



Final Report

Off Farm Series | Cotton Research & Development Corporation

FINAL REPORT

Part 1 - Summary Details

CRDC Project Number: CTFT7 OR Cotton CRC Project Number:

Project Title: Inter-laboratory Trials for Fibre Maturity
Reference Samples

Project Commencement Date: 1/7/2003 **Project Completion Date:** 30/6/2006

CRDC Program: Off Farm **OR** **CRC Program:** - Please Select One -

Part 2 – Contact Details

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Part 3 – Final Report Guide

Background

1. Outline the background to the project.

The CRDC has been funding projects at CSIRO Textile and Fibre Technology (TFT) to look at new methods of characterising fibre maturity and fineness. These projects arise as the commonly used micronaire measurement 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 if a low micronaire value is taken as indicating immaturity.

In conjunction with these projects CRDC has also been supporting CSIRO TFT to participate in an international exercise to develop carefully measured reference samples for fibre maturity and fineness. This work is being coordinated by the US Department of Agriculture research laboratories in New Orleans and the third participating laboratory is at Texas Tech University.

The maturity values are being obtained by direct measurement of fibre cross sections. This is very time consuming and detailed work that requires specialist skills in cross-sectioning and microscopy. CSIRO TFT was invited to participate in this work based on both our general interest in this area and also our suitable specialist skills.

Participation in this project is of strategic importance to the other CRDC funded projects at CSIRO TFT. It gives us both ready access to valuable reference samples that are suitable for testing our other developments in this area, and also helps build our credibility within the international cotton community which will be necessary as we move forward and try to get our other technologies adopted commercially.

Objectives

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

Objectives, Milestones, Performance Indicators Year 1:

1. Participate in the interlaboratory trial and complete the measurements of the existing set of 7 reference samples that have been obtained from the USDA.
2. Contribute to the preparation of a report & presentation of the combined data set and outcomes to the ITMF Working group meeting in March 2004.

Both of these objectives were achieved on time, as reported in the Annual report in September 2004

Objectives, Milestones, Performance Indicators Year 2:

1. Participate in the ongoing interlaboratory trial with an extended and expanded sample set.
2. To devise a new software algorithm to overcome the problem identified with the CSIRO measurement of cross-sectional area. (This additional objective was added in the annual report in September 2004.)

The first objective for the year was achieved. An expanded sample set of 11 cottons which covers a wider range of maturity and fineness values has been obtained from the US. Experimental work was commenced to undertake the detailed (and time consuming) procedures of sample preparation, mounting and sectioning of multiple replicates for the optical microscopy to obtain images of individual fibre cross sections.

The second technical objective was not fully completed. Due to the technical nature of the images of individual cross sections it transpired that the objective was a more challenging task than originally thought (to accurately undertake the image analysis to determine the cross sectional area of individual fibres). The problem centres on accurately defining the boundary of the lumen and the outside boundary of the fibre from an image where inherently these boundaries are ill-defined.

Objectives, Milestones, Performance Indicators Year 3:

1. Finalise any outstanding experimental work in the extended trial and participate in the writing up of a final report.
2. Contribute to the preparation and submission of the technique and results to ITMF and the American Standards body (ASTM) for ratification as an approved test method. (These formal approvals require the completion of satisfactory interlaboratory trials. They would significantly add to the authority and status of the measured and approved values of the reference samples to be used for future calibration purposes.)

Objective 1 was largely completed with the laboratory/experimental work leading to the capture of over 400,000 images of individual fibre cross sections of the expanded set of 11 cottons. A small number of mounted and sectioned fibre samples were not imaged within the time frame. (They have subsequently been completed in the extension of this project under CTFT17.) The full computer analysis of the data set has not been undertaken due to the outstanding software problems.

The second objective was not undertaken. The delay in resolving the software issues has meant that this objective has not be tackled.

Methods and Results

3. Detail the methodology and justify the methodology used. Include any discoveries in methods that may benefit other related research.

The methods and typical results have been published in a conference paper presented to the US Beltwide conference in January 2004. (G.J. Higerson, G.R.S. Naylor, and M. Pate, ‘

Measurement of Fibre Maturity using Image Analysis of Fiber Cross-sections: The Australian Experience.', Proc. Beltwide Cotton Conferenc, Jan 2004 (CD).

