DIRECT AND RAPID DETERMINATION OF COTTON MATURITY BY FT-MID-IR TECHNIQUE

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Abstract

FT-mid-IR (FT-MIR) spectra of seed and lint cottons were collected to explore the potential for the discrimination of immature cottons from mature ones and also for the determination of actual cotton maturity. Spectral features of immature and mature cottons revealed large differences in the 1200-900 cm^{-1} region, and such spectral distinctions formed the basis on which to develop simple three-band ratio algorithm for classification analysis. Next, an additional formula was created to assess the degree of cotton fiber maturity by converting the three-band ratios into an appropriate FT-MIR maturity (M_{IR}) index. Furthermore, the M_{IR} index was compared with parameters derived from traditional image analysis and AFIS module. Results indicated strong correlations ($R^2 > 0.89$) between M_{IR} and M_{AFIS} and between M_{IR} and M_{IA} among either International Cotton Calibration (ICC) standards or selected cotton maturity references. On the other hand, low correlations between the pairs were observed among regular cotton fibers, which likely resulted from the heterogeneous distribution of structural, physical, and chemical characteristics in cotton fibers and subsequent different sampling specimens for individual and independent measurement.

Introduction

Cotton fibers are natural products and their end-use qualities depend on their stages of growth. In general, cotton development consists of four overlapping but distinctive periods: initiation, primary wall formation (elongation), secondary cell wall thickening (cellulose synthesis), and maturation (Hsieh, 2007). Such a structural evolution suggests the heterogeneous distribution of fiber components from inner layers to outer layers, with a large portion of non-cellulosic species in primary cell wall whereas majority of cellulose in secondary cell wall. Apparently, immature fibers are composed of less cellulose component than mature species. Presence of immature fibers has been found to be prone to entanglement formation during the mechanical processing and also to alter the desired color appearance in dyed yarn and fabric products (Gordon, 2007). Therefore, a term “maturity” has been used to describe the degree of development or thickening of the secondary cell wall in fibers. Useful information about fiber maturity is desirable to cotton breeders and growers for cotton enhancement and to textile processors for quality control.

Currently, cotton maturity has been measured through direct and indirect means. Direct methods are represented by light/polarized light microscope based such tests as caustic swelling, polarized light, and cross-section image analysis (ASTM, 2006; Thibodeaux and Evans, 1986), scanning probe microscope (Maxwell et al., 2003), scanning electron microscope (Goynes, 2003), and X-ray fluorescence spectroscopy (Wartelle et al., 1995). In general, mature and immature fibers can be readily determined from the microscopic view, but degree of maturity is difficult to assess, because the procedure is subjective and depends on one’s judgment to assign the fibers into appropriate class of either immature or mature fibers. The indirect methods include the instruments such as Advanced Fiber Information System (AFIS) (Bradow et al., 1996) and Fineness and Maturity Tester (FMT) (Montalvo et al., 2005), and also optical and imaging spectroscopic approach in near infrared (NIR) and visible regions (Montalvo et al., 1989; Long et al., 2010). Clearly, the direct methods are relatively accurate and precise in the discrimination of mature against immature fibers but are time-consuming and tedious, whereas the indirect methods depend on direct methods’ references to calibrate the systems for procedures’ accuracy and performance.

A need for rapid, accurate, reliable, and routine technologies to assess the cotton maturity has been a subject of continuous and considerable interest in many years. Undoubtedly, the well-defined image analysis and AFIS instrumental are mostly acceptable, in which the former is laboratory based microscopic testing and the latter is automation and routine oriented method. Main distinctions between the two lie in the differences in the principle of testing and the way of fiber preparation. During the image analysis, a bundle of fibers (>30) were selected, embedded using a medium and mounted on a microscope slide, then the parameters (fiber wall area, *A*, and perimeter, *P*) of a single fiber cross-section was determined by analyzing its image. While in AFIS procedure, a 0.5g cotton sample was prepared into a 25 cm-long sliver, and maturity index was obtained by measuring an electro-optical signal as individualized fibers were conveyed by air through a light beam. Both image analysis and AFIS measurements are destructive, however, AFIS procedure yields several fiber quality characteristics in less than 2 min. Although they have been adapted commonly in cotton industry, it is impossible to distinguish mature fibers from immature ones with current maturity readings, and clearly, no report has been available to verify the maturity indices by other independent and direct approaches.

