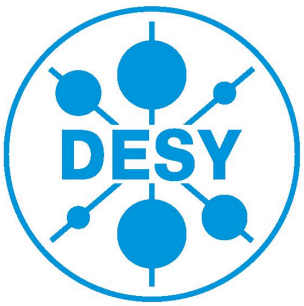

Development of a sample delivery system for use in serial crystallography at synchrotron radiation beamlines

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Abstract

A prototype sample delivery system consisting of a drop on demand system used to place protein nanocrystal-containing droplets onto an X-ray transparent conveyor belt propelled by a tape drive has been developed and tested in order to better adapt serial crystallography methods for use in synchrotron radiation beamlines, with the ultimate aim of installing the system on a beamline at PETRA III. Suggestions for future improvements, modifications and additional testing have been given.

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1 Introduction

Serial crystallography [1] is an X-ray diffraction method in which protein structures are determined from a vast amount of nano-crystals, often on the order of many hundreds of thousands, rather than from a single large crystal as with typical X-ray crystallography. These nano-crystals are transferred into the path of an X-ray beam (typically ultrafast X-ray pulses produced by a free-electron laser), resulting in the recording of diffraction patterns at a constant rate. All but single-crystal diffraction patterns are then discarded and the remaining patterns are indexed and merged in order to form a three-dimensional set of reflection intensities from which the protein structure can be determined.

Adapting such serial crystallography methods for use with synchrotron radiation sources rather than ultrafast free-electron lasers requires an efficient means of delivering samples into the X-ray beam. In previous experiments this has been achieved using a thin-walled capillary within which the suspension of crystals continously flows across the synchrotron radiation beam [2] such that the exposure time of the crystal is determined by the time it takes to transit the X-ray focus. Using a capillary in such a way has many drawbacks; there is only limited control over the speed with which the sample travels through the capillary and as such the transit time of the sample as it travels through the beamline is difficult to set. Additionally, and more seriously, the synchrotron radiation can cause damage to the capillary, altering the flow of the sample through it and significantly affecting the transit of the sample across the beamline. Such an alteration in the sample flow is extremely difficult to account for and means that the experimental conditions do not remain constant, resulting in difficulty justifying the validity of data. Suggested here is instead a system similar to the one used by Roessler *et al.* [3] at the National Synchrotron Light Source in which an acoustic drop ejection (drop on demand) system is used in order to place crystal-containing droplets onto an X-ray transparent conveyor belt which proceeds to pass the droplets into the synchrotron beam.

2 Specifications

There are three main components to the proposed method of sample delivery:

- The drop on demand system
- The tape drive system
- The conveyor tape

The drop on demand system must be able to consistently produce droplets of the same dimensions and with regular frequency, depositing them evenly in a line such that all droplets are conveyed into the beamline. The tape drive must be reliably able to be maintained at a constant speed and should be able to produce sufficient speeds for use with the synchrotron radiation beamline (discussed later). The conveyor tape must be mounted as stably as possible such that there is no movement in any dimension

other than the one travelling into the beamline and it must be rotated in order to be perpendicular to the beamline if the droplets are to be deposited vertically. The material of the conveyor tape must be as transparent as possible to synchrotron radiation (X-rays) to reduce background noise in diffraction patterns and should ideally not become damaged or deformed in any way by the X-rays.

3 Results

3.1 Drop On Demand system

The drop on demand system consists of a MicroFab MJ-AB-01-20-DLC-6MX nozzle [4] with an inner diameter of 20µm, as shown in Figure 1, connected to a MicroFab JetDrive III controller and a pressure controller. The frequency of vibration of a piezoelectric crystal within the nozzle determines the rate at which droplets are ejected. The pressure controller is present in order to exert pressure on a reservoir of liquid within which the sample nanocrystals are to be contained such that there is sufficient pressure for the release of droplets. The JetDrive III controller is connected to a laptop via USB and uses the MFJetServer software in order to control the parameters with which the nozzle's piezoelectric crystal vibrates and, consequently, the rate at which droplets are ejected. A filter is placed between the reservoir and the nozzle in order to reduce the risk of blockage since due to its size the nozzle orifice becomes clogged very easily.

