Polymer Testing 40 (2014) 4–12

Contents lists available at ScienceDirect

Polymer Testing

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

Test method

A comparison of automated and manual techniques for measurement of electrospun fibre diameter

J.J. Stanger [a], N. Tucker [a,*], N. Buunk [b], Y.B. Truong [c]

[a] The New Zealand Institute for Plant & Food Research Limited, Christchurch, New Zealand
[b] Electrospinz Ltd., Blenheim, New Zealand
[c] CSIRO Fibre Science Research Program, Melbourne, Australia

ARTICLE INFO

Article history:
Received 30 June 2014
Accepted 6 August 2014
Available online 20 August 2014

Keywords:
Electrospinning
Nano fibre
Fibre diameter
Image analysis

ABSTRACT

Electrospinning is a fibre manufacturing process, and fibre diameter is a fundamental property. We compare diameter measurements made by human operators against two automated algorithms (FibreQuant™ and SEMAnalyser™). The effects of scanning electron microscopy preparation by iridium, gold and carbon coating on fibre diameter are also examined.

A human takes 2.2 h to make 150 measurements. Automated analysis produces 9000 measurements less than 5 minutes. The automated method produces results without researcher bias and with greater consistency, but will occasionally include incorrect measurements because of the simple heuristics used. The manual method used by human operators shows larger variation in reported averages and is labour intensive.

Before obtaining scanning electron microscopy images, the fibre samples require a conductive coating to prevent charging and burning of the fibres; the effects of SEM preparation methods such as iridium, gold and carbon coating showed that iridium coating had the least impact on fibre diameter.

1. Introduction

Electrospinning is a fibre spinning process using electrostatic force to draw fibres from a liquid polymer solution or melt. The process has a long history [1] and has recently seen significant interest both in the laboratory and in industry. Many of the proposed uses for electrospun fibre depend on the high surface area to volume ratio. This makes fibre diameter a fundamental property of electrospun fibres.

The current state of the art in fibre measurement involves human operators manually measuring fibre diameter on digital scanning electron microscope (SEM) images. Without a generally accepted protocol, the methods used and, consequently, the results quoted vary considerably. A clear indicator of this is the number of measurements taken per sample, typically ranging from 5 to 300. Variation of electrospinning processing conditions certainly results in changes in fibre diameter [2], but it is difficult to quantify the effect precisely [3], although comparison of sets of measurements of electrospun fibre samples by various workers [4] indicates that the fibre diameter tends to a normal distribution within a single sample.

It is critical that in any experiment examining fibre diameter that the measurements give an accurate representation of the sample. There has, however, been little discussion within the literature of what constitutes a truly representative sample. Whilst most experiments rely on measurements by human operators, automated image analysis algorithms [5–7] are available for digital micrographs and laser diffraction [8] can be used to measure fibre diameter at the <5 micron range. It is to be expected that

* Corresponding author.
E-mail address: nick.tucker@plantandfood.co.nz (N. Tucker).

http://dx.doi.org/10.1016/j.polymertesting.2014.08.002
0142-9418/

the consistency and accuracy of an automated system would be better than those of a manual method because more measurements can be made in a given time, and a computer-implemented algorithm may be more consistent than a human operator. However, human interpretation of SEM images may be better at coping with ambiguities such as beading, co-joined fibres, or fibre crossovers.

In this paper, we examine the accuracy of automated fibre size analysis software packages against results from a panel of human operators. The variation in reported results from a panel of human operators using an identical method on an identical image set is also explored. Although the details of the commercial fibre detection algorithms cannot be discussed, a general discussion of edge detection methods is presented in the Methods section.

2. Fibre measurement methods

All electrospun fibre used in this work was produced from 8% wt poly (vinyl alcohol), average molecular weight of 118,000 $gmol^{-1}$ and degree of hydrolysis (DH) in the range of 85–90% (Chemiplas NZ limited, Wellington, New Zealand), in water on an Electrospinz Ltd ES1a electrospinning machine. Two samples were generated for analysis, Sample A at 10 kV and 100 mm distance, and Sample B at 10 kV and 120 mm distance. Microscope images were collected using a Jeol Neoscope JCM-5000 scanning electron microscope (SEM). Samples were gold coated using a Quorum Q150R S sputterer for 120 s at 20 mA. Iridium coating was performed using a Polaron range SC5750 sputter coater for 50 s at 50 mA. Carbon coating was performed using a Polaron E3700 evaporative carbon coater.

2.1. Manual fibre diameter measurement

A human operator measuring electrospun fibre must use a consistent method to ensure that the results are comparable. Before the authors had access to an automated algorithm, the following method was devised to provide a reasonably consistent result without excessive time cost. This method is the one used by all human operators throughout this paper.

Firstly, a set of ten SEM images of each sample was obtained. A single image per sample is unsuitable, as the nature of the sample will vary depending on where the image is taken. A minimum of ten images is recommended. The locations of the images were selected to give an even distribution over the entire sample, with the precise location chosen at random rather than selecting specific features, to avoid selection bias.

