Laser Scanning Confocal Microscopy Study of Dye Diffusion in Fibers

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ABSTRACT: The diffusion of fluorescein into nylon-66 fibers has been studied for the first time by laser scanning confocal microscopy (LSCM). LSCM makes it possible to noninvasively obtain high-resolution three-dimensional images of the spatial distribution of dyes (fluorescein) in fibers dyed for various length of times. Integration over the dye distribution yields the total amount of dye in the fiber, which is found to be in close agreement with that determined by UV−vis spectrophotometry after dissolving the fibers. Thus, the diffusion coefficients determined noninvasively by LSCM ($(6.9 \pm 1.0) \times 10^{-11}$ cm^2/s) and the destructive traditional means ($(7.8 \pm 1.9) \times 10^{-11}$ cm^2/s) also agree. The LSCM method has several significant advantages. Among these are its speed, nondestructive nature, and the ability not only to determine the total dye content of the fiber but also to image the dye distribution profile across the fiber diameter. This latter ability is demonstrated to be important to understanding the visual appearance of dyed fibers and fabrics. Two fibers, one ring-dyed and one uniformly dyed, each with the same over all dye content, show remarkably different shades of color. The ring-dyed fiber is lighter, an observation confirmed by the reflectivities measured for each fiber, which were in the ratio ring-dyed/uniformly dyed = 2/1. LSCM observation of dyed fibers provides us not only with a means to measure the dye diffusion coefficient in the fiber, but also the time-dependent, three-dimensional distribution of dye molecules.

Introduction

The appearance of certain kinds of defects in fabrics has been a problem for many years, in particular, the appearance of streaks. Streakiness results from a small absolute variation of lightness and is an objectionable feature for human observers. The amazing fact, however, is that the variation in lightness that causes streaks is hardly measurable by instruments. McGregor et al.,[1] observed that extremely low variations in luminance of randomly varying parallel stripes (an oversimplified model for a fabric) can cause objectionable streaky appearance (Figure 1). Such low variations in reality could be caused by a number of factors which include, variations of the dye concentration in the fiber, spatial distribution of the dye in the fiber, shape of the fiber cross-section, the diameter of the fiber, spectral shifts of the dye due to the physical environment it finds itself in, and factors related to the human visual system.

Studies of dye diffusion have been carried out in the past with the hope that some understanding can be gained about the appearance of defects. However, many of the methods are destructive techniques and are ill-suited for obtaining the spatial distribution of the dye molecules in a fiber. In a typical experiment, the fiber bundle is dyed under appropriate conditions and the amount of dye uptake as a function of time is measured. This can be easily accomplished by dissolving the fibers removed at regular time intervals, combined with the use of UV−visible absorption measurements of dye concentration. This process is rather tedious and time-consuming. Measurement of the concentration profile of the dye in the fiber using microspectrophotometry provides an alternative approach. However, there are two problems associated with this method: the difficulty of identifying the edge of the fiber and the possibility of dissolution of dye in the embedding materials. Both problems will introduce considerable error in the concentration profile and hence in the diffusion coefficient. Furthermore, these destructive techniques cannot be

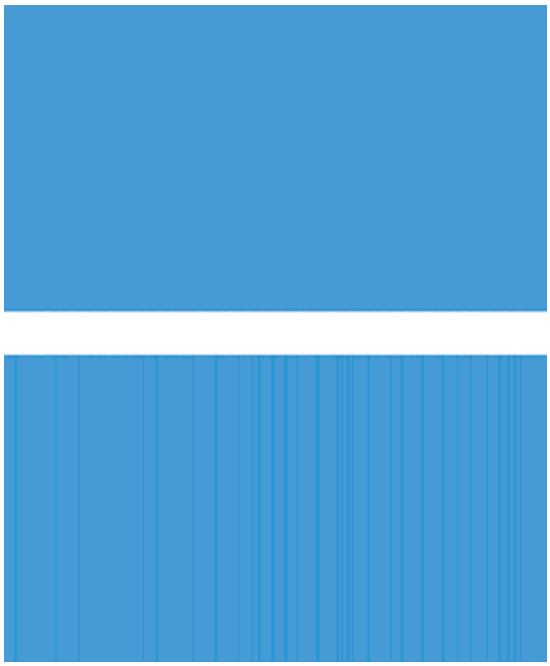

Figure 1. Demonstration of streakiness in textile fabrics. Top: reflectance variation is 0.7%. Bottom: reflectance variation is 2.1%. The appearance of streaks is due to a small variation in the reflectance, which can be affected by a variety of factors.

