In situ Insight:
Getting the best from *in situ* methods at Diamond

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CCP4 Workshop 10/12/14

Overview: What do we want from *in situ*?

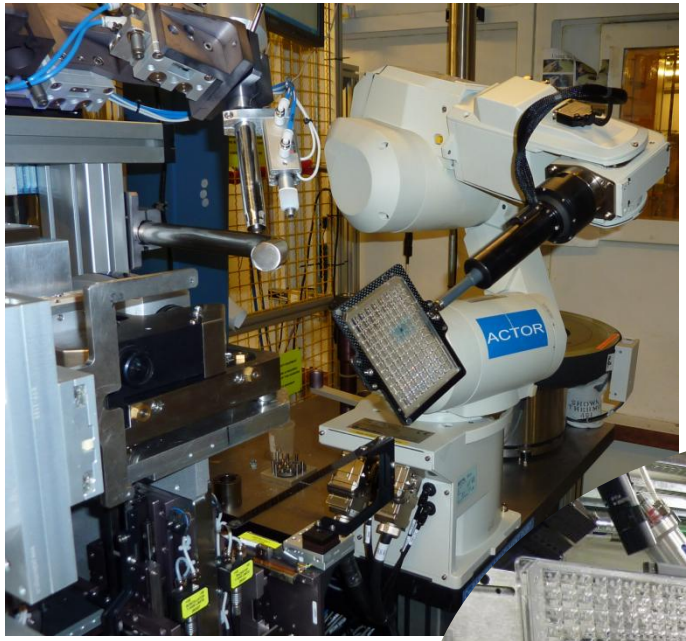
- Aims
  - Characterise early crystal hits, salt? protein? my protein?
  - Avoid manual intervention, save time, effort and preserve labile samples
  - Differentiate between different crystallisation conditions.
  - Avoid complications of cryo-cooling during crystal optimisation and data collection.
  - Investigate crystal hydration effects
  - Collect data for structure solution at room temperature with no manual step.

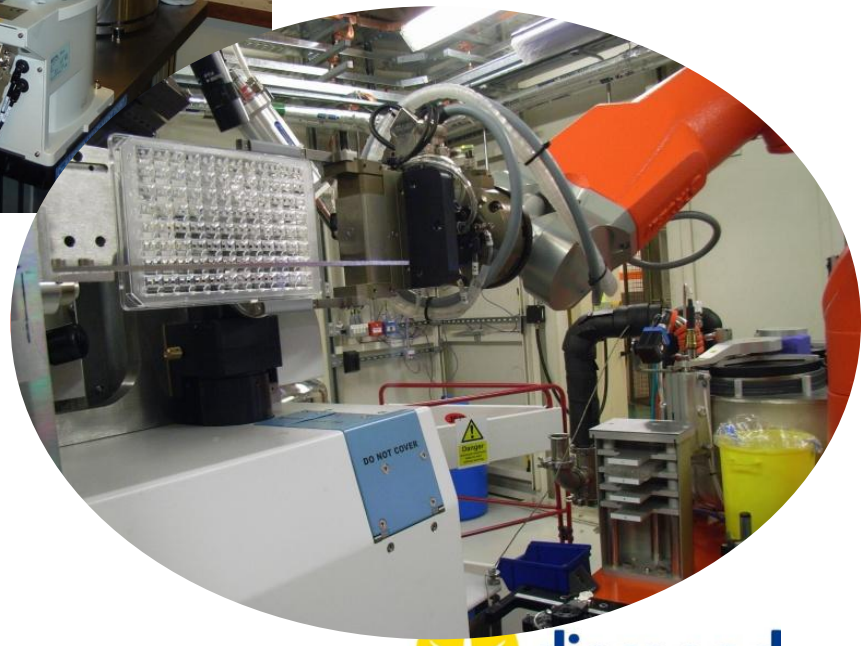
- Strategy
  - Minimise background
  - Accurately centre samples
  - Collect fast (radiation damage considerations)
  - Link laboratory information to beamline
  - Use specific data processing tools
  - Automate wherever possible

Which plate type to use?

- Many different varieties of the SBS “standard”
- Development of a sample environment that accommodates multiple types
- **Chose plates specifically designed for *in situ* diffraction.**

Holding the plate in the beam





- Robot arm or goniometer
- I24/I03 universal gripper/frame
- I03 Robot loading for Cat3
- I04-1 robot gripper