Fast maturity measurement requires minimal sample preparation, permits routine analysis at both laboratory and on-field environments, and is easy to operate. Fourier transform mid-infrared (FT-MIR) spectroscopy could be another choice, not only has it been used to obtain highly structural information on cotton cellulose (Abidi, et al., 2008; Blackwell et al., 1970; Hsieh, 2007; Liu et al., 2010), but also has been applied to investigate the structural changes of cotton fibers as a function of developmental programming (Abidi, et al., 2008; Hsieh, 2007). Especially, distinctive IR spectral features of cotton fibers at different days post-anthesis (DPA) have been reported (Abidi, et al., 2008; Hsieh, 2007), indicating both the transition from primary cell wall synthesis to secondary cell wall synthesis and the formation of inter- and intra- molecular hydrogen bonds during the cotton fiber development. Unfortunately, there has been no effort to explore the possibility of either identifying the immature cotton fibers or determining the cotton maturity.

The objectives of this study were: (1) to characterize the unique IR bands between immature and mature cotton fibers, (2) to develop simple and universal ratio algorithms for qualitative classification of immature / mature cotton fibers and further for quantitative determination of cotton maturity, and (3) to validate and compare the FT-MIR maturity with the readings from conventional image analysis and AFIS measurement. The ultimate goal is to develop this FT-MIR method for rapid, accurate, direct, non-destructive, and routine measurement of cotton fiber maturity in cotton fields, ginning sites, and classing offices.

Materials and Methods

Mature and Immature Seed Cottons

A total of 402 mature and immature dried cotton locules (one of 3 to 5 segmented compartments in a boll) were collected from 67 bags (0.90 to 9.0 kg in weight), representing 3 Upland and 1 Pima cotton varieties grown in 3 U.S. states (CA, AR, NC) in 2008 crop year and 6 Upland cotton varieties grown in six counties in one U.S. state (GA) in 2009 crop year. From an individual package, 3 mature single locules were taken randomly from differing locations whereas additional 3 immature loculus were carefully and subjectively chosen to reflect various degree of maturity or development. The condition of mature and immature bolls was identified subjectively by their appearance, size, and shape. For example, mature cottons should have a fluffy state and appear in white color, while immature ones should be in the form of unopened boll and tips, or with shinny surface. Immature bolls also could include those that resulted from premature termination of growth due to such factors as insect infestation, disease, or frost.

Lint Cottons and Maturity Readings from AFIS and Image Analysis

First set of lint cottons (i.e., cotton fibers acquired during the ginning process to remove the cotton seed) consisted of 99 samples from very diversified sources, with 55 from 2009 crop year and grown in U.S., 21 from 2001 crop year and grown in U.S., 17 from 2009 crop year and grown outside the U.S. (China, South Africa, Pakistan, Zambia, and Ivory Coast), and 6 from International Cotton Calibration (ICC) standards that were used to calibrate the HVI systems. About 0.5g of cotton fibers was measured for maturity ratio index by an AFIS Pro (Uster Technologies, Inc., Charlotte, NC), respectively. Averages of multiple readings from the instrumental were taken and used as references.

The origin and image analysis of the second cotton set were described in detail previously (Hequet et al., 2006). In brief, 104 cotton bales representing 2 cultivated species in various grown locations were selected and then blended according to the established protocol. Their image analysis was performed by the standard procedure.