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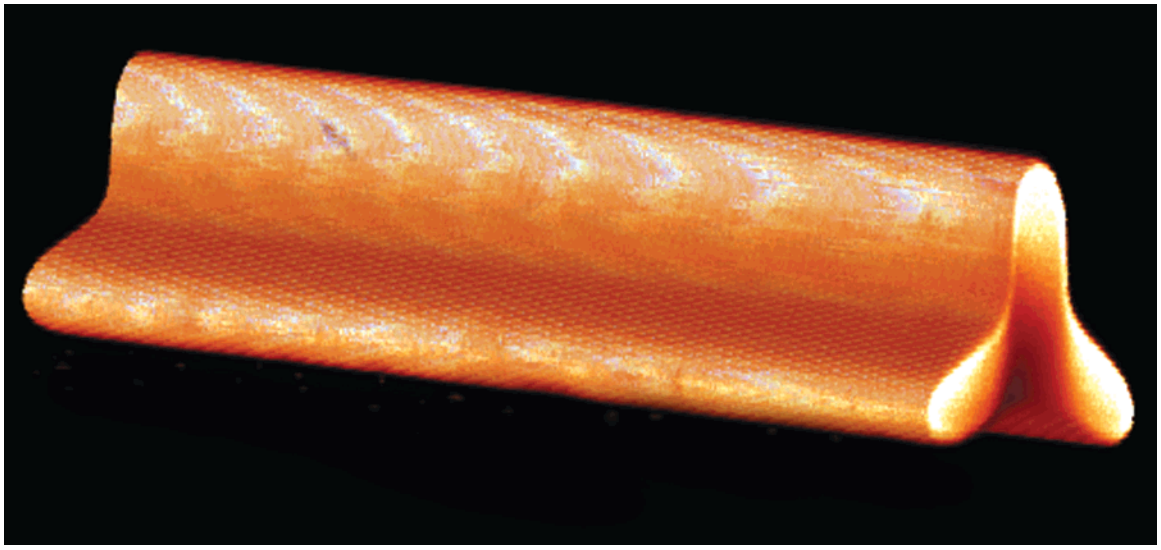

Figure 2. 3-D reconstruction of a trilobal nylon fiber dyed with fluorescein, a fluorescent dye molecule. 95*xy* sections were imaged for the 3-D reconstruction.

used in situations where fibers are under tension or during the process of forming internal or external cracks in the fiber. In conventional fiber dyeing measurements, information concerning the orientation of the dye molecules, the spectral characteristics of dye molecules, or the spatial distribution of the dye in the fiber cannot be obtained.

The appearance of a fiber depends primarily on how light interacts with the fiber. This interaction determines the amount of light that is reflected by the fiber, which in turn affects the appearance of the fiber. The shape of the fiber cross section can have a major impact on the appearance properties of the carpet or fabric made from the yarn,[2,3] and nonround fibers are frequently used because of their effect on the appearance of the final product. Therefore, it is important to have a three-dimensional image of the fiber for computations of reflectivity. The reflectivity of a fiber with a given shape can then be easily computed based on geometrical optics, primarily by the use of ray-tracing methods. In the past, it has been quite difficult to obtain a good three-dimensional image of the fiber for modeling purposes when fibers have a shape other than an infinite cylinder. Such three-dimensional images can easily be obtained using a laser scanning confocal microscope (LSCM). A three-dimensional image of a trilobal nylon fiber is shown in Figure 2, obtained using an LSCM. To obtain this image, 95 *xy* sections at different *z* positions were accumulated for the reconstruction of the three-dimensional image. Since the image was acquired using the LSCM in the fluorescence mode, the fiber was dyed with fluorescein, a commonly used and widely studied fluorescent dye.

Despite the extensive use of LSCM in the biological sciences, its use in materials science is not nearly as extensive. LSCM has been used to image colloidal crystals in 3D[4−6] and to study the morphology of polymers,[7−9] and phase separation of polymer blends.[10−14] The essential advantage of an LSCM is its ability to discriminate against unwanted out-of-focus light that limits a normal light microscope from obtaining a three-dimensional image of a transparent object.[15−17] This is accomplished by scanning a tightly focused light source in a single plane that is thin compared to the sample thickness. The ability to discriminate against out-of-focus light is due to the fact that the detection system is confocal to the illumination, thereby only a single location in the specimen is imaged at any given time. Since the confocal aperture admits light only from the focal plane of interest, a very thin slice ($x-y$ projection) can be obtained simply by scanning the beam in the $x-y$ plane. Since each point in a focal (image) plane is examined individually, the in-plane or spatial resolution is enhanced, with achievement of a ∼200 nm resolution being typical, while the axial (z) resolution is around 500 nm. The z-resolution is dependent on a number of parameters that include the size of the confocal aperture and the numerical aperture of the objective used for imaging.[15,16] Since the images of the 2D ($x-y$ image) sections are obtained in digital form, several successive optical sections can be used to construct a 3D image of the object.

Study of diffusion processes using an LSCM is still in its infancy. However, there have been a few studies devoted to the diffusion of tracer molecules[18−23] using an LSCM, primarily using fluorescence recovery after photobleaching type experiments to determine the mobility. Application of LSCM studies to fibers is almost nonexistent with the exception of a conference proceeding[24] where polyester microfibers were imaged using a confocal microscope. The observations reported were mainly qualitative in nature. Here, we report on the quantitative application of laser scanning confocal microscopy to the study of diffusion of tracer molecules into a solid (polymer) fiber. The results demonstrate that the spatial distribution of the dye is more important than the total amount of dye in the fiber with regard to the appearance of color and yield in the fiber. We are not only able to measure the diffusion coefficient of the dye but also able to discern the orientation of the dye molecules[25] and, of course, their spatial distribution, *which prove to be very important in the perception of color*.

