The High-energy Electron Xtallography Instrument

A tool for macromolecular structure determination: update

CCP4 Workshop

5th December 2023

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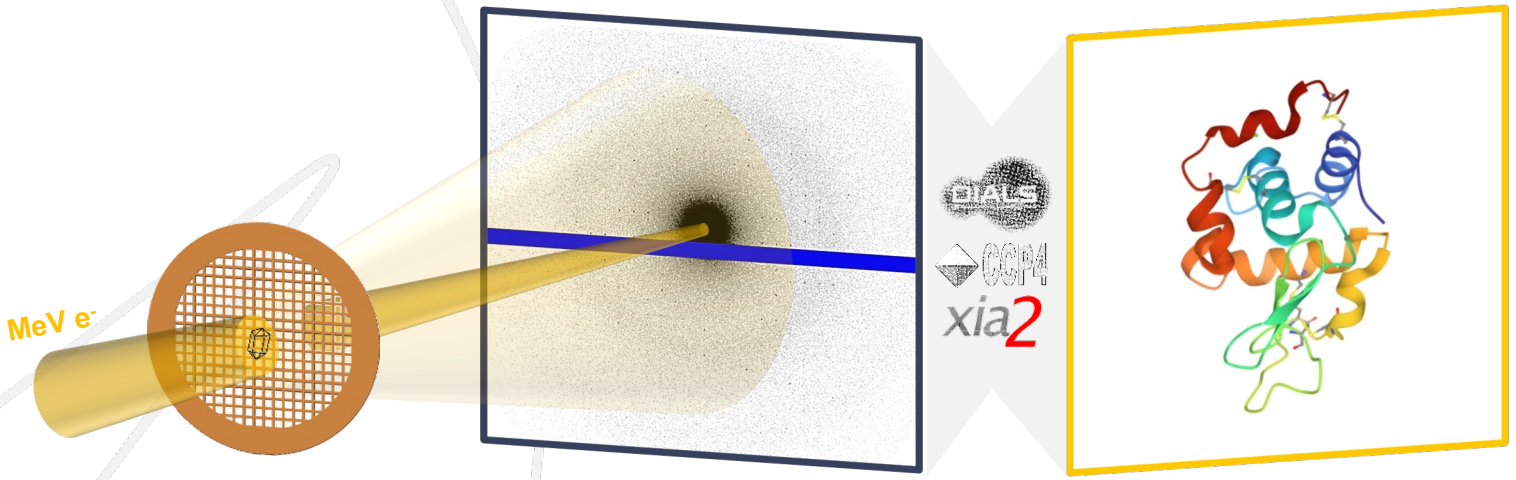
diamond

Introduction to HeXI

The **H**igh-energy **E**lectron **X**tallography **I**nstrument (**HeXI**) project aims to investigate the use of mega electron volt (MeV) electrons in macromolecular structure determination by 3DED



- Funded by The Wellcome “Electrifying Life Science” grant and MX@Diamond Light Source
- Combine the sensitivity of electrons with the performance of MX-grade goniometry

