PROGRAMA DE DOCTORADO EN CONSERVACIÓN Y USO SOSTENIBLE DE SISTEMAS FORESTALES

TESIS DOCTORAL:

Open tools for dendrochronology Advances in sample digitization and deep learning methods for image segmentation

Herramientas libres para la dendrocronología
Avances en digitalización de muestras y aprendizaje profundo para la segmentación de imágenes

Presentada por Miguel García Hidalgo
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OPEN TOOLS FOR DENDROCHRONOLOGY

Advances in sample digitization and deep learning
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Open Tools for Dendrochronology

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General Abstract
Resumen General

Open Tools for Dendrochronology
Advances in sample digitization and deep learning methods for image segmentation

Dendrochronological techniques are paramount in forest research. The current climate change scenario and the central role of forests in biogeophysical cycles enforce the importance of novel techniques to get accurate data from trees and their relationship with the environment in faster ways. Recent technological advances and the place of open source software and hardware are making free, user-developed tools for forest research available to the research community. The aim of this Ph.D. thesis is the development of tools for image acquisition and data collection in dendrochronology based on open source software and hardware. Thus, four different tools for dendrochronological research are presented in five different chapters.

The first chapter focuses on the development of a do-it-yourself tool based on open source hardware for image acquisition and wood sample digitization at high resolution. We used open hardware equipment from Arduino and Python programming to develop CaptuRING and published the entire free open source tool as: "CaptuRING: A Do-It-Yourself tool for wood sample digitization" in Methods in Ecology and Evolution, 2022; 13:1185–1191. Furthermore, the original software was registered in the Registro General de Propiedad Intelectual (00/2022/737) of Ministerio de Cultura y Deporte (Spain).

The second chapter presents "How to build and install your own CaptuRING". This contribution presents a series of videos with a step-by-step guide to promote the use of CaptuRING in the research community. The manuscript and related videos have been submitted for publication.

The third chapter describes ρ-MtreeRing. This free and open-source software, which is written in R, analyzes X-ray films from dendrochronological samples to get microdensity values automatically segmented through a graphical user interface. The open source tool and manuscript are published as: "ρ-MtreeRing. A graphical user interface for X-ray microdensity analysis" in Forests. 2021; 12(10):1405.

The fourth chapter describes the potential of deep learning methods to automatically segment xylem vessels. We trained three different convolutional neural networks to segment vessels in stained wood microsections using the Keras framework

in Python. Our results demonstrate the potential of these techniques to automatically segment xylem vessels and overcome derived problems from image illumination, which hamper segmentation using classical image segmentation methods. The manuscript is published as "Convolutional neural networks for segmenting xylem vessels in stained cross-sectional images" in: Neural Computing & Applications, 2020; 32:17927–17939.

The fifth chapter develops an algorithm to delineate annual ring limits in stained wood microsections of a species with diffuse porous wood using convolutional neural networks. We used Python for image processing and the Keras framework for the algorithm training. The results show the ability of this techniques to obtain accurate tree ring segmentation for quantitative wood anatomy, reaching similar or even outperforming conventional manual delimitation in most of the evaluated cases. The results of this chapter will be presented in the manuscript "Deep Learning for ring bordering in stained cross-sectional images".

This PhD Thesis presents four open source tools to get accurate information from wood features to unveil how trees respond to the environment. From digitization at macroscopic perspective, automatic data collection and the development of feature segmentation on microscopic samples. The presented four novel dendrochronological tools based on open source software facilitates forest research in the current climate change scenario.

Herramientas libres para dendrocronología

Avances en digitalización de muestras y métodos de aprendizaje profundo para segmentación de imágenes

Las técnicas dendrocronológicas son fundamentales en la investigación forestal. El escenario actual de cambio climático y el papel central de los bosques en los ciclos biogeofísicos subrayan la necesidad de nuevas técnicas para obtener de un modo ágil datos precisos de los árboles y de su relación con el medio ambiente. Los recientes avances tecnológicos, además de la disponibilidad actual del software y el hardware de código abierto están poniendo a disposición de la comunidad investigadora herramientas gratuitas desarrolladas por los usuarios para la investigación forestal. El objetivo de esta tesis doctoral es el desarrollo de herramientas para la adquisición de imágenes y la recogida de datos basadas en software y hardware de código abierto para el estudio dendrocronológico. Esta tesis presenta cuatro herramientas diferentes para esta rama científica en cinco capítulos diferentes.

El primer capítulo se centra en el desarrollo de una herramienta "hágalo usted mismo" basada en hardware de código abierto para la adquisición de imágenes y la digitalización de muestras de madera a alta resolución. Usamos equipos de hardware abierto de Arduino y programación de Python para desarrollar CaptuRING y publicamos la herramienta completa de código abierto como: "CaptuRING: A Do-It-Yourself tool for wood sample digitization" en *Methods in Ecology and Evolution*, 2022; 13:1185-1191. Además, el software original fue registrado en el Registro General de Propiedad Intelectual (00/2022/737) del Ministerio de Cultura y Deporte (España).

El segundo capítulo presenta "Cómo construir e instalar su propio CaptuRING" (*"How to build and install your own CaptuRING"*). Esta contribución presenta una serie de vídeos con una guía paso a paso para promover el uso de CaptuRING en la comunidad investigadora. El manuscrito y los vídeos relacionados se han enviado para su publicación.

El tercer capítulo describe *ρ-MtreeRing*. Este software gratuito y de código abierto, que está escrito en R, analiza imágenes de rayos X de muestras dendrocronológicas para obtener valores de microdensidad automáticamente segmentados a través de una sencilla interfaz gráfica de usuario. La herramienta de código abierto y el manuscrito se publicaron como: "ρ-MtreeRing. A graphical user interface for X-ray microdensity analysis" en *Forests.* 2021; 12(10):1405.

El cuarto capítulo describe el potencial de los métodos de aprendizaje profundo para segmentar automáticamente los vasos del xilema. Entrenamos tres redes neuronales convolucionales diferentes para segmentar vasos en cortes histológicos de madera utilizando el marco Keras en Python. Nuestros resultados demuestran el potencial de estas técnicas para segmentar automáticamente los vasos del xilema y superar los problemas derivados de la iluminación de la imagen, que dificultan la labor de métodos clásicos de segmentación de imágenes. El manuscrito se publicó como "Convolutional neural networks for segmenting xylem vessels in stained cross-sectional images" en: *Neural Computing & Applications*. 2020; 32:17927-17939.

El quinto capítulo desarrolla un algoritmo para delinear los límites anuales de los anillos en cortes histológicos de una especie con madera difuso-porosa utilizando redes neuronales convolucionales. Se utilizó Python para el procesamiento de imágenes y el marco Keras para el entrenamiento del algoritmo. Los resultados muestran la capacidad de estas técnicas para obtener una segmentación precisa de los anillos de los árboles para la anatomía cuantitativa de la madera alcanzando, en la mayoría de los casos evaluados, un rendimiento similar o incluso superior a la delimitación manual convencional. Los resultados de este capítulo se presentarán en el manuscrito "Deep Learning for ring bordering in stained cross-sectional images".

Esta Tesis Doctoral presenta cuatro herramientas de código abierto para obtener información precisa de las características de la madera investigar cómo los árboles responden al entorno. Desde la digitalización macroscópica de muestras, la recogida automática de datos, hasta el desarrollo de segmentación automática de características en muestras microscópicas. Las cuatro herramientas dendrocronológicas presentadas, basadas en software de código abierto, facilitan la investigación forestal en el actual escenario de cambio climático.

Introduction

ANTHROPOCENE: A new era with great challenges

We humans are ushering in a new era as a global geophysical force. Since the upsurge of industrialization, the impact of human life has led to a novel geological period in the life of Earth: the Anthropocene. A growing human population demands an amount of Earth's resources never seen before (Krausmann et al., 2013), an estimated 24.7% of the share of Earth's net primary production is appropriated by human activities (Haberl et al., 2007; Kastner et al., 2022). Furthermore, these activities modify Earth's physical, chemical and biological cycles and processes (Berlemann & Steinhardt, 2017; Osman et al., 2021). The atmospheric CO_2 concentration shows a rise without precedents in the last 4.5 million years (Friedlingstein et al., 2020) and the high rate of species loss suggests that we are immersed in a mass extinction event (Ceballos et al., 2015; Gao et al., 2020; Haddad et al., 2015). Eventually, we may reach tipping points that can modify the balanced state of different processes on Earth (Dai et al., 2012; Steffen et al., 2018). The current global change context requires different solutions to minimize the impact of human activities in nature and adapt them to new forecast scenarios. In this way, forest conservation and restoration appear as main nature-based solutions to increase atmospheric CO_2 sequestration capacity by terrestrial carbon sinks contributing to global change mitigation (Friedlingstein et al., 2020; Pyles et al., 2022), Fig.1.

Humans have been living in close relationships with forests, using them as resource providers since the emergence of the first individuals (Ritter & Dauksta, 2013). The remarkable functions of forest ecosystems to provide products (e.g., food, fuelwood or timber), but also to regulate essential environmental services (e.g., biodiversity conservation, prevention of soil degradation or habitat maintenance, water regulation, leisure spaces), highlights the importance of an adequate management of this natural resource (Chazdon, 2008).

