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Technical Note

ROXAS – A new tool to build centuries-long tracheid-lumen chronologies in conifers

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ABSTRACT

Measurements of tree-ring features (ring width, density, isotopes concentration, etc.) are well-established proxies of environmental variability and, in particular, climate fluctuation at local, regional and continental scales. In recent years, tree-ring anatomical structure (conduit size, density, cell wall thickness, ray abundance, etc.) has been recognized as a novel source of valuable ecological information. However, despite the high potential interest, these kinds of investigations have been significantly constrained by the methodological limitations and time-consuming procedures of data collection.

In this paper, we present ROXAS: an image analysis tool specifically designed to automatically recognize and measure conduit lumen area and calculate reliable statistics in a reasonable amount of time. With ROXAS, many of the aforementioned limitations in analyzing tree-ring anatomical structure can potentially be overcome. While ROXAS was previously used exclusively for angiosperm analysis, we demonstrate in this paper for the first time how it can also be used to analyze an entire sample of a conifer wood.

The use of ROXAS for the analysis of conifer anatomy is exemplified by a 120-year long *Pinus leucodermis* sample including about 75,000 tracheid cells. The results of ROXAS fully automatic tracheid detection are compared to the results obtained after using in-built manual editing capabilities. While both approaches proved to be efficient, the quality of the fully automatic tracheid detection is found to be generally sufficient for most research applications.

Introduction

It is nowadays generally accepted that tree rings are among the most widespread and reliable source of past environmental information. Indeed, tree-ring records have a special status within the kingdom of natural archives thanks to several appealing features such as the high degree of confidence in the dating, the yearly resolution and the general easiness to build extensive network of time series with a common standard (Hughes, 2002). The possibility to consider and measure several properties within the same wood sample adds depth to the quality of the information extracted, particularly when bearing in mind that different properties can provide different environmental information. Therefore tree rings can be considered as multi-proxy archives where, for example: (i) densitometric measurements are particularly suitable to extract the temperature signal usually integrated over the whole growing season (Briffa et al., 2004; Büntgen et al., 2010); (ii) isotope analyses are able to reveal several signals related to the water-cycle together with a plant's active physiological responses (McCarroll and Loader, 2004; Leavitt, 2010; Gagen et al., 2011); and (iii) ring width, although less specific with respect to the previous indicators, can provide a wealth of environmental signals at lower costs in terms of time and money (Cook and Kairiukstis, 1990; Stoffel et al., 2010; Swetnam and Brown, 2011).

Wood anatomical features represent a further set of parameters that can encode additional and novel ecological and environmental information (Fonti et al., 2010; von Arx et al., 2012). The application of the typical dendrochronological techniques to wood anatomy have paved the way to a new frontier in characterizing the relationships between tree growth and various environmental factors over time. The increasing number of papers published in the recent years confirms the promising potential of this approach (Kirdyanov et al., 2003; Fonti et al., 2009; Olano et al., 2013b; but see Fonti et al. (2010) for a thorough review). However, quantifying xylem features such as conduit size and density, or measuring cell wall thickness or tissue percentage along a series of different annual rings, are very time-consuming processes. Therefore, despite the considerable potential interest of these approaches, to date these

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types of investigations have been significantly constrained by methodological limitations and time-consuming procedures of data collection. In conifer species in particular, this results in three major limitations that significantly hamper the potential application of the tree-ring anatomy approach as an alternate source of proxy information: (i) the low number of samples (trees) processed; (ii) the low number of rings (years) considered and (iii) the low number of anatomical features per ring measured, typically along only a few radial files of cells.

The image analysis tool ROXAS presented in this paper has been specifically designed to overcome most of these constraints. After a description of the tool, we will show an example coming from an ongoing investigation aimed to build a multi-centennial chronology of tracheid lumen area of *Pinus leucodermis* from southern Italy.

