Biological laser action

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The narrowing of the spectral linewidth and the increasing of the peak intensity characteristic of laser action were observed in emission spectra of dye-infused biological tissues. The tissue was infused with a solution of Rhodamine 640 perchlorate in ethanol and then excited with frequency-doubled Q-switched Nd:YAG laser pulses. The dependence of emission linewidth on the excitation radiant exposure and dye concentration was investigated. Laser action was also observed in biologically compatible fluorescein sodium dye dissolved in phosphate-buffered saline mixed with scattering polystyrene spheres. The sharp spectral peaks of laser action in tissues may find applications in the detection of superficial disease.

Key words: Tissue optics, laser action, fluorescence, amplification of spontaneous emission, turbid media.

1. Introduction

Laser action in turbid media made of TiO_2 in methanol mixed with dye solution was first observed by Lawandy *et al.*,[1] who used a dye solution of Rhodamine 640 perchlorate dissolved in methanol. The turbid media were excited with a pulsed laser, and a surprisingly low threshold excitation energy was required to generate the laser action. The mechanism for the laser action was speculated to be scattering-enhanced amplification of spontaneous emission.[2] The scattering property of the turbid media increased the path length of light in the gain medium, which consisted of the dye molecules excited by the pulsed laser. When the path length exceeded a critical gain length, spectral narrowing of the spontaneous emission linewidth occurred. It has been shown that the spectral linewidth is inversely proportional to the square root of the light propagation length for both homogeneously and inhomogeneously broadened transitions under unsaturated gain conditions.[3]

The above experiment was conducted in physical turbid media. Knowing that most biological tissues are intrinsically scattering, we hypothesized that the laser action could be generated in biological tissues and hoped that the sharp and strong spectral peak could result in high-sensitivity diagnostic techniques in medicine. This article presents our experimental findings on laser action in biological tissue. We have replaced the methanol with ethanol as the solvent of the dye because ethanol is less biologically toxic than methanol. We have also observed laser action in biocompatible fluorescein sodium dye dissolved in a turbid solution. Other investigators have also independently studied laser action in biological tissues.[4–6]

2. Methods and Materials

Dye solutions were prepared with Rhodamine 640 perchlorate (molecular weight, 591) mixed in a 70% solution of ethanol. The concentration of dye is expressed in units of M. Muscle tissue was freshly excised from killed Fisher 344 rats and then divided into multiple pieces weighing 540 ± 10 mg each. A total of 0.2 mL of dye solution was injected into various sites of each piece of tissue and allowed to diffuse evenly for 10 min. The actual absorbed amount was 0.09 ± 0.017 mL, which was measured by weighing the tissue before and 10 min. after the dye injection. The dye solution that leaked out of the tissue was excluded.

The tissue sample was placed on a plastic dish and covered with a thin piece of glass to prevent the tissue from drying (Fig. 1). Then the dish was placed on a height-adjustable platform so the spot area of the laser beam on the sample surface could be varied. A linearly polarized frequency-doubled (532-nm) Q-switched Nd:YAG laser pulse of 10-ns duration was repeated at a rate of 10 Hz. Two prisms routed the laser light, which was focused by a lens of

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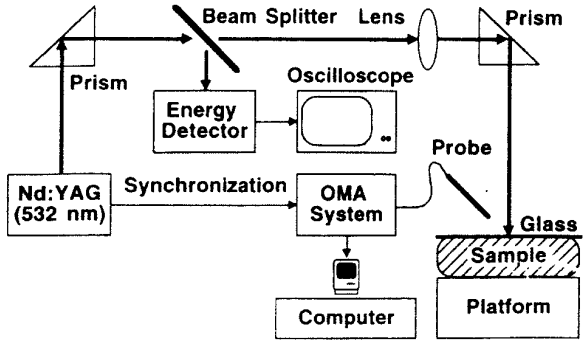

Fig. 1. Schematic of the experimental setup. The thick and thin arrows represent optical and electronic signals, respectively. The thickness of the glass layer was 0.016 cm.