FT-MIR Spectral Collection and Data Analysis

All spectra were collected with a FTS 3000MX Fourier transform (FT) IR spectrometer (Varian Instruments, Randolph, MA) equipped with a ceramic source, KBr beam splitter, and deuterated triglycine sulfate (DTGS) detector. The attenuated total reflection (ATR) sampling device utilized a DuraSamplIR single-pass diamond-coated internal reflection accessory (Smiths Detection, Danbury, CT), and a consistent contact pressure was applied by way of a stainless steel rod and an electronic load display. Two measurements at different locations for one fiber sample (as less as 0.1g) were collected over the range of 4000-600 cm^{-1} at 8 cm^{-1} and 64 co-added scans. Caution was taken to avoid the seed fraction in seed cottons. All spectra were given in absorbance units and no ATR correction was applied. Following the import to Grams/AI software (V.7, Thermo Galactic, Salem, NH), mean spectrum was taken by averaging two spectra. The data set was also loaded into Microsoft Excel 2000 to execute simple algorithm analysis.

Prior to AFIS and FT-MIR measurement, all seed and lint cotton fibers were well conditioned at a constant relative humidity of 65% and temperature of 22 ± 2 ºC for at least 48 hours.

Results and Discussion

Spectral Response to Mature and Immature Fibers: FT-MIR vs. NIR

Cross-sections of cotton fibers have been considerably analyzed for assessing the cotton maturity, an important fiber quality trait that is directly related with the amount of cellulose deposited during the secondary wall biosynthesis (Hsieh, 2007; Hequet et al., 2006). A typical cross-section overview of immature (left) and mature (right) fiber is drawn in Figure 1. Apparently, relative portion of cellulose component in secondary wall to the total mass of the fiber increases with the secondary wall thickening (or fiber maturity increasing). Hence, remarkable change in chemical structure and composition from immature fiber to mature one could be easily monitored by non-destructive molecular spectroscopic techniques, such as FT-MIR and NIR.

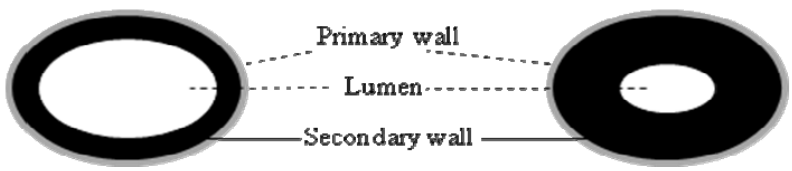

Figure 1. Schematic of immature (left) and mature (right) fiber cross-sections

Normalized FT-MIR spectra of lint mature and immature cotton fibers in the 1800-600 cm^{-1} region are given in Figure 2a. After the offset correction at 1800 cm^{-1}, the spectra were normalized by scaling the 660 cm^{-1}peak intensity to 0.1 absorbance unit. It reveals the likely significant distinctions between mature (solid line) and immature (dashed line) fibers in the 1100-900 cm^{-1} region, which could be further confirmed by the 1st derivative (Savitzky-Golay 1st function of two degrees and thirteen points) in Figure 2b. As a comparison, raw and the 1st derivative version of NIR spectra (FOSS NIRSystems Inc., Laurel, MD) from same fibers are depicted in Figure 3a and 3b, respectively. In general, intensity variations suggested that FT-MIR spectra are more sensitive to structural and compositional changes than NIR spectra. This is anticipated, because FT-MIR absorptions are attributed to the stretching and bending vibrations of such functional groups as C-H, O-H, and C-O in cotton celluloses (Blackwell et al., 1970; Liu et al., 2010), while the NIR bands arises from the overtones and combinations of fundamental bands found in IR region (Ciurczak, 2001).

