Mueller, M.; Krasnov, I.; Ogurreck, M.; Blankenburg, M.; Pazera, T.; Seydel, T.:

Wood and Silk: Hierarchically Structured Biomaterials Investigated In Situ With X-Ray and Neutron Scattering

In: Advanced Engineering Materials (2011) Wiley

DOI: 10.1002/adem.201000347

DOI: 10.1002/adem.((please add manuscript number))

Wood and silk: Hierarchically structured biomaterials investigated *in situ* with X-ray and neutron scattering

By *Martin Müller*, Igor Krasnov, Malte Ogurreck, Malte Blankenburg, Tomasz Pazera,* and *Tilo Seydel*

[*] *Prof. Dr. M. Müller, M. Ogurreck, M. Blankenburg*
Helmholtz-Zentrum Geesthacht
Max-Planck-Straße 1, 21502 Geesthacht, Germany
E-mail: martin.mueller@hzg.de
I. Krasnov, T. Pazera
Institut für Experimentelle und Angewandte Physik
Christian-Albrechts-Universität zu Kiel, 24098 Kiel, Germany
Dr. T. Seydel
Institut Laue-Langevin
B. P. 156, 38042 Grenoble Cedex 9, France

Synchrotron radiation X-ray and neutron scattering techniques are very useful tools for the non-destructive analysis of the structure of biopolymer materials such as wood and silk, making the in situ *investigation of structural changes upon mechanical stress possible. The low-divergence synchrotron radiation X-rays can be focused down to sub-micrometer size, enabling scanning studies of the wood nanostructure with (sub-)microscopic position resolution. This article highlights very recent advances in the understanding of silk and wood micro- and nanostructure, which were only possible using synchrotron radiation and neutrons. Examples include the local breakdown of cellulose fibre texture in wood cell walls, the deformation mechanism of a single wood cell, the viscoelastic properties of silk and insight into molecular mechanisms in silk upon mechanical deformation.*

Introduction

X-ray and neutron scattering techniques have become well-established in the field of non-destructive structural analysis of structural biopolymer materials such as wood and silk. The high sensitivity of diffraction methods to the crystalline properties of wood cellulose and of the protein nanocrystals in silk as well as to the fibrillar morphology of those composite materials has contributed to the success of such investigations. It is difficult, however, to access the embedding disordered matrix with diffraction; here, locally sensitive techniques, particularly inelastic neutron scattering, provide valuable complementary information. Experiments using conventional X-ray generators (i. e., sealed tubes or rotating anodes) are limited by the available X-ray flux. The largely increased flux available at synchrotron radiation sources makes it possible to carry out time-resolved *in situ* experiments e. g. during mechanical tests of specimens. The reaction of the biomaterial's nanostructure to mechanical stress thus becomes directly accessible. Furthermore, synchrotron radiation has a very low divergence enabling focusing of X-rays using various optical devices down to micrometre beam size and below. In microdiffraction experiments, the nanostructure is accessible via the atomic resolution of X-ray diffraction and the microstructure via scanning of the sample through the X-ray beam with microscopic position resolution. These parameters are even obtained simultaneously in a single experiment. — Neutron scattering experiments obviously involve the use of large scale facilities as well, i. e., the neutron sources (nuclear reactors or accelerator-based spallation sources). Their combination with e. g. mechanical tests *in situ* is possible by using specialised sample environments and by relying on the high neutron beam stability in combination with negligible beam damage induced by neutrons, making longer exposure times possible.

The aim of the current article is to demonstrate the possibilities of using synchrotron radiation and neutron scattering techniques in the materials science of wood and silk. After introducing the materials and highlighting the importance of scattering experiments to determine many

aspects of their structure, we will give an example of how the local structure of wood cells on a nanoscopic level can be probed by focused synchrotron radiation. This is followed by the presentation of an *in situ* tensile test experiment on single wood cells, giving insight into the deformation mechanisms of this unique material. A similar X-ray scattering experiment on silk fibres provides access to an understanding of silk as a nanocomposite material. We will show how neutron scattering provides complementary information, in particular with respect to the disordered structures in this protein fibre. The high sensitivity of neutrons for hydrogen and the contrast variation by hydrogen-to-deuterium exchange is crucial in this context.

