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Fiber statistics of nonwoven materials by SEM images - influence of number of images

Abstract: Electrospun nonwovens are widely applied in biomedicine and various other fields. For control of the manufacturing process and quality assurance Scanning electron microscopy (SEM) imaging is one standard practice. In this study, statistical datasets of 60 SEM images of three nonwoven samples were evaluated using Gaussian fit to obtain numerical results of their fiber diameter distributions. The question of how much effort is required for acceptable imaging and processing is being discussed. As determined here, for reliable statistics, a minimum surface area of the nonwoven has to be evaluated. The fiber diameter should be in a range of approximately 2 - 3% of the edge length of the square equivalent of the evaluated image area, using sufficiently magnified SEM images, in which the fiber diameter is imaged over at least 30 pixels.

Keywords: microfiber, nanofiber, automated evaluation, fiber diameter, reliable statistics, electrospinning

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1 Introduction

Fibrous materials are broadly used in various fields. In particular, the scope of application for electrospun nonwovens has increased. This topic is associated with a need for a high degree of stability in the manufacturing processes and indicates the necessity of quality assurance. However, for the industrial field general testing standards already exist [1]. For research in the biomedical field, an important parameter for assessing the fiber morphology of electrospun nonwovens is the fiber diameter distribution. Usually a number of fibers in single scanning electron microscope (SEM) images are measured manually. Data can also be obtained by laser scanning, optical fibre diameter

analysis, ensemble laser diffraction technique, microprojection and other techniques [2]. Here, an essential point is identifying the minimum number of SEM images (datasets) necessary to achieve reliable fiber statistics. More than 20 SEM images or 250 measurements seem to be necessary for reliable statistics [3]. To avoid individual bias of the human operator influencing the result and generating meaningful datasets, automated measuring methods are advantageous. In this study 60 SEM images of three nonwovens were statistically evaluated to point out the complexity of the topic, as well as offering a generally valid recommendation.

2 Materials and Methods

Two electrospun nonwovens of polyurethane-co-silicone were manufactured using a 4SPIN device (Contipro a.s., Czech Republic). The polymer was solved in a trichloromethane/ dimethylformamide/ trifluoroethylalcohol mixture as a 7.5% solution, electrospun with 40 kV, distance of 20 cm, and collected on a rotating drum. SEM images were taken using a Quanta FEG 250 (Thermo Fisher Scientific, USA). Three sets of SEM images of 4000x-magnification were taken at arbitrarily chosen locations on a sample, with 20 SEM images each, from the top side of nonwoven1 (NW1t), bottom side of nonwoven1 (NW1b), and top side of nonwoven2 (NW2t). The fiber diameter distributions were obtained using the self-developed General Image Fiber Tool (GIFT) [4]. In all histograms shown in this article the statistical bin width of one image pixel is presented. Each histogram of a single SEM image contains thousands of measurements which provide statistical certainty. Gaussian fits were performed on the histograms, on which the acceptability of curve fitting was rated by visual appraisal. Image processing and data handling were performed using ImageJ version 1.53a (National Institutes of Health, USA), and OriginPro version 2020 SR1 (OriginLab Corporation, USA).

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3 Results

Statistical datasets of 60 SEM images were generated, on which Gaussian fits were executed to obtain numerical results of the fiber diameter distribution. On some SEM images of fibers of multiple diameters Gaussian fit performed insufficiently (Figure 1).

Figure 1: Gaussian fit does not work sufficiently in every case, e.g. reliable curve fitting for one image of NW1t (top). Multiple distributed fiber diameters can remain unnoticed, as in the case of a curve fitting for another image of NW1t (bottom). The corresponding SEM images are displayed as insets.

Figure 2: The fiber diameter distribution in 20 single SEM images of NW1t (colors) is compared to the summation dataset (black).

Figure 3: The contributions of single fiber diameter distributions (colors) is overlaid to the summation of the dataset (black), $n = 20$. The average fiber diameter of the summation is obtained by Gaussian fit. As an inset, the mosaic display of the evaluated SEM images of NW1t is presented.

The individual SEM images revealed very different fiber diameter distributions. For clarity, they are visualized in Figure 2 and Figure 3.

The fiber diameter distributions of 20 images were summarized for three nonwoven samples each, and then Gaussian fit was performed on these summations (Figure 4).

Figure 4: Comparison of average fiber diameters on summations obtained by Gaussian fit for NW1t (top), NW1b (mid), and NW2t (bottom), $n = 20$ each.

The results of sufficient Gaussian fits (according to Figure 1 top) were averaged for the three nonwovens each. The mean values (arithmetic mean) as well as the standard deviations were averaged (Table 1) separately.

