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MEASUREMENT OF WOOL CORTICAL CELL PROPORTIONS BY IMAGE PROCESSING

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SYNOPSIS

Differences between the cell types present in the cortex of wool fibres have long been recognised. Assessment of the significance of these differences has been slow because the highly variable distributions and proportions of cortical cell types are difficult to measure. To overcome this, an image-processing algorithm has been developed which allows rapid automatic measurement of cortical cell type proportions in fibre cross-sections stained with methylene blue. The algorithm is split into three image-processing phases: image preparation; image measurement; and result verification. Measurements made with the image-processing system agree well with those made by hand drawing and digitising the same cross-sections.

INTRODUCTION

The cortex is the major component of most wool fibres and is responsible for many of the physical properties of wool. Three cell types have been distinguished in the cortex on the basis of their staining properties, structure, and keratin composition.

Orthocortical cells are distinguished by their larger cross-sectional area, inability to be stained by methylene blue following performic acid oxidation, smaller keratin aggregates (macrofibrils), the whorl arrangement of their keratin composite (i.e. the microfibril/matrix arrangement within macrofibrils), and the dispersed nature of their cytoplasmic remnants[1-6].

Paracortical cells are stained by methylene blue and have a smaller cross-sectional area, larger macrofibrils which are usually arranged around the cell periphery, centrally located cytoplasmic remnants, and a keratin composite arrangement in which the microfibrils lie parallel to the fibre axis[1-6].

Mesocortical cells are usually stained less intensely by methylene blue

than paracortical cells and otherwise have a range of characteristics intermediate between those of the other two cell types[4-6].

Other studies have indicated that paracortical cells have fewer microfibrils per unit macrofibril area, i.e. higher proportions of matrix material[5,7], and that their matrix proteins have a higher cystine content than the matrix proteins of the orthocortex[8]. Matrix high-sulphur proteins may vary in different wools[9-11] but the relationship between these variations and cortical cell type is not well defined.

The arrangement of these types of cortical cells in different fibres or along the same fibre may vary considerably[3,6,12]. While some types of arrangement, e.g. bilateral, have been associated with crimp[2], the association of other types of cell distribution with crimp or other fibre properties is not clear. There is some evidence that cell type proportions may influence tensile properties[13,14].

Overall, the evidence suggests that the proportion and distribution of cortical cell types may influence fibre properties but that further work is necessary to show this more clearly.

As a first stage in determining any relationships between cortical cell types and fibre properties it is necessary to describe the proportions of the cortical cell types in different wools. However, the available methods of making these measurements[6] are time-consuming and not suited to measuring the large number of fibres required for statistical analysis.

This paper described an image-processing algorithm designed to measure the proportions of cortical cell types in methylene blue-stained fibre cross-sections rapidly and automatically.

EXPERIMENTAL

Bundles of Romney wool fibres were cleaned in dichloromethane, ethanol, and distilled water, prior to embedment in acrylic resin. Two-μm-thick sections were cut onto glass slides and oxidised with performic acid prior to being stained in 1% methylene blue[15].

The fibre cross-sections were examined in a Zeiss light microscope using a 40× objective lens. A CCD camera (Siemens K210) attached to the microscope provided the input to a VAX image-processing system (VIPS) developed by the Dept of Electrical Engineering, University of Canterbury[16]. An image format of 256 × 256 pixels was used.

DESCRIPTION OF ALGORITHM

A wide variety of distributions and proportions of the three types of cortical cells is present in fibre cross sections. One merino fibre (the annular distribution) and some of the variations found in Romney fibres are illustrated in Figure 1. The cuticle delineates the periphery of each cross section as a dark line of varying thickness. Cells within the cross sections show varying intensities of staining.