Outcomes

4. Describe how the project's outputs will contribute to the planned outcomes identified in the project application. Describe the planned outcomes achieved to date.

As noted in the project application, as well as achieving the technical objectives outlined above, a primary strategic focus for the project has been to facilitate an important dialogue and interaction between the group at CSIRO and (a) key researchers in the area in the US namely Dr Eric Hequet at Texas Tech University and Dr Devron Thibeadeaux at USDA, (b) staff at Cotton Inc, (c) the ITMF Technical Committee on fibre fineness and maturity and (c) access to the US data on these reference cottons and the samples themselves for use in the other cotton fibre quality instrumentation developments within CSIRO. The project has been highly successful in achieving this strategy.

Conclusion

5. Provide an assessment of the likely impact of the results and conclusions of the research project for the cotton industry. What are the take home messages?

This project has indirectly added significant value to the portfolio of instrumentation work being undertaken at CSIRO for the cotton industry. It has facilitated the establishment of key relationships with our US colleagues and given access to valuable information and samples for testing our other new instruments.

6. A. List the publications arising from the research project and/or a publication plan.
(NB: Where possible, please provide a copy of any publication/s)

G.J. Higerson, G.R.S. Naylor, and M. Pate, ' Measurement of Fibre Maturity using Image Analysis of Fiber Cross-sections: The Australian Experience.', Proc. Beltwide Cotton Conferenc, Jan 2004 (CD).

- B. Have you developed any online resources and what is the website address?

No

Part 4 – Final Report Executive Summary

The commonly used Micronaire value for cotton is related to both fibre fineness (weight per unit length) 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 if a low Micronaire value is taken as indicating immaturity.

The 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 at USDA in New Orleans and Texas Tech. University have been tackling this problem by coordinating the development of a standardised set of cotton samples specifically for this purpose. 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.

Utilising our expertise in measuring cotton fibre fineness and maturity, CSIRO Textile and Fibre Technology, has participated in these trials as an independent measurement test laboratory.

G.J. Higerson, G.R.S. Naylor, and M. Pate, ‘ Measurement of Fibre Maturity using Image Analysis of Fiber Cross-sections: The Australian Experience.’, Proc. Beltwide Cotton Conference, Jan 2004 (CD).

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 (eg 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 in the optical microscopy

To check the CSIRO image capturing system in 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 $\pm 2\%$ at the 90% confidence level is estimated in Figure 9. For example, 100 cross-sections from 30 blocks ie 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 supported 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	Perimeter		Area		Theta		No of Blocks	No of Measurements
	ITC	CSIRO	ITC	CSIRO	ITC	CSIRO	CSIRO	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

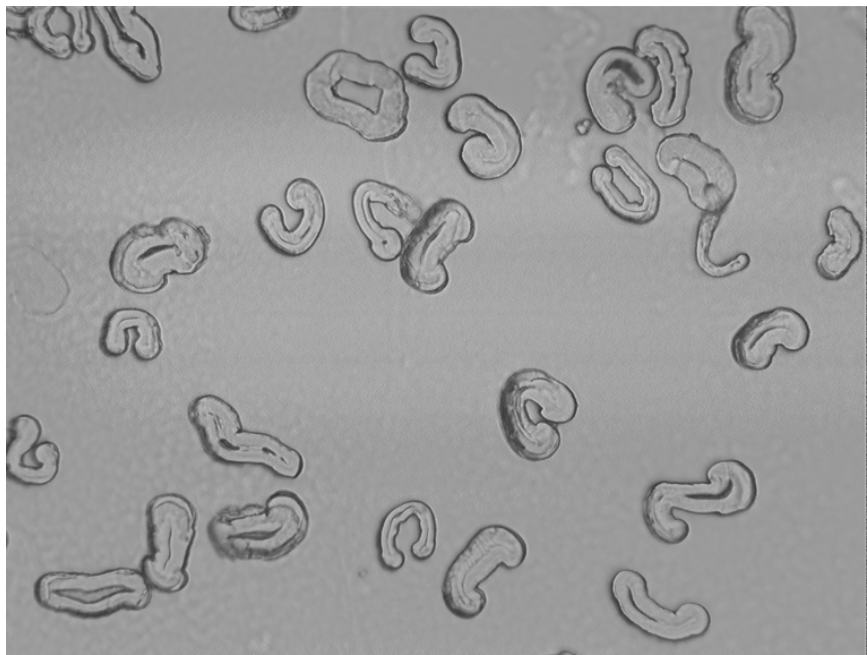


Figure 1. A typical image.

