

REPORTS

Part 1 - Summary Details

Please use your TAB key to complete Parts 1 & 2.

CRDC Project Number: CTFT3C

Annual Report: Due 30-September

Progress Report: Due 31-January

Final Report: Due 30-September

(or within 3 months of completion of project)

Project Title: Participation in an interlaboratory trial to develop standard reference cotton samples for fibre fineness and maturity.

Project Commencement Date: 1/7/2001 **Project Completion Date:** 30/6/2003

Research Program: 6 Value Chain

Part 2 – Contact Details

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Ph: **Fax:** **E-mail:**

Signature of Research Provider Representative: _____

Background

The CRDC has been and is currently funding ongoing work at CSIRO Textile and Fibre Technology to look at new methods of characterising fibre maturity and fineness (CTFT2C, CWT6C, CTFT8C). These projects arise as the commonly used micronaire measurement for cotton is ambiguous, ie the micronaire value is a combination of both fibre fineness and maturity. This is of particular importance to the Australian industry where varieties of fine, mature cotton have the potential to be wrongfully discounted commercially by misinterpreting a low Micronaire value as indicating immaturity in a coarser fibre.

The validity of any new approach developed as part of these other ongoing CRDC projects will need to be demonstrated to and accepted by the worldwide cotton trading industry as adding value commercially.

Dr Devron Thibodeaux at the USDA in New Orleans is coordinating a project to develop a set of reference cottons for use worldwide in the calibration of commercial and research instruments designed to measure fibre fineness and maturity (eg the Micromat F/MT tester). This work involves firstly selecting and preparing a set of samples by careful selection and mixing through blending and carding. The samples will then be well characterised using detailed cross sectioning and image analysis techniques that have been developed at the USDA. The fibre characterisation work was planned to be undertaken independently in three laboratories, the USDA in New Orleans, Texas Tech University in Lubbock and the Cotton Institute in Bremen. The Institute in Bremen unfortunately withdrew from the initiative.

CSIRO Textile and Fibre Technology (TFT) was invited to participate and act as the third independent laboratory to replace the Bremen Institute. Technically CSIRO TFT is in an ideal position to do that as:

(a) Stuart Gordon worked with Dr Thibodeaux at the USDA during this CRDC funded post-doctoral work and has hands on experience with the specialised fibre preparation, cross-sectioning and image analysis procedures that have been developed at the USDA for this work.

(b) CSIRO TFT has significant expertise in both microscopy and image analysis.

Finally this project was seen as an ideal opportunity to develop and maintain a good working relationship with our international colleagues.

List the project objectives and the extent to which these have been achieved.

The aim of the project was to participate in the international inter-laboratory trials as an independent laboratory as part of the development of cotton reference standards for fibre maturity and fineness. The specific objectives were:

Year 1: Establishment and validation of the required experimental protocol including fibre embedding, sectioning, imaging and data analysis.

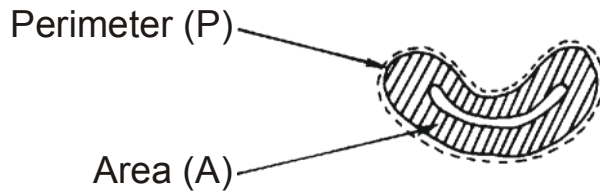
Year 2: Use of the techniques developed in Year 1, to become an active participant in the international inter-laboratory trials to characterise the fineness and maturity of the proposed standard cotton samples.

These objectives have been fully achieved.

Summary of Technical Results and ‘Take Home Messages’.

As indicated in the original proposal and in earlier progress reports, in the first year and a half of the project, work was focussed on (a) developing the sample preparation procedures etc for routinely producing optical microscopic images of fibre cross sections of adequate quality and (b) developing our in-house software analysis routines to determine fibre perimeter, area and hence fibre maturity (see Figure 1).

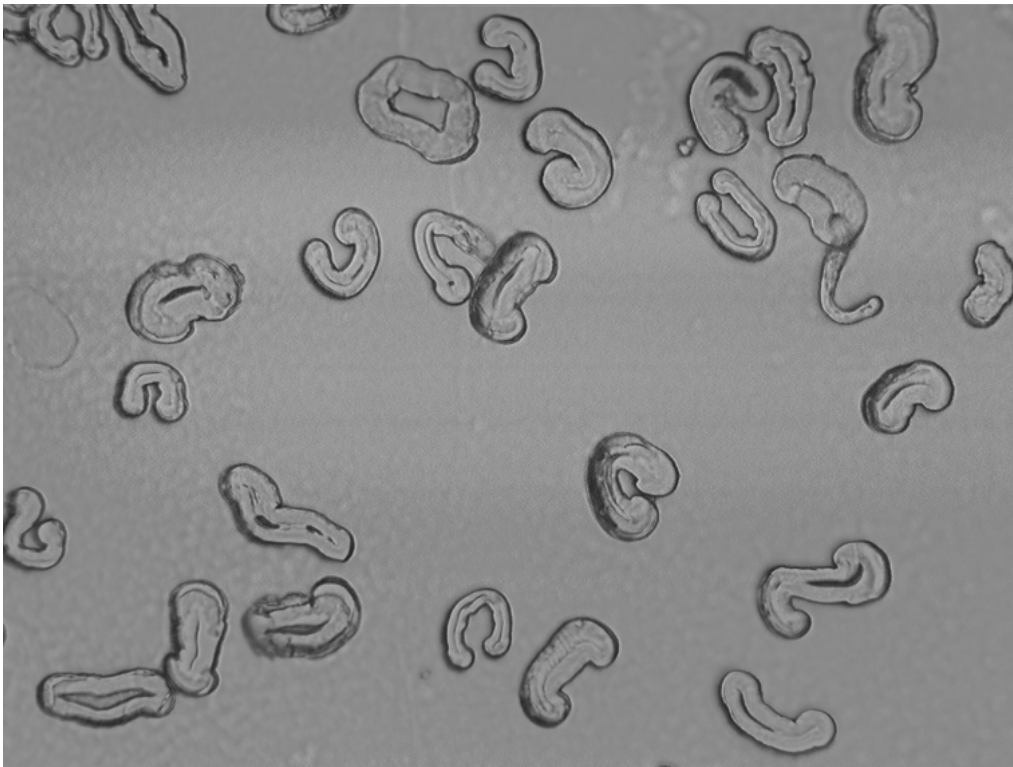
Figure 1 Schematic of a cross section.



$$\text{Maturity Ratio} = 4\pi A / (0.577P^2)$$

It transpired that fibre cross sections as thin as one micrometre were required to produce images with sufficient sharpness. Figure 2 below is an example of a typical image.

Figure 2 A Typical Image.



After this ‘set-up’ phase of the work, full details of our technical progress are listed in the three Appendices. The main points are summarised below.

A very fruitful collaboration was established with Dr Eric Hequet at Texas Tech University. (He has now largely taken over from Dr Thibodeaux at the USDA as the key researcher in this initiative in the US.) Samples, images and data were exchanged to validate our progress at CSIRO. Firstly, a block of mounted fibres was sent to Dr Hequet who confirmed that our mounting processes were satisfactory.

As noted earlier CSIRO opted to develop its own software analysis routines (using our in-house skills and avoiding the unnecessary capital costs of purchasing the system developed in the US). Appendix 1 outlines the important step of validating the CSIRO in-house software. Using the CSIRO mounted sample block mentioned above, Dr Hequet and his team prepared and analysed a series of 49 images containing approximately 457 cross-sections. These images were then reanalysed at CSIRO using the CSIRO software and the results from the two analyses compared. As detailed in Appendix 1, the between laboratory agreement in the perimeter measurement was excellent, however the CSIRO software gave cross-sectional area values consistently about 15-20% smaller than the values reported from Texas Tech University. Further as listed in Appendix 1 this has been investigated in depth and there is some evidence suggesting that perhaps the Texas Tech software is not properly subtracting the area occupied by the lumen. There is ongoing discussion between the two groups to resolve this.

The second important aspect in the validation process, was to check the CSIRO image capturing system ie the optical microscopy. This is detailed in Appendix 2. This was achieved by again utilising the CSIRO – Texas Tech University shared samples. In this case the actual microscope slide containing the sample cross-sections used at Texas Tech University 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 Texas Tech cross sections. The CSIRO images were analysed with the now validated CSIRO software and the results compared with the original Texas Tech University data. The between laboratory agreement for fibre perimeter was again excellent. The correlation between the two laboratories for fibre cross-sectional area was excellent but again the CSIRO values are about 20% smaller.

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 analysed and Appendix 3 details the results and comparison with Texas Tech University results from the similar samples. 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. Further the scatter in the data was similar for the two laboratories, consistent with the inherent variability between individual fibres in the sample.

Plan for Future Activities

CRDC has agreed to fund ongoing work in this area (CTFT7C). The planned objectives for this work are outlined in detail in that project proposal. The primary work continues the collaboration by undertaking measurements on an extended sample set.

List of Publications

G.R.S. Naylor 'Progress at CSIRO' Proceedings of the ITMF International Committee on Cotton Testing Methods, Working Group on Fibre Maturity, Bremen, 2002.

