The Automation of Protein Crystal Presentation for X Ray Diffraction Experiments using Standing Acoustic Waves in a Microfluidic Chip Environment

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ABSTRACT

As the pressure continues to grow on Diamond and the world's synchrotrons for higher throughput of diffraction experiments, new and novel techniques are required for presenting micron dimension crystals to the X ray beam. Currently this task is both labour intensive and primarily a serial process. Diffraction measurements typically take milliseconds but sample preparation and presentation can reduce throughput down to 4 measurements an hour.

With beamline waiting times as long as two years it is of key importance for researchers to capitalize on available beam time, generating as much data as possible. Other approaches detailed in the literature [1] [2] [3] are very much skewed towards automating, with robotics, the actions of a human protocols. The work detailed here is the development and discussion of a bottom up approach relying on SSAW self assembly, including material selection, microfluidic integration and tuning of the acoustic cavity to order the protein crystals.

Keywords: standing acoustic waves, X-ray diffraction, protein crystallography, micro fluidics

1 INTRODUCTION

Using a Piezo actuator that has been integrated into a microfluidic chip design a Standing Surface Acoustic Wave (SSAW) can be set up laterally across a micro fluidic channel. This creates a varied acoustic field which will encourage crystals held in suspension to align at nodes or anti-nodes. Typically this is achieved using a standing half wave, providing a stable region for particulates midway across the channel. See figure 1 and figure 2 [4] for micro graphs of a channel containing 50 μm beads before and after the SSAW wave was established across it.

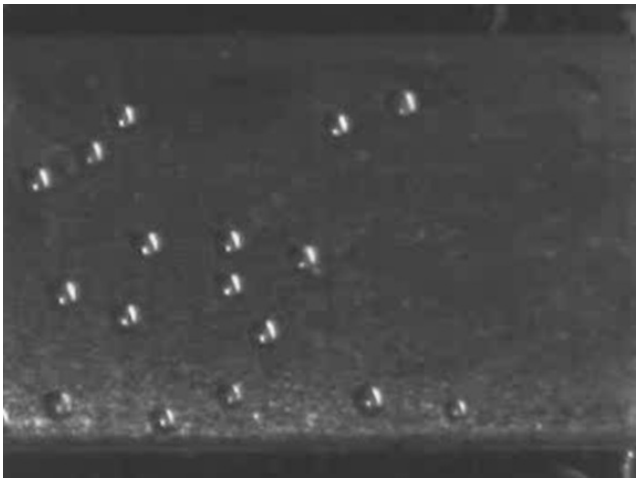

Figure 1: Channel with 50 micron beads
(image cropped for clarity)

Figure 2: After the SSAW was set running
(image cropped for clarity)

For the application of presenting and manipulating protien crystals for diffraction imaging, particles ordered by SSAW half waves in a controlled laminar flow along the channel the crystal may then be presented to the X-ray beam at a known and repeatable position aiding automation. The microfluidic cell designs comprise of an X-ray compatible window which will not attenuate the beam and subsequently the maximise the signal-to-noise ratio in the diffraction pattern that can be obtained from the sample.

2 CHALLENGES

2.1 Material selection

Many factors influence material selection for the project, these can be broken down into the materials used to characterize the micro fluidic architecture on chip during the design phase, and the material a device will be produced from for actual use in X-ray diffraction experiments. Characterisation of several X-ray compatible materials for such a device is planned. Hard materials such as silicon as they ensure the majority of the energy in the SSAW waves are reflected back into the sorting channel. However it is worth noting that the device that inspired the research into exploring this approach was seen at Nanotech 2013 and the chip was made from PDMS and facilitated alignment in 2D [5] (figure 3).

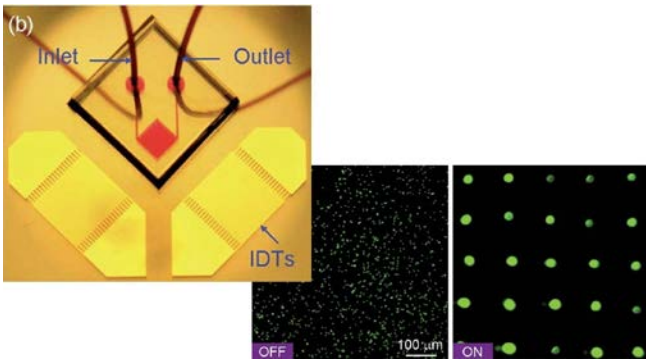

Figure 3: 2D alignment in a PDMS Device

So, there is a compromise to be evaluated in materials between diffraction capacity and manufacturability to develop an early generation design that is fit for purpose.

