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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**.

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*http://193.49.43.2:8080/smis/servlet/UserUtils?start*

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

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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.

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## 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:**<br>Sub-lamellar level mineral crystallite organisation in human femoral osteonal bone | **Experiment number**:<br>SC 1575 |
|---|---|---|
| **Beamline**:<br>ID 13 | **Date of experiment**:<br>from: 22.11.2004 to: 27.11.2004 | **Date of report**:<br>03-2005 |
| **Shifts:** | **Local contact(s)**:<br>Manfred Burghammer | *Received at ESRF:* |

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## Report:

Bone is a hierarchically structured connective tissue, whose exceptional mechanical properties depend on the structural tissue design at the micron and sub micron level. At these length scales, compact bone (which is found in the cylindrical shells of most long bones in vertebrates) consists of lamellae that are cylindrically wrapped around blood vessels (Haversian systems). A unit consisting of the cylindrical lamellar shells and the enclosed vascular channel is called an osteon, and its mechanical properties are crucial to the structural stability of the entire bone [1]. The lamellae themselves consist of several subunits [2], which contain arrays of mineralized collagen fibrils in the plane of the lamellar sheets. The orientation of the fibrils has been suggested to be longitudinal, transverse as well as at 45° to the osteon long axis [3].

Small angle x-ray scattering (SAXS) and wide angle x-ray diffraction (WAXD) provide structural information from a specific length scale each [4,5]. While SAXS gives the orientation of the mineral platelets, WAXD delivers information on the orientation of the crystalline c-axis. Jaschouz et al. [6] used a combination of SAXS and WAXD for the first time on bone to combined this information for each measured point. Quantitative texture analysis on the micrometer scale was used by Wenk [8] to characterize the crystal alignment of carbonated apatite in bone. Using a micro beam to analyse hierarchical biological structures leads to the idea of scanning SAXS experiments on osteons. Paris et al. [7] used scanning SAXS with a 20 micrometer beam on an osteon to show the circular orientation of the mineral platelets around the blood vessel. The 20 × 20 × 20 micrometer specimen volume, defined by the diameter of the X-ray beam and the

section thickness is too low to provide structural information at the level of single lamellae.To characterize the mineral nanostructure with lamellar resolution we used, in this experiment, a 1 micrometer beam, what is necessary to resolve the structure of individual lamellae. Following the basic model of Ascenzi [3], who described three different osteon types, dependent on the orientation of the fibrils within single lamellae, we choose for our investigations so called alternating osteons, where the fibril-orientation changes from lamellae to lamellae, as determined by transmission light microscopy. Due to this changes in orientation we expected maximal difference between the diffraction patterns from one lamellae to the next.

The samples are specially prepared thin (3 micrometer) sections of embedded bone from the femoral midshaft of human bone. The ID13 beamline is characterized by a 18 mm period in-vacuum undulator optimized for 13 keV and the optical setup for a submicron beam (0.5 micrometer) is defined by a Kirkpatrick-Baez (KB) mirror. The radiation wavelength is 0.9755 Angstroms and a MARCCD detector with an average pixel size of 64.45 micrometer is used for recording the diffraction patterns.
In the scanning setup the samples are fixed on glass capillaries which are attached to a goniometer head. The beam is positioned accurately on a specific point on the sample by using a high resolution microscope. From this point a reproducible scanning grid is defined on the sample and for every single grid-point a diffraction pattern is collected. By choosing an appropriate sample to detector distance (130 mm in this case) combined SAXS/WAXS experiments were done. We obtained diffraction frames in which both the SAXS and the (002) and (310) reflections from the mineral apatite (hexagonal crystal structure) axes were visible (Figure 1).

Figure 2 shows an area scan, where the total SAXS intensity clearly reflects the lamellar structure of the osteon. The transmission scan over the same area delivers only a very weak interlamellar contrast and closer inspection of the shape of the SAXS patterns shows that the contrast is actually an orientation contrast, arising from an alternating orientation of the mineral platelets with respect to the osteon axis. The WAXD data was used to calculate pole figures (stereographic projections), which delivers information on the mean orientation and texture of the principal axes of the mineral crystallites. Several osteons were scanned along vertical lines in a range from around 50 – 100 micrometer in steps of one micrometer. To derive three-dimensional information from the two-dimensional diffraction patterns it was necessary to rotate the sample around the vertical axis. The samples were rotated around the vertical axis in steps of 5° from –45° to +45° and a diffraction pattern was collected for every single step. So far azimuthal profiles were obtained by integration of a narrow radial range around the 002 reflection, and diffuse background values by integration of a small radial range on both sides of the 002 reflection. For transforming the azimuthal profiles into polar coordinates followed by a stereographic projection we developed software routines. For a full texture analysis (orientation distribution function), evaluation of several different reflections would be necessary. As seen in Figure 1, the counting statistics enabled only the 002 and the 310 reflections to be evaluated quantitatively. Figure 3 shows the polarized light microscopy image of an alternating osteon and the texture plots of five consecutive measured points. The red colour in the texture plots indicates the main orientation of the 002 axis of the hydroxyl apatite crystals. We clearly can see that the orientation of the pole shifts in a horizontally plane from one measuring point to the next. Since the lamellae on the measured position can be seen as horizontally (zenith of the circular lamella), this shows that the orientation of the mineral platelets (which corresponds with the fibril direction) is within the surface of the lamellae and varies continously. This kind of analysis will be done as well for the 310 reflection to get the texture in two complementary crystal

directions. The observed features fit well to the osteonal model of Ascenzi [3] and accordingly to Weiner's [2] rotated plywood model of lamellar bone, but our investigations show for the first time the orientation changes of the mineral crystallites with interlamellar resolution.