G. Higgerson, G. Naylor and M. Pate 'Measurement of Fibre Maturity using Image Analysis of Cross-sections: The Australian Experience.' US Beltwide Cotton Quality Conference, Jan 2004.

Appendix 1. Validation of the CSIRO Software.

The CSIRO algorithm used to determine values of Theta on slides prepared at CSIRO was used to analyse the fifty images supplied, together with individual cross-section measurements, by ITC. The algorithm comprises a watershed isolation of individual fibres 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. Because the resolution and intensity distribution of the ITC images were significantly different from those of the CSIRO images, some of the parameters used in the CSIRO algorithm had to be modified to obtain sensible results.

The results for perimeter measurements on 457 cross-sections from 49 images (Image 14 was corrupted) are shown in Fig. 1. The results in general show good agreement although there are some obvious outliers. These were examined by reference to the processed images. Those measurements highlighted in red were the result of a failure to separate touching cross-sections and those in yellow from a failure to open a cross-section where the ends had curved around to touch. These were removed from the analysis (eighteen cross-sections in total).

Table 1 Comparison of CSIRO and ITC measurements

Parameter	Slope	Intercept	Correlation (R ²)	Count
Perimeter	0.986	-1.07	0.982	439
Area	0.903	-15.0	0.958	439
Theta	0.923	-0.057	0.930	439

The subsequent measurements show good correlation with the ITC results (fig. 2, 3 and 4 and Table 1) although the area (and therefore theta) values measured by the CSIRO algorithm are some ten percent less than the ITC reported values if we assume that these have excluded the lumen from the area. When the ITC results are compared with the CSIRO results that have not been corrected for lumen removal (Fig. 5 and 6 and Table 2) the differences are greatly reduced, and the correlation has not changed significantly.

Table 2 Comparison of ITC Results with CSIRO Measurements without lumen removal

Parameter	Slope	Intercept	Correlation (R ²)	Count
Area	1.00	-7.5	0.953	439
Theta	1.01	-0.010	0.940	439

In an endeavour to resolve this apparent difference the analysed images also supplied by ITC (an example is given in Fig 7.) were processed to extract perimeter, area and lumen information. The results for perimeter measurements (fig. 8) show that there are no calibration issues involved. When the CSIRO determined areas are compared with the ITC reported area the measurement before lumen removal agrees more closely with the reported values than the area after lumen removal (+4% compared with -11% Fig. 9, Table 3). When the area measurement corrected for lumen was compared with the original CSIRO measurements a good agreement was

obtained (Fig. 10). The reduced number of cross-sections analysed was due to the occasional intrusion of the cross-section number into the cross-section itself (see, for example, numbers 1 and 11 in fig. 7).

Table 3 Comparisons based on Analysis of ITC Results Images

CSIRO Area	Slope	Intercept	Correlation (R²)	Count
Includes Lumen	1.04	2.80	0.980	421
Excludes Lumen	0.892	-12.8	0.982	421

The foregoing suggests that the supplied ITC results have been calculated without removing the lumen contribution to the area suggested by the blue highlights within each cross-section (fig. 7) although the small discrepancy shown in Table 3 suggests that some processing has been done to reduce the area. We are pursuing this with ITC.

