Dendrochronologia 60 (2020) 125687

Contents lists available at ScienceDirect

Dendrochronologia

journal homepage: www.elsevier.com/locate/dendro

AutoCellRow (ACR) – A new tool for the automatic quantification of cell radial files in conifer images

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ARTICLE INFO

Keywords:
Automated image analysis
Cell radial file
Quantitative wood anatomy
Tracheidogram
Tree ring

ABSTRACT

Quantitative wood anatomy (QWA) is a growing field of dendrochronology that allows obtaining a large number of parameters as the number, size and spatial arrangement of cellular elements, elements that highlight the adjustments of trees to their environment. In this work, we presented the free/libre open-source software AutoCellRow (ACR), a ready-to-use tool for automatic QWA in conifers. The ACR analyzes radial files of cells on cross-sections views of tree rings and provides automatic measurements of different cell parameters (e.g., lumen radial diameter, double cell wall thickness and cell radial diameter) for each cell along the selected radial file. The ACR measurements are based on high performed image analysis of xylem cells. The accuracy of the software measurements was tested in cross-sections of five conifer species from a semi-arid area of southern Siberia, and compared with measurements obtained by a semiautomatic tool. Our results suggested high accuracy in the ACR cell traits measurements, facilitating and speeding the analysis of quantitative wood anatomy in conifers over radial files of cells.

1. Introduction

Environmental conditions control tree-rings formation along the growing season, leaving a permanent imprint in the xylem structures (e.g., cell size, lumen diameter and cell wall thickness). Tree-ring width based chronologies are the most used parameter in dendrochronology, encoding environmental information in a yearly resolution (Speer, 2010), and being valuable archives of the past climatic conditions (Fonti et al., 2010). However, quantitative wood anatomy (QWA) allows extracting more detailed information (at intra-annual or seasonal resolution) by the analysis of different xylem cell traits (Olano et al., 2012; Castagneri et al., 2017; Arzac et al., 2018a). Tree-rings are formed by a complex process of consecutive new cell production (Rathgeber et al., 2016) affected by the environmental conditions at the time of its formation. This characteristic allows the identification of xylem cells in a spatiotemporal resolution within a specific tree-ring (von Arx et al., 2016). Thus, QWA quantifies xylem anatomical traits variability highlighting the adjustments of trees to their environment.

The quantification of xylem traits follows a succession of steps (see as example Gärtner and Schweingruber, 2013; von Arx et al., 2016; Arzac et al., 2018b) to achieve high-resolution wood anatomical preparations and digital images for further analysis. Recent developments in imaging technology and recognition programs have boosted the progress of QWA, offering a broad range of image-analysis software. The options range from general tools such as ImageJ (W. Rasband, National Institutes of Health, Bethesda, MD, USA) and semiautomatic tools as Lineyka (Silkin, 2010), to highly specialized wood anatomy tools such as ROXAS (von Arx and Carrer, 2014) and WinCELL (Regent Instruments Inc., Québec, Canada); leaving a choice to the user to decide the tool according to the research interests. Thus, for the measurements of basic anatomical traits (e.g., cell lumen and wall thickness) on a few radial files per ring, general or semiautomatic tools are good enough to provide stable results. However, if the volume of work is higher (e.g., number of trees, long-temporal series and the number of cells to be measured) or the study goals require more detailed information on cell traits, specialized tools as ROXAS are a better option.

In this work, we introduce the AutoCellRow (ACR), a new free/libre open-source image-analysis tool to perform measurements of xylem cell

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https://doi.org/10.1016/j.dendro.2020.125687
Received 12 September 2019; Received in revised form 26 February 2020; Accepted 2 March 2020
Available online 03 March 2020

traits in high-quality images (e.g.,. tiff,. jpg,. bmp,. png, formats). ACR was developed in Python Programming Language (https://www.python.org/) to work in MS Windows environment. The ACR is maximally automated and intuitive software orientated to quantify xylem cell traits along the radial axis of cell rows (cell radial files) in cross-sectional images of conifers. The number of xylem cell traits measured by ACR is broad, including cell lumen diameter, cell wall thickness, cell size and recognition of early- and latewood cells, among others. In addition, ACR allows to automatically plot a tracheidogram of a chosen row within a giving tree-ring, being of particular interest for the seasonal analysis of xylem cell traits. Thus, ACR offers an excellent alternative to the general and semiautomatic QWA tools currently available to analyze xylem cell traits along radial files in conifers.

