**Title:**

Flexural behavior of wood in transverse direction studied by a novel approach of computer vision and machine learning

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**Highlights**

1. The semantic segmentation model with U-Net architecture was successfully built to partition the tracheid cells in the cross-section of hinoki (*Chamaecyparis obtusa*) wood during the micro three-point bending test.
2. The Crocker-Grier linking algorithm was successfully applied to track the cell wall deformation, and 2D mapping evaluating the deformation intensity distribution at cellular level was successfully built.
3. In comparison to flat- and quarter-sawn specimens, the rift-sawn specimen showed a unique shear deformation cell wall contributing to the low modulus of elasticity and low modulus of rupture.
4. The novel approach developed in this study provides a great possibility for unveiling the relationship between anatomical features and mechanical properties of wood.

**Abstract**

A deep-learning based semantic segmentation approach (U-Net) was used to partition anatomical features in cross-section of hinoki (*Chamaecyparis obtusa*) during micro three-point bending test. With the help of the Crocker-Grier linking algorithm, thousands of cells were successfully extracted. Then, several parameters (area, eccentricity, fitted ellipse aspect ratio, bounding box aspect ratio) were used to evaluate the intensity of their deformation. Thereby, 2D mapping of a deformation intensity distribution was successfully built. By analyzing the flat-, quarter-, and rift-sawn specimens, we have confirmed the orientation of the annual ring affects the flexural behavior of wood in the transverse direction. The quarter-sawn showed the largest MOE and MOR. The ray tissue aligned against the loading might play an important role in the restriction of the cell wall deformation. The rift-sawn specimen showed the smallest MOE and MOR. Its reason might be the loading of specimen in the in-plane off-axial direction, which induces the shear deformation of the cell wall. For all three types of specimens, the fracture had a high possibility to occur at the tension part of the specimen that showed large cell deformation according to the results of K-means clustering of cell deformation pattern. Thus, the novel method developed in this study might be adapted to the fractures prediction of the wood specimen. Furthermore, with varying the test wood species such approach provides a great possibility to unveil the relationship between anatomical features and mechanical behavior of wood in the transverse direction for the improvement of effective utilization of wood resources

**Keywords:**

Flexural behavior, Cell wall deformation, Semantic segmentation, Individual cell tracking, Computer vision, Deep learning

1. **Introduction**

Wood is a natural cellular material, and it has a complex structure with different cell types (anatomical features) acting together to serve the needs of living tree [1]. Also, as an anisotropic material, wood has excellent mechanical properties parallel to the grain (longitudinal direction), while its mechanical properties perpendicular to the grain (transverse direction) are relatively weak [2] and varied among different wood species in relation to their unique anatomical features [1].

From ancient times, humans already started to use wood as a construction material considering the microstructure of wood in the transverse direction. For instance, a traditional roofing method called kokerabuki in Japanese [3]. The quarter-sawn boards with 2-3 mm of thickness, 90-150 mm of width, and 300 mm of length were stacked at the flat part of the roofs, while the rift-sawn boards were selected for the curve surface of the roofs due to its excellent flexibility in transverse direction according to the empirical knowledge of Japanese artisan. And now, understanding the relationship between anatomical features and mechanical behavior of wood is an important subject in the field of wood science for the improvement of the effective utilization of wood resources. To clarify the relationship, wood scientists have developed several approaches mainly from the two perspectives.

The first one is top-down perspective which is the direct microscopic observation of anatomical features deformation during or after the mechanical test. Ando and Onda [4] used wet-type scanning electron microscope (SEM) to observe the compression of wood cell walls. Combing with image analysis, it was found that the first fracture of cell wall occurred in one tangential row of earlywood tracheid just after the load-displacement curve exceeded the proportional limit. Müller et al. [5] observed cell deformation of both softwood (spruce) and hardwood (oak and beech) at different yielding stage of compression test by using both SEM and light microscope for concluding different fracture pattern of anatomical features in those species. Hwang et al. (2021) [6] used the replica method to intermittently analyze the cell wall deformation of flat-, quarter-, and rift-swan in the transverse direction of wood due to three-point bending test. The rift-swan of softwood exhibited a unique deformation pattern of earlywood cell wall contributing to the extremely large flexural deformation. Those direct microscopic observation methods provide important information to understand the in-situ deformation of wood microstructures.