Figure 1. Comparison of Perimeter Measurements Showing Outliers

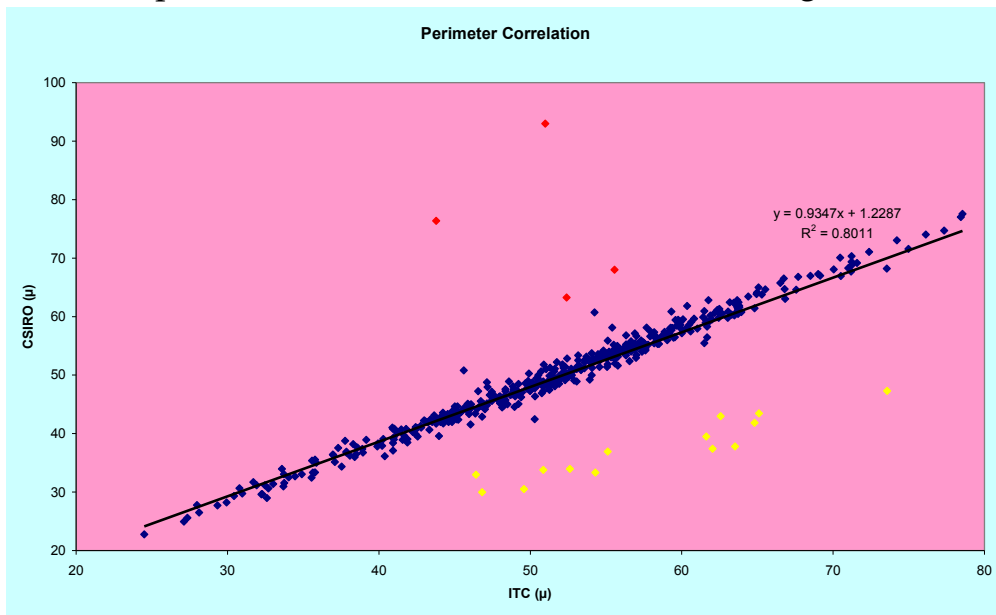


Figure 2. Comparison of Perimeter Measurements After Removal of Outliers

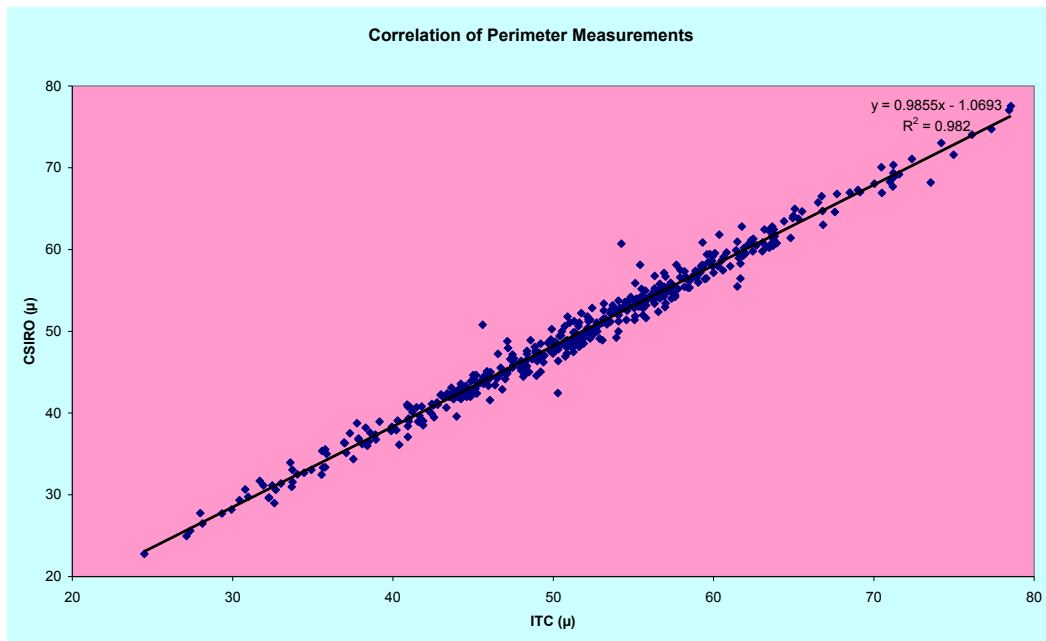


Figure 3. Comparison of Area Measurements

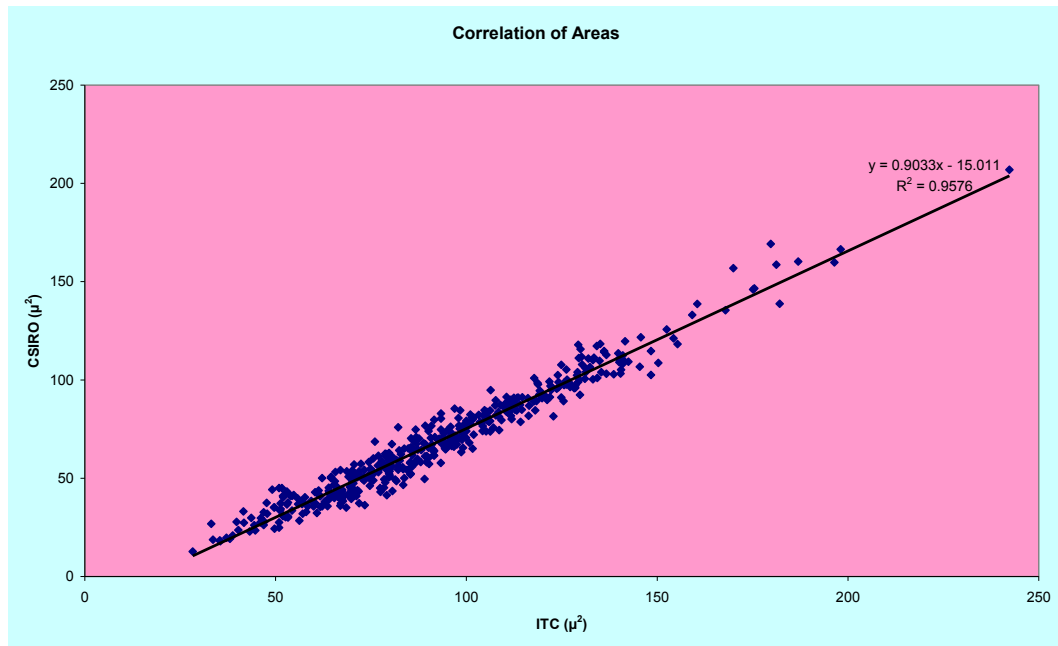


Figure 4. Comparison of Theta Calculations

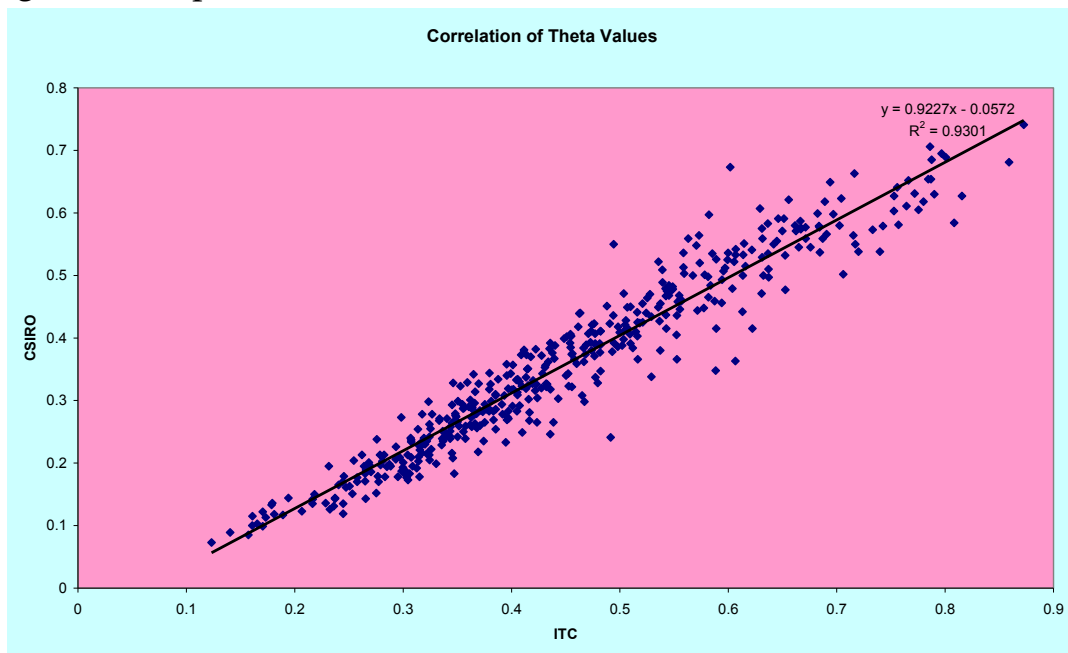


Figure 5. Comparison of Area Measurements Without Removal of Lumen from the CSIRO Measurements

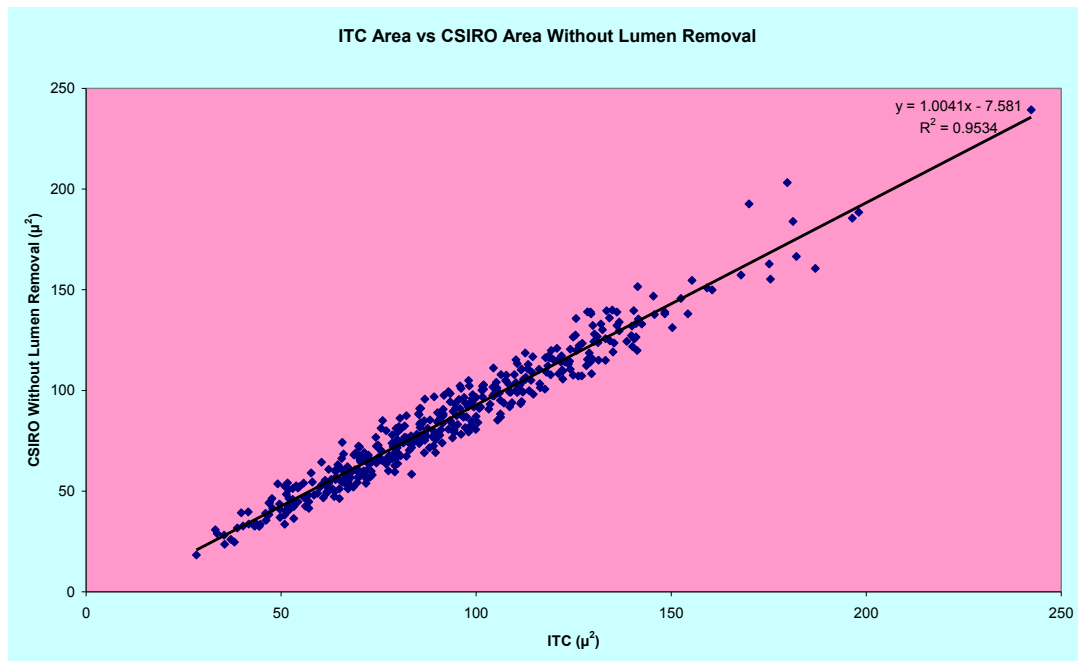


Figure 6. Comparison of Theta Calculations Without Removal of Lumen Areas from CSIRO Measurements