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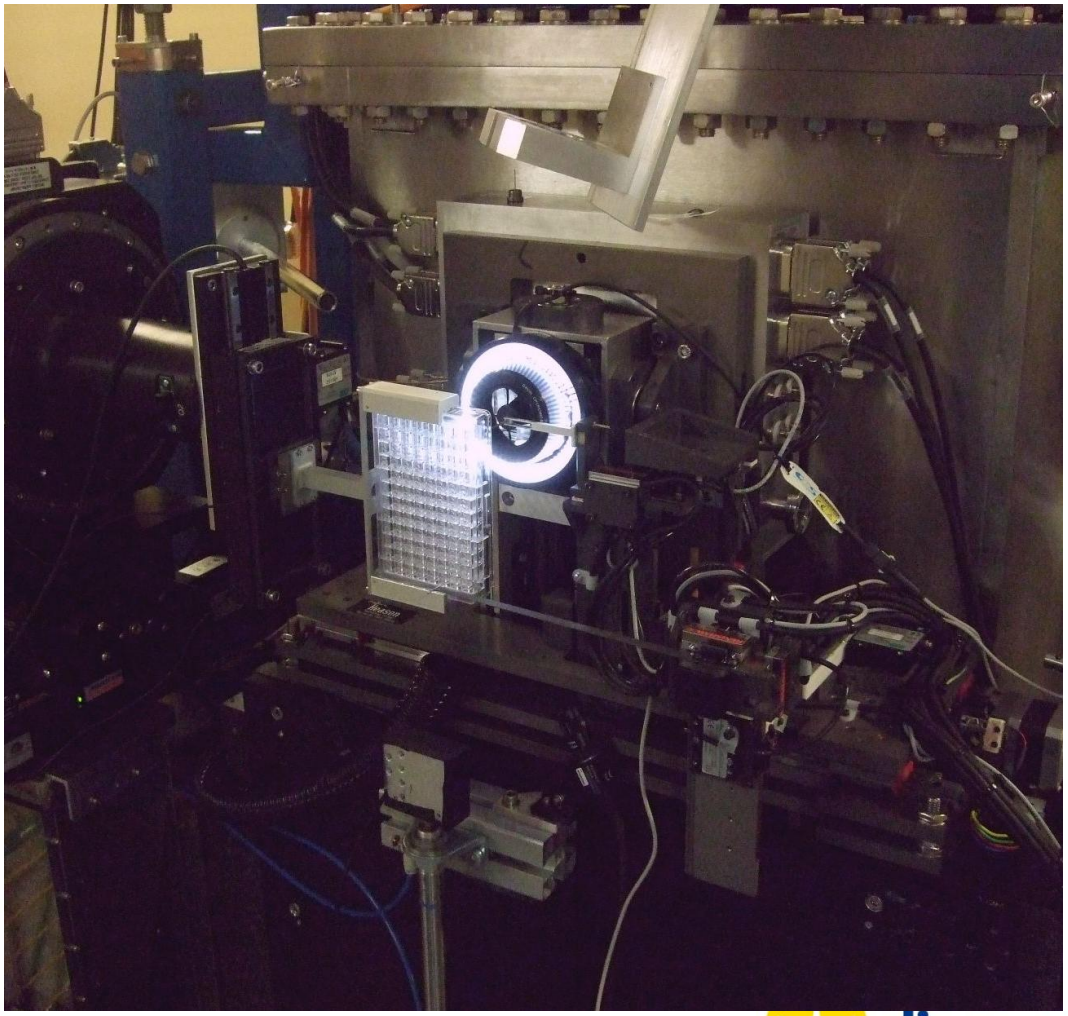
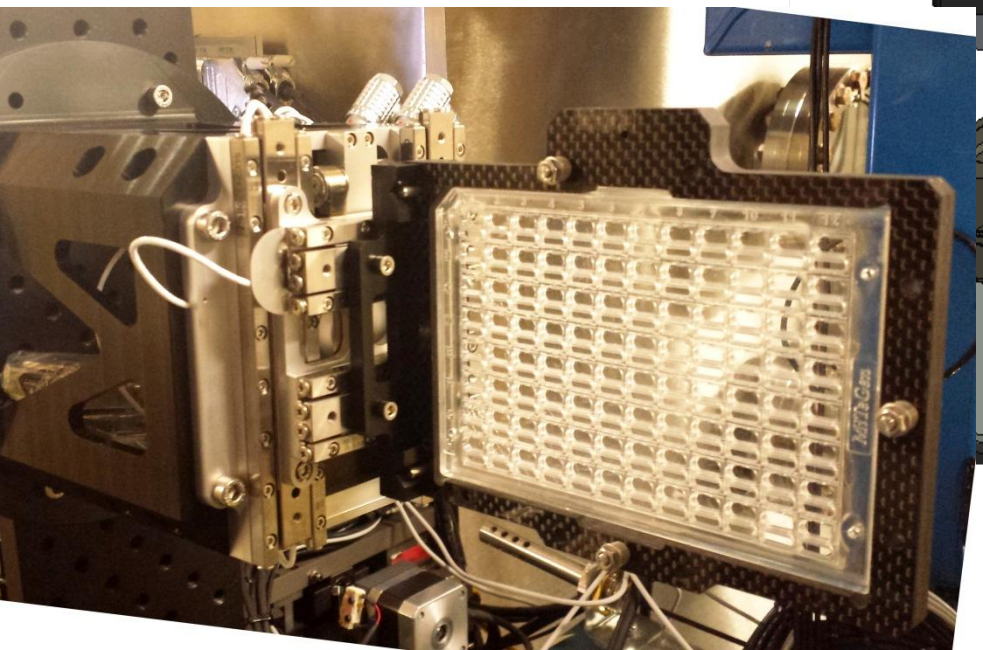
Holding the plate in the beam I24 (Old Endstation)

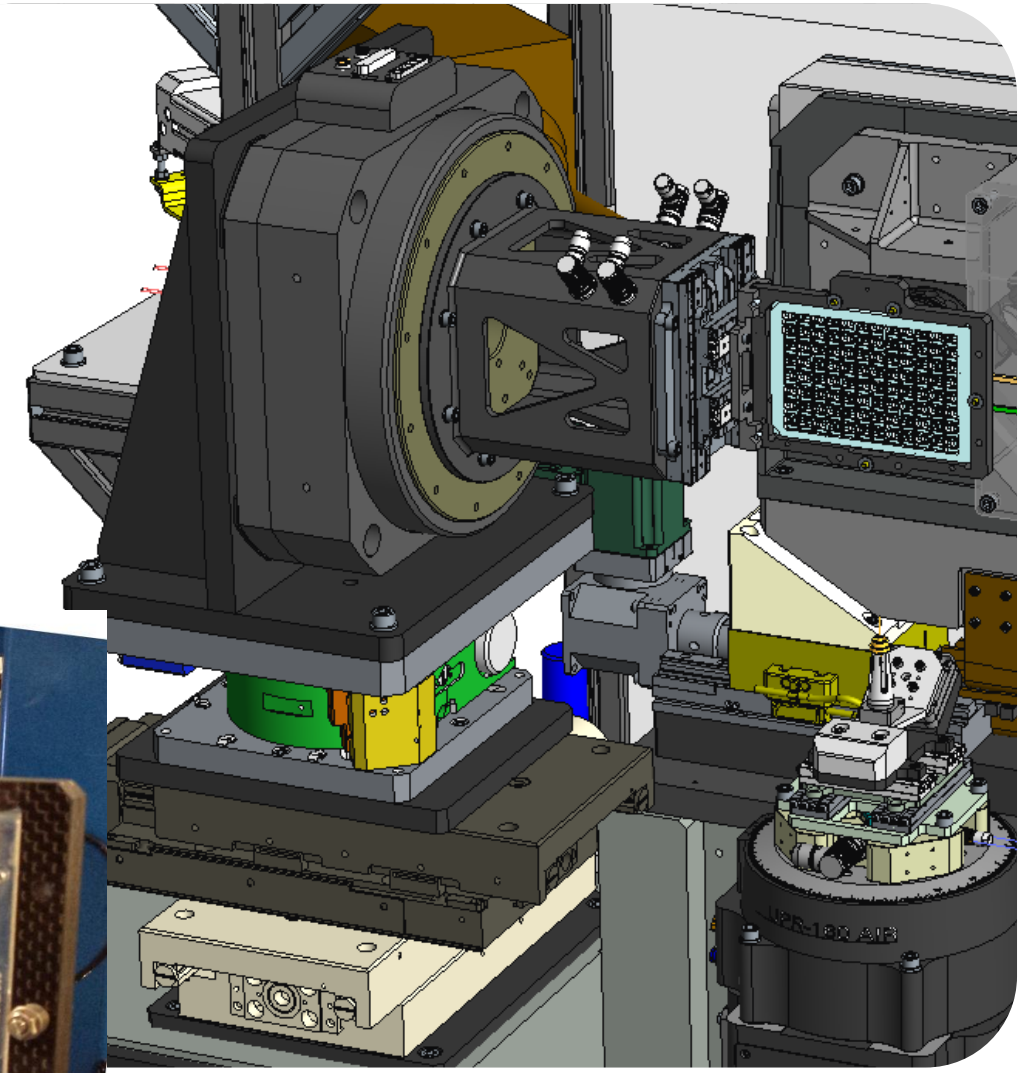
- Universal gripper designed to accommodate as many plate types as possible.
- Poor reproducibility
- Not compatible with robot loading



