MX at Diamond Light Source



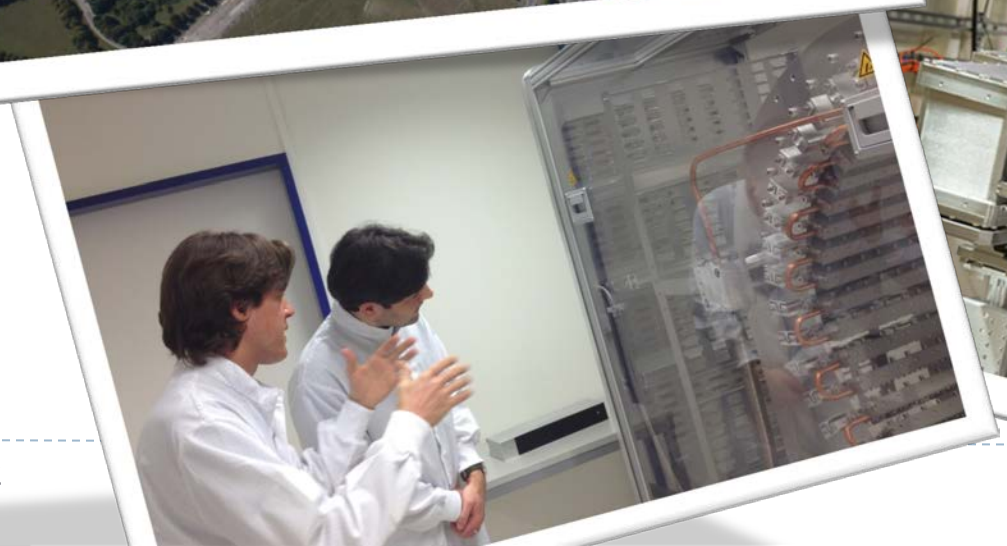
Armin Wagner
I23 PBS, Diamond

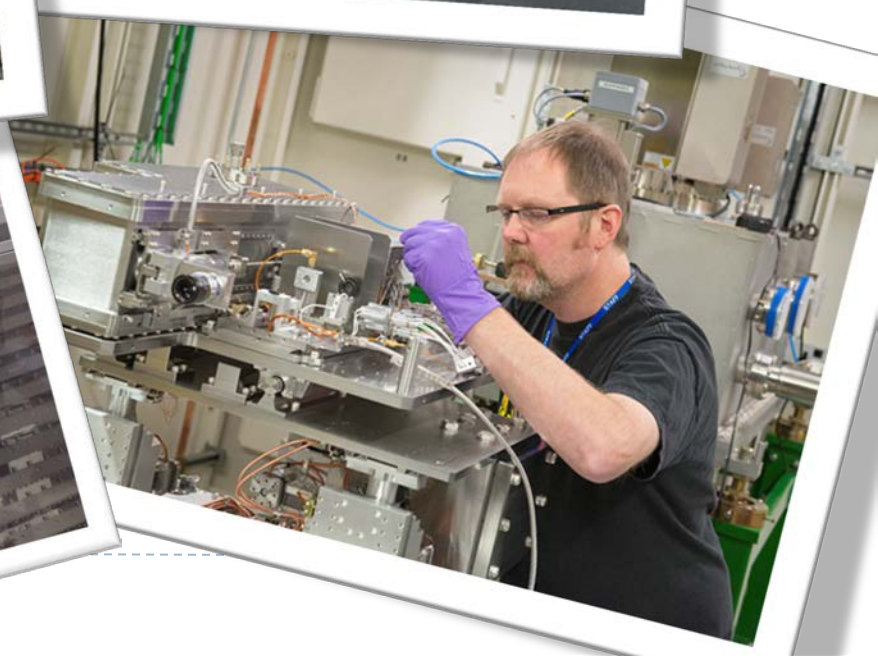
with many slides from Katherine McAuley

The aim of this talk is to give an introduction to synchrotrons and MX beamlines and how they work

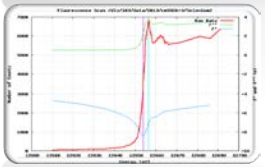




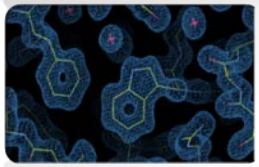




Why do you need to collect MX data at synchrotrons?



Structure solution



High resolution



High throughput



Special Conditions

What is a synchrotron?

- A synchrotron is a particle accelerator (typically electrons) which provides a source of extreme intensity light
- Can be compared to a series of ‘super microscopes’ or a gigantic x-ray machine;
- Diamond is the UK’s synchrotron light facility that provides intense light ranging from Infrared to hard X-rays



Daily Mail, Friday, February 9, 2007

It cost £260m, is the size of five football pitches and is the most remarkable British scientific project in decades

Magic doughnut: The new Oxfordshire lab will be able to study atoms in extraordinary detail

YOU CALL THAT A MICROSCOPE?

by Michael Hanlon

SCIENCE EDITOR

BY ONE reckoning this picture shows the most powerful torch in the world — a device that can create the brightest light in the known universe, ten billion times more brilliant than the sun.

It can produce a man-made beam of radiation so intense that it would be visible the other side of the cosmos, if it were to be pointed in the right direction.

Welcome to Diamond — the largest scientific instrument to be built in Britain for decades.

Constructed on the site of the old RAF Harwell airfield in Oxfordshire (from which the Pathfinder missions for D-Day took off), it has cost — so far — £260 million.

And after four years in construction it has finally opened for business, as scientists started the first of many research projects that will harness its astonishing capabilities to shed new light — quite literally — on the world around us.

Over the next 30 years, it is hoped that Diamond will revolutionise everything from the way that computer microchips are built to the manufacture of new drugs.

So what exactly is this giant building — the size of five football pitches — that sits like a beached flying saucer, near the little town of Didcot?

Well, to properly understand how the machine works you really need a degree in physics — preferably a doctorate.

Its official name is a ‘synchrotron microscope’ — the biggest, most expensive and most powerful of its kind on the planet.

But the very basic principles are simple enough. In essence, Diamond is a combination of a super-powerful torch and microscope.

It works by firing electrons, the sub-atomic particles that carry electrical charge, around a vast ring about 1,000ft in circumference. Inside this ring, they are then accelerated by gigantic powerful magnets until they are travelling at around 600 million mph — nearly the speed of light.

As the electrons reach these astonishing velocities, they are vibrated by more magnets, causing them to throw out intense and highly-focused beams of radiation — whether in the form of visible light, infra-red beams or X-rays.

These beams, just a fraction of a millimetre across, are then channelled away from the main ring down into adjacent research rooms, just like water being sent down aqueducts, where they are then used by scientists to illuminate whatever sample they want to study — for example, a human cell or a tiny dust particle.

To put it very simply, this super-bright light allows researchers to probe the most fundamental properties of the object being studied, right down to an atomic scale.

Diamond is certainly a versatile instrument. The first experiment, being conducted by scientists from Durham University, is using X-ray beams generated by the machine to find new ways of building silicon chips for machines like the iPod.

In future projects, the Natural History Museum has booked time to peer into the insides of meteorites, while police forces will be able to use Diamond as the most powerful forensic instrument in history.

A minute spatter of blood or a human hair from a crime scene can be analysed, atom by atom, to help scientists identify any evidence that conventional microscopes may have missed.

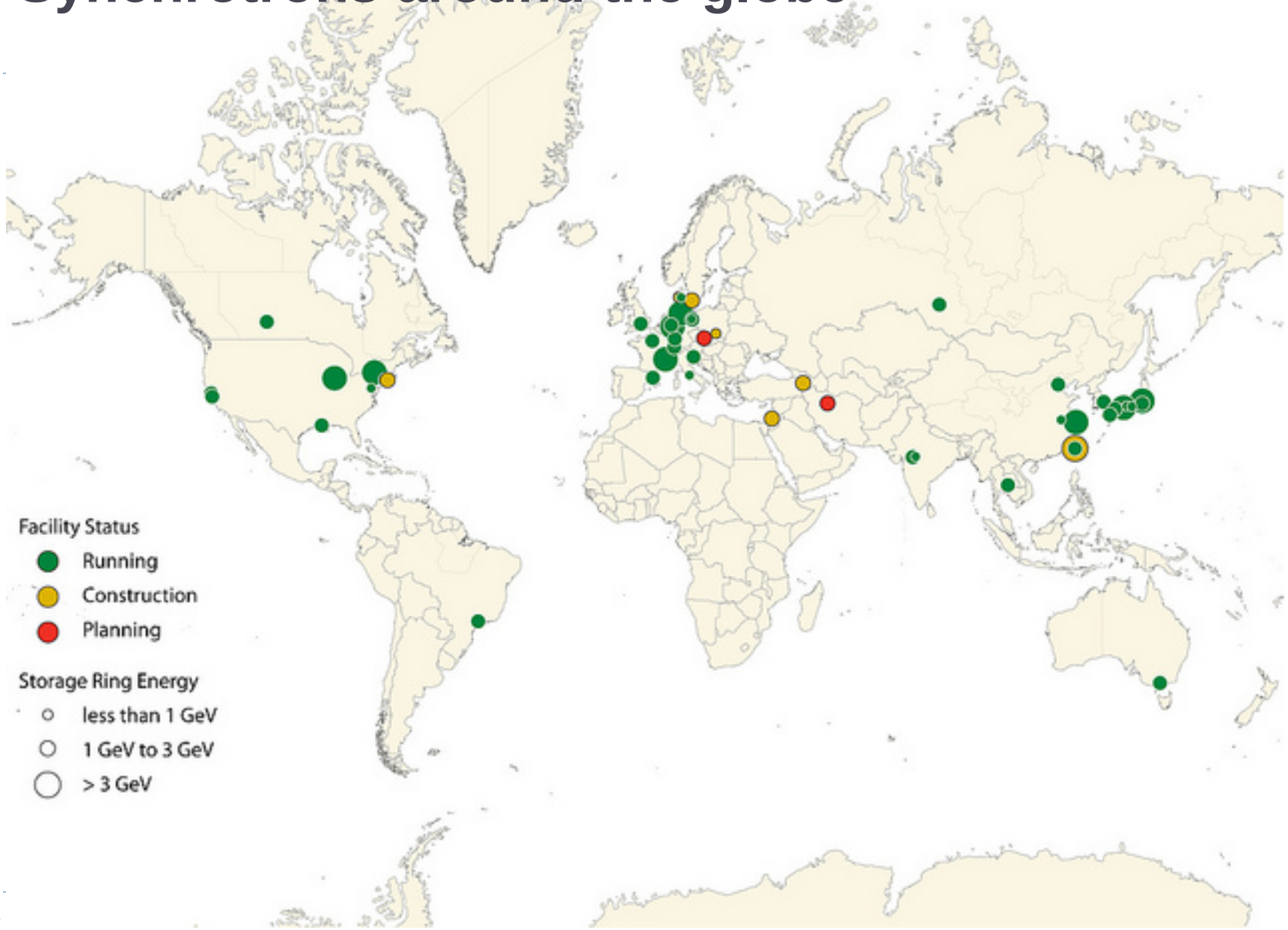
Because Diamond can peer with such precision, it will also be an invaluable tool for archaeologists.

SCIENTISTS at Cardiff University plan to examine the Domesday Book, the 11th-century Norman survey of England, to see what lies beneath the visible text. And the Dead Sea Scrolls, mysterious Biblical-era texts, will also fall under Diamond's needle-eyed scrutiny.

Jointly funded by the British taxpayer and by scientific charity the Wellcome Trust, it will also have numerous less arcane uses. Similar, less powerful, machines abroad have already been used to develop Aids drugs, and it is hoped that Diamond will be able to help analyse and develop vaccines and retroviral drugs.

Vast and costly as it may be, this is a landmark that has ushered in a whole new chapter in British scientific exploration.

Synchrotrons around the globe



Synchrotrons for MX in Europe



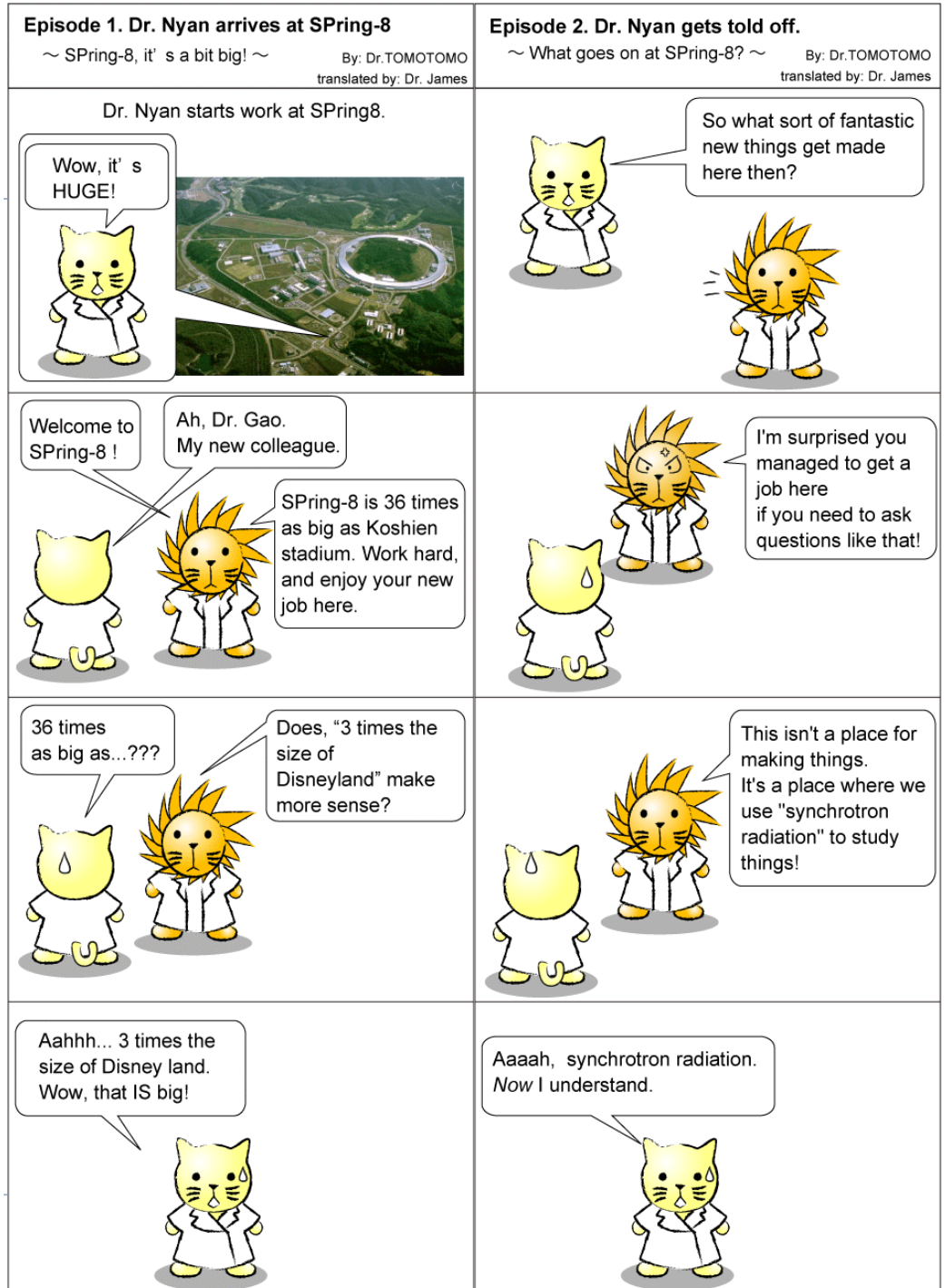
What is a synchrotron?

Lots of educational material online…

www.lightsources.org

http://www.spring8.or.jp/en/kids/8hakase/

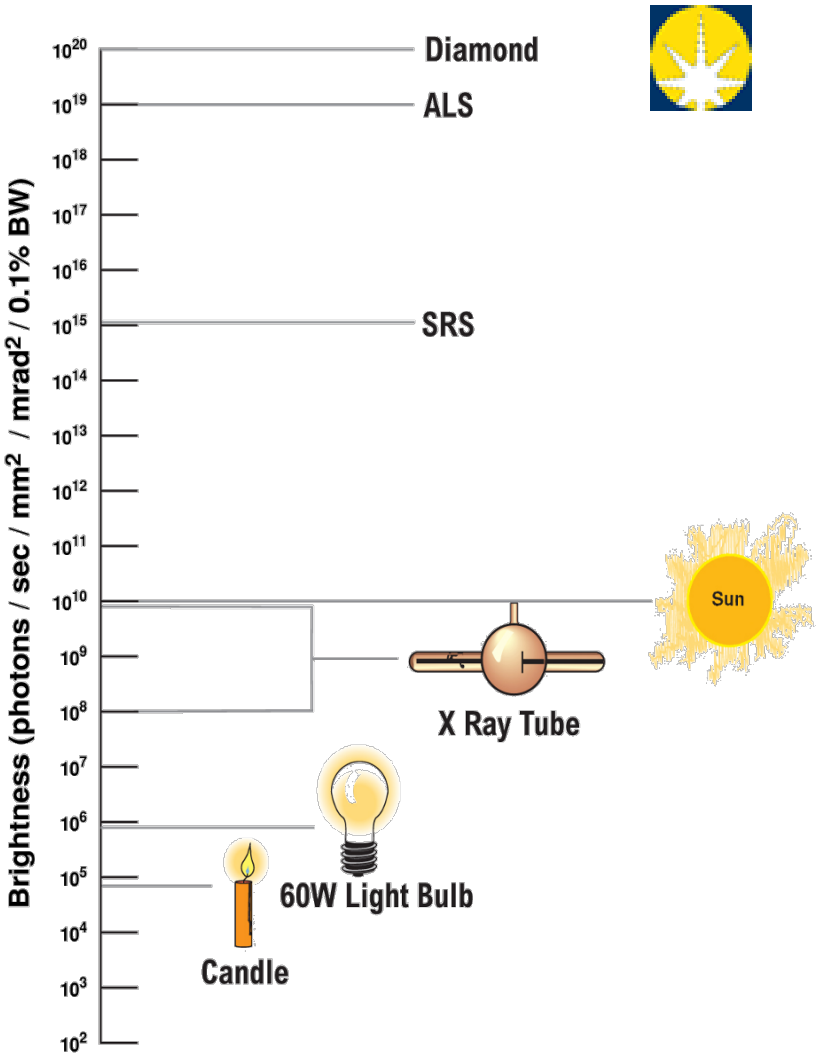
https://vimeo.com/diamondlightsource/videos



Diamond is a 3rd generation light source

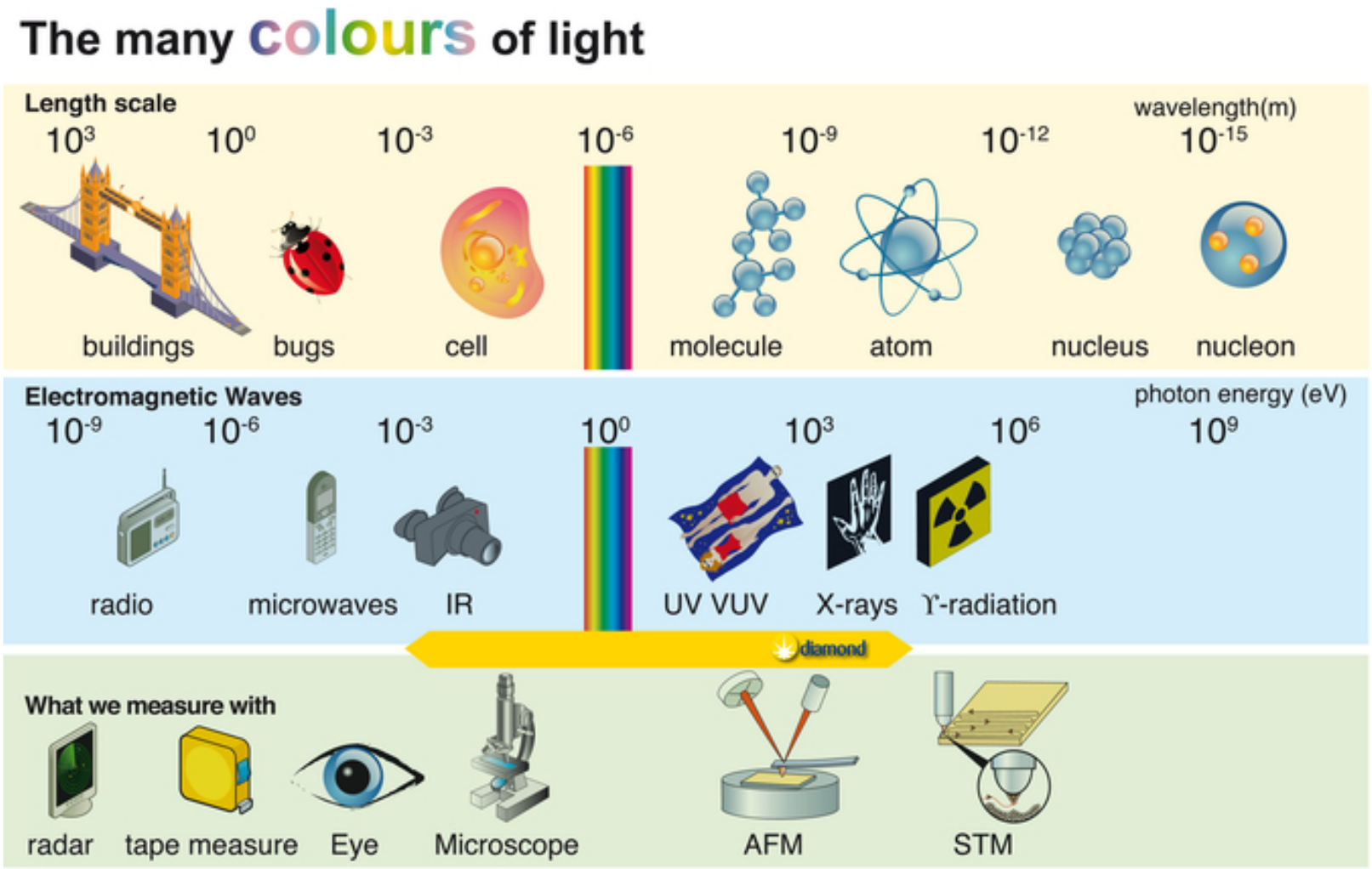
- 1st Generation sources
  - parasitic
- 2nd Generation sources
  - dedicated machines to synchrotron radiation
  - emittance typically high (150 nmrad)
- 3rd Generation sources
  - low beam emittance
  - use of insertion devices (wigglers and undulators)
  - emittance on order of 3-20 nmrad