2. Materials and methods

2.1. Fundamentals of ACR

The ACR has been developed with the application PyQt5 in Python 3 (The Python Tutorial, 2019) as a free/libre open-source software (FLOSS), ready-to-use and available at http://vs-genn.ru/acr/ and at https://github.com/vs-genn/ACR (a reserve copy in The International software depository GitHub). ACR runs in MS Windows environment and interacts with MS Excel as a data output manager. The interaction between ACR and MS Excel is by the openpyxl library, which allows the reading/writing in different Excel format files (Gazoni and Clark, 2010). The software is based on four different modules (i.e., the graphical user interface, the image analysis module, the xylem cell traits calculator and the data processing module), all packaged in a friendly user interface (Fig. 1).

Before the automatic cell recognition, the chosen image is binarized in black and white by using the Otsu's method (EQA1; Otsu, 1979), increasing the contrast between the cell lumen and the cell walls, with cell lumens resulting as light areas and cell walls as dark areas in the image (the process runs as hidden). After the binarization, the center of mass of the cell lumen (essential for the automatic cell recognition process) is calculated by the EQA2. The center of mass is primarily used to find cell lumens along a radial file of cells within the tree-ring. Thus, after the user selects the radial file to be analyzed, the software automatically identifies the center of mass of two neighboring cells lumen allowing the automatic draw of a line that extends to the following lumen center mass. If the lumen is not found on the given straight line, then the software searches the nearest lumen in a thirty degrees angle from the original line (Fig. A1A). Radial and tangential diameters of cells lumen are then calculated based on the center of mass for each cell (Fig. A1B).

The lumen area is estimated by the number of pixels contained in the lumen of a particular cell, whereas the lumen perimeter is calculated by going clockwise around eight sides of a given pixel, bypassing the outermost pixels assigned to the cell lumen. Finally, all the pixels conforming the lumen perimeter are counted. Double cell wall thickness (2CWT) is then calculated as the distance between the innermost points of the cell walls of two neighboring cells (EQA3), whereas the single-cell wall thickness is calculated as the average of the double cell wall. The differentiation between earlywood and latewood cells is based on Mork's Index (Denne, 1988). Thus, when the cell lumen area is 1.5 lower than the average cell, it is defined as latewood.

2.2. Operation of ACR

Once loaded an image file (in. tiff,. bmp,. png,. jpg formats), the ACR user must define the resolution of the original image (pixels/μm) given by the image acquisition method (e.g., resolution of camera/scanner) in the "resolution ratio" QInputDialog (graphic widget in Python environment). After the ring to be analyzed has been selected, the user must define the calendar year corresponding to that particular annual ring in the "year" QInputDialog and chose the radial file along which xylem cell traits will be measured. To analyze a particular radial file, the first cell along the radial axis should be selected by clicking with the left mouse button. As a result, all the cells along the chosen row are automatically detected (Fig. 1). Providing information about i) ring width; ii) number of cells in the selected row; iii) identification of earlywood and latewood cells; iv) radial and tangential cell lumen; v) lumen area and perimeter; vi) single and double cell wall thickness; vii) cell wall area and; viii) cell area and perimeter (see Table 1 for more details). In addition, the user has available the "threshold" tool to

