Comparison of methods to measure fibril angle in wood fibres

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Fibril angle is a key determinant of fibre mechanical properties. Two of the most useful techniques for measuring fibril angle are X-ray diffractometry (XRD), for solid wood, and confocal microscopy, for individual fibres or small groups of fibres. This paper compares fibril angles determined using XRD and confocal microscopy for one spruce and two radiata pine samples. Good agreement was obtained between the fibril angles given by the two methods.

Keywords

Fibril angle, X-ray diffraction, confocal microscopy

Cellulose polymer molecules in wood fibres are aligned in long, highly crystalline microfibrils wound helically around the lumen. The S2 layer of the fibre wall contains as much as 90% of the fibre mass, and therefore largely determines the fibre properties. The pitch of the microfibrillar helix in the S2 layer defines the microfibril angle. Theoretical and experimental work *(1-3)* has shown that while the elastic modulus and strength of fibres are approximately constant at very low angles, they fall rapidly for fibril angles beyond 5 to 10°. At fibril angles of 40° and above the tensile strength is reduced to about a third *(1)* and the stiffness is reduced to about a fifth *(2)* compared to fibres with fibril angles between 0 and 10°.

There is a wide variation in the properties of fibres from different parts of a tree and between different species of trees. During the first few years of growth, trees produce juvenile fibres with large microfibril angle (up to 45°), and the wood has low stiffness but is flexible and tough. By the age of 10 years, microfibril angle may fall below 10° (the variation is species dependant) and the fibres are stronger and much stiffer. Fibres produced after this age near the base of the tree are generally considered to be mature. The differing mechanical properties of juvenile and mature wood raise the possibility of assigning wood from different parts of the tree to different grades of paper. For example, high strength and stiffness are desirable properties for the corrugated box liner but are not as critical for the end performance of photocopy paper.

Ideally, to assess the potential usefulness of wood from different parts of the tree (or from different species) we would directly measure the distributions of mechanical properties of the single fibres. However, performing sufficient single fibre measurements remains far too time consuming to be used routinely. Thus it is essential to have fast and accurate methods for measuring the fibril angle, as this is the single factor that most strongly determines the strength and elastic modulus of the fibres.

As the fibrils in the S2 layer are tightly packed, and the S2 layer itself is not exposed, it is not possible to observe directly the fibril angle except in a few special cases (e.g. softwood compression wood). A number of different techniques have been tried to overcome this problem. These include etching and iodine staining to reveal the fibrils; phase contrast microscopy under near ultraviolet illumination *(4)*; and the observation of cracks and pits in the fibre wall. The most common methods fall into the categories of polarised microscopy and X-ray diffraction methods. Comparison of results obtained by some of these methods has been made by Meylan *(5)* and Crosby and Mark *(4)*.

Polarised microscopy techniques

Polarised microscopy techniques take advantage of the natural birefringence of cellulose. In order to measure the fibril angle by polarised microscopy it has been necessary to isolate a single cell wall since, if light passes through opposite cell walls, the effect on the light polarisation from the first cell wall is cancelled out as the light passes through the second. This isolation of a single cell wall has been achieved either mechanically, by slicing the fibre along its length *(6)*, or by impregnating the fibre lumen with mercury *(7)*. The sample is then illuminated with polarised light and the reflected (mercury impregnation) or transmitted (single fibre wall) light is passed through an analyser crossed with the polarisation direction of the incident light. The fibre is rotated until maximum extinction occurs. The angle between the fibre axis at maximum extinction and the polarisation axis of the polariser is taken as the fibril angle.

A variation on this technique that does not require isolation of a single fibre wall has been published *(8,9)*. The fibre is placed with its axis at 45° to the polarisation of the incident light. The intensity of light transmitted through an analyser is then measured, as the analyser is rotated, as a function of the wavelength of the incident light. From an optical analysis of the system it is then possible to estimate the fibril angle. Other techniques have been published which use fluorescent dyes *(10)* and micro-Raman spectroscopy *(11)*.

Recently a technique has been published that uses the depth-resolution capabilities of a confocal microscope to optically isolate one cell wall from the other, thus allowing a crossed polarisation experiment to be conducted without extensive sample preparation *(12)*. The technique is rapid and can be used on single fibres and solid wood samples. We employed it to obtain the confocal estimates of fibril angle presented in this report.

