INTRODUCING COTTONSCOPE: A RAPID AND PRECISE MEASUREMENT OF COTTON FIBRE MATURITY BASED ON SIROMAT

Mark Brims
Hy Hwang
Cottonscope Pty Ltd,
Western Australia

Abstract

Cottonscope is a rapid, direct maturity measurement instrument based on the SiroMat development. The instrument is an automated version of the polarized light microscopy (PLM) Standard Test Method (ASTM D1442, 00), which uses interference colors to identify the maturity of a cotton specimen. Cottonscope simplifies the preparation process by allowing measurement of fibre snippets without mounting medium and is at least 10 times faster than SiroMat. Performance, calibration and measurement results from two instruments are described using a sub-set of international cottons with reference values of fibre maturity measured according to the recognized fundamental method. The precision of Cottonscope is measured by assessing the repeatability of test results on mature and immature cotton. As in the case of SiroMat, it is probable that the inherent experimental error of the reference material measurements is significant in determining accuracy.

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. Several papers have been presented describing SiroMat.

Cottonscope Pty Ltd has an exclusive license to develop and market the SiroMat technology, and has combined this with the exclusive image processing technology used in the OFDA instrument that was developed by BSC Electronics Pty Ltd. The OFDA is the leading instrument for rapid and accurate measurement of the diameter of animal fibres such as wool and cashmere, with over 300 instruments sold in 20 years worldwide.

A major breakthrough is the removal of the requirement to use the mounting medium. A mounting medium such as castor oil was required by ASTM D1442 and by SiroMat to help transmit light through the fibres. Cottonscope uses custom designed optics to couple the light through dry snippets, which significantly simplifies the sample preparation and cleaning of the slides.

Materials and Methods

Description of Cottonscope

Cottonscope is a computer controlled, digital video microscope developed for fast measurement of cotton fibers. Standard microscope light sources and objectives do not provide the optical performance required by Cottonscope; hence it was necessary to develop a customized solution. Fiber images are illuminated as follows:

1. Light is emitted by an ultra-bright white light emitting diode (LED) in the microscope base. This LED is pulsed at high current for a precise interval of less than 80 micro-seconds. At this strobe period images of moving fibres are captured without blurring. The pulse-mode also allows heat from the LED to be mitigated.
2. The light passes through a focusing lens to focus most of the light onto the fiber plane
3. The light then passes through a polarizing filter
4. The light then passes through a wave plate (also known as lambda plate or tint plate) to enhance the color separation
5. The light passes through a 2 mm thick glass slide, through the cotton fiber snippets and through a 2 mm thick glass slide which acts as a cover plate to hold the snippets
6. The light passes through another polarizing filter
7. The light passes through a custom designed microscope objective which focuses the image onto a color CCD camera. The sensor resolution is 768 by 576 pixels.
8. The images are digitized and transferred by USB to the control computer

