ESRF	**Experiment title:** Sub-micrometer structure of the wood cell wall	**Experiment number**: SC-839
Beamline: ID13	**Date of experiment**: from: 23.06.01 to: 28.06.01	**Date of report**: 03.09.07
Shifts: 12	**Local contact(s)**: Manfred Burghammer	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Klaas Kölln*, Martin Müller*

Institut für Experimentelle und Angewandte Physik, Christian-Albrechts-Universität zu Kiel, D-24098 Kiel, Germany

Helga Lichtenegger, Jozef Keckes*

Erich Schmid Institute for Materials Science, Jahnstraße 12, A-8700 Leoben, Austria

Christian Riekel*, Manfred Burghammer*

ESRF

Report:

The softwood cell wall is a natural fibre composite of cellulose crystals (microfibrils) embedded in an amorphous matrix of other carbohydrates (hemicelluloses) and lignin. A mature wood cell has a layered cell wall with a primary (P) and three secondary (S1, S2, S3) walls (Fig. 1). The S2 layer comprises about 80 % of the wall thickness. The microfibrils of the S2 are well–aligned and arranged in a steep right–handed (Z–) helix around the lumen. The angle of the helix with respect to the longitudinal cell axis is defined as microfibril angle (MFA). The mechanical properties of a wood cell are, however, not only determined by the major contribution of the S2 but as well by the slow left–handed (S–) helices of the thinner S1 and S3 layers. Furthermore, there is evidence from electron microscopy studies of developing cell walls [1,2] or stained sections [3,4] that the change of microfibril orientation between neighbouring S layers is gradual. Such a helicoidal wall structure (Fig. 2) is mechanically much stronger than a simply layered system as it reduces the tendency to split axially and to fail to resist shear forces.

X-ray microdiffraction is an excellent tool to determine the local cellulose microfibril orientation as well as the MFA by scanning thin wood cross sections. When fibres (such as the cellulose microfibril bundles in the cell wall) are not measured in standard fibre geometry, i. e., with the fibre axis perpendicular to the X-ray beam, the curved Ewald sphere leads to an asymmetry of the diffraction pattern with a curved equator [5]. Using this scattering geometry effect, the helical structure in a spruce tracheid could be imaged with a 2 µm beam (produced by a glass capillary) on ID13 [6]. However, the spatial resolution was not sufficiently high to distinguish between different cell wall layers.

In order to determine details of the layered structure, we used a combined X-ray waveguide and focussing mirror optics on ID13. The set-up is extensively described in [7,8] and provides a beam of 0.1 (v) x 3 (h) μm^2, i. e., with sub-micrometer size in one direction. By choosing this direction as the scanning direction and

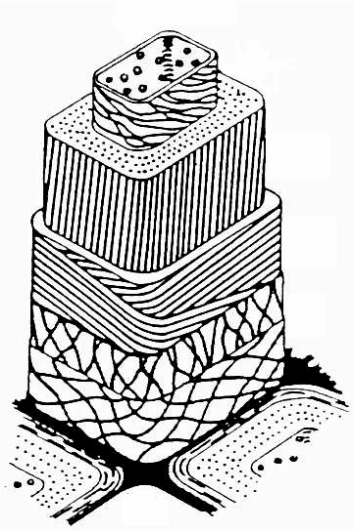


Figure 1: Schematic drawing of the layered structure of a softwood tracheid cell. The thickest layer is the S2, further outside the S1.

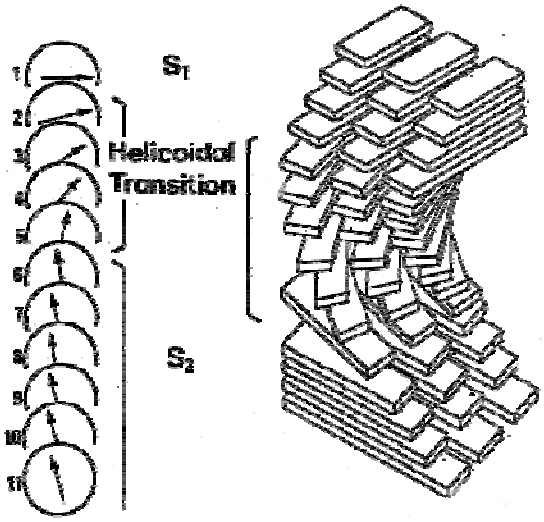


Figure 2: Proposed helicoidal transition with a gradual change of MFA from S1 to S2 [3].

