Measuring the fibril angle of bleached fibres using micro-Raman spectroscopy

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A new technique for measuring the fibril angle of bleached pulp fibres is presented. It uses micro-Raman spectroscopy and involves measuring the ratio of the 1094 to 1120 cm^{-1} peak intensities as a function of angle. It is compared with a direct method of measuring of the fibril angle (cell wall striation method) and with two indirect methods (polarized light microscopy and pit aperture angle methods). A good correlation was obtained with the direct method indicating that the Raman method can accurately determine fibril angles in pine fibres. Poor correlations with the indirect methods suggest that these methods are inherently inaccurate.

Keywords

cell wall striations, fibril angle, micro-raman spectroscopy, microscopy pit apertures, polarised microscopy

THE MECHANICAL properties of a sheet of paper are influenced to a large degree by the mechanical properties of its constituent fibres. The mechanical properties of a fibre are, in turn, related to its dimensions and internal structure. One fibre property shown to exert a strong influence on the mechanical properties of a fibre is the fibril angle. It is defined as the angle made by the cellulose microfibrils in the S2 layer and the fibre axis. It has been shown to affect tensile strength *(1)* and elastic modulus *(2)*.

Page et. al. *(1)* have briefly reviewed earlier attempts to examine the effect of species, fibre type, pulping processes, yield, chemical composition and other variables on fibre tensile strength. They noted that these studies showed little or no agreement with each other and proposed that the critical factor in determining fibre tensile strength is the fibril angle which had been largely overlooked in previous work. They showed that, when the large variation in fibre strength due to fibre defects is accounted for, fibres having the same fibril angle have similar strengths independent of fibre type (springwood or summerwood) and species (black or white spruce). The need for a method to accurately measure the fibril angle is apparent from the fact that the fibre elastic modulus drops approximately 60% as the fibril angle increases from 10° to 40°.

To be able to investigate the relationship between the mechanical properties of fibres and their fibril angles more extensively it is vital to have a quick and accurate method for measuring the fibril angle. Many different techniques have been used; they include observation of cell wall striations, orientation of cracks and splits in the cell wall, hydrolysis planes of soft rot fungi, pit aperture angles, crystal deposition, the use of replicas, polarized light microscopy and X-ray diffraction analysis *(3)*. Crosby and Mark *(3)* have come up with five criteria that an ideal fibril angle measurement technique should possess. It should:

- be specific to an individual fibre
- characterize the S2 layer only
- be accurate
- be nondestructive
- avoid wall structures which distort local fibril orientation.

They advocate a procedure employing phase contrast microscopy with near ultra violet illumination and show how it satisfies the above criteria.

Crosby and Mark also note the surprising lack of comparisons that exist in the literature between different fibril angle measurement techniques *(3)*. They point out that most fibril angle measurement techniques are indirect methods in that they measure some characteristic indirectly related to the fibril angle rather than directly observing some physical feature. Meylan *(4)* found that when direct methods (iodine staining versus optical replicas and striations) were compared there were no significant differences in the results. However there were significant discrepancies when direct and indirect methods were compared. He ascribed these differences to the contribution of the S1 and S3 layers to the measured parameter in the indirect methods.

Crosby and Mark compared the direct method of UV phase contrast microscopy with two indirect methods involving polarized microscopy *(3)*. Polarized microscopy techniques take advantage of the natural birefringence of cellulose. To measure the fibril angle it is necessary to isolate a single cell wall. This is usually achieved either mechanically, by slicing the fibre (Leney's technique *(5)*), or by impregnating the fibre lumen with mercury and observing the fibre using epi-illumination (Page's technique *(6)*). It is then placed between two crossed polarizers and rotated until extinction occurs. The angle between the fibre axis at extinction and the polarization axis of the polarizer is taken as the fibril angle. Figure 1 shows a plot of Crosby and Mark's results comparing uv phase contrast with Page's polarized microscopy method for fifteen fibres. They found that the two indirect polarized microscopy methods correlated poorly with the direct uv phase contrast method.

