Tractor beam acoustic levitation for time resolved crystallography experiments

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Abstract.

Proof of concept of serial crystallography is carried out through the translation of samples delivered by tractor beam levitation. This is achieved using arrays of low powered transducers, focused to produce acoustic traps. Contrary to traditional Langevin Horn levitators, power requirement remains in the region of $10W$, limiting the acoustic pressure on the levitated samples and hence the risk of damage to them. Automation is achieved by controlling the phase of the transducers. The traps and associated samples steadily translate with the controlled acoustic field. A translation speed of $2.8mms^{-1}$ between the nodal distance of the traps is achieved. This results in sequential delivery of sample containing droplets performed with $1.5s$ between each delivery. The results demonstrate the ability to capture automated measurements of diffraction from lysozyme micro-crystals. Our study points in the direction of an automated, acoustic levitation system for time-resolved crystallography.

1. Introduction

The determination of protein structures is routinely carried out at synchrotron light sources via the technique of macromolecular crystallography. By determining the atomic structure of proteins using X-ray beams, a greater understanding in their function and chemistry can be gained. In addition to identifying protein structure, a desirable goal is to observe substrate reactions in real time. By studying enzyme kinetics it is possible to gain insights into the catalytic mechanism of enzymes and aid the prediction of the behaviour of these enzymes in living organisms. The presentation of protein crystals to the X-ray beam used to generate high quality experimental data is an area where improvement is much sought [1]. By their nature, crystalline protein samples are difficult to manipulate to the beam, both in terms of preventing damage to the samples themselves, and in relation to the rate at which they may be presented [1].

Various methods and techniques for structural biological analysis have been developed at synchrotrons and XFELs [2, 3]. These methods range from using cryogenically cooled samples [4, 5], to sample presentation at room temperature using kapton tape [6]. However for this paper, the focus is on a sample presentation method used specifically for analysis at synchrotrons. Static protein structures are traditionally determined at cryogenic temperatures during macromolecular

crystallography. By using cryogenically cooled samples, the damaging effects of ionizing X-rays are slowed. A more desirable method for determining dynamic protein structures is to carry out structural analysis at room temperature. A room temperature environment enables normal protein motions thus making it possible to capture changes in real time. This is particularly important for the observation of ligand binding, for example.

Acoustic levitation methods are potentially suitable for the determination of dynamic protein structures. Acoustic levitation methods include Langevin horns [7] and piezo transducer arrays arranged in different configurations [8]. They are approaches by which physical supports directly surrounding crystals are removed. Data can be collected whilst levitating sample droplets, by means of an ultrasonic acoustic pressure field, within an X-ray beam [1, 9]. Recent work has been carried out to develop on-demand sample loading [10] using a Langevin transducer, crystallisation plates and an acoustic droplet ejector (ADE.) As it delivers more power to levitating droplets than digitally controlled transducer levitators [7], sample heating occurs and therefore a shorter window for data collection is made available [1]. Digitally controlled acoustic levitators have additional advantages of using low power, around $10W$, are portable and inexpensive to produce [11]. Due to these factors, digital transducer-based levitators are chosen in this research over their more conventional cousins to present samples in synchrotron X-ray beams. Previous work by Morris et al [1] introduces acoustic levitation as a sample presentation method for macromolecular crystallography at room temperature. In this work, an acoustic levitator was utilised to hold a sample within an X-ray beam at the Diamond Light Source synchrotron to successfully solve a protein structure. Manual loading was required to levitate a single stationary droplet for each new sample. Though an important stage of development, manual loading is time consuming and inefficient. An advancement of this technique is to design a levitator which provides a high throughput of samples. In doing so, samples could be presented to the X-ray beam at regular time intervals enabling rapid, repeated serial data collection for solving time-resolved protein structures.

The work covered in this paper introduces a prototype of a tunable, automated sample delivery system. The system consists of a single axis acoustic levitator and a PolyPico micro-drop dispenser [12] to eject droplets into the acoustic field. Levitated sample droplets were translated through a synchrotron X-ray beam enabling data collection. This proof-of-concept experiment showed that diffraction data can be successfully recorded from protein micro-crystals representing a first, significant step towards the automation of levitating biological samples in an X-ray beam.