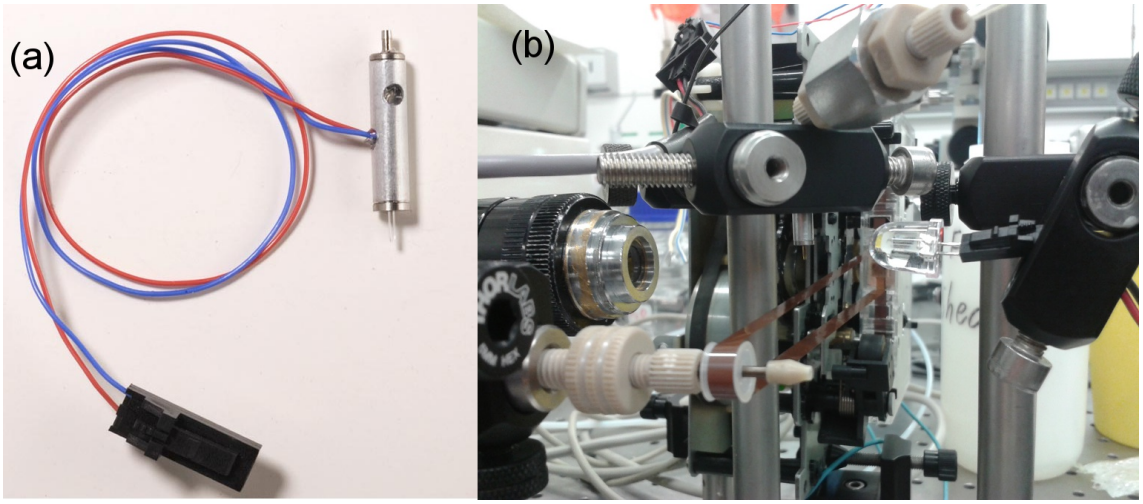

Figure 1: (a) MicroFab MJ-AB-01-20-DLC-6MX nozzle (b) Nozzle setup for testing the drop on demand system.

The drop on demand system was initially tested using deionized (DI) water. The nozzle and reservoir were flushed through with the water, ensuring afterwards that there were no air bubbles present within either the reservoir or the nozzle system itself. The nozzle and reservoir were then connected to the rest of the system. Occasionally large

drops formed on the very tip of the nozzle upon connecting it to the rest of the system, these drops were wiped off using a cleaning wipe. A Moticam 3.0MP camera was set up with a 5x magnification optic in order to image the droplets as they were ejected from the nozzle. A strobe LED was mounted pointing directly at the camera and was connected to the JetDrive III controller, ensuring that the frequency of the strobe was identical to the frequency at which the nozzle ejected droplets. This meant that, by adjusting the strobe delay relative to the time at which droplets are ejected using the JetServer software, a stationary image of the droplets was formed where the position of the droplets was dependent on the strobe delay.

Figure 2 displays an image of the water droplets produced by the drop on demand system. The JetServer was set to trigger continuously and the piezo parameters used in order to produce the droplets were as follows - rise: 3.0µs, dwell: 10.0µs, fall: 6.0µs, echo: 10.0µs, final rise: 3.0µs with dwell and echo voltages of 15.0V and -15.0V respectively and a piezo frequency of 120Hz. Applying pressure to the reservoir proved unecessary for the formation of droplets and as such the pressure was set to 0mbar.

Figure 2: Water droplets ejected from the nozzle with a strobe delay of 125µs.

The drop on demand system was subsequently tested using a buffer solution used in the crystallisation of the Complex II proteni. In order to get the drop on demand system to function with this buffer solution a different sample loading procedure was necessary. This involved starting the system running using deionized water or a low concentration of buffer and leaving the system running continuously for 5-10 minutes. After this time the reservoir was unplugged from the nozzle while the piezo was left running, the reservoir was filled with the new liquid or buffer and was then reconnected

to the rest of the system. The nozzle proceeded to drop immediately with the liquid that was initially loaded, after a duration of 6 minutes had passed this remaining liquid had finished and the new sample (the buffer solution) began dropping.

Figure 3 displays the results of using various concentrations of buffer solution with the drop on demand system. Note that due to the greater viscosity of the buffer solution significantly lengthier droplets are produced than when using water.

