Fiber-optic microsensors to measure backscattered light intensity in biofilms

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We have constructed tapered fiber-optic microsensors with a tip diameter of less than 10 μm to measure profiles of backscattered light in biofilms, which are thin layers of micro-organisms firmly attached to surfaces. The observed response agrees well with local effective diffusivity microelectrode measurements, with $R^2 > 0.85$. A strong relation between signal intensity and wavelength has been observed at 670 and 1320 nm. These sensors have the potential to replace local effective diffusivity microelectrodes for true *in situ* biofilm measurements. © 2000 Optical Society of America

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1. Introduction

Biofilms, thin layers of micro-organisms firmly attached to surfaces, are a predominant microbial growth mode in natural and engineered systems.[1] Biofilm processes are implicated in bioremediation of toxic compounds,[2] oral hygiene,[3] souring of oil formations,[4] microbially influenced corrosion,[5] and infection of prosthetic devices.[6] To evaluate metabolic activity of biofilm micro-organisms, it is imperative to measure chemical gradients of nutrient concentration across microbial deposits on surfaces. Because the thickness of most biofilms is less than a few hundred micrometers, the tools of choice to measure chemical gradients across such thin layers are microsensors with tip diameters not exceeding a few micrometers. Microsensors, electrochemical and optical, are driven across biofilms with motorized micropositioners and measure local concentration of selected chemical species, at selected locations, with high spatial resolution.[7] The results of such measurements, when plotted versus biofilm depth, form nutrient concentration profiles. Based on the shape of these profiles and biofilm structure, microbial growth rate, substrate consumption rate, and several other parameters can be calculated. Microscale fiber-optic sensors have advantages over more typical electrochemical microsensors: They are immune to electromagnetic noise; they do not need reference electrodes; they are easier to make; and, in the case of diffusivity microsensors, the nutrient solution does not need to be replaced with an electrolyte solution prior to taking measurements. For these reasons, fiber-optic microsensors will eventually replace electrically noisy and difficult to fabricate microelectrodes in biofilm studies.

An important parameter that is measured in biofilms is the local mass transport coefficient; it was defined by Yang and Lewandowski[8] for the purpose of monitoring local properties of biofilms influencing the dynamics of mass transport. The concept was refined and the measurement technique improved by Beyenal *et al.*[9] to include local effective diffusivity. Local effective diffusivity is evaluated based on the rate of diffusion of electroactive substances to the tip of a mobile microelectrode, a few micrometers in diameter, positioned at various locations on a biofilm. We demonstrated that profiles of local effective diffusivity in biofilms reflect variations in the density of a biofilm matrix, and studying this relation is important. Because measuring local effective diffusivity is rather tedious, we have been looking for an alternative technique. Measurement of local backscattered light in biofilms is promising from this aspect. The intensity of backscattered light is a function of local properties of the matrix such as the density of microbial cells and the concentration of mineral deposits. By correlating the local effective diffusivity—

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measured by the electrochemical technique—with the results of backscattered light, we can calibrate the fiber-optic microsensor measuring the intensity of backscattered light in terms of local diffusivity.

Several groups[10] have developed fiber-optic microsensors to measure the spectral characteristics of biofilms. They found that the surface reflectance of a biofilm is wavelength dependent (400–1100 nm), with the reflectance having a smooth, monotonically varying depth profile. This result contradicted other diagnostic techniques demonstrating spatial heterogeneity of biofilms.[1] Recently, a fiber-optic microsensor at 780 nm was used to measure profiles of backscattered light in biofilms[10]; these profiles indicated the spatial heterogeneity expected in the biofilms. However, the measured optical response was not benchmarked against a conventional microsensor, making it impossible to quantify the observed signal variations. The thermally tapered tips were made by use of 100–140-μm multimode fiber and suffered from poor optical efficiency when tapered to sizes smaller than 10 μm in diameter, even with a metallized coating along the taper length. The system was successfully tested by use of highly scattering or absorbing media, but it was not recommended for use with transparent or translucent films, limiting the types of biofilms that could be studied. The authors recommended use of their system only for surface detection.