Analysis of the SEM images by human operators was done using ImageJ [9], although any suitable software package could be used. The operators were instructed to identify each individual unique fibre on an image and make a single measurement of its diameter. A single measurement per fibre was done to eliminate any natural bias of the operator towards selecting larger or smaller fibres more often. Because of the limits of accuracy (see following section), each measured value has one pixel subtracted before conversion to physical units. Fibre diameter measurements are made perpendicular to the long axis of the fibre at the point of measurement. Operators were instructed to avoid measurement where the fibre was curved, to ensure the perpendicular condition was met. When the entire fibre length was curved, measurements were made at the point with minimum curvature. Operators were instructed to exclude any fibre that was visibly two or more fibres joined together; this did not include branched fibres. Where fibres were joined together, each individual component fibre was measured rather than the joined fibre. Similarly, some cases exist where a fine web of fibres exists between two fibres. Operators were instructed to exclude these fibres, as the results would certainly be bimodal and it is unclear if these are formed by the primary electrospinning process.

The operators were instructed to aim to collect 250 observations for a single sample. A higher number of observations, such as 400-1000 measurements per sample, would improve the accuracy; however, each additional measurement provides diminishing improvement. The performance of each operator was initially checked by examining the distribution of the collected measurements. If the results showed a normal or skewed normal distribution, it was assumed that sufficient measurements had been taken for the operator to characterize the sample. The average and standard deviation of the results were then calculated.

2.2. Edge detection in computer vision

The problem of detecting an edge of a feature (in this case an electrospun fibre) can be considered a fundamental technique in computer vision. Once edges are identified, then further heuristics can be used to determine the desired properties (i.e. fibre diameter) of the features in an image. There are significant difficulties in the practical implementation of automated edge detection. If an image is composed of only two tones (i.e. white and black – each pixel[1] is represented by a single data bit – either one or zero), then detecting an edge is trivial. If the way the image is captured allows for the properties of the pixels in both the feature and the background to be highly consistent and significantly different, then detecting an edge can again be trivial. Examples of this case would be good control of lighting conditions to generate a high contrast between the feature and the background or using chroma keying. The above examples are well suited to the simplest edge detection method, called thresholding. In this approach, a preset value is defined and if the transition from one pixel to its neighbour crosses that threshold then an edge has been found. Often the threshold is defined by a brightness of the pixel but it can also be defined by a colour change.

More often, a real-world image will have a somewhat gradual transition rather than abrupt changes in brightness – each pixel is represented by several bits of data allowing shades of grey between black and white.[2] In

[1] A pixel is the smallest single component of a digital image.

[2] In colour images, changes in colour are achieved by varying the relative intensity of three related pixels in the primary colours – in this report greyscale images will be considered, as this is how electron microscope pictures are received.

addition, different parts of the image may have variable contrast ratios. A visual example of how a smooth transition complicates edge detection is shown in Fig. 1. A transition from an intensity value (0 for black and 255 for white) of 4 to 152 shows a clear edge. However, from 6 to 41 to 113 to 148 would make it more challenging to define a threshold that would select the correct edge position, and the appropriate threshold for edges elsewhere on the image may be different. The complexity of real-world images can come from the finite depth of field, penumbral blur from shadows, shading on surfaces, and the digitization of the captured image. This makes thresholding less useful, as each edge has its own ideal threshold but only one can be chosen to analyse the whole image. The threshold approach can be improved by dividing up the image into smaller sub-regions and adjusting the global threshold based on how bright the pixels are in that sub-region.

Thresholding treats an edge in an image like a step change (Fig. 2). When faced with an image that is composed of gradual transitions, it is better to define the edge by the gradient (Fig. 2). Two traditional approaches to gradient-based edge detection are the Canny edge detector [10] and the Sobel filter [11]. There are many variations of these algorithms, each with their own strengths and weaknesses. They all share a similar set of criteria: minimizing multiple detections of a single edge, smoothing without minimizing maxima and localization of detection. These criteria allow the accurate detection of an edge under "real-world" conditions. Like thresholding, gradient methods still have input parameters, the results of which must be judged by a human operator as an optimal result is sought.

Finally, extracting scientific data from images using computer vision requires consideration of the ultimate degree of accuracy that can be achieved from a digital image. Any feature measurement will require the location of a minimum of two edges to be found. As a digital image is composed of pixels, this elementary unit of data is the limitation on how accurately the location of these edges can be known. The true edge will almost certainly lie somewhere within a single pixel rather than at its edge. To a first approximation, an algorithm will only be able to report the edge location in terms of whole pixels. Therefore, every feature measurement will have a minimum error of ±1 pixel. Hence, it is recommended that, when fibre diameter is measured on an image, the number of pixels from one edge to the other be reduced by 1 before converting to a physical scale. If this is not done, the reported physical diameter of the fibre will always be too large. To determine the physical size of a pixel in engineering units, the length of a scale bar must be measured.

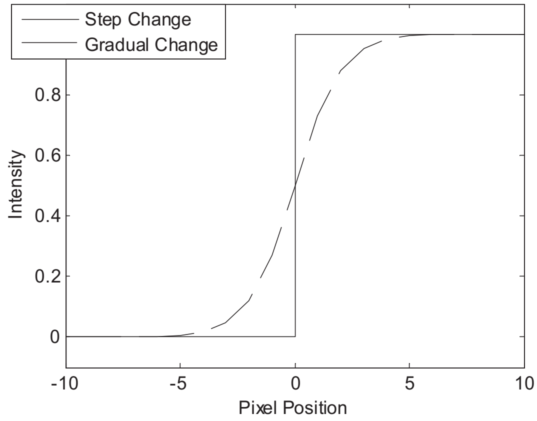

Fig. 2. An example of a step change and a gradual change. The gradual change is an idealized example of how many edges can appear in real-world situations. Here a gradient approach would be more appropriate.

