Characterising natural fibre composites with hierarchical structure

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Received 2nd January 2002; accepted in revised form 16th April 2002.

Many biological tissues, such as wood and bone, are fibre composites with a hierarchical structure. Their exceptional mechanical properties are believed to be due to an optimisation of the structure at all levels of hierarchy. Wood, for instance, is essentially made of parallel hollow tubes, the wood cells. The cell walls are a composite of cellulose fibres in a matrix of lignin and hemicellulose. Bone, on the other hand, is a composite of collagen fibrils reinforced with calcium-phosphate mineral nano-particles. These mineralised fibrils are assembled into laminates which are the basis for a large variety of structures at higher hierarchical levels in bone.

X-ray fibre diffraction and/ or small-angle scattering are ideal tools to characterise the fibre orientation (texture) and the size and arrangement of the components in the nanocomposite. Given the hierarchical nature of natural tissues, it is essential to use position-resolved diffraction methods. By such an approach, it is possible to scan a specimen and obtain information on several hierarchical levels simultaneously: the diffraction experiment yields information on the nanometer level, while the scanning procedure visualises the specimen with a resolution corresponding to the x-ray beam diameter, that is, in the micrometer range. The present paper reviews some recent experiments carried out with the goal to elucidate the relation between structure and mechanical function in wood, bone and dentin.

Introduction

Biological tissues such as wood, bone or tooth are hierarchically structured to provide maximum strength with a minimum of material. This is the reason why these materials are cellular solids (e.g., cancellous bone or wood) or gradient materials (e.g., dentin). At the lowest level of hierarchy (that is, in the nanometer range), they are fibre composites. A powerful approach to study such hierarchical materials is scanning x-ray diffraction [1] or scanning small-angle x-ray scattering [2]. The principle is shown in Fig. 1.

A thin section of the material is scanned across a narrow x-ray beam. The diameter of the x-ray beam defines the lateral resolution of the scanning procedure. It is in the order of 100 micrometer on a laboratory x-ray source [1] and in the order of 1 micrometer (or even below) at synchrotron sources [2,3]. Ideally, the thickness of the specimen should be the same as the beam diameter, d. Then the scattering volume for each individual measurement will be about d^3. Depending on the type of measurement - fibre diffraction or small-angle scattering - the evaluation of the scattering patterns yields information on the nanocomposite, within each d^3-volume separately. Such local information from x-ray scattering can be advantageously combined with local information from other techniques, e.g. microspectroscopy or nanoindentation. The following examples are chosen to illustrate the capabilities of position-resolved x-ray diffraction methods for characterising hierarchically structured materials.

Mineral particles in calcified tissue, such as bone and dentin

The mechanical properties of calcified tissues cannot be understood without taking into account all structure levels [4]. Some anatomical features known to be important for the strength of the tissue are given on a dimensional scale in Fig. 2.

Even though the list is certainly not complete, it is obvious that the structures are spread over at least eight orders of magnitude. Clearly, no single technology can cover such a wide range. While all structures down to about a micrometer in size are accessible to light microscopy, higher resolution can be achieved by other probes, such as scanning- or transmission-electron microscopy, x-ray diffraction

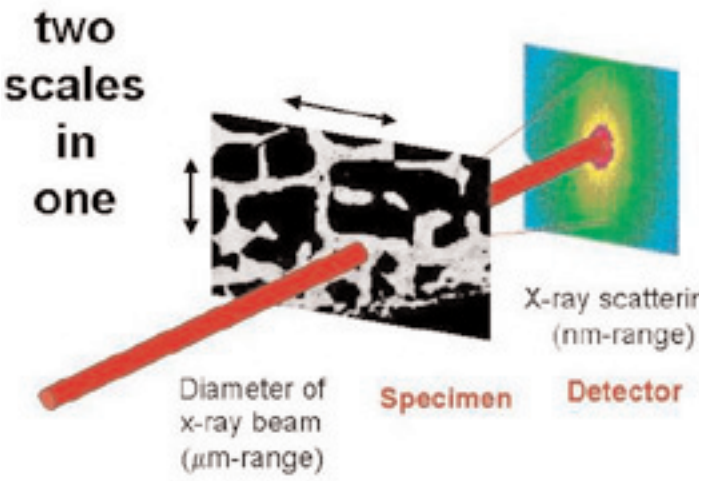

Figure1: Sketch of a scanning diffraction experiment. Structural information in the nanometer range is obtained from the evaluation of the diffraction (or scattering) pattern. The micrometer range is covered by scanning the specimen across the narrow x-ray beam.

