Variations of microfibril angle in loblolly pine: comparison of iodine crystallization and x-ray diffraction techniques

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Summary

The varied wood resource of the future will require more definitive information about the microfibril angle to improve selection and utilization. X-ray diffraction has the potential to be a much more rapid method to determine microfibril angle than is microscopic measurement. This paper discusses (I) variability and observations expected in exploratory studies when using x-ray diffraction for the first time and (2) relationship of microfibril angles estimated by x-ray diffraction to microscopically measured microfibril angles in plantation-grown loblolly pine (*Pinus taeda* L.).

Problem

Much of the future timber supply is expected to come from improved softwood and hardwood trees grown on managed plantations or from small-diameter logs removed during forest management operations, The short age-rotation resource will contain higher proportions of juvenile wood compared to the present resource. Little is known about the characteristics of small-diameter softwood thinnings or hardwoods in general.

In anticipation of this varied resource, definitive information is needed on the influence of microfibril angle on lumber properties so that selection and utilization methods can be adjusted accordingly. The microfibril angles of fast-grown wood will be substantially different from that of wood harvested in the past. The microfibril angle of the S_2 layer is a critical factor in the mechanical properties of wood (Megraw, 1986; Bendtsen and Senft 1986). In particular, the properties of composites depend on the microfibril angle of the wood fibers or particles that are used in forming them.

The microscopic technique for measuring microfibril angle is very slow and tedious. Therefore, there is little information on variation in microfibril angles in

softwoods and virtually no Information in regard to hardwoods. A much more rapid method of determining microfibril angle is needed.

Background

Early research that helped draw attention to the potential problem of plantation material was conducted on 8-year-old plantation Caribbean pine (*Pinus caribaea* Morelet) from Puerto Rico (Boone and Chudnoff, 1972). In this study of small clear specimens, the specific gravity, bending stiffness, and strength values were reduced by more than 50% from the published values for virgin timber of the same species. Not realizing that the research was based on a comparison of juvenile wood to mature wood rather than plantation-grown wood to virgin timber, scientists were surprised that the properties of plantation-grown wood were so much lower than that of virgin timber. In the study by Boone and Chudnoff (1972), differences between juvenile and mature wood were probably accentuated because the trees were very young. This study evaluated early-formed juvenile wood, which has significantly lower properties than does later-formed juvenile wood.

At about the same time, research conducted at North Carolina State University on clear wood of loblolly pine demonstrated that the problem was juvenile wood, not plantation wood (Pearson and Gilmore, 1971). This research demonstrated that juvenile wood is substantially lower in mechanical properties than mature wood, which generally accounts for the inferior properties of plantation wood compared to those of virgin timber.

Importance of Microlibril Angle

Wood cells are made up of multiple layers: a primary layer, P, and three secondary layers, S_1, S_2, and S_3. The secondary layers consist of helically arranged cellulose microfibrils oriented toward the long axis of the tracheid. The thickest of the secondary layers is the S_2 layer. Orientation of the microfibrils in the S_2 layer of juvenile wood tracheids varies widely both within and among different trees. There is a strong belief that the microfibril angle of the S_2 layer of the woody cell wall is a critical factor in the mechanical behavior of wood (Megraw, 1986). Orientation of the S_2 microfibril angle has a significant influence on tensile strength, stiffness, and shrinkage. Modeling suggests that the relative thickness of the P, S_1, and S_3 layers contributes significantly to the variability of longitudinal shrinkage (Cave, 1976).

Microfibril angle is measured as the angular deviation from the vertical cell wall of the microfrbrils in the S_2 layer. In loblolly pine (*Pinus taeda* L.), for example, the microfibril angle in mature wood is small, averaging about 5° to 10°. In juvenile wood, the microfibril angle is large; it averages 25° to 35°, is often as high as 50° in the annual rings next to the pith, and decreases outward in the juvenile core (Megraw, 1986; Bendtsen and Senft, 1986). The decrease in microfibril angle often continues well beyond the juvenile core. For example, Ying et al. (1994) found that microfibril angles in tracheids of fast-grown loblolly pines decreased from 33° at age 1 to 23° at age 10 and 17° at age 22. Bendtsen and Senft (1986) reported that microfibril angles had not yet attained stable values in 30-year-old loblolly pines.

