ESRF	**Experiment title:** Influence of growth conditions on the nanostructure of the wood cell wall	**Experiment number**: Sc2725
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Shifts: 9	**Local contact(s)**: Sebastian Schoeder	*Received at ESRF:*

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Report:

The cell wall of tracheid wood cells is a natural fibre composite of cellulose crystals (microfibrils) embedded in an amorphous matrix of lignin and other carbohydrates (hemicelluloses). Spatially, the cell wall is layered with a primary (P) and three secondary (S1, S2, S3) walls (Fig. 1). The S2 layer comprises about 80 % of the wall thickness and the cellulose content in the S layers is around 60 weight-%. 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 is mechanically much stronger than a simply layered system as it reduces the tendency to split axially and to fail to resist shear forces.

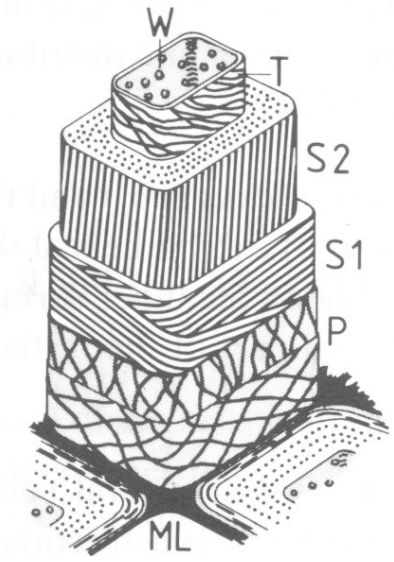

Figure 1: Schematic drawing of the layered structure in a softwood tracheid wall. The marks are explained in the text [5].

For obtaining space-resolved information about the individual cell wall layers, scans have to be performed in the plane perpendicular to the tracheid cell axis.

As the amorphous matrix does not contribute any crystal reflections, the scattering pattern of thin cross sections is the same as from a tilted fibre bundle. The curvature of the Ewald sphere in reciprocal space and

the tilted fibres lead to an asymmetric scattering pattern [6, 7]. The azimuthal distribution of the scattering intensity can be used to calculate the fibre tilt angle, which is in this geometry equivalent to the MFA. Using a refined calculation method [7], any reflection can be analyzed and the exact orientation of the scattering microfibrils in space can be resolved.

The samples originate from a nutrient optimization experiment in Southern Sweden. Norway spruce stands were planted 1975 with 2-year old seedlings. For this experiment, two types of samples have been selected. One (a12-15) tree was grown in a fertilized and irrigated plot, the other (a1-1) in a control plot without further treatment. Samples have been harvested from the trees at 1.3m height above ground. Juvenile earlywood samples – grown in the years 1986 and 1987 – have been scanned from these two trees.

The experiment has been carried out at the nanofocus endstation of the ID13 beamline. Using the installed KB-mirror system, the beam has been focused to 450x450nm² (FWHM). Scattering patterns have been acquired on a mesh of 300x300nm², using a slight oversampling. Exposure times were 0.6s at a binning of 4, using a FReLoN 2k detector.

The data shows a homogeneous electron density distribution from the diffuse scattering intensity (compare Figure 2). Since the sample is composed primarily of lignin, hemicellulose and cellulose, which have a very similar electron density, this shows the quality of the preparation of a slice with a homogeneous thickness. The cellulose 200 reflection scattering signal, – shown in Figure 3 – shows that the cellulose concentration follows the layered cell wall structure with a maximum intensity in the thick S2 layer. The orientation of the cellulose microfibrils could be determined both in the plane of the scan and perpendicular to it. Figure 4 shows the in-plane orientation of the microfibrils. The data shows that the change in orientation is very fast in the corners, which indicates that the tracheid cells prefer an in-plane alignment which follows the cell outline over a gentle change in orientation. The MFA data, shown in Figure 5, allows to separate the S1, S2, and S3 cell wall layers. The S3 layer only shows as a slight increase of the MFA, compared to the S2 layer. The S1 layer with its steep MFA of close to 80° can be well resolved, even though it is only some hundred nanometers in thickness. The change from the S2 to the S1 cell wall layer seems to be gradual, i.e. no hard boundary can be determined. The transition seems to be continuous. In addition to an increase of the mean MFA, the S1 layer also shows a wider distribution of the MFA, shown in Figure 6. This points towards an overall reduction in the organization and orientation of the microfibrils.

The comparison between the fertilized and irrigated sample (a12-15) and the control plot (a1-1) shows a variation in the S1 layer and the transition to the S2 layers. The maximum MFA is higher and the width of the transition region is wider in the fertilized sample. Figure 6 shows a plot with a position resolved plot of the microfibril angles.

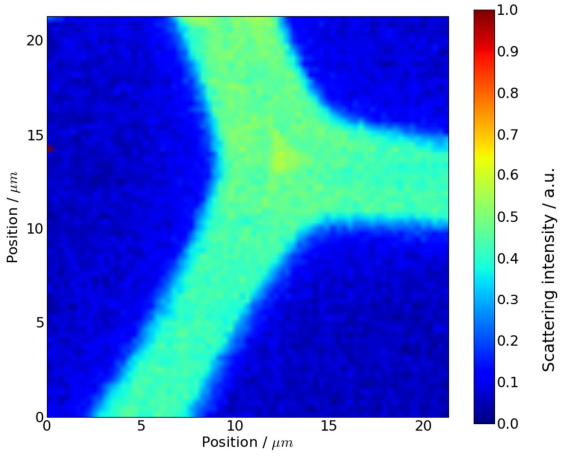

Figure 2: Diffuse scattering intensity as measure for the electron density in sample a12-15