The goals of our study are to develop fiber-optic microsensors to measure spatially resolved profiles of backscattered light in a biofilm and correlate the measurements with biofilm structure measured with a conventional local effective diffusivity microelectrode. We developed a fiber-optic microsensor based on commercially available fiber-optic telecommunications components that measure backscattered light from a tapered fiber tip having a diameter smaller than 10 μm. As opposed to local effective diffusivity microelectrodes, which are electrochemical sensors, the optical microsensors do not require replacement of the nutrient growth solution with an electrolyte solution for operation, permitting true *in situ* probing of biofilm structures. Two laser light sources at 1320 and 670 nm were used in the measurement of light backscattered from the tapered fiber tip. The fiber-optic microsensor response was compared with our local effective diffusivity microelectrode.[9] These two different microsensor measurements at the same location were used to correlate backscattered light to local effective diffusivity in biofilms.

The construction and working principles of local effective diffusivity microelectrodes were described in detail by Beyenal *et al*.[9] Briefly, they are cathodically polarized microelectrodes. In measurements, ferricyanide, $Fe(CN)_6^{-3}$, introduced into the biofilm is reduced at the tip of the microelectrode to ferrocyanide, $Fe(CN)_6^{-4}$, in a process that generates a current in the external circuit, according to the reaction: $Fe(CN)_6^{-3} + e^- \rightarrow Fe(CN)_6^{-4}$. Increasing the polarization potential applied to the microelectrode causes the current to increase until the concentration of $Fe(CN)_6^{-3}$ at the electrode surface reaches zero. The current corresponding to a zero surface concentration of the reacting species is called the limiting current. The flux of ferricyanide to the tip of the microelectrode is related to the limiting current. This limiting current generated in the polarization circuit depends on the diffusivity of electroactive species near the microelectrode tip and therefore reflects the rate of mass transport to the tip of the microelectrode. The limiting current density was related to the local effective diffusivity near the microelectrode tip by calibration of the microelectrodes in layers of agar gels of different densities and of known effective diffusivities for the ferricyanide. We normalized the local diffusivity measurements by dividing the effective diffusivity of ferricyanide by the molecular diffusivity of ferricyanide in the electrolyte solution; the results were reported as relative effective diffusivities in the biofilm.

2. Materials and Methods

A. Biofilms

The biofilm preparation and standard measurement techniques are described elsewhere.[8,9] Briefly, the biofilms were grown on a glass surface in a system shown in Fig. 1. An inverted microscope was used to observe the tip of the microsensors in the biofilm. The microsensors were mounted on a linear micropositioner equipped with a computer-controlled stepper motor and introduced from the top of the reactor, perpendicular to the biofilm surface. Custom software was used to control simultaneously the microelectrode movement and the data acquisition. In a similar fashion, we used local effective diffusivity microelectrodes; the principles of constructing and operating such microelectrodes are described elsewhere.[9]

B. Optical System

The backscatter measurement apparatus is shown in Fig. 1. A fiber-pigtailed laser at 670 nm or a connectorized 1320-nm Fabry–Perot laser diode was coupled through a nonpolarization-preserving, 50:50 single-mode fiber coupler. The laser diodes were operated below threshold (typically, $I = 0.7\ I_{th}$) for improved signal stability, reduced intensity noise, and minimal coherence effects. One arm of the coupler was monitored with a Si or InGaAs p-i-n photodiode to measure the source intensity. The other coupler output was connected to a short section of telecommunications-grade single-mode fiber, the end of which was etched to form a fiber taper. Any backscattered light was collected by the photodiode. Angled physical contact connectors were used throughout the setup to minimize spurious backreflections that interfered with signals originating at the tapered fiber tip. Experiments at 670 nm used a 4.3-μm core fiber with a N.A. of 0.11 and a cutoff wavelength of 580 nm (Newport #F-SV/F-SV-C). Experiments at 1320 nm used a 9-μm core fiber with a N.A. of 0.11 (Corning SMF-28).