A strong relationship also exists between microfibril angle and longitudinal shrinkage. In tests on juvenile wood of radiata pine (*Pinus radiata* D. Don). Harris and Meylan (1965) showed that longitudinal shrinkage increased sharply while tangential shrinkage decreased at microfibril angles greater than 25°. Specifically, longitudinal shrinkage was low as microfibril angles decreased below 25°, whereas tangential shrinkage increased as microfibril angles fell below 15°. Ying et al. (1994) showed that microfibril angles of 25° or greater corresponded to the outer boundary of the 10-year juvenile core in loblolly pine.

Data from Bendtsen and Senft (1986) based on a 30-year-old loblolly pine plantation, suggest that the differences in strength properties between juvenile and mature wood cannot be accounted for solely by differences in specific gravity. Their data also suggest that the volume of earlywood and the high microfibril angles of the earlywood tracheids in the early juvenile wood growth rings might be far more important. Cave and Walker (1994) concluded that microfibril angle was the only factor that alone could account for the pronounced decrease in stiffness (modulus of elasticity) of radiata pine wood. Yet, relatively few studies have related strength properties to wood characteristics other than specific gravity.

Methods of Measuring Microfibril Angle

The traditional methods for determining the microfibril angle of the S_2 layer have been based on the orientation of the cross-field pit apertures (Dadswell et al., 1959, 1960; Donaldson, 1991), the enhancement of checks or the maximum extinction position using polarizing light (Echols, 1955; Foracs, 1961), the use of fluorescence light microscopy to enhance checks (Marts, 1955). and the orientation of iodine crystals that form in induced checks (Senft and Bendtsen,

1985). Much of the current literature has been developed using these techniques. These methods are very slow and tedious.

X-ray diffraction techniques have also been used to determine microfibril angle (Cave, 1966; Harris and Meylan, 1965; Meylan, 1967). Recently, Evans and others (1996) did extensive work to refine x-ray diffraction as a much more rapid technique for measuring microfibril angles in a species of *Eucalyptus*. This technique uses the diffraction pattern created by the interaction of x-rays with wood tissue to determine microfibril angle. A group of fibers is irradiated perpendicular to the fiber length by a narrow, monochromatic x-ray beam. A diffraction pattern is produced by the crystalline cellulose structure and recorded on film or by an electronic detector. This pattern consists of a series of arcs that are spaced apart by a number of well-defined concentric circles. The diameters of the concentric circles are indications of the spacing of the crystalline planes within the cellulose crystalline fibrils. An example of a diffraction pattern and resulting 002 crystallographic plane arc intensity integration is shown in Figure 1. The angular distance from the equator to the point where the tangent at the point of inflection of the intensity curve cuts the zero intensity axis is T (Cave, 1966). The width T has been shown to be correlated to the microfibril angle (Meylan, 1967). X-ray diffraction has the potential to reduce dramatically the time required to determine microfibril angles.

In summary, studies of clear wood have produced a good understanding of the differences in the physical properties of juvenile ($\leq$15 years old) and mature ($\geq$15 years old) wood. Some anatomical causes of these differences have been identified. It is believed that microfibril angle plays a critical role. The methods for determining the microfibril angle of a specimen are very time-consuming and tedious. Improvements in technology, especially computers and two-dimensional detector arrays, have resulted in the development of a fast method for determining the microfibril angle, the x-ray diffraction technique. In this paper, we evaluate this technique and compare its results with those from the microscope-based iodine crystallization technique.