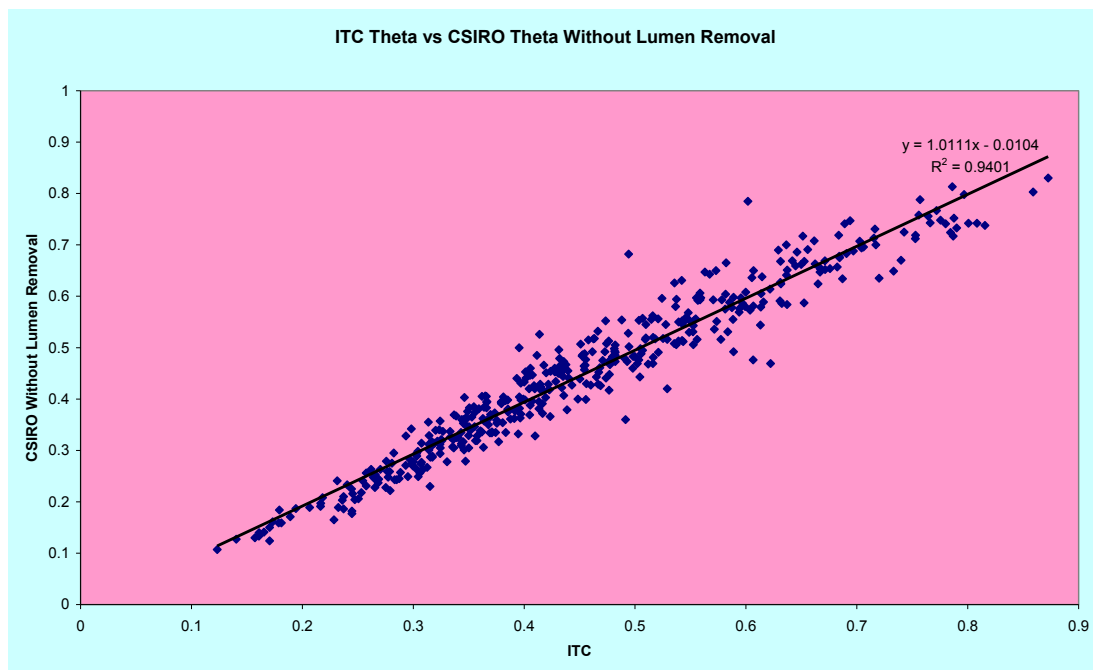


Figure 7. Example of ITC supplied Results Image

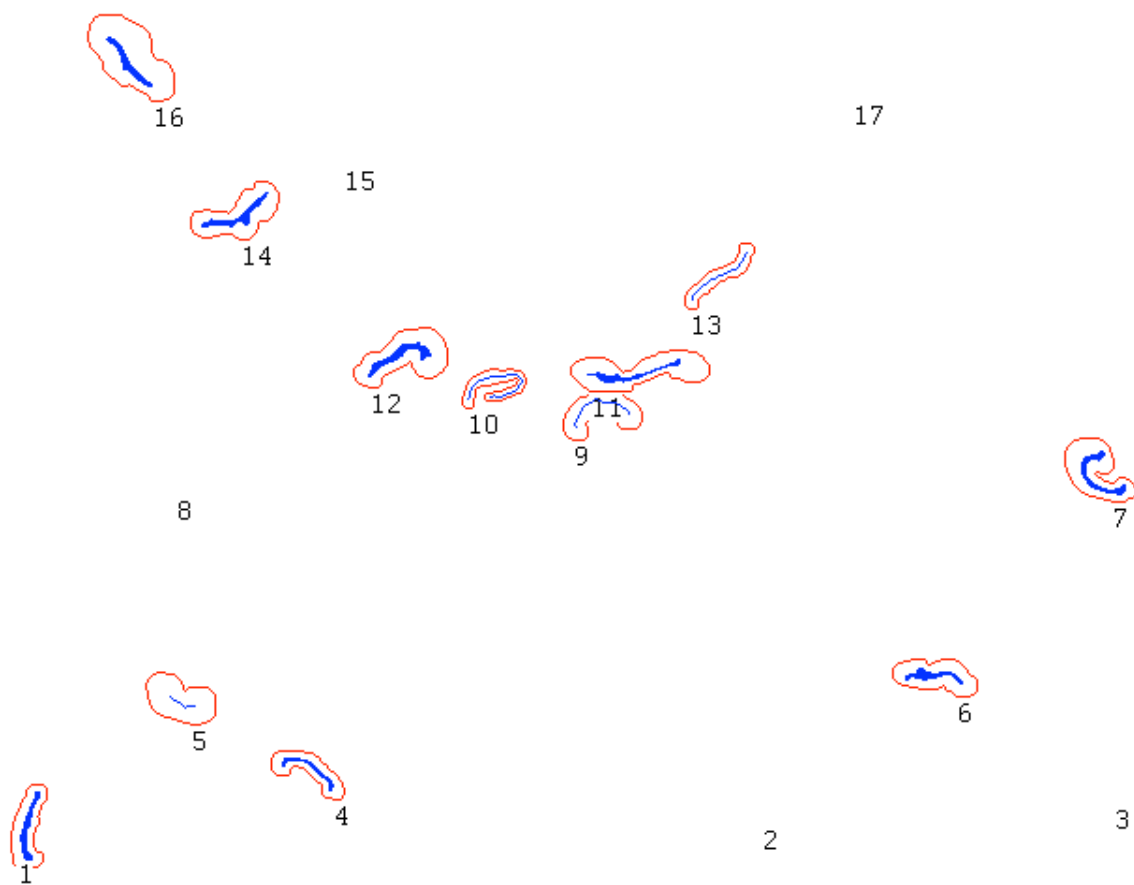


Figure 8. Comparison of Perimeter Measurements on Result Images

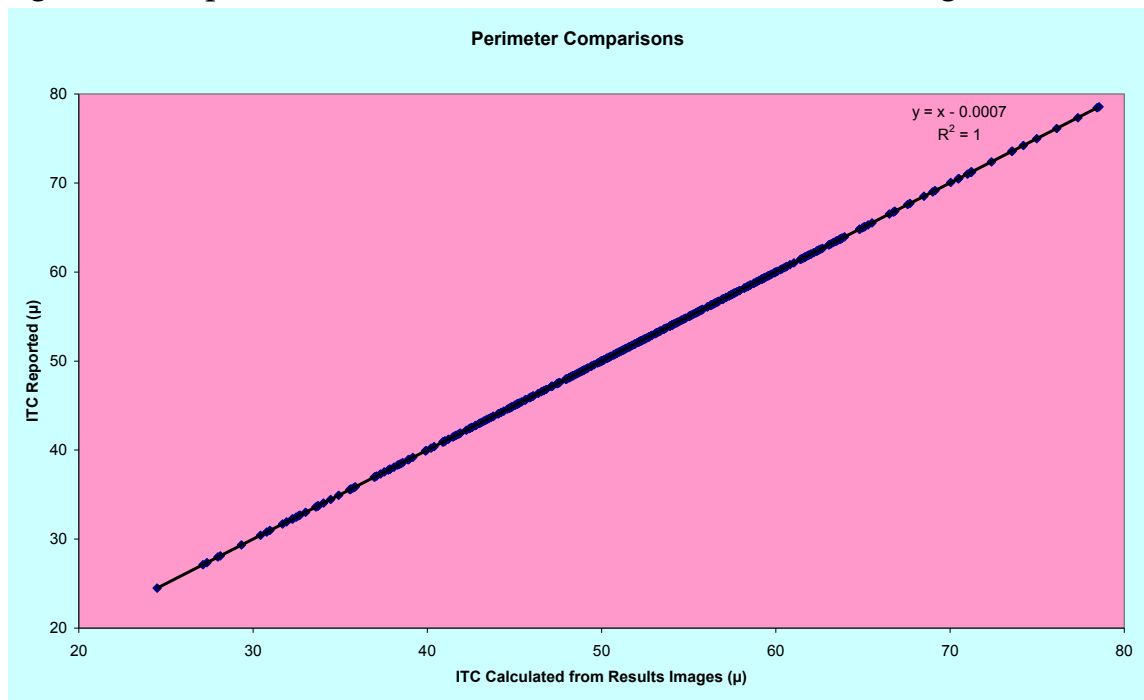


Figure 9. Comparison of Lumen and Non-Lumen Area Measurements on Results Images

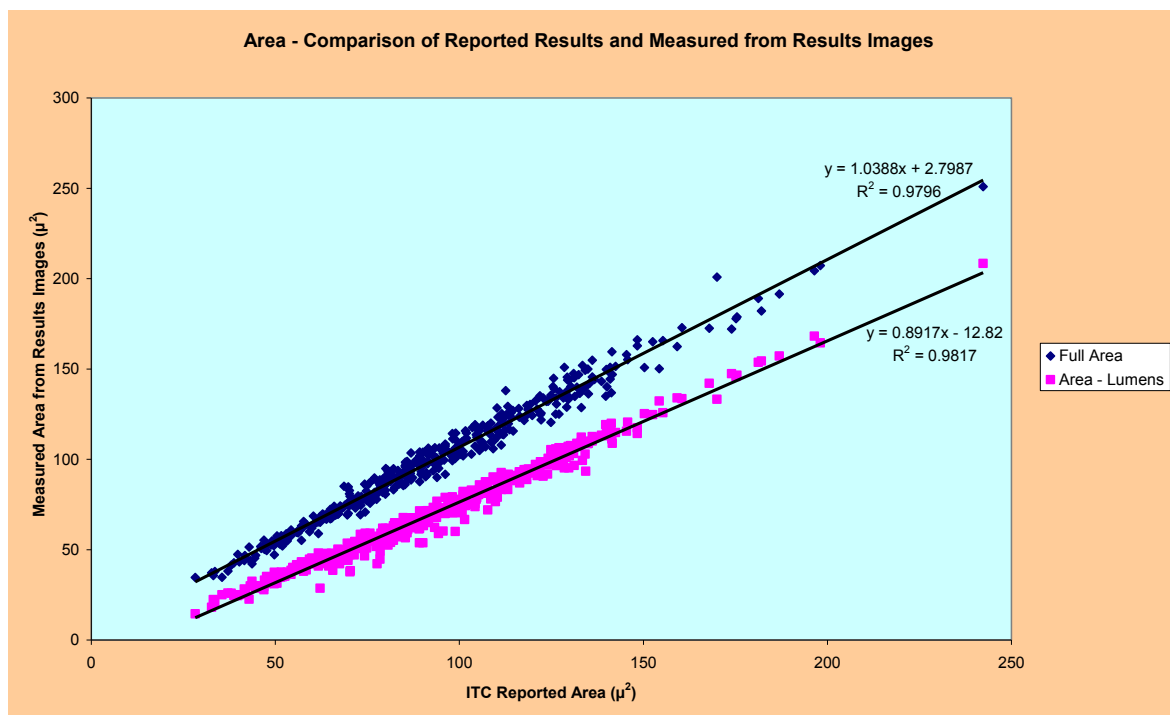
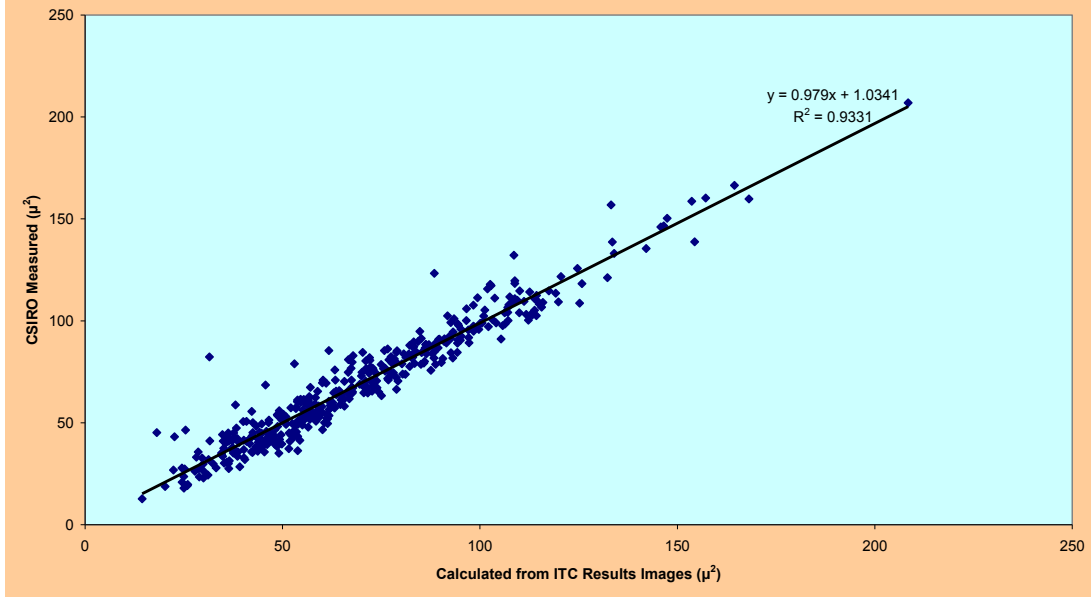


Figure 10. Comparison of CSIRO Measured Areas Excluding Lumens with Those from ITC Results Images

Area - Lumens



Appendix 2. Validation of the CSIRO Image Capturing Processes in the Optical Microscopy.