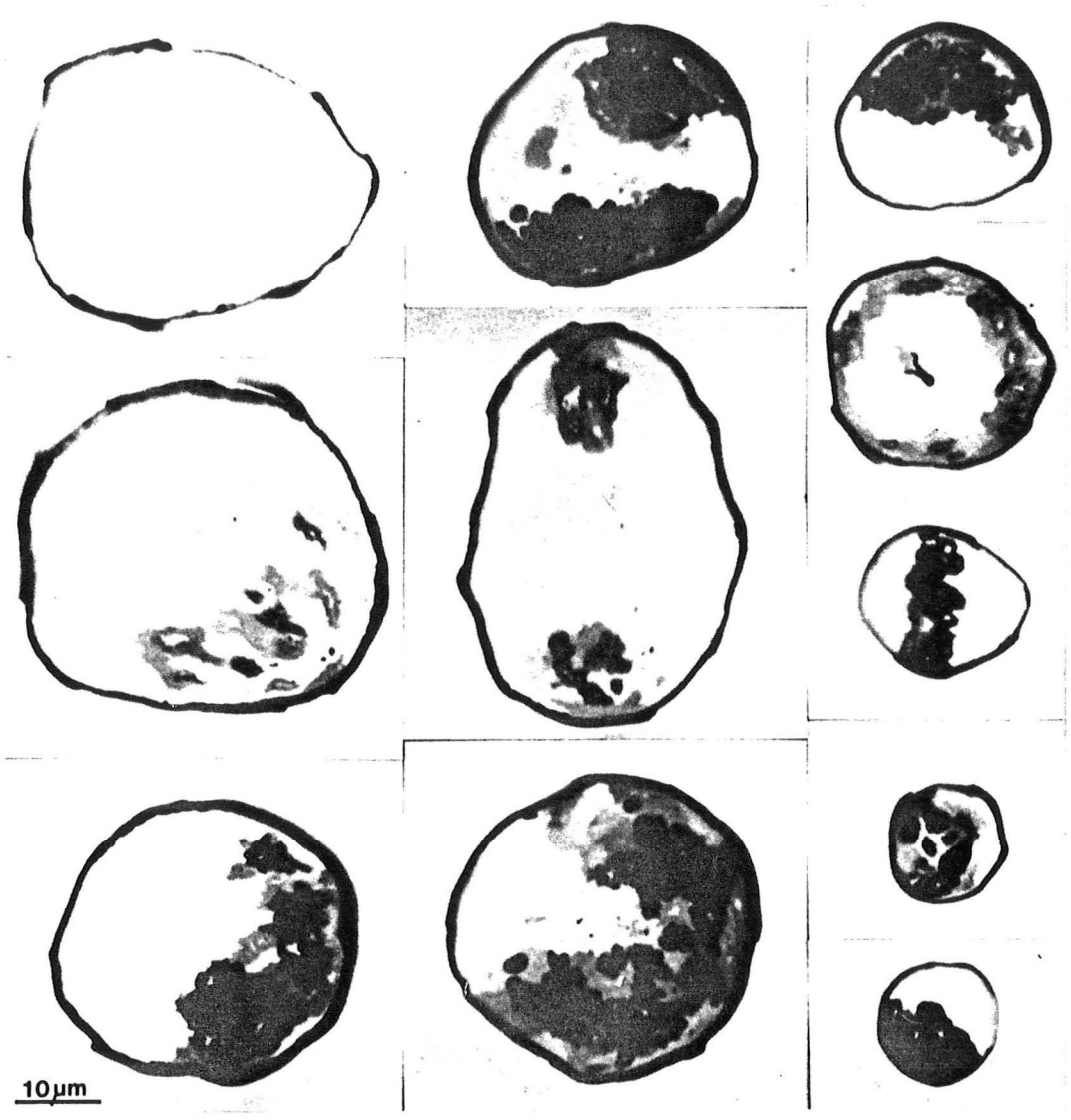

Fig. 1. Cross-sections of methylene blue-stained fibres

In addition to measuring cortical cell type proportions, the algorithm must deal with fibre cross sections which touch each other and those cut off by the frame of the image. Dirt on the slide and uneven illumination pose other problems. No solution was found to deal with cross sections

which overlapped each other.

The algorithm was split into three phases. These were:

(a) image preparation to provide suitably noise-free cross sections for measurement;

(b) image processing and measurement; and

(c) verification of the results.

A typical image of some fibre cross sections is used to illustrate the changes in the image that occur during the running of the algorithm. The grey-scale image (Fig. 2) contained some fibres in contact with each other and other noise such as dirt and uneven illumination.

