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Small-angle X-ray scattering from cellulose micro-fibrils in the S2 layers of structurally characterised softwood specimens

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A method was reported in a previous paper (Entwistle and Terrill, *J. Mater. Sci.* **35** (2000) 1675) for the measurement of the micro-fibril angle in the *S*2 layers of softwood. The small-angle X-ray scattering pattern was recorded with the beam directed at 45° to the radial direction in the cell structure. A cruciform scattering pattern was produced and the micro-fibril angle was deduced from the angle between the arms of the cross. The analysis assumed that all the cell walls lay in either the radial or the tangential direction. Real cell structures do not conform to this ideal. The objective of the work now presented was to calculate the error in the deduced value of the micro-fibril angle arising from the assumption that the specimen cell walls all lie in either the radial or in the tangential direction. To this end, the length and orientation of over 1000 cell walls was measured on two specimens using an image analyser. From these data the azimuth angle at the peak of the scattered intensity was calculated as a function of the micro-fibril angle and the standard deviation of the spread of intensity. The true micro-fibril angle was determined by comparing these data with the measured azimuth angle at the peak intensity. A value for the micro-fibril angle was also calculated from the measured value of the azimuth angle at the peak intensity using the relation for a square-section cell structure

$$M = \text{Atan}(\sqrt{2}^{*} \tan\phi).$$

The values differed from the true values by between 1° and 2° for micro-fibril angles between 25° and 30°. For many purposes this degree of error is tolerable and in those circumstances the above relation can be used to extract a micro-fibril angle value.

1. Introduction

A method has been proposed [1] for the measurement of the micro-fibril angle of the cellulose fibres in the S2 layers of softwood. The small-angle X-ray scattering pattern is recorded for a specimen which is oriented so that the X-ray beam is directed at 45° to both the radial and the tangential directions with the cell axes vertical. A cruciform pattern is produced (see Fig. 1) and the micro-fibril angle M is deduced from the angle between the arms of the cross. In the work previously reported [1] it was assumed that the cells had square cross-sections and that all the cell walls lay in either the radial or the transverse directions. We shall refer to this as the ideal structure. Real structures diverge significantly from this ideal so it is desirable to establish the degree of error that arises from the assumption, made in the derivation of M in the previous paper, that the structure is ideal. The work now described was undertaken to establish the magnitude of this error. To this end, a quantitative microscopic analysis was carried out using an image analyser of the length and the orientation of several thousand cell walls in a section of the specimen cut on the R-T plane and of dimensions comparable to that irradiated in the recording of the small-angle scattering pattern. The scattered intensity from of all these cell walls was calculated and was summed to give the total distribution of intensity round a circle concentric with the centre of the SAXS scattering pattern. The intensity distribution shows four peaks, the positions of which were compared with the peak positions calculated for