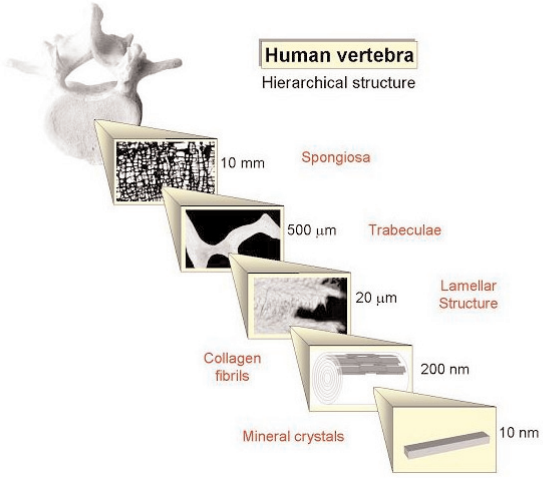

Figure 2: Hierarchical structure of the human vertebra, as an example of cancellous bone. The human vertebra is filled with a highly porous structure of spongy appearance. The trabeculae forming this cellular material are typically 200 micrometer wide and made of a composite of collagen and calcium phosphate mineral. This composite has a lamellar structure, where each lamella consists of a layer of parallel mineralised collagen fibrils. The orientation of the fibrils turns from lamella to lamella in way similar to a plywood structure. Individual collagen fibrils have a diameter of a few hundred nanometers, while the individual reinforcing mineral particles have a thickness of only a few nanometers.

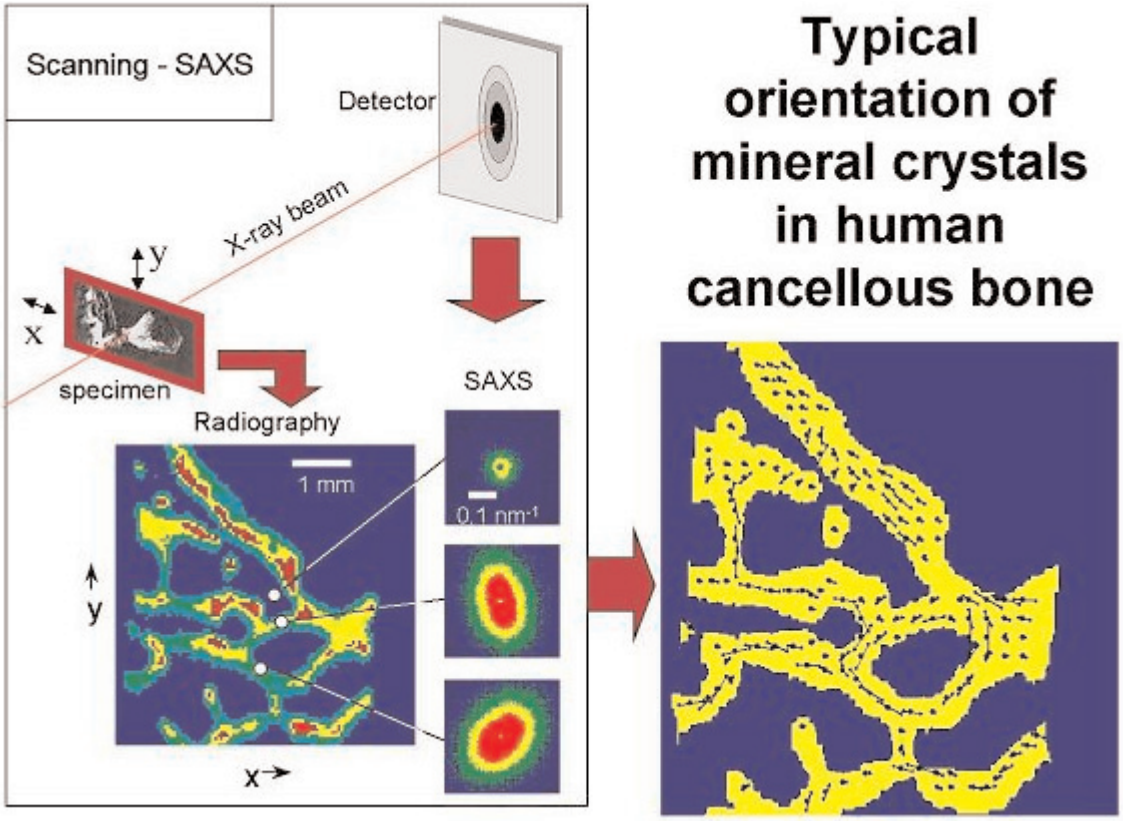

Figure 3: Scanning-SAXS investigation of human vertebra (from [5]). The specimen was a 200 micrometer thick bone section embedded in resin. First, the specimen was imaged by x-ray transmission (radiography). Transmission is high when the x-ray beam hits the (fully organic) resin and is low when it hits bone (which is a mixture of organic matrix and calcium phosphate). The trabeculae (see also Fig. 2) are clearly visible on this image. A number of positions were then chosen to perform SAXS-measurements. Clearly, SAXS-patterns are different at different positions. The evaluation of the SAXS-patterns gives the thickness and the orientation of the mineral particles in the organic matrix. As an example, the orientation is plotted by bars in the right side of the figure. Long bars mean high alignment, shorter bars less pronounced alignment of the particles within the volume of each individual measurement (in the present experiment, performed on a laboratory source, this volume was typically a cube with 200 micrometer side length).