The second one is bottom-up perspective which is the mechanical simulation of wood properties considering its hierarchical structure. Watanabe et al. [7, 8, 9] firstly used fast Fourier transform (FFT) to extract the characteristics such as axial length of tangential and radial cell wall, cell wall thickness, etc. of several conifer wood species for simulating the tangential Young's modulus by cell wall model. Ando and Onda [10] used generalized cell wall model to successfully simulate the first buckling mechanism of conifer wood cell wall under radial compression. Holmberg et al. [11] used the finite element method (FEM) to simulate the nonlinear mechanical behavior considering the irregular cell shape, anisotropic layer structure of the cell walls, and periodic variations in wood density. And the simulated deformation and fracture of wood were similar to those found in the refining process of wood. De Magistris and Salmén [12] investigated the compression and in combined shear and compression deformation of cell wall with anisotropic one-layer cell walls and orthotropic multi-layer cell wall models by FEM . Their results indicated the cell structures are the key factors influencing the deformation pattern. Recently, the multi-scale FEM is also adopted to simulate wood compression behavior under both axis and transverse loading [13]. It was found that transverse deformation of wood is gradual and uniform, while the loading velocity greatly affects wood microstructure failure modes in axial loading. Those developed approaches are quite useful and powerful for providing a comprehensive explanation of the mechanical behavior of wood.

On the other hand, in the field of computer vision, semantic segmentation has been proposed as an important approach to label each pixel of an image with a corresponding class of what is represented. With the development of artificial intelligence, the deep-learning based semantic segmentation approaches such as U-Net [14], LinkNet [15], Feature Pyramid Networks [16], and Pyramid Scene Parsing Network [17] has been developed and those technologies that have large field of application and already started to be applied into the field autonomous vehicles [18] and analysis of biomedical image for medical diagnosis [19]. If the approach of semantic segmentation can also be adapted for analyzing the wood cell wall deformation, it provides a great possibility to simultaneously analyze almost all local changes in anatomical features and their interaction during the mechanical test. Furthermore, the observed information provides more accurate and quantitative image analysis. The collected cell wall geometry can also be used for more realistic mechanical simulation for optimizing developed top-down and bottom-up approaches.

Therefore, in this study, the semantic segmentation model has been built to partition tracheids of hinoki wood, and their local deformation during the micro three-point bending test was precisely analyzed with the help of an individual cell tracking algorithm.

1. **Materials and methods**
   1. **specimen preparation**

The mature Hinoki (*Chamaecyparis obtusa*) wood was used in this study. Three types of (flat-swan, rift-swan, and quarter-swan) samples were firstly prepared only from the sapwood considering their orientation of the annual ring by visual confirmation. The annual ring aligned in horizontal direction and vertical directions were 0° and 90°, respectively. The sample with the angle of annual ring of 0° to 30° was defined as flat-swan, 30° to 60° was defined as rift-swan and 60° to 90° were defined as quarter-swan. After that, the 5 specimens of flat-swan, quarter-swan, and rift-swan, respectively, were prepared with the dimension of 10 mm (longitudinal) x 20 mm (width) x 1.5 mm (thickness). Then, the cross-section of all specimens were smoothed by a sliding microtome (TU-213, Yamato kohki industrial Co., Ltd., Japan). All specimens were then conditioned in a plastic glove box at 60% relative humidity (RH) and 25°C by using sodium bromide solution for more than two weeks.

* 1. **micro three-point bending test**

After the conditioning, all specimens were subjected to the micro three-point bending test. The customized metal jig (Fig.1 a) was used for the test. A motor (BLM230P-GFV2, ORIENTAL MOTOR Co.,Ltd., Japan) with a test speed of 1mm/min was used to horizontally bend the specimen. And a 200N load cell (LUR-A-200NSA1, Kyowa Electronic Instruments Co., Ltd., Japan) with a sensor interface (PCD-320A, Kyowa Electronic Instruments Co., Ltd., Japan) was used to record the force, the sampling speed is 1Hz. During the test, a stereo-microscope (Leica DMS300, Leica Camera AG, Germany) was set perpendicular to the cross-section to record the deformation of tracheid cells by video mode at 30 fps. The resolution was 1080p and the length of one pixel is equal to about 2.09 *µ*m. All experiment was conducted at 60% RH and 25°C.

