THE ACCURACY AND PRECISION OF THE SIROMAT™ INSTRUMENT

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Abstract

SiroMat™ is an instrument that directly measures cotton fiber maturity. In this study the accuracy of SiroMat™ is illustrated using a sub-set of international cottons with reference values of fiber maturity measured according to the recognized fundamental method. The precision of the SiroMat™ instrument is measured by assessing the repeatability of test results on mature and immature cotton. Accuracy and precision of the instrument are largely defined by the relationship between color area measurements and measured maturity values of a reference material. It is noted that the inherent experimental error of the reference material measurements is likely to be significant in determining the accuracy of SiroMat™.

Introduction

SiroMat™ is an automated version of the polarized light microscopy (PLM) Standard Test Method (ASTM D1442, 2000), which uses interference colors transmitted by cotton fibers placed between crossed polar lenses and a first order retardation plate, to identify the maturity of a cotton specimen. In previous work Gordon and Phair (2005a) surveyed a wide range of cotton fibers from different cotton plant species that had widely divergent cross-sectional fiber properties. They found the yellow hue transmitted by fibers under PLM was independent of cross-sectional wall area and perimeter and dependent only upon relative fiber wall thickening. This suggested the case for an automated and therefore objective version of the PLM test would be successful.

Previous work has illustrated how SiroMat™ data including measurements of the maturity distribution in a specimen, may be used with respect to establishing linkages between agronomy and crop physiology work and fiber quality (Gordon *et al*, 2007; Long *et al*, 2008 and Bange *et al*, 2009) and in determining and controlling mill quality with respect to fiber maturity (Gordon *et al*, 2008). This paper discusses the accuracy and precision of the SiroMat™ in relation to the image analysis processes used by the instrument to capture information about fiber maturity. The paper represents an interim report on work currently being undertaken to calibrate SiroMat™ with the 104 International Textile Center (ITC) fiber maturity reference cottons (Hequet *et al*, 2006). For this paper the accuracy of SiroMat™ is demonstrated on a small sub-set of the ITC cottons, whilst precision is demonstrated in terms of repeat measurements over time.

Materials and Methods

Selection and preparation of cotton samples

A small sub-set (N = 15) of the 104 ITC reference cottons representing a wide range of maturity (theta) and fineness (cross-sectional area & perimeter) values was selected. Theta measured from the dimensions of fiber cross-sections is regarded as the most accurate measure of relative fiber wall thickening (Lord and Heap, 1988). Table I lists the cottons in this sub-set with their cross-sectional fiber maturity and fineness values. Figure 1 provides an illustration of the range of maturity values included in the set. For convention theta values are converted to maturity ratio values using the conversion co-efficient of 0.577 determined by Pierce and Lord (1939); i.e. maturity ratio = theta/0.577.

Table I – Measured cross-sectional properties of ITC sub-set

Sample	XS Area (μm^2) Mean	Perimeter (µm) Mean	Theta Mean	Maturity ratio Mean
2684	84.6	45.8	0.52	0.90
2999	89.7	51.1	0.45	0.78
3008	82.1	48.0	0.47	0.81
3074	134.4	54.7	0.57	0.99
3089	91.5	61.3	0.33	0.57
3096	100.4	57.6	0.40	0.69
3104	106.5	56.9	0.43	0.75
3112	121.7	54.3	0.54	0.93
3115	97.2	59.0	0.37	0.64
3156	115.3	50.0	0.59	1.02
3159	125.5	58.1	0.49	0.85
3167	124.3	53.4	0.56	0.98
3183	121.0	55.4	0.51	0.89
3212	108.6	47.7	0.61	1.05
4409	124.8	54.3	0.55	0.94

