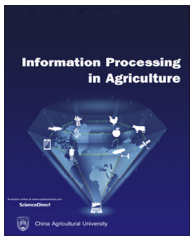
Available at www.sciencedirect.com

INFORMATION PROCESSING IN AGRICULTURE 3 (2016) 30–35

journal homepage: www.elsevier.com/locate/inpa



# Application of near infrared spectroscopy in cotton fiber micronaire measurement☆

*Yongliang Liu* [a,*], *Chris Delhom* [a], *B. Todd Campbell* [b], *Vikki Martin* [c]

[a] USDA, ARS, Cotton Structure & Quality Research Unit, Southern Regional Research Center (SRRC), New Orleans, LA, USA
[b] USDA, ARS, Coastal Plain Soil, Water and Plant Conservation Research, Florence, SC, USA
[c] Cotton Incorporated, Cary, NC, USA

ARTICLE INFO

*Article history:*
Received 9 September 2015
Received in revised form
13 October 2015
Accepted 15 January 2016
Available online 22 January 2016

*Keywords:*
Cotton fiber
Fiber quality
Micronaire
HVI™
NIR
Spectroscopy

ABSTRACT

The term "micronaire" describes an important cotton fiber property by characterizing both the fiber maturity and fineness. In practice, micronaire is regularly measured in laboratories with the well established high volume instrumentation (HVI™) protocol. In most scenarios, cotton breeders/geneticists sent cotton breeding line field trial samples to laboratories equipped to use the HVI™ systems available for fiber micronaire determination. Researchers have previously investigated the use of NIR as an alternative means of measuring micronaire either at breeding sites or in standard laboratories. As a proof-of-concept investigation, this study collected both near infrared (NIR) spectra and HVI™ micronaire from a total of 381 cottons harvested in the 2011 and 2012 crop years. Partial least square (PLS) calibration model relating NIR spectral information to fiber HVI™ micronaire was developed and then applied to both a validation sample set from identical crop years and an independent test sample set from the 2014 crop year. Results indicated an acceptable bias (or differences between HVI™ measured and NIR predicted micronaire) and an over 97% correctly predicted micronaire (within ±0.30 micronaire unit) in an independent test set. Therefore, the development of a robust and effective NIR model for rapid laboratory micronaire assessment that would be applicable to remote/breeding locations is feasible.



## 1. Introduction

Cotton, one of the most important and widely grown crops in the world, is a well-traded agricultural commodity primarily for its naturally produced textile fiber [1]. Cotton fiber's growth or development is considered to include at least four overlapping but distinctive phases: initiation, primary wall formation (elongation), secondary cell wall thickening (cellulose synthesis), and maturation [2]. The day of flowering is referred to as anthesis and the word "days post anthesis" (dpa) is commonly used to describe the cotton fiber growth. The fiber cells initiate at 0 dpa and then elongate to reach a fiber length of 22–35 mm within 20–25 dpa. The secondary cell wall synthesis starts around 15–22 dpa and continues for an additional 30–40 days until maturation, when the fibers dehydrate and collapse into flattened and twisted ribbons. Such a fiber evolution indicates a number of significant changes in fiber chemical composition, structure, and physical properties coinciding with various stages of development. The

☆ Mention of a product or specific equipment does not constitute a guarantee or warranty by the U.S. Department of Agriculture and does not imply its approval to the exclusion of other products that may also be suitable.

* Corresponding author. Tel.: +1 504 286 4455; fax: +1 504 286 4217.
E-mail address: yongliang.liu@ars.usda.gov (Y. Liu).

Peer review under the responsibility of China Agricultural University.
http://dx.doi.org/10.1016/j.inpa.2016.01.001

length of fiber growth period between floral anthesis and its harvest cannot be considered as a parameter to describe the degree of fiber maturation [3]. Fiber maturity has been accepted to reflect the degree of the secondary cell wall thickening relative to the diameter or fineness of the fiber [4].