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Fig. 1 The illustration of micro three-point bending test. (a) The illustrated apparatus for the mechanical test. (b) Cross-section of wood specimen observed with a stereo-microscope.

* 1. **Deep learning based semantic segmentation model**

For the preparation of dataset and model training, after the video taking during the bending test, the first image at every second of the video was captured to prepare the image sequence. The 12 original images with 256 pixels x 256 pixels were cropped from the image sequence. The watershed segmentation implemented by Mahotas [20, 21] was firstly applied for labeling the boundary of tracheid cells (Fig.2 a). The unlabeled part was manually modified to make their corresponding ground truth masks. The boundaries were generally labeled at the centerline of adjacent cell walls. For tracheid cell wall adjacent to the ray parenchyma cells, due to low contrast of ray parenchyma cell lumen, the boundaries were always labeled at the center part of the ray parenchyma cell in this study, which means part of a ray parenchyma cell was recognized as tracheid cells (Fig.2 b). As a future assignment, the parenchyma cell walls should be labeled separately with the methodological improvement of microscopic observation.

Finally, 12 sets of original image and corresponding ground truth mask that cell boundaries labeled in white and background labeled in black were used for building the semantic segmentation model. For model training, the symmetric U-net architecture achieved by using ‘same’ padding instead of ‘valid’ in the original model [14] was used. The network was implemented using Tensorflow framework version 1.5.0 and Keras version 2.2.4. The binary cross-entropy was used as the loss function, and Adam was used as the optimizer. The learning rate was 0.0001. During the model training, the augmentation of images by the image generator was applied.

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Fig. 2 Preparation of dataset for semantic segmentation model training. (a) Cropped patch of wood cross section; (b) Trachied cell boundary labeled mask by watershed segmentation algorithm (c) manually corrected image mask. The scale bar indicates a length of 100 *μ*m

* 1. **Metrics for model evaluation**

Four metrics were used for evaluating the trained model. They were accuracy, recall, precision, and f1-score. Those metrics were calculated from true positive (TP), false positive (FP), true negative (TN) and false negative (FN) obtained from the confusion matrix for the binary classification of cell boundary and background. The equations for the calculation were shown below:

* 1. **image prediction and individual cell tracking**

After model training, the trained model combined with the help of a patch blending algorithm [22] was used to partition all potential cells in the image sequence with 1920 pixels x 1080 pixels. After predicting all image sequences, watershed segmentation was applied to achieve the instance segmentation of all cells. Finally, the coordinates of the centroid of segmented cells were collected and a tracking algorithm (Crocker-Grier linking algorithm) [23] implemented by Trackpy [24] was used to link the same cell walls that exist in each image.

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Fig.3 Tracking the cell deformation during mechanical test. (a) watershed segmentation of predicted image by trained U-net model to achieve instance segmentation; (b) coordinates of centroids for each cell exacted as the features for individual cell tracking; (c) trajectories of centroids found by Crocker-Grier linking algorithm. The color of each trajectory was randomly generated.

* 1. **Parameters measurement for cell wall deformation analysis**

Finally, after the tracking of individual cells existing in every image sequence, the area, eccentricity, major and minor axis length of fitted ellipse (Fig.4 ①②), and length of vertical and horizontal length of bounding box (Fig.4 ③④) for each cell wall were measured. It should be noted that the eccentricity was calculated from the fitted ellipse that has the same second moments of cell wall. The eccentricity of a circle is zero, while the eccentricity of ellipse was greater than zero but less than one. Those measurements were implemented by the python package: scikit-image [25]. Furthermore, the fitted ellipse aspect ratio and bounding box aspect ratio were also calculated based on the following equations:

For evaluating the intensity of cell wall deformation, the changes in area, eccentricity, fitted ellipse aspect ratio and bounding box aspect ratio were calculated based on the following equation:

the n indicates the order of the observed image sequence and the i indicates the type of measured parameters which were shown in Fig.4.