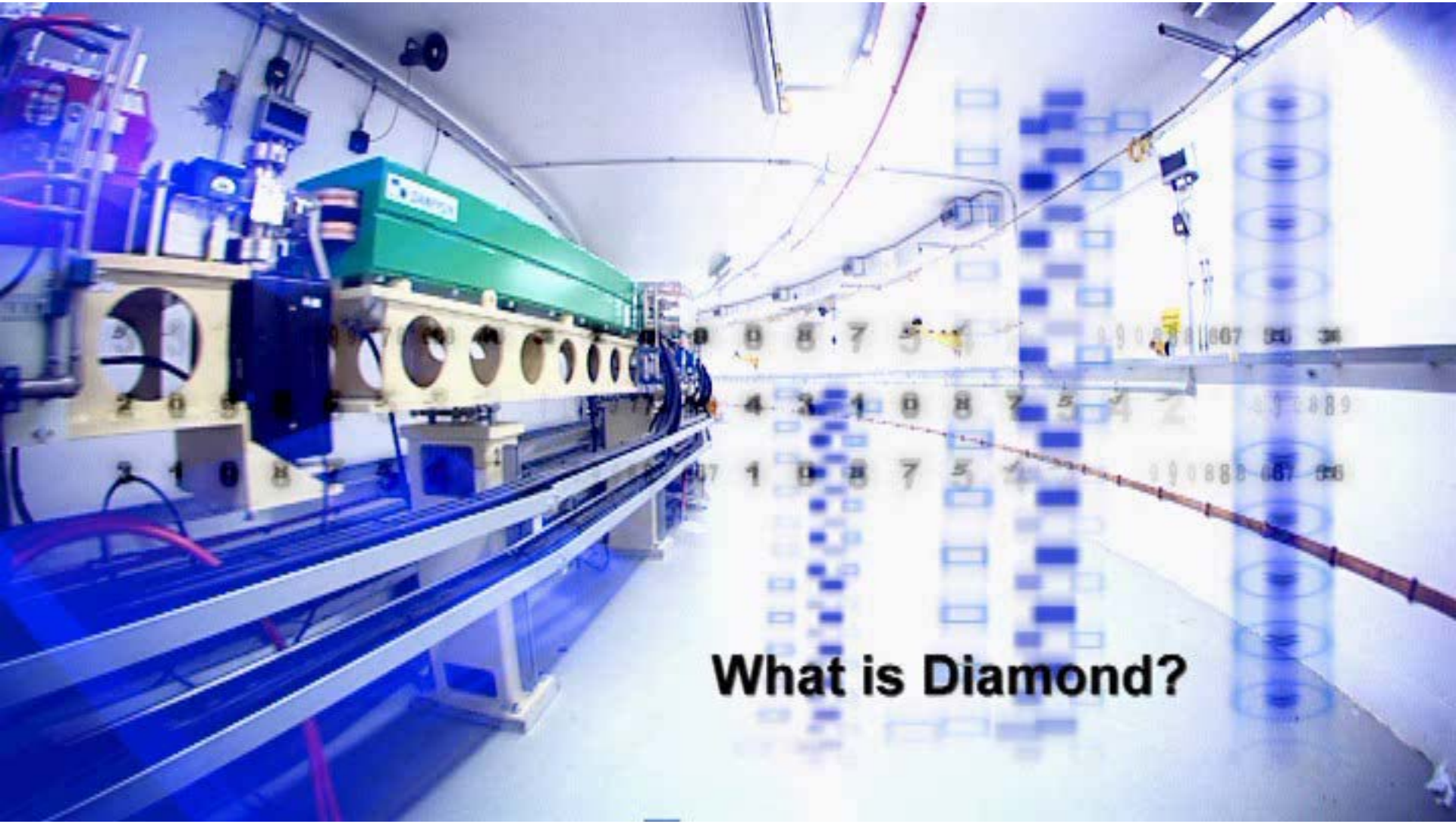
In this business, it's all about brightness !



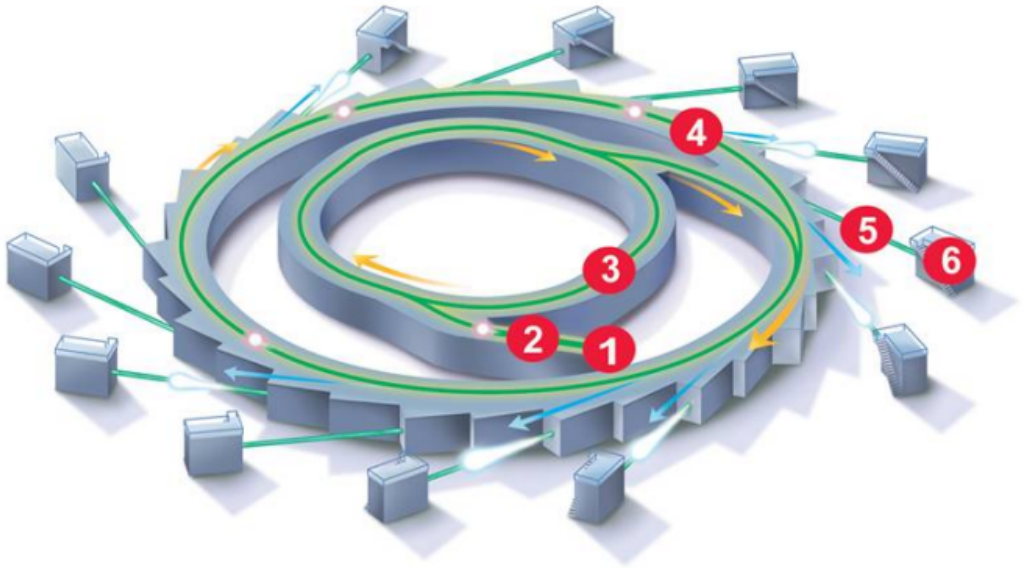
- X-rays from Diamond are 100 billion times brighter (10^{11}) than from an X-ray tube
- Or 10 billion times brighter (10^{10}) than the Sun
- >10^5 times brighter than the previous UK Synchrotron, the SRS !

And Broad Continuous Spectrum!




What is Diamond?

Synchrotron - main components



Electron gun: 90 keV

Linear accelerator: 100 MeV

Booster: 3 GeV

1 Electron Gun: produces electrons

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

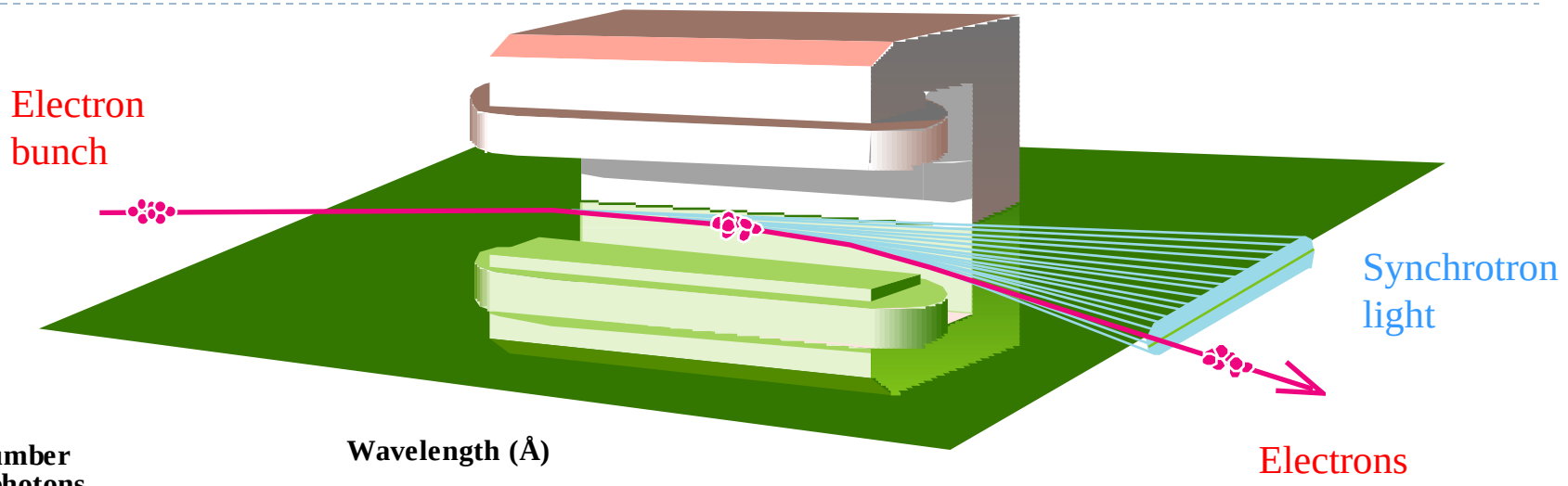
3 Booster Ring: further increases the energy of the electrons

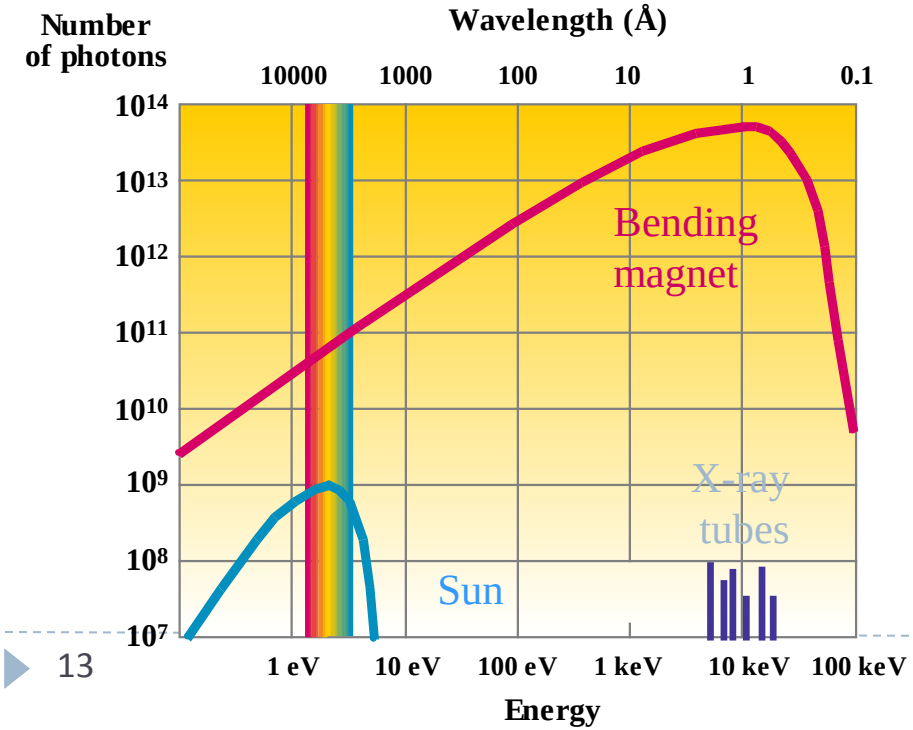
4 Storage Ring: maintains the electron energy and produces radiation

5 Beamline: set up of radiation for experiments

6 Experimental Hutch: User area

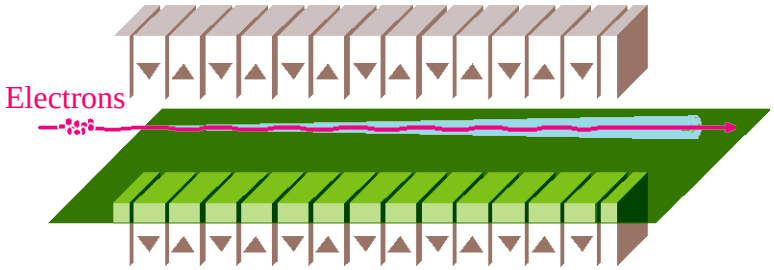
Bending Magnets curve the electron beam between adjacent straight sections and generate synchrotron light