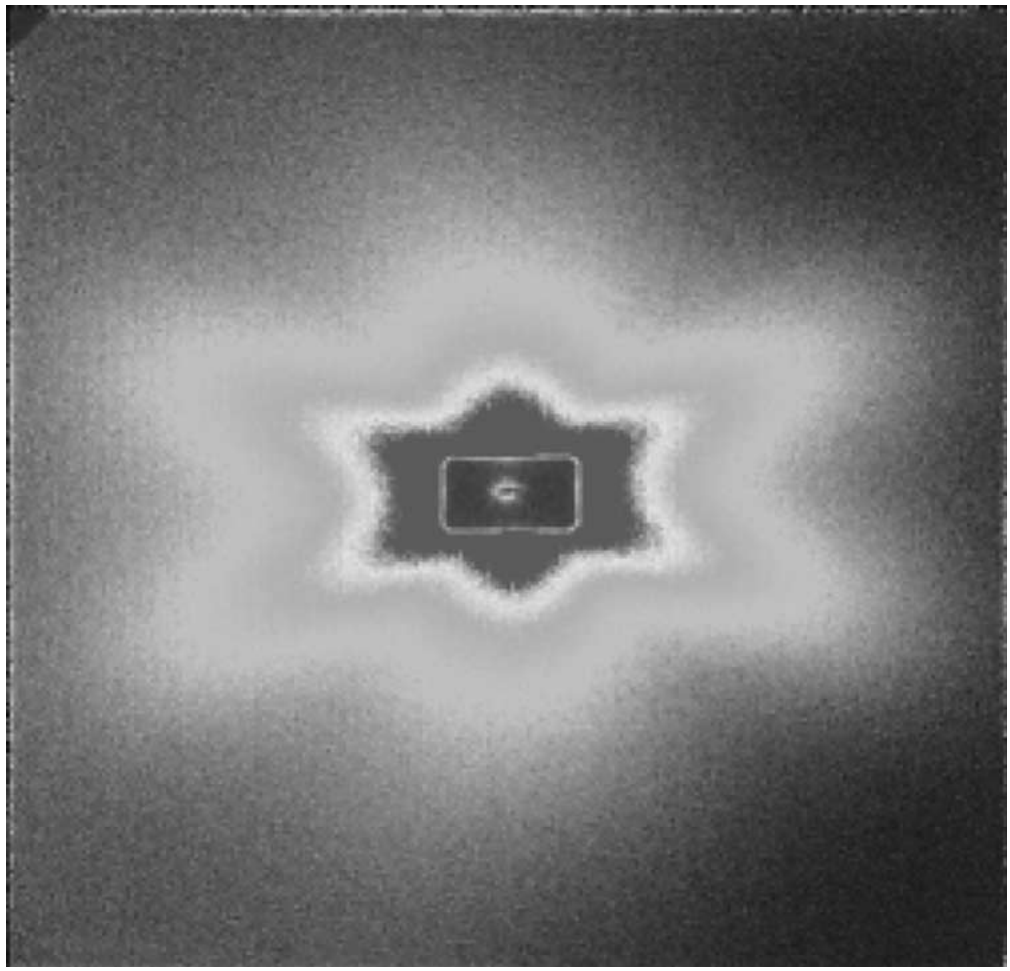


Figure 1 Typical small-angle X-ray scattering pattern from a specimen of *pinus radiata* with the X-ray beam directed at 45° to the radial direction.

the ideal structure. This work complements a parallel analysis of wide-angle scattering intensity patterns which has been submitted for publication [2].

2. Measurement of the cell wall lengths and orientations

Two specimens, for which the small-angle X-ray scattering patterns had been recorded, were prepared. A fine cut was made with a sharp blade across the cross-section of the specimen which revealed an undistorted picture of the cell structure. Five images from this section, one near each corner and another in the centre, were analysed using a Scion Image Analyser. Over 1000 cell walls were measured. Fig. 2a shows a small part of a typical pattern of the cell cross-sections.

The length and orientation of each cell wall was measured by setting up a two-dimensional co-ordinate system with the x-axis lying in the radial direction and the y-axis in the transverse direction (see Fig. 2b) . The x and y co-ordinates of each cell wall junction, for example A and B in Fig. 2a, were determined by the image analyser. The length of each cell wall (Δl) and the orientation (θ), relative to the radial direction as zero, were determined by

$$\Delta l = \sqrt{(x_j - x_i)^2 + (y_j - y_i)^2}$$

$$\text{and} \qquad \theta = \text{Atan}\left[\frac{(y_j - y_i)}{(x_j - x_i)}\right],$$

where (x_i, y_i) and (x_j, y_j) are the co-ordinates of the two ends of the cell wall.

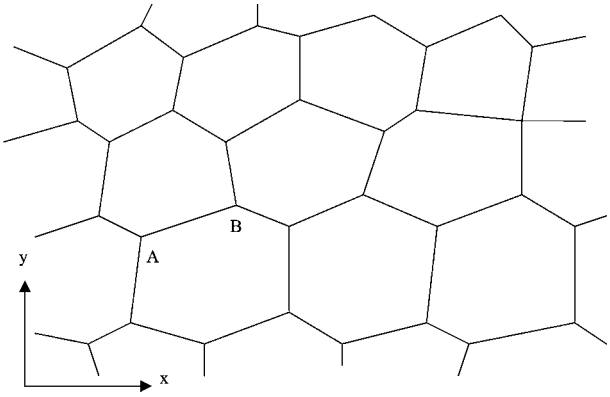
To simplify the analysis the data were collected together in 1° ranges by adding together the total length of cell wall oriented between 0° and 1°, between 1° and 2° and so on. The values of θ range between $-90°$ and $+90°$ because parallel cell walls on opposite sides of the cell are regarded as identical. Figs 3 and 4 show the distribution of cell wall lengths and orientations for two specimens for which the small-angle X-ray scattering patterns had been recorded.

We now calculate the azimuthal distribution of intensity which these cell wall populations generate when the X-ray beam is directed at 45° to the $\theta = 0$ direction and the cell axes are vertical.

