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ABSTRACT

Water transport from roots to shoots is a vital necessity in trees in order to sustain their photosynthetic activity and, hence, their physiological activity. The vascular tissue in charge is the woody body of root, stem and branches. In gymnosperm trees, like spruce trees (*Picea abies* (L.) Karst.), vascular tissue consists of tracheids: elongated, protoplast-free cells with a rigid cell wall that allow for axial water transport via their lumina. In order to analyze the over-all water transport capacity within one growth ring, time-consuming light microscopy analysis of the woody sample still is the conventional approach for calculating tracheid lumen area. In our investigations at the Imaging Beamline (IBL) operated by the Helmholtz-Zentrum Geesthacht (HZG) at PETRA III storage ring of the Deutsches Elektronen-Synchrotron DESY, Hamburg, we applied SR μ CT on small wood samples of spruce trees in order to visualize and analyze size and formation of xylem elements and their respective lumina. The selected high-resolution phase-contrast technique makes full use of the novel 20 MPixel CMOS area detector developed within the cooperation of HZG and the Karlsruhe Institute of Technology. Our results correspond well with data by light microscopy analysis and, hence, prove, that μ CT is a most appropriate method to gain valid information on xylem cell structure and tree water transport capacity.

Keywords: Synchrotron radiation, microtomography, absorption contrast, phase contrast, wood, spruce, tracheid

1. INTRODUCTION

Apart from ensuring strength and stability, the woody body of trees also provides the vascular tissue for long distance water transport from roots to shoots. Different to angiosperm trees, having developed a specialized cell type for long distance water transport, axial water transport in gymnosperm trees like spruce (*Picea abies* (L.) Karst.) occurs in tracheids. About 95% of softwood xylem is composed of this cell type, and it is both responsible for stability as well as for axial water transport. Within one growth ring tracheids vary in their appearance: in the early wood region, i.e. xylem cells built from early spring to summer, the main purpose of the tracheids is to ensure water transport pathways into the tree's crown; hence, the tracheid lumina are wide and the cell walls are much thinner than in late wood region (Fig. 1 A). Once the water transport pathway is established, tracheids change their features towards thick cell walls and small lumina. Due to the resulting increase in density, thus formed late wood region, produced between late summer and autumn, enhances strength and stability to the tree. Being a physiological process, wood formation can also be influenced by biotic as well as abiotic impacts, resulting in an altered wood tissue composition or in a change of early wood / late wood characteristics^{1,2,3}. In gymnosperm trees abiotic stress factors like drought periods most frequently result in reduced early wood formation and, consequently, reduced volume of xylem tissue designated for water transport within the respective growth ring. On only occasionally occurring drought periods spruce trees are well able to compensate this loss with adjacent growth rings, however, ongoing climate change predicting an increase of drought period frequencies in central Europe, future spruce tree's wood formation might be substantially challenged. With our investigations at PETRA III of DESY we wanted to examine non-destructive SR μ CT analysis as a valid alternative to conventional light microscopy methods when analyzing wood cell anatomy and water transport capacity of selective growth ring areas.

1.1 Conventional method of xylem element analysis by light microscopy

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 overviews of the chosen sectional plane at high spatial resolution. In softwood species early wood : late wood ratio, resin duct and wood ray arrangement, as well as cell size and formation can easily be analyzed in the respective planes (Fig. 1). However, the gained information remains at a 2D level, so when calculating volumetric information, results from different plane sections must be brought together in a meticulous and time consuming way.

Figure 1. Light microscopy images of spruce wood. A: cross section; B: tangential section through early wood region. ew-early wood; lw-late wood; rd- resin duct; r-ray; t-tracheid. Scale bars represent 200μm.

When calculating fiber lumen area of early wood tracheids, cross sectional area of an area of interest is measured on cross section samples and multiplied with tracheid lumen length of reference tangential sections. Even though those results are very accurate, they still might bear artifacts due to the following reasons: firstly, because of the destructive way at sample section preparation, cross section area and lumen length cannot be measured on identical tracheids; secondly, due to intrusive tip growth during xylem differentiation, tracheid fibers are not arranged in a strictly vertical orientation, but always show ever so slight bends and convexities in longitudinal planes; thirdly, prosenchymatic formation of tracheid cells complicates volume calculation especially with respect to the tips of the measured cells.