One of the blocks prepared at CSIRO was sent to Texas where it was sectioned and mounted. fifty images were then taken and analysed by Texas Tech and the results and the slide returned to CSIRO. Twenty of the Texas Tech images were matched as closely as possible on the CSIRO microscope using the Texas Tech prepared slide. These images were then analysed using the CSIRO software and the results compared on matched fibre cross-sections. These are presented below.

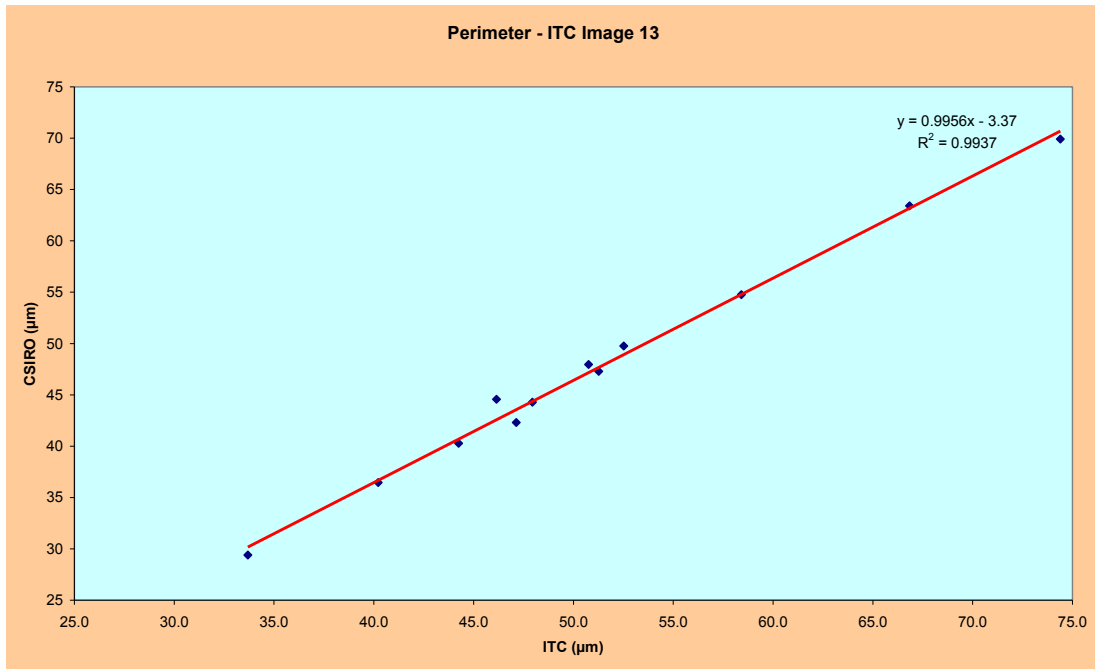


Figure 1 Comparison of Perimeters of Matched Cross-sections for ITC Image 13

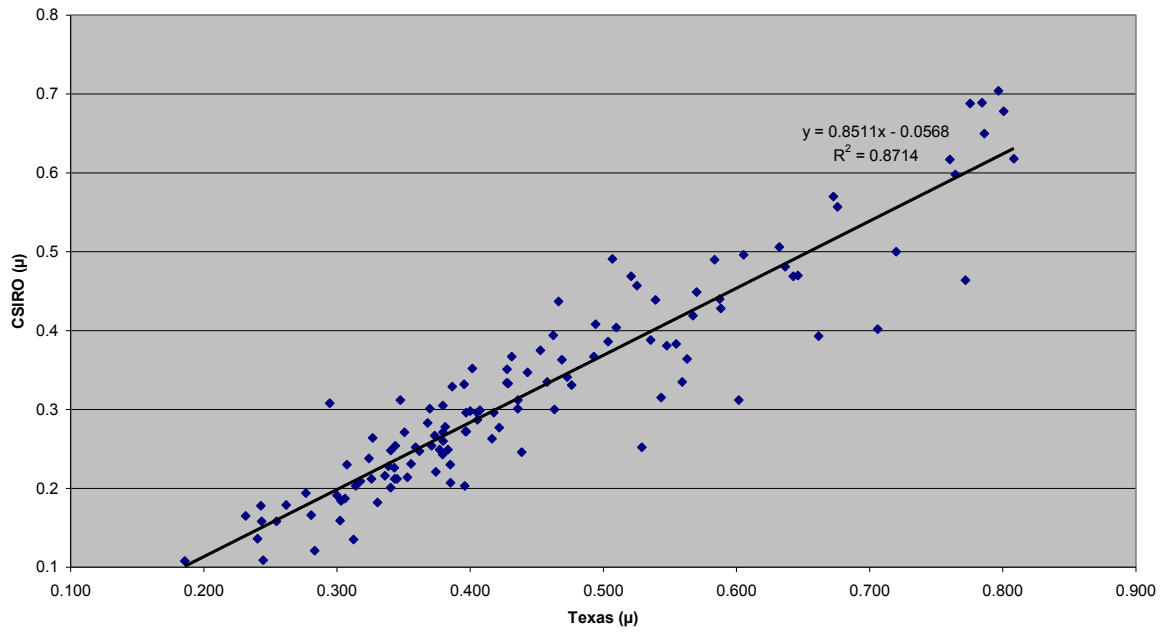
Appendix 1 contains the plots for perimeter, area and theta for all twenty images combined (after removal of obvious errors). The results are summarised in Table 1 below.

CSIRO = Slope*ITC + Offset			
Parameter	Perimeter	Area	Theta
Slope	1.03	0.80	0.85
Offset	-3.57	-11.7	-0.06
R2	0.94	0.94	0.87

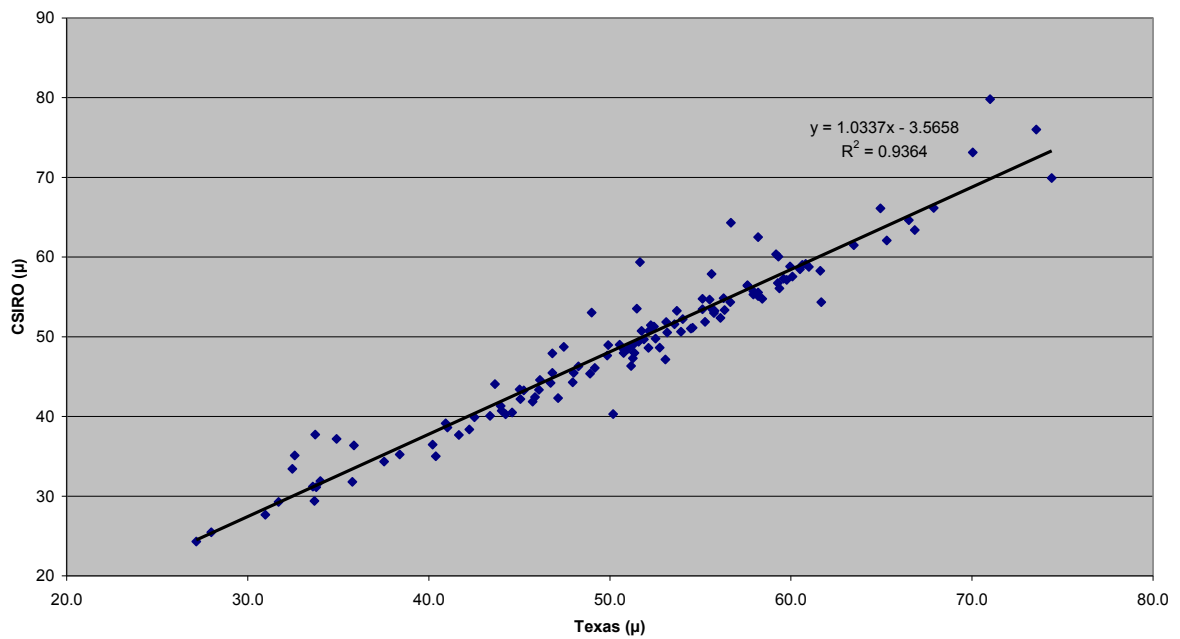
Table 4 Summary Results for CSIRO - ITC Comparisons

There is good agreement between the two techniques for perimeter measurements. The CSIRO method under-estimates the lumen area by twenty percent when compared with the ITC technique, although the correlation between the two is quite good. Since Theta is a calculated parameter, it reflects the results for perimeter and area

