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Development and Optimisation of an Optofluidic Nano Tweezers System for Trapping Nanometre Crystals in Synchrotron X-Ray Diffraction Experiments:

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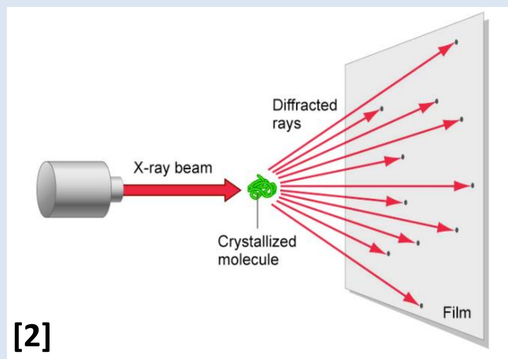
Introduction

With the Synchrotron community facing an increasing need to analyse ever-smaller protein crystals (particularly at the Diamond Light Source VMXm, I24 and I04 beamlines); new sample loading techniques are needed to present nano-dimensional samples to the beam for X-ray diffraction experiments. Although X-ray crystallography is the choice method for gathering structural data, it is an expensive, complex, and time-consuming process – with a typical processing time per structure is as follows:

- **Cloning/Purification:** 3 – 6 Month
- **Crystallisation:** 1 – 12 Months
- **Data Collection:** 1 Month
- **Phasing / Structure Solution:** 3 Months


[1]



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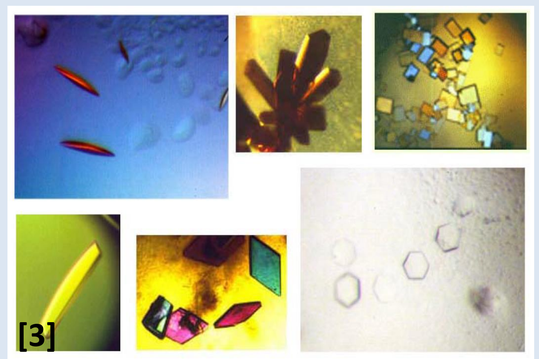
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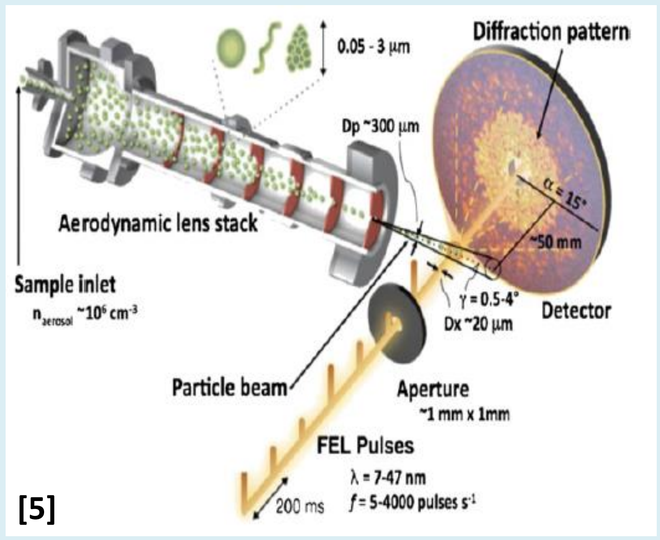
The Challenge


[4]



[5]

When processing samples in the nanometre domain, traditional approaches such as mounting on micromeshes are neither feasible nor appropriate, due to the levels of solvent around the crystals becoming unacceptable from an X-ray signal-noise perspective. Below are the current sample delivery methods:

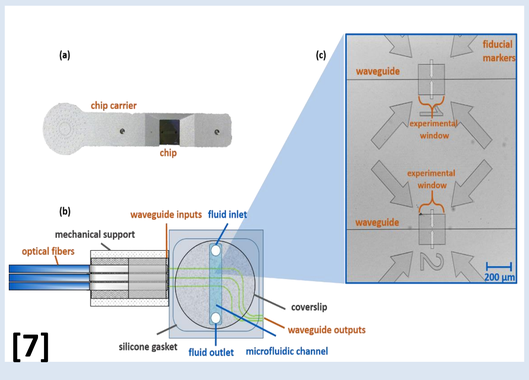
- **Robotic Handling:** The most common sample delivery solution, but this clumsy top down approach is sub-optimal, and not suitable for sub-micron crystallography.
- **Injectors:** An alternative to robotic handling, but the bulk of the sample is wasted. This method also requires imaging to take place as the sample is in motion.

The Solution

This project proposes a novel bottom up alternative, by implementing optical tweezing as a sample delivery method, capable of manipulating microscopic artefacts ranging from a few microns to tens of nanometres.

The "off the shelf" system in this project generates optical traps by harnessing the evanescent fields, which arise from the total internal reflection of a 1064nm laser carrying waveguide. Individual specimens are transported by a microfluidics channel up to the waveguide where they are trapped and manipulated into the synchrotron's beam path as it emerges from the beamline aperture. Substantially reducing sample wastage and increasing measurement accuracy.

[6]



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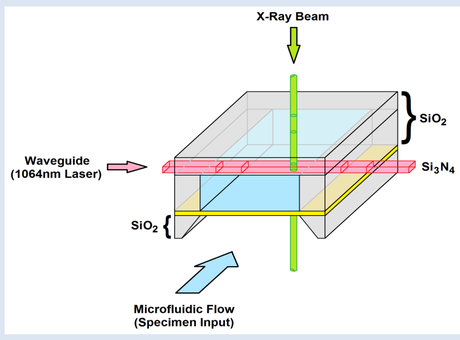

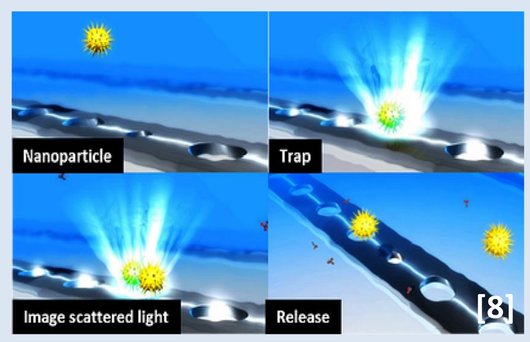

[8]

Ref.

[1] [Image taken from: http://www.diamond.ac.uk/Home/About.html]

[2] [J. C. H. Spence, U. Weierstall, H. N. Chapman; "*X-Ray Lasers for Structural and Dynamic Biology*", IOP Science, Rep. Prog. Phys. 75 (2012) 102601 (25pp)]

[3] [Strelkov, Sergei, "*Introduction to X-Ray Crystallography*"; Structure Bioinformatics Course, Basel 2004, Slide 10]

[4] [Image taken from: http://www.rigaku.com/en/products/protein/actor]

[5] (2[J. C. H. Spence, U. Weierstall, H. N. Chapman; "*X-Ray Lasers for Structural and Dynamic Biology*", IOP Science, Rep. Prog. Phys. 75 (2012) 102601 5pp]

[6, 7] [Image taken from http://docs.opfluid.com/documentation/]

[8] [P. Docker, D. Axford, M. Prince, B. Cordovez, J. Kay, D. Stuart, G. Evans; "The Development of an Automated Nano Sampling Handling System for Nanometre Protein Crystallography Experiments", Nanotech 2015, June 2015]

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