1.2 Using synchrotron radiation for 3 D wood 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 two decades the technique was applied as a user experiment using absorption contrast first at the synchrotron radiation source DORIS III at DESY. Starting 2007, the Helmholtz-Zentrum Geesthacht became responsible for setting up and the operating of the microtomography experiments at the undulator

beamlines IBL / PETRA III (using 7 to 45 keV photon energy) and at the high energy materials science beamline HEMS / PETRA III (35 - 150 keV). For low absorbing samples we use the microtomography setup at IBL allowing for tomograms not only showing high spatial resolution, but also high density resolution. Due to the partial coherent source at the 3rd generation storage ring PETRA III different kinds of contrast techniques for microtomography can be applied by varying the sample detector distance. Especially for biological samples like wood both contrast techniques, absorption contrast and phase contrast, allow for the differentiation of various cell types within the sample body together with the inspection of the porous system^{4,5}.

2. PLANT MATERIAL

Wood samples used in this investigation were taken from a 50 year old spruce stand (*Picea abies* (L.) Karst.) with standard increment core drill, a standard sampling method commonly used for dendrochronological investigations. The increment cores have a diameter of 5 mm and were taken at stem height of 1.3 m. For synchrotron radiation-based X-ray microtomography defined sections of the increment core were carefully dried in an oven at 60 °C for 24 hours. Prior to SR μ CT analysis, the core sections were glued upright onto special cylindrical sample holders with a two-component adhesive (UHU plus schnellfest, UHU, Bühl, Germany). After SR μ CT measurements, the very same samples were used for light microscopy analysis. Thereby, cross- and tangential sections were cut at a microtome (SM 2400, Leica, Wetzlar, Germany) at 15 μ m thickness. The obtained sections were stained for 6 minutes with astrablue (Astrablau FM, C. Roth, Karlsruhe, Germany), rinsed with deionized water and stained for another two minutes with safranin (Safranin O, C. Roth, Karlsruhe, Germany). Stained sections were rinsed in deionized water until the solution stayed clear, mounted on object slides and embedded in glycerin. Microscopic analysis was performed on AxioLab A1 equipped with a camera system (AxioCam 105, both by Zeiss, Germany), using the ZEN software (ZEN, Zeiss, Germany).

3. METHODOLOGY

The tomographic investigation was performed at the imaging beamline IBL operated by the Helmholtz-Zentrum Geesthacht at the storage ring PETRA III at DESY, Hamburg, Germany. The experimental setup is described in more detail in [PAPER 10391-26]. The X-ray detector was equipped with a novel 20 Megapixel CMOS image sensor (CMOSIS CMV20000). The integration of the sensor into the beamline setup was done within a cooperation of HZG and KIT. The detector uses a global shutter and has a readout time of 30 ms. For the investigation of the wood sample we used a photon energy of 23 keV.

The spatial coherence of the X-ray beam at the microtomography endstation at IBL is exploited to enhance intensity contrast by means of free-space propagation of the transmitted wave front. At a sample-to-detector distance of 60 mm, edge-enhancement occurs i.e. single fringes emerge in the recorded images at the edges of the projected object. In order to retrieve the phase, flat-and-dark field corrected projections are subject to phase retrieval prior to tomographic reconstruction. Here we employed an algebraic inversion of the linearized transport-of-intensity equation in Fourier space which is often termed Paganin phase retrieval⁶. This method acts as a low-pass filter in Fourier space i.e. at a moderate expense of spatial resolution compared to the reconstruction without phase retrieval, it removes edge-enhancement fringes and suppresses noise, see Figure 2 and Figure 3.

Because of beam instabilities (vertical drift) flat fields and projections were correlated using the structural similarity index. A combined wavelet-Fourier filtering was used to reduce ring artefacts⁷. Data preprocessing, phase retrieval, and tomographic reconstruction was implemented in MATLAB. Tomographic reconstruction was performed using the ASTRA toolbox^{8,9}.

