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Nanodrop cell for X-ray and nanoanalytic applications

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Contents

Executive Summary

In today's competitive world of science efficient analysis tools are indispensable. The tools should be instruments providing measurements of small sample amounts, being both cost and time effective. Measurements should be not only easy and fast to execute, but also reliable and precise. Here we report on a new instrument, which enables measurements in a routine and automated way for the high throughput screening of liquid samples.

The instrument is an automatic sample changer feeding a novel sample holder for volumes in the range of 5-20 µL. The general operating principle of the sample holder not only allows for precise measurements of very small volumes, but also the reduction of sample quality. High surface-to-volume effects easily occur in more conventional systems based on pumping the sample through tubing into capillaries. While avoiding such high surface-to-volume effects the automatic sample changer enables the measurement of hundreds of samples without requiring of manual intervention.

We extensively tested the instrument and showed that it satisfies the initial specifications. It quickly provides reliable, high-quality data using minimal volumes of sample. This new instrument greatly simplifies and accelerates experiments, constituting therefore a valuable contribution for accessing large scale infrastructures.

The prototype is already available for testing during general user access at the Austrian SAXS beamline at the Elettra-Sincrotrone Trieste (first experiments are scheduled within March and April 2019) and later on in the NFFA workpackage TNA4. The development of the instrument has been reported at several conferences, including MNE2017, OEPG 2018, NESY Winterschool 2017 and 2019. A publication concerning the system and its testing is currently in preparation.

1. Concept

Sample handling becomes increasingly demanding as the volume of sample that is used for measurements decreases. In additions to challenges in sample manipulation and alignment, the small dimensions may also lead to novel phenomena caused by high surface-to-volume ratios. These phenomena may have interesting applications, but they can also lead to new complications.

Pumping a sample through small channels may lead to the preferential sticking of sample on the walls, modifying the concentration of the sample in solution. It is of considerable interest to measure samples without pumping them through narrow tubes, channels or capillaries. We have developed a system based on a patent application [1], that circumvents the problems of high surface-to-volume ratio, by not relying on tubes and capillaries to load and measure samples. While the concept is called 'Nanodrop' in the patent and in the title of this deliverable, the actual device deals with drop volumes in the µL-range and will thus be referred to more accurately as 'Microdrop' throughout this report.

1.1 The Microdrop sample holder

The central part of our new system is a sample holder based on the placement of a drop of sample between two small, parallel silicon plates. A few microliters of sample are pipetted between the plates, where the drop is held by surface tension. This is the principle idea of the patent application [1].

Each of the two plates acts as a frame for an X-ray transparent silicon-nitride window, through which the sample can be investigated. This prototype was developed for and tested in Small Angle X-Ray Scattering (SAXS) experiments; however, the basic concept is also applicable after modification to other light and X-ray based methods, such as UV-vis, X-ray diffraction, Absorption Spectroscopy, X-ray Fluorescence. The distance between the plates can be adjusted according to the requirements of the sample and experiment, so that a defined and constant sample thickness is guaranteed over the duration of the experiment session.

While the sample is still transferred in and out of the pipette, the distance along which it is pumped is significantly smaller than in systems employing tubing to move the sample to the measurement position. Dispensing the sample directly in the observation area minimises the chance of sample modifications and avoids the introduction of bubbles in the tubings.

After the measurement, the sample is washed away and the cell is ready, cleaned and dried. The sample cell containing the plates is equipped with connections for both water/cleaning solution and dry air, allowing the automatisation of the cleaning process. After the entire cycle, the sample holder is ready to receive the next sample for measurement.

1.2 The automatic sample changer

The Microdrop sample holder cell is combined with a commercial robotic arm equipped with pipetting hardware (Cavro Omni Robot, Tecan Group Ltd., CH) to take small sample volumes from standard multiwell plates and place the sample drops between the silicon plates. Disposable tips are used, to avoid contamination between samples. Pumps and valve controllers for the cleaning procedure are connected to the sample cell. A software package was developed to control the robotic arm, the pumps and valves, and can be integrated into existing beamline software or other instrument software to completely automatize the measurement process.

An autonomous sample changer is perfect for experimental runs with large numbers of similar samples, such as dilution series, titration, protein investigations etc. Moreover, it enables a more efficient use of the experimental time through avoiding manual sample loading. Table 1 lists the performance characteristics of several other automatic sampling systems [2-6], as well as of our Microdrop system for comparison.

Table 1: Comparison of sample changer systems.

