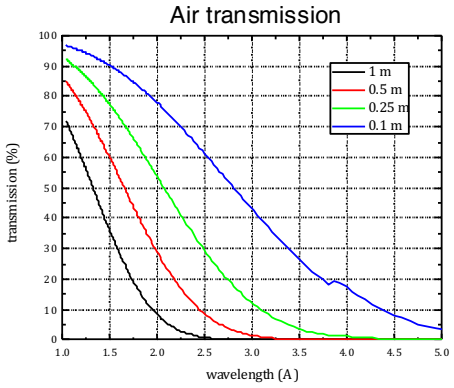
Challenges at long wavelengths

- Limited resolution:
  - Bragg's law

Pilatus 12M (Dectris)



In-vacuum crystallography:
Elimination of air scattering and absorption



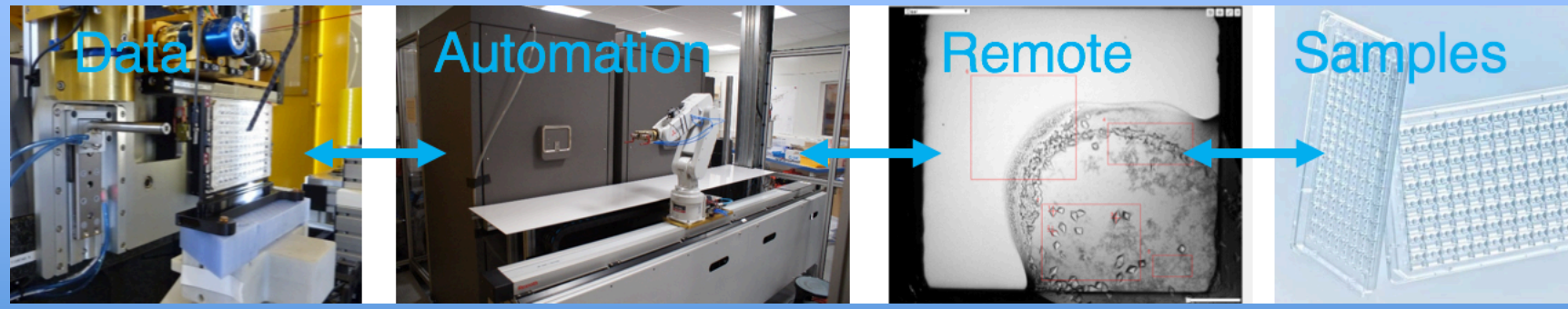
- Absorption:
  - Air absorption
  - Crystal, solvent and loop absorption



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

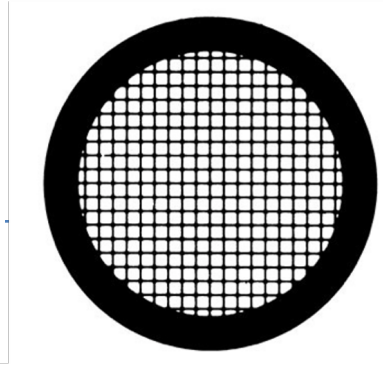
- Automated transfer of plates between storage and beamline
- Advanced imaging of drops (on-line and off-line)
- X-ray data collection and screening (automated or interactive)
- Tuneable microfocus beam (5x5 micron – 10-25 keV)
- Narrow or broad bandpass beam (2eV – 100eV)

