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

Lawrence Leney

Associate Professor
Wood and Fiber Science, College of Forest Resources
University of Washington, Seattle, WA 98195

(Received 21 April 1980)

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

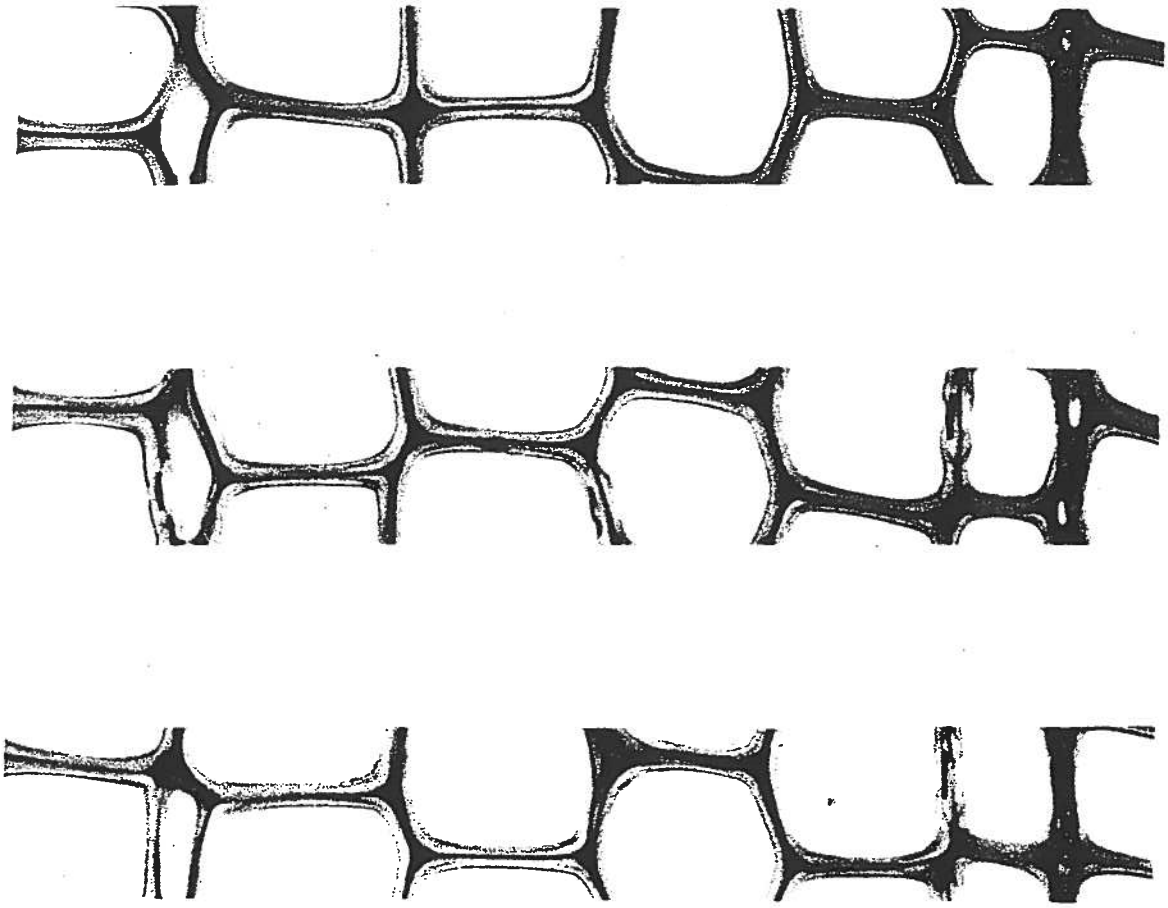

FIG. 1. Cross-sectional view of tangential sections showing cut cells that will be separated by maceration to produce the single cell-wall thickness needed for polarized-light microscopy.

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.

REFERENCES

COUSIN, W. J. 1972. Measurement of mean microfibril angles of wood tracheids. Wood Sci. Tech. 6(1972):58.

MEYLAN, B. A. 1967. Measurement of microfibril angle by X-ray diffraction. For. Prod. J. 17(5): 51–58.

PAGE, D. H. 1969. A method for determining the fibrillar angle in wood tracheids. J. Micros. 90(2):137–143.

PRESTON, R. D. 1934. Organization of the cell wall of the conifer tracheid. Royal Society, Philosoph. Trans. B 224:131–173.

———. 1952. The molecular architecture of plant cell walls. Chapman and Hall. 176 pp.

PRUD'HOMME, R. E., AND J. NOAH. 1975. Determination of fibril angle distribution in wood fibers: A comparison between the X-ray diffraction and the polarized microscope method. Wood Fiber 6(4):282–289.

GEOL 2142 - 1999

LECTURE-4

A question that remained unanswered at the end of lecture 3 was:

Why does a crystal that has an interference color go to extinction every 90 degrees of stage rotation?

