2004 BELTWIDE COTTON CONFERENCES, SAN ANTONIO, TX – JANUARY 5-9

MEASUREMENT OF FIBER MATURITY USING IMAGE ANALYSIS OF CROSS-SECTIONS: THE AUSTRALIAN EXPERIENCE

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Abstract

CSIRO has satisfactorily implemented the technique developed at the USDA to measure the maturity of cotton fibers by direct measurement of fiber cross-sections. As part of the protocol used at CSIRO, independent software has been developed in-house to undertake the necessary image analysis to derive fiber perimeter, area and maturity information for each cross-section. In a comparative test of the software between CSIRO and the ITC, the perimeter values obtained from the CSIRO software are in excellent agreement with the corresponding values from the ITC however the CSIRO area and hence maturity values are consistently some 15 to 20 percent smaller. The reason for this remains unclear. CSIRO has also undertaken a significant set of measurements on a set of seven reference cottons supplied by the USDA and the results are in good agreement with the results from ITC on the same samples, apart from the consistent difference in area and maturity values mentioned above. Further the observed range in values measured within one sample is also in good agreement with that observed at the ITC.

Introduction

A direct approach for accurate measurement of fiber maturity using fiber cross-sections has been developed at the USDA (Boylston, Thibodeaux and Evans, 1993). Whilst this is somewhat slow and requires a skilled operator, it is being used by a number of laboratories to develop reference cottons for future calibration of other techniques. For example a lot of work is being undertaken at the ITC (Texas Tech) as reported in the previous paper (Hequet, Thibodeaux and Evans, 2002). The present paper outlines the experience at CSIRO in using this technique and collaborating in the inter-laboratory trial. The results from the early stages of this work at CSIRO were reported previously (Naylor, 2002).

Materials and Methods

One small sample of each of the seven cottons forming a reference set was supplied by Dr Thibodeaux from the USDA. The history of, and results for these samples from the ITC, have been reported elsewhere (Hequet, Thibodeaux and Evans, 2002). Our understanding is that each sample supplied to CSIRO corresponds to a sub-sample in the terminology used by Hequet, Thibodeaux and Evans (2002).

Preparation of fiber cross-sections followed the technique developed by Boylston, Thibodeaux and Evans (1993). Note that in our procedure the fibers were mounted in 1/8 inch internal diameter tubing as recommended by Boylston, Thibodeaux and Evans (1993) and used routinely at the USDA whereas the group at the ITC uses 1/16 inch internal diameter tubing.

Another important difference between the current study and that used by the other two currently participating laboratories is that in the current study CSIRO developed its own image analysis software routines, rather the software used at ITC and USDA.

Results

Preparation of Cross-Sections

Following the work reported previously (Naylor, 2002), the thickness of the cross-sections was reduced to approximately one micrometer to increase the sharpness of the images. Figure 1 is a typical example.

From each cross-section the key measurements are fiber perimeter (P) and cross-sectional area (A) as illustrated in Figure 2. From these two values, the circularity or degree of wall thickening, theta, is calculated as follows

$$\text{Theta} = 4\pi A/P^2$$

Comparison of the CSIRO Software Approach with that used at the ITC and USDA

As noted earlier CSIRO opted to develop its own software analysis routines. The CSIRO algorithm comprises a watershed isolation of individual fibers, followed by the extraction of intensity histogram information from each cross-section and, finally, a threshold based on the histogram values to isolate the lumens.

Using a sample block mounted at CSIRO, Dr Hequet and his team at the ITC kindly prepared a section/slide and analyzed a series of 49 images containing approximately 457 cross-sections. The prepared section and corresponding images and results were forwarded to CSIRO. This formed a useful data set for comparing the CSIRO systems with those at ITC.

