ESRF	**Experiment title:** Structural Rearrangements of Sperm Chromatin in Mitotic Extracts	**Experiment number**: SC-1003
Beamline: ID13	**Date of experiment**: from: 07.06.2002 to: 10.06.2002	**Date of report**: 31.08.2002
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Report:

The aim of this project was to determine the possibilities for micro-SAXS analysis on a single, demembrenated nucleus of frog sperm. Experiments were performed at 100 K using the micro-goniometer of ID13 with a 5 μm beam. SAXS data were recorded in a scanning mode for a Q-range from 0.2 nm^{-1} to 2.5 nm^{-1}. In order to enhance the visibility of the nuclei on the micro-goniometer we installed a fluorescent microscopy setup on the micro-goniometer. This allowed us to identify the fluorescent labeled DNA in the nuclei. The fluorescent-labeled sample was illuminated at an excitation wavelength of 365 nm. This approach allowed us to estimate a region of interest, which was scanned with the microbeam of ID13.

The DNA was extracted from sperm of the frog *Xenopus laevis*. It was known from previous biochemical work that the transformation of pure DNA to the chromosome level can be triggered by adding a protein extract from the female egg cell. Since this extract contains the histone proteins the nucleosome core particles, as the first step of the chromatin folding, are optically visible after ~15min. Fig 1 shows an image of the standard cry-loop used at the micro-goniometer.

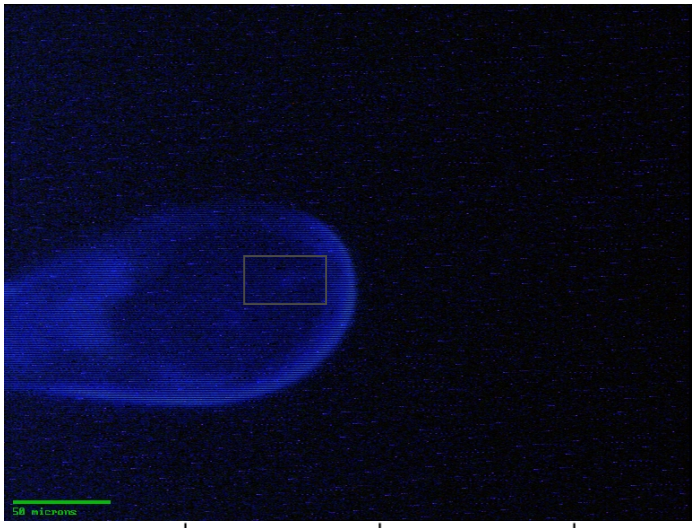

Fig1: Optical fluorescence image of the cryo-loop containing a single frog sperm nucleus of labeled DNA. The rectangular ROI containing the DNA was scanned by the microbeam.

The result of a scanning micro-SAXS experiment is shown in fig. 2. The scattering of the sperm nucleus appears in the second pattern (upper row). The layer lines correspond to a Q-value of ~1 nm^{-1}.

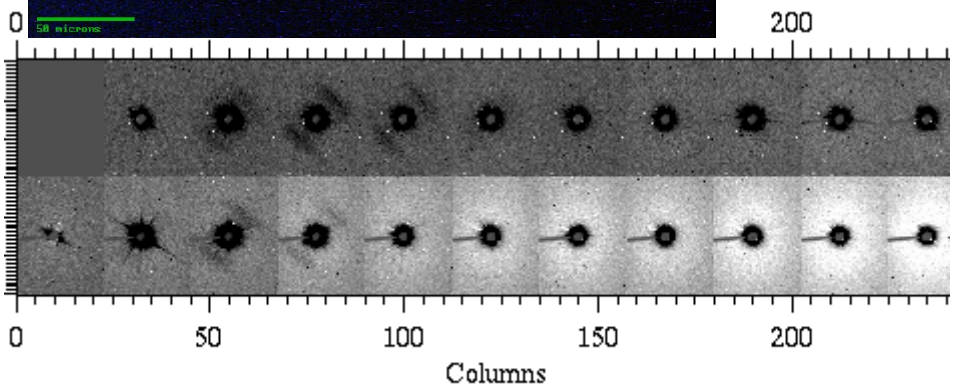

Fig 2.: Result of the micro-SAXS experiment. The exposure time for a single frame was 300s.

The origin of these layer lines is not yet clear. A possible explanation would be the organization of the chromatin in the chromosomes which start to form in this stage of the reaction. Since the Q-range of ID13 was limited to about 100 nm we performed complimentary experiments on a bulk sample of DNA/chromatin at ID02 extending to lower Q-values. We were able to investigate three different stages of the chromatin folding (see fig. 3).

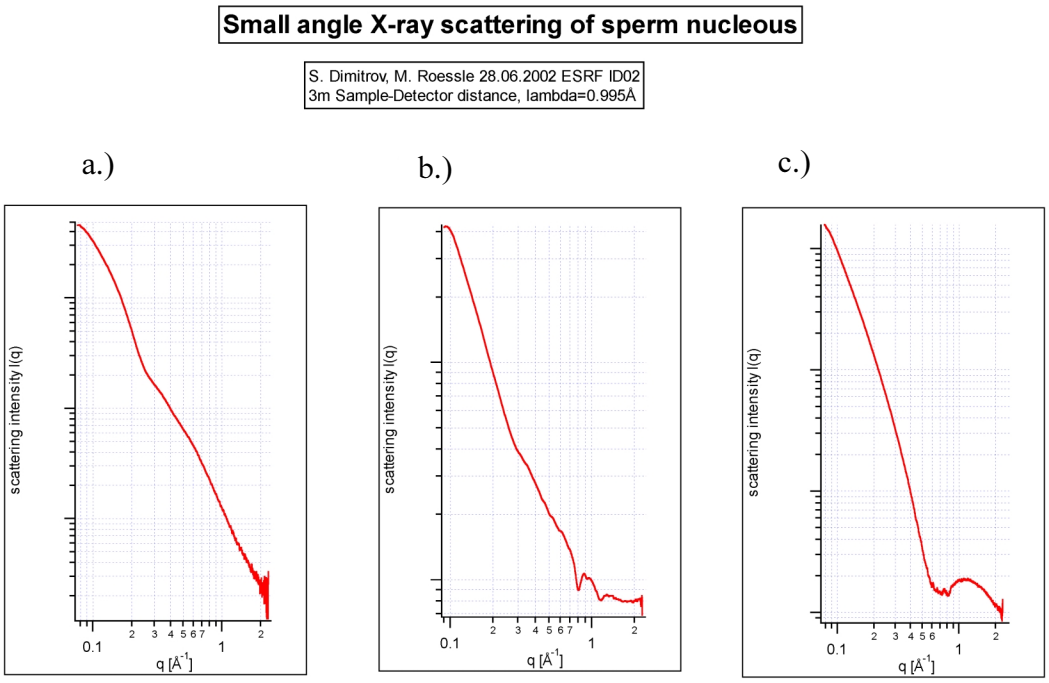

Fig. 3: Frog sperm DNA in different chromatin folding stages.
a.) Control – DNA without histone protein
b.) DNA + histone protein after ~15min of the mixing
c.) DNA + mixing with protein extract containing not all histone proteins