In Section 2, the apparatus is presented. Results are presented in Section 3 and concluding remarks are given in Section 4.

2. Methodology

2.1. System overview

The experimental set-up on beamline I24 at Diamond Light Source, is shown in Figure 1. The automated, acoustic levitation system mainly consists of a TinyLev inspired, transducer-based levitator [11] and a PolyPico micro-drop dispenser [13].

Automated, acoustic levitation system The transducer-based levitator consists of a pair of opposing hemispherical transducer arrays on a single axis. Each array comprises of thirty-six $10mm$ diameter piezo transducers arranged concentrically, in a dome with a radius of curvature of $71.5mm$. The base frequency of the transducers is $40kHz$. For this experimentation, it was observed that the characteristics of the levitated droplets are influenced by two parameters: input voltage to the acoustic system and separation of the transducer arrays. By adjusting these two parameters it is possible to tune the system to minimise oscillations, control the ellipticity of the droplet and form levitating droplets through aggregation of dispenser pico-droplets.

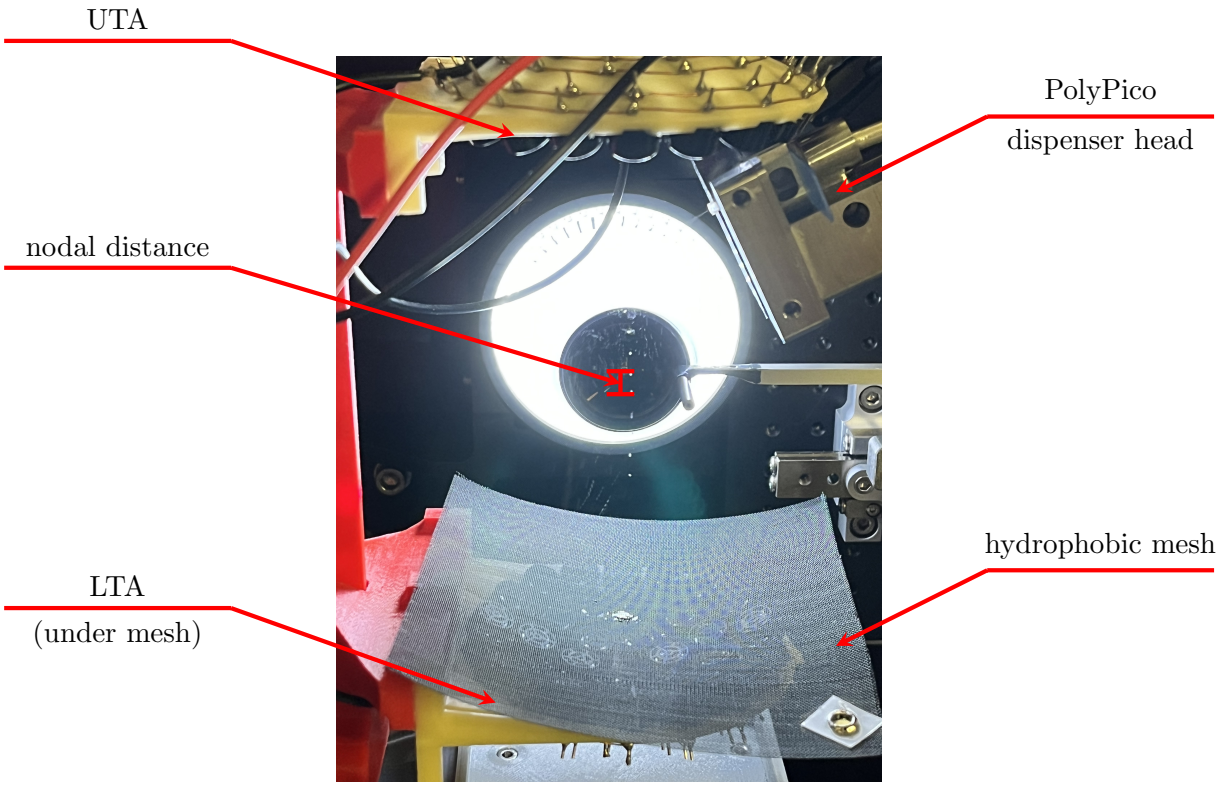


Figure 1: Automated, acoustic levitation system on I24 beamline. The Poly-Pico dispenser, the acoustic levitator's upper (UTA) and lower transducer arrays (LTA) the hydrophobic mesh and distance between nodes or traps are indicated. Levitating droplets are visible in the centre of the photograph at a nodal distance of $\lambda/2$. During data collection, the droplets translate down into the beam, and accumulate on the mesh sheet for later removal without hindering transducer performance.

The control system for the transducers allows for droplets to be held stationary in the acoustic pressure field or to translate in either direction along the system's axis. Consisting of an Arduino Nano ATmega328 microcontroller board and L298N driver board, the control system is easily replaceable and obtained at a low cost. Square wave signals, generated by the Arduino, are amplified by the driver board so the transducers receive a peak-to-peak voltage which is double the input voltage [11].

The attachment base of the system is a modular design. This allows the system to be quickly and easily configured for offline experimentation, by integration to an optical bench, or online experimentation, by easy attachment to the linear stages of the beamline end station. One of the advantages is the minimisation of disturbance to existing components of the beamline. In addition, the system may be set up offline leading to reduced cost and increased adaptability. Varying sample media and experiment types are easily accommodated to improve versatility. The ability to then re-attach the pre-set, optimised system into the beamline with no need for adjustment, maximises the time available for data collection.

A protective hydrophobic mesh was placed over the transducers on the lower array to protect the system from potential damage, due to possible contamination by the sample media.

PolyPico micro-droplet dispenser The PolyPico dispenser [13] can deliver pico-droplets whereby the size and dispensing rate are controllable, enabling the control of the levitated droplet size.

