Analysis of wood microstructure by synchrotron radiation-based X-ray microtomography (SR μ CT)

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ABSTRACT

Wood is a versatile tissue complex composed of different cell types such as tracheids, fibers, vessels and parenchyma cells, that each fulfills specific tasks within the woody body of a tree. However, even within a tree genus wood structure can differ significantly due to either biotic or abiotic stress factors occurring during the growth season, or due to cultivar specific growth characteristics. Typical tools for structural analysis of wood so far are scanning electron microscopy (SEM) and light microscopy, allowing measurements of sectional planes. However, the detection of boundary surfaces between neighboring cells and interconnectivity can only be observed in a restricted dimension. In our investigations at DESY, Hamburg, we applied SR μ CT for characterization of small wood samples of poplar (*Populus trichocarpa*) to gain a three dimensional image of a small scale sample that allows us to visualize size, volume, form and interconnectivity of the cells in a non-destructive way. Dependent on sample size, high resolution of micro-structure can be gained. This technique is appropriate to calculate volumes, e.g. of water transported in vessel cells. Since it is possible to reconstruct the vessel network within a stem section, effects of wounding or other environmental stresses on the xylem structure in general, and particularly on the water transport system, might be detected and visualized in 3D by synchrotron radiation-based microtomography. Hence this method is a most promising tool for future investigations in the field of plant physiology.

Keywords: synchrotron radiation, microtomography, absorption contrast, wood, xylem, poplar

1. INTRODUCTION

Wood is a complex anisotropic tissue derived from a lateral meristem, the cambium. Structure and cell type composition of the woody tissue determines its properties but varies within tree species. In poplar, being one of the model tree species most commonly used in tree research all over the world, and, hence, an acknowledged represent of angiosperm trees, wood is composed of three cell types each of characteristic features in form and function. Fibers, the most frequent cell type in poplar wood, are relatively thin-walled and are strung-out for an average of 1.1mm, occasionally reaching lengths up to 2 mm. They provide the stem with strength and solidity. Parenchyma cells appear as axial parenchyma, referring to their axial arrangement in the stem, as well as ray parenchyma cells, running radially from the wood forming cambium towards the inner pith. The thin walled parenchyma cells still possess a living protoplast and act as re-locating and storage points of assimilates from the leaves and minerals taken up with the transpiration flow. With about 65 μ m in poplar vessel elements have the by far largest diameter of the three occurring cell types; they enable water transport from the roots to the shoots and, by multiple elements being superimposed on another and the intermediate cell walls being disintegrated, they form a kind of tube system optimizing water transport to the leaves. Such formed axially aligned vessel tubes often can be found in radial groups of 2-3, with the contact cell walls of the adjacent vessel elements forming pits, thus facilitating a small scale horizontal translocation of the transpiration stream on its way to the shoots. Each tree species has its characteristic pattern of cell types and arrangement that makes it distinguishable from other species. Within this determined composition, however, arrangement and form might vary due to biotic or abiotic impact on wood forming tissue.

1.1 Customary methods of analyzing wood structure

On the cellular level microstructural parameters such as cell type composition, cell wall thickness, cell length and shape do influence also the macroscopic characteristics of the wood. Light microscopy analysis is one of the classical methods in wood anatomy imaging, with thin microtome sections in combination with the desired staining techniques providing explicit overviews of the chosen sectional plane (Fig. 1).

Figure 1. Light microscopy images of poplar wood. A: cross section; B: tangential section; C: radial section. v-vessel; r-ray; f-fiber. Bars represent 20 μm .

Analysis of the complex vessel network in dicotyledonous trees was first performed by Braun (1959) by reconstructing its structure from a series of transverse sections by light microscopy¹. As time-consuming and elaborate as this meticulous analysis is, it has still been the most popular way to gain information on vessel network for many years.

