LUND UNIVERSITY

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The NMX Macromolecular Diffractometer – design and expected performance

Early Science on the NMX Macromolecular Diffractometer

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Macromolecular Crystallography

Challenges for neutron crystallography

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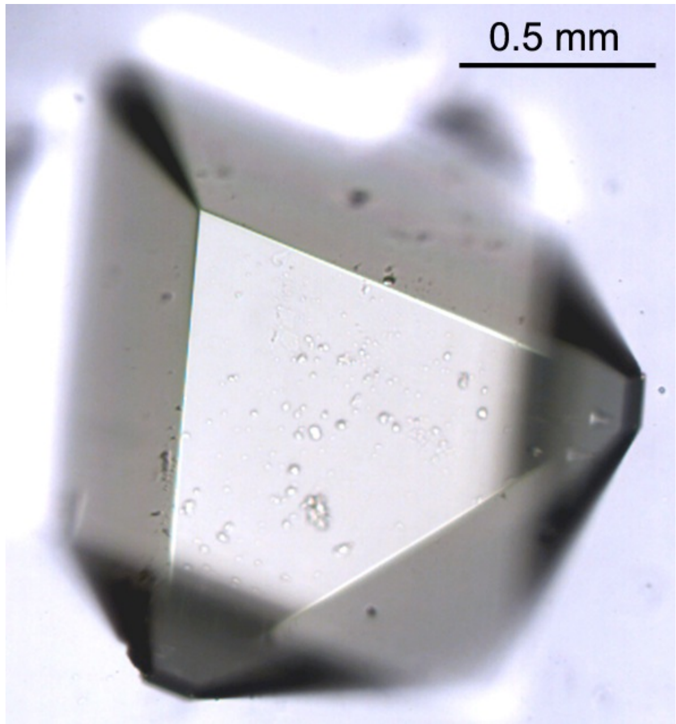
- **Weak neutron sources**
  - Bigger crystals → more diffracting volume
  - Use Laue geometry → make all neutrons count
- **Incoherent scattering**
  - Exchange ^{1}H to ^{2}H (deuterium)
  - Produce perdeuterated protein



Oksanen, E *et al. J. R. Soc. Interface* **2009**, *6 Suppl 5*, S599-610.

Incoherent scattering

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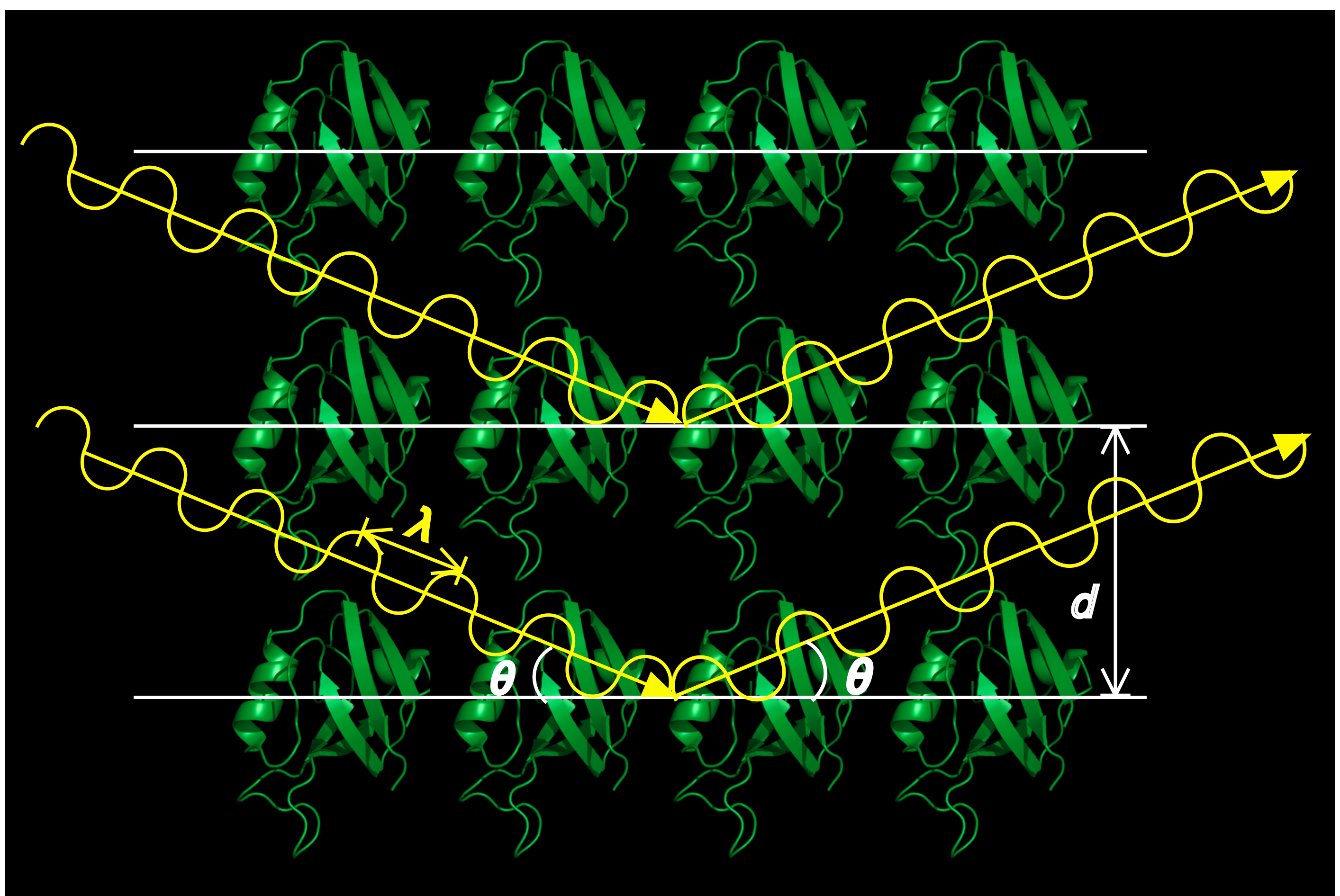
- Incoherent scattering contributes *only* to background!

Some scattering cross sections

Nucleus	σ_{coh} $(10^{-28}\ m^2)$	σ_{incoh} $(10^{-28}\ m^2)$
^1H	1,76	82,03
^2H	5,59	7,64
^{12}C	5,56	0
^{14}N	11,03	0,5
^{16}O	4,23	0



Phase change random in scattering

NMX High-level Requirements – starting point

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- A typical system to be studied would be a 30-40 kDa protein
- Unit cell edges 100-150 Å, up to 300 Å
- Realistic dmin ~1.8 Å, rarely < 1 Å
- Crystal size ~0.01-0.1 mm^3
- Data set in one day