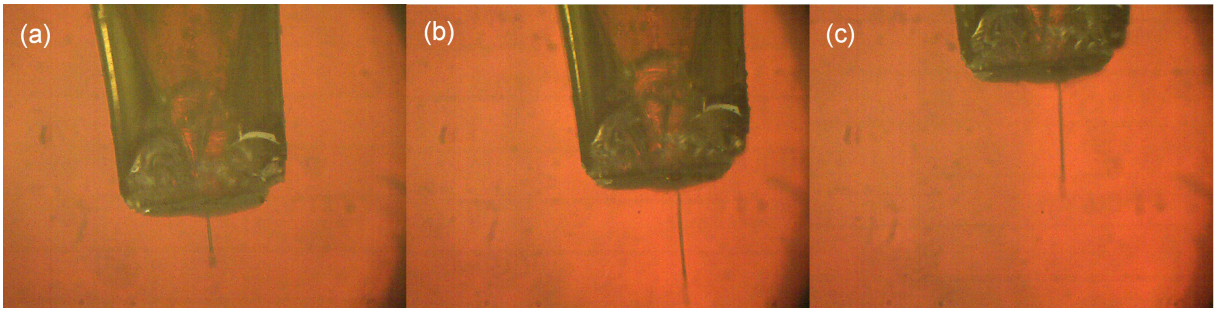

Figure 3: (a) 1% PEG 400, 0.01 M NaOAc, pH: 4.58 with dwell and echo voltages set to 20V and -20V respectively. (b) 5% PEG 400, 0.01 M NaOAc, pH: 4.58 with dwell and echo voltages set to 30V and -30V respectively. (c) 10% PEG 400, 0.01 M NaOAc, pH: 4.58 with dwell and echo voltages set to 30V and -30V respectively.

3.2 Tape Drive system

A prototype tape drive system was devised from a cassette player. This essentially functions as a motor in order to drive the conveyor tape and subsequently propel the protein samples into the beamline. The optimal speed at which the conveyor should run is dependent on the protein sample used, the transit time for the sample within the beamline should be optimised to be as long as possible without causing radiation damage to the sample in such a way that the measurements are affected. Lysozyme, for example, requires transit times of around 1 - 3ms [2] which corresponds to a conveyor velocity somewhere around the order of 10^{-2}m/s since at its widest the synchrotron beam is 9µm and the crystals have a long axis of 6µm.

The prototype system required three power supplies to run - one was used in order to operate the 'play' function of the cassette player, another to activate and deactivate the solenoid in order to connect the motor to the tape and the final one to control the fast-forward and reverse function. In order to get the tape drive system running the fast-forward was initially set to 2.0V, this was necessary or else upon attempting to run play the tape would accumulate inside the cassette (rather than returning to the reel), eventually preventing the system from operating. The 'play' power supply was set to 8.0V or higher, any lower and the system would jam upon activating the solenoid. The solenoid activated at a voltage of 7.8V, connecting the motor to the tape. The 'play'