Sample Changer	Volume	Time	Type
BioSAXS [2]	5-250 µL	50 s	quartz capillary
Soleil [3]	10-50 µL	240 s	glass capillary, SEC-SAXS
Berkeley [4]	24-30 µL	140 s	pipetted to wells, mica windows
Stanford [5]	20-30 µL	180 s	quartz capillary
AustralSynch [6]	100 µL	420 s	quartz capillary, SEC-SAXS
Microdrop	**5-20 µL**	**40 s**	**parallel SiNx windows**

2. Design specification

We developed the prototype of the Microdrop system, including the design and construction of the observation cell holder and the integration of the sample changing robot, for the use at the Austrian SAXS beamline [7] at the Elettra-Sincrotrone Trieste (Italy). The pilot experiments to examine

prototype reliability were also conducted there and consisted of measurement series of different standard proteins.

2.1 The prototype

The system we developed allows for the automatic placement of samples into the measurement cell via a robotic arm. The arm is equipped with a pipetting mechanism (seen in Fig. 1A) capable of using disposable tips to transfer samples from up to five 96-well plates. The well plates can be temperature controlled by dedicated heating /cooling modules (INHECO GmbH, DE) one of which includes a shaker to keep larger aggregates or dispersions stable in solution. The instrument can automatically detect the liquid level inside the wells, deliver and inject a predefined sample volume into the measurement cell.

The central part of the Microdrop is a set of two rectangular silicon frames, 5 mm wide and 3 mm high. Each frame has a window made from 2 µm thick silicon-nitride with a dimension of 3 mm wide and 1 mm high in its centre. Frames and windows were produced by CNR-IOM. The silicon frames are glued onto metal cylinders which are screwed into the outer cell, such that the cylinders can slide in and out. Thus, the distance between the windows can be changed adjusting the mounting screws. The optimal window distance defines the optimal sample volume, which lies in the range of 5-20 µL, and depends on the characteristics of the sample.

The outer cell for the first prototype was firstly 3D-printed and recently built from metal (Fig. 2). The cell has holes in the front and the back to receive the window holding cylinders. On the top side it has a hole for inserting the pipette tip to place the sample, as well as tubing connectors for cleaning and drying. There are openings on one side for an endoscopic camera to observe the drop placement and a second air stream to reliably dry the windows. On the other side, there is a wider opening to release the drying air, a funnel to collect the liquid waste and LEDs to provide background illumination to improve drop visibility. Holes at the bottom can be used to fix the cell with screws to the sample stage of the beamline. The cell temperature can be controlled by a water circuit, and in the future will be connected to the beamline vacuum path to reduce air scattering.

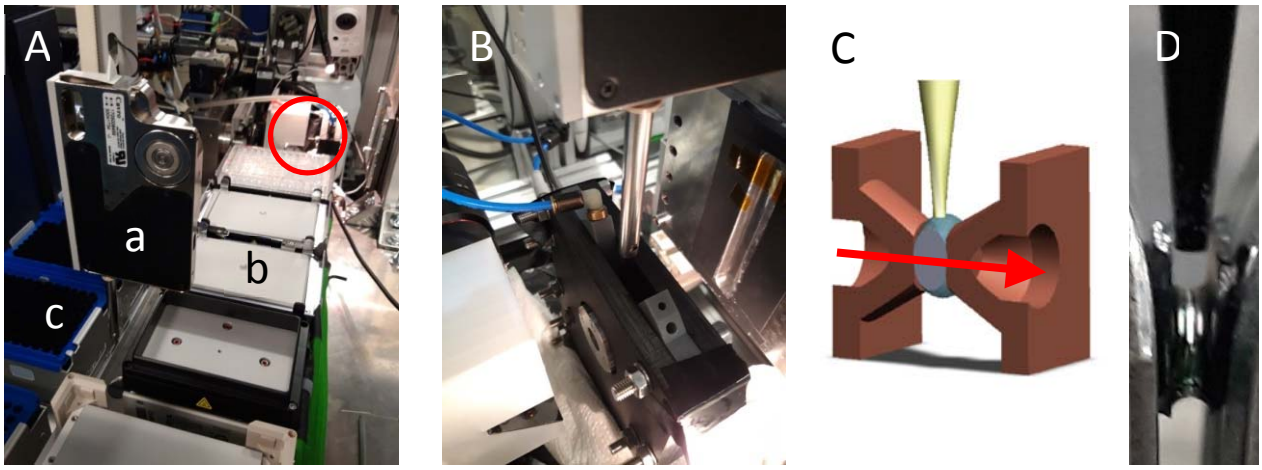

Figure 1: Overview of the Microdrop sample changer. (A) The robotic arm (a) is shown in the waste disposal position dropping a used tip. Up to five 96-well plate of samples (b) can be loaded at once (here only one is loaded). An equal number of fresh tips (c) are placed in the tip holders. The measurement cell is visible in the background, highlighted by a red circle. (B) The 3D-printed prototype of the measurement cell with the robotic arm coming from the top placing a drop. (C) Sketch of the working principle of the Microdrop cell: a drop of sample is suspended by surface tension between two observation windows made of X-ray transparent silicon-

nitride. (D) An image from an external test setup for the windows, selected for better visibility than images from inside the cell. The sample droplet between the silicon windows and the pipette tip can be seen.

