Nonlinear Self-Action of Light through Biological Suspensions

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It is commonly thought that biological media cannot exhibit an appreciable nonlinear optical response. We demonstrate, for the first time to our knowledge, a tunable optical nonlinearity in suspensions of cyanobacteria that leads to robust propagation and strong self-action of a light beam. By deliberately altering the host environment of the marine bacteria, we show experimentally that nonlinear interaction can result in either deep penetration or enhanced scattering of light through the bacterial suspension, while the viability of the cells remains intact. A theoretical model is developed to show that a nonlocal nonlinearity mediated by optical forces (including both gradient and forward-scattering forces) acting on the bacteria explains our experimental observations.

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Understanding light-matter interaction in biological suspensions is of fundamental interest in biophotonics, optofluidics, soft-matter, and life sciences [1–3], as well as of crucial importance in today's development of widespread biotechnologies. While the linear optical properties of biological media have been well studied [4,5], little is known about their nonlinear properties. Recently, there has been an increasing interest in light controlled motion of microorganisms and their hosting flows [6,7], but these controls are based on phototaxis in bacterial suspensions rather than optical nonlinearity. To efficiently propagate light through highly scattering media, it is important to study the nonlinear optical properties of soft-matter systems [8–12]. In particular, an optical nonlinearity can lead to stable low-loss propagation and deep penetration of light in scattering media such as nanoparticle suspensions, which could be employed to noninvasively initiate and control chemical or mesoscopic kinetic processes, as well as to study living organisms with high-resolution depth-resolved optical imaging [13,14]. Although nonlinear self-trapping of light was demonstrated in colloidal suspensions of stiff nanoparticles [15–17], the study of the nonlinear response of biological media has been very limited. In fact, it is commonly believed that light cannot penetrate deeply into biological environments due to strong scattering loss and weak optical nonlinearities.

In this Letter, we demonstrate deep penetration of light through scattering biological suspensions and strong nonlinear waveguiding effects arising from live cells. Specifically, we investigate nonlinear transmission of light through biological suspensions of cyanobacteria (*Synechococcus* sp. cells), while the host aqueous environments are deliberately varied. Because of nonlinear self-trapping, a light beam propagates over a remarkably long distance through the cyanobacteria suspended in seawater despite their low absorption and weak polarizability. Additionally, we have developed a theoretical model for describing the nonlinear beam dynamics. Contrary to previous models, we consider that the bacterialike particles are affected not only by the optical gradient force but also by the scattering force in the forward direction, which leads to an effective nonlocal nonlinear response along the propagation direction. Our numerical results find good agreement with experimental observations. Furthermore, we show a dramatic change of propagation dynamics from self-trapping to enhanced scattering when the background medium for the cyanobacteria is changed from seawater to a water-glycerol mixture. The viability assessment of the

cells indicates that they remain alive even after nonlinear self-action of the laser beam. These findings may herald new techniques for overcoming scattering losses in optical imaging, as well as other biological applications.

First, we report our experimental results on self-trapping of light in biological suspensions. The experiments were carried out with a linearly polarized laser beam ($\lambda = 532$ nm), which is collimated and then focused inside the medium with an input FWHM about 50 μm [15] and sent through a 4-cm-long glass cuvette filled with either a synthetic seawater medium (ASN-III) alone or with an additional colloidal suspension of *Synechococcus* cells. The *Synechococcus* cyanobacterial genus is naturally distributed in high concentration ($\sim 10^3$–10^5 cells/ml) throughout the marine photic zone [18–20], and plays a major role in global carbon cycles. The particular strain used in this work (*Synechococcus* sp. strain PCC 7002: unicellular, immotile, and about 2.5 μm long) has been chosen as a model cyanobacterium due to its low absorption for green light and high tolerance to both oxidative stress and glycerol environments [21,22]. Details about sample preparation are provided in the Supplemental Material [23].