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Fig.4 the measurement parameters to evaluate the intensity of deformation of cell wall

1. **Results and discussion**
   1. **flexural behavior of flat-, quarter-, and rift-sawn in the transverse direction**

Fig.5 showed the difference in the mechanical properties of flat-, quarter-, and rift- sawn in the transverse direction. During the micro three-point bending test, the rift-sawn specimen showed the smallest load values with the largest displacement at around 3.3 mm, resulting in the smallest modulus of elasticity (MOE) and modulus of rupture (MOR) (Fig. 5 a). If we assume the linear stage of load-displacement as the elastic region and the nonlinear stage as the plastic region. The rift-sawn specimen showed the largest plastic region. In contrast, the quarter-sawn showed the largest MOE and MOR (Fig. 5 b) with the smallest plastic region. Those results agree with the previous study [6], which suggests the orientation of the annual ring plays an important role in the flexural behavior of wood in the transverse direction. It also demonstrates that the built micro three bending test system in this study is reliable for discussing the mechanical properties of wood in the transverse direction.

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Fig.5 Mechanical properties of flat-swan, quarter-swan, and rift-swan of hinoki specimens in the transverse direction. (a): Load-displacement curve of three types of hinoki specimens during micro three-point test; (b) MOE (modulus of elasticity) and MOR (modulus of rupture) of three types of hinoki specimen. The error bars indicate the standard deviation.

* 1. **Validation of U-Net model and large image prediction**

With the development of artificial intelligence, the model of fully convolutional networks (FCN) was proposed for conducting semantic segmentation [26]. Furthermore, as an improvement of FCN, the U-Net architecture was proposed by Ronneberger et al. [14], which is designed to allow fewer training samples for model training. It is a U-shape architecture consisting of encoder blocks, decoder blocks, and skip connections. It became one of the most popular approaches for any semantic segmentation task. Recently, the U-net model has also been applied for the segmentation of plant tissues [27] and xylem vessels in stained cross-sections of wood [28] with excellent accuracy. Therefore, in this study, the U-Net has been selected for the segmentation model building.

Fig.6 a showed the evolution of binary cross-entropy loss during 100 epochs training with U-Net architecture. After about 40 epochs of training, the validation loss tended to become almost constant, while the loss continue to decrease to about 0.1. And the averaged values (standard deviation) of recall, precision, F1 and accuracy were 0.82 (0.119), 0.82 (0.017), 0.82(0.017), 0.92 (0.0006), respectively. Those measured values indicate an accurate semantic segmentation model has been built. Fig.6 b showed an example of input original image and Fig.6 c was the predicted image of original image through the trained model. The combination of patch blending algorithm and trained model worked well to predict the large image of the cross-section. The most of tracheid cells seemed to be well segmented, while the partition of latewood tracheid cells and some earlywood cell walls was not well predicted due to the low contract of cell wall lumen. To overcome the problem, the methodological improvement of microscopic observation for increasing the contrast of cell wall lumen will be needed.

To further confirm the accuracy of the segmentation, the geometry parameters of a flat-swan specimen were measured. The vertical bounding box and horizontal bounding box were regarded as cell radial diameter and cell tangential diameter. Fig.7 showed the distribution of typical parameters measured from the segmented cells. The averaged values (standard deviation) of area, cell eccentricity, cell radial diameter, and cell tangential diameter were 955 *μ*m (306), 0.596 (0.146), 37.5 *μ*m (7.63), and 34.8 *μ*m (6.62), respectively. Those measured geometry values agree with the geometry parameters reported in the past study [29].

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Fig. 6 Tracheid cell wall boundary prediction by trained U-net model. (a) binary cross entropy loss plotted against the training epochs; (b) input original image; (c) predicted image. The scale bar indicates length of 400 *μ*m.

