The Development of an Automated Nano Sampling Handling System for Nanometre Protein Crystallography Experiments

P. Docker+, D. Axford+, M. Prince*, B. Cordovez~, J. Kay+, D. Stuart+, G. Evans+

+ Diamond light Source Harwell OX110DE
*Aston University Birmingham B4 7ET
~ Optek Systems Philadelphia, PA, 19104

ABSTRACT

As the world's synchrotrons and X-FELs endeavour to meet the need to analyse ever-smaller protein crystals, there grows a requirement for a new technique to present nano-dimensional samples to the beam for X-ray diffraction experiments.The work presented here details developmental work to reconfigure the nano tweezer technology developed by Optofluidics (PA, USA) for the trapping of nano dimensional protein crystals for X-ray crystallography experiments. The system in its standard configuration is used to trap nano particles for optical microscopy. It uses silicon nitride laser waveguides that bridge a micro fluidic channel. These waveguides contain 180 nm apertures of enabling the system to use biologically compatible 1.6 micron wavelength laser light to trap nano dimensional biological samples. Using conventional laser tweezers, the wavelength required to trap such nano dimensional samples would destroy them. The system in its optical configuration has trapped protein molecules as small as 10 nanometres.

Keywords: microfluidics, protein crystallography, X rays, optical nano traps.

1 INTRODUCTION

Typically, workers in the synchrotron community look to employ a macro, top-down approach using robots to automate human protocols [1]. This is a methodology often seen in the micro and nano engineering community at large. Currently, as the handling of nano crystals is beyond top down capabilities, X-FEL facilities are currently employing an injector approach. This requires micro litre quantities of reagant containing nanocrystals to be injected in front of the X-ray beam and the diffraction pattern is imaged by the detector. Such systems have a reported 'hit' rate of less that 10 % [2].

This paper details optical nano tweezers which are very much a bottom-up approach that facilitates self-assembly, alignment and subsequently automation. This also offers the potential to inform the user that a crystal has been trapped prior to interrogation. This techneque will also present a static array of crystals to be interrogated by the X-ray beam. This technique is not to be confused with traditional optical tweezers which can be used to manipulate a single sample. Although a very useful technique it can only be used with >1 μm sized samples as the wavelength of laser light used to manipulate the sample must be of the same order of magnitude. To achieve the trapping of nano crystals using this technique would require a wavelength of laser light with too much energy thereby destroying any sample it traps.

These nanotweezers have been developed by Optofluidics (PA, USA). By using optical wave guides 1064 nm biologically friendly laser light can be used to trap samples sizes down to 10 nm at predetermined sites micro machined into the waveguides. These can be prealigned to the X-ray source and no further alignment would be required.

2 THE TECHNOLOGY

This potential solution is offered by Optofluidics, based in the Philadelphia, USA. They have developed technology which employs a microfluidic cell that uses silicon nitride waveguides to overcome the free space limitations of traditional optical traps to capture sub-micron particles with laser light using a wavelength that is compatible with biological samples (1064 nm) whilst maintaining protein sample integrity. Briefly, light is tightly confined at the near-surface of the silicon nitride waveguides, and the technology uses the evanescent wave (light outside of the waveguides) to generate strong optical gradients which are necessary to capture such small particles. If traditional optical trapping technology were to be implemented, such technologies would either be unable to optically capture the nano crystals (because of the light diffraction limit limitations of free space traps) or they would introduce a significant heating effect tothe surrounding volume, likely destroying the analyte. The flow cell allows accurate fluidic delivery (bringing the particles to the trapping locations via pressure driven flow), and once the particles are in the vicinity of the silicon nitride traps, the light is guided through the traps which results in the particles being captured (figure 1).

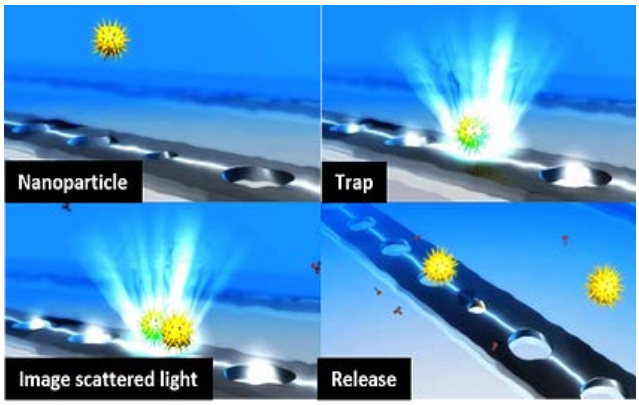

Figure 1 Trapping of nano particles

The technology has further advantages as the microfluidic flow-cell can have several waveguides each with an array of trapping sites allowing the chip to form a sample grid. This would allow for a chip to be aligned to the beam and present a 'grid' of trapped protein crystals that would also remain static during interogation. In operation, a slurry of crystals are flowed through the flow cell while the 1064 nm laser light is transmitted through the waveguides. The crystals flowing over the waveguides are then captured at the trapping sites. Given that the traps are very strong, the waveguide can continuously trap crystals while the flow is maintained, but both the flow and the trapping light can be modulated on command. If the laser light is switched off the examined crystals are released and the process can then be repeated.