High-level Scientific Requirements

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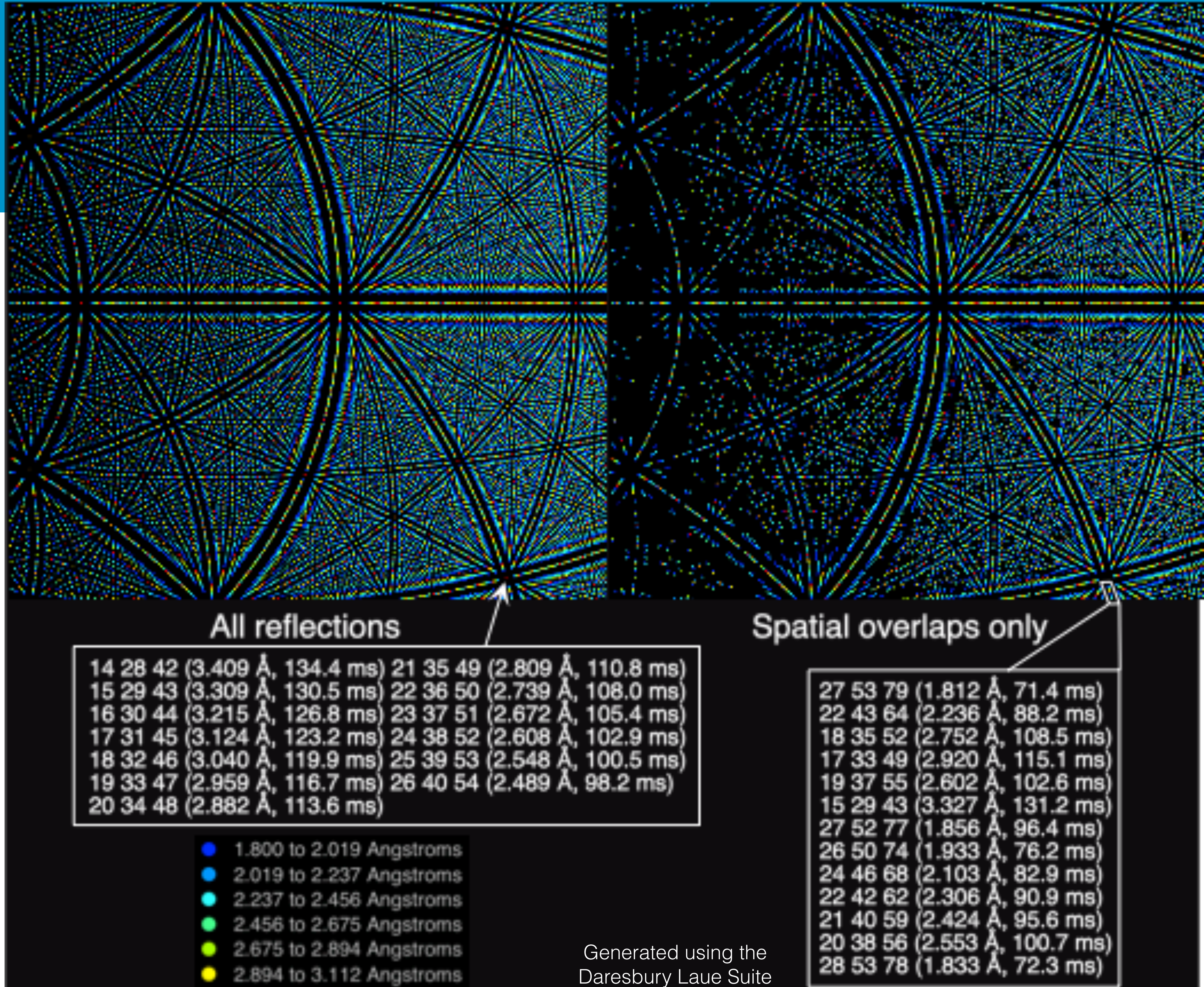
1. The instrument shall allow data collection from crystals with unit cell repeats > 300 Å.
2. The instrument shall allow data to be collected to a d_{min} of 1.5 Å.
3. The instrument shall match the size of the neutron beam to the size of the sample.
4. The instrument shall match the divergence of the neutron beam to the mosaicity of the sample.
5. The instrument should maximise the signal-to-background (S/B) ratio of the Bragg reflections.
6. The instrument should allow data collection from crystals of < 0.01 mm^3 volume

Bovine heart
cytochrome c oxidase
$P2_12_12_1$
a = 182.59 Å
b = 205.40 Å
c = 178.25 A
Detector distance 1 m



Generated using the
Daresbury Laue Suite

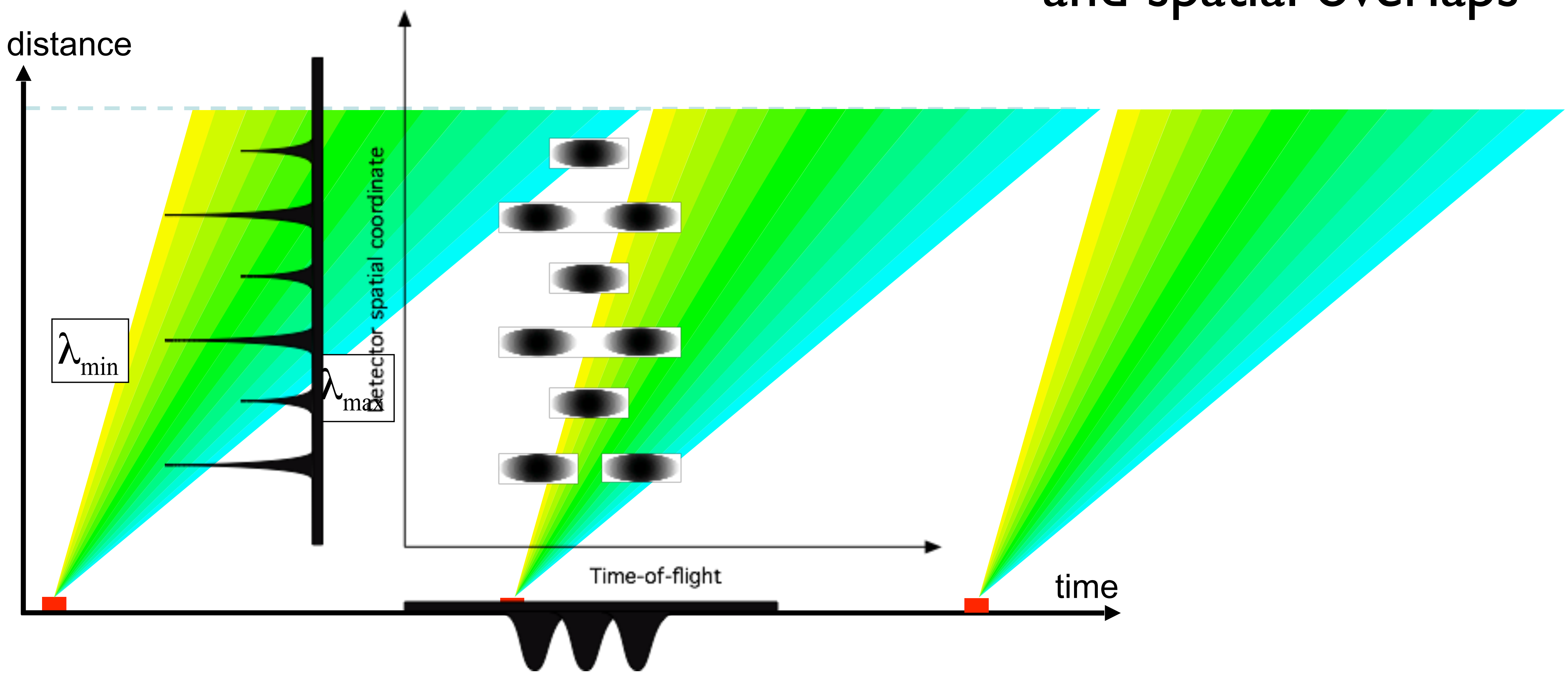
Campbell et al. J. Appl. Cryst. (1998). 31, 496-502
Artz et al. J. Appl. Cryst. (1999). 32, 554-562
Helliwell, J.R. et al. J. Appl. Cryst. (1989) 22, 483–497

TOF Laue crystallography

Spreads background in many time bins

Can resolve harmonic and spatial overlaps

At pulsed spallation sources we can resolve time-of-flight → energy



What wavelength to use?

