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Note: Non-invasive optical method for rapid determination of alignment degree of oriented nanofibrous layers

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This paper presents a rapid non-destructive method that provides information on the anisotropic internal structure of nanofibrous layers. A laser beam of a wavelength of 632.8 nm is directed at and passes through a nanofibrous layer prepared by electrostatic spinning. Information about the structural arrangement of nanofibers in the layer is directly visible in the form of a diffraction image formed on a projection screen or obtained from measured intensities of the laser beam passing through the sample which are determined by the dependency of the angle of the main direction of polarization of the laser beam on the axis of alignment of nanofibers in the sample. Both optical methods were verified on Polyvinyl alcohol (PVA) nanofibrous layers (fiber diameter of 470 nm) with random, single-axis aligned and crossed structures. The obtained results match the results of commonly used methods which apply the analysis of electron microscope images. The presented simple method not only allows samples to be analysed much more rapidly and without damaging them but it also makes possible the analysis of much larger areas, up to several square millimetres, at the same time. © 2015 AIP Publishing LLC. [http://dx.doi.org/10.1063/1.4935021]

Possibly all methods used to evaluate the morphological properties of nanofibers prepared by electrostatic spinning (ES) are based on the analysis of scanning electron microscope (SEM) images. Using these images, structural shapes and defects are observed, fiber diameters are measured, their directional preferences are determined and, potentially, interfibrous gaps (porosity) are measured. The measurement and evaluation themselves are conducted either automatically by a specially programmed software, e.g., FibraQuant™ (www.nanoscaffoldtech.com) or Electrospinz SEM Analyser (www.electrospinz.co.nz), partially automatically using image analysis software with advanced functions, such as ImageJ (imagej.nih.gov/ij, the method is described in detail, e.g., in Ref. 1), or manually, where the operator has to mark each measured property on the analysed SEM image in sufficient iterations (i.e., at least 50 times). The more accurate, automatic analysis for the characterisation of electrospun fiber alignment applies unique mathematical operations programmed in Matlab.[2] The applied image operations and functions transform a SEM image from the real domain to the frequency domain using the Fast Fourier transform (FFT). Considering that nanofibers are aligned along a single axis, parallel next to one another, creating a periodic spatial structure, a passing optical wave will create its Fourier image on the screen behind it.[3] We assume this Optical Fourier transform (OFT) can be realized by the passing beam directly on the nanofibrous layer, removing the necessity of first taking a SEM image for the Fourier transform. Should this assumption be proven correct, the method would facilitate and speed-up the whole process of nanofiber alignment analysis. We do not expect stronger interactions of radiation with the molecules of polymer nanofibers to occur or the generation of nonlinear optical phenomena,

which are utilized, for example, here.[4] The very principle of electrostatic spinning causes nanofibers to be deposited randomly[5] and in order to achieve the resulting anisotropy, special collectors need to be used.[6] Nanofibrous materials with regular structures prepared by electrostatic spinning may be used in optical and electronic applications,[7] tissue engineering, and regenerative medicine.[8] A rapid non-invasive method for the initial estimation of the degree of nanofiber alignment in materials during their production is therefore needed.

Two different methods for the determination of the degree of alignment, which utilize optical light-matter interactions with almost perfectly aligned, crossed, and randomly oriented polymer nanofiber structures are presented in this work. The first one is based on the attenuation of linear polarized light by oriented nanofibrous layers. The attenuation is highly dependent on the angle between the direction of linear light polarization and the main nanofiber alignment axis and degree of alignment in the oriented structure. The other method uses the principles of Fourier optics and an analysis of CCD captured diffraction patterns. The obtained results are compared with the usual anisotropic structural properties obtained by SEM image analysis.