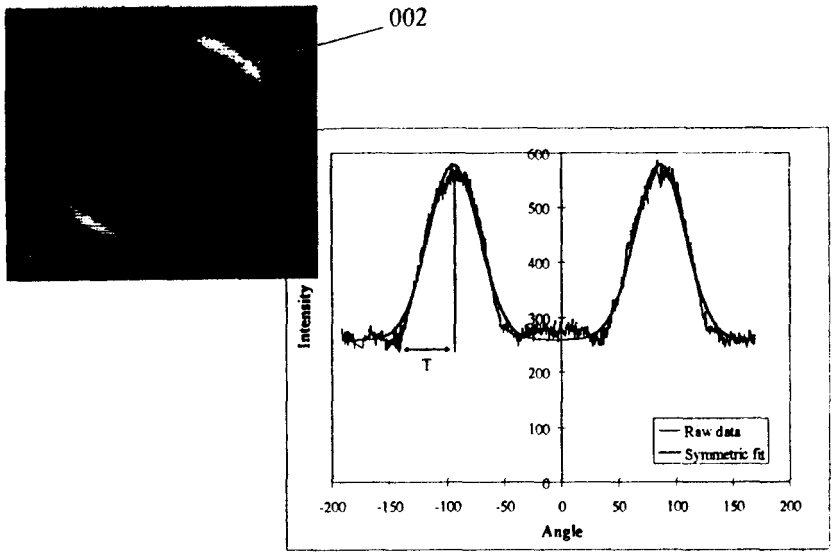


Figure 1. X-ray diffraction pattern and integration of 002 arc intensity.

Objectives

The objectives of this paper are to provide (1) information to the first-time user of x-ray diffraction about its variability and sensitivity to specimen orientation and (2) examples of the relationship between x-ray diffraction-estimated and microscopically measured microfibril angles in plantation-grown loblolly pine (*Pinus taeda* L.)

Experimental Methods

Material

Three trees from a sample of 52 trees were selected for x-ray diffraction tests. The large sample consisted of two loblolly pine trees harvested from each of 26 plantations throughout the southern United States. The large sample had already been prepared and information on a number of properties had been collected for growth rings 1 through 8 of these trees. The properties included average cell length (from a sample of about 100 cells per specimen). microfibril

angle of early- and latewood, width of early- and latewood, total growth ring width, modulus of rupture, modulus of elasticity, moisture content, and specific gravity.

The three trees were selected on the basis of previous microscopic measurements of low, medium, and high microfibril angles.

Specimen Preparation

The remains of sectioning blocks used for previous microscopic microfibril angle measurements were dissected into earlywood and latewood of individual rings. The earlywood and latewood rings varied in thickness. The resulting specimens were rectangular, approximately 2 to 5 mm thick, and 2 to 8 mm wide. The specimens were placed in specially prepared mounting brackets and attached to the equipment to determine diffraction patterns.

X-Ray Measurements

A Siemens x-ray scattering system (Kristallolflex 710D x-ray generator, two-dimensional Siemens HI-STAR area detector system, Siemens general area detector diffraction software (GADDS) version 3.310 for Windows NT (Siemens Industrial Automation, 1995))' was used to collect x-ray diffraction data (Fig. 2). Each wood sample was mounted on the goniometer (motorized three-axis positioning head) with a sample clip holder that held the wood sample perpendicular to the incident x-ray beam, which passed through the radial or tangential face near the center of the specimen. The radiation source was a CuKα (λ = 1.54 A), 40 kV, 20-mA, 0.5- or 0.8-mm aperture incident beam, with an exposure time of 60 s.

The diffracted x-ray beams passed through a beryllium window of the area detector into a chamber that held xenon gas under 4 bars of pressure. The xenon atoms were ionized by incident x-rays, producing charged particles as the x-rays passed through the gas. These charged particles were then electrically attracted to a multi-wire electrode assembly in the detector. An electrical signal was then generated, indicating the original x,y position of the x-ray on the detector.

[1] **The use of trade or firm names in this publication is for reader information and does not imply endorsement by the U.S. Department of Agriculture of any product or service.**

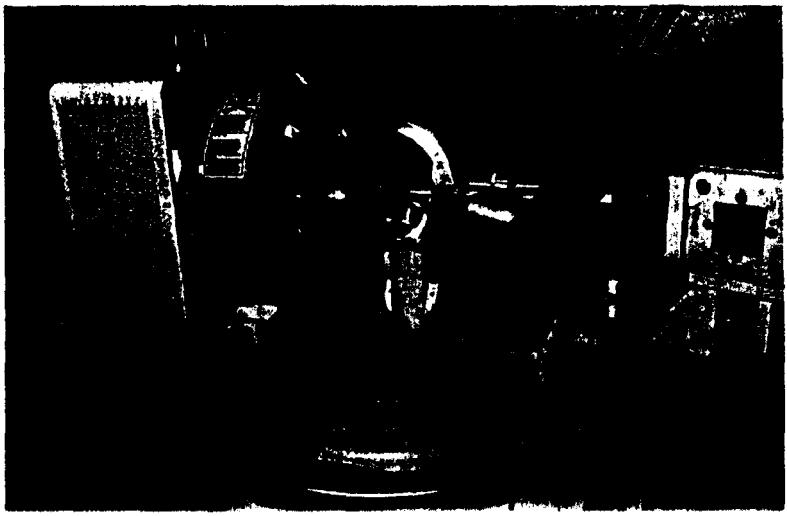


Figure 2. Testing equipment setup.