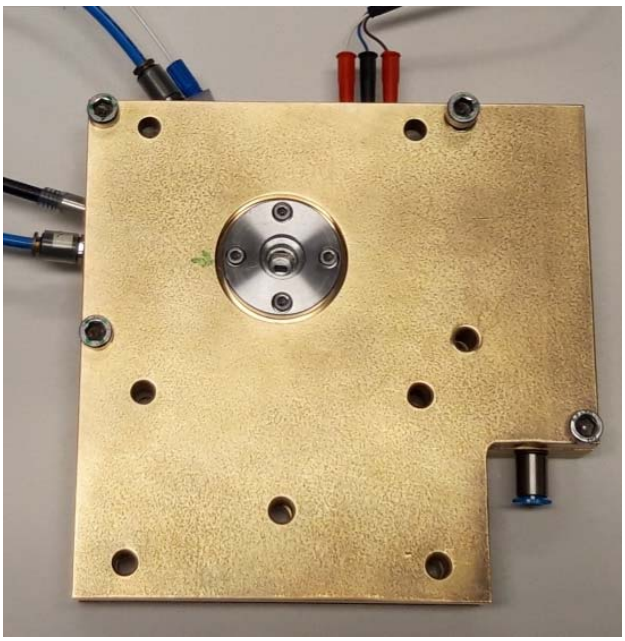

Figure 2: The final prototype cell made from brass. One of the cylinders holding the silicon-nitride windows is seen in the centre. Connections are visible for (clockwise from the left) the first drying air stream, the camera for internal imaging, the cleaning fluids, the second drying air, the power for the internal lighting and the connection for the waste drain tube.

The robotic arm takes the predefined sample volume from the sample storage, moves it to the measurement cell and dispenses it between the silicon plates, where it is held by surface tension (Fig. 1C). The X-ray beam illuminates the suspended sample and the scattering pattern is recorded by a Pilatus3 1M detector (Dectris, Switzerland). The maximum angle for a centred beam at 1 mm window distance is limited by the window aperture, and is ~14°. It can be increased by placing the beam off-centre or by reducing the window distance, and can be set up to ~63° by rotating the windows by 90° and using an off-centre beam. This configuration allows for simultaneous small- and wide angle X-ray scattering measurements.

Once the sample has been measured, the windows are washed and dried. For the cleaning cycle, the cell is connected via tubing to a peristaltic pump and an assembly of valves, controlled by an Arduino microcontroller. This provides the user to control up to three different cleaning solutions at once and individual control of two air streams. Meanwhile, the robotic arm prepares the placement of the next sample. Decoupling the cleaning and the sample loading reduces the cycling time below 40 seconds per sample, excluding the measurement time. The system can process up to 480 samples, allowing for the sample changer to run for full eight hours without intervention, assuming a measurement time of 20 seconds per sample.

2.2 The control software

The software to control the sample changer was written in LabView in order to integrate it into the existing beamline control system. The software allows to define *a-priori* and load entire measurement runs, enabling users to send samples for rapid measurement by the beamline staff. It is also possible to rapidly create and change sample sequences, permitting to quickly adjust the experimental schedule based on findings from preliminary measurements.

Figure 3 shows the most important general user interface screens of the sample changer. The Main Control Panel (A) displays the state of the instruments and information of the currently executed measurement. It is kept simple, having controls only to start, stop and abort a measurement run, to exit the program, switch to the Sample Manager (G) and to open the setup panel (B). The different parts of the instrumentation can be (re-)initialized from the setup panel, and several sub-programs can be accessed. The low level controls are available for the robotic arm (E) and the Arduino board (D), which controls the valves, the pump and the lighting. The alignment assistant (C) should be run every time the instrument has been set up to confirm the position of sample plates and tips. There is also an assistant for creating a new cleaning procedure (F), should the default one not work satisfactory. Most of these functionalities are only important for the beamline staff; the users would only use the main panel (A) and the sample manger (G) to start and monitor their measurement runs.

The main function of the Sample Manager is to store and display the information and define the parameters of the individual measurements. Both the sample and the measurement information can be loaded from a separated file that can be produced in any spreadsheet program. Templates will be provided to users prior to their beamtime.

The sample manager has in its centre a representation of a well plate, which can be clicked on to display and modify sample information (shown in the panel on the left). In the panel on the right, either the list of all the completed or pending measurements (“Done” or “Queue” menu, respectively) can be inspected. A temporary list (“Selected” menu) is also available to create new measurement runs before actually adding them to the “Queue”

New measurements can be added by double clicking the respective sample in the well-plate display. Pressing the “Upload” button appends the entire “Selected” list to the “Queue”, and the measurements will be processed by the sample changer.