FIG. 1 GLOBAL CARBON CYCLE IN THE ANTHROPOCENE (Friedlingstein et. Al., 2020).
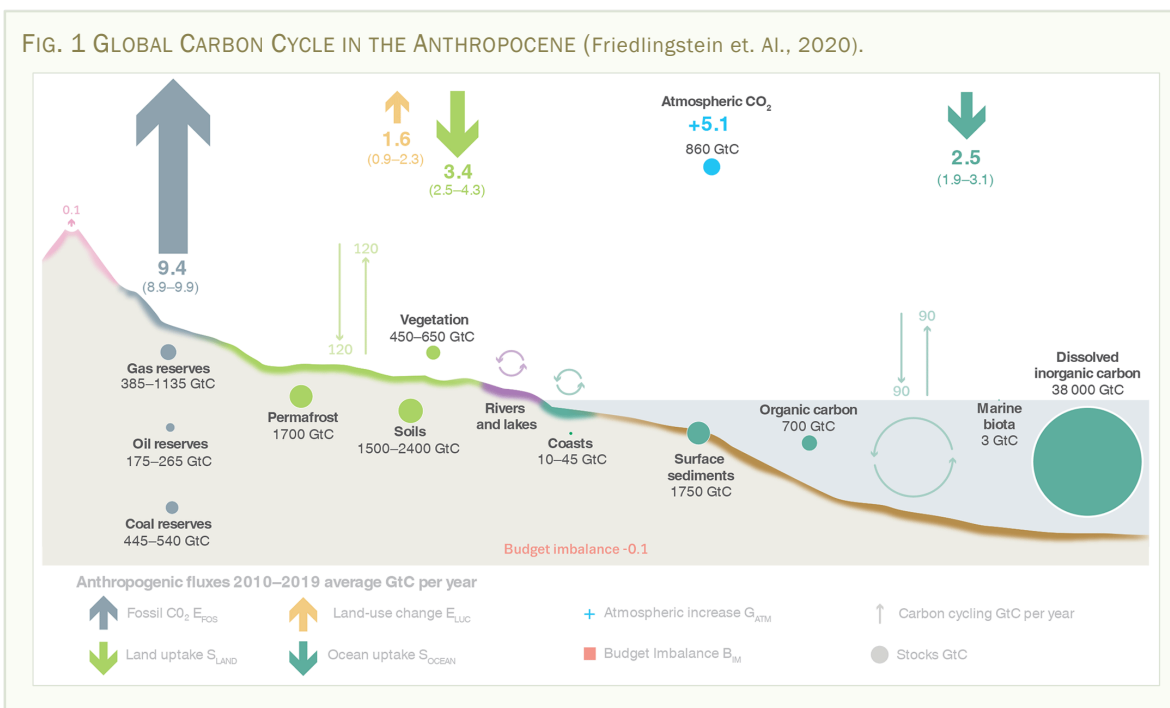
The enormous significance of forests in reducing atmospheric carbon level is critical to mitigate anthropogenic climate warming (Pan et al., 2011). Forests extract carbon from the atmosphere, store it in wood, and reload soil carbon stocks. The current increase in global mean temperature and atmospheric CO2 levels greatly affect different forest processes in complex ways (Cabon et al., 2022; Menzel et al., 2006). For example, warmer springs can result in longer growing periods in temperate forests, increasing carbon fixation rates (Allen et al., 2015; Keenan et al., 2014), but global warming also causes a higher frequency and intensity of droughts and the occurrence of late frost events that can reduce the carbon gain of forests (Green & Keenan, 2022; Sangüesa-Barreda et al., 2021). Furthermore, these novel environmental conditions could even induce tree death and forest dieback (Trumbore et al., 2015; Tumajer et al., 2022). Research is needed to solve the multiple interacting effects of global warming and its

associated disturbances on forest processes (Martinez del Castillo et al., 2022; Seidl et al., 2017).

Tree xylem is a pathway from root to leaves for water and salt transport, as well as a structural supporting tissue. Secondary growth of woody plants maintains their growth while incorporating large amounts of photosynthetically fixed carbon into the tree body. Consequently, xylem constitutes a relevant sink in the carbon biogeochemical cycle (Friedlingstein et al., 2020). Cambial activity channels this carbon input through changes in the pace and intensity of secondary xylem formation, although postcambial activity such as cell differentiation and cell wall lignification also play a relevant role in xylem growth (Huang et al., 2014; Valladares et al., 2007). Cambial and postcambial activity responds to the combined effect of multiple environmental variables (water availability, temperature, etc.) and biotic processes (inter-tree competition, herbivory...) (García-Cervigón et al., 2018; Olano et al., 2021). The interaction of environmental context with intrinsic factors (genetics, ontogeny, health status, etc.) modulates xylem cell characteristics and, therefore, wood features (Björklund et al., 2017; Schweingruber, 1996). Furthermore, the sequential nature of xylem formation incorporates time-varying effects on xylem formation that can extend along the complete tree lifespan, from decades to even millennia in long-living species (Bryukhanova & Fonti, 2013; Castagneri et al., 2017).

## Dendrochronology: From craft to science

Our ability to attribute xylem portions to the calendar years in which they grew using dendrochronological techniques allows us to access a wealth of information on tree response to changes in their environment. This annually and even intra-annually resolved information can be retrieved at different levels (annual ring length, xylem anatomy, changes in xylem microdensity or chemical composition) (Frank et al., 2022). Seasonality of climate conditions modulates cambial activity with a cessation of xylem formation in the unfavorable coldest/driest season (Schweingruber, 1996; Vieira et al., 2020). Cessation of secondary growth often produces characteristic anatomical structures in the xylem that can be identified as an observable limit between consecutive periods of cambial activity [Box 1: The variety of rings in wood]. These anatomical cues enable the identification of sequential compartments of the xylem corresponding to different periods of the growing season and, combined with interannual differences in cambial growth activity, result in the unequivocal attribution

THE VARIETY OF RINGS IN WOOD

Annual ring limits are critical for xylem temporal segmentation. Tree ring borders can be identified by the presence of differential anatomical structures at the end of the growing season. In some cases the structure is easy to identify, as the transition from latewood to earlywood in gymnosperms, but in other situations the reality is more complex. Some wood types present diverse patterns in structure and in cell dispositions that sometimes hampers ring segmentation, and some species are prone to grow even in the unfavorable season, without identifiable ring limits.

We can divide wood in three different groups according to ring anatomy.

Conifers xylem

1 mm
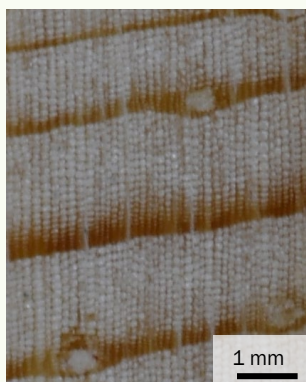
Pinus nigra

Specific of gymnosperms wood. Tracheids are homogeneously distributed along the ring with radial and sometimes axial parenchyma. Some species may present resin ducts with surrounding epithelial cells. Annual limits can be identified because tracheids decrease their lumen area and increase wood cell wall in the last period of growth (latewood).

Ring porous xylem

1 mm

Fraxinus angustifolia

First portion of ring (earlywood) is characterized by large vessels that transport the majority of sap and are functional only during one growing season. Different fiber elements, smaller vessels and different parenchyma types do conform the second part of the ring (latewood).

Diffuse-porous xylem

1 mm

Quercus ilex

Vessels lumina size does not show large variation along the ring. There is no remarkable difference in vessel size across the ring, requiring any particular structure at the end of the growing season (e.g., differential fiber features or marginal parenchyma). If these characteristics are absent, annual bordering is really complex and challenging.

We present ring-porous and diffuse-porous characteristics as discrete categories, nevertheless, this ring porosity classification (Wheeler et al., 1989) is not always so sharp and contains intermediate variations between these standard types. Furthermore, different tree ring patterns can occur in the same species under different environmental conditions or, even, in the same individual from roots to aboveground stem (Wheeler et al., 2007).

of xylem portions to distinct calendar years. The anatomical distinction of annual secondary xylem layers and its unequivocal dating to the actual calendar year of formation is the cornerstone of dendrochronological research. Ultimately, annual ring differentiation in the wood of most tree species provides a temporal window to relate such an annual growth rate to the interannual variation of past environmental conditions. Once each wood ring is unequivocally assigned to a calendar date, xylem information can be related to environmental variables at that specific time.

The association between annual growth rates and climate is the key to access to a wealth of information on how environmental conditions modulate xylem development. Secondary growth is constrained by different environmental factors, with water availability and air temperature being the main climatic drivers of secondary growth in trees (Fichtler, 2012, 2017).

In fact, the secondary xylem provides multiple layers of information (e.g., annual and intra-annual growth, intra-annual density fluctuations (IADF), isotopic content of the cell-wall cellulose, wood microdensity variation, cell anatomical properties) that are defined by a combination of endogenous and environmental conditions that affected the tree during the cambial division and xylem differentiation phases (Wilmking et al., 2020). Variance in these xylem traits can be related to environmental variability (Camarero et al., 2010; Vicente-Serrano et al., 2020).

Quantitative wood anatomy (QWA) research has emerged as a specialized area that looks at the characteristics of individual cells in xylem (Fonti et al., 2010; García-Cervigón et al., 2018) [Box 2: Quantitative Wood Anatomy]. Wood anatomy provides insight into intra-annual wood formation, due to the sequential formation of xylem along the tree ring. Advances in microsection techniques and optical instruments for image capture provide a template where cell structures can be quantified and analyzed. In combination with ongoing knowledge on the functional basis of xylogenesis, we can use high-resolution images of anatomical microsections to explore how environmental conditions modulate xylem cell division and maturation (Olano et al., 2022; Pérez-de-Lis et al., 2022). QWA has been historical a highly laborious and time-consuming technique, due to the large number of microscopical structures that must be captured and measured. Development of high capacity computing hardware and specialized image analysis software (Garcia-Gonzalez & Eckstein, 2003; von Arx & Carrer, 2014) has begun to reduce the burden needed to measure histological structures and quantify

QUANTITATIVE WOOD ANATOMY (QWA)

Metrics in wood anatomy try to answer physiological and ecological questions about plant response to the environment. QWA was initially applied to conifer samples, since its xylem structure is much simpler with just a few easily identifiable elements. Therefore, obtaining measurements from all its different cell components is relatively straightforward. With metrics ranging from number of tracheids, position and size of tracheids, resin ducts, and parenchyma rays, even individual cell wall thickness and lumen area can be quantified (Von Arx et al., 2016). Altogether, this information allows us to infer functional metrics of xylem conductive capacity (Fonti et al., 2010; Lange et al., 2020; Olano et al., 2013).

Xylem structure of broad-leaved woody species is much more complex since supporting and transport functions are performed by different cell types (fibers, vessels). Moreover, parenchyma structure is also very sophisticated, including different types of apotracheal and paratracheal parenchyma, as well as multiple ray types. Therefore, advances in cell segmentation have been much slower and mostly restricted to vessel size and arrangement, but without individual cell wall measurements. Improvements in image acquisition and segmentation opens the room to retrieve more information from xylem of broad-leaved woody species.
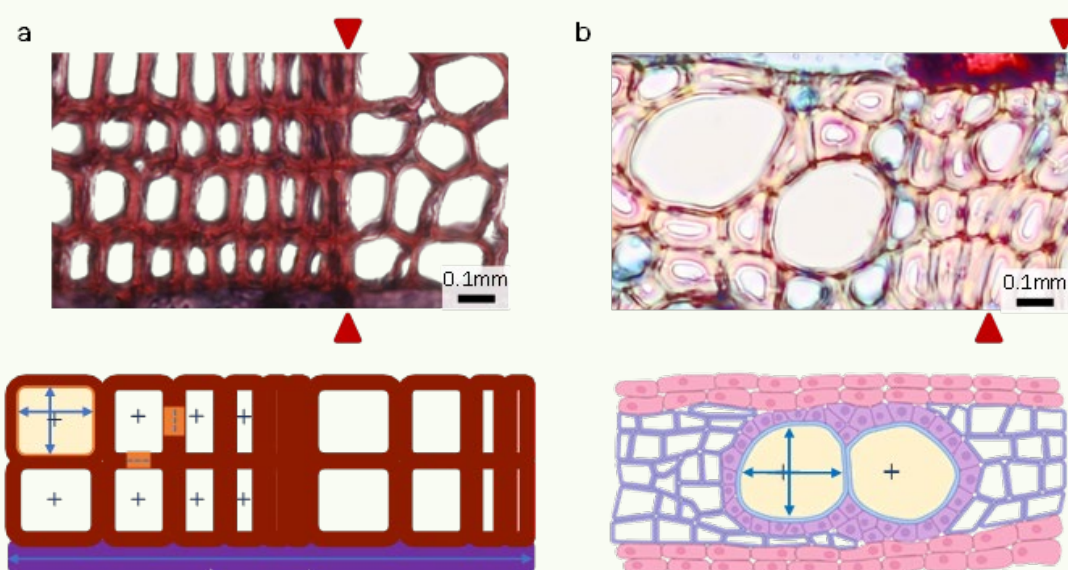
Detailed images from microsections of conifer, *Juniperus thurifera* (a), and broad-leaved, *Fagus sylvatica* (b), species with ring limits (red triangles) and graphical representation of cell pattern. QWA is further developed on conifers with automatic center detection (cross), diameter measurements (blue arrows), and derived cell wall thickness calculation (orange rectangles) due to the cell pattern homogeneity. The complexity in broad-leaved species and individual cell segmentation hampers these automatic calculations.