Structure of silk and wood

Like almost all structural biological materials, silk and wood are semicrystalline nanocomposites. Chemically, they are entirely based on organic polymers. Silk strands (baves) produced by the silkworm *Bombyx mori* are paired individual fibres (or brins) cemented together and coated by the protein sericin. Removing sericin (degumming) with warm water yields single fibres of about 10 µm in diameter consisting almost entirely of the protein fibroin. The natural spinning process leads to the aforementioned composite morphology with ordered regions (β-sheet protein nanocrystals) embedded in a less stiff matrix of disordered material.[1] All dimensions of the nanocrystals are below 10 nm.[2] To date, artificial spinning of fibroin does not reproduce the native nanostructure and thus not the unique mechanical properties combining high tensile strength with superior extensibility.[3]

In the case of wood, the cells walls are made of a number of different polymers. The crystalline units of cellulose are called *microfibrils*. Their size is characteristic for a given plant species and may also vary between different cell wall layers. In the case of softwood, typical microfibril sizes of around 3 nm laterally (i. e., perpendicular to the chain direction) are found in the secondary walls, whereas the length of the crystals may be of the order of 100 nm. [4] Lateral microfibril dimensions are slightly smaller in primary cell walls.[5,6] The

cellulose microfibrils are embedded in a matrix of pectin, other polysaccharides (hemicelluloses) and lignin. The latter is characteristic for woody material and establishes strong cross links between the components of the wood cell wall. The high specific tensile strength of wood is a result of its complex composite architecture. — In both materials, the water content plays a major role in determining the mechanical properties. Only the embedding matrix is accessible to water.[7]

Despite their completely different biosynthesis, the nanocrystals in silk and the cellulose microfibrils in wood, respectively, are similarly arranged in a more or less parallel fashion. A specific crystallographic axis of all crystals is well aligned in a particular direction. There is, however, no preferred orientation of the other directions.[8] In crystallographic terms, this arrangement of the crystals is called a fibre texture and is observed in many nanocrystalline biological systems. Usually, a fibre texture is imperfect in the sense that there is some variation of the fibre axis direction around a mean orientation.

Wood is structured on an additional hierarchical level. The parallel cellulose microfibrils in the secondary cell wall layers are wound in a helical fashion around the wood cell. The angle of the microfibril helix with respect to the longitudinal cell axis is called *microfibril angle* (MFA). A very small MFA thus means that the microfibrils are running more or less in the direction of the cell axis. The relation between mechanical properties and MFA has been proven by combining the results of tensile tests and X-ray scattering experiments.[9] The larger the MFA, the smaller is the stiffness of the wood tissue and the higher is its extensibility.

X-ray diffraction diagrams of silk and wood are dominated by the contribution of the respective fibroin and cellulose nanocrystals to the scattering signal. The fibre texture mentioned above leads to characteristic fibre diffraction diagrams (see e. g. inset in **Figure 3** for silk). They contain in particular information on lattice constants (Bragg's law), crystals sizes (Scherrer formula) and crystallinity.[10] A combination of X-ray diffraction experiments

in situ with mechanical tests allows for the determination of the dependence of the nanocrystalline structure and morphology on external load. A deformation of the crystalline lattice becomes manifest in changes of the position of the respective Bragg reflections according to Bragg's law. Any changes of the nanocrystal orientation, including the microfibril angle in wood, will alter the azimuthal reflection profiles in the diffraction pattern as outlined above.

The chemical and structural complexity of the wood cell wall and of silk means that a single analytical method will always reveal only limited information about certain aspects of the system. In the case of X-ray diffraction from nanocrystals, this inherent limitation is used as an advantage, as pointed out above. The related techniques of small-angle X-ray (SAXS) or neutron scattering (SANS) do not require structural order on the atomic level but rather a density contrast on a length scale of roughly 1 to 100 nm for an analysable signal. We will not discuss the application of SAXS for the investigation of wood here; the reader is referred to the respective publications.[11,12,13] In combination with hydrogen exchange, we will show that SANS contributes further to the understanding of silk morphology.

An adequate method to address the question of the structure of the disordered phase of nanocomposites by mainly probing the predominant hydrogen bonds is provided by neutron time-of-flight spectroscopy. This method gives information on vibrational modes in the millielectronvolt energy transfer range. Thus, while most other spectroscopic techniques are restricted to the centre of the Brillouin zone, neutron spectroscopy probes microscopic dynamics as a function of length scale. In addition, a (neutron) diffraction pattern is recorded *in situ* and visualises any structural changes as a function of tensile strain. The assignment of detectable motions to corresponding structures is of special value in samples with the complexity of biological material. Further, neutron spectroscopy can be enhanced by a selective deuteration of the samples and thus applying contrast variation techniques; this has been reported for cellulosic materials.[14] In the particular case of wood and silk,

predominantly only the amorphous regions are accessible to guest molecules like water.[7,15] A residual deuteration is possible by immersing the samples in heavy water and a subsequent drying process.[14]

