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APPLICATION OF DIRECT TRACKING METHOD FOR MEASURING ELECTROSPUN NANOFIBER DIAMETER

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Abstract - In this paper, direct tracking method as an image analysis based technique for measuring electrospun nanofiber diameter has been presented and compared with distance transform method. Samples with known characteristics generated using a simulation scheme known as μ-randomness were employed to evaluate the accuracy of the method. Electrospun webs of polyvinyl alcohol (PVA) were also used to verify the applicability of the method on real samples. Since direct tracking as well as distance transform require binary input images, micrographs of the electrospun webs obtained from Scanning Electron Microscopy (SEM) were first converted to black and white using local thresholding. Direct tracking resulted in more accurate estimations of fiber diameter for simulated images as well as electrospun webs suggesting the usefulness of the method for electrospun nanofiber diameter measurement.
Keywords: Electrospinning, Nanofibers; Fiber diameter; Image analysis; Direct tracking; μ-Randomness.

INTRODUCTION

Conventional fiber spinning (like melt, dry and wet spinning) produces fibers with diameters down in the range of micrometers. In recent years, electrospinning has gained much attention as the most efficient method to produce fibers in the nanometer diameter range classified as nanofibers. Electrospun nanofibers are notable for their very small diameter (Doshi and Reneker 1995), large surface area per unit mass (Fong and Reneker 2001), extremely small pore size (Gibson et al., 1999), flexibility and superior mechanical properties (Huang et al., 2003). These remarkable features of electrospun nanofibers have led them to be preferred materials for many applications. Proposed uses of nanofibers include tissue engineering (Lannutti et al., 2007), drug delivery (Kenawy et al., 2002), wound dressing (Khil et al., 2003), protective clothing (Gibson et al., 1999), filtration (Qin and Wang 2006), reinforcement (Huang et al., 2003) and electronic applications such as sensors (Aussawasathien et al., 2005), batteries (Lee et al., 2006) and transistors (Pinto et al., 2003).

In the electrospinning process, a high electric field is generated between a polymer solution held by its surface tension at the end of a syringe (or a capillary tube) and a collection target. Charge is induced on the solution surface by the electric field. Mutual charge repulsion causes a force directly opposite to the surface tension. As the intensity of the electric field increases, the hemispherical surface of the solution at the tip of the capillary tube elongates to form a conical shape known as Taylor cone. When the electric field reaches a critical value at which the repulsive electric force overcomes the surface tension force, a charged jet of the solution is ejected from the tip of the cone. As the jet flies in air, its diameter decreases as a result of the stretching and solvent evaporation. Decreasing the jet diameter,

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the surface charge density increases and the resulting high repulsive forces split the jet into several smaller jets. This process may be carried out several times, leading to many small jets which are finally accumulated on the surface of the collector, resulting in a nonwoven web of randomly oriented fibers with diameters on the nanometer scale (Doshi and Reneker 1995 and Fong and Reneker 2001). Figure 1 illustrates the electrospinning setup (Haghi and Akbari 2007).

Physical and mechanical properties of nonwoven textiles as well as electrospun nanofiber webs depend not only on material properties of the component fiber but also its structural characteristics. Image analysis has been used to identify fibers and measure structural characteristics such as fiber orientation (Pourdeyhimi et al., 1996), fiber diameter (Pourdeyhimi and Dent 1999), pore size (Aydilek et al., 2002), uniformity (Chhabra 2003) and other structural features (Xu and Ting 1995) in nonwoven textiles. However, these methods are generally not appropriate for measuring structural characteristics of electrospun nanofiber webs due mostly to their different size, shape and structure. Some may be employed for electrospun webs provided a number of modifications are applied.

Analyzing the electrospun nanofiber webs yields results and information which help researchers in improving the quality and predicting the overall performance of the product. Distribution of fiber diameters, which is one of the most important structural characteristics in electrospun nanofiber webs, varies depending on the process and material variables. Understanding how fiber diameter and its distribution are affected by the electrospinning parameters is essential to produce webs with desired properties. Many researchers have addressed the effects of processing variables on electrospun fiber diameters. However, there is a lack of a standard technique to measure fiber diameter and its distribution in electrospun webs. Hence, accurate and automated measurement of nanofiber diameter is useful and crucial and therefore has been taken into consideration in this contribution. The objective of the current research is thus to develop an image analysis based method to serve as a simple, automated and efficient alternative for electrospun nanofiber diameter measurement.

