ESRF	**Experiment title:** **Structure of actin networks and bundles in *Dictyostelium Discoideum* cells by nano-diffraction**	**Experiment number**: SC-2623
Beamline: ID13	**Date of experiment**: from: July 19th , 2012 to: July 24th , 2012	**Date of report**: September 30th, 2014
Shifts:	**Local contact(s)**: Manfred Burghammer	*Received at ESRF:*

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Report: We have studied freeze dried, hydrated and chemically fixed as well as living cells of the amoeba *Dictyostelium Discoideum (D.d)*, by nanobeam small angle x-ray scattering (nano-SAXS). The goal was to investigate the structure of the actin network of the cytoskeleton, in particular the actomyosin-cortex in this fully controlled model organism [1]. To this end, scanning transmission microscopy was combined with analysis of local diffraction patterns [2,3]. For comparison and as controls, we have studied the following samples in addition to the *D.d.* cells, using the same experimental conditions: *in vitro* filamentous actin suspensions, murine fibroblasts, *Deinococus radiodurans* (no actin).

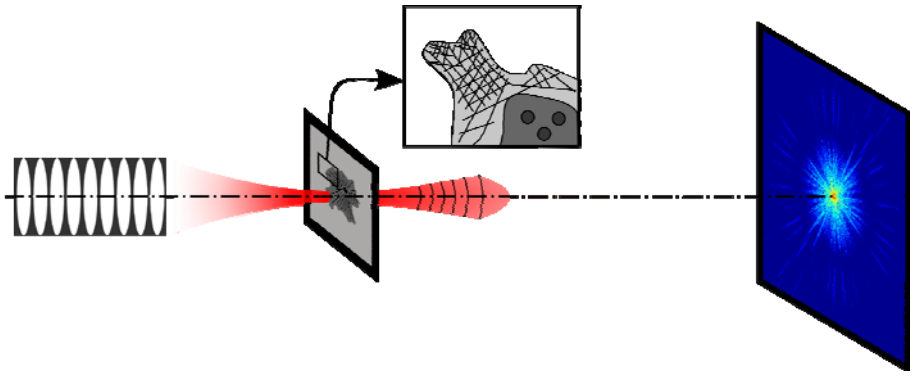

***Fig. 1**, Schematic of the experimental setup: The x-ray beam is focussed with a set of nano-CRLs yielding a focal spot <100 nm in diameter, which can be used for nano-diffraction. Scanning the sample allows probing the structure of the actin network, in particular in cellular protrusions as well as the cell periphery, where strong rearrangement of the network is expected during locomotion.*

Experimental setup: The nano-CRL setup in the extension hutch 3 (see Fig. 1) was used to focus the incoming beam (E=14.9keV) to below 100nm (h) x 70nm (v) (FWHM). The samples were placed in the focus of the beam and scanned through the beam while recording a far-field diffraction pattern at every position with a Maxipix detector (2x2 modules). Sample preparation: For freeze dried samples, cells were suspended on ultrathin Si3N4 windows (Silson), vitrified by plunge freezing (Leica GP2) followed by freeze drying. Custom manufactured microfluidic chambers (ibidi, Munich) with Si3N4-windows served as x-ray transparent sample environments for the experiments with living and formaldehyde-fixed cells. Living D.d. samples were prepared right before the experiment by bringing a cell suspension into the ibidi-channels, waiting for 5 minutes and then replacing the cell medium with buffer. The cells enter a starvation cycle during which they become motile and form pseudopodia (plasma membrane protrusions), mediated by active deformation of their actin cytoskeleton. Due to their speed of 10micron (approximately the length of a D.d. cell) per minute, the measurement of living D.d. cells is particularly challenging. We could observe sufficient signal levels for all sample types. This is remarkable as the living and chemically fixed samples are embedded in a 50 micrometer thick water layer which results in low electron density contrast as compared to the freeze dried samples. The results show significant local variations in the diffraction pattern. In particular, highly anisotropic diffraction patterns were observed at the periphery of the cell. Tentatively, we attribute

this feature to oriented fiber bundles in the actin-myosinII-network, which is involved in the contractile retraction of the cell-locomotion. The *in vitro* actin-suspensions are an important control sample which helps in the interpretation of the diffraction data obtained within the cells. On the length scale of the beam, a cell has only a few filaments that contribute to the diffraction signal, the theory of powder averaging can thus not be applied. We therefore analyze the diffraction patterns based on models of oriented filaments and bundles.

