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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.

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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.

ESRF	**Experiment title:** X-Ray Investigations of Biomaterials in Confined Geometries	**Experiment number**: SC/988
Beamline: ID10B	**Date of experiment**: from: 26-JUN-02 to: 02-JUL-02	**Date of report**: 17.10.2002
Shifts: 18	**Local contact(s)**: Bernd STRUTH	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

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Report:

The study of the interactions of stiff filamentous biopolyelectrolytes, such as DNA, actin and microtubules, with oppositely charged electrolytes (cationic lipids, multivalent cations, ….) or polyelectrolytes (polyamine dendrimers, histones, …) plays an important role for the understanding of principal biophysical processess and for the development of new materials. These long chain macromolecules can be aligned in lithographically patterend microchannel devices if the width and/or depth of the microchannels is in the order of a typical macromolecular length [1]. The structural characterization of these biomaterials, which barely show interpretative features in unoriented samples without further complications introduced e.g. by powder averaging, should be improved due to the induced orientation.

For the first x-ray experiments on microchannel samples we focused our interests on the system DNA/polyamine dendrimer (G4 - 4th generation). This system is discussed as a possible carrier for gene therapy and seems to be a model system for (artifical) chromosomes.
The influence of the patterned microchannel substrate on the scattered signal has been tested on an microchannel device (channel length: 1500µm; channel width: 5µm; channel depth: ~0.2µm) which was homogeneously coated with a thin polyelectrolyte film of 9nm. A detector scan (perpendicular) and the reflectivity data of this sample for two different orientations of the microchannels to the x-ray beam (parallel and perpendicular) are shown in figures 1 and 2. The reflectivity curve with a parallel orientation shows only Kiessig fringes due to the thin organic film of 9.2nm. In comparison, the specular reflectivity of the device with perpendicular alignment is influenced by the microchannel pattern. Additional Kiessig fringes with a spacing corresponding to a thickness of 220nm can be found.
The samples for the GID experiments were prepared in the following way. A DNA-solution was mixed with a dendrimer-solution on top of a microchannel device and was squeezed into the microchannels with a teflon bar. Using this technique, areas of ~5mm (along the channels) to ~1mm (perpendicular) of aligned DNA/dendrimer complexes

were achieved which was tested by polarization microscopy. GID experiments could be carried out, if the x-ray beam hit the microchannels in parallel orientation.

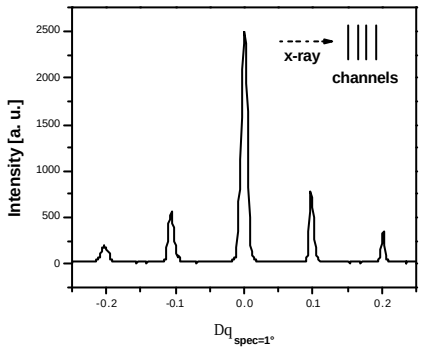

Figure 1:
Detector scan around a specular angle of 1°. The beam hits the sample perpendicular to the orientation of the microchannels.