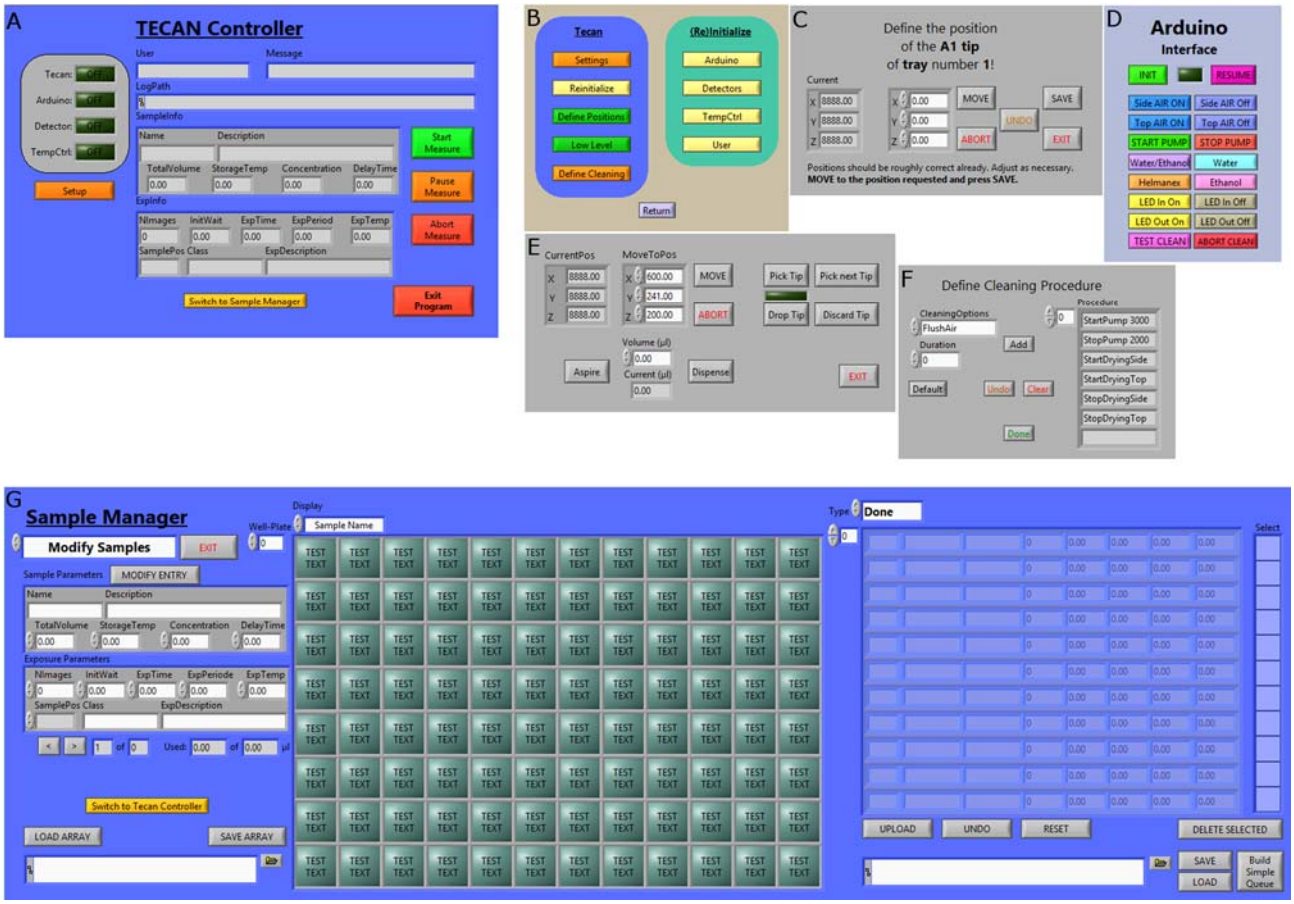

Figure 3: The different interfaces to control the sample changer. (A) The main control panel, (B) the selection window for the various sub programs, (C) the alignment assistant, (D) the low-level panel for the Arduino board controlling pump and valves , (E) the low-level control of the robotic arm, (F) the cleaning procedure creation assistant and (G) the sample manager.

2.3 The experiments

Experiments were conducted to assess the reliability of the drop placement, the efficiency of the cleaning procedure and the quality of the data measured with the Microdrop holder.

The SAXS beamline [7] was operating with an electron energy of 8 keV and a beam size of 2 x 0.4 mm^2 (determined with knife edge scans). The frame, housing the robotic arm, the cleaning system, the sample plate, the heating units and the reservoir for the new tips, was mounted at the beamline. The Microdrop sample cell was assembled, mounted on the experimental stage and connected to the tubing for cleaning and drying. The cell was placed directly downstream of the ionization chamber, aligned to the X-ray beam and the robotic arm was calibrated and aligned to the cell. The sample to detector distance was selected in order to have a q-range of 0.08 to 5.8 nm^{-1}. The program Fit2D [8] was used to average the detector images into 1D scattering patterns.