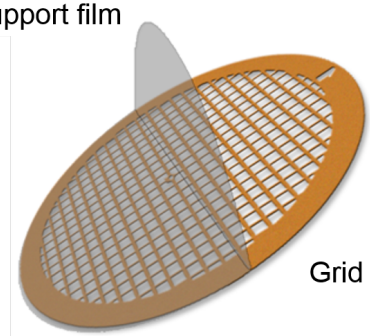


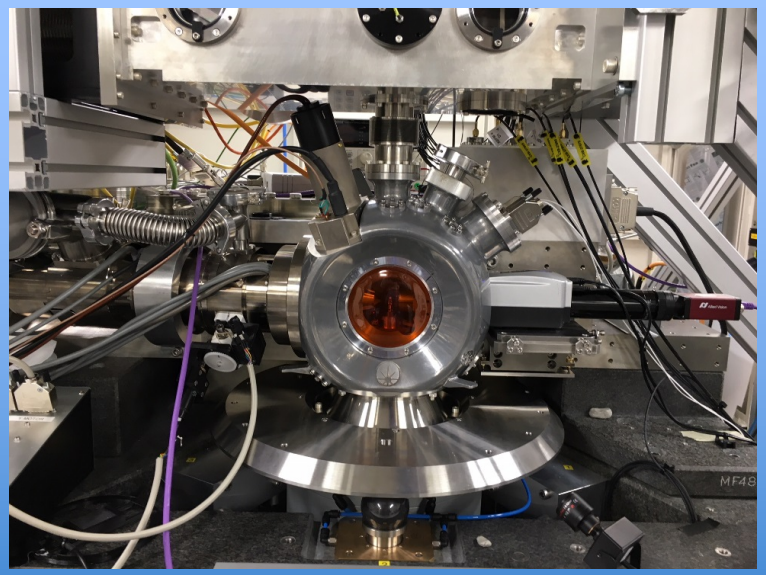


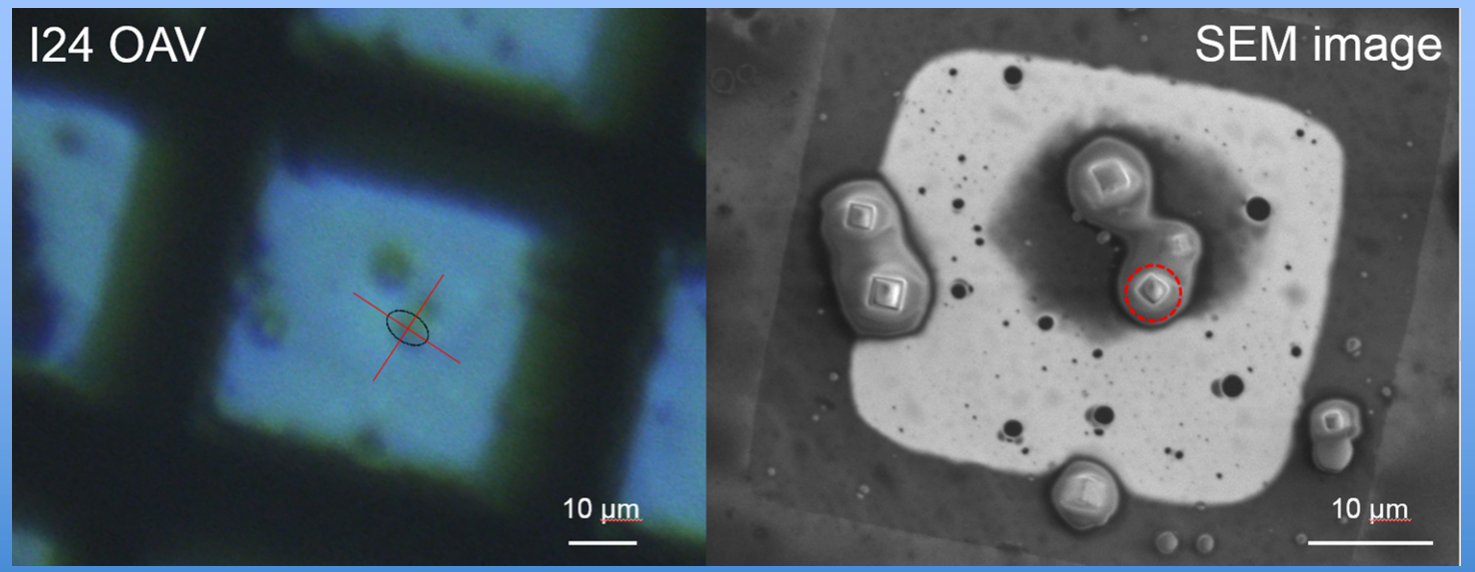
VMX_{micron} Sub micron variable focus

- < 500 nm beam size
- X-ray energies from 7 - 25 keV
- in vacuum sample environment
- Samples mounted on TEM grids
- SEM to locate crystals

