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ORIGINAL ARTICLE





Examination of Cinnamomum camphora interlocked grain adopting X-ray computed tomography combined with particle image velocimetry

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Abstract

Trees develop wood grain more or less parallel to the tree axis as they grow laterally. However, many tree species also develop interlocked grain that is expressed as a change in the wood grain orientation, periodically switching between left- and right-handed spirals over the years. Wood grain information is critical to determining the wood quality and is an important record of cellular events in a cambium. This study examined the wood grain of axial xylem cells in the semi-ring porous hardwood Cinnamomum camphora by first visualizing the three-dimensional structure using a conventional X-ray computed tomography instrument producing tomography images. We propose the use of particle image velocimetry (PIV) and the two-dimensional fast Fourier transform (2D-FFT) to analyze computed tomography images in obtaining grain angle (δ) information from the vessel deviation. We found undulation on the radial variation of the grain angle (δ) that indicates periodical changes in the wood grain orientation as left- and right-handed spirals ranging from – 25° to 16° and – 22° to 18° using the 2D-FFT and PIV, respectively. Furthermore, despite the major orientation of vessels inclining in the tangential direction, the PIV result revealed a minor deviation with vessels inclining in various orientations along the tree radius.

Keywords: Grain angle, Image analysis, Tracking algorithm, Vessel network, Water transport

Introduction

The wood grain of a living tree is not always straight or parallel to the longitudinal axis of the tree [1]. Terms have been used to define the inclined grain depending on the degree of deviation from the longitudinal axis: namely, wavy grain, spiral grain, and interlocked grain [1, 2]. An interlocked grain occurs when there is a periodic change in the grain angle between left- to right-handed spirals over the course of years [3-5]. Such grain deviation affects the woodworking process and physical-mechanical properties of the wood [1, 6-8].

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The interlocked grain of tropical tree species has been reported in numerous studies [4, 6, 9-14]. In the present study, we observed the interlocked grain of Cinnamomum camphora, which usually grows in temperate and subtropical regions [15-17]. Cinnamomum camphora wood is selected for making Buddhist images and cabinets in Japan [18]. Unlike the case for tropical species, which have a less clear growth ring boundary, the growth ring boundary of C. camphora is annual and distinct because the earlywood vessels appear to be distinctly larger than latewood vessels. Such type of vessel porosity is known as semi-ring porous.

The wood grain orientation is related to the orientation of longitudinal cells that reflect the orientation of parental fusiform cambial cells [1-3, 11, 19, 20]. Therefore, in addition to using the conventional radial splitting



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method, which is invasive [3, 6, 9], wood grain orientation can be evaluated by examining the orientation of longitudinal cells such as fibers and vessels. Fibers generally account for 30–80% on composition of angiosperm wood, by volume, which will define grain orientation. To see fibers at the cellular level and evaluate grain orientation, serial sectioning on tangential sections is commonly used [11].

Vessels also originate from the same parental cells as fibers. Thus, vessel orientation can also be used as a proxy for grain orientation [4, 11, 19, 21, 22]. Although the vessels and fibers have similar orientation, different grain orientations commonly occur in reversal zone of interlocked grain in which vessels run steeper than fibers [13, 21, 22]. Unlike fibers, vessels are actually long hollow tubes that can extend from centimeters to meters in length that have relatively larger diameters. These vessels are composed of longitudinally aligned series of vessel elements [23]. We can take advantage of this structure for examining grain orientation. The orientation can be measured by mapping vessels on consecutive transverse sections [24]. When vessels are inclined and not parallel to the longitudinal axis of the tree, there is a lateral shift of the vessel between two consecutive transverse sections at different depths. However, serial sectioning technique is time-consuming, requiring training and experience to obtain a satisfactory result. Meanwhile, cross-correlation has been adopted to automatically evaluate the grain angle from such lateral shifting in a pair of transverse images from different depths extracted from a single cross section using a confocal microscope [13]. However, this method only works on pair transverse images with narrow depth because of the inadequate intensity of the lower image of the section. Additionally, this method requires sectioning of the sample.