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Fig.7 Distribution of typical parameters measured from a flat-sawn specimen before the mechanical test. (a) cell area (*µ*m2); (b) cell eccentricity; (c) cell diameter (*µ*m). Yellow: cell radial diameter, Gray: cell tangential diameter.

* 1. **Typical deformation patterns of tracheid cell wall in three types of specimens**

Fig.8 showed typical deformation patterns of tracheid earlywood cell wall located at both compression part and tension part of three types of specimens. The changes in the shape of single cell wall located at both compression part and tension part of three types of specimen during the mechanical test were intermittently extracted and compared.

For flat-sawn specimen, the uniaxial compression and tension of tangential cell wall occurred against compressive and tensile stress during the bending test, respectively (Fig. 8 a). And because of the orthogonal orientation of the cell wall, a similar deformation was observed at the radial cell wall in quarter-sawn. As quarter-sawn was fractured at the early stage of the bending test when displacement reach around only 1 mm, the dimensional changes of the cell wall were relatively smaller than that of flat-swan (Fig.8 b).

Different from flat- and quarter-sawn specimens, the cell wall in rift-sawn seemed to show a different deformation pattern. The shear deformation of cell wall along the vertical and horizontal direction was observed at compression part and tension part, respectively. Such orientation of tracheid cells was quite similar to the uniaxial loading of honeycombs in the in-plane off-axial direction. Li et al. [30] have simulated the in-plane yield strengths of the square honeycombs in different directions under compression through both theoretical approach and FEM method. It was concluded the square honeycombs show a strong anisotropy when loaded in different orientations. And the numerical simulation indicates that the axial yield strength of the square honeycomb has minimum values at the angle of orientation with 37°to 38°, which is in the range of the orientation of the annual ring for rift-swan. Therefore, we suppose such shear deformation induced by the off-axis loading of tracheid cell is responsible for the large displacement and low MOE and MOR of rift-swan specimen.

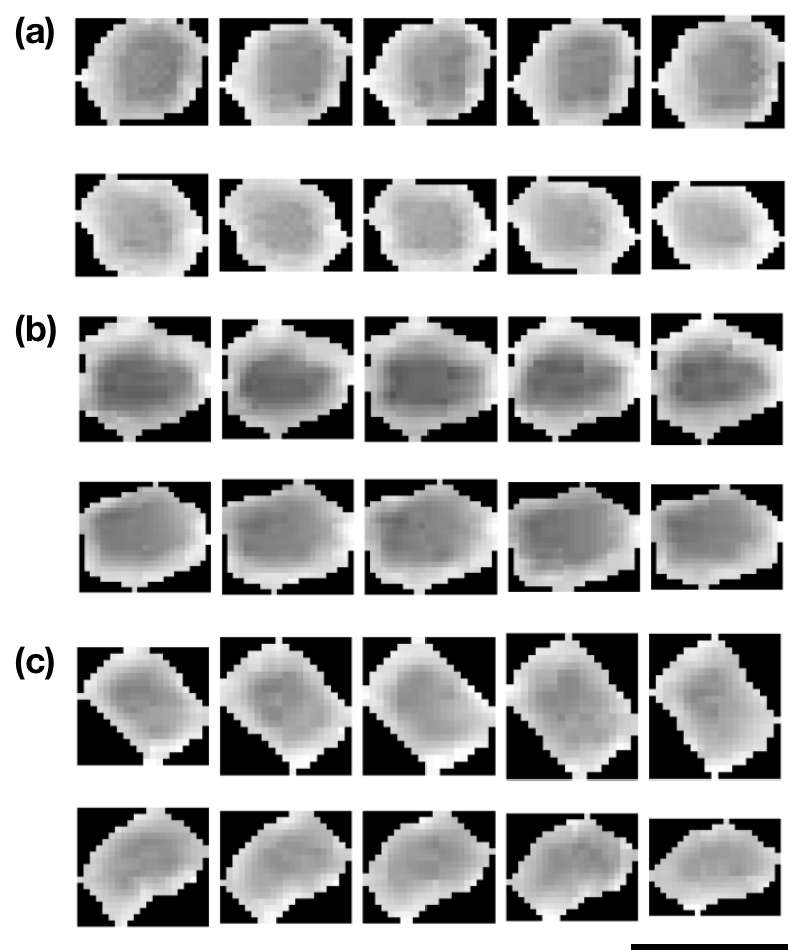


Fig.8 Typical deformation pattern of tracheid cell wall for flat- (a), quarter- (b), and rift-sawn (c) of hinoki specimens during micro three-point bending test. The upper row of each subfigure indicates the cell wall located at compression part, while the lower row of each subfigure indicates the cell wall located at tension part. The scale bar indicates 50 *μ*m.

