SHRINKAGE OF TRACHEID CELLS WITH DESORPTION VISUALIZED BY CONFOCAL LASER SCANNING MICROSCOPY

Hiroki Sakagami¹, Junji Matsumura² and Kazuyuki Oda²

SUMMARY

Confocal laser scanning microscopy (CLSM) was applied as a new method of visualizing the shrinkage of wood and its anisotropy. Control of relative humidity and temperature in a specialized environment chamber made it possible to acquire transverse images of tracheids of Akamatsu (Pinus densiflora) from the saturated condition to the dried condition. The shrinkage of tracheid cells was also determined by measuring the tangential diameter of tracheid and lumen, the radial diameter of tracheid and lumen, and the thickness of tangential and radial walls. Moreover, this technique makes it possible to discuss the relationship between moisture content and tracheid cell shape. We found the CLSM technique to be an effective method for visualizing shrinkage of tracheid cells with desorption.

Key words: Confocal laser scanning microscopy, shrinkage, anisotropy, tracheid.

INTRODUCTION

It is well known that wood shows a very complicated swelling or shrinkage behaviour with the adsorption or desorption of bound water below fibre saturation point. The increase or decrease of free water above fibre saturation point does not cause changes in wood dimensions. The swelling or shrinkage of wood shows anisotropy and the ratio is generally 10 (tangential) : 5 (radial) : 0.1–1 (longitudinal). Considerable dimensional changes that occur due to swelling or shrinkage are the cause of cracks in lumber and internal stresses and are undesirable characteristics from the point of view of timber utilization. On the other hand, swelling and shrinkage could also be considered as intelligent characteristics of wood because wood can change dimensions by itself in response to the atmosphere (Okuma 1998). Therefore it is important to obtain accurate knowledge on the swelling and shrinkage behaviour of wood.

The anisotropy in wood is broadly divided into two categories. The anisotropy on the longitudinal face of the wood relates to the difference in the shrinkage or swelling ratio in the axial and tangential directions, or axial and radial directions. The cause of the anisotropy is explained relatively easily as follows: the microfibrils of the S₂ layer

---

¹) Department of Forest and Forest Products Sciences, Graduate school of Bioresource and Bio-environment Sciences, Kyushu University, Fukuoka 812-8581, Japan.
²) Department of Forest and Forest Products Sciences, Faculty of Agriculture, Kyushu University, Fukuoka 812-8581, Japan.

Associate Editor: Lloyd Donaldson
of the secondary wall, which occupies most of the cell wall, are almost parallel to the axial direction.

Secondly, the anisotropy on the transverse face of the wood relates to the difference in the shrinkage ratio in the tangential and radial directions. There are greater numbers of factors contributing to anisotropy in the transverse face in comparison with the longitudinal face. For example, 1) the interaction of earlywood and the latewood (Pentoney 1953), 2) the effect of rays (McIntosh 1957), and 3) differences in microfibril angle (Barber & Meylan 1964) or 4) lignification (Boyd 1974) between radial wall and tangential wall in tracheids. Thus, the anisotropy in the transverse plane is a result of a complex interplay of these factors.

Anisotropy in the transverse plane has been primarily studied by conventional microscopy techniques. Nakato (1958a, b) studied anisotropy using light microscopy and reported that the lumen of latewood tracheids expanded or did not change in the radial direction but shrunk in the tangential direction during drying. The shrinkage ratio of the cell wall width was larger in the order: radial wall in latewood > tangential wall in latewood > tangential wall in earlywood > radial wall in earlywood, while the shrinkage ratio of cell wall thickness was larger in the order: tangential wall in latewood > tangential wall in earlywood ≈ radial wall in earlywood ≈ radial wall in latewood. However, the possibility exists that this method did not reflect the true swelling and shrinkage behaviour of the wood because minute cracks occurred when the thin section was cut (Bosshard 1956), and the extraction by the organic solvent used for paraffin embedding would have influenced the volume dimensions (Taylor 1974). A replica method was then proposed for observing the surface of wood blocks (Adachi et al. 1989). Using this method, Watanabe et al. (1998) reported that earlywood tracheids show anisotropic shrinkage, while latewood tracheids shrink almost isotropically, and suggested that shrinkage anisotropy depends significantly on the mechanical interaction between earlywood and latewood. According to Ishimaru and Fida (2001), cell walls swell to a much less extent in width than in thickness and the interaction between earlywood and latewood is one of the prime factors contributing to the swelling anisotropy. Recently, the change of cell shapes under dry and wet conditions was observed at the same location by low vacuum scanning electron microscopy (LV-SEM) (Mikajiri et al. 2001), and in samples from the same rod with an environmental scanning electron microscope equipped with a field emission cathode (FE-ESEM) (Gu et al. 2001). According to Mikajiri et al. (2001), the shrinkage of cell wall thickness was large, but the difference between the radial wall and the tangential wall was not so large. Noting a large difference in the lumen diameter between tangential and radial directions, they concluded that shrinkage of the cell wall in the width direction (perpendicular to cell axis) was one of the main causes of anisotropy. In the LV-SEM method, however, it is necessary to acquire an image in the swollen condition at the moment the ice on the surface sublimes, and the drying rate is fast. Murata et al. (2001a, b) visualized the tracheid cells on the transverse face in the dry and wet conditions, steamed by a heat-type humidifier using a confocal scanning laser microscope. They concluded that the anisotropy of expansion was mainly based on thickness swelling from the fact that the outside diameter of latewood tracheids swelled almost isotropically, but the lumen
diameter shrinkage was large and anisotropic. This method allows acquisition of images under dry and swollen conditions, but information on changes in cell shape with desorption or adsorption can not be obtained. It is also difficult to know the moisture content in the observed area.