Cotton micronaire is one of the most essential fiber characteristics in the cotton industry [5,6], as it reflects fiber maturity (degree of secondary cell wall development) and fineness (weight per unit length) simultaneously. In practice, automation-based high volume instrumentation (HVI™) measurement has been well established as a primary and routine tool of providing fiber micronaire and other quality properties to cotton breeders, fiber processors, and market regulators [7]. To determine the micronaire value, conditioned fiber samples with constant weight (~10 g) are measured by passing air through the fibers and then measuring the drop in pressure. Overall, the test for micronaire is very fast and accurate, therefore HVI™ measurement has been increasingly and routinely utilized in the cotton and textile industry from cotton breeding program to textile quality control [8–11], in addition to an industrial standard used internationally and domestically to classify commercial-ready cottons.

During the development and testing of advanced breeding lines and candidate cultivars, cotton breeders typically harvest thousands of fiber samples from one crop year. Fiber quality data are routinely collected on these samples, and they must be sent to outside fiber quality laboratories where HVI™ systems are available. It would be both desirable and beneficial to cotton breeders if more robust and low-cost quality measurements were available. One of the potential techniques is near infrared (NIR) spectroscopy, covering the 750–2500 nm (or 13,300–4000 $cm^{-1}$) region and representing the overtones and combination bands of the fundamental absorptions observed in the mid-IR spectral regions of cotton fiber cellulose [12].

NIR has been explored extensively for determining fiber micronaire over the years [13–19], because of its rapid, low-cost, and portable attribute that can be used away from the standard laboratories. This method largely measures the physical scattering of light from near-surface area of a fiber sample and requires a great number of training or calibration samples to develop accurate and reliable calibration equations (models) through multivariate regression procedure. Clearly, it takes time collecting the diverse samples and measuring the referenced micronaire values by the standard laboratory method in advance. Previous studies by various researchers have demonstrated the potential of NIR technique to determine micronaire with a high degree of success.

The main aim of the current study was to examine the applicability of NIR micronaire model developed from earlier crop year cottons to newly crop year fibers, by testing their micronaire predictions.

## 2. Materials and methods

### 2.1. Cotton samples

In each of 2011, 2012, and 2014 crop years, a total of 20 entries (16 elite breeding lines and 4 commercial cultivars) were grown in four replicated field tests at the Clemson University Pee Dee Research and Education Center near Florence, SC (Florence) on a Norfolk loamy sand soil, the Clemson University Edisto Research and Education Center near Blackville, SC (Blackville) on a Barnwell loamy sand soil, and the North Carolina State University Sandhills Research Station near Jackson Springs, NC (Sandhills) on a Candor sand soil. Each trial was arranged in a randomized complete block design with four replications. Each entry was planted in a two-row plot 10.7 m long with 96.5 cm spacing between rows. Plots were managed conventionally and followed the established local practices.

From each plot in each trial, 50 bolls were picked by hand. These boll samples were subsequently ginned on a 10-saw laboratory gin and lint fibers were collected. In every crop year, cotton lint fibers were conditioned at a constant relative humidity of 65 ± 2% and temperature of 21 ± 1 °C for at least 24 h, prior to routine fiber quality and NIR spectral measurement. Table 1 summarizes the fiber information about their origins and data collection at three locations over three crop years. Both fiber quality and spectral measurement were performed in August 2012, January 2013, February 2015 for the respective 2011, 2012 and 2014 crop year cottons.

### 2.2. Fiber quality measurement

Average micronaire values were obtained from five measurements on each sample by an Uster® HVI™ 900A system (Uster Technologies Inc., Knoxville, TN). All measurements were performed at the Southern Regional Research Center of USDA's Agricultural Research Service (USDA–ARS–SRRC). The same instrument was used for all fibers throughout the multiple year study.