X-ray diffraction techniques

X-ray diffractometry has been used to estimate microfibril angle (MFA) in cellulosic fibres for over 65 years *(13)*. Both the (002) and (040) cellulose I reflections have been used, each with its advantages. Although the (040) reflection

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can be used to estimate the microfibril orientation distribution directly *(14,15)*, the (002) reflection has considerably greater intensity and, by application of a suitable model, is more suited to rapid analysis *(16)*. Meylan and Cave *(5,17)* published a method and theory for the calculation of microfibril angle from the width of the (002) azimuthal diffraction arcs. The method has been widely used because of its simplicity and theoretical soundness.

More recently a method has been developed to allow the rapid scanning of large numbers of increment core samples for forest assessment and tree improvement programs *(18,19)*. The method is based on a relationship between the variance of the (002) azimuthal diffraction profile and microfibril angle, and was used to obtain the X-ray diffractometric estimates of MFA in this report.

As it seems likely that XRD and confocal measurements will be the main techniques used for microfibril angle measurements, it is the aim of this paper to compare results obtained from the two techniques.

EXPERIMENTAL METHOD

Silviscan measurements - XRD

Wood samples were prepared from increment cores by cutting to 2mm in the tangential direction and 7mm in the longitudinal direction. After extraction in acetone, they were reconditioned at 40% RH and 20°C. A copper rotating anode in point focus mode was used in conjunction with a nickel filter and a capillary focussing system to produce an X-ray beam cross-section of diameter 0.2 mm at the sample. The diffraction patterns were recorded with a CCD area detector. The samples were examined at 0.2 mm intervals with the X-ray beam in the tangential direction. Growth ring orientation had previously been measured by automated image analysis *(20)* and this information was used by the control software to maintain the growth rings parallel to the X-ray beam.

Three samples were selected for comparison of the two techniques; Norwegian spruce, a conventional radiata pine core sample and radiata pine compression wood. The two radiata pine samples were selected to give as wide a variation as possible in the fibril angle.

Confocal measurements on radiata pine samples

After X-ray diffraction analysis, the two radiata pine samples were wrapped in fine wire mesh to hold them together, then delignified by conventional kraft pulping. Six small samples were taken from various sections of the delignified wood. Each sample was separately bleached with ClO_2 for three hours at 70°C before being dispersed in water, to separate the fibres, and then dried. Between 15 and 20 fibres from each sample were randomly selected and mounted between stainless steel tabs using an epoxy glue that was allowed to set over three days. The fibril angles of the mounted fibres were then determined using a confocal microscope with an analyser crossed with the polarisation of the incident laser light. Details of the technique are given in *(12)*.

Confocal measurements on the spruce sample

For the spruce sample, measurements were made not on single fibres, but on the wood. It was found that fibril angle measurements could not be successfully made on the milled sample used for X-ray diffraction analysis, as the milling process tended to smear the walls of neighbouring fibres across each other. However, once the sample had been carefully split down the middle, fracture surfaces with largely intact fibres protruding from the surface were created. Fibril angle measurements were then made at eleven different positions along the radial direction. At each position, the measured value was an average of several fibres that were in the field of view.

RESULTS

Figure 1 shows intensity as a function of sample angle for a radiata pine fibre, together with a fit of the data with the $\sin^2$ function. The 0° angular position is the point where the fibre axis is aligned with the direction of polarisation of either the incident laser light or the analyser. It can be seen that the data are well described by the fitted curve with an R^2 value for the fit of 0.97. The fibril angle (5°) is determined from the fit and corresponds to the position of the first minimum after 0°.

X-ray diffractometry yields the azimuthal variance (peak broadening) of the cellulose I (002) peak, which is then related to the fibril angle. Figure 2 shows the X-ray diffraction measurement on the spruce sample. Firstly, it can be seen that there is a general trend of increasing fibril angle running from the bark to the pith and therefore from mature to juvenile wood. However, the variance does not increase smoothly from bark to pith.

A pattern of peaks, associated with annual growth rings, is superimposed on the trend.