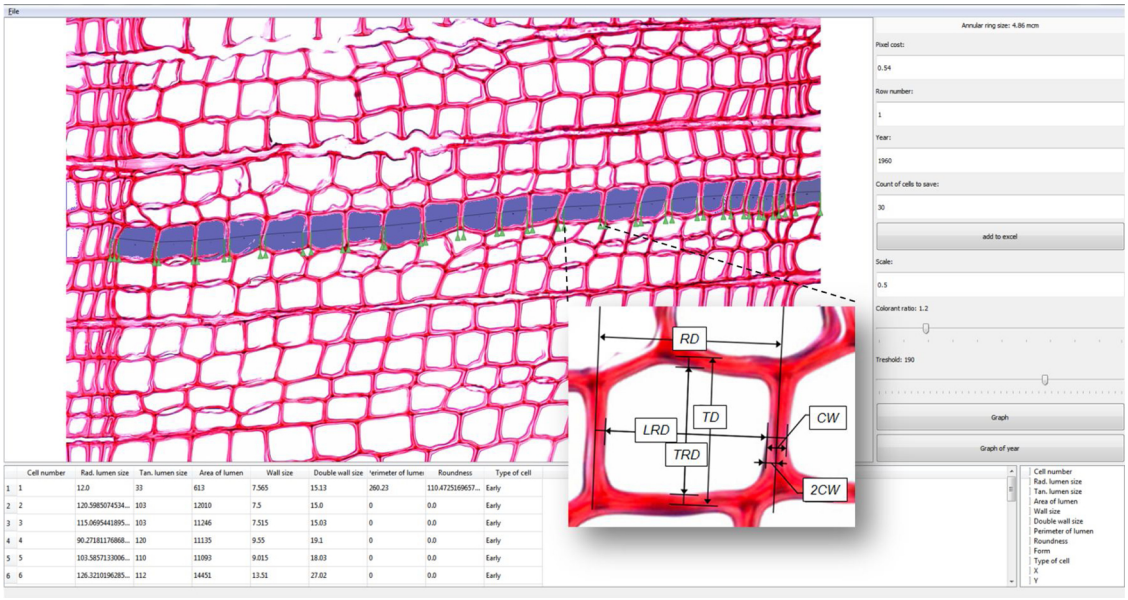

Fig. 1. ACR print screen showing the automatic identification of cells along a selected radial file. Filled cells show the identification of tracheid lumen and arrows the cell walls. The zoom in tracheid shows the measured traits. LRD, lumen radial diameter; TRD, tangential radial diameter; CW, cell wall thickness; 2CW, double cell wall thickness; RD, cell radial diameter; TD, cell tangential diameter.

Table 1
Overview of the tree-ring traits measured by ACR.

Anatomical trait	Acronym	Unit
Tree-ring width	TRW	μm
Lumen radial diameter	LRD	μm
Lumen tangential diameter	LTD	μm
Lumen area	LA	μm
Double cell wall thickness	2CWT	μm
Cell wall area	CWA	μm
Tracheid radial diameter	D	μm
Cell number in the radii	N	–

improve the automatic cell detection (see Fig. 1). Finally, total or partial calculated parameter values can be written as an MS Excel file (*.xlsx format) for further statistical analysis. Once the *.xlsx file is created, the user can continue writing new information in the file using the "add to excel" button.

Additionally, the ACR offers the option to show a tracheidogram of the analyzed radial file by clicking in the "graph" button (right lower button of the user interface, see Fig. 1). The user can scroll through the image using the right mouse button; as well, it has the option to zoom in/out the image by using the mouse scroll wheel. We have included a test image at http://vs-genn.ru/acr/ (resolution ratio = 2.26 μm/pixel), which is available for potential users of ACR.

2.3. Testing ACR

The accuracy of ACR cell detection and cell traits measurements was tested in cross-sections of five conifer species; the evergreen *Pinus sylvestris* L., *Pinus sibirica* Du Tour, *Abies sibirica* Ledeb., *Picea obovata* Ledeb and the deciduous *Larix sibirica* Ledeb. The wood material was collected in the Khakass-Minusinsk depression, southern Siberia (Khakassia Republic, Russia; see Table A1 for further details). The region is characterized by moderate continental climate conditions with a vegetation cover ranging from steppes through mixed and conifer forests to mountain tundra (Table A1). Wood cores were taken at breast height from the stem with a 5 mm diameter increment borer, labeled and taken to the laboratory where they were air-dried.