* 1. **Visualization of the distribution of tracheid cell wall deformation**

With the benefit of Crocker-Grier linking algorithm, the coordinates of centroids for each common tracheid cell that existed in each frame of image sequence were successfully linked. Thus, thousands of common cells were finally extracted. Those common cells were mainly the earlywood cells with sufficient contrast of cell wall lumen, while the latewood cells and earlywood cells with low contrast of cell wall lumen along the radial direction were not successfully linked due to their bad segmentation results. After evaluating the intensity of the deformation by calculating the changes in four parameters (Changes in area, eccentricity, fitted ellipse aspect ratio, and bounding box aspect ratio) described in materials and methods, the 2D mapping of the intensity of cell deformation for three types of specimen was successfully built. The white color means the measured parameters remain unchanged. And the darker red and darker blue color indicate larger increases and larger decreases in measured parameters, respectively. It should be noted that the changes in eccentricity and fitted ellipse aspect ratio of cell wall highly depends on the original shape of cell wall before the test to have both increase and decrease of values against same mechanical stress. On the other hand, the changes in bounding box aspect ratio didn’t depend on its original shape. So the increase in bounding box aspect ratio indicates horizontal compression of the cell wall while the decrease indicates the horizontal tension of the cell wall.

At the elastic region, all specimens showed relative slight and varied deformation for all parameters (Fig.9 a d g j, Fig.10 a d g j, Fig.11 a d g j). When entering the plastic region, the cell wall deformation distribution differed (Fig. 9 b e h k, Fig.10 b e h k, Fig.11 b e h k). And the intensity of the deformation reaches the maximum before the fracture of the specimen (Fig.9 c f i l, Fig.10 c f i l, Fig.11 c f i l). The suitable parameters for the evaluation of deformation for those types of specimens were discussed below.

For flat-swan, the area seems to be the most suitable parameter for the deformation evaluation. As shown in Fig.9 b c, the cell area increased in tension part and decreased in compression part of specimen. And the intensity of the changes at compression part is relatively smaller than that of tension part. It might be due to the existence of the latewood at compression part contributing to the restriction of deformation of cells. Both significant increases and decreases in eccentricity were concentratedly observed at the tension part of specimen (Fig. 9 e f). It is supposed that horizontal tension of wood cell wall induced both an increase in eccentricity for circle-shaped cells and a decrease in eccentricity for vertical ellipse-shaped cells resulting in the mixture of the increase and decrease in eccentricity. For the same reason, a similar result was also observed in the case of changes in fitted ellipse aspect ratio (Fig.9 h i). For bounding box aspect ratio (Fig.9 k l), the cells located in central part of the specimen showed reasonable results. The increase and decrease in bounding box aspect ratio were observed in compression part and tension part, respectively. However, the bending test also caused the curve of the specimen that also change the orientation of cells located in the surrounding part of the specimen influencing the reliability of their measured bounding box aspect ratio.

For quarter-sawn shown smallest plastic region of three types of specimen (Fig.10), the bounding box seems to be a promising parameter. The minor changes in cell area varied even at the plastic region and before the fracture (Fig.10 b c). Like flat-sawn, the changes in cell eccentricity and fitted ellipse aspect ratio highly depend on the original shape of cells, and both increase and decrease in eccentricity and fitted ellipse aspect ratio were observed at compression and tension part of specimen, which indicates those two might not be suitable parameters (Fig.10 e f h j). In the case of bounding box aspect ratio, as the specimen showed a minor curve of specimen during the bending test, the compressive stress caused the increase in the ratio, and tensile stress caused the decrease in the ratio. And a neutral axis seems to be found at the almost center part of the specimen with the smallest changes in the ratio (Fig. 10 k l).