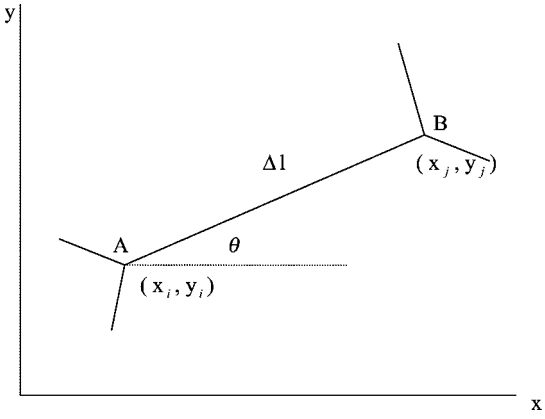
3. Small-angle X-ray scattering from a single set of parallel fibres

The cellulose fibrils in the S2 layers of softwood such as *pinus radiata* are about 25 nm in diameter and very much longer than that so the small angle scattering from them can be assumed to lie completely in the plane of the fibre cross-section. The orientation of the scattering streak in the plane of the two-dimensional detector, whose normal lies in the direction of the incident X-ray beam, is arrived at in the following way.

Fig. 5 shows the detector plane and the incident X-ray beam. An individual cell wall is shown dotted with a parallel set of fibrils f lying in the wall and oriented at



(a)



(b)

Figure 2 (a) Diagram showing a small part of the cell cross-sections revealed by the image analyser. AB is a typical cell wall, which is shown enlarged in Fig. 2b. The radial direction is x and the transverse direction is y. (b) Diagram defining the orientation θ of the cell wall AB, its length Δl and the rectangular co-ordinates of its end-points.

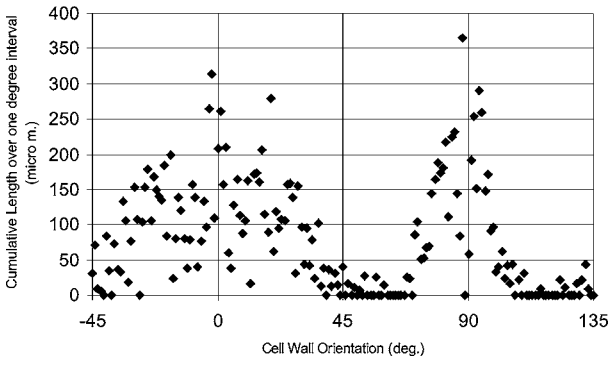


Figure 3 Specimen LO distribution of cell wall lengths and orientations.

the micro-fibril angle M to the z axis, which is the cell axis direction. The plane of the cell wall lies at an angle α to the y-direction, which is the equatorial direction in the detector plane and is perpendicular to the direction of the incident X-rays. We define α as positive if the rotation is anti-clockwise in the sense indicated in Fig. 5. Also indicated is a circle defining the edge of a plane that lies in the cross-section of the fibrils. The azimuth angle ϕ of the scattering streak in the detector plane is given by the line of intersection of the circular plane with the detector plane, which is the line S-S in Fig. 5. The angle ϕ is measured relative to the equatorial direction and is positive if the rotation is clockwise. We can

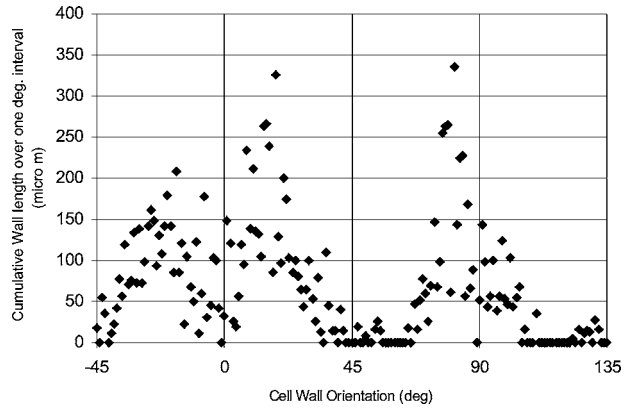


Figure 4 Specimen L12 distribution of cell wall lengths and orientations.

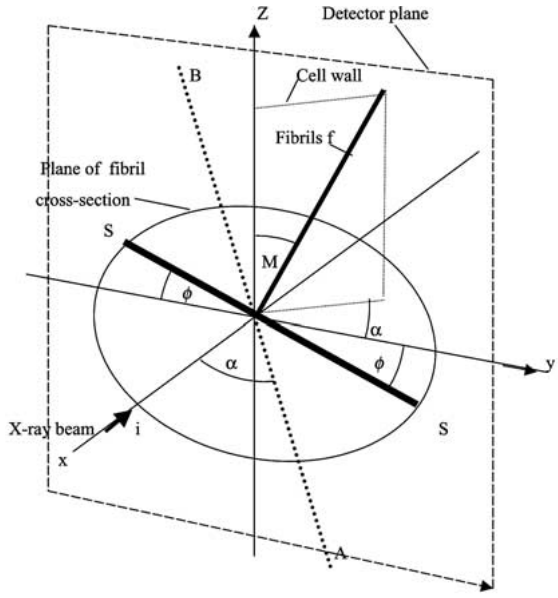


Figure 5 Diagram showing the orientation of the scattering streaks S-S generated by a set of parallel fibrils of orientation f.

