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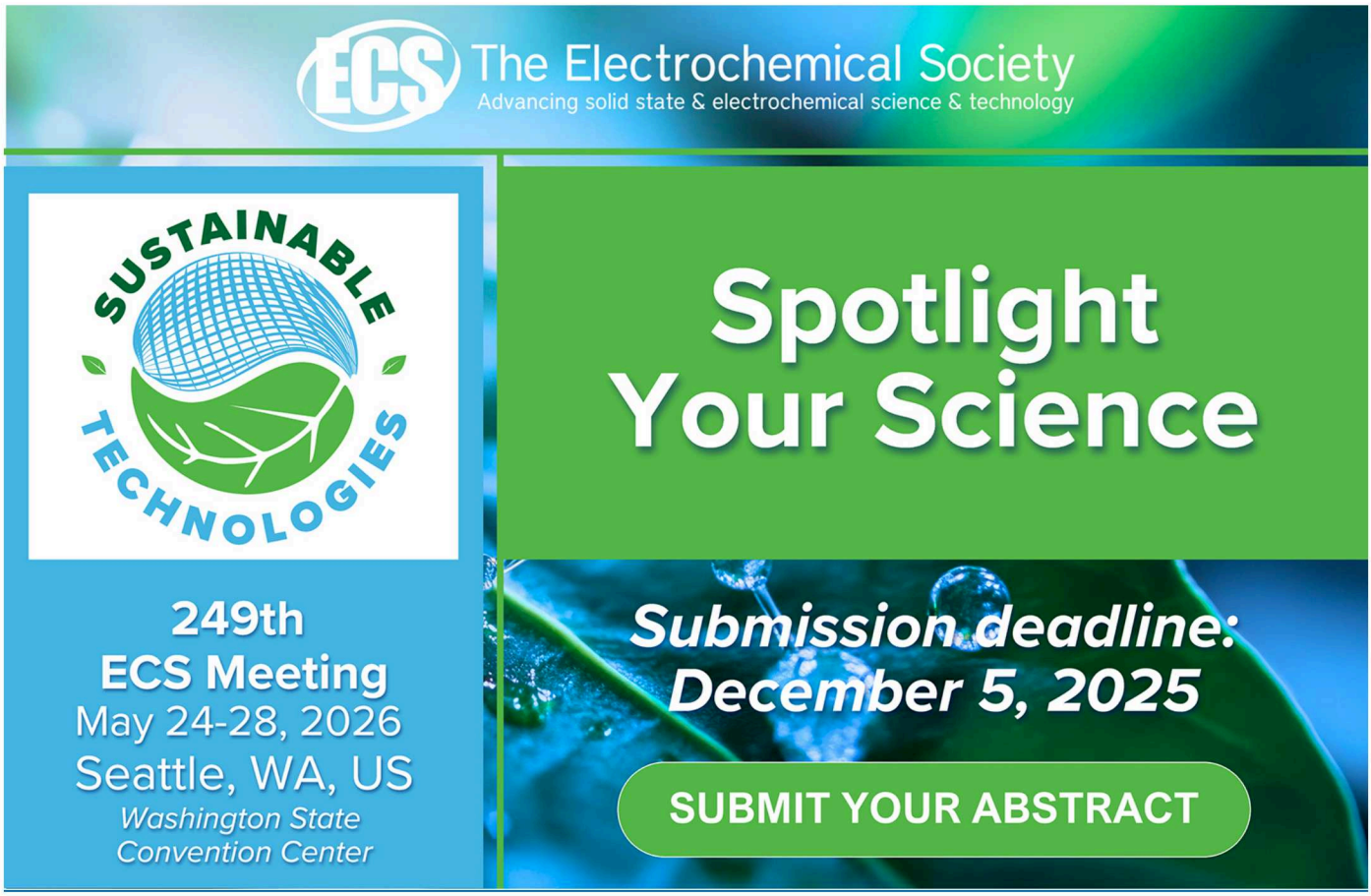

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A Versatile Sample Chamber for BioSAXS: Integration and Applications on the P12 Beamline