The general relationship between the measured variance and the fibril angle is given in Equation 1 *(19)*.

$$S^2 = \frac{1}{2}\mu^2 + \sigma^2 \qquad [1]$$

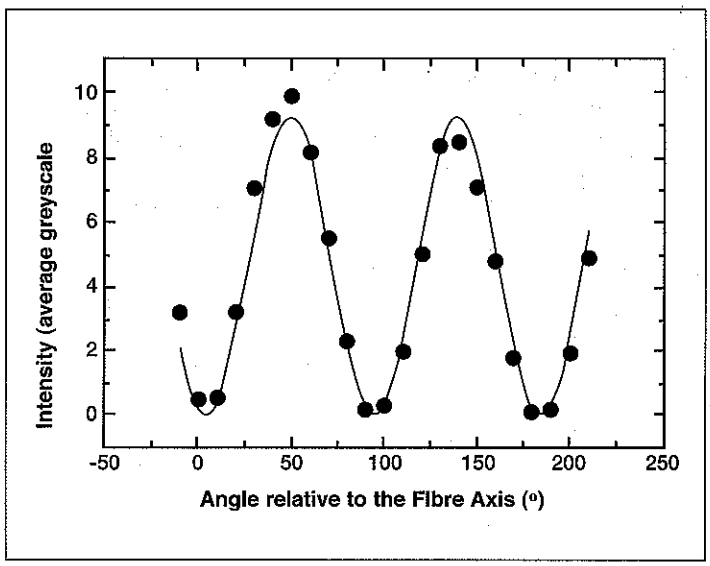

Fig. 1 Intensity data and fit for radiata pine. Fitted equation: $I = 9\sin^2[2(\theta-5)]$ where I is the intensity and θ is the angle in degrees.

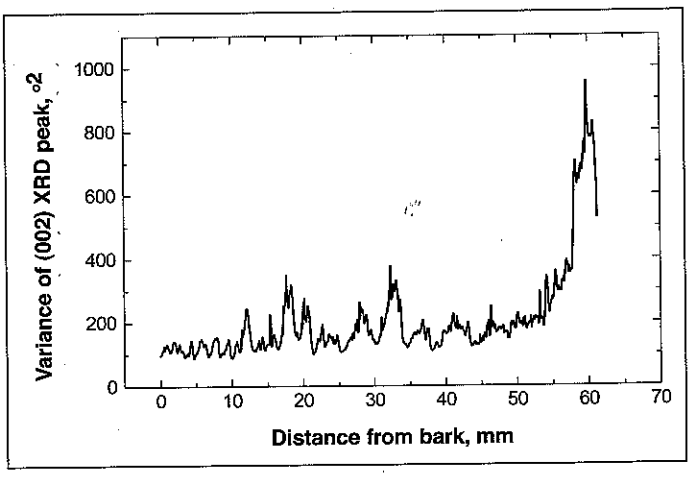

Fig. 2 Variance of the (002) XRD peak with position for a spruce sample.

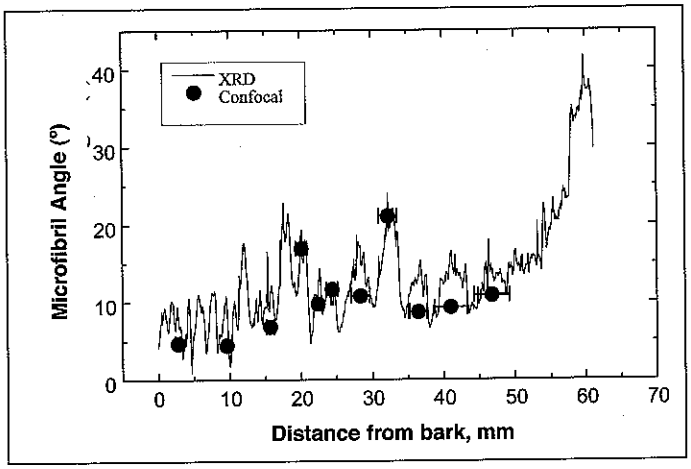

Fig. 3 XRD and confocal fibril angles with position for spruce.

where S^2 is the variance of the (002) peak, μ is the average fibril angle and σ^2 represents the variance due to sample geometry, instrumental effects, and fibril angle variance from all scales of organisation within the wood. Variation in local packing of the microfibril bundles around each other, changes in fibril angle from point to point within the fibre, and the distribution of fibre fibril angles around the average all contribute to σ.

Splitting σ into an additive component, σ_{add} which is independent of the fibril angle, and a multiplicative component, assumed to be related to the fibril angle by a constant of proportionality, k, *(19)* gives

$$S^2 = \left(\frac{1}{2} + k^2\right)\mu^2 + \sigma^2_{add} \qquad [2]$$

where σ_{add} is expected to lie in the range 0 to 10° and k is expected to lie between 0 and 1/3, with the exact values being sample dependent.