Experimental Section

Nylon 66 fibers were dyed with fluorescein (0.1 g/L, pH 6) in an infinite dyebath at 95 °C. An infinite dyebath refers to a dyebath where the concentration of the dye does not change due to the process of dyeing. The pH was maintained using a

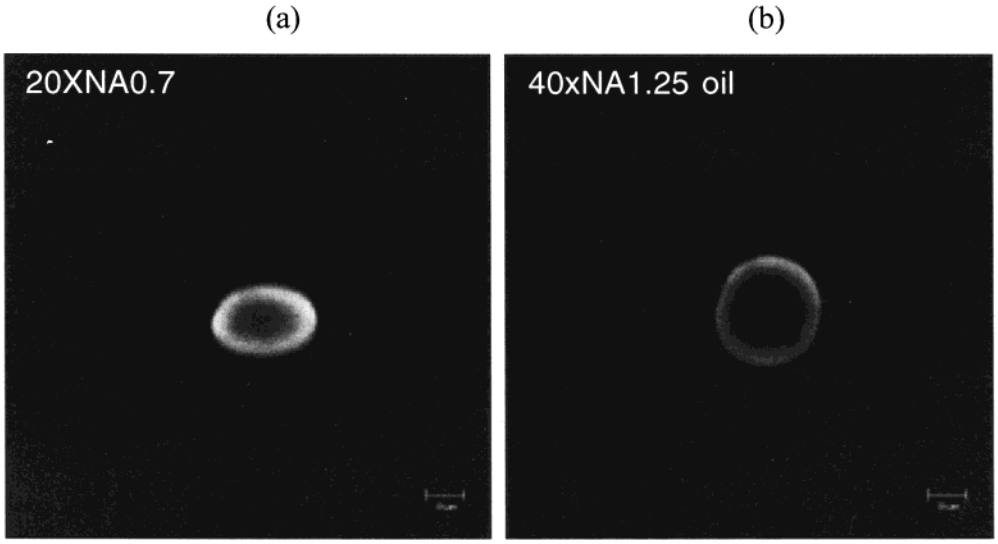

Figure 3. Cross section images of nylon 66 fibers obtained using a 20× NA0.7 objective (left) and a 40× NA1.25 oil objective (right). The perpendicular refractive index of nylon 66 fiber is the same as that of the mounting medium, 1.52, which is identical to that of the immersion oil used for the 40× objective. The scale bar represents 10 μm.

phosphate buffer (0.1 M). Nylon 66 fibers were obtained from Monsanto Chemical Corp. The fibers were subjected to an autoclave treatment to provide dimensional stability during the dyeing process. The average diameter of the fiber was 30 μm with a variation of about 1 μm.

The infinite dyebath dyeing of the fibers was carried out in an Ahiba Texomat GVIIB dyeing system. The dyeing system has 12, 400-mL dyeing beakers, with an Ahiba 1000 control unit that controls the temperature of the dyebath, agitation speed, and heating/cooling rates. The dyebath exhaustion was less than 2% and hence can be considered to be negligible. The concentration of fluorescein in the fiber was determined by dissolving the fibers in formic acid and measuring the absorbance of the formic acid solutions using a UV−visible spectrophotometer. Fluorescein is a commonly used dye with its maximum excitation at 490 nm.

The fibers were imaged using a Leica DMRBE LSCM which was equipped with three laser systems: an Ar ion (488 and 514 nm), Kr ion (568 nm), and a He−Ne (632.8 nm). For the purposes of imaging, the 488 nm line of the Ar-ion laser was used in conjunction with a 40× NA 1.25 oil immersion objective. Data provided by Leica indicate that the axial and lateral resolutions are 0.37 and 0.16 μm, respectively, although we have not independently measured the resolution. For the present work resolution is not as critical since the features that are being imaged are quite large. The images were quantified and analyzed into fluorescent intensity profiles by using Leica TCS NT software. Several fibers (~8) were imaged to ascertain that the data are reproducible, and we report typical results in this paper. Measurements were also made at several positions along the length of a given fiber to make sure that we obtained a good representation of concentration profiles.

The microscope can be used to image in various scanning modes which include a planar section (*xy* scan), a vertical section (*xz* scan), and time-dependent imaging modes. For much of the work reported in this study the images were obtained using the *xz*-scan, thus providing an optical cross section of the object under study. Each *xz*-scan data set shown in this study represents an average of four scans and sometimes the averaging was done over eight scans. However, it was found that such averaging was not essential. From the optical cross sections the intensity profile of the fluorophore was converted to a concentration profile of the dye molecules used in the dye diffusion experiments.