METHODOLOGY

Measuring Electrospun Fiber Diameters

The first step in determining fiber diameters in an electrospun web is to obtain a micrograph of the web under a suitable magnification using electron microscopy or atomic force microscopy techniques. The conventional way of measuring electrospun fiber diameter is to analyze the micrograph manually. The manual method normally consists of the following steps: determining the length of a pixel in the image (setting the scale), measuring the number of pixels between two edges of a fiber perpendicular to its axis, converting the number of pixels to nm using the scale and recording the result. Typically 100 diameters are measured by which a histogram of fiber diameter is plotted.

However, this process is tedious and time-consuming, especially when analysis of a large number of samples is required. Furthermore, it cannot be used as an on-line method for quality control since an operator is needed for performing the measurements. Hence, developing an automated technique which eliminates the use of an operator and has the capability of being employed in on-line quality control is of great importance.

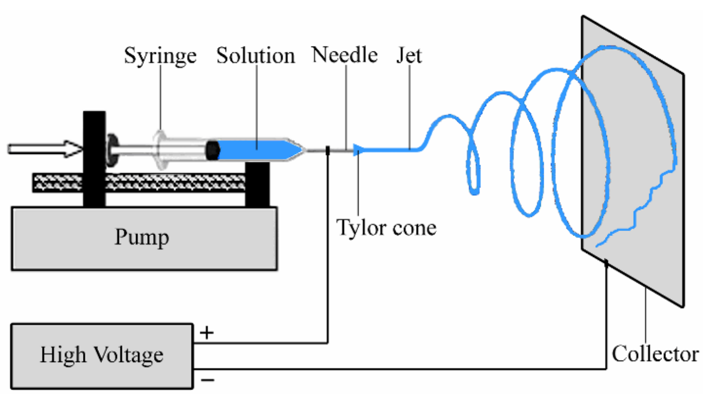


Figure 1: Electrospinning setup

a) Distance Transform Method

The distance transform of a binary image is the distance from every pixel to the nearest nonzero-valued pixel. The center of an object in the distance transformed image will have the highest value and lie exactly over the object's skeleton. The skeleton of the object can be obtained by skeletonization or thinning processes which remove pixels on the boundaries of objects but do not allow them to break apart, thereby reducing a thick object to its corresponding object with one pixel width. Skeletonization or thinning often produces short spurs which could be cleaned up automatically with a pruning procedure (Gonzalez and Woods 2001).

The algorithm for determining fiber diameter uses a binary input image and creates its skeleton and distance transformed image (distance map). The skeleton acts as a guide for tracking the distance transformed image and fiber diameters are measured from the intensities of the distance map at all points along the skeleton (Pourdeyhimi and Dent 1999). Figure 2 shows a simple simulated image, which consists of five fibers with diameters of 10, 13, 16, 19 and 21 pixels, together with its skeleton and distance map including the histogram of fiber diameter obtained by this method.

b) Direct Tracking Method

In this paper, we developed a direct tracking method for measuring electrospun nanofiber diameter. This method, which also uses a binary image as the input, determines fiber diameter based on information acquired from two scans; first a horizontal and then a vertical scan. In the horizontal scan, the algorithm searches for the first white pixel (representative of fibers) adjacent to a black (representative of background). Pixels are then counted until reaching the first black. Afterwards, the second scan is started from the mid point of the horizontal scan and pixels are counted until the first vertical black pixel is encountered. Direction will change if the black pixel is not found (Figure 3). Having the number of horizontal and vertical scans, the number of pixels in the perpendicular direction, which is the fiber diameter in terms of pixels, can be measured through a simple geometrical relationship.