10-cm focal length and normally incident to the sample surface. The pulse energy was monitored by splitting a portion of the laser beam into an energy detector connected to an oscilloscope. An optical fiber (core diameter, 600 μm; NA, 0.44) of 45° incidence collected the emission spectra near the samples, where the distance between the tip of the optical fiber and the incident point of the excitation laser beam on the tissue surface was 1.5 cm. The polarization plane of the excitation laser light was perpendicular to the plane formed by the incident laser light and the collection optical fiber. An optical multichannel analyzer (OMA) system that was synchronized with the laser pulses analyzed the spectra that passed a 570-nm long-pass filter, a narrow slit, and a spectrograph. A computer averaged the spectra over five measurements and displayed the averaged spectra.

3. Results

Figure 2(a) shows the autofluorescence spectrum of the tissue and the fluorescence spectrum of the pure dye solution contained in a quartz cuvette. Figure 2(b) compares the emission spectra of the dye-infused muscle tissues with, respectively, low- and high-excitation energy. The dye concentration in the ethanol solution was $1.69 \times 10^{-3}\,M$. The spot area of the laser beam on the sample surface was 1.47 mm^2. When the excitation energy of the laser beam on the dye-infused muscle tissue was increased from 0.07 to 3 mJ, the emission spectrum changed from a fluorescence spectrum to a laser-action spectrum, where the laser action had an ~19% full width at half-maximum (11 nm versus 58 nm) and 52 times the peak height of the fluorescence spectrum.

Figure 3 shows the emission linewidth as a function of the radiant exposure of the excitation laser while (a) the excitation laser energy was varied between 1 and 4 mJ and the spot size was held constant at 1.47 mm^2, and (b) the excitation laser spot size was varied between 0.85 and 2.6 mm^2 and the excitation laser energy was held constant at 2.5 mJ, respectively. It was apparent that the linewidth, within the experimental limits, depended primarily on the radiant exposure regardless of the

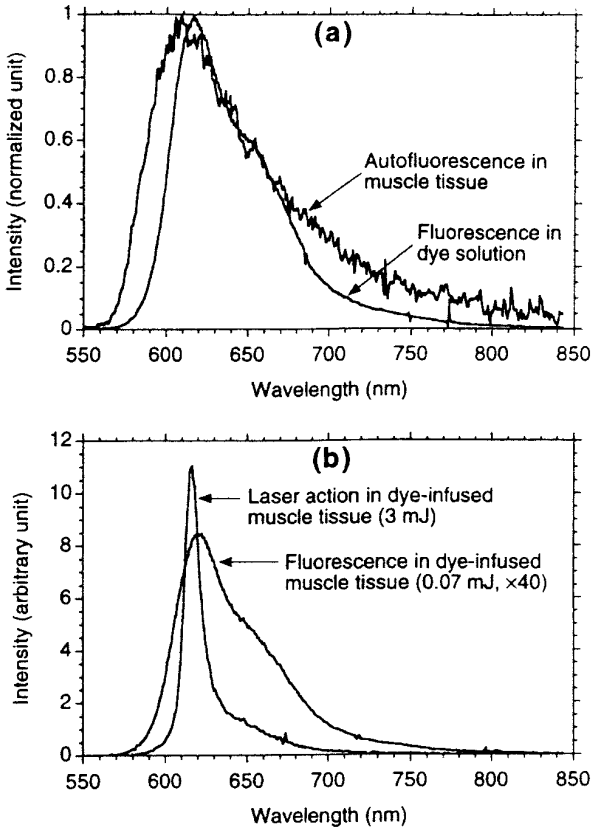

Fig. 2. Emission spectra: (a) pure Rhodamine 640 perchlorate dye solution and the autofluorescence of the rat muscle tissue, (b) Rhodamine 640 perchlorate dye-infused muscle tissue with 0.07 and 3 mJ of excitation laser energy. The excitation laser energy values and the scaling factors of the spectra are listed in parentheses.

total energy or spot size. The threshold radiant exposure causing laser action was found to be 2 mJ/mm^2, beyond which the linewidth was not affected by an increased radiant exposure.