With the aid of electron microscopy higher magnifications (transmission electron microscopy) can be achieved, allowing further insights in cell wall structure and/or a clue to the three dimensional structure (scanning electron microscopy) of the examined wood sample. However, it is mainly the two dimensional (2D) information, that is gained by those classical microscopic techniques, which then is always preceded by partial destruction of the sample body during preparation. But also non-destructive three dimensional (3D) analysis of wood has been performed in the last decades. For example stem analysis of ten to twenty year old spruce and oak has been analyzed by high-resolution computed tomography, revealing insights of water distribution relative to stem height and widths². Also other high resolution imaging techniques have been applied to gain images of wood structure non-destructive and non-invasive on the sample, such as ionizing radiation techniques, thermal techniques, microwave techniques, ultrasonic techniques or nuclear magnetic resonance techniques³. In recent years synchrotron radiation microtomography using absorption-contrast and phase-contrast were applied to wood samples, allowing a much improved resolution of the sample structure^{4,5}.

1.2 Using synchrotron radiation for soft materials analysis

Microtomography using synchrotron radiation is an established tool for the non-invasive 3D investigation in the field of materials science, medicine, biology. Within the last 14 years the technique was applied as a user experiment using absorption contrast at the synchrotron radiation source DORIS III at DESY. Starting end of 2002 the Helmholtz-Zentrum Geesthacht became responsible for operating the microtomography experiments at the wiggler beamlines BW2 / DORIS III (using 7 to 24 keV photon energy) and at the high energy materials science beamline HARWI II / DORIS III (20 - 180 keV). For low absorbing samples we use the microtomography setup at BW2 allowing for tomograms not only showing high spatial resolution, but also high density resolution. Due to the large source at the 2nd generation storage ring DORIS III showing nearly no coherence artefacts in the intensity projections we could setup the system at the optimal photon energy for pure absorption contrast. Especially for wood samples absorption contrast allows for the differentiation of different type of materials together with the inspection of the porous system⁶.

2. MATERIAL

Wood samples used in this investigation were obtained by harvesting 7 mm long stem segments of one year old poplars (*Populus trichocarpa* var. Trichobel) at the end of the growing season. For light microscopy analysis fresh samples were cut at a microtome (Hyrax, Zeiss, Germany) at 8 μm thickness, stained for six minutes with astrablue (Astrablau FM, C. Roth, Karlsruhe, Germany) and thoroughly rinsed with deionized water before stained for two minutes with safranin (Safranin O, C. Roth, Karlsruhe, Germany). Sections were rinsed in deionized water until the solution stayed clear, mounted on object slide and embedded in glycerin. Microscopic analysis was performed on Axioskop 40 (Zeiss, Germany) using the ZEN software (ZEN, Zeiss, Germany). For synchrotron radiation-based X-ray microtomography the poplar stem segments were divided lengthwise and stored in 70% ethanol until further processing. Then they were dehydrated in 99.6% ethanol for some days before being dried in an oven at 60°C for 24 hours. For the microtomography experiments itself the dried samples were trimmed with a razor blade to 2 mm (radial) x 2 mm (tangential) and glued upright onto special cylindrical sample holders with a two-component adhesive (UHU plus schnellfest, UHU, Germany).

3. METHODOLOGY

The wood samples were scanned applying SR-based microtomography in absorption mode at the wiggler beamline BW2 / DORIS III at DESY at photon energy range of 7 -24 eV, beam size of up to 20 x 3 mm² and spatial resolution of up to 2 μm . Spatial resolution is determined by measuring the projection of an edge. From this edge function the modulation transfer function (MTF) of the system is calculated. The spatial resolution is then given at that point, where the MTF decreases to 10%. The samples were scanned at with photon energy of 9 keV $\pm$ 0.002 keV. A double crystal Si(111) monochromator was used to monochromatize the incident white beam. After entering the X-ray detector, the monochromatic beam was converted by a fluorescent screen (CdWO₄, 300 μm thick) into visible light, which was then projected on the CCD camera (Apogee KX2, Kodak KAF 1600, 1536x1024 pixels, pixel length 9 μm). The sample was rotated from 0 to 180 degrees in equal steps of 0.25 degrees, resulting in a total of 720 projections. Before reconstruction the center of rotation is determined by an automatic algorithm⁷. The single slice reconstructions were calculated from the raw data (sinograms) with a filtered back-projection algorithm. These images were then subsequently stacked to create a 3D voxel data-set, which was read into visualization software (VGStudio, Volume Graphics GmbH, Heidelberg, Germany) for 3D visualization.