In addition the system will allow stationary crystals to be exposed to different types of reagents during the measurement process.

2 THE CHALLENGE

This work sets out to develop the system to meet the new challenges of X-ray protein crystallography experiments to be carried out on both Synchrotrons and the next generation of X- ray light sources, X-FEL. Often the nano crystals are constituted of up to 98 % water and suspended in an aqueous solution further complicating the selection and trapping of samples for analysis. An additional challenge is a micro engineering/fabrication one. The standard chip architecture is designed for optical microscopy where illumination and analysis measurements are carried out from one side and the system in its standard form takes place in the horizontal plane. The former requires re-engineering to the macro engineering of the chip holder and the latter the more complex issue of redesign the microfabrication route to develop chips that will cause minimal attenuation of the X-ray beam.

3 MEETING THE CHALLENGE

3.1 Trapping Experiments

The experiments required to determine whether this technique is suitable to trapping protein cryals was carried out using the standard Optofluidic chip. Figure 2 shows the microfluidic chip in its holder and micrographs of the chip architecture

Figure 2 Standard chip architecture

Trials were conducted with 400 nm beads to clarify whether submicron specimens could be consistently trapped, and then further studies were undertaken with one μm protein crystals to clarify whether such structures could be trapped and would not perish due to the laser light being used to trap them. Figure 3 shows three 400 nm nanobeads trapped on chip. The image is taken from live footage taken using a standard upright microscope stage and a stemmer IDE UI LE Camera.

Figure 3 Trapped 400 nm beads

3.2 On Chip Modifications

Figure 4 shows how the system was configured to trap 1 μm Protein crystals. This size crystal was chosen for ease of obtaining confirmation they had been trapped without attaching a flourescent tag. Three trapped crystals can be seen in Figure 4.

Figure 4 1 μm protein crystals trapped on the wave guide.

For clarity figure 5 shows a micrograph of the waveguide when it was being processed. The dark circles are the 'holes' in the wave guide where the crystals are trapped.

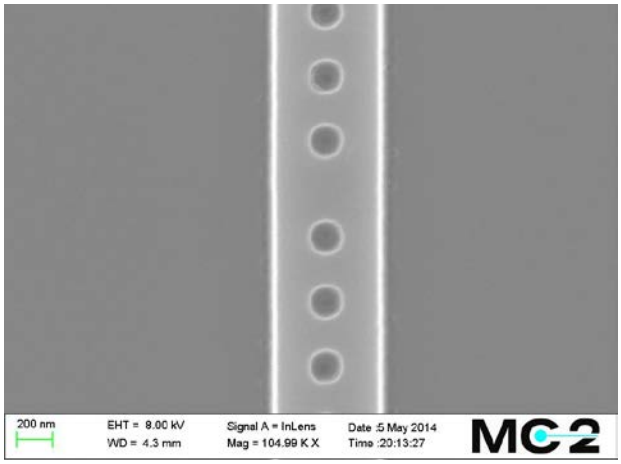

Figure 5 SEM micrograph of the waveguide. (Please note it is in the vertical orientation)

So from both a biological and size perspective the system is more than capable of meeting the demands of its new challenge. A standard chip has 320 μm of silicon below the waveguide which from standard calculations [3] would attenuate 80 % of the beam only allowing 20% to be used to obtain diffraction patterns from the sample. Predictive software was used to ensure that back-etching of this additional silicon would reduce this attenuation to an acceptable level. The new proposed chip architecture can be seen in figure 6.

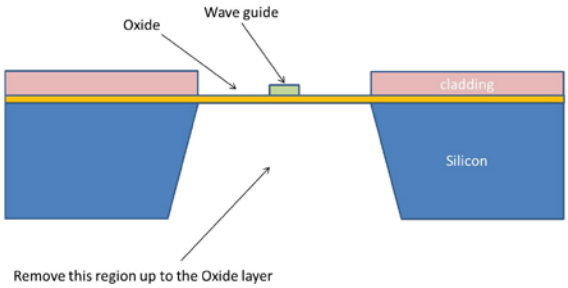

Figure 6 Proposed new chip architecture

A first run of processing the modified chips utilized wet etching as an additional stage to remove the bulk silicon below the waveguide. This approach however yielded only a small number of functional chips. The chips did however facilitate X ray tests to see if the new design had adequately reduced attenuation. The modified chip can be seen in the figure 7.