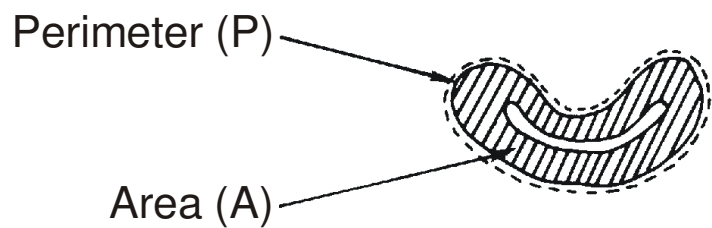


Figure 2. Schematic of a cross-section.

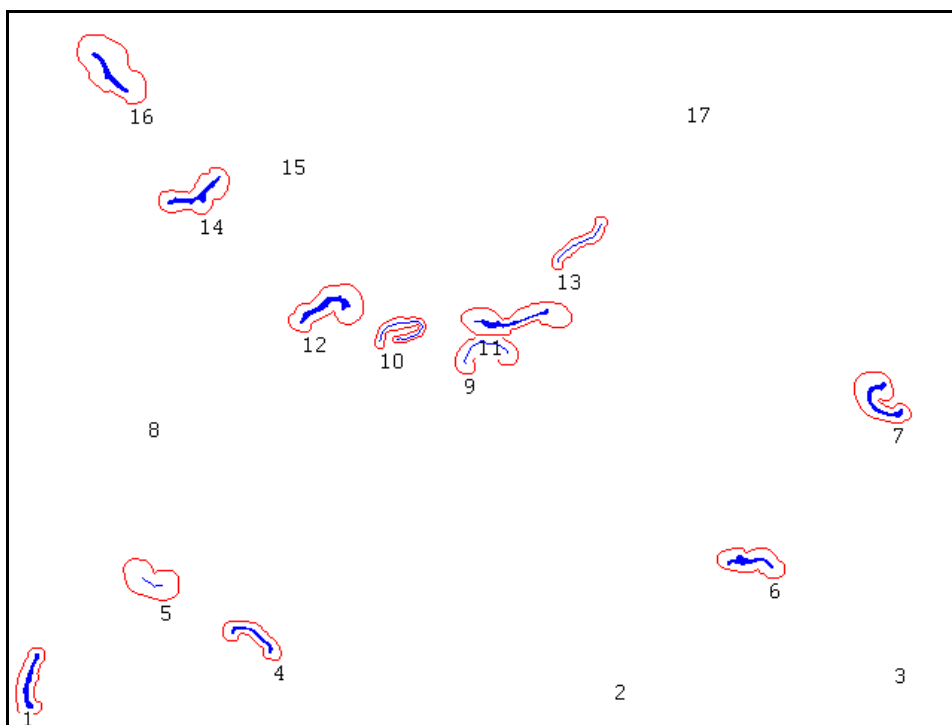


Figure 3. Example of ITC supplied 'Results' image.

