	Experiment title: Fast scanning SAXS on hydrated biological cells	**Experiment number**: SC5252
Beamline: ID13	**Date of experiment**: from: 14.06.2022 to: 20.06.2022	**Date of report**:
Shifts: 12	**Local contact(s)**: Manfred Burghammer	*Received at ESRF:*

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Report:

Overview: This experiment aimed at applying fast scanning small angle x-ray scattering (SAXS) to fixed-hydrated cells. Scanning SAXS of freeze-dried cells is well established, whereas scanning SAXS of fixed-hydrated cells remains challenging because the cells have to be kept in an aqueous state during scanning. This implies the necessity of a custom-made sample chamber that maintains a liquid volume inside, while minimizing the background scattering by the liquid. Nevertheless, this challenge is worth overcoming: hydrated cells are closer to their physiological state than freeze-dried cells, and our approach represents a significant step forward in eventually collecting data of living cells.

Experimental setup and data collection: We used SK8/K18 cells, i.e., human epithelial cells expressing fluorescently tagged keratins. 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 buffer 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 20 nm thick SU-8 spacer (figure 1**a**). The thickness of the SU-8 spacer was chosen in accordance with the typical thickness of cells (about 20 µm), allowing for a sufficiently thick layer of PBS to wet the cells at all times while minimizing background scattering. The sample chamber was placed on one part of the dedicated 3D-printed plastic frame (figure 1**b**) with an indentation to hold the chamber inside. Before placing the sample chamber on the frame, vacuum grease was applied to the indentation, to seal around the chamber as well as the gaps between the window and the frame. After placing the chamber, the counterpart of the frame was placed on the top and screwed to close the chamber tightly. The side view of the assembled frame is in figure 1**a**. The edges of the assembled frame were covered with a plastic filament using a 3D "printing" pen. Screws were removed afterwards. The chamber was clamped on a 3D-printed carbon holder designed and manufactured on the spot by Lionel Lardière (ID13). The experiment was carried out at EH III of ID13 using a nanofocused beam (200 x 200 nm^2). An Eiger 4M detector (Dectris), which was placed 1.4 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 0.1 and 0.5 µm in the horizontal and vertical directions and exposure times ranging from 3 to 10 ms. Following the scanning experiment, the fluorescence microscope (Olympus BX61) in the PSCM lab was used to take fluorescence images with the assistance of Pierre Lloria (PSCM). These images enable us to investigate structural changes to the keratin bundles, that may be induced by radiation damage.

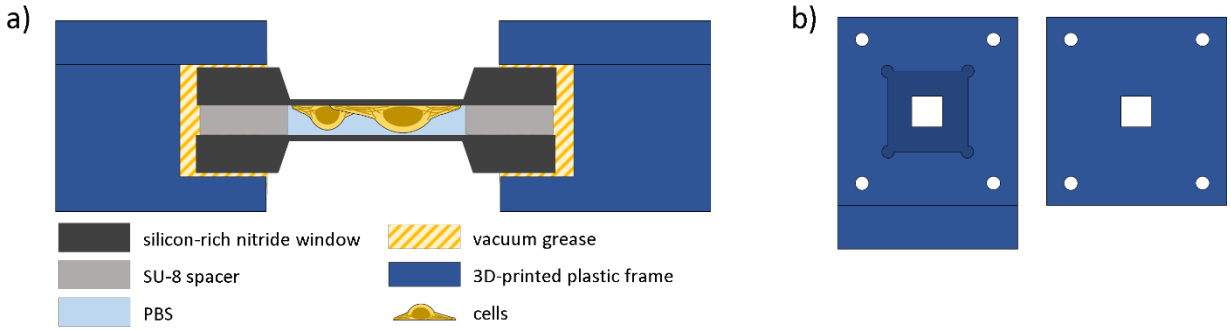

Figure 1: a) *Schematic of the wet sample chamber. The thickness of the aqueous environment (PBS) is defined by a 20 µm SU-8 spacer.* ***b)*** *Schematic of the dedicated 3D-printed plastic frame. Both parts have 4 holes for screws in the corner separately and one in the center for the x-ray pathway. The indentation is in the middle with a square shape and round corner. The stack of the silicon nitride windows was placed in the indentation.*

Results:

Our newly developed sample chamber kept the aqueous environment intact during the entire scanning time of a sample. For each sample, we selected 5 to 9 scanning locations. Scanning of a single sample took 2 to 4 hours, depending on the exposure time, the area of the scanning point, and the scan steps. We carefully looked at the sample environment using the in-line microscope before and after scanning. After 2 to 4 hours of scanning, while some bubbles formed by irradiation produced an air-water interface, most samples were still wet.