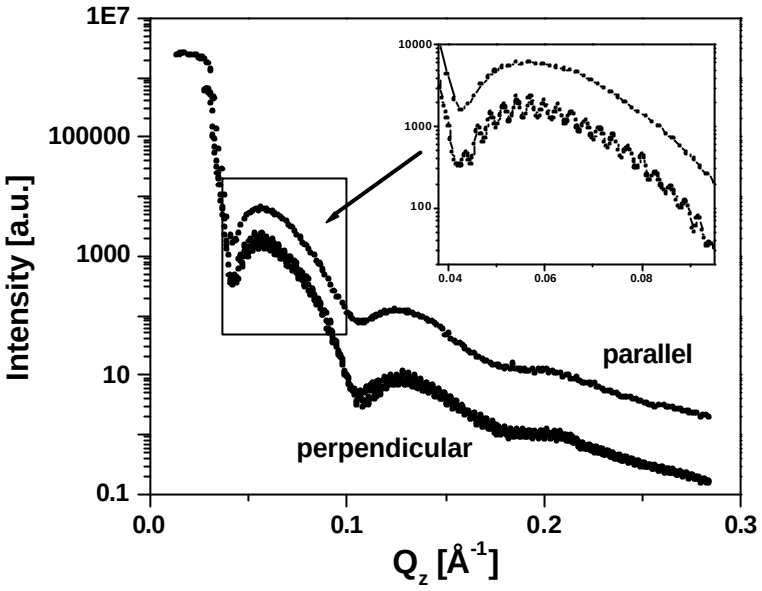

Figure 2: →
Reflectivity measurements of a polyelectrolyte coated microchannel Si-substrate parallel and perpendicular to the channel orientation.

The GID pattern of DNA/dendrimer complexes aligned in parallel microchannels (width: 5µm; depth: 0.6µm) at an angle of 150% α_c is shown in figure 3. A peak due to the alignment of the complex material can be found (arrow). However, scattering from bulk material (in the channels or on top of the channels?!) can also be observed. The peaks (figure 4) correspond to a liquid crystalline 2D-square pattern of the DNA ($Q_{11}/Q_{10} \approx \sqrt{2}$, $d_{10} \approx 3$nm) which has been found also in bulk samples of these complexes with an charge ratio dendrimer (+)/DNA (-) < 1 [2].

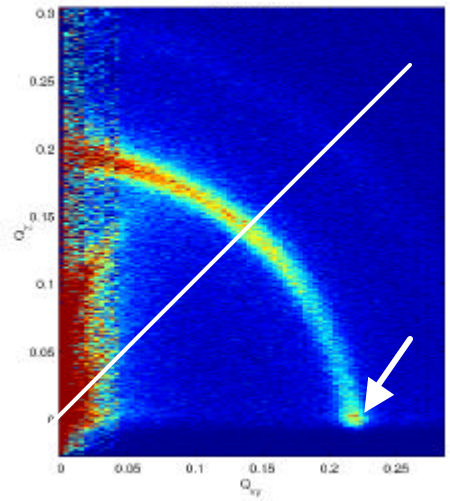

Figure 3:
GID (at 150% α_c) of DNA/dendrimer complex aligned in parallel microchannels (width: 5µm; depth: 0.6µm). The x-ray beam hits the sample parallel to the orientation of the microchannels.

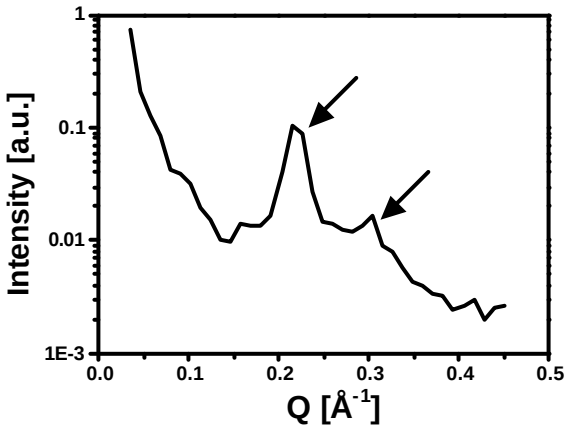

Figure 4:
Intensity along the white line of figure 3.

The used sample preparation techniques (squeezing or micromanipulating [1]) do not give access to sample areas, where a sufficiently large part of the channels is filled with well aligned material for measurements at different rotational angles to improve the characterization of the inner structure of the material (eg. pattern of the counter ions/dendrimers).
For this reason we are going to try different preparation ways. DNA and the dendrimers can be transferred alternately on a silicon substrate using the layer-by-layer transfer. In comparison to the "normal" layer-by-layer

transferred multilayer films, which show an linear thickness increase with layer number n, the thickness of the DNA/dendrimer multilayers increases exponentially [3]. Reflectivity data of a DNA/dendrimer multilayer are shown in figure 5. The thickness of the complex film depends clearly on the humidity (30nm in dry He atmosphere , 57nm in H_2O/He atmosphere).