XS = cross-sectional

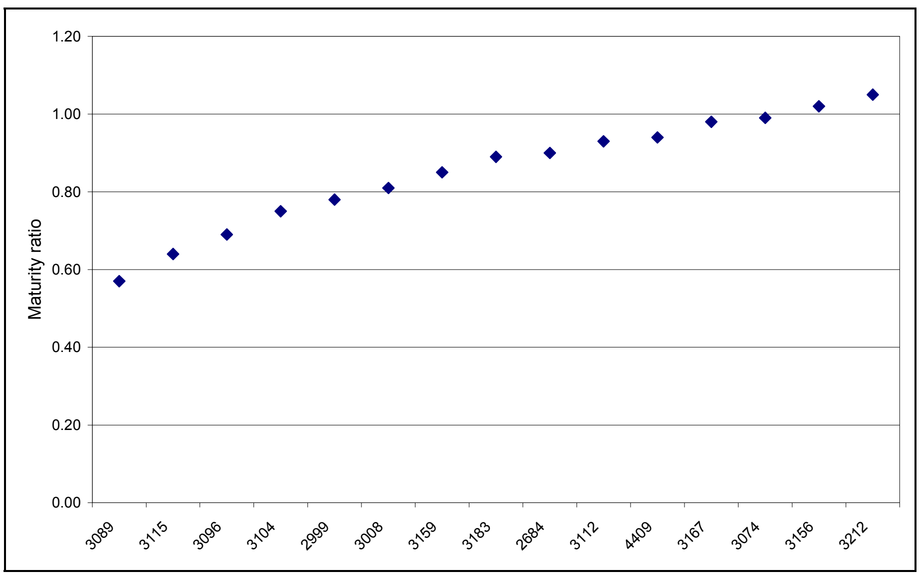

Figure 1 – Range of maturity ratio values in the tested ITC sub-set

Preparation of SiroMat™ specimens from the ITC cottons involved guillotining a fiber beard prepared using a 'Fibrosampler' to obtain between 2 to 3 mg of 1 mm snippets from two cuts near the aligned end of the beard. Snippets were collected and then spread in an annular pattern on a 5 cm x 7 cm glass slide using an OFDA™ fiber spreader. A clean 5 cm x 7 cm slide was used to cover the specimen. Castor oil (refractive index = 1.477 – 1.481) was used as the mounting medium to enhance the contrast of the fiber snippets to their background. Four specimens were prepared and tested for each of the selected ITC cottons.

Instrument testing – image analysis

Preparing the SiroMat™ instrument for testing involves allowing the light source to reach its optimum color temperature (for a halogen light source) and adjusting the digital camera settings (U balance, V balance and shutter speed) to a prescribed background (magenta) color in terms of red, green and blue (RGB) ratios. The prescribed background co-ordinates are predetermined empirically by measuring a range of fiber maturity samples to determine the greatest range in terms of percent area of yellow hue in the snippets presented. Background colors are checked at regular intervals during testing to minimize drift in instrument readings.

SiroMat™ automatically captures and analyses cotton fiber snippet images in 36 fields of view, each of approximately 9 mm^2, as shown in Figures 2a and 2b. From these images the percent area of particular interference colors assumed by the snippets under PLM is determined. These area measurements are then converted to conventional maturity values via a conversion or calibration equation.

Figure 2a

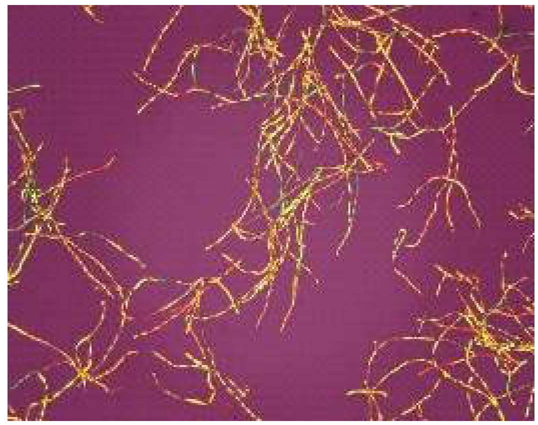

Figure 2b

Immature (2a) and mature (2b) fibers in the SiroMat™ field of view

The percent area measurement is determined using a series of standard image analysis functions starting with edge detection to find the boundaries of fibers, followed by image dilation to join small disconnected pieces (pixels) together. At this stage the image is converted to a binary image based on a grey-scale threshold, which is then subject to the processes of image dilation and erosion; these are standard image processing techniques used to close small holes in images. The resultant image is used as a mask to select the fiber area from the background.

