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AN INVESTIGATION OF THE INTERFERENCE COLOURS TRANSMITTED BY MATURE AND IMMATURE COTTON FIBRE UNDER POLARISED LIGHT MICROSCOPY

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Abstract

A study of interference colors transmitted under crossed polars by mature and immature cotton fibers has shown that the yellow hue of the 'mature' interference color is the same between cotton plant species and appears to be dependent upon relative fiber wall thickening. That the interference colors appear to be independent of cross-sectional wall area and perimeter and dependent upon relative fiber wall thickening, suggests that a case can be made to automate the standard test method for measuring cotton fiber maturity utilizing polarized light microscopy. Such a test would enable a quick and accurate direct measurement of cotton fiber maturity.

Introduction

Cotton fiber maturity is an important property to spinners and fabric manufacturers because it determines how well fibers will process both from a chemical and physical perspective. Immature cotton fiber, that is fiber with little or no fiber wall thickening, is associated with the formation of small entanglements called neps, irregularities in processed fiber assemblies including finished yarns, non-uniform dyeing of fabrics and decreased processing efficiency. The measurement of cotton fiber maturity has proved a difficult technical problem. At the moment there is no fast and accurate test method for testing fiber maturity in commerce and industry.

Measuring the wall area (A_w) and perimeter (P) of magnified cotton fiber cross-sections, and calculating the degree of wall thickening (θ) (Equation 1), has been regarded as the best reference of cotton fiber maturity. Although theoretically accurate, this direct method suffers from significant experimental error due to the fine detail involved in preparing fibers for measurement and the limited number of fibers that can be practically measured. These issues have limited the application of this reference method and left maturity measurement (and calibration) to indirect techniques, which have failed to generate industry confidence because of their lack of accuracy and precision.

$$\theta = 4\pi A_w/P^2 \qquad (1)$$

Polarized light microscopy (PLM) is a technique that has been used to investigate the crystalline structures, or birefringent nature, of inorganic and inert organic materials including textile fibers. When plane polarized light is transmitted through a birefringent object the light ray is split into fast and slow rays, which propagate through the object at two different speeds. Upon emerging from the object a phase difference occurs between the fast and slow rays. When recombined into a single ray by passage through a second polarizer (analyzer) the rays interfere with each other, which in turn create different interference colors that highlight aspects of the specimen's crystalline structure.

A standard test (American Society for Testing and Materials (ASTM) 2000) exists for determining maturity of cotton fibers by viewing them through crossed polars and a first-order retardation plate. The interference colors assumed by fibers are the result of their maturity and the interaction of this property with the optical phenomena described above. The interference colors have been classified by Grimes (1945) (Table 1) in terms of the cotton fiber maturity they represent.

Table 1 – Colors of Cotton Fibers Viewed Under Polarized Light Microscopy

Fiber Classification	Without Retardation Plate	With Retardation Plate	
	First Order Colors	Additive Colors Second Order	Subtractive Colors First Order
Mature	light yellow white	yellow green	light yellow yellow
Immature	gray-blue gray	blue purple	orange-yellow orange

The major disadvantage of the PLM test is that an operator must make an arbitrary assessment of the colors assumed by the fibers and this subjective interpretation can contribute to large discrepancies in results. The test method as it is currently described is also too slow for routine test applications. Furthermore, it is thought that the color appearance is dependent upon A_w or absolute wall thickness (Lord and Heap, 1988) or, by implication the path length of light transmitted through the fiber, rather than the degree of fiber wall thickening (θ). For this reason the test has been considered suspect when required to fully distinguish between mature fibers of small perimeter and immature fibers of large perimeter.

Whilst the subjective nature and speed of the test can be overcome by utilizing color digital cameras and computerized image analysis, the notion that the technique is biased by absolute wall thickness needs to be rejected before the test is more widely accepted. This paper reports the results of a small but revealing study of the relationship between transverse fiber properties and the interference colors transmitted by cotton fibers viewed under a PLM fitted with a first-order retardation plate.

Materials and Methods

Three species of cotton, selected on the basis that they represented a wide range of transverse fiber properties, were examined in this study (Table 2).