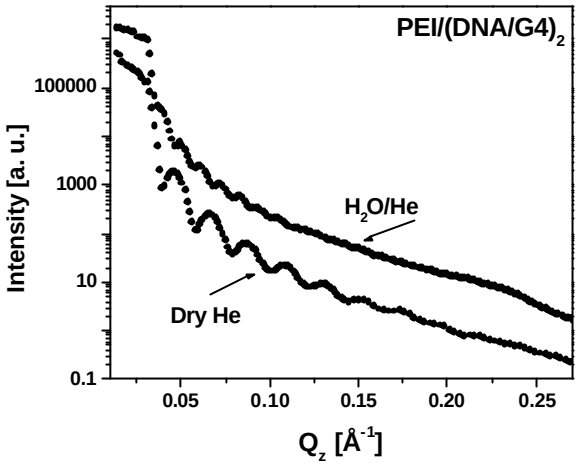

Figure 5:
Reflectivity measurements of a multilayer of DNA/Dendrimer (G4) on a flat Si-substrate in dry and wet He.

The GID pattern of this film (at 85% α_c, figure 6) contains features, which were found in bulk DNA/dendrimer materials at charge ratios dendrimer (+)/DNA (-) ≥ 1 (2D hexagonal pattern, $Q_{11}/Q_{10} \approx \sqrt{3}$, $d_{10} \approx 3$nm, figure 7) [2]. Since these thin films have more likely a bulk structure than a layered structure, we have now a technique to prepare these biomaterials in a more controlled way.

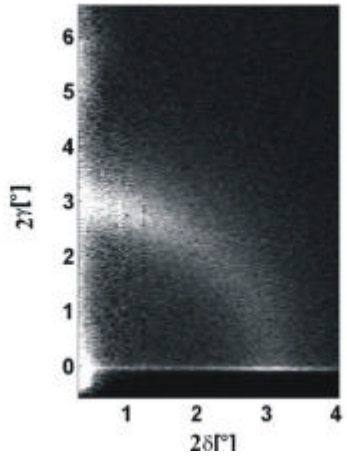

Figure 6: GID (at 85% α_c) of a DNA/Dendrimer-multilayer (see also reflectivity data in figure 5).

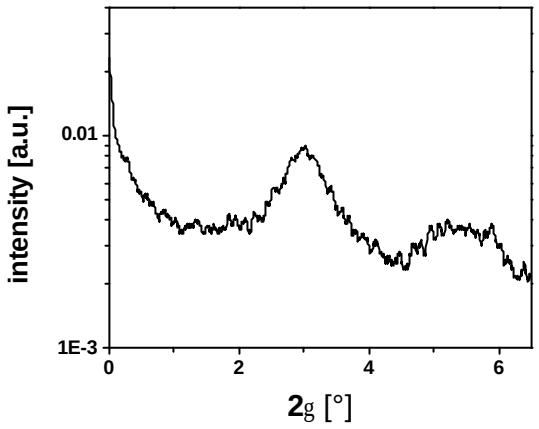

Figure 7: Intensity versus 2γ (in plane) at 2δ ≈ 0°.

Using microstructred PDMS fluidic devices (100 - 1000 parallel channels of 2-20µm width) on a flat silicon substrate during the layer by layer transfer, the macromolecules should adsorb preferentially parallel to the flow direction. Especially, multilayers containing the stiff proteins actin or microtubules will show an strong alignment. Using these alignment technique, the x-ray measurements will also not be affected by photolithographic grooves (in comparison to figures 1 and 2).

References

[1] T. Pfohl et al., *Langmuir* **17** (2001) 5343

[2] H. Evans et al., *Bull. Am. Phys. Soc.* **46** (2001), 391; H. Evans et al. in preparation

[3] J. Nie, B. Du, A. Otten, T. Pfohl in preparation