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$$I_{pk} = t\phi(\lambda)\varepsilon(\lambda)\kappa \frac{V_{cryst}\lambda^4}{2V_{cell}^2\sin^2\theta} T_{DW}\langle|F_{hkl}|^2\rangle$$

I_{pk} = integrated intensity for an average Bragg peak (n) = duration of measurement (s),

t = duration of measurement (s)

$\phi(\lambda)$= incident spectral flux at sample ($n.cm^{-2}.s^{-1}.Å^{-1}$)

$\varepsilon(\lambda)$ = detector efficiency

κ = conversion factor 1×10^{24} ($cm^2.bn^{-1}$)

N^S = no. of unit cells in sample,

2θ = Bragg angle for reflection,

v_{cell} = unit cell volume ($Å^3$)

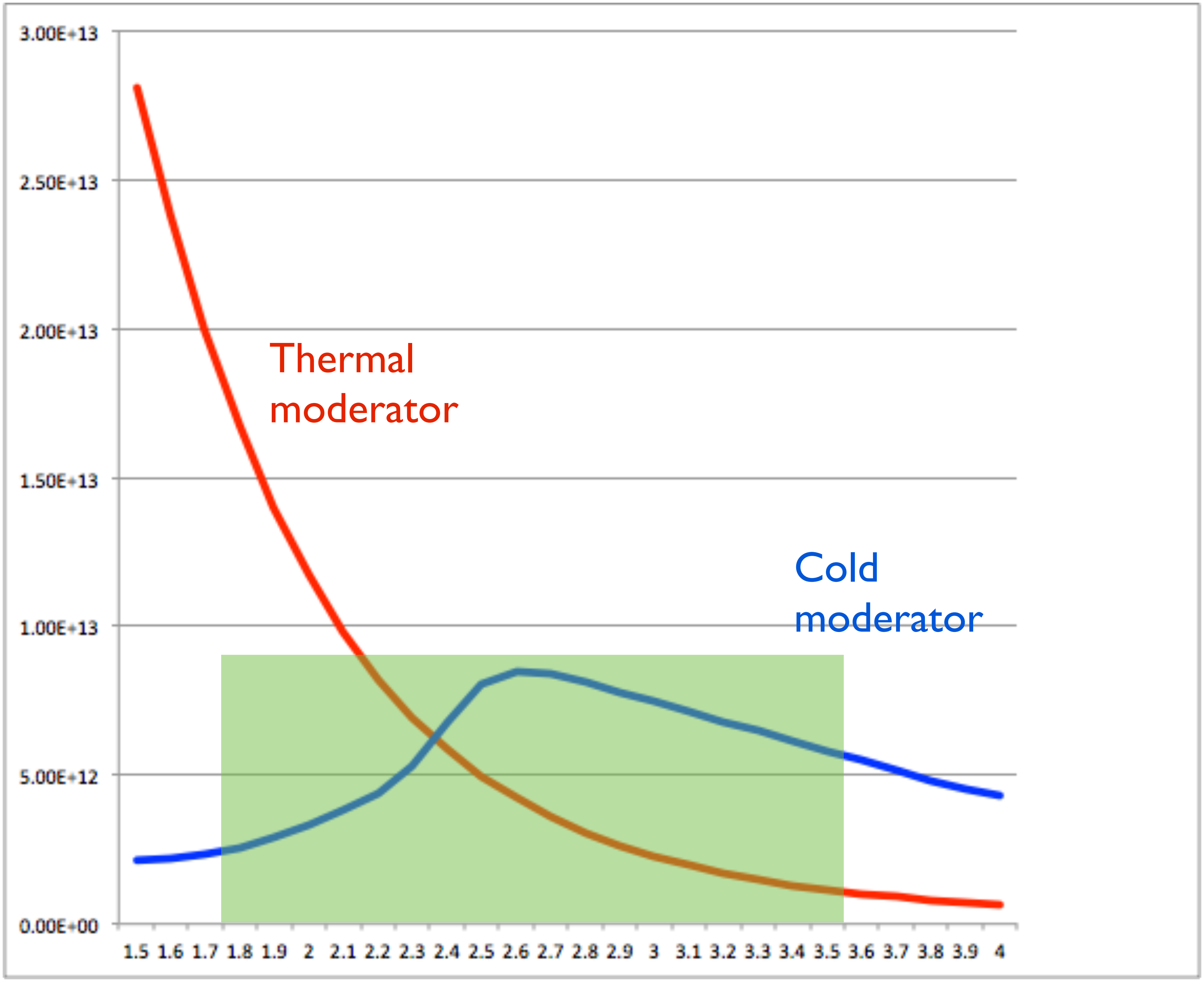
v_{cryst} = crystal volume ($Å^3$)

T_{DW} = temperature factor

$|F_{hkl}|^2$ = structure factor modulus of reflection hkl squared (bn)

Air absorption is not a problem → Go for long wavelengths!

Moderator spectra



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

For $2\theta = 180°$

↓

$d_{min} = \lambda/2$

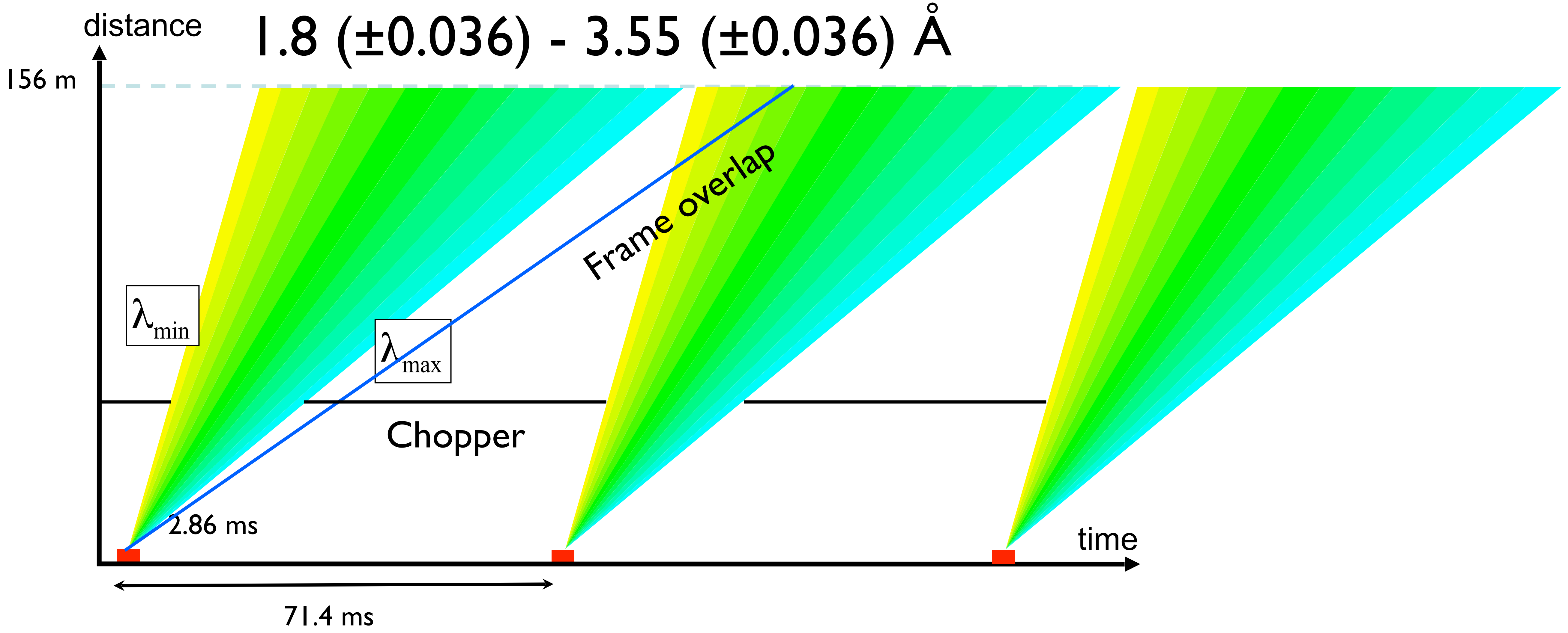
Time-of-flight Laue diffractometers – length and TOF resolution