X-ray Diffraction experiments on wood with high spatial resolution

It is not within the scope of this article to give a review of X-ray microfocusing techniques. The development towards the first dedicated X-ray microfocus experimental stations at synchrotron radiation sources has been outlined by Riekel.[16] Only synchrotron radiation sources produce X-ray beam with a sufficiently low angular divergence in order to focus the X-rays down to micrometre size beams.[17] Various techniques have successfully been used for producing micrometre size foci or even smaller ones down to 50 – 10 nm.[18,19,20,21] However, there are certain requirements for an X-ray microbeam to be used in diffraction experiments. First of all, the total flux in the (small) beam cross section has to be high enough to obtain a diffraction diagram within reasonable time (a few seconds would be desirable). Typical flux values for microbeams are of the order of 10^{10} photons/s. Background elimination is another crucial issue for diffraction experiments. This applies to intensity "tails" of the beam profile (normally eliminated by slit systems or pinhole apertures) as well as to air scattering from the primary beam passing through air. The latter can be reduced by minimising the beam path in air by a compact design of the space around the sample and by placing a very small lead beam stop immediately behind the sample.

In a recent experiment for the investigation of the local cellulose microfibril orientation in a single wood cell, thin cross sections of spruce wood (cryo-microtome cut, thickness 25 µm) were illuminated with an X-ray nanobeam of approximately 250 x 250 nm^2 FWHM at the Microfocus Beamline ID13 at the European Synchrotron Radiation Facility (ESRF Grenoble).[22] The sample was oriented with the tracheid cell axis parallel to the beam. The illuminated volume should include only one cell wall layer. As the cellulose microfibrils are

tilted by the microfibril angle (MFA ≈ 20° in this case) against the beam, the abovementioned fibre diffraction geometry (fibre axis perpendicular to the beam) is no longer ensured. Taking into account the actual scattering geometry, it can be shown that the resulting diffraction patterns are no longer symmetric to the meridian of the diagram:[8,23] E. g., the equatorial reflections move in azimuthal direction away from the equator but stay still symmetric with respect to the meridian.[23] In other words, the angular difference of the maxima on the azimuth is no longer 180° as it should be for the usual fibre geometry. As the reflection maxima move in opposite directions of the diagram, depending on whether the fibre is tilted towards or away from the beam, not only the tilt angle with respect to the beam (which, in the case of the side walls of an individual tracheid, is the MFA) but in addition the direction of the tilt is reflected in the diffraction pattern. **Figure 1** shows the azimuthal intensity distribution for the 200 reflection in the nanofocus experiment. The two reflections peaks have different heights and widths, which can only be explained by a nonparallel orientation of the cell axis and the beam and, more importantly, an asymmetry in the fibrillar structure. A fit yields an elliptical orientation distribution of the crystal orientation around the preferred (fibre) orientation; the distribution is wider in the plane parallel to the wall (representing deviations from the mean MFA) than in the plane normal to the wall (meaning how well aligned the microfibrils are with respect to the cell wall direction). Values (standard deviations) of $\sigma_1 = 6.81°$ and $\sigma_2 = 11.25°$ for the two half-axes, respectively, are obtained in a fit to the data.[22] This result should have major impact on a deeper understanding of cellulose biosynthesis in the cell wall.

The *in situ* combination of mechanical tests with structural investigations on a molecular level is clearly a domain of X-ray diffraction using synchrotron radiation. The limiting cases of well-oriented cellulose (MFA ≈ 0°) in flax fibres[24] and of compression wood slices with large MFA > 45°[25] have provided very valuable information on (i) the mechanical properties of individual microfibrils and (ii) the behaviour of the embedding matrix. In “normal” wood

with an MFA of about 20°, the interplay between these two structural parameters should determine its mechanical properties. Indeed, microfibril deformations of the order of 0.3 %[26,27] and moderate changes of the microfibril orientation in general[13,26,27,28] have been reported for thin slices of softwood xylem tissue. A model for the mechanics of the wood cell wall, based on the above experimental findings, is not available yet. As the precision of synchrotron radiation X-ray diffraction *in situ* experiments is continuously improved – this is essential in view of the minute effects to be measured –, considerable progress can be expected in the near future.

Single tracheid deformation experiments combined with X-ray microbeam diffraction experiments help distinguishing between collective deformation mechanisms in the tissue and those of a single wood cell. An example is shown in **Figure 2**.[29] The experiment was again carried out at ID13 (ESRF) but this time with a beam size of about 5 µm in diameter. During the tensile test, the 30 µm thick cell was continuously scanned through the beam in order to minimise beam damage effects. The graph shows the macroscopic stress-strain curve of the mechanically extracted dry (3 % RH in the sample cell) pine wood tracheid together with the crystal lattice strain of the cellulose microfibrils (from the 004 cellulose reflection) in fibre direction. It is possible to scale this curve such that it closely follows the macroscopic stress-strain curve, suggesting a direct mechanical stress transfer to the microfibrils. This serial arrangement of crystals and embedding matrix represents the Reuss model in polymer science.[30] It usually breaks down at higher strain in case of intact wood tissue.[29] – Our work on a comparison of the behaviour of wood slices and single tracheids is still in progress.