Calculation of *T* by Curvefitting

The intensity of the complete two-dimensional diffractogram was recorded digitally and integrated over an azimuthal angle with 0.1° resolution over the 002 crystal plane arcs of the diffractogram. A nonlinear least-squares routine was used to fit the angle-intensity data to a pair of Gaussian curves. Two models were tit to the data.

The first model was

$$y = a + b_1 \cdot \exp\left[\frac{-(x-\mu)^2}{2\sigma_1^2}\right] + b_2 \cdot \exp\left[\frac{-(x-\mu-180)^2}{2\sigma_2^2}\right]$$

where

a is	constant background,
μ, $\mu + 180$	centers of first and second peaks, respectively,
σ_1, σ_2	half-widths at inflection points, and
b_1, b_2	heights of curves above constant background.

The second model forced $b_1 = b_2$ and $\sigma_1 = \sigma_2$. Cave's (1966) *T* value was estimated as $\sigma_1 + \sigma_2$ for the first model and 2σ for the second (Stuart and Evans, 1994).

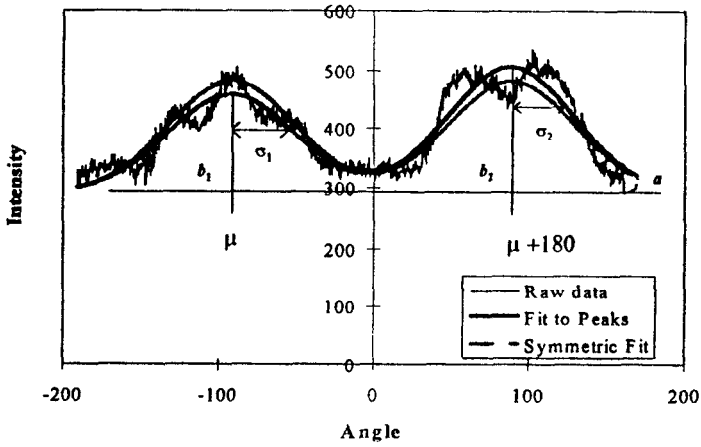


Figure 3. Curve tits to raw data.

Plots of both models are given in Figure 3. The results showed that the *T* estimates produced by the two models do not differ significantly. Other potentially more accurate modeling methods that estimate the width of arc intensity (that is, establish parameter *Q* or *W* that is not dependent on zero intensity) are being developed (Evans and others, 1996). It has been suggested that multiple Gaussians and methods independent of the intensity base may be more effective estimators.

We analyzed the results from the second model. Microfibril angle values were estimated from the Meylan's (1967) equation $\text{MFA} = 0.6T$ where MFA is microfibril angle.

Test Procedure

Potential sources of x-ray measurement variability were investigated in the first part of the study. In the second part, x-ray microfibril angle measurements were compared with measurements obtained from a microscope-based iodine crystallization technique. All specimens were taken from the three trees associated with high, medium, and low microfibril angles: trees 3a, 10a, and 12b, respectively.

In the tables, the code used to identify the specimens indicates tree number, growth ring number, type of wood (latewood or earlywood), and orientation

(radial or tangential). For example, 10ar8lr denotes tree 10a, ring 8, latewood, and radial orientation.

Results and Discussion

Instrument Variability

Instrument variability was investigated by scanning a specimen multiple times. The specimen was left in place between runs. The variability is a measure of the uncertainty that is inherent in the x-ray instrument and is the variability typically reported by an instrument manufacturer. The coefficient of variation (COV) associated with this uncertainty was approximately 2% (Table 1).

Orientation Variability

Tangential versus radial face---This portion of the study focused on the 18 specimens (three trees; earlywood and latewood; rings 3, 7, and 8) listed in Table 2. Measurements were taken on both the tangential and radial faces. The results are listed in Table 2 and plotted in Figure 4.