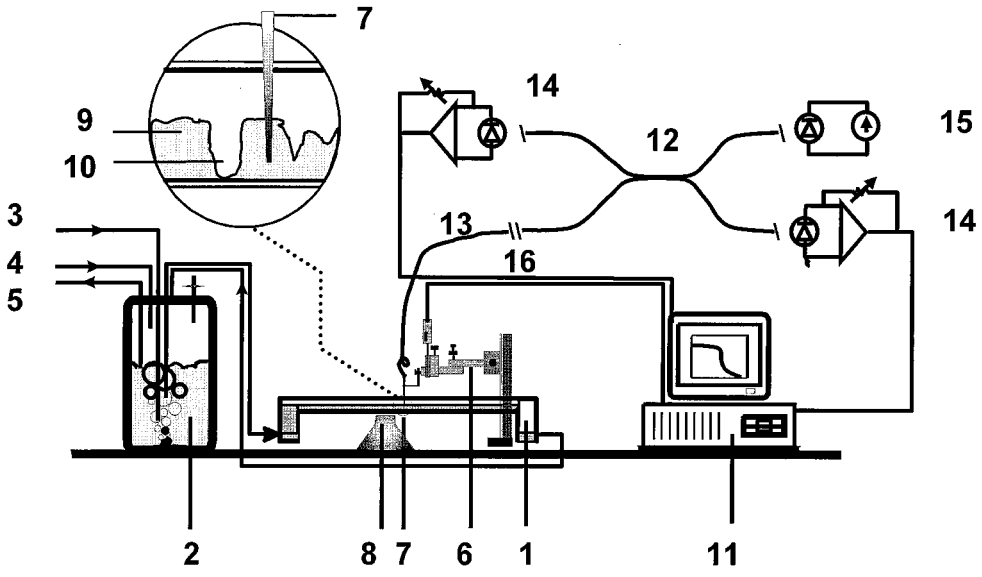

Fig. 1. Schematic diagram of experimental setup: 1, open channel, flat-plate reactor; 2, mixing chamber; 3, air conduit; 4, fresh-feed conduit; 5, effluent; 6, micromanipulator; 7, fiber-optic microsensor; 8, inverted microscope; 9, biofilm cluster; 10, biofilm void; 11, data-acquisition system; 12, 50:50 coupler; 13, single-mode fiber; 14, receiver; 15, source; and 16, angled connector.

Quantified backscattered light was recorded as the ratio between the backscattered and the reference signals. The two photodiodes were coupled into two transimpedance amplifiers having gains adjustable from 10^3 to 10^{10} V/A, followed by fine-gain adjust, offset nulling amplifiers, and a divider that provided a ratiometric output. A data-acquisition board digitized the divider output and generated control signals for positioning the microsensor in the biofilm.

C. Fiber-Optic Microsensor Tip Construction

We formed the tapered tips using a variation of previously described techniques.[11] The fiber tip was mechanically stripped, cleaned with isopropyl alcohol, and cleaved. We tested the cleave quality by measuring the backscattered signal in air and water. We corrected unreproducible results by recleaving the fiber end. The fiber was held vertically in a precision linear positioner and lowered into unstirred 37.5% hydrofluoric acid. After 15–60 min at room temperature, the fiber was removed and rinsed in deionized water. After we etched the microsensor tip, the backscattered signal was again measured in air and water. Stable, reproducible readings (albeit at much lower signal levels) indicated the successful formation of a fiber tip.

A scanning electron microscope micrograph of a typical fiber tip is shown in Fig. 2. The tips usually have a submicrometer end diameter with a smoothly tapered length of 500–1000 μm. The tapering affects only the cladding for most of the taper length. This allows the optical mode to remain well guided until it reaches the actual sensing region where the taper begins to cut into the core. The actual sensing region extends for 50–100 μm, depending on the taper angle. To reduce the sensing region size, the tapered tip can be cleaved.