In situ *X-ray and neutron scattering experiments on silk*

In the case of silk, the stiffness difference between the nanocrystals and the embedding matrix is less pronounced than in wood. The observed deformation of the crystals in the direction of an external tensile stress may thus reach up to 2 %; these lattice parameter changes (of the 002

fibroin reflection, see inset in Figure 3) can be measured with a high precision. An *in situ* X-ray diffraction experiment was carried out at Beamline A2 (HASYLAB, DESY Hamburg). Data of the macroscopic stress-strain curve of a bundle of dry silk fibres are plotted together with the microscopic lattice strain in Figure 3. Again (see above, Figure 2) the two curves can be scaled according to the Reuss model. Their agreement is excellent, in particular, the bend of the stress-strain curve, the so-called yield point at the transition from purely elastic to plastic deformation, is found in the lattice strain data as well. The scaling factor between the two curves, i. e. the linear factor between the microscopic crystal strain and the external stress (assumed to be isotropic within the composite according to the serial arrangement of crystalline and disordered regions in the Reuss model) is the Young's modulus of the nanocrystals. Its value of (26.5 ± 0.8) GPa is in good agreement with previously published data.[31,32,33]

Even deeper insight in the composite nature of dry silk can be gained by combining the *in situ* X-ray diffraction results with viscoelastic measurements on single silk fibres.[34] Viscoelastic and purely elastic properties of the embedding disordered matrix can be separated, leading to a combined elastic modulus of the crystalline and disordered regions. As those could be modeled by a serial arrangement (see above), an elastic modulus of 6.3 GPa was obtained for the amorphous matrix, about a factor of four smaller than the crystal stiffness. The relaxing modulus (6.6 GPa) in the viscoelastic model is in striking agreement. A complete description of the silk fibre as a viscoelastic nanocomposite could be achieved for the first time.

Still, it would be very interesting to learn more about the nature of the disordered regions in silk and about the morphology of the composite. Small-angle scattering could, as pointed out before, be a useful technique to probe the morphology on mesoscopic length scales. However, the required contrast between the crystalline and amorphous regions on silk seems to be weak both for X-rays (SAXS) and for neutrons (SANS). In particular, SAXS was not able to reveal any periodic structures along the fibre axis.[35] For a SANS experiment, the contrast can be

enhanced by partial deuteration of the water-accessible disordered regions.[15] By immersion in heavy water (D_2O) all protons in polar groups (mainly hydroxyl and amide groups) can be exchanged to deuterons with a markedly different scattering cross section for neutrons.[36] Experiments were carried out at the instrument SANS-2 (Geesthacht Neutron Facility GeNF, GKSS) with some of the very last neutrons provided by the FRG-1 research reactor. The result for wet silk fibre bundles is shown in **Figure 4**. The contrast variation makes distinct intensity in fibre direction visible, the position of the intensity maxima yields a periodicity of (7.2 ± 0.2) nm clearly visible. Such a structure was for the first time observed in silkworm silk.[] Its observation should have major impact on the development of models for the deformation mechanisms in silk; the analysis of *in situ* tensile test experiments using a stretching device for experiments in controlled humidity atmosphere is under way.

Finally, a so far non-standard experiment on silk with inelastic neutron scattering (INS) shall be reported. As already pointed out, this technique is sensitive to the molecular configuration of the fibroin. By using the same contrast variation technique as for SANS (selective deuteration) the contribution of the inaccessible and thus not H-D exchanged disordered regions can be enhanced in by calculation difference spectra. The principle had previously been demonstrated on various cellulose samples and had revealed the universality of the disordered surface chains of cellulose microfibrils.[14] In an experiment at the cold neutron spectrometer IN6 (ILL Grenoble) the technique was *in situ* combined with a tensile test. Surprisingly, the difference spectra of stretched and undeformed dry silk fibres were found indistinguishable.[37] This can be interpreted in terms of rubber or entropic elasticity where the molecular configuration remains unchanged and only order is increased upon stretching. Such a model had already been postulated for spider silk.[38]