System combination Combined as a system, the levitator and droplet dispenser allow for a continuous serial procession of droplets for presentation to the X-ray beam. The droplets within the container-less environment, enable collection of data at the detector, without being obscured by mechanical instrumentation.

The translation of the droplets takes place as follows. The acoustic levitator generates a pressure field capable of holding several droplets simultaneously, see Figure 1. The phase difference between transducers is manipulated by the control program, to ensure that the entire stream of droplets continually translates, whilst retaining their pitch from each other in the vertical direction.

2.2. Offline testing

Within the laboratory environment, offline testing was carried out to validate and calibrate the automated, acoustic levitator system prior to beamline experimentation. Adjustments were completed to provide the most stable levitated droplet size and position possible. The optimum input voltage for this equipment was $11.4V$ and the separation of the transducer arrays was adjusted to $120mm$. The automation of the droplet translation was initially tested by manually loading distilled water droplets of approximately $1.5\mu L$ into the acoustic field, using a gas syringe. This initial testing enabled alignment of the PolyPico dispenser so droplets can be inserted laterally. Once the position of the dispenser had been established, the next stage was to check multiple droplets were captured in the traps of the acoustic field. As multiple droplets were successfully translating between the transducer arrays, the size of ejected pico-droplets and rate of droplet ejection were ascertained. The volume was set to a maximum value of $52pL$ and a continuous rate of droplet ejection as determined by the built in calibration tools.

2.3. Online testing

The system was tested on a macromolecular crystallography beamline I24 at Diamond Light Source, UK, using a Pilatus P3-6M detector with the levitator mounted onto the beamline sample goniometer stage and the PolyPico head mounted adjacently using optical posts and clamps, with remaining elements of the beamline end-station left unchanged.

Beamline Installation A 3D printed adaptor bracket was used to attach the levitator to the vertical goniometer, replacing the usual sample mount used for single protein crystals. The vertical goniometer allowed motorised motion in 3 dimensions to facilitate alignment of the levitator nodes with the X-ray beam. The PolyPico head was mounted to the beamline using optical posts and clamps and positioned approximately 80° in-board to the X-ray beam, Figure 1, and 30° downward, with about $20mm$ between the end of the PolyPico dispensing cartridge and the central column of levitator nodes. With the detector positioned $320mm$ downstream of the sample-beam interaction point, the appearance of a shadow from the dispensing head on the detector images could be avoided but a high resolution limit of 1.75 Å at the edge of the detector (1.4 Å in the corners) could be maintained. This was deemed suitable for the expected diffracting power of the micro-crystals and enough to unambiguously identify structural changes.