As a first comparison of the CSIRO vs. ITC software approaches, the idealized fiber cross-sections in the analyzed so-called 'Results' images that are an output of the analysis used by ITC (e.g. Figure 3) were re-processed at CSIRO to extract perimeter, area and lumen information. One advantage of using these 'Results' images as a test is that they have well defined sharp edges, removing one source of uncertainty in the image analysis procedure. Figures 4 and 5 illustrate the comparison for the 457 individual sections. The perimeter results are in excellent agreement with the ITC data (Figure 4). The area values are also in very good agreement (Figure 5). The CSIRO area values are consistently approximately 5 percent smaller than the ITC values. The CSIRO software uses an area algorithm from the commercially available 'Optimas' software package and this appears to be the source of the 5 percent discrepancy. If an alternative approach of simply counting pixels is used then a virtually perfect agreement is achieved. It is documented in the software package that the area algorithm smoothes the boundary and can lead to a discrepancy similar to that observed. It is moot point whether it is more appropriate to smooth the edges or count pixels.

A second comparison of the CSIRO and ITC software was based on a reanalysis of the ITC images of real cross-sections using the CSIRO software. The results for the perimeter measurements on 457 cross-sections (49 images) are shown in Figure 6. The results in general show good agreement. (Eighteen obvious outliers explained as either a failure to separate touching cross-sections or a failure to 'open' a cross-section where the ends had curved around to touch were removed from the analysis.) Similar analyses for area and theta values are shown in Figures 7 and 8 and summarized in Table 1. There is a good correlation between the CSIRO and ITC results although the area (and therefore theta) values measured by the CSIRO algorithm are some 15 to 20 percent less than the ITC reported values. The source of this significant discrepancy is unclear and this will be the subject of further work.

Validation of the CSIRO Image Capturing Processes i.e. the Optical Microscopy

To check the CSIRO image capturing system i.e. the optical microscopy the sample block shared between CSIRO and ITC was again used. In this case the actual microscope slide containing the sample cross-sections used at the ITC to produce the data mentioned above was re-imaged at CSIRO. Somewhat like finding a needle in a haystack, from the over one thousand cross sections on the slide we were able to visually identify and match approximately 20 of the 49 image frames representing 123 of the original 457 ITC cross-sections. The CSIRO images were analyzed with the now validated CSIRO software and the results compared with the original ITC data. The comparison between the two laboratories is summarized in Table 2. The between laboratory agreement for fiber perimeter was again excellent. The correlation between the two laboratories for fiber cross-sectional area was excellent but again the CSIRO values are about 20% smaller.

Comparison of CSIRO and ITC Results for the 7 Cotton Samples

Given these very encouraging comparisons between the two laboratories, we proceeded with the main task of independently preparing cross-sections and measuring the perimeter, area and maturity of the seven cotton samples. Between 3000 and 8000 cross-sections for each sample were analyzed and the results are summarized in Table 3. Table 3 also contains the ITC summary data from measurements on similar samples (Hequet, Thibodeaux and Evans, 2002). Table 4 tabulates a comparison of the summary results between the two laboratories. In summary the actual average perimeter values were in good agreement between the two laboratories and the CSIRO area values were on average about 15 to 20% smaller.

From an analysis of variance of the data, the minimum number of cross-sections needed to obtain a variance of ±2% at the 90% confidence level is estimated in Figure 9. For example, 100 cross-sections from 30 blocks i.e. a total of 3000 cross-sections would be adequate. This is in good agreement with the values established by ITC. However, the same variance may be obtained with 16 blocks (the minimum number) and 1000 cross-sections per block. From CSIRO's experience blocks take considerable effort to prepare, and so this second option may be a more practical approach.

Conclusion

In summary, CSIRO has been able to satisfactorily implement both the sample preparation and optical microscopy associated with the prescribed technique for measuring the fiber maturity via optical microscopy of thin cross-sections. Using independently developed software, CSIRO measured fiber perimeter values were in good agreement with those obtained at ITC. However the CSIRO area values and consequently the maturity (theta) values were on average consistently about 15 to 20% smaller. The source of this discrepancy is unclear. Further the scatter in the data was similar for the two laboratories, consistent with the inherent variability between individual fibers in a sample.

