| ESRF | **Experiment title:** Fast scanning SAXS of hydrated biological cells | **Experiment number**: SC5189 |
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| **Beamline**: ID13 | **Date of experiment**: from: 11.11.2021 to: 15.11.2021 | **Date of report**: 16.2.2022 |
| **Shifts:** 12 | **Local contact(s)**: Manfred Burghammer | *Received at ESRF:* |

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**Report:**

**Overview:** This experiment was a continuation of experiment SC5002, where we aimed to apply fast scanning small angle X-ray scattering (SAXS) to hydrated biological cells. Scanning SAXS on freeze-dried cells is a well-established method [1-5], while hydrated cells remain a challenging sample type [6-7], as they need to remain in aqueous environment for the duration of the scans, which diminishes the contrast and requires an adequate sample chamber. Hydrated cells are closer to physiological conditions than freeze-dried cells, so an effective way to perform scanning SAXS on them is an important step towards the collection of more realistic data. As experiment SC5002 had to be performed remotely, the samples got dirty during their journey to the ESRF (as detailed in the experimental report for experiment SC5002), hence the need for a continuation of the experiment.

**Experimental setup and data collection:** We measured MDCK-K8YFP cells (Madin-Darby Canine Kidney cells stably transfected to express human keratin 8 with an EYFP tag [8]). The samples were prepared by growing cells on silicon-rich nitride windows with a total membrane area of 1.5 x 1.5 $mm^2$; the cells were then chemically fixed with formaldehyde and stored in phosphate-buffered saline (PBS) for transportation to the synchrotron. Once at the synchrotron, the sample chamber was prepared by sandwiching two silicon-rich nitride windows together, with their flat sides facing each other. A polydimethylsiloxane (PDMS) spacer, approximately as thick as the cells (about 20 µm) was placed on the silicon frame of one window, to make sure that the PBS wetted the cells at all times with the lowest amount of liquid possible, thus minimizing the background scattering. The PDMS spacer was sealed along its outer rim with two-component silicone to avoid water permeation and evaporation. The sandwich was kept together by metallic frames screwed together. The experiments were performed at EHII of ID13 using a microfocused beam (2.5 x 2 $\mu m^2$). The diffraction patterns were collected by an Eiger 4M detector (Dectris) placed 0.98 m away from the sample. Single cells or small groups of cells were located with the in-line optical microscope and then scanned in fast scanning mode [4-5] with a step size of either 1µm or 0.5 µm in both the horizontal and vertical direction, with exposure times ranging from 5 ms to 20 ms. A step size smaller than the beam size was deliberately chosen, in order to better

resolve cells in the dark field contrast (oversampling approach). In addition, we scanned one freeze-dried sample (NIH-3T3 cells [9]) in order to compare the signal from freeze-dried and hydrated cells.

**Results:** Because of the large amount of data collected, data analysis is still in progress. In a typical dark field contrast image, cell shapes can be distinguished in the scans of hydrated samples (Figure **1a**). Moreover, by performing an analysis of the main orientation of the scattering patterns (for a detailed description of the analysis, based on the computation of the circular mean of the azimuthal intensity profile obtained by radial averaging of the scattering pattern, please refer to [10]), we find random orientations for the empty (cell-free) positions, while the cellular positions clearly have non-random orientations (Figure **1b**). By looking more closely at single cells, the main orientation at each position appears to be in very good accord with the cell shape, especially for elongated cells (Figure **1c**). 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 sub-cellular positions.

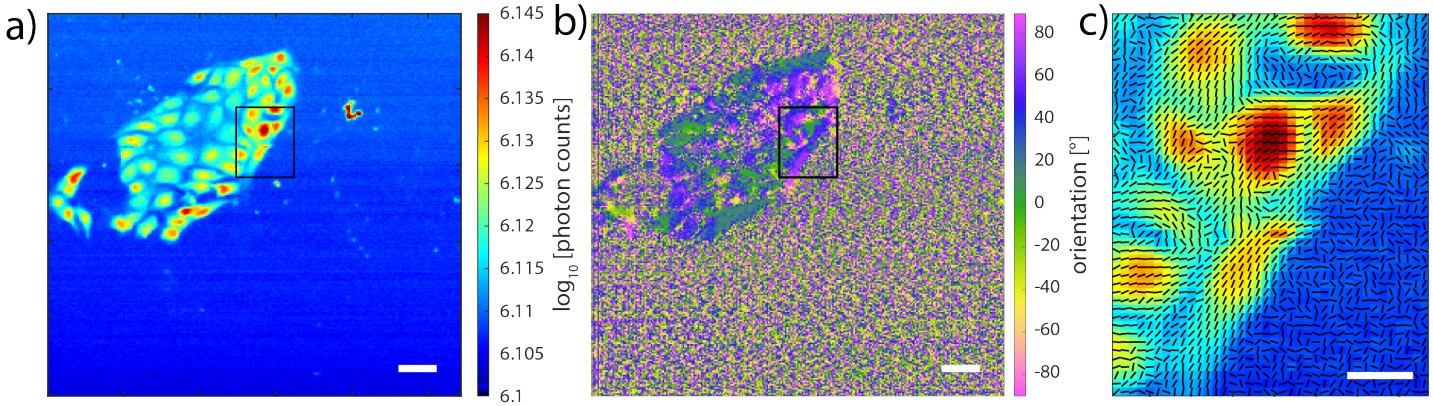


**Figure 1: a)** Dark field contrast image of a scan of a group of fixed-hydrated cells (step size 1 µm x 1 µm, exposure time 10 ms). Scale bar: 25 µm. The black box marks the area displayed in **c**. **b)** Main orientation at each scan position. Scale bar: 25 µm. The black box marks the area displayed in **c**. **c)** Dark field contrast image of the area marked by the black box in **a**, **b**. The black lines indicate the local orientation at each scan position. Scale bar: 8 µm.

**References:**


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