New Grant will Electrify Life Sciences with Breakthrough Technologies

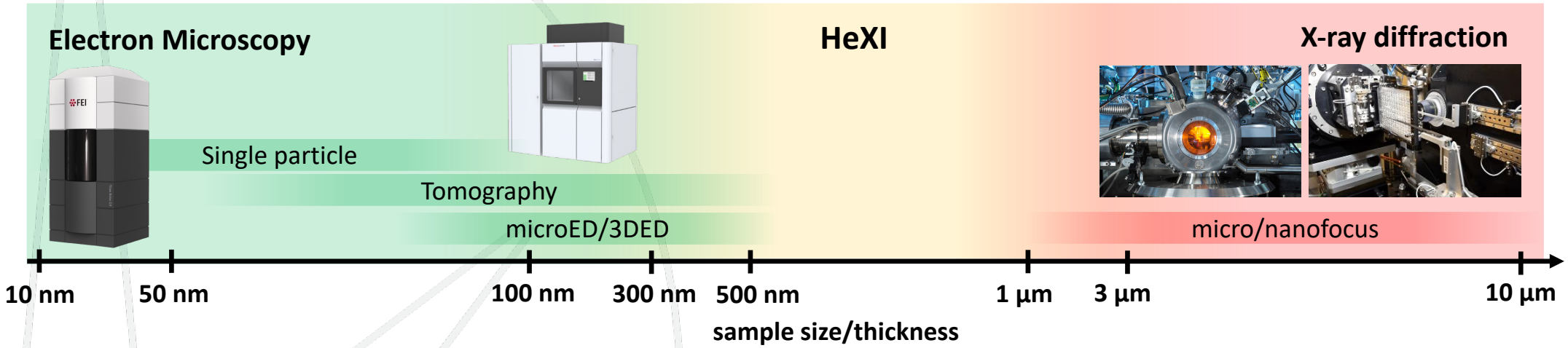
HeXI Electrifying Life Science

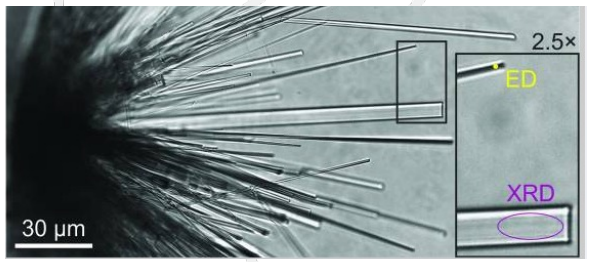
wellcome

diamond

HeXI instrument target and scope

The preferred structure determination technique varies as a function of sample size/thickness:





A. Lanza et al., IUCrJ (2019). 6, 178–188

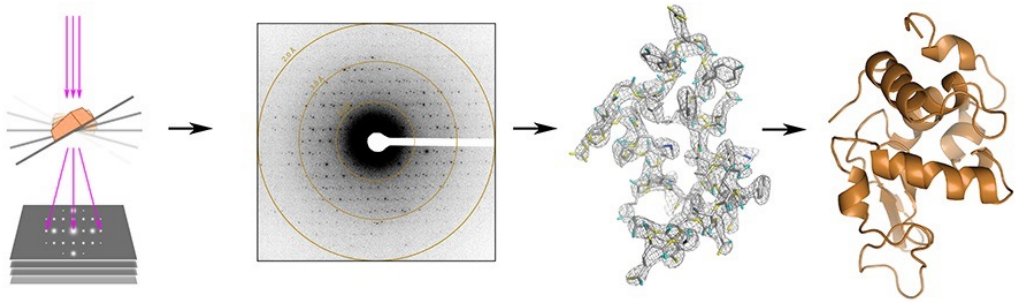
HeXI targets 300 nm to 1 µm crystals:

- Discarded crystal slurries from MX → Large number of potential samples
- Good size for ligand soaking studies → Dynamics
- Small molecules → Industry

HeXI will bridge the size gap between electron diffraction on TEMs and X-ray diffraction on MX beamlines

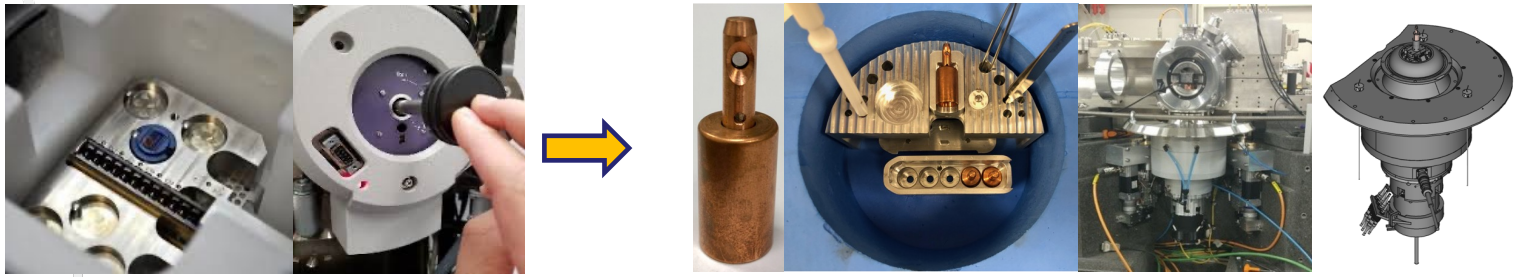
HeXI instrument goals

1. **Structure determination from 0.3 to 1 µm crystals**
   - Use the increased penetration of MeV electrons to bridge the crystal size gap between electrons and X-rays

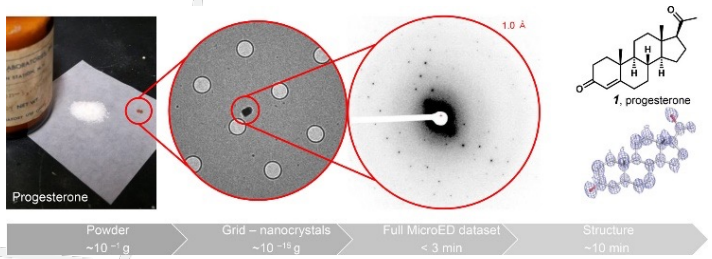


https://cryoem.ucla.edu/microed

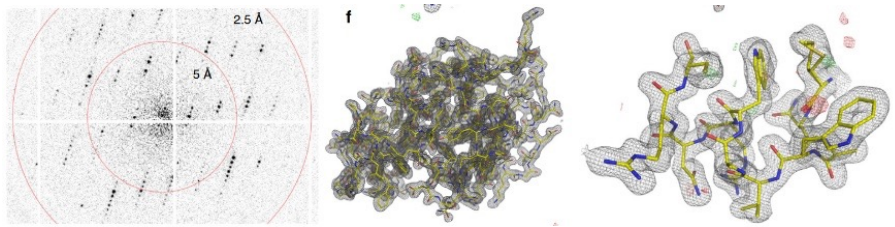
2. **Improved fidelity of 3DED data**
   - Use high performance MX-grade goniometry developed at Diamond
   - Reduced dynamic scattering