To achieve rapid validation in the design phase channels have been fabricated in two ways: complete design fabricated by 3D printing, and by 3D printing of a male mould and casting a device in PDMS. Both approaches have been fully characterized and initial flow properties through the devices investigated. Figures 4a and 5 give examples of chip designs that have been produced using both approaches.

Whilst the 3D printed devices offer many advantages in terms of complex geometrical possiblities, the requirement for manual polishing in order to achieve optically transparent surfaces prevent it from being a stand alone method. The 3D printed devices, despite being manufactured from a colourless and transparent UV-cured resin (Accura Si 60, 3D systems, Cal. USA) with highly polished outer surfaces, exhibit stepped inner faces on the inner faces of the channels which cannot be manually prepared. This leads to optical distortions when studied beneath a microscope and provides a barrier to optical validation of performance.

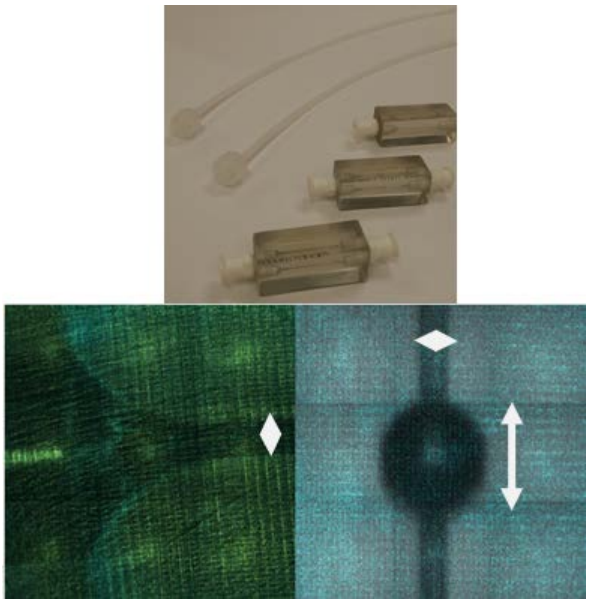

Figure 4: (a) *Top:* 3D printed chip assmblies (b) Confocal transmission scans showing dimensional accuracy of channels, scale bars show 500 micron and 2000 microns respectively

Figure 5: Cast PDMS Chip in their manifold clamp

Figure 6: KiKuchi Micropump (Scale is approximately 3:1)

The 3D printed chips in figure 4 explore Bulk Acoustic Wave (BAW) resonant cavities, each having a characteristic length and predicted acoustic mode. The devices will be explored to see if additional positional control can be achieved, and the losses that stem from the inhomogeneous structure (seen in figure 4b) can be tolerated. Figure 5 also includes with the PDMS chip a micro pump which us inder investigation with a view to integration into the final chip design. It has a dead volume of 250 nl and can run at between 1 and 350 Hz. The pump is comercially available from Kikuchi Japan. It meassures 7 mm x 7 mm (figure 6).

The small format and electronic nature of this pump is an enabling technology for the project, with rapid activation times, and full programmable control, it becomes possible to fluidically isolate desired samples, rapidly switching flow sources and velocity. Although the pumps offer a competing acoustic regime to the BAW / SSAW arrangement, the difference in operating frequency (four

orders of magnitude) is expected to leave the acoustophoresis intact.

2.2 Excitation approaches

Many authors have recently explored the excitation of SSAW devices using a variety of configurations. The two methods of interest are in-plane and out-of-plane excitation. For example the half wave, BAW, excitation in figure 2 was achieved with a commercial piezo disc bonded to the bottom of the chip. Channels fabricated from high Q materials, enable far more robust BAW excitation at lower levels of acoustic power, however and as discussed by Lenshof *et al*, this need not imply a more expensive system[6]. Fabrication of Interdigitated Transducers (IDT) activated Piezo designs are currently in progress at Nottingham Trent University (UK) using standard photolithography and lift-off fabrication techniqnues; specifically 128° YX cut lithium niobate, S1318 photoresist, Ti and Au IDT deposits. Piezo material devices are also being investigated with Phisik Instrumente (MA, USA) and Ferroperm (Humlbaek, DEN) to review off the shelf BAW exciters.