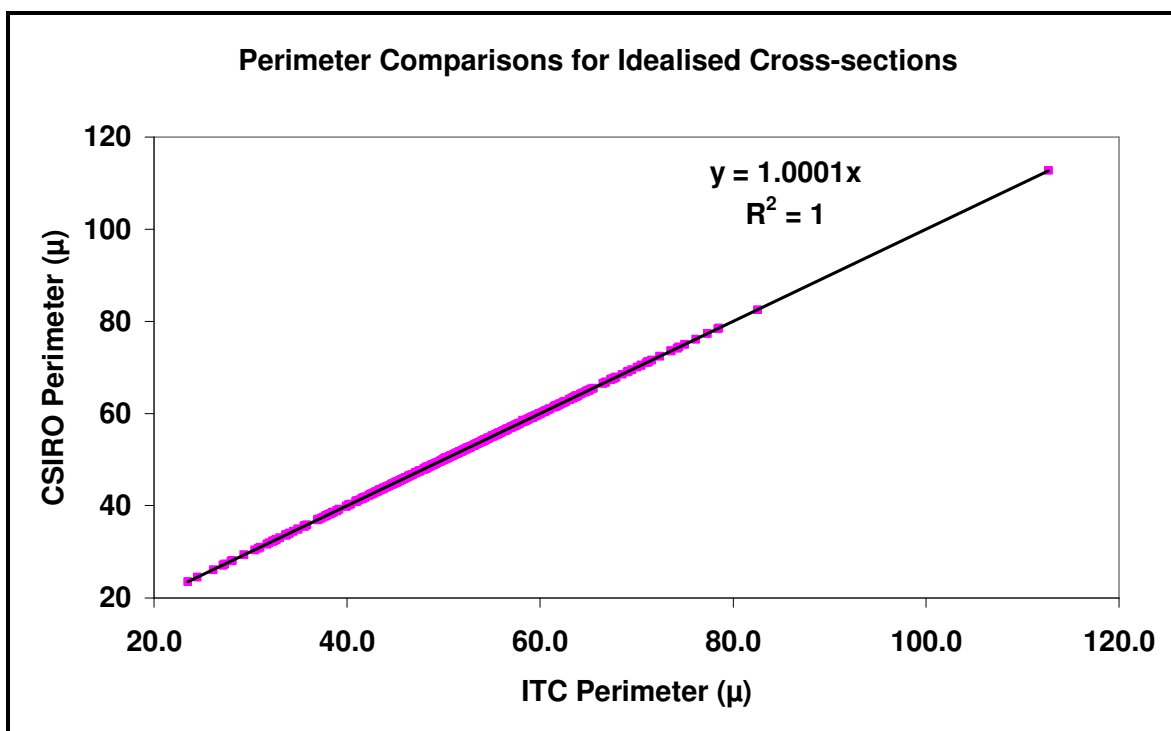


Figure 4. Comparison of perimeter values for idealized cross-sections.