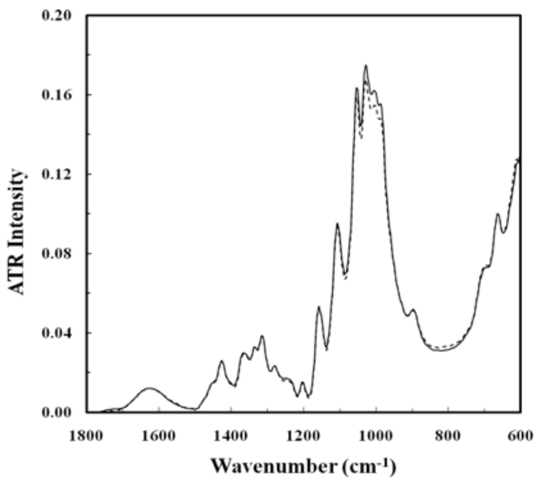

Figure 2a. FT-MIR version of mature / immature fibers.

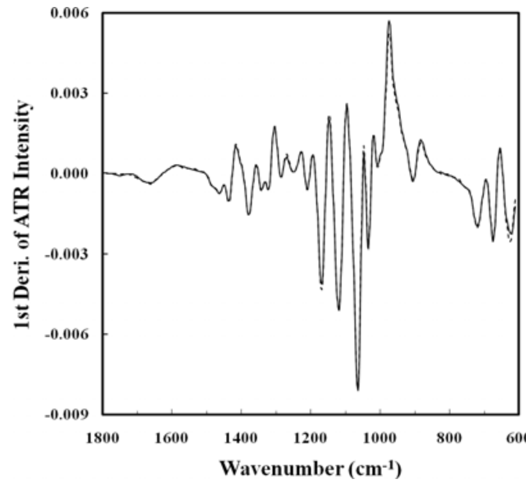

Figure 2b. 1st derivative of spectra in Figure 2a.

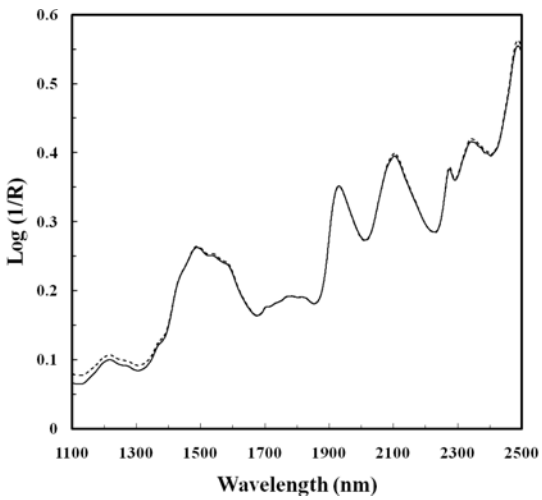

Figure 3a. NIR version of mature / immature fibers.

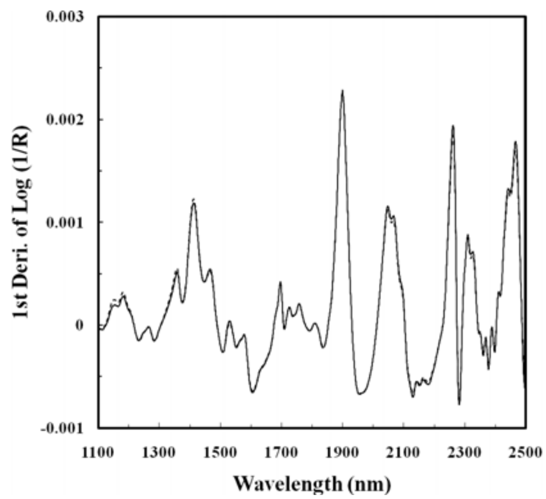

Figure 3b. 1st derivative of spectra in Figure 3a.