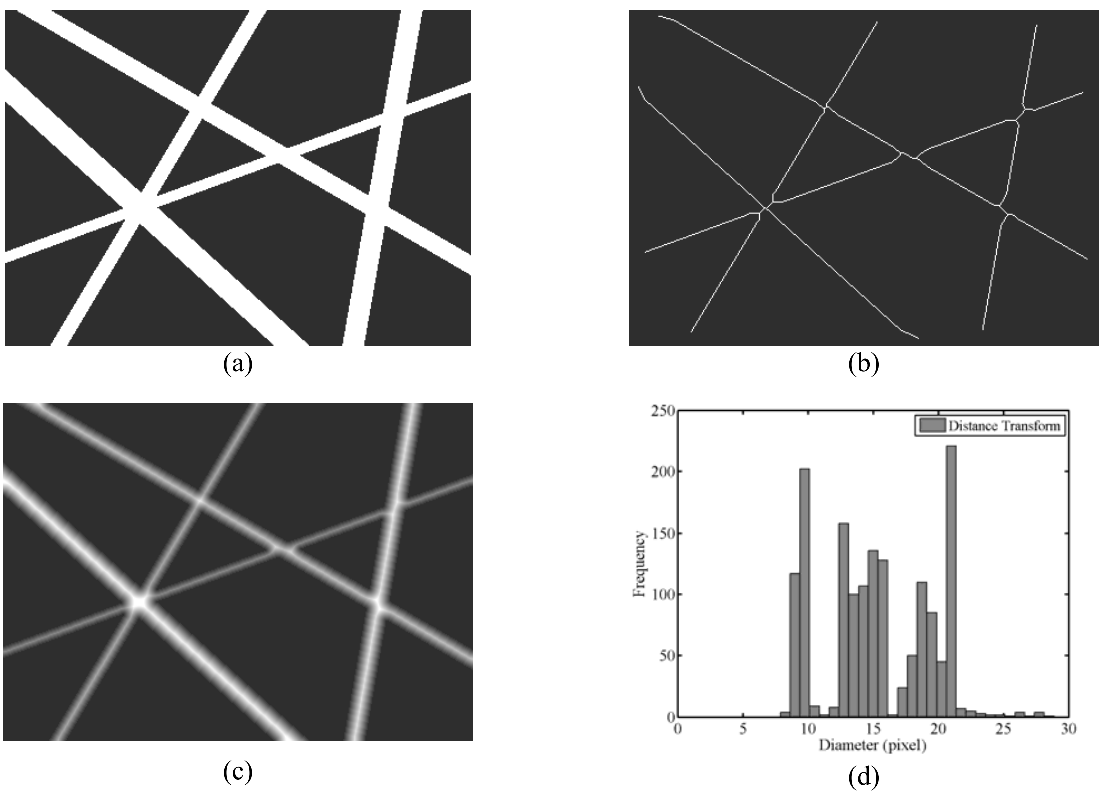
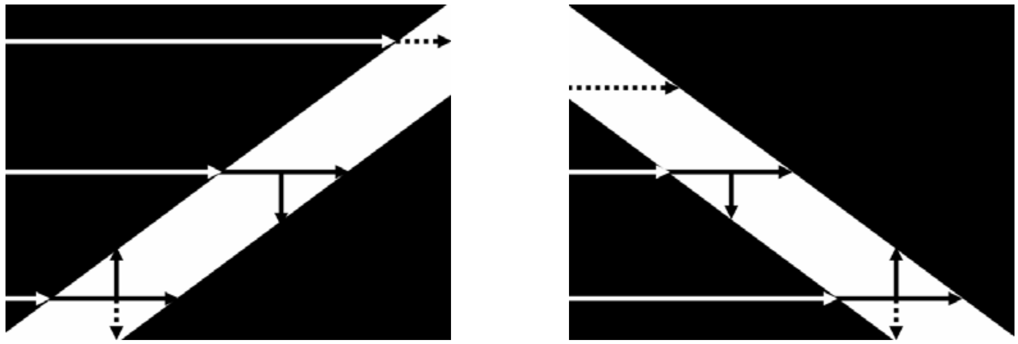


Figure 2: a) A simple simulated image, b) Skeleton of (a), c) Distance map of (a) after pruning, d) Histogram of fiber diameter distribution obtained by distance transform method



(a) (b)

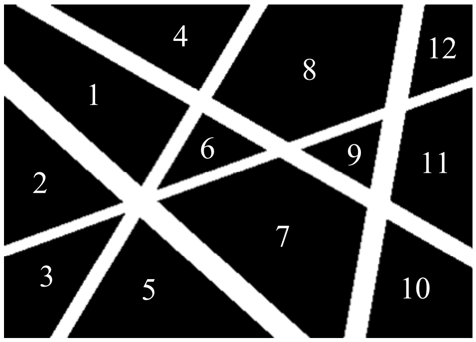
Figure 3: Fiber diameter measurement based on two scans in direct tracking method