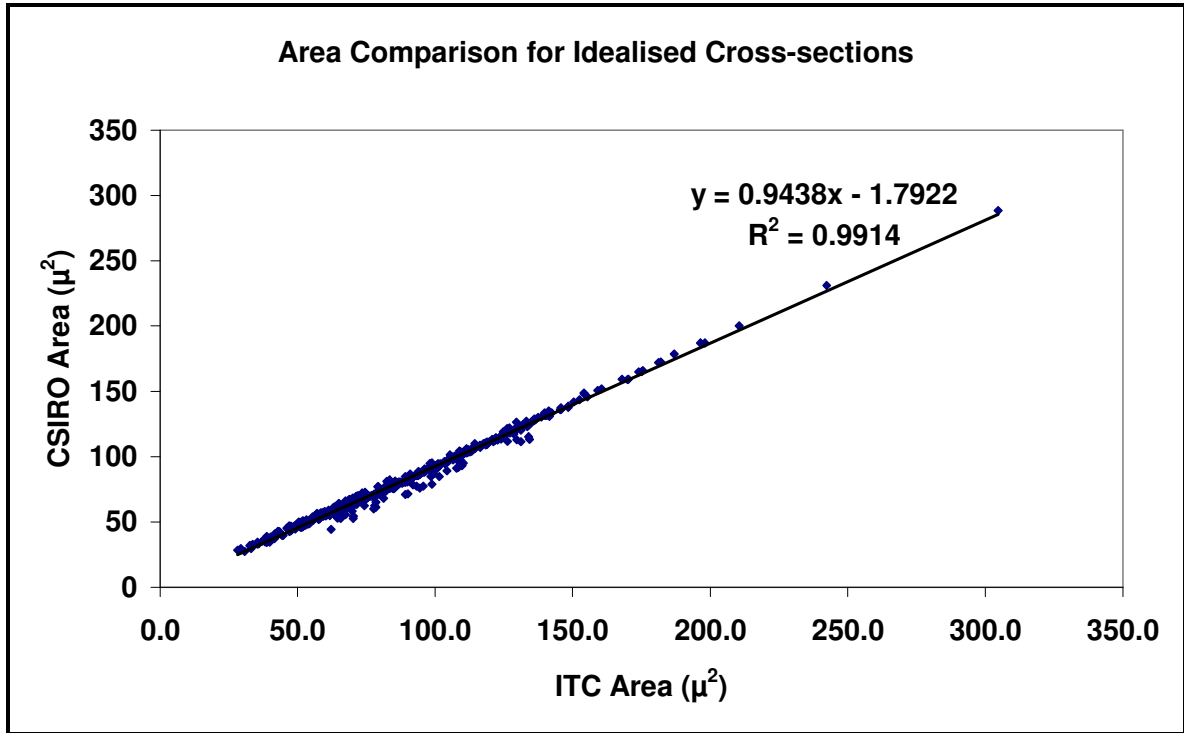


Figure 5. Comparison of area data for idealized cross-sections

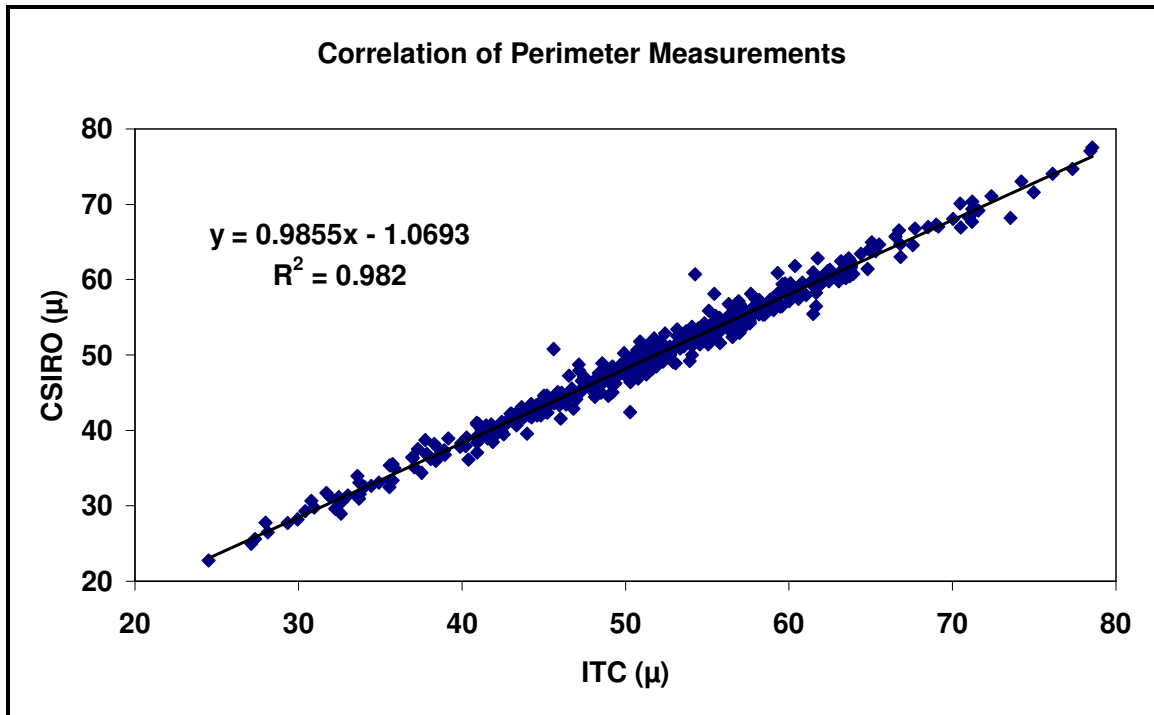


Figure 6. CSIRO/ITC comparison of perimeter measurements from the same captured image.