calculate ϕ in the following way: the vector representing the scattering streak is perpendicular to the incident X-ray beam direction i. So the direction cosines of the part of the streak on the right-hand side of the origin are

$$(0, \cos\phi, -\sin\phi).$$

The direction cosines of the direction f of the axis of the fibrils are

$$(\sin M \sin\alpha, \sin M \cos\alpha, \cos M).$$

Now S and f are perpendicular, because S lies in the cross-section of the fibrils, so their scalar product is zero i.e.

$$S \cdot f = \sin M \cos\alpha \cos\phi - \cos M \sin\phi = 0$$

or

$$\tan\phi = \cos\alpha \tan M \qquad (1)$$

4. Small-angle scattering from the real cell structure

The $S2$ layers of a cell wall have two sets of cellulose fibrils in mirror image orientation, f_1 and f_2 shown in Fig. 6. The scattering from f_1 is described by the analysis of the previous section. The fibrils f_2 will scatter identically to $f_2^{|}$ which is parallel to f_2 and will produce scattering defined by the same relation as that for f_1 but with a cell wall angle $(\alpha + \pi)$ to the y-direction. Now since

$$\cos(\alpha + \pi) = -\cos\alpha$$

then, from Equation 1, it follows that the azimuth angle $\phi^{|}$ for the scattering streak from f_2 is equal to $-\phi$. The scattering pattern from the cell wall at orientation α is therefore that displayed in Fig. 7. For a cell wall oriented at an angle α to the y-axis the two scattering streaks S and $S^{|}$ have respectively azimuth angles ϕ and $\phi^{|}$ given by

$$\tan\phi = \cos\alpha \tan M \tag{2a}$$

and

$$\tan\phi^{|} = \cos(\alpha + \pi)\tan M \tag{2b}$$

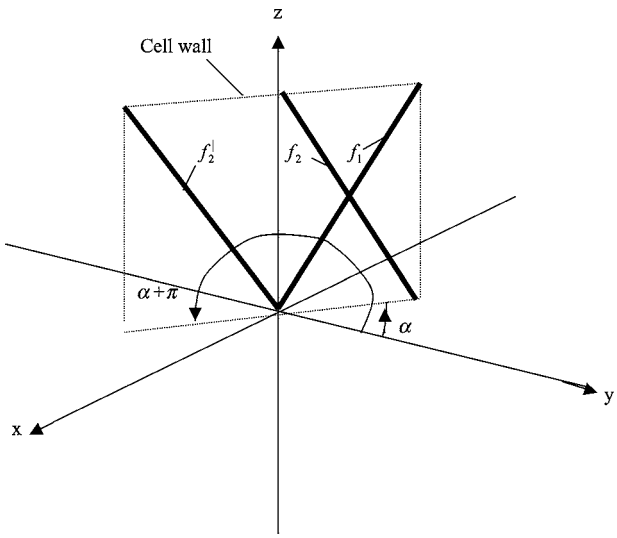


Figure 6 Diagram showing that the S2 fibrils f_2 in the cell wall at orientation α to the y-axis is equivalent to the fibrils $f_2^{|}$ in the wall at orientation $(\alpha + \pi)$.

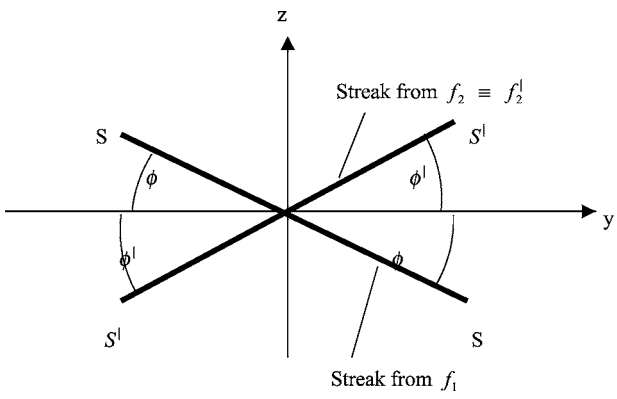


Figure 7 Diagram showing the small angle streaks from the cell wall of Fig. 6.