FT-MIR Spectral Characteristics

Figure 4 shows the representative FT-MIR spectra of mature (a) and immature (b) seed cotton fibers in the 3600-600 cm^{-1} region. Band assignments for the cotton fibers have been studied in some detail (Abidi, et al., 2008; Blackwell et al., 1970; Hsieh, 2007; Liu et al., 2010). According to these studies, features between 3600 and 2750 cm^{-1} are from the O-H and C-H stretching vibrations, a broad band centered at 1640 cm^{-1} is attributed to the O-H bending mode of water, bands in the region of 1500-1200 cm^{-1} are mixtures of CH_2 deformations and C-O-H bending vibrations, and a number of bands in the 1200-900 cm^{-1} region are assignable to coupling modes of C-O and C-C vibrations, as well as the bands between 800 and 700 cm^{-1} are likely attributable to two crystal forms (I_α and I_β) of cotton cellulose.

Comparison of the spectra reveals that immature fiber has a number of common FT-MIR bands with mature one, indicating that the dominant FT-MIR bands arise from major common chemical component in cottons, cellulose. Given the significant structural and compositional variations between immature and mature cottons, distinctive spectral differences between the two occur in several wavenumbers, for example, pronounced shoulders at 2916, 1003, and 980 cm^{-1}.

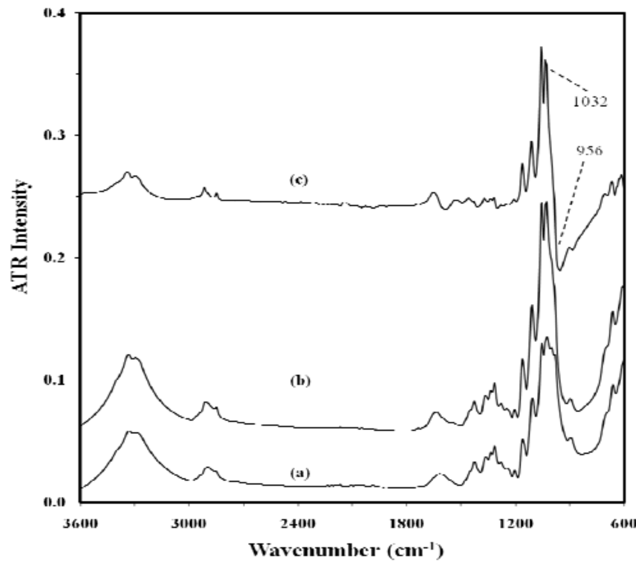

Figure 4. Typical FT-MIR spectra of mature (a) and immature (b) cotton fibers, and also difference spectrum (c, spectrum (b) – (a)). Spectra (b) and (c) were shifted vertically for direct comparison.

Relative to original FT-MIR spectra, the difference spectrum given in Figure 4 (c) reveals more subtle change of spectral intensities between immature and mature cottons. It clearly indicates the large intensity variations in the 1200-900 cm^{-1}, with at least two positive bands at 1054 and 1032 cm^{-1} and one negative band at 956 cm^{-1} that fluctuate greatly. However, there are little variations for the bands ascribed to CH groups in the 1600-1200 cm^{-1}. Although the bands at 1054 and 1032 cm^{-1} and the one at 956 cm^{-1} could be assigned to C-O stretching vibrations, they arise from different cellulose conformations and complexes in hydrogen bonded cellulose.

Algorithms for Classifying the FT-MIR Spectra of Immature and Mature Cottons

The above observation suggests that spectral distinctions between immature and mature cottons might be useful for their discrimination. Therefore, it is of interest to examine whether immature and mature cottons could be distinguished based on an spectral intensity difference. We developed a number of simple algorithms by the use of two bands at 1032 and 956 cm^{-1}, with the combination of both 2-band ratio/subtraction algorithm and other bands at 1500 cm^{-1} and 650 cm^{-1}. We found that the 3-band ratio (R_1) in Eq. 1 provides the optimal separation.

$$R_1 = (I_{956} - I_{1500}) / (I_{1032} - I_{1500}) \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots (1)$$

where R_1 represents the intensity ratio and I_{1500}, I_{1032}, and I_{956} are each a two-point average of the intensity values at each wavenumber. For example, the I_{956} readings resulted from the mean of the FT-MIR intensities at 958 and 954 cm^{-1}. The 1500 cm^{-1} band was selected as its absorbance was minimum.