To minimize the aberrations that degrade the image, the refractive index of the object needs to be matched closely with that of the immersion oil for the objective and the mounting medium for the specimen. Since we used nylon fibers which are anisotropic in nature, the refractive index of the mounting medium was matched to the ordinary refractive index of the fiber (the refractive index perpendicular to the axis of the fiber). The effect of abberations degrading the image of polymeric fibers will be discussed in detail elsewhere.[26] However, Figure 3 illustrates the outcome of poor refractive index matching, where one of the images appear to be squashed (the image on the left). This occurs because the imaging was done with the fiber in air, thus leading to spherical abberations which distorts the true shape of the cylindrical object (the fiber that is being imaged). Refractive index matching is essential for obtaining good quality images while it also prevents extraneous effects such as light guiding by the fiber.

Another source that usually degrades the image in a LSCM is fluorescence saturation. In all the measurements that are reported here, we have made the implicit assumption that fluorescence is a linear process; that is, the emitted fluorescence intensity is linearly dependent on the illumination intensity. It has been shown that it is quite easy to saturate a fluorophore in a confocal microscope simply because of the way the imaging is done.[27] Since a laser beam is focused to a diffraction limited spot for imaging, 1 mW of radiant power is enough to saturate the fluorophore, this being particularly true for a high NA objective. Much of the imaging done in this study uses 0.04 mW of radiant energy for the laser light source as measured with a laser power meter at the sample plane prior to scanning.

Measurements of the spectral reflectance of the individual fibers were done with the use of a Zeiss microspectrophotometer. The spot size for measurement was 10 μm.

Results and Discussion

The process of fiber dyeing can be considered to occur in three stages: the diffusion of dye molecules from the solution to the surface of a fiber and the adsorption of the dye molecules on the surface, which is followed by the diffusion of the dye to the fiber center. Of the three stages, diffusion of dye molecules inside the fiber is the rate-determining step, therefore making the study of dye diffusion in fibers of fundamental importance and the determination of dye diffusion coefficients essential. One of the most common methods to study dye diffusion is to perform an infinite dyebath dyeing in a very well stirred dyebath, in which the dye concentration is a

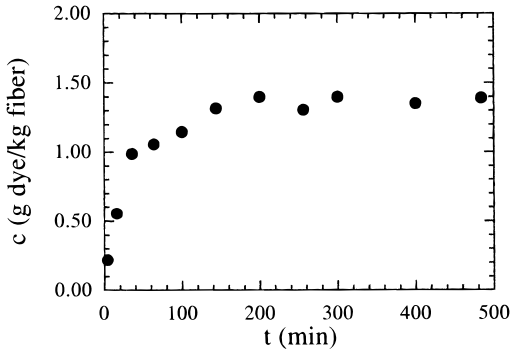

Figure 4. Plot of dye concentration in the fiber as a function of the time of dyeing. The nylon 66 fiber was dyed with fluorescein in an infinite dyebath at pH 6, 95 °C, and the dye concentration in the dyebath was 0.1 g/L.

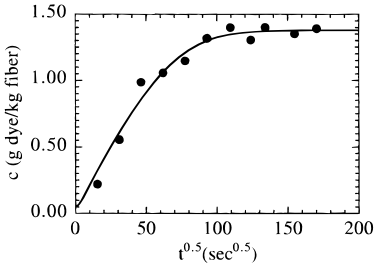

Figure 5. Direct fit using the truncated version of eq 2 for the data shown in Figure 4, limiting the truncation to 10 terms. The solid line is the fit, and the filled circles are the data points.

constant. By determination of the amount of dye uptake at given intervals of time, the diffusion coefficient can be calculated from Hill's solution to Fick's second law of diffusion and can be written as[28]

$$\frac{C_t}{C_\infty} = 1 - Ae^{-BK} - Ee^{-FK} - Ge^{-HK}... \qquad (1)$$

where C_t is the dye concentration at time t, C_∞ is the dye concentration at the equilibrium state, A, B, E, etc., are known numerical constants, and $K = Dt/r^2$, with D as the diffusion coefficient and r as the radius of the fiber. The diffusion coefficient D can be calculated from Hill's solution if the ratio of C_t/C_∞ is obtained. Traditionally C_t and C_∞ are determined individually from the absorbance of the solutions of dissolved fibers removed from the dyebath at various times t and ∞.

Hill's solution to the diffusion equation is valid under the following assumptions: diffusion from the ends of the cylinder is negligible, concentration of the diffusing species is constant during the entire dyeing process, and the adsorption of the dye onto the fiber surface is instantaneous and remains a constant during the diffusion process. Since the fibers were long (several meters in length), diffusion from the ends of a 30 μm fiber can be neglected. Diffusion measurements were carried out under the conditions of an infinite dyebath (with less than 2% change in the concentration of the dyebath), thus validating the second assumption used in Hill's solution of Fick's second law. It was, however, found that the surface concentration did not reach the value of the dyebath instantaneously.[29] However, the difference between the concentration of dye at the surface of the fiber and that of the dyebath is not substantial enough to affect the results reported here. Consequently, most of the assumptions that go into Hill's solution are valid for our study.