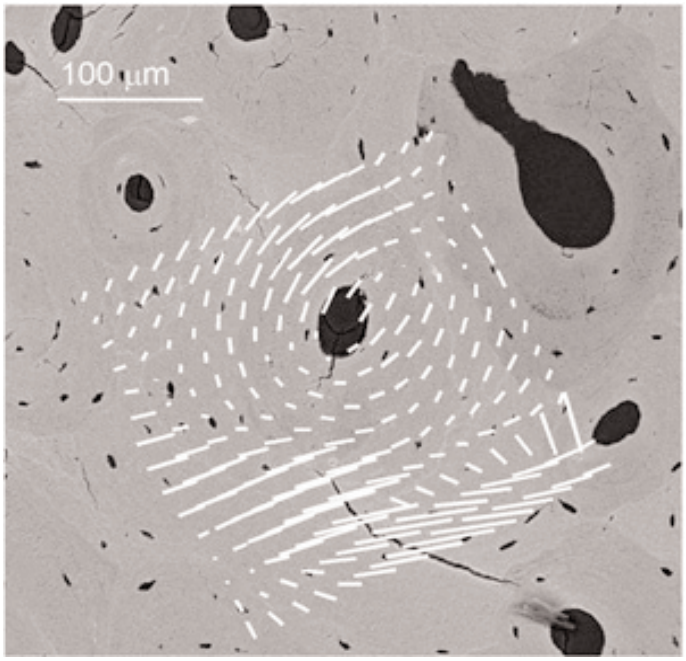

Figure 4: Orientation of mineral particle around an osteon in human compact bone (from [6]). The black ellipse in the centre is the trace of a blood vessel and there are concentric layers of bone lamellae around it, forming the osteon. Several osteons are visible on the backscattered electron image (BEI). The bars are results from scanning-SAXS, obtained at the synchrotron ELETTRA and superimposed on the BEI. They indicate the orientation of mineral platelets with the same convention as in Fig. 3. The specimen thickness and the diameter of the x-ray beam were 20 micrometers in this case.

(XRD) or small-angle x-ray scattering (SAXS) and, finally, by a variety of spectroscopic techniques, e.g., nuclear magnetic resonance or Fourier-transform infrared spectroscopy. One of the consequences of the hierarchical architecture is that nanometer-sized structures may vary systematically throughout the tissue on a larger (typically micrometer-sized) scale. This introduces the technical difficulty that the nanometer-scale structures need, in principle, be characterized in a position-resolved way. A very powerful approach in this respect is scanning XRD or scanning-SAXS. A recent review can be found in [1]. The SAXS-contrast in bone or dentin is due to the huge electron density difference between the mineral particles and the organic matrix. SAXS is particularly useful since the typical thickness of the mineral particles is in the order of a few nanometers. Typical results of scanning-SAXS investigations of human bone are shown in Fig. 3 and Fig. 4. The first figure shows the typical data collection procedure.

First, the bone section is placed on an x-y stage movable perpendicularly to the x-ray beam. Ideally, the thickness of this section should be of the same order of magnitude as the diameter d of the x-ray beam, which defines the scanning resolution on the specimen. By this choice, a specimen volume of d^3 will be probed in each SAXS measurement. Using a laboratory x-ray source, d is limited by the counting statistics in the diffracted signal and cannot be chosen smaller than about 100 mm [2]. Using synchrotron radiation, d can be as small as 1 µm [1]. In the next step of the experiment, the bone section is scanned quickly across the x-ray beam to determine the transmission at each (x,y) position. A plot of

