A TECHNIQUE FOR MEASURING FIBRIL ANGLE USING POLARIZED LIGHT[1]

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ABSTRACT

Polarized-light microscopy has been used to measure the fibril angle of plant cell walls. To do this the polarized light must pass through only a single cell wall of a wood fiber. A simplified method has been developed to produce microscope slides of half-fibers (fibers cut in half longitudinally) by maceration of microtomed wood sections. There are certain important variations from the standard methods in mounting the half-fibers on the slides.

Keywords: Microfibril angle, polarized-light microscopy, microscopy of wood, cell wall, wood cell wall.

INTRODUCTION

The fibril angle is a very important anatomical characteristic of wood and wood fibers. This indication of the general orientation of the cellulose molecules in the cell wall has been shown to relate to strength, shrinkage, and other physical properties of the cell. Although there have been a number of techniques used to measure fibril angle, it has never been simple. In consideration of space, the reader is referred to Prud'homme and Noah (1975), Page (1969), and Meylan (1967) for discussion of these techniques as they have been applied to study of the cell-wall structure.

One technique of measuring fibril angle that has proved useful over the years is polarized-light microscopy (Preston 1934, 1952). The use of polarized light requires that there be only a single cell-wall thickness in the path of the light. This precludes the use of microtome sections or whole fibers. Preston (1934) obtained the required single cell wall by mounting fibers on a slide and passing a blade parallel to the slide so as to cut off the upper walls of the fibers. It is not easy to obtain sufficient numbers of usable, properly cut fibers by this method.

Page (1969) developed a method of passing polarized light through a single fiber wall by reflecting the polarized light from a drop of mercury deposited in the fiber lumen. This technique requires a high pressure apparatus for impregnating the fibers with mercury. Cousin (1972) reported a method for obtaining the single cell-wall thickness by surfacing a wood block with a microtome and delignifying it such that the surface cells could be glued to a slide and the remainder of the block removed, leaving only a single layer of cut cells adhered to the slide. When

[1] The author wishes to acknowledge the assistance of Somkid Siripatanadilok in proving the workability of this technique in his research.

tried, this method was found to be difficult because the maceration step and the gluing procedure had to be very carefully controlled.

As these techniques of preparing wood for the polarized-light method of measuring fibril angle all proved difficult or inconsistent in some way, research into a simplification of procedure was undertaken. The objectives were (1) to obtain half-fibers (cells cut in half in the longitudinal direction), (2) to present either the radial or tangential wall for fibril angle measurement, and (3) to be able to measure fibril angle in a given growth ring or in different parts of a single growth ring. To meet these objectives, the following procedure has been perfected and tested over several years.

METHODS

A block of wood is prepared as for cutting sections with the sliding microtome when making microscope slides. The air in the wood is replaced with water by use of vacuum or boiling. Tangential or radial sections are cut, depending on the wall for which the fibril angle is to be measured. The section can be cut from a particular part of the growth ring if desired. The cut should be as parallel to the axis of the fibers as possible. If care is used in the subsequent steps, as few as four or five sections may be enough to give the number of half-fibers needed for analysis. The thickness of the sections should be approximately but not more than the diameter of the fibers. When well cut, the sections in a coniferous wood will consist of adjacent tracheids that have been cut in half longitudinally (half-fibers) held together by the highly lignified middle lamella as in Fig. 1. The separation of these cut cells by maceration gives the half-fibers with the single cell wall needed to make fibril angle measurements according to the procedure of Preston (1952).

The sections are placed in a small test tube with adequate amount of maceration solution for the number of sections. To macerate five to fifteen sections, 10 to 15 ml of maceration solution should do. The maceration solution used is a mixture of 44 parts of glacial acetic acid and 56 parts of 30% hydrogen peroxide. The test tube is heated in a water bath at 90–95 C until the sections are bleached white and easily separate into half-fibers when shaken gently. The time required is usually less than 12 h. The maceration solution must next be removed without losing any of the half-fibers. This is easily done by settling the half-fibers to the bottom of the test tube, using either time and gravity or a centrifuge. Next, most of the solution is removed by careful use of a bulb pipette without disturbing the fibers. The test tube is then filled with distilled water to dilute the remaining maceration solution. Two or three of these exchanges reduce the maceration chemicals to an acceptable level.

If the fibers are to be stored for two or three days, one should stop at the first wash and cork the tube. The residual maceration solution will retard growth of bacteria or fungi that could be a serious interference. The washes can be completed at a later time when it is time to make slides.