4. RESULTS

After data processing, the wood microstructure was observed in transverse, tangential and radial sections (Fig. 2) with VGStudio visualization software. In the cross section different cell types of the stem could easily be distinguished. The pith parenchyma cells in the center appear irregularly shaped and thin walled, as typical for this cell type. The adjacent woody body reveals all the characteristic features that can also be seen in light microscopic images; vessels are clearly distinguishable from the smaller fibers, and even the uniseriate rays are pictured explicitly. In tangential plane the three cell types of the woody body are equally distinguishable. The vessel lumen diameter discerns vessels from fibers, the superposed ray parenchyma cells show their small diameter and by being superimposed on another they reveal the height of the respective wood ray. Radial plane sections confirm the isodiametric cell shape of pith cells and also of ray parenchyma cells. Again, vessel elements can be clearly distinguished from fibers by their difference in cell lumen.

Figure 2. 2D reconstruction of the three planes of a young poplar stem. A: cross section; B: tangential section; C: radial section. In all sections the microstructure of poplar wood is clearly distinguishable. pp-pith parenchyma; r-ray; v-vessel; f-fiber.

However, the clear advantage of wood analysis by synchrotron radiation-based microtomography lies in the three dimensional visualization of the sample. The 3D rendering of the intact poplar wood sample by VGStudio software enables to spatially isolate individual tissue cell types within the complex wood matrix. In Figure 3 the cell wall material was gradually removed along with coloring of cell lumina (Fig. 3, red). Vessel forming elements can be easily distinguished from fiber lumina due to their significantly larger diameter, hence revealing the complex vessel network within the chosen region of interest (ROI). One can observe that the vessels do not run strictly parallel but occasionally deviate from their exact axial orientation in order to detach from or to contact neighboring vessels and hence forming a complex 3D vessel network (Fig. 3).

Figure 3. 3D reconstruction of a reduced region of interest (ROI, height about 1 mm) of a poplar wood sample. Identifying the vessel structure, cell wall material (grey) was gradually removed (from left to right) along with marking cell lumina in red.

5. CONCLUSIONS

To reveal an objects internal microstructure, synchrotron radiation microtomography is the method of choice. For various materials it has already been demonstrated as an interesting tool for 3D imaging. Due to its capability to select tuned monochromatic X-rays at low divergence and high flux this method enables high spatial resolution (about 2 μm) and high density resolution (about 1%). Hence, it has been found suitable for many biological materials, such as bone tissue and the investigation of insects^{8,9,10,11}. Also with wood samples μCT has been proven a promising method for non-destructive analysis of xylem architecture^{5,12,13,14}. Here it was shown, that visualization of the plane sections gained by SR μCT corresponded well with traditional light microscopic analysis methods, even though the pixel size in SR μCT is still below that of light microscopy. Improvement here could be gained by using a smaller sample size or improvement of the data rendering process. Yet, microtomography has plain advantages by its non-destructive 3D visualization of the sample. Especially the option of illustrating individual vessels within the tissue complex and observing their network formation makes this method very interesting in field of research on plant physiology, for the vessel network is not only responsible for axial water transport, but also for tangential spreading of the water flux within a year ring of a living tree¹. The more vessels are connected with each other, the more alternate paths for water flow are available in the stem. Here also the length of the overlaps and the formation of the intervessel pits determine the resistance on the transpiration flow and thus influence water availability in the leaves and ultimately physiological processes like photosynthesis. To investigate stem vessel network of the total cross sectional area on the one hand, and focalize on microstructural features like the formation of intervessel pits on the other hand, will be fields of interests which might be to explore with the help of SR μCT . With the availability of the new microtomography systems at the 3rd generation storage ring PETRA III the spatial resolution will easily be at $<1 \mu\text{m}$. Furthermore, the combination of phase-contrast and absorption contrast technique will allow for a new quality in this field of wood research.

ACKNOWLEDGEMENTS

The financial support of this work was generously provided by the Center for a Sustainable University (KNU), Universität Hamburg, granting a postdoc fellowship to S. Lautner. The authors also would like to thank Sebastian Thies and Wolfgang Drube, DESY, for their support at the beamline.

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