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ESRF

Experiment Report Form

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Once completed, the report should be submitted electronically to the User Office using the **Electronic Report Submission Application:**

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Reports can now be submitted independently of new proposals – it is necessary simply to indicate the number of the report(s) supporting a new proposal on the proposal form.

The Review Committees reserve the right to reject new proposals from groups who have not reported on the use of beam time allocated previously.

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All users must give proper credit to ESRF staff members and proper mention to ESRF facilities which were essential for the results described in any ensuing publication. Further, they are obliged to send to the Joint ESRF/ ILL library the complete reference and the abstract of all papers appearing in print, and resulting from the use of the ESRF.

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Instructions for preparing your Report

- fill in a separate form for each project or series of measurements.
- type your report, in English.
- include the reference number of the proposal to which the report refers.
- make sure that the text, tables and figures fit into the space available.
- if your work is published or is in press, you may prefer to paste in the abstract, and add full reference details. If the abstract is in a language other than English, please include an English translation.

ESRF	**Experiment title:** Imaging hydrated cells by x-ray nanodiffraction	**Experiment number**: SC3556
Beamline: ID 13	**Date of experiment**: from: 14.11.2012 to: 18.11.2012	**Date of report**: 21.03..2013
Shifts: 9	**Local contact(s)**: Emanuela DiCola	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

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Report:

In previous experiments at ID13 (SC3188, SC3199) we have studied the structure of the keratin intermediate filament (KIF) network in freeze-dried eukaryotic cells [1]. During this beamtime we have studied fixed-hydrated as well as living eukaryotic SK8/K18-2 cells (IF free SW13 cells transfected with fluorescent keratin protein [2]) in microfluidic devices [3] at the nano-beam set-up at EH3 of ID13. Performing measurements on hydrated and particularly on living cells allows us to exclude structural artifacts from plunge-freezing and freeze-drying.

The SK8/K18-2 cells were grown on silicon nitride membrane windows for 1-2 days. For the preparation of the fixed-hydrated samples, the cells were fixed with 3.7% formaldehyde solution, stored in phosphate buffered saline and imaged at an inverted microscope using phase contrast and fluorescence microscopy (KIF fluorescence signal). Note that not all cell express keratin (e.g. the cell in the center of Fig. 2 (b) does not show a network structure), which allows us to perform control experiments on the same sample at precisely identical conditions. The living cells were stored in sealed tubes with culture medium and imaged using bright-field microscopy immediately before the X-ray measurements. The silicon nitride membrane windows with either fixed or living cells were attached to a microfluidic device directly before the X-ray measurements (see Fig. 1) and the device was flushed with buffer solution or medium at a flow rate of about 100 µL/h, which corresponds to a flow velocity of about 0.4 mm/s.

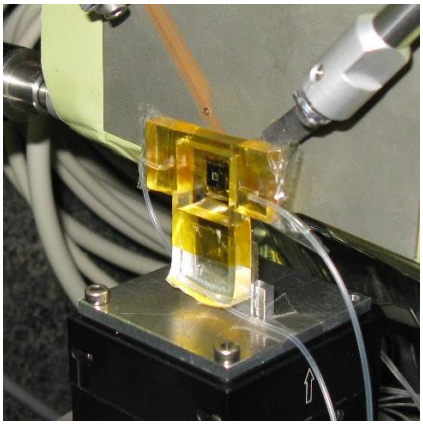

Figure 1: Picture of a microfluidic device on the sample stage at the nano-beam hutch EH3, ID13.

X-ray measurements were performed in transmission geometry at a photon energy of 14.9 keV. The beam was focussed to 170×160 nm² (horizontal × vertical) using the available crossed nano-focusing refractive lens system, yielding a primary beam intensity of ~8×10^9 photons/s. The samples were positioned in the focus of the beam using the visible-light microscope and two-dimensional mesh scans were performed on the sample.

Fig. 2 (c) shows an X-ray dark-field image taken on a fixed-hydrated sample. Visible light phase contrast and fluorescence microscopy images are shown in Fig 2 (a) and (b). Since the scattering signal from the hydrated samples was very weak, only a small ring-shaped region in reciprocal space was used for the calculation of X-ray dark-field images to enhance the contrast between the cell and the empty region of the sample. In the dark-field images in Fig. 2 (c) the cell and especially the nuclear region can be distinguished from the empty region, but the contrast is very weak and in particular some regions of the cell body show no signal difference to the empty region. To prevent spreading of the radiation damage (e.g. diffusion of radicals in the