D. Microsensor Measurement Technique

To measure the backscattered light in biofilms, the probe tip was mounted vertically in a precision positioner (0.1 μm) with a computer-controlled stepper motor. The fiber was stepped into the biofilm in 20-μm steps and data were recorded. The averaging time was approximately 3 s, and multiple data points were collected at each vertical position. After probing with the fiber tip, we probed the same location using the local effective diffusivity microelectrode. The data were normalized to the maximum variation seen as the probes penetrated the entire biofilm thickness. In all cases, the nutrient solution supporting biofilm growth was replaced with an electrolyte solution as required for operation of the local effective diffusivity microelectrode.[9]

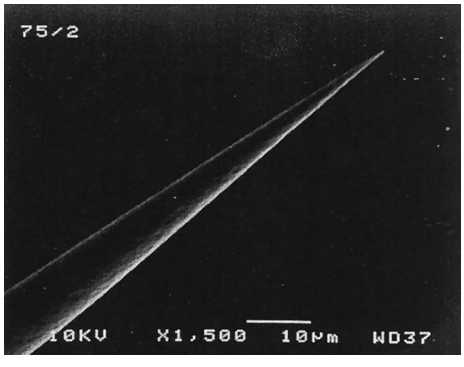

Fig. 2. Scanning electron microscope micrograph of fiber-optic microsensor tip.

3. Results and Discussion

A. Backscatter Measurements

The stability of the backscattered signal is shown in Fig. 3. Typically, approximately 10 μW at 1320 nm or 100 nW at 670 nm is launched into the 2 × 2 fiber coupler, and approximately 0.01–0.05% of this signal returns to the photodiode from the tapered fiber. The backscattered signal increases to >1% when the fiber tip is cleaved but not tapered because of a strong Fresnel backreflection at the fiber core–air interface. Figure 3 shows the variation in reflected signal of an untapered, cleaved fiber tip as it was immersed in water, back to air, into isopropyl alcohol, and finally back to air, over a period of 5 min. The ratios of reflected signals show reasonable agreement with the backreflected signals calculated with refractive-index data for these fluids. The signal-to-noise ratio of the data is >100:1 for most experiments. The fiber lead must be kept in a fixed orientation during data collection to minimize signal variations that are due to bend-induced losses. For the biofilm experiments, this is easily achieved because the fiber tip motion is limited generally to a few millimeters. We used a cleaved fiber for initial characterization of the system because of the well-defined tip geometry. Additional experiments were performed with tapered tips, and we observed results similar to those shown in Fig. 3.

B. Measurement in Biofilms

The results of a typical data run are shown in Fig. 4. The data at 670 nm was normalized to the data at 1320 nm for the probe located outside the biofilm, but still submerged in the solution. The microelectrode response is shown for comparison. The backscattered optical signal at 1320 nm increased after it entered into the biofilm, showing a large intensity variation (almost a factor of 5) as the tip moved through the biofilm toward the bottom. Moreover, the variations observed correlated well with the local effective diffusivity microelectrode. Furthermore, the measurements at 670 nm showed a much smaller (<5%) intensity variation as the probe moved through the biofilm.

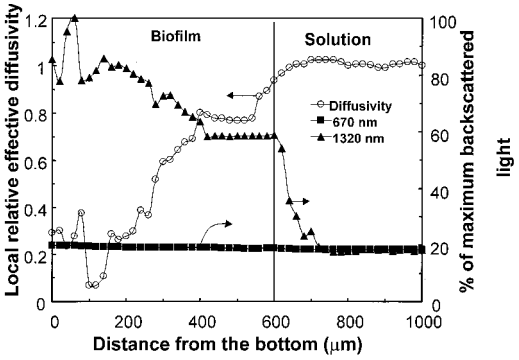

Fig. 4. Variation in the percent of maximum backscattered light intensity (670- and 1320-nm lasers) and local relative effective diffusivity by depth in a heterogeneous biofilm at the same location.