Table 2 – Cotton Samples Investigated

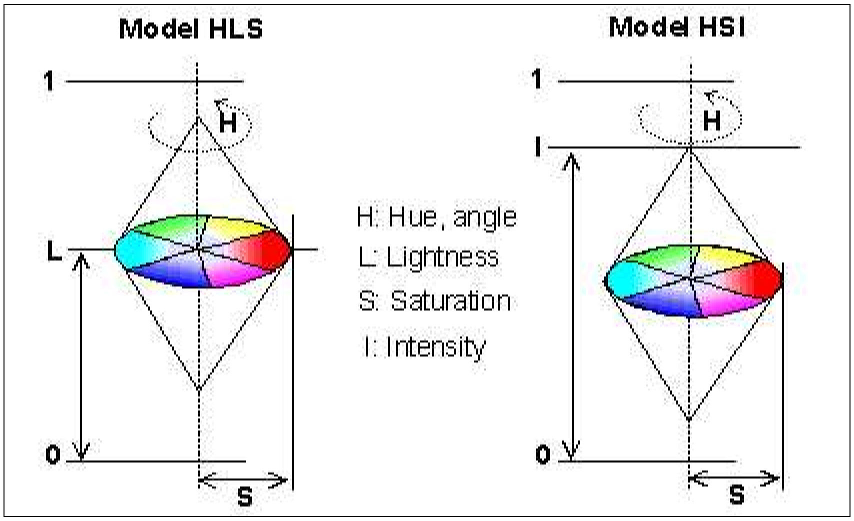
Sample	Sample description
12	*Gossypium barbadense*, International Cotton Calibration Standards (ICCS) G16 (Pima type cotton)
17	*G. arboreum*, ICCS K (very coarse tree cotton)
21	*G. hirsutum*, ICCS Dm (Upland type cotton)

A ‘Shirley’ comb-sorter was used to prepare fiber fringes for each of the three cotton samples so that they could be classified and selected according to their interference colors under a PLM with a first-order retardation plate. Initially, fibers were classed according to the color classification described by Grimes (1945). However, difficulty in interpreting fibers with mottled colors e.g., where fibers were folded and/or had large numbers of reversals, and blue-green tinged yellow fibers resulted in an ‘intermediate’ classification being designated. In most cases between 15 and 20 fibers were collected for each maturity category.

Fibers were then embedded in ‘Spurrs’ resin polymerized until firm at 60^{o}C. One micrometer cross sections were cut using a diamond histological knife. Sections were highlighted on the slide by rinsing them in a solution of 1% Methylene Blue and 1% hydrated sodium borate $Na_2B_4O_7 .10H_2O$ (‘Borax’) for five minutes at room temperature. After staining sections were permanently mounted in ‘Entellan’.

Cross-sectional images were magnified 40 times under an ‘Olympus’ polarizing light microscope and captured using a 575 x 767 pixel SONY 3 CCD color digital camera mounted to the trinocular tube on top of the microscope. Images were converted to 8-bit gray images within image analysis software ‘Optimas’ version 6.5 and transformed into binary images for analysis using a scripted set of transformations. The area morphometry command within ‘Optimas’ was then used to measure A_w and P of the binary images. Degree of fiber wall thickening (θ) for each section was then calculated from these values.

Following this the ‘mature’ (yellow) colors of longitudinal fiber sections from each sample were compared. Three ‘mature’ fibers from each sample were mounted longitudinally on a slide and magnified 40 times under the polarizing light microscope. Color digital images of these fiber sections were captured using the SONY 3 CCD digital camera and then converted to 24-bit HSI images within ‘Optimas’. The yellow color(s) of fiber sections were then sampled in ‘Optimas’ at 10 positions along their length using a 2 pixel diameter circular region of interest (ROI) cursor. Color values were expressed according to the HSI (hue, saturation and intensity) color (conical) space model (Figure 1). Hue (wavelength of light) and saturation (degree of color contrast) values were recorded and tested statistically across cotton species represented in the set.