Broad-leaved representation from biorender.com

anatomical variables, easing the segmentation of cellular elements of interest (e.g., tracheids or vessels).

QWA has contributed to improve our knowledge on how trees deal with different environmental factors such as change in water availability (Arzac et al., 2018), floods (Anadon-Rosell et al., 2022), mechanical damage (Arbellay et al., 2012) and short term events like frosts (Gurskaya & Shiyatov, 2006) or insect attacks (Hillabrand et al., 2019), contributing with information complementary to tree ring measurements (Bräuning et al., 2016; Olano et al., 2022).

The wide range of perspectives for dendrochronological studies is continuously increasing and improving. Xylem density varies within and between years, and this inter- and intra-annual variability may be related to fluctuating environmental conditions. High resolution densitometric analyses based on X-ray have become a relevant subarea of dendrochronology (Björklund et al., 2019) [Box 3: Wood Density]. In fact, xylem microdensity integrates diverse information into a rapid measurement, such as wood anatomy variations at wood opacity to X-ray derived from cell anatomy, basically lumen-wall relation and cell wall characteristics (Preston et al., 2006; Rathgeber et al., 2006; Wimmer & Grabner, 2000). From the different X-ray measurements, Maximum X-ray Density (MXD) has been profusely used in conifers due to its high correlation with thermal conditions at the end of growing season. Moreover, MXD has been preferred over tree ring width because it is less sensitive to non-climatic noise (Björklund et al., 2019). Minimum X-ray Density has received less attention, but growing research evidence shows that it is highly correlated with water availability in drought-prone ecosystems, such as Mediterranean ones (Camarero et al., 2014; Cleaveland, 1986). Moreover, a combination of ring density measurements with ring growth rates can improve our understanding of carbon storage capacity by woody plants (Babst et al., 2014; Baker et al., 2004).

## Technical advances: New tools breeding new questions

Technical innovation and scientific discoveries are continuously evolving due to reciprocal positive feedback. Implementing new techniques allows us to overcome previous obstacles and approach novel scientific questions, while scientific advance fuels technical innovation. In the last two decades, computing capacity and digitization tools have undergone a fast development, with cutting edge tools for research and becoming increasingly affordable (Schmidt et al., 2015; Zhu et al., 2021). Moreover, the

Wood Density

Microdensitometry measures wood density at small (micrometric) scale along the tree ring. This technique has proven to be a valuable proxy of the environmental conditions which xylem has been formed by (Björklund et al., 2019). Wood density captures valuable information from multiple wood traits by indirectly synthesizing anatomical features. Larger conduits and thinner cell walls decrease density values, whether smaller conduits, thicker and more lignified cell walls of fibers or tracheids increase wood density (De Micco et al., 2019).

Since its use in dendrochronology has mostly focused on latewood maximum density in conifers as a proxy of summer temperature, this proxy shows meaningfully information about the variance of cell lignification (Campelo et al., 2007). Studies with minimum density in Mediterranean conifers, however, reveal a strong potential to understand the influence of spring conditions on xylogenesis (Camarero et al., 2014).

The conventional technique is based on X-ray measurements. The sample is X-rayed and the transmitted rays are captured by a photographic plate or more recently by an electronic sensor. The captured image is then analyzed by relating the gray value of the pixel with the stepped calibration to get the density for each pixel value. This standard procedure analyzes the variation in the density for each ring (maximum, minimum, and medium). Then, these annually resolved data can be processed and related to environmental conditions time series (Serra-Maluquer et al., 2022).
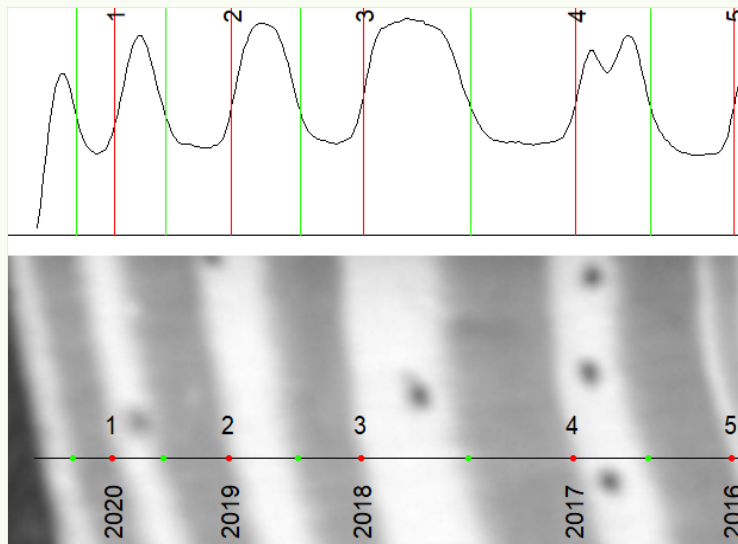
Screen caption from ρ-MtreeRing (García-Hidalgo et al., 2021)

Working with X-ray entails certain difficulties. Novel alternative techniques try to reduce costs and complexity. Blue Intensity (BI) has emerged as a possible alternative (Rydval et al., 2014). This technique extracts pixel value from the blue channel in the digitized wood sample with a flatbed scanner. However, BI is highly dependent on scanner resolution and, mainly, on sample color homogeneity, especially between sapwood and heartwood. These limitations have hampered BI techniques from replacing X-ray densitometry despite the good results found in the literature (Wang et al., 2020).

advent of open source philosophy has created a programmer community devoted to customize solutions freely available for every researcher (Technician Scientific Community Image Forum: https://image.sc) (National Academies of Sciences et al., 2018). Notwithstanding, these community-built solutions must enforce accuracy and repeatability to ensure their reliability for scientific research (Burgelman et al., 2019; National Academies of Sciences et al., 2019).

Lab infrastructure is historically based on proprietary equipment produced by well recognized companies. Instrumental for (mainly biomedical) research includes the latest technologies following the standards of scientific facilities. However, lab equipment is often very expensive owing to the smaller scale of its production, and in many cases some specific equipment cannot be acquired by research groups due to high purchase and maintenance costs. Open hardware solutions with high reliability and precision are emerging as a valuable alternative (Pearce, 2012). Furthermore, this open equipment and the whole background to build it is open access and can be tested, adapted, or even improved by the scientific or developer community (Moritz et al., 2018). Currently, open-source solutions enable affordable equipment to increase the capacity to analyze for any laboratory even in underfunded areas or developing countries (Pearce, 2016; Powell, 2012).

Open-source hardware solutions for research upsurged with the apparition of Arduino devices (arduino.cc). Arduino includes high reliability, and easy-to-use electronic components centered on the Arduino board, which collects input from different sensors and generates responses according to the user's instructions. Arduino is built under Creative Commons license, allowing circuit designers to develop the different components. At the same time, this low-cost microcontroller platform includes simple and open source software compatible with major platforms (e.g., Windows, Macintosh OSX and Linux) removing compatibility barriers.

3D printers complement Arduino electronics with the ability to build any imaginable piece to make a customized tool. 3D printers melt plastic filament and deposit it in layers along a three-dimensional structure. Furthermore, the use of different polymers can endow a wide set of characteristics to the printed pieces (e.g., magnetism) (Löwa et al., 2019). Such versatility places 3D printers as a valuable tool for a multitude of laboratory routines (Baden et al., 2015; Comina et al., 2014).

The versatility of open hardware developments is closely related to programming democratization. Computer skills are everywhere in our century. Besides, high level programming languages are increasingly used in science due to their more readable and friendlier grammar even for nonprofessional programmers. For example, R (R Core Team, 2021) or Python (Van Rossum & Drake Jr., 1995) are both widespread in the science community thanks to their possibilities for data management and analysis, and their steep learning curve. In addition, these open source languages are increasing their ability to generate not only "backend" layers of computing focused on process automation (i.e., commonly used line scripts) but also in the "frontend", including friendly interfaces thanks to new libraries or packages (*PyQt5*, 2022, p. 5; RStudio, Inc, 2013). In this way, the vast community that uses open source software and hardware is steadily increasing the variety of resources, mostly freely available, for research.

Digitization: From pixel value to deep learning

Digitization advances are switching research methods that allow obtaining information not only from the physical sample, but from its representation in high-resolution images [Box 4: Image segmentation]. Digital images collect elements with finite and quantifiable characteristics (i.e., size and intensity value), named pixels, which are organized with spatial coordinates to set a complete multidimensional array (Coppey-Moisan et al., 1994). At the same time, each digitized sample can group images obtained at different times or at different wavelengths, increasing the potentially useful information that can be acquired from its representation. New digitization devices are increasing temporal, spectral, and spatial resolution capacity, enabling sharper and more detailed digital samples. Thus, new available technologies are paving the way for digital twins for dendrochronological research as has been achieved in other areas (Björnsson et al., 2020; Fuller et al., 2020; Pylianidis et al., 2021). These kinds of samples would be identical in their response to different factors or treatments as the actual sample is in the real world (Uhlemann et al., 2017; Zobel-Roos et al., 2020).

Continuous technical improvement in pixel accuracy and detail increases the burden on data management by creating larger files. However, computational power has largely developed in recent years, setting new horizons for big-data analysis and management (Haase et al., 2022). The development of image analysis software in hand with sample digitization provides valuable and highly portable dendrochronological data from reliable data sources (Rademacher et al., 2021). Thus, the increasing role of the open-source

community is to reach the field of dendrochronology with specific free open-source software for image analysis that considers the particularities of wood digital samples (Schneider et al., 2012).