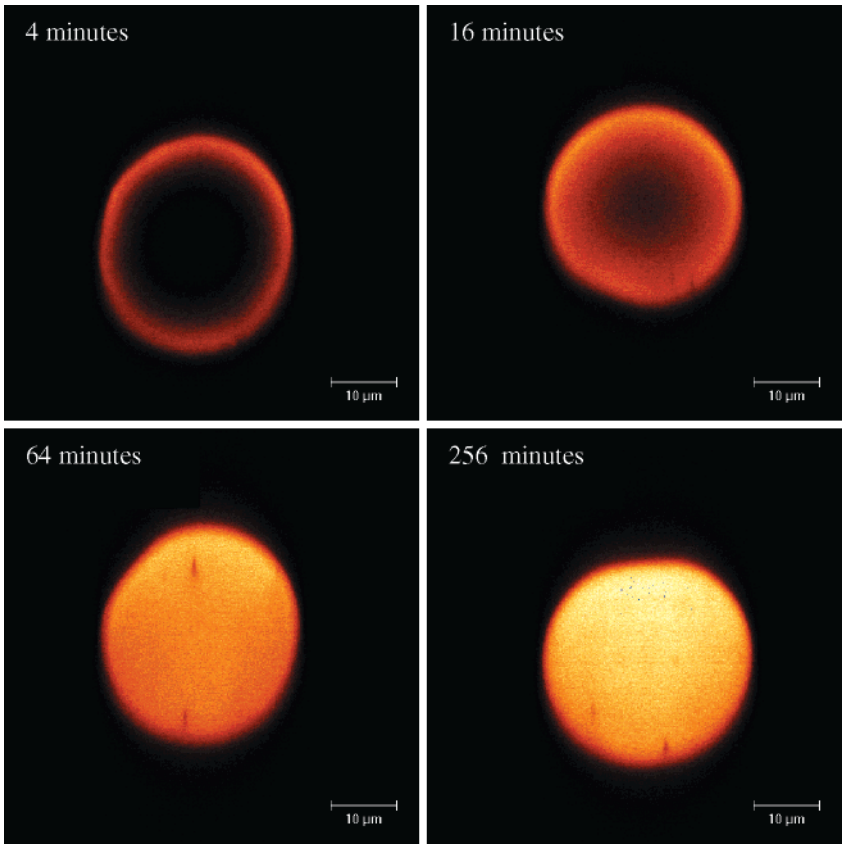

Figure 6. Images of fiber cross sections. The average diameter of the fiber is 30 μm. Nylon 66 fiber was dyed with fluorescein in an infinite dyebath for 4, 16, 64, and 256 min. The dyebath was pH 6, 95 °C, and the dye concentration was 0.1 g/L.

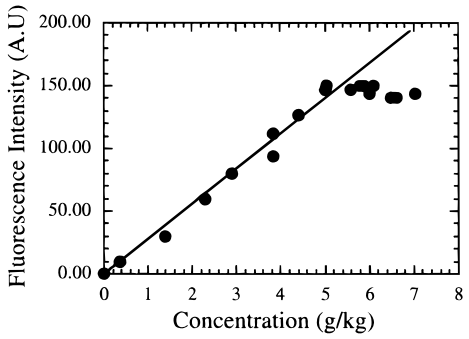

Figure 7. Plot of fluorescence intensity (arbitrary unit) as a function of fluorescein concentration in the fiber (g/kg). Nylon 66 fiber was dyed with fluorescein. Fluorescence intensity is linearly proportional to the fluorescein concentration in the fiber when the concentration is lower than 5 g/kg.

In the fiber-dyeing process, the amount of dye molecules transferred from the dyebath solution into the fiber is a function of dyeing time. As shown in Figure 4, the dye concentration in the fiber increased quickly at the beginning of dyeing and gradually reached equilibrium. The concentrations of fluorescein in the fibers were determined by measuring the absorbencies of the dissolved fibers in formic acid using an UV−vis spectrophotometer. After the fiber was dyed for about 200 min, the dye concentration in the fiber became saturated and reached equilibrium dyeing. Therefore, C_∞ can be obtained, and the diffusion coefficient can be calculated from Hill's solution of the diffusion equation. Knowing the ratio C_t/C_∞ from experiments, the value of K ($=Dt/r^2$) can be read from tables or a plot of C_t/C_∞ vs K for various K ranges for each individual reduced concentration.[28] Such a measurement provides an average diffusion coefficient of $(7.8 \pm 1.9) \times 10^{-11}$ cm^2/s. The seemingly large variation (~24%) comes from the fact that a number of fibers are weighed to determine the dye uptake in the fiber in the diffusion experiments. It is also conceivable that all the fibers used for obtaining the concentration may not have identical diameters which will lead to inaccuracies in the diffusion coefficient.