scanning across a cell wall, its structure can be obtained with sub-micrometer spatial resolution. Fig. 3 illustrates the principle of the experiment. Resolution is limited by the accuracy of the sample alignment. A tilt of the line-shaped beam with respect to the cell wall results in a "smearing" of the signal of different cell wall layers. So does a tilt of the sample surface (supposed to be parallel to the longitudinal cell axis) due to the limited thickness (10 µm) of the sample cross section.

With our set-up, a flux of 10^9 photons/s in the beam cross section is reached. Fig. 4 is an example of a diffraction diagram from a 10 µm thick *Picea abies* latewood cross section obtained in 60 s on a MAR CCD detector. An asymmetry or a bend of the equator of the diagram is clearly visible. The arrow points in the direction of the local helix direction (arrow means "up" = against the beam) derived from the position of the equatorial reflection on the azimuth. For a scan across a double cell wall, these arrows are displayed in the top of Fig. 5. There, the length of the arrow corresponds to the projection of a finite length microfibril, i. e., the longer the arrow the larger the MFA (and the slower the helix). The direction changes between adjacent cell walls of neighbouring cells. The circles in the graph give the MFA as a function of the relative position with respect to the cell border. Clearly, the MFA is very large at the transition from one cell wall to that of the neighbouring cell. Presumably, this region comprises the two S1 layers of the adjacent cells. There are slow MFA changes in the S2, and we find a transition layer (MFA changes from 30° to 80°) with a maximum thickness of 240 nm.

Figure 3: Schematics of a scan with the asymmetric waveguide/ mirror beam (white line).

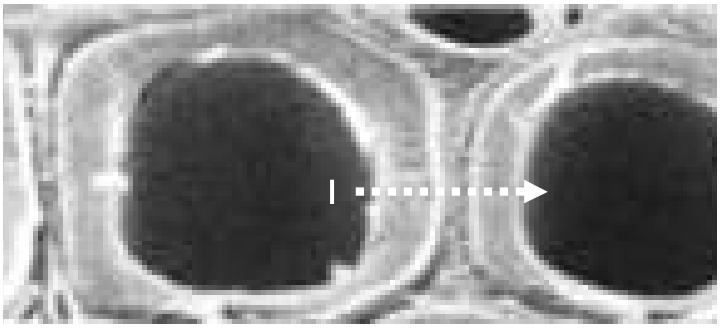
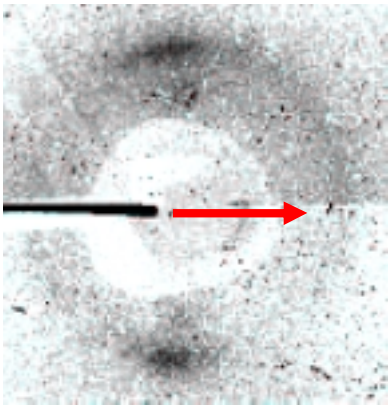


Figure 4: Asymmetric fibre pattern from a *Picea abies* cell wall (30 s exposure).