Crosby and Mark also examined another technique for measuring the fibril angle, that of pit aperture angles. Pit apertures are ellipsoidal in appearance. Many workers have estimated fibril angles by measuring the angle made between the major axis of the pit aperture and the fibre axis. Crosby and Mark note the tendency of pit aperture angles in *Pinus virginiana* pulp fibres to overestimate the fibril angle as measured by uv phase microscopy. They also point out the high variability in orientation of the pit apertures on the same fibre. They give one example where three neighbouring pits have angles of 38, 34 and 37° while the striation angle was only 21° and conclude that "measurement of the pit aperture angle is

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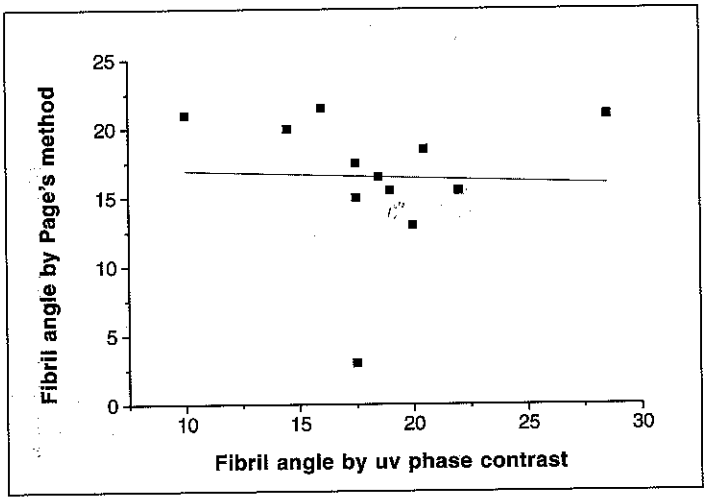

Fig. 1 Comparison of Page's polarized microscopy method with uv phase contrast method *(3)*.

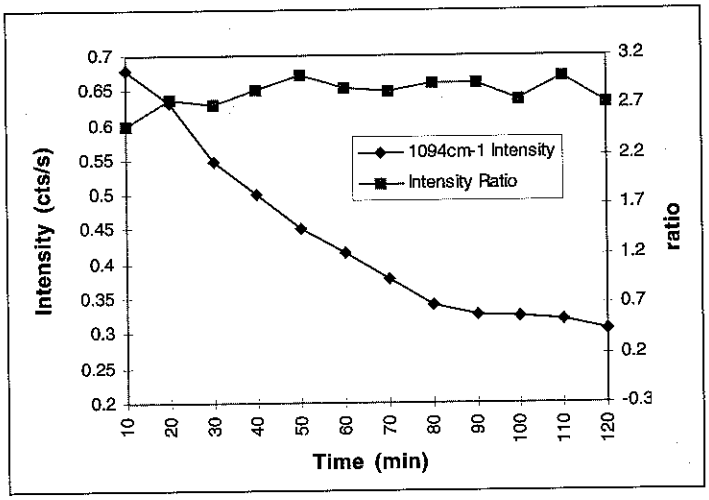

Fig. 2 Plot of 1094 cm^{-1} peak intensity and 1094 to1120 cm^{-1} peak intensity ratio against time for a pine fibre.

not satisfactory as an indicator of S2 angle".

While the uv phase contrast method meets all the criteria specified above it has the disadvantage of being time consuming and requiring painstaking technique *(3)*. To be able to test large numbers of fibres it is essential to find a technique that is rapid in addition to fulfilling the above five criteria.

With this goal in mind we investigated the possibility of using micro-Raman spectroscopy to realize these conditions. The Raman effect is caused by molecular vibrations and is thus sensitive to the orientation of the molecules in a sample. It has been used extensively to characterize the molecular orientation in uniaxially oriented synthetic polymers including poly(methyl methacrylate) *(7)*, poly-(ethylene terephthalate) *(8)* and poly (vinyl chloride) *(9)*.

Wiley and Atalla have examined the orientational dependence of the Raman peak intensities of the native cellulose fibres of ramie and *Valonia (10)*. They modelled this dependence using the equation:

$$I = a + b(\cos\theta)^2 + c(\cos\theta)^4$$

where a, b, c are constants related to the derivatives of the polarizibility tensors with respect to the normal coordinates and θ is the angle between the fibre axis and the polarization axis of the incident laser beam. They derived this equation using the theory developed by Snyder for Raman intensities of partially oriented polymers.

The purpose of the present study is to compare a technique we have developed employing micro-Raman spectroscopy with a direct method (observation of cell wall striations) and two indirect methods (pit aperture angles and polarized microscopy). The direct observation of cell wall striations is only possible in certain types of fibres such as compression wood tracheids.