Data collection The PolyPico dispenser was set to continuously eject $52pL$ droplets at $1000Hz$. These coalesced to form larger droplets in the central column of acoustic nodes and were translated downwards through the X-ray beam. Data was collected in continuous runs of 5000 frames of $10ms$ exposure. Initial runs were performed with Mille-Q pure water with the overall X-ray scattering recorded on the detector, used as a monitor for when droplets were intersecting with the X-ray beam. Next, data was collected from lysozyme crystals approximately $30\times10\times10$ cubic microns in size produced by batch crystallization [14]. The choice of micro-crystals reflects our end goal of being able to interrogate samples small enough to allow rapid diffusion of substrates through the diffracting volume. Two concentrations were tested: Run 1, whereby

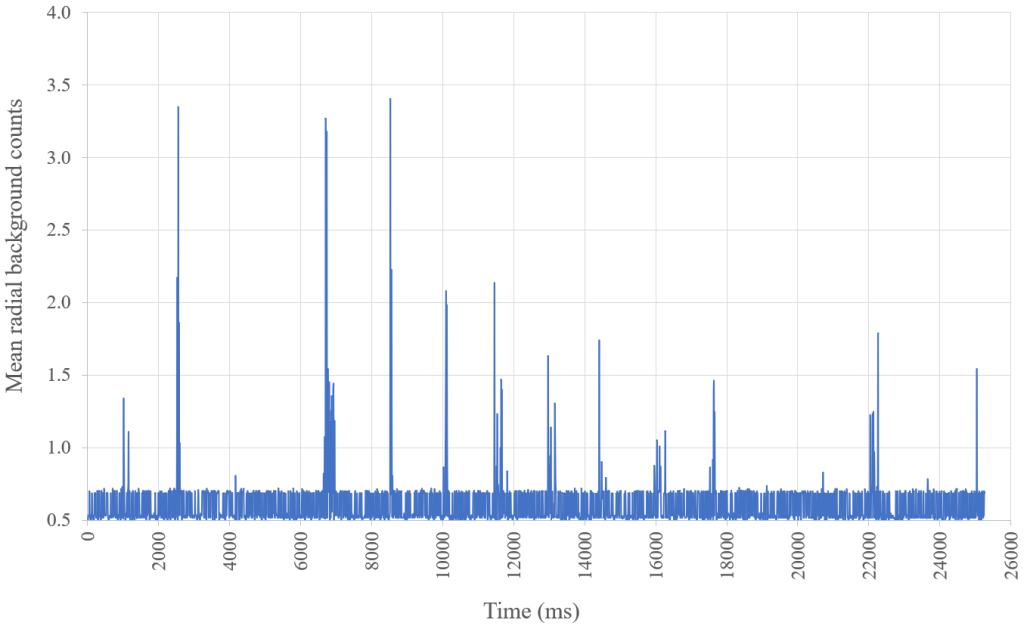


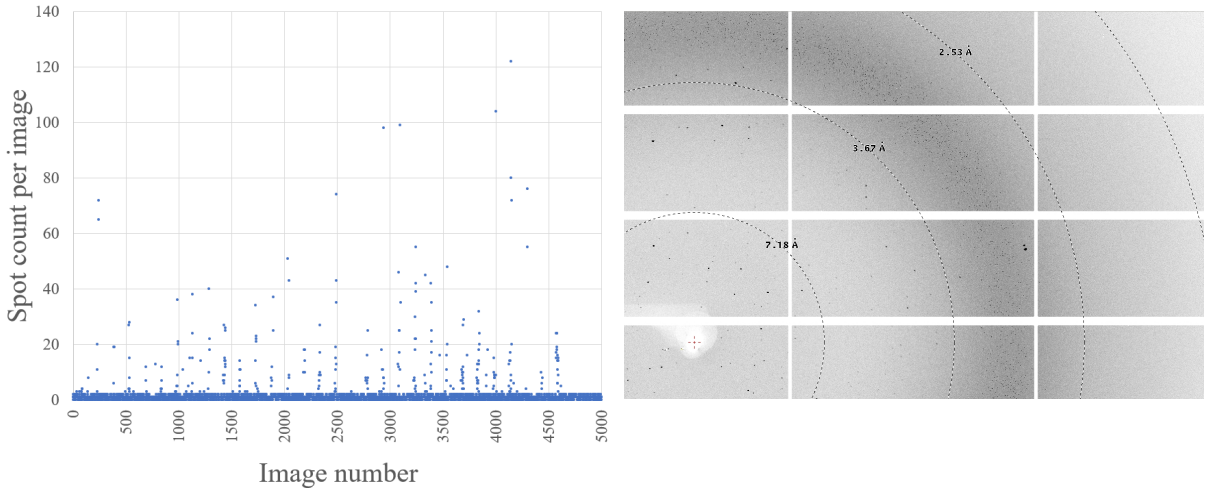
Figure 2: Frequency and duration of droplets intersecting X-ray beam.

crystals per ml was approximately in the order of 10^6 and Run 2, whereby crystals per ml was approximately in the order of 10^7. In terms of sample consumption for this proof-of-principle experiment, the droplet volume and delivery parameters yield 3.1 uL of sample per min, this puts it of the order of a viscous extruder and better than a Gas Dynamic Virtual Nozzle (GDVN) liquid injector [15].

3. Results and Discussion

In this proof of principle experiment, the frequency of intersection is an early indication of the potential of the automated, acoustic levitation system. Data displayed in Figure 2 illustrates the frequency and duration of water droplets intersecting the X-ray beam approximately every $1.5s$ for up to $360ms$. The beam intersections are consistent, notably between $6000ms$ and $18000ms$, giving a range of mean radial background counts, from approximately 1.0 up to 3.5. The duration of intersection needs to be a minimum of $10ms$ for data capture from the levitated protein crystals. The maximum beam intersection time recorded was up to $360ms$, allowing sufficient time for data collection. Average intersection time was $\tau_{int} = 205ms \pm 112$. Analysis of the