Acknowledgements

The authors gratefully acknowledge that the work presented in this paper has been support financially by the Australian Cotton Research and Development Corporation and the Australian government. The authors would also like to acknowledge

significant help from Dr Eric Hequet and his team at the ITC, Texas Tech University and also Dr Devron Thibodeaux from the SRRC, USDA in sharing data and samples etc as noted in the text.

References

Boylston, E.K., D.P. Thibodeaux and J.P. Evans. 1993. Applying microscopy to the development of a reference method for cotton fiber maturity. Text. Res. J. 63(2), 80-87.

Hequet, E., D. Thibodeaux and B. Wyatt. 2002. Update on results obtained with the proposed reference method for cotton maturity. Proc ITMF Int. C'mttee on Cotton Testing Methods, Working Group: Fineness and Maturity, Appendix FM-3, Bremen (CD).

Naylor, G.R.S. 2002. Progress at CSIRO. Proc ITMF Int. C'mttee on Cotton Testing Methods, Working Group: Fineness and Maturity, Appendix FM-5, Bremen (CD).

Table 1. Comparison of CSIRO and ITC measurements from the same captured images.

Parameter	Slope	Intercept	Correlation (R^2)	Count
Perimeter	0.986	-1.07	0.98	439
Area	0.903	-15.0	0.96	439
Theta	0.923	-0.057	0.93	439

Table 2. Comparison between CSIRO and ITC results for independent measurements from the same slide.

Parameter	Slope	Intercept	Correlation (R^2)	Count
Perimeter	1.034	-3.57	0.94	123
Area	0.801	-11.7	0.94	123
Theta	0.851	-0.056	0.87	123

Table 3. Average values obtained for the seven different cottons.

Sample ITC (CSIRO)	Perimeter		Area		Theta		No of Blocks CSIRO	No of Measurements CSIRO
	ITC	CSIRO	ITC	CSIRO	ITC	CSIRO		
2996 (42)	51.1	53.5	105.0	96.1	0.519	0.446	4	7833
2999 (43)	51.1	55.8	89.7	79.4	0.451	0.337	3	4024
3008 (44)	48.0	50.4	82.2	67.3	0.467	0.357	2	4973
3009 (45)	46.1	46.3	85.6	74.0	0.520	0.456	2	4340
3016 (46)	51.4	55.9	100.7	104.6	0.495	0.442	2	2627
3074 (47)	54.7	56.0	134.4	124.7	0.574	0.518	2	3812
3075 (48)	56.2	58.2	101.2	90.5	0.423	0.363	2	2917

Table 4. Regression Parameters for ITC/CSIRO comparisons of the average measured parameters for the seven cotton samples.

Measurement	Intercept	Slope	R^2
Perimeter	-1.46	1.08	0.847
Area	-16.1	1.07	0.912
Theta	-0.181	1.22	0.885