Following creation of the mask the image is broken up into fiber sections according to image cross-over points. To do this the mask image is skeletonised to reveal branch points, which are used to segregate and label fiber sections. The image at this point is a mask with a number of labeled segments; each segment containing a portion of a single fiber snippet. A background correction and normalization is applied to the original color image before the proportion of pixels in each color threshold bin, i.e. yellow, red, green and blue, is counted for each segment in the mask. Color threshold levels are set according to a digital color space model, e.g. HSL, which defines changes in hue according to numerical segments between the values of 0 and 255.

Total pixel number and proportion of yellow, red, green and blue color pixels per segment are counted along with the major and minor lengths from which the aspect ratio is calculated and reported. Small segments can be filtered and excluded from the data set at this stage, although the optimum segment size is still being assessed. A conversion equation is then applied to produce a maturity scale number; for convention the scale is given in terms of maturity ratio, the number reported by the ASTM Standard Test Method D1442 (2000).

The analyzed segments now with a maturity ratio value can then be sorted according to their frequency into a distribution of MR segments from which the mean, standard deviation and skewness can be calculated and reported.

Data analysis

Raw color image analysis measurements (Table II) were related to ITC reference values in a correlation matrix in order to determine the legitimacy of independent variables (IVs), i.e. percent area of the yellow, red, green and blue, for inclusion in a regression to predict the dependent variable (DV) maturity ratio. Table III lists the Pearson correlation and probability values for the relationship between each variable.

Table II – Unfiltered image analysis color results – color values represent percent of color area (pixel) measured in analysis

	Yellow	Red	Green	Blue
2684	28.87	68.03	0.39	0.93
2999	25.48	71.93	0.27	0.59
3008	19.60	78.40	0.08	0.21
3074	35.43	62.24	0.23	0.22
3089	17.29	79.98	0.22	1.03
3096	22.83	74.15	0.33	1.04
3104	34.46	62.11	0.50	1.10
3112	33.18	64.34	0.30	0.37
3115	26.29	69.73	0.47	1.83
3156	35.70	61.65	0.31	0.41
3159	30.10	67.14	0.32	0.70
3167	35.85	61.43	0.37	0.53
3183	32.84	64.44	0.39	0.61
3212	31.78	65.94	0.17	0.23
4409	33.07	64.41	0.28	0.41

Table III – Correlation matrix (Pearson correlation and *probability*) of ITC and SiroMat™ IA values

	XS Area	Perimeter	XSMR	Yellow	Red	Green	Blue
Perimeter	0.254 *0.361*	-					
XSMR	0.582 *0.023*	-0.631 *0.012*	-				
Yellow	0.778 *0.001*	-0.180 *0.521*	0.766 *0.001*	-			
Red	-0761 *0.001*	0.143 *0.612*	-0.721 *0.002*	-0.997 *0.000*	-		
Green	0.133 *0.637*	0.358 *0.190*	-0.212 *0.448*	0.383 *0.159*	-0.453 *0.090*	-	
Blue	-0.380 *0.162*	0.550 *0.162*	-0.759 *0.001*	-0.327 *0.155*	0.249 *0.235*	0.693 *0.370*	-

Maturity ratio values (DV) determined from theta values by Hequet *et al* (2006) were regressed with measured color data (IVs) from SiroMat™ in a forward stepwise regression using Minitab 15. Given the small number of samples (N = 15) and relatively large number of IV (k = 5) the F-to-enter value for inclusion of any IV into the regression equation was set at 4. Table IV lists the statistics including R-squared, adjusted R-squared, predicted sum of squares (PRESS) and Mallow's Cp, and the conversion equation coefficients and constant determined by the regression.