C. Correlation between Local Effective Diffusivity and Local Backscattered Light

These results were highly repeatable. The data in Fig. 5 show the correlation between the optical backscatter at 1320 nm and the local effective diffusivity microelectrode output signal (data extracted from Fig. 4). The correlation coefficient R^2 over many trials was always greater than 0.85 and as high as $R^2 = 0.95$ in some tests (as presented in Fig. 5). This is in remarkably good agreement considering the difficulties involved in aligning the two probes at the same biofilm location. As with microsensors, repeated testing (three to five trials) at the same biofilm location caused permanent changes in the biofilm microstructure and in the backscattered signal, leading to additional uncertainties in the location of the region being probed. We also observed different, but repeatable, calibration equations (with $R^2 > 0.85$) at different locations in the biofilm, presumably because of structural heterogeneity of the biofilm.

The dramatic wavelength dependence of the backscattered signal variations was unexpected. Optical scattering efficacy should increase strongly as the optical wavelength λ in the biofilm decreases for scattering particles having radii r_0 that satisfy $q < 5$, where $q = 2\pi r_0/\lambda$. This is contrary to the observed results. Mie scattering[12] can produce a wavelength dependence that is similar to the observed data when

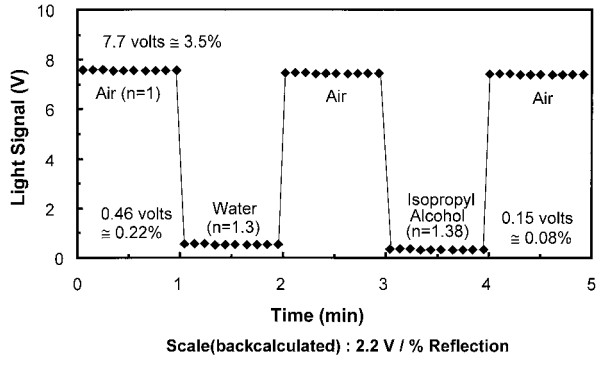

Fig. 3. Backscattered signal from the fiber-optic microsensor (1320-nm laser source) in various liquids.

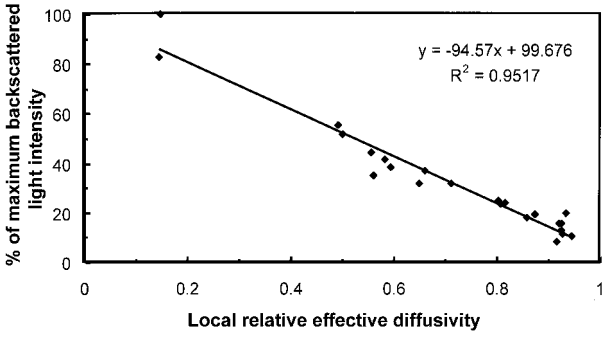

Fig. 5. Correlation between percent of maximum backscattered light intensity (at 1320 nm) and local relative effective diffusivity.

$6 < q < 10$, but the scattering cross-sectional variation is at most a factor of 2, much less than experimentally observed. The Mie effect also plays a role because we are collecting backscattered light with the fiber tip. The Mie effect predicts reduced backscatter intensity with decreasing wavelength (increasing q), with a concomitant increase in forward-scattered intensity. However, a strong wavelength dependence requires a narrow distribution of scattering particle sizes, a highly unlikely situation in the biofilm samples studied here.

The observed behavior is likely also related to a strong OH^- absorption band near 1340 nm. Regions with high water content will absorb some of the incident light, reducing the backscattered intensity. Denser, lower water concentration regions will lead to lower absorption and increased backscattered intensities. Because the optical absorption that is due to water in hydrous biological materials such as tissue is much lower at 670 nm ($\alpha_{670} = 1\ m^{-1}$) than at 1320 nm ($\alpha_{1320} = 1000\ m^{-1}$),[13] any changes in local hydration will have more pronounced effects near 1320 nm than at 670 nm.