Conclusions and outlook

Synchrotron radiation X-ray diffraction and neutron scattering techniques are powerful tools for investigating the hierarchical structure of structural biological materials such as silk and wood. Focusing down synchrotron radiation to microbeams makes it possible to obtain information locally with position resolution as good as 100 nm, i. e., better than optical resolution. In the near future, the routinely available nanofocus X-ray beams will provide excellent perspectives for more detailed experiments on the layered cell wall structure. A further strength of scattering techniques in general is the easy combination with advanced sample environments such as stretching devices, controlled humidity cells or their combination, enabling non-destructive *in situ* experiments. The dependence of structural parameters on the applied stress has already helped to link nanostructure and mechanical properties. Scattering techniques will thus significantly contribute to a deeper understanding of wood and silk mechanics on a molecular level. The new information is expected to inspire new man-made composite materials, which can in turn be investigated with the same methods.

Acknowledgements. All experiments at synchrotron radiation and neutron sources presented here were carried out in collaborations with the instrument scientists (and would not have been possible otherwise!). I would like to particularly mention the ID13 team at ESRF (Christian Riekel, Manfred Burghammer, Richard Davies, Stephan V. Roth, Sebastian Schöder), the A2 beamline staff at HASYLAB (Sérgio S. Funari, Martin Dommach), Melissa Sharp and Helmut Eckerlebe (SANS-2, Helmholtz-Zentrum Geesthacht) and colleagues at the ILL (Marek Michael Koza, Helmut Schober).

Received: ((will be filled in by the editorial staff))
Revised: ((will be filled in by the editorial staff))
Published online: ((will be filled in by the editorial staff))

_[1] Y. Shen, M. A. Johnson, D. C. Martin, *Macromolecules* **1998**, *31*, 8857.

_[2] D.T. Grubb, L.W. Jelinsky, *Macromolecules* **1997,** *30*, 2860.

_[3] F. Junghans, M. Morawietz, U. Conrad, T. Scheibel, A. Heilmann, U. Spohn, *Appl. Phys. A* **2006**, *82*, 253.

_[4] A. C. O'Sullivan, *Cellulose* **1997**, *4*, 173.

_[5] J. Laï Kee Him, H. Chanzy, M. Müller, J.-L. Putaux, T. Imai, V. Bulone, *J. Biol. Chem.* **2002**, *277*, 36931.

_[6] M. Müller, R. Hori, T. Itoh, J. Sugiyama, *Biomacromolecules* **2002**, *3*, 182.

_[7] M. Ioelovitch, M. Gordeev, *Acta Polym.* **1994**, *45*, 121.

_[8] H. Lichtenegger, M. Müller, O. Paris, C. Riekel, P. Fratzl, *J. Appl. Cryst.* **1999**, *32*, 1127.

_[9] A. Reiterer, H. Lichtenegger, S. Tschegg, P. Fratzl, *Phil. Mag. A* **1999**, *79*, 2173.

_[10] L. Azaroff, *Elements of X-ray Crystallography*, McGraw-Hill Book Co, USA, **1968**.

_[11] H.F. Jakob, D. Fengel, S.E.Tschegg, P. Fratzl, *Macromolecules* **1995**, *28*, 8782.

_[12] H. Lichtenegger, A. Reiterer, S.E. Stanzl-Tschegg, P. Fratzl, *J. Struct. Biol.* **1999**, *128*, 257.

_[13] T. Kamiyama, H. Suzuki, J. Sugiyama, J. Struct. Biol. **2005**, *151*, 1.

_[14] M. Müller, C. Czihak, H. Schober, Y. Nishiyama, G, Vogl, *Macromolecules* **2000**, *33*, 1834.

_[15] D. Sapede, T. Seydel, V. T. Forsyth, M. M. Koza, R. Schweins, F. Vollrath, C. Riekel, *Macromolecules* **2005**, *38*, 8447.

_[16] C. Riekel, *Rep. Prog. Phys.* **2000**, *63*, 233.

_[17] J. Als-Nielsen, *Elements of Modern X-ray Physics*, Wiley, Oxford, United Kingdom, **2001**.

_[18] D. H. Bilderback, D. J. Thiel, *Rev. Sci. Instrum.* **1995**, *66*, 2059.

_[19] C. G. Schroer, O. Kurapova, J. Patommel, P. Boye, J. Feldkamp, B. Lengeler, M. Burghammer, C. Riekel, L. Vincze, A. van der Hart, M. Küchler, *Appl. Phys. Lett.* **2005**, *87*, 124103.

_[20] O. Hignette, P. Cloetens, G. Rostaing, P. Bernard, C. Morawe, *Rev. Sci. Instrum.* **2005**, *76*, 063709.