For tree 3a, which had higher angles, there was no clear difference between tangential and radial measurements. For trees 10a and 12b, tangential face measurements were greater than radial face measurements. Also, the difference between tangential and radial measurements tended to be larger for latewood than for earlywood. The remainder of this report is based on data from radial measurements.

Radial face orientation-We expected that the integration of the 002 plane intensity pattern would be symmetric. However, the first series of scans resulted in a number of asymmetric patterns (Fig. 5).

The orientation of the sample was critical to the symmetry of the intensity scan that was integrated. The rotation of a specimen about the z-axis (Fig. 6) could change the symmetry of the corresponding intensity scan (Fig. 7). As a result, a second set of measurements *was* made on trees 3a, 10a, and 12b using the five-position orientation procedure (described below) to determine if symmetrization had a substantial effect. This portion of the study focused on the 11 specimens listed in Table 3.

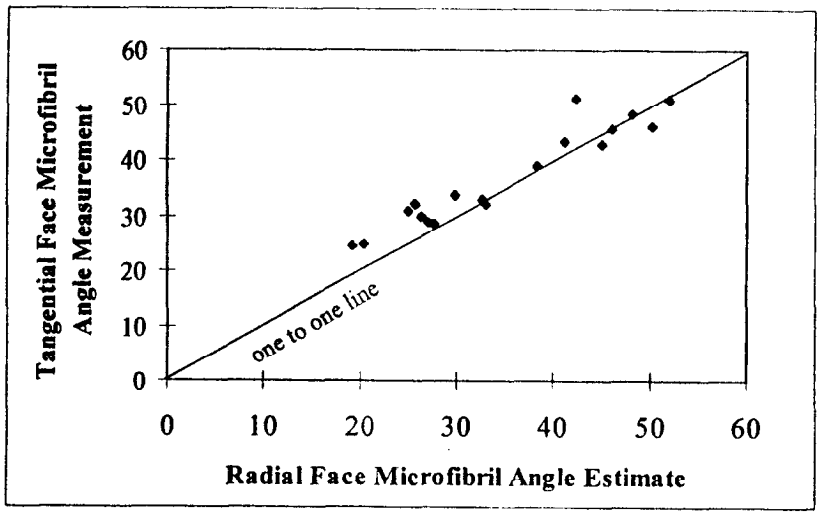


Figure 4. Radial face vs tangential face microfibril estimates and 1:1 reference line.

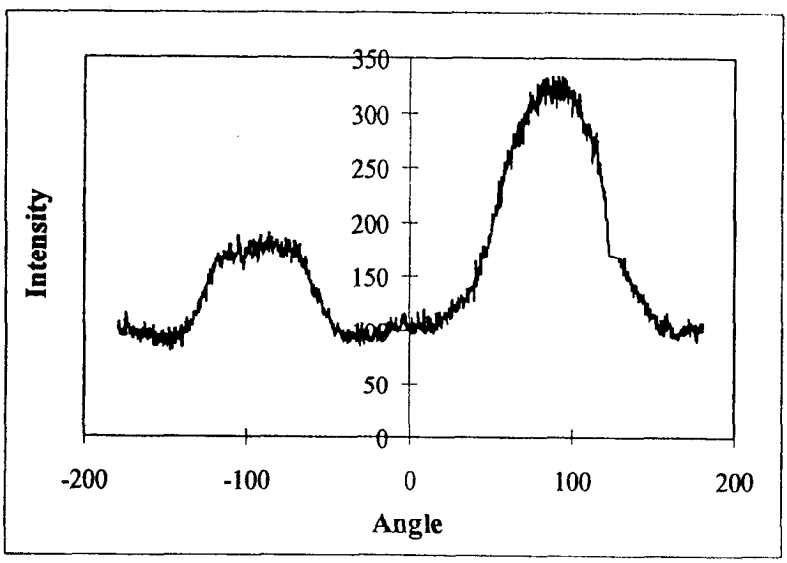


Figure 5. Integration of 002 arc showing asymmetric results.