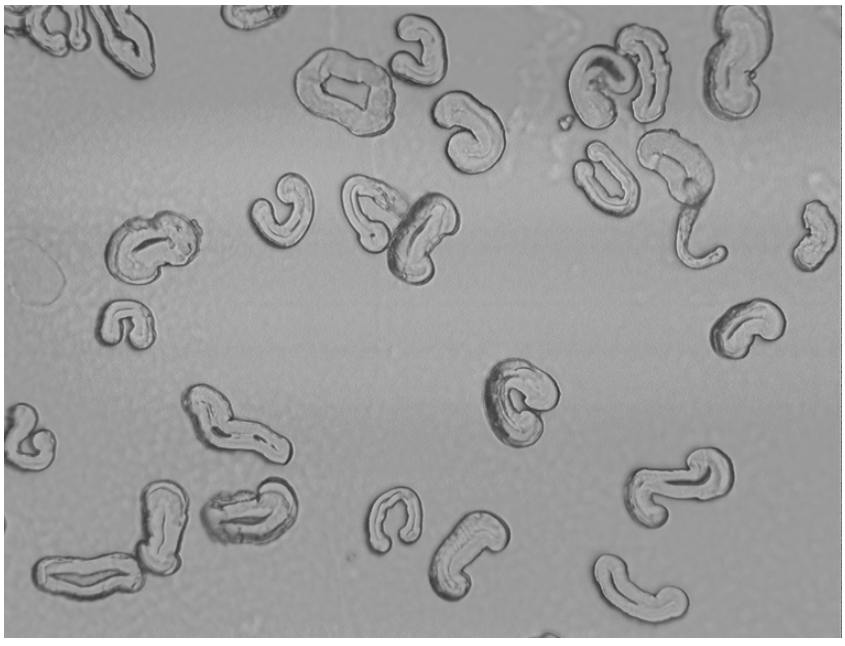

Figure 1. A typical image.

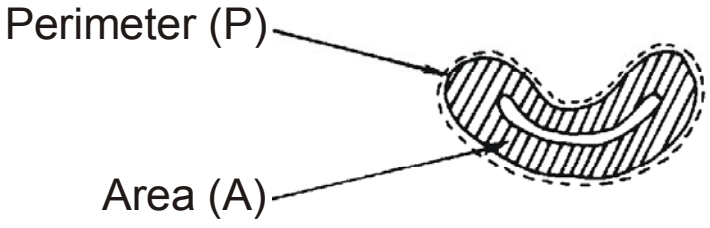

Figure 2. Schematic of a cross-section.

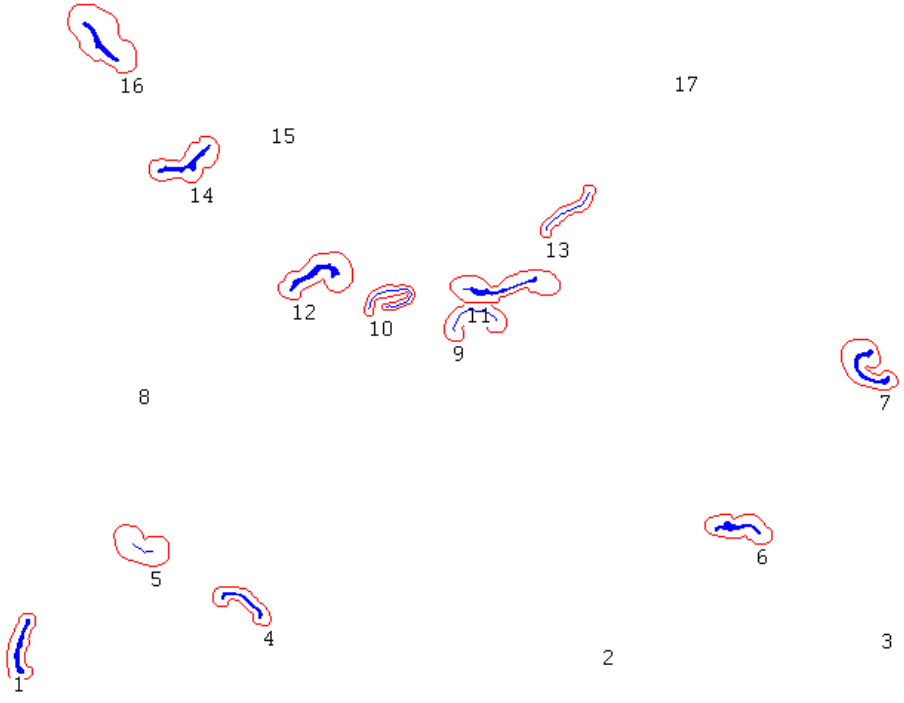

Figure 3. Example of ITC supplied 'Results' image.

