Section-Splitting Method for Fibril Angle Measurement of Cell Wall Layers

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切片剥離法による細胞壁層のフィビリル傾角計測法

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Résumé

For measurement of fibril angle using the polarizing microscope, a very effective method was devised. Longitudinal wood sections are sandwiched with epoxy resin between two glass slides, and splitted with the glass slides after curring. By this very simple preparation, a series of single walls and even respective wall layers could be obtained. These splitted-sections were shown to be really suitable in measurement of both average fibril angle and individual one of various wall layers. The former was estimated by the extinction position under crossed polars, while the latter became possible at the diagonal position to be determined from many striations along microfibrils occurred during the sample preparation.

要　旨

細胞壁のフィブリル傾角は木材の物性に非常に影響するので，これまで種々の方法で測定されてきた。偏光顕微鏡法は手軽で精度の高い測定を可能とするが，この測定に必須である細胞壁のsingle wall化が困難なために，未だ充分には活用されてはいない。そこで極めて簡単な操作で，各種の木材細胞壁のsingle wallを得る方法を考案した。その概略はまず細胞直径程度の厚さの縦断切片を乾燥し，その両面に透明なエポキシ樹脂液を塗布して2枚のスライドガラスに挟み，圧締しながら硬化させる。この時，孔をあけたポリエチレンフィルムで切片を囲んでおく。この2枚のスライドを剥すと，切片に含まれていたdouble wallは両側に引き剥されてsingle wallとなる。この時，S_1とS_2との境界で剥離する場合もある。剥離面に再び樹脂液を塗布して同様の操作を繰り返していくと，細胞壁はさらに薄片化しS_1，S_2，S_3などの個々の細胞断層を単離できる。そして剥離面をカナダバルサムで封入し偏光顕微鏡観察に供する。その結果，細胞壁各部での消光位から壁の平均フィブリル傾角が容易に測定される。これに加えて，剥離の際にフィブリルに平行な条線が多数発生した。これらは背景となる細胞壁を対角位にすると明瞭に観察できるので、個々の壁層のフィブリル傾角を非常に能率的に計測することが可能となった。

1. Introduction

Cellulose microfibril orientation in cell walls has been examined by various methods, since its orientation influences wood property to a great extent. Although electron microscopic methods show clearly the orientations of wall layers of many wood cells, the methods requiring expensive equipments and complicated preparation techniques are not so popular but also inapropriate for precise evaluation of the fibril angle which should be required in the practical aspect. On the other hand, X-ray diffraction which brings an average value of the fibril angle on a wood specimen is regarded to be very effective in a direct relation of the fibril angle with the wood properties. However, it is rather difficult to evaluate the fibril angle from the obtained diffraction pattern. That is, the diffractions are occurred not only from the normal walls to X rays but also from oblique or parallel walls of wood cells. The former diffraction indicating the fibril angle will be inevitably disturbed by the latter one. On the contrary, ordinary light microscopic method with the iodine-treated section after weak delignification has been used often because of its simplicity. The fibril angle which is estimated by striations of iodine crystaline may result in the evaluation on extremely-swollen walls by the delignification and iodine deposition.

Polarizing microscopy is another effective optical method and gives the average fibril angle on the wall through which the optical ray is passed. This method, therefore, demands inevitably the single wall preparation of wood cells. Preston cut off upper-side walls of macerated wood cells which was fixed with albumen on a glass slide[1]. Page impregnated mercury into the lumen of macerated pulps and measured the fibril angle by polarizing light reflected on the mercury surface[2]. Cousins stripped surface single walls from weakly-delignified wood blocks by glue[3]. These techniques, however, have some drawbacks such as delicate adjustments of preparation conditions and mercury recovery in the Page method.

In this report, a very simple and widely-applicable preparation technique for the polarizing microscopy is, therefore, proposed, with the Cousine method being improved.

2. Materials and Methods

Radial and tangential sections of 20-60 μm thick corresponding to diameters of tracheids, wood fibers and vessels were taken from dry or wet specimen blocks of some softwoods and hardwoods by the ordinary sliding microtomy. On the other hand, transparent epoxy adhesive, glass slides of 1.2-1.5㎜ thick, crips and polyethylene thin film were prepared. The section was coated with only a small amount of adhesive on both sides and placed on a glass slide. The section was then framed by a polyehylene film from which a little wider area than the section had been cut off from a side (Fig. 1a). The section was covered by another glass slide and put in an oven, being pressed by a crip (Fig. 1b). The projected end of the section was used to adjust between the section and the framing film. After polymerization of the adhesive, the glass slides were separated off, with a razor blade drived between them. The section is desirable to be splitted under a frozen condition of wet sections for the selective-splitting at the

intercellular layer. For this purpose, the projected part was cut after the polymerization of resin, the set was soaked in water to introduced water into the section from the cut end, and then frozen. If necessary, the procedures were repeated several times to get thinner wall layers. These splitted-sections were offered to a polarizing microscope after mounting in canada balsam.