X-ray computed tomography (CT) has been applied in wood science studies in recent decades to observe the spatial organization of xylem, which is usually obtained through time-consuming serial sectioning [25]. The CT technique allows the virtual cutting of a wood sample simulating microtomy, including obtaining serial transverse sections and other sections from any surface. The technology allows a wider region to be observed in a single image acquisition of the wood sample with relatively faster processing compared to serial sectioning method. It also provides a clear surface because X-ray attenuation addresses the problem of the inadequate intensity of the lower image when adopting confocal microscopy. In addition, interlocked grain of two African mahogany species [22] and spiral grain of radiata pine [26] have been studied recently using high-resolution CT. These studies make use of CT technique capacity to reconstruct 3D view of wood at cellular level.

Depending on the equipment, a varied resolution of CT images might be obtained [27]. Ideally, we can select the equipment based on the requirement of the investigation. However, in certain situations, we are unable to employ a CT equipment with high-resolution imaging. Meanwhile, with lower resolution CT images, it is difficult to visualize wood elements at the cellular level, especially fibers which are important to examine grain orientation. Nevertheless, due to their larger size, vessels can still be observed in lower resolution CT images. For example, we have previously used considerably lower resolution CT imaging at 50 μ m/pixel and found enough to detect vessels in *C. camphora* [28]. Therefore, we sought to develop a technique that can evaluate the orientation based on appearance of vessels on lower resolution CT images.

We propose adopting particle image velocimetry (PIV) to evaluate the interlocked grain of transverse images of C. camphora wood. PIV is often used to study liquid or gas material flow, cell imaging, and cloud motion among other applications [29-32]. We adopt PIV to evaluate the grain deviation in pairs of CT images of C. camphora transverse sections by tracking the possibility of displacement of vessels and other cells as particle-like objects in image pairs with different depths. This analysis involves dividing the images into several sub-regions and applying cross-correlation across image pairs [33]. In addition, the two-dimensional fast Fourier transform (2D-FFT) [11, 13, 22] is used to evaluate the interlocked grain in the tangential section. The present paper shows the possibility of evaluating the grain orientation from C. camphora images recorded using a low-resolution industrial X-ray CT machine by performing image analysis. We estimate the grain angle variation by applying PIV to transverse sections. The result of the PIV of transverse sections was then validated using the 2D-FFT of tangential sections. Furthermore, we demonstrate how the variation in the vessel orientation intra-annual ring can be obtained from the PIV output.

Materials and methods

Wood material

The wood block of *C. camphora* was similar to a block that was used in a previous study [28]. The block used in the present study was scanned using an X-ray CT instrument (Y.CT Modular, YXLON International GmbH, Hamburg, Germany) at the Kyushu National Museum. The CT scanning was conducted with a resolution of 0.05 mm per pixel. This process provided projection images, which were then reconstructed into two-dimensional (2D) image slices of the wood block. We used volume graphic software (VGStudio MAX 2.2, Volume Graphic GmbH, Heidelberg, Germany) to visualize the three-dimensional (3D) reconstructed volume of *C.*



camphora from the slices of 2D images and prepared a suitable image series for analysis.

Pretreatment

Images of a transverse section of the actual wood block were precisely prepared by aligning the volume of reconstructed CT images (Fig. 1). This process provided a series of transverse images with dimensions of 1433×1478 pixels. The images were rotated to align the annual rings in the vertical direction. The images were cropped to 948×344 pixels, corresponding to dimensions of $47.4 \text{ mm} \times 17.2 \text{ mm}$.

We prepared other datasets by extracting oblique transverse sections from a similar volume with the same dimensions as the transverse sections. Two types of oblique transverse section deviating in the tangential direction were prepared. The deviations were 10° in the clockwise direction and 10° in the counterclockwise direction relative to the longitudinal axis of the tree.

Tangential images of the same scale were prepared from a similar volume (Fig. 2a). A series of tangential images with dimensions of 1433×1001 pixels were generated. We then cropped the images to 427×491 pixels, corresponding to 21.35 mm × 24.55 mm.