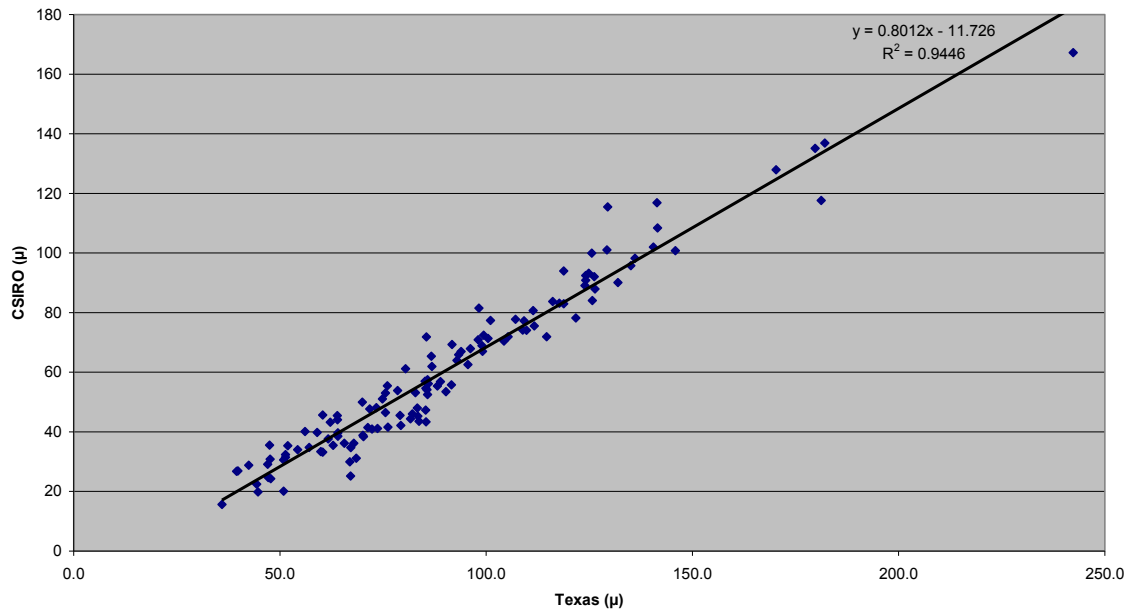
Theta - Combined Images 1-20 (Errors Removed)



Perimeter - Combined Images 1-20 (Errors Removed)



Area - Combined Images 1-20 (Errors Removed)



Appendix 3. Detailed Results from the Seven Samples and Comparison with the Data from Texas Tech. University.

Summary

The summary results for the seven samples of cotton provided are presented in Table 1 and Table 2.

Sample	Perimeter		Area		Theta		Count
	ITC	CSIRO	ITC	CSIRO	ITC	CSIRO	
2996 (42)	51.1	53.5	105.0	96.1	0.519	0.446	7833
2999 (43)	51.1	55.8	89.7	79.4	0.451	0.337	4024
3008 (44)	48.0	50.4	82.2	67.3	0.467	0.357	4973
3009 (45)	46.1	46.3	85.6	74.0	0.520	0.456	4340
3016 (46)	51.4	55.9	100.7	104.6	0.495	0.442	2627
3074 (47)	54.7	56.0	134.4	124.7	0.574	0.518	3812
3075 (48)	56.2	58.2	101.2	90.5	0.423	0.363	2917

Table 5 Summary Results for CSIRO/ITC comparison

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

Table 6 Regression Parameters for ITC/CSIRO comparisons

These results are presented graphically in Appendix 1 together with results for the individual blocks showing the between block scatter.

In addition the ITC results for Theta were compared with the CSIRO measurements made without taking the lumens into consideration. The correlation was almost the same as that when removing the lumen area before calculating Theta.

Components of Variance

The standard deviations for the within cross-section slice and between slices are given in the following table. The values were calculated using the lme (linear mixed effects) function from the statistical package SPlus.

Sample ITC (CSIRO)	Standard Deviation (lme)								
	Perimeter			Area			Theta		
	Within	Between	Mean	Within	Between	Mean	Within	Between	Mean
2996 (42)	13.0	0.85	53.5	39.6	2.47	96.1	0.171	0.017	0.446
2999 (43)	12.9	1.35	55.8	39.1	5.17	79.4	0.159	0.041	0.337
3008 (44)	13.0	1.30	50.4	33.7	1.21	67.3	0.177	0.028	0.357
3009 (45)	10.9	0.39	46.3	30.1	0.07	74.0	0.170	0.013	0.456
3016 (46)	12.6	2.07	55.9	42.3	5.24	104.6	0.169	0.012	0.442
3074 (47)	11.7	1.62	56.0	41.7	6.75	124.7	0.155	0.000	0.518
3075 (48)	15.3	1.39	58.2	45.7	2.83	90.5	0.169	0.010	0.363

Table 7 Standard Deviations for within and between blocks – CSIRO data

These results should be interpreted with some caution. Both the Perimeter and Area residuals depart significantly from the implied normal distribution (fig.1). Only in

the case of Theta do the results match the underlying assumption although a departure from normality is observed in the tails of the distribution (fig. 2).

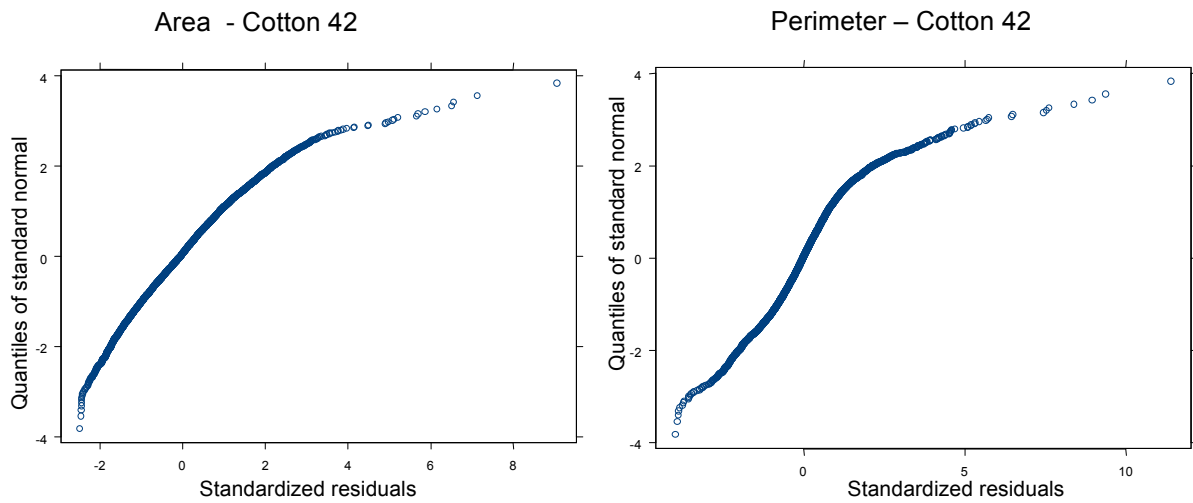


Fig 1. QQ Plots of residuals for Area and Perimeter showing non-Normal behaviour

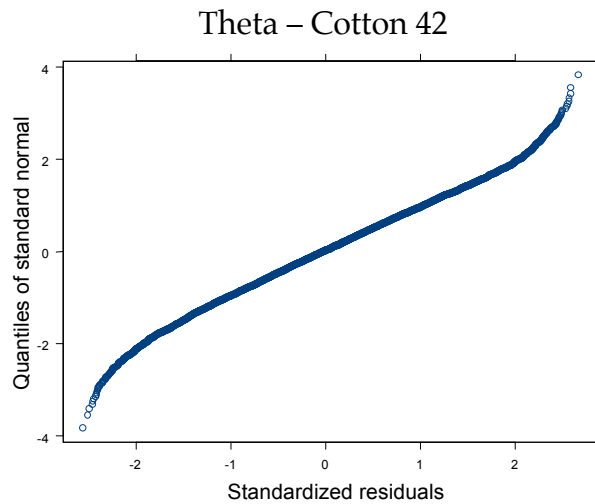


Fig 2. QQ plot of residuals for Theta

The coefficients of variation follow from these results and are compared with those obtained from the ITC (Texas Tech).

Coefficient of Variation											
Perimeter				Area				Theta			
Within		Between		Within		Between		Within		Between	
CSIRO	Texas	CSIRO	Texas	CSIRO	Texas	CSIRO	Texas	CSIRO	Texas	CSIRO	Texas
24.4	18.2	1.6	2.3	41.2	32.0	2.6	5.5	38.4	28.8	3.8	3.0
23.1	18.6	2.4	1.9	49.2	32.3	6.5	4.6	47.2	33.0	12.1	3.4
25.7	17.7	2.6	2.3	50.0	31.3	1.8	5.1	49.6	32.5	7.8	4.1
23.5	17.1	0.8	2.3	40.7	29.7	0.1	5.3	37.3	37.3	2.9	3.0
22.6	18.0	3.7	2.4	40.5	32.4	5.0	5.0	38.3	31.0	2.6	2.7
21.0	18.7	2.9	2.2	33.4	33.3	5.4	4.9	30.0	26.1	0.0	2.3
26.4	20.2	2.4	3.5	50.5	35.5	3.1	6.2	46.7	36.5	2.8	3.7

Table 8 Comparison of Coefficients of Variation

CSIRO had consistently higher within block variations than the corresponding ITC values but the between block variations showed no consistent trend. It is not clear how the samples provided to CSIRO were garnered and so it is not possible to draw

comparative conclusions about the repeatability of the two techniques – there may well be sampling implications.