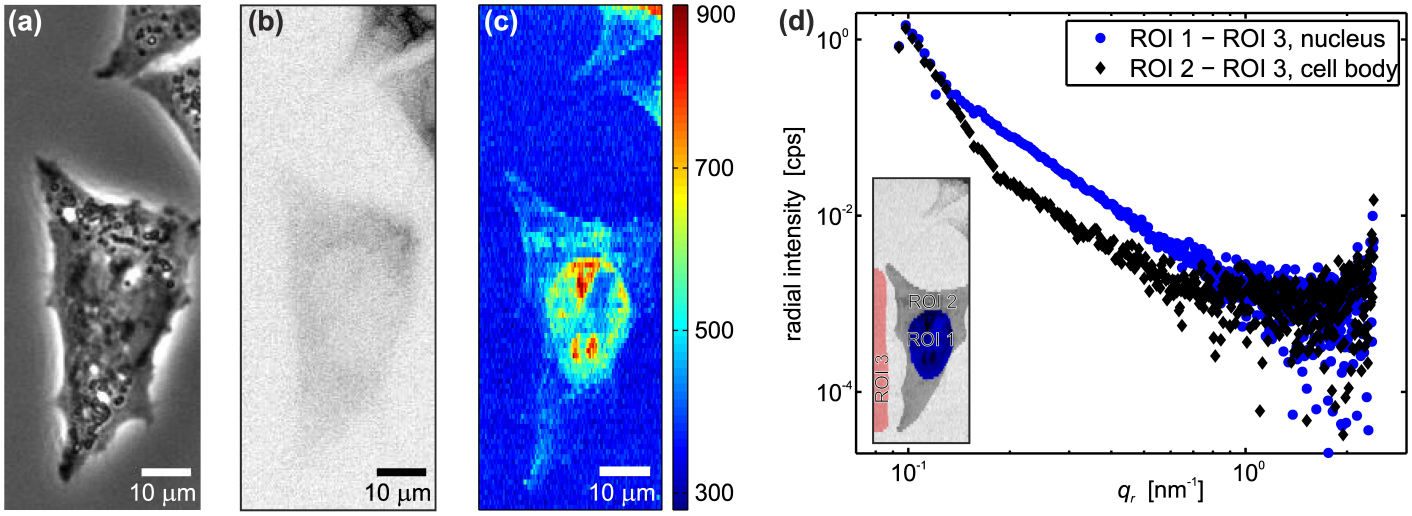

Figure 2. X-ray nano-diffraction on fixed-hydrated cells in microfluidic devices. (a) Visible light phase contrast and (b) fluorescence microscopy image of fixed-hydrated SK8/K18-2 in buffer. (c) X-ray dark-field image of the same sample region as shown in panel (a) and (b). (d) Background corrected averaged radial intensity profiles from two different regions in the cell as indicated in the inset.

buffer solution) we performed asymmetric scans with a larger step size for the slow scanning axis than for the fast axis. Radial intensity obtained from averaged scattering patterns from three different regions of the scan (nucleus, cell body, empty region) were calculated and the background corrected intensity profiles on the cell are shown in Fig. 2 (d). Here, a scattering signal up to ~1 nm^{-1} is observed.

Fig. 3 (a) shows a bright-field image taken on a sample of living SK8/K18-2 cells before the silicon nitride membrane window was attached to a microfluidic device. The cells in the center of the image exhibit a native cell shape. In the X-ray dark-field image in Fig. 3 (b) the cells can be clearly identified. However, even for mesh scans with a large step size, the total scan time was so long that the cells were completely destroyed and detached after a coarse scan. Faster scanning routines (e.g. continuous scan mode for the fast scanning axis) might help to image living cells with a higher resolution, before the cells respond to the radiation damage.

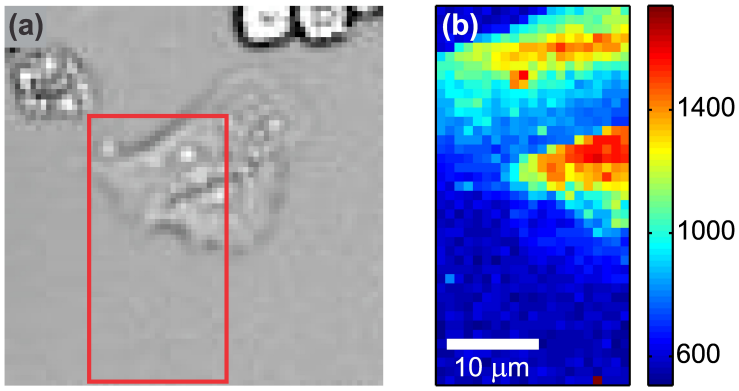

Figure 3. X-ray nano-diffraction on living cells in microfluidic devices. (a) Visible light bright-field image and (b) X-ray dark-field image on the marked region in panel (a).

During this beamtime we showed that it is possible to image fixed as well as living cells in a hydrated state in microfluidic devices using a scanning X-ray diffraction with a nano-focused beam. An improvement of the set-up in terms of scanning speed will be very helpful.

References

[1] B. Weinhausen, J.-F. Nolting, C. Olendrowitz, J. Langfahl-Klabes, M. Reynolds, T. Salditt and S. Köster. X-ray nano-diffraction on cytoskeletal networks. *New. J. Phys.* **14**, 085013 (2012)

[2] A. Leibovitz, W. B. McCombs, D. Johnston, C. E. McCoy, and J. C. Stinson. New human cancer cell culture lines. I. SW-13, small-cell carcinoma of the adrenal cortex. *J. Natl. Cancer Inst.* **51** 691 (1973); P. Strnad, R. Windoffer and R. E. Leube. Induction of rapid and reversible cytokeratin filament network remodeling by inhibition of tyrosine phosphatases. *J. Cell Sci.* **115**, 4133-4148 (2002); R. Windoffer, S. Wöll, P. Strnad and R. E. Leube. Cytoskeleton in motion: the dynamics of keratin intermediate filaments in epithelia. *Mol. Biol. Cell* **15**, 2436-2448 (2004); S. Wöll, R. Windoffer, and R. E. Leube. Dissection of keratin dynamics: different contributions of the actin and microtubule systems. *Eur. J. Cell Biol.* **84**, 311 (2005)

[3] B. Weinhausen and S. Köster. Microfluidic devices for X-ray studies on hydrated cells. *Lab Chip* **13**, 211-215 (2013)