application note

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Confocal Raman Microscopy - Characterization of Wood Cells and Cellulose Fibers

Cell walls of wood cells consist of crystalline cellulose fibrils embedded in an amorphous hemicellulose-lignin matrix with a multi-layer arrangement.The proportion of these three polymers varies between each layer. The individual layers are formed at different periods during cell differentiation. After the cell wall reaches its final structure and thickness, the mechanically crucial secondary cell wall, consisting of three different sublayers (S1, S2, and S3), is formed. The cellulose microfibril arrangement and the specific chemical composition vary between sublayers. The S2 layer is thickest (75%-85% of the total cell wall thickness) and most important for mechanical stability, with the highest carbohydrate content. The middle-lamella layer, mostly lignin, is attached to the primary cell wall and ensures the adhesion of a cell to its neighbouring cells. The chemical composition of the cell wall and the alignment of the cellulose microfibrils show significant interspecies and intraspecies variability. Confocal Raman microscopy was used to illustrate changes in molecular composition of secondary plant cell wall tissues of poplar (*Populus nigra* and *Populus deltoids*) wood.

In this experiment, a cross section of *Populus nigra* (20 µm thick) was investigated in water using the Spectal Imaging Mode of the alpha300 R confocal Raman microscope, obtaining a complete Raman spectrum at each image pixel. Fig. 1 shows an overview of 30 µm x 30 µm (150 x 150 pixels) including 22500 spectra with an integration time of 100 ms per spectrum using a 532 nm NdYag laser for excitation.

The three images in Fig. 2a-c (zoom-in at 14 µm x 14 µm) were obtained by integrating over selected Raman lines. Fig. 3 shows the color-coded image produced from the three images in Fig. 2a-c.

The corresponding spectra are shown in Fig. 4. The red spectrum represents the distribution of the G-layer, the green spectrum corresponds to the S2-layer and the yellow spectrum is mainly found in the middle lamella.

Two-dimensional spectral maps were acquired and chemical images were generated by integration of the intensities of characteristic Raman bands. The spatial distribution of the various cell components could thus be visualized without any additional chemical treatment, such as the application of molecular dyes.

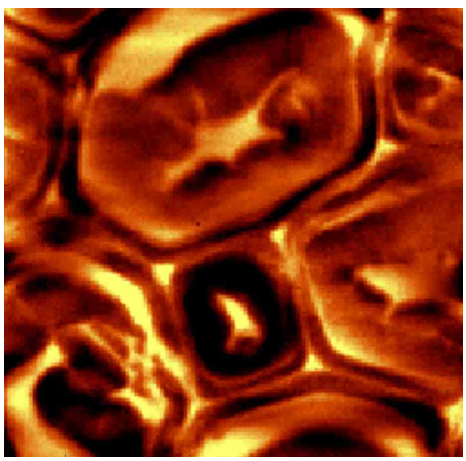

Fig. 1: 30 µm x 30 µm overview of *populus nigra* in water.

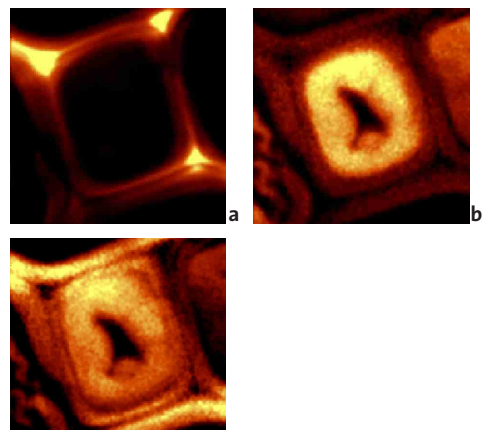

Fig. 2a-c: Zoom-in, 14 µm x 14 µm. Integrating over different Raman lines.

