

THE USE OF PIT APERTURES AS WINDOWS TO MEASURE MICROFIBRIL ANGLE IN CHEMICAL PULP FIBERS¹

L. A. Donaldson

Scientist
Ministry of Forestry, Forest Research Institute
Private Bag, Rotorua, New Zealand

(Received March 1990)

ABSTRACT

A technique for measuring microfibril angle in chemical pulp fibers using polarized light is described. Pit apertures are used as windows so that measurements can be made on single cell walls behind the pit in the absence of pit membranes. Typical data are presented using two neutral sulphite anthraquinone pulps as examples. Problems in data analysis are discussed with respect to sample size and heterogeneity of the pulp. The technique can be used on unrefined softwood chemical pulp fibers.

Keywords: Chemical pulp fibers, pit apertures, polarized light microscopy.

INTRODUCTION

There are basically three methods for measuring microfibril angle in wood cell walls; X-ray diffraction (Cave 1966; Boyd 1977), polarized light (Preston 1934; Manwiller 1966; Page 1969; Leney 1981), and direct or indirect observation (Bailey and Vestal 1937; Cockrell 1974; Senft and Bendtsen 1985). X-ray diffraction cannot be applied to pulp fibers because of the technical difficulty in obtaining a highly oriented sample.

There are three variations on the polarized light approach. The method of Manwiller (1966) uses de Sénarmont compensation to measure the birefringence on transverse sections of fibers or wood. Although tedious, this method has the advantage of being able to measure microfibril angle in each layer of the cell wall. The method of Page (1969) involves impregnation of fibers with mercury and subsequent measurement of reflectance. The mercury acts as a mirror so that only one cell-wall thickness is measured. The method of Leney (1981) involves cutting fibers in half prior to pulping, again so that only one cell-wall thickness is observed, and the measurement of maximum extinction position with respect to the fiber axis. This technique cannot be applied to industrial pulps because of the special preparation required. In addition because fibers are cut in half, pulp properties are likely to differ from those of a pulp containing intact fibers.

Both Page and Leney's techniques are subject to error from the S1 and S3 layers of the secondary wall as discussed by El-Hosseiny and Page (1973), and Page and El-Hosseiny (1974).

The method of Cockrell (1974) involves measuring the orientation of pit apertures in latewood tracheids which follow more or less the microfibril angle of the S2 layer of the secondary wall.

¹ This project was funded by PAPRO NZ. The author gratefully acknowledges the assistance of Dr. R. P. Kibblewhite (PAPRO NZ), in providing the pulps examined and for permission to publish data.

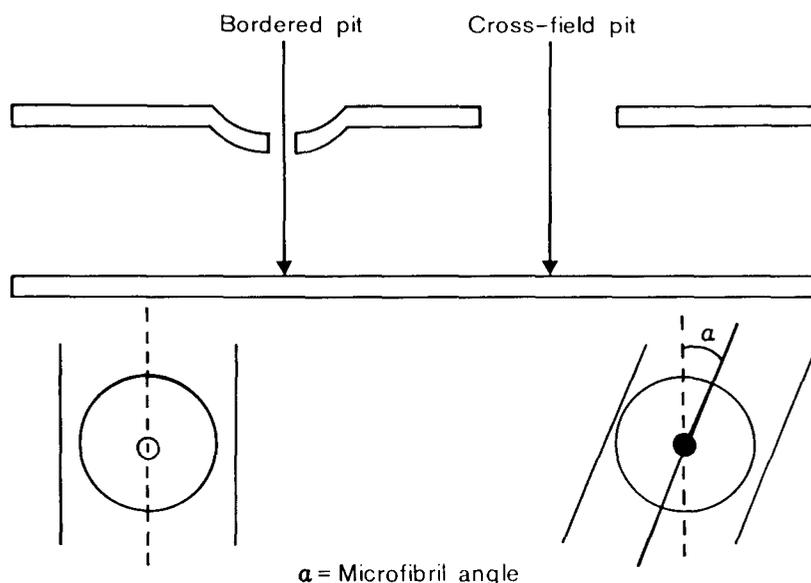


FIG. 1. A diagram illustrating the technique used to measure microfibril angle by observing single cell walls through bordered or cross-field pit apertures.