Assuming the edges of the scale bar can be detected perfectly, the scale bar length can be accurate only to within a single pixel, and so introduces an additional systematic error in reporting the physical size of a measured feature. The estimated error can be calculated using Equation (1). Therefore, given the condition that two fibre edges must be identified, the total fibre diameter error will always be greater than one pixel. For normal SEM images suitable for image analysis of electrospun fibres, typically an error is expected of between 1.5 pixels and 3 pixels for any measured fibre diameter. Knowing the expected minimum error, it is recommended that measurements of fibre diameter from a digital image be performed at a magnification corresponding to a value where most fibres are represented with a diameter of 20 pixels or above (Fig. 3).

$$\Delta d = d\left(\frac{\Delta d_{pix}}{d_{pix}} + \frac{\Delta \chi}{\chi}\right) \tag{1}$$

Equation (1): Calculation of the physical error in the measurement of a feature on a digital image. Here d is the reported diameter in physical units, d_{pix} is the same measurement in pixels, χ is the size of a pixel in physical units, and the prefix Δ represents the absolute error in that quantity.

Practically, there are numerous opportunities for additional errors to be introduced into the measurement of electrospun fibres. One of the most common is the assumption of a perfectly circular cross-section. It is well known that fibres can be deposited with an oval or flat ribbon cross-section, which cannot be described by a simple fibre diameter. Unless an algorithm is explicitly

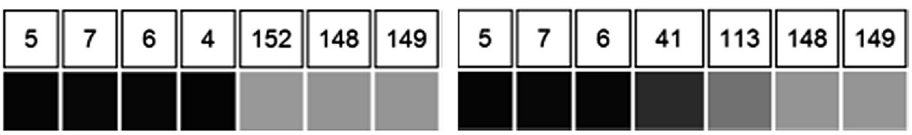

Fig. 1. Left: a trivial case of edge detection that can be handled by simple thresholding. Right: a typical case of edge detection where the exact edge location is harder to determine.

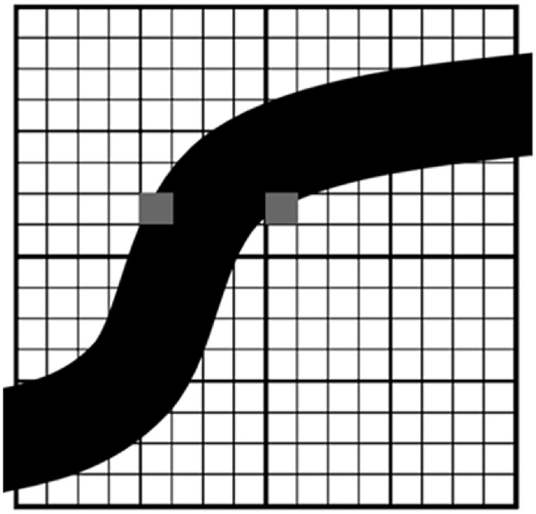

Fig. 3. The errors associated with making diameter measurements from digital images. The grey boxes represent the detected edge pixels.

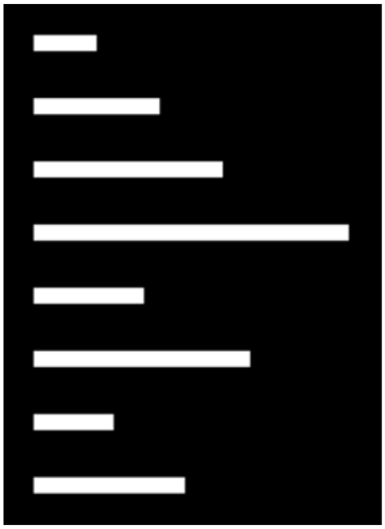

Fig. 4. Simple horizontal two-tone scale bars of known length.

designed to recognise visual indications of these cross-sections, it will report the feature size (i.e. distance between the two edges of the fibre) as the diameter of a circular fibre. Another assumption would be that the measured fibres accurately represent all the fibres in the sample. This may not be true if insufficient images of the fibre sample have been collected or if the fibre has significant variation in diameter along its length, such as when fibres are beaded, meaning that the diameter varies along the length.

3. Results

3.1. Horizontal scale bar

The first experiment was a simple comparison between two human operators and the Electrospinz SEMAnalyser™ algorithm when measuring the length of horizontal bars on a generated two-tone image. The image used (Fig. 4) was generated for the experiment. Each white bar is of known length. This experiment tests the edge detection ability of the algorithm in an ideal case and compares it with typical human performance. The human operators were asked to measure the length of the bar and the results were compared with those of the algorithm. The average difference between the reported length by the human operators and the known lengths was two pixels. The SEMAnalyser™ algorithm reported the correct length of the bars for all cases tested.

3.2. Simulated two-tone images

To further test the accuracy and robustness of the SEMAnalyser™ algorithm, another experiment was performed using simulated two-tone images of electrospun fibre. As the image is made up of two tones, the edge detection should be as accurate as the previous experiment; therefore this experiment tested the heuristics used to convert edge data into diameter data.