Figure 4 shows the emission linewidth as a function of various dye concentrations in the ethanol solution when the amount of dye solution injected

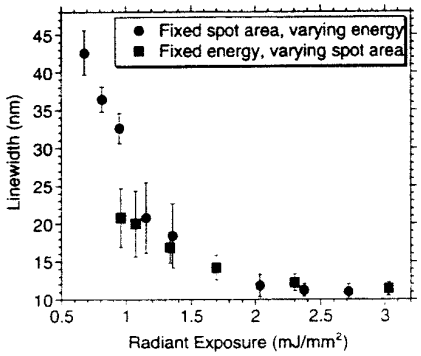

Fig. 3. Spectral linewidth of the Rhodamine 640 perchlorate dye-infused rat muscle tissue as a function of excitation laser radiant exposure while the energy or spot area was kept constant.

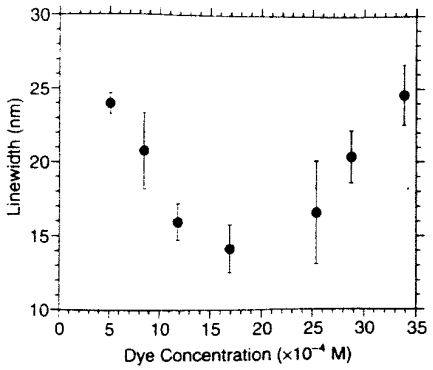

Fig. 4. Spectral linewidth of the Rhodamine 640 perchlorate dye-infused rat muscle tissue as a function of the dye concentration in the rat muscle.

into each piece of muscle tissue was held constant at 0.2 mL. The averaged values and standard deviations were based on five measurements. The excitation laser energy and spot size were held constant at 2.5 mJ and 1.47 mm^2, respectively. An optimal concentration was found to be approximately $1.7 \times 10^{-3} M$.

Monte Carlo simulations[7,8] were used to compute the excitation light fluence distribution in the tissue used in the above experiments with Rhodamine 640 perchlorate. Figure 5(a) shows the schematic of the setup that was used in the Monte Carlo simulation. Figure 5(b) shows the internal distribution of excitation light fluence in the tissue when the low-power absorption coefficient of the Rhodamine 640 perchlorate dye solution was used as the absorption coefficient of the tissue system. Figure 5(c) shows the internal distribution of excitation light fluence in the tissue when the intrinsic absorption coefficient of the tissue was used and the absorption of the Rhodamine 640 perchlorate dye was neglected. The optical properties that were used for Fig. 5(b) at the 532-nm wavelength were as follows: absorption coefficient, 18 cm^{-1} (see Section 4); reduced scattering coefficient, 10 cm^{-1}; and assumed anisotropy factor, 0.9. The optical properties that were used for Fig. 5(c) at the 532-nm wavelength were as follows: absorption coefficient, 1.0 cm^{-1}; reduced scattering coefficient, 10 cm^{-1}; and assumed anisotropy factor, 0.9. The optical properties of the intrinsic rat muscle were extrapolated values from the data in the literature.[9]

To illustrate how long photons traverse in the tissue before they escape into the air, we simulated the traverse time of photons originated isotropically inside the tissue (0.1 cm deep) by using Monte Carlo simulations (Fig. 6).[7,8] We used the intrinsic optical properties of rat muscle at the peak emission wavelength of 617 nm, i.e., absorption coefficient of 0.08 cm^{-1}, the reduced scattering coefficient of 10 cm^{-1}, and the assumed anisotropy factor of 0.9.[9] The number of diffusely reflected photons per unit time interval (diffuse reflectance) increased sharply at the beginning and then decreased approximately expo-