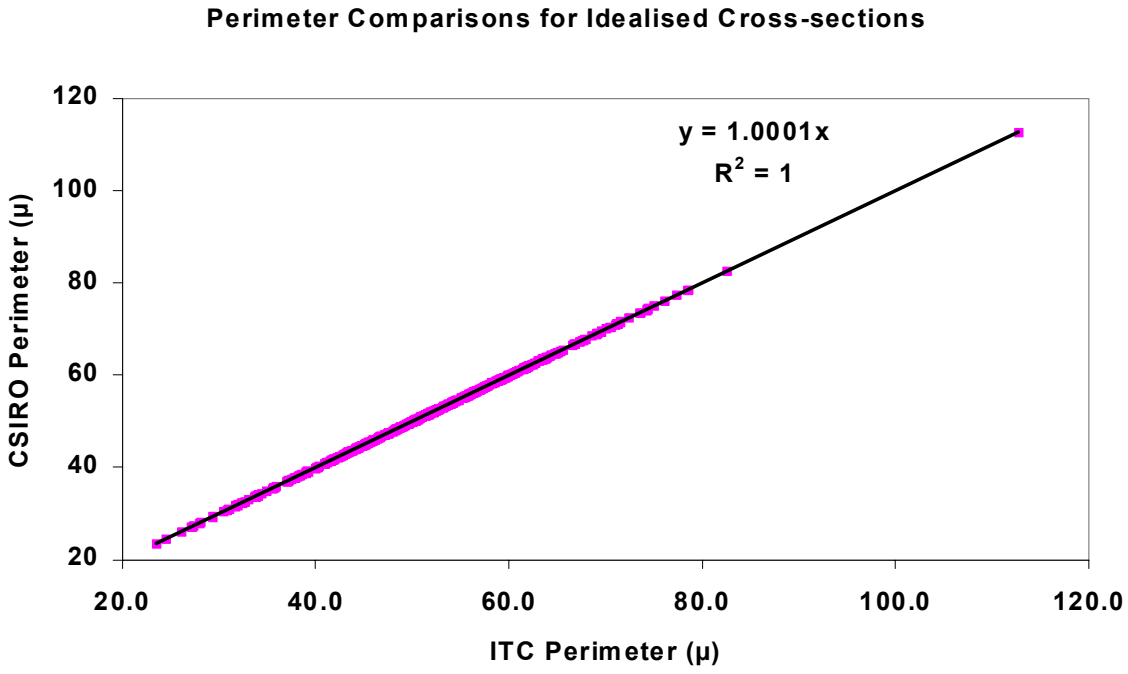

Figure 4. Comparison of perimeter values for idealized cross-sections.