### 2.3. NIR reflectance spectral acquisition

NIR reflectance spectra were acquired on a Foss XDS rapid content analyzer (Foss NIRSystems Inc., Laurel, MD). Approximately 10 g of cotton fibers were pressed into a Foss coarse granular cell (3.8-cm wide × 15.2-cm long × 4.8-cm deep). Background was recorded with the use of an internal ceramic reference tile before scanning the samples. The log (1/Reflectance) readings were acquired over the 400–2500 nm wavelength range at 0.5 nm interval and 32 scans. At least two spectra were collected for each of the cotton samples by repacking and the mean spectrum was obtained.

### 2.4. Micronaire model development

All NIR spectra were imported into GRAMS IQ application in Grams/AI (Version 9.1, Thermo Fisher Scientific, Waltham, MA) for partial least squares (PLS) regression model development. On the order of the smallest to largest in micronaire property within each crop year fibers, two-thirds of spectra (or samples) were selected for calibration equation development and the remaining one-third (every 3rd sample) spectra were used for model validation. To optimize the accuracy of prediction models, the spectra were subjected to different combinations of both the spectral ranges (e.g., full and narrow regions) and the spectral pretreatments (e.g., mean centering

**Table 1 – Fiber origin and data collection at three locations over three crop years.[a]**

| | Florence SC | Blackville SC | Sandhills NC | |
|---|---|---|---|---|
| 2011 crop (total no. = 238) | 80 | 78 | 80 | August 2012[b] |
| 2012 crop (total no. = 143) | 0 | 63 | 80 | January 2013[b] |
| 2014 crop (total no. = 240) | 80 | 80 | 80 | February 2015[b] |

a 2013 crop year cottons were not collected in this study.
b In which dates fiber quality and spectral measurements were taken.

(MC), multiplicative scatter correction (MSC), and the first and second derivatives). Full (one-sample-out rotation) cross-validation method was used, and the number of optimal factors chosen for the regression equation generally corresponded to the minimum of the predicted residual error sum of squares (PRESS). The saved regression equations were subsequently applied to (1) the validation samples that were harvested from the same crop year and (2) the test samples that were harvested from differing crop year or different locations. Model accuracy and efficiency were assessed in the calibration, validation, and independent set on the basis of the coefficient of determination ($R^2$, $r^2$, $r^2$), root mean square error of calibration (RMSEC), validation (RMSEV), or test (RMSET), and also the bias (or differences) between referenced and NIR predicted micronaire values.

## 3. Results and discussion

### 3.1. *Cotton fiber micronaire component and NIR spectral response*

Fig. 1 shows the typical log (1/R) spectra of cotton fibers in the spectral region between 1100 and 2500 nm. For the purpose of only exhibiting the NIR spectral response to fiber micronaire, these spectra were obtained by averaging the spectra of neighboring micronaire values in the respective range of <3.50, 3.50–4.20, 4.30–5.00, and >5.00. In general, spectra of cotton fibers with low micronaire have common NIR bands with those of fibers having high micronaire, but there is some degree of intensity changes induced by rising micronaire in the entire spectral region. Characteristic bands in this region are mainly due to the (1st and 2nd) overtones and combinations of OH and CH stretching vibrations of cotton fiber cellulose [12]. The broad absorptions between 1150 nm and 1300 nm are from the 2nd overtones of CH stretching modes and their 1st overtones appear in the 1675–1860 nm region. Features in the 1300–1400 nm region are ascribed to combination bands of the CH vibrations. Broad and intense bands in the 1400–1675 nm region are due to the overlap of the 1st overtones of the OH stretching modes in hydrogen bonded forms. The strong bands at 1935 and 2105 are most likely attributed to the combination of OH stretching and deformation mode and the combination of OH and CO stretching vibrations in cellulose, respectively.

