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ESRF

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

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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.

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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:** Fibrillar and mineral level strains measured simultaneously in bone	**Experiment number**: SC 1814
Beamline: ID 13	**Date of experiment**: from: 03.11.2005 to: 07.11.2005	**Date of report**:
Shifts:	**Local contact(s)**: Peter Boesecke	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

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Wolfgang Wagermaier*

Jong Seto*

Paul Zaslansky*

Report:

The hierarchical structure of bone (Weiner and Wagner 1998) has been long implicated as a crucial reason for its outstanding mechanical properties, specifically a combination of stiffnes (modulus 10 – 20 GPa) along with a large toughness of 1 – 10 kJ/m^2, but the precise mechanisms have remained, until recently, unclear. At the nanometer level the bone matrix consists of mineralized collagen fibrils. These fibrils are a combination of type I collagen triple helical molecules impregnated with nanometer sized particles of carbonated apatite. Along with 1 – 2 wt. % of noncollageneous proteins (like osteopontin), the mineralized collagen fibrils aggregate into fiber bundles, lamellae, osteons and trabeculae to form the skeletal elements. Understanding how bone deforms and breaks – and how its nano- and microstructure contribute to these mechanisms – requires a simultaneous quantification of the effects of the different levesl of structural hierarchy. However, at the length scale below 1 microns, conventional macroscopic and light –microscopic techniques are unable to resolve the structural changes taking place during deformation. In this context, high brilliance synchrotron X – ray small angle scattering (SAXS) and wide angle diffraction (WAXD) can measure the changes in fibrillar (SAXS) and mineral strain (WAXD) in real-time, when combined with *in-situ* tensile testing(Gupta, Messmer et al. 2004; Gupta, Wagermaier et al. 2005). Using SAXS alone, we have recently shown that in bone, shearing between fibrils is a fundamental deformation mechanism(Gupta, Wagermaier et al. 2005). In this experiment, we took a significant further step toward understanding the deformation mechanisms one step further by combining a new sample preparation method (laser microdissection) with simutaneous measurements of the SAXS and WAXD signals on the same sample at the same time, to directly access three levels of hierarchical deformation – tissue, fibril, and mineral.

The small angle scatering siganl arising from the 67 nm periodicity in the collagen axial molecular packing, and the percentage change in this D – period is a measure of the fibril strain. The apatite mineral has a hexagonal cubic crystallographic texture, with the (0002) *c*-axis aligned along the fibril direction. Percentage shifts in the Bragg diffraction *c*-axis peak at d = 0.344 nm correspond, likewise, to changes in strain of the mienral nanoparticles along the fibril direction. As our experimental system, we used the parallel fibered bone from bovine femur. Bone blocks were sectioned into 200 μm sheets (Buehler ISOMET), which were then ground and polished down to 50 μm thick sheets (Logitech PM5). Using a ultraviolet laser microdissection system (P.A.L.M. Technologies, Germany), single bone packets were sectioned out from the thin bone sheets (Figure 1). These samples were fixed with cyanoacrylate to plastic tabs, with reference markers on the tabs. The tabs (with the sample) were clamped in the tensile grips in a specially designed tensile tester (250 gram maximum load) (Figure 2). The plastic tabs were clamped instead of the bone itself in order to reduce damage to the tissue. Machine compliance was calibrated as ~ 0.5 μm/N by testing progressively thicker layers of aluminium foil composites with the same tab system. To keep the sample hydrated during testing, a drop of saline was put on an X – ray transparent foil (Kalle GmbH, Germany) which was then gently touched to the clamped bone sample. By surface tension, the water film / plastic foil system stuck to the bone with negligible friction during tensile testing. A complementary foil on the other side of the sample was used as a lid to prevent evaporation. An example stress – strain curve is shown in Figure 3. By rotating the tensile tester on a tilt mounting stages available at the ID2 beamline (ESRF, Grenoble) to half the Bragg angle for the (0002) *c*-axis peak ($2\theta = 16.7°$), we access only the shift in *c* –axes arising due to those crystallites (fibrils) oriented along the stretch direction (taking into the account that every sample has some proportion of fibers oriented away from the tensile axis). SAXS frames were acquired with a Mar CCD detector positioned at 10.0 m downstream from the sample, while WAXD reflections are acquired using a Photonics detector mounted vertically and off axis from the direct X – ray beam. Continuous stretch to failure measurements at a (slow) strain rate of 0.0033 $\%.s^{-1}$ were carried out simultaneously with successive SAXS/WAXD measurements at different stress levels up to tissue failure. Data analysis is being done with a suite of software tools developed by Peter Boesecke for data evaluation at the ID2 beamline. The resultant parameters include macroscopic stress, macroscopic strain, fibril D spacing, and (0002) lattice spacing at different stress levels. An example of the variation of the fibrillar and (0002) *c*-axis peak positions on application of tissue strain is shown in Figure 4.