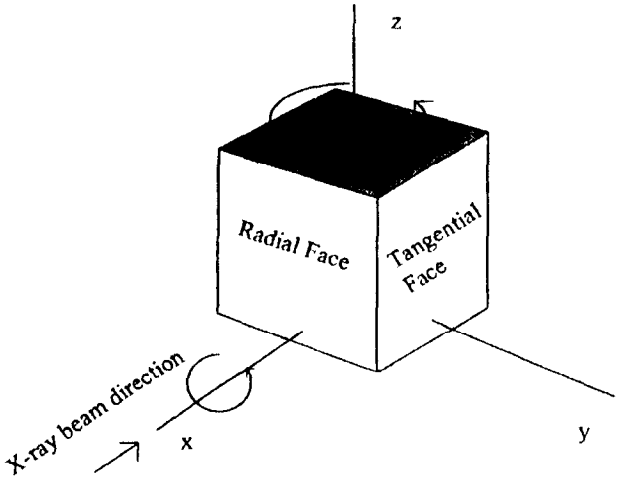
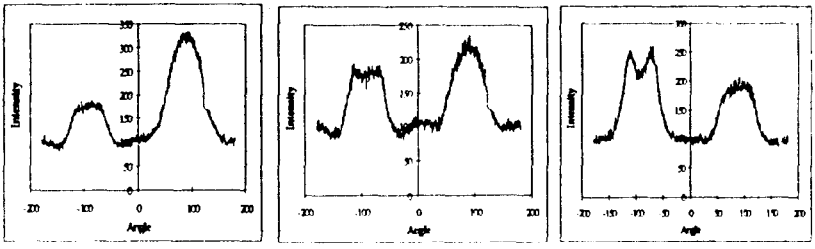


Figure 6. Sample rotation definitions.



a b c

Figure 7. Effect of specimen rotation about longitudinal axis on integration of 002 arc intensity: (a) 10°, (b) 0, and (c) 10°.

We suspected that the symmetrized peak measurements might be less variable than the non-symmetrized peak measurements. This did not appear to be the case (see Table 3). When the estimated microfibril angle for the symmetric peaks was large (>40°), the variability of the symmetrized data was greater than that of the non-symmetrized data. When the estimated angle was lower, the non-symmetrized data tended to have greater variability. Also, the mean microfibril

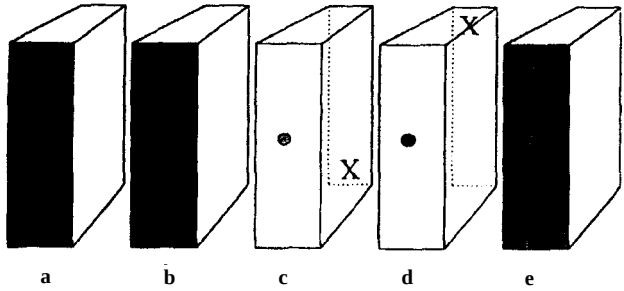


Figure 8. Sample positions examined to determine variability resulting from specimen positioning. X shows change in orientation of each specimen.

angle estimates calculated from the symmetrized peaks were uniformly larger than those calculated from the non-symmetrized peaks.

Specimen positioning–The specimens were systematically oriented in the beam path. Five readings were taken for each specimen, as shown in Figure 8.

The averages, standard deviations, and COVs based on specimen positions (a to d) are reported in Table 3. These results, together with the results reported in Table 1, indicate that the expected COV associated with specimen positioning was about 6%. These results are comparable to the average COV of 5.5% that was observed with the microscopic measurements for more than 830 groups of 10 readings each taken previously from the large sample of 52 trees.

Effect of Calibration

To determine the bias introduced by calibration of detector distance and field warping, tests were conducted on eight specimens following three different calibrations. Calibration was shown to have a minor impact on the values that resulted from x-ray diffraction (Table 4). The observed change in the estimated value for microfibril angle was primarily due to the observed 6% to 7% difference that resulted from specimen positioning. A 1% to 2% change in microfibril angle might have been due to calibration.

Scan Characteristics

A wide variety of scans was observed; a sample is shown in Figure 9. Most scans of tree 3a (high microfibril angle) and the rings closest to the pith (rings 1 to 3) of the other trees looked like Figure 9a and 9b, with a clear superposition of

multiple peaks from front, back, and side walls. Quite a few of the rings closest to the pith also showed superimposed peaks (Fig. 9c and 9d). The scans of most rings further from the pith (rings 6 to 8) looked more like Figure 9e and 9f.

