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UPDATE ON PROPOSED REFERENCE METHOD FOR COTTON MATURITY PART 1: SAMPLE PREPARATION AND IMAGE ANALYSIS

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Abstract

The proposed reference method for measuring the maturity of cotton fiber includes procedures for: sampling the cotton; forming a bundle of well-aligned fibers; embedding the bundle in a polymer matrix; cutting a thin cross-section of the bundled fibers; imaging the cross-sections with a light microscope; and using image analysis software to characterize the size, shape, and degree of development (maturity) of the cotton. The Southern Regional Research Center (SRRC) and the International Textile Center (ITC) applied the method to seven bales of specially selected cottons. The maturity results from the two groups were in excellent agreement.

Introduction

Two years ago at the Beltwide Conference, Thibodeaux, et al., (2000) reported on the continuing effort to define well values of fineness and maturity for large quantities of diverse cottons. The reference method for measuring fiber maturity based on microscopic image analysis of thin sections of fiber bundles was applied to fiber samples covering the range of properties of U.S. cottons. These samples had been obtained from cooperating cotton breeders who harvested the cotton from selected positions on the plant. Two different software packages for assessing fiber maturity by image analysis, one from Leica International and another from Professor Bugao Xu, University of Texas, were evaluated. We found that: a) the image analysis reference method gives reliable data with a minimum of problems and can be used in other laboratories; b) selective harvesting gives reasonable differences in fiber area, perimeter, and micronaire, but only marginal differences in maturity; c) results from Leica and Xu are compatible but Xu's software is faster and easier to use; and d) micronaire measurements alone are not good predictors of fiber maturity. The following documents cooperative research effort at the Cotton Fiber Quality Research Unit of the Southern Regional Research Center (SRRC) and the International Textile Center (ITC) that extends the original work so as to include bales purchased commercially that represent the diversity of the U.S. crop.

Materials and Methods

Seven bales of cotton were purchased and blended into card web at the ITC. Samples of the card web from the seven bales were shared between SRRC and ITC. At the respective centers the fibers were separated into small bundles, embedded with a methyl-butyl methacrylate prepolymer basically according to the method developed at SRRC (Boylston, et al., 1993). The embedded bundles were trimmed, mounted on a microtome, and cut into thin (~1 μm) sections that were mounted on microscope slides. Each section contained between 500 and 1000 single fiber cross-sections. The cross-sections were imaged on a transmission microscope equipped with a video camera interfaced to a personal computer. Both SRRC and ITC utilized the FIA (fiber image analysis) software developed by one of the authors (Xu). A typical video image from one of the fields of fiber cross sections is shown in Figure 1. When the FIA software is used to analyze such an image, it converts the gray level image into a binary image as shown in Figure 2. The binary image detects the outer boundary (perimeter) of each fiber cross-section and clearly delineates the difference between the inner lumen and the cell wall of the fiber (i.e. the portion that lies between the outer perimeter and inner lumen. The FIA software yields results that include but are not limited to the area and perimeter of the cell wall and the lumen and also the degree of thickening or circularity ($\theta = 4\pi A/P^2$) where A is the area of the cell wall and P is its outer perimeter.

Results and Discussion

Results from the image analysis of the seven bales by the ITC are given in Table I. Data is given on the number of cross sections measured, and the average perimeter, area, and theta. As will be seen in this report, the ITC was able to process about 40,000 cross-sections per bale. This represents about 80 bundles of fibers each that were embedded, sectioned and

scanned for image analysis. The range of fiber perimeters is from about 46 to 56 μm. The range of fiber cross-sectional areas is from about 80 to 130 μm^2. The range of θ measurements is from about 0.42 to 0.57.

Results from the image analysis of the seven bales by the SRRC are given in Table 2. The numbers of cross sections measured at SRRC was much smaller than was carried out at ITC. On average, for each bale, this represents about 12 bundles of fibers that were embedded, sectioned and scanned for image analysis. In general the SRRC fiber perimeters and areas are larger than the ITC values. However, the θ values are quite close. This observation is confirmed by comparisons of the average values from the seven bales. The range of fiber perimeters measured by SRRC is from about 49 to 59 μm or about 3 μm greater on the average. The range of fiber cross-sectional areas is from about 93 to 160 μm^2, or about 14 μm^2 greater on the average. The range of θ measurements is from about 0.42 to 0.58 (almost identical to ITC).