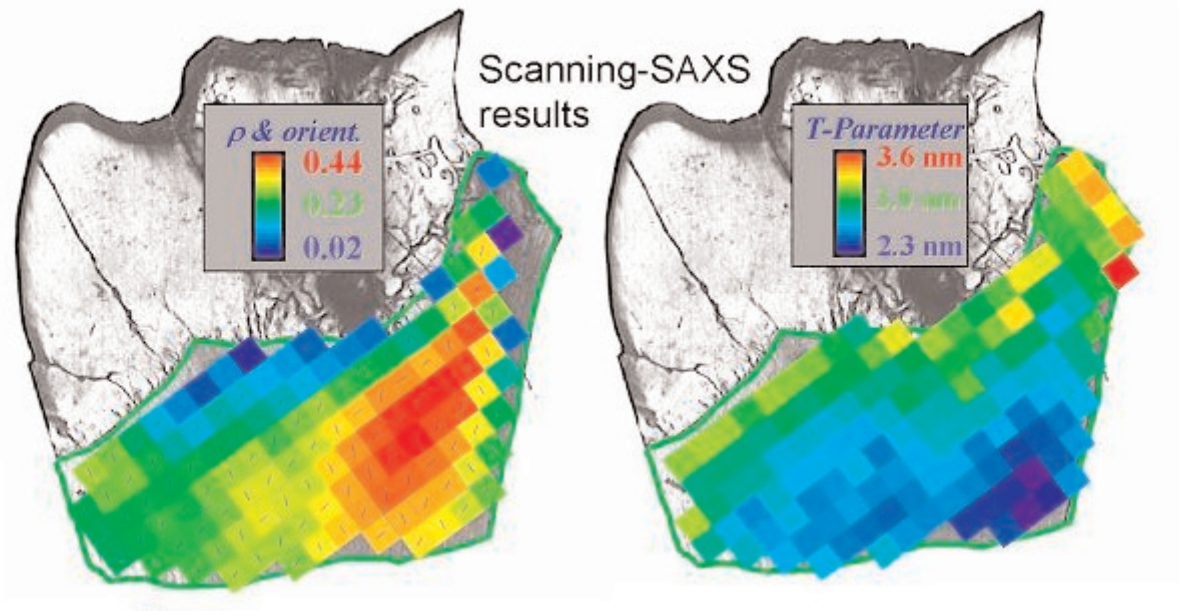

Figure 5: Thickness (right) and orientation (left) of mineral particles in human dentin (from [8]). The T-parameter, which is a measure for the particle thickness, varies from 2.3 to 3.6 nanometers. The degree of alignment r (a parameter which is =1, if all the mineral platelets are parallel, and =0, if they are randomly oriented) is larger further away from the enamel (top). The thickness of mineral particles correlates well with the elastic modulus measured (in position-resolved way) on the same specimen by nanoindentation [8].

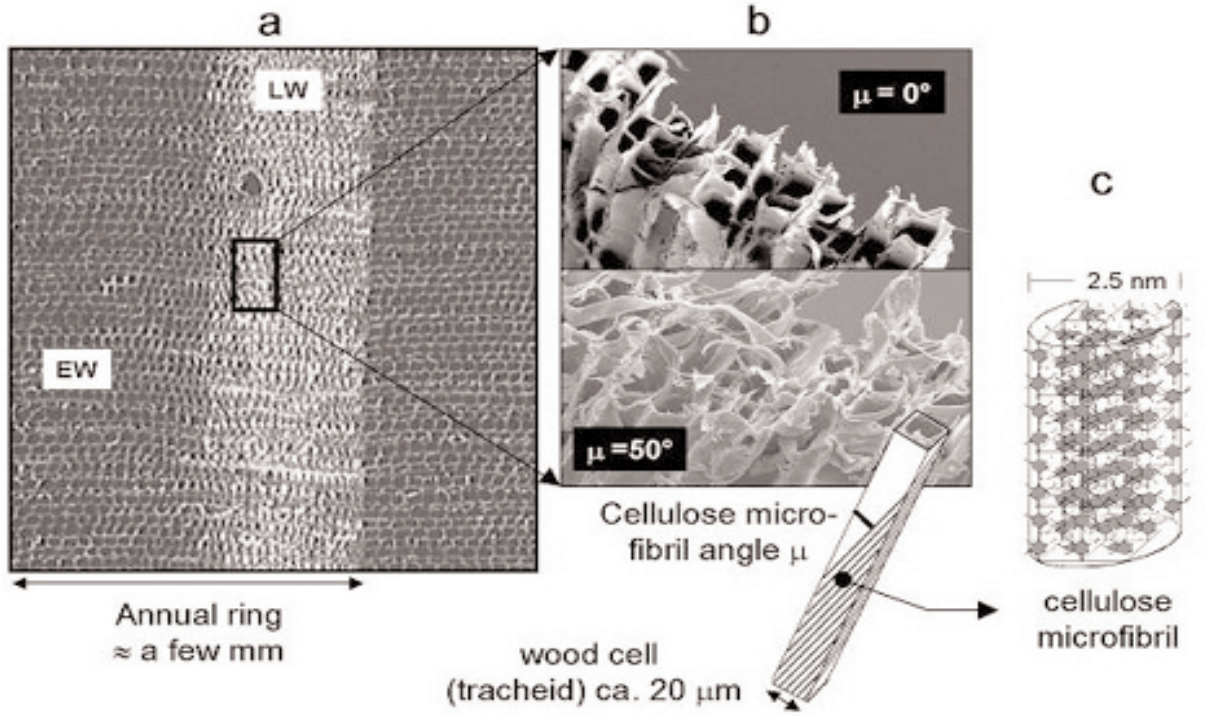

Figure 6: Hierarchical structure of spruce wood. (a) is a cross-section through the stem showing earlywood (EW) and latewood (LW) within an annual ring (from [9]). Latewood is denser than earlywood because the cell walls are thicker. The breadth of the annual rings varies widely depending on climatic conditions during each particular year. (b) shows scanning electron microscopic pictures of fracture surfaces of spruce wood with two different microfibril angles (from [10]). One of the wood cells (tracheids) is drawn schematically showing the definition of the microfibril angle between the spiralling cellulose fibrils and the tracheid axis. (c) is a sketch of the (crystalline part) of a cellulose microfibril in spruce (from [11]).