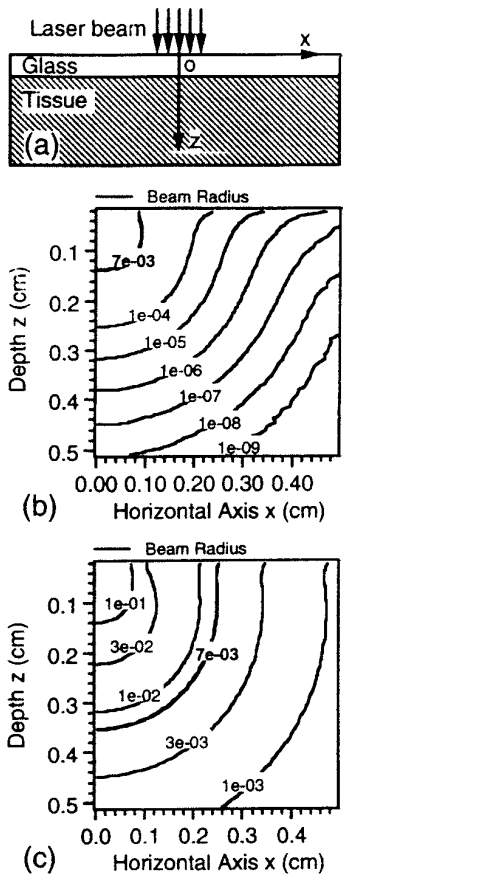

Fig. 5. (a) Setup that was used for Monte Carlo simulations of excitation light distribution in Rhodamine 640 perchlorate dye-infused rat muscle. The diameter of the laser beam was 0.137 cm, corresponding to the 1.47-mm^2 beam area. The pulse energy and pulse width were 3 mJ and 10 ns, respectively. The thickness of the glass layer was 0.016 cm. (b), (c), Fluence (J cm^2) of excitation light at a 532-nm wavelength, using the low-power absorption coefficient of the dye as the absorption coefficient of the tissue system (b), and using the intrinsic absorption coefficient of the tissue system and neglecting the dye absorption (c).

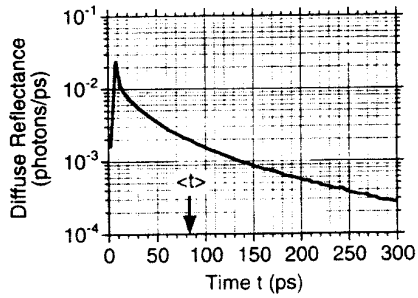

Fig. 6. Monte Carlo simulated diffuse reflectance of light (photons ps) from the glass surface as a function of time. The geometric setup is the same as that shown in Fig. 5(a). An impulse isotropic point source of light was buried 0.1 cm below the tissue surface on the z axis. The average time (t) that photons travel in the tissue system was 82 ps.

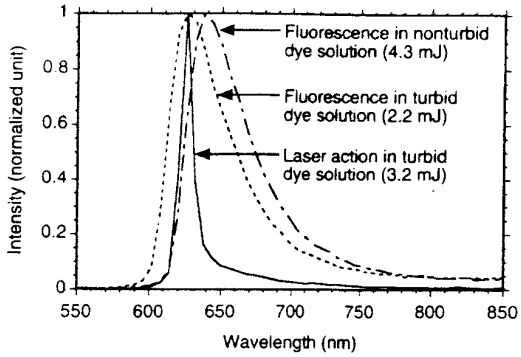

Fig. 7. Emission spectra of biocompatible fluorescein sodium dye dissolved in two solutions: PBS (nonturbid), and PBS mixed with scattering polystyrene spheres (turbid). The excitation laser energy values are listed in the parentheses.

nentially with time. The average flight time in the tissue or glass was 82 ps, corresponding to approximately a 1.8-cm path length in tissue.