Obviously, the data in Figure 9a-d do not follow either of the paired Gaussian models that were discussed earlier in this paper. This does not preclude fitting these models and obtaining estimates of microfibril angle. The resulting estimates may still correlate reasonably well with those obtained via microscopic techniques. In fact, this was the case in our study. However, a poor fit sometimes leads the nonlinear least-squares routine to produce unreasonable estimates of the parameters (the tip of a peak with a lower *a* value and higher *b* and σ values can roughly match a peak with a higher a value and lower *b* and σ values). This can have a great effect on the estimate of microfibril angle. Consequently, if x-ray diffraction is to be used further on young Southern Pine samples, it will be necessary to use improved models to tit data such as that displayed in Figure 9a to 9d.

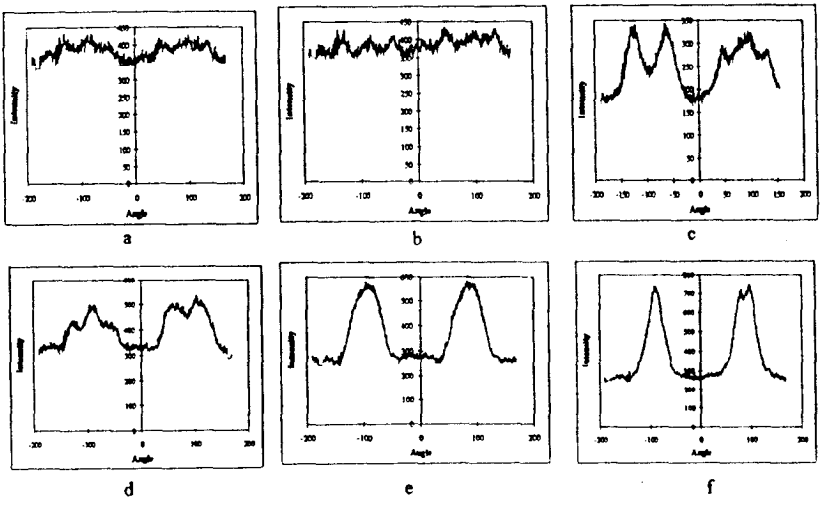


Figure 9. Different types of integration of 002 arc.

Comparison of X-Ray Diffraction and Microscopic Techniques

To compare x-ray diffraction and microscopic techniques for measuring microfibril angle, individual microfibril angle measurements were taken on earlywood and latewood samples from rings 1 to 8 of the three test trees. Our previous trials of radial and tangential face exposures indicated little difference between predicted microfibril angles. Therefore, the radial faces (the most likely scan orientation in a commercial device) of these samples were exposed.

The relationship between the estimated x-ray diffraction microfibril angle (based on MFA = 0.6*T* symmetrized x-ray data) and the microscopically measured microfibril angle is shown in Figure 10. The correlation between the two sets of measurements was 0.8.

For these Southern Pine data, a regression of the iodine crystallization microfibril angle measurements on the estimated x-ray diffraction microfibril angle yields the equation $14.5 + 0.25T$.

Concluding Remarks

In summary, the results of this study on the use of x-ray diffraction to measure microfibril angle were as follows:

- Instrument precision was approximately 2%.
- For the instrument and specimen positioning, the combined coefficient of variation (COV) was approximately 6% to 7%.
- For equipment recalibration, the associated COV was approximately 1% to 2%.
- X-ray diffraction and iodine crystallization had similar COVs.
- Results of x-ray diffraction measurements correlated reasonably well with iodine crystallization results.

Higher-angle material produced more variable results, noisier signals, and more difficulty in curvefitting compared to lower-angle material.

Future Work

The results of this initial study are promising. As a follow up, we intend to scan the remaining material from the 26 different Southern Pine plantations. Other species will also be investigated. Further work will also address the improvement of theoretical models, methods of calibration, and sample preparation.