Furthermore, we tested a ratio algorithm R_2 by linking it with relative portion of crystalline (I_β) to amorphous (I_α). In which R_2 is the intensity ratio and I_{800}, I_{730}, and I_{708} are two-point average of the intensities at 800, 730, and 708 cm^{-1}, respectively. It employed an offset to zero at 800 cm^{-1} wavenumber due to its minimum absorbance in low-wavenumber side (< 900 cm^{-1}).

$$R_2 = (I_{708} - I_{800}) / (I_{730} - I_{800}) \quad \ldots\ldots\ldots\ldots\ldots (2)$$

Both algorithms were applied to the FT-MIR spectral set consisting of 201 immature and 201 mature seed bolls, and the plot of R_1 vs. R_2 value is shown in Figure 5. Notably, there is not a clearly cut in both R_1 and R_2 value between immature and mature fibers. In general, R_1 values increase with R_2 values, which is expected because both values should be associated with cotton growth. Especially for mature fibers, R_2 increment is more apparent when $R_1 > 0.50$ than when $R_1 < 0.50$. Probably, at the maturation stage, desiccation induced drying process (or moisture loss) within fibers not only reduces the cellulose I_α fraction but also augment the crystalline regions through the rearrangement and orientation of cellulosic OH groups via intra- and inter- molecular hydrogen bonding.

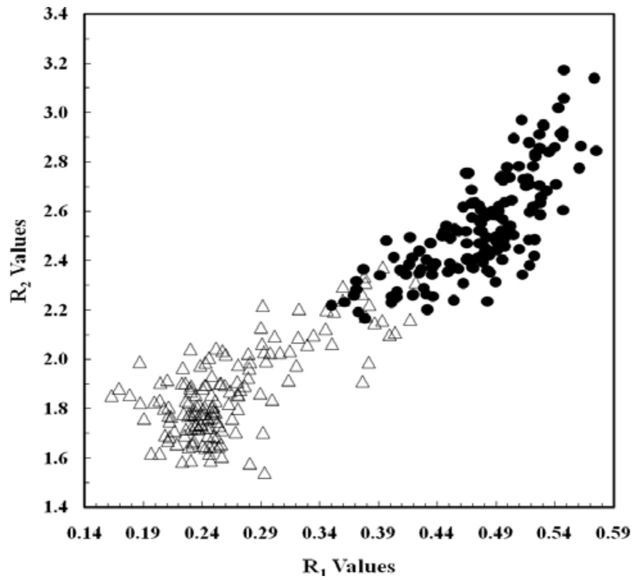

Figure 5. Plot of R_1 vs. R_2 values from seed cottons, immature (Δ) and mature (●).

Examination of the R_1 values suggests that R_1 readings were in the range of 0.35 and 0.58 for mature fibers and 0.16 to 0.41 for immature samples. It is reasonable that some mature and immature fibers had the R_1 value in the range of 0.35 to 0.41, as it is very difficult to classify fibers into mature or immature class by visual inspection once they were fluffed. Actually, mature and immature fibers co-exist even in one cotton ball. With a set R_1 threshold value of 0.40, i.e., a sample was classified as immature class when $R_1 < 0.40$ and as mature class otherwise, 197 of 201 (98.0%) immature fibers and 190 of 201 (94.5%) mature samples were correctly classified with an average of 96.0%. Similarly, R_2 values were distributed from 1.54 to 2.37 for immature fibers and 2.10 to 3.17 for mature species. By setting a R_2 threshold value of 2.20, 7 immature fibers and 10 mature samples were misclassified.