Materials and methods

ROXAS in theory

ROXAS is an image analysis tool specifically designed to quantify the xylem structures in cross-sectional view of trees (angiosperms and conifers), shrubs and herbaceous plants. It is built around the image processing and analysis capabilities of Image-Pro Plus ≥v6.1 (Media Cybernetics, USA) and also interacts with MS Excel ≥XP for data output. Digital images are taken of microscopic slides, and photographs and scans of the sample surface. The more than 25,000 code lines of the ROXAS tool control Image-Pro Plus functionality, automatically identify anatomical structures and calculate output parameters, and manage user interaction. Data output is saved into well-organized spreadsheets comprising statistics on the level of the entire sample, each annual ring and individual conduits. The basic output parameters are the courses of annual ring borders and the position, shape and lumen area of conduits. From these data, about 50 further parameters are calculated. These parameters include ring width, conduit density, vessel grouping (in angiosperms only), theoretical hydraulic conductivity (based on Hagen-Poiseuille' law), and the radial conduit position within the corresponding annual ring. As ROXAS mainly focuses on conduit lumen area, automated detection of other anatomical features such as cell wall thickness, parenchyma rays, and IADFs are not yet implemented, but is on the potential development agenda. Because of obvious technical limitations, ROXAS is not able to detect tracheids that are not visible in the image due to collapse or insufficient image quality. However, in contrast to most other image analysis tools specialized for conifer xylem analysis, ROXAS quantifies all tracheids in a cross-section with additional possible restriction to radial files. For more information on output parameters, applications and availability refer to www.wsl.ch/dienstleistungen/produkte/software/roxas/index_EN.

Typical ROXAS analysis workflow consists of three primary phases: (i) automatic ROXAS analysis, (ii) manual editing, and (iii) automatic data output. In the automatic analysis step ROXAS first increases and homogenizes image contrast, then extracts conduits based on size and shape, and finally detects annual ring borders if possible (see von Arx and Dietz (2005) for details). All visual and numerical results are stored in separate files, which allow the user to re-access and edit previously analyzed images at any time while not modifying the original image. During manual editing, the user can modify (delete, add, change, undo, etc.) ring borders and conduit outlines that are represented as vector overlays on the original image. The user can exclude sample deficiencies such as cracks by defining areas of interest (AOIs). Several batch-processing utilities for analysis and creation of data output summaries are also implemented. The pixel-per-unit resolution of the images is specified before using ROXAS for a new data set to obtain correct absolute measurement values. Furthermore, a set of about 130 configuration settings can be tailored for each new data set, which typically takes an experienced user 2–4 h. Starting from suitable, existing configuration settings, the configuration time can be further reduced. An appropriate configuration improves tolerance against unequal illumination, inhomogeneous staining, out-of-focus image regions and sample pollution. An optimal configuration therefore increases the reliability and robustness of automatic results.

ROXAS in practice

To test the accuracy and efficiency of ROXAS measurements for conifers, we selected a single cross-sectional thin cutting from a core of *Pinus leucodermis*. The sample was prepared following the standard protocol for wood anatomical analysis: (i) the cores were cut into small (3–5 cm) pieces; (ii) thin sections (ca. 20 μm) were cut with a microtome; (iii) these sections were then stained with safranin and permanently fixed with Canada balsam. Image acquisition was performed with a digital camera (Nikon Digital Sight DS-5M) mounted on a light microscope (Nikon Eclipse80i; Nikon, Tokyo, Japan); images were captured at 40× magnification. Multiple overlapping images were taken from each sample and stitched together using PTGui v8.3 (New House Internet Services B.V., Rotterdam, NL) to obtain high-resolution images (0.833 pixels/μm) of the entire thin section as a low-compression JPG file (Fig. 1a; von Arx et al., 2012). Final image size was 2000 × 23,000 pixels. The selected image was of average quality, and ROXAS analysis was restricted to 120 years (1702–1821). Images were automatically analyzed using ROXAS v1.6, and tree-ring borders were manually drawn on the images.

We compared the results from two different approaches: ROXAS automatic conduit detection ($ROXAS_{auto}$) and ROXAS but with manual improvement ($ROXAS_{edited}$). For $ROXAS_{auto}$, the only manual editing carried out was the drawing of ring borders. For $ROXAS_{edited}$, the majority of the misinterpreted pseudo-tracheid cells (resin ducts, parenchyma cells around resin ducts and in radial rays) were deleted and the undetected tracheids were subsequently added as long as their lumen was clearly visible in the image (see Fig. 1b,c). Annual data from both approaches were compared with respect to several parameters that were all automatically generated by ROXAS: number of tracheids, maximum tracheid size (MaxCA), mean size of the three largest tracheids (Max3CA), tracheid size representing the largest 95 and 99 percentiles (CA95, CA99), mean tracheid size (MCA) and median tracheid size (MedCA). The statistics of the widest tracheid cells were chosen because these tracheids are most critical when investigating hydraulic efficiency (and arguably safety). In addition, their size responds most sensitively to environmental conditions (Eilmann et al., 2006), and measurement mistakes are typically more problematic due to their lower relative abundance within an individual.