3. **A tool for rapid high-resolution structure determination**
   - 3DED → Small molecules
   - Cryo-3DED → "" + proteins
   - SerialED → "" + Dynamics



C. G. Jones et al. *ACS Cent. Sci.* 2018, 4, 11, 1587–1592



R. Bucker *et al. Nature Communications*, **11**, 996 (2020)

HeXI instrument specifications

Design considerations

- Diffraction optimised instrument
- Low-resolution STEM imaging
- 1 Å resolution (<200 Å unit cells)
- No post-specimen lenses
- Cryo-capabilities
- Samples mounted on TEM grids

Parameter	Value
Electron beam energy (MeV)	0.1 – 1
Electron beam timing	CW
Energy spread (ΔE/E FWHM)	$<10^{-4}$
Beam current at source (μA)	15
Beam current at sample (pA)	0.6 - 400
Beam size at the sample (μm)	0.3 - 3
Rotation speed (deg/s)	60
Environment temperature (K)	77 and 293
Sweep data collection rate (Hz)	0.5
Serial data collection rate (Hz)	100

Continuously tunable

High flux

Match crystal size

10 x faster than TEM

Cryo-capabilities

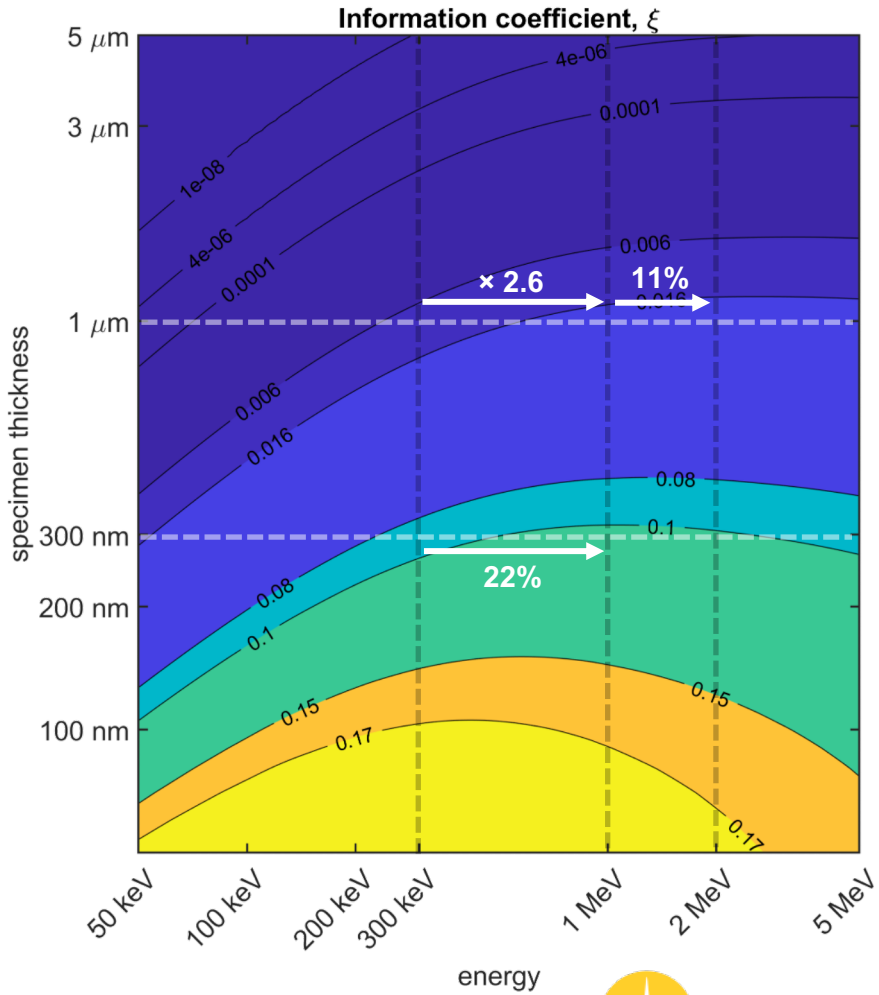
Information per unit damage as a function of electron energy and crystal thickness:

Information coefficient:

$$\zeta \equiv T\frac{\sigma_e}{\sigma_i}$$ ← Elastic cross-section / Inelastic cross-section

$$T \simeq e^{-t/\lambda}$$ ← Total mean free path; t: Specimen thickness

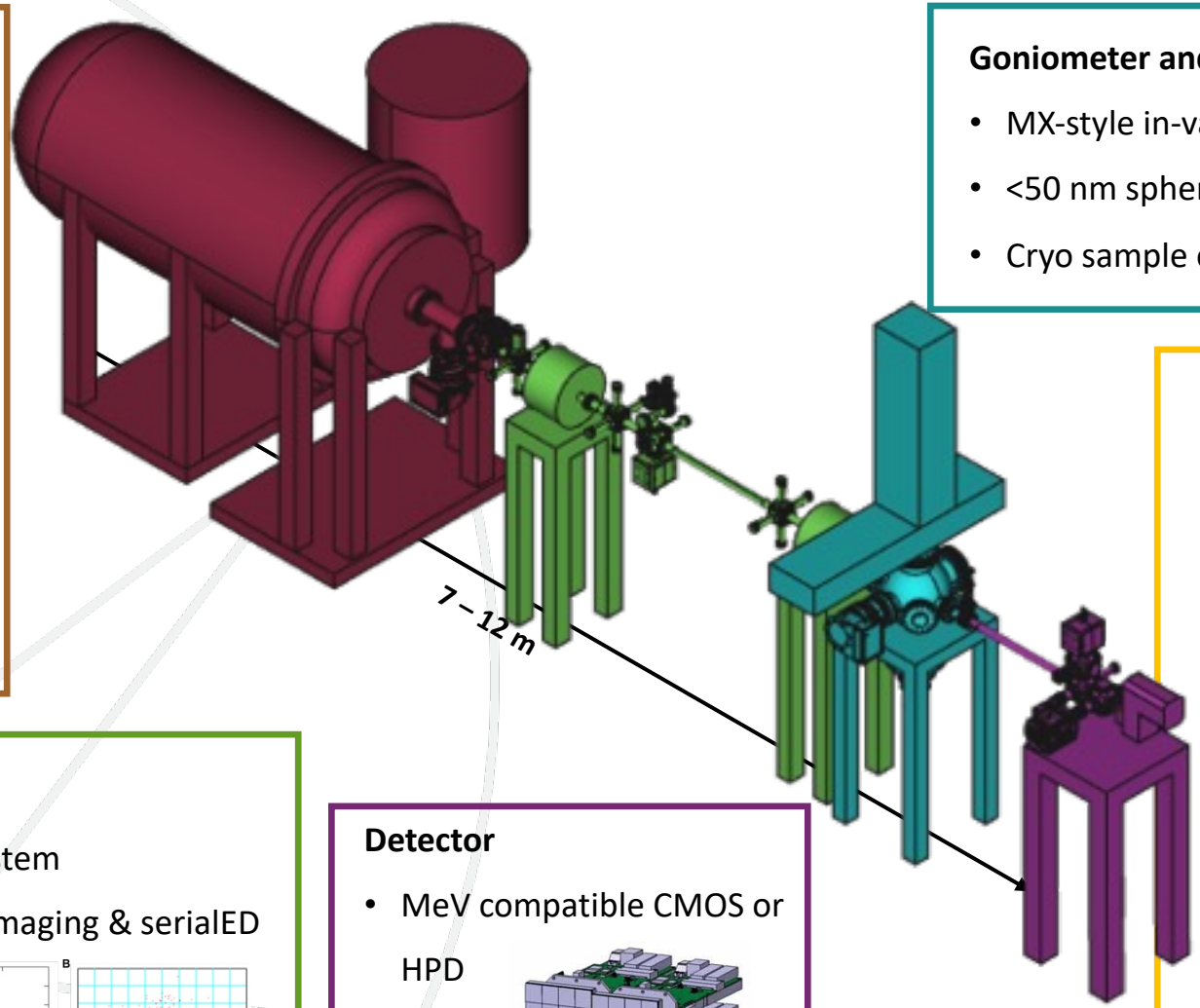
M. J. Peet, R. Henderson, C. J. Russo, Ultramicroscopy 203 (2019)



HeXI instrument design concept

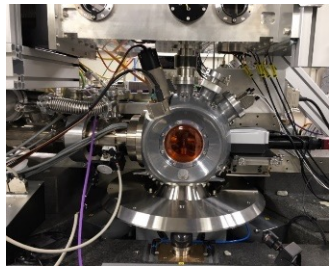
1 MeV electron source

- Cockcroft-Walton generator
- Electrostatic accelerator
- LaB_6 emitter
- Continuously tunable