We observed that for increased window distance (e.g. >1.2 mm for water), the drop placement becomes increasingly unreliable as the drops fail to stick between the windows. Another critical factor is the precision of the placement of the pipette tip above the windows, which becomes more difficult with decreasing window distance. Due to the robotic arm tip placement variation of ±0.5 mm, we added an additional tip alignment mechanism to the cell, in order to guarantee precise drop placement. We found window distances in the range from 0.5 – 1 mm to be the most reliable. We

worked with a window distance of 0.6 mm and a sample volume of 11 µL for the subsequent measurements.

As a cleaning procedure, we defined a sequence of flushing with water to remove the sample, flushing with ethanol to improve the removal of organic materials and rinsing with water for several seconds to guarantee removal of the ethanol. More aggressive cleaning using Hellmanex™ (Sigma-Aldrich) is not necessary, except for very highly concentrated proteins (observed at >90 mg/mL). Hellmanex in particular was found to modify the surface of the silicon-nitride and inhibit the adhesion of the drop to the sample cell.

We measured dilution series of bovine serum albumin (BSA) dissolved in HEPES (50 mM, pH 7.5) and lysozyme dissolved in Tris HCl (100 mM, pH 8), (all reagents were purchased from Sigma-Aldrich). The samples were prepared on site and their effective concentration was determined with a Cary60 spectrophotometer (Agilent Technologies, Santa Clara, CA), measuring the UV absorbance at 280 nm. The concentrations were 2, 1, 0.5, 0.3, 0.25, 0.17, 0.15 and 0.1 mg/mL for the BSA and 4, 2.4, 1, 0.5, 0.21 and 0.11 mg/mL for the lysozyme. Repeated SAXS measurement were performed for each concentration (eight for the BSA, 12 for the lysozyme), with a buffer measurement preceding each sample measurement. For each measurement, ten detector images were collected and averaged after checking for the emergence of radiation damage in the sample. Poorly placed drops were identified by searching the scattering patterns for clear outliers and those data were discarded. The scattering patterns were normalised for fluctuations of the primary beam intensity and transmission using the signal of a photodiode placed on the beamstop. Then the respective backgrounds were subtracted from each sample. The resulting scattering patterns were calibrated to absolute scattering units by rescaling the forward intensity, I(0), of the highest concentrated lysozyme measurement (4 mg/mL) to the literature value [9].

3. Results

3.1 Minimum volume and stability

We used sample drops of 11 µL, which is the maximum volume that could be placed between the windows without overflowing. This was done to get the best possible data, since lower sample volumes could lead to partially covered windows and consequently edge effects in the scattering pattern. Subsequent analysis of the minimum volume tests showed that with a window distance of 0.6 mm, drops as small as 7 µL could be placed reliably (Fig.4) to give quality and reproducible data. We measured varying volumes of water several times and examined the stability of the measured signal in order examine the volume dependence on the repeatability. The number of measurements per volume was 50 for the 10 µL and ten for the other drop sizes. Misplacement impacts the lowest volume (<4µL), because the drop is not placed between the windows. In all other cases a misplaced drop can be measured; however, the volume is not enough to fully cover the entire window, leading to scattering patterns unsuitable for a correct background subtraction.

The reuse of the pipette tips was found to slightly reduce the placement reliability. New tips were used for the protein measurements. Another significant influence on the reliability was the stability of the well plate placement. We affixed the well plates to the thermostatic module by the provided clamping mechanism and covered with a protective foil to avoid sample evaporation. We found out that the well plate moves slightly as the robotic arm punctures the foil and pulls out afterwards. This misplaces the sample within the well causing parts of it to stick to the cover foil, and leads to an

increased number of cases where the sample is not drawn up correctly (almost 10%). As a temporary solution to this problem the well plates were fixed with tape to the thermostatic module. The final version will be adapted to involve a more elegant solution. The misplacement rate using new tips and taped well plates was ultimately 0.85%.

It should be noted that since not all sample can be taken from the sample wells, the actual minimal volume required for measurements with this instrument is higher than the minimum volume needed for measurement. To facilitate correct acquisition of the set sample volume, a slight surplus has to be present in the sample well, which is dependent on the type of well plate used. Generally, tapered well requires approximately 5 µL more, while a flat-bottomed well may require 20 µL more, to allow at least one successful measurement.