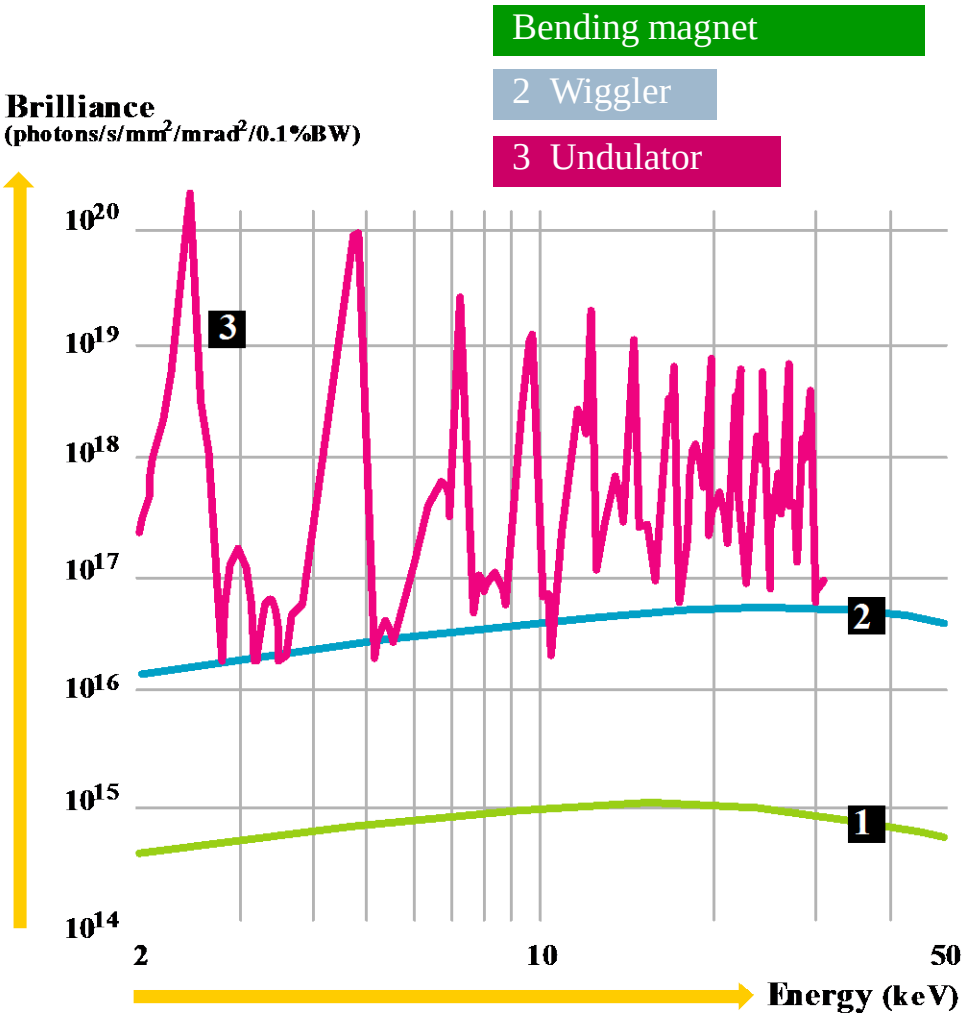


Emission spectrum

The MX beamlines at Diamond use insertion devices called undulators

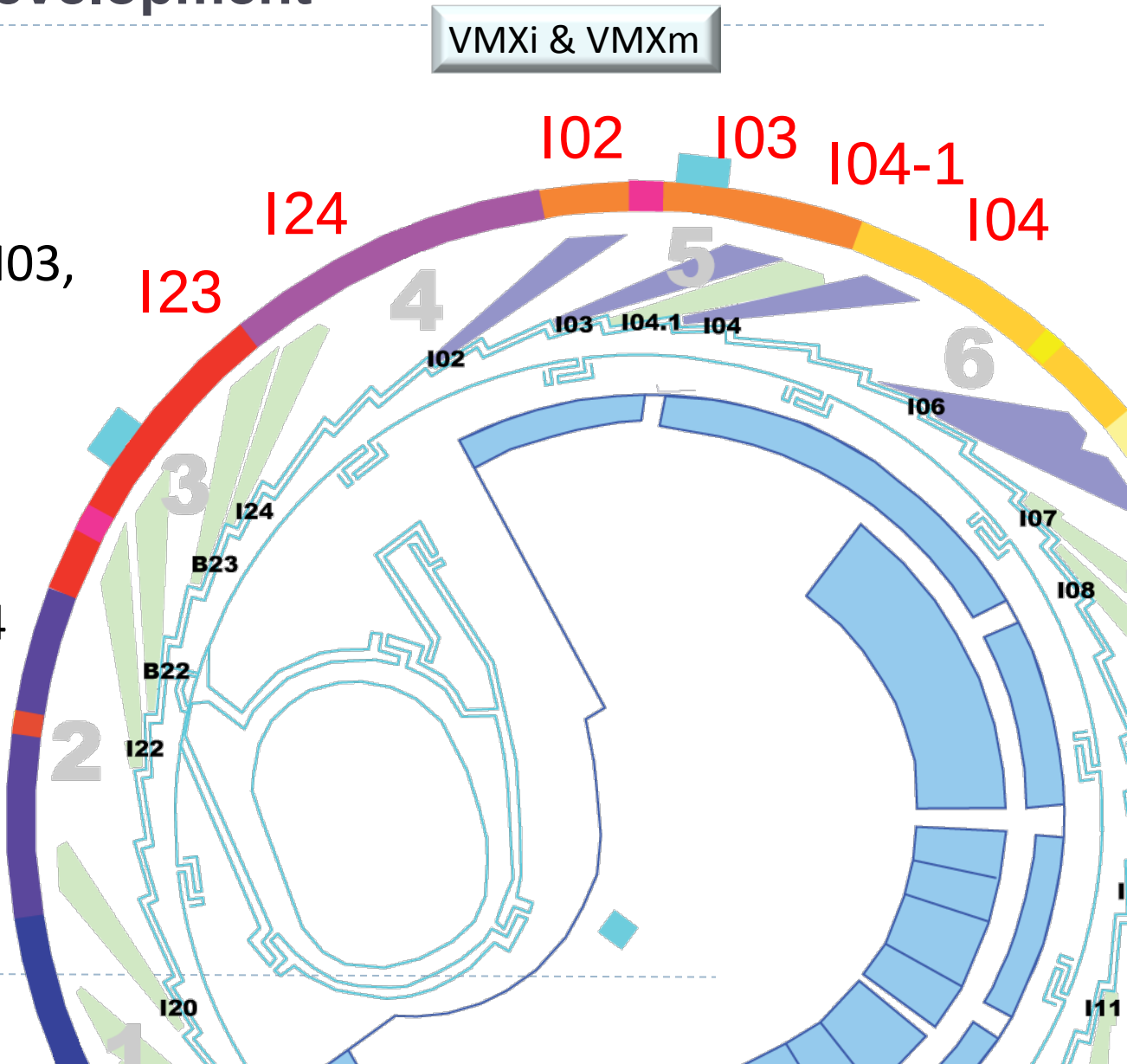




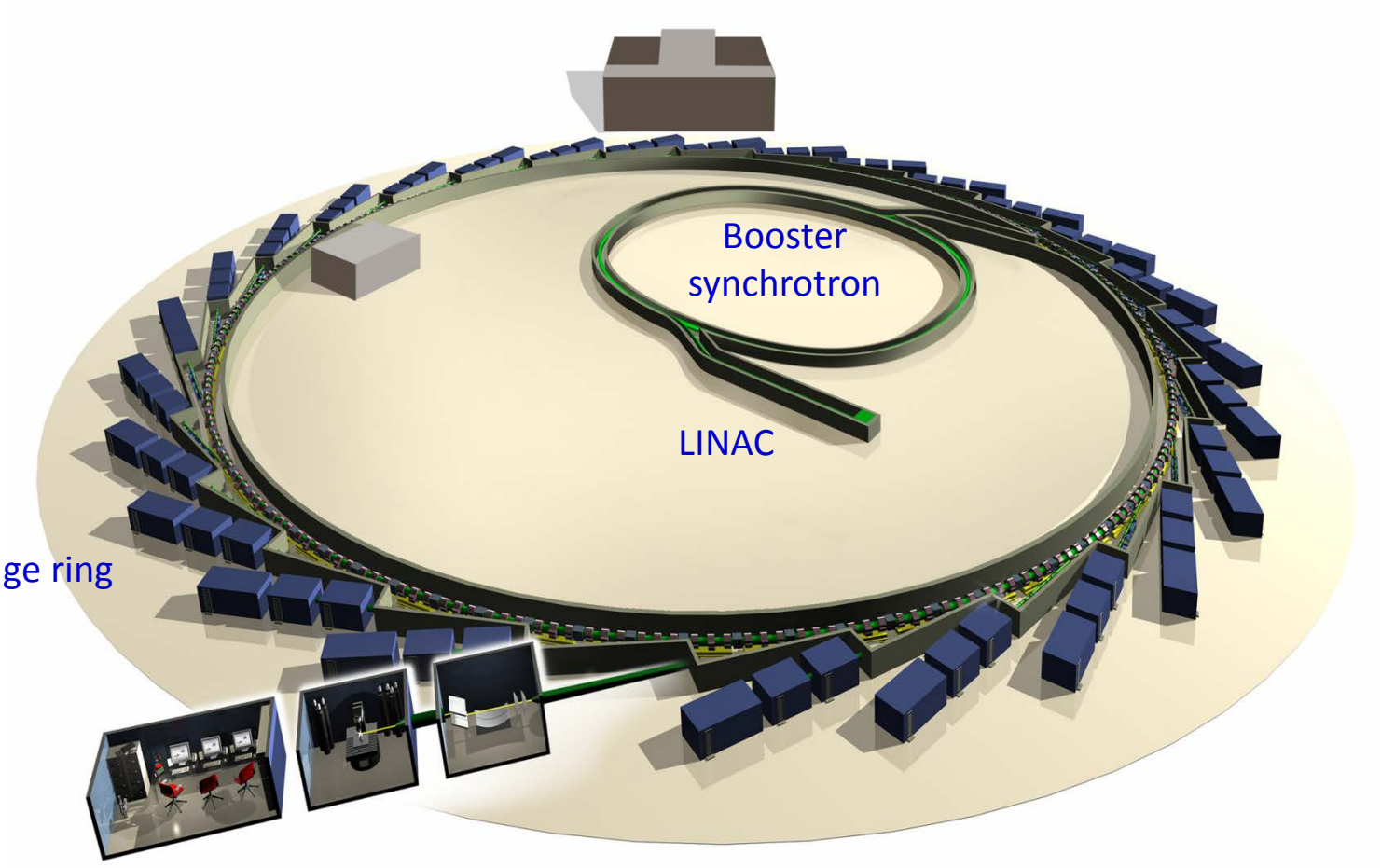


MX Village consists of 5 operational beamlines and several under development

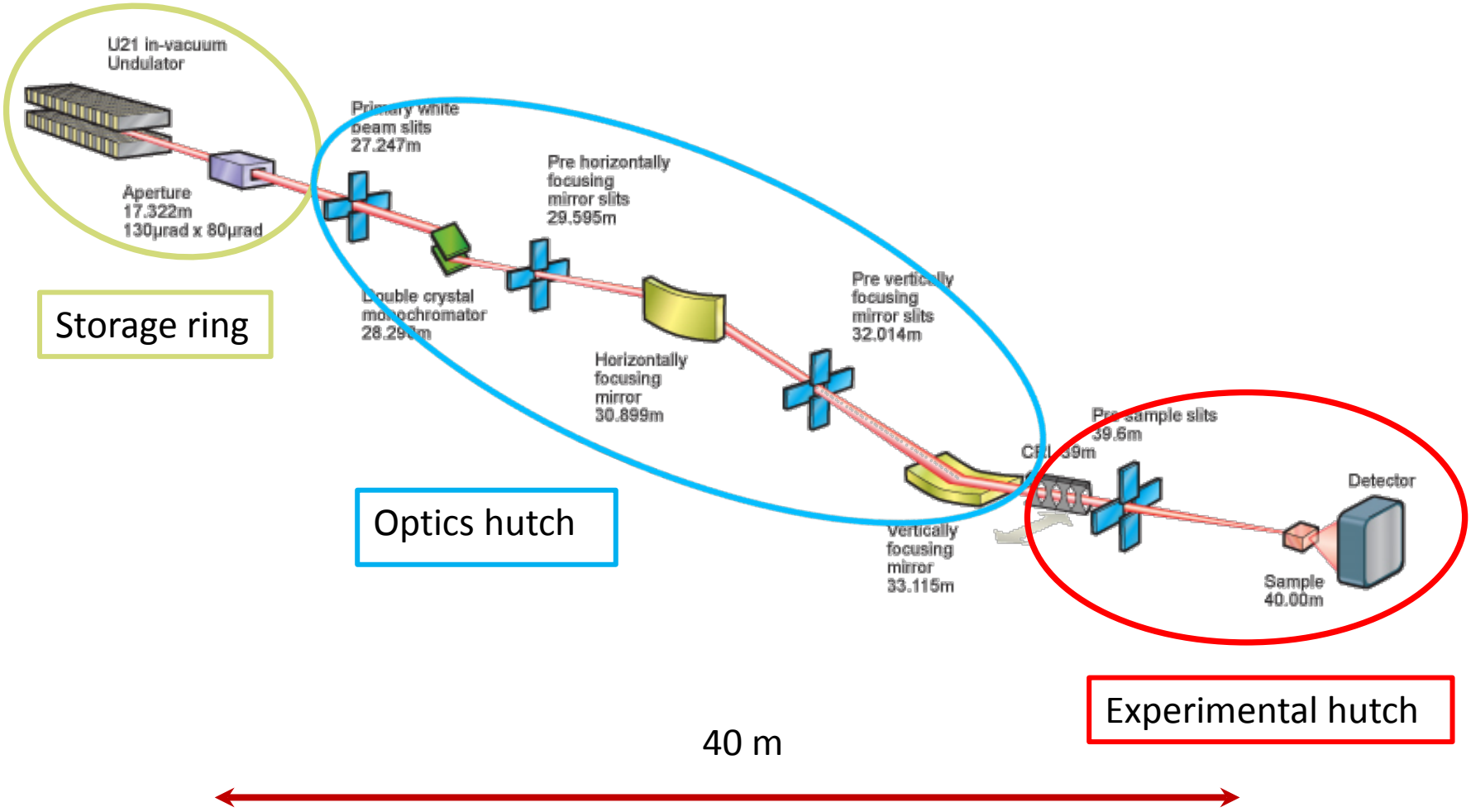
- **Operational**
  - **Phase I**
    - Tuneable I02, I03, I04
  - **Phase II**
    - Fixed λ I04-1
    - Tuneable microfocus I24
- **In Development**
  - **Phase III**
    - Long λ I23
    - VMX *m* and *i*



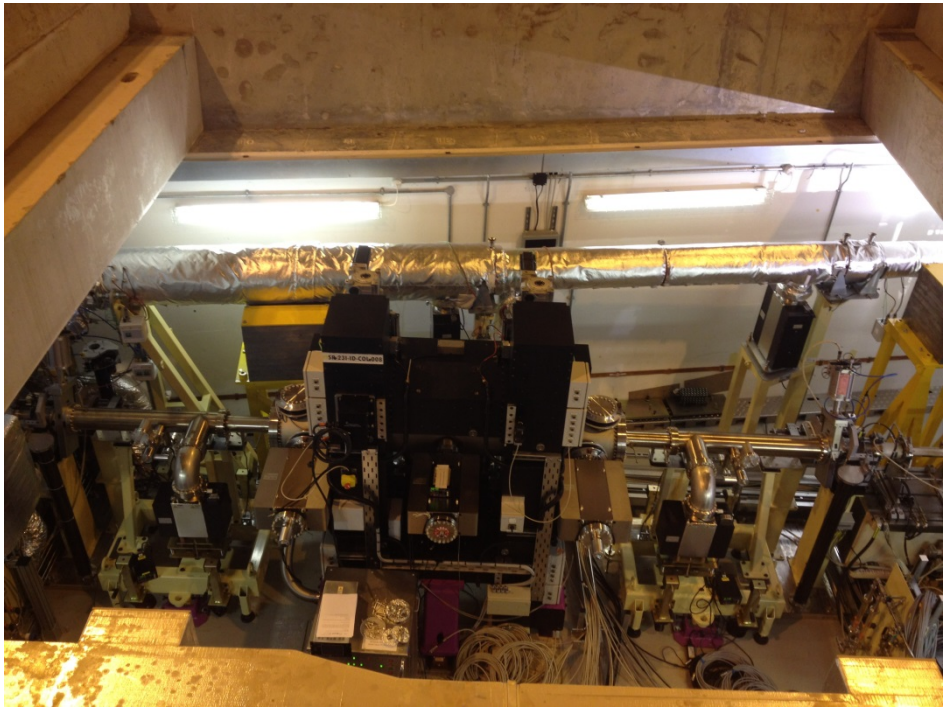
Beamlines consist of an optics hutch, experimental hutch and a control area



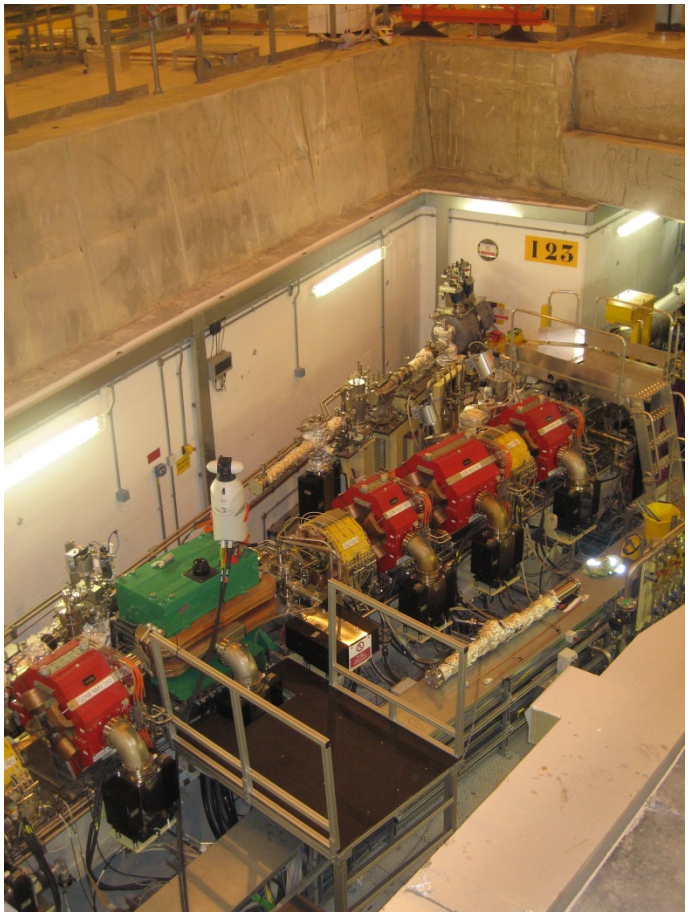
The components in the optics hutch are used to select the
X-ray energy and adjust the beam size



Insertion device and front end

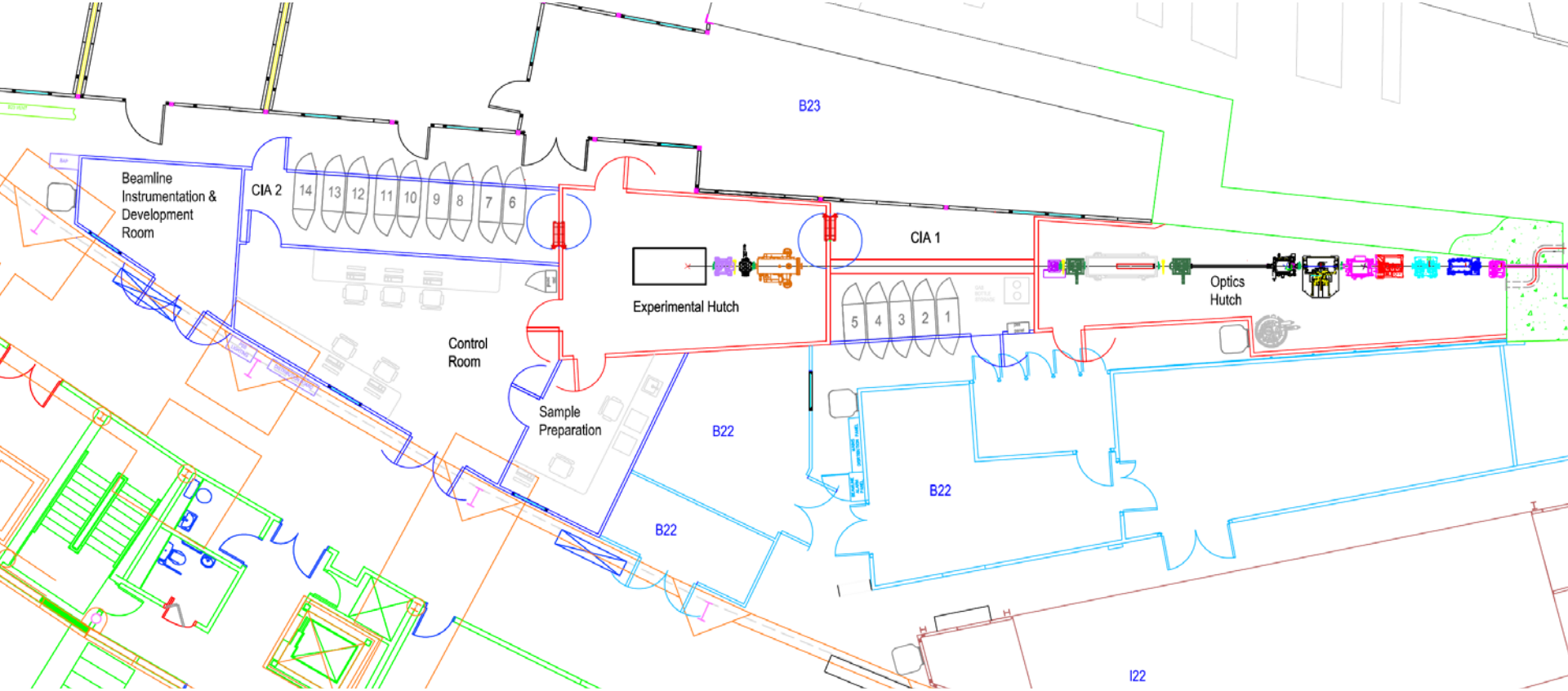


In-vacuum undulator



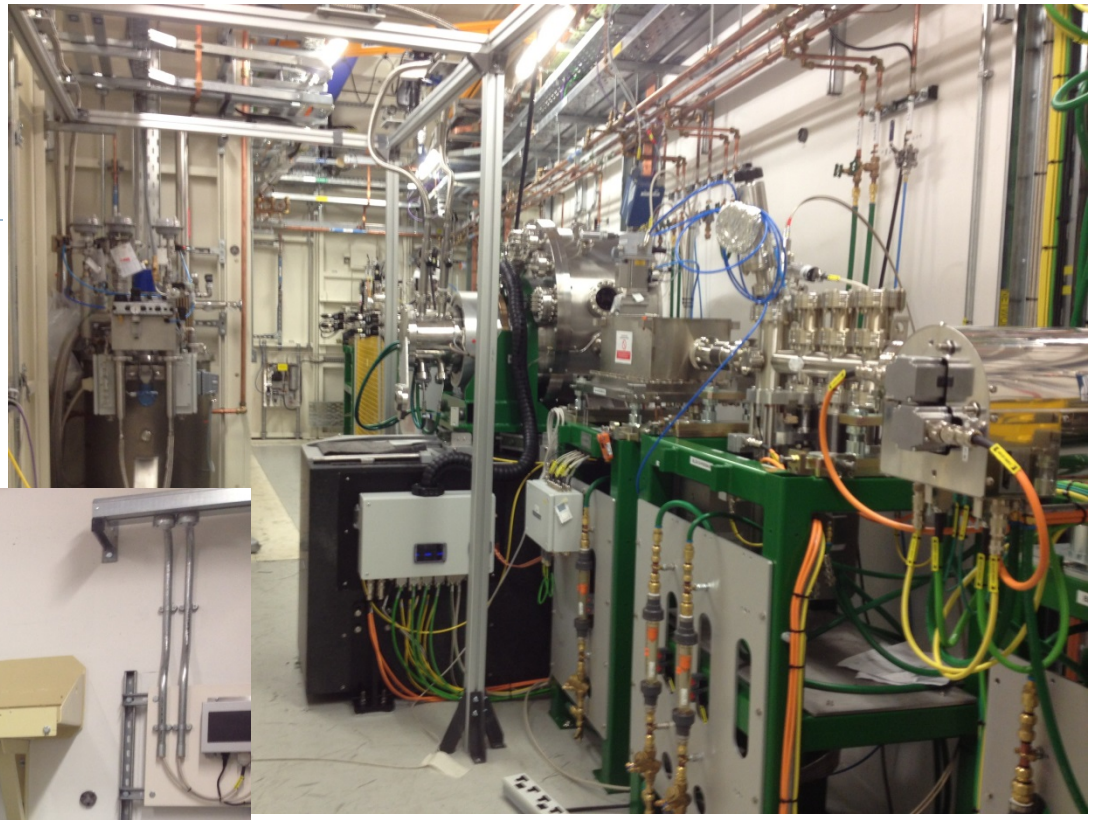
Front end

Beamline Layout



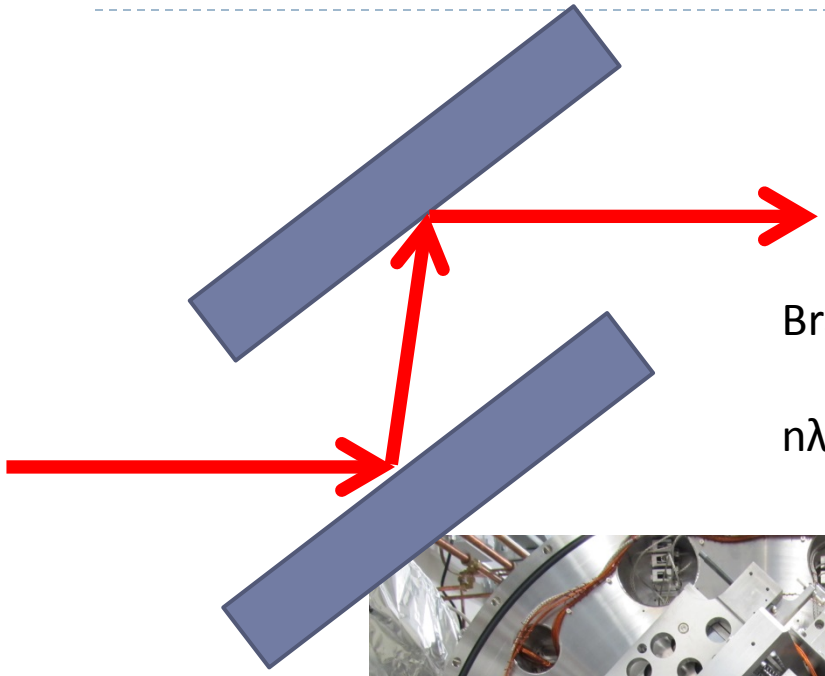
Sample position 40 – 50 m from insertion device