From *http://semmix.pl/color/models/emo18.htm*

Figure 1 – Diagrams Showing the Spatial Relationships of Components in the Hue, Luminescence and Saturation (HLS) and Hue, Saturation and Intensity (HSI) Color Models

Results

Table 3 lists the mean cross-sectional properties of fibers from the three maturity groups. For the samples classed in this study mean θ values for 'immature' fibers ranged from 0.17 to 0.28. 'Intermediate' fiber θ values ranged from 0.49 to 0.63 and 'mature' fiber θ values ranged from 0.60 to 0.71. Fibers selected by the operator as being 'intermediate' were those where it was difficult to distinguish the fibers as being 'mature' or 'immature'. The overlap between 'immature' and 'intermediate' and 'intermediate' and 'mature' fiber classes illustrates the difficulty in subjectively determining where each maturity class starts and finishes.

Table 3 – Cross-Sectional Fiber Properties of Three Cotton Species (as per Table 2)

Sample	Immature				Average				Mature			
	A_w μm^2	P µm	θ	θ SD*	A_w μm^2	P µm	θ	θ SD	A_w μm^2	P µm	θ	θ SD
12	54.7	50.9	0.28	0.15	74.9	44.1	0.49	0.12	96.7	44.9	0.60	0.10
17	79.2	76.7	0.17	0.04	154.2	54.9	0.63	0.18	208.0	59.9	0.71	0.10
21	55.0	58.5	0.21	0.06	123.3	56.3	0.50	0.15	145.4	53.4	0.65	0.11

** SD = standard deviation of degree of thickening values*

Figure 2 shows θ and A_w results for individual fibers from the three species of cotton determined directly from cross-sectional analysis. The results are plotted according to the fiber maturity class i.e., 'immature', 'intermediate' and 'mature' fibers, as perceived by the operator using the PLM technique. It is significant that fibers classed as 'mature' under PLM can have a wide range of transverse fiber properties e.g., individual perimeter values ranging between 38 and 76 µm and A_w values ranging between 91 to 331 μm^2 and still be classed as 'mature' according to the color perceived by the operator. Also significant is the fact that obviously 'immature' and 'mature' fibers are significantly different across the three species of cotton examined.