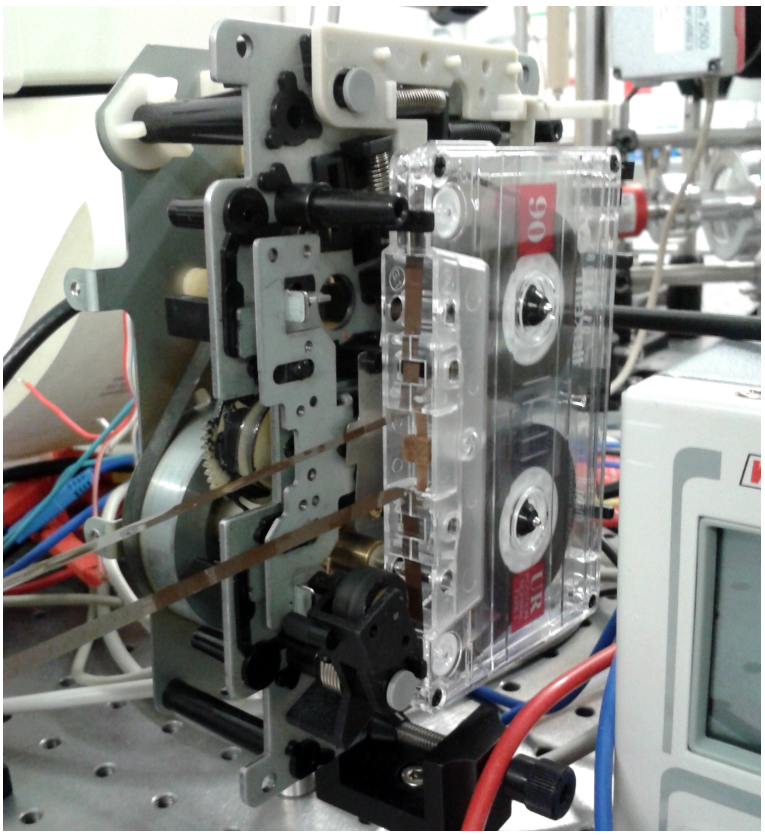

Figure 4: Prototype tape drive system devised from a torn apart cassette player.

voltage could then be reduced (or increased) in order to achieve the desired velocity, though it would stop altogether below 3.6V. For the solenoid to be deactivated once more the tape had to be running or else the system would jam.

Images of water droplets deposited on the conveyor tape can be seen in Figure 5, the water droplets are small enough that they evaporate over the course of less than a second. This would be ideal if used with samples given that we would just have diffraction from the crystal itself and the conveyor tape without any additional liquid, though it is unlikely that buffer solution would evaporate in a similar fashion. The water droplets close to the point of deposition are estimated from the image to be around 32± 8µm in diameter where the uncertainty largely arises from a perceived discrepancy in droplet size, though a fastcam would be necessary in order to determine the exact dimensions of the droplets.

Attempts were made to determine the speed at which the prototype tape drive travelled for a given velocity by marking a line on the tape using a pen and measuring how long it took that mark to travel a certain distance however the device proved too unreliable and could not reproduce measurements for velocity at the same voltage on consecutive days. For example, the velocity was determined to be 0.0087± 0.0004m/s at a voltage of 3.4V however the subsequent day 3.4V was insufficient to get the tape

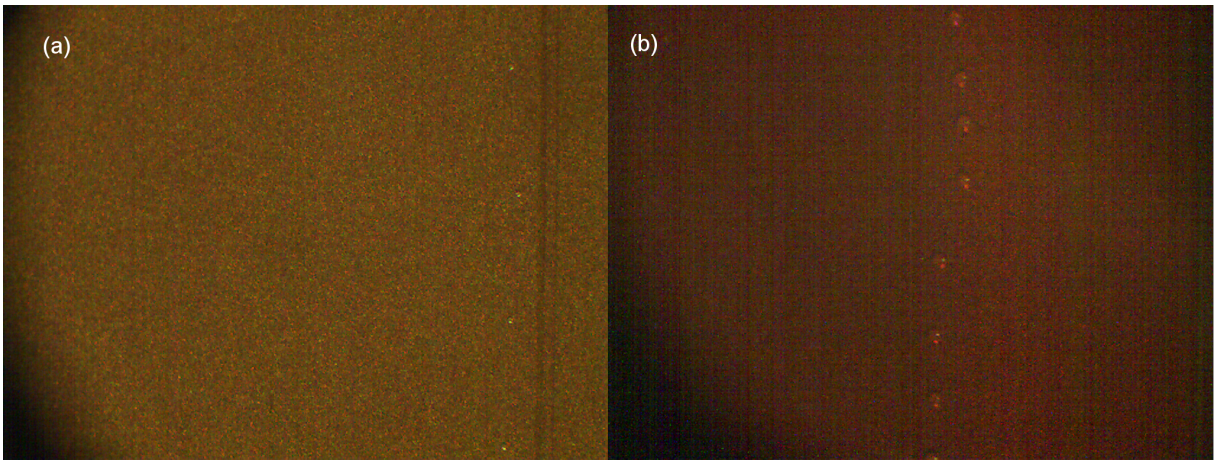

Figure 5: (a) Water droplets imaged on the cassette tape further from the nozzle, they are particularly small since they are subject to evaporation. (b) Water droplets imaged on the cassette tape closer to the nozzle, the shift of droplets in the middle of the image is due to movement of the tape rather than because of the way they have been deposited.

drive running at all. This meant it was not possible to accurately reproduce the same speeds with the prototype device, thus it is not suitable for delivering samples into the beamline, we instead require a motor over which we have a significantly greater control and with which we can reliably produce a given velocity.