There were six images generated where white lines were randomly drawn over a black background. The diameter of these lines was randomly generated from a normal distribution with an average diameter of 40 pixels and a standard deviation of 10 pixels. Each image contained 25 simulated fibres to ensure there was sufficient chance for many junctions and near parallel fibres to be represented. The analysis of these images was performed by an operator who was unaware of the desired fibre diameter distribution. Examples of the images generated can be seen in Fig. 5.

As the fibre diameters were randomly generated, the generated average diameter and standard deviation will not be exactly the same as the specified values used to generate the six images. The results of the analysis can be seen in Table 1. The SEMAnalyser™ algorithm is able to generate a very similar average fibre diameter but has a significantly larger standard deviation. From Fig. 6, it can be seen that this is due to the algorithm reporting a trailing tail of larger diameters along with a small leading tail of smaller diameters than was originally generated.

3.3. Measuring electrospun fibre

Obtaining a traceable standard sample of known diameter distribution at the nano-scale to take SEM photos of proved to be difficult because of manufacturing limitations at that scale. Therefore, to test the SEMAnalyser™ algorithm in a real world environment, the results from the algorithm were compared with the results reported by a panel of human operators measuring identical images. The operator of the SEMAnalyser™ algorithm was not aware of the human results at the time the images were analysed by the algorithm. Each sample (A and B) had 10 SEM photos taken according to the specifications in the manual

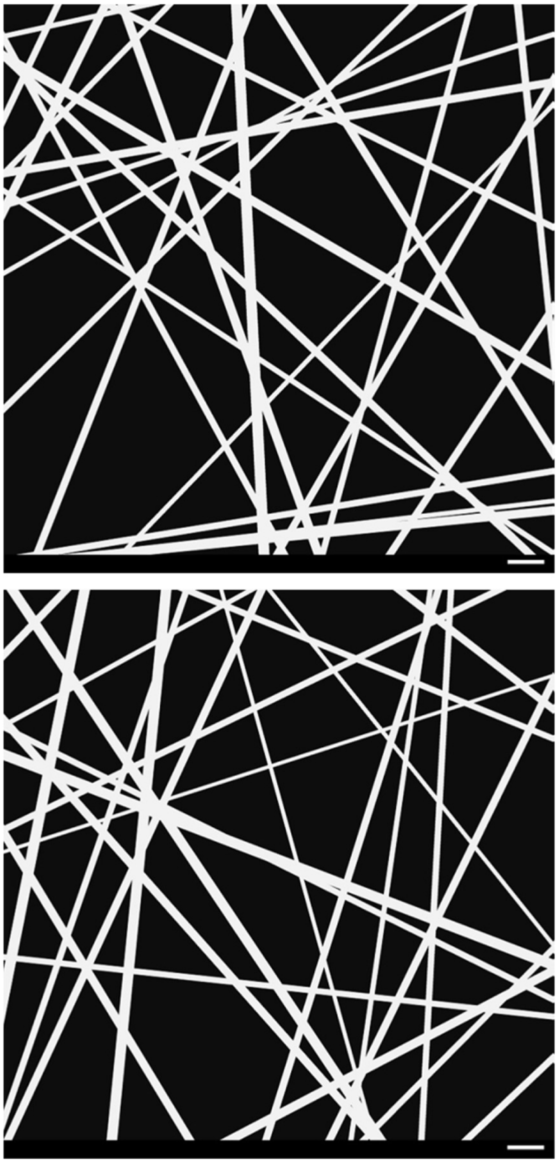

Fig. 5. Examples of the simulated two-tone electrospun fibre SEM images. These images were generated with an average fibre diameter of 40 pixels and a standard deviation of 10 pixels. The scale bar is 200 pixels long.

measurement method. Both samples were gold coated. An example of each set can be seen in Fig. 7.

The total set of 20 images was then given to each of the six human operators along with the manual measurement method specification. A human operator took on average 2.5 hours to complete the specified task. This included time to load the image, calibrate it, make the measurements then export the data to an MS® Excel spreadsheet before starting the next image. A similar process could be completed in less than 5 minutes (includes user interaction time) using the Electrospinz SEMAnalyser™ software. From sample A, five images were also sent to nanoScaffold Technologies LLC to be analysed using their FibreQuant™ software.

Table 1
Performance of the SEMAnalyser™ algorithm in measuring simulated electrospun fibre. The generated and measured columns represent the entire dataset generated from all six images used.

	Specified	Generated (over all images)	Measured (over all images)
Mean	40	39.9	39.4
Standard Deviation	10	9.7	14.2

The reported diameters were then tabulated and the average fibre diameter per operator/method per image was calculated along with the overall average diameter per method per sample (see Table 2). This overall average combined all the measurements taken from the 10 images of a given sample for a given method. Here, each human operator is assumed to be the same method. This allows the performance of the different measurement methods to be compared. An example of the cumulative distribution for Sample A is shown in Fig. 8.

The standard deviation of the overall average was also calculated, along with the range in the per-image average diameter. The higher standard deviation reported for the SEMAnalyser™ software is due to the very large number of measurements reported and the long tail for high diameters seen in Fig. 8. For the human operators it was possible to calculate the range of the average diameter reported for a single image across operators. This gives an indication of the repeatability of a human operator, which could be as large as 35 nm (approximately 5 pixels). The physical size of a single pixel in these images was 7.2 nm. The settings used with the Electrospinz SEMAnalyser™ software for sample A are given in Table 3 and for sample B are given in Table 4. The average diameter measured for each image can be seen in Fig. 9.