so

$$\phi^{|} = -\phi.$$

We wish to calculate the variation of scattered intensity round a circle located on the centre of the small-angle scattering pattern, which is the point at which the incident X-ray beam strikes the detector plane. The scattered intensity falls off with the radial distance from this point. At a particular distance along a scattering streak, the intensity will be proportional to the strength of the scattering source, that is to the length of the particular cell wall which scatters at that azimuth angle. Further, the scattered intensity from a single cell wall will be spread circumferentially because the orientation of the micro-fibrils that make up the cellulose fibres wanders somewhat about the average direction. This in effect causes the micro-fibril angle to spread over a range of values and, through Equation 1, will generate a range of values for the azimuth angle ϕ of the scattering intensity. We assume that this distribution of scattered intensity can be described by a Gaussian curve so the azimuthal distribution of the scattered intensity ΔI_{n1} from a single set of fibrils f_1 in a cell wall of length Δl_n lying in a cell wall at an angle α_n to the y-axis is given by

$$\Delta I_{n1} = \Delta l_n \exp -\frac{1}{2}\left[\frac{\phi - \phi_{n1}}{\sigma_\phi}\right]^2 \tag{3a}$$

where σ_ϕ is the standard deviation of the intensity distribution and ϕ_{n1} is the azimuth angle of the scattered streak from fibrils f_1 given by Equation 2a that is

$$\tan\phi_{1n} = \cos\alpha_n \tan M$$

The same length of cell wall will, in addition, generate scattering from fibre f_2 . The intensity of this component will be

$$\Delta I_{n2} = \Delta l_n \exp -\frac{1}{2}\left[\frac{\phi - \phi_{n2}}{\sigma_\phi}\right]^2 \tag{3b}$$

where

$$\tan\phi_{n2} = \cos(\alpha_n + \pi) \cdot \tan M.$$

The distribution of the total scattered intensity from the nth cell wall is

$$\Delta I_n = \Delta I_{n1} + \Delta I_{n2} \tag{4}$$

The total scattered intensity I from the specimen is calculated by summing terms like Equation 4 for all the measured cell walls. Formally

$$I = \sum_{\alpha=1}^{\alpha=180} \Delta l_n \exp -\frac{1}{2}\left[\frac{\phi - \phi_{n1}}{\sigma_\phi}\right]^2 + \sum_{\alpha=181}^{\alpha=360} \Delta l_n \exp -\frac{1}{2}\left[\frac{\phi - \phi_{n2}}{\sigma_\phi}\right]^2 \tag{6}$$

Where ϕ_{n1} and ϕ_{n2} are given by Equations 2a and 2b respectively and values of Δl_n are obtained from the data which yield Figs 3 and 4.

The calculation of the individual terms in Equation 6 and their summation was carried out in an EXCEL spreadsheet.

We recall from the previous publication (1) that the azimuth angles at the peaks of the scattered intensity for the square cell structure irradiated at 45° to both sets of cell walls are

$$\phi = \pm \text{Atan}[\cos 45^\circ \tan M] \qquad (5)$$

We discuss in the next section the choice of appropriate values of the micro-fibril angle M and of the standard deviation of the intensity distribution σ_ϕ to feed into the calculations.

5. Calculation of the small-angle scattered intensity from two specimens

The first specimen we examine had the laboratory designation LO. The measured separation of the small-angle scattering peaks for this specimen is 37.5° so the measured scattering angle is $\phi = 18.75^\circ$.

The measured distribution of cell wall lengths and orientations is given in Fig. 3, where the cell wall orientation θ is measured relative to the radial direction in the cell structure. The total length of radial walls, that is lying between $\theta = \pm 45^\circ$, is almost exactly twice that of the transverse walls lying between 45° and 135°. It is evident that the radial walls cover a wider range of orientations than do the transverse walls.

Using the cell wall geometry of Fig. 3 and the analysis of the previous section, we can calculate the distribution of scattered intensity round a circle located at the centre of the scattering pattern. For this purpose we need a value for the standard deviation σ_ϕ of the intensity distribution and for the micro-fibril angle M. A typical calculated intensity distribution is shown in Fig. 8 for $M = 28^\circ$ and $\sigma_\phi = 8^\circ$. The total intensity peaks occur at $\phi = \pm 20.05^\circ$. The upper curve is the total intensity. Below it are two curves that give the contributions to the total intensity from each of the two sets of fibrils in the S2 layers. One curve is the exact mirror image of the other.