3.3 Conveyor Tape

A range of materials were tested to determine which would be most suitable for use with the sample delivery system. In order to see which material provided the least background noise for X-ray radiation they were subjected to a Cu k-alpha X-ray source from a Rigaku MicroMax-007HF [5] with X-rays of wavelength 1.54178Å. The materials chosen for testing were Kapton film, Kapton tape, cassette tape, leader tape - i.e. cassette tape without the magnetic coating, and sellotape. A glass capillary was additionally tested for the sake of comparison.

The material samples were prepared by taping them to a mount and aligning them perpendicular to the source of X-rays. The HKL-3000 software was used in order to set the X-ray parameters. For each measurement the detector was set to be 80mm from the sample with the phi-axis at 90°and for every sample material 3 different measurements were made with exposure times of 30s, 45s and 60s. Figure 6 shows how the samples were mounted in the X-ray machine and Figure 7 displays the images taken at the detector for each of the materials tested.

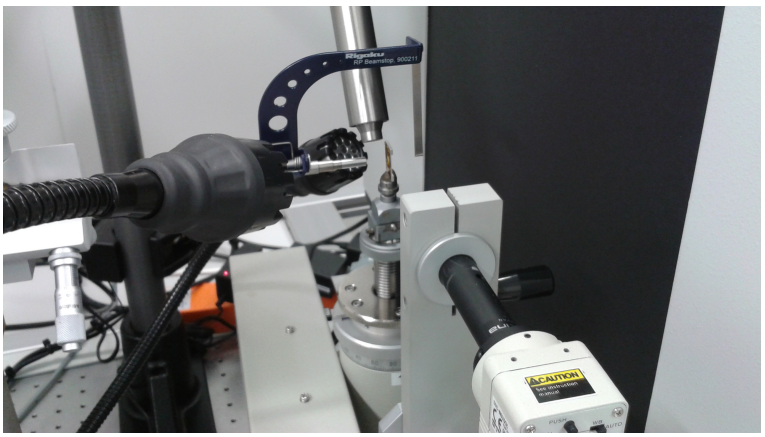

Figure 6: A sample of Kapton tape is aligned perpendicular to the X-ray source, demonstrating the X-ray setup.

The data was subsequently converted to 1d profile microfiles using the software 2DP. X-ray images of the air were taken using the same parameters as for the materials and subtracted from these profiles in order to reduce the background noise such that only the Bragg peaks of the materials are given. This data was then exported to the ascii format and imported into a data processing tool (Microsoft Excel) in order to plot the intensities against the resolution, determined using Bragg's law. Figure 8 displays plots of the intensity against both the resolution and Q, the reciprocal lattice.

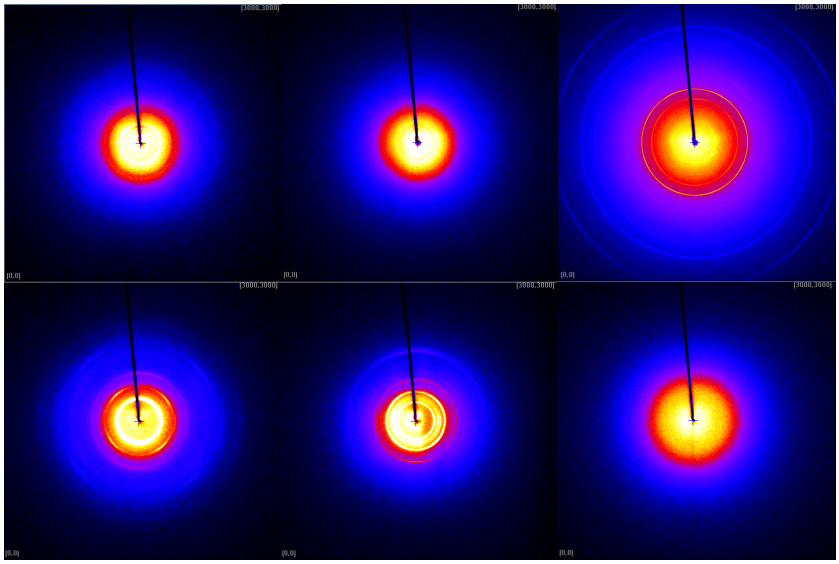

Figure 7: False colour x-ray images of the materials tested at an exposure time of 60s. From left to right - Top row: Kapton film, Kapton tape, cassette tape. Bottom row: leader tape, sellotape, glass capillary.

