	Experiment title: Small Angle Scattering of Chromatin in Single Cell Nuclei	**Experiment number**: SC-1004
Beamline: ID13	**Date of experiment**: from: 06.06.2002 to: 10.06.2002	**Date of report**: 31.08.2002
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Report:

The aim of the experiments was threefold:

1. To repeat and improve the previous data
2. To investigate, if the chromatin fiber has a alignment in the cell nucleus
3. To test the conditions for study single steps of the mitosis reaction

In previous experiments we have shown, that the chromatin in a single cell can be investigated at 100K by using the ID13 micro-goniometer with the SAXS setup. With this technique we found a structured scattering function showing a well-pronounced maxima at ~Q=1 nm^{-1}. The same setup was used for the experiments described below. The SAXS option allowed to investigate a Q-range from Q=0.2nm^{-1} to Q=1.6nm^{-1}.

We collected scattering data by used the micro-SAXS (beamsize 5µm) scanning approach. A bundle of cells frozen to 100K in a standard-cryo loop were mapped. The cells were scanning in steps of 5µm whereas at every step a exposure of 300s was taken. No radiation damaging was detected. The set of data we obtained using this technique shows a scattering pattern with strong reflections at Q=1 nm^{-1} (see figure 1a.). Further analysis of the data show that further maxima can found at the qQvalues of 0.23 nm^{-1}, 0.31nm^{-1} and 0.41nm^{-1}(see figure 1b.). For the strong reflection at 1 nm^{-1} a varying azimuthal distribution from 360° (Bragg ring) to a single peak with a lower limit of ~14° was found (see figure 1c.). The experiments have been repeated several times with similar results.

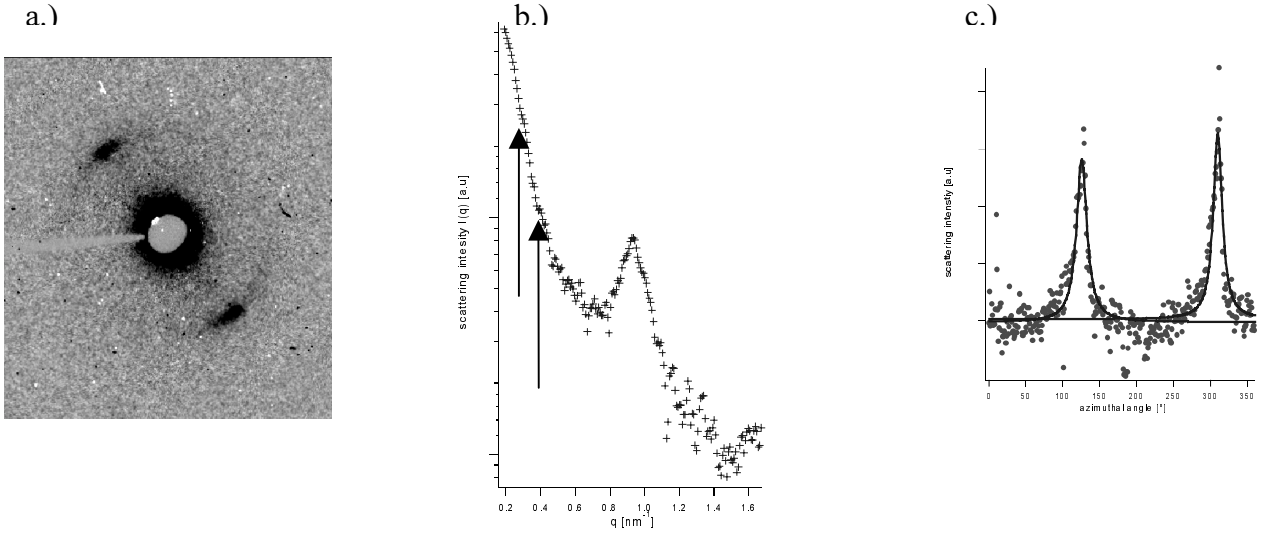

Fig 1: a. scattering pattern obtained by scanning of a bundle of HeLa cells, cooled to 100K.
b. radial averaging of the scattering pattern from a. In addition to the strong maxima at Q~1 nm^{-1} additional side maxima can be found
c. azimuthal scan of the pattern. The smallest peak fwhm was found at ~14°. (Lorentzian fit)

We investigated in addition single HeLa-cells in a defined state of the cell mitosis. Using the chemical reagent *Colcemid* the mitotic spindle apparatus of the cell was inhibited. The function of these spindles is to divide the formed double chromosomes in the metaphase of the cell division. According the experimental setup described above we were able to obtain quite different scattering pattern (see Fig. 2).

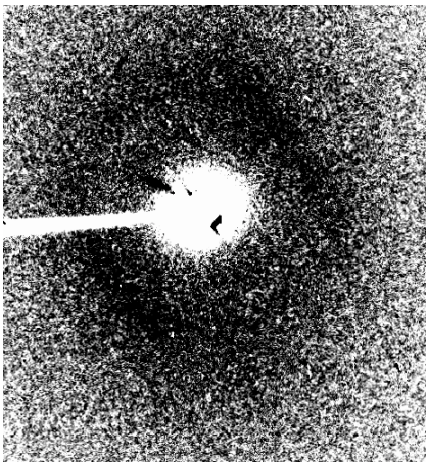

Fig 2: Scattering pattern of a single mitotic HeLa cell trapped in the metaphase of the cell division. The layer line appears at a Q-value of ~1 nm^{-1}.

For the scattering of the mitotic cell a layer-like structure was found. Since in the metaphase of the cell division the high organized and densely packed chromosomes are formed this layer lines are maybe related to the organization of the chromatin and the nucleosome core particles in the chromosome.