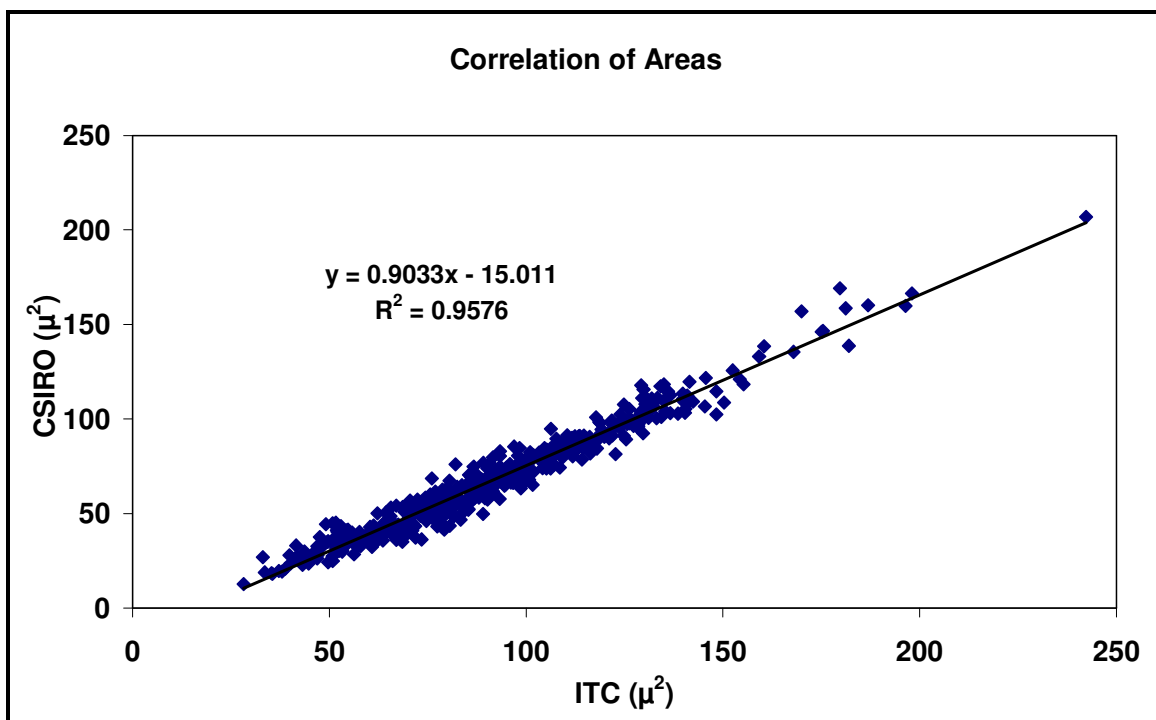


Figure 7. CSIRO/ITC comparison of area measurements from the same captured image.