We attempted without success to determine values for σ_{add} and k by performing regression analysis on a plot of S^2 versus μ^2 (taken from confocal measurement). The associated problems were many. Firstly, it can be seen from Figure 2 that there is wide variation in the fibril angle within a single annual growth ring. Unfortunately, the original location of the fibres used for the confocal measurements could only be determined within a growth ring. Thus a single X-ray variance value for each average value measured on the confocal microscope could not be assigned. Furthermore, while at high values of μ, σ^2_{add} is insignificant and the relationship between S^2 and μ^2 is linear, the coefficient $1/2+k^2$ varies only between 0.50 and 0.61, for k ranging from 0 to 1/3. The accuracy of our data was insufficient to determine a slope with the precision required to set k.

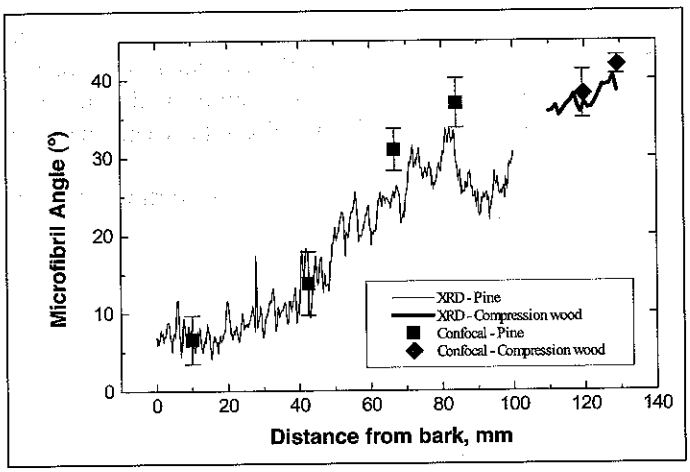

Fig. 4 XRD and confocal fibril angles with position for radiata pine sample and pine compression wood.

In the absence of sufficient accuracy in the data to quantitatively determine σ^2_{add} and k, we manually calculated fibril angles from the X-ray diffraction peak variance using a number of different values of σ_{add}. For the sake of simplicity a value of $k=0$ was used in all the calculations. After comparing the fibril angles calculated using the different values of σ_{add} with the fibril angles measured using confocal microscopy, $\sigma_{add}=9.4$ was selected as giving a reasonable fit between all three data sets. This was the largest value that could be selected without producing some negative fibril angles, as calculated from the X-ray diffraction peak variance.

Fibril angles for spruce and pine, measured by the two techniques, are compared in Figures 3 and 4 as a function of distance along the sample. For both figures $\sigma_{add}=9.4$ and $k=0$ were used to calculate the fibril angle from the measured diffraction peak variance. The horizontal error bars in Figure 3 reflect the fact that the samples for confocal measurement could have originated from anywhere within each growth ring.

Figure 3 shows a very good match between the two sets of data. Both show an increasing trend moving from the bark to the pith, and the fibril angle measured with confocal microscopy generally lies within the range of fibril angles measured by XRD for each location.

Figure 4 shows the results from both the radiata pine core sample and the radiata pine compression wood sample. The compression wood results have been offset by 110 mm so that they can be displayed on the same graph. The error bars were calculated from a statistical analysis of the 15 to 20 measurements contributing to each data point. The data show good agreement between the XRD and confocal measurements for the first

two points of the core sample and for the compression wood. The fibril angles measured with the confocal microscope are significantly higher (~5°) than corresponding values determined with XRD only for the last two points on the core sample. This discrepancy is considered acceptable, as the confocal measurements were made on dried and mounted pulp fibres and the XRD measurements were made on solid wood. Given that both pulping and drying would be expected to affect the fibril angle, the agreement between the two sets of data is reasonable.

CONCLUSIONS

X-ray diffraction measurements of (002)-peak variance were made as a function of distance along a radiata pine core sample, a radiata pine compression wood sample and a Norway spruce core. The results were compared with fibril angle measurements using confocal microscopy. Confocal measurements on the spruce sample were made on the solid wood, while the pine samples were pulped and dried and the measurements made on single fibres. Excellent agreement between the fibril angles given by the two methods was found when the fibril angle was calculated from the measured (002) peak variance using the formula $\mu^2 = (S^2 - 9.4^2)/2$.

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