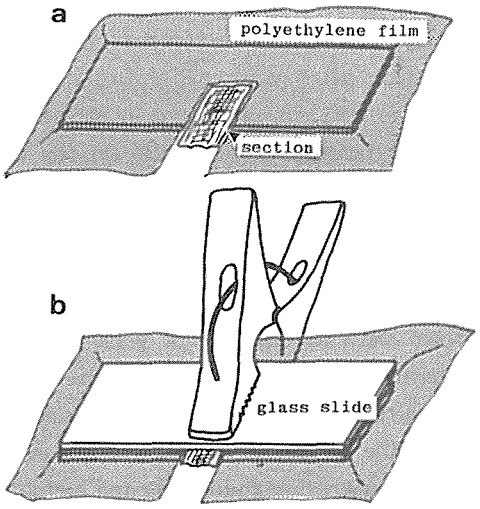

Fig. 1. Preparations for the section-splitting. Dry sections are coated with epoxy mixture, placed put on a glass slide, and framed by a trimmed polyethylene film (a). The section is covered by another glass slide, and adhered to both glass slides, with pressed by a crip. Such set was heated in an oven at 80℃ for polymerization of the resin (b).

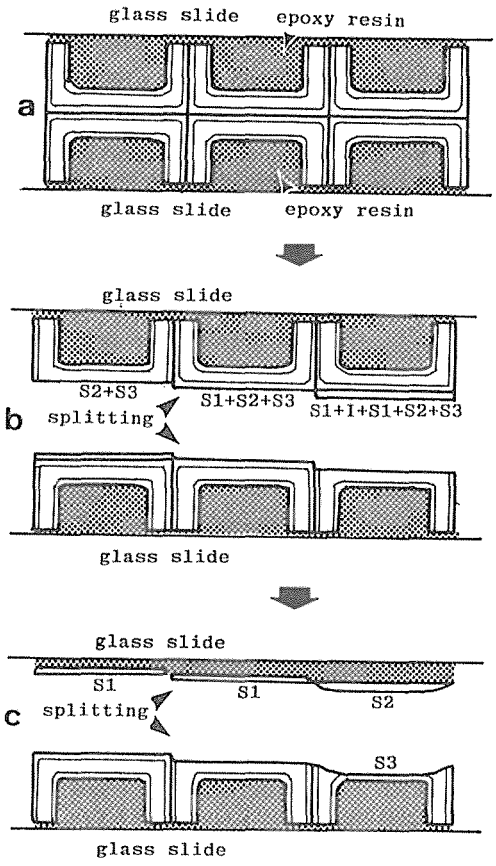

Fig. 2. Section-splitting to take single walls and cell wall layers. Original double walls contained in a section (a) are splitted mainly at intercellular layer (I) or at the transition between S_1 and S_2 by the first splitting (b). After the subsequent splittings, cell walls are fractured to respective wall layers (c).

3. Results and Discussion

When a longitudinal section which was sandwiched with glue and glass slides was splitted mechanically after the hardening of glue, original double cell walls in the section were separated off at the intercellular layer or sometimes at the transitional region between S_1 and S_2 (Fig. 2a and b). When one side of splitted-section was observed

under crossed polars, the average fibril angle of single walls or that of S_2 and S_3 can be measured by the extinction position. If anyside of the splitted-section was splitted again after covered with epoxy-resin and a glass slide, single walls could be splitted to each wall layer, succeedingly (Fig. 2c). The slides were broken sometimes, if the polyethylene frame was not used or too big sections were prepared.

As the splitting procedure described above always makes a pair of fractured walls all over the section area, the single wall or wall layers can be reverted to the original position of wood cells by comparing the paired-ones with one another. Single walls and also cell wall layers of various wood cells such as vessels and parenchyma cells (Fig. 4), needless to say about tracheids (Fig. 3) and wood fibers (Fig. 4), could be observed clearly, and the average value of fibril angle could be measured by the extinction