Holding the plate in the beam I24 (New Endstation)

- Dedicated and permanently mounted high precision goniometer
- Move to universal frame system
- Solves issues of repeatability and robot loading





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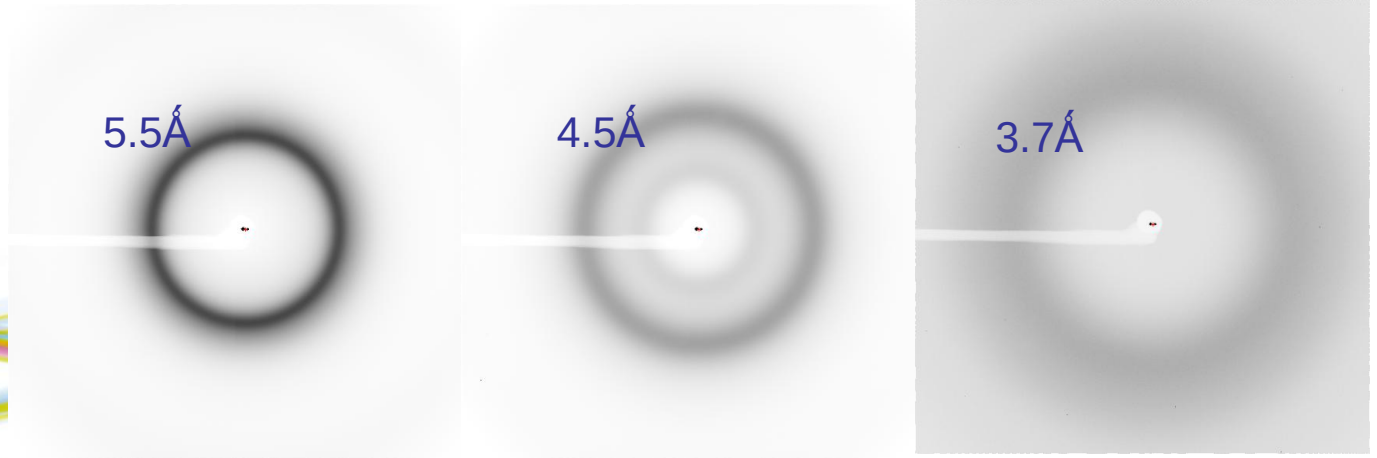
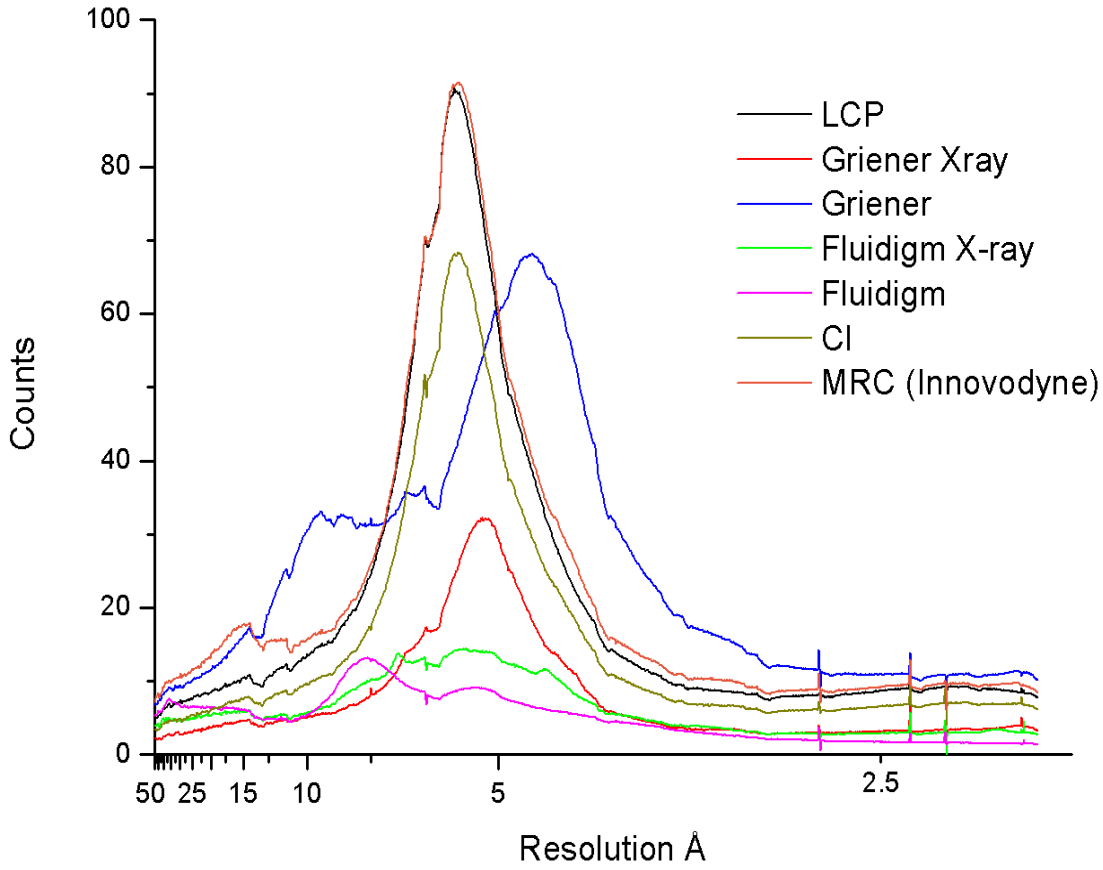
Background: Plate material

Innovodyne/MRC | Greiner | Glass LCP



- I24: 1s exposures at 12.68keV with 10^{12} ph/s
- The glass LCP plate absorbs ~90% of beam(!)

Background: Comparison of different plate types



- Plastics give peak scatter between 3-7 Å
- The thinner the better!