The suitability of a material for the conveyor tape is dependent on the protein that we wish to investigate. It is immediately evident from the graphs in Figure 8 that neither the Kapton tape nor the cassette tape would be a viable choice of material given that both maintain high intensities throughout the range of resolutions, they would obscure Bragg peaks of protein samples tested and obscure measurements made.

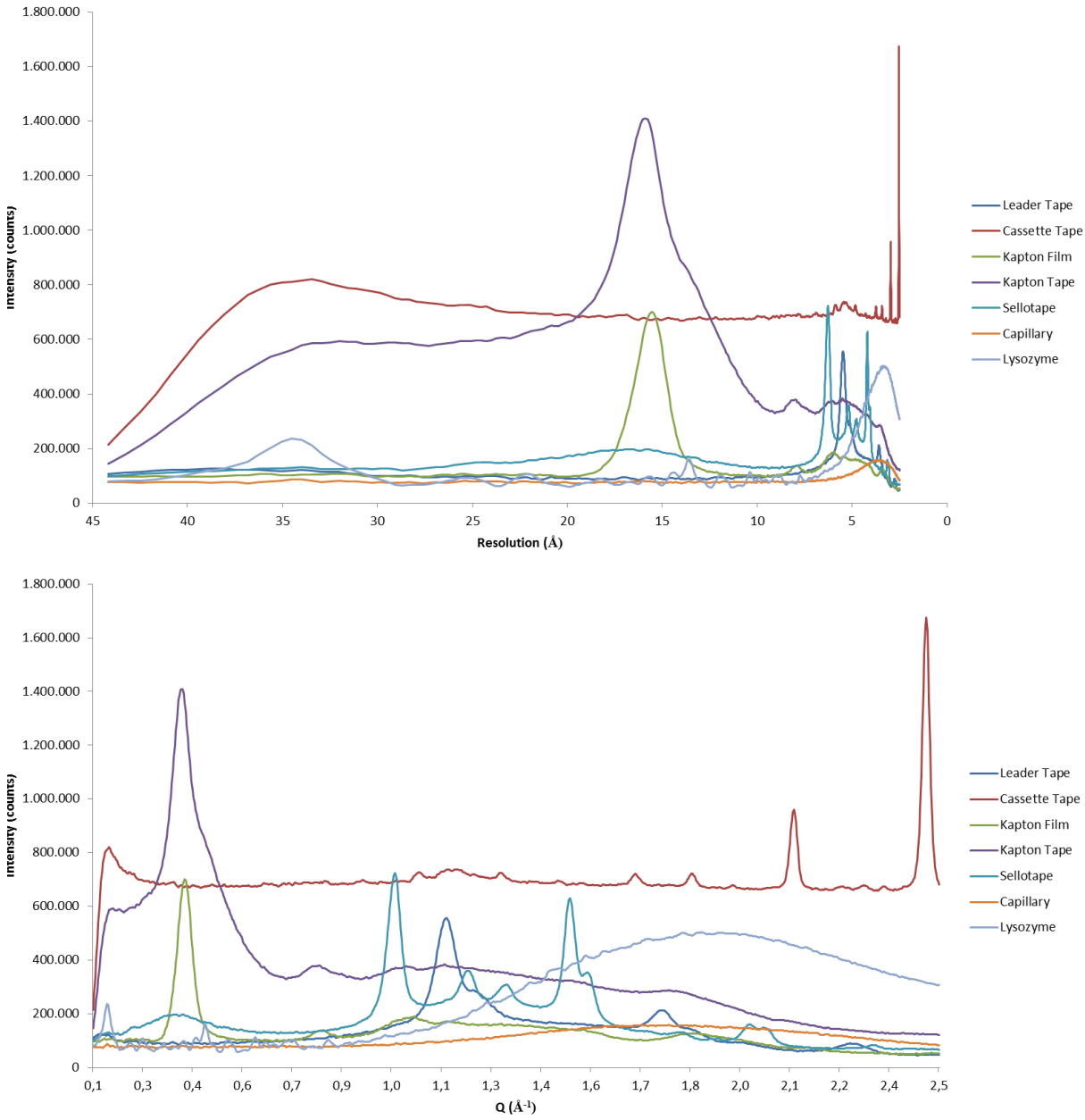

Figure 8: Intensity is plotted against both the resolution and against Q, the reciprocal lattice, for all materials tested at an exposure time of 60s.

A powder diffraction of lysozyme was performed using identical parameters as for the X-ray imaging of the materials. This can be seen plotted against the potentially viable materials for the conveyor tape in Figure 9.