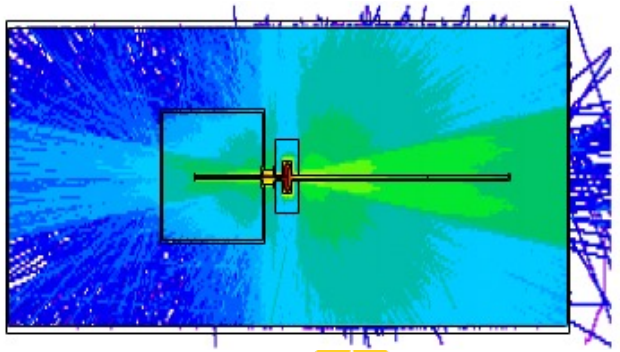
Goniometer and sample environment

- MX-style in-vacuum goniometer
- <50 nm sphere of confusion
- Cryo sample exchanger and hotel



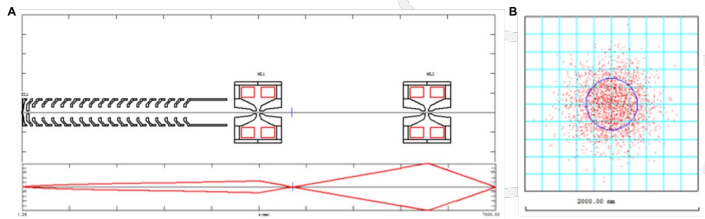
Shielding & Location

- ~820 mm concrete or ~160 mm lead (assuming 15 µA 1 MeV beam)
- B10 wedge on Experimental Hall floor
- Typical “optics hutch” - 50mm lead construction



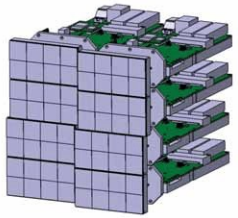
Electron optics

- Bespoke 2 or 3-condenser system
- Scanning capabilities: STEM imaging & serialED

