	Experiment title: Fast scanning SAXS of living biological cells	**Experiment number**: SC5331
Beamline: ID13	**Date of experiment**: from: 04.11.2022 to: 07.11.2022	**Date of report**:
Shifts: 9	**Local contact(s)**: Manfred Burghammer, Jiliang Liu	*Received at ESRF:*

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Report:

Overview: The original aim of this experiment was to study living cells in aqueous environments by fast scanning small angle x-ray scattering (SAXS). However, due to delays in organizing the culture of our living cells at ESRF previous to the experiment, we turned to fixed-hydrated biological cells. As a study object, we chose SK8K18 cells. These cells contain a highly interesting, somewhat ordered network of keratin bundles as part of their cytoskeleton, which are believed to play an important role in determining the mechanical properties of the cells and the corresponding tissues. In order to move an important stop towards our ultimate goal, i.e., to investigate living cells in a close-to-physiological environment, we employed a specifically developed flow chamber that will eventually also host living cells. The flow chamber serves three purposes: first, cells are supplied with nutrients, and waste is flushed away during the (hour-long) scans. Second, the continuous flow of buffer or medium cools the sample slightly and this decreases damage by reducing the input energy on the sample. Third, radiation-induced gas bubble formation inside the sample chamber, as we previously observed them (in experiment SC5252), are diminished. Whereas purpose (1) is not relevant for fixed-hydrated cells and will only become important for living cells, purposes (2) and (3) were investigated during the beamtime. This implied the development of a custom-made flow-through-sample chamber while minimizing the background scattering by the liquid, in order to optimized the signal-to-noise ratio for our data.

Experimental setup and data collection: The cells were grown on silicon-rich nitride (Si_3N_4) windows with a total membrane area of 1.5 x 1.5 mm^2. Afterwards, they were chemically fixed with 3.7% formaldehyde and stored in phosphate buffered saline (PBS) for transport to the synchrotron. Just before scanning, the sample chamber was prepared by sandwiching two silicon-rich nitride windows, one with cells and the other one with a latex structure to guide the flow. To do so, the window containing the cells was placed on a dedicated 3D-printed frame (polyactic acid (PLA), figure 1b) with two pieces of tubing attached for the inlet and the outlet; next, the window with the latex guide was placed on the counter part of the 3D-printed frame. As the last step of the chamber assembly, we covered the top and bottom of this sandwich structure by a pair of metal frames with screw holes, which were then mechanically clamped to ensure that the appropriate level of pressure was evenly applied over the frame. A side view of the whole assembly is shown in figure 1a. The flow chamber was connected to microfluidic syringe pumps (Nemesys by Cetoni, supplied by Peter van der Linden/ESRF): one syringe was completely filled with degassed PBS for the inlet and the one was filled with around 0.5 mL of degassed PBS for the outlet (figure 1c). The flow rate of the inlet was set to 100 µL/h and for the outlet to -100

µL/h for a leakage test. After testing the chamber, we screwed the metal frame together, removed the clamp, and installed the flow-chamber in the beamline. The chamber was mounted on a 3D-printed carbon holder designed and manufactured on the spot by Lionel Lardière/ESRF, ID13. The experiment was carried out at EH III of ID13 using a microfocused beam (20 x 20 µm^2). An Eiger 4M detector (Dectris), which was placed 0.9 m away from the sample, was employed to collect the diffraction patterns. The in-line optical microscope was used to select single or multiple cells, which were then scanned in fast scanning mode with a step size between 0.5 and 2.0 µm in the horizontal and vertical directions, exposure times ranging from 3 to 10 ms, and flow rates ranging from 0 to 200 µL/h.

