ESRF	**Experiment title:** Scanning x-ray diffraction of hierarchical biological tissues	**Experiment number**: SC-1579
Beamline: ID13	**Date of experiment**: from: 15.11.2004 to: 21.11.2004 from: 31.10.2005 to: 06.10.2005 from: 12.04.2006 to: 17.04.2006 from: 10.11.2006 to: 13.11.2006	**Date of report**: 14.02.2007
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Report:

The aim of the long term proposal was to improve and to further develop scanning microbeam diffraction towards a real imaging technique and to apply the method to actual scientific questions on complex biological tissues. Several scientific experiments were conducted at ID13 by the research consortium, i.e., the three Research Groups from Potsdam, Cardiff and Grenoble within four common scientific beamtimes in the time frame of 3 years. In the meanwhile several papers have been published or are in press from these experiments and are listed below together with the abstracts. Three scientific meetings closely related to the LTP activities have been organized by members of the consortium, with all partners being present (see below). Beside the scientific achievements, a series of important technical and software developments have been made which are available for the microbeam diffraction user community at ESRF. A Postdoc from the Max Planck Institute Potsdam (A. Gourrier) has spent eight months (January 2006-August 2006) at ID13, working primarily on software developments for online data evaluation in close collaboration with the beamline staff. A close methodical collaboration has been established between the ID13 team (Riekel, Burghammer) and the team at the BESSY μ-Spot beamline in Berlin (Paris, Li). Instrument control software based on the ODAK library (M. Burghammer) was developed within the LTP and is now running routinely at ID13 and at the μ-Spot beamline. Furthermore, data evaluation software for the automated reduction and evaluation of 2D SAXS patterns was developed and is available for the partners, and upon request, also for other ESRF and BESSY users. A batch-processing software (Windows) was optimized by the ID13 beamline scientist R. Davies for the data reduction of large mesh-scanning data sets. This software is now routinely used at ID13. The LTP activities allowed the development of new sample environments for thin corneal sections and microfocus studies of skin sections after a series of treatments; these have both been parts of the Ph.D. thesis of Donna Lammie and Clark Maxwell (Cardiff). Other new sample environments such as cryo-stream cooling in connection with simultaneous scanning SAXS/WAXS experiments have been successfully implemented and tested, and are now available for users at ID13 and at the μ-Spot beamline.

Scientific papers originating from experiments performed in SC-1597

[1] W. Wagermaier, H. S. Gupta, A. Gourrier, M. Burghammer, P. Roschger, P. Fratzl, *Spiral twisting of fiber orientation inside bone lamellae*, Biointerphases 1 (2006) 1-5.

Abstract: The secondary osteon - a fundamental building block in compact bone - is a multilayered cylindrical structure of mineralized collagen fibrils arranged around a blood vessel. Functionally, the osteon must be adapted to the in-vivo mechanical stresses in bone at the level of its microstructure. However, questions remain about the precise mechanism by which this is achieved. By application of scanning X-ray diffraction with a micron-sized synchrotron beam, along with measurements of local mineral crystallographic axis direction, we reconstruct the three-dimensional orientation of the mineralized fibrils within a single osteon lamella (≈ 5 μm). We find that the mineralized collagen fibrils spiral around the central axis with varying degrees of tilt, which would - structurally - impart high extensibility to the osteon. As a consequence, strains inside the osteon would have to be taken up by means of shear between the fibrils.

[2] W. Wagermaier, H. S. Gupta, A. Gourrier, O. Paris, P. Roschger, M. Burghammer, R. C, P. Fratzl, *Scanning texture analysis of lamellar bone using microbeam synchrotron X-ray radiation*, J. Appl. Cryst. 40 (2007) 115-120.

Abstract: Texture analysis with microbeam scanning diffraction enables the local mapping of three - dimensional crystallite orientation in heterogeneous natural and synthetic microcomposite materials. Cortical (compact) bone is an example of a hierarchically structured biocomposite, which is built mainly of cylindrical osteons, having a lamellar texture at the micron level. In this work, we use a combination of microbeam synchrotron X - ray texture analysis with thin sections of osteonal bone to measure the three - dimensional orientation of the *c* - axis of the mineral apatite in bone with positional resolution of 1 μm. We describe the data reduction procedure needed to go from the stereographic projection of X - ray intensity to the local determination of a principal angle and the degree of crystalline ordering in the mineralized collagen fibrils. The procedure can be applied to other mineralized tissues (like trabecular bone and chitin) with micron scale and biologically controlled fibrillar texture.

[3] A. Gourrier, W. Wagermaier, M. Burghammer, D. Lammie, H. Gupta, P. Fratzl, C. Riekel, T. Wess, O. Paris, *Scanning X-ray imaging with small-angle scattering contrast,* J. Appl. Cryst. 40 (2007) in press.

Abstract: An X-ray scanning imaging technique using the integrated intensity of the small-angle X-ray scattering (SAXS) signal is presented. The technique is based on two-dimensional scanning of a thin sample section with an X-ray microbeam, collecting SAXS patterns at every scanning step using a two-dimensional detector. The integrated intensity within pre-defined regions of interest of the SAXS patterns is used to image bulk nanostructural features in the specimen with micrometer resolution, which are usually not accessible by other methods such as light microscopy or scanning electron microscopy. The possibilities and limitations of the method are discussed with particular emphasis on the sources of contrast in the SAXS region for three biological specimens: cortical bone, egg-shell and hair. Two main sources of image contrast are identified in the form of orientation effects for strongly anisotropic systems like cortical bone, and differences in the local volume fraction of the scattering entities in egg-shell. Moreover, other parameters than the integrated intensity can be quantitatively deduced from the SAXS patterns, for instance, the mean thickness of mineral platelets in bone or the strain distributions in a hair deformed plastically by microindentation.

