



Experiment Report Form

The double page inside this form is to be filled in by all users or groups of users who have had access to beam time for measurements at the ESRF.

Once completed, the report should be submitted electronically to the User Office using the **Electronic Report Submission Application:**

<http://193.49.43.2:8080/smis/servlet/UserUtils?start>

Reports supporting requests for additional beam time

Reports can now be submitted independently of new proposals – it is necessary simply to indicate the number of the report(s) supporting a new proposal on the proposal form.

The Review Committees reserve the right to reject new proposals from groups who have not reported on the use of beam time allocated previously.

Reports on experiments relating to long term projects

Proposers awarded beam time for a long term project are required to submit an interim report at the end of each year, irrespective of the number of shifts of beam time they have used.

Published papers

All users must give proper credit to ESRF staff members and proper mention to ESRF facilities which were essential for the results described in any ensuing publication. Further, they are obliged to send to the Joint ESRF/ ILL library the complete reference and the abstract of all papers appearing in print, and resulting from the use of the ESRF.

Should you wish to make more general comments on the experiment, please note them on the User Evaluation Form, and send both the Report and the Evaluation Form to the User Office.

Deadlines for submission of Experimental Reports

- 1st March for experiments carried out up until June of the previous year;
- 1st September for experiments carried out up until January of the same year.

Instructions for preparing your Report

- fill in a separate form for each project or series of measurements.
- type your report, in English.
- include the reference number of the proposal to which the report refers.
- make sure that the text, tables and figures fit into the space available.
- if your work is published or is in press, you may prefer to paste in the abstract, and add full reference details. If the abstract is in a language other than English, please include an English translation.



	Experiment title: Small Angle Scattering of Chromatin in Single Cell Nuclei	Experiment number: LS-1857
Beamline: ID13	Date of experiment: from: 6.6.2001 to: 8.6.2001	Date of report: 20.8.2001
Shifts: 9	Local contact(s): C. Riekkel, M. Roessle	<i>Received at ESRF:</i>
Names and affiliations of applicants (* indicates experimentalists): * C. Riekkel ESRF * J. Langowski Division Biophysics of Macromolecules, H0500 German Cancer Research Center (DKFZ) Im Neuenheimer Feld 280 D-69120 Heidelberg, Germany		

Report:

We have continued the examination of chromatin in single cell nuclei by small angle X-ray scattering using the ID13 scanning μ SAXS setup at **room temperature**. In addition we used the new micro-goniometer of ID13 at **100 K**. In the latter case we expected to be able to irradiate the cells longer than at room temperature without loss of SAXS pattern. In both cases a 5 μ m beam was used. The slit system, used by both setups, has now been stabilized and no further beam drifts were observed. It will nevertheless be necessary to add an on-line beam intensity monitor in order allow more quantitative data analysis.

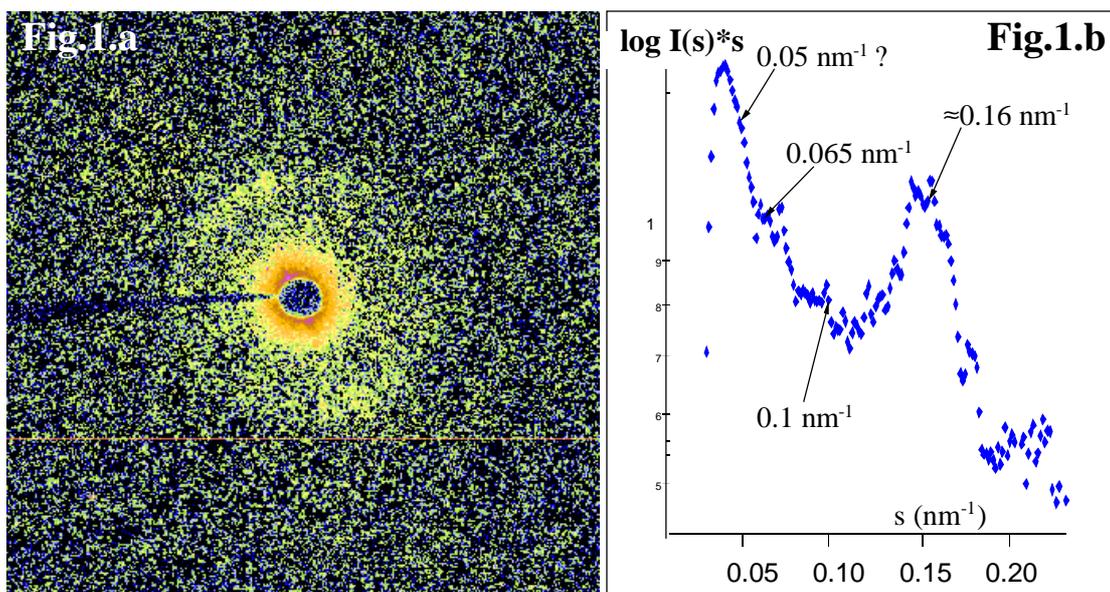
In the case of the microgoniometer we determined a resolution limit of 30 nm based on rats tail collagen. This value could probably be further improved. In order to reduce background scattering, we placed a Helium filled tube between detector and sample. We used human HeLa cells, which were, however, quick-frozen using standard biocrystallography techniques. Thus we transferred a cell in a mixture of glycol and buffer and placed it inside a Hampton nylon loop. The size of the cell nucleus is less than 10 μ m diameter. This was then quick-frozen to **100 K** using the standard Oxford cryoflow system. The on-line observation of the sample (1) allowed positioning the cell inside the beam with μ m precision.

In the case of scanning μ SAXS experiments we scanned again single human HeLa cells which had been grown on the walls of 300 μ m diameter glass capillaries. The cells were kept inside the buffer and experiments were performed at **room temperature**. We also examined T7 cells. The resolution of the SAXS camera was optimized so that the first order of rats tail collagen could be separated from the beamstop (65 nm). A MAR CCD was used for data collection. We performed both line-scans and mesh scans across whole cells.

Fig.1.a shows the SAXS pattern of a HeLa cell recorded with the microgoniometer within 120 sec. No visual sample damage was evident after this experiment. It was verified that the Nylon loop did not contribute to the pattern. Fig.1.b shows the corresponding azimuthally averaged pattern. We note that the pattern corresponds very well to patterns reported in literature (2). In particular we observe a very strong band at about $s \approx 0.16 \text{ nm}^{-1}$ which has been attributed to intranucleosomal scattering. It is also interesting to note that

the 2D-pattern (Fig.1.a) shows already an on-set of texture of this band which will be interesting to follow up during mitosis.

The experiments with HeLa cell using the scanning μ SAXS setup resulted in a destruction of the sample within about 15 sec as evident from the optical images. We nevertheless observed basically the same features as at 100 K which suggests that the chromatin had retained its organization sufficiently long to allow the formation of a SAXS pattern. The statistics was, however, worse and features like the $s \approx 0.16 \text{ nm}^{-1}$ band have become very weak. We were able, however, to see an additional band at $s \approx 0.025 \text{ nm}^{-1}$ which has been associated with an interfibre interference (2). We probably could get better statistically more significant patterns by improving the sample environment (less buffer solution, flat sample container instead of capillary and somewhat lower temperatures). This issue should be further explored in future experiments.



References

- (1) http://www.esrf.fr/exp_facilities/ID13/index.html
- (2) J. Bordas et al., Eur. J. Bioph. 13:157 (1986)