For rift-swan specimen (Fig.11), the area, eccentricity, and fitted ellipse aspect ratio seem to be robust parameters for its deformation evaluation. In comparison to flat- and quarter-sawn, the more concentrated and intensive deformation was observed at the innermost of the compression part and outermost of the tension part of rift-sawn. A decrease in cell area for both compression part and tension part was found (Fig. 11 b c), which is corresponding to the region showing an increase in cell eccentricity and fitted ellipse aspect ratio (Fig. 11 e f h i). It is because the shear formation of cell wall is the dominant deformation pattern, which is responsible for the increase in eccentricity, major axis length and the decrease in minor axis length. For bounding box aspect ratio, same with the case of flat-sawn, the changes in orientation of cells cause unreliable results for cell deformation (Fig. 11 k l).

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Fig. 9 Intensity of cell wall deformation of flat-sawn specimen during micro three-point bending test evaluated by four parameters. (a,b,c) Changes in area (%); (d,e,f) Changes in eccentricity (%); (g,h,i) Changes in fitted ellipse aspect ratio (%); (j,k,l) Changes in bounding box aspect ratio (%). (a, d, g, j) Elastic region; (b,e,h,k) Plastic region; (c,f,i,l) Before the fracture.

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Fig. 10 Intensity of cell wall deformation of quarter-sawn specimen during micro three-point bending test evaluated by four parameters. (a,b,c) Changes in area (%); (d,e,f) Changes in eccentricity (%); (g,h,i) Changes in fitted ellipse aspect ratio (%); (j,k,l) Changes in bounding box aspect ratio (%). (a, d, g, j) Elastic region; (b,e,h,k) Plastic region; (c,f,i,l) Before the fracture.

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Fig. 11 Intensity of cell wall deformation of rift-sawn specimen during micro three-point bending test evaluated by four parameters. (a, b, c) Changes in area (%); (d, e, f) Changes in eccentricity (%); (g, h, i) Changes in fitted ellipse aspect ratio (%); (j, k, l) Changes in bounding box aspect ratio (%). (a, d, g, j) Elastic region; (b, e, h, k) Plastic region; (c, f, i, l) Before the fracture.

* 1. **Clustering analysis of deformation pattern of individual cell and its relationship with stress-strain curve**

After choosing the suitable parameter for the deformation evaluation, the changes in area, bounding box aspect ratio, and fitted ellipse aspect ratio was selected for discussing the cell deformation of flat-, quarter-, and rift-sawn, respectively. The k-means clustering algorithm implemented by the Python package: scikit-learn [31] was then applied to summarize the deformation pattern.

The clustering algorithm worked well to summarize 8 clusters that correspond to the intensity of cell deformation for three types of specimen (Fig.12 a b c). The distribution of the clusters for three types of specimen were shown in Fig.12 d e f. And the relationship between 8 summarized deformation patterns and both strain and stress for three types of specimen was shown in Fig.12 g h i j k l. At elastic region, the linear changes in cell area, bounding box, and fitted ellipse aspect ratio for flat-, quarter, and rift-sawn specimen were observed with the increase of strain and stress. However, the cell deformation pattern of those specimen differed at the plastic region.