By using the collated dataset prepared for Table 2, it is possible to investigate the number of measurements required to report a precise and accurate result. This can be done by randomizing the order of measurements and then calculating a cumulative average, effectively treating the randomized order as a time-ordered series of measurements. Two criteria are employed here: first, how many measurements before the cumulative average converges towards the overall average, and second, how many measurements before the cumulative average represents an accurate value? Given the size of the dataset, convergence with the overall average is typically fast, requiring only a visual inspection of the plotted results. To determine if the reported average is accurate, the criteria of the minimum measurement error of one pixel can be employed. Once the cumulative average is within the minimum measurement error of the overall average, additional measurements are not really improving the accuracy. Because of the randomization element of the analysis, an example of a worst-case scenario for the SEMAnalyser™ algorithm and for the human operators is presented in Fig. 10 and Fig. 11 respectively. In these specific cases both the SEMAnalyser™ algorithm and the human operator appear to reach an accurate and precise measurement within approximately 150 measurements.

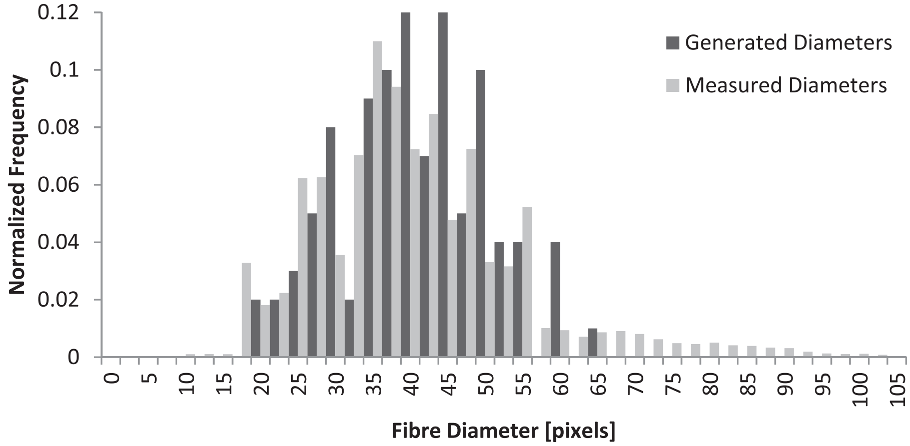

Fig. 6. Measured fibre diameter for simulated images of electrospun fibre compared with the parameters used to generate the images.

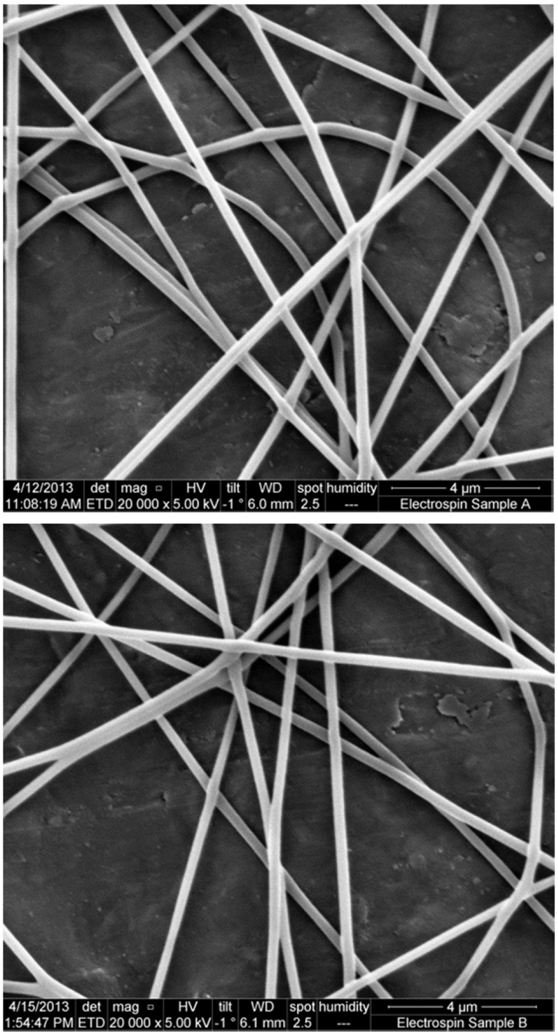

Fig. 7. Example SEM images of electrospun 8% PVOH. Sample A is 10 kV at 100 mm distance. Sample B is 10 kV at 120 mm distance.

3.4. *Effects of coating*

A final experiment was carried out to examine the effects of coatings applied for electron microscopy on the measured fibre diameter. A replicate of Sample A was prepared and divided into three pieces and the samples were coated with iridium, gold and carbon. At three different fixed magnifications, images were taken of the sample in four different locations. The identical fibres in these images were measured using the SEMAnalyser™ algorithm. It was found that gold and carbon results were reported within the minimum measurement error, while iridium coating resulted in a much smaller fibre diameter (see Table 5). The three magnifications also demonstrated the importance of reporting the size of the pixels in the image. As the magnification was increased, an error of a single pixel could result in the mistaken interpretation that the fibres examined were of different diameters, whereas they are exactly identical (see Table 6).