Assessment of Cotton Fiber Maturity from FT-MIR Measurement (M_{IR})

In addition to differentiate the immature fibers from mature species qualitatively, it is of significance to determine the degree of cotton maturity from a simple FT-MIR measurement. R_1 value was taken as an example. On the basis of the smallest and largest R_1 values, we developed the Eq. 3 to assess the degree of cotton maturity by representing the R_1 values.

$$M_{IR} = (R_1 - R_{1,\,sm}) / (R_{1,\,lr} - R_{1,\,sm}) \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots \ (3)$$

where M_{IR} stands for the degree of fiber maturity through FT-MIR measurement, R_1, $R_{1,\,lr}$, and $R_{1,\,sm}$ are the R_1 value for unknown sample, the largest R_1 and the smallest R_1 values , respectively. If the most immature and mature fibers are assigned the M_{IR} value of 0.0 and 1.0, respectively, the corresponding R_1 values should be the smallest and largest ones in Figure 5. Hence, the $R_{1,sm}$ and $R_{1,lr}$ were determined to be 0.14 and 0.59, respectively, which are approximately 0.02 units smaller and larger than those observed in Figure 5. In order to verify the acceptability of $R_{1,sm}$ and $R_{1,lr}$ values, FT-MIR spectra of more than 400 additional lint cotton fibers from different crop years, varieties, locations within and outside the U.S. as well as ICC standards were collected and analyzed, and none was recognized to have the $R_{1,sm}$ value of < 0.14 or $R_{1,lr}$ value of > 0.59. Going back to Figure 5, if R_1 = 0.14, 0.40, and 0.59, then the responding M_{IR} = 0, 0.58, and 1.0, respectively. In other words, immature fibers whose $R_1 < 0.40$ might have $M_{IR} < 0.58$, and vice versa.

Validation of M_{IR} with other Instrumental Approaches

To compare the efficiency of fiber maturity from direct FT-MIR measurement, 2 sets of cotton fibers with different maturity readings as determined by AFIS and image analysis were used. The first set included 99 cotton samples that represent diverse variations in crop year, variety, and grown locations. Table 1 summarizes the determination of correlation (R^2) between the pair of maturity indices from FT-MIR and AFIS, with a R^2 of 0.403.

Table 1. Determination of correlation (R^2) from 2 sample sets [a]

	Correlation	Correlation	R^2
All 99 Cottons	M_{AFIS} vs. M_{IR}		0.403
6 ICC Cottons	M_{AFIS} vs. M_{IR}		0.920
All 104 Cottons		M_{IA} vs. M_{IR}	0.596
50 Cottons		M_{IA} vs. M_{IR}	0.894

[a] M_{AFIS} from AFIS; M_{IR} from FT-MIR; M_{IA} from image analysis.

Due to the nature of the inhomogeneous distribution of cotton fibers in structural, chemical, and physical properties and also the subsequent concern of different sampling species for 2 independent measurements, it is possible to observe low R^2 between them. In this regard, only 6 ICC standards were considered and the resultant R^2 was significantly improved to be 0.920 (Table 1). This is much desired and expected, since each independent measurement should possess some kind of relationship on common interests and the resultant correlations between them should be stronger.

With an effort to establish standard cottons, Hequet et al. have reported the creation of 104 reference materials for cotton fiber maturity measurements (2006). It shows an R^2 of 0.596 between M_{IR} and M_{IA}, which is much improved compared to that of 0.403 between M_{IR} and M_{AFIS} (Table 1). Because these cottons were blended from various bales and also the subsequent concern of micro-sampling in two independent measurements (FT-MIR vs. image analysis), it is reasonable to observe a number of outliers that exhibited large differences between two values. In this regard, the ratios (M_{IA} / M_{IR}) were calculated and then utilized to determine the outlier samples. Only those samples with the ratio range of 0.60 to 0.66 were subjectively considered to possess close (or appropriate) maturity values between two testing methods. This procedure resulted in a selection of 50 samples that yielded an expected increase in R^2 (0.89). Figure 6 shows the plot of maturity ratios from image analysis against maturity values from FT-MIR measurement.