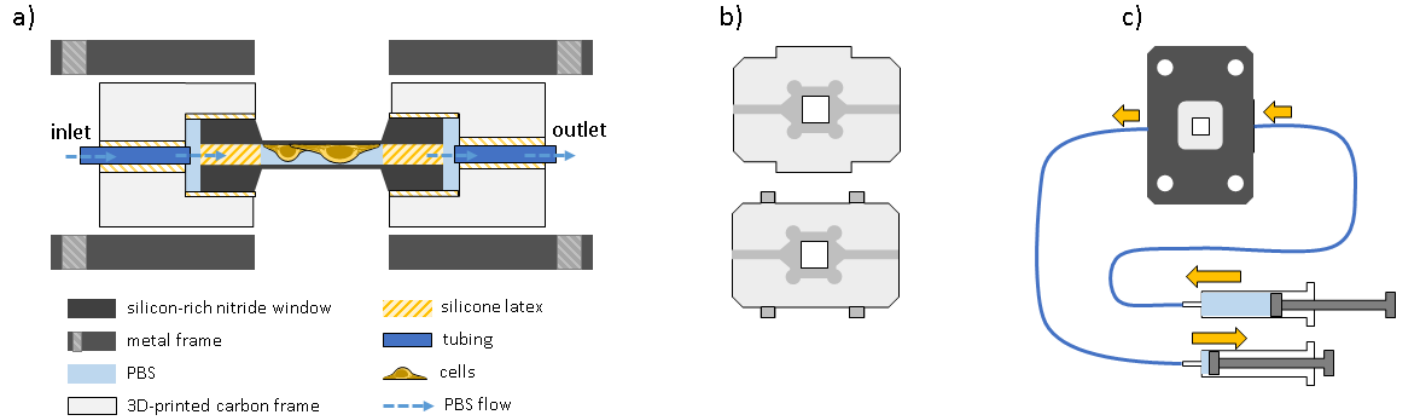

***Figure 1**: Schematic of the flow chamber. **a)** Side view of the sandwich structure after assembly. **b)** Schematic of the dedicated 3D-printed carbon frame. Both parts were coated with natural latex **c)** Simplified flow device setup. Syringe pumps are omitted in this representation.*

Results: We compared the wet chamber we previously developed (experiment SC5252) with the new flow chamber. Whereas in the wet chamber without flow radiation-induced gas bubbles formed, grew and persisted, thus interfering with the measurement, an instrumental advantage of the flow chamber is that by the continuous flow these bubbles can be flushed out. For each sample, we selected 6 to 12 scan regions where interesting cells or groups of cells were visible with the in-line microscope. Once scanning all regions in a single sample was completed, we carefully looked at the sample environment using the in-line microscope. When no gas interface was found in the microscope image, we repeated the scans several times to check for radiation damage on the sample. Figure 2a, b shows bright field microscopy images of two samples with and without flow, respectively, recorded after scanning. As a result, we found no gas formed inside the chamber (figure 2a), and small bubbles were captured in the outlet tubing and the syringe. By contrast, in the wet chamber without flow, a distinct liquid-air-interface formed and interfered with the data collection. In addition, the quality of the darkfield contrast images with flow present is increased, with much higher contrast, even after five consecutive scans (figure 2c), than the no-flow data (figure 2d). These results imply that the flow inside the chamber flushes out the radiation-induced gas and reduces the radiation damage to the cells. Further data analysis is still ongoing as changes in the structure of the recorded data on the ESRF side now forced us to adapt our in-house software for data analysis.

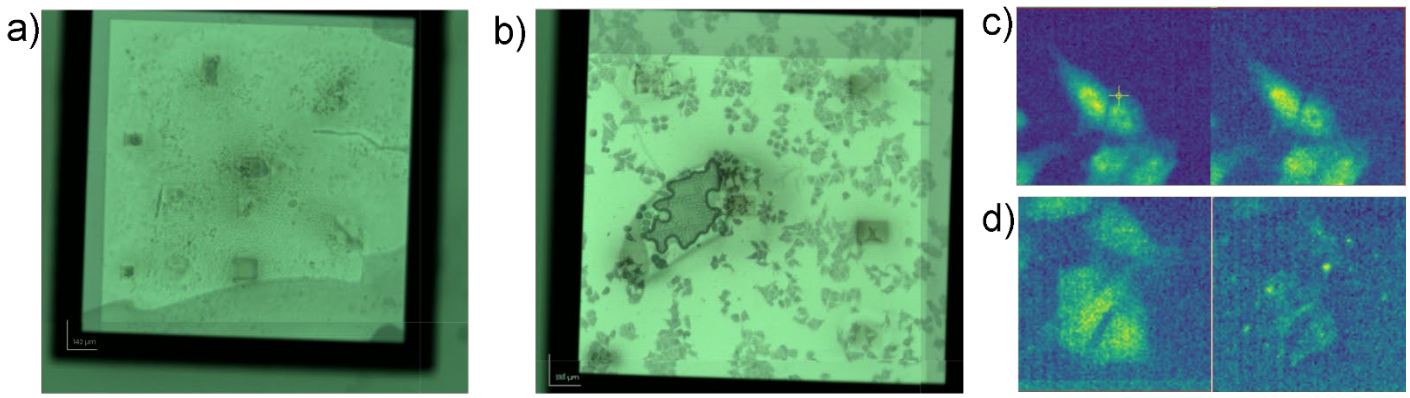

***Figure 2**: **a**, **b)** In-line bright-field microscopy images recorded after scanning SAXS with **a)** a flow of 25 to 50 µL/h, and **b)** no flow. **c,d)** Darkfield contrast images of biological cells: left hand side corresponds to the first scan of a series; right hand side corresponds to the fifth scan; **c)** flow rate 200 µL/h; **d)** no flow.*