The objective of this study was to visualize the shrinkage behaviour of the tracheid cell with time using confocal laser scanning microscopy (CLSM). CLSM enabled us to obtain information in the Z-axis. The added advantage was that sample preparation was minimal, and the technique allowed the analysis of three-dimensional changes with time in wood cells with decreasing moisture content.

**MATERIALS AND METHODS**

A stick specimen (30 × 30 mm) of *Pinus densiflora* was prepared so that the growth rings were either parallel or perpendicular to the surface. The mean growth ring width was 1.6 mm. The stick specimen cross-cut to a length of 5 mm and shrinkage specimens for conventional methods and specimens for CLSM observation were obtained. The specific gravity was 0.60. The shrinkage from saturated to oven-dry conditions was 9.03% in the tangential direction and 5.97% in the radial direction.

From the specimen for CLSM observation, 5 mm cube specimens were obtained and examined using a BIO-RAD Radiance 2000 CLSM. Two end-matched specimens were used as a set. One was a specimen for CLSM and the other for moisture content measurement. Nine sets were prepared in this study. Transverse faces were planed with a sliding microtome. Specimens were then stored in a controlled environment room for two weeks. The initial moisture content was 25–30% when the experiment started.

The CLSM system used for visualization is shown in Figure 1. Temperature and relative humidity in the environment chamber (see light grey zone in Fig. 1) were con-

---

**Figure 1.** Schematic diagram of CLSM system for visualization of shrinkage behaviour.
trolled. A dry lens (×60/n.a. 0.95) was used for all observations. The theoretical resolution is 0.26 μm and the minimum spot size of laser must be 0.13 μm. Images were acquired at 1280 × 1024 pixel resolution and the pixel size was 0.16 μm without an optical zoom. Therefore, maximum useful zoom factor was estimated at 1.2 times. Images were recorded using an Argon ion laser with excitation wavelengths at 488 and 514 nm and an emission filter with 500 nm longpass (HQ500LP). The scan speed was 25 lps (lines per second). The images in the saturated condition were taken immediately after placing the specimens on the stage. Subsequently, relative humidity in the controlled environment chamber was gradually decreased. An example of change in temperature and relative humidity in the environment chamber is shown in Figure 2. Images at identical locations and the specimen weights were obtained during drying. The images were optical sections 5 μm below the surface. When the final image was obtained, the moisture content was 8 to 10%.

The latewood selected was located near the annual ring boundary on the transverse face. Tangential and radial diameters of the lumina and the thickness of tangential and radial cell walls were measured by NIH image software. Areas of tracheid and lumen were also obtained. Line analysis was used to measure each dimension. In this study, only latewood was analysed because of limited resolution. This study requires a dry lens and not an oil lens because the possibility exists that immersion oil affects shrinkage behaviour. The highest magnification of a dry lens is ×60 at the moment. We did not think that this CLSM system was suitable for measurement of dimensional changes of earlywood with desorption.
RESULTS AND DISCUSSION

Control of moisture content
Changes in moisture content of nine specimens during the experiment are shown in Figure 3. It took 12–15 hours to reach the air-dry condition from the fibre saturation point. The initial moisture content of specimens ranged from 25 to 30% and the moisture content slowly decreased linearly with a decrease in the relative humidity within the environment chamber. It can be assumed that there was little difference in moisture content between surfaces and the interior of specimens.