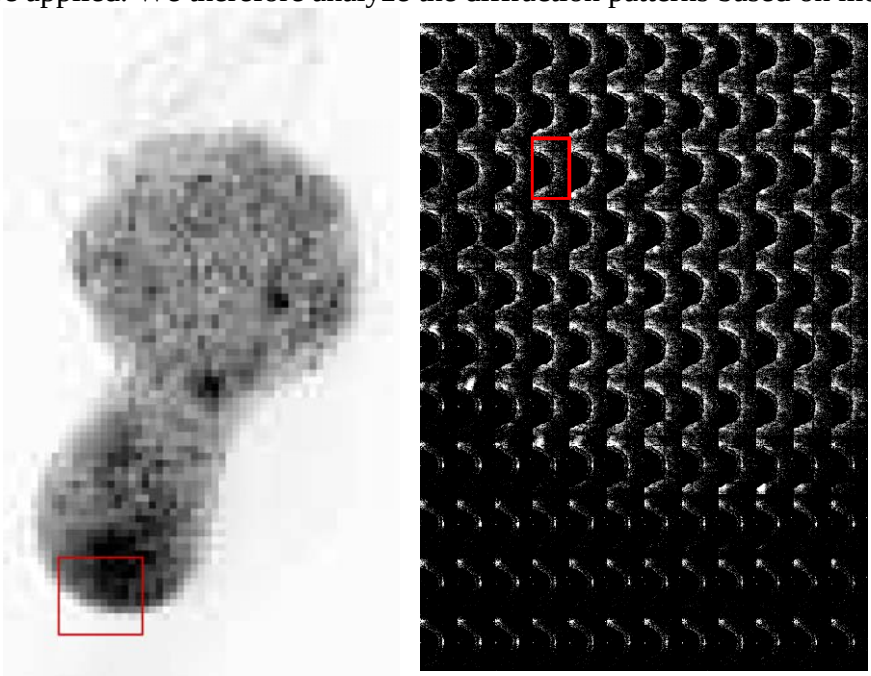

Fig. 2: *(left) Darkfield map and (right) composite image of D.d. (freeze dried), showing a grid of diffraction pattern (one outlined in red for illustration), arranged in the same order as in the corresponding region of interest (ROI), indicated by the red square on the left. Arrangement as seen in beam direction. Unfortunately, the beam stop (round shadow) limits the q-range of the signal. 250nm step size, 0.4s dwell time, I_0=1.6 10^9 , 14.9 keV photon energy.*

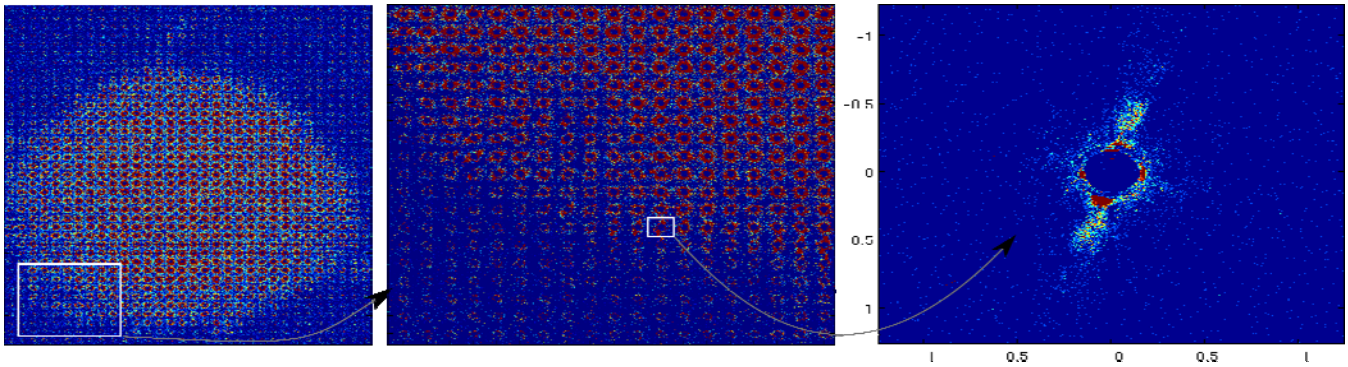

Fig.3: *Examples for nano-diffraction data taken on a Dictyotelium D. cell (left) A typical composite image of the measured signal covering one cell with a stepsize of 250nm and 1s per pixel. In this representation, each pixel shows a miniaturized version of one diffraction pattern. (middle) Finer mesh of indicated ROI , stepsize 100nm, 1s per pixel. (right) Single diffraction pattern recorded at the rim of the cell. The streak-like diffraction patterns are found to be typical and are attributed to bundle structures of actin, as quantified by ongoing model building and analysis. Settings: I_0 =3 10^9cps, 15.25keV photon energy.*

References

[1] Pollard, Science 326:1208 (2009); Koestler, Nat. Cell Biol. 10:306, (2008).
[2] B. Weinhausen, O. Saldanha, R. N. Wilke, C. Dammann, M. Priebe, M. Burghammer, M. Sprung, and S. Köster, *Scanning X-ray Nano-Diffraction on Living Eukaryotic Cells in Microfluidic Environments*
Phys. Rev. Lett. 088102, 112 (2014).
[3] R. N. Wilke, M. Priebe,M. Bartels,K. Giewekemeyer, A. Diaz, P. Karvinen, T. Salditt
Hard X-ray imaging of bacterial cells: nano-diffraction and ptychographic reconstruction
Optics Express 20, 19232 (2012).
[4] 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 Journal of Physics 14, 085013 (2012).