

RAPID COMMUNICATION

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Observation of microscopic swelling behavior of the cell wall

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Key words Swell anisotropy · Cell wall · Sputter etching · Confocal laser microscope · Digital image correlation**Introduction**

The swelling or shrinkage behavior of a cross section of wood is complicatedly affected by its anatomical structure and anisotropic elasticity.¹ Therefore, no theory that explains exactly the cause of swelling anisotropy has been reported. Recently, application of the digital image correlation method (DIC) to microscopic or mesoscopic strain measurements has been studied,^{2,3} and DIC was found to be a promising tool for that purpose. We also observed the transverse swelling behavior of tracheids of Douglas fir by means of confocal scanning laser microscopy and DIC and succeeded in measuring the change of cell shape and distribution of expansion in cell walls with absorption of moisture.⁴ However, the cross section surface planed by a sliding microtome was so smooth that we could not measure strain distribution in cell walls using DIC. In this study, therefore, the smooth surface of the cross section of cell walls was made rugged by sputter etching; and subsequently the minute swelling distribution of cell walls of latewood tracheids was observed with confocal scanning laser microscopy and DIC.

Materials and methods

Douglas fir (*Pseudotsuga menziesii* Franco) specimens 7 mm (L) × 15 mm (R) × 7 mm (T) were prepared. After

being softened by boiling in hot water for a few minutes, their surfaces (RT) were planed by a sliding microtome. We believe that boiling had little effect because the loss in weight after softening was $1.6\% \pm 0.3\%$ (16 samples). They were dried at 60°C for 2 days and then were sputter-etched with an ion sputter (JFC-1100E; JEOL).⁵⁻⁷ Sputter-etching was performed at an ion current of 10 mA for 10 min and at 15 mA for 5 min. Swelling experiments were conducted after the specimens were conditioned in a desiccator with silica gel for 1 week. The dried specimens were observed with a confocal scanning laser microscope (1LM21H; Lasertec). The image obtained on the 1LM21H has little distortion and is suitable for strain measurements because the detector in its optics is a one-dimensional CCD image sensor.⁸ Digital images of 640 × 480 pixels were then obtained with a magnification of 7000 on a 14-inch television monitor. After observations in the dry condition the specimens were steamed by a heat-type humidifier (ML-400; Morita Denko) for 60 s on the stage of the laser microscope and then observed in the wet condition. The average moisture content of the specimens increased about 3% owing to the steaming. The digital images obtained before and after steaming were analyzed using DIC.

Results and discussion

Sputter-etching generated microcraters on the cross sections of latewood tracheids. The image of the microcraters at 10 mA for 10 min had patterns suitable for measuring the minute swelling distribution of cell walls using DIC, but the image at 15 mA for 5 min lacked contrast. For the following experiments, therefore, sputter-etching was performed at an ion current of 10 mA for 10 min. Nodes with 20-pixel intervals were set on the cross section of the observed cell wall (black meshes in Figs. 1, 2). Normal strains of each element were calculated from vectors of the nodes⁹ and were transformed into the principal strains. The image in Fig. 1 was that of the cross section of tracheids after steaming. The center triangles show the change in the lumen in

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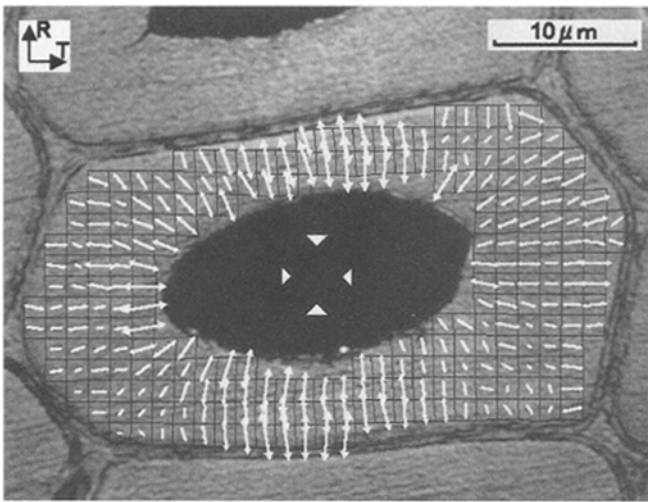


Fig. 1. Image of a latewood tracheid after steaming. Center triangles show shrinkage of the lumen. White arrows show directions of principal strain ϵ_1