![Figure 3. Change of moisture content of all specimens. Initial moisture content ranged from 25 to 30% and the final moisture content ranged from 8 to 10%.](image)

Shrinkage by CLSM method
We succeeded in animating the shrinkage behaviour from near fibre saturation point to 8%. Six images with decreasing moisture content from 28.2 to 8.9% are shown in Figure 4. The relationship between moisture content and the dimensional change of tangential and radial lumen diameters, the thickness of tangential and radial cell walls, and the change of areas of tracheid and lumen are also shown in Figure 4. Tracheid area, tracheid diameter and cell wall thickness in both tangential and radial directions decreased linearly with decreasing moisture content. Other specimens also showed the same tendency. This is well known behaviour. Thus, CLSM is a powerful tool for studying shrinkage behaviour with time. However, the lumen showed complicated shrinkage behaviour. The radial lumen diameter and lumen area decreased after repeated shrinkage and expansion. Tangential lumen diameter also repeated shrinkage and expansion with decreasing moisture content, and three types of dimensional change, that is, expansion shrinkage and no change was observed. In the case of Figure 4, the tangential diameter of the lumen increased in comparison with the initial and final moisture con-
tents and the radial diameter of the lumen decreased. This suggests interaction between adjacent cells which requires further investigation. Some dimensional changes between plots in Figure 4 were less than the theoretical resolution (0.26 μm). However, CLSM makes it possible to visualize shrinkage behaviour with time. More detailed information could be obtained in comparison with conventional images of the swollen and dried conditions.

Figure 4. Changes in cell shapes with desorption.
Visualization of localized behaviour by CLSM

Sequential observation with time by CLSM made it possible to capture interesting features. Figure 5 shows that the thickness of the radial wall in the right-hand tracheid increased in spite of the decrease in moisture content. The moisture content decreased from 11.74% to 10.97% and the left-hand cell shrank from 7.20 µm to 6.88 µm, while the right-hand cell increased from 9.92 µm to 10.08 µm. This may be evidence of complex interaction between adjacent cells in relation to drying stresses. However, it may be difficult to discuss the dimensional change in the right-hand cell because of limited resolution in this CLSM system. It will be necessary to show more unequivocal evidence.

Shrinkage of the tangential wall in the width direction is shown in Figure 6. In spite of the decrease in moisture content from 19.93% to 19.45%, the radial diameters of tracheid and lumen did not change but the tangential lumen diameter shrank from 20.96 µm to 20.64 µm. This suggests that the cell wall shrank not only in thickness but also in width. It is difficult to explain this behaviour by the interrupted lamella model of Kerr & Goring (1975). According to this model, the cellulose microfibrils are ribbon-like structures consisting of 2–4 protofibrils bonded on their radial surfaces, with their tangential surfaces co-planar and parallel to the middle lamella, and shrinkage or swelling occurring only in the thickness direction because water enters or exits between the layers. Alternatively, the fibril agglomeration model proposed by Sell and Zimmermann (1993) may be able to explain this behaviour. According to this model, the structure of
the secondary wall appears like a sandwich and the $S_2$ layer acts as a core with fibril agglomeration perpendicular to the face layers, and radial relative to the longitudinal axis of the cell. Gu et al. (2001) studied anisotropic transverse shrinkage using the fibril agglomeration model and concluded that a difference in oriented fibril agglomerations between the radial and tangential wall on the transverse face was a major factor for the anisotropy. This model has the possibility to explain that the cell wall shrinks not only in the thickness direction but also in the width direction, but the change in the width direction in this study was not enough to cause anisotropic shrinkage.

CONCLUSIONS

The ability to control relative humidity and temperature in an environment chamber in combination with CLSM is an effective approach for visualizing the shrinkage or swelling of wood cells with desorption or adsorption. The technique provides valuable new information, which makes it possible to understand a relationship between moisture content and wood cell shape.

ACKNOWLEDGMENTS

The authors thank Dr. Adya Singh and Dr. Lloyd Donaldson for their suggestions. This research was supported in part by Grant-in-Aid for Scientific Research (16780126) from the Ministry of Education, Culture, Sports, Science and Technology of Japan.
REFERENCES


McIntosh, D.C. 1957. Transverse shrinkage of red oak and beech. For. Prod. J. 7: 114–120.


