ESRF	**Experiment title:** Small-Angle Scattering of Chromatin in Single Cell Nuclei	**Experiment number**: LS 1571
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Report:

The feasibility to record small-angle scattering (SAXS) from chromatin in single cell nuclei was examined. Experiments were performed at ID13 at a wavelength of 0.0948 nm using a 5 μm beam. Resolution tests with dry rats tail collagen showed that the first meridional order (65 nm) could be resolved from the backstop in vertical direction. The condensing mirror of ID13 introduces a factor 10 larger horizontal divergence than vertically. By reducing the horizontal divergence with a recently installed slit system we obtained a similar horizontal resolution. The background at the beamstop showed, however, fluctuations in horizontal direction which suggest to further stabilize the slit system.

We spent about 2 days in improving the SAXS alignment and testing different sample preparations. Human cancer cell (HeLa) cultures of about 20 μm cell-diameter had initially been prepared in Heidelberg. Cells adhered well to the inner surface of tinwalled glass capillaries of 0.1, 0.2 and 0.3 mm inner diameter, which allowed to maintain a physiological buffer during X-ray experiments. Cells transported to Grenoble prooved, however, to be too old as they had mostly fallen from the walls of the capillaries. We were able, however, to transfer fresh cells to the walls of glass capillaries during the experimental period. The capillaries were fixed on a goniometer head, transferred to the scanning setup of the ID13 beamline and aligned initially optically before transfer into the beam. The capillary axis was oriented vertically so that dead cells would fall down, which is a good criterium for the assessment of survival of the cells in the beam. The limited depth-of-field of the optical microscope made it initially difficult to align individual cells. A proper alignment protocol could, however, be developed. Fig.1 shows two HeLa cells on the surface of a 300 μm glass capillary (within rectangle).

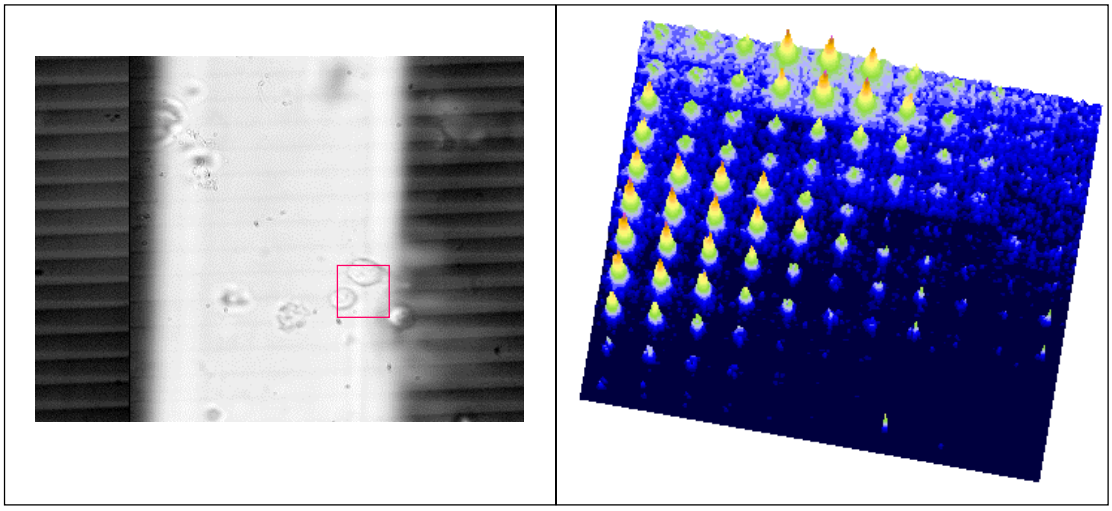

Fig.1 **Fig.2**

Fig.2 shows a scanning SAXS experiment for a 2D-mesh of 5x5 μm^2 mesh-width. At every position of the mesh a 60 sec frame was recorded with a MAR-CCD. The figure shows a pseudo-3D plot of all frames. Only scattering close to the origin of each frame has been plotted and the background has been subtracted. We find a significant increase in intensity in two areas which we assign to SAXS from the two nuclei which were in the mapped area. Note that mesh-scan did not completely cover both HeLa cells (rectangle).

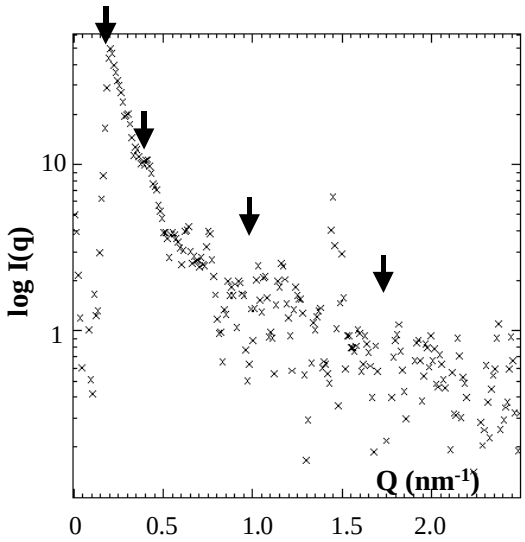

Fig.3

Fig.3 shows an azimuthally averaged pattern of 3 frames from the upper HeLa cell in Fig.2. The azimuthal angle covered was about 30 degrees in the top part of each frame in order to avoid background contribution in the horizontal direction. The arrows design expected positions of interfiber, internucleosomal and nucleosomal core scattering from chicken erythrocyte nuclei (*Bordas et al., Eur. J. Bioph. 13,p.157,1986*). We find evidence for interfiber and internucleosomal scattering but statistics is not yet sufficient for nucleosomal core scattering. We believe, however, that statistics can be significantly improved by repeating the scans and improving the horizontal background. Furthermore, a second undulator segment (35 mm period) has been installed at ID13 in summer 2000, which will increase the brilliance at 13 keV.

In conclusion, we believe to have demonstrated that scattering from chromatin in single cells is technically feasible. Further improvements of the setup, but also an increase of the size of the cells, are required to improve the statistics.