Evaluation of the interlocked grain and vessel network through PIV

To measure interlocked grain, we extracted 36 sequential transverse images from the serial transversal images, where images were spaced at intervals of 10 pixels (500 μ m). For each image, the next sequential image was its pairing image. A total of 35 pairs of transverse images were thus analyzed in the study. Each pair of images was analyzed adopting PIV. The PIV analysis was programmed in Python [34] using the OpenPIV package [35, 36]. The program splits each image into sub-regions. These sub-regions are called interrogation windows in the first image and search windows in the second image. We used a sub-region of 16×16 pixels for the interrogation window and a larger sub-region of 32×32 pixels for the search window. The sub-regions, either interrogation windows or search windows, overlapped each other at a distance of eight pixels from the center of the sub-region.

The PIV program provides a displacement vector of elements within the pair of images by demonstrating cross-correlation of the windows using the fast Fourier transform (FFT) function and statistical analysis to validate the vectors. The program tracks the most feasible displacement of elements in the sub-region that are assumed to be related to the wood grain orientation



Fig. 1 Left: part of a transverse section of a CT image of *C. camphora* used in the present paper. Right: optical microscopy image of *C. camphora* anatomical features for a different specimen. White arrowheads indicate annual ring boundaries (left). The optical microscopy image (right) is inverted from a brightfield microscopy image to get similar black and white pattern with CT image



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arrow indicates the grain angle (δ) measurement



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based on the movement of a group of vessels in two consecutive images, as illustrated in Fig. 3.

The output of the image analysis adopting PIV is the coordinates of sub-region vectors (x, y) and the vector displacement (u, v). Values u and v correspond to the deviation of the sub-region representing the grain inclination in radial and tangential directions, respectively. We measure the grain angle (δ) according to the displacement of a group of vessels with respect to the axial direction on the tangential plane as seen from the bark side. To obtain the grain angle (δ) , we adapt an equation proposed by Ogata and Fujita [13] for examining the grain angle (δ) of *Hopea odorata* Roxb.:

grain angle(
$$\delta$$
) = $tan^{-1} \left(\frac{\nu}{DD} \right)$, (1)

where *DD* is the depth difference in pixels in the longitudinal direction between two consecutive images used for the measurement. A positive value corresponds to righthanded spiral grain and a negative value to left-handed grain. The variation in the radial grain angle obtained from PIV is measured by averaging the grain angle for 35 pairs of transverse images arranged in the longitudinal direction. The value of the grain angle is then also averaged in the tangential direction.

The value of the grain angle (δ) is limited because it only gives the inclination of the grain in the tangential plane. We present another calculation from the PIV output, as illustrated in Fig. 3, to show the displacement vector of grain on a transverse surface representing the grain orientation and the inclination angle (ϕ) at the given orientation. The inclination angle (ϕ) represents the tilt of the grain from the direction of axial growth (Eq. 2). We use

inclination angle(
$$\phi$$
) = tan⁻¹($\frac{R}{DD}$), (2)

$$R = \sqrt{u^2 + v^2},\tag{3}$$

where R (Eq. 3) is the magnitude of the displacement and DD is the depth distance of the virtual sections.

Interlocked grain analysis using the 2D-FFT

We evaluated the grain angle (δ) from a series of tangential images and conducted 2D-FFT analysis by measuring





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the dominant orientation of streaks from the tangential images. The grain angle (δ) was evaluated from the tangential images automatically using the 2D-FFT algorithm provided in the Directionality plug-in for Fiji (http://fiji. sc/Fiji, Ashburn, VA) [22, 37, 38]. This program generated a power spectrum (Fig. 2b) with a streak representing the dominant grain orientation from a tangential image (Fig. 2a). The program then transformed the power spectrum into a histogram. The highest peak in the histogram was used to determine the grain angle (δ).