Permanent histological preparations were processed according to standard anatomical techniques (Schweingruber and Poschlod, 2005) and embedding in starch before cutting (Schneider and Gärtner, 2013) to reduce cell damage while sectioning. Cross-sections thinner than 20 μm were cut with a sledge microtome (MICROMHM 430) and stained with a safranin solution, resulting in lignified cells turning red. Thin-sections were dehydrated using a series of ethanol solutions of increasing concentrations, washed with xylol, and permanently preserved by embedding them into Canadian balm. Digital images were captured with an AXIOCam MRc5 digital camera (Zeiss, Germany) mounted on a Zeiss Axio Imager D1 optical microscope (Zeiss, Germany) with 200x magnification.

To validate the ACR output data, we compared cell traits measurements with the measurements produced by the semiautomatic software Lineyka 2.01 (Silkin, 2010). Lineyka is a semiautomatic tool that allows the measurement of radial lumen diameter and cell wall thickness along radial files of cells (see Fonti and Babushkina, 2016; Arzac et al., 2018a; Popkova et al., 2018 as examples). For this task, lumen radial diameter (LRD), tracheid wall thickness (CWT), and the resulting tracheid radial diameter (D = LRD + 2CWT) were measured along the radial axis of five radial files per annual ring in four tree-rings per species. A one-way ANOVA was used to evaluate differences in measurements between methods, with the measurement methods as a fixed factor.

3. Results

The total number of measured cells was 2369 (across the 100 radial files) per measuring method. ACR showed high accuracy in the cell lumen and cell walls recognition along the selected row (Fig. 1), including both earlywood and latewood cells. Additionally, the differentiation between large and thin-walled earlywood cells and small and thick-walled latewood cells was accurate among the radial files and species. However, we found a decrease in the accuracy of cell recognition according to the quality of the micro-sections. Fig. 2 shows how the ACR accuracy in cell detections varies according to the image/section quality, thus in images where artifacts occurred while sectioning (Figs. 2B and 2C), the automatic cell recognition process decreased, whereas in high-quality micro-sections (Fig. 2A) improved. Nevertheless, independently of the species, the measurements performed with ACR were considerably less time consuming than with the semiautomatic tool Lineyka. Thus, ACR requires around two to three seconds on average (according to the image quality) to identify and measure the cell traits along a cell radial file.

The automatized cell detection offered by ACR revealed to be a much faster and accurate method (non-dependent of the user for cell limits definition) in comparison with the semiautomatic tool, mostly improving the cell walls borders identification. Semiautomatic measurements systematically slightly overestimate LRD and underestimate CWT in all the cases (Table 2). However, the resulting tracheid radial diameter was similar in all the species and rings analyzed, overlapping the values produced by the two methods in all the tree-rings measured (Fig. 3). In this sense, the one-way ANOVA revealed no significant differences between the ACR and semiautomatic approach in LRD and D ($P > 0.05$) for all the species (Table 3). Nevertheless, 2CWT was significantly different in all the species but *Pinus sylvestris* (see Table 3 for further details).

4. Discussion

The detection of cells and anatomical traits measurements performed by the ACR showed high accuracy in the five conifer species