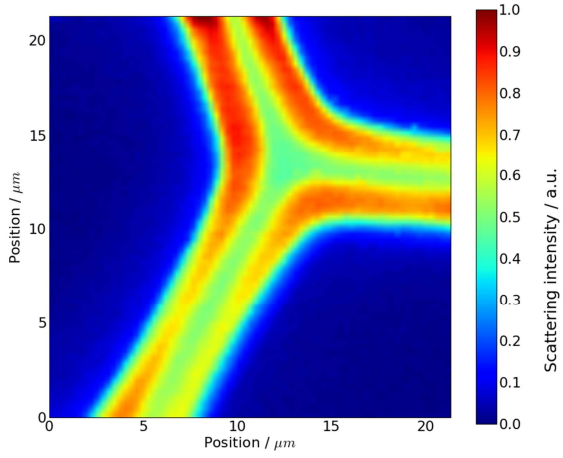

Figure 2: Cellulose 200 scattering signal strength (sample a12-15), normalized for the orientation-dependence of the the scattering strength.

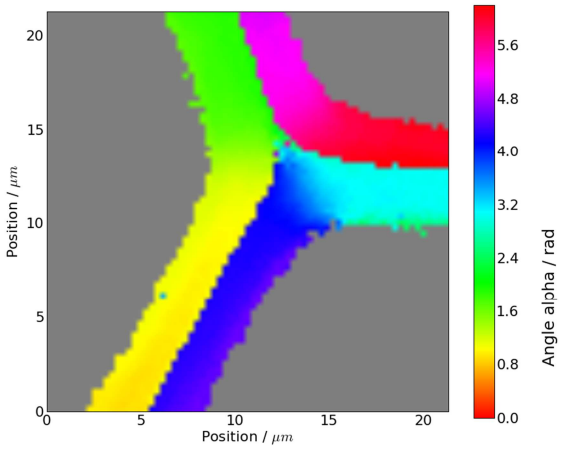

Figure 4: Microfibril orientation in the scan plane (i.e. perpendicular to the tracheid cell axis), sample a12-15

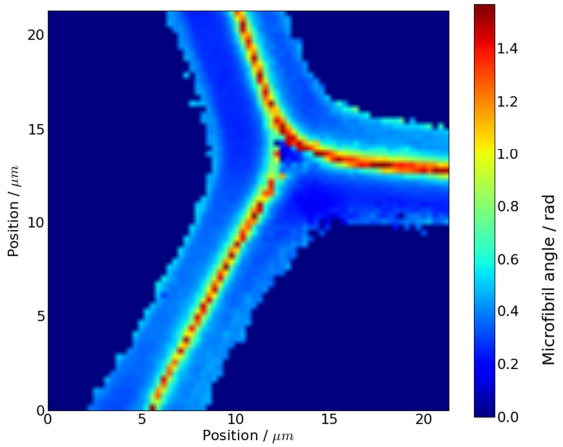

Figure 3:Local microfibril angle (sample a12-15). The layered structure with the steep S1 is very pronounced.

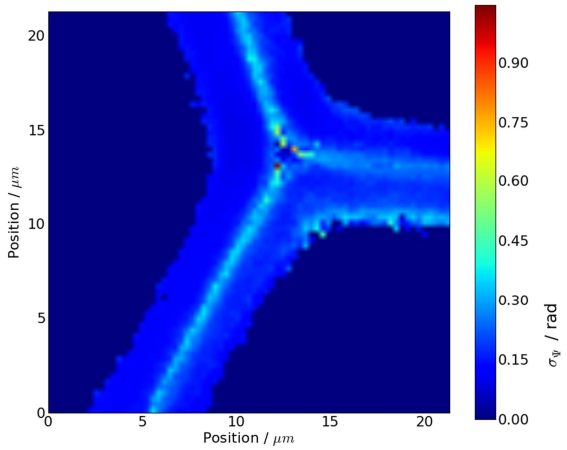

Figure 4: Distribution width of the local MFA angle distribution

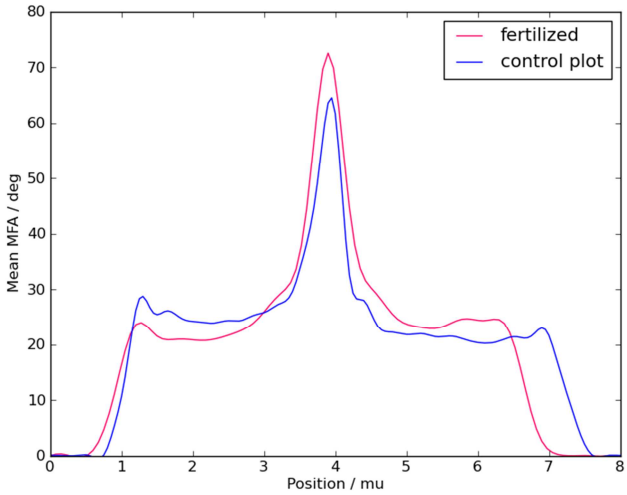

Figure 6: Comparison of the wall structure of fertilized sample (a12-15) and untreated control plot sample (a1-1). The S1 layer thickness seems to be increased in the case of fertilization, as well as the thickness of the transition region S1-S2

This data is interesting in two ways. First, the influence of environmental conditions on wood growth and wood structure is of major importance for predicting how forests will react to climate changes, especially global warming. If, as first results suggest, the composition of the cell walls changes and individual cell walls become thinner, trees might be more susceptible to extreme conditions which might, in addition, occur more often in the future. Also, as the wood structure changes, the mechanical properties change as well. Industrial use of wood as building material and foresting relies on unvarying mechanical properties. If these cannot be guaranteed in the future, substitute woods or different growing techniques have to be found.

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