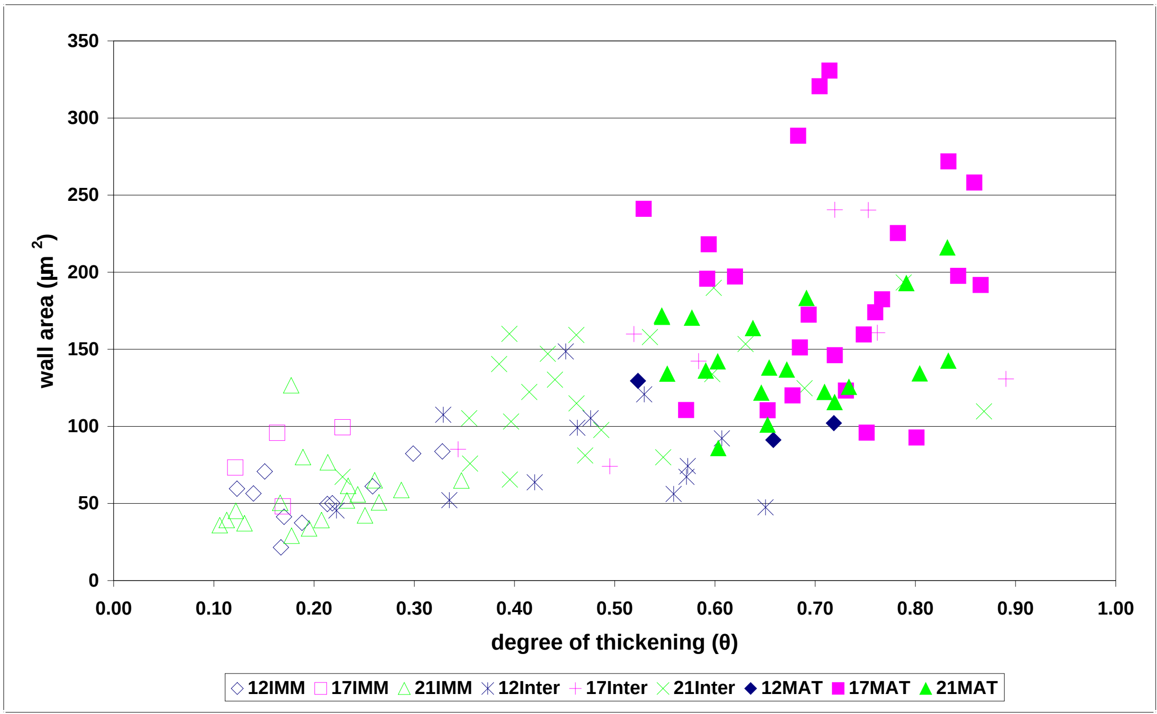


Figure 2 – The Relationship between Cross-Sectional Wall Area (A_w) and the Degree of Fiber Wall Thickening (θ) for Three Classes of Maturity Determined by Polarized Light Microscopy: 'Immature', 'Intermediate' and 'Mature'.

To test whether 'mature' (yellow) colors of fibers differ between cotton species of different cross-sectional fiber properties mean yellow hue and saturation values of 'mature' fiber samples were sampled and compared. Table 4 lists mean yellow hue and saturation values for 'mature' fibers from each cotton sample. Within the HSI model the general assignation of the frequency of light waves (hue) results in a division of the 0 to 255 scale into 3 color sectors of roughly equal size; red, green and blue. Along the scale the numbers from 30 (orange-yellow) to 70 (yellow-green) encompass both additive second order and subtractive first order yellow colors. Saturation values are also scaled from 0 to 255 with values approaching 0 corresponding to lighter pastels and values approaching 255 representing increasing richness of the particular hue. 'Low' and 'high' hue and saturation values describe the minimum and maximum values sampled each time by the ROI cursor.

Table 4 – Mean Hue (Yellow) and Saturation Values for 'Mature' Cotton Fibers of Three Cotton Species (as per Table 2)

Sample	Hue						Saturation					
	Start			End			Low			High		
	Av.	SD*	e**	Av.	SD	e	Av.	SD	e	Av.	SD	e
12	39	2.4	2.35	48	3.6	2.88	201	30.8	5.80	255	3.0	0.45
17	40	2.7	2.54	47	2.3	1.89	206	27.8	5.11	254	0.5	0.08
21	40	2.2	2.12	47	3.1	2.50	200	29.5	5.61	255	0.4	0.04

** SD = standard deviation*

*** e = precision = (t_{95}.CV)/√n-1 where CV = coefficient of variation of hue/saturation values*

Student t-tests (n = 30) confirm no significant difference in terms of yellow hue or saturation between the samples measured in this study. The reason for this has not been explored in this work although it is thought that the cellulose layers, which become more crystalline towards the center of the fiber, change the optical phenomenon commonly assumed for birefringent samples that are uniformly crystalline. These results support the results of a more extensive study by Grimes (1945) that the PLM test is able to differentiate between coarse immature fibers and fine mature fibers.

Moreover, the results allow the application of a universal (yellow) color threshold value in an automated PLM test to ‘measure’ the number and/or area of ‘mature’ fibers presented in a field of view to be applied with confidence. The application of such a threshold is illustrated in Figures 3(a), (b) and (c). In these photos the same threshold values are used to define and measure the area of yellow seen by the camera in a given field of view. The areas shaded in black in these Figures represent yellow areas selected by the same threshold. Application of a universal yellow threshold and analysis of the area it highlights can therefore effectively be used as a measure of cotton fiber maturity. Gordon *et al* (2004) have confirmed the significance of the relationship between percent area of yellow color in a field of view, calculated by applying a similar threshold, and fiber maturity as measured by the ‘Shirley’ Fineness and Maturity Tester (Figure 4).