Optics hutch



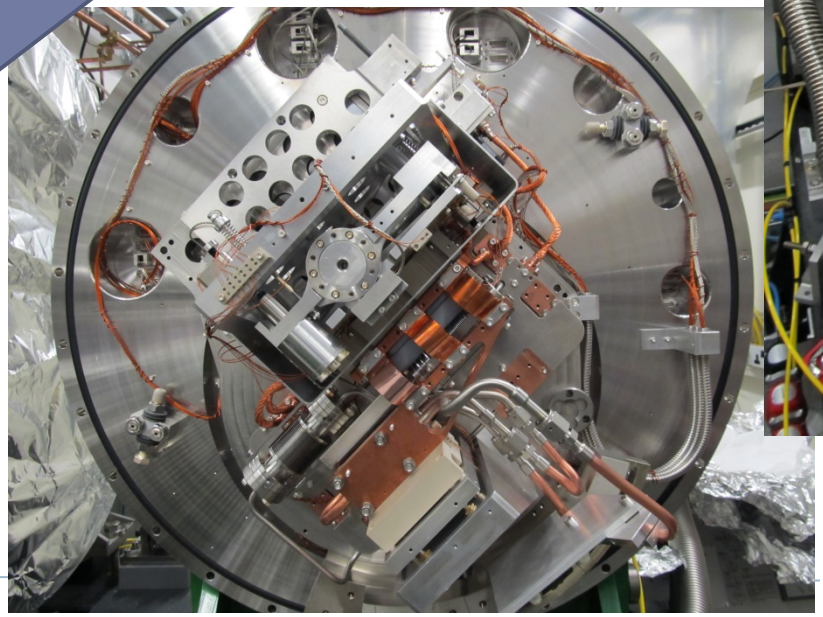


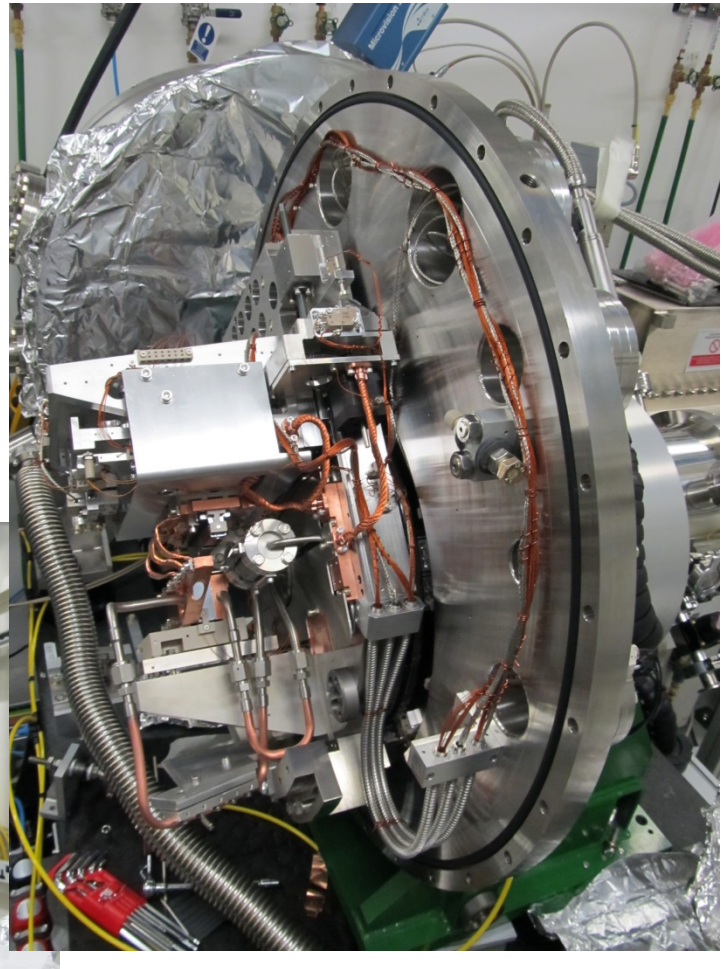
A double crystal monochromator is used to select the X-ray energy (wavelength)



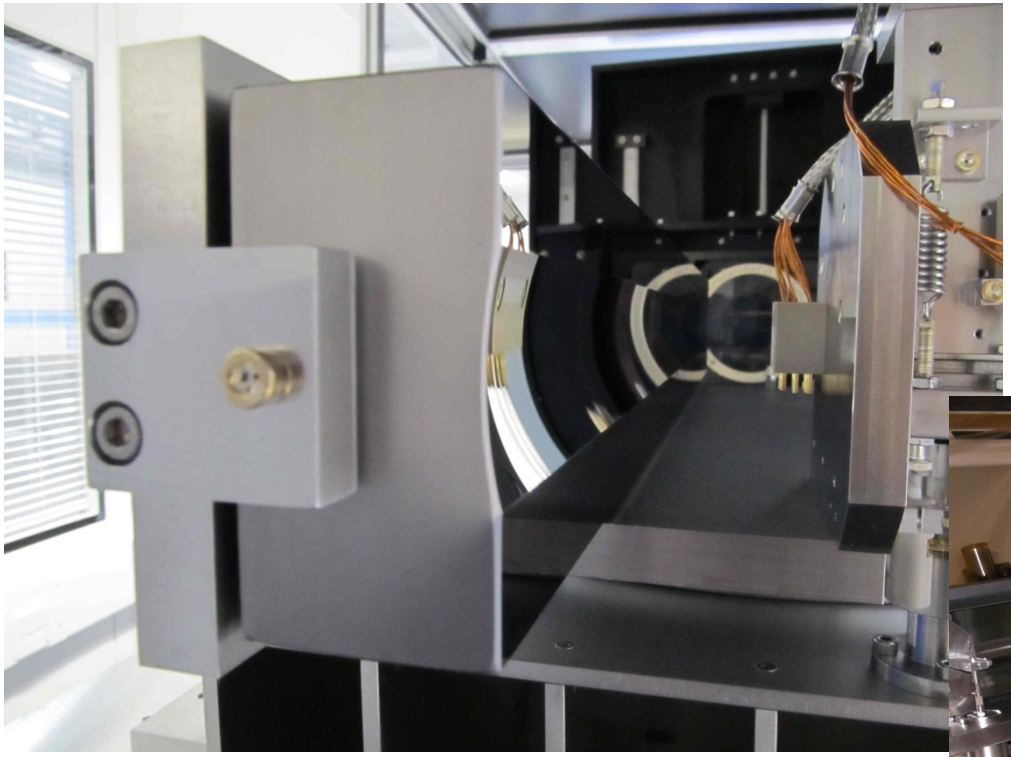
Bragg's law:

$n\lambda = 2d \sin\theta$





One mirror is used to focus the X-ray beam horizontally and another to focus vertically



Total reflection at very small angles



Typically 2 – 5 mrad

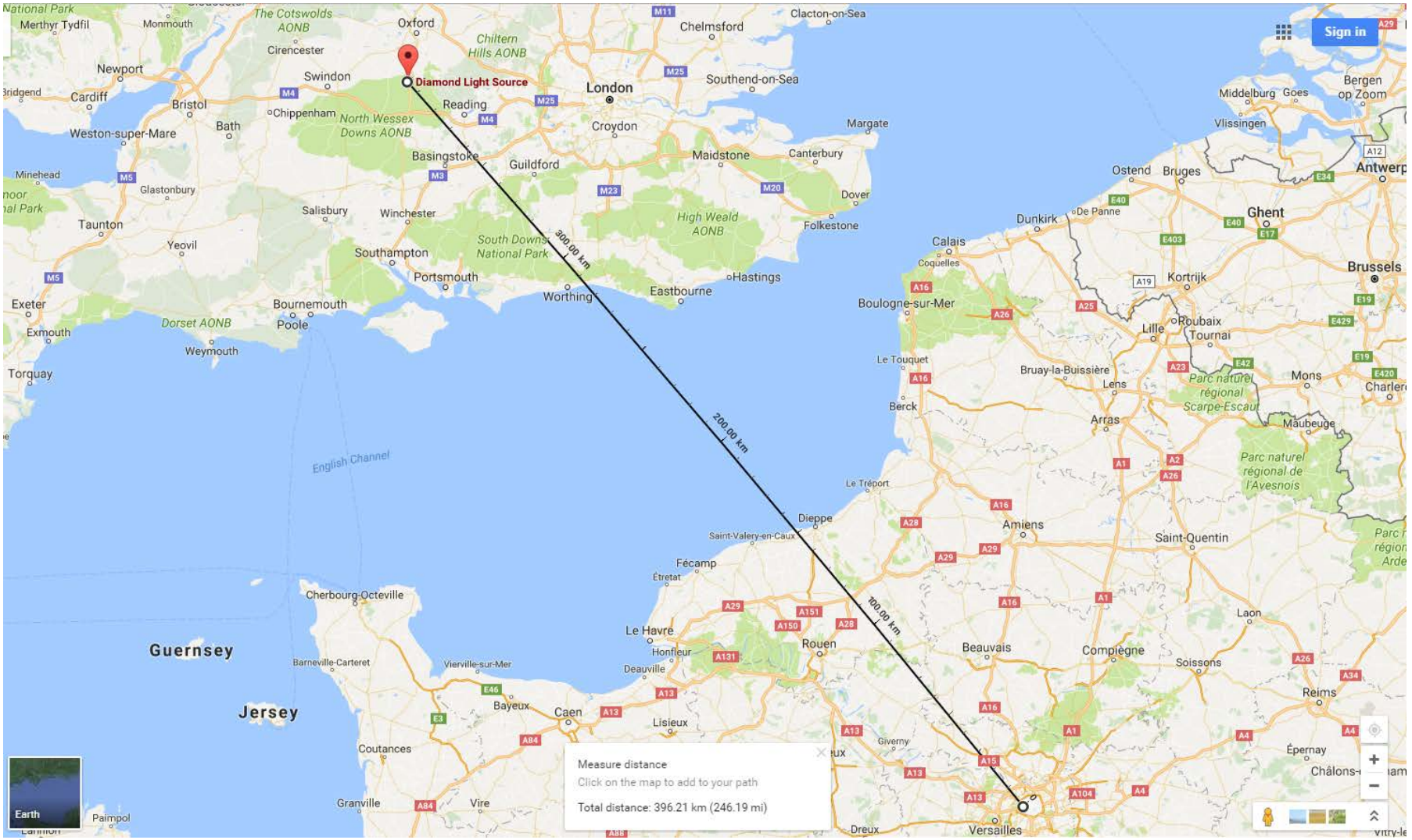
Figure errors: < 0.5 µrad

Diamond – Eiffel tower: 396.21 km

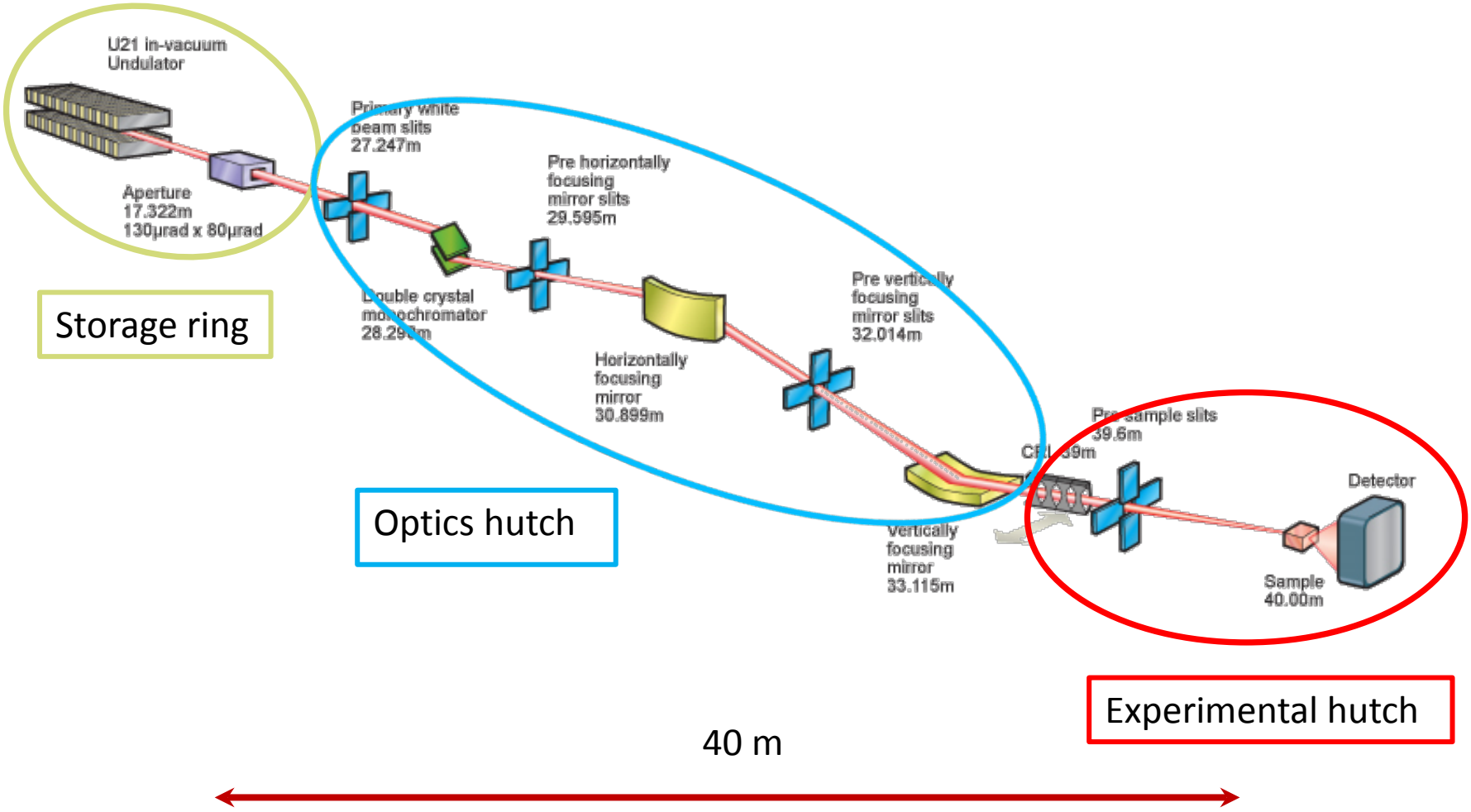
0.5 µrad over 400 km: 200 mm

Eiffel tower (300 m): 0.75 mrad

Earth curvature: 200 mm over 1.6 km!

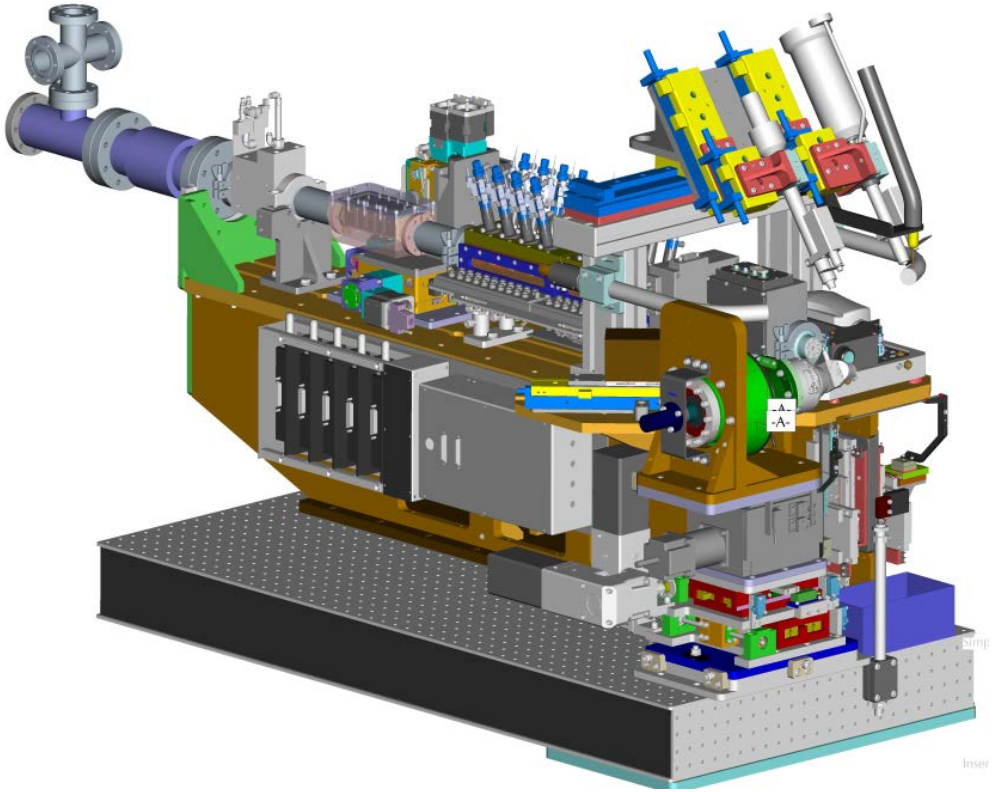


The experiment and final beam conditioning happens in the experiment hutch



The end-station table supports:

- CRLs
- Attenuators
- Beam diagnostics
- Slits
- On-axis viewing system
- Sample environment

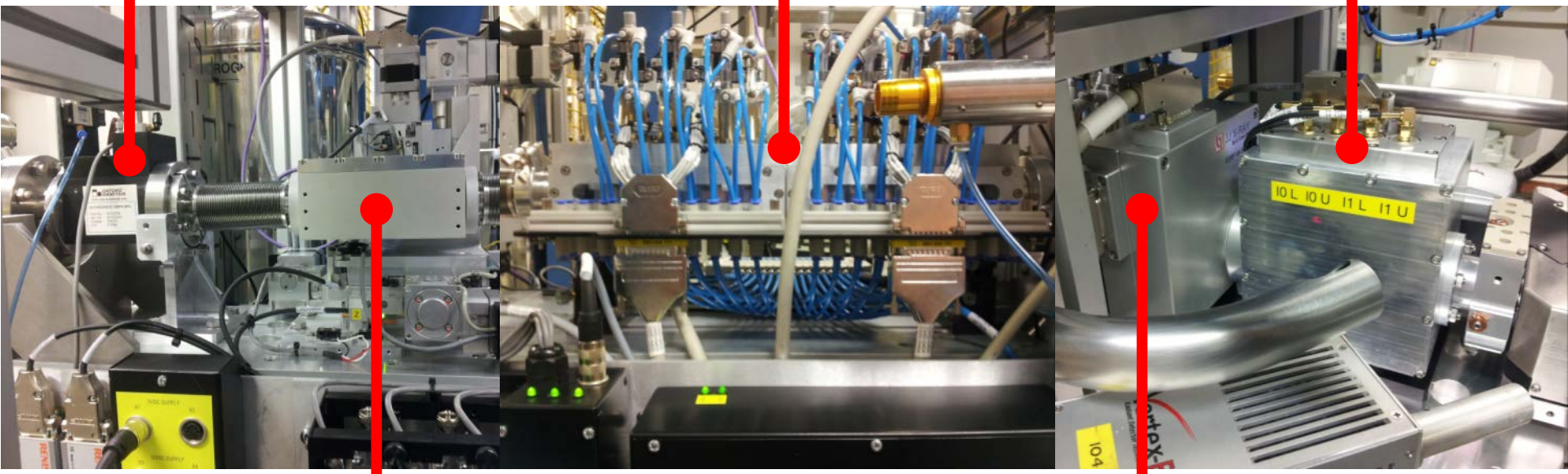


Beam conditioning

QBPM

16 Foil Attenuators

Diode – Shutter - Diode

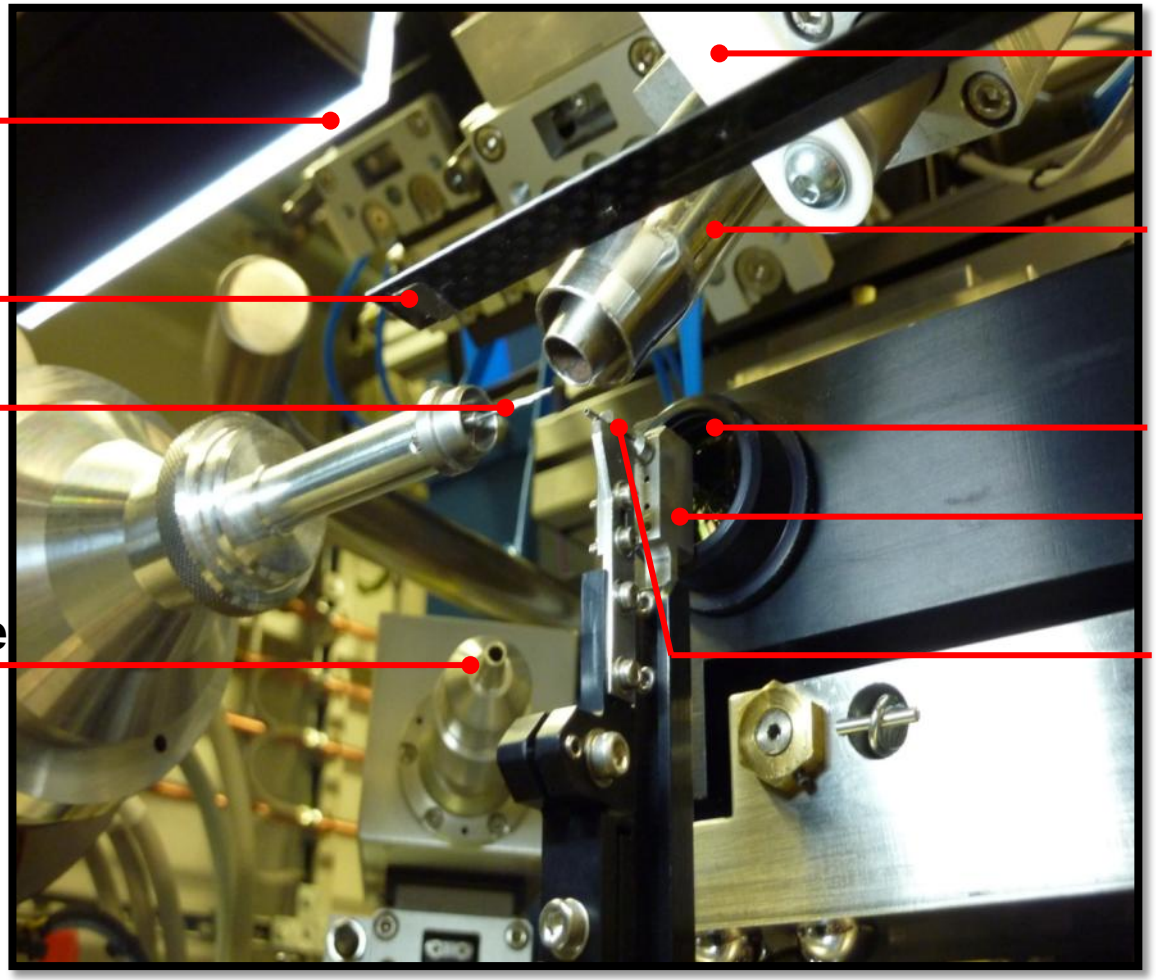


Compound Refractive Lenses

Slits

X Rays

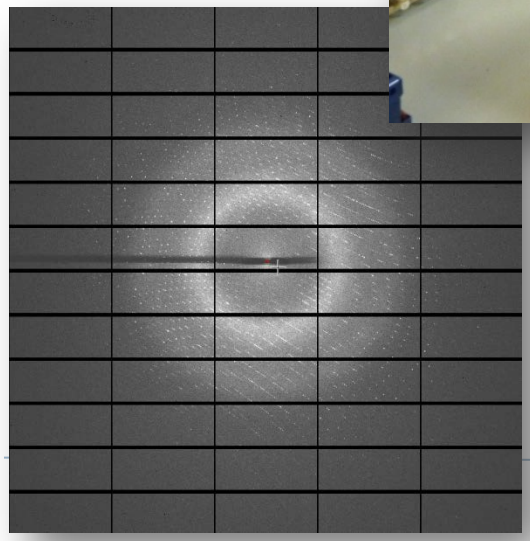
The sample environment



Fast pixel array detectors record the diffraction images

- Advantages
  - S/N much better than CCD
  - Higher throughput
  - Fine phi slicing – better data
  - Beating radiation damage – go even faster with high dose can collect more data at room temp
  - Key for CL3, protein dynamics, etc
- On all beamlines since April 2013
- 100 Hz on I03 and I24





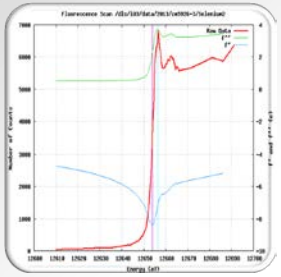


Automation!