_[21] A. Jarre, C. Fuhse, C. Ollinger, J. Seeger, R. Tucoulou, T. Salditt, *Phys. Rev. Lett.* **2005**, *94*, 074801.

_[22] M. Ogurreck, M. Müller, *J. Appl. Cryst.* **2010**, *43*, 256.

_[23] R. Hori, M. Müller, U. Watanabe, H.C. Lichtenegger, P. Fratzl, J. Sugiyama, *J. Mater. Sci*. **2002**, *37*, 4279.

_[24] K. Kölln, *PhD Thesis*, Kiel, Germany, **2004**.

_[25] J. Keckes, I. Burgert, K. Frühmann, M. Müller, K. Kölln, M. Hamilton, M. Burghammer, S. V. Roth, S. Stanzl-Tschegg, P. Fratzl, *Nature Mater.* **2003**, *2*, 810.

_[26] I. Grotkopp, *PhD Thesis*, Kiel, Germany, **2006**.

_[27] M. Peura, K. Kölln, I. Grotkopp, P. Saranpää, M. Müller, R. Serimaa, *Wood Sci. Technol.* **2007**, *41*, 565.

_[28] K. Kölln, I. Grotkopp, M. Burghammer, S.V. Roth, S.S. Funari, M. Dommach, M. Müller, *J. Synchr. Rad.* **2005**, *12*, 739.

_[29] T. Pazera, *Diploma Thesis*, Kiel, Germany, **2008**.

_[30] I. Sakurada, K. Nakamae, *Makromol. Chem.* **1964**, *75*, 1.

_[31] A. Sinsawat, S. Putthanarat, Y. Magoshi, R. Pachter, R. K. Eby, Polymer **2002**, *43*, 1323.

_[32] K. Nakamae, T. Nishino, H. Ohkubo, *Polymer* **1989**, *30*, 1243.

_[33] A. Sinsawat, S. Putthanarat, Y. Magoshi, R. Pachter, R. K. Eby, *Polymer* **2003**, *44*, 909.

_[34] I. Krasnov, I. Diddens, N. Hauptmann, G. Helms, M. Ogurreck, T. Seydel, S. S. Funari, M. Müller, Phys. Rev. Lett. **2008**, *100*, 048104.

_[35] Z. Yang, D. T. Grubb, L. W. Jelinski, *Macromolecules* **1997**, *30*, 8254.

_[36] M. Bée, *Quasielastic Neutron Scattering*, Adam Hilger, Bristol, United Kingdom, **1988**.

_[37] T. Seydel, K. Kölln, I. Krasnov, I. Diddens, N. Hauptmann, G. Helms, M. Ogurreck, S.-G. Kang, M. M. Koza, M. Müller, *Macromolecules* **2007**, *40*, 1035.

_[38] J. M. Gosline, M.W. Denny, M. E. DeMont, *Nature* **1984**, *309*, 551.

_[39] M. Blankenburg, *Diploma Thesis*, Kiel, Germany, **2010**.