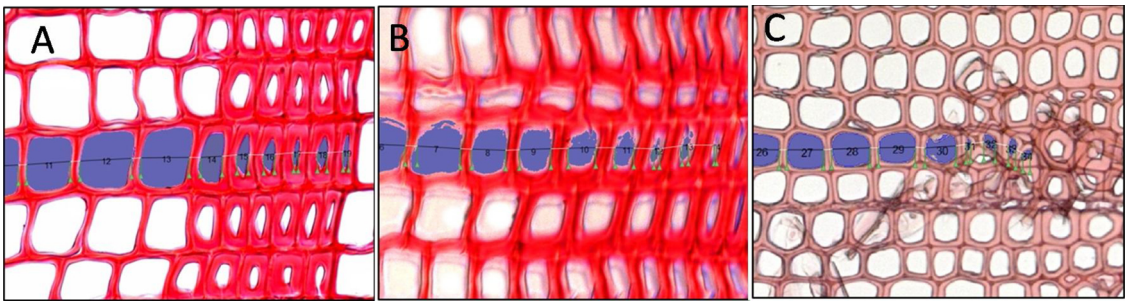

Fig. 2. Effect of the image and sectioning quality on the automatic cell detection by ACR. Good quality section and image (A), blurred image (B) and bad quality section (C).

Table 2
Anatomical traits measurements by ACR and the semiautomatic tool Lineyka. Values are averages (mean ± SD) of five radial files measured in four years per species. LRD, lumen diameter; 2CW, double cell wall thickness; D, tracheid radial diameter.

		Pinus sylvestris	*Pinus sibirica*	*Abies sibirica*	*Picea obovata*	*Larix sibirica*
ACR	LRD	21.14 ± 12.31	24.10 ± 10.35	20.52 ± 10.19	28.15 ± 12.32	20.26 ± 18.10
	2CW	6.04 ± 3.46	5.74 ± 1.43	6.80 ± 1.93	6.97 ± 3.89	10.74 ± 5.20
	D	27.18 ± 10.81	30.16 ± 9.92	27.32 ± 9.32	35.12 ± 11.36	31.00 ± 15.35
Semiautomatic (Lineyka)	LRD	21.89 ± 11.98	25.51 ± 10.48	22.32 ± 10.22	30.43 ± 11.98	22.22 ± 18.14
	2CW	5.42 ± 2.04	4.90 ± 0.91	5.18 ± 1.45	5.06 ± 1.76	8.74 ± 5.10
	D	27.31 ± 10.82	30.40 ± 10.17	27.50 ± 9.48	35.49 ± 10.97	30.96 ± 15.69