Typical experimental results are presented in Fig. 1. In the absence of bacteria, the input beam diffracts normally to about 650 μm (FWHM) in seawater, irrespective of the laser power, as seen in Figs. 1(a), 1(e). With the inclusion of *Synechococcus* cells (1.3×10^7 cells/ml), and for low laser powers, the beam dramatically expands to about 1.25 mm (FWHM) due to linear scattering [see Figs. 1(b), 1(f)]. However, as the laser power is increased, the beam undergoes a transition from normal diffraction to nonlinear self-trapping, as illustrated in Figs. 1(c), 1(g). The side-view pictures clearly show solitonlike self-guiding [15,16,24] exclusively induced by the presence of live cells. It should be pointed out that Fig. 1(d) is taken by filtering out the green beam. The bacteria trapped along the beam path actually exhibit the red autofluorescence associated with chlorophyll *a*. The persistent red autofluorescence under green excitation indicates low rates of chlorophyll degradation in the trapped *Synechococcus* cells, as is typically seen when examined under an epifluorescence microscope [Fig. 1(h)] [25]. A series of measurements are performed to determine how the size of the output beam and the power transmission depend on the input laser power and the cell concentration. We found that the normalized transmission increases slightly with the laser power due to self-guiding under nonlinear propagation [Fig. 1(i)], but decreases dramatically at high cell concentrations due to enhanced scattering losses from the cells [Fig. 1(j)].

Intuitively, the cyanobacteria suspended in seawater may be modeled as dielectric particles with a positive polarizability because the refractive index of *Synechococcus* ($n_p \sim 1.38$) is slightly higher than that of seawater ($n_b \sim 1.33$). Like particles, the cells in a positive polarizability environment tend to be attracted against the diffusive Brownian motion towards the center of the light beam due to the optical gradient force [10,26,27]. This attraction leads to an increase in cell density along the beam path, which in turn creates an effective waveguide due to

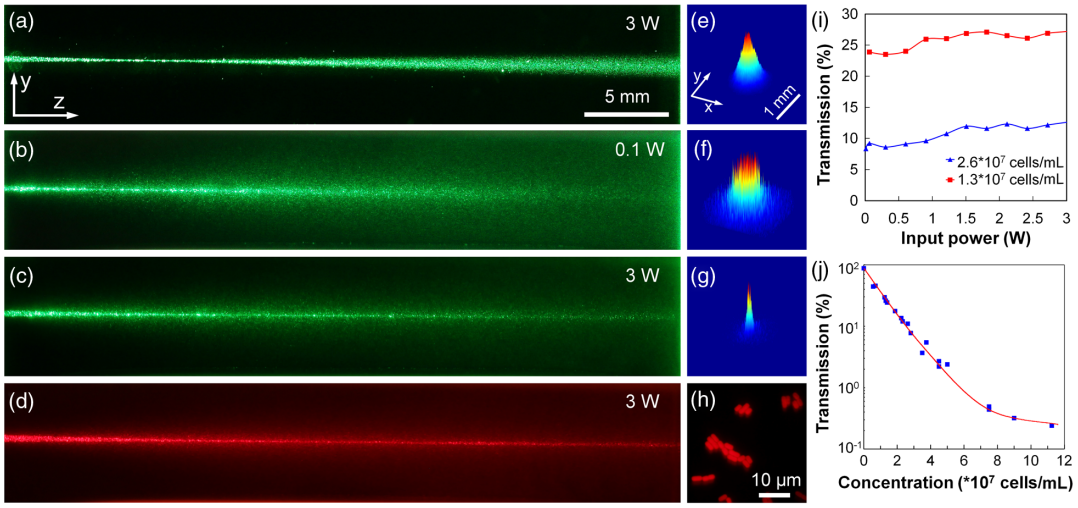

FIG. 1. Nonlinear self-trapping of light through cyanobacteria in seawater. (a) Side view of normal diffraction of an intense laser beam in seawater, showing no self-action of the beam when no bacteria are present. (b),(c) When *Synechococcus* cells are suspended in seawater, the beam undergoes linear diffraction or scattering at low power (b) yet it experiences nonlinear self-trapping at high power (c). (d) Side view of the same beam in (c) imaged using autofluorescence of the cells (in red) when the green light is partially blocked, thus indicating survival of the trapped cells under laser illumination. (e)–(g) Corresponding 3D plots of the beam's normalized intensity profiles after 4 cm of propagation, captured by the CCD camera. (h) Image of the *Synechococcus* cells taken with an epifluorescence microscope using 100X objective when excited by green light. (i) Transmission percentage measured as a function of input power for two different cell concentrations. (j) Semilogarithmic plot of transmission percentage as a function of cell concentration at a fixed input power of 3 W.

the higher index of the cyanobacteria compared to the host environment. However, this approximation cannot fully explain the interaction between light and algae cells.