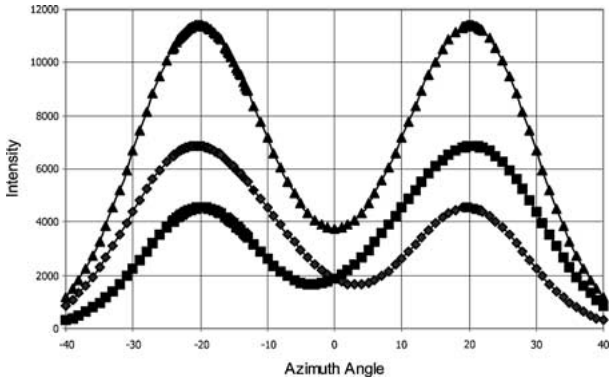


Figure 8 Specimen LO. Azimuthal distribution of intensity calculated from the real cell wall geometry assuming $M = 28^\circ$ and $\sigma_\phi = 8^\circ$. The two lower curves (squares and diamonds) show separately the scattering from the two sets of fibrils in each cell wall. The upper curve (triangles) is the total scattering with peaks at ±20.05°.

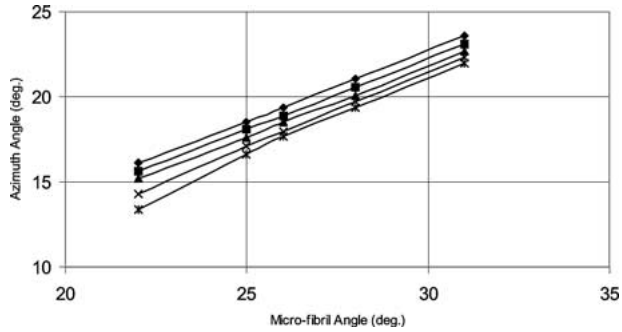


Figure 9 The values of σ_ϕ for the five curves are, from the top curve down, 4°, 6°, 8°, 10° and 11°.

In order to establish the most likely value of the micro-fibril angle corresponding to the measured scattering angle of $\phi = 18.75^\circ$, we proceed in the following way. We calculate the angular position ϕ of the scattered intensity peaks as a function of the micro-fibril angle M for a given value of σ_ϕ and then repeat this for a number of values of σ_ϕ. This produces the family of curves shown in Fig. 9. We can now read off from the data of Fig. 9 the combination of values of M and σ_ϕ which yield a calculated value of ϕ equal to the measured value of 18.75°. The results are as follows:

σ_ϕ	M
4°	25.2°
6°	25.7°
8°	26.35°
10°	26.8°
11°	27.2°

Evidently M is not very sensitive to the value of σ_ϕ. In order to narrow down the most likely value of M within this table, we need to establish a favoured value for σ_ϕ. One way forward is to compare the measured and the calculated widths of the intensity peaks. We define the peak width as that at half the peak height which for the measured peak is 29°. The width of the calculated peak for $M = 26^\circ$ is 29.4° for $\sigma_\phi = 11^\circ$. So if we use that value as the most likely, then the table gives

$$M = 27.2^\circ.$$

and this is the best value to attach to the micro-fibril angle.

Had the measured value of $\phi = 18.75^\circ$ been used to deduce the micro-fibril angle by assuming that all the cell walls lay in the radial or the tangential directions then the value of M would be

$$M = \text{Atan}(\sqrt{2}^* \tan 18.5^\circ)$$
$$= 25.6^\circ.$$

The error arising from the assumption of this ideal cell structure is therefore 1.6°.

An alternative parameter that is sensitive to the value of σ_ϕ is the ratio between the height of the scattering peaks and the height of the minimum between them, because this ratio is sensitive to the extent of adjacent peak

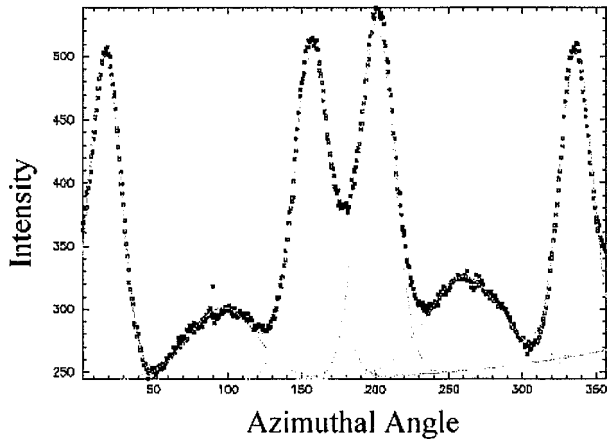


Figure 10 Azimuthal variation of small-angle scattered intensity for specimen L12 with the X-ray beam directed at 45° to the radial direction. The scattering angle $\phi = \pm 20.4°$.