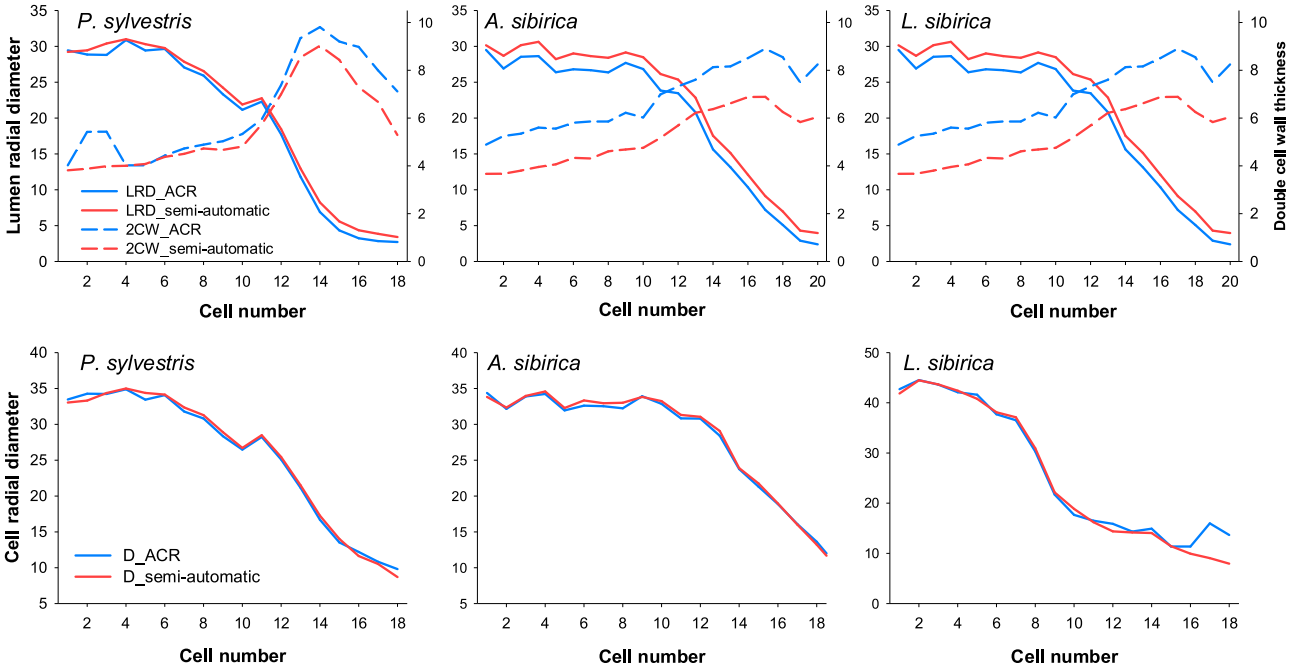

Fig. 3. Comparison of cell traits measurements (lumen radial diameter, double cell wall thickness and cell radial diameter) between ACR (blue lines) and semi-automatic Lineyka tool (red lines) in three species. The plots show the average of measurements in five radial files in four years per species (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

Table 3
Results of the one-way ANOVA comparing measurements of cell traits by the automatic ACR and the semiautomatic approaches. LRD, lumen diameter; 2CW, double cell wall thickness; D, tracheid radial diameter.

	Pinus sylvestris	*Pinus sibirica*	*Abies sibirica*	*Picea obovata*	*Larix sibirica*
LRD	F = 0.047	F = 0.131	F = 0.351	F = 0.467	F = 0.129
	P = 0.83	P = 0.72	P = 0.55	P = 0.49	P = 0.72
2CW	F = 1.16	F = 22.45	F = 17.7	F = 24.67	F = 6.187
	P = 0.28	P < 0.001	P < 0.001	P < 0.001	P < 0.05
D	F = 0.001	F = 0.006	F = 0.004	F = 0.002	F = 0.033
	P = 0.97	P = 0.93	P = 0.95	P = 0.96	P = 0.85

tested, considerably reducing the working time for quantitative wood anatomy (QWA) measurements in comparison to semiautomatic methods as Lineyka, proving to be an excellent option for QWA analysis at seasonal resolution.

Although both methods showed no significant differences in the measurements of LRD and D, the differences found in 2CWT might respond to the difficulties to correctly define the cell walls limits by the user in the semiautomatic tool. Whereas ACR limits the cells according to the information (in pixels) contained in the image, being the ACR a more accurate approach. The ACR allows the user to work with different image formats (e.g.,. tiff, bmp,. png,. jpg) and long series of tree-rings, facilitating seasonal analysis in chosen years. Since radial files might differ from ring to ring, the current version of ACR works for independent years, relaying in the user the selection of the best radial files to be measured in each target ring. The ACR has proved to be an efficient tool for automatic cell recognition and measurement; however, the accuracy in automatic cell detection is linked with the quality of the cross-sectional view. Thus, the accuracy in cell detection increased with the quality of the sectioning and image, which is a common characteristic limitation in the automatic tools (von Arx et al., 2016).

Even though ACR is an accurate tool that facilitates measurements of different xylem cell traits, providing different options to the users in order to facilitate seasonal analysis of cell development, the software is under continuous improvement to include new features to fulfill the requirements of wood anatomists. In this sense, the ACR is a dynamic software that will be continually upgraded according to the users' necessities. Additionally, we are developing an online version of ACR in a sense than users can work uploading an image to a server to be directly analyzed without the requirement of the installation of any additional software, in the same way this option is already available for software as the VS-Oscilloscope (Shishov et al., 2016; Peresunko et al., 2018; Tychkov et al., 2019).