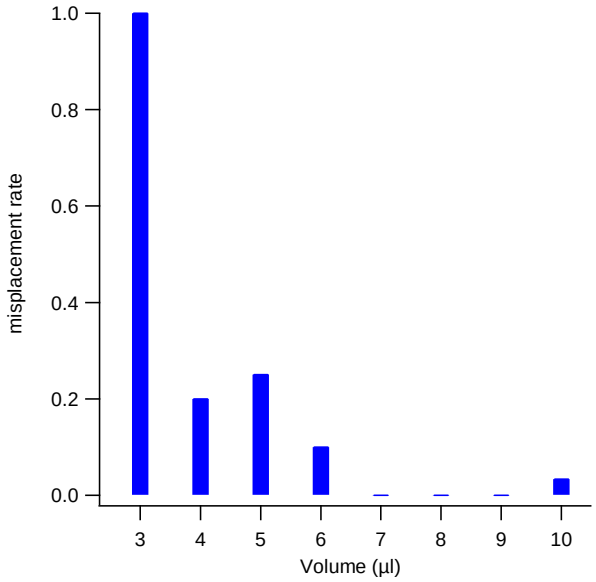

Figure 4: Misplacement rate (misplaced drops/number of tries) for different volumes at a window distance of 0.6 mm. At volumes below 7 µL drops often don't cover the whole window anymore. At 3 µl the volume is too low even to be placed between the windows.

3.2 Repeatability and precision

The results of the BSA and lysozyme measurements are shown in Figures 5 and 6, respectively. Repeated measurements of the same concentration are highly reproducible. The scattering patterns for each concentration were averaged and a Guinier fit was performed ($q=0.478$ nm^{-1} to $q=0.697$ nm^{-1} for the BSA and $q=0.298$ nm^{-1} to $q=1.998$ nm^{-1} for the lysozyme) to extract the radius of gyration, R_g, and the forward intensity, $I(0)$. The found values for R_g and $I(0)$ are listed in Table 2 and 3. The values are very consistent between different measurements and concentrations. The R_g of the BSA is virtually the same as the value of 2.99±0.08 nm given in [9]. The R_g of the lysozyme deviates from the value of 1.43±0.04 nm reported in [9], but is consistent over all measurements so that we attribute this deviation to the influence of the different buffer composition [10], rather than to a measurement error. The found concentration/absolute scattering deviates slightly from the value expected from the UV-vis measurement; however, the UV-vis measurement itself is increasingly inaccurate for lower concentrations. A comparison is shown in Fig. 7.

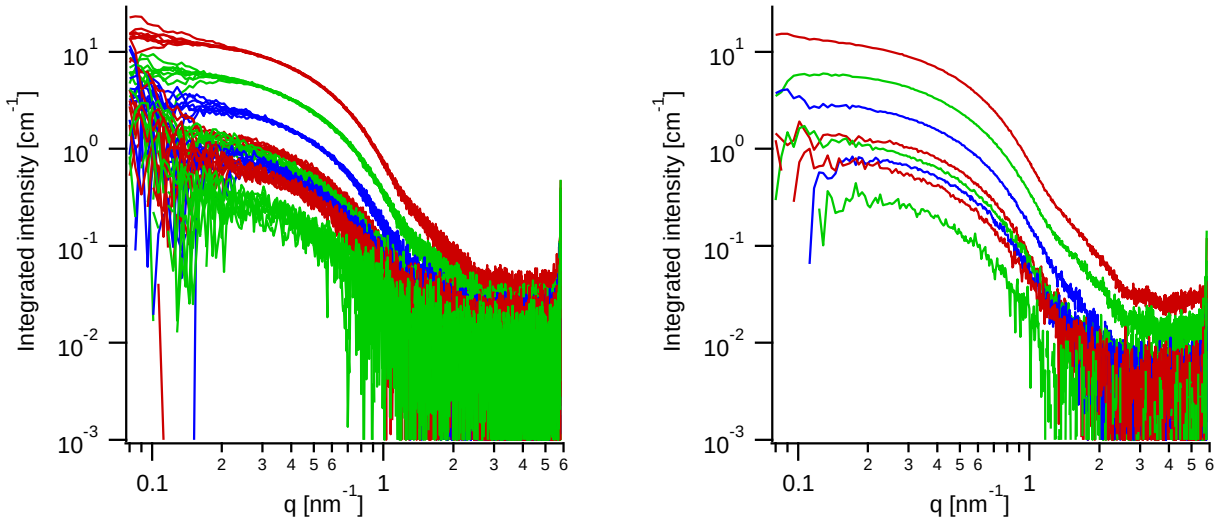

Figure 5: Scattering patterns of the BSA measurements (left) and their respective average (right). The concentrations are, top to bottom: 2.0, 1.0, 0.5, 0.3, 0.25, 0.17, 0.15 and 0.1 mg/mL.

