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Raman imaging to reveal components and metabolites in wood cells and tissue

Biology

Plant cell walls form multifunctional complex structures, enabling various plant functions to be fulfilled. These functions are not only limited to the transport of nutrients, but also to mechanical durability, resistance to decomposition and other forms of biological attack. Wood tissues are a good example of complex natural composites. The structural architecture consists primarily of cellulose, hemicelluloses, and lignin, forming the shell of the tree. Decomposition, and other forms of biological attack of the wood tissue, are resisted by the presence of secondary metabolites named 'extractives'.

Raman imaging is an ideal technique to spatially resolve chemical information at the cellular level. Fresh and decayed Scots pine wood were investigated revealing biochemical information of the cell structure and decay processes. Biochemical images were generated, without damaging the sample, with high spatial resolution using the inVia™ Qontor® confocal Raman microscope.

Analyse with ease

Cut sections of fresh and decayed Scots pine wood were analysed using a Renishaw inVia Qontor confocal Raman microscope, equipped with 532 nm and 785 nm laser excitations. Sample preparation was minimal, and non-invasive, enabling the sample to be analysed as it would be in the environment. Raman data was collected using Renishaw's StreamHR™ mapping. Biochemical images were generated from intensities of selected Raman bands, using Renishaw's WiRE™ software.

Reveal cell structure from biochemical images

Raman images enabled a deeper understanding of the structure and organisation present in wood cell walls. Cellulose, hemicelluloses, and lignin are predominant components in this structure. Nevertheless, there are significant differences in cell wall polymer composition between, and within, cell wall layers. Additionally, some of the components, such as cellulose, show changes in the orientation of their molecules within cell wall layers. Raman imaging enables us to observe and track these differences.

Figure 1 shows two high resolution images of a fresh Scots pine section. Image (A) shows the variation in the Raman band intensity corresponding to the lignin band at 1595 cm^{-1}. This image highlights the compound middle lamella (CML) consisting of middle lamella and adjacent primary walls and the cell corners (CC), where there is a higher concentration of the lignin than in the sublayer of the secondary cell wall (S2). Image (B) represents the variation in the intensity of the orientation sensitive cellulose band at 1095 cm^{-1}. Two small layers, adjacent to the secondary wall (S2), exhibit high intensity of the cellulose band resolving the CML into the two adjacent the primary cell walls (S1) separated by middle lamella.

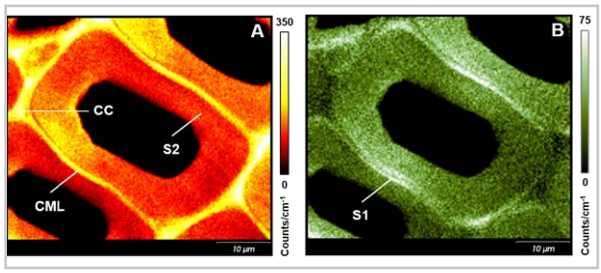

Figure 1. Raman images of cross section of Scots pine wood showing intensity changes of Raman band at 1595 cm^{-1} (A, Lignin) and the orientation sensitive cellulose band at 1095 cm^{-1} (B, Cellulose).

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Study stress and degradation

Extractives, belonging to the group of secondary metabolites, are biologically active chemical components present in the wood cells at low concentration. These compounds, which are not very abundant, significantly affect the properties of wood; particularly its resistance to decay and other forms of biological attack[1]. High specificity of Raman spectroscopy to chemical composition enables us to record the changes in extractives distribution during decay. Pinosylvin is an antifungal phenolic extractive present in Scots pine, and is responsible for the natural durability of the wood. Raman images of the distribution of lignin and pinosylvin, in a decayed Scots pine wood section, are presented in Figure 2.

Image (A) shows the lignin distribution in the decayed wood section using the intensity of Raman band at 1595 cm^{-1}. This is comparable to Figure 1A, but the cell decomposition impacts image definition. Like in the fresh wood (Fig. 1A), the highest concentration of lignin occurs in the compound middle lamella (CML) and cell corners (CC). Image (B) represents the distribution of pinosylvins using the variation in the intensity of the band at 1634 cm^{-1}. The antifungal phenolic compound appears co-located to the lignin and is visibly more concentrated at the cell corners (CC) and compound middle lamella (CML). The potential binding of pinosylvins to lignin has been discussed previously in literature[1].

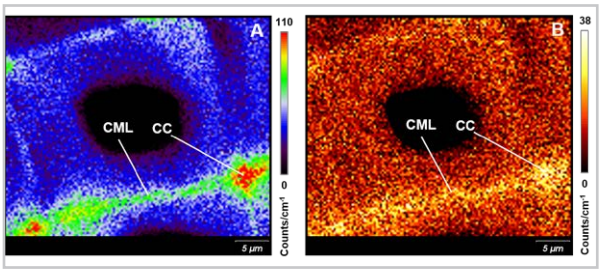

Figure 2. Raman images of cross section of decayed Scots pine wood showing A - lignin concentration changes (intensity changes of Raman band at 1595 cm^{-1}), and B - the increased concentration of pinosylvins (Raman band at 1634 cm^{-1}).

The ideal tool for plant biology

High resolution Raman images of fresh and decayed wood cells have provided insight into the composition, structure and orientation of the wood primary and secondary components. The high specificity of Raman enables different components in the complex multifunctional structure to be distinguished. Observing extractives can help our understanding of the natural wood durability. From this, we can develop more effective and less toxic wood preservatives. Optimised for throughput and sensitivity, the fast imaging of the inVia microscope makes it ideal for plant biology. inVia provides the chemical and spatial detail needed to study these key bio-materials.

inVia Qontor confocal Raman microscope

Acknowledgements
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References
(1) T. Belt, T. Keplinger, T. Hänninen, L. Rautkari, *Cellular level distributions of Scots pine heartwood and knot heartwood extractives revealed by Raman spectroscopy imaging*, **Industrial Crops & Products** 108 (2017) 327–335 (http://dx.doi.org/10.1016/j.indcrop.2017.06.056)

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Renishaw manufactures a wide range of high performance optical spectroscopy products, including confocal Raman microscopes with high speed chemical imaging technology, dedicated Raman analysers, interfaces for scanning electron and atomic force microscopes, solid state lasers for spectroscopy and state-of-the-art cooled CCD detectors.

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