Because higher effective diffusivity correlates with higher water content, the local effective diffusivity microelectrode response decreases as it penetrates denser regions of the biofilm. The observed complementary responses of the optical sensor and the local effective diffusivity microelectrode are consistent with this explanation of the wavelength dependence. Further experiments are under way to resolve the mechanisms involved in the measured optical response and to develop a unique correlation between local effective diffusivity and backscattered light intensity.

4. Conclusions

We have built tapered fiber-optic microsensors using telecommunications components. The relatively long taper length compared with angle-polished tips makes the fiber ideally suited for probing biofilms having a thickness of a few hundred micrometers. The simple fabrication involves straightforward wet chemistry processes and lends itself to creating fiber bundles having thousands of parallel tapered tips suitable for three-dimensional mapping of biofilm properties. We have observed a large optical response using fiber-optic microsensors in biofilms. The observed response agrees well with local effective diffusivity microelectrode measurements, with $R^2 > 0.85$. We have also observed a strong wavelength dependence using measurements at 670 nm and 1320 nm.

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References

1. J. W. Costerton, Z. Lewandowski, D. E. Caldwell, D. R. Korber, and H. M. Lappin-Scott, "Microbial biofilms," Annu. Rev. Microbiol. **49,** 711–745 (1995).
2. A. B. Cunningham and B. M. Peyton, "Bench-scale/field-scale interpretations: an Overview," in *Applied Bioremediation of Petroleum Hydrocarbons*, R. E. Hinchee, J. A. Kittel, and H. J. Reisinger, eds. (Battelle, Dayton, Ohio, 1995), pp. 97–100.
3. P. D. Marsh, "Dental plaque," in *Microbial Biofilms*, H. M. Lappin-Scott and J. W. Costerton, (Cambridge U. Press, New York, 1995), pp. 282–300.
4. C. I. Chen, M. A. Reinsel, and R. F. Mueller, "Kinetic investigation of microbial souring in porous media using microbial consortia from oil reservoirs," Biotechnol. Bioeng. **44,** 263–269 (1994).
5. W. Lee, Z. Lewandowski, P. H. Nielsen, and W. A. Hamilton, "Role of sulfate-reducing bacteria in corrosion of mild steel: a review," Biofouling **8,** 165–194 (1995).
6. A. G. Gristina, "Biofilms and chronic bacterial infections," Clin. Microbiol. News. **16,** 171–178 (1994).
7. N. P. Revsbech, "An oxygen microsensor with a guard cathode," Limnol. Oceanogr. **34,** 474–478 (1989).
8. S. Yang and Z. Lewandowski, "Measurement of local mass transfer coefficient in biofilms," Biotechnol. Bioeng. **48,** 737–744 (1995).
9. H. Beyenal, A. Tanyolaç, and Z. Lewandowski, "Measurement of local effective diffusivity and cell density variations in heterogeneous biofilms," Water Sci. Technol. **38,** 171–178 (1998).
10. I. Klimant, G. Holst, and M. Kuhl, "A simple fiber optic sensor to detect the penetration of microsensors in the sediments and other biogeochemical systems," Limnol. Oceanogr. **42,** 1638–1643 (1997).
11. H. Gao, Z. Chen, J. Kumar, S. Tripathy, and D. Kaplan, "Tapered fiber tips for fiber optic biosensors," Opt. Eng. **34,** 3465–3470 (1995).
12. M. Born and E. Wolf, Principles of Optics, 6th ed. (Pergamon, Oxford, UK, 1980).
13. M. Yarborough, Photonics Design and Applications Handbook (Laurin, Pittsfield, Mass., 1996), pp. H287–H290.