these data gives an image called "radiography" in Fig. 3. This image can serve two purposes: first, it is very useful to decide at which positions to collect SAXS data. The radiography in Fig. 3 shows a typical section through spongious bone, as one would find, e.g., in human vertebra or in clinical biopsies from the iliac crest. Clearly, there are many positions where one would only find the embedding resin and, therefore, no signal from bone (blue regions). Useful positions for the SAXS measurements (red or yellow areas which reveal bone) can easily be identified on this image.

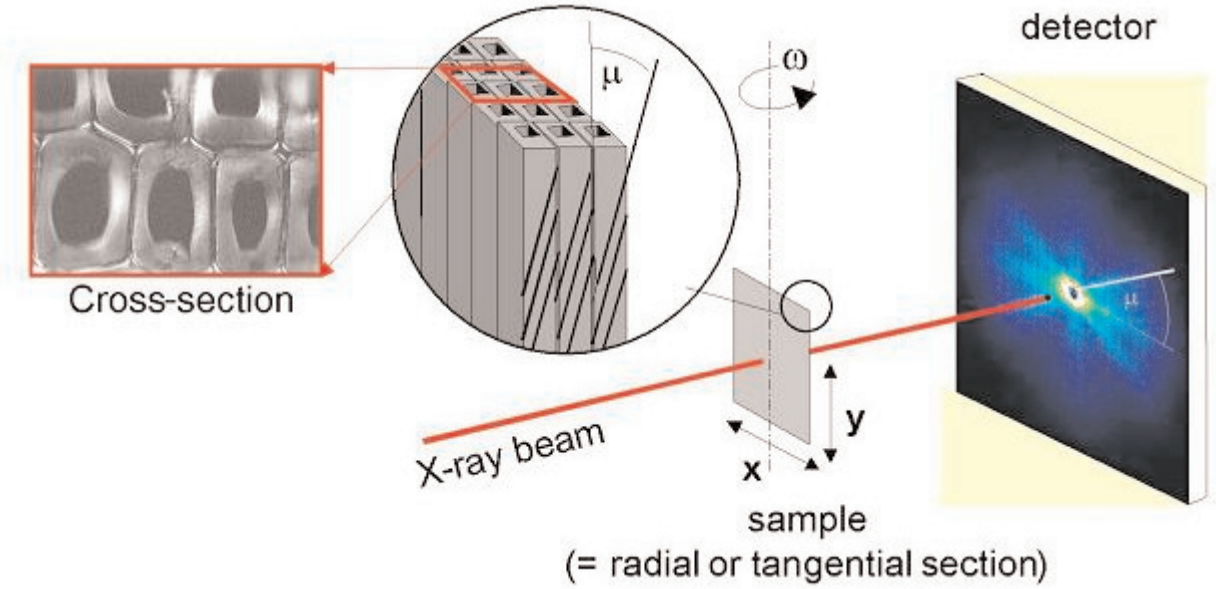

Figure 7: SAXS-signal from radial or tangential wood sections (the example shown is spruce wood), where the macroscopic fibre direction (the axis of the wood cells) is within the plane of the section (vertical in the example). The SAXS pattern shows several streaks originating from the cellulose fibrils tilted by an angle μ with respect to the macroscopic fibre direction. In normal spruce wood (see microscopic image on the left), the cross-section of the cells is rectangular. Hence, there are four different streaks on the detector, corresponding to cellulose fibrils in the four sides of the rectangular cells. If the distance specimen to detector is reduced sufficiently, it is also possible to measure the diffraction pattern from the crystalline parts of cellulose (see Fig. 8).