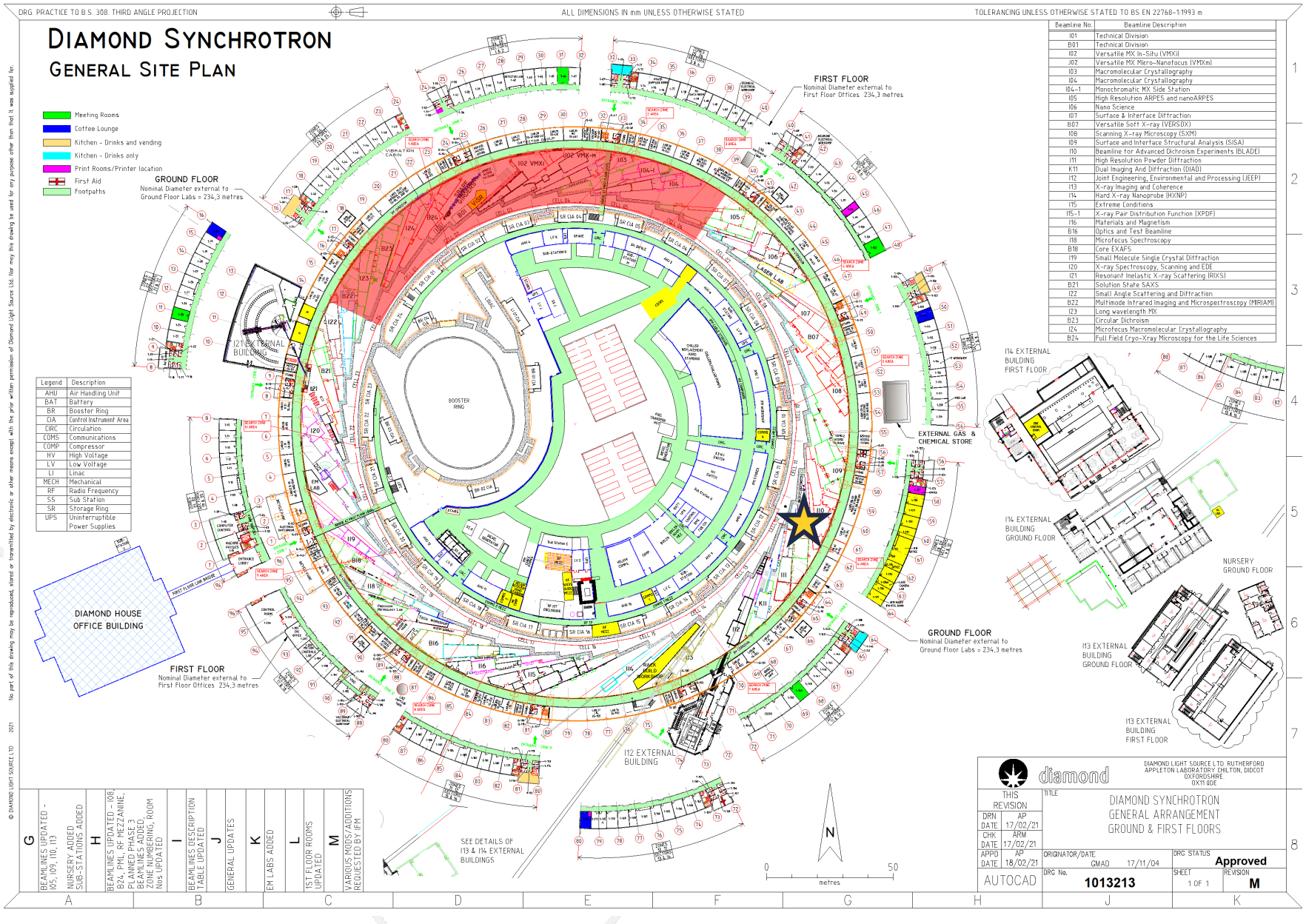


Detector

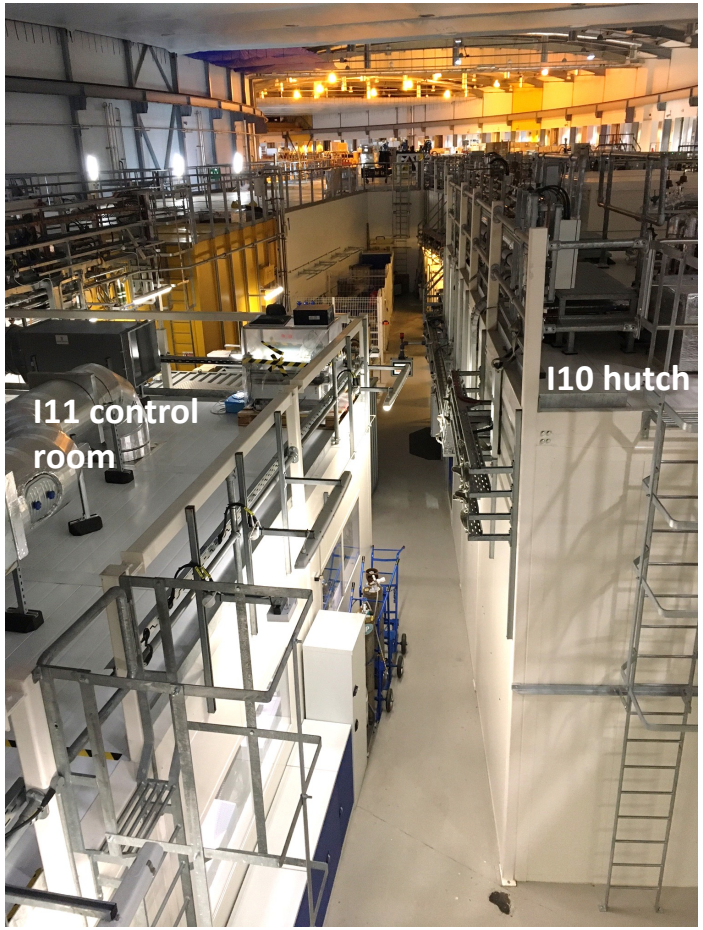
- MeV compatible CMOS or HPD



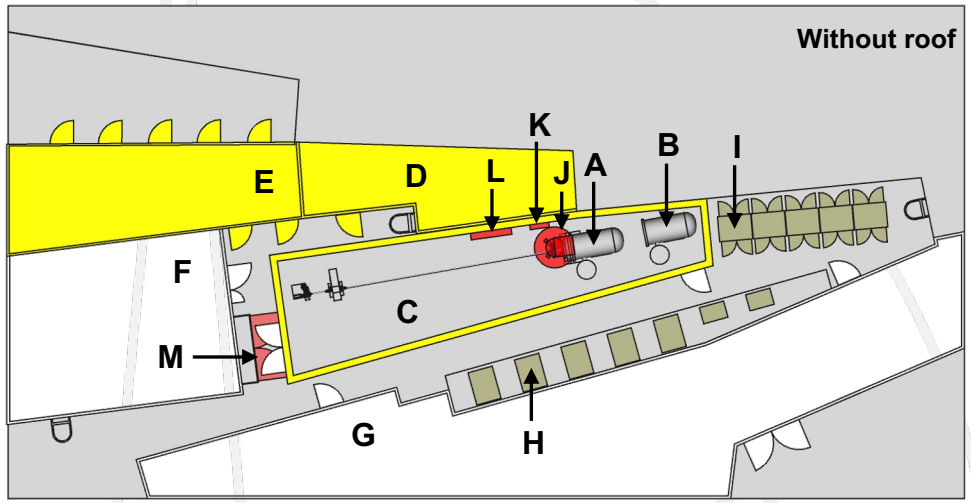
HeXI location

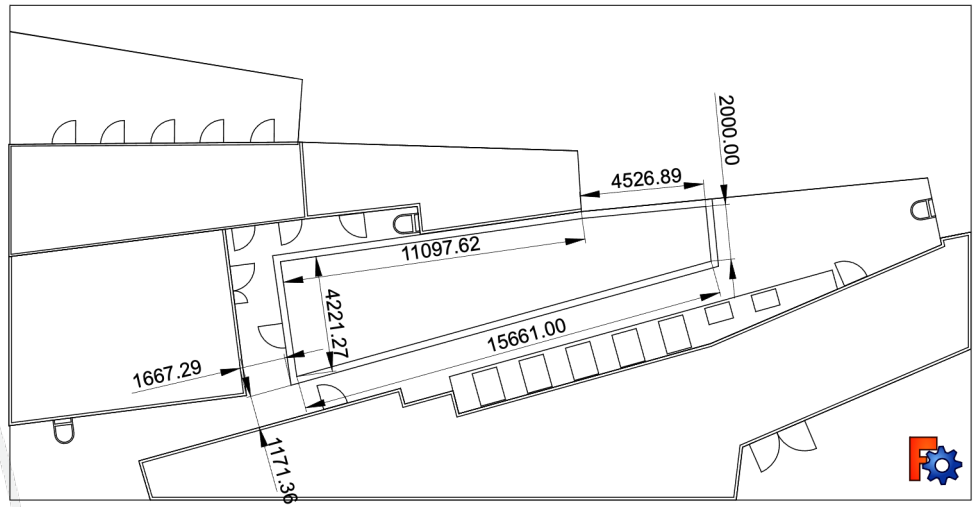


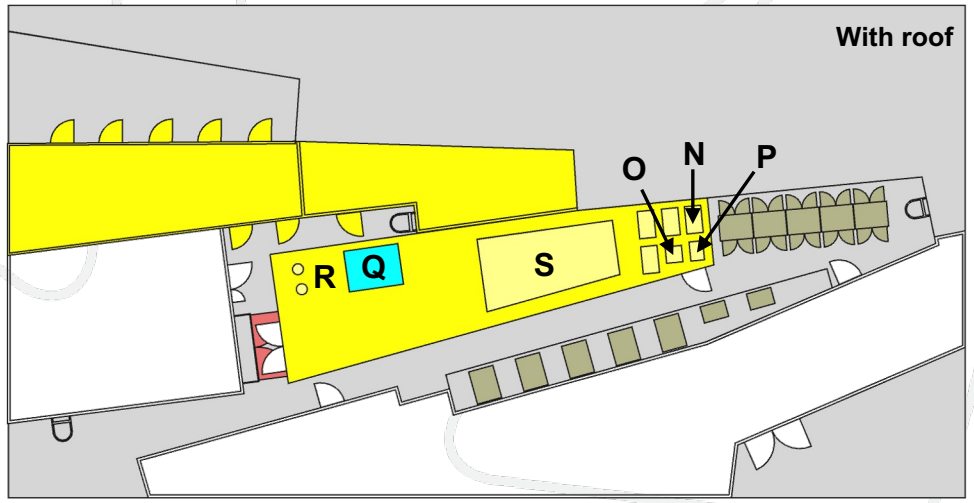
B10 wedge

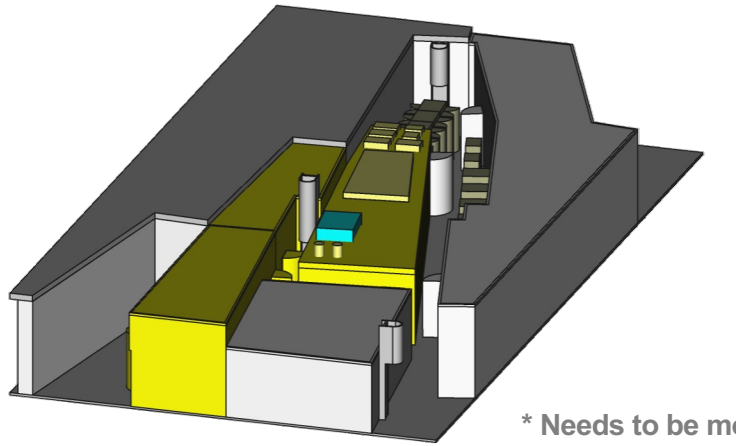


HeXI hutch design concept









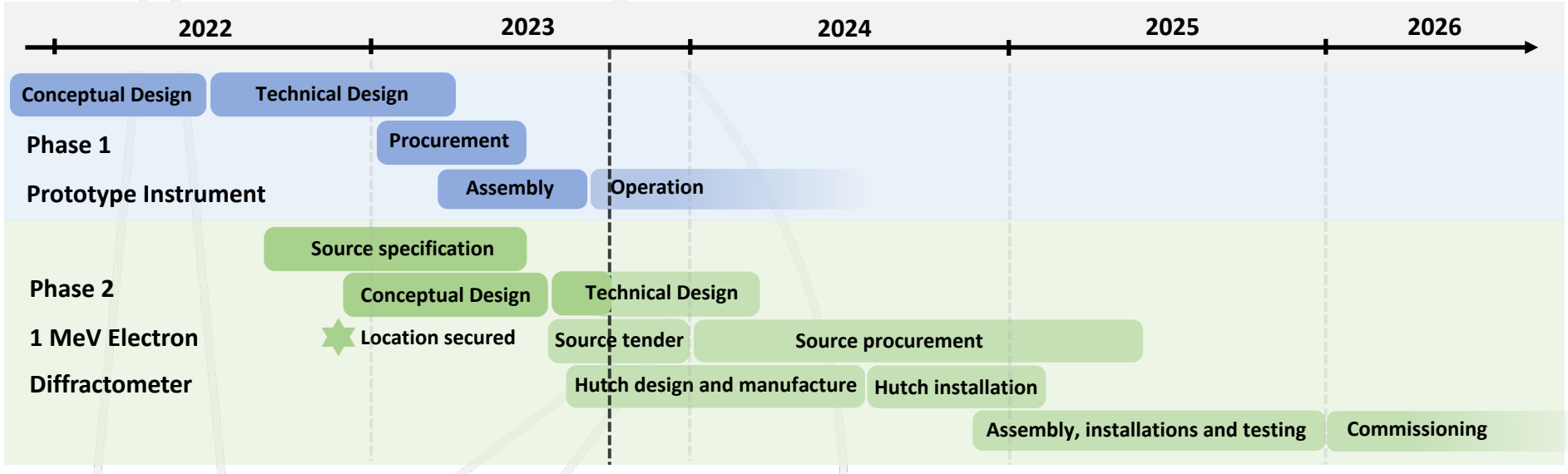
* Needs to be modified

A – HeXI instrument
B – HeXI service position
C – HeXI hutch
D – I11 OH1
E – I11 CIA
F – I11 CC2
G – I10 Cabin
H – I10 racks
I – HeXI racks
J – I11 cryo-cooler*
K – I11 chicanes*
L – I11 radiation monitor*
M – I11 cylinder cage*
N – HeXI electronics chicanes
O – HeXI services chicanes
P – HeXI LN2 chicanes
Q – HeXI air handling