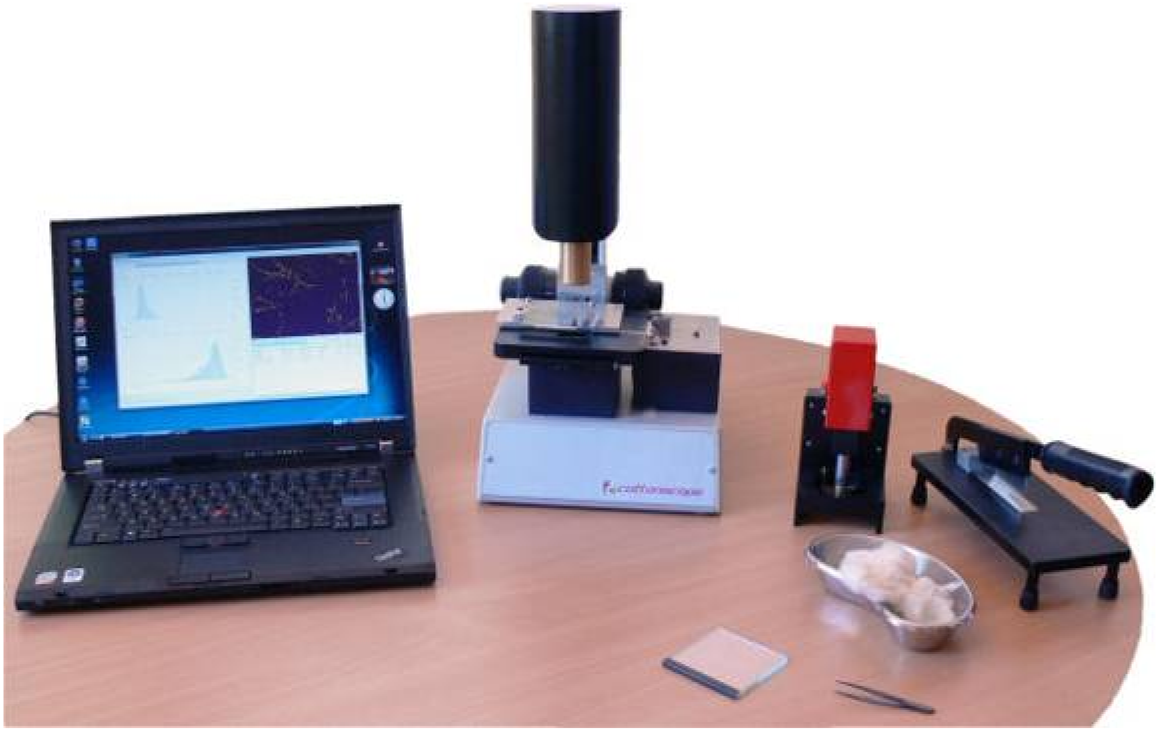

Figure 1. A typical laboratory setup with PC, Cottonscope, fibre spreader and guillotine

Preparation and measurement of cotton fibers for the test has also been simplified. The process is as follows:

1. Cotton snippets are cut from the sample using a minicore or guillotine. The guillotine cuts snippets that are approximately 1 mm long.
2. Approximately 7 to 10 mg of snippets are placed in an automatic spreader and spread onto a 70mmx70mmx2mm thick glass slide that has a hinged slide attached to act as a cover. After 30 to 40 seconds of spreading, the glass slide with the fibres on it is withdrawn and the hinged cover glass is slowly laid down.
3. The slide containing the snippets is placed onto the microscope stage, the sample name is entered into the computer and the measurement begins.
4. The computer checks and adjusts the light and then scans the slide and analyses the digital images of the fibers to produce the maturity number.
5. The scanning of the slide takes less than 25 seconds.
6. The slide is removed and the snippets are vacuumed off and the slide is then ready for the next sample.

<u>Maturity Measurement Principle</u>

The measurement of maturity is similar to that detailed in the SiroMat papers (see www.cottonscope.com). Against the magenta background, immature fibres appear red or blue whilst mature fibres appear yellow. Figure 2 illustrates a typical Cottonscope image. In previous work Gordon and Phair (2005) 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.