In electrospun webs, nanofibers cross each other at intersection points and this brings about the possibility for some untrue measurements of fiber diameter in these regions. To circumvent this problem, a process called fiber identification is employed. First, black regions are labeled and a couple of regions between which a fiber exists are selected. In the next step, the two selected regions are connected, performing a dilation operation with a large enough structuring element. Dilation is an operation that grows or thickens objects in a binary image by adding pixels to the boundaries of objects. In the following process, an erosion operation with the same structuring element is performed and the fiber is recognized. Erosion shrinks or thins objects in a binary image by removing pixels on object boundaries. The specific manner and extent of thickening or shrinking in dilation or erosion is controlled by the size and shape of the structuring element used (Gonzalez and Woods 2001). In order to enhance the processing speed, the image is then cropped to the size of selected regions. Afterwards, fiber diameter is measured according to the aforementioned algorithm. This trend continues until all the fibers in the image are analyzed. Finally, the data in pixels may be converted to nm and the histogram of fiber diameter distribution is plotted. This process was applied on the simulated image shown in Figure 2a. Figure 4 depicts the labeled simulated image and the histogram of fiber diameter obtained by direct tracking method.

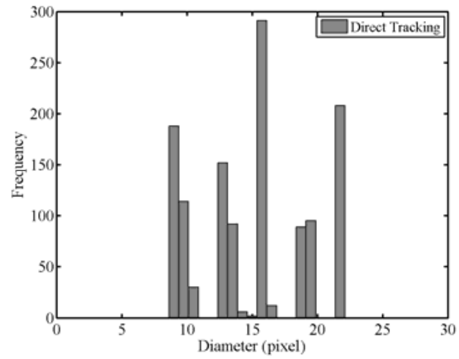
Simulation

Reliable evaluation of the accuracy of the developed methods requires samples with known characteristics. Since it is neither possible to obtain real electrospun webs with specific characteristics through experiment nor there is a method which measures fiber diameters precisely with which to compare the results, the method will not be well evaluated by using just real webs. To that end, a simulation algorithm has been employed for generating samples with known characteristics. The physical characteristics of simulated images are known exactly, hence they could be employed to evaluate the usefulness of algorithms used in characterizing diameter and other structural features. Simulation algorithms for generating nonwovens with both continuous and discontinuous fibers were presented elsewhere (Abdel-Ghani and Davis 1985).

The most important component of simulation is the way in which lines or curves are generated. The aim of simulation is to obtain unbiased arrays which are spatially homogeneous. It was found that the best way to simulate nonwovens of continuous fibers is through μ-Randomness procedure (Pourdeyhimi et al., 1996). In this case, it is assumed that the lines are infinitely long, so that in the image plane they intersect the boundaries. Under this scheme, which is shown in Figure 5, a line with a specified thickness is defined by the perpendicular distance d away from a fixed reference point O located in the center of the image and the angular position of the perpendicular α. Distance d is limited to the diagonal of the image (Abdel-Ghani and Davis 1985). Several variables are allowed to be controlled during simulation; line thickness, line density, angular density and distance from the reference point. These variables can be sampled from given distributions or held constant.



(a)



(b)

Figure 4: a) The labeled simulated image, b) Histogram of fiber diameter distribution obtained by direct tracking method

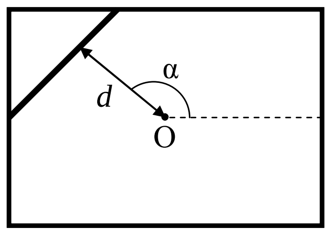


Figure 5: μ-randomness procedure

Thresholding

Distance transform and direct tracking algorithms for measuring fiber diameter both require binary images as their input. Hence, the micrographs of electrospun webs first have to be converted to black and white. This is carried out by a thresholding process (known also as segmentation) which produces a binary image from a grayscale (intensity) image (Gonzalez and Woods 2001 and Petrou and Bosdogianni 1999). This is a critical step because the segmentation affects the result significantly (Pourdeyhimi et al., 1999). Prior to the segmentation, an intensity adjustment operation and a two dimensional median filter are often applied in order to enhance the contrast of the image and remove noise.

In the simplest thresholding technique, called global thresholding, the image is segmented using a single constant threshold. One simple way to choose a threshold is by trial and error. Each pixel is then labeled as object or background depending on whether its gray level is greater or less than the value of the threshold, respectively (Gonzalez and Woods 2001 and Petrou and Bosdogianni 1999).