- ep loop centering using
  on and OAV image
  able.
- m units
- size can be used for
  tion from crystals.
- ing grid scans

Choose which beamline is appropriate to your experiment requirements



Energy/wavelength?

- Fixed energy – suitable?
- Tuneable
- Long-wavelength?



Microfocus?

- Use of apertures?
- Dedicated microfocus beamline



Special Apparatus?

- Dehydration equipment
- Spectroscopy
- Biocontainment
- *In-situ* experiments

Use the web pages to compare the MX beamlines at Diamond

I02 MX

High throughput and highly automated beamline for optimised MAD and SAD experiments.

More information...

Beamsize: 90 x 20

Wavelength: 0.5Å - 2.5Å

Detector: Pilatus 6M-F

I03 MX

High throughput and highly automated beamline for optimised MAD and SAD experiments. Capable of accepting CL3 type experiments on crystals of pathogens.

More information...

Beamsize: variable

Wavelength: 0.5Å - 2.5Å

Detector: Pilatus3 6M

I24 Microfocus MX

High throughput variable microfocus beamline for optimised MAD and SAD experiments on crystals down a few microns in size.

More information...

Beamsize: 5x5 - 50x40 (variable aspect ratio)

Wavelength: 0.62Å - 1.77Å

http://www.diamond.ac.uk/Beamlines/Mx.html

I04-1 Fixed wavelength MX

High throughput and highly automated fixed wavelength SAD beamline for macromolecular crystallography.

More information...

Beamsize: 60 x 50

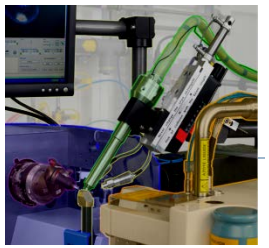
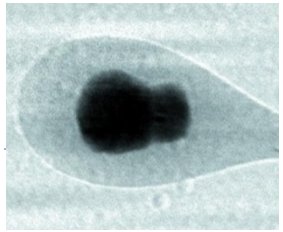
Wavelength: 0.92Å (fixed)

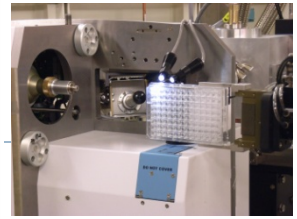
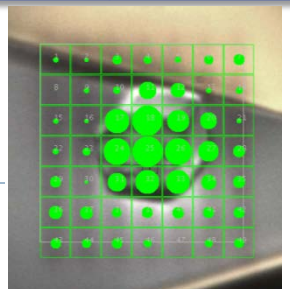
Detector: Pilatus 2M

- Microfocus
- Sample Humidity Control
- Mini Kappa
- *In situ* Data Collection
- Spectroscopy
- Biocontainment

Beamline	I02	I03	I04	I04-1	I24	I23	VMXi	VMXm
Type	Tuneable			Optimised fixed	Tuneable µ-focus	Tuneable long λ	Tuneable	Tuneable µ-focus
Beamsize [µm]	70 x 20 (mirrors) down to 8 x 3 (CRLs)			40 x 60	5 – 60	120 x 120	5 x 5	4 x 0.5
Flux [ph/s]	> 10^{12}			10^{11}	> 10^{12}	> 10^{12}	> 10^{12} (DCM) > 10^{14} (DMM)	> 10^{13}
Pilatus Detector	P6M (25 Hz)	P3-6M (100 Hz)	P6M (25 Hz)	P2M (30 Hz)	P3-6M (100 Hz)	Custom P12M	TBD	TBD
Sample changer	Rigaku ACTOR (< 35 s)			Irelec CATS (< 45 s)		Custom made	TBD	TBD
Containment	CL 1	CL 3	CL 1	CL 1	CL 2	CL 2	CL 2	CL 2
Humidity Controller	On request					No	TBD	No
Multiaxis goniometry			Mini-kappa	Mini-kappa		Custom kappa	No	No
Minibeam apertures	Yes				Development			
In situ	Soon	Yes		Yes	Yes	No	Yes	TBD
Crystal Washer and Annealer	Yes	Yes	Yes	Yes	Soon	No	No	No
Remote Access	Yes					No	Yes	TBD



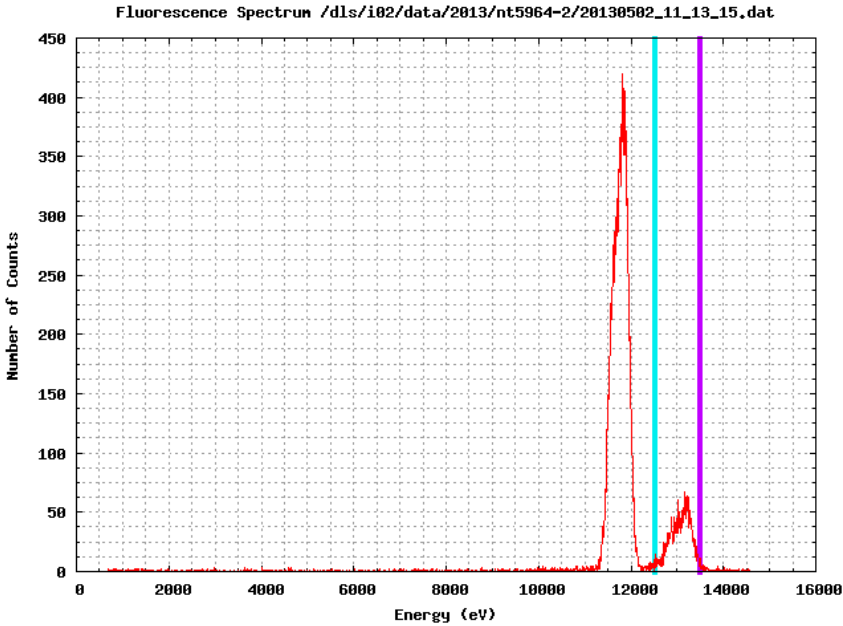



Techniques available on the MX beamlines



Using fluorescence spectra to identify metals in your sample

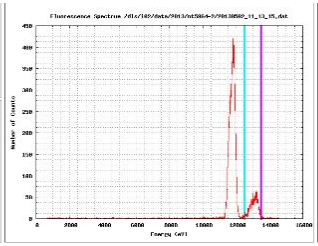
- All beamlines have fluorescence detectors
- Take a fluorescence spectrum of your sample in seconds
- Automatic identification of your sample with summary in webpages:



Thu May 2, 2013 - 11:13:54
20130502_11_13_15.dat

Fluorescence Spectrum

Beamline Energy:	13488.99eV	Omega:	0.0°
Acq Time:	1.0s	Trans:	0.05%
Sample Position:	(-329,-530,276)		





Automated PyMca results

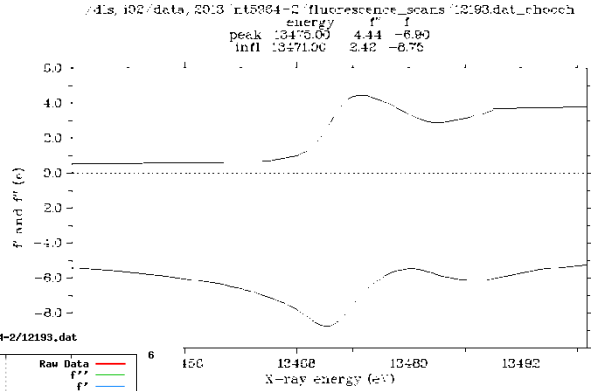
Element	Counts	Percentage
Br-K	16689.7	70.5
Se-K	68.0	0.3
W-L	40.2	0.2
Os-L	39.8	0.2
I-L	19.7	0.1
As-K	17.9	0.1

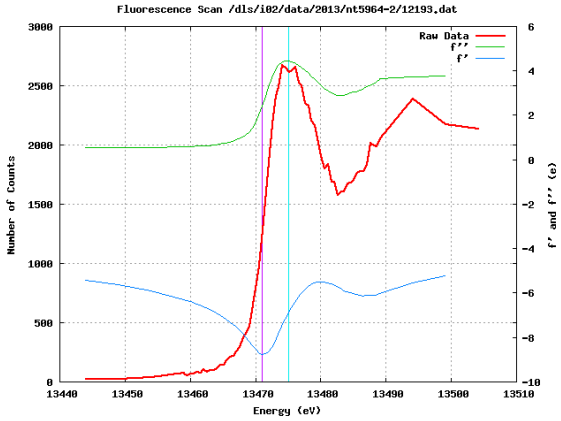
Counts (total): 23690.0 (background): 861.0

Guide to AutoPyMCA (pdf)

If there is a metal present:

- On the tuneable beamlines (i.e. not I04-1) collect fluorescence scan around the edge
- e.g. Signal from Br soaked lysozyme
- Results stored in webpages/ISPyB:



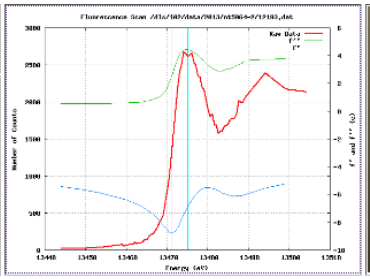


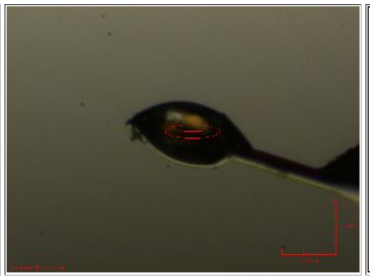
Thu May 2, 2013 - 11:02:29
12193.dat

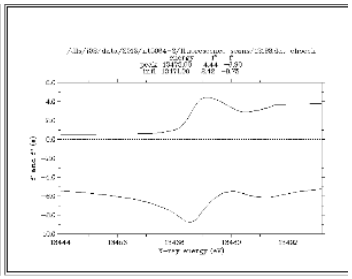
Fluorescence Scan

Element:	Br	Edge:	K
Omega:	0.0°	Transmission:	0.0125%
Sample Position:	(-329,-530,276)		

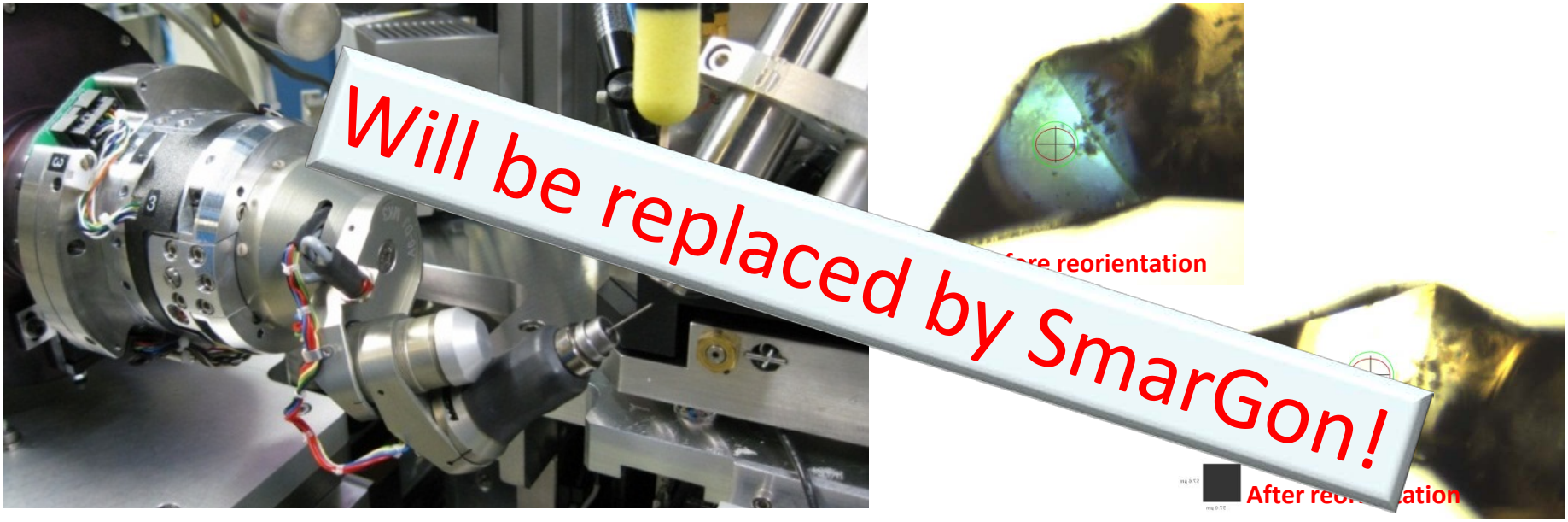
	Energy	f''	f'
Peak	13475.0eV	4.44	-6.9
Inflection	13471.0eV	2.42	-8.75





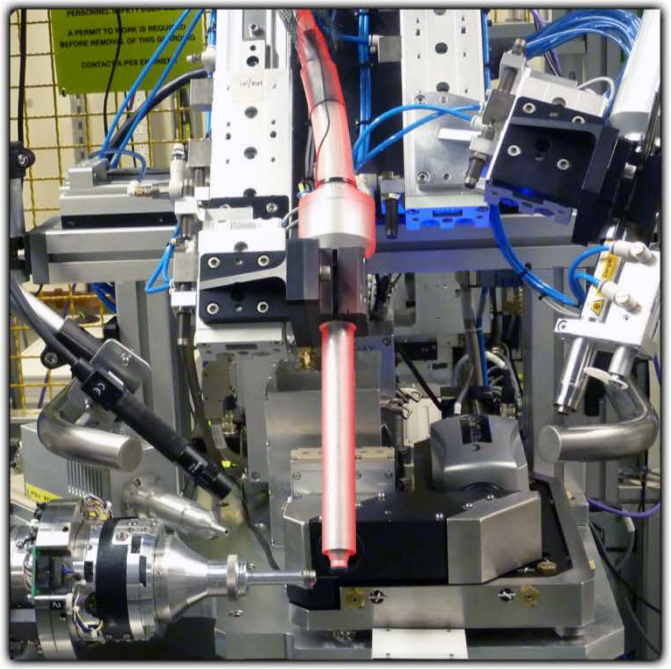


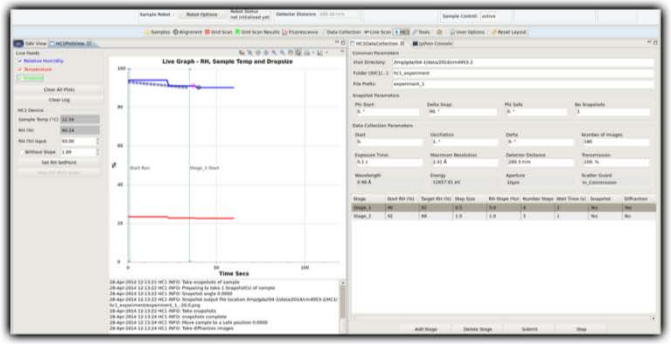
The Mini Kappa can be used to reorientate crystals for data collection



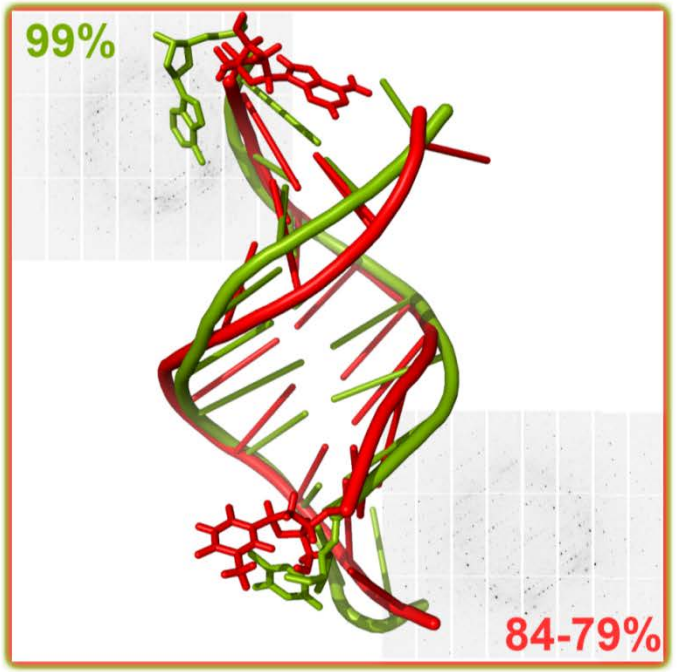
- Optimised MAD data collection; Bijvoet pairs on the same frame
- Improved high-multiplicity SAD data collection protocols
- Smart data collection strategies, better completeness of data, especially P1
- Reducing/avoiding spot overlap, better spot separation (long unit cell axes)
- Multi-crystal data collection: collect missing data
- Help in point group determination
- Comparing crystals in the same orientation