Preliminary results show clearly the existence of three levels of hierarchy in the deformation: the largest (tissue) deformation, followed by a reduced deformation at the fibrillar level (due to shear in the intervening matrix (Gupta, Wagermaier et al. 2005), and a reduced deformation also at the mineral nanoparticle level. While a detailed quantitative evaluation is underway, the mineral particles are seen to stretch even less than the fibrils, as expected. We have therefore shown directly the hierarchical nature of bone deformation at different length scales by means of a novel combination of SAXS, WAXD and tensile testing, made possible due to the high flux due to synchrotron radiation, which enables time – resolved measurements.

We thank Peter Boesecke, T. Narayan, and Pierre Panine for their outstanding scientific and technical assistance during the beamtime.

References:

Gupta, H. S., P. Messmer, et al. (2004). "Synchrotron Diffraction Study of Deformation Mechanisms in Mineralized Tendon." Physical Review Letters **93**(15): 158101.

Gupta, H. S., W. Wagermaier, et al. (2005). "Nanoscale deformation mechanisms in bone." Nano Letters **5**(10): 2108-2111.
Weiner, S. and H. D. Wagner (1998). "The material bone: Structure mechanical function relations." Annual Review of Materials Science **28**: 271-298.

Figures :

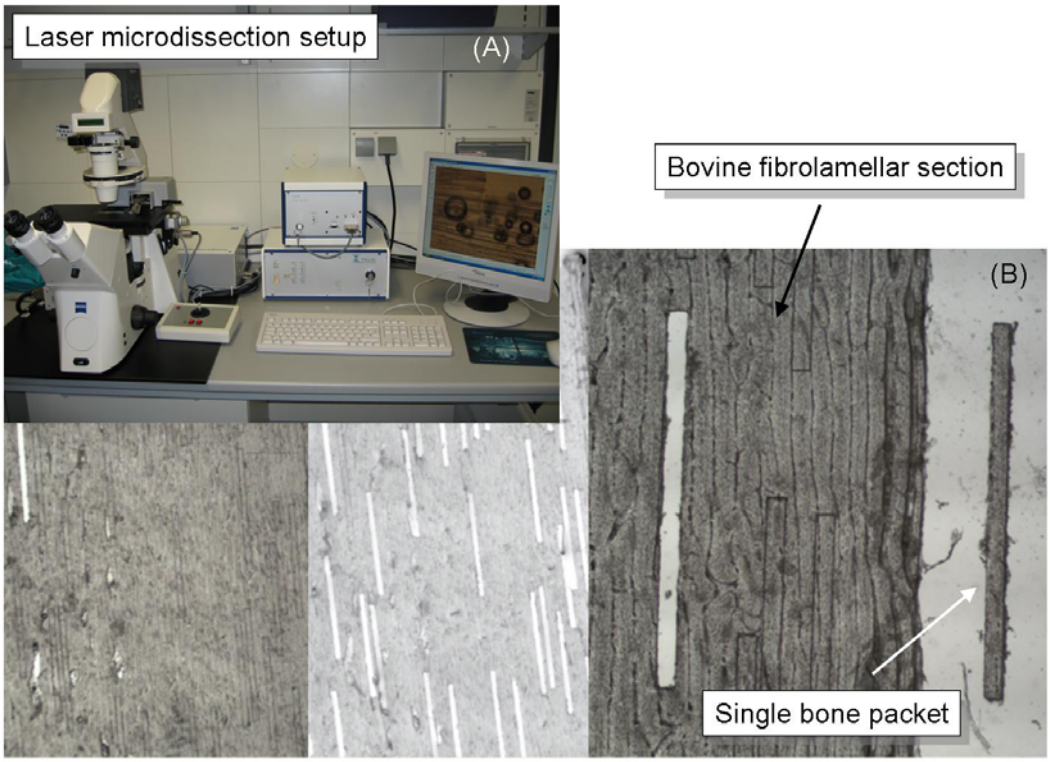

Figure 1: Samples are prepared using a laser microdissection apparatus shown in (a). Using this setup, microtensile test specimens can be sectioned out of 50 μm thick sheets of bovine fibrolamellar bone, as shown in (b)