To better understand our observations, we developed a model for beam propagation mediated by an optical force-induced nonlinearity in a colloidal suspension. In our model we do not *a priori* assume any particular form for the nonlinearity, but we rather let the beam propagate in a waveguide due to the spatial variation of the particle (i.e., cells) concentration and the associated changes to the effective refractive index distribution. The particles are driven not only by an optical gradient force, but also by a scattering force in the forward direction, which is pivotal to the beam dynamics. The modified nonlinear Schrödinger equation used to simulate the beam propagation through the nonlinear medium can be written as [10,16]

$$i\frac{\partial\phi}{\partial z}+\frac{1}{2k_0n_b}\nabla_\perp^2\phi+k_0V(n_p-n_b)\rho(\vec{r})\phi+i\frac{\sigma}{2}\rho(\vec{r})\phi=0, \tag{1}$$

where φ is the electric field envelope, $k_0=2\pi/\lambda_0$ denotes the vacuum wave number, and σ is the scattering cross section. Meanwhile, V represents the volume of an individual particle and n_p its refractive index, n_b stands for the refractive index of the background medium, and ρ denotes the intensity-dependent particle concentration. The spatial variation of ρ was, contrary to previous models [8–12,15–17,28], assumed to be driven not only by an optical gradient force but also by a scattering force in the forward direction of propagation. The temporal evolution of the particle concentration was modeled by a diffusion-convection equation,

$$\frac{\partial\rho}{\partial t}+\vec{\nabla}\cdot[\rho\vec{v}(\vec{r})-D\nabla\rho]=0, \tag{2}$$

where D is the diffusion coefficient, t is time, $\vec{v}=\mu\vec{F}(|\varphi|^2)$ is a velocity field determined by the intensity-dependent optical forces and μ is the particle mobility. In particular, we take the optical forces acting on the particle as $\vec{F}(I=|\varphi|^2)=\alpha\vec{\nabla}I+\beta I\hat{z}$, including both the gradient force with a polarizability coefficient α and a forward-scattering force along z depending on a coefficient β. Without the scattering force, the model reduces to the exponential nonlinearity in a steady state previously used to model dielectric nanosuspensions [10]. The model was solved numerically using a $(2+1)$D split-step algorithm that also included additional scattering effects modeled by random fluctuations of the refractive index. A self-consistent solution was obtained by repeatedly propagating the field through the entire medium and then calculating the particle distribution after a short time step for the corresponding optical force. The new particle distribution was then used in the next iteration to propagate the field again, and the process was repeated until no significant modification of the field or the particle distribution was observed.

To highlight the necessity of including the forward-scattering force in simulations of the beam dynamics, in Fig. 2 we show a direct comparison of the transverse beam profiles and the corresponding distributions of particle concentrations obtained by numerical simulations using both an exponential growth model (which considers gradient forces only) [10] and our new forward-scattering model. The inclusion of forward-scattering force arising from radiation pressure is essential, and it accounts for the deep penetration of the light beam observed in our experiment. With only the optical gradient force present, the beam either experiences additional diffractive broadening or undergoes catastrophic self-focusing and collapse (Fig. 2, top panels). The scattering force causes particles to

