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STXM Chemical Mapping of Norway Spruce Knotwood Lignans

Marko Huttula[1,*], Minna Patanen[1], Riikka Piispanen[2], Takuji Ohigashi[3], Nobuhiro Kosugi[3], Sufal Swaraj[4], Rachid Belkhou[4], Andrey Pranovich[5], Tuula Jyske[2], Petri Kilpeläinen[2], Anna Kärkönen[2], Risto Korpinen[2], Tapio Laakso[2], Sauli Valkonen[2] and Pekka Saranpää[2]

1. Nano and Molecular Systems Research Unit, University of Oulu, Oulu, Finland
2. Natural Resources Institute Finland (Luke), Helsinki, Finland
3. UVSOR Facility, Institute of Molecular Science, Okazaki, Japan
4. SOLEIL Synchrotron, Saint-Aubin, France
5. Åbo Akademi University, Turku, Finland
* Corresponding author, marko.huttula@oulu.fi

The aim is to find out the applicability of the scanning transmission X-ray microscopy (STXM) technique to study the knotwood cells of Norway spruce (*Picea abies*), and especially to locate lignans in their tissue. Lignans are wood extractives and strong antioxidants with anti-tumor, wound healing effects, and hormonal activity. Norway spruce knots are known to be a rich source of extractable lignans (mainly hydroxymatairesinol (HMR)) [1,2]. Axially along the tree stem, lignan concentration is highest in knots located near the crown base. Based on pilot studies with confocal Raman IR microscopy, lignan molecules are hypothesized to be packed in specific structures in the cell lumens of knotwood [3]. In order to understand the biosynthesis and role of lignans in vascular plants and in different tissues, it is important to localise them on cellular level.

The multidisciplinary and intersectoral team consists of biologists, chemists, and physicists from Natural Resources Institute Finland (Luke), Åbo Akademi University, and University of Oulu. Feasibility trials related to present work were performed at HERMES beamline [4] at SOLEIL synchrotron. The first beamtime obtained was recently at the beamline BL4U [5] at UVSOR-III.

In order to get fresh samples, a Norway spruce tree was felled in southern Finland, and a branch whorl was sawn off at the height of 2.6 m. A small sample (1x1x1 mm^3) sample was prepared from the knotwood and fixed for 4.5 h in 2.5 % glutaraldehyde and 2.2 % para-formaldehyde in 0.1 M Na-phosphate buffer (pH 7.4). After a 3-day storage in 0.1 M Na-phosphate buffer, the sample was washed in ascending ethanol and acetone series, and embedded in LV resin. Transverse sections (60-120 nm in thickness) were cut with an ultramicrotome and placed on TEM copper slots with a Pioloform foil.

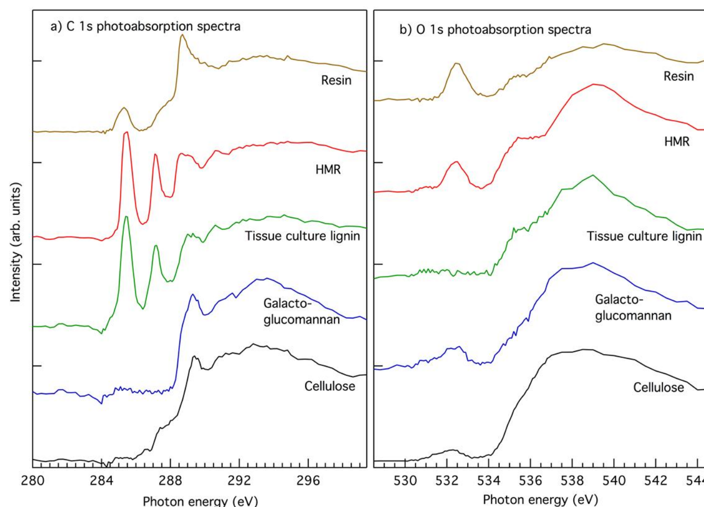

Figure. 1. a) C 1s and b) O 1s XAS of typical knotwood cell compounds and embedding resin.