4. Discussion

If an automated algorithm is to be of use in scientific research, it must be at least as accurate as a human operator. The results in Table 1 demonstrate that the SEMAnalyser™ algorithm can accurately measure fibre diameter under the ideal conditions of a two-tone image. The use of a two-tone image ensures that correct edge detection is trivial. In this case, it is the heuristics used by the algorithm to convert detected edges into fibre diameters that is being tested. Unlike a human operator, the algorithm will occasionally mistake non fibre-edge image features for fibre, reporting very small diameters that are non-physical. Similarly, the algorithm is not always able to identify and avoid measurements where two fibres have merged together or junctions where two fibres cross. Therefore, some large fibre diameters are reported that do not represent the fibre produced. Despite this, the SEMAnalyser™ algorithm is able to make such a high volume of measurements in a short period of time that the proportion of these erroneous measurements to valid measurements is

Table 2
Comparison of the performance of different fibre diameter measurement methods.

	Overall Average Diameter [nm]	Standard Deviation [nm]	Range of Averages [nm]	Range Between Operators [nm]	Number of reported diameters
Sample A					
Human Operators	282	37	82	35	1026
SEMAnalyser™	303	64	25	–	9926
FibreQuant™	296	29	27	–	4060
Sample B					
Human Operators	271	35	82	35	949
SEMAnalyser™	292	64	42	–	9389

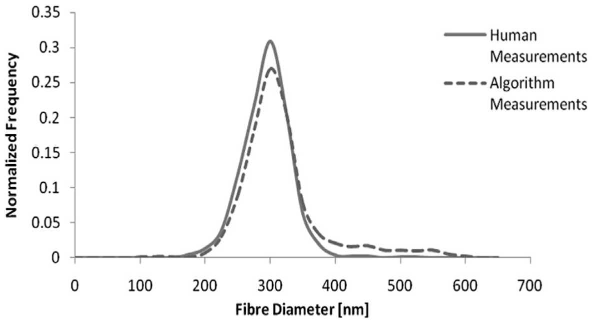

Fig. 8. Cumulative fibre diameter distribution for all 10 images from sample A.

very low. This is why the peak in the normal distribution, i.e. the average fibre diameter, does correspond to the correct measurement. These erroneous measurements will result in a higher standard deviation than the true fibre distribution because of the leading and trailing long tails, as seen in Fig. 6.

Although the heuristics of a human operator are superior to those of the SEMAnalyser™ algorithm, humans suffer from difficulties judging edge locations. Whereas an automated algorithm has a rigid specification allowing it to detect edges repeatedly in exactly the same location (even if that location is incorrect), humans must use their judgement every time they select an edge. Human error can creep into each edge measurement, the perpendicular nature of the measurement, and the location along the fibre where the measurement is taken. The last is a problem that is introduced by the method used to eliminate operator bias towards a given fibre size by measuring each fibre only once.

If the errors were minor, then a human operator would be superior to the current generation of automated algorithms. Fig. 9 demonstrates that this is not the case. For a given image, the reported averages by human operators varied by more than the ± 2 pixel estimated error (Fig. 9). Often, measurements reported for the same image are well outside the bounds of the intrinsic measurement error. This wide variation between different human operators that were given identical instructions demonstrates that the average human is a poor judge when trying to measure fibre diameter in a repeatable manner. If a human operator were given infinite time, they may be able to match the performance of an automatic process. Practically, research time is limited and thus only normal operation will be considered. For a human operator, taking a large number of measurements as demonstrated in Fig. 11 is the most reasonable option to compensate for human error. If the number of measurements taken for a single sample is in the hundreds, then the measured diameter can be expected to be reliable within the limits of precision dictated by pixel size. The time required to make this number of measurements, although not infinite, is generally impractically large, with simple experiments consuming days of measurement time.

The SEMAnalyser™ algorithm was able to report an average diameter per image that was in all but three cases within one pixel of the overall average diameter; similarly with the FibreQuant™ algorithm. This shows that automated algorithms are able to consistently report values within the natural variation range due to the intrinsic measurement error. With further consideration of the sheer number of measurements that can be made in a short time and the rigid heuristics used by the algorithms to reliably measure without bias, automated algorithms are clearly superior to human operators. Both algorithms tested performed sufficiently similarly to be considered interchangeable in their superiority to human operators.

A final aspect to accurately reporting fibre diameter is the effect of SEM preparation. Tables 5 and 6 show that both the preparation of samples and the capturing of the SEM images can affect the reported fibre diameter. Whereas gold and carbon coating gave comparable fibre diameters, Table 5 shows that iridium coating resulted in a smaller fibre diameter. As these SEM preparation methods

Table 3
Settings used for the Electrospinz SEMAnalyser™ software in the analysis the SEM images of sample A.

Max Feature Size	Min Feature Size	Line Spacing	Running Average	Detection Threshold
700 nm	100 nm	15 pixels	7 pixels	0.015

Table 4
Settings used for the Electrospinz SEMAnalyser™ software in the analysis the SEM images of sample B.