The main problem of global thresholding is its possible failure in the presence of non-uniform illumination or local gray level unevenness. An alternative to this problem is to use local thresholding instead. In this approach, the original image is divided into subimages and different thresholds are used for segmentation. A more common variant of this approach, which has been used in this study, consists of estimating the background illumination by using a morphological opening operation, subtracting the obtained background from the original image and applying a global thresholding to produce the binary version of the image. The morphological opening is a sequential application of an erosion operation followed by a dilation operation (i.e., opening = erosion + dilation) using the same structuring element (Gonzalez and Woods 2001 and Petrou and Bosdogianni 1999). The selection of the appropriate threshold can also be automated using Otsu's method (Otsu 1979), which chooses the threshold to maximize the interclass variance and minimize the intraclass variance of the black and white pixels. As it is shown in Figure 6, global thresholding resulted in some broken fiber segments. This problem was solved using local thresholding.

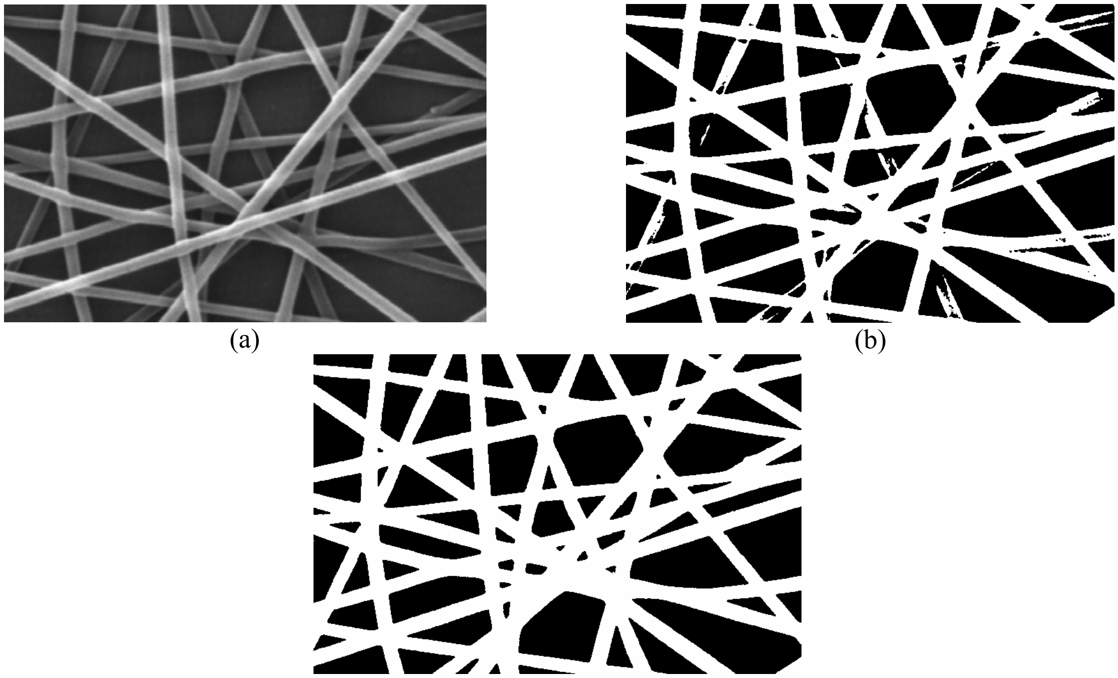


Figure 6: a) A typical electrospun web, b) Global thresholding, c) Local thresholding

EXPERIMENTAL

Electrospun nanofiber webs used as real webs in image analysis were prepared by electrospinning aqueous solutions of PVA with average molecular weight of 72000 g/mol (MERCK) under different processing parameters. The micrographs of the webs were obtained using a Philips (XL-30) environmental Scanning Electron Microscope (SEM) under magnification of 10000X after gold sputter coating.