The HC1 device controls sample humidity and can improve crystal diffraction

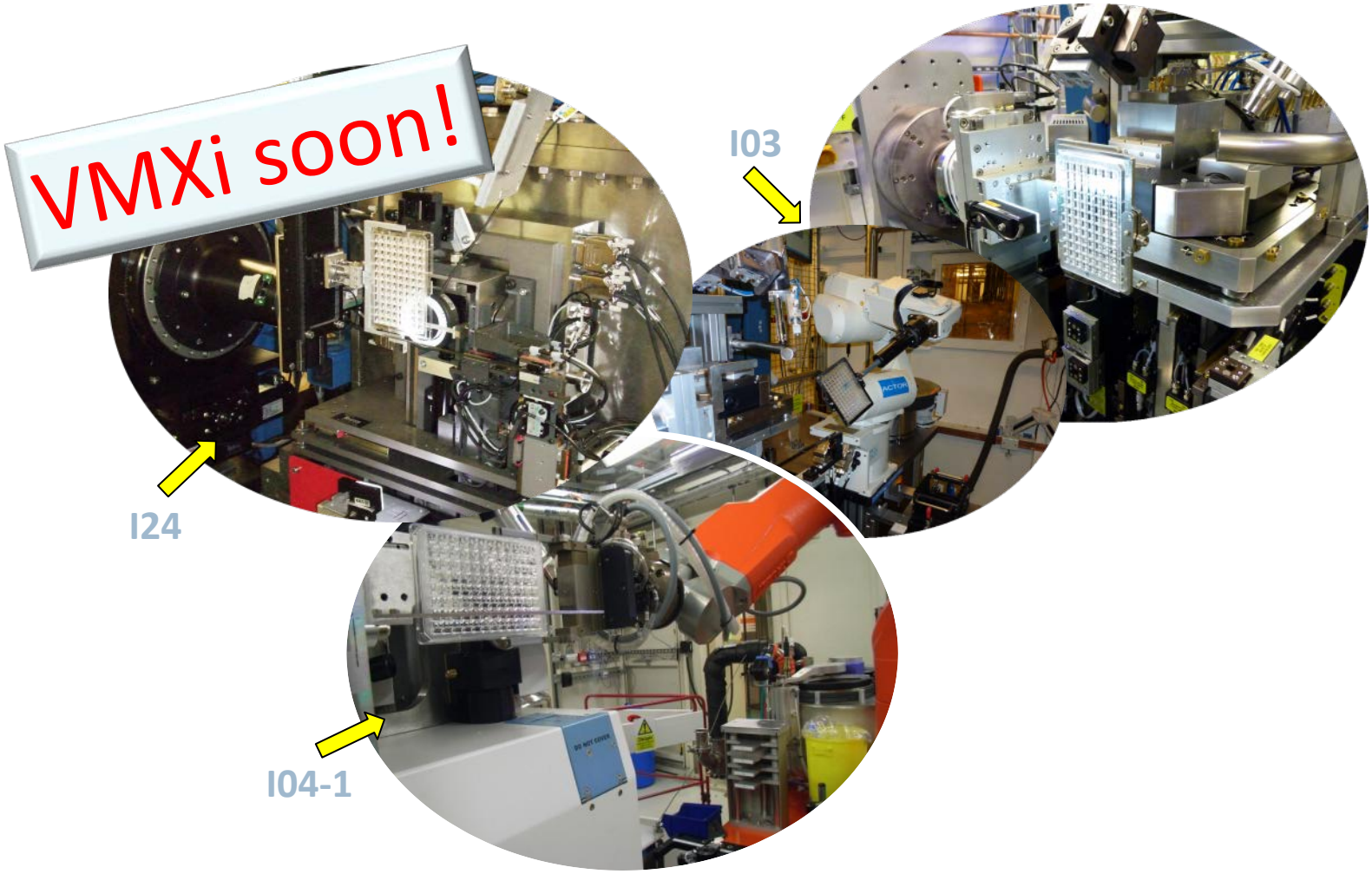






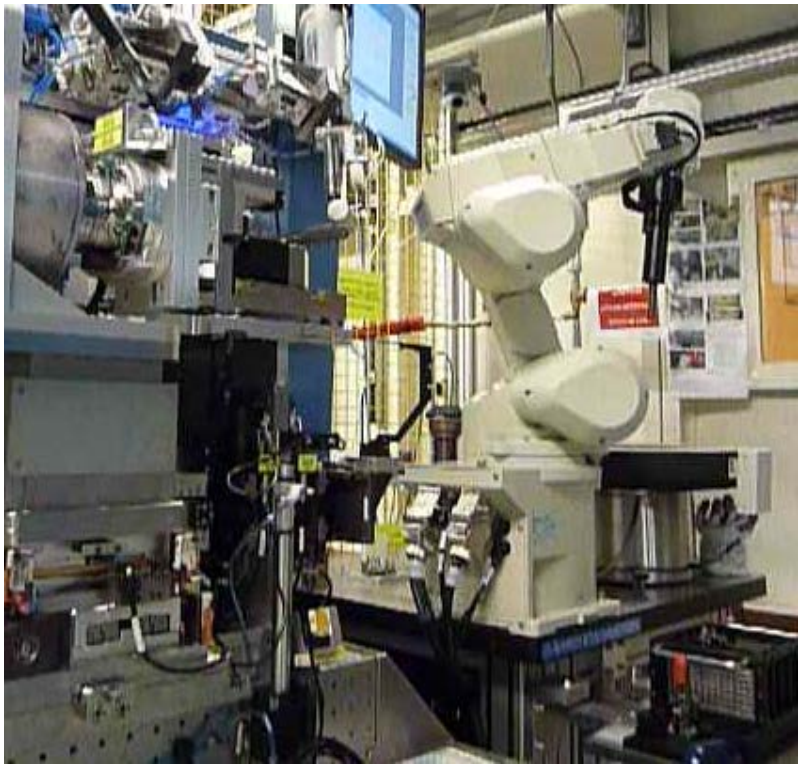


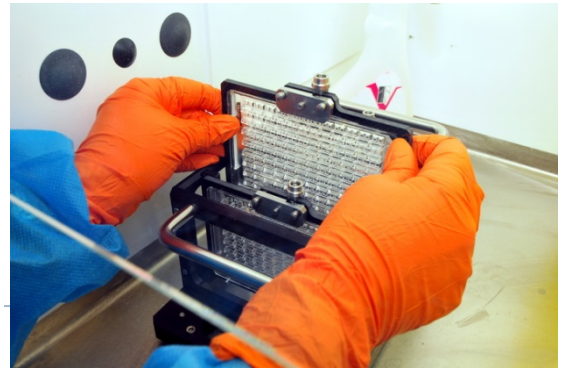
In-situ crystallography: data collection from crystals in crystallisation plates



Pathogenic samples can be studied on I03 and I24

- All beamlines level 1
- I03 and I24 up to level 2 biological containment
  - Cryo and *in-situ* samples
- I03 containment level 3 compliant
  - *In-situ* samples only
- Request via standard beamtime application process





Coming soon to the MX Village (new developments)



Performance: Crystal preparation

500 crystals in 2-day experiment

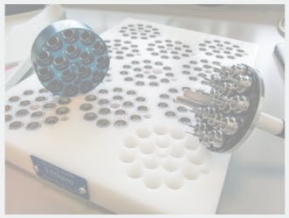
now

Echo + fragment libraries





Mitogen loops, trays, wand



IRELEC robot, loop autocentring



Add fragments

Add cryoprotectant

500 crystals

Drop evaluation | Soaking – wait | harvesting

Soaking lab: >2 days

Setup | Data collection | Puck change | Data collection | Puck change | Data collection | Puck change | Data collection

Beamline – 26 hours – *babysitting*

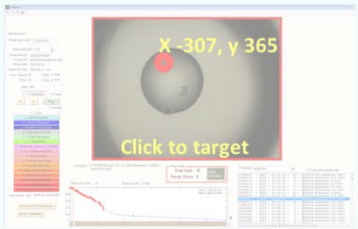
future

Soaking lab: <12hrs

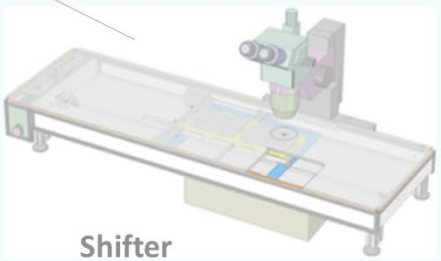
drop targeting

drop selection

Robot-assisted harvesting



TeXRank

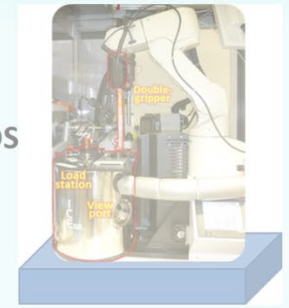


Shifter

Setup | Continuous data collection

Beamline – 20 hours – *unattended*

HPDS



Recent experiment:

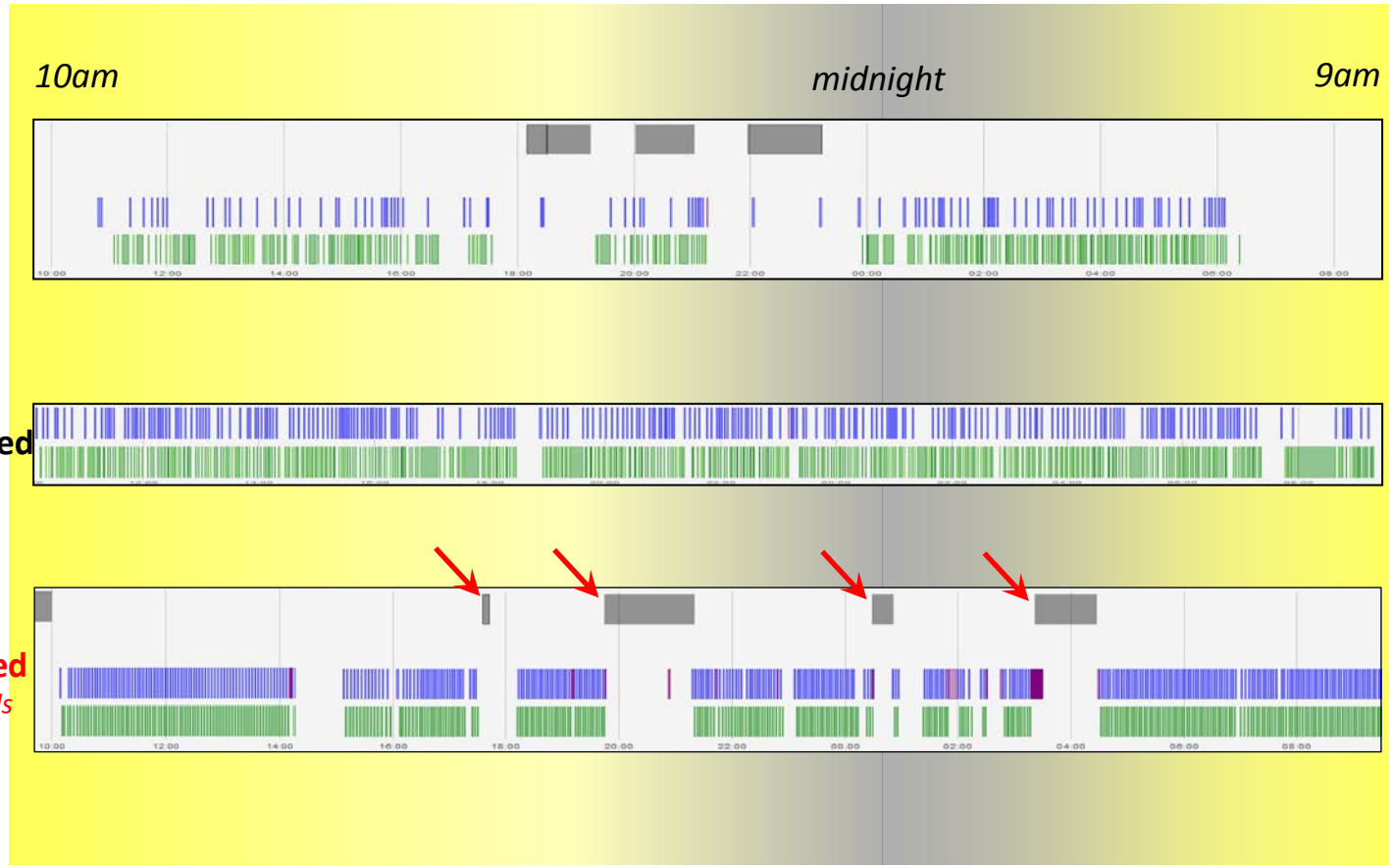
Preparation
200 crystals in 6 hours

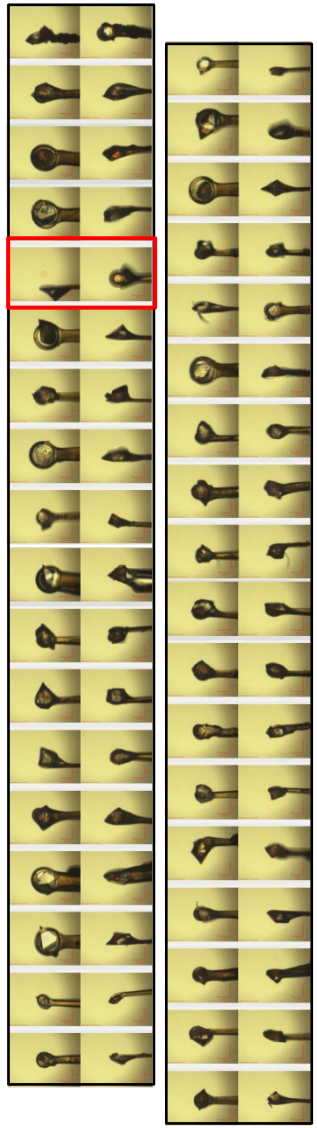
Collection
Overnight

(Full analysis: 2-7 days)

Unattended data collection queues

Sample auto-centring: >97%

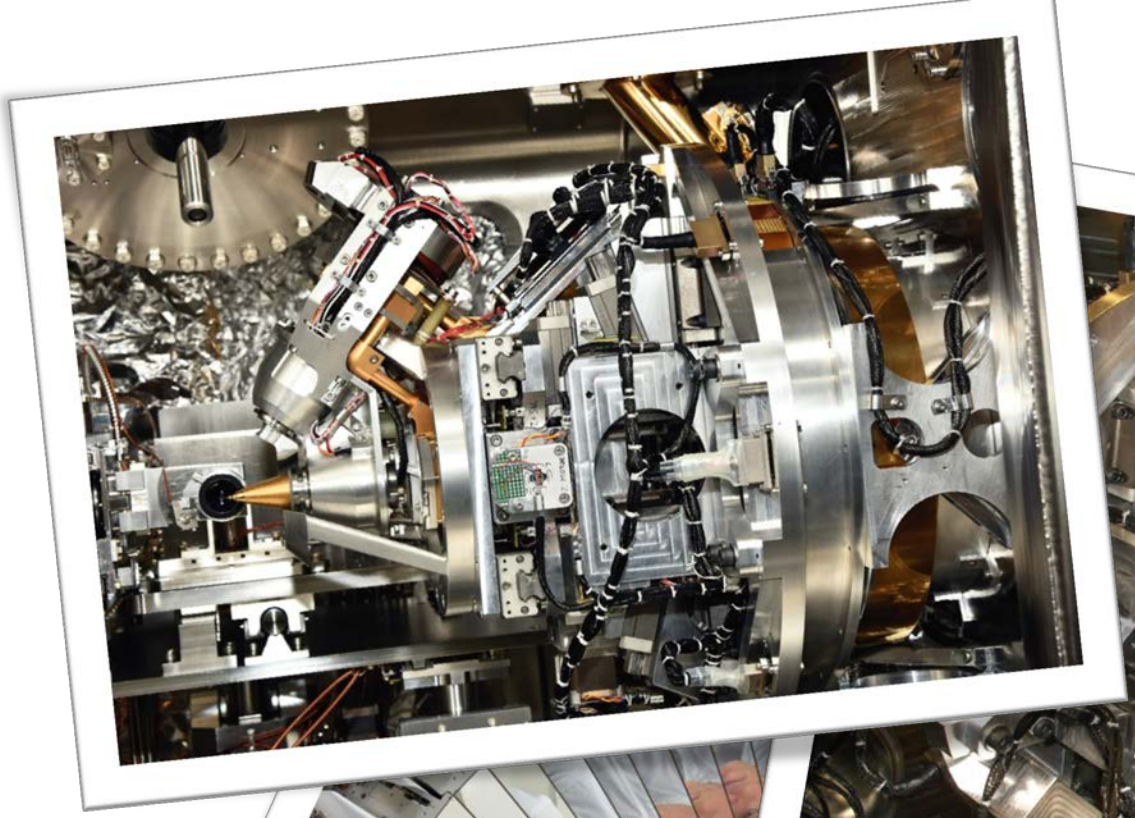


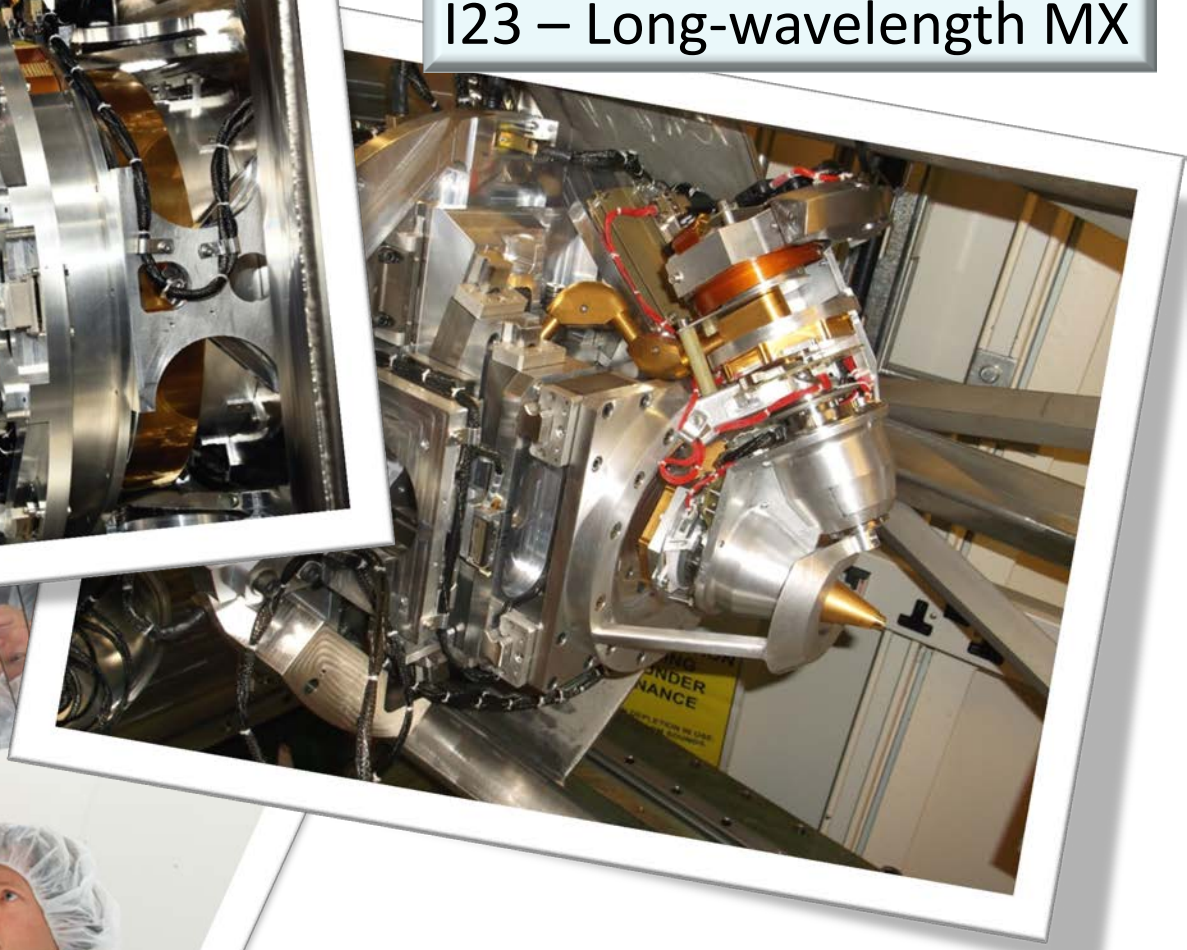


Coming soon to the MX Village (new beamlines)



I23 – Long-wavelength MX

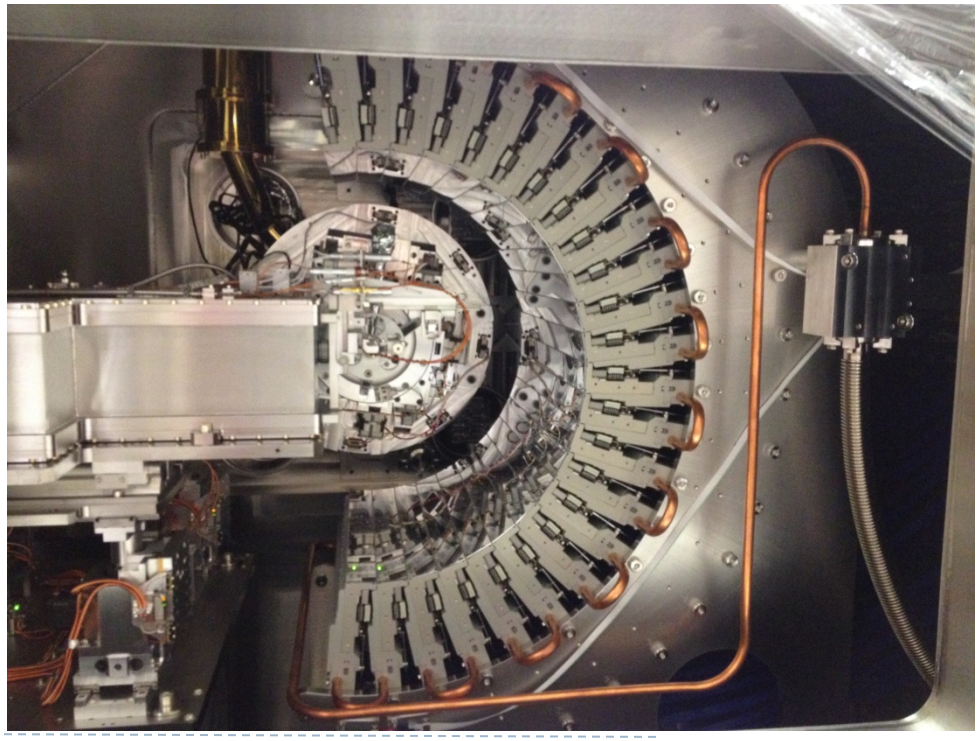






I23 – First beamline for in-vacuum long-wavelength MX

- Experimental Phasing
  - SAD phasing from native proteins and DNA/RNA crystals by using anomalous signal from sulphur and phosphorus.
  - SAD and MAD phasing for large complexes by using enormous anomalous signals from M-edges.
- Assist model building at low resolution
- Element specific analysis



- Beamline parameters:

Energy (wavelength) range:

2.1 - 12 keV (1 - 5.9 Å)

optimized for 3 - 8 keV (1.5 - 4 Å)

Band-pass (ΔE/E):

2×10^{-4}

Beam size at sample:

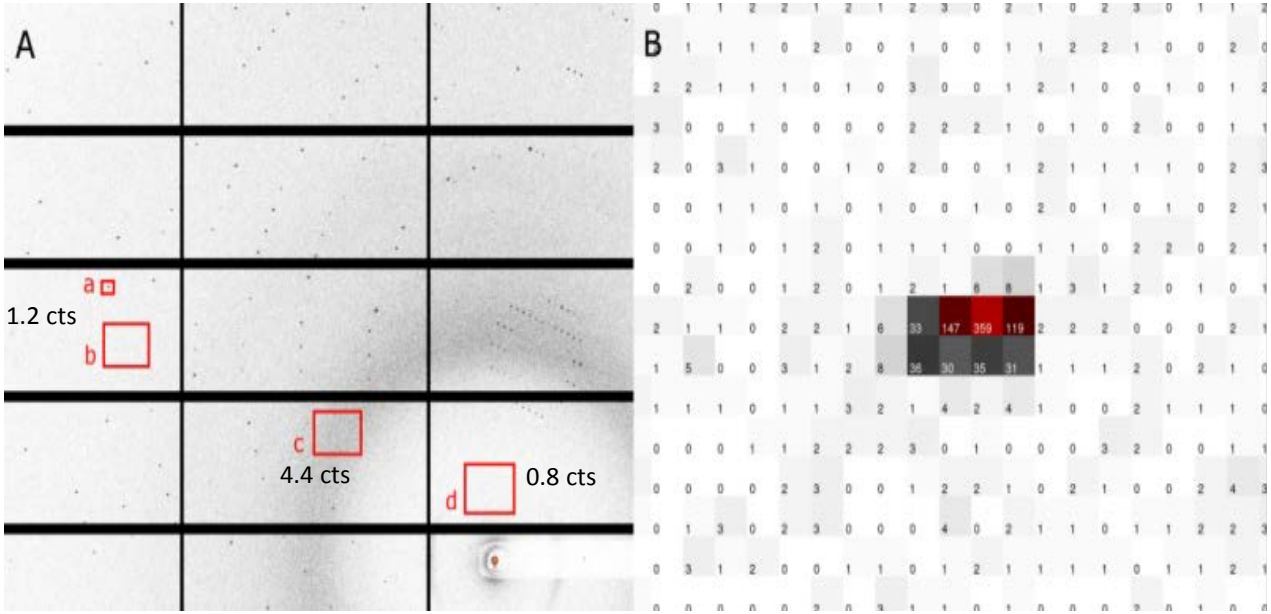
100 - 500 µm

Photon flux:

5×10^{12} ph/s in 100 x 100 µm @ 4 keV



Superior background



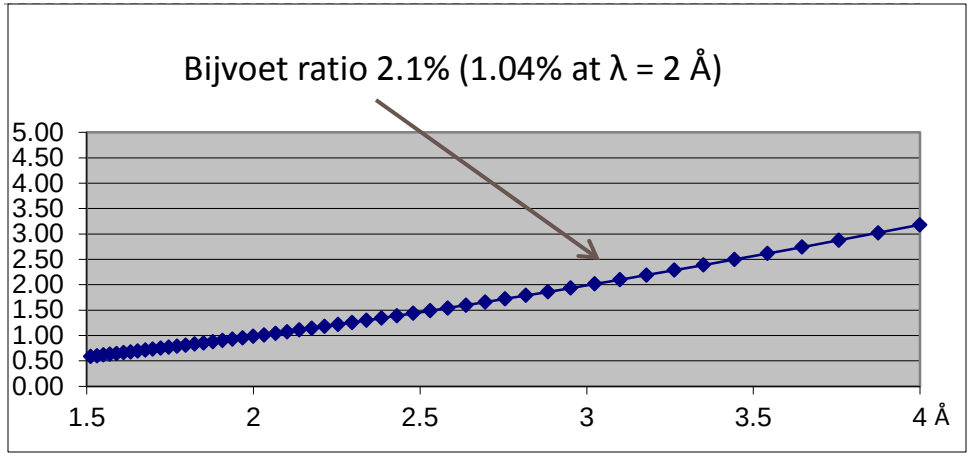
- In-vacuum setup on I23
- Every count on detector from sample (or sample mount)!

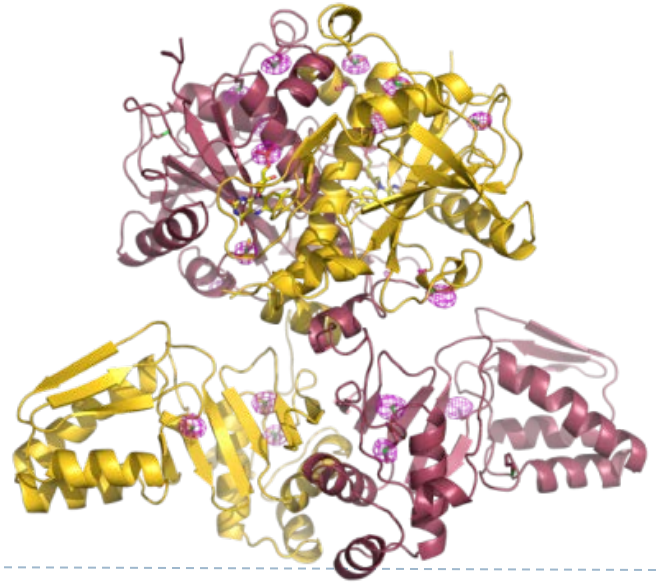
- Optimisation of signal-to-noise (I/σ) by background reduction rather than increase of primary intensity
- More efficient as experiments are limited by radiation damage!

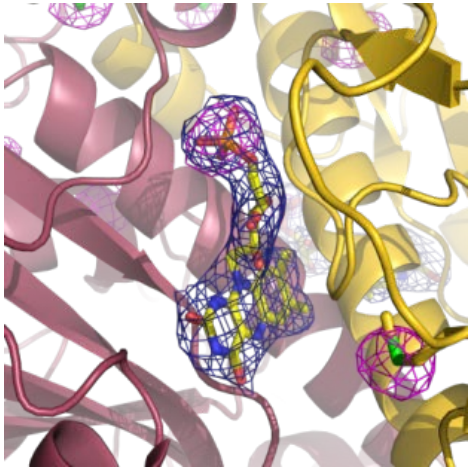
CyaGox

University of St Andrews

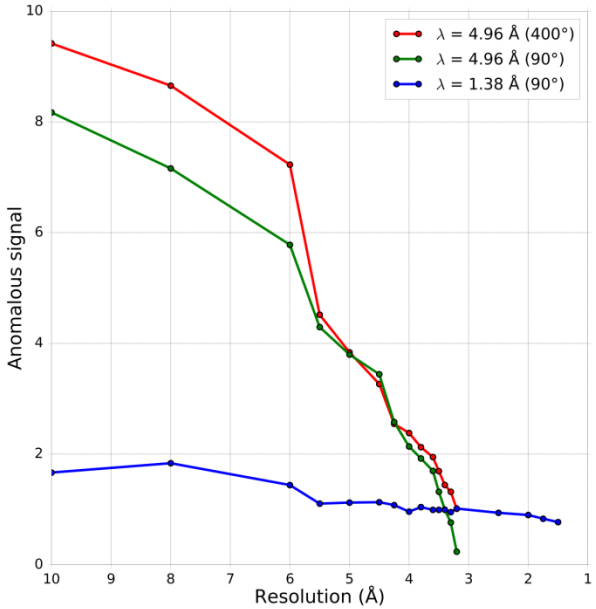
- 10 sulphurs in 477 amino acids
- $P4_12_12$
- λ = 3.1 Å
- Inverse beam 2 x 200 deg
- In total 48 crystals were mounted
- Large sample to sample variations
- Native data to 3.2 Å resolution
- Anomalous data to 4.2 Å resolution

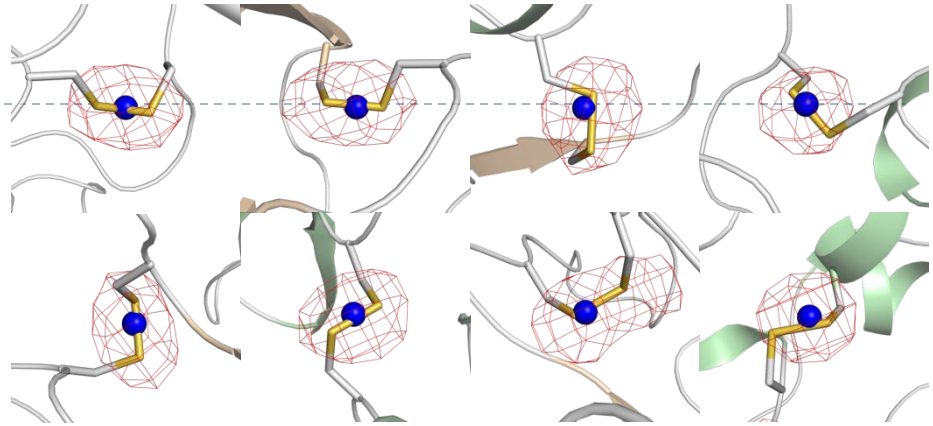






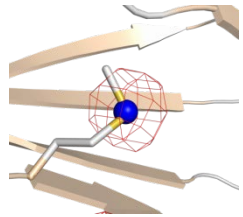
Towards the edge
λ = 4.96 Å

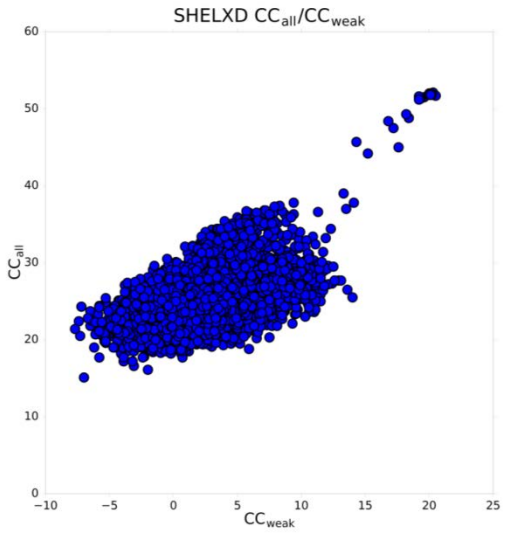


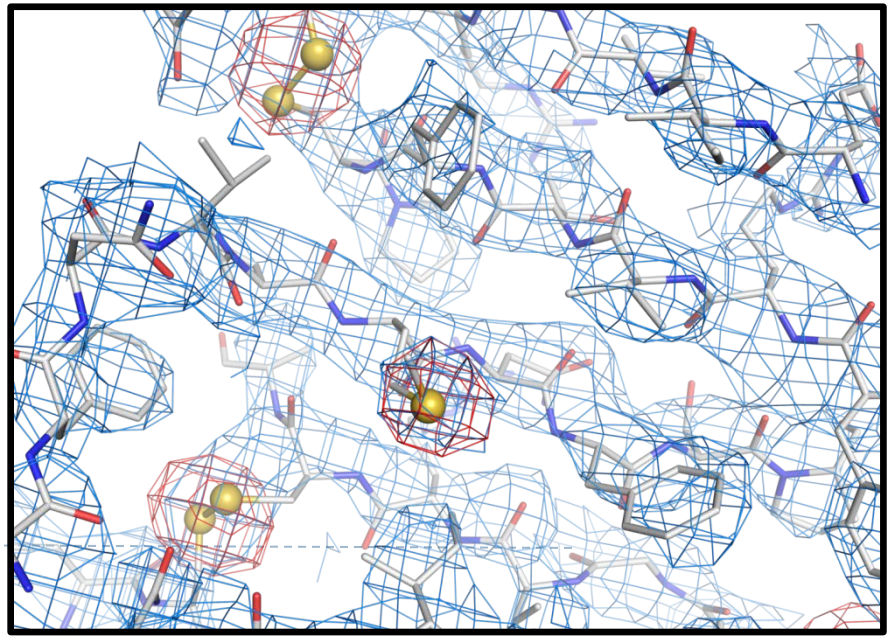


Thaumatin

Red (5.0 σ) – anomalous difference map

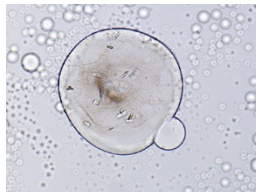


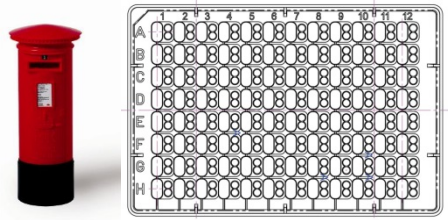




VMX$_i$ - dedicated to *in-situ* (plate) crystallography

- This beamline will provide
  - Plate storage facility
  - Automated transfer between storage and beamline
  - Advanced laser 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)
  - Diffraction data available less than 48 hours after shipping plate to Diamond - during run time…





VMX_{micron} Sub micron variable focus

Aims

- 0.5 x 0.5 um – 5 x 5 um beam
  - variable aspect ratio
- rapid beam size change
- 5 – 30 keV (dependent on ID choice)
- fast sample exchange/delivery
- fast crystal location

Challenges

- sample delivery
- sample visualization
- DCM
  - 13nrad pitch stability
- optics
  - facilitating fast beamsize changes is the real challenge