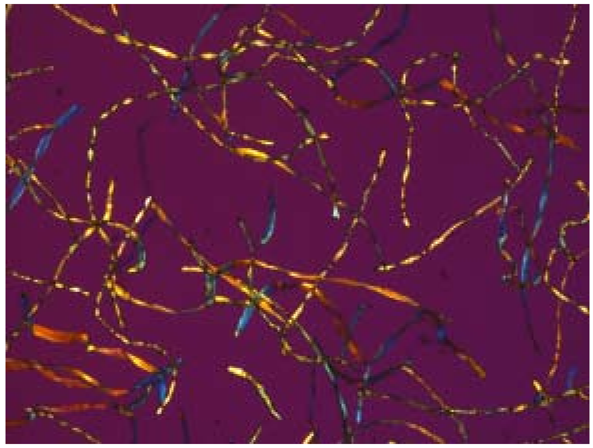

Figure 2. Cottonscope image showing mature and immature cotton snippets

The analysis of an image begins with the capturing of fibres using a wide spectrum white LED, chosen to maximize the color separation between the fibres. Fibres are captured using 32-bit color CCD. The image is converted to an 8-bit gray scale to speed up the processing. The image is first tested for maximum density to ensure the fibre spread is optimized. The fibres are located on the image and traced using threshold edge detection and following algorithm. The length of the fibre is then measured. The centre of the fibre has the highest illumination and therefore an area around the centre and along the length of the fibre is used to record the hue and intensity. The maturity ratio is calculated from the hue using the yellowness equation and calibration coefficients. The width and focus is calculated using a sub pixel interpolation of the three RGB colors from the two edges of the fibre. The fibre is finally tested to meet the minimum specification of length, focus, width, intensity and hue saturation before it is accepted.

An average image with 100 fibre snippets will take less than 20 milliseconds to analyze. The analysis code runs in parallel with the image acquisition. During a scan across the slide, the analysis time will vary in proportion with the density of fibres on the slide and typically at the end of each across axis scan, the analysis will have completed as well, making the Cottonscope an extremely fast maturity measuring system.

Typically 3000 to 7000 of these fibre segments are identified on the slide. The calibration of Cottonscope has been simplified by using only one variable known as “yellowness” or yn.

$$\text{Maturity} = \text{slope}*\text{yn} + \text{offset}$$

Each fiber is assigned a maturity number based on the yellowness of the fiber.

This was found to give a similar correlation of measured maturity on the standard cotton samples when compared against the multiple linear regression used by SiroMat. A major advantage is that the single linear regression gave much closer slopes and offsets of the lines of best fit between calibrations.

A critical part of the measurement is the setting and adjustment of the light. The red, green and blue gains of the video camera are adjusted at the beginning of the measurement to obtain a precise magenta background hue, and if this is not within tolerance, the measurement will not proceed. The intensity of the light is then set by adjusting the flash length of the white LED. This procedure will typically take less than 5 seconds to complete. Cottonscope is designed to operate in normal lighting conditions. Variation in ambient lights has tested to show no effect on measurement. However, direct sunlight falling on the measurement point must be avoided.

Results and Discussion

Calibration

Cottonscope was calibrated using a subset of the 104 International Textile Center (ITC) fiber maturity reference cottons (Hequet *et al*, 2006). A subset of 6 samples was chosen to represent the widest range of cotton maturity ratio (MR) values from this set, i.e. from 0.577 to 1.07.

Approximately 8 mg of snippets was spread onto each slide. Each slide was measured once and a line of best fit of the mean yellowness value versus the standard MR was calculated.

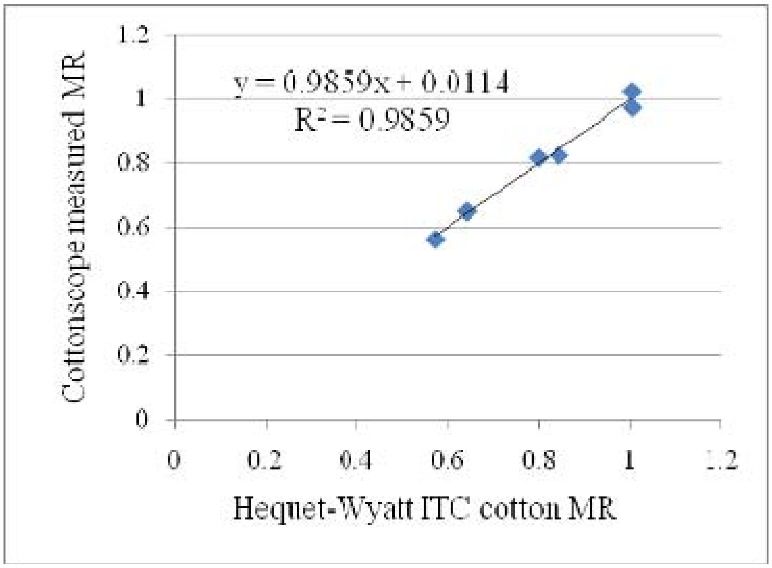

Figure 3. Calibration graph

Validation

A different subset of the 34 ITC cotton samples was chosen to validate the calibration. Using the same procedure, a new set of data was measured and the results can be seen in Figure 4. The r^2 value of the linear regressed line is 89%, which indicates the Cottonscope principle is an excellent way of measuring maturity ratio based on the subset of the standard cottons.