Consider now the individual parameters measured on each bale at SRRC versus at ITC. The average fiber perimeter values measured at SRRC versus at ITC are plotted in Figure 3. The correlation between the two sets of measured perimeters is excellent ($R^2 = 0.976$) but, the slope deviates slightly from unity (1.12) and the offset is about 3.5 μm. The average fiber wall area values measured at SRRC versus those measured at ITC are plotted in Figure 4. The correlation between the two sets of measured areas is quite good ($R^2 = 0.949$) but there is a non unity slope (1.31) and an offset of about 18 μm^2. The average values for degree of thickening θ as measured at SRRC versus those measured at ITC are plotted in Figure 5. The correlation between the two sets of measured thetas is good ($R^2 = 0.914$) and the slopes and offsets are near unity and zero, respectively.

Despite the fact that SRRC and ITC agree so well for θ, there remains the question as to why SRRC has slightly higher values of perimeter and area than does ITC. This, in fact remains one of the unanswered questions of this study. Since this first seemed that it might be a problem of calibrations, we checked the calibrations and found no problems. We have also exchanged physical samples and measurements at the two centers also agreed. The one area where the two laboratories differ is the fact that SRRC embeds the fiber bundle in a 1/8" i.d. tube while ITC saw fit to use a 1/16" i.d. tube. Given this, it might be possible that pulling the fiber bundle through a smaller diameter tube caused the fibers to be better aligned and thus leave a smaller projection or footprint when cross-sectioned. The plans are to embed some of the cottons in a 1/16" diameter tube at SRRC. In addition the statistical analysis of the data to determine how many sections and how many subsamples are required to assure a reasonably accurate characterization of the maturity of a bale of cotton is being addressed. Once we are successful in these last two endeavors we will seek approval for the method by the ISO.

Conclusions

- It is feasible to apply the proposed reference method for maturity to analyzing large numbers of samples.
- In comparing the two laboratories, there is excellent agreement as regards maturity measurements and good correlation for all three parameters.
- There are all indications that the present method can produce sufficient reference cottons to calibrate or standardize such indirect methods as F/MT, NIR, AFIS, and any other instruments under development.

References

Boylston, E.K., Thibodeaux, D. P., and Evans, J. P., *Textile Res. J.* **63,** 80-87 (1993).

Thibodeaux, D. P. and Rajasekaran, K., 2000 Proceedings Beltwide Cotton Conferences,748-751 (2000).

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Table 1. Average Values from ITC.

Bale ID	Fibers Tested	Perimeter (µm)	Area (µm)^2	Theta
2996	41,844	51.10	105.00	0.52
2999	42,237	51.10	89.70	0.45
3008	43,428	48.00	82.20	0.47
3009	43,785	46.10	85.56	0.52
3016	41,165	51.40	100.70	0.50
3074	40,055	54.70	134.40	0.57
3075	40,312	56.20	101.20	0.42
Mean Values		**51.23**	**99.82**	**0.49**

Table 2. Average Values from SRRC.

Bale ID	Fibers Tested	Perimeter (µm)	Area (µm)^2	Theta
2996	3,830	53.65	113.53	0.50
2999	4,491	54.05	102.06	0.43
3008	8,335	50.28	92.90	0.47
3009	5,362	48.99	95.50	0.50
3016	6,301	54.74	110.35	0.47
3074	3,082	58.89	160.06	0.58
3075	1,618	59.31	122.00	0.42
Mean Values		**54.27**	**113.77**	**0.48**