[4] R. Seidel, A. Gourrier, M. Burghammer, C. Riekel, G. Jeronimidis, O. Paris, *Mapping fibre orientation in complex-shaped biological systems with micrometre resolution by scanning X-ray microdiffraction*, Micron (2007) in press.

Abstract: A fully automated procedure to extract and to image local fibre orientation in biological tissues from scanning X-ray diffraction is presented. The preferred chitin fibre orientation in the flow sensing system of crickets is determined with high spatial resolution by applying synchrotron radiation based X-ray microbeam diffraction in conjunction with advanced sample sectioning using a UV micro-laser. The data analysis is based on an automated detection of azimuthal diffraction maxima after 2D convolution filtering (smoothing) of the 2D diffraction patterns. Under the assumption of crystallographic fibre symmetry around the morphological fibre axis, the evaluation method allows mapping the three-dimensional orientation of the

fibre axes in space. The resulting two-dimensional maps of the local fibre orientations - together with the complex shape of the flow sensing system - may be useful for a better understanding of the mechanical optimization of such tissues.

[5] A.C. Hermes, R.J. Davies, S. Greiff, H. Kutzke, S. Lahlil, P. Wyeth and C. Riekel, *Characterization of decay of ancient Chinese silk fabrics by microbeam synchrotron radiation diffraction,* Biomacromolecules 7 (2006) 777-783

Abstract: Scanning synchrotron radiation microdiffraction with an approximately 1x1μm^2 beam has been used as a novel method for characterizing the decay of several T'ang dynasty (618-907 AD) silk fabrics. The crystalline fraction could be visualized based on β-sheet 210 reflections intensities extracted by recursive peak fits from several thousand diffraction patterns recorded during mesh-scans. The azimuthal width of the 210 reflection -which is related to the orientation distribution of the crystalline domains within nanofibrils and the macroscopic orientation of the fibers traversed by the beam- was found to be sensitive to the overall state of decay of the fabric. The fine structure of the histogram of azimuthal width was related to fiber hierarchical microstructure and the fabric morphology. SAXS/WAXS analysis supports the assumption of an initial loss of random chain network with decay. At a subsequent state of ageing, decay proceeds into the nanofibrils and the silk fibers break up into even smaller fractions.

[6] J. Schoeck, R. J. Davies, A. Martel and C. Riekel, *Na-cellulose formation in a single cotton fiber studied by synchrotron radiation microdiffraction, Biomacromolecules*, ASAP, January, 2007

Abstract: A cotton fiber was kept under slight tension and exposed locally to a stream of aqueous 1N NaOH microdrops of 50 μm diameter. The resulting "macrodrop" of about 300 μm size was at the origin of the formation of Na-cellulose I domains extending about 550 μm from the centre of the macrodrop along the fiber. The phase transformation zone between cellulose I and Na-cellulose I was mapped by scanning synchrotron radiation microdiffraction using a 300nm*300 nm beam. In order to limit radiation damage a stitching technique was used. Subsequent exposure of the NaOH containing macrodrop to a stream of H_2O or HCl microdrops converted part of the Na-cellulose I back into cellulose I.

[7] R. Davies, *A new batch-processing data-reduction application for X-ray diffraction data*, J. Appl. Cryst., 39 (2006) 267-272

Abstract: Modern synchrotron radiation facility beamlines offer high-brilliance beams and sensitive area detectors. Consequently, experiments such as scanning X-ray microdiffraction can generate large data sets within relatively short time periods. In these specialist fields there are currently very few automated data-treatment solutions to tackle the large data sets produced. Where there is existing software, it is either insufficiently specialized or cannot be operated in a batch-wise processing mode. As a result, a large gap exists between the rate at which X-ray diffraction data can be generated and the rate at which they can be realistically analysed. This article describes a new software application to perform batch-wise data reduction. It is designed to operate in combination with the commonly used *Fit2D* program. Through the use of intuitive file selection, numerous processing lists and a generic operation sequence, it is capable of the batch-wise reduction of up to 60 000 diffraction patterns during each treatment session. It can perform automated intensity corrections to large data series, perform advanced background-subtraction operations and automatically organizes results. Integration limits can be set graphically on-screen, uniquely derived from existing peak positions or globally calculated from user-supplied values. The software represents a working solution to a hitherto unsolved problem.

Related scientific meetings

6th Elba Max Planck Forum: ***Nanoscale Science and Technology, Synchrotron Radiation and Nanobiosciences,***
Porto Conte - Sardinia (Alghero), Italy, 9-12. September 2004.
Organized by C. Niccolini, C. Riekel, P. Laggner and O. Paris.
http://www.fondazione-elba.org/nsito/portoconte04.html
The conference proceedings were published in a special issue of J. Synchr. Radiation (2005) 12, 711-785

CCP13: 14th Annual Fibre diffraction and Non-crystalline Diffraction Workshop
Cardiff University, 22th-24th June 2005
Organized by T. Wess
http://www.ccp13.ac.uk/workshop/workshop.htm

From Diffraction to Imaging: International Symposium on Scanning Microbeam Small- and Wide-Angle Scattering of Hierarchically Structured Materials,
Satellite Symposium of the BESSY User Meeting,
BESSY Berlin, 6th December 2006,
Organized by O. Paris and P. Fratzl
http://www.mpikg.mpg.de/aktuelles/veranstaltungen/archiv/index.html