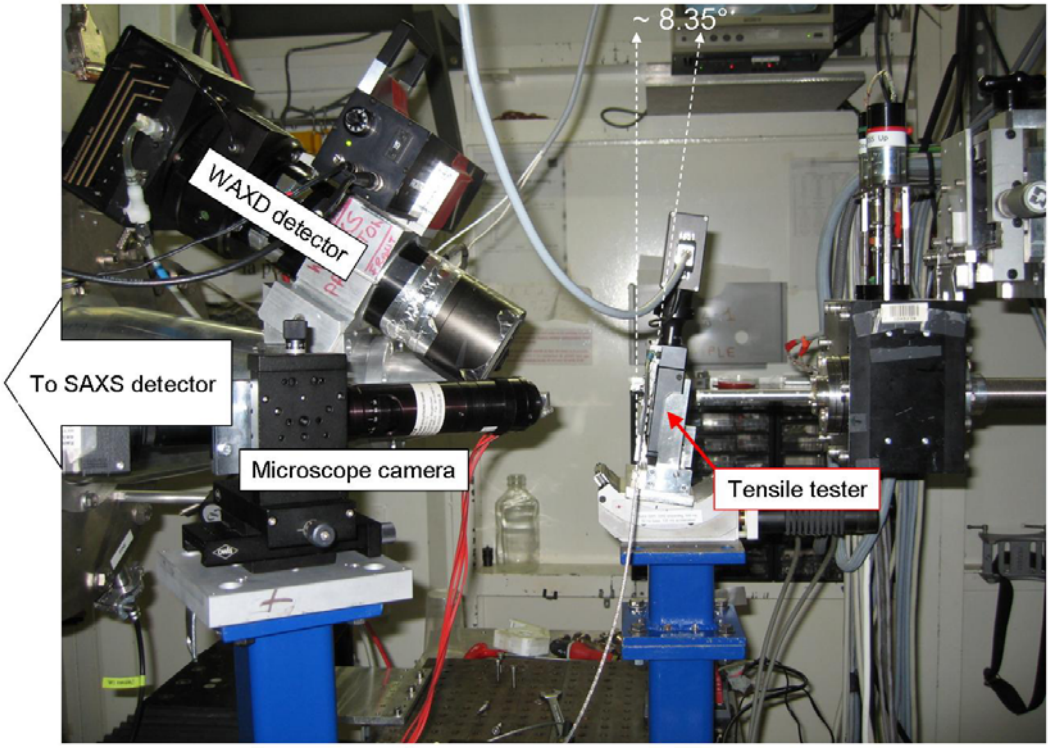

Figure 2: Microtensile setup at ID2, showing the tilt in the tensile tester required to visualize the (0002) apatite c-axis reflections along the tensile direction. The incident beam is from the right and the SAXS detector is 10 m downstream from the sample (left in the picture).