Source parameter

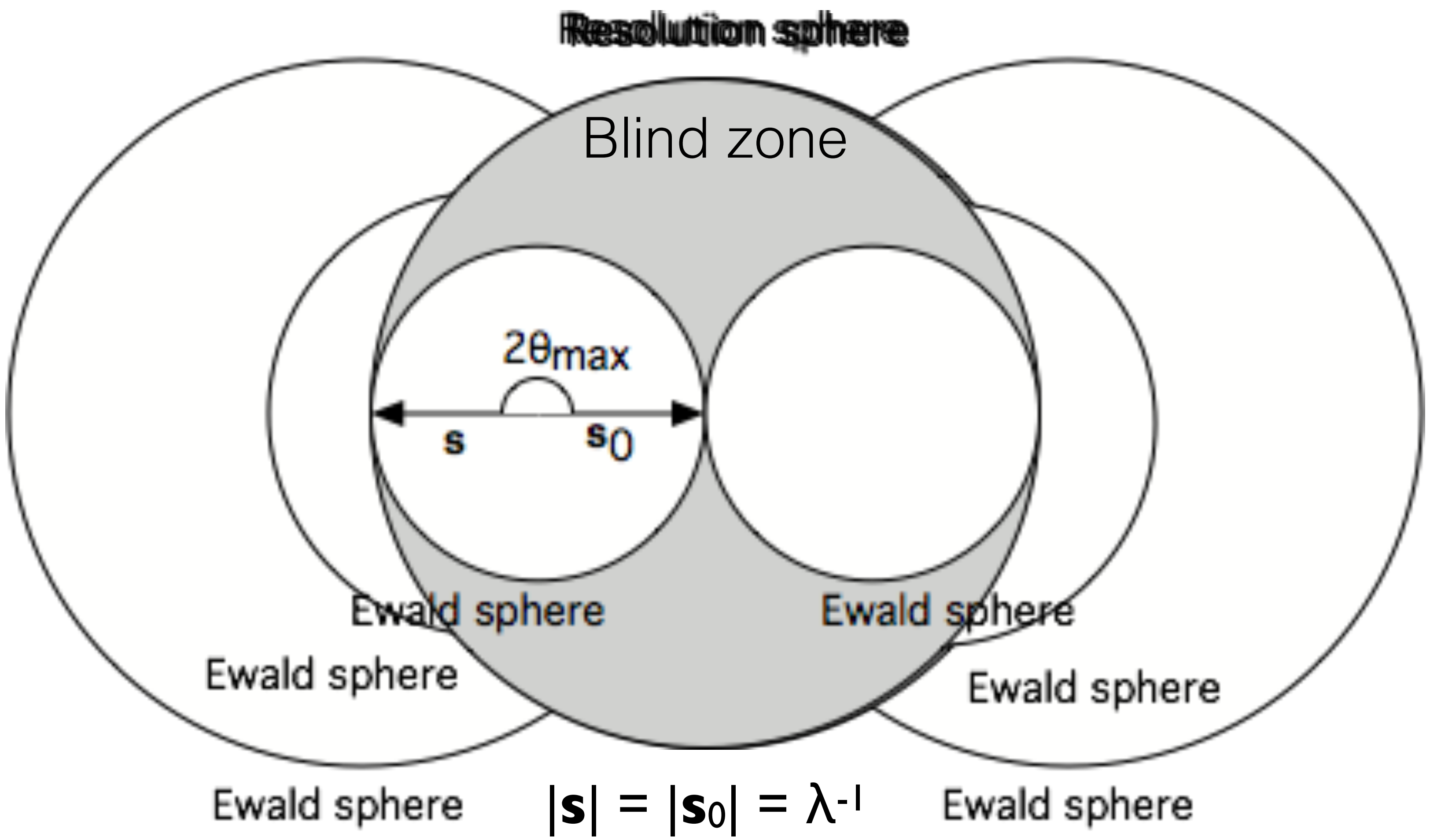
Instrument parameter

- Time-of-flight uncertainty → λ uncertainty
- Longer pulse → larger λ uncertainty
- Longer flight path → smaller λ uncertainty → narrower λ band

1.8 (±0.036) - 3.55 (±0.036) Å



Crystallography with long wavelengths



Multi-axis goniometry

Large solid angle detectors

The instrument shall allow data to be collected to a d_{min} of 1.5 Å

- Scattering power increases with wavelength
- Air scattering/ absorption is not a problem with neutrons
- Long wavelengths require large 2θ
- Blind zone gets large

NMX – conceptual view

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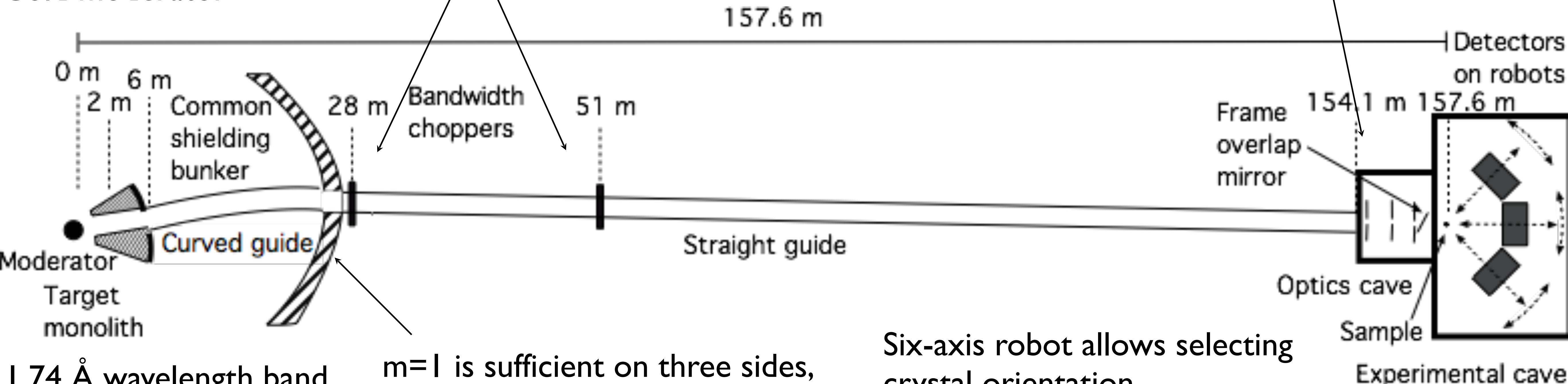
Adjustable opening chopper allows flexible bandwidth selection.

Collimation section giving flexibility in beam size and divergence

Cold moderator



1.74 Å wavelength band (1.8-3.55 Å)

m=1 is sufficient on three sides, m=2.2 for curving

Six-axis robot allows selecting crystal orientation

Three 60 x 60 cm detectors with 0.2 mm spatial resolution Variable sample-detector distance (0.2-1.0 m)
Variable 2θ angle (0-110°)

- **NMX** @ ESS, Lund, Sweden
  - Cold, TOF-Laue, Δλ<1.75 Å
  - 158 m length, λ-range 1.8-10 Å
  - Flexible geometry (robots)
  - Gd-GEM detector