The technique of Senft and Bendtsen (1985) is a modification of I. W. Bailey's technique (Bailey and Vestal 1937). It involves inducing checking in the cell wall and precipitation of iodine crystals within the checks that follow the microfibril angle. According to Senft and Bendtsen (1985), it is possible to measure all three layers of the secondary wall by selective focussing of the microscope.

The technique described in this report was developed in order to study the influence of microfibril angle on pulp properties.

MATERIALS AND METHODS

Water-saturated neutral sulphite anthraquinone (NSAQ) pulp fibers were mounted in glycerol on microscope slides. Microfibril angle was obtained by determining the maximum extinction position using a 1st order red retardation plate as described by Leney (1981). In order to make measurements on a single cell-wall thickness, observations were made through either bordered pit apertures or cross-field pit apertures, on the wall behind the pit as shown in Fig. 1. Two southern pine NSAQ pulps were examined with five repeat measurements on each fiber, and two groups of 20 fibers were examined for each pulp. Data were analyzed by both parametric and nonparametric methods to determine the effect of deviations from normality. Estimations of the sample size required for different levels of sensitivity to differences between pulps were made based on analysis of variance.

RESULTS AND DISCUSSION

Although a number of techniques are available for estimating microfibril angle in wood, there are difficulties in applying these to pulp fibers. Such difficulties include lack of orientation if X-ray diffraction is to be used (Cave 1966). For polarized light, the problem of double cell walls must be overcome (Page 1969).

TABLE 1. *Summary of microfibril angle data.*

Pulp	Sample	Mean	Median	Range
A	1	19.83	15.48	4.28–39.28
	2	14.70	14.82	4.94–25.16
B	1	15.42	14.34	4.44–35.80
	2	23.68	25.29	3.44–43.13

This problem has been solved in the past by sectioning fibers either before (Loney 1981) or after pulping (Preston 1934), or by infiltrating fibers with mercury and measuring extinction position under incident illumination (Page 1969). By making measurements through pit apertures, it is possible to avoid the technical difficulties involved in sectioning fibers or infiltration with mercury. The problem of error due to the birefringence of the S1 and S3 layers of the secondary wall is inherent in all techniques involving optical measurements on pulp fibers (Page and El-Hosseiny 1974) except that of Manwiller (1966) where sections are used. Values reported in Table 1 do not therefore correspond exactly to the S2 microfibril angle.

Typical data for a NSAQ pulp are shown in Fig. 2 and summarized in Table 1, indicating a range of angles from 0–40 degrees. The precision (least significant difference) of each mean angle measurement based on the mean variance (1.17) of all the fiber angles shown in Fig. 2 is ± 2.0 degrees based on five repeats per fiber. Analysis of the data from both pulps is shown in Table 2.

The results of all three methods of analysis are in good agreement with both Mann-Whitney and Kolmogorov-Smirnov giving a more conservative result than conventional analysis of variance. However, the results based on analysis of variance are realistic, indicating that this technique can be applied to microfibril angle data with reasonable confidence. In cases where pulps contain high levels of compression wood leading to greater bimodality in the frequency distribution of data, it would be wise to try all three methods of analysis to avoid incorrect interpretation of results. In any case, it is recommended that a plot of frequency distribution be examined prior to analysis. The usefulness of frequency distributions in biological data analysis has recently been examined by Burke et al. (1988).

The comparison of the two samples of pulp B indicates the presence of a real difference, which suggests that the sample size is too small to give a representative sample of the population. The following procedure is recommended for determining the sample size required to give consistent results. Two random samples of 25 fibers should be measured for each pulp. If the samples are similar, they can be pooled to enable comparison with other pulps. If not, then a further 50 fibers should be measured and a comparison made. This process can be continued until results are consistent.