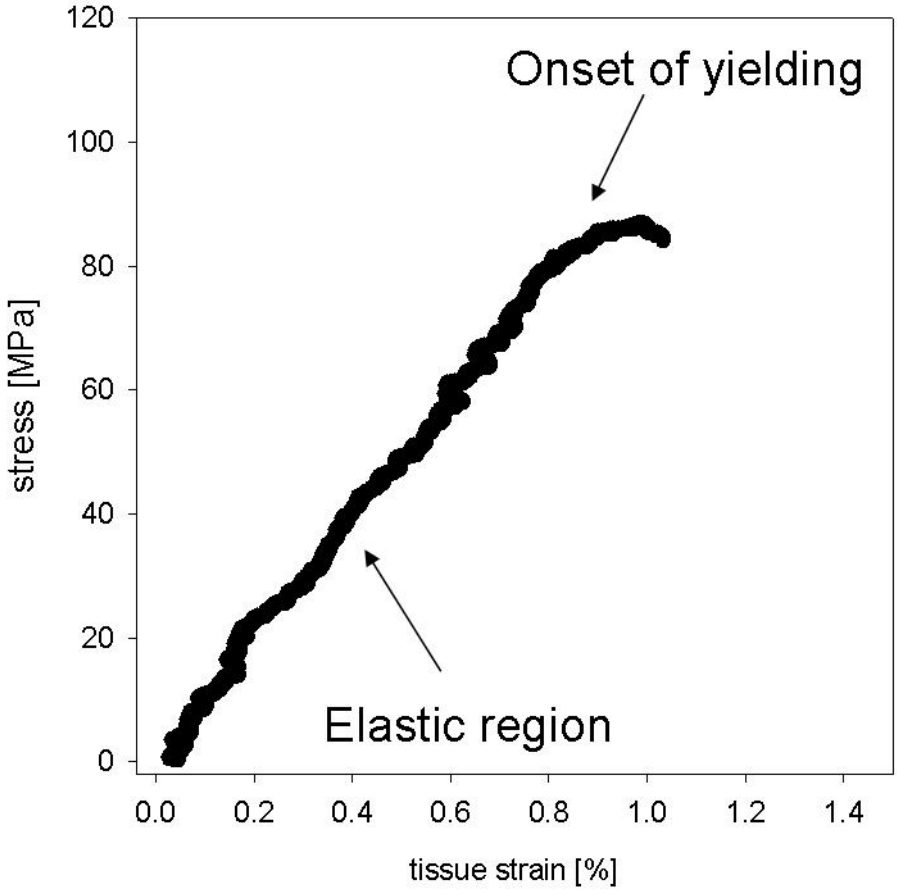

Figure 3: Example stress strain curve for bone, showing the yield region

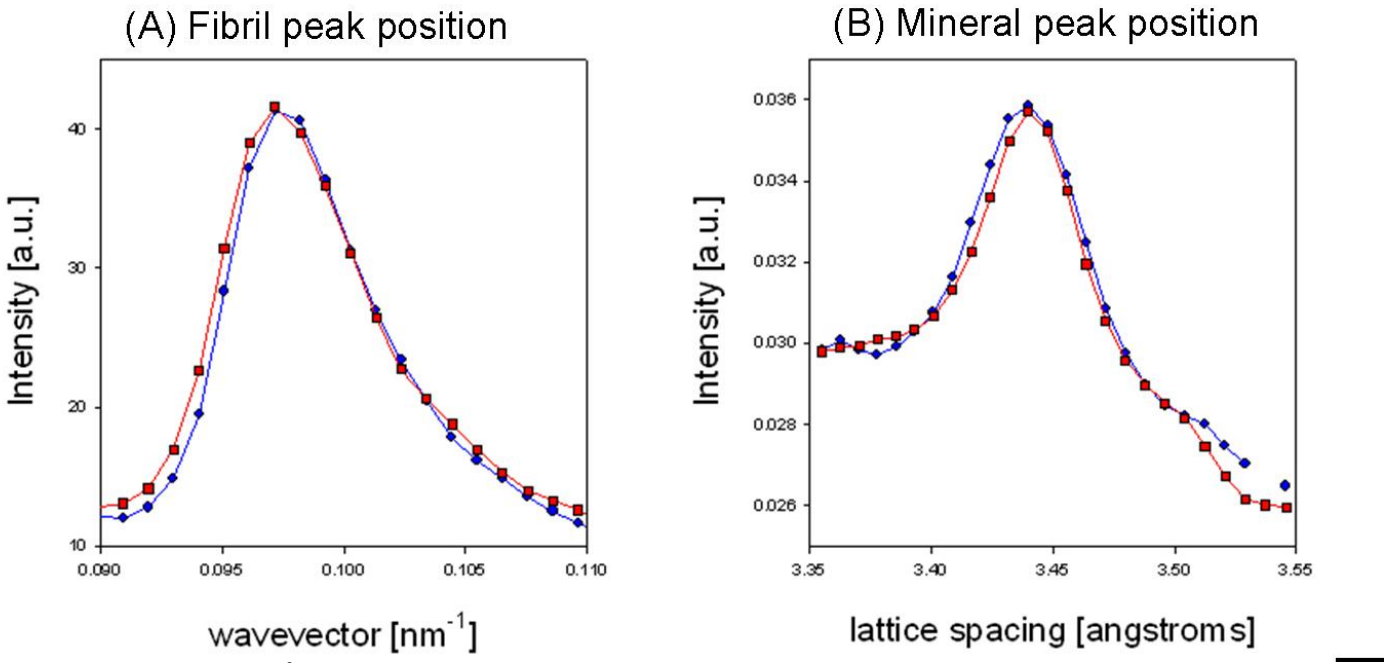

Figure 4: (a) Shift of 3rd order collagen peak to lower wavevector on application of strain. Blue curve shows the unstrained peak position and red curve shows the position of the fibril peak when the tissue is strained upto ~ 0.5 % (b) Analogous plot for the same sample for the mineral c-axis (0002) reflection, showing a shift to larger lattice spacing when strained. Because the second plot is plotted against lattice spacing instead of wavvector, the curve shifts to the right instead of the left when strained, in contrast to (a).