Results and discussion

Information on the anatomical structure from CT data

We present the analysis of the interlocked grain applying the PIV and 2D-FFT methods to CT images of C. camphora. Figure 1 shows the CT image and optical micrograph of C. camphora for a transverse section. Compared with the optical micrograph, the CT image with a resolution of 0.05 mm per pixel was unable to reproduce the detailed shape of xylem cells. However, we could recognize the vessel and surrounding cell lumen distribution as dark dots with a diffuse arrangement on the transverse plane. As expected, the differences in density between the vessel lumen and other cell tissue provided contrast sufficient to support further image analysis. Vessels are used as markers because they can be considered as particles in a transverse section for PIV analysis. We could also determine annual ring boundary based on radial density variation (Fig. 1). Abrupt changes of gray levels from bright to dark, indicating a transition between the end latewood zone into new earlywood zone, become the boundary between annual rings. Meanwhile, within annual ring, a part with brighter pixel is the earlywood part, and another part with darker pixel is the latewood part. However, we could not exactly demarcate the border between earlywood and latewood within annual rings. Moreover, we could see the vessel network as dark streaks on tangential planes that undergo periodical changes in orientation along the radial direction (Fig. 2a). Therefore, in the case of C. camphora, vessel elements could be important features in predicting the grain angle (δ) through X-ray CT.

Extracting 2D images from 3D volume CT observations mimics conventional serial sectioning without physically cutting the object. The serial section of the transverse plane of *C. camphora* shows vessels, implying that the continuity of vessel networks could deviate to a certain degree rather than follow a straight course (Additional file 1: Fig. S1). As seen in the present study, PIV is a useful image analysis tool with which to analyze the existence of interlocked grain in transverse images by tracking the displacement of the vessel network at different depths in



the longitudinal direction. Meanwhile, serial sections on the tangential plane explicitly show a periodical change in the grain angle according to the vessel network alignment (Additional file 2: Fig. S2). Therefore, the 2D-FFT is a suitable option for evaluating the grain angle (δ) as exhibited by the arrangement of longitudinal elements on different tangential surfaces as performed in previous works on *Khaya ivorensis, K. senegalensis, Hopea odorata* and *Acacia mangium* [11, 13, 22].

annual ring to the n + 19 annual ring

The advantage of adopting X-ray CT is that we can perform virtual cutting at any surface position on a wood block. We can obtain transverse and tangential sections from a similar CT volume as demonstrated in this study. 京都大学

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We can even provide other datasets for other sections, such as the oblique transverse section. Additionally, the alignment of the section axis, which is usually required when using conventional serial sectioning techniques [11, 13], is not necessary. This advantage can increase accuracy and save much time in the observation. Moreover, because CT scanning is a non-invasive and non-destructive technique, it is possible to keep the sample intact for further observation [28, 39].

Radial variation of the grain angle

We present two image analysis methods with which to evaluate the grain angle (δ) of *C. camphora* from CT images (Fig. 4). Figure 4 shows the grain angle (δ) variation evaluated using the two methods, PIV and 2D-FFT, along the radial profile with a distinctive undulation pattern representing fluctuation of the grain angle. The fluctuation might be promoted by the inclination of longitudinal cells toward the tree axis as seen from the bark side, where a positive value represents right-handed spirals and a negative value represents left-handed spirals. The age of the observed region was approximately 22 years according to the number of annual ring boundaries corresponding to peaks in the gray level profile (Fig. 4). Figure 4 shows that there was only one complete period of interlocked grain in the 22 annual rings. One period of interlocked grain means the length between peaks in the same direction [9], which is the 16 years from annual ring n + 3 to ring n + 19 in this case.

The radial variations of the grain angle measured using the two methods were similar. The grain angle (δ) of C. camphora ranged from - 25° to 16° when adopting 2D-FFT based on the serial tangential section and from -22° to 18° when adopting PIV based on transverse sections. In previous work, the maximum amplitude of the C. camphora interlocked grain was from 10° to 15° [17]. Figure 4 shows three peaks: two peaks of lefthanded grain and one peak of right-handed grain. The first peak was located in growth ring n+3, the second in n + 10 and the third in n + 19. Both 2D-FFT and PIV performed well in the analysis of the grain angle variation.