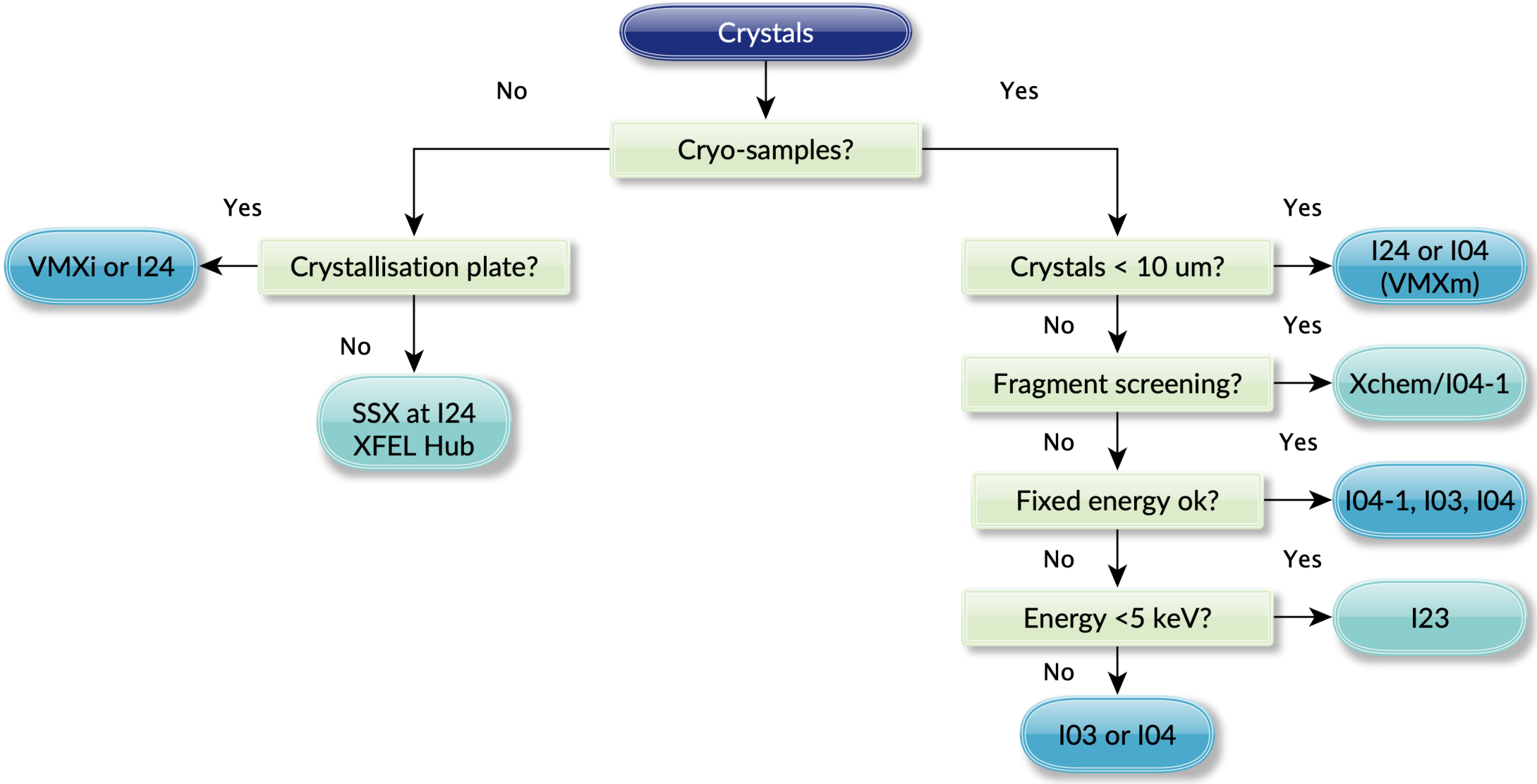






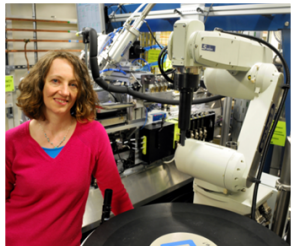


Choosing a beamline for your samples



MX Group

I03



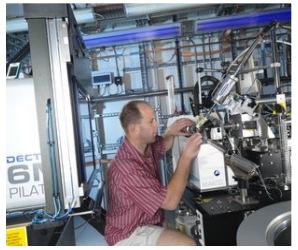
- **Katherine McAuley**
- Neil Paterson
- Mark Williams

I04



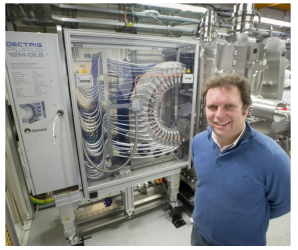
- **Ralf Flaig**
- David Aragao
- Pierpaolo Romano

I04-1 / XChem



- **Frank von Delft**
- Jose Brandao-Neto
- Alice Douangamath
- Louise Dunnett
- Anthony Aimon
- Romain Talon

I23



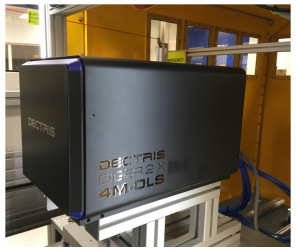
- **Armin Wagner**
- Kamel el Omari
- Vitaliy Mykhaylyk
- Ramona Duman
- Christian Orr

I24



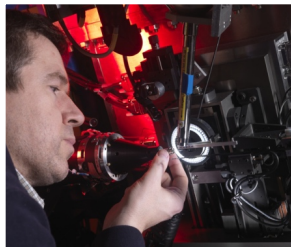
- **Robin Owen**
- Danny Axford
- Selina Storm
- Sam Horrell

VMXi



- 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

MPL



- **Andrew Quiqley**
- James Birch
- Harish Cheruvara
- Nadisha Gamage

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- Nicholas Devenish
- James Beilsten-Edmands
- Melanie Vollmar
- Luis Fuentes-Montero

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Marco Mazzorana
Elliot Nelson

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Alex Dias
Jitka Waterman

Controls

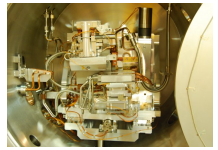


Technicians



Software

Engineers



The End

- With thanks to Diamond MX Group