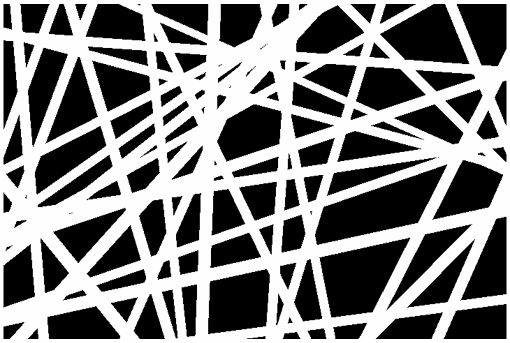
RESULTS AND DISCUSSION

Three simulated images generated by the μ-randomness procedure were used as samples with known characteristics to demonstrate the validity of the techniques. They were each produced by 30 randomly oriented lines with varied diameters sampled from normal distributions with a mean of 15 pixels and standard deviation of 2, 4 and 8 pixels respectively. Table 1 summarizes the structural features of these simulated images which are shown in Figure 7.

Mean and standard deviations of fiber diameters for the simulated images obtained by direct tracking as well as distance transform are listed in Table 2. Figure 8 shows histograms of fiber diameter distribution for the simulated images obtained by the two methods. In order to make a true comparison, the original distribution of fiber diameter in each simulated image is also included. The line over each histogram is related to the fitted normal distribution to the corresponding fiber diameters. Note that the true mean and standard deviation of fiber diameters for the simulated images are slightly different from those set as the simulation parameters (see Table 2). This deviation may be attributed to using a discrete normal distribution for generating 30 fibers with varied thicknesses as well as differences of line lengths in the image, as longer lines contribute more than shorter ones to the fiber diameter distribution.

Table 1: Structural characteristics of the simulated images generated using μ-randomness procedure

No.	Angular range	Line density	Line thickness	
			M	Std
1	0-360	30	15	2
2	0-360	30	15	4
3	0-360	30	15	8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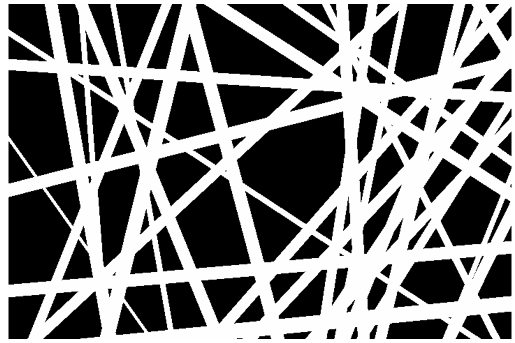
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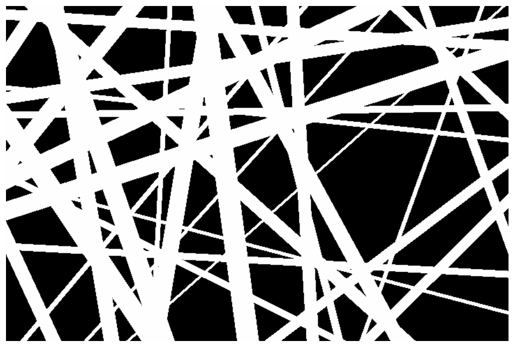
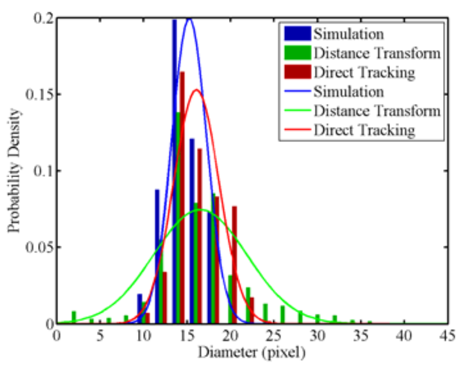

(3)

Figure 7: Simulated images generated by using the μ-randomness procedure



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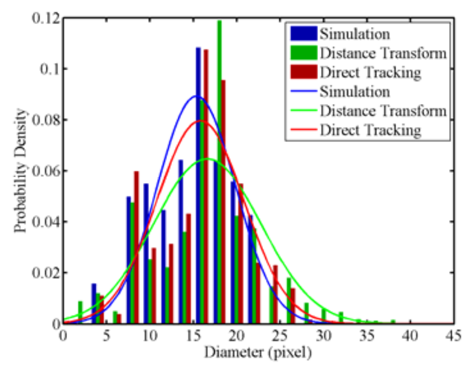

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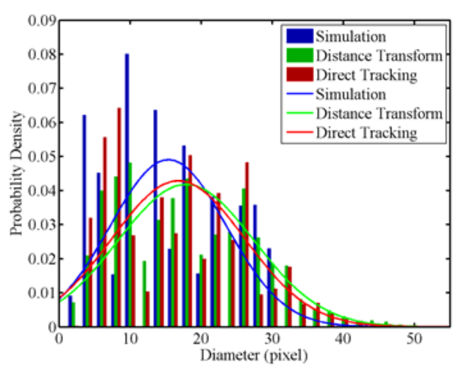