Hill's solution to Fick's second law is based on the following equation from Crank[30]

$$\frac{C_t}{C_\infty} = 1 - \sum_{n=1}^{\infty} \frac{4}{r^2 \alpha_n^{\,2}} \exp(-Dt\alpha_n^{\,2}) \qquad (2)$$

where the α_n's are defined through

$$J_o(r\alpha_n) = 0 \qquad (3)$$

J_o is the zero-order Bessel function of the first kind, and $r\alpha_n$ are the roots of J_o. An alternative method for extracting a diffusion coefficient from C_t vs t data (as in Figure 4) involves a direct fit of a truncated version of eq 2 to the data, from which D and C_∞ are obtained. Such a fit to a 10-term version of eq 2 is displayed in Figure 5, and yields $D = (11.7 \pm 1.8) \times 10^{-11}$ cm^2/s and $C_\infty = 1.36$ g dye/kg fiber. This D value compares well with the average value obtained from Hill's solution. The uncertainty in the D value is typical for a sparse

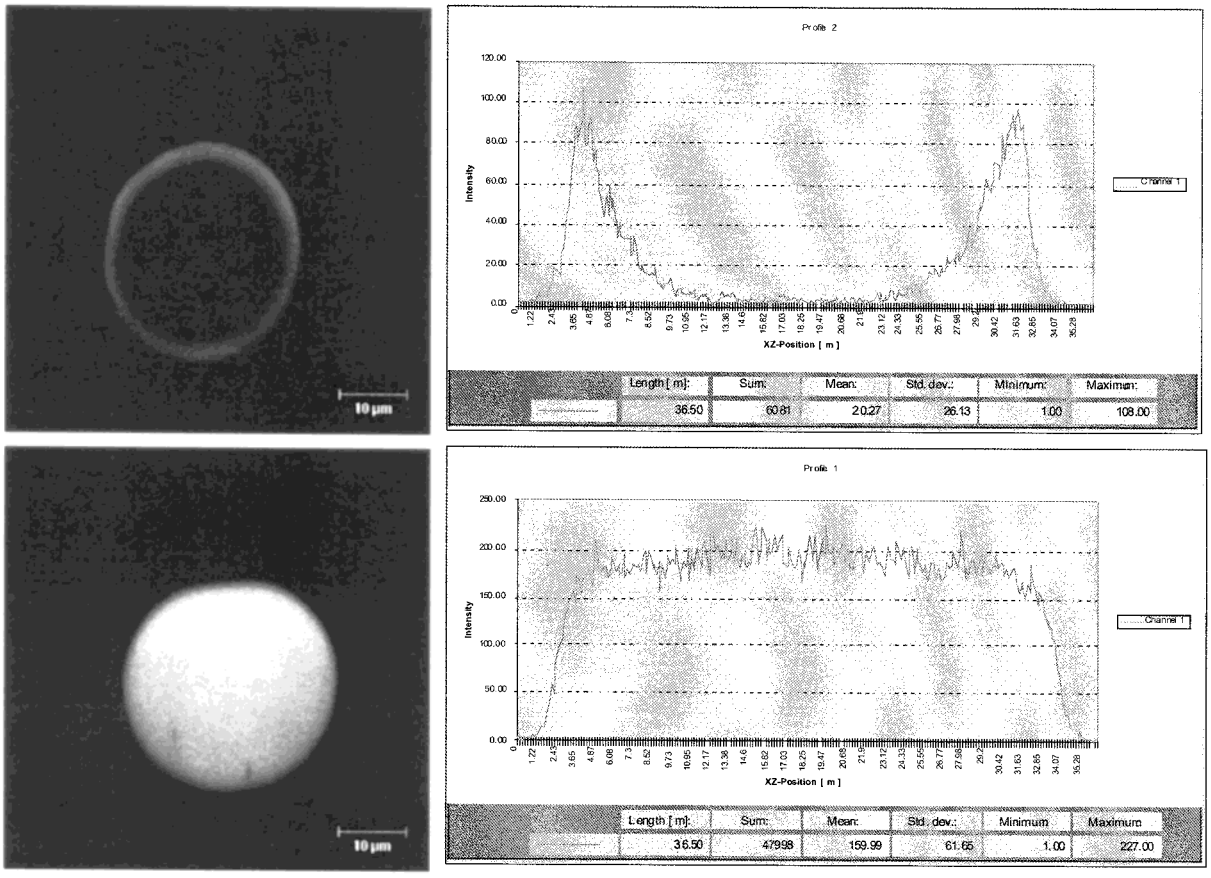

Figure 8. Top: image of fiber cross section and the fluorescence intensity across the fiber diameter when fiber dyed for 4 min. Bottom: image of fiber cross section and the fluorescence intensity across the fiber diameter when fiber dyed for 256 min. Nylon 66 fiber was dyed in an infinite dyebath at pH 6 and 95 °C. The dyebath concentration was 0.1 g/L.

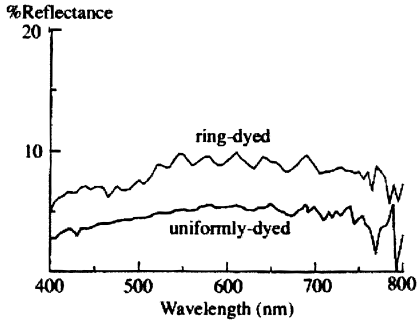

Figure 9. Measurement of reflectance from nylon 66 single fibers dyed with fluorescein shows that the ring-dyed fiber has higher reflectance than the uniformly dyed fiber with the same concentration in the fiber.

and scattered data set such as this. The direct fit of C_t vs t data to eq 2 probably yields a more reliable value of D than the point-by-point method, since all the data is regressed simultaneously, and errors associated with reading values from the graph of Hill's solution are avoided. It also provides a best-fit value for C_∞.