Background: Cover tape makes a difference

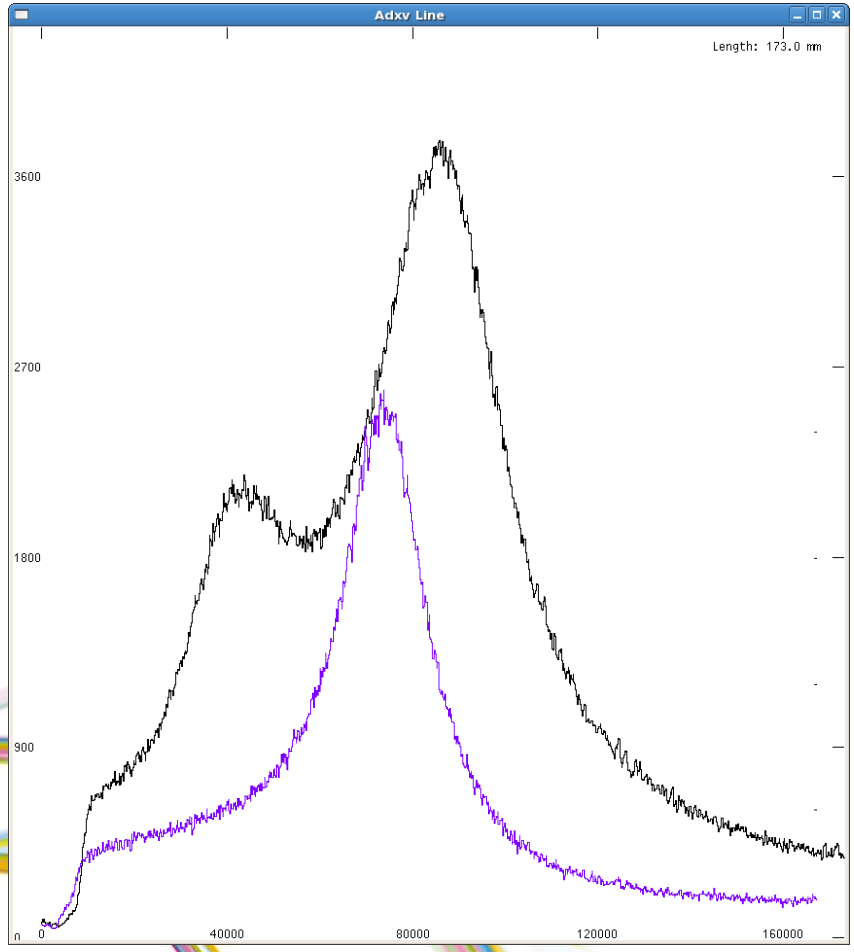
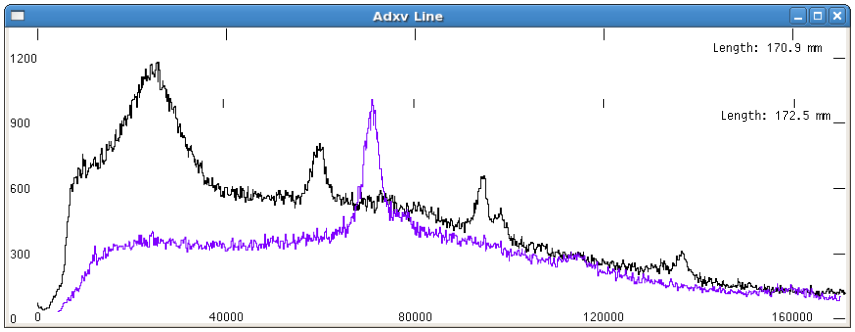


Plate Scatter

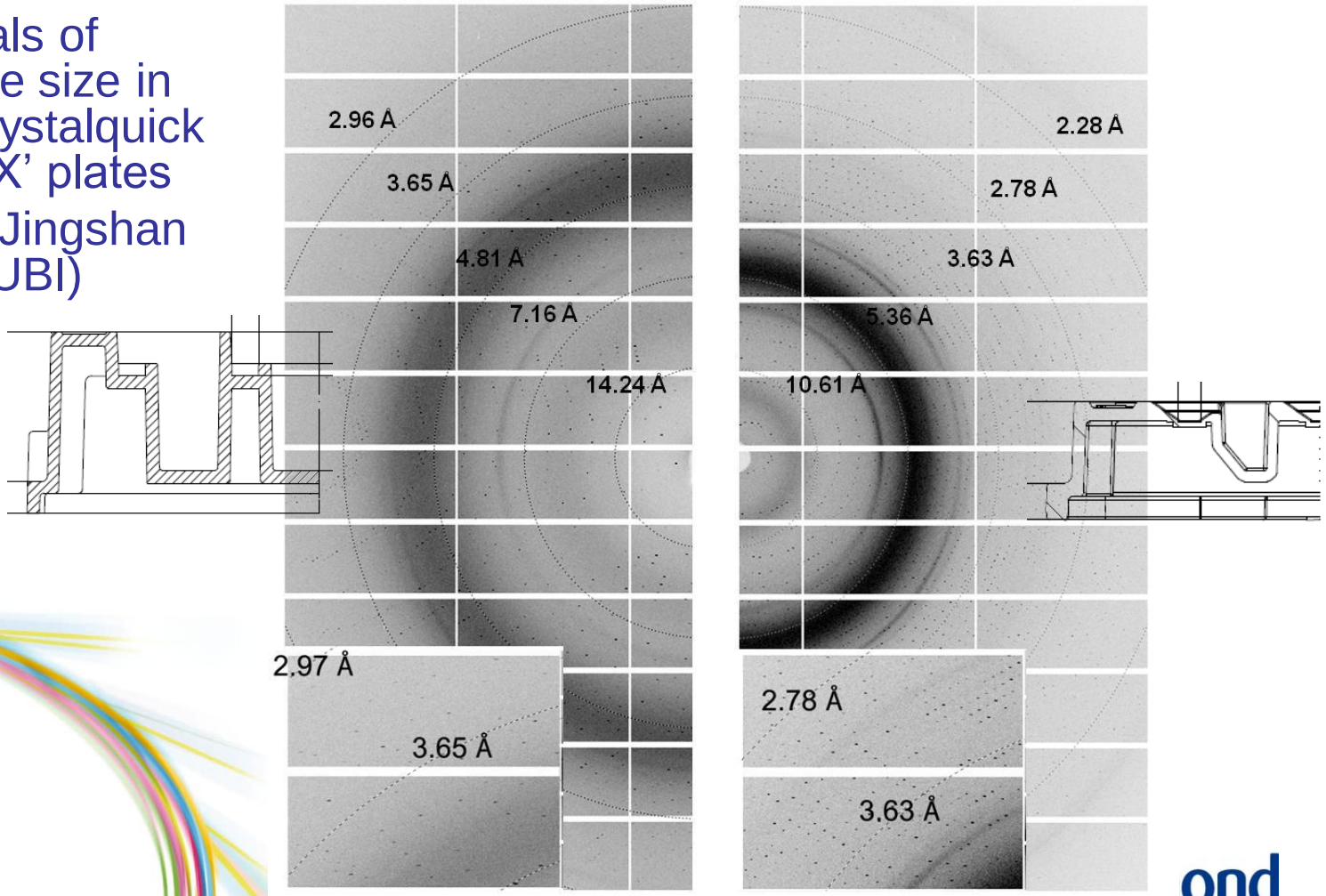
- Data from Karl Harlos (STRUBI) Greiner Viewseal tape (3M) scatters more than Greiner X ray specific plates (<3.5 Å & 7 Å>). Thermal seal RT tape is better.