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Abstract. We present the integration of a sample chamber for advanced BioSAXS measurement on the P12 beamline of EMBL Hamburg. The sample chamber is designed to be installed in the beamline P12 at PETRA and BM29 at ESRF with minimal effort to swap the standard sample environment, allowing for a fast and efficient beamline configuration change. The new sample chamber is equipped with an on-axis microscope, including front and back illumination, for sample visualization; an XYZ piezo stage for fine alignment of sample into the beam; an optical port for in situ spectroscopy; and fluidics feedthroughs. This sample environment has been fully integrated on the P12 beamline and employed in users project for scanning SAXS and in vacuum microfluidics applications.

1. Introduction

Over the last few decades, several beamlines have been developed and optimized specifically for measuring SAXS signals from biological molecules in solution. These efforts focus on minimizing instrument background to detect the low-intensity signals from biological samples, whose electron densities are only slightly higher than that of the surrounding buffer, resulting in weak contrast and low signal. This optimization often includes fully in-vacuum setups, vacuum flow-through cells, and scatterless slits.

Additionally, specialized sample environments are required to handle the fragile and often limited quantities of biological samples. Most BioSAXS beamlines are equipped with sample changers, in-situ purification, and spectroscopic characterization tools. For example, the dedicated sample environments at P12 (PETRA III, Hamburg, Germany) [1] and BM29 (ESRF, Grenoble, France) [2] consist of in-vacuum, temperature-controlled, flow-through capillary sample chambers. These chambers can be connected either to a liquid handling robot [3] or a size-exclusion chromatography system [4].

While these specialized setups have improved the quality and robustness of experiments, automated measurements, and increased throughput, they significantly limit the range of experiments that can be performed. Non-standard experiments often require a complete rebuilding of the sample environment.

The sample chamber presented here aims to overcome these limitations. It can be installed on the beamline with minimal modifications to the existing beamline configuration and is versatile enough to accommodate different types of experiments, such as in-situ microfluidics or scanning SAXS. It is equipped with a piezo stage for precise sample alignment and an on-axis microscope with on-axis and backlighting to provide a direct view of the sample in the beam direction. The chamber also features fluidic feedthroughs for in-vacuum microfluidic experiments, ports for optical fibers for in-situ illumination or spectroscopy, and customizable walls that can be exchanged for specific experimental needs.

2. Sample chamber description

2.1 Vacuum chamber

The vacuum chamber is designed for easy exchange with the standard chambers used at beamlines BM29 and P12 (Figure 1). To replace the chamber, the capillary tubes are disconnected, and the chamber is removed by unscrewing four bolts. Other components of the sample environment, such as the auto-sampler, remain in place without modification. The chamber's front and bottom walls consist of removable plates that can be customized as needed.