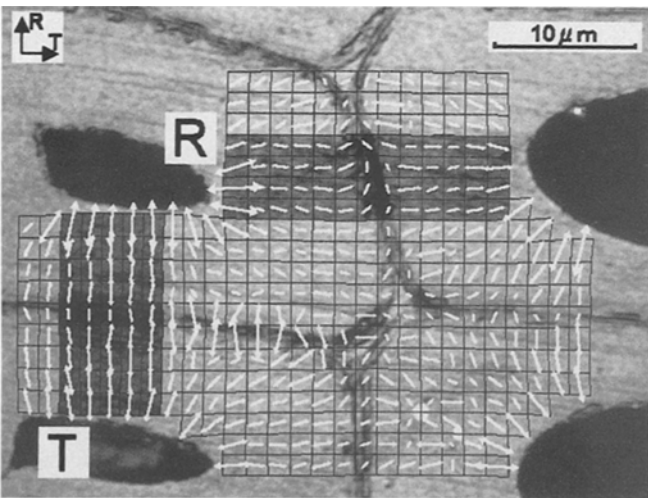


Fig. 2. Directions of principal strain ϵ_1 of four adjacent cells. T, tangential cell wall; R, radial cell wall

the radial and tangential directions, and the white arrows show the directions of the principal strain ϵ_1 on a tracheid after absorption of moisture. It was observed that the lumen shrank and the cell wall swelled mainly in the thickness direction. We think that sputter-etching did not influence the swelling behavior of cells because lumen shrinkage is the same behavior as seen in the cell without sputter-etching.⁵ Figure 2 shows the directions of the principal strain ϵ_1 of four adjacent cells. The principal strain was small in many of the elements near the compound middle lamella. Figure 3 shows the distribution of the swelling expansion along the thickness directions of tangential and radial cell walls (dark areas in Fig. 2), where swelling expansion means the radial or tangential normal strain caused by absorption of moisture. It was found that the more distant it is from the

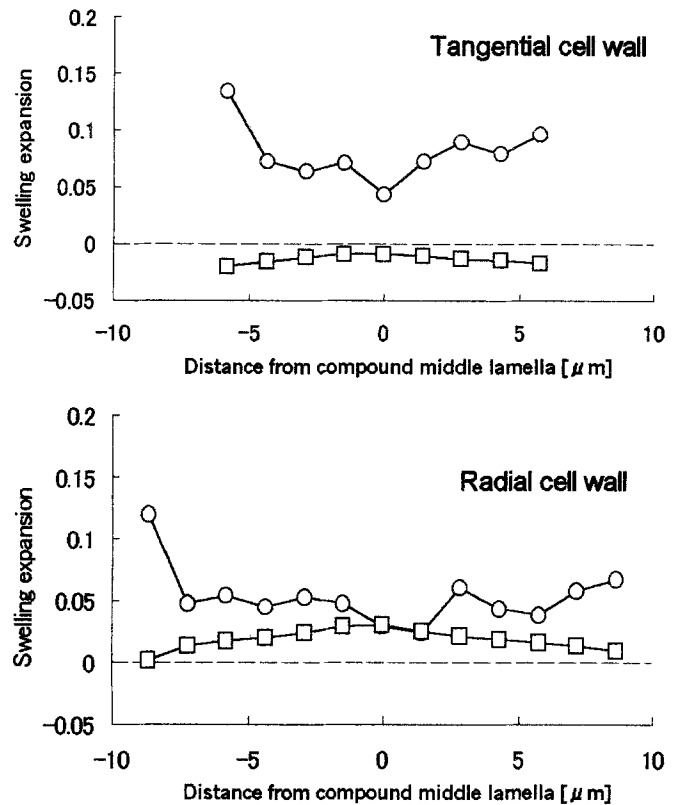


Fig. 3. Distributions of swelling expansion along thickness direction of the tangential and radial cell wall (dark areas in Fig. 2). Circles, thickness expansion; squares circumferential expansion

compound middle lamella, the greater is the expansion in the thickness direction. This phenomenon was observed in both radial and tangential cell walls. Furthermore, the difference between thickness expansion and circumferential expansion of tangential cell walls was larger than that of radial cell walls, and the tangential cell walls shrank a little in the circumferential direction, which is similar to that in the tangential direction of the specimen during absorption of moisture. It is generally said that swelling anisotropy of isolated latewood is minimal¹⁰ when observing the bulk, especially that of Douglas fir, which is 0.81 (tangential/radial).¹¹ In our microscopic observations, it was confirmed that parts of a cell wall in latewood had large swelling anisotropy.

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