5. Conclusions

Our results showed that AutoCellRow (ACR) can perform automatic measurements of different xylem cell traits along the radial axis of cell rows on cross-sections views of conifers. ACR has proven a high accuracy (contrasted with a semiautomatic tool) in the cell detection and measurement of anatomical traits in five conifer species. The automatic cell detection by ACR considerably reduces the time spending in measurements, proving to be an excellent alternative to the general and semiautomatic quantitative wood anatomy tools currently available. Besides, ACR provides reliable data on traditionally used parameters in QWA (e.g., cell radial lumen and cell wall thickness), being of particular relevance in the study of seasonal patterns.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This work was carried out on the basis of the Laboratory for integral studies of forest dynamics of Eurasia" of the Siberian Federal University [FSRZ-2020-0014], with financial support from the Ministry of Education and Science of the Russian Federation [Project 5.3508.2017/4.6, software development] and the Russian Science Foundation [Grant18-74-10048, software testing and upgrade, experiment design and paper writing]. VS appreciates the support of the Russian Science Foundation [Grant 18-14-00072, data analysis] and LE STUDIUM/Marie Skladowska-Curie Research Fellowship.

Appendix A

Equations

EQA1. Otsu's method for binarization of images.

$$\sigma_{\omega}^2(t) = \omega_1(t)\sigma_1^2(t) + \omega_2(t)\sigma_2^2(t)$$

where, ω_1 and ω_2 are the probabilities of two classes separated by the threshold t and σ_i^2 represents the variance of these two classes.

EQA2. Calculation of the center of mass of the cell lumen.

$$x_c = \frac{\sum_{i=1}^{n} xi}{n},\ y_c = \frac{\sum_{i=1}^{n} yi}{n}$$

where x_i and y_i are the coordinates of the pixel related to the lumen and x_b and y_b coordinates of the center of mass of the lumen.

EQA3. Calculation of double cell wall thickness

$$W = \frac{DW_1 + DW_2}{2}$$

where DW_1 and DW_2 are the width of the double wall to the left and right of the cell lumen,.

Table A1
Description of sampling sites.

Species	Sampling year	Latitude	Longitude	Altitude (m asl)	Site description
Pinus sibirica Abies sibirica	2017	52.91 °N	E91.36°	1600	Upper tree line of Western Sayan,
Larix sibirica	2012	N54.00°	E91.01°	690	Forest-steppe zone, foothills of the Batenevsky Ridge near Vershino-Bidja
Picea obovata	2012	N54.31°	E89.91°	610	Forest-steppe zone, foothills of the Batenevsky Ridge along the Tuim River
Pinus sylvestris	2013	N53.71°	E91.84°	380	Steppe zone, close to the Yenisei River

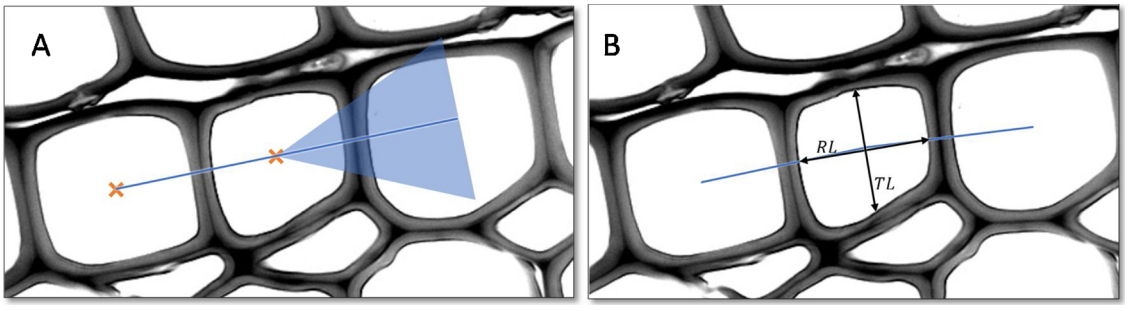

Fig. A1. Tracheid's center of mass and cell row identification (A). Determination of tangential and radial lumen diameter from the center of mass.

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