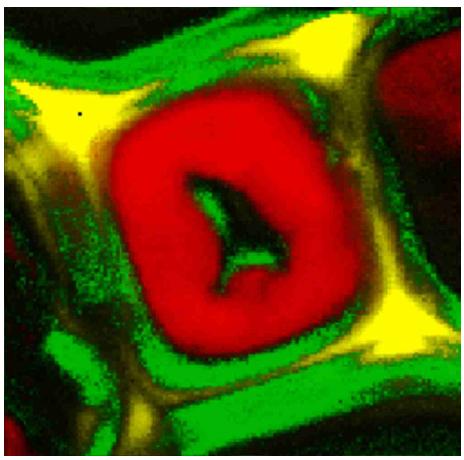

Fig.3: Color-coded image created of the three images in Fig. 2a-c.

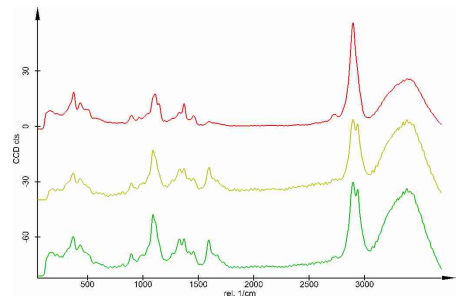

Fig. 4: Corresponding spectra. Red= G-layer, green= S2-layer, yellow= middle lamella.

Conclusion

In summary, the application of high resolution confocal Raman imaging allows the spatial resolution of different components of wood cells by their chemical fingerprints in all three dimensions. Analyses were carried out in vivo, no additional chemical treatment was required.

For further reading see:

Gierlinger N. and Schwanninger M. (2006) Chemical imaging of poplar wood cell walls by confocal Raman microscopy. *Plant Physiology*, 140, 1246-1254.

Gierlinger N., Schwanninger M., Reinecke A. and Burgert I. (2006) Molecular changes during tensile deformation of single wood fibers by Raman microscopy. *Biomacromolecules*, 7, 2077-2081.

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Cellulose

Today over 20 percent of the industrial wood harvest is used in paper manufactering. Wood mainly consists of cellulose fibers (about 50 %), which represents the structural component of wood and the most important basic material of paper.
Nearly 30 % of the wood is lignin, a type of biological cement that interlinks the cellulose fibers. The remaining 15 -30% are hemicelluose, which along with cellulose and lignin belong to a group of structural carbohydrates.
Paper is made of three components: pulp, filler and glue. For the production of fine and print paper, a chemical process based on acids, the so-called sulphite process, is applied. Lignin and hemi-cellulose are thus removed from the wood cells.

Such chemically treated cells are investigated with Confocal Raman Microscopy.
A video image of a wood cell is depicted in Fig. 5, whereas Fig. 6 shows the corresponding Raman image generated by the Spectral Imaging Mode of the alpha300 R by integrating over selected Raman lines with a scan range of 40 µm x 40 µm and 128 x 128 pixels (=16384 spectra) and an integration time of 100 ms per spectrum.
Different levels of cellulose and lignin can be seen from the corresponding spectra(Fig. 7). The green spectrum is an averaged spectrum of the green regions in Fig. 6, representing the cell walls of the cells in Figs. 5 and 6, whereas the red spectrum is an averaged spectrum of the red regions in Fig. 6.
Fig. 8 shows a single cellulose fiber and Fig. 9 the corresponding spectrum in blue. The red spectrum belongs to the fiber after rotation of 90 degrees. The difference in the spectra indicates a dependence on the polarization of the excitation light.

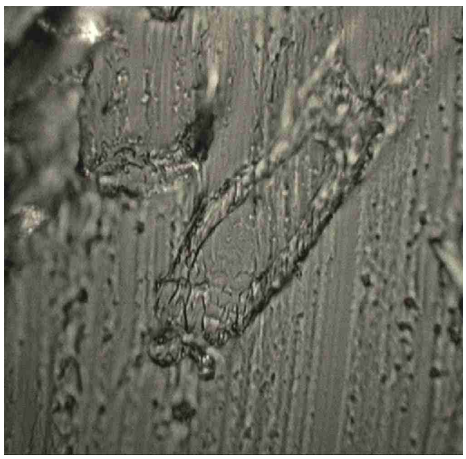

Fig. 5: Chemically treated wood cells.