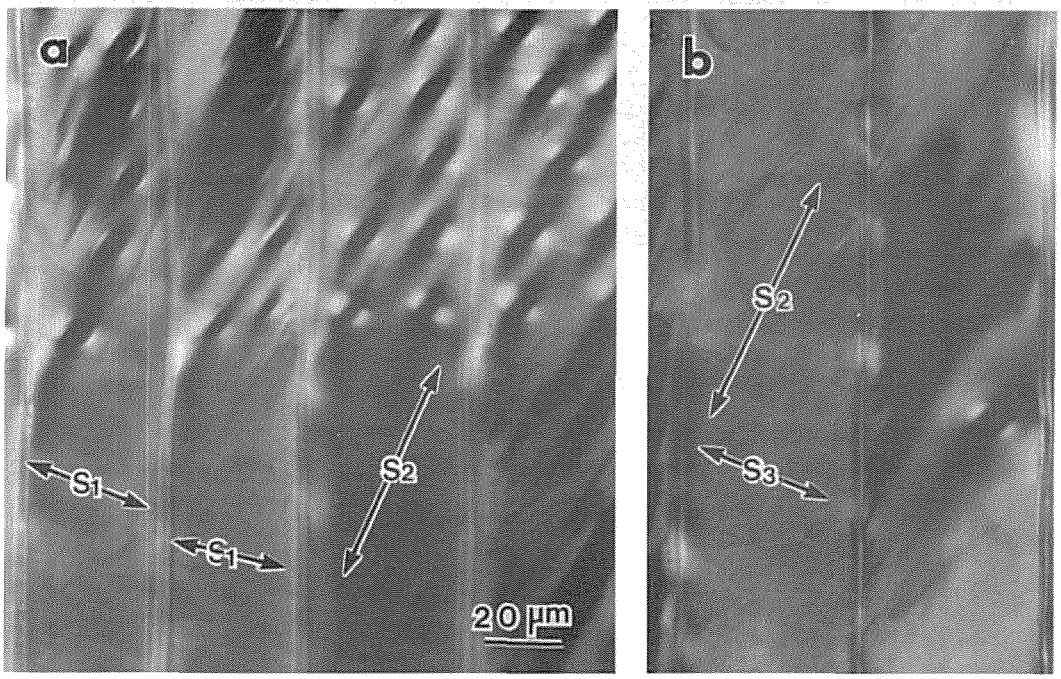

Fig. 3. Radial wall layers of HINOKI (*Chamaecyparis obtusa* Endl.) tracheids showing S_1, S_2 and a cross-field area (a), and S_2 and S_3 (b) on the three-times splitting. Fibrillar orientations on respective wall layers are indicated by fine striations under their diagonal position.

position of the respective wall parts. It is noteworthy that there were many striations or ripped-edge line, which are caused by the mechanical splitting and run parallel with microfibrils of wall lamellae, were very useful to determine the fibril angle at respective wall layers. They were clearly indicating the fibril angle, being observed even under the diagonal position (Figs. 3, 4). Therefore, both the respective and average fibril angles could be evaluated at a time on the splitted-section.

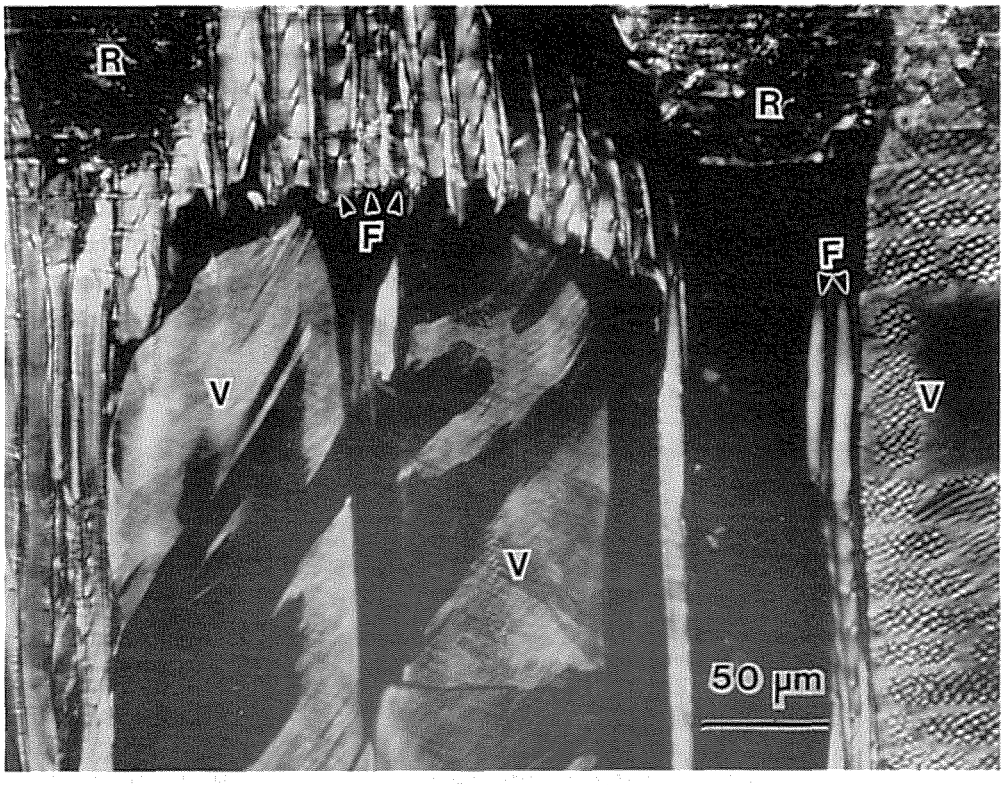

Fig. 4. Slitted-radial section of MAKANBA (*Betula maximowicziana* Regel) containing single walls of vessel elements (V), fibers (F) and ray parenchyma cells (R).

References

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