Max Feature Size	Min Feature Size	Line Spacing	Running Average	Detection Threshold
700 nm	100 nm	15 pixels	7 pixels	0.016

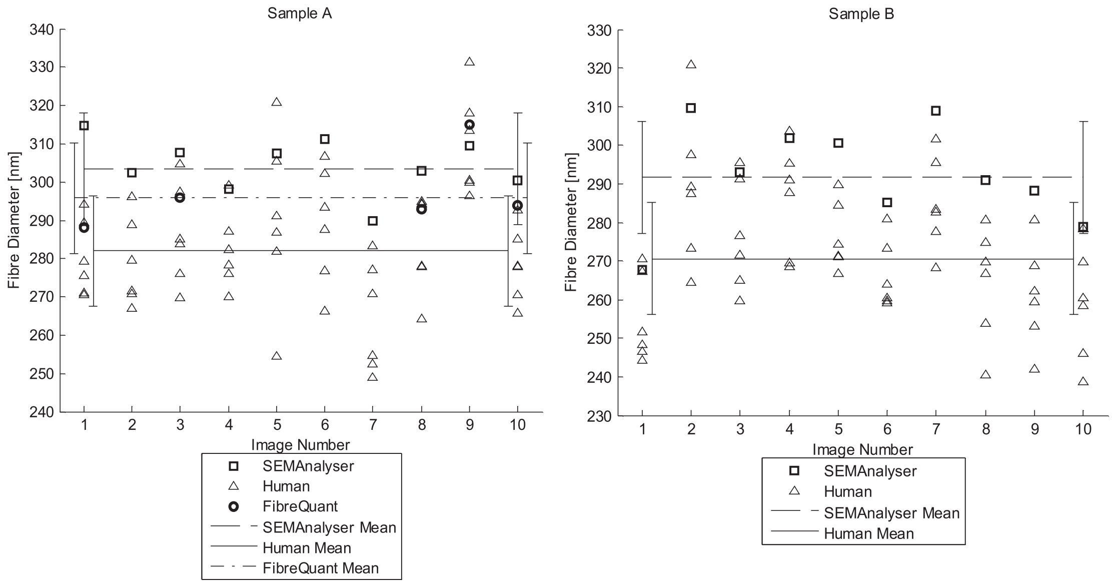

Fig. 9. Average diameter measured by human operators and automated algorithms. The circles are the average fibre diameter measured for each image. The horizontal lines represent the total average for all 10 images of the same sample, with the error bars representing the equivalent of ±2 pixels from the total average.

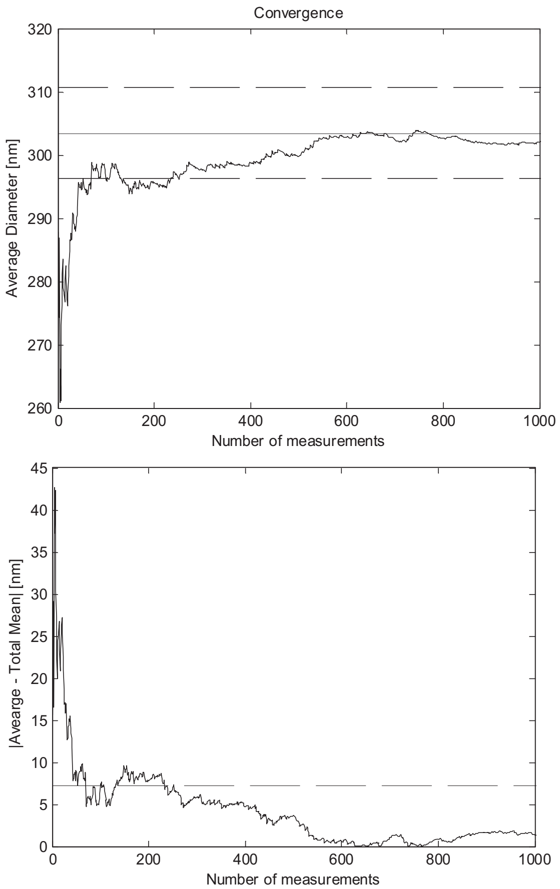

Fig. 10. Top: Cumulative average of a randomized set of measurements taken from sample A by Electrospinz SEMAnalyser™ software. The horizontal dashed line represents ±1 pixel from the total average. Bottom: The absolute value of the total mean subtracted from the same cumulative average as shown on the left. This shows the magnitude of the deviation from the mean. The horizontal dashed line marks a 1 pixel deviation from the total mean (the total mean is at zero).

involve the addition of a coating to the fibre surface, the smaller fibre diameter must be closer to the diameter of the originally deposited fibre. If reported results in literature are to be comparable, the SEM preparation methods must be reported. Similarly, Table 6 shows that it is vital to report the physical size of a pixel in the images that are measured. Reporting the magnification is insufficient, as each instrument may have a different intrinsic error associated with a given magnification. Considering the publication of results, the final piece of information required is the number of measurements taken. If fewer than 150 measurements per sample were taken, it should be assumed that the potential error in the measurement is larger than the minimum error associated with the pixel size.

5. Conclusions

This work presents an in-depth analysis of the suitability of using image analysis algorithms for automatically determining electrospun fibre diameter from SEM images. It is argued that any measurement of features made from a digital image must have a minimum error of ±1 pixel, although practically will be higher.