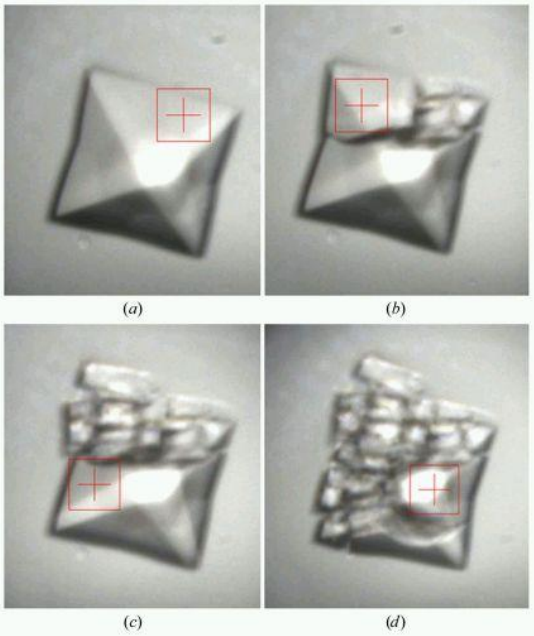
Tape Scatter

Background: Diffraction specific plates make a difference

- BEV crystals of comparable size in Greiner Crystalquick ‘SW’ and ‘X’ plates
- Data from Jingshan Ren (STRUBI)



In situ data collection facilitating science



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NATURE STRUCTURAL & MOLECULAR BIOLOGY | ARTICLE

More-powerful virus inhibitors from structure-based analysis of HEV71 capsid-binding molecules

Luigi De Colibus, Xiangxi Wang, John A B Spyrou, James Kelly, Jingshan Ren, Jonathan Grimes, Gerhard Puerstinger, Nicola Stonehouse, Thomas S Walter, Zhongyu Hu, Junzhi Wang, Xuemei Li, Wei Peng, David J Rowlands, Elizabeth E Fry, Zihe Rao & David I Stuart

Affiliations | Contributions | Corresponding authors

Nature Structural & Molecular Biology **21**, 282–288 (2014) | doi:10.1038/nsmb.2769

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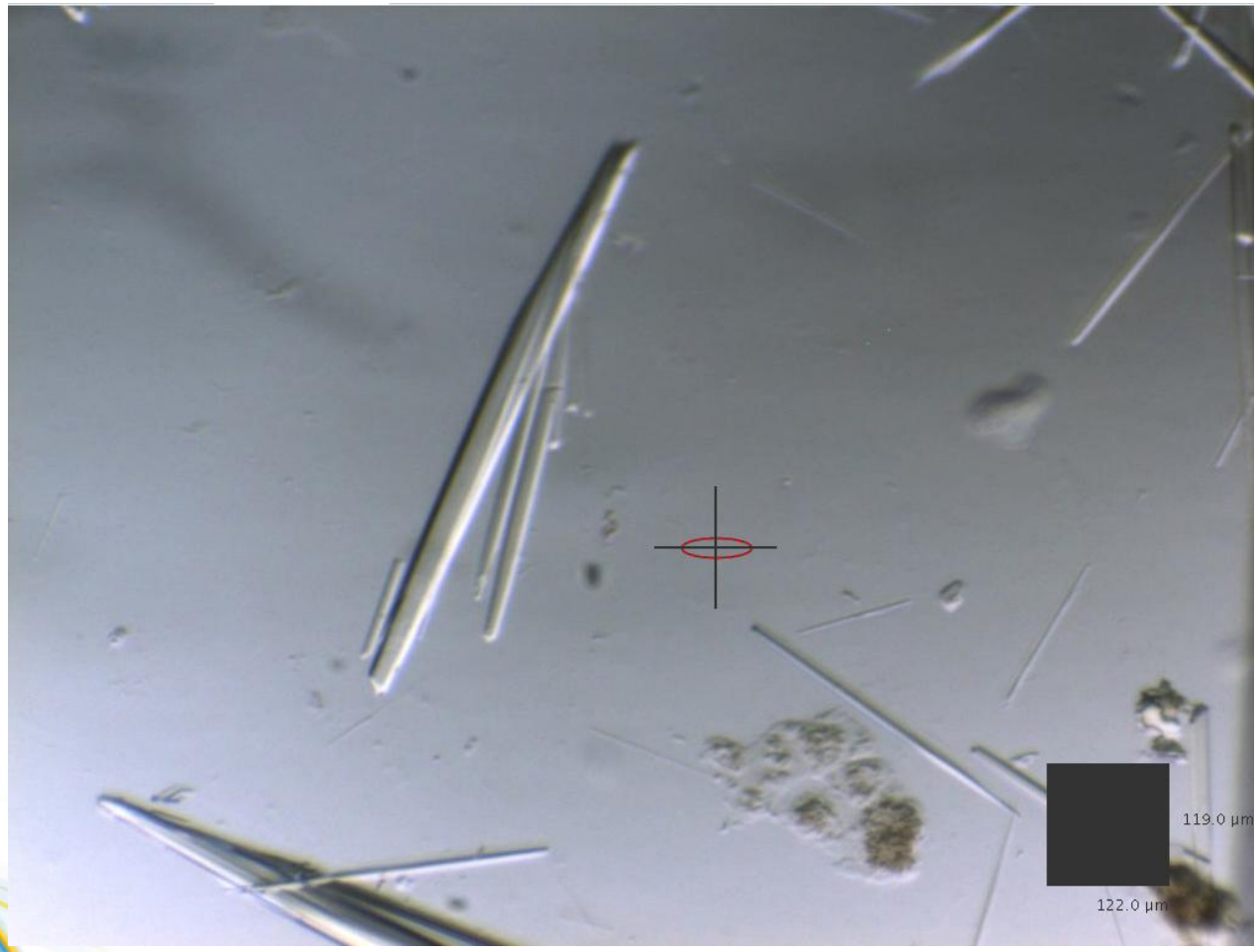
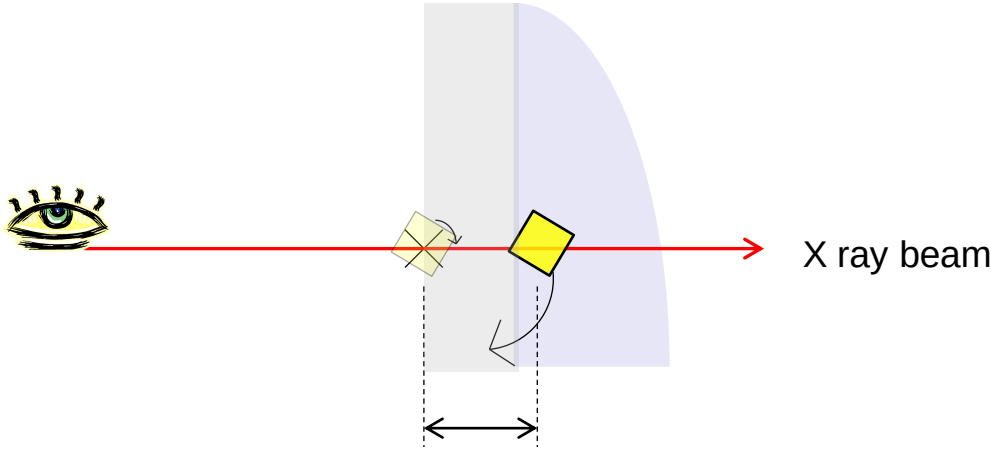
Sample alignment issues

