ESRF	**Experiment title:** DNA compaction during cell division studied by combined ptychography and nano-diffraction	**Experiment number**: SC3959
Beamline: ID13	**Date of experiment**: from: 12/11/2014 to: 15/11/2014	**Date of report**:
Shifts: 9	**Local contact(s)**: Manfred Burghammer Britta Weinhausen	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):
Clément Hémonnot*
Sarah Köster*
Oliva Saldanha*
Juliane Reinhardt*
Jens Patommel*

Motivation: The proposed experiment involved studying the structural changes of DNA/chromatin in cell nuclei for different stages of the cell cycle. The DNA in the nucleus is reorganized and compacted in a highly controlled manner on length scales ranging from nm to µm, which renders x-rays an ideal probe for this system. We proposed to combine ptychography and nano-diffraction [1] in order to obtain high resolution real space data including quantitative phase contrast, as well as reciprocal space structural information from the cellular structures.

Experimental setup and data collection: Cells were grown on Si_3N_4 windows, chemically fixed and freeze-dried [2, 3]. We performed the ptychography measurements at a sample position 1 mm out of focus to obtain a large enough beam of 850 x 950 nm^2 to minimize the number of scan positions required and thus the dose imposed on the sample (D = 1.3 x 10^6 Gy per scan point). The detector distance was 2.2 m, no beam stop was employed and flux was reduced by closing the vertical and horizontal slits. The nano-diffraction (D = 3 x 10^8 Gy per scan point and a beam size of 100 x 150 nm^2), by contrast, was performed in focus at a detector distance of 1 m with beam stop. Using the beamline microscope, we were able to precisely (re-)position the sample and perform both ptychography and nano-diffraction on the same regions.

Results: Surprisingly and contrary to preliminary tests at the cSAXS beamline /SLS, Villigen, the nuclei of the cells were not visible in the ptychography data. However, we observed a dense and highly structured network (see Fig. 1a) which we identified as the keratin network in these cells [2, 3, 4]. In nano-diffraction with a small step size of 100 nm and 1 s exposure time per scan point, the network is directly visible (Fig. 1c) and corresponds well to the ptychography image (corresponding region shown in Fig. 1d). Each scan position in Fig. 1c contains a complete 2D scattering signal (for an example of such a single diffraction pattern see Fig. 1d). These patterns are highly anisotropic and when radially integrated (Fig. 1f) characteristic modulations are observed. In order to keep the signal-to-noise high, the orientation of the signal is determined and only those segments, which are aligned with the anisotropic signal, are included in the integration. We are still in the process of analyzing the remaining data sets in the same way. Furthermore, we are working on an interpretation of the modulations seen, e.g., in Fig. 1f.