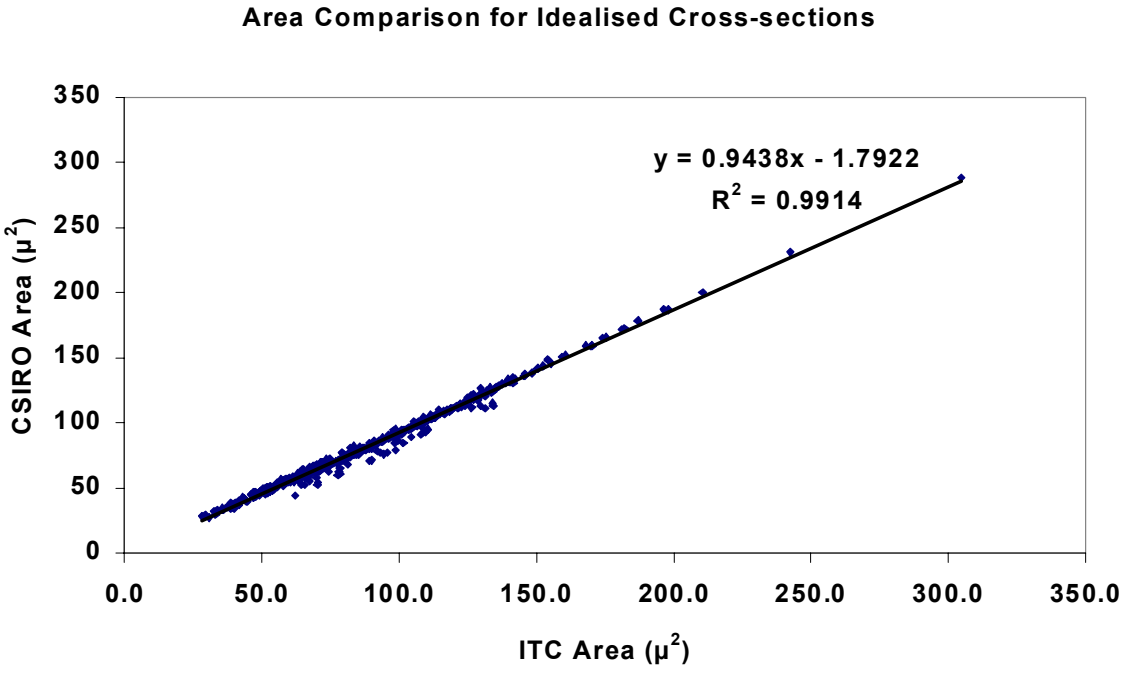

Figure 5. Comparison of area data for idealized cross-sections.

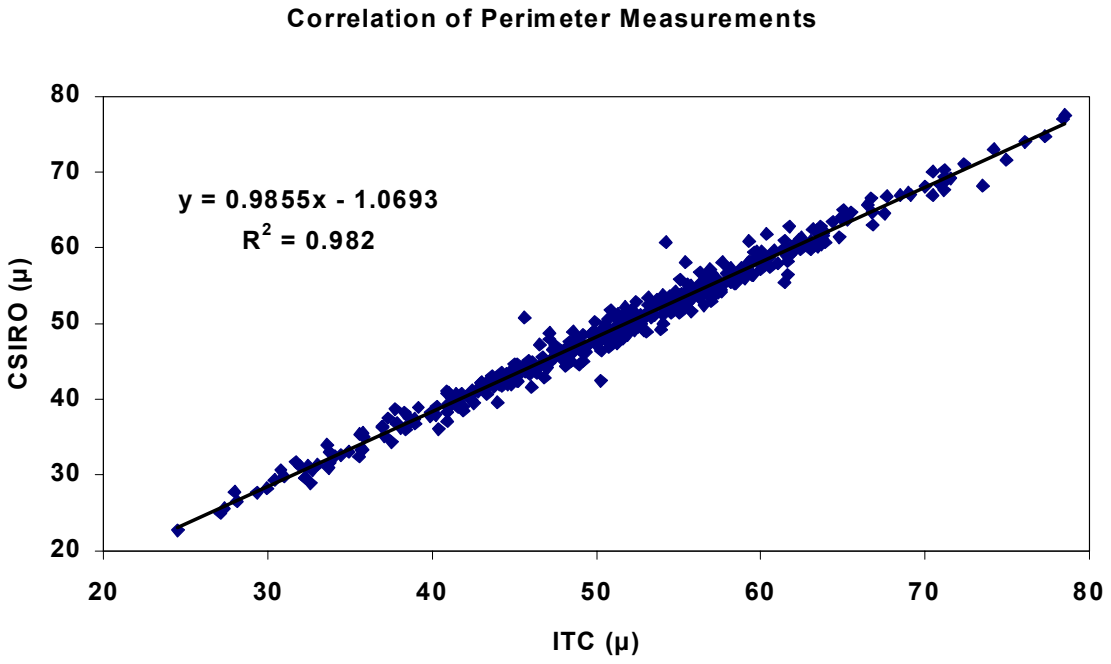

Figure 6. CSIRO/ITC comparison of perimeter measurements from the same captured image.