R – HeXI in/out vents
S – HeXI removable roof

- Completed the preliminary room datasheet and specification for the HeXI hutch
- Started the design brief documentation for hutch tender

Project milestones

Project plan:



HeXI Phase 1 Prototype Instrument

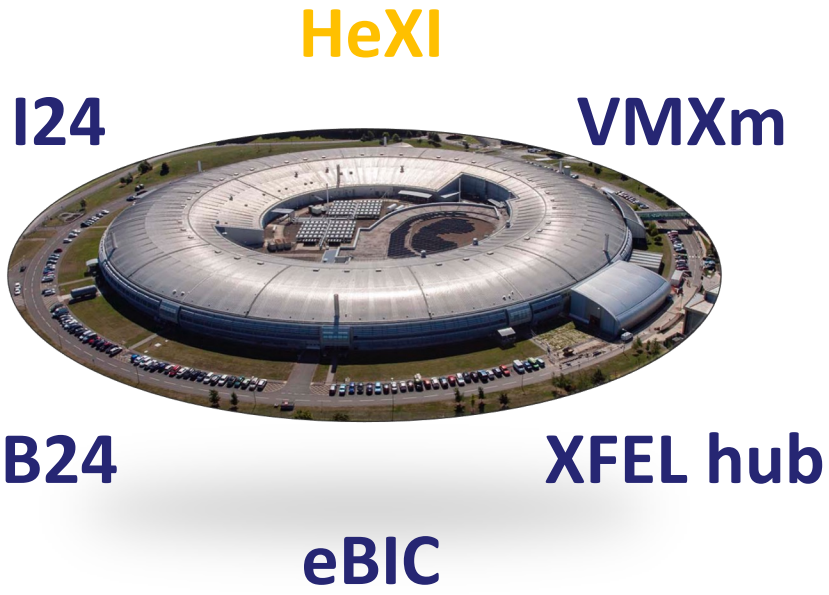
- Test optical configurations
- Investigate crystal targeting
- Prototype diagnostic tools
- De-risk phase 2 instrument

Recent and upcoming milestones:

- Conceptual design report submitted and signed-off – August 2023
- Functional specification completed and signed-off – September 2023
- Technical design report on track for submission by December 2023
- Technical design report sign-off scheduled for March 2024

Project outlook

1. **Deliver an instrument ready for commissioning in 2026**
    - Unique addition to Diamond's suite of crystallography instruments
2. **Perform systematic multi-modal structural determination studies**
    - Synergy between HeXI and VMXm
    - Leverage the inherent complementarity of electrons and X-rays
3. **Perform time-resolved serialED on slurries of sub-micron sized crystals**
    - Future synergies with XFEL hub and I24
4. **Explore micron-scale MeV electron imaging**
    - Future synergies with eBIC and B24



Acknowledgments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Richard Littlewood

Mark Lunnon

Andrew Foster

Adam Prescott

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Richard Doull

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