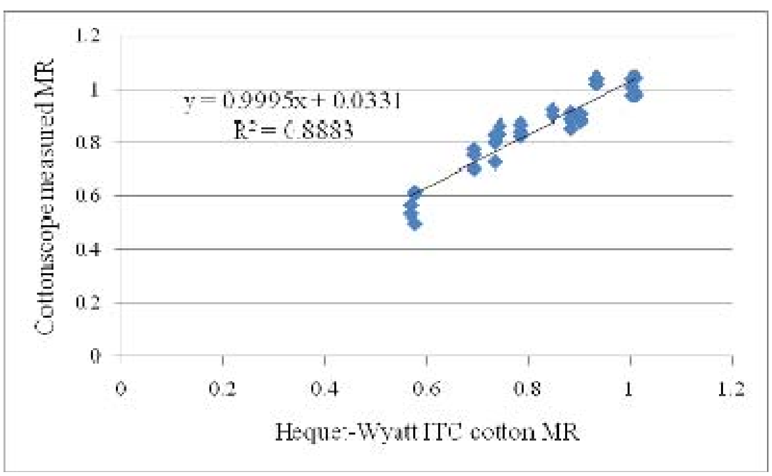

Figure 4. The validation graph of measured samples after calibration

Repeatability over Time

To test the repeatability of measurement over time, a Cottonscope was set to measure the same slide repeatedly over 24 hours. The instrument was not in a conditioned room and there was considerable variation in temperature, humidity and background light over the period of the measurements. The slide had a MR of 0.97 and a fibre count of 3400. . The plots in Figures 5 and 6 show the variation in MR values and conditions over the 24 hour period.