The answer to that question comes from recalling that there are two priviliged vibration directions linked to the crystallographic axes of anisotropic crystals and that those vibration directions are perpendicular to one another. Furthermore, since the orientations of the vibration directions are tied to the ccrystal structure, rotating the stage, and consequently the crystal, also rotates the orientations of the vibration directions. When neither vibration direction is oriented parallel to the privilived direction of the lower polarizer, light vibrating parallel to the lower polarizer has vector components of its vibration direction parallel to each of the crystals vibration directions. Thus double refraction and consequently retardation occur as light passes through the crystal as fast and slow rays. However, if the crystal is rotated so that one of its vibration directions is oriented parallel to the priviliged direction of the lower polarizer, then the orientation of the other vibration direction is perpendicular to that of the polarizer (remember that the two vibration directions are at right angles to one another). In this case, there is no vector component of the incident light's vibration direction parallel to the vibration direction oriented perpendicular to the polarizer and the light passes through the crystal as a single ray vibrating parallel only to the crystals vibration direction oriented parallel to the polarizer. Consequently, double refraction does not occur, no retardation is produced and the crystal is extinct. Rotating the crystal 90 degrees repeats this situation, but aligns the crystal's other priviliged vibration direction with that of the polarizer and extinction occurs again. It is also important to note that when one of the priviliged directions of a crystal is oriented parallel to the polarizer's priviliged direction, all of the light passes through the crystal as a single ray and experiences only the refractive index associated with that vibration direction. This feature allows properties of the crystal, such as pleochroic color, which are associated with a specific vibration direction (refractive index) to be determined.

Knowing that one of the crystals vibration directions is oriented in a particular direction is also useful when it is necessary to make measurements relative to specific vibration directions. For example, extinction angles are generally measured relative to a specific vibration direction (*i.e.* the fast direction or the slow direction). Thus it is necessary to be able to determine which of the crystals' vibration directions corresponds to the larger refractive index (and is the "slow direction") and vice versa. The accessory plate is used to make this measurement. In order to understand how anaccessory plate works, it is necessary to know what one is. There are three types of accessory plates in common use. They are: (1) first order red plate; (2) quarter wave plate; and (3) quartz wedge. What these three types of accessory plates have in common is that they are all made from anisotropic crystals and consequently produce retardation, and they are very carefully constructed so that one of their principal vibration directions (usually the slow direction) is oriented at a 45 degree angle to the priviliged direction of the polarizer when the accessory is inserted into the light path of the microscope. In more detail, a first order red plate is constructed so that it produces 550 nm or retardation and generates the interference color called first order red. Similarly, a quarter wave plate produces 137.5 nm of retardation and generates a first order gray color. A quartz wedge, is what it sounds like, a piece of wedge shaped quartz which varies in thickness from about 0.01 mm to about 0.08 mm. As a quartz wedge is inserted into the accessory slot, it produces progressively higher retardations (depending on the thickness of the wedge in the light path) and progressively higher order interference colors. A typical quartz wedge produces interference colors from about first order gray to the top of the third order. The most common accessory plate that you will use is the first order red plate. accessory slot on a petrographic microscope is located above the sample, but below the upper polaroid. Thus, when an accessory plate is inserted with the upper polarizer in the light path, the accessory will produce retardation and the corresponding interference color. What is

important to understand is the relationship between the orientation of the sample crystal's principal vibration directions and the effect caused by inserting the accessory plate.

Like the sample crystal, the accessory plate has both fast and slow vibration directions. Unlike the sample crystal, the orientation of theses vibrations is known *i.e.*, labelled) for the accessory plate. Consider what happens when the sample crystal is rotated until the orientation of its slow vibration direction is parallel to the slow direction of the accessory plate. First, the light from the polarizer is split into fast and slow rays as it passes through the sample crystal and the slow ray becomes retarded relative to the fast ray. When the light exits the sample, it then must pass through the asccessory plate. SInce the slow vibration direction of the plate is parallel to the crystal's slow direction, the light ray that passed through the crystal as the slow ray also passes through the accessory as the slow ray. The already retarded slow ray is then further retarded by passage through the accessory plate. The total retardation produced by the sample and the accessory plate is the sum of their individual retardations (*i.e.*, the retardation of the accessory adds to that of the sample) and the order of the sample's interference color increases (by one order if a first order red plate is used). On the other hand, if the crystal is rotated so that its fast vibration direction is parallel to the slow direction of the accessory, then the light ray that passed through the crystal as the slow ray, passes through the accessory as the fast ray and vice versa. In this case, the former slow ray (sample reference frame) can catch up with the former fast ray and the effect of the accessory plate is to reduce the total retardation. The net effect of inserting the accessory plate in this orientation is to decrease the retardation by the amount produced by the accessory (*i.e.*, one order for a first order red plate). This effect is called subtraction. We will use this effect to determine the orientation of the fast and slow rays for sample crystals in this week's lab. This add/subtract behavior of the accessory plate can also be used to help determine the order of an interference color under observation since adding or subtracting an order of interference color usually permits the correct order of the color being observed to be determined.

This document: http://www.unb.ca/courses/geol2142/lec-4-99.htm