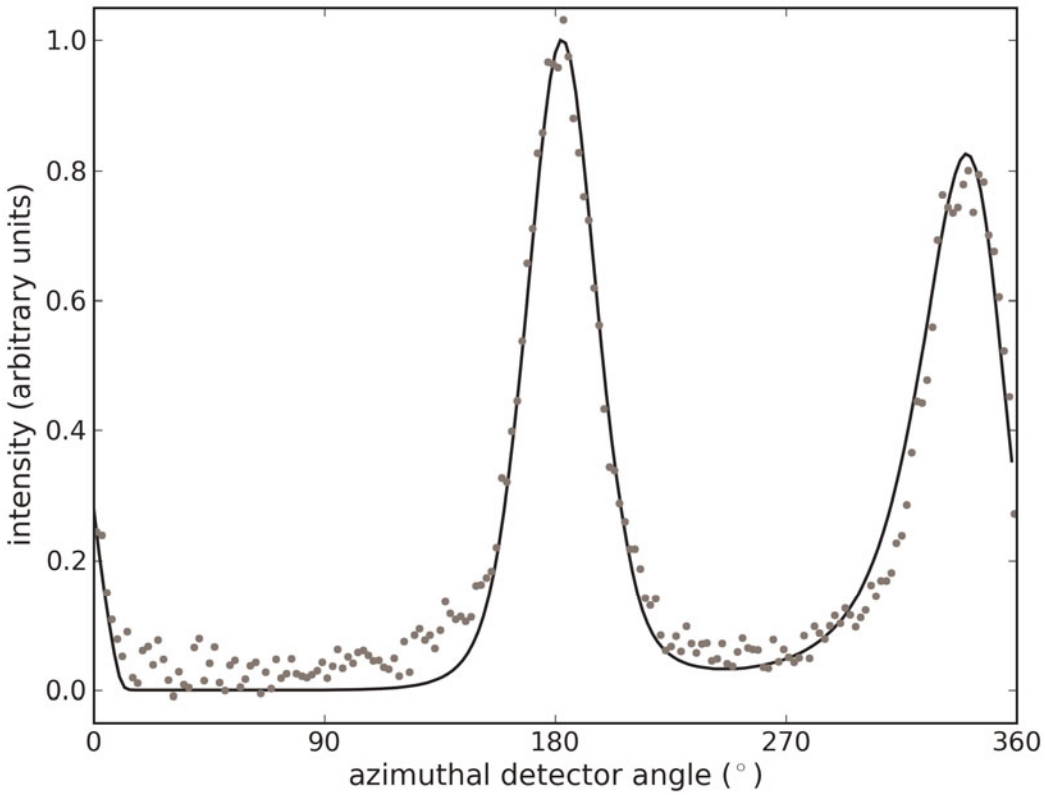

Fig. 1. Azimuthal equatorial reflection intensity profile from a Picea abies *softwood sample (dots). As a result of a tilt of the cell axis relative to the beam, the width and intensity differ in the two 200 peaks. For an explanation of an asymmetric pattern, two factors have to be considered: (a) the sample must be tilted relative to the beam and (b) the microfibril orientation distribution around the preferred orientation has to have an elliptical shape. Reproduced with permission from [22].*

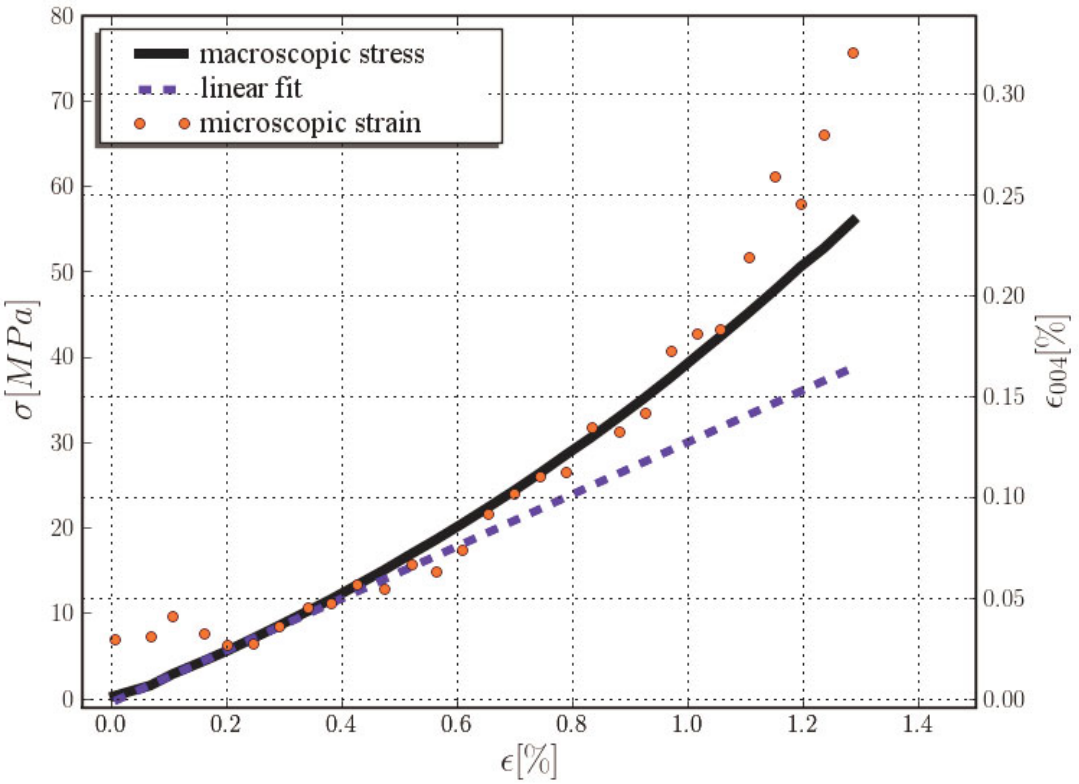

Fig. 2. Stress-strain curves of a single pine wood tracheid cell (solid lines, right-hand scale). The symbols give the strain ε_{004} (right-hand scale) of the crystal lattice in axial direction, as determined from the meridional 004 reflections. From [29].