Table IV – Stepwise regression statistics

Response is XSMR on 4 predictors, with N = 15		
Step	1	2
Constant	0.3102	0.3875
Yellow	1.84	2.38
P-value	*0.001*	*0.000*
Green		-77
P-value		*0.000*
S	0.0956	0.0521
R-Sq	58.72	88.68
R-Sq (adj)	55.55	86.80
Mallows Cp	32.9	3.0
PRESS	0.16237	0.053105

To determine precision one slide each with mature (no. 3074) and immature specimens (no. 3089) were run for 40 consecutive tests; a period covering around 3.5 hours. The respective variation in values over the test period is shown in Figures 3 and 4. A summary of the mean and variation in values appears in Table V.

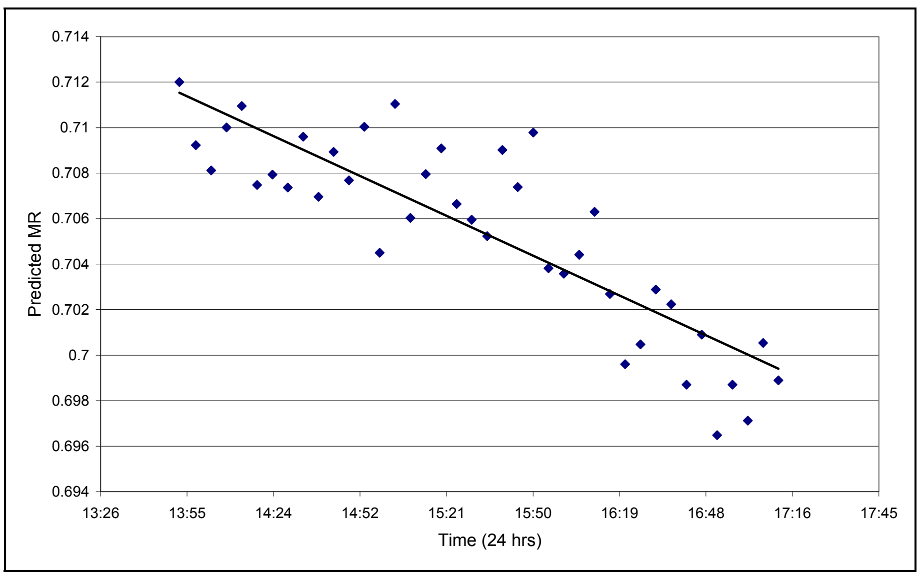

Figure 3 – MR test results over time ($t_1 – t_{40}$) for immature fiber specimen

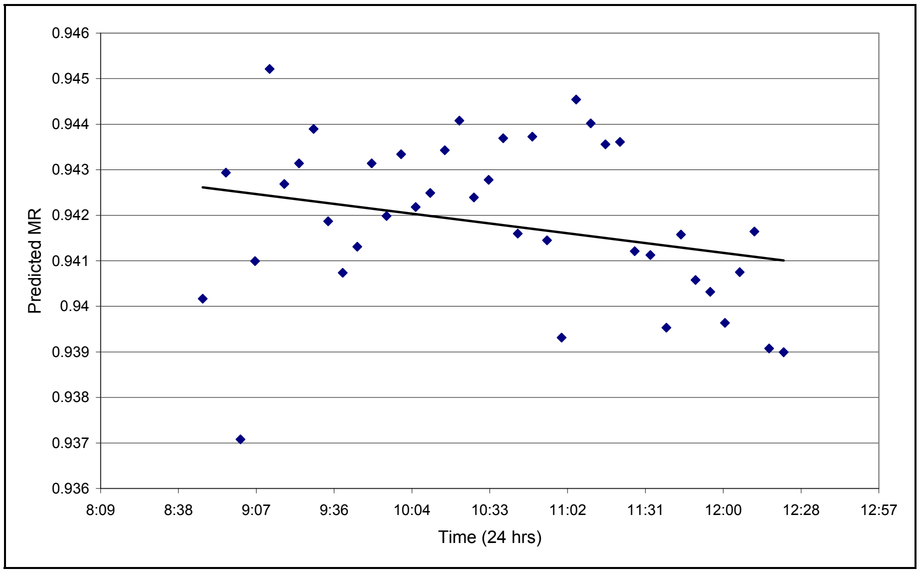

Figure 4 – MR test results over time ($t_1 – t_{40}$) for mature fiber specimen

Table V – Mean and variation in SiroMat™ repeat measurements (n = 40) over time

	ITC sample no.	
	3074 Mature	**3089 Immature**
Mean	0.942	0.705
Std	0.002	0.004
CV%	0.19%	0.60%