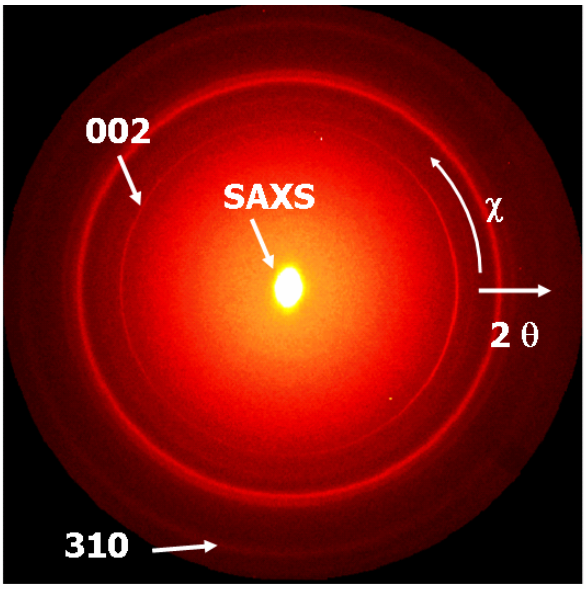

Figure 1. Diffraction pattern, 002 and 310 reflection.

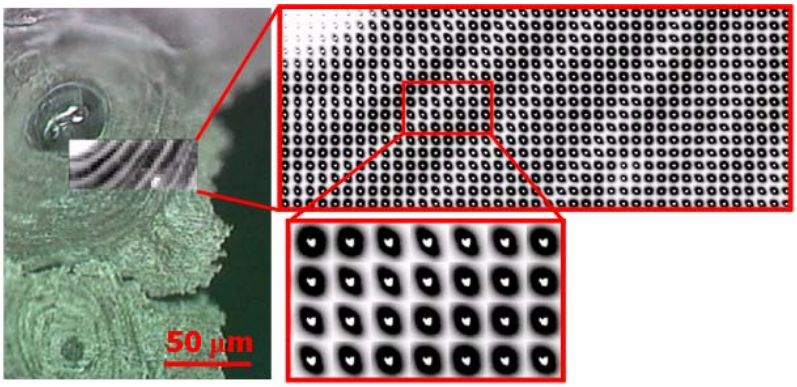

Figure 2. Area scan on osteonal bone, SAXS signal.

*Key publications:*

[1] J. D. Currey, "Bones: Structure and Mechanics", Princeton University Press, Princeton (2002).
[2] S. Weiner and HD Wagner, Annu Rev Mater Sci 28, 271 (1998).
[3] A. Ascenzi, Bone 8, 319–325 (1987).
[4] P. Fratzl et al., J. Appl. Cryst., 30, 765 (1997).
[5] I. Zizak et al., J. Appl. Cryst., 33, 820 (2000).
[6] D. Jaschouz et al., J. Appl. Cryst. 36, 494-498 (2003).
[7] O. Paris et al., Cell. Mol. Biol. 46, 993-1004 (2000).
[8] H.-R. Wenk et al., Bone 24, 4, 361-369 (1999).

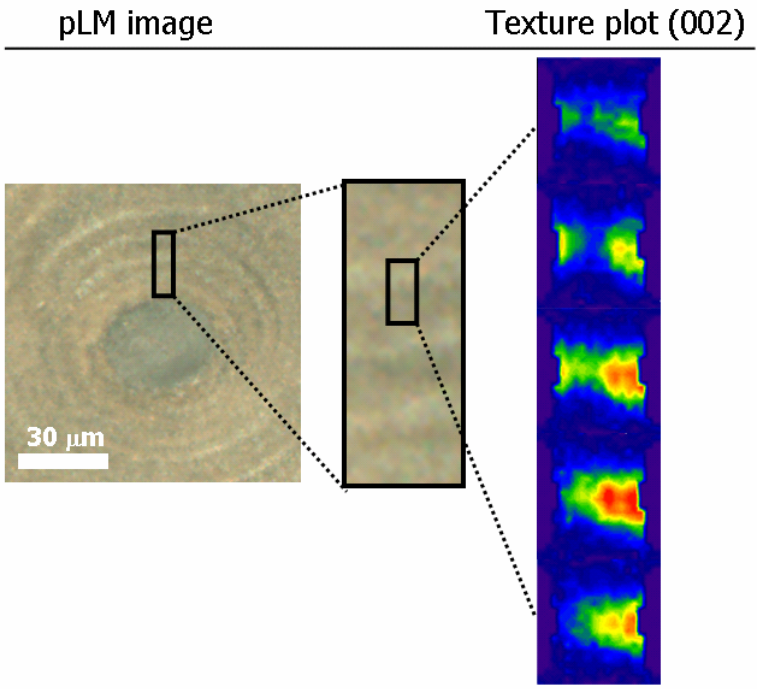

Figure 3. Orientation changes of the 002 reflection.