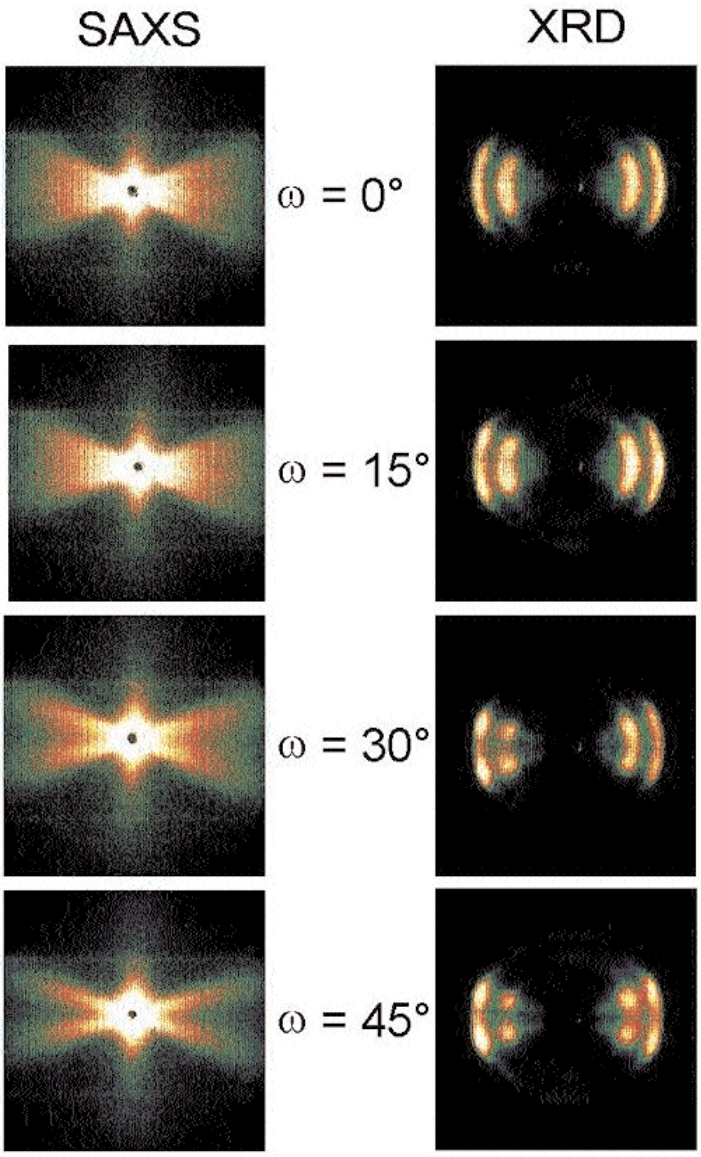

Figure 8: SAXS (left) and XRD (right) from cellulose fibrils in the wood cell wall of spruce wood, measured in the geometry shown in Fig. 7, for different rotation angles ω of the wood section. In all cases, the axis of the wood cells is oriented vertically. For ω=0, the x-ray beam hits the rectangular tubes perpendicularly to one side , for ω=45°, the x-ray beam hits the (nearly square) tubes perpendicularly to an edge. Both, SAXS and XRD-signals can be used to extract the MFA (from [16]).

Secondly, radiographs can also be used to correlate the exact positions where SAXS data were collected to results from other measurements, such as light or scanning-electron microscopy [5]. The principle is to match the radiography to the corresponding light-microscopy image from the same specimen. The experience shows that the matching procedure works well, down to a precision of a few microns. Typical SAXS patterns obtained from bone are anisotropic (see Fig. 3) and it is possible to extract information on thickness and orientation of the (usually plate-shaped) mineral particles embedded in the organic matrix [5]. The rightmost image in Fig. 3 shows the typical orientation of the elongated mineral nano-particles at many positions within the bone section. The direction of each bar gives the orientation of the longest axis of the particles within the plane of section. The length of the bar is a measure for the degree of alignment which is reflected in the anisotropy of the SAXS pattern. It is obvious in Fig. 3 that the orientation of the mineral particles closely follows the orientation of the bone trabeculae in the spongy structure.

Fig. 4 shows similar kind of data, but collected within compact (and not spongious) bone, using an x-ray beam diameter of 20µm (that is, with a ten times better scanning resolution than in Fig. 3).

The orientation of the nano-particles reflects the layered structure of an "osteon" which is the tissue surrounding a blood vessel embedded in bone. The concentric arrangement of the layers is a mechanical protection of the blood vessel [1].

More recently, the structure of dentin (the bone-like

body of teeth) was studied by scanning-SAXS [7,8]. A gradient from the enamel towards the root was found both for the structure and for the mechanical properties [8]. Fig. 5 shows the orientation and the thickness of mineral particles in dentin as a function of position.

The figure shows that the T-parameter (which is a measure of the thickness of mineral particles) increases systematically from the enamel towards the root. The same section was also investigated by nano-indentation in an atomic force microscope, providing the elastic modulus of the tissue as a function of position. This mechanical parameter also exhibited a gradient from the enamel towards the tooth and was plotted against the particle thickness (as measured by the T-parameter) determined at the same position on the specimen. The excellent correlation between these two parameters is also shown in Fig. 5. This provides some insight on how the mechanical properties of mineralised tissue can be tuned by the type of reinforcing mineral particles. The probable reason for the grading of properties is a better long-term stability against failure of the tooth [8].