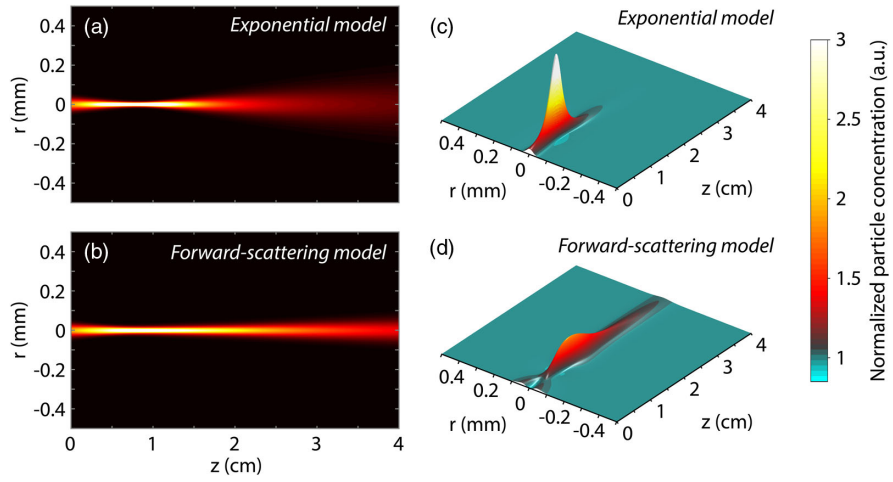

FIG. 2. Comparison of two different models to describe nonlinear beam dynamics in biological suspensions. (a),(b) Side view of beam propagation (normalized linear scale) obtained numerically using (a) an optical gradient force only (exponential model) and (b) an optical gradient along with a forward-scattering force (forward-scattering model). (c),(d) Corresponding theoretical distributions of the normalized concentrations of bacterialike particles induced by the respective types of light-particle interactions.

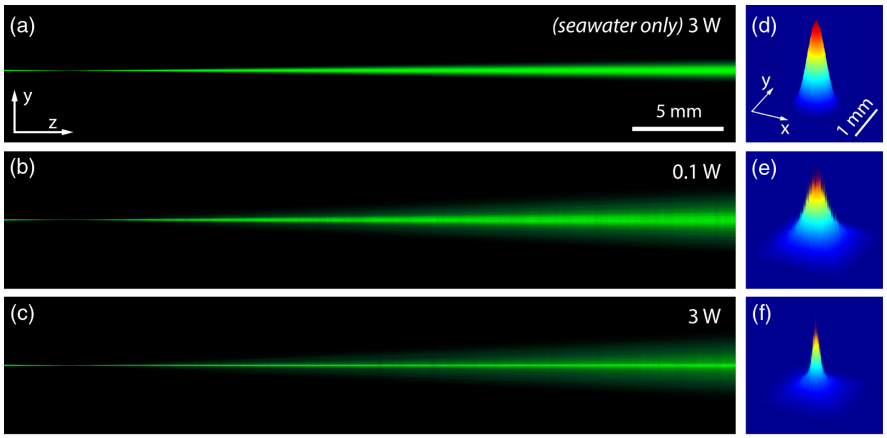

FIG. 3. Numerical simulations of nonlinear beam propagation in biological suspensions. (a)–(c) Side view of the laser beam and (d)–(f) corresponding output intensity profiles simulated with parameters obtained from the experimental results shown in Fig. 1. (a),(d) Case of seawater only without suspended particles, exhibiting linear diffraction. (b),(e) Case of a low power beam in the presence of suspended particles, showing linear diffraction with additional broadening due to random scattering. (c),(f) Case of a high power beam in the presence of suspended particles, where nonlinear self-trapping of the beam is achieved.

be pushed out of the beam's focus, accumulating in front of the high intensity region where they form an effective waveguide to prevent the beam from collapsing (Fig. 2, bottom panels).

Typical numerical results obtained using the forward-scattering model are presented in Fig. 3. If we consider only diffraction in pure seawater, a 50 μm wide input beam broadens to an output size of 600 μm [see Figs. 3(a), 3(d)]. With the inclusion of particles, the beam widens to 740 μm at a low power level of 100 mW [see Figs. 3(b), 3(e)]. However, at a high power level of 3 W, the beam self-focuses strongly back to 270 μm as shown in Figs. 3(c) and 3(f) due to the formation of a nonlinear self-induced waveguide *in front* of the beam focus (see movie in the Supplemental Material [39]). In the simulations where particles are present, we have also included additional scattering effects that are modeled by random fluctuations of the refractive index. However, we have, for simplicity, neglected effects such as the drag force acting on the particles as well as particle-particle interactions [28]. Despite the omission of such additional perturbative effects in our model, there is evidently a good qualitative agreement between results from experiments (Fig. 1) and simulations (Fig. 3). One can also compare our numerical parameters with those expected from the theory of optical forces [26]. For instance, considering a 1-μm particle (about the measured bacterial size), we obtain $\beta_{\mathrm{th}} = 4.5 \times 10^{-19}$ m s and $\alpha_{\mathrm{th}} = 6.9 \times 10^{-28}$ m^2 s. In this case, we find a ratio between scattering and gradient forces of $R_{\mathrm{th}} = 6.4 \times 10^{8}$ m^{-1}, which is close to the value $R_{\mathrm{th}} = 1.2 \times 10^{8}$ m^{-1} obtained from our numerical fit. Thus, the ratio of the optical forces determined by the numerical fit is in agreement with analytical estimates, even based on a nonideal approximation assuming spherical dielectric particles in the Rayleigh scattering regime.