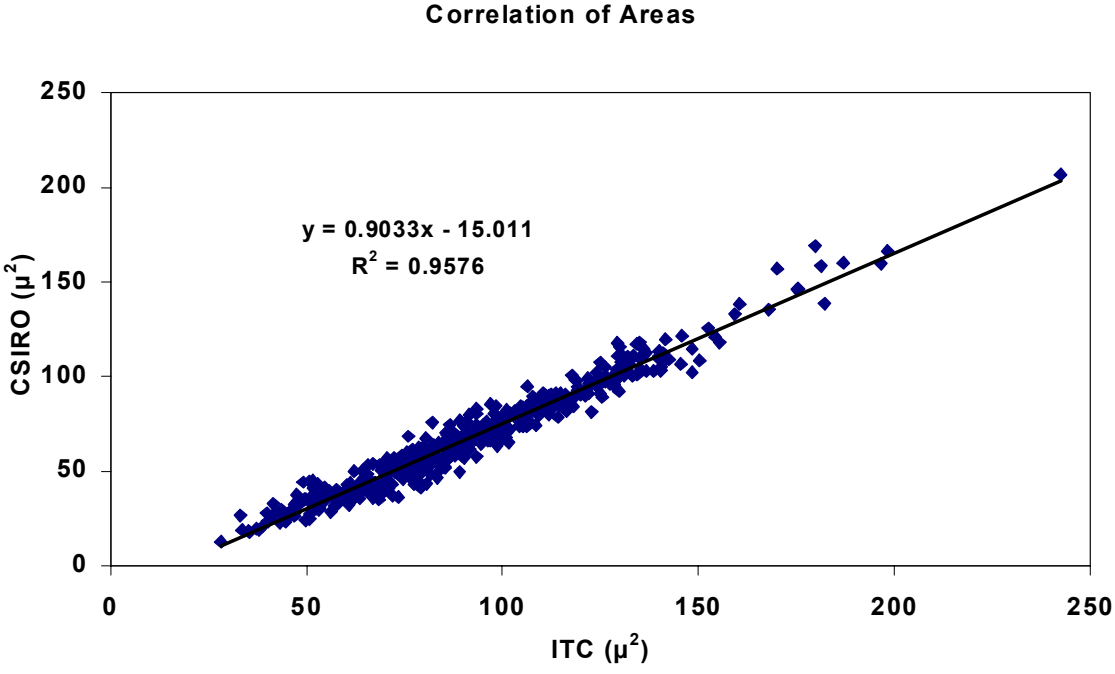

Figure 7. CSIRO/ITC comparison of area measurements from the same captured image.