Cellulose microfibril angle in wood

The hierarchical structure of wood is shown in Fig. 6. At the lowest level, wood is a composite of cellulose microfibrils with a diameter in the order of a few nanometers and a matrix of lignin and hemicellulose. The cellulose fibrils are wound around tube-like wood cells with an angle called the microfibril angle (MFA, see Fig. 6). The MFA determines to a large extent the elastic modulus and the fracture strain of wood: When the MFA varies from 0 to 50°, the elastic modulus decreases by about an order of magnitude and the fracture strain increases by a similar factor [12].

Both XRD [11,13,14,16,17] and SAXS [9,11,15,16,18,19] have been used widely for the investigation of wood and, in particular, for the measurement of the MFA. Three geometries have been employed. First, radial or tangential wood sections were investigated as shown in Fig. 7.

In this case, the axis of the wood cells (that is, the

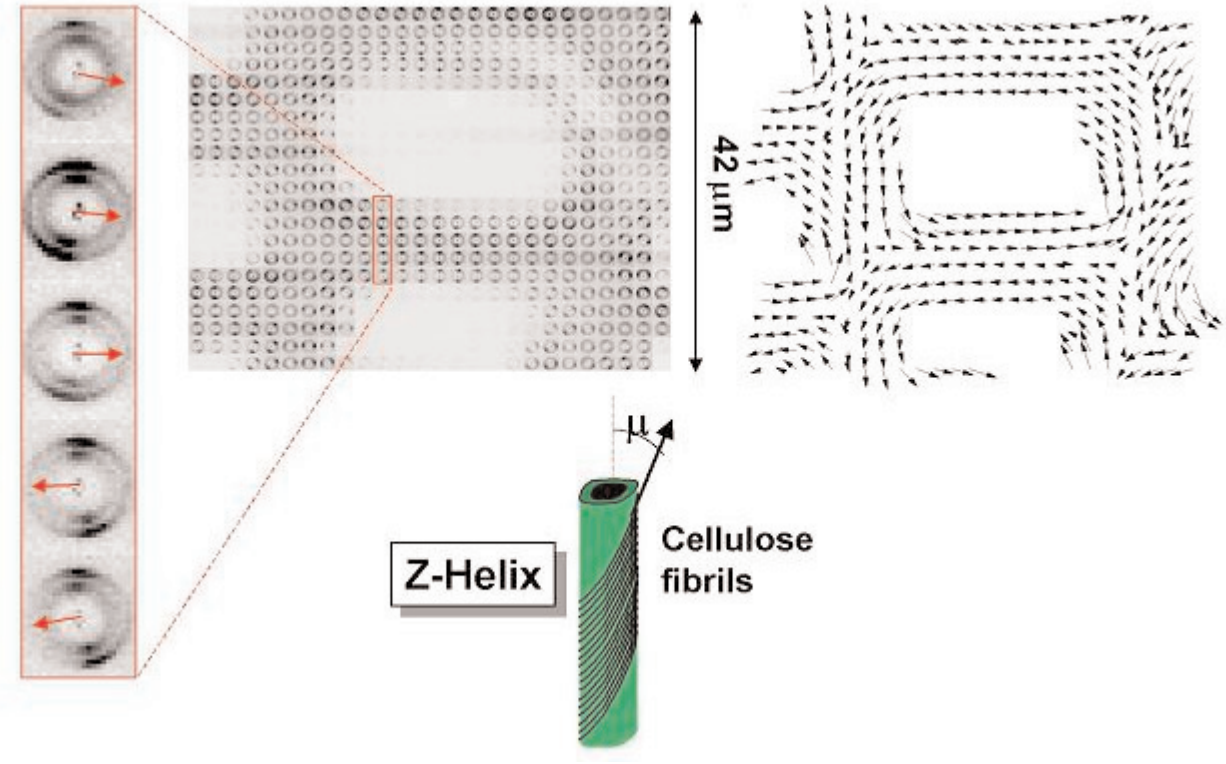

Figure 9: X-ray microdiffraction experiment with a 2 mm thick section of spruce wood embedded in resin (from [17]). Left: typical XRD-patterns from the crystalline part of the cellulose fibrils. Each pattern has been taken with a 2mm wide x-ray beam (at the European Synchrotron Radiation Source). The diffraction patterns are drawn side by side as they were measured. They reproduce several wood cells in cross-section. Note the asymmetry of the patterns in the enlargement (far left) which can be used to determine the local orientation of cellulose fibrils in the cell wall (arrows) [17]. The arrows are plotted in the right image with the convention that they represent the projection of a vector parallel to the fibrils onto the plane of the cross-section. The picture clearly shows that all cells are right-handed helices