Another consideration in determining sample size is the level of precision required. The sample size required to give a specific level of precision can be calculated from the following formula:

$$n = \frac{2t^2s^2}{d^2} \quad \text{Steel and Torrie (1981)}$$

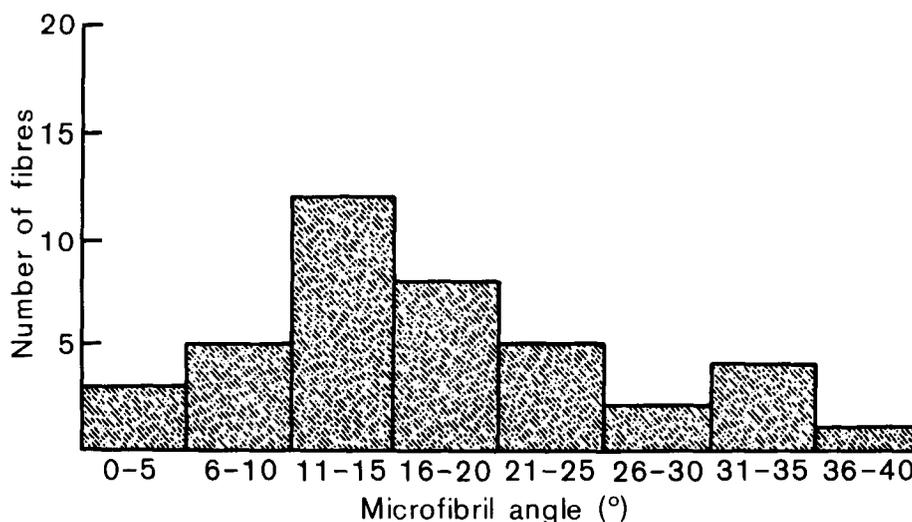


FIG. 2. Frequency distribution of microfibril angle for a NSAQ pulp based on measurement of 40 fibers.

where

- n = sample size
- t = t value at desired probability
- s = standard deviation of sample
- d = the minimum difference to be detected.

Assuming a standard deviation of 10.5 (the value for pulp B), and a difference of 5 degrees, a sample size of 40 fibers would be required. For a smaller difference of 2 degrees to be detected, 250 fibers must be measured. These values fit in well with the discussion on sample size required to give a representative sample of microfibril angle.

In the authors' experience, it should be possible to measure 50 fibers in an 8-hour day. Because of the tedious nature of the measurement procedure, it may be advisable to only spend half a day on measurements or to have two operators each working half a day.

An important factor in determining the sample size required will be the natural heterogeneity of the pulp. A pulp made from many trees and containing compression wood will naturally vary more than, for example, a pulp made from a single growth ring, and will therefore require a larger sample size.

During the present work, it was observed that microfibril angle in shives (un-separated fibers) showed very little variation among fibers, suggesting that values within a growth ring may be relatively uniform. It would therefore be an advantage to use single growth ring pulps with small confidence intervals to enable the clear detection of treatment effects.

Microfibril angle was found to be identical when comparison was made between measurements through pit apertures and on single cell walls exposed by fracture of the fiber where available. Measurements of the orientation of bordered pit apertures, where possible, also gave similar results. In rare cases, observations

TABLE 2. *Statistical analysis of microfibril angle.*

Comparison	Analysis of variance	Mann-Whitney	Kolmogorov-Smirnov
Pulp A			
Sample 1 vs. Sample 2			
20 fibers each	0.05*	0.14	0.10
Pulp B			
Sample 1 vs. Sample 2			
20 fibers each	0.02	0.06	0.05
Pulp A vs. Pulp B			
40 fibers each	0.30	0.43	>0.10

* Probability that the variation is due to random sampling error. Values less than 0.05 indicate a significant difference between the samples or pulps being compared.

made through bordered pit apertures showed partial or no extinction. It is assumed that this was due to pit membranes obscuring the underlying cell wall. Most pit membranes were destroyed by the pulping process.