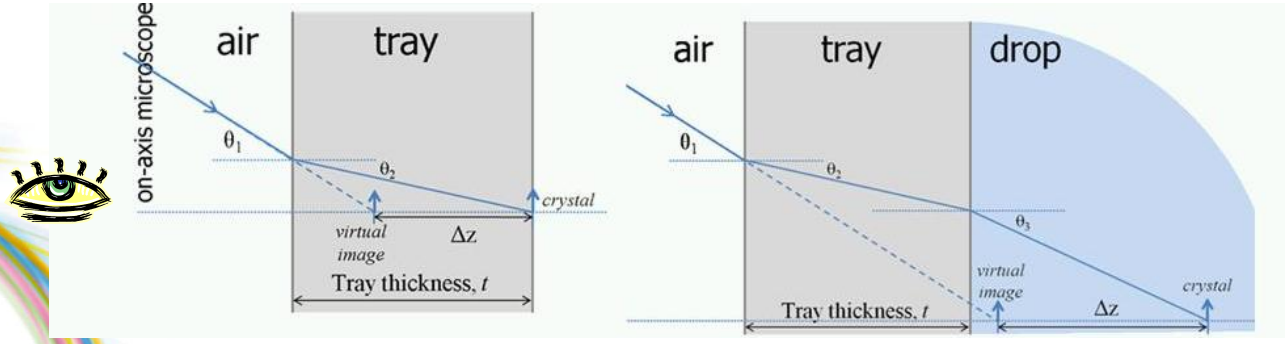
- No option to rotate crystal 90 degrees!
- The plate media acts as a lens, appearing to displace the crystal from its actual location.
- A real problem when going to microfocus



Sample alignment issues

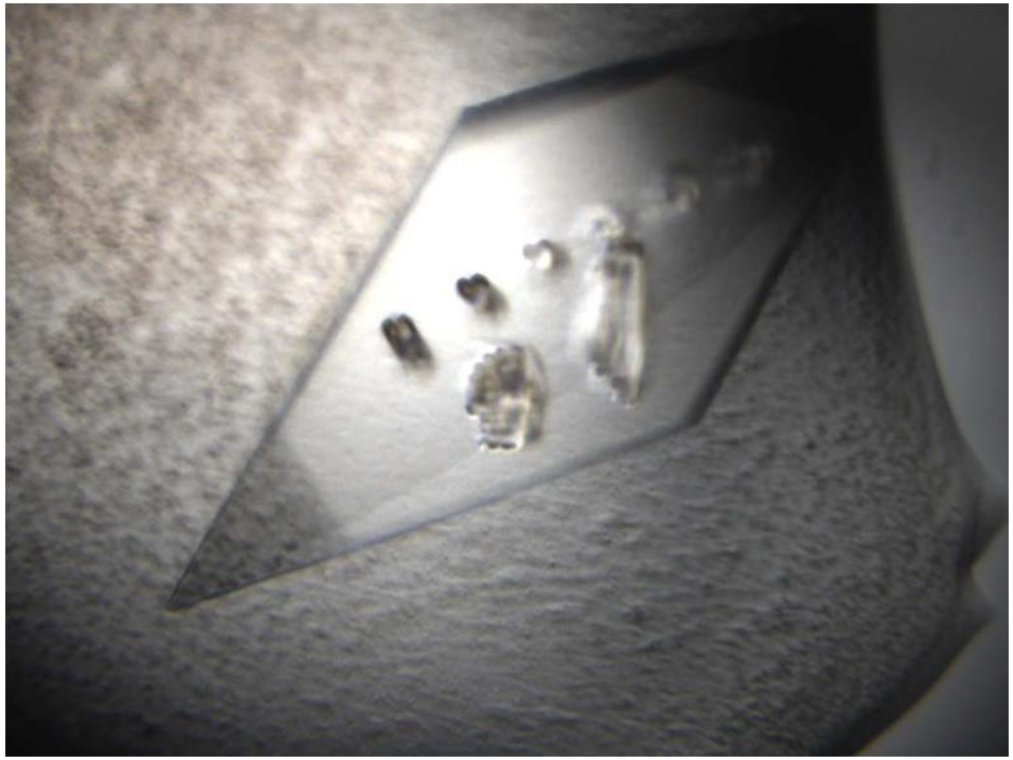
- Although the sample appears to be in focus and centred, it is not coincident with the axis of rotation.