The curve fit for the 10-term approximation in Figure 5 does not go through the origin due to series truncation errors. This solution to Fick's second law is designed to converge rapidly at long times, and many more terms would have to be added to the equation to move the intercept closer to the origin. However, this does not affect the value of D obtained. Fitting a 4-term approximation to the series to the data yields the same D value to three significant figures.

Conventional study of dye diffusion determines the total amount of dye uptake or the average dye concentration in the fiber without details of the dye diffusion process, such as the local dye concentration or the spatial distribution of dye molecules in the fiber and their changes during the dyeing process. This process is tedious, time consuming and unable to provide the spatial distribution of dye in the fiber. In this work, we provide an elegant alternative method to study dye diffusion in fibers by using an LSCM. In this method, the spatial distribution of dye molecules in fibers and the diffusion coefficient of the dye can be quickly determined. To our knowledge this is the first report of using a LSCM to study dye diffusion in polymeric fibers.

The most significant advantage of LSCM is the ability of optical sectioning. By using an LSCM, the optical cross section of a fiber can be easily obtained as well as the dye spatial distribution in the fiber if the fluorescent intensity is considered linearly proportional to the fluorescein concentration in the fiber. Figure 6 shows dye spatial distribution in fibers during the dyeing process. At the beginning of dyeing, the fluorescent intensity is higher near the fiber surface and becomes uniform only when dyeing reaches equilibrium.

To quantify the data shown in Figure 6, one must convert the intensity profile to a concentration profile. The measured intensities were converted to a concen-

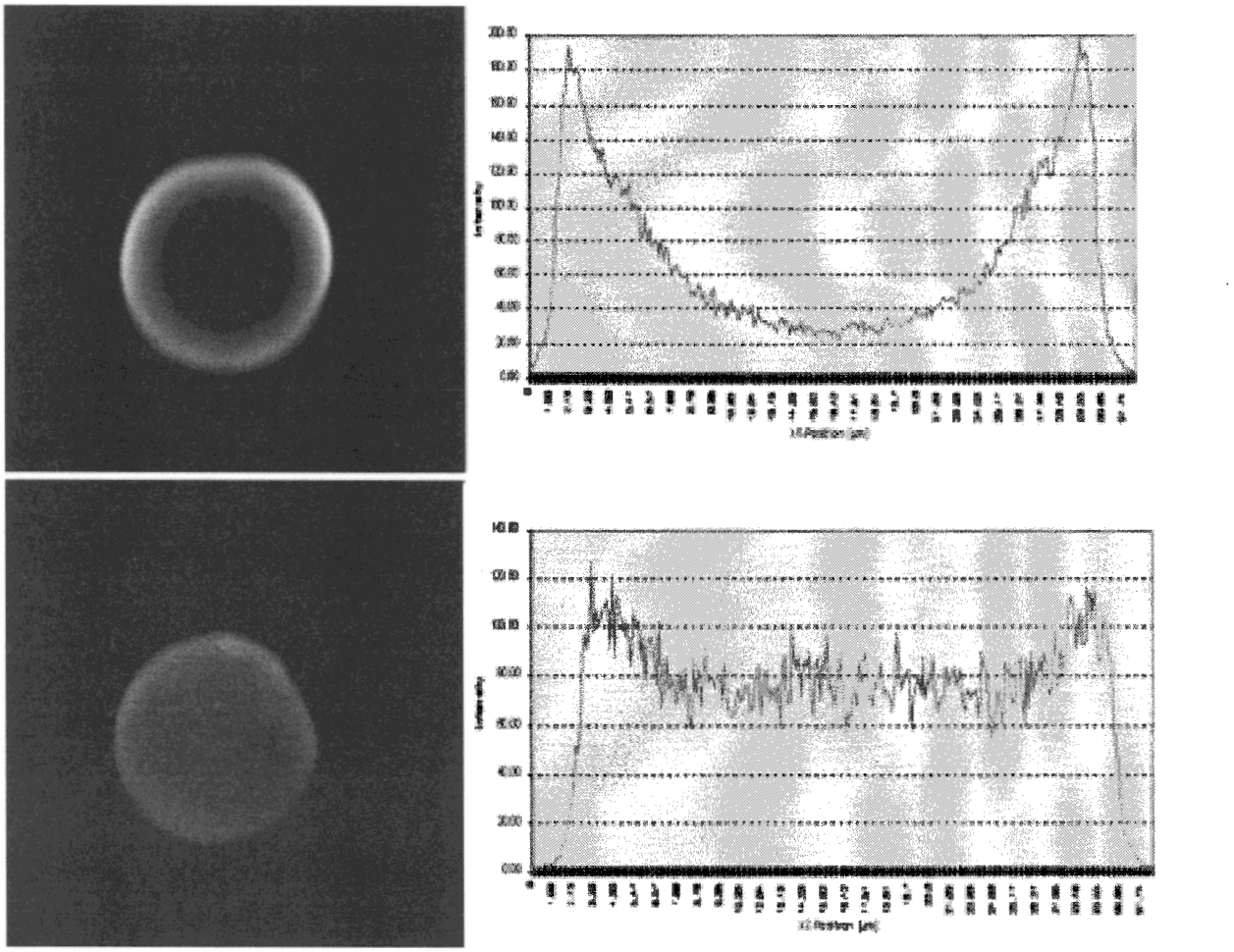

Figure 10. Images of fiber cross sections and fluorescence intensity profiles across fiber diameter for ring-dyed (top) and uniformly dyed (bottom) fibers. Both fibers have the same total dye concentration. The ring-dyed fiber appeared lighter than the uniformly dyed fiber.