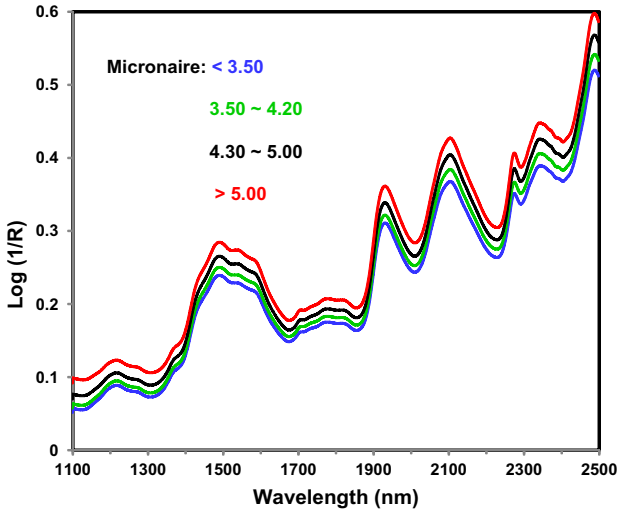



**Fig. 1 – Mean NIR log (1/R) spectra of cotton fibers with various micronaire readings, by averaging the spectra of neighboring micronaire values in the respective range of <3.50, 3.50–4.20, 4.30–5.00, and >5.00 micronaire unit.**

### 3.2. *Referenced micronaire values*

The range, mean, and standard deviation (SD) of referenced micronaire values for 2011, 2012, and 2014 cotton fibers in calibration, validation, and independent test sets are summarized in Table 2. Fiber micronaire readings covered from 4.10 to 5.68 micronaire unit for the 2011 cottons, 3.44–4.84 micronaire unit for the 2012 cottons, and 3.92–5.60 micronaire unit for the 2014 cottons. Apparent discrepancies in fiber micronaire over three crop years were expected due to differences in growth environments.

### 3.3. *Micronaire prediction models*

Within the 2011 and 2012 cottons, variation of referenced micronaire values in either crop year was in a narrow range and could not represent the variability in commercial cotton bales or in cotton breeding programs. Hence, the two crop year cottons were divided into calibration and validation samples, and then were combined into respective calibration or validation sets. PLS models were developed from combinations of such spectral pretreatments as MC and 1st derivative in the 1105–2495 nm region. The use of 2nd derivative, along with other data processing, yielded relatively poor results (not shown). The statistics in calibration, validation, and independent test sets are compared in Table 3.

The model built from this 2-year (2011 + 2012 crop year) data set exhibits acceptable $R^2$ (0.95) and $r^2$ (0.93) as well as low RMSEC (0.124) and RMSEV (0.134) in calibration and validation sets, respectively. When applying the model to independent 2014 crop year cottons, $r^2$ (0.83) decreases

**Table 2 – Summary of range, mean, and SD for cotton micronaire component (micronaire unit) in calibration and validation sets.**

| Micronaire | | Range | Mean | SD |
|---|---|---|---|---|
| 2011 crop | Calibration set ($n$ = 160) | 4.10–5.68 | 5.00 | 0.32 |
| | Validation set ($n$ = 78) | 4.26–5.59 | 5.01 | 0.30 |
| 2012 crop | Calibration set ($n$ = 96) | 3.44–4.84 | 4.12 | 0.28 |
| | Validation set ($n$ = 47) | 3.56–4.66 | 4.13 | 0.26 |
| 2-year (2011 + 2012) | Calibration set ($n$ = 256) | 3.44–5.68 | 4.67 | 0.52 |
| | Validation set ($n$ = 125) | 3.56–5.59 | 4.68 | 0.51 |
| 2014 crop | Calibration set ($n$ = 162) | 3.92–5.60 | 4.84 | 0.32 |
| | Validation set ($n$ = 78) | 4.16–5.53 | 4.84 | 0.30 |
| 3-year (2011 + 2012 + 2014) | Calibration set ($n$ = 418) | 3.44–5.68 | 4.74 | 0.46 |
| | Validation set ($n$ = 203) | 3.56–5.59 | 4.74 | 0.45 |

expectedly but RMSET is nearly unchanged compared to those in calibration/validation set.