NMX engineering design – neutron guide

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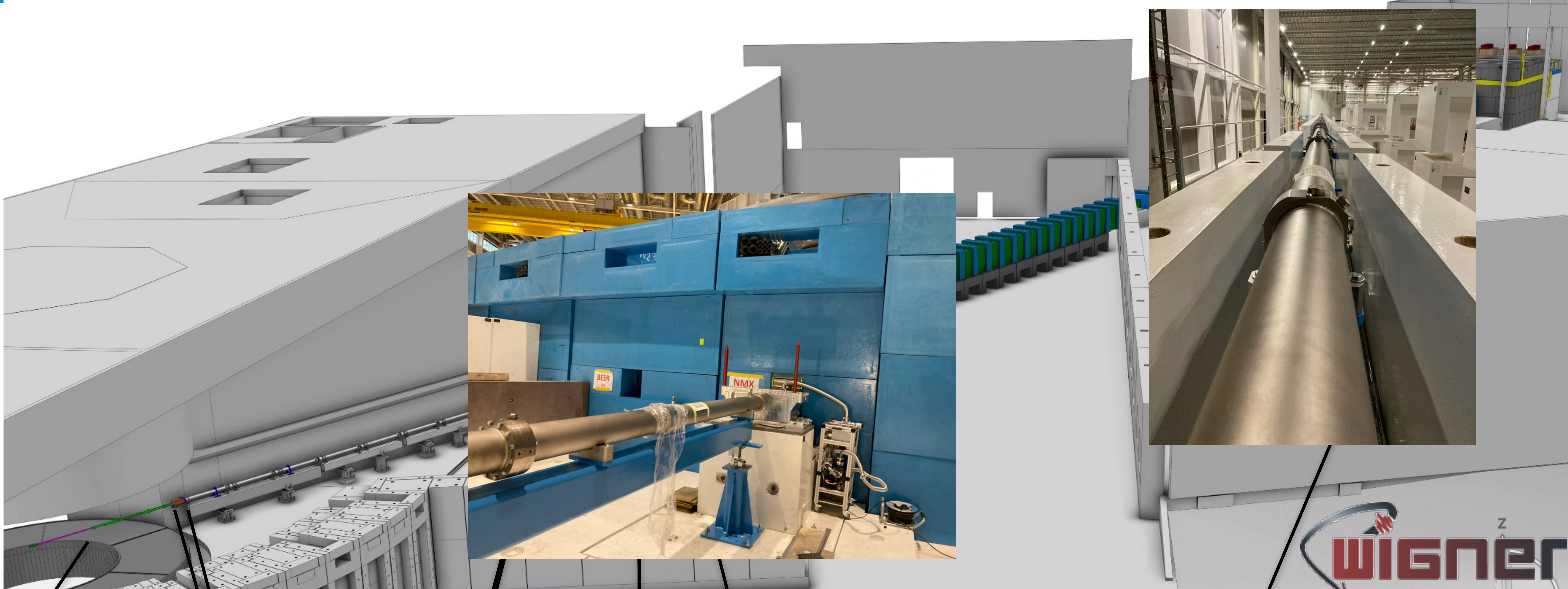
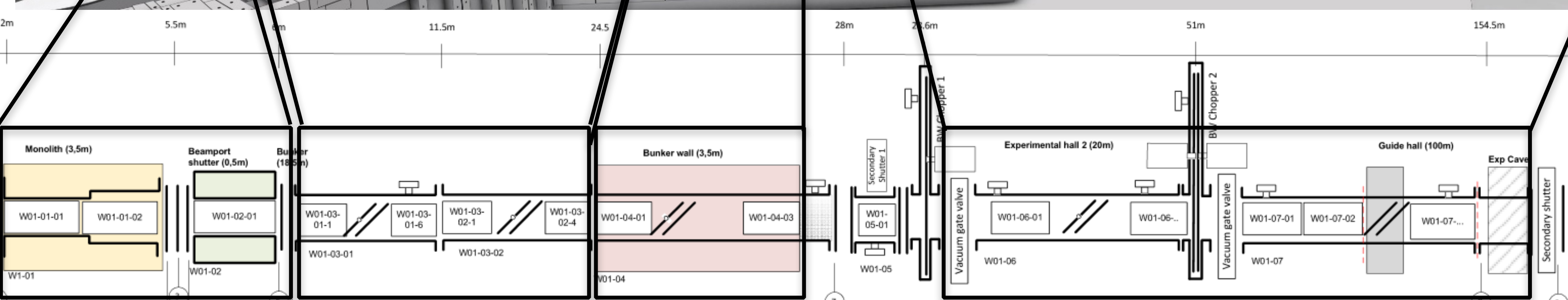