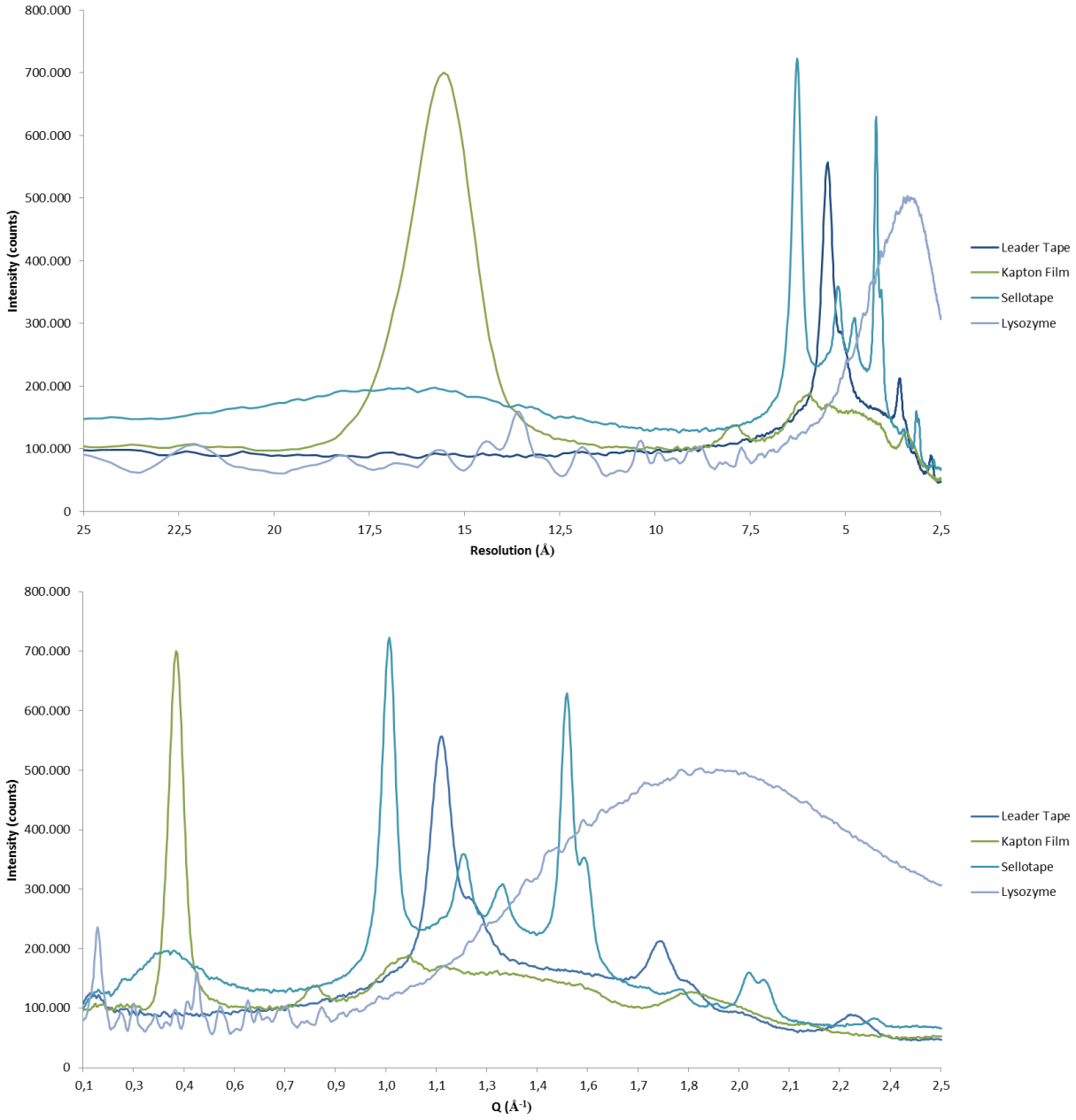

Figure 9: Intensity is plotted against both the resolution and against Q, the reciprocal lattice, for leader tape, Kapton film, sellotape and a powder diffraction sample of lysozyme

We want the peaks of the conveyor tape material not to coincide with peaks from protein samples that are being tested, however this is entirely dependent on the protein sample being investigated. Both the Kapton film and the leader tape would be viable materials for use in the conveyor tape (sellotape less so given its adhesive surface), Kapton film would be useful for a protein that has minimal Bragg peaks for the resolution limit of around 13.5 - 17.5Å and likewise the leader tape would be useful were we not interested in the limit 3.5 - 6.5Å.

4 Conclusion

A prototype sample delivery system consisting of a drop on demand system used to place protein nanocrystal-containing droplets onto an X-ray transparent conveyor belt for use in serial crystallography at synchrotron radiation beamlines has been developed and tested. The parameters for successfully attaining droplets with the drop on demand system have been determined and it has been found to produce water droplets with a diameter of 32± 8µm. It has additionally been successfully tested with a buffer solution used in crystallising the Complex II protein. Various materials have been tested for use as a conveyor tape and both Kapton film and leader tape have proven to be viable options depending on the protein being investigated. Kapton film would be ideal for proteins without Bragg peaks in the resolution range of 13.5 - 17.5Å while leader tape would be more useful for proteins without peaks in the resolution range 3.5 - 6.5Å.

In order to get the sample delivery system working in the synchrotron beamline it will be necessary to replace the cassette player prototype with a motor with which we can control the speed of the conveyor to a very high degree of accuracy, ideally through the use of software. With this we should be able to achieve the velocities necessary for suitable protein transit times within the beamline. Using this motor we