Figure 2: Horizontal slice through tomographic reconstructions where flat-and-dark field corrected projections (left) or retrieved phase maps (right) served as input. The white square indicates the zoomed-in region of interest depicted in Figure 3. Comparing reconstructions without (left) and with phase retrieval (right), contrast is enhanced, noise is reduced, and ‘unphysical’ density variations in the reconstruction stemming from propagation effects are removed. This comes at the expense of spatial resolution, see Figure 3.

Figure 3: Zoomed-in region of interest as shown in Figure 2 using intensity (left) or phase maps (right), respectively, as input for the tomographic reconstruction.

Images were then subsequently stacked to create a 3D voxel data-set, which was read into visualization software (Avizo, FEI, Thermo Fisher Scientific, Hillsboro, Oregon, USA) for 3D visualization.

4. RESULTS

The results of our light microscopy analysis on tracheids revealed an average lumen volume of 0.005 mm^3 per early wood tracheid, compared to 0.0005 mm^3 in late wood tracheids (Fig. 4), with the ten times higher volume in early wood tracheids reflecting the importance of this tissue for axial water transport capacity. Likewise, increase of cell wall

volume in late wood regions of a growth ring points towards the enhancement of strength and stability for the woody body in this part of the growth ring. Analysis of SR μ CT on the identical spruce wood samples revealed a very similar volume ratio of early wood : late wood tracheid lumina (Fig. 5).

Figure 4. Calculated volumes of spruce wood tracheid's lumina and cell wall by light microscopy analysis. Early wood tracheid lumen clearly exceeds late wood lumen, emphasizing its importance for axial water transport. For volume calculations of early wood 20 tracheids were measured in transverse plane (cell wall as well as lumen cross section area), 16 in tangential plane, for late wood 20 tracheid's cross section areas and 18 lengths were measured accordingly.

Figure 5. Calculated ratio of spruce wood tracheid's lumina and cell wall of a chosen region of interest of synchrotron radiation-based microtomography. Early wood tracheid lumen clearly exceeds late wood lumen, emphasizing its importance for axial water transport. ROI: 0.13 mm³

Additionally to the good correspondence of the calculated volumes, SR μ CT also allows for the 3D visualization of intact tracheid volumes. Via a segmentation process, the visualization software allows for removal of cell wall material in favor of presenting the respective cell lumina. As shown in Figure 6, the lumen visualizations can display the actual volumes very graphically. Axial orientation and local irregularities in circumference are reliably displayed and the above mentioned artifacts in the course of light microscopy analysis therefor can be excluded. Furthermore, 3D imaging of xylem elements not only allows a more precise calculation of individual cells, but also allows for reliable information on intra-tissue arrangement and conjunctions within different cell types.

Figure 6. Visualization of cell wall (yellow, left) and lumina (blue, right) volumes of representative 3D areas of spruce's early wood region. ROI: 0.13 mm³

5. CONCLUSIONS

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^{10,11}. Also with wood samples SR μ CT has been proven a promising method for non-destructive analysis of xylem architecture^{4,12,13}. Here it was shown, that volume calculation of selected wood tissues gained by SR μ CT corresponded well with traditional light microscopic analysis methods, even though the pixel size in SR μ CT is still below that of light microscopy. By the use of the fast 20 MPixel CMOS system, together with the application of the Paganin phase retrieval method using 23 keV photon energy, we were able to reduce the dose on the sample and to increase the total sample throughput without losing contrast in the tomograms for the selected specimens. Also, compared to light microscopy, microtomography has plain advantages by its non-destructive 3D visualization of the sample. Especially the option of illustrating individual cells and cell types within a chosen tissue complex and analyzing their interconnectivity and special arrangement makes this method very interesting in field of research on tree physiology: especially when built under abiotic or biotic stress not only can the general shift in wood tissue composition be observed, but also strategies of trees to bypass the thereby occurring challenges in order to keep their physiological functioning intact.

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