In-monolith optics

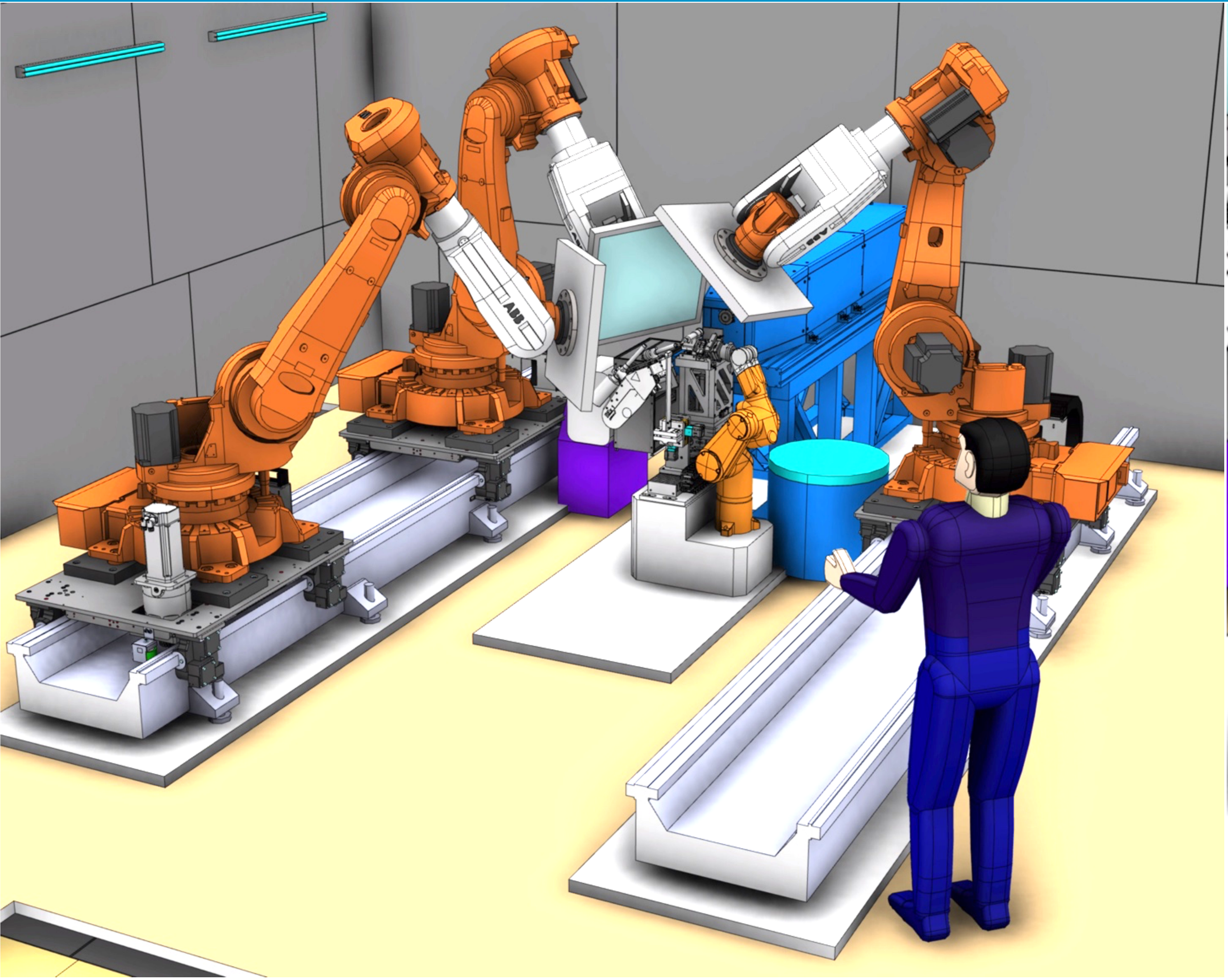
Curved guide in bunker

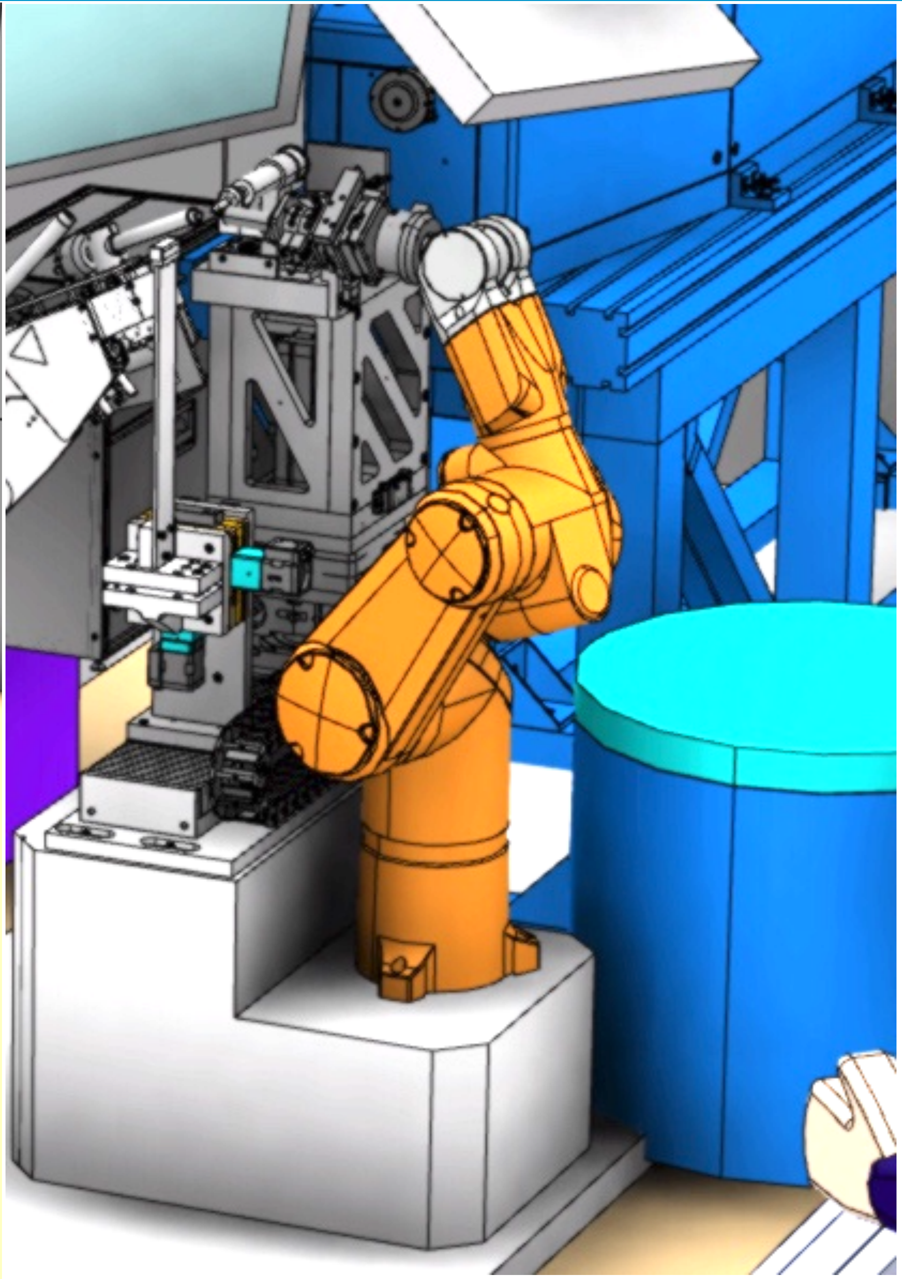
Bunker wall insert

Straight guide outside bunker

Gergely Nagy
Márton Markó

Endstation – Robotics





A molecular vision of life
Une vision moléculaire du vivant

Endstation

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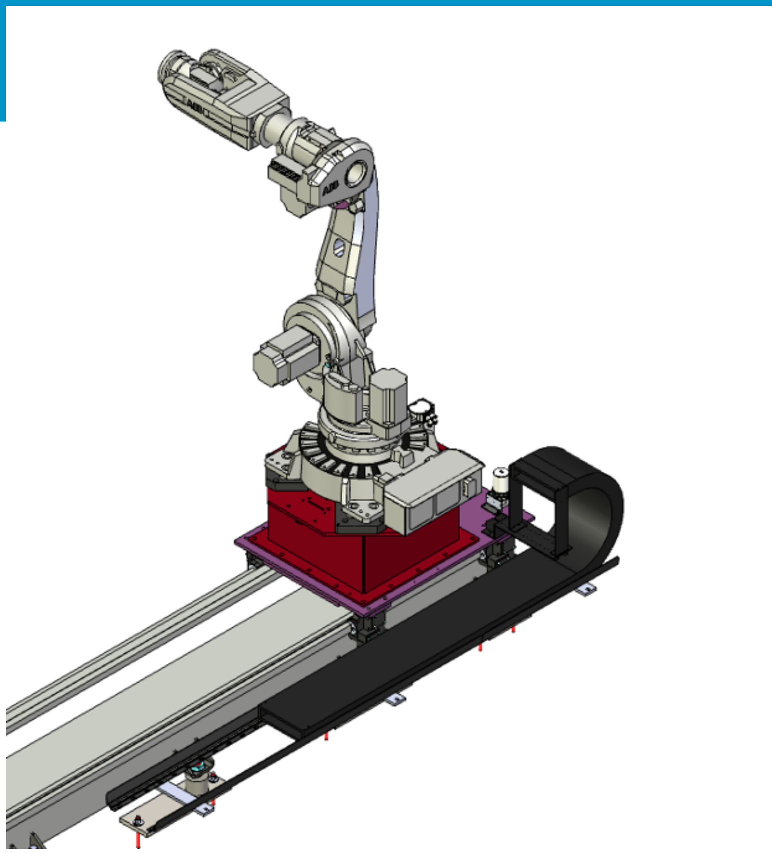
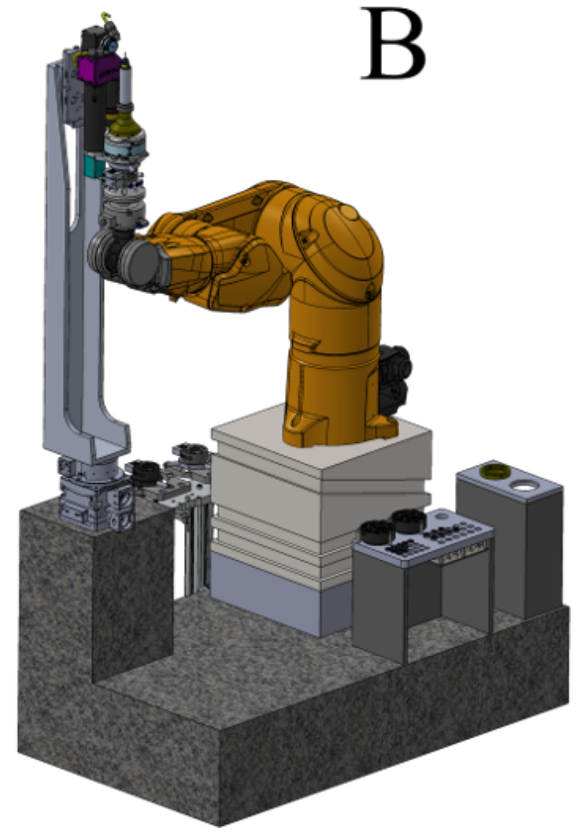


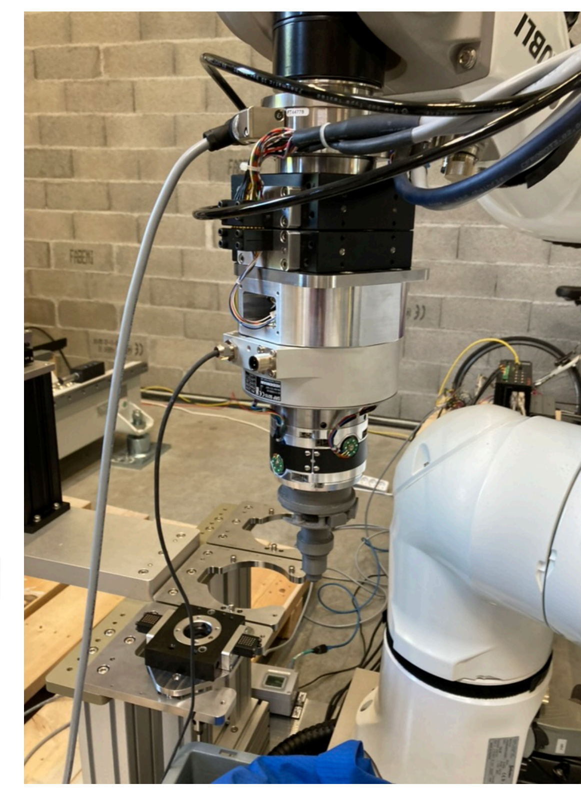
ARINAX

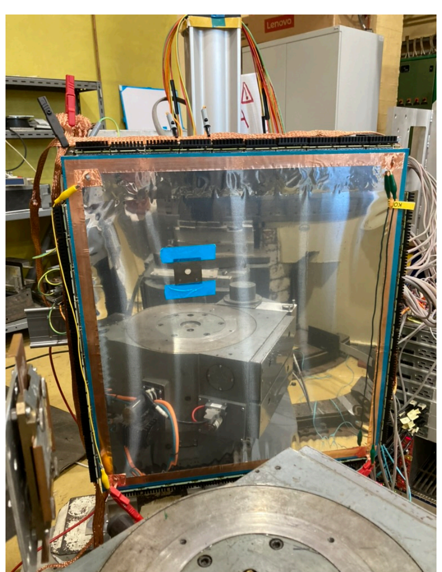
Advanced Research Instrumentation for Neutrons & X-rays