Overall, strong correlations between M_{IR} and M_{AFIS} as well as between M_{IR} and M_{IA} from small sets of samples in Table 1 confirm the equivalent cotton maturity measurements between FT-MIR and AFIS and also between FT-MIR and image analysis. It suggests that current maturity results from image analysis and AFIS are reliable. This might be understood easily by the fact that FT-MIR spectra are more sensitive to the change in relative cellulose amount of cotton fibers during the growth (or the thickening of secondary cell wall), compared to image analysis's theoretical approach and AFIS's instrumental assessment. Undoubtedly, more diversified samples in varieties and locations and also additional techniques are necessary to validate the FT-MIR observations.

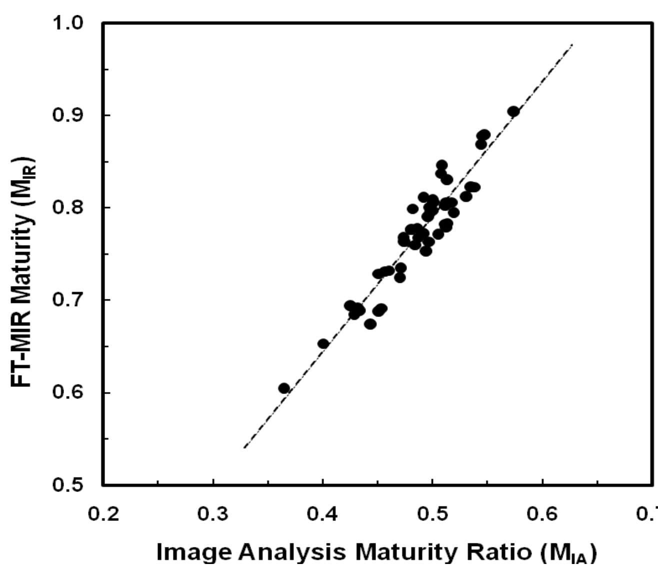

Figure 6. Relationship between M_{IA} from image analysis and M_{IR} from FT-MIR measurement.

As a differing approach to FT-MIR, imaging spectroscopy in this region could be developed. Imaging technique covers a large sampling area and the spectrum is composed of thousands of pixels, in which a pixel originates from a one-dimensional spectrum discussed above. Hence, the algorithms could be migrated for analyzing the imaging spectra of cotton fibers.

Summary

This study demonstrates the usefulness and effectiveness of FT-MIR spectroscopy in rapid, non-destructive, and direct determination of cotton fiber maturity (M_{IR}). FT-MIR spectral differences between immature and mature cotton fibers revealed a number of significant bands, which were used to develop simple three-band ratio algorithm for discriminant analysis. The result suggests that the ratio algorithm could classify mature fibers from immature ones with a success of over 96.0%. Next, on the basis of magnitude in R_1 value, the degree of fiber maturity was assessed directly by converting the R_1 value into the M_{IR} range of 0 to 1.

In order to verify the acceptability of FT-MIR procedure, the M_{IR} index was compared with results from image analysis and AFIS. Low correlations were observed between any pair of M_{IA}, M_{AFIS}, and M_{IR}. The correlations were improved significantly with sole consideration of either ICC standards or 50 selected cottons, suggesting the importance of cotton homogeneity and subsequent sampling in accurate and precise maturity determination. Strong correlations between M_{IR} and M_{AFIS} as well as between M_{IR} and M_{IA} indicated the equivalence and effectiveness of the three techniques in maturity assessment.

Notably, the algorithm approach is the most attractive and interesting since, in its simplest form, there is no calibration model which is commonly built from a large number of sample set. In addition, the use of intensity ratio at different wavelengths can reduce the influences from diverse samples; hence it could be universally applied for fast, accurate, routine, and direct determination of cotton fiber maturity. The finding of three mid-IR bands is most promising in the development of simple optical and imaging IR systems for *in situ* measurement of cotton maturity at various surroundings.

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