Although the orientation of pit apertures can be used to determine microfibril angle, there are a number of problems in using this approach. For bordered pits, apertures can be used only in either latewood tracheids or in compression wood tracheids, thus limiting the sample to these two types of fiber. For cross-field pits, estimation of angle is often difficult and can vary with species. For example, cupressoid, piceoid, and taxodioid pits give a clear indication of angle because of the shape of the aperture, while pinoid and fenestriform pits do not. In addition, cross-field pits are relatively rare compared to bordered pits, thus making measurement even more time-consuming. Because microfibril angle may be distorted by the presence of a pit, this may also lead to biased results. By measuring through the pit onto a pit-free area of wall, such bias can be avoided.

An important factor limiting the ability to measure a particular fiber was the orientation of the fiber with respect to its tangential or radial wall. Only fibers lying on their radial wall, thus exposing the pits to view, could be measured. In practice, more time was spent searching for suitable fibers than actually making measurements. Attempts to use this technique on refined fibers were unsuccessful because such fibers lay exclusively on their tangential walls so that pit apertures were not visible. It is important to note that only the radial wall can be measured using observations through pit apertures. If any differences exist between radial and tangential walls, then they cannot be assessed by this technique.

Areas of cell wall distorted by compression failure or twisting were avoided during this study. It is assumed that fibers were in equilibrium with the mounting fluid and that changes in moisture content did not occur during the period of measurement.

The technique is applicable only to softwood fibers as hardwood fibers do not have large pit apertures through which measurements can be made.

SUMMARY

Microfibril angle of pulp fibers can be measured using polarized light by making measurements of maximum extinction position on single cell walls exposed beneath pit apertures. This eliminates the need for technically difficult procedures

such as sectioning of fibers or infiltration with mercury. The technique can be applied to unrefined softwood chemical pulp fibers and requires measurement of at least 50 fibers to detect differences of 5° or more. It is necessary to check the consistency of measurement by comparing two samples of 25 fibers from the same pulp. If agreement is poor a larger sample is required.

REFERENCES

- BAILEY, I. W., AND M. R. VESTAL. 1937. The orientation of cellulose in the secondary wall of tracheary cells. *J. Arnold Arboretum* 18(3):185-195.
- BOYD, J. D. 1977. Interpretation of X-ray diffractograms of wood for assessments of microfibril angles in fibre cell wall. *Wood Sci. Technol.* 11:93-114.
- BURKE, P. V., B. L. BULLEN, AND K. L. POFF. 1988. Frequency distribution histograms for the rapid analysis of data. *Plant Physiol.* 87:797-798.
- CAVE, I. D. 1966. Theory of X-ray measurement of microfibril angle in wood. *For. Prod. J.* 16(10):37-42.
- COCKRELL, R. A. 1974. A comparison of latewood pits, fibril orientation and shrinkage of normal and compression wood of giant sequoia. *Wood Sci. Technol.* 6:58.
- EL-HOSSEINY, F., AND D. H. PAGE. 1973. The measurement of fibril angle of wood fibers using polarised light. *Wood and Fiber* 5(3):208-214.
- LENEY, L. 1981. A technique for measuring fibril angle using polarized light. *Wood and Fiber* 13(1):13-16.
- MANWILLER, F. G. 1966. Sénarmont compensation for determining fibril angles of cell wall layers. *For. Prod. J.* 16(10):26-30.
- PAGE, D. H. 1969. A method for determining fibrillar angle in wood tracheids. *J. Roy. Microsc. Soc.* 90(2):137-143.
- , AND F. EL-HOSSEINY. 1974. The birefringence of wood pulp fibres and the thickness of the S1 and S3 layers. *Wood and Fiber* 6(3):186-192.
- PRESTON, R. D. 1934. Organisation of the cell wall of the conifer tracheid. *Royal Society, Philosophical Trans. B* 224:131-173.
- SENFT, J. F., AND B. A. BENDTSEN. 1985. Measuring microfibrillar angles using light microscopy. *Wood Fiber Sci.* 17(4):564-567.
- STEEL, R. G. D., AND J. H. TORRIE. 1981. *Principles and procedures of statistics* 2nd ed. McGraw Hill Inc., Auckland.