Manual segmentation by the user has been a particularly difficult problem to solve in dendrochronological images. Ring delimitation or cell segmentation is a labor-intensive, repetitive and time-consuming task that constitutes a potential error source. However, the advent of artificial intelligence techniques is changing working strategies. Novel open source development using deep learning is replacing traditional image segmentation methods with a huge scientific community behind (Fernandez-Gonzalez et al., 2022; Hallou et al., 2021). Specifically, the use of supervised learning with Convolutional Neural Networks (CNNs) is highly outperforming methods based on pixel thresholding, mathematical morphology, or clustering techniques (Bai et al., 2021). CNNs are based on calculus structured in layers to obtain complex information inspired by how neurons works in the visual cortex of mammals (Singh et al., 2022). CNNs use an array (tensor) that considers both pixel value and its position to get a derived array from a computation with a defined array (kernel) to obtain a model which can auto-detect the desired areas of interest (Goodfellow et al., 2016) [Box 4: Image segmentation]. In this process, the model is trained by using segmented images (i.e., annotated) as samples to learn the relationships between the network inputs and the segmented images. Subsequently, the adjusted model is tested with annotated images not previously used for training (Borowiec et al., 2022). The current advance in the state of the art of CNNs provides a plethora of options at data pre-processing steps, neural network model election, and post-processing transformations applied images for diverse uses in wood anatomy, from species recognition in trade control to more basic research in dendrochronology and quantitative anatomy (Garcia-Pedrero et al., 2018; Olschofsky & Köhl, 2020; Wu et al., 2021).

Deep learning is still scarcely applied in dendrochronological research and wood structure analysis with a great future potential for development in this field. The simplicity of xylem tissue in conifer species promotes adequate segmentation by thresholding the pixel value or by traditional image filters. However, the complexity of xylem tissue in non-conifer species requires more sophisticated methods to segment individual structures. In this way, the first CNN applications on ring segmentation outperformed the application of traditional preprocessing methods in ring porous

IMAGE SEGMENTATION

Digital images collect a finite number of pixels in layers. Each pixel has a value determined by bit depth. The most used values for bit depth usually range from 1 to 16, resulting in $2^{bit\ depth}$ possible values, thus from 2 levels (black and white images) to 65356 levels. The image sharpness is determined in the actual size represented by each pixel. In general, image quality is determined by bit depth and pixel size.

The image file stores each pixel value and, if the digitization device can capture data from different channels (e.g., Red Green Blue), there will be one layer for each channel.
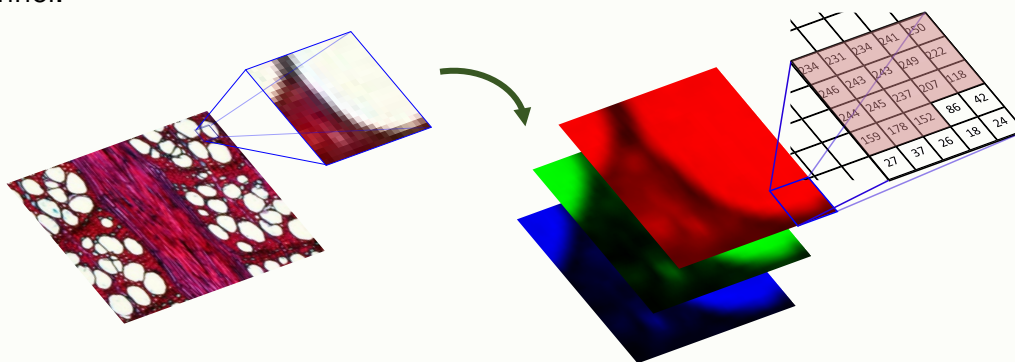
RGB image in 8 bit and detailed image segmentation by threshold (values >90) in the red channel.

Classical segmentation methods analyze images from each pixel value. The basic approach is using a pixel value threshold to determine regions of interest. This method can be sophisticated, usually taking into account the values of adjacent pixels to enhance certain regions from the background (Brunel et al., 2012). Classical segmentation has been successfully used in xylem anatomy to identify structures, but they are sensible to individual image characteristics (e.g., brightness, intensity, color, etc.) (von Arx & Carrer, 2014).

Deep learning is an alternative to the above mentioned mechanistic ways, because it has the ability to improve the algorithm when it is trained. Convolutional neural networks are supervised methods with flexible training from annotated images to obtain a generalized method for image segmentation (Goodfellow et al., 2016). These learning algorithms train with tagged annotated images (e.g., RGB image indicating segmented areas) which should represent the variability of the entire population of images. The images are sequentially filtered to extract increasingly summarized features from the previous filters, resulting in a first step of convolution reducing at each step the size of the input and, therefore, the process requires a latter deconvolution to obtain an array with the same size as the original image with the primary pixels classified. The derived trained model with the extracted features is recalibrated and continuously improved with new training images.
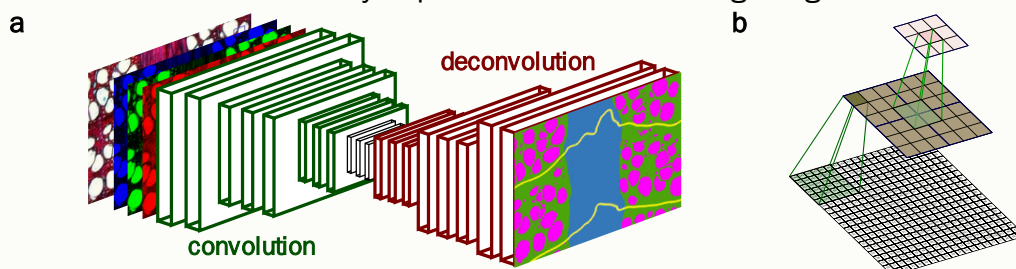
Representation of Convolutional Neural Network for quantitative wood anatomy in (a), and sequential filtering steps in convolution in (b).

species (Fabijańska et al., 2017; Fabijanska & Danek, 2018) with macroscopic images. From a microscopic point of view, ring delimitation and cell segmentation in broadleaved species with CNN has emerged after the pioneer work of García-Pedrero et al. (2018) (Hwang & Sugiyama, 2021; Resente et al., 2021). Nevertheless, there are two main obstacles to the application of CNN techniques. First, the knowledge required to apply appropriate pretreatments to images and, afterwards, designing a suitable neural network, at least until the application of CNN is shared among the scientific community. On the other hand, there is a lack of image data sources for training deep learning algorithms. In this way, the application of deep learning algorithms is restricted to labs counting with a huge quantity of data and with specialists in the current methods for image segmentation.

The aim of this thesis is to develop new open-source tools for dendrochronological analysis from image acquisition to data segmentation and extraction. The use of open-source tools in science with low cost infrastructure and high and contrasted accuracy promotes forest research in the current scenario of global change. These chapters are not presented in chronological order of creation, rather, they follow the standard path for dendrochronological analysis. The first step is sample collection by digitization, then manual segmentation and analysis, and finally training the algorithms from these manually segmented samples.

In the first chapter, entitled *CaptuRING: A do-it-yourself tool for wood sample digitization*, we present CaptuRING which streamlines wood sample digitization in high quality resolution surpassing standard methods in terms of resolution and agility (Garcia-Hidalgo et al., 2022). We propose this tool based on low-cost components and free open-source software as an alternative to the commonly used flatbed scanners in dendrochronological research. As open-source tool, CaptuRING can be developed for different lab requirements by any user.

The second chapter presents *"How to build your own CaptuRING"*. Here, we show the step-by-step process to construct CaptuRING to ease the assembly and configuration for the dendrochronological research community. This video-article opens the production and use of CaptuRING to people with minimal knowledge in electronics.

The third chapter, *ρ-MtreeRing: A graphical user interface for X-ray microdensity analysis*, is devoted to present a free open source tool for densitometry data acquisition and analysis from dendrochronological samples (García-Hidalgo et al., 2021).

Furthermore, we compare the tool results with one of the standards for dendrochronological analyses.

The fourth chapter, *"Convolutional neural networks for segmenting xylem vessels in stained cross-sectional images"* is focused on the automatic segmentation of vessels using deep learning methods (Garcia-Pedrero et al., 2019) . We demonstrate the potential of convolutional neural networks to automatically segment xylem vessels and overcome derived problems from image illumination which hamper segmentation using classical image segmentation methods.

Finally, the fifth chapter explains preliminary results of the automatic segmentation of rings in stained wood microsections. We trained convolutional neural networks with annotated images from a diffuse porous wood using Python and the Keras framework. The results confirm the potential of this methods for ring detection comparing with conventional manual delimitation. This chapter will be presented in the manuscript *Deep Learning for ring bordering in stained cross-sectional images*.

The five chapters of this PhD thesis present four usable and open source tools for dendrochronological research. Open tools, maintaining accuracy and reliability, pave the way for affordable and state-of-the-art equipment reducing economic barriers and promote research in the current climate change scenario.

Objectives

Objectives

The main objective of this PhD Thesis is to develop new tools for image acquisition and data collection in dendrochronology based on open-source software and hardware. The specific objectives distributed in the five chapters are:

1. To create a do-it-yourself tool based on open source for image acquisition and wood sample digitization at high resolution.

2. To promote the open-source use of the do-it-yourself tool for high resolution image acquisition.

3. To develop an open-source software for image segmentation and the extraction of microdensity values from X-ray images.

4. To train a segmentation algorithm for vessel segmentation based on Convolutional Neural Networks.

5. To develop a segmentation algorithm for tree-ring segmentation based on Convolutional Neural Networks.

Methods

Methods

This Ph.D. Thesis compiles various advances in tools for digitization and image segmentation using open-source resources including both hardware and software. We built hardware from widely available and affordable elements and developed open freeware to provide reliable tools that can be freely used by the dendrochronological research community.

Arduino hardware tools are well recognized for their reliability and ease of use by laymen with a growing use in ecology (Capri et al., 2021; Vallejo et al., 2020). We built the CaptuRING tool (chapters 1 and 2) using the Arduino board MKS Gen L v1.0 as the basic tool for hardware systems. This control board was primarily designed for a 3D printer control with the capacity to output orders to three different motors (one for each axis) and associated inputs for travel sensors. In this way, this board presents multiple controls available for further developments of the CaptuRING tool. The associated components selected are compatible with this Arduino board and follow the open-source hardware guidelines.

We used high level and interpreted languages for programming. Python (Van Rossum & Drake Jr, 1995) and R (R Core Team, 2021) are computing languages which work similarly with the use of object-oriented programming. Furthermore, there is a large collection of freely available functions in specific compilations, libraries, or packages that eases their use for nonspecialists. R is commonly used in science due to its high possibilities for data analysis and its specialized packages in statistics. On the other hand, Python is currently one of the most used programming languages in data science owing to its compatibility robustness, computation capacity, and ease of use. The development of specific packages for the front-end (i.e., interface) enables the use of R and Python as languages to build programs entirely including user-friendly interfaces.

CaptuRING programming was designed using Python 3.7. The library pyserial (https://pypi.org/project/pyserial/) operates with the Arduino board, gphoto2 (http://www.gphoto.org/) controls the DSLR camera, and PyQt5 (https://pypi.org/project/PyQt5/) was used to design and execute the tool interface.

ρ-MtreeRing is based on the graphical design of MtreeRing (Shi et al., 2019). We used R and its Shiny (RStudio, Inc., 2013) package for the front-end. The required image

manipulation was designed using the packages raster (Etten, 2012), rgdal (Bivand et al., 2015), magick (Ooms, 2022) and imager (Barthelmé & Tschumperlé, 2019). Finally, data analysis features use measuring (Lara et al., 2015), dplR (Bunn, 2008), dplyr (Wickham, 2017) and openxlsx (Walker & Braglia, 2018).