EXPERIMENTAL

Pinus radiata compression wood fibres were used for the comparison with the cell wall striation method while *Eucalyptus nitens* fibres taken from near the pith were used for the comparison with the polarized microscopy and pit aperture angle methods. Both samples were placed in boiling water overnight and then sliced with a microtome. The cuts were made parallel to the fibre axes so that longitudinal fibre sections were produced. The pine samples was sliced to a thickness of 15 µm while the eucalypt sample was sliced at 10 µm. The sections were extracted (pine in a 2:1 benzene-ethanol mixture, eucalypt in methanol) for 8 hours. They were bleached using the modified nitric acid method of Schorning and Jacepian *(11)*. It was necessary to remove the lignin from the fibres by bleaching since lignin causes fluorescence which competes with the Raman process. The bleached fibre sections were then dried on to a glass slide.

The fibril angle of individual pine fibres was found by measuring the angle between the striations on the cell wall and the fibre axis under a microscope.

With the eucalypt fibres a site was chosen which had a pit aperture in the field of view. The angle that the pit aperture made relative to the fibre axis was measured. The fibril angle was also estimated by polarized microscopy using Leney's method. *(5)*.

The same measurement technique was used for both pine and eucalypt fibres to measure the fibril angle by micro-Raman spectroscopy. The Raman spectra were acquired with a T64000 Jobin Yvon confocal micro-Raman spectrometer with 514.4 nm laser excitation polarized in the vertical plane at the laser. An analyser, with its polarization axis also in the vertical plane, was placed in front of the entrance slit of the spectrometer. The slide was placed on the rotating stage of the microscope coupled to the spectrometer and a fibre was selected. The laser was focused a micrometre beneath the surface of the fibre which was rotated in 5° intervals over a 40° range centred on the measured fibril angle. A laser power of 50 mW was used which gave approximately 3 mW at the sample. An integration time of 10 minutes was employed. Spectra were acquired at each angle and then curve-fitted using Galactic's Peaksolve curve-fitting package. The ratio of the 1094 to 1120 cm^{-1} peak intensities was plotted against angle relative to the fibre axis. This plot was fitted with a parabola and the angle at which the parabola had a maximum was taken to be the estimated fibril angle.

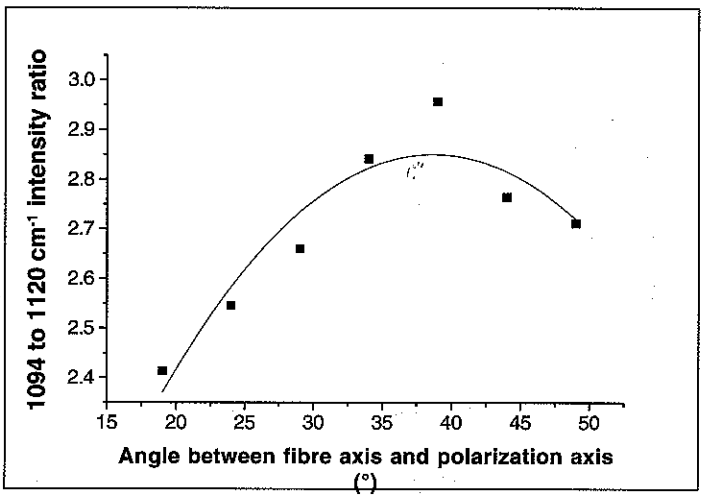

Fig. 3 Plot of 1094 to 1120 cm^{-1} Raman peak intensity ratio against angle for a pine fibre.

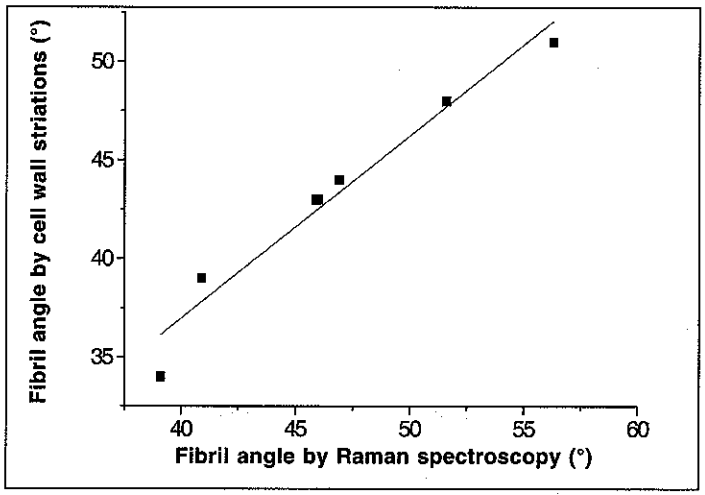

Fig. 4 Comparison of Raman method with cell wall striation method for measuring the fibril angle of pine compression wood fibres.