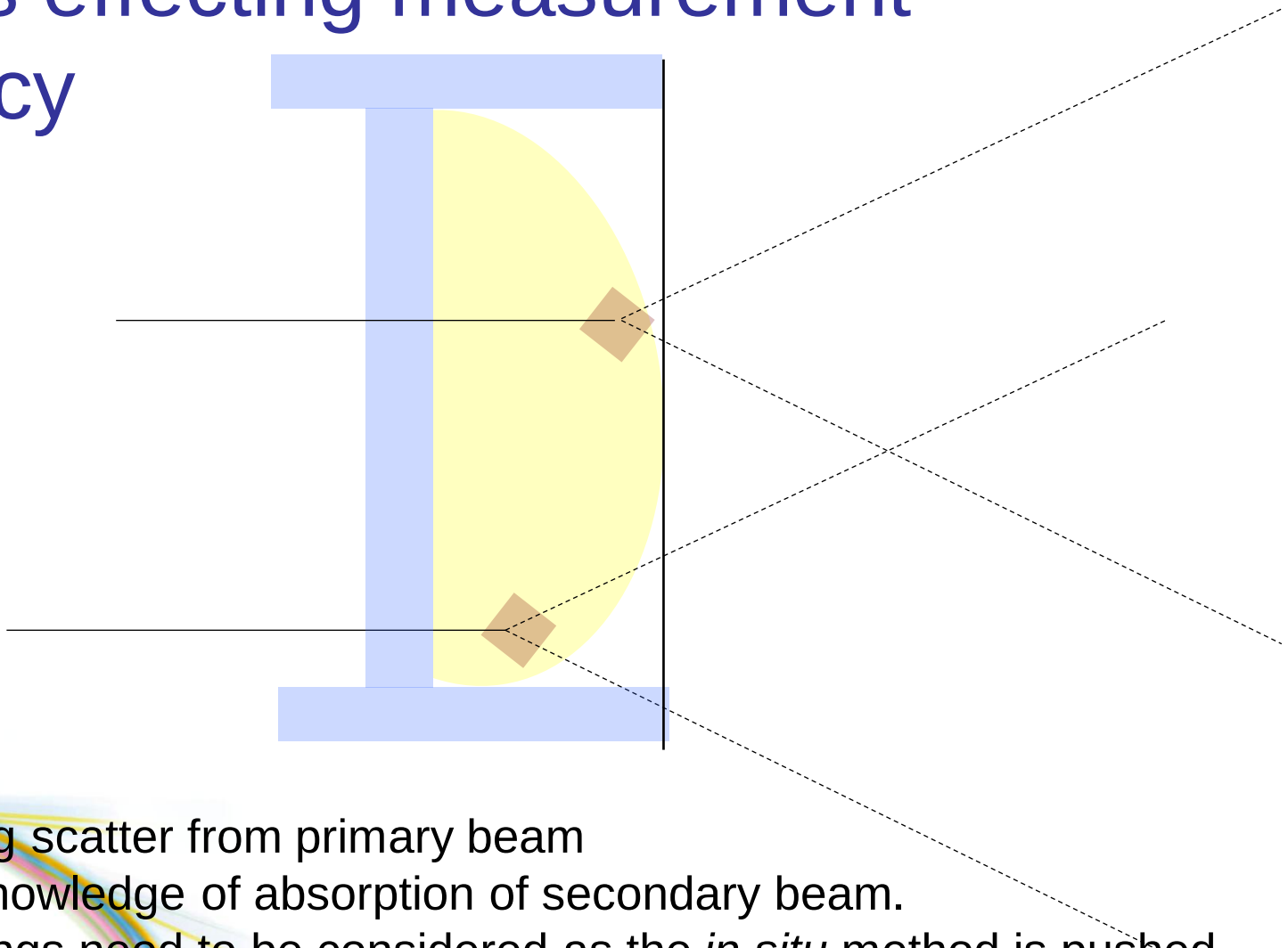


Sample alignment issues

- Although the sample appears to be in focus and centred, the pattern of radiation damage shows is not coincident with the axis of rotation.

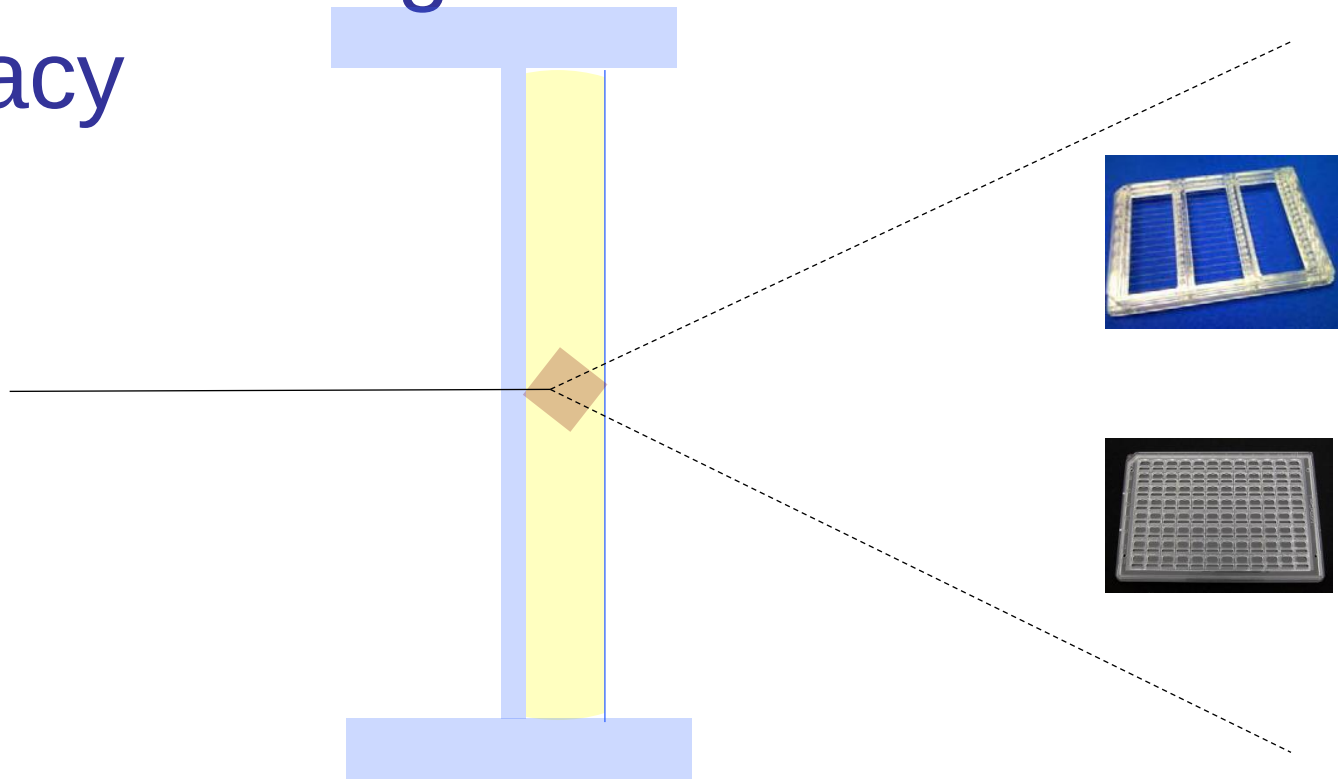


Factors effecting measurement accuracy



- Minimizing scatter from primary beam
- Control/knowledge of absorption of secondary beam.
- These things need to be considered as the *in situ* method is pushed further.