Image segmentation algorithms were completely developed using Python and Keras framework (Chollet et al., 2015) framework with a NVIDIA TITAN Xp GPU with 3840 cores and 12 Gb of memory. Convolutional Neural Networks are under high development in this language due to the ability to manage large data arrays. The architecture of these networks requires high computation capacity for the repeated processes of encoding and decoding in the convolution.

We followed the current dendrochronological standards in wood sampling to develop and test the tools. Wood samples for digitization with CaptuRING (chapter 1 and 2) were collected with a 0.5 mm increment borer at breast height from conifers, ring-porous, and diffuse-porous species, mounted and glued in a wood holder, flattened and progressively sanded from 200 to 800 grain per mm^2. Microdensity studies (chapter 3) used 2 mm cross sections from samples obtained with increment borer at breast height and washed from resins with pentane. After that, these samples were X-rayed (Polge, 1978) and the generated film was scanned at 1000 dpi. Segmentation methods (chapters 4 and 5) used 10 µm cross-sections stained with Astra blue and Safranin (1%) from branch samples of *Nothofagus pumilio* (Poepp et Endl) and *Fagus sylvatica* (L.) following standard procedures (Schweingruber et al., 2005). The stained sections were digitized using a Nikon D90 camera attached to a Nikon Eclipse 50i microscope at 40x magnification.

A more detailed explanation used methods is developed in each chapter.

1

CaptuRING: A do-it-yourself tool for wood sample digitization

CaptuRING: A do-it-yourself tool for wood sample digitization

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Abstract

1. Standard procedures to obtain high-quality images of wood samples have become a bottleneck in the digitization of dendrochronology. Digitization is currently dominated by flatbed scanners, but the use of these devices is limited by sample length and surface flatness. Although several solutions based on digital photography have been published, they lack effective digitization processes or are too expensive to be widely adopted.

2. Free open-source software and hardware has emerged as an alternative to create research tools that combine reduced costs with high reliability. Here we present CaptuRING, an open-source tool for wood sample digitization combining a do-it-yourself hardware based on Arduino® with a DSLR camera and a free open-source software with an easy-to-use graphical user interface.

3. We compared CaptuRing with image acquisition from a standard flatbed scanner Epson® V750PRO. CaptuRING outperforms scanner image resolution and sharpness, while it removes sample size limitations. Moreover, CaptuRing performs this task in less than half of the time needed by Epson® V750PRO flatbed scanner.

4. CaptuRING emerges as a reliable and low-cost tool to capture high-resolution images of wood samples boosting current digitization processes. The combination of free open software and hardware empowers dendrochronology to advance in wood sample digitization.

Keywords: dendrochronology, DIY, hardware, open source, sample digitization, software, wood cores, wood slices.

2

How to build and install your own CaptuRING

submitted

How to build and install your own CaptuRING

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Abstract

CaptuRING is a reliable and affordable tool to digitize tree-rings combining open source software and do-it-yourself philosophies. Here, we present three video tutorials to construct and install CaptuRING from scratch. First video tutorial explains how to construct the CaptuRING platform, second tutorial covers Arduino board and Raspberry Pi connections, and the third tutorial is devoted to software installation and CaptuRING use.

Keywords: Digitization, DIY hardware, Open source software, Tree rings, Tutorial video

1 Introduction

Digitizing samples is already a common task in many tree-ring laboratories worldwide. The ease of storing, processing, and exchanging images (wood samples) is moving dendrochronology towards digitization. Initially, high-resolution flatbed scanners led this transformation, but digital photography has emerged as a solid alternative due to its many benefits over scanning. First, digital photography is less constrained by sample size, and large tree cores as well as slices can be photographed. Second, image quality increases while processing times for digitization are reduced. Third, autofocus option in digital photography enables sharp captures even in non-perfectly flat surfaces. All in all, different photography-based devices have appeared in the last years (Levanič, 2007; Griffin et al., 2021; Garcia-Hidalgo et al. 2022), promoting digitization in dendrochronology.

CaptuRING tool is based on a DIY hardware and open-source software for wood cores digitization (García-Hidalgo et al. 2022), while an adaptation for wood slices is coming soon. The greatest advantage of CaptuRING is its relatively low cost (approximately 1,700 €), affordable for small-budget tree-ring laboratories and, being open-source hardware and software, it empowers users to customize their devices to their requirements. CaptuRING can be assembled with just basic skills. However, detailed video tutorials on CaptuRING

hardware and software set up and installation makes this task much easier for the community.

Here, we present three video tutorials with associated subtitles to show step by step 'How to build and install your own CaptuRING' from hardware assembly to electronic connection and configuration.

2 Tutorial one overview

In the first video, we show how to construct the CaptuRING platform. The first part of the video shows all the hardware components needed to build CaptuRING: 1 enlarger base, 1 C-Beam actuator with step motor, 1 or 2 endstop switches, 2 linear rails, 1 Arduino controlling board for a 3D printer, 1 power supply, 4 wood screws for the linear rails, 8 nuts, and 12 machine screws. In addition, we need to 3D print the sample holder and 2 rail

http://tiny.cc/CRO1

adapters. 3D designs and further information on required materials can be consulted directly at CambiumRG github repository (https://github.com/CambiumRG/CaptuRING). In the second part of the video, we show step by step how to assemble all the components. At the end of this video, we will have built the CaptuRING structure.
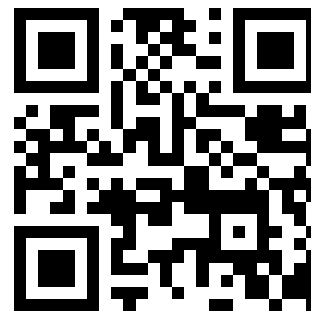
2.1 Tutorial two overview

In the second video, we show how to connect the electronic components with the Arduino board and Raspberry Pi . In this phase, we need: 1 Arduino board with and associated power supply, 1 Raspberry Pi kit and the components cited in the previous video. After this video, we will be done with CaptuRING hardware.

http://tiny.cc/CR02

2.2 Tutorial three overview

In the third video, we show step-by-step how to configure all the electronic components and install and launch CaptuRING from scratch. We follow specific guidelines on the terminal window to configure the caption system. Once we have completed this task, we can acquire our dendrochronological samples in high resolution.
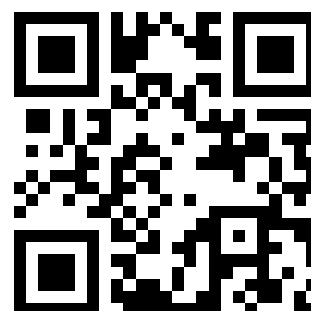
http://tiny.cc/CR03

Conflict of interest

Authors have no competing interests to declare.

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3

ρ-MtreeRing: A graphical user interface for X-ray microdensity analysis

ρ-MtreeRing: A graphical user interface for X-ray microdensity analysis

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Abstract

Wood microdensitometry provides an integrated measurement of inter and intra-annual changes in wood anatomy and lignification. Although it can be acquired through a wide array of techniques, X-ray based techniques are still the standard. Conversion of X-ray image grayscale to density and annual ring boundaries delimitation is performed through image analysis software. Proprietary software has dominated these applications, albeit Free Open Source Software (FOSS) has been developed recently. We present ρ-MtreeRing, a user-friendly FOSS that streamlines the entire microdensitometry analysis process through a Graphical User Interface based on Shiny -R Software without any programming knowledge. We compared the results of this program with the most widely used commercial software (WinDendro) showing the validity of the results. ρ-MtreeRing can be personalized and developed by the microdensitometry research community.

Keywords: Dendrochronology; Densitometry; Shiny Graphical user interface (GUI); Tree Ring; Wood Density.

4

Convolutional neural networks for segmenting xylem vessels in stained cross-sectional images

Convolutional neural networks for segmenting xylem vessels in stained cross-sectional images

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Abstract

Xylem is a vascular tissue that conducts sap (water and dissolved minerals) from the roots to the rest of the plant while providing physical support and resources. Sap is conducted within dead hollow cells (called vessels in flowering plants) arranged to form long pipes. Once formed, vessels do not change their structure and last from years to millennia. Vessels' configuration (size, abundance, and spatial pattern) constitutes a record of the plant–environment relationship, and therefore, a tool for monitoring responses at the plant and ecosystem level. This information can be extracted through quantitative anatomy; however, the effort to identify and measure hundreds of thousands of conductive cells is an inconvenience to the progress needed to have solid assessments of the anatomical–environment relationship. In this paper, we propose an automatic methodology based on convolutional neural networks to segment xylem vessels. It includes a post-processing stage based on the use of redundant information to improve the performance of the outcome and make it useful in different sample configurations. Three different neural networks were tested obtaining similar results (pixel accuracy about 90%), which indicates that the methodology can be effectively used for segmentation of xylem vessels into images with non-homogeneous variations of illumination. The development of accurate automatic tools using CNNs would reduce the entry barriers associated with quantitative xylem anatomy expanding the use of this technique by the scientific community.

Keywords: Convolutional neural networks · Dendrology · Xylem cells · Image segmentation

5

Deep Learning for ring bordering in stained cross-sectional images

in preparation

Deep Learning for ring bordering in stained cross-sectional images

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Abstract

Intrinsic and environmental features trigger and regulate tree ring growth. Xylem cell formation controls tree functioning, modulates growth, and therefore, conditions the role of trees in the geophysical carbon flux dynamics. Sequential growth of wood allows the study cell formation retrospectively, due to the stop of cambial function in the unfavored season. Quantitative wood anatomy focuses on the study of wood formation from the cellular perspective using high-resolution images from wood micro sections. Obtaining meaningful cellular information is hindered by suitable tree ring delimitation on the images. This time-consuming task requires experienced operators to manually delimit ring borders and limits data acquisition. We present an automatic methodology for tree ring delimitation using convolutional neural networks on images from stained cross microsections. The trained model was tested considering discrepancies with manual segmentation and for quantitative wood anatomy analyses. Automatic segmentation equaled or improved manual segmentation in most cases (90% approx.) and right cell attribution to annual rings was similar when manual segmentation was considered better. Further advances in the application of models based on convolutional neural networks and precise evaluation methods would reduce costs of massive and accurate data collection for quantitative wood anatomy.

Keywords: Convolutional Neural Networks, Image segmentation, Quantitative Wood Anatomy, Tree ring, Xylem

Introduction

Forests are a crucial in terrestrial carbon fluxes with a decisive role in the future trajectory of atmospheric carbon dioxide concentration (Cabon et al., 2022; Pan et al., 2011). Therefore, understanding tree responses to environmental conditions and

forecasting their behavior to future climate scenarios is a key priority(Seidl et al., 2017). Fortunately, forests can contribute to decipher these questions through the information stored in wood (Martinez del Castillo et al., 2022; Olano et al., 2021). Tree xylem constitutes a temporal record of their individual growth, as well as providing a gamut of characteristics that stores information that reveals how different factors shaped their features. Therefore, studies of temporally resolved information stored in tree rings have become a powerful tool to forecast the impact of global change on terrestrial ecosystems (Sangüesa-Barreda et al., 2021; Wilmking et al., 2020).