graphical data gives an approximation of the translation speed of the droplet. Based on an average time between beam intersection peaks of $1.5s$ and nodal distance of $\lambda/2$ an estimate of $v_{tr} = 2.8mms^{-1} \pm 0.35mms^{-1}$ calculated at $20C$ standard atmosphere, can be made. The average radius r_{drop} of droplet can be estimated as $r_{drop} = v_{tr} \times \tau_{int}/2 = 0.29mm \pm 0.05$. The volume is estimated at $V_{drop} = 0.10mm^3 \pm 0.03$, which corresponds to approximately 1900 polypico droplets, where one polypico droplet is defined as 52pL.

Lysozyme diffraction was visible at hit-rates between 1% and 2% (images where the DIALS software [16] found 15 or more spots, includes periods between droplets, Figure 3a) with the best diffraction extending to around 3 Å, Figure 3b. We deduce the limiting factor in measurable diffraction resolution being the background water scatter, as estimated above, coming from a maximum path length of 0.58mm.



(a) (b)

Figure 3: (a) Droplet intersection as recorded by diffraction spot counts per image. (b) Lysozyme diffraction data collected from Run 2.

4. Conclusions

A prototype for an automated system for serial sample delivery using acoustic levitation has been demonstrated. Droplets frequently intersect the X-ray beam for a sufficient amount of time to enable data collection. From the beamline experimentation carried out, further work is required to optimise the system for serial data collection for time-resolved protein structure solution and more general measurements at room temperature. However, early promise is evident and there is scope to improve both sample consumption rate and sample hit rate. Some of the design modifications that would address this include reducing droplet speed, to increase levitation time in traps, and improved stability of traps, by minimising lateral motion of droplets. In addition, a reduction in droplet volume would reduce background scatter, improving signal-to-noise and measurable diffraction from micro-crystals. Finally, we plan the introduction of substrates via a second droplet ejector for time resolved mixing experiments.

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