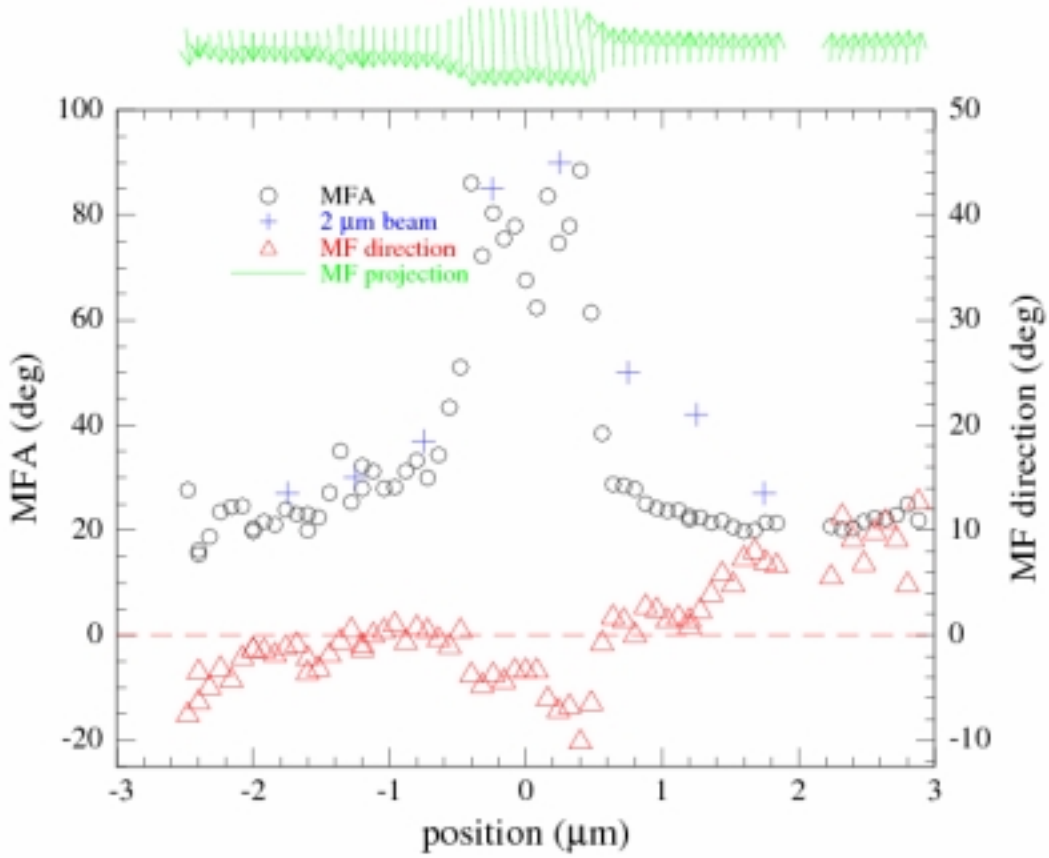


Figure 5: Local microfibril angle (MFA; circles) and microfibril direction (triangles) as a function of the position in a lineat scan across two adjacent cells walls (see Fig. 3).

Unfortunately, only this one and even incomplete scan could be carried out. As the spatial resolution with a line-shaped beam depends crucially on the alignment of the cell wall to the beam as well as on the straightness of the cell wall, no accurate thickness of the transition layers could be determined. They do not even appear much thinner than those found with a 2 µm beam (crosses; taken from [9]). As the microfibrils are apparently not always parallel to the cell wall (triangles; 0° means parallel) cutting artefacts cannot be excluded either.

References

[1] H. Abe, J. Ohtani, K. Fukazawa. *IAWA Bull.* **12**, 431-438 (1991).
[2] H. Abe, R. Funada, J. Ohtani, K. Fukazawa. *Trees* **11**, 328–332 (1997).
[3] A. C. Neville, S. Levy. *Planta* **162**, 370–384 (1984).
[4] B. Vian, D. Reis, M. Mosiniak, J. C. Roland. *Protoplasma* **131**, 185–199 (1986).
[5] O. Paris, M. Müller. *Nucl. Instr. Meth. B* **200**, 390-396 (2003).
[6] H. Lichtenegger, M. Müller, O. Paris, C. Riekel, P. Fratzl. *J. Appl. Cryst.* **32**, 1127–1133 (1999).
[7] M. Müller, M. Burghammer, D. Flot, C. Riekel, C. Morawe, B. Murphy, A. Cedola. *J. Appl. Cryst.* **33**, 1231-1240 (2000).
[8] ESRF Highlights 2001 (http://www.esrf.eu/UsersAndScience/Publications/Highlights/2001/methods/METH2.html)
[9] H. Lichtenegger, A. Reiterer, S. E. Tschegg, M. Müller, C. Riekel, O. Paris, and P. Fratzl. In: H. Ch. Spatz and Th. Speck, editors, Proceedings of the 3rd Plant Biomechanics Conference, pages 436-442. Georg Thieme, Stuttgart, New York, 2000.