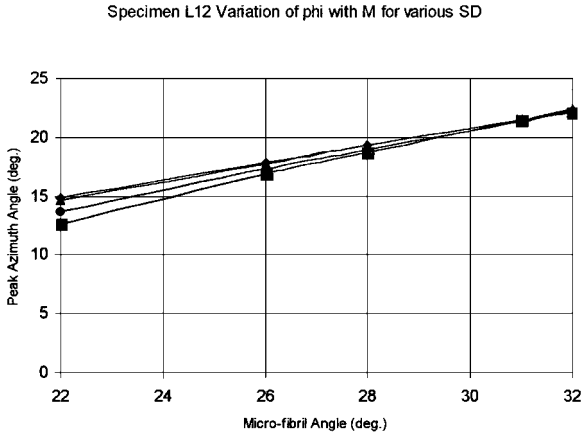


Figure 11 The values of σ_ϕ for the four curves are, from the top down, 6°, 8°, 10° and 11°.

overlap. The ratio for the measured curves is 0.42. This ratio is achieved in the calculated curves for $\sigma_\phi = 9°$ for $M = 26°$. For this value of σ_ϕ the above table gives

$$M = 26.6°$$

so the error arising from the assumption that the cell structure is ideal is, on this basis, 1.0°.

We now carry out a similar analysis for specimen L12 for which the cell wall geometry is presented in Fig. 4. The total length of radial walls is about 1.8 times the length of the transverse walls and, as with specimen LO , the radial walls show a wider range of orientation with, in addition, evidence of a double peak in the length distribution. Fig. 10 shows the measured circumferential variation of scattered intensity with the X-ray beam directed at 45° to the radial direction. The measured scattering angle $\phi = 20.4°$.

We use the data of Fig. 4 to calculate the variation of the peak scattering angle ϕ with M at a number of different values of σ_ϕ. The results are given in Fig. 11. A typical calculated intensity distribution curve for $M = 28°$ and $\sigma_\phi = 8°$ is presented in Fig. 12. It is apparent from Fig. 11 that the effect of σ_ϕ on the relation between ϕ and M is less than for specimen LO (Fig. 8) and the variation is more complex. Around M = 31° the peak angle is almost independent of σ_ϕ in the range 6°–11°. The effect of σ_ϕ on the peak movements is conditioned by the

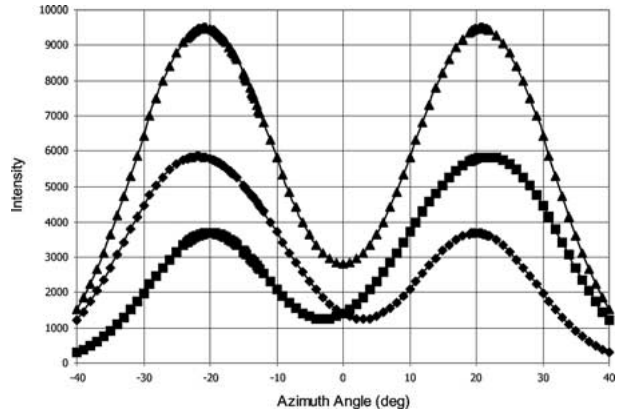


Figure 12 Azimuthal distribution of intensity calculated from the real cell wall geometry for specimen L12 assuming $M = 30°$ and $\sigma_\phi = 8°$. The two lower curves give the scattered intenssity generated by each of the two sets of fibrils in each cell wall. The upper curve (with triangular markers) is the total scattering with peaks at ±20.8°.

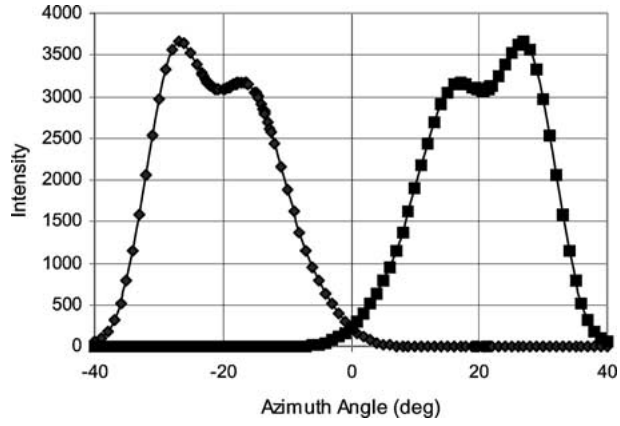


Figure 13 Specimen L12. Azimuthal distribution of intensity by the two sets of fibrils in the radial walls, that is those with values of alpha between 1°–90° and 181°–270°. $M = 30°$ and $\sigma_\phi = 4°$.