Next, we investigate how light propagates in the cyanobacterial suspension when the host seawater solution is altered, motivated by achieving a tunable optical nonlinearity in soft matter [9]. The effective refractive index of the background medium is varied by adding different concentrations of glycerol ($n_{\mathrm{eff}} \sim 1.47$ at 532 nm) to the seawater solution. Results of output beam size as a function of input power are presented in Fig. 4(a) for different concentrations, along with side-view images and output transverse intensity profiles. For these results, the *Synechococcus* cells are prepared in glycerol-water mixtures with varying ratios (0:1, 1:3, 1:1, and 3:1), thus directly impacting the effective refractive index of the hosting medium (1.33, 1.37, 1.40, and 1.44). At low concentrations of glycerol (first two cases), the bacteria are attracted towards the beam due to gradient forces under a positive polarizability, and the beam size decreases dramatically as nonlinear self-focusing takes place [see red or green curves in Fig. 4(a)]. At high concentrations of glycerol (last two cases), the polarizability is negative, but one would expect self-trapping to still take place. This is due to particles with an index of refraction lower than the background medium being repulsed away from the beam path, leading to an effective waveguide [15], akin to self-induced spatial cleaning effects recently observed in optical multimode fibers [29]. However, in our experiments with living cells, the beam undergoes enhanced broadening at high power rather than self-trapping [see blue or black curves in Fig. 4(a)].

To explain this enhanced light scattering, we discuss how different optical forces acting on *Synechococcus* cells redistribute due to cell shrivel and increased viscosity of the ambient medium. *Synechococcus* sp. is in isotonic condition in normal seawater, meaning that there is no net water flow into or out of the cells [Fig. 5(a)]. When a

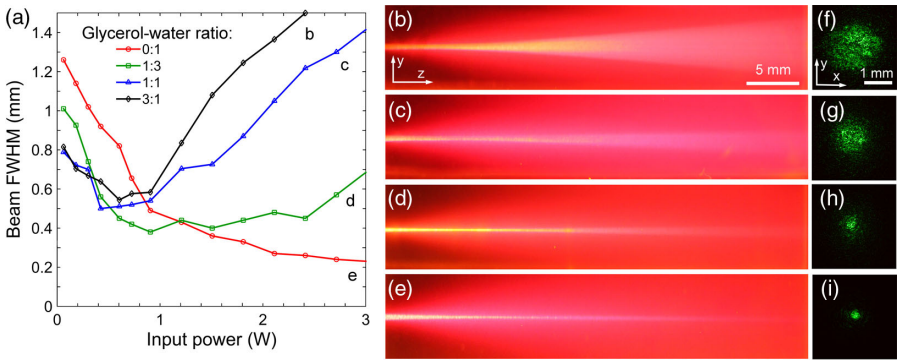

FIG. 4. Enhanced scattering of light through cyanobacteria in glycerol-seawater mixtures. (a) Measured beam size after 4 cm of propagation as a function of input power through *Synechococcus* suspensions of varying glycerol-seawater ratios. (b)–(e) Side view of the beam taken from auto fluorescence of the cells when excited by laser light at 3 W input power, corresponding to the four samples of cyanobacterial suspensions in (a). (f)–(i) Corresponding transverse intensity profiles taken at the output of the samples.

high concentration of glycerol is added, the osmotic pressure around the cells changes and, consequently, the solution becomes hypertonic [Fig. 5(d)]. In this case, a *Synechococcus* cell releases its internal water while absorbing a small amount of glycerol [30]. At a 3:1 glycerol-seawater ratio (i.e., 75% glycerol in seawater), the estimated volume of the cells (averaged over 50 cells) changes from about 3.8 to 2.6 μm^3, as measured from the bright-field images using an optical microscopy system [Figs. 5(b), 5(e)]. This 30% of shrinking in cell volume, along with a slight absorption of glycerol, could lead to an increase in the refractive index of the bacteria, consequently suppressing the effective negative polarizability and the associated repelling gradient forces. In addition, since glycerol has a very weak absorption at 532 nm, it can lead to slight self-focusing of light without bacteria (see Fig. S3 in the Supplemental Material [23]). When bacteria are added in the glycerol-seawater mixture, the thermophoresis effect (or the Soret effect) [31–37] also comes to play a role which would affect the redistribution of bacteria. Finally, due to the larger concentration of glycerol, the suspension's viscosity is expected to increase dramatically (about 47 times larger [38]), which prevents the cells from being attracted or pushed far away from the beam's focus. Under these combined effects, the cells tend to form clusters in glycerol-rich environments that behave like light diffusers [Fig. 5(f)] rather than forming a light guide [Fig. 5(c)] along the beam path.