Efficient Experimental Design

In two-stage sampling the variance of the primary unit (slides or blocks) is σ_1^2 and the variance of the within block (residuals) measurements is σ_2^2 . The variance of the sample mean \bar{y} is given by

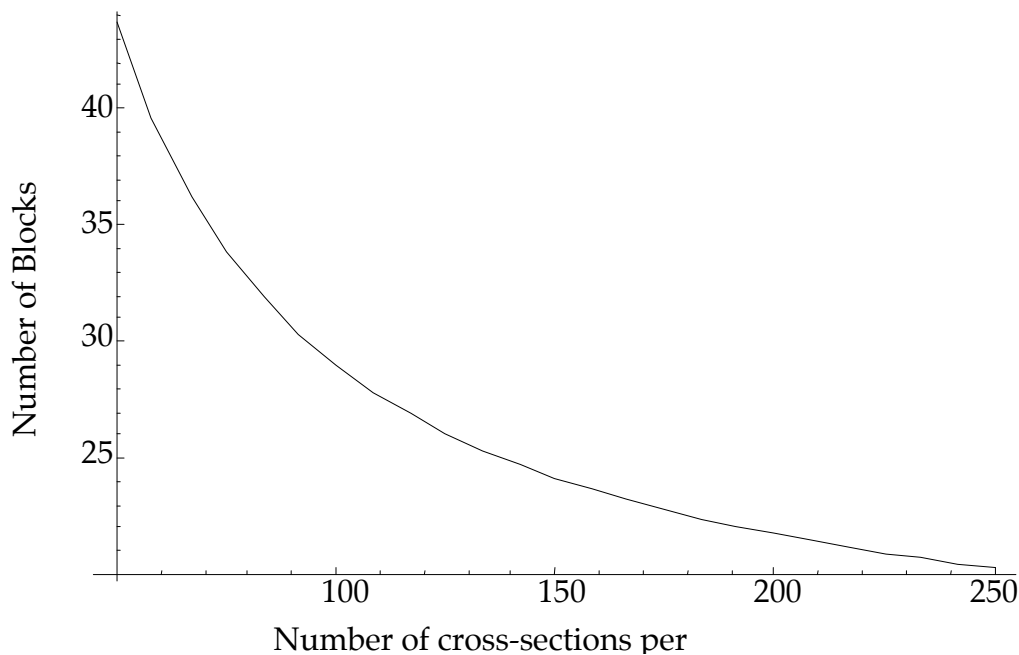
$$\sigma_{\bar{y}}^2 = \frac{\sigma_1^2}{n_1} + \frac{\sigma_2^2}{n_1 n_2}$$

The question remains as to the most efficient choice of n_1 and n_2 to achieve a desired experimental variance.

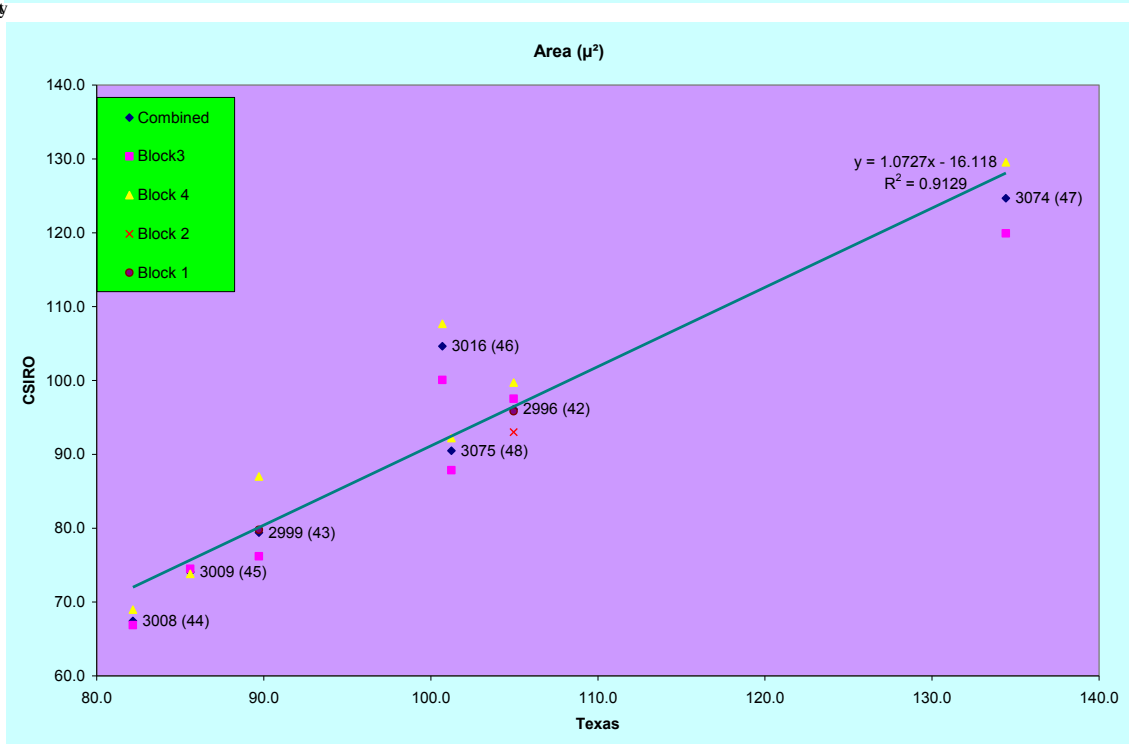
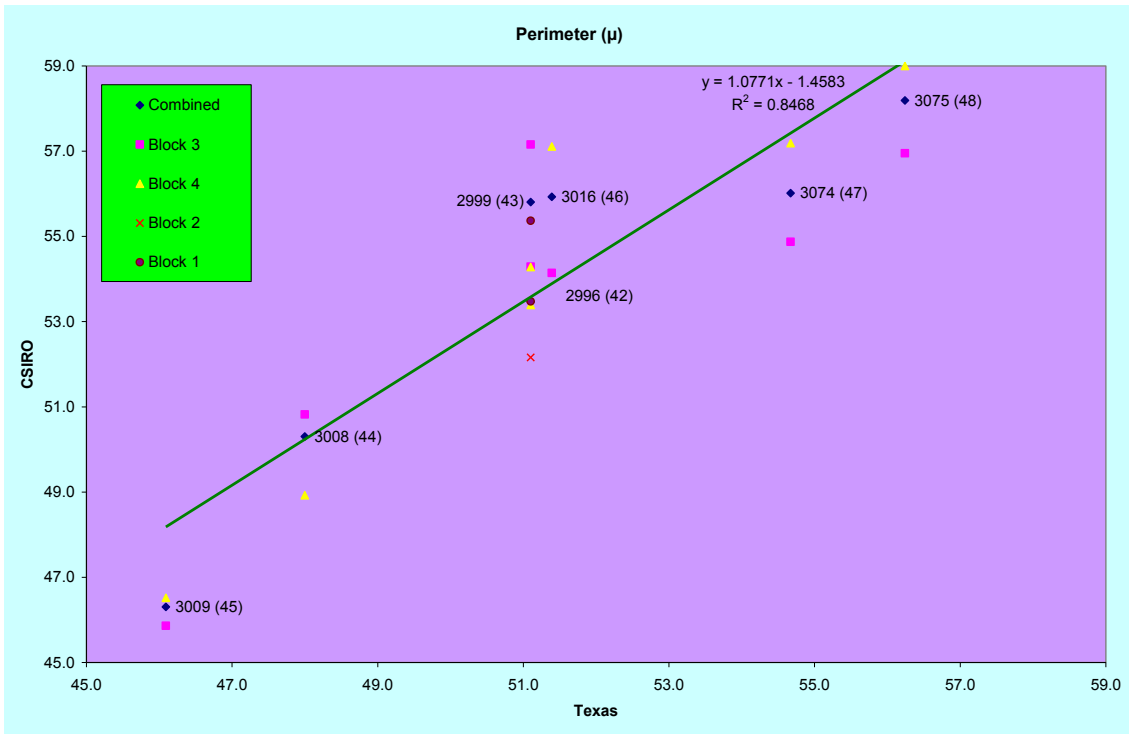
For sample 2996 (42) $\sigma_1=0.017$ and $\sigma_2=0.171$. If we choose $2\sigma_{\bar{y}} = 0.446/50$ ($\pm 2\%$ at the 90% confidence level) then