Results and discussion

The generation of a 120-year long time series of about 75,000 tracheids, with roughly 70 files of tracheids per ring, took 4 h (including sample preparation and image acquisition) in the approach using automatic ROXAS output ($ROXAS_{auto}$; Table 1). Deleting about 2000 erroneously identified tracheids for the $ROXAS_{edited}$ data set (corresponding to less than 3% of all tracheids) took an additional 2.5 h and adding undetected tracheids required about 1.5 h. The data produced in $ROXAS_{auto}$ were generally very accurate in correctly identifying the tracheids and in

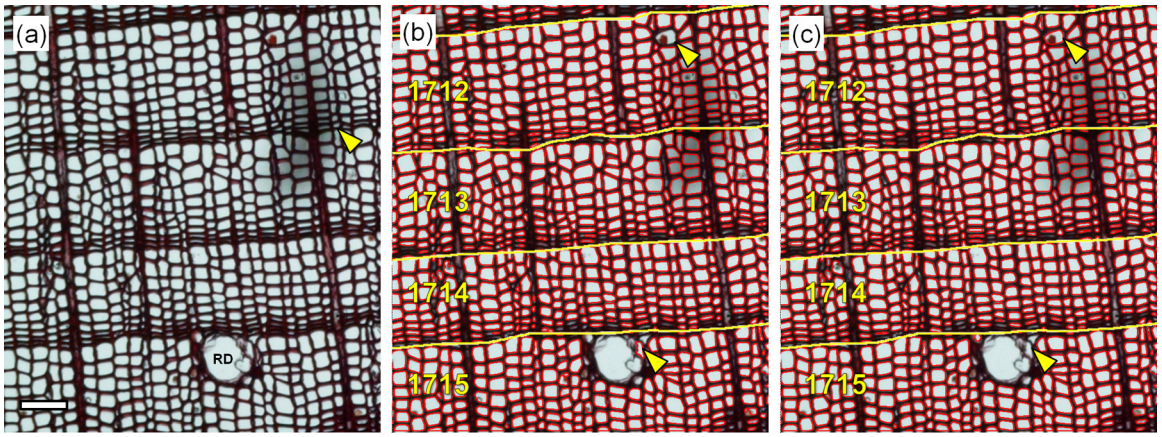

Fig. 1. Cut-out image of the evaluated cross-section of *Pinus leucodermis*. (a) Original image, (b) recognized tracheid outlines after automatic ROXAS analysis (ring borders manually added), (c) tracheid outlines after reasonable editing. The arrow in (a) depicts a sample pollution that was successfully handled by ROXAS (see b). The upper arrow in (b) depicts a tracheid cell that was not recognized in automatic analysis because of the irregular shape of the lumen but added during manual editing (see corresponding arrow in c); the lower arrow in (b) depicts a parenchyma cell of a resin duct that was erroneously recognized as a tracheid cell and removed during manual editing (see corresponding arrow in c). The red tracheid outlines in (b) and (c) are thickened here for better visibility. RD, resin duct. Scale bar = 100 μm.