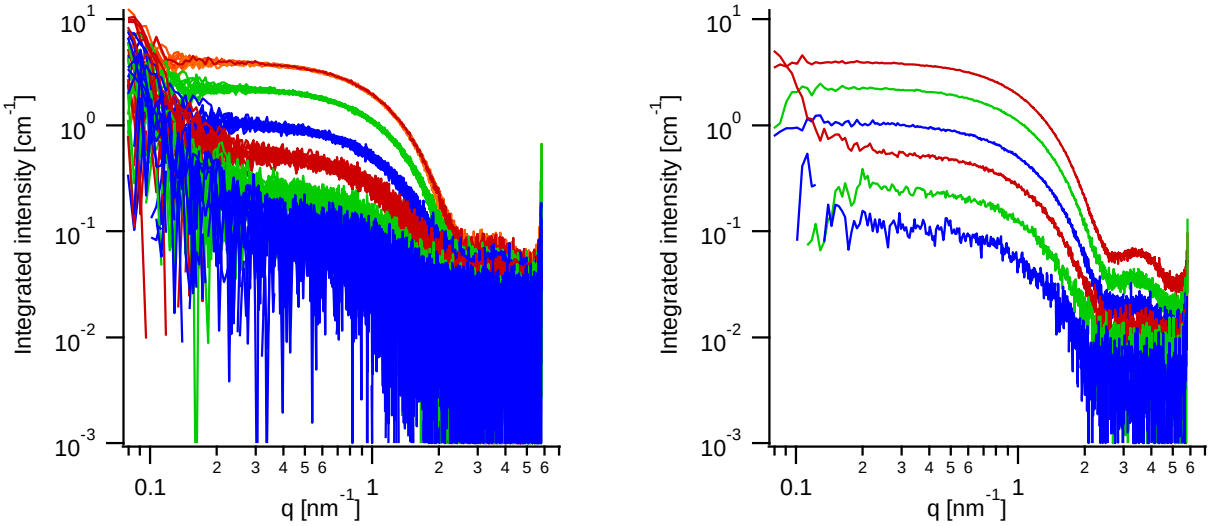

Figure 6: Scattering patterns of the lysozyme measurements (left) and their respective average (right). The concentrations are, top to bottom: 4.0, 2.4, 1.0, 0.5, 0.21 and 0.11 mg/mL.

Table 2: Comparison of parameters extracted from the BSA measurements

Concentration [mg/mL]	Rg [nm]	Iabsolute(0) [cm-1]	Iliterature(0) [cm-1]
2 ± 0.05	2.997 ± 0.011	10.617 ± 0.084	9.68 ± 0.26
1 ± 0.05	2.989 ± 0.017	5.003 ± 0.063	4.84 ± 0.13
0.5 ± 0.05	3.012 ± 0.033	2.432 ± 0.051	2.42 ± 0.065
0.3 ± 0.05	3.023 ± 0.068	1.262 ± 0.056	1.452 ± 0.039
0.25 ± 0.05	2.996 ± 0.080	0.978 ± 0.053	1.210 ± 0.033
0.17 ± 0.05	2.931 ± 0.103	0.780 ± 0.054	0.823 ± 0.022
0.15 ± 0.05	2.963 ± 0.143	0.622 ± 0.054	0.726 ± 0.020
0.1 ± 0.05	2.989 ± 0.279	0.287 ± 0.051	0.484 ± 0.013

Table 3: Comparison of parameters extracted from the lysozyme measurements

Concentration [mg/mL]	Rg [nm]	Iabsolute(0) [cm-1]	Iliterature(0) [cm-1]
4 ± 0.05	1.494 ± 0.003	4.093 ± 0.010	4.12 ± 0.24
2.4 ± 0.05	1.498 ± 0.003	2.323 ± 0.008	2.472 ± 0.144
1 ± 0.05	1.494 ± 0.007	1.062 ± 0.009	1.03 ± 0.06
0.5 ± 0.05	1.499 ± 0.017	0.571 ± 0.008	0.515 ± 0.030
0.21 ± 0.05	1.477 ± 0.018	0.260 ± 0.009	0.216 ± 0.013
0.11 ± 0.05	1.527 ± 0.088	0.117 ± 0.008	0.113 ± 0.007