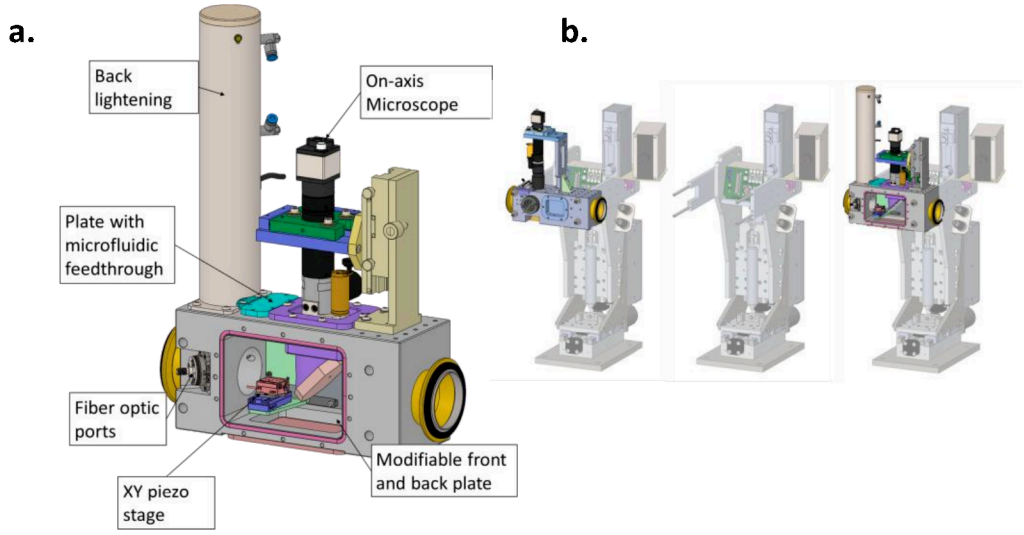

Figure 1. *(a)* CAD model of the vacuum sample chamber, showing its different components. *(b)* Replacement of the standard sample chamber with the versatile sample chamber.

2.2 On-axis microscope and illumination:

The on-axis microscope consists of a camera and an objective lens aligned with a 45-degree mirror with a central hole to allow the incoming X-ray beam to pass through. The microscope is focused on the sample to provide a magnified view (0.90X).

The sample can be illuminated using either front or back illumination. Front illumination is provided through a light guide port in the camera objective, but it offers less control over unwanted reflections. Back illumination, which reduces reflections and provides clearer images, is achieved with a light source and a diffusive screen positioned behind the sample.

The diffusive screen can be moved in and out of the X-ray beam path. Since the screen must be positioned directly behind the sample (in the X-ray trajectory), back illumination is not possible during X-ray measurements. The screen is retracted from the beam path during data collection.

2.3 Piezo positioning stages

The sample is mounted on vacuum-compatible piezo motors (combination of Physik Instrument Q-545 (z-axis) and Q521 (x and y axis) linear stages), which allow precise positioning in all directions: within the plane perpendicular to the beam for alignment (13mm x 32mm) and along the beam path (12mm) to accommodate various experimental setups. The piezo motors provide high precision (sub-micron accuracy) and can handle a load of up to 500g.

2.4 Optics and fluidics feedthrough

The sample chamber is equipped with fiber optic ports (Thorlabs PAF2S-11A) that can be used for illuminating the sample, for example, in light-triggered experiments or in-situ spectroscopy, such as Dynamic Light Scattering (DLS) [5]. Fluidics feedthroughs, compatible with standard HPLC connectors (1/16”), are available on the top plate of the chamber. These feedthroughs connect the microfluidic chip, mounted on the piezo holder, to the microfluidic pump located outside the vacuum chamber.

3. Integration on the P12 Beamline

The control system for the sample chamber is structured into three layers. At the first layer, an E-873 servo controller (Physik Instrumente) provides nanometer-precision positioning to control the piezo stage axis. Additionally, a Beckhoff I/O system combined with TwinCAT 3 software actuates the focus motor and backlight.

At the mid-layer, a Device Server running on a Spectra PowerBox edge device integrates low-level signals with online data from a CMOS camera. This setup enables fast-feedback tasks such as focusing, achieved through locally executed image processing using gradient filters. The software was implemented using a plug-in architecture [6] and integrated into the TINE-distributed control system.

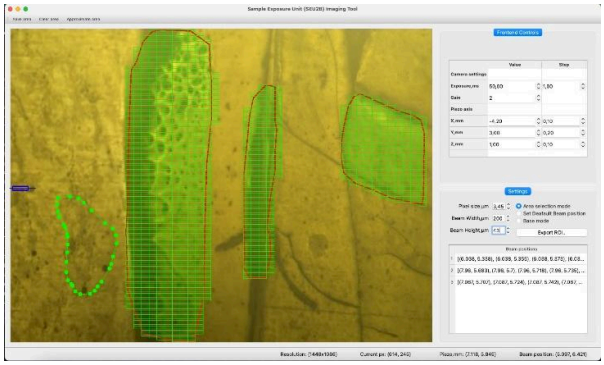

Figure 2. Imaging Tool user interface for real-time sample visualization and positioning, enabling freeform selection of scanning areas and precise piezo stage movements.