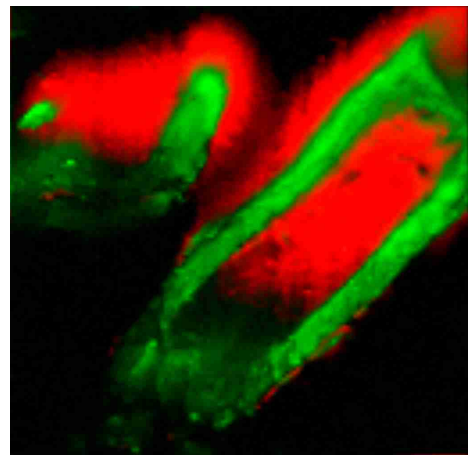

Fig. 6: Integrating over selected Raman lines. 40 µm x 40 µm.

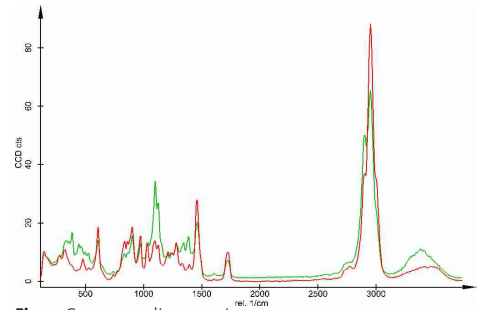

Fig. 7: Corresponding spectra.

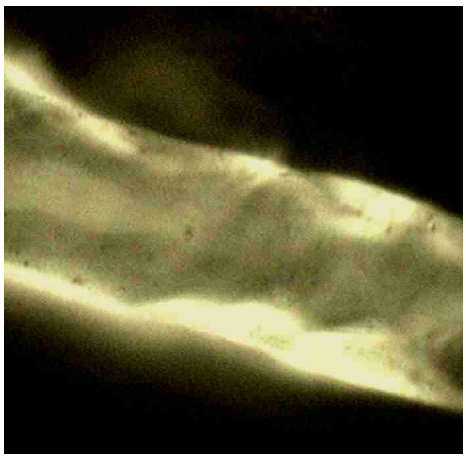

Fig. 8: Video image of a single cellulose fibre.

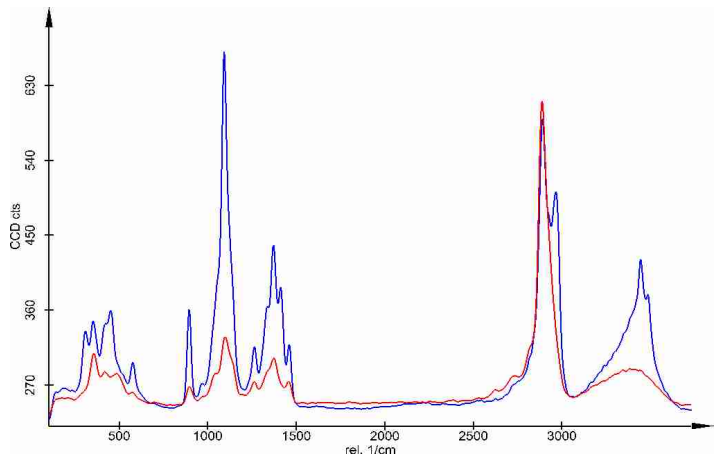

Fig. 9: Corresponding spectrum in blue. The red spectrum belongs to the fibre rotated 90 degrees which shows the dependence of the polarisation of light.

Samples courtesy of Dr. Thomas Röder, Lenzing AG, Austria