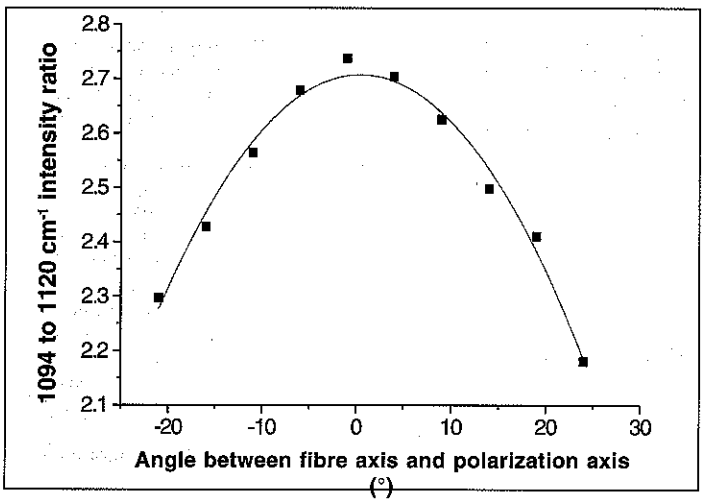

Fig. 5 Plot of 1094 to 1120 cm^{-1} peak intensity ratio against angle for a eucalypt fibre.

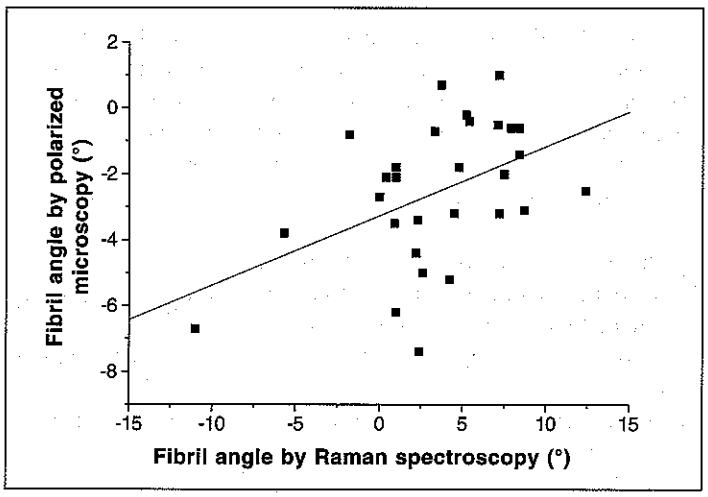

Fig. 6 Comparison of Raman method with Leney's polarized microscopy method for measuring fibril angle.

RESULTS AND DISCUSSION

Before looking at how the spectrum of a fibre changed as the fibre was rotated it was necessary to see if there were any changes to the spectrum when the fibre was held stationary. It was noted that for some fibres there was a considerable drop in the intensity of the spectrum over a period of a few hours. This is shown in Figure 2 for a pine fibre. Here the intensity of the 1094 cm^{-1} peak drops to about half its initial value over two hours.

It is not clear what causes this effect. It could be due to local heating of the sample by the focused laser beam. However similar reductions in intensity have been observed for fibres completely immersed in water so this explanation would seem to be unlikely. Direct photo-degradation of cellulose is not thought to occur since the laser wavelength of 514.5 nm is well below that required to rupture the bonds in cellulose *(12)*. The decrease in intensity is most noticeable in fibres which have been recently bleached and so it may be due to the bleaching method employed.

But regardless of what produces the drop in intensity, the ratio of the 1094 to 1120 cm^{-1} peak intensities remains constant, as can be seen from Figure 2. This means that any change observed in this ratio on rotating the fibre must be due to orientation effects alone.

Figure 3 shows a typical plot for the ratio of the 1094 to 1120 cm^{-1} peak intensities for a pine fibre. The ratio goes through a maximum and can be fitted by a parabola.

Figure 4 shows the comparison between the Raman method and the direct cell wall striation method for seven pine compression wood fibres. There is an excellent correlation ($R^2 = 0.95$) between the two methods. It is apparent, however, that the fibril angle measurements made by the Raman method are significantly higher than those obtained by the cell wall striation method. The Raman method gave a mean fibril angle of 46.6° while the cell striation technique had a mean of 43.1°. This systematic error is most likely due to inaccurate measurement of the laser polarization axis. A more accurate method for measuring this was developed for later experiments.

Figure 4 is a highly significant since it shows that even though the micro-Raman spectroscopy method is indirect for measuring the fibril angle it has the same degree of accuracy as a direct method, in this case the cell wall striation method.