Results

The statistical data presented in Tables III and IV shows the strong relationship between the yellow color and the ITC reference values of maturity. The significant correlation between the yellow and the measurement of maturity ratio, and the poor relationship between the yellow and perimeter ratifies earlier work by Gordon and Phair (2005a), who showed that the yellow hue of fibers varied only with fiber maturity or relative wall thickening. The poor relationship between cross-sectional area and perimeter, a result of including a number of samples with the same maturity but different perimeter and cross-sectional area combinations, further enhances the legitimacy of the set's predictive power. Indeed, a poor relationship between absolute wall thickening and perimeter is a preferred condition in a maturity 'calibration' set, as the relationship between any IV predicting maturity and maturity ratio can be compromised by a high degree of co-linearity between these properties.

The high degree of co-linearity between red and yellow colors is regarded as inconsequential as the red color is not considered unique in the conversion of measured color area to a maturity result. Indeed the color references for the ASTM Standard Test Method do not include red as a color to differentiate mature from immature fibers (Grimes, 1945; ASTM, 2000), and differentiation of the small amount of orange hue associated with very immature fibers from the red hue range is considered too difficult in the application of the (SiroMat™) test. It is interesting to note that the proportion of red analyzed in SiroMat™ images can be reduced by inserting a day light filter across the field of view. A day light filter also increases the proportion of yellow and green analyzed although not the range of values.

Considering the small set tested here, the resulting regression shows abundantly the significance of the yellow and green colors as the primary IVs used to predict fiber maturity; the two term (yellow and green) multiple linear regression has an adjusted R-squared of 86.8 for the relationship with theta. The goodness of this relationship is reflected in the selection of the yellow color as the most significant variable and the green color as the second most important variable. Whilst a stepwise regression includes IVs only upon their statistical strength in the model, both the yellow and green are important physical variables is deciding the relative maturity of a fiber as per the original descriptions of the PLM test (Grimes, 1945; ASTM, 2000). Further, the Mallow's Cp statistic (3.0) calculated for this small subset is consistent with the ideal Mallow's Cp value, which should indicate the number of predictors (2) plus the constant (1); a value close to this number indicates the model is relatively precise and unbiased in predicting future response (Minitab 15, 2006).

Interestingly, the standard error of estimate (S) (0.052) and the PRESS (0.053) results whilst not directly comparable are within the same range as standard error of estimate values calculated when using SiroMat™ results to predict the percent of immature fibers in purposely constructed mature and immature fiber blends (Gordon *et al*, 2007). At that time SiroMat™ was calibrated using maturity ratio values determined from an IIC 'Shirley' FMT; the conversion equation for this calibration set also containing the yellow and green color values (Gordon and Phair, 2005b). It remains to be seen what effect a larger set of ITC reference samples has on this regression equation and what experimental error effects associated with SiroMat™ and the ITC reference values can be highlighted and partitioned.

Whilst repeat measurements over time showed no significant statistical differences between mean results measured at t_1 and t_{40}, there were significant trends away from the value recorded at t_1 for the immature sample. Longer examination and consideration of the light source used to illuminate the SiroMat™ specimen is required to understand these trends further.

Conclusion

In this paper a small sub-set of ITC with accepted reference values of fiber maturity (theta) measured according to the fundamental method were measured by SiroMat™. Raw color values measured by SiroMat™ were cast as IVs and subject to a competitive stepwise regression process with the maturity reference values. The results of this analysis reflected previous unpublished work (Gordon and Phair, 2005b), which showed the yellow and green hues of fibers under PLM were the most appropriate variables, both from statistical and physical perspectives, to predict fiber maturity.

Longer examination and consideration of the light source used to illuminate SiroMat™ specimens is required to understand the variation seen between repeat tests particularly for immature fiber specimens.

Acknowledgements

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