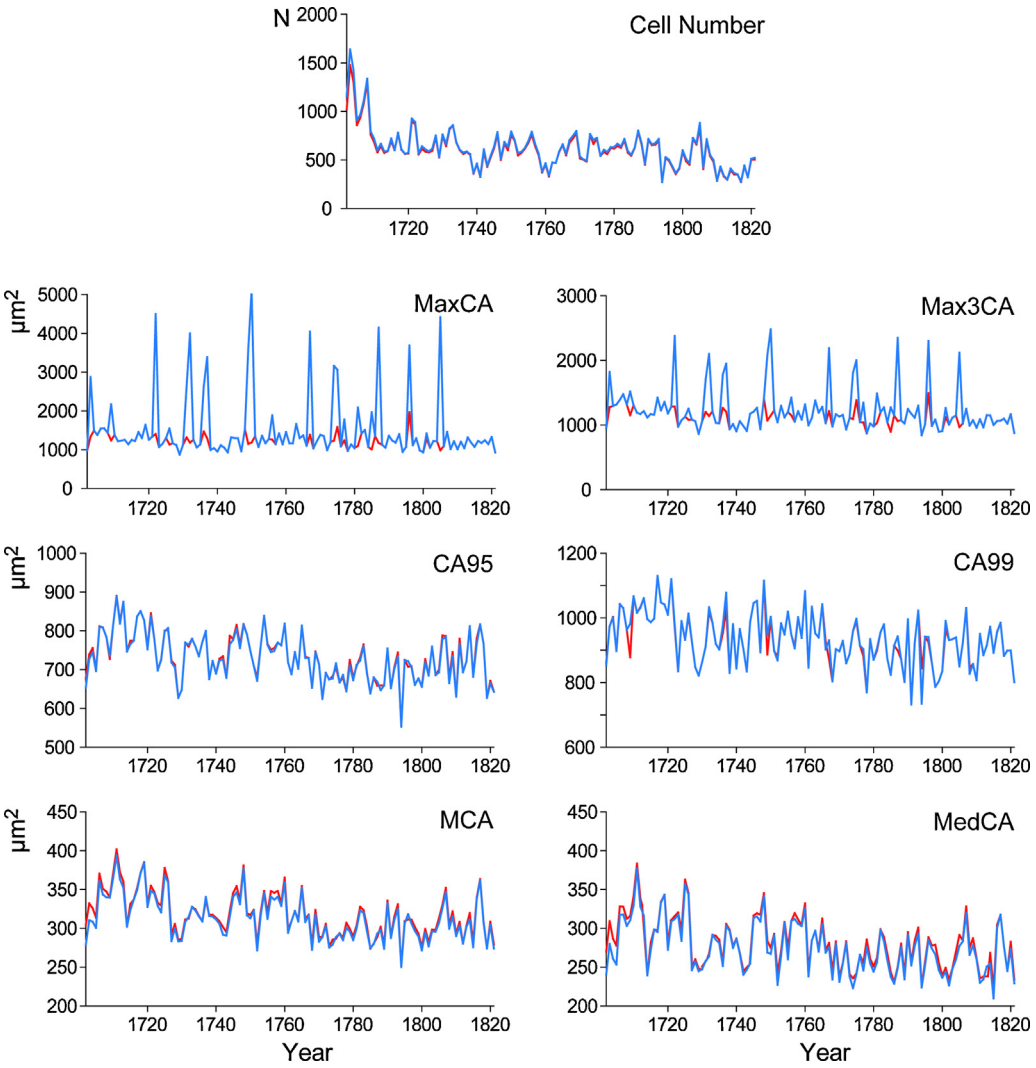

Fig. 2. Comparison of 120-year chronologies of tracheid cell counts, maximum tracheid size (MaxCA), mean size of the three largest tracheids (Max3CA), tracheid size representing the largest 95 and 99 percentiles (CA95, CA99), mean tracheid size (MCA) and median tracheid size (MedCA) as obtained with $ROXAS_{auto}$ (blue lines) and $ROXAS_{edited}$ (red lines).

Table 1
Comparison of time requirements and summary statistics (mean ± 1 SD, where applicable) between automatic ($ROXAS_{auto}$) and manually refined ($ROXAS_{edited}$) ROXAS analysis. See text for explanations of the acronyms.

	$ROXAS_{auto}$	$ROXAS_{edited}$
Time requirements (h)		
Sample preparation	1	
Creating high-resolution image	1	
Drawing annual rings	2	
Deleting pseudo-tracheids	–	2.5
Adding missing tracheids	–	1.5
Total time	4	8
Overall statistics		
Number of tree rings	120	120
Number of tracheids	75,170	74,164
Number of deleted pseudo-tracheids	–	2128
Number of added missing tracheids	–	1122
Mean ring width (±1 SD) (mm)	0.228 ± 0.072	0.228 ± 0.072
Mean number of tracheids	621 ± 219	618 ± 207
MCA (μm^2)	316 ± 33	317 ± 28
MedCA (μm^2)	276 ± 33	278 ± 33
CA95 (μm^2)	734 ± 67	732 ± 72
CA99 (μm^2)	937 ± 82	934 ± 98
Max3CA (μm^2)	1237 ± 350	1123 ± 170
MaxCA (μm^2)	1525 ± 843	1221 ± 198

defining the tracheid outlines (Fig. 1b). Significant improvement by manual editing was only observed in the one to three widest tracheids (Table 1 and Fig. 2). Indeed, we did not detect any significant change in the overall means (Table 1) and only minor deviations in the 120-year time series (Fig. 2) at the 99 percentile of tracheid lumen area (CA99), corresponding to the six largest tracheids or about a tenth of the number of tracheids in one tangential row. The apparent weak overestimating tendency of $ROXAS_{auto}$ in the time series of mean and median tracheid area was due to the deletion of mainly very small pseudo-tracheid cells (about 80% of deleted cells were below the mean tracheid size).

A pre-requisite to obtain such convincing automatic results are high quality thin sections and images, and a tailor-made ROXAS configuration. The time invested in these preparation steps usually pays off several fold by reducing the necessary efforts for any potential manual editing.