(3)

Figure 8: Histograms of fiber diameter distribution for the simulated images

Table 2: Mean and standard deviation of fiber diameters for the simulated images

		No. 1	No. 2	No. 3
Simulation	M	15.247	15.350	15.367
	Std	1.998	4.466	8.129
Distance transform	M	16.517	16.593	17.865
	Std	5.350	6.165	9.553
Direct tracking	M	16.075	15.803	16.770
	Std	2.606	5.007	9.319

Table 2 and Figure 8 clearly demonstrate that, for all simulated webs, the direct tracking method resulted in mean and standard deviations of fiber diameters that are closer to those of the corresponding simulated image (the true ones). The distance transform method is far away from making reliable and accurate measurements. This may be due to some remaining branches in the skeleton even after pruning. The thicker the line, the higher the possibility of branching during skeletonization (or thinning). Although these branches are small, their orientation is typically normal to the fiber axis; thus causing a widening of the distribution obtained by the distance transform method.

Furthermore, in the distance transform method, it is for just one single fiber that the value of the center of the object in the distance map could be related to fiber diameter. At intersections, however, two or more fibers cross each other. Therefore, the value of the center of the object corresponds to more than a fiber and is no longer related to fiber diameter. In addition, both the distance transformed image and the skeleton are broken at intersections and this problem becomes more severe as fibers get thicker and for points where more fibers cross each other. Hence, the method fails in measuring fiber diameters at intersections. In the direct tracking method, since the image is divided into parts where single fiber exists, the effect of intersections, which cause inaccurate measurement of fiber diameter, is eliminated. Therefore, there will be a more precise estimation for fiber diameter.

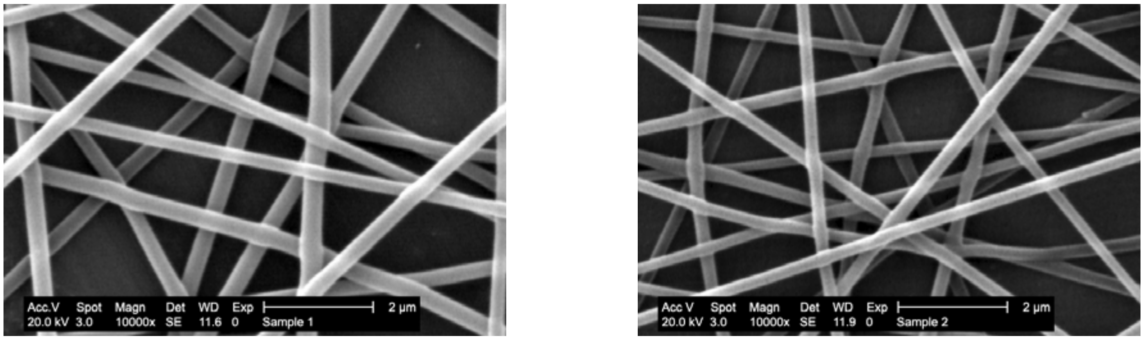
There are several reasons for the deviation of the computed results using direct tracking and true gathered results. A one-pixel error occurs in the selection of the mid point pixel (as a starting point for the second scan) when the number of pixels in the first scan is even. Furthermore, fiber segments must be of minimum lengths so that the diameter can be measured.

There are a series of issues concerning both of the methods. First, the thresholding techniques fail to correctly distinguish between multiple fibers being joined together and a single fiber, which causes an overestimation of fiber diameter. Further advancements in thresholding techniques may overcome this problem. Second, if the nanofiber web is thick, so that there is little or no free space between fibers, the methods will fail to identify fibers and therefore to perform the diameter measurement. Third, it is possible in the simulation process that one fiber overlays the other. As was mentioned before, at these intersecting points, distance transform results in some untrue fiber diameters and direct tracking makes no measurements. Nevertheless, fiber diameters at these points are counted in simulation and this could be another source of the differences observed in the case of simulated images.