Three differently structured samples were prepared using the laboratory device 4SPIN® LAB[9] (Contipro Biotech). A polymer solution of 16% Polyvinyl alcohol (PVA) (Sigma Aldrich) dissolved in H_2O was dosed with a needle (g17, Hamilton) at a rate of 12 μl/min for 10 min and at a high voltage of 15 kV. The distance of the spinning needle from the collector was set to 18 cm. Nanofibers were deposited on collectors C1 (plate), C2 (drum), and C3 (patterned) in order to achieve random, single-axis, and crossed alignments, respectively. The C2 rotating collector of 120 mm in diameter was
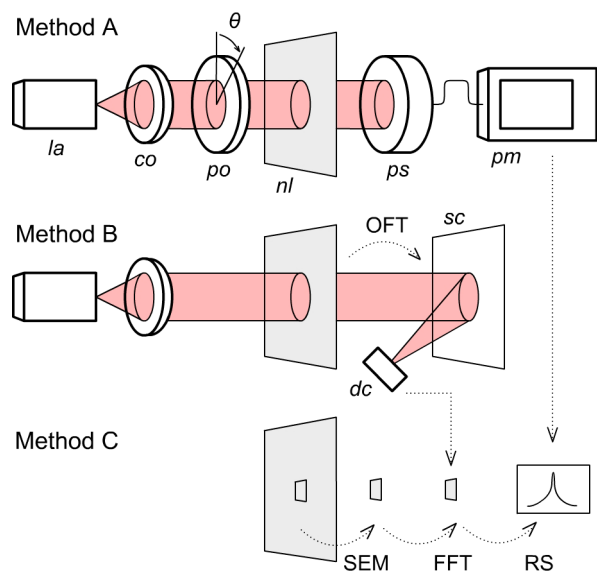
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Method A

Method B

Method C

FIG. 1. A schematic representation of the compared methods. Abbreviations: la—laser, co—collimator, po—polarizer, nl—nanofibrous layer, ps—photodiode sensor, pm—power meter, sc—screen, dc—digital camera, RS—radial summation of pixel intensities.
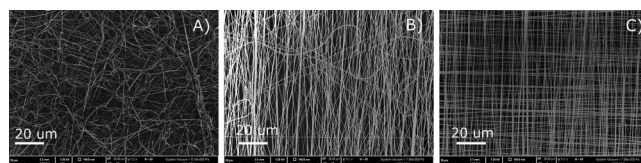

FIG. 2. Scanning electron microscope images of nanofibrous layers with (a) random, (b) single-axis, and (c) crossed alignment; these were deposited on the C1, C2, and C3 collectors, respectively.

usual anisotropic structural properties obtained by analysing SEM images of the samples were compared with the results obtained by method B.

Nanofibrous samples with isotropic and anisotropic properties were prepared for optical measurements; SEM images of these samples are shown in Figure 2. The diameters of these fibers ranged from 336 to 578 nm.

The results of method A for linear dichroism dependency between the angle of sample rotation and normalized transmission of polarized light are shown in Figure 3. As expected, the highest levels of orientation were obtained from the aligned and crossed structure samples.

In the case of the crossed structure, a slightly lower level of orientation in a 180° area, compared to the aligned structure, was caused by the perpendicularly aligned layers. Figures 4(a)-4(c) show a set of diffraction patterns of the same samples obtained with method B. Figures 4(d)-4(f) show the results of the FFT of the SEM images shown in Figure 2, which were obtained with method C. When passing through the sample with random structure, the laser beam was diffused and its projection on the screen was hardly visible (see Fig. 4(a)).

The other projections corresponded to the results of the FFT of parallel and perpendicular structure SEM images. For the purpose of a thorough comparison of the optical method B and the standard method C, the dependencies obtained with both these methods are shown in the graphs in Figure 5. The graphs show radial summations (RSs) of pixel intensities of all images from Figure 4 having a regular structure (i.e., Figs. 4(b) and 4(c) and Figs. 4(e) and 4(f)) processed according to the procedure reported in the paper.[1] All data were normalized. The graphs demonstrate a good match between the final results obtained with methods B and C.

set to rotate at 5000 rpm. The air gap between the conductive wires of the C3 collector was 20 mm.[10] The collection was carried out using a sliding tool which rotated by 90° after every movement.[11]