We took phase contrast and fluorescence images in our in-house laboratory in Göttingen before transportation. After the scan, the samples were brought to the PSCM laboratories to retake phase contrast and fluorescence images. When comparing the phase contrast images before and after scanning, we observed that the cell outlines remained similar; the irradiated region was clearly visible on the Si_3N_4 membrane, indicating radiation-induced changes. The fluorescence images taken after the scan are not fully comparable due to the focus restriction coming from the wet sample chamber. But the overall appearance of the keratin in the fluorescence images was still in good accordance with the respective cell outlines.

During this particular beamtime, we measured 30 samples and collected over 400 data sets in total. Due to the large amount of data collected, data analysis is still ongoing. In a typical dark field contrast image, cell shape can be distinguished in the scans of hydrated samples (Figure 2**a**). We performed a radial integration from the individual scattering patterns to obtain the azimuthal intensity profile. The main orientation was determined as the circular mean of the azimuthal intensity profile. By analysing the main orientation of the scattering patterns, we find random orientations for the empty (cell-free) positions, while the cellular positions clearly have non-random orientations (Figure 2**b**). By looking at details of the cell, the main orientation at each position is in good accordance with the subcellular structure (Figure 2**c**). This reveals that, despite the low electron density difference between the cells and their aqueous environment, we have sufficient contrast to detect the orientation of nanometric structures at subcellular positions.

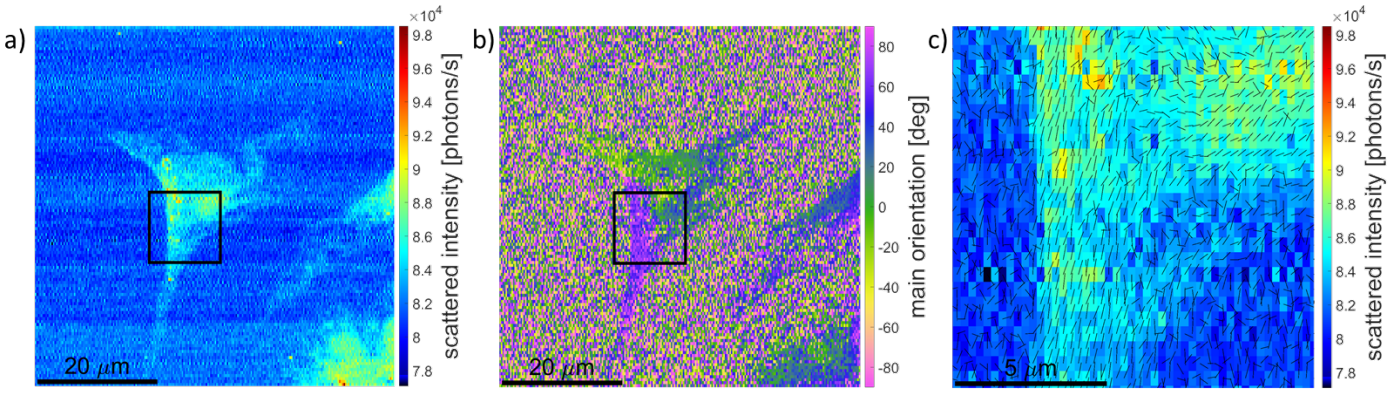

Figure 2: a) *Dark field contrast image of a scan of a fixed-hydrated cell (step size 0.25 µm x 0.5 µm, exposure time 10 ms).* ***b)*** *Main orientation at each scan position.* ***c)*** *Dark field contrast image of the area marked by the black box in* ***a*** *and* ***b****. The black lines indicate the local orientation at each scan position.*