Factors effecting measurement accuracy



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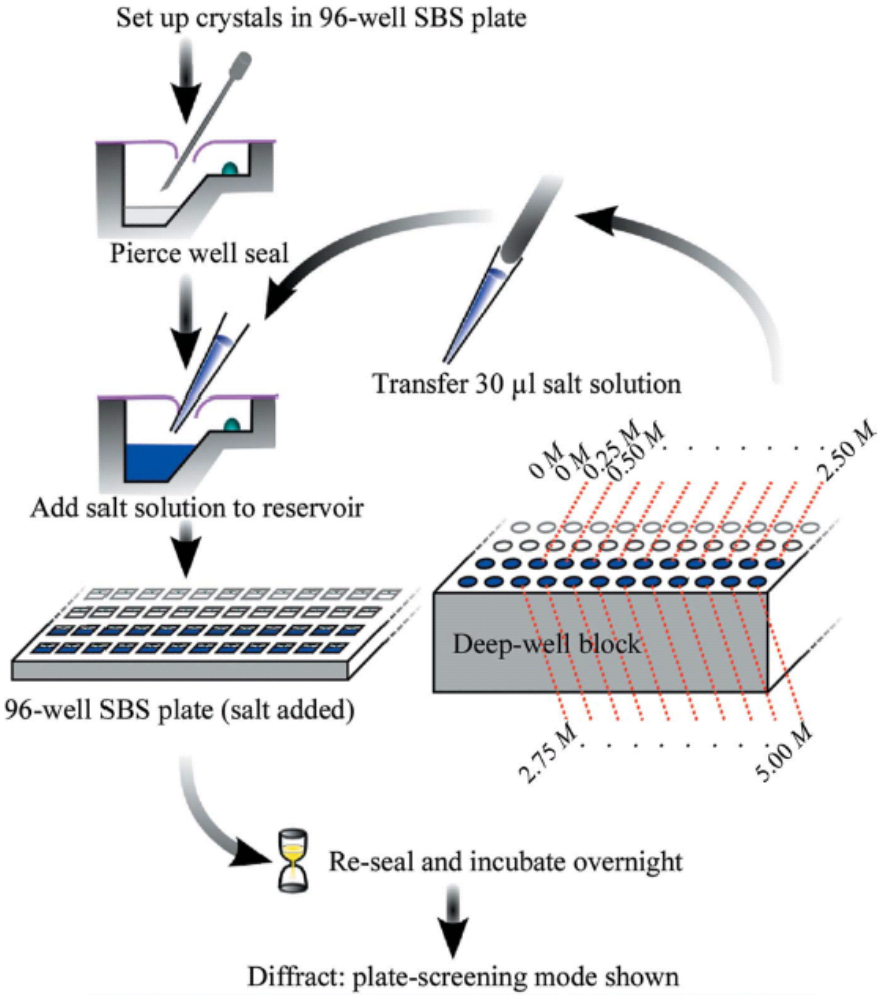
Extending the method: *In situ* crystal dehydration



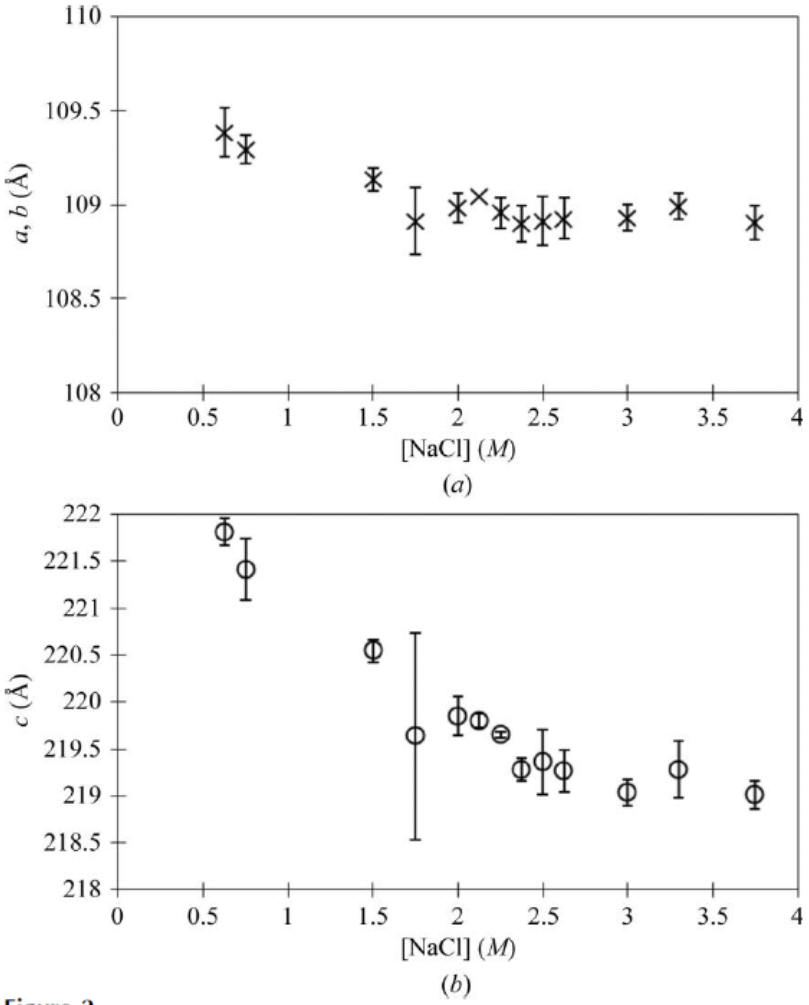
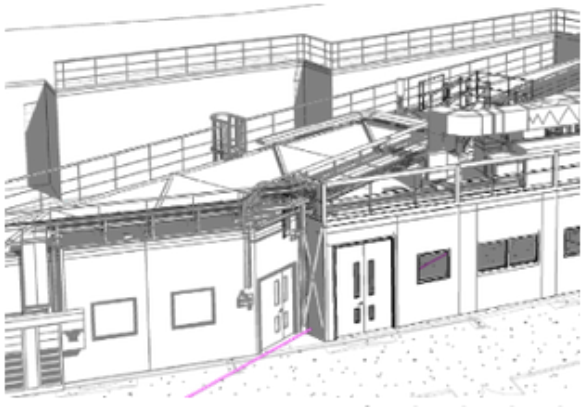


Figure 2

Douangamath *et al.* (2013)

Future

- VMXi will be a beamline dedicated to *in situ*
- Extensive automation will enable high throughput
- Designed to give rapid and comprehensive feedback on crystals down to <5µm



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Take home messages

- If you can use a diffraction specific plate then do so.
  - Simply identifying your sample and getting it in the beam
  - Ability to record (weak) reflections
- Cover taper scatter should not be ignored.
- Thinner and flatter is better!
- Think about refractive effects and rotation axis offset when centring samples. (Particularly with a small beam and/or sample.)
- Many improvements currently under development at Diamond with user collaboration.

Acknowledgements and Contacts

- I24: Robin Owen, Darren Sherrell
- I04-1: Frank Von Deft, Jose Brandao-Neto, Alice Douangamath
- I03: Katherine McAuley, Stuart Fisher, Mark Williams
- STRUBI: Liz Fry, Ren, Karl Harlos, Abhay Kotecha
- MPL: Isabel De Morales, Kostas Beis,

References:

Axford *et al*. Acta Cryst D, 2012, 68:592-600
Douangamath *et al*. Acta Cryst D, 2013, 69:920-923
Wang *et al.* Nat Struct Mol Biol, 2012, 19:424-429

Other practical issues

- In typical small volume drops (200nl) no drop movement is seen and crystal movement is infrequent.
- Increasing drop volumes beyond this e.g. ion soak can cause problems.
- Reservoir movement not an issue with well sealed plates.
- Crystal imaging systems (LIMS) typically work in landscape format, whereas on I03 and I24 plates are held in portrait.

Abstract

- Many different versions of the 96 well ‘standard’ plate are available and currently in use in laboratories around the world. Although diffraction specific plates are available, most types can be handled and used for *in situ* work at Diamond. When collecting *in situ*, careful consideration should be taken of X-ray absorption and scatter, and optical refraction effects, to optimise the quality of collected data and to avoid false negatives when screening. The behaviour of specific plate types is described, along with methods to ensure the chance of obtaining useful data is maximised.