Figure 5 shows a typical plot of the peak ratios as a function of the angle between the fibre axis and polarization axis of the laser obtained for a eucalypt fibre. It

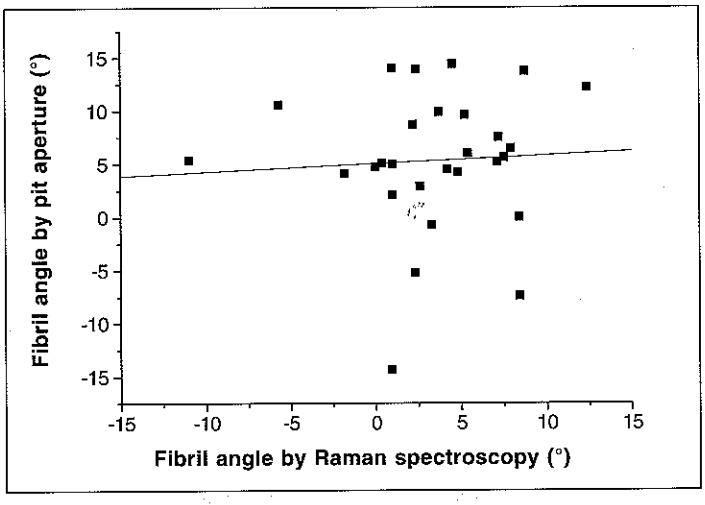

Fig. 7 Comparison of Raman method with the pit aperture angle method for measuring fibril angle.

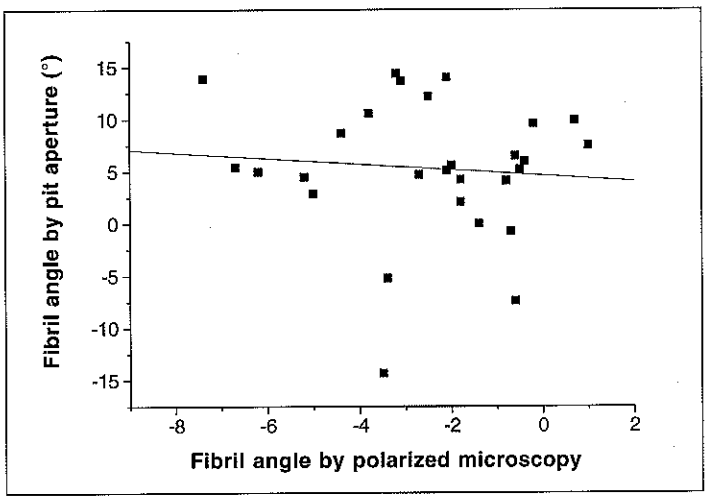

Fig. 8 Comparison of Leney's polarized microscopy method with the pit aperture angle method for measuring fibril angle.

is similar to those obtained for pine fibres and can be fitted well with a parabola.

Figure 6 shows the comparison between the Raman method and the polarized microscopy method. It shows no correlation between the two techniques. No correlation is obtained for the pit aperture method (Fig. 7).

There are two possible explanations for these poor correlations. The first is that the Raman method gives inaccurate results for eucalypt fibres. The main difference between eucalypt and pine fibres is that the cell walls of eucalypt fibres are generally considerably thinner than those of pine fibres. This means that under the same conditions eucalypt fibres will give a lesser Raman signal than pine fibres. This was compensated for by using a longer integration time for eucalypt fibres. The resulting spectra had comparable signal to noise ratio. As pointed out previously the plots of the peak intensities versus angle between fibre and polarization axis were similar for both pine and eucalypt fibres. The worst fit with a eucalypt fibre had an R^2 of 0.85 with most having an R^2 greater than 0.95.

The other possible explanation for the poor correlations is that the polarized microscopy and pit aperture measurement techniques are inherently inaccurate, both being indirect techniques. Figure 8 shows a comparison between the two techniques revealing that there is no correlation between them over the range of angles examined. This would imply that at least one of the two techniques is inaccurate.

This explanation is also in accord with Crosby and Mark's finding that neither technique compared well with the direct method of uv phase-contrast microscopy. To be able to show conclusively that this was the case it would be necessary to compare the Raman method and a direct method such as uv polarized microscopy for measuring the fibril angle of eucalypt fibres.

CONCLUSIONS

The micro-Raman spectroscopy method described in this paper can accurately determine the fibril angle of pine fibres as demonstrated by the good correlation obtained with the cell wall striation method.

The poor correlations with the polarized microscopy method and pit aperture angle method for eucalypt fibres supports Crosby and Mark's assertion that these are inherently inaccurate methods for measuring the fibril angle.

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