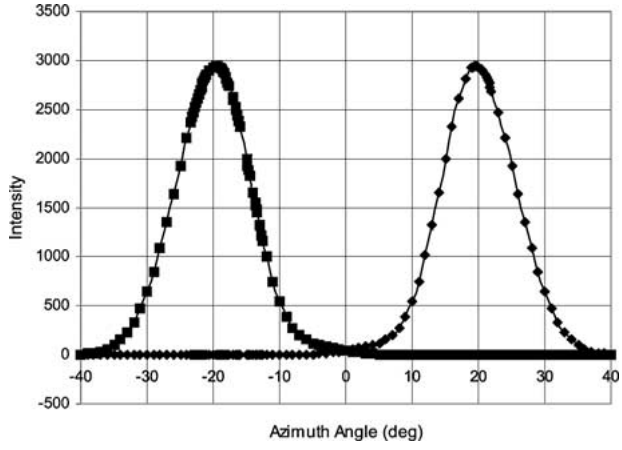


Figure 14 Specimen L12. Azimuthal distribution of intensity scattered by the two sets of fibrils in the tangential walls, that is those with values of alpha between 91°–180° (diamonds) and 271°–360° (squares). $M = 30°$ and $\sigma_\phi = 4°$.

asymmetry of the cell wall geometry. We plot separately in Figs 13 and 14 the contributions to the total scattered intensity from the transverse walls (that is those lying between $\alpha = 91°$–180° and 271°–360°) and the radial walls (lying between 1°–90° and 181°–270°) for $M = 30°$ and $\sigma_\phi = 4°$. The intensity distribution from the transverse walls is seen to be symmetrical and the

peak value moves only slightly with changes of σ_ϕ. The intensity distribution from the radial walls is asymmetrical and shows evidence of a double peak reflecting the double peak distribution of cell wall lengths evident about $\theta = 0$ in Fig. 4. The movement of this peak structure with changes of σ_ϕ is more complex than is that of the transverse wall peak.

We use Fig. 11 to establish the combination of values of M and σ_ϕ that give the measured peak angle of $\phi = 20.4^\circ$, with the following results:

σ_ϕ	M
6°	29.5°
8°	29.5°
10°	29.7°
11°	30.0°

The width of the measured peak is 27°. The value of σ_ϕ which generates a calculated peak of this width is 10° for $M = 30^\circ$. Assuming this value for the standard deviation, the preferred value of M from the table above is

$$M = 29.7^\circ.$$

Had the measured value of $\phi = 20.4^\circ$ been interpreted assuming that all the cell walls lay in either the radial or the transverse direction then the deduced value of the micro-fibril angle would be

$$\begin{aligned} M &= \text{Atan}(\sqrt{2} * \tan 20.4^\circ) \\ &= 27.7^\circ. \end{aligned}$$

so the error arising from the assumption of ideal cell wall orientation is 2.0°.

The measured ratio of the peak height to the height if the minimum between adjacent peaks is 0.39. The value of σ_ϕ that gives this ratio for the calculated peaks is 9° which corresponds to a value of

$$M = 29.6^\circ$$

in the above table, so this approach gives nearly the same value as does the use of peak width and the corresponding error is 1.9°.

6. Conclusions

We have measured the cell wall geometry of two specimens and calculated the expected azimuthal distribution of small-angle scattered intensity which would be generated by this real cell wall population, with the X-ray beam directed at 45° to the radial direction. We used the measured SAXS patterns to deduce the parameters needed for these calculations. The azimuthal variation of scattered intensity shows peaks at the points corresponding to the axes of the scattering cross (Fig. 1) which is generated by this irradiation geometry.

We calculated the values of the micro-fibril angle which would be deduced from the angle between the scattering peaks assuming that all the cell walls lay in the radial or in the tangential directions, that is using

$$M = \text{Atan}(\sqrt{2} * \tan \phi)$$

where 2ϕ is the azimuth angle between adjacent scattering peaks calculated from the real cell wall structure. These calculations gave values that differed from the true values by between 1° and 2° for micro-fibril angles between 25° and 30°. For many purposes this error would be tolerable.

The analysis leads to the conclusion that an approximate value for the micro-fibril angle, which would be acceptable for many purposes, can be measured by determining the azimuth angle ϕ at the scattering peak for a specimen irradiated in a direction at 45° to the radial and the tangential directions. M is then calculated from the following relation for the peak position for square section cells

$$M = \text{Atan}(\sqrt{2} * \tan \phi)$$

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