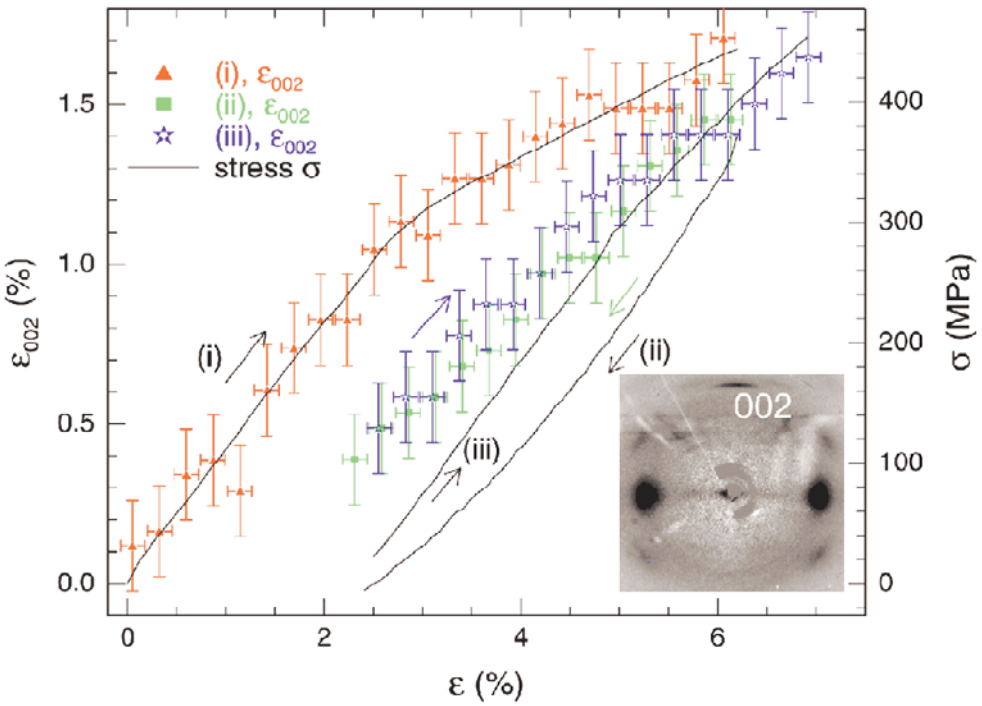

Fig. 3. Stress-strain curves of a bundle of silkworm silk fibres (solid lines, right-hand scale). (i), (ii) denote data of the unstretched fibres (increasing and decreasing strain) whereas (iii) stands for the prestretched fibre bundle. The symbols give the strain ε_{002} (left-hand scale) of the crystal lattice in axial direction, as determined from the meridional 002 reflections of the fibre diffraction diagram (inset). Reproduced with permission from [34].

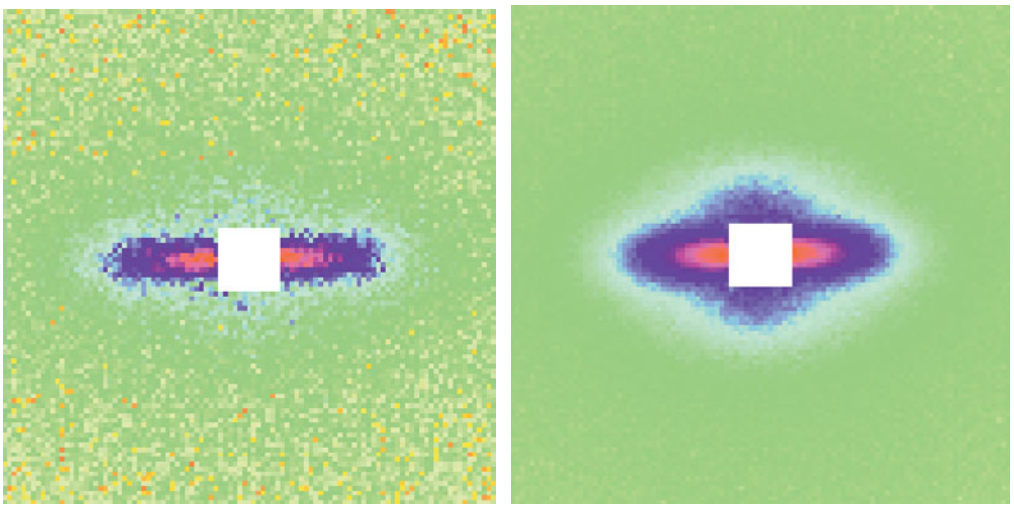

Fig. 4. Two-dimensional small-angle neutron scattering patterns from a bundle of silkworm silk fibres in a saturated water atmosphere (left) and of selectively deuterated silkworm silk fibres in a saturated D_2O atmosphere. From [39].