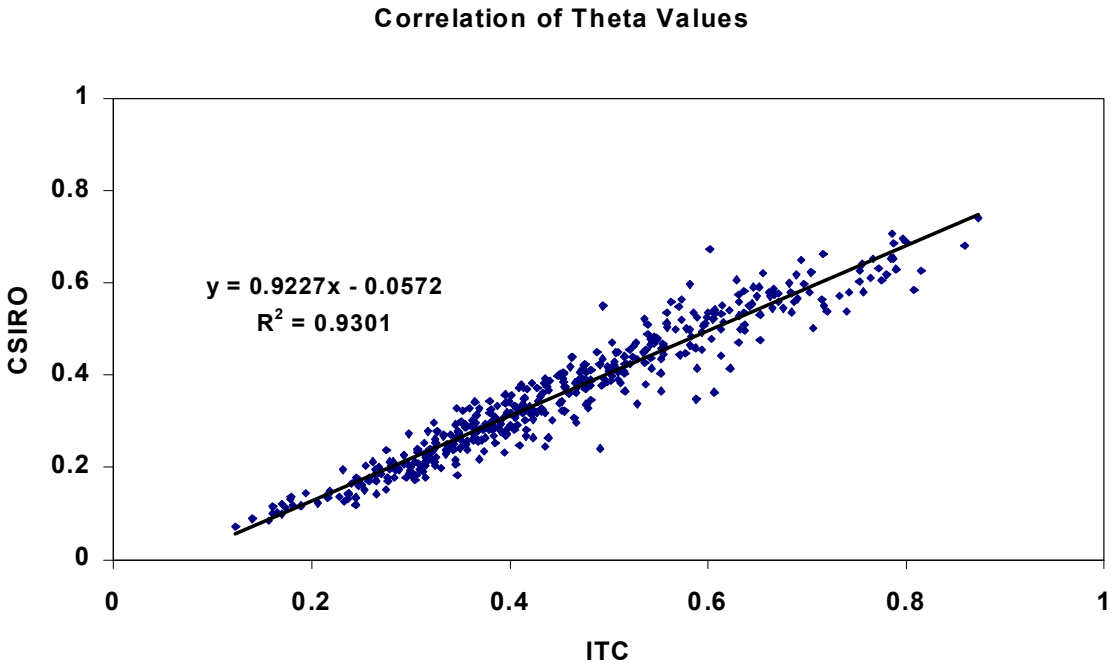

Figure 8. CSIRO/ITC comparison of theta measurements from the same captured image.

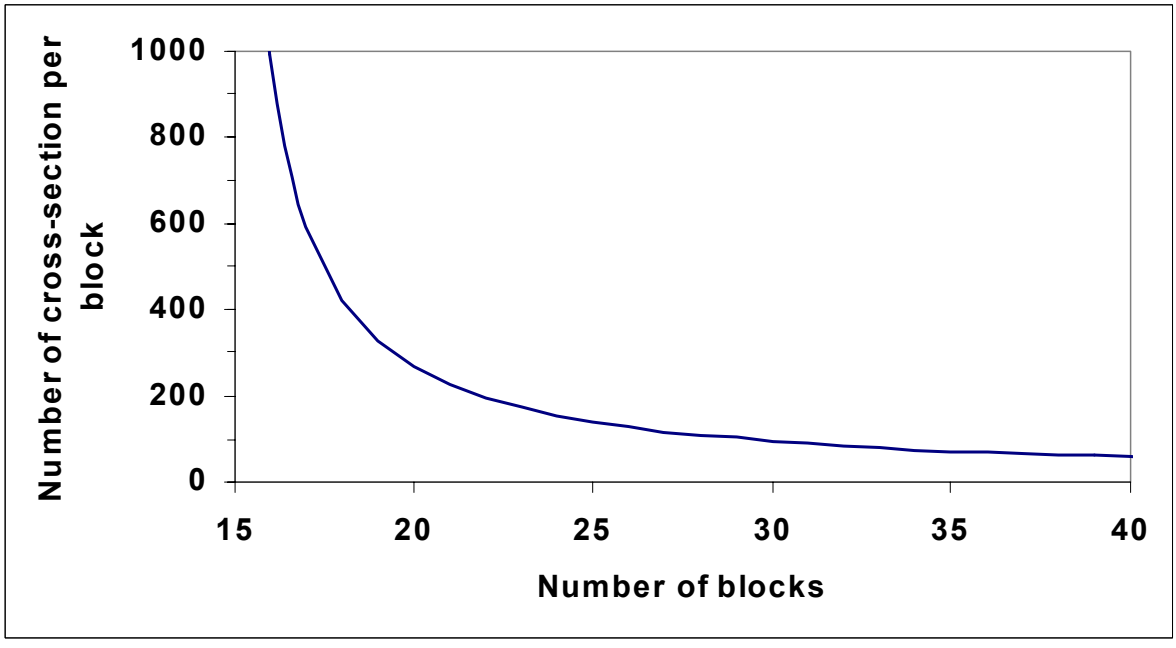

Figure 9. Estimation of the minimum number of blocks and cross-sections per block to achieve the required precision in the mean value per sample.