To assess the performance of the calibration model, a bias parameter (defined as the difference between measured and NIR predicted micronaire) was used. It is very reasonable to observe a greater deviation in bias within the independent test set than among calibration or validation sets (−0.044 in test set vs. 0.000 in calibration set or −0.003 in validation set), mostly because these test samples were measured at least two-year apart from calibration/validation samples and they were not included in this model development. Very likely, bias in the test set is insignificant when comparing it (−0.044) to either the mean value (4.84) or the ±0.30 micronaire unit discussed in the following. Comparative scatter plots of measured and NIR predicted micronaire in validation and independent test sets are given in Figs. 2 and 3, respectively.

Another approach to examine the robustness and efficiency of the 2-year model was the use of ±0.30 micronaire unit role [20]. Within the 256 calibration samples, 125 validation samples and 240 test samples, there are 4 (1.6%), 3 (2.4%), and 7 (2.9%) samples that had absolute prediction errors (or differences) greater than the permitted range of 0.30 unit, respectively. In other words, this model results in over 97% of micronaire predictions that were within the acceptance range of ±0.3 micronaire unit.

Therefore, statistics ($r^2$, RMSET, bias, and ±0.3 micronaire unit role) in independent test set suggest the feasibility and suitability of implementing an NIR micronaire model developed from earlier crop year cottons to new crop year fibers.

Similarly, the 2014 crop year cottons were divided into calibration and validation samples, and compiled into the previous 2-year model set. As anticipated, $R^2$, $r^2$, and bias in the recalibrated 3-year (2011 + 2012 + 2014 crop year) model were similar to those in the 2-year model, but RMSEC and RMSEV are smaller in the 3-year model than in the 2-year model. In the line of expectation, the majority of the calibration samples (411 of 418, or 98.3%) and validation samples (200 of 203, 98.5%) were within ±0.3 micronaire unit, reflecting an over 98% correct predictions of micronaire. Comparative

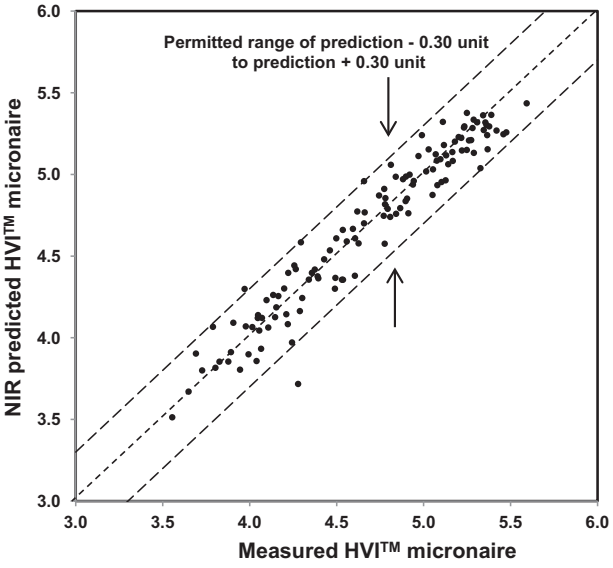



**Fig. 2 – Correlation between measured and 2-year NIR model predicted micronaire in validation set ($n$ = 125).**

**Table 3 – Statistics of NIR model for micronaire prediction in calibration, validation, and test sets.[a]**

| Micronaire | Calibration set | | | Validation set | | | Test set | | |
|---|---|---|---|---|---|---|---|---|---|
| | $R^2$ | RMSEC[b] | Bias[c] | $r^2$ | RMSEV[b] | Bias[c] | $r^2$ | RMSET[b] | Bias[c] |
| 2-year model | 0.95 | 0.124 | 0.000 | 0.93 | 0.134 | −0.003 | 0.83 | 0.135 | −0.044 |
| 3-year model | 0.94 | 0.115 | −0.000 | 0.93 | 0.121 | −0.002 | | 0.080 | 0.033[d] |

a All spectral processing with mean centering (MC) and the first (1st) derivative. 6 optimal factors were used for 2 models.
b Root mean square error of calibration (RMSEC), validation (RMSEV), and test (RMSET).
c Bias = HVI™ measured − NIR predicted.
d Based on 6 independent fibers with a narrow micronaire range of 4.71–4.87. These fibers were obtained from routine quality test at the USDA ARS's Cotton Structure & Quality Research Unit, with known breeding lines, growing location, and crop year.