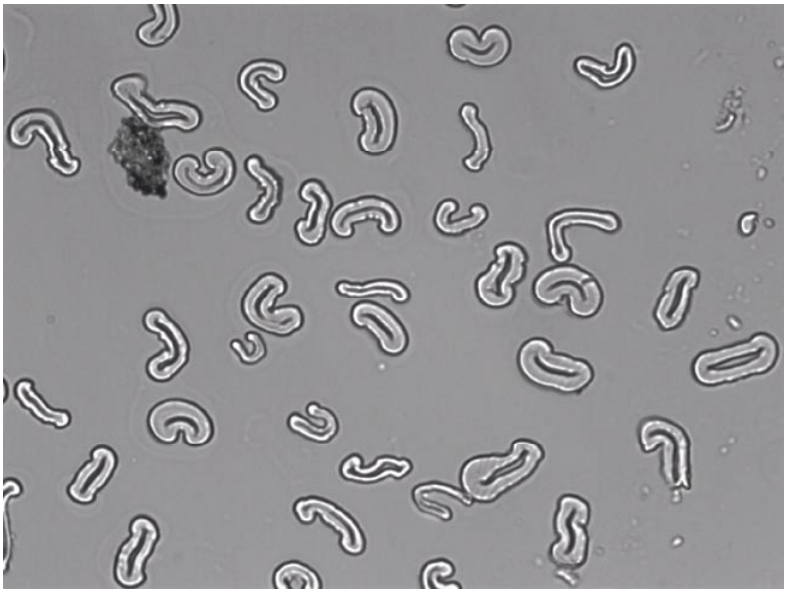

Figure 1. Video image of cotton fiber cross-sections.

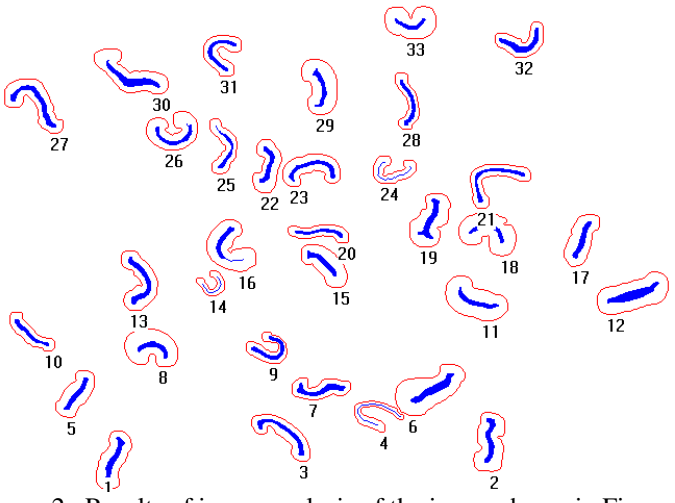

Figure 2. Results of image analysis of the image shown in Figure 1.

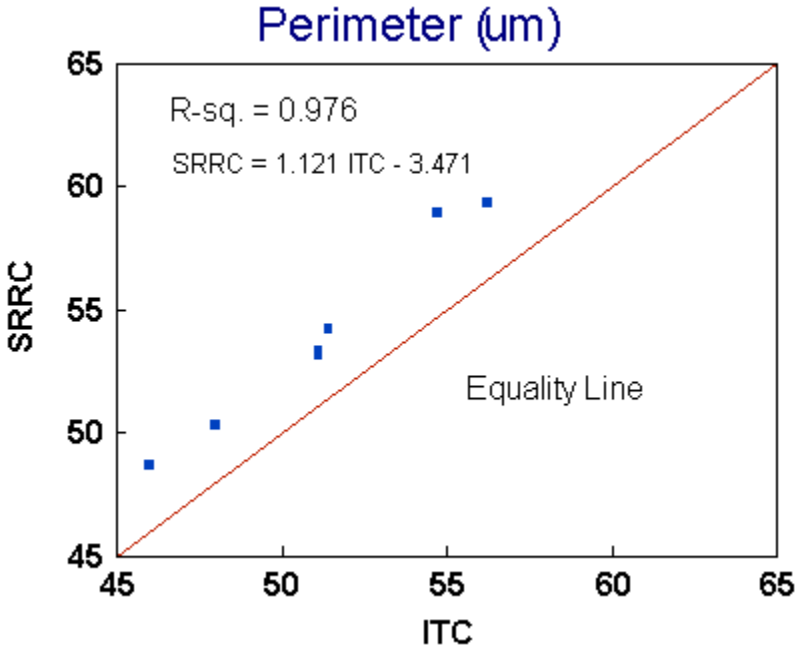

Figure 3. Comparison of perimeter values as measured by SRRC and ITC.