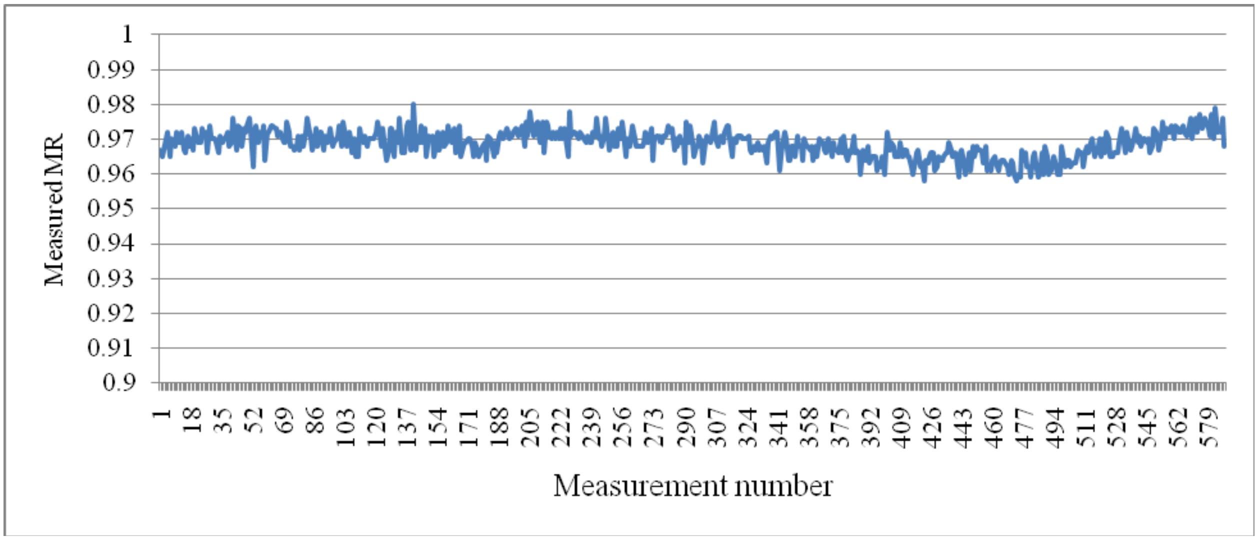

Figure 5. MR repeat measurement over 24 hours

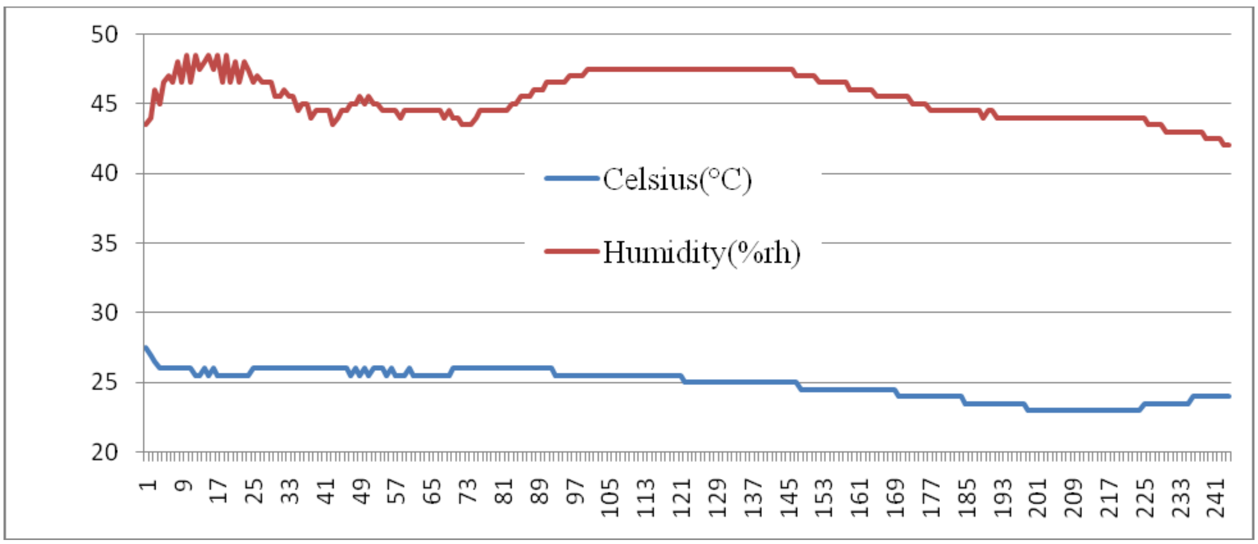

Figure 6. Temperature and humidity over the same 24 hours as the MR repeat measurement

The standard deviation of the repeat measurements was 0.0038, which indicates a 95% confidence limit of ± 0.0075 for repeat measurement of this single slide.

Between Slide Repeatability

To determine the between slide repeatability, 3 slides were prepared from each sample in a subset of the standard cottons, covering the full range of MR, Figure 7. It appeared that the between slide variability is generally higher for lower MR samples.