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Fig. 7 a Illustration of oblique transverse sections extracted from the *C. camphora* volume. Two types of oblique transverse section, with deviations of 10° counterclockwise and 10° clockwise with respect to the longitudinal axis of tree, were extracted from the *C. camphora* volume. Vector field plot showing the PIV output of the oblique transverse section inclined. **b** 10° counterclockwise and **c** 10° clockwise. The arrow color indicates the inclination angle (φ) relative to the oblique transverse section axis. Zones 1 and 2 are dominated by vessels with an inclination angle (φ) of 10° in right-handed spirals. Not all vectors are displayed to avoid overplotting

In this study, the maximum grain angle of *C. camphora* was 25° and 22° when measured using the 2D-FFT based on the serial tangential section and PIV based on two transverse sections, respectively. These values were lower than the maximum amplitude of 31° for the tropical species *Bagassa guianensis* [14], but higher than those for other tropical species [6, 8, 11, 13, 40, 41].

The change in grain angle had a certain intra-annual ring pattern (Fig. 4). We can see the pattern in n+13 annual ring to the n+19 annual ring. As illustrated in Fig. 5, near the earlywood, the grain angle changed

with constant increment, away from the longitudinal axis. Subsequently, the grain angle increment decreases abruptly and even reverses direction in the earlywood–latewood transition zone heading into the latewood area. This variation may be related to the ability of the vessel to adjust its inclining position rapidly during secondary growth to make contact with adjacent cells [11]. Further observation is needed to confirm the intra-annual ring variation with a higher-resolution imaging technique.



Vessel deviation in the transverse sections

The vector plot in Fig. 6b shows the variation in the grain orientation of C. camphora vessels in an example pair of transverse sections (Fig. 6a) with a depth difference of 500 µm. The arrows in Fig. 6b show the orientation of the tilted vessel as seen from a transverse view. Most vessels were oriented in a tangential direction (left-handed spiral or right-handed spiral) with high inclination angle. However, the vessels oriented nearly perpendicular to the transverse section (zones 1 and 2 in Fig. 6b) shows minor orientation that differed from the major orientation. The vessels were inclined towards each other, and some were moving away from each other, following the tangential direction. Some vessels tilted in the radial direction. The same phenomenon also occurred in the oblique transverse sections (Fig. 7a) that can be observed in zones 1 and 2 (Fig. 7b) with inclined vessels at 10° in left-handed spirals as well as zones 3, 4, and 5 (Fig. 7c) with inclined vessels at 10° in right-handed spirals.

Therefore, such vessel orientations continuously existed along the radial direction from the pith to bark despite the wood grain orientation changing periodically along the radial direction. This phenomenon suggests that the vessels maintain their connection to other cells by inclining at various orientations during secondary growth. The arrangement might be useful for physiological functions such as water transport in *C. camphora* trees.

Conclusion

We evaluated the interlocked grain of semi-diffuse porous hardwood C. camphora from images obtained using an industrial X-ray CT instrument combined with image analysis. The technique allows obtaining volumetric datasets of wood from which we are able to make virtual sections from any direction without damaging the samples. Firstly, we examined the grain angle variation from those consecutive transverse images by adopting PIV analysis to track grain displacement. Secondly, 2D-FFT analysis was performed for serial tangential sections of the same datasets. Examination of the grain angle was consistent between the PIV and 2D-FFT, with the maximum amplitude from S to Z spiral being ca. 40°. PIV can also expand our understanding of the minor orientation of vessels of the interlocked grain of C. camphora, which is related to the continuity of the vessel network. Such evaluation is important in the examination of mechanical properties as well as hydraulic function in woody plants.

Abbreviations

X-ray CT: X-ray computed tomography; 2D-FFT: Fast Fourier transform; PIV: Particle image velocimetry.

Supplementary Information

The online version contains supplementary material available at https://doi. org/10.1186/s10086-022-02064-z.

Additional file 1: Fig. S1. Serial transverse sections of *Cinnamomum* camphora obtained using X-ray computer tomography.

Additional file 2: Fig. S2. Serial tangential sections of *Cinnamomum* camphora obtained using X-ray computer tomography.

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Author contributions

HC performed all the experiments and was a major contributor in writing the manuscript. KK supported numerical analysis throughout the study. SC aided in interpreting the results. JS edited the manuscript and supervised the work. All of the authors read and approved the final manuscript.

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Availability of data and materials

The datasets used and/or analyzed in the current study are available from the corresponding author on reasonable request.

Declarations

Competing interests

The authors declare they have no competing interests.

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