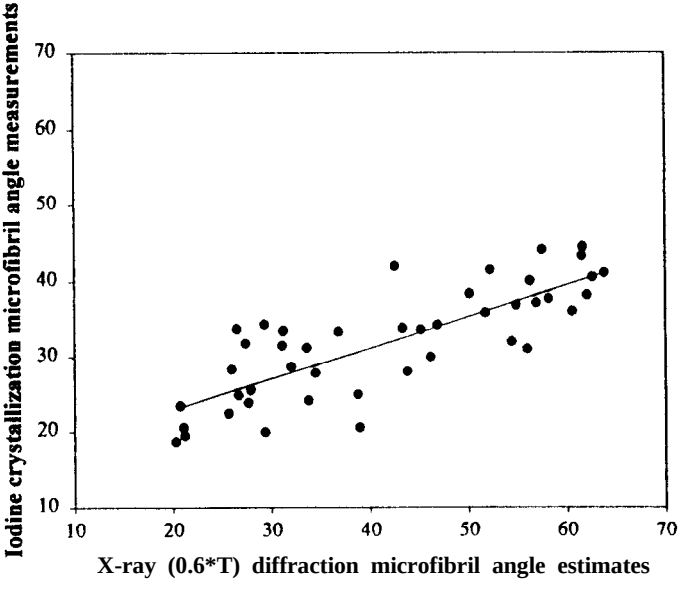


Figure 10. Comparison of MFA measurements by iodine crystallization and x-ray diffraction. For these Southern Pine data, regression of iodine crystallization measurements on estimated x-ray diffraction MFA yielded 14.5 + 0.25*T*.

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Table 1. Results of instrument variability scans

Tree[a]	Estimated MFA[b]	COV (%)
10ar8lr	25.93	2.0
10ar8lr1	25.02	
10ar8lr2	25.07	
10ar8lt	30.16	0.4
10ar8lt2	30.42	
10ar8lt3	30.29	
10ar7lrW	29.38	2.0
10ar7lrX	29.35	
10ar7lrY	28.42	
10ar7lrZ	28.28	
12br8lrX	21.20	1.7
12br8lrY	21.93	
12br8lrZ	21.71	

[a]Identification code: tree number, growth ring number, type of wood (latewood or earlywood), and orientation (radial or tangential); 10ar8lr denotes tree 10a, ring 8, latewood, and radial orientation.

[b]MFA is microfibril angle.

Table 2. Comparison of radial and tangential microfibril angle measurements

Tree	Mean MFA		Tangential-radial
	Radial	Tangential	
03ar3e	45.0	42.8	-2.2
03ar3l	48.1	48.4	+0.3
03ar7e	50.2	46.1	-4.1
03ar7l	42.4	51.3	+8.9
03ar8e	46.1	45.6	-0.5
03ar8l	52.0	50.9	-1.1
10ar3e	38.3	39.1	+0.8
10ar3l	41.2	43.3	+2.1
10ar7e	32.6	33.1	+0.5
10ar7l	25.7	32.2	+6.5
10ar8e	27.0	29.1	+2.1
10ar8l	24.9.	30.9	+6.0
12br3e	29.8	33.8	+4.0
12br3l	32.9	32.4	-0.5
12br7e	26.3	30.1	+3.8
12br7l	20.4	25.0	+4.6
12br8e	27.6	28.6	+1.0
12br8l	19.2	24.6	+5.4

Table 3. Results of symmetric vs asymmetric scans

Tree	Mean MFA		Standard deviation		COV (%)	
	asym	sym	asym	sym	asym	sym
03ar3er	45.4	64.1	3.82	5.27	8.4	8.2
03ar3lr	47.6	60.1	2.99	4.40	6.3	7.3
03ar8lr	52.5	56.6	1.82	3.54	3.5	6.3
10ar3er	38.5	47.4	1.50	8.98	3.9	18.9
10ar3lr	41.5	50.6	2.33	3.18	5.6	6.3
10ar8er	27.0	28.8	0.78	0.37	2.9	1.3
10ar8lr	24.7	25.8	1.36	0.87	5.5	3.4
12br3er	29.8	31.2	1.39	1.54	4.7	4.9
12br3lr	33.2	38.7	2.99	2.74	9.0	7.1
12br8er	27.3	29.3	2.35	0.43	8.6	1.5
12br8lr	18.9	21.0	0.94	1.14	5.0	5.4

Table 4. Effect of calibration

	Microfibril angle		
Tree	Calibration 1	Calibration 2	Calibration 3
10ar7er	32.6	34.9	32.6
10ar7et	33.1	35.5	33.7
10ar8er	27.0	28.8	29.4
10ar8et	29.1	30.6	30.4
12br7er	26.3	26.1	28.8
12br7et	30.0	29.9	29.9
12br8er	27.3	29.3	28.2
12br8et	28.6	28.8	29.9
Average	29.3	30.5	30.4

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