The primary task was to identify the applicability of the STXM method on spatially and chemically separate knotwood extractives, cell wall macromolecules (lignin and cellulose), and locate HMR-rich regions in the knotwood cells. Our further aim was to recognize the compounds associated with lignan

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molecules (e.g. hemicelluloses and lipophilic molecules). Localization of lignans is a challenge, because both, dimeric lignans and the cell wall lignin polymer, are composed of phenylpropanoid units and give very similar X-ray absorption spectra (XAS) with overlapping regions. At C K-edge, both aromatic lignan and lignin polymer exhibited two sharp peaks around 285–287 eV, and the spectra were clearly different from those of non-aromatic cellulose and galactoglucomannan (hemicellulose), as shown in Fig. 1 a). The O K-edge revealed differences between tissue culture lignin [6] and HMR (Fig.1 b), confirming the chemical feasibility of the STXM method on the knotwood cell research. The key is to be able to use low enough photon flux in order to record the same cell without any radiation damage at both C and O K-edges, and cross-correlate the spatial and spectral findings between them.

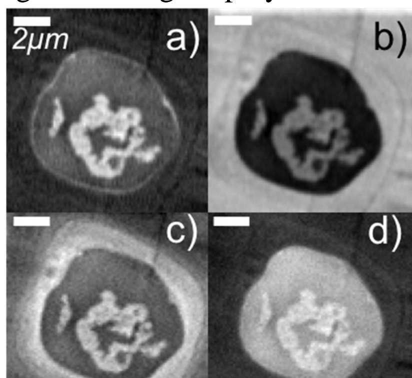

Figure. 2. Selected optical density images obtained at energy ranges a) 532.1–532.9 b) 537.0–540.0 c) 285.35–287.65 d) 288.6–288.9 eV highlighting the complementary information obtained from O and C K-edges.

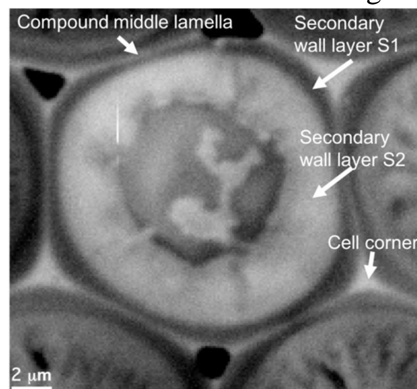

Figure. 3. A typical cell of an early wood knotwood recorded with 285 eV photon energy.

The STXM images of cells in knotwood have been recorded with 50 nm spatial resolution and varying energy steps (from 0.05 eV at regions with rapid spectral changes to 1 eV used above the ionization thresholds) at O and C K-edges. Figure 2 presents an example of a preliminary component analysis with aXis2000–program. In Fig. 2 a), a spectral region at O K-edge, where mainly lignan and resin have a resonance, is selected, whereas the region in b) contains also strong signals from cellulose, hemicellulose and lignin. In Fig. 2 c), the selected energy region corresponds to signals in both lignan and lignin spectra. Figure 2 d) indicates the embedding resin to contribute as a continuous background especially inside the cell. In the future, we will investigate the use of cryosections in order to avoid effects from organic fixatives and embedding. The attempt to directly fit the reference spectra to the stack images revealed a residual component that could be a lipophilic compound, probably sterol, fatty and/or resin acid, which are typical wood extractives.

Results indicate that lignans are linked with extractives like resin and fats in knotwood, and that the torus of bordered pit, located in the tracheid cell wall, consists mainly of lignin. Simultaneously with the chemical data, the STXM technique will provide morphological information on cell wall layers, and of cell organelles, where lignans are located. The spatial resolution of around 30 nm available at the present facilities was noted to provide resolution sufficient to imaging of the cells (Fig. 3). Increased structural resolution could enhance the contrast, and possibly, with spectral resolution, reveal structural features of cells.

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