Table 1: Gaussian fit results of 20 summarized SEM images each, compared to the average results obtained from sufficient Gaussian fits of single SEM images

Parameter	NW1t	NW1b	NW2t
Average fiber diameter, summation of 20 SEM images	$1.39 \pm 0.25 \mu\text{m}$	$1.31 \pm 0.38 \mu\text{m}$	$1.34 \pm 0.45 \mu\text{m}$
Number of sufficient Gaussian fits	14	13	13
Average fiber diameter of sufficient Gaussian fits	$1.40 \pm 0.25 \mu\text{m}$	$1.32 \pm 0.46 \mu\text{m}$	$1.30 \pm 0.44 \mu\text{m}$
Variation of sufficient Gaussian fits, average mean value, and average standard deviation respectively	mean value $1.400 \pm 0.091 \mu\text{m}$ standard deviation $0.25 \pm 0.13 \mu\text{m}$	mean value $1.32 \pm 0.19 \mu\text{m}$ standard deviation $0.46 \pm 0.17 \mu\text{m}$	mean value $1.302 \pm 0.092 \mu\text{m}$ standard deviation $0.44 \pm 0.15 \mu\text{m}$

4 Discussion

In this study, fiber diameter datasets of 60 SEM images of three nonwoven samples ($n = 20$ each) were statistically evaluated using Gaussian fits on histograms. Each histogram of a single SEM image contains thousands of measurement points which provide statistical certainty. In the case of inhomogeneous diameter distribution, a Gaussian fit may generate insufficient results (Figure 1), which have to be excluded from final result. A comparison of different SEM images of the same nonwoven reveals remarkable differences between the individual SEM images (Figure 2), e.g. images 1 and 2 of NW1t. Hence incorrect conclusions could be drawn from numerical results, if only singular SEM images are considered for stating results. Combining several datasets increases statistical certainty. The summation of only two datasets (e.g. images 1 and 2 of

NW1t) can be sufficient for results close to that of much higher numbers of summarized datasets (Figure 5).

Figure 5: Distinct numbers of fiber diameter distribution datasets of SEM images of NW1t are summed up, and the results of Gaussian fits are compared.

Data from sufficient Gaussian fit curves of NW1t revealed a mean value of $1.400 \pm 0.091 \mu\text{m}$. The mean values of NW1t and NW2t are of low statistical spread, whereas the mean values of NW1b have a wider distribution. The distribution of the standard deviations was found in a range 0.13 to 0.15 μm , i.e. the fitted curves vary in a similar range for the individual nonwoven samples each. However, although the results of individual images differ from each other, their average is close to the results of summarized datasets of several SEM images, e.g. NW1t (Figure 5).

Based on this example, it can be stated that at least two or three SEM images of different locations are necessary to obtain reliable numerical results. But fibers are imaged very differently, depending on magnification, fiber density, and overlap. In the SEM images shown here, fibers are densely packed in several layers. The area of one SEM image is $31.8 \mu\text{m} \times 27.4 \mu\text{m} = 871 \mu\text{m}^2$. A square of the area of one SEM image has an edge length of 30 μm , or 42 μm for two images, or 51 μm for an area of three images respectively, so the fiber diameter ranges from 2.7% to 4.7% of those edge lengths. For reliable results of densely packed fibers it seems reasonable that the fiber diameter should be roughly 2% to 3% of the edge length of the evaluated square equivalent image area, which means roughly three to six SEM images of e.g. 4000x magnification. For less densely

imaged fibers a larger image area is needed to evaluate enough data. This must not be misunderstood by evaluating less magnified SEM images. Insufficiently magnified SEM images lead to deficient results due to a less detailed dataset (Figure 6).

Figure 6: The comparison of differently magnified SEM images reveals loss of measuring accuracy with increasing imaged area, despite an increasing quantity of measurements. Datasets of different magnifications, Gaussian fits, and insets of the corresponding SEM images of the same nonwoven are displayed.

Another interesting aspect is revealed. Despite a large quantity of measurements, peaks in fiber diameter distribution appear that do not average out, for example, the remarkable peak at 0.7 μm for NW2t (Figure 4). This peak seems to be an actual accumulation, which would be very difficult to be discovered by manual image evaluation.

5 Summary and Outlook

Fiber diameter statistics are often affected by the randomness inherent in arbitrarily chosen SEM image datasets. A high number of measurements, which can be obtained by automated image evaluation tools, increase the

statistical certainty, but also the processing effort and time required. In this work we determined that to obtain reliable results, a minimum surface area has to be evaluated considering thousands of measurement points. The example images, shown in this work, provided about 6000 - 10000 measurement points per image, including fiber data and statistical noise. The fiber diameter should be in a range of 2 - 3% of the edge length of the square equivalent of the evaluated image area, using sufficiently magnified SEM images. In other words, three to six SEM images of a commonly used magnification (e.g. 4000x) provided a reasonable tradeoff between processing time and accuracy. In further studies SEM images of fibers in single layers or a distinct alignment can be brought into focus.

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