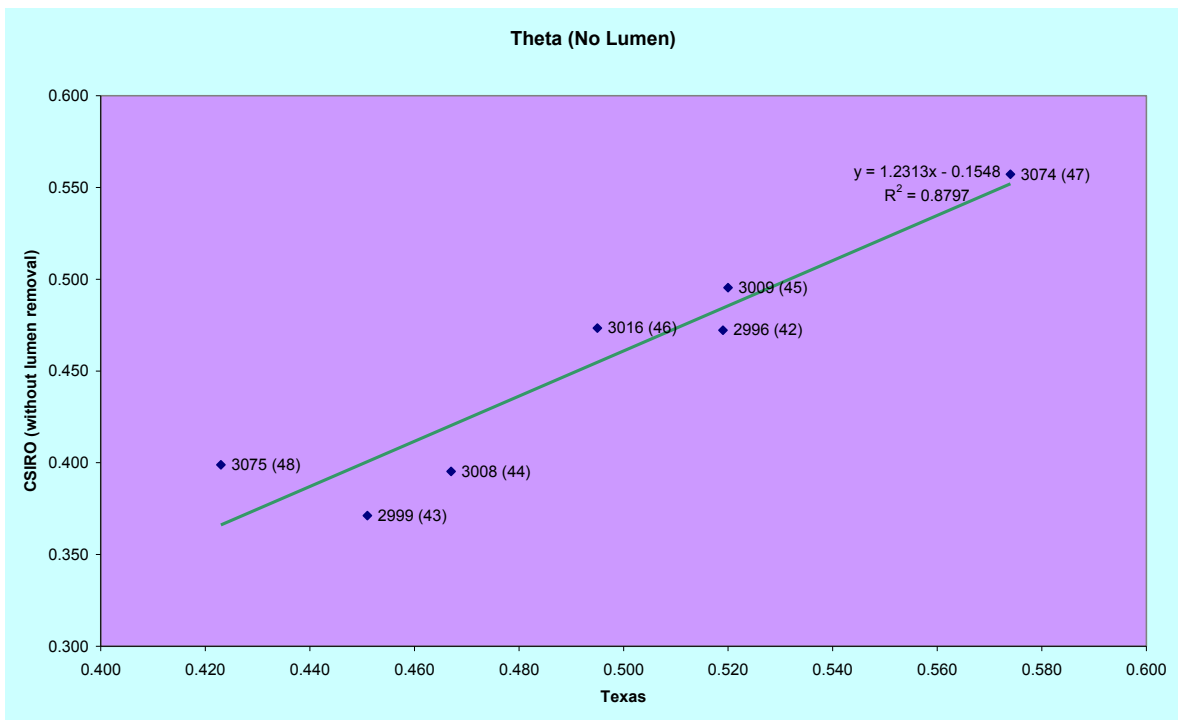
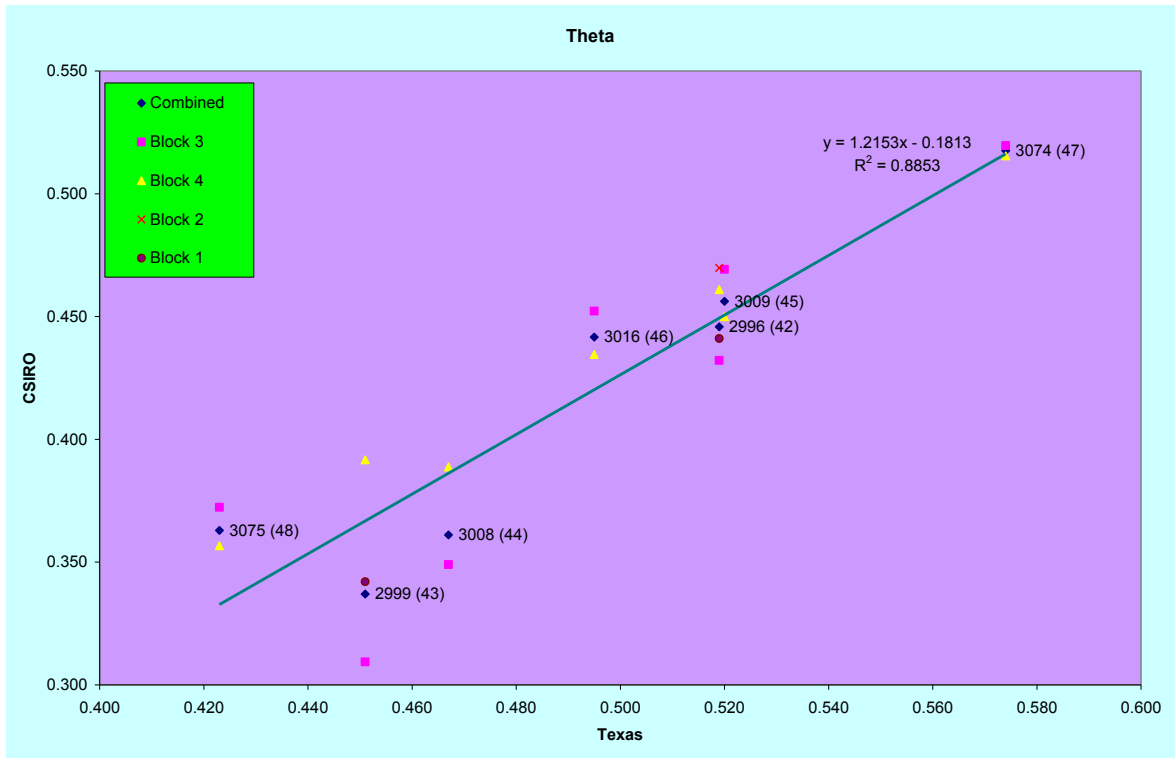
$$1.99 \times 10^{-5} = 2.89 \times 10^{-4} / n_1 + 0.0292 / n_1 n_2$$

where n_1 = number of blocks; n_2 = number of cross-sections per block



The plot shows that the minimum number of cross-sections needed to obtain the desired variance is about 3000 – 100 cross-sections from 30 blocks. This is in good agreement with the values established by ITC. However, since the blocks take considerable effort to prepare, the same variance may be obtained with 16 blocks (the minimum number) and 1000 cross-sections per block.





Sample	Perimeter					
	Texas	CSIRO	Block 3	Block 4	Block 1	Block 2
2996 (42)	51.1	53.5	54.3	53.4	53.5	52.2
2999 (43)	51.1	55.8	57.2	54.3	55.4	
3008 (44)	48.0	50.3	50.8	48.9		
3009 (45)	46.1	46.3	45.9	46.5		
3016 (46)	51.4	55.9	54.1	57.1		
3074 (47)	54.7	56.0	54.9	57.2		
3075 (48)	56.2	58.2	56.9	59.0		

Sample	Area						
	Texas	¹ Full	² Lumen	Block 3	Block 4	Block 1	Block 2
2996 (42)	105.0	106.1	96.1	97.5	99.7	95.8	93.0
2999 (43)	89.7	87.8	79.4	76.2	87.0	79.8	
3008 (44)	82.2	74.1	67.4	66.9	69.0		
3009 (45)	85.6	80.9	74.0	74.5	73.8		
3016 (46)	100.7	112.7	104.6	100.1	107.7		
3074 (47)	134.4	134.7	124.7	119.9	129.6		
3075 (48)	101.2	99.9	90.5	87.8	92.2		

Sample	Theta						
	Texas	Full	Lumen	Block 3	Block 4	Block 1	Block 2
2996 (42)	0.519	0.472	0.446	0.432	0.461	0.4	0.470
2999 (43)	0.451	0.371	0.337	0.309	0.392	0.342	
3008 (44)	0.467	0.395	0.361	0.349	0.389		
3009 (45)	0.520	0.495	0.456	0.469	0.450		
3016 (46)	0.495	0.473	0.442	0.452	0.435		
3074 (47)	0.574	0.557	0.518	0.520	0.515		
3075 (48)	0.423	0.399	0.363	0.372	0.357		

¹ The area including the lumen. For Theta, the value calculated using this area

² The area excluding the lumen. For Theta, the value calculated using this area

Part 4 – Final Report Executive Summary

Provide a one page Summary of your research that is not commercial in confidence, and that can be published on the World Wide Web. Explain the main outcomes of the research and provide contact details for more information. It is important that the Executive Summary highlights concisely the key outputs from the project and, when they are adopted, what this will mean to the cotton industry.

The commonly used Micronaire value for cotton is related to both fibre fineness and maturity. There is a need for a new measurement technique to separate these. This is of particular importance to the Australian industry where varieties of fine, mature cotton have the potential to be wrongfully discounted commercially by misinterpreting a low Micronaire value as indicating immaturity in a coarser fibre.

CRDC is currently funding research at CSIRO Textile and Fibre Technology aimed at developing techniques to measure fibre fineness and maturity to overcome this deficiency in the Micronaire measurement.

One difficulty with research in this area is that there are no internationally recognised standard cotton samples that can be used for checking the accuracy of new measurement approaches or for that matter for checking the calibration and accuracy of existing instrumentation.

Researchers in the US are tackling this problem by coordinating the development of a standardised set of cotton samples specifically for this purpose. Once this work is completed, the cottons in this set will each have well characterised values of both fibre fineness and maturity and the set will cover a wide commercial range in these parameter values. This set will then be of extensive value to the cotton community.

In order to accurately determine the correct fineness and maturity of each sample bale, samples will be measured by a variety of techniques and in a variety of laboratories and CSIRO Textile and Fibre Technology (TFT) in Geelong, were invited to participate in these trials as an independent measurement test laboratory.

During this project CSIRO became proficient in the specific techniques of sample preparation and developed software for the analysis of the images of fibre cross sections. After detailed validation trials of the CSIRO techniques, measurements were undertaken on a set of seven different cottons. In total more than 30,000 individual fibre cross-sections were imaged and analysed. The results from these trials have been satisfactorily correlated with the data from another participating laboratory. This is a major step towards establishing accepted values for these reference cottons.