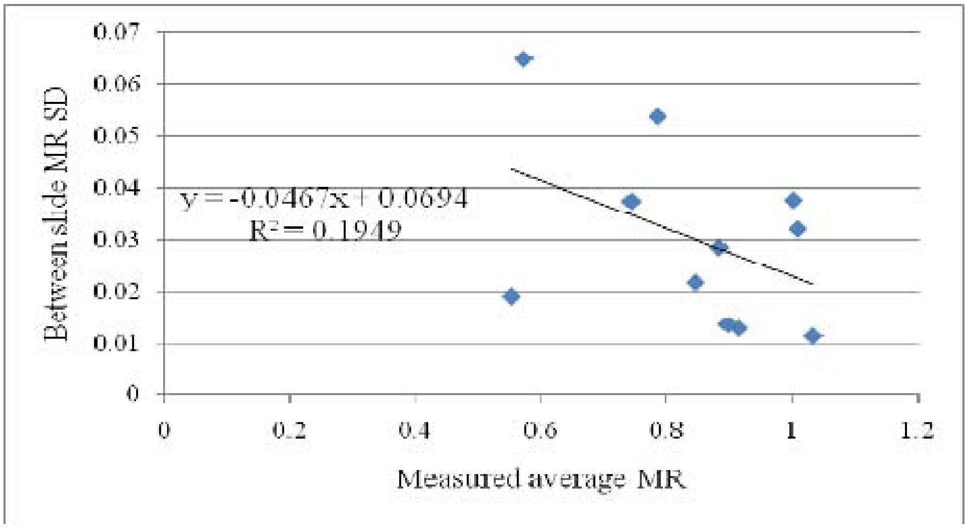

Figure 7. Between slide SD of MR

The average between slide standard deviation of MR was 0.03. As expected, this is higher than the SD of the between measurement of the same slide. It is probable that a large part of this variation is due to the difference between sample sites. To improve repeatability, results from several slides can be averaged by software.

Between Instrument Repeatability

Two Cottonscopes were calibrated using the same slides from the subset. A different set of slides from the subset was then measured on both instruments.

The correlation coefficient r^2 is 98% indicating an excellent agreement between the 2 instruments.

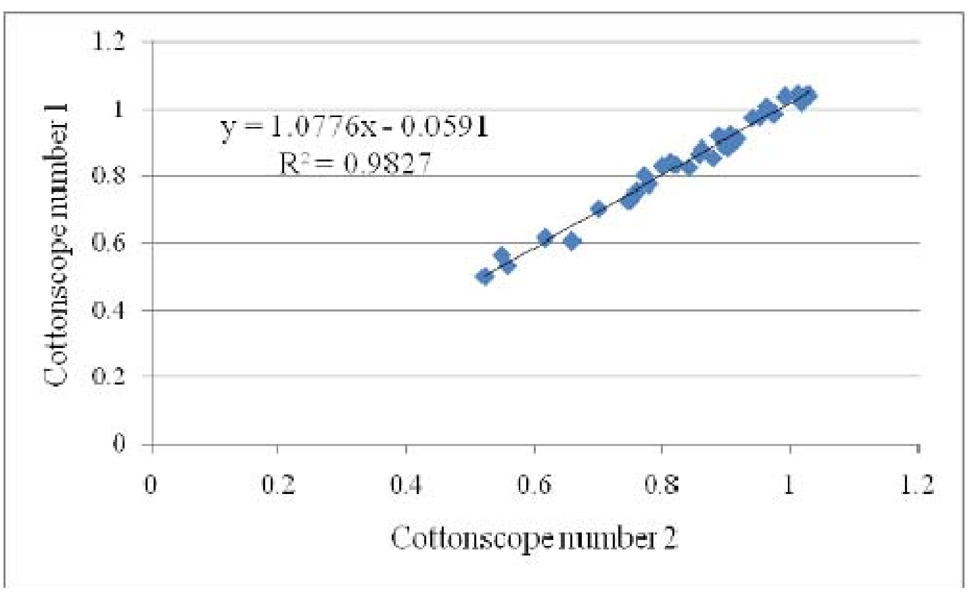

Figure 8. Between instrument repeatability

Conclusion

Cottonscope is an automated microscope that measures cotton maturity directly from color images. Using a sub-set of the ITC cottons with accepted reference values for maturity, the results show linear calibration can be achieved with an excellent correlation between with the predicted values. The 24 hour continuous run showed no significant drift in the MR value under uncontrolled condition such as temperature and ambient light. Using the same sub-set of ITC to calibrate a second instrument, there was no significant statistical difference.

Acknowledgements

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