The study of tree rings, by means of dendrochronology, comprises a panoply of techniques to extract the information in wood (Schweingruber, 1996). Novel technical advances in dendrochronological studies have improved data collection and preparation with a wide range of tools, valid from the traditional macroscopic perspective to the microscopic one (Garcia-Hidalgo et al., 2022; von Arx et al., 2016). Initially, tree rings studies mainly focused on the observation of wood samples using a stereoscope set to provide total ring width and eventually early and latewood measurements when this annual division could be established, resulting in annually resolved time series of tree growth investment (Schweingruber et al., 2005). Advances in wood microsectioning, staining, and the current development of high-resolution digitization tools have contributed to move into the microscopic domain, with research focused on identifying and measuring xylem cells, exploring in a wide array of opportunities and research goals at intraanual scale (Fonti et al., 2010; Olano et al., 2013). The term quantitative wood anatomy (QWA) has been coined to this approach that collects high-detailed information on xylem anatomy, opening appealing opportunities to explore intraannual resolution and approaching xylem functional characteristics. These advances have allowed QWA and dendrochronology to evaluate functional traits of trees (e.g., tree physiology or ecophysiology) (Cuny et al., 2012; Pérez-de-Lis et al., 2022).

QWA provides valuable data from measurable characteristics of conductive xylem cells (e.g., lumen area, cell wall dimension, cell pattern), but also from other wood cells-(parenchyma, fibers, resin ducts, etc.), and related traits (e.g., density fluctuations) (Anadon-Rosell et al., 2018; Arzac et al., 2018). Furthermore, these anatomical features can in some cases be translated into functional features (Borghetti et al., 2020; Hérault et al., 2011). Since wood growth is characterized by a sequential development due to the growth arrest occurring when a limiting season exists, wood anatomy usually

enables the identification of annual rings. Recognition of these annual structures is basic to accurately date samples, i.e., assign a portion of the xylem to a specific calendar year of formation. When rings are properly detected and dated, any measured wood feature can be related to other sources of temporal data (e.g, climatic features, environmental disturbances, water availability, etc.).

Anatomical features are mainly studied from wood-stained microsections according to standardized protocols (von Arx et al., 2016). Software advances in QWA enable moving from individual cell measurement to semiautomatic identification of a massive number of cells (e.g., 20 600 xylem vessels in just one core) reinforcing the validity of the obtained data. Furthermore, some of the available software offers the option to automatic ring detection (von Arx & Carrer, 2014), but the potential for improvement in this task is still ample. Currently, most of the processes designed for automatic ring segmentation computes the variation of pixel value through a selected path and find the points that overcome an arbitrary threshold. This approach has been commonly used in dendrochronological studies based on macroscopic wood samples (e.g., increment cores or wood slides) with satisfactory results (Fabijanska et al., 2017). However, these ring detection techniques are less accurate in wood microsections and, most of the time, a thorough expert supervision is required. In fact, the poor reliability associated with this automatic segmentation forces most experts to segment ring borders from scratch without using any automatic detection option, due to time-consuming correction.

To circumvent these limitations, we propose to explore the opportunity of supervised learning techniques applied to ring-border detection in stained wood microsection. From the wide palette of techniques, convolutional neural networks (CNNs) are widely used for image segmentation with notable results in different areas (Singh et al., 2022; Sultana et al., 2020). The basic structure of CNNs is inspired on how mammal neural networks works (Bai et al., 2021; Schmidhuber, 2015). Convolutional layers process the image to detect a specific feature, its location and strength. The larger number of convolutional layers, the more complex and abstract the information obtained. However, computation costs are considerable when many additional layers are incorporated (Goodfellow et al., 2016).

Recent novel hardware development (e.g., Graphic Processing Units, GPUs) enables the high computation tasks required for image segmentation and high-resolution image analysis leading remarkable applications for dendrochronological samples. CNN

methods reach noteworthy ring detection rate for macroscopic samples (Fabijanska & Danek, 2018). Further developments on ring detection and model validation for dendrochronological samples are recently handled considering ring borders as segments rather than polylines (Poláček et al., 2022). Despite these approaches in automatic ring segmentation and evaluation of models for dendrochronological samples, QWA is focused on cellular and anatomical characteristics of tree rings, requiring specific segmentation models and robust methods of model evaluation. Consequently, specific models must be trained according to cell pattern characteristics due to the wide disparity between different kinds of wood, and then maintain high accuracy at ring segmentation. Furthermore and considering QWA purposes, ring border evaluation must follow specific criteria according to image characteristics at microscopic scales.

In order to fill this gap, we aim to generate a CNNs model for automatic ring delimitation on stained microsections of wood, evaluating whether the delineations made by two humans and the CNN model have the same validity for QWA analyses.

Materials and Methods

European beech (*Fagus sylvatica* L.) is a deciduous tree species widely distributed throughout Europe that constitutes the broadleaved tree of highest economic relevance for European forestry (Geßler et al., 2007). This species has received considerable interest from dendrochronological studies, including QWA (Sánchez de Dios et al., 2021; Zimmermann et al., 2021). Rings describe semiring-porous structure due to the decreasing lumen diameter in xylem vessels across the annual tree ring with distinct rings identifiable by a band of narrower fibers, Fig. 1.

We developed a CNN model using a collection of 426 transversal microsections from tree cores collected at different climate conditions and elevations derived from a previous research (Olano et al., 2022). These microsections were stained with safranin and Alcian blue according the standard procedures (Garcia-Pedrero et al., 2018) and digitized as RGB images using a Nikon D90 digital camera mounted on a Nikon Eclipse 50i optical microscope with 40x magnification resulting in a resolution of 1.63 μm per pixel. Individual images were stitched using PTGUI v8.3.10 Pro (New House Internet Services B.V., Rotterdam, The Netherlands).

Ring borders were manually segmented by two different operators on 214 digitized samples using ROXAS software and following common QWA standards and guidelines (von Arx et al., 2016).
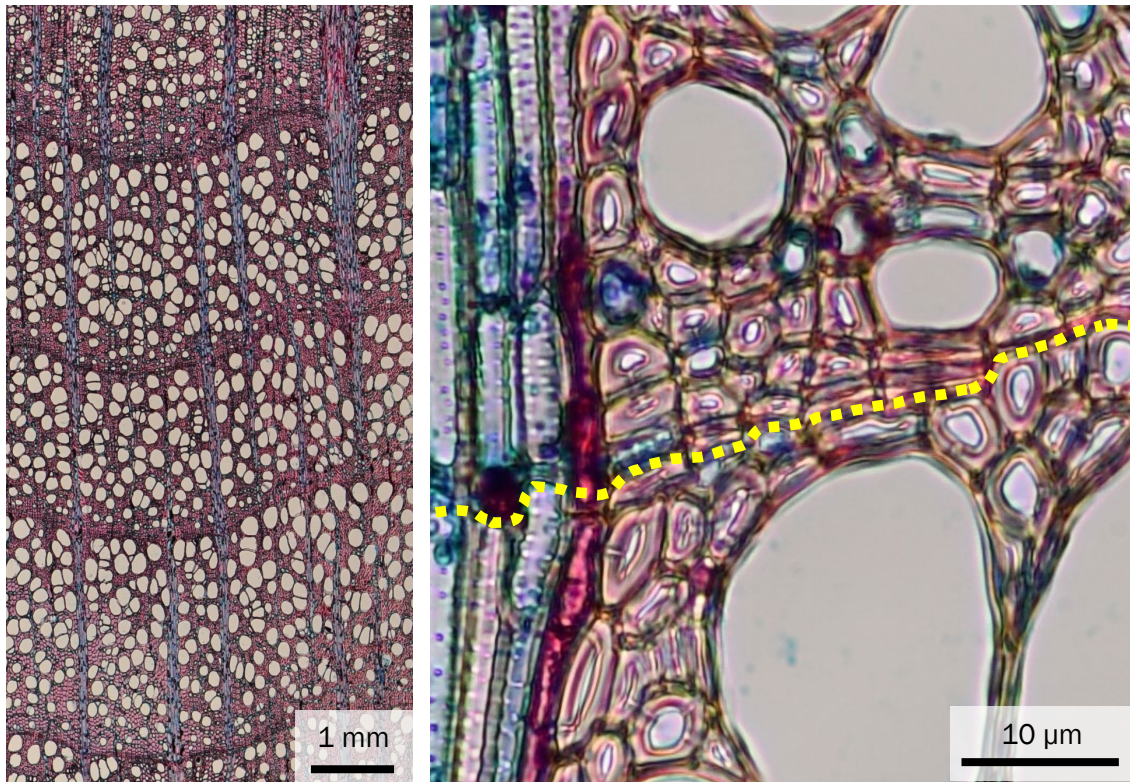

Fig. 1. Images of stained cross microsections from beech (*Fagus sylvatica*) wood cores. 4x image with four complete tree rings (left) and 40x detailed image with ring limit drawn in yellow dotted line (right).

Data preprocessing

To feed the models, images were cropped into 256 x 256 pixels, hereafter called data patches. Data augmentation techniques were performed over these patches during training to increase data variability. This included horizontal flipping operations (left-to-right flipping) and rotation with −20º and 20º angles were used. Furthermore, the patches were randomly blurred with a maximum and a minimum Gaussian kernel size of 3 and 10, respectively. Finally, each data patch was normalized in the range [0, 1]. Data augmentation was performed using the library called Albumentations (Buslaev et al., 2020).

Finally, the delineated rings were binarized to obtain masks, where the number 1 corresponded to the pixels limiting the rings and 0 the pixels of the background. These masks were subjected to the same processes as the data patches, except for normalization, so that the lines matched the rings.

CNN model

To distinguish if a pixel belonged to the ring border or to the background, we considered the semantic segmentation model. UNEt TRansformer (UNETR) (Hatamizadeh et al., 2022), which is a state-of-the-art deep learning network for semantic segmentation. UNETR combines the well-known UNET network (Ronneberger et al., 2015) with Visual Transformers (ViT) (Dosovitskiy et al., 2020) (Fig. 2). ViT outperforms state-of-the-art CNNs in terms of accuracy and computational efficiency. It uses mechanisms of attention by differentially weighing the importance of each part of the image independently. Transformers are made up of multiple layers of self-attention. The self-attention layer (Vaswani et al., 2017) of ViT allows information to be embedded globally throughout the image. ViT divides an image into fixed-size patches, linearly embeds each of them, and adds positional embedding as input to the Transformer Encoder. In this way, the model learns about the training data to encode the relative location of the image patches to reconstruct the complete image structure.

The transform encoder included three layers:

• Multi-head Self-attention Layer: This layer concatenates all attention outputs linearly into the appropriate dimensions. The numerous attention heads help train the local and global dependencies of an image.