Before closing, several issues merit further discussion. The above observations of stable self-trapping of light through the *Synechococcus* cells in pure seawater is achieved with a cw 532 nm laser, despite that these blue-green-colored bacteria have relatively low absorption (thus low thermal effects) at this wavelength. Therefore, thermal effects are not the main drive for the self-action of light observed in our experiment. Such a role belongs to the optical force-induced nonlinearity, in contrast to the case of solitons in "hot" particle nanosuspensions [37]. More evidently, thermal effects typically occur at much slower time scales (seconds), while the response time of soliton formation in our bacterial suspensions is at the millisecond level. When the seawater background solution was replaced with a glycerol-water mixture, *Synechococcus* cells were observed to be slightly attracted towards the high intensity region under the microscope. No significant photo damage was observed due to laser illumination or health degradation due to the presence of glycerol. (Movies for bacterial motion and viability assessment are included in the Supplemental Material [23,39]). We want to mention that we have also observed nonlinear self-focusing of light in a

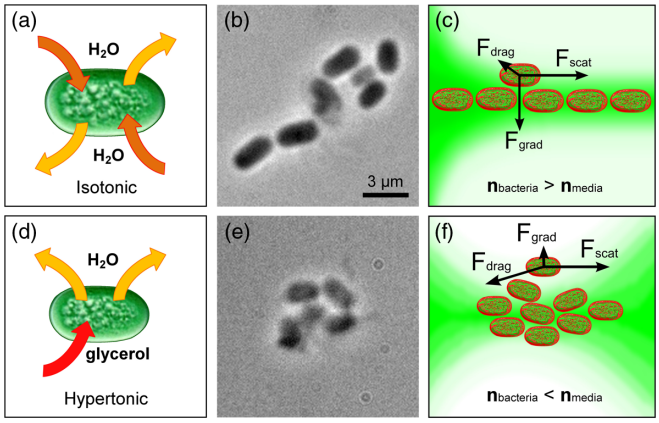

FIG. 5. Impact of environment on force distribution and cell mobility dynamics. Comparison of cell size and force distribution between cyanobacteria in seawater (top row) and in the glycerol-water mixture (bottom row). (a),(d) Schematic illustration of a *Synechococcus* cell under isotonic (as in seawater) and hypertonic (as in glycerol-water mixture) conditions. Red and orange arrows denote an influx of glycerol and water, respectively, while yellow arrows denote a water outflux. (b),(e) Bright-field images of *Synechococcus* cells taken when they are in seawater (b), and in a 3:1 glycerol-water mixture (e), where the average volume of individual cells has decreased by 30%. (c), (f) Schematic force diagrams showing that the cells form a light guide in seawater (c), but behave like a light scatterer in glycerol-rich environments (f). The laser light is represented in green.

few other biological suspensions, including *P. marinus* cyanobacteria growing in seawater, *E. coli* bacteria growing in the lysogeny broth media, as well as human red blood cells under different osmotic conditions. The precise impact of the cell type, size, and concentration as well as viscosity and absorption on the optical forces are yet to be determined, which may be critical for tuning the optical nonlinearity in biological suspensions.

In summary, we have demonstrated self-action of light in biological suspensions. Nonlinear interaction between light and cyanobacteria leads to deep penetration or enhanced scattering of a light beam through otherwise lossy biological environments. Our results may open up various possibilities towards exploring nonlinear optical properties of microorganisms in aqueous suspensions or other optofluidic environments. In the long term, bio-optical materials may prove useful for performing noninvasive medical diagnosis, deep-tissue imaging, and engineering of environmentally friendly bio-optical components with tunable properties.

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