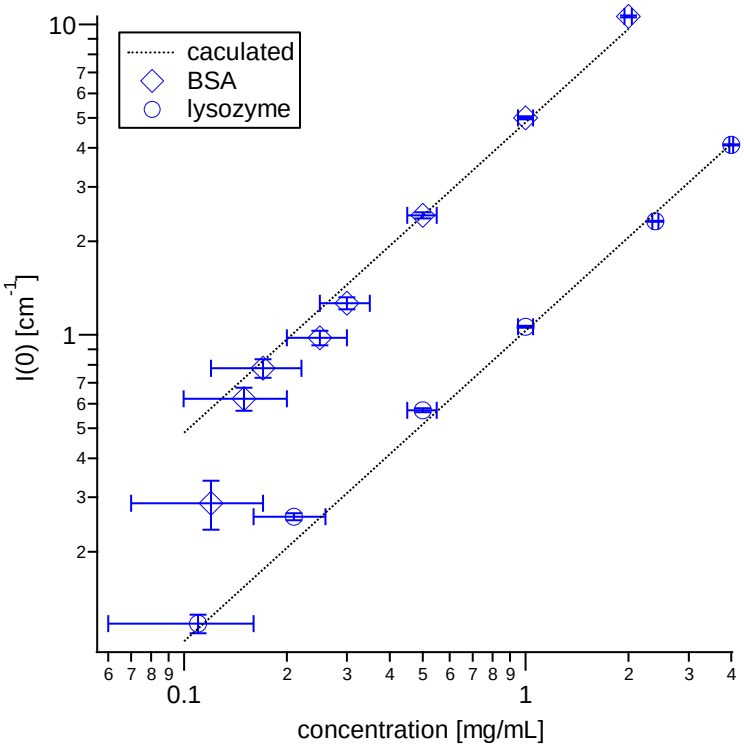

Figure 7: The measured, absolute forward intensities, I(0), of the different concentrations of both proteins, plotted against their concentration measured with UV-vis. The dotted lines are the expected values calculated from the absolute scattering in the forward direction, I(0), given in [9].

4. Conclusions and perspectives

A Microdrop sample holder has been developed and tested to provide a novel way of sample placement. The advantage of the instrument is the circumvention of induced sample modifications that occur in conventional capillary based sample holders due to high surface to volume ratio when the sample is pumped through the loading circuit and measurement capillary. The Microdrop holder has been combined with a robotic arm and other equipment to produce a fully automatic sample

changer. The system has been tested and improved over several iterations resulting in a simple, fast and reliable operation.

Based on the obtained results, the sample placement is reliable and measurements with the Microdrop holder give repeatable results of high quality. The system allows fast measurements of minute sample volumes providing good results even with very low concentrations. This makes it a valuable tool for the investigation of biological samples such as proteins.

The prototype presented here is already available at the Austrian SAXS beamline. The first usage of the system with external users will be conducted in March-April 2019.

As a next step to further improve its capabilities, a new sample cell made of metal has been constructed tested. This will enable control of the sample temperature, adding an additional feature. Furthermore, this sample cell will be directly connected to the vacuum path of the beamline, significantly reducing the background of the set-up by avoiding air scattering.

References

[1] H. Amenitsch, B. Marmiroli, P. Laggner, US PATENT 9164047: *Apparatus and method for supporting a liquid sample for measuring scattering of electromagnetic radiation* (2015)

[2] A. Round, F. Felisaz, L. Fodinger, A. Gobbo, J. Huet, C. Villard, C. E. Blanchet, P. Pernot, S. McSweeney, M. Roessle, D. I. Svergun & F. Cipriani, *Acta Cryst.* **D71** (2015) 67-75, DOI: 10.1107/S1399004714026959

[3] G. David & J. Perez, *J. Appl. Cryst.* **42** (2009) 892-900, DOI: 10.1107/S0021889809029288

[4] S. Classen, G. L. Hura, J. M. Holton, R. P. Rambo, I. Rodic, P. J. McGuire, K. Dyer, M. Hammel, G. Meigs, K. A. Frankel & J. A. Tainer, *J. Appl. Cryst.* **46** (2013) 1-13, DOI: 10.1107/S0021889812048698

[5] A. Martel, P. Liu, T. M. Weiss, M. Niebuhr. & H. Tsuruta, *J. Synchrotron Rad.* **19** (2012) 431-434, DOI: 10.1107/S0909049512008072

[6] T. M. Ryan, J. Trewhella, J. M. Murphy, J. R. Keown, L. Casey, F. G. Pearce, D. C. Goldstone, K. Chen, Z. Luo, B. Kobe, C. A. McDevitt, S. A. Watkin, A. M. Hawley, S. T. Mudie, V. Samardzic Boban & N. Kirby, *J. Appl. Cryst.* **51** (2018) 97-111, DOI: 10.1107/S1600576717017101

[7] H. Amenitsch, M. Rappolt, M. Kriechbaum, H. Mio, P. Laggner and S. Bernstorff, *J. Synchrotron Radiat.* **5** (1998) 506, DOI: 10.1107/S090904959800137X.

[8] Hammersley, P. *Fit2D Reference Manual V3.1.* (1998) Internal Report ESRF98HA01T. ESRF, Grenoble, France.

[9] E. Mylonas & D. I. Svergun, *J. Appl. Cryst.* **40** (2007), 245-249, DOI: 10.1107/S002188980700252X.

[10] M. Hirai, Y. Hagiwara, K. Takeuchi, R. Kimura, T. Onai, Teruaki, R Hirai, N Tohta & M. Sugiyama, (2012), *Thermochimica Acta* **532** (2011) 15–21, DOI: 10.1016/j.tca.2011.11.019.