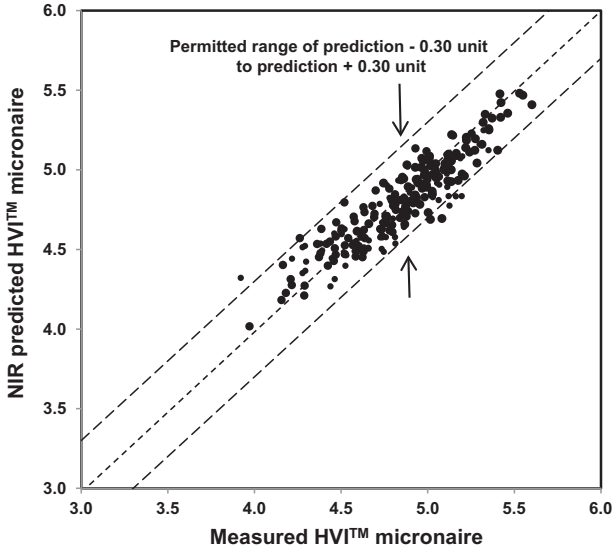



**Fig. 3 – Correlation between measured and 2-year NIR model predicted micronaire in test set ($n$ = 240).**

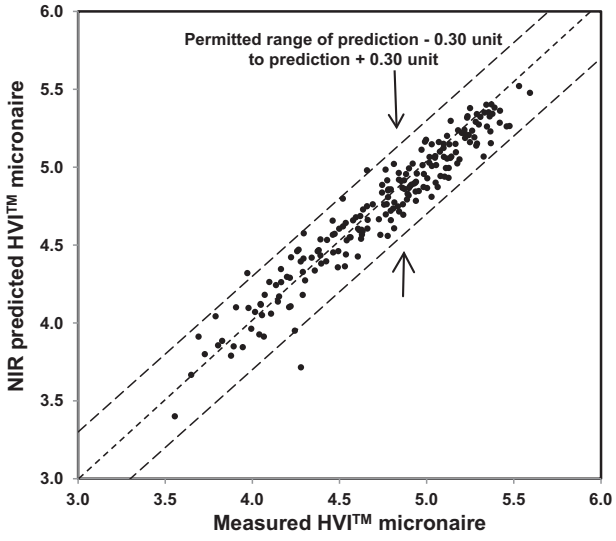



**Fig. 4 – Correlation between measured and 3-year NIR model predicted micronaire in validation set ($n$ = 203).**

scatter plot of measured and NIR predicted micronaire in the validation set is given in Fig. 4. Furthermore, the model was applied to six independent 2014 crop year cottons that were known to have different breeding lines and growing locations, and the result is very promising with RMSET = 0.080 and bias = 0.033. Clearly, more diversified fibers are necessary to verify the robustness and efficiencies of this model.

## 4. Conclusion

NIR spectroscopy is generally considered as a cost-effective alternative to traditional laboratory methods and systems. In this study, an NIR model developed from earlier crop year cottons enables the accurate and quantitative determination of fiber micronaire in new crop year cottons. The laboratory model is sufficiently robust to indicate that NIR could be an appropriate cost-effective methodology for quantitative analysis of cotton micronaire component in early testing at remote sites.

Practical implementation of this NIR procedure for rapid and routine micronaire screening will require that a small subset of fiber samples be conditioned at a controlled environment and measured with HVI™ to examine the NIR model performance. The NIR model must be updated by including the data from new crop year (or location) cottons.

## Acknowledgement

The authors would like to acknowledge all technical supporters at both ARS locations (New Orleans, LA and Florence, SC) for their diligent work in the field and laboratory operations.