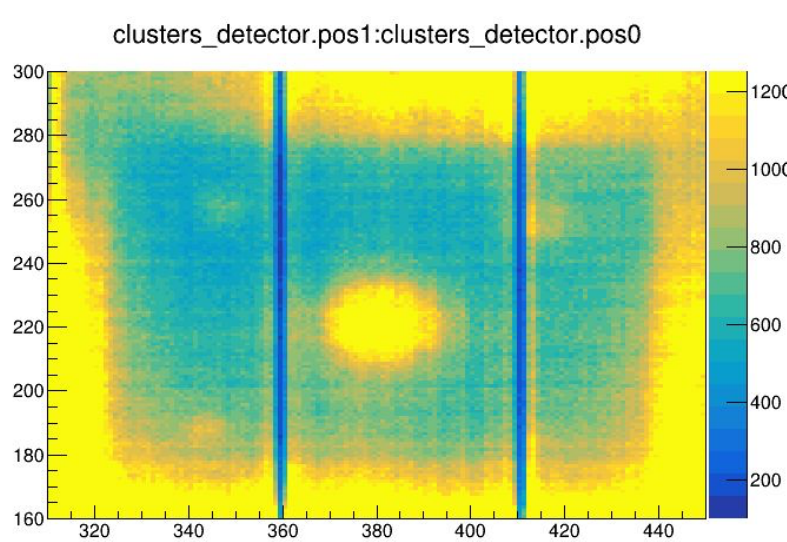
Large, adjustable solid angle

B









ibs

A molecular vision of life

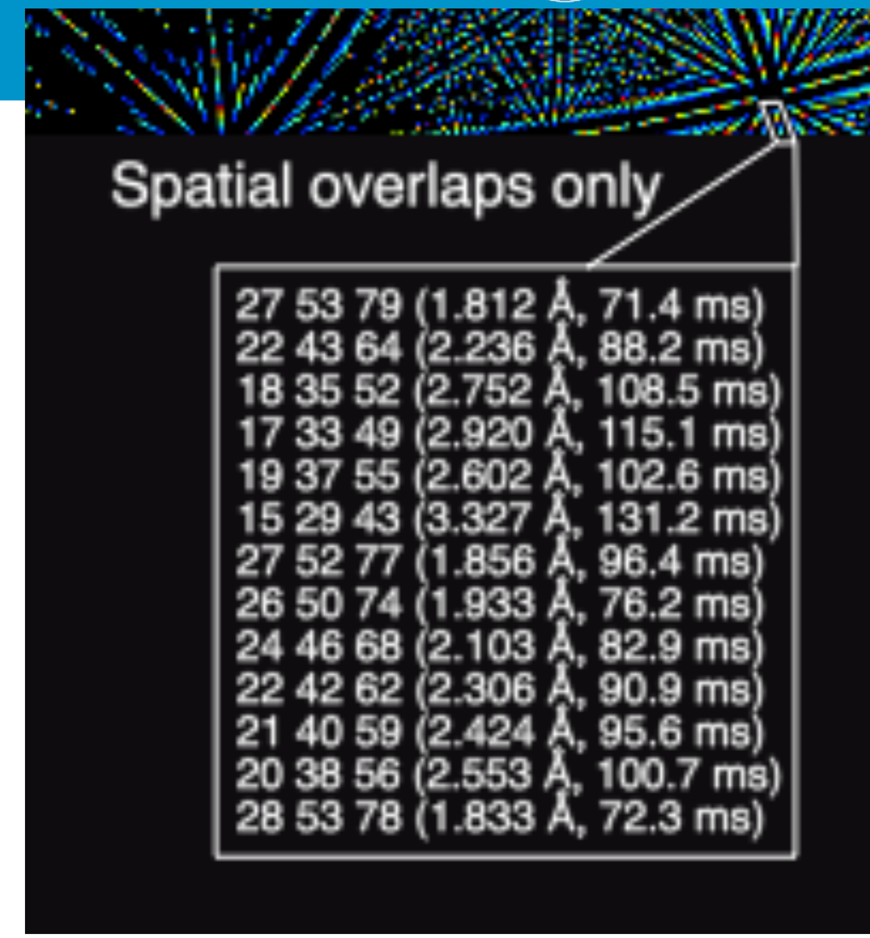
Une vision moléculaire du vivant

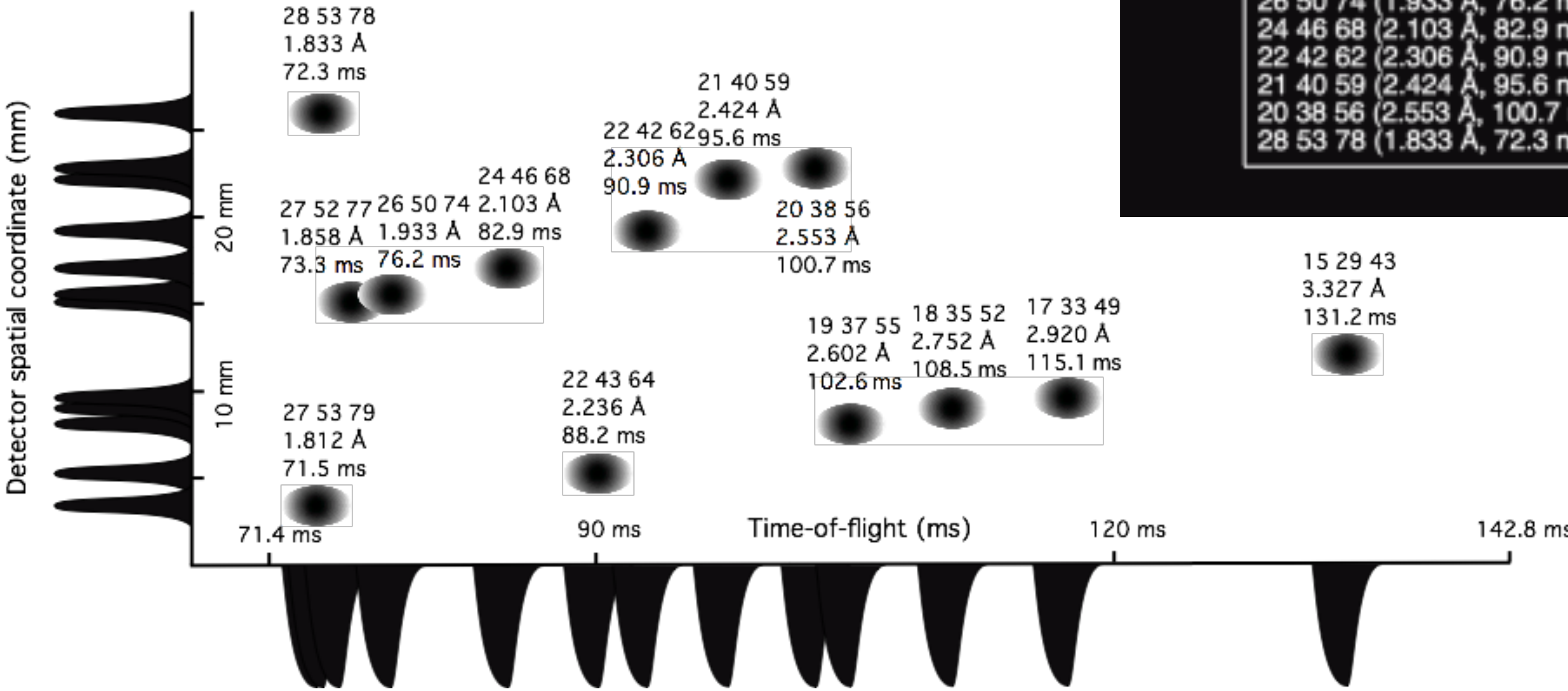
50 x 50 cm, effective pixel size 400 μm

Multi-axis goniometry

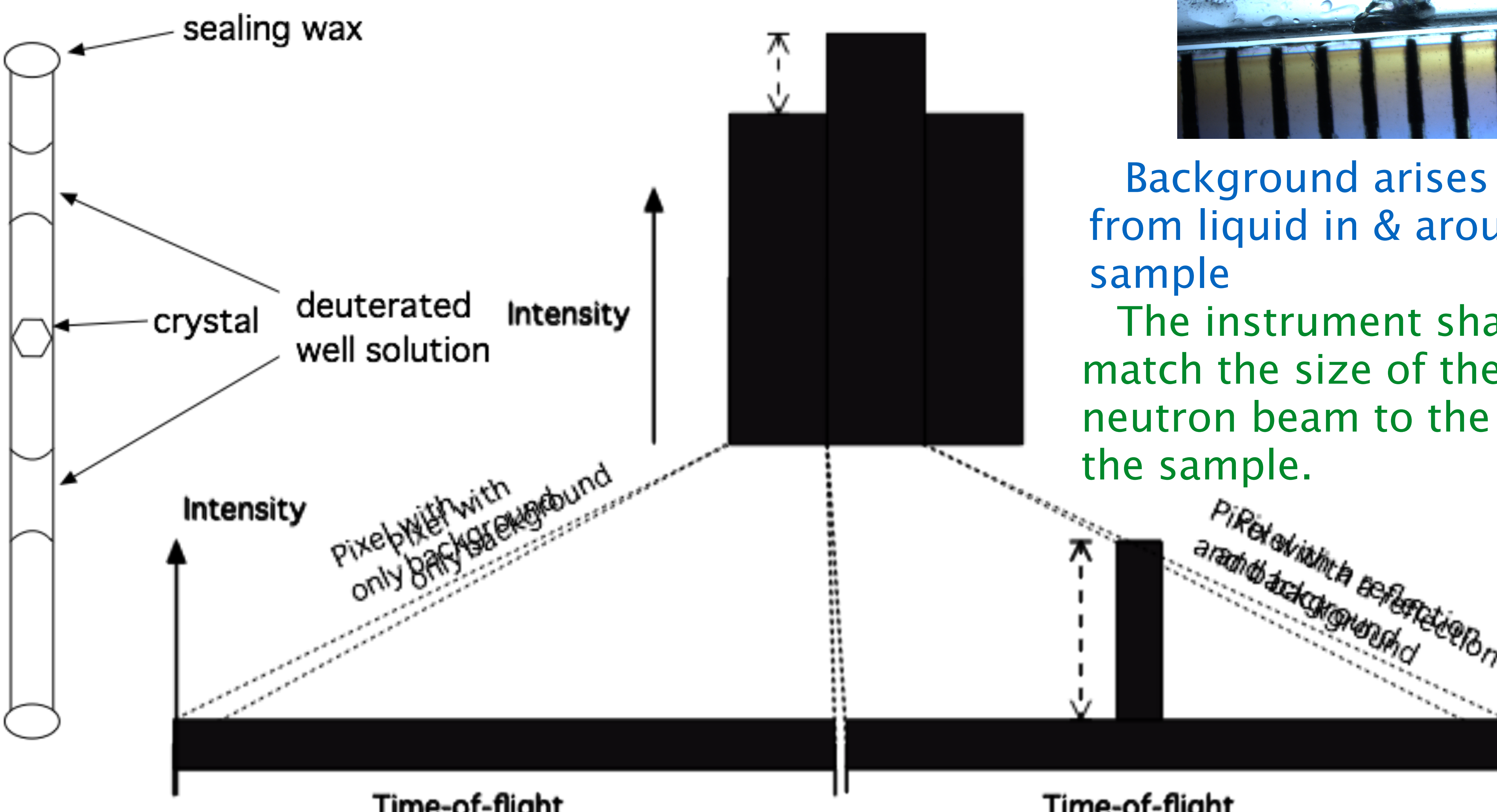
Overlap separation with TOF

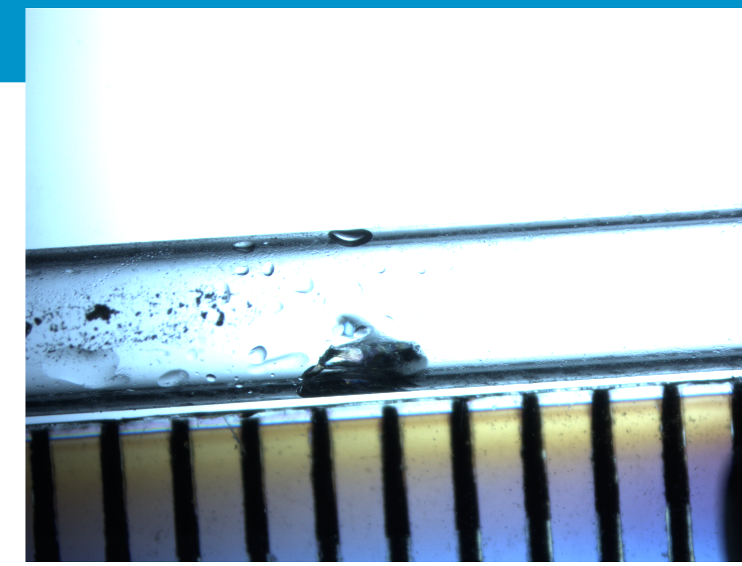
The instrument shall allow data collection from crystals with unit cell repeats > 300 Å





Spreading background over time-of-flight





Background arises mostly from liquid in & around the sample

The instrument shall match the size of the neutron beam to the size of the sample.

Flux at sample – time averaged

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- By Monte Carlo simulation 5 x 10^8 n/s/cm^2 at ±0.2° divergence
- LADI-III 5 x 10^7 n/s/cm^2, divergence unclear

Factor 10

NMX makes full use of the long pulse and high-brilliance moderators

Signal/background gain from TOF is difficult to quantify – depends on crystal, but at least factor 10

The instrument should allow data collection from crystals of < 0.01 mm3 volume

Should be realistic to collect 0.1 mm^3 crystal in < 1 day

Crystal size or collection time?

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The instrument should allow data collection from crystals of < 0.01 mm3 volume

Should be realistic to collect 0.1 mm^3 crystal in < 1 day

1. Do we want to push minimum crystal size and collect data for weeks?
2. Do we want to push unit cell size and collect data for weeks (on a large crystal)?