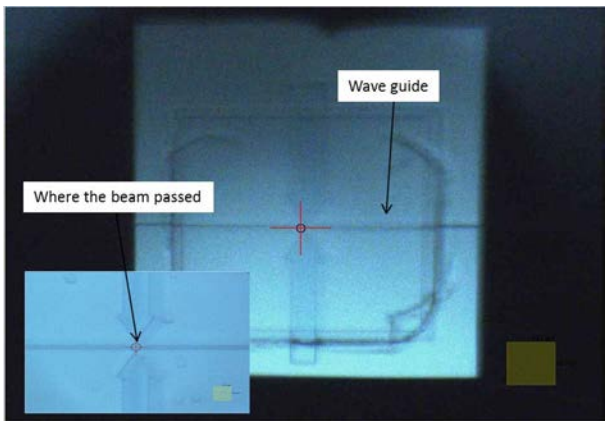

Figure 7 Modified chip

The chip was tested using 12.8 keV X-rays and proved the chip facilitated transmission of X-rays >99.9 %. It also caused negligible scatter comparable to that the air presents between the sample and the detector.

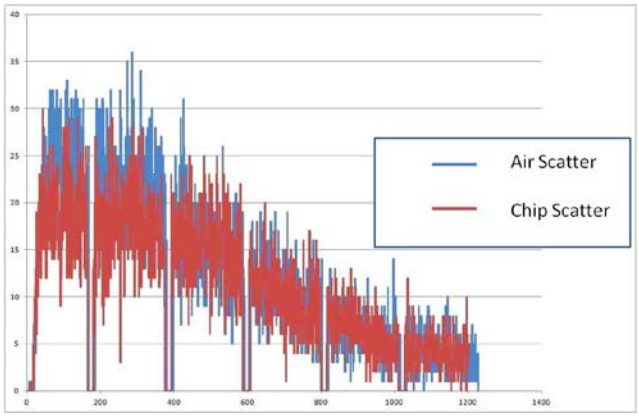

Figure 8 Scatter comparable with air between chip and detector

3.3 Hardware Modifications

The majority of the hardware is already fully compatible for X-ray diffraction experiments, the only component requiring modification is the chip holder. This component is responsible for making the fluid tight seal between the chip and the sample see figure 9.

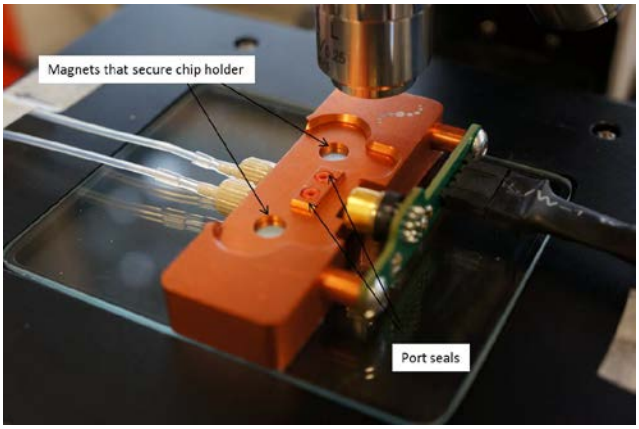

Figure 9 Standard chip holder

This chip holder requires a hole to be machined through the aluminuim between the two port seals as the X ray diffraction experiments are carried out in transmission. The beam will pass through the the hole, difract through the trapped crystals and onto the diffraction detector.

In service, laser light will pass throught the wave guides and a slurry of crystal will pass over the waveguides and will be attracted to the array of traps. The chip can then be indexed and patterns may be determined from each crystal in turn. The laser will then be turned off and the spent crystals sent to waste and the fresh analyte containing the crystals will re-enter the chip and the process repeated.

4 FUTURE CHALLENGES

The key driving challenge is to develop a completely dry etch process route in to improve yield and ensure it integrates with the modified chip holder. The last process run used wet etching to hollow the chip below the waveguides,moving forwards the next batch will employ deep reactive ion etching (DRIE) to remove the excess silicon. This approach is more expensive but more controlled. There is also no requirement to freeze dry the devices to negate issues from surface tensions caused by using wet etching techniques

A more 'pure science' challenge will investigate using manipulation of the laser light to orientate the crystals that are trapped in different or known orientations. To get the full structure of a protein the sample will need to be imaged in different orientations to obtain its full structure. By polarizing the light at the laser traps it is hypothesized that the crystals could be trapped and rotated with careful control of the laser light.

5 SUMMARY AND CONCLUSIONS

This paper details experiments that have been carried out which prove that 'typical' protein crystals can be trapped, with potential for trapping sub-micron crystals. A modified chip architecture has been developed specifically for use with a synchrotron X-ray beam. The chip is manufactured using standard semi-conductor technology which has been revised to produce a chip with a transmission of 99.98 % of the beam at energies between 6.4 - 20.0 KeV whilst maintaining the structural integrity of the flow cell part of the device. Standard chips were only capable of transmitting 20%.

This technology when fully developed for X-ray protein Crystalography experiments will truly be a game changing methodology for the nano protein crystallography community. Allowing these crystals to be held in position whilst they are imaged and allow for automation by default.

When Synchrotron and X-FEL beam time can take users two years to obtain an 8 hour slot, the ability to obtain as much high-quality data as possible is of paramount importance.

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Dr Peter Docker The Diamond light Source
Peter.Docker@Diamond.ac.uk