It was found that an automated algorithm was able to accurately measure simulated two-tone images. The difference between the known dimensions and the measurement was 0.5 pixels. Images taken of samples of PVOH electrospun fibre were measured by six human operators. The reported difference between the algorithm and the human operators was 21 nm or 2.9 pixels. The inter-image variation in average fibre diameter reported by human operators was >80 nm (11 pixels), compared with <45 nm (6.2 pixels) for the image analysis software. The variation between human operators for the same image was approximately 35 nm (4.8 pixels). Examining the convergence of a cumulative average, it was found that a minimum of 150 measurements would be needed to ensure the reported total average was within the ±1 pixel error limit. It

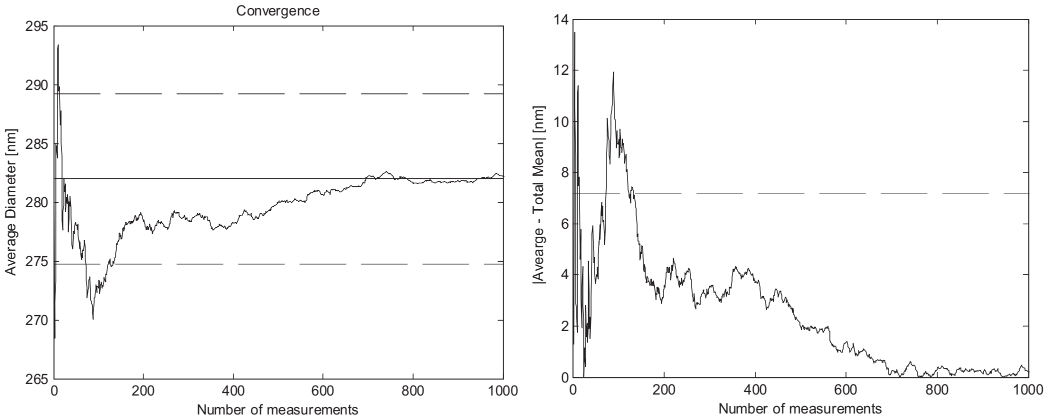

Fig. 11. Left: Cumulative average of a randomized set of measurements taken from sample A by human operators. The horizontal dashed line represents ±1 pixel from the total average. Right: The absolute value of the total mean subtracted from the same cumulative average as shown on the left. This shows the magnitude of the deviation from the mean. The horizontal dashed line marks a 1 pixel deviation from the total mean (the total mean is at zero).

Table 5
Measured identical electrospun fibres under different coating regimes before imaging with electron microscopy.

Coating	Mean Diameter [nm] Pixel Size: 8.5 nm
Gold	359
Carbon	356
Iridium	324

Table 6
Measured identical electrospun fibres under different magnifications.

Relative Magnification	Mean Diameter [nm]	Mean Diameter [pixels]	Pixel Size [nm]
1x	315.9	9	34.2
2x	347.5	20	17.1
4x	359.4	42	8.5

is recommended that 250-500 measurements be made per sample, as the ±1 pixel error limit is a best-case scenario, and so other errors would delay convergence.

Acknowledgements

Ismael Ferrer of nanoScaffold Technologies LLC performed comparison automated measurements using FibreQuant™. Electrospinz Ltd provided the sample copy of SEMAnalyser™ used in this work. Pablo Lepe, Jacob Boutrup, Julie Anderson, Dr Nurfaizey Hamid, Yoan Camaret and Kevin Devarenne kindly donated their time to perform manual measurements. The work performed at The New Zealand Institute for Plant & Food Research Limited was funded by a New Zealand government Ministry of Business, Innovation & Employment Pre-Seed Accelerator Fund grant P/215006/01 arranged by Carmella Lee.

References

[1] N. Tucker, J.J. Stanger, M.P. Staiger, H. Razzaq, K. Hofman, The history of the science and technology of electrospinning from 1600 to 1995, J Eng Fibers Fabr 7 (2012) 63–73.
[2] M.M. Demir, I. Yilgor, E. Yilgor, B. Erman, Electrospinning of polyurethane fibers, Polymer 43 (2002) 3303–3309.
[3] J. Stanger, N. Tucker, S. Fullick, M. Sellier, M. Staiger, Insights into the power law relationships that describe mass deposition rates during electrospinning, J Mater Sci 47 (2012) 1113–1118.
[4] J.S. Lee, K.H. Choi, H.D. Ghim, S.S. Kim, D.H. Chun, H.Y. Kim, W.S. Lyoo, Role of molecular weight of atactic poly(vinyl alcohol) (PVA) in the structure and properties of PVA nanofabric prepared by electrospinning, J Appl Polym Sci 93 (2004) 1638–1646.
[5] M. Ziabari, V. Mottaghitalab, A. Haghi, Simulated image of electrospun nonwoven web of PVA and corresponding nanofiber diameter distribution, Korean J Chem Eng 25 (2008) 919–922.
[6] M. Ziabari, V. Mottaghitalab, A. Haghi, Application of direct tracking method for measuring electrospun nanofiber diameter, Braz J Chem Eng 26 (2009) 53–62.
[7] M. Ziabari, V. Mottaghitalab, S. McGovern, A.K. Haghi, A new image analysis based method for measuring electrospun nanofiber diameter, Nanoscale Res Lett 2 (2007) 597–600.
[8] C.-T. Li, J. Tietz, Improved accuracy of the laser diffraction technique for diameter measurement of small fibres, J Mater Sci 25 (1990) 4694–4698.
[9] M.D. Abràmoff, P.J. Magalhães, S.J. Ram, Image processing with ImageJ, Biophotonics Int 11 (2004) 36–43.
[10] J. Canny, A computational approach to edge detection, IEEE Trans Pattern Anal Mach Intell PAMI-8 (1986) 679–698.
[11] I. Idris, NumPy Cookbook, Packt Publishing Ltd, Birmingham, U.K, 2012.