3. Do we want to push throughput and collect data in a day from larger crystals? Software!

Choice not limited by design decisions

Software & instrument throughput

- Data collection strategy software can save significant beamtime (or get better completeness in the same time)
- Work flow automation can save considerable time when testing larger numbers of crystals (e.g. evaluate resolution from test images)

What limits neutron crystallography today?

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- Crystal size needed is still big – 0.01 mm^3 is a lot easier to get to than 0.1 mm^3
- Data collection is long wrt available beam time – only few data sets can be collected world-wide
- Beam time allocation is not always matching availability of crystals

Support labs vs. user expertise

- Perdeuteration (in *E. coli*) is relatively easy for most crystallographers
- Growing large crystals is easy in some systems, but hard to know if size could not increase further
- Increasing crystal volume increases instrument throughput **a lot!**

LADI–III @ ILL

Crystal volume (mm^3)	4	2	1.5	1
Number of images / Number of crystal settings / Exposure time	35 / 3 / 4 hours	5 / 1 / 12 hours + 2 / 1 / 8 hours	21 / 2 / 6 hours	24 / 3 / 24 hours + 5 / 1 / 6 hours

Acknowledgements

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Choppers

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Markus Olsson

Nikolaos Tsapatsaris

Optics

Márton Markó (EK)

Robotics

Jean-Luc Ferrer (ibs Institut de Biologie Structurale)

Controls

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Questions?

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