A further challenge from a system and excitation perspective is the characterisation of the coupling of the SAW waves with the specific protein crystals to be acoustophoretically ordered when in service. Presented sample crystals may typically span two orders of magnitude in size and require time consuming handling to achieve cryoloop mounting. The system must also be able to autonomously prevent particle aggregation, since this may lead to multiple crystal structures being present in the diffraction data.

2.3 Excitation On/chip Off/Chip

Currently two methods are proposed for developing the desired acoustophoretic ordering effect, SSAW and BAW. The advantage of the BAW approach, externally exciting any device, is the ability to use ruggedised mount – demount proceedures, and standardised components. The core challenged posed by an otherwise ideal method is an increase in the required acoustic power to achieve the desired ordering effect, the implication of this is scheduled to be as part of the project.

Selecting an ‘on-chip solution’ requires that the SSAW device must be robustly connected to the fluidised channel. This connection and the fragility of the IDT microstructures may prevent re-usability, the project will investigate the effect on system reliability.

Due to the competing factors it is not decided whether excitation and fluidic manifold will be incorporated in to a single device or whether the microfluidic manifold, and exciter, may be seperated, allowing the manifold to become disposable, the BAW/SSAW exciter then being reused. The latter appears to be the most desirable due to the significantly lower power levels. In all configurations the fluidic flow section of the device will need to be disposable between experiments, allowing the processing of hazardous protiens, circumventing blockages and cleaning downtime, and most importantly providing a robust and contamination-free imaging environment.

3 PREDICTED PERFORMANCE

Resolution - From the work of Shi et al [7], 10 μm particle focusing was achieved in 2009, and more recently this was achieved in three dimensional particle streaming [8]. While theoretically far higher resolution can be achieved with SSAW techniques at higher excitation frequencies, the practical limitation of the maximum particle size approaching the wavelength of the excitation signal, means a 10 μm particle spread after ordering remains realistic.

Flow throughput – comparing the requirements of the synchrotron to a cytometer, Chen et al were able to detect flowing blood cells at a rate of 1000 events s^{-1}, analysing fluid at a rate of 15 μl min^{-1} [9]. The impact of this already achieved figure, would be the ability to process a 1 ml sample of crystals in a little more than an hour (67 minutes). Given only 10 viable samples in this volume, it becomes evident that the intended system should out-perform hand loading of samples by an order of magnitude or better.

Low force handling – The need for samples to be conveyed at power levels that are below what is needed to cause structural change is one of the confounding factors for optical tweezing. The work of Ding et al successfully demonstrated that a living organism (C. elegans) may be restained by ultrasonic acoustic forces without causing damage [10]. The added success of Zeng et al in enhancing the output of interdigitated transducers [11] through the use of Reflective Bragg Gratings (RBG) demonstrates it is possible to actuate particles at reduced levels of piezo excitation, reducing system noise, and absorbed energy by the crystal sample. Figure 7 and 8 are taken from their work and demonstrates the absorbtion loss reduction based on RBG. Further to this the work highlighted the benefits of sub millimeter wall section between the exciter and the fluid medium.

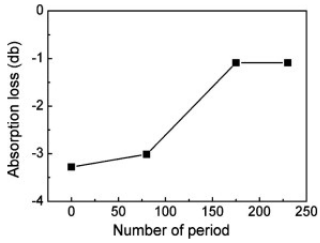

Figure 7: Absorbtion losses

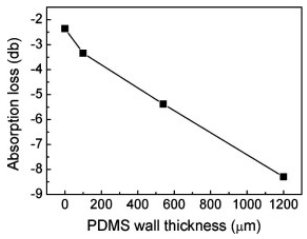

Figure 8: Wall thickness parameter

4 CONCLUSIONS

The paper covered the initial conclusions on process viability for continous mode acoustic ordered sample presentation, in-line with synchrotron beam imaging. No contra-indications were found for use, while many results demonstrate that a low complxity system will achieve desired throughput with no anticipated sample degradation.

In the feasibility assessment of the project from the literature, it is evident that the proposed system can offer useful ordering of samples for the synchrotron crystallography community. The current state of the art, developed in literature significantly since 2009, offers a robust set of tools to achieve the desired process automation and throughput improvement.

Reviewing sample handling data suggests that control of the crystals is attainable and further the construction methods are known, if customised, for each application.

Fabrication of the system has begun and the authors look forward to reporting integration and optimisation studies in the near future.

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