Figure 7 shows the emission spectra of biocompatible fluorescein sodium dissolved in phosphate-buffered saline (PBS) and in PBS mixed with scattering polystyrene spheres, respectively. The first dye solution was nonturbid and made of 60 mg of fluorescein sodium per milliliter of PBS, which corresponds to 0.16 M because the molecular weight of the fluorescein sodium is 376.3. The second dye solution was turbid and made of 60 mg of fluorescein sodium per milliliter of PBS, with an addition of a 0.05-mL, 8% polystyrene-spheres solution (diameter of spheres, 579 ± 21 nm). The low-power extinction coefficients of the fluorescein sodium dye at 532 nm and 626 nm were 350 and 20 M^{-1} cm^{-1}, respectively. Hence, the low-power absorption coefficients of the fluorescein sodium dye solution at 532 nm and 626 nm were 129 cm^{-1} and 7.4 cm^{-1}, respectively. The reduced scattering coefficients of the turbid dye solution at 532 and 626 nm were 15 cm^{-1} and 13 cm^{-1}, respectively. A 1-cm-thick quartz cuvette containing the dye solution was placed where the sample was in Fig. 1. The spot area of the laser beam on the solution surface was 1.47 mm^2. When the excitation laser energy increased from 2.2 to 3.2 mJ for the turbid dye solution, a sudden narrowing of the emission spectrum from 51 nm (full width at half-maximum) to 10.5 nm was observed. The excitation energy for laser action in the turbid dye solution (3.2 mJ) was less than the excitation energy that yielded only fluorescence in the nonturbid dye solution (4.3 mJ).

4. Discussion and Conclusions

Figure 2(b) shows that laser action can be obtained similarly in dye-infused biological turbid media as in physical turbid media. The dye concentration was 1.69×10^{-3} M in the ethanol solution, and it was reduced to 2.4×10^{-4} M in the tissue because of the additional tissue volume (see Section 2). The extinction coefficient of Rhodamine 640 perchlorate at the excitation wavelength (532 nm) was $\sim 3.2 \times 10^4$ M^{-1} cm^{-1}. The low-power absorption coefficient of the dye in the tissue was then $\ln(10) \times (2.4 \times 10^{-4}\ M) \times (3.2 \times 10^4\ M^{-1}\ \text{cm}^{-1}) = 18\ \text{cm}^{-1}$, which was much higher than the intrinsic absorption coefficient of the rat muscle (~ 1 cm^{-1}). The reduced scattering coefficient of the rat muscle at the excitation wavelength (532 nm) was approximately 10 cm^{-1}, and hence the transport mean free path length in the native tissue was 0.09 cm. At a low excitation intensity, the light energy will be limited to a small zone. However, when the excitation intensity approaches the saturation intensity of the dye (0.7 MW/cm^2),[1] the saturated dye becomes transparent, and the excitation light energy will be distributed into a larger volume in the tissue.

Based on the pulse width of the excitation laser beam (10 ns), the saturation intensity of the dye (0.7 MW/cm^2) can be converted into a saturation fluence of 7.0×10^{-3} J/cm^2, for which the contour curves were bold in Figs. 5(b) and 5(c). The saturation region enclosed by the contour curve of the saturation fluence provides a gain medium for amplification of spontaneous emission. The computation for Fig. 5(b) assumed the low-power absorption coefficient of the dye solution as the absorption of the tissue system; hence the saturation region in Fig. 5(b) represents the minimal volume of the gain medium. In contrast, the computation for Fig. 5(c) ignored the absorption of the dye solution and used the intrinsic absorption of the rat muscle; hence the saturation region in Fig. 5(c) represents the maximal volume of the gain medium.

In a nonturbid Rhodamine 640 perchlorate dye solution, the saturation region would be a narrow cylinder. Therefore, the shape of the saturation region in the turbid media will facilitate the amplification of spontaneous emission because photons can travel longer in an approximately semispherical region than in a narrow and long region.