The applicability of the techniques to real samples was also investigated using two nanofiber webs obtained from electrospinning of PVA at concentration of 12 and 10%, applied voltage of 15 and 10 kV, flow rate of 0.3 and 0.2 mL/h and spinning distance of 10 and 15 cm respectively. SEM micrographs of the webs (Figure 9) were first thresholded for diameter measurement by image analysis methods. The fiber diameter distributions were determined for each image by using distance transform and direct tracking methods and the results were compared to those obtained by the manual method.

Table 3 shows the results for real webs in terms of pixels and nm. The corresponding fiber diameter distribution histograms are given in Figure 10. In this case, mean and standard deviations of fiber diameters for direct tracking were closer to those of the manual method, which concurs with the trends observed for the simulated images.

In addition to the reasons mentioned previously, the small discrepancies between these results may also be attributed to the different number of measurements utilized in each technique. Distance transform and direct tracking measure over 1000 diameters. In the manual method, however, the number of measurements is limited to mostly 100 due to the time-consuming nature of the procedure.

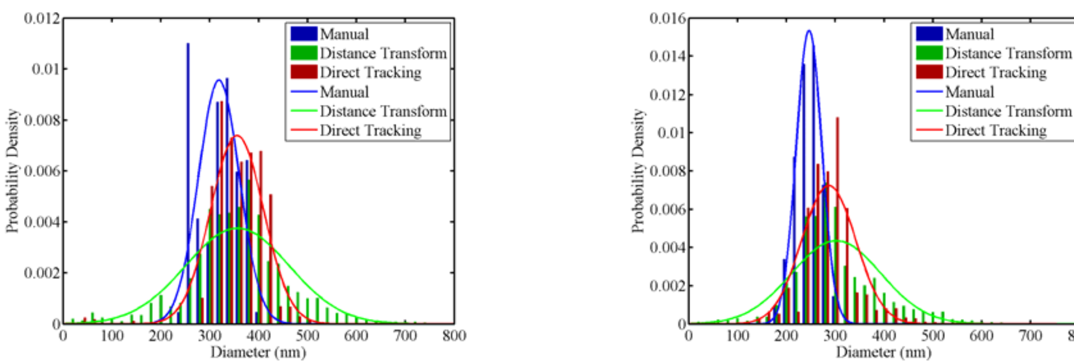


(1) (2)

Figure 9: Micrographs of the electrospun nanofiber webs

Table 3: Mean and standard deviation of fiber diameters for the electrospun webs

			No. 1	No. 2
Manual	pixel	M	24.358	18.827
		Std	3.193	1.984
	nm	M	318.67	246.31
		Std	41.77	25.96
Distance transform	pixel	M	27.250	23.079
		Std	8.125	7.005
	nm	M	356.49	301.94
		Std	106.30	91.64
Direct tracking	pixel	M	27.195	21.913
		Std	4.123	4.214
	nm	M	355.78	286.68
		Std	53.94	55.14



(1) (2)

Figure 10: Histograms of fiber diameter distribution for the electrospun webs

CONCLUSION

Fiber diameter is one of the most important structural characteristics in electrospun nanofiber webs. Electrospun nanofiber diameter is often measured by the manual method – a labor intensive, time consuming, operator-based technique that utilizes only a low number of measurements, thereby inefficient for automated systems, e.g., online quality control. In this study, an automated technique called “Direct Tracking” for measuring electrospun nanofiber diameter has been developed that is fast and has the capacity for automation, enabling improved quality control of large scale electrospinning operations. Serving as a novel technique, the accuracy of the method needs to be evaluated by using samples with known characteristics. To that end, the µ-randomness procedure was employed to generate three simulated webs. The results obtained by direct tracking were compared to those of the distance transform method. For all the simulated webs, the direct tracking method resulted in mean and standard deviations of fiber diameters closer to the simulation. The general applicability of the method to real samples was also demonstrated by using two electrospun nanofiber webs obtained by electrospinning of PVA. Due to the necessity of binary images as inputs for image analysis based methods of fiber diameter measurement, the images first had to be segmented. In order to do that, local thresholding was employed and, with the aid of Otsu's method, the appropriate threshold was automatically selected. The results obtained for real webs are in complete agreement with the trends observed in the case of simulated images. Mean and standard deviations of fiber diameters measured by direct tracking were closer to those of the manual method, suggesting that direct tracking could generally perform superior than distance transform. The results indicate that the attempt to develop the direct tracking method as an automated and fast alternative to the manual method for determining fiber diameter in electrospun nanofiber webs has been successful.

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