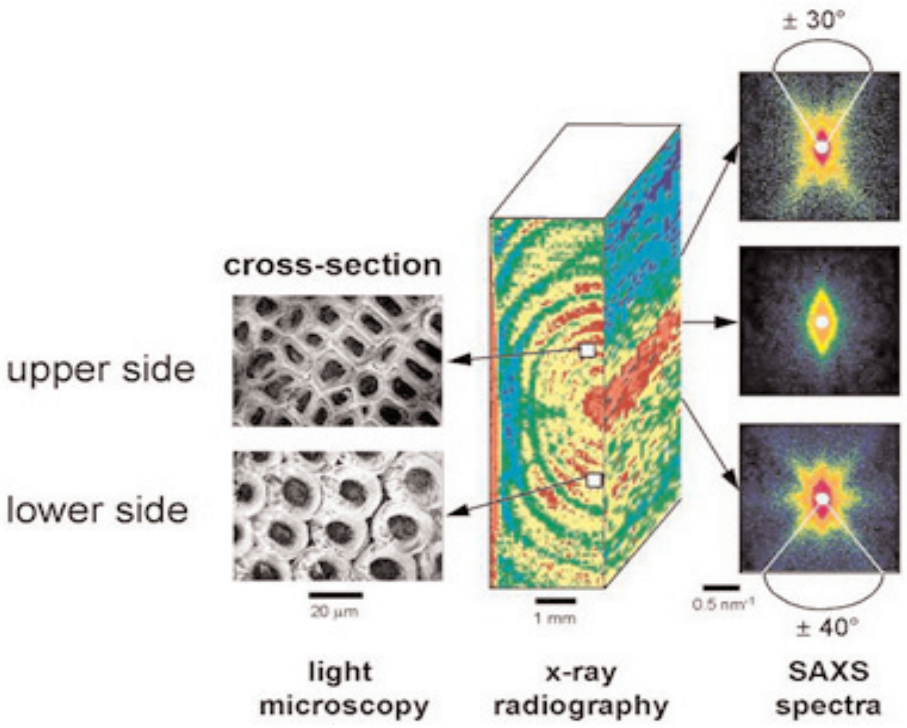

Figure 10: SAXS-patterns from a spruce branch (from [18]). Left: light-microscopic images of cross-sections. Note the round cell shape on the compression side (lower side) of the branch. Centre: X-ray transmission micrograph showing the annual rings. Right: SAXS-patterns showing an MFA of 30° on the upper side and an MFA of 40° on the lower side of the branch.

macroscopic fibre direction) is within the plane of the section. The direction of the cellulose fibrils is tilted by an angle m (the MFA) with respect to the cell axis. In SAXS, the contrast is due to the difference in electron density between cellulose and the surrounding matrix. In XRD, the contrast arises from the (partly) crystalline nature of cellulose, while the matrix is amorphous. For both types of measurement, a strongly anisotropic signal is expected from the cellulose fibrils, which may be used to determine µ. The difficulty is that the cellulose fibrils are actually spiralling around the wood cells, which means that several orientations of the fibrils superimpose in the scattering pattern. For cells with rectangular cross-section (as usually found in normal spruce wood, for instance) this means that the scattering pattern depends on the orientation angle ω of the cells with respect to the x-ray beam (the angle ω is defined in Fig. 7). This is demonstrated in Fig. 8, where both SAXS and XRD-signals are shown for different values of ω. The simplest case is when the x-ray beam hits wood cells with nearly square shaped cross-section perpendicularly to an edge of the tube (ω=45° in Fig. 8). Then, only two streaks appear in the SAXS-pattern and the smallest angle between them is simply 2µ [16].

The third possibility which has been exploited to measure µ, is shown in Fig. 9. This technique is suited for investigating cross-sections (rather than radial or tangential sections). It has been used to determine the orientation distribution of cellulose fibrils within individual cells, with micrometer resolution [17]. The principle is based on the fact that - due to the curvature of the Ewald sphere - the diffraction pattern from cellulose fibrils becomes asymmetric in this geometry (see Fig. 9, left). Since the direction of the asymmetry depends on the local orientations of the fibrils by a simple formalism (given in detail in [17]), a map of fibril orientations can be drawn, demonstrating that the cellulose fibrils form right-handed helices around the spruce wood cells.

A final example is given in Fig. 10, where the MFA was determined in a branch of spruce. Clearly, not only the cell shape is different between the upper and the lower side of the branch (left side in Fig.10), but also the MFA (right side in Fig. 10). It was shown recently that new wood, growing on the outside of an existing branch, has an MFA which tends to accommodate the mechanical properties to the increasing weight and length of the branch [20].

The author thanks all collaborators involved during

the last few years in the reported studies, in particular: J. Färber, H. Lichtenegger, O. Paris, W. Tesch, I. Zizak (Erich Schmid Institute, Leoben, A); M. Müller, Ch. Riekel (ESRF, Grenoble, F); H. Amenitsch, S. Bernstorff (ELETTRA, Trieste, I), A. Reiterer, S. Tschegg (Univ. Agriculture, Wien, A); P. Roschger, K. Klaushofer (Ludwig-Boltzmann Institute of Osteology, Wien, A).

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