## REFERENCES

[1] Wakelyn PJ, Chaudhry MR. Cotton: technology for the 21st century. Washington DC: International Cotton Advisory Committee; 2010.
[2] Hsieh Y-L. Chemical structure and properties of cotton. In: Gordon S, Hsieh Y-L, editors. Cotton: science and technology. Cambridge, UK: Woodhead Publishing Limited; 2007. p. 3–34.
[3] Bradow J, Davidonis G. Quantitation of fiber quality and the cotton production-processing interface: a physiologist's perspective. J Cotton Sci 2000;4:34–64.
[4] Boylston EK, Thibodeaux DP, Evans JP. Applying microscopy to the development of a reference method for cotton fiber maturity. Text Res J 1993;63:80–7.
[5] Lord E. Air flow through plugs of textile fibers part II. The micronaire test for cotton. J Text Inst 1956;47:T16–47.
[6] Montalvo JG. Relationships between micronaire, fineness and maturity. Part I. Fundamentals. J Cotton Sci 2005;9:81–8.
[7] American Society for Testing and Materials (ASTM) D 5867-12. Standard test method for measurement of physical properties of cotton fibers by high volume instruments. In: Annual book of ASTM standards. West Conshohocken, PA: ASTM International; 2012.
[8] Frydrych I, Thibodeaux D. Fiber quality evaluation – current and future trends/intrinsic value of fiber quality in cotton. In: Wakelyn PJ, Chaudhry MR, editors. Cotton: technology for the 21st century. Washington DC: International Cotton Advisory Committee; 2010. p. 251–95.
[9] Kelly C, Hequet E, Dever J. Interpretation of AFIS and HVI fiber property measurements in breeding for cotton fiber quality improvement. J Cotton Sci 2012;16:1–16.
[10] Kim HJ, Rodgers J, Delhom C, Cui X. Comparisons of methods measuring fiber maturity and fineness of Upland cotton fibers containing different degrees of fiber cell wall development. Text Res J 2014;84:1622–33.
[11] Paudel D, Hequet E, Noureddine A. Evaluation of cotton fiber maturity measurements. Ind Crops Prod 2013;45:435–41.
[12] Burns DA, Ciurczak EW. Handbook of near-infrared analysis. New York: Marcel Dekker; 2001.
[13] Montalvo JG, Von Hoven TM. Analysis of cotton. In: Near-infrared spectroscopy in agriculture. In: Roberts C, Workman J, Reeves J, editors. Agronomy monograph No. 44. Madison, WI: American Society of Agronomy; 2004 [chapter 25].
[14] Thomasson JA, Shearer SA. Correlation of NIR data with cotton quality characteristics. Trans ASAE 1995;38:1005–10.

[15] Rodgers J, Montalvo JG, Gayle D, von Hoven TM. Near infrared measurement of cotton fiber micronaire, maturity and finesses – a comparative investigation. Text Res J 2010;80:780–93.
[16] Rodgers J, Fortier C, Montalvo J, Cui X, Kang SY, Martin V. Near infrared measurement of cotton fiber micronaire by portable near infrared instrumentation. Text Res J 2010;80:1503–15.
[17] Rodgers J, Kang SY, Fortier C, Cui X, Davidonis G, Clawson E, Boquet D, Pettigrew W. Preliminary field measurement of cotton fiber micronaire by portable NIR. Spectrosc Mag 2010;25:38–44.
[18] Vogt F, Luttrell RD, Rodgers J. New approaches for field analyses of cotton quality by means of near infrared spectroscopy supported by chemometrics. Anal Lett 2011;44:2466–77.
[19] Liu Y, Gamble G, Thibodeaux D. UV/visible/near-infrared reflectance models for the rapid and non-destructive prediction and classification of cotton color and physical indices. Trans ASABE 2010;53:1341–8.
[20] US Department of Agriculture (USDA). Cotton classification: understanding the data. Washington DC: USDA Agricultural Marketing Service; 2005.