At the higher layer, BECQUEREL [7], a beamline control and data-acquisition software, provides supervisory control over all beamline device servers. The in-house developed Imaging Tool offers a real-time interface for sample visualization and positioning (figure 2), allowing users

to define scanning areas directly on images using freeform selection tools. This tool converts user-defined coordinates into piezo stage movements, optimizing scan precision and reducing data collection time by focusing only on relevant regions of interest.

4. Application

4.1 Scanning SAXS on bones

The chamber was tested with scanning SAXS measurements on bone slices to characterize various structural parameters, including hydroxyapatite (HAp) platelet size and the degree of orientation.

Sample originated from an animal experiments which was conducted at the Molecular Imaging North Competence Centre (MOIN CC) Kiel under the approval number: V 241–26850/2017(74-6/17)) in the framework of the BMBF project MgBone (Projektnummer 05K16CGB). The experimental and anaesthetic protocols are described in [8]. After a healing time of 20 weeks bone samples were extracted and embedded in Methyl methacrylate. Following this, the samples were cut in the centre and slices with a thickness of 10 µm were prepared by LLS Rowiak, Hanover. The samples were cut with a laser technique and transferred to 40 µm thick Kapton tape

The bone section was placed in a dedicated holder mounted on the piezo stage. The on-axis microscope was used to align the sample with the X-ray beam and to select the region of interest for scanning. The X-ray beam was collimated to a size of 50 x 50µm (FWHM), and the sample was raster-scanned in 50µm steps over a 2x2 mm area.

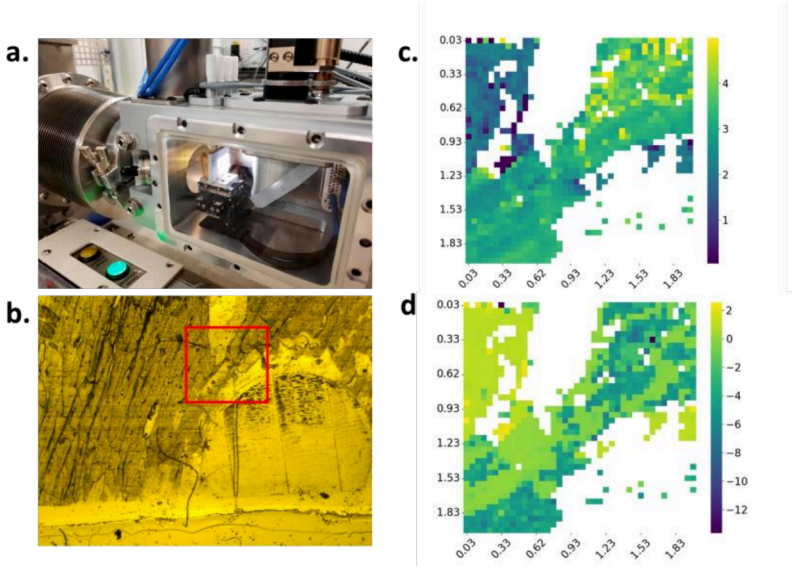

Figure 3. *(a)* Bone section mounted in the sample chamber. *(b)* Images from the on-axis microscope, with the scanned area indicated by the red square. *(c)* Map of the T-parameter (thickness of hydroxyapatite crystals). *(d)* Map of the κ-parameter describing the degree of ordering of the Hap/collagen matrix

The bone sample was analysed to determine the ordering of the HAp/collagen matrix and hydroxyapatite platelet size using the "Stack of Cards" model. A Matlab script was employed to fit the model function over a range from 0.026 to 3 nm^{-1}. Figures 3 c and d show the distribution of

the T-parameter (thickness of the hydroxyapatite crystal) and the parameter κ (ordering of Hap/collagen matrix), respectively.

The average T-parameter was 2.89 nm ± 0.75 nm. The analysis shows that the diagonal bone structure has an average platelet size of about 3 nm, while other regions, depending on the orientation, exhibit a smaller size of approximately 2 nm. Interestingly, both regions display different degrees of orientation, as indicated by the higher κ-parameter in areas with smaller platelet sizes.

This sample was initially provided for the test commissioning of the device. The chamber has since been successfully used in several projects to characterize bone regeneration.