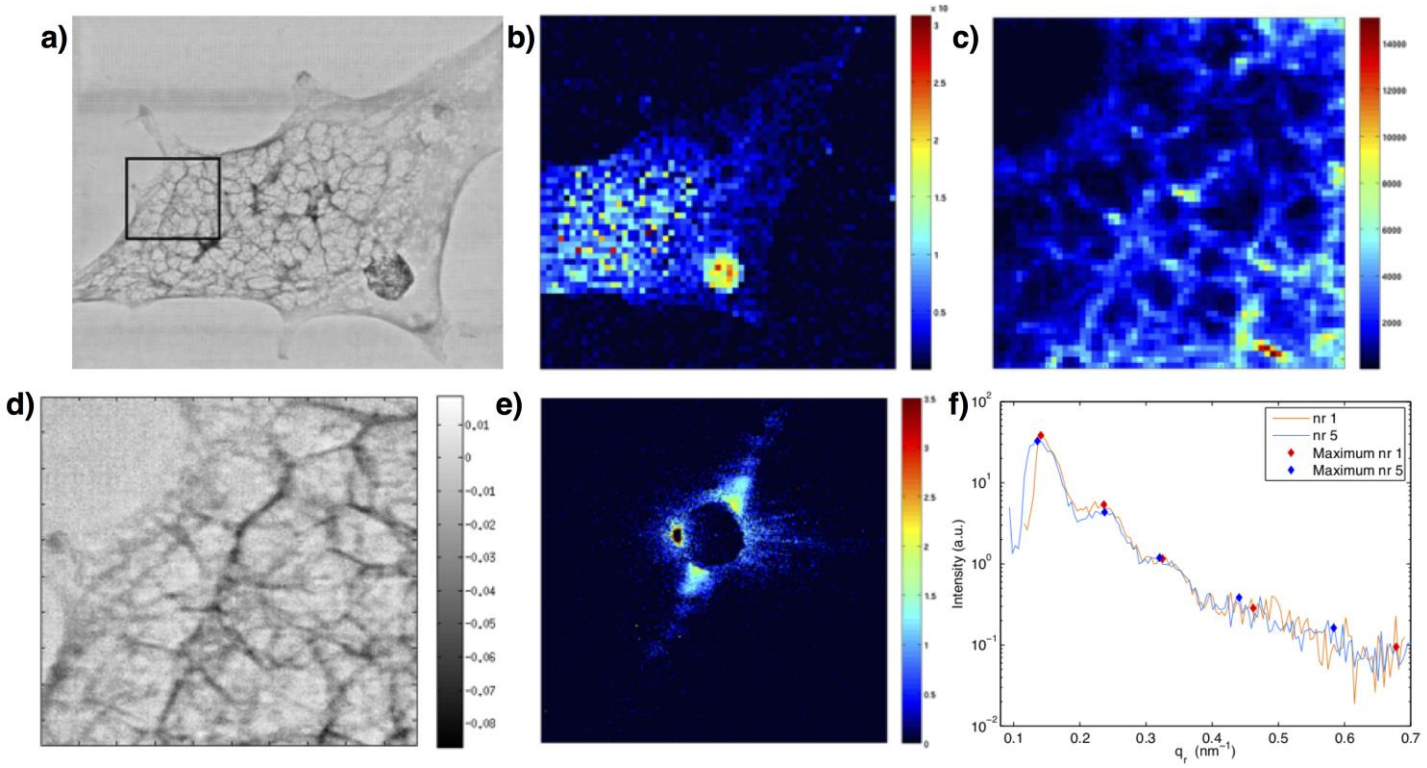

Figure 1: a) Ptychogram of one cell; 100 x 100 scan points, step size 300 mn, exposure time 40 ms per scan point. **b)** Coarse nano-diffraction scan, dark-field representation, of the same cell. **c)** Fine nano-diffraction scan of the ROI highlighted in a), exposure time per scan point 1 s, 100 nm step size. **d)** Detail of the ptychogram corresponding to the region shown in c). **e)** One individual diffraction pattern showing strong anisotropy. **f)** Azimuthally integrated intensity plotted over the scattering vector *q*.

Thus, we were able to demonstrate the feasibility and the advantages of such a combined ptychography/nano-diffraction imaging approach for subcellular components. By combining high-resolution imaging in real space and in reciprocal space, quantitative phase contrast structural information from scattering data we obtain a wealth of information about the system. The technique used here will in the future be applicable to other biological and soft matter systems.

References:

[1] V. Piazza, B. Weinhausen, A. Diaz, C. Dammann, C. Maurer, M. Reynolds, M. Burghammer and S. Köster , *ACS Nano*, 2014, *8* (12), pp 12228–12237, doi: 10.1021/nn5041526

[2] B. Weinhausen, J-F. Nolting, C. Olendrowitz, J. Langfahl-Klabes, M. Reynolds, T. Salditt and S. Köster, New J. Phys. 14, (2012), doi:10.1088/1367-2630/14/8/085013.

[3] B. Weinhausen, O. Saldanha, R. N. Wilke, C. Dammann, M. Priebe, S. Köster, M. Burghammer and M. Sprung, Phys. Rev. Lett., 112, (2014), doi:10.1103/PhysRevLett.112.

[4] B. Weinhausen and S. Köster, Lab on Chip 13, (2013), doi:10.1039/c2lc41014a.