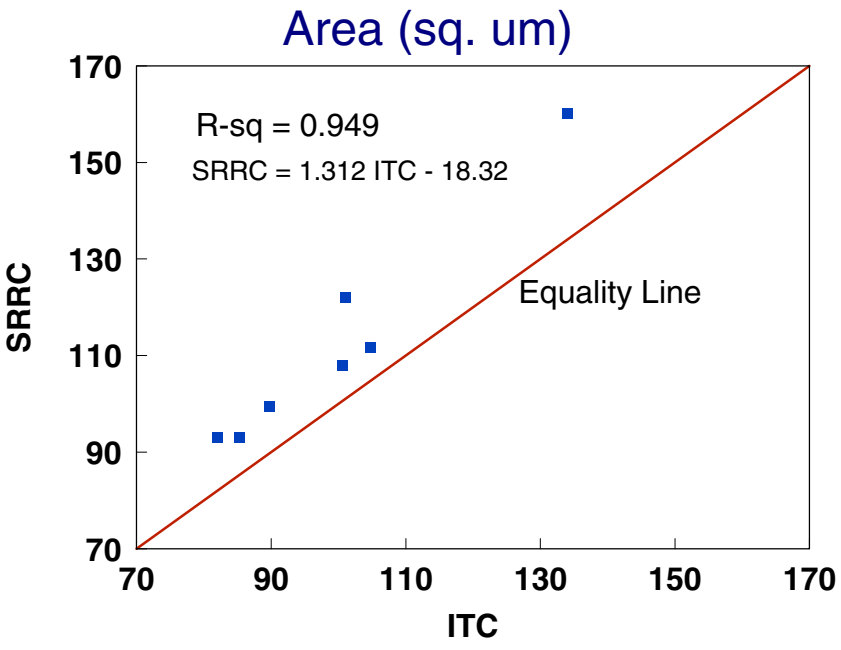

Figure 4. Comparison of area values as measured by SRRC and ITC.

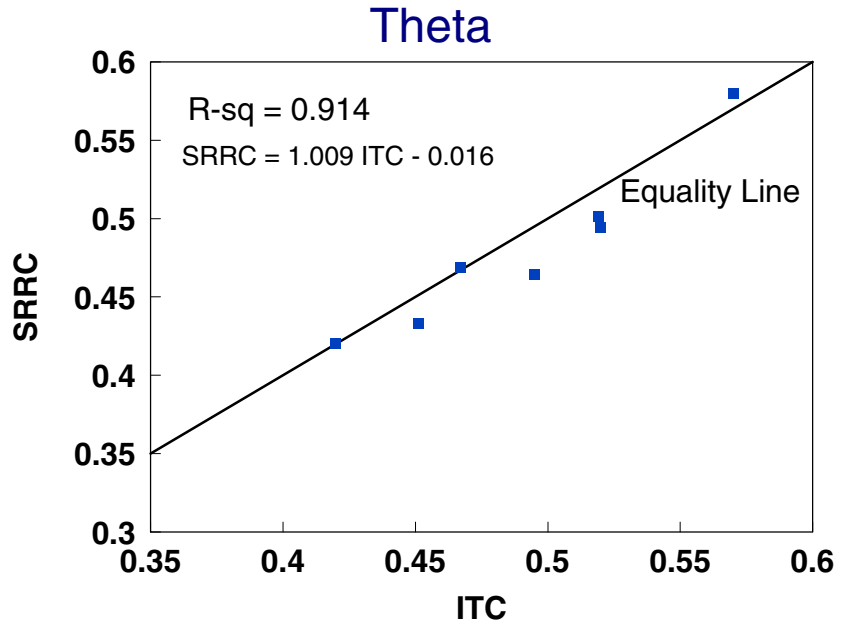

Figure 5. Comparison of theta values as measured by SRRC and ITC.