4.2 Microfluidics SAXS.

The in-vacuum microfluidics SAXS capabilities were tested using a continuous flow mixer chip inspired by the design presented in [9]. This chip combines mixing and co-flow features, where diffusion allows a smaller component from the buffer solution to mix with the larger biomolecules in the sample solution while maintaining a buffer layer to minimize radiation.

This setup enables time-resolved experiments by measuring the SAXS patterns at different points along the capillary, which correspond to different reaction times. The buffer layer helps maintain sample integrity during measurements by reducing the effects of radiation damage and by preventing sample buildup on the cell wall [10].

The microfluidic chip was mounted on an adapter on the piezo stage and connected to a flow pump through the microfluidic feedthrough. The on-axis microscope was used to precisely align the chip with the X-ray beam.

Initial test measurements were conducted to verify the chip's performance in a vacuum environment, ensure its vacuum tightness, and evaluate the data quality. All fluidic lines were

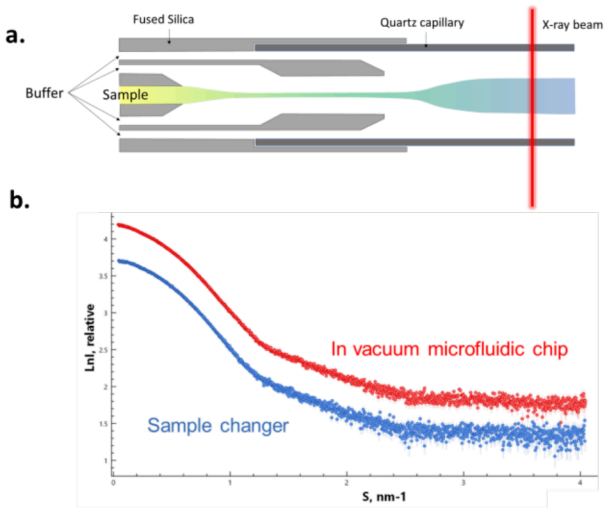

Figure 4. *(a)* Schematic of the microfluidics chip. *(b)* SAXS curves collected from 20 µL of BSA solution at 4 mg/mL using the in-vacuum microfluidic chip and the standard sample changer.

connected to the same sample solution, and the chip's co-flow and mixing features were not utilized during these tests.

Additional tests were conducted with a sample stream of 4mg/ml BSA and a sheath stream of buffer at flow rates of 5 µL/min for the sample and 10 µL/min for the sheath to maintain a sample stream diameter of 400 microns inside the 700 microns diameter tubing.

The data collected during the tests are shown in Figure 4, along with data obtained using the standard sample changer. Both curves were measured using 20 µL of BSA solution at a concentration of 4 mg/mL. Notably, the data from the microfluidic chip exhibit a similar noise level to that of the standard sample changer, indicating comparable performance.

These initial results are very promising for further optimization, demonstrating the potential advantages of using a microfluidic chip in a vacuum environment with low scattering background. Additional measurements with this setup are currently being conducted to monitor intermediate reaction states of anaerobic samples.

Conclusion

In this work, we have presented the integration of a versatile sample chamber for advanced BioSAXS measurements on the P12 beamline at EMBL Hamburg. The chamber was designed to be compatible with the existing infrastructure at beamlines P12 and BM29, enabling easy exchange with minimal modifications to the standard sample environment. It is equipped with an on-axis microscope, piezo positioning stages, optical ports for in-situ spectroscopy, and fluidics feedthroughs, providing a flexible platform for a variety of experimental setups.

The sample chamber has demonstrated its capabilities in scanning SAXS experiments, such as analyzing bone samples to determine collagen orientation and hydroxyapatite crystal size. It has also been successfully applied in in-vacuum microfluidics SAXS, allowing measurements with low scattering backgrounds. Initial tests have shown comparable performance to standard sample changers, highlighting the potential advantages of this versatile setup.

The flexibility and adaptability of this sample chamber make it a valuable addition to BioSAXS facilities, supporting both routine measurements and innovative experimental approaches.

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