At the wavelength of the laser action peak (617 nm), the extinction coefficient of the Rhodamine 640 perchlorate dye was $\sim 920\ M^{-1}$ cm^{-1}. The low-power absorption coefficient of the dye in the tissue system of Fig. 2 was then 0.51 cm^{-1} and was greater than the intrinsic absorption coefficient of the tissue (0.08 cm^{-1}). The reduced scattering coefficient of the rat muscle at the 617-nm wavelength was approximately 10 cm^{-1}, and hence the transport mean free path length was 0.1 cm.[9] When the excitation intensity approaches the saturation intensity of the dye, the path length of spontaneous emission photons may be stretched long enough to observe laser action. Figure 6 illustrates that a photon originated from 0.1 cm, which is one transport mean free path length, below the tissue surface traversed on average ~ 1.8 cm in the tissue. Therefore, turbid media can increase the path length of photons to facilitate amplification of spontaneous emission.

Figures 3 demonstrates that the spectral linewidth depends primarily on the radiant exposure,

which is the total excitation energy divided by the spot area of excitation on the tissue surface. The data taken at a fixed spot area of excitation indicate that the spectral linewidth decreases with an increased excitation energy. The higher the excitation energy, the higher the gain and the larger the gain volume, which leads to a narrower spectral linewidth.

The data taken at a fixed excitation energy indicate that the spectral linewidth decreases with a decreased spot area of excitation. Simulations that are similar to those in Fig. 5 show that, when the excitation energy is held constant at 2.5 mJ and the spot area of excitation increases from 0.5 to 3.0 mm^2, the minimum and maximum saturation volumes in the tissue stay approximately the same, which cannot explain the decreased linewidth with a decreased spot area of excitation. More accurate models are needed to simulate the saturation volume in tissue.

Figure 4 indicates that there exists an optimal dye concentration for laser action. This phenomenon can be explained by the excitation capability of the laser pulse with a given radiant exposure, if the dye solution is assumed to be absorbing only and the tissue provides the scattering. When the concentration is increased from a low value, more dye molecules are excited to act as the gain medium; hence, the linewidth narrows. However, when the concentration reaches a point at which more dye molecules are provided than the laser pulse can excite, the ground-state dye molecules act as absorbers; hence, the linewidth increases with the increased dye concentration.

The toxicity problem of the dye solution has to be solved before we can test laser action in biological tissues *in vivo*. The toxicity of Rhodamine 640 perchlorate was not well studied. The 70% ethanol solution has known toxicity to cells, although it is less toxic than the methanol originally used in our experiments to test laser action in biological tissues. The fluorescein sodium dye dissolved in PBS is biologically compatible, and laser action was observed when scattering polystyrene spheres were added into the solution (Fig. 7). Unfortunately, the absorption peak of fluorescein sodium is near 460 nm, and the absorption at 532 nm is ~30-fold less than the peak absorption. Therefore, the dye concentration that was used in our experiment was very high to produce a gain medium for laser action. This problem may be solved by the choice of an appropriate excitation wavelength. Other biocompatible dyes and solvents should also be investigated.

When dye molecules are injected into biological tissues, they are affected by the host tissue through binding or collisions. Therefore, the emission spectra of the dye may have different spectral peak positions in different tissues, such as diseased versus normal tissues. Because the spectral peaks of laser action are much sharper than those of fluorescence, the laser action should be more sensitive in detecting any shift of the spectral peak than fluorescence. This unique feature may find applications in the diagnosis of superficial lesions. A comparison of laser action spectra between normal and abnormal tissues will test this hypothesis.

5. Summary

Laser action in dye-infused biological tissues was observed and was speculated to be caused by scattering-enhanced amplification of spontaneous emission. The sharp spectral peaks of laser action may carry information of the host tissue that can be used for high-sensitivity diagnoses of disease.

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