To mount the half-fibers, the water in the test tube is reduced so that the number of fibers present will give an optimum consistency for producing the desired spacing of fibers on the slide. This is an experience judgment easy to develop. The test tube is agitated to produce an even distribution of the fibers in the water. The end of a wide-mouth bulb pipet, 8-mm inside diameter, is put into

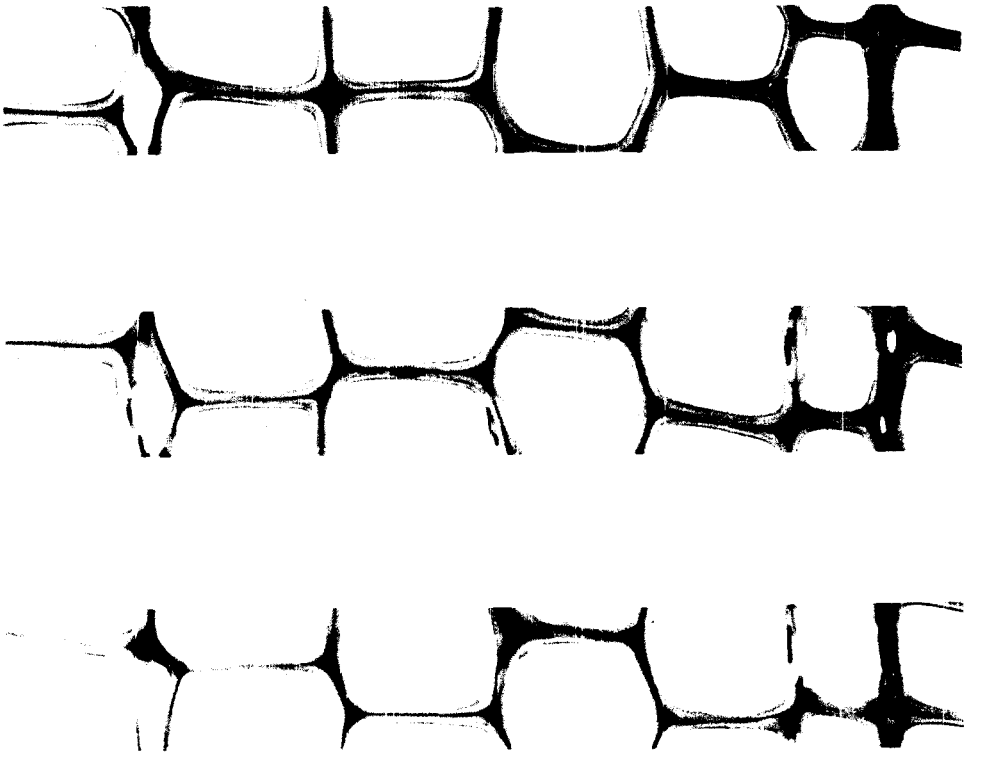

FIG. 1. Cross-sectional view of tangential sections showing cut cells that will be separated by maceration to produce the single cell-wall thickness needed for polarized-light microscopy.

the suspension and two bubbles are squeezed out. The bulb of the pipet is then released quickly to draw a random sample of fibers into the tube. The pipet is held vertically and moved to a position over the slide on the hot plate. The fiber suspension is squeezed out onto the slide, which was prepared similar to TAPPI Standard T401. We apply the fibers to the center section of the slide rather than to the ends. This is for convenience in handling.

It has been found that when some types of these half-fibers are dried on the slide on a hot plate, as in TAPPI Standard T401, they will twist or the edges will curl inward and close up because of the surface tension of the water. To prevent this, a variation from the standard procedure is used.

The fibers are allowed to settle well onto the slide, and part of the water is allowed to evaporate either on the hot plate at 80–90 C or at room temperature. The amount of water remaining should be small enough so that the fibers will not move out from under the coverglass when it is applied but large enough so that numerous bubbles will not be trapped among the fibers. The coverglass is not depressed, but is allowed to settle of its own weight. The optimum condition is when the coverglass lowers slowly by further drying so that it presses on the half-fibers. This flattens them in the open condition.

This water mount may be examined immediately with the polarizing microscope. A more permanent mount can be made by applying glycerol to one edge

of the coverglass so that it will take the place of the water, which evaporates. When the glycerol has replaced the water, the slide can be stored indefinitely if kept in a horizontal position.

DISCUSSION OF POLARIZED-LIGHT METHOD

Some points of procedure for using the polarized-light microscope to measure fibril angle may be worth a brief discussion here. With the polarizer and analyzer in the crossed position (darkest), a first order red wave plate is introduced into the beam below the analyzer at a 45° position. This gives a red field.

The half-fiber is introduced into the field and rotated clockwise through an angle where its color changes from yellow to red to blue, in that order. This indicates that the red position (matches color of red background) is the major extinction position (MEP). Then the red plate is removed and slight adjustment is made for best extinction position in black and white until the central part of the half-fiber is darkest. The angle of the rotary stage is recorded. Then the fiber axis is aligned parallel to the vertical cross hair line in the eyepiece. Again, the angle of the stage is recorded. The difference between these two readings is the fibril angle for the cell wall as measured by polarized light.

The MEP is easier to determine in black and white than in color. The color is only necessary to ascertain that it is the major and not the minor extinction position. This is necessary when working with compression wood tracheids having fibril angles greater than 45 degrees.

SUMMARY

The section-maceration method for preparing slides of half-fibers with a single cell wall has been used successfully in our laboratory when measuring fibril angles by use of polarized-light microscopy. It has been possible to measure fibril angle in a particular part of a growth ring. The fibril angle for either radial or tangential walls can be easily measured by this technique.

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