• Multilayer Perceptron Layer (MLP): It contains two layers of neurons with Gaussian error linear unit (GELU) (Hendrycks & Gimpel, 2016).

• Norm Layer: It is added before each block as it does not include any new dependencies between the training images. This helps improve training time and overall performance.

In UNETR (Fig. 2) input data is divided into a sequence of uniform, nonoverlapping patches and projected into an embedding space, using a linear layer and projected into an embedding space by means of a linear layer. The sequence is then added with a positional embedding and used as input to a transformer model. The encoded representations of the different layers of the transformer are extracted and merged with a decoder through hopping connections to predict the final segmentation. The output

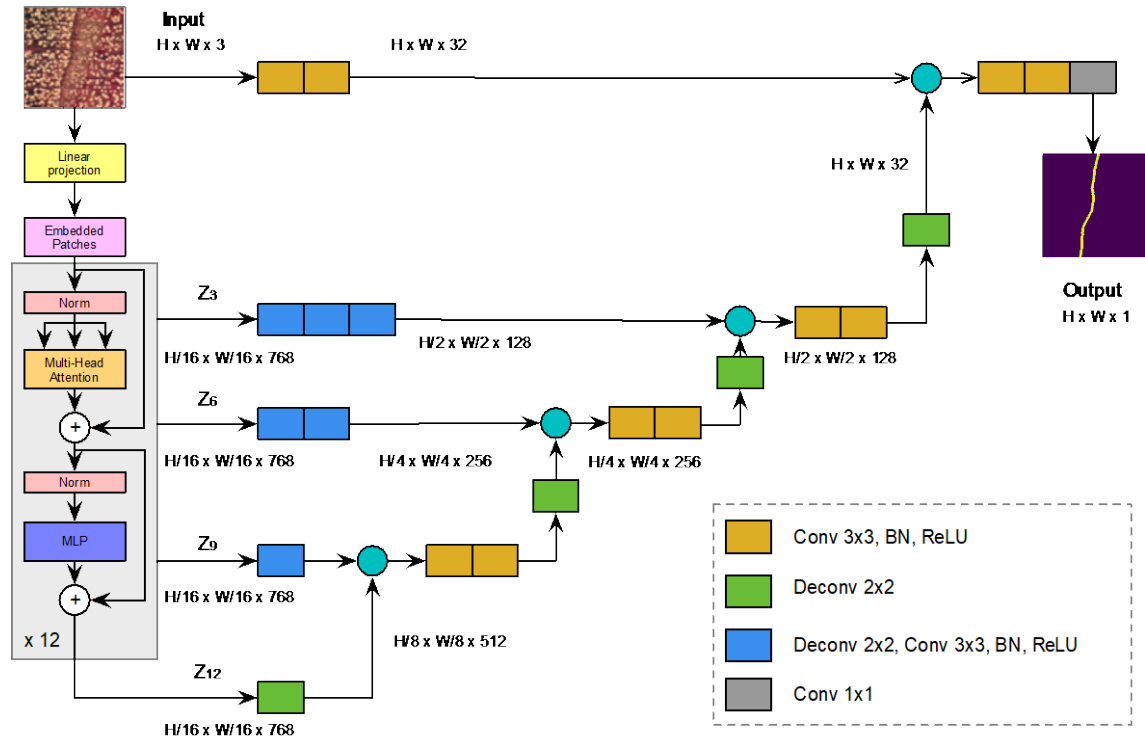sizes are given for a patch resolution P = 16 (giv total of 256 patches) and an embedding size K = 768.

Fig. 2. UNETR architecture. Modified from (Ronneberger et al., 2015). The yellow block represents a 3x3 convolution layer, followed by a batch normalization and a ReLU layers. A Green Block represents a 2x2 deconvolution layer. A blue block represents a 2x2 deconvolution layer, followed by a 3x3 convolution, a batch normalization and a ReLU layers. Finally, the gray block represents a 1x1 convolution.

To train neural model, we used Focal Tversky Loss (Abraham & Khan, 2019) as the loss function, which is given as:

$$\mathcal{L}(Y, \widehat{Y}) = \left(1 - \frac{\sum_i y_i \widehat{y_i}}{\sum_i y_i \widehat{y_i} + \alpha \sum_i y_i (1 - \widehat{y_i}) + \beta \sum_i (1 - y_i) \widehat{y_i} + \epsilon}\right)^{\gamma}$$

where $y_i \in Y$ and $\hat{y}_i \in \hat{Y}$ denote the ground truth and the predicted image of the ith pixels for the class C $(c \in \{ring, no\ ring\})$ respectively, α and β are the penalty parameters, and ε is a smoothing factor used to avoid zero division error. In this work, the following values were used: α = 0.7, β = 0.3, γ = 0.75, and ε = 1e−12. The network was trained over 25 epochs, cycles that the complete training dataset was run.

Post-processing

Each high-resolution image was split into N small subimages, each of the same size than a data patch. The subimages were created using an overlap-tile strategy to an extent of 90% (Ma et al., 2018). A test-time-augmentation (TTA) strategy was applied to

improve the detection of rings (Gonzalo-Martín et al., 2021). Geometric transformations belonging to the dihedral group of order 4 were used as TTA augmentations, obtaining eight different versions of the same subimage. The probability that a pixel belongs to a ring is obtained by calculating the maximum response of the eight values that correspond to the location of the same pixel. This value is normalized to be in the range [0,1]. A pixel is considered to belong to a ring if it exceeds a threshold of 0.2 (estimated experimentally).

Since prediction networks tend to draw thicker rings than those drawn by hand, a skeletonization process has been carried out. This process reduces binary objects to 1-pixel wide representations, providing more suitable lines for ring delineation (Zhang & Suen, 1984). Since skeletonization generally produces noisy or redundant branches (spurs), a pruning process was performed to preserve only the "trunk" lines rich in information with the Discrete Skeleton Evolution (DSE) model (Bai & Latecki, 2007). DSE iteratively eliminates the final branches of the skeleton with less relevance according to their contribution to the shape reconstruction. In this work, branches with an area smaller than 100 pixels were eliminated. All experiments have been done using an NVIDIA RTX3090 GPU with 10496 CUDA cores and 24 GB of memory.

Evaluation

To evaluate the results obtained by the segmentation model, a 3-fold cross-validation was performed. In this prospective evaluation we selected 12 images with both objective and specialist-based analysis of ring delimitation. We analyzed four images from each fold, including varied parts of the tree stem from pith to bark and varied illumination.

Ring borders are linear paths of 1.63 μm width (1 pixel) delimiting tree rings of 1110 ± 780 μm width. Thus, the discrepancies between the linear paths of ring delimitations pixel by pixel highly penalize the results. For example, two complete equally segmented lines 1 pixel apart result in a 0% of agreement, albeit the error range in manual segmentation is commonly larger and practical results for QWA would be equivalent. To reinforce the fidelity of the validation method, we compared each ring limit derived from manually segmented images with those segmented by the CNN model according to four criteria (Fig.3). (1) We compared pixel coincidence between tree ring limits at pixel level according to the common evaluation methods on image segmentation. On a second step, (2) we calculated the mean position of each segmentation for each ring and

calculate their distance. (3) We counted the number of vessels mismatch depending on the ring bordering strategy. Finally, (4) we counted with an expert to judge which of the ring delimitations followed better performance according to the ring growth with a trinomial criterion: 0 = better performance of manual delimitation, 1 = no differences, 2 = better performance of CNN model delimitation. Finally, we divided model prediction into three folds to examine potential deviation between data dispersion and then possible biased results.
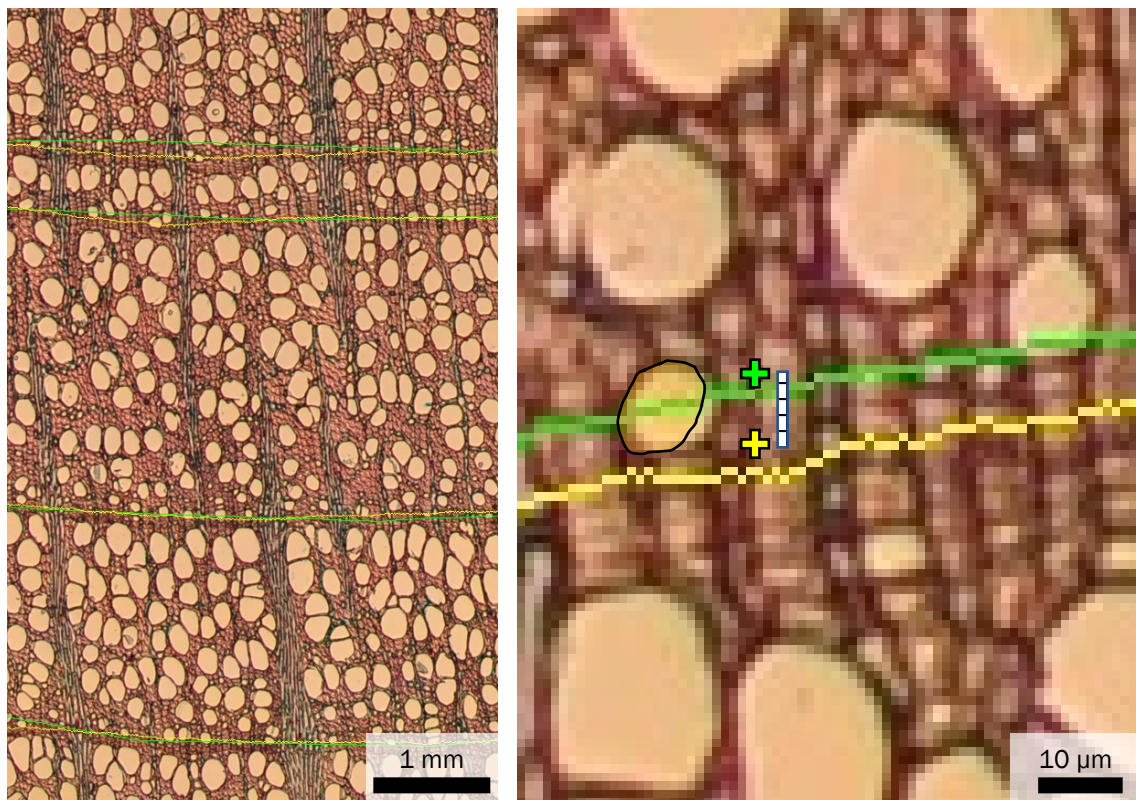
Fig.3. Images of beech (*Fagus sylvatica*) cross microsections with ring delineations with manual (green) and model (yellow) approaches. Right: Detailed image with mean ring center (cross), distance between mean ring centers (white stripped segment, and vessel with center mismatched (light yellow area) between tree ring borders.

Results

Two different operators manually segmented 5928 rings on 214 images which were used to train the model. From this data set we randomly extracted 446 ring segmentations in the 12 selected images, image width 1468±13.74 pixels (mean±SE), and mean height 23975±48.61 pixels for the evaluation of the results.