Pseudo-colour was used in interactive viewing to indicate more accurately the differentiation of the cross sections into cuticle, orthocortex, mesocortex, and paracortex. For instance, less intensely stained cells at grey levels between 78 and 94% of the background level normally gave an accurate representation of mesocortex in pseudo-colour (Fig. 3).

The next step corrected for uneven background grey levels due, for example, to uneven illumination, by subtracting a cross section-free background image from the image to be processed. A binary image was then obtained by thresholding the image using the mesocortex to orthocortex grey level (94%). Everything darker was set to white and everything lighter to black (Fig. 4).

The binary image was then chain-coded[17]. Short chains (in terms of both length and area) were discarded as noise. The remaining chains were drawn into an empty image and all areas within the chains were filled, i.e. set at white (Fig. 5).

Fibres in contact were separated at this stage after locating them by the concavities between the fibres. A separation line was drawn between the nearest concavities. This image was chain-coded again and any remaining short or small chains were eliminated. Any cross-sections intersected and cut off by the image frame were also eliminated, leaving the remaining fibres ready for measurement (Fig. 6).

The measurement of the areas in real terms required a calibration of the pixel dimensions, i.e. their aspect ratio. A 100-μm-diameter circle was used for this purpose. This typically gave pixel heights of 0.50 μm and pixel widths of 0.79 μm, i.e. an aspect ratio of 0.676 in our imaging system.