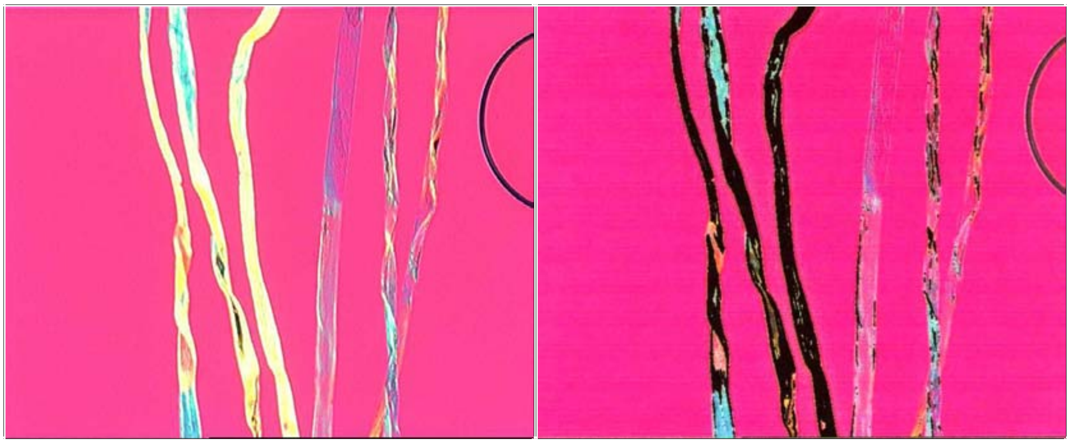


Figure 3(a) – Sample Number 12, *G. barbadense* Fiber Samples Showing Mature and Immature Fibers under Polarized Light Microscopy (left) and with Universal Yellow Threshold Applied (right).

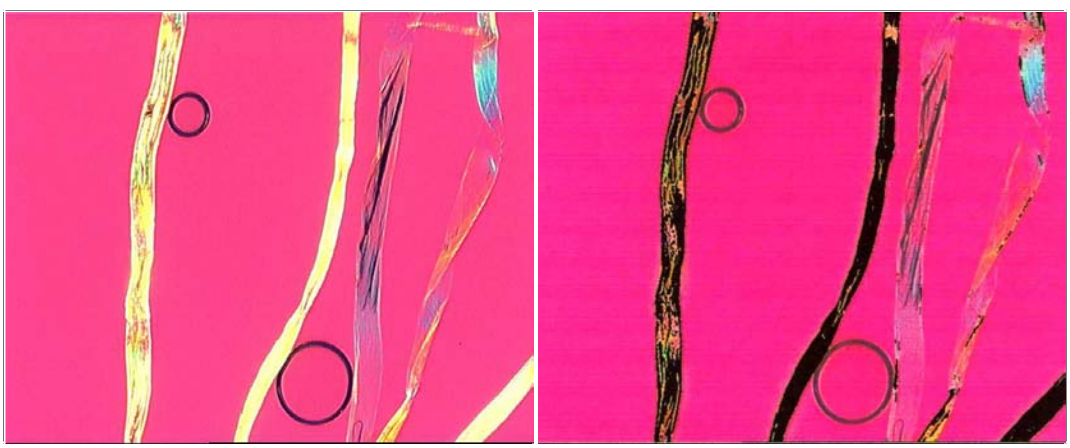


Figure 3(b) – Sample Number 17, *G. arboreum* Fiber Samples Showing Mature and Immature Fibers under Polarized Light Microscopy (left) and with Universal Yellow Threshold Applied (right).

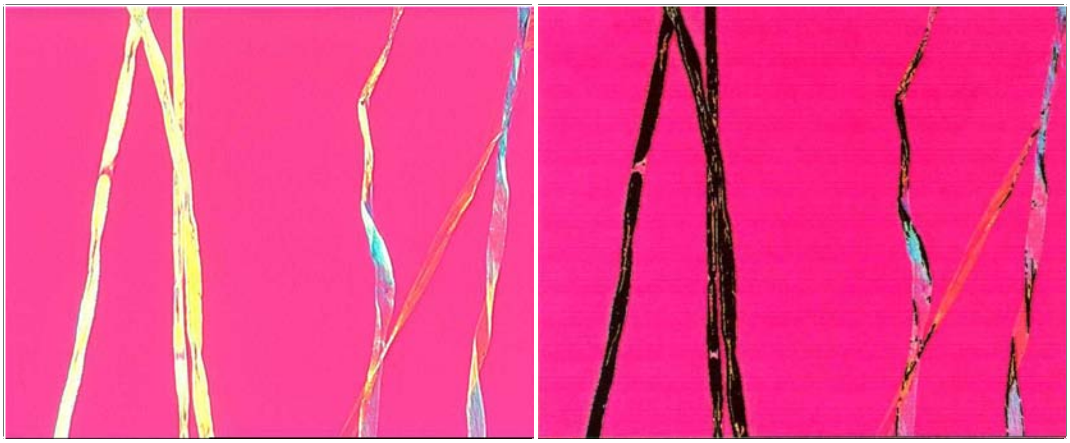


Figure 3(c) – Sample Number 21, *G. hirsutum* Fiber Samples Showing Mature and Immature Fibers under Polarized Light Microscopy (left) and with Universal Yellow Threshold Applied (right).