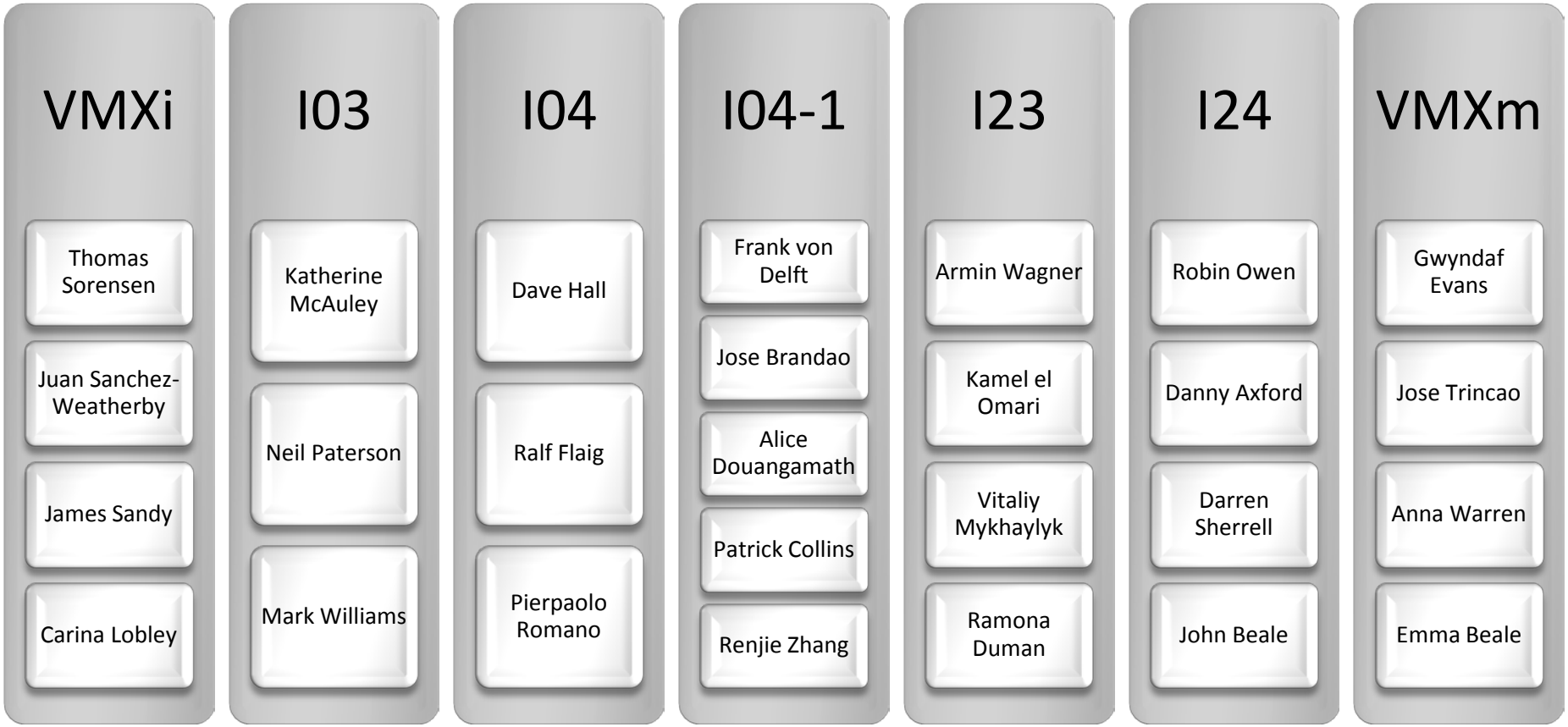
2014

- Technical Design Report
- Prototyping

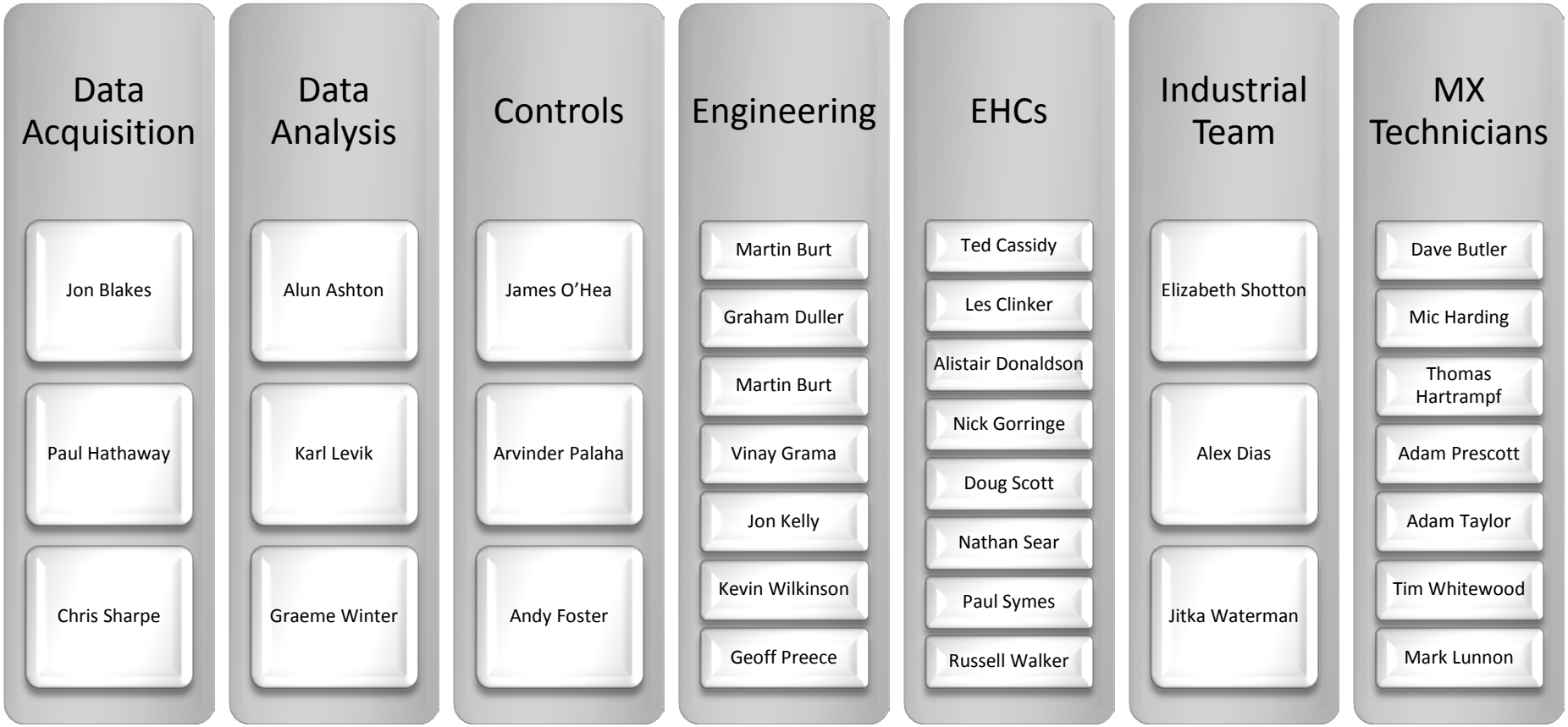
Team

- Gwyndaf Evans
- Jose Trincao
- Anna Warren
- Emma Beale

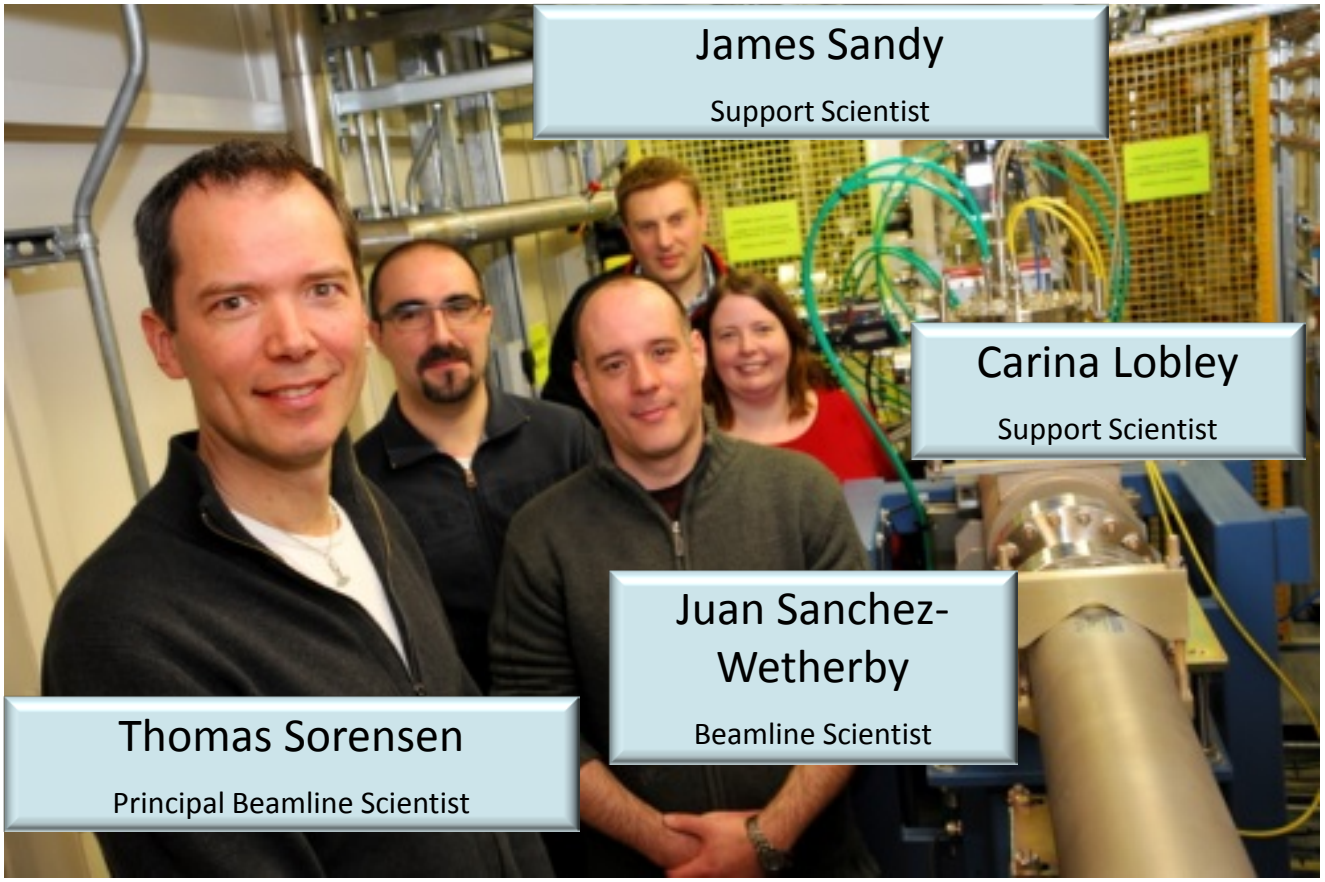
MX Village Support Team



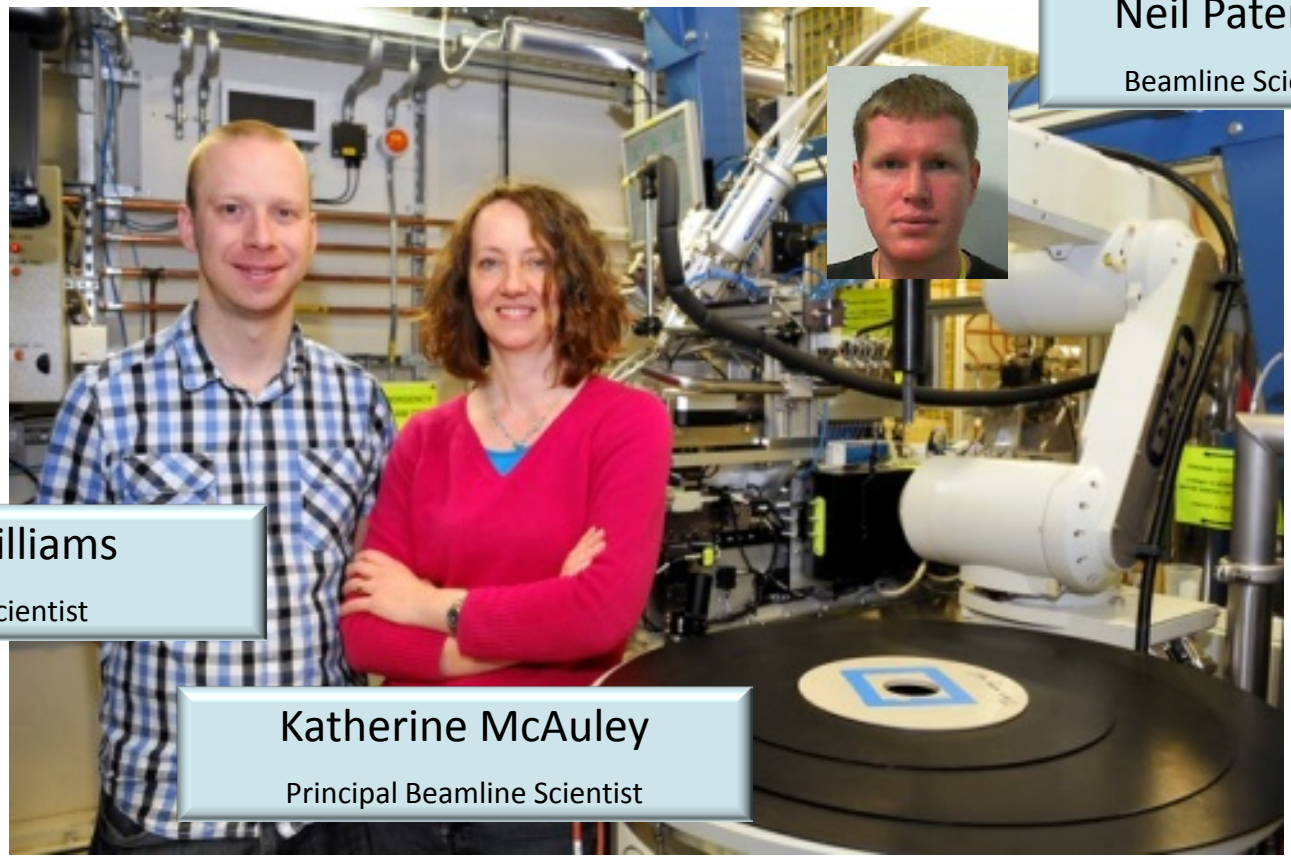
MX Village Support Team



I02/VMXi Scientists



I03 Scientists



Neil Paterson

Beamline Scientist

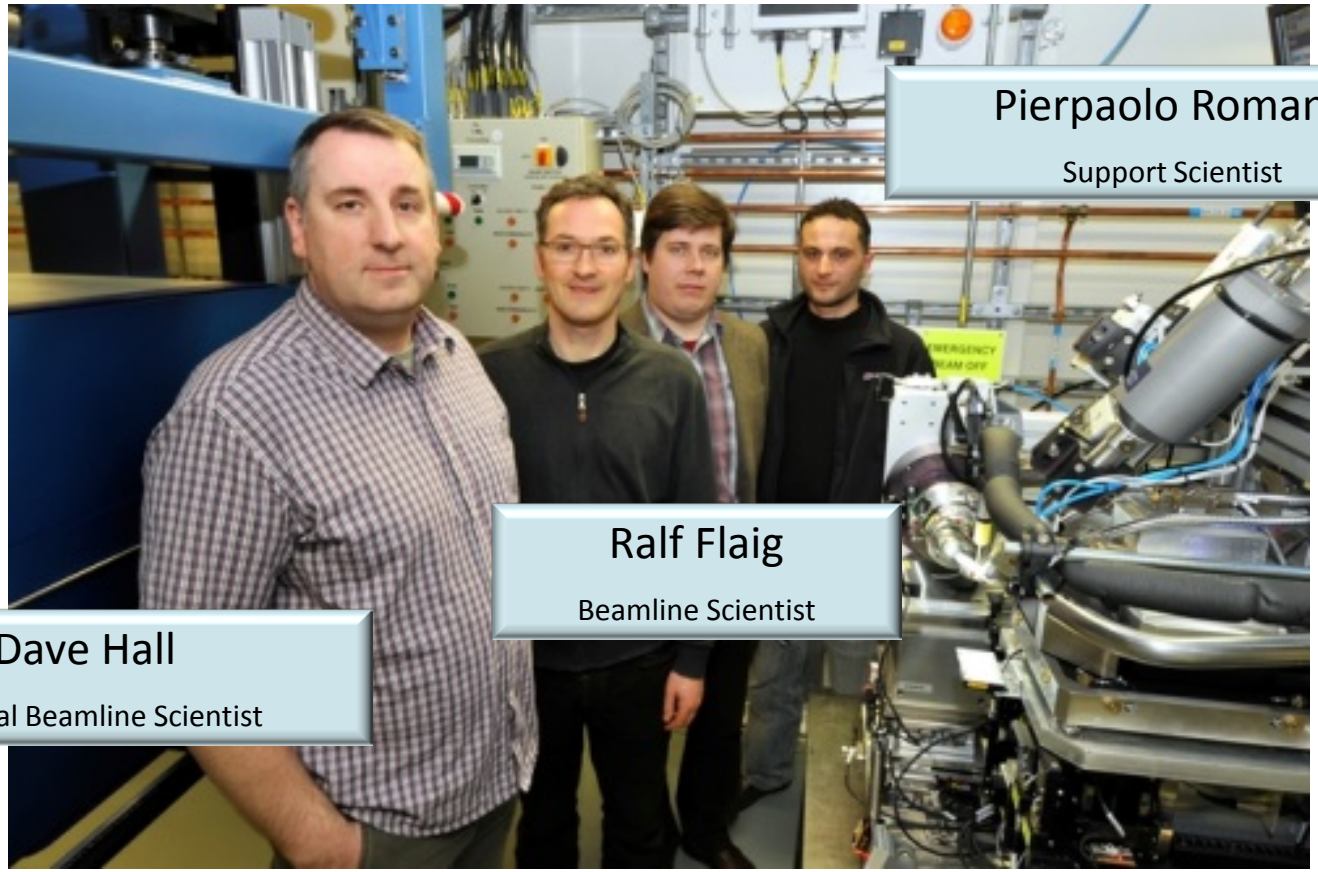
Mark Williams

Support Scientist

Katherine McAuley

Principal Beamline Scientist

I04 Scientists



Pierpaolo Romano

Support Scientist

Ralf Flaig

Beamline Scientist

Dave Hall

Principal Beamline Scientist

I04-1 Scientists



Frank von Delft
Principal Beamline Scientist

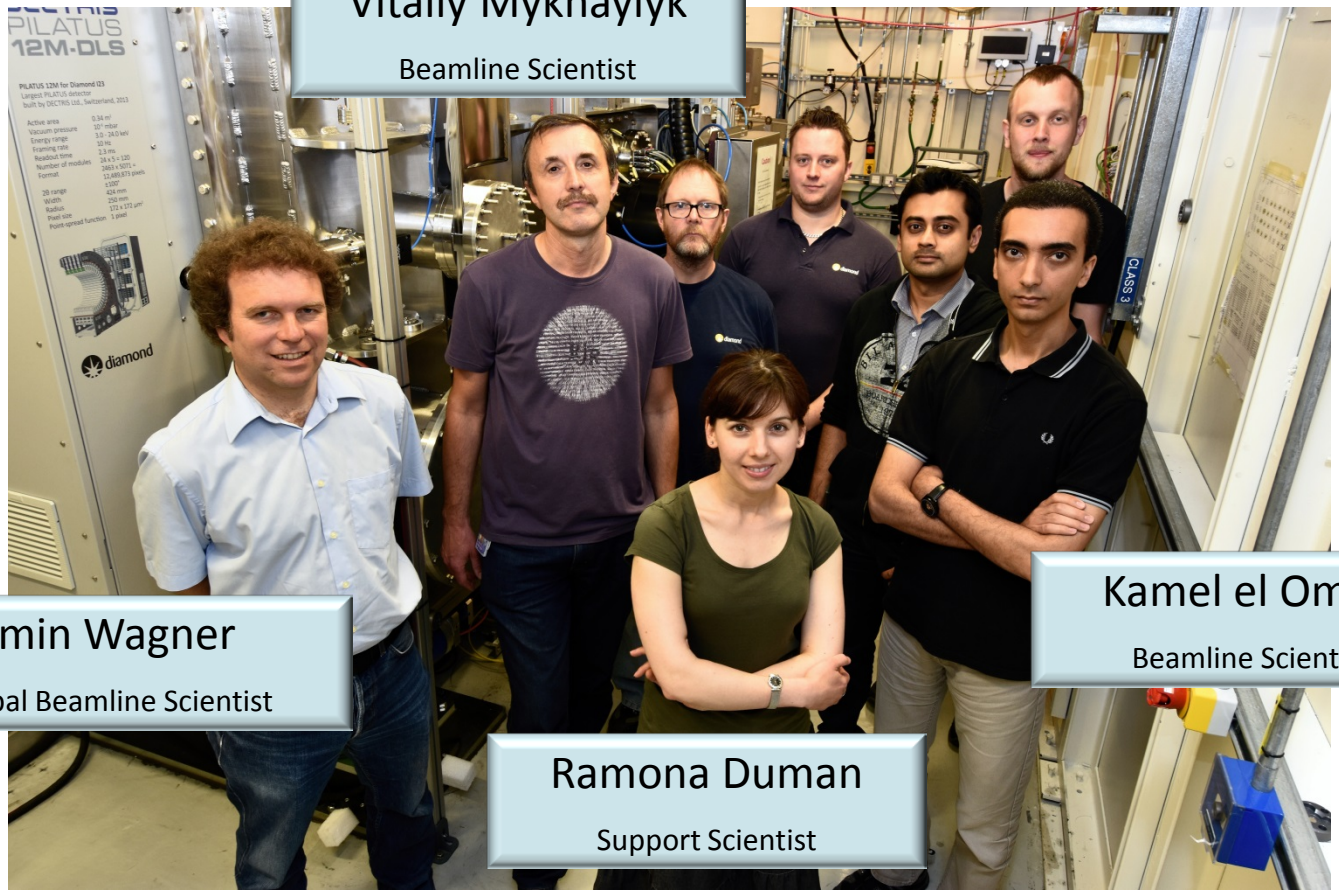


Jose Brandao
Beamline Scientist



Alice Douangamath
Support Scientist

I23 Scientists



Vitaliy Mykhaylyk

Beamline Scientist

Armin Wagner

Principal Beamline Scientist

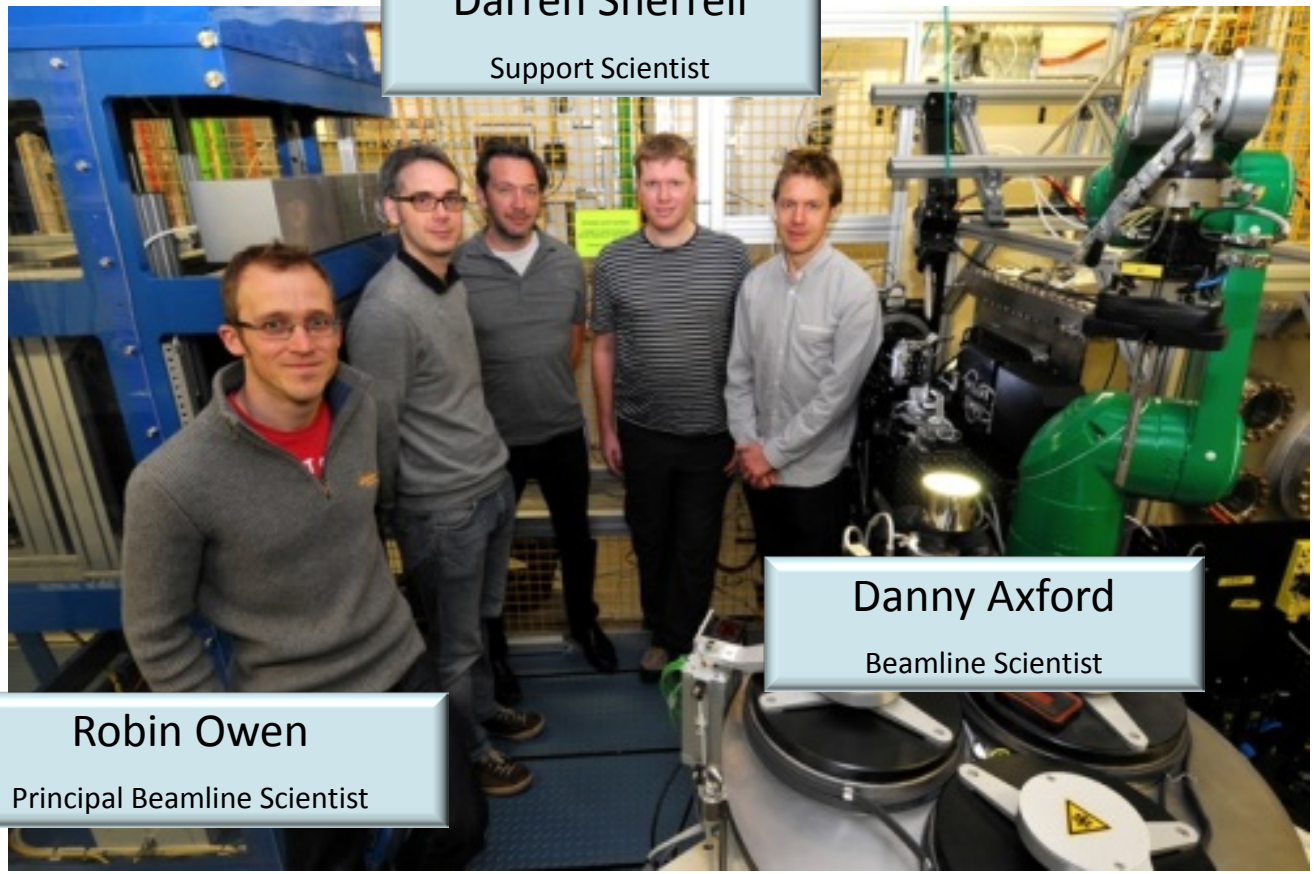
Kamel el Omari

Beamline Scientist

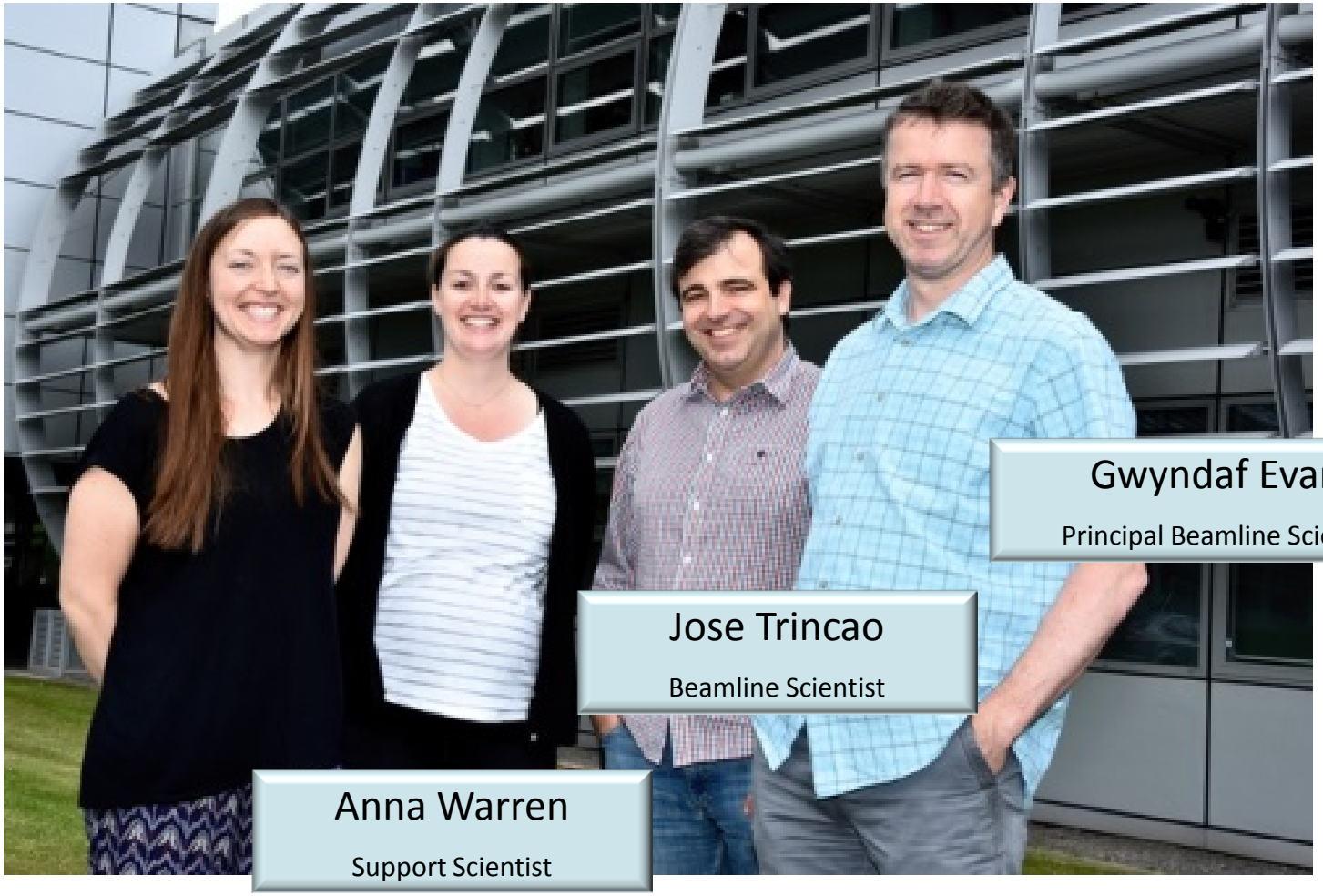
Ramona Duman

Support Scientist

I24 Scientists



VMXm Scientists



The End