For flat-sawn, when entering the middle stage of plastic region, the clusters with red and vermilion colors showed a slight monotonic increase in cell area against the evolution of strain and stress was observed (Fig.12 g j). As shown in Fig. 13 (a), the ray parenchyma cells seem to be a defect for the induction of the fracture, then detachment of the tangential cell wall between the cells was also observed. For quarter-sawn, a monotonical increase in bounding box aspect ratio was observed at late stage of the plastic region (Fig.12 h k). Interestingly, the significant increase and decrease in bounding box aspect ratio mainly occurred at earlywood region near to the previous latewood region (Fig.12 b). As the earlywood cell wall located at that region showed thinner cell wall thickness with large cell area resulting in weaker mechanical properties, we suppose it is the reason why the fracture of specimen induced by the detachment of the radial cell wall between cells started to occur at the earlywood region of the tension part (Fig.13 (b)). Furthermore, as the ray parenchyma cells of quarter-sawn was aligned against the mechanical load, it is possible that ray parenchyma cells play an important role in the restriction of cell wall deformation resulting in the larger MOE and MOR than that of flat-swan. For rift-swan, two clusters with red and vermilion colors along the radial files showed an exponential increase with the increase of strain and stress. (Fig.12 i l). Such drastically large shear deformation plays an essential role in the flexibility of the rift-sawn specimen. Furthermore, due to the orientation of the annual ring around 44.5°, the ray tissue seems to have a minor restriction for the cell walls. And the detachment of tangential cell walls between cells along the radial direction dominated the fracture pattern of the specimen (Fig.13 (c)).

According to the above discussion, the fractures of three types of specimen seem to have a high possibility to occur at the corresponding tension part of their clustered image that showed large cell deformation. Therefore, the novel method developed in this study might be adapted to the fracture prediction of the wood specimen.

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Fig.12 The k-mean clustering results of deformation pattern and their relationship with strain and stress of specimen. (a, b, c) Clusterized images of flat-, quarter-, and rift-sawn specimen, respectively; (d,e,f) Distribution of clusters of changes in area, bounding box aspect ratio, and fitted ellipse aspect ratio for flat-, quarter- and rift-sawn specimen, respectively; (g,h,i) Clusterized changes in area, bounding box aspect ratio, and fitted ellipse aspect ratio during mechanical test for flat-, quarter- and rift-sawn specimen, respectively, and their relationship with strain. The light green and skyblue areas indicate the assumed elastic and plastic regions; (j,k,l) Clusterized changes in area, bounding box aspect ratio, and fitted ellipse aspect ratio during mechanical test for flat-, quarter- and rift-sawn specimen, respectively, and their relationship with stress. The light green and skyblue areas indicate the assumed elastic and plastic regions.

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Fig.13 The typical fracture of the (a) flat-, (b) quarter-, and (c) rift- swan specimen after micro three-point bending test. The scale bar indicates 400 *μ*m.

1. **Conclusion**

In this study, a deep-learning based semantic segmentation model with U-Net architecture was successfully built to partition tracheid cells in cross-section of hinoki during the micro three-point bending test. With the help of Crocker-Grier linking algorithm, thousands of cells were successfully extracted. Then, several parameters (area, eccentricity, major/minor axis length, vertical/horizontal bounding box length) were used to evaluate the intensity of their deformation. Finally, 2D mapping of a deformation intensity distribution at cellular level was successfully built. And the main conclusions for analyzing the flat-, quarter- and rift-sawn specimens are as follow:

1. The area and bounding box aspect ratio were suitable for evaluating the cell wall deformation of flat- and quarter-sawn specimen, respectively. As a relatively large shear deformation of cell wall was induced for the rift-sawn specimen, the area, eccentricity, and fitted ellipsed aspect ratio are robust parameters for deformation evaluation.

2. The quarter-swan showed the largest MOE and MOR. The ray parenchyma cells aligned against the loading might play an important role in the restriction of the cell wall deformation. The rift-sawn specimen showed smallest MOE and MOR and its reason might be the loading of specimen in the in-plane off-axial direction, which induces the shear deformation of the cell wall.

3. For all three types of specimens, according to the k-means clustering results of cell wall deformation pattern, the fracture has a high possibility to occur at the tension part of specimen that showed large cell deformation. Therefore, the novel method developed in this study might be adapted to the fracture prediction of the wood specimen.

By increasing the test wood species with different anatomical features, the novel approach developed in this study provides a great possibility for clarifying the relationship between anatomical features and mechanical behavior of wood in the transverse direction.

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1. **Author contributions**

SC and JS designed experiments. SC mainly conducted experiments and wrote the manuscript. TA, AY, JS supervised the work and all the authors approved the final version of the manuscript.

1. **Data Availability**

All codes and a set of test images in this study are available online on Github.com (https://github.com/pywood21/Tracking\_cell\_wall\_deformation).