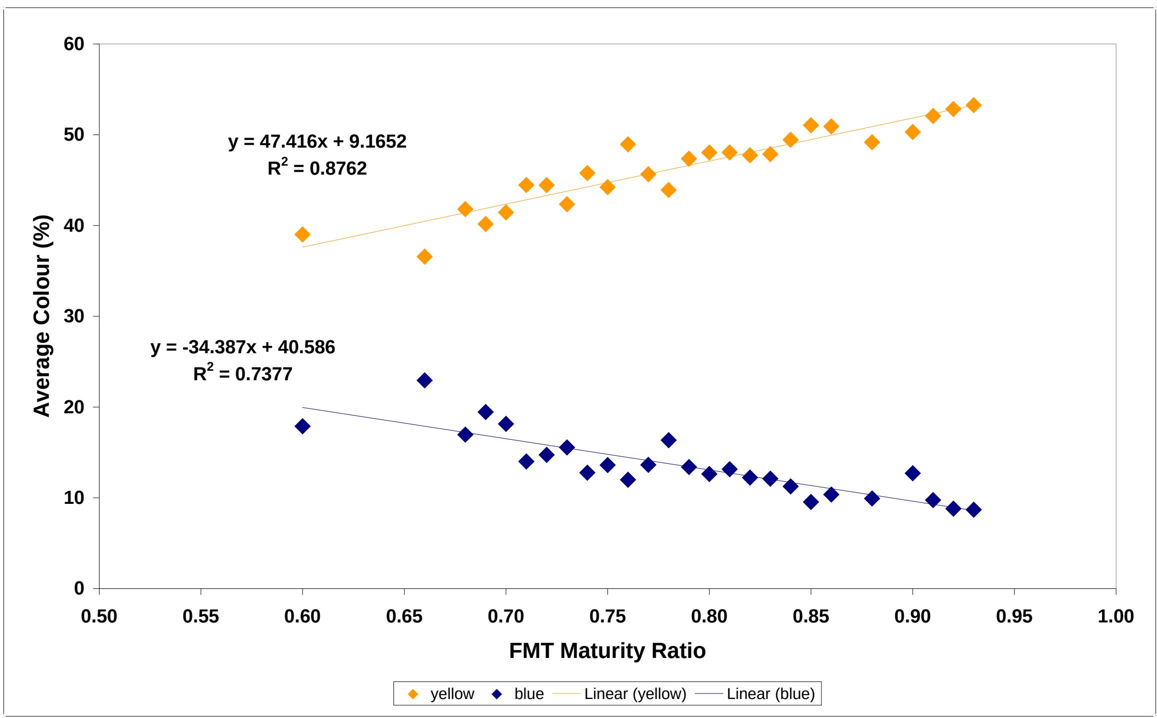


Figure 4 – The Relationship between Average Yellow & Blue Percent Area of Longitudinal Fiber Sections, Viewed Under PLM and Distinguished by Universal Yellow & Blue Thresholds, and the Maturity Ratio as Measured by the 'Shirley' Fineness & Maturity Tester (FMT). Relationships Defined by Linear Regression.

Conclusion

In this work we have found that 'mature' cotton fibers are represented by the same yellow hue irrespective of species and cross-sectional dimensions. The same yellow hue was transmitted by 'mature' fibers with small A_w and P and those with large A_w and P. This work confirms that a universal yellow threshold can be applied in the analysis of color images to distinguish and measure 'mature' fiber area as part of an automated PLM test method. This work supports more recent work (Gordon *et al*, 2004) showing that a good relationship exists between the percent yellow fiber area as measured by a universal yellow color threshold and cross-sectional measures of the fiber maturity.

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