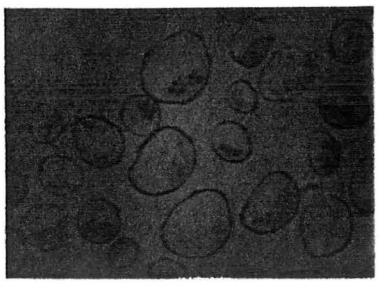

Fig. 2

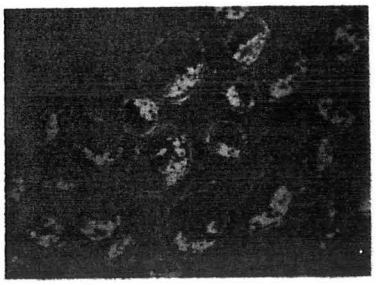

Fig. 3

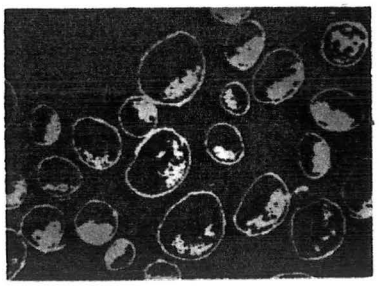

Fig. 4

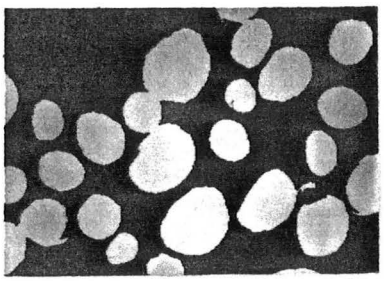

Fig. 5

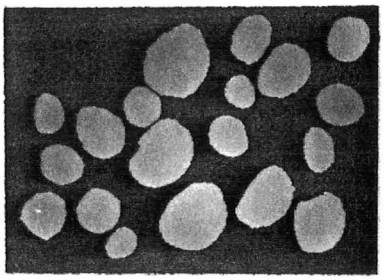

Fig. 6

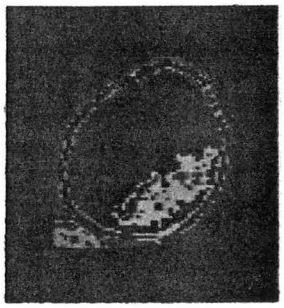

Fig. 7

Fig. 8

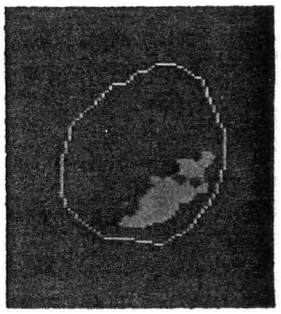

Fig. 9

Fig. 10

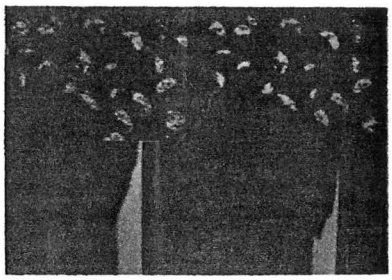

Fig. 11

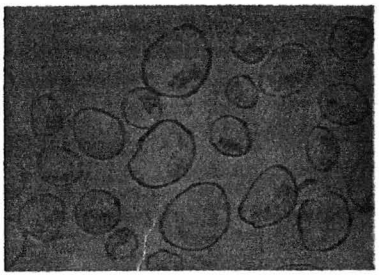

Fig. 12

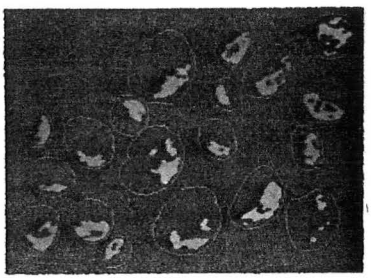

Fig. 13

Individual cross sections remaining after processing were then prepared for measurement. The raw image of one cross section showed noise around the cuticle and within the cortex (Fig. 7). The image was first median-filtered to reduce point noise (Fig. 8). An edge-enhancement filter was used to sharpen the edges of the fibre and the boundary between the orthocortex and the meso-/paracortex.

The fibre was then measured; the cuticle edge was marked (Fig. 9) and

whole-fibre measurements were made. These could include fibre cross-sectional area and maximum/minimum diameter. In the next step, the pixels corresponding to the cuticle were removed (Fig. 10) and cortical areas and diameters were measured. In addition, the areas of orthocortex, mesocortex and paracortex present were measured according to their grey level settings. The data from these measurements were stored in a separate VAX file while awaiting further analysis. The processing and measurement of each cross section took about one minute.

Result verification involved simultaneous viewing of pre- and post-processed images (Fig. 11). The images could be checked against the light microscope preparations as well. Typically, processing was carried out in BATCH overnight and the pre- and post-processed images were kept in a file so that they could be viewed at the convenience of the operator. This system allowed incorrectly processed fibres to be readily detected and their data to be deleted from the VAX data file subsequently. Additional information about the cross sections was obtained from a histogram of grey level intensities, colour-coded to indicate cortical cell types (Fig. 11).

The classification of cortical cell types in methylene blue-stained cross sections by eye and by the image-processing system correlated well (*cf.* Figs 12 and 13). Measurements of orthocortical cell proportions, obtained by the image-processing system and by hand drawing and digitising the same cross sections also agreed well. For example, 60 fibres from four Romney sheep were compared; these fibres had a good range of cortical cell type distributions and stain intensities and the results were:

$$\frac{\text{VIPS area (ortho)}}{\text{VIPS area (total)}} \quad \frac{23\,724}{29\,690} \times 100 = 79.9\ \%$$

$$\frac{\text{Drawn area (ortho)}}{\text{Drawn area (total)}} \quad \frac{23\,418}{29\,937} \times 100 = 78.2\ \%.$$

DISCUSSION

The image-processing system described here represents a much faster and more reliable way of obtaining information about fibre cross sections than the more tedious hand drawing and digitising method previous employed [6,14]. The accuracy of the VIPS system seems to be at least equal to that of the hand-drawing method and is actually probably more accurate given that hand drawing does not really differentiate the inner and outer edges of the cuticle. A more reliable assessment of varying stain intensities is also

made. The image-processing system represents an improved way of gaining information about the fibre cortex with sufficient speed and with statistically significant numbers of fibres to enable the role of cortical cell types in fibre properties to be explored further.

Although the object of this study was to determine cortical-cell proportions, the algorithm is capable of measuring a wide range of fibre cross-section characteristics. As well as the classification of wools according to cortical cell type, the system will allow comparisons to be made between fibre types from different species.

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