could additionally test to see the whether the droplets form a straight line on the conveyor tape such that they are all conveyed into the beamline, this could be achieved by setting a strobe to the frequency of droplets which pass a point in the course of a second - this frequency can be determined using the velocity of the conveyor tape and the distance between droplets on the conveyor tape (i.e. the velocity divided by the distance between droplets gives the number of droplets per second). Additional future endeavours should involve testing the drop on demand system using a sample of protein nanocrystals within the buffer solution in order to determine whether this functions correctly. In the event that it does not there is the option to use a piezo nozzle with an orifice of 50µm in diameter as opposed to 20µm, however this is not ideal given that it would result in larger droplets which would have more effect on diffraction background noise. A fastcam could also be used in order to determine the dimensions of the droplets of water to a greater degree of accuracy in addition to the dimensions for the buffer solution and the buffer solution with sample (or water with sample, depending on the sample used). Ultimately this should lead to installing the system into the synchrotron beamline at PETRA III and performing tests using the synchrotron radiation itself.

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References

[1] H. Chapman *et al.* Femtosecond X-ray protein nanocrystallography. *Nature*, **470**, 73-77 (2011)

[2] F. Stellato *et al.* Room-temperature macromolecular serial crystallography using synchrotron radiation. *IUCrJ*, **1**, 204-212 (2014)

[3] C. Roessler *et al.* Acoustic methods for high-throughput protein crystal mounting at next-generation macromolecular crystallographic beamlines. *J. Synchrotron Rad.*, **20**, 805-808 (2013)

[4] MicroFab Technologies Inc. `http://www.microfab.com/index.php?option=com_content&view=article&id=8&Itemid=10` (date accessed: September 11, 2014)

[5] Rigaku Corporation. `http://www.rigaku.com/products/protein/micromax007` (date accessed: September 11, 2014)