Conclusion

Our results suggest that ROXAS automatically produces data of sufficient accuracy for most research questions without need for any manual editing. The efficiency of ROXAS analysis opens the door towards completely new approaches of whole-sample anatomical studies in conifers as demonstrated for angiosperm trees and herbaceous plants (Fonti et al., 2009; von Arx et al., 2012, 2013; Olano et al., 2013a; Wegner et al., 2013). The strength of ROXAS therefore lies in its reliability and the massive number of tracheids it measures automatically. Implementation of additional parameters traditionally used in conifer wood anatomical analysis such as cell wall thickness and wood density would make ROXAS an even more powerful tool.

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References

Briffa, K.R., Osborn, T.J., Schweingruber, F.H., 2004. Large-scale temperature inferences from tree rings: a review. Global Planet. Change 40, 11–26.
Büntgen, U., Frank, D., Trouet, V., Esper, J., 2010. Diverse climate sensitivity of Mediterranean tree-ring width and density. Trees – Struct. Funct. 24, 261–273.
Cook, E.R., Kairiukstis, L.A., 1990. Methods of Dendrochronology: Applications in the Environmental Sciences. Kluwer Academic Publishers, Dordrecht, the Netherlands, 394 pp.
Eilmann, B., Weber, P., Rigling, A., Eckstein, D., 2006. Growth reactions of *Pinus sylvestris* L. and *Quercus pubescens* Willd. to drought years at a xeric site in Valais, Switzerland. Dendrochronologia 23, 121–132.
Fonti, P., Eilmann, B., Garcia-Gonzalez, I., von Arx, G., 2009. Expeditious building of ring-porous earlywood vessel chronologies without loosing signal information. Trees – Struct. Funct. 23, 665–671.
Fonti, P., von Arx, G., Garcia-Gonzalez, I., Eilmann, B., Sass-Klaassen, U., Gartner, H., Eckstein, D., 2010. Studying global change through investigation of the plastic responses of xylem anatomy in tree rings. New Phytol. 185, 42–53.
Gagen, M., McCarroll, D., Loader, N., Robertson, I., 2011. Stable isotopes in dendroclimatology: moving beyond 'potential'. In: Hughes, M.K., Swetnam, T.W., Diaz, H.F. (Eds.), Dendroclimatology. Springer, Dordrecht, pp. 147–172.
Hughes, M.K., 2002. Dendrochronology in climatology – the state of the art. Dendrochronologia 20, 95–116.
Kirdyanov, A., Hughes, M., Vaganov, E., Schweingruber, F., Silkin, P., 2003. The importance of early summer temperature and date of snow melt for tree growth in the Siberian Subarctic. Trees – Struct. Funct. 17, 61–69.
Leavitt, S.W., 2010. Tree-ring C—H—O isotope variability and sampling. Sci. Total Environ. 408, 5244–5253.
McCarroll, D., Loader, N.J., 2004. Stable isotopes in tree rings. Q. Sci. Rev. 23, 771–801.
Olano, J.M., Almeria, I., Eugenio, M., von Arx, G., 2013a. Under pressure: how a Mediterranean high-mountain forb coordinates growth and hydraulic xylem anatomy in response to temperature and water constraints. Funct. Ecol. 27, 1295–1303.
Olano, J.M., Arzac, A., García-Cervigón, A.I., von Arx, G., Rozas, V., 2013b. New star on the stage: amount of ray parenchyma in tree rings shows a link to climate. New Phytol. 198, 486–495.
Stoffel, M., Bollschweiler, M., Butler, D.R., Luckman, B.H. (Eds.), 2010. Tree Rings and Natural Hazards: A State-of-Art. Springer, New York, p. 505.
Swetnam, T.W., Brown, P.M., 2011. Climatic inferences from dendroecological reconstructions. In: Hughes, M.K., Swetnam, T.W., Diaz, H.F. (Eds.), Dendroclimatology. Springer, Dordrecht, pp. 263–295.
von Arx, G., Archer, S.R., Hughes, M.K., 2012. Long-term functional plasticity in plant hydraulic architecture in response to supplemental moisture. Ann. Bot. 109, 1091–1100.
von Arx, G., Dietz, H., 2005. Automated image analysis of annual rings in the roots of perennial forbs. Int. J. Plant Sci. 166, 723–732.
von Arx, G., Kueffer, C., Fonti, P., 2013. Quantifying vessel grouping – added value from the image analysis tool ROXAS. IAWA J. 34, 433–445.
Wegner, L., von Arx, G., Sass-Klaassen, U., Eilmann, B., 2013. ROXAS – an efficient and accurate tool to detect vessels in diffuse-porous species. IAWA J. 34, 425–432.