Optical measurement was performed with a single-axis optical rail system: a randomly polarized light of a HeNe laser (632.8 nm, Thorlabs) was transferred by a single-mode optical fiber (SM600, Thorlabs) and collimated by a triplet collimator (TC12FC-633, Thorlabs), see Figure 1. For transmitted light intensity detection, a linear polarizer was also added—the optical beam after passing through this part was collimated, had almost Gaussian intensity profile, and was linearly polarized. The diameter of the collimated laser beam was approximately 3 mm. The polarizer was attached to a rotatable mount to allow the changing of the incidence angle between the polarization preference of the incident light and the main axis of the aligned nanofibers. Typical output power on the sample setup was several milliwatts. *Method A*: The intensity of the light scattered perpendicularly to the sample surface as a function of the incidence angle θ (by rotating the polarizer in its plane) was simultaneously measured by a power meter (PM100D with the S120C sensor, Thorlabs). Optical power values of the passing beam were measured in 10° steps within the 360° polarizer rotation interval. The final optical intensity value was obtained by averaging ten values for each set degree. The main output of this approach is, as linear dichroism predicts, represented by the difference between the parallel T_1 and the perpendicular T_2 transmissions ($\Delta T = T_1 - T_2$). The greater the degree of alignment of an oriented structure, the higher the observed difference between these absorptions. *Method B*: This method used the same optical system (without the polarizer) and the principles of Fourier optics to capture, using a camera, diffraction patterns projected by the structured samples on a projection screen. The patterns were analysed in the ImageJ software by the same procedure as is used for SEM images converted by FFT.[1] *Method C*: The
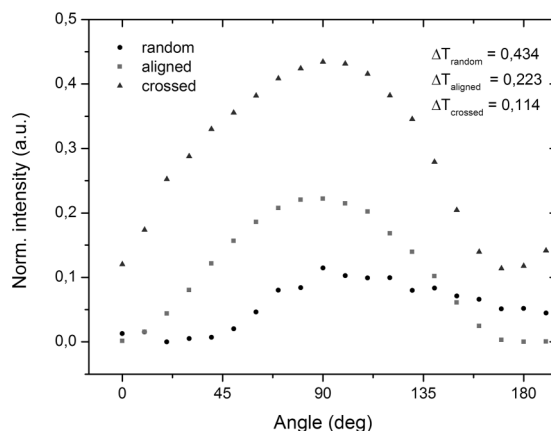

FIG. 3. The dependency of detected optical intensity on the angle of rotation of the plane of polarisation of the passing laser beam for random, single-axis aligned, and crossed structures.
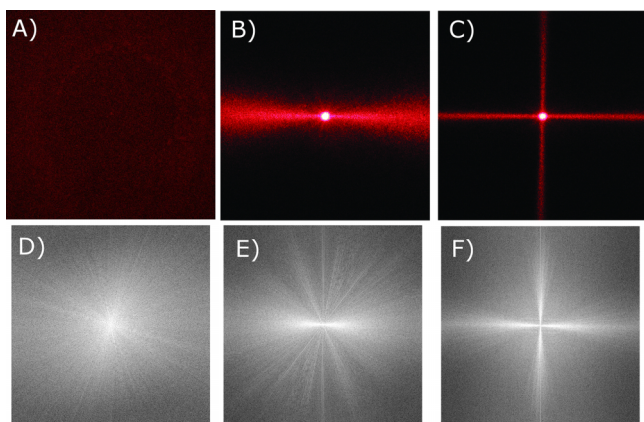
FIG. 4. The images show patterns projected on the screen by polarized laser beam passing through a sample with (a) random, (b) single-axis aligned, and (c) crossed structures. Results of the FFT analysis ((d)–(f)) of SEM images of the same structures obtained with method C.

The obtained results prove that the time-consuming and technically demanding (costly) analysis using an electron microscope can be substituted with a cheap optical method. One of the advantages of optical methods is that they allow rapid acquisition of information about the anisotropic
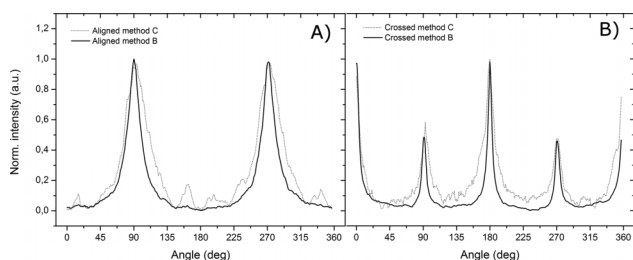

FIG. 5. Comparison of the sum intensities dependent on the angle of rotation of a notional line in the images obtained with methods B and C. (a) Comparison of results obtained from the single-axis aligned structure (corresponds to images in Figs. 4(b) and 4(e)). (b) Comparison of results obtained from the crossed structure (corresponds to Figs. 4(c) and 4(f)).

character of a sample—obtained, moreover, from a relatively large area (7 mm^2 in our case). Their disadvantage is that methods based on the transmission of light exhibit high dependency on the thickness of the analysed sample. However, method C is destructive and also allows only the surface of the sample (i.e., upper nanofibrous layers) to be analysed and only in a small area (in our case 0.03 mm^2 for one image).

A comparison of the three different approaches confirmed the possibility of a simple way of estimating the degree of alignment of oriented nanofibrous layers. A simple, non-invasive optical method allows to immediately determine whether a nanofibrous sample has some anisotropic properties, which would be otherwise impossible without analysing its SEM images. It also allows a larger area of the sample to be analysed much faster. And if a collector with an air gap (type C3 patterned) is used, the method can be applied without manipulating with the nanofibrous layer in any way as well as directly in the production process.

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