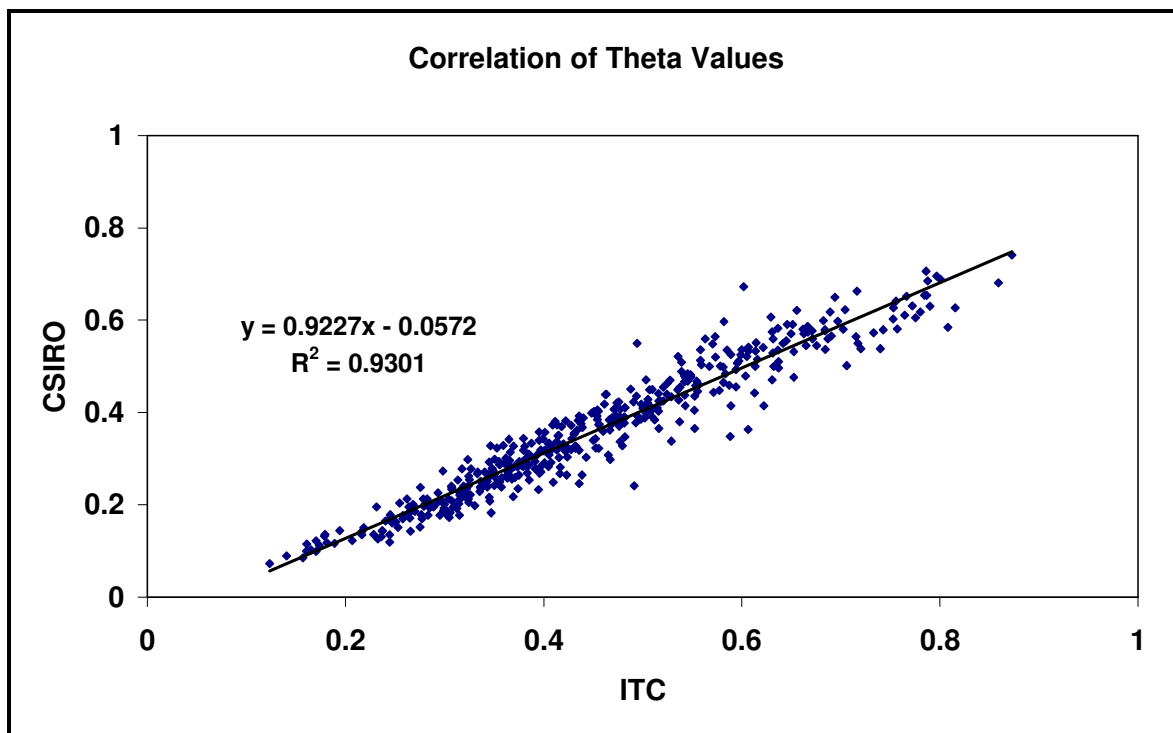


Figure 8. CSIRO/ITC comparison of theta measurements from the same captured image.

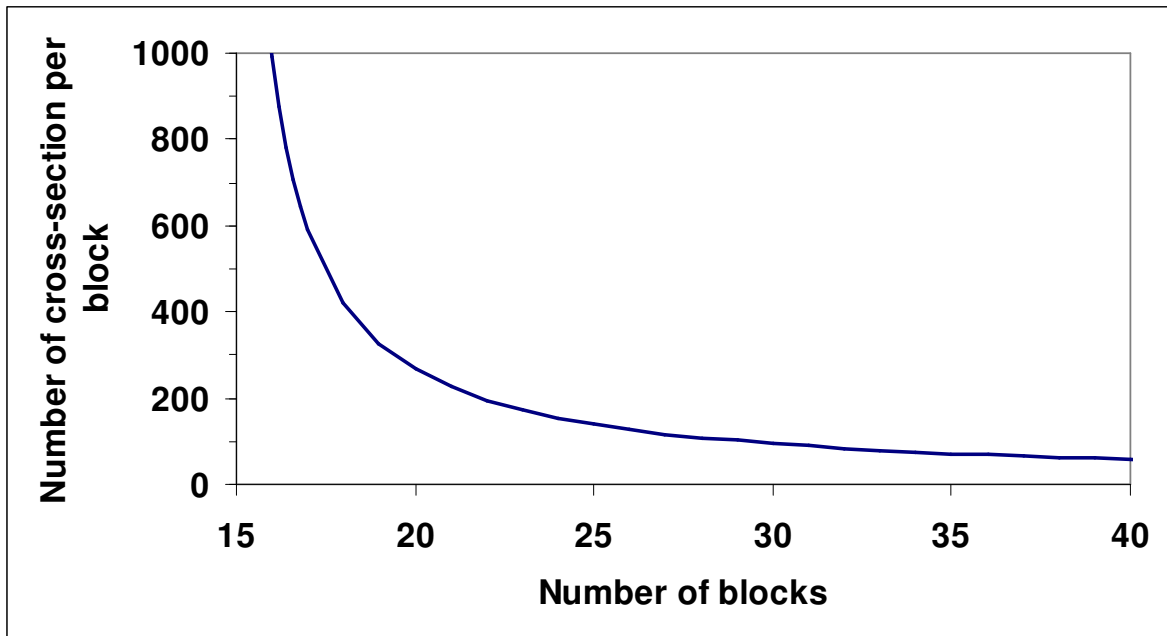


Figure 9. Estimation of the minimum number of blocks and cross-sections per block to achieve the required precision in the mean value per sample.