Comparison between segmented borders considering pixel value coincidence revealed very low results (< 1 %) as expected. However, overall mean ring position distance between manual and artificial segmentation was 17.87±0.84 pixels that

correspond to 29.12±1.38 μm. Such a small difference was reflected in the low disparity in vessel attribution between delimitations, the mean discrepancy in vessel assignation between models was less than a vessel per ring (0.91±0.04 vessels). The assessment of the results by an expert opinion revealed a better performance for CNN prediction (Table 1), being better than manual segmentation in 203 (45.5 %) cases. In fact, manual segmentation only improved CNN in 48 (10.7 %) of the ring delineations.

Based on the expert evaluation, discrepancies between mean ring center were higher when one model was preferred: 23.64±3.41 pixels when manual delimitation was considered better, and 21.89±1.54 pixels when CNN model was selected. The largest vessel mismatch between segmentations occurred when CNN model prediction was considered better. In this cases, the trained model included 1.33±0.09 vessels more than the manual segmentation. On the other hand, there was less than one vessel center mismatch 0.9±0.13 when manual segmentation was considered better.

Table 1 Results of the analysis of discrepancies between segmentations.

Better performance by expert	Number of rings (overall %)	Mean ring center difference (SE) in pixels	Mean vessel mismatch (SE)
Manual	48 (10.7 %)	23.64 (3.41)	0.9 (0.13)
Similar	195 (43.7 %)	12.57 (0.9)	0.47 (0.03)
Model	203 (45.51 %)	21.89 (1.54)	1.33 (0.09)

A separate analysis by folds confirmed the best performance of CNN model segmentation in all the folds (Table 2). In fact, a higher performance of manual segmentation was marginal in all the folds, between 8.26% and 12.24% of the segmented rings.

Table 2. Results of the discrepancies between tree ring limits by each fold in the evaluation.

Fold	Better performance by expert	Number of rings (inner fold %)	Mean ring center difference (SE) in pixels	Mean vessel mismatch (SE)
1	Manual	13 (8.26%)	14.04 (3.89)	0.92 (0.25)
1	Similar	53 (35.09%)	11.02 (1.51)	0.49 (0.67)
1	Model	85 (56.29%)	12.33 (1.33)	1.95 (0.21)
Overall		151	11.99 (0.97)	1.35 (0.11)
2	Manual	18 (12.24%	4.89 (1.15)	0.56 (0.13)
2	Similar	75 (51.02)	5.47 (0.63)	0.35 (0.04)
2	Model	54 (36.73)	9.1 (1.23)	0.63 (0.09)
Overall		147	6.72 (0.55)	0.48 (0.04)
3	Manual	17 (11.48%)	48.06 (11.65)	1.24 (0.3)
3	Similar	67 (45.27%)	21.63 (2.64)	0.6 (0.07)
3	Model	64 (43.24%)	43.94 (5.49)	1.09 (0.14)
Overall		148	34.25 (2.81)	0.89 (0,07)

Discussion

The application of CNNs is progressively advancing in QWA analyses automatization since the development of different models for cell delimitation (Garcia-Pedrero et al., 2018, 2019; Resente et al., 2021). The required effort in both training and processing time for any operator opens the necessity to develop useful methods for ring delimitation in QWA. Our model was equal or better than manual tree ring delimitation on stained wood microsections in 89,21% of cases. Albeit, more advances in model evaluation are required, these preliminary results settle CNNs as a valuable tool for accurate ring delimitation in QWA.

Beech ring limits are characterized by the presence of a band of narrower fibers at the end of the growing season, crossed by parenchymatic rays that show less evident limits. This limit can be neatly delineated at first sight (at least for the tracheids), however conventional ring segmentation is not performed following each cell wall limit pixel by pixel, but using polylines consisting of segments linking multiple points marked by the operator. Thus, ring limits slightly differ between operators or even between different attempts of a given operator depending on where the segment boundaries are placed. This poses no problems in most QWA approaches since most effort is based on vessel adscription and differences are very small in terms of percentage of the area of the ring or vessel adscription, not affecting the robustness or reproducibility of QWA

analyses. In this way, our results showed an averaged distance of 17.87 (SE = 0.84) pixels with less than one vessel mismatch between borders.

Artificial segmentation evaluation usually contrasts ground truth with the segmentation predicted by the model comparing differences in pixel value (border vs. not border) between the two methods of segmentations used. However, that is not the case in dendrochronological studies focused on tree ring delimitation (Fabijańska & Danek, 2018). In this work, the authors compared the detection of tree ring limits on macroscopic samples, but did not evaluate the discrepancies between both methods of segmentations. Poláček et al. (2022) proposed advances in model evaluation by widening the drawn line to transform it in a polygon and comparing the filled areas.

The abovementioned particularities of QWA analysis require a different method of evaluation. The mere detection of the tree ring boundaries in just one point will stablish the limit on a horizontal line when the ring limits rarely show this arrangement, producing a noteworthy vessel mismatch between rings. On the other hand, the specific drawn limit comparison in pixel value highly penalizes the results as shown with < 1 % coincidence between borders, even when there is not any vessel or cell difference. Ring is manually bordered by a polyline according to the different points marked by the operator, meanwhile CNN model marks borders running through the image pixel by pixel. These different ways of drawing the limit and the severe penalty when comparing pixel by pixel between lines recommend the use of complementary approaches. We considered useful the extraction of the mean position of the pixels belonging to each ring border and compared this value between manual and automatic segmentation.

The discrepancies among our results and the delineated tree rings were within those expected between different delineations of the same ring, producing a valuable framework for QWA analysis and allowing the study of tree response to the environmental conditions. The very low discrepancy on the number of vessels attributed to a ring (< 1 vessel center mismatch on average), considering the 120 vessels per ring on average from this data set (Olano et al., 2022), would not change QWA results.

CNNs are currently under full development for image segmentation to streamline time-consuming processes mainly in biomedical imaging (Sultana et al., 2020). The use of CNNs techniques in stained images with similar characteristics of wood samples could be useful for QWA application. However, CNNs are really sensitive to image characteristics, mainly resolution and pixel pattern. CNNs models require training with

a considerable amount of segmented images to learn and modulate the species-specific parameters for reaching optimal results. In this way, the large variety of cell patterns and wood ring characteristics involves different models for ring segmentation, at least for the main anatomical patterns of wood (Wheeler et al., 1989). The tree-ring delimitation discrepancies between operators and the wide range of wood types highlight the importance of considering not only pixel value but other relevant metrics for QWA, including expert opinion. The compilation of different parameters proposed in this manuscript helps to confront different segmentation methods for QWA.

Our results show that CNNs can reach similar and even better accuracy than manual segmentation in most cases on ring delimitation, with a trivial divergence on vessel center mismatch when CNNs models are surpassed by the manual operator. The better results of CNNs can be almost partially explained by the inherent constraint of manual delimitation by polylines, whereas CNN can match closely annual ring limits to pixel value and pattern Nonetheless, there is still ample room of improvement for more specific models according to wood anatomical patterns, staining procedures, different approaches for model evaluation (i.e., considering the complete ring as a polygon and studying differences in the segmented area), or even retraining options with human corrected results to reinforce model accuracy. Further advancing through this avenue requires compiling large image banks of delineated annual rings on a wide variety of wood microscopic samples.

Conclusions

CNN models can significantly reduce costs in QWA ring segmentation with potential to outperform human operators. Higher development in CNNs application and the development of accurate evaluation methods for wood ring segmentation in QWA may allow the collection of accurate and massive data on the functioning and response of trees to the environment in the current global change context.

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Conclusions

Conclusiones

Open Tools for Dendrochronology
Advances in sample digitization and deep learning methods for image segmentation

1. We created an open source tool to digitize wood samples. This tool enables to obtain high quality images with a reliable method at a price much lower than existing alternatives and that could be assembled by the final user. The development of reliable open source tools as ours promotes further technical advances by the impulse of a community of users and developers.

2. Wood sample digitization at high resolution images expands the opportunities to get different environmental variables from wood proxies and disentangle how trees respond to the surrounding factors. Furthermore, the use of digital samples creates an opportunity for raw data exchange among researchers and fosters active and long-distance collaborations.

3. Image and data management with a user friendly graphical user interface for microdensitometry analysis eases the obtention of microdensity data from X-ray images.

4. Automatic vessel segmentation with deep learning methods was able to achieve results of equal or better quality as images edited with proprietary software. The use of Convolutional Neural Networks is therefore an advance over existing techniques in quantitative wood anatomy due to the reduction of working time on the method calibration and manual editing required by classical techniques.

5. Deep learning methods for ring segmentation face the main existing barrier for the complete quantitative wood anatomy automatization. The inner capacity for getting abstract information across the neuron layers of deep learning methods opens the way for highly reduce times in image analysis for quantitative wood anatomy.

6. Overall, this PhD thesis shows that there is an avenue for the digitization of dendrochronological work by the creation of open software and do-it-yourself hardware. The development of these tools does not only eases work and increases productivity, but also enables the share of data among researchers.

Herramientas libres para la dendrocronología
Avances en digitalización de muestras y aprendizaje profundo para la segmentación de imágenes

1. Hemos creado una herramienta de código abierto para digitalizar muestras de madera. Esta herramienta permite obtener imágenes de alta calidad con un método fiable a un precio muy inferior a las alternativas existentes y que, además, puede ser montada por el usuario final. El desarrollo de herramientas fiables de código abierto promueve nuevos avances técnicos mediante el impulso de una comunidad de usuarios y desarrolladores.

2. La digitalización de muestras de madera en imágenes de alta resolución amplía las oportunidades de obtener diferentes indicadores ambientales de la madera y desentrañar cómo los árboles responden a los factores del entorno. Además, el uso de muestras digitales crea una oportunidad para el intercambio de datos brutos entre los investigadores y fomenta las colaboraciones activas y a distancia.

3. La gestión de imágenes y datos a partir de imágenes de rayos X con una interfaz gráfica fácil de usar facilita la obtención de datos de microdensidad.

4. La segmentación automática de vasos con métodos de aprendizaje profundo es capaz de lograr resultados de igual o mayor calidad que las imágenes editadas con software propio. El uso de Redes Neuronales Convolucionales supone, por tanto, un avance respecto a las técnicas existentes en anatomía cuantitativa de la madera debido a la reducción del tiempo de trabajo en la calibración y la edición manual que requieren las técnicas clásicas.

5. Los métodos de aprendizaje profundo para la segmentación de anillos se enfrentan a la principal barrera existente para la completa automatización de la anatomía cuantitativa de la madera. La capacidad interna de obtener información abstracta a través de las capas neuronales de los métodos de aprendizaje profundo abre el camino para reducir bruscamente el tiempo de análisis de las imágenes para la anatomía cuantitativa de la madera.

6. Esta tesis doctoral muestra que existe una vía para la digitalización del trabajo dendrocronológico mediante la creación de software abierto y hardware basado en

modelos de "hágalo usted mismo". El desarrollo de estas herramientas no sólo facilita el trabajo y aumenta la productividad, sino que también permite compartir los datos entre los investigadores.

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