tration with the assumption that the intensity is linearly proportional to the concentration. The linear relationship between the integrated fluorescent intensity and the concentration of fluorescein in a fiber is demonstrated in Figure 7. It is clear that the above assumption is valid as long as the concentration is less than about 5 g/kg. In the experiments reported herein, the maximum dye concentration was 1.4 g/kg, thus providing data in the linear region of Figure 7.

However, there are at least two important factors that contribute to inaccuracies in quantification of the intensity data to concentration: (a) fluorescence saturation and (b) photobleaching. To make quantitative measurements, we have implicitly made the assumption that the fluorescence is a linear process in that the emitted fluorescence is linearly proportional to the illuminating laser intensity. It has been well documented that saturation of a fluorophore can affect the quality of the image and lead to false quantification data.[27] Fluorescence saturation occurs when the exciting illumination is so intense that a significant fraction of the fluorophores are in the excited state and are no longer able to respond to the incident intensity. In our imaging care was taken to keep the intensity of the illuminating laser below the saturation threshold of fluorescein by ascertaining that the emission was twice as much when the illumination intensity was doubled. On the basis of the observation of linearity of emission with respect to the illumination intensity, we believe that the phenomenon of saturation is not a problem in our imaging and quantification.

Effects of irreversible photobleaching were avoided by using multiple scanning at low laser powers and also with the use of the photomultiplier tube of the LSCM at fairly high gains so that the signal-to-noise ratio was high.

When the integrated fluorescent intensity is linearly proportional to dye concentration in the fiber, the ratio of concentrations C_t/C_∞ will be equal to the ratio of integrated fluorescent intensities, which can be obtained from the total integrated area of the fluorescent intensity profiles. Therefore, the concentration ratio C_t/C_∞ was obtained from the fluorescent intensity profiles of the fibers dyed for 4 min and 256 min (Figure 8), and a diffusion coefficient of $(6.9 \pm 1.0) \times 10^{-11}$ cm^2/s is calculated from Hill's solution. This result is also in close agreement with the values cited above, thus demonstrating the validity and the value of the approach presented here.

The diffusion coefficient measured by the conventional method is based on yarn, which consists of a number of individual fibers. The rate of dye diffusion can be different between the individual fibers and that may contribute to the streakiness of textile fabrics. The diffusion coefficients measured by LSCM are based on the individual nylon fibers, however, they are in close agreement with the diffusion coefficients measured from the nylon yarn. Therefore, differences in the rates of dye diffusion between individual yarn fibers is unlikely a major factor to influence the streakiness of a fabric.

It has been reported that ring-dyed fibers have higher color yield and appear deeper in shade than homogeneously dyed fibers with the same overall dye concentration.[31−33] This indicates that the dye spatial distribution in the fiber can cause differences in the perceived color. We obtained two pieces of nylon yarns, both dyed with fluorescein and having the same total dye concentration, but the color appearance is different. The reflectance measured from the single fibers by a microspectrophotometer is shown in Figure 9, and LSCM images of cross section of these two fibers are shown in Figure 10. The fiber with the higher reflectance has the ring-dyed spatial distribution and the fiber with lower reflectance has the uniformly dyed spatial distribution. This implies that the ring-dyed fiber with the same concentration of dye as the uniformly dyed fiber absorbs less light. The uniformly dyed fiber was obtained by placing the ring-dyed fiber in a blank dye bath containing no dye at 95 C for 24 h. Measurements of refractive index, birefringence, and orientation distribution of dye molecules and polymer chains in each fiber indicated no significant difference between them, and no fluorescence spectral shift for the dye was found either.[25] This suggests that the color difference between the ring-dyed and uniformly dyed fibers, with the same total dye concentration in the fiber, is unlikely to be caused by variations in the physical properties or morphologies of the fibers. Instead, the spatial distribution of dye molecules in these fibers is the apparent reason for their different color yields and possibly a cause of streakiness in textile fabrics.

Conclusions

We have applied LSCM as a new nondestructive method to study dye diffusion behavior in fibers. This technique is simple and quick. It is able to provide high-resolution images of the fiber cross-section, quantitative measurement of the diffusion coefficient and 3D images of the dye distribution inside the fiber. The diffusion coefficient of fluorescein in nylon 66 fiber measured by LSCM agreed with the result from the conventional method. We have provided evidence that spatial distribution of dye molecules in a fiber can cause differences in the perceived color even if the fibers have the same dye concentration, and this has significant implications for dyed fabrics.

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